American Chemical Society  
Division of Biochemical Technology  

S. Banta, Program Chair; R. Venkat, Program Chair

SUNDAY MORNING

**Downstream Processes**
a. hesslein, Organizer; A. Lenhoff, Organizer; A. Lenhoff, Presiding; a. hesslein, Presiding Papers 1-8

**Stem Cells and Regenerative Medicine**
B. Harley, Organizer; H. Kong, Organizer; B. Harley, Presiding; H. Kong, Presiding Papers 9-16

**Recent Advances in Biotechnology Product Development**
J. Maynard, Organizer; S. Vunnum, Organizer; J. Maynard, Presiding; S. Vunnum, Presiding Papers 17-23

**The Alan S. Michaels Award for the Recovery of Biological Products Lecture**
S. Banta, Organizer; R. Venkat, Organizer; R. Venkat, Presiding; S. Banta, Presiding Papers 24

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A. Lenhoff, Organizer; a. hesslein, Organizer; A. Lenhoff, Presiding; a. hesslein, Presiding Papers 25-31

**Biophysical and Biomolecular Processes**
J. Laurence, Organizer; Y. Gokarn, Organizer; J. Laurence, Presiding; Y. Gokarn, Presiding Papers 32-39

**Stem Cells and Regenerative Medicine**
I. Banerjee, Organizer; R. Brandenberger, Organizer; I. Banerjee, Presiding; R. Brandenberger, Presiding Papers 40-47

**Recent Advances in Biotechnology Product Development**
R. Tatichek, Organizer; G. Miro-Quesada, Organizer; G. Miro-Quesada, Presiding; R. Tatichek, Presiding Papers 48-55

**The David Perlman Lecture**
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**Biophysical and Biomolecular Processes**  
J. Laurence, Organizer; Y. Gokarn, Organizer; J. Laurence, Presiding; Y. Gokarn, Presiding Papers 65-72

**Upstream Processes**  
O. Lara, Organizer; S. T. Sharfstein, Organizer; O. Lara, Presiding; S. T. Sharfstein, Presiding Papers 73-80

**Recent Advances in Biotechnology Product Development**  
J. Otero, Organizer; P. M. Alves, Organizer; P. Reddy, Organizer; J. G. Aunins, Organizer; J. G. Aunins, Presiding; J. Otero, Presiding; P. M. Alves, Presiding; P. Reddy, Presiding Papers 81-88

**Stem Cells and Regenerative Medicine**  
A. Engler, Organizer; B. Rao, Organizer; A. Engler, Presiding; B. Rao, Presiding Papers 89-95

**BIOT Young Investigator Award Lecture**  
R. Venkat, Organizer; S. Banta, Organizer; R. Venkat, Presiding; S. Banta, Presiding Papers 96

MONDAY AFTERNOON

**Downstream Processes**  
A. Potty, Organizer; L. Pampel, Organizer; C. Haynes, Organizer; A. Potty, Presiding; C. Haynes, Presiding; L. Pampel, Presiding Papers 97-104

**Biophysical and Biomolecular Processes**  
J. Laurence, Organizer; Y. Gokarn, Organizer; J. Laurence, Presiding; Y. Gokarn, Presiding Papers 105-112

**Upstream Processes**  
S. T. Sharfstein, Organizer; O. Lara, Organizer; O. Lara, Presiding; S. T. Sharfstein, Presiding Papers 113-119
Recent Advances in Biotechnology Product Development  
T. Seewoester, Organizer; C. Goochee, Organizer; C. Goochee, Presiding; T. Seewoester, Presiding Papers 120-127

Stem Cells and Regenerative Medicine  
E. Tzanakakis, Organizer; Y. Nie, Organizer; E. Tzanakakis, Presiding; Y. Nie, Presiding Papers 128-135

MONDAY EVENING

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K. Kao, Organizer; M. L. Dickson, Organizer Papers 214, 421, 463, 241, 408, 264, 413, 417, 211, 428, 216, 452, 239, 458, 283, 277

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Biophysical and Biomolecular Processes  
C. Roberts, Organizer; S. Hershenson, Organizer; C. Roberts, Presiding; S. Hershenson, Presiding Papers 144-150

Upstream Processes  
H. Dorai, Organizer; K. Jones Prather, Organizer; H. Dorai, Presiding; K. Jones Prather, Presiding Papers 151-158

Biofuels  
J. Dueber, Organizer; U. Lao, Organizer; J. Dueber, Presiding; U. Lao, Presiding Papers 159-166

Stem Cells and Regenerative Medicine  
J. Butcher, Organizer; T. McDevitt, Organizer; J. Butcher, Presiding; T. McDevitt, Presiding Papers 167-173

TUESDAY AFTERNOON

Downstream Processes  
J. Hubbuch, Organizer; J. Vogel, Organizer; J. Vogel, Presiding; J. Hubbuch, Presiding Papers 174-181

Biophysical and Biomolecular Processes
C. Roberts, Organizer; S. Hershenson, Organizer; C. Roberts, Presiding; S. Hershenson, Presiding Papers 182-189

**Upstream Processes**
C. Komives, Organizer; M. D. Hilton, Organizer; C. Komives, Presiding; M. D. Hilton, Presiding Papers 190-197

**Biofuels**
U. Lao, Organizer; J. Dueber, Organizer; J. Dueber, Presiding; U. Lao, Presiding Papers 198-204

**TUESDAY EVENING**

**Poster Session**
K. Kao, Organizer; M. L. Dickson, Organizer Papers 205-300

**WEDNESDAY MORNING**

**Downstream Processes**
D. Roush, Organizer; J. Pieracci, Organizer; D. Roush, Presiding; J. Pieracci, Presiding Papers 301-307

**Biophysical and Biomolecular Processes**
K. Mallela, Organizer; S. Krishnan, Organizer; K. Mallela, Presiding; S. Krishnan, Presiding Papers 308-315

**Upstream Processes**
R. Srivastava, Organizer; G. Sriram, Organizer; G. Sriram, Presiding; R. Srivastava, Presiding Papers 316-323

**Biofuels**
J. A. Morgan, Organizer; Q. Hu, Organizer; J. A. Morgan, Presiding; Q. Hu, Presiding Papers 324-331

**Emerging Topics in Protein Engineering**
W. Chen, Organizer; D. Ercek, Organizer; D. Green, Organizer; D. Ercek, Presiding; D. Green, Presiding; W. Chen, Presiding Papers 332-339

**WEDNESDAY AFTERNOON**

**Downstream Processes**
A. Hunter, Organizer; K. Goklen, Organizer; A. Hunter, Presiding; K. Goklen, Presiding Papers 340-347
Biophysical and Biomolecular Processes
K. Mallela, Organizer; S. Krishnan, Organizer; K. Mallela, Presiding; S. Krishnan, Presiding Papers 348-355

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R. Srivastava, Organizer; G. Sriram, Organizer; G. Sriram, Presiding; R. Srivastava, Presiding Papers 356-363

Biofuels
D. Bond, Organizer; H. Beyenal, Organizer; P. de Figueiredo, Organizer; D. Bond, Presiding; H. Beyenal, Presiding; P. de Figueiredo, Presiding Papers 364-371

Emerging Topics in Protein Engineering
M. Raab, Organizer; P. Heinzelman, Organizer; G. Kannan, Organizer; G. Kannan, Presiding; M. Raab, Presiding; P. Heinzelman, Presiding Papers 372-379

WEDNESDAY EVENING

Poster Session
M. L. Dickson, Organizer; K. Kao, Organizer Papers 380-467

THURSDAY MORNING

Emerging Topics in Protein Engineering
E. Shusta, Organizer; R. Kelley, Organizer; E. Shusta, Presiding; R. Kelley, Presiding Papers 468-474

Biophysical and Biomolecular Processes
T. Przybycien, Organizer; W. Liu, Organizer; T. Przybycien, Presiding; W. Liu, Presiding Papers 475-482

Upstream Processes
I. Aldor, Organizer; P. Cirino, Organizer; I. Aldor, Presiding; P. Cirino, Presiding Papers 483-489

Biofuels
X. Lin, Organizer; z. Fan, Organizer; X. Lin, Presiding; z. Fan, Presiding Papers 490-497

THURSDAY AFTERNOON
Emerging Topics in Protein Engineering
I. Kwon, Organizer; J. Champion, Organizer; I. Kwon, Presiding; J. Champion, Presiding Papers 498-505

Biophysical and Biomolecular Processes
A. Dumetz, Organizer; J. Cochran, Organizer; A. Dumetz, Presiding; J. Cochran, Presiding Papers 506-513

Upstream Processes
G. Seth, Organizer; M. Antoniewicz, Organizer; G. Seth, Presiding; M. Antoniewicz, Presiding Papers 514-521

Biofuels
M. Lynch, Organizer; V. Rajgarhia, Organizer; M. Lynch, Presiding; V. Rajgarhia, Presiding Papers 522-529
Understanding and predicting protein adsorption in multimodal chromatographic systems

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Multimodal chromatographic systems offer improved selectivity over traditional separation methods such as ion exchange or hydrophobic interaction chromatography. Chromatography, nuclear magnetic resonance, and isothermal titration calorimetry experiments were used in conjunction with molecular simulations to probe the binding interactions in multimodal chromatographic systems. This multifaceted approach provided significant insight into the interactions taking place at the molecular level. In order to improve the selectivity in these systems, a variety of mobile phase modifiers were employed to probe the binding mechanisms of protein libraries to several multimodal resins. Experiments were carried out using high-throughput batch experiments as well as column experiments with various classes of mobile phase additives, pH, and salt types. The results clearly demonstrate how the appropriate use of mobile phase modifiers and multimodal resins can enable unique separations to be performed that cannot be achieved with traditional methods.

Multimodal polishing resins and the effect of ligand concentration

Kristina Nilsson-Välimaa(1), kristina.nilsson-valimaa@ge.com, Björkgatan 30, Uppsala Uppsala 75184, Sweden; Hans Rogl(2); Susanne Konrad(2); Linda Pell(1); Hans J Johansson(1). (1) GE Healthcare, Sweden (2) Roche Diagnostics GmbH, Germany

Since the development of combinatorial chemistry and corresponding ligand libraries in the eighties significant efforts have been made on development of affinity mimetics targeting e.g. immunoglobulins. However, most of the work has been focusing on ligand diversity and very little is published on the effect of ligand concentration on purification performance. With the appearance of high throughput technologies for screening of chromatographic conditions it is now possible to study the effect of ligand densities and running conditions efficiently. In this study two different multi-modal ligands, N-Benzyl-N-methyl ethanolamine (adhere) and N-benzoyl-homocysteine (MMC) were immobilized to
an agarose based resin at different ligand concentrations. The prototypes were investigated for the polishing of a monoclonal antibody using both 96-well filter plates and Design of Experiment studies in column format. This presentation will demonstrate the effect of ligand concentration on binding capacity, yield and removal of critical contaminants e.g. aggregates and host cell proteins.

BIOT 3

Clustered-charge anion exchange adsorbents: Studies of adsorption at the macro and single-molecule levels

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Traditionally, charged ligands are introduced into ion-exchange matrices by random chemical processes, producing a heterogeneous charge distribution. In previous work we demonstrated the improved protein-binding capacity and selectivity of ion-exchange adsorbents displaying a “clustered” rather than random, distribution of surface charges. We also found that clustered adsorbents selectively favor proteins with inherent charge clustering. The current work shows that “clustered” penta-arginamide adsorbents show DNA binding capacity comparable to that of conventional dispersed adsorbents with 10- to 100-fold higher ligand density. We also observed that at moderate ionic strength the DNA affinity of all adsorbents tested increased with salt while RNA affinity decreased, so that selectivity for DNA over RNA was enhanced as salt concentration increased. Early results of single-molecule studies of protein ion-exchange adsorption are also presented.

BIOT 4

Hyperthermophilic affinity ligands for protein purification

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Affinitychromatography has great potential in reducing the cost and complexity of protein purification steps in the production of biopharmaceuticals. However,
availability of robust affinity ligands for use in industrial chromatography remains a challenge. Here we show that the DNA binding 7 kDa Sso7d protein from the hyperthermophilic archaean Sulfolobus solfataricus can be used as a scaffold to generate stable affinity ligands. We successfully isolated binding proteins for a wide spectrum of model targets from combinatorial library of Sso7d mutants. The Sso7d-based ligands show remarkable stability against thermal and chemical denaturation as well as extended exposure to extreme conditions of pH. Further, we also describe Sso7d-based proteins binding the Fc portion of human IgG (hFc) that have been additionally selected for mild elution conditions. These hFc binding proteins can be used for purification of humanized antibodies, as well as human IgG from plasma for use in intravenous immunoglobulin therapy.

BIOT 5

WITHDRAWN

BIOT 6

New, robust methods for protein elution from ceramic hydroxyapatite (CHT)

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Ceramic hydroxyapatite (CHT) is used for protein purification owing to its superior removal of all process stream impurities (aggregates, HCP, viruses, etc.). At commercial scale CHT can exhibit fewer cycles than desired. This is due to the release of protons during elution, lowering mobile phase pH and decreasing the stability of CHT. We have developed a novel technology, called the Surface Neutralization System (SNS), which desorbs protons from the surface of CHT prior to elution. We have demonstrated that the SNS technique significantly extends the useful life of CHT and prevents pH drop during elution, without desorbing the target protein, when the target protein is a monoclonal antibody or polyclonal human IgG. Data will be presented demonstrating that this technology does not impact the common quality outputs usually monitored during antibody purification.

BIOT 7

Salt tolerant membrane adsorbers for large scale polishing in flow-through mode

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In anion exchange flow-through (FT-AIEX) applications membrane chromatography offers higher throughput, less buffer consumption and more convenient handling than traditional bead columns. Conventional quaternary amine based chemistries – although established as a standard FT polishing step in Monoclonal Antibody (MAb) purification processes – typically require low feed conductivity. This often involves dilution offeedstreams and can result in facility fit limitations when high titer processes are accommodated in existing plants. Furthermore, high impurity levels limit load densities and would require larger membrane adsorber volumes and lead to increased production cost. To address these limitations and facilitate a wider design space for FT-AIEX membrane chromatography at commercial scale, a novel membrane concept based on a weak anion exchange chemistry that is less sensitive to increasing salt concentration than standard Q membranes was developed. In this study, the performance of Sartobind STIC® (Salt Tolerant Interaction Chromatography) was explored for host cell protein (CHOP) clearance using industrially relevant MAb feedstreams at different experimental conditions. The novel membrane adsorber was also tested for clearance of relevant bacteriophages and model viruses.

**BIOT 8**

Performance of a novel salt tolerant membrane adsorber in flow-through mode

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A salt tolerant membrane adsorber provides trace contaminant clearance without feedstock dilution and can be sized much smaller than bead adsorbers. This paper will show performance data for CHOP and virus clearance under a variety of operating and solution conditions. Large and small devices will be compared along with the effect of membrane variability.

**BIOT 9**

Biodegradable conducting polymer-based scaffold for electric and magnetic field controlled tissue engineering
Abstract: Electrically conducting and biodegradable copolymers, containing both self-doped conducting polymer and biodegradable polymer segments, were enzymatically synthesized and characterized. These copolymers display unique characteristics such as conductivity, biocompatibility and biodegradability. Various conducting scaffolds were prepared through different processing methods. Experiments demonstrate that these copolymers are not only biodegradable, but also biocompatible, by supporting the growth of osteoblasts. Electric field stimulation of various cells of the osteoblastic lineage grown on these copolymers revealed positive effects on not only cellular proliferation, but also differentiation, into a more mature, bone-forming phenotype. Our findings suggest that these copolymers have beneficial effects on bone formation, which may have clinical utility in treating various musculoskeletal pathologies.

Keywords: Electric-magnetic tissue engineering, biodegradable, self-doped conducting polymer, osteoblasts.

BIOT 10

Assessment of a new biomaterial designed to restore pliability to scarred vocal folds

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A majority of unresolvable hoarseness is due to vocal-fold (VF) scarring that permanently decreases the pliability of the phonatory mucosa and degrades voice quality. We created a novel polyethylene glycol-based injectable hydrogel
(PEG30) and investigated its biocompatibility and function in vivo. Biocompatibility of PEG30 was evaluated in ferret and canine VFs. Functional impact of PEG30 on normal VF vibration was assessed by injection into canine VFs followed by periodic assessments of in vivo VF function using high-speed-videos (HSV) of VF-vibration induced by intratracheal airflow. HSV-analysis showed no reductions or only slight reductions in the maximum vibratory amplitudes of the injected VFs. No endoscopic signs of significant inflammation were observed in any animal. Histology revealed macrophage-mediated resorption of PEG30 with minimal to no fibrosis. PEG30 is a promising candidate to restore vibration to scarred VFs since it exhibits in vivo biocompatibility, pliability, and does not impede endogenous VF function.

BIOT 11

Gradient hydrogel systems to assess biophysical regulation of hematopoietic stem cell fate decisions

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Hematopoietic stem and progenitor cells (HSPCs) are an ideal platform for studying extrinsic regulation of stem cell behavior. We have developed biomaterial systems to assess the effects of cell-matrix and cell-cell cues on HSPC biophysical parameters and early fate decisions. For these experiments we isolate HSPCs as Lin−cKit+Sca1+ cells from murine bone marrow and use MC3T3-E1 pre-osteoblasts as niche cells. We have developed a microfluidic platform to create combinatorial collagen hydrogel microenvironments (niche-mimics) comprised of uniform vs. bidirectional, opposing gradients of cells and/or matrix. Regions in these microenvironments correspond to distinct hydrogel properties or ratios of HSPC:niche cells, allowing imaging or post-culture isolation and analysis of HSPC fate decisions. We have shown a significant influence of matrix microenvironment on HSPC cytoskeleton and viability. Ongoing work is quantifying differences in HSPC viability, proliferation, and functional capacity under defined niche conditions.

BIOT 12

Direct biophotolithography to create instructive biomaterials: Spatially-controlled biomolecular functionalization of porous collagen scaffolds for applications in tissue regeneration
We have developed a biomolecularly general method to attach multiple biomolecules within porous collagen-GAG scaffolds into spatially well-defined geometric patterns and continuous gradients. In our approach, scaffolds modified with benzophenone moieties are exposed to ultraviolet light in the presence of biomolecules and the resulting photochemical crosslinking reaction facilitates the covalent immobilization of the biomolecules. This attachment only occurs at spatial locations where light interacts with the scaffold, and the amount of immobilized biomolecule can be carefully tuned by controlling exposure parameters. We find that our chemical modification scheme does not significantly affect the compositional or microstructural properties of the scaffold, and that photochemically-attached proteins can increase the rate of cell attachment and cell metabolic activity relative to native scaffolds. We feel that the biomolecular generality of this technique, coupled with the ability to create multi-component patterns/gradients, make this is versatile methodology well suited to a range of tissue regeneration studies.

**BIOT 13**

**Fabrication of multicompartmmenthydrogel scaffold using density gradient method**

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We report a new method, a density gradient method (DGM), as a simple and versatile technique to fabricate multicompartmmenthydrogels. Multicompartmment hydrogels with distinct layers of mechanical/chemical cues can mimic complex 3D structural organization or provide a gradient of cellular cues existing in native tissue. However, the requirement of integrating heterogeneous materials in a mechanically robust composite provides a challenge for fabrication. Unlike conventional methods, DGM allows integration of heterogeneous layers before gelation thereby resulting in improved cohesion between layers. We will highlight the DGM technique with respect to formation of a number of layers, their discreteness and the mechanical properties of the composite. Furthermore, we will discuss the ability of DGM to produce discrete and cross gradients of a variety of proteins based on diffusion in multilayers. Finally, we will show the ability of
multilayered hydrogels to produce a chemically distinct environment by allowing cell growth in specified layers.

BIOT 14

Tunability and tailorability of cell-triggered DNA release from a substrate-mediated delivery system

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The inherent limitations of bolus gene delivery may be avoided by surface immobilization of DNA onto biomaterial scaffolds. As an alternative to current immobilization approaches, we chemically bind plasmid DNA to a substrate via an enzymatically-labile peptide sequence, allowing for cell-responsive gene delivery. In our design, DNA is functionalized using a peptide nucleic acid (PNA) clamp. Coupling peptides with a matrix metalloproteinase-1 (MMP-1) degradable sequence are attached to this conjugate forming DNA-PNA-peptide (DPP) conjugates. In the past, we have demonstrated formation, immobilization, and MMP-1-responsive release of DPP conjugates from a model gold surface. Tunability of DNA release is being investigated by altering the number of DNA-surface tethers, altering the size of the polycation used during complexation, and decreasing surface coverage of the DPP conjugates. Simultaneously, we are transitioning the tethering mechanism from a model gold substrate to a polylysine-coated substrate to demonstrate the tailorability of the system.

BIOT 15

Collagen peptide-based hydrogel for cell encapsulation

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Collagen is a structural protein with the ability to self-assemble into fibers and networks as one of the main components of the ECM. This feature has inspired the creation of innovative biomaterials for tissue engineering. For this purpose, we have developed new type of collagen-based material by attaching a CGG-(POG)₈ collagen mimetic peptide to an 8-arm PEG-MAL star polymer to afford a 4% 8-arm PEG-CGG(POG)₈ hydrogel. This material possessed the ability to melt into a liquid-like state around the Tₘ of the peptide and reform back into an elastic-solid at RT, as determined by CD and rheology. In addition, the hydrogel demonstrated to function as a three dimensional matrix for hMSC culture as shown by MTS.
assays and fluorescence microscopy. Cryo-SEM images were taken to reveal a highly cross-linked network with pores that surrounded the cells providing physical support and a desirable scaffold for cell growth.

**BIOT 16**

**Combinatorial biomaterials screening for cardiac epithelial-to-mesenchymal transformation**

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In early cardiac valvulogenesis, cells in the atrioventricular (AV) cushion undergo epithelial-to-mesenchymal transition, invade the extracellular matrix (ECM) and begin remodeling the surrounding tissues into valve leaflets. Similar interactions occur between the epicardium and epicardial in the development of coronary vessel. Here, we use high throughput biomaterials screening methods to identify the contributions of different ECM components and signaling molecules to activate cardiac EMT. Cardiac-ECM biomaterials libraries were generated based on the results of proteomic studies of the developing heart as well as analysis of the gene regulatory networks pre- and post-cardiac EMT. Our screening methods reveal the relative importance of fibronectin over other ECM proteins present in the developing heart in cell adhesion and the promotion of EMT in endothelial progenitors. We further explore the links between fibronectin, and other ECM molecules, in epicardial EMT with a high throughput functional EMT assay. The methods developed here comprise a general model for high throughput cardiac EMT screening.

**BIOT 17**

**Process and product quality improvements of a challenging monoclonal antibody**

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During the initial implementation of a mAb producing CHO cell culture, limitations were found to exist around titer and aggregate formation. As a result, a process development project was initiated to investigate ways of increasing the titer and improving the aggregation characteristics of the mAb. This work was able to yield a process capable of a >100% increase in the ICA and a >50%
increase in specific productivity. Specific productivity and ICA increases resulted in an >100% improvement in the process titer. During the initial bioreactor runs, the unpaired cysteine content in the mAb product was found to be significantly higher than that reported for other antibodies. Special focus was directed at looking at covalent aggregation between the mAb molecules as well as between the mAb and host cell proteins. As a result, a more robust, enhanced CHO cell culture process was established with improved characteristics.

BIOT 18

Dissecting contributors to particle formation in drug product sourced from alternative drug substance processes

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During the development of an alternative process for a monoclonal antibody, particle formation in the drug product (DP) was observed in Process B but not in Process A. Materials from the two processes were analytically comparable by standard stability-indicating assays. The focus of this talk will be on the impact of the cell culture and purification processes (Process A vs. B), on atypical particle formation. The process was segregated to determine if the DP differences were due to the cell culture and/or purification process. Process A purification led to particle-free drug products, regardless of cell culture conditions. Drug product sourced from Process B purification formed particles over time and the kinetics differed slightly between A and B cell culture conditions, therefore the effect from cell culture on particle formation was less pronounced than the purification process. Segregating the purification process at the second chromatography step identified the second half of Process B, specifically the third chromatography step and viral filtration, as contributing to DP particle formation. Further characterization evaluated the individual effect from these two unit operations in Process A or B by including or excluding the corresponding step, and also the effect of the order of these steps. The impact on particulation of the resin and buffers for the third column from the two processes was also explored. The detailed results of the primary contributors from the drug substance process will be discussed. Formulation and primary container were also found to affect the formation of these particles in separate studies. The multi-faceted contributions to this issue highlight the link between process and product stability and the need for effective cross-functional collaboration.

BIOT 19
Caught by surprise: Unexpected lyophilization induced compound in a protein formulation

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Will enter abstract upon approval from legal department

BIOT 20

Predicting shelf life stability of monoclonal antibodies

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Biologics undergo modifications through physical and chemical reactions under various conditions such as solution pH, temperature and ionic conditions that ultimately affect shelf life. These modifications, are manifest as product qualities (such as size, charge variants, and activity), could present safety and efficacy concerns during human clinical trials. Using mathematical models, we analyze the modification of monoclonal antibodies under stressed conditions (elevated temperatures) and use the model to predict product stability at real time storage conditions (lower storage temperatures).

BIOT 21

Analytical lifecycle for marketed protein therapeutics

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Marketing approval for a protein therapeutic marks the culmination of years of development effort, and it is tempting to consider the bulk of the analytical work as complete. However, the drug will be on the market for a significantly longer period than the development time, and more work will come. During the lifespan of the product, it is likely that there will be process improvements and transfers of the manufacturing site. The lifecycle of the product similarly will require periodic analytical updating. Advances in technology, new analytical strategies, new understanding of the molecule and its mechanism of action, and a history of manufacturing results may lead to updates of the protein characterization and modifications of the control system. In this presentation, we will examine the analytical lifecycle strategy for marketed products.

BIOT 22
Crystallization: A specialized form of aggregation which can be exploited to develop high concentration monoclonal antibody formulations

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High concentration formulations are required for the efficacy of the monoclonal antibodies especially in metabolic disorder treatments and for the comfort of patients subjected to high volume injections. To ameliorate the viscosity issues and volume considerations of a liquid monoclonal antibody formulation, we have developed a crystalline formulation with a concentration of 330 mg/ml. The process can be scaled to a commercial formulation process with 95% recovery of protein in the crystalline form. The crystalline process can be made isotonic allowing direct injection of the crystals with its mother liquid. The viscosity of the crystalline formulation is less than 10 cP and the monoclonal antibody in the crystal form is stable over six months at room temperature. Animal studies are in progress to compare the efficacy of the crystalline formulation to that of the commercial liquid formulation.

BIOT 23

Development of follow-on biologicals: Challenges and opportunities

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Biotechnology derived protein therapeutics or biologicals have become a critical part of modern healthcare landscape. The substantial cost associated with the use of protein therapeutics, however, has resulted in causing significant financial burden to patients and the healthcare system and there is considerable pressure for introduction of cheaper or generic versions of these therapeutic agents. The expiry of patent protection for some protein therapeutics has resulted in the arrival of follow-on protein products or biosimilar version of the licensed reference product. Patents on more protein products are expected to expire in the near future and cheaper versions of these products are expected to be made available by several manufacturers. Nonetheless, there is considerable debate concerning the scientific and regulatory issues associated with introduction of a biosimilar product. The inherent complexities associated with biologicals and lack of clear guidelines has been a major hurdle in the approval of biosimilar version of a reference biological product. This presentation will focus on the challenges and opportunities in the development of follow-on or biosimilar biologics for marketing approval. In particular, technical considerations related to analytical, stability, pre-clinical, clinical and regulatory aspects in establishing equivalency of a reference protein therapeutic and its biosimilar version will be discussed. Finally,
challenges posed by issues related to intellectual property rights in the introduction of a biosimilar are also discussed briefly.

BIOT 24

Recovery of biological products research: Interplay between science and technology

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What do recombinant DNA techniques, fluid mechanics, interfacial science, biophysical methods and structural biology have in common? They are topics investigated by the Belfort Group that are either intimately connected with the recovery of biological products or have led to new fundamental understanding as a result of such research. Examples demonstrating novel approaches to the separation of biological molecules, such as using intein technology for fusion affinity separation, secondary flow for new self-cleaning module design and high throughput synthesis and screening of new surfaces for anti-protein fouling membranes, will be presented. Realizing that proteins lose conformation and can aggregate during filtration and adsorption onto synthetic membranes, fundamental questions relating to protein stability in solution and at interfaces will be discussed. Mimicking nature is the quintessential complement to evolution. So, together with others, we have embarked on a detailed effort to understand and then mimic the transport and exquisite selectivity of biological molecules passing through the nuclear pore complex (NPC). Implicit here is collaboration with colleagues, many of whom have different backgrounds to that of the Belfort Group and have made seminal contributions to the work described here. Mention will be made of Alan S. Michaels' influence on and his personal support of Georges Belfort's academic and research career.

BIOT 25

Countercurrent tangential chromatography for large-scale purification of high value proteins

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Countercurrent Tangential Chromatography (CTC) is a new column-free purification and capture technology that holds great promise for large-scale protein purification. CTC can provide a scalable, disposable, and continuous unit
operation that overcomes many limitations of packed bed chromatography. This talk will examine the underlying principles of CTC, including different modes of operation and potential advantages for large-scale capture applications. CTC replaces the stationary phase of a packed column with a moving slurry that is continuously pumped through several cascades of static mixers and hollow fiber membrane modules. Chromatographic operations of binding, elution, washing, and equilibration are performed directly on the moving slurry. The buffers required for each operation are introduced in a countercurrent direction to the slurry flow by recycling permeate solution from later stages. The concentration gradients created in this configuration ensure robust and predictable operation while reducing buffer volume requirements.

BIOT 26

Enhancement of MAb purification using simulated moving bed chromatography with HIC adsorbents

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Hydrophobic interaction chromatography (HIC) is frequently used as a polishing step to remove product-related impurities from monoclonal antibodies (MAbs) because of its high resolving power. This high resolution, however, is often achieved by sacrificing capacity and productivity which may demand larger columns and/or increased column cycles. Simulated moving bed chromatography (SMB) provides one solution for this. The main advantage of SMB lies in its counter-current nature, which can result in more efficient separation and increased productivity. In this study, HIC adsorbent was utilized in a multi-column SMB system to assess the clearance of high molecular weight (HMW) impurities from a MAb stream. Various system configurations were examined and the fractionation mode showed promising results. A comparison of SMB and conventional process performance will be presented in terms of load capacity, productivity, yield, and HMW clearance. Overall, SMB HIC demonstrated improved process productivity compared to conventional chromatography while maintaining impurity clearance.

BIOT 27

Preparative insulin purification by continuous countercurrent chromatography (MCSGP)

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Innovative purification technologies are of high interest, if they can be scaled up for industrial large scale production processes. Especially for the chromatographic purification steps, which are one of the largest cost drivers in the production of therapeutic proteins, it is desired to improve the existing processes and to develop less cost intensive strategies for the future. For the large scale production of human insulin for example, even small improvements in the downstream processing can significantly decrease the manufacturing costs.

One innovative approach to improve the current batch chromatographic purification steps is the MCSGP process. It is a continuous countercurrent chromatographic purification process, which applies solvent gradients in order to isolate the target component from a multi-component mixture with high yield, purity and productivity. In this work, a lab-scale MCSGP unit has been used for a reversed phase chromatographic purification step of human insulin. Compared to the current production process using batch chromatography, the MCSGP process experimentally shows a strong increase in the productivity. Simulations based on a lumped-kinetic model with a Moreau adsorption isotherm have been used as supportive tool in order to verify the design space for the operation of the MCSGP unit.

BIOT 28

Purification of PEGylated protein by hydrophobic interaction membrane chromatography

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In a recent paper we have reported the use of hydrophilized polyvinylidene fluoride (PVDF) membrane as environment-responsive stationary phase for the fractionation of unmodified lysozyme and its different PEGylated forms (i.e. mono-, di- and tri-PEGylated lysozyme) \(^{[1]}\). In the current study, a similar hydrophobic interaction based technique is applied to purify mono-PEGylated human serum albumin (HSA) prepared by N-terminus PEGylation reaction of the protein with m-PEG propionaldehyde. This reaction results in the formation of mainly mono-, and di-PEGylated and smaller amounts of tri-PEGylated HSA. The reaction mixture is separated at both analytical and preparative scales. The fractionated samples are analyzed and characterized using techniques such as SDS-PAGE and 2-D electrophoresis and multiple angle light scattering (or MALS) technique. References\(^{1}\). Deqiang Yu, Xiaojiao Shang, Raja Ghosh,

**BIOT 29**

**Insights into protein selectivity, ligand design and methods development for multimodal chromatography**

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Recent advances in the design of multi-modal chromatographic systems has produced materials which provide alternative and improved selectivity as compared to single mode interaction systems. We have used a strategic approach to the development of simulations and experiments to examine the relevant interactions in these systems. Molecular dynamics protein-ligand simulations were performed to determine multimodal ligand binding sites on the protein surface. Importantly, these simulations were found to corroborate experimental data obtained with both NMR and chromatography experiments for a library of homologous protein mutants. Further, these MD simulations were conducted with a combinatorial ligand library to study the role of synergy in the determination of ligand binding sites for these “pseudo-affinity” ligands. All-atom MD simulations were also performed to examine the binding of several proteins to self-assembled monolayers presenting relevant multimodal ligands. Finally, the effect of mobile phase modifiers and the development of efficient methods development protocols are presented.

**BIOT 30**

**Out with the old and in with the new: Mixed-mode chromatography as an alternative to anion exchange as a polishing step**

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While AEX chromatography is typically a good choice as a polishing step, it can sometimes be challenging for antibodies that bind with high affinity to AEX resins. This type of antibody binds under a wide pH range, at low load capacities, and requires high salt concentration elutions. This process can lead to ineffective impurity removal and lower viral clearance. An alternative to AEX adsorbent is mixed-mode adsorbent, which contains anionic and hydrophobic properties. Mixed-mode adsorbents can be operated in bind-elute, or flow-through mode, under a wide range of salt concentrations and pH’s. High step yields, and significant HCP and aggregate reduction can be achieved. We will present
several case studies employing mixed-mode adsorbents in bind-elute and flow-through mode. One example demonstrates a continuous process, connecting mixed-mode chromatography in flow-through mode with CEX chromatography in overloaded mode. High throughput of over 300 g antibody / L of resin can be achieved.

BIOT 31

Proteome-based development of novel affinity tail(s) for immobilized metal and hydrophobic interaction chromatographies

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We are in the process of developing a bioseparation system comprised of a suite of affinity tags used to facilitate purification and a set of Escherichia coli strains deficient in certain genomic proteins that either reduce column capacity or complicate gradient design. Our research team previously identified E. coli alpha-galactosidase as a protein which, under conditions of salt promoted adsorption, binds both Immobilized Metal Affinity Chromatography (IMAC) and Hydrophobic Interaction Chromatography (HIC) resins. The entire protein and individual domains of the protein have separately been tested as affinity tags based on a separation scheme that consists of IMAC followed by HIC. Results demonstrate that the combination of one of the newly developed affinity tail and simple loading and elution conditions can promote binding and shift [elution of] the model proteins into regions of few contaminants. The use of E. coli deletion mutants that do not express alpha-galactosidase and other nonessential, coeluting proteins completes the minimization of the contaminant pool for the model proteins. Combining affinity tail design based on proteomic data and homologous recombination to alter genomic protein expression, within the context of improving bioseparation, has placed this work on a path towards creating a novel platform strategy with E. coli.

BIOT 32

Thin film voltammetry of wild type and mutant reaction center proteins from photosynthetic bacteria

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Direct thin film voltammetry was used to study the electrochemical properties of the wild type Rhodobacter sphaeroides reaction center (RC) and those of specific mutants. Thin films of RC mixed with the lipid dimyristoylphosphatidyl choline (DMPC) or as layer-by-layer (LbL) films with polycations on pyrolytic graphite electrodes were constructed. Cyclic voltammetry (CV) and square wave voltammetry (SWV) of RC films revealed similar chemically irreversible electrochemical processes. The shape and appearance of the oxidation peak is affected by the starting potential. The previously observed oxidation peak at 0.95V vs. NHE was assigned as the primary donor (P), based on ferricyanide oxidized P leading to the disappearance of this peak. The reduction peak at -0.18V vs. NHE was assigned as the quinone on basis of our previous work. SWV of site-directed mutants RC films showed shifts in oxidation peak potential of P that correlated reasonably well with those reported from electrochemical titration experiments.

BIOT 33

Creation of a panel of glucose indicator proteins for continuous glucose monitoring

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The development of implantable glucose sensors for use in diabetes treatment has been pursued for decades. However, enzyme-based glucose sensors often fail in vivo. In our previous work, we engineered a novel glucose indicator protein (GIP) that can sense glucose without relying on any enzymes and cofactors. Nevertheless, this GIP is unsuitable for blood glucose monitoring due to its low dissociation constant. Here, we report a novel approach to creating a new GIP that can be used to monitor blood glucose level. By disrupting pi-pi stacking around GIP's glucose binding site through site-directed mutagenesis, we showed that GIP's dissociation constant can be manipulated from 0.026 mM to 7.86 mM. This approach yielded several GIP mutants. We showed that one of the mutants can be used to detect glucose from 0 to 32 mM, while another mutant can be employed to visualize intracellular glucose (~200 mM) within living cells through FRET imaging microscopy measurement.

BIOT 34
Exploring the trafficking, ligand-binding activity, and unfolding of a model GPCR

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G protein-coupled receptors (GPCRs) are integral membrane proteins vital for cellular signaling and constitute one of the major drug targets. Despite their importance, relatively little information regarding their structure, folding, and stability has been published. This work describes the impact of disulfide bonds on the expression and structural stability of the human adenosine receptor, A2A (hA2AR). The crystal structure of this receptor revealed four disulfide bonds present in extracellular loops that could contribute to expression, stability, or ligand binding or to a combination of these. To test the role of these residues, cysteine to alanine mutants of hA2AR were created; expression and ligand-binding activity of the constructs were tested in mammalian and yeast cells. Once purified from yeast, unfolding of the hA2AR through thermal and chemical means was monitored via intrinsic tryptophan fluorescence and circular dichroism. The effect of ligand addition and reduction of disulfide bonds was also investigated.

BIOT 35

Gauging the flexibility of fluorescent markers for an accurate interpretation of fluorescence resonance energy transfer (FRET)

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The spectroscopic ruler is a noninvasive technique exploiting fluorescence resonance energy transfer (FRET) to determine intramolecular distances in proteins and other bio-molecules [1,2]. Because FRET scales strongly with the separation distance and orientation between the optical dipole moments of the donor and acceptor molecules, the accuracy of the technique is limited when the molecules undergoes thermal fluctuations in physiological conditions. We introduce a statistical model based on the Fisher-von Mises distribution for the interpretation of fluorescence decay dynamics in donor-acceptor FRET pairs that allows us to retrieve both the mean orientation and the extent of directional fluctuations of the involved dipole moments [3,4]. We verify the method by

BIOT 36

Intraparticle fluorescent-based probe for tracking intracellular dissociation of ionic siRNA-complexes

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One of the critical barriers facing the delivery of polymer/lipid-siRNA complexes to their target tissue/cell is their untimely and timely disassembly in extracellular and intracellular environments. As such, imaging and analytical tools that can be used to probe the integrity of these complexes in a non-invasive manner are highly desired. To meet this demand, we have designed an intraparticle fluorescent-based probe that can be used to determine relative pseudo-dissociation constants (kd) for polymer/lipid-siRNA complexes and in addition, non-invasively track their intracellular and extracellular half-lives (t1/2). We demonstrate proof-of-concept by showing excellent correlation between our fluorescence data and the commonly used gel retardation assay. In addition, we
are able to measure extracellular and intracellular t_{1/2} of several commercially available and in-house siRNA transfection agents and we see good correlation between intracellular RNAi activity and t_{1/2} of the siRNA complexes. Information that can be obtained with this new probe will aid the development of structure-function relationships that will improve the design of future siRNA delivery vehicles.

BIOT 37

3D tracking of single mRNA particles in S. cerevisiae using a double-helix pointspread function

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The precise subcellular localization of mRNA-protein complexes plays a critical role in the spatial and temporal control of gene expression, and a full understanding of mRNA localization precise characterization of mRNA transport dynamics beyond the optical diffraction limit. In this paper, we describe three-dimensional tracking of single mRNA particles with 25 nm precision in the x and y dimensions and 50 nm precision in the z dimension in live budding yeast cells using a microscope with a double-helix point spread function. Two statistical methods to detect intermittently confined and directed transport were used to quantify the three-dimensional trajectories of mRNA using ARG3 mRNA as a model. The quantitative methods detailed in this paper can be broadly applied to the study of mRNA localization and the dynamics of diverse other biomolecules in a wide variety of cell types.

BIOT 38

Site-specific labeling of transcription factors using noncanonical amino acids and strain-promoted [2+3] cycloaddition via copper-free Click Chemistry

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Bioorthogonal chemical functionalities followed by copper-free click chemistry is promising tool. The labeling of transcription factor proteins (TFPs) by incorporation of non-natural amino acids was achieved. The cyclooctyne promotion [3 + 2] dipolar cycloaddition with azides, “copper-free click chemistry” provides rapid, site-specific labeling of TFPs. With successive chemical reactions, TFPs retain their sequence-specific DNA-binding activity under native conditions, whereas the activity of TFPs labeled by copper-mediated click chemistry was ablated. Copper-free click chemistry exhibited high reaction yields, even at low temperatures or in the presence of high salt concentrations, generally unfavorable reaction conditions for other common protein modification methods. Residue-specific replacement of methionine by azidohomoalanine was used to achieve mono- or multi-functionalization of engineered, “synthetic” TFPs. Overall, we anticipate that copper-free click chemistry mediated by non-natural amino acid incorporation will provide a robust and viable route for site-specific labeling of metallic proteins, which comprise a universal class of TFPs.

BIOT 39

Bottom-up assembly and characterization of multienzyme complexes

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Dynamic proximity between sequential enzymes is proposed to regulate metabolic pathways such as the purine de novo biosynthesis pathway and the Krebs cycle. Progress toward the bottom-up assembly and characterization of enzyme-nanoparticle bioconjugates where proximity can be controlled will be discussed. Gold nanoparticle scaffolds were used for immobilization of fluorescently-labeled sequential enzymes. The bioconjugates were characterized by dynamic light scattering, zeta potential, fluorescence and UV-vis spectroscopy to determine size and charge of the scaffolds, as well as stoichiometry and activity of the enzymes. The amount of enzyme attached to the particle was determined and the specific activity was calculated. The kinetic advantages of co-localization of sequential enzymes are also discussed. This approach allows for the study of sequential enzyme kinetics as a function of proximity alone.

BIOT 40

Human stem cell-derived blood-brain barrier platform for screening CNS-penetrating therapeutics

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Drug delivery to the CNS is hampered by the inability of neuropharmaceuticals to cross the blood-brain barrier (BBB), which is comprised of brain microvascular endothelial cells (BMECs) possessing specialized barrier properties. These cells can be cultured ex vivo to serve as a platform for high throughput screening of brain-penetrating compounds. Previous human BMEC platforms have intrinsic problems that prevent their widespread use, including insufficient yield upon isolation and poor barrier properties. We have addressed these issues by developing a novel human pluripotent stem cell (hPSC) differentiation scheme that yields endothelial cells expressing combinations of tight junction and transport proteins exclusive to the BBB. Upon purification, these BMECs exhibit many other hallmarks of the BBB, including a selective response to cultured astrocytes, high trans-endothelial electrical resistance, low passive permeability, and polarized transporter expression. These hPSC-derived BMECs should serve as a robust platform for the discovery of new CNS-penetrating agents.

BIOT 41

Drug-encapsulated super stiff poly(ethylene glycol) hydrogel for stem and progenitor cell mobilization

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Stem cell mobilization therapy is commonly implemented by repeatedly administering granulocyte colony stimulating factor (GCSF). Recently, GCSF is often encapsulated in injectable and biodegradable hydrogels to sustain stem cell
mobilization without repeating drug administration. Hydrogels used for this therapy should be rigid, to prevent uncontrolled release due to local tissue pressures, but degrade an appropriate rate to continually release drugs. However, conventional hydrogels are often plagued by limited controllability of drug release rate with increasing material stiffness. This study presents a new hydrogel system with extremely high elastic modulus, similar to that of polystyrene, but with controllable drug release rates. The hydrogel was formed from Michael reaction between acrylate groups of poly(ethylene glycol) diacrylate (PEGDA) and amine groups of poly(ethylene imine). Intramuscular injection of GCSF-encapsulating hydrogels led to sustained mobilization of stem cells into circulation over four days with a single administration and significantly enhanced CD34+ cell mobilization.

**BIOT 42**

**Corning® Synthemax™ surface: A synthetic, xeno-free surface for long-term self-renewal of hESC in defined medium**

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hESCs have two distinct properties that render them suitable for development of cell therapies: self-renewal and the potential to differentiate into all major lineages of somatic cells in the human body. To enable commercialization of hESC-derived therapeutic cells, low-cost culture methods must be developed that are robust, scalable and that use chemically-defined materials. Here we describe synthetic peptide-acrylate surface (Synthemax Surface) that support self-renewal of hESC in chemically-defined, xeno-free medium. H1 and H7 hESCs were successfully maintained on Synthemax Surface for >10 passages. Cell morphology and phenotypic marker expression were similar for cells cultured on Synthemax Surface or Matrigel™. Cells on Synthemax Surface retained normal karyotype and pluripotency and were able to differentiate to functional cardiomyocytes. Finally, we demonstrate scale-up of Synthemax Surface to large culture vessel formats. To our knowledge, this is the first report of synthetic surface that support self-renewal of hESC and differentiation to cardiomyocytes in defined medium.

BIOT 43

Mineralized electrospun nanofibrous scaffolds for directing osteogenic differentiation of human mesenchymal stem cells

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To mimic the structure of native bone matrix, electrospinning technique was employed to fabricate poly(l-lactic acid)/Collagen (PLLA/Col) nanofibres, and nano-HA minerals were deposited on the nanofibrous surfaces by mineralization in vitro. These biomimetic nanocomposites were used to study their effects on osteogenic differentiation ability of human mesenchymal stem cells (hMSCs). The results of SEM/EDX and calcium deposition showed that significant bone minerals were formed by the cells on the surface of nanofibrous PLLA/Col/HA at very early time point (10 days after culturing), even without any osteogenic supplement in the cell culture media. Additionally, the gene expression of specific markers of osteogenic differentiation (osteocalcin and osteopontin) was remarkably higher on mineralized scaffolds than on non-mineralized scaffolds. As such, sustainable supply of Ca/P ions locally effectively induced the osteogenic
differentiation of hMSCs. To our best knowledge, this is the first time to demonstrate osteoinduction controlled by the biomimetic nanocomposites.

BIOT 44

Effect of early endoderm induction in late pancreatic commitment

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Strategies for directed pancreatic differentiation involve a multi-step protocol in which the in-vivo developmental stages are replicated in the in-vitro culture. The first step in pancreatic differentiation thus involves inducing the ESCs to endoderm lineage. While we concentrate on deriving optimum endoderm in the first stage of differentiation, little study has been devoted to the impact of the quality of initial endoderm on late stage pancreatic differentiation and islet maturation. Our research focus is centered on two unanswered questions: How does initial endoderm commitment affect late stage islet maturation? Should we concentrate on \( \beta \) cell or try to reproduce islets? To answer the first question we are analyzing different pathway mediated endoderm and subsequent islet differentiation. While this reveals interesting dynamics between endoderm and pancreatic commitment, what is striking is the effect of different pathways on \( \beta \) cell versus islet regeneration.

BIOT 45

Unraveling the emergence of bistable behavior in metabolism during liver differentiation

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Stem cell differentiation is accompanied by a switch from a fastproliferating to slow proliferating phenotypes. As the two phenotypes have distinct energy requirements, this transition is likely to be accompanied by changes in metabolism. We examined alterations in metabolic characteristics during the directed differentiation of human embryonic stem cells towards the hepatic lineage at both the flux and transcriptome levels. Changes in the isozyme profiles of glycolysis as well as signaling pathways components were observed. Given the different allosteric regulations associated with different
isoforms and extensive crosstalk between signaling and metabolic networks, we utilized akinetic model to dissect the complexity of the liver differentiation on metabolism level. We show that bistability in the glycolytic flux arises under certain conditions, and that such bistable behavior is affected by isozyme combinations. These findings suggest that the possibility of enhancing liver differentiation through manipulations of this bistable behavior is worth exploring further.

BIOT 46

Culture methods can influence the genetic stability and phenotypic properties of human embryonic stem cells

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Human embryonic stem cells (hESCs) hold great potential for cell therapies but subchromosomal genetic changes undetectable by standard karyotyping that can negatively impact their safety profiles. In this study the effects of commonly used culture methods of hESCs were examined on the genetic and phenotypic stability of hESCs. The WA09 hESC line was cultured under four different culture conditions with six replicates per condition for over 80 passages. The genotyping data was analyzed for CNVs and the overall rate of accumulation of new CNVs and SNPs among the four culture conditions was compared to detect specific regions of the genome that accumulate CNVs in more than one sample. Our results suggest that culture methods can significantly influence the properties of human pluripotent stem cells and the genetic changes discovered in this study can be used in the future to screen out cell preparations not appropriate for clinical use.

BIOT 47

EGF receptor family ligands differentially modulate single-cell migration in subpopulations of primary human Multipotent Stromal Cell (MSCs)

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We, and others have reported that ligands of the EGFR family can control the migration, proliferation, and trafficking of bone marrow-derived multipotent stromal cells (MSCs), processes important for therapeutic applications. Human MSCs express three members of the epidermal growth factor receptor (EGFR) family -- EGFR/HER1, HER2, and HER3 -- that are implicated in bone development, homeostasis, and healing in vivo, and that regulate cell migration and motility in several cell types. In this study, we analyzed behavior of primary human MSC migrating on defined extracellular matrices in the basal and growth-factor stimulated states, using time-lapse video microscopy of individual cells to derive population distributions of cell speed, persistence, path length, and total displacement along with cell morphological parameters. Basal cell motility and sensitivity to growth factor stimulation were correlated with cell projected area, and evidence of EGFR family autocrine stimulation in the motility response was observed.

BIOT 48

Enabling technologies for implementation of Quality by Design for biopharmaceuticals

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Out of considerable efforts over the last five years from the regulators, especially the US FDA, Quality by Design (QbD) is being steadily adopted in the biotech industry. While the underlying concepts are becoming clear and widely accepted, the realization that technological advancements are necessary to facilitate QbD implementation is also dawning on the industry. This talk will focus on presenting some of the key technologies that have emerged in the last five years and that are likely to play significant roles in the future success of the QbD initiative. These include use of multivariate data analysis (MVDA) for analysis of process data, use of design of experiments (DOE) approaches towards experimentation, use of high throughput tools for process development, mechanistic modeling of different unit operations and use of computational fluid dynamics (CFD) as a tool for gaining process understanding in complex systems. Real case studies will be presented to highlight the potential of these tools.

BIOT 49

Design space for cell culture production stage

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This presentation discusses how to construct a cell culture design space for a late phase biopharmaceutical molecule in Eli Lilly and Company. Both lab scale design of experiments (DOE) data and manufacturing scale data are used to construct a knowledge space. A Bayesian approach is used to calculate the probability that two critical quality attributes (CQAs) meet their specifications within the knowledge space. A cell culture design space is defined by several mathematical equations. A control space is proposed for commercial manufacturing processes, which considers not only CQAs but also business attribute like titer. Different types of variability regarding biopharmaceutical manufacturing processes are included in Monte Carlo simulation. Experiences and future applications are also discussed.

BIOT 50

Application of quality risk assessment and design space concepts to early phase production fermentation of recombinant protein

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Typically, early fermentation process development (PD) is performed using univariate and designed experiments without clearly defined targets for process performance. Not only is this approach time consuming but also puts huge demands on scarce resources. Here we present an alternative approach, which is based on quality risk assessment and design space concepts, to optimize an early phase fermentation growth medium and induction conditions for expressing a recombinant protein. To direct the PD effort, input parameters were analyzed based on their potential impact (severity) on process performance (protein expression, specific protein productivity, and product purity). Using a risk scoring matrix, input parameters identified as high risk were evaluated using a combination of multivariate and univariate designed experiments. With this systematic approach, the optimized fermentation production process achieved 166 and 100% increase in recombinant protein expression and specific protein productivity, respectively, in comparison to the performance in the non-optimized fermentation medium while maintaining the product purity, in a much shorter development time.

BIOT 51

Structured approach to identify the control and design spaces of a cell culture process

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This presentation will describe the approach currently being employed to systematically define the design space for an antibody producing cell culture process. The presentation will emphasize on the importance of process definition as it relates to quality risk and describe the approach that was taken to identify the process and product related risks associated with different unit operations. The risk assessment was then utilized to identify various univariate, multivariate, and linkage studies. Also, the list of process and product attributes to be tested and the capture step used to generate product quality data at small scale were predefined. Ranges of various parameters were set based on the desired manufacturing flexibility as well as equipment capability. The scale down models mimicked the manufacturing equipment and procedures closely. Finally, appropriate statistical designs were chosen and experiments executed. Data from key risk assessments and resulting screening and optimization studies will be presented and discussed.

BIOT 52

Discerning critical parameters influencing bioprocess performance through pattern recognition in manufacturing data

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Recent QbD implementation advocates a quality-by-design approach to “design, analyze, and control” manufacturing processes for biopharmaceuticals. Multivariate data analysis has emerged as a critical tool to unearth any hidden patterns within bioprocess data and identify key parameters for enhanced process performance and product quality. The time dynamics of more than one hundred process parameters acquired from 243 production runs from the Genentech’s Vacaville manufacturing facility were investigated in this study. A kernel-based support vector regression was used to construct multivariate models to predict critical process performance attributes. Furthermore, a differential weighting scheme was incorporated into the models to rank both measured and calculated process parameters according to their relevance to process performance. The analysis reveals several parameters with significant influence
on process outcome, notably lot-to-lot variability of soyhydrolysate and air sparge rate - the later points out to the possibility for intervention. A transition stage wherein lactic acid concentration starts to differentiate among runs was further examined to seek for possible corrective actions early in the process. This approach represents an important step towards implementing QbD principles to facilitate a dynamic control strategy for improved process robustness.

BIOT 53

Practical use of QbD: Defining the design space of an anion exchange chromatography unit operation by multi-variant experimentation

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Anion Exchange chromatography is a key step in the purification of an Fc fusion protein requiring precise resolution from particular related substances and impurities. Early risk assessments had identified elution gradient slope, incoming related substance level and g/L resin load as potentially important parameters that might impact critical quality attributes (CQAs). These were studied using a design of experiments (DOE) approach and the results later transferred to large-scale operations. However, during the large-scale runs the upper limit of the column pressure drop was exceeded and after investigation, a number of changes were made to packing technique and the upper limit of the packed column bed height was reduced. It was not known if bed height was a new factor that could impact CQAs. Another risk assessment and DOE were performed that evaluated contact time, g/L resin load and incoming levels of the related substance. The result of this analysis redefined our operating ranges. Large scale work was continued and thirty two consecutive runs were completed that performed flawlessly from both the standpoint of pressure and resolution performance. The strategy associated with this work and the results will be discussed.

BIOT 54

PAT in bioprocessing - current status & future directions

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One of the important outcomes of CDER’s GMP initiative was the 2004 PAT guidance: “PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance.” The essence of PAT consists of real-time measurement of process intermediates and materials for feed-back control...
of the process to maintain quality and consistency of the final product. In pharmaceutical world, PAT concepts were adopted early on by the small molecules industry; the progress in biotech community may be more incremental and gradual but optimistic. We have recently reviewed the progress of implementation of PAT in bioprocessing (Read et al., 2010). PAT seems to be following the traditional incremental pattern of technology development in the bioprocess world. This evolutionary process involves process control based on real-time measurement of (a) parameters that confirm that a unit operation/piece of equipment continues to be fit for purpose, move on to (b) those that directly correlate with a CQA, and then to finally (c) actual product (or raw material) critical quality attributes (CQAs, i.e. the traditional conception of PAT). Examples were found of all three types of control, but (a) & (b) were much more common than (c). Achievement of (c) will require surmounting significant technology barriers by intense and purposeful R & D. PAT has the capacity to revolutionize the biopharmaceutical industry, but only if the opportunity is seized. The development and implementation of such technological advances have, and will continue to receive, strong support from the FDA.

**BIOT 55**

**Application of process analytical technologies (PAT) in biopharmaceutical chromatography processes**

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Process analytical technologies (PAT) are a key piece to realizing quality by design (QbD) principles in biopharmaceutical processes. Traditional purification chromatography processes have relied heavily upon offline measurement to quantify critical quality attributes of process intermediates and final drug substance and for assessing process performance. New approaches utilizing online and at-line analytics hold the promise of delivering more consistent product quality while maximizing process performance and is a key component of QbD. This presentation will address two aspects of PAT capable of controlling product quality and process performance. One approach involves the use of online analytics that directly measure process performance or product related impurities. The direct measurement of product impurities enables the process to adapt key process parameters to deliver a specified product quality with adaptive control. The second approach involves a feed forward process control strategy where multivariate analysis was used to establish critical process parameters and develop a predictive model. Application of the predictive model to the downstream process can deliver consistent product quality regardless of varying load impurity levels by adjusting one of the critical chromatographic parameters.
Building better medicines: Biotherapeutics in the 21st century

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Strategies to address aggregate removal during antibody purification process development

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Antibody purification processes are normally designed to employ orthogonal separation mechanisms to achieve the purity required. Reduction of High Molecular Weigh (HMW) and/or aggregate protein has always been challenging to downstream development. In this case study, multiple chromatography mechanisms were evaluated on the ability to remove substantially high level of aggregate from cell culture fluid. By using linear pH gradient elution, Protein A chromatography can reduce aggregate efficiently. Cation exchange chromatography was demonstrated not to reduce aggregate at these high levels without compromising yield, indicating marginal charge difference between monomer and aggregate. In addition, hydrophobic interaction chromatography (HIC) resins, particularly with high capacity, high resolution and ease of scale-up properties, were screened and proven highly efficient in removing aggregate. Results will be presented demonstrating utilization of different separation mechanisms can deliver an overall improved purification process to address the challenge of aggregate removal.

Development of streamlined high capacity two-column platform process for mAb purification

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Recent advances in upstream technologies have significantly increased cell culture titers of monoclonal antibodies. This requires more efficient and high throughput downstream process. To address the downstream challenges from high titer cell culture materials, we have developed a streamlined high capacity two-column platform for monoclonal antibody purification. The work presented here will focus on development of a single column as a polish chromatography step in a two-column mAb platform process. Anion exchange resins and mixed mode resins have been appraised for their impurity removal capabilities. To align buffer system with capture step, the effects of ion strength and counter ion components on impurity removals were evaluated. DOE studies were applied to define process design space with streamlined buffer system. Multiple monoclonal antibodies were performed in the study to establish a platform process targeting high titer cell culture.

**BIOT 59**

**Exploration of overloaded cation exchange chromatography**

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Cation exchange is typically operated in bind-and-elute mode with loading capacity of up to 100 g/L chromatography resin. We have been able to load up to 1,000 g monoclonal antibody per L matrix in flow-through mode under isocratic conditions to remove the majority of host cell protein, DNA, Protein A leachate and reducing aggregate, while maintaining high yields. Experimental data suggests that the removal of impurities during the overloaded step may be the result of impurities displacing monomer. The mechanism of this phenomenon was investigated and optimization of load conditions can improve impurity removal. In addition to being a highly effective mode of operation, overloaded chromatography would allow for decreased raw materials requirements. By implementing overloaded chromatography conditions, the size of the cation exchange column can be reduced by up to 90%, thus reducing buffer volumes. Purity, throughput, and yield data for MAbs comparing overloaded cation exchange chromatography with resins will be presented.

**BIOT 60**

**Utilizing mAb adsorption isotherms to direct process development of a strong-cation exchange chromatography step**

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As the industry is developing alternative strategies to deal with monoclonal antibody (mAb) downstream bottleneck concerns, the use of ion-exchange chromatography is still considered a proven workhorse of the industry. Using two separate mAbs, adsorption isotherm and linear gradient elution studies were conducted to understand the relationship between loading, washing, and elution conditions on the dynamic binding capacity and process yield obtained during preparative CEX chromatography. Although adsorptive capacities were relatively salt insensitive at low pH values, optimal salt concentrations that maximize adsorption capacity were observed at higher pH values. MAb yield from the preparative chromatographic runs was found to be highly dependent upon both the mAb characteristic charge and the time the mAb was bound to the chromatographic resin. A theory will be presented that will help explain the salt and pH dependent adsorption capacity trends as well as the resulting mAb yield data. Finally, the use of adsorption isotherm information in the development of a strong CEX chromatography step will be discussed within the context of both direct capture and polishing of mAbs.

**BIOT 61**

**Exploring limits of affinity resins**

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Affinity chromatography purification is commonly used in downstream processing of monoclonal antibodies. Since the introduction of Protein A affinity chromatography in the early 80s significant improvements have been achieved in design of both Protein A affinity ligands and chromatography matrices. The increased pressure to match the high product titers achieved in upstream processing in a cost efficient manner, is guiding the development of a third generation Protein A resin. This paper will present performance data from a new series of experimental resins with equilibrium capacities close to 100 mg/l. We will discuss the theoretical calculation with respect to ligand type, residence time, particle size, and ligand density to reach maximum capacity. The influence of the binding domain will be addressed. In addition economical models looking at the effect of the improved performance in a platform scenario will be presented.

**BIOT 62**

**Molecular perspective on the role of eluents in affinity chromatography**

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Due to the increasing demand for monoclonal antibodies, the production processes for manufacturing these antibodies need to be improved. From the expression to the purification of the antibody, nearly half of the product is lost. The first and the most critical step in the purification process is affinity chromatography. A ligand is used to bind the antibody to a column, and eluents are then used to elute the bound antibodies. Arginine and citrate salt are two commonly used eluents for elution of antibodies. Arginine and citrate both work well at low pH but at high pH, arginine improves the recovery of antibodies considerably as compared to citrate, which gives negligible recovery. Milder elution conditions are desired because at low pH, a lot of product is lost due to aggregation. The focus of this paper is to understand the role of the eluents in Protein A affinity chromatography. This paper studies the role of arginine and citrate as eluents computationally. A molecular insight into the mechanism by which arginine promotes elution of antibodies is obtained. Arginine facilitates the dissociation of antibody-Protein A complex and inhibits the aggregation of eluted antibodies whereas citrate works in an exactly opposite manner. These observations explain the low recovery of antibodies in the presence of citrate and improved performance in the presence of arginine. The current work also provides guidelines for design of eluents which provide enhanced recovery.

BIOT 63

Study of antibody diffusion into controlled pore glass using confocal Raman spectroscopy

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Controlled pore glass (CPG) based Protein A affinity resin possesses the highest antibody binding capacity at short residence time (e.g. 4 min). While this phenomenon is routinely demonstrated, there has been limited understanding of IgG diffusion at an individual particle scale of CPG. We incorporated confocal Raman Spectroscopy and fluorescence to study IgG diffusion into CPG-based Protein A resin particles by “seeing through” CPG. IgG diffusion into the resin particles at different contact times was studied. This work shows that IgG diffusion in CPG-based chromatography resin is almost instantaneous, and is much less hindered than that in the agarose-based resin. This result correlates well with the high antibody dynamic binding capacities that we have observed for CPG based affinity media, such as, ProSep Ultra Plus, which renders
significantly higher productivity for antibody capture processing. Modeling analysis is applied to help understand these experimental observations.

BIOT 64

Recovery and concentration of low level of HCPs from purified monoclonal antibody product for 2D gel analysis

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During a monoclonal antibody (MAb) process development, host cell protein (HCP) is monitored by ELISA to ensure sufficient clearance. The use of 2D gel electrophoresis and Western blotting complements ELISA testing by providing a visualization of HCP species present, and may permit enhanced understanding of impacts of the process on HCP distribution. However, high product concentrations (typically 50-150 mg/mL for MAb therapeutics) coupled with low HCP levels (often at low ppm levels) hinders 2D gel analysis. A product depletion procedure was developed and optimized to remove the MAb product, maximize HCP recovery and concentrate HCPs to detectable levels.

BIOT 65

Gelation of a monoclonal antibody

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Although extensively studied, the mechanism of protein-protein interactions remains highly elusive and is of increasing interest in drug development. We show the assembly of a monoclonal antibody, using multivalent carboxylate ions, into highly-ordered structures. While the presence and function of similar structure in vivo is not known, this may present a possible unexplored scope of structure-function relationship of antibodies. Using a variety of analytical tools, we characterize the physical and biochemical properties of these structures.

BIOT 66

High-throughput, nanoparticle-based analysis of antibody self-association
Currently there are staggering demands on biopharma to process and deliver high-concentration antibody therapeutics (≥100 mg/mL). Such high antibody concentrations present two important challenges, namely protein aggregation and high viscosity. Measurements of antibody self-association are critical to understand and overcome both issues, yet they are notoriously difficult to obtain in a high-throughput manner. We seek to exploit the separation-dependent optical properties of gold nanoparticles as a novel approach for rapidly characterizing self-interactions between antibodies immobilized on nanoparticles. In this presentation, we will demonstrate that antibody self-interactions can be characterized using Plasmonic measurements of protein-gold conjugates in qualitative agreement with measurements of osmotic second virial coefficients, and discuss our current efforts to make this method more quantitative. Moreover, we will discuss our use of this method to rapidly analyze the self-association behavior of a library of homologous antibody fragments with the goal of improving the design and selection of aggregation-resistant antibodies.

BIOT 67

Prediction and accelerated test of protein aggregation

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In biotechnology, irreversible protein aggregation is a frequently encountered problem in the production, formulation, and storage of therapeutic proteins. Aggregates reduce protein efficacy and can cause toxicity. Typical aggregation studies require monitoring for weeks or months. This study presents a convenient, accurate and quick (~30min) way of inferring information about medium-specific protein aggregation tendencies from stable protein samples. Salt-induced protein aggregation is studied with dynamic light scattering (DLS) in solutions of lysozyme and BSA containing different sodium salts. The same ions are used in a second measurement series assessing the effect of more dilute electrolytes on protein diffusivity in non-aggregating dispersions. Both aggregation and stablediffusion exhibit strong ion specificity along the Hofmeister series. Within this common qualitative trend, lysozyme and BSA solutions show marked differences in nature. Despite the differences, a strong correlation is
found in both cases between ion-specific diffusivity and the proteins' aggregation tendency.

BIOT 68

Advancements in high-throughput formulation screening technologies: The role of conformational and colloidal stability in predicting stability of monoclonal antibodies

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The application of two different high-throughput screening (HTS) techniques, differential scanning fluorescence (DSF) and differential static light scattering (DSLS), are demonstrated in order determine the conformational and colloidal stability of therapeutic monoclonal antibodies. DSF utilizes an extrinsic fluorescence probe, SYPRO® Orange, which is sensitive to changes in polarity, to monitor protein unfolding, allowing it to be used as a rapid formulation screening tool for conformational thermal stability. The DSLS methodology evaluates the colloidal protein stability during thermal denaturation in a multi-well format. The effects of various excipients on accelerated stability are correlated to stability rankings obtained by a HTS utilizing both DSF and DSLS, which can be conducted in a fraction of the time. The role of both conformational and colloidal stability in controlling monoclonal antibody aggregation, as well as the ability of the HTS process to accurately predict the stability of several therapeutic monoclonal antibodies will also be discussed.

BIOT 69

Impact of glycosylation on IgG stability and physical properties

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Antibody glycosylation is a common post-translational modification and has a critical role in ADCC function. Producing an antibody with a particular glycoform can significantly impact efficacy, however, stability might be impacted and therefore lead to increased challenges in formulation development. In this study, human IgG1 antibodies were generated using \textit{in vivo} or \textit{in vitro} glycosylation
modification to produce glycosylation variants. LC-MS was used to confirm glycoform structure. FTIR and intrinsic fluorescence spectra indicate no structural changes after glycosylation modification. As previously reported (Mimura et al, Molecular Immunology 37: 697-706, 2000), DSC shows a significant alteration in the thermal transition of the CH2 domain of the Fc region upon glycosylation modification. Data are also confirmed with CD and fluorescence measurements during thermal unfolding. IgG individual domains exhibit pH dependency during thermal unfolding. Stability during storage is evaluated at two pHs and no major alteration was observed for most of the glycoforms. Protease susceptibility was also evaluated as a probe to detect structural changes in the IgGs.

BIOT 70

Identifying residue-specific contributions to protein stability

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The overall stability of a protein depends not only on its composition but also on the solution environment in which it resides. Many techniques exist that identify the outcome that changing solution conditions has on a protein's stability, but little information is available to explain how or why these changes in solution composition affect stability. We have undertaken a study using high-resolution solution NMR to explore the mechanism by which a protein is affected by its environment. A simple approach involving a two-dimensional 1H-15N NMR experiment was used to detect changes in the chemical shift positions and lineshapes of individual residues within the protein during titrations. This data was cross-correlated to standard biophysical and analytical assay results to identify portions of the protein where structure and dynamics are altered by specific changes in the solution environment. This study provides evidence that different perturbations may be experienced at different sites.

BIOT 71

Diffusional interaction parameter as a high throughput tool to screen for low viscosity monoclonal antibody solutions during lead candidate selection

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Development of high protein concentration formulations often leads to manufacturing, analytical, stability and delivery challenges associated with high viscosity solutions. To circumvent these challenges, it is often possible to select for molecules with desirable viscosity properties in addition to the other attributes routinely considered during lead candidate optimization. However, viscosity measurements of high protein concentration formulations using conventional techniques (e.g., cone-and-plate techniques, falling sphere method, etc.) are time-consuming and often require quantities of material not readily available during drug discovery and lead optimization. In an attempt to reduce the material requirements and increase the throughput of viscosity screening, the utility of the diffusional interaction parameter was assessed as a tool to screen for molecules with undesirable viscosity properties. Diffusional interaction parameters for 16 IgG1 monoclonal antibodies were measured in dilute solutions using a dynamic light scattering plate reader. Diffusional interaction parameters were compared to solution viscosities measured at high protein concentration using a cone-and-plate rheometer. In general, the results demonstrate a good correlation between the diffusional interaction parameter and solution viscosity. This suggests that the diffusional interaction parameter, a dilute solution parameter, could be used to screen for antibodies with desirable viscosity properties during the lead candidate selection process.

BIOT 72

Connecting the aggregation behavior of human growth hormone to its colloidal and conformational stability

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Development of formulations that stabilize against protein aggregation is often dependent upon combinatorial methods because the mechanisms for stabilization are not well understood. As protein aggregation produces deleterious consequences, there is a need for improved methods for developing formulations. The objective of this study was to relate the colloidal stability and conformational stability of a well-characterized therapeutic protein to its aggregation behavior. The conformational stability of human growth hormone was characterized by measuring the Gibbs energy of unfolding via thermal unfolding studies for various solution conditions. These values were then coupled to $B_{22}$ measurements of colloidal stability made via self-interaction chromatography to explain real-time and accelerated stability behavior. A weak positive correlation between the resultant composite rate and the measured aggregation rate suggests that this approach can be effective in forecasting protein aggregation. This work therefore provides a framework for relating the
colloidal and conformational stability of a protein for application in formulation development.

**BIOT 73**

**Understanding of C-terminal lysine variants in antibody production using mammalian cells**

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C-terminal lysine variants are commonly observed in monoclonal antibodies and recombinant proteins. Heterogeneity of C-terminal lysine residues is believed to be the result of proteolysis by endogenous carboxypeptidase(s) during the cell culture process. Understanding the potential parameters affecting the extent of C-terminal lysine provides valuable insights to the cell culture process. The extent of change of C-terminal lysine has been reported in the different cell types (Hybridoma vs. CHO) and process conditions (i.e. feeding strategy). A CHO cell line E was selected as the model cell line in this study due to the exhibited sensitivity of its C-terminal lysine level on the process conditions. The different analytical methods such as charge variant analysis and LC-MS peptide mapping for determining the extent of variation were evaluated. The effects of operation conditions and media components on C-terminal lysine variants were studied.

**BIOT 74**

**Development and scale-up of a high titer cell culture process**

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This presentation will detail the development and scale-up of a cell culture process yielding 14+ g/L titer for an antibody. Process development involved dissecting the media and feeds into individual components/subgroups, evaluating their effects by conducting univariate as well as multivariate studies, and subsequent enrichment with important components. In addition, the effects of various process conditions such as bioreactor temperature, pH, feed volumes, and feed dynamics were evaluated. This development work resulted in a cell culture process where the cells utilized nutrients very efficiently and produced very low amounts of lactate during antibody production phase. Process performance at bench and large scale, including cell growth, metabolic profiles, product yield, and product quality will be discussed.
Epigenetic influences on recombinant antibody production in CHO cells

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The ever increasing demand for monoclonal antibodies has led to an interest in understanding productivity rates in CHO cells. Previous work in our laboratory has indicated that differences in specific productivity rates are directly proportional to the elevated levels of transcripts in higher-producing cell lines. In this study, we aimed to understand the causes for RNA enhancement. mRNA stability was observed to be consistent between higher and lower producing cell lines. However, upon investigating the epigenetic differences between parental and MTX amplified progeny cell lines via chromatin immunoprecipitation (ChIP), an increased DNA-protein interaction in the higher producing cell lines was observed. AP, NFkB and CREB transcription factors showed 2-3 fold increased association with the CMV promoter in higher-producing cell lines. Improved accessibility of the transgene inserts to the transcriptional machinery may explain transcriptional enhancement. In most industrial applications, the strong, viral CMV promoter is used to drive recombinant protein expression. Methylated DNA IP (mDNA-IP) was performed on the parental and progeny clones to identify differences in the methylation state of the CMV promoters between the parental and amplified progeny clones. It is our hypothesis that some combination of methylation, acetylation, and other chromatin modifications may influence the interaction of the transgene with the transcriptional machinery, resulting in altered expression of the recombinant biotherapeutics. Identification of these factors will guide development of selection methods and strategies for cell engineering, thus reducing costs and timelines for development, and hastening critical therapies to market.

Utilizing nonlinear experimental design methods to optimize CHO cell culture processes with high dimensionality and multiple responses in chemically-defined media

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Chinese Hamster Ovary (CHO) cell culture in chemically-defined medium is generally a non-linear process with high dimensionality and multi-responses. Optimization of base/feed formulations generally applicable to multiple CHO cell lines can be very complicated and preferably be obtained using highly efficient, nonlinear design of experiment (n-DOE) methods. The n-DOE methods have been applied to build a representative and informative database to find optimal operating conditions for the higher desirability of single or multiple responses of interest (e.g., volumetric productivity, titer, integrated viable cell density, viability, ammonium, lactate). The n-DOE method, based on radial basis function neural network and truncated genetic algorithm, can utilize information accumulated from all previous experimental runs and suggest new experimental conditions towards the local or global optimum of the complex CHO cell culture process. Specifically, deficient test technique was successfully applied to multiple cell lines for an exhaustive search of the essential/significant nutritional requirements of CHO cells from more than 80 potential medium components in a starting chemically-defined medium. To further dynamically eliminate insignificant variables possibly existing in this multi-dimensional problem, data mining techniques have also been applied to the informative database to identify critical variables and suggest optimal variable ranges for the next set of experimental design.

**BIOT 77**

**Metabolic modeling of cell culture**

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The construction of a metabolic network and an associated mathematical model for antibody producing cells is discussed. A compositional analysis of cells in conjunction with metabolite data for key reaction pathways (Glycolysis, TCA, and amino acid metabolism) as well as cellular energy requirements was performed. Weighted least squares analysis was used in conjunction with experimentally determined metabolite consumption and production rates to solve the overdetermined system of equations for the reaction fluxes. The fluxes generated by the metabolic model closely matched experimental values. The same model approach was used to identify metabolic fluxes for a different cell culture process for the same cell line. In addition, fluxes that were directly and indirectly correlated with the lactate, biomass and antibody production were identified. These correlations were then applied to construct a predictive cell culture mathematical model which was further used for performing in-silico risk assessments for process sensitivity.
**BIOT 78**

**Strategies to reduce protein aggregation through controlling cell culture conditions: A case study of IgG fusion proteins produced by Chinese hamster ovary cells**

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The presence of aggregated forms of proteins can be problematic for therapeutics due to the potential for immunogenic and pharmacokinetic issues. Although downstream processing can remove the aggregated forms, inhibiting aggregate formation upstream at the cell culture stage will reduce the burden on downstream processing and potentially improve process yields. This case study first examined the effects of environmental factors (temperature, pH, and dissolved oxygen) and medium components (bivalent copper ion, cysteine, and cystine) on the aggregation of two different recombinant fusion proteins expressed by Chinese hamster ovary (CHO) cells. Any strategy to reduce protein aggregation during cell culture must then consider potential effects on other parameters such as cell growth, harvest titer, and protein sialylation levels. Manipulating the culture temperature shift and cystine concentration in the medium were identified as effective and practical strategies for reducing protein aggregation in both CHO-cell expression systems. Furthermore, a combination of both strategies was more effective in reducing protein aggregation levels compared to either approach individually; and without any negative effects on cell growth, harvest titer, or protein sialylation. This study demonstrates a practical methodology for decreasing protein aggregation during upstream processing and emphasizes the importance of process understanding to ensure production of recombinant glycoprotein therapeutics with consistent product quality.

**BIOT 79**

**Challenges in developing process analytical technologies for a cell culture unit operation**

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In 2004, the FDA published the Process Analytical Technology (PAT) Guidance for Industry to “encourage the voluntary development and implementation of
innovative development, manufacturing and quality assurance”. Currently, we are developing a PAT system for timely bioreactor process control that is informed by measurement of important process parameters and critical quality attributes of in-process materials. Although we have achieved an intermediate level of success, we have also encountered a variety of technical challenges. We will present lessons learned regarding the sampling, monitoring for PAT control of a bench scale model bioreactor. The on-going development of near real-time at-line technology for assessing antibody glycosylation will be discussed.

BIOT 80

Film electrodeposition for on-chip cell culture and analysis at defined addresses

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Advancement in cell culture processes is attainable by employing microelectronics for small-scale culture, simplistic analysis, and automation. Our work uses electrodeposited biocompatible film interfaces on microelectronic chips for cell entrapment and in-film bioprocessing for analysis. Specifically, the process exploits the natural materials chitosan and alginate for cell-entrapped film formation; cells have been shown to survive the electodeposition process and proliferate in the film environment upon incubation. Electrochemical parameters and electrode patterning allow the deposition to be precisely controlled and spatially defined. The self-contained device allows deposition work, incubation, and analysis to be performed at a single electrode surface. We have further demonstrated detection of a cell-released biological signal on-chip as a proof-of-concept of the ease and convenience of chip-based cell probing. Outcomes of this work include promising possibilities for the organization of micro-scale biological samples via chip patterning by microfabrication techniques and automated on-chip analytical capability.

BIOT 81

Life cycle management for vaccine assays

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Life cycle management for biotech products has historically focused on process changes based on biochemical comparability facilitated by the application of modern analytical tools to better understand production processes and to facilitate process changes. However, those biotech products, particularly vaccines, which had been developed and licensed many years ago, often have dated and widely variable assays associated with them. In many cases, the original potency assays were animal based and susceptible to wide variation. Often the instrumentation used in the original license application is obsolete and no longer supported by the vendor forcing development of a more modern assay. Furthermore, as older processes are themselves being modernized or revalidated (perhaps because they are being moved to new facilities), appropriate analytical tools with higher levels of precision are expected. Introducing new technology brings with it many challenges – bridging, understanding offsets, reacting to new data, and resetting specifications.

BIOT 82

Challenges in transferring a mature process

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For a biopharmaceutical process that was approved a decade ago, changing the manufacturing site and obtaining regulatory approval has its unique challenges. With limited process characterization data on hand compared to current standards, any changes to the process due to facility fit constraints present a risk to the success of the transfer. This presentation will showcase the strategy used for transferring a mature mammalian cell culture process; including facility fit analysis, risk mitigation approach and process performance evaluation plan. We will also discuss details around addressing facility differences between the approved manufacturing site and proposed future site while leveraging new process technologies, evaluating a vendor initiated raw material change for a complex cell culture medium component, and acquiring additional process understanding during the transfer to increase the likelihood of success at the proposed manufacturing site.

BIOT 83

New mechanistic look at a vintage cell cultivation method

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Microcarrier culture was once regarded as a true scalable method for anchorage-dependent cells. The adoption of suspension culture for the vast majority of recombinant cell culture process has confined its applications to a few production processes of viral vaccines. With the resurgence of both human and veterinary vaccines, and especially the need of producing large quantity of cells in many stem cell applications, microcarrier culture has regained attention. Key to the success of large-scale cultivation is the successful cell adhesion and direct inoculation from microcarriers to microcarriers. We dissected the mechanistic steps involved in establishing successful cell-substrate adhesion, and carrier-carrier collision-induced agglomeration, as well as the process of cell migration in those agglomerates. Time-lapse microscopy was applied to establish various rates of each mechanistic step and the data were used to establish kinetic models. An optimization schema for different kinetic behaviors of cell and microcarrier combinations was proposed and discussed.

BIOT 84

Data mining builds process understanding for vaccine manufacturing

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Biologic products licensed more than 10 years ago pose distinct challenges but also bring decided advantages to the manufacturer. Critical process parameters were initially selected based on limited data primarily from small scale studies, measured using the best analytics available at the time of development. Years of manufacturing experience produce a rich source of data for hundreds of manufacturing lots, which can be mined to develop a deeper level of process understanding. In addition, important technology advances in the quality of biological raw materials, the precision of analytical methods, and equipment and controls may be incorporated over time. Full understanding of the impact of these enhancements may only be possible through thorough analysis of multiple full-scale manufacturing lots. Techniques for mining process data will be introduced and illustrated with a case study. As process understanding builds, critical process parameters may be refined and updated over the product lifecycle.

BIOT 85

Improvements on Peste des Petits Ruminants Vaccine stability during production and storage
Peste des Petits Ruminants (PPR) is an acute, highly contagious and fatal disease of sheep and goats and is considered one of the major constraints to the productivity of small ruminants in Africa and Asian countries. In this work, we examined the effect of Tris based formulations with trehalose or sucrose on the preservation of a PPR vaccine (PPRV) in contrast to the Weybridge formulation. The stability during storage at both refrigerated and clinically relevant storage temperatures, and their ability for protection upon lyophilization was evaluated. The consequence of increasing osmolality on production and stability of PPRV was also assessed. The information gathered here showed that it is possible for the PPRV vaccine to have adequate short-term stability at non-freezing temperatures to support manufacturing, short-term shipping and storage. The identification of a more stable formulation should significantly enhance the utility of the vaccine in the control of a PPRV outbreak.

BIOT 86

Mathematical modeling of a polysaccharide-protein conjugation reaction

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Polysaccharide based vaccines are available for the prevention of disease caused by certain pathogens. For children under 2 years of age, these polysaccharide vaccines are relatively ineffective due to the infant's naive immune system. Polysaccharide conjugate vaccines have been developed to circumvent this problem. There are many ways to covalently link polysaccharides to carrier proteins, e.g., direct coupling or use of bi-functional linker chemistries. We have developed an aqueous chemistry technique with a commercially available bi-functional cross-linker to covalently attach polysaccharide antigens onto protein carriers. Conjugation reaction parameters were evaluated for their effect on reaction efficiency, and overall resultant conjugate quality. These factors included polysaccharide concentration, polysaccharide to protein reaction charge ratio, reactant activation level, conjugation time, pH, ionic strength, and starting molecular weight of the polysaccharide antigen. Using the empirically derived results, a mathematical model was developed affording a better understanding of the conjugation reaction.

BIOT 87
**Optimizing baculovirus/insect cells-based bioprocesses: Combining experimental and computational tools for metabolic investigation**

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Significant molecular advances in the past decades have pushed the baculovirus (BV)-insect cells system into a versatile technology for manufacturing a variety of bioproducts, from recombinant proteins to vaccines and gene therapy vectors. However, a critical bottleneck remains to be the decreased specific productivities obtainable from high cell density culture, with implications for overall bioprocess costs. Our group has tackled this so-called cell density effect by a combination of experimental and computational tools to assess the metabolic determinants of BV production. Extracellular metabolite profiling was used in conjunction with metabolic flux analysis to assess the major alterations of *Spodoptera frugiperda Sf-9* cells metabolism with increasing cell density, identifying the tricarboxylic acids cycle and ATP synthesis as the main limiting pathways. In addition, biochemical methods were used to screen for possible enzyme capacity limitations in different culture conditions. Taken together, these results highlight the importance of the cellular energetic state as a reliable sensor of system productivity. Our latest work demonstrates that individual supplementation of energy generating metabolites at high cell density infection results in 6 to 7-fold increases in specific BV yields, representing a promising strategy for efficient manufacturing.

**BIOT 88**

**Vaccine manufacturing for the developing world: Bioprocess opportunities and challenges**

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This talk will review the unique bioprocess opportunities and challenges presented by vaccine manufacturing for the developing world. Global vaccine demand is dominated by the developing world due to the significant birth cohort and disease burden. Significant manufacturing is already being conducted in the developing world, with one developing country vaccine manufacturer being responsible for more than 50% of global infant immunization. Though narrowing, there is still a significant delay in vaccine introduction in the developing world. As
vaccines become more complicated (e.g. virus-like particles, multi-valency), the need to have simple, low-cost manufacturing processes is ever more important. In addition, these vaccines still need to be low-cost, thermo-stable, and amenable to multi-dose presentations. This presents a rich menu of opportunities and challenges to the bioprocess engineer/scientist.

BIOT 89

Pore-poring injectable alginate hydrogels for therapeutic stem cell deployment

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Deployment from macroporous biomaterials improves stem cells' ability to repair damaged tissues, but also subjects them to host inflammatory responses. We hypothesized that co-encapsulating cells along with degradable porogens within hydrogels would allow pore formation to occur in situ, so that cells would initially be protected from inflammation and subsequently be deployed as pores form. To accomplish this, we formed gel porogens using blends of poorly degradable alginate (MVG) with alginate that was oxidized to facilitate hydrolytic degradation. Porogens were encapsulated into hydrogels comprised only of poorly degradable MVG “bulk” gels modified with adhesive (RGD) peptides. Both porogens and bulk gels were crosslinked with calcium. Pore formation kinetics could be manipulated by altering the degree of porogen oxidation, and were correlated with mesenchymal stem cell (MSC) deployment. In preliminary studies, delivering MSC with pore-forming hydrogels led to improved regeneration in cranial defects at 4 weeks, whereas little improvement was observed when cells were delivered in saline or standard hydrogels.

BIOT 90

Using the epigenetic code to promote unpackaging from a non-viral, gene delivery vector

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Non-viral gene delivery biomaterials must be able to release DNA inside the nucleus of a cell. Unfortunately, the majority of non-viral vehicles unpack prior to nuclear entry or do not unpack at all, promoting either premature degradation or inefficient transcription. Thus, we have created DNA polyplexes that are designed to release DNA upon arrival in the nucleus. These vehicles mimic the natural histone biochemistry that promotes chromosomal DNA activation, and consist of H3 histone tail peptides that are trimethylated at the fourth position (H3K4Me3) and grafted onto a PEG backbone. The H3K4Me3 modification has been associated with actively transcribing DNA in a variety of eukaryotes. We have explored the effects of the N:P packaging ratio on DNA protection and transfection efficiency. Upon insertion of the H3K4Me3 polyplexes into the nucleus, we observe protein production approximately 50% faster than with polyplexes containing nontrimethylated H3 peptides or with conventional gene delivery materials.

BIOT 91

**Autocatalytic drug delivery vehicle based on targeting extracellular DNA**

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In this presentation, we present a new cancer drug targeting system, termed H-gemcitabine, which can upregulate the tumor receptor it targets and has autocatalytic drug targeting properties. H-gemcitabine is composed of the DNA binding agent Hoechst conjugated to gemcitabine. H-gemcitabine initially targets tumors by binding extracellular DNA (E-DNA) via its Hoechst moiety, which is overproduced in tumors due to their necrotic core. After localizing in tumors, H-gemcitabine releases free gemcitabine, which diffuses into neighboring tumor cells and kills them, which triggers the release of more E-DNA. Therefore on subsequent rounds of therapy increased levels of H-gemcitabine are targeted to the tumor, due to the cell killing from the previous rounds of H-gemcitabine therapy. We show here that H-gemcitabine has autocatalytic drug targeting properties in tumor spheroids, and is significantly more effective than free gemcitabine at treating xenografted tumors in nude mice. We anticipate numerous applications of H-gemcitabine, given its unique autocatalytic drug targeting ability.

BIOT 92

**Effects of substrate mechanics on yield and contractility of cardiomyocytes generated from pluripotent stem cells**

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The mechanical microenvironment plays a role in proper development and function of heart cells, but implementing mechanical cues in an appropriate context during differentiation of human pluripotent stem cells (hPSCs) to cardiomyocytes remains challenging. We have used polyacrylamide hydrogels as a model system to investigate the effects of substrate mechanics on cardiogenesis of hPSCs. We have observed that substrate stiffness affects cardiogenesis of hPSCs undergoing embryoid body-based differentiation. We have also used traction force microscopy to quantify contractility of hPSC-derived cardiomyocytes. We have observed that substrate stiffness affects the magnitude of contractile force generated by these cells. This work demonstrates that hPSCs and their cardiomyocyte derivatives can respond to substrate mechanics. It introduces an approach for quantifying this response which may aid in the development of novel culture systems for effective differentiation of functional cardiomyocytes from hPSCs.

BIOT 93

Of newts and niches: Regenerating tissues by mimicking natural processes

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We are exploiting natural mechanisms to derive mammalian cell sources for regenerative medicine: (1) by recapitulating pathways used by newts and zebrafish, (2) by mimicking biophysical cues to which adult stem cells are exposed in the body, (3) by gaining insights into the mechanisms such as DNA demethylation by which adult cells are reprogrammed to pluripotency (iPS) by cell fusion in heterokaryons. These approaches provide fundamental mechanistic insights into stem cell fate determination and should enable clinical applications.

BIOT 94

Dynamic mechanical properties improve cardiomyocyte differentiation in vitro and in vivo

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Tissue-specific elastic modulus (E), or 'stiffness,' arises from developmental changes in the extracellular matrix (ECM) and suggests that differentiation may be optimal when physical conditions mimic development. For cardiomyocytes, maturing from mesoderm to myocardium results in a 9-fold stiffening originating in part from a change in collagen expression. To mimic temporal stiffness changes, thiolated-hyaluronic acid hydrogels were crosslinked with poly(ethylene glycol) diacrylate, and their dynamics were modulated by crosslinker molecular weight. With the hydrogel appropriately tuned to stiffen like myocardium, pre-cardiac cells grown on collagen-coated HA hydrogels form up to 60% more maturing muscle fibers than they do when grown on compliant but static polyacrylamide hydrogels over 2 weeks. Ester hydrolysis does not substantially alter stiffening over 2 weeks in vitro, but model predictions and in vivo tests indicate that ester hydrolysis will eventually degrade the material, implying its appropriateness for applications where temporally changing material properties enhance cell maturation.

BIOT 95

Osteoblast cell behavior on titanium surface coated with mussel adhesive protein and hyaluronic acid by layer-by-layer method

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Recombinant mussel adhesive protein (MAP) fp151 has excellent adhesion ability on various surfaces. Based on its property, fp-151 is possibly suitable for use as a biocompatible coating material in tissue engineering field. Surface modification of biomaterials using extracellular matrix (ECM) molecules such as hyaluronic acid (HA) usually has performed in tissue engineering field for endowment of biocompatibility. In this aspect, we designed oppositely charged Layer-by-Layer (LbL) of fp151 (+) and HA (-) as a platform surface to improve complication of titanium-based implantation such as aseptic loosening. Each layer was deposited using spin coater, and the built-up of both fp151 and HA layer were confirmed by contact angle analyzer and quartz crystal microbalance. Then, adhesion and proliferation of MC3T3-E1 pre-osteoblast cells were examined on the sample-coated surfaces by MTT method, and spreading of cells was measured by SEM and immunostaining methods. Differentiation of cells was performed by alizarin red s staining and colorimetric assay. As results, we observed that fp-151 and HA were well fabricated on titanium surface, and adhesion, proliferation, differentiation and spreading properties of the MC3T3-E1 cells were superior on the surface-treated titanium, especially fp-151-HA coating layer to bare titanium surface. Our results suggest that LbL strategy using fp-151 and HA can be applied as an efficient coating method for titanium-based implant.
and extended to other negative charged glycosaminoglycan (GAG) coating method to improve biocompatibility of biomaterials.

BIOT 96

Understanding and engineering natural product biosynthesis

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Natural products biosynthesized by microorganisms represent an important source of bioactive compounds and pharmaceuticals. Uncovering the biosynthetic pathways, followed by elucidating the mechanistic basis of the enzymatic assembly, can therefore lead to new engineering opportunities for drug discovery and synthesis. Our laboratory has been focused on the biosynthesis of polyketides and nonribosomal peptides from both bacterial and fungal species. In this presentation, I will present our recent work on several natural product targets, including tetracycline, lovastatin and quinazoline-containing fungal alkaloids. I will also discuss the discovery, characterization and engineering of an acyltransferase from Aspergillus terreus that has been commercially used to produce simvastatin, which is the active pharmaceutical ingredient of the blockbuster drug Zocor®.

BIOT 97

Connected-processing: Filtration is just as important as chromatography

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Connected downstream processing facilitates greater facility flexibility by avoiding the need for large fixed tanks. However, linking operations in a way that targets near continuous processing, poses unique process and operational challenges. For example, filtration operations begin to arise as the critical constraints on process throughput and flow matching across the process stream. This paper will discuss a number of considerations relating to the implementation of downstream “connected-processes.” We will also present results from large-scale proof-of-feasibility runs.

BIOT 98

Membrane separation of polysaccharide from its conjugate: The role of fouling and electrostatic interaction
Tangential flow filtration (TFF) was employed to remove unconjugated polysaccharide (Ps) from its conjugate (with protein complex carrier). When using a 0.1 micron hollow fiber membrane, the linear Ps with Mw about 200kD exhibited poor sieving (S < 0.1) along with low permeate flux. This was in contrast to excellent sieving (S > 0.9) and high permeate flux when conjugate was not present in the feed solution. To further understand the TFF process and underlying mechanisms, three distinct feed solutions including free Ps, free Ps with free protein complex carrier, and free Ps with conjugate were compared. The TFF performance was evaluated against different ionic strength and pH. Such systematic study deconvoluted the contributions of free Ps, protein complex carrier, and conjugate to membrane fouling and electrostatic interactions. The results indicate that ionic strength and pH have different effects on permeate flux and Ps sieving, and that these effects vary across different feed solutions. Overall, the TFF flux decay is mainly attributed to fouling by protein complex carrier deposited on the membrane surface. Meanwhile, the sieving behavior is mostly governed by electrostatic repulsion within the fouling layer. In addition to the above studies, the zeta potential of various feed solutions provides further insights to the observed phenomena.

BIOT 99

Salt tolerant ligands and polymers for membrane absorber applications

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The use of cationic polymers for the flocculation of cells and/or cell debris, as well as for the precipitation of proteins, has long been known. Similarly, cationic polymers have been used to modify filtration media to enhance the removal of impurities from process streams in depth filtration or membrane absorber type applications. The effectiveness of these approaches, however, typically declines as the conductivity of the media being processed increases. In recent work on salt tolerant ligands for membrane absorber applications1, we described several ligands that display excellent performance for DNA and virus removal in high conductivity media, but only modest removal of negatively charged proteins. We now report that when appropriately functionalized and subsequently attached to membranes and other filtration media, these ligands display dramatically improved capacities for impurity removal, especially for negatively charged proteins. We will describe developments that led up to these findings, our studies with model systems, and recent results on the removal of host cell impurities.

BIOT 100

Evaluation of UV-C irradiation as an alternative technique for virus inactivation in biopharmaceutical manufacturing

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UV-C irradiation was evaluated for its effectiveness on viral inactivation and impact on product quality attributes. The inactivation efficacy was evaluated by subjecting enveloped and non-enveloped viruses to a UV-C dose of 100 J/m² in samples containing monoclonal antibody (mAb). Treatment at this dose resulted in effective inactivation of the non-enveloped virus (> 7 log reduction value, LRV), but ineffective inactivation of the enveloped virus (≤ 1 LRV). Effect on product quality was assessed by comparing a mAb that was purified with a process using UV-C irradiation to the same mAb purified with a process using low pH inactivation. Irradiation caused some oxidation, but the oxidized form was removed in subsequent purification steps. The quality of the purified product was therefore comparable to the mAb subjected to low pH inactivation. These results suggest that UV-C irradiation may be a viable viral inactivation technique, but requires careful evaluation of product quality.

BIOT 101

Qualification of a novel inline spiking method for virus filter validation

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Validation of virus clearance from filters is often affected by filter plugging that does not occur in mfg. Protein solutions reaggregate after prefiltration. A novel inline spiking method was developed to allow protein prefiltration and virus spiking. It allows direct measurement of virus filter performance and improves virus filter capacity. This paper will describe the method in detail and show performance with several proteins, virus spikes, and operating parameters.

BIOT 102
Impact of precipitation steps on process performance

**Sascha Keller**

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While recent advances in cell culture expression levels have led to a renewed interest in protein precipitation as a downstream processing step, high expression rates are characteristic for microbial sources. DSP technologies for microbial processes have therefore always been adapted to work around the limitations of chromatographic separations. Thus, at least one precipitation step is performed in almost all of the current microbial GMP manufacturing processes at Sandoz Biopharmaceuticals in Kundl (Austria). The precipitants used include polyethylenimines and polyethylenglycols as well as pH and temperature shifts. Especially the pH precipitation is part of our N^pro autoprotease fusion technology (NAFT) platform process. In the majority of these cases impurities are precipitated, but in some instances product characteristics allow for a selective precipitation of the target protein. This presentation will demonstrate the particular challenges in the development and scale-up of these precipitation steps for GMP manufacturing. Based on process data we will show how precipitation steps can be used to increase the binding capacities or the robustness of chromatographic steps.

**BIOT 103**

PEG precipitation for monoclonal antibody purification

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Various precipitation techniques have been used for the industrial purification of proteins for many years. Precipitation processes can be segregated into two main categories: impurity precipitation and product precipitation. Impurity precipitation is, in general, operationally simpler but carryover of the precipitants can challenge subsequent unit operations. Product precipitation may have a higher risk of damage to the target molecule, but in addition to product purification, product precipitation also enables buffer exchange and concentration during the resolubilization step. An antibody precipitation step has been developed using statistical design of experiments to optimize product yield and HCP removal. With the appropriate precipitation conditions developed, two methods to capture the antibody pellet were evaluated: depth filtration and microfiltration. A wash step was incorporated in both methods to improve the reduction of soluble impurities. The final process resulted in a product yield of 90% and HCP reduction of approximately 1 LRV. The method of pellet capture
was shown to have a significant impact on the purity of the redissolved product. The precipitation step developed is readily scalable and fits a fully disposable downstream process.

**BIOT 104**

Purification of monoclonal antibodies by affinity precipitation using thermally responsive elastin-like polypeptides (ELPs) fused to IgG binding domains: A high throughput approach

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A robotic high throughput screening protocol was developed to investigate the inverse transition behavior of various ELP-IgG binding domain constructs under a wide range of solution phase conditions using turbidity measurements. Several operating conditions with appropriate pH, salt concentrations, modifiers and transition temperatures were identified. These conditions were then employed in a subsequent high throughput assay to determine their performance with respect to antibody binding, elution and precipitation efficiency. Dynamic light scattering was employed to evaluate ELP aggregate sizes at various stages of the process. The results were also quantified using size exclusion chromatographic analysis of the IgG in the supernatant after each precipitation step and antibody activity was determined using ELISA. This work sets the stage for the industrial implementation of this technology.

**BIOT 105**

Biophysical analysis reveals that preferential hydration and exclusion mediate the solution viscosity of high protein concentration formulations containing sugars

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Sugars, which are commonly used for formulating proteins, typically result in increased viscosity in high concentration protein formulations, posing many challenges to the pharmaceutical industry. The mechanism through which this occurs has not been delineated. We have employed a novel high throughput
dynamic light scattering method to systematically evaluate the effect of seven sugar molecules on protein solution viscosity. All of the sugars examined increased viscosity at high protein concentrations, with the degree of increase being determined by the sugar and whether it was a mono- or disaccharide. In addition, the viscosity enhancement by sugars was significantly decreased by increased ionic strength or temperature, consistent with the preferential hydration/exclusion mechanism. Thermal melting measurements further revealed that the conformational stabilization by sugars correlates with viscosity at high protein concentrations. The observations and the biophysical techniques presented here will help guide the viscosity mitigation of high concentration formulation of protein therapeutics.

BIOT 106

Capillary size exclusion and ion-exchange chromatography methodology with picogram sensitivity to support biopharmaceutical development

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Size exclusion and ion-exchange chromatography are widely used in the characterization and quality control of therapeutic proteins. We will talk about the development and application of capillary size exclusion and capillary ion-exchange methodology for biopharmaceutical development. We have demonstrated an automated platform to perform the methodology after making several key modifications to a commercially available capillary liquid chromatograph. While conventional scale SEC and IEC methods require milligrams of protein per injection, capillary SEC and IEC require injection amounts on the order of nanograms, with detection sensitivity on the order of picograms. Thus, sample amounts that are too low for conventional scale chromatography can be analyzed with capillary chromatography methodology. Applications for capillary SEC and IEC will be discussed, including cell culture development, molecular assessment, formulation development, recovery development, fraction analysis and product characterization.

BIOT 107

Dynamics and interactions of VEGF - DNA aptamer association: Ensemble and single molecule studies

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Surface plasmon resonance (SPR), fluorescence anisotropy (FA), isothermal titration calorimetry (ITC), sedimentation velocity, and single-molecule fluorescence resonance energy transfer (SM-FRET) were used to characterize the association of a DNA aptamer and its binding partner, recombinant human Vascular Endothelial Growth Factor (VEGF165). FA, ITC and sedimentation velocity show that, at equilibrium, a single aptamer molecule binds to each VEGF homodimer. Kinetic studies with mass-transfer-compensated Biacore SPR were used to determine association and dissociation rates of VEGF. Recently, single-molecule intramolecular FRET was used to extensively investigate the Mg²⁺-dependent conformational dynamics within the VEGF-DNA aptamer pair. In the absence of VEGF, the aptamer favors a closed conformation, but in a manner which is highly dependent on Mg²⁺ concentration. The same dynamics occur in the presence of VEGF, but interaction with VEGF shifts the aptamer equilibrium toward a more open conformation.

BIOT 108

Single pores on ultra-thin silicon nitride membranes for viral sensing

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A fast and reliable method of sizing and counting biologically relevant particles was introduced roughly six decades ago by William Coulter. When a particle suspended in electrolyte passes through a narrow channel, the ion transport through the aperture is temporarily hindered, causing a transient decrease in ionic current through the device, with a shape correlated to both particle size and transit time. While Coulter's counter is best known for its application to microscopic blood cell counting, recent advances in fabrication have enabled the extension of this principle to the nanoscale. As most fabrication efforts have focused on creating channels with molecular sizes, a gap remains in the intermediate area of bacterial and viral particle counting. We will discuss the fabrication of single nanopores in ultrathin silicon nitride membranes using Focused Ion Beam (FIB), and the correlation between the physical parameters of the apertures and the shape of the electrical events transducing the presence of virus-sized particles. In addition to biological-particle sizing and counting, our ultimate goal is to develop localized surface functionalization schemes to enable specific bio-particle detection. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.
BIOT 109

Determination of protein secondary structures and orientation at interfaces by chiral sum frequency generation spectroscopy and computational modeling

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Determination of protein secondary structures and orientation at interfaces is central for the development of novel biomaterials and biosensors. We identify a set of optical vibrational signatures for distinguishing protein secondary structures at interfaces by combining chiral sum frequency generation (SFG) and computational modeling. We observe the N-H stretch (3280 cm\(^{-1}\)) from \(\alpha\)-helices and the amide I stretch (1620 cm\(^{-1}\)) from beta-sheets. Because chiral SFG is surface-specific and background-free from solvent, vibrational signatures are optically clean. Therefore, spectral deconvolution is not needed for quantitative analysis of secondary structures. The analysis of vibrational features based on \textit{ab initio} quantum chemistry calculations of hyperpolarizabilities of beta-sheets, formed by islet amyloid polypeptide at the lipid/water interface, shows that the beta-strands orient at \(\sim 45^\circ\) relative to the interface. The reported results demonstrate the potential of the combined methodology, integrating chiral SFG spectroscopy and computational modeling, for determination of orientation of protein secondary structures at interfaces.

BIOT 110

Characterization of turbid syringes using automated inspection

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Detection of turbid syringes is a new capability of commercial automated inspection technology. These syringes are considered defects due to presence of unexpected particulate or protein aggregation due to the presence of component tungsten. Human capability using certified operators was first determined using EP opalescence standards (0 – 30 NTU). The results using opalescence standards was used to interpret the threshold of turbidity detection by manual inspection. The characterization of turbid syringes was assessed using a new camera based unit that uses LED sourced light detection at defined angles. The characterization philosophy and approach allow qualification of syringes for defined turbidity ranges in automated inspection high-speed equipment. The
characterization results for trials with syringes show that the automated equipment can detect defective turbid drug product syringes for biologic products.

BIOT 111

Protein self-association: Identification of association sites and related conformational changes by hydrogen exchange

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Self-association of proteins can impede purification processes. In this work, hydrogen exchange mass spectrometry (HXMS) coupled with proteolytic digestion was used to probe the self-association of a multidomain protein. Differences in solvent protection between monomers and oligomers were used to identify a region of residues as the self-association site for both dimers and higher order oligomers. The solvent protection difference was greater for the higher order oligomers indicating a stronger interaction between the molecules at the self-association site. Studies on protein fragments and size-exclusion chromatography were used to provide complementary information about dimer and oligomer formation. HXMS further identified regions that unfold during the association process, indicating that self-association of the protein also involves a conformational change.

BIOT 112

Biophysical form is important to adjuvant activity in vaccines

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The biological activity of amphiphilic Toll-like receptor 4 agonists shows a dependence on the supramolecular structural form. Such associated states are not easily discernible by classical analytical methods such as chromatography and divide and conquer sample preparation schemes; nor are processing methods able to necessarily guarantee the desirable forms are present. To measure such higher order structures we have employed both calorimetric (differential scanning calorimetry) and spectroscopic methods that are sensitive to particular phases and polymorphic forms, and have undertaken efforts to correlate these structures to biological innate immune activation in vitro. The
consequence of such findings to immunogenicity of aggregated forms is also considered.

BIOT 113

Combining high-throughput screening of caspase activity with anti-apoptosis genes for development of robust CHO production cell lines

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A set of anti-apoptotic genes were over-expressed, either singly or in combination, in an effort to develop robust Chinese Hamster Ovary host cell lines suitable for manufacturing biotherapeutics. High-throughput screening of caspase 3/7 activity enabled a rapid selection of transfectants with reduced caspase activity relative to the host cell line. Transfectants with reduced caspase 3/7 activity were able to withstand the apoptotic-inducing property of Sodium Butyrate. Apoptotic-resistant transfectants were then tested for improved integrated viable cell count (IVCC), a function of peak viable cell density and longevity. The maximal level of improvement in IVCC could be achieved by over-expression of either single anti-apoptotic genes, e.g. Bcl-2Δ (a mutated variant of Bcl-2) or Bcl-XL, or a combination of two or three anti-apoptotic genes, eg. E1B-19K, Aven and XIAPΔ. One cell line expressing Bcl-2Δ with low caspase 3/7 activity and a greatly enhanced IVCC generated a stable clone (BΔ31-1). In a 5L, 14-16-day fed-batch bioreactor system, BΔ31-1 had a product titer that was 140% as compared to an optimal clone (Con-1) derived from the control host cell line. Importantly, the lactate and glucose consumption profiles of BΔ31-1 were significantly different from that of control cell line, confirming our earlier finding that apoptosis and metabolism are linked intracellularly. Addition of 2mM Sodium Butyrate on day-4 post-inoculation, in certain media formulations, increased the productivity of apoptotic-resistant cell lines an additional 115%-120%, as compared to untreated cultures. To the best of our knowledge, this is the first study to utilize the high throughput caspase screening method to identify CHO host cell lines with superior anti-apoptotic characteristics.

BIOT 114

Understanding the acute pH sensitivity of GS-CHO cell lines

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We explored the sensitivity of multiple mAb-producing GS-CHO cell lines to changes in pH setpoints. Cells grown at pH ranges of 6.8 to 7.1 had increased lactate and alanine production with increasing pH and an abrupt decrease in ammonia production at lower pH. Use of metabolic flux analysis (MFA) found that, while glycolytic flux decreased with lower pH, the fraction of pyruvate converted to lactate was also reduced at lower pH. Based on these observations, we reexamined our method of feed addition at larger scales in order to minimize pH heterogeneity within the bioreactor. We compared submersed and dripping methods of non-neutral pH feed addition and found that subsurface feed delivery at the 45L scale increased antibody titers by 25% and reduced lactate production by 60%. The sensitivity of GS-CHO cells to pH identified here was exploited through an engineering solution, leading to improved culture performance.

BIOT 115

Factors that influence the synthesis and consumption of lactate in CHO cell culture

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Two case studies, which show that lactate metabolism is a function of both environmental factors such as culture media as well as inherent cell line specific factors, will be presented. With the first study, we show that the rate of synthesis of lactate for a cell line is sensitive to the concentration of copper in the culture medium. Lower initial concentration of copper led to higher rate of lactate synthesis and lower rates of lactate consumption. Since copper plays a key role in oxidative phosphorylation, we hypothesize that these effects are due to respiratory capacity impairment. The second study will focus on metabolomic profiling of the relative concentration of more than 400 metabolites in two representative cell lines that exhibit different lactate accumulation phenotypes even when copper concentrations were not limiting. We will also discuss the benefits and challenges of utilizing systems biology tools in process development.

BIOT 116

Insight into CHO host cell protein profile differences observed in cultures in chemically defined vs. peptone-containing media

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The biotech industry is moving towards chemically defined media as the platform media for mammalian cell culture bioprocess engineering. The amount of host cell proteins in the harvested cell culture fluid for Chinese hamster ovary (CHO) cell line A was significantly higher for cultures in a chemically defined medium compared to in a peptone-containing medium when process conditions were optimized for each medium. The differences in CHO cell protein level were still apparent, although to a lesser degree, when cell culture process conditions were held constant except for media type. Two-dimensional gel electrophoresis was performed to identify whether this observed difference stemmed from variation in the magnitude of certain species of CHO cell proteins or from the presence of different species of CHO cell proteins for the two media conditions.

**BIOT 117**

**Bioprocess engineering: From genome, cell to reactor**

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In the past decade and half biochemical engineering profession has played a major role in propelling the biologic therapeutics into a major class of medicine. This success was brought about by enhanced productivity and product quality, and achieved by a better understanding of cell physiology and a more robust control of process variables. The accomplishments and maturity is inevitably accompanied by an agony that further process innovation may be of less significance. As the field increasingly sets sight on new potential technologies, such as cellular therapy, it is also prudent to contrive innovations that may further enhance or even transform process technology. This presentation will forward the notion that one decade after genomics time is ripe to focus scale-up at data and information level, as opposed to reactor size level. A fundamental transformation of bioprocess can be brought about by integrating designs at genomic and cellular levels with the control at reactor level.

**BIOT 118**

**Modulating autophagy to increase productivityin CHO cell fed-batch processes**
Autophagy is a eukaryotic cell process which targets intracellular components for lysosomal degradation as part of a survival response to metabolic and other physiological stresses. This mechanism was activated under fed-batch conditions, upon glutamine limitation along with a decrease in cell-specific productivity. The mRNA levels of several autophagy genes increased 4-fold during the process. Glutamine deprivation also changed mitochondrial physiology and cell proliferation, but with no apparent loss of viability. To engineer the cellular response during fed-batch a chemical inhibitor of autophagy, 3-methyladenine (3MA), was used and yielded a 2.5-fold increase of tissue plasminogen activator (t-PA) production (>0.5 g/L). Treatment with 3MA did not impair the glycosylation capacity of the cells. Combining manipulation of glutamine metabolism for increased proliferation with inhibition of autophagy for enhanced t-PA production, a 3.5-fold increase was obtained relative to control fed-batch processes. Current work is expanding these findings to CHO cell lines producing monoclonal antibody.

BIOT 119

Differential effect of reduced culture temperature on the expression and biophysical properties of monoclonal antibody variants

The use of reduced culture temperature is becoming an increasingly popular practice to improve recombinant protein yields in CHO cells. Recent studies have attributed the enhancement of protein titers at sub-physiological temperatures to increased mRNA levels and extended stationary phase. In this study, we observed that reducing the culture temperature resulted in arrest of cell growth, prolonged viability, and increased cell size. However, the reduced culture temperature had a differential effect on protein and mRNA expression of closely
related antibody mutants from stable cell lines. The high-expressing mutant (277 Ala) exhibited similar or decreased specific productivity and decreased volumetric productivity over the culture lifetime at 32°C compared to 37°C. In contrast, the specific and volumetric productivity of the poorly expressing mutant (277 Gly) was enhanced when cultured at the lower temperature. The difference in specific productivity was reflected in the amounts of heavy and light chain mRNA. Analysis of the secondary and tertiary configurations of the purified antibodies by circular dichroism revealed fundamental structural differences imposed by the Ala to Gly mutation as well as reduced culture temperature. We propose that the effect of reduced culture temperature on expression is protein-dependent; protein-folding fidelity and assembly is improved at lower temperatures, therefore enhancing the expression of proteins that have a propensity to misfold.

BIOT 120

High throughput system for cell culture process development

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Process development efforts in cell culture including clone screening, media development and process optimization are performed at various scales of shaker flasks and controlled bioreactors. These efforts require significant resources in both equipment and personnel. In addition, the above listed cell culture systems allow only for low (bioreactor) to medium (shaker flasks) experimental throughput. In an attempt to overcome these limitations, we have developed and implemented a novel high-throughput system for cell culture (HTS-CC) to improve the efficiency of cell culture process development. This automated system, based on commercially available as well as newly developed equipment, is used as a complementary tool to our existing cell shake flask and bioreactor culture systems. Real world performance will be highlighted, including how cell culture performance in the high throughput system compares to shake flasks and 2L bioreactors and ongoing efforts to improve the comparability of the two systems.

BIOT 121

Platform development strategy for glycoengineered *Pichia* for the production of monoclonal antibodies and therapeutic proteins

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Efforts continue on reducing the time from discovery to market with mammalian cell culture, while maintaining desired product quality attributes. In addition, an alternative being investigated is the use of faster growing microbial systems which offer the ability to reduce development timelines and production costs compared to slower growing mammalian cell lines. The use of glycoengineered *Pichia pastoris* libraries, that replicate the most essential glycosylation pathways found in mammals, is now becoming a viable option. This paper will demonstrate a systematic and efficient approach to building the *Pichia* technology as a standard platform. This includes the use of a combination of statistical design of experiments, molecular profiling and metabolite analysis. This achieved a fundamental understanding of the impact of process operations to control process consistency and product quality.

**BIOT 122**

**Modeling industrial centrifugation of mammalian cell culture utilizing a small-scale system**

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Continuous-flow centrifugation is widely utilized as the primary clarification step in the recovery of biopharmaceuticals from cell culture. However, it is a challenging operation to develop and characterize due to the lack of small-scale systems that can be used to model industrial processes. As a result, pilot-scale, continuous centrifugation is typically employed to model large scale systems requiring significant resources. In an effort to reduce resource requirements, a capillary shear device, capable of producing energy dissipation rates equivalent to those present in the feed zones of industrial disk stack centrifuges, was developed. Laboratory-scale parameters that are analogous to those routinely varied during industrial-scale continuous centrifugation were identified and evaluated for their utility in emulating disk stack centrifuge performance. Use of the scale-down system enabled small-scale modeling of the centrifuge and downstream filtration processes and reduced the resources required for clarification development and characterization.

**BIOT 123**

**Comparison of antibody purification conditions for anion-exchange resins using high-throughput screening**

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In order to streamline identification of chromatographic operating conditions for antibodies with high affinity for anion exchange (AEX) resins, binding and non-binding conditions were studied on four AEX resins as a function of pH and chloride concentration. Resins were screened in batch-binding mode on a TECAN robot. Based on the partitioning of the antibody and host cell proteins, the best resin for the antibodies was predicted, trends in binding were identified, and the robustness of a potential chromatography operation was estimated. Optimal conditions identified using robotic high-throughput screening were tested using packed-bed column chromatography to verify the validity of the batch-binding screening results.

BIOT 124

Improving a high productivity protein A resin performance via high-throughput screening process development

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To de-bottleneck the downstream processing, a high-capacity protein A resin was evaluated and adopted for clinical phase monoclonal antibody manufacturing. This new resin showed significantly improved productivity and process economy relative to other protein A resins. In order to further enhance the purification performance of this new resin and extend its reuse lifetime, we have applied 96-well plate based high-throughput screening (HTS) approach to develop improved wash and/or cleaning conditions for this new resin. The methods will be illustrated along with the results and findings from this work.

BIOT 125

Characterization of virus spike preparations used in viral clearance studies

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Monoclonal antibody and antibody-derived products are generally produced in mammalian cell culture. These cultures generally produce endogenous retroviruses and are susceptible to contamination by adventitious viral agents. Among other viral safety measures, viral clearance validation studies are a requirement for clinical use and for marketing of biopharmaceuticals. We recently reported a high-level overview of viral clearance practices in industry, which
included ranges and median clearance values by common unit operations for two commonly used viruses (MMV and X-MuLV; Miesegaes et al 2010). We found that a number of data records had contained clearance values which substantially deviated from the mean (and were therefore deemed as outliers). One plausible explanation for these variations could be unit operation performance impacts by the different virus preparations themselves, which can vary either cross-batch or cross-vendor (PDA, 2010). From a total of 18 unique virus preparations, we tested whether variations in the quality of spike preparations do exist, and whether any differences identified could impact viral clearance by two scale down unit operations. Attributes we assessed included host cell protein and DNA content, infectious-to-total virus particle count, and level of aggregates. Low pH inactivation of X-MuLV and small virus retentive filtration of PPV was performed from a subset of these preparations.

BIOT 126

Mechanisms and novel approaches to stability prediction: Osmolyte effects on IgG aggregation

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Non-native aggregation is a common degradation route for therapeutic proteins. Formulation screening often involves generation of accelerated stability data at one or more temperatures. A temperature-scanning approach for measuring non-native aggregation rates as a function of temperature is proposed and evaluated here for a monoclonal antibody across a range of different formulation conditions. Observed rate coefficients of aggregation ($k_{obs}$) were determined from isothermal kinetic studies for a range of pH and osmolyte conditions at several temperatures, corresponding to shelf lives spanning multiple orders of magnitude. $k_{obs}$ values were efficiently and quantitatively predicted by the temperature-scanning approach across a range of solutions conditions – suggesting a new approach to rapid and quantitative shelf life prediction and formulation screening. Mechanistically, both stabilizing and de-stabilizing osmolytes affected aggregation rates primarily via changes in conformational stability – at least putatively through stability of the Fab domains.

BIOT 127

From lab to manufacturing: Turning lab data into plant design space

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During the development of a cell culture process, statistical screening designs are used with lab based process models to develop an understanding of the relationships between process factors and the Critical Quality Attributes. For commercialization, this information must be turned into confirmed relationships that can be relied upon to inform the process design space in the manufacturing facility. A challenge associated with this opportunity is to attain sufficient confidence that the models are suitably predictive of responses at commercial manufacturing scale. This case study will describe the model transfer philosophy and experimental approach taken to tackle this challenge.

BIOT 128

Binding of a cationic protein to the cell surface is insufficient for cellular uptake and bioactivity: Arginine-rich sequences are necessary

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Proteins tagged with arginine-rich (R9) protein transduction domains (PTDs) cross the cell membrane and act on the cell's intracellular processes. The mechanism of internalization is unknown, but it is hypothesized that the cationic PTD fusion proteins bind non-specifically to the cell surface, which is rich in negatively-charged moieties such as heparan sulfate. The bound protein is then internalized through non-specific endocytosis. However, recent studies have suggested that the internalization process may be more specific than previously thought. Our work supports that observation. First, we expressed and purified a cationic protein, the SOX2 transcription factor, with and without the R9 PTD. We then showed that R9SOX2 and SOX2 bind equally well to heparin and cells. Both also exhibited similar binding characteristics to their cognate DNA sequence. However, only the R9SOX2 exerted transcriptional activity when administered to cells. On closer inspection, we discovered that the majority of the cell-associated SOX2 was not actually internalized, but rather tightly bound to the outside of the cell. Though the SOX2 is cationic by nature and binds well to cells without the R9 PTD, it still requires the PTD for bioactivity. Thus, our observations support the claim that a specific receptor(s) may be responsible for protein transduction via arginine-rich PTDs.

BIOT 129

Freezing dynamics and chamber scale-up challenges in the controlled rate freezing of cells in cryobags and cryovials
Variability can exist in the viability and recoverability of cells frozen in a controlled rate freezer (CRF). This may be attributed to several factors (e.g., sample handling, transfer times, CRF position). We explored several parameters related to freezing cells in cryobags and cryovials. Two critical challenges exist when freezing cells in this environment: intra-sample thermal gradients and inter-sample temperature variability during the freezing phase transition. Due to thermal gradients that can develop across the cell suspension in flat cryobags, there is a risk for spatial heterogeneity in cell survival. We examined a fully loaded industrial CRF chamber. Individual vials and cryobag profiles were studied using thermocouples to assess deviations from the chamber probe. Six bag locations in a loaded chamber were profiled for cell viability and recoverability post-thaw to assess the effect of freeze cycle variability. Additionally, alternate cryobag orientations are being explored to improve sample temperature homogeneity.

BIOT 130

Automated cell culture and screening towards cell-based therapeutics

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Production of cell-based therapeutics requires safe and standardized manufacturing procedures. To accomplish that, we have developed a closed and automated bench scale system for the production of cellular therapeutic agents. In this new cell processing device we integrated filtering, temperature-controlled centrifugation, magnetic separation and cell culture. A new centrifugation chamber was designed to enable in-process liquid exchange, cell fractionation and culture, tested with standard cell lines, stem, progenitor, dendritic, T- and NK-cells. We found significant lower process variations compared to other reference cell manufacturing systems. Building on this technology, we extend our applications to guided differentiation or maturation of stem cells. Optimized culturing technology for induced pluripotent cells and progenitor cells will enable translational research in these emerging technologies. We present how we are using automated cell culture and cell-based screening solutions to optimize culture media improving basic and translational research enable direct into clinical applications.

BIOT 131
Combination cell therapy for diabetes using recombinant non-β cells

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A living biological substitute for treatment of insulin-dependent diabetes has significant potential in providing a less invasive, more physiologic regulation of blood glucose levels than insulin injections. Cell therapies based on potentially autologous, non-β cells genetically engineered to secrete insulin are advantageous due to immune acceptance. As it is unlikely that a single recombinant non-β cell can replicate the complex biphasic secretion kinetics of normal islets, we investigate a combination cell therapy based hepatic and enteroendocrine cells. Results show that hepatic cells secrete insulin in a more sustained manner, whereas enteroendocrine cells, which possess a regulated secretory granule system, exhibit rapid secretion upon stimulation with metabolic secretagogues. Combinations of the two cell types better approximate the β cell secretion dynamics than either cell type alone. Insights into in vivo preclinical experiments and clinical translation of the dual cell therapy will be discussed.

BIOT 132

Mass production of cardiomyocytes from murine embryonic stem cells in 3D bioreactor culture system

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Heart disease is one of top causes of death in the US and other developed countries. Many heart diseases are related to the progressive loss of functional cardiomyocytes. Cardiomyocytes cannot proliferate after birth; therefore, differentiation of embryonic stem cells (ESCs) to cardiomyocytes has become a promising method for heart repair and regeneration. In this study, a novel 3-dimensional (3-D) bioreactor culture system using polyethylene terephthalate (PET) fibrous matrices as cell supports were developed. The PET fibrous matrices provided large surface areas and an in vivo-like 3-D environment, and murine ESCs were expanded in this culture system with a significant fraction of cells showing cardiogenesis as confirmed with immunostaining, flow cytometry and reverse transcriptase polymerase chain reaction. This 3-D bioreactor culture system provided highly facilitated cell growth and cardiac differentiation of ESCs, exhibiting great potential in mass producing ESC derived cardiomyocytes that can be used for cell transplantation therapies.
BIOT 133

Enrichment of hepatic progenitor cells from directed differentiation of pluripotent stem cells

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Stem cell derived hepatocytes hold great potential for applications in the treatment of liver failure and the development of pharmaceuticals. We have previously demonstrated that pluripotent stem cells can be guided towards a hepatic fate, yielding cells that exhibit key hepatocyte functions. A major step in the differentiation process is the emergence of hepatic progenitor cells (HPCs) which are capable of self-renewal and differentiation into both hepatocytes and biliary epithelial cells. We explored the potential of enriching HPCs through surface marker delta-like-protein (DLK1) based sorting using flow cytometry and by use of an EFGP based promoter-reporter line. These HPCs were further differentiated into hepatocytes in a three-dimensional aggregate culture system, which enhanced their functional maturity. Studies on enriched HPCs can provide insights into the liver development process and differentiated hepatocytes can be used as a cellsource in microfluidic devices, which serve as a platform for high-throughput drug toxicity screening.

BIOT 134

Assembly of functional neovessels using a stereolithographic hydrogel matrix

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Hydrogels are increasingly being used as cell encapsulation devices for both fundamental biology studies and cell transplantation therapies because of their structural similarity to the natural extracellular matrix. The successful use of a hydrogel greatly relies on an ability to control hydrogel stiffness which affects structural integrity and regulates cellular phenotypes. This study presents a novel strategy to decouple the inversed dependency of permeability on the stiffness of a hydrogel by chemically cross-linking methacrylic alginate (MA) with poly(ethylene glycol) dimethacrylate (PEGDA). In addition, incorporating micro-sized channels into a cell encapsulating hydrogel by a stereolithography (SLA) would improve the cell viability and subsequently secretion of multiple proangiogenic growth factors from cell encapsulated. In this study, fibroblasts were encapsulated into the vascularized 3D hydrogel by the SLA based in situ photo cross-linking reaction. The role of micro-sized channels by combining a hydrogel formulation in enhancing cell-viability and regulating cell-functions was investigated. The roles of incorporation of MA and microchannels in regulating fibroblasts function to secret proangiogenic growth factors and subsequently promote the neovascularization in a connective tissue was evaluated using chorioallantoic membrane (CAM). Implantation of PEGDA hydrogel onto CAM stimulated inflammation within two days likely because of extravasation of dead cells' debris. In contrast, the PEGDA/MA hydrogel minimally stimulated host inflammation likely because of its ability to let the encapsulated cells remain viable. Interestingly, fibroblast-encapsulated PEGDA/MA containing microchannels increased the density of mature capillaries with certain patterns, depending on the geometry. Taken together, the results of this study will be an invaluable paradigm of a 3D cell encapsulation device prepared with a broad array of gel-forming polymers.

BIOT 135

Enhanced neuronal differentiation of mouse embryonic stem cells cultured on PET membranes coated with multi-wall carbon nanotubes

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Embryonic stem cells (ESCs) capable of self-renewal and differentiation into various cell types are generally regarded as good cell sources for cellular transplantation and tissue engineering. Neurons differentiated from stem cells can be exploited as promising cell therapy tools in treating neurological pathologies of the central and peripheral nervous systems. Although ESCs are already successfully used in repairing spinal cord injury in small animal models, in vitro differentiation of ESCs into neuronal cells has always been a challenge. In this work, we found that multi-wall carbon nanotubes (MWCNTs) coated on microporous polyethylene terephthalate (PET) membranes promoted the differentiation of mouse embryonic stem cells into neuronal cells. Flow cytometry
and RT-PCR were used to characterize cell differentiation. Besides cell differentiation, neurites outgrowth and cell viability were also investigated. MWCNTs-coated PET membranes can serve as potential scaffolds for cell differentiation in tissue engineering.

BIOT 136

Molecular design of affinity ligands for MABs purification

Carlo Cavallotti

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One of the most critical steps in Monoclonal Antibody (MABs) production is their purification, usually performed through protein A affinity chromatography. Because of the cost of this process, alternative approaches have been investigated in the last years. In the present work we present the computational study of an affinity material aimed at elucidating how each material component (support, spacer, surface chemistry) affect its performances for MABs capture. Material properties were investigated developing a molecular model of support (agarose), spacer, and ligand (the bio-mimetic triazine-based A2P affinity ligand). The computational predictions were validated through comparison with experiments performed both for mock feed solutions as well as real cell culture supernatants. The results indicate that the optimization of the ligand support interaction is as important as that of the ligand protein interaction. The main result of this study is a design criteria for affinity ligands for MABs capture. Some examples are given.

BIOT 137

Prediction of protein chromatographic behavior in multi-modal chromatographic systems

Ying Hou

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Multi-modal chromatographic systems offer unique selectivities over traditional single mode chromatography. However, the complex protein-resin interactions make the prediction of chromatographic behavior challenging. In this work, predictive tools were developed in multi-modal ion exchange and hydroxyapatite chromatographic systems. A large set of proteins with a wide range of physicochemical properties such as size, charge and hydrophobicity were investigated in these systems. Molecular dynamic simulations were employed to
provide fundamental insight into the binding behavior in these systems. The insights from these simulations were then used to design novel molecular descriptors for these systems and the descriptors were employed in QSPR models to predict key thermodynamic parameters. Finally, column models were used to predict protein elution behavior in these multimodal systems. This work will have significant implications for methods development in industrial bioprocesses and for improving for quality-by-design (QbD).

BIOT 138

Elution band prediction for isoelectric chromatofocusing and its application to protein purification

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Although it theoretically achieves greater peak capacities than ion exchange chromatography, isoelectric chromatofocusing (ICF) has not found widespread use in industry, due in part to difficulties in predicting and reproducing protein elution behavior. We present a new model and associated isotherm for ICF that accounts for key determinants of protein partitioning, including the influences of pH, ionic strength and the hydrogen-ion Donnan equilibrium at the stationary phase surface, and then show that it accurately predicts ICF elution chromatograms for b-lactoglobulin A and b-lactoglobulin B. Retention times are predicted and experimentally found to be strongly dependant on mobile phase ionic strength. Protein elution profiles can be altered by adjusting the ionic strength in accordance with model predictions to improve the resolution of similar protein isoforms. For preparative separations, throughput can be improved by using the model to define ICF operating conditions that minimize product retention while achieving required purity and yield.

BIOT 139

Effect of protein concentration on the pH and composition of protein solutions: Implications for protein formulation and TFUF process development

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The pH and buffer composition of protein solutions prepared by ultrafiltration, diafiltration and dialysis change with increasing protein concentration. Even though the physical principles that underlie this phenomenon are well known, i.e. Gibbs-Donnan and excluded volume effects, they are not commonly considered in the development of high concentration formulations. In this work, experimental pH data for two different proteins is presented as a function of protein and salt concentrations for acetate, succinate and phosphate buffers. Ion species and sugar concentrations are also measured experimentally for selected conditions. These experimental results are used to test a simple model based on the Gibbs-Donnan equilibrium. The effect of temperature is also measured experimentally and compared to theoretical predictions. This approach is used to identify formulation conditions that would lead to a robust TFUF (Tangential Flow Ultra Filtration) process and to understand the pH shift often observed during TFUF at high protein concentrations.

BIOT 140

Excipient partitioning under non-equilibrium ultrafiltration conditions: Qualification of a model for late-stage process development

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Highly concentrated protein formulations can experience significant deviations of the retentate composition from the buffer used during diafiltration. While the underlying causes of these deviations are generally known, existing models suffer from limited applicability or require the use of empirical corrections. This lack of reliable predictive tools can put a strain on the interface between purification and formulation development. We will report on an extensive qualification of an excipient partitioning model incorporating volume exclusion and electrostatic interaction effects. The predictive capacity of the model was tested against 5 monoclonal antibodies of widely diverging properties and a range of process conditions. We report on model performance under both equilibrium and non-equilibrium ultrafiltration conditions as well as the use of protein sequence-derived information for further refinement of model predictions. The application of the resulting model to studies of process robustness and parameter criticality in characterization situations will be discussed.

BIOT 141

Model based design of experiments – case studies
Implementing QbD for process development, the use of statistical methods and Design of Experiments has become the method of choice in many pharmaceutical companies. In this presentation we will show how mathematical models based on chemical engineering principles can be used to get a better understanding of unit operations and to improve the experimental design when performing a process challenge. Using simulation for process challenge a full factorial set up may be performed without considering the number of physical experiments. Based on the results of the factorial simulation study or Monte Carlo simulations we define our design space and select a set of relevant conditions for experimental verification. This type of model reduced design of experiments has the big advantage that experimental conditions may be selected based on the output variables (CQAs) instead of only the input variables. Case studies showing the complete set up from calibrating the models to a summary of how we may report the work to regulatory agencies will be discussed.

BIOT 142

Preparative chromatography modeling for process verification and control

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The development and use of mechanistic models in design and verification of preparative chromatographic processes is illustrated by two case studies. The objective is to use mechanistic modeling as a tool to implement the ideas of quality by design and improve process understanding. Modeling is used to predict process performance under process parameter variability which allows the identification of critical process parameters and probability of batch failure. This information can be used to in the design of experiments for process verification and to give a quantitative ranking of process parameters based on the sensitivity of the process to the parameter and the quality requirements. The knowledge gained about process performance under variability is also used to design control strategies to ensure that the process is maintained within the quality requirements. The implications of the choice of control strategy on the process performance are discussed.
Spatial homogeneity analysis of packed bed chromatography

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Packed bed chromatography is usually modeled in one or two spatial dimensions, for example by the general rate model. Such models assume that fluid flow and solute molecule concentrations are homogeneously distributed over column cross sections. Moreover, concentration gradients within the beads are at most considered along the radial coordinate. These homogeneity assumptions are studied with a three-dimensional model of the involved convection, diffusion and adsorption processes. Due to the complex geometry only bed sections with up to several hundred spheres can be computed without using supercomputers. Hence, a sphere packing algorithm was modified for cuboids with periodic boundaries perpendicular to the fluid flow. The resulting concentration profiles indicate that the studied homogeneity assumptions are valid in many cases, even though complex flow profiles are observed in the interstitial volume. However, dominant wall effects justify the computational effort of spatially resolved simulations for small columns with volumes on the micro-liter scale.

BIOT 144

Prediction of aggregation propensities of therapeutic proteins

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Methods for in silico prediction of the aggregation propensities of proteins are reviewed. A new technology termed SAP (spatial aggregation propensity) is presented which permits the identification of aggregation hot-spots based on the dynamic exposure of spatially–adjacent hydrophobic amino acids. Monoclonal antibodies will be ranked by SAP scoring and the results compared with characterization and long-term stability data. The utility of the SAP technology in protein engineering and in the design of antibody drug conjugates will be demonstrated.

BIOT 145

Engineering and characterizing aggregation-resistant antibodies
Monoclonal antibodies and antibody fragments are critical for numerous detection and therapeutic applications, yet their utility is limited by their variable and unpredictable aggregation propensities. Therefore, determining the sequence determinants that differentiate aggregation-resistant antibodies from aggregation-prone ones is critical to improving their activity. We are investigating the molecular origins of aggregation resistance for human VH domain antibodies that differ only in the sequence of their complementarity determining regions (CDRs), yet possess dramatically different aggregation propensities. We find that the propensity of domain antibodies to aggregate after being transiently unfolded is not a distributed property of the three CDR loops, but can be localized to a single hydrophobic residue at the edge of one CDR. We are currently exploring the impact of hydrophobic-to-charged mutations at the edge of specific CDR loops in several human antibodies to evaluate if resistance to aggregation can be rationally engineered into antibodies without altering their affinity.

BIOT 146

Predictive tools towards screening monoclonal antibodies with optimal physicochemical properties

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Predicting physical behavior of mAbs in solution (viscosity and solubility), especially at high concentration remains a challenge. This talk will focus on approaches that we have utilized towards prediction of such behavior. Specifically, we have developed the concept of theoretically quantifying relative hydrophobicity (with and without inclusion of net charge) of mAbs, known as “hydrophobicity index” from their sequence/structure. The utility of this index along with charge and pl and correlation to physical properties will be presented. Utilization of homology modeling towards generating 3-D structures of Fv domains will be presented in the context of hydrophobicity index and correlations to physical behavior. Additionally, data will be presented to demonstrate utilization of residue SASA towards developing a risk-based model for chemical degradation, especially, Met/Trp oxidation. We believe that such in silico approaches will aid in an early evaluation and screening of molecules towards their physical behavior and chemical stability.

BIOT 147
Characterization of protein-protein interactions at high concentrations by selective conjugation of small molecules to antibodies

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High concentration formulations of some monoclonal antibodies can be problematic as they are highly viscous. The high viscosity in these mAbs is thought to be caused by weak protein-protein interactions. The nature of these protein-protein interactions and the surfaces that interact is not understood. We have selectively conjugated hydrophobic and charged small molecules to mAbs using maleimide chemistry on engineered cysteines. The first two mAbs tested elicit high viscosity at high concentrations while a third mAb does not demonstrate a large increase of viscosity at high concentration. Conjugation of a hydrophobic molecule to the first two mAbs greatly increases the measured viscosity at high concentrations; however, this small molecule does not affect viscosity of the third mAb. The differences in CDR regions between these three mAbs and the nature of the interactions of the small molecules (charged and hydrophobic) with the CDR regions as well as the effect of different excipients will be discussed.

BIOT 148

Modeling protein degradation processes and the development of rational approaches to stabilization

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We describe a new strategic approach to the formulation and stabilization of biotherapeutics. The approach is based on applying both molecular and macroscopic modeling tools in order to gain an understanding of degradation processes with unprecedented detail and accuracy. The microscopic modeling approach can be used to visualize key regions of proteins, including antibodies, that lead to degradation or are otherwise important for a variety of technological applications. Such applications include screening molecules for developability and manufacturability during discovery, identifying key sites that are responsible for degradation for the purpose of removing them, identifying sites for conjugation of payloads, and identifying binding regions. The macroscopic modeling approach can be used to screen formulations and generally understand better degradation phenomena. We also show how molecular-level simulations can lead to an understanding of protein-cosolute interactions with unprecedented
detail and therein the rational design of new formulations. Our group works on a variety of degradation processes, such as aggregation, oxidation, deamidation, and hydrolysis. We combine modeling with experimental approaches, always with the objective of aiding in the development of biopharmaceuticals. Emphasis for this talk is on protein/antibody aggregation.

BIOT 149

**Novel technique to measure particle load in protein solutions based on product filterability**

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A new method has been developed that characterizes the particle content in a product based on the filter pore plugging propensity of the solution. The method estimates pore-plugging of a filter by measuring the impact on extrusion force needed for constant flow rate through a syringe filter. This technique provides a mechanism to fingerprint the particle load in a protein solution using a sample volume as small as 3mL. Unlike HIAC, the method is shown to be more accurate at measuring sub-visible particles in high concentration products. The pore-plugging data also shows improved correlation with visual observations and can be used to develop a predictive model to assess kinetics of particle growth. The sensitivity of the method allows it to measure the impact of various stresses including freeze-thaw, shear and transportation on product quality at a resolution higher than visual inspection and other analytical methods.

BIOT 150

**Auto-oxidation: A potential pathway for antibody degradation**

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Protein oxidation can occur by multiple mechanisms including the generation of reactive oxygen species (ROS), free radicals, and hydrogen peroxide (H$_2$O$_2$) by either intrinsic (e.g. light induced auto-oxidation) or extrinsic pathways (e.g. Fenton reaction). A decrease in potency under real time storage conditions for an IgG1 monoclonal antibody (MAb1) drug product was observed and correlated to oxidation of a solvent exposed tryptophan residue in the CDR. The purpose of this study was to determine if the observed oxidation in MAb1 was the result of ROS generated through an auto-oxidation pathway. Our results indicated that
MAb1 generated H$_2$O$_2$ via an auto-oxidation pathway and that this was dependent upon time of light exposure as well as MAb1 concentration. However, H$_2$O$_2$ production was independent of formulation conditions including surfactant and excipient concentrations. Our studies also indicated that the H$_2$O$_2$ generation occurred through ROS, predominantly singlet oxygen. Concurrent to the production of H$_2$O$_2$, results from RP-HPLC and LC/MS confirmed that the solvent exposed tryptophan in the CDR was the primary site of oxidation. A possible reaction pathway and its implication on formulation development of MAbs will be discussed.

BIOT 151

Engineered biosynthesis of hydroxytyrosol, a potent antioxidant from olive

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Hydroxytyrosol, one of the most potent antioxidants, is found in olive oil extract and presents several interesting aspects for human health. Currently, hydroxytyrosol is produced mostly as the form of enriched olive extracts which is extracted from olive as an ester (oleuropein) and hydrolyzed chemically or enzymatically. In this report, we engineered *Escherichia coli* to produce hydroxytyrosol from inexpensive and readily available starting materials such as tyrosine and glucose. In engineered *E. coli* strain, tyrosine which is exogenously supplemented or endogenously synthesized from glucose was converted into hydroxytyrosol via 4 steps; (i) hydroxylation of the tyrosine, (ii) decarboxylation of L-DOPA, (iii) deamination of dopamine, (iv) reduction of dihydroxyphenylacetaldehyde. We have developed efficient conversion step of tyrosine to L-DOPA without overoxidation into o-quinone and dopachrome which polymerizes to melanin. This finding can be further investigated and applied toward the biosynthesis of more advanced natural product such as morphine, serotonin, and melatonin.

BIOT 152

Optimizing functional production of human adenosine A$_2$b receptor in *S. cerevisiae*

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Biophysical and structural characterization of G protein-coupled receptors (GPCRs), a diverse class of seven transmembrane domain-containing receptors that mediate signaling across the plasma membrane, have been limited by obstacles to functional production. Our group has previously shown that human adenosine A2b receptor can be expressed in S. cerevisiae, where it exhibits whole-cell ligand binding. However, immobilized ligand affinity chromatography of purified receptors shows that only a fraction of the receptors maintain ligand binding outside of the cell. We hypothesize that tuning A2bR expression level may reduce cellular responses and will result in increased functional yields as shown previously for the trypanosomal H+/adenosine cotransporter protein. We have developed a generalized high-throughput strategy to evaluate single cells that differ in gene copy number based on an integrated fluorescent unfolded protein response reporter. Current work focuses on appropriate choice of promoter strategy and sorting for desired properties by fluorescence-activated cell sorting (FACS).

BIOT 153

Engineering gene knockdown in mammalian cells by transcriptional interference

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Many metabolic engineering applications require controlled suppression of gene expression. Transcription interference, the hindrance of RNA polymerase functions at targeted site(s), offers a direct means of repressing even highly expressed metabolic genes. This work aims to reduce transcription by engineering a large DNA-binding protein to specifically bind to the target gene, thus retarding RNA polymerase transcription of the target gene. The extent of gene expression suppression depends on the affinity of the DNA-binding protein to the gene sequence and also the number of target sites along the gene. This technique is demonstrated in mammalian cells by targeting it at a model gene, GFP. This novel method for gene knockdown has potential for widespread applications in metabolic engineering of recombinant protein producing mammalian cells.

BIOT 154

Metabolic network reconstruction and \(^{13}\text{C}\)-metabolic flux analysis for the extremophile Thermusthermophilus HB8
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*Thermus thermophilus* is a thermophilic bacterium that thrives at temperatures around 70-80°C. It has immense biotechnological potential, especially for production of low-boiling biofuels. A major limitation for the application of this organism is the limited knowledge regarding its metabolism. Here, we reconstructed and validated the metabolic network model for *T. thermophilus* HB8 using $^{13}$C tracer experiments and metabolic flux analysis. *T. thermophilus* HB8 was successfully grown in custom-designed mini-bioreactors (10 mL) on a fully defined medium. The metabolic network model was extracted from KEGG and was validated using $^{1-13}$C, $^{2-13}$C, $^{3-13}$C, $^{4-13}$C, $^{5-13}$C, $^{6-13}$C, $^{1, 2-13}$C glucose. For each tracer, enrichments of 29 amino acid fragments were measured using GC-MS. In addition, enrichment of CO$_2$ in the off-gas was measured. Fluxes were obtained by fitting the isotopomer data to the reconstructed metabolic model. The results obtained from this work will allow future metabolic engineering of *T. thermophilus* for biofuels production.

**BIOT 155**

Toward a bacterial dirigible: Autonomous localization and actuation

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Synthetic biology is often attributed to the rewiring of a cell's genetic circuitry for the synthesis of novel products using heterologous processes. A less common but equally innovative view makes use of the reprogrammed cell as the product. We have created a genetic circuit that endows *E. coli* with targeting, sensing & switching capabilities. The resultant cell is a bacterial dirigible – a cell that autonomously navigates and carries or deploys important “cargo”. The bacteria target desired locales on mammalian cell surfaces by “homing” functions which bind cell surface biomarkers, receptors or other ligands. Specifically, a IgG Fc region binding domain, protein G, is displayed on the outer membrane of bacteria which allows targeting. Upon accumulation at the targeted surface they trigger a “switch” in response to the biomarker density. This serves as a phenotype focusing system and maintains the switch in an “off” state until the desired threshold is reached. Several potential applications are discussed.
**BIOT 156**

**CAP-Technology: Production of biopharmaceuticals in human amniocytes**

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Human CAP (CEVEC’s Amniocyte Production) cells allow for stable and high yield production of recombinant proteins, with excellent biologic activity and therapeutic efficacy, as a result of authentic posttranslational modification. Based on CAP cells a human transient expression system has been developed, that enables extremely high production yields of recombinant proteins within a few days. Thus, CAP and CAP-T technologies offer the use of only one unique platform for early preclinical development through to clinical supply of recombinant biotherapeutics. The platform represents also an efficient tool for virus and vaccine production. *Hartmut Tintrup, Ph.D.*, Director, Marketing and Business Development, *Cevec Pharmaceuticals GmbH, Germany*

**BIOT 157**

**Enhanced propionic acid production from sucrose by metabolically engineered propionibacteria**

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Propionic acid is a widely used chemical currently produced almost exclusively through petrochemical route. There are high interests in producing propionic acid from renewable biomass through fermentation. Propionyl-CoA:succinate CoA transferase (CoA T) of *Propionibacterium acidipropionici* catalyzes the key and last step of propionic acid biosynthesis pathway. In this work, CoA T was overexpressed by using its native promoter in *P. acidipropionici* and heterologously in *P. freudenreichii* to study its effects on cell growth and propionic acid production. Batch fermentations with mutants were performed in serum bottles and 5-liter fermentors with sugarcane juice containing mainly sucrose as the substrate, and the results showed that overexpressing CoA T increased cell growth and propionic acid production. Further analysis of metabolic flux distributions in the mutants and wild-type parent strains was performed and the results can be used to elucidate the effects of CoA T overexpression on cell growth and propionic acid production.

**BIOT 158**
Directed evolution of quorum-sensing-dependent transcriptional repressors

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A key challenge in the field of synthetic biology is to engineer communities of cells that exhibit coordinated, population-level behaviors. Components from acyl-homoserine lactone (AHL)-based quorum-sensing (QS) systems have been used to engineer communication in synthetic multicellular systems. To date, the QS regulators available for engineering intercellular communication have been limited to transcriptional activators, such as LuxR. To expand this toolbox, we targeted the AHL-dependent transcriptional repressor, EsaR. Characterization of wild-type EsaR showed that it requires micromolar concentrations of AHL to turn on gene expression, compared to nanomolar concentrations for LuxR. Therefore, we built libraries of esaR mutants and identified EsaR variants with increased AHL sensitivity using an ON/OFF screening system. We have identified EsaR variants that respond to a range of AHL concentrations, from 10 to 1000 nM. These new parts are enabling work exploring the roles of regulatory mechanisms and network architectures for intercellular communication in multicellular systems.

BIOT 159

Biohydrogenation from biomass sugar mediated by in vitro synthetic enzymatic pathways

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Future transportation fuels might mainly consist of hydrogen for most vehicles, electricity for short-distance vehicles, and high-energy density liquid biofuels for jet planes. Therefore, it would be important to efficiently produce high-energy density liquid jet fuel (e.g., hydrocarbons, fatty acid esters or butanol) suitable for jet planes from less costly biomass sugars, because energy application usually decides its production. Cell-free synthetic pathway biotransformation (SyPaB) is the in vitro assembly of a number of enzymes and coenzymes for implementation of complicated biochemical reactions that a single enzyme cannot do. We demonstrate high-yield generation of NAD(P)H from a renewable biomass sugar – cellobiose through an in vitro synthetic enzymatic pathway consisting of 12 purified enzymes and coenzymes. When the NAD(P)H generation system was coupled with its consumption reaction mediated by xylose reductase, the NADPH yield was as high as 11.4 mol NADPH per cellobiose (i.e., 95% of theoretical yield – 12 NADPH per glucose unit) in a batch reaction. Consolidation of endothermic reactions and exothermic reactions in one pot results in a very high
energy-retaining efficiency of 99.6% from xylose and cellobiose to xylitol. The combination of this high-yield and projected low-cost biohydrogenation and aqueous phase reforming may be important for the production of sulfur-free liquid jet fuel in the future.

BIOT 160

Evaluation of HMG-CoAReductase variants for improved isoprenoid production in Escherichia coli

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Mevalonate pathway has been explored and engineered as the major biosynthetic pathway for the production of isoprenoids in both E.coli and yeast. HMG-CoA reductase (HMGR) is one of the key enzymes in this pathway and it is involved in the reduction of toxic intermediate HMG-CoA into non-toxic intermediate mevalonate, which has been known as a crucial bottleneck of mevalonate pathway engineering. In this study, we examined the effect of perturbing enzymatic properties of HMGR while maintaining existing features of the engineered mevalonate pathway. In particular, we examined the kinetic properties of five HMGR variants and the impact of them on amorphadiene production in a well-characterized engineered strain of E. coli. These results emphasize the importance of both enzyme kinetics and enzyme abundance in pathway optimization efforts to achieve high product yields.

BIOT 161

Efficient optimization of synthetic metabolic pathways with the RBS Library Calculator

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Synthetic metabolic pathways enable microbes to manufacture high-value chemicals from low-value feedstock. However, identifying the optimal enzyme
expression levels that maximize a pathway's productivity remains a major challenge. We have developed the RBS Library Calculator, a predictive design method that enables efficient combinatorial optimization of synthetic metabolic pathways. The method employs our thermodynamic model to predict a protein coding sequence's translation initiation rate in bacteria (Salis et al., Nature Biotech, 2009). We demonstrate the RBS Library Calculator's utility by optimizing a many-protein pathway responsible for biodetoxification of lignocellulosic feedstock. The RBS Library Calculator designs synthetic, degenerate ribosome binding site sequences to vary a protein's expression level in uniform steps across a 100,000-fold range, while minimizing the library size. The method also allows you to "zoom in" on a selected expression level range and control the search resolution. Using the method, degenerate RBS sequences can be designed to simultaneously vary the expression levels of a 10-enzyme pathway in 12 uniform steps across a 100,000-fold range, for a total of $10^{12}$ combinations. In contrast, the same goal using a random mutagenesis approach is entirely unfeasible. After using screening or selection to identify the best pathways, their enzyme expression levels can then be determined from their sequences. By successively "zooming in" on these near-optimal expression level ranges, the globally optimal enzyme expression levels can be identified. The RBS Library Calculator offers an unprecedented ability to efficiently search the expression level space of a many-protein pathway, while quantifying the activity-sequence relationship along the way. We experimentally quantify the method's capabilities using fluorescent protein reporters, spectrophotometry, and steady-state protein expression level measurements. We use both single-protein and multi-protein operon systems to simulate a typical genetic system. We then employ the RBS Library Calculator to optimize a many-enzyme biodetoxification pathway for lignocellulosic feedstock. Throughout, we use new in vitro DNA assembly techniques to construct large, synthetic, multi-protein genetic systems.

BIOT 162

Design-driven approaches for engineering RNA-regulated pathway controls

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Models and simulation tools for designing and assembling components into functional devices are the hallmarks of most engineering fields. However, the design tools available to engineer synthetic biological devices are very limited. We formulated design-driven approaches that
employ mechanistic modeling and kinetic RNA folding simulationsto engineer RNA-regulated genetic expression devices. In this presentation, I will describe methods for engineering ribozyme and metabolite-controlled, aptazyme-regulated expression devices from components characterized in vitro, in vivo and insilico. We validated the underlying models and overall strategy by constructing 28 E. coli expression devices with excellent agreement between the predicted and measured expression levels ($r=0.95$). We have also demonstrated the application of these technologies for building pathway biosensors and for optimizing production from engineered strains. I will discuss these results and the broader potential for using biochemical and biophysical models to develop biological design tools that rival those found in other engineering disciplines.

BIOT 163

Engineering a synthetic microbial consortium for efficient production of biofuels

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Present approaches to engineering microbes for cellulosic biofuel production are based on a “superbug” paradigm of incorporating all required functionalities into a single organism, an endeavor that has hitherto proven to be difficult. Inspired by the widespread occurrence of synergistic microbial communities in nature, we explore a novel alternative direction for microbial engineering: the design and construction of a synthetic microbial consortium consisting of multiple species which cooperate to directly convert cellulose to biofuel. Three microbial specialists are utilized in the consortium: a cellulolytic specialist, which secretes cellulase enzymes to hydrolyze lignocellulose into component hexose and pentose mono/oligosaccharides, and engineered hexose and pentose specialists, which each ferment their respective carbon sources to biofuel products. We use ecology and evolutionary theory as a framework to design stability and tunability into the consortium. As a proof-of-concept, we construct a consortium capable of directly converting cellulose to isobutanol, a promising next-generation biofuel.

BIOT 164

Comparative transcriptomic and proteomic profiling of high ethanol-producing Clostridium thermocellum

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Clostridium thermocellum, an anaerobic, thermophilic, and cellulolytic bacterium, is an attractive ethanogenic organism for renewable biomass conversion. However, its low ethanol tolerance has been an obstacle for the process industrialization. A strain tolerant of 4% (w/w) ethanol obtained by serial transfer was capable of producing twice higher concentration of ethanol than wild type. Transcriptomic and proteomic analysis were performed on exponentially growing and stationary phase wild type and tolerant strain. Wild type and tolerant strain exhibited similar dynamics in transiting from growth to stationary phase in many gene classes including cellulosome, glycolysis and protein folding. Whereas many genes involved in membrane transport underwent different responses between them. Combining transcriptomic data with iTRAQ-based quantitative proteomic results, genes and gene classes related to high ethanol producing feature emerged as potential targets for genetic manipulation.

BIOT 165

Using genome-wide and targeted tools to engineer acetate tolerance in E. coli for improved cellulosic biofuel production

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Biofuels from cellulosic biomass are important in the transition to the next generation of renewable energy, but there are more obstacles when compared to first generation biofuels. These include inhibitory compounds formed during pretreatment, the most prevalent of which is acetate. Here we utilize multiple genetic engineering tools to identify mechanisms of acetate tolerance and engineer an improved strain. The SCalar Analysis of Library Enrichments (SCALEs) and the TRackable MultiplexRecombineering (TReMR) genome-wide methods were employed to identify good candidates for mutating ribosomal binding sites using a method similar to Multiplex Automated Genome Engineering, developed by Wang et al. (2009). Presented here are SCALEs, TReMR, and RBS mutation selections with acetate. Mutants were identified via microarray analysis and traditional colony picking. Mutants identified after the process of selections showed significantly better growth characteristics compared to the parent strain. Various genetic modifications and supplementation strategies that increase tolerance were identified.
Engineering *Clostridium tyrobutyricum* for n-butanol production

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Butanol production in acetone-butanol-ethanol fermentation suffers from low butanol yield, titer and productivity. In this work, a novel solventogenic *Clostridium* was developed by introducing butanol biosynthesis pathway and blocking acid producing pathways in non-solventogenic acid-forming *Clostridium tyrobutyricum* which has relatively simple metabolic pathways with high flux toward butyryl-CoA, the precursor for butanol biosynthesis, and high butanol tolerance, rendering it an ideal host for butanol production. Plasmids with different Clostridial replicons were used to overexpress butanol-forming enzymes. Various mutants with knockout of acetate kinase, phosphotransacetylase and phosphotransbutyrylase were studied for their effects on butanol production. Strategies to improve NADH level *in vivo* by using substrates with different reduced states and overexpressing formate dehydrogenase were also investigated for increasing butanol production. A high butanol titer (>10 g/L) with a high yield (0.33g/g substrate) was obtained, indicating that the engineered *C. tyrobutyricum* can be used for butanol production.

Effects of 3D microwell confinement on Wnt/β-catenin signaling in human embryonic stem cells

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We have developed a 3-D microwell culture system that promotes long-term self-renewal of hESCs and can generate uniform embryoid bodies (EBs) with enhanced cardiogenic potential. Elucidating the mechanisms underlying these effects can provide effective targets for controlling self-renewal and differentiation. Here we evaluated the effect of microwell confinement on Wnt/β-catenin signaling in hESCs. Our results showed that the microwell environment led to downregulation of Wnt signaling, as evidenced by a lack of nuclear β-catenin and downregulation of Wnt target genes in microwells. Additionally, flow cytometry demonstrated that hESCs cultured in microwells had higher expression of E-cadherin per cell. Although there was reduced Wnt signaling in
microwells, data from a Wnt reporter line showed that EBs formed from microwells contained higher levels of Wnt signaling than EBs from 2-D controls. These differences in Wnt/β-catenin signaling suggest a potential mechanism by which microwell confinement affects hESC self-renewal and cardiac differentiation.

BIOT 168

3D cell culture microarray for high-throughput studies of stem cell fate

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We have developed a miniaturized 3D stem cell culture array on a functionalized glass slide for spatially addressable high-throughput screening. A microarray spotter was used to deposit cells onto a modified glass surface to yield an array consisting of cells encapsulated in alginate gel spots with volumes as low as 60 nL. A method based on an immunofluorescence technique scaled down to function on a cellular microarray was also used to quantify specific cell marker protein levels \textit{in situ}. Our results revealed that this platform is suitable for studying the expansion and neural commitment of mouse embryonic stem (ES) cells as they retain their pluripotent and undifferentiated state, and are able to generate neuroectodermal precursor cells characterized by expression of the neural marker Sox-1. In addition, the high-throughput capacity of the platform was tested using a dual-slide system that allowed rapid screening of the effects of tretinoin and fibroblast growth factor-4 (FGF-4) on the pluripotency of mouse ES cells. This high-throughput platform may represent a powerful new tool for investigating cellular mechanisms involved in stem cell expansion and differentiation, and provides the basis for rapid identification of signals and conditions that can be used to direct cellular responses.

BIOT 169

Engineering stem cell microenvironments for directed differentiation and morphogenesis
Pluri- and multipotent stem cells represent robust sources for the development of cell therapies and diagnostics, however effective differentiation of stem cells is limited by morphogen presentation methods. Microspheres fabricated from different biomaterials can be engineered to control the spatiotemporal presentation of biomolecules to cells, thus the objective of our work is to introduce engineered microspheres into stem cell microenvironments for controlled, local delivery of differentiation factors. Microsphere materials can be incorporated within 3D stem cell aggregates without adversely affecting cell viability and directly impact differentiated stem cell phenotype. In addition, microspheres regulating the kinetics and dose of morphogen can affect the relative proportions of differentiated stem cell phenotypes. Overall, controlling stem cell extracellular microenvironments via engineered biomaterials is a novel route to direct the differentiation of stem cells through controlled, local presentation of molecules, a broadly applicable principle that can be integrated into the development of stem cell technologies.

**BIOT 170**

**Efficient isolation of sub-cellular fractions in hESCs: Applications to quantitative analysis of hESC self-renewal and differentiation**

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Efficient isolation of sub-cellularorganelles such as nuclei and the plasma membrane remains a primary challenge in the study of human embryonic stem cells (hESCs). These sub-cellularfractions are often required in high purity for cell signaling studies as wellas for proteomic analysis through mass spectrometry. Current protocols,especially for the membrane fraction, rely on enrichment rather than isolation and compromise severely on purity. We have developed a discontinuous sucrose gradient-based protocol for efficient isolation of the nuclear, cytoplasmic and membrane fractions from the same sample of hESCs with high purity. Here we describe the quantitative analysis of sub-cellular localization in the Transforming Growth Factor – beta (TGF-beta) and Wnt...
signaling pathways, in the context of self-renewing hESCs. Further, we also report the proteomic analysis of sub-cellular factions during differentiation to the trophodermal lineage by inhibiting the Activin/Nodal pathway.

BIOT 171

Synthetic stem cell niche engineering in vitro and in vivo

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Enabling stem cell–targeted therapies requires an understanding of how to create local microenvironments (niches) that stimulate endogenous stem cells or serve as a platform to receive and guide the integration of transplanted stem cells and their derivatives. In vivo, the stem cell niche is a complex and dynamic unit. Although components of the in vivo niche continue to be described for many stem cell systems, how these components interact to modulate stem cell fate is only beginning to be understood. Using the blood and pluripotent stem cell niche as a models, this presentation will overview our efforts in the use of computational and microscale engineering strategies to systematically interrogate and reconstruct individual niche components. Synthetic stem cell–niche engineering may form a new foundation for regenerative therapies.

BIOT 172

Dynamics of the pluripotent gene network in stem cells

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The pluripotency of stem cells is maintained by a robust gene network consisting of interactions between key transcription factors and signaling associated genes. Recent advances in cellular reprogramming have provided a new tool to better understand this network but this is limited by the stochasticity of the process. Multipotent adult progenitor cells (MAPCs) express key pluripotency markers like Oct4 and Sall4, and, can differentiate into multiple cell types in vitro. Their unique gene expression profile separates them from pluripotent cells of the epiblast by a single differentiation event. These characteristics, along with the ease of genetic manipulation of MAPCs, have allowed us to probe the dynamics of the pluripotency gene network at the
population level though high frequency genetic events. The experimental results from these gene over-expression and knockdown studies were combined with a mathematical model to provide us with novel insights into how pluripotency is established and maintained in stem cells.

BIOT 173

Biclustering algorithm in deciphering mechanical induction of ESC towards endoderm

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Differentiation of embryonic stem cells is typically induced by either chemical or genetic perturbations. Recently there has been an increased interest in inducing stem cell differentiation by modulating the mechanical microenvironment. Our own experiments reveal the effect of substrate mechanical properties on early germ layer commitment by the ES cells. While our experiments reveal that softer substrates preferentially induce endoderm differentiation, a systems approach is required in understanding the mechanism behind mechanical induction of endoderm. We differentiate the ESCs on substrates with varying mechanical stiffness and analyze the differentiated cells for endoderm markers. We use a biclustering algorithm in identifying transcription factors that have been co-regulated across specific conditions. We take into account the variability of the experimental data using a bootstrapping approach, which leads to identification of robust sets of co-regulated transcription factors. Comparison of the co-regulated transcription factors across different culture conditions reveal interesting dynamics of endoderm induction.

BIOT 174

High throughput process development: Advantages and limitations of different formats used

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In the past development of chromatographic separation has been performed by tedious experimental methods involving series of experiments on either a manual or a fully automated chromatographic system. However, these systems were designed for fairly large columns and, therefore, large sample volumes to be used. This in turn, made a screening of a vast experimental space very expensive, if not impossible. To address these issues high throughput miniaturized methods employing different operating principles have been introduced to simultaneously investigate effect of process conditions on
chromatographic separations. In this presentation a technical review of the most common formats used for development of chromatographic purification steps will be given. The formats considered will include flow based systems, such as micro-columns or pre-filled pipette tips, and techniques utilizing a batch incubation principle such as micro-titer plates pre-filled with chromatography resins. Advantage and limitations of each format will be discussed through the prism of engineering principles, chromatographic theory and mass transfer mechanisms. A roadmap for applicability of the different formats for process development purposes and implementation of Quality by Design initiative into process for designing/optimization of chromatography steps will be given. A brief overview of future trends and development needs in the field of high throughput protein purifications will be also provided.

BIOT 175

High-throughput process development methods for chromatography of proteins by using 96-well format micro-plates and small columns

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Fully automated (robotic) systems using a 96-well micro-plate format have been developed by many researchers as high-throughput process development (HTPD) methods for chromatography of proteins. In most cases HTPD methods are employed for screening of suitable binding conditions and elution conditions. However, methods for predicting the column performance based on the information obtained with the micro-plate have not yet been fully developed. In this study, we first investigated various different HTPD methods by using the 96-well micro-plate. Firstly, the plates with ion-exchange chromatography (IEC) monolith disks and membranes were employed for elution experiments. Linear gradient elution (LGE) experiments were carried out and the data were in good agreement with the column data in terms of Yamamoto model. A simplified method for predicting the elution behavior was also developed. Next, the batch adsorption experiments with various different chromatography gels (particles or resins) were carried out to determine the isotherm. It was found that accurate transfer of the gels into each well is critical and the transfer method must be carefully developed based on the properties of individual gels. After we established the suitable method, the isotherms were successfully determined. In most cases the working liquid volume was 0.1-0.2 mL and the gel volume 0.004-0.010 mL. The mixing of the well was also important to assure the complete mixing of the gel suspension. A simple method for predicting the dynamic binding capacity (DBC) was proposed, which uses a correlation between the DBC/SBC and the residence time (SBC: static binding capacity) determined with small columns along with the isotherm data.
Prediction and optimization of chromatographic performance using high throughput 96-well plates

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Traditional purification process development involves screening of adsorbents and operating conditions using packed chromatography columns. The increasing need for rapid, low cost process development for biopharmaceutical proteins would benefit from the implementation of high throughput development methodologies. Furthermore, the potential for using high throughput techniques to screen a wide variety of conditions early in development could better provide an early glimpse into the manufacturing design space. Several case studies will be presented that explore the utilization of a 96 well plate format for accelerating chromatography process development. The high throughput methodologies in these cases were used to rapidly identify the significant factors that could impact process yield, the clearance of product related impurities or the resolution of product isoforms. The plate data was utilized to predict the performance of the packed chromatography columns. Experimental results obtained from packed chromatography columns were then compared with the preliminary high throughput format results.

Rapid optimization and sensitivity analysis of a cation exchange chromatography step using 96-well high throughput techniques

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High throughput techniques that make use of miniaturization and automation are powerful not only for resin screening but for mapping process sensitivities and developing robust operating conditions. Often their conservation of limited feedstock and high data productivity leave HT techniques the only practical means of getting sufficient data with the ever continuing pressure to shorten development timelines. By employing these techniques on a Tecan platform we have been able to routinely capitalize on the increased data for better process understanding. As a case study, we will discuss the optimization of a difficult CEX step with many apparent sensitivities (to temperature, pH, residence time, loading), and how Tecan HT data from static and dynamic studies was used to
guide process development decisions. Ultimately, the additional process knowledge gained led to better decisions on cost, yield, purity, capacity, and productivity trade-offs.

BIOT 178

Integration of screening, rapid analytics and model based development

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In the downstream area of biopharmaceutical production, the demand for fast process development with limited material is everyday life. To meet this demand, high throughput experimenting (HTE) strategies for application in downstream process development have been developed. The HTE approach is based on a miniaturized experimental setup performed automated and parallelized on robotic workstations. In order to gain maximal benefit from this experimental approach, an integrated strategy of rapid analytical methods applied and the use of model based development strategies should be applied. Here, a new method for selective protein quantification which meets HTE-requirements is presented. This new method is based on protein absorption spectra in the range from 240 - 300 nm coupled with a multivariate calibration using projection to latent structures regression. This application allows a quantitative fingerprint of multi-component systems with the ease and speed inherent spectroscopic measurements. In combination with the analytical method a model based development approach is presented which reduces timelines and material consumption dramatically. We will present an example for the optimization of an ion exchange chromatographic multi-component separation performed on a robotic platform, combining rapid and designed experimentation with 'in-silico' model based processing of data.

BIOT 179

Integration of high throughput screening and analytical tools to improve development timelines and process understanding

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Time to clinic is currently a significant focus for all development organizations. To improve the timelines in development, many companies have developed a "one size fits all" platform for early clinical material. The strategy of Downstream
Development, Biopharmaceuticals at Boehringer Ingelheim GmbH & Co. KG is to develop a customized purification process for each therapeutic protein that goes into the clinic and to develop this process with the same or better timeline than a platform process. The development and implementation of a specific and optimized process early in the development timeline allows Boehringer Ingelheim to maintain a high safety standard in early clinical stages and integrates Quality by Design strategies from the beginning of development to give significant advantages in scale-up and later development phases. For this purpose, the Downstream Development Department has developed the BI-PurEx Strategy. This strategy insures a rapid and competitive development timeline through the application of high-throughput biophysical and biochemical assays, BI's RAPPTor® automated screening platform, and an integrated technology platform from vector construction to clinical material. This presentation will demonstrate how the integration of data from high-throughput biophysical assays and automated purification screening is used to develop critical process knowledge and a fast track to success before a single experiment would be run in standard or platform development processes. This early process knowledge gained with Quality by Design strategies results in the rapid and efficient development of a safe, scale-able and robust process for material supply from clinic to market.

**BIOT 180**

**Bioprocess data and knowledge framework for chromatography design**

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Bioprocess design may require substantial experiments to investigate the options for each bioprocess step. With the aim of reducing the amount of experimentation that is undertaken, we propose that data and knowledge about bioprocess design can be systematically exploited. We present a new general framework called the Bioprocess Data and Knowledge Framework (BDKF), for representing and reasoning with data and knowledge on bioprocess design in order to make predictions about output of bioprocess steps and to make suggestions about experiments to be done in order to better understand the landscape of possible bioprocess steps. In the chromatography case study, we establish a database with 1060 experiments described by 68 parameters, a knowledgebase with ontological, theoretical and empirical knowledge about chromatography and explain how they are used for search, prediction and suggestion. We conclude that BDKF is a promising approach to gain value from data and knowledge for bioprocess development.

**BIOT 181**
Investigating downstream processing options for monoclonal antibody manufacture

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Increasing productivities in mammalian cell culture for therapeutic antibody (mAbs) production have triggered interest in more efficient and robust downstream processing platforms capable of handling higher mass product and impurity mass loads. This presentation will describe a decisional tool that integrates process economics and uncertainty analysis in a database-driven Java platform. Case studies will be presented to illustrate the application of the stochastic process economics model to predict the impact of increasing titre on the feasibility of alternative downstream process operations in terms of process throughput, impurity clearance capabilities and ultimately cost of goods per gram (COG/g). Results will be presented for alternative flowsheets containing precipitation operations; these include precipitation prior to Protein A chromatography and as a replacement to the capture step and identify the performance levels required to be competitive with the generic platform. The robustness of the strategies to process fluctuations and equipment failures will be presented.

BIOT 182

Rheological characterization of proteins at fluid interfaces

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The stability of proteins at interfaces is an important topic in biopharmaceutical development. It addresses common problems like the stability and shelf life of formulations in the presence of different interfaces, lubricants or surfactants. In our study, the interfacial shear rheology of BSA, lysozyme and anti-atreptavidin-IgG1 and -IgG2 was investigated at the air/water, the decane/water and the PDMS/water interface. In addition, we studied the effect of the pH of the aqueous buffer solution on the rheology of the adsorbed protein films as well as the influence of the surfactants polysorbate 20 and polysorbate 80. In experiments using a Du Noüy ring and a custom build 2d double-Couette cell, the denaturing or rearrangement of the proteins at the different interfaces could be monitored over a period of about 24 h. In this respect we present a sensitive method for the
acquisition of information on the stability and the prediction of possible storage problems of protein solutions. Atomic force microscopy was used to visualize the films and to provide further insights about the fine structure of the films.

**BIOT 183**

**Effect of surface exposure on the stability of monoclonal antibodies**

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During the manufacturing, storage, and delivery process, biotherapeutics are exposed to a variety of surfaces and interfaces. Interactions between proteins and both solid-liquid as well as liquid-gas interfaces have been shown to have deleterious effects on the protein and depend on a variety of factors including surface chemical properties and solution conditions. In this work, we investigate the quality of monoclonal antibody (mAb) solutions immediately after transient exposure to two model solid surfaces as well as the longer term impact on protein stability after samples have been exposed to these surfaces. In particular, we examine the behavior of two mAbs in contact with silicon dioxide and polystyrene surfaces, which represent hydrophilic and hydrophobic surfaces typically encountered during manufacturing and storage processes. We show that exposure of two different antibodies to high surface areas can lead to differences in the stability profile even after surface contact has been removed.

**BIOT 184**

**Glass dissolution and delamination in formulation development: Implications for neutral pH product development**

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Interaction of neutral pH formulations with the primary drug product container resulted in particle formation due to glass degradation. The occurrence of glass dissolution or glass delamination depended on pH, ionic strength, type of excipient present in the formulation, the surface composition of the glass, and the
storage conditions of the drug product. Case studies of glass dissolution and glass delamination (resulting in the presence of visible lamellae) will be presented. Container surface and particle characterization methods as well as ion quantitation will be discussed. Early screening of the container compatibility of formulation candidates (that have a neutral pH) may identify the potential for glass dissolution or delamination.

BIOT 185

Manufacturing challenges for high concentration formulations: Implications of protein-solute interactions on the control and operation of large scale ultrafiltration/diafiltration processes

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High concentration formulations are becoming increasingly common with subcutaneous administration of therapeutic antibodies. We observed that when ultrafiltration and diafiltration operations are performed at elevated protein concentrations, the unequal partitioning of buffer solute components, caused by protein solute interactions, is exacerbated, resulting in a significant offset in pH and excipient concentrations between the final composition of the retentate and the diafiltration buffer. For the initial implementation at scale, additional product pool adjustments were necessary to achieve the target pH and molarity of the formulated product. This increased processing time and introduced operational complexity. A study was undertaken to provide fundamental insight into the role of protein-solute interactions in high concentration formulations, incorporating a systematic scale-down approach to optimize the diafiltration buffer pH and excipient concentration, to consistently meet target pH and excipient concentration values at the end of ultrafiltration/diafiltration operations. Data from development studies with two different antibody formulations will be presented. The results presented in this study highlight the importance of interactions between protein and buffer solute components and indicate that these interactions should be taken into consideration while designing a suitable diafiltration buffer for high concentration formulations.

BIOT 186

Effect of protein and solution properties on the Donnan effect during the ultrafiltration of proteins

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Formulation of protein biopharmaceuticals as highly concentrated liquids can improve the drug substance storage and supply chain, improve the target product profile and allow greater flexibility in dosing methods. The Donnan effect can cause a large offset in pH from the target value established with the diafiltration buffer during the concentration and diafiltration of charged proteins with ultrafiltration membranes. In this study new equations for the Donnan effect during the diafiltration and concentration of proteins in solutions containing monovalent and divalent ions were derived. The new Donnan models obey mass conservation laws, account for the buffering capacity of proteins, and account for protein-ion binding. Data for the pH offsets of an Fc-fusion protein and a monoclonal antibody were predicted in both monovalent and divalent buffers using these equations.

BIOT 187

High concentration mAb formulation development: A case study

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Development of a high-concentration liquid formulation for a mAb required careful balancing of viscosity and stability requirements. Excipients and solution conditions which reduced viscosity were identified in screening experiments. A DOE experiment was used to simultaneously optimize the parameters for both viscosity and thermal stability; however, this design yielded a large number of samples. Development of high-throughput viscosity and stability assays was required to complete the experiment in a reasonable amount of time. The most promising candidates were then tested in a long term stability study, which used DOE principles to define the formulation space with respect to excipients and pH. We found that conditions that minimized viscosity, such as higher levels of salt, tended to reduce stability at low pH. Data will be presented that highlights these issues and demonstrates how to integrate statistical analysis into the formulation development pathway.

BIOT 188

Vaccine stabilization strategies
The majority of commercially-available vaccines are stored under refrigerated conditions, if not frozen. Such stringent conditions place a strain on the storage and transport of vaccines, in addition to the added complexities of mass vaccination campaigns in regions lacking sufficient cold chain infrastructure. Although new vaccines are developed or re-engineered to improve safety and efficacy, the development of thermally stable vaccines has lagged behind. Stabilization of vaccines in the solid state, typically achieved by lyophilization, is the method of choice, although their storage stability leaves a lot to be desired. Improvements in vaccine stability were attained through a combination of spray drying or foam drying, a modified lyophilization method, and formulation optimization, which is not only specific to the labile biological, but against stresses unique to the processing method employed. The formulation and processing challenges involved in stabilizing both virus- and bacteria-based vaccines will be presented.

BIOT 189

Real time protein release and biomaterials characterization by in-situ vibrational spectroscopy

Advances in therapeutic development rely on the ability to characterize therapeutics during the formulation process as well as probe their in vitro release kinetics. Typically analyses rely on methods lacking real time capabilities and provide little information regarding the integrity of the therapeutic. Infrared spectroscopy (IR) is a label free, non-destructive, chemical specific technique providing valuable information regarding the state of the system under investigation. This talk illustrates the power of coupling fiber-optic probe technology with IR for use as a tool in the therapeutic delivery field by examining protein release from two different delivery systems, hydrogels and nanoparticles. Exposure to mildly acidic conditions, as are found in subcellular compartments and diseased tissue, triggers release. IR parameters specific to both the polymeric material degradation and release of encapsulated protein are followed, in realtime, thereby simultaneously monitoring all delivery components and providing a more complete picture of these important systems.

BIOT 190
Elucidating mechanisms of heterologous neurokinin 2 receptor expression and trafficking in *S. cerevisiae* through receptor chimeras

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Biophysical and structural characterization of G protein-coupled receptors (GPCRs), a class of integral membrane proteins that mediate signaling across the plasma membrane, have been limited by obstacles to functional production. The budding yeast *S. cerevisiae* has been utilized for functional expression of heterologous GPCRs, but its use has been limited by host cellular responses. We propose that improving heterologous GPCR trafficking may be required to reduce cellular responses and have developed a novel and efficient system for generating and evaluating receptor chimeras to test this hypothesis. Using this system, we have constructed chimeras of rat and human neurokinin 2 receptors, which show differential expression and whole-cell ligand binding in yeast. As judged by confocal microscopy, chimera localization is determined by a single domain. Current work focuses on determining localization using imaging at nanometer resolution using EM, and investigating differences in cellular responses such as the unfolded protein response (UPR).

BIOT 191

Development of a new R1-based plasmid for DNA vaccine production

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Interest in plasmid DNA (pDNA)-based vaccines has recently been stimulated by approval of several veterinary pDNA-based therapies, improvement of delivery methods, and disappointing results from clinical trials of viral-vectored DNA vaccines. The increase in demand for pDNA underscores the need for robust, high-yielding vector platforms. We have constructed a DNA vaccine vector, pDMB02-GFP, containing a runaway R1 origin of replication that offers an alternative to pUC-based plasmids. The runaway R1 origin has been used previously for recombinant protein production, and is characterized by a temperature-inducible loss of plasmid copy number control. We have demonstrated that in *E. coli* DH5α our vector gives high yields, even without a temperature shift. We have also shown that pDMB02-GFP often out-performs a similar vector with a pUC replicon in terms of specific pDNA yield. Ongoing work is focused on designing a bench-scale fed-batch fermentation process for production of pDMB02-GFP.
Heparosan production and modification towards a bioengineered generic heparin

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Heparin, a polysaccharide that is widely used as an anticoagulant drug, is currently produced from porcine intestine extract. The animal source production of current heparin imposes some disadvantages, which are evidenced by the heparin contamination crisis in 2008. The in vitro chemoenzymatic modification of E. coli K5 heparosan polysaccharide showed as a promising alternative approach to produce heparin. In this study, E. coli K5 heparosan fermentation process was investigated and improved, the fermentation yield was increased and the process was scaled up; strain improvement possibilities by genetic engineering were investigated; the heparosan modification process was also controlled towards the goal of producing a bioengineered generic version of USP heparin.

New strategies to overcome glucose repression in mixed sugar fermentation in Saccharomyces cerevisiae

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One of the main limitations in the fermentation of mixed sugars to fuels and chemicals using recombinant Saccharomyces cerevisiae strains is glucose repression, i.e. utilization of pentose sugars is inhibited by glucose. This results in delayed utilization of pentose sugars and significantly reduced overall ethanol productivity. Various approaches have been attempted to overcome glucose repression, such as evolutionary engineering and deletion of key genes involved in glucose repression such as MIG1 and MIG2. However, these approaches met with limited success. In this presentation, I will discuss two new strategies that we have been working on to overcome glucose repression in mixed sugar
fermentation. One strategy is based on the coexpression of a cellobiose-transporter and b-glucosidase (Li, et al. Molecular Biosystems, 6, 2129 (2010)), and the other is is based on the overexpression of pentose-specific transporters (Du, et al. Molecular Biosystems, 6, 2150 (2010)).

BIOT 194

Expeditious strain and fermentation development for quality recombinant protein production in *Pseudomonas fluorescens*

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The ability to produce a high quality protein product in a time and cost efficient manner is of particular value throughout the discovery to commercialization pathway of therapeutic and vaccine proteins. To this effect, a suite of toolboxes spanning strain, analytics, fermentation, and downstream process development have been established for a *Pseudomonas fluorescens*-based expression platform. Seamless development of robust protein expression strains result from combining off-the-shelf toolboxes of expression plasmids that utilize a wide range of gene expression strategies with host strains of diverse phenotypes that are screened via high throughput, parallel screening methods. Subsequent simultaneous strain and far-reaching fermentation evaluation in a mini-bioreactor system which can be predictably scaled, results in high levels of soluble, properly folded protein being produced. Case studies of successful implementation of this platform and subsequent scale-up will be presented.

BIOT 195

Cellular response to isooctane in *Escherichia coli*: An integrated systems biology approach

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The toxicity of organic solvents decreases the productivity of biochemical processes involving microorganisms. The cytotoxicity of water-miscible organic solvents such as ethanol and butanol has been extensively studied; however, cellular responses to hydrophobic organic solvents have not been well characterized in bacterial cells. Given that isooctane (2,2,4-trimethylpentane) is an important component of gasoline fuels and a widely used hydrophobic organic solvent, herein, we report the cellular response of *Escherichia coli* to isooctane using transcriptome profiling, gene ontology and network component analyses,
gene-knockout, and electron microscopy. Our results suggest that in response to isoctane, (i) outer membrane and iron transport mechanisms were disrupted; (ii) fimbriae-like structures and altered cell membrane were developed; (iii) the activity of some transcription factors were significantly perturbed; and (iv) the knockout of these transcription factor genes led to a significant decrease in viability. This outcome implies that those genes are essential elements in the genetic regulatory network of cellular responses to isoctane in *E. coli*. We further demonstrate that engineering of the identified genetic regulatory network can increase the tolerance of *E. coli* against isoctane. We envision that engineered bacteria with high tolerance to isoctane can be exploited for improved biochemical processes, fuel oil bioremediation, and metabolic engineering for biofuel production.

**BIOT 196**

**Development of a small scale system for the creation of a Biolsoprene™ production process**

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With a growing demand for biobased solutions to industrial problems, it is essential to develop a bench scale system that will effectively predict large scale processes. The Micro-24 MicroReactor System has provided a method of process optimization and strain characterization prior to high cost, low throughput large scale experimentation. This poster will give examples of how small scale development has provided large scale results in the creation of a Biolsoprene™ production process.

**BIOT 197**

**Performance comparison of various ethanologenic microbes for SSF of pure sugars and cellulosic biomass**

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Currently, several microbes are promising for fermentation of sugars derived from cellulosic biomass to ethanol, and many perform well in terms of sugar (mainly glucose) consumption, ethanol production rate, and ethanol tolerance.
when applied to pure sugars. Many researchers favor the simultaneous saccharification and fermentation (SSF) for conversion of cellulosic biomass to reduce the powerful inhibition of cellulase enzymes by glucose, cellobiose, and other hydrolysis products that limits sugar concentrations and yields. However, application of SSF results in a much different environment than for fermentation of pure sugars due to the slow release of sugars by enzymatic hydrolysis of cellulose coupled with the presence of inhibitors, operation at higher temperatures to accommodate enzymatic hydrolysis, and the accumulation of ethanol with conversion. Because some organisms can better tolerate such highly stressful conditions better than others, evaluation of organisms based on simple sugar fermentations is inadequate for selection of ethanologens for SSF. Therefore, in this study, promising SSF microbes including the NREL yeast strain D5A, the industrial yeast Ethanol® Red, and the NREL bacterium Z. mobilis are evaluated for their fermentation performance with pure sugars and in the SSF of pure cellulose and pretreated cellulosic biomass.

BIOT 198

Clostridial biotechnologies for biofuel and biorefinery applications

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Clostridia are anaerobic, endospore forming prokaryotes of major importance to cellulose degradation, human and animal health, and acidogenesis. Clostridial genetics and biotechnology have been frequently misunderstood or ignored. In the last 5 years however, there has been enormous growth in clostridial-based industrial processes. For example, solventogenic clostridia can produce a large array of metabolites, while metabolic engineering could enhance these native capabilities for production of additional chemicals. Such chemicals can serve as biofuels directly or indirectly. A major advantage, compared to E. coli and yeast systems, is their ability to utilize a large variety of substrates: mono-, oligo- and polysaccharides, including starches, most pentoses and hexoses, while some can use directly cellulosic or CO and CO₂ as substrates. I will review past advances in metabolic engineering of clostridia, and will highlight recent developments in genetic and genomic tools for advancing clostridial biotechnologies, as well as new and novel applications.

BIOT 199

Engineering synthetic yeast consortia for consolidated bioprocessing

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In this era of pollution and energy source deficit, there is an urgent need of finding new cost and energy effective methods that can efficiently convert celluloses into simple sugars and eventually ethanol. Consolidated bioprocessing (CBP) that combines simultaneous saccharification with fermentation into a single step is attractive as it avoids a separate step for cellulase production. Compared to a single-strain approach, the use of a consortium can evade possible unwanted consequences of gene instability, metabolism burden and other negative effects on cell performance due to the expression of a large number of foreign genes. Recently, we have developed a synthetic yeast consortium capable of displaying mini-cellulosomes on the cell surface by intracellular complementation. The feasibility of using the consortium for CBP was demonstrated under anaerobic conditions using phosphoric acid swollen cellulose (PASC) as a sole carbohydrate source with a final ethanol yield corresponded to 87% of the theoretical value. The final ethanol level was 4-fold higher than a similar consortium secreting only cellulases, again highlighting the synergistic effect of the cellulosome structure. The population dynamic of the yeast consortium was quantified by real time PCR, and a similar ratio was maintained throughout the fermentation. To our best knowledge, this is the first report of using a yeast consortium approach for cellulosic ethanol production by CBP.

BIOT 200

Trackable recombination-based directed evolution

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Directed evolution is a powerful and proven method for the optimization of desirable functions in biocatalysts and proteins. Despite the successes of directed evolution, improvements in library design, construction and analysis can significantly enhance its utility. The incorporation of directed evolution with statistical analyses, such as ProteinSequence Activity Relationships (ProSAR), have shown great promise but are often labor intensive and limited to the study of an individual enzyme. Here we describe an extension of our previously reported TRackable MultiplexRecombineering (TReMR) technique that harnesses the power of recombineering and molecular barcoding technology to generate libraries of proteins with each mutation linked to a trackable barcode. This approach enables the rapid determination of each mutation's impact on protein function via a selectable or screenable phenotype. Current status of this work will
be presented with emphasis on its application towards evolvingproteins useful in the production of next generation biofuels.

BIOT 201

Engineering modular control over metabolic pathways for improved flux

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Synthetic biology strives to introduce control over biological systems. In particular, metabolism is a process of value that would gain from such control. Complex formation between metabolic enzymes was engineered using modular protein-protein interaction domains and their corresponding ligands. Synthetic scaffolds were constructed from these interaction domains to co-localize pathway enzymes as a means of increasing the effective concentration of each pathway component. Further, the scaffold’s modular design was employed to optimize the stoichiometry of the recruited enzymes of the mevalonate biosynthetic pathway for improved flux. Due to the increased pathway efficiency, product titers were increased even as the expression level of pathway enzymes was lowered, thereby providing a means for reducing the metabolic load to the production host. This design strategy should prove generalizable to other pathways, particularly those requiring optimal flux through multiple enzymatic steps such as next generation biofuels.

BIOT 202

Sustainable production of advanced biofuels

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The cost-effective production of biofuels from renewable materials will begin to address energy security and climate change concerns. Ethanol, naturally produced by microorganisms, is currently the major biofuel in the transportation sector. However, its low energy content and incompatibility with existing fuel distribution and storage infrastructure limits its economic use in the future. Advanced biofuels, such as long chain alcohols and isoprenoid- and fatty acid-based biofuels, have physical properties that more closely resemble petroleum-derived fuels, and as such are an attractive alternative for the future supplementation or replacement of petroleum-derived fuels. Here, we discuss
recent developments in the engineering of metabolic pathways for the production of known and potential advanced biofuels by microorganisms.

BIOT 203

Synthetic biology for the production of fatty acid based fuels and chemicals

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Increasing energy costs and environmental concerns have emphasized the need to sustainably produce renewable fuels and chemicals. Fatty acid biosynthesis provides a renewable route to many products that could replace their petroleum-derived counterparts. These energy rich molecules are today isolated from plant and animal oils for a diverse set of products ranging from fuels to oleochemicals. A more scalable, controllable, and economic route to this important class of chemicals would be through the microbial conversion of renewable feedstocks, such as biomass-derived carbohydrates. Although this complete production scheme has been demonstrated, increases in titer, productivity, and yield are necessary for industrial transition. Strategies employed for increasing yields of biodiesel include balancing the enzymes in the pathway, condensing the pathway onto a triple-operon, single-plasmid system, and subsequent chromosomal integration. These efforts guided towards understanding fermentation scalability, pathway stability, and balancing pathway enzymes for biodiesel production will be discussed.

BIOT 204

Hydrocarbon-based fuels from yeast: The industrialization of synthetic biology

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Living systems have an enormous capacity to synthesize diverse chemical structures. Demonstrating the ability to make new and useful compounds via microbial fermentations has become a common and relatively facile endeavor. Reaching maximum yield and productivity has become the major challenge for industrial microbiology because such efficiency is required to reach the cost targets of fuels and chemicals. Engineering a highly productive strain can be accomplished by multiple rounds of strain design, testing, analysis and re-design – a process that is dramatically enhanced by the ability to construct thousands of
strains in a short time frame. To rationally construct thousands of strains per week, we have employed a novel platform to make DNA construction standard and modular. We then coupled the modular molecular biology underpinning DNA construction with robotics to automate the unit operations of strain engineering. The system has enabled a 100-fold increase in our ability to create new, rationally designed yeast for the production of hydrocarbon-based fuels. With this capability we are able to investigate tens of thousands of strain constructions, using combinatorial solutions to enhance the productivity of strains making our fuels and chemicals. The platform of automated strain engineering represents a significant step in the industrialization of strain construction.

BIOT 205

ThYme: A database for thioester-active enzymes

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The ThYme (Thioester-active enzYme) database brings together amino acid sequences and three-dimensional (tertiary) structures of enzyme groups present in the fatty acid synthesis and polyketide synthesis cycles, which can be used to make many chemicals from biorenewable materials. The enzyme groups are acyl-CoA synthases, acyl-CoA carboxylases, acyl transferases, ketoacyl synthases, ketoacyl reductases, hydroxyacyl dehydratases, enoyl reductases, and thioesterases. These groups are classified into families, members of which are similar in sequences, tertiary structures, and catalytic mechanisms, implying common protein ancestry. The database is continually updated as new sequences and tertiary structures become available. At present it contains approximately 300,000 sequences and about 1,000 tertiary structures. ThYme should be a useful source of information on these enzymes in helping researchers to find or predict active sites, catalytic residues, and mechanisms of individual sequences, as well as in providing a standardized nomenclature for enzymes in the same family and group.

BIOT 206

Determining positional isomer distributions of antibody drug conjugates

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Antibody drug conjugates are designed to deliver potent antitumor agents directly to cancerous cells. The chemical conjugation of cytotoxins to monoclonal
Development of enzyme-based biosensors using self-assembled, photoluminescent peptide hydrogel

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The self-assembly of peptides has much drawn attentions as fabricating novel nanostructured materials because of their unique properties (i.e., molecular recognition, biocompatibility, versatile functionalities, and not-requiring harsh conditions). Here, we present that a self-assembled peptide hydrogel consisting of Fmoc-diphenylalanine has been employed as a sensing platform through in-situ immobilization of enzyme bioreceptors (e.g., glucose oxidase or horseradish peroxidase) and fluorescent reporters (e.g., quantum dots; QDs). We observed that the self-assembled peptide hydrogel had a three-dimensional network of nanofibers of about 80 nm that physically hybridized with QDs and encapsulated enzyme bioreceptors with a minimal leakage. We found that the self-assembled hydrogel co-encapsulating QDs and enzymes have a high quenching efficiency for the detection of glucose and phenolic compounds. These results suggest that the peptide hydrogel is an alternative optical biosensing platform with practical advantages such as simple fabrication via self-assembly, efficient diffusion of target analytes, and high encapsulation efficiencies for fluorescent reporters and
BIOT 210

Characterization of disulfide bond scrambling in human growth hormone induced by thermal stress, freeze-thaw cycling, agitation and lyophilization

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**Purpose:** Thiol-disulfide exchange and disulfide scrambling are common routes of protein covalent aggregation, which is a common instability in protein drugs that has been associated with reduced pharmacological activity and increased immunogenicity. The goal of this study was to characterize the effects of thermal stress, freeze-thaw cycling, agitation and lyophilization on thiol-disulfide exchange and disulfide scrambling in human growth hormone (hGH). These laboratory scale processes serve as models for stresses that may be experienced during manufacturing. **Methods:** Purified hGH was subjected to thermal stress and agitation, freeze-thaw and lyophilization, respectively, followed by alkylation with iodoacetamide (IAM). Tryptic digestion was performed on the alkylated samples and the fragments were analyzed and quantified by LC-MS using an Agilent Q-TOF. The percentage of native and scrambled disulfide bonds, free thiol and IAM-labeled cysteine were determined. **Results:** All of the stressed samples show an increased level of scrambled disulfide bonds than non-stressed protein. In particular, 24-h thermal stress at 60°C induced the greatest increase in scrambled disulfide bonds, while freeze-thaw cycling has a minimum impact. Except for the lyophilized sample, the other stressed proteins display a similar level of free thiol. Native hGH was more easily digested with trypsin than the stressed proteins, and preferential cleavage was also observed among the stressed samples. **Conclusion:** The results show that thermal stress, freeze-thaw, agitation and lyophilization increase the scrambling of disulfide bonds in hGH, suggesting that common manufacturing stresses may damage disulfide bonds.

BIOT 211

Dual-function, inline platinum-tripeptide tag for targeted anticancer therapy

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We recently discovered a novel tripeptide (NCC) that is capable of coordinating a metal ion and are investigating its use in targeted delivery of platinum to cancer cells via incorporation into a therapeutic protein. It has been shown that co-administration of therapeutic proteins and cytotoxins, such as cisplatin, leads to improved outcomes. The conjugation chemistry typically employed generates an extremely heterogeneous mixture of species that are difficult to characterize well. In contrast, our unique system provides the ability to rationally design and site-specifically encode the tripeptide into any position within a targeting protein, allowing complete control over positioning the tag within the protein to generate a homogeneous product. To deliver metals, a chelator is conjugated; chelation is reversible, favoring dissociation upon dilution within the patient. Platinum binding to our tag is irreversible at physiological pH, but the metal is released upon acidification, overcoming the dilution problem.

BIOT 212

Screening microorganisms for plasminogen activator secretion

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Plasminogen activators (PAs) are proteolytic enzymes clinically administered to treat strokes and myocardial infarctions. Mammalian PAs such as tissue-type PA and urinary-type PA are used for treatment but are expensive for large-scale therapy. Streptokinase and staphylokinase are PAs isolated from bacteria mostly used for cleaning catheters because they trigger an undesired immune response. Short half-life of these PAs adds significantly to the cost of treatment. Hence, there is an acute need for stable PAs which could lead to a cost effective treatment for these diseases. We hypothesize that enzymes isolated from halophilic organisms are more stable under non-standard conditions of temperature and pH and will thus have longer half-lives. Halophiles were isolated from the salines in Cabo Rojo, Puerto Rico and screened for PA activity. Intracellular and extracellular PA activity was assayed using Fibrin Plate Assay. The halophilic bacterial species identified as positive is now being isolated and characterized.

BIOT 213

Performance and qualification of a fast Surface Plasmon Resonance based MAb quantification method
Monoclonal antibodies are large complex molecules with many possible modifications that may affect safety and efficacy. Hence, many different analytical methods and techniques are needed to characterize and analyze the product. A key attribute is the concentration of monoclonal antibody (MAb), equally important is the specific activity of the molecules. These are attributes that need to be followed all the way from cell culture to the final formulation and from development to the final manufacturing of the product. With the evolvement of high throughput process development the number of samples are continuously increasing and automated, fast, robust and precise analytical methods are essential. In this work we show how Surface Plasmon Resonance (SPR) can be used for MAb quantification. Performance such as robustness, linearity, specificity and preciseness of the method will be shown.

Ultra-sensitive multiplex detection of lung cancer biomarkers using immuno-magnetic particles

Early detection of non-small cell lung cancer (NSCLC) is correlated with a significantly better outcome for patients, and several studies have reported the ability to distinguish lung cancer from normal tissue based on specific protein markers. In order to allow the sensitive and parallel detection of these biomarkers, we have developed immuno-magnetic nanoparticles decorated with target-specific antibodies or aptamers and unique, amplifiable DNA labels. Multiple biomarkers can be detected in parallel using TaqMan™-like DNA reporters. Magnetic force discrimination is employed to significantly reduce non-specific background binding. Furthermore, we have employed a photochemical immobilization method for antibodies and amine-PEG immobilization to create a low adsorptive surface to maximize specificity. Our goal is to engineer a parallel protein detection system with better sensitivity and specificity than current ELISA-based technologies. We have shown the concentration-dependent detection of cancer biomarkers. We can detect the presence of less than 100 immuno-magnetic nanoparticles by real-time PCR.
BIOT 215

Electrocatalytic signal amplification of DNA-modified electrodes with covalent redox reporters

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DNA-modified electrodes, using DNA-mediated charge transfer, allow for the electrochemical detection of perturbations to the base-stack as subtle as single-base mismatches. Target specificity is obtained through the covalent attachment of the redox reporter to the DNA, such that the redox reporter is well-coupled to the base-stack. Electrocatalysis is used to improve the sensitivity of detection. Covalent attachment/confinement of redox reporters disrupts the kinetics of electrocatalysis. However, varying the length, rigidity, and placement of the reporter can restore electrocatalysis.

BIOT 216

Developing an electrochemical system for reducing CO\(_2\): Immobilization of carbonic anhydrase for accelerated CO\(_2\) capture

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Although there has been a wealth of research focused on strategies for oxidation of methanol completely to carbon dioxide, the reverse electrocatalytic reaction of producing methanol from carbon dioxide has shown interest but, as of yet has lacked efficiency. This project focuses on understanding the possible role of
carbonic anhydrase to efficiently facilitate uptake of CO₂, which can be the rate determining step in producing fuels from carbon dioxide. The three oxidoreductase enzymes responsible for CO₂ reduction to methanol are formate dehydrogenase, formaldehyde dehydrogenase, and alcohol dehydrogenase. This synthetic pathway for methanol production is coupled to a poly(neutral red) modified electrode that continually regenerates the NADH coenzyme so the process can run continuously. We have found that while the dehydrogenases alone can achieve reduction of carbon dioxide, the process is accelerated by the addition of the carbonic anhydrase. As researchers try to increase the efficiency of electrochemical production of fuels, carbonic anhydrase will likely have a critical role.

BIOT 217

Rational design of additives for inhibition of protein aggregation

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Protein aggregation is the most common degradation pathway for proteins. Aggregation leads to a decrease in efficacy of protein drugs and could elicit an immunological response. A common approach to solving the aggregation problem is to add low molecular weight cosolvents, such as sugars, salts, polyols etc. While ubiquitously used, this approach can be inefficient and does not always enable the discovery of stable protein solution formulations. Therefore, the rational design of cosolvents, which offers a significant improvement over commonly used cosolvents is highly desired. Arginine, a naturally occurring amino acid has been widely used to suppress protein aggregation, purify proteins using Protein-A affinity resins, and to improve the refolding efficiency of proteins. In this study, we show that arginine strongly self-associates in solution which leads to the enhanced crowding around macromolecule. Due to the crowding around the macromolecule, the free energy of the encounter complex formed when two proteins are associating increases. In this study, several different approaches based on the molecular level understanding of Arginine mechanism are used to design novel cosolvents. These approaches are tested both computationally and experimentally. The designed cosolvents offer an order of magnitude improvement over aqueous arginine solutions.

BIOT 218

Specific targeting of [¹⁸F]-FDG-metal chelate to DAbR1 reporter gene for In-vivo tumor imaging
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The DOTA-Antibody Reporter gene 1 (DAbR1) binds to DOTA chelates with infinite affinity (J Nucl Med 2008;49:1828). To facilitate the use of DAbR1 for PET, 2-[18F] Fluoro-2-deoxy-D-glucose ([18F]FDG), a widely available 18F PET tracer, was directly radio-labeled on a synthetic yttrium DOTA chelate, (S)-2-{4-[2-(2-(Aminooxy-acetylamino)-3-mercapto-propionylamino]benzyl}-1,4,7,10-tetraazacyclododecane- N,N',N'',N''' tetraacetic acid (AOCD(Y)). The AOCD(Y) chelate, containing aminobenzyl-DOTA attached to a cysteine residue, was prepared as a disulfide with an aminooxy group on the N-terminal. The aminooxy group reacts with [18F]FDG under acidic conditions at elevated temperatures, and the disulfide can undergo displacement to form a covalent bond with the free sulfhydryl on the DAbR1 reporter gene. Glucose was removed from commercial [18F]FDG by preparative HPLC before reacting [18F]FDG with the Y-AOCD probe. This probe reaction mixture was purified chromatographically with good radiochemical purity. In-vitro and in-vivo studies were done on stably transfected DAbR1-U87 glioma with promising results.

BIOT 219

Case study in sustainable operations of commercial drug substance manufacturing through knowledge management and use of analytical tools

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Process knowledge management is essential to ensure sustainable operations. An out-of-trend, low process performance was observed during commercial manufacturing of an antibody drug substance at Genentech's biopharmaceutical production facility at Oceanside. This led to an investigation to identify possible causal factors and to better understand the factors that contribute to variability during commercial production. A rigorous, data-driven root cause methodology was used for problem solving, which covered evaluation of cell culture performance, operations, raw materials, and equipment. This presentation will discuss the subtle cell culture changes during commercial manufacturing that can impact process performance and corrective action taken to resolve the issues. This case study will also discuss how effective controls were introduced in the
process to improve process robustness with the goal to produce the drug substance with consistent yield and quality across multiple product campaigns.

BIOT 220

Evaluation of sub-visible particles in recombinant humanized monoclonal antibody formulations

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Protein aggregates are significant degradation products in monoclonal antibody formulations. Recently concerns have been raised on the affect of subvisible protein aggregates or particles, especially those below 10 µm, on product safety and efficacy. Subvisible particles may range from approximately 1 µm to 100 µm in size. Therefore, protein aggregates in this size range are too large for measurement by size exclusion chromatography and too small to be visibly observed. Methods that have been proposed for the analysis of subvisible particles include light obscuration and Coulter counter techniques. In the following study we have applied these methods to the characterization and stability analysis of subvisible particles in liquid antibody formulations prepared at a low protein concentration suitable for IV delivery and high protein concentration for subcutaneous delivery. The results show that the majority of observed subvisible particles are between the 2 and 10 µm size range. However, a high degree of assay variability was observed in the measurement of particles in this size range that made trends on stability difficult to discern. In addition, differences in subvisible particles counts occurred depending on the method used. For example, increased particles occurred in the analysis of the same samples by the Coulter principle than that measured by the light obscuration method. The evaluation of these methods for characterization and stability testing of antibody formulations is on-going.

BIOT 221

Stabilization of lipase in the ethanol solvent by a computational surface design

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In this study, the stability of lipase in ethanol cosolvent was improved by computational optimization of ethanol-contacting surface residues. Preferred ethanol-contacting sites on the lipase surface were investigated by solvent mapping methods and their flexibility changes in ethanol solvent were predicted by molecular dynamics simulations. In silico saturated mutation was performed for target sites in flexible regions and mutants with fewer ethanol-contacts were selected for experimental validation. For mutants showed the improved stability in 50 % (v/v) ethanol solvent with comparable thermal stability and specific activity to the wild-type lipase. This computational strategy could be used as a practical tool to design organic solvent-stable mutants of industrial enzymes.

BIOT 222

Understanding intercellular signaling of biofilms in logistics fluids

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Microbial induced corrosion (MIC) is an electrochemical process where microorganisms initiate or enhance a corrosion reaction at a metal surface, primarily via formation of biofilms to increase microbial persistence. Counter-intuitively, these biofilms can exist in petroleum transport, storage and delivery systems causing corrosion and other mechanical failures; an estimated 40% of internal pipeline corrosion in the petroleum industry is caused by MIC. Biofilm formation is coordinated by a phenomenon known as quorum sensing (QS). One QS molecule, autoinducer-2 (AI-2), seems to be universal in the bacterial world. In this study we engineer E. coli to report the presence of (AI-2) in a fuel-water mix for detecting the presence of biofilms in fuel storage containers or pipelines. Demonstrated are the effects of increasing and/or deleting AI-2 manufacture, transport, modification, and breakdown, as well as the detection limits of AI-2 in a fuel-water mixture through biological reporter and electrochemistry assays.

BIOT 223

Discovery and isolation of novel lasso peptides through genome-mining

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The unique structure, tremendous stability, and broad substrate specificity of lasso peptides make them extraordinary subjects for research. In addition to an elegant fold, these short, cyclized and looped peptides possess a broad spectrum of function. Antimicrobial and anti-HIV agents are among the eight that have been structurally confirmed as lasso peptides thus far, and some are also potent receptor antagonists (Severinov et al. 2007). Unfortunately, the list of lasso peptides is still short and the genetic systems that encode their biosynthesis have been elucidated for only microcin J25 and capistruin. We will present our efforts toward identifying novel peptides with the lasso fold through genome-mining based on conserved domains and gene-cluster architecture as well as preliminary results on isolation of these targets from their host organisms.

BIOT 224

Transcriptional analysis of pectin utilization in Clostridium acetobutylicum

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Clostridium acetobutylicum is a gram positive anaerobic bacterium that can ferment a variety of carbohydrates into butanol, acetone and ethanol. Pectin is a component of plant cell walls, found in many agricultural wastes, and readily fermented by C. acetobutylicum. In this study, DNA microarrays were used to compare the transcriptional profiles of C. acetobutylicum grown on pectin and glucose with the goal of identifying genes responsible for pectin degradation, uptake and metabolism. There were 248 genes that were induced greater than two fold with 99% confidence by pectin. The majority of the genes appear to be involved in pectin degradation, carbohydrate (galacturonic acid, pentose) utilization or part of a chromosomally located prophage. The potential physiological roles of the gene products in pectin metabolism and entry into central metabolism will be discussed.

BIOT 225

Characterization of a novel tetracycline tailoring pathway in SF2575 biosynthesis

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SF2575 is a tetracycline family compound which is naturally produced by Streptomyces sp. SF2575. In vitro and in vivo testing has shown it to have potent anticancer properties. We have previously reported the gene cluster and proposed biosynthetic pathway of this fascinating molecule in the *Journal of the American Chemical Society*, 2009. This presentation further illuminates previously unknown tailoring steps leading to the glycosylated tetracycline intermediate, and further characterizes a novel C4 salicyl transferase, SsfX3 which is important for biological function of this compound. Additionally, SsfX3 has been shown to have a broad substrate specificity which has been exploited to generate a library of SF2575 analogs. We have also solved the crystal structure of SsfX3 revealing a didomain structure consisting of an SGNH hydrolase domain and a putative substrate recognition domain. This pathway therefore offers new opportunities to diversify tetracycline scaffolds and shed further light on tetracycline biosynthesis.

**BIOT 226**

**Implantable microprobe with microelectrode array sensors for simultaneous monitoring of glutamate and dopamine**

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Platinum (Pt) microelectrode arrays on an implantable microprobe micromachined from silicon are modified with overoxidized polypyrrole, Nafion, and enzyme as need be for simultaneous detection of both glutamate (Glut) and dopamine (DA) by constant potential amperometry. When testing *in vitro*, the glutamate oxidase (GlutOx)-based Glut microbiosensor on one array site has a sensitivity of ~50 nA·μM⁻¹·cm²⁻¹, a detection limit of ~5 μM, and a ~1 second response time. The DA microsensor on another site on the same microprobe has a sensitivity ~1280 nA·μM⁻¹·cm²⁻¹, a detection limit of ~100 nM, and a ~1 second response time. No diffusional interaction among closely arrayed microelectrodes (100 μm and 40 μm separation) is observed and no interferent response from ascorbic acid, uric acid, 3,4-dihydroxyphenylacetic acid, and L-3,4-dihydroxyphenylalanine is detected. Studies of Glut and DA changes in the brains of live rodents using this microprobe currently are underway.
BIOT 227

Production of bioethanol and biodiesel from renewable resources: Cost analysis and process optimization using simulation tools

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Over the past three decades there has been intense investigation on the development of fuel producing processes that are based on the use of renewable agricultural materials as feedstock. The main effort has been concentrated on bio-ethanol and bio-diesel which have been shown to give motor engine performance similar to that of conventional petroleum based fuels. In addition to product characteristics, however, process economics play an equally important role in any successful product commercialization. In this work, realistic process simulation models have been developed in order to analyze the economics of “corn-to-ethanol” and “soybean-oil to bio-diesel” production. The models developed include process flow diagrams, material and energy balances, equipment sizes and estimates of capital and operating costs. This presentation will illustrate how such models can facilitate the design of new manufacturing facilities and the optimization of existing ones.

BIOT 228

Biofuel production from acid hydrolysates of the construction and demolition wood waste

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The feasibility of biofuel production from the construction and demolition (C&D) wood waste acid hydrolysates was investigated. Concentrated sulfuric acid hydrolysis was used to obtain the saccharide hydrolysates and the composition including inhibitors in the hydrolysates was analyzed. The C&D wood waste composed of lumber, plywood, particleboard, and medium density fiberboard (MDF) had polysaccharide (cellulose, xylose, and glucomannan) fractions of 60.7 – 67.9%. The sugar composition (glucose, xylose, and mannose) of the C&D wood wastes varied according to the type of wood. The additives used in the wood processing did not appear to be released into the saccharide solution under acid hydrolysis. The hexosesugar-based ethanol yield and theoretical yield by P.
stipitis were 0.42-0.46 and 0.82-0.91, respectively. Butanol production by *C. acetobutylicum* was enhanced with detoxified hydrolysate by ion exchanger. Therefore, the C&D wood wastes dumped in landfill sites could be used as a raw material feedstock for the production of biofuels.

**BIOT 229**

**Functional expression and optimization of formate dehydrogenase in *Escherichia coli* by coexpression of selenocysteine insertion genes**

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Much interest has been recently focused on the production of large quantities of hydrogen, due to its potential importance in our economy and needs in the petroleum and chemical industries. Formate dehydrogenase H (FDH-H) from *Escherichia coli* containing selenocysteine that oxidizes formate to carbon dioxide with the release of a hydrogen, is a component of the anaerobic formate hydrogen lyase complex of *E. coli*. In this approach, the *fdhF* gene was subcloned into expression vector, pET-22b(+), and a 6xHis tag was fused to FDH-H at the C-terminus and overexpressed in *E. coli*. Then, the activity of FDH-H were analyzed by employing benzyl viologen as electron mediator. However, overexpression of FDH-H in *E. coli* resulted in the formation of inclusion body. Several efforts including low temperature for induction, optimization of inducer concentration and coexpression of selenocysteine insertion genes were tried to improve the functional expression of FDH-H.

**BIOT 230**

**Production of α-olefins using an engineered strain of *E. coli***

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Terminal olefins are the starting material for the bulk of the polymer industry. As a family, these compounds represent hundreds of millions of tons per year of chemical manufacturing. Traditionally, these compounds have been derived from
petroleum feedstocks. Assuch, their costs are continuing to trend upwards, while their supplies diminish. In an effort to find a price stable and sustainable replacement for these commodities, we have engineered a strain of E. coli to convert agricultural feedstocks (sugar) into α-olefins. As a proof of concept we have produced 1-hexene. We are currently assessing our system for the production of propene, butane, and styrene. However, given the flexibility of our technology, we believe that its greatest potential may lie in the ability to produce novel olefins currently too cost prohibitive to generate using traditional synthetic chemistry.

BIOT 231

Biosynthesis of a tetracycline-like polyketide-isoprenoid hybrid compound from Penicillium aethiopicum

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Viridicatumtoxin 1 is a polyketide-isoprenoid hybrid compound produced by Penicillium aethiopicum. It is moderately nephrotoxic and exhibits potent antimicrobial activity against gram positive bacteria including MRSA. The tetracyclic carboxamide core in 1 highly resembles the tetracycline intermediate, anhydrotetracycline. This provided a unique opportunity to study their biosynthetic convergence in bacteria and fungi, and may lead to discovery of novel tetracycline modifying enzymes. The gene cluster for biosynthesis of viridicatumtoxin has been identified recently by targeted gene deletion of vrtA encoding a non-reducing polyketide synthase (NRPKS). Based on the biosynthetic logic, the pathway for 1 is proposed to involve at least 13 genes. Here, we report our recent investigations on the two most interesting aspects of the biosynthetic pathway: i) the biosynthesis and incorporation of the malonamate starter unit to form the carboxamide moiety, and ii) the prenylation and cyclization of the geranyl moiety in 1.

BIOT 232

Improving specific activity of pretreatment enzymes on alternate substrates

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Enzymatic hydrolysis of delignified biomass requires the synergistic activity of diverse and thermostable glycosylhydrolases, making pretreatment economically challenging. Our research focuses on evaluating a rational mutagenesis strategy (site-directed followed by saturation mutagenesis) to improve hydrolytic activity on a broader range of substrates. We have chosen an endo-β-1,4 glucanase and an endo-β-1,4 xylanase from thermophilic fungi, and are secreting them from Pichia pastoris. Our strategy is based on extensive bioinformatics analyses involving sequence-based scoring functions of several cellulase and hemicellulase sequences deposited in the literature, to determine key amino acid positions along the substrate binding groove. Of the potential sites identified based on catalytic residue and substrate proximity, and solvent accessibility, we have picked the top two positions for mutagenesis. The mutant enzymes secreted from P. pastoris have been studied for biochemical activity on diverse substrates. The strategy developed will be applicable for protein engineering of other pretreatment enzymes of interest.

BIOT 233

Combination of stability prediction methods to complement SCHEMA structure-guided recombination

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SCHEMA structure-guided recombination is highly efficient for producing diverse sets of thermostable enzyme chimeras. The additive nature of SCHEMA sequence blocks allows for simple regression analysis to accurately model experimental data. By combining these regression models with information from large multiple sequence alignments (MSAs) and FoldX predictions, we were able to identify mutations that gave a stability enhancement comparable to that of the entire SCHEMA library. The 470 possible point mutations between five thermophilic fungal celllobiohydrolase I (CBH I) enzymes were modeled in 39 CBHI structures using the FoldX force field and compared in a MSA of 40 naturally occurring CBH Is. Mutations with large FoldX stability predictions, high MSA frequencies (consensus), and large regression data values were chosen for evaluation. Of the 13 tested, five were significantly stabilizing with a combined
stability increase of nearly 5 °C. A comparison to FoldX and consensus alone will be presented.

BIOT 234

Monitoring aggregation of human copper/zinc superoxide dismutase in cultured cells

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that inevitably leads to the death of motor neurons. Approximately 10% of familial ALS (fALS) cases are linked to point mutations in copper/zinc superoxide dismutase (hSOD). There is supporting evidence that the development of hSOD aggregates in the spinal cord and brain of fALS patients is pathologically linked to fALS. Cell culture model of fALS is a convenient system to study and understand the mechanism behind aggregate formation. Utilizing green fluorescent protein (GFP) fusion, we observed formation of mutant hSOD aggregates in HEK293T and NSC-34 (neuroblastoma-spinal cord) cell lines via fluorescence microscopy and a substantial reduction in cellular fluorescence measured by flow cytometry while wild-type hSOD fused to GFP did not aggregate. These findings could lead to the development of a screening assay for drug candidates inhibiting mutant hSOD aggregation for treating fALS.

BIOT 235

Aggregation modulating effect of methylene blue on amyloid-beta(1-40)

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Formation of extracellular plaques from amyloid-beta is a pathological hallmark of Alzheimer's disease (AD). Amyloid-beta aggregation is considered to be the cause of AD. One therapeutic strategy to avert disease progression is to modulate amyloid-beta aggregation using small molecules. Methylene blue (MB) is an FDA-approved drug with a variety of pharmacological activities. The recent Phase-II clinical trial reported that administering MB to AD patients slowed down cognitive degeneration. To understand the therapeutic mechanism behind MB, we investigated its modulating effects on amyloid-beta(1-40) aggregation. Our results showed that co-incubation of amyloid-beta(1-40) with MB led predominantly to high-molecular weight non-fibrillar aggregates and to a small population of fibrils. This result differs from amyloid-beta(1-42) fibrillization
promoted by MB. It is proposed that the different MB-modulating effects on the two major amyloid-beta isoforms, amyloid-beta(1-40) and (1-42), is due to their unique aggregation pathways.

BIOT 236

Characterization of heparan sulfate biosynthetic pathways in Chinese hamster ovary cells

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Chinese hamster ovary (CHO) cells are routinely used for production of recombinant glycoproteins, leading us to hypothesize that they could be used for biosynthesis of medically important polysaccharides such as heparin (HP), which is widely used as an anticoagulant. CHO cells naturally produce heparan sulfate (HS), which is synthesized in the same biosynthesis pathway as HP. CHO-S (suspension) cells were characterized with the goal of establishing levels of expression of genes required for HP biosynthesis using qRT-PCR and Western blot. Our preliminary results indicate that several genes required for HP biosynthesis are expressed in CHO-S cells, including those which code for Exostosin-2, C5-Epimerase, 2-O-sulfotransferase, and 6-O-sulfotransferase-1. We have also shown that CHO-S cells express 6OST-3, which plays an important role in refining the sulfated structure of HS. This has not been reported for previously investigated CHO cell lines. Genes coding for N-deacetylase/N-sulfotransferase-2 (NDST2) and 3-O-sulfotransferases (3OST), key enzymes in the anticoagulant HP synthesis pathway, are not naturally expressed in these cells. NDST2 and 3OST1 were stably transfected into cells and expression was confirmed. Several core proteins, upon which HS chains are attached in cells, are expressed in CHO-S cells including syndecan-1, glypican-1, and glypican-5. Core proteins are key, because cells use them to transport biosynthesized HP out of the cell. This will streamline an industrial-scale purification process. The HS/HP biosynthetic pathway will be characterized in additional CHO cell lines with the aim of identifying those lines that have the greatest potential for anticoagulant HP production.

BIOT 237

Analyses of the distribution of carbon products in Hydrothermal Pretreatment (HTP) of comingled wood wastes and biosolids
A Hydrothermal Pretreatment (HTP) has been developed to prepare a pumpable co-mingled wood wastes and biosolids slurry with an initial solid content of over 40 wt.%. A redistribution of carbon in the gas, liquid and solid products occurs due to hydrolysis and pyrolysis reactions during the HTP process. Analytical analyses of the carbon content in the products show that: the carbon loss through the gas phase, which is in the forms of non-condensable volatiles, was lower than 1.1% of the initial carbon loading; Over 90% of carbon present in the liquid phase was organic carbon, and the overall carbon in the liquid phase was lower than 1.4% of initial carbon loading; fixed carbon in the solid phase increased by 20%-30% after HTP, and the overall carbon recovery in the solid phase after HTP was over 95%. The pretreated wood wastes and biosolids slurry has favorable properties for applications as a feedstock for gasification.

**BIOT 238**

**Modeling the entire process: Finding the production and cost limits**

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Although the upstream and downstream processes are separated from an organizational standpoint, optimizations in one area may not necessarily lead to reduced costs or improved productivity in the overall process. The results of a series modeling studies of the production of monoclonal antibodies are presented. The results indicate that there is an economic limit to the value of increasing product titer in the bioreactor. The studies also show that production bottlenecks may develop in support areas, especially buffer preparation. The effectiveness of several debottlenecking strategies, including in-line buffer dilution and single-use containers, are presented.

**BIOT 239**

**Bioproduction of a broad spectrum antimicrobial molecule: Structure-function characterisation in the soluble and tethered state**

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Human beta defensin 28 (hBD28) is highly cationic and hypothesized to have a high membrane permeabilization efficiency on negatively-charged pathogens. The scarcity of hBD28 in vivo, however, impedes structure-activity studies of hBD28. This study reports a rapid and scalable production route to produce bioactive hBD28 in Escherichia coli (E. coli), thus opening the way for detailed structure-function characterization of the antimicrobial peptide (AMP). A dual fusion tag expression construct was pivotal in enhancing soluble expression and enabling recovery of hBD28 at a final purity of >95%. Purified hBD28 displayed dose-dependent killing kinetics activity against a model gram negative microorganism. Circular dichroism spectroscopy confirmed the presence of both β-sheet and α-helix conformations in the secondary structure of hBD28. The binding kinetics of hBD28 on different chemically modified surfaces having negatively charged, hydrophobic and zwitterionic properties were quantitatively studied using surface plasmon resonance (SPR). Correlation between hBD28 binding behavior and its antimicrobial property in the tethered state was studied, leading to new knowledge in structure-function elucidation of defensins. The outcome of this study opens the way for rapid production of AMPs for tethering applications. Importantly, understanding AMP structure-function relationship in the tethered conformation will enable rational improvements of immobilisation parameters.

**BIOT 240**

**Identify melanogenesis inhibitors from Cinnamomum subavenium by in vitro and in vivo screening systems**

**Hui-Min Wang**

Tyrosinases are known to be the first two rate-limiting enzymes in the synthesis of melanin pigments responsible for coloring hair, skin and eyes. The inhibition of tyrosinase is one of the major strategies to treat hyper-pigmentation. In human skin melanocytes, the cellular tyrosinase inhibition was examined by the conversion of L-tyrosine and oxidation of L-dopa to dopaquinone. Linderanolide B and Subamolide A exhibited metal-coordinating interactions with Cu2+ ions in a virtual model of molecular docking with human tyrosinase. We evaluated the skin pigmentation inhibitor effects with both in vitro and in vivo systems to find skin whitening agent without cytotoxic concerns. Linderanolide B and Subamolide A were isolated from the stems of Cinnamomum subavenium. These two herbal compounds were proved with good pigmentation inhibitory abilities and showed free cytotoxicities at low doses to human skin melanocytes and
zebrafish systems. The results obtained from biological assays showed that Linderanolide B and Subamolide A possessed anti-tyrosinase properties, which exhibited potential application in medical cosmetology and food supplementation. To our knowledge, this is the first study to reveal these bioactivity evidences of Linderanolide B and Subamolide A from this species plants.

BIOT 241

Directed evolution of CotA laccase for increased substrate specificity using Bacillus subtilis spores

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Directed evolution is an effective strategy to engineer and optimize protein properties and microbial cell-surface display is a successful method to screen protein libraries. To date, spores have not been demonstrated as a vehicle to screen for improved protein properties. Protein surface display on spores offers several advantages compared to other cell surface display systems. By virtue of the natural sporulation process, protein folding issues are avoided. In addition, chaperone proteins are present during sporulation to assist in protein folding. Finally, spores can endure extreme conditions. Therefore, spore display may provide an opportunity to search a region of protein space that is currently inaccessible with other display systems. We illustrate for the first time that spores are useful for directed evolution. Tapering the substrate specificity of the naturally present B. subtilis outer spore coat enzyme CotA, which is a laccase, shows this. After one round, a CotA variant was identified that was improved 120-fold.

BIOT 242

New preparation method of biocompatible collagen by using enzyme treatments and a reducing agent

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The purified collagen is produced by a new method contained steps of contacting collagen with a first proteolytic enzyme followed by contacting a reducing agent and second proteolytic enzyme
Biomechanical properties of cartilage extracellular matrix

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Cartilage is mainly composed of extracellular matrix, whose constituents are synthesized by the chondrocytes. The major components of cartilage extracellular matrix are the fibril-forming collagen (primarily type II collagen) and the large proteoglycan aggregates. Many biomechanical properties are governed by the combined functions of these constituents. Collagen fibers are strong in tension and reinforce the proteoglycan gel. The highly charged proteoglycan assemblies exhibit a large osmotic swelling pressure and provide compressive resistance to external load. The composition of cartilage, the organization of the collagen network, and the interaction between collagen and proteoglycans have important consequences on the load bearing, transport and diffusional properties of the tissue. Our objective is to gain insight into the biomechanical behavior of cartilage extracellular matrix by a systematic investigation of the physico-chemical properties of proteoglycan assemblies in near physiological salt solution using an array of techniques that probe statistically representative volumes of the sample.

Directed evolution of a thermophilic cellulase for biomass hydrolysis

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Lignocellulose is the most abundant biomass in the world and provides an abundant source for renewable production of biofuels. A key step in the production of biofuels from lignocellulose is the enzymatic hydrolysis of cellulose to glucose, which is carried out by cellulases. Cellulases from thermophiles have higher temperature optima, making them good candidates for industrial biofuels production; however, they often have lower than desired activity. Here we describe the directed evolution of a thermophilic endoglucanase (\(T_{\text{opt}} = 70^\circ\text{C}\)) for improvement of its specific activity. Mutant libraries from error-prone PCR were
screened based on two criteria: 1) expression of full length proteins as reported by a fused C-terminal GFP using FACS; and 2) activity using a robotic HTP screening platform. Potential mutants from initial screens (activity vs. GFP) were further purified and confirmed by specific activities. We report here on a mutant with 3-fold increase in specific activity.

BIOT 245

Benchtop high throughput screening: Chemical library and live-cell microarrays for combinatorial drug screening

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High throughput screening (HTS) technologies have been used to successfully identified proteins, small molecules, and bioactive compounds across a broad spectrum of biological fields; however, screening technologies have not kept pace with an expanding number of potential targets. Here, we present a microarray system for screening chemical libraries and combinatorial libraries in cell-based assays at the bench-top. The microarray platform exposes isolated cell cultures to chemical libraries by 'sandwiching' cell seeded microwells with matching chemical-laden arrayed posts. In this way, arrays of sealed cell-based assays are generated without cross-contamination. We demonstrate the efficacy of the system by identifying four hits from a library of hundreds of natural compounds in toxicology screens towards MCF-7 breast cancer cells. Additionally, we rank the capacities of various chemicals to induce apoptosis in MCF-7 cells. The bench-top microarray system miniaturizes HTS systems and allows for the screening of various chemical libraries and combinatorial libraries in a reproducible manner. We anticipate application of the system across many different experimental investigations in biology, as it is amenable to drug, small molecule and cytokine screening in a simple, robust and portable platform.

BIOT 246

Bi-specific antibody derived fusion proteins with tunable pharmacokinetic properties for cancer pretargeting using a covalent probe capture approach

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We designed bi-specific, antibody derived fusion proteins for a cancer pretargeting approach by fusing an anti-cell surface antigen antibody fragment (scFv BC8), and an anti-metal-chelate antibody fragment, scFv 2D12.5 G54C, to either end of a human IgG Fc fragment, making an "scFv-Fc-scFv" fusion. MAb BC8 binds to CD45, a potential target in the treatment of Acute Myeloid Leukemia. 2D12.5 G54C is capable of capturing and irreversibly binding to metal-DOTA probes. Neonatal Fc receptor point mutations have been made on the Fc of the fusion protein, making it possible to tune the serum half-life and modulate pharmacokinetic properties of the fusion. (NIH Grant CA016861 and NIH CA13663901)

**BIOT 247**

**Novel fusion expression partner: Halophilic proteins**

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Halophilic proteins have structural properties that are considered to be critical as fusion partner for soluble expression of recombinant proteins. We have isolated a few candidate genes, including β-lactamase (BLA). When each of these proteins was fused to the target protein to be expressed, the soluble expression in *E. coli* was greatly improved. We have characterized the physicochemical properties of BLA using circular dichroism (CD). It showed reversible melting. When BLA was thermally unfolded either with incrementally increasing temperature or rapid shift to a high temperature in 0.2 to 0.65 M NaCl solution, it was soluble even at 70 °C and regained the native structure upon cooling. Refolding from the heat-denatured structure was fast. These results demonstrate that BLA is highly soluble both in the native and thermally unfolded states and readily refolds upon cooling, as long as the salt concentration is moderate. Such high solubility and folding fidelity may support the folding of nascent target protein by keeping it soluble for a sufficient time.

**BIOT 248**

**Versatile monomer for preparing functional polycarbonates and poly(ester-carbonate)**

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Despite the increasing demands for degradable biomaterials, strategies for generating libraries of these materials with modular compositions and well-defined functionalities from common building blocks are still lacking. Here we report an azido-functionalized cyclic carbonate monomer that exhibited pseudo-living polymerization kinetics in both homopolymerization and copolymerizations with L-lactide. Well-defined random and block copolymers with number-average molecular weight more than 20k and polydispersity lower than 1.1 were precisely synthesized as characterized by gel permeation chromatography, \(^1\)H and \(^{13}\)C nuclear magnetic resonance spectra with differential scanning calorimetry. Side-chain functionalizations of the polymers prepared by this method were accomplished under facile conditions via copper-catalyzed or strain-promoted azido-alkyne cycloaddition. Complete conversion of azido groups were achieved without backbone degradation. This versatile building block, obtainable in two steps without chromatographic purifications, provides a practical solution to the preparation of functional polycarbonates and poly(ester-carbonates).

BIOT 249

Mimicking the leukocyte adhesion cascade by nucleic acid aptamer programmed cell-cell interactions


Nature has evolved complex yet effective cell adhesion mechanisms to deliver leukocytes to inflamed sites. This includes three types of cell-cell interactions between: 1) circulating leukocytes and endothelial cells (ECs), 2) circulating leukocytes and leukocytes arrested on ECs, and between 3) two or more circulating leukocytes in blood circulation which form cellular complexes and subsequently adhere on EC. Here we show cells engineered chemically with artificial nucleic acid aptamer ligands can recapitulate all above-mentioned cell-cell interactions under dynamic flow conditions. We demonstrate P-selectin binding aptamer engineered mesenchymal stem cells can tether and adhere to P-selectin coated substrates or P-selectin expressing endothelial cells. We show that L-selectin binding aptamer engineered MSCs can tether to L-selectin-coated substrates or L-selectin expressing neutrophils that are either freely flowing or arrested on a P-selectin-coated substrate. Our approach can be broadly applied
for building models to study the biology of cellular interactions, cell targeting, and tissue engineering.

BIOT 250

Employment of a process analytical technology (PAT)-based system to generate a linear pH gradient for protein separation and purification

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Chromatography is often used for downstream bioprocessing steps, and the buffered mobile phase properties such as ionic strength and pH are often critical process parameters (“CPP”). Ion exchange chromatography is therefore commonly used to separate proteins with different ionic characters. However, protein recovery may be poor and the de-salting steps could be cumbersome and time-consuming. In this presentation, we offer a linear pH gradient method through a process-ready PAT-based system, which is capable of making reproducible in-line pH adjustment. Examples include a linear pH gradient control and separation of two proteins with similar pI. Process analytical data will show that the use of real-time in-line PAT also provides automatic correction of pH and ionic strengths. By correctly identifying the CPP respective to the final process buffer blend composition, PAT should facilitate the implementation of Quality by Design (QbD) approach.

BIOT 251

Affinity chromatographic purification of human IgM from Human B lymphocyte cell culture supernatant

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Although monoclonal antibodies of the G class can be conveniently purified by affinity chromatography using immobilized protein A or G, even on a large scale, scaling up IgM purification still presents several problems since specific and cost-effective ligands for IgM purification are not available. Hexamer peptide ligand HWRGWV initially screened against the Fc portion of human immunoglobulin G (hlgG) also exhibited potential for use in the purification of human immunoglobulin M (hlgM). In this study, the ligand was employed for affinity chromatographic purification of human IgM from human B Lymphocyte cell culture supernatant. Different loading, wash, and elution conditions were tested based on previous studies in order to achieve high recoveries and purities. The HWRGWV
peptide ligand proved useful for a very convenient one-step purification of monoclonal IgM from the cell culture supernatant. The loading of the samples was done in phosphate buffers saline with 1 M NaCl, the washing step consisted of 0.2 M acetate buffer at pH 5, and the elution buffer was 0.2 M acetate buffer at pH 3. Other conditions included loading with 0.2 M acetate buffer at pH 5 with 0.2 M arginine and eluting adsorbed IgM by 0.2 M acetate buffer at pH 3. Both protocols achieved recoveries of 80% and purities of 24%, which were determined by human IgM enzyme-linked immunosorbent assay and densitometric analysis of sodium dodecyl sulfate polyacrylamide gels of purified fractions. The purities of hlgM improved about 300 times in one step from an initial value of 0.086%. The absolute purities were not high due to the low initial concentration of hlgM from un-optimized cell line. Much higher purities were achieved from complex mixture consisting of 1 mg/ml hlgM, in Minimum Essential Media, fetal calf serum and tryptose phosphate broth which contains bovine serum albumin as major contaminant.

**BIOT 252**

Integrated, two-component buffer system for mAb purification

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At GSK, a downstream platform process that has been used to manufacture mAbs in early/mid-stage clinical development over the past decade has recently been streamlined and updated with line-of-site to a specific large-scale facility for late-stage production. Among other improvements, a key development focus was to minimize raw materials and select buffer components that would more readily allow the use of concentrates and inline dilution at large-scale. In this work, we present our approach to select an integrated, two-component buffer system that encompasses the entire downstream process. Well-established buffer models were used to determine activity coefficients, predict pH robustness, conductivities, and capacities of various conjugate acid/base pairs under relevant chromatographic conditions. Mixing studies were conducted to confirm theoretical predictions and determine ratios for in-line dilution of concentrates. A comparative impurity clearance and performance assessment based on bench- and pilot-scale data was conducted for five mAbs fitted to the updated platform.

**BIOT 253**

Rates of Fc and Fab deamidation in mAb as determined by ion exchange chromatography
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The deamidation of asparagine residues into aspartate and isoaspartate moieties is a major pathway for chemical degradation of mAbs. A new approach to detect deamidation using ion exchange chromatography has been developed that separates papain digested mAbs into Fc and Fab regions; from this the deamidation rate of each region can be calculated. The effect of buffer species and pH on the rate of deamidation, in both the Fc and Fab regions of an IgG1 mAb, was determined using this IEC method. These findings will help predict deamidation rates of other IgG1 mAbs in specific buffer species and pHs. The degradation observed in the Fab region may potentially be used as a predictor of potency loss.

BIOT 254

Platform approach to evaluating impurity clearance in downstream chromatography operations

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The removal of process-derived impurities is critical to ensure the safety and efficacy of final bulk drug substance. CHO cell-line derived mAb products produced in the platform manufacturing process share a number of common process related impurities. These include host cell proteins and DNA, residual Protein-A from affinity chromatography, and fed-batch media components. To capture any interactions between impurities that might occur in the manufacturing process and to streamline clearance studies, it is desirable to evaluate the clearance of these impurities simultaneously. A series of spiking studies was performed on CEX and HIC/AEX chromatography columns to demonstrate the feasibility of combining these spiked impurities in the load material and quantifying the log reduction of each impurity in the final eluate. Combined spiking runs, wherein all impurities where spiked simultaneously, were compared to runs spiking only one classification of impurity, host cell impurities, residual Protein A, or media components. With only one exception, the comparison of these runs did not indicate that the interactions between impurities impacted the clearance achieved. An interaction between a recombinant growth factor, added as a non-platform media supplement, and host cell impurities was observed. This interaction is thought to affect the clearance of the recombinant growth factor.
These results indicate that for impurities common to the platform CHO processes, excluding the recombinant growth factor, clearance can be evaluated in a combined spiking study. This combined spiking approach mimics interactions in the process while reducing the total number of experiments in clearance studies.

BIOT 255

Utilization of pH gradient elution during Protein A capture for improving impurity clearance in antibody purification process

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Purification processes for monoclonal antibodies (mAbs) generally begin with a Protein A chromatography step using an isocratic elution followed by one or more polishing chromatography steps. However, processes with high impurity clearance demands may benefit by utilizing this capture step to deliver additional purification benefits. In this work, we investigated the use of a pH gradient elution to allow for significant removal of aggregate with Protein A chromatography. In addition, fractionation of host cell proteins, glycan isoforms and charge isoforms during the pH gradient elution was studied. Results will be presented demonstrating that utilization of a pH gradient elution during the Protein A capture step can provide additional impurity clearance and an overall improved purification process.

BIOT 256

Chromatography column integrity assessment, an analysis in transition

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The transition analysis (TA) method is a robust alternative to the pulse injection method for assessing column chromatography integrity given its ability to provide real time detection of small changes in column performance. However as a newer method, best practices for TA data interpretation and use are still evolving. The use of TA has been adapted for use at a large scale mammalian and microbial production facility for GMP monitoring and troubleshooting purposes. Examples are presented from large scale production experience to show different methodology for interpretation of TA results.
BIOT 257

Treatment of phenol in the organic solutions by a hybrid partition and biodegradation process with two-phase dispersions

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A combined process of partition and two-phase biodegradation was proposed to treat phenol in organic solution by Pseudomonas putida BCRC 14365 in aqueous mineral salt medium at 30°C in an agitated vessel equipped with a six-blade disk turbine. Kerosene was selected as the organic solvent because of its non-biodegradability and suitable partition coefficient for phenol (0.35). The initial cell concentration in mineral salt medium was fixed at 25 g/m³. The effect of initial phenol concentration in the organic solution (300-2500 g/m³) on phenol removal and cell growth was experimentally examined. Under the conditions studied, phenol could be completely removed from the organic solution and biodegraded in the cell medium within 78 h even though the initial phenol concentration was high up to 1800 g/m³. A simple model that combines steady mass-transfer equations and dynamic growth kinetics of suspended cells was proposed to follow the whole treatment process. The mass transfer characteristics between organic/aqueous dispersions were first determined by measuring the fluxes of simple diffusion of acetic acid and iodine. The proposed model was shown to satisfactorily describe the process as long as phenol concentration in the cell medium did not exceed the toxicity limit of P. putida cells in freely suspended condition.

BIOT 258

Development of a simple and efficient Mab polishing platform to replace the traditional anion exchange chromatograph or membrane adsorber using Sartorius Sartobind STIC

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In many mAb purification platforms, traditional anion exchange column chromatography or, increasingly, anion membrane chromatography is used as a polishing step in a product flow-through mode to bind trace levels of process or
product related impurities and assure efficient viral clearance. Anion exchange chromatography is however limited by the requirement for low loading buffer conductivity to efficiently remove impurities, which necessitates buffer exchange or dilution of the Protein A column eluate. In this study we developed a mAb polishing step using Sartorius Sartobind Salt Tolerant Interaction (STIC) membrane chromatography. Utilizing a HTS approach we identified the initial chromatographic parameters for acceptable step recovery and product quality. We then confirmed these conditions in small STIC capsules. Utilizing a combination of HTS screening and DOE optimization we developed a mAb polishing platform which demonstrated high step recovery and efficient clearance of impurities (HCP, HMW, host DNA, leached Protein A) for multiple antibodies at higher loading buffer conductivity. This simple and efficient polishing step can be easily integrated into most current mAb purification platforms, which may shorten mAb purification processes and accelerate development programs.

BIOT 259

Spatial homogeneity analysis of stacked membrane chromatography

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Stacked membrane chromatography is usually modeled in one spatial dimension, and external hold-up volumes are accounted for by a continuously stirred tank and a plug flow region. Such models assume that fluid flow and solute molecule concentrations are homogeneously distributed over membrane cross sections. However, good radial distributions are practically hard to achieve in membrane capsules with extreme length to width ratios. The previously published zonal rate model (ZRM) allows to quantitatively analyze the impact of radially inhomogeneous membrane loadings on measured chromatograms by virtually partitioning hold-up volumes and the membrane stack. The semi-empirical ZRM results are now validated by mechanistic modeling of both the flow field and the concentration gradients in two and three spatial dimensions. Finite element simulations are computationally expensive and require detailed knowledge of the internal capsule geometry, including inlet, distributor, membrane stack, collector and outlet regions. However, this model is solely based on the governing mechanisms and reproduces experimental breakthrough curves without empirical factors.

BIOT 260

New high resolution ion exchange resins for polishing
The main challenge for the polishing step is often to combine high resolution with sufficient throughput. We have developed two high resolution resins, one cation exchanger and one anion exchanger, to address these needs. The combination of high flow agarose technology and a small particle size resulted in novel ion exchange (IEX) resins with very good pressure-flow properties as well as high resolution. In this poster, characteristics of the experimental resins, comparisons with other IEX resins, throughput calculations and examples of protein purifications are presented. One example describes the optimization of a separation of a truncated form of a recombinant protein from the intact protein.

BIOT 261

Novel high capacity protein A resin

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We present results from work on a novel high capacity Protein A resin based on high flow agarose, optimized towards higher dynamic binding capacity (DBC) at longer residence time. Depending on the specific antibody, 20 – 50% higher DBC (50-60 g/L) was obtained compared to MabSelect SuRe™. DBC was independent of MAb concentration in the range 1 – 10 g/L. Optimization of elution conditions (pH and flow rate) was performed by design of experiments. Results from a lifetime study performed with a clarified CHO cell culture supernatant with a MAb titer of 1 g/L, will be shown. In addition the alkaline stability was tested by repeated cycling.

BIOT 262

Comparison of endotoxin removing capabilities of commercially available resins and filters from new biological candidates

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The removal of contaminating endotoxin is a significant concern during downstream process development as well as in the generation of pre-clinical material for initial animal studies. The generation of new biological candidates is often developed quickly and in a non-GMP facility setting. This involves the risk of endotoxin contamination as well as insufficient endotoxin removal by utilized
downstream process steps from E. coli generated material. In order to accommodate aggressive timelines it is highly valuable to specify a strategy for endotoxin removal from new biological compounds when needed. To facilitate a platform set-up for endotoxin removal regardless of endotoxin contamination level or nature of new biological candidates such as pI and MW, a comparison of different endotoxin removing filters and resins was performed. Here we report the comparison of different commercially available resins and filters with regard to endotoxin reducing capabilities of different new biological candidates purified from either mammalian cell-cultures or E. coli fermentations.

BIOT 263

VEGF inhibitor with infinite affinity: Covalently modifying VEGF with a peptide

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Peptide v107 has been found to inhibit vascular endothelial growth factor (VEGF) from binding to its receptors, a mechanism important in inhibiting tumor angiogenesis and macular degeneration. An approach to improve inhibition activity is to improve the affinity of v107 for VEGF. v107 was modified to provide a site of conjugation of a small library of cross-linkers while maintaining the affinity of the peptide for VEGF. This affinity is necessary to catalyze the reaction of the linker to VEGF, so that the binding is covalent and irreversible. The reactivity of these cross-linkers for VEGF was compared using Western blots. The modified peptide with the most reactive linker was tested for its inhibition activity by cell proliferation assays. Supported by NIH research grant CA016861

BIOT 264

Affinity membrane based separation of plasminogen activators

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Plasminogen activators (PAs) are enzymes responsible for conversion of plasminogen to plasmin by cleaving the Arg-Val peptide bond. The resulting plasmin dissolves fibrin blood clots. PAs are thus very important clinical agents. The objective of this project is to develop an affinity membrane based separation process for the isolation of plasminogen activator from mammalian cell culture broth. As compared to traditional chromatographic methods, affinity membranes are characterized by a high surface area, reduced diffusion distance, low operating pressure, and higher cost effectiveness. In this project regenerated cellulose membranes were chemically modified through linkage to affinity ligands via spacer arms of different lengths (5C, 7C, 12C). The affinity ligand used was p-aminobenzamidine. The membrane modified with 12-C spacer arm linked p-aminobenzamidine was found to bind PA with higher efficiency as compared to other spacer arms. Other affinity ligands for PAs are now being synthesized based on reports available in literature.

BIOT 265

Density as an alternative measure of protein concentration and its use as an in-line monitor for downstream purification unit operations

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In-process monitoring of protein concentration is typically conducted by measuring the absorbance at \(\lambda=280\) nm. When higher concentrations are encountered, sample dilution is required in order to attain measurement accuracy. This action becomes a source of error and limits the utility of performing in-line measurements and process analytical technology (PAT) applications. To address this problem, density measurement was investigated as an alternative approach for concentration evaluation. A direct and linear correlation was identified between density and protein concentration providing for an accurate concentration assessment. Using this relationship, an in-line density transducer was successfully implemented as a monitor of protein concentration in ultrafiltration and chromatography processes. The in-line density monitor allowed for the accurate assessment of process completion for ultrafiltration and the maximum protein concentration of the elution peak for chromatography. This technology may provide a means for increased process control and better process understanding in the production of protein therapeutics.

BIOT 266

Protein purification using caprylic acid precipitation
Increasing cell cultured densities and productivities during therapeutic protein production are placing a larger burden on downstream clarification and purification operations due to higher product and impurity levels. Caprylic acid precipitation is a low-cost, high-throughput process that can replace chromatography steps used for HCP clearance and result in significant cost savings during protein production. Caprylic acid precipitation works to selectively remove acidic proteins (HCP) from solution by forming hydrophobic complexes which precipitate out of solution, while basic proteins (most monoclonal antibodies and fusion proteins) remain in solution. The goals of this project were to examine the feasibility of caprylic acid precipitation as an alternative purification method in our protein production processes. Laboratory-scale precipitation experiments were run to determine optimal conditions for HCP clearance, product yield, and product quality. Multiple products were evaluated to examine the robustness, prior to scaling up to our pilot scale production process.

BIOT 267

Development of a robust and simplified DNA removal unit operation for non-mAb recombinant protein purification using a disposable Q membrane adsorber

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In the current purification process for a Bayer recombinant therapeutic protein, an anion exchange column operated in flowthrough mode was used to specifically remove host cell DNA. This step, however, has two limitations: time-consuming feed stream adjustment and significant yield loss. When developing the next generation purification process, we replaced the anion exchange column with a disposable Q membrane adsorber for DNA removal. This new unit operation eliminated the need for feed stream adjustment and demonstrated robust DNA removal capability with significantly higher yield. Other benefits of using disposable Q membrane adsorbers include simplified operation, reduced buffer usage, and savings on capital expenditure. Q membrane adsorber DNA removal is a platform technology currently being implemented for the purification of other Bayer non-mAb therapeutic proteins. A cost analysis will also be presented to compare operational costs between using disposable Q membrane adsorbers and using anion exchange columns.
Development of capture and intermediate chromatography steps for insulin purification

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This presentation will describe the development of a capture step for pro-insulin expressed in \(E.Coli\) and an intermediate step for the purification of insulin following the capture step. Resin screening and chromatography condition screening using an HTPD approach in a 96-well filter-plate format were performed. The results indicated that the media of choice for the capture step was Capto\(^{TM}\) MMC, a cation-exchange multi-modal resin. Binding and elution conditions were further optimized in packed columns using the DoE functionality in an ÄKTATM avant 25 chromatography system. The optimal chromatographic conditions were identified and followed by a robustness study. The capture step was then scaled to pilot scale. After enzymatic cleavage of the purified pro-insulin from the capture step the formed insulin was subjected to cation exchange chromatography. A similar approach for development of this step as for the capture step was followed. Results from the cation exchange step will be presented.

**BIOT 269**

In vitro production of anticarbohydrate antibodies: Purification of antitrimannose single chain antibodies (scFvs) from inclusion bodies

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We previously reported isolation and characterization of anti-trimannose scFvs by phage display technology (Biochemistry 46:253 & 263, 2007). Since scFv protein expression levels were too low, the pET expression system was used to express scFv proteins. 1A4, 1G4 and 5A3 scFv genes were inserted into pET22b to generate bacterial expression vectors. Expression of scFv proteins was induced by IPTG in \(E. coli\) BL21 transformed with pET/1A4, pET/1G4 or pET/5A3. Inclusion bodies prepared from bacterial cells were solubilized in 3.5M Gdn-HCl. 1A4, 1G4 or 5A3 scFv protein was purified by Ni\(^{2+}\)-Sepharose chromatography. The eluted scFv proteins in 3.5M Gdn-HCl were refolded by stepwise dialysis. From 80 ml of culture media, 612, 586 and 271 mg of 1A4, 1G4 and 5A3 scFv proteins, respectively, were purified to homogeneity. SPR
analyses revealed $K_D = 2.5 \times 10^{-6}, 1.8 \times 10^{-6}, 3.8 \times 10^{-6} \text{ M}$ for 1A4, 1G4 and 5A3 scFv proteins, respectively.

BIOT 270

Understanding the flux decay of syringe viral filters and improvement of viral filtration performance

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Viral clearance validation of purification processes is a critical component in regulatory filings in the biotech industry. Observed inconsistency of virus filtration flux during scale down and spiking studies have been problematic in viral clearance validation. Despite efforts by manufacturers to improve the quality of syringe viral filters, it is still common to observe up to 70% flux decay at bench scale using manufacturing process intermediates without any virus spiking, compared to minimal decay at manufacturing scale. A systematic study was performed to identify a key mechanism of flux decay in the scale down model. A simple method was implemented to overcome the flux decay problem, without modification to the existing downstream processes. The new method is applicable to a variety of viral filters.

BIOT 271

New high capacity anion exchanger for bioprocessing

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UNOsphere Q High Capacity (Nuvia Q) is a new high capacity anion exchange resin designed for optimal bio-processing applications. Mass transport is rapid at flow rates of 300-600 cm/hr, with a dynamic binding capacity (10% breakthrough) of 190-240 mg/ml for bovine serum albumin. Performance of UNOsphere Q High Capacity (Nuvia Q) as a function of protein load, pH, conductivity and flow rate will be discussed. Alkaline resistance of this Q resin will also be reported.

BIOT 272

Tips and hints for purification of GST-tagged proteins using prepacked columns
Purification using a GST-tag has many advantages, such as high specificity, increased target protein solubility and usage of mild elution conditions. Concerns regarding yield and recovery have been reported when working with low expressed and/or large proteins. The objective for this study has been to evaluate purifications with the prepacked columns, GSTrap™ HP, GSTrap FF and GSTrap 4B which differ with respect to matrix properties. GST tagged proteins with different molecular weights and expressed at high and low levels have been used in the investigation. GST has a tendency to dimerize and glutathione has stronger binding for dimeric GST compared to monomeric. The results of this work indicate that when applying low expressed GST tagged proteins, endogenous glutathione may inhibit the binding of the GST tag to the immobilized glutathione. GSTrap 4B prepacked with Glutathione Sepharose™ 4B showed the best results after applying both high and low expressed GST tagged proteins resulting in recoveries of >60 % and yields up to 40 mg purified protein/ml medium independent of the size of the protein to be purified.

BIOT 273

Model supported design and optimization of preparative chromatographic multi component purifications

Model-based optimizations of the preparative purification of valuable pharmaceutical drugs become more and more important as regulatory organizations move towards model-based understanding of purification processes. In addition, simulation based optimizations become increasingly attractive, as the computational resources and numerical algorithms are steadily improving and the computer power is not the bottleneck anymore. Instead the evaluation of accurate physical properties like the adsorption isotherms and mass transport resistances is expensive and it requires a high level of knowledge and experience. In order to estimate the competitive multicomponent adsorption isotherm of polypeptides and small proteins on reversed phase resins, a procedure has been developed, which allows to reduce the experimental effort to a minimum. After the adsorption isotherm of the product to be purified has been evaluated, the isotherm parameters of the impurities can be easily estimated from a few chromatograms of the multicomponent mixture. The prediction of overloaded solvent gradient chromatograms of the pure component, pure impurities and of the multicomponent mixture even on different resins and for
different organic modifiers is demonstrated. The chromatographic models are further verified for continuous multi-column chromatographic processes, i.e. the MCSGP process. Comparisons of experimental results with dynamic simulations are shown.

BIOT 274

Importance of osmolality in cell culture process design & optimization

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The biotech industry spends significant effort on developing cell culture processes with high antibody titers to diminish the cost of goods. One approach is to modify the feed concentration. An unintended consequence of this approach is a subsequent change in the osmolality of the culture. In this study, we demonstrate that increased osmolality results in suppressed growth accompanied by improved antibody secretion for certain mammalian cells. We present a case study, wherein initial modification of the feed nutrients levels resulted in improved antibody titer in a fed-batch process. Using a statistically designed experiment, we then explored several methods to balance the culture osmolality and were able to identify conditions that resulted in further optimization of antibody titer. In conclusion, the impact of osmolality on the performance of cell culture processes may be significant and must be taken into account during process development.

BIOT 275

Carbon dioxide inhibitory effect on uninfected and baculovirus-infected insect cell culture and the role of intracellular pH

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Large scale production recombinant protein and biopesticide with the baculovirus-insect cell system requires the use of bioreactors with high cell densities and large working volumes. This increases the potential for CO2 accumulation in the culture medium to inhibitory levels. The aim of this study was to quantify the effect of elevated CO2 concentration on cellular metabolism in cells, before and after infection with wild type baculovirus AcMNPV; and to determine the mechanism leading to the inhibitory effect of CO2 accumulation. It has been hypothesized that the inhibitory effect of elevated CO2 concentration
could result either from (i) intracellular acidification (ii) oxidative stress. It was demonstrated that oxidative stress did not contribute to the inhibitory effect of uninfected Sf-9 cells. A protocol is currently being developed using 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester to quantify the intracellular pH. Furthermore, the effect of CO₂ concentration on the metabolism of baculovirus-infected Spodoptera frugiperda (Sf-9) cells and its effect on intracellular pH and oxidative stress are currently being investigated.

BIOT 276

Using response surface methodology to understand and optimize a commercial cell culture process

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With increased process understanding over a product lifecycle, process optimization is continually applied in Genentech's antibody manufacturing processes. Here we present a case study where response surface methodology was used to optimize and increase the robustness of a commercial, Chinese hamster ovary (CHO)-based cell culture process. Multiple cell culture process parameters were screened utilizing a scale-down model within the established, acceptable parameter ranges using a fractional factorial experimental design. Subsequent experiments were performed using a subset of parameters deemed to have a significant impact on cell culture performance. Statistical designs including linear and quadratic models were explored to quantify the impact of these process parameters on cell culture performance. This presentation will discuss the results of this case study.

BIOT 277

Determining the impact of pH, temperature and dissolved oxygen changes in high density mammalian cell cultures using metabolic flux analysis

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Quality by Design (QBD) is increasingly becoming an integral component of biopharmaceutical process development given the industry shift towards including design space data in biological license applications. Typically, screening experiments are first performed to reduce the process variable space and also to classify variables based on criticality. In this study, we describe the application of metabolic flux analysis to screening experiments performed with
CHO cells in high density perfusion cultures. Bioreactor pH, temperature, and dissolved oxygen were varied between predefined low and high limits and cellular response to these changes was quantified using metabolic flux analysis. Since typical cell culture response variables both in screening and subsequent design space studies have been limited to cell specific rates and protein productivity, metabolic flux data substantially extend the response variable space. The additional quantitative characterization of cell physiology through metabolic flux analysis can help in the development of more robust process which can ultimately produce consistent quality product.

BIOT 278

Estimating respiration rates for mammalian cells in perfusion culture

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Oxygen uptake and carbon-dioxide production rates (OUR and CPR, respectively) provide information on cell metabolism and physiology and are also inputs for metabolic flux analysis. While OUR estimation is relatively straightforward, CPR estimation is complicated because of the reversible dissociation of CO₂ into H₂CO₃⁻, HCO₃⁻, and CO₃²⁻ in solution. The equilibria of these dissociation reactions are strong functions of pH, temperature, and ionic strength that must be accounted for during CPR estimation. Here we present methods to estimate OUR and CPR in mammalian cell perfusion cultures using global mass balances. This method relies on O₂ and CO₂ measurements in the inlet and outlet gas streams and kLa data and reactor perturbations are not necessary. Our approach allows real-time OUR and CPR estimation, which in addition to being indicators of cell physiology and metabolism, enable real-time estimation of metabolic fluxes thereby substantially enhancing the cell physiological state vector.

BIOT 279

Effect of culture conditions on N-glycan profiles of a monoclonalantibody produced by mammalian cell culture

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Consistent production of product of a predefined quality is a goal of the QbD and PAT regulatory initiatives. High-throughput mini-bioreactors have a great potential to aid in process development studies in order to achieve this goal. Studies were carried out to see the effect of culture parameters on monoclonal antibody (IgG3) titers and its glycosylation profile using high-throughput mini-bioreactors, 250ml spinner and 5L bioreactor. We use a two step scheme to purify IgG3 antibody from the cell culture broth and carry out N-glycan analysis using high pH anion exchange chromatography with pulsed amperometric detection. It has been found that the glycosylation profile of IgG3 produced in a 250 ml spinner differed significantly from that produced in the bench scale bioreactor. Here, we further investigate the effect of different culture conditions on N-glycan profile of monoclonal antibody produced by serum free mammalian cell culture using high throughput mini-bioreactors.

BIOT 280

Optimization study on propagation of Influenza A virus in Vero cells by using Cytodex™ microcarriers in WAVE Bioreactor™ systems

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As the threat of an influenza pandemic has become more apparent, there is an increasing need for responding quickly to mass produce influenza vaccine. The shift from egg-based to cell culture-based vaccine production is seen as one way to secure a more rapid response. Furthermore, one additional obstacle is to overcome the need to greatly reduce time for clinical trials & approval i.e. to reduce time to market, where cleaning validation is of major importance. Here, we present a way for eliminating cleaning validation by using a disposable bioreactor alternative. We have performed an optimization study on production of live Influenza A/Solomon Islands/3/2006 in Vero cells by using Cytodex™ microcarriers in WAVE Bioreactor™ systems. Parameters such as inoculation conditions, additives and composition of media etc. have been considered. Thus, the data shows that production of the influenza virus in WAVE Bioreactor™ system is a fast and convenient alternative to conventional systems.

BIOT 281

Process development for cell-based live influenza virus
Present influenza vaccine production faces several challenges to guarantee support when a severe pandemic occurs. The main obstacles to overcome is to greatly reduce time for clinic/approval, manufacturing and to increase global production capacity. Our team aim is to develop the tools, technologies and services required by the vaccine industry. Here we present work up to pilot-scale in upstream, downstream and analysis focusing on reduction of critical host cell derived impurities such as genomic DNA. Hands-on time for analytics, and thus cost, is one of the main bottlenecks during influenza vaccine development and production. For this purpose, we have developed assays for virus and HCP quantitation based on Surface Plasmon Resonance (SPR) technology a more accurate and sensitive alternative compared to current methods. Additionally, a prototype purification process with modern technologies was tested for multiple live influenza strains with retained TCID50 levels established under a standard set of conditions.

**BIOT 282**

**Biacore™ quantification analyses for process development: Influenza vaccine and plasma fractionation**

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Reliable analytical tools are important for economical process development, production and batch release of vaccines and human plasma proteins. For *influenza virus titer determination* the recommended method, single radial immuno-diffusion assay (SRID), is straightforward but leaves room for improvement when it comes to assay variability, sensitivity and speed. A Biacore assay was compared with SRID for concentration determination of influenza virus from vaccine process development. The Biacore assay had higher sensitivity (0.5 - 10 µg/ml), higher precision and significantly shorter analysis time compared with SRID. Biacore analysis was also used for *quantification of human plasma proteins* from all steps during plasma fractionation. The Pharmacopoeia recommends techniques as Biuret and immuno electrophoresis. Our initial studies show reliable quantification and identification of total IgG and albumin and the distribution of the IgG subclasses, performed with high precision, accuracy and sensitivity. Further, quick in-process control quantification is easily obtained within < 20 min.
BIOT 283

Impact of coordinating viral systems biology and personalized-medicine for optimizing HIV treatment

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Systems biology has had its greatest successes in the study of microbe populations. Attempts to extend this approach to human pathology have yielded limited success. We hypothesize that an individualized approach to the study of human disease yields more beneficial results that a population-based strategy. A genetic algorithm was used to optimize Highly Active Antiretroviral Therapy (HAART) based on the average dynamic behavior of a cohort of simulated HIV subjects based upon a published model of HIV viral dynamics, as well as for two selected members the cohort. We report that treatment optimized for the individual theoretical subjects resulted in behavior that when compared to the application of the “one-size-fits-all” treatment could be related to clinically significant differences in treatment outcomes. The example of optimizing HAART for HIV treatment suggests that an individualized analysis is necessary when using systems biology to study human disease as opposed to a population-based approach.

BIOT 284

Expression of recombinant green fluorescent protein in B. methanolicus

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Industrial enzymes is currently a multi-billion dollar industry. The substrate most commonly used for these processes is glucose or sugar mixture. This work explores the use of a methylotroph, B. methanolicus, for the production of a recombinant protein. The combination of methanol substrate and thermotolerant nature of the strain offers an alternative for low cost processing to the use of carbohydrates. A plasmid was constructed that incorporates the methanol dehydrogenase promoter from the strains native plasmid and the gene for green fluorescent protein. Electroporation was used for the transformation of the strain. The analysis of protein production under various growth conditions will be presented.
Collagen nanoscale structure and disease: The relationship between D-axial spacing, osteoporosis, and osteogenesis imperfecta

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Quantitative assessments of the distribution of Type I collagen fibril D-axial spacings made using AFM and analyzed using two-dimensional Fourier transforms indicate that this marker of nanoscale morphology changes as a function of disease state for both osteogenesis imperfecta and estrogen-depletion related to osteoporosis.

The effects have been investigated in a variety of tissues including bone, teeth, and tendon. A population of shorter D-axial spacings is observed for the tissue in the diseased states.

Structural confirmation of a novel nondisulfide covalent cross-linked dimer in uricase family

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Uricase [EC 1.7.3.3] is an enzyme that catalyzes the hydrolysis of uric acid into allantoin. Uricase from all species is functionally active as a homo-tetramer, which was maintained by non-covalent interactions among monomers. Slight amount of dimeric isomers were widely observed in different species, even
include the marketed rasburicase proteins. However, little information was available for its detail structure. Recombinant canine uricase were successfully expressed and purified in our labs and about 1% of dimeric isomers were also observed in the purified tetrameric canine uricase that detected by reducing SDS-PAGE. The above uricase was further purified by size-exclusion and affinity chromatography, but the dimeric isomers were still existed and its content was constant, indicating that the dimeric isomers were active isomers and formed within the tetramer. Given the results observed in reducing SDS-PAGE, size-exclusion and affinity chromatography, we speculated that such dimeric isomers were active non-disulfide covalent cross-linked dimers that existed in the homotetramers. The above active uricase was denatured to monomers by adding urea and the denatured samples were loaded onto a HiLoad 26/60 Superdex 75 column. The dimeric isomers were successfully separated. The normal monomers and the covalent dimers were digested by trypsin and analyzed by capillary HPLC-ESI-MS systems, respectively. A novel peptide that cross-linked between N and C terminus were observed in dimeric isomers, indicating that such dimers may covalently cross-linked between the N-terminal amino group and C-terminal carboxyl group. Moreover, homology modeling results showed that the distance between the N-terminus from one monomer and the C-terminus from another monomer within the same tetramer allowed such covalent interaction. In conclusion, we considered that a novel active covalent cross-linked dimer, which was widely existed in uricase family, was first structurally confirmed in our lab.

BIOT 287

Implementation of electronic lab notebooks for downstream process validation experiments

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Electronic lab notebook templates have been developed for downstream experiment preparation, execution, and data collection purposes. The use of electronic lab notebooks in process development and validation experiments allows the datamanagement process to be more streamlined and transparent. This leads to increased experiment efficiency and productivity, and therefore the ability to meet accelerated timelines. However, designing and implementing electronic lab notebooks for process validation presents many unique challenges. Adapting the existing templates used in development for use in process validation requires stricter guidelines and data tracking, along with increased cross-department functionality. This talk will focus on the key features and benefits of the electronic lab notebook, as well as the challenges of
implementing and creating electronic lab notebook guidelines and templates for downstream process validation unit operations at Genentech.

BIOT 288

Extracellular polymeric substances of *Shewanella* biofilms contain redox active components with potential roles in extracellular electron transfer

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Dissimilatory metal-reducing bacteria (DMRB) are an important group of microbes that can directly or indirectly catalyze the reduction of redox-reactive metal ions (such as Fe, Mn, and U) and can interact with solid electrodes through donating or accepting electrons. DMRB such as *Shewanella* species have been extensively used as model organisms to study electrochemically active biofilms (ECABs). However, it remains unclear whether the EPS of DMRB biofilms are redox active and have a role in extracellular electron transfer. We characterized the composition of EPS from *Shewanella* sp. HRCR-1 biofilms, especially protein components, to provide insight into potential interactions of EPS with redox-active metals and solid electrodes. Both bound and loosely-associated EPS were extracted from *Shewanella* sp. HRCR-1 biofilms prepared using a hollow-fiber membrane biofilm reactor (HfMBR). FTIR spectra revealed the presence of proteins, polysaccharides, nucleic acids, membrane lipids, and fatty acids in both EPS fractions. A total of 58 extracellular and outer membrane proteins were identified in the EPS through a global proteomics approach. These included homologues of multiple *S. oneidensis* MR-1 proteins that potentially contribute to key physiological biofilm processes. In addition, 20 redox proteins were found in extracted EPS, including the homologues of two *S. oneidensis* MR-1 c-type cytochromes, MtrC and OmcA, which have been implicated in extracellular electron transfer reactions. The presence of these homologues has also been confirmed by immunoblot analysis. Given their detection in the EPS of *Shewanella* sp. HRCR-1 biofilms, c-type cytochromes may contribute to the redox activity of the biofilm matrix and play important roles in extracellular electron transfer process.

BIOT 289

Cellular electron transfer rates quantified on a per cell basis for single cells on electrodes
Fundamental understanding of electron transfer mechanisms in biofilms can be achieved using electrodes as electron acceptors which enable quantitative real-time measurements of extracellular electron transfer rates. We recently developed a continuous flow, optically accessible electrochemical system allowing real-time microscopic imaging of anode population development from single cells to a mature biofilms. This system was used to correlate electron transfer rates for the first time on a per cell basis and with overall biofilm development for *Shewanella oneidensis*. During early biofilm development, estimates of current per cell reached a maximum of 204 fA/cell. Intriguingly, since the publication of these results, several research groups, using very different approaches have reported very similar values. We have now extended these dynamic experiments further and observed and quantified the cellular growth characteristics and metabolic response of cells to the introduction of various electron acceptors and soluble metals on electrodes poised at accepting potentials.

**BIOT 290**

**Improving the isoprenoid production in yeast one gene at a time**

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Mevalonate pathway in *S. cerevisiae* is essential for the synthesis of two valuable precursors; isopentenyl pyrophosphate (IPP) and its isomer, dimethylallyl pyrophosphate (DMAP). IPP and DMAP are used for sterol synthesis as well as a diverse class of isoprene based molecules (isoprenoids). Beside their essential cellular functions, isoprene derived molecules have great industrial value as they are precursors to many compounds that have medical, nutritional, and agricultural importance. More specifically, the same pathway has the potential to be exploited for biofuel production. To improve the production of isoprenoids in yeast, we screened the yeast deletion collection using carotenoid synthesizing plasmids. About 200 gene deletions showed significant improvement in colony color and of those with direct relevance to the isoprenoid pathway are being tested in a yeast strain that was previously engineered for enhanced isoprenoid production.

**BIOT 291**
Enabling process scouting devices (PSDs) with low cost dissolved oxygen and pH sensors for bioprocess development

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The bioprocess design at every step of the process development cycle is a complex task that requires a complete understanding of the engineering of the process (e.g. mass transfer, mixing, CO2 removal, process monitoring and control) and its effect on cell biology and product quality. Despite their widespread use in bioprocess development (i.e. process optimization and process validation), process scouting devices, (PSDs) are considered by some as “black boxes” from a monitoring standpoint because they are generally not equipped with sensors. In this research study we equipped two of the most commonly used PSDs, T-flasks and spinner flasks, with low cost patch-based optical sensors for monitoring dissolved oxygen and pH during early cell culture development settings. We observed that, during extended cell passaging in these PSDs, our murine hybridoma cell line showed oscillatory-sinusoidal trends for oxygen uptake and pH slope shift curves. These trends showed some correspondence to standard cell culture metrics such as viable cell density (VCD). We then carried out a mass transfer characterization of T-flasks and spinners flasks under different operational conditions. We observed that orbital shaking in T-flasks can provide volumetric mass transfer coefficient ($k_{L}a$) values as high as 22.6h$^{-1}$ but under rocking agitation, the highest $k_{L}a$ observed decreased to 14.8h$^{-1}$. We also observed that T-flasks can be operated at similar $k_{L}a$ values as spinner flasks thus allowing us a cell culture comparability study between two cell culture trains during scale up. We benchmarked these scale-down studies by a demonstration that at constant $k_{L}a$, spinner flasks and a 5L standard lab-scale stirred bioreactor displayed cell culture similarities in terms of VCD and product titer. We believe that equipping these PSDs (and others) with low cost dissolved oxygen and pH sensors can significantly improved upstream bioprocess optimization.

BIOT 292

Exploration for an effective and low cost chemical method to convert hemicellulose oligomers to monomers with high yields

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Hemicellulose makes up about 15-40% of the dry weight of cellulosic biomass, and its effective utilization is vital to low cost conversion of cellulosic biomass to fuels/chemicals. Among the leading pretreatment technologies essential to high yields from biological conversion of cellulosic biomass, several of them, especially hydrothermal pretreatments such as steam explosion, flowthrough, and some base and acid pretreatments, produce hemicellulose oligomers during pretreatment. The portion of these oligomers can vary from 10 to 80% of the total sugars released from hemicellulose in the raw biomass depending upon solids loading, pretreatment severity, and chemical addition and type. Because most organisms cannot ferment these oligomers directly, they must generally be converted into monomeric sugars prior to fermentation, but biological pathways are not yet economical due to the high cost of enzymes. Therefore, chemically catalyzed hydrolysis is still a leading option, but it is necessary to develop a low cost processing method which can give yields close to theoretical with the least amount of energy and chemicals input. In this study, the effects of dilute sulfuric acid, oxalic acid, maleic acid, and acetic acid on oligomer hydrolysis are evaluated at various processing conditions (acid loading, temperature, and time) to define pathways to high yields at low costs. Key words: hemicellulose; oligomers; yields

BIOT 293

Substrate channeling in biofuel cells: Synthetic Metabolon electrodes

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Enzymatic biofuel cells though useful, have been plagued by limitations due to incomplete oxidation of fuel. In order to combat this limitation researchers have explored the use of multi-enzyme cascades to more completely oxidize a readily available fuel in a similar manner in which metabolism occurs in organisms. However, organisms have been shown to optimize cascade performance through the formation of complexes that improve the substrate channeling through a given pathway. This research effort attempts to more accurately mimic metabolic pathways as they occur in vivo by examining nature’s key enzymatic energy producing cycle (The Krebs Cycle), through covalent linkage of metabolic enzymes to utilize improvements in efficiency through substrate channeling while immobilized on a carbon electrode. In addition, to improve upon this effort through increased catalyst density, in vitro cross-linked methodologies have also
been explored to examine the oxidation of glucose utilizing a substrate channeling multi-enzyme cascade.

**BIOT 294**

**Exploring the viability and challenges of using biocatalysts for the synthesis of electrically conducting polymers**

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Growing interest in the use of conjugated polymers in organic photovoltaics, organic light-emitting diodes, chemi-resistant & fluorescence-based sensors is driving demand for new and innovative synthetic schemes. While there has been interest in green synthetic methodologies in other areas research, a safe and environmentally benign approach to synthesizing conjugated polymers has been traditionally overlooked. The use of biocatalytic enzymes (oxidoreductases) for the oxidative polymerization of conjugated monomers offers several advantages over traditional chemical oxidants. Oxidoreductases facilitate the synthesis to occur in an aqueous environment, in air, at moderate temperatures, and in special cases can offer enhanced specificity. In this presentation, we will review recent advances in the use of oxidoreductases for the polymerization of pyrrole, aniline, indole, and their derivatives. The role of redox potential in evaluating reaction feasibility and methods to influence the initiation of polymerization will be emphasized. A generalized discussion will be presented on the appropriate selection of materials and reaction conditions (pH, temperature, and hydrogen peroxide concentration) that are necessary for successful polymerization. Specific examples from a new class of biocatalytically synthesized derivatives of polypyrrole will be given.

**BIOT 295**

**Large volume, high density cell cryopreservation improves efficiency in biopharmaceutical manufacturing**

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Expansion of cells for biopharmaceutical manufacturing requires multiple weeks in the production facility, and variability in this process disrupts scheduling of manufacturing operations. Cryopreservation of sufficient cells to inoculate a seed bioreactor would allow for decoupling of early and late expansion stages. Methodology will be presented on the cryopreservation of large volumes of high cell density CHO cells followed by inoculation of lab-scale bioreactors. Logistics for implementing this procedure in a GMP facility will be presented, including freezing container types, storage temperature, and thaw methods. This approach shortens the time from thaw to harvest by two weeks, decouples seed train variability from the bioreactor stages, and allows for maximum manufacturing scheduling flexibility.

**BIOT 296**

**Transitioning from an established peptone-containing process to a chemically defined process: A case study**

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Chemically defined (CD) media are becoming increasingly desirable for monoclonal antibody (MAb) production in mammalian cell culture. A project was undertaken to transition an established animal-based peptone-containing process into a CD process. Initial results were discouraging, as both growth and titer decreased by 50%. Several standard process modifications were implemented (e.g., production temperature and batch feed timing), but the greatest productivity improvement came from increasing production culture inoculation densities while minimizing inoculum train carryover. After proof of concept studies using media exchange, process modifications were implemented which enabled increased growth in the inoculum train and, ultimately, decreased medium carryover. These modifications include addition of a media supplement that accelerated cell growth, and increasing both the seeding densities and growth periods in the inoculum train. The net result was a process that outperformed the productivity of the established peptone-containing process, and delivered 2.5X the growth and titer of that achieved with the platform CD process.

**BIOT 297**

**Evaluation of ethanol production from renewable cellulosic resources using process simulation tools**
Over the past three decades there has been intense investigation on the development of fuel producing processes that are based on the use of renewable agricultural materials as feedstock. This activity is driven primarily by the quest for fuel self-reliance and carbon oxides emission reductions. The main effort has been concentrated on bio-ethanol and bio-diesel which have been shown to give motor engine performance similar to that of conventional petroleum based fuels. In addition to product characteristics, however, process economics play an equally important role in any successful product commercialization. In this work, realistic process simulation models have been developed in order to analyze the economics of corn-stover to ethanol conversion. This presentation will illustrate how such models can guide R&D work and facilitate process optimization.

BIOT 298

Using simulation for bioprocess technology transfer and process-facility fit

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Technology transfer is, in large part, transfer of process knowledge from the development group to a manufacturing site. Because process simulation models represent a succinct, functional repository of process knowledge, they are a natural addition to the technology transfer process. This paper focuses on the capabilities and limitations of simulation models for technology transfer. Process-Facility fit is part of the technology-transfer process and is ultimately a determination of whether and how well a given process can be executed in a particular facility. Gap analysis is often the technique of choice for evaluating a facility. This presentation primarily focuses on the use of process simulation and scheduling tools to add quantitative information to gap analysis. A series of examples demonstrate how simulation can aid in determining gaps in primary equipment, buffer preparation equipment, purified water, utilities, labor and staffing. Techniques for managing uncertainties in process facility fit will also be covered.

BIOT 299

WITHDRAWN

BIOT 300
Functionalization of 5-hydroxyisophthalic acid via its phenolic hydroxyl group

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The primary focus of this research project is to synthesize a nanocrystalline organic/inorganic magnetic composite to be used as a drug carrier for targeted medical treatment. Dimethyl 5-hydroxyisophthalate (HIP), a derivative of isophthalic acid, is a suitable precursor in the synthesis of organic capping ligand and was reacted with Allyl Glycidyl Ether (AGE) at 100°C, without additional solvent, to produce a product capable of stabilizing an inorganic magnetic core in physiological fluids. In a nucleophillic addition the oxirane ring by the hydroxyl group of HIP, an HIP-AGE ester product was synthesized and then hydrolyzed to produce a functionalized isophthalic acid. To enhance the hydrophilic properties of the acid, the terminal vinyl group of the acid was oxidized using cold potassium permanganate to thereby produce a diol. The crude product yield of the unoxidized isophthalic acid was calculated based on the moles of HIP and the crude acid was recrystallized. The synthesis was successful and yielded 95% crude unoxidized acid and 84% unoxidized pure acid. Additionally, during the synthesis of the HIP-AGE ester, it was discovered that the nucleophillic addition to the oxirane ring takes place in the presence of a basic catalyst. Further testing of the ester synthesis was done by incorporating a 10%, 1%, 0.1%, and 0.05% induced basic contamination of HIP with NaOCH₃ before reacting HIP with AGE. The modified procedure, specifically in the 1% basic contamination of HIP, led to the production of a more highly branching structure of the ester, believed to be a derivation from a molecule of HIP reacting with more than one molecule of AGE. This was supported by the HIP-AGE ester spectra obtained from mass spectrometry. The structures of all intermediate and final products were supported by proton and C-13 NMR spectra.

BIOT 301

Particle distribution and cholesterol level as predictors of cell culture flocculation and filterability performance

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Recent advances in increasing monoclonal antibody titer using high density mammalian cell culture process have led to challenges in harvest operations (centrifugation and depth/sterile filtration). Cell culture flocculation has improved the performance of the centrifugation and depth filtration steps. Understanding
how these flocculating agents improve clarification efficiency is important to define a robust process. Thus factors that may be contributing to filter plugging such as particle size distribution and lipids were investigated. In general settled or centrifuged supernatants of flocculated cell culture have higher mean particle size compared to untreated cell culture fluid. Increased mean particle size consistently correlated with increased filterability. The increase in mean particle size was attributed to decreased counts in the lower particle size ranges indicating that these sizes are primarily responsible for filter fouling. Interestingly, increased filtration capacity was correlated to lower cholesterol level in the filter feed material regardless of flocculation method used. Therefore, cholesterol level may be promising marker in flocculation/filtration studies.

BIOT 302

PDADMAC flocculation of CHO cells: A centrifuge-less harvest process for mAb’s

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Recent advances in mAb cell culture processes have increased product titer while maintaining higher viable cell densities over longer culture duration. A corresponding exponential increase in the density of non-viable cells and cellular debris is also observed. This higher burden of cells and cellular debris can exceed the capabilities of the typical centrifugation followed by depth and membrane filtration harvest process. Addition of the polycationic polymer Polydiallyldimethylammonium chloride (PDADMAC) to the cell culture fluid flocculates negatively charged cells and cellular debris into larger particles which rapidly settles producing a supernatant with a higher depth/membrane filter throughput by decreasing the fouling agents. The floc sedimentation rate and the settled volume are dependent on temperature, time, cell and cellular debris density, PDADMAC size and concentration. Pilot scale PDADMAC flocculated harvest recovery of >90% was achieved without impacting product quality and stability. Clearance of PDADMAC for a mAb harvest and purification was demonstrated since it is known that both natural and synthetic polycations are toxic to all types of cells. PDADMAC was cleared from mAb to the assays LOD prior to the second column step. Detection was achieved via a cytotoxicity assay at 2 ppm and QPCR amplification inhibition at 5 ppb. A Protein A lifetime study
was performed and the impact of the load's PDADMAC will be discussed over the 100 cycled study

BIOT 303

**Fully disposable primary recovery step for harvesting mammalian cell culture systems: Technology comparisons and economics**

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The advent of highly productive cell-culture processes for the production of recombinant proteins has driven smaller batch processing that provides opportunity for transforming conventional bioprocess trains into single-use operations. We will be discussing a number of options for implementing a 'fully' disposable primary recovery step for harvesting recombinant proteins from high cell density cell cultures containing mammalian cells. We will highlight the pros and cons for implementing clarification technologies such as depth filtration, TFF, and centrifugation including discussion of experimental results and process economics.

BIOT 304

**Protease characterization in purification intermediates from CHO processes**

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Proteases are key regulators of a wide range of biological processes. In this study, zymography coupled with densitometry techniques were developed to identify and quantify proteases present in cell culture harvest and purification intermediates, in order to establish the normal protease patterns for different processes. Zymograms were customized for unpurified and purified intermediates. Visualized patterns on zymograms identified MMP-9, MMP-2 and MMP-12 as the most common proteases and determined process-specific protease levels and relative ratios. Matrix metalloproteases (MMPs), a class of zinc-dependent proteolytic enzymes, are responsible for the degradation of extracellular matrix proteins in healthy CHO cell cultures but may play a deleterious role by degrading monoclonal antibodies (mAb) during manufacturing. Protease spiking studies were performed to determine the impact on mAb stability with respect to fragmentation patterns.
BIOT 305

Exploiting insights to facilitate the integration of upstream and downstream

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Advances in titres and scales can make the downstream processing a bottleneck. This paper considers two complementary approaches researched at University College London (UCL) to resolve. In the first the creation of small scale process mimics now makes it possible to investigate process designs in a far more systematic and holistic fashion. An example might be pinpointing harvest point decisions from the combined views of titre but also ease of clarification. The result is better integrated processes with enhanced process robustness and performance. The second and more radical approach is to re-engineer the cell host to remove problematic downstream process elements ie removal of nucleic acids prior to process. This presentation will provide examples of both and highlight some of the challenges for the future as we move toward a position of better informed decision making equipped with new tools and methods.

BIOT 306

New purification concepts to address the interface between up- and downstream processing

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While upstream productivity has increased in line with demand, downstream processing is facing a disconnect. The accommodation of high titer processes in existing downstream suites is limited by issues around high biomass and contaminant levels. In this context the interface between up- and downstream processing, but also the contaminant removal steps later in the process, gain more and more importance. Process integration, disposable concepts, new enabling technologies and innovative solutions have to improve the productivity and efficiency of downstream processes significantly in order to address today’s challenges. During this presentation alternative ideas for early-on contaminant removal will be discussed.
Feasibility study to integrate perfusion cell culture processes to continuous downstream processing

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This work investigates the concepts, techniques and applications involved in moving toward a continuous downstream processing that can be linked seamlessly to a continuous cell culture process (perfusion cell culture) for a non MAb protein. The paper will examine the design and use of multi-column periodic continuous chromatography (PCC) system for target molecule capture, and use of disposable products such as depth filters, bags for buffers and process intermediates and the incorporation of membranes in place of resins for product polishing. Particular emphasis will be devoted to the experimental results on the capture PCC system. The PCC system will be compared to batch mode chromatography in respect to facility infrastructure, target molecule capacity, buffer usage and system productivity and processing time. An examination of target molecule recovery and purity between PCC and batch chromatography will also be conducted.

BIOT 308

Misfolded polymorphic amyloid ion channels present mobile beta-sheet subunits in contrast to conventional ion channels

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Truncated Alzheimer's disease (AD) amyloid beta-peptides resulting from alpha-secretase and BACE cleavage are often found in amyloid plaques of AD and in preamyloid lesions of Down syndrome. Using complementary techniques of molecular dynamics simulations, atomic force microscopy (AFM), channel conductance measurements, calcium imaging, neuritic degeneration, and cell death assays, we show that truncated amyloids, form heterogeneous misfolded ion channels with loosely attached subunits that elicit single-channel conductances. The subunits appear mobile, suggesting dynamic channel assembly and dissociation. Similar to the highly polymorphic oligomers and fibril aggregates observed in solution, Abeta channel morphologies in the lipid bilayer
are also polymorphic. All channels are ion-permeable, and their heterogeneous morphologies are consistent with AFM images. The emerging picture from our large-scale simulations is that toxic ion channels formed by beta-sheets spontaneously break into loosely-interacting dynamic units leading to toxic ionic flux. Funded by NCI Contract HHSN261200800001E and NIH (NIA) extramural program.

BIOT 309

**Thermodynamic model for amyloid fibril and oligomer formation**

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We present a model describing the equilibria between amyloid fibrils, soluble oligomers, and monomeric peptides. The free energy of beta structure formation is described using Zimm-Bragg-like propagation parameters, while the solution equilibrium is described in the grand canonical formalism. The model explains the extreme sensitivity of fibril formation rates to sequence and environmental perturbations, and predicts that the concentration of oligomers in equilibrium with fibrils will be equally sensitive. We compare our model to a broad range of experiments on the influence of peptide concentration, pH, salt concentration, and denaturants.

BIOT 310

**Paucity of amyloid nuclei defy isolation and toxicity evaluation**

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One of the main difficulties in characterizing amyloid oligomers, most likely the toxic species, is the fact that these entities are transient species present in very low concentration. Researchers have used different techniques and more recently have developed fluorescent methods to capture the intermediate oligomers. Here, we offer the first attempt to “reverse-estimate” the concentrations of nuclei, starting from a distribution of fibril lengths. Assuming the nucleation model is valid, with a few reasonable assumptions, a fibril length distribution and a set of seeding experiments, we estimated the in vitro concentration of nuclei for the model hormone, recombinant human insulin, to be in the picomolar range. Because of their propensity to form aggregates (non-
ordered) and fibrils (ordered), this very low concentration could explain the difficulty in fractionating, isolating and blocking nuclei toxicity. Moreover, this theoretical approach, based on our measurements and a structural fibril model recently published by others, is general and could be used for other amyloid proteins.

BIOT 311

Unmasking structures and structural dynamics in dimers of amyloid-β peptide

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Alzheimer's disease is linked to the self-association of amyloid-β peptide (Aβ), a protein of 39-43 amino acids that is found in brain plasma and cerebrospinal fluid. Historically, research focused on the Aβ fibrils found in extracellular senile plaques; however, recent evidence has linked oligomers as small as dimers and trimers to disease symptoms, including impaired long-term potentiation and synapse loss. We have used Förster resonance energy transfer (FRET) to investigate structures in dimers of Aβ40, both in bulk solution and in single, surface-tethered species. In both cases, we observe two characteristic FRET efficiencies, which may represent two preferred dimer structures; a fraction of single dimers exhibit time-dependent changes in FRET efficiency indicative of structural dynamics. Comparisons will also be made to Aβ40 dimers in the presence of zinc. Collectively, these empirical insights into oligomer structures may lead to new approaches for the prevention and treatment of Alzheimer's disease.

BIOT 312

Aromatic small molecules remodel toxic soluble oligomers of amyloid β through three independent pathways

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In protein conformational disorders such as Alzheimer's disease, substantial evidence suggests that prefibrillar oligomers are the most toxic aggregated species. To what extent such oligomers can be selectively targeted and remodeled into non-toxic conformers using small molecules is poorly understood. We have evaluated the remodeling pathways employed by a diverse panel of
aromatic small molecules against toxic oligomers of the Aβ42 peptide associated with Alzheimer's disease. We find that small molecule antagonists can be grouped into three classes based on the distinct pathways they utilize to remodel Aβ oligomers into multiple conformers with reduced toxicity. In the presentation, we will discuss our characterization of each remodeling pathway and the conformational specificity of aromatic small molecules for remodeling toxic oligomers relative to other Aβ conformational variants. Unexpectedly, we find that relatively subtle differences in small molecule structure encipher dramatic differences in the pathways they employ to remodel toxic Aβ soluble oligomers.

BIOT 313

Modulation of amyloid-beta aggregation using a new family of small molecules

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Alzheimer's disease (AD) is the most common form of dementia. The accumulation of insoluble amyloid aggregates, composed primarily of the neurotoxic amyloid-beta peptide, is a hallmark of AD. Although a soluble monomeric amyloid-beta is not toxic, amyloid-beta oligomers are known to be the primary toxic species. Therefore, reduction of amyloid-beta oligomers is considered a promising therapeutic strategy to reduce neurotoxicity associated with amyloid-beta oligomers. In the search for safe, effective aggregation modulators, we screened a family of compounds that already demonstrated safety profile. One molecule identified reduced oligomers by promoting fibril formation. Furthermore, its analog, which is known to cross blood brain barrier, substantially slowed down formation of amyloid-beta oligomers. Modulating effect of these small molecules on amyloid-beta neurotoxicity is being evaluated. Ultimately the small molecules identified are expected to serve as drug leads to cure AD.

BIOT 314

Aggregation kinetics of interrupted polyglutamine peptides

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Abnormally expanded polyglutamine domains lead to aggregation and are associated with at least nine neurodegenerative diseases, of which the best
known is Huntington’s disease. Studies of synthetic polyglutamine peptides have confirmed that residues surrounding or interrupting the glutamine region modulate the aggregation process. In this study, interrupting residues were inserted into polyglutamine peptides containing 20 glutamines, and the impacts on conformational and aggregation properties were examined. A peptide with 2 alanine residues displayed conformations and aggregation properties which were similar to the uninterrupted Q20 peptide. Insertion of 2 proline residues resulted in soluble, nonfibrillar aggregates. Insertion of a β-turn template, consisting of βPG, rapidly accelerated aggregation and resulted in an aggregate morphology lacking the lateral alignment between fibrils observed in the uninterrupted peptides. The data support a mechanism of polyglutamine aggregation by which monomer collapse drives formation of soluble oligomers which undergo slow structural rearrangement to form sedimentable aggregates.

BIOT 315

Missense mutations in N-terminal actin binding domain of dystrophin that trigger muscular dystrophy decrease protein stability and lead to cross-beta aggregates

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A deficiency of functional dystrophin protein in muscle cells causes muscular dystrophy (MD). More than 50% of missense mutations that trigger the disease occur in the N-terminal actin binding domain (N-ABD or ABD1). We examined the effect of four disease-causing mutations - L54R, A168D, A171P, and Y231N - on the structural and biophysical properties of isolated N-ABD. Our results indicate that N-ABD is a monomeric, well-folded alpha-helical protein in solution, as is evident from its alpha-helical circular dichroism spectrum, blue shift of the native state tryptophan fluorescence, well-dispersed amide crosspeaks in 2D NMR 15N-1H HSQC fingerprint region, and its rotational correlation time calculated from NMR longitudinal (T₁) and transverse (T₂) relaxation experiments. Compared to WT, three mutants - L54R, A168D, and A171P - show a decreased alpha-helicity and do not show a cooperative sigmoidal melt with temperature, indicating that these mutations exist in a wide range of conformations or in a ‘molten globule’ state. In contrast, Y231N has an alpha-helical content similar to WT and shows a cooperative sigmoidal temperature melt but with a decreased stability. All four mutants experience serious misfolding and aggregation. FT-IR, circular dichroism, increase in thioflavin T fluorescence, and congo red absorption spectral shift and birefringence show that these aggregates contain intermolecular cross-beta structure similar to that found in amyloid diseases. These results indicate that disease-causing mutants affect N-ABD structure by
decreasing its thermodynamic stability and increasing its misfolding, thereby
decreasing the net functional dystrophin concentration.

BIOT 316

Metabolic pathway databases for 1,000 organisms

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Comprehensive knowledge of metabolic pathways is required in a variety of applications including biofuels and drug discovery. The BioCyc family of 1,000 Pathway/Genome Databases (PGDBs) exists for organisms with sequenced genomes. Common to these PGDBs is their derivation from the MetaCyc PGDB. MetaCyc contains 1,400 experimentally elucidated metabolic pathways that were curated from 21,000 publications. Also common to these PGDBs is the Pathway Tools software, which contains a large suite of algorithms for manipulating biological networks and genome data. Pathway Tools includes inference modules for inferring the metabolic pathways of an organism, and for predicting which genes fill missing reactions in the predicted pathways. It includes extensive visualization tools for individual metabolic pathways and for complete metabolic networks. Pathway Tools contains algorithms for systems biology analyses of metabolic networks, including detection of dead-end metabolites and reachability analysis.

BIOT 317

Metabolic flux analysis of the central metabolism of laboratory and industrial \textit{Saccharomyces cerevisiae} strains using \textsuperscript{13}C-labeling experiments

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The central metabolism of \textit{Saccharomyces cerevisiae} is highly regulated in a cooperative manner. The metabolic responses to exogenous metabolic pathway including cell growth and substrate utilization depend on the metabolic functionality of wild type strain. Recombinant yeast strains expressing identical xylose catabolic pathway show differences in cell growth, xylose uptake, and ethanol production. To elucidate the metabolic background conferring those differences, the central metabolism of a laboratory strain and an industrial strain are compared by determining metabolic fluxes. Two yeast strains were grown on
$^{13}$C-labeled substrate and flux distributions were calculated by simulating isotopomer distributions and fitting simulated mass isotopomer distributions to those obtained experimentally. These two yeast strains showed distinctive flux distributions and partitioning in the central metabolic pathways. These results combined with the strain-dependent rearrangement of flux distribution in recombinant *Saccharomyces cerevisiae* would provide valuable metabolic engineering strategies to create recombinant yeast strains efficiently utilizing xylose for bioethanol production.

**BIOT 318**

Measuring carbon flow patterns in a marine diatom and its implications for metabolic engineering

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Diatoms are unicellular photosynthetic microorganisms that contribute over 20% of global photosynthetic CO$_2$ fixation and also contain abundant lipid reserves. Therefore, understanding carbon flow patterns in diatom metabolism has implications on metabolic engineering for improved CO$_2$ fixation and biofuel production. *Phaeodactylum tricornutum* is a model diatom whose sequenced genome reveals that its biochemistry may be unusual compared to other algae or plants; however, this biochemistry remains uninvestigated. Toward this goal, we performed isotope-assisted metabolic flux analysis on *P. tricornutum* by supplying combinations of $^{13}$C/$^{12}$C carbon sources, measuring isotope labeling patterns by mass spectrometry and NMR and employing metabolic modeling to quantify fluxes from the labeling patterns. Interesting metabolic flux patterns have surfaced from this study, including an atypical path traced by CO$_2$ through central carbon metabolism to lipids. We anticipate the knowledge generated from this investigation to be pivotal towards developing metabolic engineering strategies for *P. tricornutum* and other diatoms.

**BIOT 319**

Application of orthogonal biochemical methods to understand metabolic changes that occur in CHO production cultures

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Growth of transformed mammalian cell lines, including CHO, is associated with overflow metabolism and production of metabolites such as lactate and ammonia which can be detrimental to performance and productivity of cells in fed-batch production bioreactors. Distinct metabolic stages consisting of lactate production, subsequent lactate consumption, and mAb production are observed in typical CHO fed-batch production cultures. Reports in the literature indicate that majority of the lactate in transformed cells is generated from glucose. Our studies using media supplemented with $^{13}$C labeled metabolic tracers revealed alternative pathways for lactate production and changes in the dynamics of intracellular glucose metabolism during fed-batch productions. These results were corroborated by our results on the regulation and genetic manipulation of pyruvate kinase in fed-batch productions. We will discuss our findings using orthogonal methods and approaches to probe cell metabolism for the identification of new regulatory mechanisms and targets for modulation of cell growth and productivity.

**BIOT 320**

How to tie a peptide knot

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Lasso peptides are short peptides (~20 aa) produced by bacteria that have a unique knotted topology in which the N-terminus of the peptide forms an isopeptide bond with a glutamic acid or aspartic acid sidechain. The C-terminal "tail" of the peptide is fed through the resulting macrocycle resulting in the characteristic lasso structure. Lasso peptides have tremendous resistance to chemical and thermal denaturation and exhibit resistance to most proteases making this scaffold attractive for therapeutic and diagnostic uses. This talk will focus on work in my group exploring the metabolic engineering of lasso peptide production and the engineering of the lasso peptide framework. In addition, basic studies aimed at understanding the folding of lasso peptides and elucidating the biosynthetic pathways bacteria use to make these peptides will be discussed.

**BIOT 321**

Rapid characterization and engineering of natural product biosynthetic pathways via DNA assembler

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The majority of existing antibacterial and anticancer drugs are natural products or their derivatives. However, the discovery and sustainable production of these compounds are often hampered owing to limited ability to manipulate the corresponding biosynthetic pathways. Here we report a genomics-driven, synthetic biology-based method, PANDA (Pathway chAracterization and eNgineering via DNAAssembler), for discovery, characterization, and engineering of new natural product biosynthetic pathways. By taking advantage of the highly efficient yeast \textit{invivo} homologous recombination mechanism, this method synthesizes the entire expression vector containing the target biosynthetic pathway and the genetic elements needed for DNA maintenance and replication in \textit{Saccharomyces cerevisiae}, \textit{Escherichiacoli}, and a target heterologous expression host in a single-step manner. By using three distinct biosynthetic pathways, we demonstrate that PANDA offers unprecedented flexibility and versatility in pathway manipulations such as heterologous expression, introduction of point mutation(s), scar-less gene deletion and replacement, and generation of new products.

BIOT 322

Modulating bacterial signal processing through the directed evolution of the AI-2 global regulator protein, LsrR

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Metabolic pathway regulators act as molecular “tuners” that potentiate gene expression and enzyme activity in cells. By altering these regulators, essential cellular processes can be targeted for enhancement or attenuation. One such key regulator is LsrR, the repressor of the \textit{E. coli} bacterial communication, or quorum sensing, system. This repressor protein has been identified in many other bacterial species, and has been shown to control the expression of more than one hundred genes in \textit{E. coli}. Through the process of directed evolution, LsrR was engineered to have both altered substrate and DNA binding capabilities. A suite of novel LsrR proteins was identified through a dual screening process using a reporter plasmid containing the evolved \textit{lsrR} genes and the promoter region of the quorum sensing \textit{lsr} operon. This technique enabled us to examine the effects of evolved LsrR on the \textit{lsr}-mediated quorum sensing response. By engineering LsrR to respond to alternate substrates, and bind alternate DNA sequences, we will develop synthetic circuits that process extracellular signals to produce specific intracellular responses.
Production of straight-chained higher alcohols from renewable resources

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1-Butanol, an advanced biofuel and important chemical feedstock, has been traditionally synthesized by Clostridium species through acetone-butanol-ethanol (ABE) fermentation. Production of 1-butanol in recombinant Escherichia coli has been previously demonstrated via the threonine biosynthetic pathway, the citramalate pathway, and the CoA-mediated Clostridial 1-butanol pathway with the highest titer achieved at 1g/L from the threonine pathway. Characteristics associated with the native threonine pathway and the non-native citramalate pathway will be compared in terms of their oxygen demand, redox requirement, and long term production efficiency. On the other hand, a high-titer (15g/L), high-flux (0.1g/L/h/OD) production of 1-butanol, comparable to the levels demonstrated by native producers, from the Clostridial 1-butanol pathway driven by NADH accumulation in E. coli will also be discussed. These results demonstrate the feasibility of high-flux production of straight-chain alcohols using the CoA-mediated pathway.

Photochemical NAD(P)H regeneration for redox-enzymatic catalysis under visible-light

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Oxidoreductases can catalyze difficult redox reactions that conventional chemical catalysts are unable to catalyze. Despite the capability of oxidoreductases to catalyze complex reactions, they require a stoichiometric amount of cofactors that have two contrary states (i.e., oxidized and reduced). Numerous efforts have been made for in situ regeneration of cofactors from its consumed counterparts towards the practical application of oxidoreductases in industrial biosynthesis. Among the technologies developed for the cofactor regeneration, the photochemical route, mimicking the very idea of natural photosynthesis, provides an opportunity to utilize clean and abundant solar energy for artificial photosynthesis converting the solar energy into stored energy resources. However, the photochemical approach is still at its infancy, suffering from extremely low turnover frequency and synthetic yield due to the lack of efficient light harvesting components and energy transduction system. The present talk will discuss about our recent efforts for efficient visible-light driven photochemical
NAD(P)H regeneration coupled to biochemical transformations. Efficient cofactor regeneration was achieved through several organic dyes and inorganic materials and the photo/bio chemical energy transfer mechanisms were investigated. We anticipate that our studies for the photoenzymatic synthesis provides a foundation for developing efficient artificial photosynthetic systems driven by solar light.

Our Recent Publications Related to This Presentation:
5. C. B. Park et al., Chemical Communications 5423-5425 (2008).

BIOT 325

Enhancement of hydrogen production in recombinant Escherichia coli expressing [NiFe]-hydrogenase by introduction of light acceptor

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Hydrogen production in biological way can be one of solutions for energy crisis. In previous work, we demonstrated successful production of biohydrogen in recombinant Escherichia coli expressing [NiFe]-hydrogenase. Although recombinant [NiFe]-hydrogenase showed relatively high oxygen-tolerance property compare to other hydrogenases, hydrogen production efficiency was still low. In the present work, we introduced proteorhodopsin, light acceptor works as proton pump in prokaryote, in recombinant E. coli for utilizing light energy to produce biohydrogen. Proteorhodopsin need a chemical named retinal to work as proton pump by using light energy but E. coli does not have a synthesizing pathway for retinal. In order to give E. coli an ability to synthesize retinal, we expressed five foreign genes for retinal synthesis pathway. By introducing light accepting system into recombinant E. coli expressing [NiFe]-hydrogenase, biohydrogen production was enhanced.

BIOT 326

Control strategy for optimizing algal growth rates in open systems

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Temperature and dissolved inorganic carbon concentration (DIC) are two key variables that control algae biomass production in outdoor cultivation ponds. In this study, a turbidostat photobioreactor was used to independently determine the effect of temperature and DIC concentration on the specific growth rate ($\mu$) of a microalgal species (*Nannochloris*, UTEXLB1999). Although $\mu$ generally increased with increasing DIC and temperature, growth inhibition was observed when the DIC level reached 5.8 mM. At low temperature or low DIC, growth rates were less sensitive to increases in DIC or temperature, respectively. This suggests that control strategies should focus on moderate DIC and temperature scenarios. For typical water chemistry conditions (i.e. pH and alkalinity ranges), it was possible to control DIC through addition of CO$_2$ to the system. This strategy can be used to optimize algae productivity by holding DIC concentration within an optimal range.

**BIOT 327**

**Determination of metabolite concentrations in algae by non-aqueous fractionation**

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Transient labeling of cells with $^{13}$CO$_2$ is the critical experimental methodology in determination of metabolic fluxes under photoautotrophic conditions. Solving for the metabolic fluxes requires measurements of several metabolite concentrations and their respective isotopic labeling patterns. In eukaryotes, the presence of some metabolites in multiple compartments makes this determination problematic. We are using a technique known as non-aqueous fractionation which separates disrupted cells and their metabolites in a gradient of water immiscible organic solvents. After centrifugation, the fractions are assayed for compartment specific marker enzymes to determine the contribution of each subcellular compartment. Finally, the metabolites and the isotopic labeling patterns are quantified by liquid chromatography/mass spectrometry. We will present our findings of cell disruption techniques, choice of solvent and ratio for the gradient, and selection of marker enzymes and metabolites.

**BIOT 328**

**Temporal transcriptomics of Chlamydomonas reinhardtii during triacylglycerol accumulation**
Despite widespread interest in algae derived biofuels, little is known about the regulatory mechanisms that induce triacylglycerol (TAG) accumulation. It has been reported in a variety of algae that certain nutrient deficiencies, such as nitrogen, sulfur or phosphorous, cause the accumulation of TAGs. Therefore, we chose *C. reinhardtii*, a reference green alga, to investigate gene expression during nitrogen starvation induced lipid accumulation. Roughly 16% of the genes in the genome are regulated during nitrogen starvation, showing increases/decreases of 4 fold or more during a 48 hour time course. Some genes, such as the stress response genes *HLA3*, *LHCSR2* and *LHCSR3*, show dramatic changes in gene expression in just a few minutes after transfer to nitrogen deplete media. RNA-seq results from three time course experiments on different time scales (1 hour, 1 day and 3 days) will be presented and discussed.

**BIOT 329**

**Progress and challenges in algae biofuels research and commercialization**

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Microalgae are typically aquatic, photosynthetic microorganisms, many of which possessing the ability to produce substantial amounts (e.g. 20–50% dry cell weight) of storage neutral lipids/oils mainly in the form of triacylglycerols (TAG). Since TAG can be converted to biofuels, microalgae have been considered a promising alternative, renewable source of feedstock for biofuels. In this presentation, an overview of the current status of research on selection of high TAG-producing microalgal strains, the synthesis and regulation of TAG, and the effects of environmental and biological factors on cellular TAG accumulation will be provided. Production of algal feedstock by various types of mass culture systems will be assessed and the technical limitations associated with existing culture systems and processes will be described. Finally, the path forward for microalgae-based biofuels with respect to both challenges and opportunities will be discussed.
Algae-based production of ethanol: Direct to Ethanol technology

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Algenol Biofuels' DIRECT TO ETHANOL° technology has several advantages over competing methods for ethanol production. It does not compete with food, nor require arable land, nor require large amounts of fresh water. It consumes large amounts of carbon dioxide with a large positive net energy balance, leading to a substantial reduction in net greenhouse gas emissions compared to gasoline. The technology over-expresses the genes for fermentation enzymes in cyanobacteria. The metabolically enhanced hybrid algae carry out photosynthesis and utilize CO₂ to make ethanol inside each cell. The ethanol diffuses through the cell wall into the culture medium and then evaporates, along with water, into the headspace of an enclosed, sealed photobioreactor, and is condensed, collected as a liquid, and distilled into fuel grade ethanol. This presentation will cover the technology, life cycle analysis, and various projects underway to bring Algenol's DIRECT TO ETHANOL° technology to commercialization.

Cyanobacteria as biocatalysts for solar-driven biofuel production

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Traditionally algal biofuel largely involves extraction from biomass. We have changed this paradigm to have cyanobacteria secrete biofuel, which then can be collected without a need to harvest or significantly propagate the cyanobacteria themselves. This not only allows the cyanobacteria to put their energy into biofuel production, but also minimizes use of minerals and production of biomass waste. As a first example of this concept we have generated strains of Synechocystis sp. PCC 6803 that contain a thioesterase and that produce free fatty acids that subsequently are secreted into the medium. While optimization is still in progress, we demonstrate that native fatty acid biosynthesis in the cell can be diverted to free fatty acid production that is secreted, and fatty acids continue to
be produced even when stationary phase is approached. Produced fatty acids can subsequently be converted to n-alkanes by decarboxylation via the Centia™ process. This concept of photosynthetic microbes as biocatalysts producing secreted products independent of biomass growth represents an important step forward in economical production of biofuels from CO₂ and solar energy.

BIOT 332

Cellulase engineering from individual components to their complexes – cellulosome

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Biomass saccharification is still the last technical and economical obstacle to commercialization of second generation biorefineries. Complicated relationship between heterogeneous cellulosic materials and different action mode cellulase components results in great challenges in evaluating cellulase performance on solid substrates. We hope to develop recombinant cellulosolytic (Geo)bacillus strains that can co-express secretory glycoside hydrolase family 5 endoglucanase (Cel5), family 9 processive endoglucanase (Cel9), and family 48 cellobiohydrolase (Cel48), hydrolyze pretreated biomass with fast rate and high digestibility, and ferment hexoses and pentoses to desired biofuels or value-added chemicals in a single step. In this talk, we will talk about our latest progress for this goal: (i) simple, fast and high-efficiency transformation system for Bacillus subtilis so that cellulase mutants can be screened on pretreated solid cellulose; (ii) optimization of ternary mixture of Cel5, Cel9 and Cel48; (iii) construction of tri-functional mini-cellulosome containing Cel5, Cel9 and Cel48; and (iv) one-step lactate production from recombinant cellulolytic Bacillus subtilis.

BIOT 333

SCHEMA structure-guided recombination in engineering cellulase families for biomass-to-biofuel conversion processes

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We have demonstrated that SCHEMA crystal structure-guided recombination is an effective approach for creating families of industrially relevant fungal
cellulases from which application-specific enzyme mixtures can be formulated. A family of fungal cellobiohydrolase class II (CBHII) enzyme chimeras containing ~6,600 sequences was designed. A linear regression model for CBHII chimera thermostability was 100% accurate in predicting the sequences of 31 CBHII chimeras, which contain an average of 50 mutations, with stabilities greater than those of the parents from which the chimeras were constructed. Selected CBHII chimeras had broad pH activity profiles and hydrolyzed significantly more solid cellulose than their parental counterparts over long time intervals. Subsequently applying a modified chimera family design strategy to the recombination of cellobiohydrolase class I (CBHI) enzymes allowed prediction of what are likely the most stable sequences among a family of nearly 400,000 CBHI chimera sequences from just 28 sample set CBHI chimera thermostability measurements.

BIOT 334

Engineering chimeric cellulases for enhanced hydrolysis of solid substrates

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Fossil fuels are the primary source of energy worldwide. In addition to the environmental implications, a finite existence and an increased demand guarantee an unsustainable dependence on these sources. Cellulosic biofuels are a promising alternative. Cellulose is renewable, carbon neutral, and can be converted to a variety of liquid fuels with desirable properties. Biomass recalcitrance presents one of the largest barriers to the commercialization of cellulosic biofuels. To address this limitation, we have fused a library of natural cellulose-binding domains to the three most promising thermophilic catalytic domains at JBEI, with the purpose of enhancing the hydrolysis of real-world pretreated cellulosic substrates into fermentable sugars. Here we show the feasibility of our approach as well as promising results that demonstrate that the chimeras have enhanced activity over the catalytic domain alone. Further research will be directed towards the screening of large libraries of engineered chimeric cellulases.
BIOT 335

New screening method for cellulase engineering using in-vitro protein expression

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Efficient enzymatic hydrolysis of lignocellulosic biomass remains one of the major challenges to cost-effectively convert biomass to ethanol. Protein engineering and bioprospecting represent promising approaches to finding cellulases with improved properties under conditions of practical interest. A major bottleneck in screening cellulases is the development of efficient protein expression methods and techniques for rapid assay of proteins expressed from variant libraries. Conventional cell-based cellulase expression methods are time consuming and laborious. On the other hand, cell-free protein expression is an attractive alternative because of its flexibility and suitability for high-throughput automation. In this study, we developed a high-throughput expression and screening platform for cellulase engineering. This methodology was applied in metagenomics research and in directed evolution experiments to improve the specific activity of beta-glucosidase. The new method represents a powerful platform for high-throughput screening of cellulases and related enzymes.

BIOT 336

Engineering non-native intein-modified cell wall-degrading enzymes with controllable activity

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Inteins are self-excising protein domains that modulate the activity of their host enzyme. Agrivida utilizes this switching mechanism to control the activity of cell wall-degrading enzymes (CWDE), allowing them to be expressed in plants without detrimental impacts and reducing costs for downstream cellulosic biofuels production. Significant features of native insertion sites in their host enzyme were identified and used to predict non-native insertion sites in CWDE. These included local sequence conservation, local secondary structure, and the proximity to the active site and binding interface. These properties were used to
rank order non-native insertion sites in CWDE's predicted to have the most native-like switching properties. Several intein-modified CWDE were developed that showed intein dependent switchable activity. The insertion sites of the successful designs were enriched with the features of native intein insertion sites. The results suggest that the predictive models improve our ability to screen for intein-modified CWDE's with native-like switching.

BIOT 337

Synthetic “+1” metabolic pathway for biosynthesis

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Biological synthesis of chemicals is limited by the length of carbon backbone of the metabolites cells can produce. Here we report the engineering of a “+1” metabolic pathway to extend the carbon chain of keto acids. This biosynthetic module enables the synthesis of high volume industry chemicals such as 1-heptanol and 1-octanol directly from glucose. The success of this module to catalyze 5 elongation cycles involving 25 chemical reaction steps relies on engineering the gatekeeper enzyme to have similar catalytic efficiency towards C4-C8 substrates and the remarkable promiscuity of downstream enzymes. The “+1” chemistry developed is analogous to the “+5” chemistry for biosynthesis of isoprenoids and “+2” chemistry for biosynthesis of fatty acids and polyketides used in nature. This result demonstrates the plasticity of the keto acid pathways and provides a useful tool to biosynthesize nonnatural keto acids and their derivatives.

BIOT 338

Engineering a thermostable dehydrogenase to utilize biomimetic cofactors to improve enzymatic biofuel cell performance

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Dehydrogenase enzymes are commonly used in a wide range of biotechnology applications, including chemical synthesis and bioelectrocatalysis. These enzymes typically require a nicotinamide cofactor, which is responsible for shuttling electrons between the enzyme active site and the electrode surface.
However, these natural cofactors are not optimal for use in immobilized systems due to their high cost and low stability. Thus it is desirable to utilize alternate biomimetic cofactors which are cheaper, and have a size, stability, and redox potential optimized for the application. In this work, we have engineered the cofactor binding pocket of a thermostable alcohol dehydrogenase to improve activity with biomimetic cofactors. The enzyme was used to construct a bioanode capable of oxidizing D-arabinose, and is the first example of an enzyme utilizing a minimal cofactor for bioelectrocatalysis. Additionally, a novel directed evolution selection technique has been developed to identify enzymes with desired cofactor and substrate specificities.

**BIOT 339**

**Development of the radical-robust *Coprinus cinereus* peroxidase by blocking suicide pathway to improve the polymerization of phenolics**

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The peroxidase has been commonly used in many biotechnology fields but such potentially applications are limited because peroxidase loses activity with a mechanism-based (suicide) mode. Our study elucidated that the formation of a covalent bond between *Coprinus cinereus* peroxidase (CiP) and the phenol could be a major factor in the inactivation of CiP. Mass spectrometry analysis of inactivated CiP revealed that amino residue F229 was modified by phenol through phenol oxidation reaction. Among F229-mutant CiP, F229A led to a peroxidase with 70 times the residual activity and showed 14-fold higher turnover capacity than that of wild-type. As a modeling result, removal of F229 residue at the entrance of the enzyme led to greater resistance against phenoxyl radical interaction. The lower HOMO energy and binding free energy of F229A with phenol supported this result. Therefore, F229-mutant CiP has the radical-resistant and can be efficiently used in the polymerization of various phenolics.

**BIOT 340**

**Systematic approach for novel molecule purification process development**

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Compared to mAbs, there is a lack of platform knowledge that can be drawn on for early stage process development of novel protein biopharmaceutical products. This leads to less predictable timelines and may place drug supply manufacture on the critical path for entry into the clinic. This paper describes a systematic approach to novel molecule purification process development. Protein structure-function relationship is used to identify quality and stability indicating attributes to guide process development. Conventional and custom made separation matrices are systematically investigated and combined in a rational sequence to successfully achieve process development goals at both the laboratory and manufacturing scale. Case studies presented include a refolded recombinant protein and a single chain glycoprotein. Purification problems encountered include aggregate removal and the separation of product related charge variants. Also discussed are different approaches to viral clearance challenges, for example instability at low pH, more typically encountered with novel molecules.

BIOT 341

Chromatographic immunoaffinity displacement of Factor IX Gla isoforms

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In recombinant human coagulation factor IX (rhFIX), complete γ-carboxylation of 12 glutamic acids (Glu) in the Gla domain is a known challenge, which is addressed in the course of development of a glycoPEGylated variant. Separation of partially γ-carboxylated species Gla_{30,33,36,40}Glu, Gla_{33,36,40}Glu,Gla_{36,40}Glu and Gla_{40}Glu and fully γ-carboxylated rhFIX (i.e. Gla^8 - Gla^12) was performed using a resin immobilized Gla-directed antibody. Enrichment of Gla^{11} and Gla^{12} was accomplished by frontal column overload leading to displacement of lower γ-carboxylated isoforms Gla^8, Gla^9 and Gla^10. Even a modest overload of ~120% resulted in a significant enrichment of the Gla^{11} and Gla^{12} with a high yield of ~95%. Incremental calcium dependence for the antibody with K_{0.5} of 2.9±0.1 mM, 1.72±0.01 mM, 0.98±0.03 mM, 0.81±0.01 mM and 0.65±0.04 mM for Gla^8, Gla^9, Gla^{10}, Gla^{11} and Gla^{12} was observed. Decreased calcium-dependent cooperativity was observed for Gla^8 and Gla^9 with Hill coefficients of 1.5±0.0 and 2.15±0.02, while Gla^{10}, Gla^{11} and Gla^{12} all displayed a high cooperativity with Hill coefficients of ~3.2. Molecular dynamic simulations indicated that the ω-loop of the partially γ-carboxylated isoforms becomes unfolded compared to the fully γ-carboxylated isoform Gla^{12}. The method has been implemented in production scale for
development of a long-acting glycoPEGylated rhFIX variant with longer t½ than marketed FIX-products.

BIOT 342

Single-step purification of a PEGylated protein

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A single-step column chromatography method was developed to purify process and product-related impurities (aggregates, multi-PEGylated protein, low molecular weight impurities, unmodified protein, un-reacted mPEG, and a reductant) for a PEGylated protein. This was accomplished by first screening various chromatography resins (i.e., hydrophobic interaction chromatography, size-exclusion chromatography, and cation-exchange chromatography (CEX)), and ultimately choosing CEX for purification. Following further CEX resin screening and selection, input parameters were screened for those that affect product critical quality attributes (CQAs) and yield using a design of experiment (DOE) approach. After elimination of those parameters that did not impact CQAs and yield, broader ranges were explored for those parameters that control CQAs and yield to find edges of failure. Based on these findings, ranges and action limits for key and critical control parameters were established for manufacturing.

BIOT 343

Application of QbD and PAT methods to an *E. coli* protein purification process development project

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Quality by Design (QbD) and Process Analytical Technology (PAT) are receiving a lot of attention in biopharmaceutical and biotechnological industries subsequent to the regulatory guideline. Biopharmaceuticals are complex, which translates into complex manufacturing processes. QbD, when properly applied during process development, yields a process map to get from raw material to finished product. PAT can potentially act as a guide, e.g., like a GPS, to successfully reach the destination (final drug substance manufacturing process). These methods were utilized to accelerate the downstream process development of an *E. coli* protein. The paper discusses the implementation in downstream process development during tangential flow filtration and chromatography steps.
functioning to remove impurities and maximize overall recovery of active protein (drug substance) for an early stage manufacturing process.

**BIOT 344**

Downstream processing for monoclonal antibodies produced in *Pichia pastoris*: Challenges and solutions

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Because of differences between glycoengineered *Pichia Pastoris* and mammalian cell culture, monoclonal antibody purification from *Pichia Pastoris* has demonstrated some unique challenges and required process modifications to the platform processes previously developed for mammalian cell culture. Due to significantly higher cell densities reached during fermentation, some of the less common primary recovery approaches such as EBA and ATPS gained interest and were shown to be effective in addition to modified schemes involving centrifugation and depth filtration. Product specific antibody quality issues around fragmentation and glycosylation were experienced as well. The use of mixed mode chromatography, further evaluation of Protein A and cation exchange chromatography operating conditions were shown to be effective in dealing with the impurity profile. This presentation will highlight some of these process challenges and their resolution.

**BIOT 345**

Application of Ambrx's EuCode™ technology on making homogeneous ADC drug product: Case studies of downstream process development

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Antibody-drug conjugates (ADCs) are potent cytotoxic drugs linked to antibodies through chemical linkers, and allow specific targeting of drugs to neoplastic cells by combining the unique targeting feature of mAbs with the cancer-killing ability of cytotoxic drugs. It is now very well known that the chemistry of coupling mAbs to cytotoxic drugs as well as an accurate control of the payload to protein ratio are crucial to a successful drug candidate with desirable safety and therapeutic function profiles. Ambrx's technology, which has been successfully proven at clinical stage for non-ADC products, also offers unique advantages in the ADCs field in terms of site-specific drug conjugation and precise control of payload per molecule. Ambrx's EuCode™ technology will be presented in detail as to its
superior application in making fully controlled and homogeneous ADC products. Case studies on development of purification process for making ADCs with high efficiency and purity, especially strategies on removing free drugs post conjugation will be presented. Additionally, in vitro and in vivo data will also be presented to demonstrate the impact of conjugation site, type of drug and payload to protein ratio on ADC potency and pharmacokinetics.

BIOT 346

Purification of vaccine antigens expressed in *Pseudomonas fluorescens*

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In recent years, development of vaccines has attracted increasing attention, with launch of several blockbuster vaccines. Threats of pandemics and bioterrorism, and the need to reduce healthcare costs, has resulted in increased interest in this area from government agencies. Microbial expression of some vaccine antigens/carrier proteins results in inability to express properly folded full-length antigen and/or degradation resulting in production of undesirable fragments, in many cases due to presence of proteolytically-sensitive sites required for the protein's natural function. Pfēnex Expression Technology™ employs a toolbox of defined strains containing custom-designed combination of genetic elements, including a broad array of protease-deficient strains to minimize proteolytic degradation. High level expression of a broad array of vaccine antigens/carriers has been achieved. Results from strain engineering, purification process development and analytical characterization would be presented. Unlike platform processes commonly used for antibody purification, the diversity of vaccine antigens requires purification processes tailored to each protein. Proper choice of strains, use of automated resin/parameter screening and conventional purification sequence of ion exchange capture followed by HIC was able to result in highly pure full-length antigenic -proteins.

BIOT 347

Purification and characterization of glycoproteins expressed in the PER.C6® human cell line

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Mammalian cell lines are a widely used expression system for the production of monoclonal antibodies and other recombinant proteins due to their capacity for proper post-translational modifications and protein folding. These attributes create a biologically similar protein to the naturally occurring protein and may lead to comparable \textit{in vitro} and \textit{in vivo} properties. PER.C6® cell lines are human cell lines that have recently gained attention due to the high expression levels of recombinant monoclonal antibodies and for its good growth characteristics, e.g. greater than 27 g-IgG/L in an intensified cell culture process. We have successfully used these cell lines to also express various recombinant proteins at high levels and with desired quality characteristics. This presentation will describe our experiences in expression, purification, and characterization of recombinant proteins expressed in PER.C6® cell lines.

BIOT 348

**Novel classification system based on biophysical characterization for biotherapeutic aggregates**

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The characterization of protein aggregates as caused by various stress conditions is an essential step to understanding the properties, causes and potential safety issues of these particles. A variety of different stress conditions were applied to a monoclonal antibody (IgG\(_2\)) to yield a population of protein aggregates with varying morphologies. Aggregated solutions were biophysically characterized for percent aggregation, particle counts, size distribution, gross morphology, changes in secondary and tertiary structure and surface hydrophobicity, metal content, reversibility, and chemical modifications. Aggregates were classified into 7 discrete classes, based on identified properties, in order to assist with identifying attributes that pose potential risks. A second IgG\(_2\) molecule was tested for one stress condition in each class and found to be classified similarly. Class members generated by harsh mechanical stress showed the greatest number of subvisible particles, whereas classes treated with thermal stress displayed the largest number of visible particles. Most classes showed a disruption of the native secondary and tertiary structure, where the severity of the disorder depended on the stress method. The exception is high pH aggregates which retained a native-like structure and were highly reversible, suggesting that this condition affects the colloidal stability of the protein. Particles in all classes tested (except thermal stress) were found to be reversible over time upon dilution in pH 5 buffer. High copper content was detected in metal catalyzed aggregates, a stress technique previously shown to
produce immunogenic aggregates. These results demonstrate that protein aggregates can be a very heterogeneous population, whose properties depend on the exact conditions used to generate them. Classification of aggregate groups could help to develop protein formulation strategies as well as to identify the aggregate classes and attributes that pose problems in terms of safety (including immunogenicity) and efficacy.

BIOT 349

Mutational analysis of aggregation: Pitting conformational stability vs. predicted aggregation propensity

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γD crystallin is a 21 kDa, two-domain eye lens protein associated with hereditary cataracts that serves as a model aggregating system for multi-domain, β sheet-rich proteins. We have used this system to evaluate three strategies for reducing aggregation: stabilizing the least stable domain, stabilizing the domain-domain interface, or mutating aggregation-prone sub-sequences. The protein design algorithm, RosettaDesign, was implemented in combination with several aggregation calculators to predict various point mutations that would improve protein stability. The conformational stability and aggregation kinetics for several mutants were experimentally tested against the wild type using intrinsic fluorescence spectroscopy, hydrogen-exchange mass spectrometry, chromatography, and static light scattering. The computational tools have proven useful for identifying mutants with increased conformational stability and reduced aggregation. Kinetic modeling of the observed aggregation has been used to help parse the relative impact of conformational stability and predicted aggregation propensity on the resulting aggregation rates, and to evaluate the effectiveness of the different strategies for reducing aggregation in multi-domain proteins.

BIOT 350

Minimizing self-association: Application of conformational and colloidal stability analyses in developing an optimal formulation

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Reversible self-association (RSA) of protein therapeutics may have the potential to adversely impact drug product quality, long-term stability, manufacturability, product safety and efficacy. RSA in MabA detected by orthogonal techniques, including HPSEC, DLS and AUC, is characterized as: temperature, pH, and protein concentration dependent. An increase in the apparent MW, as confirmed by various size-distribution techniques, of the shoulder on the leading edge of the monomer peak indicates a transient species with rapid dissociation to the monomeric form following room temperature equilibration, followed by a slow reformation of the self-associated monomer at 2-8°C. This study demonstrates a comprehensive formulation approach involving high-throughput excipient screening and biophysical characterization, to develop an alternative formulation that not only minimizes the undesired reversible self-association but also meets acceptable stability criteria. Formulation factors influencing protein-protein interactions and colloidal and conformational stability will also be discussed.

BIOT 351

Artificial nucleating centers for studying aggregation mechanisms of monoclonal antibodies

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We are developing “artificial nucleating centers” (ANC) to understand mechanisms that drive particle formation of mAbs. The ANC’s were developed using gold nanoparticles (GNP) with a 20 nm diameter by either coating directly onto the GNPs or indirectly through ionic interactions after first coating the GNPs with 3-mercaptopropionic acid. Fourier transform infrared spectroscopy showed a significant loss in secondary structure for the directly coated mAb while the indirectly coated mAb retained the majority of its native structure. Stressing the system with NaCl induced aggregation in the directly coated GNP but had little effect on the system with indirectly coated mAb. This data provides evidence that the mechanism of protein aggregation is driven through nucleation centers containing structurally perturbed protein and that conformationally native protein is likely not involved in the aggregation process. Other methods for capturing partially unfolded mAb are being developed and correlated with their propensity to aggregate.

BIOT 352

Characterization of sub-visible and visible monoclonal antibody aggregates
As the number of FDA-approved monoclonal antibody therapeutics increases, the need to understand and control aggregation of these therapeutics becomes more critical. Aggregation of therapeutic proteins is a common phenomenon and can occur during bioprocessing, shipping, storage or delivery to the patient. Aggregation is a major concern because it is generally believed that aggregates are immunogenic and can cause adverse responses in patients. In this study we employ size exclusion chromatography (SEC) and electrospray differential mobility analysis (ES-DMA) to characterize nanometer sized aggregates, micro-flow imaging (MFI) and atomic force microscopy (AFM) to characterize sub-visible and visible macroscopic particles, and confocal fluorescence microscopy to determine the density and refractive index of sub-visible and visible macroscopic particles. Results are presented for four antibodies of the IgG class; a polyclonal antibody (IgG-A), the monoclonal antibody Rituxan (Rmab), and a monoclonal antibody that is glycosylated (IgG-B) and deglycosylated (IgG–C) in the Fc region.

**BIOT 353**

**Analysis of subvisible particles in protein therapeutics using Micro-flow Imaging**

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The subvisible particles that might be present in protein therapeutics have been identified by the regulatory agencies as a potential safety issue. Some of these particles can be highly transparent, fragile and unstable. In addition, for much of the size range of concern, no practical measurement method with adequate sensitivity and accuracy has been available. The need has therefore been identified for new analytical methods which can accurately measure these particle types. Micro-Flow Imaging (MFI) is one such technique that has been shown to provide improved sensitivity and characterization potential over a wide range of sample types. This paper will describe MFI technology and compare results to those obtained by light obscuration. Case-Studies will be presented showing how MFI is used to characterize protein formulations for the purposes of studying aggregate formation and isolating particle sub-populations of interest using MFI software filters.
BIOT 354

Use of divalent cations to mitigate viscosity in monoclonal antibodies

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High concentration formulations are required for the efficacy of the monoclonal antibodies especially in metabolic disorder treatments and for the comfort of patients subjected to high volume injections. In one of our therapeutic candidates there is a highly negatively charged amino acid sequence region which we have determined to be the core region which causes extremely high viscosities in formulations above 100 mg/ml. Using Molecular Modeling, amino acid substitution and empirical screening methods, we have determined that small amounts of divalent cations in our formulations can lower the viscosity by as much as 80% thus allowing high concentration formulation feasible for manufacturing and patient dosing.

BIOT 355

Elucidating the aggregation suppression mechanism of arginine and a novel class of additives

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Protein aggregation is arguably the most common and troubling manifestation of protein instability, occurring in almost all phases of development. The current approach toward stabilizing therapeutic proteins against aggregation is by adding to the solution a cosolute or additive which inhibits aggregation. One of the most common and most studied additives is arginine. Even though it has received much attention, its mechanism of action still remains unclear. Furthermore, better performing additives are highly desired. In response to this, we have explored the arginine mechanism and have developed and tested a new class of additives based on what we have discovered. Here we report what we have elucidated about the arginine mechanism and how our new additives perform in comparison to commonly used additives.

BIOT 356

Glucose valves: Tuning primary metabolism for heterologous production
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In engineering production pathways, competition between desired and endogenous processes frequently creates a bottleneck that limits productivity. Common flux manipulation strategies, however, are infeasible when the heterologous pathway competes directly with central metabolism. In this study, we have engineered a viable \textit{E. coli} host that decouples glucose transport and phosphorylation allowing us to independently control the flux of glucose through control of glucokinase (\textit{glk}) expression. Through the use of antisense RNA, we are able to inhibit \textit{glk} activity by up to 25\% and perturb central metabolism with an attendant increase in the specific productivity of a model glucose-consuming pathway. In this talk, I will compare these constructs with simple gene circuits as alternative strategies for creating an analog, tunable 'glucose valve'. Such devices allow for fine control of glycolytic flux and create novel opportunities for pathway optimization and development.

BIOT 357

Metabolic engineering to increase production of malonyl-CoA derived products

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As the demand for fuels and chemicals increases, their production from renewable feedstocks by fermentation becomes a more critical means of supplementing or even replacing traditionally petroleum-based products. Many commercial products may be derived from the core metabolic precursor, malonyl-CoA, including fatty acids (and hence long chain alkanes), polyketides including pharmaceuticals, and 3-hydroxypropionic acid (3-HP), a precursor of the industrial monomer acrylic acid. However, metabolic engineering is often required to construct microorganisms with the ability to produce compounds not normally the end-product of metabolism and to redirect metabolic flow towards the desired endpoint. We have engineered a microorganism for the production of 3-HP. A key focus of the strain development centered on increasing the cellular pools of malonyl-CoA, the first committed intermediate for the 3-HP production pathway. This engineered microorganism and bioprocess is capable of producing commercially relevant rates, titers, and yields of 3-HP, which enable the large scale production of biologically derived acrylic acid at production costs competitive with petroleum based sources.
BIOT 358

Biosynthesis of 3-hydroxy-γ-butyrolactone and 3,4-dihydroxybutyric acid in \textit{Escherichia coli} from glucose as a sole feedstock

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3-hydroxy-γ-butyrolactone (3-HBL), one of the top ten value added chemicals from biomass in a 2004 Department of Energy report, is a versatile chiral building block, frequently used in the pharmaceutical industry for synthesis of blockbuster drugs like Zetia®, most cholesterol reducing statins and antibiotics. Our group established the first complete biosynthetic pathway towards 3-HBL and its hydrolyzed form, 3,4-dihydroxybutyric acid (DHBA) in \textit{Escherichia coli} using glucose and glycolate as feedstocks. Glycolate itself may be synthesized from glucose in \textit{E. coli}, using the endogenous glyoxylate shunt, eliminating the need to supply it as a separate feedstock. Here we present work on the engineering and integration of this endogenous glycolate synthesis pathway with the 3-HBL and DHBA pathway in \textit{E. coli} and report for the first time the direct synthesis of 3-HBL and DHBA from glucose as a sole feedstock. This opens avenues for synthesis from other simple sugars derived from biomass.

BIOT 359

Production of heparin in Chinese hamster ovary cells by metabolic engineering

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Heparin (HP), a highly sulfated glycosaminoglycan (GAG), is the most widely used anticoagulant drug in modern medicine. A health crisis that took place in 2008 led to a demand for production of HP from non-animal sources. Chinese hamster ovary (CHO) cells are capable of producing heparan sulfate (HS), a related polysaccharide naturally. Since HP and HS share the same biosynthetic pathway, we hypothesized that HP could be produced in CHO cells by metabolic engineering. Human \textit{N}-deacetylase/\textit{N}-sulfotransferase (NDST-2) and mouse 3-
O-sulfotransferases 1 (3-OST-1) genes were transfected sequentially into CHO cells growing in suspension culture. Out of 60 clones expressing NDST-2, 14 cell lines were selected and a cell line (CHO-NDST2-1) which showed stable expression over 10 passages was used as a host cell line for 3-OST-1 transfection. Out of 120 clones expressing 3-OST-1, 54 clones were selected. Preliminary analysis of the GAGs from clones stably expressing NDST-2 and stable NDST-2 clones, transiently transfected with 3-OST-1, showed an increase in sulfation in the transfectants. Moreover, an antithrombin III (ATIII) binding assay using flow cytometry, designed to recognize a key sugar structure characteristic of HP, indicated that 3-OST-1 transfection was capable of increasing ATIII binding. Critical issues currently under investigation are the yields of HP and whether overexpression of HP core proteins and optimization of culture parameters will be necessary to obtain a sufficient yield of HP.

BIOT 360

Development and application of the Visualizing Evolution in Real-Time (VERT) method

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We have developed a novel adaptive evolution (Visualizing Evolution in Real-Time or VERT) method for the evolutionary engineering of microbial systems for complex phenotypes. With traditional evolutionary engineering, the evolution dynamics cannot be determined in real-time. VERT addresses this deficiency in evolutionary engineering and allows one to determine when a beneficial mutant arises in the population and helps to facilitate the isolation of beneficial mutants from the population for further analysis and characterization. We applied VERT to evolve E. coli for enhanced tolerance to the biofuel, butanol. Butanol is highly toxic to microorganisms, with most strains being able to tolerate up to 2% (v/v) of this organic solvent. Wild-type E. coli can tolerate up to approximately 1% (v/v) of n-butanol. We successfully evolved E. coli for enhanced butanol tolerance using VERT. The butanol tolerant mutants were isolated from the population and analyzed to elucidate the molecular mechanisms involved in tolerance.

BIOT 361

Vector set for systematic metabolic engineering in Saccharomyces cerevisiae

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A unique vectors series, designed to facilitate rapid and systematic combinatorial expression of pathway genes, was constructed for metabolic engineering in *Saccharomyces cerevisiae*. For differential expression of genes, six different promoters and six different reusable selection markers were included on both high and low copy plasmids. Unique restriction sites between promoter and terminator allow convenient insertion of gene cassettes. Furthermore, a fragment for integration into the genome can be readily generated via PCR, and markers can be recycled following cassette integration. We have fully characterized expression from these vectors using reporter genes. In addition, a group of specific genomic sites was replaced with the reporters and expression was comparable to that at standard integration loci. This expands the number of characterized loci and demonstrates that such sites provide an extensive pool of targets for stable expression of multiple pathway genes. Current metabolic engineering applications using these vectors will be described.

**BIOT 362**

**Alterning communication networks of multispecies microbial systems by engineering signal transduction**

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The bacterial cell-cell communication network or quorum sensing (QS) is mediated by signatures of small molecules. These molecules represent targets for “quenching” communication to prevent the emergence of virulent phenotypes. As environmental systems typically consist of a variety of bacteria and secreted or adsorbed proteins, polysaccharides, etc., we use the prevailing molecular cues as “beacons” to silence or “quench” the QS regulon in multispecies synthetic ecosystems. We have devised two strategies that interrupt bacterial communication in these ecosystems that target the interspecies signaling molecule autoinducer-2 (AI-2), which is thought to exert phenotypic control of over 70 species of bacteria. Our first approach is to bring the native intracellular
AI-2 signal processing mechanisms to the extracellular surroundings, specifically we deliver the AI-2 kinase, LsrK, to *Escherichia coli* populations *ex vivo* and phosphorylate the extracellular AI-2. This significantly attenuates the native QS response in *E. coli* and in a tri-species synthetic ecosystem. In our second quenching strategy, we have explored a panel of C1-alkyl analogs of AI-2. We have observed both species-specific and cross-species quorum quenching. Notably, we tested these in a quaternary synthetic ecosystem comprised of *E. coli*, *Vibrio harveyi*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*. Importantly, we examine the effects of these strategies on the specific intracellular pathways they are predicted to modulate. Our results suggest entirely new modalities for interrupting or tailoring the network of communication among bacteria and identifying drug targets.

BIOT 363

**Influence of Autoinducer-2 on *E. coli* swimming motility**

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Quorum Sensing (QS) signal autoinducer 2 (AI-2) is considered an universal regulator that mediates both intra- and inter-species bacterial communication. Most previous studies of AI-2-mediated motility, including our own, have examined swarming – or motility on or within semi solid agar substrates. Swimming analysis represents another mode of motility that is also reflective of the natural environment, particularly for *E. coli*. We analyze *Escherichia coli* swimming motility in a variety of genetic backgrounds and in the presence of a panel of AI-2 analogs. Specifically, we examined signal repressor (*lsrR*), kinase (*lsrK*), transporter (*lsrACDBFG*) and synthase (*luxS*) gene knockouts for cells grown to mid exponential phase. Motility responses were also compared in conditions with and without of ex-vivo AI-2 addition. Finally, effects of C1-alkyl analogs of AI-2: QS agonist methyl-DPD and antagonist isobutyl-DPD were quantified. AI-2 signal relay effects were analyzed by direct high speed video imaging wherein individual cell tracks were calculated and statistically quantified. We report AI-2 signaling effects in both trajectory persistence and overall cell speed distributions.

BIOT 364

**Investigation of deoxyribozymes as catalysts in biofuel cells**
Our goal is to develop novel biofuel cell catalysts using DNA. Catalytic DNA sequences called deoxyribozymes or DNAzymes offer the advantages of lower production costs and potentially higher stability compared to protein enzymes. We are using in vitro selection to identify new redox deoxyribozymes capable of catalyzing reactions with potential biofuels. Our current efforts towards identifying and characterizing these new catalysts will be discussed. We are also investigating a previously identified peroxidase deoxyribozyme as a biocathode catalyst, thus expanding the application of this deoxyribozyme from sensors to biofuel cells. Our progress on constructing this deoxyribozyme-modified bioelectrode and improving the electrochemical response will also be presented.

**BIOT 365**

**Direct electron transfer by multicopper oxidases: Application in biofuel cathode design**

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Multicopper oxidases (MCO) have been studied as catalysts for direct electro-reduction of oxygen to water in biofuel cells. In this study, three MCO (laccase from Trametes versicolor, bilirubin oxidase from Myrothecium verrucaria and ascorbate oxidase from Cucurbita sp.) were investigated. Protein film voltammetry and electrochemical characterization of the MCO electrodes showed that direct electron transfer had been successfully established with each MCO. The redox potential of the T1 center was strongly modulated by physiological factors including pH, anaerobic and aerobic conditions and the presence of inhibitors. Cyclic voltammetry (CV) was used to evaluate MWCNT electrodes conjugated with MCO. The CV results depicted obvious electrocatalytic activity for oxygen reduction with a Tafel slope of 18 mV/decade in the kinetic region above 0.57 V in agreement to the theoretical value for a four-electron-transfer reaction. The MCO electrodes were successfully employed in gas-diffusion, air breathing biofuel cell cathodes.

**BIOT 366**

**Mitoplast bioelectrocatalysis immobilized in modified nafion membranes**
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Among biocatalyzed fuel cells, there has been extensive research into enzymatic reactions, but little research has been devoted to mitoplasts in biofuel cells. Mitoplasts isolated from tubers using conventional methods of centrifugation and digitonin treatment were immobilized on a quaternary ammonium modified Nafion membrane on a carbon paper electrode to study the power produced through the oxidation of pyruvate in a pyruvate/air biofuel cell. Extensive characterization of samples was performed through spectrophotometric enzyme assays as well as TEM imaging before being immobilized onto the anode to be analyzed using electroanalytical methods against a 20% platinum-carbon cloth cathode. Studies included the effect of pH, temperature, and type of fuel using linear sweep voltammetry.

BIOT 367

Integrated experimental and modeling studies of extracellular electron transfer mechanisms in electrochemically-active biofilms

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Biofilms interact with their environment through redox reactions, consuming substrates and producing metabolic products. Many of these metabolic products are electrochemically-active, and are generally believed to play an important role in electron transfer processes in biofilms. As a special subset of biofilms, electrochemically-active biofilms (ECABs) utilize these redox reactions involving electrochemically-active products to transfer electrons to inert, conducting surfaces. ECABs are utilized in microbial fuel cells, biomineralization, and bioremediation of contaminated soils, although the use of ECABs apply to bioelectrochemical systems as a whole. Although many applications of ECABs have been studied, the mechanism by which electrons are transferred from the microorganisms has been less studied. This presentation focuses on extracellular electron transfer (EET) processes in ECABs and demonstrates the use of microelectrodes to understand electron transfer processes in biofilms. In addition, new proposed EET mechanisms are integrated to mathematical models. Modeling of ECABs redox activity is important for determining rate limiting steps in bioelectrochemical systems and for testing hypotheses, especially regarding electron transfer mechanisms. Two dominant theories have been proposed for the mechanism of transferring electrons from the microorganism to the inert electrode: direct electron and mediated electron
transfer. Currently, evidence exists for both theories and it appears that both mechanisms may each play a role in electron transfer. We have developed a mathematical model that considers direct and mediated electron transfer and have integrated it to micro-electrochemical environments observed experimentally within ECABs.

BIOT 368

Development of self-assembling enzymatic biomaterials for methanol oxidation to carbon dioxide in a biofuel cell

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Protein engineering provides a toolset to modify proteins for desired improvements. Recently, we have reported that enzymes can be rationally engineered to be bi-functional so that they retain their catalytic activity while gaining the ability to self-assemble into hydrogels. This is accomplished by adding alpha-helical leucine zipper domains to the termini of the proteins. In this study, we have extended this approach by engineering 3 dehydrogenase enzymes to form a hydrogel that supports a metabolic pathway. Three NAD\(^{+}\)-dependent dehydrogenases were modified for self-assembly: alcohol dehydrogenase, aldehyde dehydrogenase, and formate dehydrogenase. When combined these enzymes produce a synthetic metabolic pathway capable of the complete oxidation of methanol to carbon dioxide. These bifunctionalized enzymes were used to create an anode for an enzymatic biofuel cell which utilizes methanol as a power source. Examination of the power curves for the cell using different intermediates in the pathway as fuels demonstrates that all three enzymes are active in the mixed hydrogel. The power densities and current densities obtained from the biofuel cell will be compared to those obtained in a conventional biofuel cell configuration.

BIOT 369

Engineering and improvement of an electrode-attached biocatalyst as a platform for biofuel production

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Fermentations produce undesired endproducts, and respiration requires addition of electron acceptors. The use of electrode-based electron acceptors/donors to balance biotransformations or drive reductive reactions within the cell may
overcome these constraints. To test this hypothesis, *Shewanella oneidensis* was engineered to convert glycerol into ethanol. Multiple modules were combined to alter *S. oneidensis* metabolism: a glycerol module (*glpF, glpK, glpD*, and *tpiA* from *E. coli*), and an ethanol module (*pdc* and *adh* from *Z. mobilis*). Furthermore, an increase in product yields was accomplished via deletion of genes for acetate production (*ack, pta, ald, acs*) and serine/glycine pathway (*gcvT*). Electrode-attached biofilms oxidized glycerol to ethanol with 95% coulombic yield, and volumetric rates were on par with fermentation technologies using engineered *E. coli*. Linking microbial biocatalysis to electrodes can eliminate redox constraints by shifting unbalanced reactions to yield pure products and serve as a platform for next-generation bioproduction strategies.

**BIOT 370**

**Electrochemical and microbial properties of the biocathode of a solar microbial fuel cell (SMFC)**

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In a solar microbial fuel cell (SMFC), it is postulated that fuel and oxygen are regenerated internally from the electrode products by photosynthesis. To enhance power output and advance fundamental understanding of SMFCs, we analyzed by cyclic voltammetry and microbial community characterization biocathodes of SMFCs derived from a salt-marsh inoculum. These biocathodes exhibited a sigmoid-shaped catalytic current-potential dependency with a fairly positive midpoint potential of 0.200 V vs. Ag/AgCl that correlates with oxygen reduction. Voltammetry of SMFC biocathodes kept in darkness exhibit this same feature, indicating that non-photosynthetic members of the cathode community catalyze the electrode reaction. Subsequent 3-electrode electrochemical cells in which working electrodes were maintained at 0.100 V and inoculated from a biofilm of a SMFC biocathode enriched in darkness developed the same voltammetric feature with substantially higher limiting current density. 16S rDNA libraries revealed a non-photosynthetic, iron-oxidizing marine bacteria belonging to the gamma-proteobacteria dominated these biofilms.

**BIOT 371**

**On the conductive nature of biofilms of Geobacter sulfurreducens**

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The electrical conductivity and catalytic activity of biofilms of *Geobacter sulfurreducens* (DL1) grown across gold-on-glass interdigitated microsensor electrode microarrays has been studied. Biofilms between adjacent electrodes produced different current–voltage characteristics at the various stages of biofilm formation. Biofilm conductivity was characterized *in situ* following the first onset of catalytic current, during growth, and when fully grown; and under turnover condition (when acetate was present) and under non-turnover condition (when acetate was absent). The biofilms were also characterized by cyclic voltammetry, by small amplitude a.c. impedance spectroscopy and by scanning electrochemical microscopy. The results are used to further expand understanding of the nature of biofilm conductivity and its role in biofilm electrode-catalytic activity. We have also studied the conductivity of DL1 biofilms through the incorporation of an exogenous redox enzyme to explore the ability of the biofilms to transport electrons from the enzyme to the underlying electrode.

**BIOT 372**

**Yeast-based high-throughput cellulase expression and its application in DNA family shuffling**

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Conversion of lignocellulosic crops to biofuels may provide an alternative to fossil fuels. A major cost associated with ethanol production arises from the enzymes used to degrade cellulosic biomass to glucose. Thus, there is strong incentive to increase catalytic activity to reduce enzyme loading and to increase thermostability to allow a higher process temperature and enzyme recycle. However, cellulase engineering has been hindered by difficulties associated with enzyme expression and with screening a library of mutants on a solid lignocellulosic substrate. This work enhances the recombinant expression of the cellulase Cel7A in *Saccharomyces cerevisiae* by upregulating the protein disulfide isomerase and removing the Golgi membrane ATPase Ca²⁺ and Mn²⁺ pump PMR1. This strain was applied in the DNA family shuffling of 11 Cel7A genes that were synthesized to improve nucleotide homology. For each round of evolution, 10,000 hybrids from the library were screened for improved thermostability using a roboticized system.

**BIOT 373**
Microbial synthesis of Xylitol from hemicellulose hydrolysates

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Xylitol is used as a natural sweetener in food, pharmaceutical, and confectionery industries and is also considered as one of Department of Energy’s top 12 platform chemicals for biorefinery. The current processes for xylitol manufacture, based on either chemical synthesis or fermentation, all rely on the use of pure D-xylose as a feedstock, resulting in relatively high cost of production. To address this limitation, we first cloned and characterized a novel xylose reductase from Neurospora crassa and used protein engineering to create a xylose reductase (XR) mutant with decreased specificity toward L-arabinose, while maintaining its high activity toward D-xylose. Then we used metabolic engineering to create an E. coli strain containing the engineered XR to efficiently produce xylitol from D-xylose with minimal production of L-arabinitol byproduct. Notably, we were able to eliminate L-arabinitol formation and produce xylitol to near 100% purity from an equiweight mixture of D-xylose, L-arabinose, and D-glucose.

Directed evolution of non-natural ligand-activated enzymes

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A protein switch uses an input signal such as ligand concentration to modulate its output function (e.g. enzyme activity). Protein switches have potential applications as biosensors and selective protein therapeutics. We have constructed ribose- and glucose-activated beta-lactamases through the fusion of periplasmic binding proteins and circular permuted variants of TEM1 beta-lactamase. We have explored random mutagenesis and cassette mutagenesis to improve the properties of these proteins. In addition, we have introduced mutations designed to alter the identity of the activating ligand for these switches. The implications of these studies for protein switch design rules will be discussed.

Solution dynamics of monoclonal antibodies: Experimental and computational approach
Recombinant humanized monoclonal antibodies are a major class of protein therapeutics. It has been shown that the efficacy of these multi-domain macromolecules can be affected by changes in their flexibility defined by the hinge region (a linker connecting Fab and Fc domains). We are using time-resolved fluorescence anisotropy in conjunction with molecular dynamics simulations (MD) to probe antibody solution properties. In the experimental part of this study, we conjugate fluorescence probes to the antibody molecules to determine rotational correlation times of different parts of the molecule (Fab and Fc regions); in the computational part we employ all-atom molecular dynamics simulations to obtain atomic-detail structure of antibodies and observe conformational changes over time. MD shows existence of various domain-domain (Fab-Fab and Fab-Fc) interactions and how these are affected by the presence of salt ions. We also characterize the flexibility of the antibody in terms of conformational entropy and correlation of motions.

BIOT 376

Computational design of carbohydrate-binding proteins

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The ability to rationally engineer novel functionality into carbohydrate-binding proteins could find use in a wide range of potential applications, including diagnostic identification of microbes, prevention of retroviral infection, and targeted delivery of protein-therapeutics to specific tissue-types. However, the design of carbohydrate binding interfaces is challenging both due to the intrinsic conformational flexibility of carbohydrate targets and the highly polar nature of the binding interface, which necessitates accurate treatments of solvation effects. Here, we will highlight some of the methodological advances we have made in order to make the computational design of carbohydrate-binding protein tractable; these include the use of optimized solvation models, the application of statistically-derived rules for hierarchical screening methods, and the combination of molecular dynamic simulation with the Dead-End Elimination and A* algorithms for discrete conformational search. The applicability of these methods will be presented using examples from the engineering of a number of algal lectins with potent virucidal activity against the Human Immunodeficiency Virus (HIV).
BIOT 377

Focused library design via combinatorial optimization of degenerate codons

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Conventional computational protein design methods yield a single low-energy sequence, or a list of low-energy sequences. I have developed methods for performing direct library optimization, where degenerate codons (e.g. 'NNK') take the usual place of sidechain rotamers. The key requirement is an accurate approximation for the energy of any candidate sequence using only 1- and 2-body terms. To create such an approximation, I employ the SHARPE model platform, the all-atom Rosetta energy function, and regression methods similar to cluster expansion. The resulting method allows efficient identification of focused libraries that are Pareto optimal for low total library size and high coverage of low-energy sequences. Rapid calculations and ease of use are important if optimized codons are to compete with the blind use of 'NNK' codons for routine protein engineering screens.

BIOT 378

Engineering of P450pyr monooxygenase as highly enantioselective catalyst for asymmetric hydroxylation

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Regio-and stereo-selective biohydroxylation of non-activated carbon atom is a useful reaction for chemical and pharmaceutical synthesis, but remains as a significant challenge. Cytochrome P450pyr monooxygenase from Sphingomonas sp. HXN-200 was found to be an unique enzyme which catalyzes the hydroxylation of n-alkanes, cyclic alkanes, N-heterocycles, spirooxazolidine, and benzoxazole derivatives with high activity, good to excellent enantioselectivity. It is a class I P450 enzymes with 3 components P450 hydroxylase, Ferredoxin and Ferredoxin Reductase. To further improve enantioselectivity of P450pyr monooxygenase, P450pyr hydroxylase was subjected to directed evolution. 22 key amino acids within the active site of the enzyme were selected based on P450pyr hydroxylase X-ray structure. Iterative targeted site saturation mutagenesis was applied to create mutant library, coupled with a high throughput MS screening method to screen mutant for high enantioselectivity of the hydroxylation of N-
benzyl pyrrolidine. Excellent enantioselectivity was achieved after 3 rounds. Further biochemistry characterizations are conducting with the best mutants.

BIOT 379

Improvement of the thermostability of *Candida antarctica* lipase B through in silico Design for Engineering of disulfide bridge

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Lipase B from *Candida antarctica* (CalB) is a versatile biocatalyst for various bioconversions. In this study, the thermostability of CalB was improved through introducing new disulfide bridges based on Modeling of disulfide bond in proteins (MODIP) and B-factor. Among residue pairs in Grade A of MODIP, four candidates (S50-A273, Q156-L163, A162-K308, N169-F304) were selected based on B-factor of its residues. As a result, CalB A162C-K308C showed greatly improved thermostability with preserved catalytic efficiency compared to its wild type. Remarkably, temperature at which 50% of its activity remains after 60 minute incubation (T₅₀) of CalB A162C_K308C was 8.5°C improved compared to that of CalB wild type (55°C and 46.5°C, respectively). Additionally, half-life at 50°C of CalB A162C_K308C was 4.5 fold higher than that of CalB wild type (220 minutes and 49 minutes, respectively). Studying at molecular level using Molecular dynamic simulation demonstrated that rigidity of CalB A162C_K308C was enhanced compared to its wild type, which may be the major cause for the thermostability improvement.

BIOT 380

Production and recovery of bio-based fumaric acid using a novel process

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Fumaric acid is a 4-carbon unsaturated dicarboxylic acid that has potential as a biobased monomer for production of polymers, such as unsaturated polyester resins (UPRs). Currently, fumaric acid is produced from petrochemicals, however the biological production of fumaric acid by filamentous fungi is known. The best published performance was achieved when calcium carbonate was used as base
to control the pH in the fermentation process, which produces an insoluble calcium fumarate product. The presence of excess calcium carbonate and the insoluble fermentation product lead to a thixotropic broth that makes high demands on agitation requirements, affects fermentation control, and complicates product recovery. A modified, proprietary fermentation process using R. oryzae that was developed by MBI will be presented. The novel process produces a soluble furmarate salt, achieves titers >70 g/L and 1.7 g/l-h productivity. This provides improved fermentation control and simplifies the product recovery.

**BIOT 381**

Novel crosslinker material based on mussel adhesive protein-fused BC domain of protein A for efficient immobilization of antibodies on diverse surfaces

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Protein A is an antibody binding protein, which specifically targets the Fc portion of antibody. Mussel adhesive proteins (MAPs) are able to form strong bonds to diverse substrates. In the present work, we constructed a novel fusion protein, BC-MAP, as an immobilizing agent by genetically fusing MAP with two domains (B and C) of protein A for effective immobilization of antibody on surface. Sole BC domain without MAP was also constructed as a comparative control. Color image analyses showed BC-MAP is completely coated on diverse surfaces (glass slide, polypropylene, and aluminum) when compared to sole BC, antibody is immobilized on BC-MAP coated diverse surfaces, and immobilized antibody interacts with the corresponding antigen. Quartz crystal microbalance (QCM) analyses showed that BC-MAP has an excellent antibody binding ability compared to sole BC protein. Thanks to unique adhesive property of MAP, BC-MAP-based antibody immobilization does not require any chemical modification procedures for surfaces. Thus, the proposed BC-MAP fusion protein could be a valuable linker material for efficient immobilization of antibodies onto diverse surfaces.

**BIOT 382**

Reducing the allowable kinetic space by constructing ensemble of dynamic models with the same steady-state flux

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Dynamic models of metabolism are instrumental for gaining insight and predicting possible outcomes of perturbations. Current approaches start from the selection of lumped enzyme kinetics and determine the parameters within a large parametric space. However, kinetic parameters are often unknown and obtaining these parameters requires detailed characterization of enzymekinetics. In many cases, only steady-state fluxes are measured or estimated, but these data have not been utilized to construct dynamic models. Here, we extend the previously developed Ensemble Modeling (EM) methodology by allowing various kinetic rate expressions and employing a more efficient solution method for steady states. We show that anchoring the dynamic models to the same flux reduces the allowable parameter space significantly such that sampling of high dimensional kinetic parameters becomes meaningful. The methodology enables examination of the properties of the model's structure, including multiple steady states. Screening of models based on limited steady-state fluxes or metabolite profiles reduces the parameter space further and the remaining models become increasingly predictive.

**BIOT 383**

**Mitigating peptone lot-to-lot variability**

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The use of hydrolysates (peptones) in CHO cell culture media usually provides the benefits of higher cell growth and productivity in protein production processes. However, the drawbacks of using peptones include their undefined nature and also the potential for lot to lot variability. A plant based peptone was used during the initial process development for a CHO produced monoclonal antibody. After initial process development work with one lot of the selected peptone, it was observed that a different lot of the peptone altered cell culture process performance significantly. Further examination of several lots of the peptone indicated high lot to lot variability which translated into inconsistent cell culture performance. Analysis of the different peptone lots pointed to variability in copper content; the lots that were associated with poor performance had a lower measured concentration of copper. Supplementing additional copper into the basal medium led to more consistent performance in the peptone containing process.

**BIOT 384**

**Higher density fed-batch culture through the use of lactate-adapted cells**
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In 2009, we introduced a novel strategy to reduce lactic acid production and control pH in animal cell culture. We were able to control the balance between lactate consumption versus production, as well as the pH trajectory in shaker flask cultures, through the use of CHO cells adapted to lactate-supplemented medium. This year, we applied our novel technology to the development of higher density, fed-batch CHO cell cultures in pH-controlled bioreactors. Our approach successfully reduced lactic acid production and base additions by eight fold and allowed more concentrated nutrient feeds to be added without excessive increases in osmolality. We have achieved viable cell densities of 35 million cells per ml, among the highest ever reported for a fed-batch animal cell culture. Furthermore, we maintained high viabilities for an extended period, resulting in an integral viable cell density (IVCD) level of 273 million cell-days per ml, again one of the highest ever reported for a fed-batch animal cell culture.

BIOT 385

High titer and high-throughput cell culture process development

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As the number of candidate biotherapeutic drugs increases, the need for high titer yielding, efficient production processes to supply the clinic and maximize clinical manufacturing capacity has become apparent. A fed batch process is routinely used throughout the industry because of its ability to deliver high product concentration and its operational simplicity. Common fed-batch development approaches focus on maximizing specific productivity (qp) and/or integral of viable cells (IVC), although it is not atypical for a decrease in qp to accompany an increase in IVC. MedImmune’s cell culture platform feeds were enriched, and reformulated in order to achieve an improved balance of the nutrients and optimization of the overall feed volume. The current study with a representative cell line focused on feed development optimization and process set-points to increase volumetric productivity and optimize process throughput. Additionally, modulation of the viable cell density on day of inoculation was used in order to increase the daily volumetric productivity to > 600 mg/L/day, with a peak daily volumetric productivity of over 1000 mg/L/day. These approaches increased antibody productivity by increasing the qp by approximately two-fold while still maintaining high IVC. This resulted in an increase in volumetric productivity from approximately 4 g/L to 8 g/L while shortening the duration of the process by four days.
BIOT 386

Effects of buffer systems on Protein A capture of monoclonal antibodies

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In striving for lean manufacturing and operational excellence, the biopharmaceutical industry is constantly looking into ways to reduce costs and waste with respect to buffers, including minimizing the number of buffers and their components used in downstream steps, as well as making them online for immediate use. In order to develop a more cost-efficient and buffer-streamlined downstream process for therapeutic monoclonal antibody manufacturing, several buffer systems were evaluated on different types of commercial Protein A resins at laboratory-scale. Blank runs tracing pH and conductivity transitions between different process steps of Protein A affinity chromatography to evaluate ion-ion and ion-resin interactions of the different buffer systems on each of the resins will be presented. Additionally, buffer effects on capture performance in terms of impurity clearance, eluate volume and recovery will be presented.

BIOT 387

Development of chromatofocusing techniques employing mixed-mode column packings for biomolecule separations

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Recent studies using mixed-mode column packings have shown that multiple modes of interactions between the column packing and the biomolecules of interest can be usefully exploited to yield excellent resolution as well as salt-tolerant adsorption of the target biomolecule. In this study, we extended this past work to develop a novel mixed-mode separation method which combines the techniques of hydrophobic-interaction chromatography and chromatofocusing. Several commercial mixed-mode column packings were investigated in this regard using internally generated, retained pH gradients to separate proteins. In addition, a novel mixed-mode column packing that can be used for chromatofocusing was synthesized which incorporated both hydrophobic and weak-acid cation exchange functionalities.

BIOT 388
Encapsulation of protein into a biodegradable polymer using high pressure homogenization

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Herein we report on the encapsulation of proteins into triggered release nanoparticles. Nanoparticles were prepared from a novel dual-response polymer using high pressure homogenization1. Nanoparticles were prepared using double emulsion method incorporating a model protein. Tangential flow was used to purify the resulting nanoparticles. The particles were characterized by dynamic light scattering (DLS) in pH 7.4 and 5. Protein integrity was analyzed by gel electrophoresis and measuring its ultravilot and fluorescence emission spectrums and was found to be stable. Degradation kinetics show that these nanoparticles have the ability to release their encapsulated payload when exposed to mildly acidic pH resembling the pH levels of early endosomes and diseased tissue. Furthermore, the nanoparticles were stable for 24 hours in normal cellular pH of 7.4. Our results indicate the potential of this vehicle to deliver therapeutics and diagnostics to cells and diseased tissue.1 Sankaranarayanan et al, ACS Nano, Article ASAP DOI: 10.1021/nn100968e.

BIOT 389

Extended MCSGP process for continuous four-fraction separation: Case study of monoclonal antibody variants

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Many protein and peptide purification challenges encountered in the biopharmaceutical industry consist of the purification of the product from a mixture of impurities with similar adsorptive properties. The MCSGP process, which represents a highly efficient continuous countercurrent chromatographic purification technology platform, is particularly suited for these “difficult” separations as it is capable of performing linear gradients and three-fraction separations and achieving high yield and purity simultaneously. In certain purification challenges a three-fraction separation is not sufficient e.g. for the purification of charged monoclonal antibody (mAb) variants for pre-clinical
studies. However, by extending the MCSGP process with further sections, additional fractions may be separated. In this presentation, the general approach for the extension of the MCSGP process for multi-fraction separations is outlined. Experimental data for a four-fraction separation of charged mAb variants of Erbitux® is presented and the performance of the extended process and batch chromatography is compared.

BIOT 390

Implications of capacity dependence on feed pressure for the proper development and scaling of multi-stage filtration processes

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It has been observed that the capacity of a membrane filter for a given feed can show significant dependence on feed pressure. In these cases, filters typically show increased capacity with increased feed pressure. This is especially true when the feed contains deformable particles as in cell culture media. This capacity variability with feed pressure becomes especially troublesome when trying to develop and scale multi-stage filtration trains, where the pressure drop across each filter changes with time and loading over the course of the process. Traditionally, each filter in a train is sized individually at constant pressure in a manner that does not take into account the variable feed conditions seen during the process. This leads to unpredictable scaling and a risk of process deviation or failure at manufacturing scale. In this study, the filter capacity dependence on feed pressure was characterized for a cell culture media. Several test methods were then explored in an attempt to more accurately size the media filter train, testing filters both sequentially and simultaneously. For each method, process simulations were executed at constant pressure with the filters in series at the recommended sizing for each filter. Based on the results of these trials, each approach’s ability to accurately size and scale the filtration process is assessed, and recommendations are made for an improved test method for sizing of multi-stage filtration trains.

BIOT 391

Mussel-derived underwater bioadhesive based on complex coacervation

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Marine mussels attach to substrates using adhesive proteins. It has been suggested that complex coacervation (liquid-liquid phase separation via concentration) might be involved in the highly condensed and non-water dispersed adhesion process of mussel adhesive proteins (MAPs). However, as purified natural MAPs are difficult to obtain, it has not been possible to experimentally validate the coacervation model. We demonstrate complex coacervation in a system including recombinant MAPs. Our recombinant hybrid MAPs can be produced in large quantities, and are readily purified. We observed successful complex coacervation using cationic MAP and an anionic partner. Importantly, we found that highly condensed complex coacervates significantly increased the bulk adhesive strength of MAPs in both dry and wet environments. Especially, underwater adhesion was possible using the coacervated MAPs. Collectively, our results indicate that a complex coacervation system based on MAPs shows superior adhesive properties, combined with additional valuable features including liquid/liquid phase separation and appropriate viscoelasticity. Our coacervated mussel adhesive could be useful in the development of underwater adhesion for use in biomedical applications.

BIOT 392

HIC in the faster lane: Capture and polishing

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Common challenges in the construction of porous media for bioseparations are to achieve rigid resin media which does not exhibit significant non-specific adsorption while also offering fast process flow rates and higher column beds. GE Healthcare recently developed a range of cross-linked agarose media which offer superior mechanical and surface properties. The base matrix was first used for ion exchange and affinity media. Recently hydrophobic affinity chromatography (HIC) media was added for early stage purification. For polishing applications there is a need for HIC media offering better resolution properties than previous HIC media. We are presently involved in designing and testing a newer generation of HIC media for polishing. Some chemical and material design considerations will be presented together with application results.

BIOT 393

Single-pass TFF processing

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While single pass TFF has been widely used in other industries, its application for bioprocessing has been limited. Operation and design will be described along with a comparison to conventional batch operation. Several applications will be considered including volume reduction for process de-bottlenecking.

BIOT 394

Correlating raw material fingerprints from Near Infrared (NIR) Spectroscopy to cell culture process performance

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Near Infrared Spectroscopy (NIRS) can be used for raw material analysis in biotechnology production. In cell culture production, NIRS provides an orthogonal method for analyzing complex non-defined raw materials that contribute to fermentation processes variability. By capturing the characteristic overtone and combination bands of the fundamental vibrations of organic functional groups, NIRS can observe small differences in chemical composition between raw material lots. Although the individual contributors to the NIR spectra and their inherent variation cannot be deconvoluted, raw material lots can be clustered according to their chemical similarities as determined by their unique NIR fingerprint. Additionally, correlations between NIR spectra and process performance can describe the variability in fermentation performance that is due to the lot-to-lot variability of the complex raw materials used. In this study, NIR spectral preprocessing of the peptone lots and partial least squares regression models quantitatively predicts the main pre-harvest performance parameters of a well controlled manufacturing fermentation. Such analyses can be used to screen for less than optimal raw material lots, reduce batch-to-batch variability within a fermentation process, and indirectly increase manufacturing production capabilities.

BIOT 395

Formation process and structure of polyproline self-assembled monolayer on gold surface

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Formation of monolayer of functional peptide on solid surface has attracted much attention because one can introduce wide range of functionalities. Polyproline is an interesting peptide as its conformation varies dramatically with solvent. For example, it takes pπI and pπII conformation in water and methanol, respectively. In this study, we have investigated the formation process and the structure of self-assembled monolayer (SAM) of polyproline on Au(111) surface in water and methanol using various techniques including electrochemical reductive desorption, ellipsometry and infrared (IR) spectroscopy. The formation process of SAM of polyproline was investigated by immersing gold coated silicon prism in 1 uM polyproline D_2O solution. IR spectra showed amide I band at 1628 cm\(^{-1}\) and C-N stretching band at 1453 cm\(^{-1}\). Both bands were increased and kept constant with immersion time. Peak shift was observed by changing solvent. The formation process of SAM of polyproline was also followed by reductive desorption by dipping Au(111) electrode in 10 uM polyproline aqueous solution and then sweeping the potential negatively in 0.5 M KOH aqueous solution.

BIOT 396

Antibodies purification using ELP-zz domain fusions

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Antibodies are immune system-related proteins called immunoglobulins (IgGs) which have applications for medical diagnostics and research. However, their purification from different sources has always been a challenge because of low antibody concentration and higher purity requirements for usage. The unique capability of Elastin like Proteins (ELPs) to reversibly precipitate at a relatively modest temperature has been utilized for purification of antibodies. This feature of ELPs to purify antibodies has already been explored using larger fusion domains such as Protein L and Protein G. However, the usage of larger Protein G/L fusions with ELP resulted in 10-fold lower expression when compared to ELP or ELP fusions with shorter peptides. In the current work, ELP fusions with a small IgG-binding peptide i.e. zz domain (a synthetic IgG binding domain derived from the Staphylococcus aureus protein A) were generated. The production of ELP[VPGVG]-78-zz fusion in E. coli (~500 mg/L) was found to be five-fold higher than ELP[VPGVG]-78-ProL (~100 mg/L). In addition, the ELP[VPGVG]-78-zz fusion showed excellent binding affinity toward human, mouse, and rabbit IgGs, enabling simple purification of the different antibodies by reversible thermal
precipitation. In order to recover antibody from the ELP-zz-IgG complex, different elution conditions were investigated. Close to 90% recovery was achieved using 0.5 M arginine pH 3.8 buffer. To further increase the production of ELP-zz fusion protein, three different ELP domains (VPGXG)-39 (where X= K:V:F=1:7.8:1); (VPGXG)-59 (where X= K:V:F=1:7.8:1), (VPGXG)-79 (where X= K:V:F=1:7.8:1), were generated. Production of ELP(KV7.8F)-zz fusions were increased by two fold, while maintaining similar binding affinity for IgGs. Due to its high level production and affinity for different IgGs, we believe that these ELP-zz fusions will be useful as an economical, highly efficient, and universal platform for the purification of antibodies.

BIOT 397

Affinity chromatography of therapeutic protein variants: Impact of protein size, resin backbone properties, ligand density, and orientation on purification performance

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Immuoaffinity Chromatography is the key unit operation in purification of a class of therapeutic proteins in clinical development. Purification performance can be impacted by mechanical and chemical properties of the resin backbone. This was explored in detail by coupling an antibody (directed against a common antigen) to different commercial resins. Results can be used in process optimization / lifecycle management. The investigated variables are protein size, resin backbone properties, ligand density and orientation resulting from different coupling chemistries. Evaluated performance outputs are binding capacities, affinities and selectivity. Packed bed resin properties like accessible resin volume and apparent pore size distribution were determined through size exclusion chromatography with polymer and protein standards. Static and dynamic binding capacities and affinities were obtained in batch and column experiments. Based on the fundamental characterization using one-component solutions, differences in yield, host cell protein clearance and product aggregation using process feed stream material were evaluated.

BIOT 398

Evaluation of cation exchange chromatography as a capture step for monoclonal antibody purification

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Monoclonal antibody (mAb) manufacturing involves multiple unit operations designed to clarify and purify the drug substance. One of the most common unit operations is affinity chromatography, particularly protein A, used for capturing the mAb from clarified supernatant. Despite the fact that protein A is highly specific for IgGs, robust, and capable of being re-used up to 100 cycles (Lute, 2008), it still remains the most expensive step in mAb manufacturing. In an effort to reduce the cost of manufacturing, researchers have been investigating the use of cation exchange (CEX) chromatography as an efficient replacement for capture chromatography via protein A. One concern with using CEX for capture is that the capacity for mAbs may be significantly impacted by host cell proteins competing for binding sites. The reliability of viral clearance is also of concern. However, with the recent production of high capacity CEX resins, the use of CEX as a replacement capture step for protein A is likely more plausible. Here we evaluated five different CEX resins for their ability to capture a model mAb from harvested cell culture fluids spiked with a model mammalian virus. We assessed multiple process parameters including: mAb yield, host cell protein, viral clearance, and host cell DNA. The results from these studies were compared to the same model load run across a protein A resin. Our findings will be discussed for the feasibility of using CEX as a capture step in mAb manufacturing.

BIOT 399

Separation of isoform proteins using optimized charged membranes

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Downstream processing and recovery of a specific protein often involves a membrane-based unit operation such as ultrafiltration. The separation of proteins with similar molecular weight and charge remains very difficult, especially for membrane filtration which is a primarily size-based method. Here, combining customized charged membranes with tight control of solution conditions, we optimized the separation of two pairs of proteins: Lysozyme - RNase A (MW 14.3 and 13.7 kDa, pI 11.3 and 9.6, respectively) and BSA - hemoglobin (MW 69 and 67 kDa, pI 4.8 and 6.8, respectively). We used our patented photo-induced graft polymerization method to modify the surface of poly(ether sulfone) membranes and to control the charge density. The membranes were tested in filtration experiments under separate pH and conductivity (ionic strength) gradients, using a scaled-down industrial buffer mixer (Asahi Kasai, Glenview, IL).
The effect of TMP was also taken into account in order to maximize protein purity and yield.

**BIOT 400**

**Viresolve pro Parvovirus filtration development of a high titer CHO derived protein**

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A filtration process using the Viresolve Pro parvovirus filter was shown to achieve low mass throughput when filtering a challenging CHO derived IgG1 antibody. Though the Phase III process of this molecule used an adenovirus filter and achieved high mass throughput, switching to the parvovirus filter resulted in substantially reduced throughput. Initial studies evaluated feedstock stability, pre-filters and the configuration design but nothing improved capacity. To understand the causes of the filter fouling, a DOE approach was used to assess impact of feedstock properties (pH and conductivity) in combination with different pre-filters. The study tested two pool intermediates and showed mass capacities ranged from 0 to 4 kg/m². Results led to appropriate parameter selection and gave insight of parameter ranges to evaluate for future robustness testing. Antibody self-association and aggregation were considered when analyzing the data, as well as the effect of a characteristic and prominent CHO host cell protein.

**BIOT 401**

**Rational approach for depth filter sizing to achieve performance consistency for a commercial process**

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Depth filtration has long been used in the clarification of mammalian cell culture, either directly for primary clarification, or as a secondary clarification step following centrifugation or microfiltration methods for primary clarification. Cellulosic depth filters embedded with diatomaceous earth have historically been the depth filter of choice for these applications. The nature of these depth filters and the methods in which they are manufactured impart some variability based on raw material sources, manufacturing methods, and scale up of devices. Mammalian cell culture fluid also imparts significant variability based on media components, cell line, and cell culture conditions. Variability with both the separation technology and the incoming feed material, as well as the high cost associated with process failure in a GMP environment highlights the need for a
rational method to establish process specifications for a commercial harvest process. A robust process design for sizing a depth filtration harvest step should incorporate an assessment of four key criteria: 1. Filter media selection 2. Filter media variability 3. Feed (Process) variability and 4. Performance variability upon scale up. This presentation will detail a rational approach for characterizing and defining filter sizing for the harvest step and give some examples of data generated to assess each of these factors using mammalian cell culture fluid. The approach involved minimizes external sources of variability to ~2% using custom test devices to increase statistical significance. The data shows that depth filters scale and perform more consistently than generally recognized.

BIOT 402
WITHDRAWN
BIOT 403

High-throughput screening and evaluation of a multimodal chromatography resin for purification of monoclonal antibodies

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Mixed-mode chromatography has become an increasingly popular tool in recent years, as it offers a means of separating a variety of impurities in a single step. One such multimodal resin, Capto adhere (produced by GE Healthcare), functions as a strong anion exchanger with additional selectivity based on hydrogen bonding and hydrophobic interactions. Purification process development for two different monoclonal antibodies incorporated Capto adhere as a second chromatography step, following Protein A affinity chromatography. The objective was to remove as many impurities (aggregated/clipped antibodies, DNA, host cell proteins, and residual Protein A ligand) as possible while maintaining a step yield and operating conditions that would be feasible for large-scale manufacturing. In an effort to save time and material, a high-throughput screen of operating conditions was conducted using a Tecan liquid handling system and custom-made pipet tips packed with resin. Promising leads from the scale-down model were identified and successfully scaled-up to column runs. Results demonstrated that the mixed-mode step could give a yield of 85% with significant reductions in aggregates, Protein A ligand, and host cell proteins. Removal of clipped species was deemed to be more challenging such that a third column needed to be included for further impurity removal. Further development included comparing the performance of the mixed-mode resin to several traditional anion exchangers in terms of operating window and robustness.
BIOT 404

Formation of nanoconjugates of chicoric acid assemblies as drug delivery vehicles

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Chicoric acid, a derivative of caffeic acid aids in the efficiency of immune cells and is a known HIV-1 integrase inhibitor. It also acts as an anti-oxidant. In this work, we examined the ability of chicoric acid to form supramolecular assemblies at varying pH at room temperature. The shape and sizes of the assemblies formed were dependent upon the pH. In order to prepare efficient drug delivery vehicles, the nanotubular and nanovesicle assemblies were coated with polyarginine. The intermolecular interactions between the positively charged polyarginine and the negatively charged carboxylate groups of chicoric acid allowed for the formation of nanoconjugates. Upon association, we explored the ability of the nanostructures to interact with mammalian cells such as mouse embryonic fibroblasts and normal rat kidney cells. The cytotoxicity and cell proliferation were studied. We observed that the materials were associated with the cells depending upon the concentration of the pre-cursors used.

BIOT 405

PEGylation of E2 virus-like nanoparticles

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The structural core of the E2 subunit of B. stearothermophilus pyruvate dehydrogenase (E2) has been engineered to display antigens on its surface. For potential therapeutic applications, we have PEGylated a variant of E2 (E2-E279C) on introduced surface cysteines with methyl-PEG24-maleimide. We verified PEGylation on the scaffold with mass spectrometry and dynamic light scattering. Using circular dichroism, we showed the PEGylated scaffold remained correctly folded and that PEGylation does not decrease the scaffold's high thermostability, with $T_m$ remaining near 90°C. Correct assembly was confirmed with transmission electron microscopy. We have also evaluated classical/lectin
complement pathway activation and in vitro macrophage phagocytosis to determine the biological effect of PEGylation on the E2 nanoparticle.

**BIOT 406**

**Fabrication and characterization of novel electrospun composite nanofibrous membrane using polycaprolactone and mussel adhesive protein for tissue engineering**

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Electrospinning is a simple method for fabricating a nanofibrous membrane, which is considered as a bio-mimicking scaffold for tissue engineering because of its structural similarity with extracellular matrix (ECM). As materials of nanofiber scaffold, biodegradable synthetic polymers, which have a good mechanical property and are able to be easily manipulated, have been conventionally used. For biomedical uses, however, these polymer materials have limitations such as poor cell adhesion ability and no biological activity. Recombinant mussel adhesive protein (rMAP), fp-151-RGD, showed a great cell adhesion, spreading, and proliferation abilities when it was coated on tissue culture plate surface. In the present work, we used the typical electrospinning procedure to fabricate the novel composite nanofibrous membrane by blending polycaprolactone (PCL) and rMAP solution with various ratios. Characterization of nanofibrous membrane was performed by scanning electron microscopy, FT-IR, contact angle, and tensile strength. In addition, the cell behaviors on these nanofibrous membranes were investigated using MC3T3-E1, mouse pre-osteoblast cell line.

**BIOT 407**

**Molecular insights into interactions between multimodal chromatographic ligands and proteins**

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The recent advances in multimodal (MM) chromatography have the potential to offer unique selectivities for proteins and enable separations that were hard to achieve with traditional chromatographic systems. The optimization of these processes is limited by the lack of understanding of the mechanisms by which these ligands and resins interact with different protein surfaces. Molecular dynamics simulations have been applied to gain molecular insights into these mechanisms and to examine the effects of various coupled modes of interactions with the non-homogeneous protein surface. The effect of mobile-phase modifiers on the local environment and their effect on the behavior of these systems have also been studied. A deeper understanding of the nature of the interactions between MM ligand and proteins can help in further exploration and determination of characteristic properties that will guide in development of predictive tools for challenging industrial separations.

BIOT 408

Self-assembly and display of cell targeting moieties on a caged protein complex

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Our laboratory is exploring the applications of a 60-mer caged protein nanoparticle that is derived from the E2 subunit of the pyruvate dehydrogenase multienzyme complex. We have shown drug encapsulation and pH-triggered dissociation, which potentially possesses the ability to release drug payloads. Modification of the protein to display defined peptides on its surface would enhance the particles' ability to target specific cells or sites in a physiological system. A peptide known to bind to breast cancer cells has been genetically fused to the N-terminus of the protein and expressed in E. coli cells. Experiments indicate that incorporating the targeting peptide in each subunit via cassette mutagenesis results in improper folding and assembly of the protein complex. We attempted different methods to promote properly-folded proteins, including codon optimization, protein refolding with E2 subunits which do not contain the targeting ligands, expression in the presence of chaperonins, and coexpression of the peptide-containing and non-peptide-containing subunits. Our results demonstrated that two strategies, refolding of the targeting subunits with unmodified subunits and coexpression of the two different subunits within the same cell, resulted in a properly assembled complex displaying the peptide of interest on the surface. This protein was then used to examine uptake and binding to target cells.

BIOT 409
Purification and characterization of anti-LNFPIII single chain antibody (scFv) conjugated with Fc from CHO cells transfected with 3F1 scFv-Fc gene

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LNFPIII is an oncodevelopmental cancer-associated antigen. We previously isolated and characterized anti-LNFPIII (lacto-N-fucopentaose III) scFvs by phage display technologies. Although 1F12 scFv-Fc proteins were secreted from a NS0 stable clone into media and successfully purified, production of stable clones for 3F1 scFv-Fc was never established (J. Biol. Chem. 285: 30587, 2010). Thus, pCIneo/3F1 scFv-Fc expression vector was introduced into CHO cells by lipofection. From transfected CHO cells, one clone 8C7 was established. Expressed 3F1 scFv-Fc proteins were mostly accumulated inside cells although some were secreted into media. 3F1 scFv-Fc proteins were purified from the cells by protein-A affinity chromatography. The purified scFv-Fc protein showed binding affinity for LNFPIII. It was shown to be a dimer by SDS-PAGE as expected. Since the amount of purified scFv-Fc proteins was insufficient for further analyses, suspension culture is now being set up for a large scale preparation of cells and purification of scFv-Fc proteins.

BIOT 410

Protein and peptide purification by continuous countercurrent chromatography (MCSGP)

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The MCSGP process is a continuous countercurrent chromatographic process suitable for protein and peptide downstream purification. By combining the advantages of discontinuous and simulated moving bed chromatography, MCSGP is capable of running buffer or solvent gradients and performing three- or multifraction separations in a continuous manner. In contrast to batch chromatography, MCSGP is not limited by a yield / purity tradeoff. In this presentation, recent progress in made in the development of the MCSGP process will be shown in the format of case studies for various purification challenges, such as the a multi-fraction monoclonal antibody (mAb) variant purification and a peptide purification.
Empirical design of continuous chromatography (MCSGP process)

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Continuous chromatographic purification processes can significantly improve the efficiency of downstream processing (DSP) with respect to yield, product purity, solvent requirement and productivity. But in comparison to common batch purifications on single columns the design of continuous chromatography is most often more complex, if the molecules to be separated have nonlinear isotherms [Ind. Eng. Chem. Res. 2009, 48, 7733–7752]. Especially when mobile phase gradients are used [Journal of Chromatography A, 1026 (2004) 47–55, AIChE Journal, Volume 49, Issue 3 (p 665-674)], the SMB design is very complicated and a detailed simulation model is necessary to properly design operating parameters. For the MCSGP process, which applies buffer strength / pH gradients for countercurrent purifications of large biomolecules, a pure empirical design was developed, which allows for proper evaluation of operating parameters, even when no isotherm data and no simulation tool is available. The operation parameters are evaluated from a simple overloaded single column gradient chromatogram. In this way the single column batch gradient is reproduced inside the MCSGP unit. But in contrast to the original single column batch elution the final product yield is much higher in MCSGP mode than in batch mode. In this poster the empirical MCSGP design procedure is introduced and the MCSGP performance is experimentally compared with the original single column batch chromatograms from which the design was made. Process relevant details and the evaluation of the design space are discussed.

Investigation of protein binding interactions in multimodal chromatographic systems: Understanding selectivity and implications for process development

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Recent advances in the multi-modal chromatographic systems have shown significant potential for protein purification. A deeper understanding of the nature of selectivity in these systems can result in more efficient process development.
A large set of proteins with a variety of physicochemical properties such as size, surface area, charge and hydrophobicity was investigated in multi-modal ion exchange and hydroxyapatite chromatographic systems. Experiments were carried out under different conditions (e.g. salt, gradient, mobile phase modifier) and protein retention data was analyzed to determine unique selectivity trends and binding behavior. Quantitative structure property relationship (QSPR) classification and prediction models based upon custom-made a priori molecular descriptors were generated to provide better understanding and further insights into protein selectivity in these systems. Geometry constrained synergistic binding interactions were found to play important roles. Finally, novel mixed-gradient experimental techniques are presented to facilitate methods development for industrial bioprocesses.

BIOT 413

Novel hybrid liquid-phase gradients for biomolecule separation by chromatofocusing

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In this work, innovative liquid-phase composition gradients are studied with regards to their use in ion exchange HPLC. Biomolecule separations are accomplished by a combination of ionic strength and pH gradients which are, respectively, externally and internally produced. Gradients are studied in three different scenarios: when the gradient is formed due to an increase in salt concentration, an increase on the buffer species concentration or an increase in the organic solvent content. In order to rationally design and optimize these complex phenomenological chromatographic applications, full numerical simulations are carried out which predict the detailed behavior of the separation process. The results of this study are useful not only to aid in the development of novel hybrid chromatographic separations processes, but also to gain a better understanding of separations with competing and/or complex separation mechanisms.

BIOT 414

Effects of fibronectin and vitronectin on human fetal osteoblast cell attachment and proliferation on nanostructured titania surfaces

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Improvements in osseointegration require focusing efforts on the bone-implant interface, which is complex and involves numerous factors, including surface topography and composition. Nanostructured surfaces have been shown to promote serum protein adsorption and osteoblast adhesion when compared to microstructured substrates. The influence of the serum proteins fibronectin and vitronectin on human fetal osteoblast cell line (hFOB 1.19) attachment, proliferation and extracellular matrix production was studied on nanoporous TiO$_2$ templates fabricated by an anodization process. The nanoporous TiO$_2$ templates were coated with 1, 10 and 100 $\mu$g/ml of fibronectin and/or vitronectin for 24 hrs prior to seeding with hFOB 1.19. Initial cell attachment and spreading was determined after 6h through the evaluation of morphology via image analysis of immunofluorescence micrographs of labeled F-actin fibers (cytoskeleton) and the vinculin (focal adhesion complex). Cell adhesion and proliferation was characterized after 1, 4 and 7 days using a standard BrdU assay, and viability was characterized via the two-color fluorescence assay after 4 days. Total protein content, alkaline phosphatase activity, and extracellular matrix production was quantified using standard colorimetric assays for up to 2 weeks of culture. Extracellular matrix production was also characterized by measuring surface concentrations of calcium and phosphorus using X-ray photoelectron spectroscopy. Results indicate that nanostructured TiO$_2$ significantly affect serum protein adsorption and osteoblast response.

BIOT 415

Antitumor activity of chloroquine-conjugated gold nanoparticles

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We have conjugated chloroquine, which has been used as an antiviral (against influenza, HIV-1) and antitumor drug in addition to malaria treatment,
ontothiol-stabilized gold nanoparticles and studied its antitumor activity. Gold nanoparticles have been synthesized using sodium borohydride as reducing agent and 11-mercaptoundecanoic acid to stabilize them in aqueous medium. The formation of gold nanoparticles was confirmed from characteristic surface plasmon absorption band at 520 nm and transmission electron microscopy; the average size of gold nanoparticles was found to be ~ 7 nm. The chloroquine was conjugated to thiolated gold nanoparticles using EDC/NHS chemistry and the binding was analyzed using Fourier Transform Infrared spectroscopy. Optical density measurement suggested the release of chloroquine from chloroquine-conjugated gold nanoparticles (GNP-Chl) at acidic pH and is attributed to the cleavage of amide bond linkage of the drug to nanoparticles. The anti-tumor activity of GNP-Chl has been demonstrated on MCF-7 breast cancer cell line.

BIOT 416

Evaluation of polyethyleneimine based chromatographic supports

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Ion-exchange chromatography is routinely chosen for aggregate removal due to its gentle conditions and ease of use. However, when high levels of aggregate are present, traditional ion-exchangers often require unrealistically tight control of critical process parameters such as conductivity and pH, making their use operationally unfeasible. In this work, we investigate new process scale resins that incorporate polyethyleneimine (PEI) derived ion-exchange ligands. PEI alone is a weak anion exchanger with a high charge density. The ligand can be further derivatized to add strong or weak anion or cation functionality. These resins have demonstrated improved selectivity for aggregate and charge variant separation. In this work we quantitatively compare the performance of PEI and traditional ion-exchangers using techniques including linear gradient elution, stepwise elution, and titration curve analysis. The potential of PEI ligands for the generation of retained pH gradients and pH elution is also explored.

BIOT 417

Novel affinity tails, based on genomic data, for immobilized metal affinity and ion-exchange chromatographies

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The purpose of this effort is the development of novel affinity tails with the ability to promote binding to multiple stationary phases, using adsorption and elution characteristics of genomic proteins as a guide. We constructed Green Fluorescent Protein (GFP) fusions with natural chromatography tails based on the *Escherichia coli* peptidyl prolyl cis/trans-isomerase (SlyD). SlyD is a known Immobilized Metal Affinity Chromatography (IMAC) and Ion-Exchange Chromatography (IEC) contaminant that significantly reduces column capacity and complicates gradient design. The candidate amino acid sequence of SlyD for promoting adsorption in IMAC and IEC consists of the 55 C-terminal amino acids rich in histidine, glutamic acid, and aspartic acid. Two affinity tail sequences have been tested: an un-modified form comprised of the full sequence, and a truncated form that emphasizes the histidine, glutamic acid and aspartic acid rich region while removing a glycine rich region. This poster will discuss the process leading to the design of the SlyD-based affinity sequences, present the cloning strategy for the GFP construct, and compare binding characteristics and solubility in the presence and absence of the [affinity] tails.

**BIOT 418**

**Expression and purification of single chain antibodies (scFvs) isolated from a phage-displayed library with LNFP III (Le⁺) probe**

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LNFP III is an oncodevelopmental cancer-associated antigen. We previously reported isolation and characterization of anti-LNFP III (lacto-**N**-fucopentaose III) scFvs by phage display technologies. Although 1F12 scFv-Fc proteins expressed in NS0 cells were secreted into media and successfully purified, production of stable clones for 3F1 scFv-Fc was never established (*J. Biol. Chem.* 285: 30587, 2010). In addition, 1F12 scFv-Fc production in the stable clone declined during early passages. Thus, anti-LNFP III 3F1 and 1F12 scFv genes were inserted into pET22b (+) to generate bacterial expression vectors. Expression of scFv proteins was induced by IPTG in *E. coli* BL21 (DE3) transformed with pET/3F1 or pET/1F12. 1F12 and 3F1 scFv proteins were purified from cytoplasmic and periplasmic fractions by Ni²⁺-Sepharose chromatography. Alternatively, insoluble fractions were solubilized in 3.5M Gdn-HCl, then purified by Ni²⁺-Sepharose chromatography. Eluted scFv proteins in 3.5M Gdn-HCl were refolded by
stepwise dialysis. Affinity and specificity of the purified scFvs proteins are in progress.

**BIOT 419**

**High throughput screening in downstream process development: Clone selection and optimization of an antigen purification process in 96-well plates and Robocolumns™ with compressed timelines**

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High Throughput Process Development (HTPD) for chromatographic protein purification has many benefits over traditional process development including low material requirements, shortened timelines and the generation of large data sets. With a Tecan automated liquid handling system, we have developed methods in the 96-well filter plate format for screening large operating windows of process conditions. These methods include resin selectivity screening of product from aggregates and trace impurities, adsorption isotherms and static binding capacities, salt solubility screens, and pseudo-chromatograms. These techniques were applied to aid in clone selection and optimize current purification process conditions for a CHO cell line-derived antigen. Once the optimal operating windows were determined, the resins and processing conditions were scaled up into RoboColumns, run eight at a time on the Tecan, for optimization of each unit operation. The purification conditions, performance and product quality of the HTS processes were then compared to preparative scale columns.

**BIOT 420**

**Calorimetric study of protein adsorption and enzyme immobilization on organic composite mesostructured cellular foam silica materials; Effect of salt concentration and surface functionality**

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Understanding the interaction between biomolecule and surface is of great importance in biotechnology such as design of immobilized enzyme and protein chromatography because the interaction affects the extent of biomolecule adsorption on the solid and the adsorption process, which accompanies with heat events, can determine system performance. Mesoporous silica materials have shown great potential as enzyme host and protein separation media because properties such as pore size, pore volume and surface area can be controlled. In this study, heat energies during protein adsorption on mesostructured foam silica
grafted with organic functional groups are measured by using flow microcalorimetry to investigate the influences of salt concentration and surface functionality. Calorimetric data expect to reveal underlying thermodynamic mechanism for protein adsorption and help us to design optimal interaction between protein and surface for design of highly efficient immobilized enzyme and improvement of stationary phase technology in protein chromatography.

BIOT 421

Olfactory biosensor based on olfactory neurons for odorant detection

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Olfaction plays a great part in insects, animals, especially for mammals. Recently great progress has been made in the research of olfactory transduction and biomimetic olfactory biosensors since Buck and Axel’s great work in finding of gene super-family encoding olfactory receptors and they got their Nobel Prize in 2004. A new kind of olfactory biosensor was based on mammals olfactory neurons cultured on the surface of field effect transistors (FETs). The olfactory neurons were attained from mammal neural cells, and can survive on FETs for about a month. When the odorant specific to the corresponding receptor was induced, the sensor can detect electric signal through the electrodes. The magnitude of signal depends on concentration of odorant, after amplification and noise-filter and high signal-to-noise ratio (SNR) signal was obtained. Biosensor based on olfactory neurons is sensitive to the specific odorant and can be used for the quantitative measurement of the odorant.

BIOT 422

WITHDRAWN

BIOT 423

Ralstonia eutropha as a chemolithoautotrophic chassis for biofuel production

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Ralstonia eutropha is an aerobic bacterium that grows with CO2 as the sole carbon source and H2 as the sole electron donor while producing copious
amounts of polyhydroxybutyrate. This poster discusses the development of Ralstonia eutropha as a chemolithoautotrophic chassis for the production of biofuels from CO2 and electrogenic H2. We demonstrate the application of synthetic biology tools to divert intermediates and metabolic flux from existing R. eutropha pathways into engineered, recently discovered pathways to produce favorable biofuels. Novel Mo-polypyridine catalysts that can convert water to hydrogen in neutral aqueous media were employed as chemical mediators to generate H2 from electrodes in the presence of engineered strains of R. eutropha. The engineered chemolithoautotrophic chassis provides a transformational new source of renewable liquid transportation fuels that extends beyond biomass-derived substrates.

BIOT 424

Intergenomic interrogation of signal transduction reveals horizontal gene transfer and amelioration

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Most informatics or genomics based approaches characterizing gene transfer focus on specific genes or metabolic pathways. Few, if any, reports attempt to characterize Eubacterial signal transduction pathways, which is particularly surprising given the discovery of "quorum sensing" pathways that alter virulence in human pathogens. We developed a method for delineating the gene transfer of signal transduction modules, starting with the Lsr subsystem of E. coli, a quorum sensing module. Extending prokaryotic operon identification methods, module fingerprints are identified by gene organization homology on the level of relative gene order and relative direction, with a nucleotide level E-value filter. Results show Lsr homologues extant in numerous bacteria. The subsystem is dispersed and ameliorated widely amongst 112 strains in 30 species along the Eubacteria phylogenetic tree from Spirochaete to Pasteurella. Eccentricity of the module organization is evident with various canonical components rearranged or altogether missing from certain homologues.

BIOT 425

WITHDRAWN

BIOT 426

Chemometric modeling of HPLC-UV data for prediction of hydrolysate fermentability
A method for prediction of hydrolysate fermentability via chemometric modeling of chromatographic data is reported. Chromatographic data were obtained from analysis of 20 hydrolysate samples using high-performance liquid chromatography (HPLC) with UV detection at four discrete wavelengths. Previous work in our laboratory demonstrated that a chemometric model is capable of providing rapid and accurate prediction of hydrolysate fermentability using only UV-visible (190-450 nm) spectroscopic data of hydrolysate samples. Moreover, this model was able to identify four specific wavelengths that accounted for a majority of variation in fermentability between samples. Subsequent model development using these four wavelengths resulted in a prediction accuracy that was equivalent to the original model. In the current study, we evaluate the potential for chemometric modeling to not only predict hydrolysate fermentability based on chromatographic data, but also to identify the retention time(s) that are most strongly correlated with hydrolysate fermentability. The chemometric model was developed using biomass hydrolysates of variable composition, obtained from twenty different pretreatment conditions. Each hydrolysate sample was analyzed via reversed-phase HPLC with UV detection using a nonlinear gradient. Fermentability of each hydrolysate sample was determined independently in batch fermentation experiments. Chromatographic and fermentability data were regressed using a chemometric software. The potential for this model to predict fermentability of new hydrolysate samples and to identify the retention time(s) that are most strongly correlated with fermentability will be presented. The utility of this approach towards accurately identifying compounds that may be most responsible for observed microbial endpoints will also be discussed.

**BIOT 427**

**Highly sensitive microfluidic pathogen detectorsystem based on micro-retroreflector immunoassays**

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Many bioanalytic techniques rely upon a label to signal the presence of analyte and while these labels are common and well-developed, label detection sensitivity often limits assay sensitivity. This work introduces magnetic sample-prep particles and nanoparticles as light-blocking labels in optical assays based on micron-scale microfabricated retroreflectors. Retroreflectors return light directly to its source and are readily detectable with inexpensive optics; the assay can easily detect the presence of a single 1.0 μm particle bound to the surface. The magnetic properties of the particles are useful in sample preparation and concentration, and magnetic force, as well as microfluidic fluid flow shear, are used to increase specificity by discriminating against non-specific interactions. Shear force discrimination, reproducibility, and convenience are enhanced by implementation in a microfluidic cartridge format and early results show that $10^3$ Rickettsia conorii bacteria per mL can be detected.

**BIOT 428**

**Single molecule gene expression profiling using atomic force microscopy**

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The goal of our research is to dissect, understand, and control the biology of single cells in complex tissues, such as brain, or in malignant tumors. Furthering this body of work requires that we address a problem in single cell molecular analysis: the lack of a method to routinely, reliably and inexpensively determine global gene transcriptional activity. To solve this problem we aim to directly identify individual cDNA gene transcript molecules via atomic force microscopy (AFM). Each molecule is 'coded' by in situ digestion with sequence-specific restriction enzymes which produce detectable breaks in individual polynucleotide backbones, whose locations correspond to particular enzyme recognition sequences. The advantage of this approach stems from: (1) the chemistry involves many fewer biochemical steps than do the alternatives, and (2) AFM's single molecule sensitivity, which allows enzymatic amplification to be eliminated. These improvements will significantly reduce time, cost and complexity of small sample transcriptional profiling.

**BIOT 429**

**Applications for electrically wired mitochondria: High throughput mitochondrial drug screening**
Mitochondria, typically dubbed the “powerhouse of the cell”, are in every cell of the human body and are solely responsible for the metabolism of energetic substrates and the production of energy. Mitochondrial drug toxicity is recognized as one of the leading issues of drug development spanning a wide range of diseases from diabetes to cancer. During drug development it is often difficult and time consuming to correlate a physiological effect of a drug at the cellular or organism level and its exact mechanism of action or inaction on the mitochondria. We have developed a method of wiring mitochondria to an electrode to analytically monitor their metabolic function in real time which can allow for the addition of a drug compound to evaluate its effect on the mitochondria.

**BIOT 430**

WITHDRAWN

**BIOT 431**

Multi-photon optical image guided spectroscopy for characterization of collagen materials modified by genipin

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We applied multiphoton microscopy to investigate the effects of a cross-linking reagent, genipin on the collagen materials assembled *in vitro*. Materials were incubated with genipin. We observed an increase in the *in situ* fluorescence within the materials during the course of genipin cross-linking. The fluorescence emission maximum was at 630 nm with the excitation wavelength at 590 nm. The emission maximum shifted to 590 nm when the excitation wavelength was at 543 nm. The rate of cross-linking with 10 mM genipin was 3.5 times faster than with 1mM genipin. Second harmonic generation (SHG) and transmission electron microscopy (TEM) imaging revealed that the structural morphology of collagen materials was modified by genipin. For example, genipin remodeled 10 µm fiber-like collagen structures to be about 28 µm in length as observed with SHG imaging. Also as observed with TEM, genipin eliminated the fibrillar striation - a characteristic of native collagen.
BIOT 432

Metabolomics for bioprocessing: Study design, method development and data analysis

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In recent years there has been a rise in –omics approaches to understand cellular phenotype with applications in bioprocessing. In particular, metabolomics involves the identification of small molecule metabolites which offer a more accurate snapshot of the cellular metabolism as a result of process and environmental parameters. Few instances in the literature have looked at quench and extraction methods to better preserve the cell metabolism. This work will emphasize the importance of a well hypothesized study design as a first step for the successful application of global metabolite profiling. Further, the development of a robust method for sample collection will be reviewed by discussing the impact of factors such as cell pellet handling, quenching of metabolism, time of exposure and cell pellet resuspension. The amount of data generated from such a study is typically large and insights will be provided on data analysis from sample studies.

BIOT 433

Correlating Raman identification of dental biofilm pathogenic traits using a spatially overlapping multimodal measurement

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Current methods in evaluating the capacity to quell corrosive acidogenic bacterial biofilm pathogenesis are primarily focused on either evaluating overall biofilm presence and/or viability. In the interest of time and sample preparation costs, most preliminary analyses of acidogenic biofilm pathogenesis are conducted in the absence of their targetted substrate. Although this approach saves time and money, it implements a bulk evaluation approach that limits the ability discern if a live biofilm is indeed exhibiting pathogenic properties, or is surviving in a non-pathogenic capacity. In an attempt to evolve analytical techniques to distinguish living biofilms in pathogenic and non-pathogenic modes, acidogenic dental pathogen Streptococcus mutans was used as a model. As the exhibition of pathogenic properties can be controlled by nutritive suplements, this study was
able to identify unique chemical signatures using Raman spectroscopy that are highly correlated to pathogenesis evaluated by other conventional techniques. These techniques require minimal sample preparation and may offer a rapid, low-cost, high-throughput system for evaluation of the capacity to affect bacterial biofilm pathogenesis.

BIOT 434

Heterogeneous amyloidosis between YE8 synthetic peptide and insulin

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Amyloid diseases are characterized by the aggregation of normally functioning proteins into ordered β-sheet rich fibrils, the process of which is not well understood: Researchers have shown that specific aminoacid segments are responsible for protein fibrillation and characterize the fibril spine, while others suggest that amyloid fibril formation and amyloidogenic oligomerization is a generic property of the polypeptide backbone. To address this, we have used YE8 (GH6[(GA)3GY(GA)3GE]8GAH6), a synthetic amyloid-like peptide, as a seed in solution of native insulin, a model amyloid protein. YE8 addition increased the fibrillation magnitude and decreased the pre-fibril incubation period (lag-time) by 2- and 0.9-fold, respectively. The aggregation was followed by both absorbance at 600 nm and Thioflavin-T measurements, with good agreement. Fibrils were characterized using atomic force microscopy and deep UV resonance Raman spectroscopy: The seeded fibrils showed features intermediate between the unseeded insulin and YE8 fibrils. Fluorescent probes were also used to understand the interaction between the two peptides.

BIOT 435

Electrochemical investigation of Shewanella spp. using electrodes as electron donors and electron acceptors

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Shewanella spp. catalyze electrochemical current generation by transferring electrons to electrode surfaces. Although it is postulated this occurs in a manner analogous to electron transfer to insoluble metal oxides, the molecular
mechanism of electron transfer to electrodes remains poorly understood. Other electrode-respiring bacteria (e.g. *Geobacter* spp.) appear to directly transfer electrons to electrodes via outer membrane cytochromes and/or conductive cellular appendages. In addition to direct electron transfer via redox-active proteins, *Shewanella* spp. have also been postulated to use endogenously produced electron shuttles (e.g. flavins) to indirectly transfer electrons to insoluble electron acceptors as well as electrodes. Here, we report on our study of multiple *Shewanella* spp. and corresponding deletion mutants tested for the ability to respire using electrodes as both terminal electron acceptors and donors. Voltammetric analysis is shown to provide insight into the molecular mechanisms of electron transfer utilized by *Shewanella* spp. at the microbe-electrode interface.

**BIOT 436**

**Chronoamperometric investigation of electro-active biofilms in biorefinery MFCs**

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Determination of current density in microbial fuel cells (MFCs) is usually done via closed circuit current measurements or via voltammetry techniques. As improvements are being made in bioelectrochemical systems (BES) for bioenergy production, a variety of electrochemical techniques are being investigated. Use of chronoamperometry (CA) to measure current densities of electro-active biofilms can reveal important information relevant for understanding steady state as well as dynamic behavior in these systems. Here, we report use of CA to measure maximum current densities in continuous flow systems suitable for practical implementation of BES systems. Use of MFCs to convert residual organics in biorefinery process water to bioelectricity has been shown. Current production as a function of substrate loading was investigated in the biorefinery process. Implications of the current densities achieved in high performance MFCs for water recycle, increasing energy efficiency and bioenergy production will be discussed.

**BIOT 437**

**Incorporating computational fluid dynamics methods for Quality By Design framework for exploring design space of a fermentor**

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In the Quality by Design (QbD) framework, there is a significant emphasis on the robust characterization of manufacturing processes and prospectively identifying the engineering design space that ensures product quality. Although the QbD approach is expected to bring about significant long-term benefits, there are concerns regarding the increased amount of characterization work that will be required upfront, which can be very resource intensive. In cases where the number of input variables is large, performing a design of experiments (DOE) with statistical significance may be impractical. Computational fluid dynamics (CFD) provides a first principles approach to gain insight into the hydrodynamics and mass transfer. The scope of traditional CFD methods can be extended to explore the engineering design space of the mixing process by coupling to statistical tools. In this talk, the authors summarize the results of a multivariate study of a prototypical fermentation process. And while a direct link to product quality cannot be provided by CFD alone, the authors hope to illustrate how CFD coupled with statistical methods can lower the risk of this unit operation having an adverse impact on product quality.

**BIOT 438**

**Lipase-catalyzed caffeic acid phenethyl ester synthesis in ionic liquids and its optimization**

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Although caffeic acid phenethyl ester (CAPE), an active flavonoid, which has an important role in the antioxidant activity, enzymatic synthesis of CAPE in organic solvents often suffers from low yields and productivity because of the poor solubility of caffeic acid (CA). In this study lipase-catalyzed synthesis of CAPE in ionic liquids (ILs) and the effect of reaction conditions on the yield of reaction were investigated. Among tested hydrophobic ILs, *[Emim][Tf₂N]* showed to be the best reaction media. The solubility of CA in *[Emim][Tf₂N]* was 4.6 times higher than in organic solvent (isooctane), which resulted in 2.5 times higher CAPE synthesis productivity compared to in organic solvent. Reaction conditions were optimized by response surface methodology which showed an optimal synthesis yield of 99.8% after 60 hrs at 73.7°C with the molar ratio of phenyl alcohol and CA of 27.1:1 and enzyme: CA weight ratio of 17.8 :1, respectively.

**BIOT 439**

**Preliminary experimentation for in-vitro cell culture of Watercress** *(Nasturtium officinale R.Br.)*
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Watercress (Nasturtium officinale R.Br.) is a plant with medicinal properties that could be a soil and sweet aquatic bodies bioremediator. An utility could be as tool in the biotechnological area, permitting the terpenoids biotransformation to compounds medicinally potentials. The objective in the work realized was find the ideal \textit{In vitro} cell culture conditions for the Watercress reproduction, which was employed for subsequent experiments in the environmental and biotechnological area. For established the \textit{In vitro} conditions were made studies varied the agar, culture salts and sucrose concentration observing the changes in the plants growing. After five observation weeks was determine that the best growing medium for Watercress plant was one medium containing eighty percent agar, 1.6250g/L Murashige & Skoog salts and 2ppm IAA/1ppm Kinetin hormones concentration ratio. The results suggest that this medium composition could be an option to obtain a good propagation of \textit{In vitro} Watercress.

BIOT 440

Physical and chemical requirements for entry into the nucleus of a cell during mitosis: Implications for non-viral gene delivery

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One of the major barriers facing non-viral gene delivery is the nuclear membrane. While it isthought that the majority of the DNA enters the nucleus during cell division, this hypothesis contradicts the fact that non-native macromolecules that are over 40 kDa are excluded upon nuclear reassembly. We explored the requirements for nuclear entry during mitosis with fluorescently labeled polystyrene particles that ranged in size from 50 to 200 nm in diameter. By functionalizing these particles with -COOH, -OH, and -NH\(_2\) terminated poly(ethylene glycol), we have evaluated how the surface chemistry and size of these particles alters access to the nucleus. These particles were compared with PEI/DNA complexes of similar sizes to examine the role of DNA in this process.

BIOT 441

Efficiency of laccases produced by \textit{Trametes} sp I-62 and \textit{Pycnoporus sanguineus} applied to Kraft pulps bleaching

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Nowadays, the application of laccases to the bleaching processes is widely studied because not only are they able to degrade lignin with high selectivity, but also to reduce the pollution levels in the bleaching effluents by means of increasing the efficiency during subsequent bleaching steps. To ensure optimal conditions during the enzymatic bleaching of the laccase produced by *Trametes* sp I-62, the enzyme stability at different pH and temperature values was studied. The enzymatic bleaching was then applied on *Eucalyptus globulus* kraft pulp using a laccase-mediator system. Then, an alkaline extraction and a hydrogen peroxide steps were carried out to evaluate the efficiency of enzymatic bleaching. Finally, a comparison using a laccase from *Pycnoporus sanguineus* is shown. Although both bleaching processes showed a reduction in the consumption of hydrogen peroxide, laccase from *Trametes* sp I-62 was more efficient in brightness increase than in delignification compared to laccase from *Pycnoporus sanguineus*.

**BIOT 442**

Collagen binding domains (CBD) fused to novel therapeutic proteins: Expression and isolation of CBD-parathyroid hormone

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Recent interest in the expression of a recombinant protein consisting of collagen binding domains and a parathyroid hormone has increased due to the therapeutic capabilities of the fusion as applied to the repair of eardrum tear / perforation. The production of this construct was investigated by testing both batch and fed batch cultures of *Escherichia coli* combined with glutathione-S-transferase (GST) affinity chromatography. Different medium formulations were tested in order to develop a defined medium lacking yeast extract or other difficult to validate materials. GST chromatography was optimized to provide purified product. Data indicate that high productivity of this novel fusion protein can be achieved by combining this expression and isolation strategy.

**BIOT 443**

Protein structure in solid-state environments
Proteins are widely used in the biotechnology industry as therapeutics to treat disease. A large fraction of proteins undergo a plethora of covalent and non-covalent changes upon storage in buffered solutions and require the use of solid-state formulations to prevent such deleterious modifications. Short-term storage strategies often rely upon freezing protein solutions and long-term formulation strategies usually utilize carbohydrate glasses as a matrix to maintain native protein structure and function. Methods to elucidate tertiary structure in such environments are limited. Small Angle Neutron Scattering (SANS) can be used to study the tertiary structure of proteins in solid materials and in this work we discuss recent measurements and modeling efforts to understand the structural changes of model proteins in ice and hydrogen-bonding carbohydrate glasses. The interaction distances measured by SANS and proposed model protein structures in different solid environments will be compared.

BIOT 444

Computer simulations of protein subdiffusion in the cell membrane

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The rate of protein diffusion in the cell membrane is related to some diseases and to medicinal uptake because it affects the rates of chemical reactions inside the cell. Proteins in the cell membrane have been found to be subdiffusive due to obstacles in the cell membrane and other factors. Our goal is to find a quantitative model of protein subdiffusion in the cell membrane that accounts for various membrane properties, and to use this model to predict protein subdiffusion in different types of membranes. To reach this goal, we simulate subdiffusion in membranes having different properties (such as obstacle density, excluded volume, and hydrodynamic interactions), and compare the different simulation's subdiffusion. We have found that independent increases in obstacle density and size both increase subdiffusion. The functional form of excluded volume has been found to be negligible in affecting subdiffusion. Preliminary results indicate that hydrodynamic interactions, surprisingly, decrease subdiffusion.

BIOT 445
Identification of SsfT1 as a pathway-specific transcriptional regulator for SF2575 production in heterologous host *Streptomyces lividans* K4-114

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SF2575 is an aromatic polyketide with potent anticancer activity produced by *Streptomyces* sp. 2575. The *ssf* biosynthetic gene cluster was cloned and successfully heterologous expressed in *Streptomyces lividans* K4-114. Based on bioinformatics analyses, two putative regulatory genes, *ssfT1* and *ssfT2*, were found in *ssf* gene cluster, and their products belong to *Streptomyces* antibiotic regulatory protein (SARP) family and TetR family respectively. Here we confirmed SsfT1 is a SARP transcriptional activator. Inactivation of *ssfT1* abolished SF2575 biosynthesis, while overexpression of SsfT1 led to 6-fold improvement of SF2575 titer relative to that of “wild-type” heterologous strain. Reverse transcription-PCR transcriptional analyses showed that at least 11 genes were under the positive control of SsfT1. These studies demonstrate the role of SsfT1 is the “key” to ignite the SF2575 biosynthetic machine.

**BIOT 446**

Development of a 5-(stearoylamino) fluorescein-based assay for studying the interaction of Hsc-70 chaperone with the endosomal compartments

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In order to understand the molecular basis for Hsc-70-mediated translocation of cytosolic proteins into the late endosomal compartments, we developed a fluorescence assay which uses 5-(stearoylamino) fluorescein to label the lipid bilayer of the late endosomal (LE) compartments isolated from human dendritic cells. This fluorescein derivative is an amphiphilic fluorescent probe used for monitoring membrane lipid behavior and its fluorescence is sensitive to the fluidity, the physical/chemical composition and the integrity of the lipid bilayer. We tested different LE: 5-(stearoylamino) fluorescein, and LE: Hsc-70 ratios and determined the maximum fluorescence emission at 525 nm upon excitation at 497 nm, as a function of the probe incorporation into the LE bilayer. An increase in the fluorescence signal at 525 nm was correlated with a higher amount of Hsc-70 incorporated win the lipid bilayer, attaining the saturation at 2:1 hsc-70/LE protein ratio. The incorporation of Hsc-70 into the LE membrane was validated by immunoprecipitation of the His-tagged-Hsc-70 bound to the LE and further
analysis of the lipid composition of the bound LE. We proposed that the 5-(stearoylamino) fluorescein based assay is a reliable tool for labeling the LE membrane and for further study of the proteins interactions or translocation into the endosomal compartments.

BIOT 447

Triggered complement activation using liposome flip-flop

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The complement system is a biochemical cascaderesulting in the lysis of an offending cell. The complement system is an important part of the innate immune system and is thought to play a role in cancer therapy and other diseases. Detailed is a method of preparing liposomes to unmask a hapten labeled lipid by mild hyperthermia. Heating liposomes to the phase transition temperature increased the flip-flop rate of the lipids, moving the hapten-lipid from the protected, inner layer to the exposed, outer surface. Antibody accumulation on the liposomes triggered the classical complement pathway, which was monitored through a hemolysis assay. It was observed that PEGylated lipids on the liposomes did not prevent antibody binding to the surface and subsequent complement activation. The effects of spacer length between the hapten and the liposomes on complement activation was examined. The kinetics of the flip-flop by the hapten-lipid was modeled by UV-Vis spectroscopy.

BIOT 448

Novel organic solvent stable biocatalysts from hypersaline environment

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Biocatalysis requires the use of stable and versatile enzymes that can be exposed to a vast range of reaction conditions and high concentrations of organic solvents. One of the most useful classes of biocatalysts is hydrolases due to their
high operational stability, chemo- and stereo-selectivity. Four metagenomic libraries and 150 microorganisms isolated from tropical solar salterns at Cabo Rojo, Puerto Rico were screened for the presence of hydrolases (proteases, lipases, esterases, and epoxy hydrolases). Microorganisms were cultivated in marine broth while metagenomic in LB medium containing 4.5% NaCl. The positive microorganisms were identified by colorimetric estimation of the released para-nitro phenol or para-nitro aniline using a microplate reader. Positives were tested for different hydrolase activities in organic solvents and stability over a period of 24 hours in 15%, 30% and 50% (v/v) concentrations of (dimethylsulfoxide, dioxane and acetonitrile). Results showed correlation between optimum salt concentration and stability to organic solvents.

BIOT 449

Carbohydrate response element binding protein, an important transcription factor

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Carbohydrateresponse element binding protein (carbohydrate response element binding protein, ChREBP) is a recently discovered an important transcription factors, it directly activates multiple genes involved in glycolysis and fat synthesis in the transcription, regulation of glucose metabolism and fatty acids synthesis.

BIOT 450

WITHDRAWN

BIOT 451

Lab scale and pilot demonstration scale studies of hydrothermal pretreatment of wood wastes and biosolids for slurry preparation for the steam hydrogasification (SHG) process

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A Hydrothermal pretreatment (HTP) process for wood wastes and biosolids was studied at both laboratory and demonstration scales. Wood wastes and biosolids were pretreated in a mini hydrothermal reactor, with an initial wood/biosolids ratio of 1:1.5. The resultant slurries had a viscosity of lower than 1.5 Pa·s at a shear rate value of 102 s⁻¹. Wood wastes and biosolids were also pretreated in a 5 gallon stirred batch hydrothermal reactor with a convective cooling system. The viscosity of resultant slurries was 10% lower than that of the comparable slurries from the mini HTP reactor. A continuous feedstock preparation system with a pretreatment capacity of 10 lb/hr was simulated using ASPEN Plus based on these experimental data. This system included 2 parallel operated HTP reactors. The energy cost of a Steam Hydrogasification (SHG) process with or without a HTP was then calculated. The economic benefit of using HTP in SHG process will be presented.

BIOT 452

Biomimics for the replacement of NAD/NADH mediators in biofuel cells

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Biofuel cells using immobilized cascades of dehydrogenase enzymes have been shown to produce exceptional power densities ranging in the milliwatts per square centimeter. One drawback to these biofuel cells is that they need the biological cofactor NAD to be dissolved in the fuel solution to act as a diffusive mediator between the enzyme and electrode. The compounds 1-benzylnicotinamide (BNA) and 1-benzyl-1,4-dihydronicotinamide (BNAH) have been shown to act as NAD/NADH mimics in the reduction of various ketones with alcohol dehydrogenase, and could be viable alternatives to the NAD/NADH couple in a biofuel cell. This study will investigate the use of the BNA/BNAH redox couple as a diffusive and/or immobilized mediator for NAD/NADH in biofuel cells. Also, because many dehydrogenase enzymes are reversible, the BNA/BNAH couple will be investigated for use in an enzymatic CO₂ reduction reactor to produce methanol.

BIOT 453

Genome-scale metabolic reconstruction of the homoacetogen Clostridium ljungdahlii and its application to microbial electrosynthesis

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Microbial Electrosynthesis (ME) is the process where microbes reduce carbon dioxide to multi-carbon compounds with energy donated from an electrode as the electron donor. Microbial electrosynthesis differs significantly from photosynthesis in that carbon and electron flow is primarily directed to the formation of extracellular products, rather than biomass. In this study, we describe a systems biology approach to characterize this process.

Reconstruction of genome-scale metabolic networks has become a common denominator in systems biology. Following the established protocol, we have reconstructed the metabolic network of an electrosynthetic organism, *Clostridium ljungdahlii*. The draft network comprises of 965 metabolic reactions encoded by 846 genes and 921 metabolites. This reconstruction captures all the major central metabolic, biosynthetic, and carbon dioxide fixation pathways among others. Furthermore, this reconstruction represents one of the first descriptions of key electrosynthesis pathways. The genome-scale model is interrogated using established computational approaches and will be validated based on physiological data. We will further employ *in silico* strain-design tools on the validated metabolic model in order to optimize butanol production under electrosynthetic conditions. This is the first reconstruction of a gram-positive acetogen and it will serve as a platform for strain design for other organisms capable of microbial electrosynthesis.

**BIOT 454**

Development of biosensor and their measurement

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This paper represents a biosensor system can be modeled by using system of enzymatic and analytical techniques.

**BIOT 455**

Lipase-catalyzed enantioselective synthesis of R-lactide from alkyl lactate to produce PDLA (poly D-lactic acid) and stereocomplex PLA (poly lactic acid)

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R-lactide, a pivotal monomer for the production of PDLA or stereocomplex PLA was synthesized from alkyl R-lactate through lipase-catalyzed reaction without racemization. Enantio-pure lactide needs to determine physical property of PLA. Among lipases, only lipase B from Candida antarctica (CALB; Novozym 435) was active in reaction for R-lactide synthesis. Extremely enantiopure R-lactide was synthesized by the catalysis of CALB but alkyl S-lactate is no consumption. Result of molecular dynamics (MD) simulation demonstrates enantiopreference of CALB in synthesis of lactide. The effect of the molecular sieve was significant on the conversion of lactide. The lactide conversion was elevated with increasing pore size of the molecular sieve. Other reaction conditions such as temperature, substrate, and organic solvent were optimized and reached 60 % conversion of R-lactide. This novel synthetic method can supply the important monomer R-lactide that is required for the production of the widely recognized bioplastic PDLA and stereocomplex PLA.

BIOT 456

Functional expression of recombinant anti-BNP scFv in methylotrophic yeast Pichia pastoris to detect the extremely low concentration of BNP

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Recent studies have revealed the potential of BNP as a prognostic marker for patient with heart failure. Antibodies against BNP are expected to be usefully employed in diagnosis of heart failures. We established a method to produce functional anti-BNP scFv using Pichia pastoris system. Although the N-terminal sequence of expressed anti-BNP scFv was analyzed that two Ste13 sites were not cleaved, the specificity of anti-BNP scFv was not affected significantly. Binding activity of anti-BNP scFv against other antigens was 10-3
times less compared with that of original BNP antigen. The anti-BNP scFv based electrochemical immunoassay exhibited excellent analytical performance in terms of a detection limit of 0.18 fg/mL and a wide linear detection range of 1 to 10,000 fg/mL. This study will be used from the efficient production of scFv, and anti-BNP scFv engineered antibodies have promised to be further view of application in the diagnosis of heart failure.

BIOT 457

Novel approach to characterize dissolved oxygen reduction in an anaerobic fermentation system using the Mass Transfer Theory

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The rate at which fermentation medium reaches an anaerobic state can be impacted by many variables. To test this impact, an inefficient empirical approach is frequently used in order to demonstrate that the system has reached a sufficiently low reduction level required to support anaerobic growth. A highly efficient, fundamental approach based on mass transfer theory is presented here. A mass transfer model was built and validated in order to describe the reduction process. A series of experiments were performed at small scale (spinner flask) in order to demonstrate model accuracy and predictability power. The anaerobic fermentation reduction process was successfully characterized by using this fundamental approach. This concept of applying first-principle science to process development can eliminate the trial and error approach, thus saving resources, time, costs, and can be tested using only medium without the necessity for anaerobic culturing. The predictive model can be easily utilized for process technology transfer, scale-up, and scale-down at different sites and laboratories.

BIOT 458

Genetic and biochemical investigation of tryptoquialaninebiosynthesis in Penicillium aethiopicum

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Tryptoquialaine is a tremorgenic mycotoxin which belongs to quinazoline-containing indole alkaloids. Tremorgenic mycotoxins are capable of eliciting intermittent or sustained tremors in vertebrate animals and are a major source of mycotoxin contamination in grains and livestock feeds. The biosynthesis of tremorgenic quinazoline alkaloids, such as Tryptoquialanine, is poorly understood. Recently, we used 454 sequencing technology to gain partial genome information of Penicillium aethiopicum. We identified a compound in the extracts of P. aethiopicum that has the same UV absorbance and mass to tryptoquialanine. Therefore, the availability of the genome sequence data presented an excellent opportunity to study the biosynthesis of this family of fungal indole alkaloids. In this abstract, we present the identification and verification of the tryptoquialanine gene cluster, the functional assignment of the individual genes through genetic and biochemical approaches, and insights into the unique structural features of tryptoquialanine.

BIOT 459

Application of mass transfer theory to characterize oxygen reduction in anaerobic fermentation medium

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The mass transfer of oxygen using kLa is a commonly used method for estimating the amount of dissolved oxygen in an aerobic fermentation, such as the production of expressed proteins from recombinant E. coli. In contrast, the cultivation of anaerobic microorganisms requires growth medium that is free of oxygen. In order to achieve an oxygen free state, the anaerobic medium must go through reduction to decrease the redox level prior to fermentation initiation. Redox values give an indication of the relative reduction potential of the fermentation medium and can be measured and monitored using an online redox probe. Based on the mass transfer theory, a reduction model was created which successfully described and predicted the decrease in redox level during the medium reduction process. The model successfully predicted and characterized the growth medium reduction process, and was confirmed and verified through experimentation. In this presentation, the anaerobic fermentation medium reduction model is presented, and the results obtained during experimentation are described.

BIOT 460

Enhanced production of fungal polyketides in Saccharomyces cerevisiae
The yeast *Saccharomyces cerevisiae* is a promising host for the synthesis of fungal polyketides. Using 6-methylsalicylic acid (6-MSA) and dihydromonacolin L (DML), a precursor to lovastatin, as model polyketides, we have studied the effects of host strain attributes and precursor availability on the level of synthase production and product synthesis. Native metabolic pathways in *S. cerevisiae* were up-regulated to enhance precursor production, and the expression of heterologous polyketides synthase genes as well as other accessory genes were evaluated and optimized. The various expression systems and their effects on polyketide production have been characterized.

**BIOT 461**

**Optimizing MAB and vaccine processes with simulation and scheduling tools**

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The successful development, scale-up, design, and optimization of integrated biochemical processes is a challenging task that requires the collaboration of professionals from many disciplines. Process simulators and other computer aids can facilitate this task by introducing a common language of communication among the various teams involved in process development and product commercialization. They can facilitate answers to the following questions: What is the impact of product titer increase (through media change) on the capacity load of the downstream section, the overall throughput of a plant, and the total manufacturing cost? Can the cost of expensive chromatography steps be reduced through changes in preceding concentration steps? What is the impact of changes in scheduling and cycles times on the demand for resources (e.g., labor, utilities, raw materials, etc.) and the overall throughput of a plant? Our experience in addressing the above questions will be presented using industrial examples in which we evaluated alternative technologies for producing monoclonal antibodies and vaccines.

**BIOT 462**

**Production planning, scheduling, and debottlenecking practices in the biopharmaceutical industries**
This paper presents industrial experience with a resource-constrained batch process scheduling tool. The scheduling algorithm is a non-optimization approach that proceeds in two steps. First a bottleneck analysis is done to determine a lower bound on the process cycle time, and all the batches are scheduled accordingly. Second, if conflicts remain, they are resolved by applying progressively aggressive modifications to the schedule. This approach to scheduling was tested on several biotech processes. The scheduling challenges in biotech processes lie in the ancillary operations: media and buffer preparation, vessel and line cleaning, and chromatography column preparation. Such operations may use shared resources that can couple process suites with otherwise dedicated equipment. Three case studies, which are based on a process for the manufacture of monoclonal antibodies (MABs), illustrate the value of a constrained-resource scheduling tool for biotech processes.

**BIOT 463**

Use of computational fluid dynamics to calculate flow patterns inside bioreactor headspace for evaluation of impact on vent filter plugging

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This presentation describes use of bioreactor headspace computational fluid dynamic model to evaluate vent filter location and liquid level effects on the flow patterns created by the sparge and overlay air. These flow patterns help (or prevent) entrainment of liquid droplets generated by bursting bubbles at the liquid surface causing wetting out and plugging of sterile vent filters. Goal of this study was to evaluate the effect of operating level, air overlay inlet and bioreactor exhaust nozzle location on gas flow patterns and droplet entrainment to identify nozzle locations with less potential for vent filter fouling. Singlephase turbulent computational fluid dynamic models of headspace gas were created and velocity vectors of the converged flow field were closely examined. The study showed that a vent filter located either on the top of the bioreactor or above the overlay air inlet would minimize flow patterns expected to transport liquid to the vent filter thus minimizing the risk of vent filter plugging. Also, it was found that an 18 cm change in liquid level could change the flow pattern from one expected to entrain to one expected to de-entrain droplets. Learnings from this study proved to be critical in mitigating vent filter plugging issue at pilot scale.
Development of *Saccharomyces cerevisiae* strains for the high-level synthesis of short chain fatty acids

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The need to shift from petroleum feedstock to a renewable carbon source is becoming more evident to maintain long term sustainability within the chemical industry. The Center for Biorenewable Chemicals (CBiRC) is investigating polyketide / fatty acid biosynthesis pathways in *Escherichia coli* and *Saccharomyces cerevisiae* as a source of renewable carbon precursors. These pathways have the ability to produce short chain carbon molecules; which through chemical catalysis, can substitute for current petroleum based chemicals. In our lab, we are using *Saccharomyces cerevisiae* as a recombinant host for the production of short chain fatty acids. Currently, we strive to increase fatty acid production by eliminating degradation pathways and by overexpressing substrate and co-factor molecules. We have up- and down-regulated key control elements, as well as expressed exogenous synthase systems with unique properties for the optimization of fatty acid production. Our goal is to harness this yeast for the high-level synthesis of short chain fatty acids.

Quantitative screening system of β-glucuronidase genes using unilamellar liposomes and cell sorter

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Here we report a quantitative gene screening system using unilamellar liposomes as micro-compartment and a fluorescence activated cell sorter (FACS) as a tool for analysis and selection. In order to assemble the chemical system for gene screening, a PURE system of cell-free gene transcription/translation system, DNA molecules encoding β-glucuronidase (GUS; wild-type GUS or SNAP-tagged GUS), and a fluorogenic substrate for detection and selection were encapsulated in unilamellar liposomes. FACS analysis of the GUS synthesis showed that the reaction efficiency of wild-type GUS (3% of the whole observed events) was 30
times higher than that of SNAP-tagged GUS. Liposomes encapsulating wild type GUS-DNA were sorted selectively using FACS on the basis of fluorescence intensity of reaction products and the liposome size. Wild-type GUS genes were enriched to several tens of times from a 1:9 mixtures of liposomes, in which wild-type GUS genes or SNAP-tagged GUS genes were enclosed separately.

BIOT 466

Disposable vs. conventional reusable glass bench-scale bioreactors: Performance assessment and economical analysis

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Driven by advantages such as lower upfront capital investments and fewer requirements for process validation, an increasing number of biotech companies, especially new start-up and new entering companies, are building large-scale (200L to 2000L) clinical manufacturing facilities using disposable Stirring Tank Reactors (STRs). Compared to the large-scale disposable STRs, however, bench-scale disposable stirring tank reactors available on the market for the process development stage are relatively immature in terms of its design and application. There has not been much literature reported on the performance of bench-scale disposable stirring tank reactors as well as economical analysis of applying them for cell culture process development. From an operational perspective, disposable stirring tank reactors offer the unique advantages of eliminating the need for expensive autoclaving equipments, shortening turnaround time and reducing labor efforts. Agensys Process Development group evaluated the first generation Millipore Mobius® CellReady disposable bioreactors. After encountering various technical and operational challenges such as unstable agitation shaft and lack of sufficient addition lines and gas overlay, feedbacks were given to Millipore for improvement for the design of the second generation Mobius® CellReady. To continue evaluating the possibility of replacing the conventional glass bioreactors, a study was done using the second generation CellReady to fully characterize bulking mixing time, oxygen transfer and carbon dioxide removal. Bioreactor runs were also performed side by side using the conventional glass bioreactors and the second generation CellReady to compare their cell culture performance with three independent cell lines. Finally, we will demonstrate the preferable situation of applying the bench-scale disposable STR over the conventional glass bioreactors through cost analysis.

BIOT 467

Evaluation of scale-down to novel stirred high-throughput mini-bioreactors using genomic profiling of a hybridoma cell-line
The novel stirred mini-bioreactors with non-invasive optical sensing, and ability of parallelization, could be an economical alternative to the existing bench-scale bioreactors. Although several methods exist for process scale-down such as matching $k_{La}$, P/V, mixing times, and oxygen transfer rates, the choice of the physical parameter is empirical and cell-line dependent. In our study using hybridoma cells, we have evaluated a 150-fold scale-down by matching the oxygen-transfer rates between 5L bench-top reactor and mini-bioreactor. Discrepancies in final product titer and to a lesser extent in cell growth were observed, which were further examined using time-course DNA microarrays to probe at the cellular level. We present here a novel method for evaluating scale-down using profiles of signature genes that are related to various cellular functions. The expression of these identified genes in scale down devices can then subsequently be compared using PCR to profiles in a control 5L system.

BIOT 468

Two-in-one antibody: From proof-of-concept to therapeutic candidate

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To expand the utility and potential of antibody as therapeutic, we explored whether antigen-binding site of an antibody can bind two unrelated protein antigens with high affinity. We took the approach of evolving a mono-specific antibody into dual specific antibodies and validated a strategy of repertoire selection by first generating a phage-displayed library of antibody variants with mutations in the light chain CDRs and next selecting those variants that can bind to a new antigen while retaining its original binding specificity. The first such dual specific antibodies are evolved from Herceptin and bind both HER2 and VEGF. Crystallographic and mutagenesis studies of a HER2/VEGF dual specific Fab revealed that 1) the antibody surface areas contacting HER2 and VEGF overlap extensively and 2) the antigen-binding residues contribute energetically for the two antigens are highly distinct. The dual affinity in nanomolar and sub-nanomolar range was achieved and translated into dual action in vitro and in vivo. The results demonstrate 1) the capability of antibody-combining site to interact with two unrelated antigens with high affinity and 2) a strategy that can be applied to generate other dual specific antibodies toward two defined targets.
The application of the strategy to generate a therapeutic candidate that target HER3 and EGFR with a single molecule as conventional IgG will also be discussed.

BIOT 469

Development of therapeutic proteases with novel substrate specificities by directed evolution and genetic selection

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Site-specific proteases hold enormous potential as therapeutic agents due to their ability to initiate the degradation of peptides and proteins responsible for pathogenic states in human diseases. However, endowing proteases with tailor-made substrate specificities is the principle challenge to developing such therapeutic interventions. We have developed proteases possessing enzymatic activities towards novel substrates by using a bacterial genetic selection system and principles of directed evolution. This system was initially validated and characterized by evolving a Tobacco Etch Virus protease mutant targeting a substrate weakly recognized by the wild-type enzyme. The candidates that emerged were characterized by qualitative growth tests, as well as by quantitative assays that employ transcriptional reporters and fluorogenic substrates. We have implemented this strategy to develop therapeutic proteases that recognize the amyloid-beta peptide, a peptide catabolite linked to the pathogenesis of Alzheimer's Disease.

BIOT 470

Mutagenesis of the lasso antibiotic peptide Microcin J25: Discovery of variants with increased potency and new insights into the structure-activity relationship

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Infections caused by drug-resistant Gram-negative bacteria pose a rising threat as some strains become ever more resistant to virtually all existing antibiotics. Microcin J25 (MccJ25) is a ribosomally-synthesized antibacterial peptide isolated from E. coli that exhibits potent mechanisms of action against pathogenic strains of Salmonella, Escherichia coli, and Shigella. MccJ25 has an unusual threaded
lasso structure in which the C-terminal “tail” of the peptide is fed through a macrocyclic “ring” formed by the N-terminal residues. Production of MccJ25 in *E. coli* is dependent on a four-gene cluster encoding the structural gene *mcjA*, two maturation enzymes *mcjB* and *mcjC*, and an immunity factor, *mcjD*, in the form of an MccJ25 export pump. Here we have developed a system for orthogonal control of the expression of the structural gene and the export pump thus permitting independent control of MccJ25 production and export/immunity in *E. coli*. We used this system to screen saturation mutagenesis libraries targeted to the ring and tail portions of MccJ25. While multiple amino acid substitutions in the tail portion of the peptide were well-tolerated, mutagenesis of the ring portion of the peptide was detrimental to the antimicrobial function of MccJ25. We demonstrated that substitutions in the ring portion of the peptide likely disrupt the transport of these variants into the cytoplasm of susceptible strains. Additionally, we found several MccJ25 variants from the tail library with up to nearly 5-fold improvement in potency toward the strains *E. coli* and *Salmonella enterica* serovar Newport.

BIOT 471

Proteolytic silencing of intracellular targets by engineered ubiquitin ligases with expanded substrate specificity

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The ubiquitin-proteasome system (UPS) is the main route of protein degradation in eukaryotic cells and is a common mechanism through which numerous cellular pathways are regulated. Protein clearance by the UPS involves covalent tagging of target proteins with ubiquitin chains that are specifically recognized by the proteasome. We hypothesize that the UPS can be intentionally redirected to accelerate the degradation of otherwise stable cellular target proteins. To test this, we have developed a general protein silencing technology whereby the last enzyme in the UPS pathway, E3 ubiquitin ligase, is genetically fused to targeting antibodies that bind tightly and specifically to targets of interest. The resulting chimeras, which we call “ubiquibodies”, enable the targeted degradation of virtually any intracellular protein. This technology should enable: (i) dissection of protein function in somatic cells; (ii) identification of novel pharmacological targets; and (iii) selective targeting of disease proteins that underlie numerous human disorders.

BIOT 472

Engineering cells to death
A group of cellular proteases known as caspases are responsible for a number of fate-determining steps in higher eukaryotic cell biology including cell death, innate inflammatory responses, cell differentiation, and many other cellular remodeling events. My lab has been developing engineered enzymes to identify the proteins that caspases cleave, and generating new enzymes and small molecules control specific caspase activities in cells. These new tools allow us to dissect the roles these cellular remodelers play, as well to generate new protein and small molecules that may be useful in diagnosing and controlling cancer and inflammation.


BIOT 473

Combinatorial methods for engineering antagonists of the Axl receptor tyrosine kinase

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The Axl receptor tyrosine kinase and its activating ligand Gas6 have been implicated in the progression and metastasis of many forms of solid tumors. Disrupting the Gas6-Axl interaction offers a new strategy for cancer treatment. Using two separate approaches, we developed Axl variants with increased binding to Gas6, creating “decoy” receptors that bind to and neutralize Gas6 ligand. First, we used yeast surface display to engineer Axl variants that bind with 20-fold better affinity to Gas6 than wild-type Axl. Second, we developed a novel technique for improving native binding interaction through domain addition and evolution. We fused a small, folded peptide domain to Axl and used yeast surface display to engineer this synthetic domain for binding to Gas6, generating Axl-peptide fusions with 4-fold improvements in Gas6 binding over wild-type Axl. We are currently testing the ability of these engineered proteins to inhibit metastasis in murine tumor models. Funding: Coulter Foundation

BIOT 474
Motif-grafted, conformation-specific antibodies for selectively targeting toxic misfolded proteins

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The misfolding of proteins in several neurodegenerative disorders results not in a single folded structure, but rather a spectrum of aggregated conformers with unique secondary and tertiary structures. Antibodies that target specific aggregated conformers are of broad interest, yet generating such antibodies is extremely challenging. We are developing a motif-grafting strategy whereby peptide segments known to regulate condensation of several aggregation-prone proteins are inserted into the complementarity determining regions (CDRs) of antibodies as a novel approach to generating conformation-specific antibodies. In this presentation, we will discuss our development of motif-grafted antibodies specific for targeting toxic aggregates of the amyloid β peptide associated with Alzheimer's disease and infectious amyloids of the yeast prion Sup35. We find that motif-grafted antibodies are powerful structural probes for illuminating the conformational differences between toxic and non-toxic protein aggregates. We expect our motif-grafting approach will be useful for generating conformation-specific antibodies against diverse toxic misfolded proteins.

BIOT 475

Future advances in development of chromatography platforms for purification of monoclonal antibodies

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Downstream processes might soon be required to rapidly recover and purify 100 kg lots in an economical and robust way. This presentation will focus on the development of new chromatography resins and their ability to handle these challenges from both a technical and productivity perspective. Real application data from a new generation of chromatography resins in development, including ion exchange, multi-modal, and high capacity protein A resins, will form the basis of the presentation.

BIOT 476

Formulation factors affecting metal leachables from stainless steel
Purpose: Evaluate the effect of biologics formulation and process factors on metal leachables from 316L stainless steel (SS). Methods: Passivated 316L SS coupons and 20 mM buffers were utilized. Four major factors were investigated: 1) Buffer species; 2) The relation between fill volume and SS contact surface area (SA); 3) Metal chelators of Na$_2$EDTA and DTPA; and 4) pH. Levels of iron, chromium and nickel were determined by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Na$_2$EDTA and DTPA concentrations were determined by RP-HPLC. Results: Among the three monitored metal leachates, iron is the most abundant. For most conditions, chromium and nickel were below their limit of quantitation. Thus, iron was used to evaluate the impact of each factor. Buffer species exhibited a temperature dependence in their impact on metal leachates. At -40°C, phosphate exhibited the highest impact. At 25°C, the iron leachates were highest for citrate followed by phosphate > succinate > histidine-HCl > histidine-Acetate. At -20°C and 2-8°C, no difference was observed among the buffer species. Similarly, pH also exhibited a temperature dependence. At 25°C, pH 5.0 extracted the highest level of iron while no difference was observed at pH 6.0-9.0. At 2-8°C, the impact was pH 5.0 >> pH 9.0 > pH 6.0 ~pH 7.0. The metal leachates decreased when the ratios of fill volume to SS contact SA increased but plateau was observed when the ratio reached 3:1. Na$_2$EDTA and DTPA produced the strongest effect in facilitating metal leaching from SS. Furthermore, Na$_2$EDTA showed stronger impact than DTPA at equal molar concentrations. Conclusions: Each studied factor has exhibited its own effect on the ability to leach metal from SS. This study elucidates the impact of various factors that would be important to consider when developing formulations, manufacturing and storage options for biologics.

BIOT 477

Distinct aggregation mechanisms of monoclonal antibody under thermal and freeze-thaw stresses revealed by hydrogen exchange

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Aggregation of monoclonal antibodies (mAbs) is a common, yet poorly understood issue in therapeutic development. In this study, we have used hydrogen exchange mass spectrometry (HX-MS) to investigate the structure and
aggregation mechanism of bevacizumab under freeze-thaw (F/T) and thermal stresses. F/T operation led to aggregation of bevacizumab dependent on number of cycles and protein concentration. HX-MS showed the aggregates to be made up of native-like monomers. Direct measurements of HX in the frozen state demonstrated that the molecule retained its native structure in the freezing step. Conversely, thermal stress triggered non-native aggregation at temperatures below the melting point of the least stable domain. Notably, the Fab fragment was implicated to be critical for the non-native aggregation by the pronounced HX changes observed only in this domain after aggregation. Fluorescence spectroscopic analysis supported the structural differences between aggregates formed by F/T versus thermal stresses.

BIOT 478

Frozen state aggregation of a monoclonal antibody: A consequence of excipient crystallization?

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The temperature for frozen state storage of therapeutic protein is an important parameter. Over the last few years, erratic increase in soluble aggregates levels was observed for several antibodies when stored at -20°C over long term. In order to understand the cause of this erratic increase and to understand the fundamentals of the solute freezing process in large scale systems, we perfomed systematic mapping studies of the frozen material. Stability studies of frozen cores, based on this mapping, were initiated to understand the cause of aggregate formation at various frozen storage temperatures. Our results over 15 months show that the aggregation occurred at -20°C irrespective of protein concentration. On the other hand, no substantial increases in aggregates was observed for other frozen storage temperatures (-10°C and -40°C). Excipient (trehalose) crystallization was investigated as a possible cause of aggregation observed at -20°C. No change were seen at -40°C, while growth of crystals was apparent at -20°C and -10°C in all samples, indicating trehalose crystallization. These results as well as possible hypotheses for the observations on aggregation will be presented. It is also important to note that the significant technical difficulties in studying a system in the frozen state, has limited the fundamental studies in this area.

BIOT 479

Aggregation of monoclonal antibodies in purification process intermediates during frozen storage
Process intermediates from purification of therapeutic proteins are often frozen prior to sample analysis or further experiments, as it is assumed that the samples do not change over time while they remain frozen. Several monoclonal antibody programs showed increased aggregation in some frozen intermediates, so the effects of various parameters associated with sample freezing, storage, and thawing were investigated. Duration of the frozen storage, as opposed to freeze-thaw cycling, appears to be the main cause of the aggregation. While it is frequently assumed that samples frozen at a lower temperature are more stable, we found that process intermediates often aggregate during storage at -70 °C, but not at -30 °C. Above 10-20 g/L, aggregation declined with increasing protein concentration in the cases that were examined. Results from differential scanning calorimetry suggest that crystallization of buffer components and salts plays a key role in the aggregation. Sample volume, amount of headspace, and air-liquid interface area were less significant. One solution identified to prevent aggregation during frozen storage is cryoprotectant addition. In cases where sample conditions cannot be modified, a properly designed hold stability study can indicate the optimal storage temperature.

BIOT 480

Characterization of product rheology and syringe component variability for delivery systems

Prefilled syringes and auto-injectors are becoming increasingly common for parenteral drug administration primarily due to the patient convenience they offer. High concentration products however challenge the functionality of such delivery systems and require thorough characterization for successful commercialization. In this work, we characterized the impact of various parameters on the delivery forces associated with syringe injection. More specifically, the effect of barrel size, needle size, frictional forces and product interaction with syringe barrel has been evaluated. Rheological behavior of high concentration products is also explored. Data suggest significant non-Newtonian behavior for high viscosity products. An analytical model based on underlying physics has been developed.
that can be used to fully characterize the design space for a product delivery system.

**BIOT 481**

**Challenges and opportunities of MAbs delivery**

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Generally, MAbs require a higher dosage (say, 100-1000 mg/dose) than typical protein therapeutics. It is commonly accepted that subcutaneous (SC) injections over 1 mL cause skin distortion and pain. As a result, most biotech companies spend much effort concentrating MAbs to 100 mg/mL or more and then try to stabilize these formulations to avoid aggregates and particulates. Halozyme’s Enhanze™ technology permits subcutaneous dosing much greater than 1 mL per injection, which enables bypassing the formulation challenges associated with achieving high concentrations. Enhanze technology enables conversion of intravenous (IV) drugs to SC administration, which may benefit patients’ convenience and compliance. A prototype 10-mL SC delivery device, based on an established disposable IV-infusion pump technology, will also be presented as an example for how patients could potentially self-administer MAbs.

**BIOT 482**

**Technology transfer to a new drug product manufacturing facility: A case study in quality by design**

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In the Quality by Design (QbD) framework there is increased expectation of robust characterization of manufacturing processes. At Genentech, we are adopting sound risk-based approaches that leverage mechanistic understanding of unit operations and experimental designs to identify the relevant design space. We present an application of the above methodology to technology transfer of a liquid and a lyo monoclonal antibody formulation to a new drug product manufacturing facility. The approach enables identification of robust design space and excursion spaces for the relevant unit operations.

**BIOT 483**

**Rational design of recombinant phytochemical microbial producers**
For several years, an important source for drug leads has been natural products: more than half of the drugs currently in clinical use are either natural products or their analogues with a significant fraction of these compounds derived from plant sources. Our group focuses on utilizing the richness, versatility but also simplicity of microbial organisms in order to make them ideally suited to convert cheap, renewable resources into high-value, high-quality chemicals with enormous potential as nutraceuticals and pharmaceuticals. For the purpose of reprogramming the cellular network in order to achieve optimal phenotypes supporting high-yield production, we have developed in silico models of the genome-wide metabolism of the two industrially important microorganisms such as Escherichia coli. Through the application of Metabolic Flux Analysis, we can predict genetic modifications such as deletions and gene expression attenuations that lead to dramatic increases in production levels. Such Systems Biology approaches, in combination with traditional genetic engineering have resulted in robust production levels that can result in the commercially viable processes for the synthesis of important molecules, in particular ones that belong to the plant polyphenol category. Combination of biochemical processes through the engineered microbial strains with classical mutasynthesis feeding experiments have also led to the generation of novel polyphenol molecules with promising therapeutic properties, in particular antifungal activities. Overall, our work has allowed us to develop microbial Systems Biology and Metabolic Engineering approaches for the efficient production of natural products that can be extended to other small molecules of pharmaceutical and industrial importance.

BIOT 484

Enzyme evolution of an alpha-keto decarboxylase

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The thiamin diphosphate-dependent decarboxylase family of enzymes can be exploited to develop biological pathways for the production of a variety of renewable products including biofuels and commodity chemicals. This family of enzymes has a broad substrate specificity that can be extended to produce new metabolites. We report the formation of malonate semialdehyde via non-oxidative alpha-decarboxylation by an engineered decarboxylase. Furthermore, we have used this enzyme in conjunction with an alcohol dehydrogenase to produce 3-hydroxypropionic acid, a precursor of the industrial monomer acrylic acid.
Azide-bearing amino acid incorporation into recombinant proteins in *E. coli*

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Unnatural amino acids can be incorporated into recombinant proteins *in vivo* through the use of engineered aminoacyl-tRNA synthetases (aaRSs). In studies conducted to screen methionyl-tRNA synthetase (MetRS) mutants that allow for the incorporation of the unnatural amino acid azidonorleucine (ANL), a MetRS variant with a leucine to glycine (L13G) mutation in its methionine binding pocket was identified. Methionine auxotrophic and prototrophic bacteria were engineered by replacing their genomic MetRS allele, *metG*, with the mutant synthetase gene, *metG*\(^{*}\), which encodes for the MetRS L13G variant. The resulting engineered strains were able to replace methionine with ANL at high levels. Both engineered auxotrophic and prototrophic strains produced more ANL substituted protein than the wild-type strains with plasmid-borne copies of *metG*\(^{*}\) in addition to genomic copies of native *metG*. The incorporation of azide-bearing amino acids can be utilized for various applications using the engineered MetRS L13G.

**BIOT 486**

Design and applications of customized regulatory proteins as molecular reporters in high throughput screening of novel biocatalysts

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Customized metabolite-responsive gene regulators find applications as molecular reporters and as novel gene control tools in synthetic biology and metabolic engineering. In this study the AraC regulatory protein of *E. coli* was engineered to control gene expression in response to mevalonate and to triacetic acid lactone (TAL; 4-Hydroxy-6-methyl-2-pyrone). Residue positions in the AraC binding pocket were first randomized and mutants were expressed in *E. coli* cells containing a pBAD-GFP reporter construct. AraC variants having desired regulatory properties in the presence and absence of the effector of interest were isolated via FACS. Our customized mevalonate reporter system was used to screen for improved mevalonate production in *E. coli* harboring a heterologous mevalonate synthesis operon by randomizing gene ribosomal binding sites. Colony screening yielded an RBS mutant resulting in ~10-fold improved mevalonate production. Similarly, the TAL reporter was used to screen for
improved TAL synthesis in *E. coli* expressing the *Gerbera hybrida* 2-pyrone synthase (2-PS). Random mutagenesis of the 2-PS gene led to improved TAL production and identification of substrate binding pocket residues tolerant to mutation. Subsequent saturation mutagenesis at those positions and screening resulted in further enhanced TAL production. Random chromosomal transposon insertions were also screened and yielded a mutant with ~15-fold improved TAL production. Biochemical characterization of the 2-PS mutants and host chromosome modifications will be discussed.

BIOT 487

Role of structure in engineering an improved commercial glucoamylase

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Glucoamylases (glucan 1,4 alpha glucohydrolases are exo-acting catalyzing the removal of successive glucose units from the non-reducing ends of starch. The glucose produced from starch using glucoamylase are used in large scale fermentations to produce fuel ethanol. There is a need to produce new glucoamylases with improved specific activity. The glucoamylase from Hypocrera jecorina (aka Trichoderma resei) is an excellent starting point for such an engineering effort, having a higher specific activity that is twice that of the glucoamylase from Aspergillus awamori. The three-dimensional structure of this enzyme with an intact starch binding domain was recently determined. The starch binding domain forms a stable complex with the catalytic domain to form an extended binding site. The variant, comprised of substitutions at sites selected on the basis of this structure and related structures, having the best overall performance included a combination of sites from both the catalytic and starch binding domains.

BIOT 488

Characterization and directed evolution of FrbF for combinatorial biosynthesis of FR-900098

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FR-900098 is a novel antimalarial agent that blocks isoprenoid biosynthesis in the parasite *Plasmodium falciparum* via inhibition of DXP reductoisomerase. Our recent studies have focused on FrbF, which catalyzes acetyl transfer from acetyl-CoA to the growing phosphonate compound. Kinetic parameters have been determined with multiple substrates, and alanine scanning has elucidated the catalytic mechanism. To generate novel FR-900098 derivatives, three strategies are being investigated. First, we have selected FrbF as a target for combinatorial biosynthesis by directed evolution. We have developed a high throughput screening method using Ellman's reagent to detect FrbF activity toward novel CoA substrates, including malonyl-CoA. Second, we are employing a metabolically engineered *E. coli* strain to increase intracellular levels of alternate FrbF substrates. Third, we are utilizing the methyltransferase Dhpi from the dehydrophos biosynthetic pathway to further diversify product formation. To evaluate novel FR-900098 derivatives, DXP reductoisomerase from *P. falciparum* has been heterologously expressed and characterized.

**BIOT 489**

**Substrate specificity change of a dehydrogenase**

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Biocatalysts are increasingly used in industry to create enantiomerically pure compounds. Enantiomeric compounds have a broad range of uses including an utmost importance in pharmaceuticals. Biocatalysis employs enzymes to produce enantiomerically pure compounds circumventing the difficulties of current production methods. Contemporary methods of protein engineering, such as applying rational design guided by mechanistic and structural knowledge, have greatly increased the ability to create novel enzymefunctionality. We have developed a novel amino acid dehydrogenase (AADH) with broad substrate specificity. Thenovel amino acid dehydrogenase was developed from an existing amino acid dehydrogenase scaffold. Expansion of its substrate specificity was achieved through several rounds of iterativesaturation mutagenesis. Constraint ofmutant library size was guided by mechanistic and structural knowledge, ultimately reducing the screening requirements while maintaining an increased chance of generating alternate substrate specificity.

**BIOT 490**
Behaviors and impacts of lignin during bioconversion of lignocellulose to ethanol

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Lignin is the major obstacle to enzymatic hydrolysis of lignocellulose. This study comprehensively discusses the impacts and changes of lignin during bioconversion of lignocellulose. Lignin retards the hydrolysis of lignocellulose by acting as both a physical barrier, restricting the access of cellulases to cellulose, and an irreversible adsorbent to cellulases, resulting in non-productive binding. Irreversible adsorption of enzymes also limits the enzyme recycle and reuse. It was apparent from the studies that lignin content and distribution have an impact on enzymatic hydrolysis. It has also been demonstrated that the inhibitory effect of lignin is not only depending on its content but also its structural features, such as hydrophilicity and functional groups. Novel approaches for removing the recalcitrance are briefly discussed. One key step in the bioconversion of lignocellulosic materials is the pretreatment. Chemical reactions, structural changes, and physicochemical properties of lignin during the pretreatment are addressed.

BIOT 491

Integrated production of levulinic acid and furfural from cellulosic biomass

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Levulinic acid and furfural have been identified as leading reactive intermediates to produce from cellulosic biomass and support conversion of these versatile building blocks into a wider range of derivatives. In this study, these two products were targeted as the main products from acid pretreatment and hydrolysis of C6 and C5 sugars, respectively, in cellulosic biomass. The kinetics of levulinic acid and furfural production were studied and applied to optimization of reactor designs. Reactions of glucose, galactose, mannose, purecellulose, and cellulose in biomass to levulinic acid were studied in succession to systematically build up the key rate constants to describe the reaction of cellulose in cellulosic biomass to levulinic acid. Similarly, reactions of xylose, arabinose, and xylan to furfural were followed in that sequence to develop rate constants for the breakdown of hemicellulose to furfural. The difference in optimal reaction conditions for furfural and levulinic acid described suggested application of a two step process to
pretreat, hydrolyze, and dehydrate cellulosic biomass, and product distributions were compared for single step and two step processes and yields optimized for each. The result is very high yields of both targeted intermediates that can support emergence of new routes to make drop in hydrocarbon fuels as well as chemicals that can readily integrate with the existing fuels and chemicals infrastructure.

BIOT 492

Comparison of laboratory delignification methods, their selectivity, and impacts on physiochemical characteristics of cellulosic biomass

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Most often, two leading delignification methods (using sodium chlorite/acetic acid at 70°C and peracetic acid at 25°C) are used to delignify cellulosic biomass for various research purposes. However, the effects of these techniques on the carbohydrate portion, especially cellulose, and whether their impact varies with feedstock and pretreatments is not well understood. In this study, the selectivity of these two methods for lignin removal with minimal disruption of the remaining solids was examined for two different types of cellulosic biomass, switchgrass and poplar, in their untreated form and following application of various pretreatment technologies. A new method is also presented to improve lignin removal with less modification of the remaining carbohydrates. Compositional analysis, GPC, FT-IR, and other tools are applied to characterize the physiochemical nature of the materials before and after pretreatment and lignin removal and evaluate the ability to remove lignin with minimal impact on the remaining solids. Key words: delignification; cellulosic biomass; selectivity

BIOT 493

Application of a high throughput system to investigate the effects of hemicellulose and lignin removal on enzymatic hydrolysis of cellulosic biomass

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Because biomass recalcitrance is considered as the main hurdle to low cost conversion of lignocellulosic biomass to fuels, overcoming recalcitrance is essential to economically feasible and environmentally sustainable biomass conversion. Hemicellulose and lignin are both considered as contributing to biomass recalcitrance in terms of the intricate carbohydrate-lignin network surrounding the cellulose microfibrils and restricting access by enzymes. Dilute acid pretreatment has shown excellent promise in removing hemicellulose with high yields while base pretreatment is capable of removing lignin. However, both have proved to be effective in increasing cellulose digestibility in pretreated biomass by enzymes, even though their application leads to different compositions of pretreated material. Because this outcome can reveal different effects in the following step, enzymatic hydrolysis of biomass, this research studied the effect of pretreatment on enzymatic hydrolysis by comparing sugar release yields from pretreated materials with different hemicellulose and lignin contents obtained from the two pretreatment technologies. To achieve this goal, a high throughput pretreatment and co-hydrolysis system was extended to low pH operation and applied to collect large amounts of data from various combinations of dilute acid and base pretreatment conditions and enzyme loadings. The results showed that substrate with the hemicellulose removal or lignin removal can give similar performances in enzymatic hydrolysis.

BIOT 494

Comparison and optimization of hemicellulose extraction from maple wood by different acid pretreatments

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Dilute hydrochloric, sulfuric, and oxalic acid along with hydrothermal conditions were applied to pretreat maple wood and release hemicellulose sugars. The maximum combined xylose and xylooligomer yields in the hydrolysates for the different pretreatments were 84%, 87.5%, and 84.4% of the maximum possible for hydrothermal, oxalic acid, and dilute H2SO4 pretreatment respectively, all significantly higher than the total xylose yield of 73.8% for dilute HCl pretreatment. In addition to differences in the total xylose and xylooligomers yields, these different pretreatments resulted in different xylose monomer to oligomer ratios. Oxalic acid pretreatment appears attractive in not only realizing high hemicellulose sugar recovery but also produced xylose solutions that are more compatible with subsequent direct hydrogenation or other catalyzed reactions than the strong inorganic acids.
BIOT 495

Neutron reflectometry and QCM-D study of the interaction of cellulase enzymes with films of amorphous cellulose

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Cellulase enzyme cocktails include exoglucanases that digest cellulose chain ends and endoglucanases that cleave randomly at interior points along the chains. While it is known that these enzymes work synergistically, the details are not fully understood. In addition, cellulose binding domains (CBDs) are known to play an important role in the digestion of crystalline cellulose but much less is known about the benefit of CBDs in the digestion of amorphous cellulose. Amorphous cellulose is of interest as it results from ionic liquid pretreatment of biomass, a promising pretreatment technology. To unravel the actions of endoglucanases and the role of cellulose binding domains in enhancing activity on amorphous cellulose, we have combined studies of the profile of water through cellulose films during digestion by neutron reflectivity, measurements of changes in mass and film stiffness using a quartz crystal microbalance (QCM), and visualization of the motion of individual enzymes by TIRF microscopy.

BIOT 496

Simultaneous saccharification and fermentation (SSF) of hydrothermal pretreated corn stover by mixed culture of Saccharomyces cerevisiae D5A, Pichia stipitis and Brettanomyces custersii

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Integration of pretreatment, enzymatic hydrolysis and fermentation is the key to low cost conversion of cellulosic biomass to ethanol. Simultaneous saccharification and fermentation (SSF) is a favorable configuration for producing ethanol from cellulosic biomass due to the reduced end-product inhibition to cellulases. However, pretreatment greatly impacts SSF performance. Pretreatment conditions must be optimized according to SSF performances for maximizing the digestibility of cellulose and hemicellulose and ethanol yield and minimizing the accumulation of fermentation inhibitors. In this study, we investigated the effect of hydrothermal pretreatment at less severe 'sub-optimal' and 'optimal' conditions in terms of sugar yields on SSF of pretreated corn stover by co-culture of *Saccharomyces cerevisiae* D5A and *Pichia stipitis*. In addition, *Brettanomyces custersii*, a cellobiose-fermenting yeast, was also cultured in SSF for further reduction of cellulases usage. Results showed that (1) less severe 'suboptimal' pretreatment condition was superior to 'optimal' pretreatment because of its low fermentation inhibitor levels; (2) Co-culture of *B. custersii* improved the higher yield and reduced beta-glucosidase use, (3) the adaptation of the yeasts to pretreatment hydrolysate improved SSF ethanol yield.

**BIOT 497**

**Fungal pretreatment of pine wood to reduce the emission of volatile organic compounds**

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Certain microorganisms are known to selectively degrade nonstructural wood components that are responsible for VOC (volatile organic compounds) emissions from softwood (strictly regulated by the AgBB norm of the Federal Environment Agency of Germany) without negatively impacting the characteristics of the wood end-product. Gamma-sterilized pinewood strands were incubated with the well known biocontrol fungi *Trichoderma harzianum* LC3 and *Ophiostoma piliferum* (Cartapip 97®) for 6 weeks, dried and pressed to laboratory boards. VOC emissions of these boards were measured over 28 days using a Markes μCTE Chamber by sampling on Tenax and subsequent TDAS/GCMS analysis. *T. harzianum* was found to extensively degrade nonstructural softwood components, such as glycerides, and resin acids, which act as precursors of volatile aldehydes. Detailed results on the effect of fungal pretreatment on time course and composition of VOC emissions from laboratory boards as well as the degradation of wood extractives will be presented in this work.
**BIOT 498**

**Site-specific chemical modification of phage particles**

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The ability to chemically modify phage display libraries bypasses the functional limitations imposed by the 20 canonical amino acids. Site-specific modification of a displayed peptide in the context of the forest of competing functional groups in the phage coat requires a uniquely reactive chemical group. The so-called “21st amino acid” selenocysteine can be cotranslationally incorporated into the phage coat via context-dependent opal suppression. The enhanced nucleophilicity of selenocysteine permits specific chemical modification under conditions where the typical targets of modification, cysteine and lysine, are unreactive. We have demonstrated quantitative incorporation of selenocysteine into M13 coat protein pIII and subsequent specific chemical modification with reporter groups and pharmacophores. Applications include a) incorporating the modification in the context of a displayed random peptide library as a route to chimeric semisynthetic peptide ligands, b) the use of phage particles as medical imaging reagents, and c) catalysis-based screening for novel enzyme activities.

**BIOT 499**

**Controlling the size of thermally responsive micelles using mixtures of linear and three-armed star polypeptides**

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We have studied the formation and control of environmentally responsive micellar particles based on mixtures of linear elastin-like polypeptide (ELP) and a three-armed star ELP. Stable particles having a narrow polydispersity form above the transition temperature, and reversibly dissociate when the temperature drops below the transition temperature. The size of these responsive nano particles can be controlled by pH, salt concentration, and the ratio of the linear to trimer concentration in the solution. The characterization of the micelles was done using lightscattering, UV absorbance, AFM, and NMR and it was found that the size can be controlled over a fairly wide range, from below 20 nm to above 200
The ELP molecules are based on the repeats of the pentapeptide (Gly-Val-Gly-Val-Pro) and the star polypeptide was formed by combining foldon (a trimer-forming oligomerization domain) and an ELP chain using recombinant molecular biology techniques. An E. coli system was used for cloning and expressing the polypeptides. Purification was done by thermal cycling. These responsive micellar particles are being designed for use in applications such as drug delivery of poorly soluble drugs.

BIOT 500

Drug-encapsulating protein nanoscaffold as a controlled release delivery system

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Delivery of bioactive antitumor therapeutics to the site of action is a key objective of drug delivery. We have exploited a 25-nm dodecahedron protein scaffold from the E2 subunit of pyruvate dehydrogenase of \textit{B. stearothermophilus} to serve as a drug carrier. Through protein engineering, we encapsulated the antitumor drug doxorubicin into the hollow internal cavity of this scaffold utilizing the chemical conjugation and showed drug release to be pH-dependent. Additionally, we created a hydrophobic microenvironment within the cavity that significantly increased the molecular encapsulation capacity. The cellular response to the drug-protein complex was investigated. We confirmed the uptake of doxorubicin-loaded E2 nanoparticles via cellular fluorescent imaging and observed significant therapeutic efficacy against breast cancer cells. These results demonstrate the promise of the E2 nanoscaffold as a robust platform for drug encapsulation and controlled release.

BIOT 501

Molecular imaging of the Epidermal Growth Factor Receptor in rodent colon via Affibody-functionalized surface enhanced Raman scattering (SERS) nanoparticles

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Affibodies are powerful ligands for molecular imaging due to their small size, high affinity, and rapid reprogramming to different biomarkers. We report Raman-based imaging of the epidermal growth factor receptor (EGFR) via SERS gold-silica nanoparticles and a three-helix affibody monomer (Ac-Cys-Z(EGFR:1907)). Affibodies coat the NPs via a hetero-bifunctional, PEG linker with ~200 ligands per NP. With cultured A431 cells (EGFR+) and EGFR-targeted NPs, 6.3-fold and 55-fold more signal was observed versus isotype and non-targeted NPs, respectively. Competitive inhibition with unlabeled affibody decreased signal by 7.3. The EGFR-targeted NPs labeled EGFR on xenografted A431 tumors excised from Nu/Nu mice with 11 times higher signal than affibody-free particles (n=3; p<0.05). Tumors blocked with unlabeled affibody produced signal 6 times lower (n=3; p<0.08). To model colonoscopy applications, we examined murine colon sections known to overexpress EGFR on induced tumors. Raman imaging of cancerous animals produce twice the Raman signal (p<0.05) than untreated animals.

BIOT 502

Enhanced tumor cell separation using biomimetic combinations of adhesive proteins

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Detection of circulating tumor cells (CTCs) is of clinical importance in diagnosis/prognosis of cancer metastasis. However, CTCs are rare, being estimated as few as one in 10\(^9\) hematologic cells, presenting a tremendous challenge for effective detection and separation. We have investigated a novel separation method of tumor cells using surfaces functionalized with biomimetic combinations of adhesive proteins, i.e. anti-epithelial-cell adhesion molecule (anti-EpCAM) and E-selectin. The concurrently induced dynamic rolling (E-selectin) and stationary binding (anti-EpCAM) under flow, which mimics the physiological interactions between CTCs and endothelium, significantly increased capture efficiency. The experiments using in vitro cell lines - MCF-7 cells as a CTC model and HL-60 cells as a leukocyte model - revealed that the surface with the two proteins co-immobilized enhanced MCF-7 cell isolation by 3-fold as compared to the surface with anti-EpCAM alone. This biomimetic combination technique presents potential as a device with enhanced CTC detection and separation.

BIOT 503
Enzymatic assembly and protein engineering for advancing molecular detection techniques

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Progress in molecular detection capabilities can be expanded upon through the use of in-film bioprocessing, which enlists biofabrication and protein engineering to enzymatically assemble molecular probes on microelectronic chips. These probes consist of fusion proteins engineered for covalent binding to a film interface. This film is formed by electric deposition of chitosan, a natural biocompatible material, on a patterned electrode. Enzymatic assembly of the engineered proteins results in a well-defined protein-covered area and allows for specificity in biological signal detection. The functionalized films can be used as parts of multi-component biomimetic devices on which the signal is both produced and detected. Here we present a small-scale, reusable, and programmable platform with individual biologically-active components for production and detection. This novel approach can be applied towards high throughput disease marker screening, immunoanalysis, and pharmaceutical drug discovery.

BIOT 504

Biofunctionalization of nanopatterned surfaces and their integration with DNA nanostructures

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The ability to control biomolecules on surfaces with nanometer resolution is of great interest in the fields of nanoscience and nanotechnology. DNA nanoarrays, in particular, are of interest in the study of DNA-protein interactions, for biodiagnostic investigations and as a tool to drive self-organization of nanomoieties on surfaces. Here we describe different strategies to control the
immobilization of single- and double-stranded DNA, as well as DNA nanostructures (DNA "origami"), on nanopatterned surfaces with features down to the sub-10nm regime. Using electron-beam and nanoimprint lithography we fabricated sub-10nm metal dots arranged in multiple configurations. The selective bio-functionalization of these nanopatterns allowed us to follow the activity (kinetics) at surfaces of a restriction enzyme in real time and with single-molecule resolution. We will highlight the broader utility of such nanopatterned surfaces for the self-organization of DNA nanostructures (as functional templates): the ordered placement of DNA-origami on surfaces will be discussed.

**BIOT 505**

**Probing peptide-membrane interactions using microcantilever sensors**

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We report the use of the lipid–membrane coated microcantilevers to probe the interactions between phospholipid membranes and membrane-active peptides. This sensing method integrates two well developed techniques: The solid-supported lipid bilayers (SLBs) and the microcantilever (MC) sensors. As molecules physisorb or chemisorb onto the surface of the microcantilever, the microcantilever bends due to induced compressive or tensile stresses, which result from the surface free energy change. SLBs are prepared on the silicon dioxide surface of the MCs using a vesicle fusion method. By monitoring the deflection values of the MCs, the real-time surface free energy change during the SLB formation can be detected. We investigate the insertion mechanism of PEP1 (AnaSpec, CA), a synthetic amphipathic peptide resembling a nonstructural proteins NS5A of hepatitis C virus, into SLBs. A threshold at 4mM of free PEP1 in the bulk solution is observed. Before the threshold is reached, the PEP1 insert into and crowd the lipid membranes; for concentration greater than the threshold, the PEPs start to aggregate leading to membrane pore formation. The membrane coated microcantilever sensor is capable of characterizing the kinetics and dynamics of membrane-peptide interactions with great sensitivity.

**BIOT 506**

**Virus-PEDOT nanowires for biosensing**

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Real-time, reagent-free detection of disease markers in biological fluids could transform clinical diagnostics. For example, early detection of cancer markers could guide improved anti-cancer treatments. Virus-based biosensors include viruses for specific molecular recognition and conducting polymer-based electrodes for reagent- and optics-free analyte sensing. Nanowire arrays with M13 viruses grafted into organic, electrically conducting polymers provide an easily synthesized hybrid material. Lithographically patterned nanowire electrodeposition (LPNE) allows for arbitrary designs and configurations of nanowires during synthesis. For cancer marker detection, peptides specific for prostate-specific membrane antigen (PSMA), a protein found in the urine of prostate cancer patients, were displayed by viruses. Incorporation of affinity-matured PSMA-binding phage into the polymer nanowires could allow detection of PSMA in urine. Such simple and low-cost biosensors could be widely deployed for cancer and disease marker detection.

BIOT 507

Disulfide-bond mutant of human keratinocyte growth factor: Its therapeutic potential with structural confirmation

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Human keratinocyte growth factor-1 (KGF-1) is a multiple regulator involving in epithelial proliferation, differentiation and migration, its N23 mutant developed by Amgen has been approved for preventive treatment of mucositis in leukemia or lymphoma radiotherapy. It is known that mature KGF-1 consists of five cysteine, which form two disulfide bonds between Cys1-Cys15 and Cys102-Cys106 with free Cys40. Recombinant N23 KGF-1 was expressed in E.coli and purified by SP and heparin sepharose chromatography, however, its S-S analysis using trypsin enzymatic cleavage and MASS spectroscopy demonstrated that N23 KGF-1 contained Cys102-Cys106 and no disulfide-bond subtypes. Firstly, Cys102-Cys106 studies aimed to construct different mutants of N23 KGF-1 on C40S, C102S and C106S. In result, N23 KGF-1 C40S and N23 KGF-1 C106S were impossible to expressed in bioactive forms, indicating the Cys40 and Cys106 are necessary for KGF bioactivity and conformation. N23 KGF-1 C102S displayed relatively equal bioactivity as N23 KGF-1. Interestingly, N23 KGF-1 C102S could be transformed to form a novel disulfide-bond between Cys40-Cys106 by 1,10-phenanthroline and Cu2+ addition and reconstruction. The novel Cys40-Cys106 N23 KGF-1 C102S mutant was then prepared for structural, bioactive and conformational analyses. Bioinformatic date of the docking with human KGFR and human KGFR demonstrated that Cys40-Cys106 N23 KGF-1 C102S showed stronger combination with human KGFR. The CD, HPLC retention, immunogenicity, stability in solution were totally
different between Cys40-Cys106 N23 KGF-1 C102S and Cys102-Cys106 N23 KGF-1. Meanwhile, Cys40-Cys106 N23 KGF-1 C102S displayed inhibitory activity on fibroblast and endothelial proliferation in vitro, prevented acute and chronic hepatic injury induced by CCl4, reduced hepatic and pneumatic fibrosis in rats and rabbit model. All those spectacular bioactivities of Cys40-Cys106 N23 KGF-1 C102S might be potential for anti-fibrosis therapeutic value, and its further molecular mechanism are in process.

BIOT 508

Mimicking antibodies: Peptoid-based affinity reagents

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We are developing peptoid-based affinity reagents for use in sandwich enzyme-linked immunosorbent assay (ELISA) microarray. Peptoids have close structural similarity to peptides, with the side chain on the nitrogen rather than the alpha-carbon. They are ideal candidates for use as affinity reagents due to low cost and ease of synthesis, the ability to incorporate unique reactive sites into the sequence, highly stable helical structures, and protease resistance. Peptoid affinity reagents have been designed to have similar structural attributes to monoclonal antibodies, while being smaller in size. That is, they contain a highly stable, conserved backbone region and a variable, protein-binding region. The extent of helical structure is determined using circular dichroism and the affinity of the peptoid is analyzed using sandwich ELISA microarray. Peptoid-based affinity reagents have the potential to accelerate the validation of protein biomarkers for complex diseases such as cancer, as well as be used as therapeutic reagents.

BIOT 509

Selecting genetic suppressor elements against Hepatitis C Virus

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Hepatitis C virus (HCV) infection represents a major public health problem, and is the leading cause of cirrhosis and cancer of the liver. The current standard of care for HCV infection is costly, time-consuming and cures only 50% of the patients infected with the most common genotype. We describe the efficient selection of anti-HCV genetic suppressor elements (GSEs). GSEs are sequences derived from a gene or genome of interest that acts transdominant inhibitors of a
particular biological function. In this study, N4mBid hepatoma cells expressing a library comprising a fragmented HCV genomewere subjected to a growth-based selection via infection with cytopathic HCV. N4mBid and the parental Huh-7.5 cells transduced with genetic fragments obtained from the 4th round of selection showed significant resistance to HCV-induced cytotoxicity and lower levels of permissivity to HCV replication/infection, respectively. These studies represent the first reported unbiased selection for anti-HCV genetic elements. We are currently isolating individual GSEs from the library and characterizing their specific effects on the HCV cycle.

BIOT 510

Creating molecular recognition from scratch: Stepwise engineering of knottin peptides to bind tumor marker CA IX

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Cystine-knots (knottins) are small peptides consisting of at least three interwoven disulfide bonds and multiple solvent exposed loops. We engineered knottin peptides with nanomolar affinities to tumor marker Carbonic Anhydrase IX (CAIX) for use as non-invasive molecular imaging agents. First, a phage-displayed library was screened to identify cyclic peptides with weak (μM) CAIX binding affinity. Second, these cyclic peptides were grafted into a knottin loop with similar structure, and a neighboring knottin loop was engineered using yeast surface display, resulting in knottin mutants with high affinity CAIX binding. We showed that both mutated loops contributed to CAIX binding. This work demonstrates that a small knottin scaffold is suitable for engineering high affinity binders from naïve libraries, and multiple loops of a knottin peptide can be engineered to bind target. We are currently preparing labeled knottin peptides and will test their ability to image tumors in living subjects. Funding: NIH R21CA143498

BIOT 511

Beta roll motifs as a novel scaffold for engineering biomolecular recognition

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Most protein scaffolds used for directed evolution experiments, including antibodies, result in molecules that bind in a nearly irreversible fashion to their targets. This limits their potential applications in purification, diagnostic and sensing systems, where it can be essential for the scaffold to have the ability to both bind and release the target. We have previously demonstrated that the repeat-in-toxin motif (RTX) can reversibly switch from intrinsically disordered to a beta roll structure in response to the presence of calcium ions. This allows for the controlled formation of a binding interface which suggests that, if the beta roll can be engineered to bind to a target in one state, binding can be reversed by switching to the other state. In this presentation, we will discuss the critical factors for optimization of this scaffold for directed evolution and results of directed evolution experiments on the optimized scaffold. Our results demonstrate that the beta roll motif is a novel allosterically-regulated scaffold that can be utilized for biomolecular recognition.

BIOT 512

Binding proteins derived from a "super-library" of hyperthermophilic protein scaffolds

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We describe an ensemble of scaffold proteins derived from hyperthermophilic bacteria and archaea to generate stable binding proteins for a wide spectrum of targets. Based on structural data, we randomized 10-15 residues on surface accessible regions of each scaffold to create a "super-library" of \( \sim 4 \times 10^8 \) mutant proteins. Binding proteins for a wide spectrum of targets can be isolated from this "super-library". Through comparative studies with a library of \( 1 \times 10^{12} \) mutants derived from a single hyperthermophilic scaffold, we address the question: does library diversity matter? Further, we discuss the use of binding proteins derived from the super-library in the context of three different applications: generation of multi-subunit binding proteins through "stitching together" individual scaffold protein mutants, development of engineered proteins for culture and differentiation of human embryonic stem cells, and design of adaptor proteins to facilitate virus-mediated drug delivery.

BIOT 513

Characterization of particle formation for a peptidomimetic under thermal stress
A peptidomimetic is a compound designed to mimic the biological function of a natural peptide. The peptidomimetic being developed was stable at intended storage condition (2-8°C), but formed particles under prolonged thermal stress (>25°C). It was important to understand the nature of particle formation and how to control it as most of the drugs fill-finish operations occur at controlled room temperature (15-30°C). The particles were composed of product and trace metals, based on data from ICP mass spectroscopy, FTIR, and RP-HPLC. Particle formation was pH and temperature dependent. A model based on a temperature kinetics study enabled the prediction of particle formation at intended and accelerated storage conditions. By limiting exposure to accelerated temperatures, and controlling pH, risk of particle formation was reduced during fill-finish operations and temperature excursions throughout the product's shelf life.

**BIOT 514**

Transcriptome profiling of BHK cells for bioprocess characterization using cross-species hybridization on CHO microarrays

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It is often said that, for biologics – especially mammalian cell based therapeutic proteins, the product is the process. While bioprocess characterization has traditionally relied on monitoring a limited set of process variables that make up the physiological state vector, there remains a significant disconnect between process parameters and associated outcomes. The advent of 'omics' tools allows for molecular profiling of cells which are likely much more reflective of cell physiology and hence product characteristics. This presentation will focus on the application of DNA microarrays and other 'omics' technologies for a Baby Hamster Kidney (BHK) based perfusion process characterization. With limitations in the availability of genomic resources for BHK cells, cross-species hybridization using Chinese Hamster Ovary (CHO) microarrays was performed. Preliminary proof-of-concept experiments suggested that, although only a fraction of probes on the CHO array displayed high degree of homology with BHK sequences, careful analysis and data filtering can provide reproducible results accurately reflecting the process changes. Some potential applications of such 'omics' tools in process development will then be discussed with examples from specific case-studies.
Identification of genes related to differentiation in *Clostridium acetobutylicum* by genome-wide analysis

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The anaerobic, endospore-forming bacterium *Clostridium acetobutylicum* is best known for its Acetone-Butanol-Ethanol (ABE) fermentation, in which production of solvents occurs during stationary phase. Also during stationary phase, the cells undergo differentiation to form a mature endospore. Despite the close relationship between solventogenesis and differentiation, still relatively little is known about the specifics of differentiation in clostridia, while the metabolism is fairly well characterized. In order to gain insight into clostridial differentiation, and how it compares to the model organism *Bacillus subtilis*, we identified the regulons of Spo0A and three sporulation-specific alternative sigma factors (σ<sup>F</sup>, σ<sup>E</sup>, and σ<sup>G</sup>) using genomic microarrays. By comparing strains with a wild-type transcription/sigma factor against strains mutant for that protein, we identified potential genes influenced by that transcription/sigma factor. Interestingly, these identified regulons in *C. acetobutylicum* shared very little similarity with their respective regulons in *B. subtilis* potentially indicating significantly different differentiation processes.

Metabolomic analysis of lactate metabolism in a recombinant monoclonal antibody producing CHO cell line

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Net lactate production in industrial monoclonal antibody-producing cell lines can often result in poor growth and productivity. In recent years, significant advances have been made to better understand energy metabolism in cultured mammalian cells, as the potential for improved metabolic control of cells could lead to further process optimization and increased productivity. In the study highlighted here, the concentration of a single component, copper sulfate, was varied in a chemically defined medium formulation to generate the desired lactate profile. The resulting lactate-producing (LP) and lactate-consuming (LC) phenotypes
were observed in fed-batch culture after the initial growth phase. To further characterize the shift in cellular biochemistry associated with glucose and lactate metabolism by this single component, metabolomic analysis was conducted. Statistical data analysis of both cell pellet and supernatant samples shows impacts to multiple metabolic pathways associated with energy production. The metabolomic data show substantial differences well before the lactate divergence, suggesting the cells in the LC condition are primed for greater oxidative metabolism. This early distinction likely exacerbates the difference in lactate metabolism later in culture, due to improved maintenance of mitochondrial health in the LC condition. In addition, correlations with cell cycle progression and overall process performance will be highlighted as well as specific examples of altered pathways.

BIOT 517

Systems biology of filamentous fungi for understanding cell wall development

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The impact of filamentous fungi on human activity is enormous. While pathogenic fungi are responsible for numerous deaths and billions of dollars in crop damage each year, fungi used in the bioprocessing industry are used to produce billions of dollars in beneficial products. In both cases the fungal cell wall, and its material properties, plays a critical role. Yet, little is known about how cell-wall regulatory mechanisms, or downstream effectors, impact its material properties. We are using a systems biology approach to better understand fungal cell-wall synthesis, its regulation and its impact on morphological development. As a first step in this process, we have systematically deleted a number of putative cell-wall related genes from the model fungus Aspergillus nidulans, and are using a sophisticated set of experimental tools to assess subsequent phenotypes providing insight regarding gene function. Proteomic analysis, of both cytoplasmic and cell-wall protein fractions, shows that these gene deletions affect a wide range of cellular processes. Atomic force and electron microscopy indicate that particular gene deletions impact both cell-wall material properties and morphology. In addition, there appears to be a significant impact of some of these genes on overall mycelial morphogenesis, in particular regulation of fungal branching. The implications of these findings for the bioprocess industry will be discussed.
Quantitative Bcl-2 family interactome analysis using bacterial display

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Bcl-2 protein family members play crucial roles in programmed cell death. The fate of the cell relies on interactions between anti- and pro-apoptotic members of the Bcl-2 family. We have developed a new tool to study these interactions by displaying a 16-mer peptide containing the Bcl-2 homology 3 (BH3) region of the pro-apoptotic Bcl-2 family members on the E. coli cell surface, and examining their binding affinity toward anti-apoptotic proteins in solution through fluorescent labeling and flow cytometry in a systematic fashion. We present quantitative data for a near-complete set of 17 BH3 domains from the human genome binding to each of the 5 anti-apoptotic Bcl-2 family members. From this dataset, we calculated several quantitative structure-activity relationship (QSAR) metrics, providing new molecular level insight into Bcl-2 family interactions. This quantitative dataset will enable further systems-level analysis of apoptosis.

GeneForce: A computational tool to improve integrated metabolic and regulatory models used in metabolic engineering

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Integrated genome-scale models of metabolism and transcriptional regulation can identify genetic perturbations needed to improve chemical production. The construction of such models is currently a challenge since computational methods that automate the refinement of such models are not available. We recently developed an algorithm, GeneForce, that pinpoints what aspects of the model cause incorrect phenotype predictions. We used the approach to refine integrated models of Escherichia coli metabolism and regulation, and experimentally confirmed some of the algorithm's suggested refinements. After making the model refinements the model's accuracy improved to (~80.0%) when comparing predictions to over 50,000 measured phenotypes. We believe that this general computational approach will enable the rapid development and improvement of integrated genome-scale metabolic and regulatory network models. Since such models can be constructed from genome annotations and
cis-regulatory network predictions, they can be developed for less characterized microorganisms with significance to metabolic engineering.

**BIOT 520**

Essential regulatory determinants that link oxidative stress response and metabolic redirection in *Escherichia coli* under anaerobic conditions

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Oxidative stress tolerance can improve fermentation productivity and simplify fermentation processes. Despite prior studies on oxidative stress response in bacteria, our understanding of oxidative stress response under anaerobic conditions is limited. Herein, we report an integrative systems biology view of cellular response to oxidative stress in *Escherichia coli* under anaerobic conditions. Further, we show molecular determinants that re-direct metabolic states in response to oxidative stress under anaerobic conditions. Briefly, we identified genetic regulatory networks and metabolic pathways underlying the anaerobic oxidative responses of *E. coli* using transcriptome, bioinformatics, and phenotype analyses. The network component analysis was further utilized to identify the transcription factors perturbed by oxidative stress. The involvement and specific roles of the perturbed transcription factors were elucidated using gene knockout and phenotype analyses. Notably, the transcription factors that regulate energy derivation and cellular respirations were essential regulators of cellular response to oxidative stress under anaerobic conditions. Oxidative stress also changed availability of reduced cofactors, which was confirmed by quantifying NADH/NAD+ ratio and a relative amount of NADH and NAD+. Based on these results, we propose essential regulatory determinants that link oxidative stress response and metabolic redirection in *E. coli* in response to oxidative stress under anaerobic conditions. We envision that engineering of the genetic regulatory network identified in this study may help to develop oxidative stress-tolerant microbial cells under anaerobic conditions.

**BIOT 521**

Sequence, assembly and analysis of the Chinese Hamster genome: A context for comparative genomics in CHO cells

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High-throughput sequencing was employed to provide an in-depth coverage of the Chinese Hamster genome. Hybrid assembly of the data generated in this effort resulted in a draft genome (~2.5 Gb), with more than 1.7 Gb in specific hamster chromosomes. The current assembly was further strengthened by the synergistic integration of an existing CHO EST collection. Annotation identified more than 13,000 genes, across a range of functional classes, as well as numerous microRNA precursors. Comparative analysis to the mouse genome permitted the examination of sequence orthology and the expansion of a draft hamster-mouse synteny map. Furthermore, this work aims to study the genetic variation underlying economically-important productivity traits in CHO cells through a comparative genomics approach, with diploid hamster DNA as reference. The availability and application of these genomic resources will facilitate physiological comprehension of cellular behavior, providing further opportunities to engineer and enhance CHO cells in their role as recombinant protein producers.

BIOT 522

Starch production through artificial photosynthesis mediated by cell-free synthetic enzymatic pathway

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A number of biofuels have been investigated for the transportation sector. Hydrogen plus fuel cells is believed to be the best combination in the future. According to our previous breakthrough -- complete conversion of carbohydrate and water to hydrogen and CO2\(^{1}\), we propose the use of carbohydrate (e.g., starch) as a high energy density hydrogen carrier with a gravimetric density of 14.8 H2 mass % and suggest sugar fuel cell vehicles\(^{2}\). To produce starch without relying on living plants, we design a non-natural CO2 fixation pathway that can fix CO2 by using hydrogen or electricity and produce water insoluble starch. This pathway would be scalable because it involves neither membrane nor membrane protein. A combination of this chemical synthesis pathway with commercial solar cells would have overall solar energy-to-chemical energy efficiencies of more than 20% and decrease water consumption by more than 1000 fold as compared to those of natural plants. This artificial photosynthesis would solve key challenges, such as CO2 fixation, electricity storage, feed and food production, transportation fuel production, water conservation or maintaining an ecosystem for space travel. Therefore, collaborations are urgently needed to

BIOT 523

Characterization of hydrothermal liquefaction biocrude oils produced from primary sludge, swine manure and algal feedstocks

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Hydrothermal liquefaction is a promising technology for biofuel production from high-moisture biomass processed in wastewater treatment facilities. This study explores the conversion of primary sludge, swine manure, and algal feedstocks representative of municipal, agricultural, and next-generation wastewater biomass resources. Hydrothermal process conditions were varied to determine the dependence of biocrude oil yield and chemical composition on operating temperature (260-300°C) and feedstock selection. Advanced chemical characterization techniques were used to analyze the resulting biocrude oil and included high temperature simulated distillation (HT-SimDist), Fourier Transform infrared spectroscopy (ATR-FTIR), ¹H- and ¹³C-nuclear magnetic resonance (NMR), gas chromatography-mass spectroscopy (GC-MS), and gel permeation chromatography (GPC). The biocrude oil yield, bulk properties (e.g., elemental analysis, heating value) and physico-chemical characteristics (e.g., boiling point distribution, molecular weight distribution, functional group allocation, molecular constituents) were highly dependent on feedstock selection and highlight the promise of microalgae as a feedstock for wastewater remediation and bioenergy production.

BIOT 524

Novel biological conversion of hydrogen and carbondioxide directly into biodiesel
There is increasing pressure to reduce dependence on foreign petroleum sources. As such, the development of green chemistry routes to produce fuels from renewable feedstocks has been the focus of significant research. Traditional bio-refining processes rely on microbial fermentation of renewable carbon sources such as sugar into higher value products. More recently, work has focused on the use of non-traditional feedstocks in bio-processing such as cellulosic biomass, pyrolysis of waste biomass, or gasification of organic municipal solid waste, to name a few. OPXBIO is developing a novel, engineered microorganism that directly produces a biodiesel-equivalent fuel from renewable hydrogen (H₂) and carbon dioxide (CO₂). The proposed process will fix CO₂ utilizing H₂ to generate an infrastructure-compatible, energy-dense fuel. The proposed process is scalable, the initial economics are favorable, and the liquid fuel can be used directly as a blending stock in the existing diesel infrastructure.

**BIOT 525**

Butanol production from wood pulp hydrolysate in integrated fermentation with in-situ butanol recovery

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ABE fermentation using corn, cassava or sugar cane for biobutanol production is uneconomical due to the high cost of food-based substrates. Renewable lignocellulosic feedstocks including crop residues and woody biomass are available in abundance for biofuels production but present some technical challenges. In this work, we studied the feasibility of using wood pulping waste as the substrate for butanol production by *Clostridium beijerinckii* immobilized in a fibrous bed bioreactor. Wood pulping waste containing mainly xylose and glucose obtained after alkaline extraction and acid hydrolysis of hemicellulose was detoxified with ion-exchange resin and activated carbon. About 8 g/L butanol were produced from sugars present in the hydrolysate with ~72% sugar conversion. When gas stripping was integrated with the fermentation to recover butanol in situ, ~12 g/L butanol were produced with >90% sugar conversion. The integrated fermentation-gas stripping process thus can be used to produce butanol from wood pulping waste.

**BIOT 526**
Biofuels from CO₂ using ammonia-oxidizing bacteria in a reverse microbial fuel cell

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Nitrifying bacteria, such as *Nitrosomonas europaea*, have the ability to oxidize ammonia to nitrite while fixing CO₂ into biomass. These bacteria can be genetically engineered to produce biofuels, such as isobutanol, in a reverse microbial fuel cell where ammonia, electrical energy and CO₂ are fed to the system, and biofuels and biomass are produced. The excess biomass created by the system can also be used in downstream fermentors to increase the biofuel yield. The microbial reactor for fixing CO₂ can be coupled with an electrochemical process to convert nitrite back to ammonia, allowing for a recycling of the mediator. This new concept has the potential to dramatically improve the cost-effectiveness and viability of reverse microbial fuel cells because ammonia is an inexpensive mediator. Studies on genetic modification and growth kinetics of *N. europaea* and optimization of electrochemical reduction of nitrite to ammonia will be presented.

BIOT 527

Engineering crop processing traits for the production of cellulosic biofuels and chemicals

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Agrivida, Inc., is an agricultural biotechnology company developing industrial crop feedstocks for the fuel and chemical industries. Agrivida’s crops have improved processing traits that enable efficient, low cost conversion of the crops’ cellulosic components into fermentable sugars. Currently, pretreatment and enzymatic conversion of the major cell wall components, cellulose and hemicellulose, into fermentable sugars is the most expensive processing step that prevents widespread adoption of biomass in biofuels processes. To lower production costs we are consolidating pretreatment and enzyme production within the crop. In this strategy, transgenic plants express engineered cell wall degrading enzymes in an inactive form, which can be reactivated after harvest.
We have engineered protein elements that disrupt enzyme activity during normal plant growth. Upon exposure to specific processing conditions, the engineered enzymes are converted into their active forms. This mechanism significantly lowers pretreatment costs and enzyme loadings (>75% reduction) below those currently available to the industry.

BIOT 528

Application of high throughput systems to assess agave as a biofuels feedstock for semi-arid lands

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An important issue slowing the development of technologies for making biofuels is concern about possible competition for land and the impacts on food supply. However, because agave offers high productivity with low water and nutrient demands, it can thrive on semiarid lands not suitable for conventional agriculture, making it a promising lignocellulosic feedstock for biofuels production. However, biomass recalcitrance is the key impediment to commercial applications of cellulosic biomass, and there is a critical need for systematic research to identify features of agave species and their impact on sugar release performance. Thus, high throughput systems have been applied to determine carbohydrate compositions of agave, screen pretreatment conditions, and define enzyme formulations. First, the new rapid analysis method that we developed was applied to estimate the hemicellulose and cellulose contents of two agave samples, *A. americana* and *A. lechuguilla*. Then, our novel high throughput pretreatment and hydrolysis (HTPH) system was employed to evaluate the effect of enzyme loadings and formulations in combination with hydrothermal pretreatment conditions on sugar yields. In addition, the HTPH system enabled screening of leaves from *A. americana* and *A. lechuguilla* to investigate variations in sugar release with plant age and location and the importance of sampling technique. Thermochemical pretreatments with dilute acid or sodium hydroxide were then applied in the HTPH system to determine their impact on the performance of these two agave samples in combination with enzyme loadings and formulations. Results from these studies will be applied to draw a baseline of performance in sugar release, understand the recalcitrance features of different agave species, and identify promising directions for more detailed studies.

BIOT 529

Gas fermentation: No such thing as waste
The use of gases in place of sugars as the carbon and energy source for fermentation allows a broad spectrum of resources to be considered as an input for product synthesis. LanzaTech has developed gas fermentation system for the production of biomass, alternative transport fuels and commodity chemicals. The company has focussed initially on the use of industrial waste gases, and specifically from the steel industry, for fuel ethanol production. Today, 50% of the world steel is produced in China, where a rapidly growing economy has been coupled with dramatic increases in vehicle sales, an increasing dependence on foreign oil imports, and a commitment to minimising greenhouse gas emissions. Gas fermentation offers an efficient route to add much greater value to steel industry gas streams than established technologies, while also reducing CO2 emissions and providing a strategically important alternative to food or farmed resources for domestic fuel production.