Understanding Gel Structural Properties to Control Macromolecule Mobility in Self Assembling Peptide Hydrogels

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Employing self assembly of peptides to construct hydrogels can lead to materials that have potential use in drug delivery and tissue engineering applications. We present a family of de novo peptide designs that links the intramolecular folding of amphiphilic β-hairpin peptides to their propensity to self assemble, affording hydrogel materials. These peptides adopt a random coil conformation in aqueous solutions and are freely soluble. However, when subjected to a stimulus, such as a change in pH, ionic strength, or temperature, the peptides fold into a β-hairpin, and subsequently, self-assemble to form a structurally rigid hydrogel stabilized by non-covalent cross-links. The gelation rate and mechanical rigidity of the hydrogels have been found to be directly influenced by the peptide primary sequence and concentration. Rheology reveals that increasing the peptide concentration results in materials with higher rigidity. As a result, these physio-chemical changes also affect the porous morphology within the hydrogel system, which directly determines the ability of macromolecules to move within the peptide fibrillar network. Here, we investigated the influence of the peptide network in the gels formed from two peptide sequences at different concentrations on the mobility of large molecules in static systems to assess the feasibility of using these gels for macromolecule delivery. Using confocal microscopy combined with fluorescence recovery after photo-bleaching (FRAP) to measure the diffusion coefficients of FITC-dextran macromolecules in the hydrogel, it was observed that the mobility of the probes, in particular those of larger in size, was greatly influenced by the rigidity of the gel. Release of the dextrans from the hydrogels over a month period further verified the influence of the gel rigidity and mesh size on the mobility of the probes. Interestingly, the release studies also demonstrated the presence of two characteristic diffusion rates of the macromolecules, suggesting the hydrogel may be heterogeneous with two distinct phases of cross-linking density. By understanding the effect of material properties on the movement of multiple sized species within and out of the hydrogel, better and specific design of self-assembling peptide materials can be achieve, allowing for predictable and controlled delivery of small molecule drugs, proteins, and large antibodies for wide range of biomaterial applications.
G-protein coupled receptors (GPCRs) represent the largest family of integral membrane proteins. This class of receptors is composed of seven $\alpha$-helical transmembrane domains, which mediate the transduction of extracellular signals across the cellular membrane via interaction with heterotrimeric G-proteins. Despite the role of GPCRs in a variety of physiological functions and interest from the pharmaceutical industry which generates $50$ billion in sales per year by targeting GPCRs [1], our knowledge of this class of proteins is limited by the lack of structural and functional data.

The aim of this project is to express and purify fragments of GPCRs and to characterize the GPCR-G$\alpha$ interaction via nuclear magnetic resonance (NMR). To this end, a novel system has been developed that utilizes our experience with the expression of human GPCRs as inclusion bodies in *Escherichia coli* [2]. We have used this system to express and purify fragments of the human adenosine GPCRs. In addition, we have expressed and purified G$\alpha_i$, the G$\alpha$-protein that interacts with the Adenosine 1 receptor (A$1\text{R}$) and Adenosine 3 receptor (A$3\text{R}$). Ultimately, the results of this work will illuminate the structure and binding behavior of the A$1\text{R}$/A$3\text{R}$-G$\alpha_i$ interaction.


The Effect of Promoters on Ag Catalysts for Ethylene Epoxidation

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The direct gas phase epoxidation of ethylene over Ag has been widely studied in an attempt to create more active and selective catalysts. Computational and experimental studies have led to many patents and scientific publications which have shown improvements of ethylene oxide (EO) selectivity with the addition of many different materials. Most selectivity-enhancing materials have been discovered though empirical methods. In contrast, Linic, Jankowiak and Barteau proposed an oxametallacycle mechanism and used computational chemistry to predict more selective bimetallic catalysts for this process\(^1\). They predicted and verified an increase in the EO selectivity for Cu-Ag bimetallic catalysts. Complementing expanded computational studies, we have carried out reactor studies of additional promoters. In particular, two materials, Re and Cd, will be discussed in detail.

Reactor studies of Re-promoted Ag catalysts (Re-Ag) have shown that Re promotes the catalyst with or without the presence of Cl in the feed stream. The catalyst preparation conditions were found to be particularly important in dictating performance. Without Cl in the feed, a catalyst prepared by sequential impregnation showed an increase in the EO selectivity from \(~30\%\) to \(~45\%\) at a constant conversion of 1.8\%\(^2\). The sequentially impregnated catalyst showed further improvements with the addition of Cl as well. However, sequentially impregnated Re-Ag was significantly less active than the unpromoted Ag. Co-impregnation of Re with Ag showed different behavior. A decrease in the EO selectivity to \(~18\%\) was seen without the addition of Cl to the feed stream. Conversely, a sharp increase in the selectivity to \(>65\%\) was seen when Cl was added to the feed. Characterization of these catalysts with SEM shows a change in the catalyst morphology. Kinetic studies indicate that the change in morphology reduces the number of step sites available for reaction. This leads to the large increases in selectivity (since terrace sites are more selective to EO) but also the decrease in conversion seen upon the addition of Re.

The sequential addition of Cd to Ag also produces a catalyst with dramatically different catalyst morphology when compared to the unpromoted case. However, the change in morphology has a different effect than that produced by Re addition, even though the SEM images look similar. Cd addition causes a large increase in the catalyst activity, increasing the conversion from \(~6\%\) to \(~25\%\) at 267°C. The increase in conversion is also accompanied by an improvement in the catalyst selectivity at 1.8% conversion from \(~30\%\) to \(~55\%\). This material, which was also predicted by computational models to improve the catalyst performance, represents another example of model-driven catalyst design and will be studied further to understand the mechanism of promotion.

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[1] Linic, Jankowiak, and Barteau. Journal of Catalysis 224(2) 2004
There has been increasing interest in the development of new portable power generation devices. Based on the relatively high gravimetric energy density of liquid fuels, an integrated thermoelectric/microreactor system with relatively low chemical to electrical energy conversion efficiency has an overall energy density on par with conventional batteries. It has recently been shown that effective thermal management of the released heat can improve this efficiency such that these systems can surpass their traditional counterparts [1]. One method of achieving this is utilizing “excess enthalpy” burners, suggested first by Lloyd and Weinberg [2], where the hot exiting products are used to exchange heat with the incoming cool reactants. Heat recirculation is an example of these “excess enthalpy” reactors and was used in this study of the enhancement of catalytic microcombustor stability with respect to single channel systems.

The heat loss stability of both gas-phase [3] and catalytic [4] microcombustion of propane/air mixtures was observed in single channel and heat recirculation reactors. Using the commercial, computational fluid dynamics (CFD) package Fluent, the effect of wall thermal conductivity on the critical heat loss coefficient was determined. It was observed in both gas-phase and catalytic systems that the heat recirculation benefit is only observed in the limit of non-conductive walls. Stainless steel, single channel and heat recirculation microreactors were fabricated and tested with Pt/anodic alumina catalysts. Consistent with CFD results, the conductive wall heat recirculation system was not observed to demonstrate a significant increase in heat loss stability.

A Mechanism for Shear Banding in Wormlike Micellar Solutions

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Self-assembled surfactant solutions are used in a wide variety of consumer products and industrial applications, where product performance depends critically on fluid rheology. It is the goal of this work to link the meso-scale properties of these fluids to their macroscopic flow behavior to better aid in material design and formulation. Specifically, many such complex fluids undergo “shear banding”, a flow instability whereby the flow-field segregates into two distinct regions of shear characterized by two different shear rates. Shear banding has also been experimentally observed in a variety of other systems, including dense colloidal suspensions, entangled polymers, and DNA solutions. However, the structural mechanisms driving shear banding remain a source of controversy [1].

Perhaps the most well-studied class of materials exhibiting shear banding are wormlike micellar solutions (WLMs), in which self-assembled surfactant micelles form an entangled network, much like polymer solutions. Our novel approach to investigating these materials combines measurements of flow kinematics and fluid microstructure with rheological characterization to develop a more complete understanding of shear banding surfactant solutions. The use of our unique flow-small angle neutron scattering (flow-SANS) instrument [2] enables the first spatially-resolved study of surfactant microstructure through the shear banding transition. We combine these techniques with rheological constitutive models to identify the underlying microstructural mechanism for shear banding.

Above the micellar entanglement concentration, WLM solutions display linear viscoelasticity and nonlinear rheology that is well-described by the Giesekus constitutive model [3]. The model includes an anisotropy parameter that provides a predictive criterion for discrimination between banding and non-banding fluids. Incorporating segmental diffusion into the model allows for prediction of flow-kinematics through the shear banding transition that are in quantitative agreement with experimentally measured steady-state flow profiles. The model also yields predictions for segmental orientation of WLMs under shear through the shear banding transition. These predictions are validated by 1-2 plane flow-SANS measurements, and enable formulation of structure-property relationships for shear banding WLMs. In doing so, we observe a critical segmental alignment for the onset of shear banding. This critical behavior is explored in relation to the underlying equilibrium phase behavior of the surfactant network, resulting in methods to rationally engineer shear banding by tailoring the meso-scale self-assembly of surfactant solutions.

Activity and Selectivity of Hydrogenation Reactions on Pt-3d Bimetallic and Pt- and Ni-modified WC Surfaces

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Bimetallic surfaces often show novel properties that are not present on either of the parent metal surfaces. To gain insight into these novel properties, the electronic and chemical properties of model bimetallic surfaces were studied using a combination of experimental methods and theoretical modeling. Temperature programmed desorption (TPD) experiments were performed with various probe molecules, and density functional theory (DFT) was utilized as a tool to provide insight into the interaction between these molecules and the model surfaces. Ultimately, it was desired to determine if activity can be determined from first-principles.

Extensive characterization has been performed on Pt-Ni bimetallic surfaces\(^1\). It is known that Ni deposited on Pt(111) at 300 K results in a surface configuration of Ni where the first atomic layer is concentrated in Ni (Ni-Pt-Pt). In contrast, Ni deposited at 600 K diffuses into the bulk resulting in a subsurface Ni configuration where the second atomic layer is enriched in Ni and the surface layer in Pt (Pt-Ni-Pt). This has also been found to be the case for several other 3d-Pt surfaces\(^2\)\(^-\)\(^4\), and this work is extended to these bimetallic surfaces. The general results of the TPD experiments are that hydrogen binds weaker on Pt-3d-Pt surfaces and stronger on 3d-Pt-Pt in comparison with Pt(111). This is consistent with DFT calculations\(^5\), which further show that several hydrocarbons, including ethylene and cyclohexene, also bind strongly to 3d-Pt-Pt and weakly to Pt-3d-Pt. These results explain the unique activity and selectivity on the Pt-3d-Pt surface as determined by TPD experiments with cyclohexene and acrolein, respectively.

With this knowledge, and the idea that WC has similar electronic properties to Pt\(^6\), it was desired to replace bulk platinum in the Pt-Ni-Pt(111) surface with WC, producing a Pt-Ni-WC surface. Using WC to replace Pt has two important benefits. The obvious one is cost, which is a large driving force for this research. Perhaps more important, however, is the fact that WC is an effective diffusion barrier layer\(^7\). This property prevents the diffusion of Pt and Ni into the bulk of a carburized tungsten foil substrate at elevated temperatures, which is crucial to applications in heterogeneous catalysis. TPD experiments have been performed on WC and Pt- and/or Ni-modified WC substrates, and these results are compared to those on Pt(111) surfaces.

Micromechanics of Shear Thickening Fluids (STFs) and Consequences for Applications in Protective Materials

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Concentrated suspensions undergo many types of complicated flow behavior, including shear thinning, shear thickening, and yielding. Shear thickening is of interest in industry as it can pose a problem in coating processes and can even damage process flow equipment. More recently, shear thickening fluids have been used in composites with protective fabrics such as Kevlar® and Nylon to improve their response to ballistic and stab threats (Lee, J. Mat. Sci, 2003). Our goal is to develop a predictive understanding of how particle properties affect the onset, severity, and ultimate limit of shear thickening, which should be controlled only by the hydrodynamics of the system according to the hydrocluster theory (Bergenholtz, JFM, 2002). Scaling theories exist to predict the onset of shear thickening and prior simulations have shown the structure in the shear plane, but until now, no experiments have measured the microstructure of a concentrated suspension undergoing shear thinning or shear thickening in the plane of shear. Additionally, the internal structure of hydroclusters which drive shear thickening is not known from prior measurements, nor is the overall size. These results are important for the rational engineering of STFs for commercial applications.

Flow-Ultra Small Angle Neutron Scattering (USANS) measurements on concentrated silica dispersion in the shear thinning and shear thickening regimes provide evidence for the hydrocluster correlation length and its dependence on shear rate and particle concentration. The results are compared to Stokesian Dynamics simulations. These results suggest the correlation length is on the order of 2-3 particle diameters in the flowing hydroclustered state. Lower volume fraction suspensions show larger fluctuations, consistent with packing arguments. To study the internal structure of the hydroclustered fluids, a novel 1-2 plane flow-SANS instrument is employed, which provides the first quantitative measurements of the internal structure in the plane of shear. Using statistical mechanical theories that link the microstructure to the stresses (Wagner, J. Chem. Phys, 1992), it is shown that the thermodynamic stresses control shear thinning. These measurements also confirm previous measurements of the hydrodynamic stresses that drive shear thickening (Maranzano, J. Chem. Phys, 2002), providing a complete picture of the rheology of a hard-sphere suspension.

The ultimate limit of shear thickening and its dependence on particle hardness is studied by comparing shear thickening behavior across a broad range of particles, including soft PMMA dispersions synthesized for this research. The results agree with predictions of an elastohydrodynamic model that includes the particle modulus. Compression-Shear Split-Hopkinson Pressure Bar (CS-SHPB) testing is used as a rheometric technique to gather ultra-high strain rate data and test the theory for the ultimate limit of shear thickening. Finally, we test variations in the particle hardness in composite materials to determine the effect on the stab and ballistic properties and help elucidate the mechanism for shear thickening fluid improvement of stab and ballistic properties of body armor.
Knowledge of membrane proteins (MPs) lags far behind that of soluble proteins; fewer than 1% of the structures contained in the Protein Data Bank are of membrane proteins. Surfactants are typically used to solubilize membrane proteins in the form of protein-detergent complexes (PDC). However, the underlying mechanism for the stabilizing role of surfactant is not well understood. The interactions leading to crystallization are also complicated by the addition of surfactant and precipitants. Several studies suggest that detergent interactions and phase behavior play a major role in protein crystallization.

Most MP crystals have been obtained by vapor diffusion, in which a protein solution is mixed with a highly concentrated precipitant solution in the form of a small drop. The drop is then suspended above a reservoir filled with the precipitant solution and allowed to equilibrate, concentrating the contents of the drop. In this work, the phase behavior of surfactant-precipitant solutions known to lead to MP crystallization was explored. The vapor diffusion paths for these conditions were observed to exist within or near surfactant-precipitant phase boundaries. Solution phase microstructures were visualized using cryogenic-transmission electron microscopy (cryo-TEM). Micellar networks and elongated micelles were observed in surfactant-precipitant solutions that lead to MP crystallization. Crystals and/or aggregates were also observed along vapor diffusion paths. Detergent microstructure and phases may play a role in MP crystallization and the mechanism for detergent crystallization may be similar to that proposed for crystallization in lipidic mesophases.

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Excessive alcohol intake over a prolonged time period can lead to physical dependence, where the body requires alcohol for normal function, but the processes by which the brain recognizes, regulates, and adapts to this new state of dependence are largely unknown. Furthermore, physically dependent alcoholics experience serious physical and emotional consequences upon the cessation of alcohol intake including: increased blood pressure, increased heart rate, anxiety, seizures, and in some cases, cardiac arrhythmias or sudden death. These symptoms appear to stem from a dysregulation of homeostatic and emotional control. As a result, we have chosen to study two interconnected brain regions that are known to regulate homeostasis and emotion: the nucleus tractus solitarius (NTS) and the central nucleus of the amygdala (CeA), respectively. We use a systems approach and employ several experimental techniques to gain insight into the molecular mechanisms underlying chronic alcoholism and alcohol withdrawal in these two brain regions. One of our previous studies toward uncovering molecular mechanisms in chronic alcoholism identified more than 500 genes that were differentially expressed in the NTS of chronic alcoholic rats. We have built upon these results with our current studies to determine the dynamics of gene expression changes during alcohol withdrawal and to elucidate mechanisms that enable sustained gene expression in chronic alcoholism. To study alcohol withdrawal, we use an animal model of alcoholism with rat triplets, and at prescribed intervals before each experiment, one of the alcohol-fed animals is withdrawn involuntarily. We then microdissect the NTS and CeA from each animal and take measurements of the regulatory transcriptomic response with cDNA microarrays or quantitative real time polymerase chain reaction (qPCR). With this design, we are able to capture the gene expression dynamics of withdrawal, and we will present our current results and future plans for these gene expression studies. Our other goal is to elucidate the molecular mechanisms that allow for the sustained gene expression during chronic alcoholism that we measured in our previous study. We hypothesize that epigenetic modifications allow sustained expression, and our efforts to test this hypothesis have focused on differential promoter methylation within a subset of differentially expressed genes known to be influential in alcoholism. We will discuss our approach to these epigenetic studies and present the results we have obtained to date. Finally, while performing our experiments, we discovered an unexpected subpopulation of rats that voluntarily withdrew from the alcohol diet and subsequently displayed an array of behavioral abnormalities and homeostatic dysregulation to the point of death. Our analysis of the lifespans of these rats shows that our rats are most susceptible to these fatal episodes of voluntary withdrawal in the initial 10-20 days of the alcohol diet. In this presentation, we will discuss these results and the implications for additional experiments to determine the molecular mechanisms of chronic alcoholism at this time point. The compilation of all of these studies will enable us to propose a model of the molecular mechanisms of chronic alcoholism and withdrawal in the NTS and CeA. A model of this type will help us to generate testable hypotheses about methods of treatment or prevention of alcoholism and alcohol withdrawal.
Bimetallic catalysts have been shown to exhibit chemical properties, such as activity and selectivity that are not simply the weighted sum of the chemical properties of the pure parent metals. One such example is the self hydrogenation of cyclohexene to cyclohexane on Pt-Ni bimetallic surfaces\cite{1-4}. While pure Pt and pure Ni show negligible activity for this reaction, the Pt-Ni bimetallic catalyst exhibits significant activity. These novel properties have been directly related to the configuration of the bimetallic structure in the first few layers of the bimetallic catalyst\cite{2,3}.

In this study, model monolayer bimetallic surfaces (MBS) are studied which are comprised of a host metal A with one monolayer of admetal B present in the first few atomic layers of the surface. There are three ideal MBS configurations which could be considered: the surface configuration, the mixed surface configuration, and the subsurface configuration. The surface configuration is where the 1\textsuperscript{st} atomic layer of the catalyst is purely composed of the admetal B and the remaining layers are composed of the host metal A (denoted B-A-A(hkl)). The mixed surface configuration is where both host metal A and admetal B are present within the 1\textsuperscript{st} layer. The subsurface configuration is where the 1\textsuperscript{st} atomic layer is the host metal A, the 2\textsuperscript{nd} layer contains the admetal B, and the remaining layers beyond the 2\textsuperscript{nd} layer are the host metal A (denoted A-B-A(hkl)). It has been shown experimentally that it is the presence of the surface and subsurface configurations that lead to the novel chemical properties.

However, while a bimetallic catalyst may exhibit increased activity for a given reaction, it may only occur for a specific MBS configuration. For example, the oxygen reduction reaction (ORR), which combines oxygen with hydrogen to form water on the cathode of proton exchange membrane fuel cells (PEMFC), is predicted to have increased activity only for the subsurface Pt-Ni-Pt(111) configuration\cite{5}. The surface Ni-Pt-Pt(111) configuration is predicted to bind oxygen too strongly and decrease the activity below that of pure Pt(111). Therefore, an important parameter for bimetallic catalysts is the thermodynamically stability of a desired MBS configuration within the operating conditions of a given reaction.

It will be shown that the thermodynamic stability of MBS configurations can be correlated with the surface d-band center. First the trend for the adsorbed-induced segregation is discussed for a few common reaction environments (atomically adsorbed O, H, C, N, S, and P) on Pt-3d-Pt(111) systems (where 3d=Ni, Co, Fe, Mn, Cr, V, or Ti). Next, the effect of switching the admetal or the host between the 3\textsuperscript{rd}, 4\textsuperscript{th}, and 5\textsuperscript{th} row of the periodic table is presented.

Expression, Purification, and Biophysical Characterization of Mammalian G-Protein Coupled Receptors Expressed from Saccharomyces cerevisiae

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The G-protein coupled receptors (GPCRs) represent the largest family of integral membrane proteins, many of which have been identified as important pharmaceutical targets. Despite their importance, structural and conformational studies of these proteins have been hindered by their relatively low abundance in native tissues, their generally low levels of expression in recombinant systems, and difficulties associated with their isolation and stabilization in membrane-mimetic environments.

Previously, we have achieved high-level expression and membrane localization of the human adenosine A2a receptor in the yeast Saccharomyces cerevisiae at levels of approximately 10 milligrams per liter of culture. The development of a suitable purification scheme has facilitated the purification of greater than 6 milligrams per liter of culture ligand-binding A2aR, which has allowed for extensive characterization of this receptor. Circular dichroism and intrinsic fluorescence spectra of receptors reconstituted in surfactant micelles indicate that the protein is highly alpha-helical, and that A2aR’s seven tryptophan residues reside in a highly non-polar environment, as expected. Heating the protein samples to 90°C led to an ~80% loss of alpha helical content, a substantial decrease of fluorescence intensity, and a small red shift of the fluorescence emission maximum. These changes, which were largely irreversible, are consistent with thermal denaturation of receptor secondary and tertiary structure. No detectable changes in CD spectra of A2aR are seen upon binding agonist or antagonist ligands, implying that A2aR’s alpha helices may rearrange but otherwise remain unaffected during ligand binding. Thermal denaturation studies in the presence of agonist reveal an increase in the midpoint of unfolding; suggesting that the agonist-bound form of A2aR is more stable than the unliganded receptor.

Although high-level expression of A2aR can be accomplished in Saccharomyces cerevisiae, proper folding and membrane localization of these proteins proves GPCR-dependent. Overexpression of the human adenosine A3, neurokinin NK1, cannabinoid CB2, and dopamine D2S receptors in S. cerevisiae results in low-level expression (~ µg/L of culture), extremely low cell densities, and intracellular protein localization. N-terminal sequencing has suggested that these problems may arise during translocation across the ER membrane. Furthermore, activation of the unfolded protein response (UPR) is observed during the expression of these GPCRs whereas expression of A2aR does not activate this pathway. Immunoprecipitation experiments have also verified a long-term association of misfolded receptors with BiP, an ER-resident chaperone. Currently, we are investigating the trafficking of these problematic GPCRs through the secretory pathway of Saccharomyces cerevisiae through confocal microscopy. To complement these methods, immunoprecipitation is being used to identify other molecular players which associate with mis-localized receptors in order to provide insight into cellular mechanisms which impact localization.
Characterization of Catalytic Materials using Electron Microscopy

William Pyrz

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Advances in both transmission and scanning electron microscopy allow the determination of morphology (BF/DF imaging, HREM, secondary electrons), structure (SAED, HR-STEM, HREM), and composition (EELS, EDS) of materials using highly integrated instrumentation. In the TEM, the point resolution now extends down to 1.0Å or even better, where it is now possible to image individual atoms. In this work, electron microscopy has been used to study the following catalytic systems: (i) Ru-based NH₃ decomposition catalysts for H₂ production, (ii) MoVNbTeOₓ complex oxides for selective propane (amm)oxidation (iii) Pt-Re bimetallics for glycerol processing and (iv) Pt/Au dendrimer-encapsulated nanoparticles for the electrocatalytic O₂ reduction reaction for fuel cell applications. The majority of this talk will focus on NH₃ decomposition and the MoVNbTeOₓ catalysts.

Due to rising costs of fossil fuels and associated environmental concerns, alternative energy solutions are becoming more important. One option is environmentally-friendly H₂ since its use only yields H₂O as a waste by-product. Ammonia is an attractive carrier of H₂ since it has high relative storage capacity (17.7wt%) and its decomposition is COₓ-free. The best catalysts for ammonia decomposition are alkali and alkaline earth promoted ruthenium [1]. Using electron microscopy, several morphologies were observed on the K-promoted Ru catalyst during the various calcination, pretreatment, and reaction steps. It was discovered that best performing catalysts at low temperatures all contain hollandite-structured KRu₄O₈ nanowhiskers. Long-term exposure to the reaction environment led to catalyst deactivation and whisker degradation, but activity and whisker regeneration was observed following an O₂ cycle.

The second system to be discussed is the MoVNbTeOₓ complex oxide catalyst (“M1/M2”). These catalysts are a possible candidate for commercial-scale processes that directly convert propane to acrylonitrile by ammoxidation or to acrylic acid by selective oxidation [2]. Current production of acrylonitrile has been accomplished using a propene feedstock, but a switch to propane would lead to significant cost savings (~300-500M $/yr). The switch to propane requires a multifunctional catalyst that can selectively activate propane C-H bonds, abstract H, and finally insert an adsorbed N atom to form acrylonitrile [2]. The M1/M2 catalyst can accomplish these steps, but the true structure, composition, and the fundamental function of the component M1 and M2 phases is under debate. The metal frameworks have been solved previously by our group using simultaneous Rietveld analysis of powder neutron and X-ray diffraction [2]. Recently, we have used Cs-corrected STEM to directly image the metal frameworks and confirm the structure. For the M1 phase, we clearly show that Te occupies both hexagonal and heptagonal channels, and that the occupancy, which correlates with catalytic yield, can vary based on the synthesis procedure employed. We also estimate the metal site occupancies using the scattering contrast from HAADF images and find reasonable agreement between the measured values and the occupancies predicted from the refined M1 model.

Aqueous solutions of silica with PEO or PVP at $R_g/R=0.7$ and 1.8 were investigated. An experimental complication is the potential adsorption of polymer to the surface of the particle. The PEO–silica system can be modeled with depletion theories because phase separation occurs at polymer concentrations above silica surface saturation. On the other hand, phase separation for the PVP–silica system depends on the choice of buffer. In DI water, separation was observed at polymer concentrations below silica surface saturation which indicates that PVP bridges two colloidal particles rather than causes a depletion effect. In acidic buffer, depletion flocculation is the method of phase separation hence, depletion models should be appropriate. The ratio of polymer concentration to polymer entanglement concentration, $c/c^*$, increases with increasing $R_g/R$ for all four polymer–particle systems in buffer. This trend opposes classical depletion theories but is in agreement with the PRISM theory of athermal polymer–colloid suspensions.
Characterizing Cell–Mediated Erosion in Heparin–Based Hydrogel Networks

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Cell-responsive hydrogels have been developed for clinical applications in wound healing and tissue regeneration. These bioactive materials are designed to mimic characteristics of natural extracellular matrices and therefore elicit cell migration into the networks via specifically engineered signals, including the incorporation of cell-attachment domains, cell-degradable cross-link substrates, and growth factors. The aim of this work is to rationally engineer hydrogel properties, including gel stiffness, binding ligand density, growth factor loading, and cell-mediated degradation, for optimal biological responses in vascular tissue engineering scaffolds. Cellular migration into the scaffolds is studied via cell-loaded fibrin clots suspended in the hydrogel network. Three-dimensional radial cellular invasion into the surrounding matrix is observed over several days. Methods for assessing local material deformation and local cell-induced strain fields are being developed through the tracking of particles embedded in the matrix.

In this work, we investigate mouse fibroblasts (NIH3T3) and human dermal microvascular endothelial cells (HDMVEC) interacting with heparin–polyethylene glycol (PEG) hydrogel scaffolds with PEG as the covalent cross-linker. Both basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) can be sequestered into the hydrogel via known heparin-GF interactions. In this manner, VEGF functions as a dimeric, heparin-binding cross-linker and serves as a cell receptor mediated erosion site. The addition of fibronectin and RGD peptides as binding ligands facilitates cell attachment to the network. The hydrogel stiffness is controlled via variation of the polymer concentration and cross-linking density. Cell-mediated scaffold degradation is made possible through cross-linking peptide substrates that are targets for the local cell-secreted enzyme, matrix metalloproteinase (MMP), in addition to cell receptor mediated erosion provided by dimeric cross-linking VEGF. Radial cell invasion rate is monitored as the network degrades and growth factor is released. Ligand density, MMP peptide concentration, gel modulus, and growth factor loading influence the cell invasion rate. From this study, we have attained a better understanding of how to engineer materials for optimal biological responses in vivo.
Active microrheology is an emerging method for quantifying the behavior of complex fluids. In active microrheology, micrometer-sized probe particles are dispersed within a complex fluid, and their motion is manipulated by an external force using magnetic tweezers or laser tweezers. This external forcing affords increased flexibility in the kinds of fluid behavior that can be probed. For instance, small forces can be used to characterize the fluid linear rheological response, while the application of larger forces can potentially be used to measure non-linear fluid behavior, such as shear thinning [1]. Here, we investigate the active non-linear microrheology of colloidal suspensions. Suspensions are used due to the richness of non-linear rheological behavior they exhibit, and correspondingly large theoretical framework that has been developed with regard to macro-rheology, and increasingly, microrheology.

Recently, several contributions to the suspension microviscosity have been identified that suggest both important similarities and differences with macrorheological measurements. These contributions arise from both direct and indirect probe-bath interactions. The latter is directly related to the macrorheological behavior of the suspension. Therefore, by evaluating these contributions, one can develop accurate comparisons between microrheology and bulk rheology for measurements of non-linear rheology.

To resolve these issues, we investigate the behavior of a colloidal suspension using small amplitude active oscillatory microrheology. The experimental system is an aqueous suspension of index matched fluorinated ethylene propylene (FEP) particles embedded with either 2 um diameter silica or 3 diameter um polystyrene probe particles. Probes are trapped and oscillated using laser tweezers at frequencies of between 5-1000 Hz and at amplitudes of 25-400 nm. The oscillation amplitude and phase of the probe are measured using a photo diode and lock-in amplifier, and these values are used to compute the frequency dependent microviscosity of the suspension. Frequency thinning is observed at all concentrations and is most significant for volume fractions above 0.3. The results are in quantitative agreement with both previous measurements of the suspension microviscosity [1] and recently developed theory.

Protein misfolding and aggregation hamper the production of pharmaceutical products by decreasing shelf-life and product quality or safety. Liquid-liquid and liquid-solid phase behavior of protein solutions are mitigated by a related but alternative aggregation pathway in which proteins maintain their folded structure. In both cases, qualitative and quantitative prediction of the macroscopic behavior by molecular simulations is desirable to aid in controlling the kinetics and thermodynamics of aggregation. However, current algorithms developed primarily for small-molecule or simple colloidal systems are often hindered by computational burden because of the complex, anisotropic interactions between protein molecules and the relatively short range of those interactions. The project presented here focuses on development and assessment of hybrid methods that would allow accurate simulations of colloidal protein aggregation by treating interactions exactly at local length scales and coarse-graining over longer length scales. The results for aggregation and liquid-liquid phase behavior of simple, colloidal particles with short or long-range isotropic interactions are presented here as test cases to assess the accuracy and applicability of our hybrid, cell-based approach before extending it to more complex interactions that more closely mimic real proteins.

The cell-based approach incorporates a Wang-Landau biased Monte Carlo algorithm\(^1\) to exactly enumerate the degeneracies within abutting subvolumes (cells) within a larger system. The canonical partition function for an arbitrary, macroscopic system can then be represented by a Potts-type lattice model where each lattice site is a cell that can interact with its neighbors. While this cell-based approach is very efficient when cell-cell interactions are ignored, the method becomes cumbersome as more detailed interactions are employed. Thus, coarse-graining is introduced in terms of the level of sophistication (and computational expenditure) one applies for treating the cell-cell interactions. The main focus of this talk will be on how the level of coarse-graining affects the ability of such cell-based models to qualitatively and quantitatively capture the thermodynamics of prenuclei or cluster formation in the dilute phase, as well as the equilibrium between dilute and concentrated liquid phases.

Analyzing Protein Dynamics: Molecular Chaperone and Co-Chaperone Interactions in the Endoplasmic Reticulum of *Saccharomyces cerevisiae*

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The objective of this research is to develop a systematic approach to elucidate the mechanisms associated with chaperone/co-chaperone binding interactions in the endoplasmic reticulum (ER) of *S. cerevisiae*. Protein-protein interactions of this system are highly dependent upon the timescales of diffusion and kinetics. Effective diffusion calculations must incorporate the physiological conditions of the cell, specifically the inhomogeneous spatial environment. Recent advances in confocal light microscopy (CLM) combined with the use of green fluorescent protein (GFP) allow the continuous monitoring of protein dynamics in living cells. An estimation of species population and diffusion coefficients can be achieved using CLM to visualize fluorescently tagged proteins *in vivo*. Examining the kinetics of a system requires *in vitro* techniques to obtain a mechanistic-level understanding of protein interactions. These experiments provide a direct measurement of equilibrium and reaction rate constants. Collectively, *in vivo* and *in vitro* experimental data has been integrated with computational design accounting for spatial and temporal effects of chaperone/co-chaperone interactions.

BiP/Kar2 is the molecular chaperone of the ER and a member of the Hsp70 family of chaperones that resides in the lumen of *S. cerevisiae*. BiP has been identified in multiple critical processes through biochemical and genetic experiments, including protein folding and ER translocation. These various processes of BiP are associated with selective co-chaperones. Our hypothesis is that chaperone/co-chaperone interactions are a result of spatial distribution, regulated by co-chaperones, and serves as a means of dictating cellular functions. The low resolution of traditional immunofluorescence techniques combined with BiP’s relative high abundance within the ER has previously inhibited the determination of molecular gradients. The experimental set-up of our research will re-examine this causal relationship.

Our approach is to understand chaperone/co-chaperone interactions in the ER of *S. cerevisiae* by combining elements of *in vitro* and *in vivo* experimentation to develop a coherent computational model. Binding experiments provide insight into the kinetics of BiP’s interaction with the co-chaperone Sec63. At present, recombinant protein expression and purification of BiP and Sec63 have been accomplished. *In vitro* preliminary studies have determined estimates of kinetic parameters. Protein dynamics and spatial localization were examined by utilizing *in vivo* fluorescent microscopy techniques. Fusion proteins of BiP and Sec63 have been created with multiple fluorescent variants to facilitate visualization of *in vivo* interactions. Using these variants, results have established that the ER of *S. cerevisiae* is continuous and structurally dynamic. Advancing our understanding of chaperone/co-chaperone interactions requires a dual approach, incorporation of *in vitro* and *in vivo* experimentation combined with additional insight provided by computational studies.