Biophysical Characterization of Critical Physicochemical Properties of Therapeutic Monoclonal Antibody Formulations

Jannat Nayem

Advisors: Norman J. Wagner and Yun Liu

Committee Members: Abraham M. Lenhoff, Christopher J. Roberts, and Antonio Faraone

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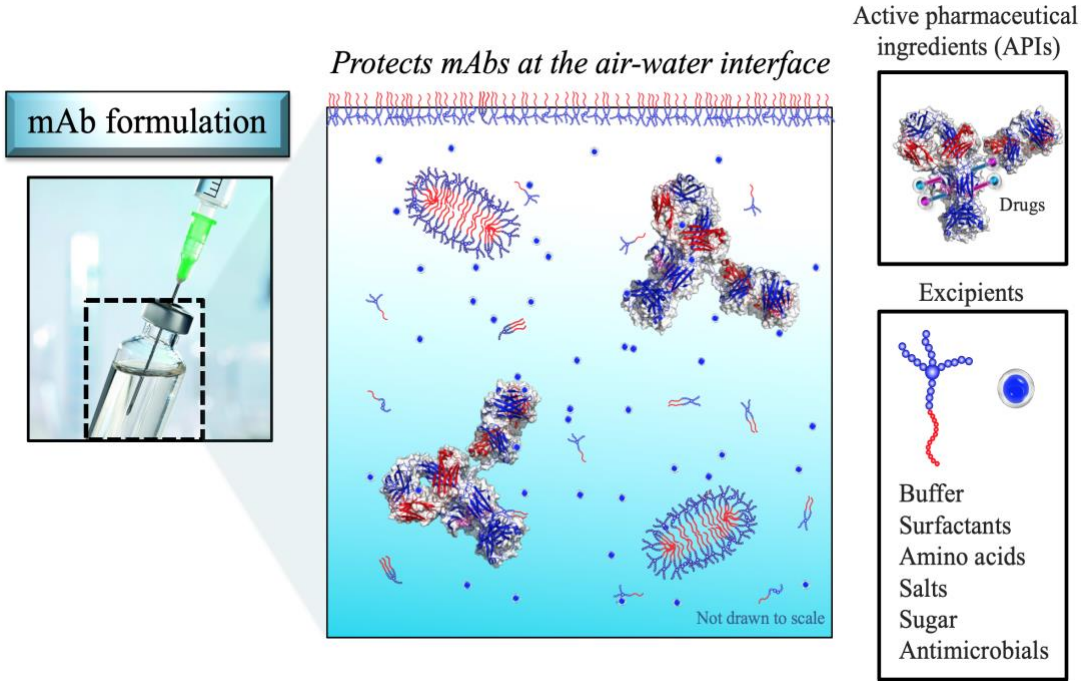

An artistic rendition of the primary components of a therapeutic mAb formulation.

Manufacturing challenges and pharmacokinetics often limit the development of new mAb therapeutics. While the characterization of primary through quaternary structure is crucial for understanding their biochemical functionalities and efficacy, the quaternary structure, interactions, and dynamics of therapeutic mAb proteins and the formulation excipients in both solid state and solution are crucial for understanding their stability during manufacturing, formulation, long-time storage, and drug delivery.

In the first part of this thesis, we investigated mechanistic degradation pathways of two commonly used surfactants, polysorbate 20 (PS20) and polysorbate 80 (PS80), in therapeutic mAb

formulations. Typically, PS20 and PS80 are added in formulations to protect mAbs against interfacial stresses which in turn impart solution stability. However, the inherent heterogeneity of these surfactants and their propensities to preferentially degrade via hydrolysis and oxidation pose a lot of stability related challenges in the biopharma. To determine the role of individual components in the overall micellar aggregation behavior of the structurally heterogeneous commercial surfactants, we investigated the morphologies of primary components PS20 and PS80 and their mixtures at physiological and pharmaceutical temperatures via SANS. To understand degradation, we systematically investigated the micellar morphologies of the undegraded and degraded PS20 systems with a variety of techniques including small angle neutron scattering (SANS), dynamic light scattering (DLS), and evaporative light scattering detector (ELSD).

In the second part, we studied the internal dynamics pertaining to the hinge-bending domain motions of therapeutic mAbs, protein-protein interactions, and the hydrodynamic properties that are crucial for bio-functionalities and the stability of concentrated protein formulations. In particular, a direct measurement of the hinge-bending domain motions using traditional biophysics techniques is highly nontrivial. In this dissertation, we developed a substantiated methodology using a unique combination of techniques including the neutron spin echo (NSE) spectroscopy, dynamic light scattering (DLS), an accurate dynamic decoupling theory, analytic colloidal theories, and all atom molecular dynamic simulations to probe the internal dynamics and hydrodynamics of the standard NISTmAb and several industrial mAbs in both dilute and concentrated formulations. We also determined the nanosecond internal motions of NISTmAb as a function of temperatures, especially during partial unfolding. In addition, other techniques including differential scanning calorimetry (DSC), SANS, and DLS are used to investigate the thermodynamic unfolding profiles, solution structure, interactions, and free diffusion coefficients of NISTmAb proteins. Together, a correlation between these measured fundamental solution properties and the intrinsic conformational stability is established. In summary, the work presented in this thesis provides a mechanistic understanding of the fundamental solution properties of biopharmaceutical surfactants and mAbs. Therefore, the knowledge gained from this work can aid the development of mAb based therapeutics in biopharma.