MOLECULAR-SCALE UNDERSTANDING OF PROTEIN INTERACTIONS AND SOLUTION VISCOSITIES

Mahlet Asfaw Woldeyes
Advisors: Christopher J. Roberts and Eric M. Furst
Committee Members: Abraham M. Lenhoff, Yun Liu, and Vladimir I. Razinkov

Protein-based products, such as monoclonal antibodies (MAbs), dominate the pharmaceutical market share as the top selling drugs, a trend that is expected to continue for the foreseeable future. MAbs are therapeutic proteins that are used to effectively treat many chronic illnesses such as various forms of cancer and autoimmune disorders. These proteins are effective due to their ability to bind selectively and tightly to drug targets, however high doses (on the order of 1 mg of drug per kg of patient body weight) are necessary. This high dose requirement creates challenges both in the production and delivery of MAbs. Mainly, high protein concentrations can lead to undesirable solution behaviors such as high solution viscosity, aggregations, and protein instability. Consequently, improved understanding of the causes of such undesirable behaviors may lead to new strategies to alleviate current challenges when formulating, manufacturing, and delivering high-concentration protein therapeutics.

Various studies suggest that these undesirable solution behaviors such as elevated viscosity are caused by short-lived transient "clusters" that result from attractive protein-protein interactions (PPI). The protein interaction parameter (k_D) calculated from low-protein-concentration dynamic light scattering (DLS) measurements is often used to relate PPI to solution viscosity at high protein concentration. These empirical relations involve some degree of extrapolation as k_D is determined at dilute conditions, while high solution viscosities are not evident until high protein concentrations are reached. At these high concentrations, the solution is crowded, and short-ranged and multi-protein interactions are dominant. Small angle neutron and x-ray scattering along with neutron spin echo and molecular simulations of concentrated MAb solutions have been used to propose a correlation between effective cluster formation and viscosity of MAbs. These studies, however, were limited to a small set of MAbs and formulation conditions.

This dissertation provides systematic and large studies of PPI and solution viscosities of various proteins and formulation conditions to fill the gap in the understanding of the relation between PPI and high solution viscosities. Experimentally determined PPI for *alpha*-chymotrypsinogen A (aCgn) were used in combination with colloidal models and molecular simulations to predict high concentration PPI from low concentration measurements. The results highlight the need for using experimental data when predicting high concentration PPI. The correlations between protein interaction and solution viscosities of MAbs were determined for a wide range of MAbs, formulation conditions, and protein concentrations. Static and dynamic light scattering (SLS and DLS) were used to measure PPI in terms of the osmotic second virial coefficient (B_{22}), diffusion protein interaction parameter (k_D), zero-q limit structure ($S_{q=0}$) and hydrodynamic ($H_{q=0}$) factors. Various viscometry techniques were used to determine the solution viscosities. The combined results are discussed within the context of challenges and approaches to best model and/or predict the correlation of PPI and solution viscosity at various concentrations.

Biophysical characterization techniques such as SLS, DLS, and microrheology were used to examine solution behavior of the next generation therapeutic proteins, i.e. bispecific antibodies. These techniques were also used to examine the effect of temperature on the solution viscosity and PPI. The results demonstrated that PPI and viscosity are sensitive to changes in temperature for proteins where the net-PPI were dominated by strong, short-ranged, and anisotropic attractions. Furthermore, effect of temperature on PPI provided insight into solution conditions where specific configurations that lead to non-ideal solution behavior, such as high viscosity, dominate the behavior. Finally, small angle x-ray scattering, and neutron spin echo were used to further gain insight into the structure and dynamics of the MAbs and molecular origins of high solution viscosity.