Insights into Stability, Heterogeneity, and Self-Interactions of Biopharmaceuticals

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Within the biopharmaceutical industry, biologics encompass a wide range of drug products that are used to treat conditions such as various cancers, infectious diseases, and rare or orphan disorders. A large sector of this industry comprises protein-based therapeutics, which are inherently susceptible to physical and chemical degradation. These processes introduce product heterogeneity that can compromise purity, efficacy, or safety. Elucidating the factors that drive degradation, and characterizing the resulting heterogeneity, provides the mechanistic insights that are necessary for optimizing formulations and ensuring the long-term stability and safety of the final drug product. This work examines the structural heterogeneity and self-interactions of monoclonal antibodies (MAbs) and adeno-associated viruses (AAVs), two leading but structurally distinct protein-based therapeutic modalities.

MAbs are natively glycosylated, exhibiting heterogeneity on both a macro- (presence versus absence of glycans) and micro- (specific glycan composition) level. While macro-heterogeneity impacts the conformational stability of the Fc region, its role in mediating protein self-interactions has not been widely quantified in the literature. In the first part of this thesis, the second osmotic virial coefficient (B_{22}) and dynamic light scattering interaction-parameter values (k_D) were used to characterize the solution behavior of two fully deglycosylated MAbs and their native counterparts across a range of industry-relevant pH and ionic strength conditions. A domain-level coarse-grained molecular model was also considered to potentially provide additional insight. The results demonstrate that protein self-interactions measured via light scattering can be sensitive to changes in glycosylation, and that the exclusion of glycans limits existing models in making quantitatively accurate predictions of self-interactions.

Despite their clinical success, AAV vectors lack the thorough biophysical characterization of more established modalities like MAbs. Electrostatic interactions have been

reported to influence AAV formulation stability, but their impact on capsid-genome integrity remains poorly defined. The impact of electrostatics on the aggregation behavior and capsid-genome integrity of AAV9 capsids carrying an enhanced green fluorescent protein (EGFP) transgene was explored by modulating solution pH and ionic strength. Thermal stress studies revealed pH-dependent differences in genome release and the extent of aggregation, while dynamic light scattering measurements demonstrated that low ionic strength led to the formation of reversible aggregates. Overall, the data demonstrate the significant role of electrostatic interactions in driving AAV9 capsid stability, particularly in relation to aggregation and genome release.

Although AAV capsids demonstrate a propensity to self-associate at low ionic strength, experimental measurements of protein self-interaction parameters have not been widely reported. Static light scattering measurements demonstrated that AAV9 capsid-capsid interactions can be driven by strong electrostatic attractions at 150 mM ionic strength, with increased attractions observed at acidic pH. AAV9 self-interactions also exhibited a strong dependence on genome loading; full capsids displayed electrostatically-driven attractions potentially attributed to charge anisotropy, whereas empty capsids behaved as charge-screened colloidal particles with global net repulsions. In contrast, results for the AAV5 serotype showed a diminished effect of genome loading and pH on self-interactions, illustrating that colloidal behavior depends not only on solution conditions but also the underlying serotype.

Additionally, small-angle X-ray and neutron scattering were employed to provide *in situ* measurements on particle size, shape, and interparticle interactions. A form factor model for a core-shell spherical particle was found to be a reasonably suitable approximation for empty capsids; however, the high heterogeneity related to typical full capsid formulations presents an ongoing challenge in defining model parameters. These techniques also demonstrated potential in extracting experimental structure factors to further elucidate AAV self-interactions.

Overall, this thesis provides valuable insight into the heterogeneity and self-interactions of both MAb and AAV formulations. The findings presented herein advance the fundamental understanding of the biophysical and colloidal behavior of AAV vectors. Furthermore, this work expands the analytical toolbox by applying small-angle scattering techniques to AAV systems, providing recommendations and laying the foundation for further *in situ* characterization.