Small Angle X-Ray Studies of Protein-Protein Interactions at High Concentration to Understand Viscosity, Opalescence, and Phase Separation

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Whereas monoclonal antibodies (mAbs) are popular candidates for pharmaceutical therapies, viscosities of mAb solutions often become too large for subcutaneous delivery above 150 mg/ml. Few analytical techniques can directly probe concentrated mAb solutions and consequently relatively little is known about protein-protein interactions (PPI) at these conditions. A key goal is to understand PPI at the molecular level and determine how they influence viscosity, phase separation, and opalescence. Small angle X-ray scattering (SAXS) is an emerging technique for determining structure factors (S(q)) of proteins at high concentrations. Herein, we present experimental structure factors that are modelled with coarse-grained molecular dynamics (MD) simulation. The MD model represents mAbs as 12 beads, with short range attraction between specific beads to capture relevant Fab-Fab and Fab-Fc interactions, along with long range electrostatic repulsion, and van der Waals attraction. We show that the proteins form small clusters and demonstrate how the cluster morphology influence the viscosity of the solution. Furthermore, the opalescence of the solutions as well as phase separation are analyzed in terms of S(q). In summary, the interpretation of experimental SAXS S(q) curves with MD simulation to discern the strength of PPI in solution is an invaluable tool for understanding opalescence, phase separation and viscosity needed for pharmaceutical development of mAb therapies.