Proteins therapeutics have emerged as an important class of biological macromolecules with the potential to improve survival rates and quality of life of patients suffering from a variety of rare or difficult-to-treat diseases. Recombinant protein therapeutics are produced in living organisms and are prone to physical and chemical degradation throughout all stages of production, storage, and administration. Non-native aggregation, or the net-irreversible formation of higher molecular weight oligomers and multi-mers, is a problematic form of degradation, especially during storage and administration. Aggregation can lead to loss of purity and efficacy of the therapeutic substance and cause immunogenic response in a patient during administration.

“Weak” or “colloidal” protein-protein interactions (PPI) have been previously related to the aggregation rate and mechanism of protein solutions. PPI are typically considered to be non-specific and are influenced by the solution conditions (pH, ionic strength, types of cosolutes) as well as a protein’s amino acid sequence. In this work, PPI are investigated as a design target for modulating protein aggregation. Molecular simulations are also utilized in this work as a tool to evaluate the effects of changes to amino acid sequence or solution conditions on PPI.

The effects of modulating PPI through changes to amino acid sequence of a model protein were investigated in this work. Molecular simulations were used to predict single-charge amino acid substitutions of human gamma-D crystallin (γDc) expected to alter PPI without significantly impacting conformational stability. A subset of potential variants was produced for biophysical characterization of PPI, conformational stability, and aggregation rates. With one exception, variants with increased net-repulsive PPI where observed to have decreased aggregation rates and variants with decreased net-repulsive PPI had increased rates. Also, the predicted values of the second osmotic virial coefficient, $B_{22}$, of the variants showed semi-quantitative agreement with experimentally measured values.

This engineering approach was also applied to the 4-4-20 anti-fluorescein single-chain variable fragment (scFv). Molecular simulations were applied to predict single-charge variants
expected to modulate PPI without significantly perturbing conformational stability. Experimental characterization of WT 4-4-20 and selected variants showed unexpected strong electrostatically-driven net-attractive PPI and self-association at near-neutral pH and low ionic strength. Influence of the highly flexible polypeptide linker connecting the domains of the scFv on PPI was hypothesized as a potential reason for the disparity between predicted and experimental results. PyRosetta was used to generate a small subset of 4-4-20 structures containing different linker conformations. The effects of the different linker conformations on $B_{22}$ were evaluated using molecular simulations. Results showed that the configuration of the linker has a significant effect on the calculated $B_{22}$ values, and can result in strong attractive interactions between oppositely charged residues of two protein monomers.

Finally, the PPI of three different monoclonal antibodies (mAbs) were investigated using biophysical characterization. Light scattering experiments showed distinct dependence on ionic strength (or lack thereof) of PPI for each mAb. Calculated values of $B_{22}$ from parameterized molecular simulations were in quantitative agreement with experimentally measured values for the IgG1 and captured qualitative trends in $B_{22}$ with ionic strength for the two IgG4s characterized here. The behavior of PPI with ionic strength was also compared to the trends observed in relative aggregate formation at 35 °C for each mAb. Overall, the results suggest that PPI may serve as a design target for aggregation resistance and molecular simulations can be tools to assess and predict changes to PPI.