Aggregation Prediction in Therapeutic Protein Formulations for Excipient Design

Brock C. Roughton[1], Anthony I. Pokphanh[1], T. Steele Reynolds[1], J. Laurence[2], E. M. Topp[3], and K. V. Camarda[1]

1 Department of Chemical and Petroleum Engineering, University of Kansas, Lawrence, KS

2 Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, KS

3 Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, IN

Protein Aggregation and Excipients

•Proteins can interact with each other through electrostatic or hydrophobic/hydrophilic interactions to form bound groups called aggregates[1].

•Aggregation is a major concern when developing protein therapeutics. Significant aggregation can lead to product loss and may elicit an immune response in the patient[2].

•Aggregation may be limited by the use of excipients in the therapeutic formulation. Excipients are molecules used as additives in pharmaceuticals[3].

•This project is concerned with predicting the aggregation of proteins, specifically the regions most prone to aggregation.

•Once the aggregation prone regions are known, excipients can be designed that will favorably interact with the regions. An ideal excipient will minimize the possible interactions between proteins.

•Preliminary experimental data has been collected for the percent aggregation observed in various protein-excipient formulations. The excipients considered were poly(vinylpyrrolidone) (PVP), trehalose, and guanidine hydrochloride.

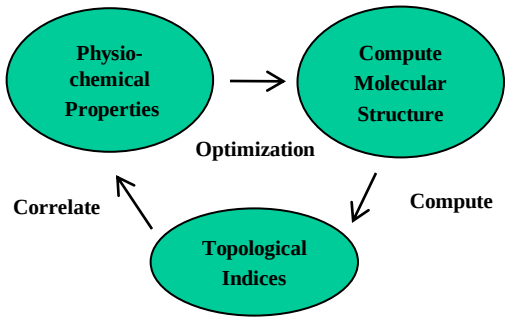
•The data collected indicates that trehalose is most effective in preventing protein aggregation. Sugars, such as trehalose, are widely used in practice to stabilize protein formulations. Sugars may interact with hydrophobic regions on the protein's surface.

Computational Molecular Design of Excipients

•In industry, trial-and-error approaches or heuristics are often used to determine which excipients are included in the pharmaceutical formulation.

•Many common excipients are not effective for many emerging biologics and novel drug delivery systems[4].

•This project aims to use Computational Molecular Design to determine optimal excipients for protein formulations.



Computational Molecular Design Methodology

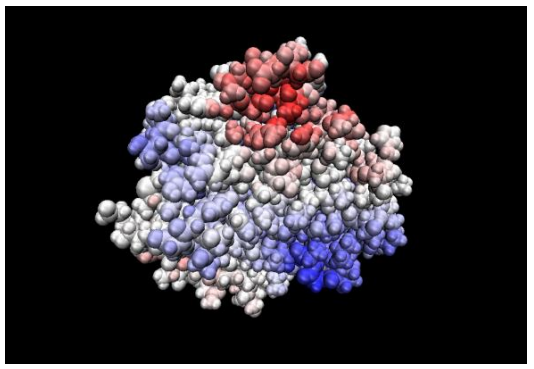
•Computational Molecular Design is a methodology to develop novel structures for molecules based on specific physical chemical and biological property targets.

•Models are created to relate properties to the structure of known molecules, in this case excipients and proteins.

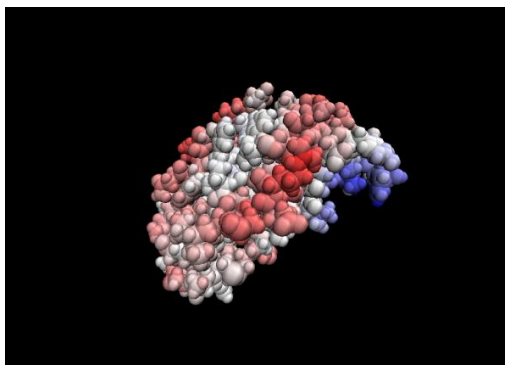
•The models used to find optimal molecules that match a desired property set. For this project, the optimal excipients will be found that minimize protein aggregation.

•To use Computation Molecular Design for protein-excipient systems, aggregation must be modeled.

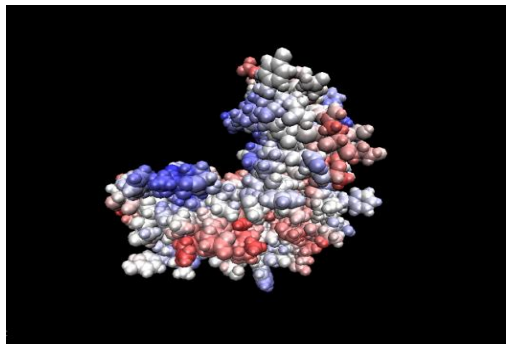
Computational Aggregation Prediction



SAP Mapping for Bovine DNase I (14 Hotspots Predicted by Aggrescan)



SAP Mapping for Bovine Insulin (6 Hotspots Predicted by Aggrescan)



SAP Mapping for Recombinant Human Insulin (9 Hotspots Predicted by Aggrescan)

•Surface Aggregation Potential (SAP) uses the hydrophobicity , solvent accessible surface area, and proximity of residues in the folded state to determine aggregation prone regions or "hot spots"[5].

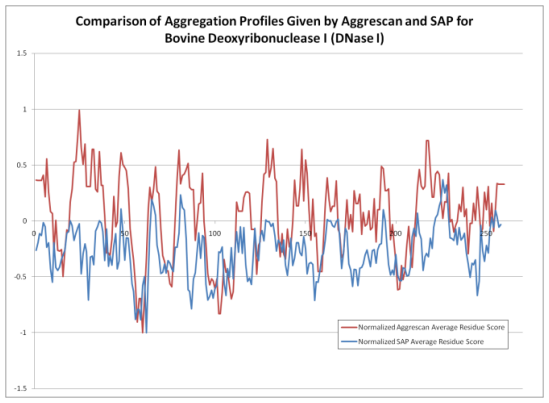
•SAP considers the protein's tertiary structure and assumes only the solvent accessible surface area is available to interact with other proteins during aggregation.

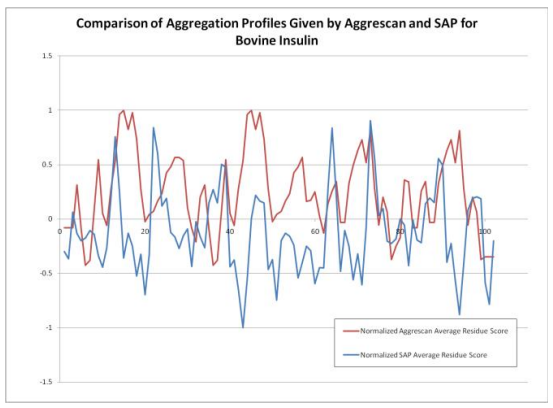
•Aggrescan looks at the amino acid sequences for regions (hot spots) where the amino acids have high aggregation propensities[6].

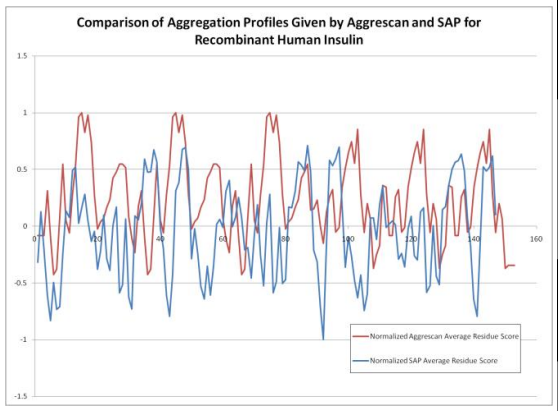
•Aggrescan uses experimental data to determine aggregation propensities for each residue.

•Aggrescan is only concerned with the primary sequence.

Comparison of Aggregation Prediction Methods







•Aggregation profiles were calculated for Bovine DNase I, Bovine Insulin, and recombinant human insulin using both Aggrescan and SAP. The profiles were normalized by the residue's aggregation propensity with the highest magnitude for each protein.

•The aggregation profiles of each protein show similar trends overall for both methods

•Any major discrepancies are likely due to differences in how the protein is modeled by the computational programs. Regions considered aggregation prone by Aggrescan may not be part of the exposed surface area and therefore are not considered aggregation prone by SAP.

•Aggrescan takes seconds to run through an internet browser, while SAP takes anywhere from 10 minutes to an hour on a cluster of 16 x AMD 1.2 GHz MP processors , depending on protein size.

Experimental Aggregation Prediction

•FTIR experiments are being conducted on protein systems previously studied via H/D exchange.

•H/D exchange shows which regions exchange deuterium for hydrogen. The exchange regions are exposed and should prove to be most susceptible to aggregation[7].

•Experiments are also being performed on proteins that have had part of their amino acid sequence truncated. The FTIR results will be compared to the results of the wild type protein to determine how the removed regions affect the exposed surface area.

•Further work will compare the exposed regions to the predicted aggregation prone regions.

Conclusions and Future Work

•Protein aggregation can be computationally modeled using Aggrescan and SAP. The prediction will be used to create models for designing excipients via Computational Molecular Design.

•Aggrescan may work best for a quick view of the aggregation tendencies of a protein, especially if many different proteins need to be quickly evaluated. SAP can be used to provided a more refined estimate of aggregation for a protein of interest.

•Molecular simulations will study the interactions of proteins and excipients. The regions of interaction should match the predicted aggregation prone regions.

•FTIR analysis will be performed on protein-excipient mixtures to determine the excipient's effect on the exposed surface area of the protein.

Acknowledgements

- Dr. Gerry Lushington
- Lynn Villafuerte
- Dr. Naresh Chennamsetty
- Natalie Ciaccio
- IMSD – NIH 5 R25GM62232
- NIH R01GM085293

References

1. Ajit, Joseph M., Schowen, Richard L., Borchardt, Ronald T., Salsbury, Jonathon S., Munson, Eric J., Topp, Elizabeth M. Reaction of a Peptide with Polyvinylpyrrolidone in the Solid State, *Journal of Pharmaceutical Sciences*, **92**, *3*, (2003).
2. Andya, J., Hsu, C., Shire, S. Mechanisms of Aggregate Formation and Carbohydrate Excipient Stabilization of Lyophilized Humanized Monoclonal Antibody Formulations, *AAPS PharmSci*, **5** *2*, (2003).
3. Cromwell, M., Hilario, E., and Jacobson, F. Protein Aggregation and Bioprocessing, *The AAPS Journal*, **8,** *3*, (2006).
4. Gibson, Mark. Pharmaceutical Preformulation and Formulation, CRC Press, Boca Raton, (2004).
5. V. Voynov, N. Chennamsetty, V. Kayser, B. Helk, and B. L. Trout, "Predictive tools for stabilization of therapeutic proteins", mAbs, 1, 580 (2009).
6. Aggrescan, http://bioinf.uab.es/aggrescan, accessed 2010.
7. Sinha, S., Li, Y., Williams, T., and Topp, E. Protein Conformation in Amorphous Solids by FTIR and by Hydrogen/Deuterium Exchange with Mass Spectrometry, *Biophysical Journal*, **95**, *12*, (2008).