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Understanding and predicting aggregation in biopharmaceuticals.

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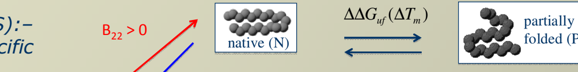
The aim is to develop a combined experimental/computational toolkit for assessing the manufacturability of proteins. One of the main obstacles we address is preventing protein aggregation throughout protein production, processing, and in formulations. In addition, in the final steps of filling and formulation, high protein concentrations are often required leading to problems associated with solution cloudiness and phase separation, as well as high viscosities, all of which need to be avoided.

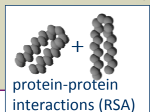
Overview of protein self association, partial unfolding and aggregation

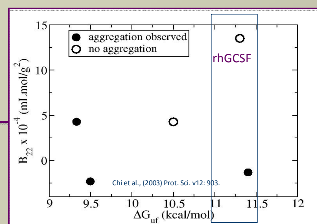
Static Light Scattering (SLS):– used to measure non-specific protein-protein PPIs from B_{22}

$B_{22} > 0$ repulsive PPIs

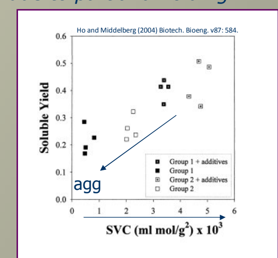
$B_{22} < 0$ attractive PPIs





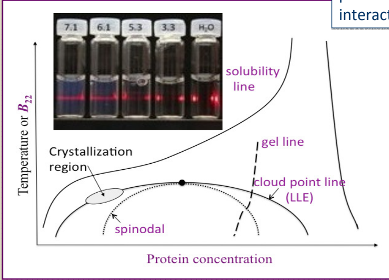


Aggregation propensity is often assessed by examining changes in T_m upon changing solution conditions (pH, ionic strength, buffer type and strength) which works well when rate limiting step is due to partial unfolding.



partially folded PPIs

aggregate



In some cases, there is a correlation between B_{22} and aggregation propensity implying that interactions between native proteins correlate with interactions between partially folded proteins, which in turn, are a controlling feature in aggregation pathways.

Reduced refolding yields occur due to aggregation. Here a correlation is observed with B_{22} measured under denaturing conditions and aggregation with minimal amounts of denaturant. This implies that protein-protein interactions between partially folded proteins can be extrapolated from measurements taken under denaturing conditions.

Attractive protein-protein interactions (or reversible self association) correlate with properties of concentrated protein solutions, such as phase separation, opalescence and high viscosities

- A better understanding of the structural prerequisites for aggregation can provide insights into mutations that engineer out aggregation hot spots
- Improved predictive and experimental methods are needed for screening excipient conditions to find optimal formulations, where aggregation upon storage is minimized, viscosities are reduced, and phase separation is avoided.

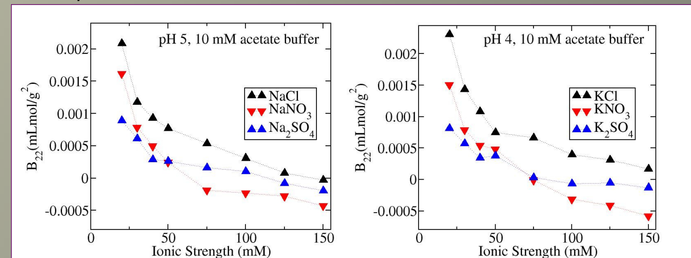
Choice of materials to be studied:

Our main focus lies on antibodies – whole immunoglobulin monomer and fragments. However we will benchmark methods by studying behaviour of model proteins in aqueous solutions, such as lysozyme. All proteins we currently study are listed in table below.

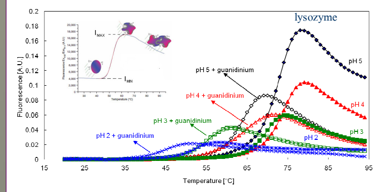
Protein	Reason for studying
Lysozyme, BSA and Ribonuclease A – initial stages only	Proteins well characterised in literature. Stable globular structure and predictive behaviour in broad range of pH. Cheap enough to use for improving experimental methods
Human Single Chain Fv (ScFv)[1]	Antibody fragment with variable regions. Model fragment of antibody for studying self-association behaviour. Easily produced in laboratory scale
Human V_H domains	Model system for mutagenesis studies, easy to produce in laboratory scale
Human monoclonal antibodies	Full structure of antibody – 2 light and 2 heavy chains. Monoclonal well purified sample. Samples donated by Pall, MedImmune, and Arecor (Genzyme)

Experimental methods:

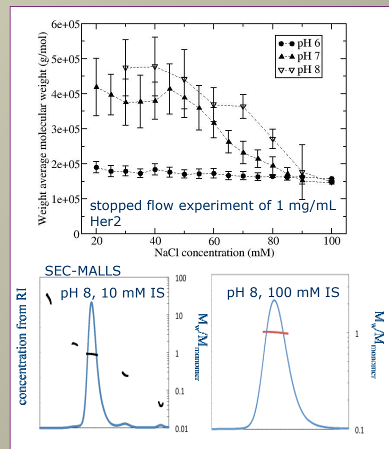
***Static and dynamic light scattering:** We are developing high throughput methods for measuring protein-protein interactions. Below we show results of B_{22} studies on lysozyme where we analyze specific ion effects as a function of ionic strength and pH. Understanding specific ion or buffer effects are especially relevant, as aggregation upon storage is sensitive to the buffer used in the liquid formulation.*

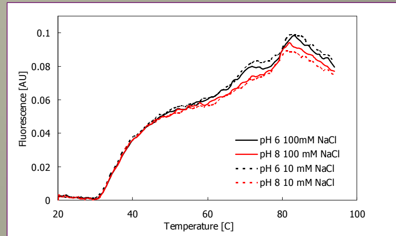


***Differential Scanning Fluorimetry** (DSF) is a HT inexpensive method for screening. The protein melting temperature is related to increase of fluorescence when dye binds to exposed hydrophobic parts of protein.*

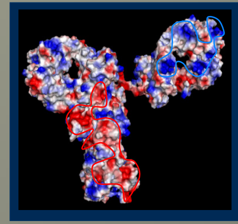


Aggregation studies of herceptin binding antibody (Her2) – IgG1





Aggregation occurs readily at pH 7 and 8 in low ionic strength solutions (i.e. 10 mM). Aggregation is reduced when decreasing pH to 6 or increasing ionic strength to 100 mM. DSF studies indicate the protein conformational stability does not depend on ionic strength, indicating aggregation is due to self-association. Consistent with B_{22} studies at 100 mM (= -3.5 x 10^{-4} mLmol/g²) reflecting strong reversible self association.



Aggregation behaviour can be rationalized by an electrostatic attraction mechanism. Here, we have calculated the electrostatic potential for a homology model of Her2. Preliminary results indicate that the patchiness of the surface is very important for controlling the electrostatic attraction.