Assess aggregation risk at higher protein concentrations with G_{22}

Introduction

Weak, nonspecific interactions between protein molecules can have a large impact on colloidal stability during the development of protein-based drugs. The diffusion interaction parameter (k_D) and the second virial coefficient (B_{22}) enable early characterization of colloidal stability for dilute protein solutions. While these measurements typically use low protein concentrations (< 10 mg/ml), aggregation risk can vary rapidly at higher protein concentrations (> 10 mg/ml), which is the relevant range for most biopharmaceuticals. At high protein concentrations, short-range intermolecular interactions become more prominent and can increase the risk of protein aggregation. For these conditions, the Kirkwood-Buff Integral (G_{22}) serves as the better measure of colloidal stability because it accounts for protein crowding effects that give rise to strongly attractive or repulsive interactions.

Using dynamic and static light scattering detection methods, Uncle measures both k_D and B_{22} simultaneously and allows you to identify the aggregation propensity of your protein under dilute conditions. Uncle can re-analyze the same k_D and B_{22} dataset to independently calculate G_{22} values for high protein concentrations, providing multiple self-interaction measurements within one experiment. As a guiding principle, B_{22} is valid when the following inequality holds true; otherwise, G_{22} should be used,

$$|| c \times B_{22} \times MW || < 0.05$$

where c is the protein concentration and MW is the molecular weight of the protein.[1] Positive values for k_D and B_{22} indicate weak, net-repulsive intermolecular forces between protein molecules while negative values indicate net-attractive interactions. The interpretation of the sign of G_{22} is opposite to that of B_{22}, such that a positive value indicates net-attractive interactions and a negative value indicates net-repulsive interactions. A B_{22} or G_{22} value of zero indicates net-neutral intermolecular forces between protein molecules.

Figure 1: Uncle: a one-stop protein stability platform.

Uncle is a one-stop protein stability platform that uses fluorescence, SLS and DLS detection to enable 12 different applications (Figure 1). Multiple measurements such as thermal melting, aggregation, and sizing can be performed with the same samples in just one experiment, allowing you to obtain more information on your biologics faster than before. Uncle uses only 9 µL of sample and can measure up to 48 samples simultaneously, enabling greater flexibility for characterizing your proteins.

Methods

The scattering intensity of Toluene was measured in a Uni to calibrate the standard parameters for the instrument. Commercial alpha-chymotrypsinogen was first dialyzed into 10 mM sodium citrate buffer (4 x 1L) at pH 3.5 to remove excess salts and then treated with PMSF to inhibit trypsin.[2] Alpha-chymotrypsinogen was then formulated at 80 mg/mL in 5 mM sodium phosphate buffer at pH 7 or 40 mM sodium acetate buffer at pH 5 with or without 300 mM NaCl. The exact concentrations were verified by absorbance and dilutions were made to obtain seven protein concentrations down to 20 mg/mL. Nine µL of each sample were loaded in triplicate in a Uni and run with the G_{22} application using 4 DLS acquisitions of 5 seconds each. Uncle software uses the light scattering intensity from each sample to calculate the R_{90}/K values, which are used in conjunction with the expected molecular weight of the protein (~25.7 kDa) to calculate G_{22} values for each protein concentration.

For experiments using a human monoclonal antibody, Adalimumab (~144 kDa) was formulated at 100 mg/mL in its commercial formulation at pH 5.2. The exact concentration was verified by absorbance and dilutions were made to obtain seven protein concentrations down to 14 mg/mL. Sample loading and Uncle analysis were performed as described previously.

Results

A strong upward trend for R_{90}/K suggests net attractive interactions for alpha-chymotrypsinogen in phosphate buffer in the presence or absence of NaCl (Figure 2). Both sodium acetate buffer conditions also display positive slopes for R_{90}/K, although the buffer condition without NaCl indicates smaller net attractive interactions between protein molecules (Figure 3). These results are consistent with the reported trends in scattering intensity for alpha-chymotrypsinogen under these buffer conditions with increasing ionic strength.[2]

The value of G_{22} is concentration-dependent, according to the following equation,

$$\frac{R_{90}}{K} = MW_{app}c + MW \times G_{22}c^2$$

where c denotes concentration and MW and MW_{app} denote the molecular weight and apparent molecular weight, respectively.[1] The G_{22} values for each concentration were calculated by Uncle and these values were plotted for the 50-80 mg/mL protein concentrations for the different formulations (Figure 4).

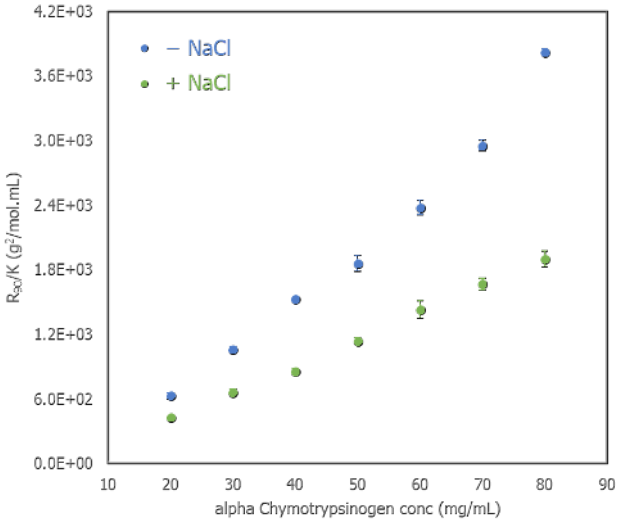

Figure 2: Scattering intensity as a function of protein concentration in phosphate buffer at pH 7 with or without NaCl.

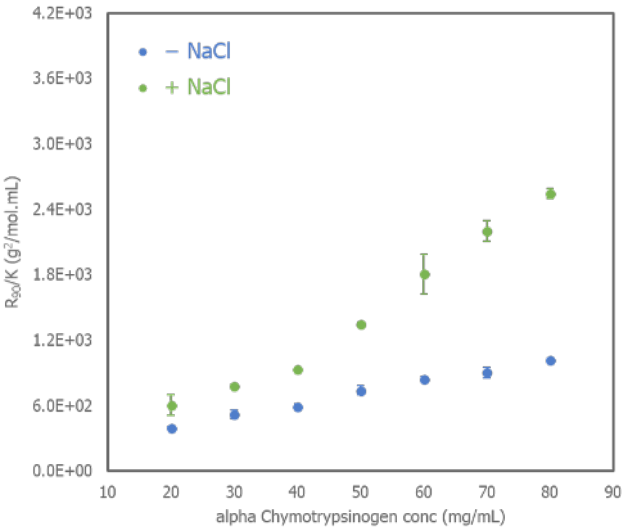

Figure 3: Scattering intensity as a function of protein concentration in sodium acetate buffer at pH 5 with or without NaCl.

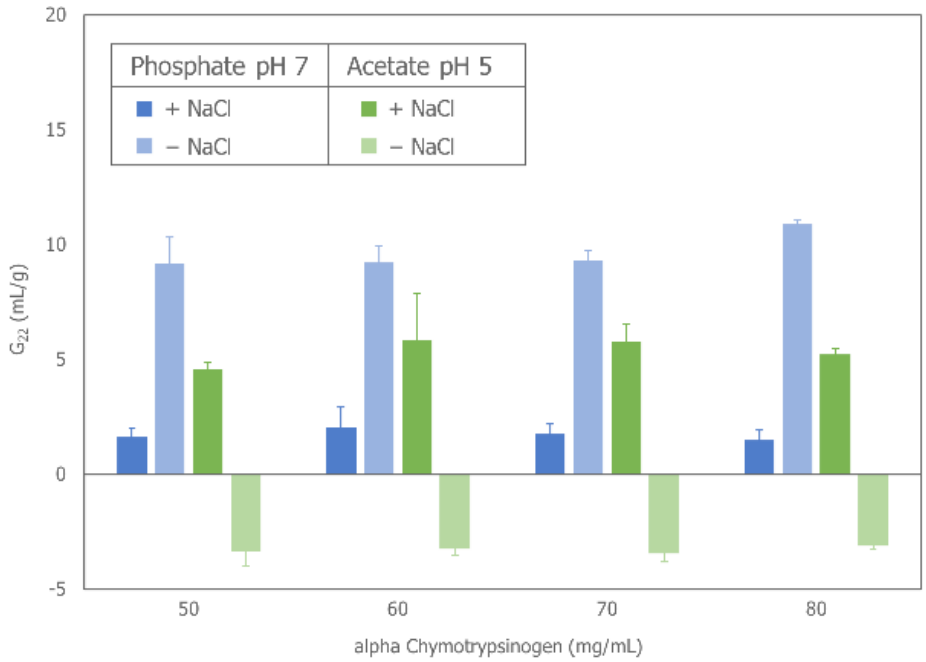

Figure 4: Calculated G_{22} values for each protein concentration for all four formulations. Shown are the average values of three replicate measurements. Error bars denote the standard deviation.

The calculated positive G_{22} values for all conditions, apart from sodium acetate buffer without salt, indicates that the protein has a propensity to self-associate under these conditions. The magnitude of the G_{22} value indicates the strength of the protein-protein interactions, and in the case for phosphate buffer with 300 mM NaCl, the attractive forces are particularly weak. The formulation without salt for sodium acetate buffer shows a modest increase in R_{90}/K along with negative G_{22} values at the highest protein concentrations, indicating net-repulsive intermolecular forces between protein molecules.

Uncle can convert and analyze the same G_{22} dataset in the k_D and B_{22} application, or vice versa, providing additional information on colloidal stability with no additional experimental work (Table 1). Note that by using the inequality mentioned above, the values of k_D and B_{22} were determined to be valid for one condition.

Next, the aggregation propensity of a therapeutic antibody, Adalimumab, was evaluated at high protein concentrations in its commercial formulation at pH 5.2 (Figure 5). As expected, the negative G_{22} value at the highest protein concentration indicates net-repulsive interactions under these optimized conditions. At these high protein concentrations, it is not advisable to use B_{22} values. By checking the calculated B_{22} value with the inequality provided in the introduction, we determined that the calculated B_{22} was invalid for this protein concentration range.

Formulation	G_{22} (mL/g)*	k_D (mL/g)	B_{22} (mol mL/g^2)
Phosphate pH 7	10.97	-**	-
Phosphate pH 7, 300 mM NaCl	1.58	-	-
Acetate pH 5	-3.00	0.17	2.24E-04
Acetate pH 5, 300 mM NaCl	5.33	-	-

Table 1: Data from all formulations of alpha-chymotrypsinogen showing three independently calculated parameters of colloidal stability collected from a single experiment. *The value reported is the average of three replicates for the 80 mg/mL protein concentration. **Values for k_D and B_{22} were determined to be invalid according to the inequality provided in the introduction.

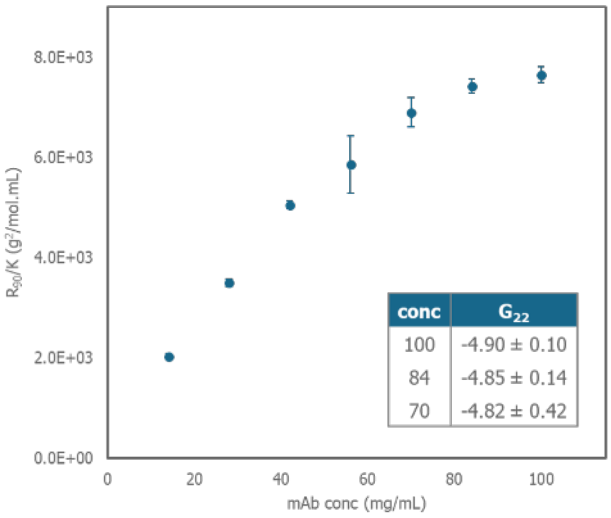

Figure 5: Scattering intensity as a function of mAb concentration. The table inset shows the calculated G_{22} parameters obtained for several concentrations from one experiment. Error bars denote the standard deviation from triplicate measurements.

Conclusion

To assess colloidal stability across both low and high protein concentrations, Uncle can measure and independently calculate three different parameters, k_D, B_{22}, and G_{22}. As illustrated in this technical note, this information provides researchers with additional flexibility to evaluate the colloidal stability of their constructs or formulations at multiple points in their workflow. Following previously reported results, the aggregation propensity of alpha-chymotrypsinogen was evaluated with two formulations at high and low salt conditions, which showed opposite solution behaviors for the two formulations. For the therapeutic antibody, a negative value for G_{22} was measured, indicating net-repulsive interactions for its commercial formulation at high protein concentrations. The built-in analysis of Uncle therefore provides wide-applicability for selecting constructs or formulations that minimize protein aggregation risk.

References

1. O'Brien CJ, Blanco MA, Costanzo JA, Enterline M, Fernandez EJ, Robinson AS, Roberts CJ. 2016. Modulating non-native aggregation and electrostatic protein-protein interactions with computationally designed single-point mutations. Protein Engineering, Design & Selection. 29(6): 231-243.

2. Woldeyes MA, Rubio CC, Furst EM, Roberts CJ. 2017. Predicting protein interactions of concentrated globular protein solutions using colloidal models. Journal of Physical Chemistry B. 121: 4756-4767.

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