The use of small angle X-ray scattering for studying excipient modulated physical stability and viscosity of monoclonal antibody formulations

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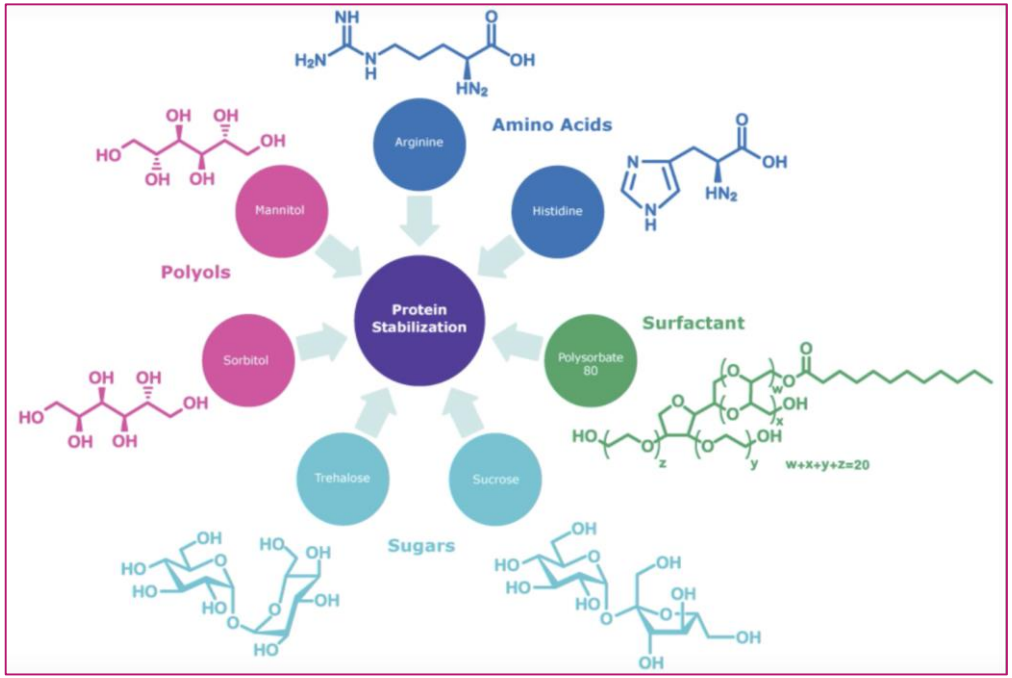
Challenges of monoclonal antibody (mAb) formulation

- High concentration is required to achieve therapeutic dosage
- High concentration leads to increased non-specific **protein-protein interactions (PPI)** that could lead to self-association and solution viscosity
- Excipients are used to improve protein colloidal stability (tendency to remain monomeric form)
- Selection of excipients involves laborious empirical screening due to limited knowledge of the effects of excipients on PPI



*Commonly used excipients**

*Image cited from: https://www.youtube.com/watch?v=LMGo7V2wgVQ

Aims of this study

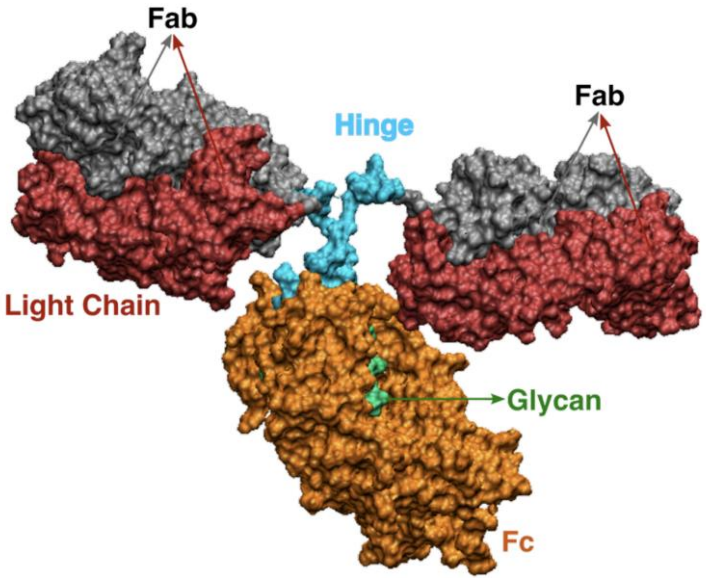
- To characterize the effects of excipients on a particular monoclonal antibody (NISTmAb)
- To evaluate different techniques for studying excipient modulated PPI in concentrated mAb formulations
  - *Physical stability:*
    Dynamic Light Scattering (DLS) vs Small Angle X-ray Scattering (SAXS)
  - *Solution viscosity:*
    DLS, SAXS (predicted) vs Viscosity measurements (experimental)

Excipient Class	Excipients	Buffer	Ionic Concentration (mM)	pH
Sugars	300 mM Glucose	25 mM Histidine	12.5	6
	300 mM Sucrose	25 mM Histidine	12.5	6
	300 mM Trehalose	25 mM Histidine	12.5	6
	300 mM Mannitol	25 mM Histidine	12.5	6
Amino Acids	171 mM Arginine	25 mM Histidine	196	6
	200 mM Proline	25 mM Histidine	12.5	6
	200 mM Glycine	25 mM Histidine	12.5	6
	200 mM Alanine	25 mM Histidine	12.5	6
Non-ionic Surfactants	0.06 mM Polysorbate 20	25 mM Histidine	12.5	6
	0.12 mM Polysorbate 80	25 mM Histidine	12.5	6
Salts	150 mM NH_4Cl	25 mM Histidine	162.5	6
	150 mM Na_2SO_4	25 mM Histidine	312.5	6
	150 mM NaCl	25 mM Histidine	162.5	6
	150 mM $NaClO_4$	25 mM Histidine	162.5	6
pH	-	67 mM Phosphate	82.8	6
	-	67 mM Phosphate	148.3	7
	-	67 mM Phosphate	196	8

mAbs in 25mM histidine buffer (without excipient) is used as control sample

NIST monoclonal antibody reference material (NISTmAb)

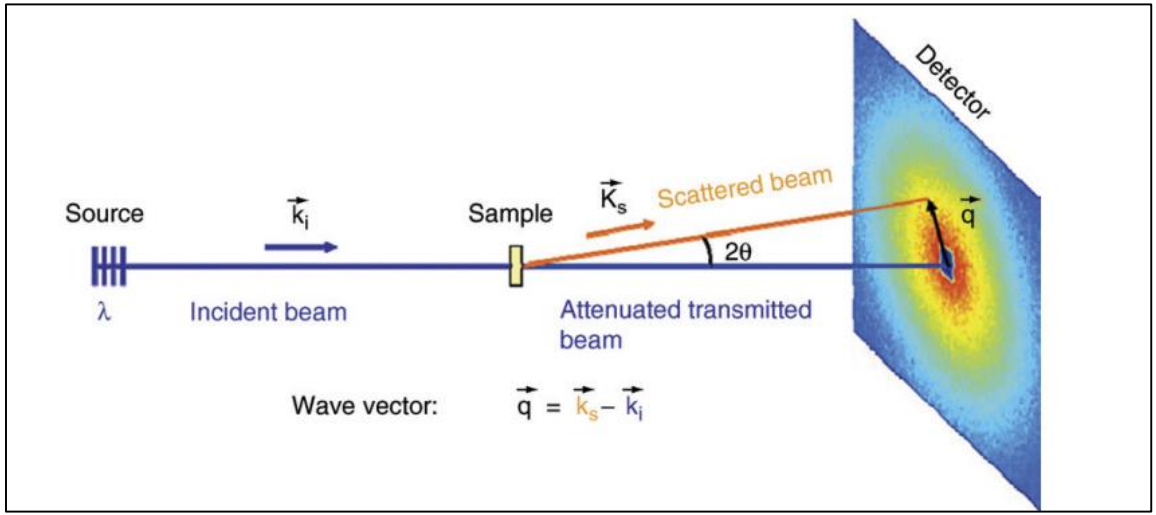
- First mAb (IgG1) reference material, representative of the largest class of biological therapeutics
- Standard reference material for analytical characterization of biopharmaceutical products, facilitates the assessment of existing analytical methods and promotes faster adoption of new technologies
- Used as representative mAb for this study



*3D structure of NISTmAb**

*Image cited from: Characterization of the NISTmAb Reference Material using small-angle scattering and molecular simulation. Analytical and Bioanalytical Chemistry 2018, 410, 8, 2141–2159

Small Angle Scattering



The scattered intensity is expressed as:

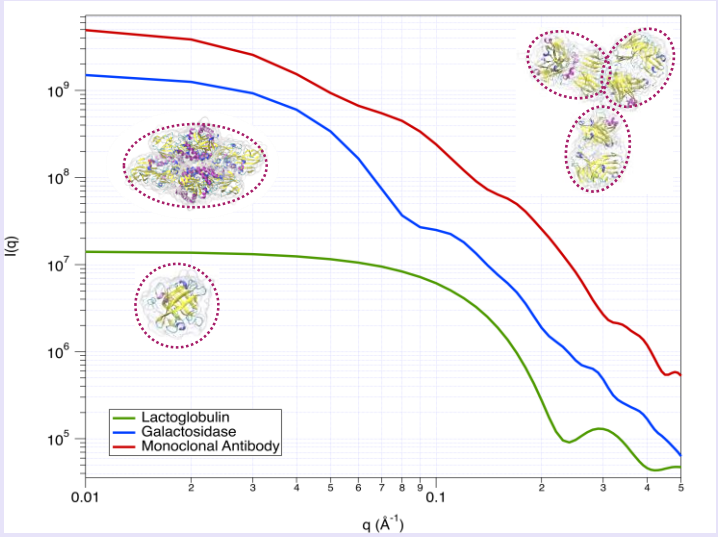
$$\boldsymbol{I(q) = (\Delta\rho^2 \phi V) * P(q) * S(q)}$$

Where $\Delta\boldsymbol{\rho}$ is the difference in scattering length density, $\boldsymbol{\phi}$ is the volume fraction, $\boldsymbol{V}$ is the volume of the scattered objects, ***P(q)*** is the **form factor** and ***S(q)*** is the **structure factor**

Small Angle Scattering

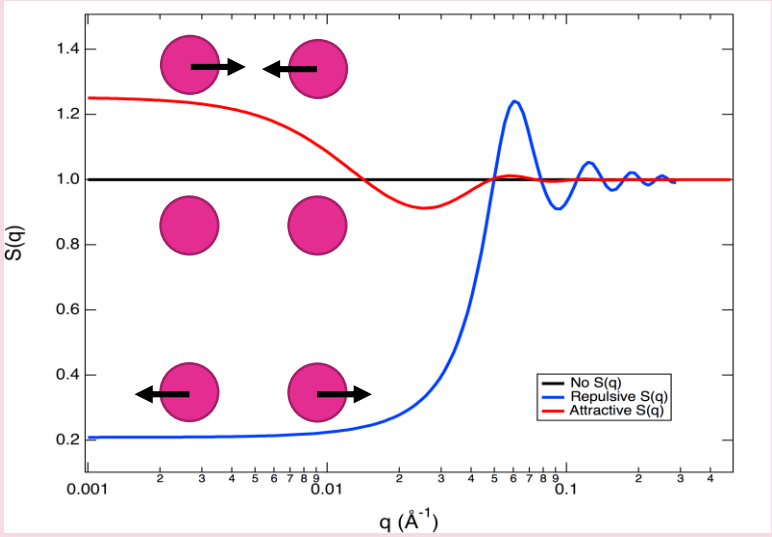
Form Factor *P(q)*

- Measured from dilute solution, where intermolecular interactions are negligible
- Contains information on the size and shape of scattering objects

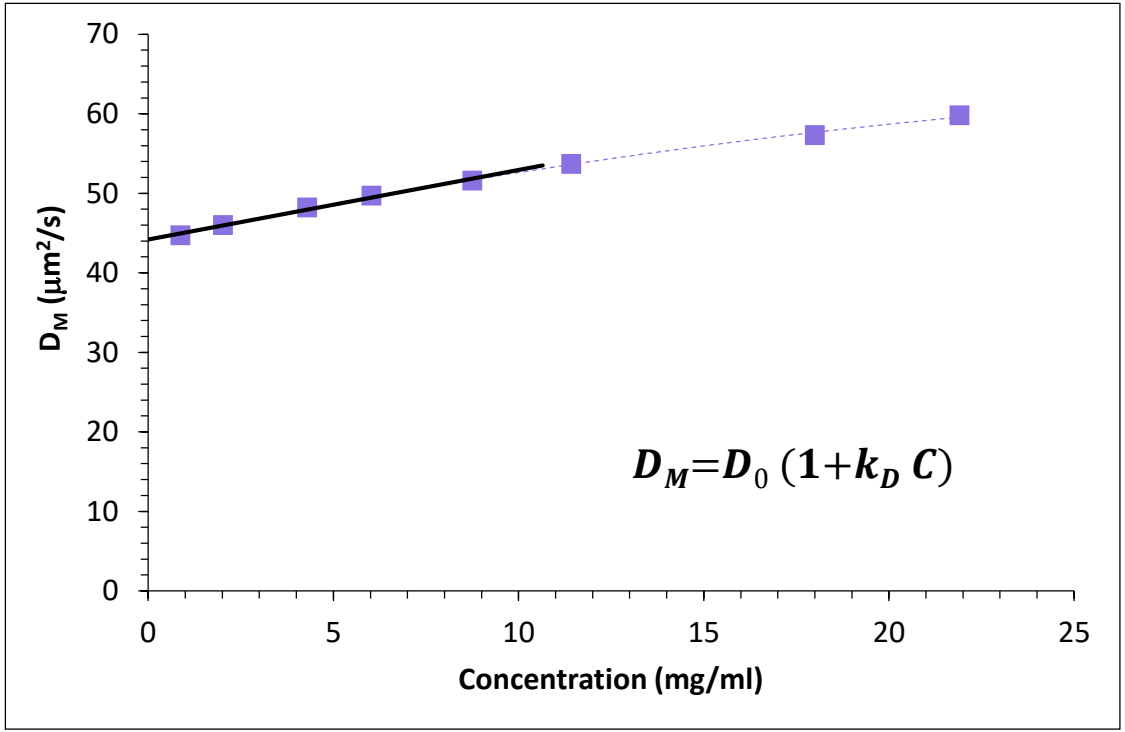


Structure Factor *P(q)*

- Arise due to intermolecular interactions with increasing concentration
- Contains information on the relative position/spatial correlation of scattering objects



Protein colloidal stability: DLS vs SAXS



- **Dynamic light scattering (DLS)**
  - Measured at low concentrations, (<10mg/ml), but used to predict properties of concentrated formulations
  - Interaction parameter k_D is obtained from DLS measurements:

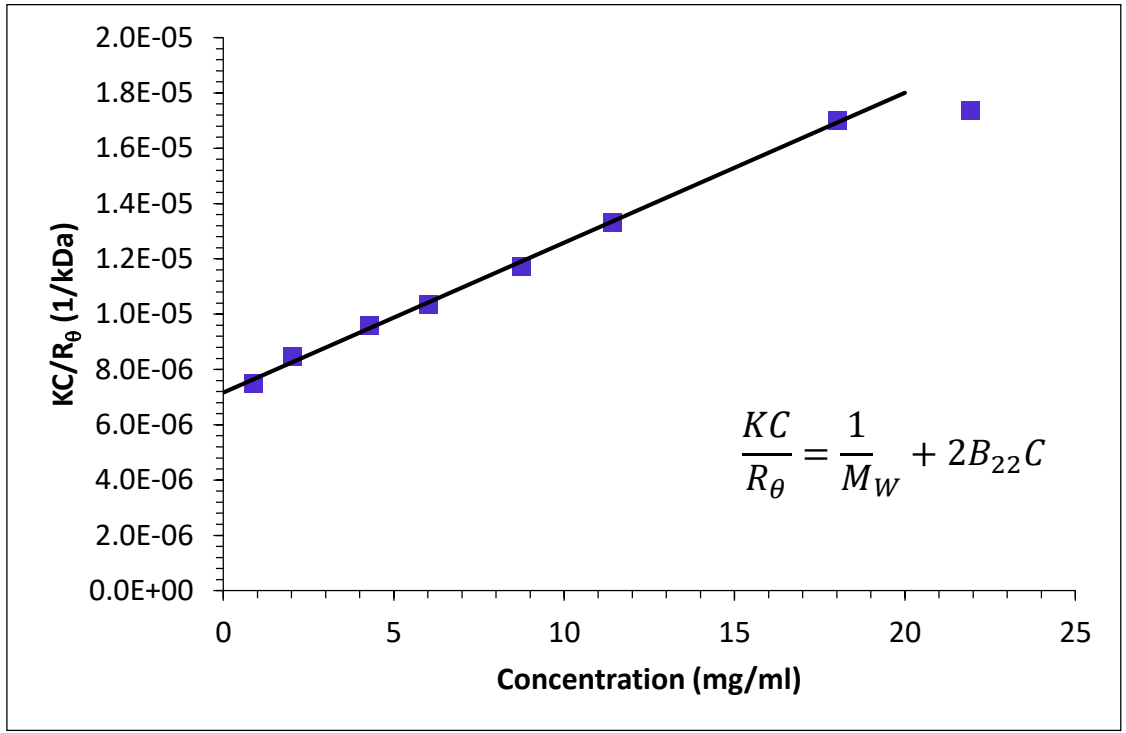
$$k_D = 2B_{22}M_W - (k_f + 2v)$$

Where $B_{22}M_W$ is the thermodynamic component, $k_f + 2v$ is the hydrodynamic component

  - ***k_D > -8 ml/g*: Net Repulsive PPI***
    k_D < -8 ml/g*: Net Attractive PPI

*Jayaraman, J. , et.al. (2014), Plasmonic Measurements of Monoclonal Antibody Self-association using Self-interaction Nanoparticle Spectroscopy. Biotechnol. Bioeng., 111: 1513-1520.

Protein colloidal stability: DLS vs SAXS



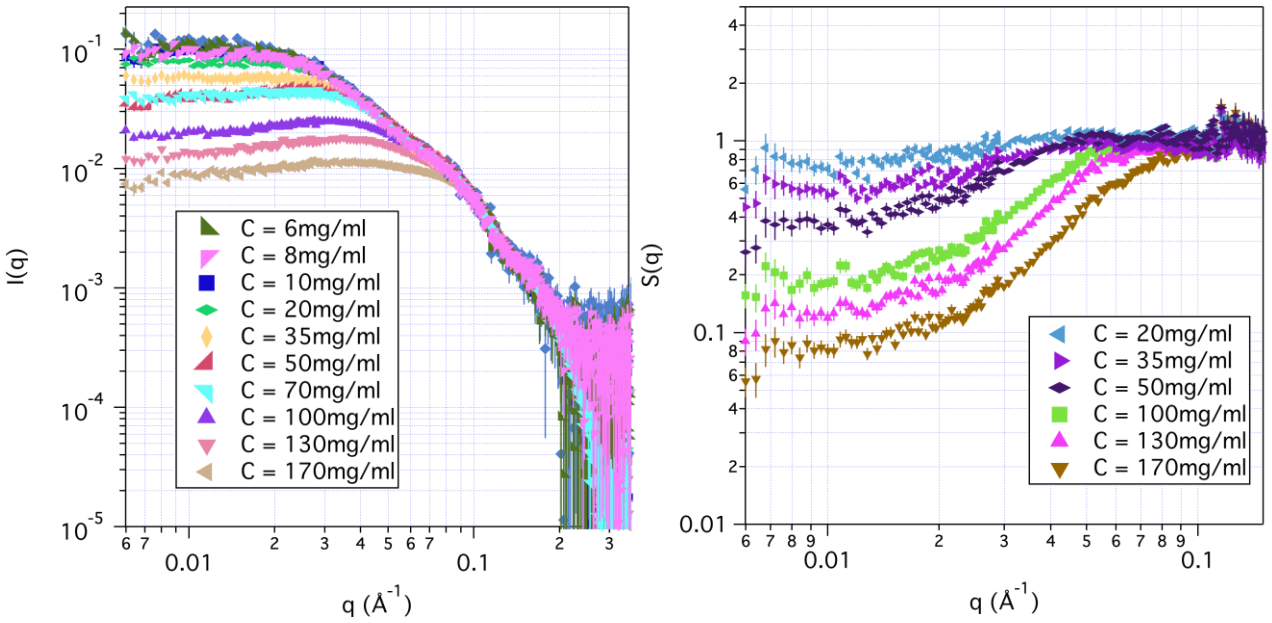
- **Dynamic light scattering (DLS)**
  - Measured at low concentrations, (<10mg/ml), but used to predict properties of concentrated formulations
  - 2nd virial coefficient B_{22} is obtained from DLS measurements:

$$\frac{KC}{R\theta} = \frac{1}{M_W} + 2B_{22}C$$

Where K is an optical constant, $R\theta$ is the Rayleigh ratio of scattered to incident light intensity, M_w is the weight average molecular weight

- ***B_{22} > 0 mol ml/g²: Net Repulsive PPI***
  B_{22} < 0 mol ml/g² : Net Attractive PPI

Protein colloidal stability: DLS vs SAXS



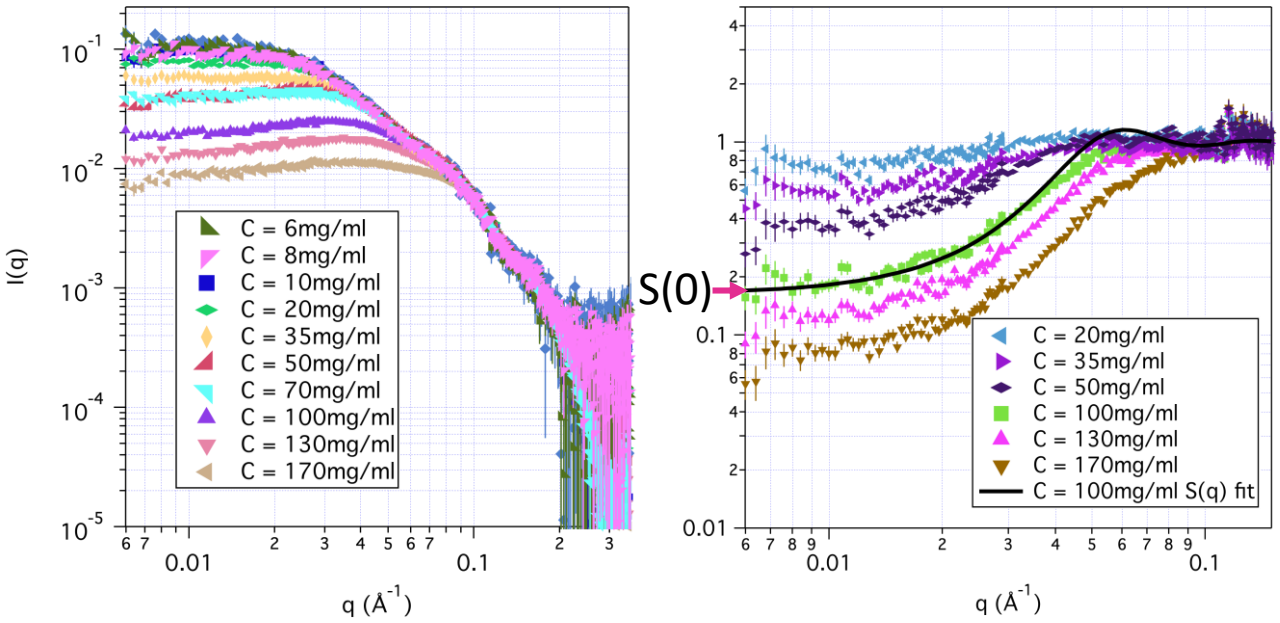
SAXS spectra and *S(q)* measured from NISTmAb in Alanine solution as a function of protein concentration

- **Small Angle X-ray Scattering(SAXS)**
  - Measured at both low and high concentrations

$$I(q) \propto P(q)S(q)$$

P(q) is measured from dilute solutions
S(q) is measured from concentrated solutions

Protein colloidal stability: DLS vs SAXS



SAXS spectra and *S(q)* measured from NISTmAb in Alanine solution as a function of protein concentration

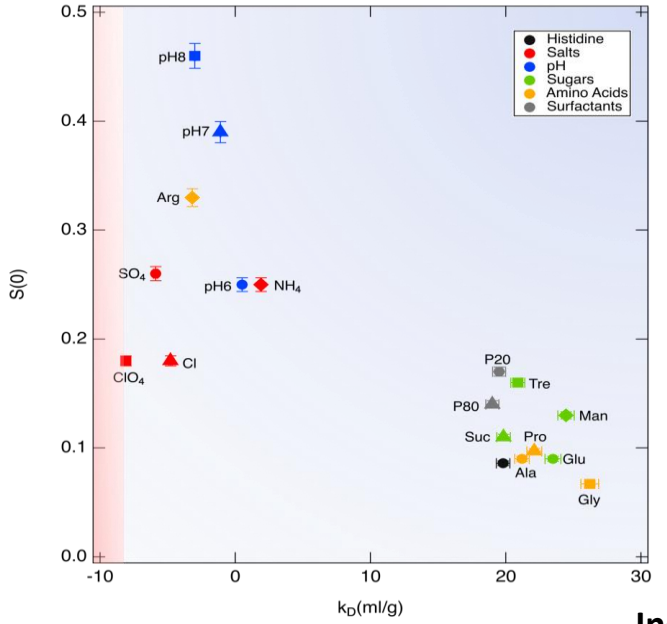
- **Small Angle X-ray Scattering(SAXS)**
  - *S(q)* at $q \rightarrow 0$, i.e. *S(0)* is obtained from fitting S(q) profile, it is used to study nature of PPI

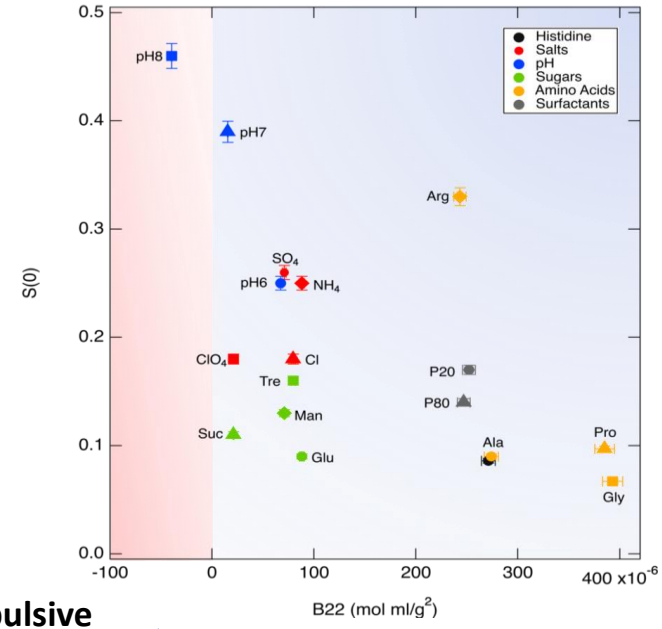
    S(0) < 1 : Net Repulsive PPI
    S(0) > 1 : Net Attractive PPI

Comparison between k_D / B_{22} and *S(0)*

- S(0) value less than 1 was measured from all excipient conditions, suggesting the net PPI was of repulsive nature
- Close agreement was found between *S(0)* and k_D values

Increasingly Repulsive

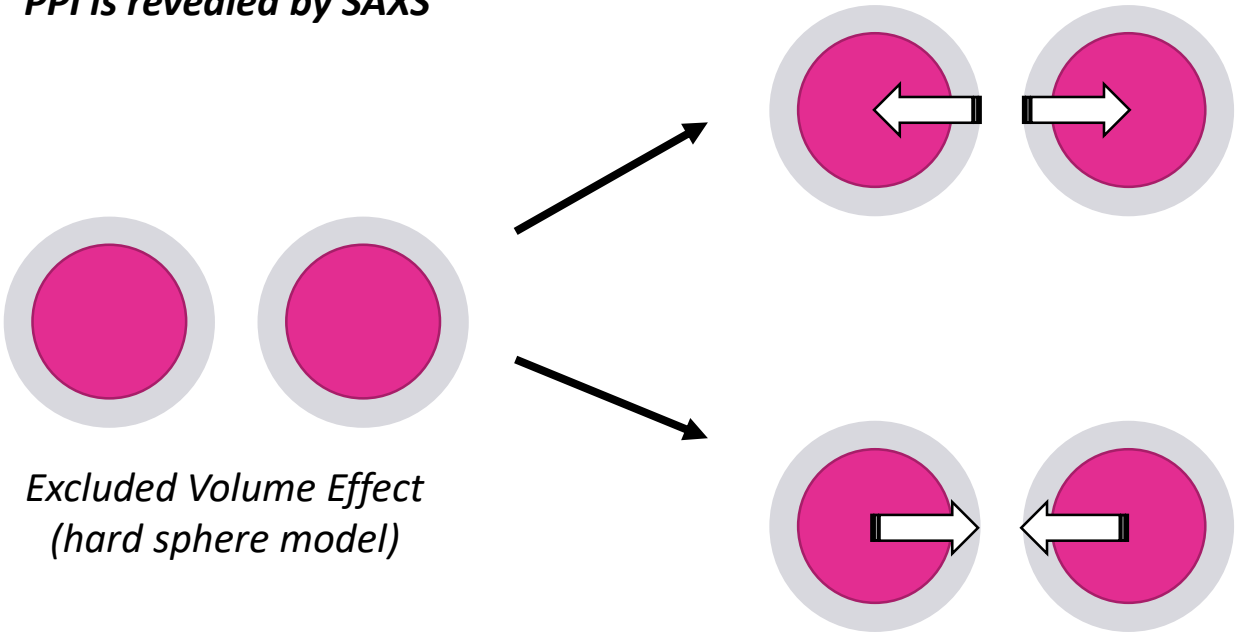




Increasingly Repulsive

Analysis of S(q) reveals various energetic components towards the net PPI

- ***Compared to DLS, more information on PPI is revealed by SAXS***



Additional Repulsive Interactions (More stable)

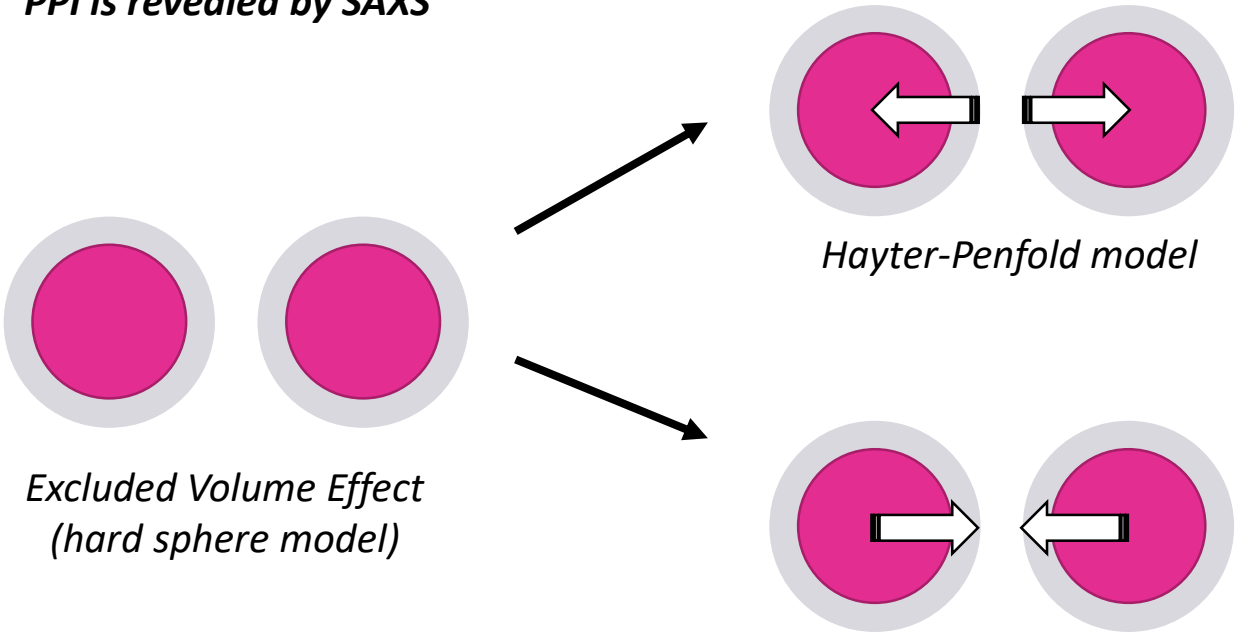
Excluded Volume Effect (hard sphere model)

Additional Attractive Interactions (Less stable)

Different contributors toward net PPI can be resolved by fitting S(q) profile to different models

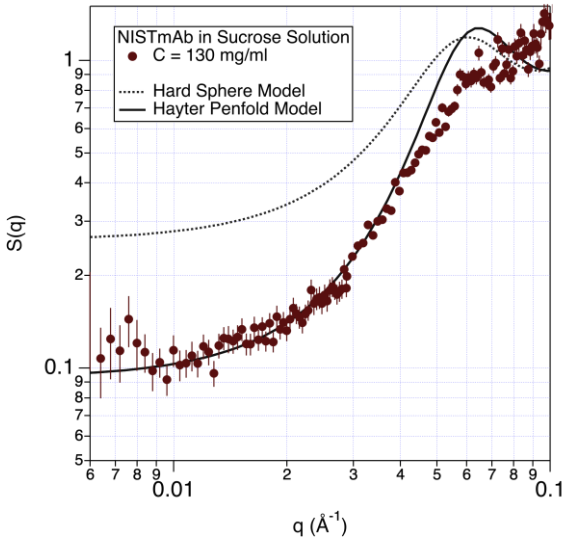
Analysis of S(q) reveals various energetic components towards the net PPI

- ***Compared to DLS, more information on PPI is revealed by SAXS***



Excluded Volume Effect (hard sphere model)

Hayter-Penfold model

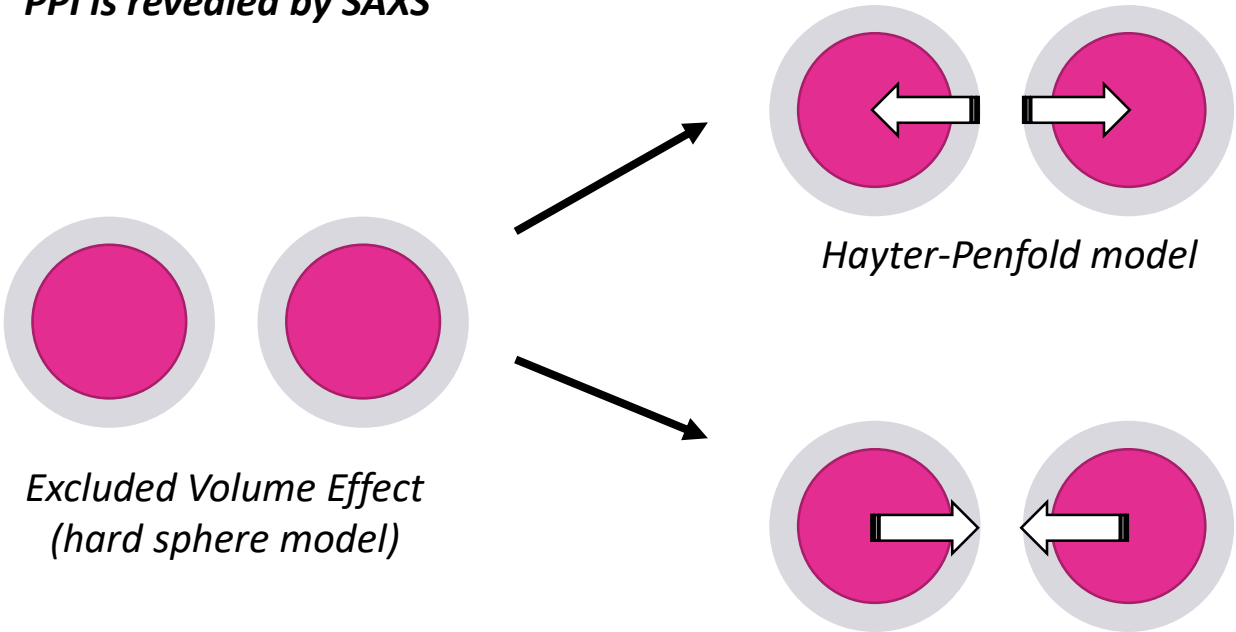


Additional repulsive forces lead to smaller S(0)

Different contributors toward net PPI can be resolved by fitting S(q) profile to different models

Analysis of S(q) reveals various energetic components towards the net PPI

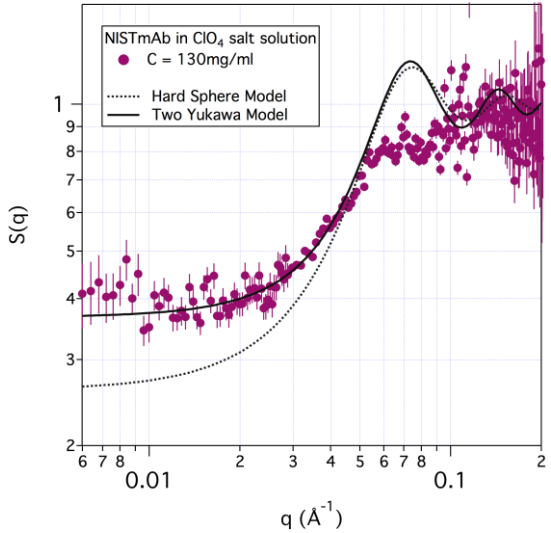
- ***Compared to DLS, more information on PPI is revealed by SAXS***



Excluded Volume Effect (hard sphere model)

Hayter-Penfold model

Two Yukawa model



Additional attractive forces lead to larger S(0)

Different contributors toward net PPI can be resolved by fitting S(q) profile to different models

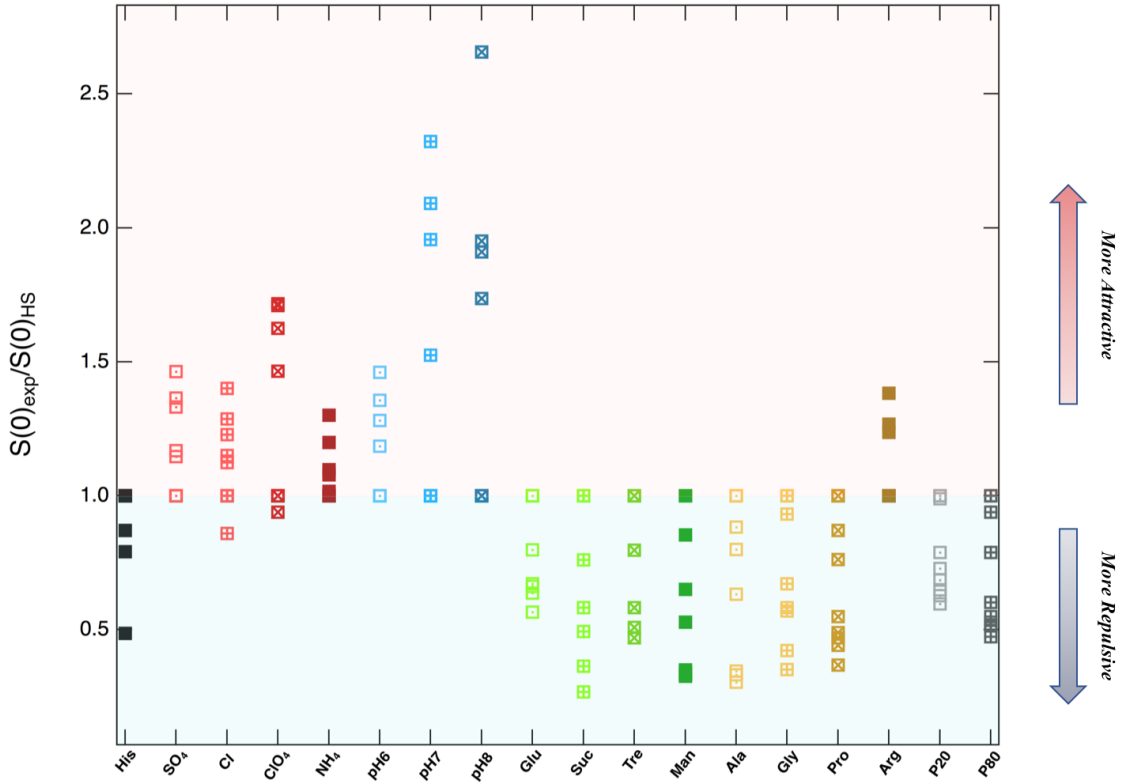
Analysis of S(q) reveals various energetic components towards the net PPI

- $S(0)_{exp}/S(0)_{HS}$

  < 1: improved colloidal stability

  >1: reduced colloidal stability

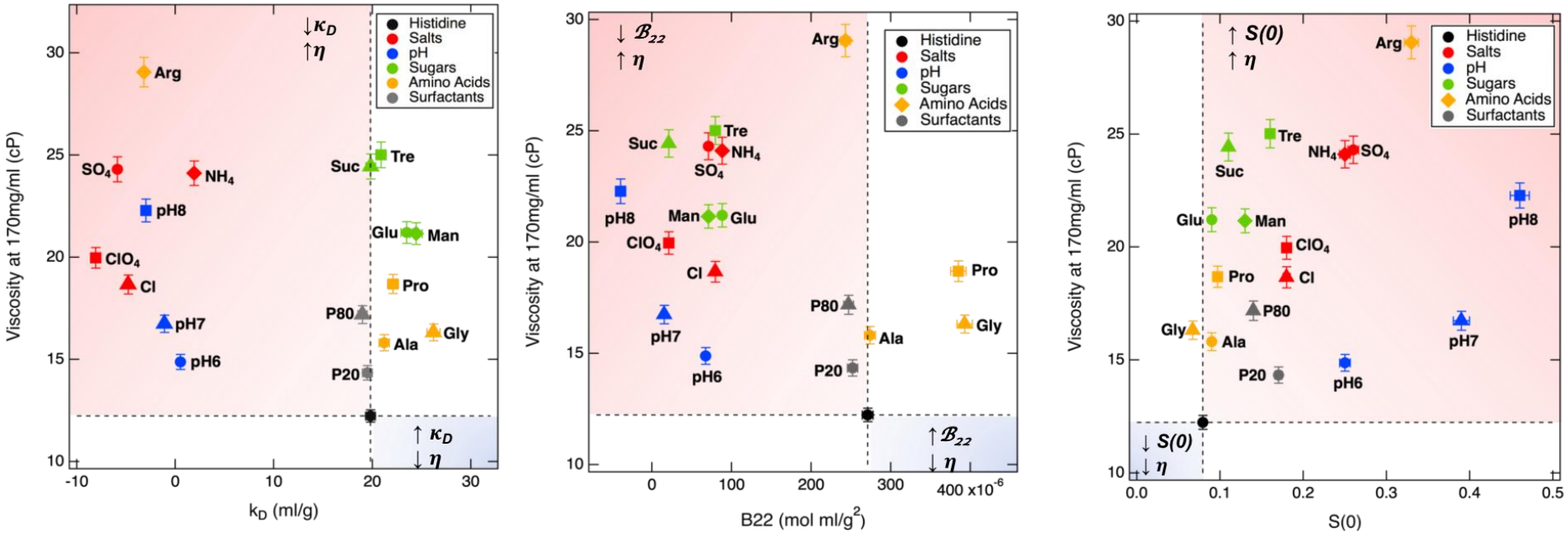
- Further analysis of *S(q)* reveals the presence of attractive intermolecular interactions even though the net PPI is repulsive



Summary of $S(0)_{exp}$ /$S(0)_{HS}$. Each point represents the ratio obtained for a particular protein concentration in given excipient condition

Solution Viscosity: Predicted vs Experimental results

- Measurements were made to obtain the viscosity (η) of concentrated NISTmAb formulations (170mg/ml), whereas k_D, B_{22} and $S(0)$ were used to predict the viscosity (η) of concentrated NISTmAb formulations



Shaded area highlights samples from which a decrease in k_D/B_{22} or an increase in S(0) is correlated with an increase in η, and vice versa

Conclusions

- NISTmAb is colloidally stable in all of the examined excipient conditions. Although the net PPI is repulsive, elevated solution viscosity was measured with the presence of excipients
- The close agreement between k_D and *S(0)* results suggests DLS could be used to provide reliable information on the colloidal stability of mAbs in concentrated formulations.
- Detailed analysis of S(q) reveals various energetic components towards the net PPI, hence provides valuable insights in guiding the excipient selections
- B_{22} and *S(0)* appeared to be better viscosity predictors than k_D. Disagreement between predicted and measured results suggests other factors apart from PPI contribute to the bulk rheological properties of concentrated protein solutions.

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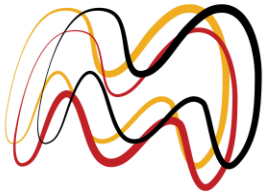

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