Approaches and challenges for quantifying multi-body interactions as higher-order structure for predicting product attributes

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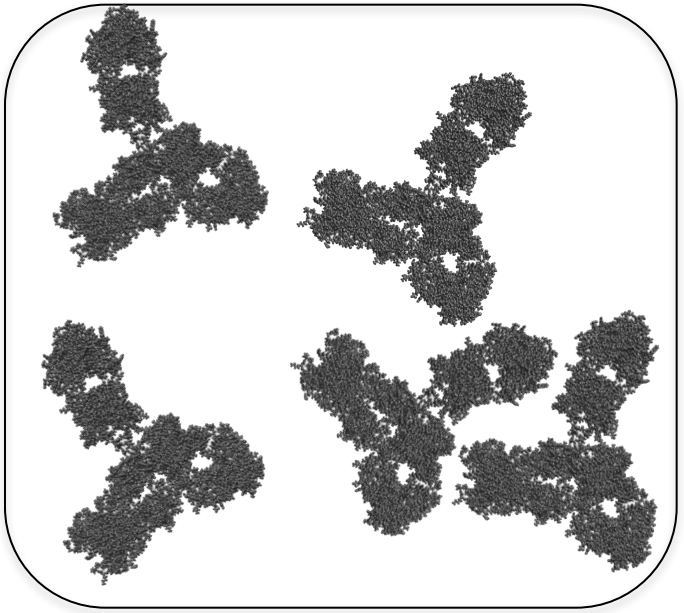

SAVE THE DATE: May 16th – 17th, 2018
NIIMBL National Meeting

http://www.niimbl.us/2018NationalMeeting/index.php

Welcome | Agenda | Travel/Directions | Accomodations | Register

Welcome

The 2018 National Meeting is your opportunity to learn about NIIMBL activities, celebrate the institute's progress this past year and network with the biopharmaceutical community.

Time: May 16-17, 2018, Program begins at 9:00 am. Check-in and breakfast at 8:00 am.

Location:

National Academy of Sciences Building (May 16, 2018)

SAVE THE DATE: January 6th – 11th, 2019

Inaugural GRC-Biotherapeutics and Vaccines Development

Frontiers of Science

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Biotherapeutics and Vaccines Development

Gordon Research Conference

January 6 - 11, 2019

Apply Now

Chairs
Jai A. Pathak and Christopher J. Roberts

Contact Chairs

Vice Chairs
Michael J. Tarlov and Linda O. Narhi

Hotel Galvez
2024 Seawall Boulevard
Galveston, TX, US

Venue and Travel Information

Venue
Office Manager: Beth Mancuso
Office Manager Email: Galvez_SM@grc.org
Office Phone: 409-765-7721 ext. 227

Application Information

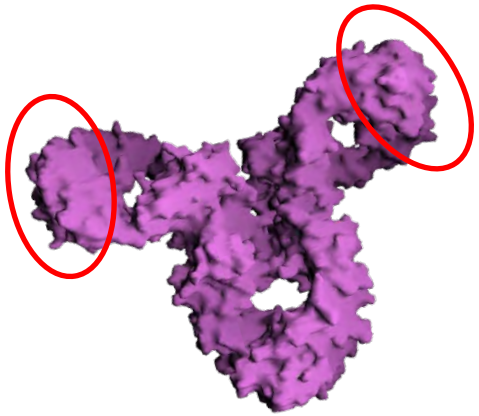
Applications for this meeting must be submitted by **December 9, 2018**. Please apply early, as some meetings become oversubscribed (full) before this deadline. If the meeting is oversubscribed, it will be stated here. *Note*: Applications for oversubscribed meetings will only be considered by the conference chair if more seats become available due to cancellations.

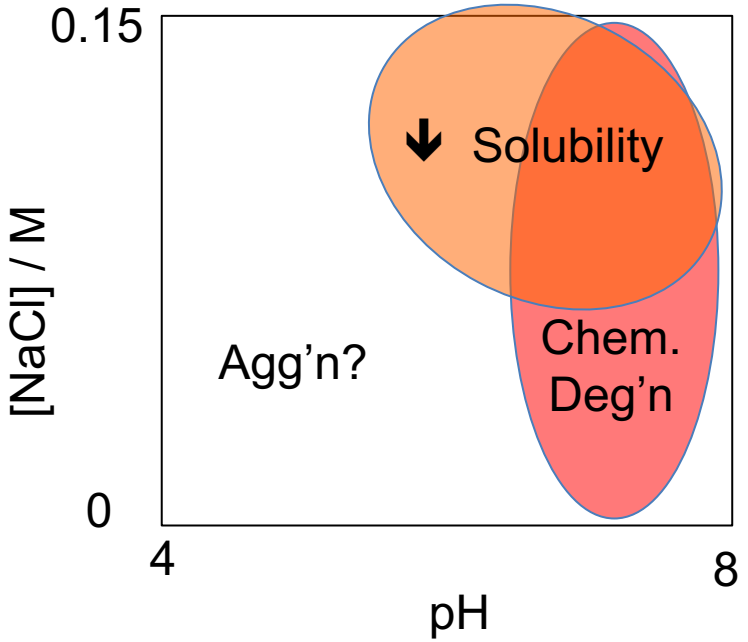
Conference Description

Conference Links

Context: Protein formulation, delivery, & selection

Monoclonal Antibody





- Very large molecules (~ 1.5 x 10^5 Da)
- Only small fraction of structure is "active"
- Net: large doses needed:
  ~ 1 mg protein : kg of patient body wt.
- Market share → Patient self administration

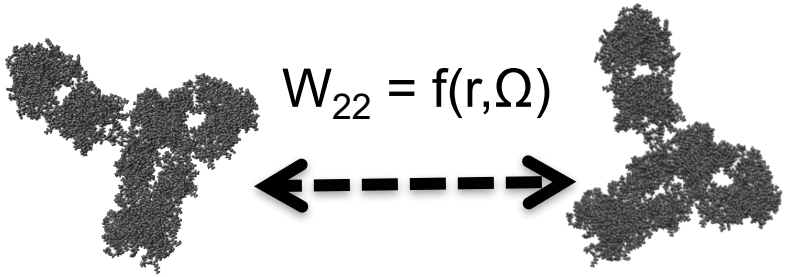
Protein
Conc'n
~ 10^2 g/L

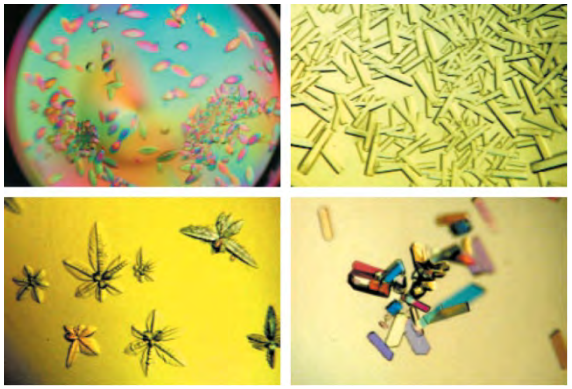
http://img.en.china.cn/0
/0,0,171,20469,640,433,83b95ef1.jpg

Protein-protein interactions and phase behavior

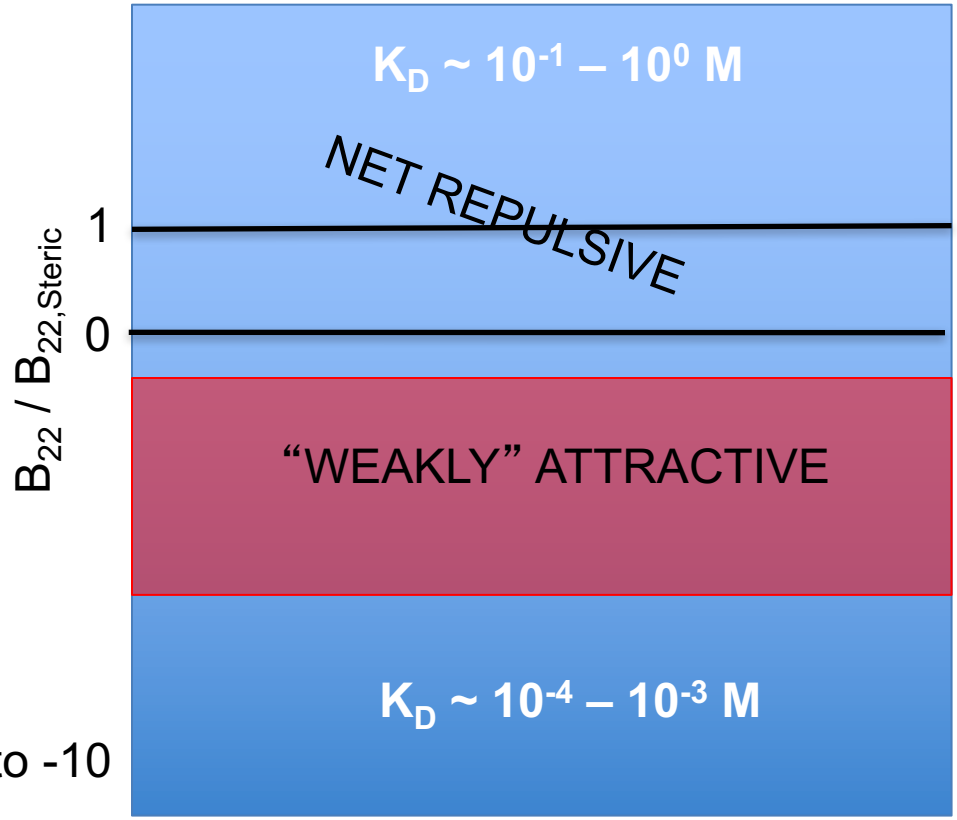
$$B_{22} = -\frac{1}{2}\int 4\pi r^2 \int [e^{-W_{22}(r,\Omega)/kT} - 1] d\Omega dr$$

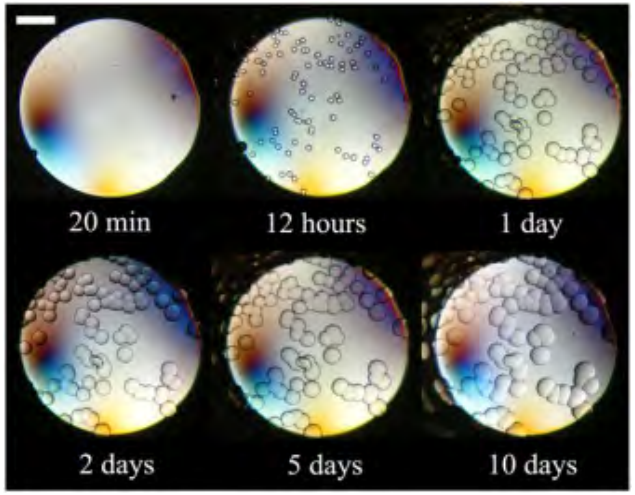
$$B_{22} = -\frac{1}{2}\int 4\pi r^2 [g(r) - 1] dr$$





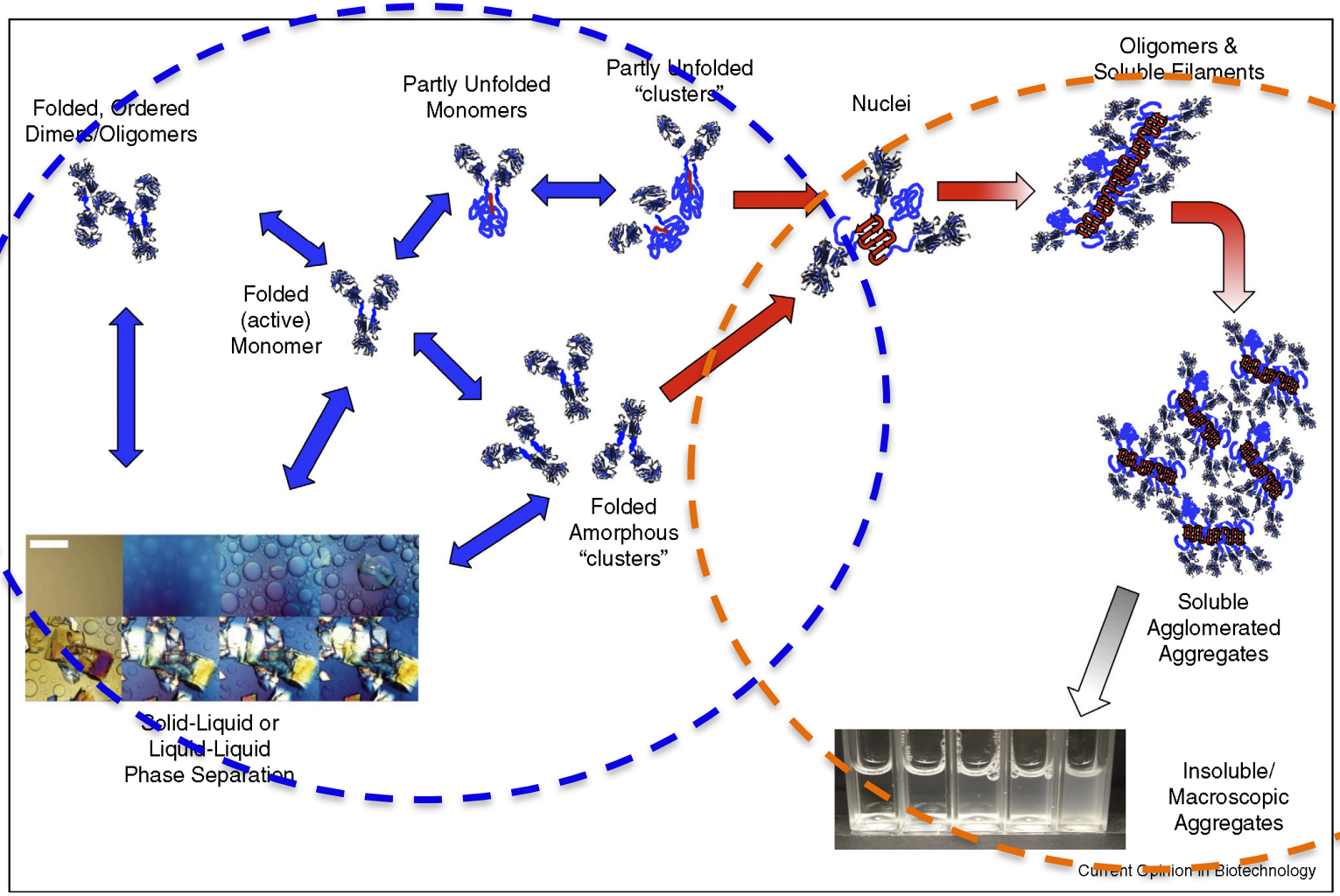
http://www.bayertechnology.com/typo3temp/fl_realurl_image/proteinkristallisation-bieten-01-pr.jpg

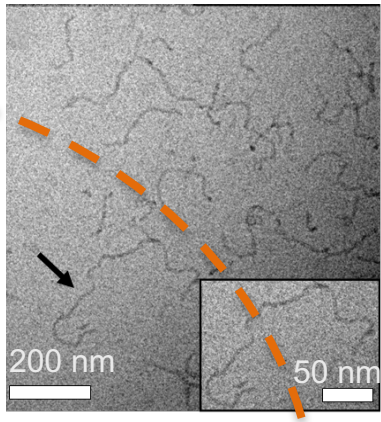




Dumetz et al., Biophys. Journal (2008)

Protein-Protein Interactions (PPI) influence a number of stages in aggregation pathways



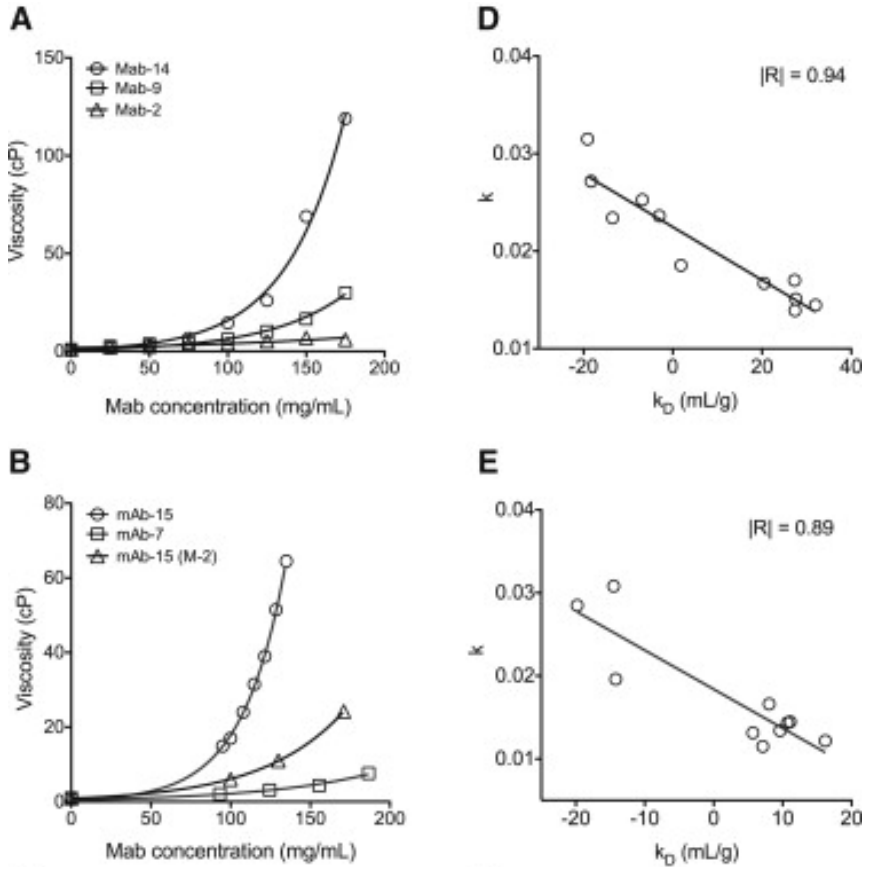


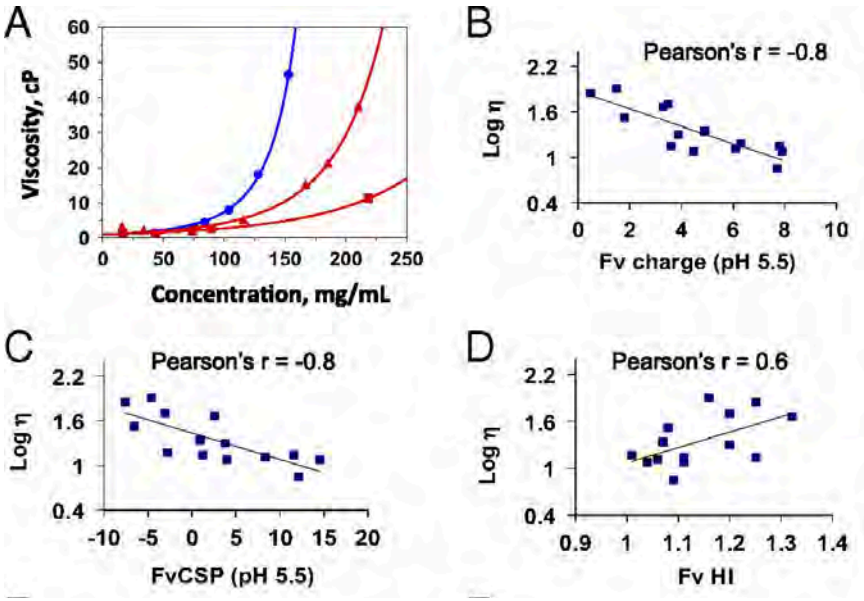
Weiss WF IV, Hodgdon T, et al. *Biophys J* (2007).

Roberts CJ, *Curr. Opin. Biotech.* (2014).

Viscosity and "weak" interactions / "cluster" formation

Viscosity has shown correlations with "weak" interactions (k_d) (?)



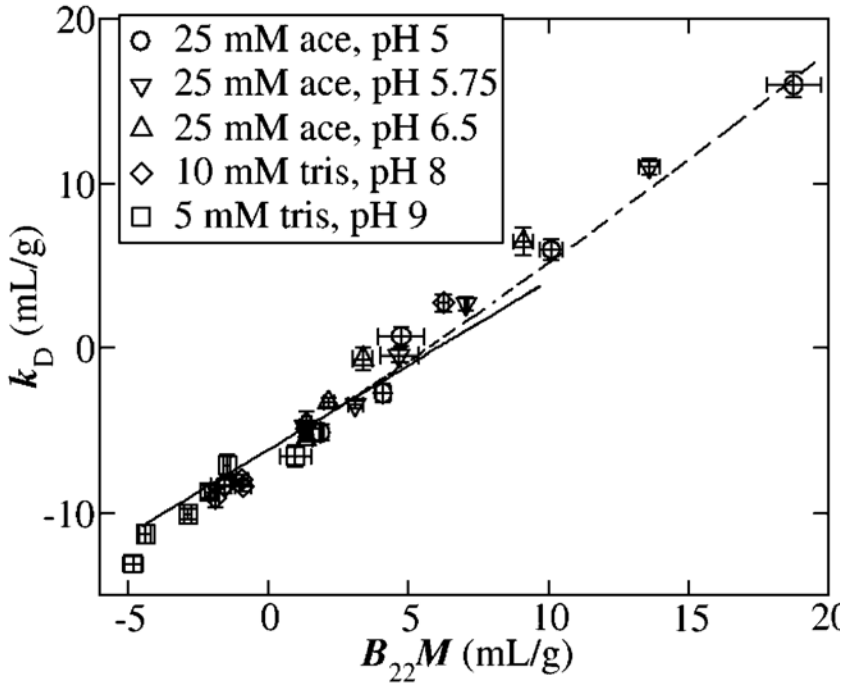


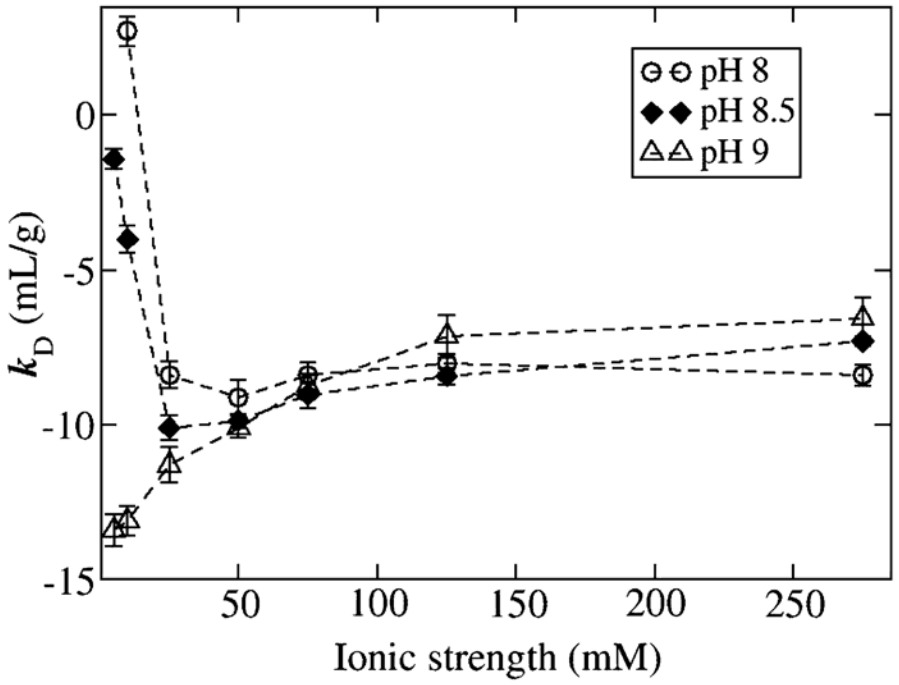
If one is in the dilute limit,

$$k_D = h_0 + 2B_{22}$$

Connolly, B.; *et. al. Biophys. J.* **2012**, *103*, 69–78.
Sharma, V. K.; *et. al. Proc. Natl. Acad. Sci.* **2014**, *111*, 18601–18606.

B_{22} and k_D are often used "interchangeably" to capture "colloidal" interactions





If one is in the dilute limit, theoretically

$$k_D = h_0 + 2B_{22}$$

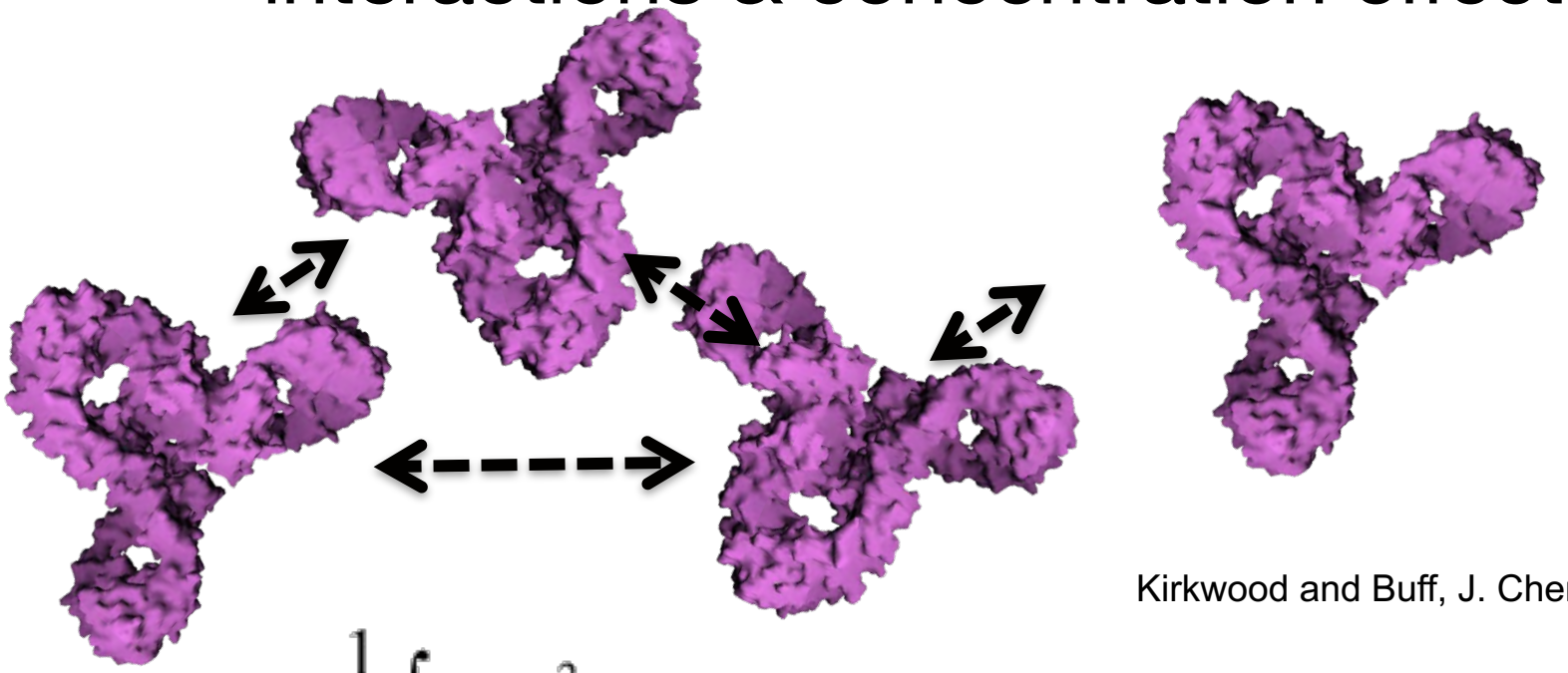
Electrostatic interactions can be repulsive or attractive, but it is difficult to actually predict when this will occur

Roberts D, Keeling R, Tracka M, van der Walle C, Uddin S, Warwicker J, Curtis R. *Mol. Pharm.* 2014

1-1 Interactions: Spanning the scales from "weak" to "strong" interactions…

- K_D ~ nanomolar spanning to Kd ~ mM or higher conc
- For strongly attractive conditions, Kd values scale ~ $-1/B_{22}$
- K_D ~ micromolar ➔ "B_{22}" or k_D ~ 1000 mL/g
- At low conc, $G_{22} = -2B_{22}$ (See next few slides)

Multi-body interactions: G_{22} vs. B_{22}..."weak" interactions & concentration effects



Kirkwood and Buff, J. Chem. Phys (1951)

$$B_{22} = -\frac{1}{2}\int 4\pi r^2 [g(r) - 1] dr$$

$B_{22} \neq$ f([protein]); **dilute** solution
"weak" interac'n

$$G_{22} = \int 4\pi r^2 [g(r) - 1] dr$$

G_{22} = f([protein]); **any conc**.
"weak"/"strong" interac'n

Kirkwood-Buff Solution Theory…EXACT, but not originally developed for proteins.

At low concentrations, these are ”equivalent” measures

Dynamic Light Scattering

$$D_c(q \to 0, c_2) = D_0 \frac{H(q \to 0, c_2)}{1 + c_2 G_{22}}$$

Equilibrium AUC

$$\left(\frac{\partial \mu_2}{\partial c_2} \right)_{T,V,\mu_{j \neq 2}} = \frac{kT}{c_2 (1 + c_2 G_{22})}$$

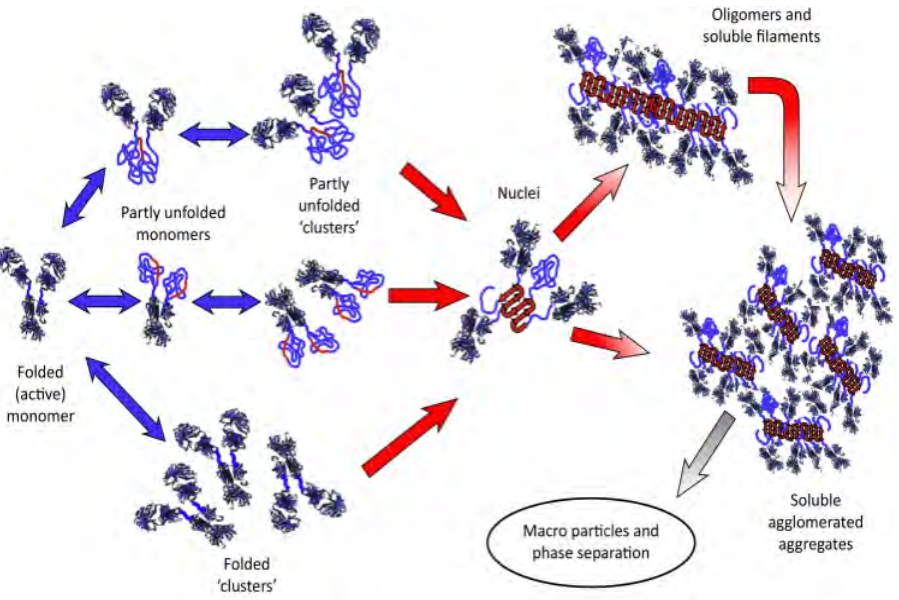
Neutron / x-ray small-angle scattering

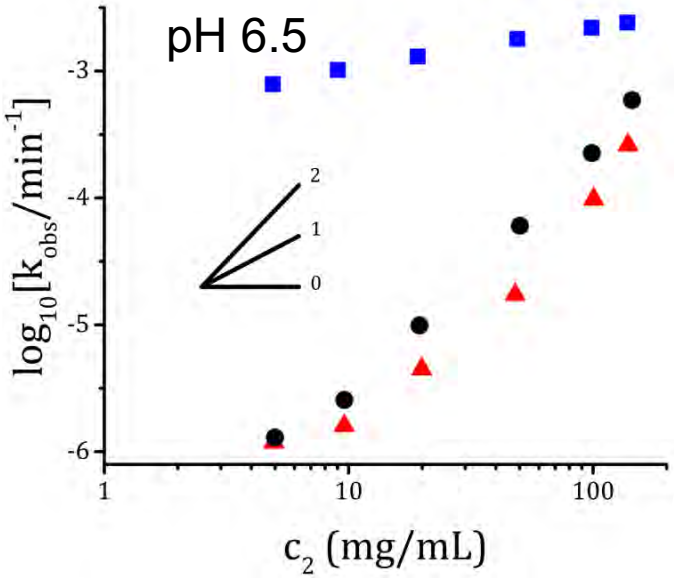
$$I(q \to 0) \sim c_2 (1 + c_2 G_{22})$$

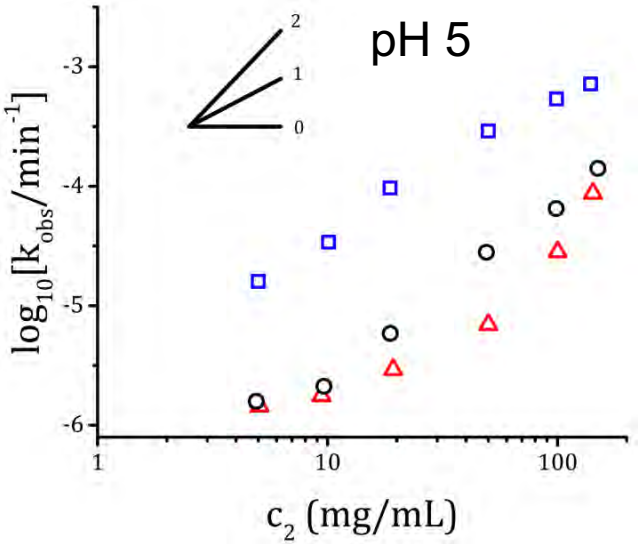
Do not need to be in the dilute limit…G_{22} is valid at <u>any c_2</u> (for a 1-phase system)

$$S_0 = 1 + c_2 G_{22}$$

Experimental protein aggregation (SEC) from low to high concentration



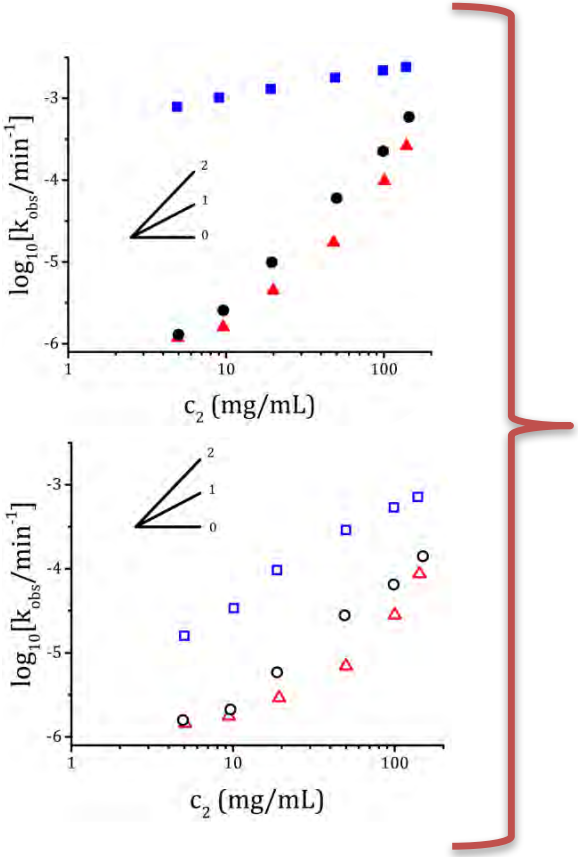


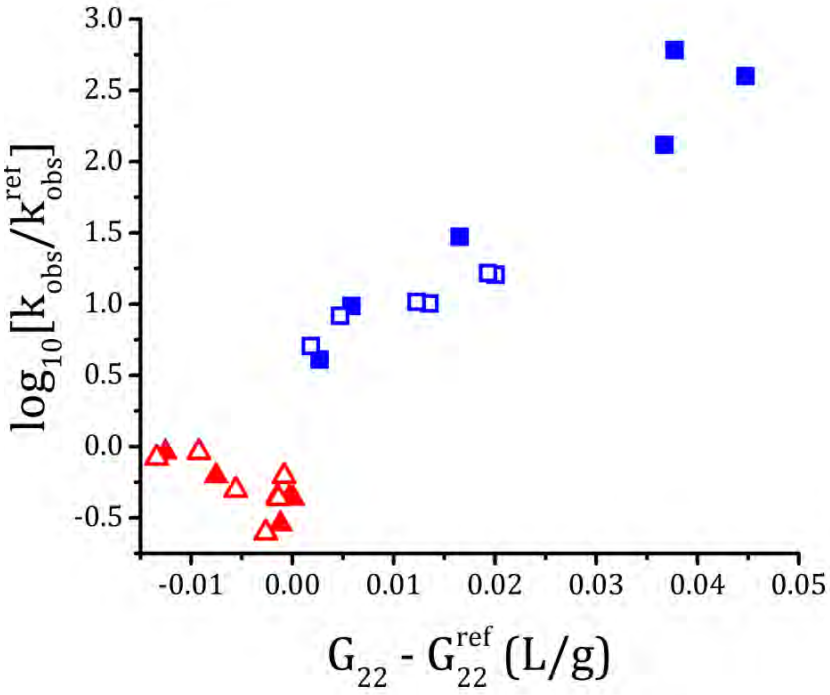


k_{obs} = rate coeff. for monomer loss via SEC

Ghosh, R., *et. al. J. Pharm. Sci*. **2016**

Protein aggregation and "weak" interactions





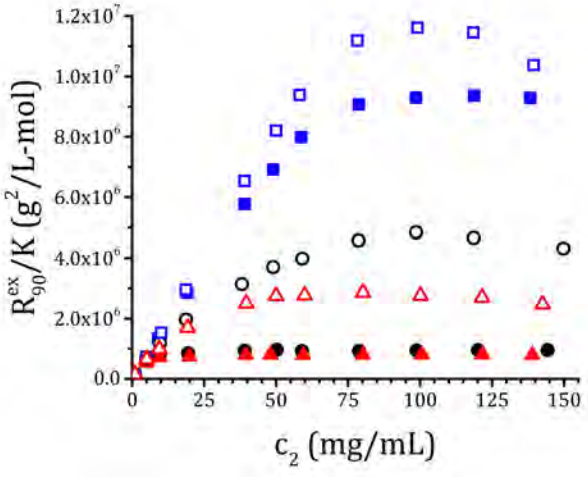
Ghosh, R., *et. al. J. Pharm. Sci*. **2016**

More attractive / less repulsive conditions (higher ionic strength) have higher aggregation rates

Protein-Protein "weak" interactions quantified with Rayleigh scattering

c_2 = protein concentration
B_{22} = protein-protein osmotic 2nd coefficient (independent of c_2)
G_{22} = protein-protein KB integral (depends on c_2)

Experimental Rayleigh profiles



Subindeces:
1 - Solvent (water) **3 & on -** Excipients
2 – Protein

$$\frac{R_{90}^{ex}}{K} = M_{2,app}c_2 + M_2 G_{22}^{(m)} c_2^2$$

$$S_{q=0} = 1 + c_2 G_{22}^{(m)}$$

$$M_{2,app} \cong M_2$$

$$G_{22} = \int_V [\bar{g}(r) - 1] dV$$

If one uses B_{22}

$$B_{22} = -\frac{1}{2}\lim_{c_2 \to 0} G_{22}$$

If one uses k_D

$$k_D = \omega + 2B_{22}$$

Could be obtained from molecular simulations

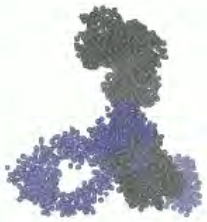
Ghosh, R., *et. al. J. Pharm. Sci*. **2016**
Blanco, M., *et. al. J. Chem. Phys.* **2011,** *134*(22), 225103

Molecular simulations for protein interactions – low protein concentrations

Mayer Sampling with Overlap Sampling (MSOS): B_{22}

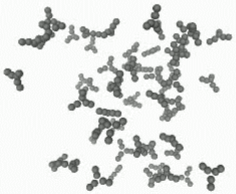
- Open 2-particle system (N=2, V→∞, T)
- Mayer functions integrated with respect to a known reference:

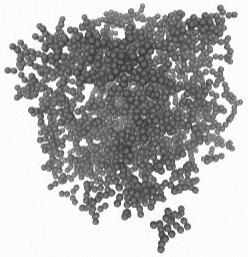
$$B_{22} = B_{22,ref}\left[\frac{\left\langle \gamma_{22}/\pi \right\rangle_{\pi} / \left\langle \gamma_{22}^{over}/\pi \right\rangle_{\pi}}{\left\langle \gamma_{22}^{ref}/\pi \right\rangle_{\pi,ref} / \left\langle \gamma_{22}^{over}/\pi \right\rangle_{\pi,ref}}\right]$$



Rubio, C.C. et al. *J. Phys. Chem B* ***2016***
Shaul, K.R.S., *et. al. J. Chem. Phys.* **2011**
Errington, J.R. *J. Chem. Phys.* **2003**
Ben-Naim, A. Statisitical Thermodynamics for Chemist and Biochemists *Plenum Press.* **1992**

Molecular simulations for protein interactions – High concentrations



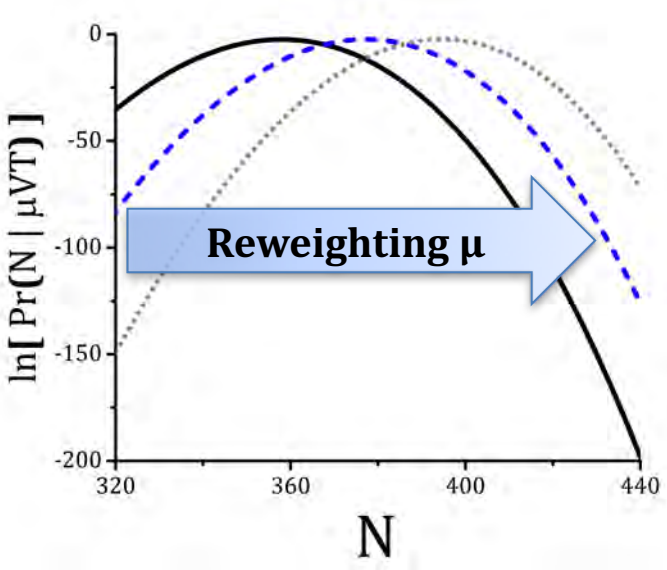


Transition matrix Monte Carlo (TMMC): G_{22}

- Grand-canonical ensemble
- **Pr(N | μVT)** is reconstructed and reweighted

$$\left(\frac{\partial \mu_2}{\partial \langle N_2 \rangle}\right)_{T,V,\mu'} = \frac{kT}{\langle N_2 \rangle \left(1 + \langle N_2 \rangle G_{22}^{(m)} / V\right)}$$

$S_{q=0}$ = f(protein conc.)



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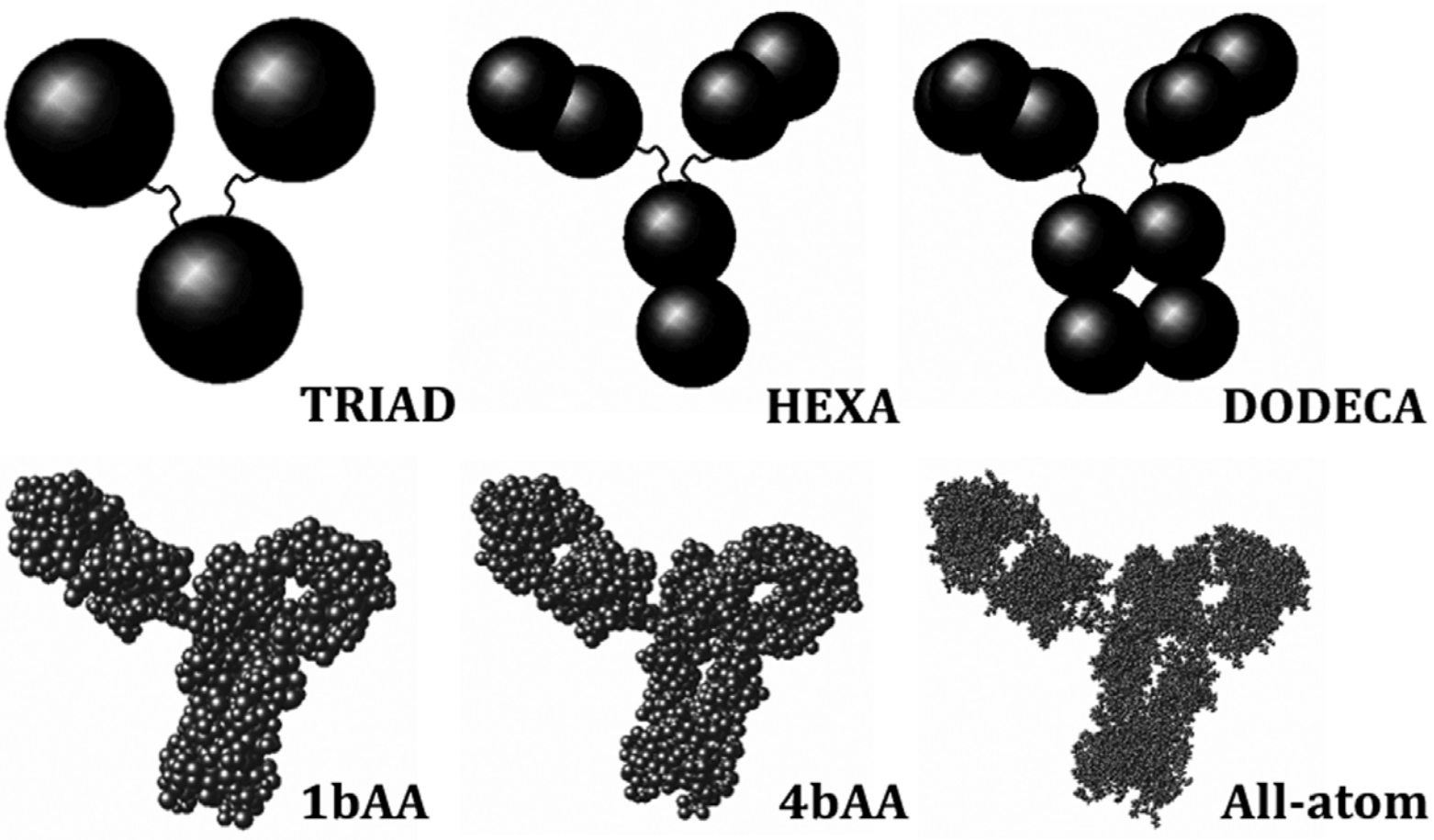
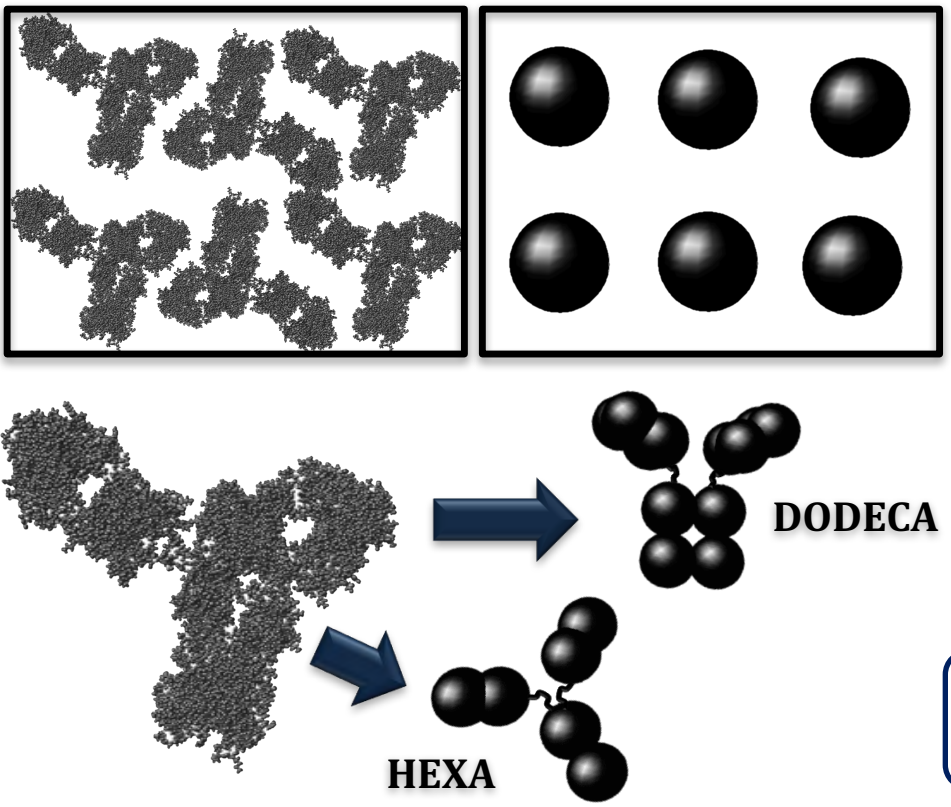
Different ranges of CG models

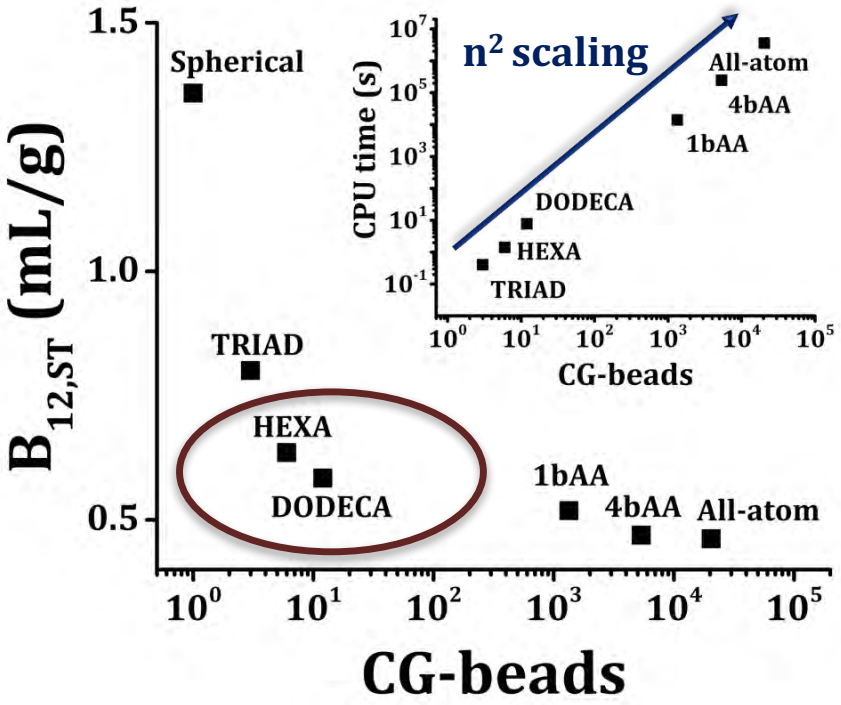


Calero-Rubio et al. *J Phys Chem B 2016*

Coarse-graining: balancing computational cost & accuracy

Same volume, different packing

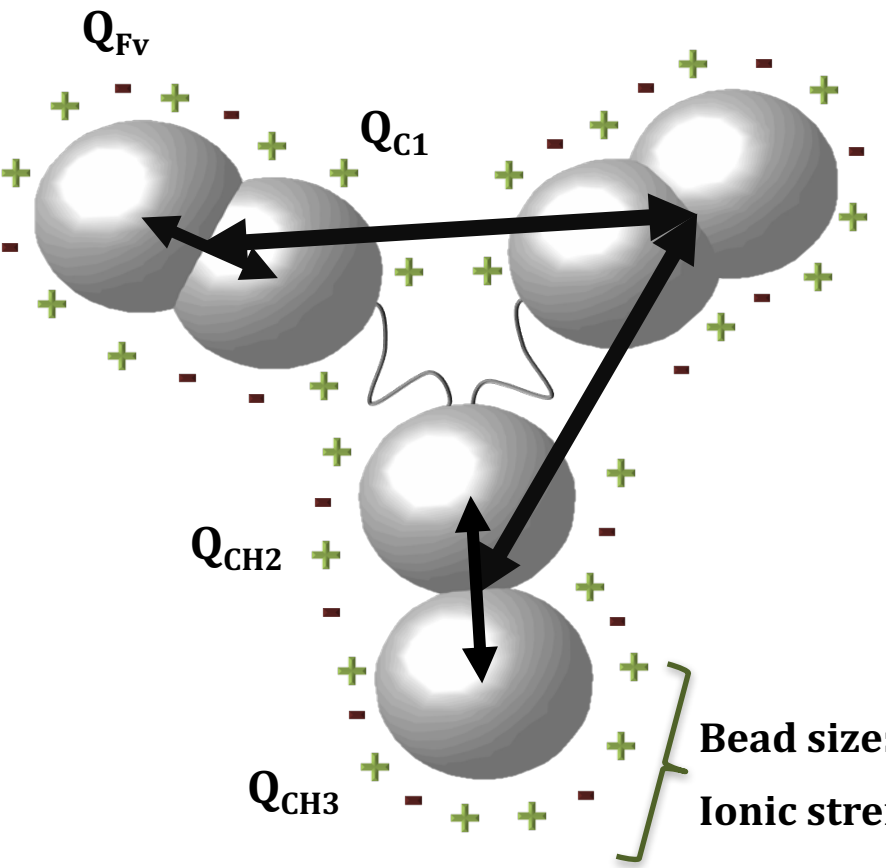




Asymptotic behavior towards all-atom result.
HEXA and DODECA balance accuracy + speed

Calero-Rubio et al. *J Phys Chem B 2016*

Overview of interaction model (illustrated with HEXA example)



Sterics:
Hard-sphere potential

Short-range:
Modified Lennard-Jones potential

Electrostatics:
Modified screened-Coulomb potential

Hinge:
Harmonic potential

Bead size: σ_{ST} (nm)

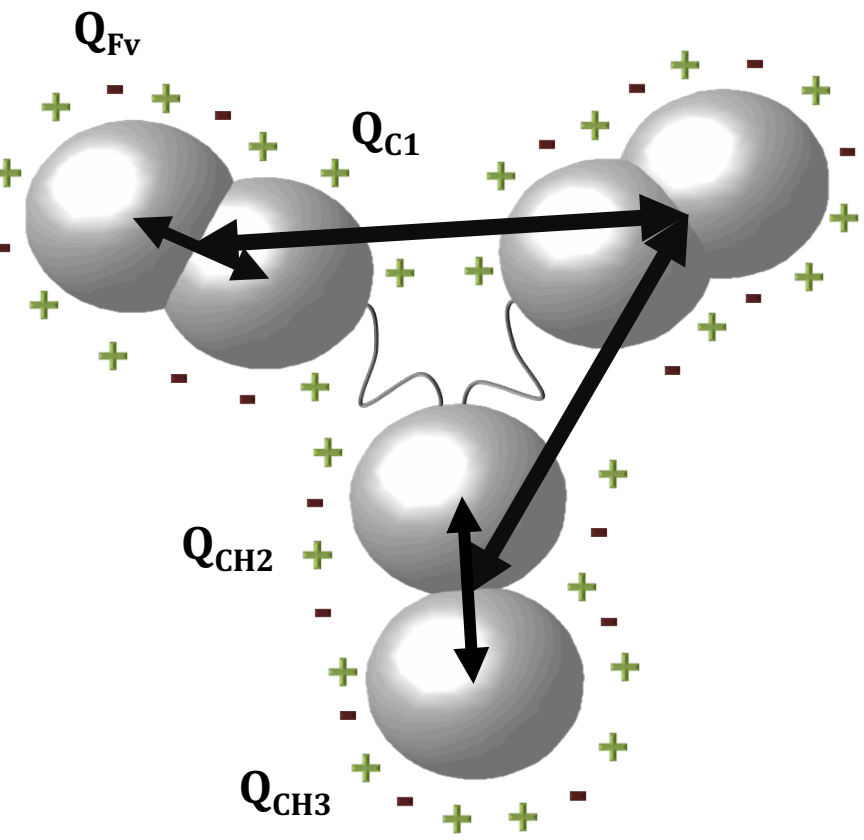
Ionic strength: I (mM)

Bead hydrophobicity: ε_{SR} (k_BT)

Structural INPUT:
1 – sequence
2 – 3d struc if available

Calero-Rubio et al. *J Phys Chem B 2016*

Selecting physically realistic parameters



- **σ_{ST} :** matching $B_{22,ST}$ from all-atom simulations
- **Charges (Q_i):** theoretical charge (sequence + pH)
- **Flexibility:** rigid molecule (speed reasons)
- **Ionic strength:** experimentally determined
- **Bead-bead distances:** crystal structure

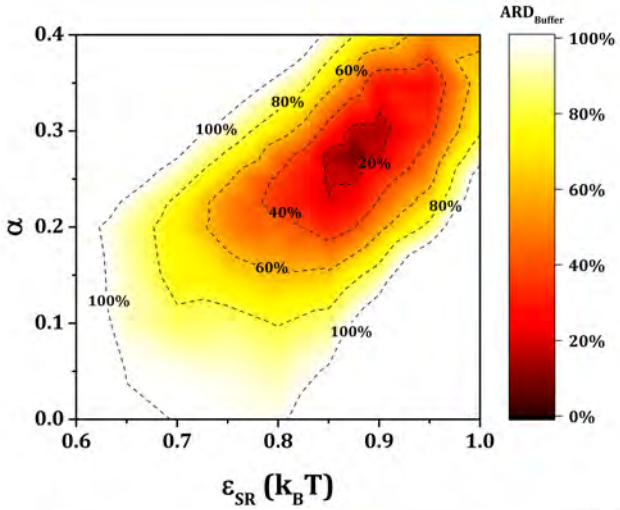
This leaves two parameters to tune:

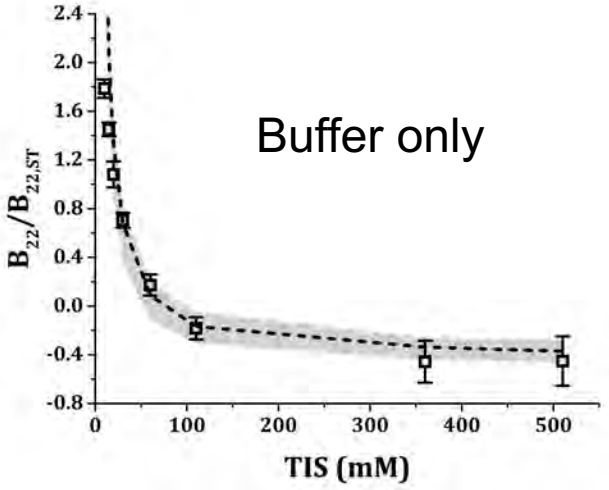
1. **Bead hydrophobicity / van der Waals attractions: "well depth", ε_{SR}**
2. **Effective charge/Theoretical charge: ε_{cc}**

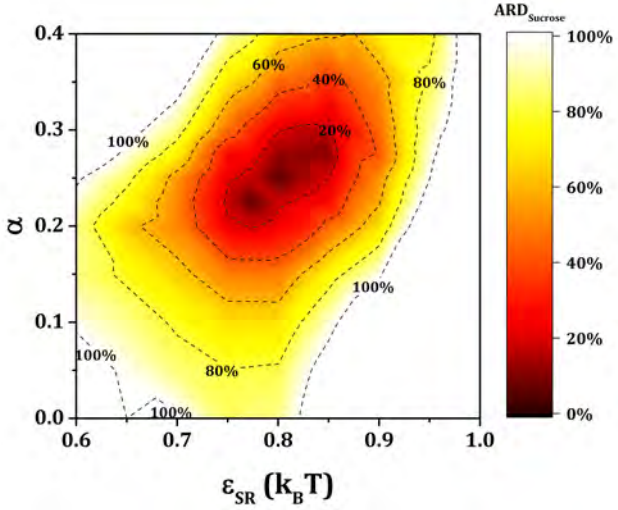
Calero-Rubio et al. *J Phys Chem B 2016*

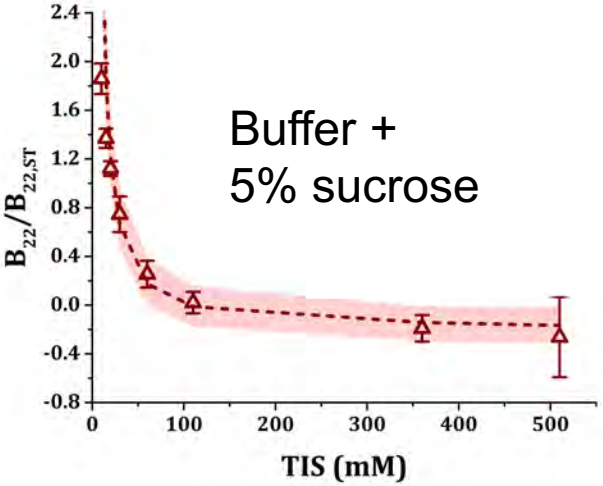
Training the model: B_{22} vs total ionic strength (TIS)

pH 5.0



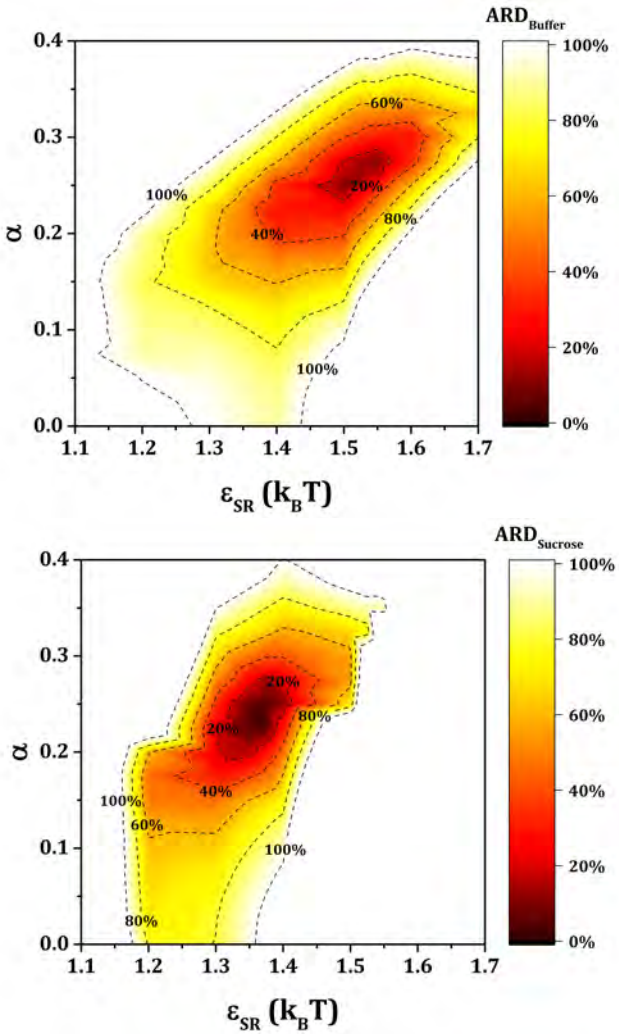




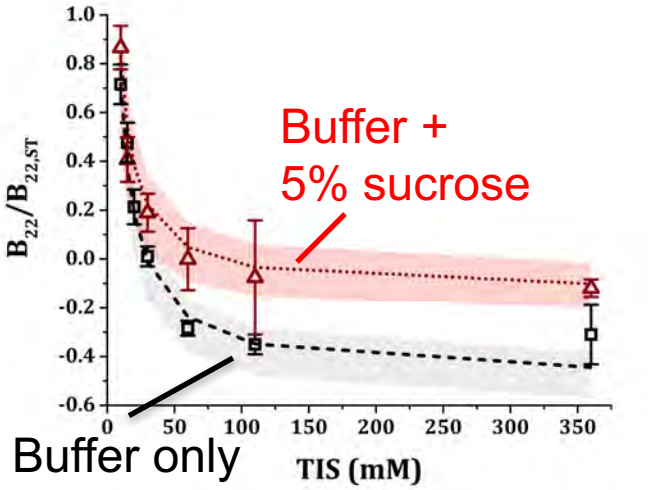


Calero-Rubio et al. *J. Pharm. Sci. (2018)*

Training the model: B_{22} vs total ionic strength (TIS)

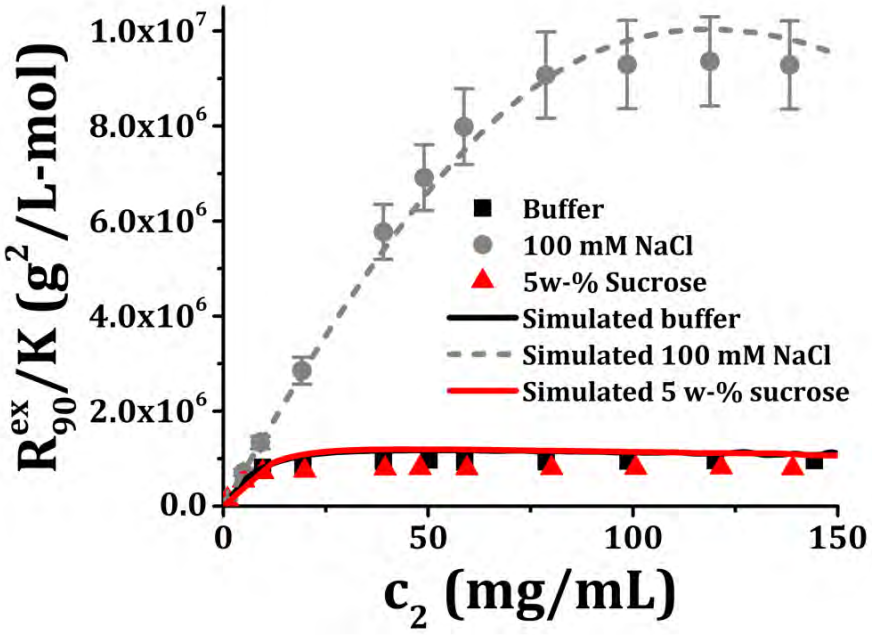


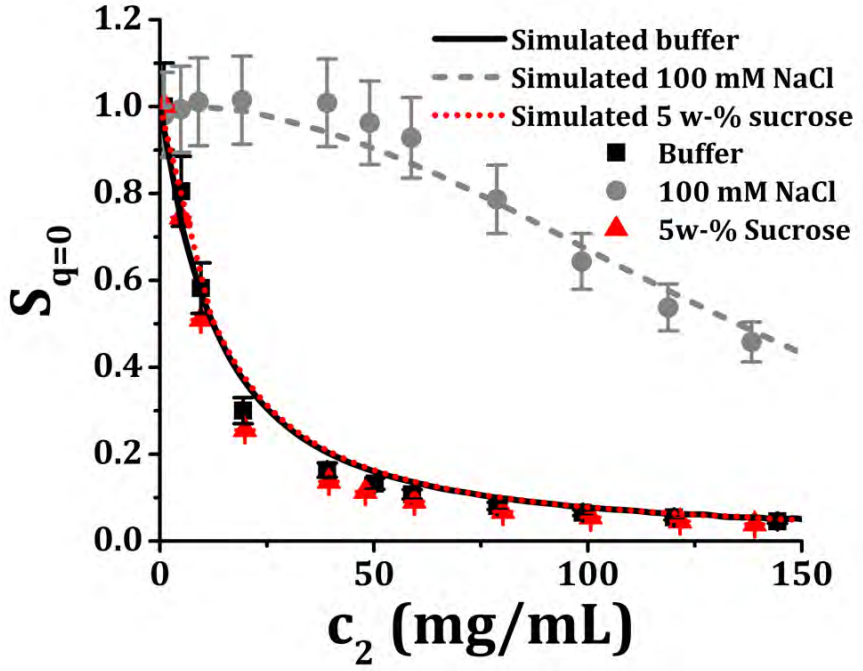
pH 6.5



Calero-Rubio et al. *J. Pharm. Sci. (2018)*

Predicting high c_2 with only low-c_2 data
pH 5.0



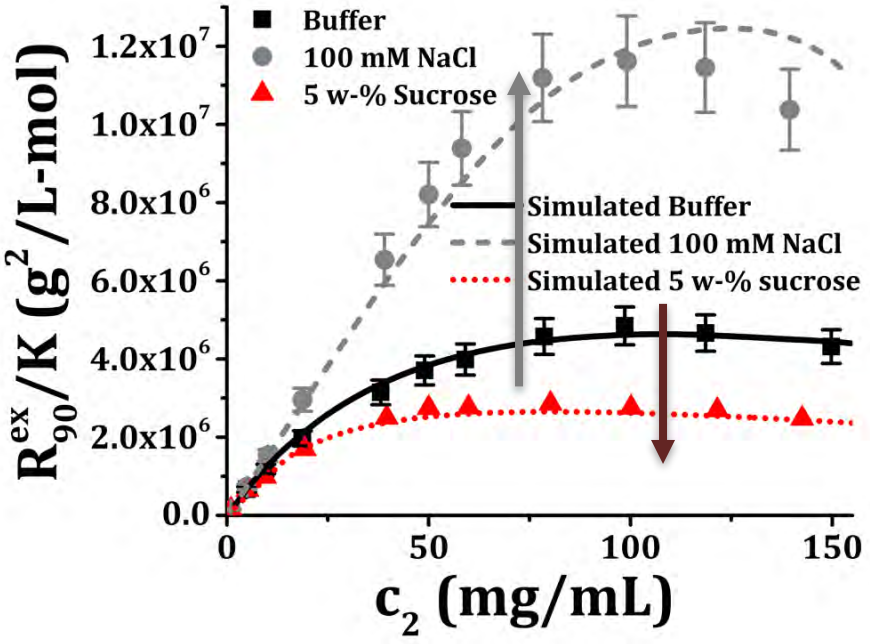


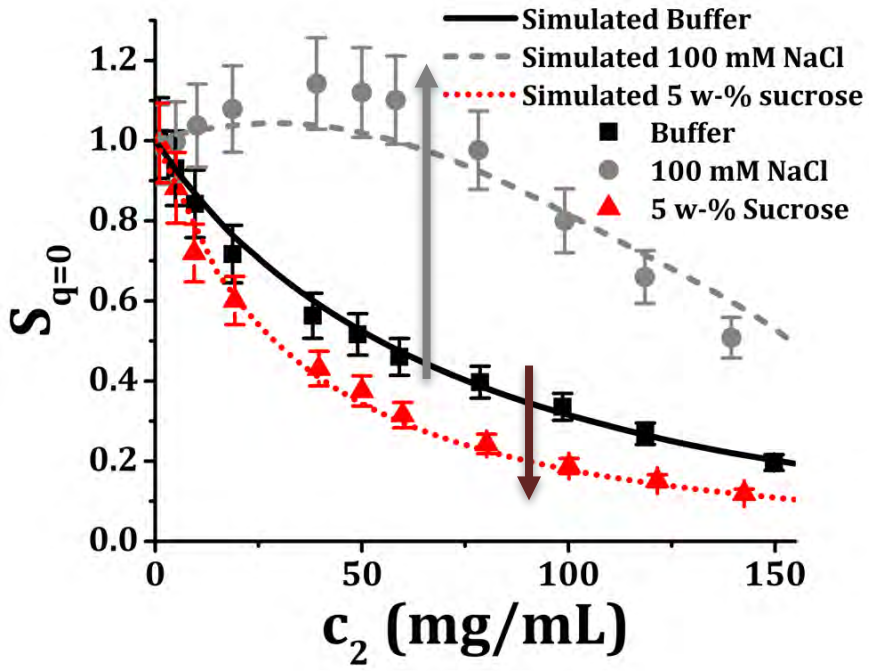
Lines are predictions of high c_2 behavior based on low c_2 behavior

Higher c_2 are achievable with fancier sampling algorithm

Calero-Rubio et al. *J. Pharm. Sci. (2018)*

Predicting high c_2 with only low-c_2 data
pH 6.5





Repulsive conditions: easier to model, more accurate (faster convergence)
Attractive conditions: required more configurations (slower to converge)

Calero-Rubio et al. *J. Pharm. Sci. (2018)*

What about when electrostatic interactions are strongly attractive?

Manuscript in preparation; please contact C. Roberts (cjr@udel.edu) for requests for preprints once they have been cleared internally

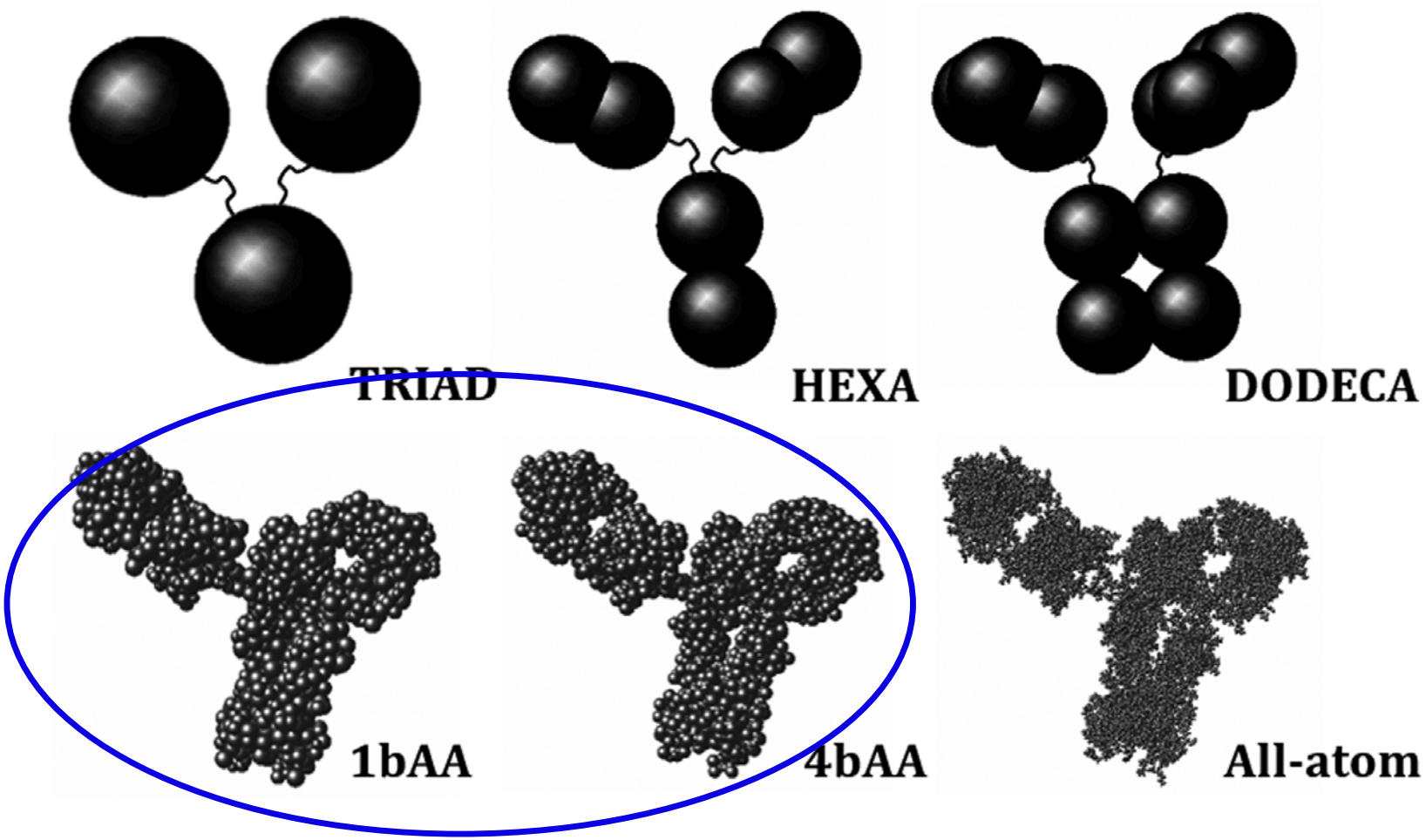
Surface charge distributions – attractive dipole and higher multi-pole contributions can dominate

Manuscript in preparation; please contact C. Roberts (cjr@udel.edu) for requests for preprints once they have been cleared internally

Predictions of high concentration interactions from B_{22} and MC simulations

Manuscript in preparation; please contact C. Roberts (cjr@udel.edu) for requests for preprints once they have been cleared internally

What about higher resolution CG models?

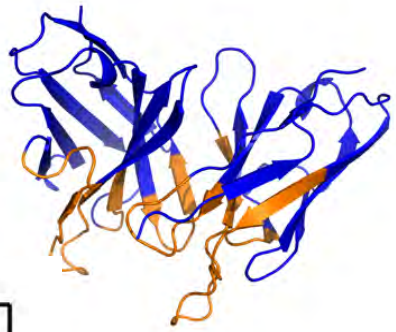


Calero-Rubio et al. *J Phys Chem B 2016*

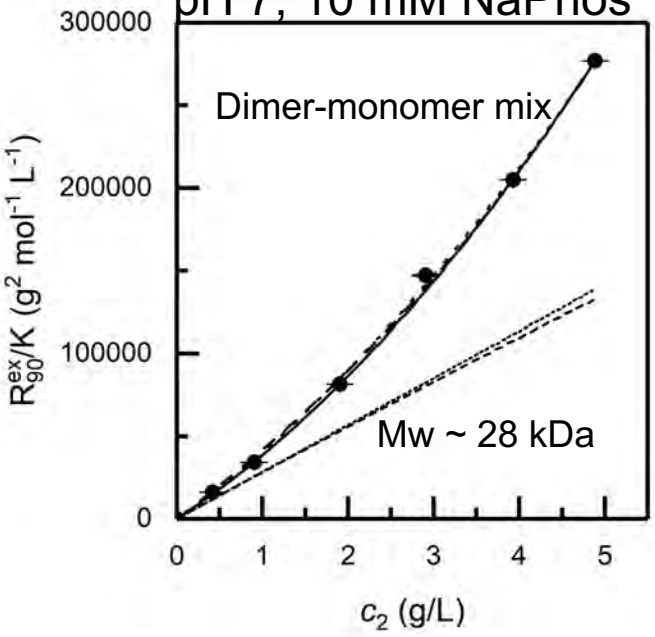
B_{22} response surfaces: potential “developability” index

Manuscript in preparation; please contact C. Roberts (cjr@udel.edu) for requests for preprints once they have been cleared internally

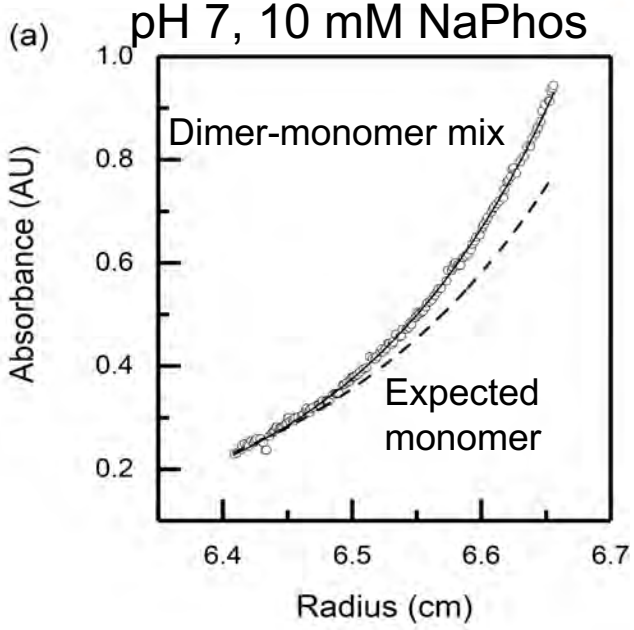
Single Chain Variable Fragments (scFv) can pose a different challenge



Static light scattering
pH 7, 10 mM NaPhos



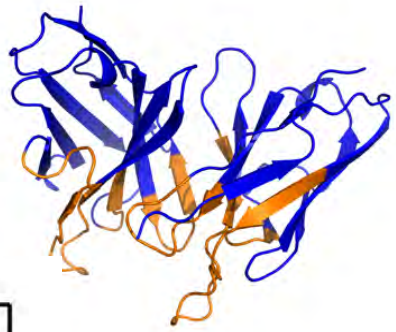
Equilibrium AUC
pH 7, 10 mM NaPhos



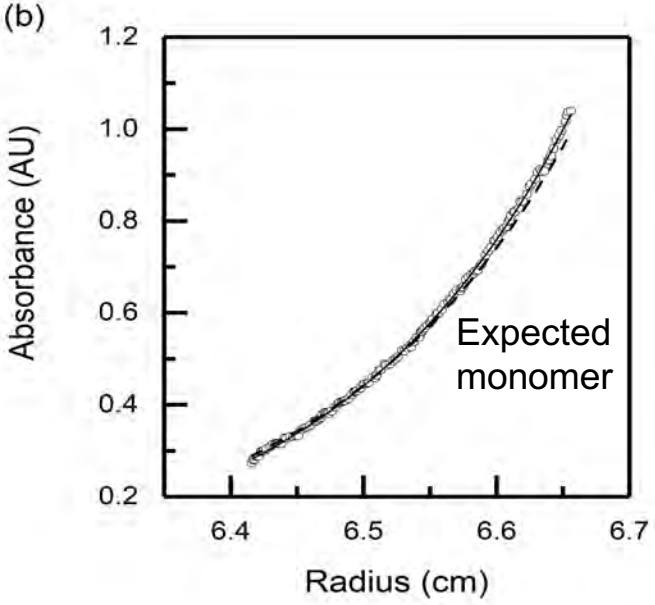
LS and AUC data to show dimerization at typical formulation pH / ionic strength; but reverts to monomer at higher TIS

O'Brien, Calero-Rubio et al., *Protein Science* (2018)

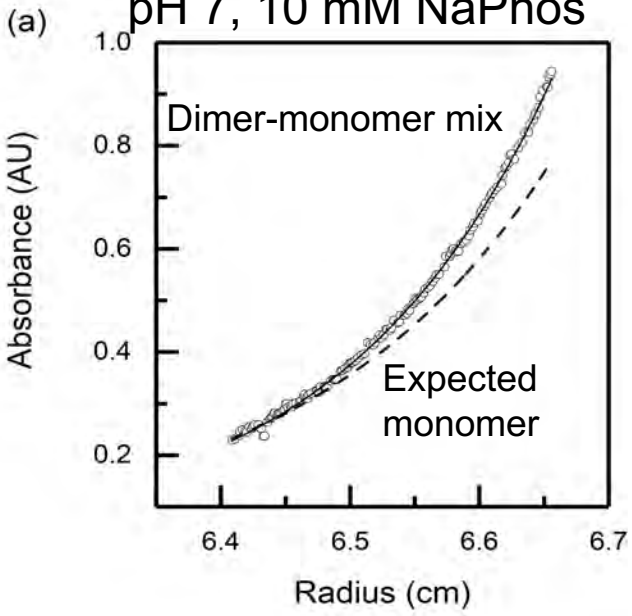
Single Chain Variable Fragments (scFv) can pose a different challenge



Equilbrium AUC
pH 7, buffer + 200 mM NaCl



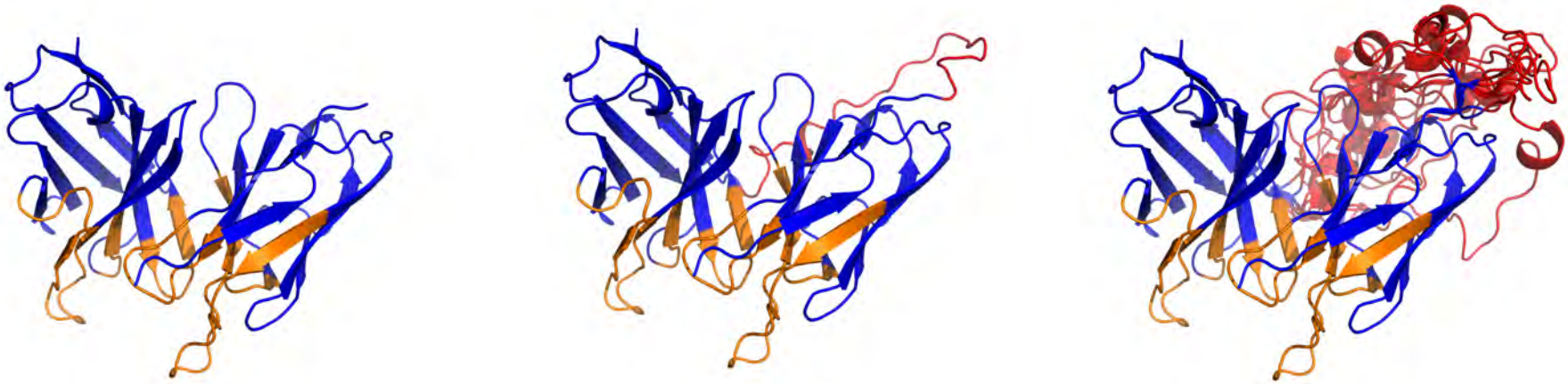
Equilibrium AUC
pH 7, 10 mM NaPhos



LS and AUC data to show dimerization at typical formulation pH / ionic strength; but reverts to monomer at higher TIS

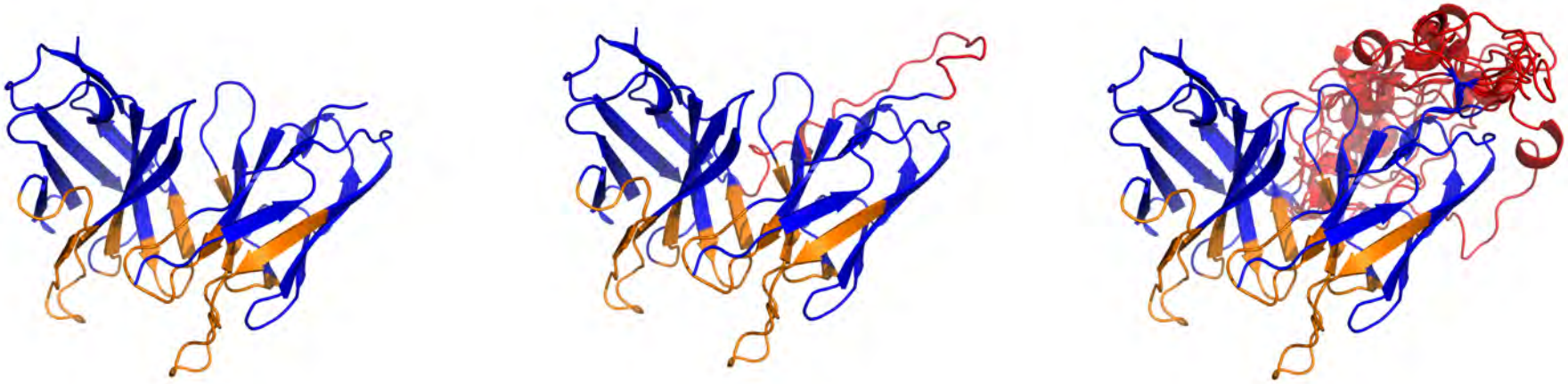
O'Brien, Calero-Rubio et al., *Protein Science* (2018)

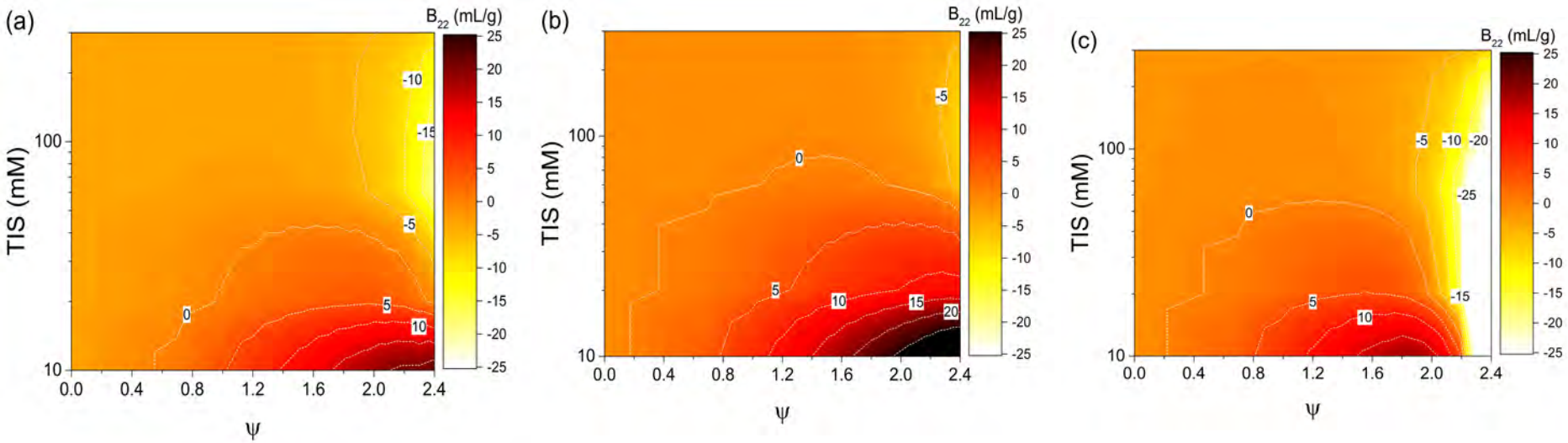
scFv has a net neutral but charged linker, with multiple possible configurations



O'Brien, Calero-Rubio et al., *Protein Science* (2018)

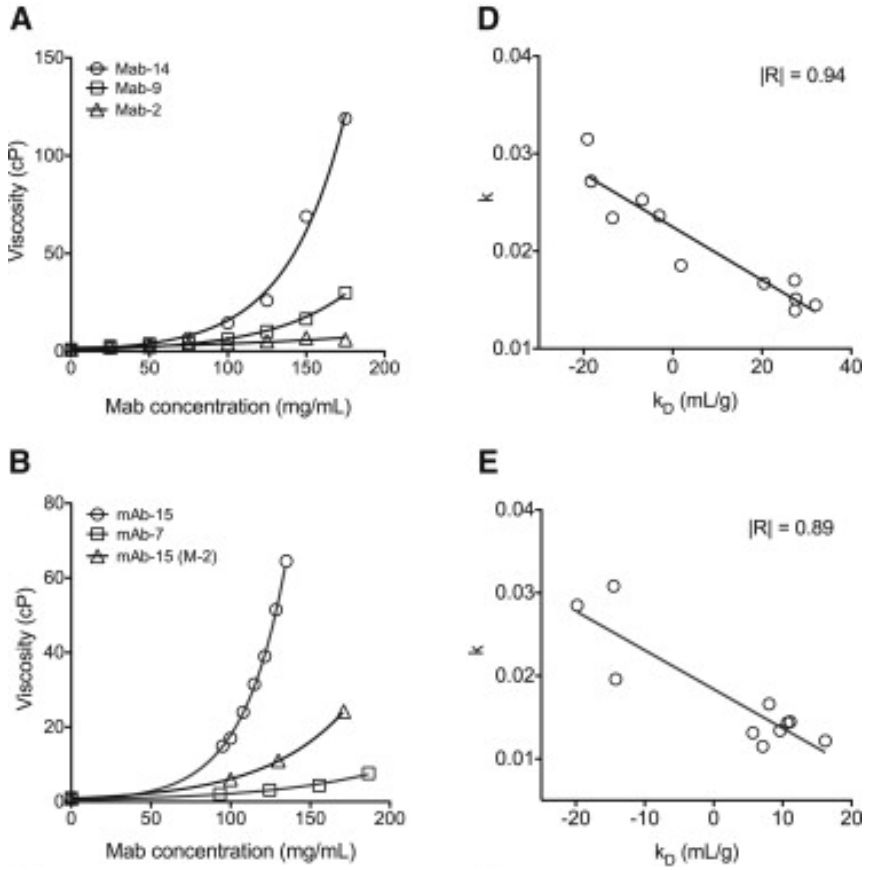
The flexible linker is able to cause strong electrostatically driven attractions between the linker and the VH and VL domains





O'Brien, Calero-Rubio et al., *Protein Science* (2018)

Viscosity and “weak” interactions



How predictive are these experimental or simulated interactions of potential problems with high viscosity?

Current practice = assume B_{22} (~ k_D) correlates with high viscosity

Protein Conc’n ~ 10^2 g/L

http://img.en.china.cn/0/0,0,171,20469,640,433,83b95ef1.jpg

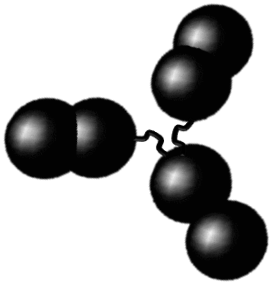
Connolly, B.; *et. al. Biophys. J.* **2012**, *103* (1), 69–78.
Sharma, V. K.; *et. al. Proc. Natl. Acad. Sci.* **2014**, *111* (52), 18601–18606.

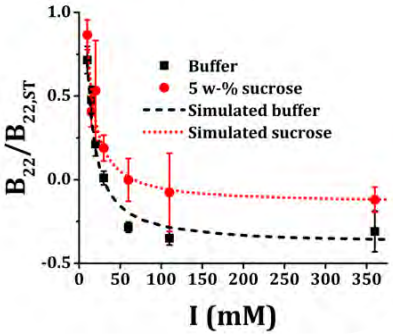
IgG candidates can display a wide range of electrostatic attractive / repulsive behavior

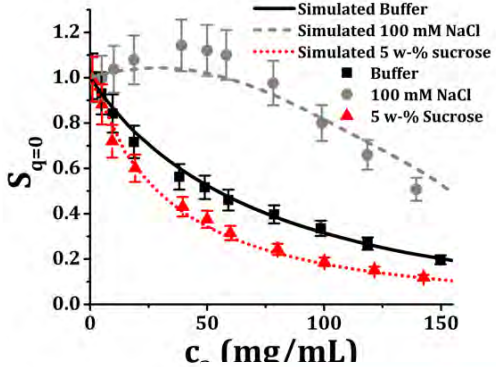
Manuscript under review; please contact C. Roberts (cjr@udel.edu) for requests for preprints once they have been approved by the journal

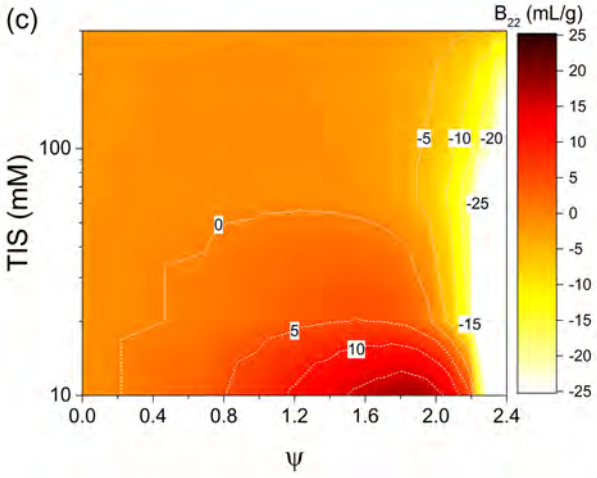
Woldeyes, Razinkov, Qi, Battistoni, Furst, Roberts *under review*

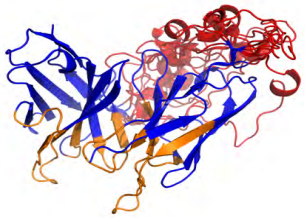
Summary











Funding: Amgen, BMS, NIH, NSF, BITC