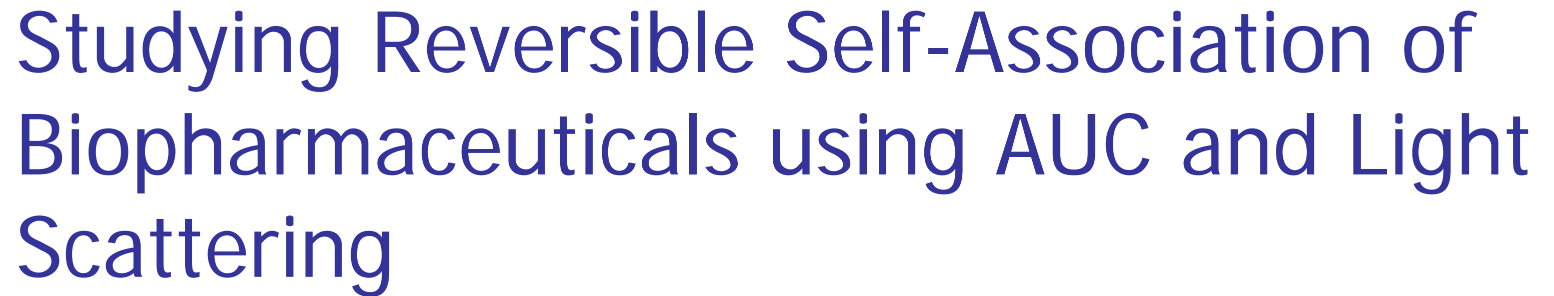


Studying Reversible Self-Association of Biopharmaceuticals using AUC and Light Scattering

John Philo

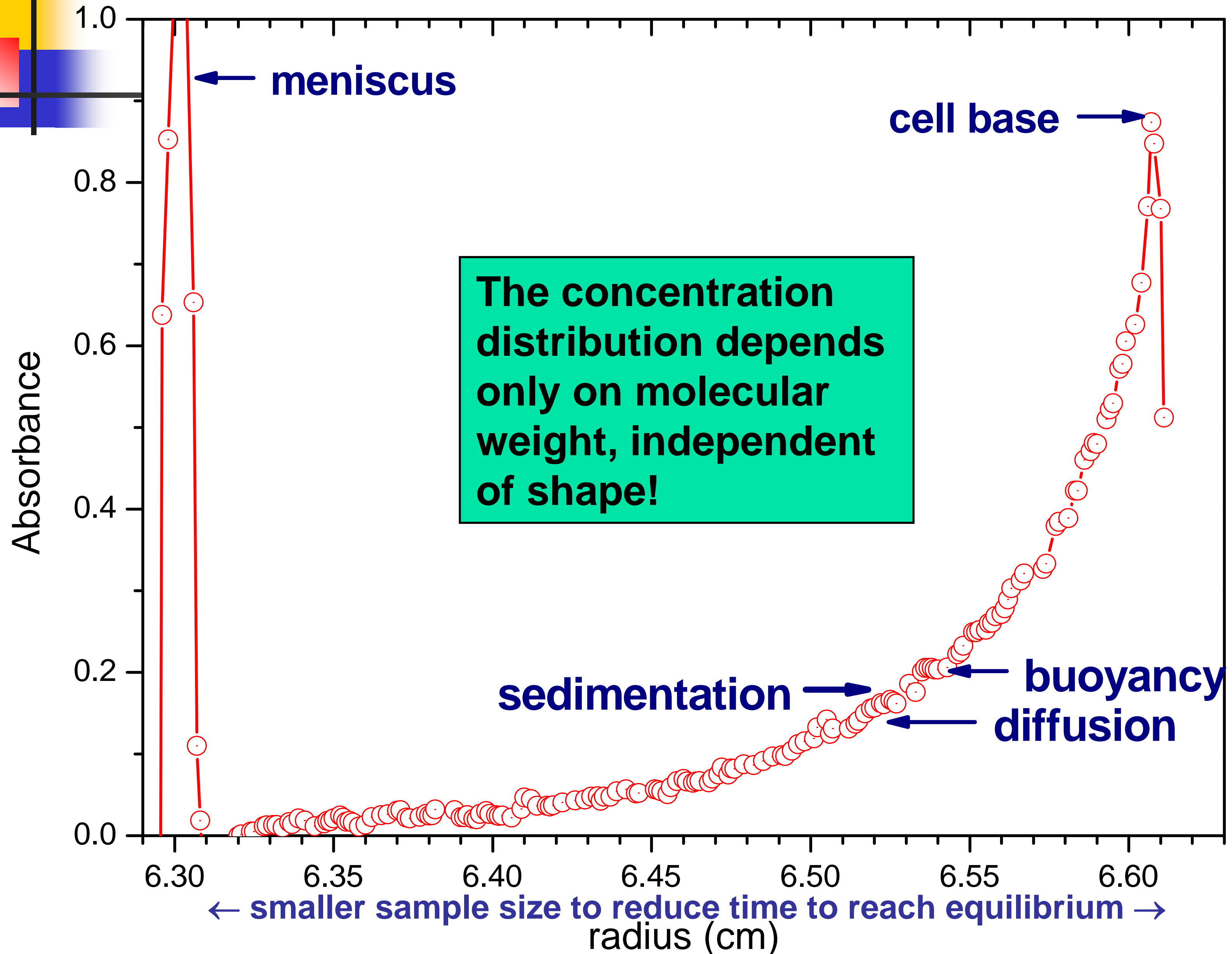
HOS2014, February 2014

© Copyright 2014 Alliance Protein Laboratories

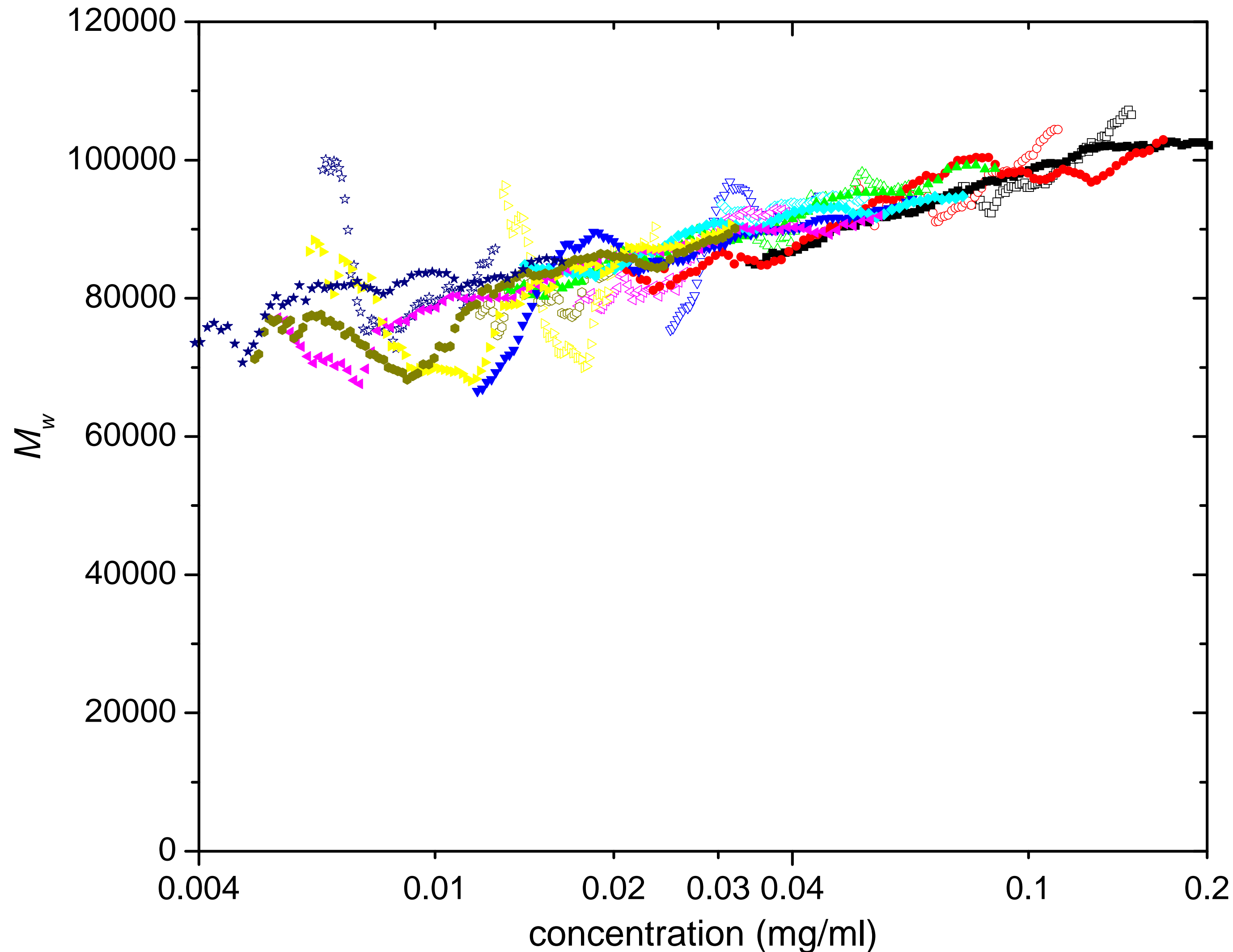
Outline

- Some examples of applying sedimentation equilibrium (SE) and sedimentation velocity (SV) to study reversible self-association
  - for each, how do we distinguish reversible from irreversible association/aggregation?
- Using classical light scattering to study reversible interactions (CG-MALS)
  - the complex world of "crowded solutions" at ~100 mg/mL

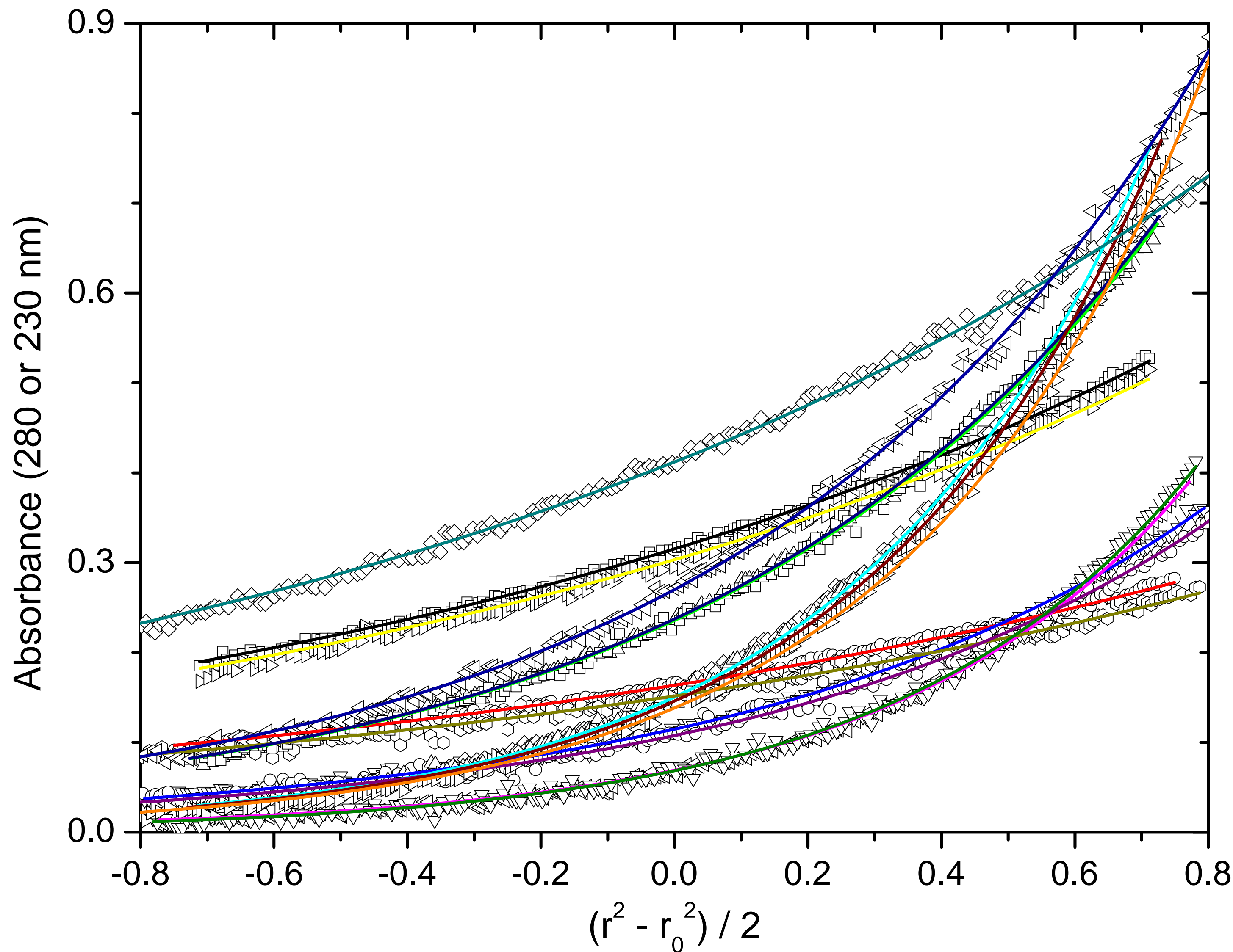
The fundamentals of sedimentation equilibrium



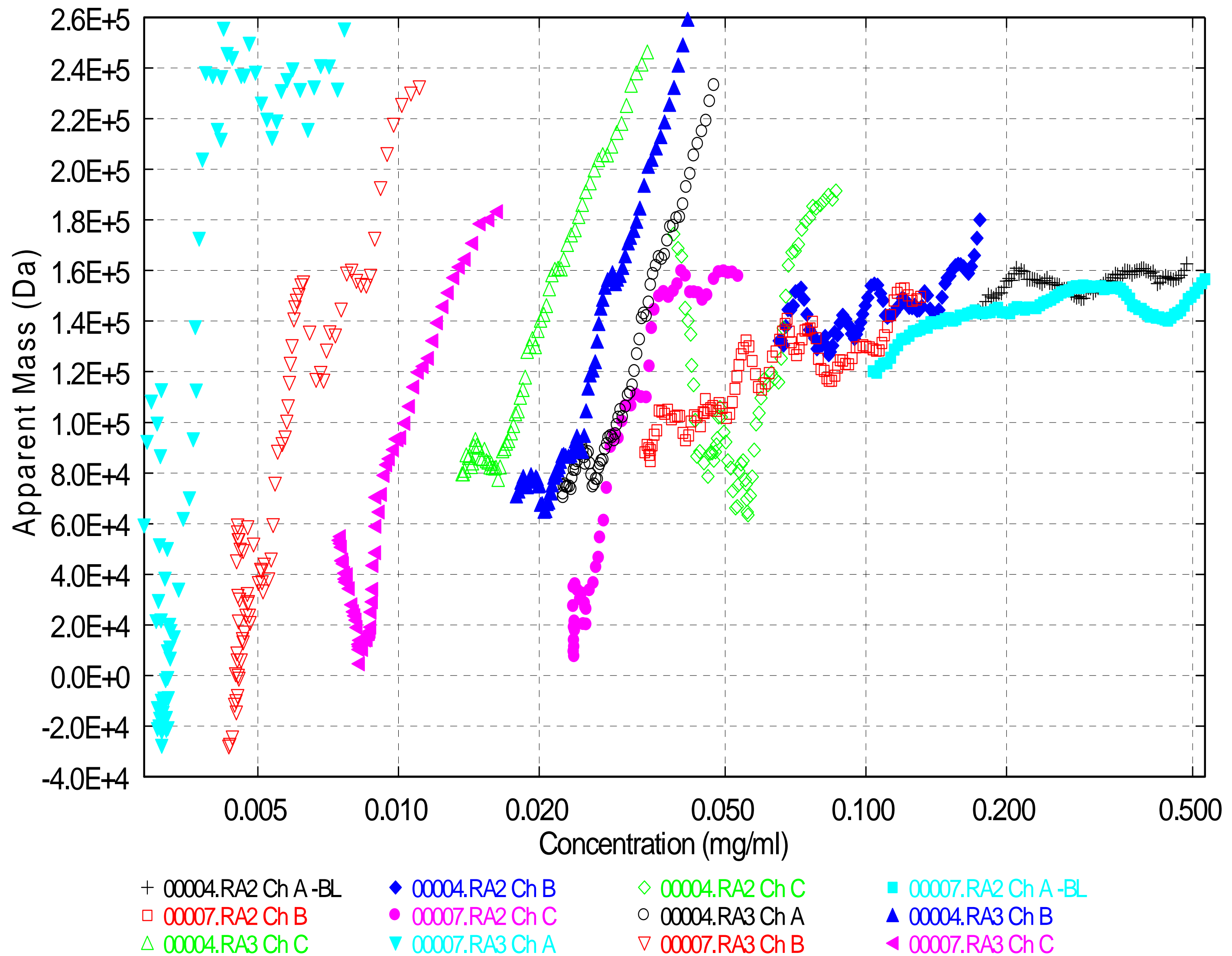
Apparent mass *vs.* concentration plots for the soluble extracellular domain (ECD) of the atrial natriuretic peptide receptor (monomer mass 58 kDa)



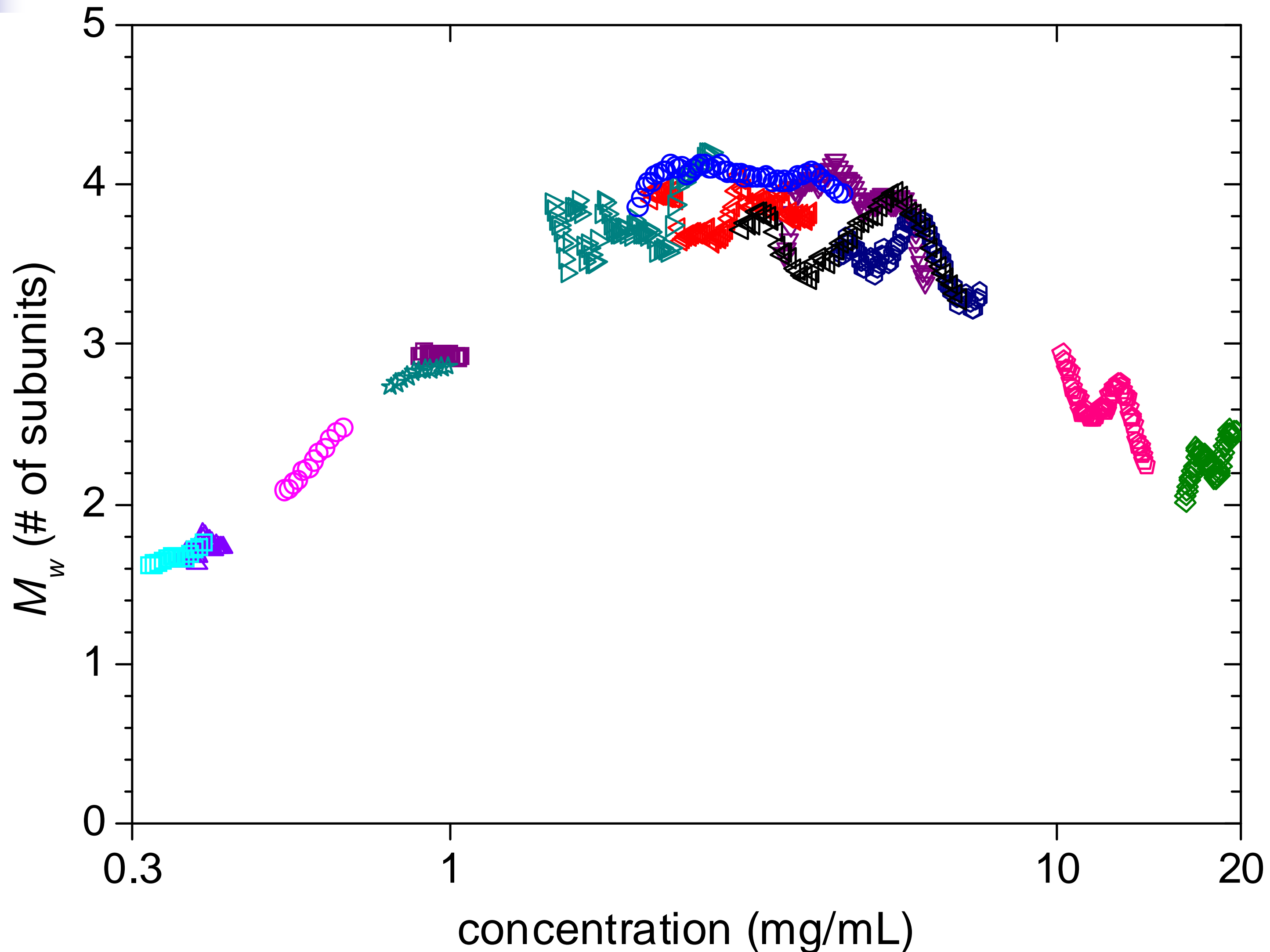
Global analysis of all the data shows the ECD dimerizes with K_d = 520 +/- 20 nM (ΔG = -8570 +/- 25 cal/mol)



Reversible or not? This monomer-dimer-tetramer system acts as a mixture at low concentrations (? due to dilution of its cofactor)

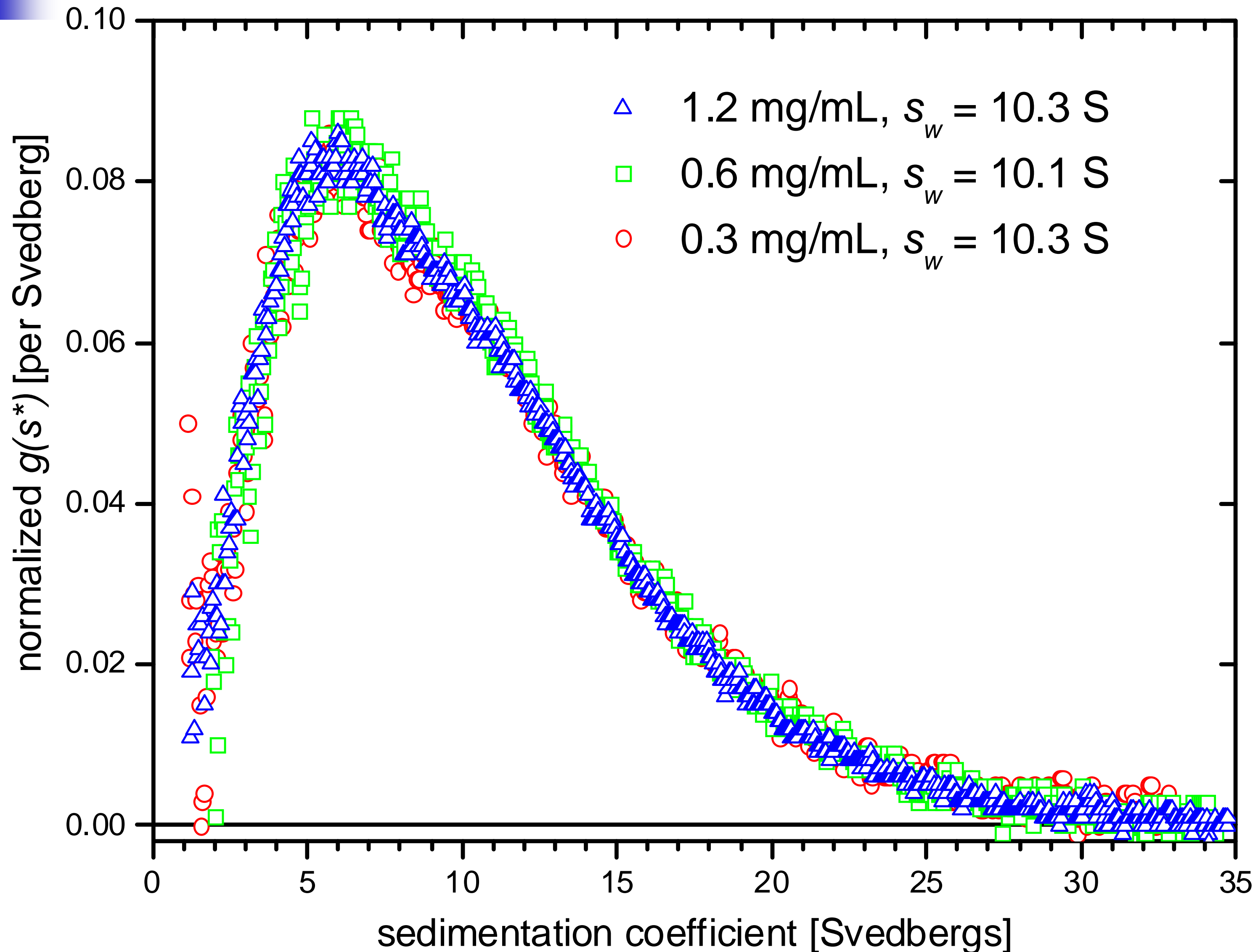


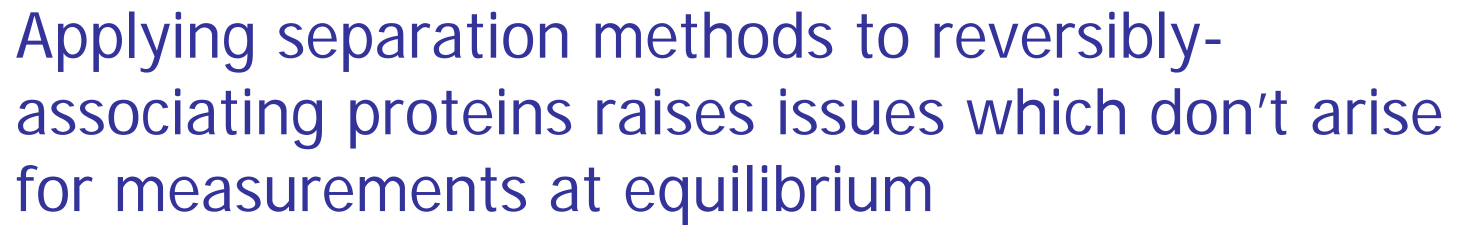
SE data for a peptide (3-5 kDa) which strongly reversibly self-associates; above ~3 mg/mL repulsive solution non-ideality causes the apparent mass to decrease



Sedimentation velocity of reversibly-associating proteins/peptides

First we must test for reversible association by doing a dilution series; here is such a test for a vaccine drug substance that contains a wide range of different oligomers (up to ~25-mer)

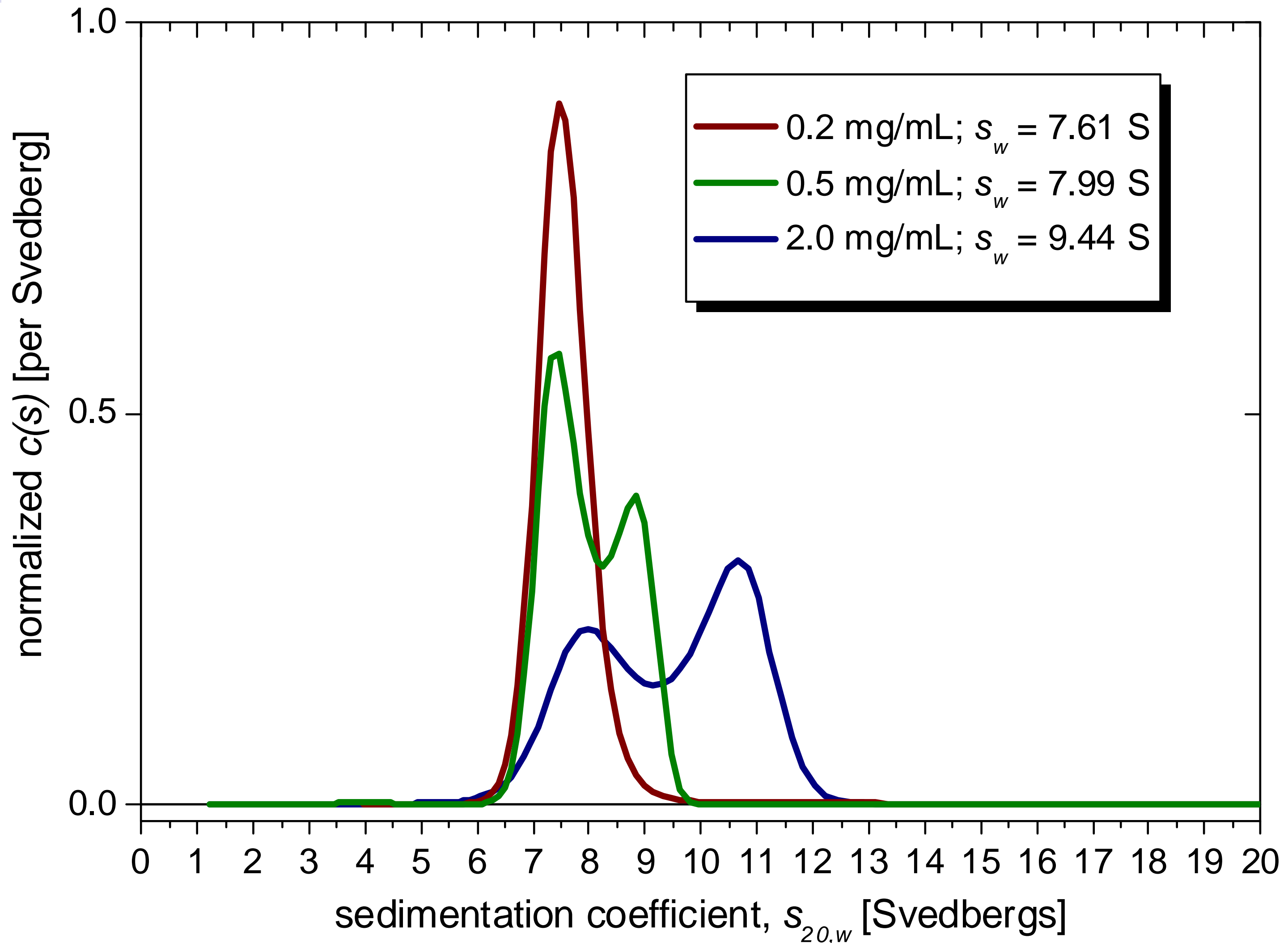


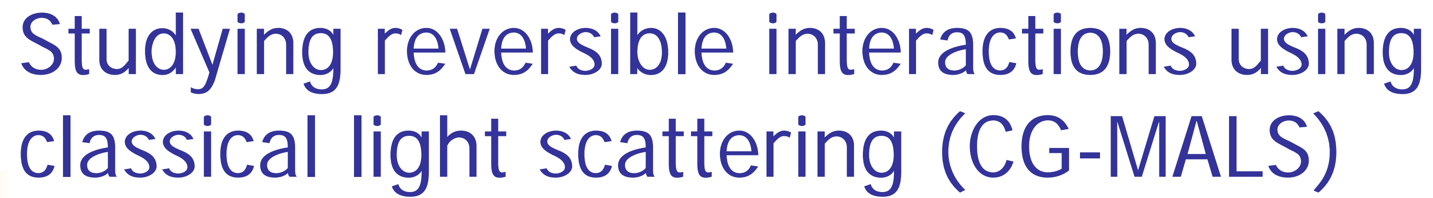


Applying separation methods to reversibly-associating proteins raises issues which don't arise for measurements at equilibrium

- Separation methods (SEC, sedimentation velocity, FFFF, electrophoresis) typically will <u>not</u> resolve individual oligomer species for a molecule in rapidly-reversible association equilibrium
  - what is detected will depend on the rates of association and dissociation compared to the speed of separation
  - for associations to oligomers larger than dimer, from theory multiple peaks may be seen even for infinitely-fast kinetics, but those peaks usually do <u>not</u> represent single oligomers
  - theory says it is possible to see more peaks than the number of different species that are present!
- The bottom line: your so-called "dimer" or "trimer" peak may really represent a dynamic mixture of different rapidly-reversible oligomers

Here is a SV dilution series for a monoclonal antibody that self-associates strongly in a high ionic strength SEC mobile phase



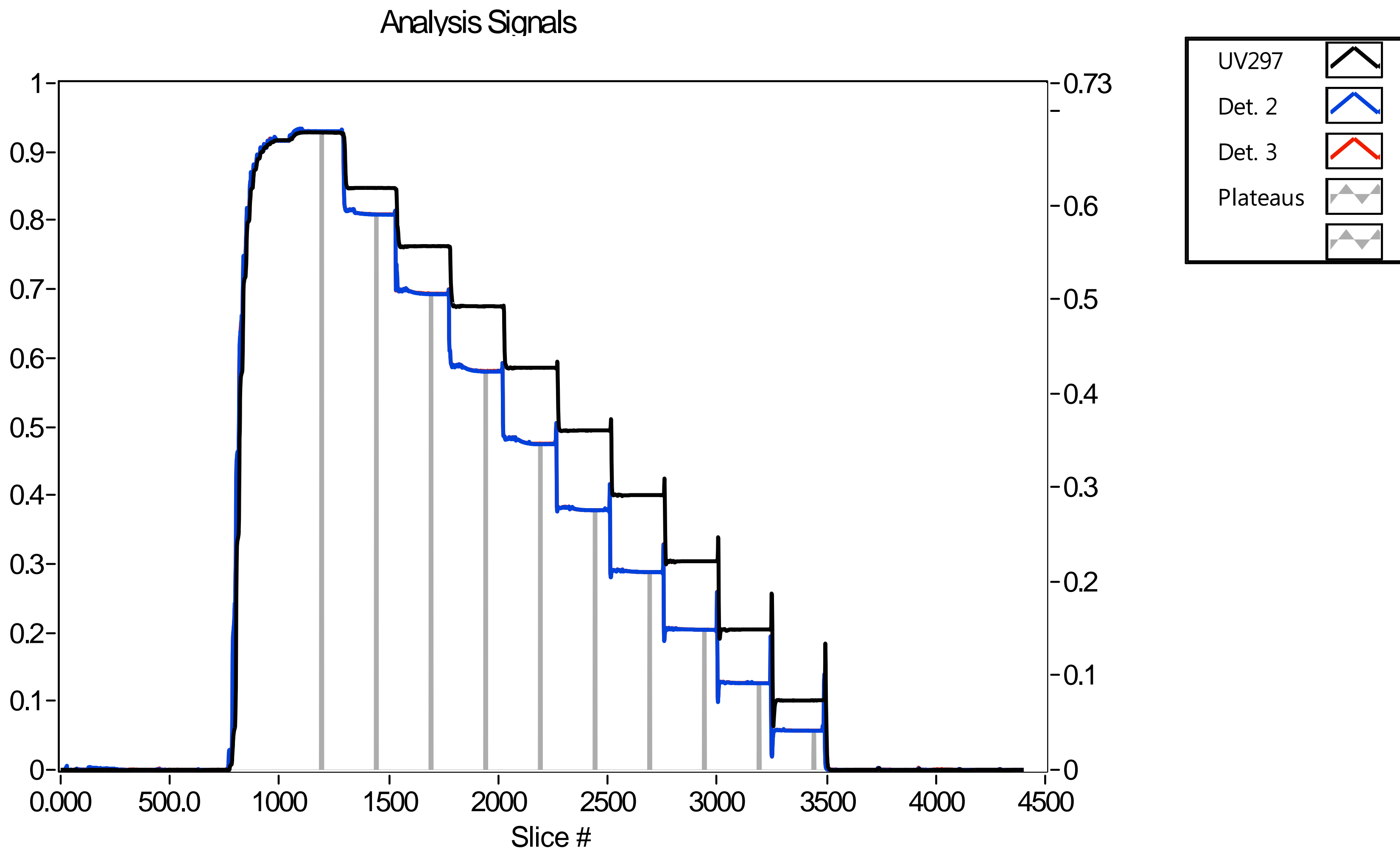


Studying reversible interactions using classical light scattering (CG-MALS)

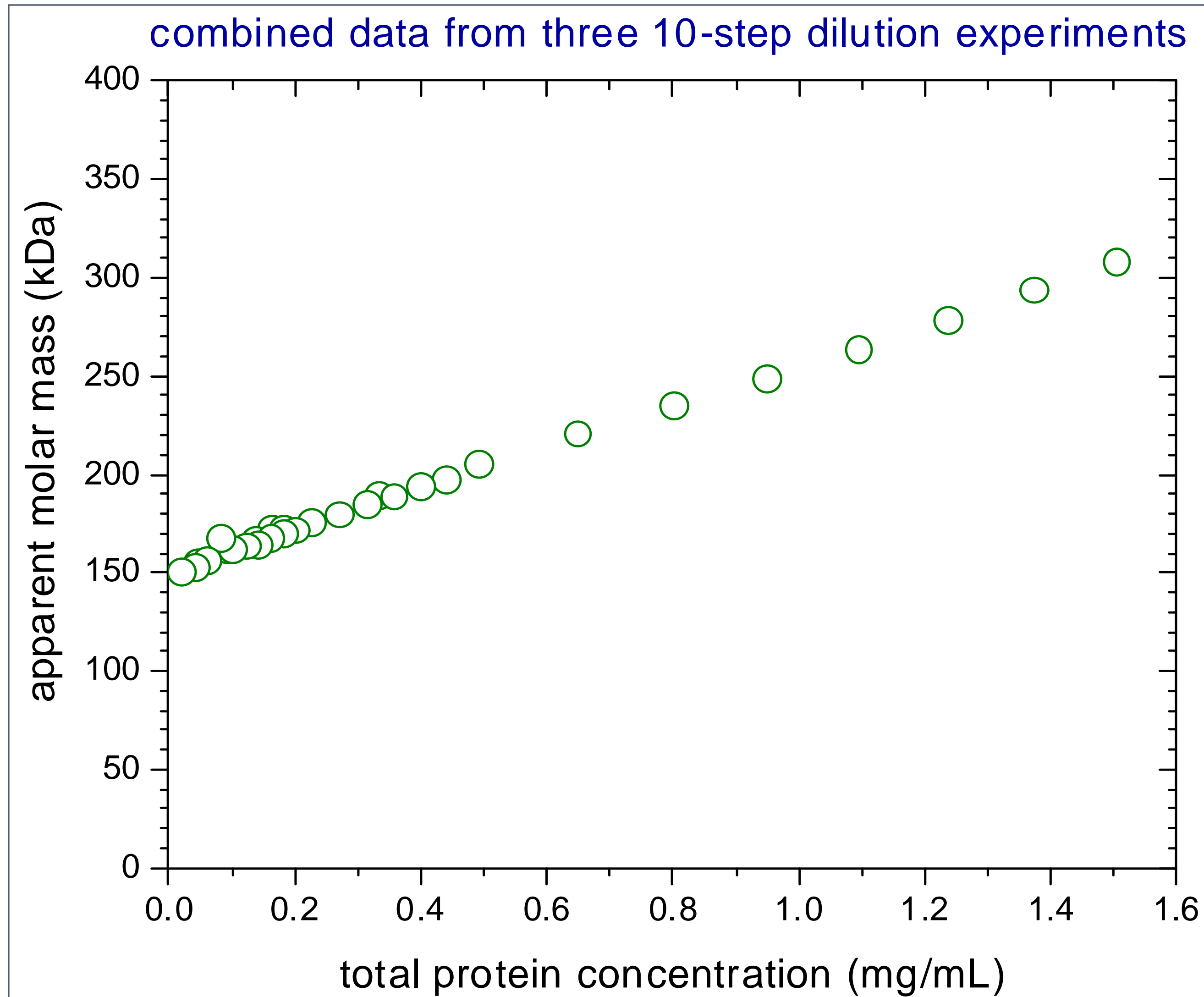
Concentration Gradient MALS combines computerized syringes and a mixing system that flows the mixed solutions into a standard MALS detector and then on into a concentration detector

concentration detector (RI or UV)

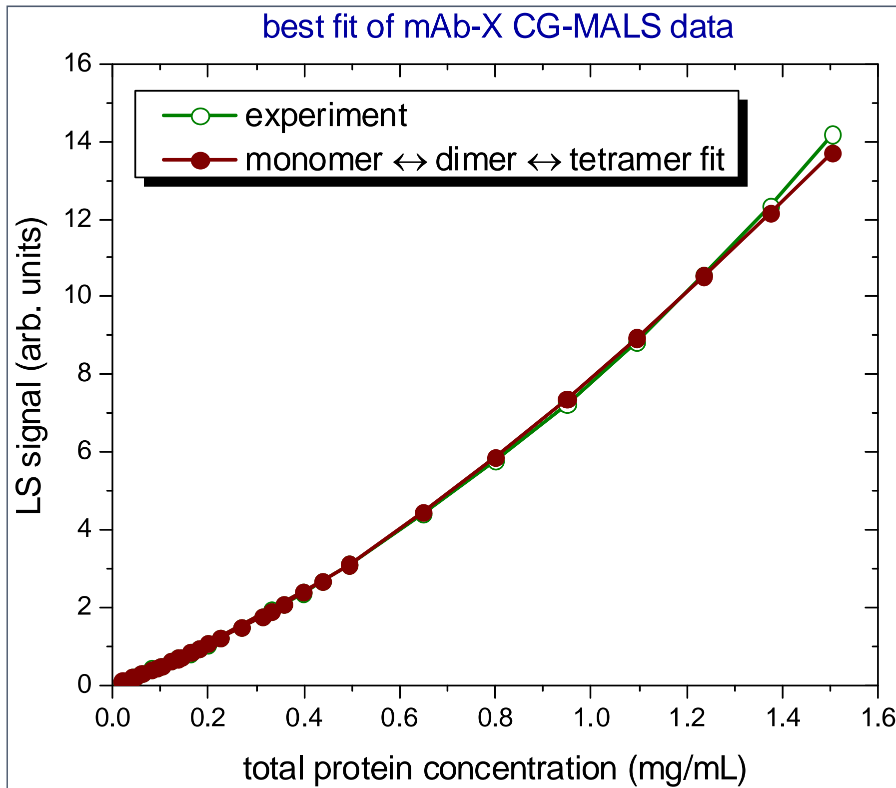
This is a 10-step dilution from ~1.5 mg/mL of that strongly associating mAb in its SEC mobile phase

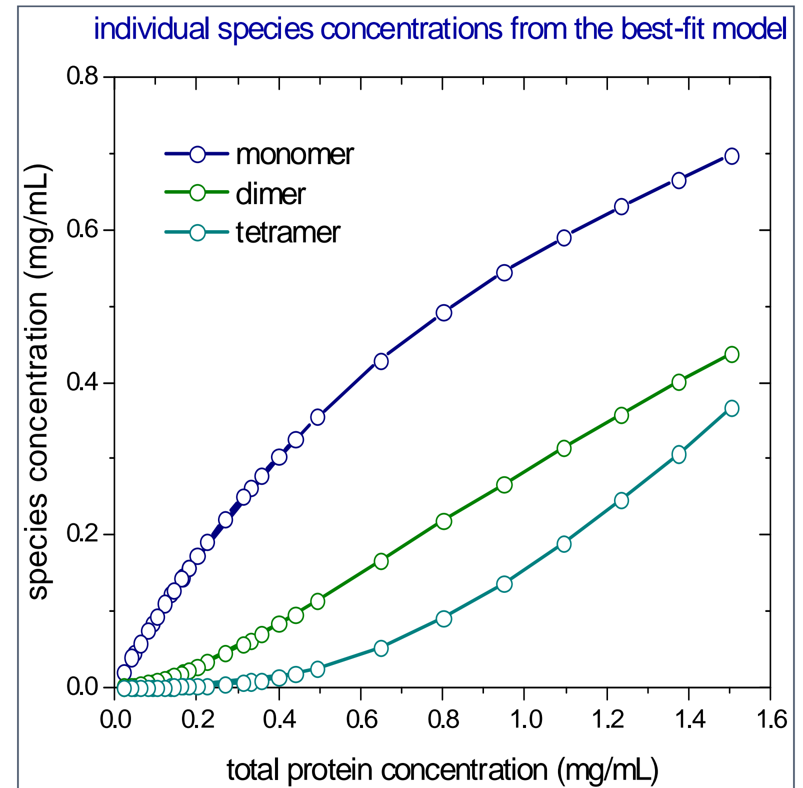


CG-MALS results from 3 such dilution series show that the weight-average molar mass is actually above that of dimer at 1.5 mg/mL and falls continuously as the concentration falls



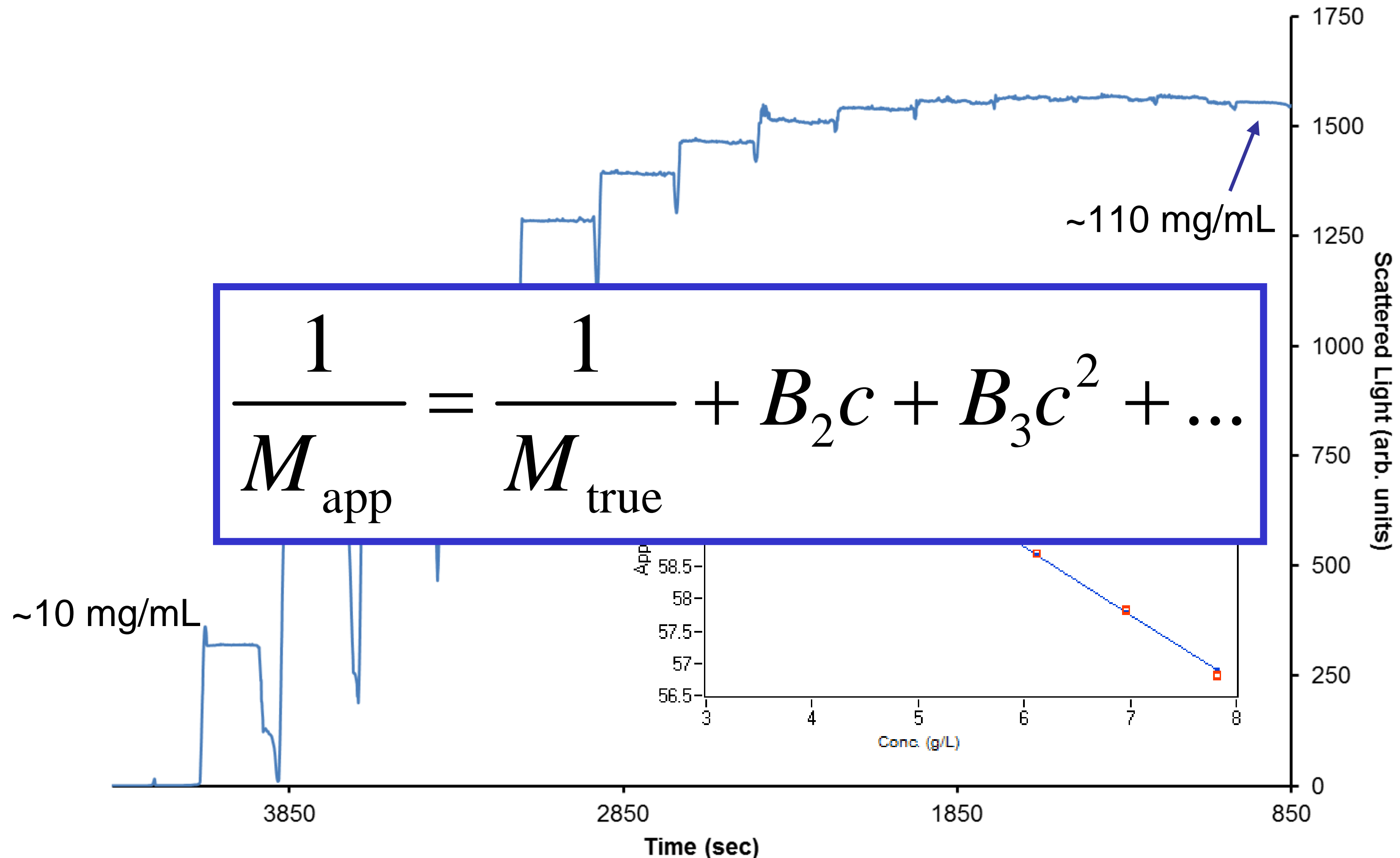
The CG-MALS data fit fairly well to a monomer-dimer-tetramer equilibrium





The next frontier---weak associations at
very high concentrations (50-200 mg/mL)

Light scattering of monomeric HSA up to ~110 mg/mL (physiological pH, ionic strength) shows very strong non-ideality

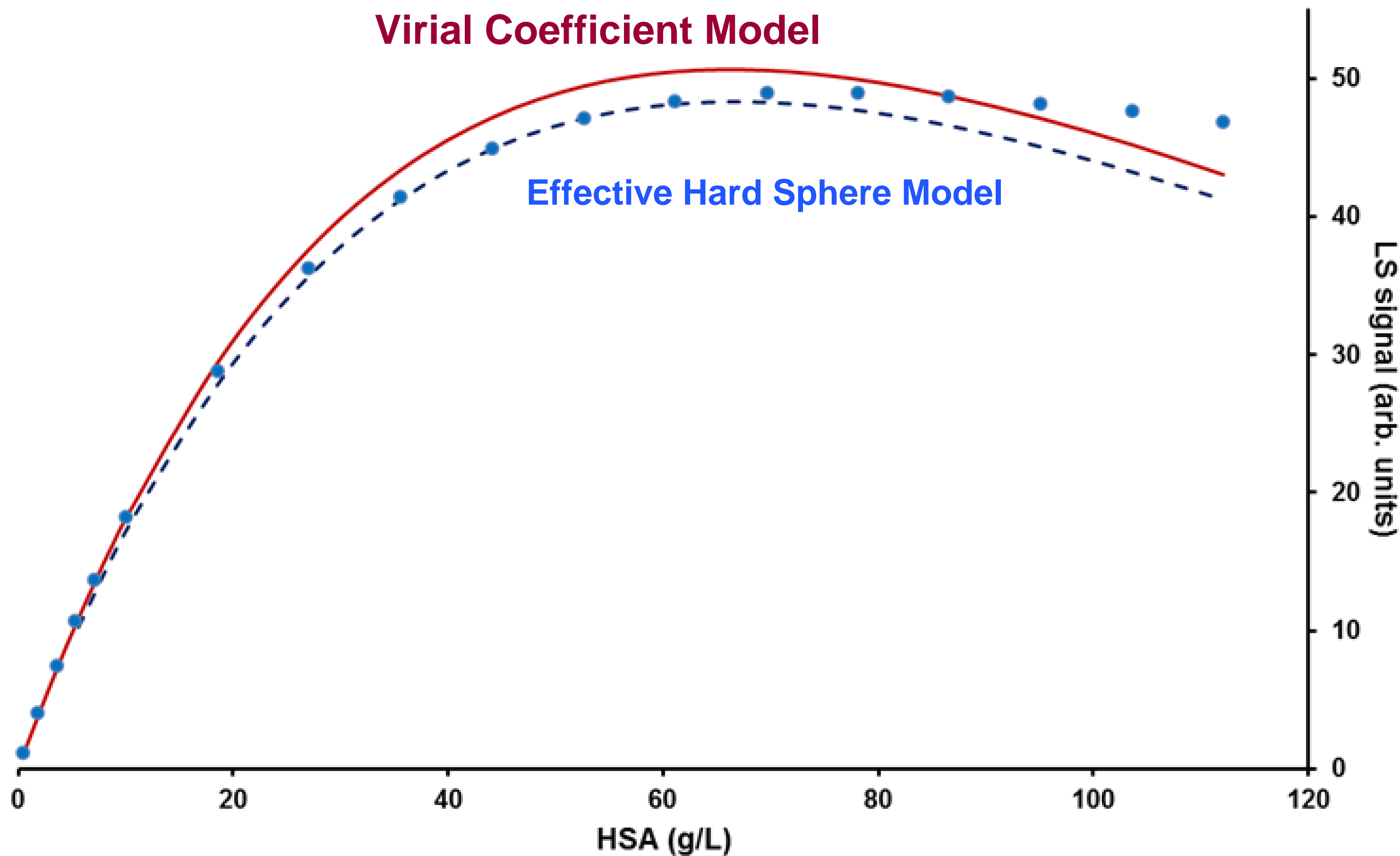


$$\frac{1}{M_{app}} = \frac{1}{M_{true}} + B_2 c + B_3 c^2 + \ldots$$

REGENERON

slide courtesy Mike Marlowe

Monomeric fit with non-ideality is clearly insufficient; indicates presence of higher order species

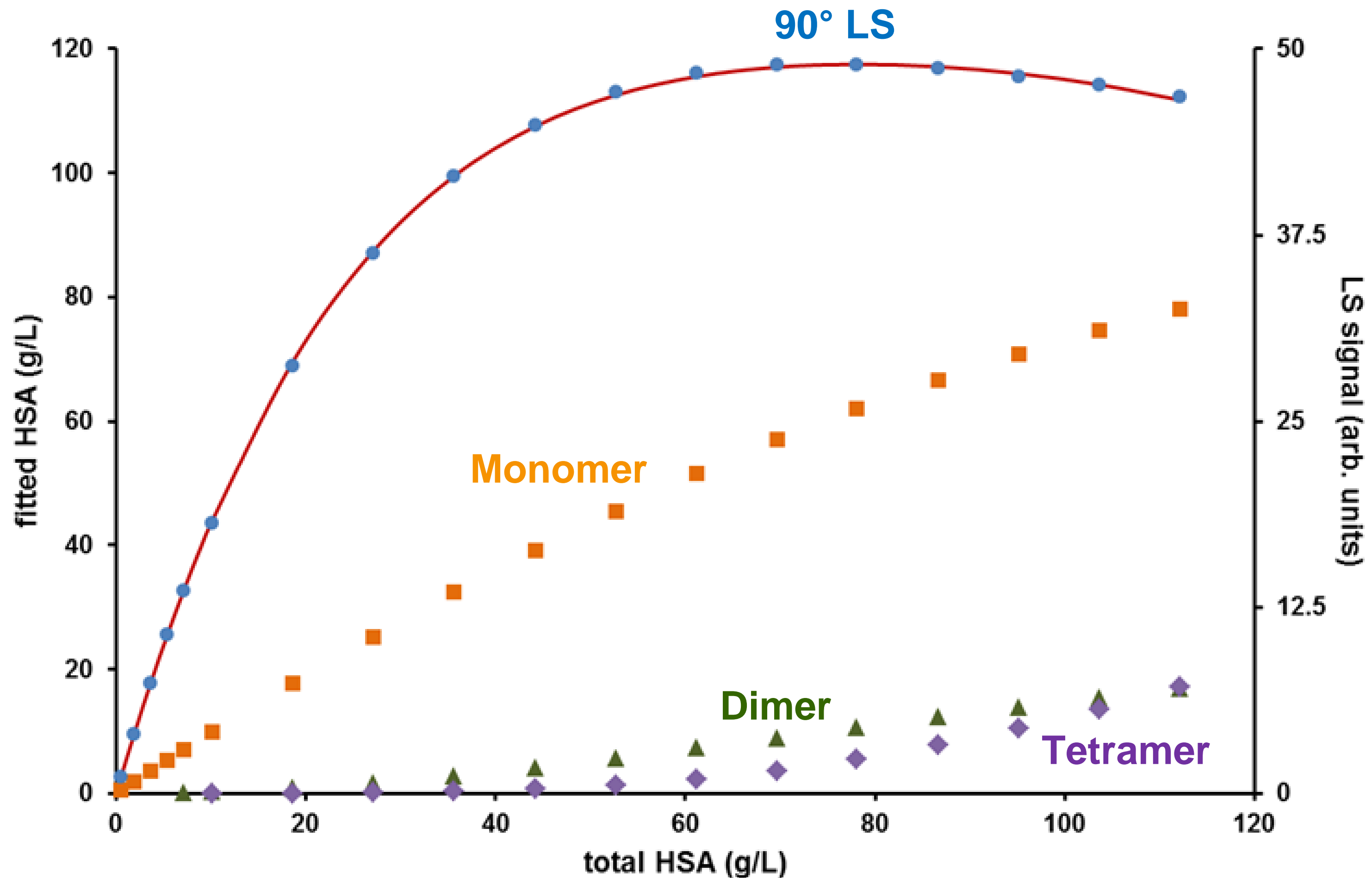


REGENERON

slide courtesy Mike Marlowe

Weak self-association of HSA monomers into dimers starts around 30 mg/mL (~0.5 mM), and then higher oligomers also form

$$LS = \Sigma M_{w,i} c_i$$



REGENERON

slide courtesy Mike Marlowe

Some remaining challenges

1. None of the analytical methods are good at handling samples containing both reversible and irreversible oligomers (which is often the real situation)
2. For these samples at very high concentrations can we really quantitatively account for the non-ideality well enough to reliably measure weak reversible interactions? How can we cross-check such results to prove they are right?
3. CG-MALS requires ~5-10 mL of the solution at high concentration, which is likely prohibitive except for late stage development
   - can sedimentation equilibrium get us the same information with much less material?