Reversible Association of a Monoclonal Antibody Studied by Concentration-Gradient Light Scattering (CG-MALS) and Sedimentation Velocity (SV-AUC)

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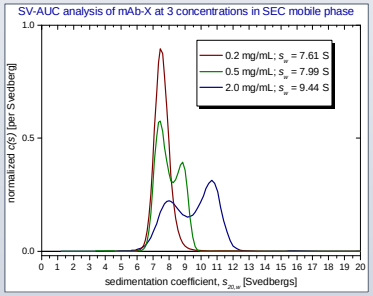
Does mAb-X reversibly self-associate?

One of our clients observed unusual behavior during size-exclusion chromatography (SEC) analysis of a monoclonal antibody (mAb-X), which suggested it might exhibit substantial reversible self-association in the SEC mobile phase at rather low protein concentrations (below 1 mg/mL).

Some initial sedimentation velocity experiments were done at concentrations from 0.2 to 2.0 mg/mL. The systematic increase in the weight-average sedimentation coefficient, s_w, with concentration (see graph below) is definitive proof of reversible association.



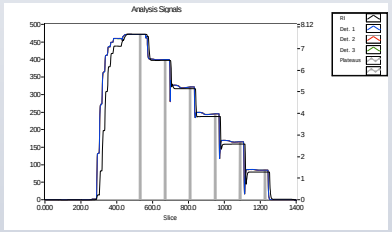
As the concentration increases new peaks are resolved, and the peak at 10.7 S seen in the sample at 2.0 mg/mL is sedimenting at a rate close to that expected for a dimer. However the continuous shifting of the peak positions indicates that these peaks represent dynamic mixtures of different oligomers rather than single species. Further, a reversible monomer ↔ dimer system normally does not resolve into two peaks, so the peak seen at 10.7 S actually suggests that reversible oligomers larger than dimer are also present.

In summary, these initial SV experiments clearly established that substantial reversible association does occur in this concentration range, but at that time we did not attempt to characterize the association stoichiometry or strength.

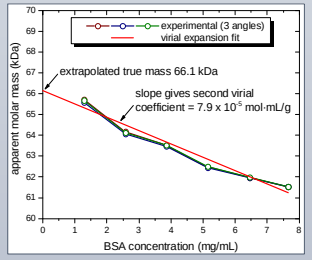
Introduction to CG-MALS: Analysis of BSA

CG-MALS combines a classical light scattering detector with computer-controlled syringe pumps, degassers, and filters. The pumps can mix together combinations of buffer and up to two different protein solutions to create a stepped series of different concentrations and/or mixing ratios, and then supply those mixed solutions to the scattering detector, and then to a concentration detector (RI or UV) in series.

The graph below shows a CG-MALS experiment where a solution of BSA at ~8 mg/mL in DPBS was diluted to zero concentration in six equal steps. We see an initial build-up of the scattering and RI signals to a plateau level as the flow system is saturated with the BSA stock, and then a series of six downward stair-steps as that stock is mixed with increasing amounts of buffer.



The drop in scattering intensity with each drop in concentration can then used to calculate an apparent molar mass at that concentration, as shown in the graph below.

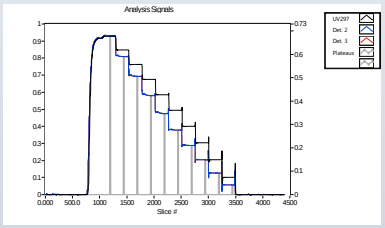


The slight drop in apparent mass as the concentration increases is due to repulsive solution non-ideality ("molecular crowding") effects. Fitting to a straight line gives the expected 66 kDa mass at zero concentration and also the second virial coefficient.

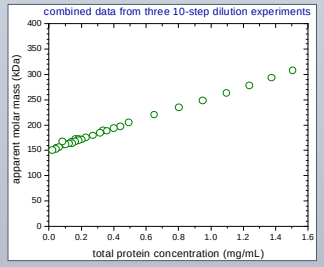
Recent mAb-X results from CG-MALS

Our new 'Calypso' CG-MALS system has allowed us to re-investigate the association of mAb-X in a more detailed and quantitative way. The graph below shows the raw data for a 10-step downward dilution from a starting concentration of ~1.5 mg/mL (with UV concentration detection at 297 nm in this case), obtained ~10 days ago.

Clearly the scattering and concentration traces are much more divergent than for the BSA data (and in the opposite direction). Here we see that the initial downward LS steps are much larger than the later ones, which means the effective molar mass is falling as the concentration falls. Another important observation is that the light scattering reaches a plateau quickly after each dilution, with no evidence of slow dissociation of reversible oligomers.

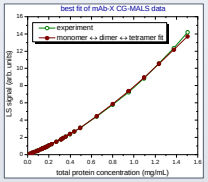


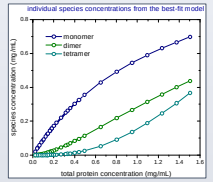
Two additional similar titrations were done starting from ~0.5 and ~0.2 mg/mL, giving 30 total concentration points. The graph below summarizes all those data as an apparent molar mass *vs.* concentration plot. This plot shows that near 1.5 mg/mL the weight-average mass exceeds that of dimer, and hence oligomers at least as large as trimer must be present.



Fitting the CG-MALS data to assembly models

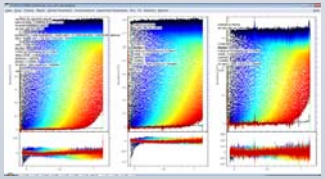
Attempts were made to fit the CG-MALS data to both non-specific indefinite association (isosdesmic) models and to specific assembly stoichiometries. A monomer ↔ dimer ↔ tetramer model gives a reasonably good fit (summarized in the graphs below) and returns dimer → monomer and tetramer → dimer dissociation constants of 15 μM (4.5 mg/mL) and 3.5 μM (2.1 mg/mL), respectively.





This assembly model works for the SV-AUC data too

Because the CG-MALS results imply there is a specific assembly pathway, an attempt to globally fit the existing SV data at 3 concentrations seemed worthwhile. A monomer ↔ dimer ↔ tetramer model does provide a reasonably good fit, returning similar dissociation constants and sedimentation coefficients that are hydrodynamically reasonable.



Note that a high quality fit is not expected for the 2 mg/mL data because the model cannot account for the large hydrodynamic solution non-ideality at that concentration. The dissociation constants also may differ from the CG-MALS values due to differences in temperature and buffer composition.

Advantages & drawbacks of CG-MALS *vs.* SV-AUC

- data interpretation at high concentrations is much easier for CG-MALS because *hydrodynamic* non-ideality effects are much stronger and more complex than *thermodynamic* ones
- SV data depends on molecular shape as well as mass; CG-MALS is independent of shape
- SV requires an order of magnitude less sample volume
- SV data sets are much more information-rich, but only 3-7 samples/day can be run
- fitting SV data to association models requires more fitting parameters because the sedimentation coefficients for different species cannot be accurately predicted