Beckman Coulter Webinar Series
Exploring the Stoichiometry of Macromolecular Complexes Using Multi-Signal Sedimentation Velocity Analytical Ultracentrifugation
View the On-Demand Recording Here

Presented by: Chad Brautigam, PhD
Associate Professor, Biophysics, Director, Macromolecular Biophysics Resource,
The University of Texas Southwestern Medical Center
October 22nd, 2015
Q&A Session

Question: Can this technique used for preparation purpose? What is the dynamic range of this technique?
Answer: No, there is no means of recovering the sample that would not involve extensive mixing, abrogating the preparative utility. The dynamic range is determined by the extinction properties of the materials under study. The concentrations must be such that their respective signals do not exceed the limits of the XL-A/I absorbance detection system (ca. 0.1 to 1.0 AU). That said, there are some ways to extend dynamic range through the judicious use of different wavelengths and centerpieces with different path-lengths.

Question: The SEDPHAT analysis is giving a 'matrix allocation error' and crashing - any advice?
Answer: I believe this is a memory error. Questions like this, which specifically deal with the inner workings of SEDPHAT, would best be addressed on the SEDPHAT user list (https://list.nih.gov/cgi-bin/wa.exe?A0=SEDPHAT-L).

Question: Can samples/complexes that do not apsobsorb in the UV/VIS area be analyzed?
Answer: In general, no. The technique is dependent on the use of at least two signals, and in the current centrifuge one of those must be UV/VIS.

Question: How much can the method tolerate the polydispersity? Can we use average extinction coefficient especially for polydisperse nanoparticles?
Answer: I think the method should tolerate polydispersity well, but such systems may not yield stoichiometry, but rather molar ratios. The average extinction could be used, but one would require that the dispersion in extinction coefficients be not large at all.

Question: If I collected A280, 250 and IF for a 2-protein complex system, is the use of all three best or focus on A280 and IF? Does using more signals make the analysis take longer?
Answer: Overall, I think the best approach is to use all three signals. Because using more signals does take longer, analyzing all three will minimize the lost information. Just keep in mind that it is possible if not likely that one pair of signals may be providing most of the discriminatory power of the method.

Question: the Ck peaks do not have the exact same perfect S-value overlap - is that an artifact in the fitting?
Answer: The origin of this phenomenon is obscure. It may arise from imperfect radial calibration of the two different optical systems. It may reflect fast off-rates/lack of complex saturation. Or it may be artefactual, as you suggest. The answer will likely be dependent on the system studied.

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Question: Any tips on the software use to avoid very long analyses/crashes?

Answer: Save your work often. For MSSV, I find the Marquardt-Levenberg minimization technique to be more efficient. I have found that, when increasing the resolution of an MSSV analysis, it is best to work in integral multiples, meaning that if a segment has a resolution of 50 and you want to increase it, do so to 100 or 150. If your analyses are really time-consuming you could load less data, but that has the effect of reducing the certainty of the analysis, of course.

Question: Is there a sample volume recommendation? Is it ok to fill the SV cells to 300ul instead of 400ul if limited by samples?

Answer: I recommend 400 uL. That said, you could use less. The problem with smaller volumes is that they will yield less data, reducing the amount of information you can provide to the analysis.