Global Analysis of AUC data

Why perform a global analysis?

A global analysis allows the investigator to combine signal from multiple experiments. This has several benefits:

- **Signal averaging improves the signal-to-noise ratio and improves the quality of the model by suppressing noise.**
- **Signals from different experiments can provide different information. For example, a velocity experiment may be performed at different speed, and all speeds could be compared in a global fit. This would enhance diffusion signal from the slow experiments and enhance sedimentation signal for fast sedimenting species.**
- **When multiple experiments are globally fitted, outliers are more easily identified, and systematic changes are more readily detected (e.g., oligomerization)**
- **A global analysis provides an excellent opportunity to compare the quality of different instruments, AUC cell components, etc.**

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How does it work?

- Multiple sedimentation velocity experimental data sets are used to fit a single model, which finds the best compromise solution for the fit. This model is called the SuperGlobal model (SG)
- To better interpret the results from individual datasets, additional models are generated. The total number of models generated is 2n+1, where n is the number of datasets.
- The two additional models generated for each individual dataaset are called Scaled (SC) and Variable Ratio (VR). They contain the same components as the SG model, but the amplitudes describing the amount of each species in the model could be different.
- The SC model maintains the same amplitude ratio between all species in the model, but scales it to the concentration observed in each individual experiment.
- The VR model allows the amplitude ratios between the individual components in the model to be different than in the SG model

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How can the results of the global analysis be interpreted?

- **By comparing the RMSD values between the various models, important conclusions can be drawn about the globally fitted system. The models are increasing constrained in going from VR → SC → SG.**
- **The SuperGlobal model provides the overall best average for all datasets. If its RMSD is close to the RMSD of any of the 2DSA-IT fits to individual datasets, then all datasets included in the global fit are of identical composition, concentration, and all cells and centerpieces are identical, and should produce random residuals and low RMSD values when fitted with the SG model.**
- **The Scaled model will produce good RMSD values, even when the concentrations are different for separate datasets, but the composition is constant.**
- **The Variable Ratio model will always show the best fit and lowest RMSD because it is the least constrained model. It allows for the composition to change between the different datasets, as well as the concentration.**

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Application Example 1:

- **Provide additional information from different speeds.**
- **High speed provides best separation and therefore highest resolution for sedimentation signals.**
- **Low speed gives the same more time to diffuse before pelleting, hence improves the signal for diffusion**
- **Combine low and high speed measurements of the same sample in a global fit to extract additional signal that would not be available from a single speed experiment.**

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Application Example 2:

Test the sameness of the centerpieces:

- **If all samples contain the same concentration of the same sample, and all centerpieces are identical, the superglobal model will fit well for identical samples. If the cells or centerpieces have different pathlengths, only the scaled and variable ratio models will work and provide low RMSD values.**

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Application Example 3:

Test for non-interacting or reversibly self-associating samples:

- **If multiple concentrations of a sample are measured, and the composition stays constant, the scaled model will provide good RMSD values.**
- **If the sample is reversibly self-associating, the composition of the sample changes as more oligomer is formed at higher concentration, and only the variable ratio model will work correctly.**

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DEMO