Experimental Design

The quality of the experimental data is highly correlated with the quality of the design of the experiment. To improve the design, it is important to understand all aspects of the data collection process.

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- **Is the molecule homogeneous/pure?**
- **Is the molecule self-associating (change mass)?**
  - **Reversibly or irreversibly (aggregate)?**
  - **Are there hydrophobic or electrostatic interactions?**
- **Does the molecule change conformation?**
  - **Does the molecule conformation and molar mass?**
- **Does the molecule interact/bind to something else?**
  - **What is the k_D and the stoichiometry?**
  - **Are there multiple chromophores?**
- **Do molecules have a different density? What is their density?**

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2) **What do I want to learn from the experiment?**

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- **Molar parameters?**
  - **sedimentation/diffusion coefficients, molar mass, friction, density?**
- **How does the molecule react to different buffers (ionic strength, pH, additive)?**
- **How many different components are in the mixture?**
- **Does a small molecule bind to my protein?**
- **What is the order of assembly?**
- **Is my viral particle loaded with nucleic acid?**
- **What is the loading of a lipid nanoparticle?**

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- **If you need precise molar mass, use mass spectrometry**
- **If you need precise vbar (density) of a pure sample, use densitometry (not density matching)**
- **If you don't have enough sample, consider other methods like MST**
- **Consider pre-fractionating using column chromatography if the sample is too heterogeneous**
- **If you need precise structure, use X-ray crystallography, NMR or cryoTEM**
- **SAXS is better at defining shape**
- **Speed selection and length of an experiment, concentration selection, temperature considerations, Instrument settings, optical system considerations**

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- **Do I have sufficient sample to measure different conditions with enough sensitivity? 0.6 OD is optimal – consider baseline contributions from buffer**
- **Can I spin fast enough for very small molecules?**
- **Does the sample pellet by gravity alone**
- **Always check absorbance in a UV spectrophotometer before the experiment!**
- **Is there enough/too much OD? Am I using the right wavelength? Is there any light scattering?**

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- **SEC, gel electrophoresis, densitometry, mass spec or sequencing**
- **Interaction studies with MST, ITC, electrophoretic mobility shift assay (EMSA)**
- **PDB structure, Alphafold.**

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6) **Do I have enough sample?**

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- **Titration series**
- **Concentration series**
- **PH, ionic strength series**
- **Aim for 0.6 OD (0.1-1.1 OD) at whatever wavelength is reasonable**

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8) **What results need to be presented?**

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- **Overlay plots, s-value distributions, molar mass/density distributions**

Experimental Design

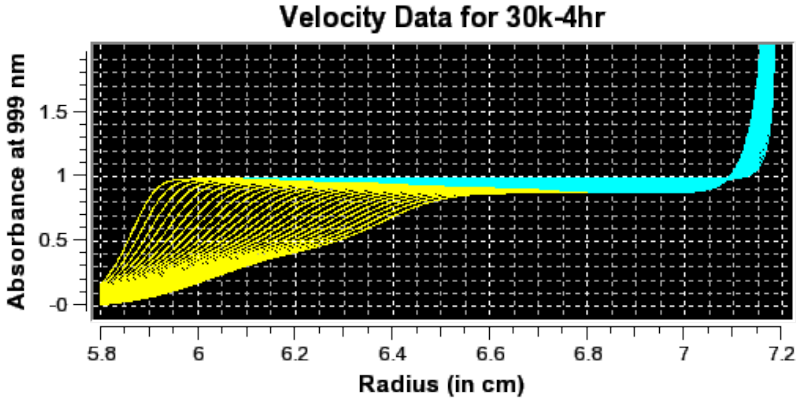
ALWAYS collect scans from the first seconds of the early experiment all the way until most material is pelleted. Remember, you can always discard scans later, but repeating the experiment to obtain missed data is not desirable

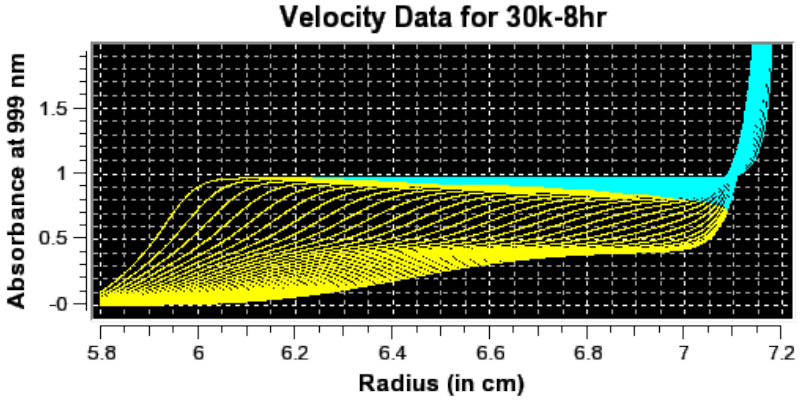
Use the finite element simulation routine to simulate all expected components in a system. You should model all components by shape, MW, s and D using the "Simulation:Model s, D and f from Molecular Weight for 4 basic shapes" and then use the "Simulation:Finite Element Simulation" module to predict how long to run the experiment and what speed should be selected.

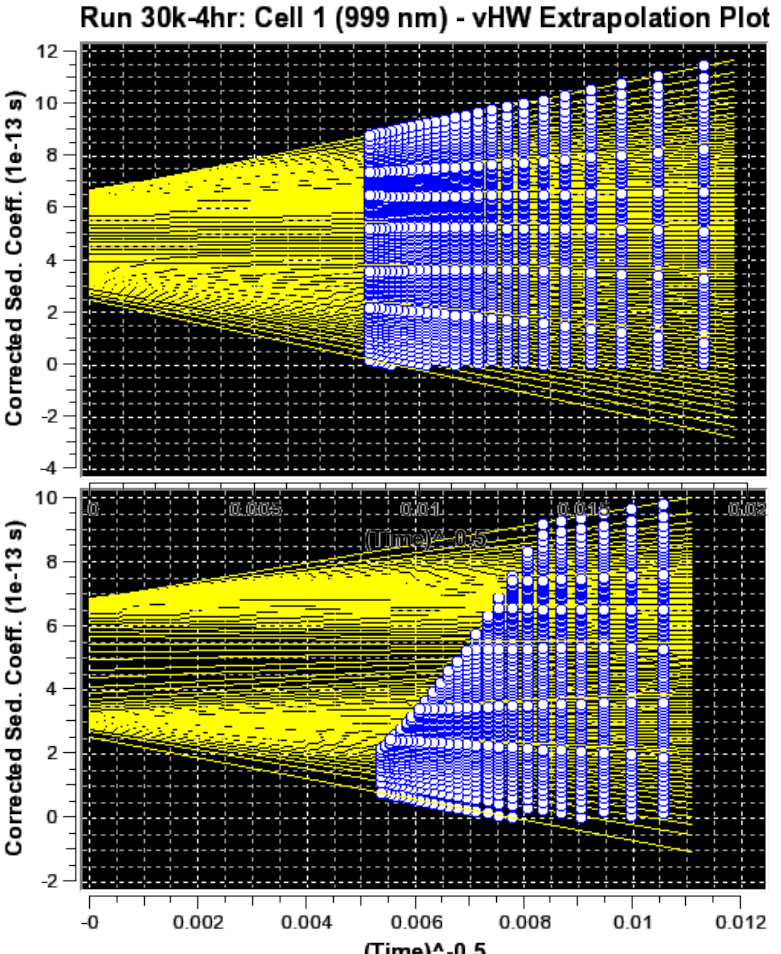
Determine the number of scans to get at least 80-120 scans. UV-absorbance: Speed dependent, Interference: 5 seconds per scan.

Experimental Design

Effect of Time on Resolution

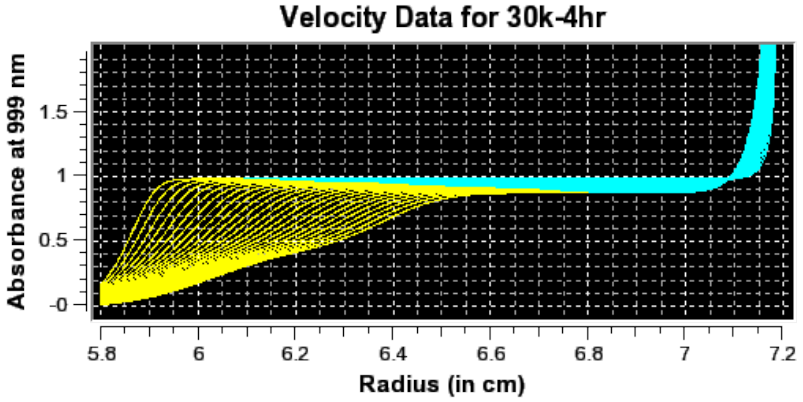


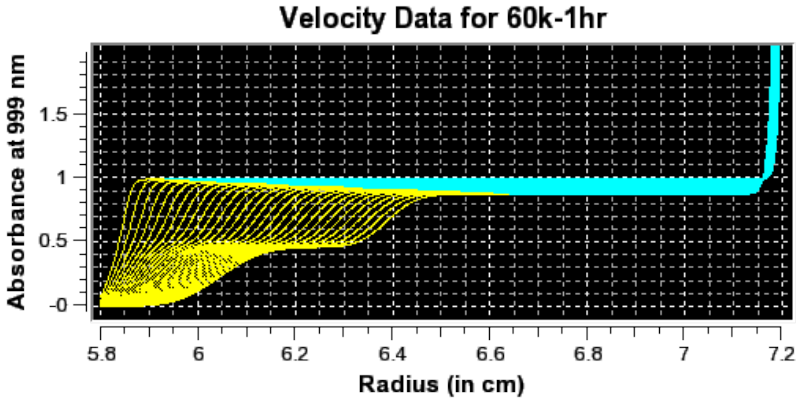


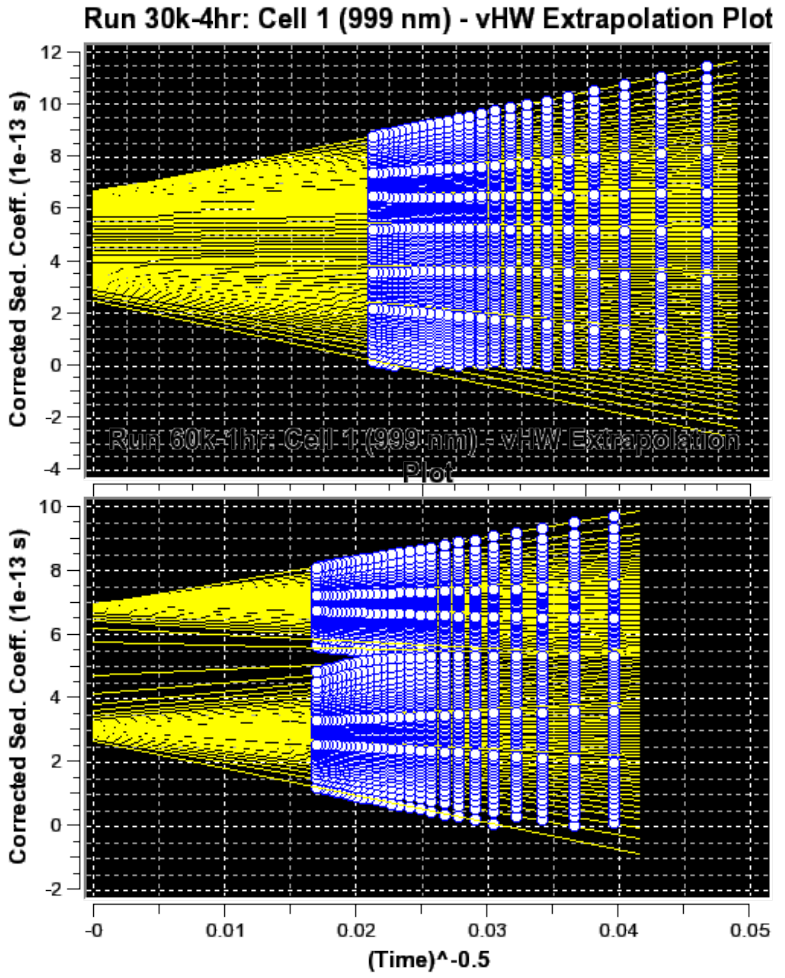


Experimental Design

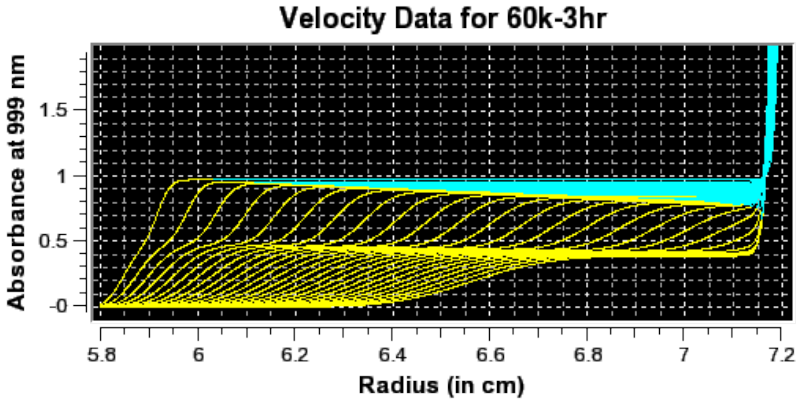
Effect of Rotorspeed with constant $\omega^2 t$ on Resolution:

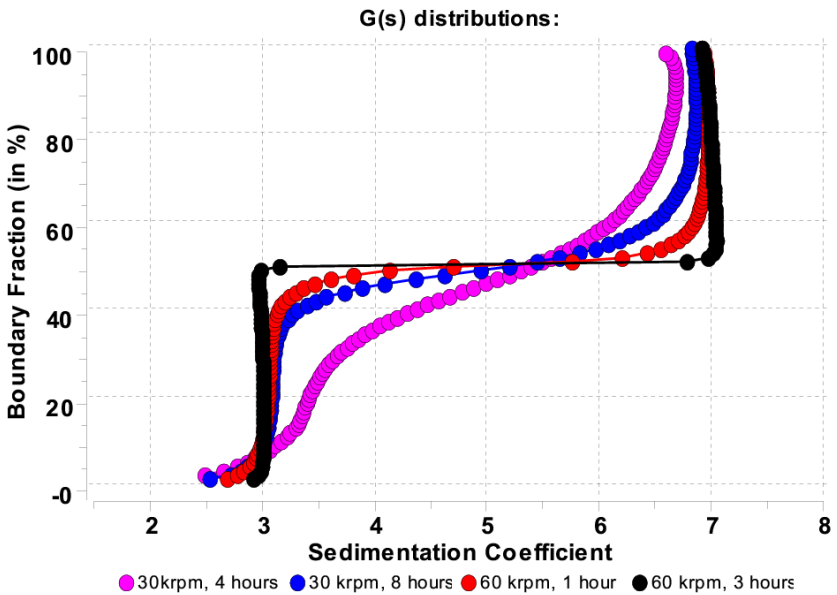


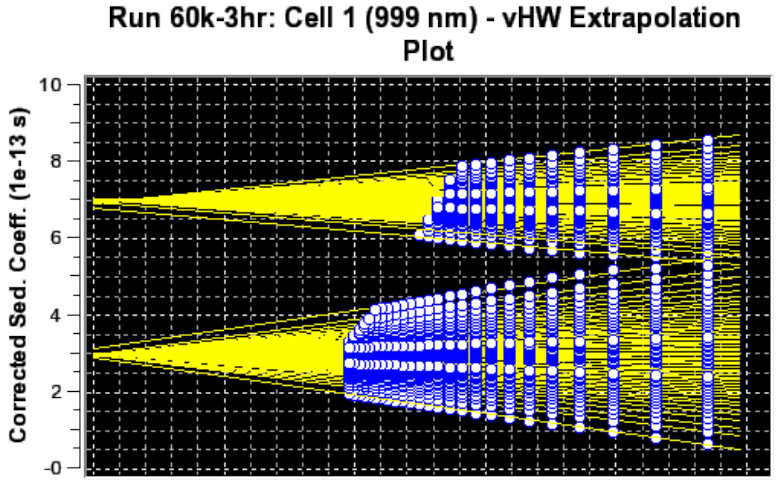


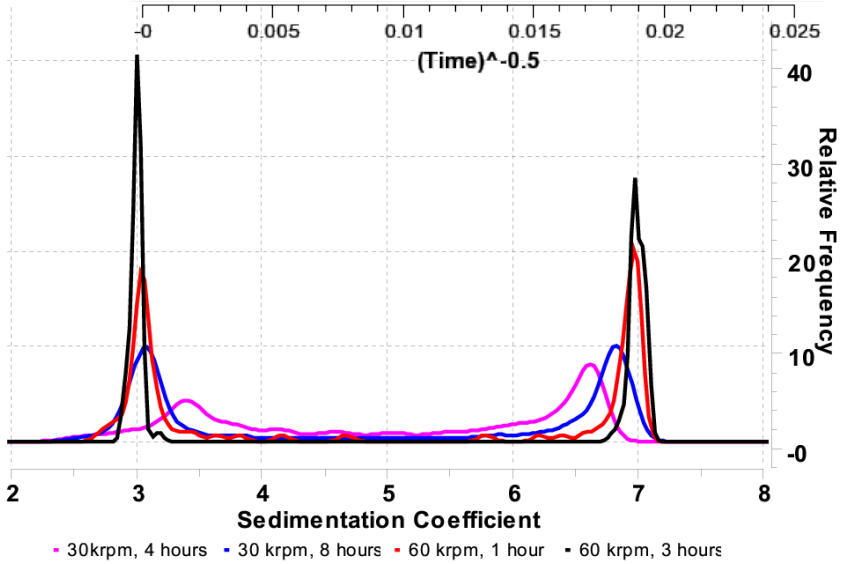


Experimental Design - *Resolution Comparison:*









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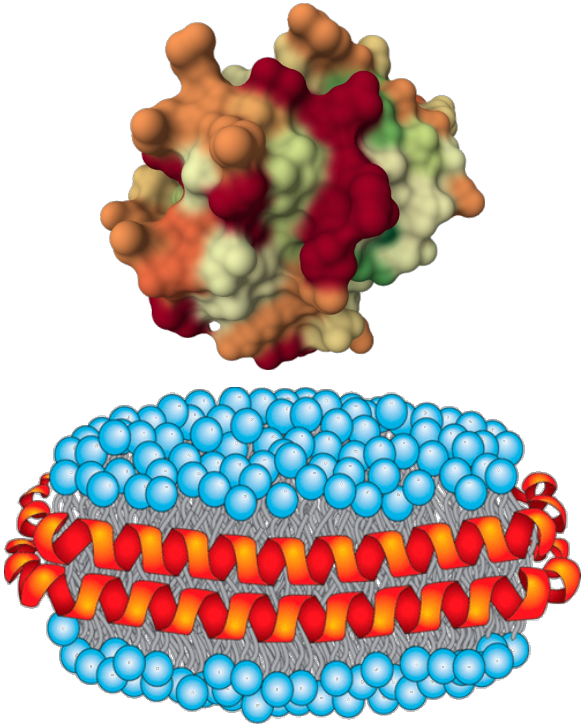
Design the following systems:

1) measure the relative concentration of monomer, dimer and trimer of BSA.
2) Cytochrome C (contains a heme group with 410 nm absorbance) binding to a lipid nanodisk



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