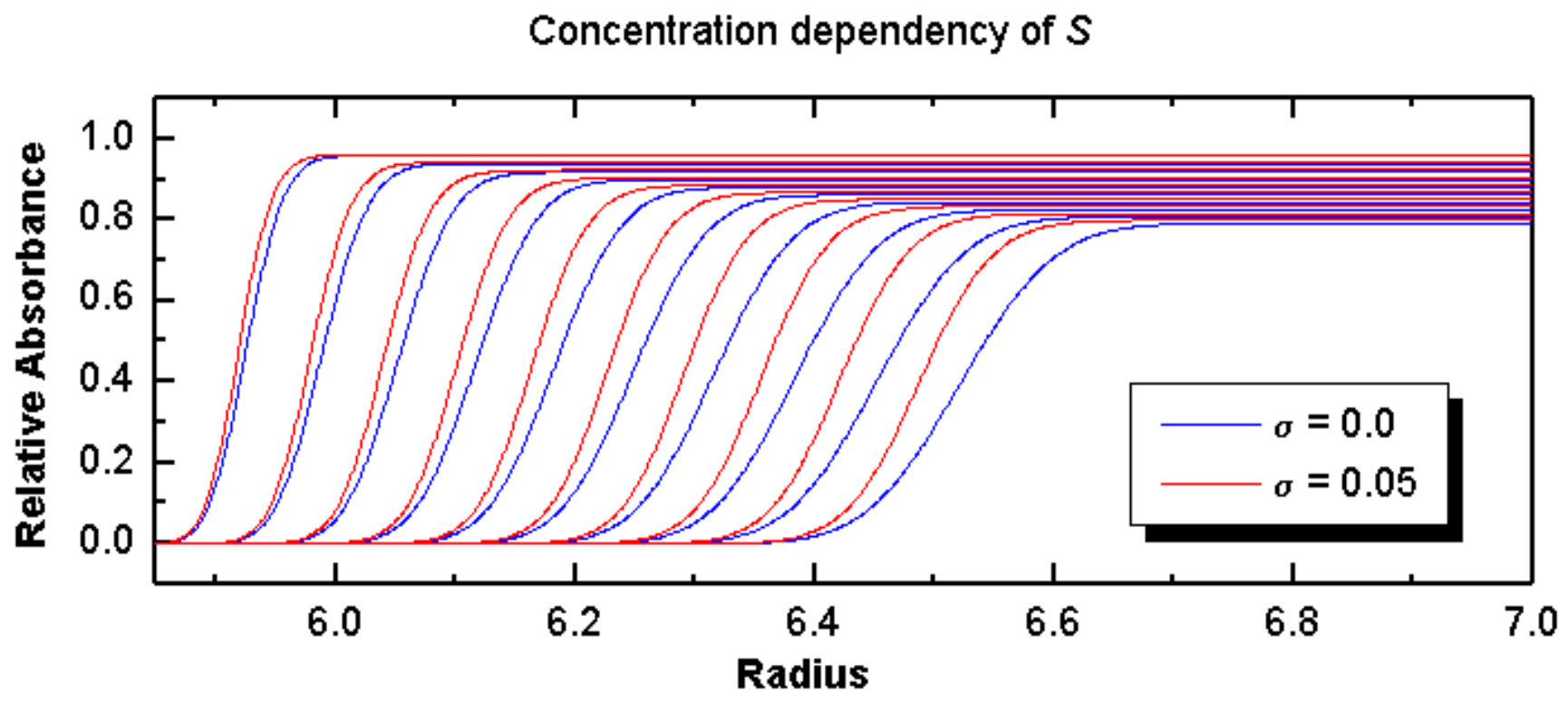
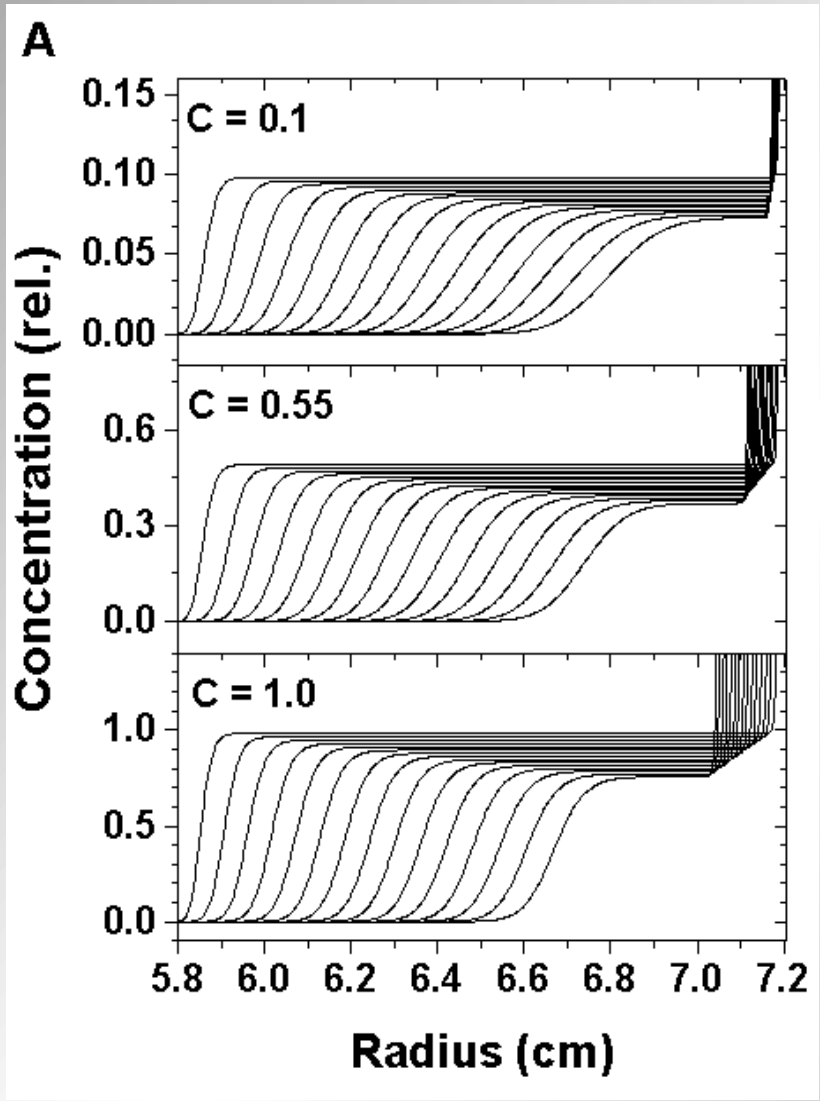
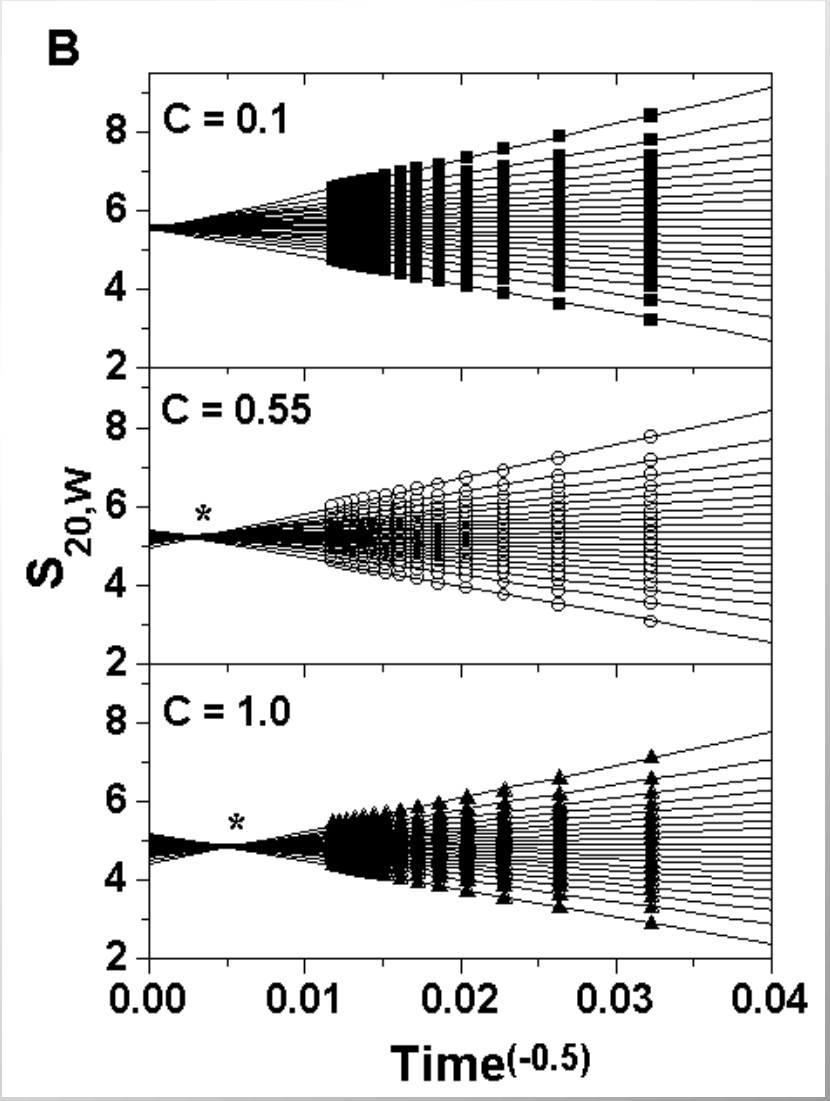
Concentration Dependency of the Sedimentation Coefficient

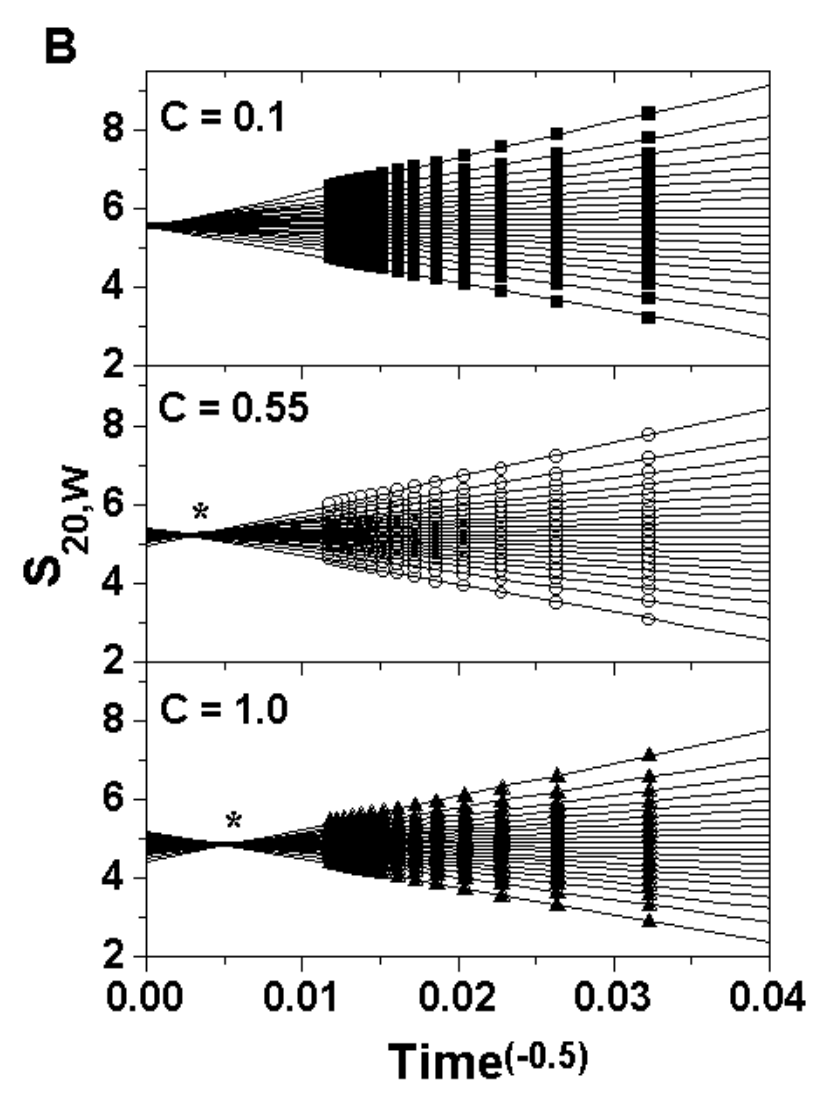


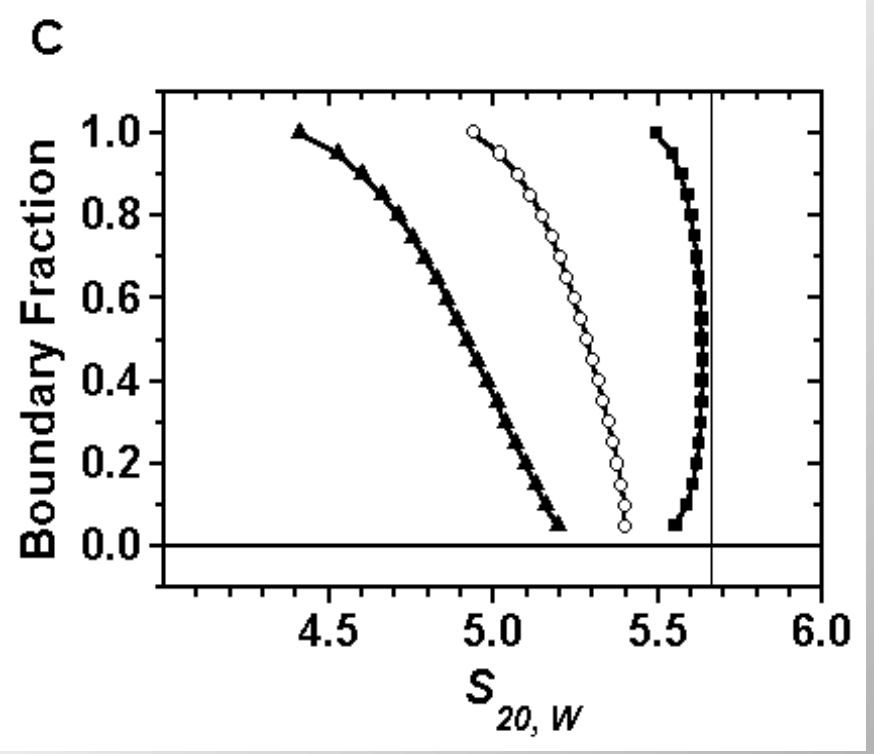
Concentration Dependency of the Sedimentation Coefficient



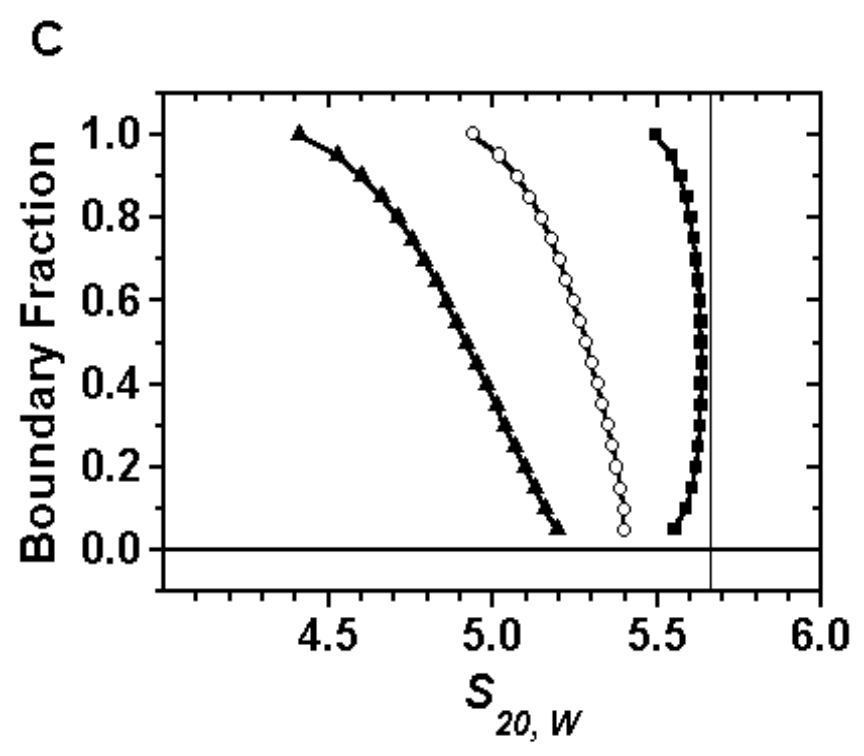


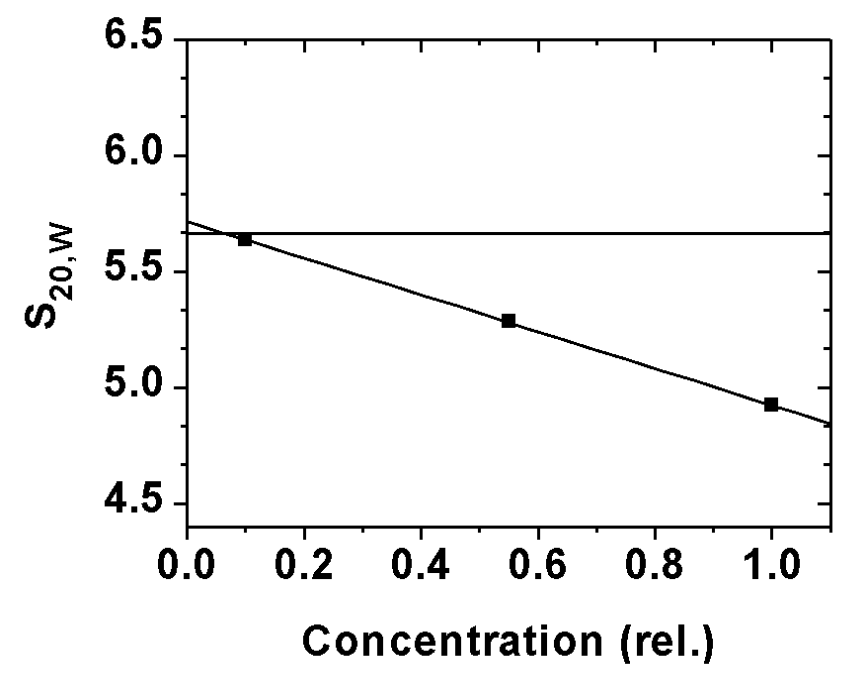
Concentration Dependency of the Sedimentation Coefficient



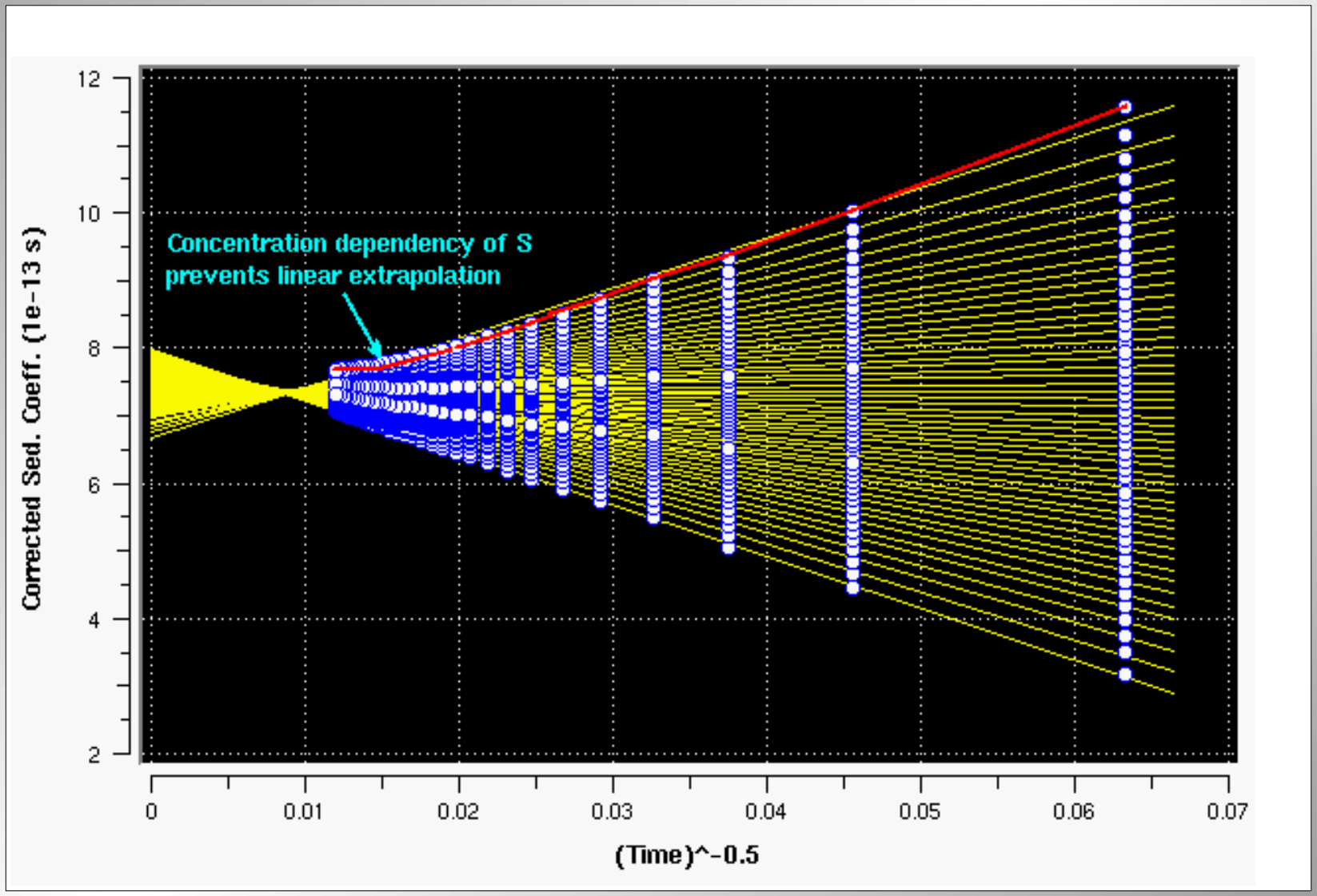


Concentration Dependency of the Sedimentation Coefficient

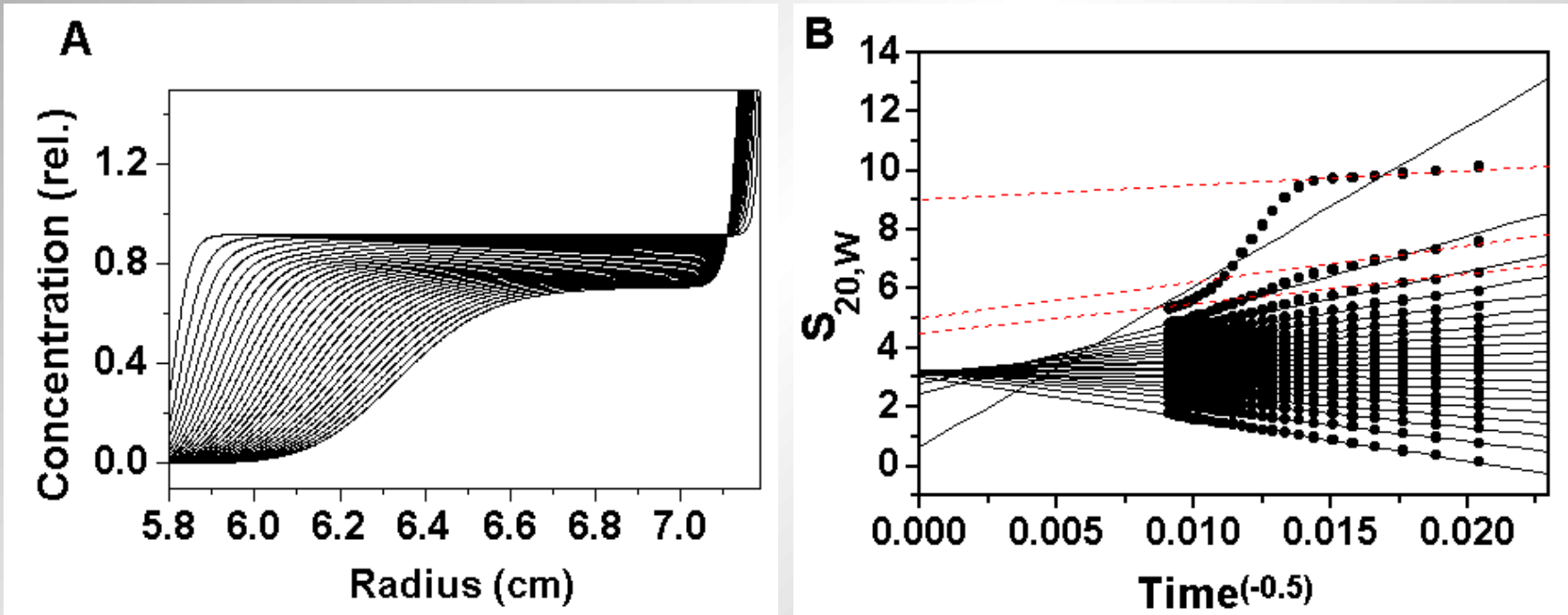




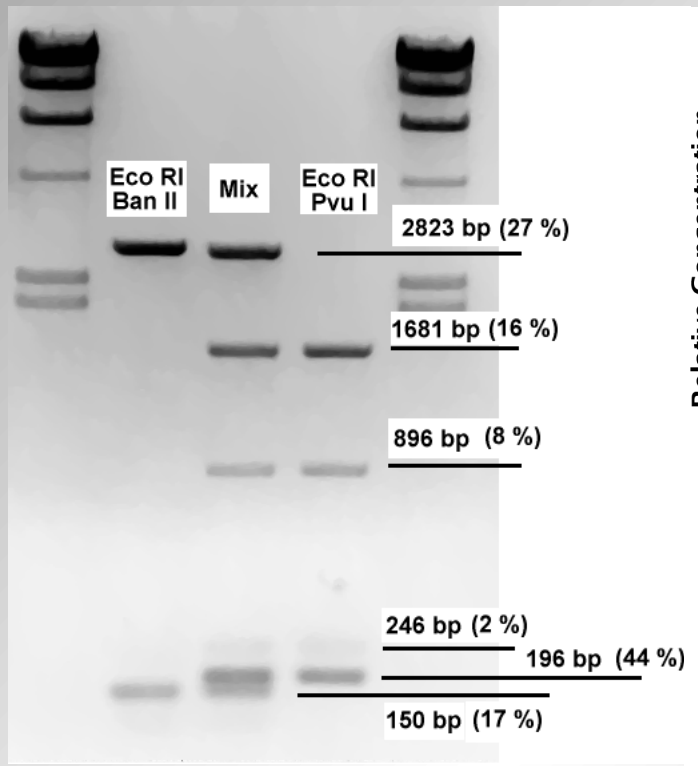
Concentration Dependency of the Sedimentation Coefficient

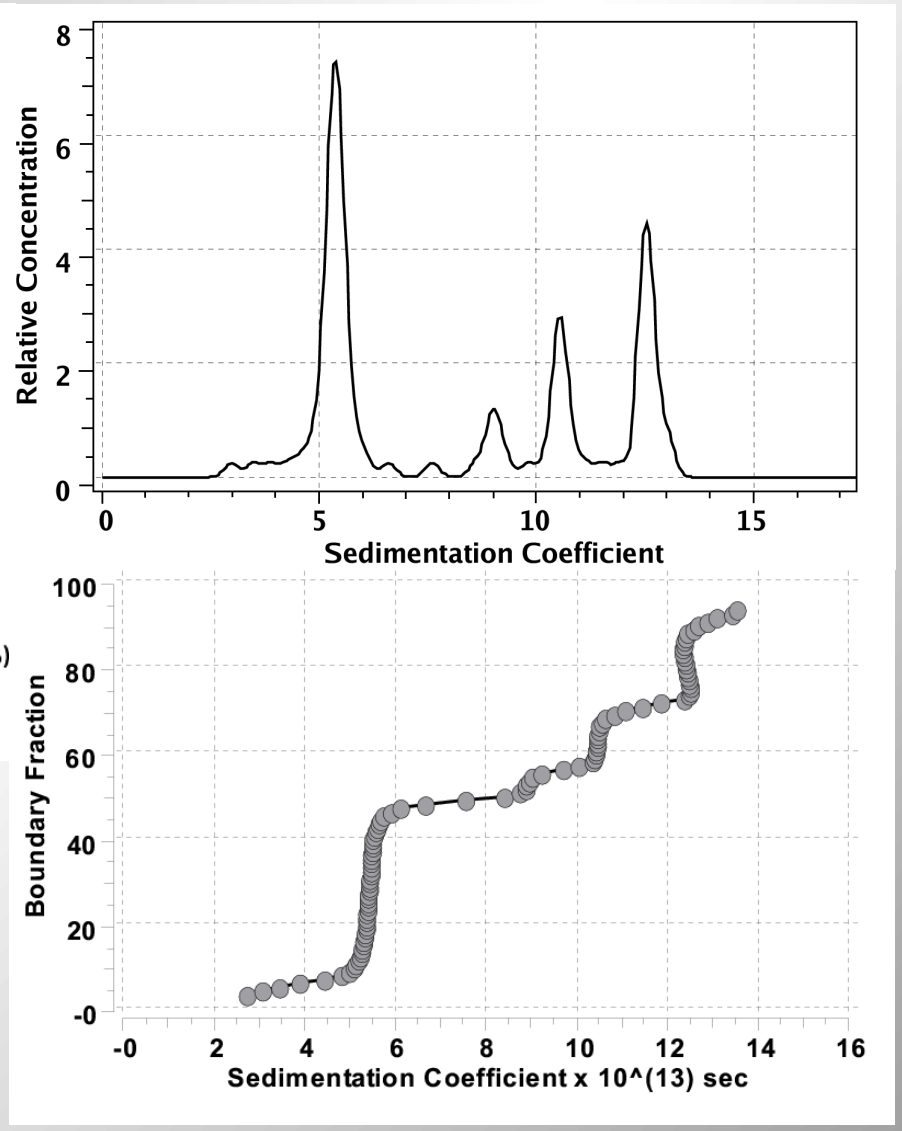


Aggregation:



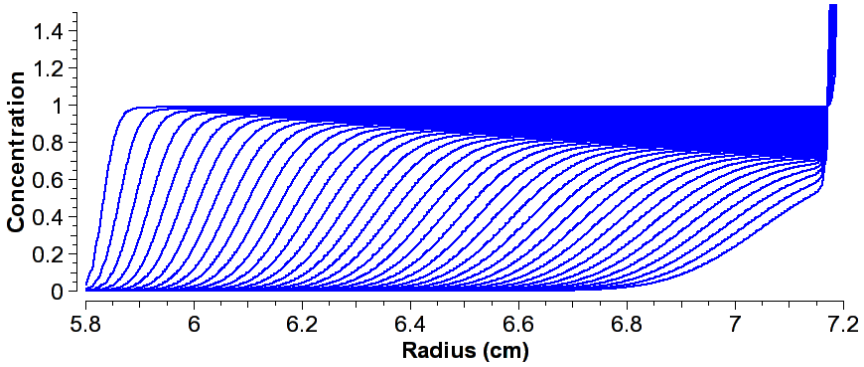
Composition Analysis:





A Model for Reversible Reactions

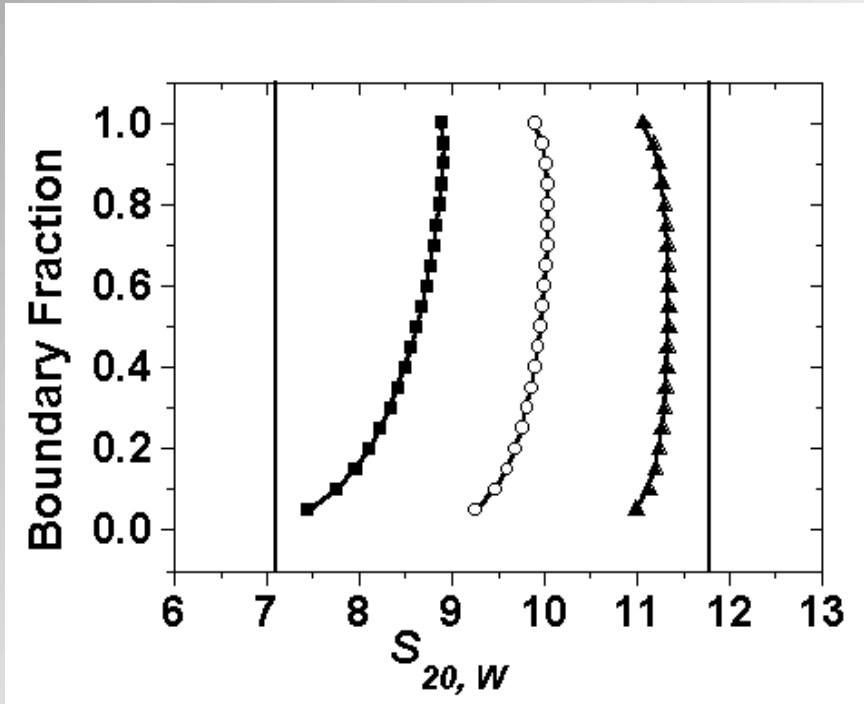
In the gradient, the *weight-average* sedimentation coefficient and the *gradient-average* diffusion coefficient are observed:

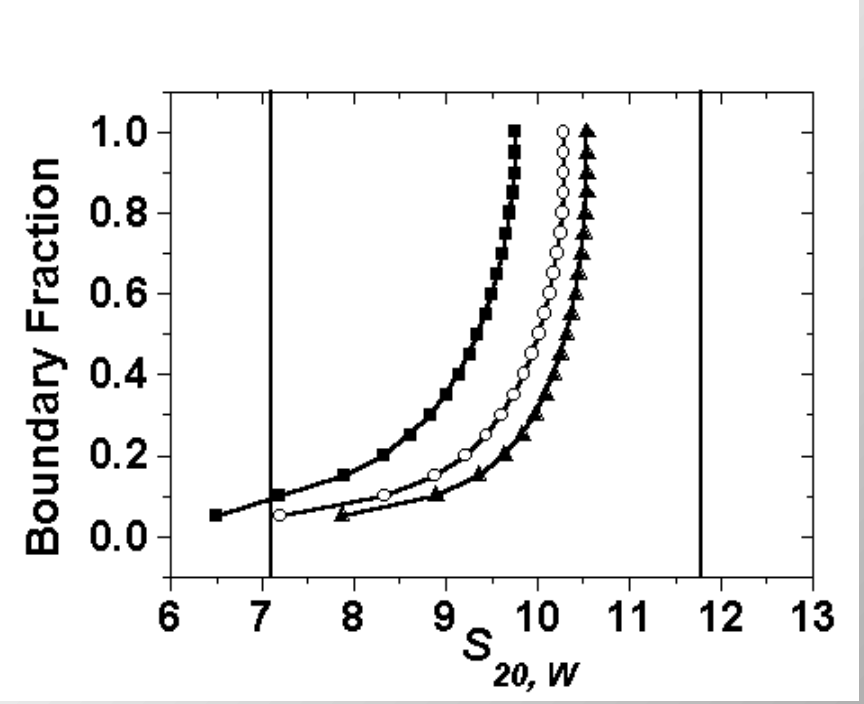


$$\bar{s} = \frac{\sum_{j=1}^{m} s_j C_j}{C_T} = \frac{\sum_{j=1}^{m} s_j K_j C_1^j}{C_T}$$

$$\bar{D} = \frac{\sum_{j=1}^{m} D_j \left(\partial C_j / \partial r\right)}{\sum_{j=1}^{m} \left(\partial C_j / \partial r\right)} = \frac{\sum_{j=1}^{m} j D_j K_j C_1^{j-1}}{\sum_{j=1}^{m} j K_j C_1^{j-1}}$$

Self-Associating Equilibrium:

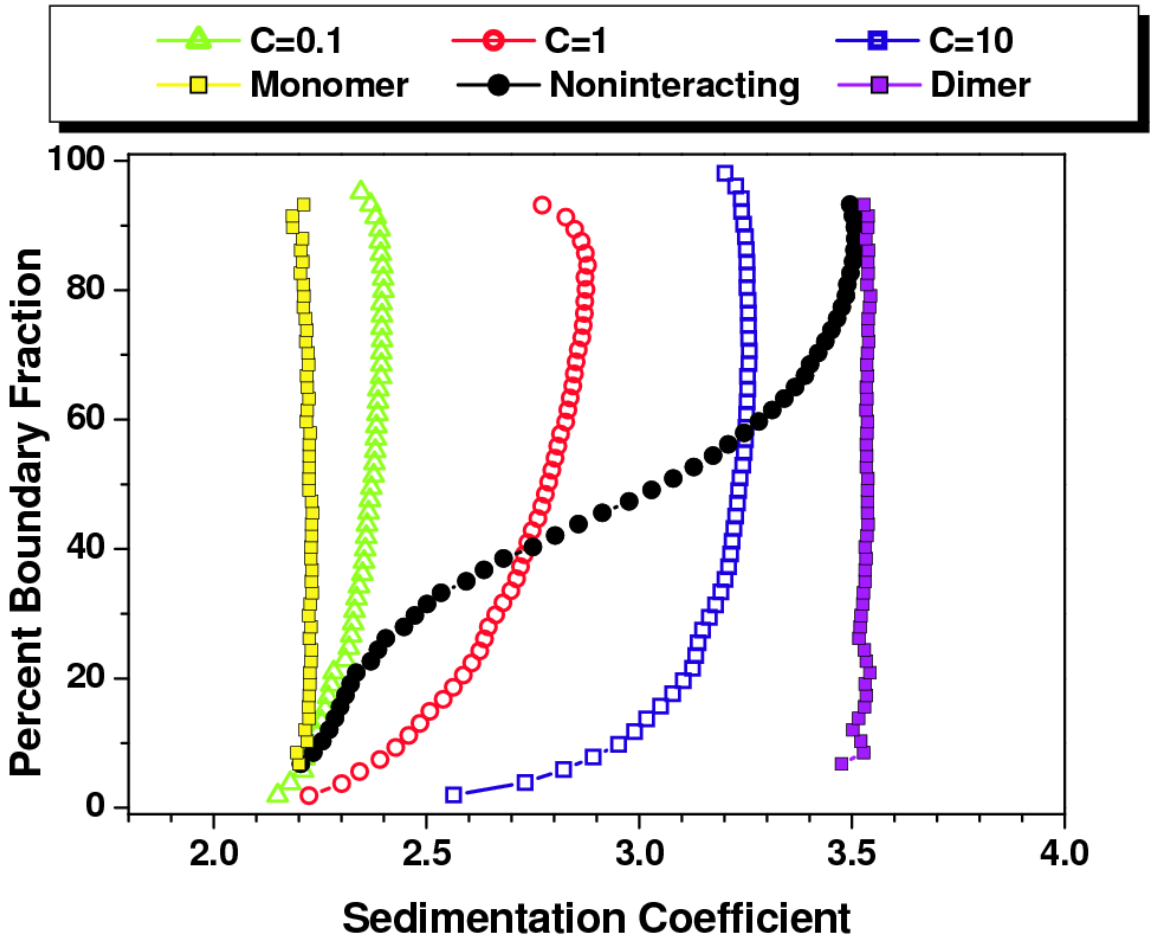




Three different Loading Concentrations for two Different Association Equilibrium Constants

Reversible Associations

Example 1: Simulated Monomer – Dimer Equilibrium

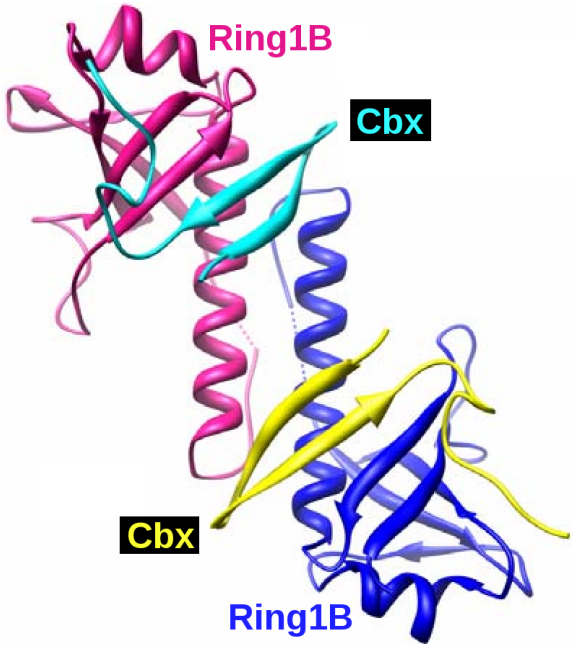


Monomer MW = 20 kDa, K_d = C x 1, k_{off}= 1x10^{-3}/sec, f/f0 = 1.25 (both)

Monomer-Dimer Interface Mutation Analysis

Example 2: Ring1B mutation analysis (Dr. Chong Kim, UTHSCSA)

Assembly of Polycomb Repression Complex 1 (PRC1) (Wang et al., 2009)
- involved in chromatin packaging and responsible for gene silencing during differentiation



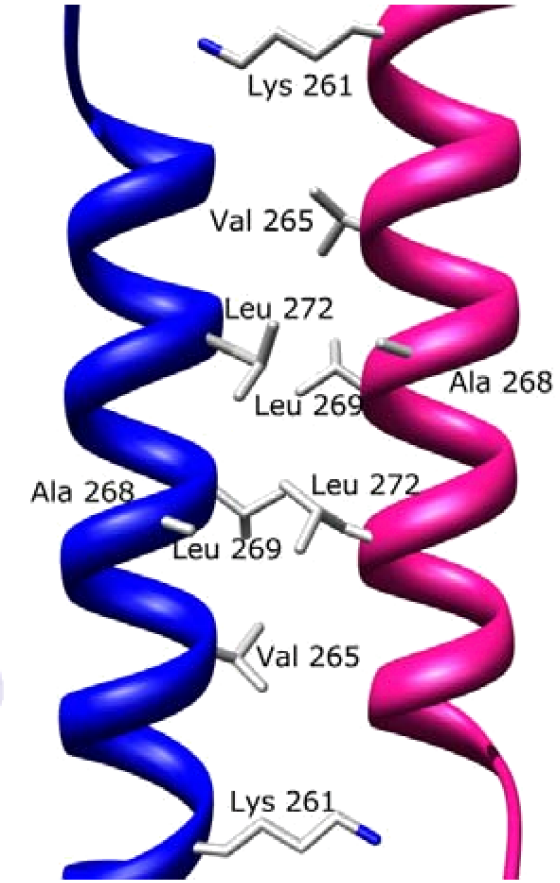
PRC1 contains 4 proteins: Ring1B, Polyhomeotic, Polycomb, and BMI1.
What is the stoichiometry in PRC1?
It is thought to be 1:1:1:1

Observations:

Ring1B binds the C-terminal domain of Polycomb, but crystallizes as a hetero-dimer. In solution without c-polycomb, Ring1B is a dimer. Is the crystal dimer interface the same observed in solution?

Monomer-Dimer Interface Mutation Analysis

Example 2: Ring1B mutation analysis (Dr. Chong Kim, UTHSCSA)

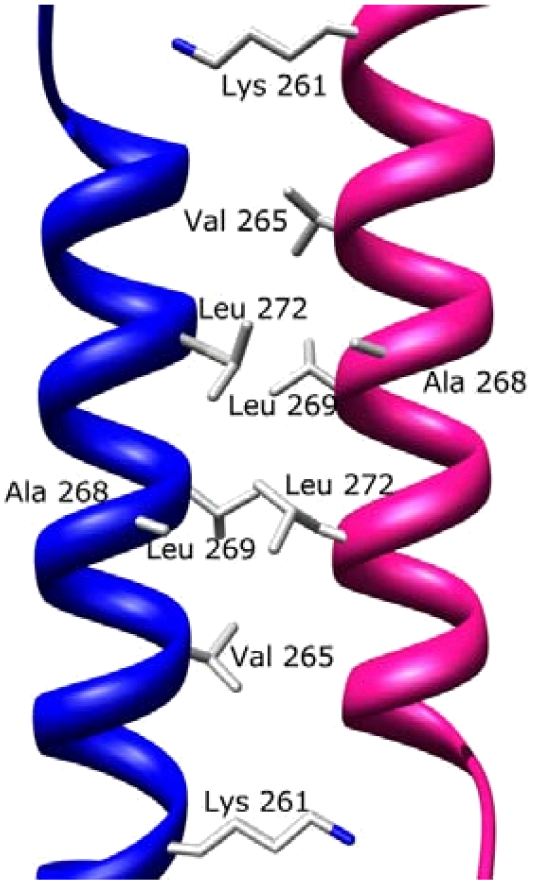


Question: Is the dimerization interface observed in crystal structure responsible for dimerization in solution?

Approach: mutate non-polar residues to charged residues to see if the dimer interface is disrupted.

Monomer-Dimer Interface Mutation Analysis

Example 2: Ring1B mutation analysis (Dr. Chong Kim, UTHSCSA)

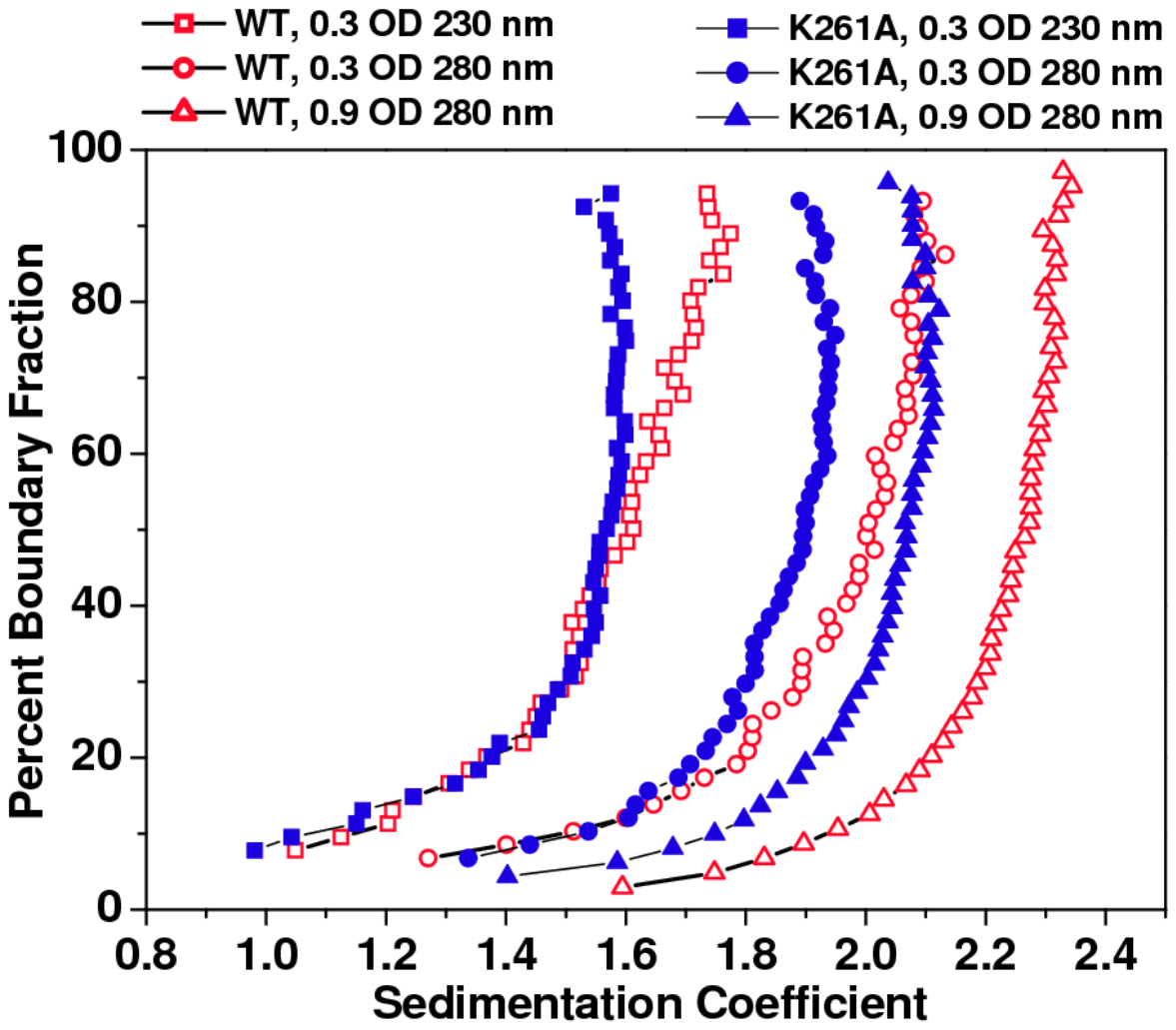


Hydrophobic residues were replaced by polar residues in dimerization study:

	Dimerizes?
Wildtype	**yes**
Val 265 Glu	**no**
Leu 269 Glu	**no**
Leu 272 Arg	**no**
Lys 261 Ala	**yes**

Answer: acidic residues seriously disrupt the dimer interface, while non-polar or basic residues have a slighter effect. But clearly the dimer interface observed in the crystal is present in solution as well.

Monomer-Dimer Interface Mutation Analysis



Self -Associating Equilibrium Experimental Design:

Always run several concentrations of your sample!

Use 3 different loading concentrations at the same wavelength

Increase concentration range by measuring at different wavelenths such as 280 nm, 230 nm and ~210 nm, check absorbance spectrum!

If interference optics are available, use them to extend concentration range.

van Holde – Weischet Applications: Summary

Model independent analysis

Initial characterization of an unknown sample

Composition analysis:
- **Homogeneous or heterogeneous?**
- **Aggregation?**
- **Relative quantification of individual components**

Qualitative information about diffusion

Identify concentration dependency:
- **Self-association or non-interacting?**
- **Reversible or irreversible?**
- **Concentration dependent solution nonideality?**

Application Examples:

van Holde – Weischet Analysis Application Examples:

- **Concentration dependent nonideality of *s*.**
- **Aggregation and irreversible self-association**
- **Composition Analysis**
- **Reversibly Self-Associating Systems vs. non-interacting systems**
- **Stoichiometry of Association**
- **Relative quantification of individual components**
- **Conformational information**