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Multi-speed Sedimentation Velocity Simulations with Ultrascan-III

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Presentation Outline

1. Background

- Multi-speed SV
- Complex biological molecules & research gap
- ASTFEM

2. Methods

a) **Simulation** – designing a speed profile

b) **Experimental design** – finite element analysis

c) **Fitting** – comparing data

3. Fitting Results

- Combined: 2DSA & GA-MC
- 2DSA-MC (individual speeds)
- Percent error (2DSA-MC & GA-MC)
- 95% CI for 2DSA-MC & GA-MC
- 95% CI comparison – single speed vs. multi speed

4. Summary

Comparison of single-speed to multi-speed experiment

Multi-speed Sedimentation Velocity – Pros and Cons

Cons

1. Beckman Proteomelab XLA or XLI AUC only records scans at a constant speed
2. Solvent compressibility

Pros

1. Exploits *S* and *D* signals for individual solutes
2. Rotor stretch factor accounted for each speed step
3. UltraScanIII detects when a solute has pelleted out of view and automatically excludes it from the fit
4. UltraScanIII applies a correction to boundary conditions for each experimental data

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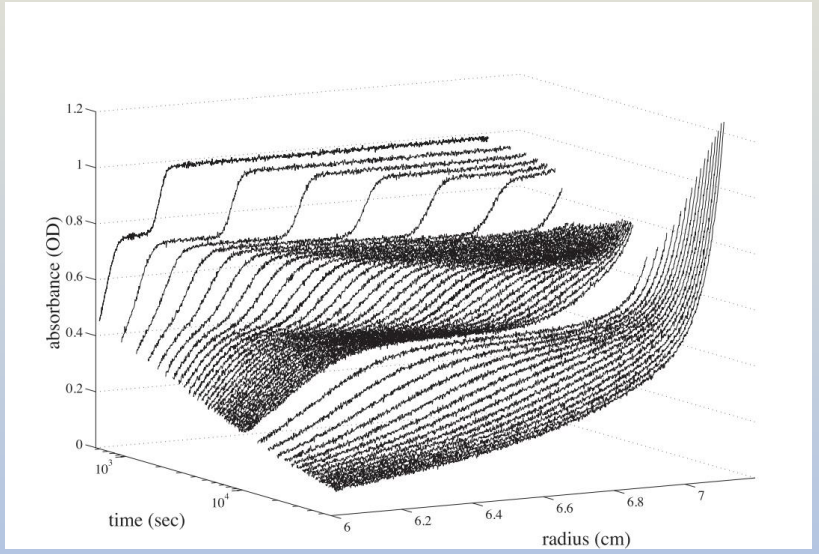
Complex Biological Molecule

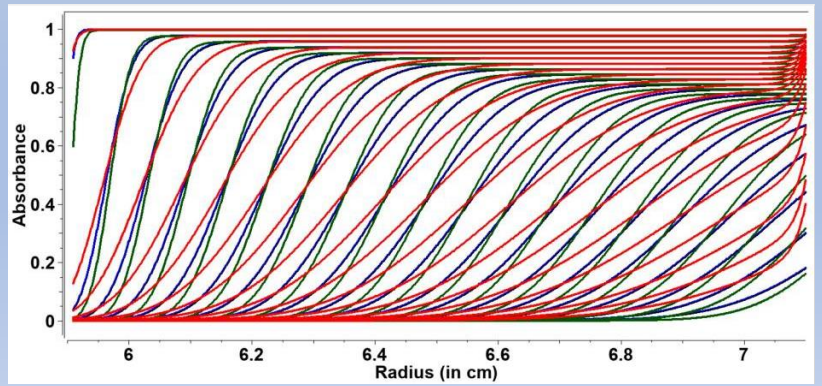
Figure 1. Chromatin self-association at increasing [MgCl2]

Research gap

Single speed SV



multispeed SV



Heterogeneous samples may exhibit unique sedimentation coefficients and move at different speeds in different environments. Thus, their composition cannot be resolved at only one speed!

Adaptive Space-Time Finite Element Solution (ASTFEM)

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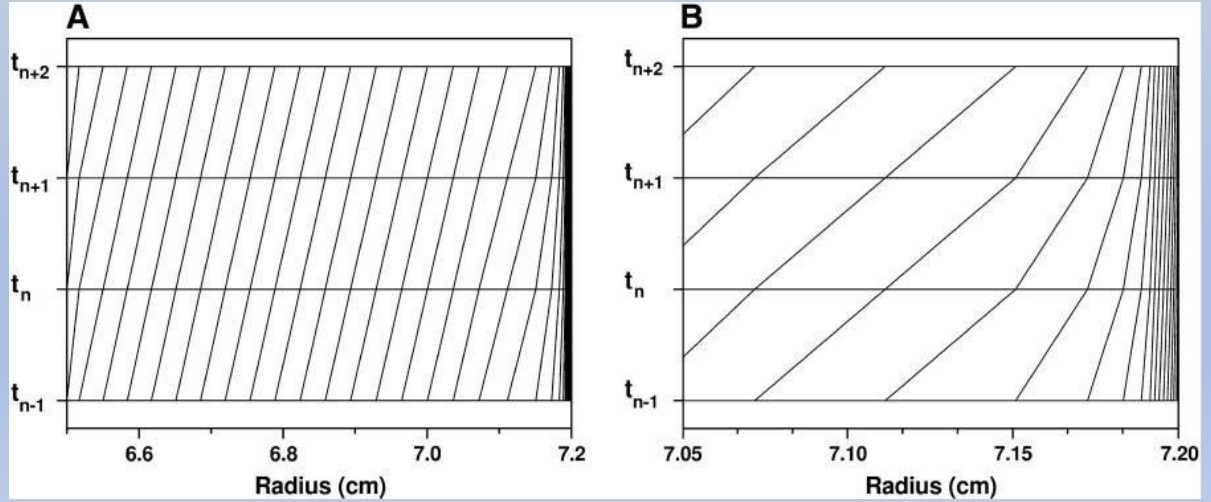
AUC experiments are modelled by the finite element solution of the Lamm equation

$$\left(\frac{\partial C}{\partial t}\right)_r = \frac{-1}{r}\frac{\partial}{\partial r}\left[s\omega^2 r^2 C - D r \frac{\partial C}{\partial r}\right]_t$$

Concentration | Sedimentation | Diffusion

ASTFEM is a major improvement from its counterparts (e.g., the Moving hat finite element method by Schuck et. al)

ASTFEM removes oscillation at the bottom by using an adaptive grid



ASTFEM grid distribution for the entire cell (A) and at the bottom of the cell (B)

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a) Simulation

Aim: To design a speed profile for heterogeneous solutes

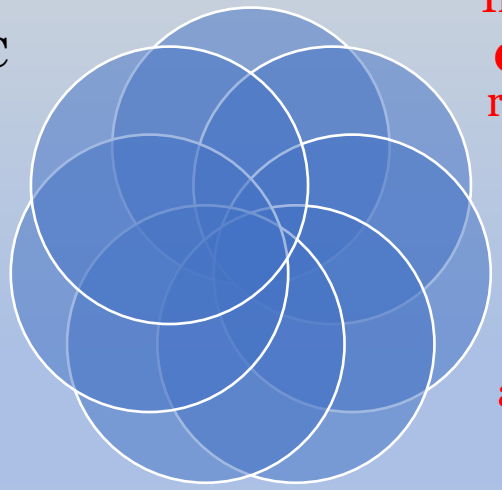
5 distinct solutes with different anisotropies

Density and viscosity of water at 20°C

Increasing speeds

Control 15,000 rpm single-speed experiment

Rotor stretch added for each speed step



Speed step using acceleration time delay

Artificial stochastic noise of 0.4% of total concentration

Finite element solution simulated with ASTFEM

Parameter/Solute	Solute 1	Solute 2	Solute 3	Solute 4	Solute 5
s ($\times 10^{13}$ sec)	1.0111	2.3464	7.2608	25.276	93.858
D ($\times 10^{7}$ cm^2/sec)	18.862	4.3774	1.3545	0.47154	0.17509
Molar mass (Da)	5.0×10^3	5.0×10^4	5.0×10^5	5.0×10^6	5.0×10^7
f/f_0	1.0	2.0	3.0	4.0	5.0
Absorbance	0.2	0.2	0.2	0.2	0.2
$\bar{\nu}$(ml/g)	0.74	0.74	0.74	0.74	0.74

Simulation speeds and durations.

Speedstep:	1	2	3	4	5
Rotor speed (rpm):	3,800	7,500	15,000	30,000	60,000
Duration (hh:mm)	37:13	25:56	22:03	16:10	07:54
Delay (minutes):	0.167	0.167	0.317	1.875	1.875

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b) Experimental design

Aim: To generate a model for all possible *S* and *D* in the solution

$$t = \ln\left(\frac{b}{m}\right)\frac{1}{s\omega^2} \quad \text{Equ. 1}$$

Determining the theoretical duration for a multi-speed AUC SV experiment

Speed: determine *S* value distribution using a single-speed experiment

- The theoretical **duration**, *t* is set to the time required for the midpoint to reach past the cell bottom
  - Equ. 1 is used to find all other *t* values.

Each species acquires a new *m* from each speed step

```
int next_rpm (int);
double calc_time(int, double);
double calc_b   (double, int, double);
double omega_s  (int);
double m, meniscus=5.9, b=7.2, largest_s=4e-12;
int first_speed=6000, rotor_max=50000;
```

Figure 5. C++ program to predict appropriate rotor speed and experimental duration

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c) Fitting – UltraScanIII

Aim of fitting: to compare experimental data with simulated data

UltraScanIII separates each speed step into a separate experimental dataset

Pseudo-global analysis (addresses limitation)

1. Each speed step is analyzed individually over the expected sedimentation and anisotropy range
2. Merged into a global model
3. Parameters are refined for each speed step

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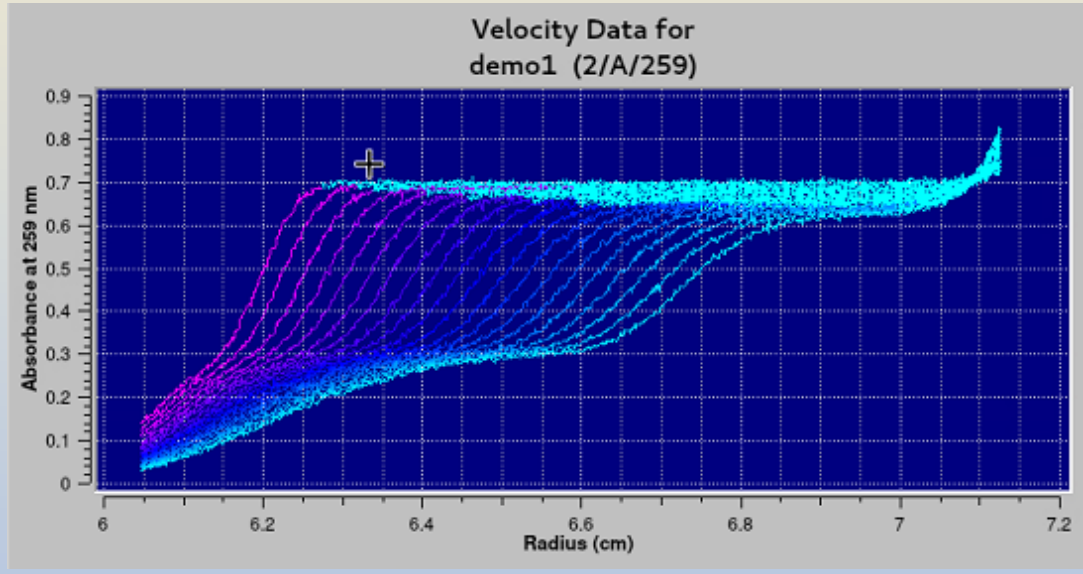
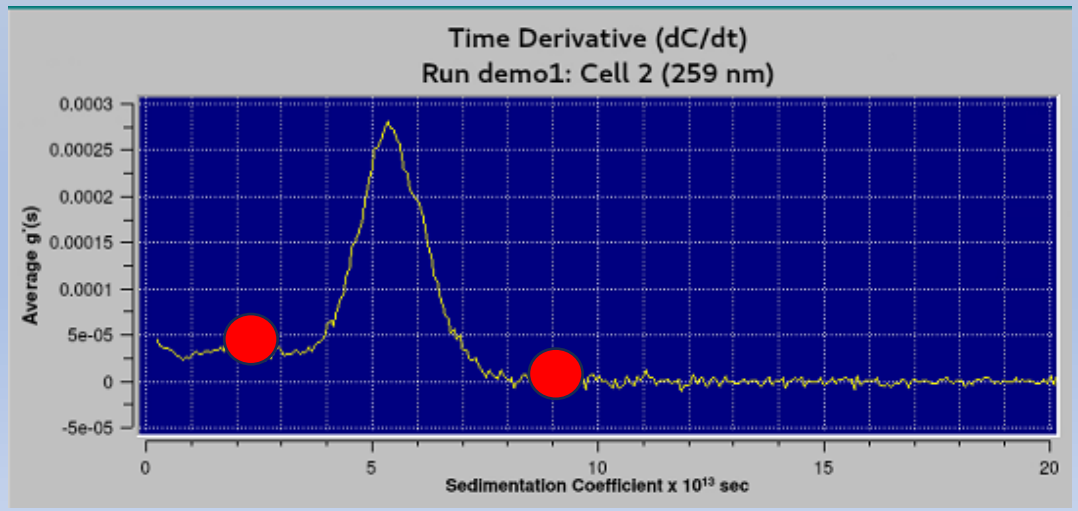
c) Fitting – Time Derivative Method

1. **Identify the maximum *S* value for the range to be fitted**
   a. The upper *S* limit is estimated by taking a group of scans at the end of the first speed step
   b. Cut-off**:** where the time-derivative distribution approaches zero on the high end of the sedimentation coefficient spectrum

Before 2-DSA: Choose f/f_0 based on knowledge about the solutes





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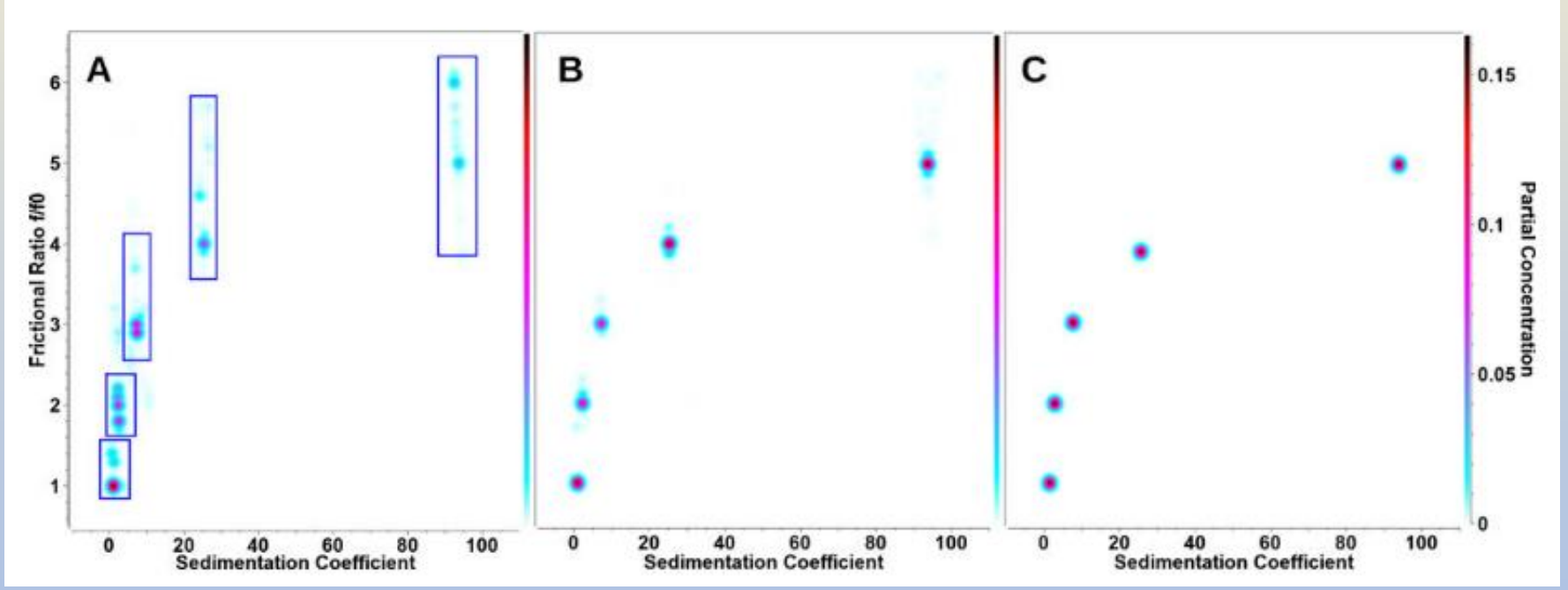
Result 1 – 2-DSA and GA-MC

Combined 2DSA & GA-MC result



Pseudo-3D representation

Results

1A) Determine solute regions **(2DSA-MC)**

1B) Species with the smallest 95% confidence limit shown from manually combined solutes in 1A **(2DSA-MC)**

1C) GA-MC for discrete species from each speed step

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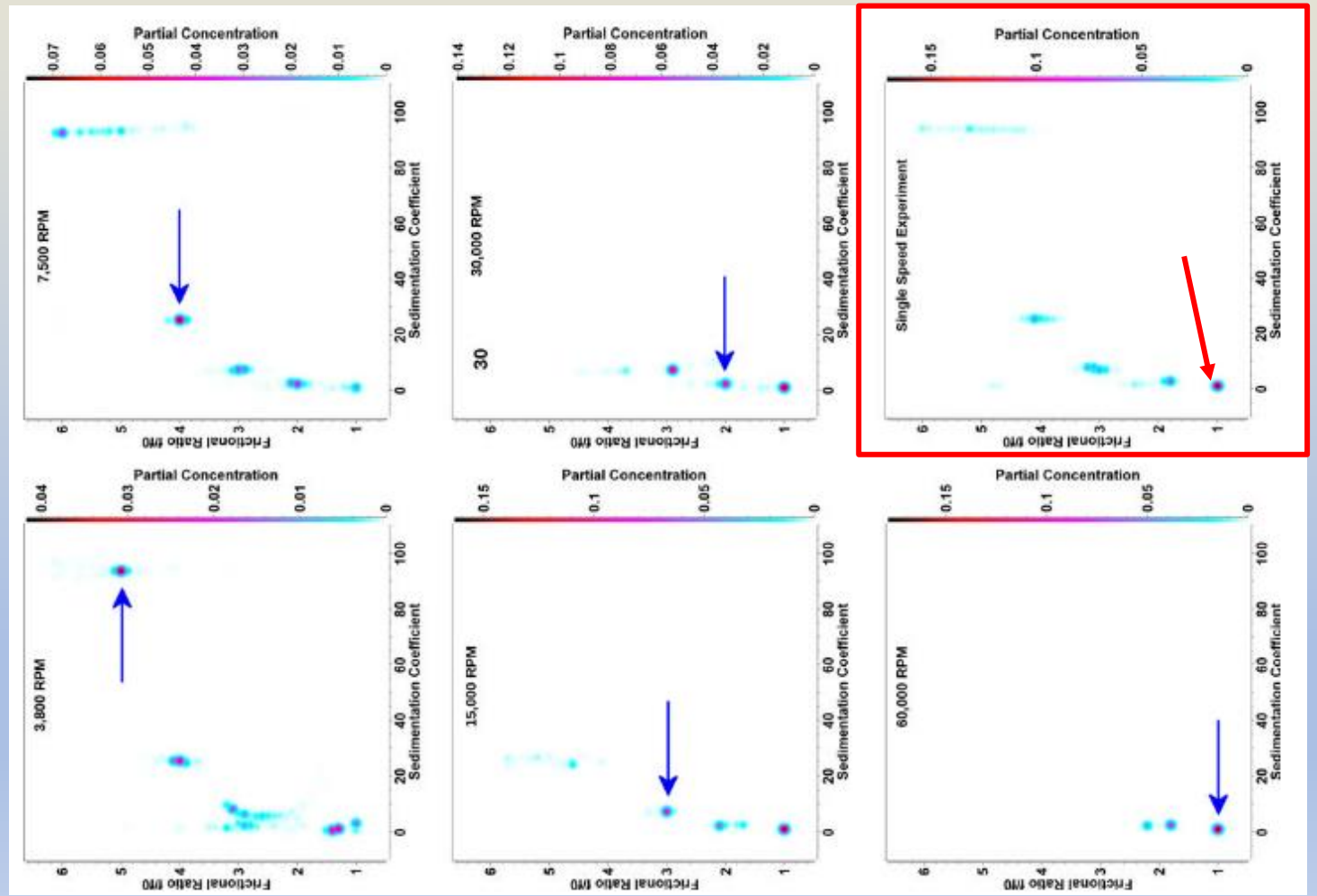
Result 2 – 2DSA-MC for each speed step

Purpose
Time - and radially invariant noise removal

Result
Each solute is resolved at a different speed step

Partial concentration of solutes at each speed – if present, how much of a solute is available at each speed



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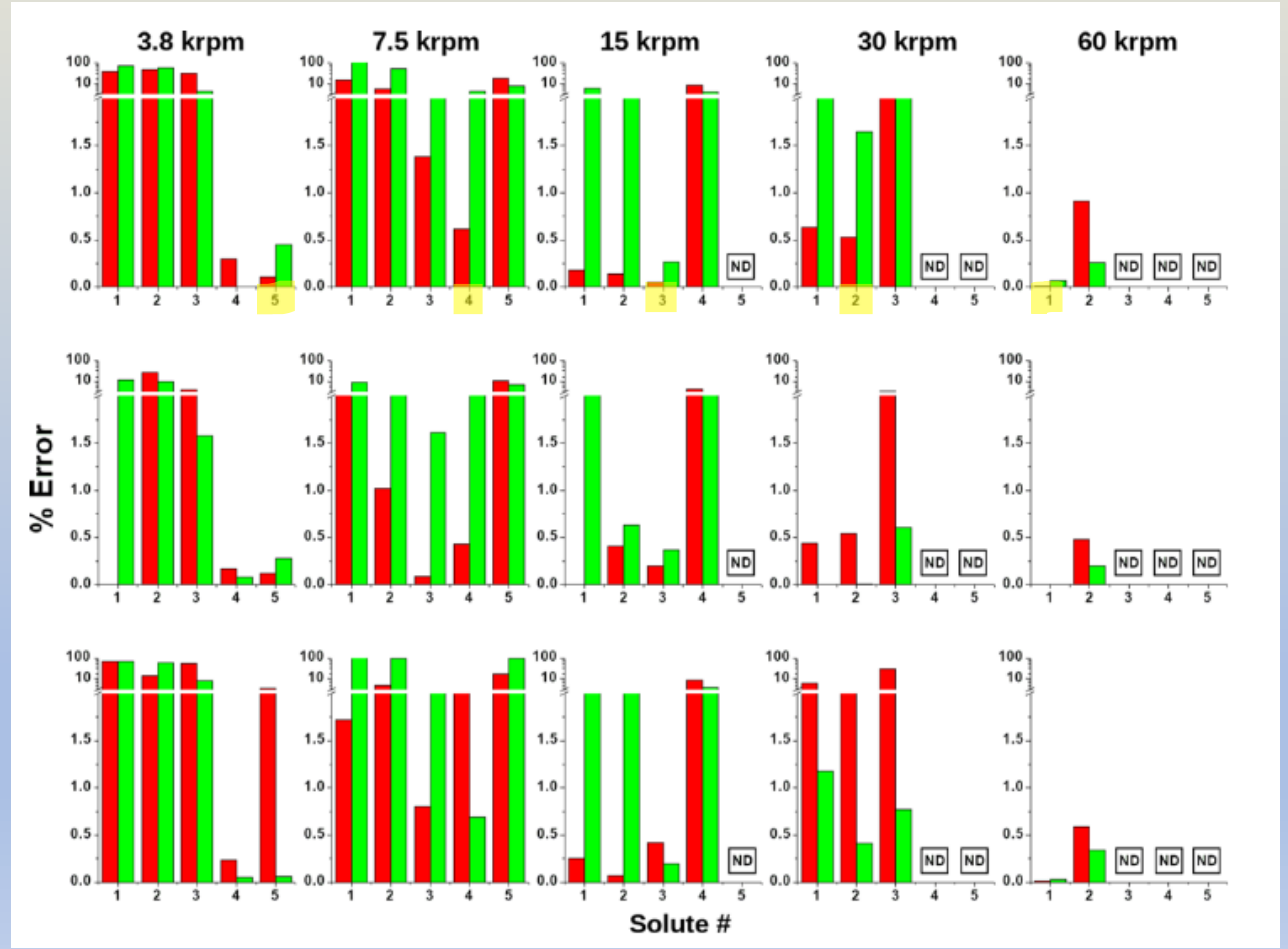
Result 3 – Percent error 2DSA-MC & GA-MC

Result

Generally, GA-MC illustrates a lower percent error than 2DSA-MC for the resolved solute at each speed step

At each speed step, only one solute procures a <1% percent error

$$\%Error = 100\% \cdot \left[\frac{\sqrt{(X_e - X_m)^2}}{X_e} \right] \quad \text{Equ. 4}$$



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Result 4 – 95% CI for 2DSA-MC & GA-MC

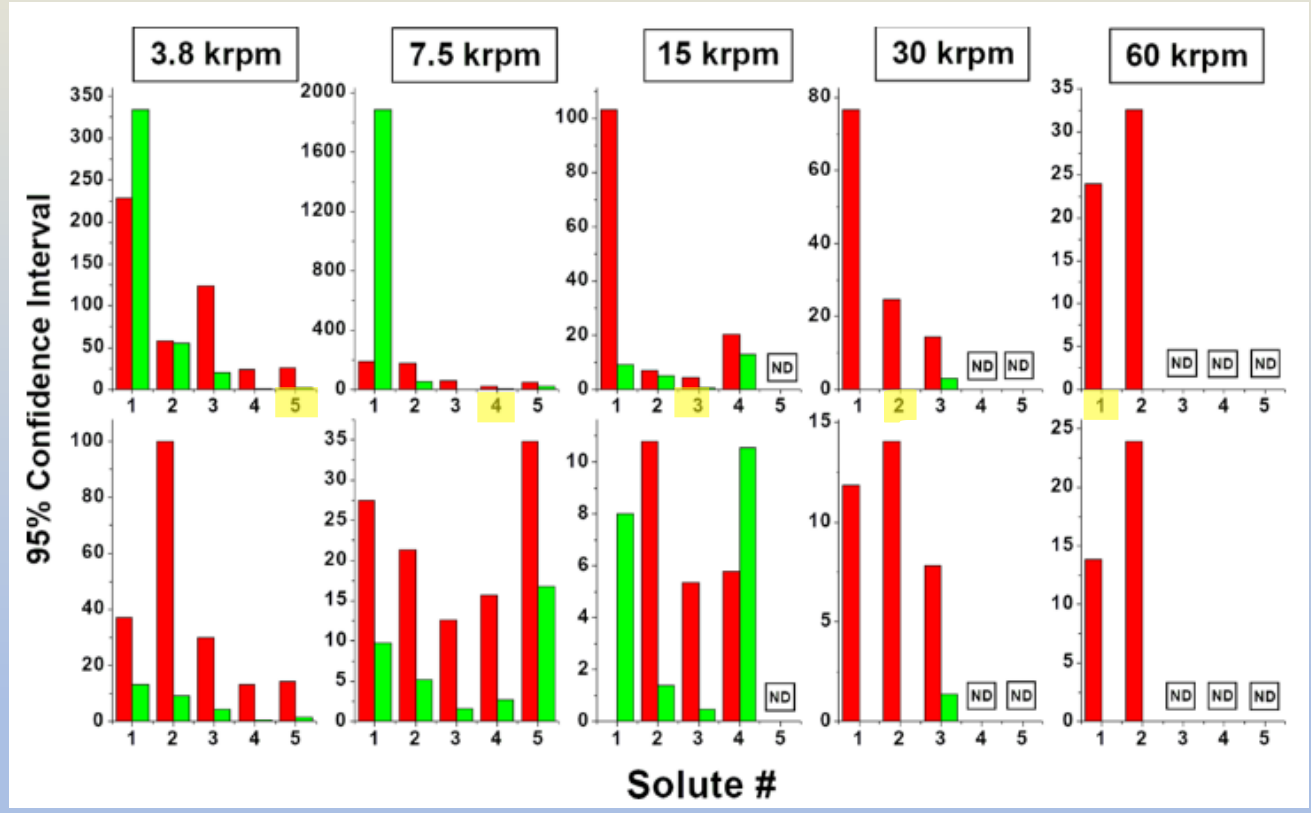
Purpose

Increases confidence that values will fall between the upper and lower values for the CI

Corroborates the percent error and two-dimensional analysis methods used

Result

Low 95% CIs are observed for GA-MC and 2DSA-MC for each speed step.



$$95\%CI\ Error = 100\% \cdot \left[\frac{\left(\sqrt{(X_e - X_{95l})^2} + \sqrt{(X_e - X_{95h})^2} \right)/2}{X_e} \right] \quad \text{Equ. 5}$$

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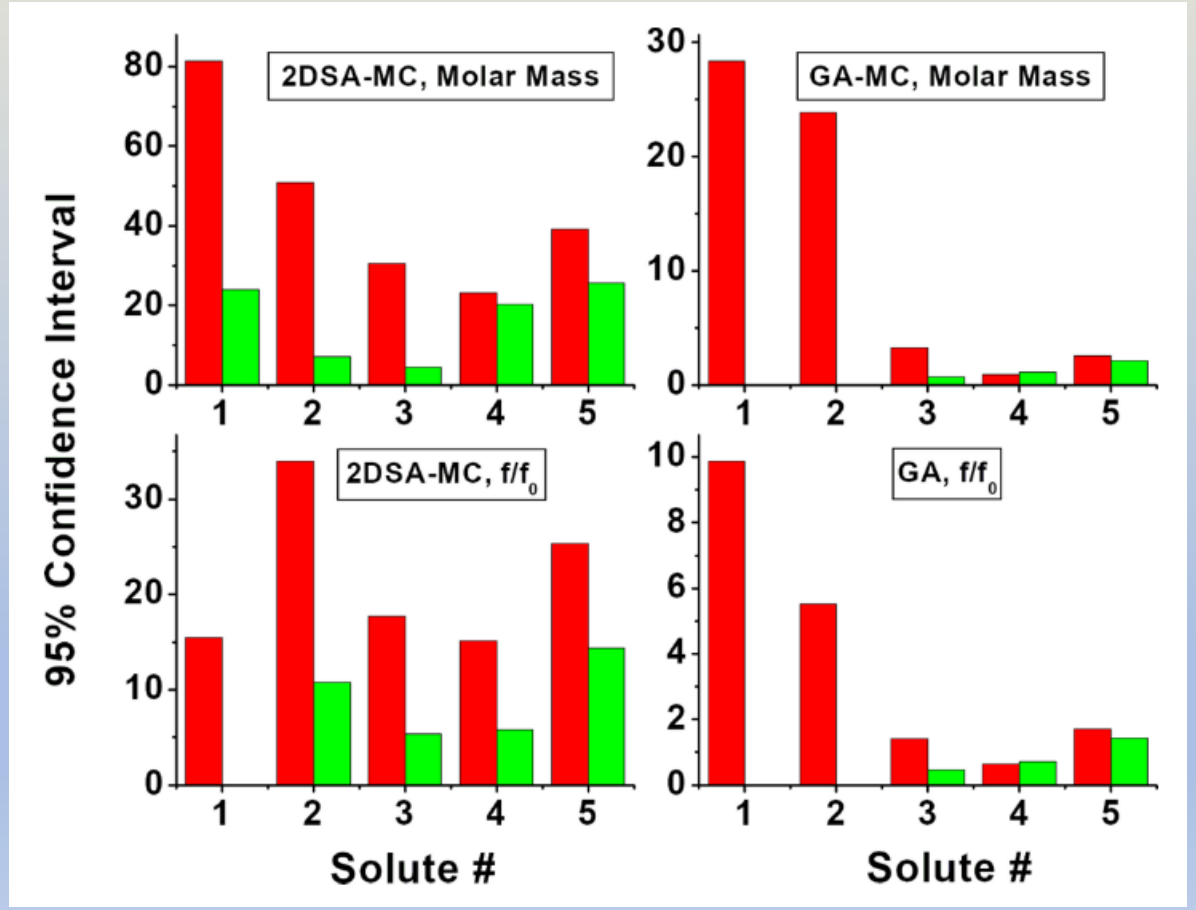
Result 5 – 95% CI comparison of single-speed to multi-speed

Red – single speed **Green – Multi speed**



Conclusion

Multi-speed experiment showed good statistics results compared to the single-speed experiment

2DSA – 54% better

GA-MC – 60% better

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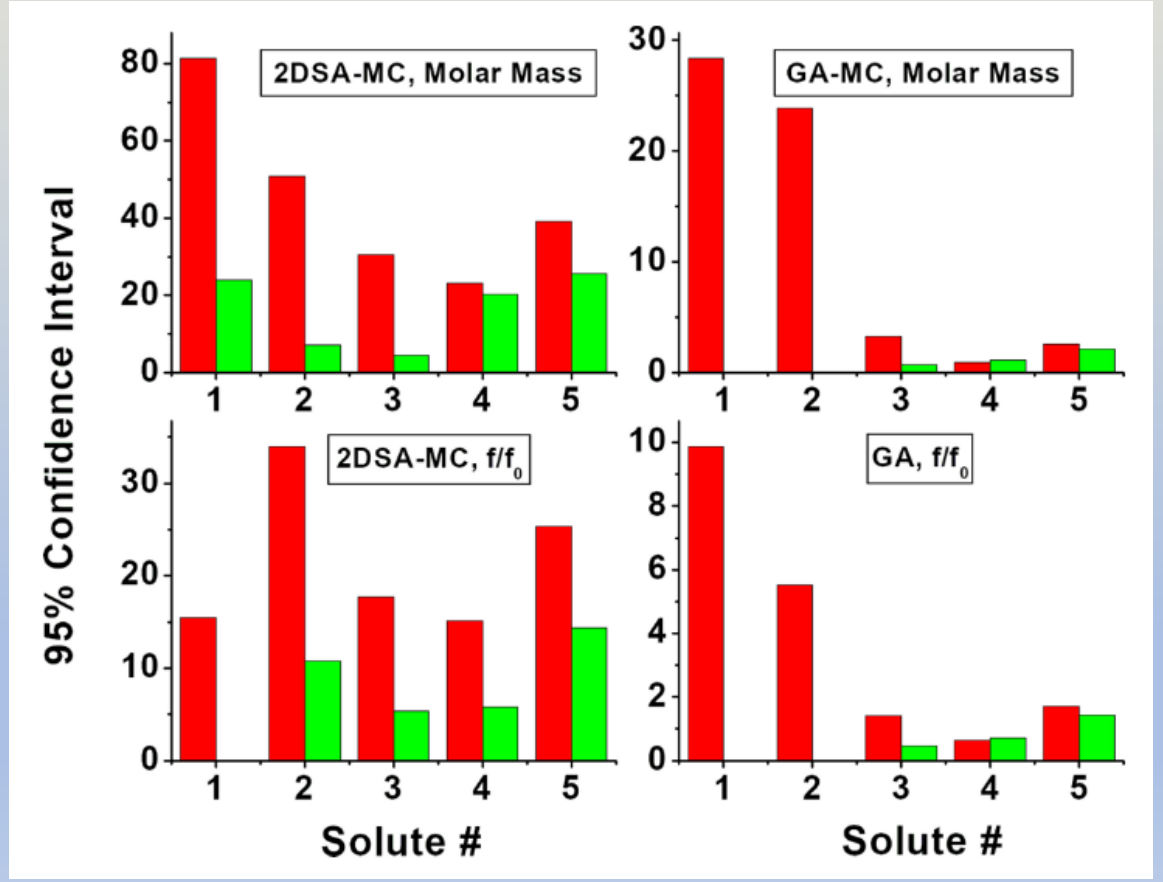
- The single-speed SVE did not procure confident MW and f/f_0 results for heterogeneous solutes
- However, heterogeneous solutes can be resolved individually using different rotor speeds and duration based on sedimentation coefficients
- GA-MC and 2DSA-MC multi-speed SVE results procured significantly lower 95% CI for each solute resolved at different speeds than the single-speed experiment
- Therefore, multi-speed SVE is a reliable approach to resolve non-interacting heterogeneous solutes



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Questions