

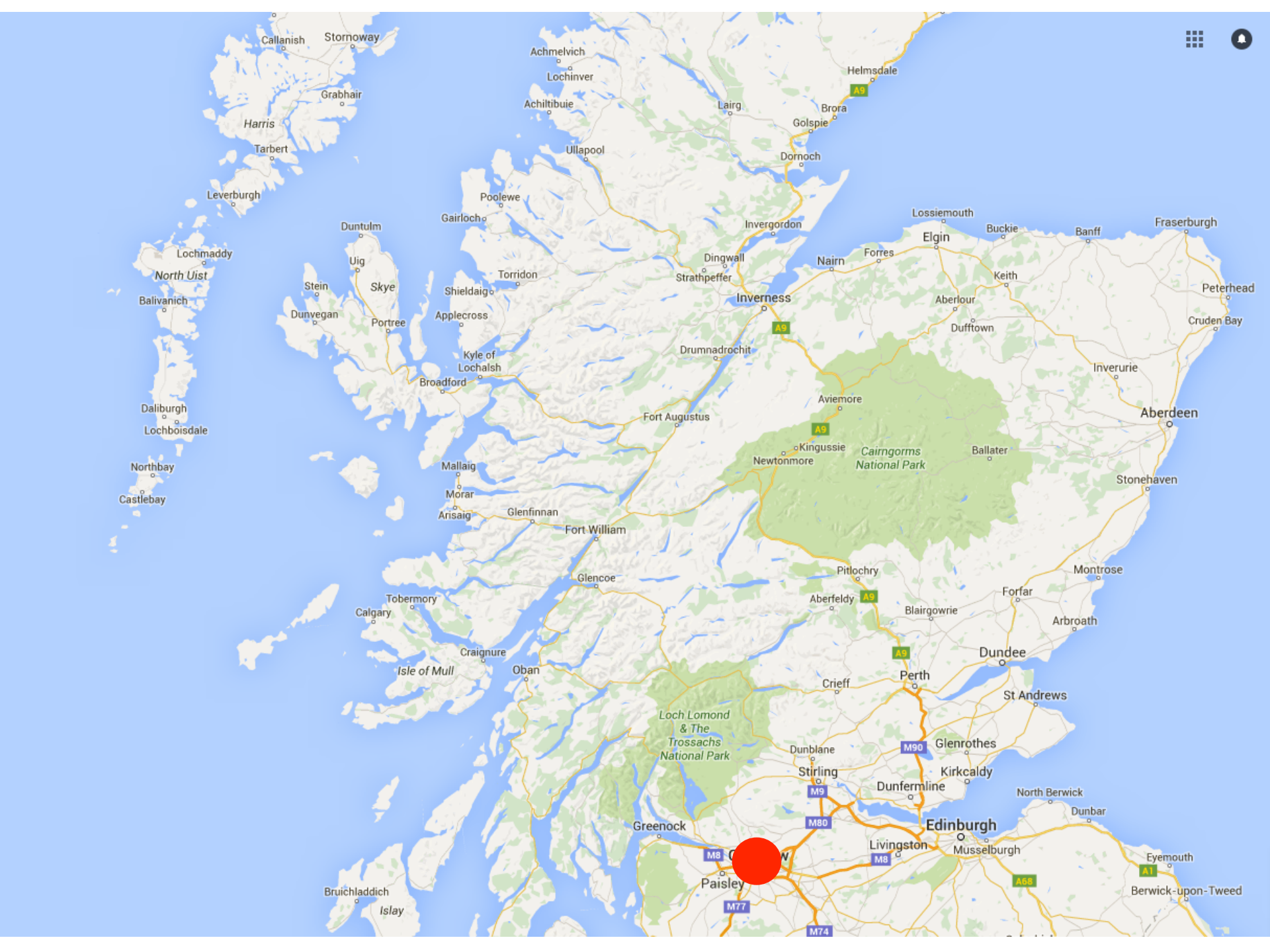
Analytical ultracentrifugation

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Outline

- AUC background
- How AUC experiments are performed
- Data analysis
- Example: simple model-independent investigation of a hetero-association
- Detergent solubilised systems
- Hydrodynamic bead modelling (HBM)
- Example: oligomerisation of synthetic polyvalent integrin $\alpha_5\beta_1$ ligands

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Questions that can be answered by AUC

- Is sample heterogeneous?
 - in molecular weight, shape, or both?
 - does heterogeneity depend on pH, salt, buffer, etc?
- Is sample pure enough for MX, SAXS, SANS, NMR?
- What is sedimentation & diffusion coefficient?
 - Globular or unfolded/disordered?
 - Is conformation dependent on salt, pH, ligand concentration, deuteration, tagging, mutations etc?

Questions that can be answered by AUC

- Does sample...
 - ...self-associate?
 - ...aggregate?
- What is M of sample?
- Does sample bind to a ligand?
- What is stoichiometry of binding?
- What is K_d ?
 - Is K_d affected by salt, pH, ligand concentration, deuteration, tagging, mutations etc?
- Is sample affected by crowding?



Advantages of AUC

- In solution
- Non-destructive
- Self-cleaning
- Absolute
- Complementary
- Can analyse (nearly) anything
 - Proteins
 - Nucleic acids
 - Carbohydrates
 - Polymers
 - Colloids
 - Complexes



AUC: a high speed preparative UC with optics

- Choice of 3 instruments

- Beckman Coulter

- ProteomeLab XL-A/XL-I
 - Optima AUC

- <https://www.beckman.com/centrifuges/analytical-ultracentrifuges>

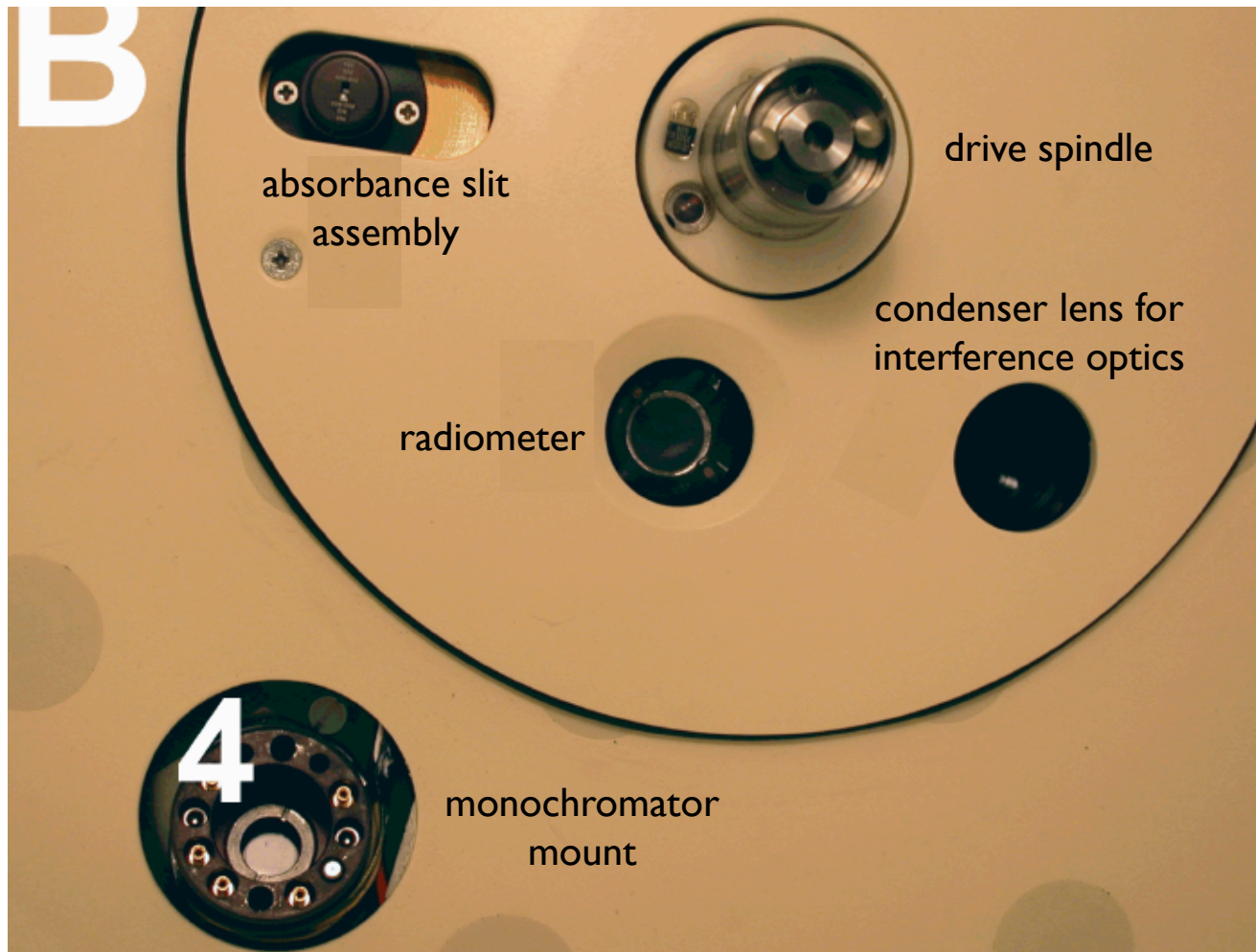


- Spin Analytical CFA

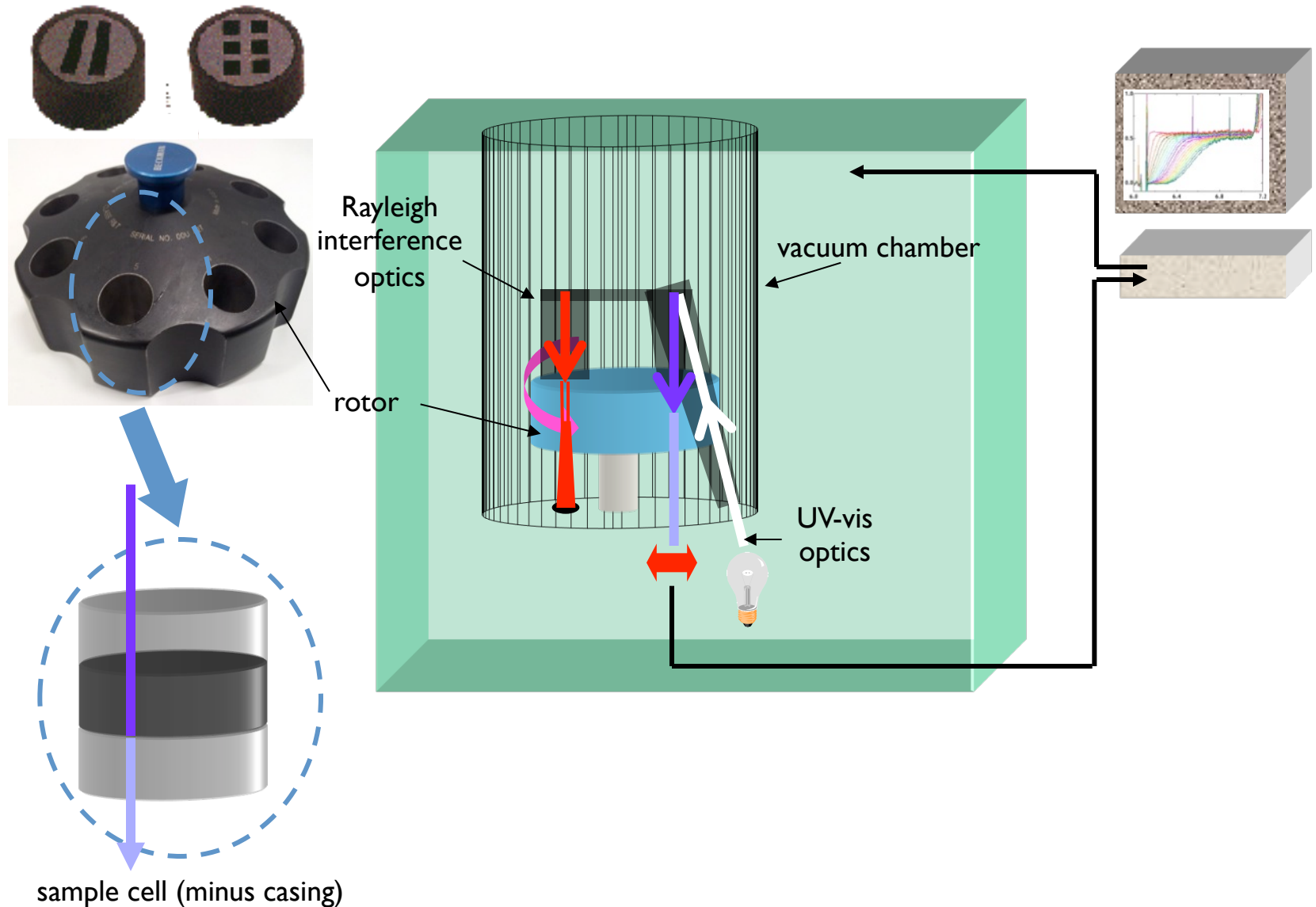
- <http://www.spinanalytical.com/cfa.php>



Inside the Beckman Coulter XL-I rotor chamber



Inside the Beckman Coulter XL-I



Relationship between data and sample

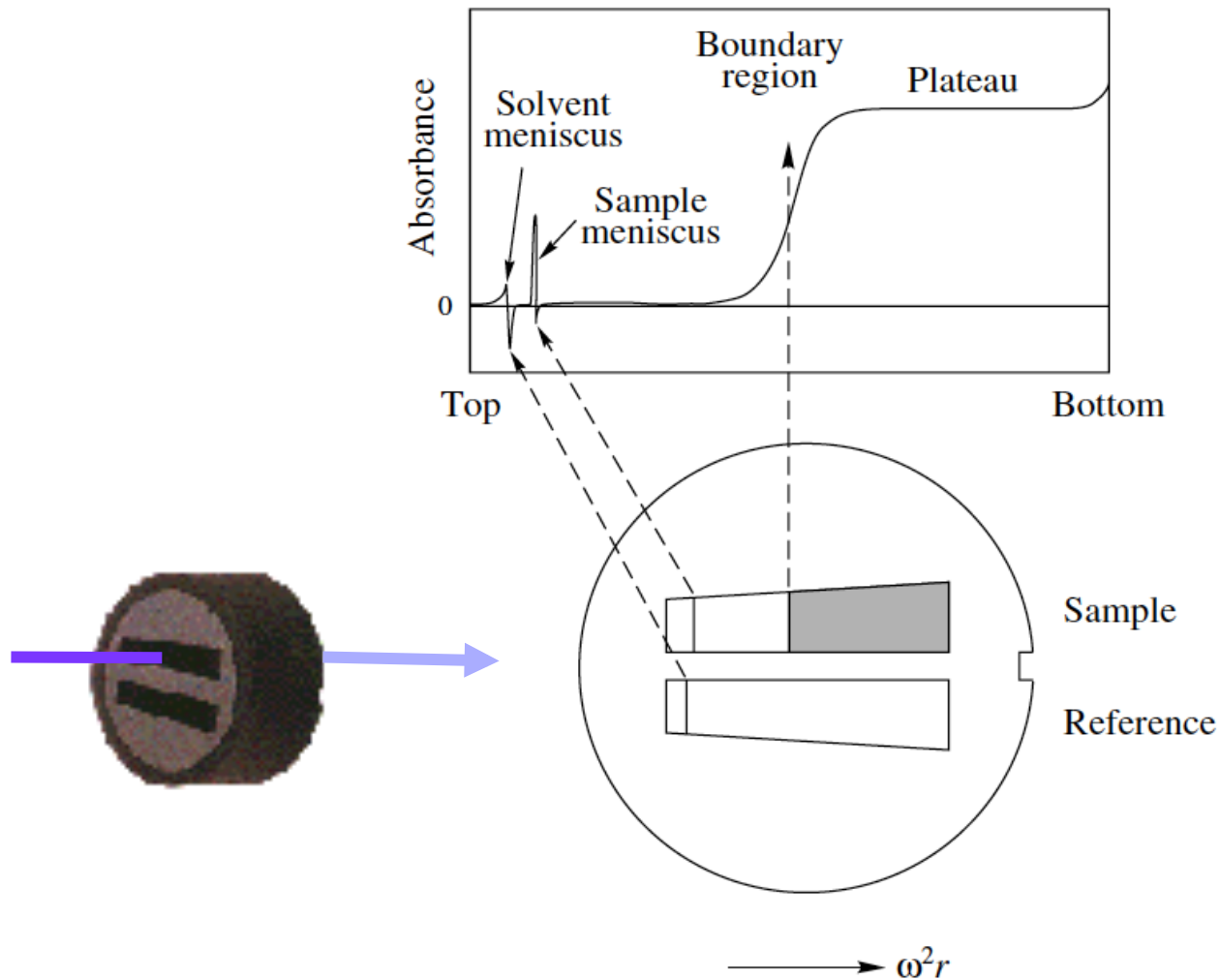


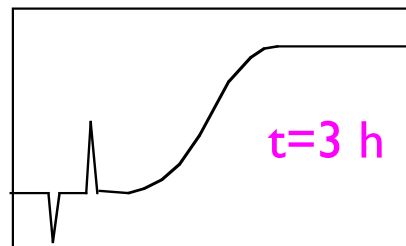
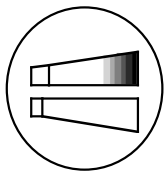
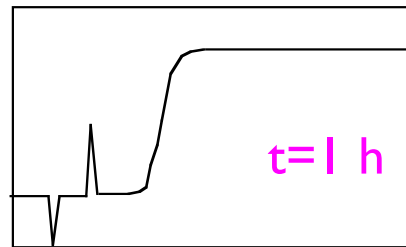
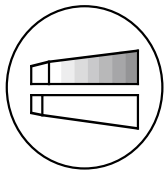
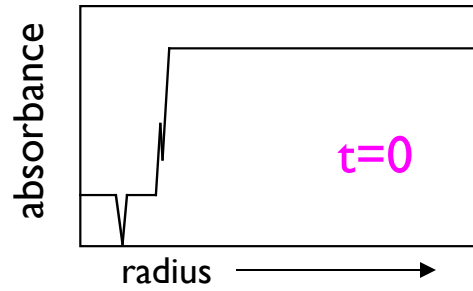
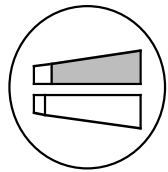
image from Ralston, 1993

<https://www.beckman.com/centrifuges/analytical-ultracentrifuges/proteomelab-xl-a-xl-i>

Outline

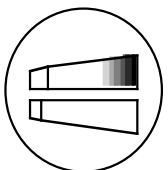
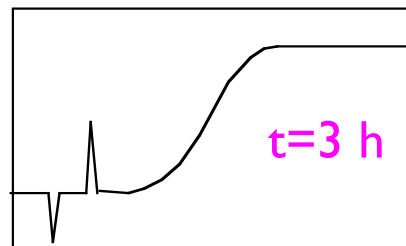
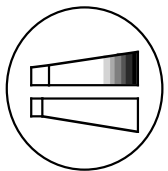
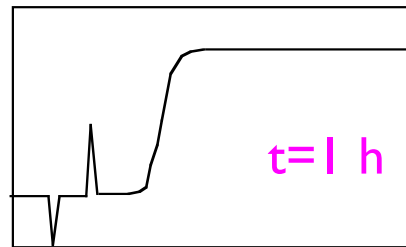
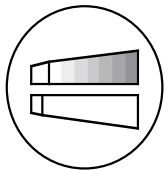
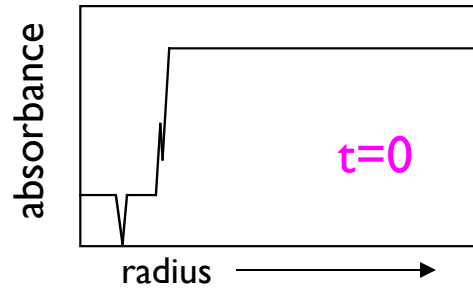
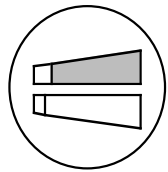
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Sedimentation velocity (SV): shape & homogeneity



heterogeneity determination
sedimentation (s) & diffusion (D)
coefficients (shape)
association/dissociation constant (K_a/K_d)
stoichiometry

Sedimentation equilibrium (SE): mass & self-association



M
association/dissociation constant (K_a/K_d)
stoichiometry
non-ideality (B)

Which optical system?

	Absorbance	Interference	Fluorescence
Lowest conc	$A_{\lambda} = 0.1$	0.05 mg/ml	100 pM _{fluorescein}
Dynamic range	2-3 logs	3-4 logs	6-8 logs
Radial res'n (μm)	20-50	10	20-50
Scan time (s/cell)	≈ 60	1	≈ 15
Utility	• Selectivity • Sensitivity • Non-dialysables	• Buffer absorbs, sample doesn't • Variable extinction coefficient • Short column equilibrium • Rapid sedimenters	• Selectivity • Sensitivity • Non-dialysables • Limited sample

Sample preparation

- Purify by gel filtration or similar
 - Unless you want to know what is in solution in its entirety
- Estimate concentration
 - Using e.g. NanoDrop
- (Dialyse sample against the desired solvent)
 - Possible problems with detergents
 - Required for interference optics only
- Choose windows
 - Sapphire windows
 - Necessary for interference optics
 - Good for all AUC optics
 - Quartz windows
 - No good for interference



Sample requirements

- **Sample volume**

- SV
 - 360 μ l (12 mm pathlength)
 - 90 μ l (3 mm pathlength)
- SE
 - 80 μ l (2- or 6-channel centrepiece)
 - 20 μ l (8-channel centrepiece - interference optics only)

- **Sample concentration**

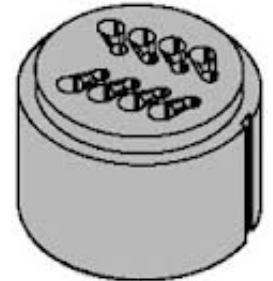
- Absorbance optics: $A_{\lambda} \approx 0.1-1.0$ (12 mm pathlength)
 - $\lambda = 180-800$ nm
- Interference optics: typically 0.05-30 mg/ml

- **Sample reference**

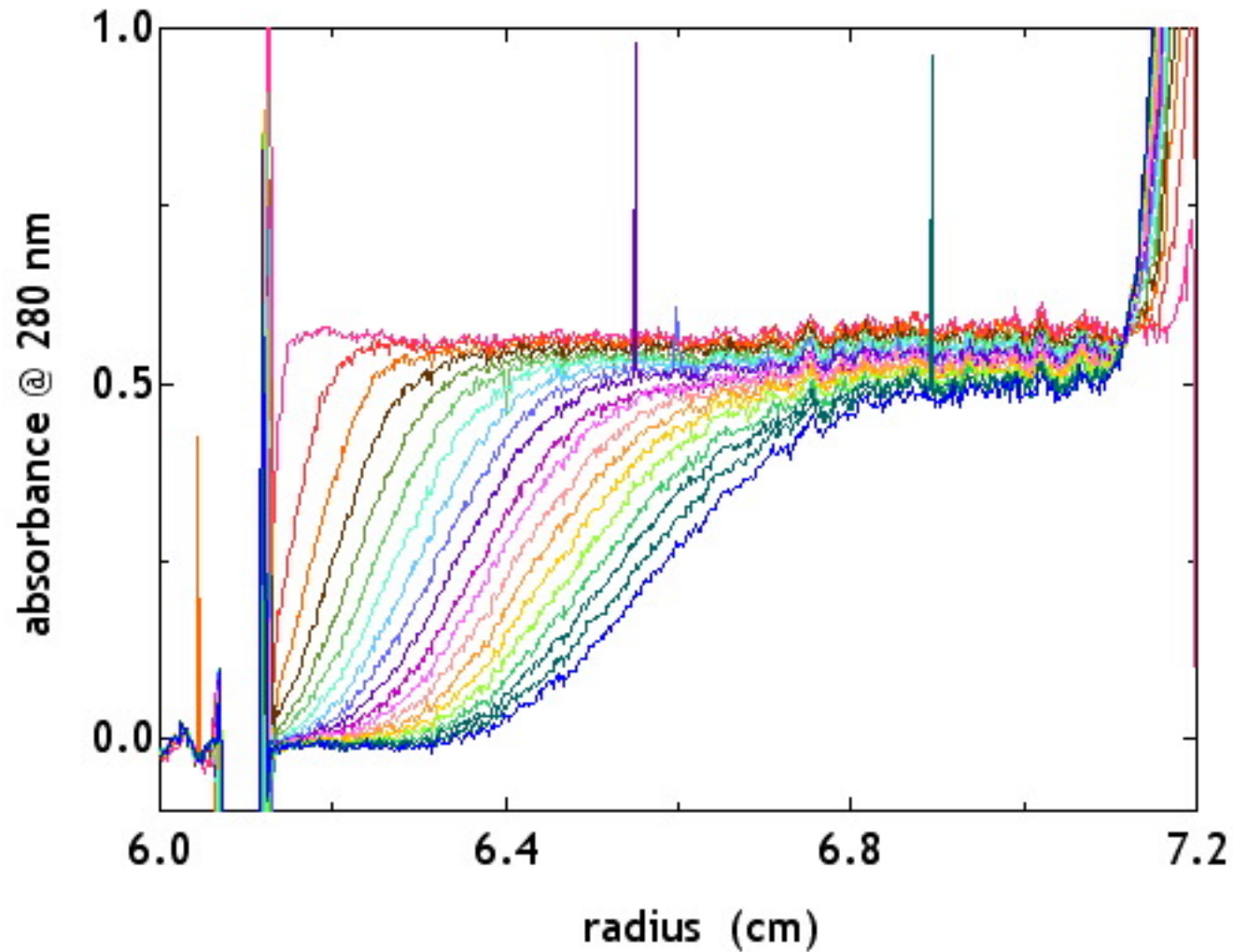
- Absorbance optics: can be column eluant or dialysate better
- Interference optics: must be dialysate

- **Typical multiplexing: 3 or 7 sample holders (“cells”)/run**

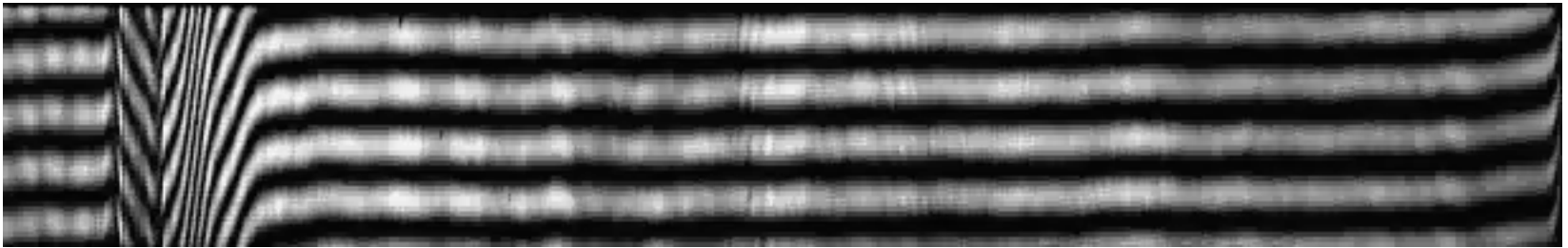
- Up to 28 samples per run



SV: radial movement recorded as function of time

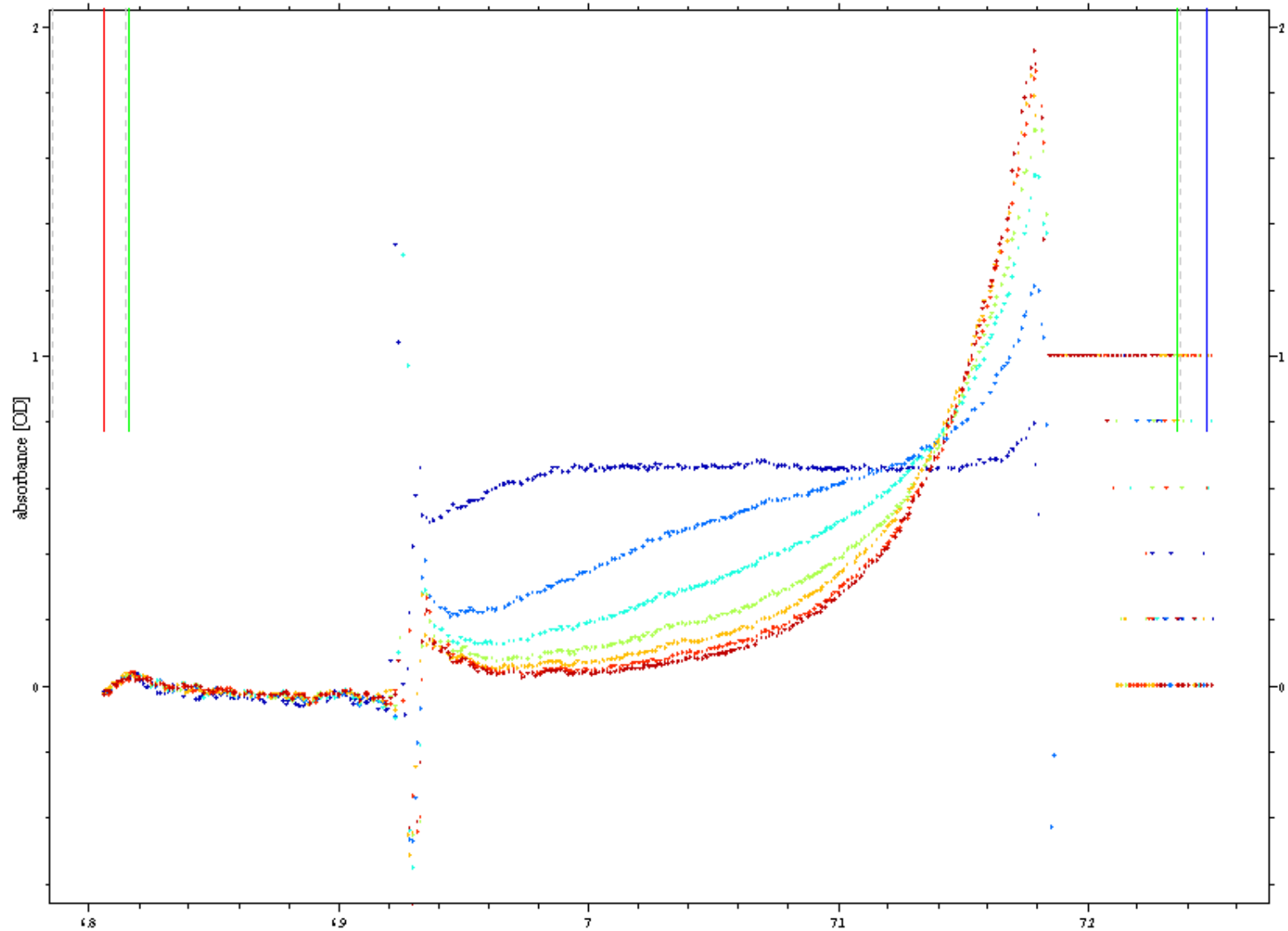


Interference optics acquire refractive index data rapidly, independent of chromophores



hemocyanin sedimentation observed with interference optics

SE: data recorded until no change...

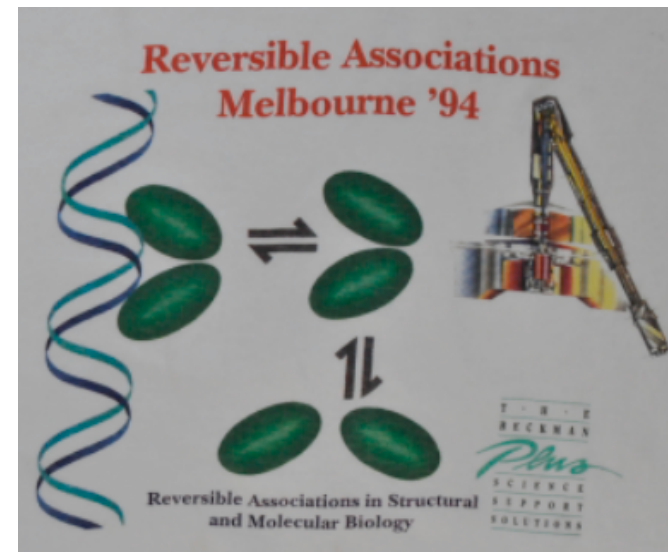


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Almost all AUC data analysis software is freely available – here are the most widely used

- The RASMB website
 - “Reversible Associations in Structural and Molecular Biology”
 - <http://www.rasmb.org/>
 - Access to freely available software
 - Subscription to AUC-related discussion group
- Schuck lab (SEDFIT, SEDPHAT)
 - <http://www.analyticalultracentrifugation.com/default.htm>
- Demeler lab (UltraScan III)
 - including US-SOMO
 - <http://www.ultrascan.uthscsa.edu/>



SV important equations

- The Lamm equation describes SV

$$\frac{\partial c}{\partial t} = -\frac{1}{r} \frac{\partial}{\partial r} \left[r \left(cs\omega^2 r - D \frac{\partial c}{\partial r} \right) \right]$$

- s is particle velocity per unit centrifugal field

$$s = \frac{v}{\omega^2 r}$$

- Svedberg equation relates s , D and M

$$s = \frac{D}{RT} M(1 - \bar{v}\rho)$$

- Stokes-Einstein equation

$$D = \frac{RT}{N_A f} = \frac{kT}{6\pi\eta R_s}$$

- Combining Svedberg with Stokes-Einstein

$$s = \frac{M(1 - \bar{v}\rho)}{N_A 6\pi\eta R_s} = \frac{M_b}{N_A f}$$

s is influenced by solvent density & viscosity and sample partial specific volume (psv or $\bar{v}$)

sample partial
specific volume
 $\approx 1/\text{density}$

solvent density

solvent viscosity

$s_{20,w}$

$$= \frac{(1 - \bar{v}\rho)_{20,w}}{(1 - \bar{v}\rho)_{T,b}} \frac{\eta_{T,b}}{\eta_{20,w}} s_{T,b}$$

$s_{T,b}$

sedimentation coefficient
standardised to solvent of
water @ 20°C

≈ 1.5 for typical aqueous
solvent at 4°C

experimental sedimentation
coefficient determined in e.g.
buffer (b) at T°C

SEDNTERP: Calculation of ρ , η & p_{sv}

Sednterp/Applications/Sednterp.app/Contents/MacOS/./Database/sednterp.db

☒ **Calculate Buffer Density**

Density Density Corrected for Temperature & Isotopes of Water

☒ **Calculate Buffer Viscosity**

Viscosity Viscosity Corrected for Temperature

Components

Sodium carbonate
Sodium chloride
Sodium citrate
Sodium diatrizoate
Sodium dichromate
Sodium ferrocyanide
Sodium hydroxide
Sodium molybdate
Sodium nitrate

Buffer Components

Buffer Components	Concentration	Units
Sodium chloride	0.15	molar
Sodium phosphate, di-basic	0.20	molar

Heavy Isotopes of Water

☐ **Heavy Isotopes of Water**

H₂O

D₂O

H₂O¹⁸

D₂O¹⁸

Search

pH

Buttons: Compute, Read Composition from File, Save Composition to File, Save Solvent to Database, Cancel, OK

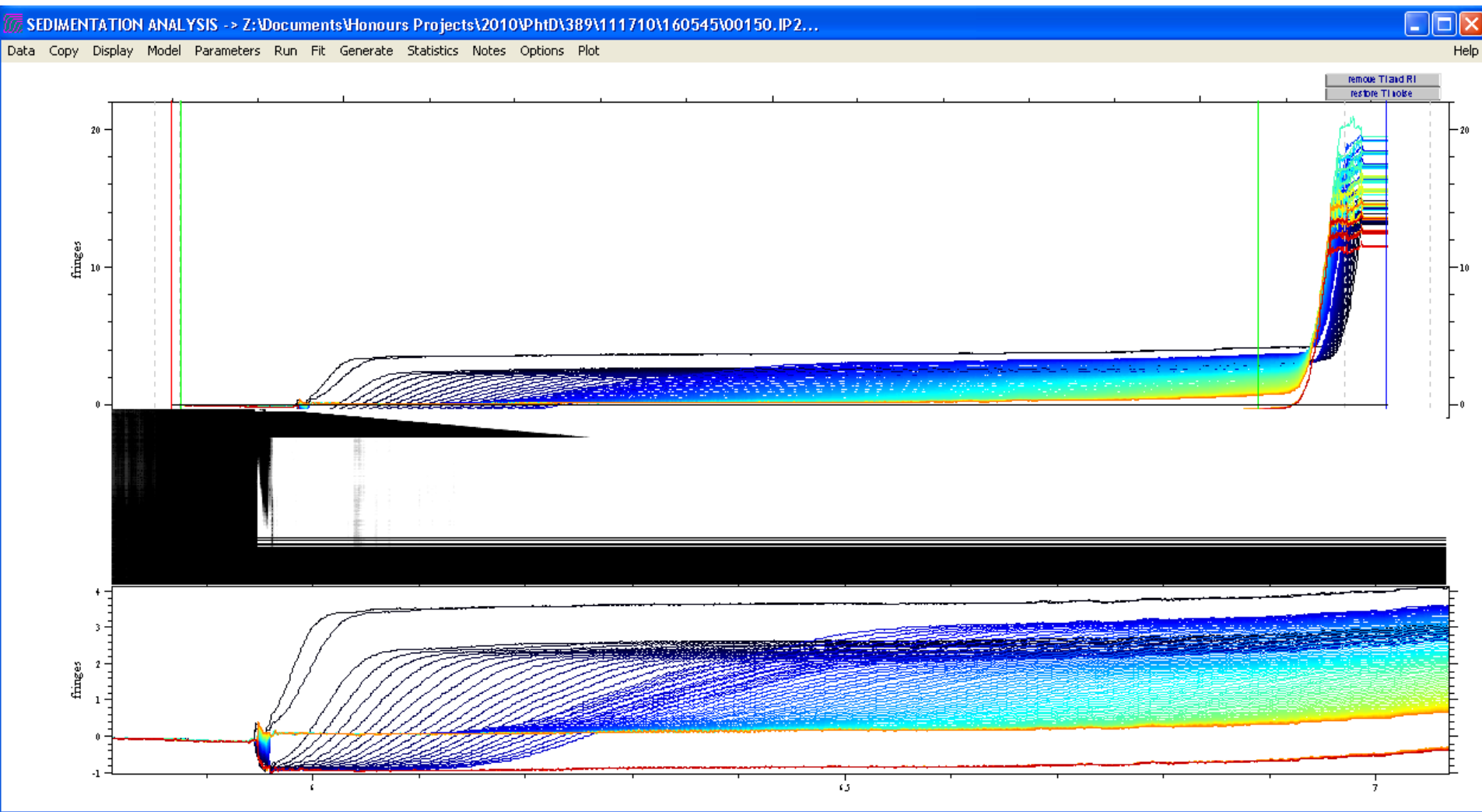
Ready

Values for $\bar{v}$

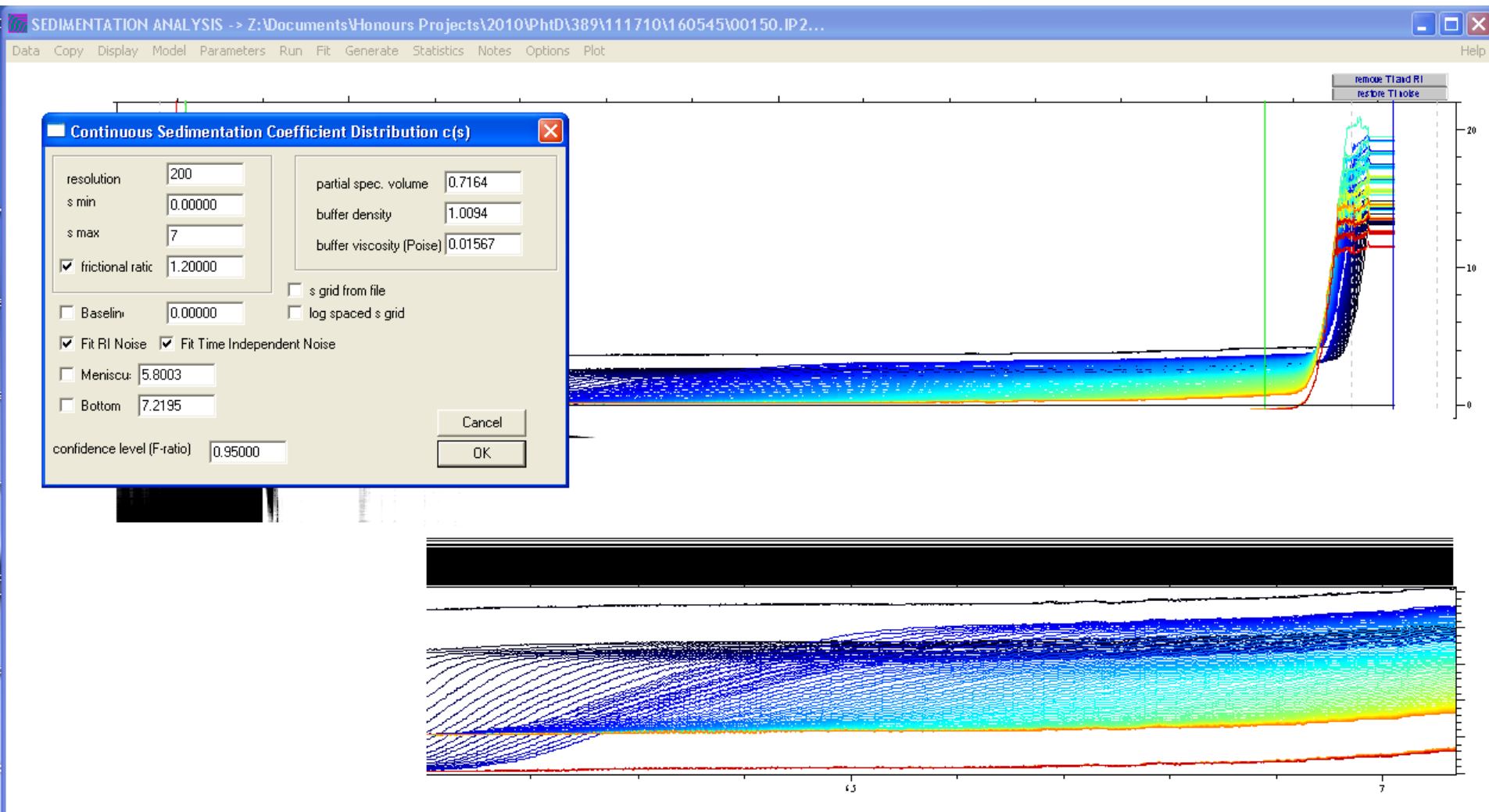
Component	$\bar{v}$ (ml/g)	Comment
Protein	0.73	Can be reliably calculated from sequence
Carbohydrate	0.63	
Lipid	1.02	An average value Close to value for solvent, therefore almost invisible
Detergent	0.7-1.2	Can sediment or float

SEDFIT c(s) analysis: how many species + s of species

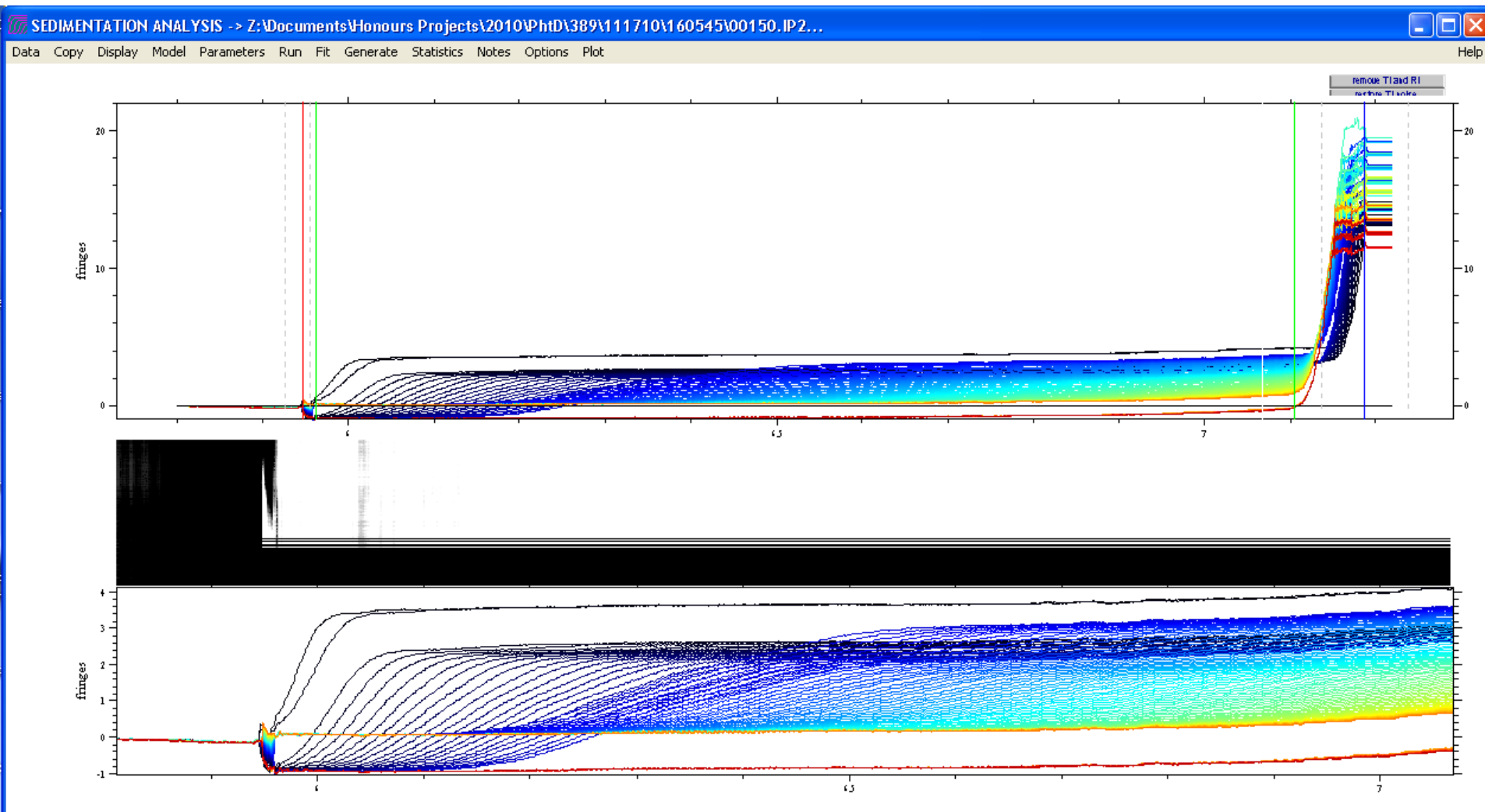
I: Load SV data



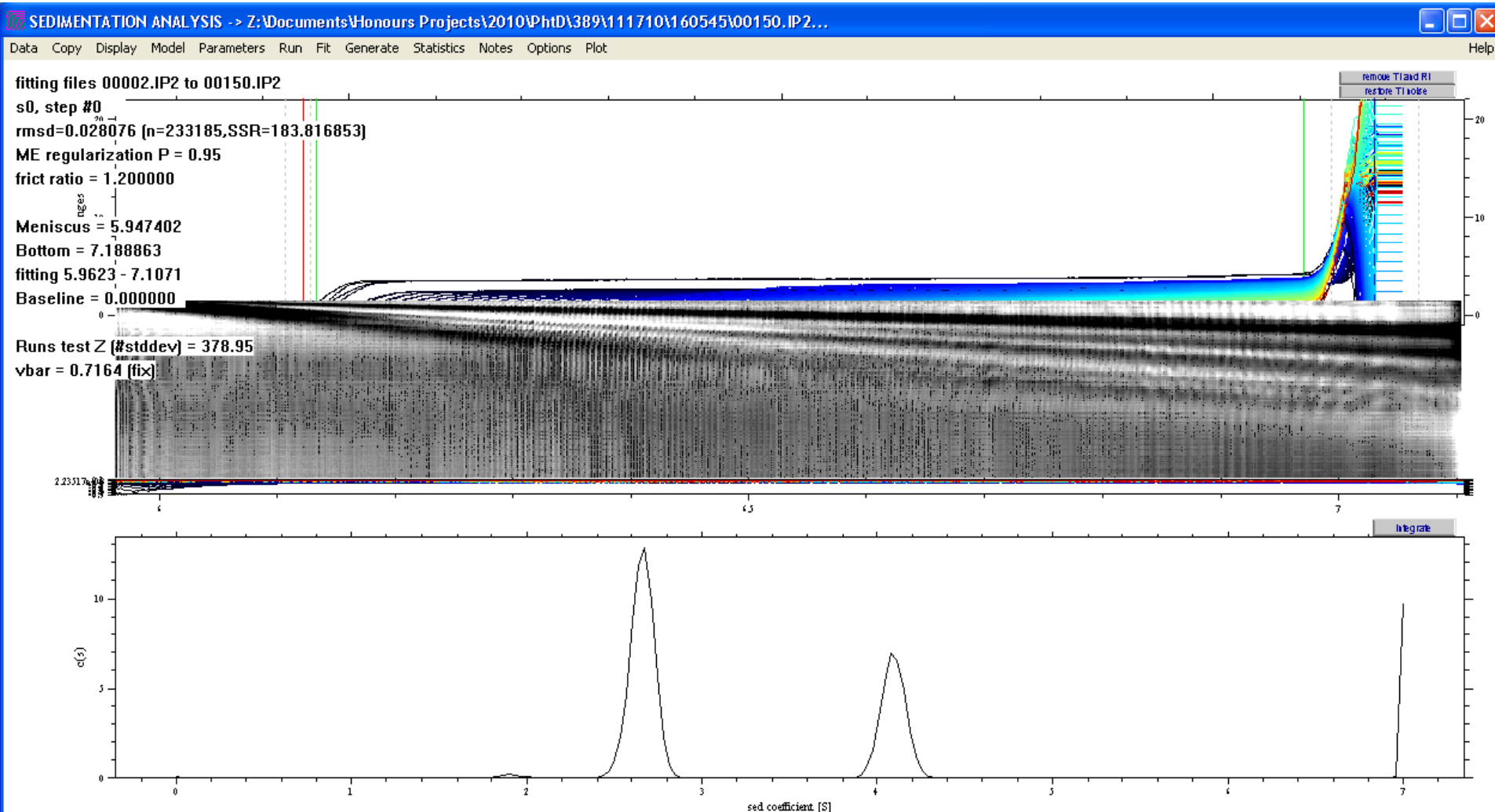
2: Specify parameters



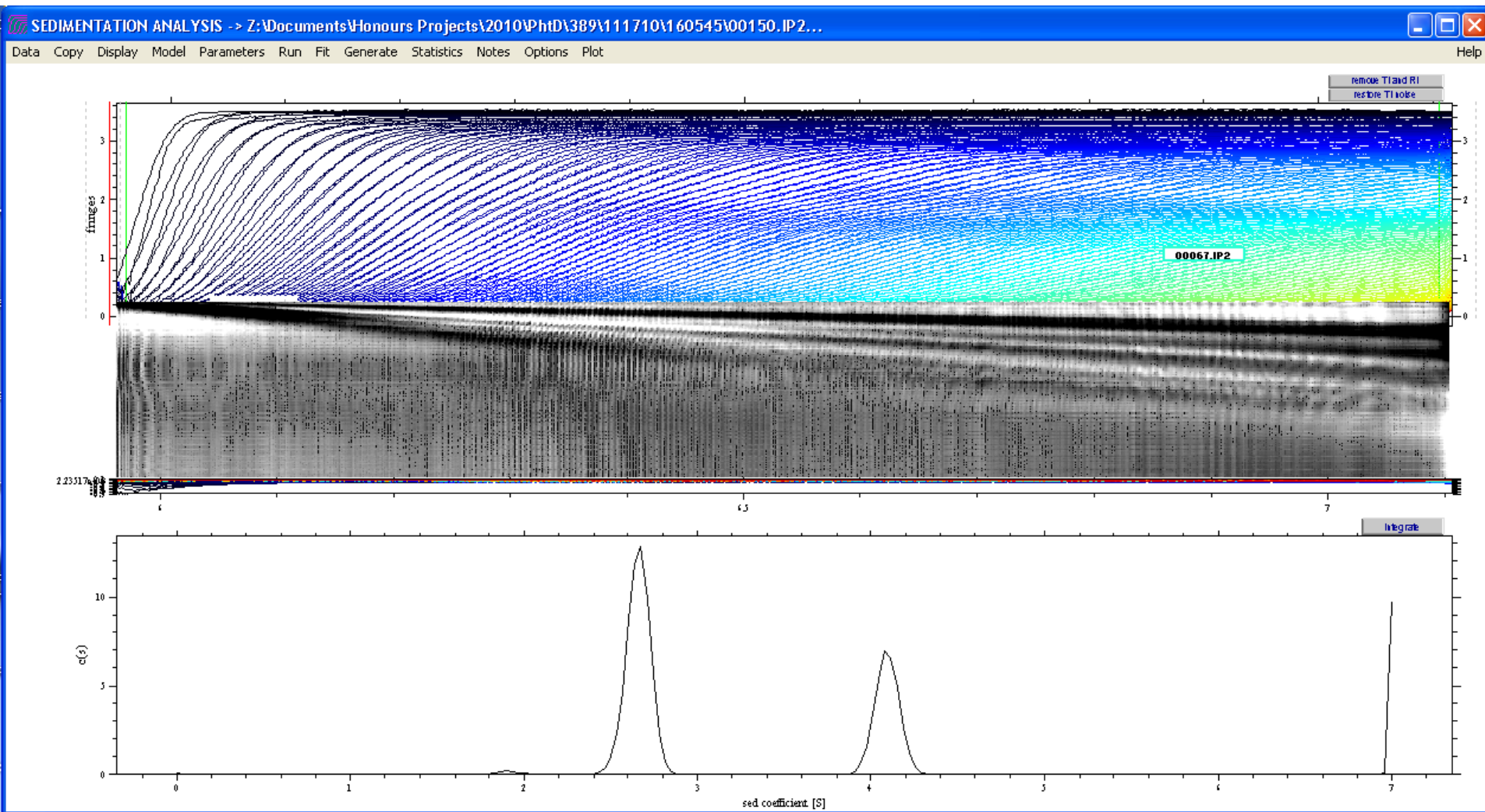
3: Set meniscus, cell base and analysis limits



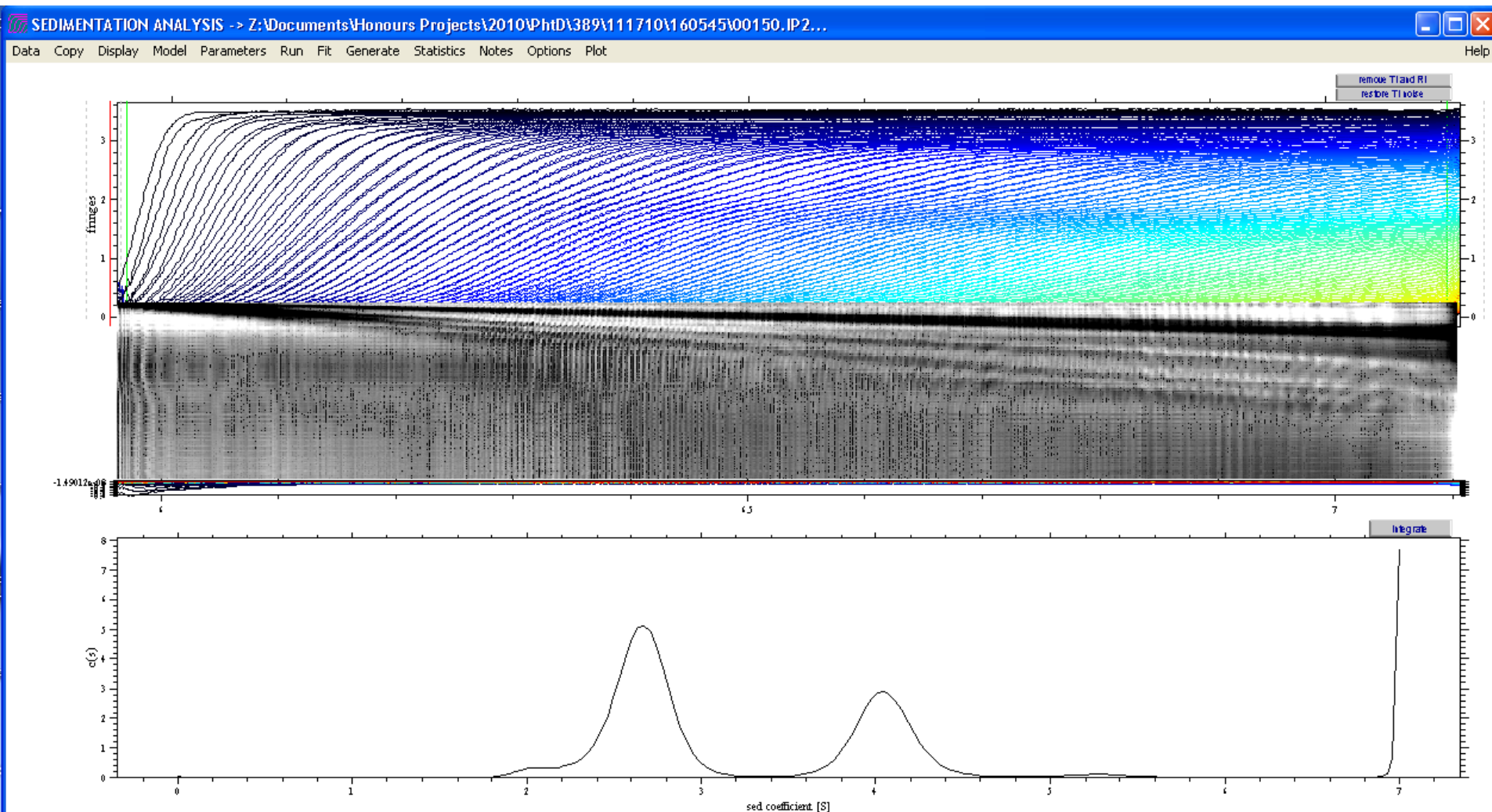
4: Run



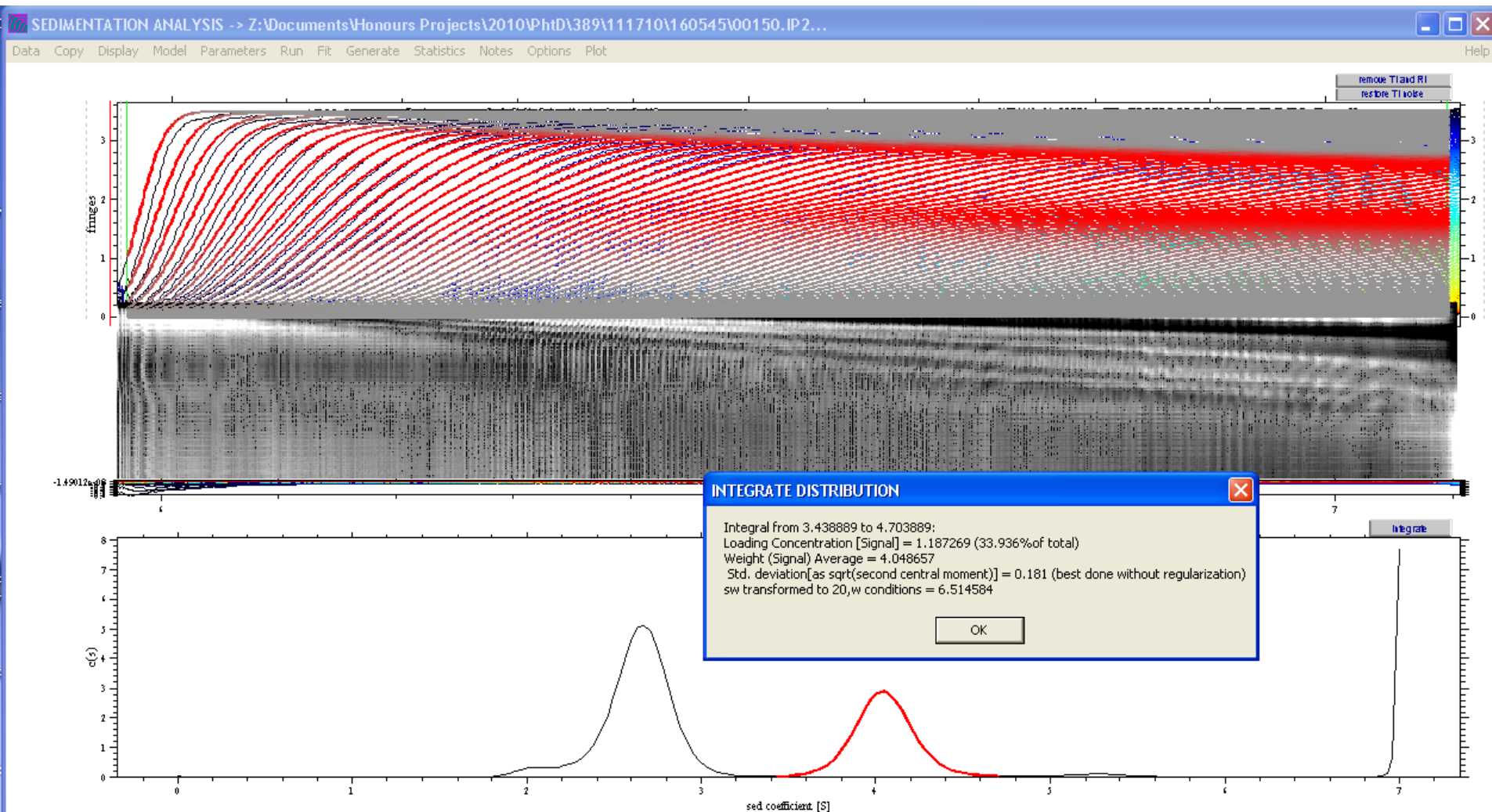
5: Subtract time and radial invariant noise



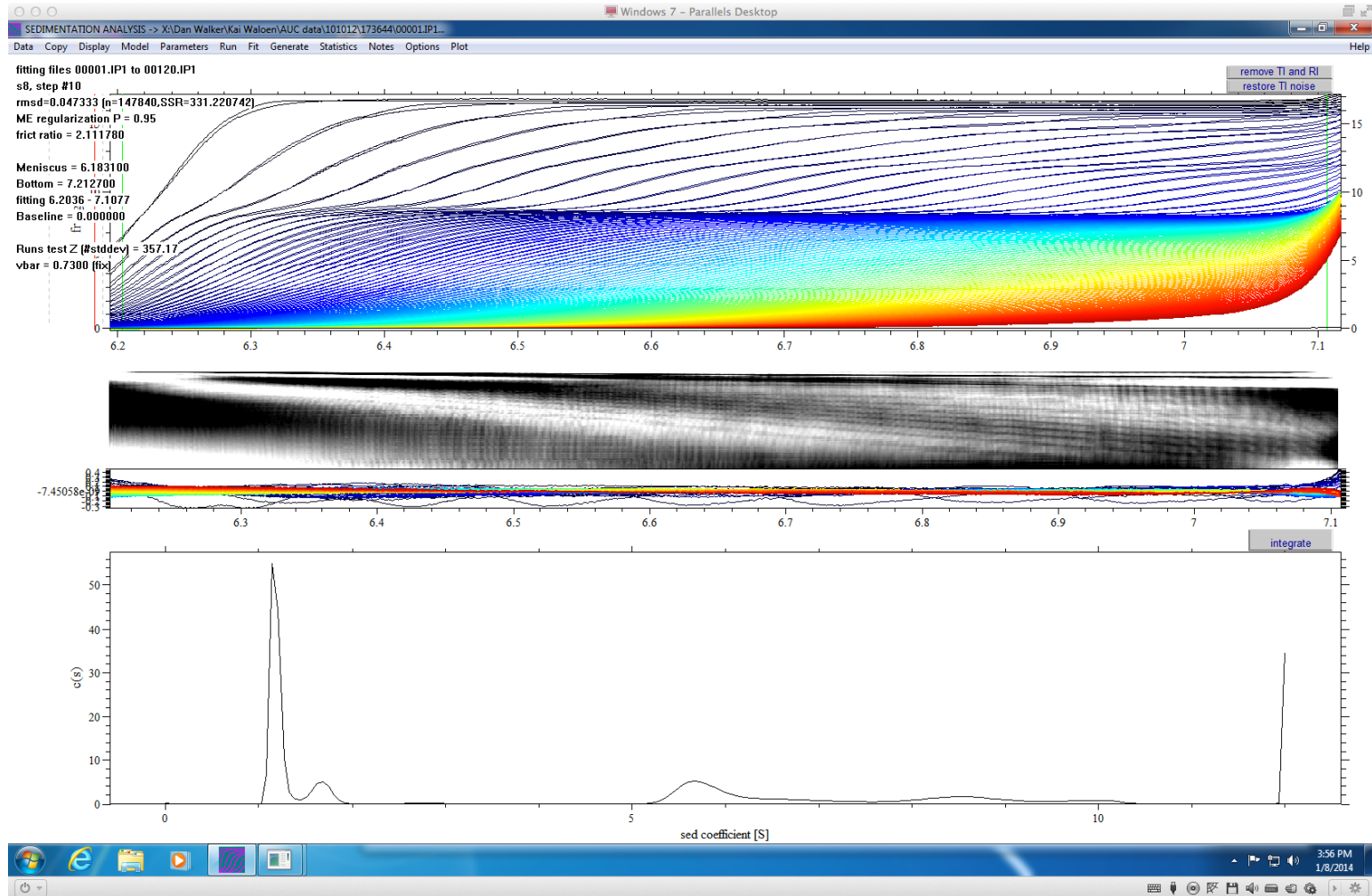
6: Fit (with solutions to the Lamm equation)



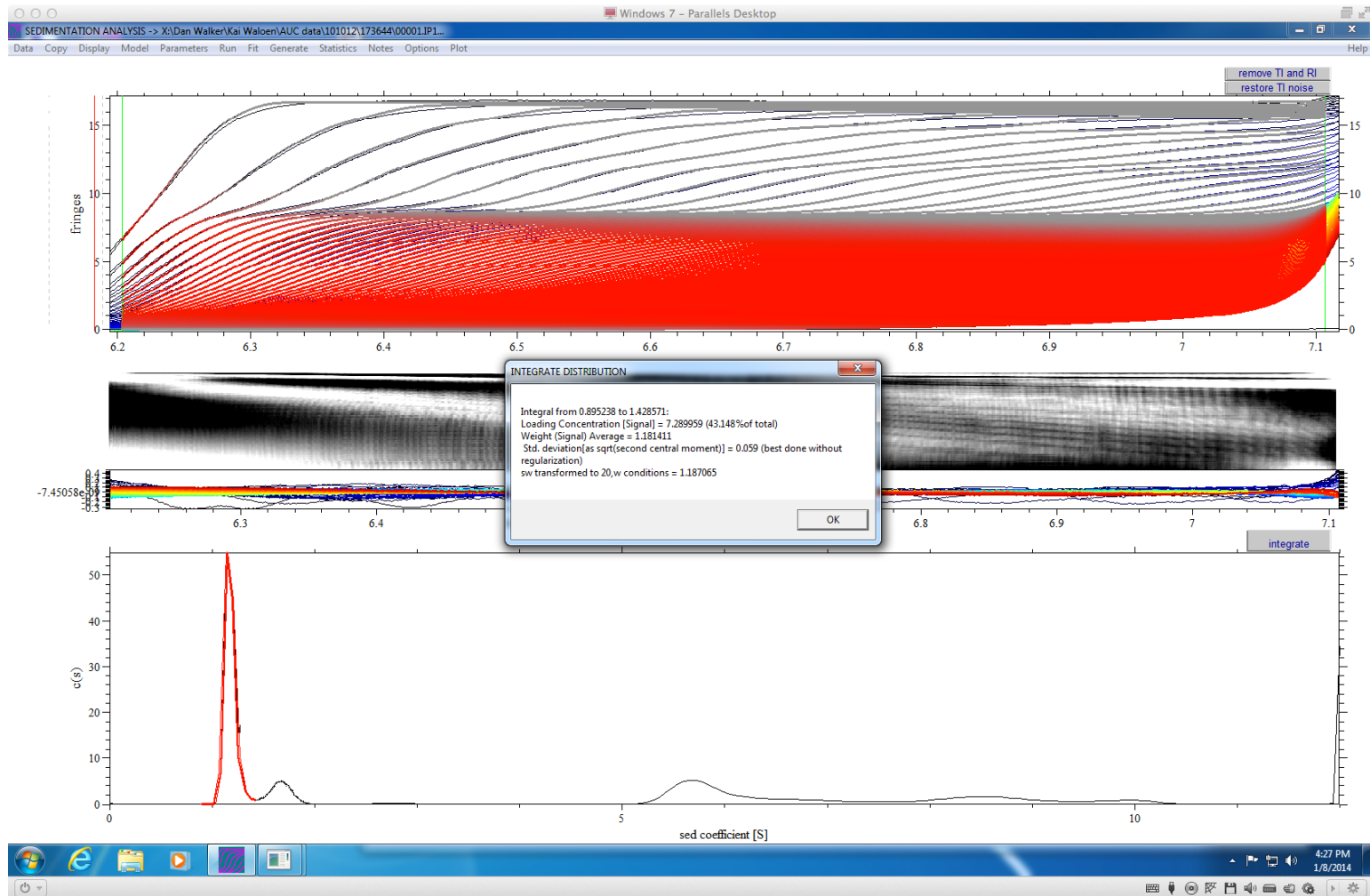
7: Integrate to obtain estimate of concentration of species and weight-average values



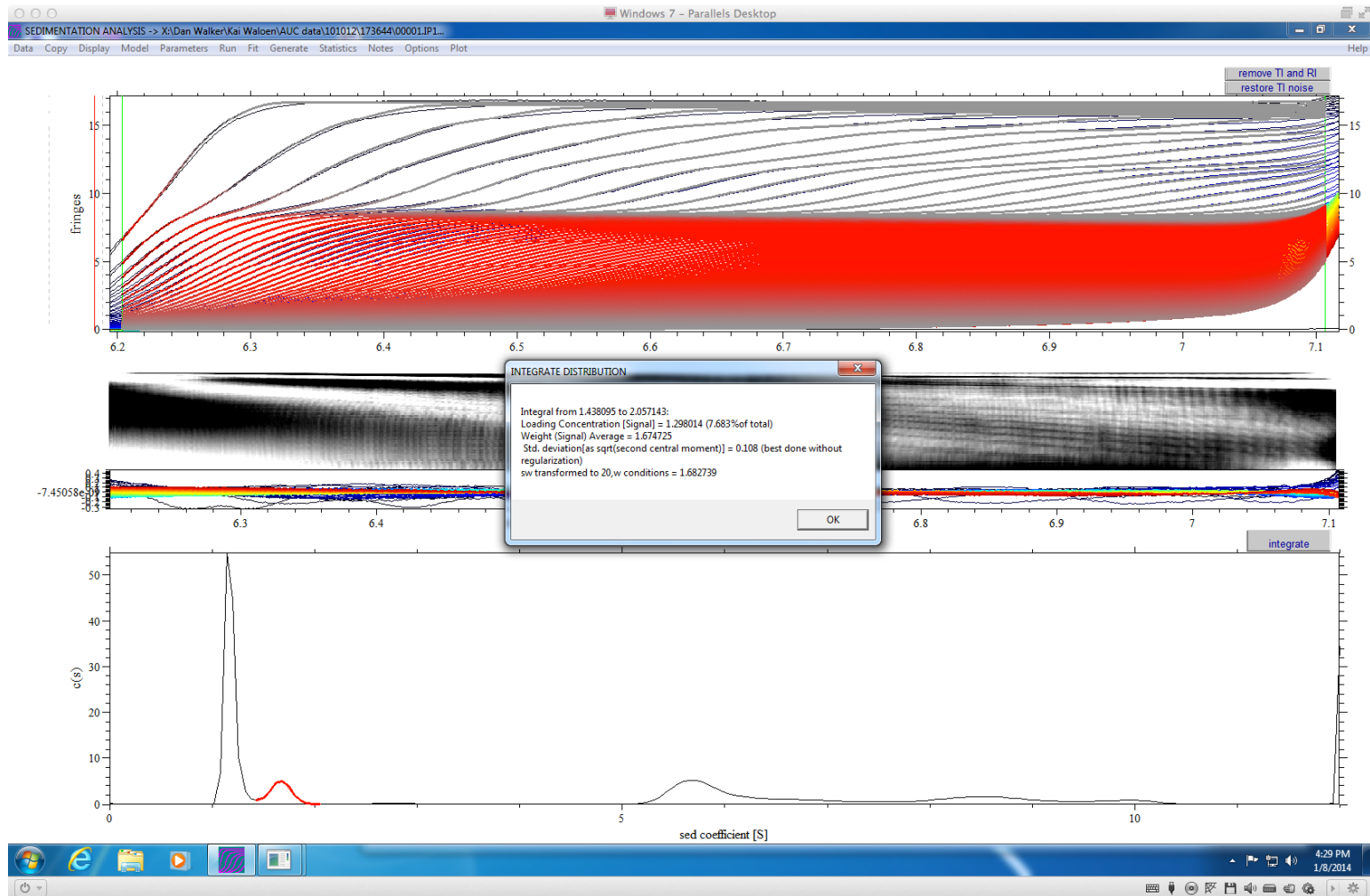
Sum of Lamm equations $0 \leq s \leq 12$ S discretised by 200



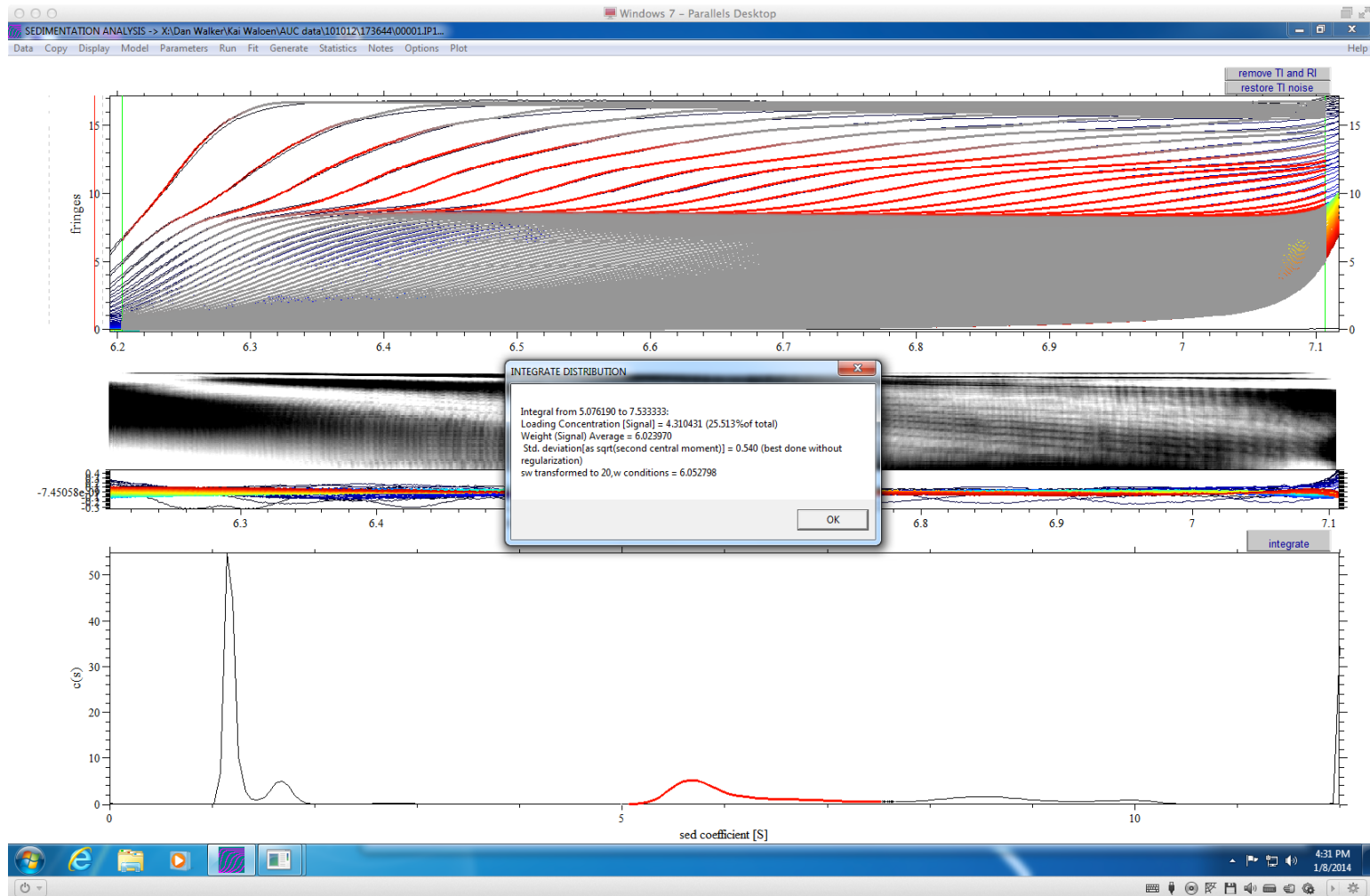
Integrating $c(s)$ peaks reveals region of boundary that contains species



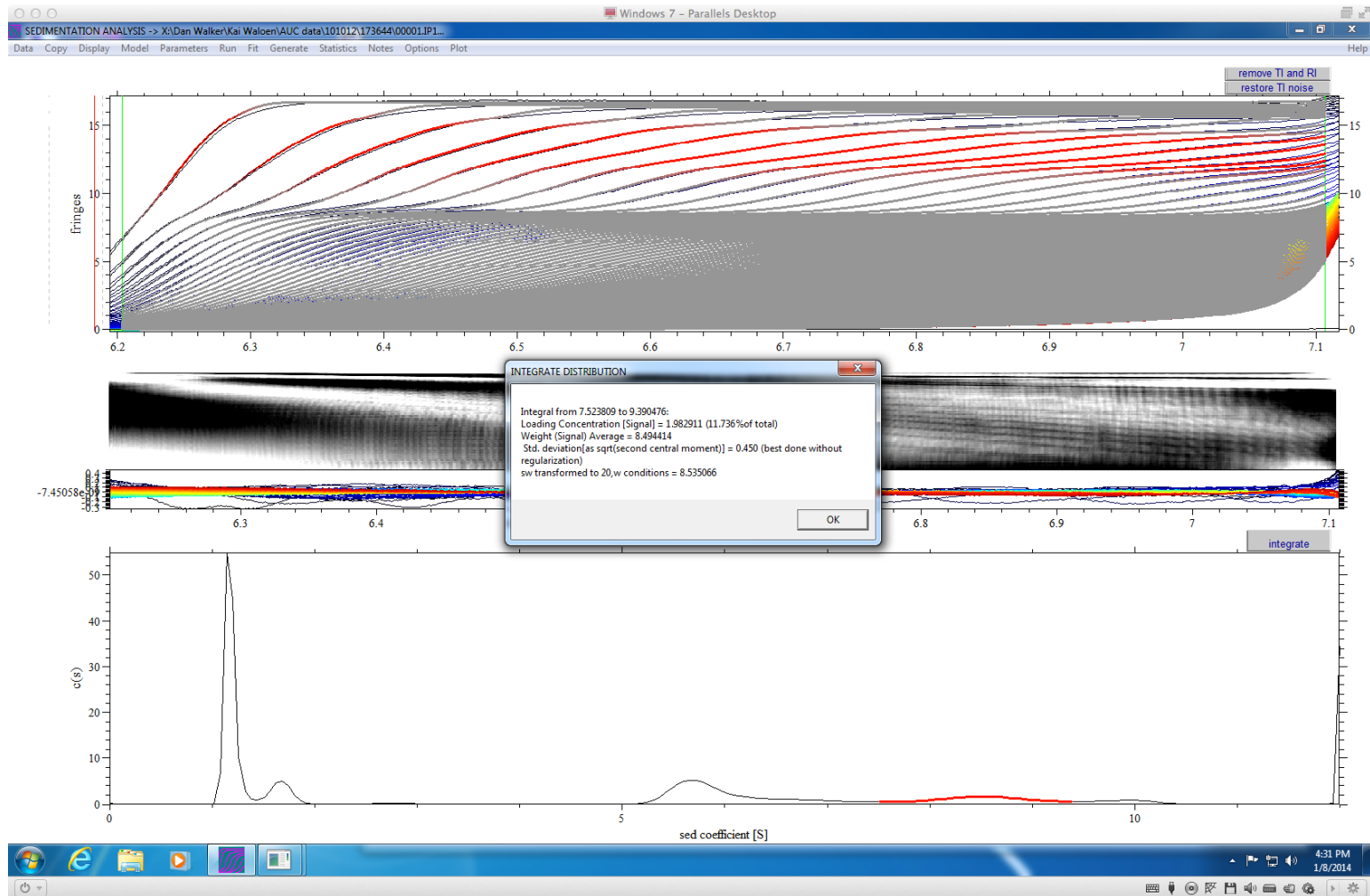
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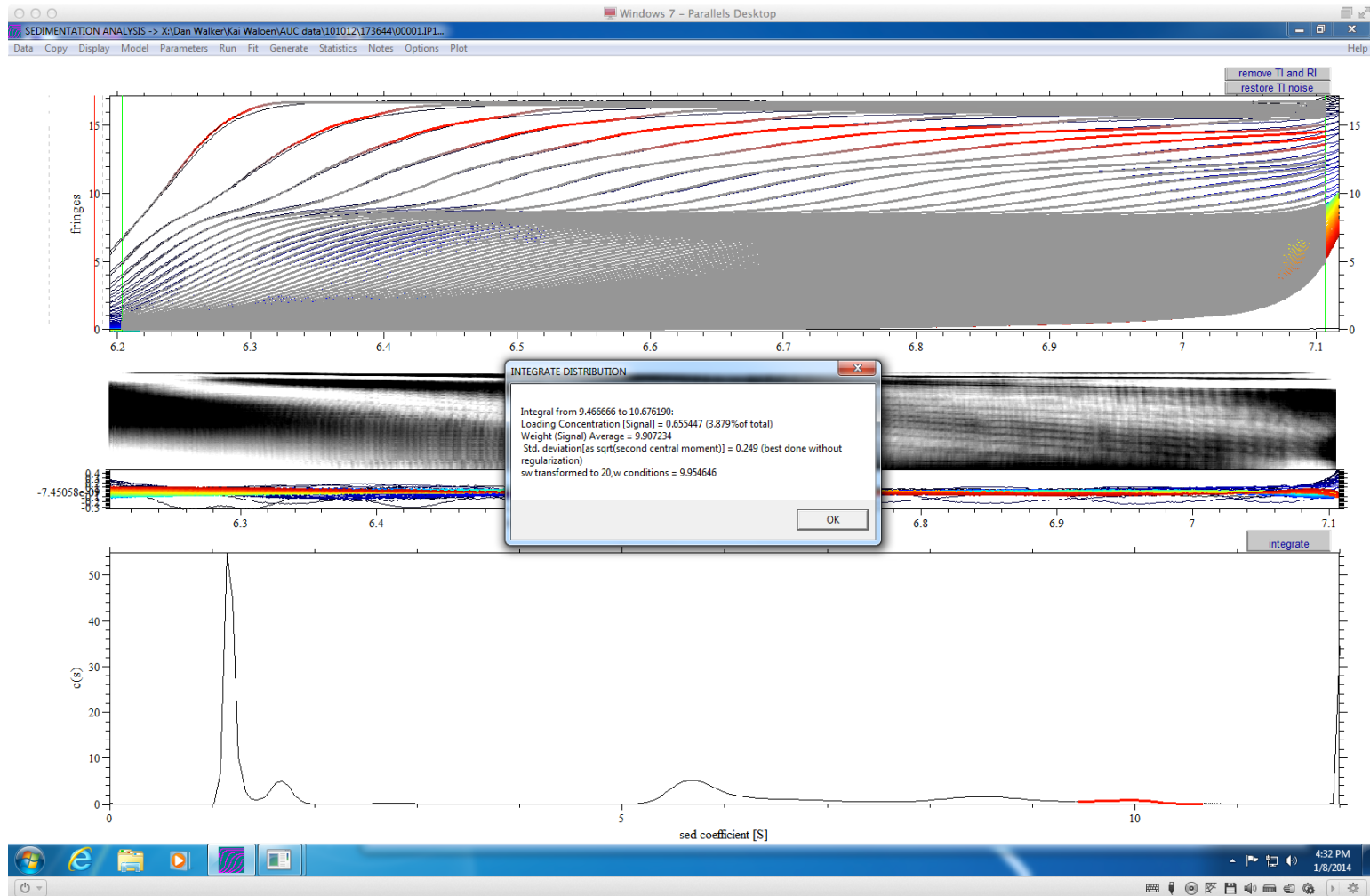
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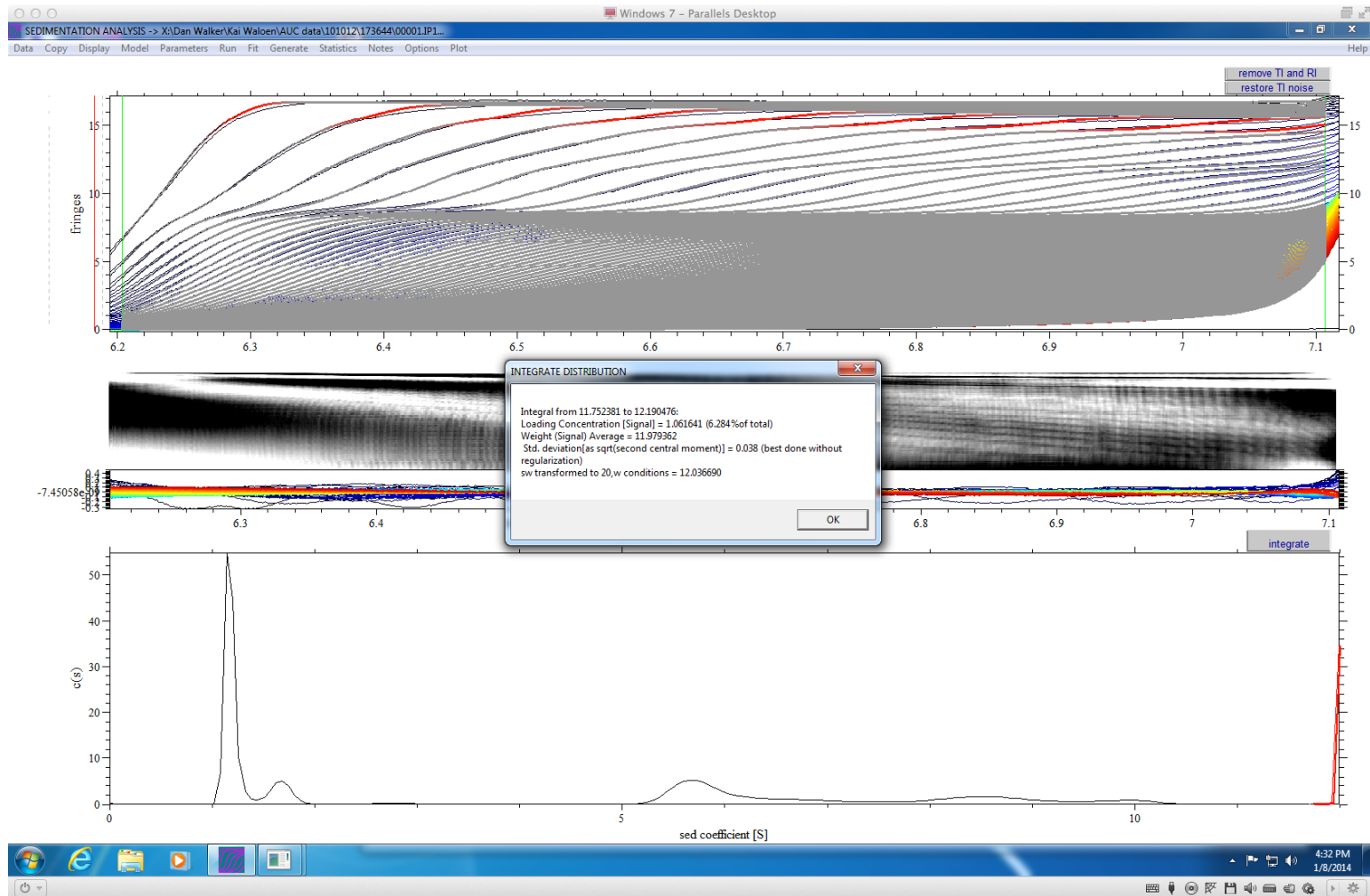
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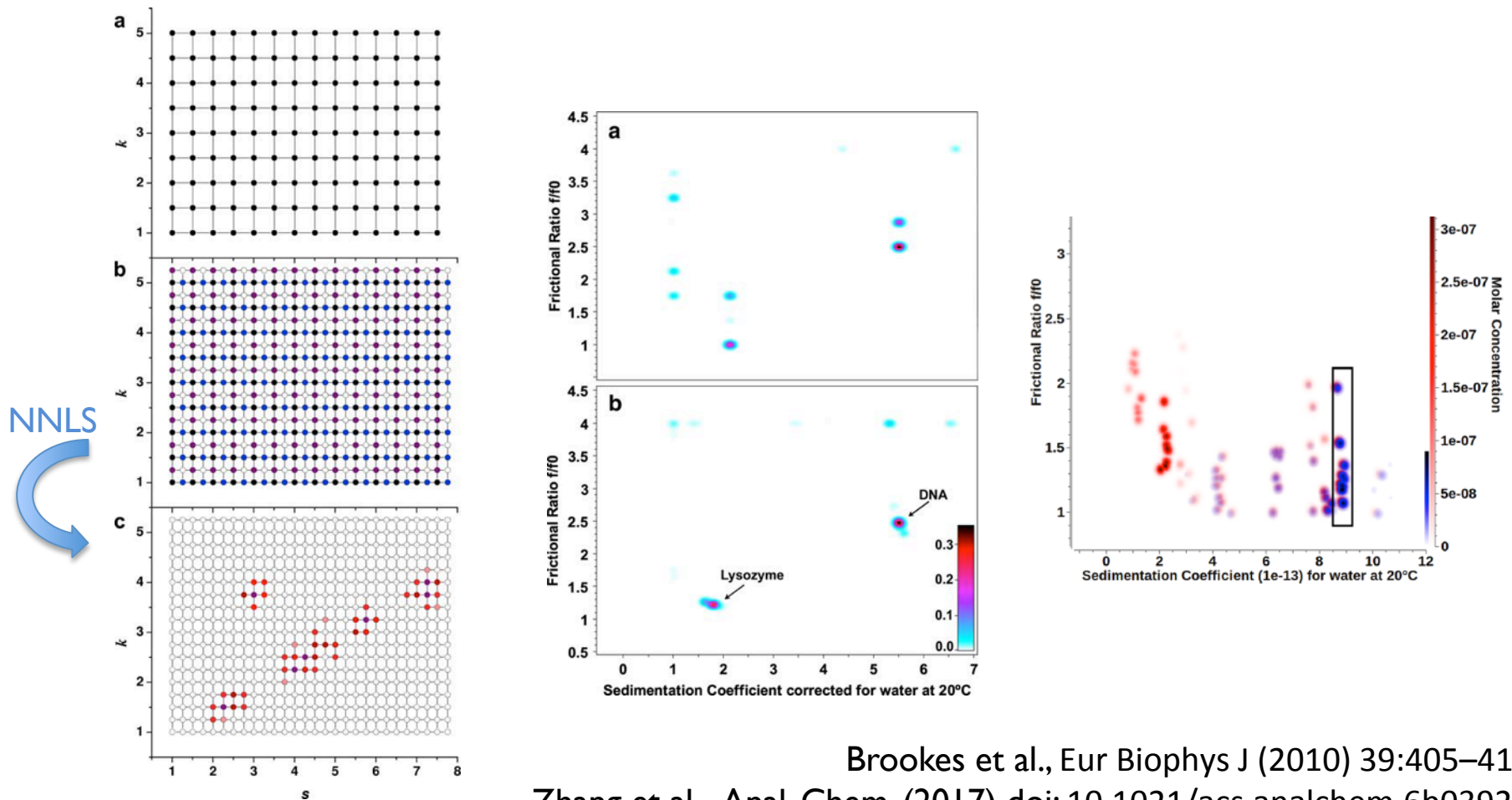


Integrating $c(s)$ peaks reveals region of boundary that contains species



Two-dimensional spectrum analysis (2DSA) of SV data with UltraScan: model independent fitting giving s & M

- Important when f/f_0 varies for components

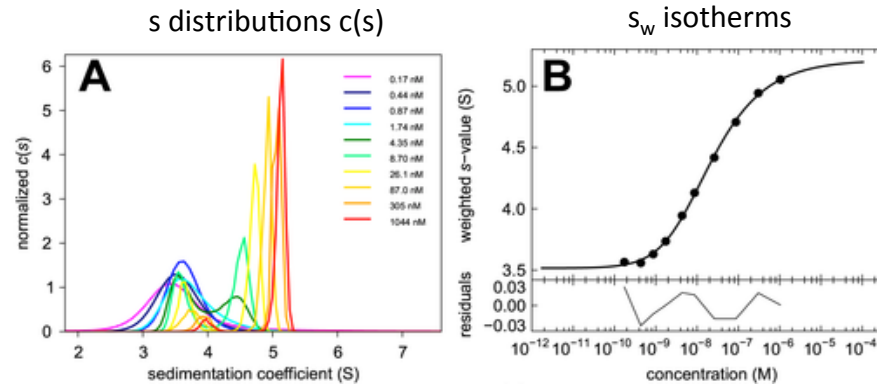


Interacting systems & monodispersity

- Rapid monomer-dimer
 - SV will show 1 symmetrical boundary
 - Can be confused with monodispersity
 - Position will correspond to average of s_{monomer} & s_{dimer}
- Slow monomer-dimer
 - Indistinguishable from mixture of monomer and dimer
 - Therefore 2 peaks, or asymmetric single peak
 - Except if proportion of species depends on loading concentration

Life-time of FAM-GluA2 ATD dimer is significantly longer than that of Dylight488- or EGFP-GluA2 ATD

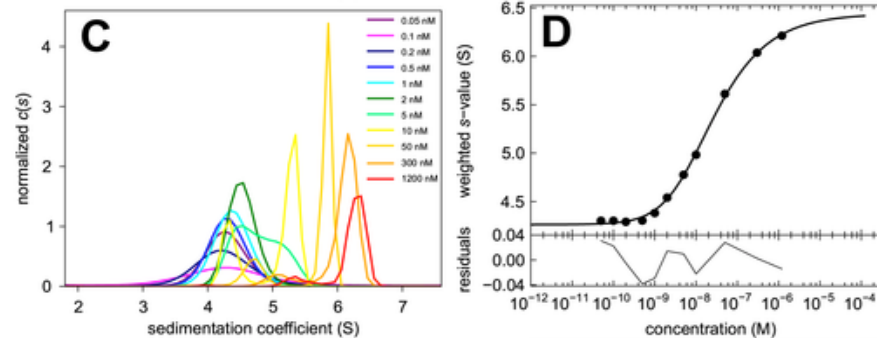
Dylight488-GluA2 ATD



$$K_D = 20.5 \text{ nM}$$

$$s_1 = 3.52 \text{ S}, s_2 = 5.21 \text{ S}$$

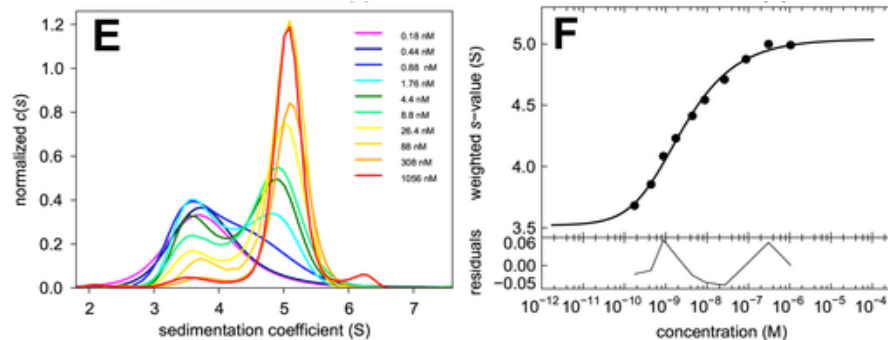
EGFP-GluA2 ATD



$$K_D = 25.4 \text{ nM}$$

$$s_1 = 4.26 \text{ S}, s_2 = 6.44 \text{ S}$$

FAM-GluA2 ATD



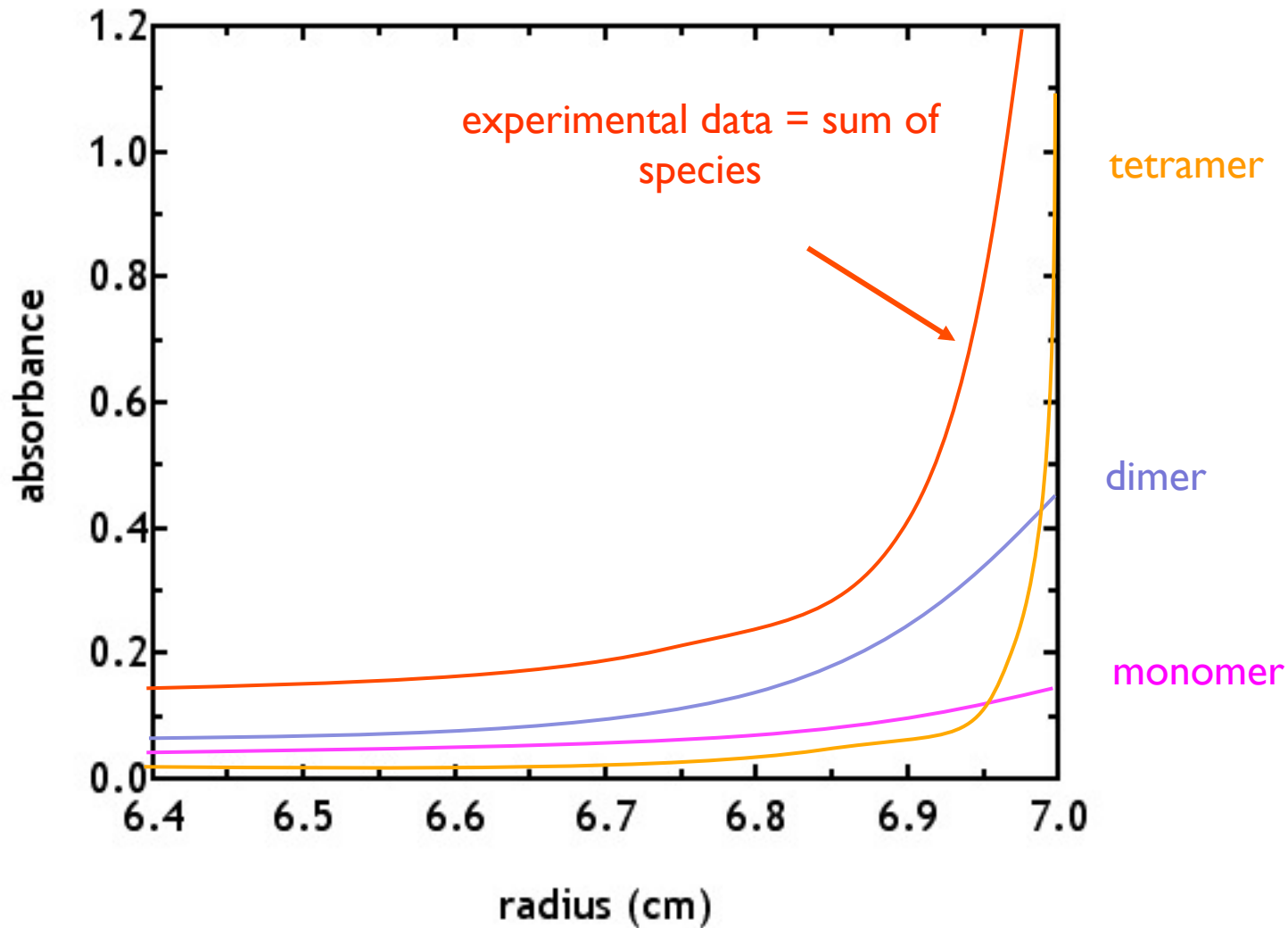
$$K_D = 2.3 \text{ nM}$$

$$s_1 = 3.52 \text{ S}, s_2 = 5.04 \text{ S}$$

Take a break.
You deserve it!



Self-association: “deconvolution” of SE data into individual components



Self-association: SE data are the sum of exponentials

$$A_r = \exp[\ln A_0 + H.M(r^2 - r_0^2)] \leftarrow \text{monomer}$$

$$+ \exp[n_2 \ln A_0 + \ln K a_2 + n_2.H.M(r^2 - r_0^2)]$$

$\leftarrow I - n_2$

$$+ \exp[n_3 \ln A_0 + \ln K a_3 + n_3.H.M(r^2 - r_0^2)]$$

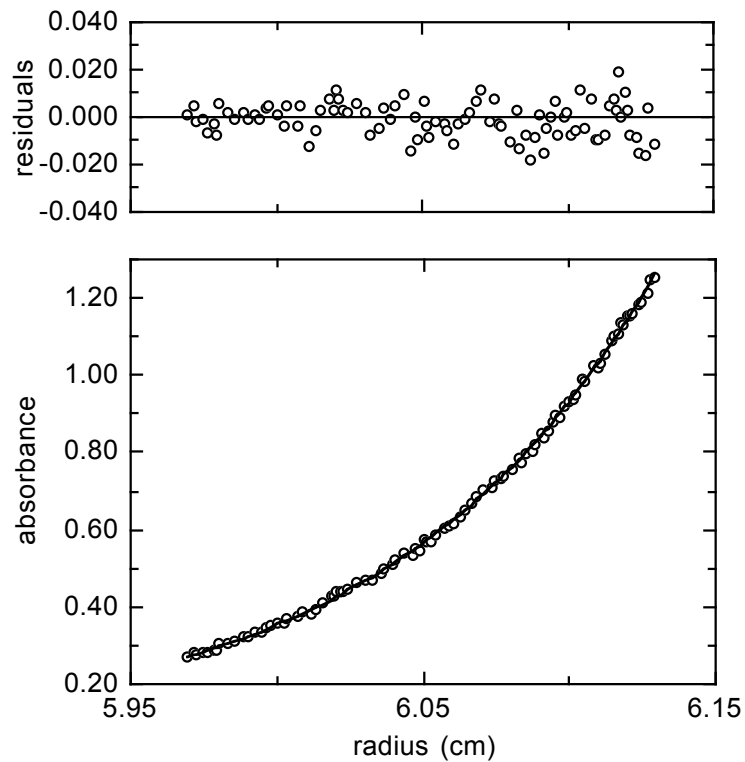
$\leftarrow I - n_3$

$$+ \exp[n_4 \ln A_0 + \ln K a_4 + n_4.H.M(r^2 - r_0^2)] + E$$

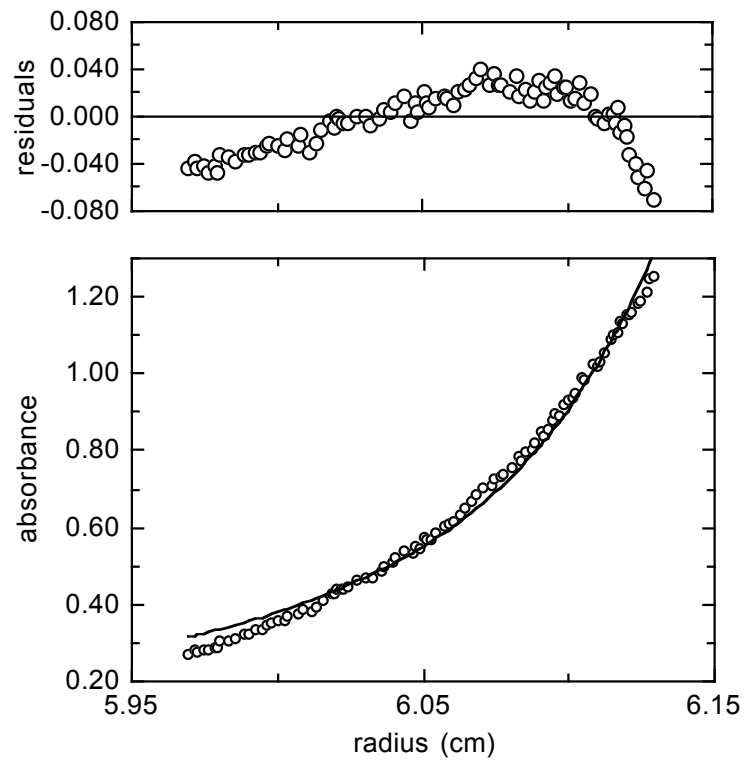
$\leftarrow I - n_4$

Self-association: best model revealed by residuals

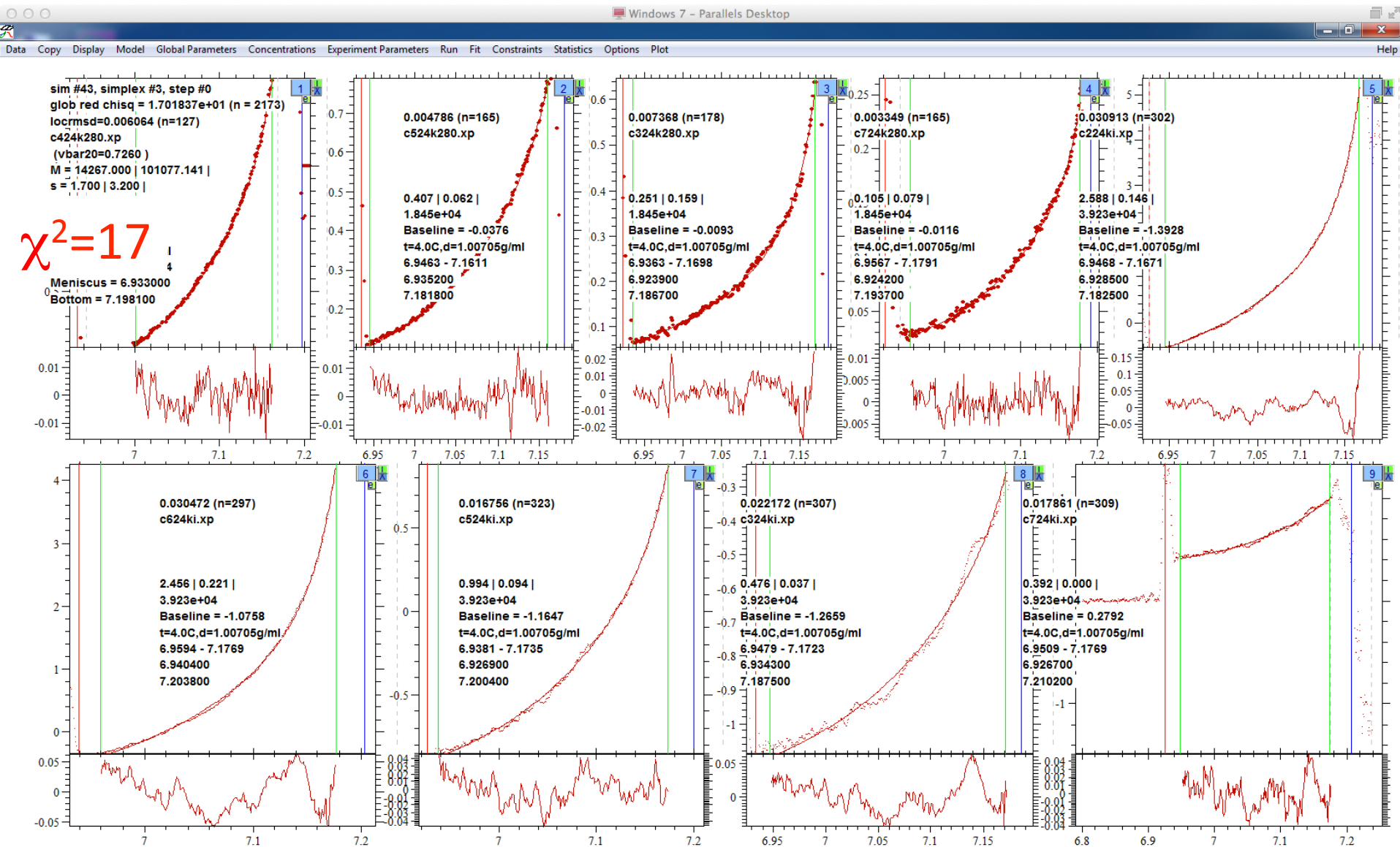
2-4



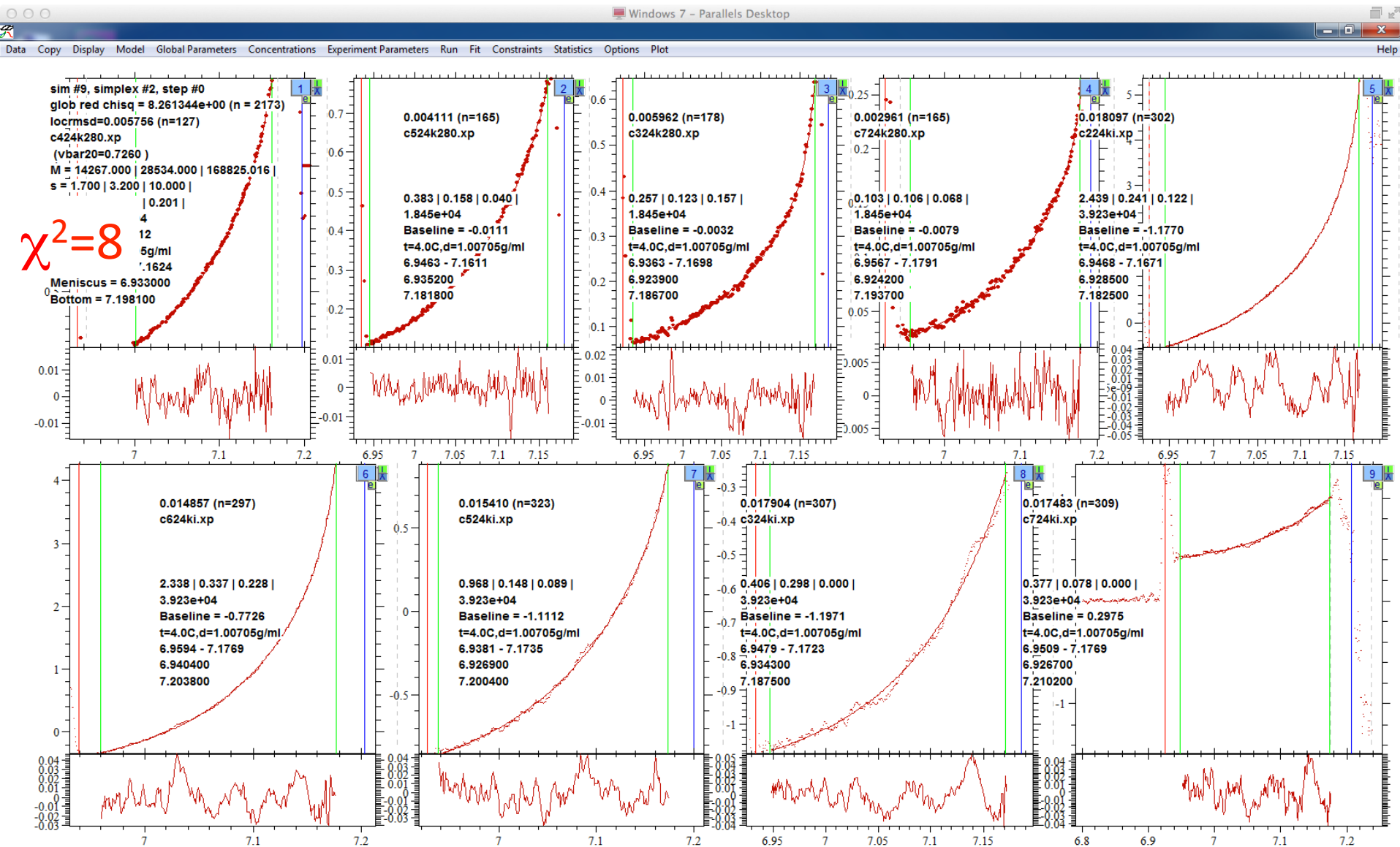
1-4



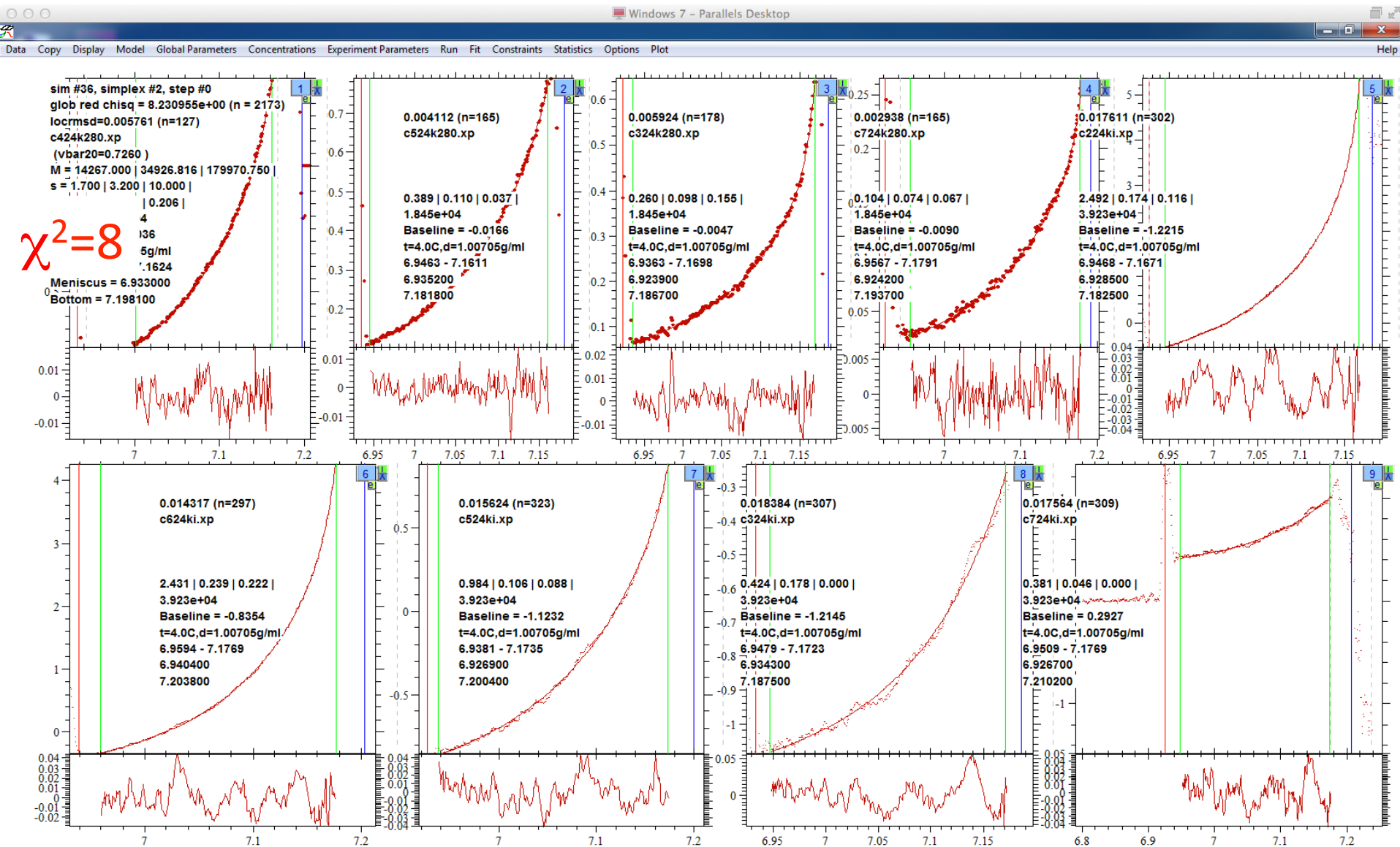
SEDPHAT: species analysis: monomer + heavy



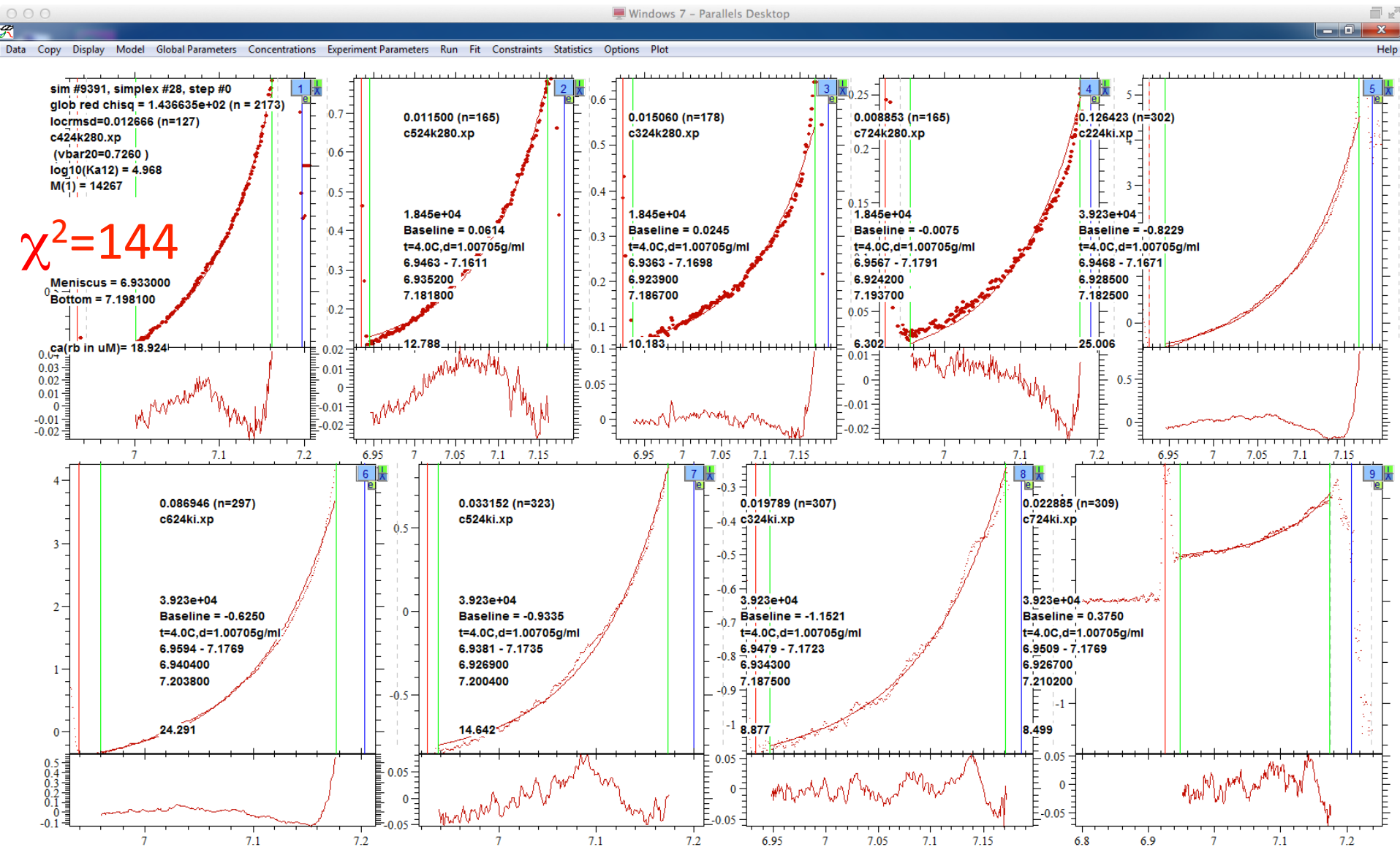
SEDPHAT: species analysis: monomer + “dimer” (both fixed) + heavy



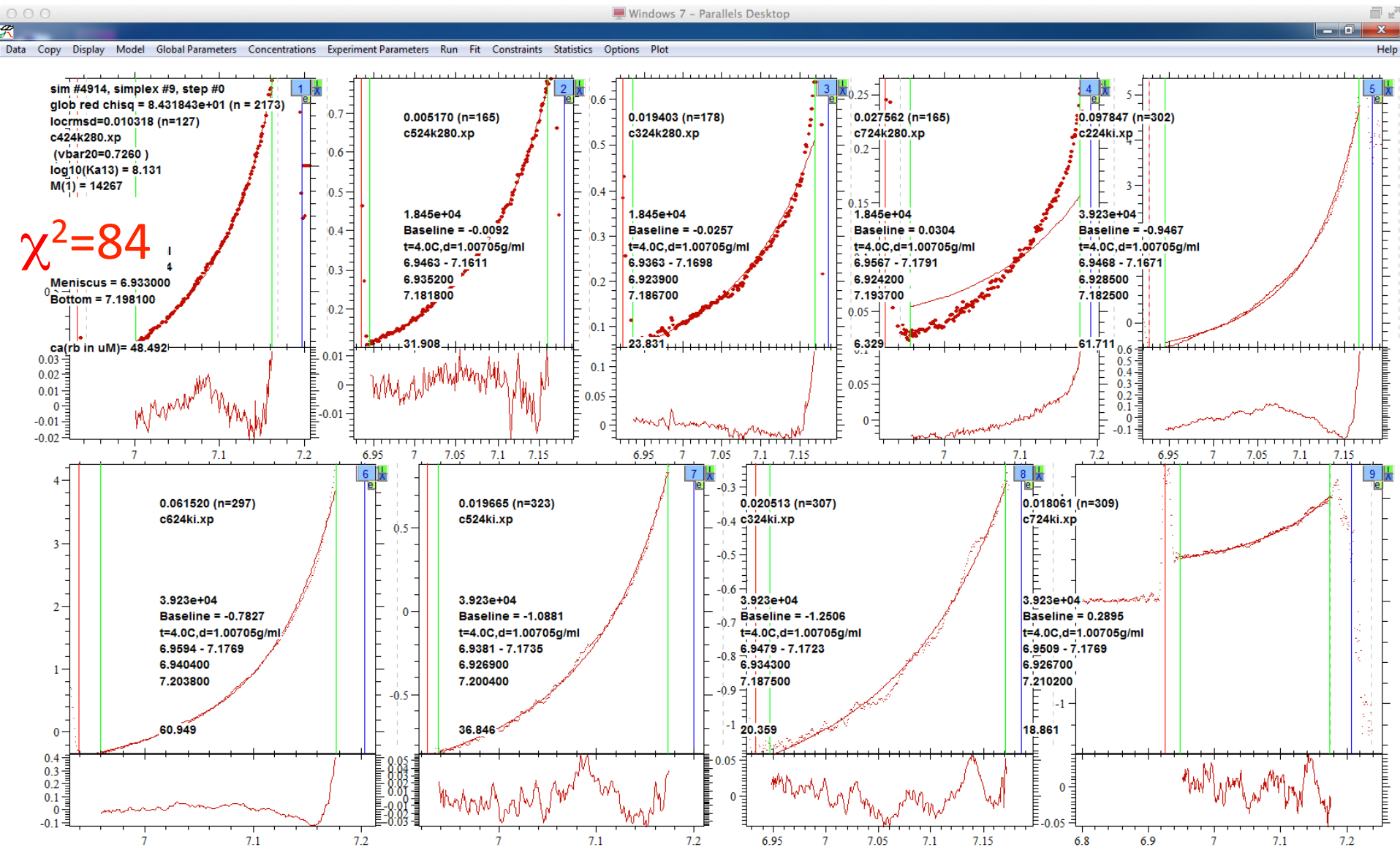
SEDPHAT: species analysis: monomer (fixed) + “dimer” + heavy (both free)



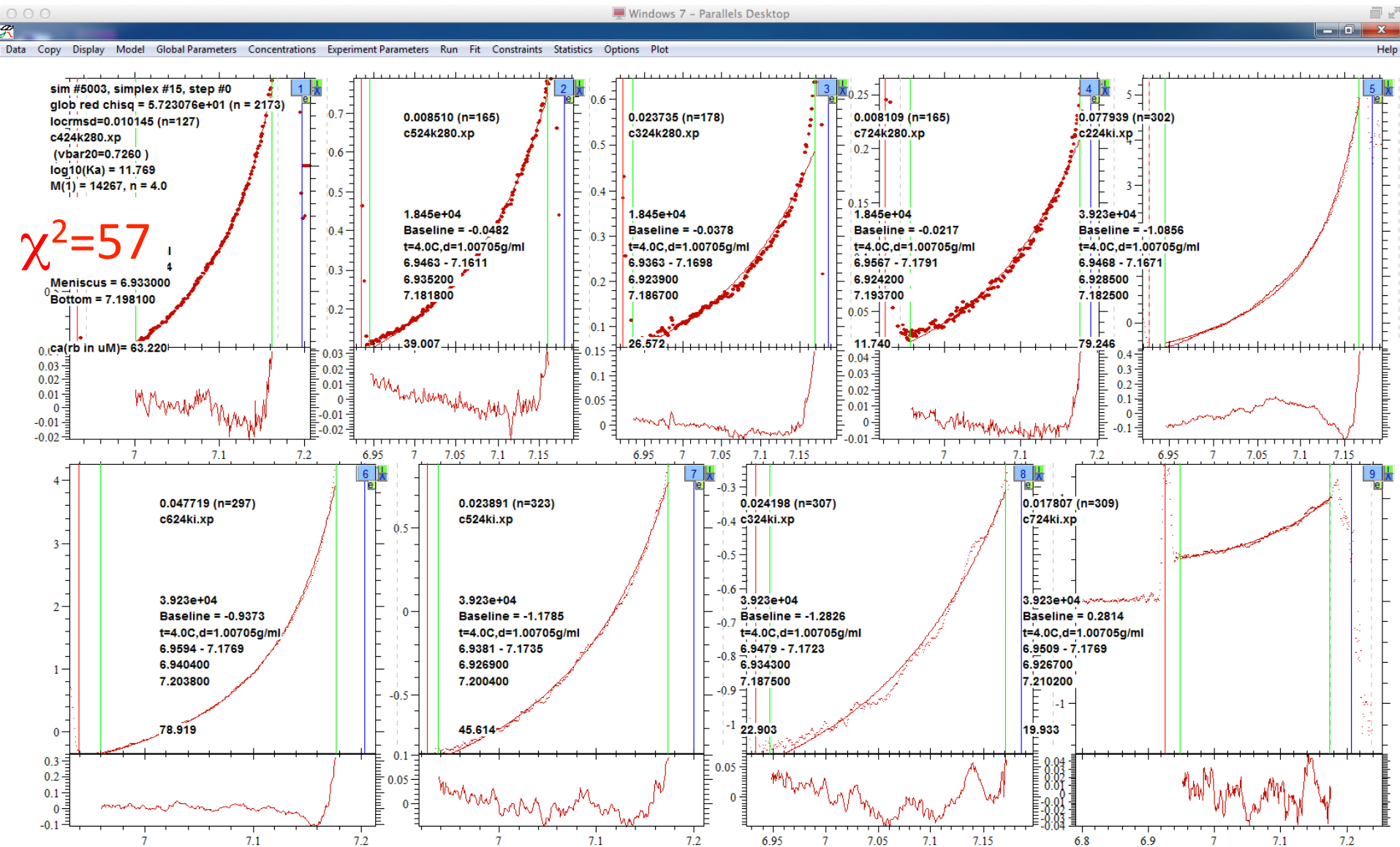
SEDPHAT: Abs + interference, 5.4 – 54 μM , $K_d = 10.8 \mu\text{M}$



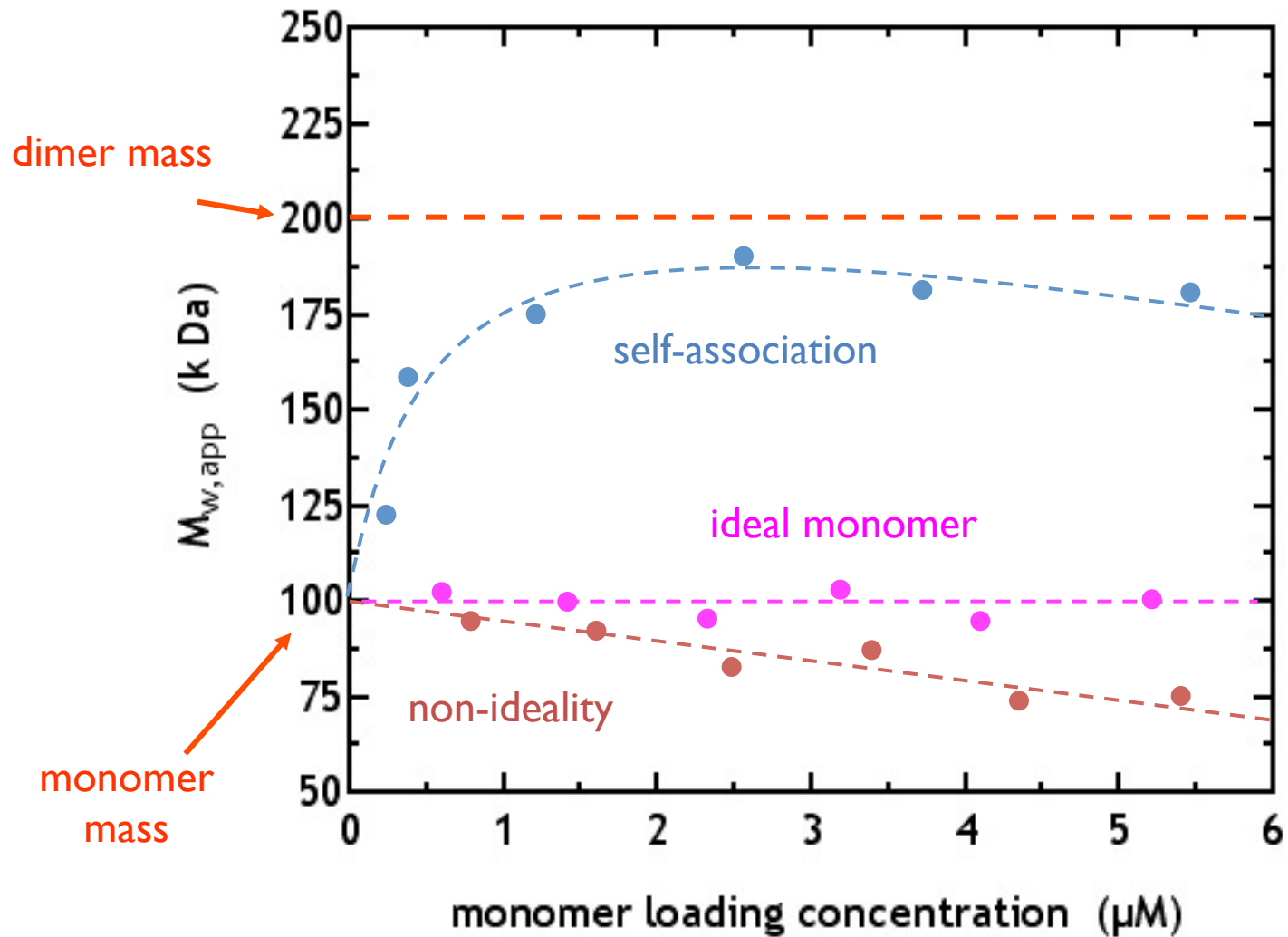
SEDPHAT: Abs + interference, 5.4 – 54 μM , $K_d = 7.4 \times 10^3 \mu\text{M}^2$



SEDPHAT: Abs + interference, 5.4 – 54 μM , $K_d = 1.7 \times 10^6 \mu\text{M}^3$

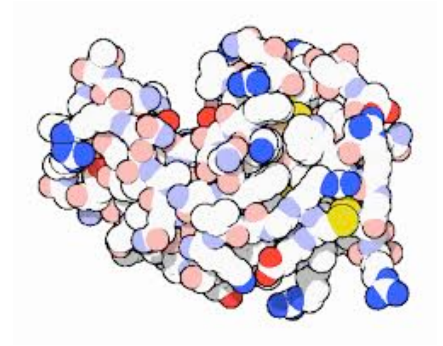


SE: more than just mass

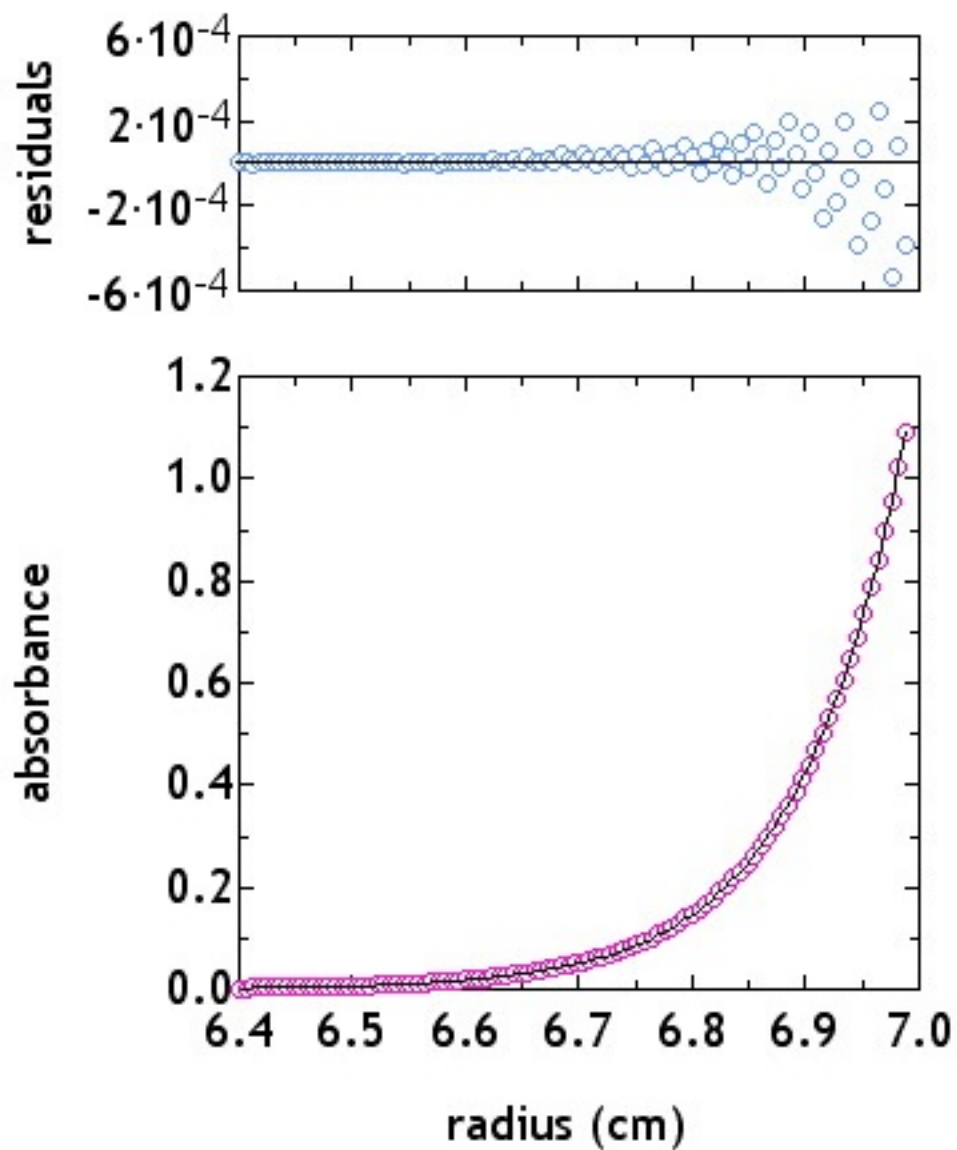


Ideal monomer

- Globular molecule
- Not highly charged
- Areas of charge neutralised by salt in buffer
- Sediments with monomer mass at most normal concentrations
- Examples: lysozyme, Fab, etc.

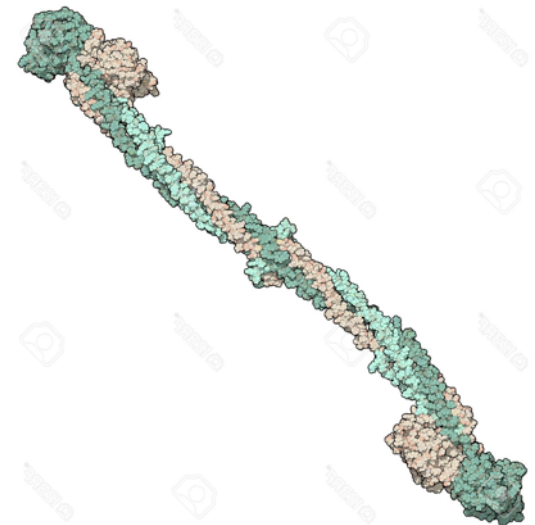


Ideal monomer data

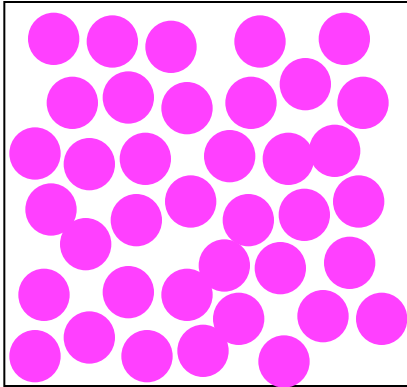


Non-ideal monomer

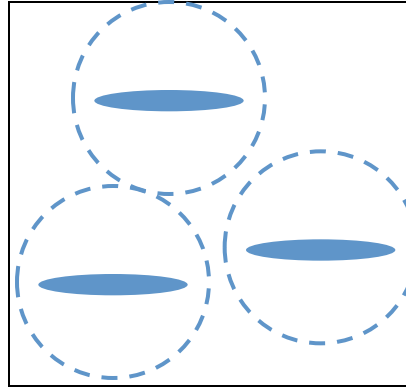
- Asymmetric molecule
and/or
- Highly charged
- Areas of charge **not** neutralised by salt in buffer
- Sediments with mass **below** that of monomer
- Mass is dependent on concentration
- Examples: myosin, fibrinogen etc.



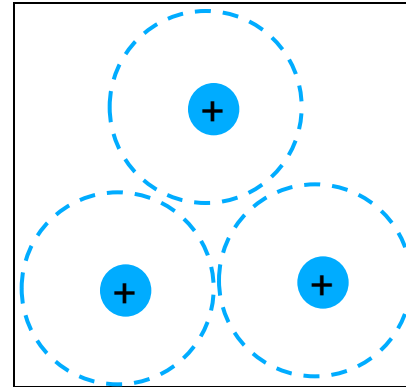
What is non-ideality ???



ideal



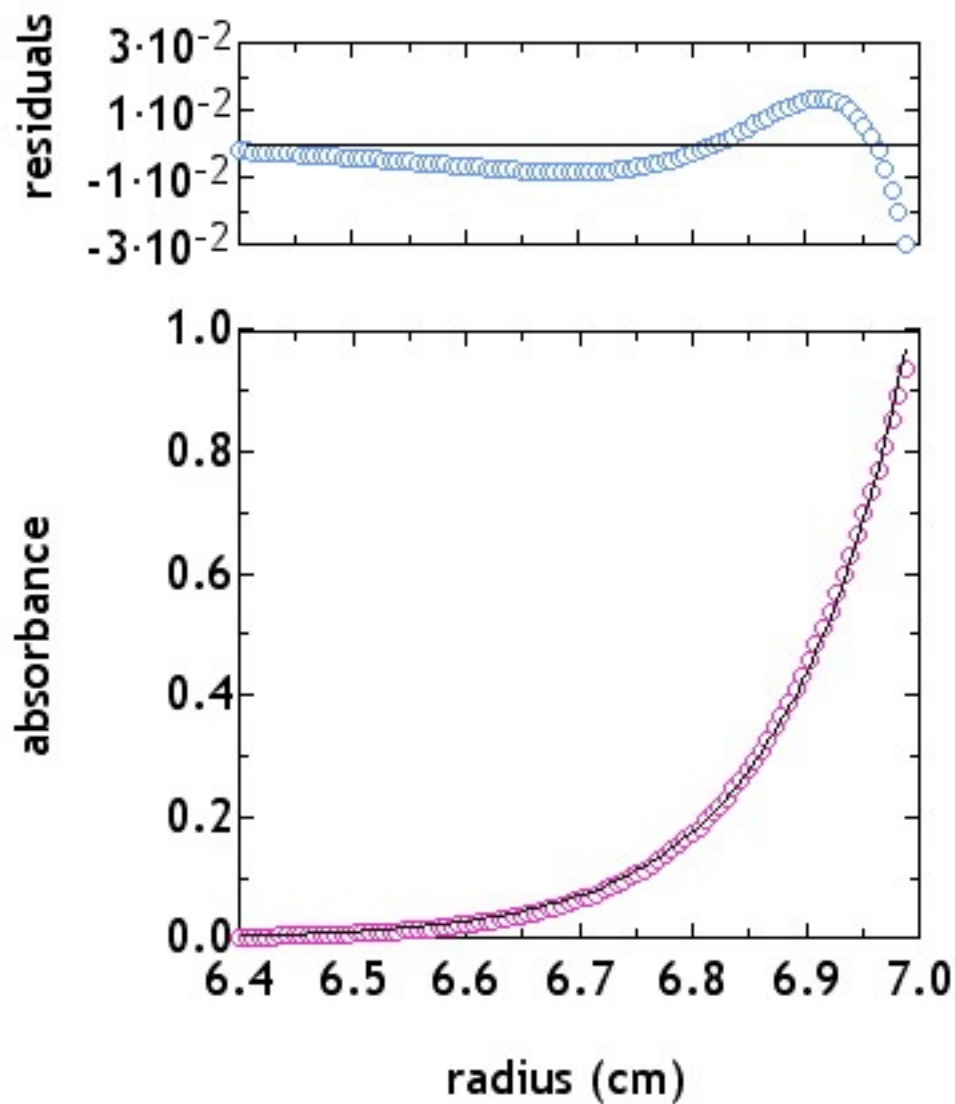
elongated



charged

non-ideal

Non-ideal monomer data

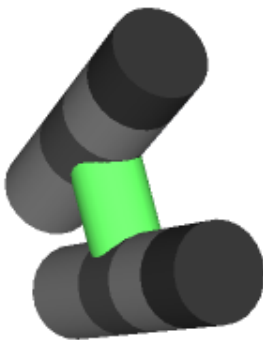
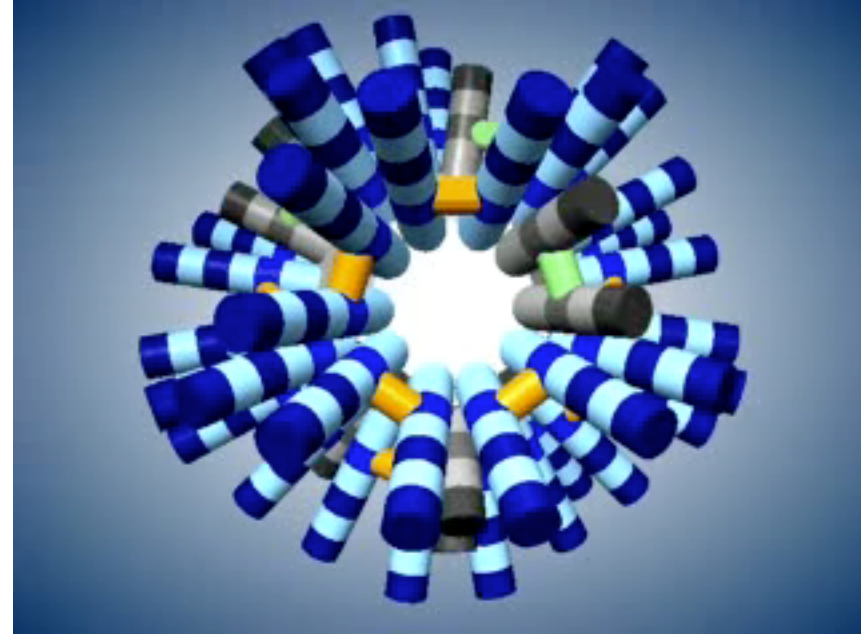


Outline

- AUC background
- How AUC experiments are performed
- Data analysis
- **Example: simple model-independent investigation of a hetero-association**
- Detergent solubilised systems
- Hydrodynamic bead modelling (HBM)
- Example: oligomerisation of synthetic polyvalent integrin $\alpha_5\beta_1$ ligands

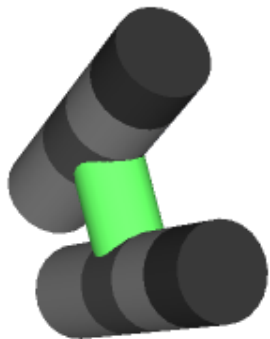
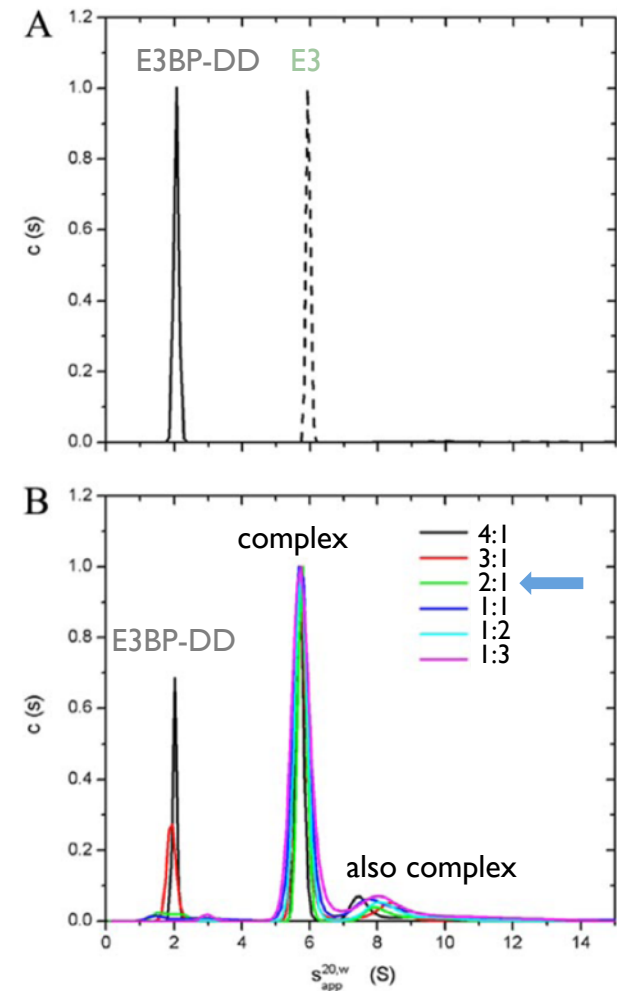
Hetero-association example: PDC E3BP:E3 sub-complex

- E3 forms a homo-dimer
- E3BP binds to E3
- Native PAGE & ITC shows 2:1 complex
- Needed confirmation



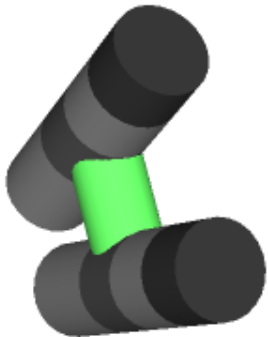
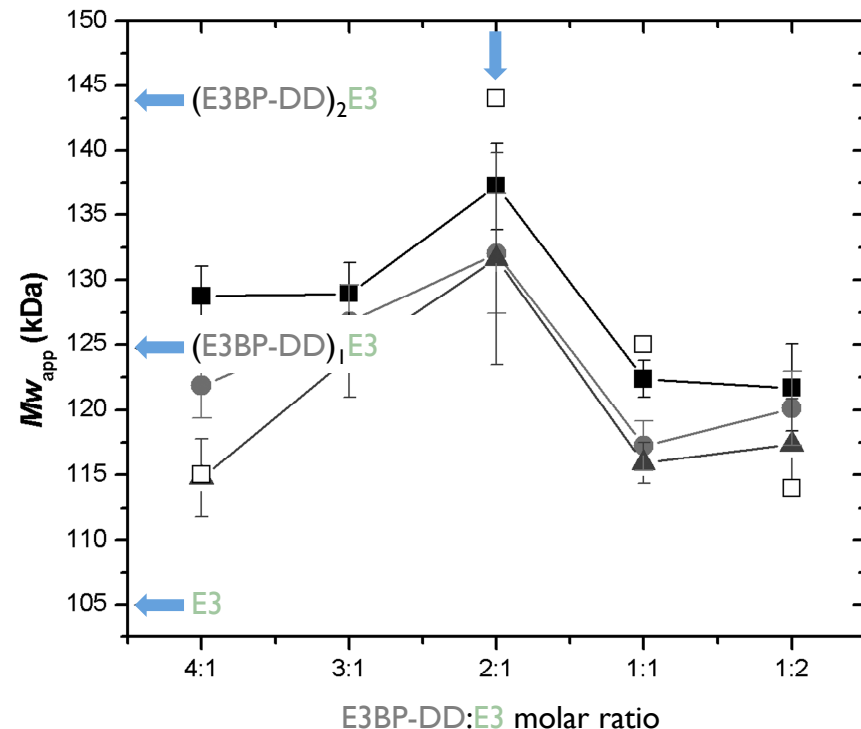
SV titration: stoichiometry is 2:1

- Expt 1: SV of E3 alone; SV of E3BP-DD alone
 - Determine their s
- Expt 2: SV of different E3BP-DD+E3 ratios
 - At what ratio does E3BP-DD peak vanish?
 - This reveals stoichiometry: 2:1
 - Note 2 complex peaks
 - Different conformations
 - $s \approx 6$ S peak less compact
 - $s \approx 8$ S peak more compact



SE titration: stoichiometry is 2:1

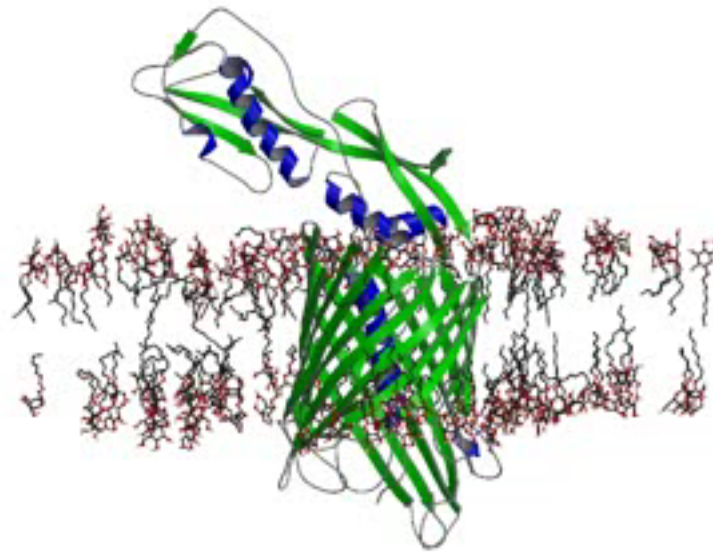
- Whole-cell weight-average M ($M_{w,app}$) determined
 - e.g. using species analysis in SEDPHAT with 1 species only
 - No model assumed
- When E3BP-DD is in excess
 - $M_{w,app} < M_{complex}$ until complex is formed
- When E3 is in excess
 - $M_{w,app} < M_{complex}$ because excess E3 lowers $M_{w,app}$
- ??? Why $M_{w,app} \neq M_{complex}$ at 2:1???



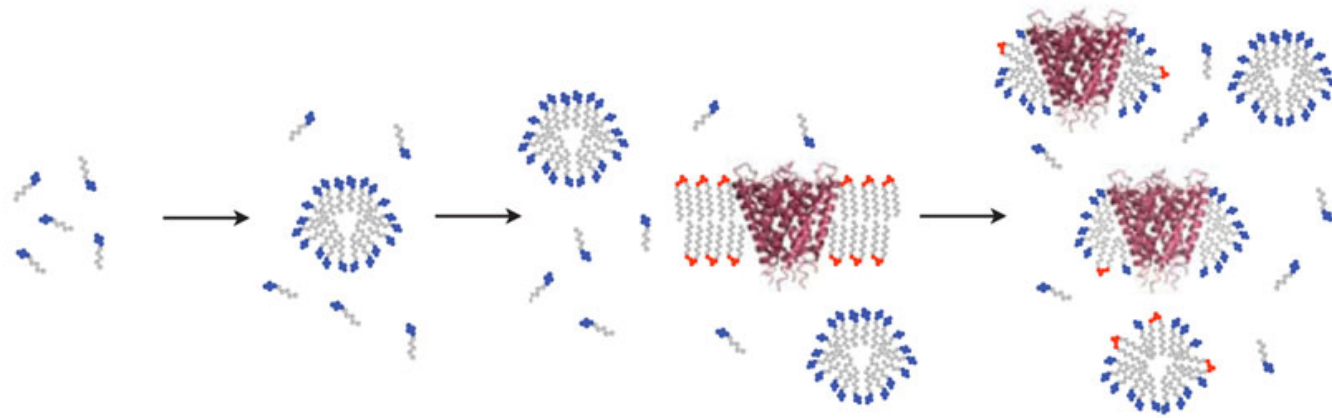
Outline

- AUC background
- How AUC experiments are performed
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Illustrative movie of association of 140 detergent molecules with membrane protein FhaC



Detergent solubilised proteins are complex mixtures



Sedimentation velocity of proteins solubilised in detergent

Methods 54 (2011) 56–66



Contents lists available at [ScienceDirect](#)

Methods

journal homepage: www.elsevier.com/locate/ymeth



Review Article

Sedimentation velocity to characterize surfactants and solubilized membrane proteins

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^bCNRS, UMR 5075, IBS, 41 rue Jules Horowitz, F-38027 Grenoble, France

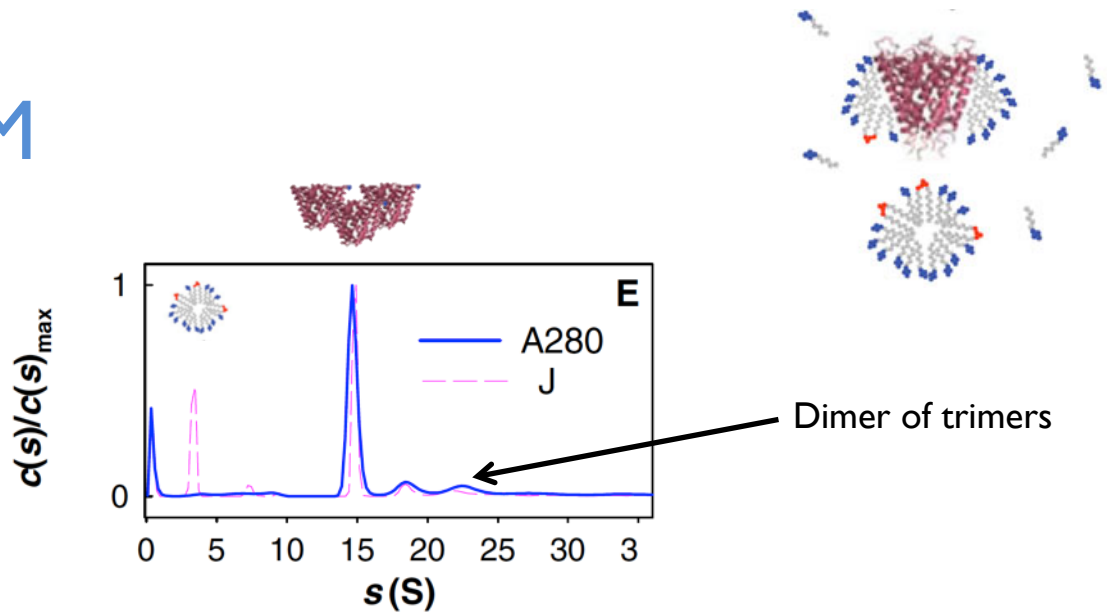
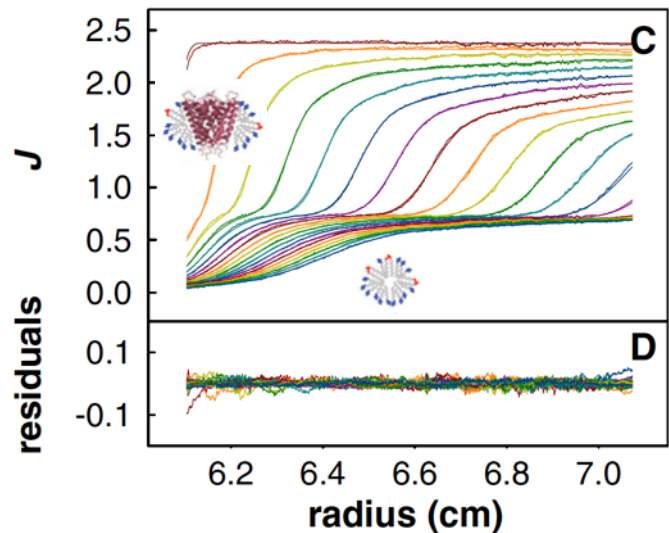
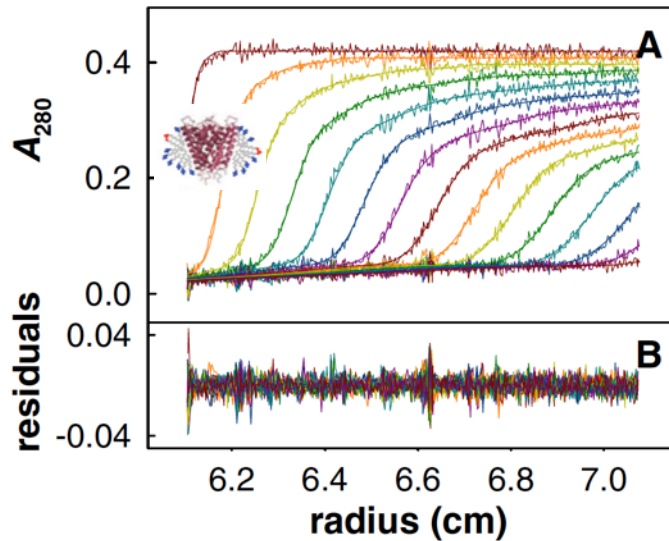
^cUniversité Joseph Fourier, IBS, 41 rue Jules Horowitz, F-38027 Grenoble, France

Values for $\bar{v}$ (psv)

Component	$\bar{v}$ (ml/g)	Comment
Protein	0.73	Can be calculated from sequence quite reliably
Carbohydrate	0.63	Can be calculated from sequence quite reliably
Lipid	1.02	An average value Close to value for solvent, therefore almost invisible
Detergent	0.7-1.2	Can sediment or float

Detergent	$\bar{v}$ (ml/g)	Comment
C ₈ E ₅	0.95	
LAPAO	1.002	Doesn't sediment
Octyl-POE	0.997	
Triton X-100		Absorbs @ 280 nm
LDAO	1.13	Floats
DDM	0.82	Aggregation number $\approx$ 130

SV of AcrB in DDM



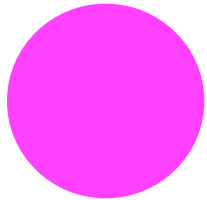
- $s \approx 14.9$ S for 66% of material (protein-detergent)
- $s \approx 3.3$ S (observed from ΔJ) (micelles)

Outline

- AUC background
- How AUC experiments are performed
- Data analysis
- Example: simple model-independent investigation of a hetero-association
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- **Hydrodynamic bead modelling (HBM)**
- Example: oligomerisation of synthetic polyvalent integrin $\alpha_5\beta_1$ ligands

s = deviation from sphericity + hydrodynamic hydration

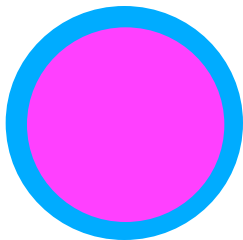
$$s = \frac{M(1 - \bar{v}\rho)}{N_A f}$$



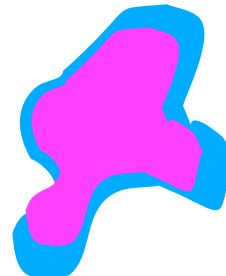
M, f_0



$M, f > f_0$

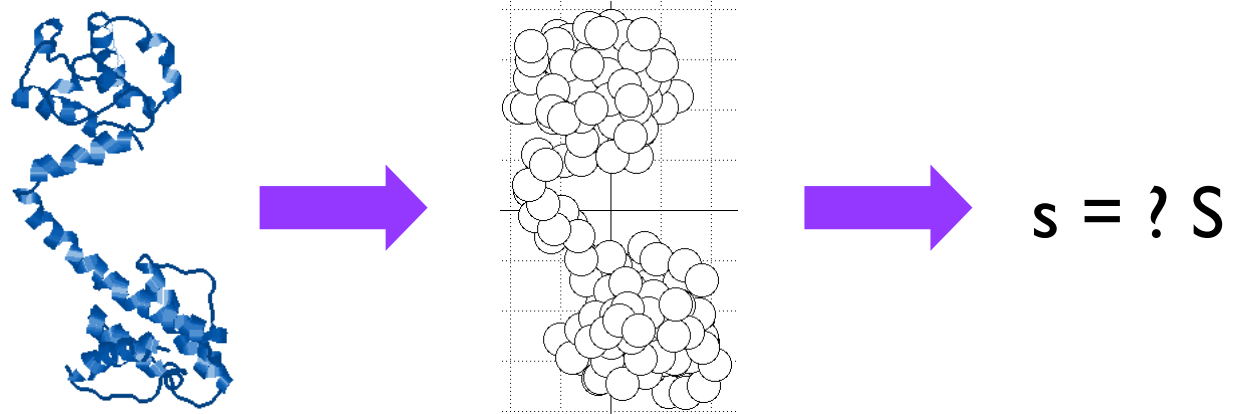


$M, f > f_0$



$M, f \gg f_0$

Sedimentation coefficient is a constraint for SAS modelling



- For one sphere

$$f_0 = 6\pi\eta_0\sigma$$

- For an assembly of N spheres

$$\mathbf{F}_i = -f_i (\mathbf{u}_i - \mathbf{v}_i^0) - f_i \sum_{j=1}^N \mathbf{T}_{ij} \mathbf{F}_j$$

- where

$$\mathbf{T}_{ij} = \frac{1}{8\pi\eta_0 R_{ij}} \left(\mathbf{I} + \frac{\mathbf{R}_{ij} \mathbf{R}_{ij}}{R_{ij}^2} \right)$$

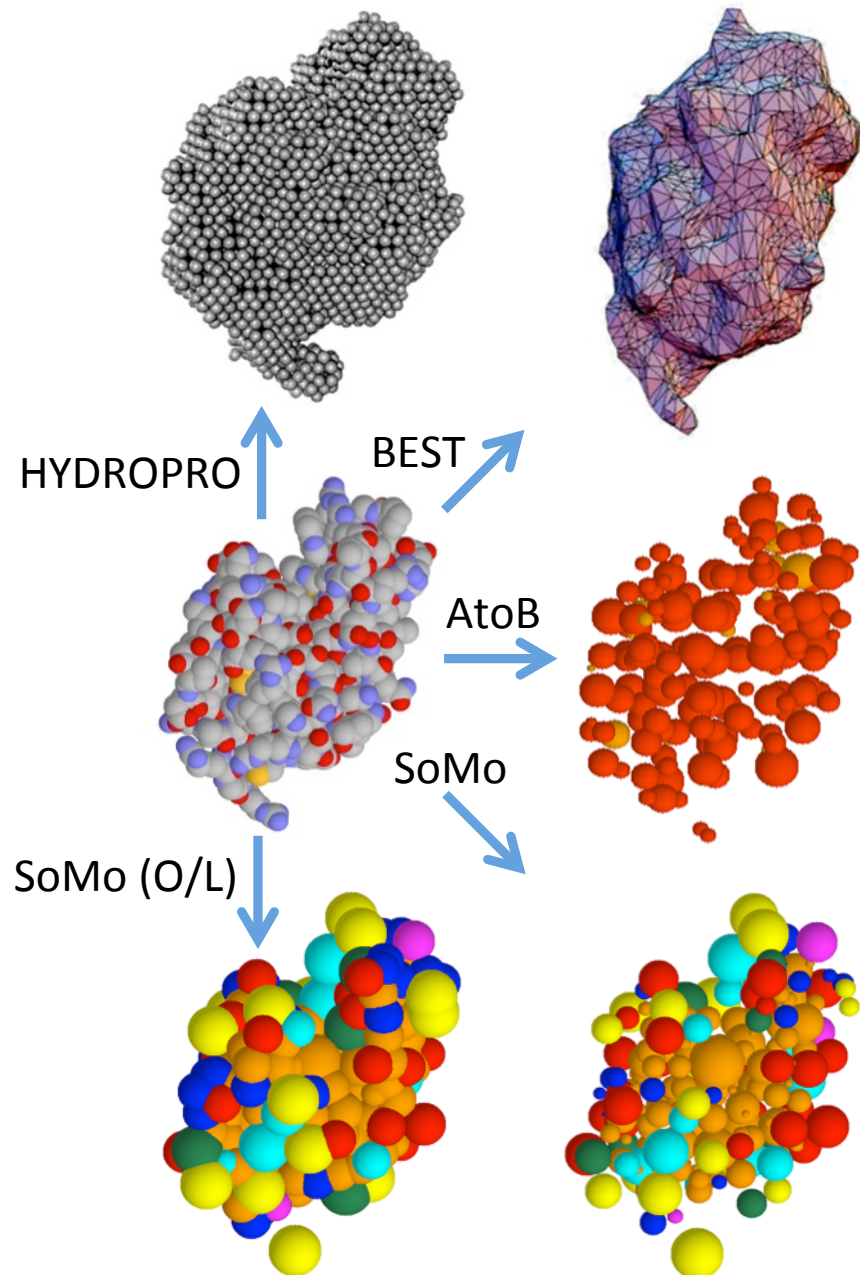
Several freely available programs for HBM

- José García de la Torre *et al.*
 - <http://leonardo.inf.um.es/macromol/programs/programs.htm>
 - HYDRO++
 - Computes hydrodynamic & other parameters for any bead model
 - HYDROPRO
 - Computes hydrodynamic & other parameters for models constructed from pdb files
 - And many other programs....
- Mattia Rocco, Emre Brookes
 - <http://somo.uthscsa.edu/>
 - US-SOMO
 - AtoB and SoMo
 - Generates HBMs from pdb files, computes hydrodynamic & other parameters with “realistic” hydration
 - BEST (Sergio Aragon)
 - Boundary element modeling – very precise, computationally intensive
 - Zeno
 - Electrostatic-hydrodynamic analogy

Reviewed in Byron (2008) *Methods in Cell Biology* 84, 327-373

See also Rocco & Byron, *Methods in Enzymology* (2015) 562, 81-108

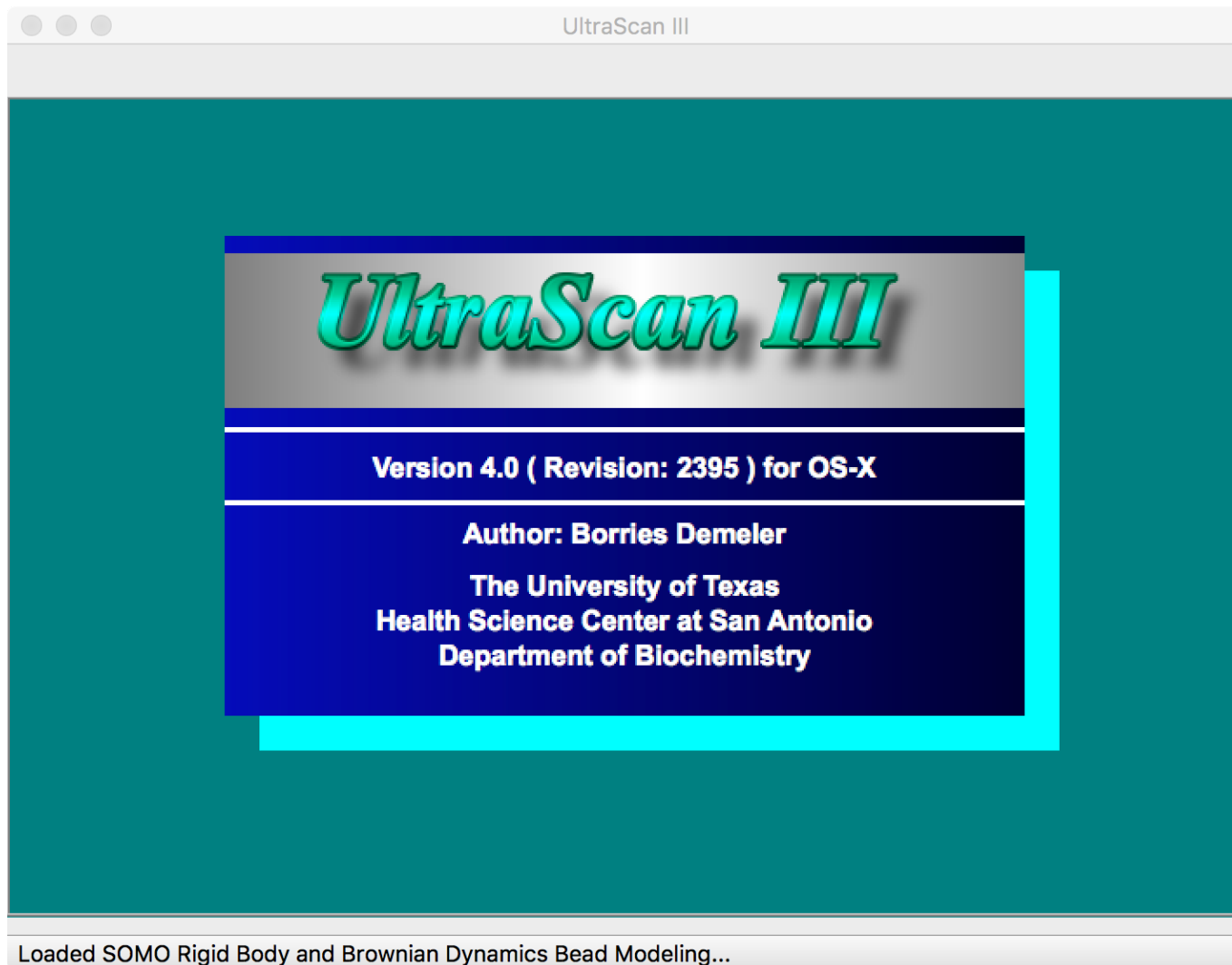
Different HM methods have their own pros and cons



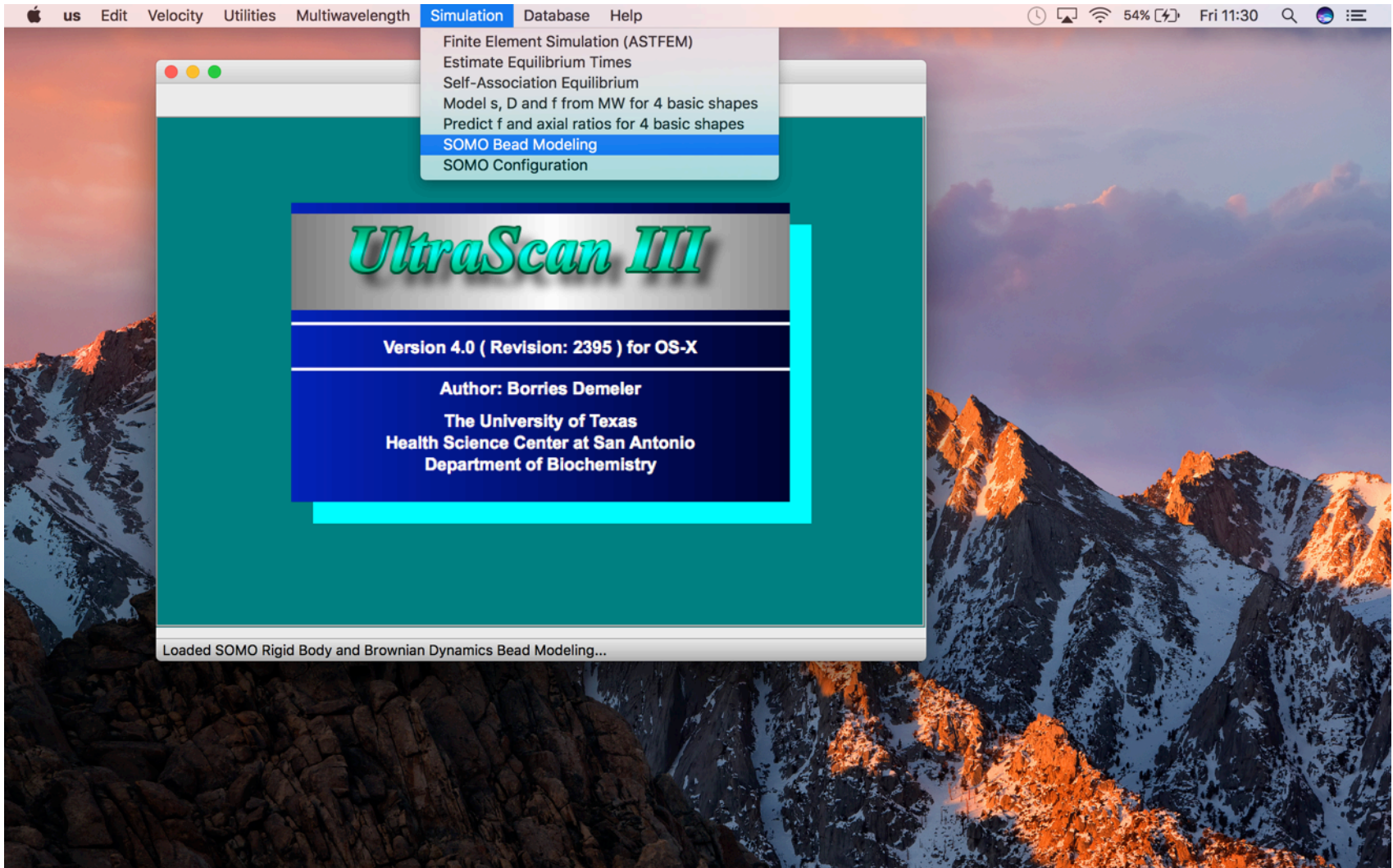
Rocco & Byron, *Methods in Enzymology* (2015) 562, 81-108

Rocco & Byron, *European Biophysics Journal* (2015) 44, 417-431

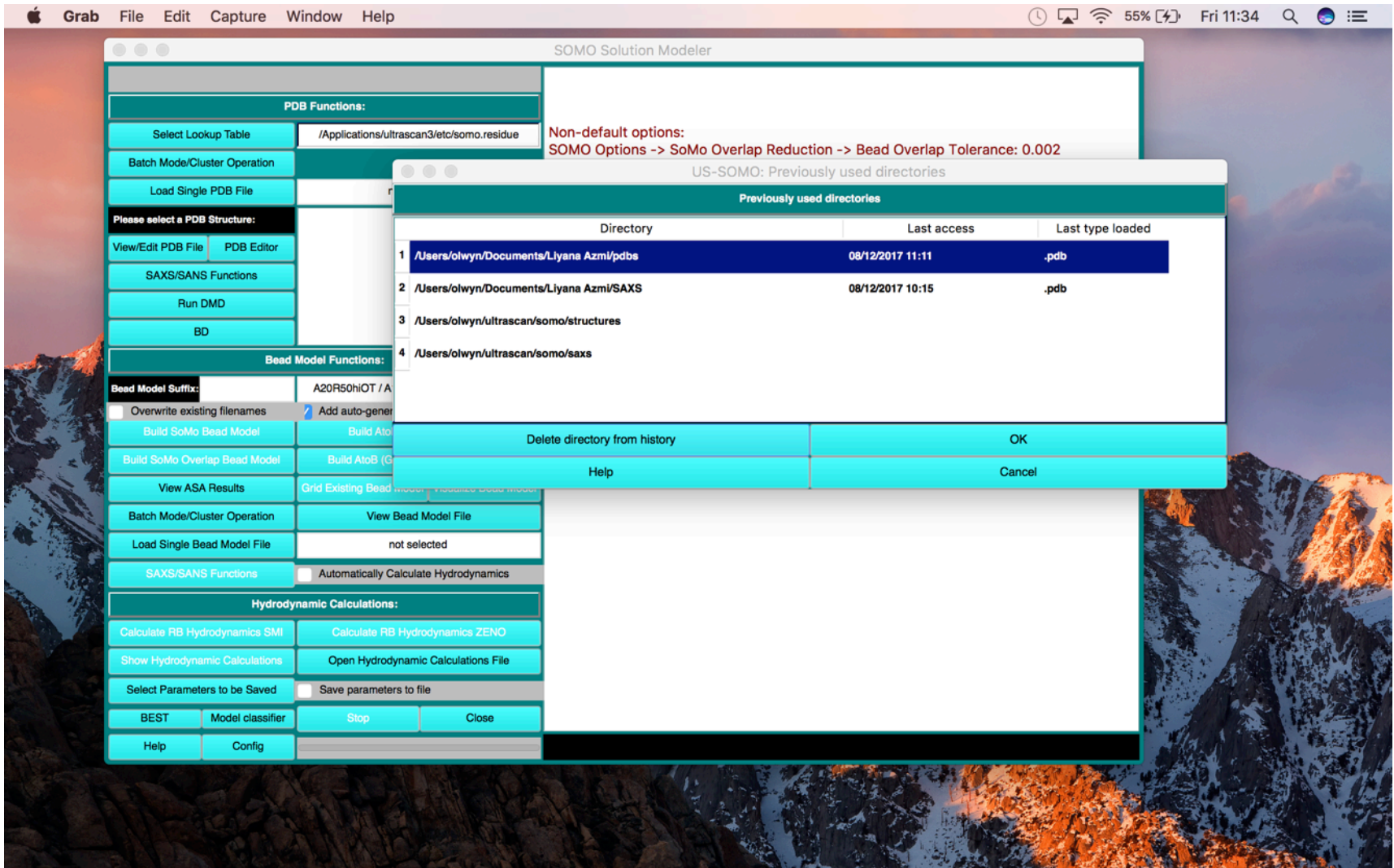
SOMO is a subprogram of UltraScan III



Select from Simulation drop-down menu



I: Load PDB file



2: File read, checked for compatibility, displayed with RasMol

The screenshot displays the SOMO Solution Modeler application window. The interface is divided into several sections:

- PDB Functions:** Includes buttons for 'Select Lookup Table' (set to '/Applications/ultrascan3/etc/somo.residue'), 'Batch Mode/Cluster Operation', 'Load Single PDB File' (set to 'yana Azmi/pdbs/AdhE_D1_predicted_171207.pdb'), 'Please select a PDB Structure:' (set to 'Model: 1'), 'View/Edit PDB File', 'PDB Editor', 'SAXS/SANS Functions', 'Run DMD', and 'BD'.
- Bead Model Functions:** Includes 'Bead Model Suffix:' (set to 'A20R50hIoT / A10R30syOTHyG5 / A20R50'), 'Overwrite existing filenames' (checked), 'Add auto-generated suffix' (checked), 'Build SoMo Bead Model', 'Build AtoB (Grid) Bead Model', 'Build SoMo Overlap Bead Model', 'Build AtoB (Grid) Overlap Bead Model', 'View ASA Results', 'Grid Existing Bead Model', 'Visualize Bead Model', 'Batch Mode/Cluster Operation', 'View Bead Model File', 'Load Single Bead Model File' (set to 'not selected'), 'SAXS/SANS Functions', and 'Automatically Calculate Hydrodynamics' (checked).
- Hydrodynamic Calculations:** Includes 'Calculate RB Hydrodynamics SMI', 'Calculate RB Hydrodynamics ZENO', 'Show Hydrodynamic Calculations', 'Open Hydrodynamic Calculations File', 'Select Parameters to be Saved' (checked), 'Save parameters to file' (checked), 'BEST', 'Model classifier', 'Stop', 'Close', 'Help', and 'Config'.

The right panel displays a list of warnings and model information:

volume
Warning: hybrid_map name missing for hybrid_name C4H1, not added to excluded volume
Warning: hybrid_map name missing for hybrid_name C3H0, not added to excluded volume
Warning: hybrid_map name missing for hybrid_name O1H0, not added to excluded volume
Warning: hybrid_map name missing for hybrid_name C4H3, not added to excluded volume
Warning: hybrid_map name missing for hybrid_name N3H1, not added to excluded volume
Warning: hybrid_map name missing for hybrid_name C4H1, not added to excluded volume
Warning: hybrid_map name missing for hybrid_name C3H0, not added to excluded volume
Warning: hybrid_map name missing for hybrid_name O1H0, not added to excluded volume
Warning: hybrid_map name missing for hybrid_name C4H2, not added to excluded volume
Warning: hybrid_map name missing for hybrid_name C4H2, not added to excluded volume
Warning: hybrid_map name missing for hybrid_name C3H0, not added to excluded volume
Warning: hybrid_map name missing for hybrid_name O1H0, not added to excluded volume
Warning: hybrid_map name missing for hybrid_name O2H1, not added to excluded volume

Model: 1 Chain: Molecular weight 47685 Daltons, Volume (from vbar) 58833.8 A³, atomic volume 0 A³ average electron density nan A⁻³

Model 1 Rg: 2.26 nm

Model: 1 Molecular weight 47685 Daltons, Volume (from vbar) 58833.8 A³, atomic volume 0 A³ average electron density nan A⁻³
AdhE_D1_predicted_171207 model 1 47.69 kD, Rg 22.58 A, (Rg/6.5)³: 41.92 12.1 %

The bottom right corner shows a smaller window titled 'RasMol - AdhE_D1_predicted_1712...' displaying a 3D molecular structure visualization.

3: Compute & display bead model

The image displays two software windows on a desktop. The primary window is the 'SOMO Solution Modeler' application, which features a menu bar (Grab, File, Edit, Capture, Window, Help) and a toolbar. The interface is divided into several functional sections:

- PDB Functions:** Includes buttons for 'Select Lookup Table' (pointing to '/Applications/ultrascan3/etc/somo.residue'), 'Batch Mode/Cluster Operation', 'Load Single PDB File' (with a file path 'yana Azmi/pdbs/AdhE_D1_predicted_171207.pdb'), 'Please select a PDB Structure:' (with 'Model: 1' selected), 'View/Edit PDB File', 'PDB Editor', 'SAXS/SANS Functions', 'Run DMD', and 'BD'.
- Bead Model Functions:** Includes 'Bead Model Suffix:' (set to 'A20R50hiOT-so'), 'Overwrite existing filenames' (checked), 'Add auto-generated suffix' (checked), 'Build SoMo Bead Model', 'Build AtoB (Grid) Bead Model', 'Build SoMo Overlap Bead Model', 'Build AtoB (Grid) Overlap Bead Model', 'View ASA Results', 'Grid Existing Bead Model', 'Visualize Bead Model', 'Batch Mode/Cluster Operation', 'View Bead Model File', 'Load Single Bead Model File' (with a file path 'AdhE_D1_predicted_171207_1'), 'SAXS/SANS Functions', and 'Automatically Calculate Hydrodynamics' (unchecked).
- Hydrodynamic Calculations:** Includes 'Calculate RB Hydrodynamics SMI', 'Calculate RB Hydrodynamics ZENO', 'Show Hydrodynamic Calculations', 'Open Hydrodynamic Calculations File', 'Select Parameters to be Saved' (with 'Save parameters to file' unchecked), 'BEST', 'Model classifier', 'Stop', 'Close', 'Help', and 'Config'.

The central text area of the SOMO window displays the following status messages:

- There are 3351 atoms in 1 chain(s) in this model
- Creating beads from atomic model
- Computing ASA via ASAB1
- Return from Computing ASA
- Anhydrous volume 59506.41 Å³
- There are 864 beads in this model before popping
- Begin popping stage 1
- Beads popped 0.
- Begin radial reduction stage 1
- Begin popping stage 2
- Beads popped 0.
- Begin radial reduction stage 2
- Begin popping stage 3
- Beads popped 0.
- Begin radial reduction stage 3
- Finished with popping and radial reduction
- Rechecking beads
- 3 previously buried beads are exposed by rechecking
- Finished rechecking beads
- Build bead model completed
- Visualizing model 1

The second window, titled 'RasMol - AdhE_D1_predicted_171207_1-A20.bms', shows a 3D visualization of the bead model as a cluster of multi-colored spheres. Below it, a smaller window displays a wireframe representation of the same molecular structure.

4: Compute & display hydrodynamic parameters

The screenshot displays the SOMO Solution Modeler software interface. The main window is titled "SOMO Solution Modeler" and features a menu bar (Grab, File, Edit, Capture, Window, Help) and a status bar (Fri 11:45). The interface is divided into several panels:

- PDB Functions:** Includes buttons for "Select Lookup Table" (pointing to "/Applications/ultrascan3/etc/somo.residue"), "Batch Mode/Cluster Operation", "Load Single PDB File" (pointing to "yana Azmi/pdbs/AdhE_D1_predicted_171207.pdb"), "Please select a PDB Structure:" (with "Model: 1" selected), "View/Edit PDB File", "PDB Editor", "SAXS/SANS Functions", "Run DMD", and "BD".
- Bead Model Functions:** Includes buttons for "Bead Model Suffix:" (set to "A20R50hiOT-so"), "Overwrite existing filenames" (with "Add auto-generated suffix" selected), "Build SoMo Bead Model", "Build AtoB (Grid) Bead Model", "Build SoMo Overlap Bead Model", "Build AtoB (Grid) Overlap Bead Model", "View ASA Results", "Grid Existing Bead Model", "Visualize Bead Model", "Batch Mode/Cluster Operation", "View Bead Model File", "Load Single Bead Model File" (pointing to "AdhE_D1_predicted_171207_1"), "SAXS/SANS Functions", and "Automatically Calculate Hydrodynamics".
- Hydrodynamic Calculations:** Includes buttons for "Calculate RB Hydrodynamics SMI", "Calculate RB Hydrodynamics ZENO", "Show Hydrodynamic Calculations", "Open Hydrodynamic Calculations File", "Select Parameters to be Saved" (with "Save parameters to file" selected), "BEST", "Model classifier", "Stop", "Close", "Help", and "Config".

On the right side, a panel titled "SOMO Hydrodynamic Results" displays a 3D visualization of a protein structure (AdhE_D1) and a table of results:

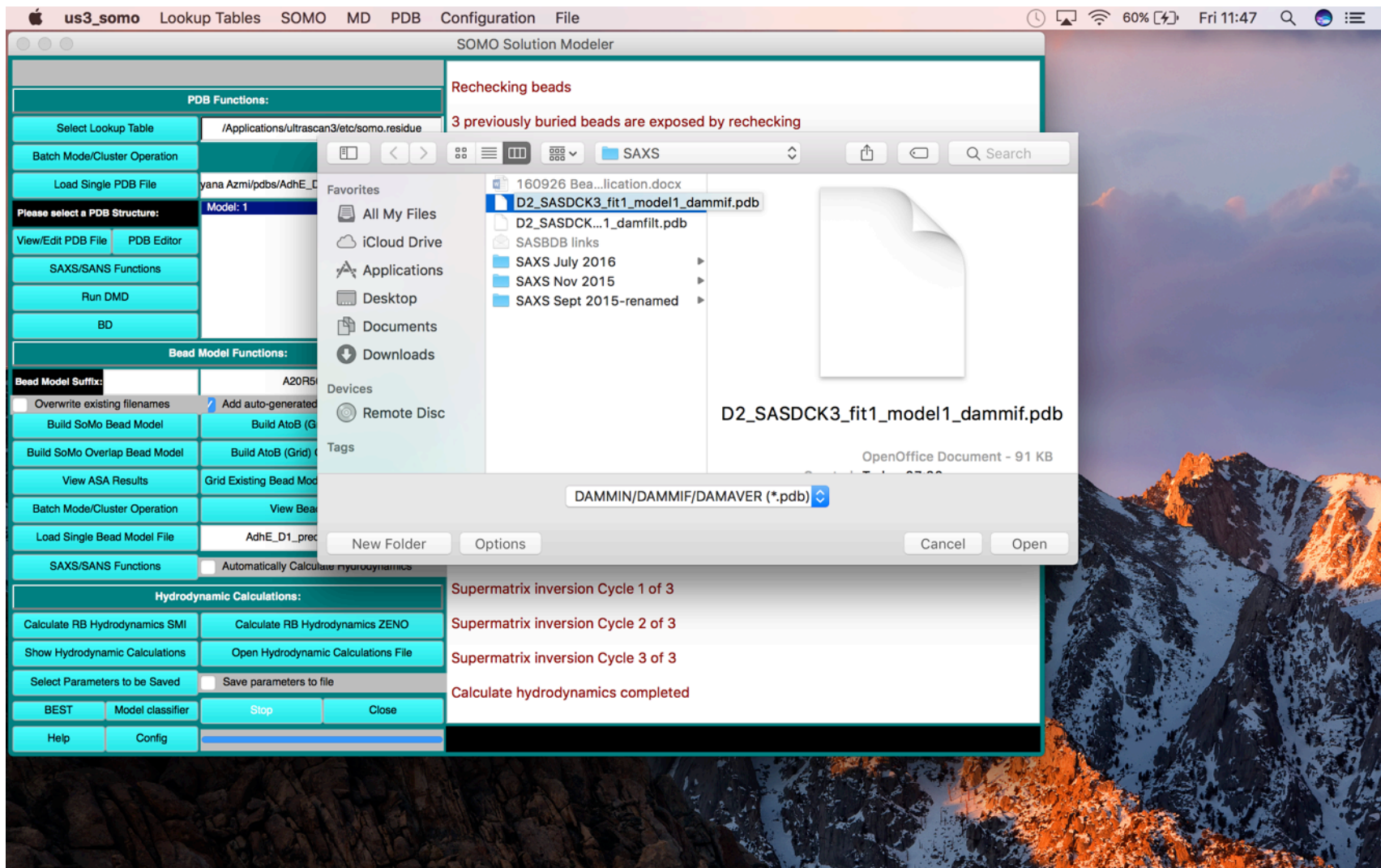
SOMO Hydrodynamic Results (Water at 20°C) (Density 1.00194 cP, Viscosity 0.998234 g/ml)	
Model:	dhE_D1_predicted_171207-A20R50hiOT-so
Method:	SMI
Total Beads In Model:	864
Used Beads In Model:	260
Molecular Mass:	4.7685e+04 Da
Part. Specif. Volume:	0.743 cm³/g
Sedimentation Coefficient s:	3.70e+00 S
Tr. Diffusion Coefficient D:	7.33e-07 cm²/sec²
Stokes Radius:	2.93e+00 nm
Frictional Ratio:	1.21
Radius of Gyration:	2.30e+00 nm
Relaxation Time, tau(h):	3.18e+01 ns
Intrinsic Viscosity:	3.77e+00 cm³/g

Below the table, there are buttons for "View ASA Results File", "View Bead Model File", "View Full Hydrodynamics Results File", "Help", and "Close".

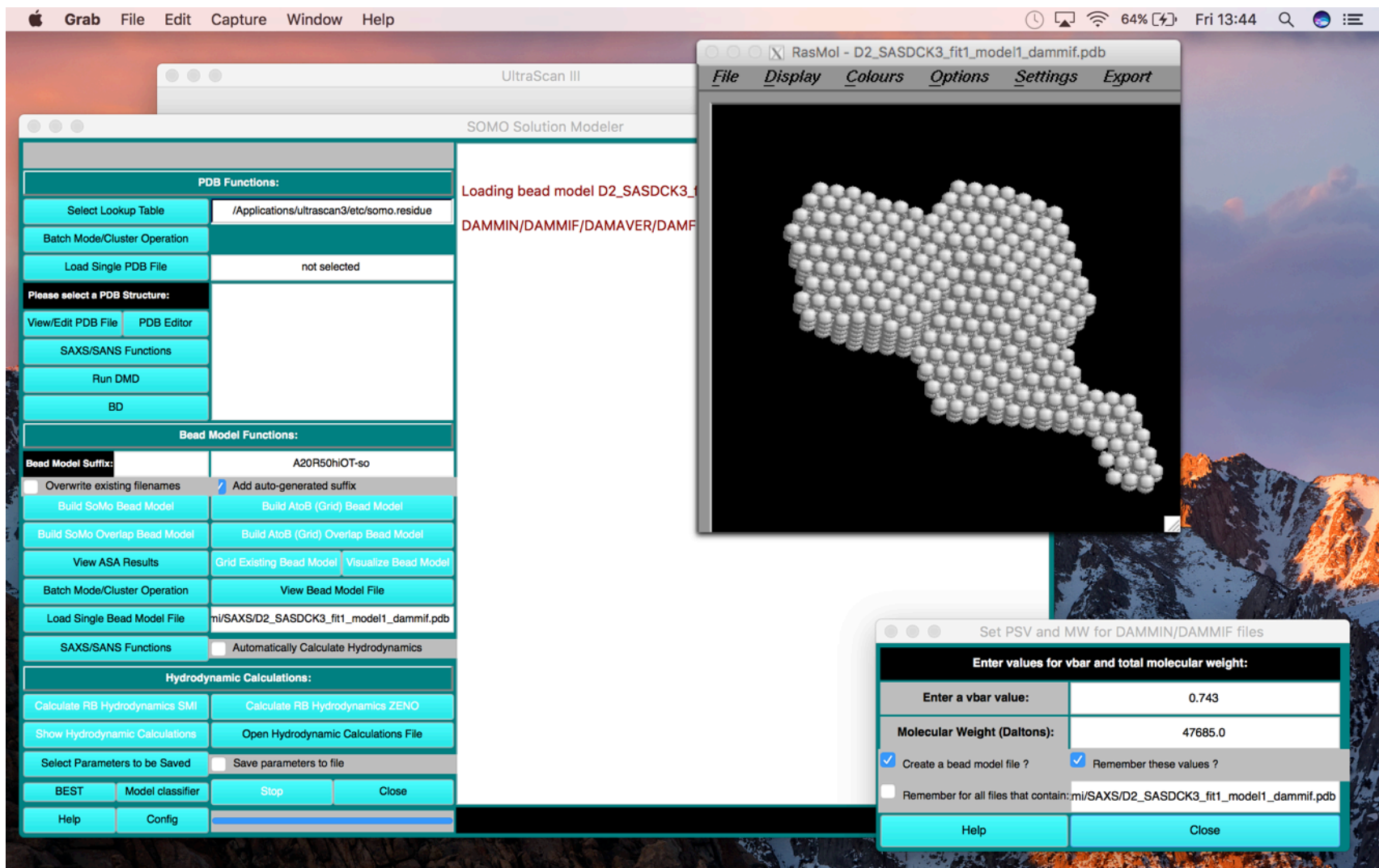
Text overlays on the interface indicate the following steps and results:

- Rechecking beads
- 3 previously buried beads are exposed by rechecking
- Finished rechecking beads
- Build bead model completed
- Visualizing model 1
- Non-default options:
SOMO Options -> SoMo Overlap Reduction -> Bead Overlap Tolerance: 0.002
- To reset to default: Menu bar -> Configuration -> Reset to Default Config
- Begin hydrodynamic calculations
- Model 1 will be included
- Processing model 1 bead count 864 vbar 0.743
- Using 260 beads for the matrix
- Supermatrix inversion Cycle 1 of 3
- Supermatrix inversion Cycle 2 of 3
- Supermatrix inversion Cycle 3 of 3
- Calculate hydrodynamics completed

Can also upload DAMs to SOMO



Input psv and M for model



Convert DAM to HBM

The screenshot displays the SOMO Solution Modeler application window. The interface is divided into several sections:

- PDB Functions:** Includes buttons for 'Select Lookup Table' (pointing to '/Applications/ultrascan3/etc/somo.residue'), 'Batch Mode/Cluster Operation', 'Load Single PDB File' (not selected), 'Please select a PDB Structure:' (Model 1 (from bead model)), 'View/Edit PDB File', 'PDB Editor', 'SAXS/SANS Functions', 'Run DMD', and 'BD'.
- Bead Model Functions:** Includes 'Bead Model Suffix:' (A10R30syOTHyG5-a2bg), 'Overwrite existing filenames' (checked), 'Add auto-generated suffix' (checked), 'Build SoMo Bead Model', 'Build AtoB (Grid) Bead Model', 'Build SoMo Overlap Bead Model', 'Build AtoB (Grid) Overlap Bead Model', 'View ASA Results', 'Grid Existing Bead Model', 'Visualize Bead Model', 'Batch Mode/Cluster Operation', 'View Bead Model File', 'Load Single Bead Model File' (D2_SASDCK3_fit1_model1_dammif_1), 'SAXS/SANS Functions', and 'Automatically Calculate Hydrodynamics' (checked).
- Hydrodynamic Calculations:** Includes 'Calculate RB Hydrodynamics SMI', 'Calculate RB Hydrodynamics ZENO', 'Show Hydrodynamic Calculations', 'Open Hydrodynamic Calculations File', 'Select Parameters to be Saved' (Save parameters to file), 'BEST', 'Model classifier', 'Stop', 'Close', 'Help', and 'Config'.

The right panel shows the status of the conversion process:

- Rechecking beads
- 39 previously buried beads are exposed by rechecking
- Volume of bead model 29119.5
- Build bead model completed
- Non-default options: SOMO Options -> SoMo Overlap Reduction -> Bead Overlap Tolerance
- To reset to default: Menu bar -> Configuration -> Reset to Default Co
- Begin hydrodynamic calculations
- Model Model 1 (from bead model) will be included
- Processing model 1 bead count 584 vbar 0.743
- Using 471 beads for the matrix
- Supermatrix inversion Cycle 1 of 3
- Supermatrix inversion Cycle 2 of 3
- Supermatrix inversion Cycle 3 of 3
- Calculate hydrodynamics completed
- Visualizing model 1

The bottom right window, titled 'RasMol - D2_SASDCK3_fit1_model1_dammif.bms', displays a 3D visualization of the bead model, showing a cluster of orange and yellow beads.

Compute hydrodynamics for DAM-HBM: compare with experimental values

The screenshot displays the SOMO Solution Modeler software interface. The main window is titled "SOMO Solution Modeler" and shows a file named "D2_SASDCK3_fit1_model1_dammif.bms". The interface is divided into several panels:

- PDB Functions:** Includes buttons for "Select Lookup Table" (set to "/Applications/ultrascan3/etc/somo.residue"), "Batch Mode/Cluster Operation", "Load Single PDB File" (not selected), "Please select a PDB Structure:" (Model 1 (from bead model)), "View/Edit PDB File", "PDB Editor", "SAXS/SANS Functions", "Run DMD", and "BD".
- Bead Model Functions:** Includes "Bead Model Suffix:" (A10R30syOThyG5-a2bg), "Overwrite existing filenames" (checked), "Add auto-generated suffix" (checked), "Build SoMo Bead Model" (Build AtoB (Grid) Bead Model), "Build SoMo Overlap Bead Model" (Build AtoB (Grid) Overlap Bead Model), "View ASA Results" (Grid Existing Bead Model, Visualize Bead Model), "Batch Mode/Cluster Operation" (View Bead Model File), "Load Single Bead Model File" (D2_SASDCK3_fit1_model1_dammif_1), "SAXS/SANS Functions" (Automatically Calculate Hydrodynamics checked), "Hydrodynamic Calculations:" (Calculate RB Hydrodynamics SMI, Calculate RB Hydrodynamics ZENO, Show Hydrodynamic Calculations, Open Hydrodynamic Calculations File, Select Parameters to be Saved, Save parameters to file), "BEST" (Model classifier), "Stop", "Close", "Help", and "Config".
- Log/Status Panel:** Displays the progress of the calculation, including "Supermatrix inversion Cycle 1 of 3", "Supermatrix inversion Cycle 2 of 3", "Supermatrix inversion Cycle 3 of 3", "Calculate hydrodynamics completed", "Visualizing model 1", "Non-default options: SOMO Options -> SoMo Overlap Reduction -> Bead Overlap Tolerance: 0.002", "To reset to default: Menu bar -> Configuration -> Reset", "Begin hydrodynamic calculations", "Model Model 1 (from bead model) will be included", "Processing model 1 bead count 584 vbar 0.743", "Using 471 beads for the matrix", and "Calculate hydrodynamics completed".
- Visualization:** A 3D visualization of the bead model, showing a cluster of orange spheres.
- SOMO Hydrodynamic Results:** A pop-up window showing the results of the calculation, including the model name, method, total beads, used beads, molecular mass, part. spec. volume, sedimentation coefficient, tr. diffusion coefficient, Stokes radius, frictional ratio, radius of gyration, relaxation time, and intrinsic viscosity.

The "SOMO Hydrodynamic Results" window displays the following data:

SOMO Hydrodynamic Results (Water at 20°C) (Density 1.00194 cP, Viscosity 0.998234 g/ml)	
Model:	1_model1_dammif-A10R30syOThyG5-a2bg
Method:	SMI
Total Beads in Model:	584
Used Beads in Model:	471
Molecular Mass:	4.7684e+04 Da
Part. Specif. Volume:	0.743 cm³/g
Sedimentation Coefficient s:	3.39e+00 S
Tr. Diffusion Coefficient D:	6.70e-07 cm²/sec
Stokes Radius:	3.20e+00 nm
Frictional Ratio:	1.32
Radius of Gyration:	2.81e+00 nm
Relaxation Time, tau(h):	4.38e+01 ns
Intrinsic Viscosity:	5.20e+00 cm³/g

Buttons at the bottom of the results window include "View ASA Results File", "View Bead Model File", "View Full Hydrodynamics Results File", "Help", and "Close".

Outline

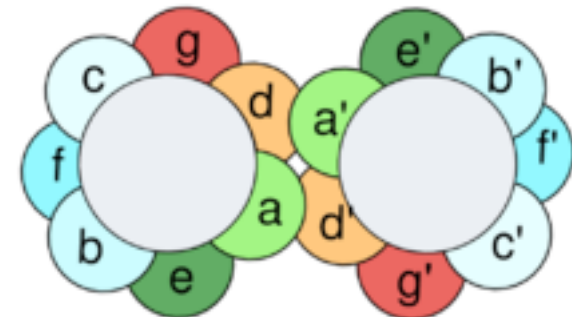
- AUC background
- How AUC experiments are performed
- Data analysis
- Example: simple model-independent investigation of a hetero-association
- Detergent solubilised systems
- Hydrodynamic bead modelling (HBM)
- Example: oligomerisation of synthetic polyvalent integrin $\alpha_5\beta_1$ ligands

Example: Oligomerisation of synthetic polyvalent integrin $\alpha_5\beta_1$ ligands

MRGSHHHHHHGMASGLDSPTGIDFSDITANSFTVHWIAPRATITGYRIRHHPEHFSGRPREDRVPHSRNSIT
 LTNLTPGTEYVVSIVALNGREESPPPLIGQQSTVSDVPRDLEVVAATPTSLLISWDAPAVTVRYRITYGETG
 GNSPVQEFTVPGSKSTATISGLKPGVDYTTITVYAVTGGRGDSPASSKPISINYRTSKLEPKSSDTPPGSPRSP
 EPKSSDTPPGSPRSGRIKQLEDKIEELLSKIYHLENEIARLKKLIGELEDKIENTLGC

↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑

- $\alpha_5\beta_1$ ligands used to immobilise cells on surfaces via
 - 9th type III FN domain synergy site (PHSRN)
 - 10th type III FN domain RGD site
- $\alpha_5\beta_1$ ligand oligomers facilitate increased binding
- Oligomerisation accomplished via 5 heptad repeats based on GCN4 leucine zipper
 - I/L placed variously @ a and d positions to promote di-, tri- & tetramerisation
- Thiol-linked immobilisation to surface achieved via C-terminal Cys
- Question: do the ligands oligomerise as designed?

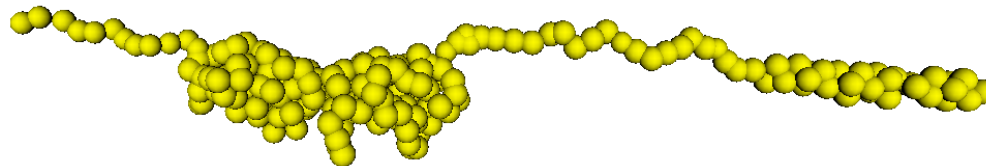


Construction of hydrodynamic bead models

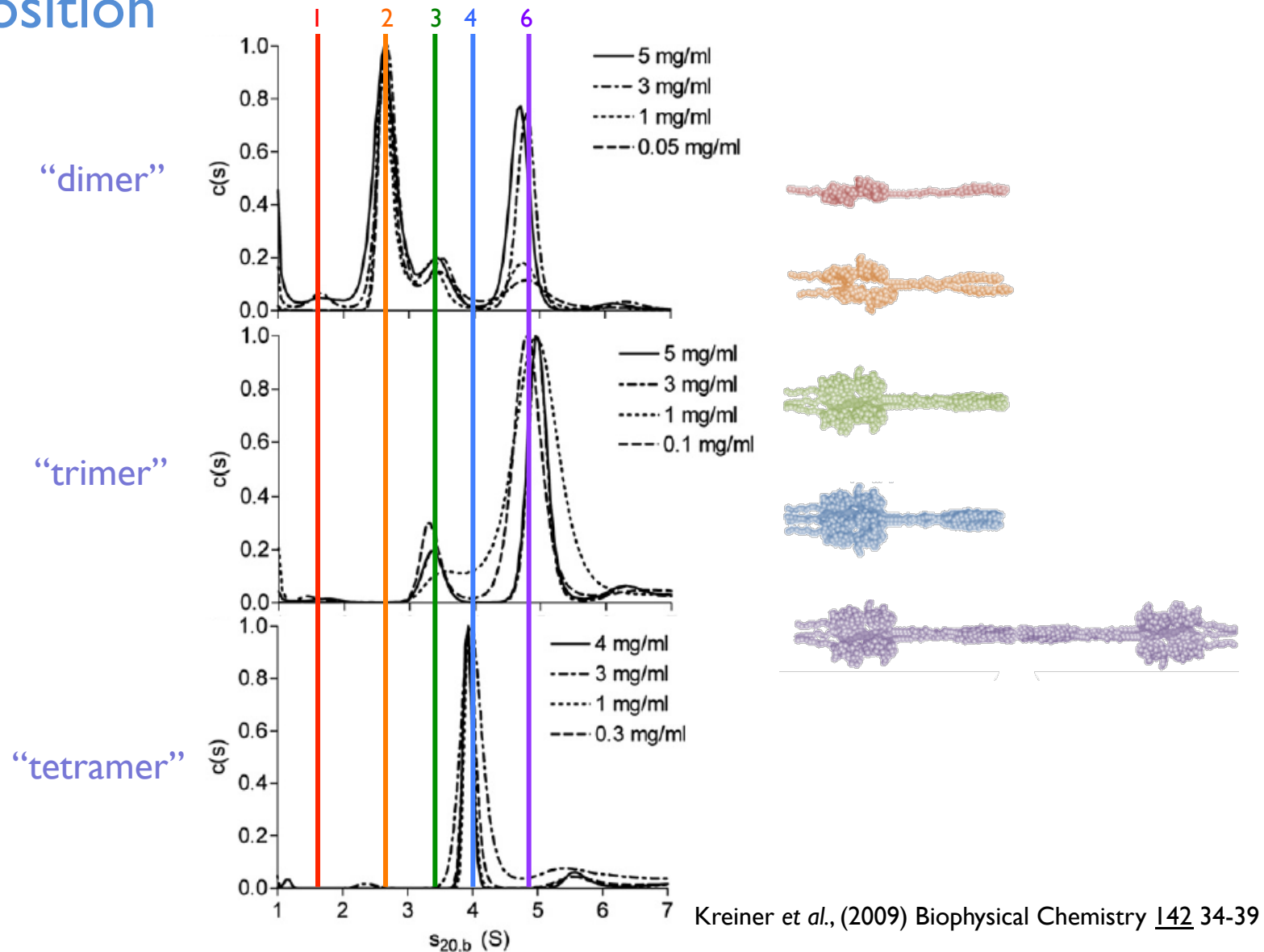
MRGSHHHHHHGMASGLDSPTGIDFSDITANSFTVHWIAPRATITGYRIRHHPEHFSGRPREDRVPHSRNSIT
LTNLTPGTEYVVSIVALNGREESPPPLIGQQSTVSDVPRDLEVVAATPTSLLISWDAPAVTVRYRITYGETG
GNSPVQEF TVPGSKSTATISGLKPGVDYTITVYAVTG RGD SPASSKPISINYRTSKLEPKSSDTPPGSPRSP
EPKSSDTPPGSPRSGRIKQLEDKIEELLSKIYHLENEIARLKKLIGELEDKIENLGC

↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑

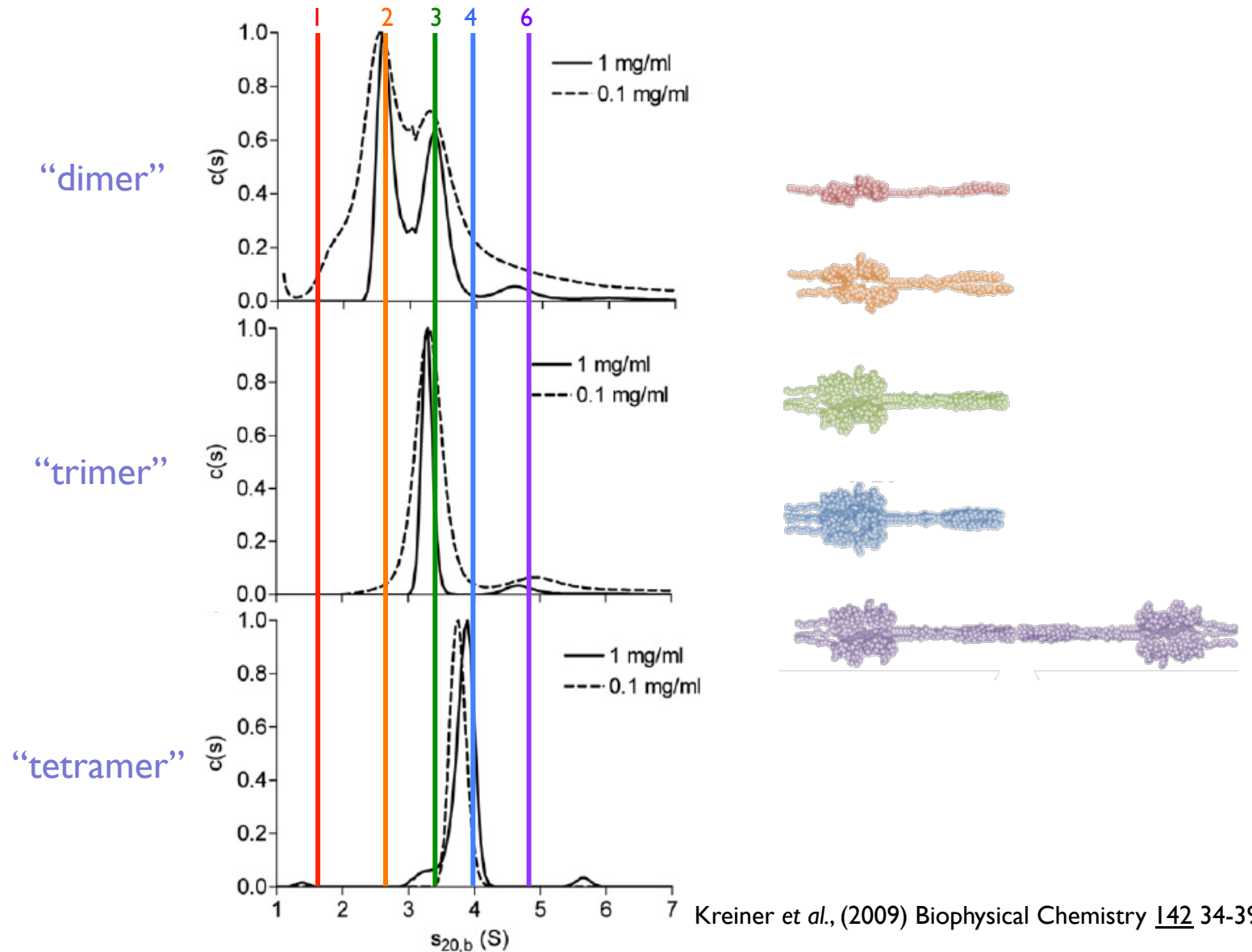
- From vector (including His-tag) – too short for e.g. SWISSMODEL
- FN III 9-10 domain pair homology model (SWISSMODEL)
- Synthesised “missing beads”
- Coiled-coil (42 a.a.) – SWISSMODELS generated for underlined segment



Oligomerisation of synthetic polyvalent integrin $\alpha_5\beta_1$ ligands - AUC SV: c(s) reveals complex composition



AUC SV + DTT: c(s) composition simplified



Outline

- AUC background
- How AUC experiments are performed
- Data analysis
- Example: simple model-independent investigation of a hetero-association
- Detergent solubilised systems
- Hydrodynamic bead modelling (HBM)
- Example: oligomerisation of synthetic polyvalent integrin $\alpha_5\beta_1$ ligands

AUC tutorials

- Setting up and running AUC experiments
 - Tutorial paper
 - Lebowitz, J., M.S. Lewis, and P. Schuck, Modern analytical ultracentrifugation in protein science: A tutorial review. Protein Science, 2002. 11(9): p. 2067-2079.
 - AUC user guide from Demeler lab
 - <http://www.uslims.uthscsa.edu/AUCUserGuideVolume-I-Hardware.pdf>
- Data analysis
 - Using SEDFIT & SEDPHAT
 - <http://www.analyticalultracentrifugation.com/default.htm>
 - Using UltraScan
 - <http://www.ultrascan.uthscsa.edu>

AUC tutorials



- Alexander Bepperling
 - Aggregation analysis and beyond analytical ultracentrifugation in the biopharmaceutical industry
 - <https://www.youtube.com/watch?v=liERbl-Xz4c>
- Borries Demeler
 - Advances in sedimentation analysis
 - <https://www.youtube.com/watch?v=zuAwWOJZtkM>
- Chad Brautigam
 - Exploring the stoichiometry of macromolecular complexes using multi signal sedimentation velocity analytical ultracentrifugation
 - <https://www.youtube.com/watch?v=ea6tvKF8zkA>
- John Burgner
 - Quantitative determination of reaction stoichiometry, interaction energies, and something else
 - <https://www.youtube.com/watch?v=ivRodzqVjS8>
- Andrew Herr
 - Analytical ultracentrifugation as a complementary technique for structural analysis of proteins and macromolecular complexes
 - <https://www.youtube.com/watch?v=Kw72fyaiQsw>

thank you

terima kasih

谢谢

நன்றி



Questions?



If Apple
made a car...

would it have
Windows!?

If a dog wore pants would he wear them
like this or like this?

