

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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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-1-Hardware.pdf>
- Data analysis
 - Using SEDFIT & SEDPHAT
 - <http://www.analyticalultracentrifugation.com/default.htm>
 - Using Ultrascan
 - <http://www.ultrascan.uthscsa.edu/>

Questions that can be answered by AUC

- Is sample heterogeneous?
 - If yes, is it in molecular weight, shape, or both?
 - If yes, does it depend on pH, salt, buffer, etc?
- Is sample pure enough for X-ray crystallography, SAXS, SANS or NMR?
- Does sample...
 - ...self-associate?
 - ...aggregate?
- What is molecular weight of sample, or a mixture of samples?
- Does sample bind to a ligand?
- What is stoichiometry of binding?
- What is K_d ?
- Is association state/conformation affected by tagging?

More questions that can be answered by AUC

- What is sedimentation & diffusion coefficient?
 - Globular or unfolded/disordered?
 - Is conformation dependent on salt, pH, ligand concentration, deuteration, etc?
- Do mutations affect K_d , conformation, stoichiometry, etc?
- Is sample affected by crowding?

The analytical ultracentrifuge (AUC) was invented by Theodor (The) Svedberg

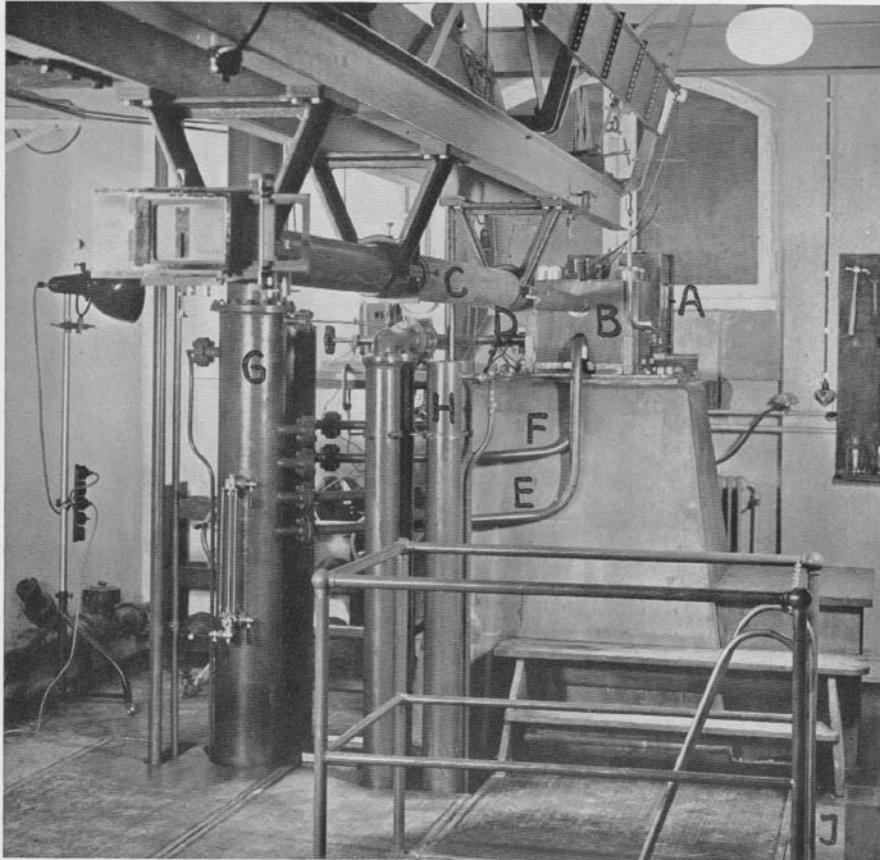


FIG. 68. Oil-turbine ultracentrifuge laboratory at Upsala

- | | |
|------------------------|--------------------------------------|
| A. Lamp-house; | F. Bearing oil outlet and oil drain; |
| B. Centrifuge; | G. Main oil container; |
| C. Camera; | H. Oil coolers; |
| D. Turbine oil inlet; | I. Steps to compressor pit. |
| E. Turbine oil outlet; | |



Nobel Prize in Chemistry 1926 awarded to The Svedberg *"for his work on disperse systems"*

Svedberg was an interesting man...

- Married 4 times
- 12 children!
- Liked to paint
 - “Atomics”



Svedberg in front of his textile print Atomics. (Gustaf Werner Institute archives)



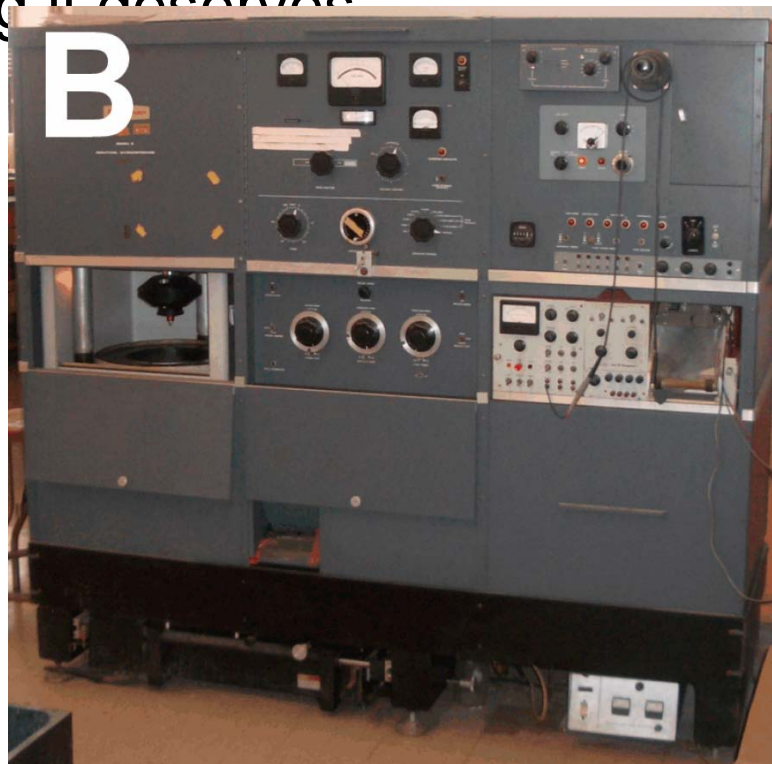


Svedberg in front of his textile print Atoms. (Gustaf Werner Institute archives)

1960's-80's

AUC = core biochemical/biophysical technology

- Advice from the Beckman Model E AUC 1964 manual:
- “The Model E, like a woman, performs best when you care. But you needn't pamper it - just give it the understanding it deserves.”

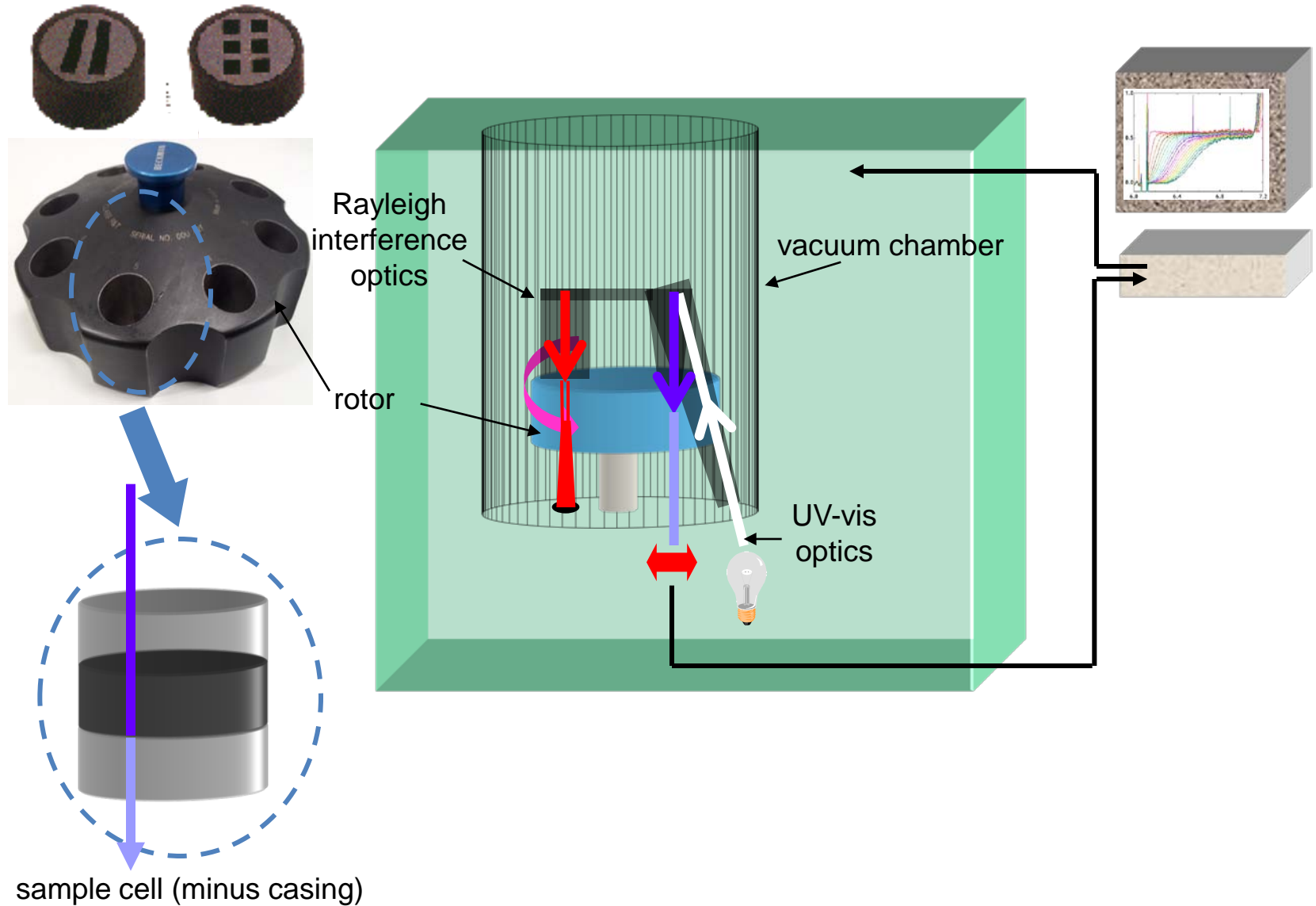


The modern AUC a high speed preparative UC with optics



Beckman Coulter ProteomeLab XL-A/XL-I; €250-350 k

Inside the Beckman Coulter XL-I



Relationship between data and sample: absorbance

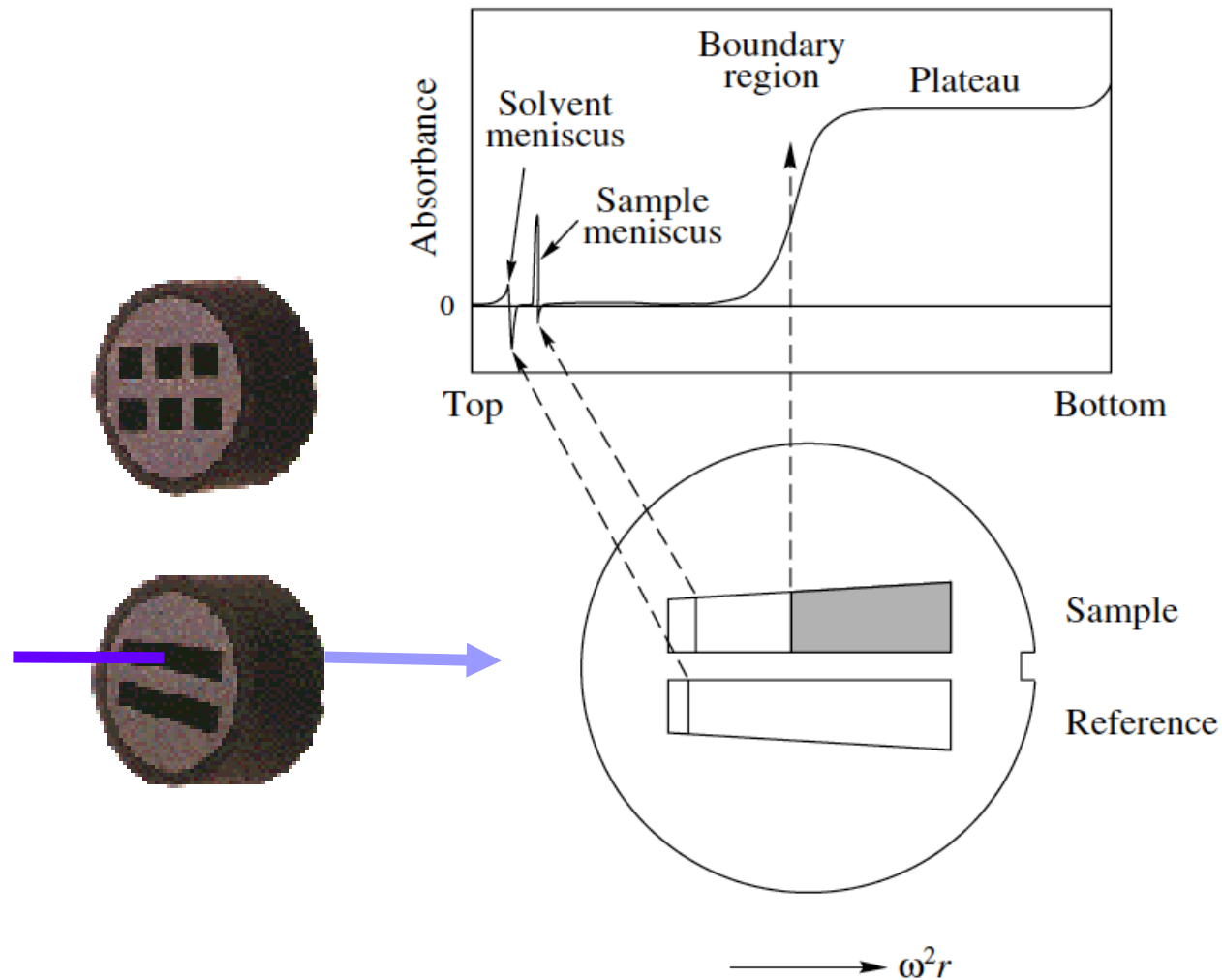


image from Ralston, 1993

<https://www.beckmancoulter.com/wsrportal/bibliography?docname=361847.pdf>

Inside the rotor chamber

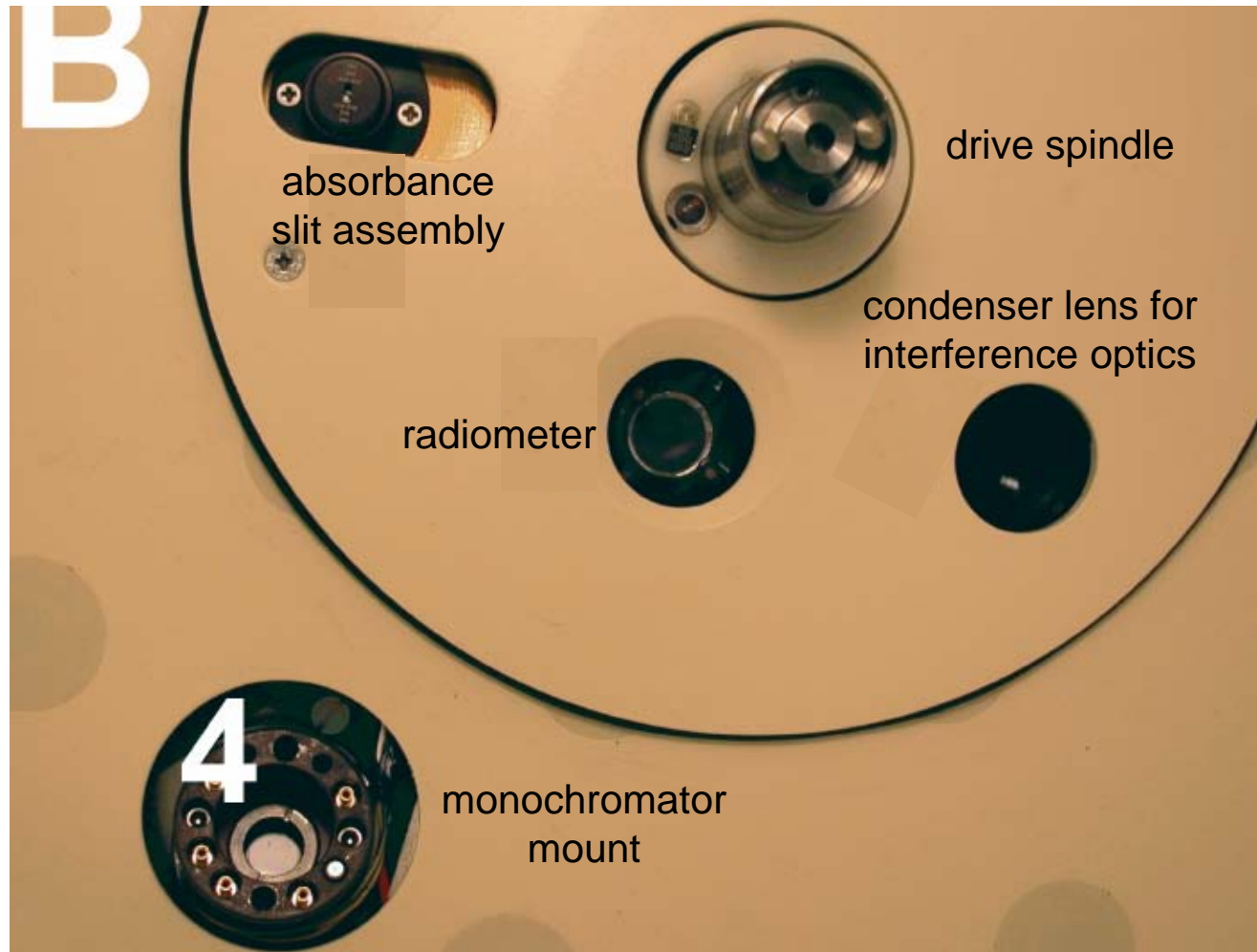
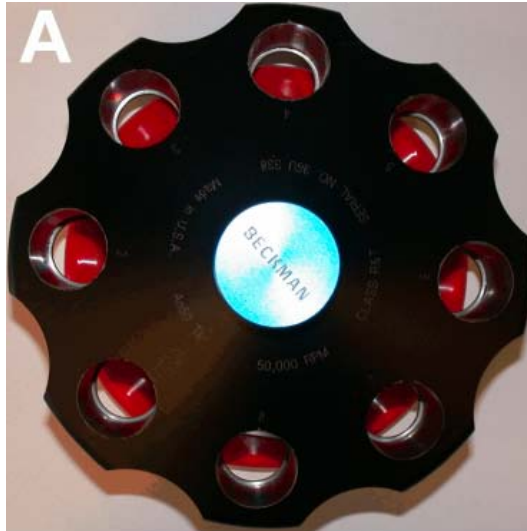


image from Analytical Ultracentrifuge User Guide Volume 1: Hardware, K. L. Planken & V. Schirf, 2008
(<http://www.ultrascan.uthscsa.edu/>)

Sample holders sit in holes in the AUC rotor

50k rpm



60k rpm

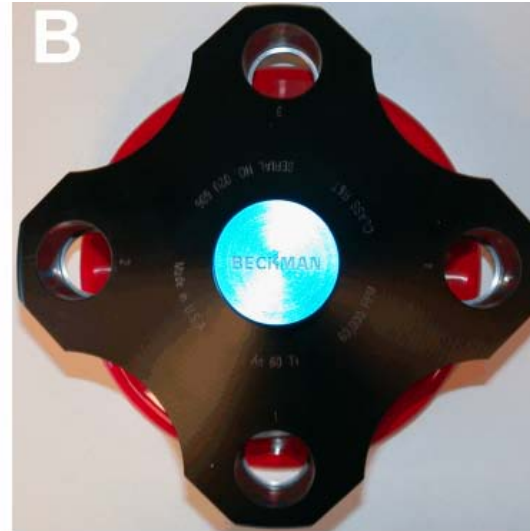
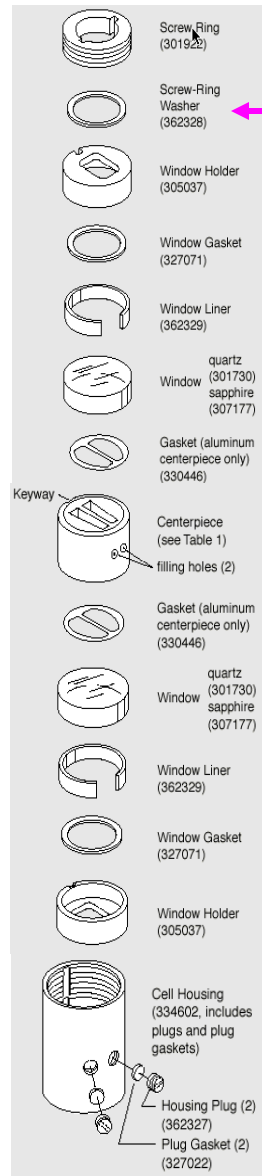


image from Analytical Ultracentrifuge User Guide Volume 1: Hardware, K. L. Planken & V. Schirf, 2008
(<http://www.ultrascan.uthscsa.edu/>)

The most difficult part of an AUC experiment: assembling the sample holders



Nose grease !!!

image from Beckman AUC manual
http://www.beckmancoulter.com/resourcecenter/labresources/resource_xla_xli.asp

Loading a sample

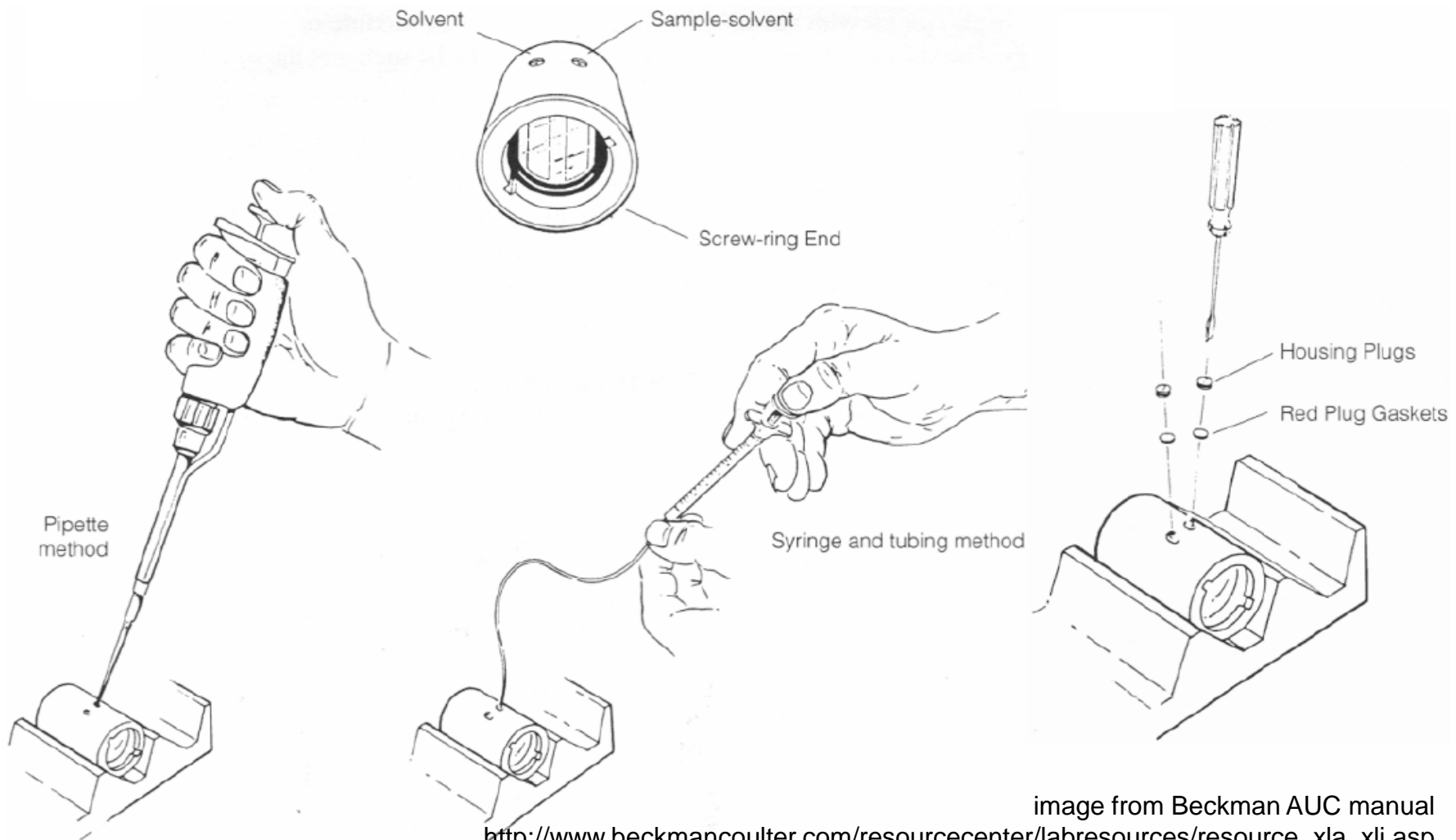


image from Beckman AUC manual
http://www.beckmancoulter.com/resourcecenter/labresources/resource_xla_xli.asp

Absorbance optics: the AUC is like a spinning double-beam spectrophotometer

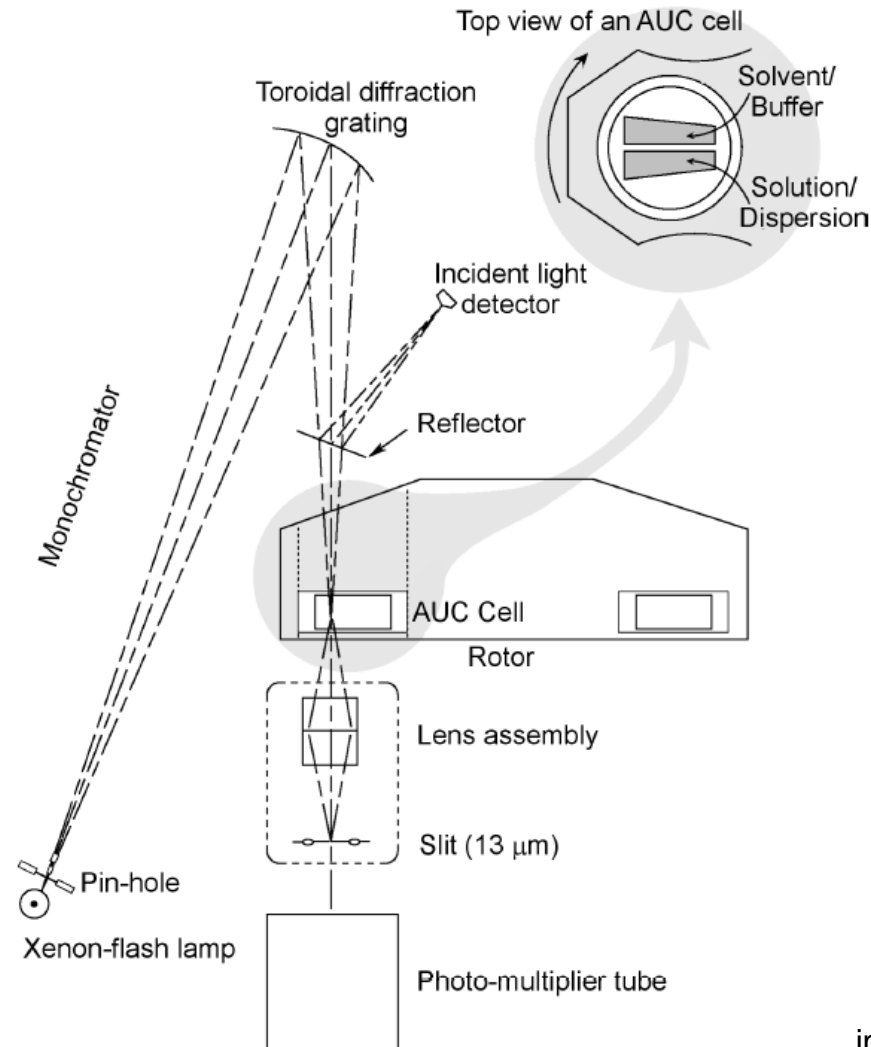


image from Beckman AUC manual

http://www.beckmancoulter.com/resourcecenter/labresources/resource_xla_xli.asp

Interference optics acquire refractive index data rapidly, independent of chromophores

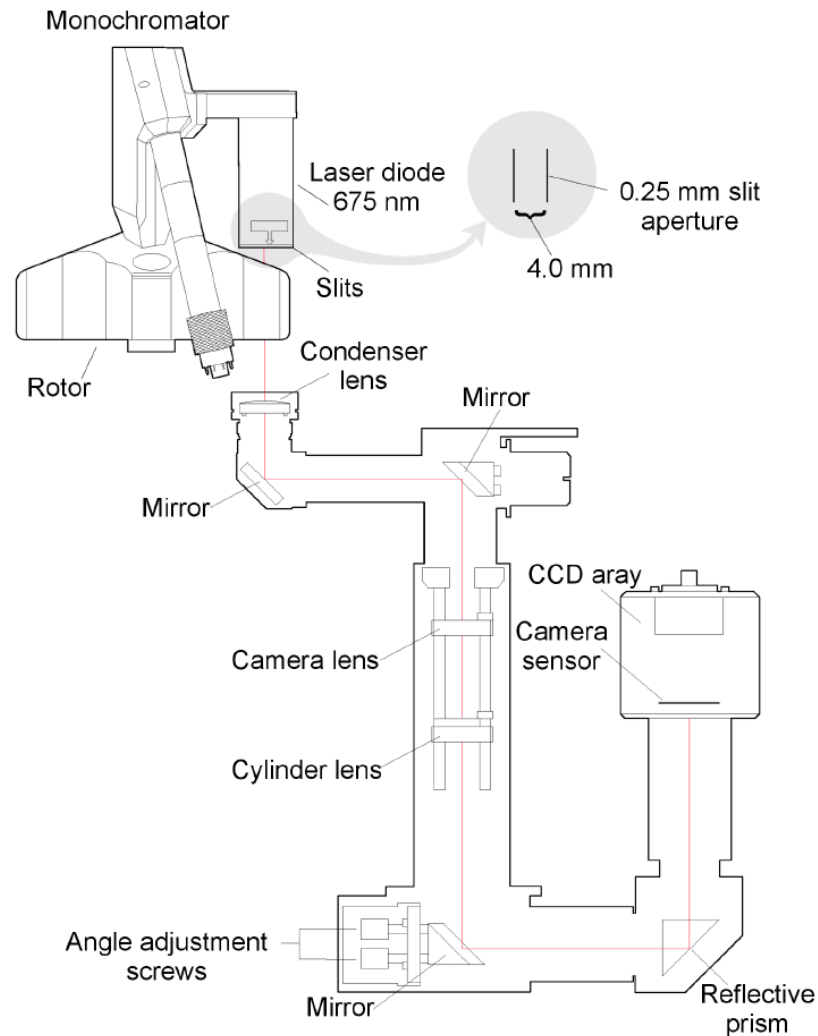


image from Beckman AUC manual

http://www.beckmancoulter.com/resourcecenter/labresources/resource_xla_xli.asp

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2 modes of operation - several data types

- Sedimentation velocity (SV)
- Sedimentation equilibrium (SE)
 - In solution
 - Non-destructive
 - Self-cleaning
 - Absolute

Comparison of all optical systems

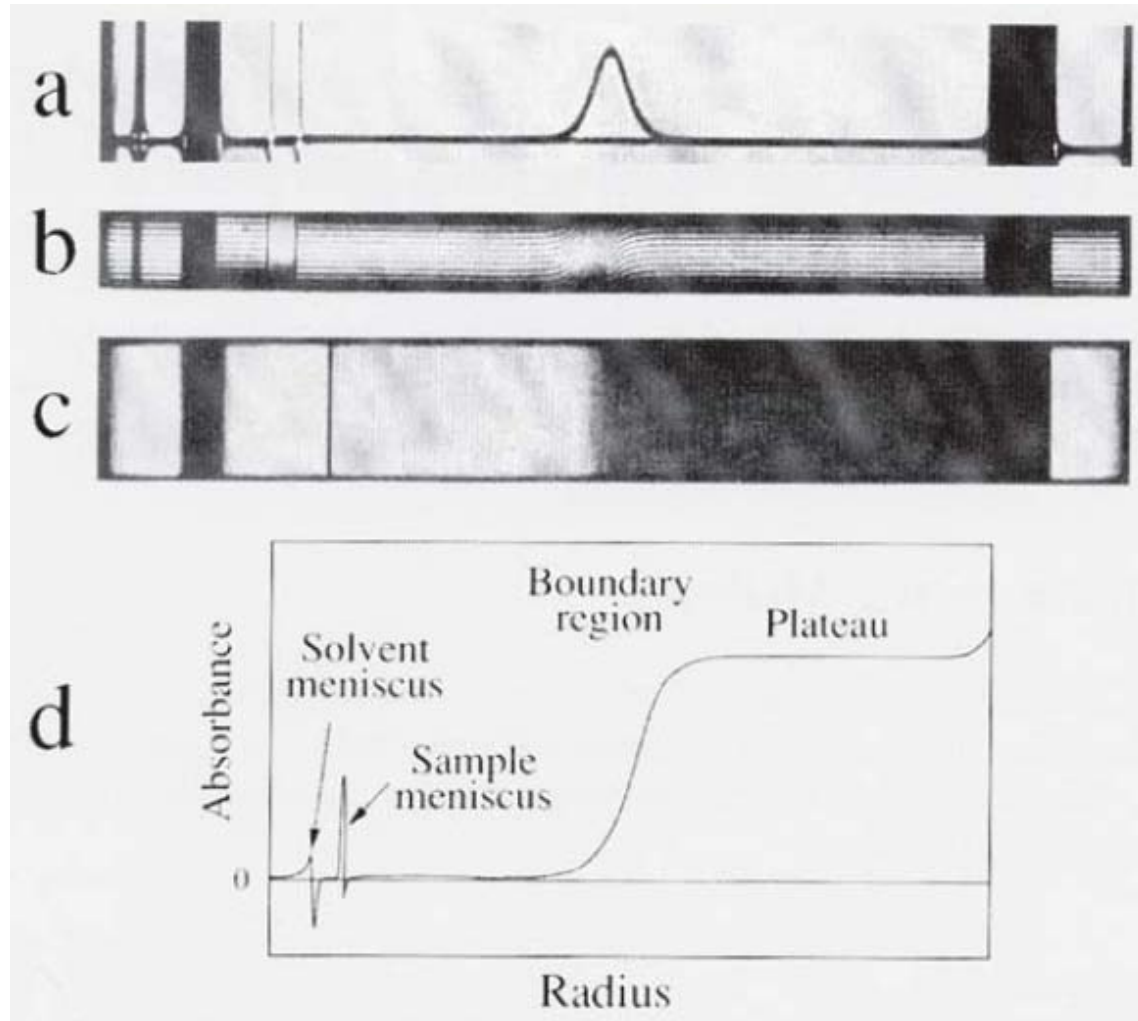


image from Ralston, 1993

<https://www.beckmancoulter.com/wsrportal/bibliography?docname=361847.pdf>

Wanna buy an AUC?

- Choice of 2 instruments
 - Beckman Coulter ProteomeLab™ XL-A/XL-I (≈ €250k)
 - Spin Analytical CFA (available 3rd quarter 2014) (≈ \$200k)
 - <http://www.spinanalytical.com/cfa.php>



CFA: Centrifugal Fluid Analyser – part of the Open AUC Project

Eur Biophys J (2010) 39:347–359

DOI 10.1007/s00249-009-0438-9

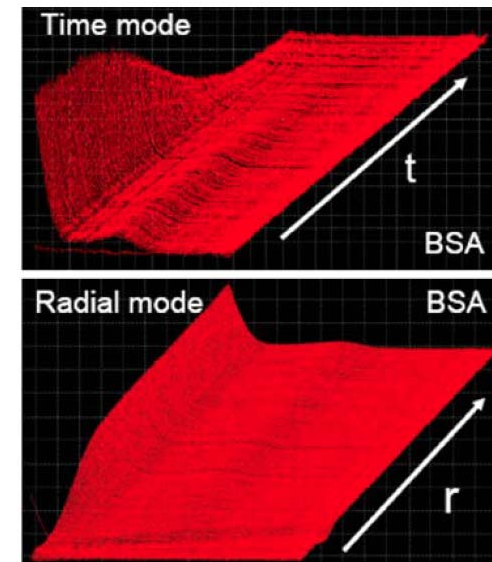
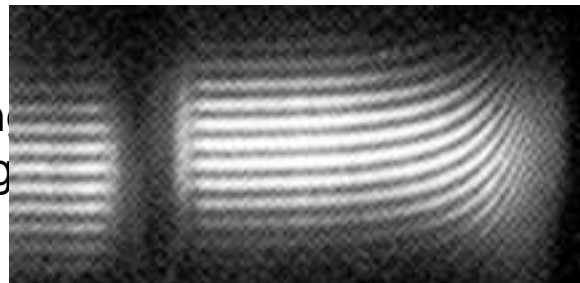
REVIEW

The Open AUC Project

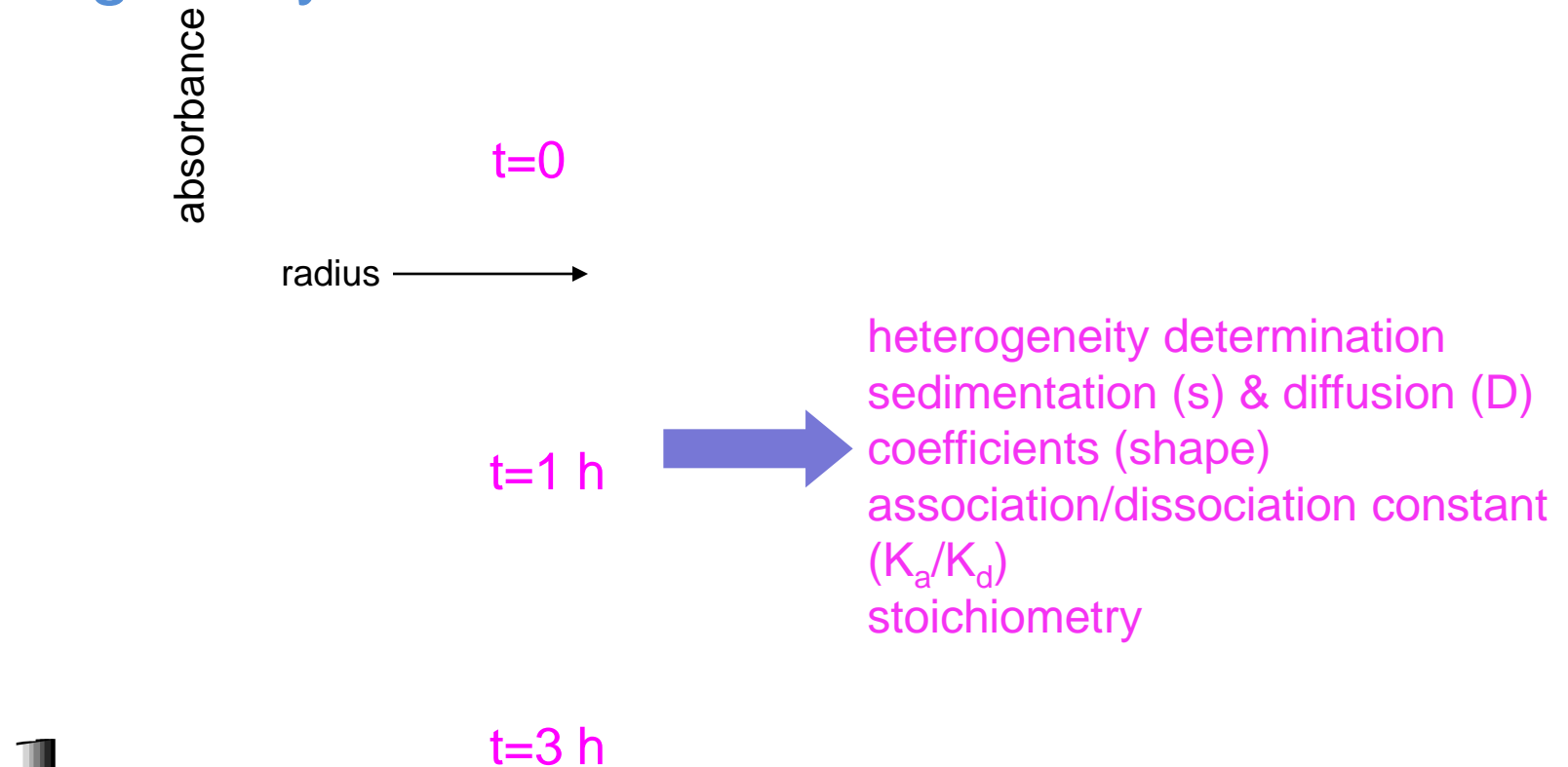
**Helmut Cölfen • Thomas M. Laue • Wendel Wohlleben • Kristian Schilling •
Engin Karabudak • Bradley W. Langhorst • Emre Brookes • Bruce Dubbs •
Dan Zollars • Mattia Rocco • Borries Demeler**

The CFA is an entirely new AUC

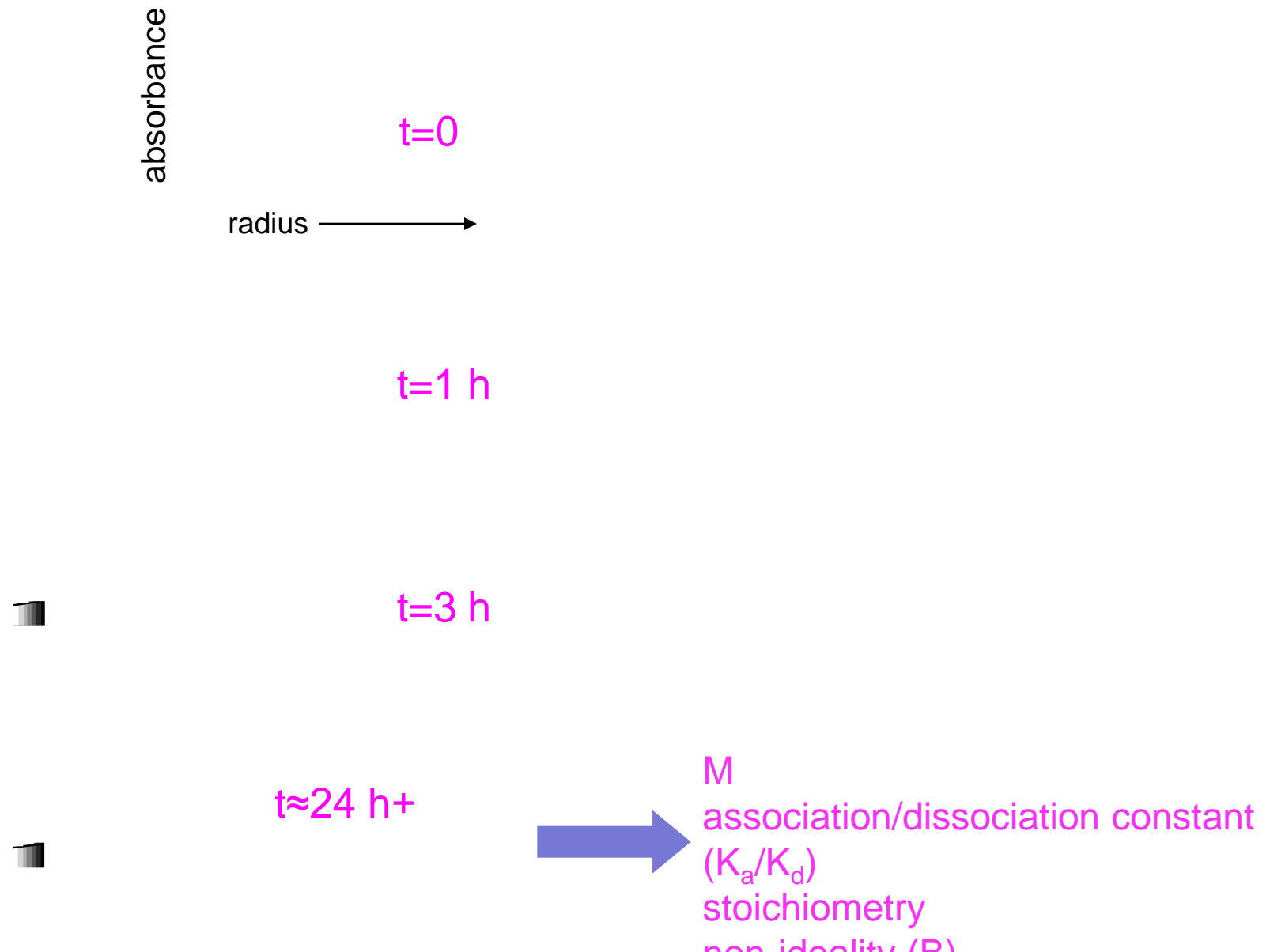
- Capacity for 3 optical systems
 - Detectors outside vacuum system
- Current
 - Dual Wavelength Fluorescence (DWF); permits:
 - 2 different fluorescently tagged molecules to be monitored simultaneously
 - FRET detection of molecular proximity of co-sedimenters
 - Multi-wavelength Absorbance (MWA); permits:
 - separation of components by absorbance spectrum & s
- Planned
 - Rayleigh interference
 - Schlieren refraction
 - Small-angle light scattering
 - Multi-angle light scattering
 - (SAXS?)



Sedimentation velocity (SV): shape & homogeneity



Sedimentation equilibrium (SE): mass & self-association



SV versus SE

- SV: observe movement of sedimentation boundary
- Change in (sometimes complex) boundary over time is due to
 - Sedimentation
 - Diffusion
- SE: rotor spun more slowly so diffusion can balance sedimentation - system reaches thermodynamic equilibrium
- Observe no change in boundary over time
 - Unless sample is degrading or changing in some other way

Sample requirements

- Sample volume

- SV
 - 360 μ l (up to 480 μ l) in 12 mm pathlength
 - 90 μ l (up to 120 μ l) in 3 mm pathlength
- SE
 - 20 μ l (8-channel centrepiece - interference optics only)
 - 80 μ l (2- or 6-channel centrepiece)

- Sample concentration

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

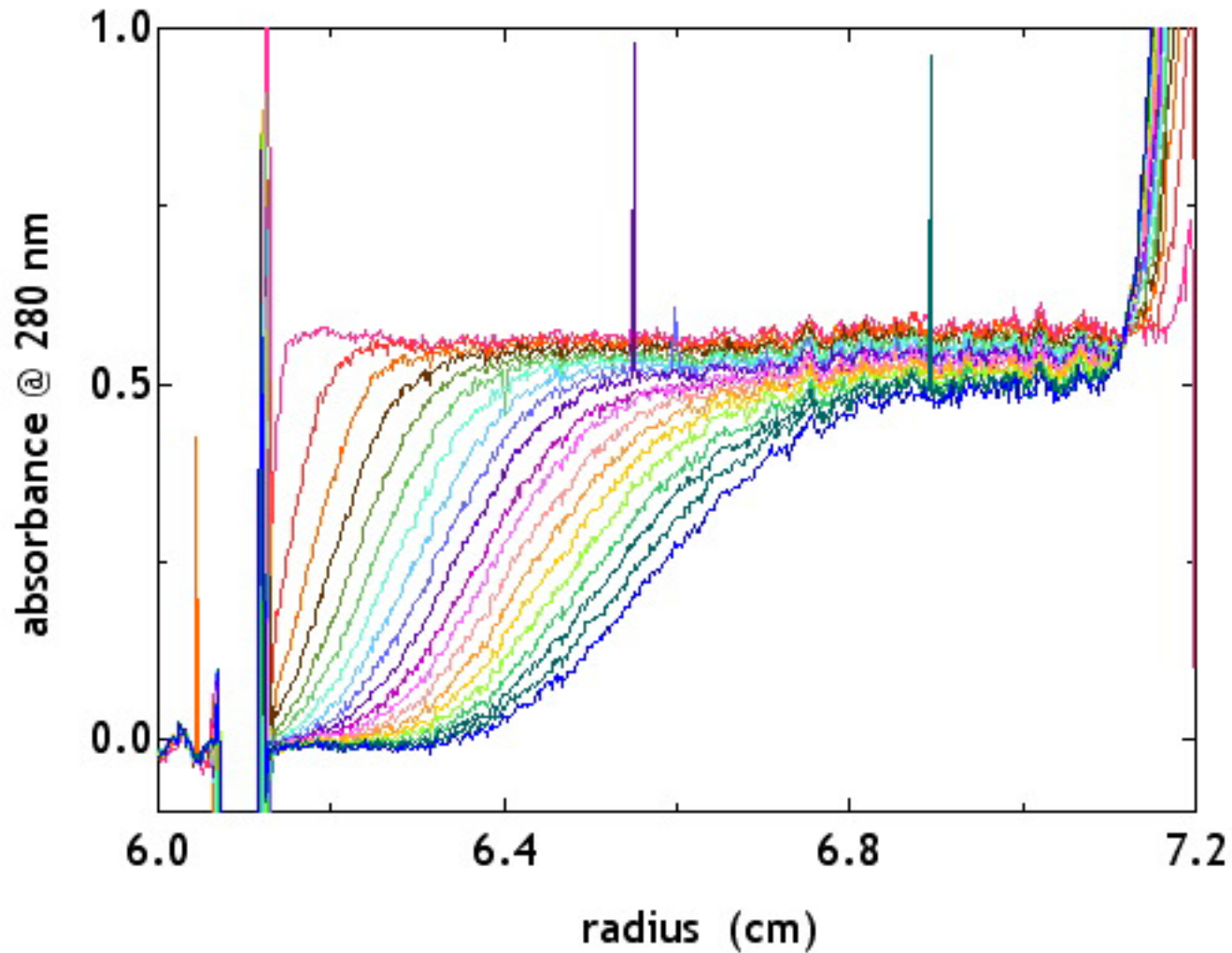
- Sample reference

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

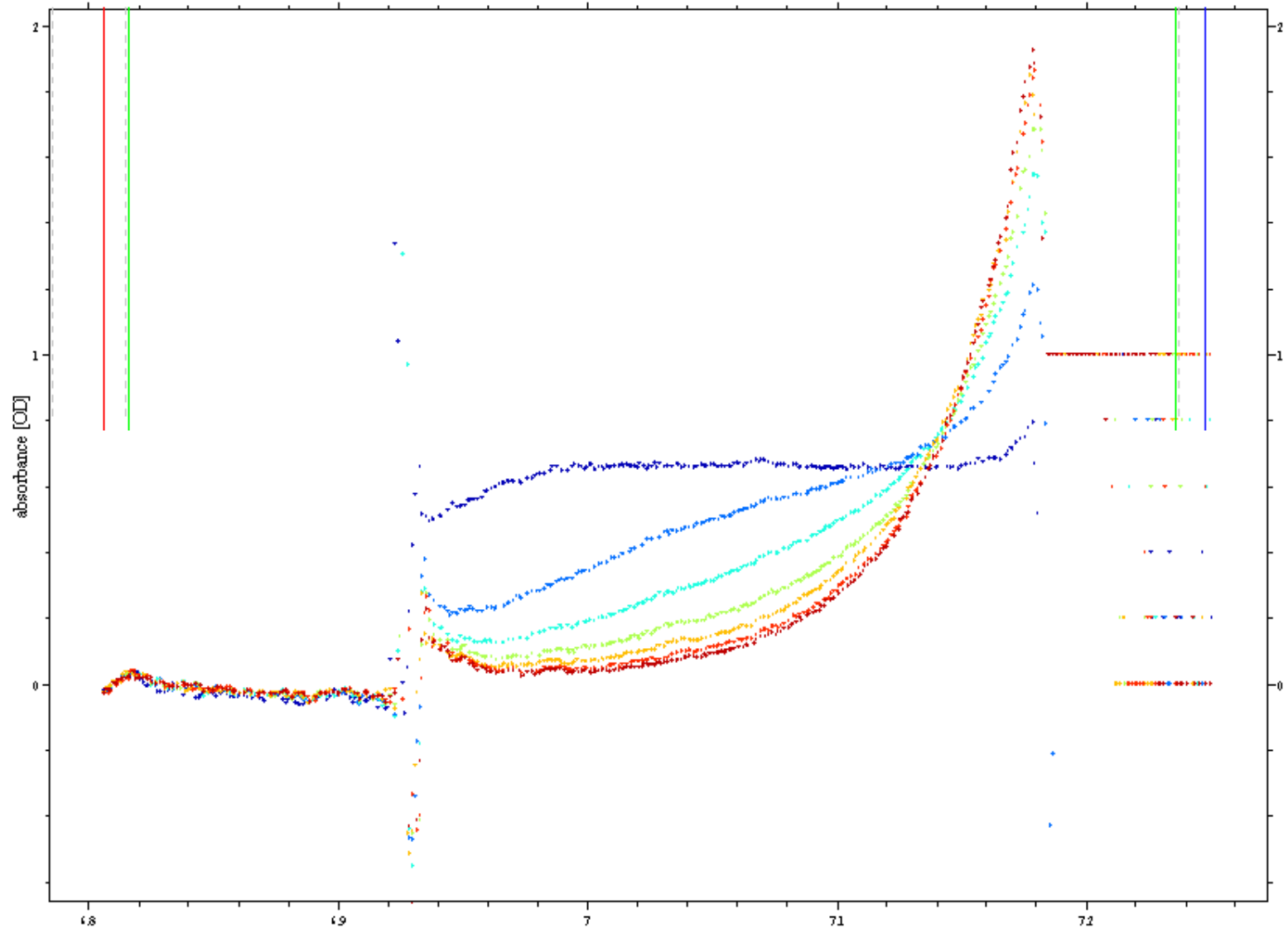
- Typical multiplexing: 3 or 7 sample holders (“cells”)/run

- Up to 28 samples per run

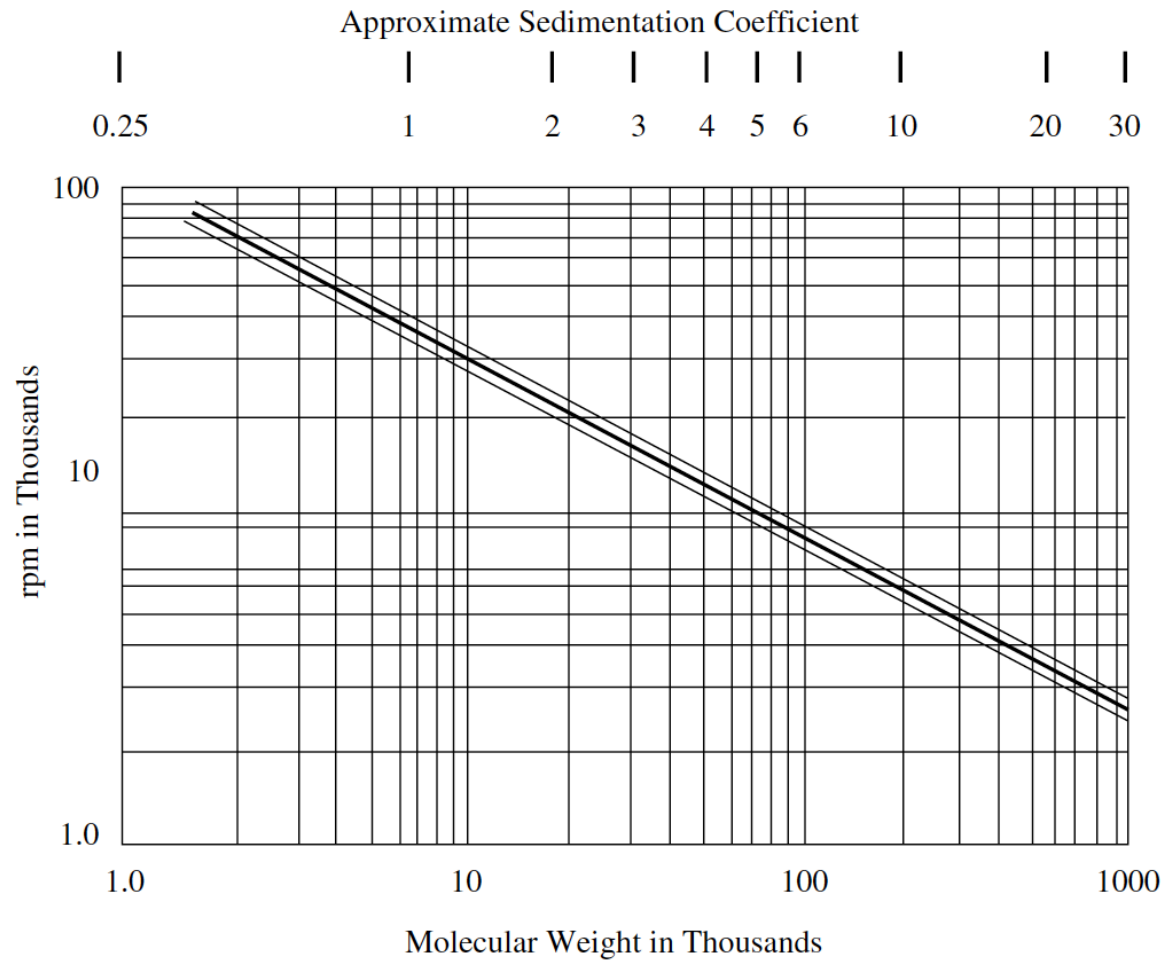
SV: radial movement recorded as function of time



SE: data recorded until no change



Which speed?



Chervenka, C. H. *A Manual of Methods for the Analytical Ultracentrifuge*.
Spinco Division, Beckman Instruments, Palo Alto, 1969

Which speed?

- Rotor speed chosen to optimise shape of equilibrium distribution
- Rule of thumb: at lowest chosen rotor speed, effective molecular weight (σ) = 1

$$\sigma = \frac{M(1 - \bar{v}\rho)\omega^2}{RT}$$

- At subsequent speeds, speed factor = 1.5

$$\text{Speed Factor} = \frac{\omega^2 \text{ for Speed2}}{\omega^2 \text{ for Speed1}}$$

- Ensures that in global fitting of data at different speeds, data are different from each other

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3 important equations

$$s = \frac{u}{\omega^2 r} = \frac{M(1 - \bar{v}\rho)}{N_A f}$$

Svedberg equation

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

Lamm equation

$$\frac{dc}{dt} = \frac{l}{r} \frac{d}{dr} \left[rD \frac{dc}{dr} - s\omega^2 r^2 c \right]$$

Almost all AUC data analysis software is freely available

- The RASMB website
 - “Reversible Associations in Structural and Molecular Biology”
 - <http://www.rasmb.bbri.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 SOMO))
 - <http://www.ultrascan.uthscsa.edu/>

Many methods & programs for SV data analysis

- Too many for comprehensive review here
- Model independent:
 - dc/dt (Stafford, SedAnal)
 - Eliminates time invariant noise. Resultant curves can be fitted with Gaussians to reveal species content and sedimentation coefficients.
 - c(s) (Schuck, Sedfit)
 - Good for “first look” at data to get an idea of number of species. Not a proper fit to data.
 - van Holde-Weischet (Demeler, UltraScan III)
 - Diffusion corrected s distribution. Good for detection of aggregates and identification of underlying model.
- Model dependent:
 - Non-interacting discrete species (Schuck, Sedfit)
 - Up to 4 separate species can be fitted.
 - Self-association (Stafford, SedAnal; Demeler, UltraScan III)
 - Determination of K_d , k_{on} , k_{off} , stoichiometry

s is influenced by solvent density & viscosity and sample density

sample partial
specific volume solvent density solvent
viscosity

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

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 and partial specific volume online

The screenshot shows the SEDNTERP web application interface. The browser address bar displays sednterp.unh.edu. The application has a navigation bar with tabs: Sample, Solvent (active), Experiment, and Results. The main content area is divided into two panels. The left panel, titled 'Buffer Data', contains a dropdown menu 'Select from list to retrieve from database' with 'New Solvent' selected. Below this are sections for 'Density' and 'Viscosity', each with 'Direct Entry', 'Standard' (highlighted in green), and 'Compute' buttons. The 'pH' section is also present. The right panel, titled 'Calculate Buffer Density' and 'Calculate Buffer Viscosity', contains input fields for 'Density' (1.00790) and 'Viscosity' (0.01002), and corresponding corrected values. Below these is a table for 'Buffer Components' with columns for 'Components', 'Concentration', and 'Concentration Units'. The table lists 'Sodium chloride' (0.2 molar) and 'Tris(hydroxymethyl)aminomethane' (0.05 molar). At the bottom right, there are 'Cancel' and 'Ok' buttons.

Buffer Data
Select from list to retrieve from database
New Solvent

Density
Direct Entry Standard Compute

Viscosity
Direct Entry Standard Compute

pH

☒ **Calculate Buffer Density**
Density 1.00790 Density Corrected for Temperature & Isotopes of Water 1.00790

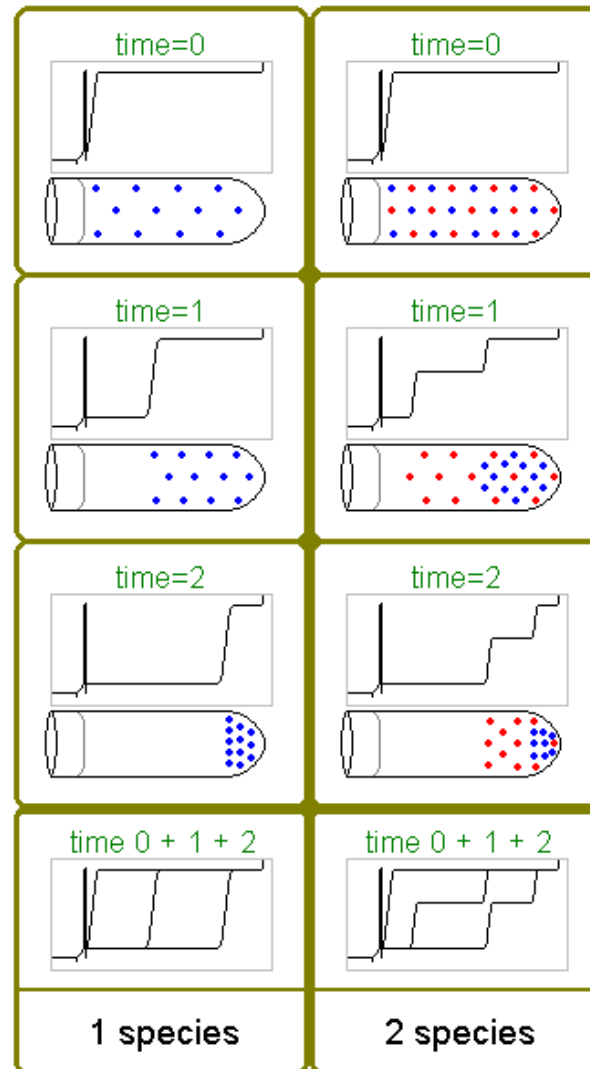
☐ **Calculate Buffer Viscosity**
Viscosity 0.01002 Viscosity Corrected for Temperature 0.01002

| Components | Concentration | Concentration Units |
|---------------------------------|---------------|---------------------|
| Sodium chloride | 0.2 | molar |
| Tris(hydroxymethyl)aminomethane | 0.05 | molar |

☐ **Heavy Isotopes of water**
H₂O 100 % Volume
D₂O 0 % Volume
H₂O₁₈ 0 % Volume
D₂O₁₈ 0 % Volume

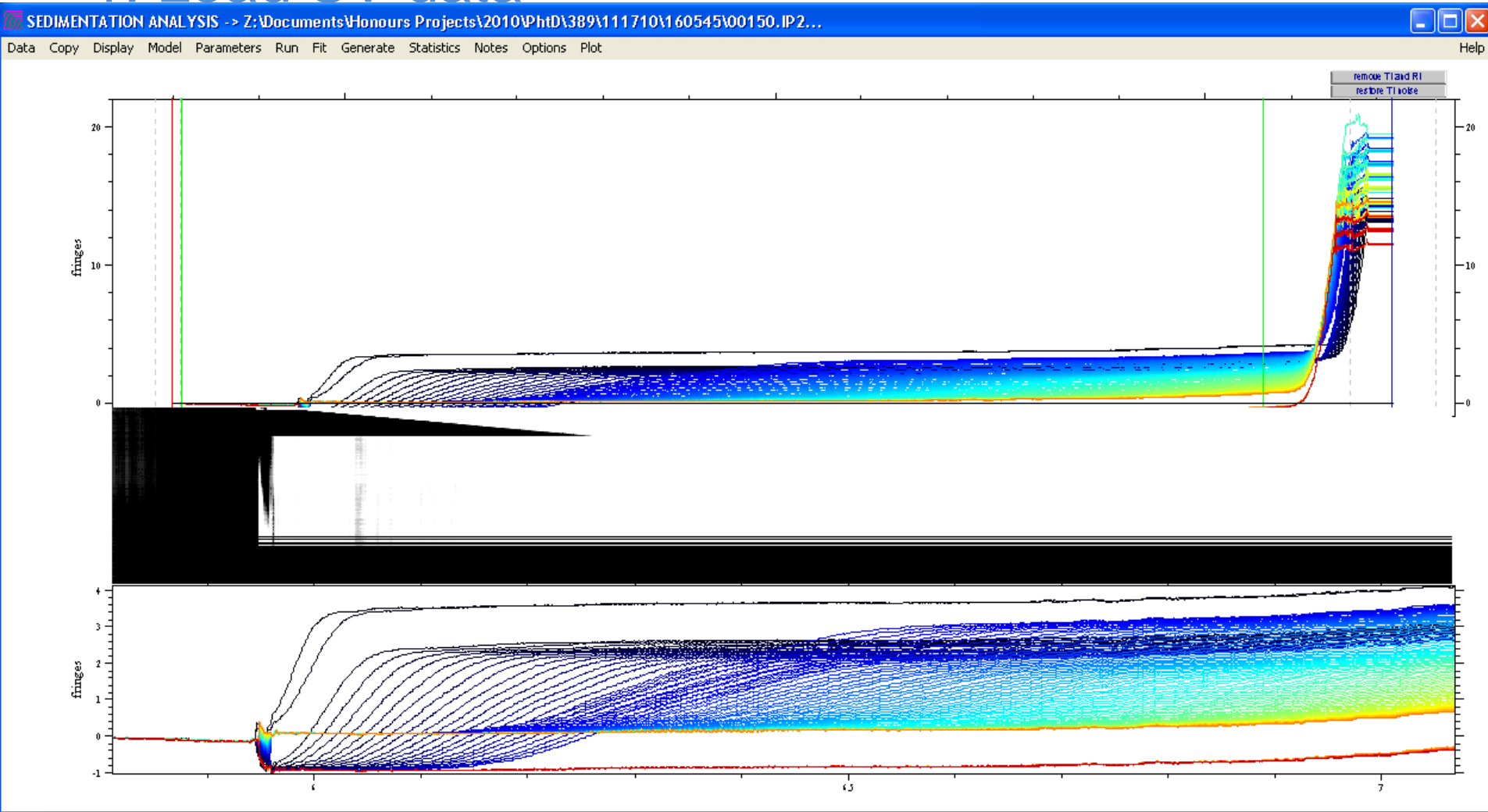
Cancel Ok

SV: species can resolve into separate boundaries

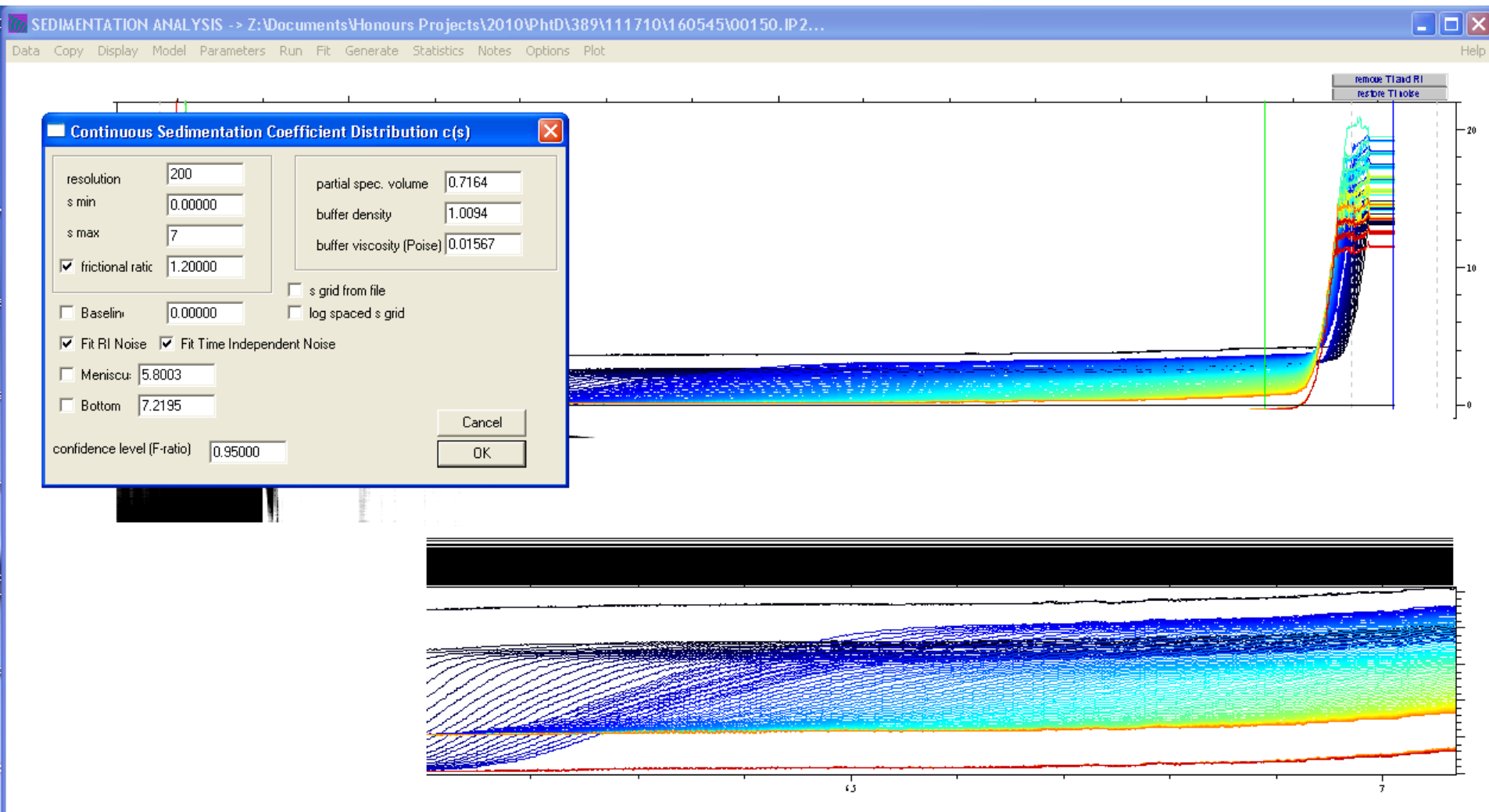


SEDFIT c(s) analysis: how many species + s of species

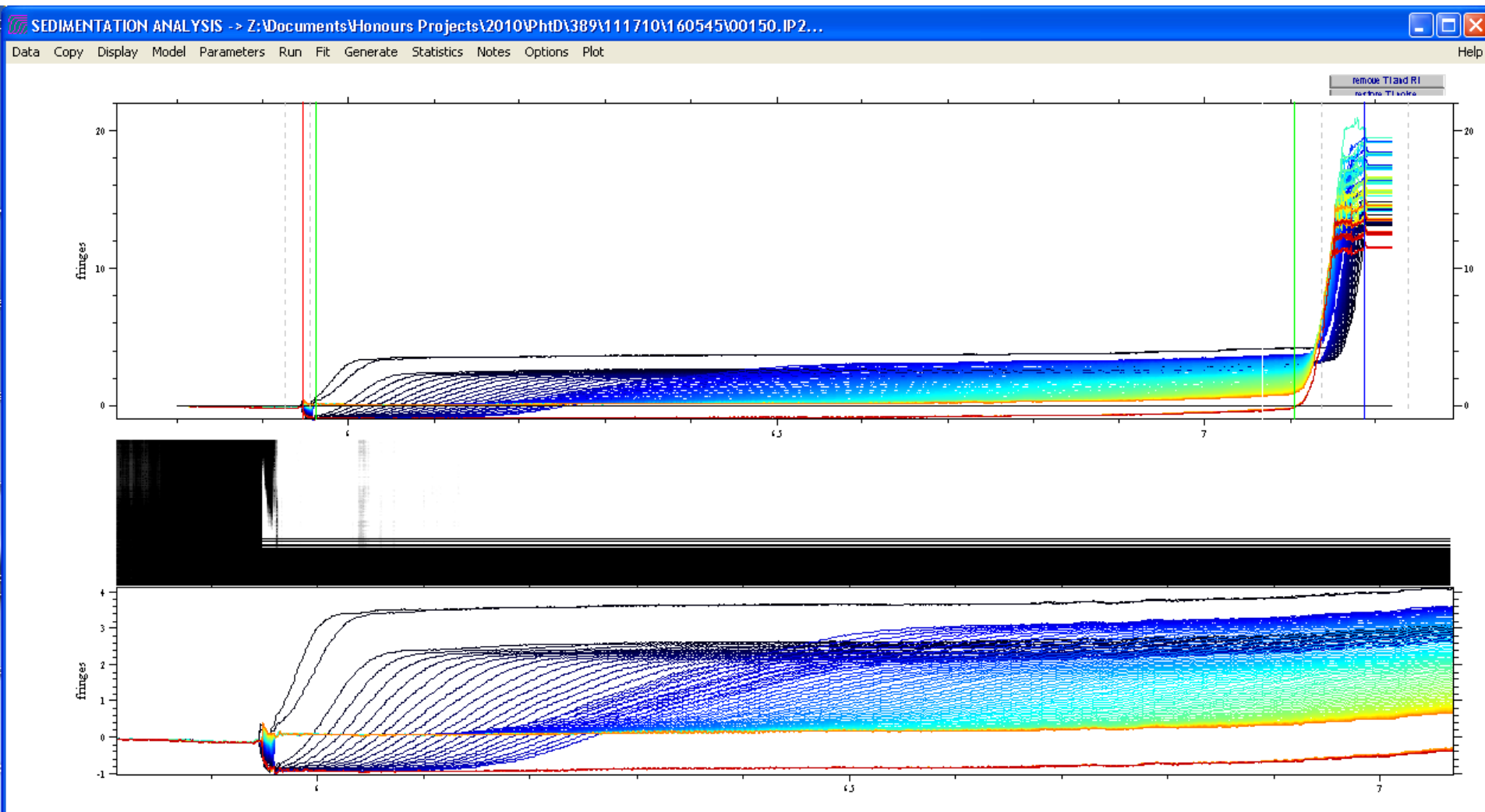
1: Load SV data



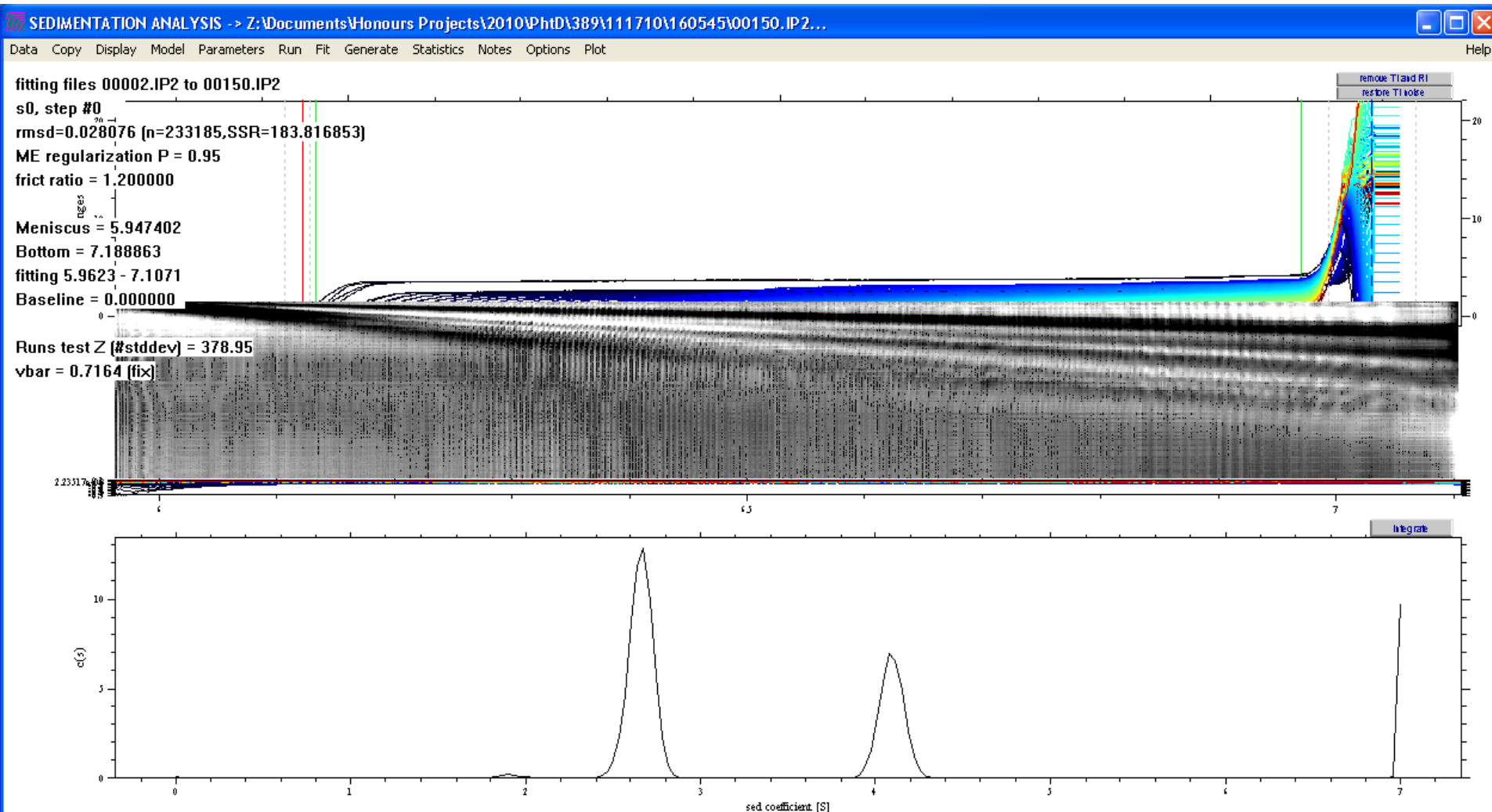
2: Specify parameters



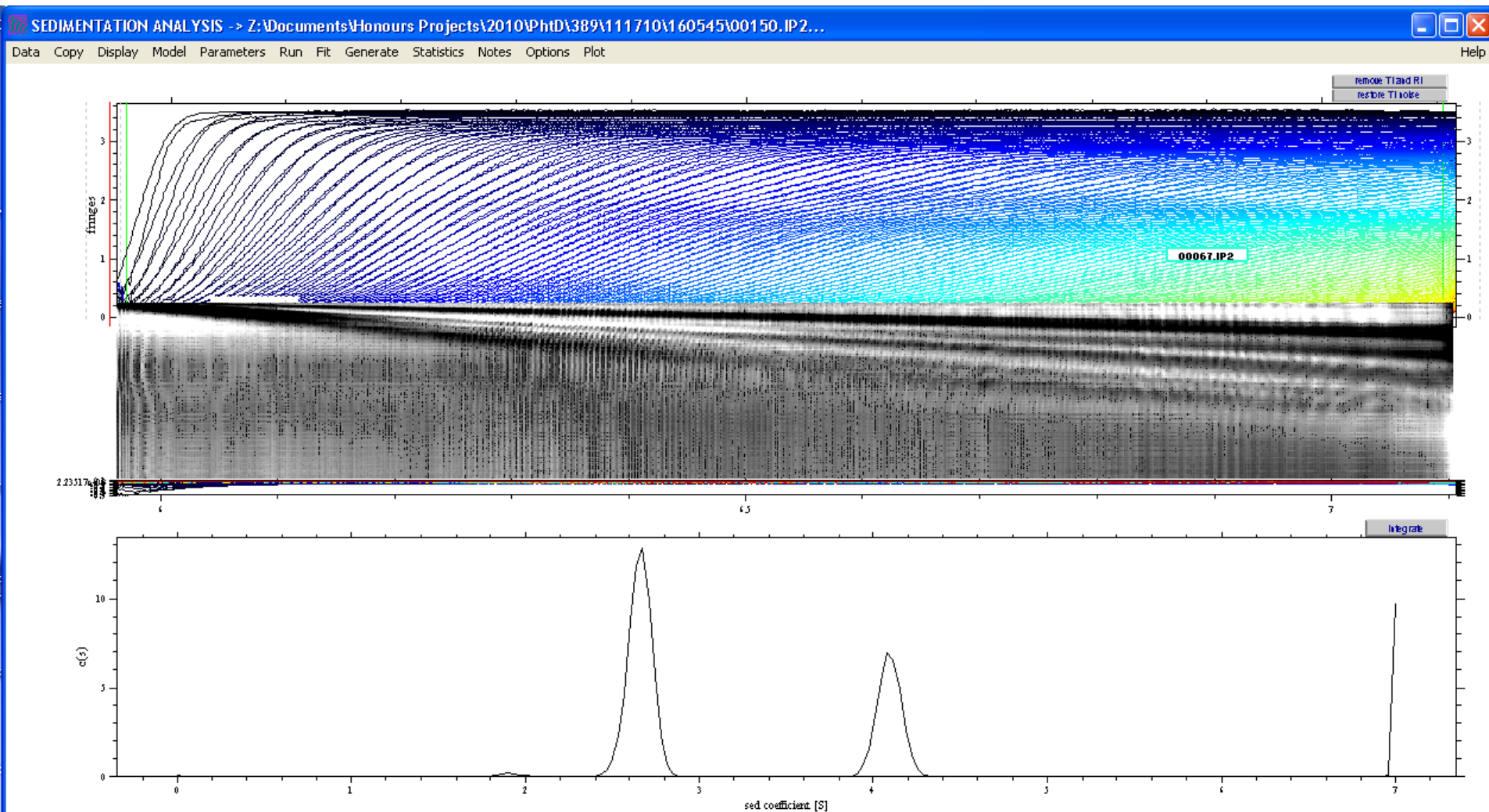
3: Set meniscus, cell base and analysis limits



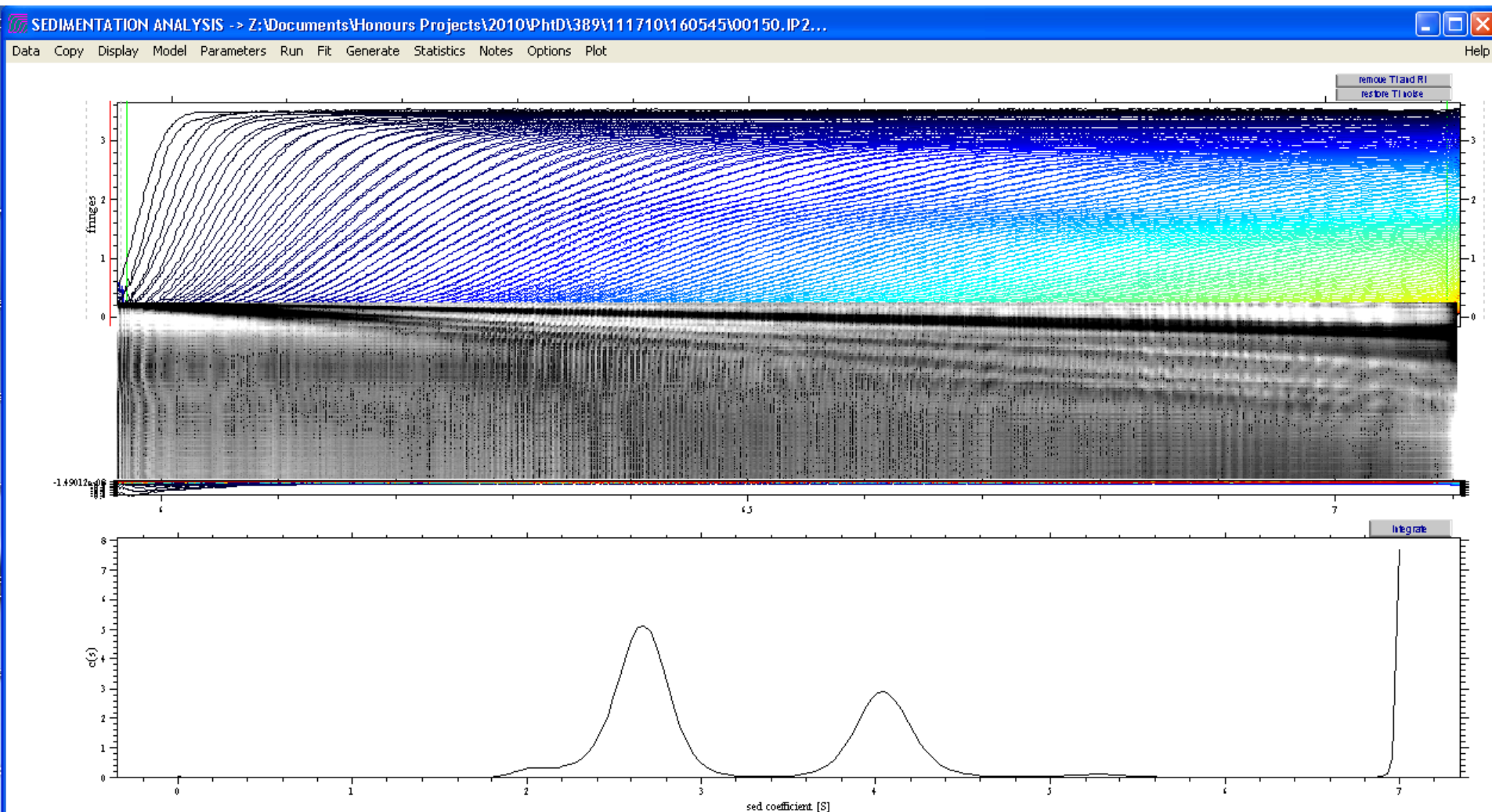
4: Run



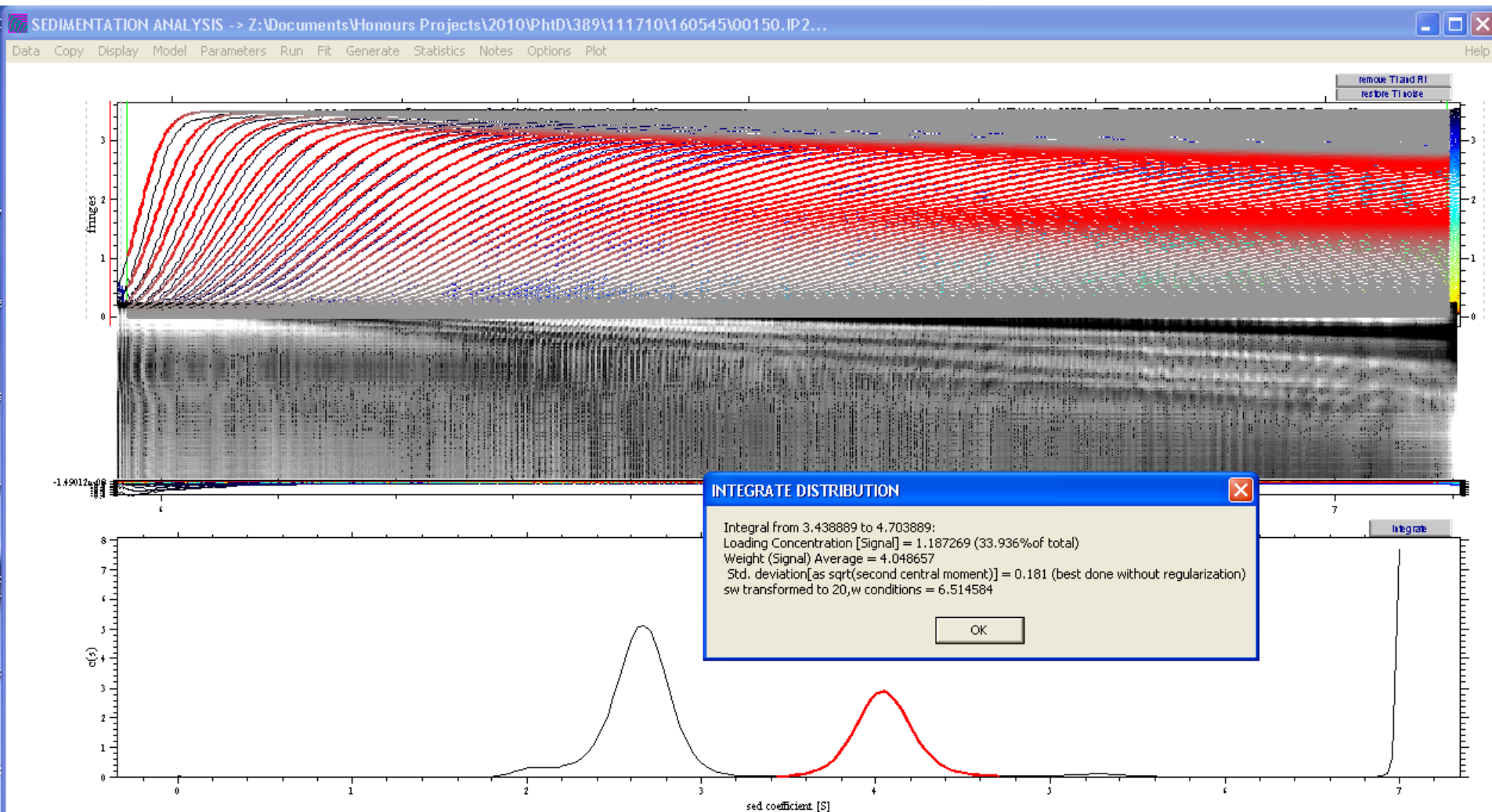
5: Subtract time and radial invariant noise



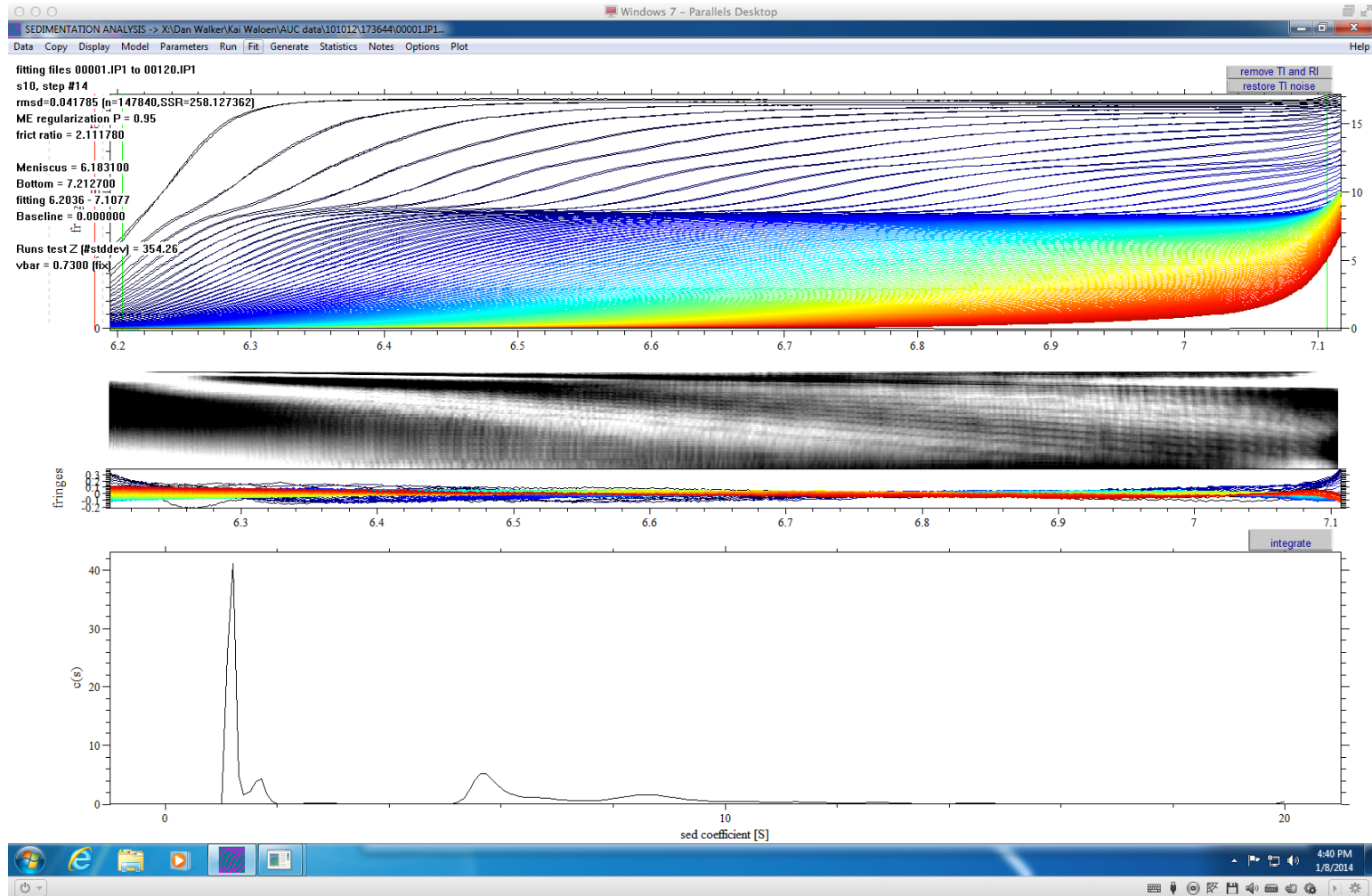
6: Fit (with solutions to the Lamm equation)



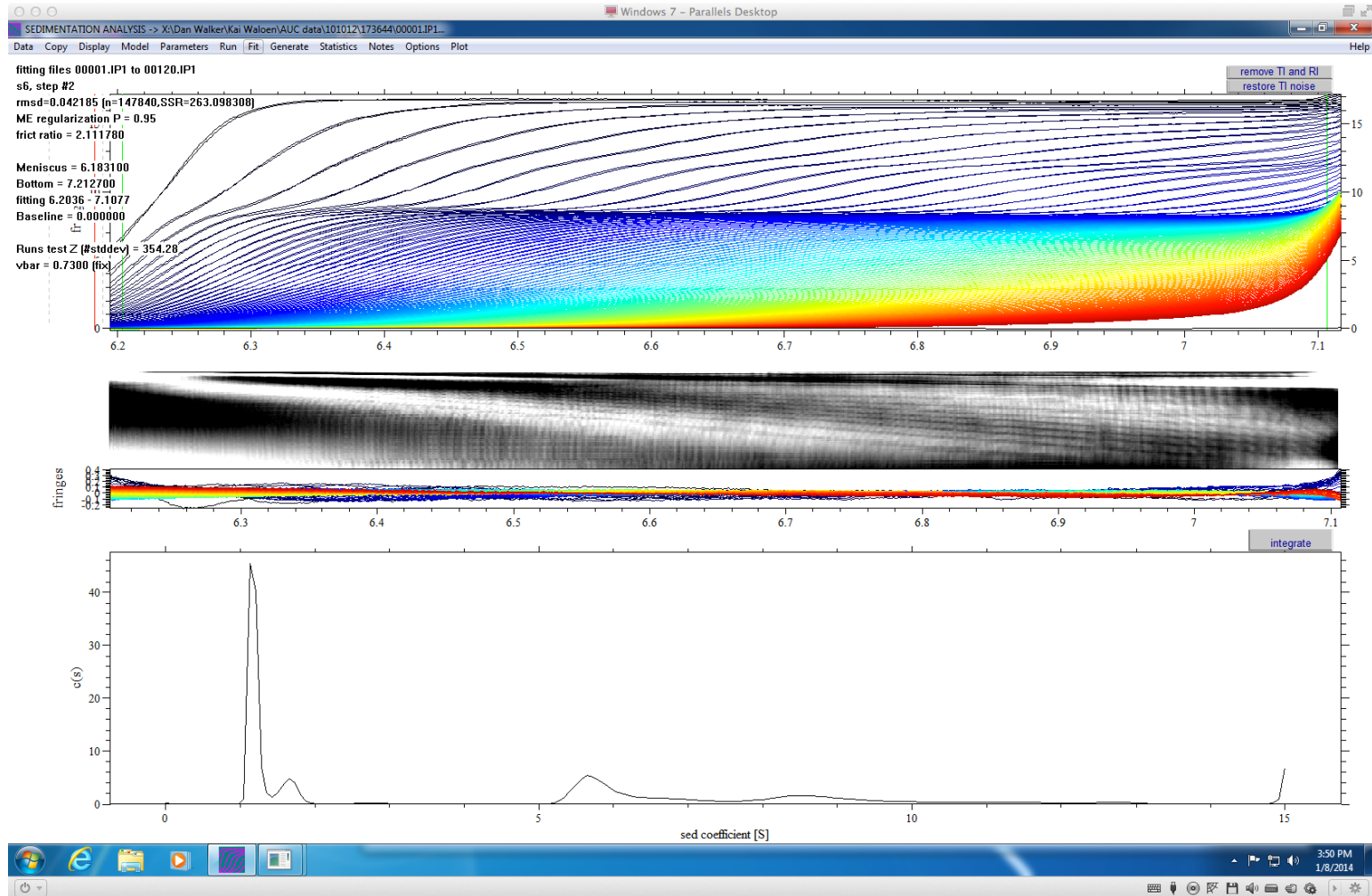
7: Integrate to obtain estimate of concentration of species and weight-average values



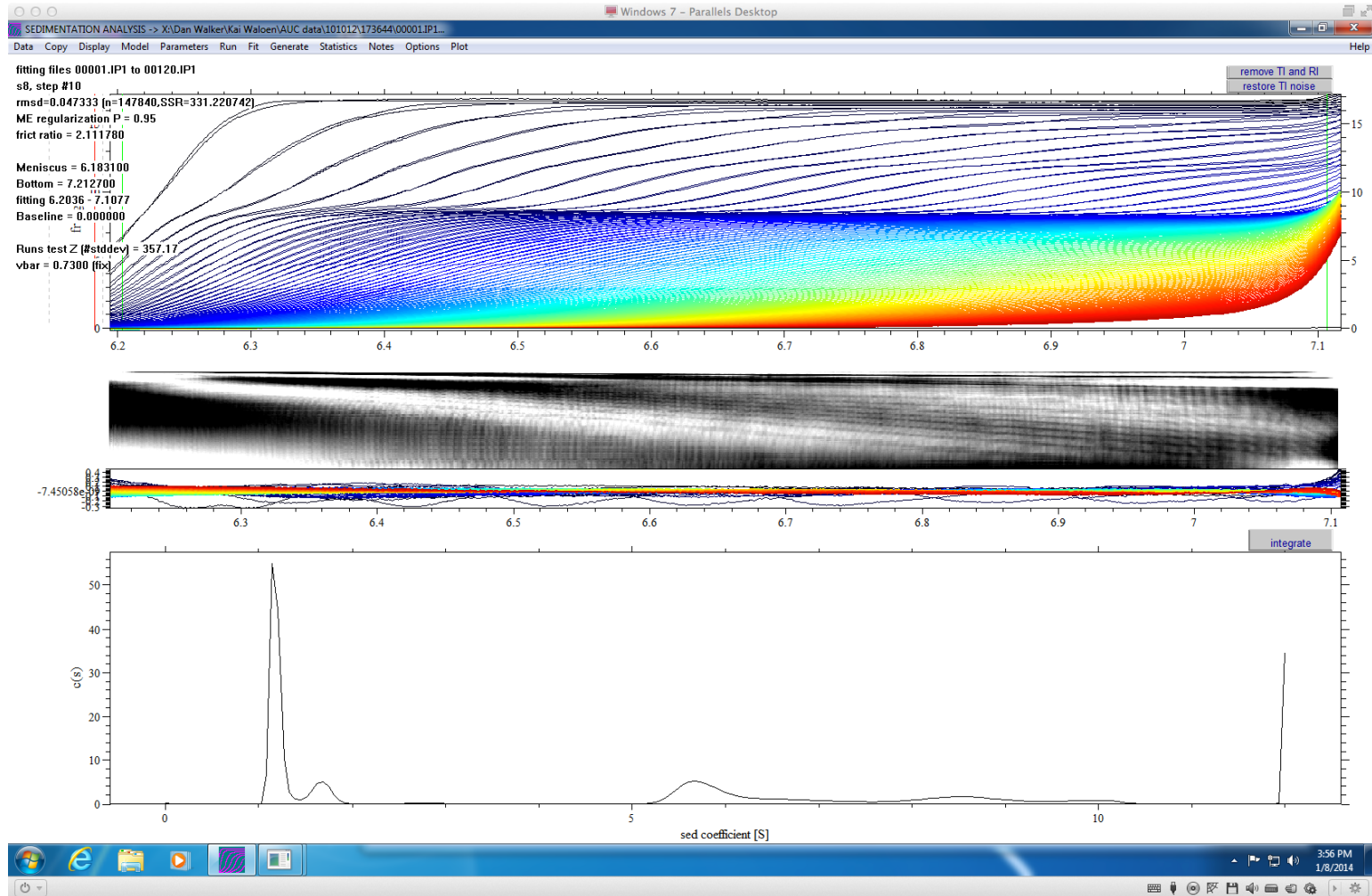
Sum of Lamm equations $0 \leq s \leq 20$ S discretised by 200



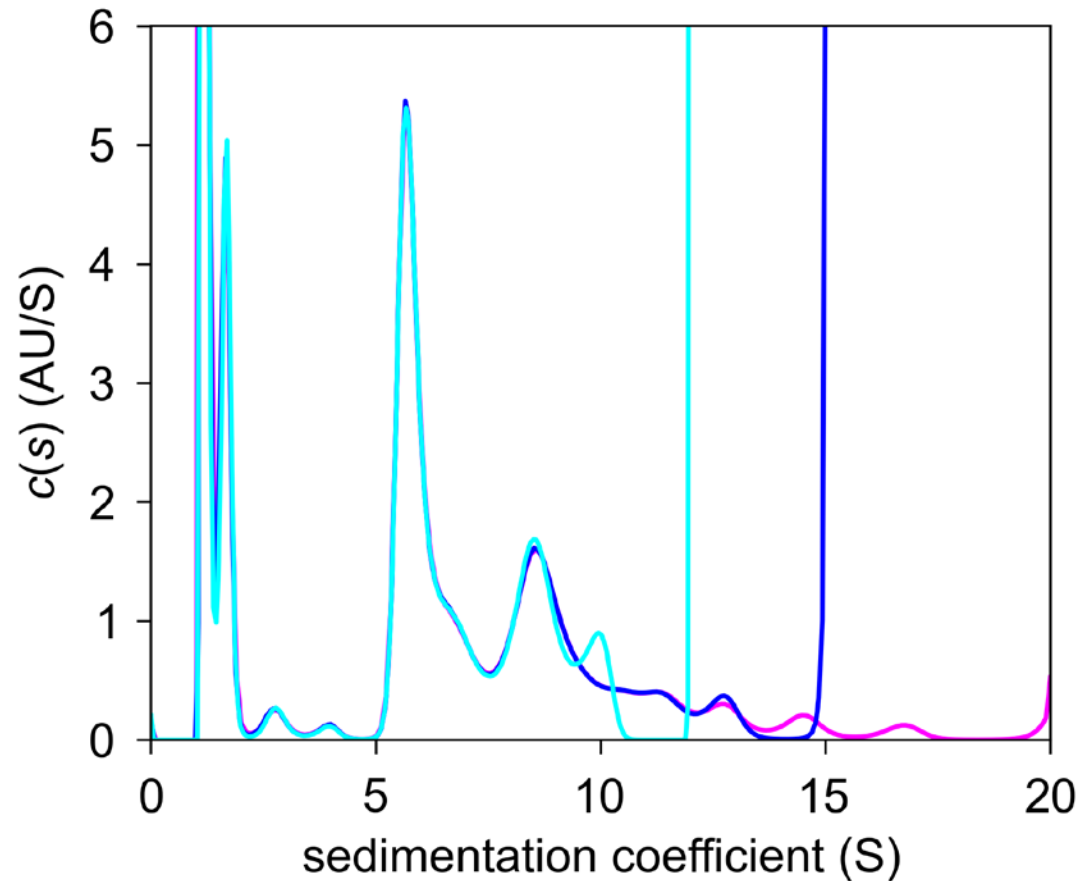
Sum of Lamm equations $0 \leq s \leq 15$ S discretised by 200



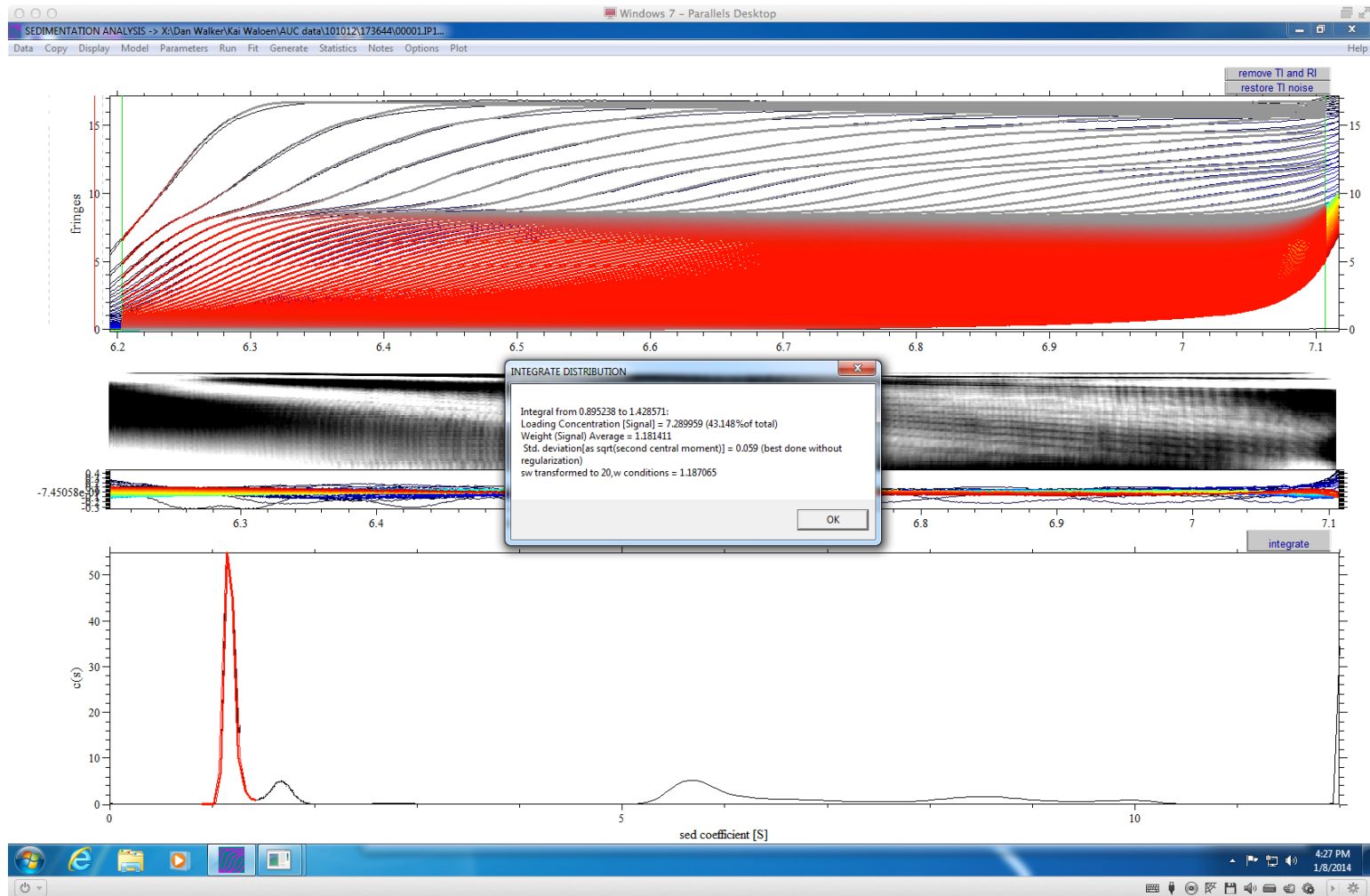
Sum of Lamm equations $0 \leq s \leq 12$ S discretised by 200



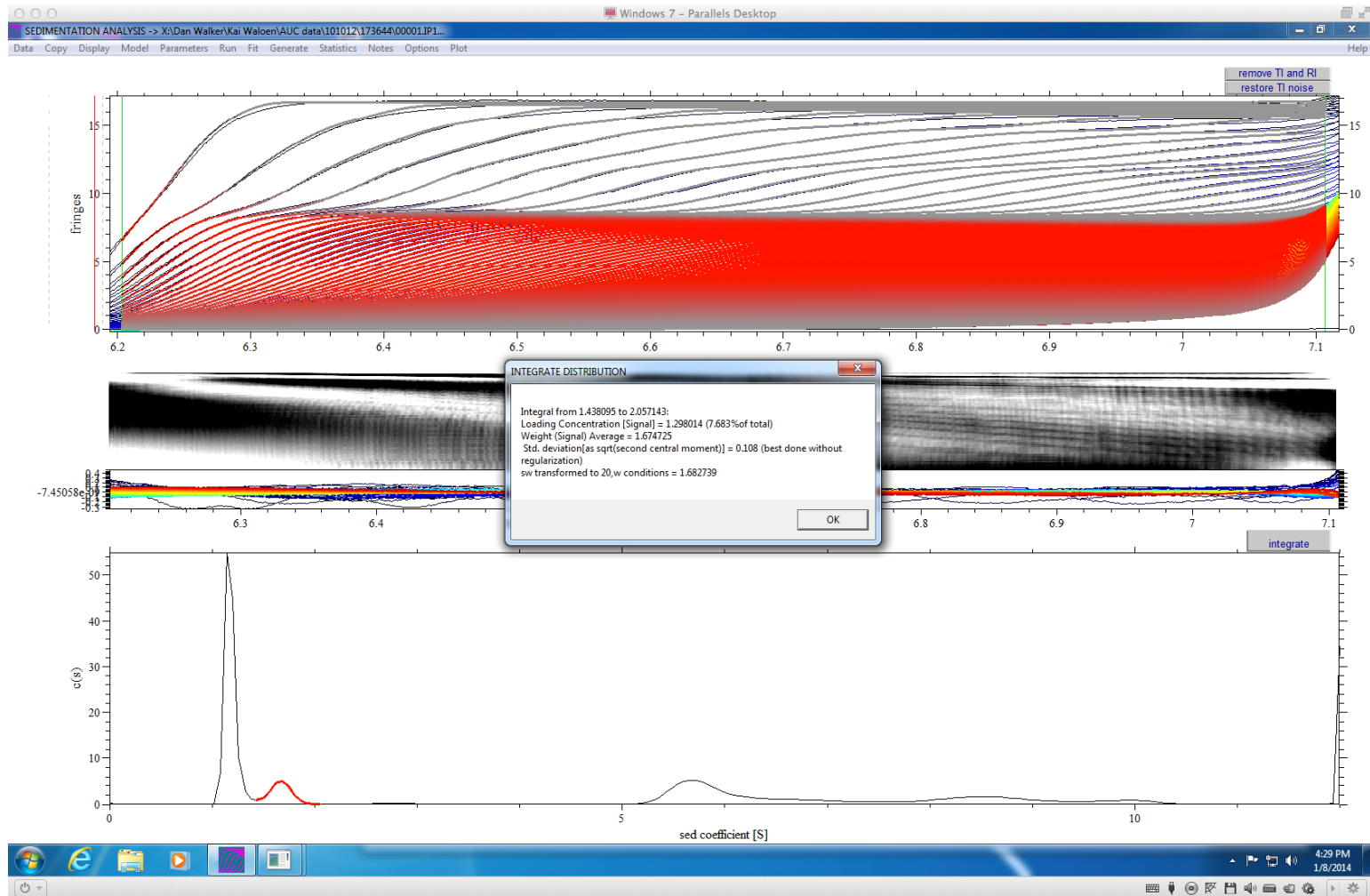
Truncating upper fit limit does not increase the resolution at lower s



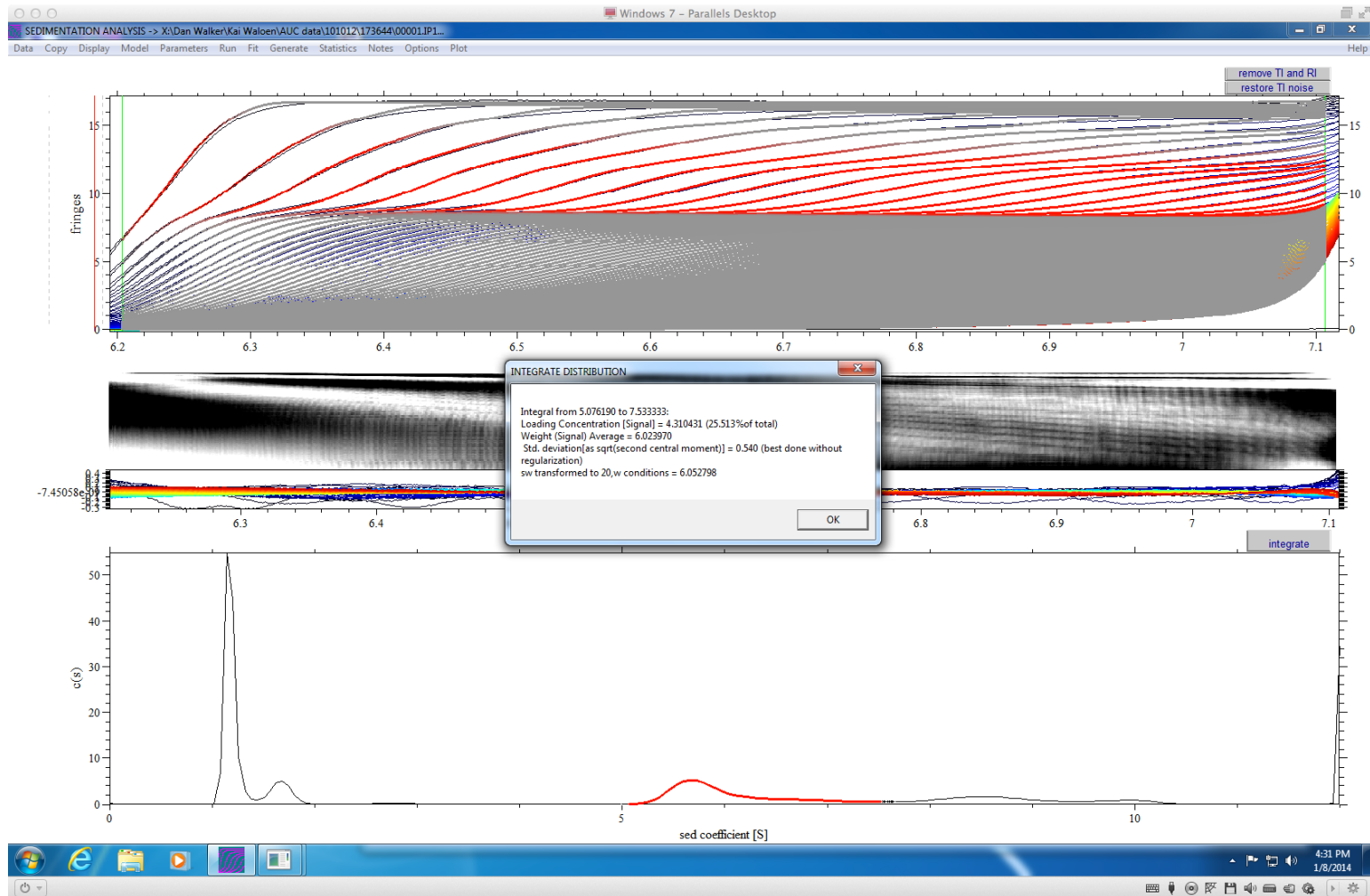
Integrating c(s) peaks reveals region of boundary that contains species



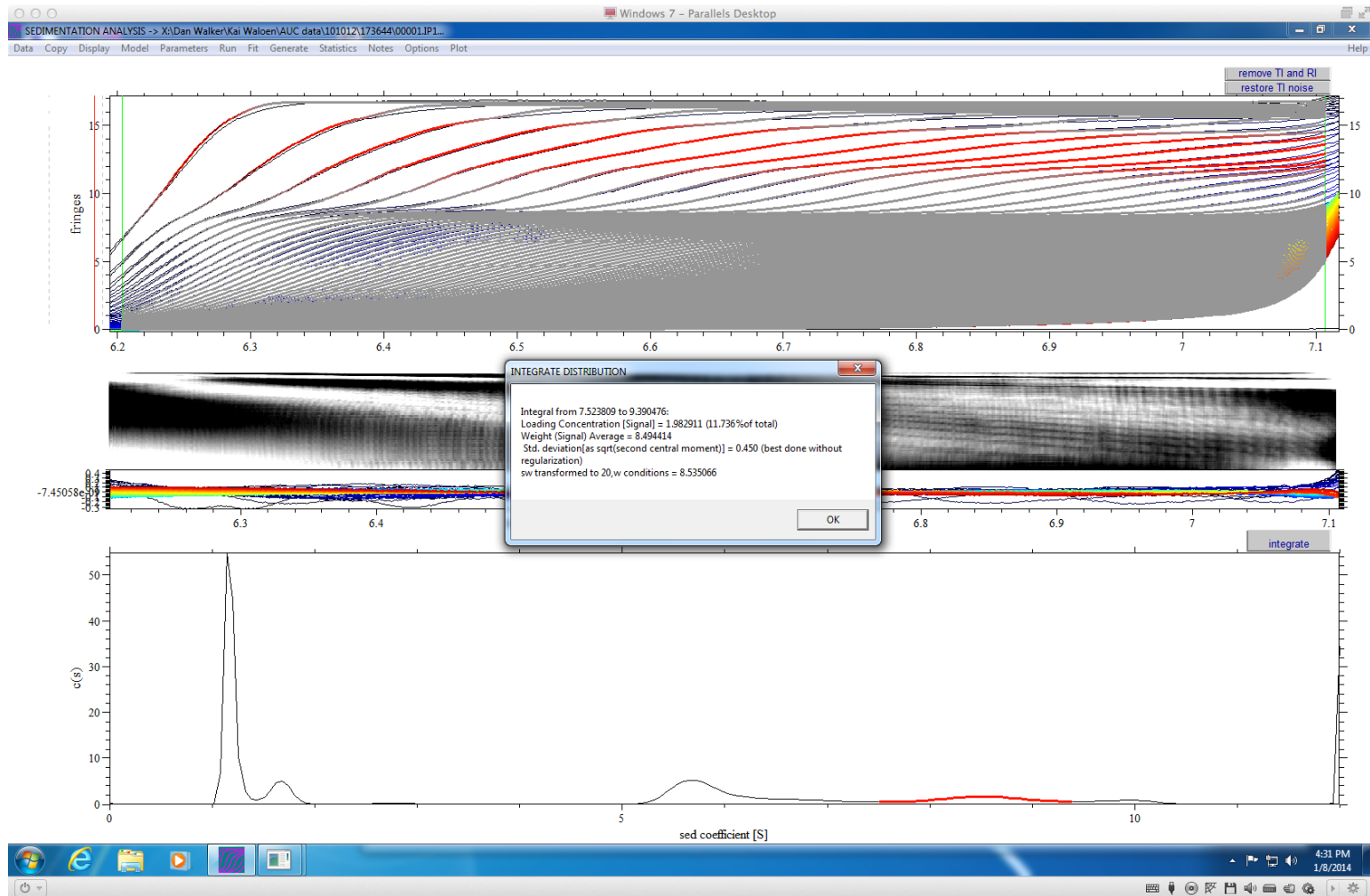
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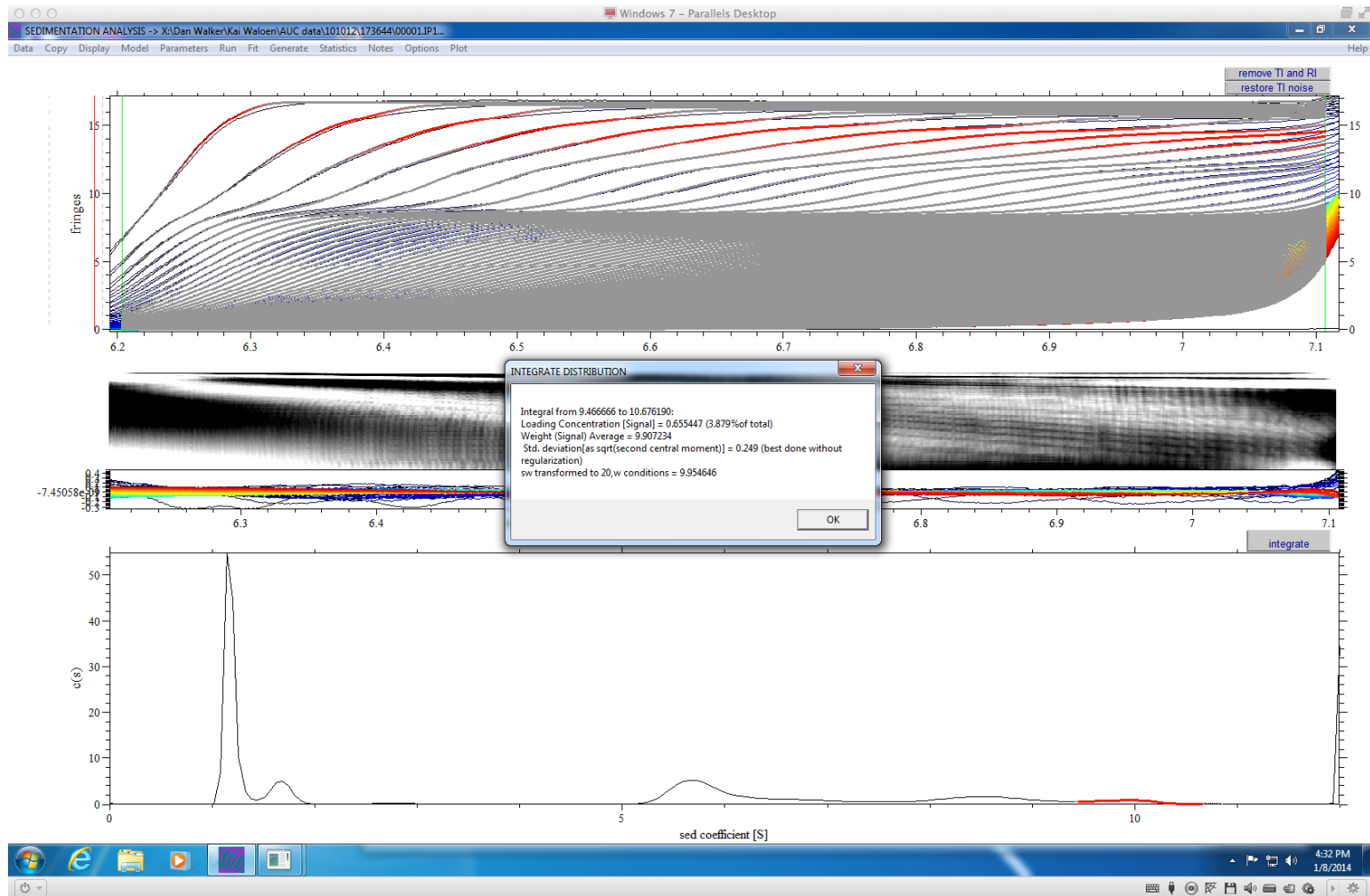
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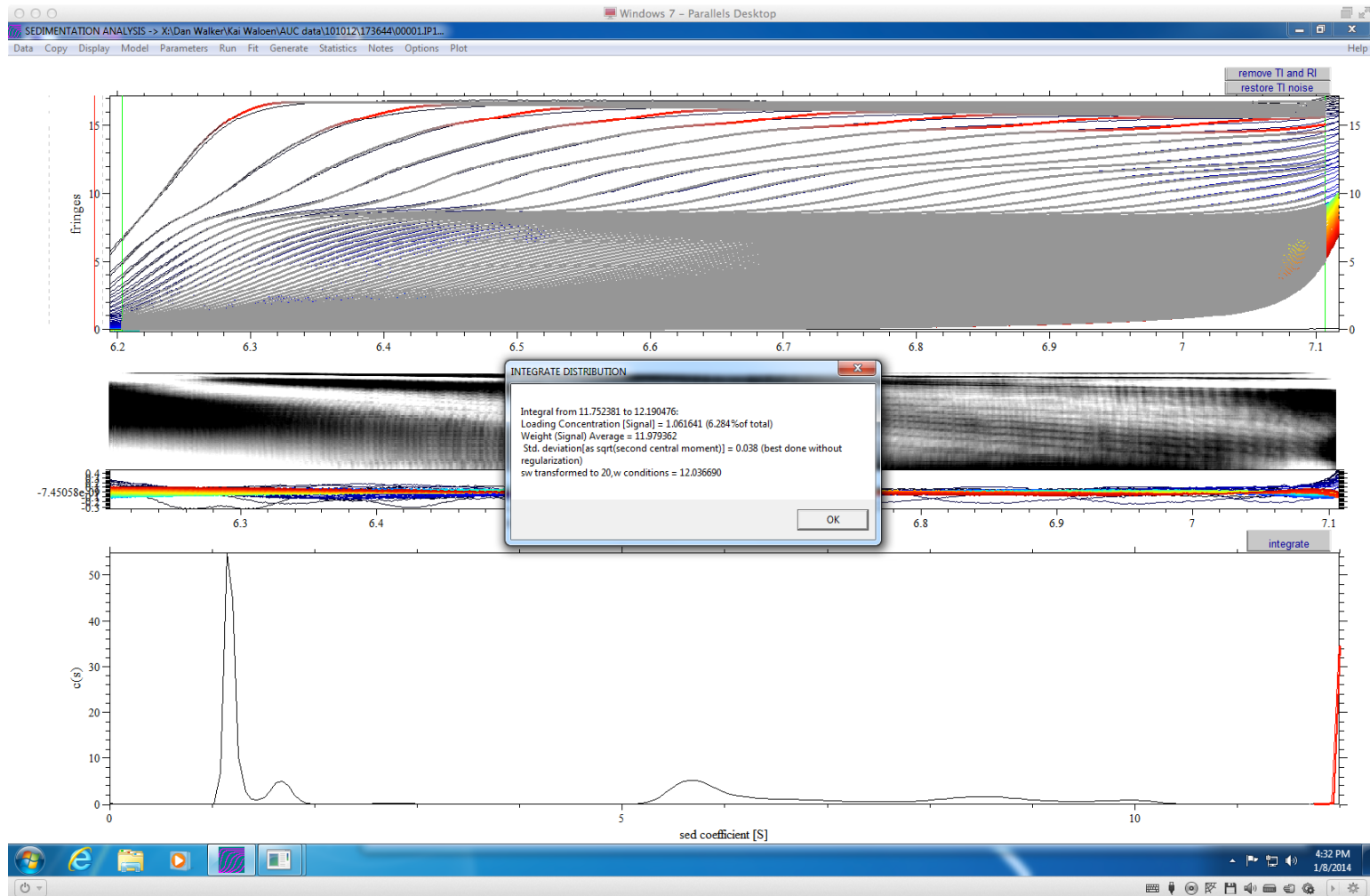
Integrating c(s) peaks reveals region of boundary that contains species



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



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



Self association: SE data are the sum of exponentials

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

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

$\leftarrow 1-n_2$

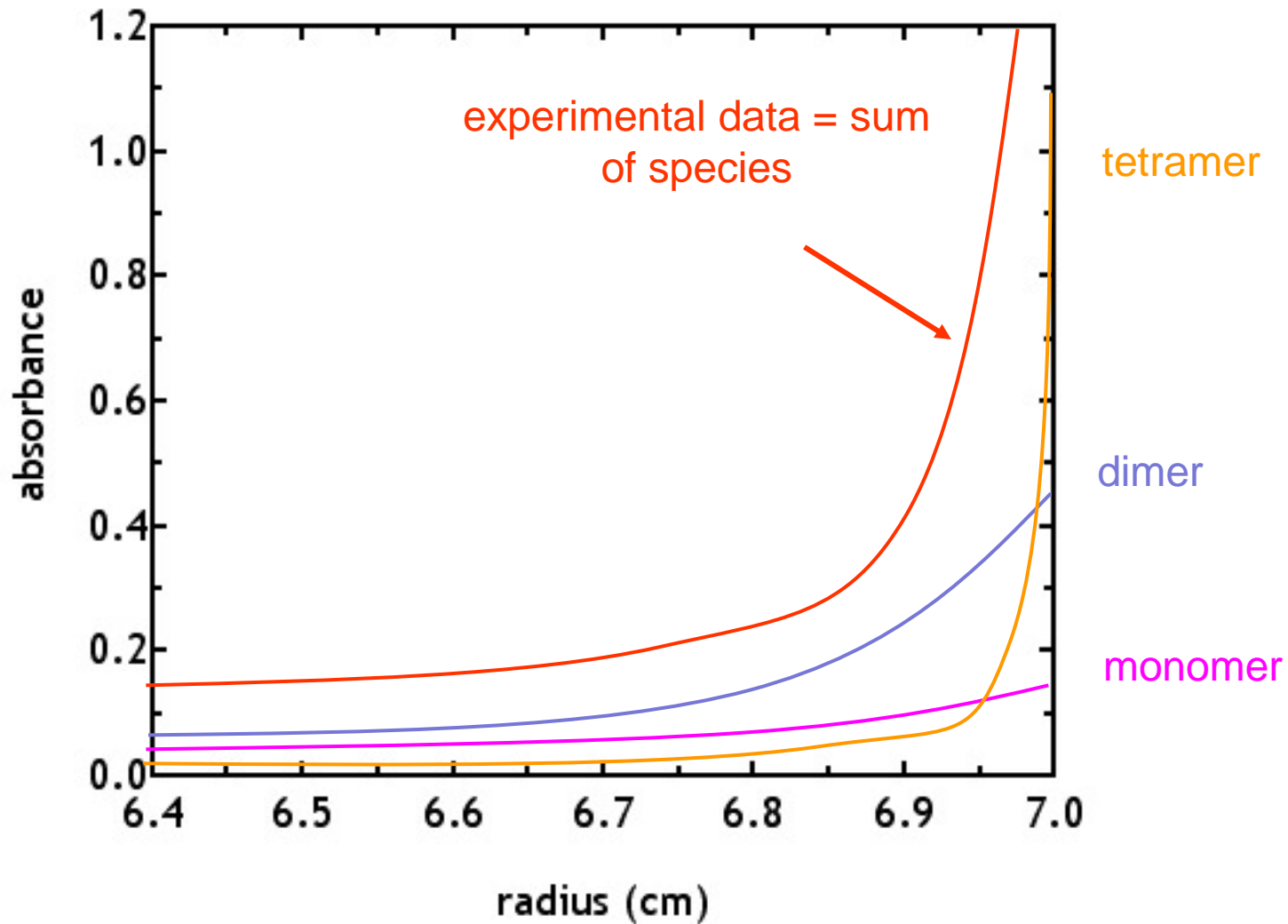
$$+ \exp[n_3 \ln A_0 + \ln K a_3 + n_3.H.M(r^2 - r_0^2)]$$

$\leftarrow 1-n_3$

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

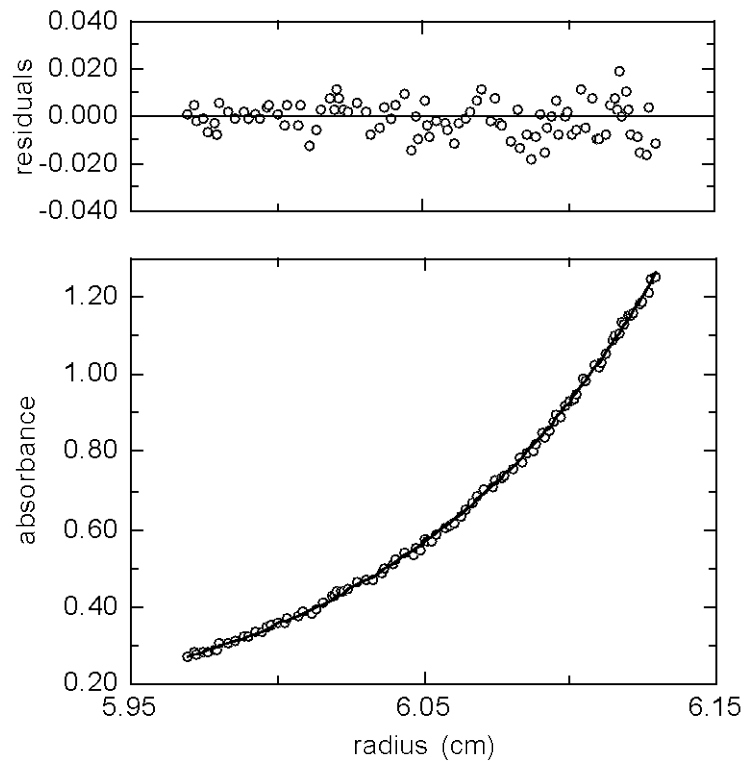
$\leftarrow 1-n_4$

Self-association: “deconvolution” into individual components

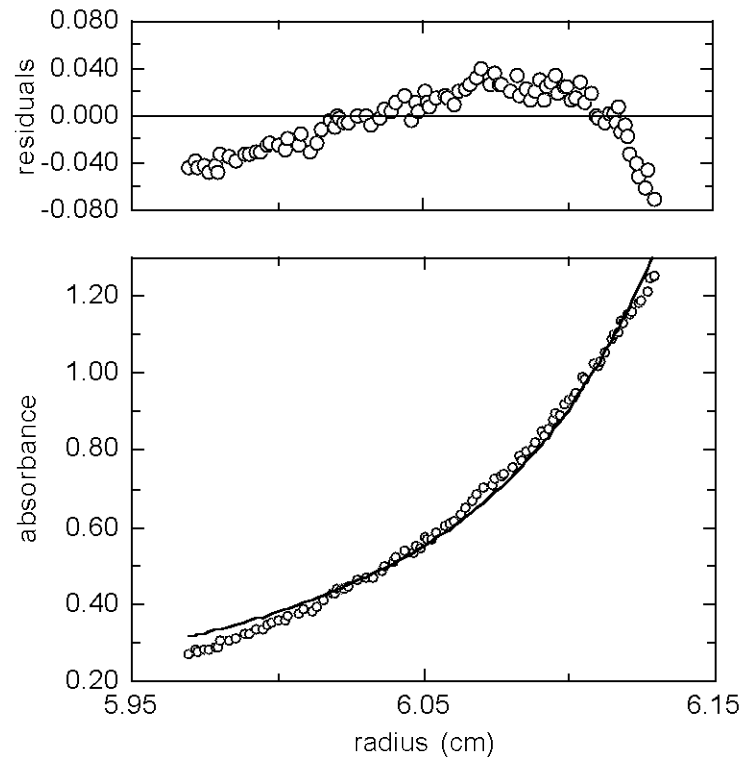


Self-association: best model revealed by residuals

2-4



1-4



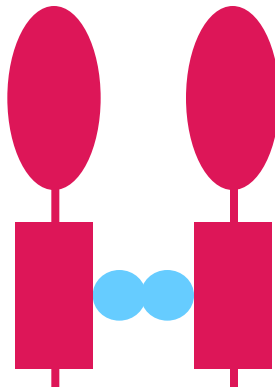
LET'S HAVE A BREAK!

Outline

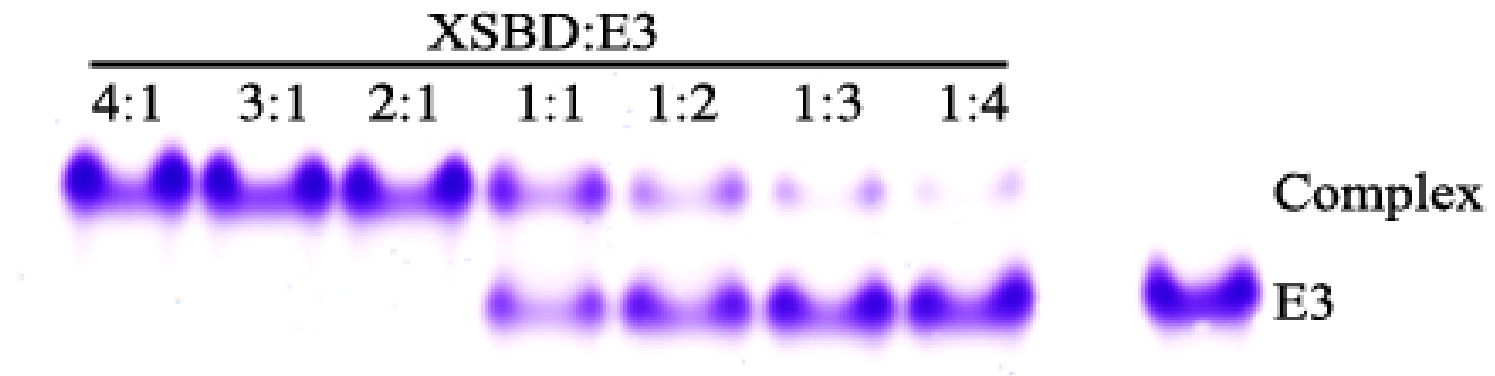
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Hetero-association example: PDC E3BP-DD:E3 sub-complex

- E3 forms a homo-dimer
- E3 binds to E3BP-DD

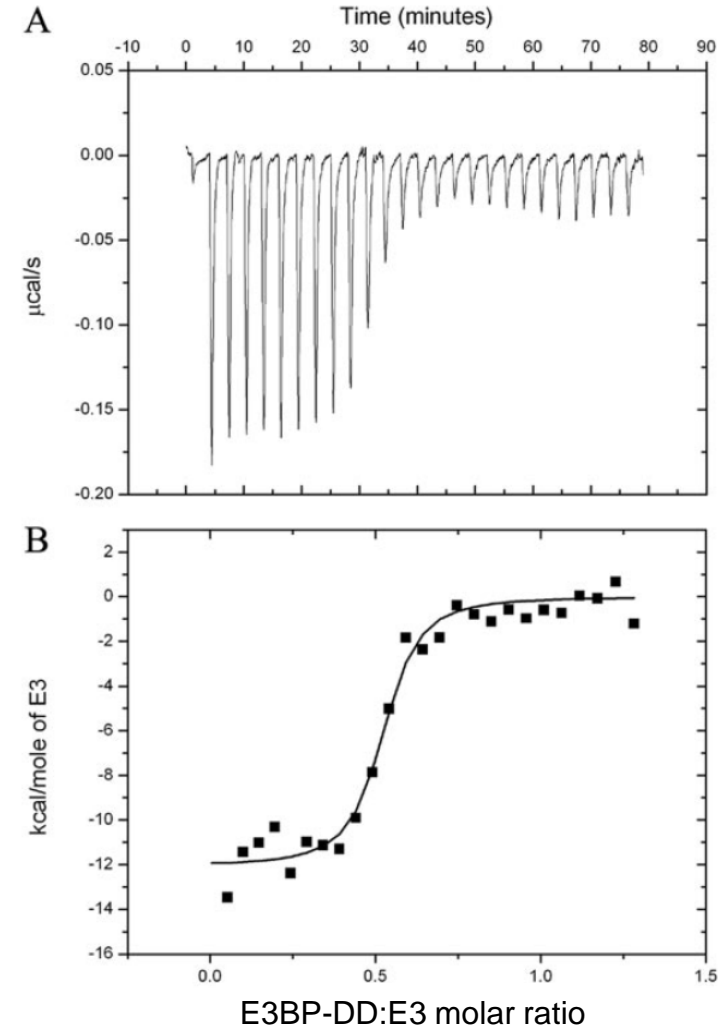


Native PAGE: stoichiometry is 2:1



ITC: stoichiometry is 2:1

- Microcal VP-ITC
- $T = 25^{\circ}\text{C}$
- Proteins dialysed o/n vs ref buffer
- 10 μl aliquots E3 (40.7 μM) titrated into 6.2 μM E3BP-DD
- Data fitted with non-linear regression model (Microcal software)
- $K_d = 36 \text{ nM}$
- $\Delta H = -12.1 \text{ kcal/mol}$
- $T\Delta S = -1.7 \text{ kcal/mol}$
- $N = 0.5 \text{ molecules E3 bind/molecule E3BP-DD}$
 - equivalent to 2 E3BP-DD/E3

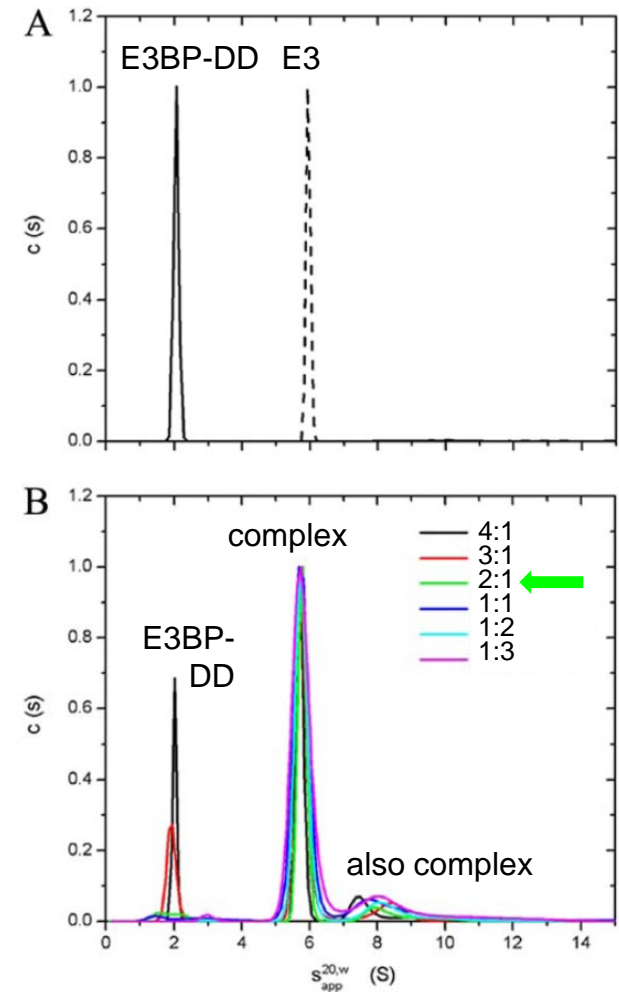


SV titration

- $T = 4^{\circ}\text{C}$
 - Must ensure that T is constant
 - Takes *hours* to thermally equilibrate
- Rotor speed 45k rpm
- Interference optics used
 - Scan interval 1 minute
- $[\text{E3}] = 4.9 \mu\text{M}$
- Sample volume 380 μl
- Pathlength 12 mm

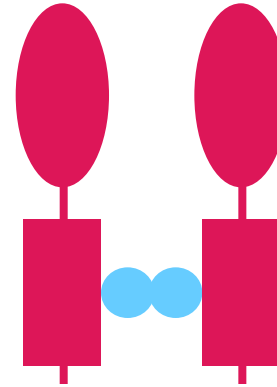
SV titration: stoichiometry is 2:1

- Expt 1: SV of E3 alone; SV of E3BP-DD alone
 - Determine their s
- Expt 2: SV of E3BP-DD+E3
 - 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



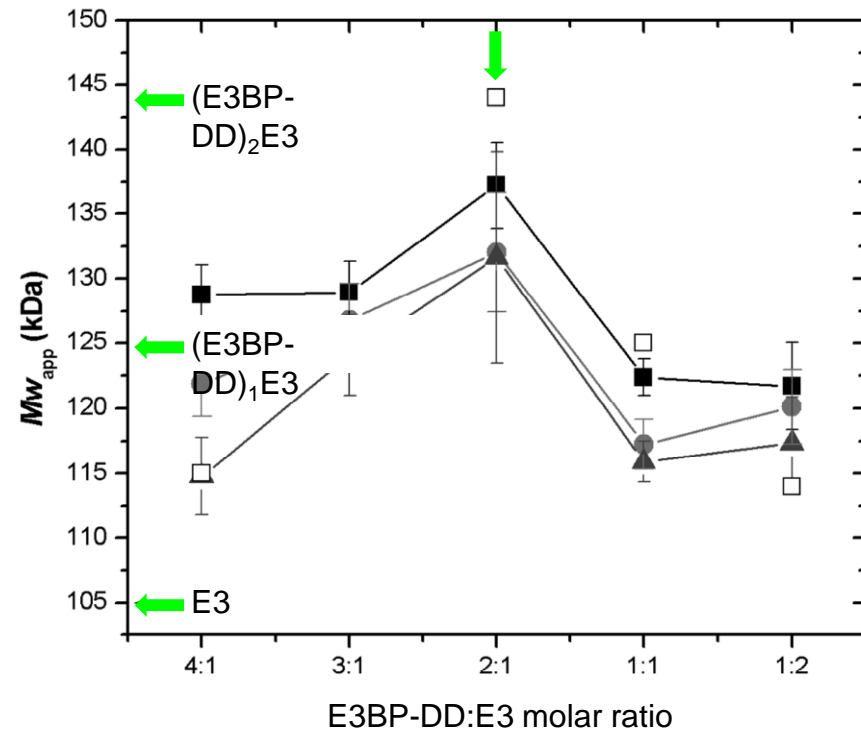
SE titration

- From amino acid sequence:
 - E3BP-DD M = 19.5 kDa
 - E3 M = 105 kDa
- Sample volume = 30 μ l
- Path-length 3 mm
- SE performed at 3 rotor speeds
 - 8.5, 12, 16k rpm
 - Appropriate for different complexes
- Absorbance data (280 nm)
- Radial step size 0.001 cm
- Program WINMATCH used to demonstrate attainment of equilibrium
 - Comparison of scans 3 h apart



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

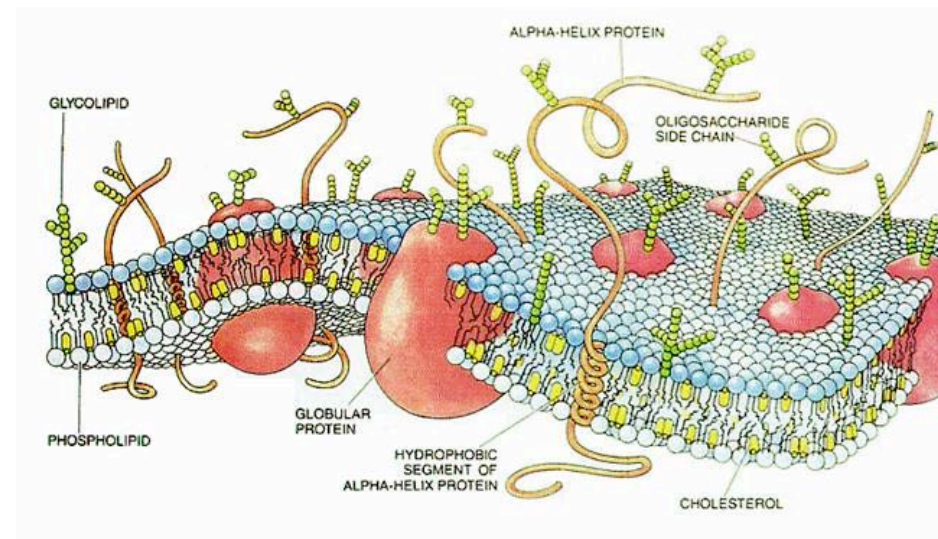
- AUC background
- How AUC experiments are performed
- Data analysis
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- **Detergent solubilised systems**
- Hydrodynamic bead modelling (HBM)
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Detergent solubilised proteins: density matching SE

- In SE bouyant molecular weight is determined:

$$M_p(1 - \phi' \rho) = M_p[(1 - \bar{v}_p \rho) + \delta_{\text{Det}}(1 - \bar{v}_{\text{Det}} \rho)]$$

- In many AUC expts we want to observe self-association
- Density matching is a good method for self-associating membrane proteins



Burgess, N. K., Stanley, A. M. & Fleming, K. G. (2008). Determination of membrane protein molecular weights and association equilibrium constants using sedimentation equilibrium and sedimentation velocity. In *Methods in Cell Biology* (J. Correia & H. W. Detrich, III, eds.), **84**, 181-211. Academic Press

Density matching SE: experimental conditions

- Experimental conditions adjusted such that:

- solvent ρ = effective ρ of bound detergent

$$\rho = 1/\bar{v}_{\text{Det}}$$

$$M_p(1 - \phi'\rho) = M_p[(1 - \bar{v}_p\rho) + \delta_{\text{Det}}(1 - \bar{v}_{\text{Det}}\rho)]$$

→ 0

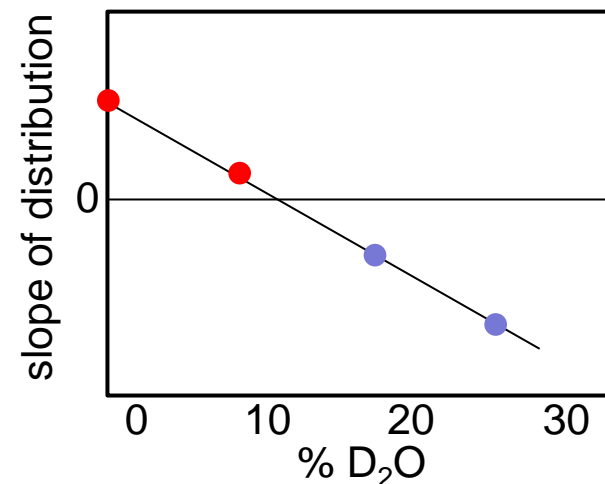
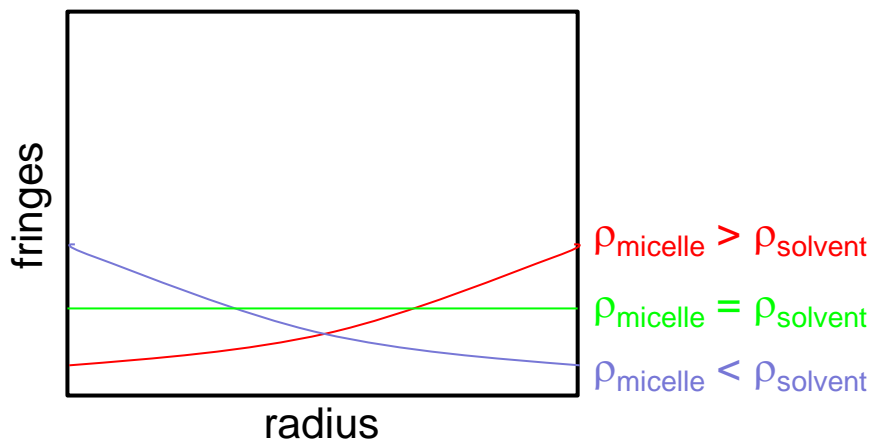
- So detergent becomes effectively invisible to centrifugal field
- SE data can be analysed with standard methods

BUT.....this method works only in certain conditions

- The solvent density must be adjusted with D₂O or D₂¹⁸O
 - Alternatives would be e.g. sucrose or other co-solvent
 - Affect chemical potential
 - Lead to preferential binding and/or exclusion of water or additional co-solvent at protein surface
- But use of D₂O or D₂¹⁸O limits detergents that can be used
 - $\rho \bar{v}_{D_2O} = 1.1 \text{ g/ml}$
 - of the detergent must be between that of water and D₂O
 - i.e. $0.9 \leq \bar{v} \leq 1.0 \text{ ml/g}$
 - Eliminates:
 - dodecylmaltoside ($\rho = 1.21 \text{ g/ml}$, $\bar{v} = 0.83 \text{ ml/g}$)
 - β -octylglucoside ($\rho = 1.15 \text{ g/ml}$, $\bar{v} = 0.87 \text{ ml/g}$)
 - Suitable:
 - C8E5 ($\bar{v} = 0.993 \text{ ml/g}$)
 - C14SB (density matched by 13% D₂O in 20 mM Tris-HCl, 200 mM KCl)
 - Dodecylphosphocholine (DPC, density matched by 52.5% D₂O in 50 mM Tris-HCl, 100 mM NaCl)

Determination of density-matching point for C14SB

- Determine % of D₂O required to density match C14SB micelles in background of other buffer components
 - 30 mM C14SB in 20 mM Tris-HCl, 200 mM KCl made in 0, 10, 20, 30% D₂O
 - Reference solvent the same minus detergent
 - SE observed with interference optics
 - Collect “buffer blanks” for subtraction to reduce noise
 - Then replace buffer with micelle solution in sample channel
 - Rotor speed 50k rpm
 - T = 25°C



SE of systems solubilised by C14SB: OMPLA

- Outer membrane phospholipase A (OMPLA)
 - Gram negative bacteria
- Beckman XL-A, $T = 25^{\circ}\text{C}$
- 20 mM Tris-HCl, 200 mM KCl
- 13% D_2O
 - $[\text{OMPLA}] = 0.3, 0.6, 0.9 \text{ A}_{280}$ (12 mm pathlength)
 - Rotor speed = 16.3, 20, 24.5k rpm
 - $[\text{C14SB}] = 5 \text{ mM}$
 - Increased [detergent] \rightarrow dilution of protein that is solubilised in detergent phase thus promoting dissociation
 - Monomer mass determined

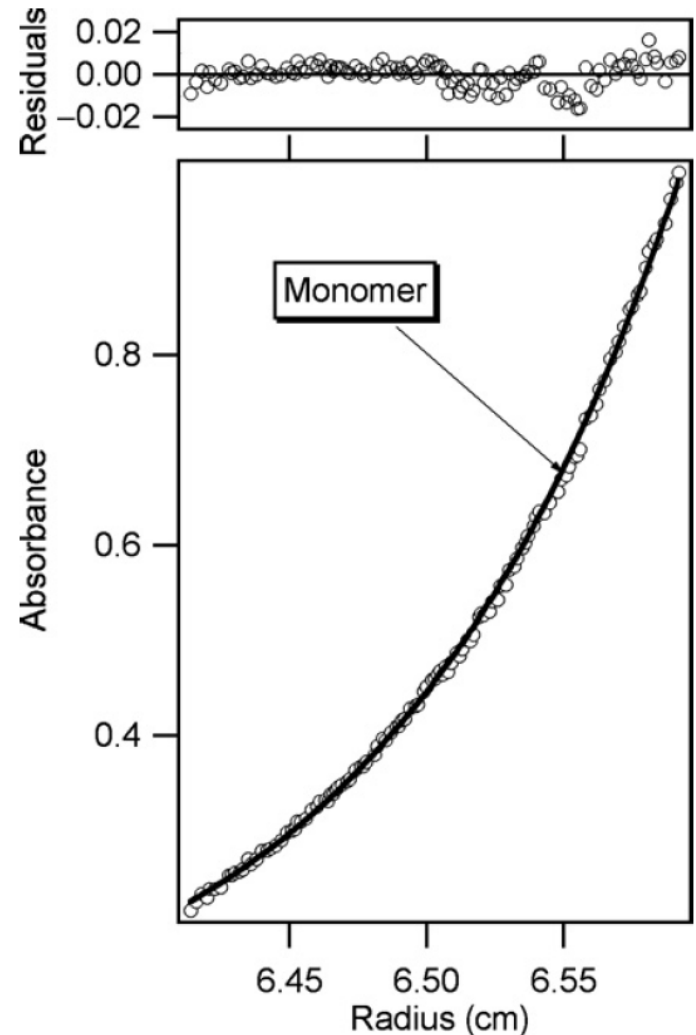


OMPLA studied at 3 concs, 3 rotor speeds for each of 4 conditions

1. OMPLA
2. OMPLA + 20 mM CaCl_2
3. OMPLA + covalently bound fatty acyl chain substrate analogue
4. OMPLA + covalently bound fatty acyl chain substrate analogue + 20 mM CaCl_2

SE results: 1. OMPLA

- SE data first globally fitted with equation for single ideal species
- Good fits
 - $\sqrt{\sigma^2} \approx$ instrument noise (≈ 0.005)
 - Residuals randomly distributed about 0
- M for all 9 data sets within 5% of monomer M
- Conclusion: OMPLA monomeric in absence of co-factors

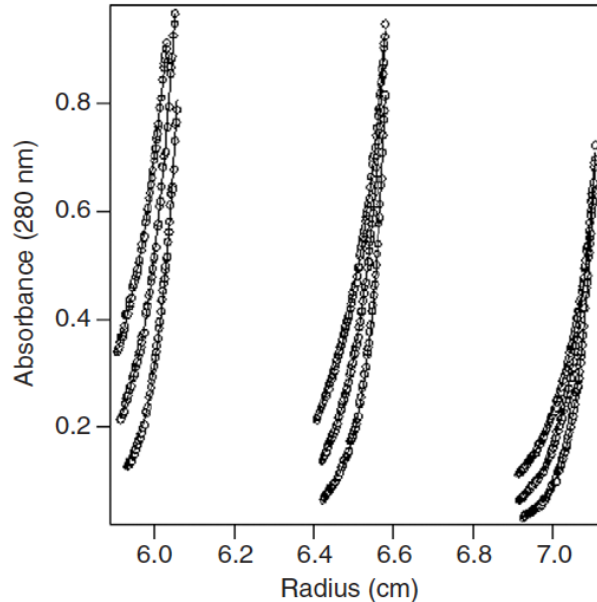
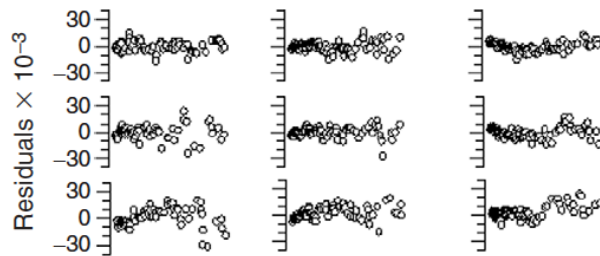


SE results: 4. OMPLA + covalently bound fatty acyl chain substrate analogue + 20 mM CaCl_2

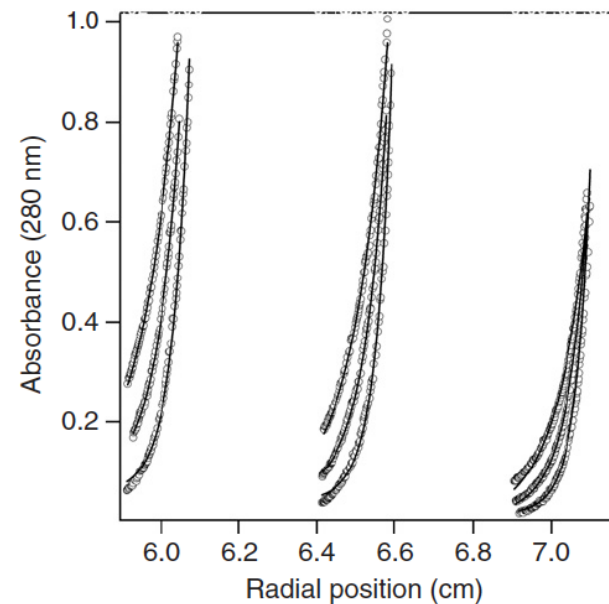
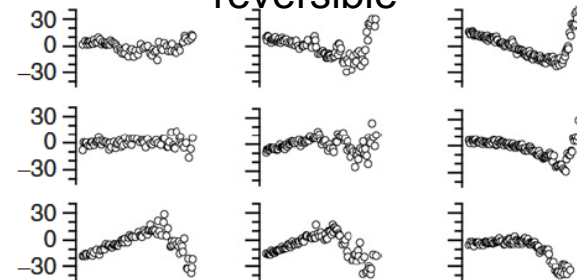
- 2 fatty acyl chain analogues tested:
 - decylsulfonylfluoride (DSF)
 - perfluorinated octylsulfonylfluoride (pOSF) (all H replaced by F)
- For both analogues, single species fits returned $M > M_{\text{monomer}}$
- Therefore tried
 - Monomer-dimer
 - Monomer-trimer
 - Monomer-tetramer
- Fitting parameter is K_d

OMPLA-DSF reversibly dimerises

DSF: monomer-dimer reversible



pOSF: monomer-dimer not reversible



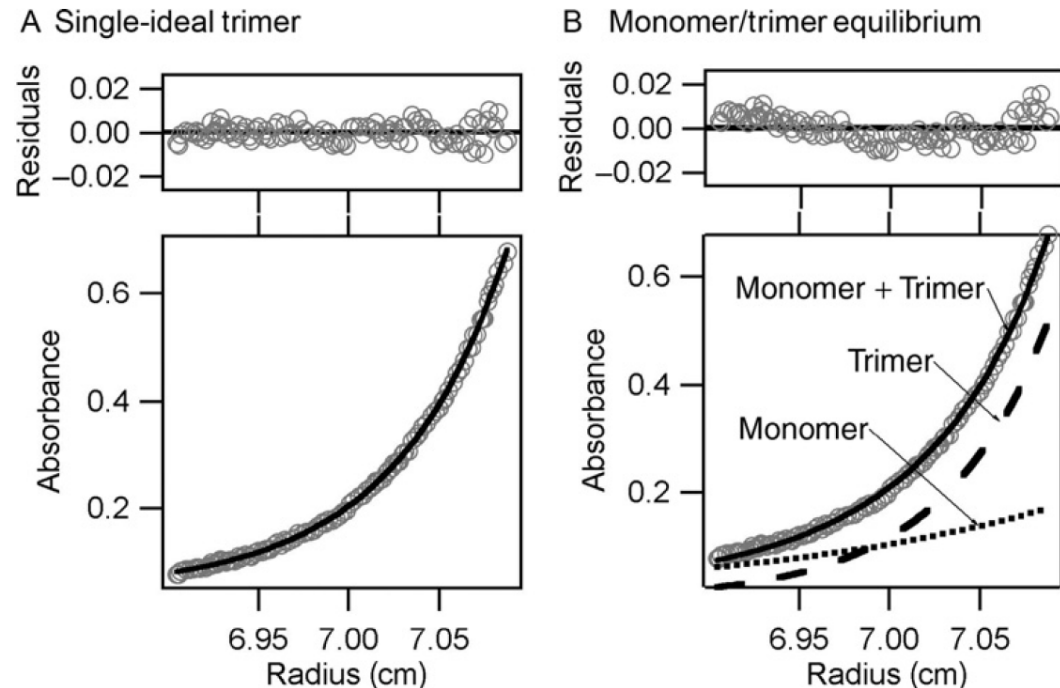
SE of systems solubilised by C14SB: OmpF

- *E. coli* OmpF
- Beckman XL-A, $T = 25^{\circ}\text{C}$
- 20 mM Tris-HCl, 100 or 200 mM KCl
- 13% D_2O
- OmpF normally trimer
- Collected 36 data sets:
 - $[\text{OmpF}] = 0.3, 0.6, 0.9 \text{ A}_{230}$ (12 mm pathlength)
 - $[\text{C14SB}] = 5, 12 \text{ \& } 30 \text{ mM}$
 - Rotor speed = 9, 11, 13.5, 16.3k rpm



OmpF

- Self-association probed in 2 ways:
 - Working at low [protein]
 - Increasing [detergent]
- At each [detergent], SE data globally fitted
 - For 4 rotor speeds & 3 [protein]

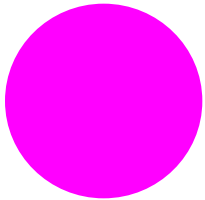


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s = deviation from sphericity + hydrodynamic hydration

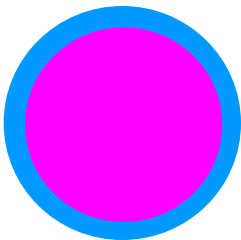
$$s = \frac{M(1 - \bar{v}\rho)}{N_A f}$$



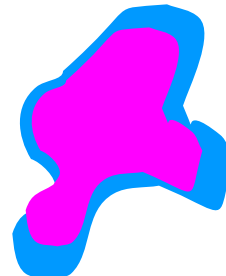
M, f_0



$M, f > f_0$

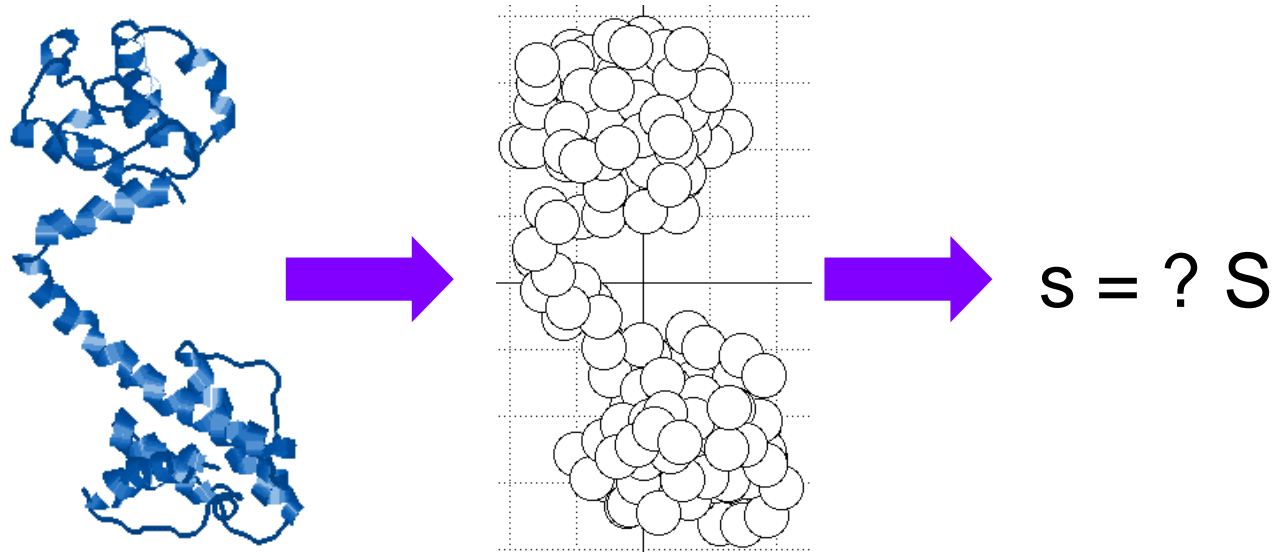


$M, f > f_0$



$M, f \gg f_0$

Sedimentation coefficient is a constraint for SAS modelling



- For one sphere $f_0 = 6\pi\eta R_0$
- For an assembly of N spheres an approximate solution is

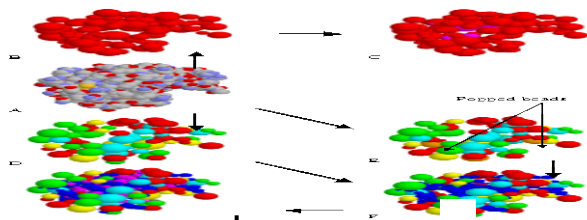
$$f_t = \frac{\sum_{i=1}^N \zeta_i}{1 + (6\pi\eta_0 \sum_{i=1}^N \zeta_i)^{-1} \sum_{i \neq j}^N \sum_j^N \zeta_i \zeta_j r_{ij}^{-1}}$$

- where $\zeta_i = 6\pi\eta_0\sigma_i$

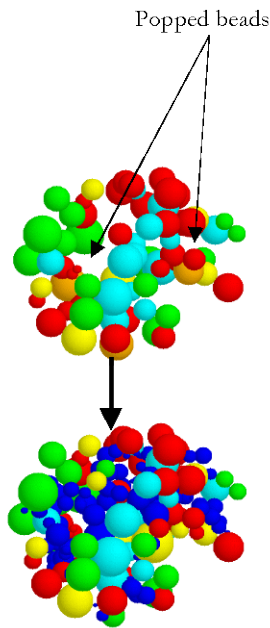
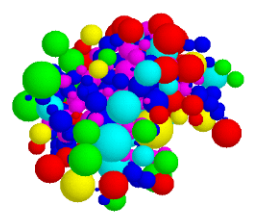
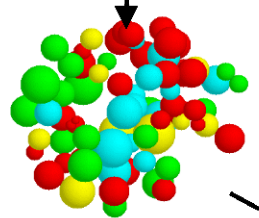
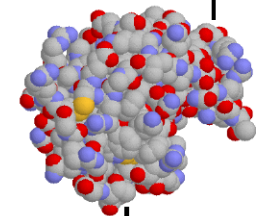
Several freely available programs for HBM

- A more exact expression for f_t together with expressions for other hydrodynamic and related parameters are encoded in HBM software:
- José García de la Torre *et al.* (Universidad Murcia, Spain)
 - <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/>
 - Generates HBMs from pdb files, computes hydrodynamic & other parameters with realistic hydration

SOMO - construction of “intelligently” hydrated bead models from atomic coordinates



AtoB

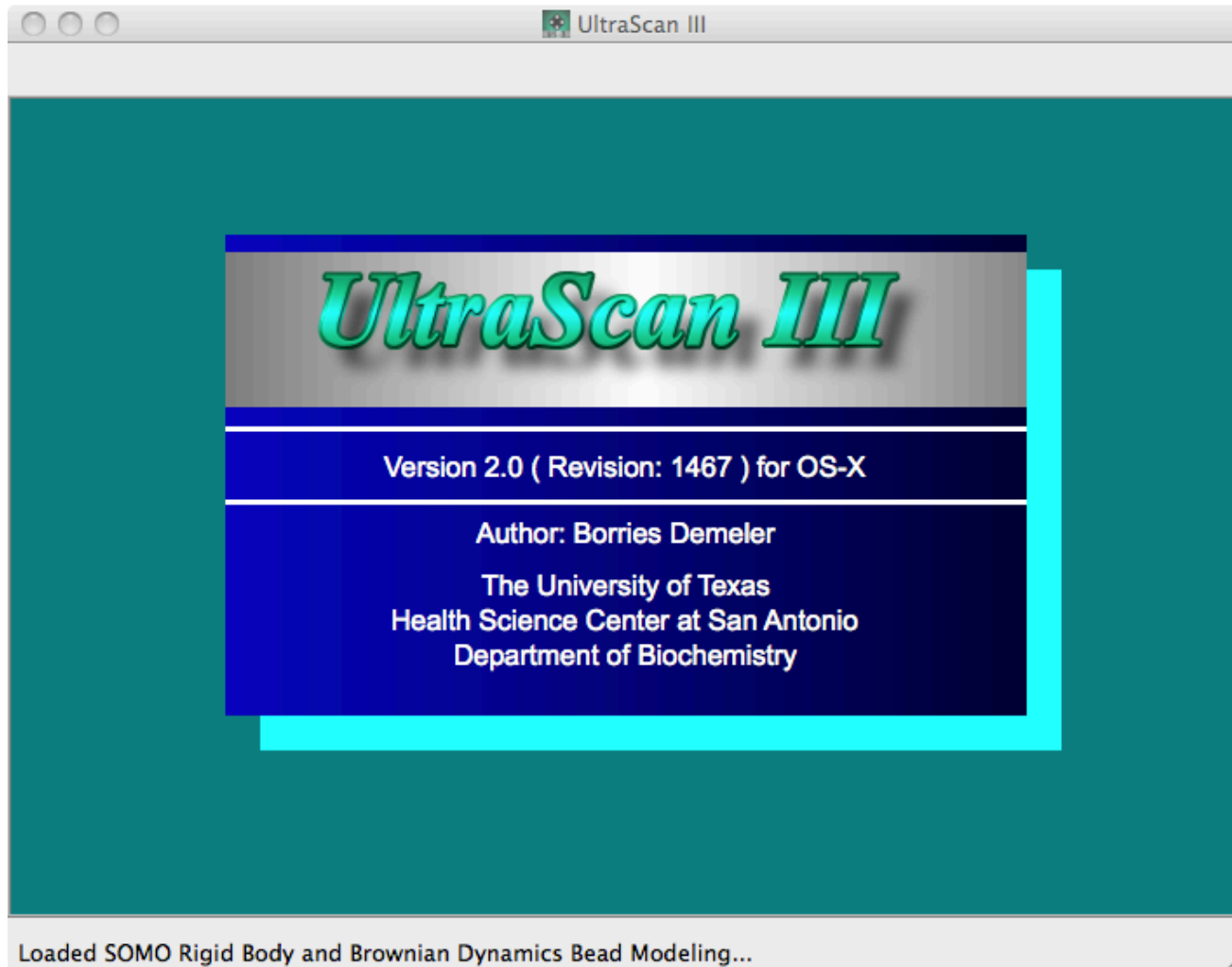


Trans

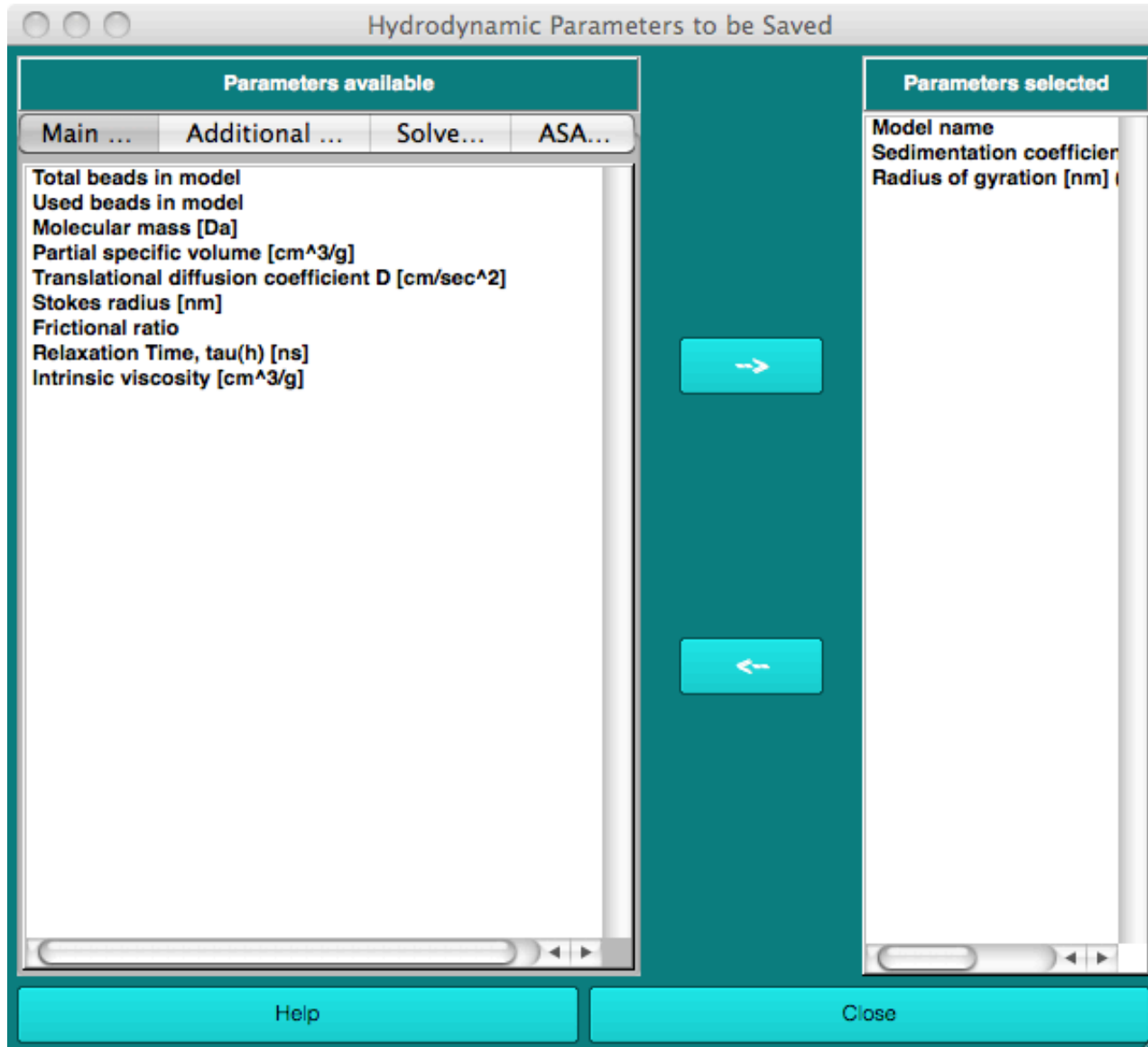
exposed acidic
exposed basic
exposed polar
exposed
nonpolar/hydrophobic
mainchain
buried

- Water of hydration included in each bead
- Bead overlaps removed hierarchically
 - Reducing radii + translating bead centres outwards
- Beads overlapping by > preset threshold are fused (“popped”)
- Buried beads excluded from hydrodynamic calculations
 - Reduces cpu time

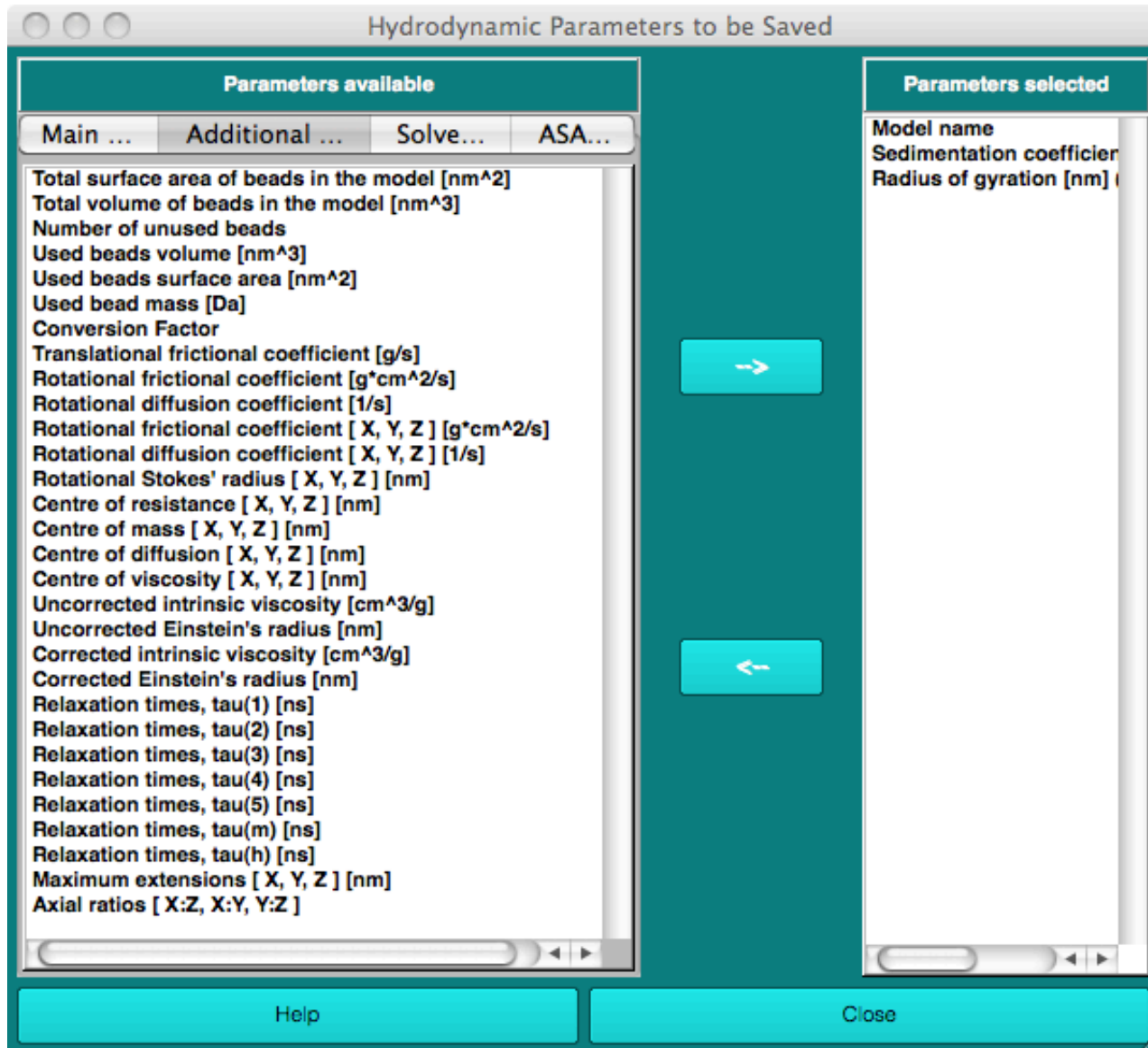
SOMO is a subprogram of UltraScan III



Parameters computed by SOMO (1)



Parameters computed by SOMO (2)



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

- $\alpha_5\beta_1$ 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 TVPGSKSTATISGLKPGVDYTTITVYAVTG RGD SPASSKPISINYRTSKLEPKSSDTPPGSPRSP
EPKSSDTPPGSPRSGRIKQLEDKIEELLSKIYHLENEIARLKKLIGELEDKIENLGC

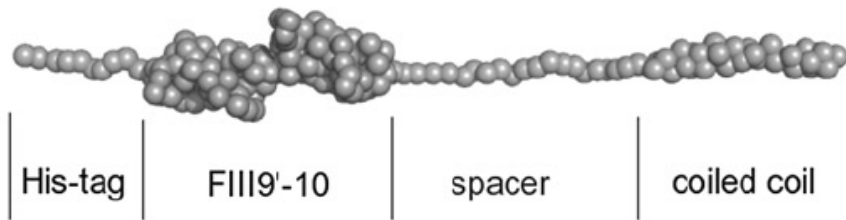
↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑

- 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

Oligomer models generated

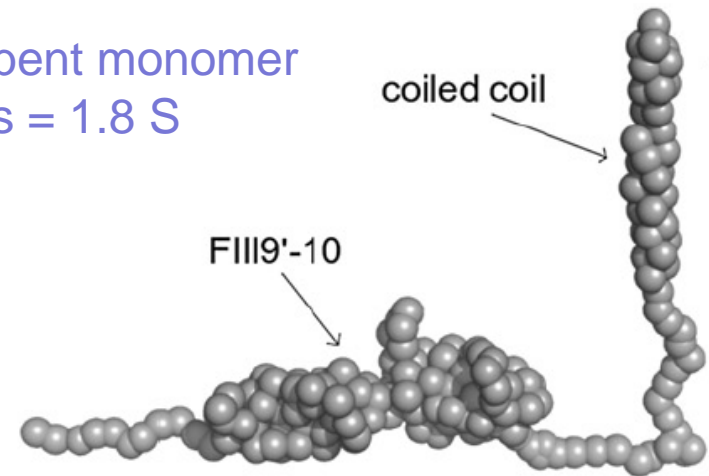
linear monomer
 $s = 1.7 S$

A(i)



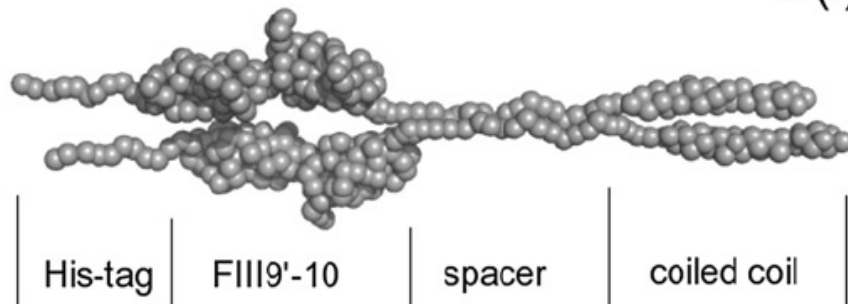
bent monomer
 $s = 1.8 S$

A(ii)



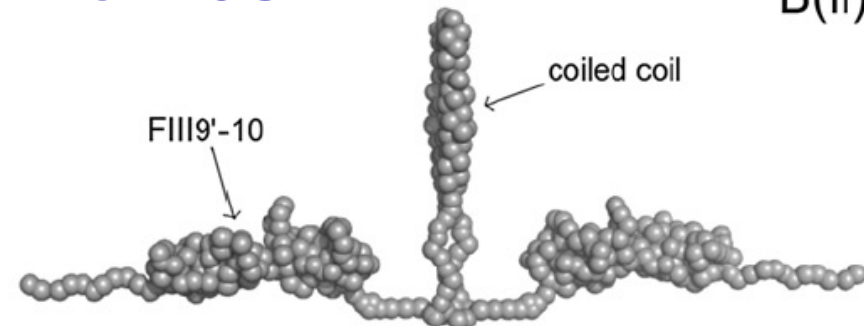
linear dimer
 $s = 2.7 S$

B(i)



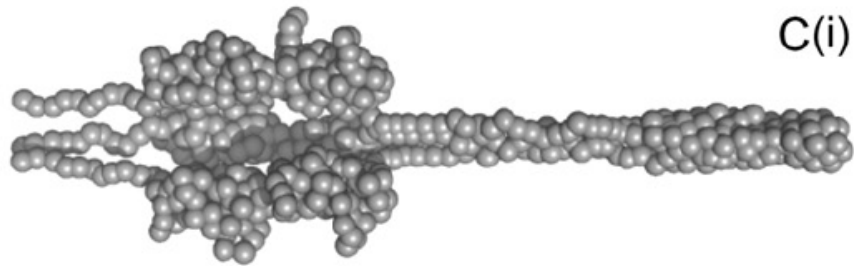
bent dimer
 $s = 2.5 S$

B(ii)

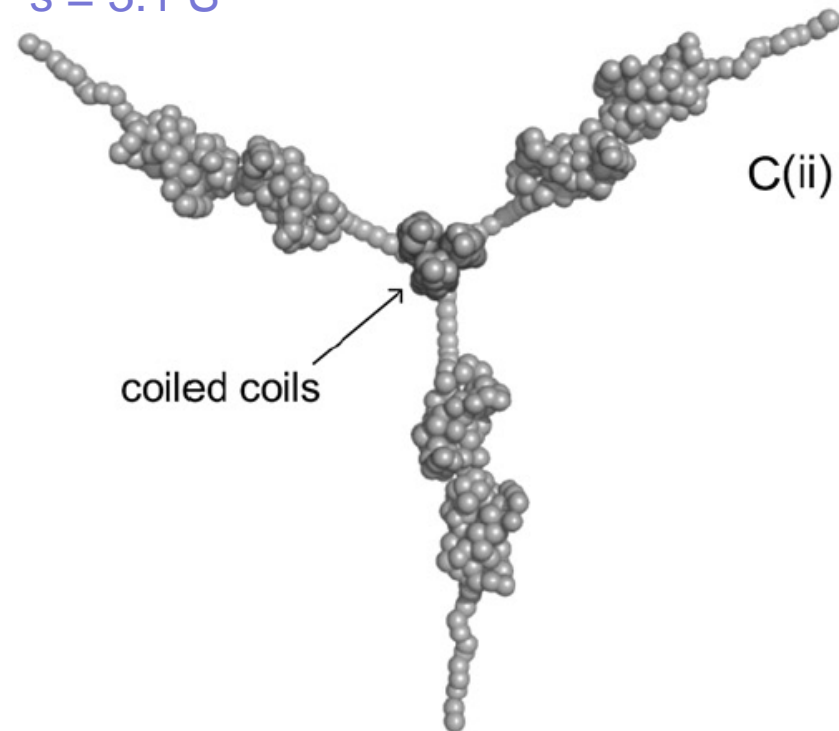


Oligomer models generated

linear trimer
 $s = 3.9 \text{ S}$

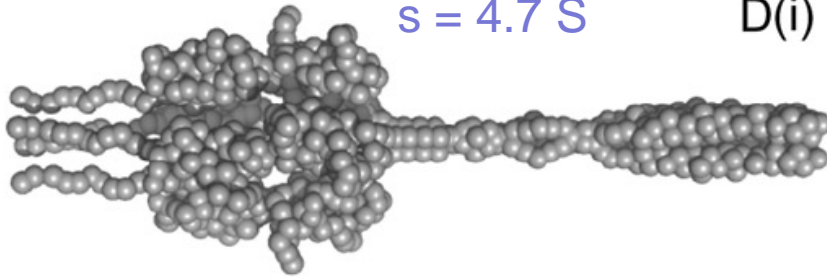


bent trimer
 $s = 3.1 \text{ S}$

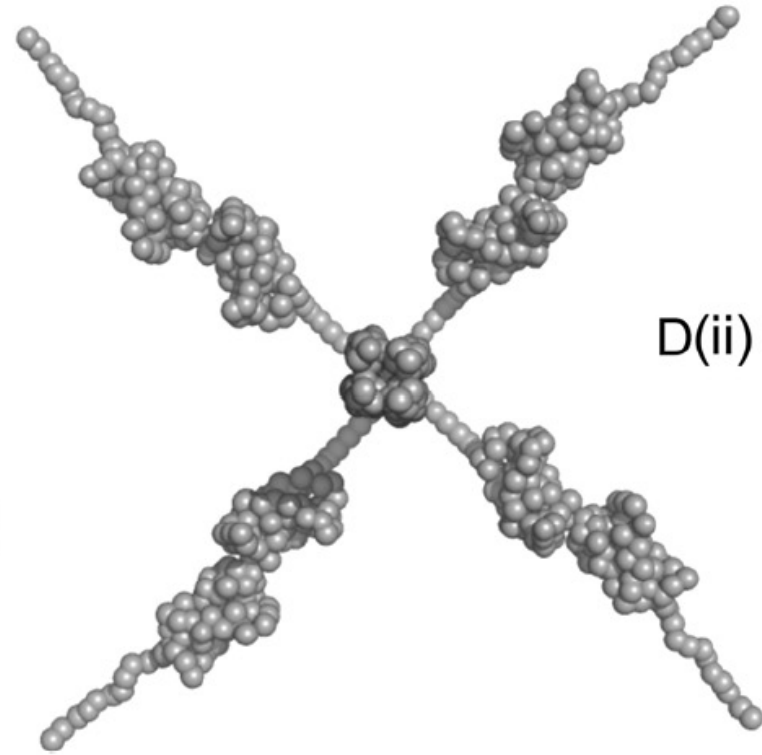


Oligomer models generated

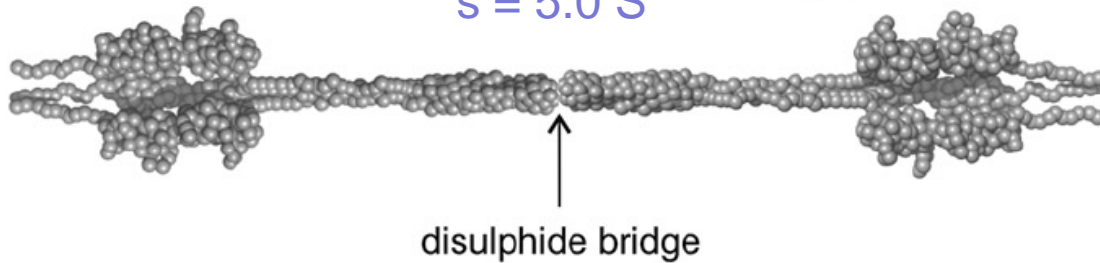
linear tetramer
 $s = 4.7 \text{ S}$ D(i)



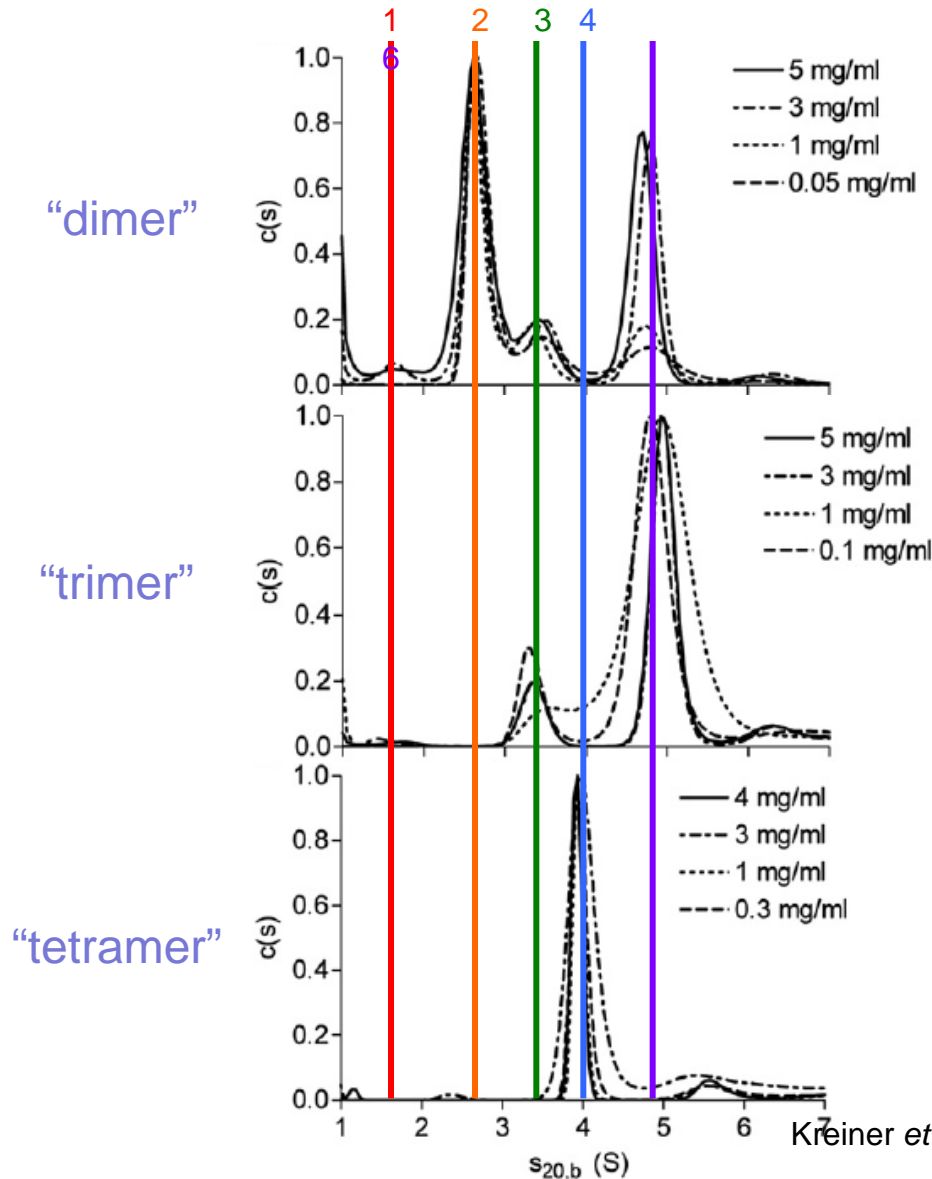
bent tetramer
 $s = 3.7 \text{ S}$



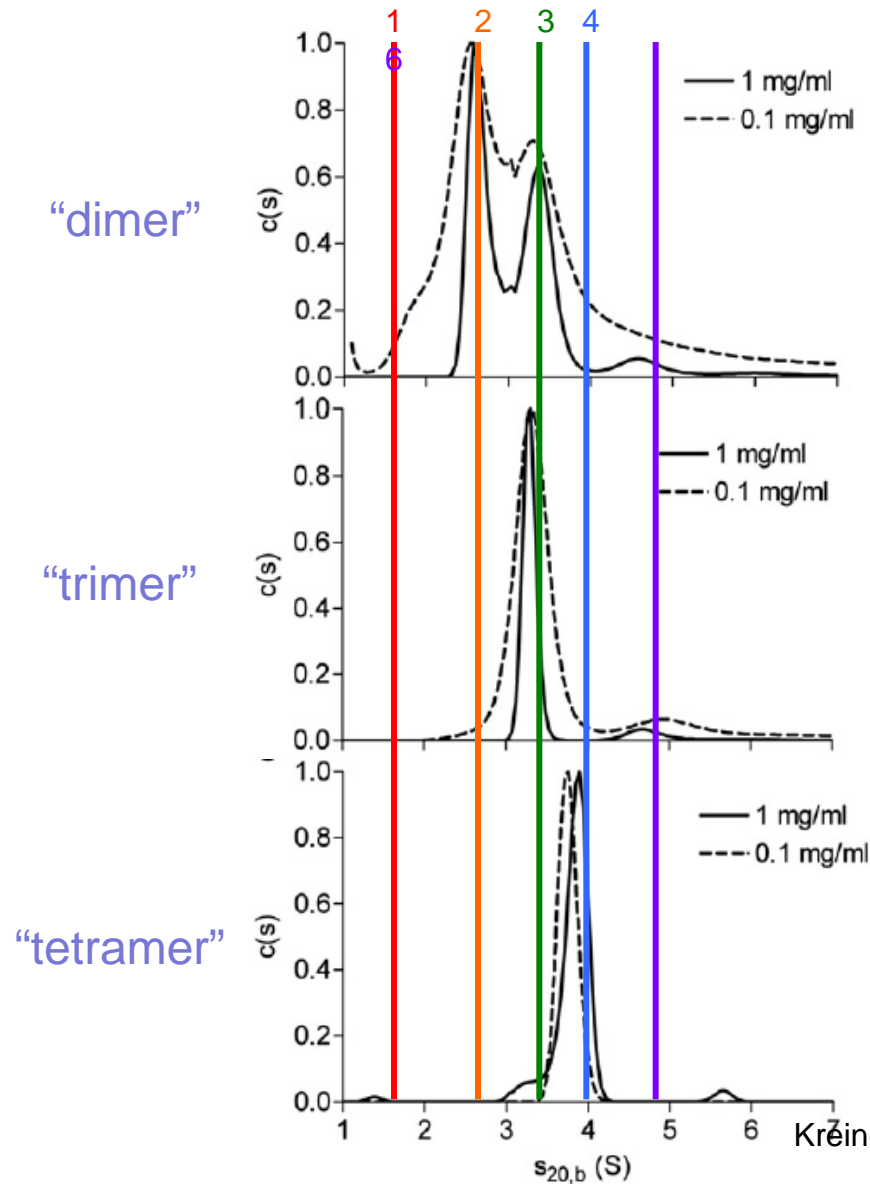
linear hexamer
 $s = 5.0 \text{ S}$ E



AUC SV no DTT: c(s) analysis reveals complex composition



AUC SV + DTT: $c(s)$ analysis reveals simplified composition



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

- $\alpha_5\beta_1$ ligands used to immobilise cells on surfaces via
 - 9th type III FN domain synergy site (PHSRN)
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 - 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?**
 - They do not!
 - AUC allows model-free observation of unexpected species
 - HMB allows interpretation of these species

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