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

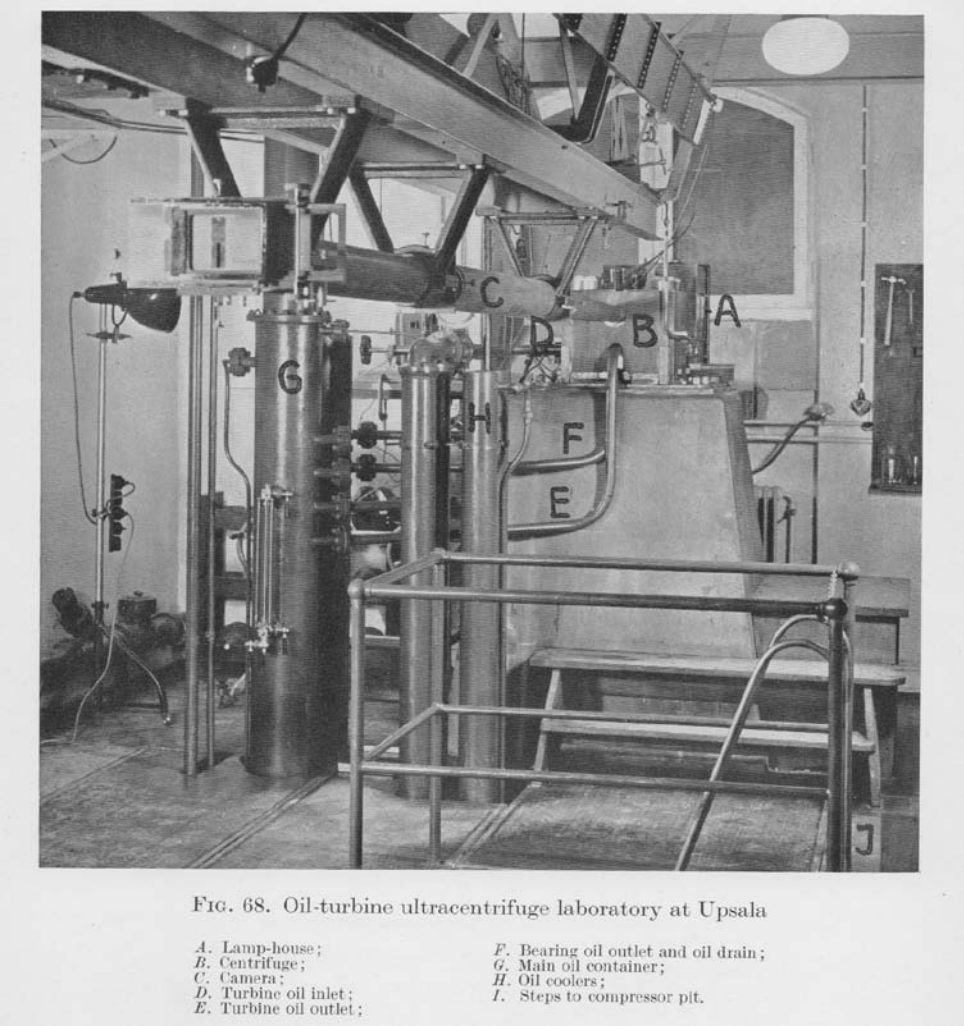


FIG. 68. Oil-turbine ultracentrifuge laboratory at Upsala

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

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 Atomics. (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."

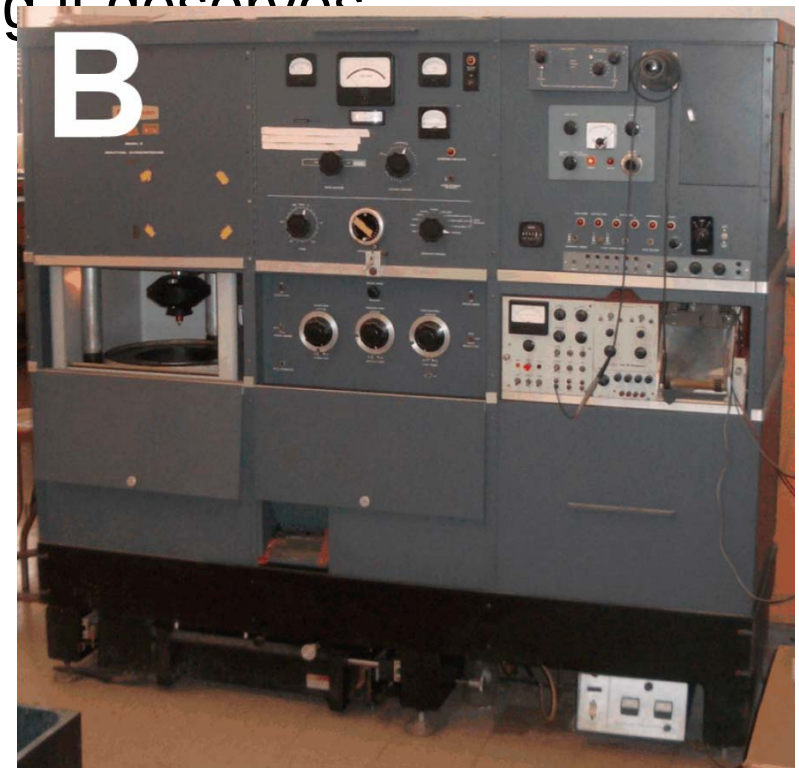
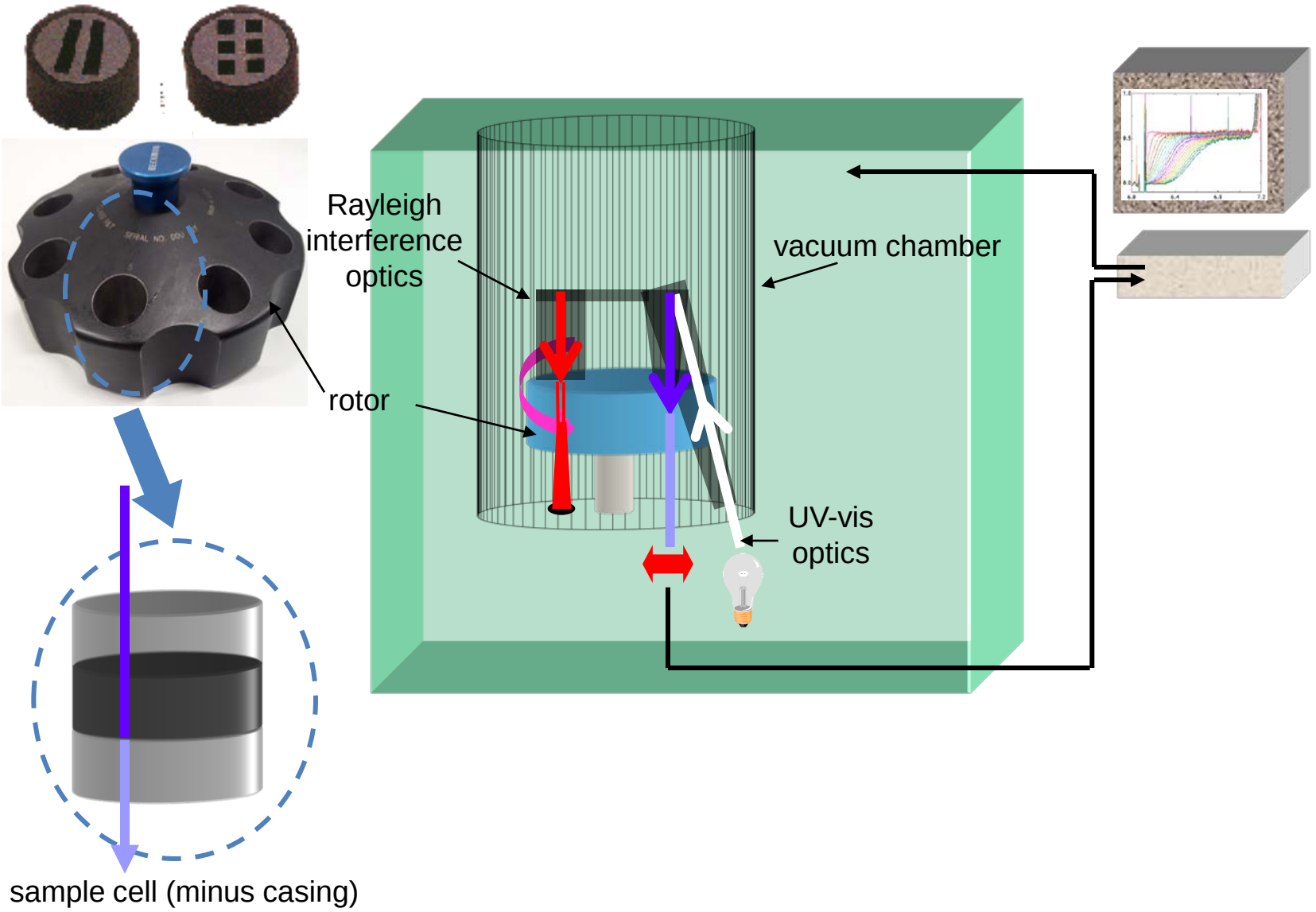


image from Analytical Ultracentrifuge User Guide Volume 1: Hardware, K. L. Planken & V. Schirf, 2008 (http://www.ultrascan.uthscsa.edu/)

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

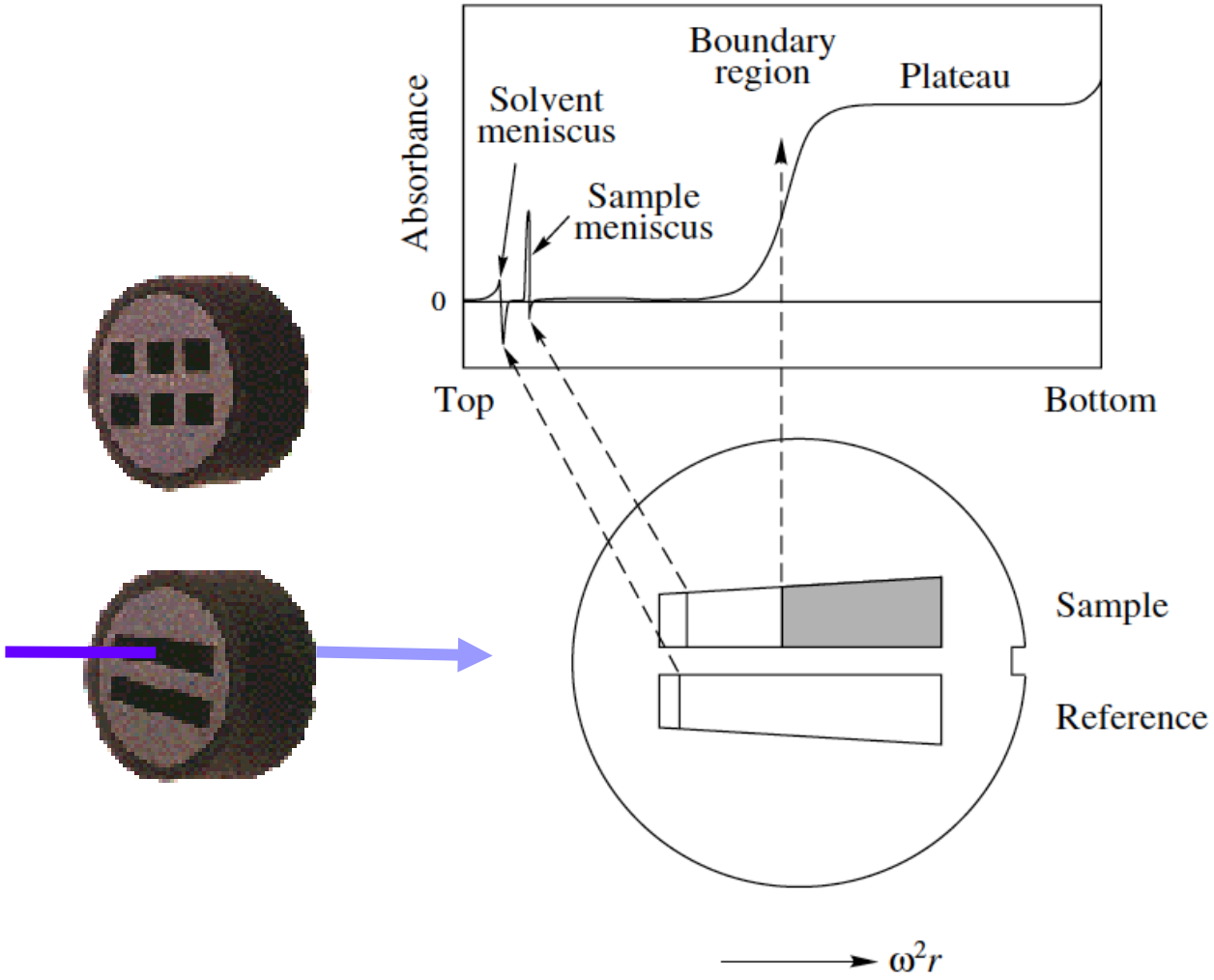


image from Ralston, 1993
https://www.beckmancoulter.com/wsrportal/bibliography?docname=361847.pdf

Inside the rotor chamber

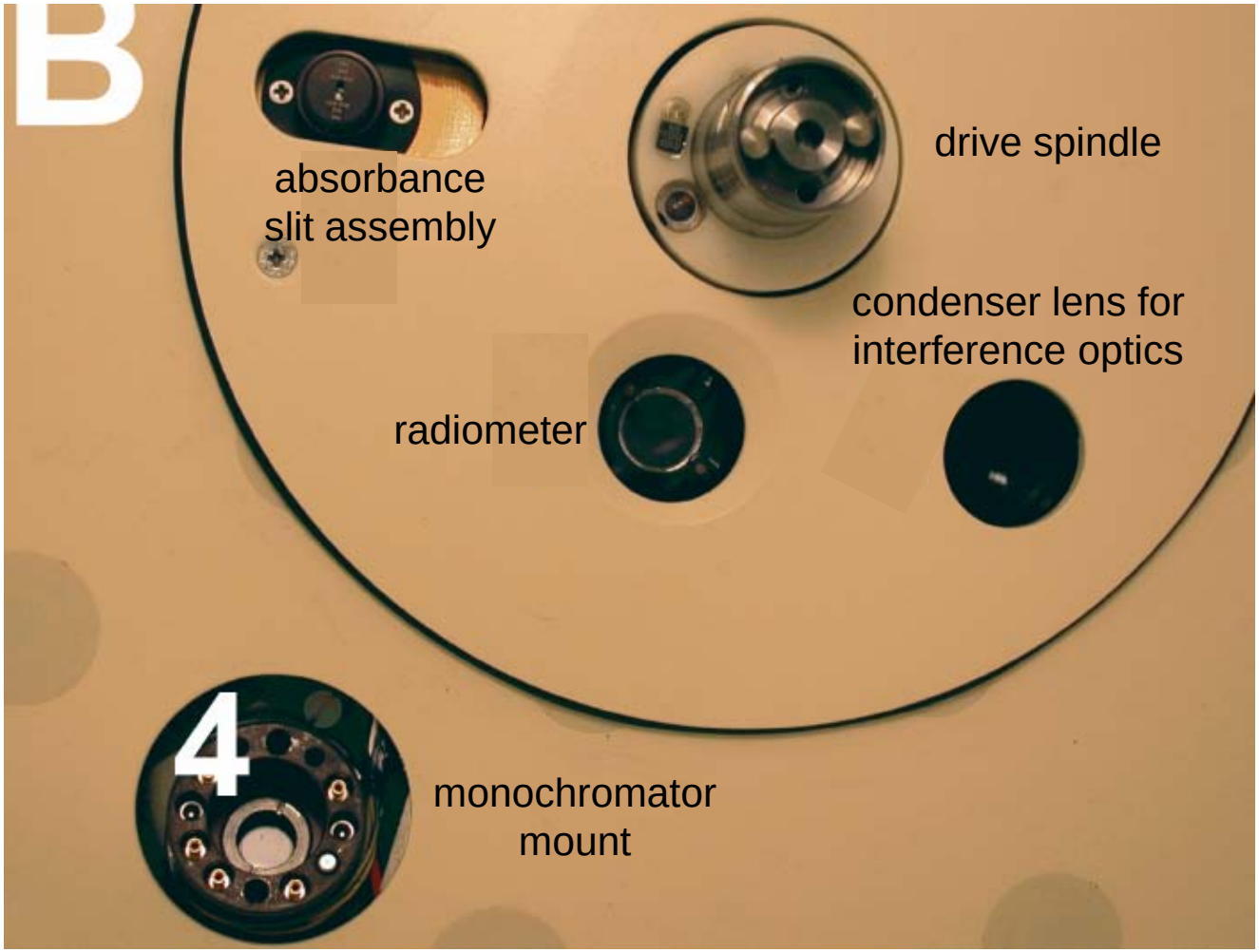
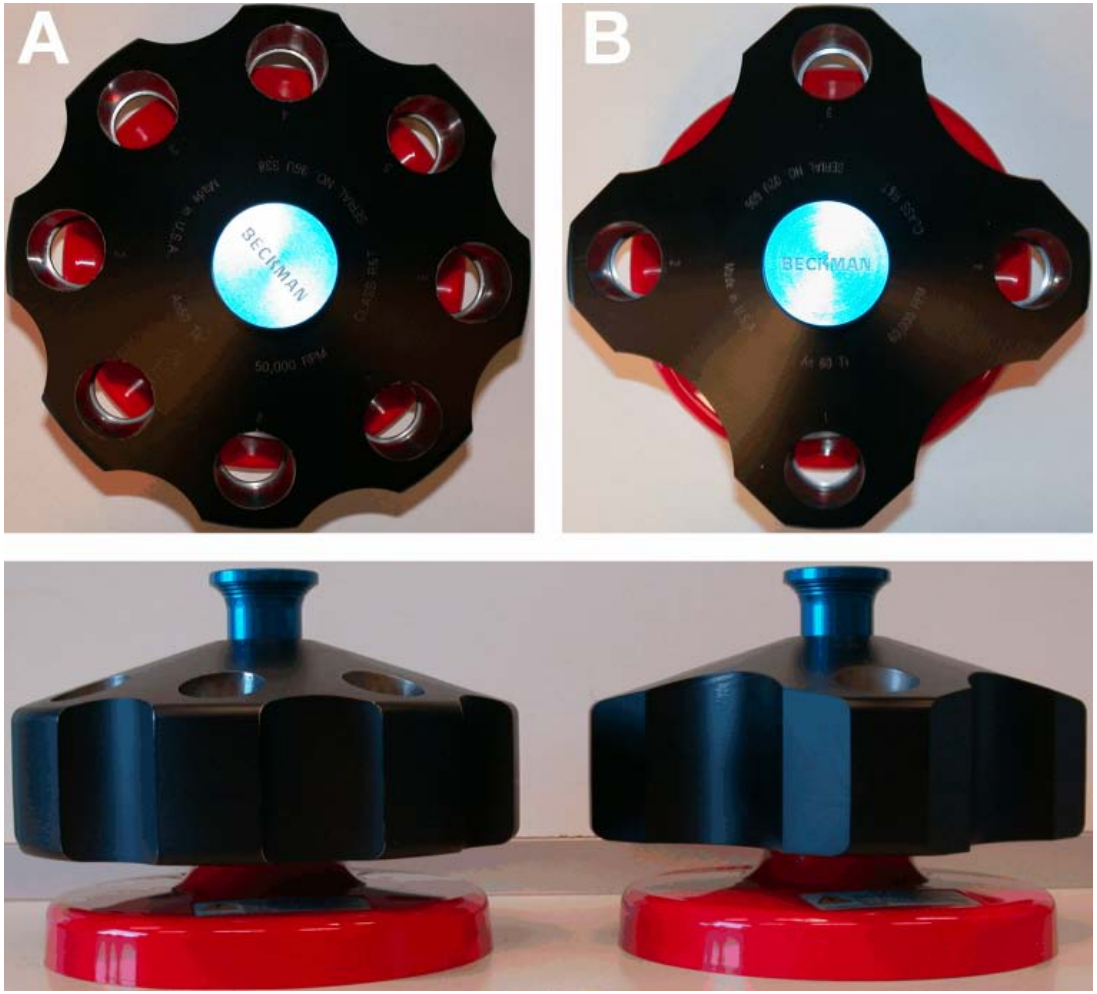


image from Analytical Ultracentrifuge User Guide Volume 1: Hardware, K. L. Planken & V. Schirf, 2008
(http://www.ultrascan.uthscsa.edu/)

Sample holders sit in holes in the AUC rotor

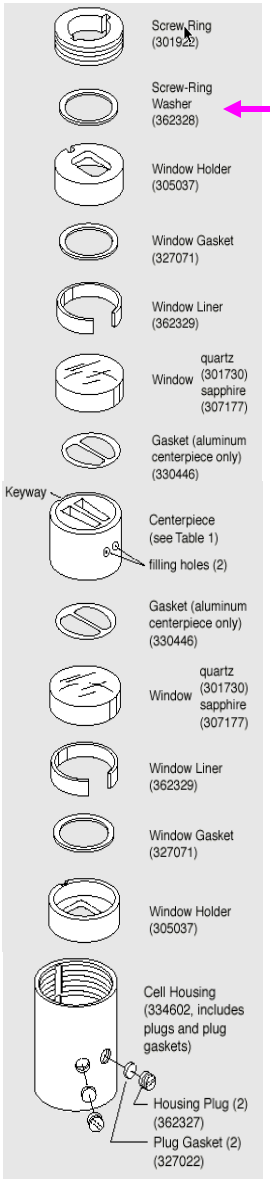
50k rpm

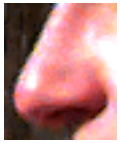


60k rpm

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

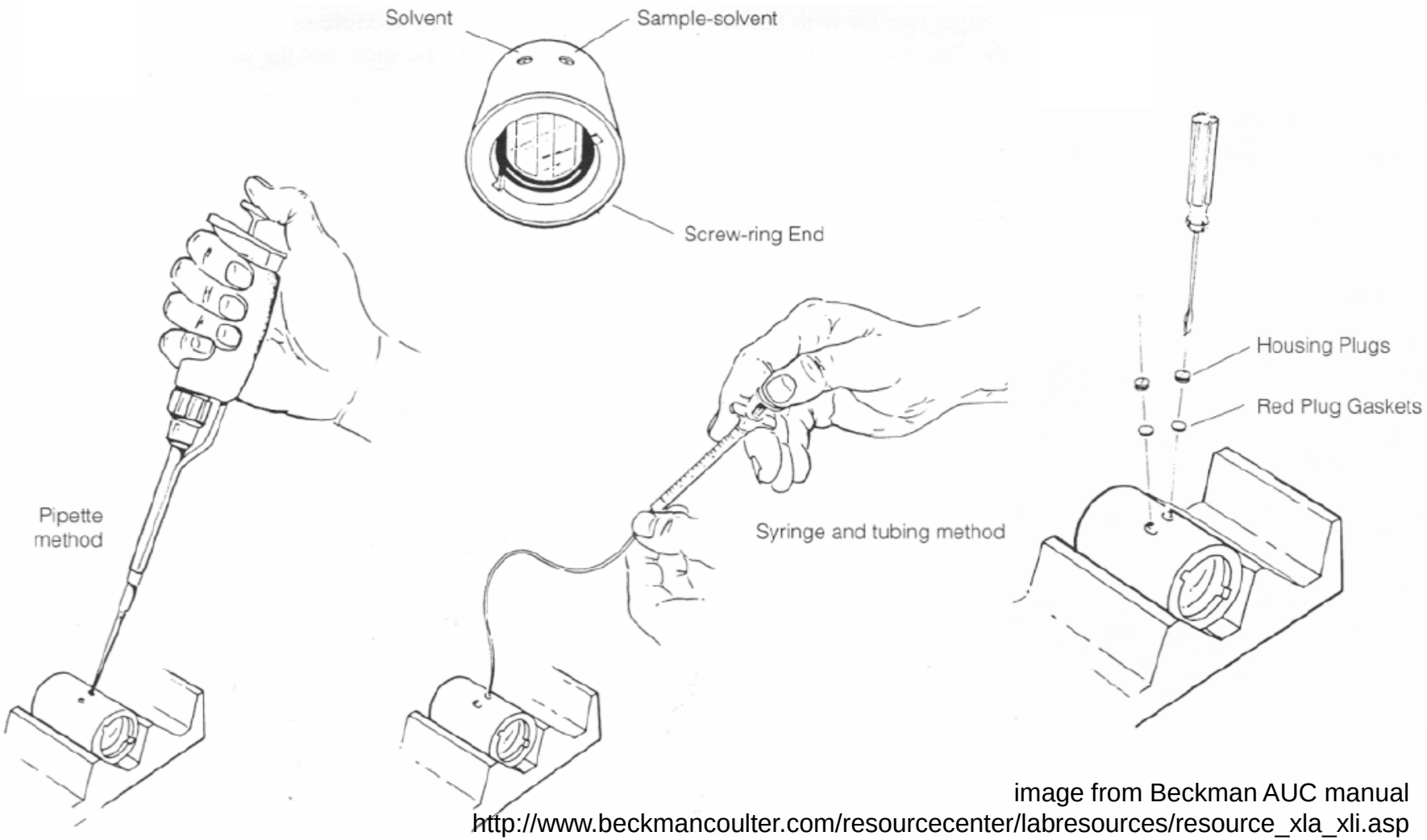


image from Beckman AUC manual
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Absorbance optics: the AUC is like a spinning double-beam spectrophotometer

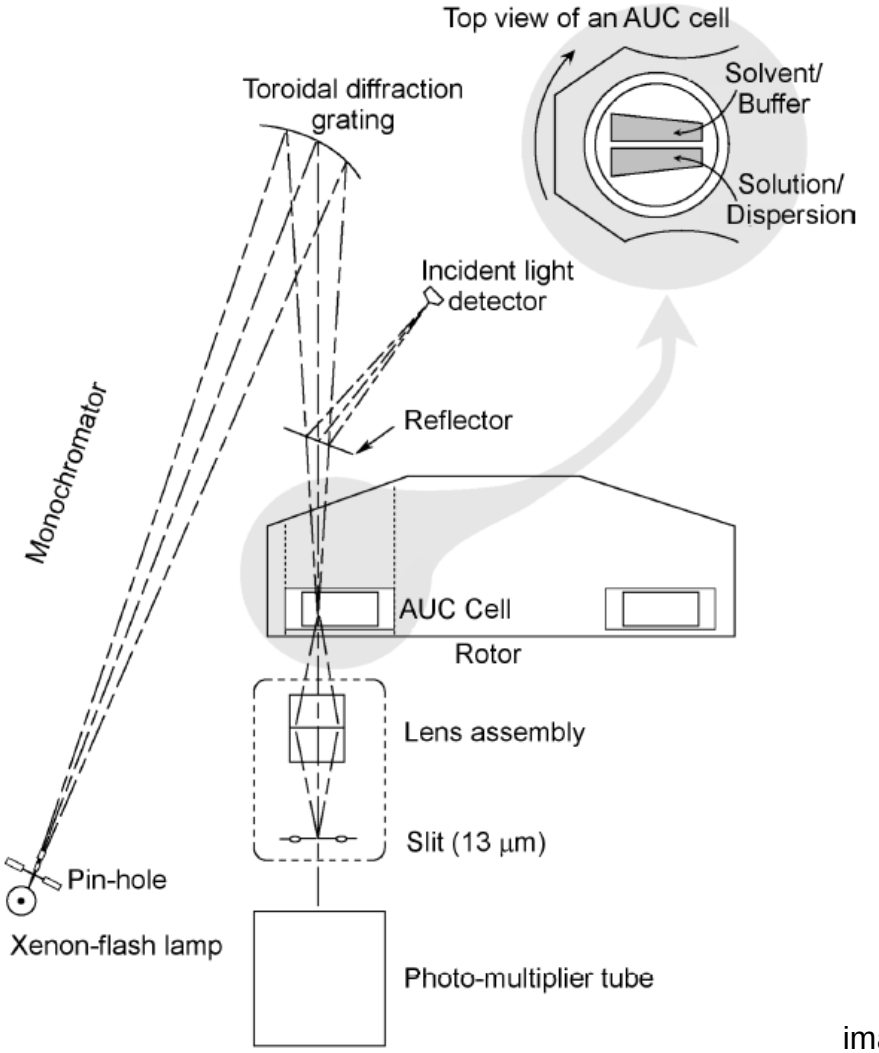

image from Beckman AUC manual
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Interference optics acquire refractive index data rapidly, independent of chromophores

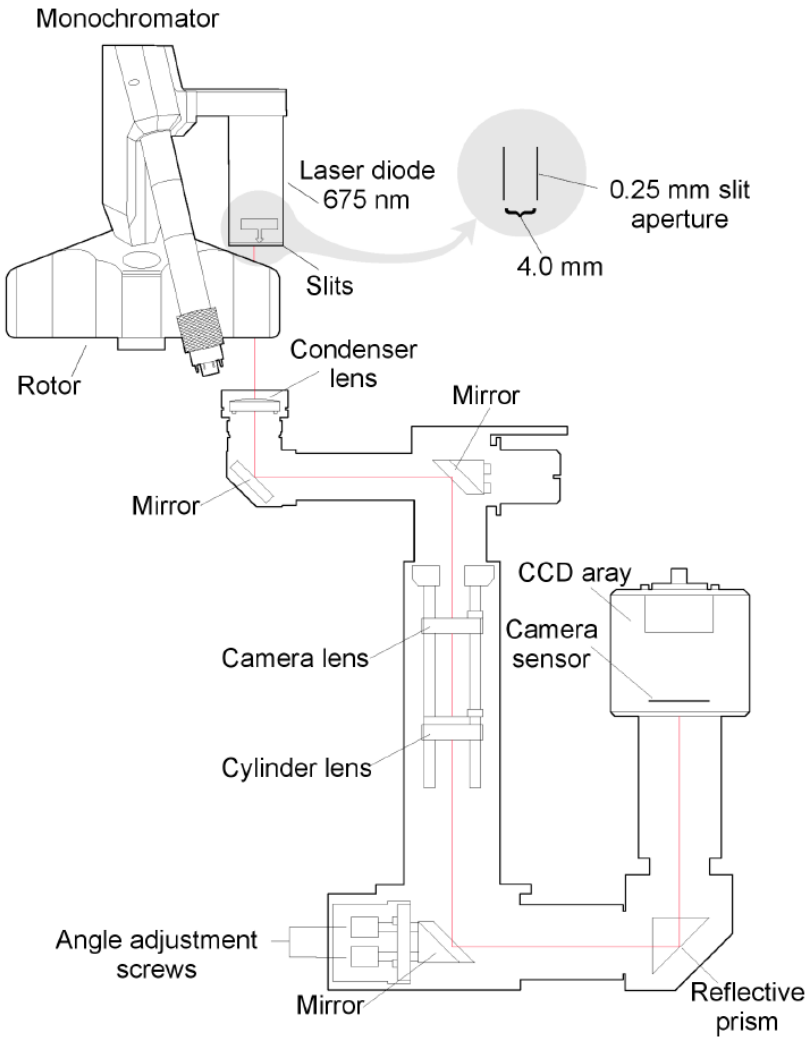

image from Beckman AUC manual
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2 modes of operation - several data types

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

Comparison of all optical systems

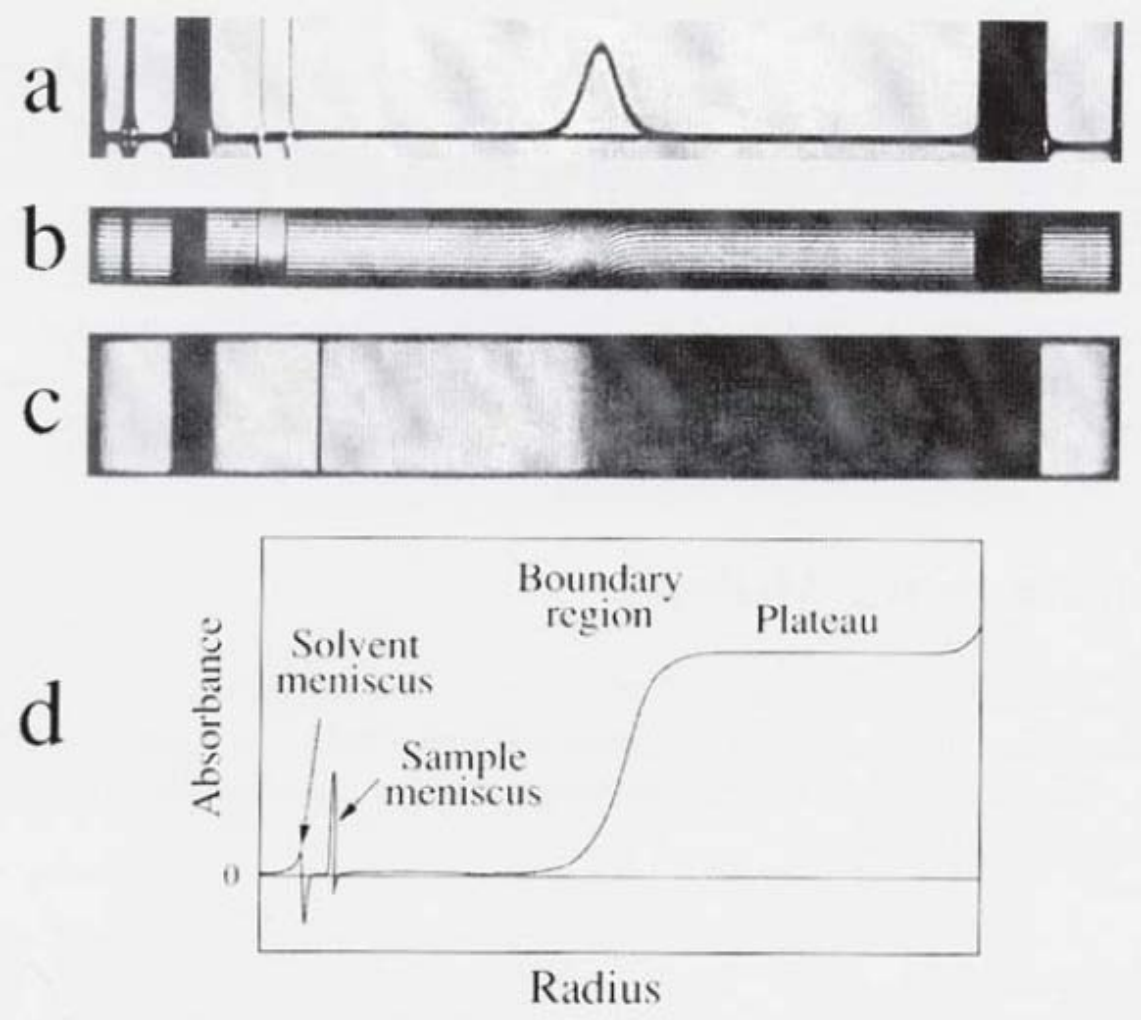


image from Ralston, 1993
https://www.beckmancoulter.com/wsrportal/bibliography?docname=361847.pdf

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

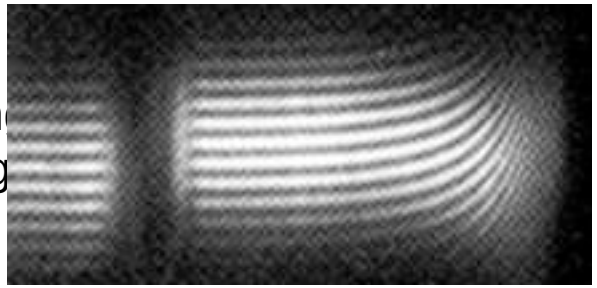
REVIEW

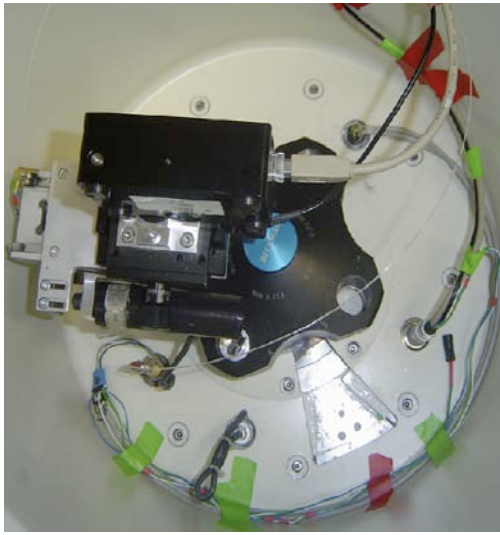
The Open AUC Project

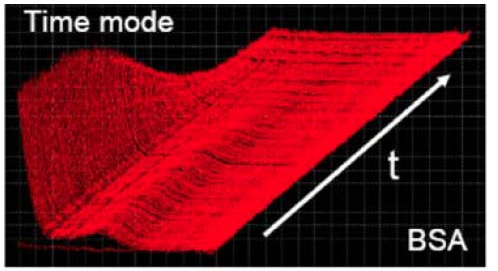
Helmut Cölfen · Thomas M. Laue · Wendel Wohlleben · Kristian Schilling · Engin Karabudak · Bradley W. Langhorst · Emre Brookes · Bruce Dubbs · Dan Zollars · Mattia Rocco · Borries Demeler

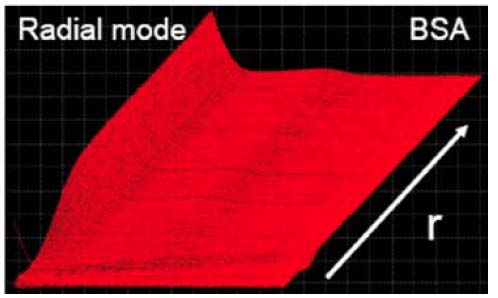
The CFA is an entirely new AUC

- 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 scatterin
  - Multi-angle light scattering
  - (SAXS?)

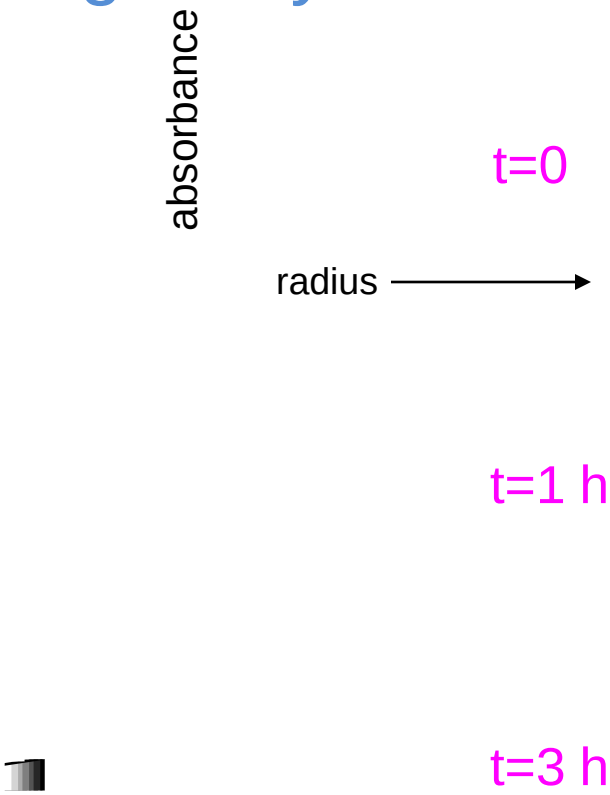






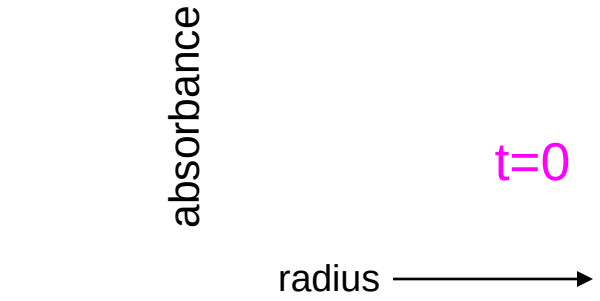


Sedimentation velocity (SV): shape & homogeneity



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

Sedimentation equilibrium (SE): mass & self-association



t=0

t=1 h

t=3 h

t≈24 h+

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

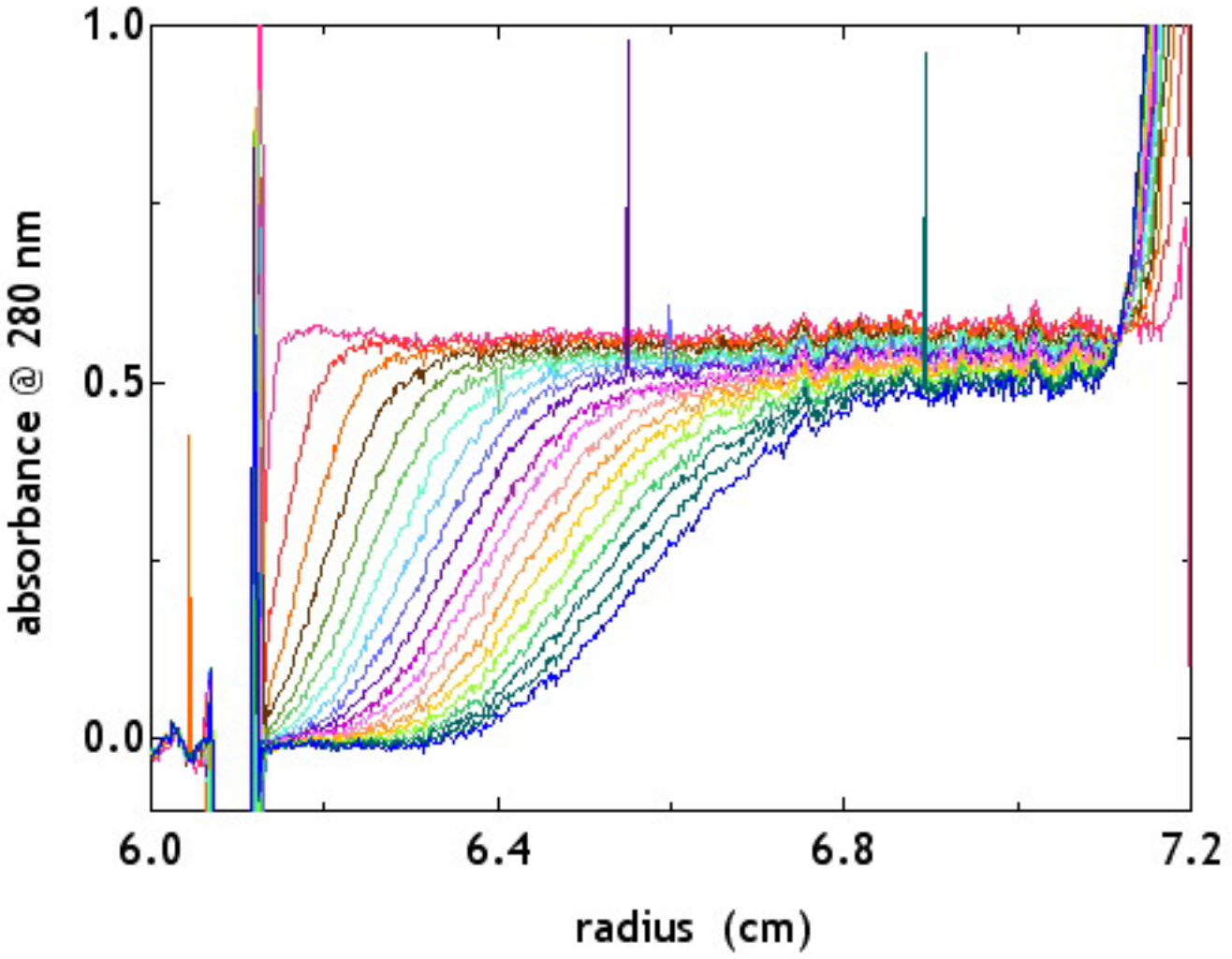
SV versus SE

- 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

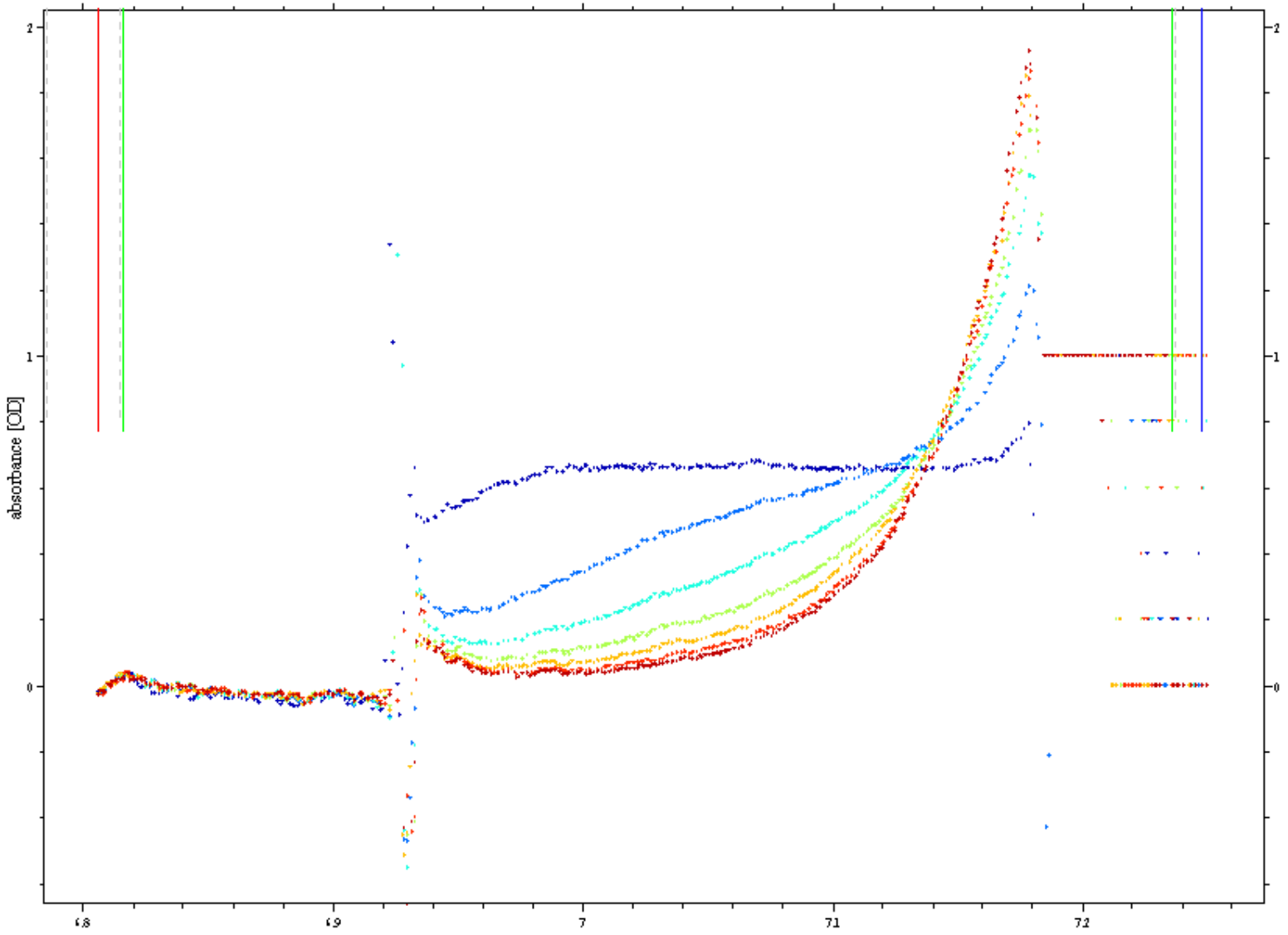
Sample requirements

- Sample volume
  - SV
    - 360 µl (up to 480 µl) in 12 mm pathlength
    - 90 µl (up to 120 µl) in 3 mm pathlength
  - SE
    - 20 µl (8-channel centrepiece - interference optics only)
    - 80 µl (2- or 6-channel centrepiece)
- Sample concentration
  - Absorbance optics: $A_\lambda \approx$ 0.1-1.0 in 12 mm pathlength cell
    - λ = 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

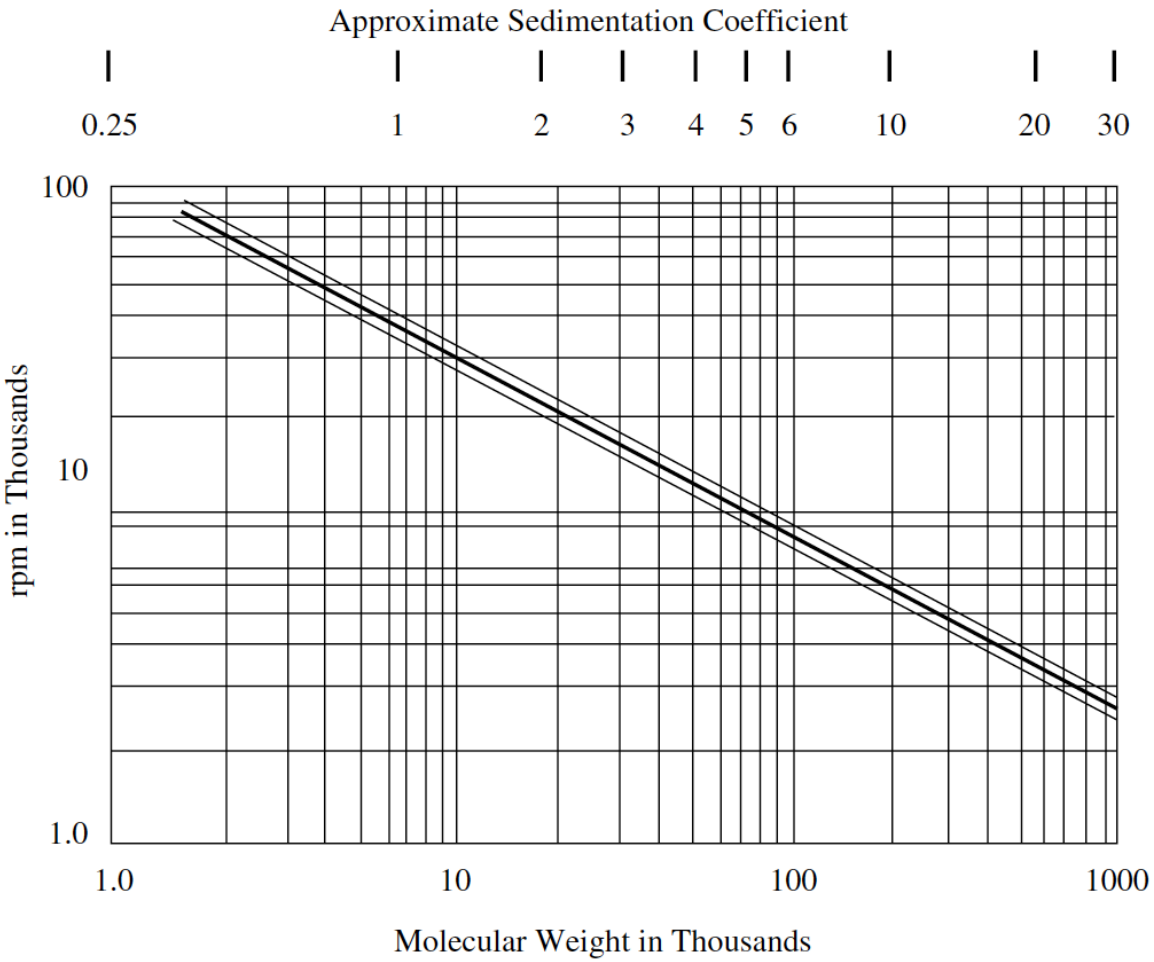
SV: radial movement recorded as function of time



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 $$\frac{dc}{dt} = \frac{1}{r}\frac{d}{dr}\left[rD\frac{dc}{dr} - s\omega^2 r^2 c \right]$$

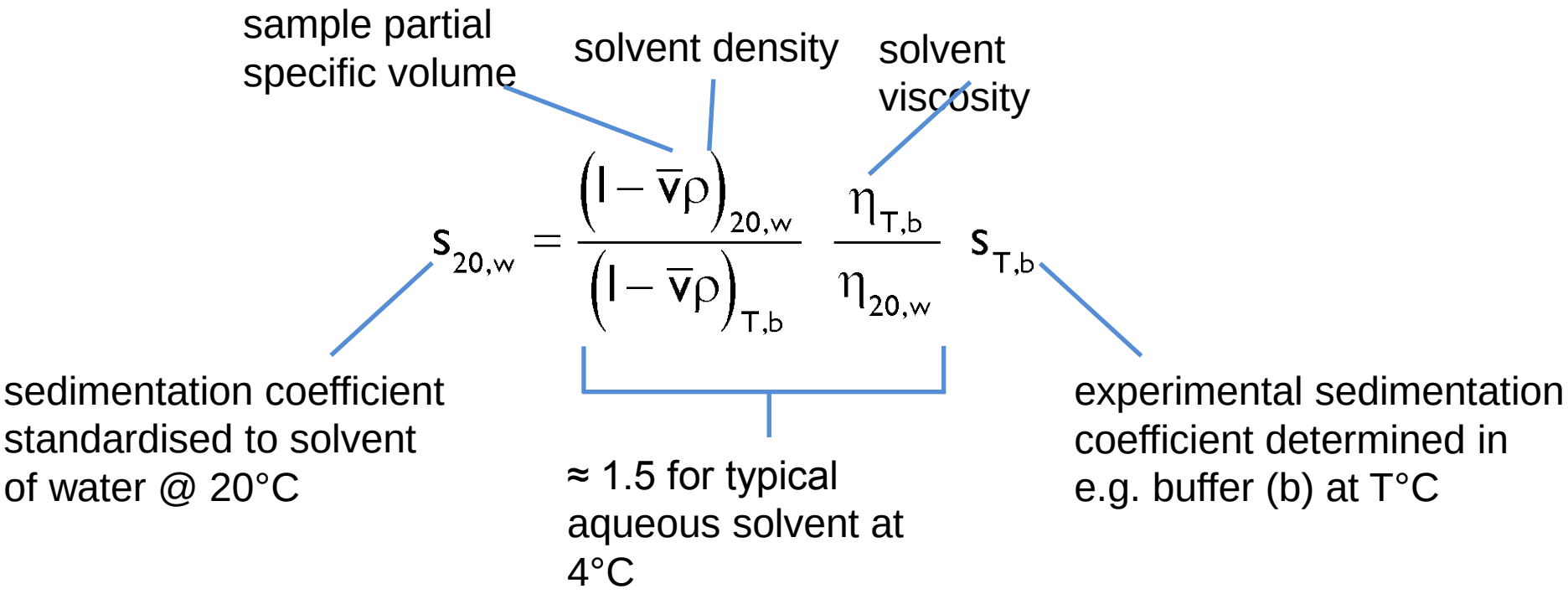
Almost all AUC data analysis software is freely available

- 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/

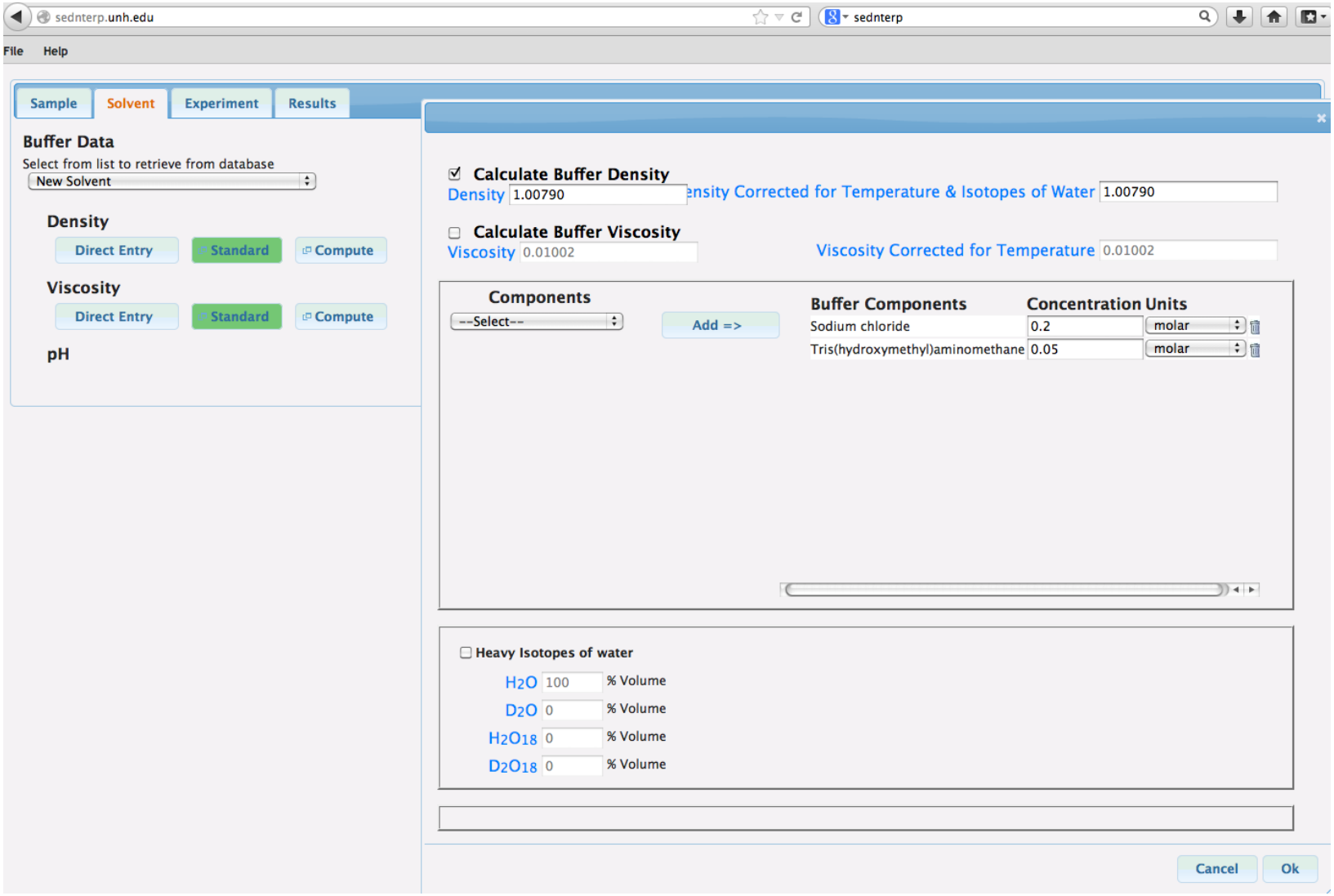
Many methods & programs for SV data analysis

- 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

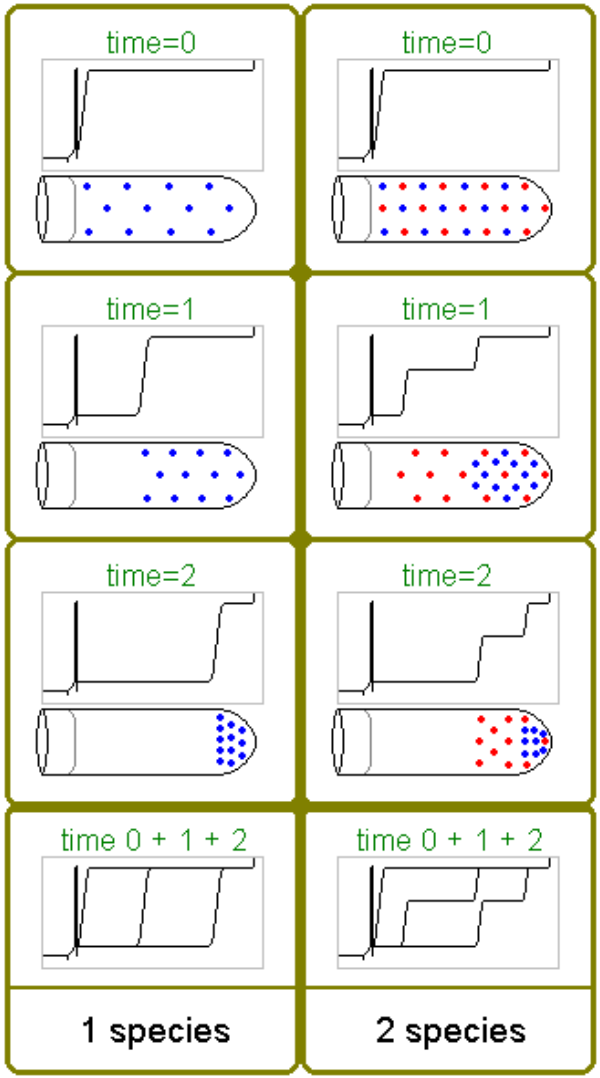


SEDNTERP : Calculation of ρ, η and partial specific volume online



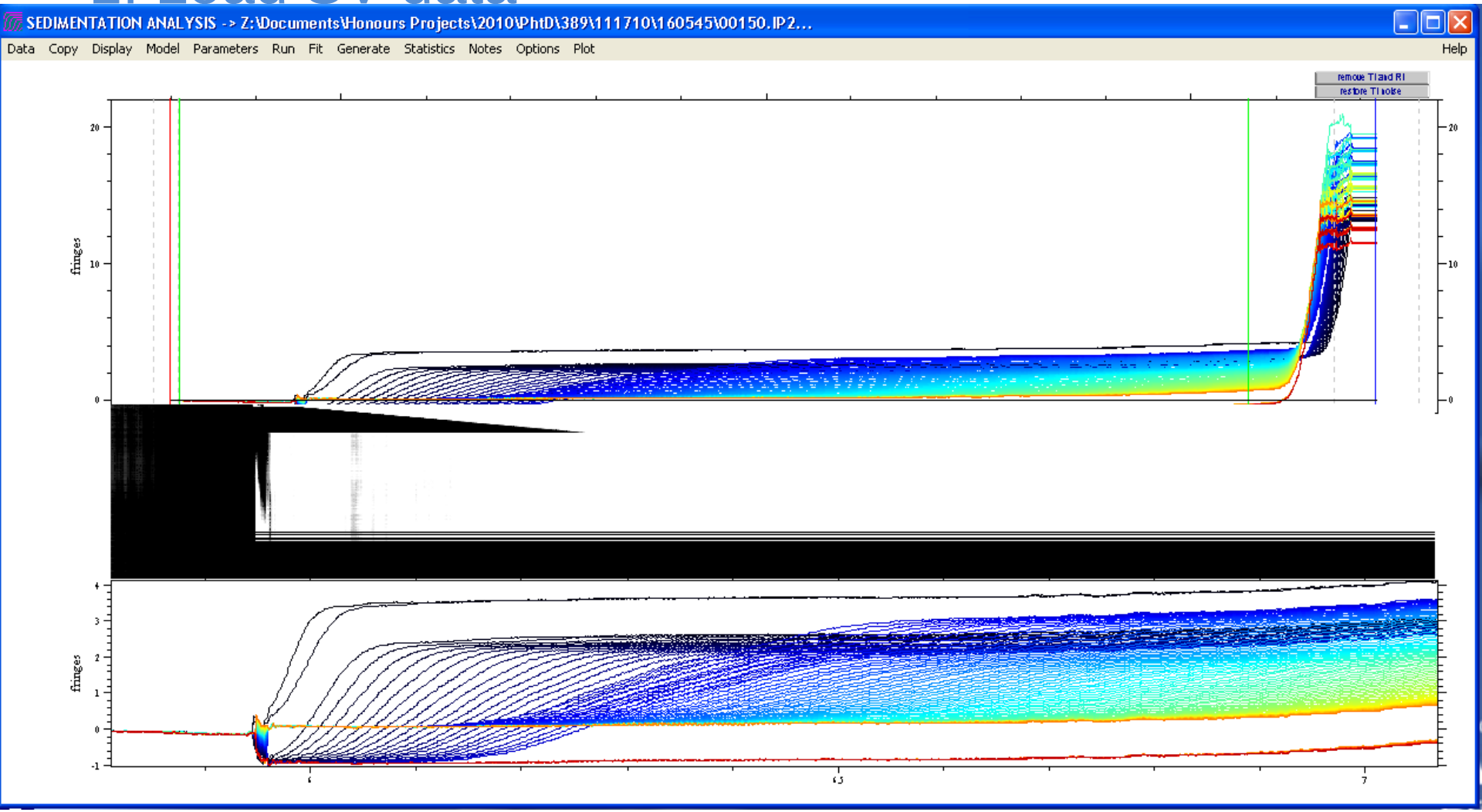
http://sednterp.unh.edu/

SV: species can resolve into separate boundaries



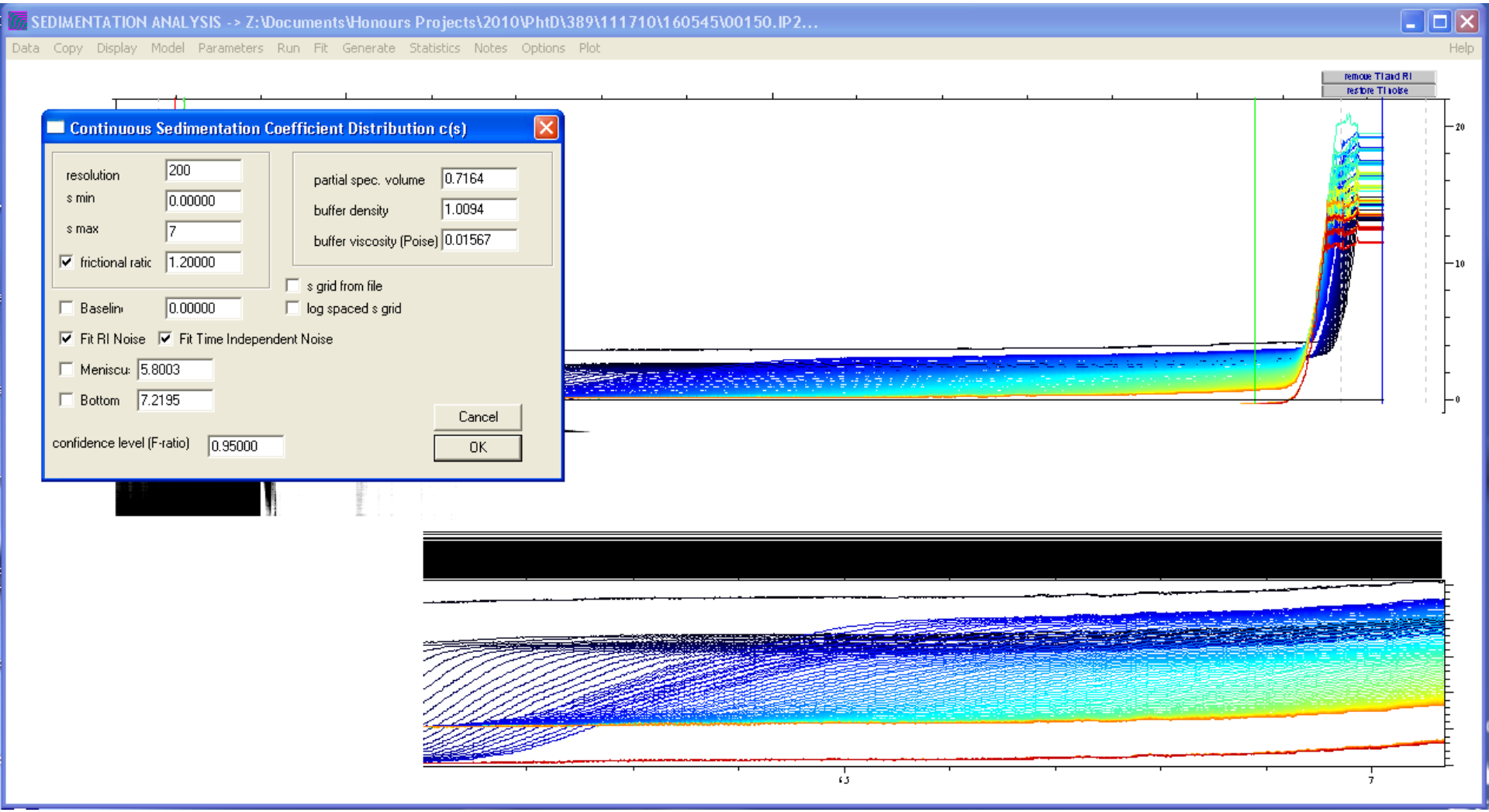
SEDFIT c(s) analysis: how many species + s of species

1: Load SV data



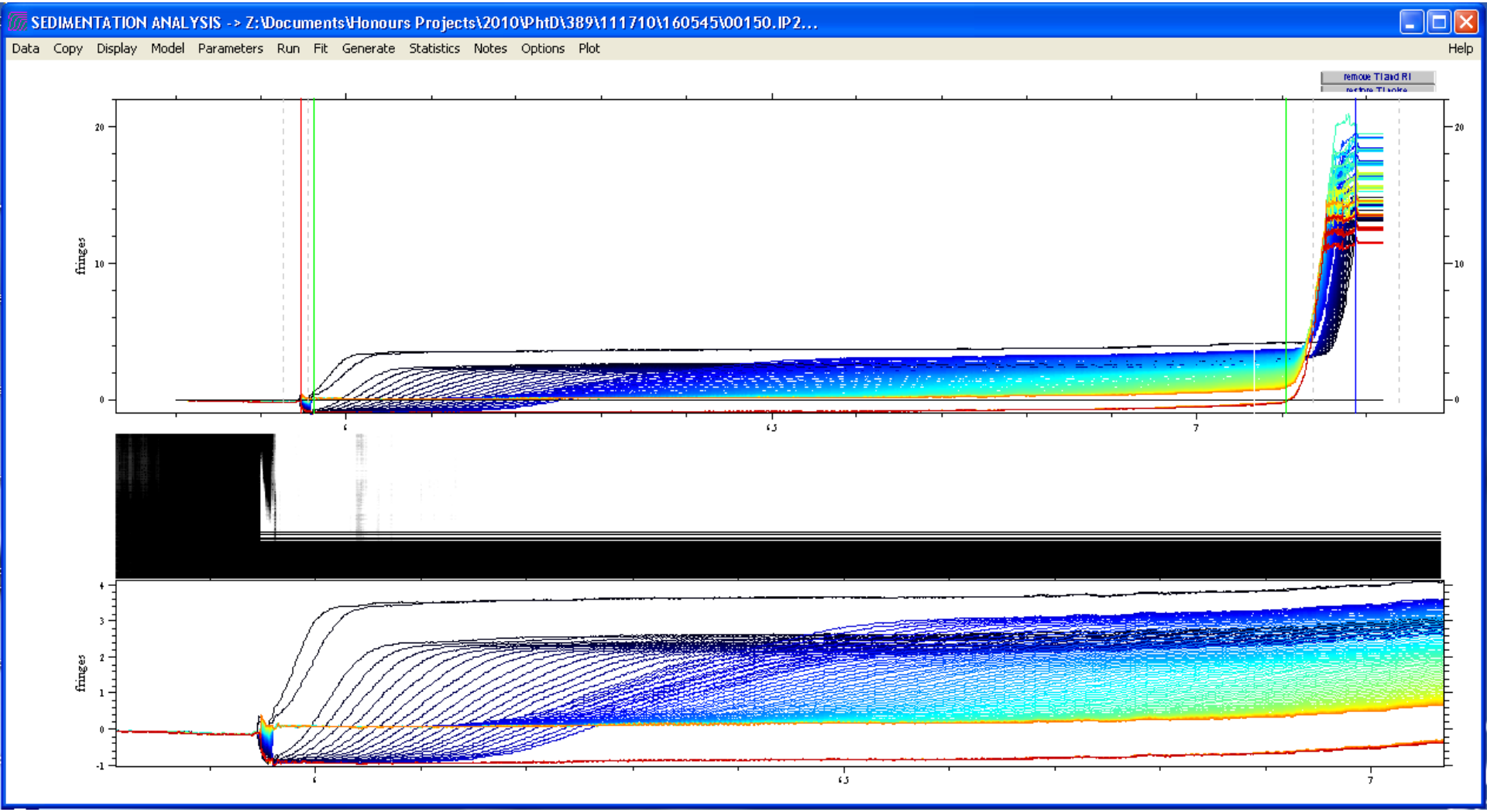
http://www.analyticalultracentrifugation.com/default.htm

2: Specify parameters



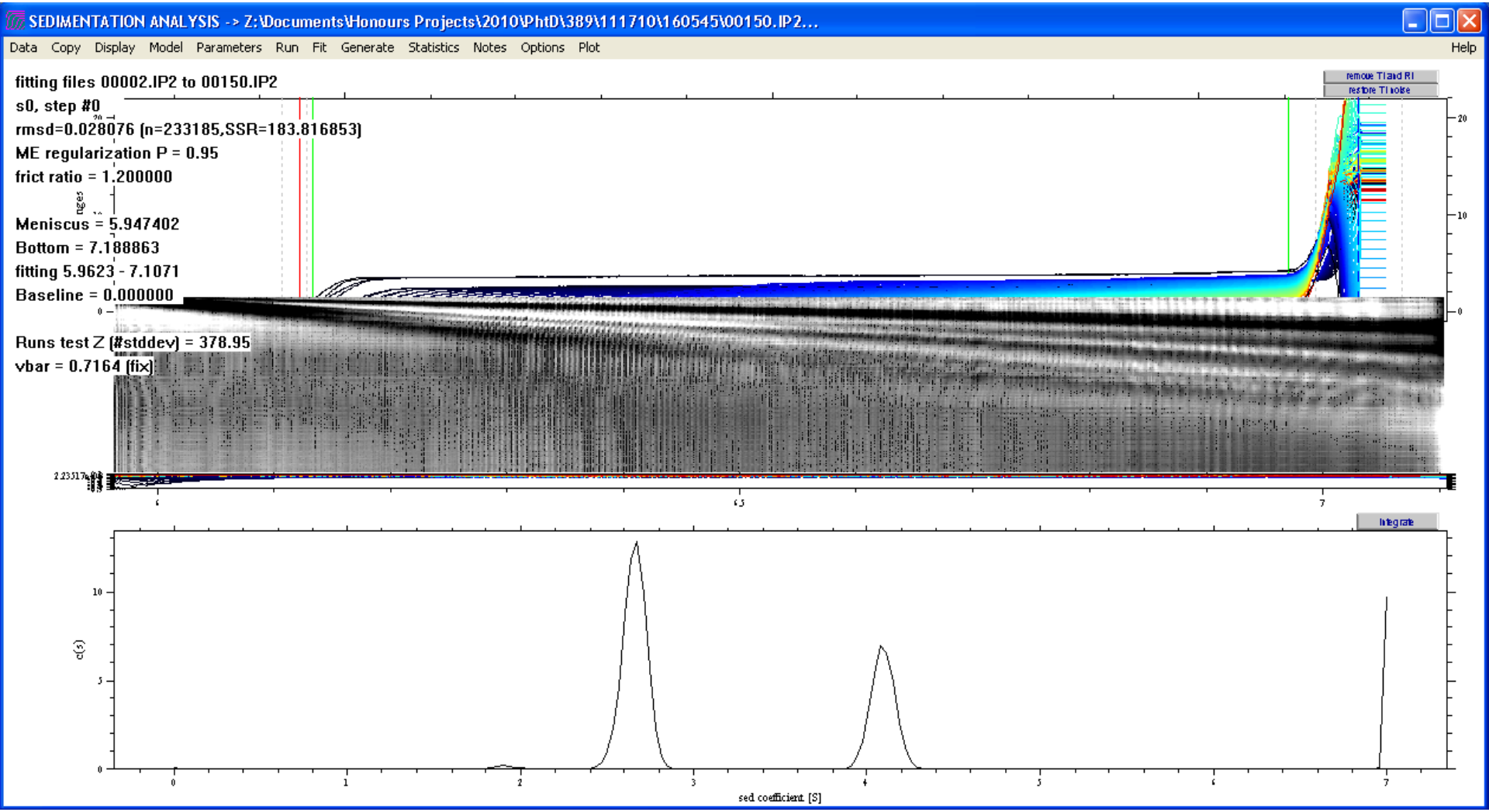
http://www.analyticalultracentrifugation.com/default.htm

3: Set meniscus, cell base and analysis limits



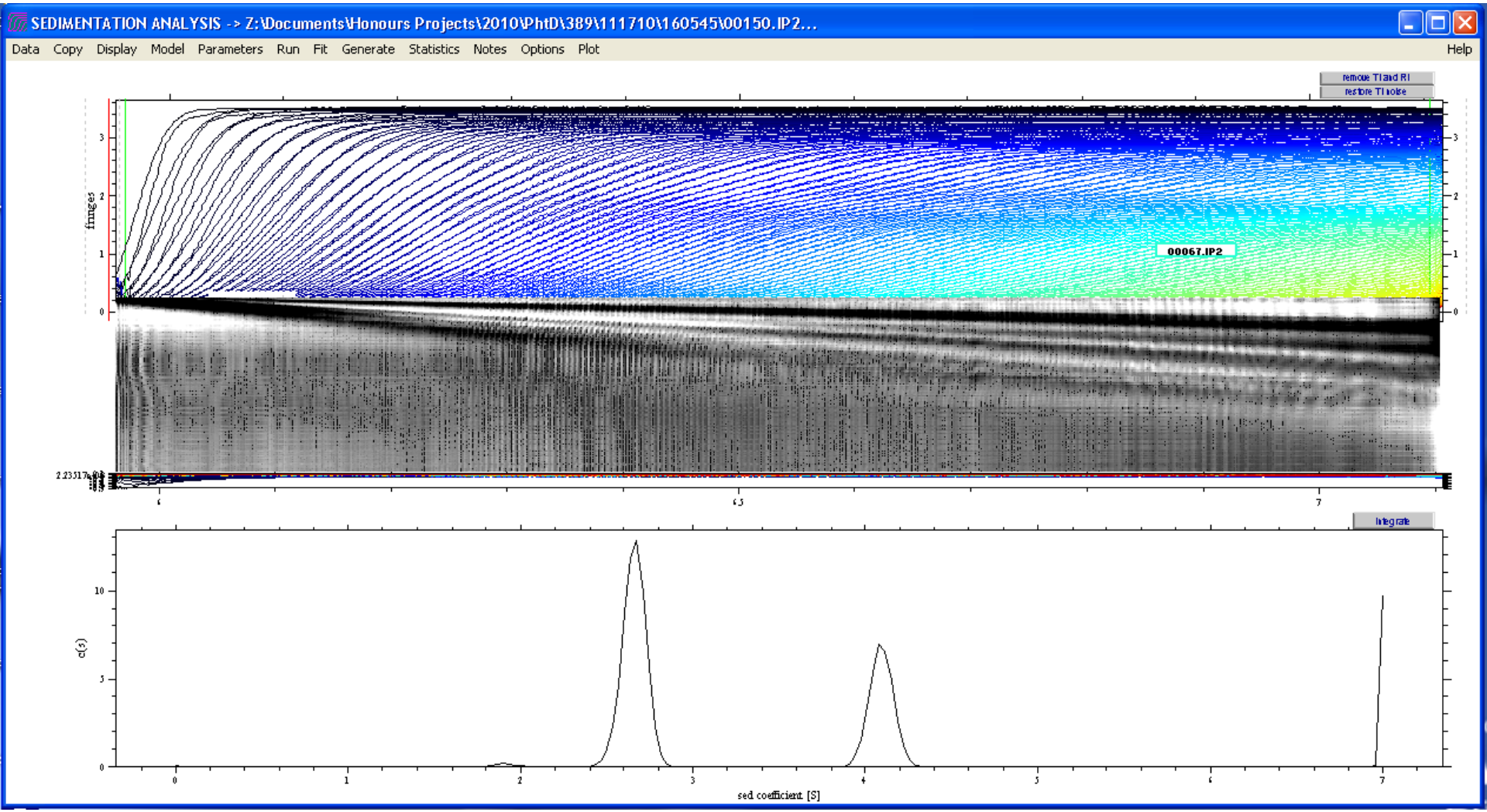
http://www.analyticalultracentrifugation.com/default.htm

4: Run



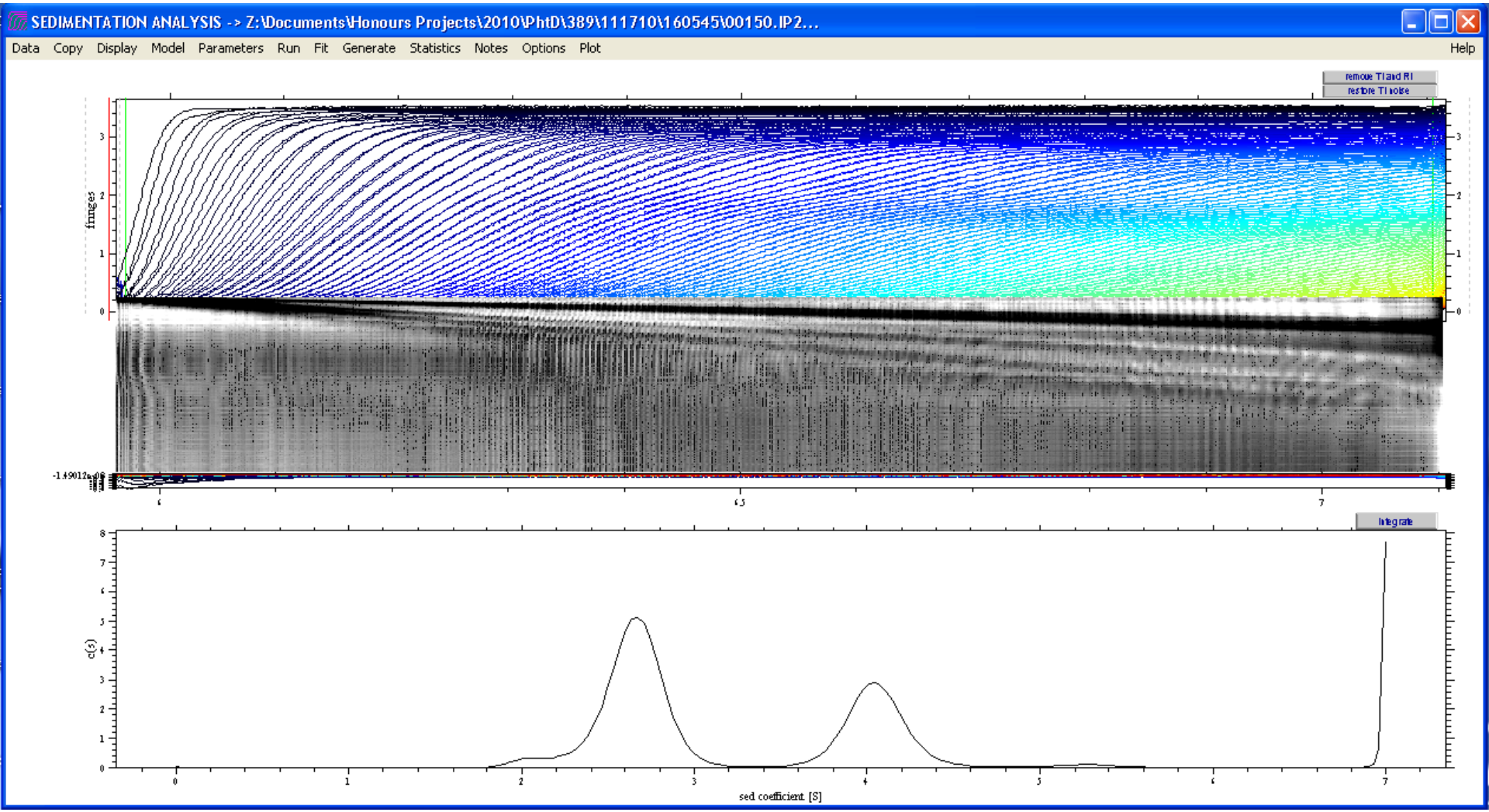
http://www.analyticalultracentrifugation.com/default.htm

5: Subtract time and radial invariant noise



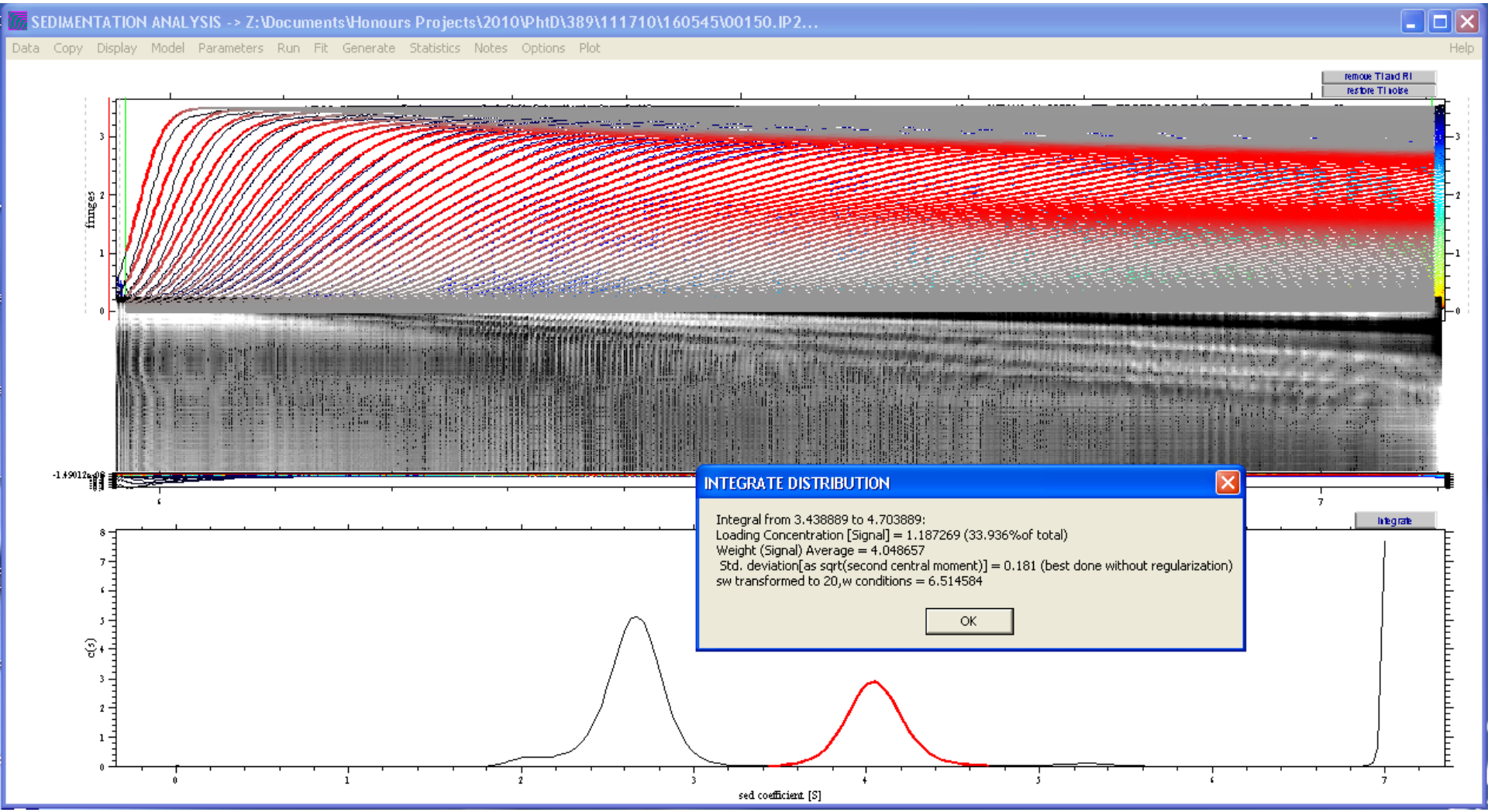
http://www.analyticalultracentrifugation.com/default.htm

6: Fit (with solutions to the Lamm equation)



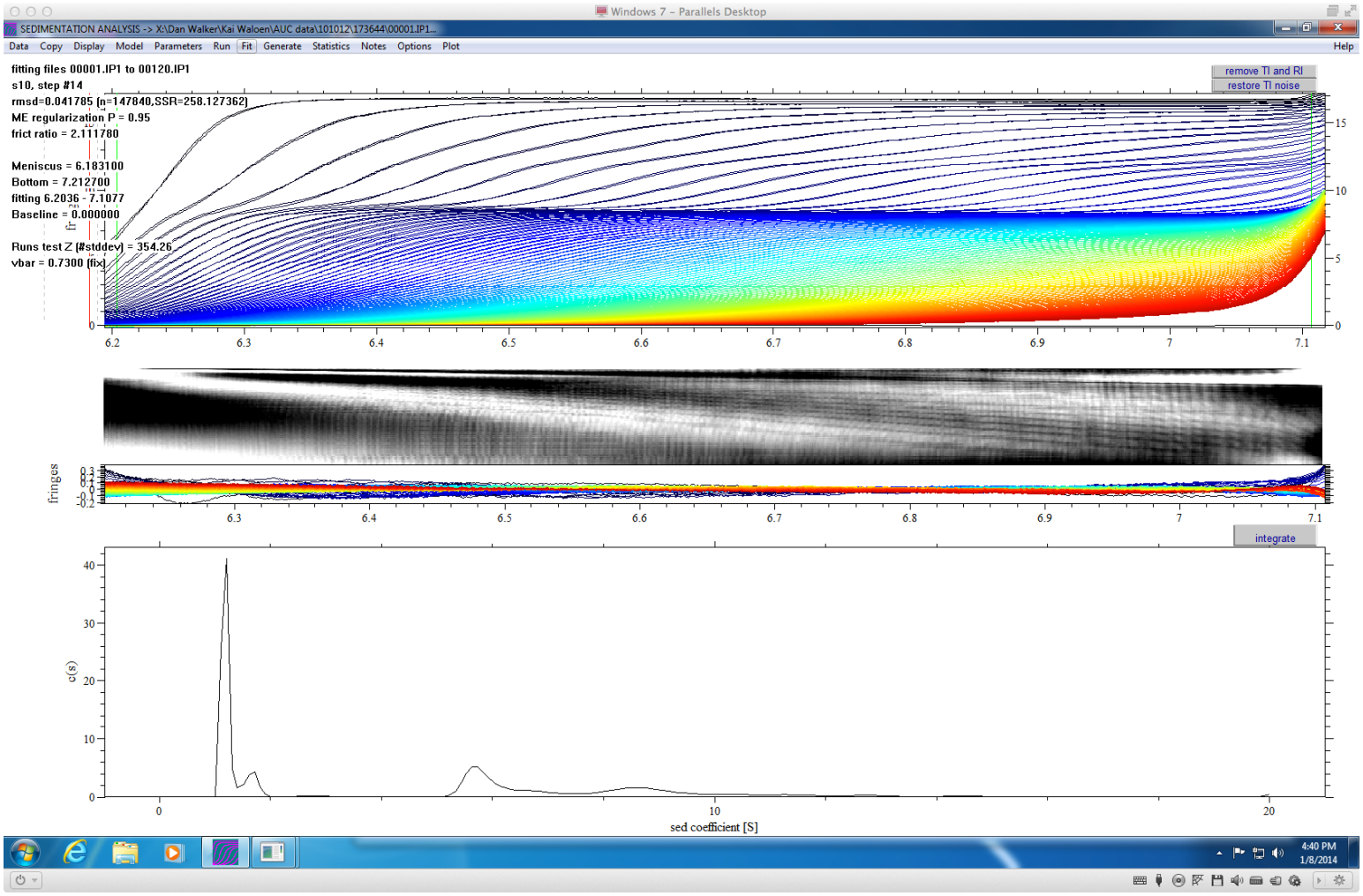
http://www.analyticalultracentrifugation.com/default.htm

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

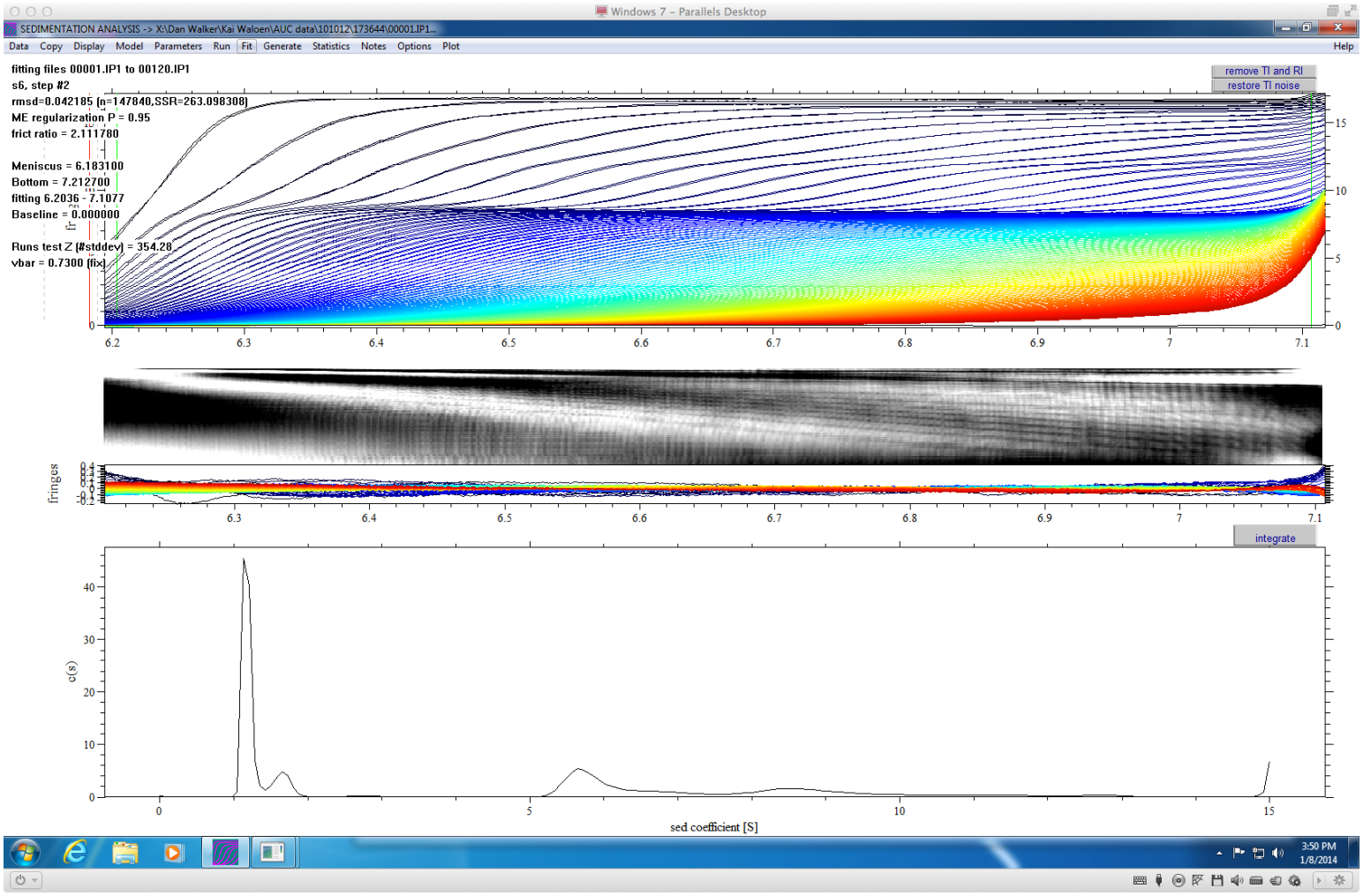


http://www.analyticalultracentrifugation.com/default.htm

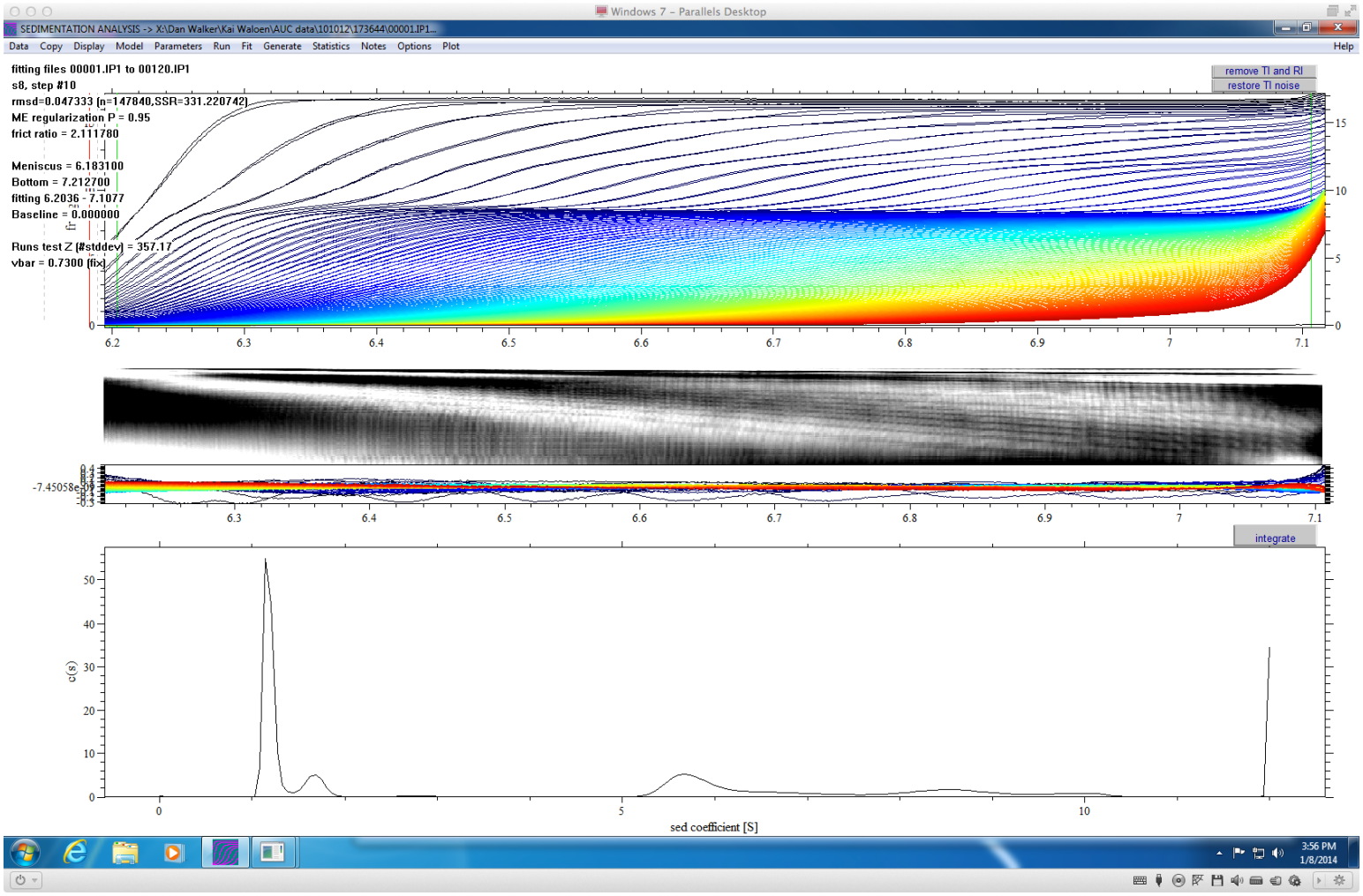
Sum of Lamm equations 0 ≤ s ≤ 20 S discretised by 200



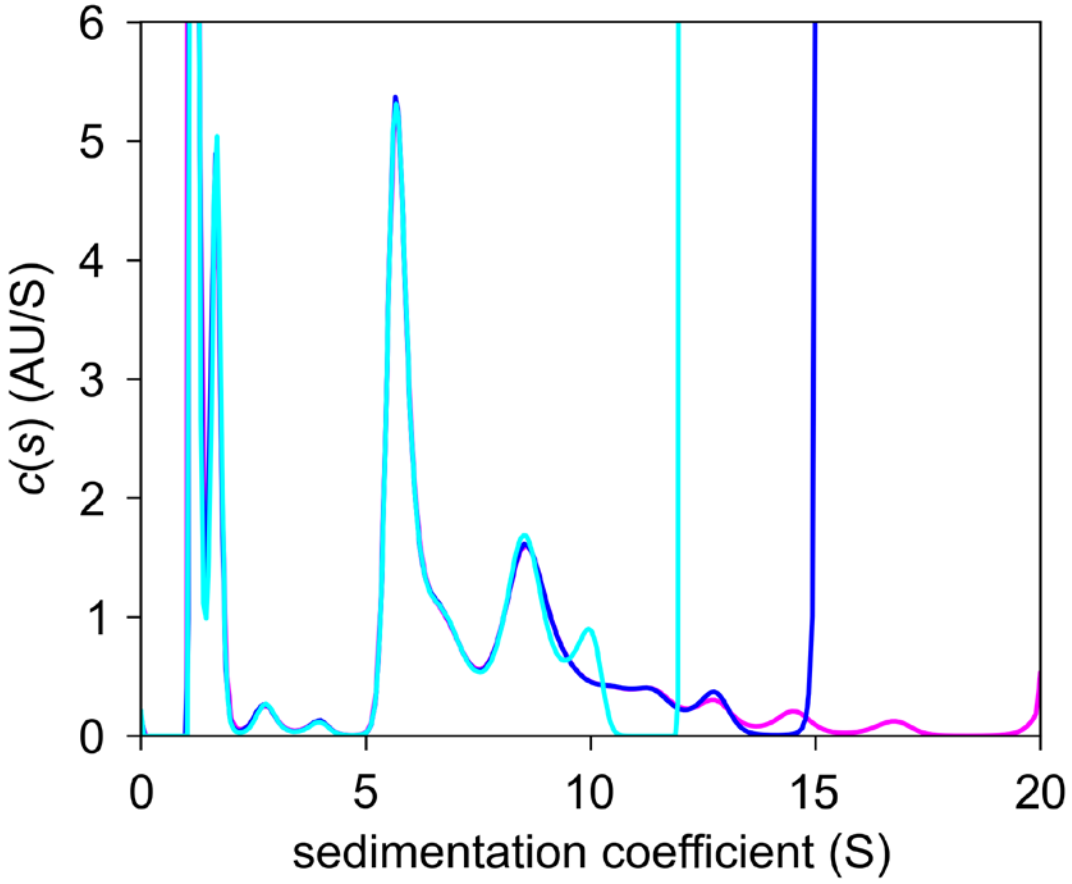
Sum of Lamm equations 0 ≤ s ≤ 15 S discretised by 200



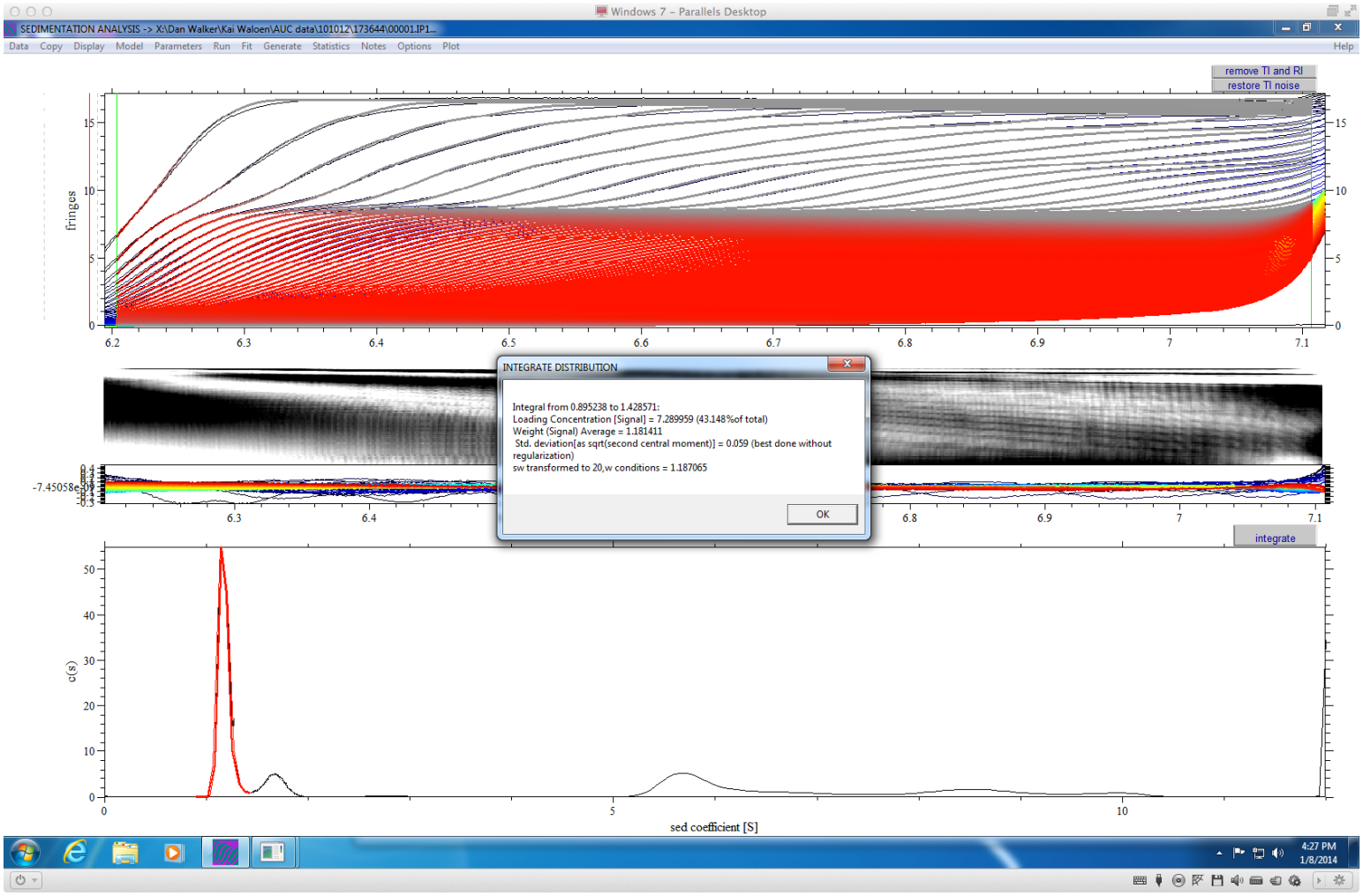
Sum of Lamm equations 0 ≤ s ≤ 12 S discretised by 200



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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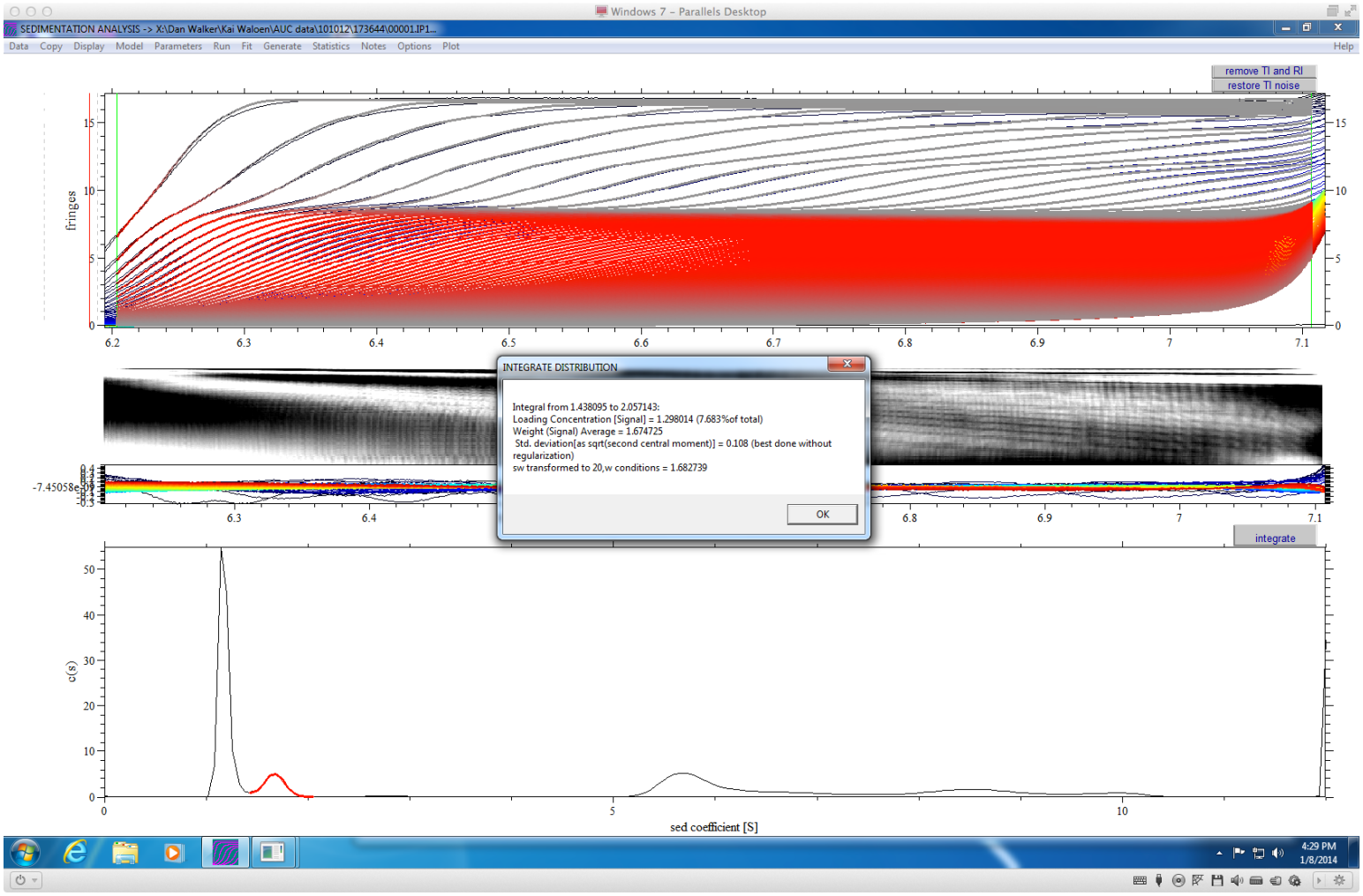

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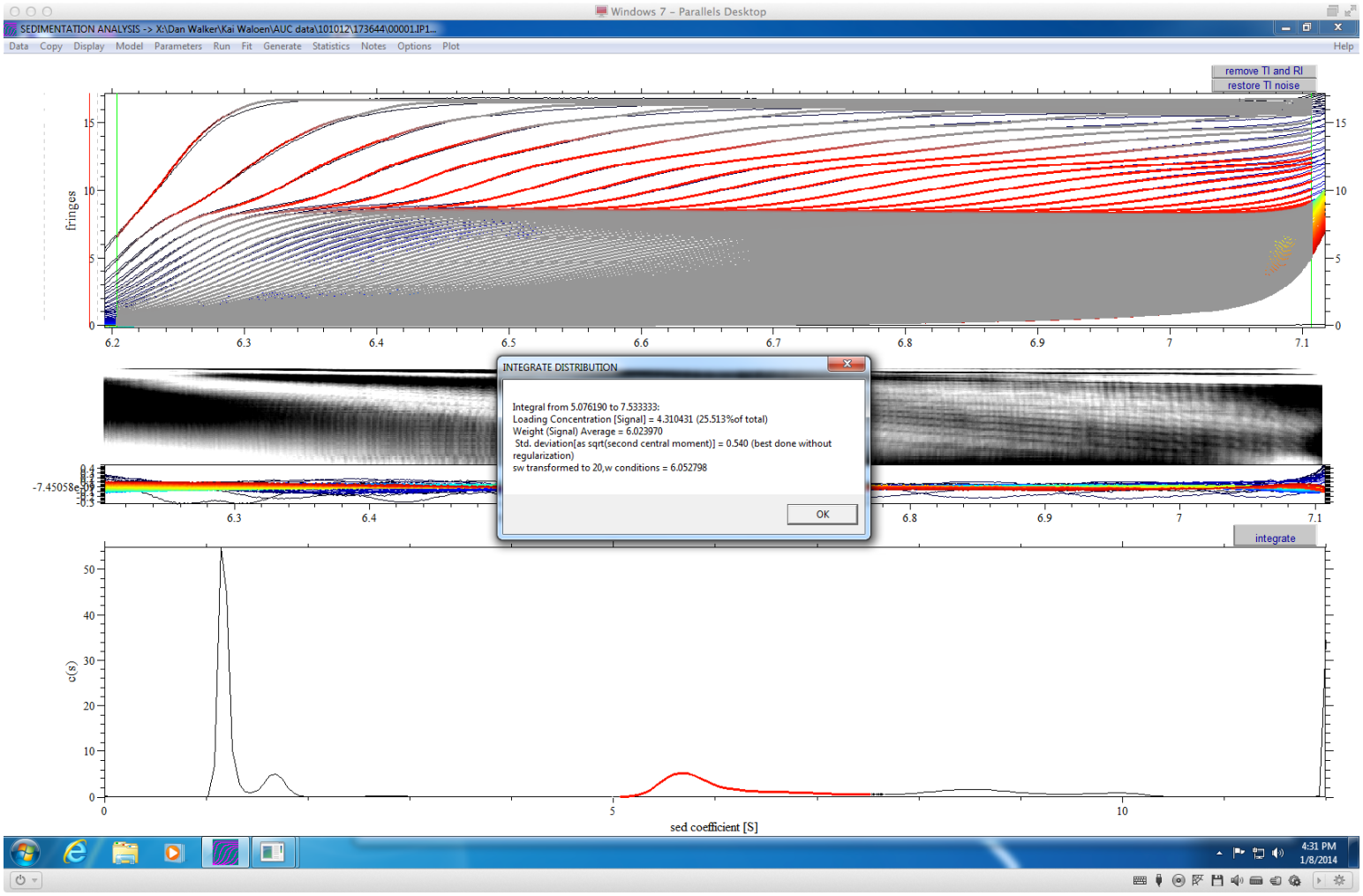
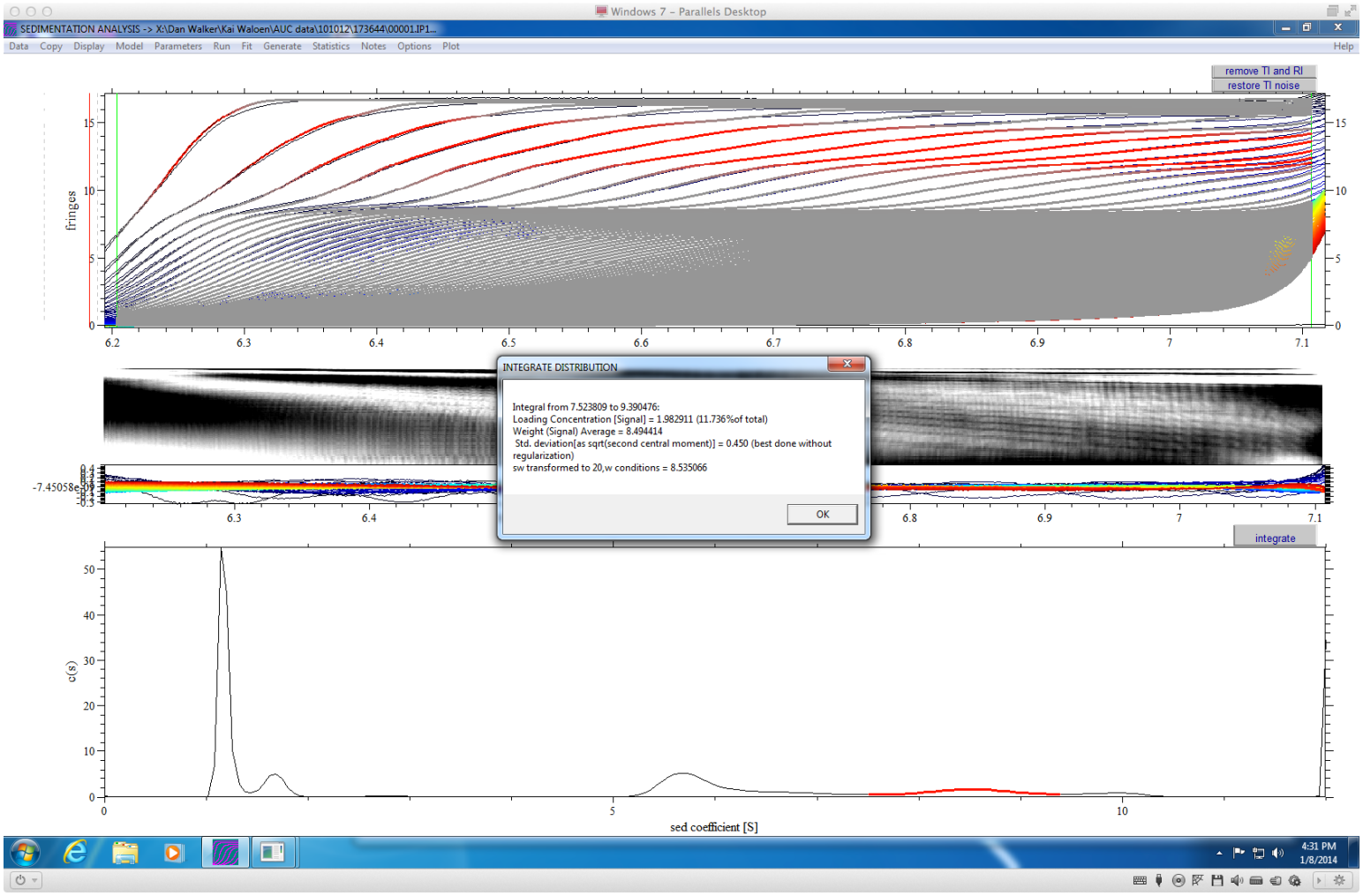
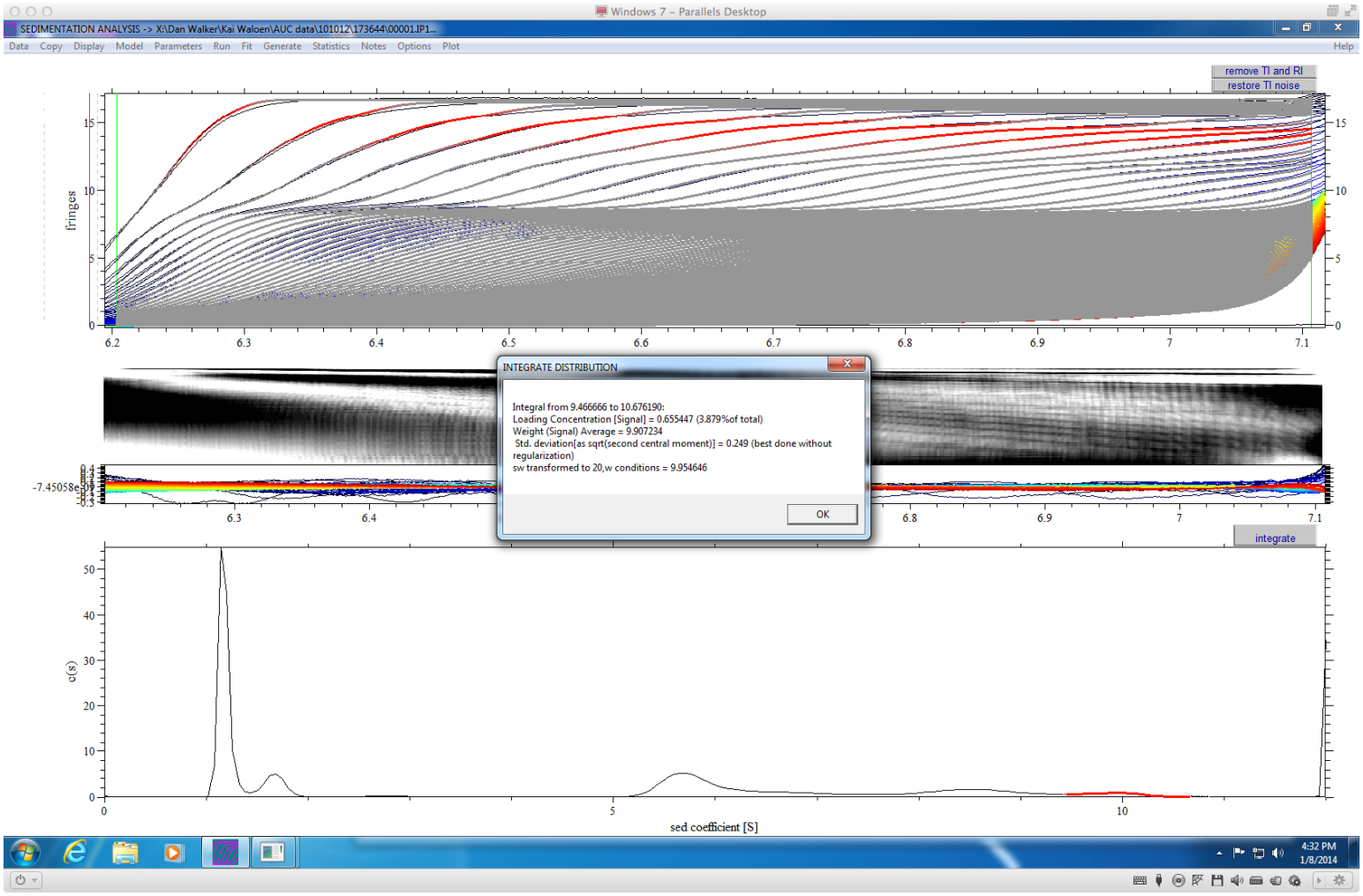


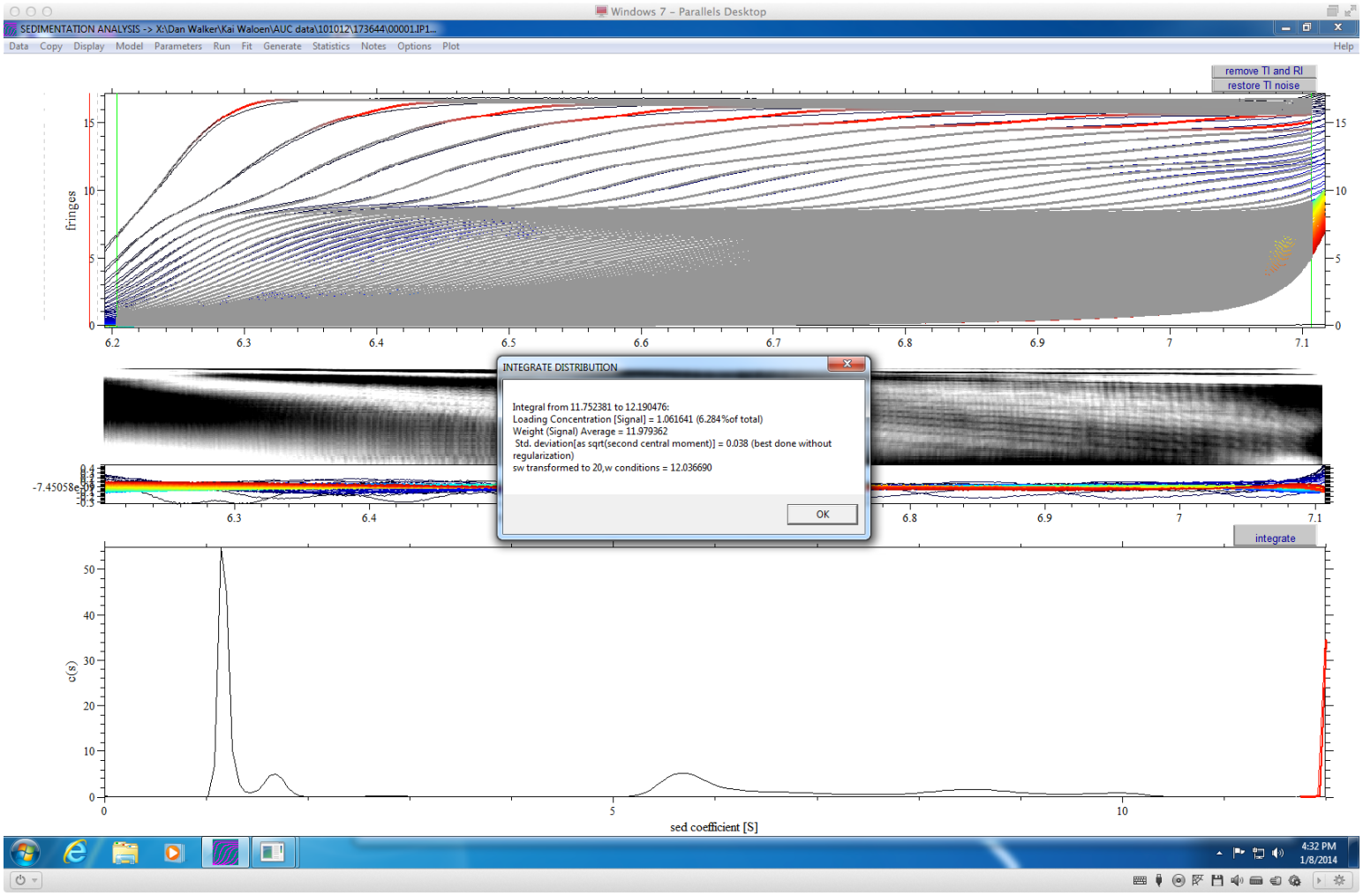
Integrating c(s) peaks reveals region of boundary that contains species



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

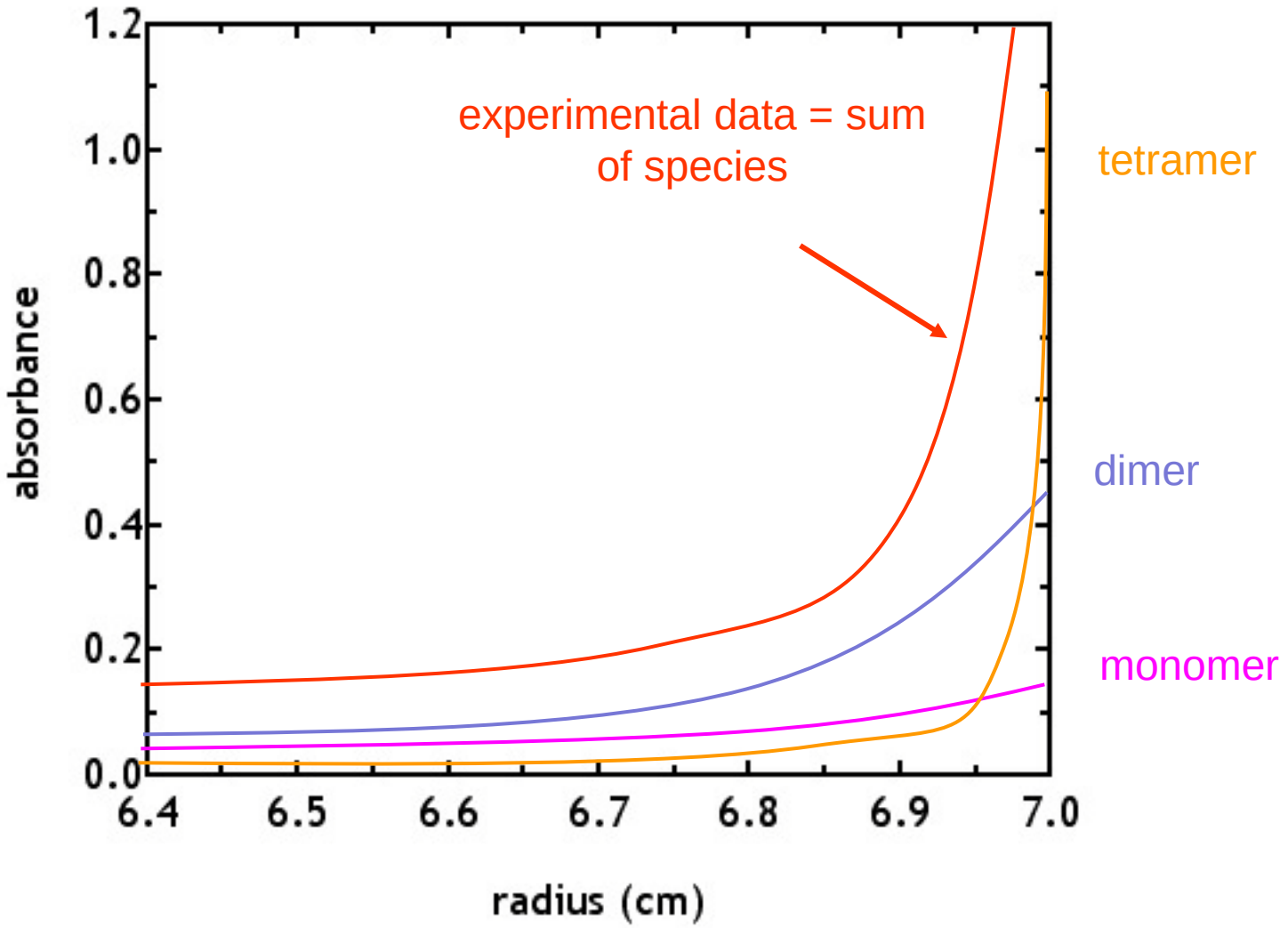
$$A_r = \exp[\ln A_0 + H.M(r^2 - r_0^2)] \leftarrow \text{monomer}$$

$$+\exp[n_2 \ln A_0 + \ln Ka_2 + n_2.H.M(r^2 - r_0^2)] \leftarrow 1\text{-}n_2$$

$$+\exp[n_3 \ln A_0 + \ln Ka_3 + n_3.H.M(r^2 - r_0^2)] \leftarrow 1\text{-}n_3$$

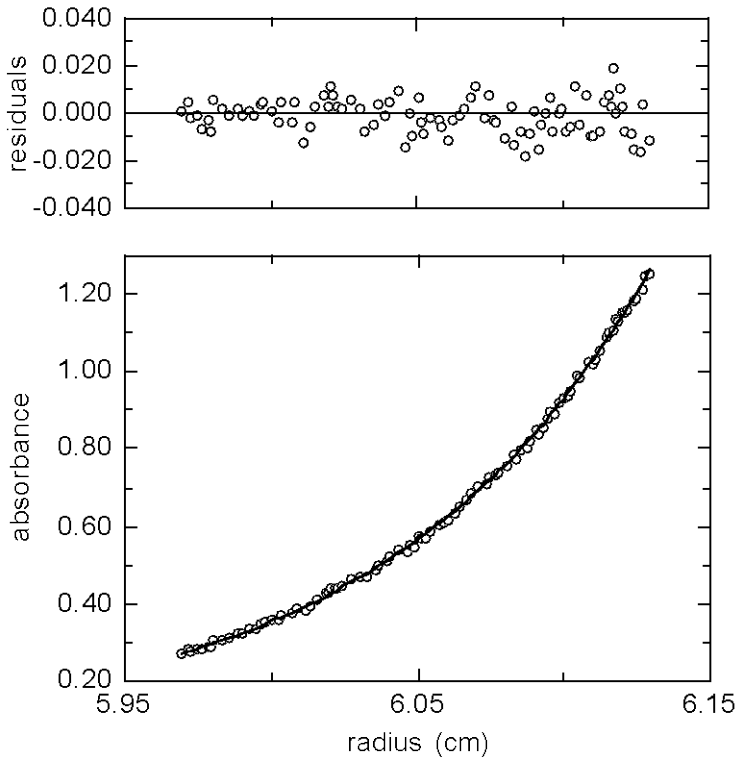
$$+\exp[n_4 \ln A_0 + \ln Ka_4 + n_4.H.M(r^2 - r_0^2)] + E \leftarrow 1\text{-}n_4$$

Self-association: “deconvolution” into individual components

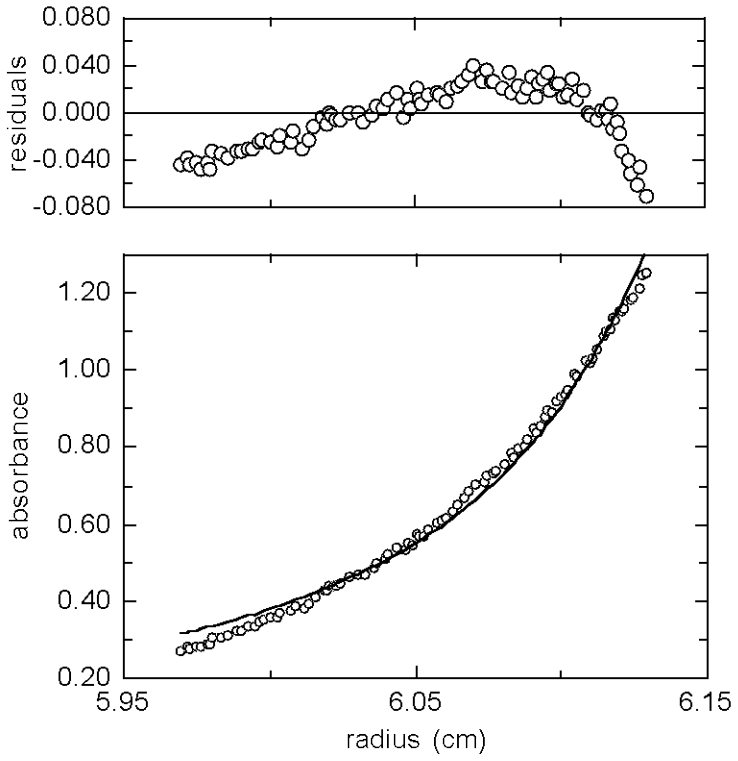


Self-association: best model revealed by residuals

2-4



1-4



LET’S HAVE A BREAK!

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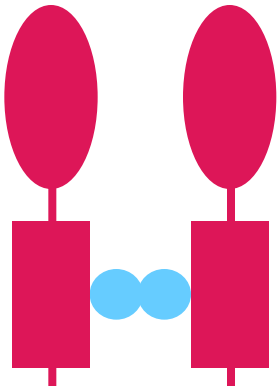
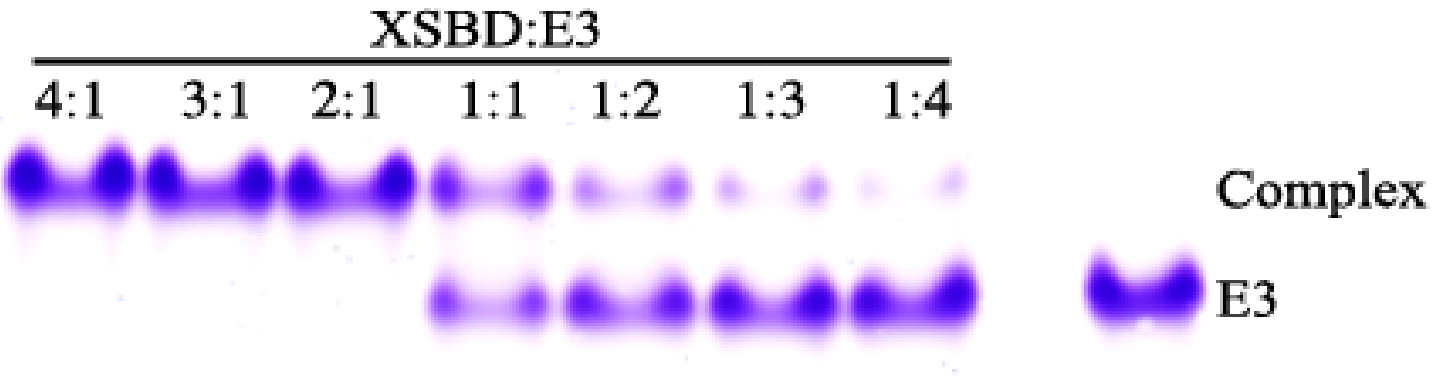
Hetero-association example: PDC E3BP-DD:E3 sub-complex

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



Smolle et al., JBC *281* 19771-80 (2006)

Native PAGE: stoichiometry is 2:1

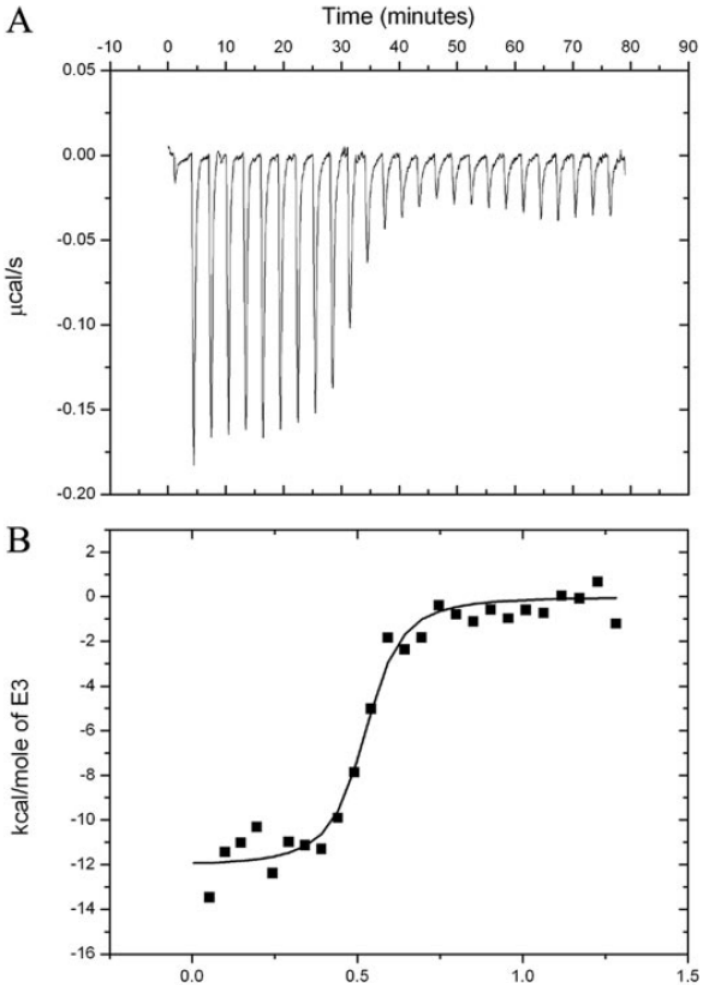


Mischa Smolle
Smolle et al., JBC *281* 19771-80 (2006)

ITC: stoichiometry is 2:1

- Microcal VP-ITC
- T = 25°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)

- Kd = 36 nM
- ΔH = -12.1 kcal/mol
- TΔS = -1.7 kcal/mol
- N = 0.5 molecules E3 bind/molecule E3BP-DD
  - equivalent to 2 E3BP-DD/E3



Mischa Smolle, Alan Cooper
Smolle et al., JBC *281* 19771-80 (2006)

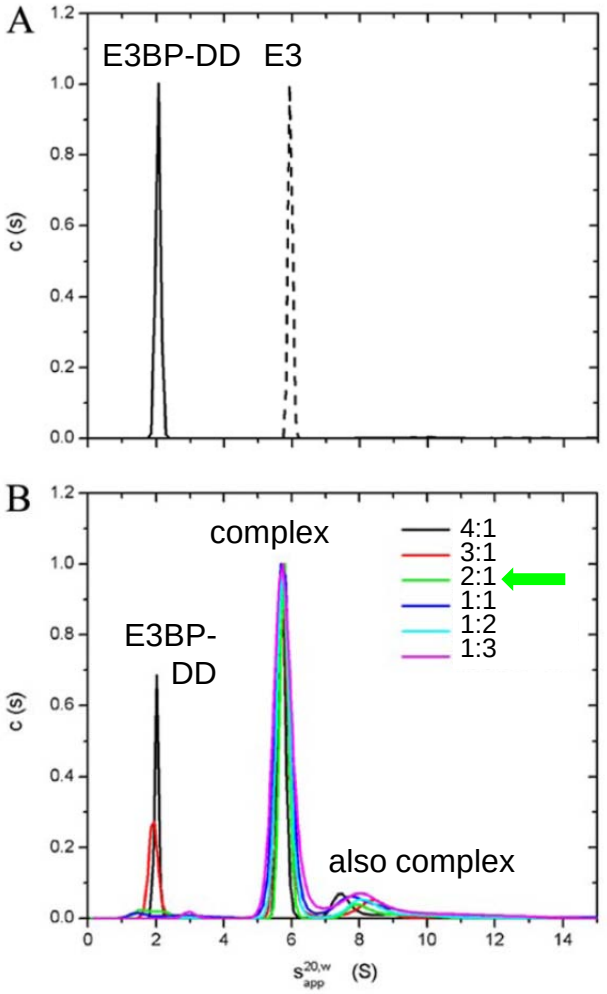
SV titration

- T = 4°C
  - Must ensure that T is constant
  - Takes *hours* to thermally equilibrate
- Rotor speed 45k rpm
- Interference optics used
  - Scan interval 1 minute
- [E3] = 4.9 µM
- Sample volume 380 µl
- Pathlength 12 mm

Mischa Smolle
Smolle et al., JBC *281* 19771-80 (2006)

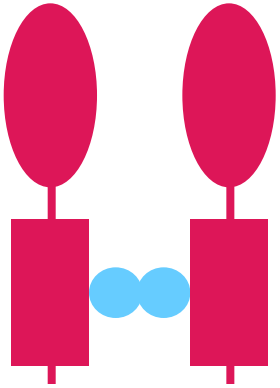
SV titration: stoichiometry is 2:1

- 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 ≈ 6 S peak less compact
      - s ≈ 8 S peak more compact



Mischa Smolle
Smolle et al., JBC *281* 19771-80 (2006)

SE titration

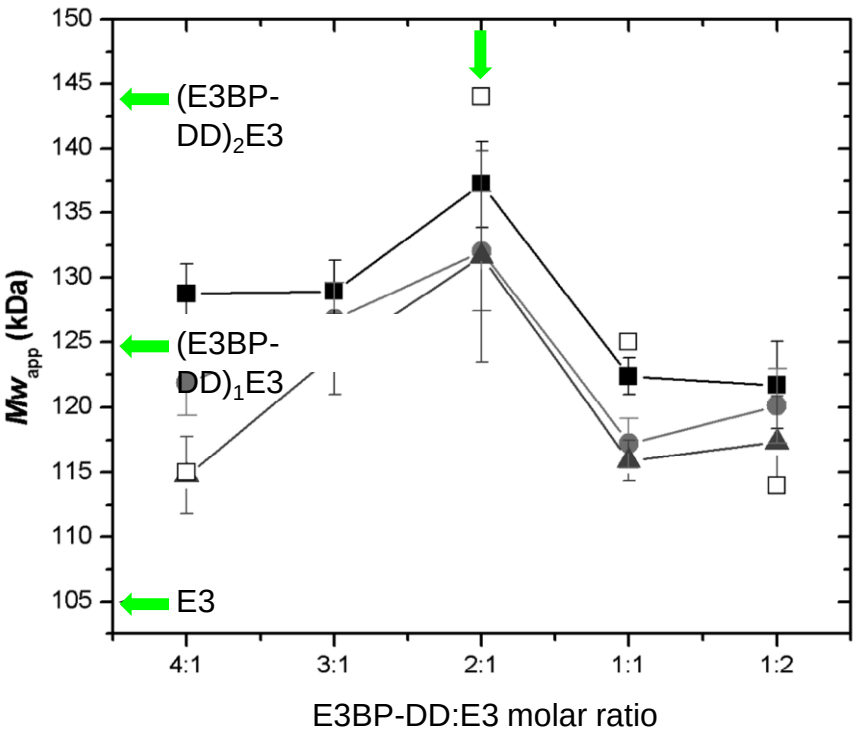


- From amino acid sequence:
  - E3BP-DD M = 19.5 kDa
  - E3 M = 105 kDa
- Sample volume = 30 µl
- Path-length 3 mm
- SE performed at 3 rotor speeds
  - 8.5, 12, 16k rpm
    - Appropriate for different complexes
- Absorbance data (280 nm)
- Radial step size 0.001 cm
- Program WINMATCH used to demonstrate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

Mischa Smolle
Smolle et al., JBC *281* 19771-80 (2006)

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???



Mischa Smolle
Smolle et al., JBC *281* 19771-80 (2006)

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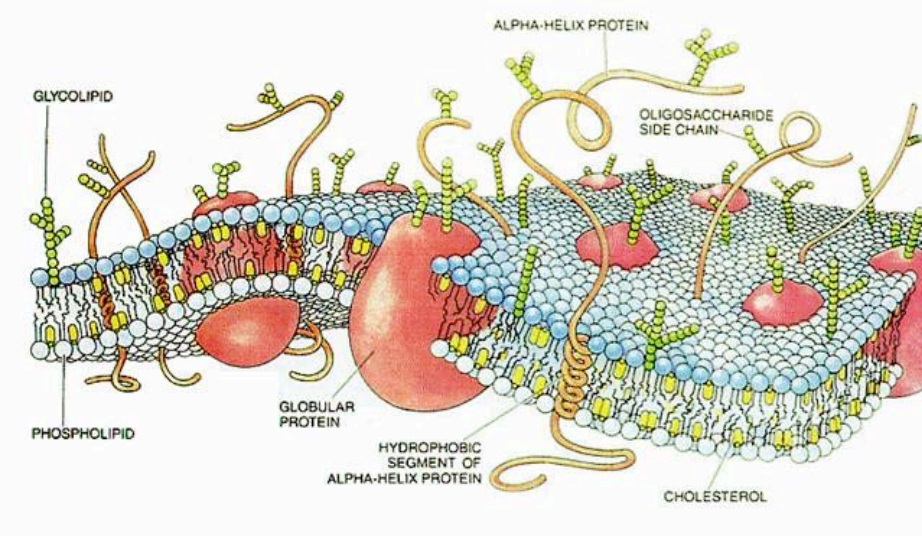
Detergent solubilised proteins: density matching SE

- In SE bouyant molecular weight is determined:

$$M_{\mathrm{p}}(1-\phi'\rho) = M_{\mathrm{p}}[(1-\bar{v}_{\mathrm{p}}\rho) + \delta_{\mathrm{Det}}(1-\bar{v}_{\mathrm{Det}}\rho)]$$

- 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

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

$$M_{\mathrm{p}}(1 - \phi'\rho) = M_{\mathrm{p}}[(1 - \bar{v}_{\mathrm{p}}\rho) + \delta_{\mathrm{Det}}(1 - \bar{v}_{\mathrm{Det}}\rho)]$$

0

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

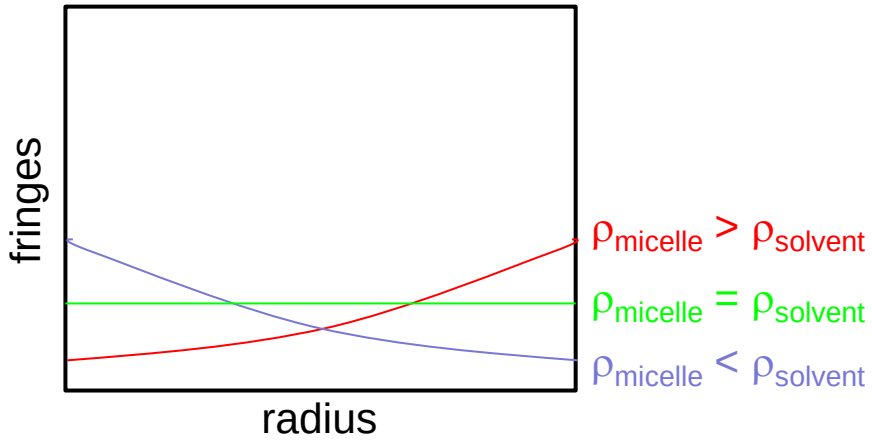
BUT…..this method works only in certain conditions

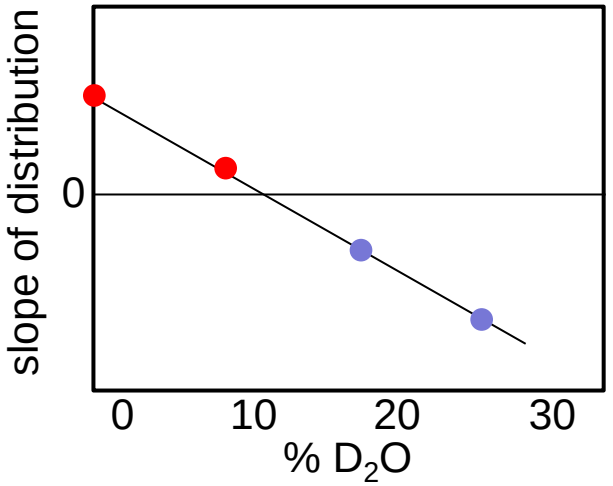
- The solvent density must be adjusted with D_2O or $D_2{}^{18}O$
  - Alternatives would be e.g. sucrose or other co-solvent
  - Affect chemical potential
  - Lead to preferential binding and/or exclusion of water or additional co-solvent at protein surface
- But use of D_2O or $D_2{}^{18}O$ limits detergents that can be used
  - ρ D_2O = 1.1 g/ml
  - $\bar{v}$ of the detergent must be between that of water and D_2O
  - i.e. 0.9 ≤ $\bar{v}$ ≤ 1.0 ml/g
  - Eliminates:
    - dodecylmaltoside (ρ = 1.21 g/ml, $\bar{v}$ = 0.83 ml/g)
    - β-octylglucoside (ρ = 1.15 g/ml, $\bar{v}$ = 0.87 ml/g)
  - Suitable:
    - C8E5 ($\bar{v}$ = 0.993 ml/g)
    - C14SB (density matched by 13% D_2O in 20 mM Tris-HCl, 200 mM KCl)
    - Dodecylphosphocholine (DPC, density matched by 52.5% D_2O in 50 mM Tris-HCl, 100 mM NaCl)

Burgess, Stanley & Fleming (2008). In *Methods in Cell Biology* (J. Correia & H. W Detrich, III, eds.), **84**, 181-211. Academic Press

Determination of density-matching point for C14SB

- Determine % of D_2O 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% D_2O
  - 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





Burgess, Stanley & Fleming (2008). In *Methods in Cell Biology* (J. Correia & H. W Detrich, III, eds.), **84**, 181-211. Academic Press

SE of systems solublised by C14SB: OMPLA

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

Burgess, Stanley & Fleming (2008). In *Methods in Cell Biology* (J. Correia & H. W Detrich, III, eds.), **84**, 181-211. Academic Press

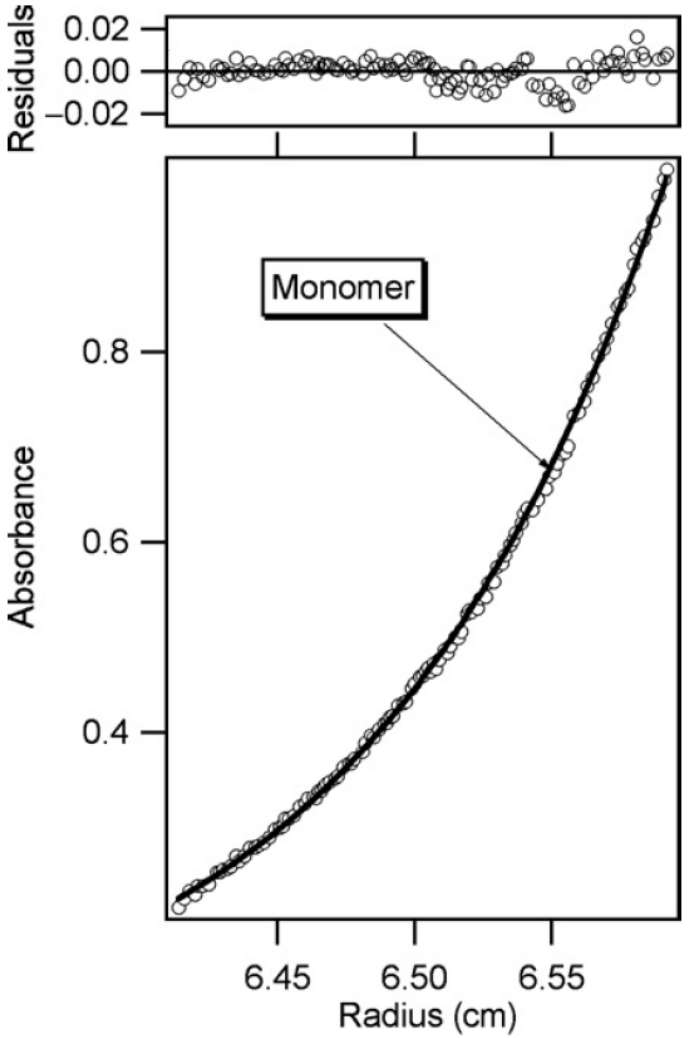
OMPLA studied at 3 concs, 3 rotor speeds for each of 4 conditions

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 $CaCl_2$

Burgess, Stanley & Fleming (2008). In *Methods in Cell Biology* (J. Correia & H. W Detrich, III, eds.), **84**, 181-211. Academic Press

SE results: 1. OMPLA

- SE data first globally fitted with equation for single ideal species
- Good fits
  - $\sqrt{\sigma^2}$ ≈ 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



Burgess, Stanley & Fleming (2008). In *Methods in Cell Biology* (J. Correia & H. W Detrich, III, eds.), **84**, 181-211. Academic Press

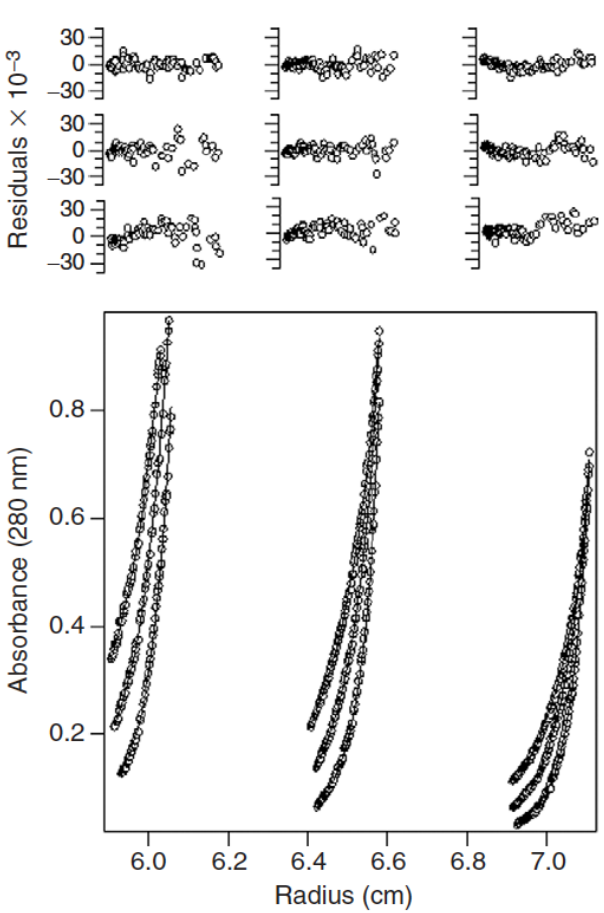
SE results: 4. OMPLA + covalently bound fatty acyl chain substrate analogue + 20 mM $CaCl_2$

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

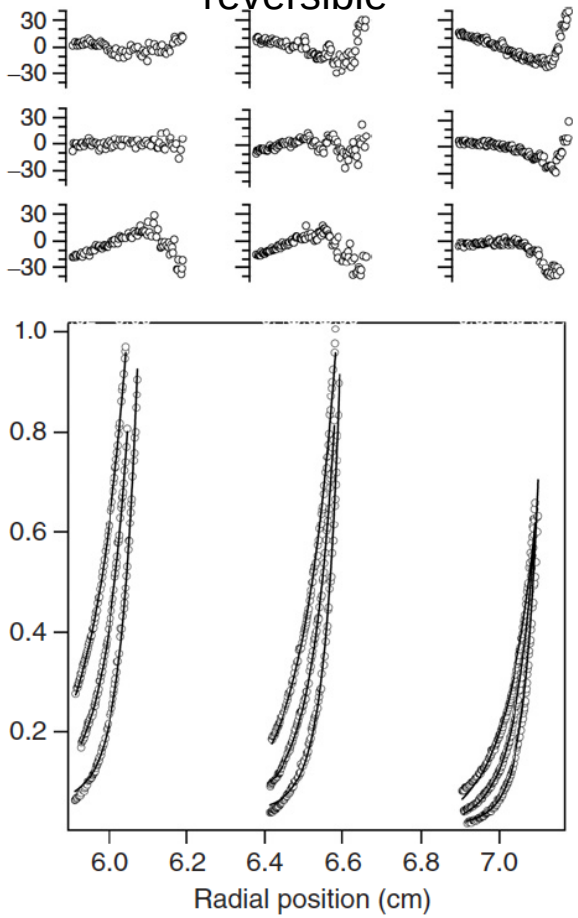
Burgess, Stanley & Fleming (2008). In *Methods in Cell Biology* (J. Correia & H. W Detrich, III, eds.), **84**, 181-211. Academic Press

OMPLA-DSF reversibly dimerises

DSF: monomer-dimer reversible



pOSF: monomer-dimer not reversible



Burgess, Stanley & Fleming (2008). In *Methods in Cell Biology* (J. Correia & H. W Detrich, III, eds.), **84**, 181-211. Academic Press

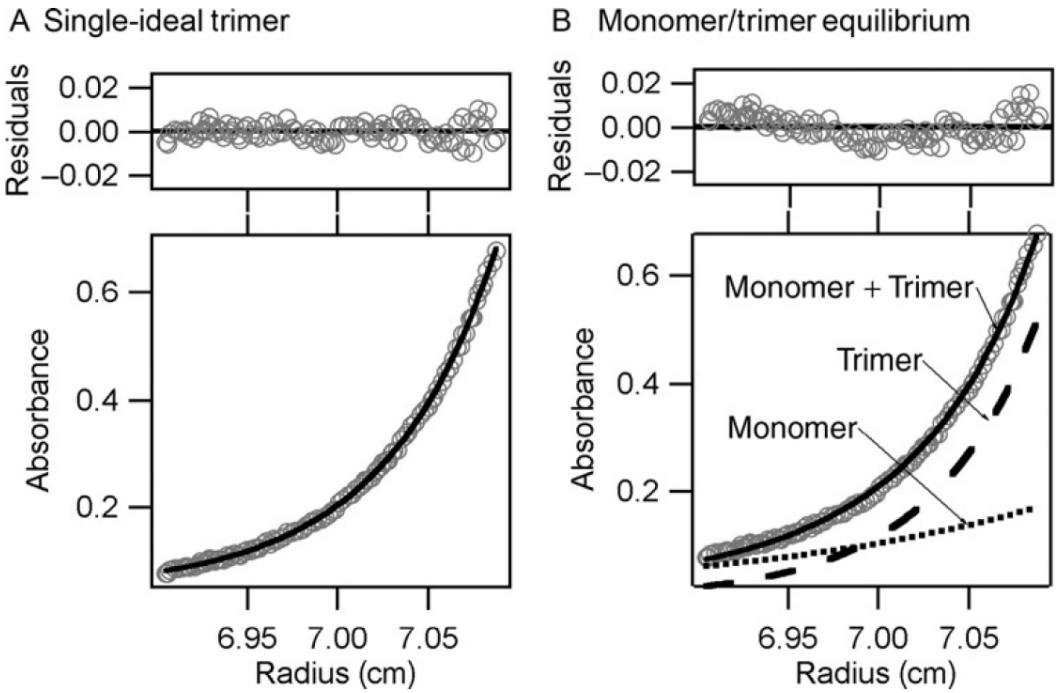
SE of systems solublised by C14SB: OmpF

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

Burgess, Stanley & Fleming (2008). In *Methods in Cell Biology* (J. Correia & H. W Detrich, III, eds.), **84**, 181-211. Academic Press

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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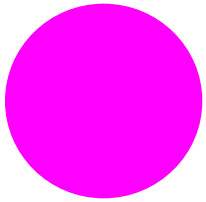
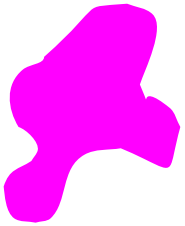
s = deviation from sphericity + hydrodynamic hydration

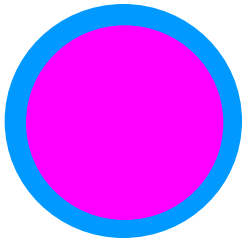
$$s = \frac{M(1-\bar{v}\rho)}{N_A f}$$



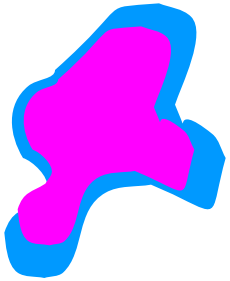
M, f_0



M, $f>f_0$

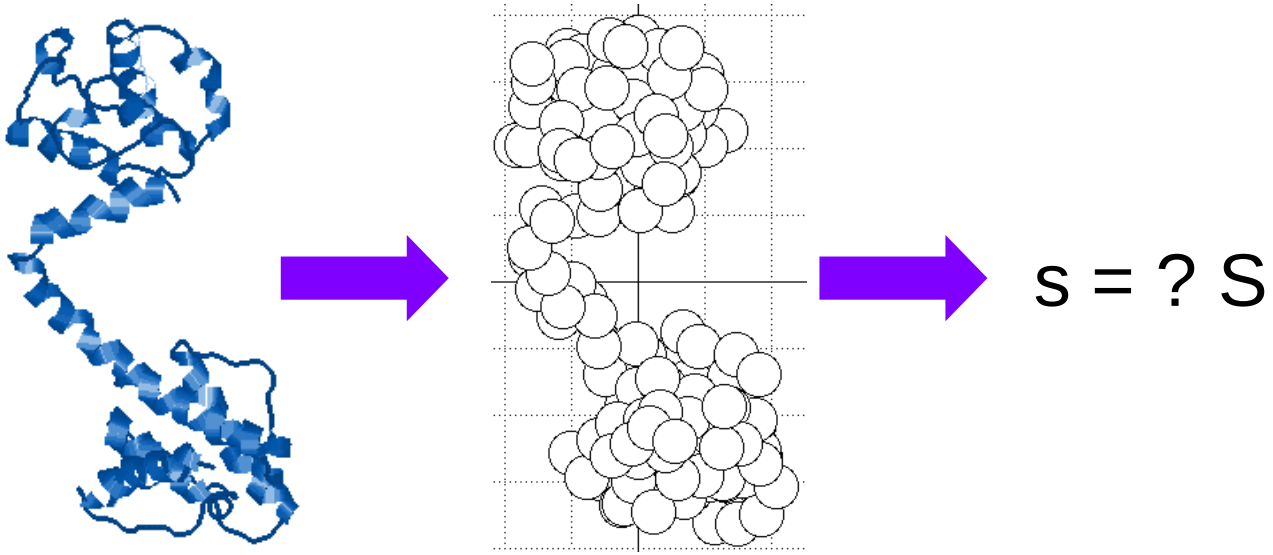


M, $f>f_0$



M, $f>>f_0$

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

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

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

Several freely available programs for HBM

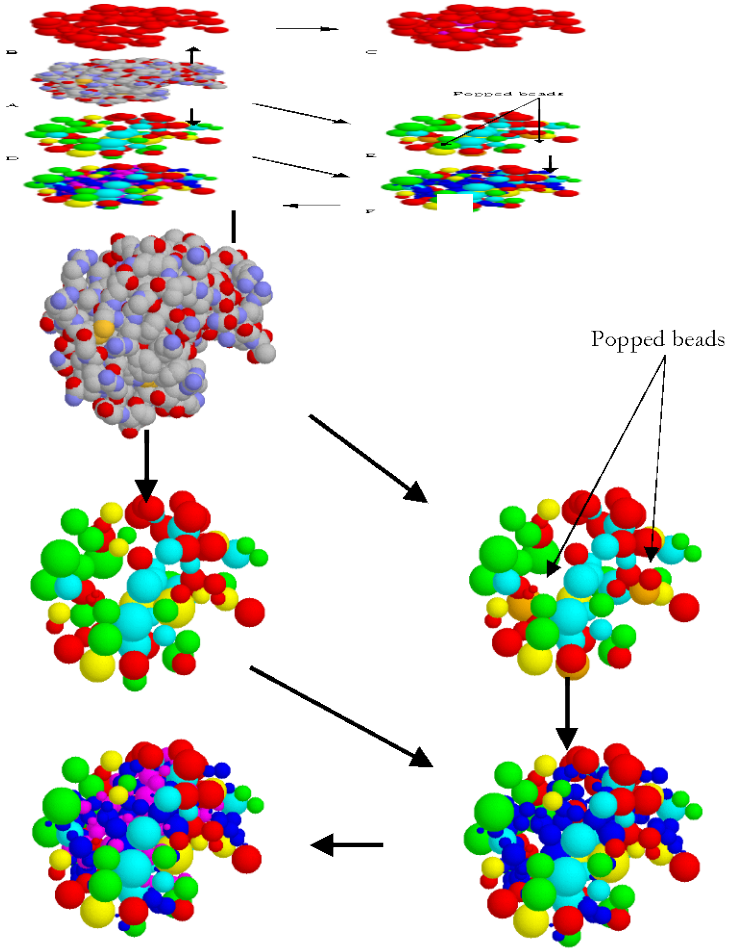
- A more exact expression for f_t together with expressions for other hydrodynamic and related parameters are encoded in HBM software:

- 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

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

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



AtoB

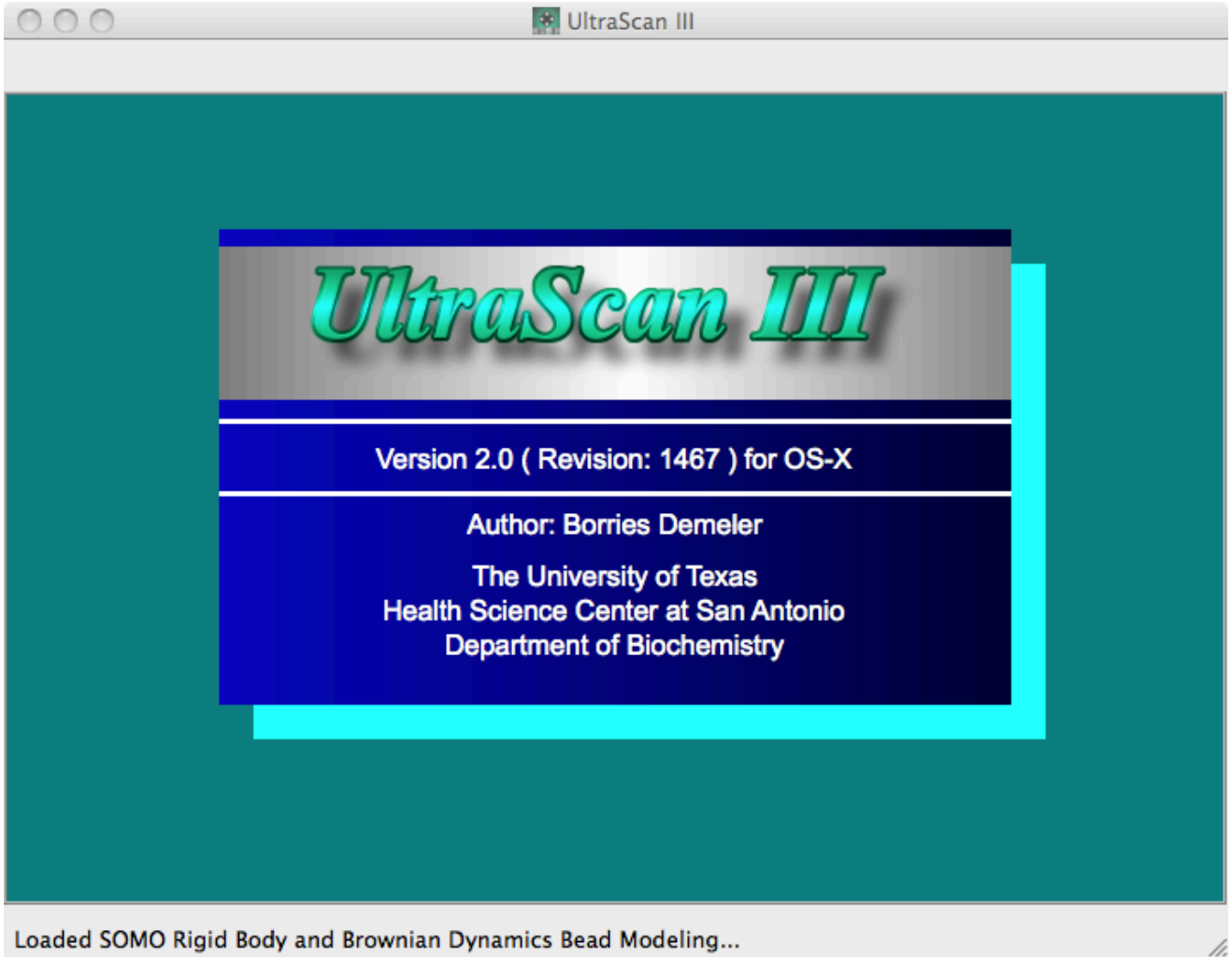
Trans

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

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

Rai, Nöllmann, Spotorno, Tassara, Byron & Rocco, Structure (2005) 13, 723-734

SOMO is a subprogram of UltraScan III

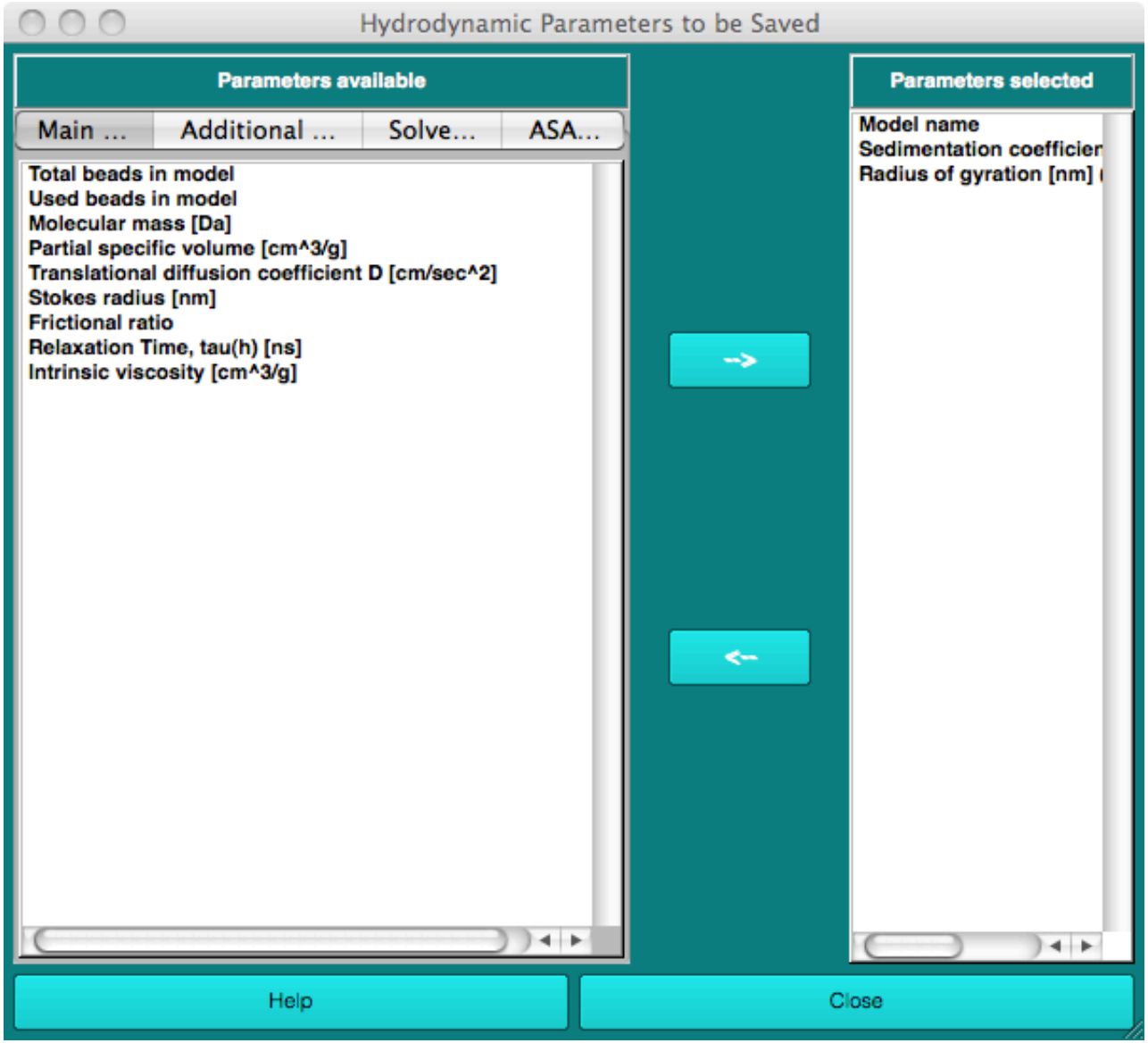


Mattia Rocco/ Borries Demeler/ Emre Brooks

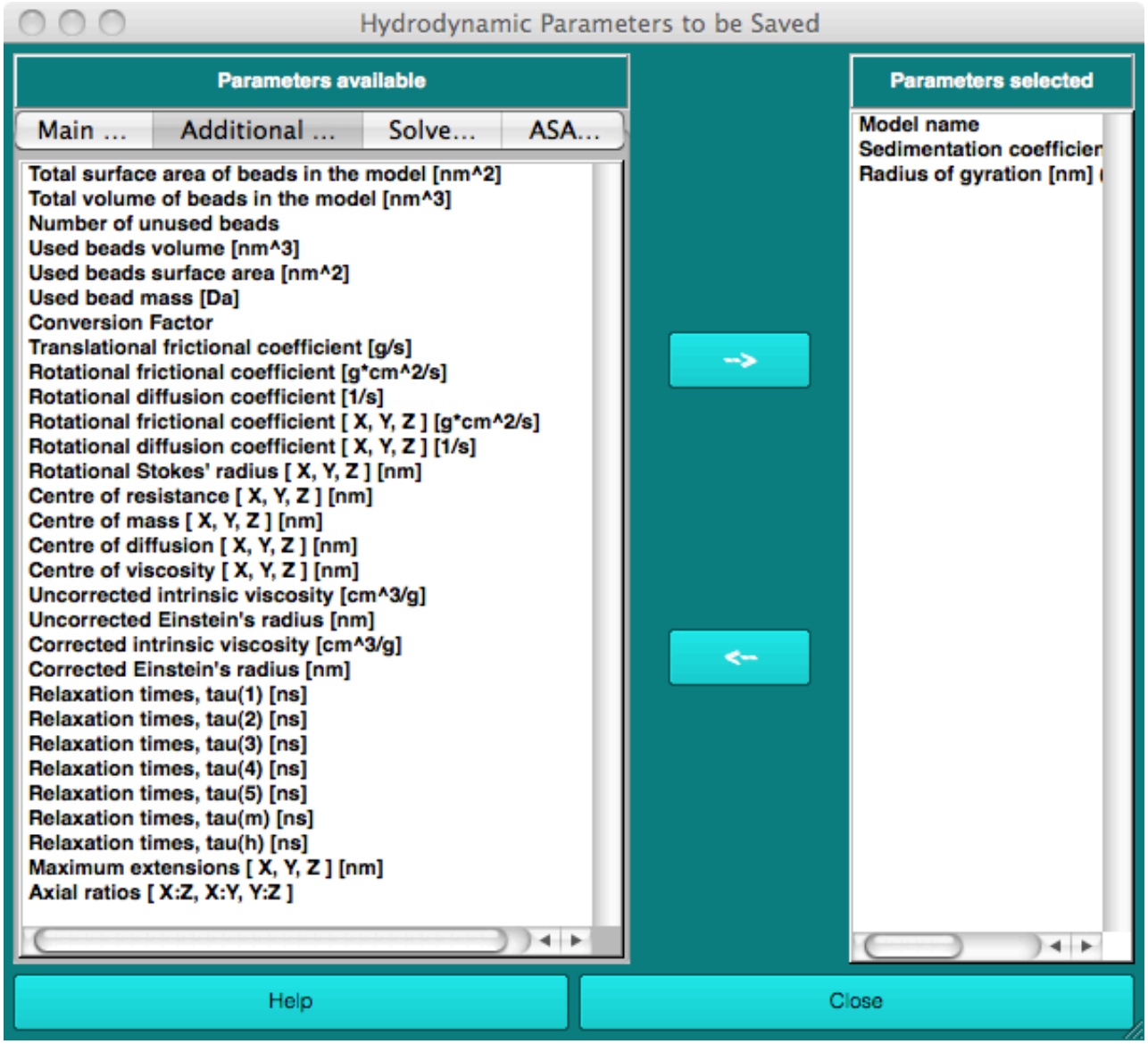
Rai et al. (2005) Structure *13* 723-34; Brookes et al. (2010) Eur. Biophys. J; Brookes et al., (2010) Macromol. Biosci.

http://somo.uthscsa.edu/

Parameters computed by SOMO (1)



Parameters computed by SOMO (2)



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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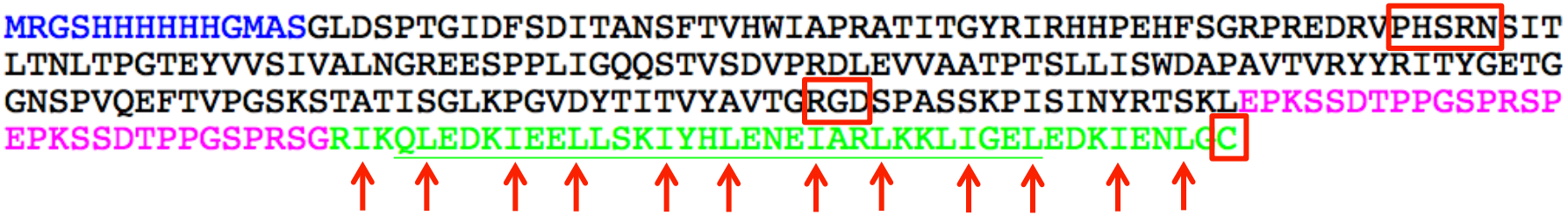
- Question: do the ligands oligomerise as designed?

Construction of hydrodynamic bead models

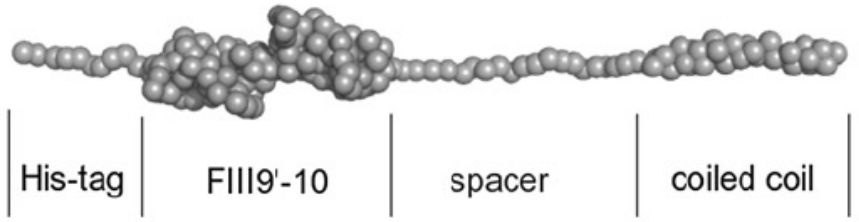


- From vector (including His-tag) – too short for e.g. SWISSMODEL
- FN III 9-10 domain pair homology model (SWISSMODEL)
- Synthesised “missing beads”
- Coiled-coil (42 a.a.) – SWISSMODELs generated for underlined segment

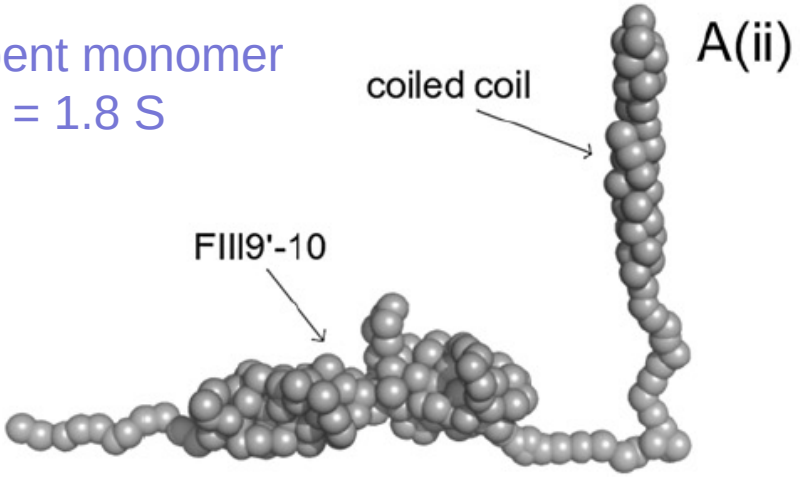
Kreiner *et al.*, (2009) Biophysical Chemistry 142 34-39

Oligomer models generated

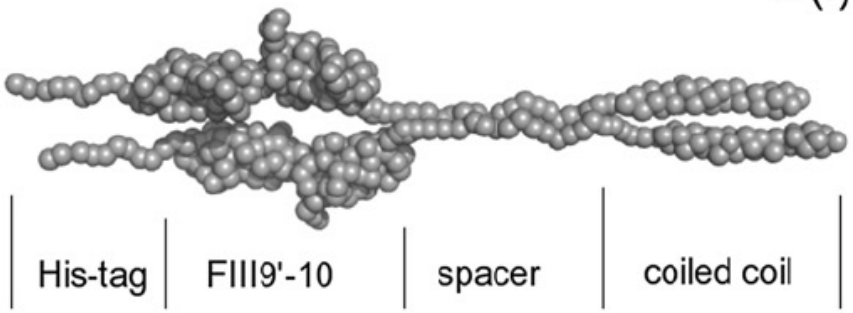
linear monomer
s = 1.7 S



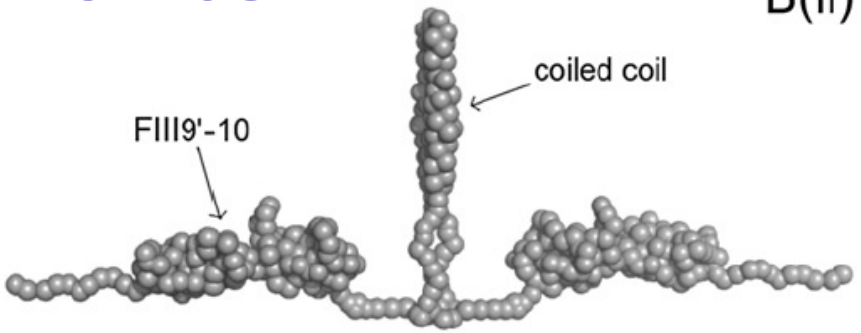
bent monomer
s = 1.8 S



linear dimer
s = 2.7 S



bent dimer
s = 2.5 S



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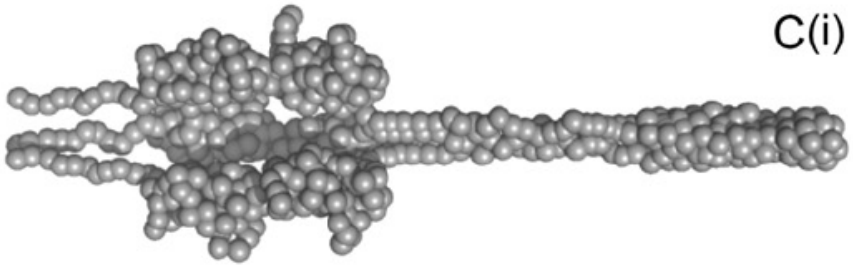
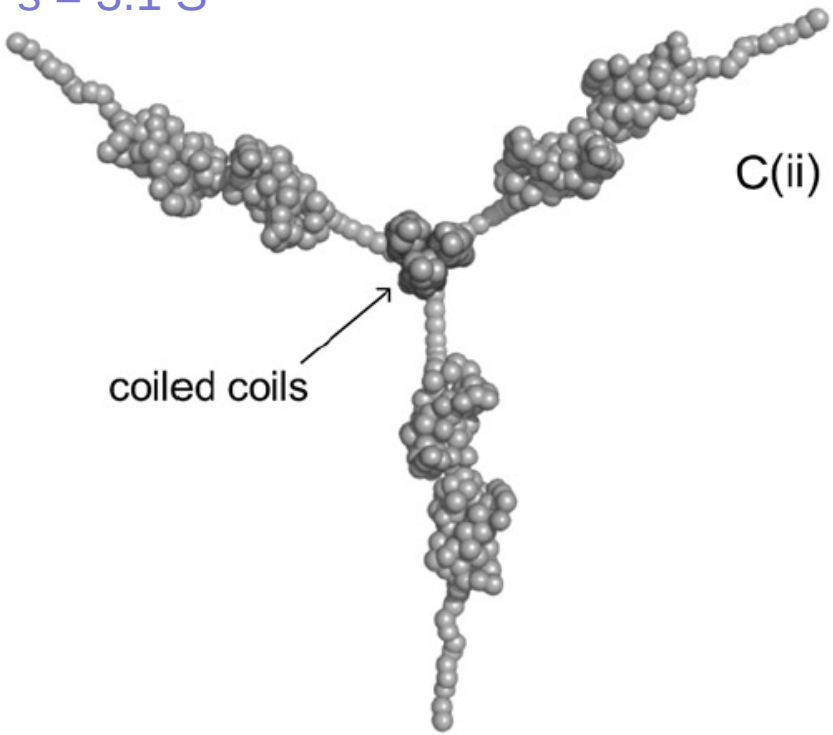
Oligomer models generated

linear trimer
s = 3.9 S



bent trimer
s = 3.1 S



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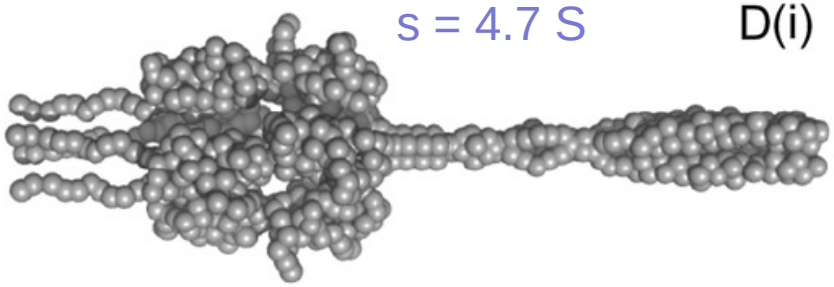
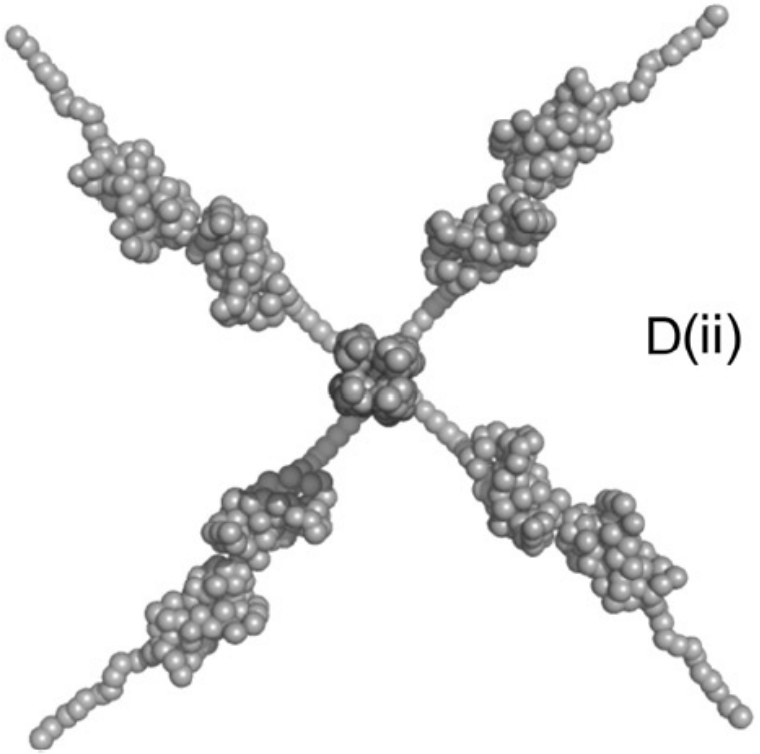
Oligomer models generated

linear tetramer
s = 4.7 S

D(i)



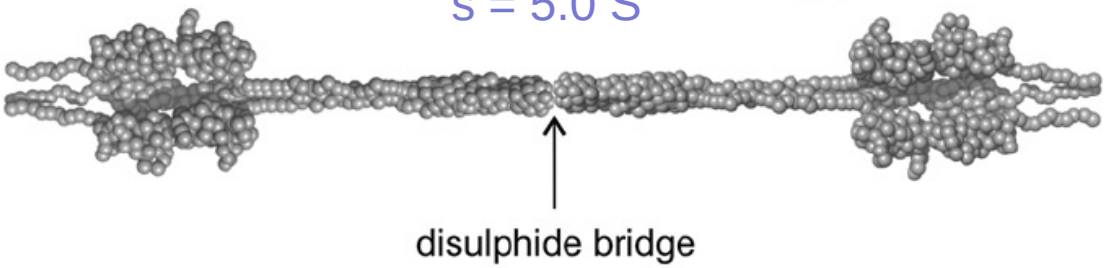
bent tetramer
s = 3.7 S



D(ii)

linear hexamer
s = 5.0 S

E



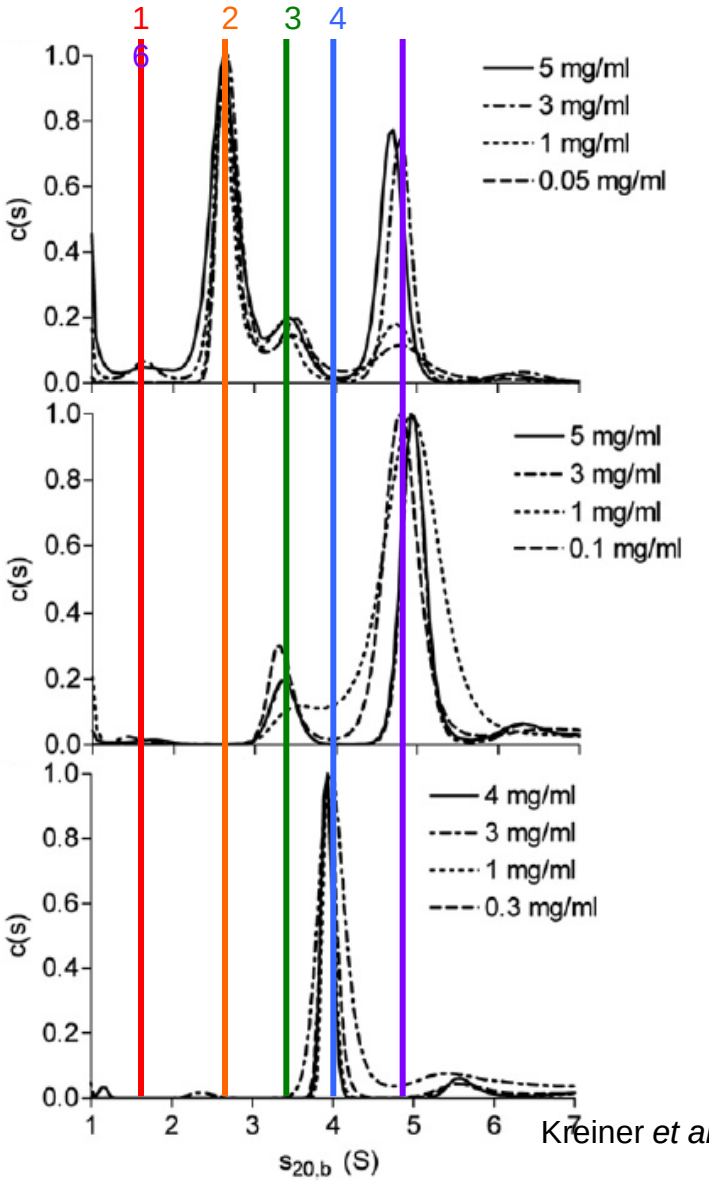
Kreiner *et al.*, (2009) Biophysical Chemistry 142 34-39

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

“dimer”

“trimer”

“tetramer”



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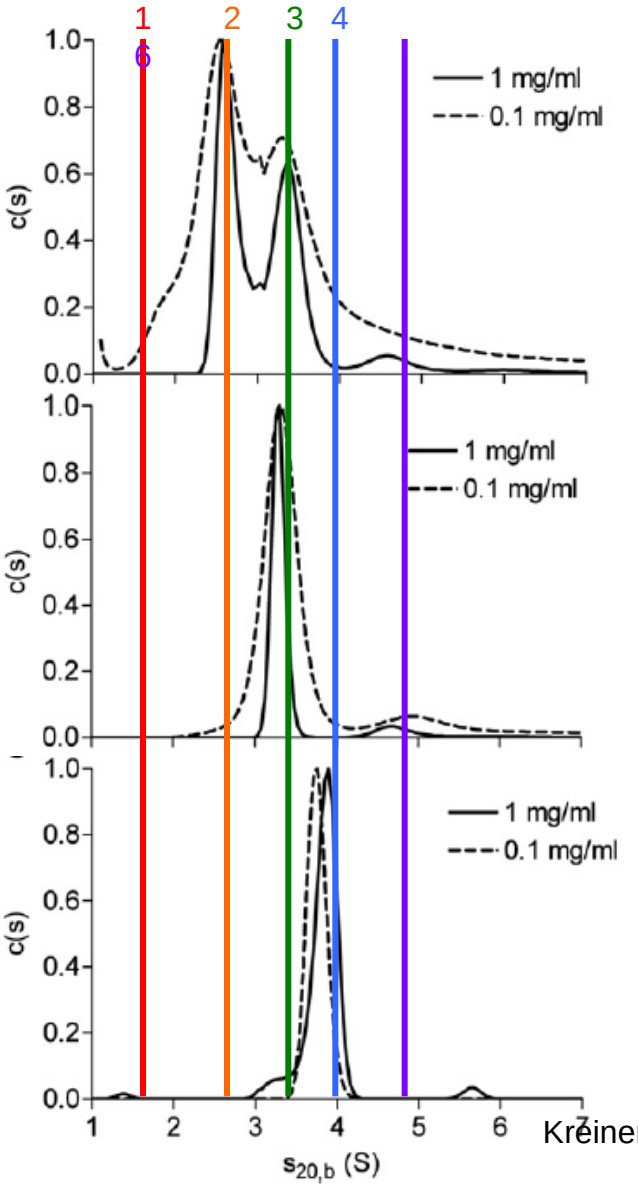

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- Thiol-linked immobilisation to surface achieved via C-terminal Cys

- 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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