Analytical ultracentrifugation

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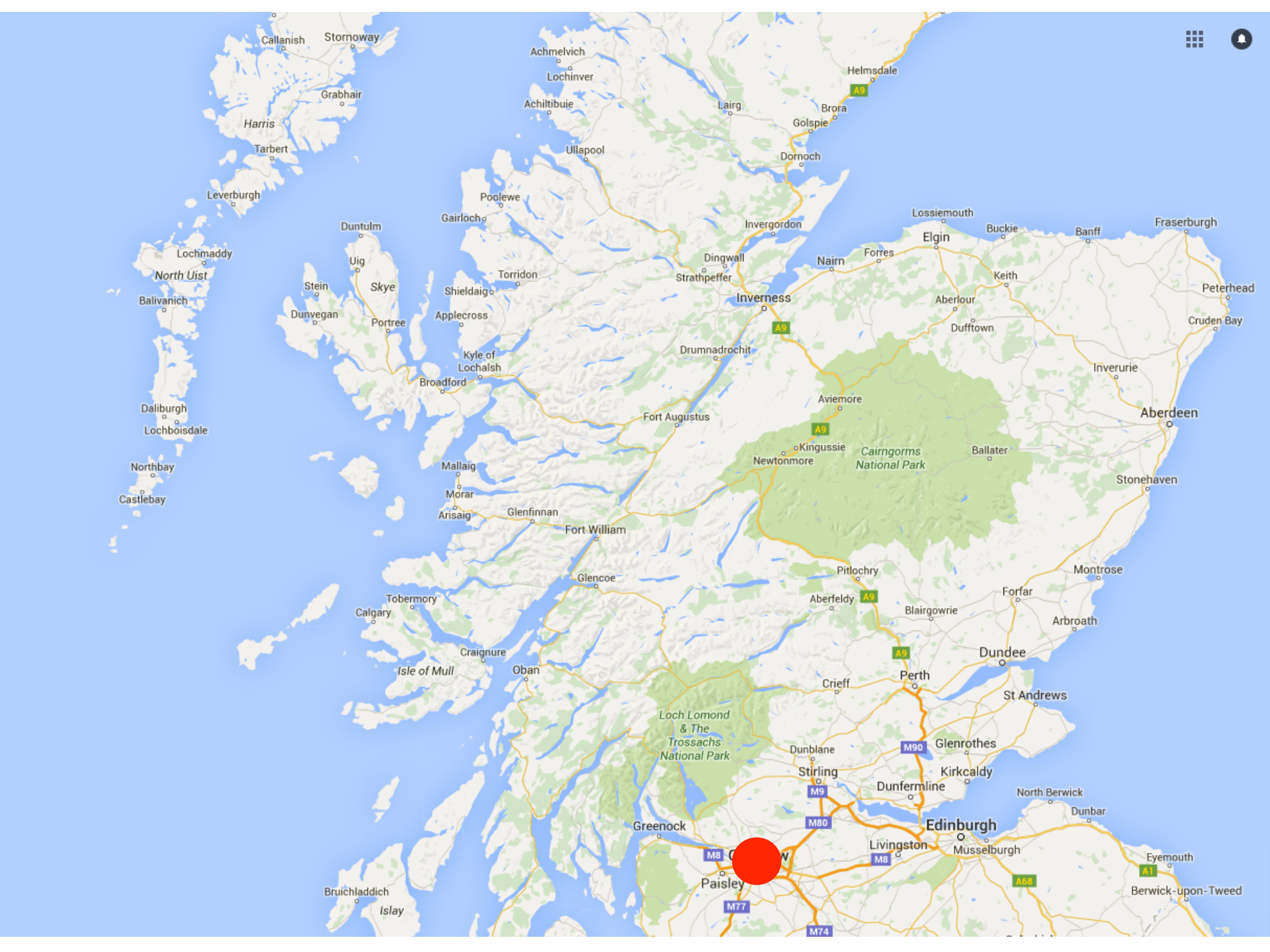
Callanish
Stornoway
Achmelvich
Lochinver
Helmsdale
A9
Grabhair
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Lairg
Brora
Harris
Golspie
Tarbert
Ullapool
Dornoch
Leverburgh
Poolewe
Gairloch
Lossiemouth
Duntulm
Invergordon
Buckie
Banff
Fraserburgh
Elgin
Lochmaddy
Uig
Dingwell
Nairn
Forres
North Uist
Torridon
Strathpeffer
Keith
Stein
Skye
Shieldaig
Inverness
Peterhead
Balivanich
Aberlour
Dunvegan
Portree
Applecross
Cruden Bay
Dufftown
Drumnadrochit
Kyle of Lochalsh
Inverurie
Broadford
Aviemore
Aberdeen
Daliburgh
Fort Augustus
Lochboisdale
Kingussie
Cairngorms National Park
Ballater
Newtonmore
Northbay
Stonehaven
Mallaig
Castlebay
Morar
Arisaig
Glenfinnan
Fort William
Pitlochry
Montrose
Glencoe
Forfar
Tobermory
Aberfeldy
Calgary
Blairgowrie
Arbroath
Craignure
Dundee
Isle of Mull
Oban
Perth
Crieff
St Andrews
Loch Lomond & The Trossachs National Park
Glenrothes
Dunblane
M90
Kirkcaldy
Stirling
M9
Dunfermline
North Berwick
Dunbar
M80
Greenock
Edinburgh
Livingston
Musselburgh
M8
Eyemouth
A1
Paisley
A68
Bruichladdich
Berwick-upon-Tweed
M77
Islay
M74

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

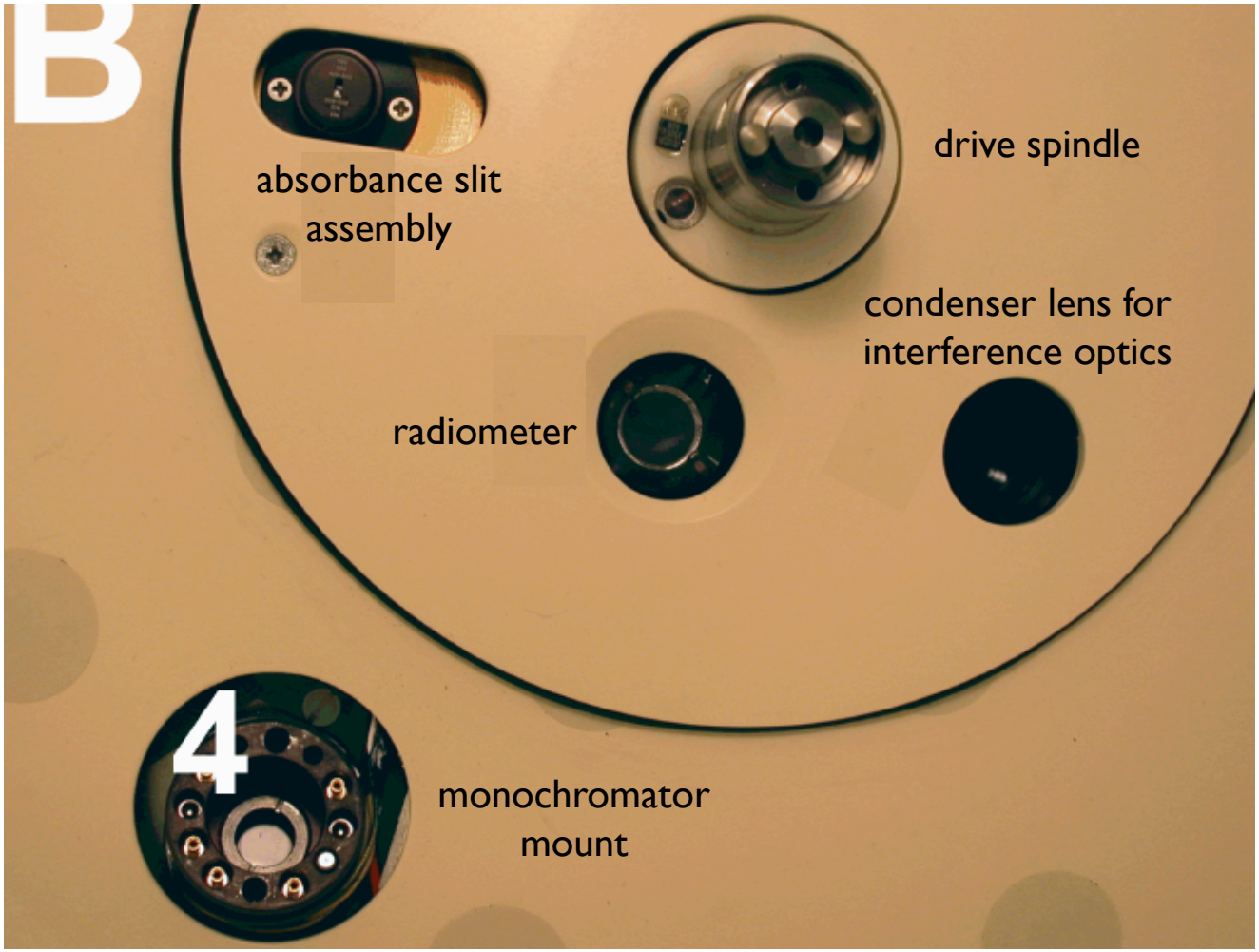
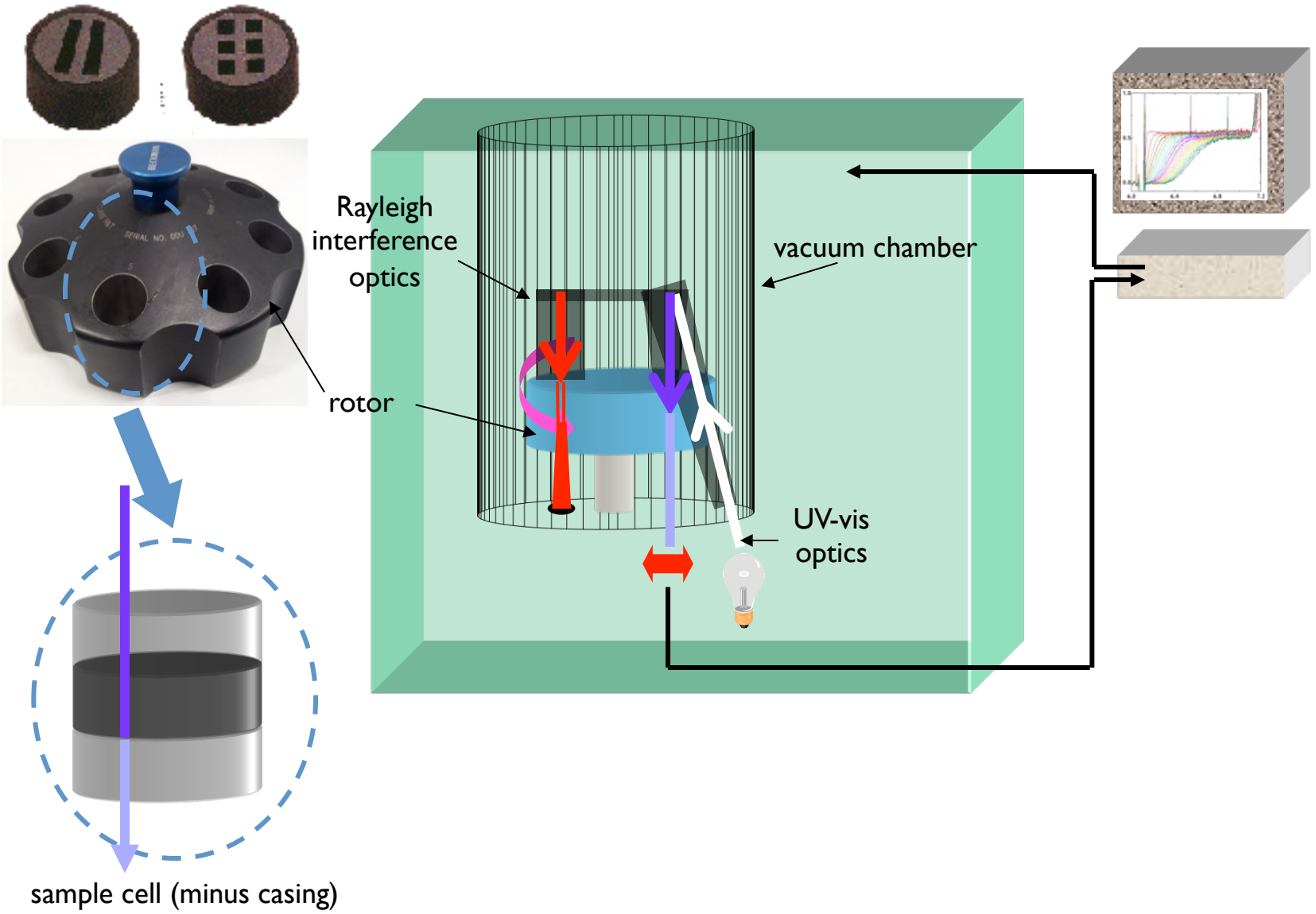


image from Analytical Ultracentrifuge User Guide Volume 1: Hardware, K. L. Planken & V. Schirf, 2008 (http://www.ultrascan.uthscsa.edu)

Inside the Beckman Coulter XL-I



Relationship between data and sample

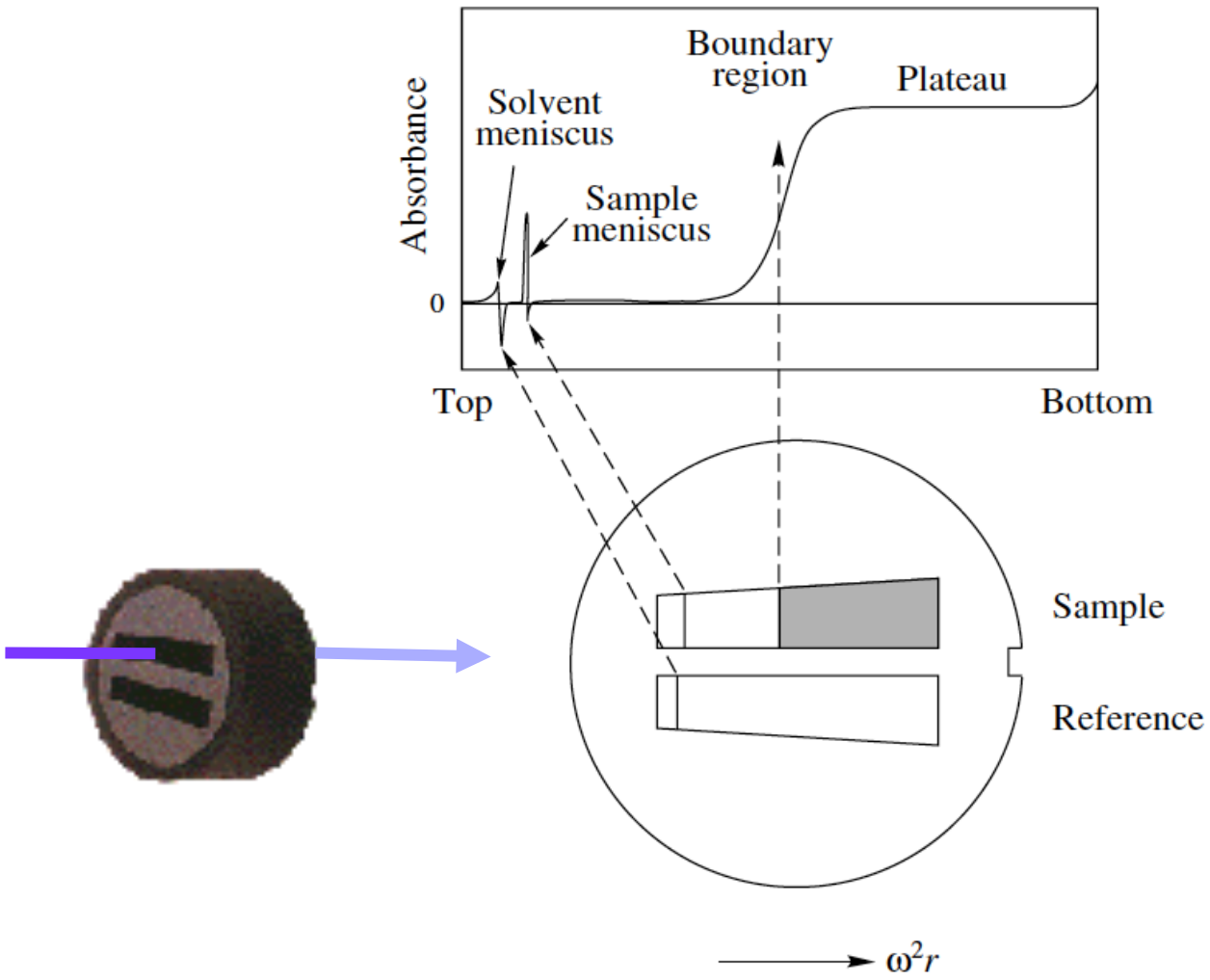


image from Ralston, 1993
https://www.beckman.com/centrifuges/analytical-ultracentrifuges/proteomelab-xl-a-xl-i

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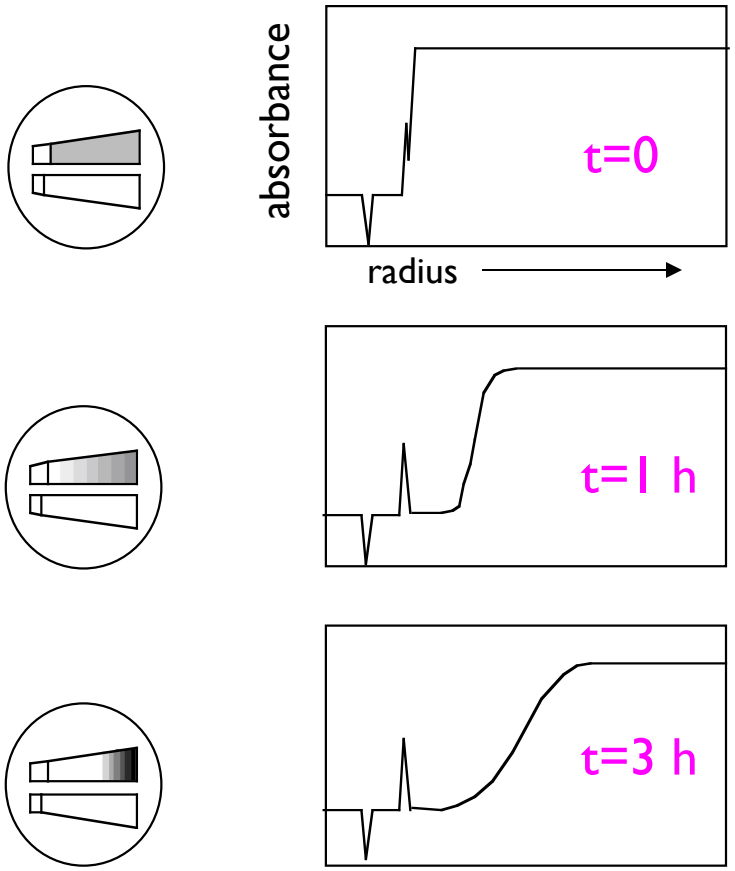
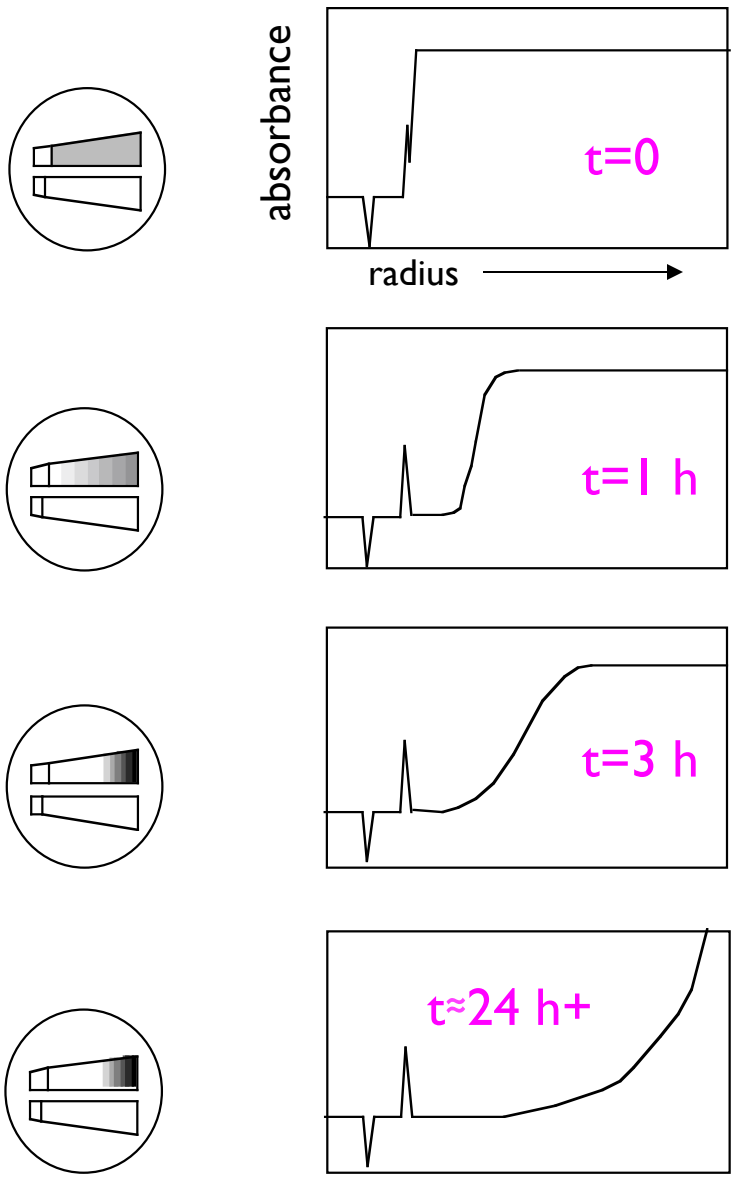
Sedimentation velocity (SV): shape & homogeneity



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_λ = 0.1	0.05 mg/ml	100 pM $_{\text{fluorescein}}$
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

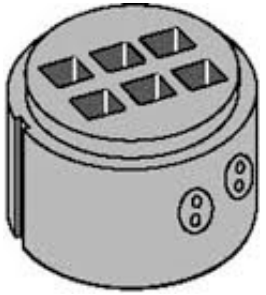
Adapted from a slide by Tom Laue

Sample preparation

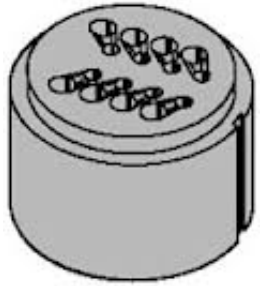
- Purify by gel filtration or similar
  - Unless you want to know what is in solution in its entirety
- Estimate concentration
  - Using e.g. NanoDrop
- (Dialyse sample against the desired solvent)
  - Possible problems with detergents
  - Required for interference optics only
- Choose windows
  - Sapphire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

Sample requirements

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

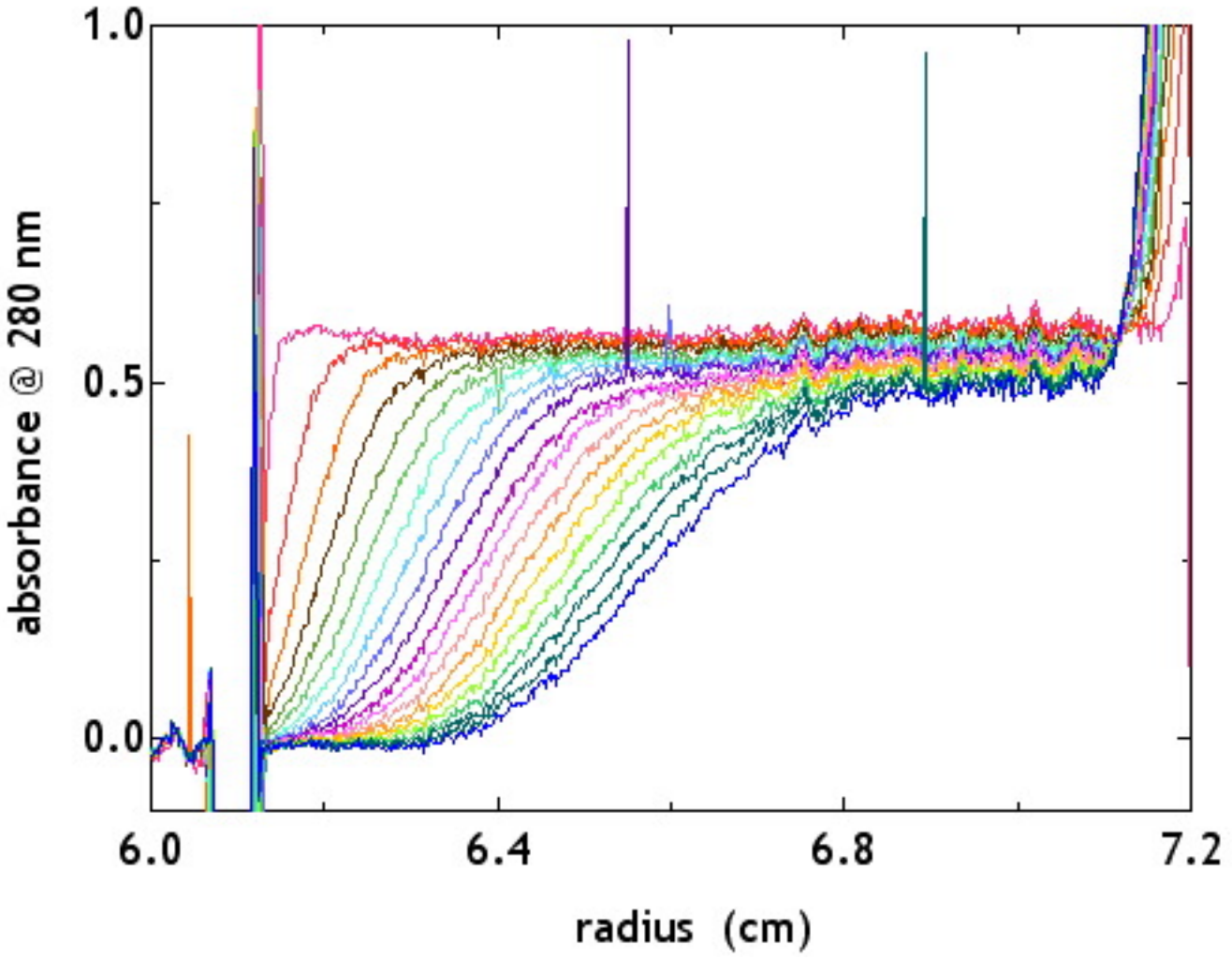


- Sample concentration
  - Absorbance optics: $A_\lambda \approx 0.1\text{-}1.0$ (12 mm pathlength)
    - λ = 180-800 nm
  - Interference optics: typically 0.05-30 mg/ml

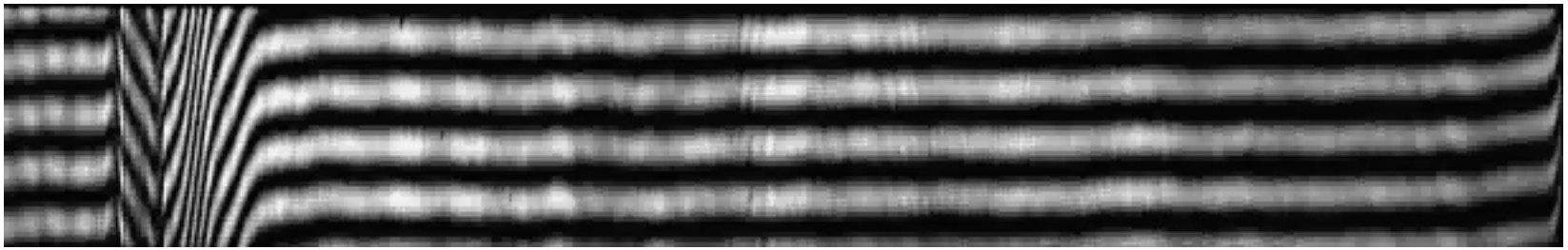


- Sample reference
  - Absorbance optics: can be column eluant or dialysate better
  - Interference optics: must be dialysate
- Typical multiplexing: 3 or 7 sample holders ("cells")/run
  - Up to 28 samples per run

SV: radial movement recorded as function of time

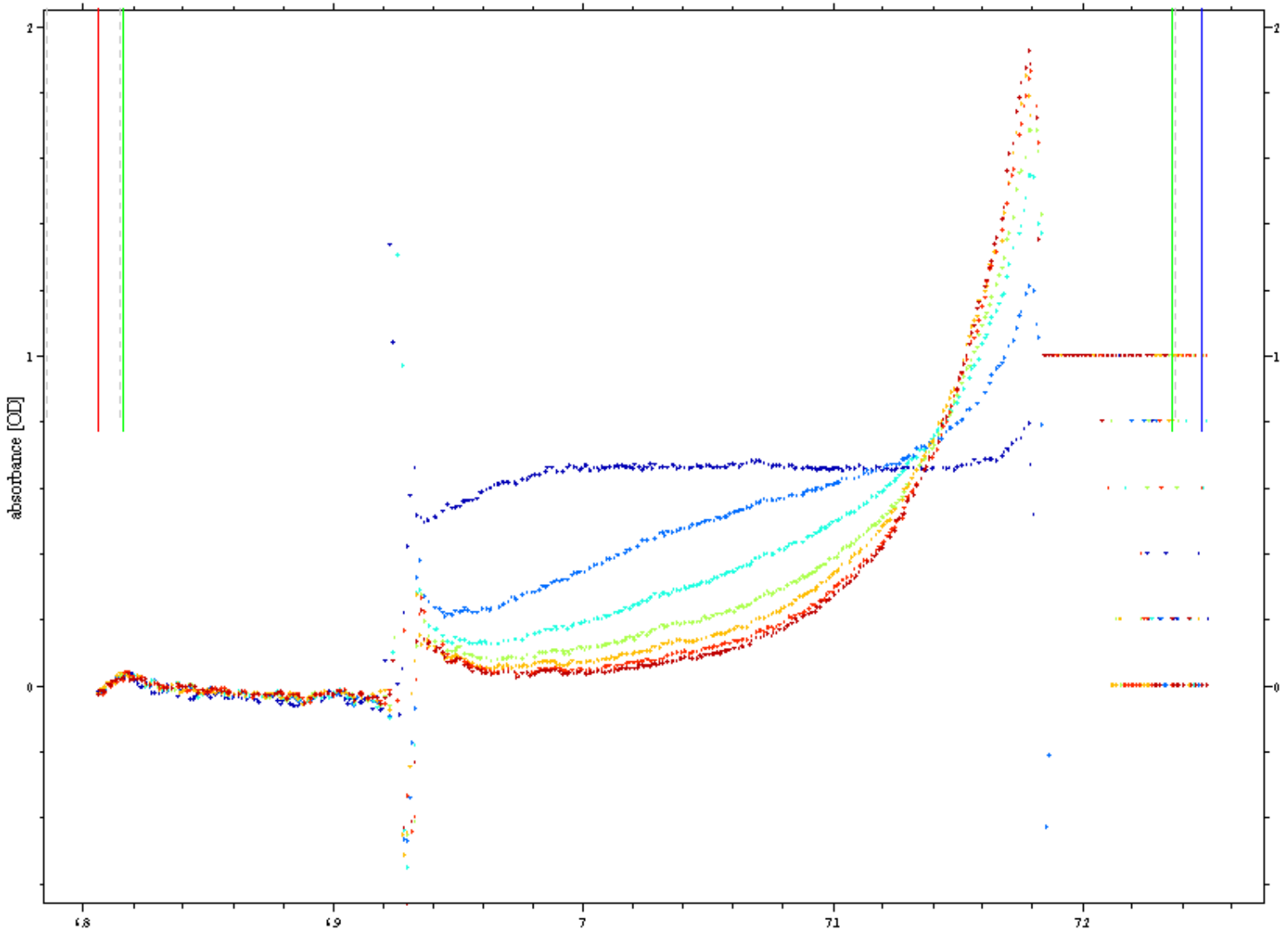


Interference optics acquire refractive index data rapidly, independent of chromophores



hemocyanin sedimentation observed with interference optics

SE: data recorded until no change…



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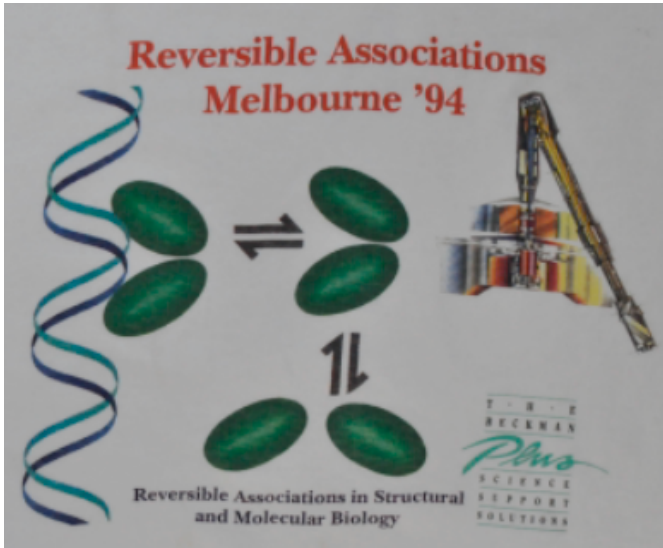
Almost all AUC data analysis software is freely available – here are the most widely used

- 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

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

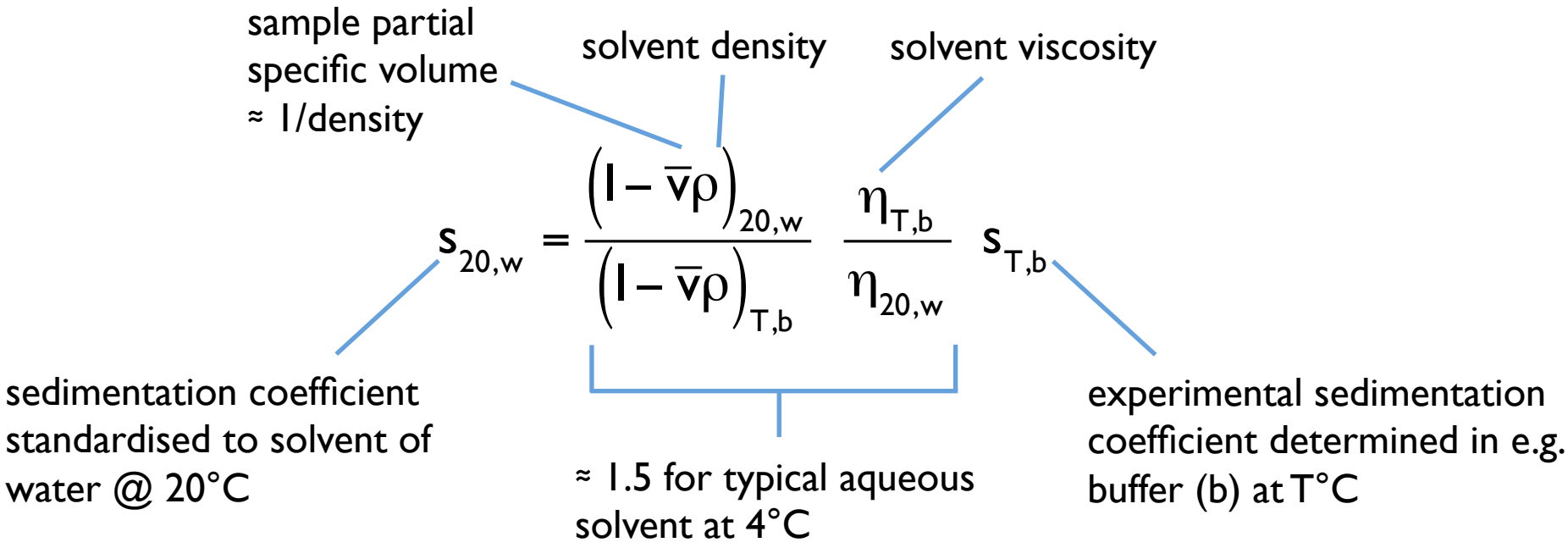
- Stokes-Einstein equation

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

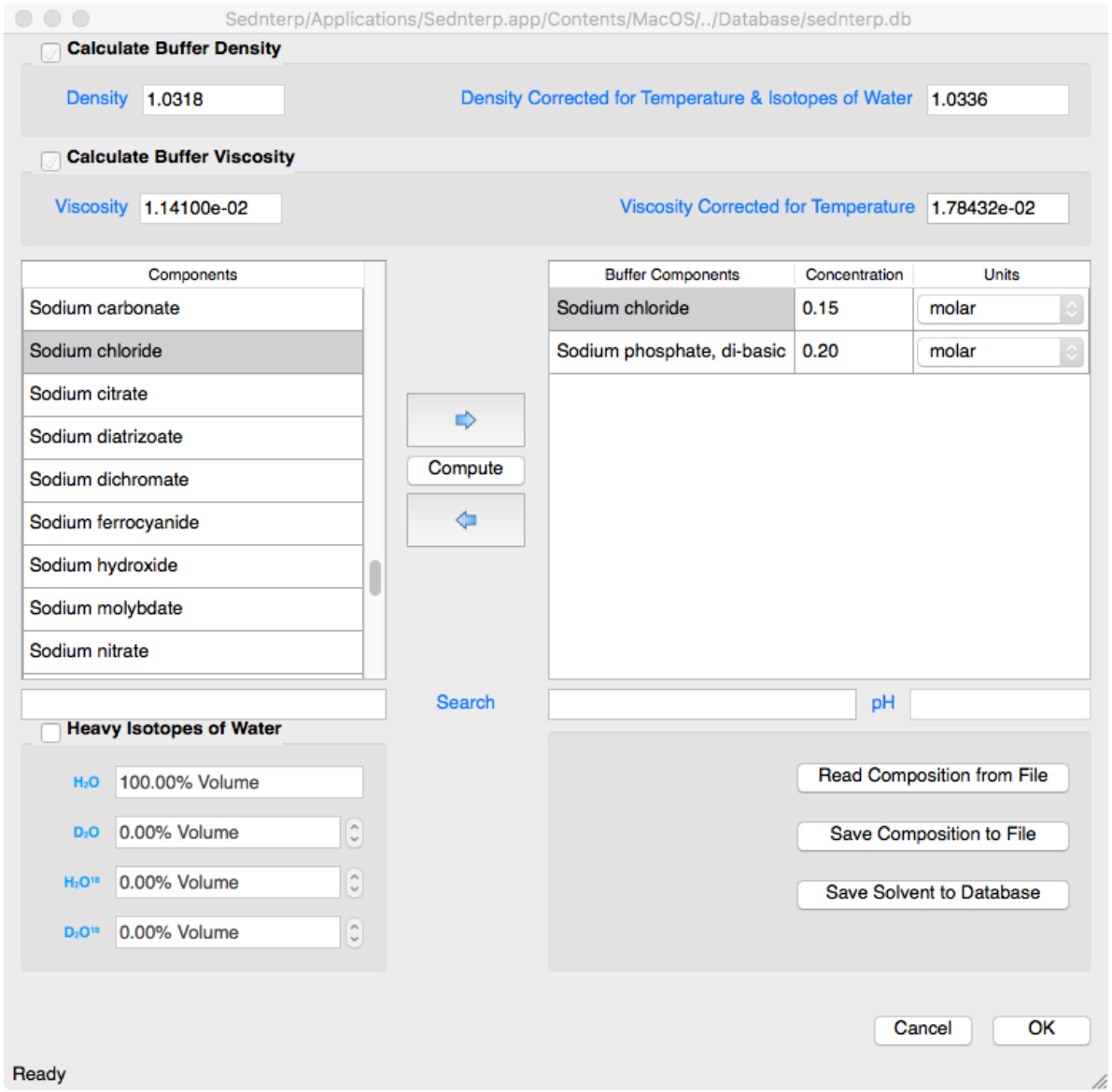
- Combining Svedberg with Stokes-Einstein

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

s is influenced by solvent density & viscosity and sample partial specific volume (psv or vbar)



SEDNTERP: Calculation of ρ, η & psv



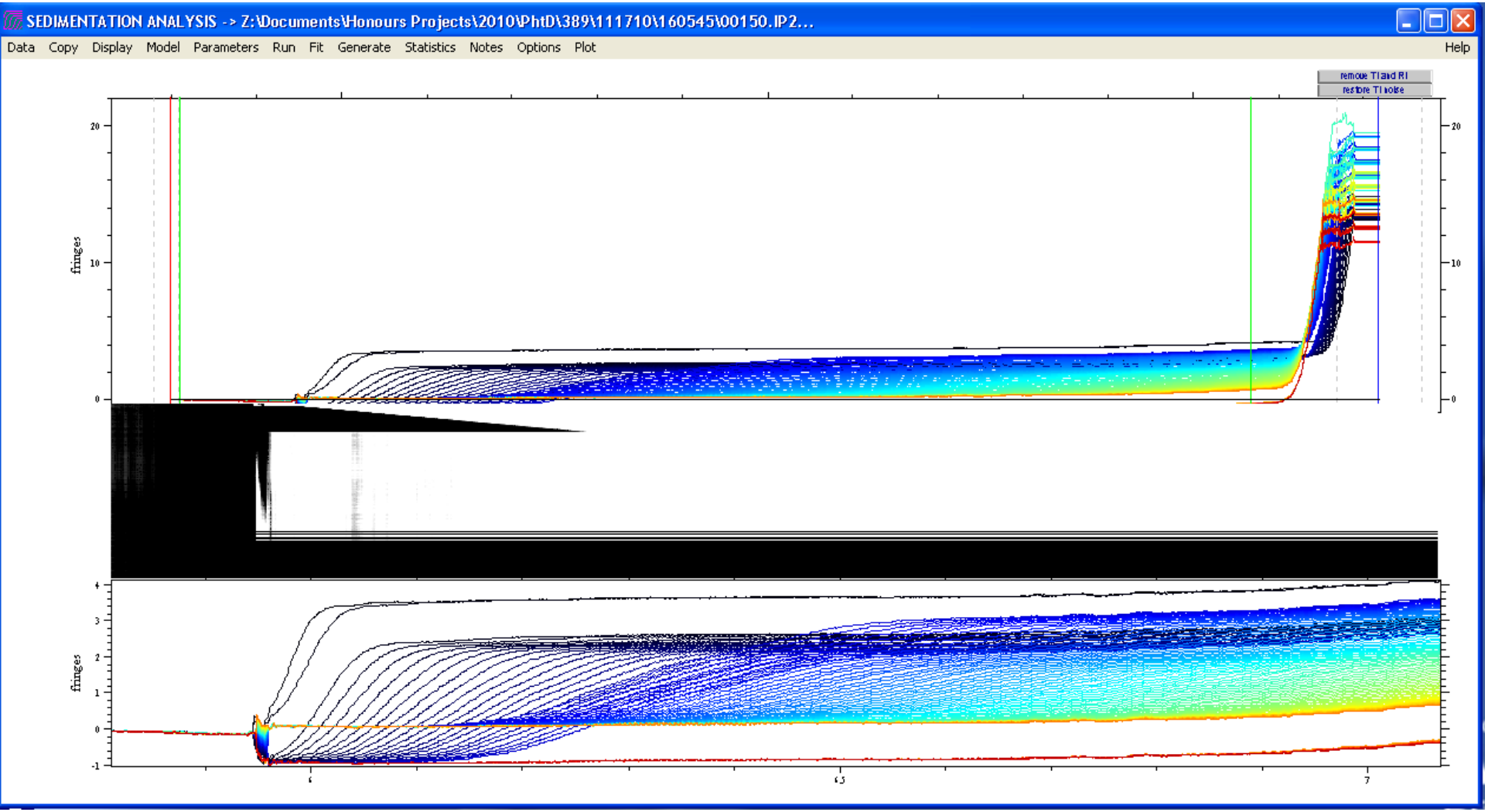
http://rasmb.org/sednterp/

Values for vbar

Component	vbar (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

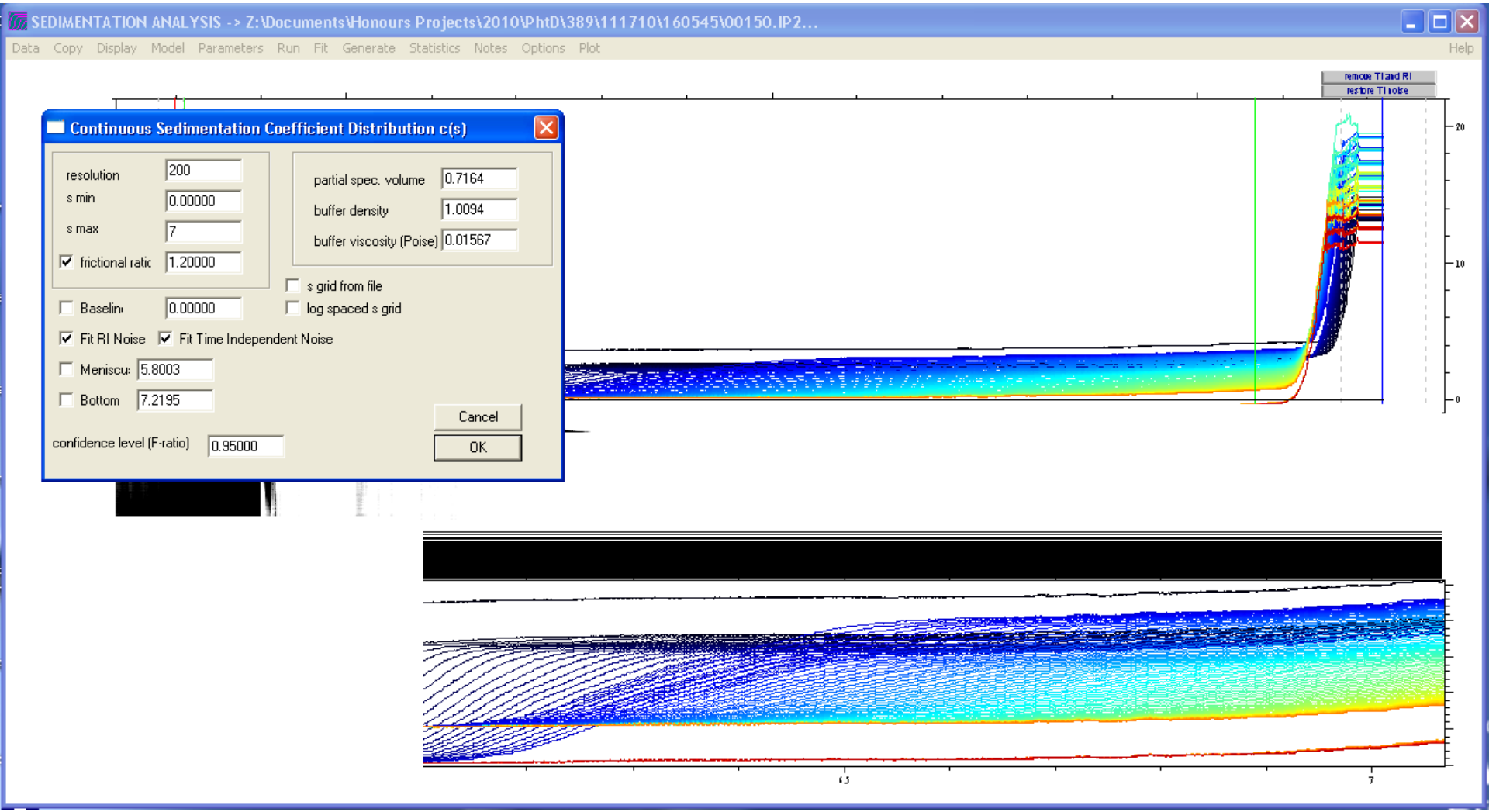
SEDFIT c(s) analysis: how many species + s of species

1: Load SV data



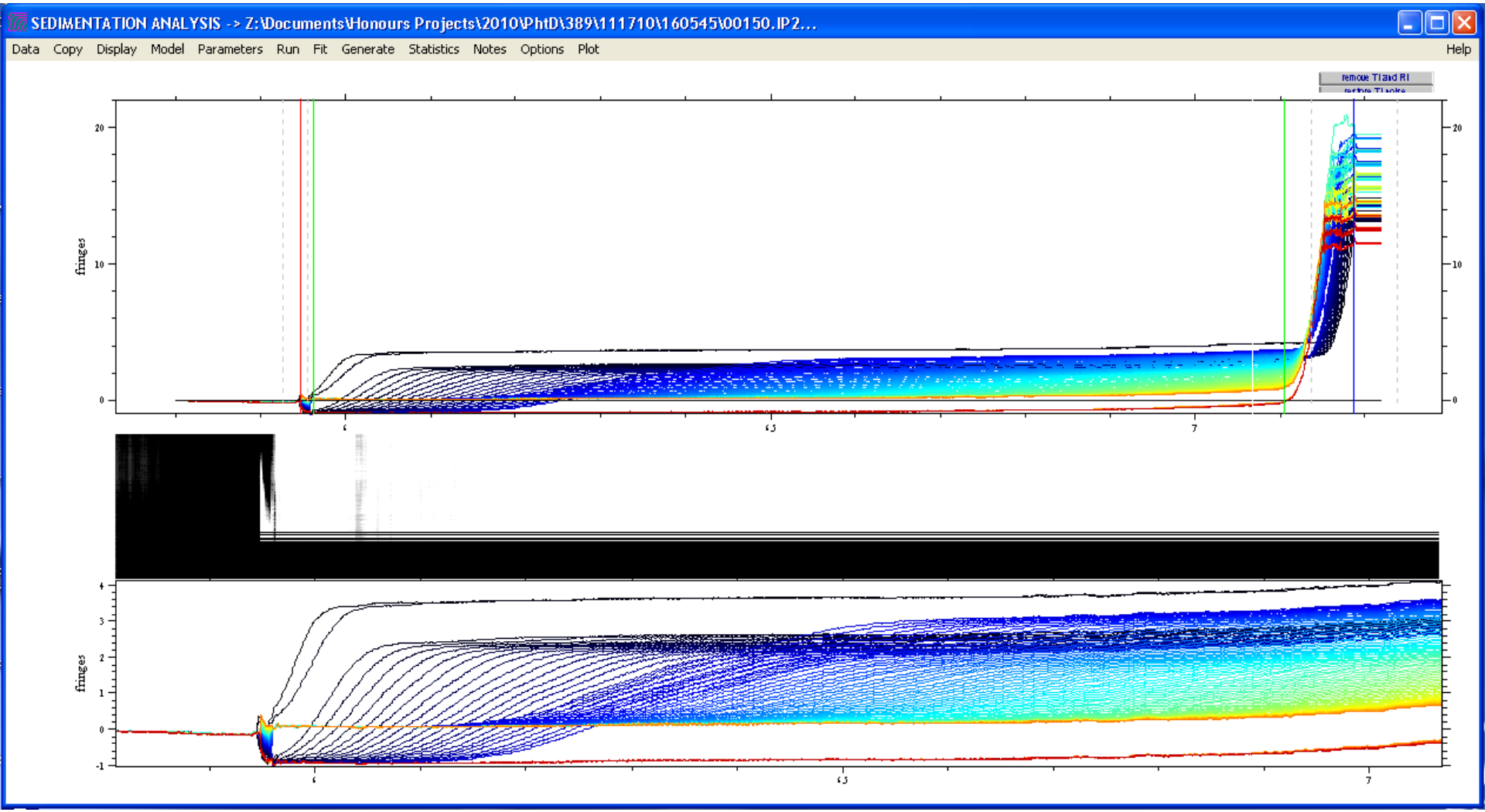
http://www.analyticalultracentrifugation.com/default.htm

2: Specify parameters



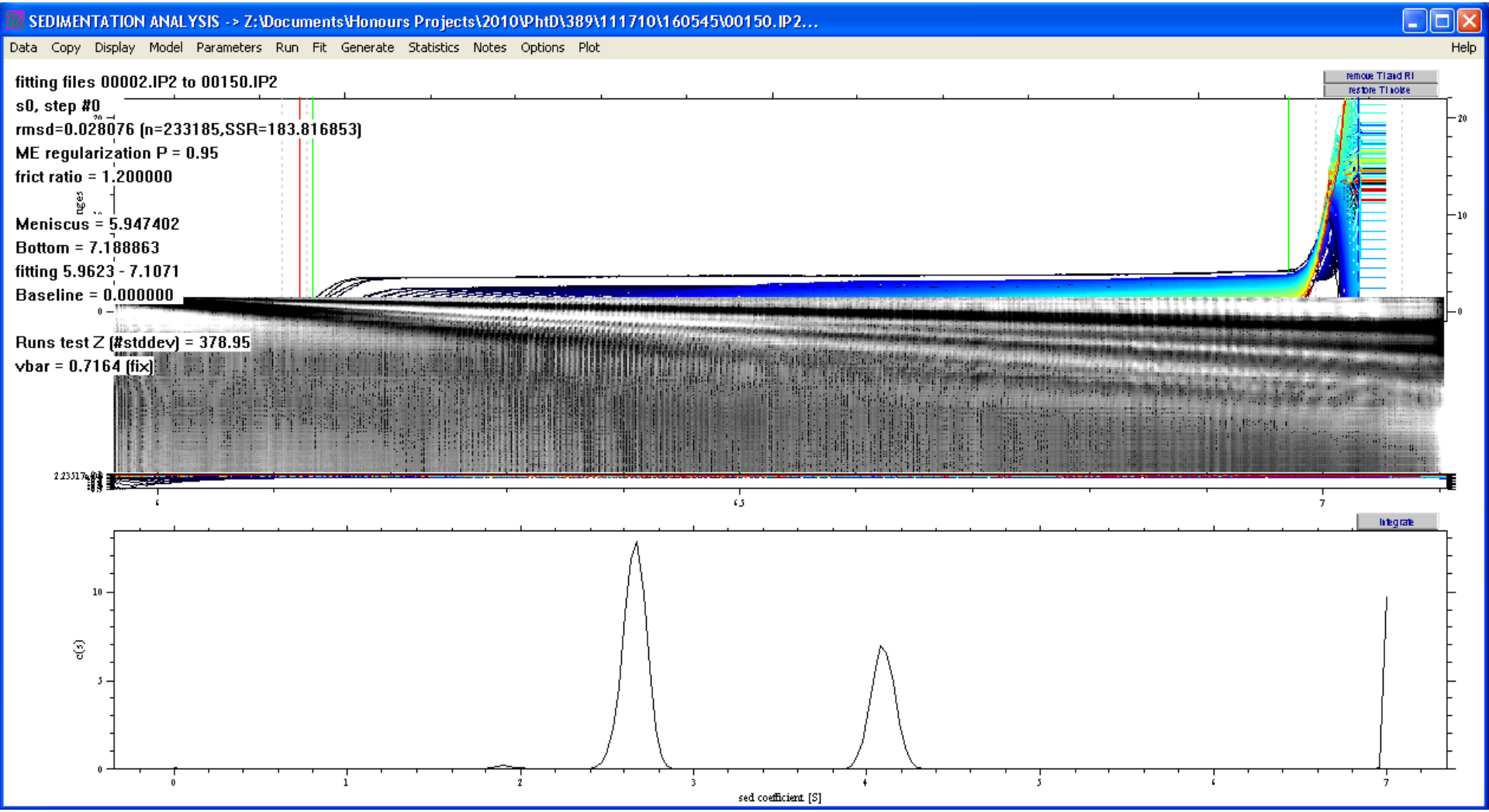
http://www.analyticalultracentrifugation.com/default.htm

3: Set meniscus, cell base and analysis limits



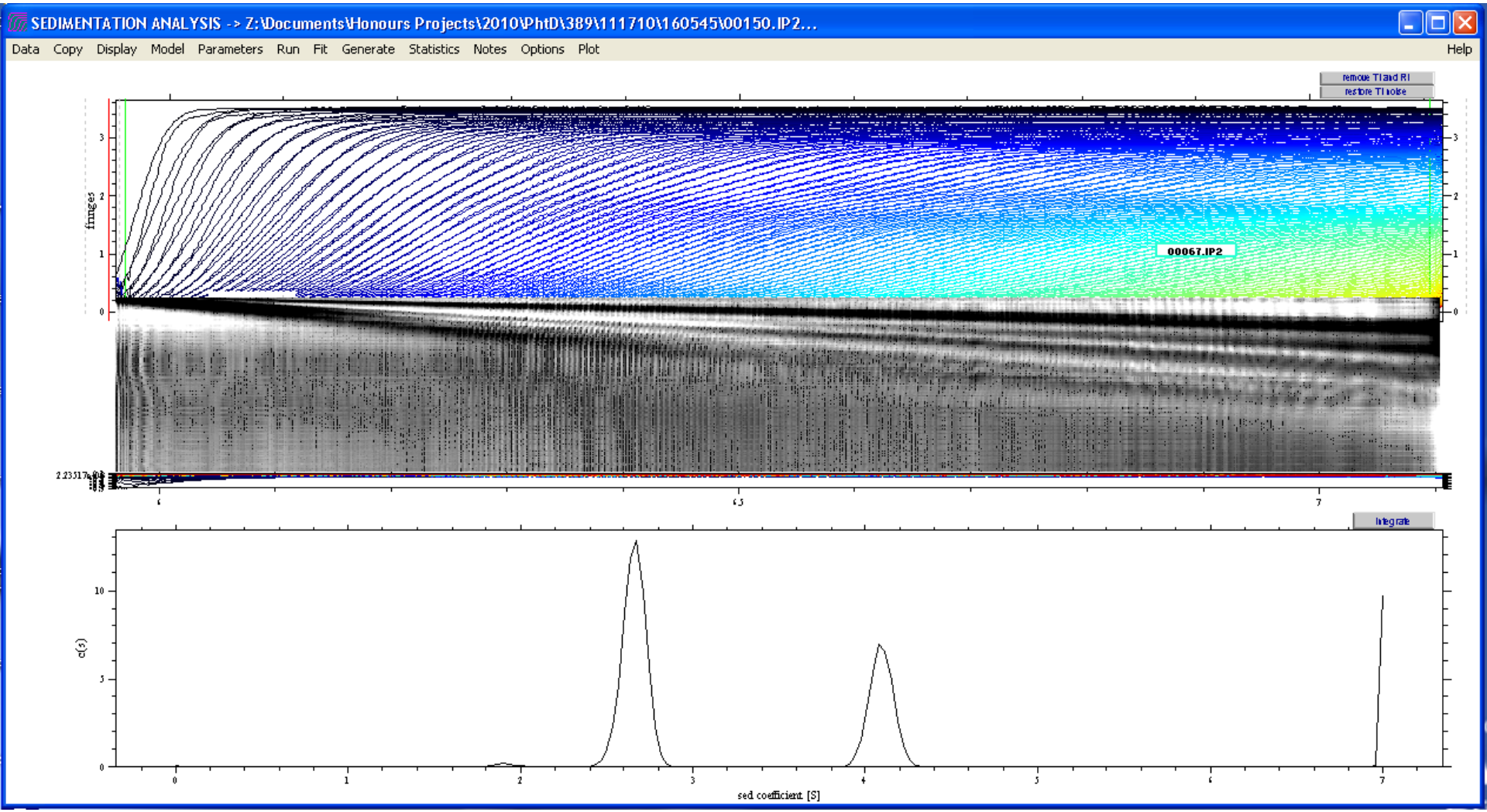
http://www.analyticalultracentrifugation.com/default.htm

4: Run



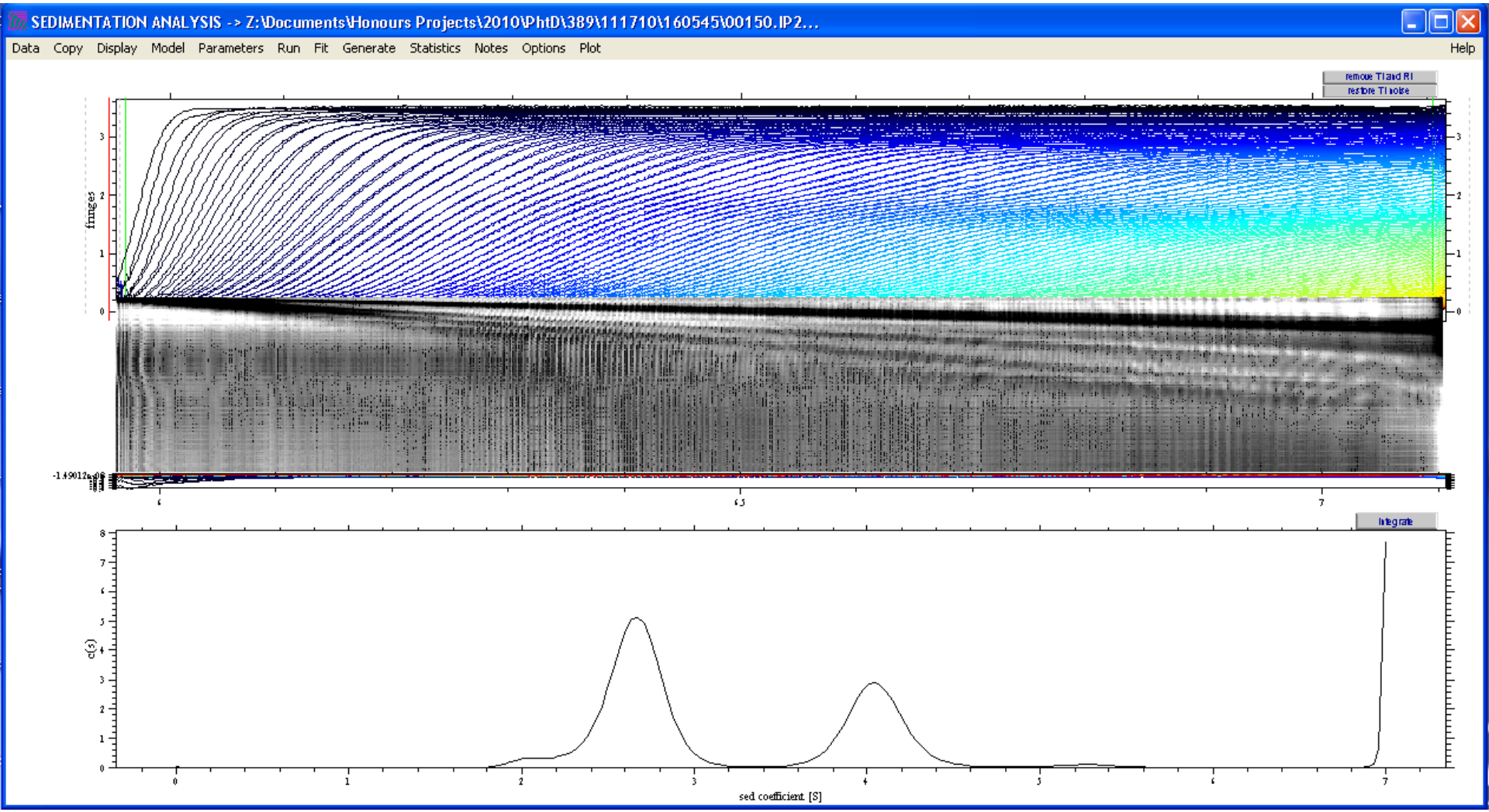
http://www.analyticalultracentrifugation.com/default.htm

5: Subtract time and radial invariant noise



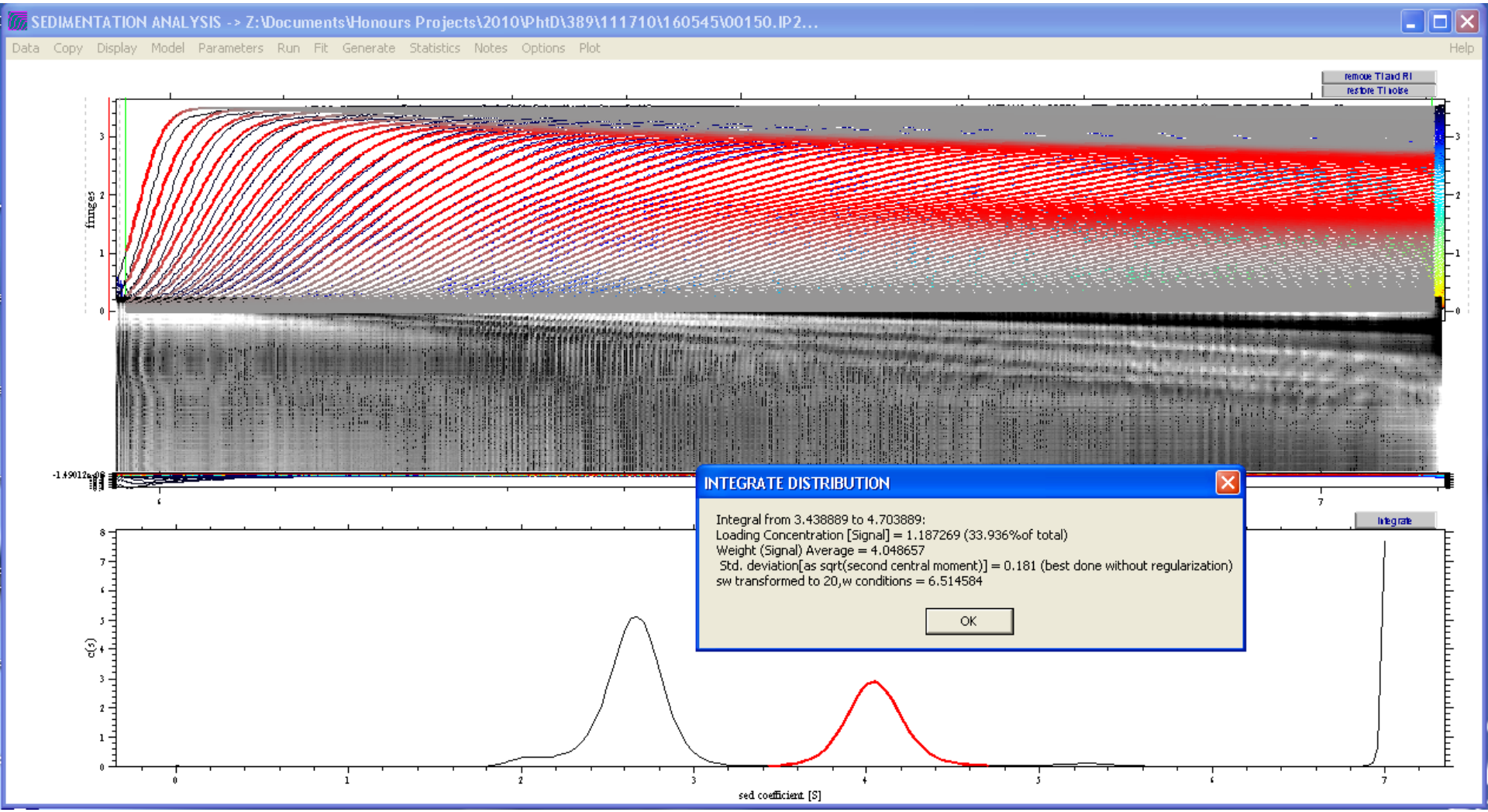
http://www.analyticalultracentrifugation.com/default.htm

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



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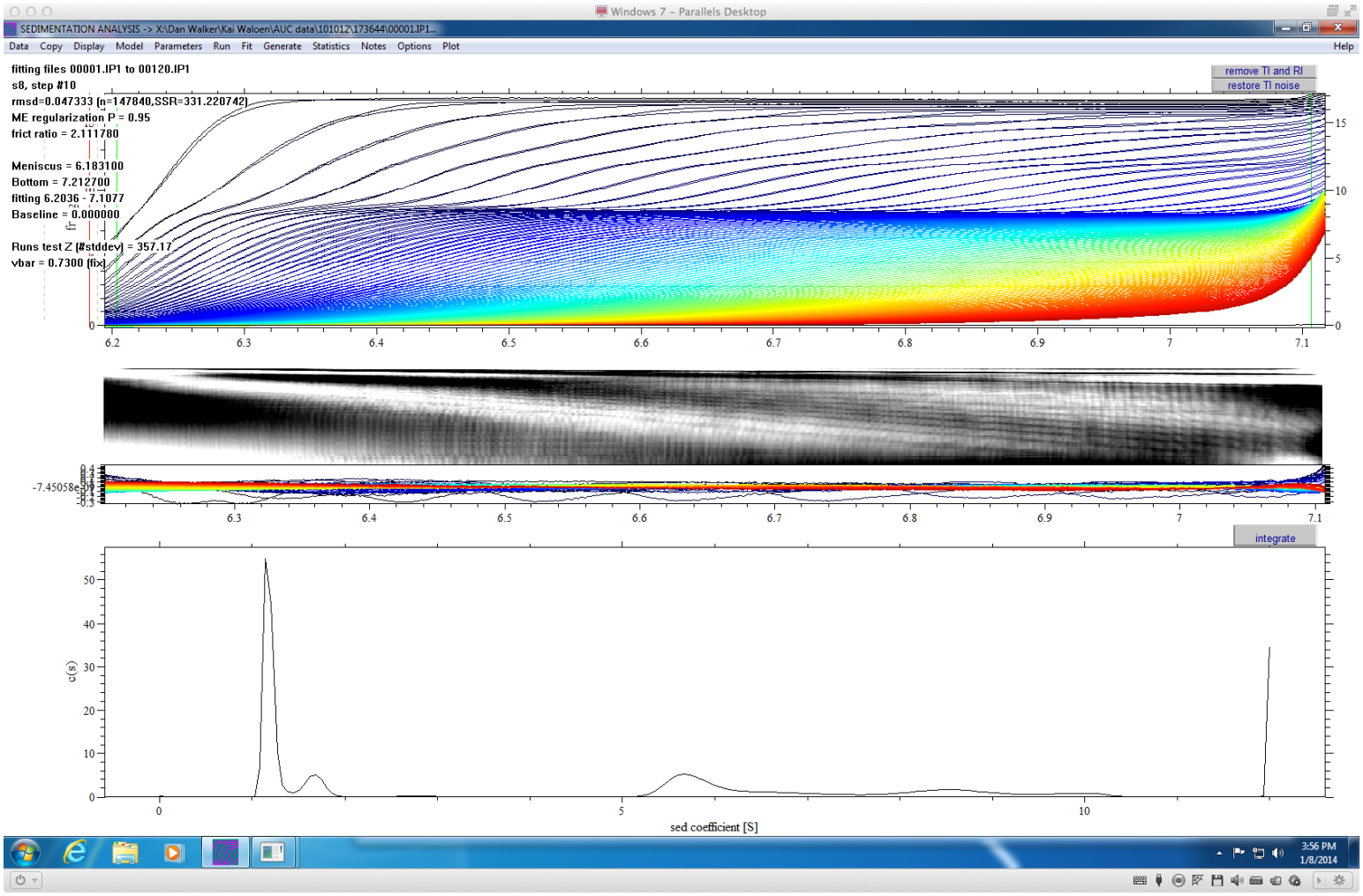
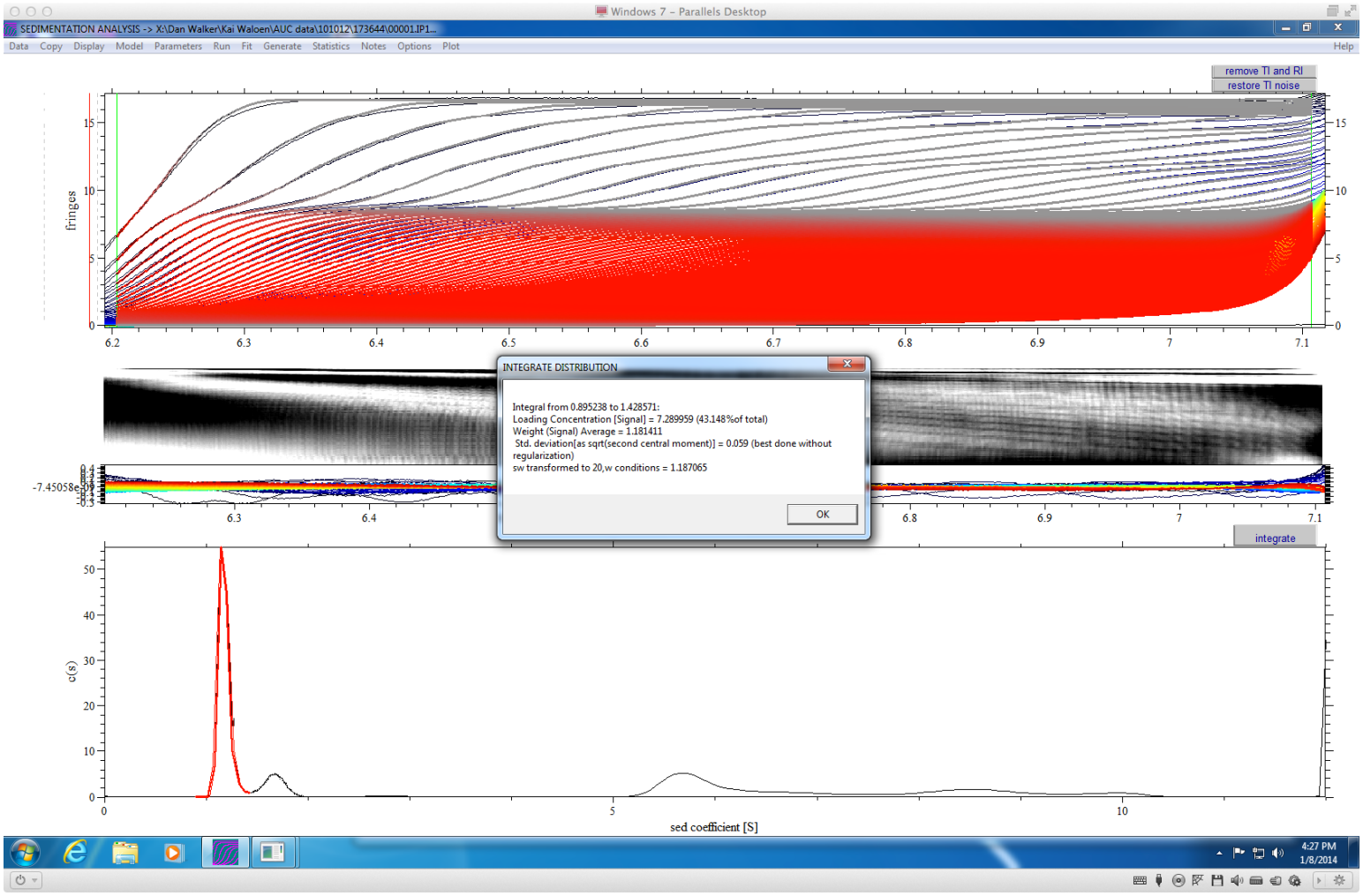
Sum of Lamm equations $0 \leq s \leq 12$ S discretised by 200



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



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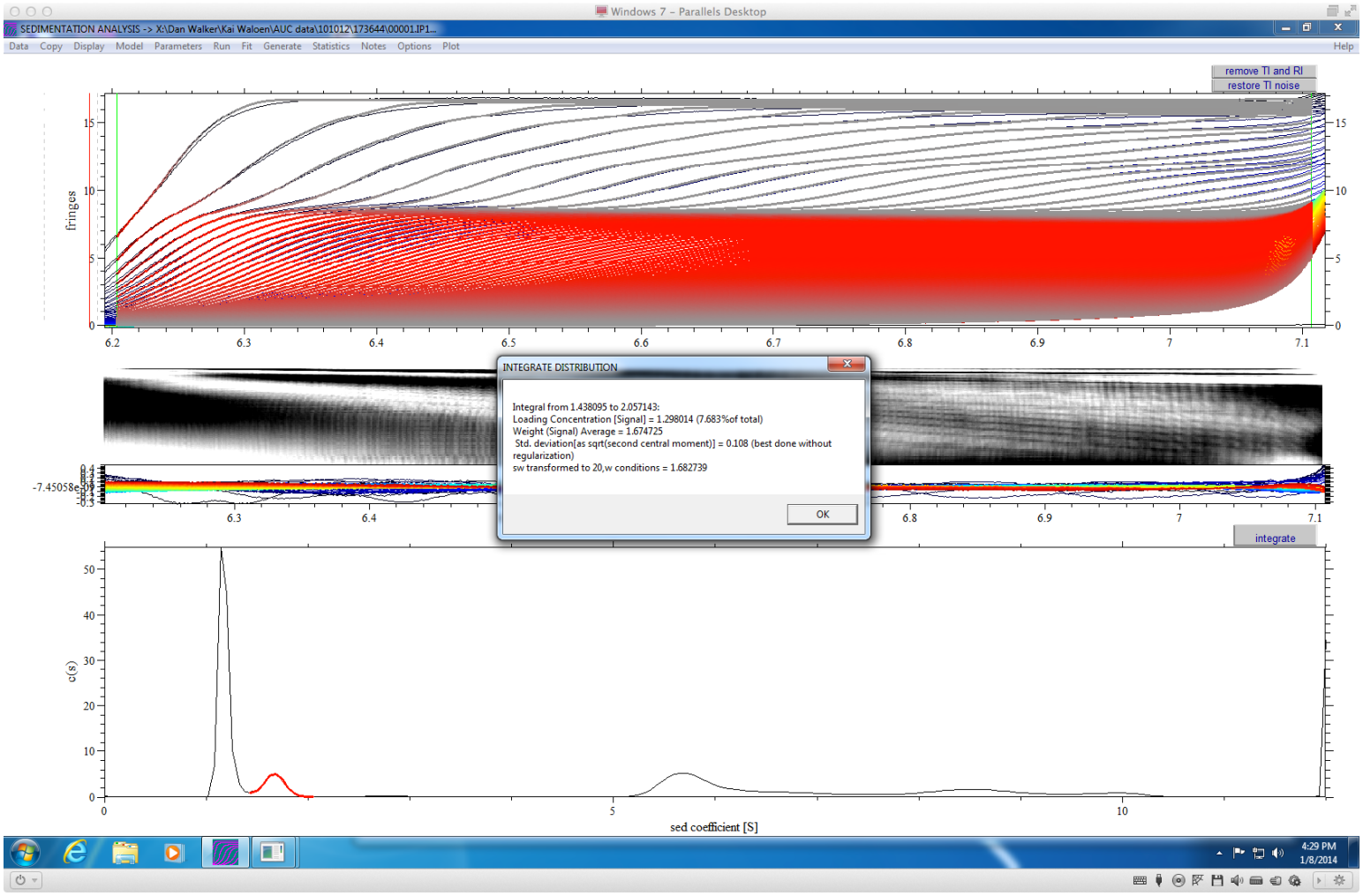

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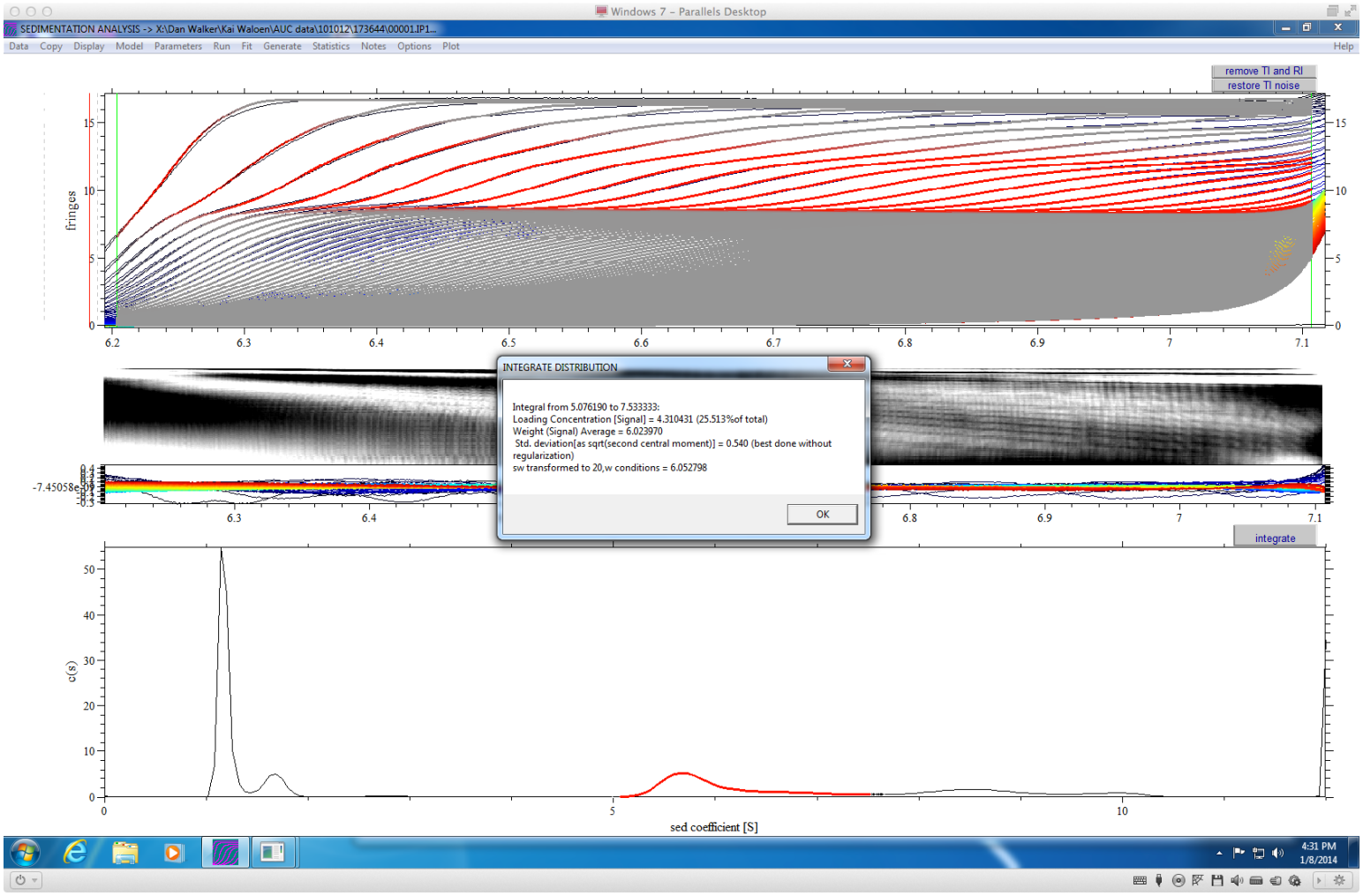

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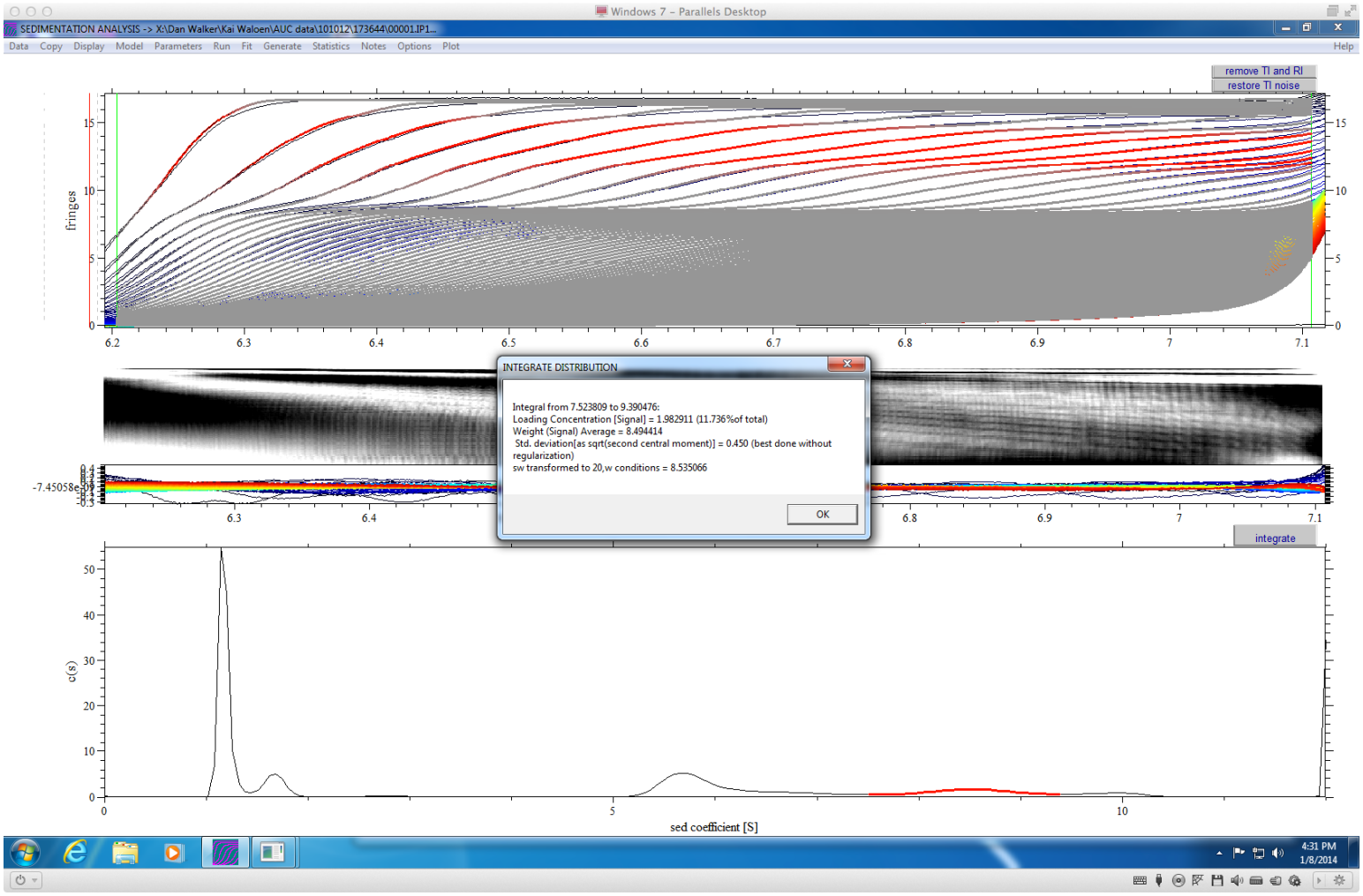

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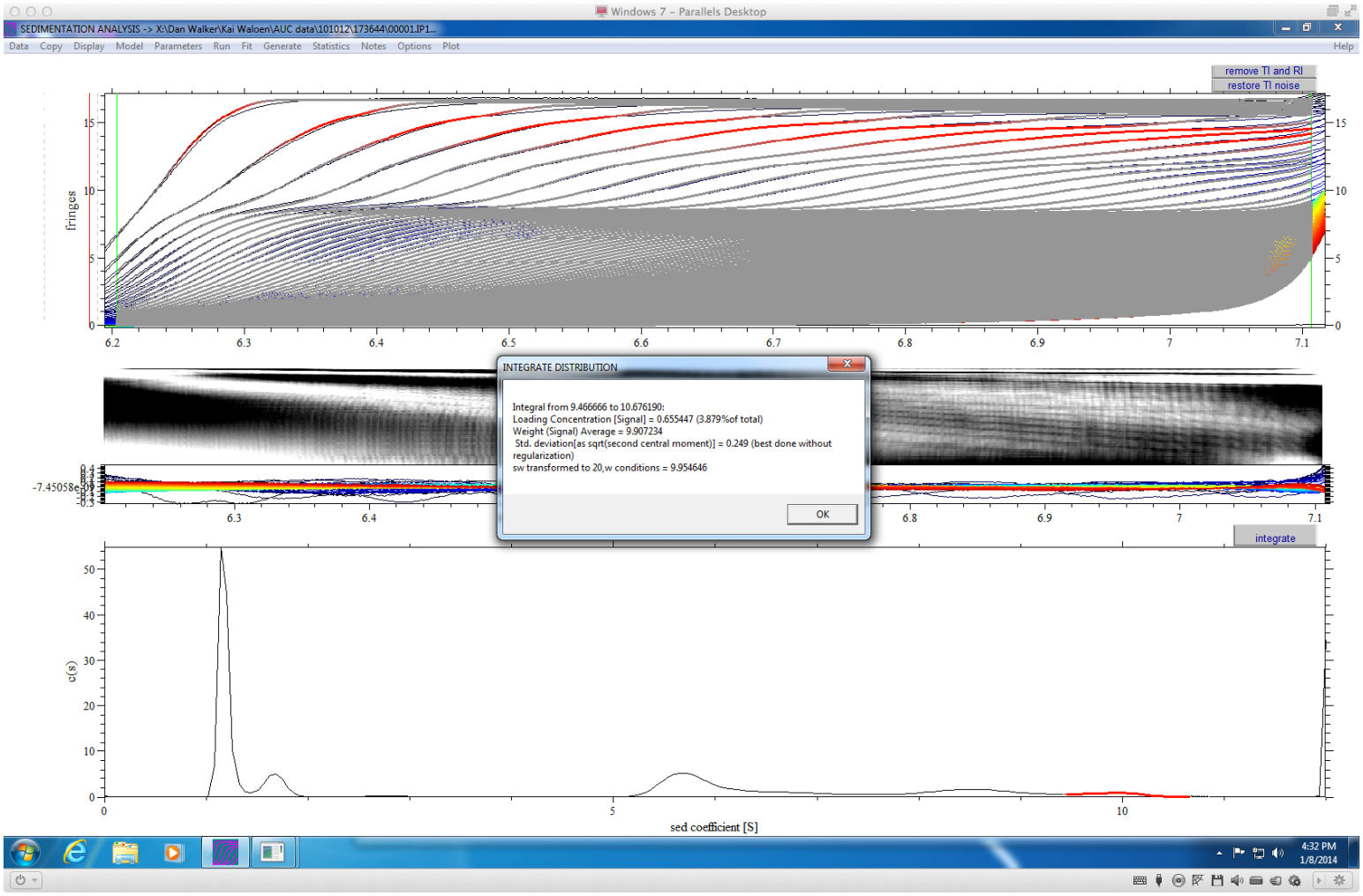

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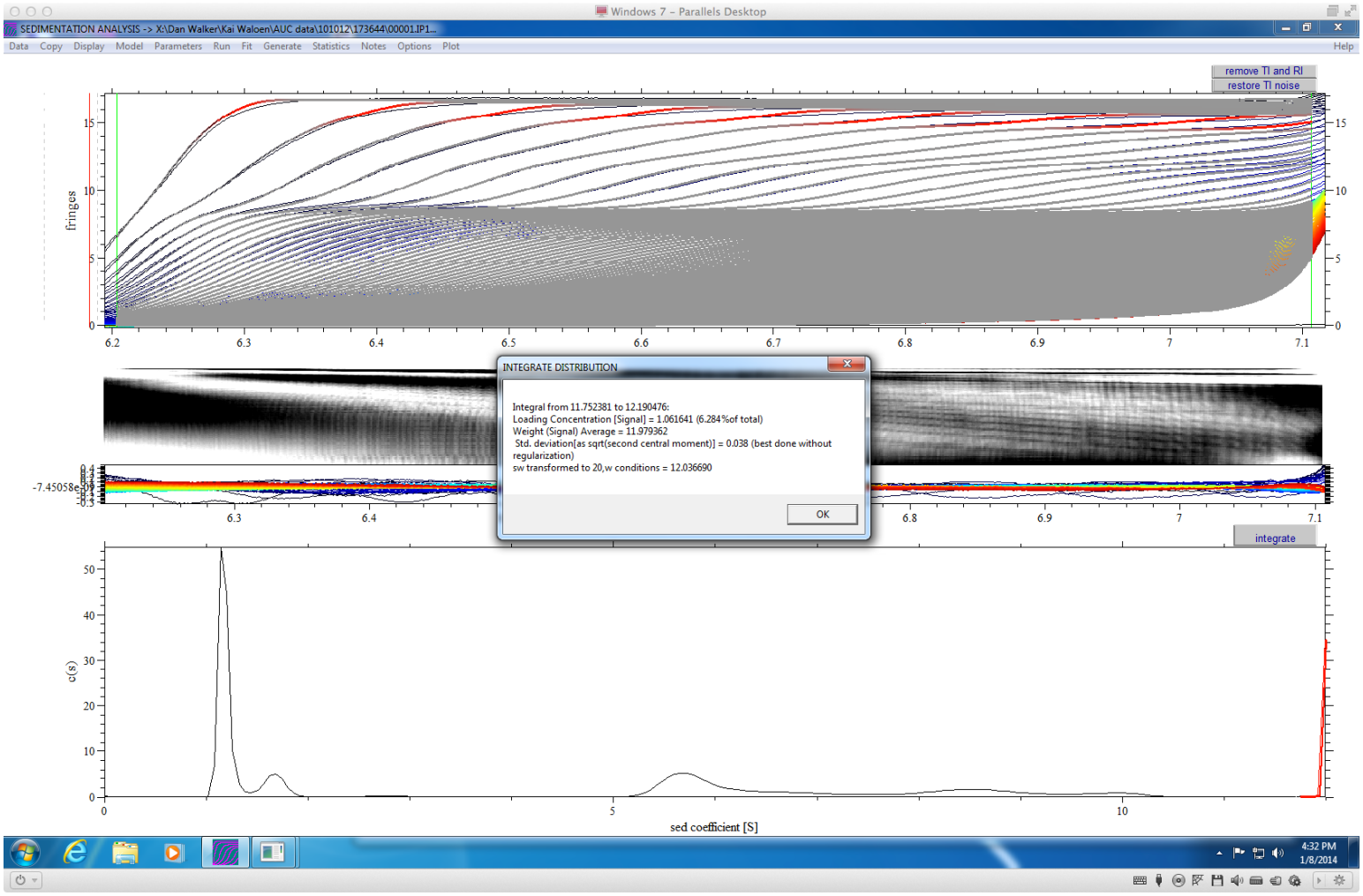
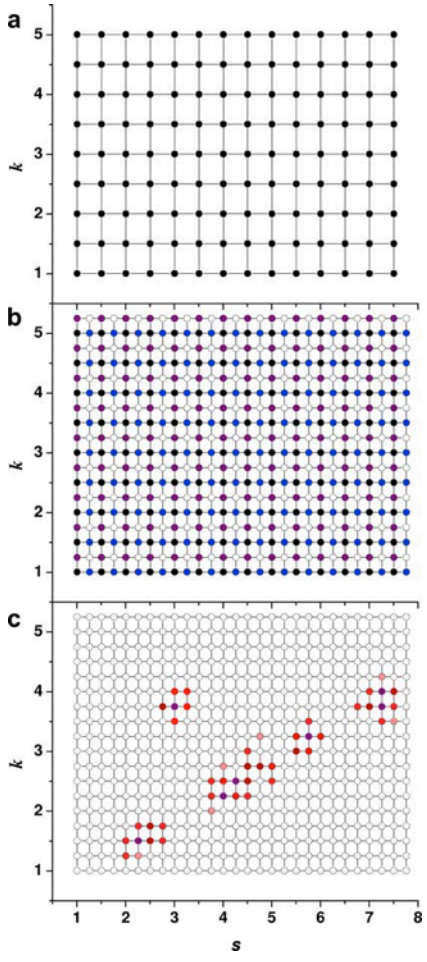
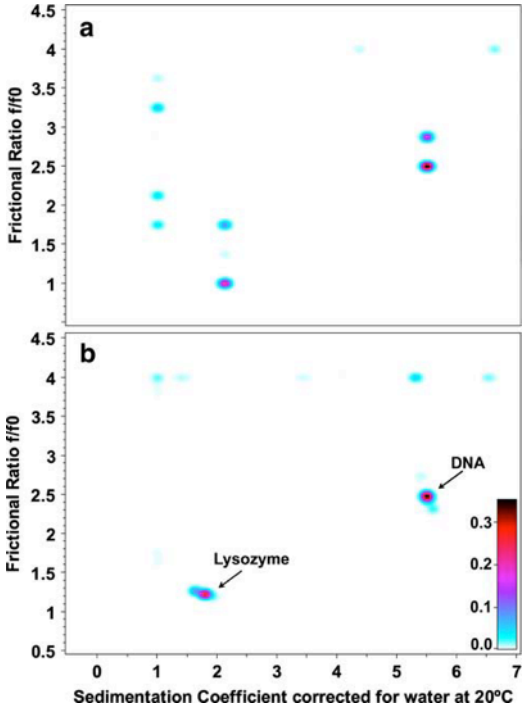


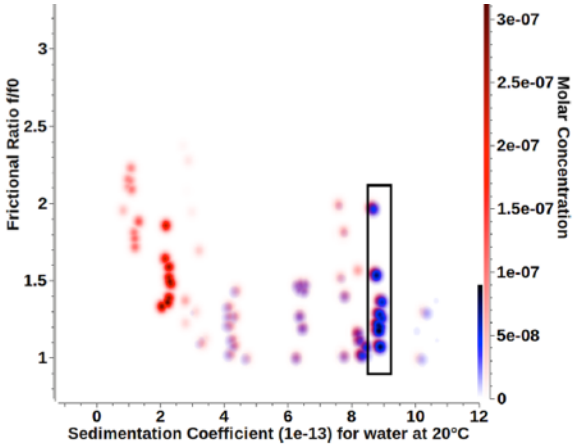
Two-dimensional spectrum analysis (2DSA) of SV data with UltraScan: model independent fitting giving s & M

- Important when f/f_0 varies for components

NNLS







Brookes et al., Eur Biophys J (2010) 39:405–414

Zhang et al., Anal. Chem. (2017) doi: 10.1021/acs.analchem.6b03926

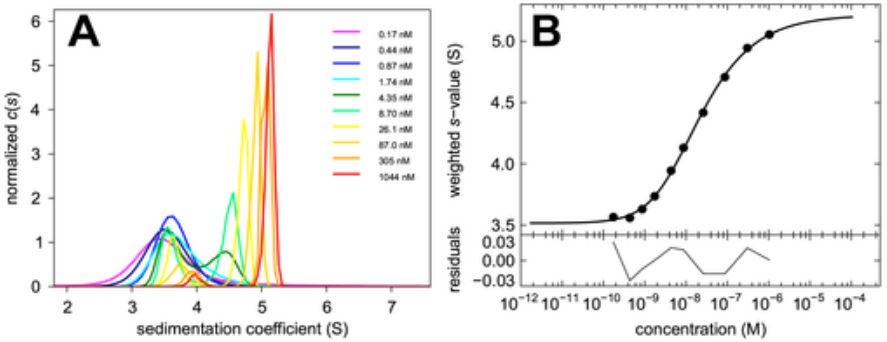
Interacting systems & monodispersity

- 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

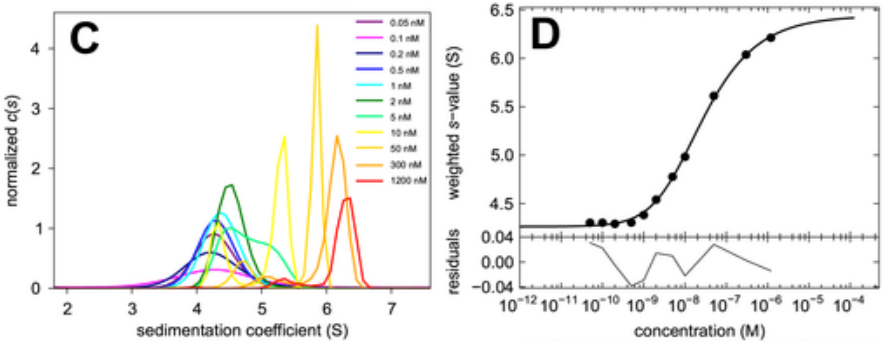
s distributions c(s) | s_w isotherms

Dylight488-GluA2 ATD



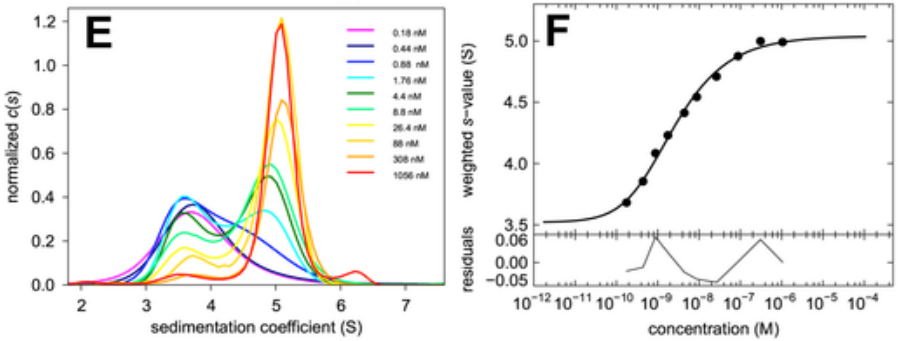
K_D = 20.5 nM
s_1 = 3.52 S, s_2 = 5.21 S

EGFP-GluA2 ATD



K_D = 25.4 nM
s_1 = 4.26 S, s_2 = 6.44 S

FAM-GluA2 ATD

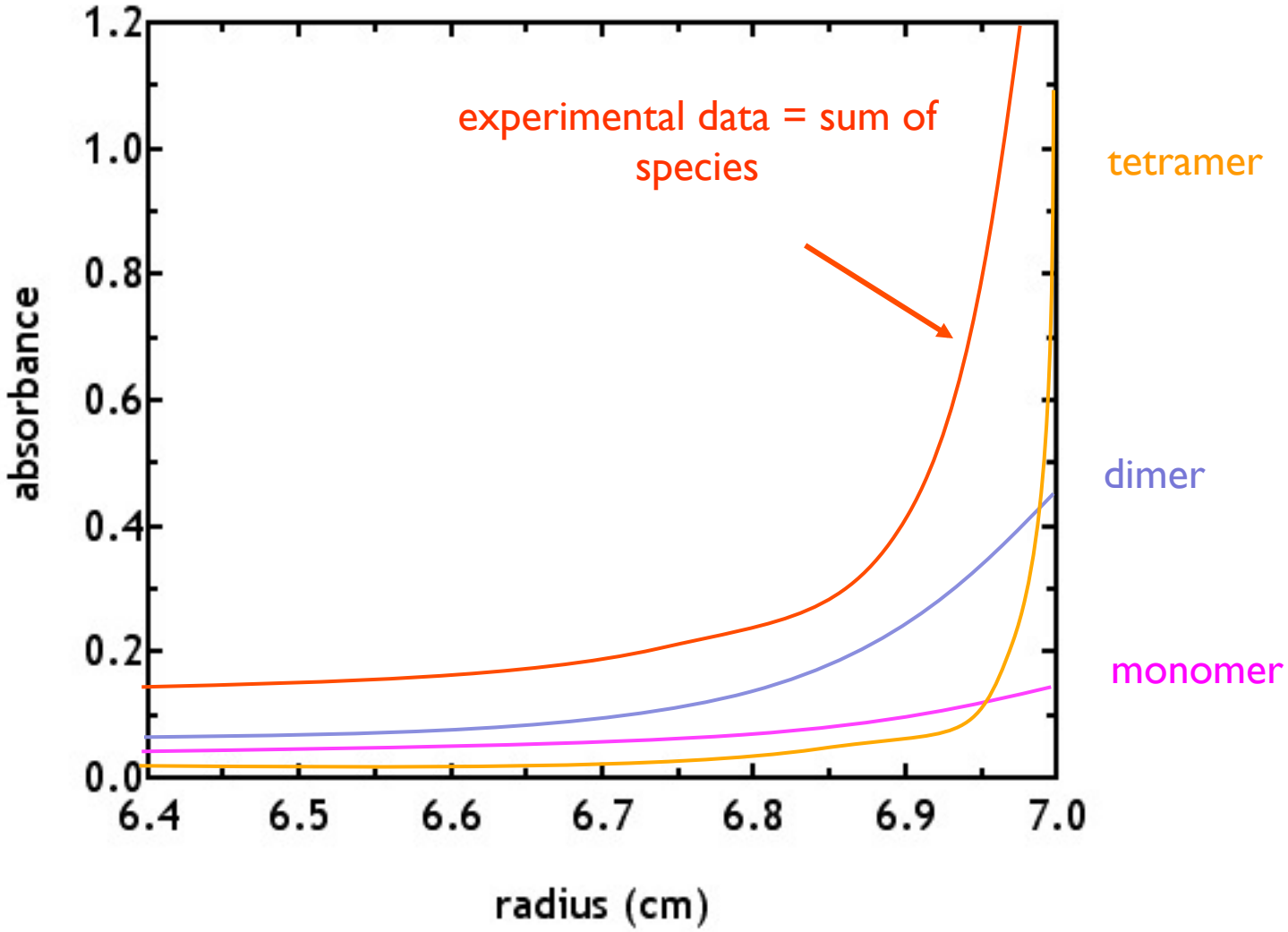


K_D = 2.3 nM
s_1 = 3.52 S, s_2 = 5.04 S

Zhao H, Lomash S, Glasser C, Mayer ML, Schuck P (2013) PLoS ONE 8(12): e83439. doi:10.1371/journal.pone.0083439


Take a break.
You deserve it!

Self-association: "deconvolution" of SE data into individual components



Self-association: SE data are the sum of exponentials

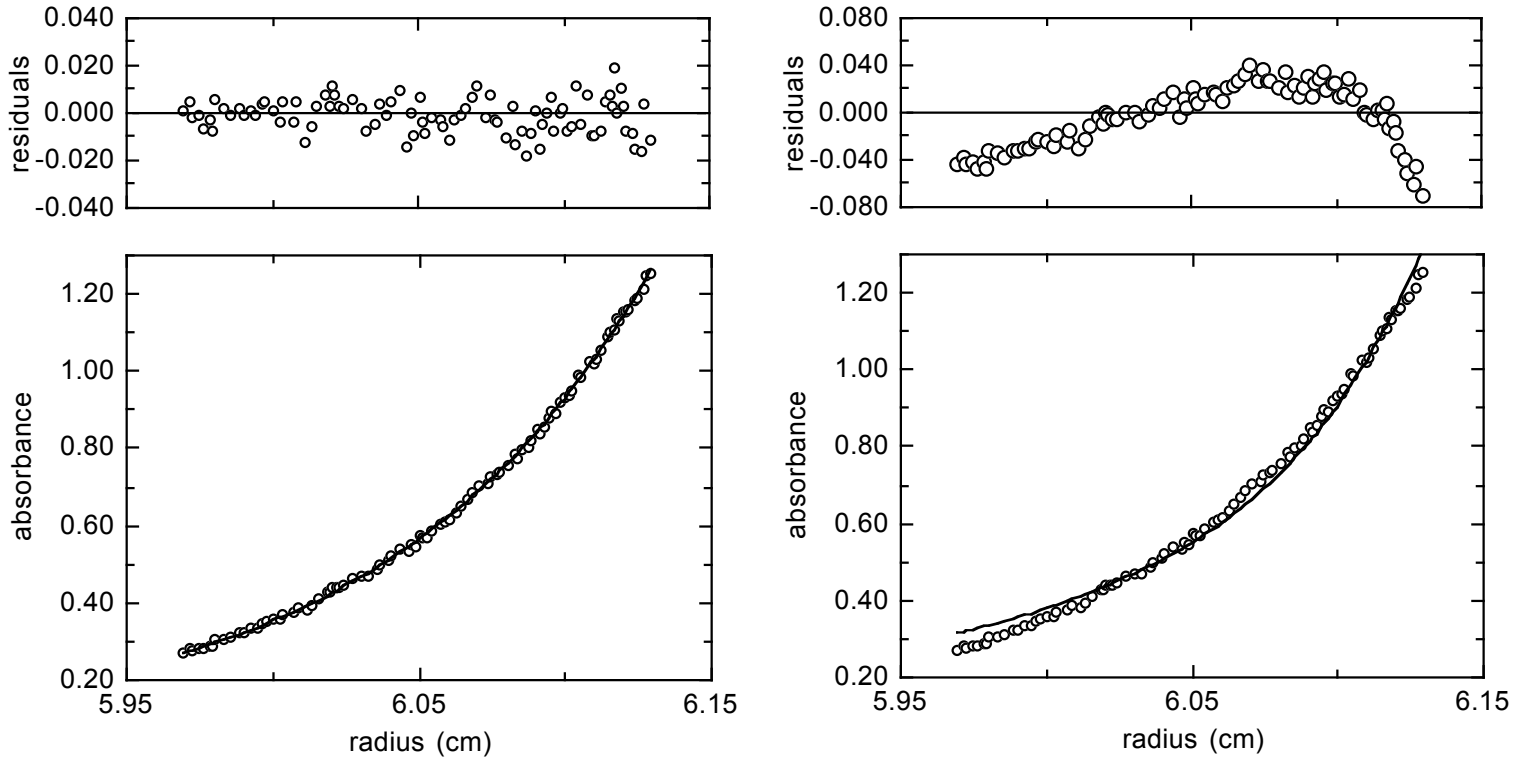
$$A_r = \exp[\ln A_0 + H.M(r^2 - r_0^2)] \leftarrow \text{monomer}$$

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

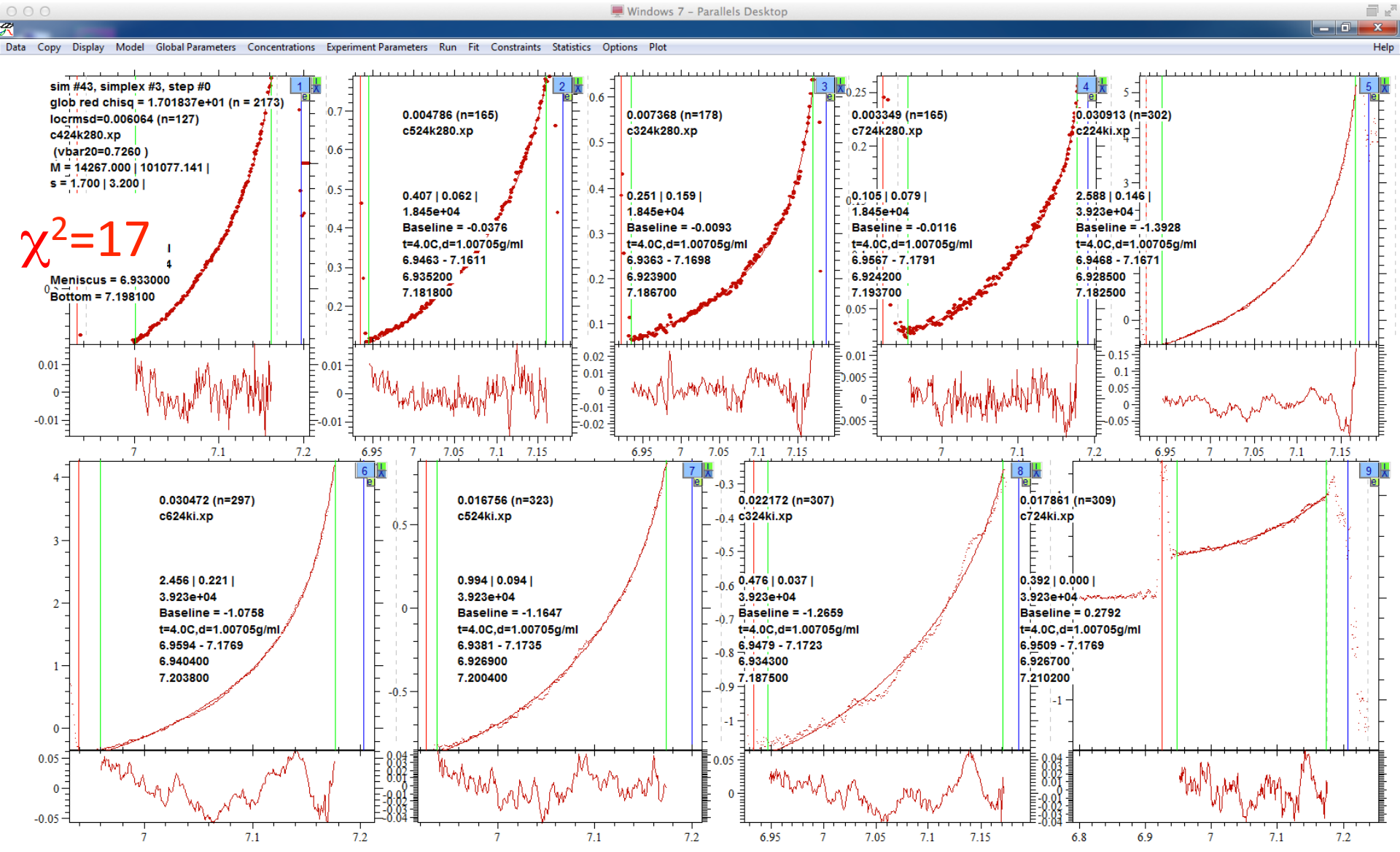
$$+\exp[n_3 \ln A_0 + \ln Ka_3 + n_3.H.M(r^2 - r_0^2)] \leftarrow I\text{-}n_3$$

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

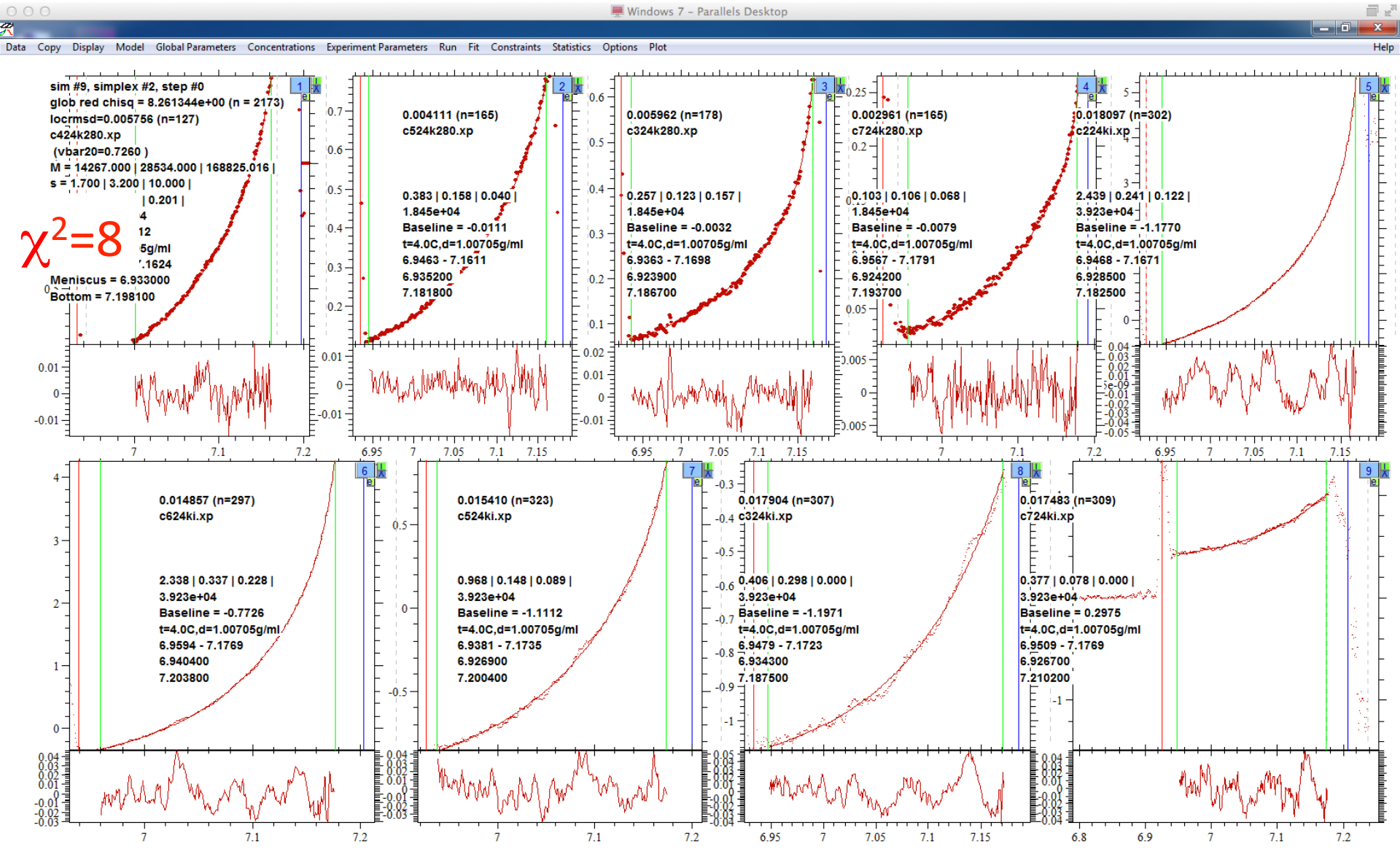
Self-association: best model revealed by residuals



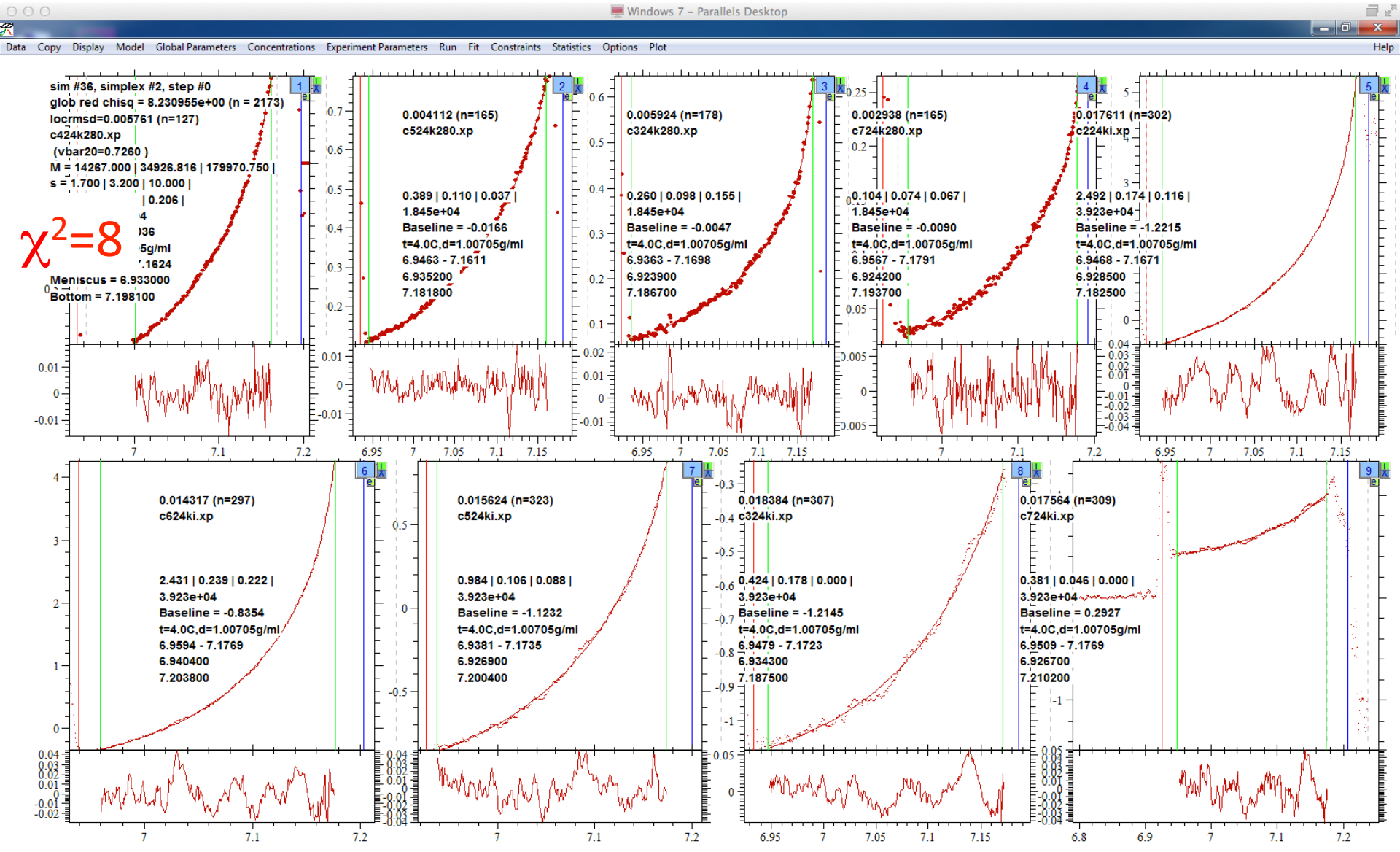
SEDPHAT: species analysis: monomer + heavy



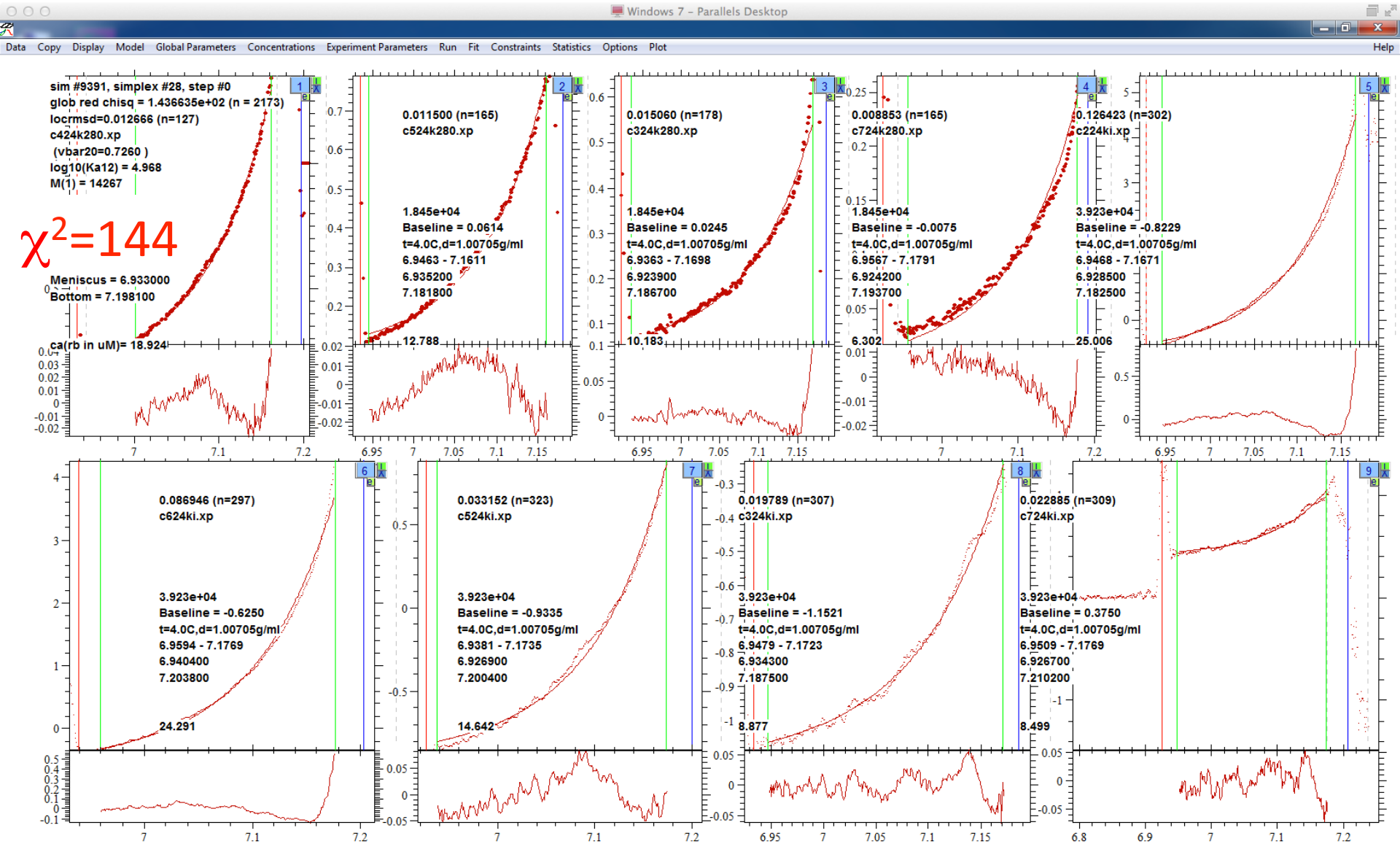
SEDPHAT: species analysis: monomer + "dimer" (both fixed) + heavy



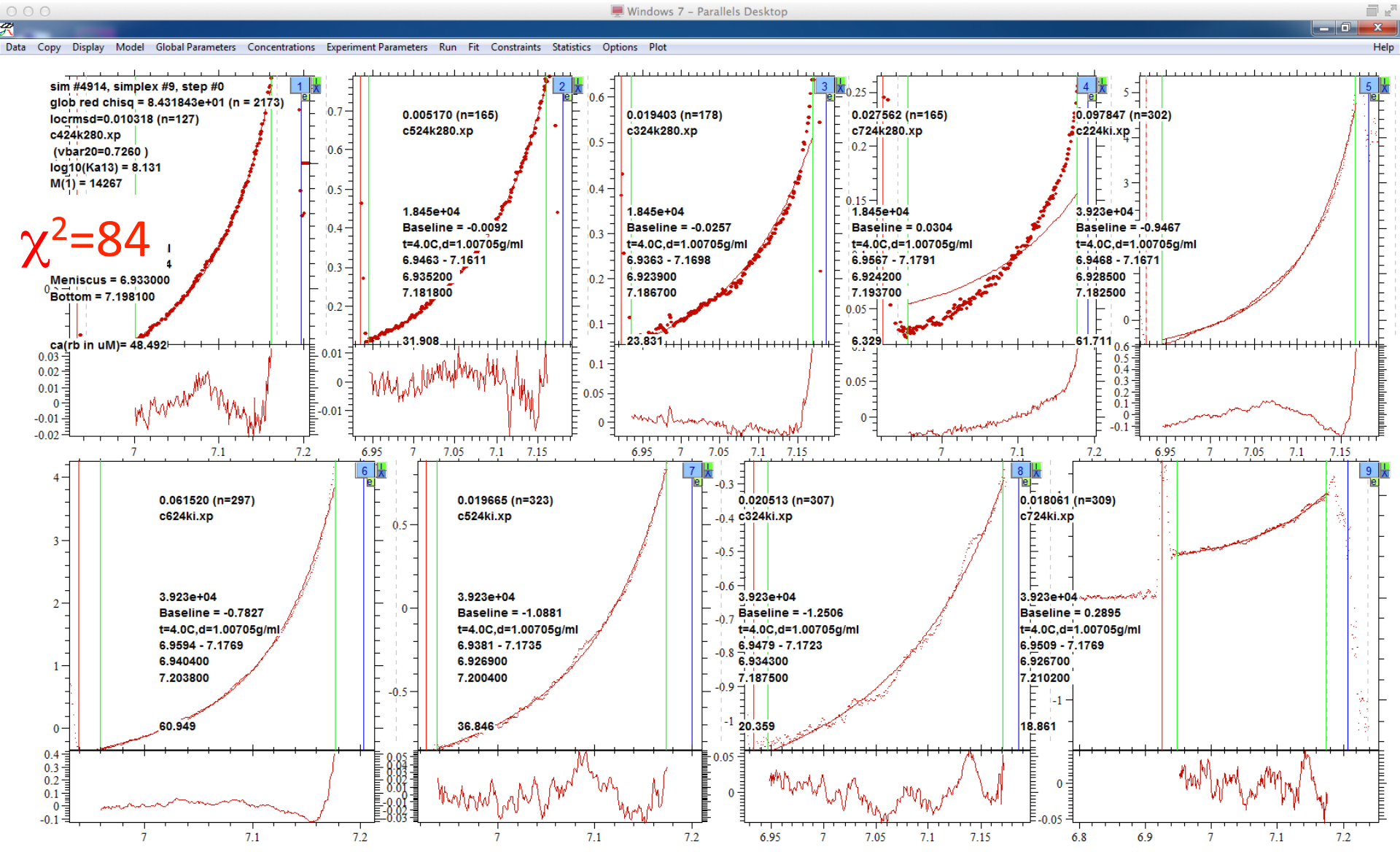
SEDPHAT: species analysis: monomer (fixed) + "dimer" + heavy (both free)



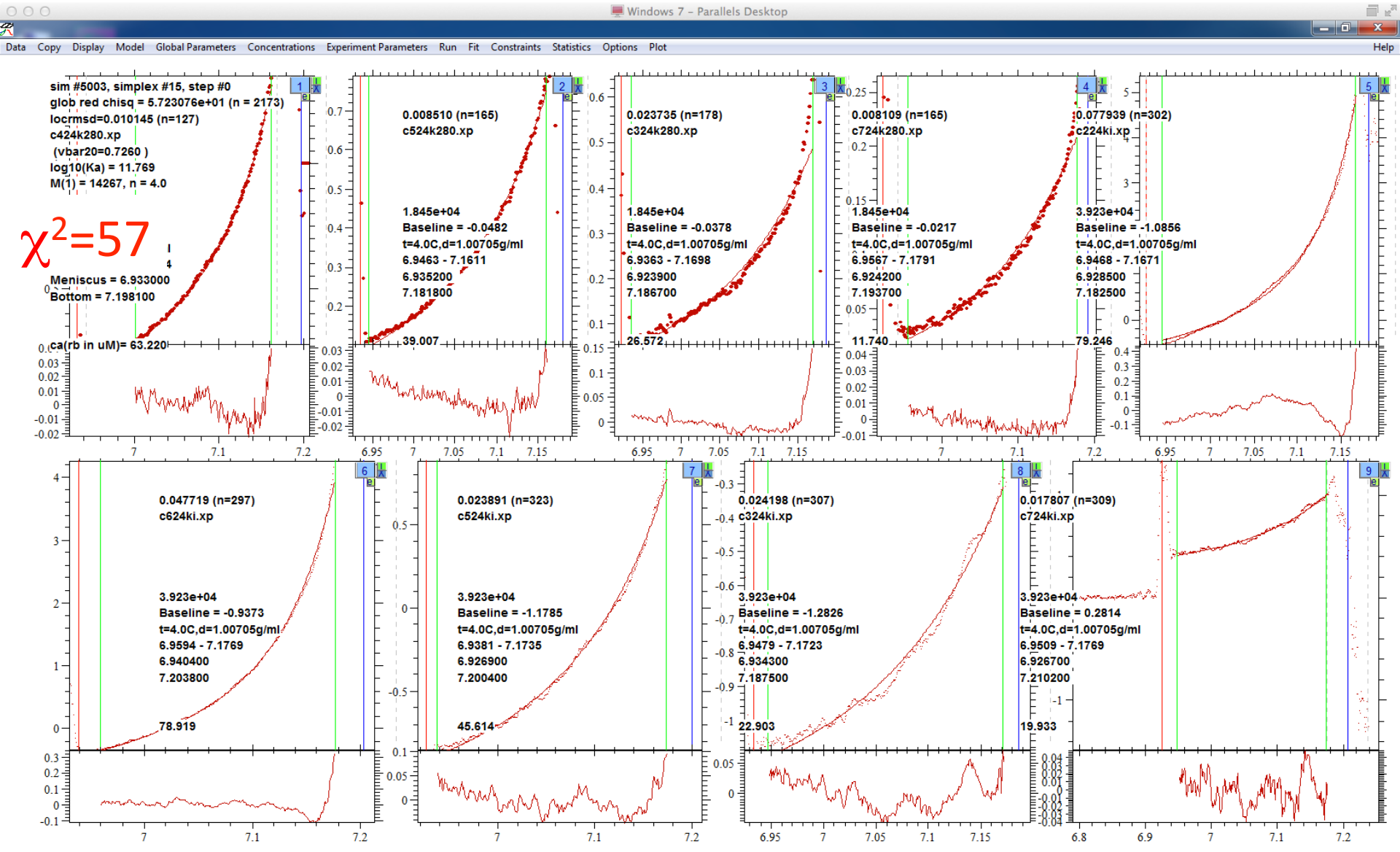
SEDPHAT: Abs + interference, 5.4 – 54 µM, Kd = 10.8 µM



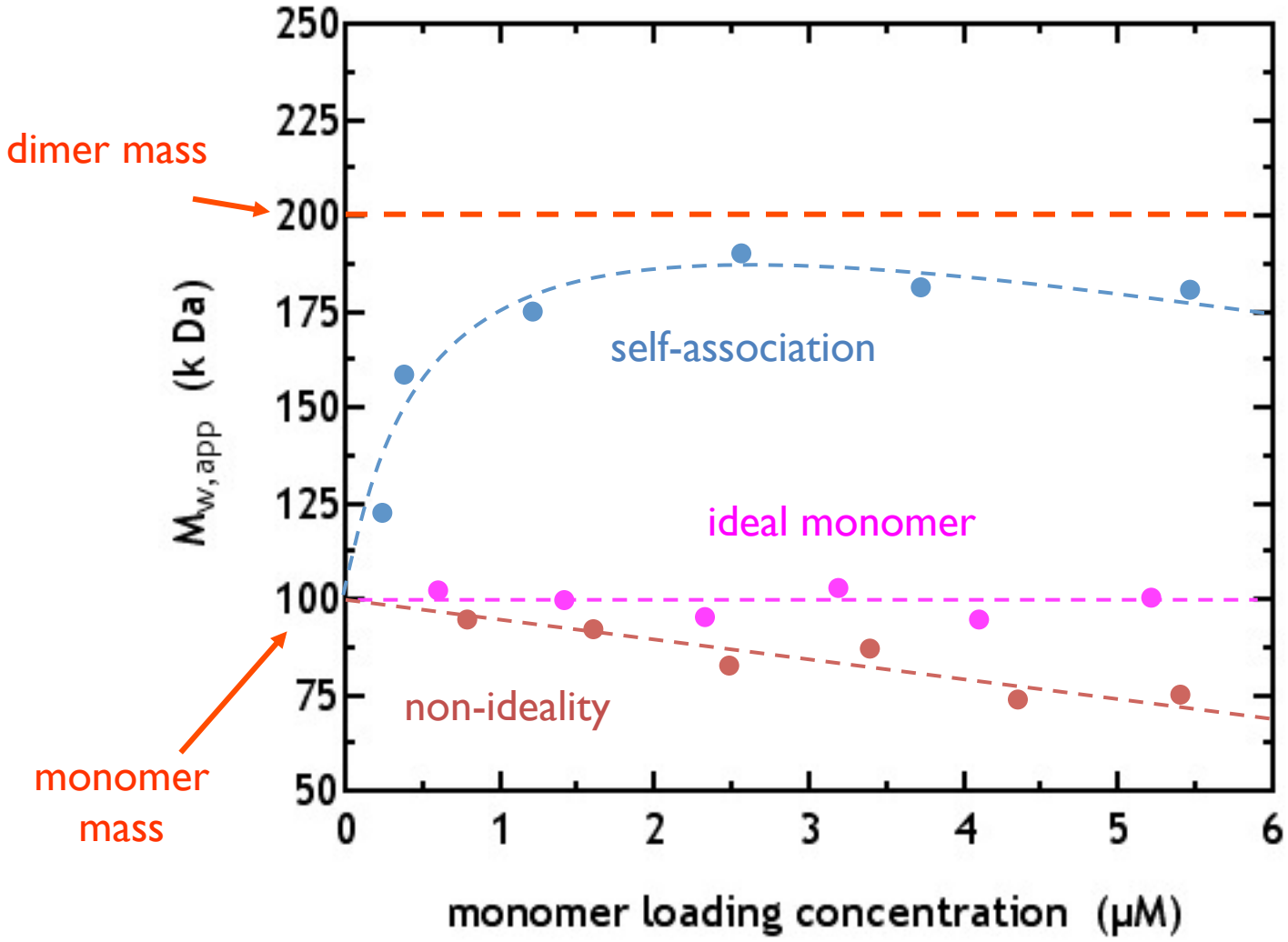
SEDPHAT: Abs + interference, 5.4 – 54 µM, Kd = 7.4 x10^3 µM^2



SEDPHAT: Abs + interference, 5.4 – 54 µM, Kd = 1.7 x10^6 µM^3

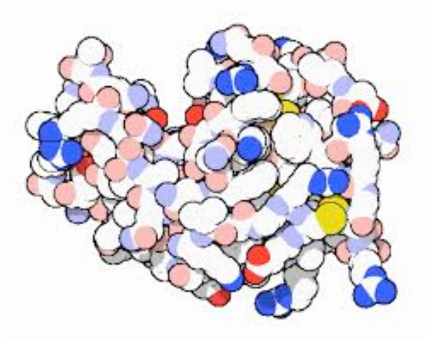


SE: more than just mass

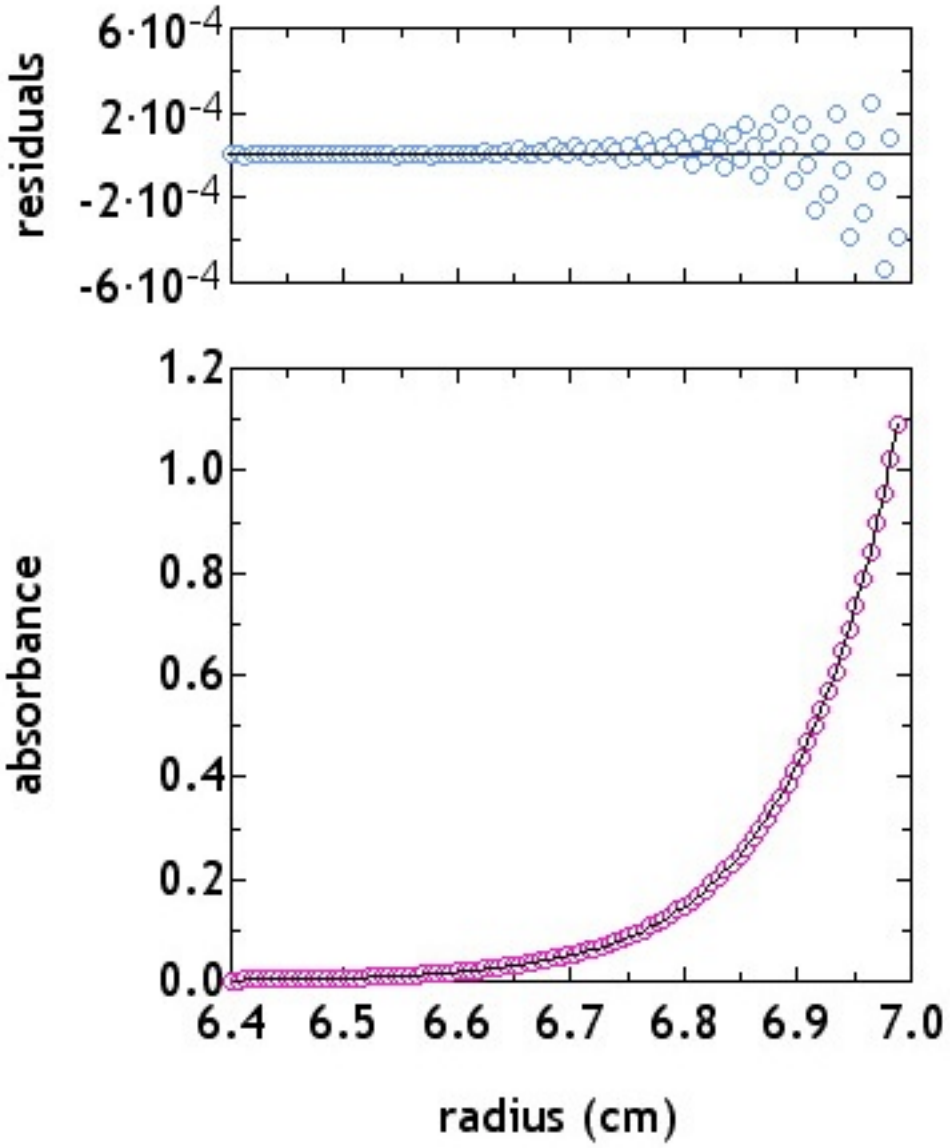


Ideal monomer

- 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

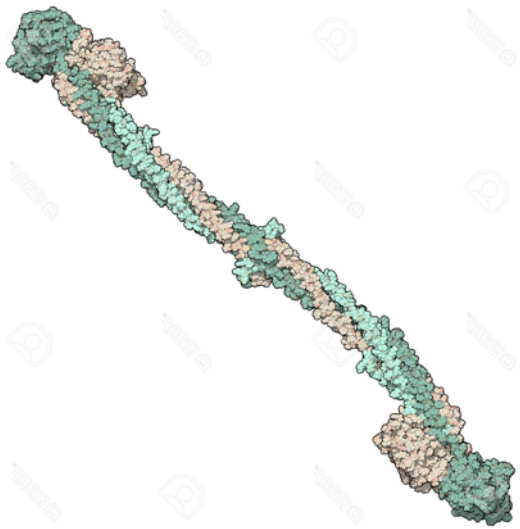


Non-ideal monomer

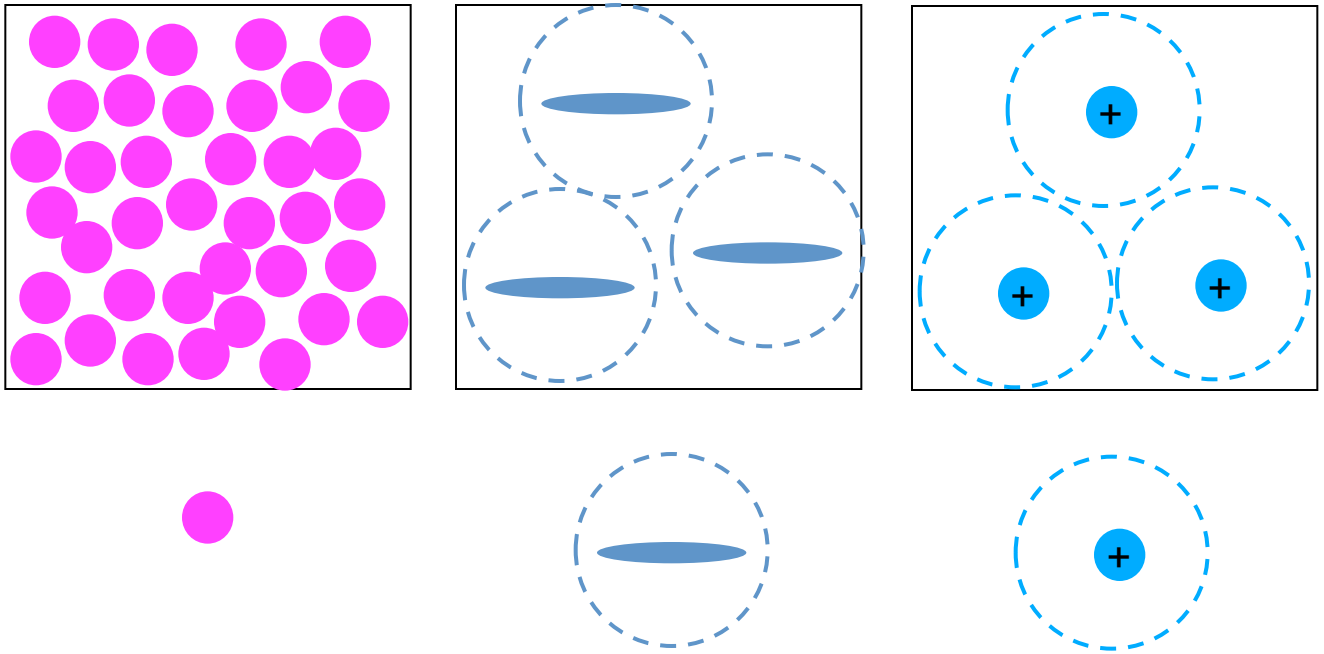
- Asymmetric molecule

  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 ???



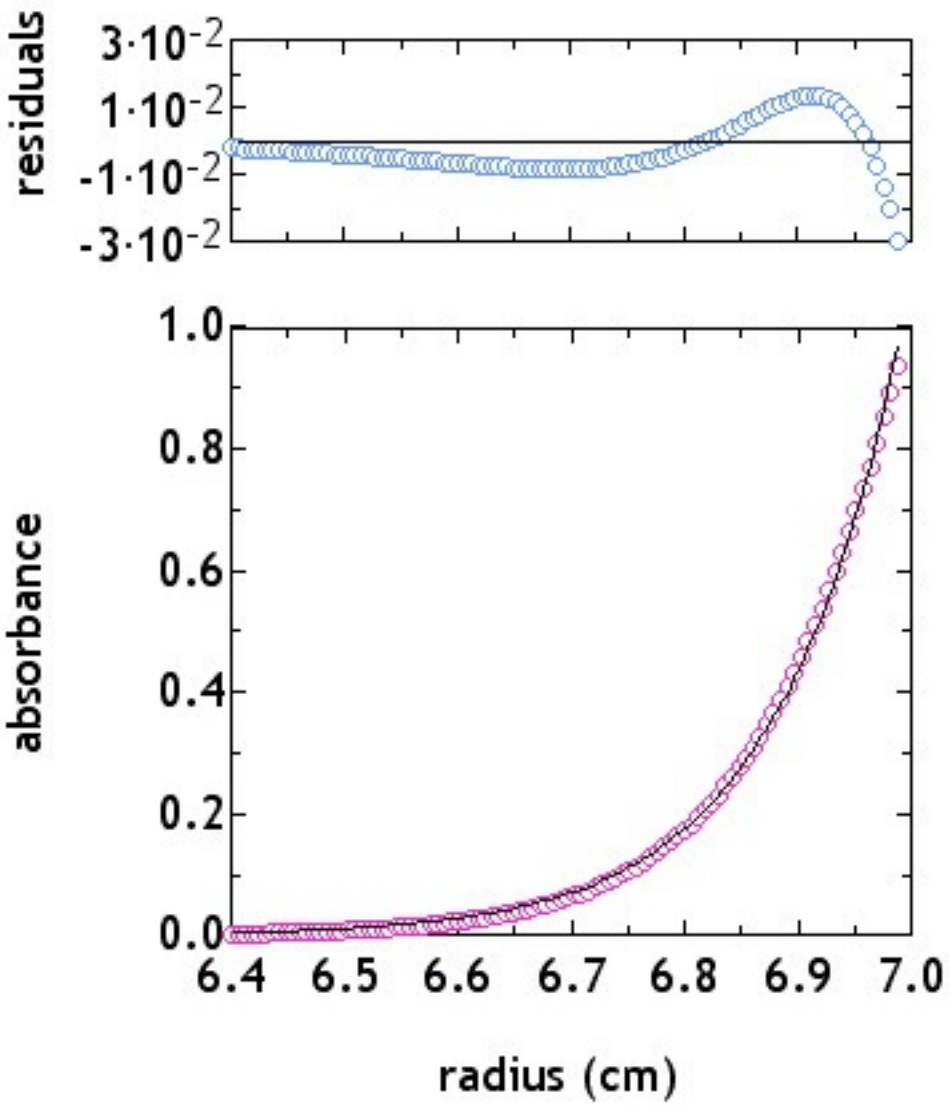
ideal

non-ideal

elongated

charged

Non-ideal monomer data



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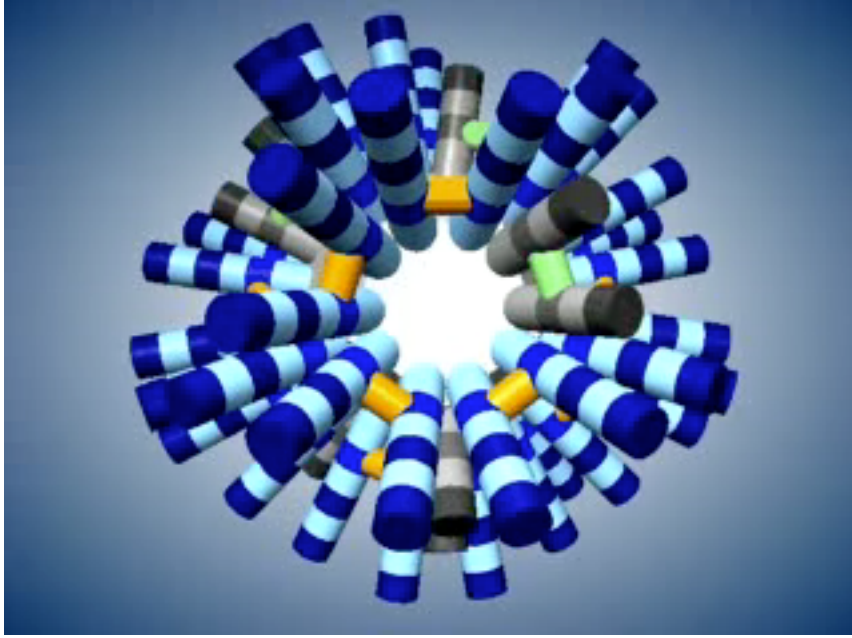
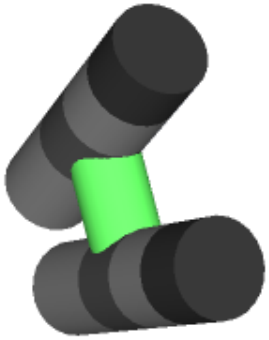
Hetero-association example: PDC E3BP:E3 sub-complex



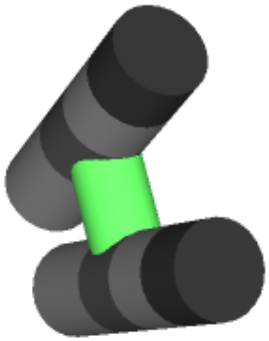
- E3 forms a homo-dimer
- E3BP binds to E3
- Native PAGE & ITC shows 2:1 complex
- Needed confirmation

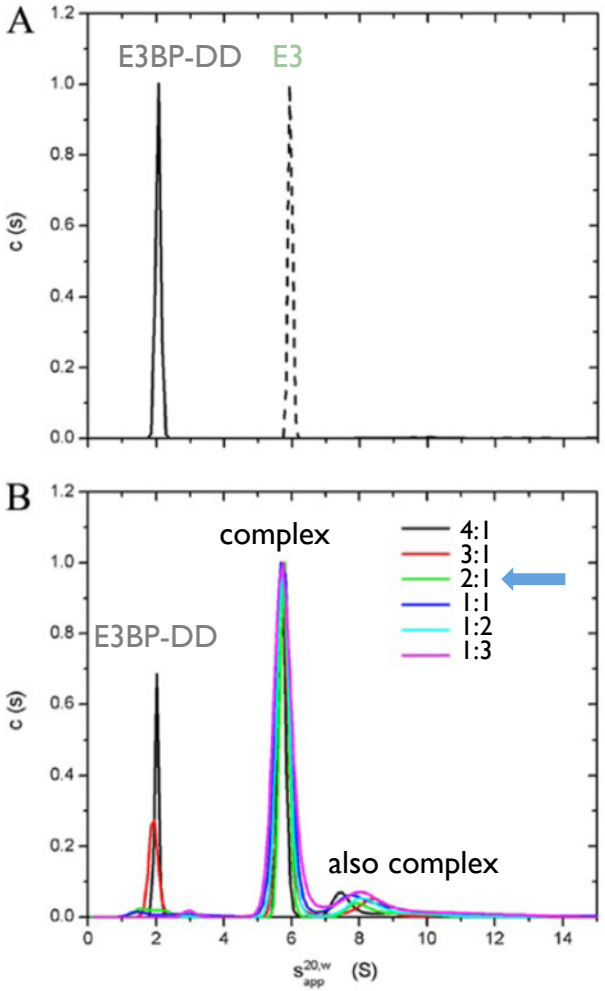


Smolle et al., JBC *281* 19771-80 (2006)

SV titration: stoichiometry is 2:1

- Expt 1: SV of E3 alone; SV of E3BP-DD alone
  - Determine their s
- Expt 2: SV of 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 ≈ 6 S peak less compact
      - s ≈ 8 S peak more compact

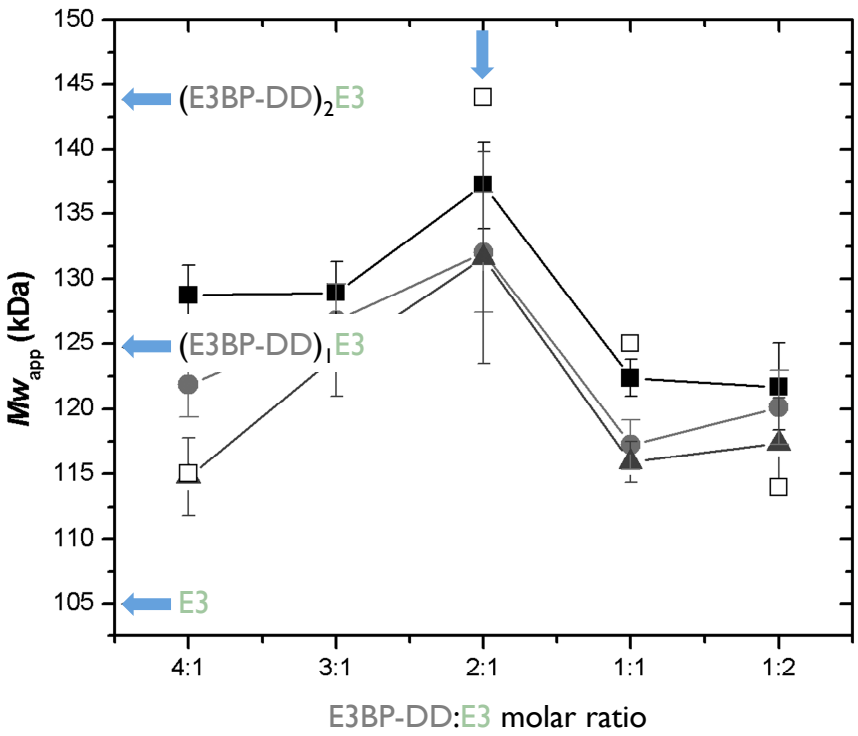


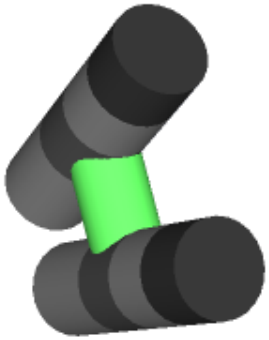


Mischa Smolle
Smolle et al., JBC *281* 19771-80 (2006)

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???
  - Non-ideality





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

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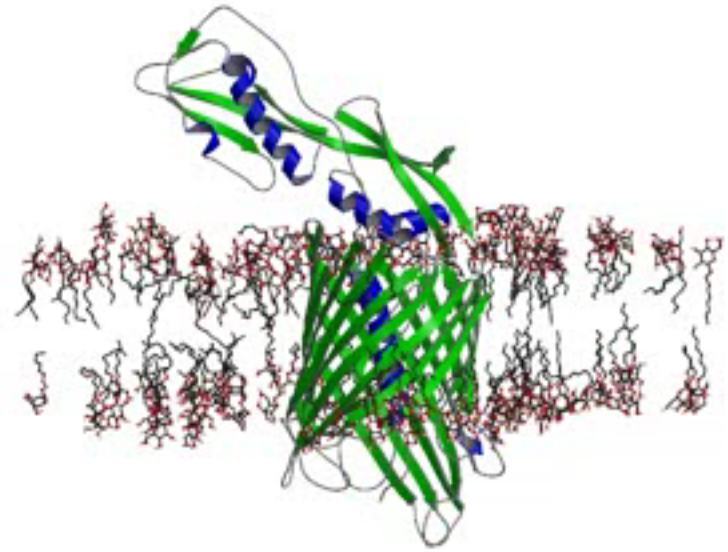
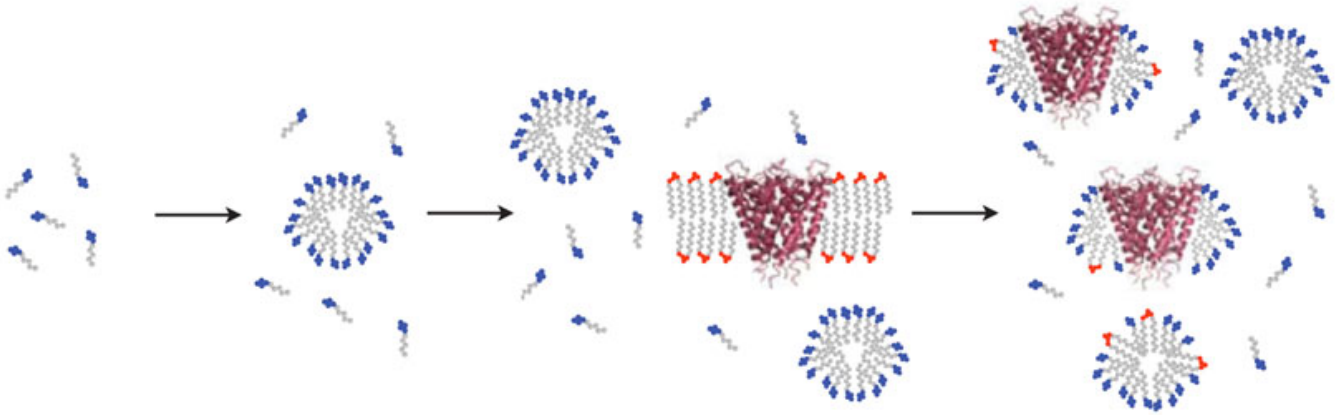
Illustrative movie of association of 140 detergent molecules with membrane protein FhaC



Gabel, Lensink, Clantin, Jacob-Dubuisson, Villeret & Ebel, C. (2014). *Biophys. J.* **107**, 185-196.

Detergent solublised 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

Christine Ebel [a,b,c,*]

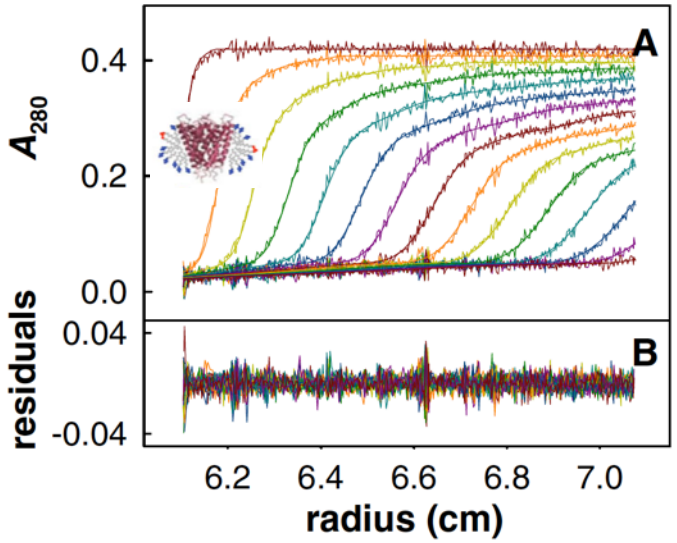
[a] CEA, Institut de Biologie Structurale, IBS, Grenoble, 41 rue Jules Horowitz, F-38027 Grenoble, France
[b] CNRS, UMR 5075, IBS, 41 rue Jules Horowitz, F-38027 Grenoble, France
[c] Université Joseph Fourier, IBS, 41 rue Jules Horowitz, F-38027 Grenoble, France

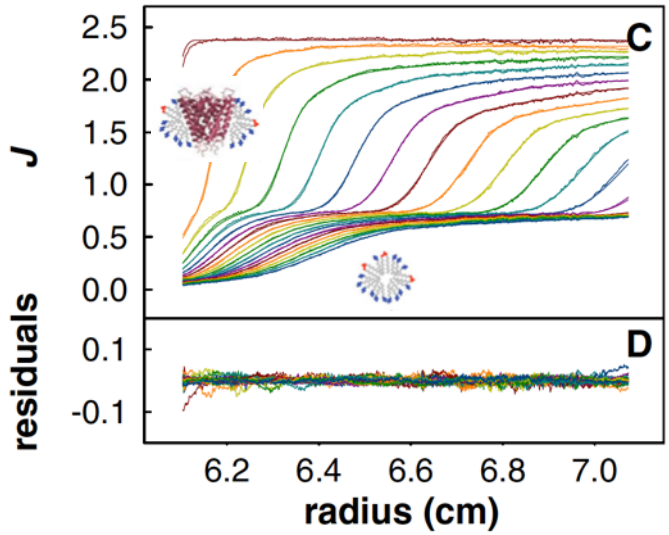
Values for vbar (psv)

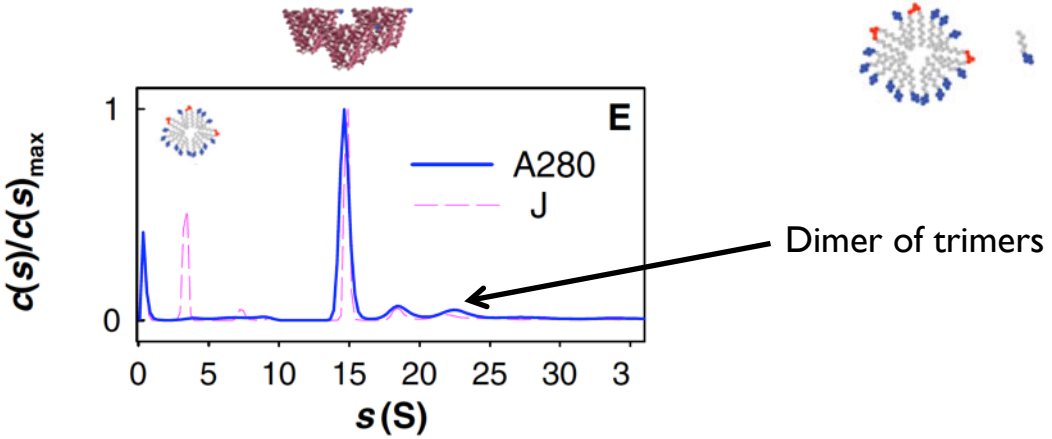
Component	vbar (ml/g)	Comment
Protein	0.73	Can be calculated from sequence quite reliably
Carbohydrate	0.63	Can be calculated from sequence quite reliably
Lipid	1.02	An average value Close to value for solvent, therefore almost invisible
Detergent	0.7-1.2	Can sediment or float

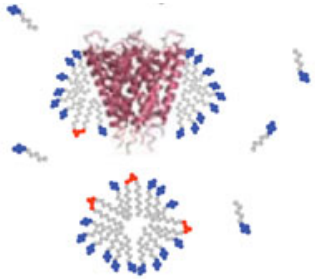
Detergent	vbar (ml/g)	Comment
C_8E_5	0.95	
LAPAO	1.002	Doesn't sediment
Octyl-POE	0.997	
Triton X-100		Absorbs @ 280 nm
LDAO	1.13	Floats
DDM	0.82	Aggregation number ≈ 130

SV of AcrB in DDM









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

Ebel, Methods (2011) doi: 10.1016/j.ymeth.2010.11.00

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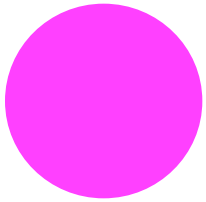
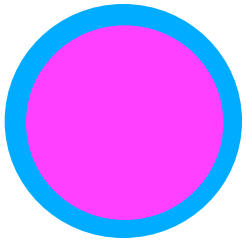
s = deviation from sphericity + hydrodynamic hydration

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

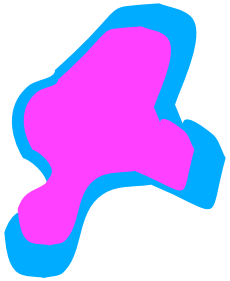


M, f_0

M, $f>f_0$

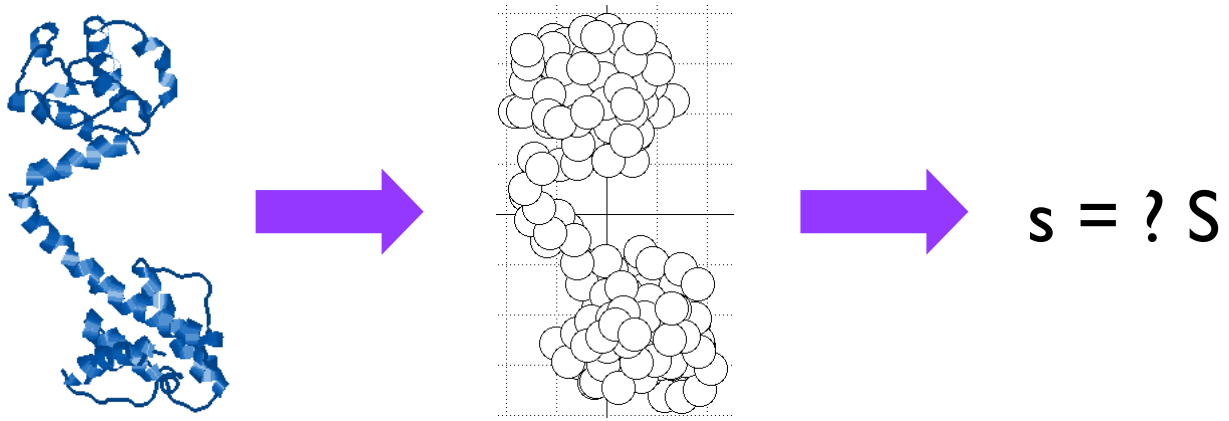


M, $f>f_0$



M, $f>>f_0$

Sedimentation coefficient is a constraint for SAS modelling



- For one sphere $$f_0 = 6\pi\eta_0\sigma$$
- For an assembly of N spheres $$\mathbf{F}_i = -f_i\left(\mathbf{u}_i - \mathbf{v}_i^0\right) - f_i\sum_{j=1}^{N}\mathbf{T}_{ij}\mathbf{F}_j$$
- where $$\mathbf{T}_{ij} = \frac{1}{8\pi\eta_0 R_{ij}}\left(\mathbf{I} + \frac{\mathbf{R}_{ij}\mathbf{R}_{ij}}{R_{ij}^2}\right)$$

Byron, O., *Introduction: Calculation of hydrodynamic parameters* in *Analytical Ultracentrifugation: Instrumentation, Software and Application* (S. Uchiyama, F. Arisaka, W. F. Stafford and T. Laue, editors), 2016, Springer.

Several freely available programs for HBM

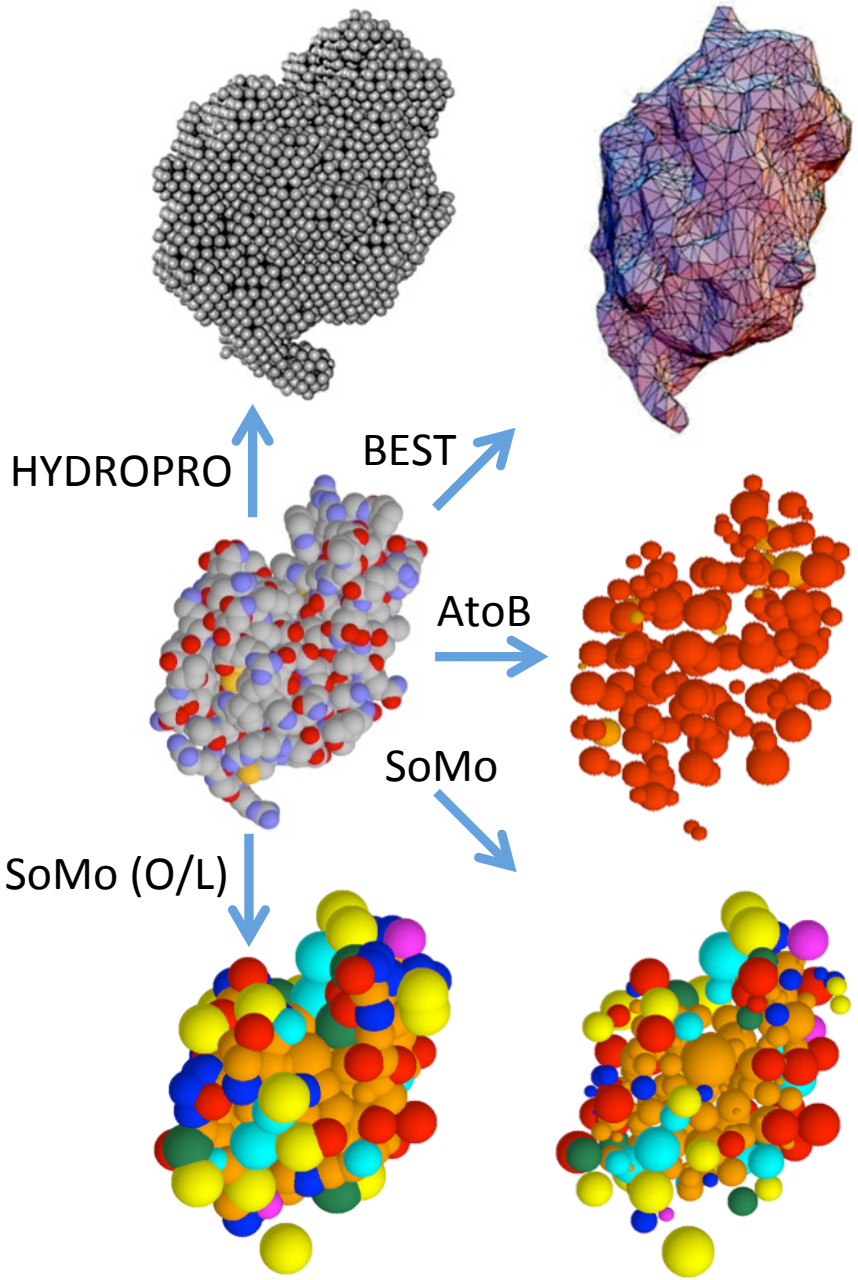
- José García de la Torre *et al.*
  - http://leonardo.inf.um.es/macromol/programs/programs.htm
  - HYDRO++
    - Computes hydrodynamic & other parameters for any bead model
  - HYDROPRO
    - Computes hydrodynamic & other parameters for models constructed from pdb files
  - And many other programs….

- 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

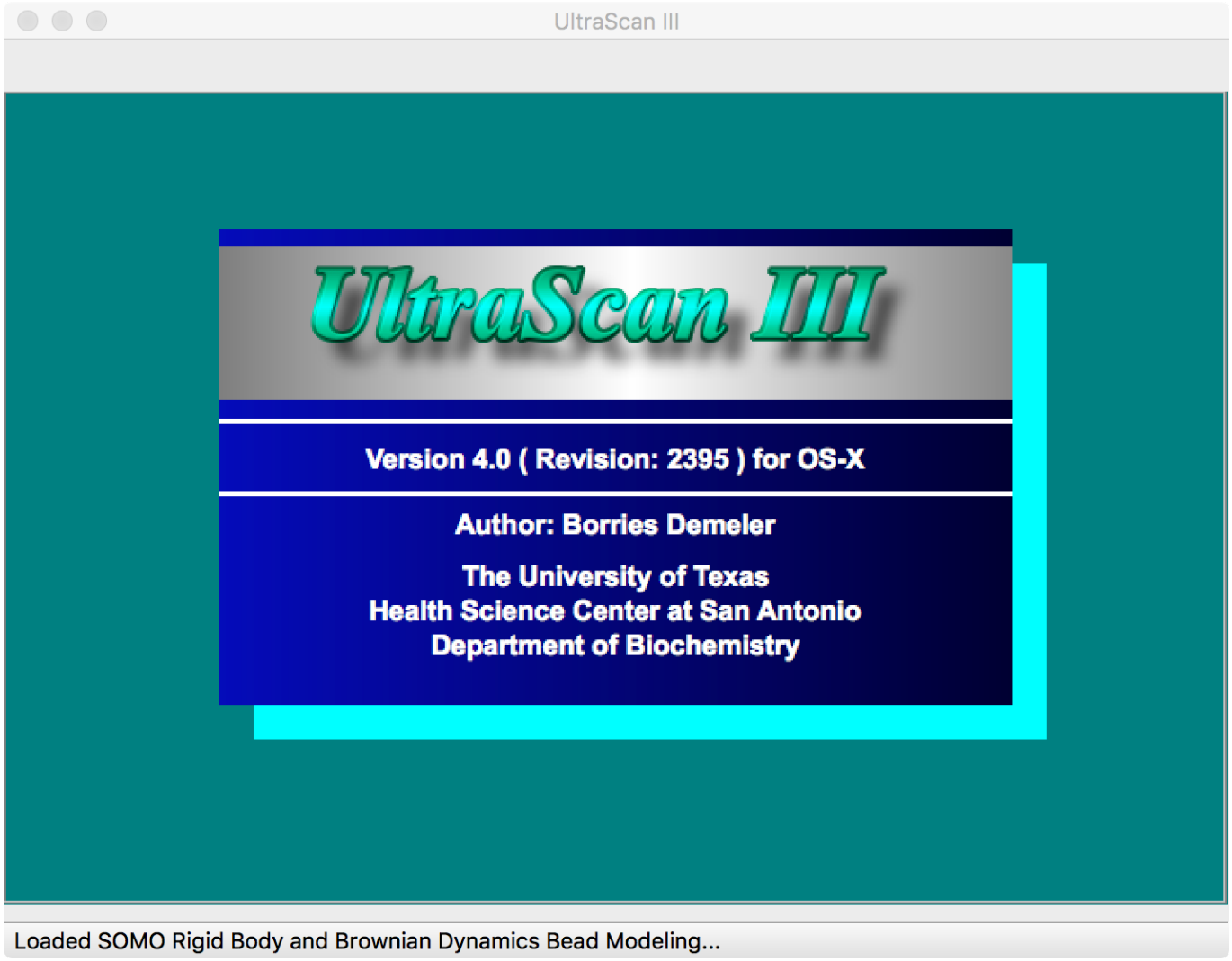
Different HM methods have their own pros and cons



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

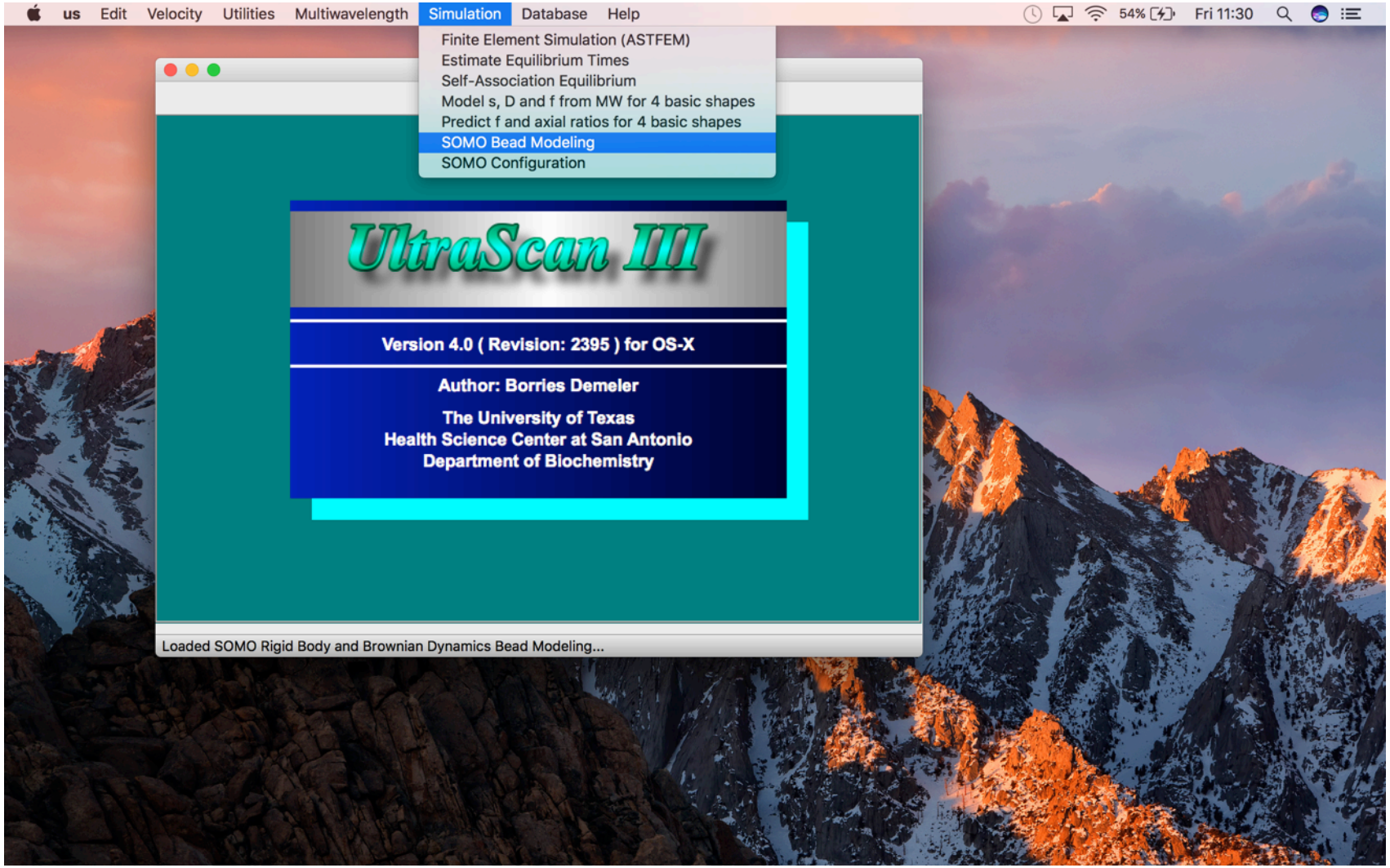


Mattia Rocco/ Borries Demeler/ Emre Brooks

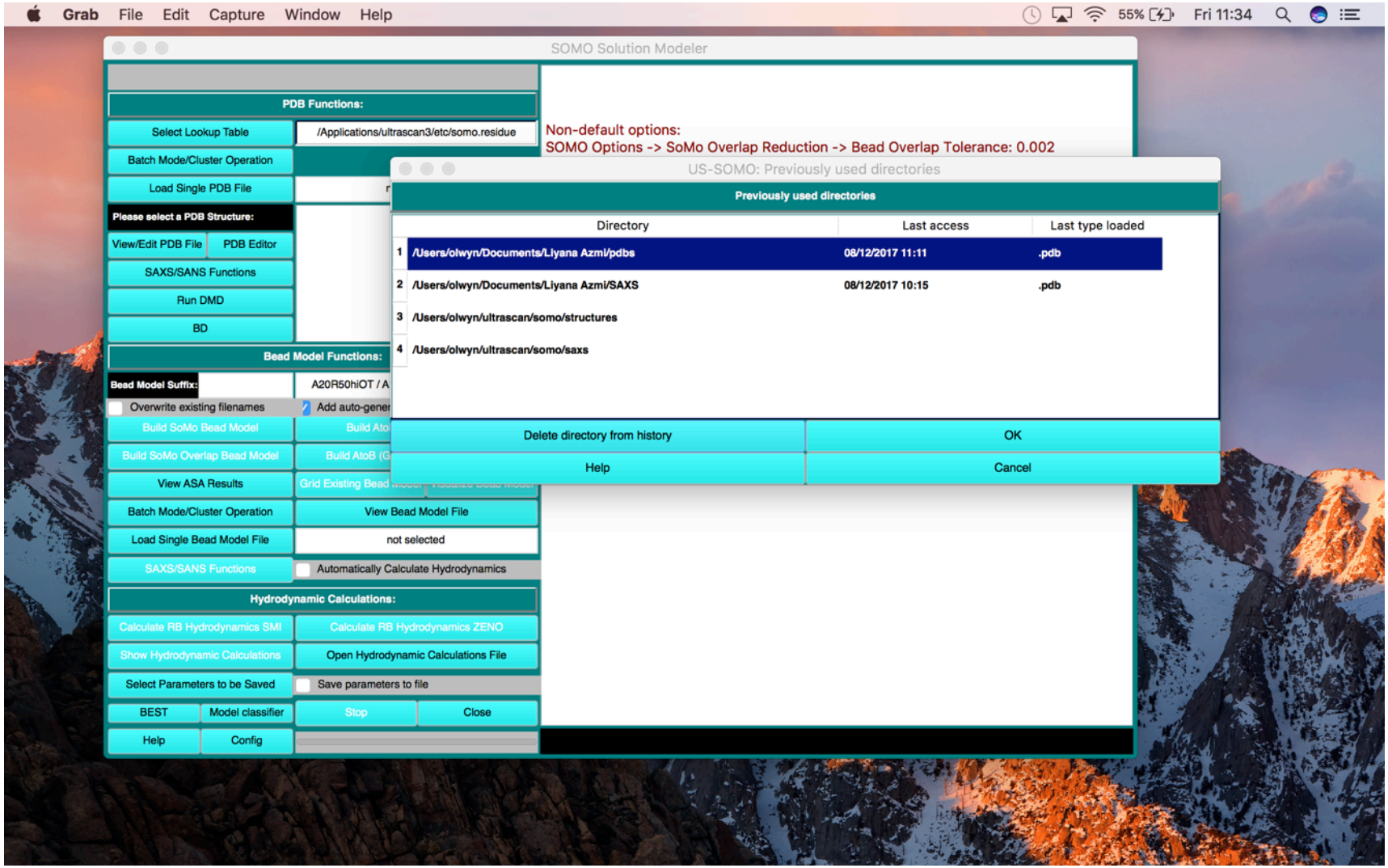
Rai et al. (2005) Structure *13* 723-34; Brookes et al. (2010) Eur. Biophys. J; Brookes et al., (2010) Macromol. Biosci.

http://somo.uthscsa.edu

Select from Simulation drop-down menu

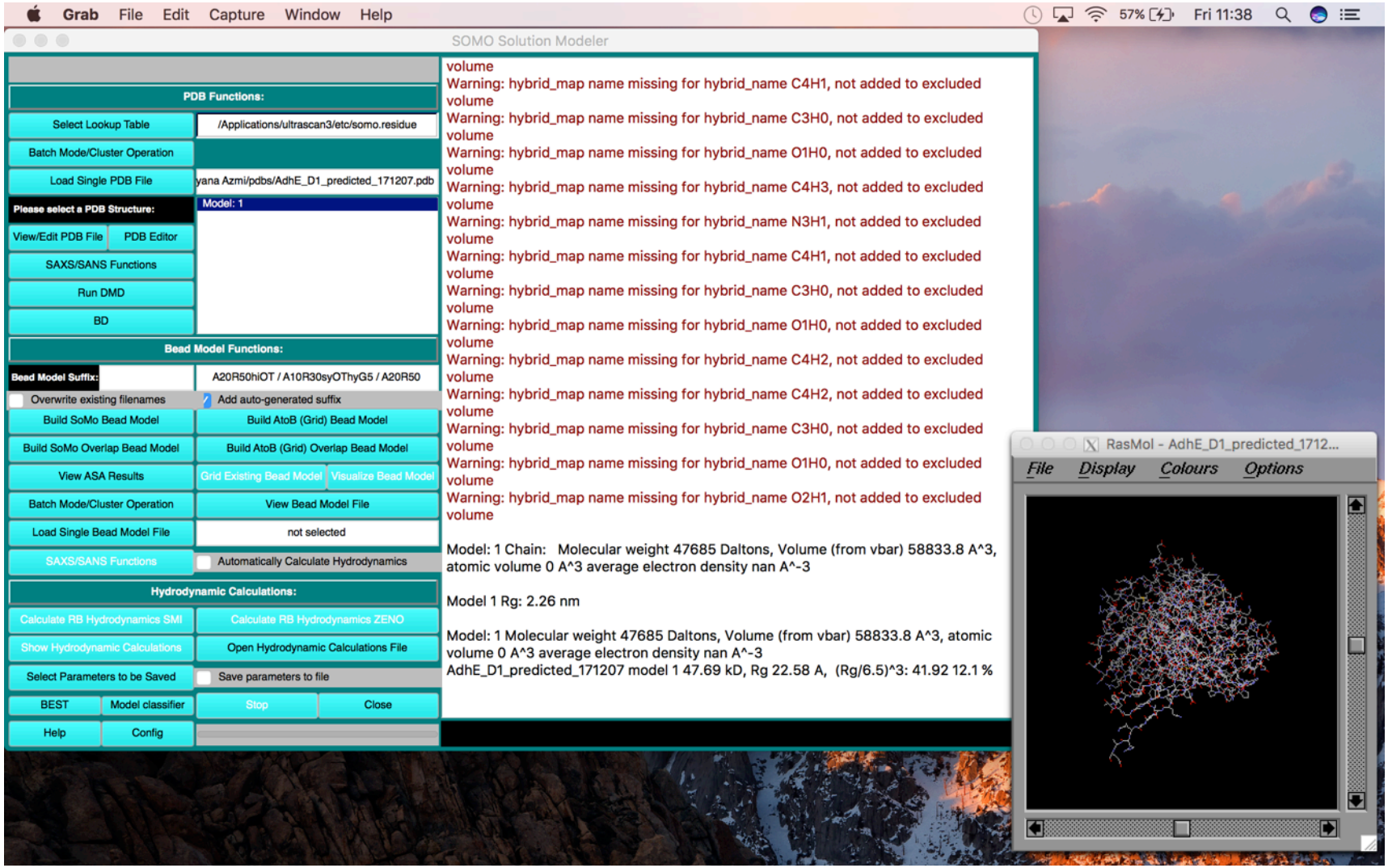


1: Load PDB file



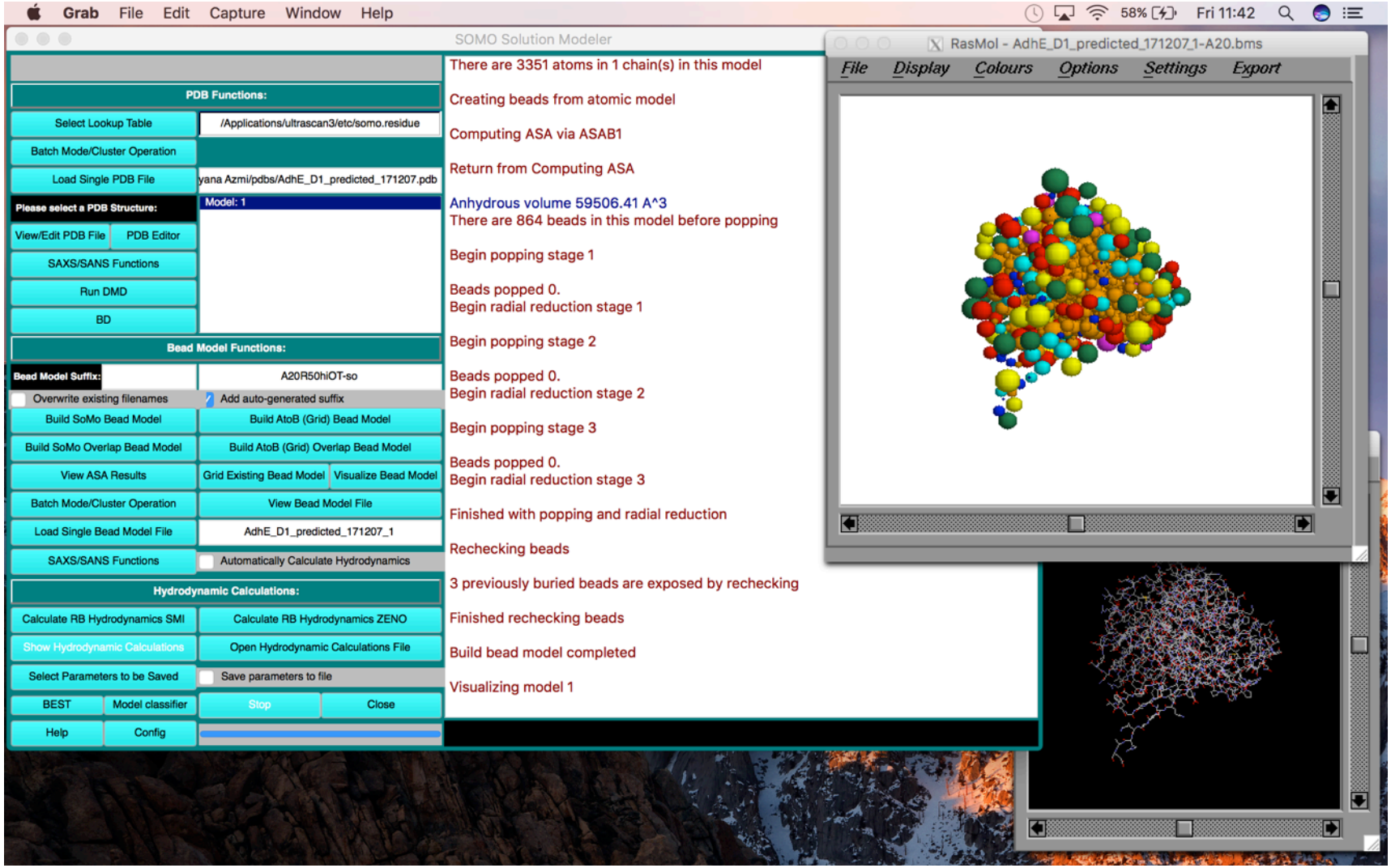
http://somo.uthscsa.edu

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



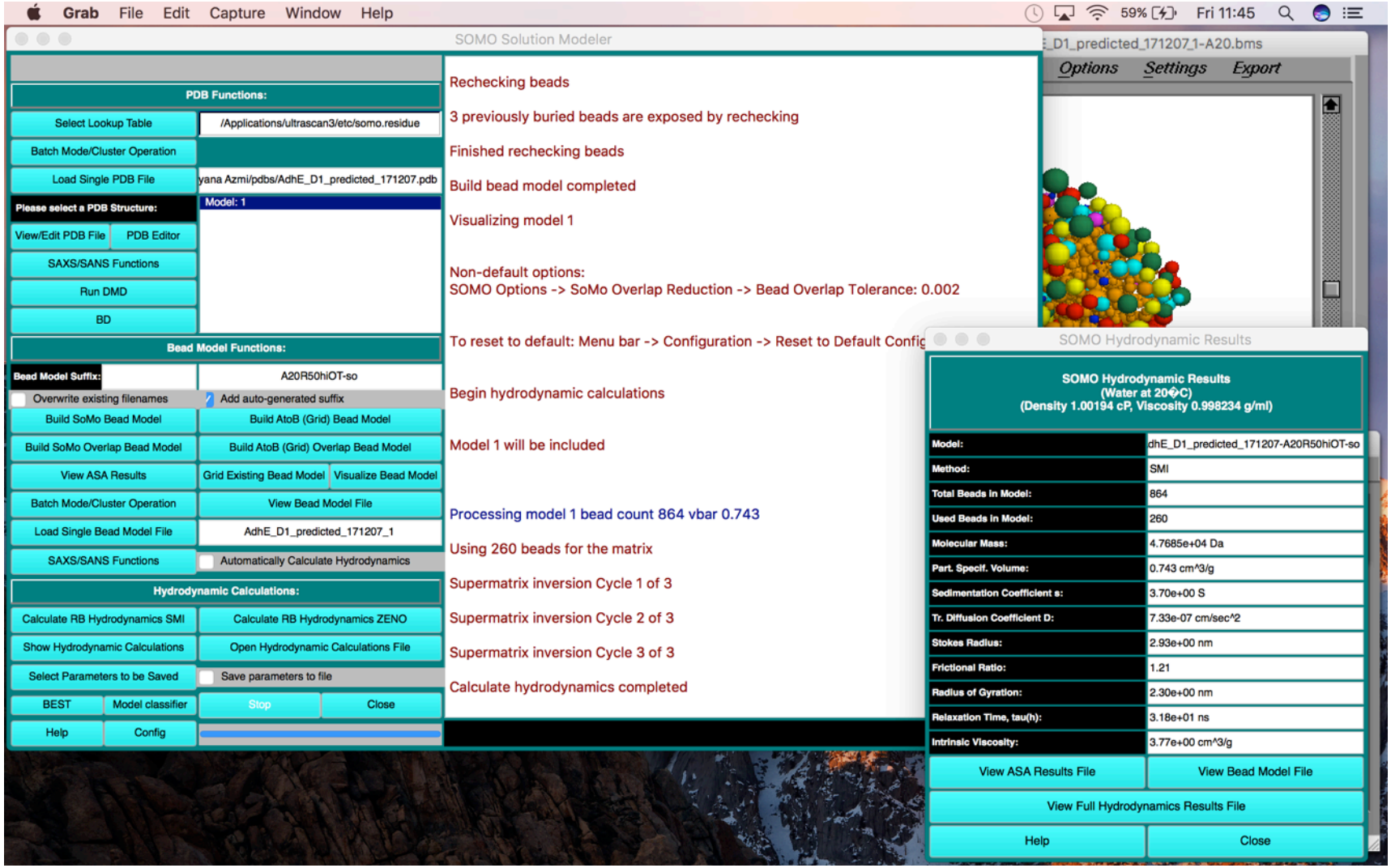
http://somo.uthscsa.edu

3: Compute & display bead model



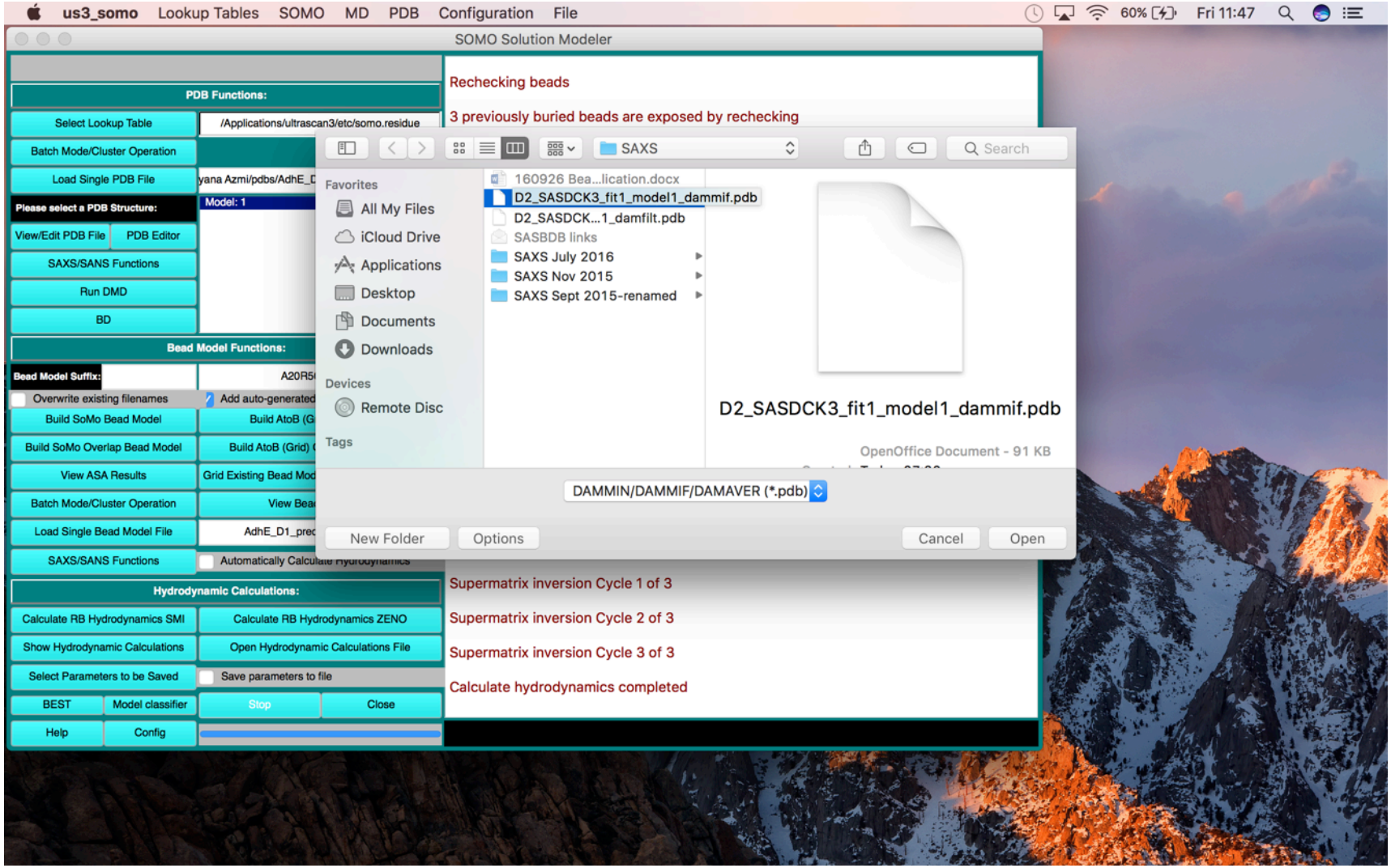
http://somo.uthscsa.edu

4: Compute & display hydrodynamic parameters



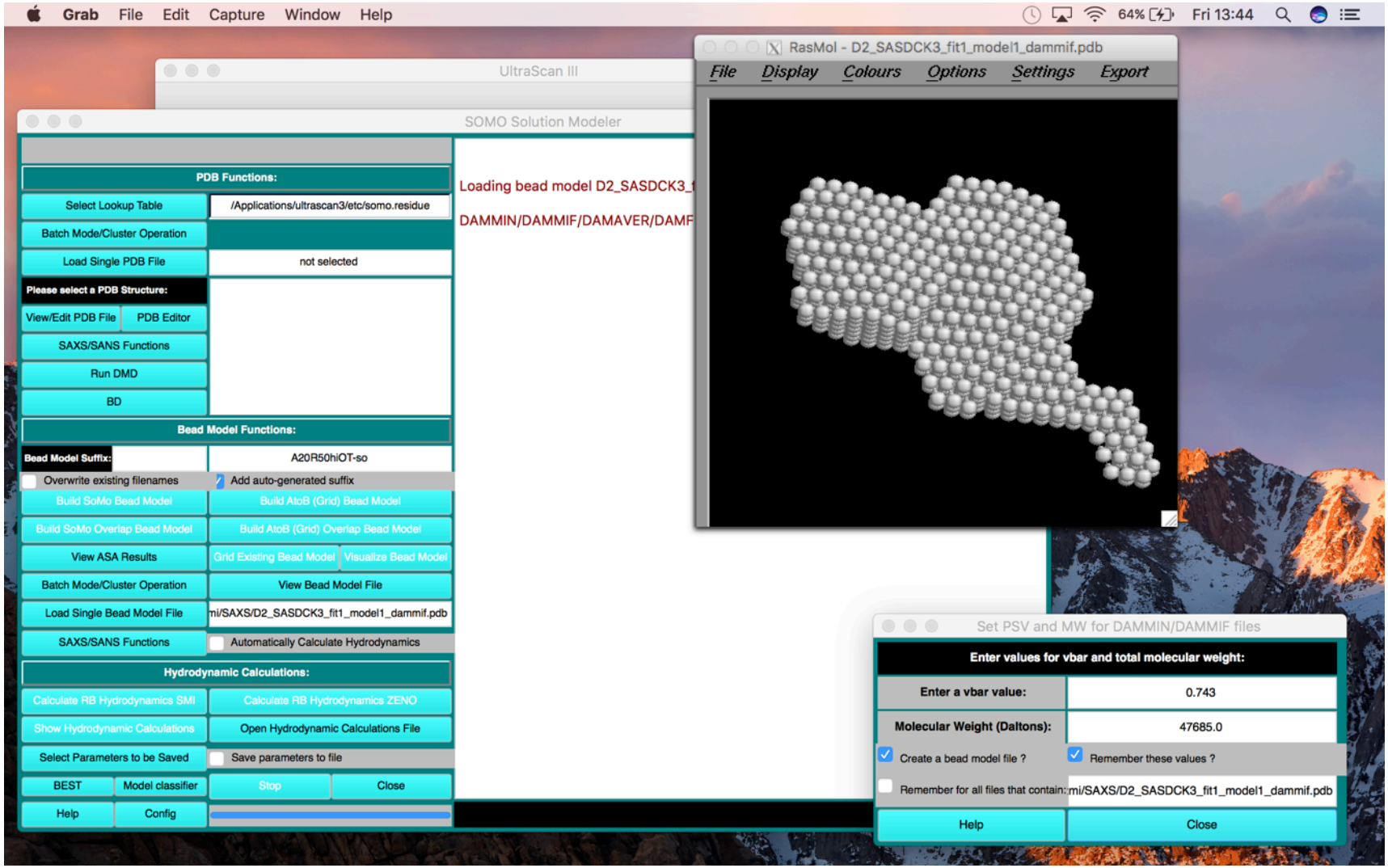
http://somo.uthscsa.edu

Can also upload DAMs to SOMO



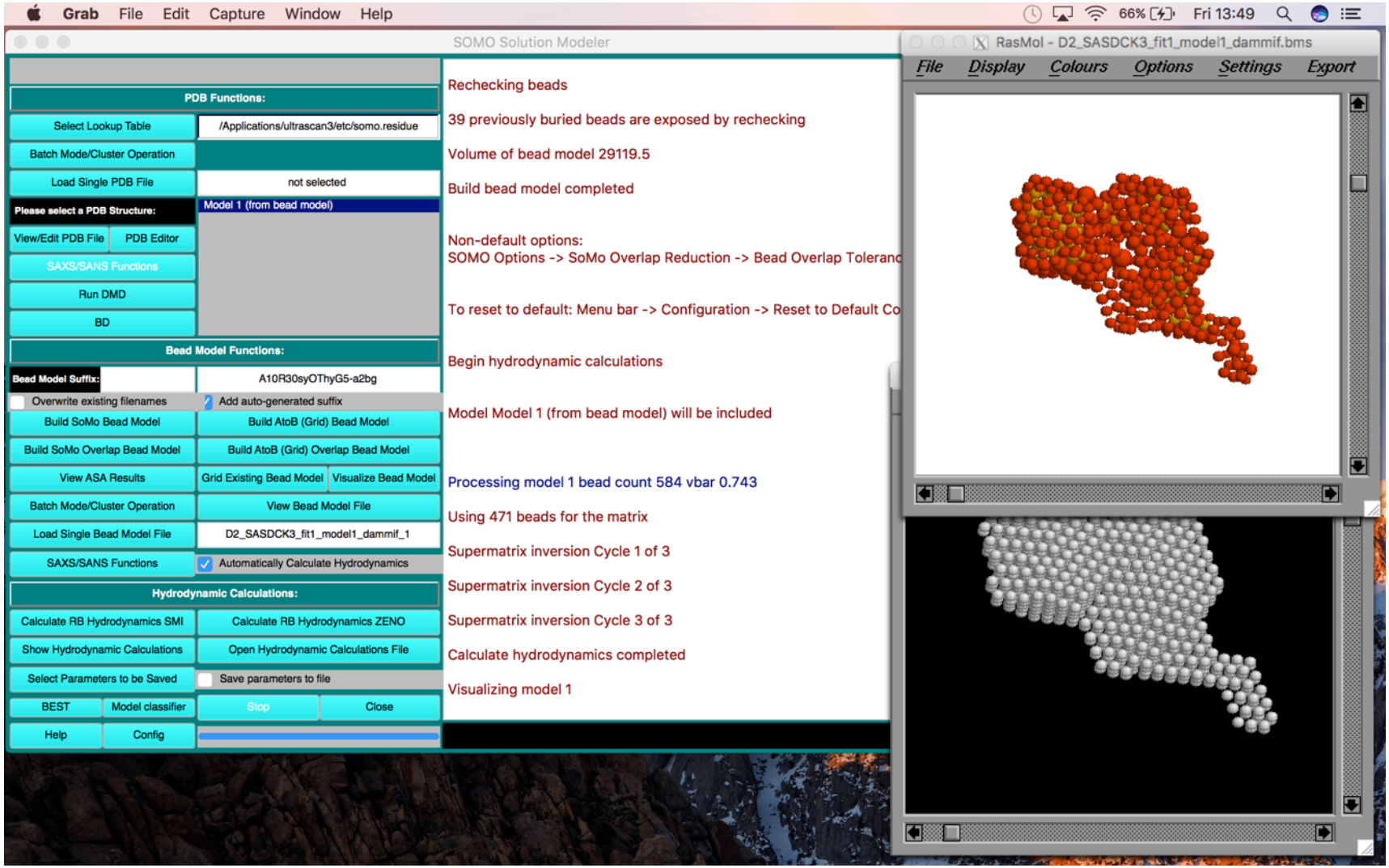
http://somo.uthscsa.edu

Input psv and M for model



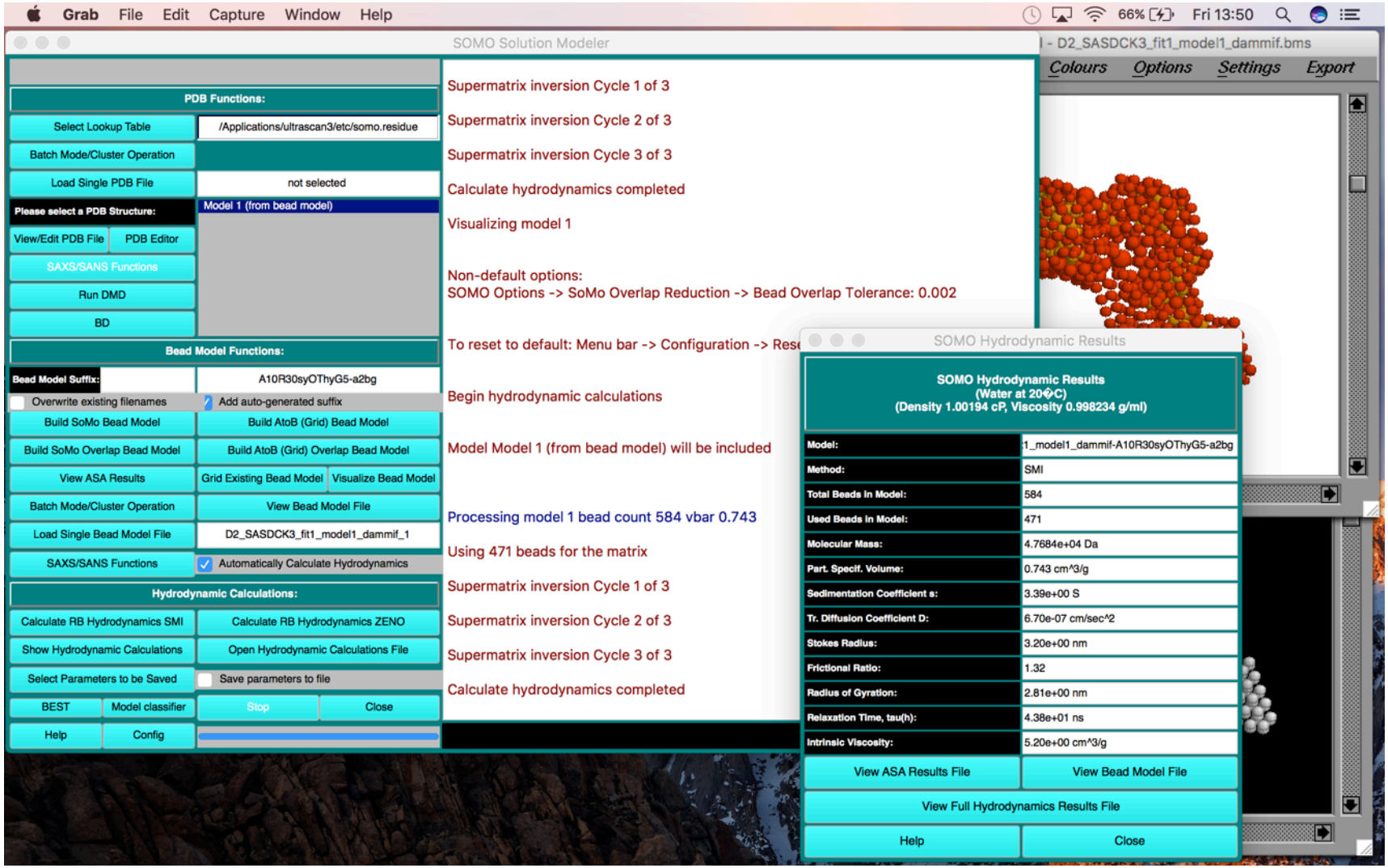
http://somo.uthscsa.edu

Convert DAM to HBM



http://somo.uthscsa.edu

Compute hydrodynamics for DAM-HBM: compare with experimental values



http://somo.uthscsa.edu

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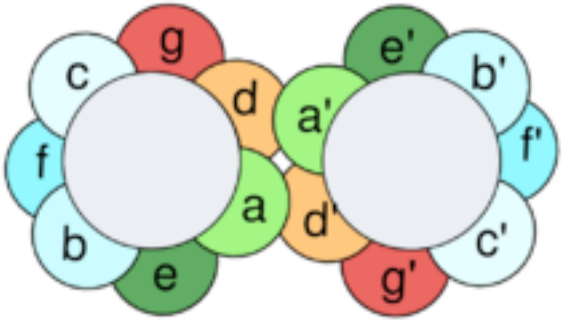
Example: Oligomerisation of synthetic polyvalent integrin $\alpha_5\beta_1$ ligands

MRGSHHHHHHGMASGLDSPTGIDFSDITANSFTVHWIAPRATITGYRIRHHPEHFSGRPREDRVPHSRNSIT
LTNLTPGTEYVVSIVALNGREESPPLIGQQSTVSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETG
GNSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSKPISINYRTSKLEPKSSDTPPGSPRSP
EPKSSDTPPGSPRSGRIKQLEDKIEELLSKIYHLENEIARLKKLIGELEDKIENLQC

- $\alpha_5\beta_1$ 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

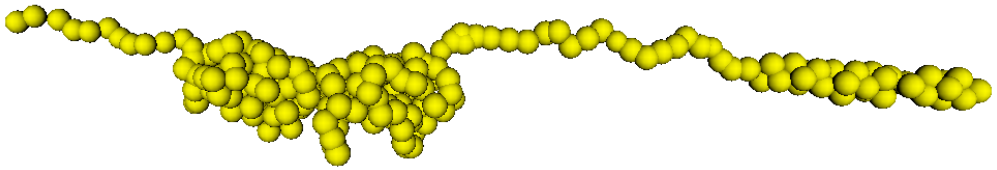
- Question: do the ligands oligomerise as designed?



Construction of hydrodynamic bead models

MRGSHHHHHHGMASGLDSPTGIDFSDITANSFTVHWIAPRATITGYRIRHHPEHFSGRPREDRVPHSRNSIT
LTNLTPGTEYVVSIVALNGREESPPLIGQQSTVSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGETG
GNSPVQEFTVPGSKSTATISGLKPGVDYTITVYAVTGRGDSPASSKPISINYRTSKLEPKSSDTPPGSPRSP
EPKSSDTPPGSPRSGRIKQLEDKIEELLSKIYHLENEIARLKKLIGELEDKIENLQC

- 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



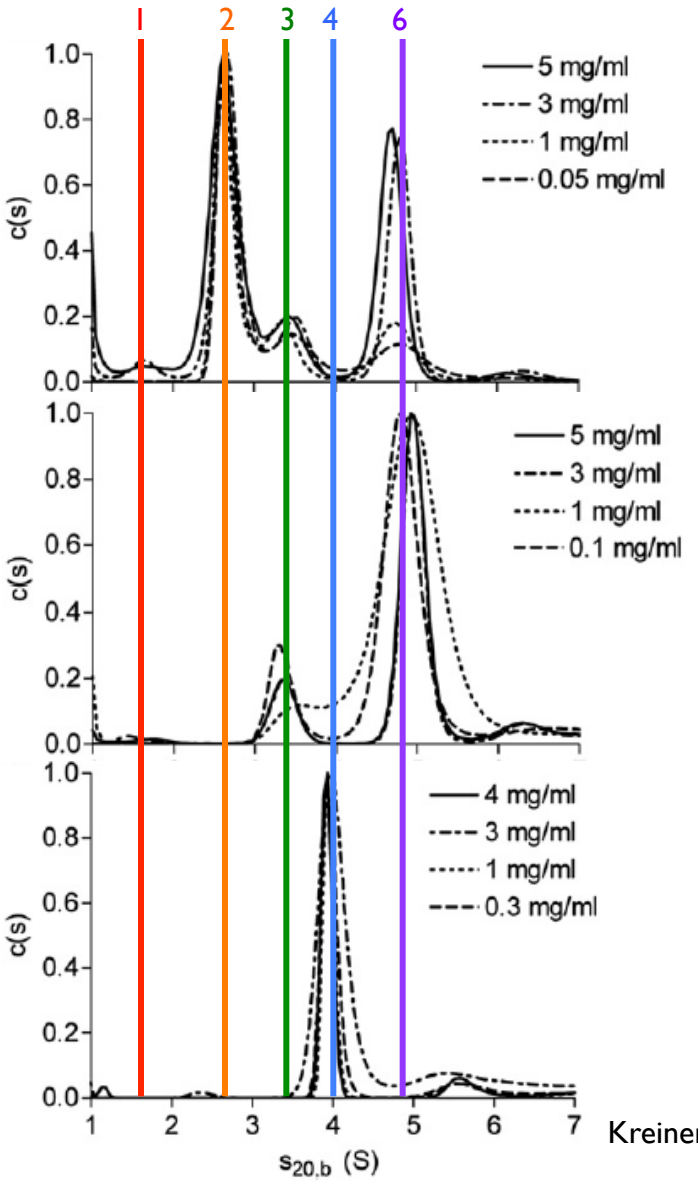
Kreiner *et al.*, (2009) Biophysical Chemistry 142 34-39

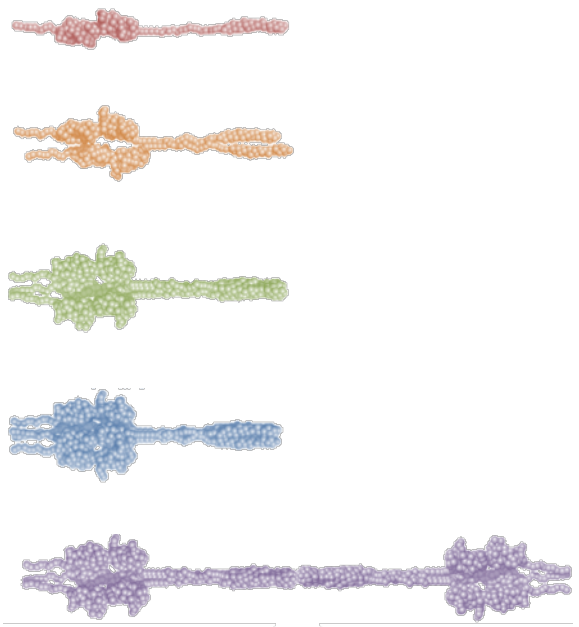
Oligomerisation of synthetic polyvalent integrin $\alpha_5\beta_1$ ligands - AUC SV: c(s) reveals complex composition

"dimer"

"trimer"

"tetramer"





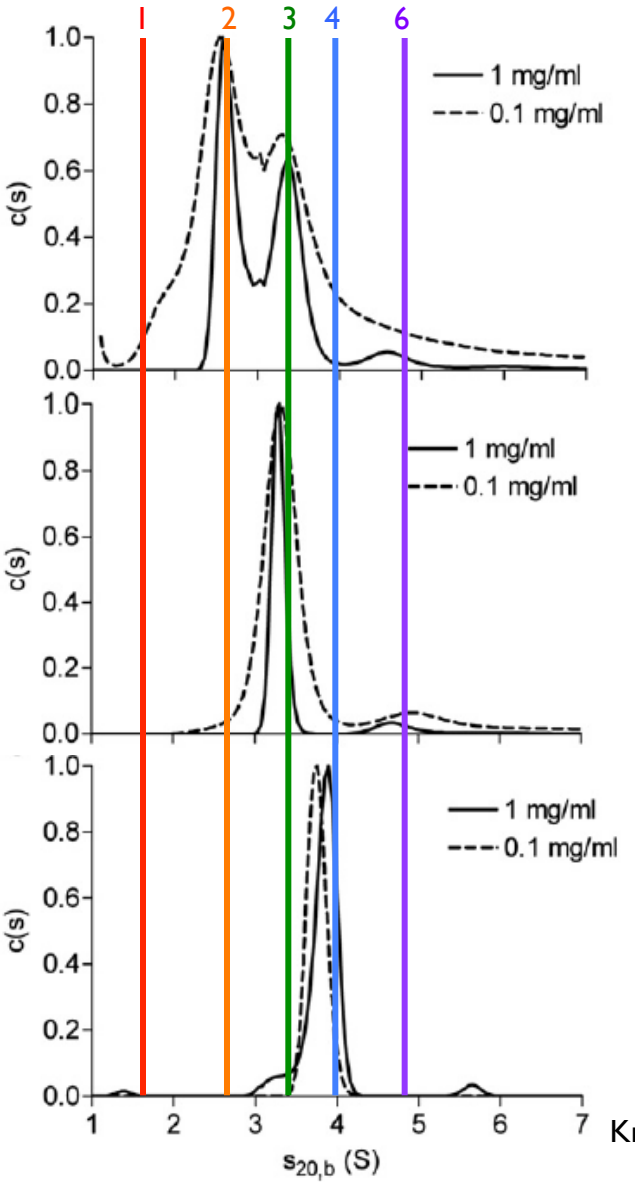
Kreiner *et al.*, (2009) Biophysical Chemistry 142 34-39

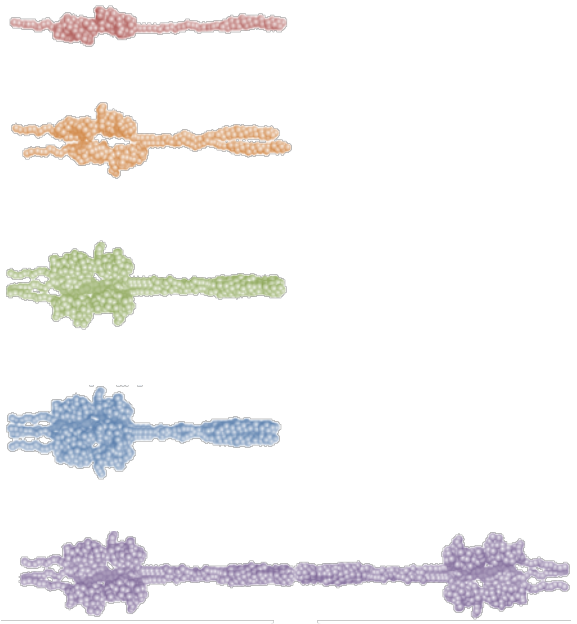
AUC SV + DTT: c(s) composition simplified

"dimer"

"trimer"

"tetramer"





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

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

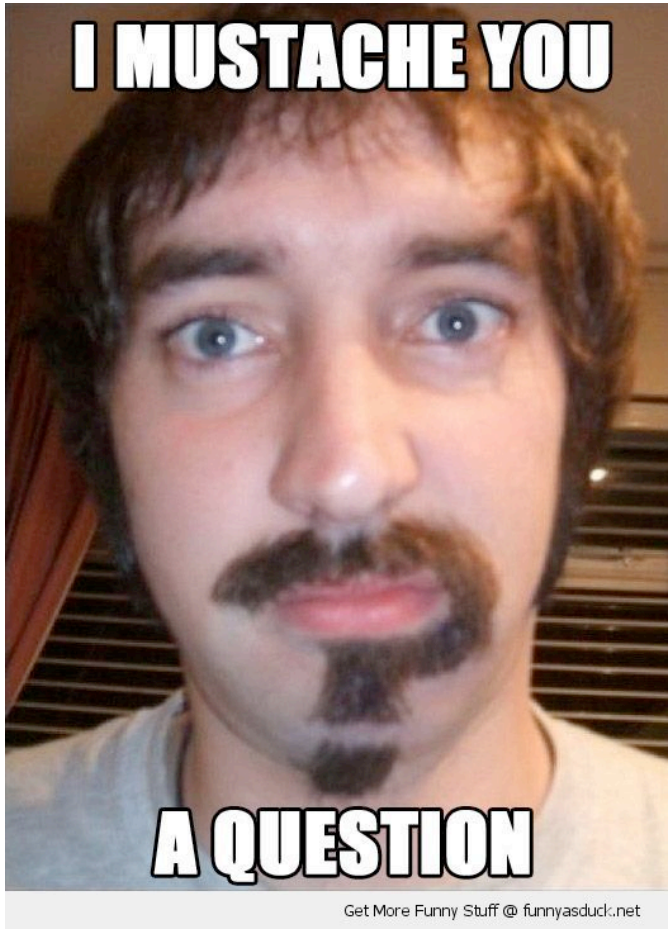
- Setting up and running AUC experiments
  - Tutorial paper
    - Lebowitz, J., M.S. Lewis, and P. Schuck, Modern analytical ultracentrifugation in protein science: A tutorial review. Protein Science, 2002. 11(9): p. 2067-2079.
  - AUC user guide from Demeler lab
    - http://www.uslims.uthscsa.edu/AUCuserGuideVolume-1-Hardware.pdf
- Data analysis
  - Using SEDFIT & SEDPHAT
    - http://www.analyticalultracentrifugation.com/default.htm
  - Using UltraScan
    - http://www.ultrascan.uthscsa.edu

AUC tutorials

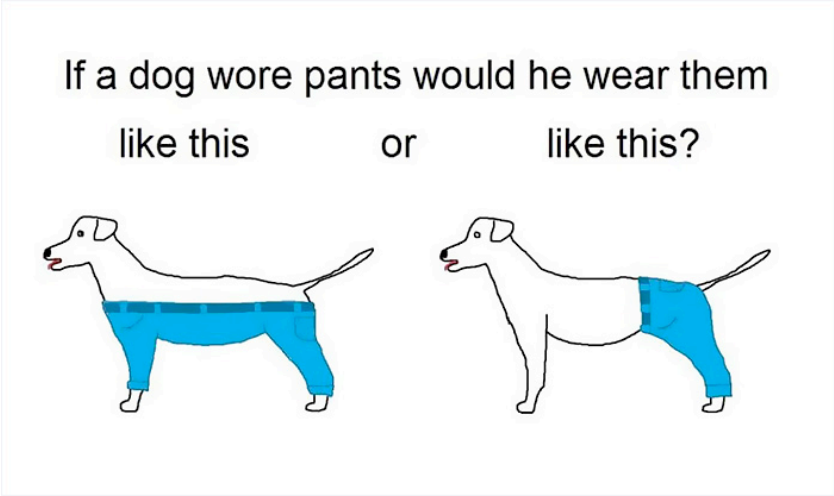
- YouTube
  - Alexander Bepperling
    - Aggregation analysis and beyond analytical ultracentrifugation in the biopharmaceutical industry
    - https://www.youtube.com/watch?v=IiERbI-Xz4c
  - Borries Demeler
    - Advances in sedimentation analysis
    - https://www.youtube.com/watch?v=zuAwWOJZtkM
  - 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=ivRodzqWjS8
  - 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?


I MUSTACHE YOU
A QUESTION
Get More Funny Stuff @ funnyasduck.net


If Apple made a car...
would it have Windows!?


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