

Fitting the MSSV Data of the  
Interaction of GST-VCA and Arp2/3.  
*Supplemental Protocol to Padrick &  
Brautigam, Methods, 2011*

Chad A. Brautigam

Shae B. Padrick

The University of Texas

Southwestern Medical Center at Dallas

**Background:** The fusion protein GST-VCA (a dimer of about 72,000 Da) interacts with the Arp2/3 complex (a 224,000 Da heteroheptamer). Three experiments were performed in a single run of the ultracentrifuge.

Cell 1: GST-VCA alone

Cell 2: Arp2/3 alone

Cell 3: A mixture of the two.

The run was performed at 20° C and 42,000 rpm.

**The goal:** Determine the molar ratio of the complex between the two proteins.

**The software:** SEDPHAT v. 8.1

(<http://www.analyticalultracentrifugation.com/sedphat/default.htm>)

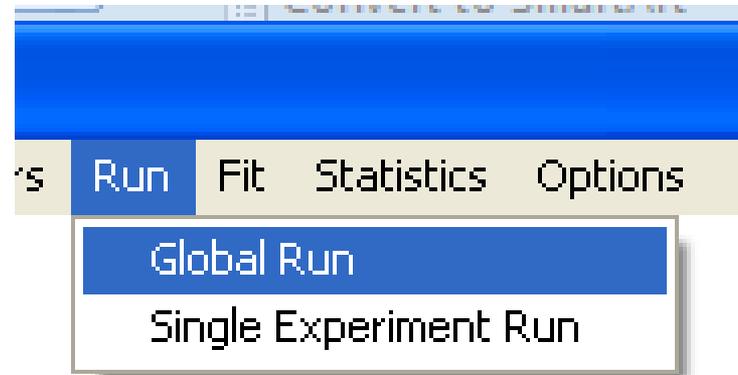
# Summoning Parameter Windows in SEDPHAT



When Global Parameters need to be summoned, click here.

When Experimental Parameters need to be summoned, click here.

# How to “Run” and “Fit”



To perform a “Global Run”  
(only linear parameters fit)



To perform a “Global Fit”  
(linear and non-linear parameters fit)

# Step 1

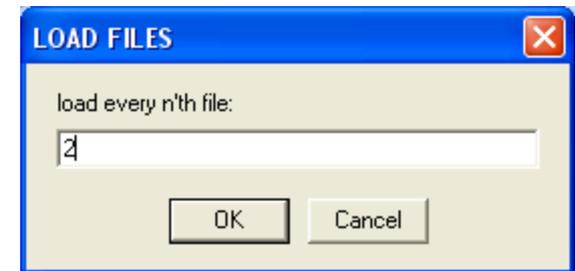
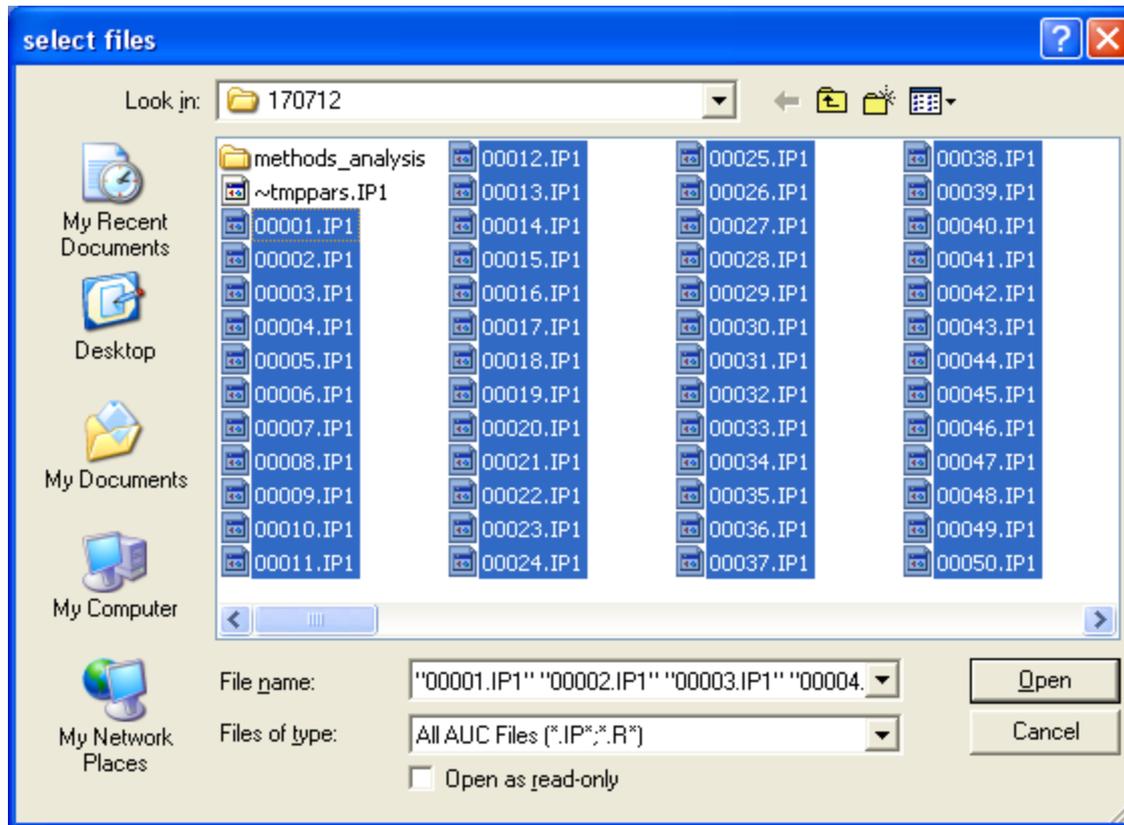
The IF data acquired from the GST-VCA-alone sample (Cell 1) were loaded into SEDPHAT. This task is initiated by selecting “Load New Sedimentation Velocity Data” from the “Data” menu of SEDPHAT. All of the “\*.IP1” files were displayed, and files 1-101 were selected. After pressing “OK,” the user is prompted by a dialog box to “load every n’t h file”. The numeral “2” was entered; thus every 2<sup>nd</sup> IP1 file between 1 and 101 will be loaded. See the next 2 pages.

# Load New Data

The image shows a screenshot of a software application's menu system. The title bar at the top reads "C:\sedffit\sedphatstartupconfig.ini". Below the title bar is a menu bar with the following items: "Data", "Copy", "Display", "Model", "Global Parameters", "Concentrations", and "Experiment Parameters". The "Data" menu is open, displaying a list of options. The option "Load New Sedimentation Velocity Data" is highlighted in blue. Other options include "Load Experiment", "Save Experiment", "Save All Experiments", "Remove Experiment", "Load New Sedimentation Equilibrium Data", "Load New Multi-Speed Equilibrium Data", "Load New DLS Data", "Load New AUC Isotherm Data", "Load New ITC Data", "Load New Surface Binding Equilibrium Data (Flow)", "Load New Competitive Surface Binding/Solution Equilibrium Data (Flow)", "Load New Steady-State Anisotropy Data", "Load New Spectroscopy Data", "Edit Data Files", "Save Fit Data", "save TI Noise in file", "Model", "Update Current Configuration", "Save Current Configuration As", "Read Configuration From File", "Set Current Configuration As Startup Default", "Reset Default Configuration", "Copy All Data And Save As New Config", and "Exit".

Menu Item	Shortcut
Load Experiment	^E
Save Experiment	
Save All Experiments	^A
Remove Experiment	
<b>Load New Sedimentation Velocity Data</b>	<b>^L</b>
Load New Sedimentation Equilibrium Data	
Load New Multi-Speed Equilibrium Data	
Load New DLS Data	
Load New AUC Isotherm Data	
Load New ITC Data	
Load New Surface Binding Equilibrium Data (Flow)	
Load New Competitive Surface Binding/Solution Equilibrium Data (Flow)	
Load New Steady-State Anisotropy Data	
Load New Spectroscopy Data	
Edit Data Files	
Save Fit Data	^S
save TI Noise in file	
Model	
Update Current Configuration	^U
Save Current Configuration As	^W
Read Configuration From File	^C
Set Current Configuration As Startup Default	
Reset Default Configuration	
Copy All Data And Save As New Config	
Exit	

# Load the Interference Data First



Every other file was loaded

Interference files 1-101 were selected

# Step 2

A dialog box appears prompting the user for Experimental Parameters (Table 3). SEDPHAT considers each data set loaded as one “experiment.” The first data set loaded is deemed Experiment 1, the second Experiment 2, etc. The buffer parameters, i.e. density ( $\rho$ ) and viscosity ( $\eta$ ) were entered. Their values were 1.00079 g/cm<sup>3</sup>, and 0.010024 Poise, respectively. Ordinarily, the protein’s  $v_{bar}$  would be entered here as well. However, the default value of 0.73 cm<sup>3</sup>/g was accepted here. The  $v_{bar}$  does not strongly bear on the goal of this analysis, which is the molar ratio of the proteins in the GST-VCA:Arp2/3 complex. Therefore, a value that is sensible for most proteins was chosen. By default for IF SV data, the RI noise, TI noise, and baseline checkboxes are activated, which turns on their optimization. This is appropriate. Another parameter to note at this point is the “noise” box. This is the expected root-mean-square error for the experiment; it is by default 0.01 for SV data. This default was accepted in this case. Also, for SV data, dual-sectored centerpieces with a 1.2-cm optical path length are assumed and were accepted in this case.

# Step 2 (continued)

Another area of the dialog box accepts the meniscus and bottom parameters. The default values were accepted, and the checkboxes allowing for the refinement of these values are left unchecked for now. The reasons for this choice are both practical and lazy. The meniscus is better chosen graphically, not entered numerically, and there is no opportunity to choose it before this dialog appears. Further, if the meniscus checkbox were activated (as it will be later), the program would ask for fitting limits based on the current, default position of the meniscus. Because the default limits that SEDPHAT sets are the current meniscus  $\pm 0.03$  cm, the defaults will be unacceptable, and would have to be input manually. See Step 11 for easy ways around these problems. There is also a section for the extinction properties of the proteins. Because these are both vital to the analysis and global to all experiments in MSSV, they are input later in the Global Parameters dialog (see Step 8). See the next page.

# Change solution parameters

Experimental Parameters

(1) INTERFERENCE data for SEDVELOCITY

C:\AUC Data\Rosen\120106\170712\mssv\cell1\_if.xp (00001.IP1 ...)

Comment

active

noise 0.0100  \*sqrt (N1/Nx)

Centerpiece 2

Pathlength 1.200000

Rotor type 0

no backdiffusion neces

v-bar (ml/g) 0.7300

buffer density (g/ml) 1.00079

buffer viscosity (P) 0.010024

Temperature 20.0

fit baseline

fit RI Noise

fit TI Noise

Meniscus 5.9999

Bottom 7.2004

redirect men./bot. 1

For Associating Systems:

extinction coefficient A 1.0000

extinction coefficient B 1.0000

extinction coefficient C 0.0000

redirect xt A 1

redirect xt B 1

redirect xt C 1

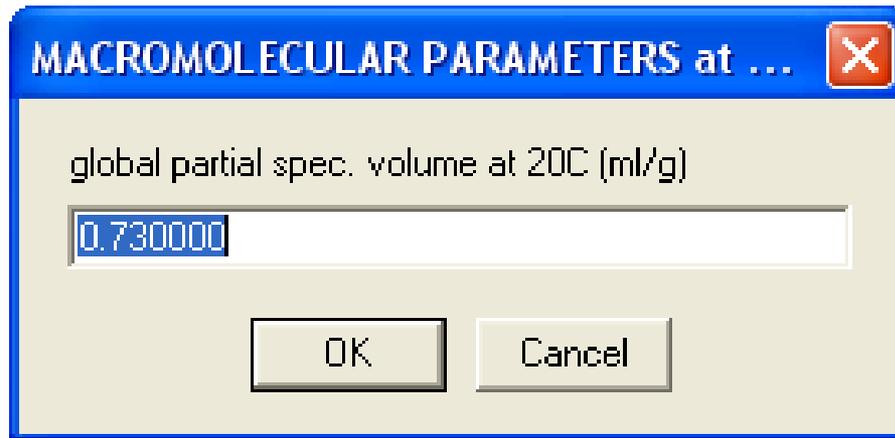
partial boundary fitting smin 0.00 smax 100.00

use for sigma of MC sims: 0.0100  use local rmsd

Throughout, the boxes show parameters that are altered in the course of the step.

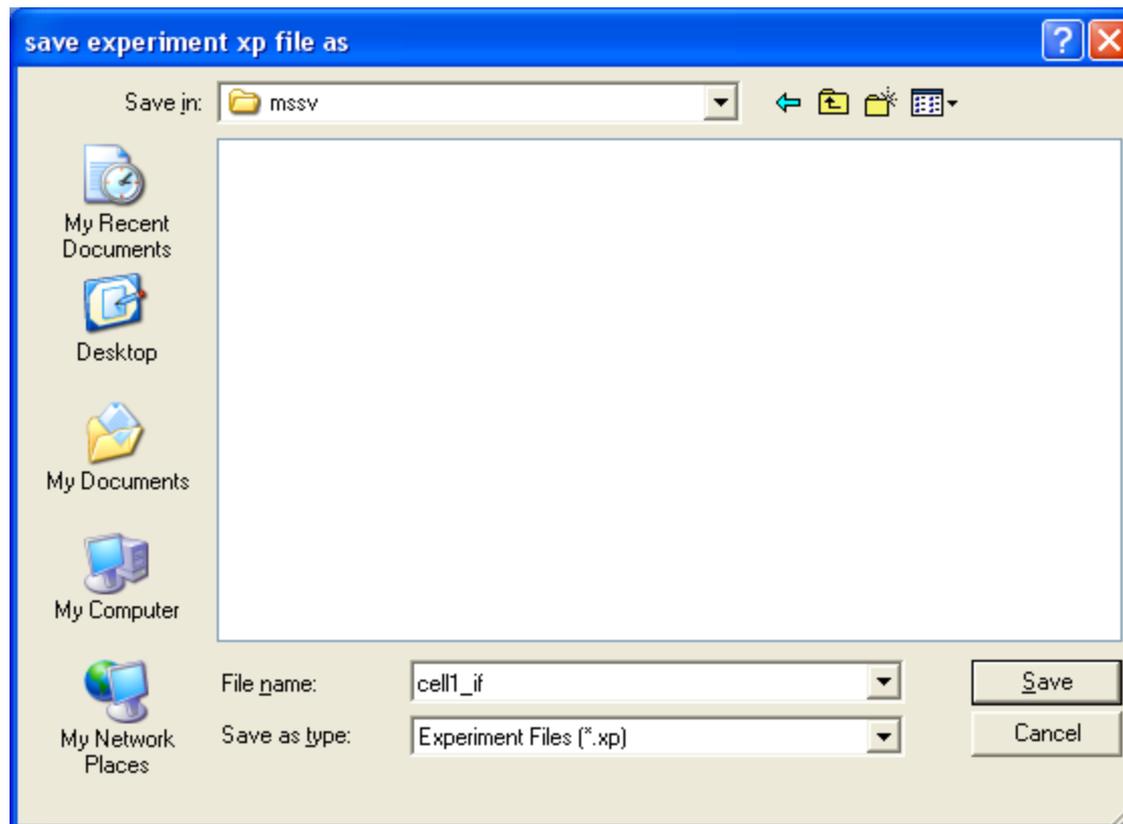
# Step 3

The user was prompted to input a global vbar.  
The default of 0.73 is accepted.



# Step 4

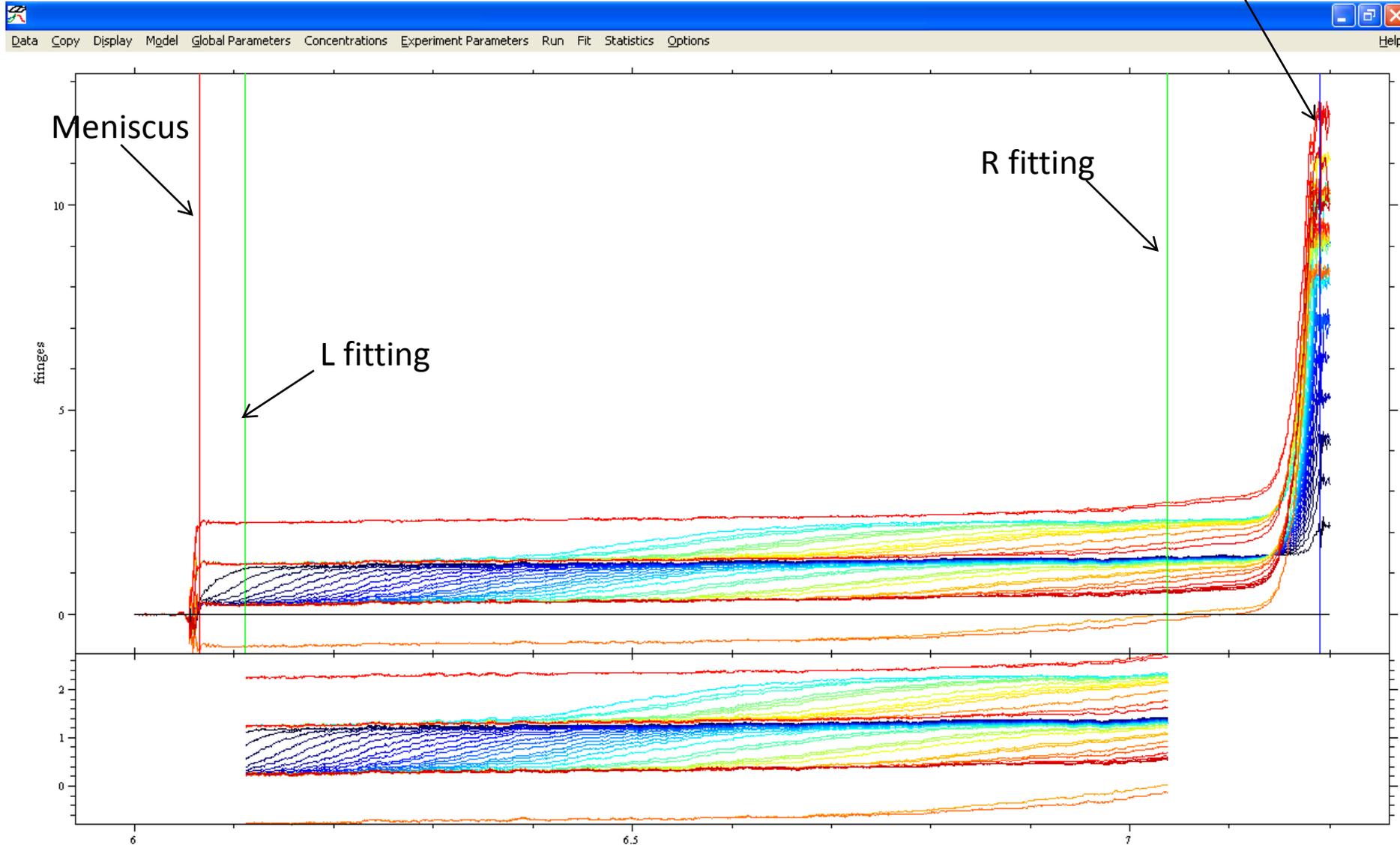
A dialog opened for the purpose of saving the experiment file (file extension .xp). This is a text file that contains information about the location of the data files and all of the parameters input in the Experimental Parameters dialog.



# Step 5

The data were displayed by the program, and the meniscus (red line), bottom (blue line), left and right fitting limits (green lines) were set graphically therein. These values can be set by dragging them from their default positions. See the next page.

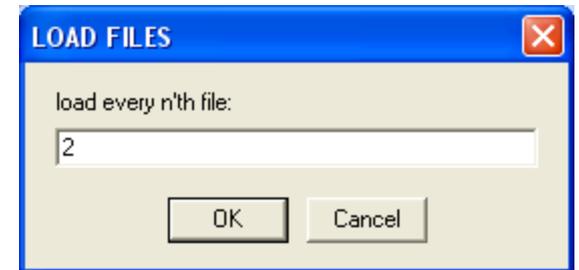
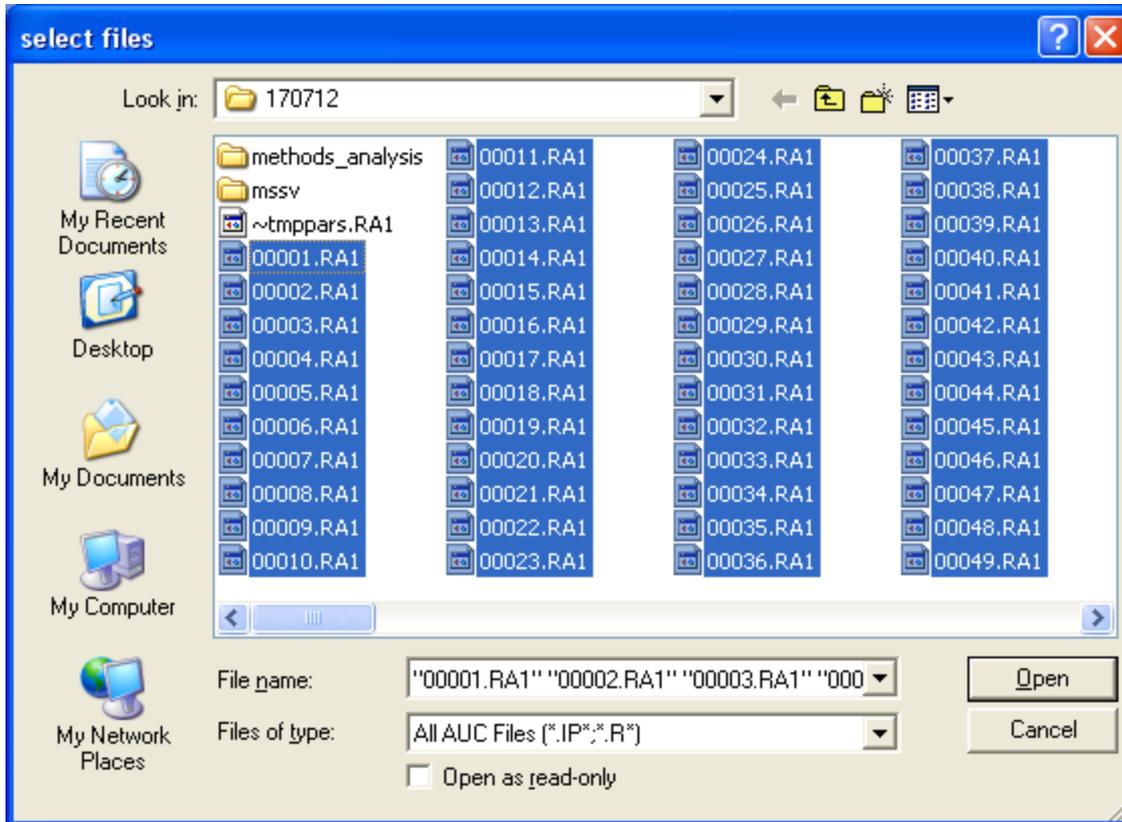
# Set the Meniscus, Bottom, and Fitting Limits



# Step 6

Steps 1-5 were repeated for the “\*.RA1” data, i.e. the absorbance data acquired from Cell 1 (the same data range was used). There were a few differences (see pg. 17). In the Experimental Parameters box, the RI noise and TI noise boxes are unchecked for absorbance data by default. The TI noise box was therefore activated by the user; time-invariant noise correction is routinely applied to absorbance data to compensate for optical imperfections that manifest in such data. RI noise is inappropriate for absorbance data unless time-dependent baseline changes are observed. Also, the checkbox near to the “noise” input box labeled “\*sqrt (N1/Nx)” was activated. The calculation of the global reduced chi-squared (see Text for definition) allows data sets with larger numbers of data points to dominate parameter refinement. Checking this box compensates for this imbalance. The compensation is necessary because the IF experiment had approximately three times as many data points as the absorbance experiment. These substeps are described on the next four pages.

# Load the ABS280 Data



...every other file was loaded.

Absorbance files 1-101 are selected...

# Experimental Parameters #2

Experimental Parameters

(2) ABSORBANCE data for SEDVELOCITY

C:\AUC Data\Rosen\120106\170712\mssv\cell1\_abs.xp (00001.RA1 ...)

Comment

active

noise 0.0100  \*sqrt (N1/Nx)

Centerpiece 2

Pathlength 1.200000

Rotor type 0

no backdiffusion nece:

fit baseline

fit BI Noise

fit TI Noise

Meniscus 6.0000

Bottom 7.1940

redirect men./bot. 2

For Associating Systems:

extinction coefficient A 1.0000

extinction coefficient B 1.0000

extinction coefficient C 0.0000

redirect xt A 2

redirect xt B 2

redirect xt C 2

partial boundary fitting smin 0.00 smax 100.00

use for sigma of MC sims: 0.0100  use local rmsd

v-bar (ml/g) 0.7300

buffer density (g/ml) 1.00079

buffer viscosity (P) 0.010024

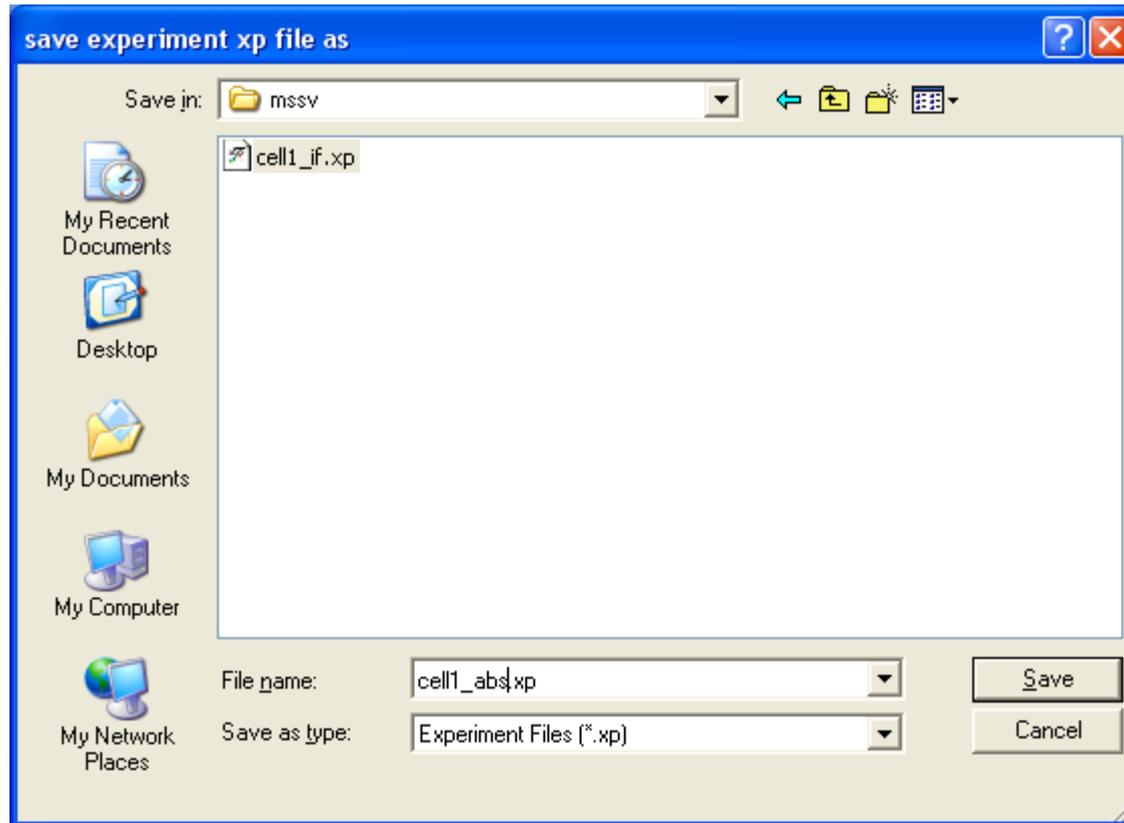
Temperature 20.0

Cancel

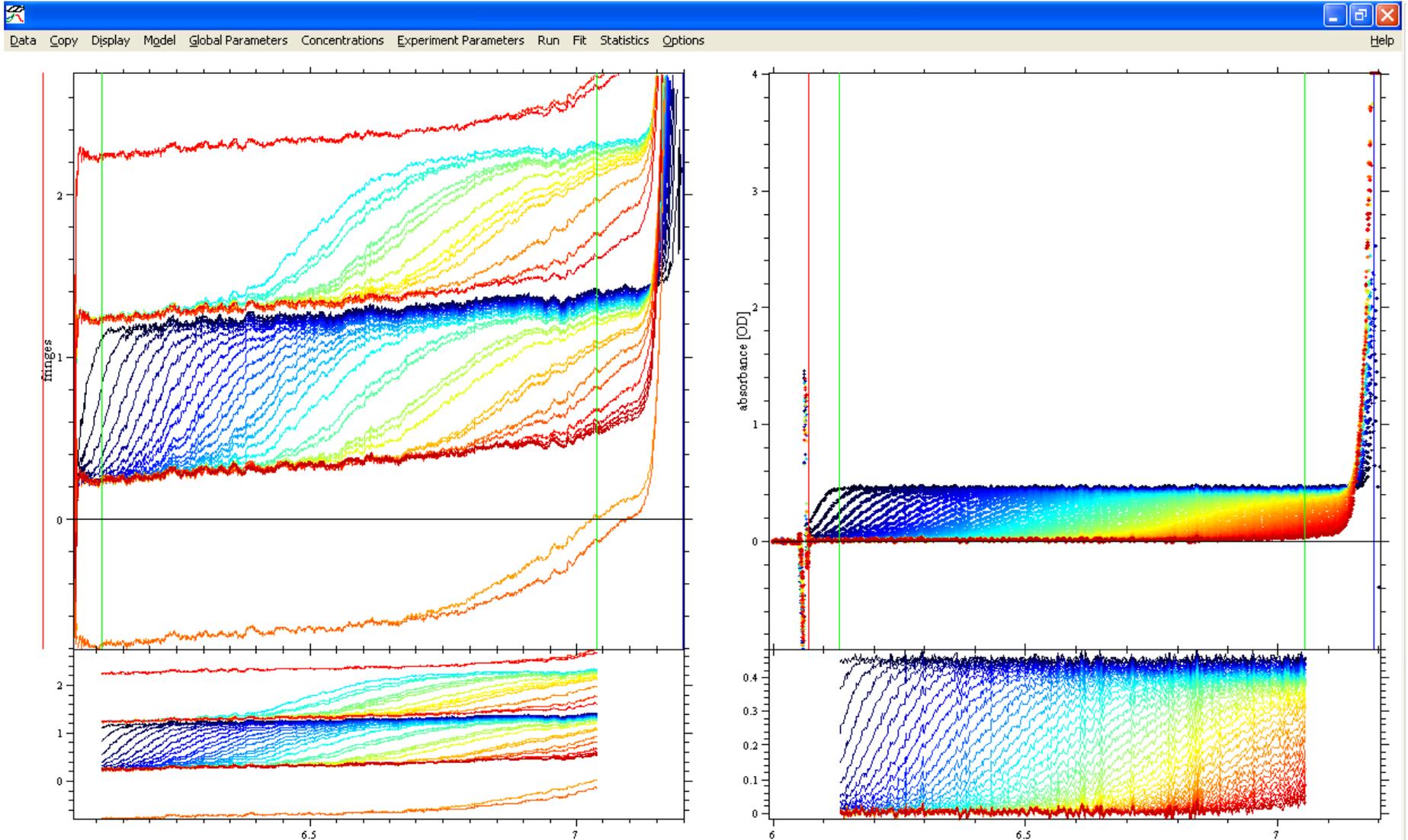
OK

These values should already be input for you by SEDPHAT.

# Save the Experiment



# Set Meniscus, Bottom, Fitting Limits for ABS Data



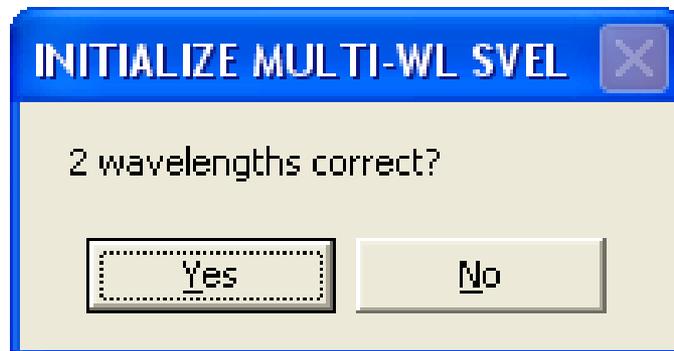
# Step 7

At this point, under the “Model” menu, the model “Multi-Wavelength Discrete/Continuous Distribution Analysis” was chosen. The user was prompted “2 wavelengths correct”; Yes was chosen. The program then asked the user if the data sets were in the “standard configuration,” i.e. if the data sets were loaded in the order wavelengths (N.B.—here, an IF data set is considered a “wavelength”). Yes was chosen. See the next two pages.

# Choose Model

Model	Global Parameters	Concentrations	Experiment Parameters	Run	Fit	Statistics	Options
Species Analysis							
Linear Fractional Boundary Model							
Species Analysis with Mass Conservation Constraints							
Global Discrete Distribution							
Hybrid Local Continuous Distribution and Global Discrete Species							
Hybrid Global Continuous Distribution and Global Discrete Species							
Multi-Wavelength Discrete/Continuous Distribution Analysis							
Single Nonideal Species							
A (Single Species of Interacting System)							
A <-> A* (Single Species in Two Conformations)							
Monomer-Dimer Self-Association							
Monomer-Trimer Self-Association							
Monomer-n-mer Self-Association							
Monomer-Dimer-Tetramer Self-Association							
Monomer-Tetramer-Octamer Self-Association							
Monomer-'m-mer'-'n-mer' Self-Association							
A+B <-> AB Hetero-Association							
A+B+B <-> {AB}+B <-> ABB; with 2 symmetric sites, macroscop K							
A+B+B <-> AB+B <-> BA + B <-> BAB; with 2 non-symmetric sites, microscop K							
A+B+B+B <-> AB+B+B <-> ABB+B <-> ABBB; with 3 symmetric sites, macroscop K							
A+B+C <-> AB + C <-> AC + B; competing B and C for A							
A+B+B+C forming complexes AB, BA, BAB, BC, CB, BCB; competing A and C for B, microscop K							
A+B+B+C+C forming complexes AB, BA, BAB, AC, CA, CAC, BAC, CAB; competing B and C for 2 Sites on A, microscop K							
A+B+C <-> AB + C <-> AC + B <-> ABC; triple complex							
A+B+B+C forming complexes AB, BA, BAB, AC, ABC, BAC, BABC; quadruple complex, microscop K							
A+B+B+C+C forming complexes AB, BA, BAB, CB, CBA, ABC, CBAB, BABC, CBABC, quintuple complex, microsc. K							
(A+A)+B+B forming complexes (AA), AB, (AA)B, (AA)BB; self-asso. A with 2 symmetric sites, macroscop K							
(A+A)+(B+B) <-> A+AB+B <-> (AA)B+B <-> A+A(BB) <-> (AA)(BB); self-assoc A and B, macroscop K							
(A+A)+(B+B) forming complexes (AA), (BB), AB, (AB)(AB); self-assoc w heterodimer of homodimers							

# Choose Yes in Both Dialogs



# Step 8

The Global Parameters portion of the menu bar was clicked, opening the Global Parameters dialog box. The upper part of the box is for discrete species. This part was not used in this analysis; only continuous species were used. The lower part of the box allows up to three segments of  $s$ -space to be modeled with continuous distributions. See the next slide

# Introducing the MSSV Global Parameter Box.

Parameters for Multi-Wavelength Hybrid Local Continuous/Global Discrete Model

species 1  
 species 1  
 M1 0.0000  
 S1 0.000000  
xt wl 1 0.000  
xt wl 2 0.000

species 2  
 species 2  
 M2 0.0000  
 S2 0.000000  
xt wl 1 0.000  
xt wl 2 0.000

species 3  
 species 3  
 M3 0.0000  
 S3 0.000000  
xt wl 1 0.000  
xt wl 2 0.000

species 4  
 species 4  
 M4 0.0000  
 S4 0.000000  
xt wl 1 0.000  
xt wl 2 0.000

species 5  
 species 5  
 M5 0.0000  
 S5 0.000000  
xt wl 1 0.000  
xt wl 2 0.000

species 6  
 species 6  
 M6 0.0000  
 S6 0.000000  
xt wl 1 0.000  
xt wl 2 0.000

species 7  
 species 7  
 M7 0.0000  
 S7 0.000000  
xt wl 1 0.000  
xt wl 2 0.000

species 8  
 species 8  
 M8 0.0000  
 S8 0.000000  
xt wl 1 0.000  
xt wl 2 0.000

species 9  
 species 9  
 M9 0.0000  
 S9 0.000000  
xt wl 1 0.000  
xt wl 2 0.000

link molar mass  
 link molar mass  
in multiples of # 1  
for species from #  
2 to # 4

link s values  
 link s values  
in multiples of # 1  
for species from #  
2 to # 4

continuous  
 segment 1  
resolution 50  
s min 0.200  
s max 10.000  
frictional ratio 1.500  
xt1/chr 1.000  
xt2/chr 0.000  
PP  
fit ffo  
 spectrum 1  
 spectrum 2 0.000 0.000 PP

discrete spectra in multiples of chromophore xt  
contin. spectra in multiples of chromophore xt  
xt wl 1 xt wl 2  
chromophore #1 193089.0 96000.0  
chromophore #2 0.000 0.000

segment 2  
 segment 2  
resolution 0  
s min 17.000  
s max 60.000  
frictional ratio 1.200  
xt1/chr 0.000  
xt2/chr 0.000  
PP  
fit ffo  
 spectrum 1  
 spectrum 2 0.000 0.000 PP

with Tikhonov Regularization P= 0.700  
 normalize distributions

segment 3  
 segment 3  
resolution 0  
s min 17.000  
s max 60.000  
frictional ratio 1.200  
xt1/chr 0.000  
xt2/chr 0.000  
PP  
fit ffo  
 spectrum 1  
 spectrum 2 0.000 0.000 PP

Cancel  
OK

The upper part of this box will not be used in these analyses; it is omitted in the following.

# Step 8, continued

The checkbox next to “segment 1” was checked, activating this segment. This segment, which will be evaluated as a  $c_{\text{GST-VCA}}(s)$  distribution, was given a resolution of 50, and the “smin” and “smax” values were set to 0.2 and 10, respectively. The frictional ratio was left at the default value of 1.5, and the “fit ff0” checkbox, which would allow the refinement of the frictional ratio, was deactivated.

The screenshot shows a software interface with three segments and a right-hand panel. The first segment is active and highlighted with a red box.

segment	continuous	resolution	s min	s max	frictional ratio	fit ff0
segment 1	<input checked="" type="checkbox"/>	50	0.200	10.000	1.500	<input type="checkbox"/>
segment 2	<input type="checkbox"/>	0	17.000	60.000	1.200	<input type="checkbox"/>
segment 3	<input type="checkbox"/>	0	17.000	60.000	1.200	<input type="checkbox"/>

Additional parameters for segment 1:

xt1/chr.	xt2/chr.
1.000	0.000
0.000	0.000

Right-hand panel options:

- discrete spectra in multiples of chromophore xt
- contin. spectra in multiples of chromophore xt
- chromophore #1:  193089.  96000.0
- chromophore #2:  0.000  0.000
- with Tikhonov Regularization P= 0.700
- normalize distributions

Buttons: Cancel, OK

# Step 8, continued

Up to two “spectra” may be used in a single segment (for a two-signal experiment). Each spectrum is a single  $c_k(s)$  distribution. Because only one component was evaluated here, only a single spectrum is necessary. Therefore, the checkbox to the left of “spectrum 2” was deactivated. The two boxes to the right of “spectrum 1” describe the molar ratio of up to two chromophores (macromolecules) that were to be modeled by the “spectrum 1”  $c_k(s)$  distribution. Because only one chromophore is present, a 1:0 molar ratio of chromophore 1 (GST-VCA) to chromophore 2 (not present) was to be modeled. In other words, spectrum 1 will represent a  $c_{\text{GST-VCA}}(s)$  distribution. Therefore, “1” and “0” were entered into the left and right boxes, respectively, next to spectrum 1.

The screenshot shows a software interface with the following configuration options:

- Segment 1 (checked):**
  - continuous:
  - resolution: 50
  - lineal/log:  lineal
  - s min: 0.200
  - s max: 10.000
  - frictional ratio: 1.500
  - fit fff
- Spectrum 1 (checked):**
  - xt1/chr: 1.000
  - xt2/chr: 0.000
  - PP: PP
- Spectrum 2 (unchecked):**
  - xt1/chr: 0.000
  - xt2/chr: 0.000
  - PP: PP
- Segment 2 (unchecked):**
  - continuous:
  - resolution: 0
  - lineal/log:  lineal
  - s min: 17.000
  - s max: 60.000
  - frictional ratio: 1.200
  - fit fff
- Chromophore Settings:**
  - discrete spectra in multiples of chromophore xt
  - contin. spectra in multiples of chromophore xt
  - chromophore #1: xt wl 1 = 193089, xt wl 2 =  96000.0
  - chromophore #2: xt wl 1 = 0.000, xt wl 2 = 0.000
- Other Settings:**
  - with Tikhonov Regularization P= 0.700
  - normalize distributions



# Step 8, continued

Finally, the “extinction” information for GST-VCA must be entered in the area of the box above the Tikhonov regularization section. The four boxes in this area represent a 2x2 extinction matrix possible for 2 species with data obtained at 2 wavelengths, which is the matrix E in Equation 7. Because only one “chromophore” is present, only the “chromophore #1” row of the matrix will be used. The left-hand column, labeled “xt wl 1,” is for the signal increments for experiment 1. Here, we use the straightforward and very reliable estimate of the IF signal increment for GST-VCA ( $\epsilon_{\text{IF}}^{\text{GST-VCA}}$ ) to be 2.75 multiplied by the molar mass of the protein [2,3]. For GST-VCA,  $\epsilon_{\text{IF}}^{\text{GST-VCA}} = 193,089 \text{ fringes}\cdot\text{M}^{-1}\cdot\text{cm}^{-1}$ . Note that this calculation was based on the dimer molecular mass of GST-VCA (70,214 g/mol, calculated from the sequence of the protein). Thus, all concentrations for this protein in this protocol will be for the dimer, not for the monomer. The  $\epsilon_{\text{IF}}^{\text{GST-VCA}}$  was fixed in the analysis, and so the checkbox to its left remained unchecked. An estimate for the molar extinction coefficient of GST-VCA at 280 nm ( $\epsilon_{\text{ABS280}}^{\text{GST-VCA}}$ ) is  $96,000 \text{ M}^{-1}\cdot\text{cm}^{-1}$ , obtained from SEDNTERP. This value was input into the right-hand box, and the checkbox to its left was activated, allowing this value to refine. See the next page.

# Extinction Info

<input checked="" type="checkbox"/> continuous <input checked="" type="checkbox"/> segment 1	resolution <input type="text" value="50"/>	<input checked="" type="radio"/> linear <input type="radio"/> log	s min <input type="text" value="0.200"/>	s max <input type="text" value="10.000"/>	frictional ratio <input type="text" value="1.500"/>	<input type="checkbox"/> fit ffo
<input checked="" type="checkbox"/> spectrum 1	xt1/chr <input type="text" value="1.000"/>	xt2/chr <input type="text" value="0.000"/>	<input type="text" value="PP"/>			
<input type="checkbox"/> spectrum 2	<input type="text" value="0.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>			

<input type="checkbox"/> segment 2	resolution <input type="text" value="0"/>	<input checked="" type="radio"/> linear <input type="radio"/> log	s min <input type="text" value="17.000"/>	s max <input type="text" value="60.000"/>	frictional ratio <input type="text" value="1.200"/>	<input type="checkbox"/> fit ffo
<input checked="" type="checkbox"/> spectrum 1	xt1/chr <input type="text" value="0.000"/>	xt2/chr <input type="text" value="0.000"/>	<input type="text" value="PP"/>			
<input checked="" type="checkbox"/> spectrum 2	<input type="text" value="0.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>			

<input type="checkbox"/> segment 3	resolution <input type="text" value="0"/>	<input checked="" type="radio"/> linear <input type="radio"/> log	s min <input type="text" value="17.000"/>	s max <input type="text" value="60.000"/>	frictional ratio <input type="text" value="1.200"/>	<input type="checkbox"/> fit ffo
<input checked="" type="checkbox"/> spectrum 1	xt1/chr <input type="text" value="0.000"/>	xt2/chr <input type="text" value="0.000"/>	<input type="text" value="PP"/>			
<input checked="" type="checkbox"/> spectrum 2	<input type="text" value="0.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>			

<input checked="" type="checkbox"/> discrete spectra in multiples of chromophore xt						
<input checked="" type="checkbox"/> contin. spectra in multiples of chromophore xt						
chromophore #1	<input type="checkbox"/> xt wl 1 <input type="text" value="193089."/>	<input checked="" type="checkbox"/> xt wl 2 <input type="text" value="96000.0"/>				
chromophore #2	<input type="checkbox"/> <input type="text" value="0.000"/>	<input type="checkbox"/> <input type="text" value="0.000"/>				

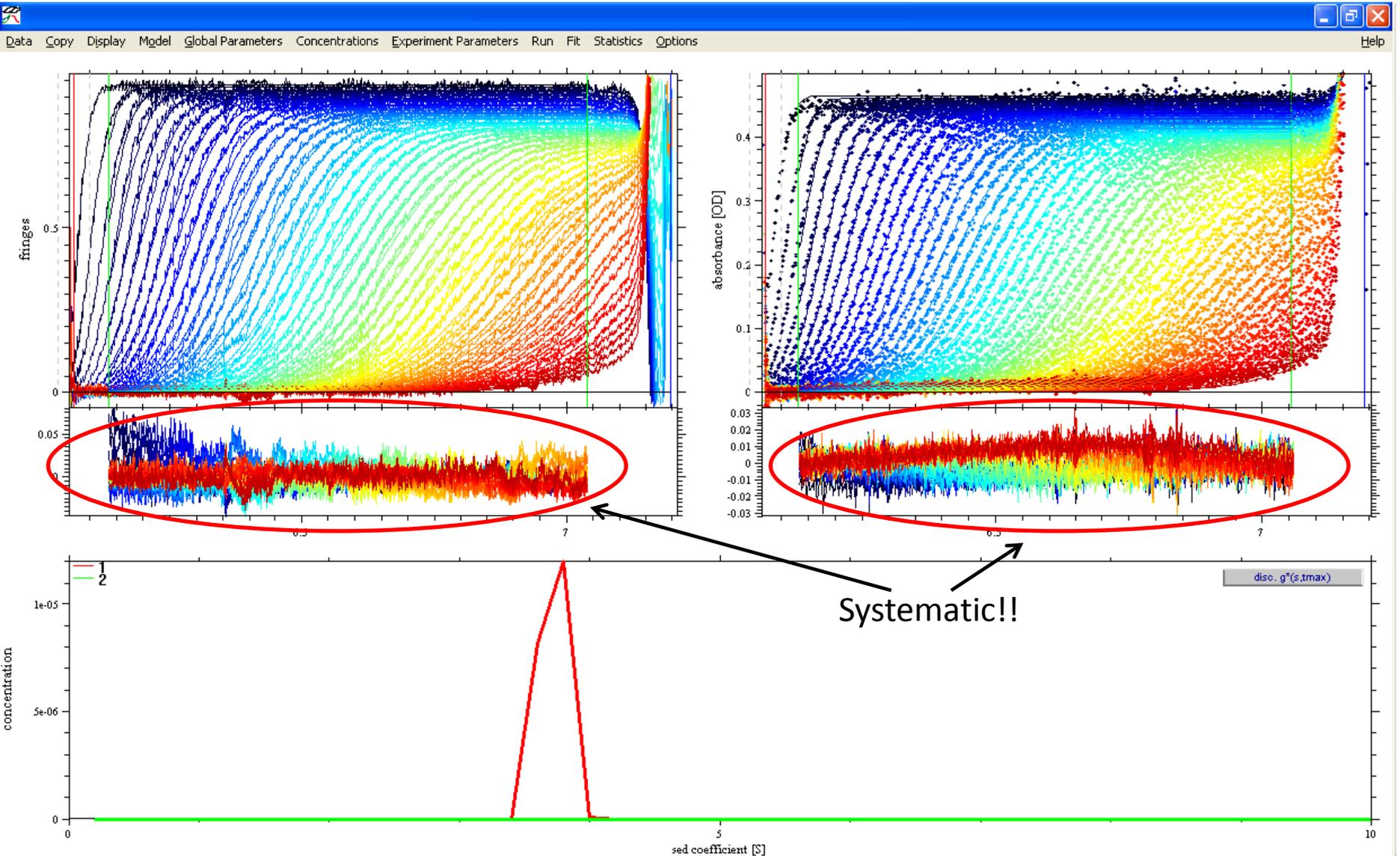
  

<input checked="" type="checkbox"/> with Tikhonov Regularization P=	<input type="text" value="0.700"/>
<input checked="" type="checkbox"/> normalize distributions	

# Step 9

A Global Run was performed by selecting the “Global Run” item in the “Run” dropdown menu. This action optimized the linear parameters, both global (the  $c_{\text{GST-VCA}}(s)$  distribution) and local (the noise elements) to each experiment. For the ease of visually inspecting the quality of the fits to the experimental data, the RI and TI noise were subtracted from the data. The quality of the fit (rmsd’s of 0.01 and 0.006 for the IF and  $A_{280}$  data, respectively) was deemed to be acceptable to proceed. There is very significant systematicity in the residuals at this point (see circled areas on next page); we hoped that this would be resolved by parameter refinement. The  $c_{\text{GST-VCA}}(s)$  distribution shows a single peak at  $\sim 3.7$  S. See the next page.

# After a Global Run



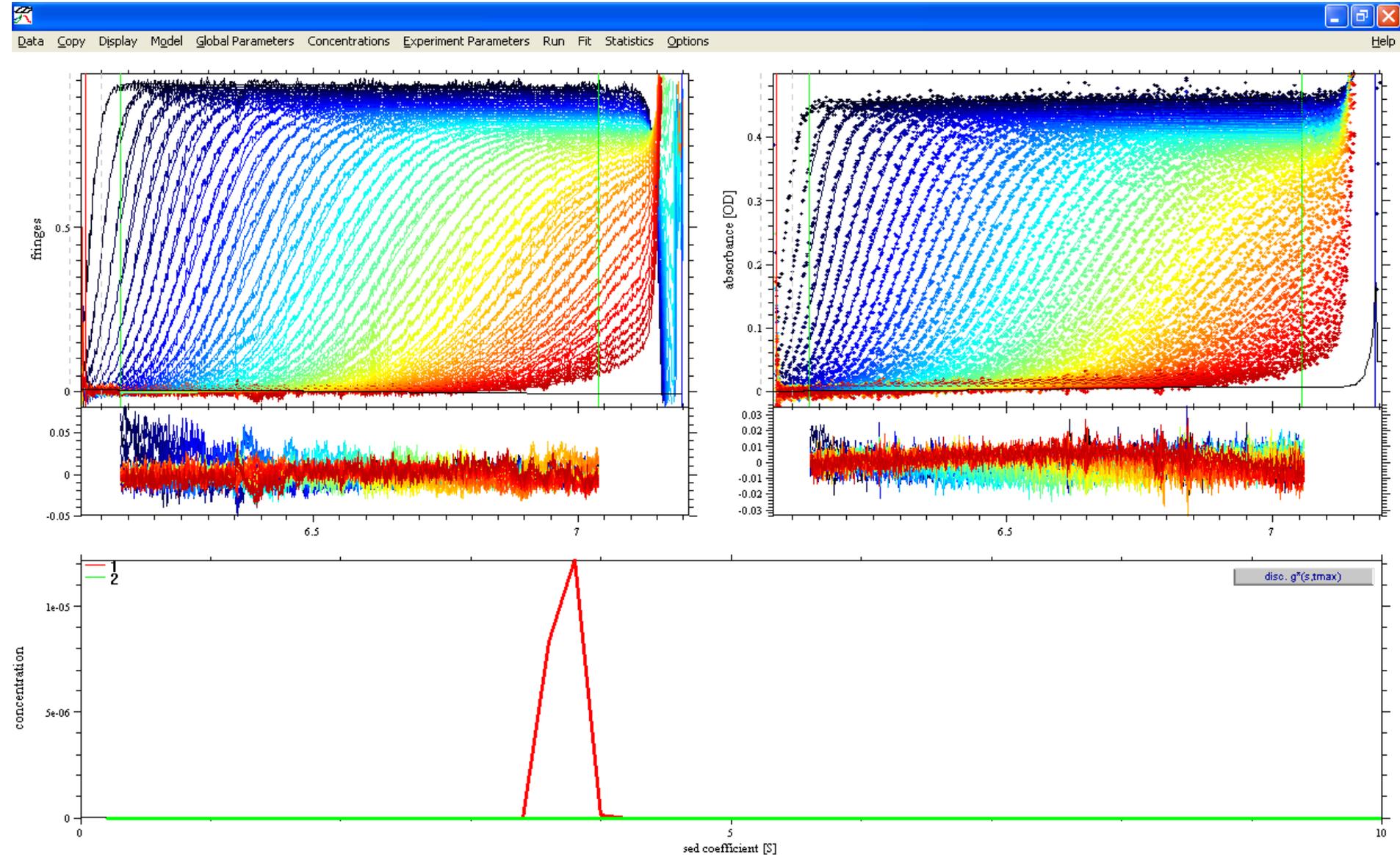
# A Note on Noise Subtraction

On the previous page, radially invariant (RI) and time-invariant (TI) noise had been subtracted from both of the experiments. The subtraction can be accomplished in one of two ways: one can go to the “Display” menu item, click on it, then choose “Subtract All Systematic Noise From Raw Data”; or, one can use the simple keyboard shortcut of pressing “Ctrl-N.”

# Step 10

A Global Fit was initiated by selecting the menu item Fit and clicking on “Global Fit.” This sequence iteratively optimized the nonlinear parameters. Because the menisci and frictional ratio are fixed, the only nonlinear parameter that was optimized during this analysis was  $\epsilon_{\text{ABS280}}^{\text{GST-VCA}}$ . The fit improved slightly, but the rounded rmsd’s were still 0.01 and 0.006. There is still significant systematicity to the residuals. Examination of the Global Parameters demonstrated that  $\epsilon_{\text{ABS280}}^{\text{GST-VCA}}$  had refined to  $92268.2 \text{ M}^{-1}\cdot\text{cm}^{-1}$ . SEDPHAT can use one of three nonlinear optimization algorithms: Simplex, Marquardt-Levenberg, and Simulated Annealing. Simplex is set by default, and it was this method that was used for this initial optimization. The result is shown on the next page.

# After a Global Fit



# Step 11

The remaining appropriate nonlinear parameters were set to refine by checking the “fit ff0” box for segment 1 in the Global Parameter box, and clicking on the checkbox to the left of “Meniscus” in the Experimental Parameter boxes of both experiments. After accepting each of the Experimental Parameter boxes, the user was prompted for the fitting limits for the respective meniscus. These limits are hard; the program does not allow for the refinement of the meniscus outside these bounds. The default limits were the current meniscus  $\pm 0.03$  cm. Because the menisci had been visually chosen to be at acceptable values, the defaults were accepted. Experience has shown that the Marquardt-Levenberg fitting algorithm is the most efficient means to a converged fit in such analyses, so it was selected by clicking on “Options,” then “Fitting Options,” then “Marquardt-Levenberg.” The pictorial view of the substeps of Step 11 are illustrated on the next four pages.

# Fit ff0 Now (check its box)

continuous	resolution	s min	s max	frictional ratio	
<input checked="" type="checkbox"/> segment 1	<input type="text" value="50"/>	<input type="radio"/> lineal <input checked="" type="radio"/> log	<input type="text" value="0.200"/>	<input type="text" value="10.000"/>	<input type="text" value="1.500"/>
<input checked="" type="checkbox"/> spectrum 1	<input type="text" value="1.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>	<input checked="" type="checkbox"/> fit ff0	
<input type="checkbox"/> spectrum 2	<input type="text" value="0.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>		

<input type="checkbox"/> segment 2	resolution	s min	s max	frictional ratio	
	<input type="text" value="0"/>	<input type="radio"/> lineal <input checked="" type="radio"/> log	<input type="text" value="17.000"/>	<input type="text" value="60.000"/>	<input type="text" value="1.200"/>
<input checked="" type="checkbox"/> spectrum 1	<input type="text" value="0.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>	<input type="checkbox"/> fit ff0	
<input checked="" type="checkbox"/> spectrum 2	<input type="text" value="0.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>		

<input type="checkbox"/> segment 3	resolution	s min	s max	frictional ratio	
	<input type="text" value="0"/>	<input type="radio"/> lineal <input checked="" type="radio"/> log	<input type="text" value="17.000"/>	<input type="text" value="60.000"/>	<input type="text" value="1.200"/>
<input checked="" type="checkbox"/> spectrum 1	<input type="text" value="0.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>	<input type="checkbox"/> fit ff0	
<input checked="" type="checkbox"/> spectrum 2	<input type="text" value="0.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>		

<input checked="" type="checkbox"/> discrete spectra in multiples of chromophore xt				
<input checked="" type="checkbox"/> contin. spectra in multiples of chromophore xt				
	xt wl 1	xt wl 2		
chromophore #1	<input type="text" value="193089"/>	<input checked="" type="checkbox"/> <input type="text" value="92268.2"/>		
chromophore #2	<input type="text" value="0.000"/>	<input type="checkbox"/> <input type="text" value="0.000"/>		

<input checked="" type="checkbox"/> with Tikhonov Regularization P=	<input type="text" value="0.700"/>
<input checked="" type="checkbox"/> normalize distributions	

Cancel  
OK

# After Clicking on Experimental Parameters, Activate Meniscus Fitting

The image displays a software interface for configuring experimental parameters. The main window, titled "Experimental Parameters", contains the following fields and options:

- Experiment name: (1) INTERFERENCE data for SEDVELOCITY
- File path: C:\AUC Data\Rosen\120106\170712\mssv\cell1\_if.xp (00001.IP1 ...)
- Comment: [Empty]
- Active:
- Noise: 0.0100 (with  \*sqrt (N1/Nx))
- Centerpiece: 2
- Pathlength: 1.200000
- Rotor type: 0
- no backdiffusion neces:
- v-bar (ml/g): 0.7300
- buffer density (g/ml): 1.000790
- buffer viscosity (P): 0.010024
- Temperature: 20.0
- fit baseline:
- fit RI Noise:
- fit TI Noise:
- Meniscus:  (highlighted with a red box)
- Bottom:
- redirect men./bot: 1
- For Associating Systems:
  - extinction coefficient A: 1.0000
  - extinction coefficient B: 1.0000
  - extinction coefficient C: 0.0000
- redirect xt A: 1
- redirect xt B: 1
- redirect xt C: 1
- partial boundary fitting:  (smin: 0.00, smax: 100.00)
- use for sigma of MC sims: 0.0100 (radio button)
- use local rmsd:

Two dialog boxes are shown to the right, both titled "DATA #1: set range for meniscus var...":

- The top dialog box prompts for the "enter upper limit" and has the value 6.099084 entered.
- The bottom dialog box prompts for the "enter lower limit" and has the value 6.093083 entered.

On the left, a smaller window titled "EDIT EXPERIMENTAL PARAMETERS" shows "edit experiment#" with the value 1.000000 and "OK" and "Cancel" buttons.

# Activate Meniscus Fitting for Experiment #2

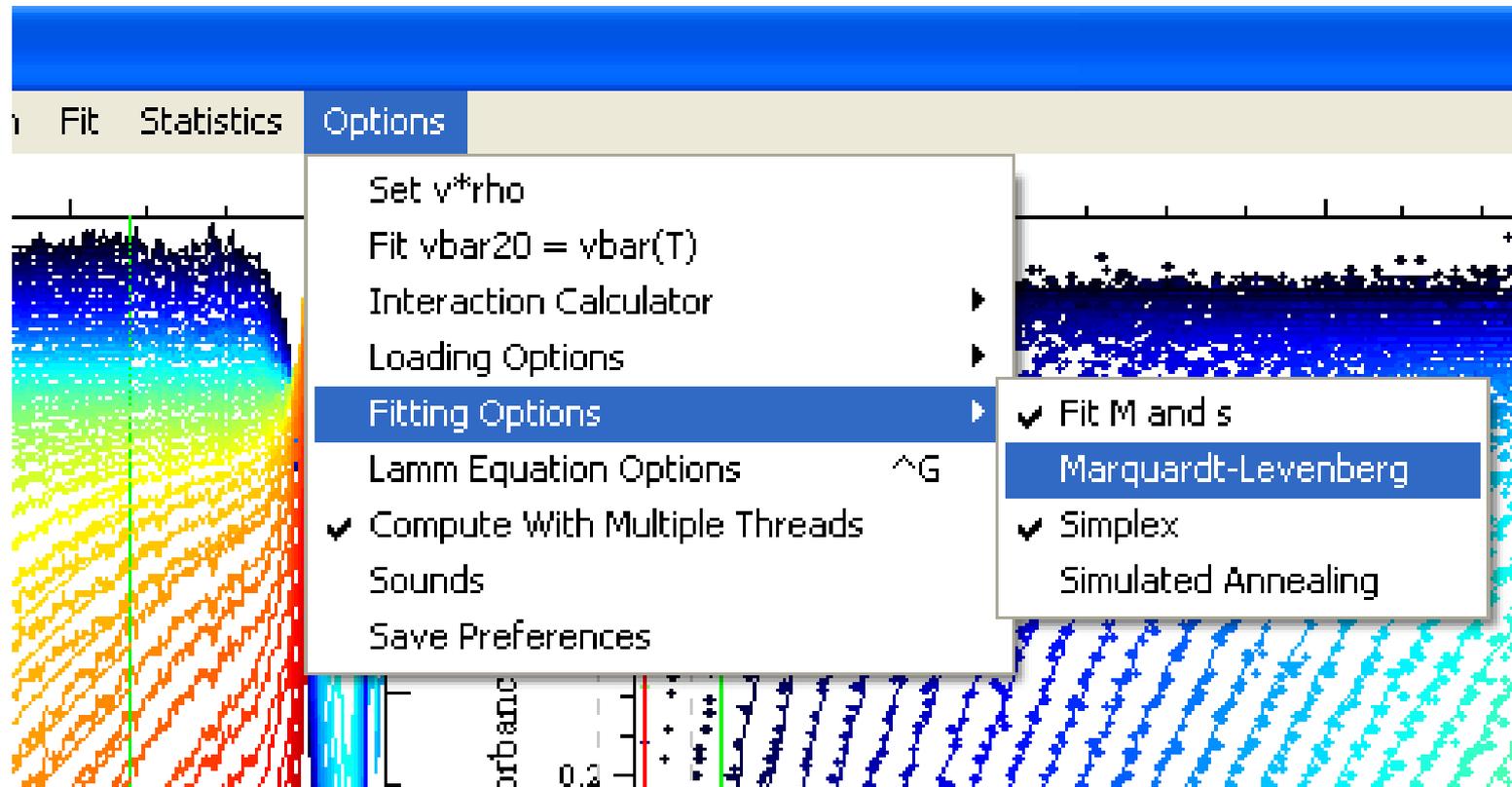
The image displays a software interface for editing experimental parameters. The main window, titled "Experimental Parameters", shows the following settings:

- edit experiment#: 2.000000
- File path: C:\AUC Data\Rosen\120106\170712\mssv\cell1\_abs.xp (00001.RA1 ...)
- Comment: [empty]
- active:
- noise: 0.0100  \*sqrt (N1/Nx)
- Centerpiece: 2
- Pathlength: 1.200000
- Rotor type: 0
- no backdiffusion neces:
- v-bar (ml/g): 0.7300
- buffer density (g/ml): 1.000790
- buffer viscosity (P): 0.010024
- Temperature: 20.0
- fit baseline:
- fit RI Noise:
- fit TI Noise:
- Meniscus:  (highlighted with a red box) 6.0712
- Bottom:  7.1940
- redirect men./bot:  2
- For Associating Systems:
  - extinction coefficient A: 1.0000
  - extinction coefficient B: 1.0000
  - extinction coefficient C: 0.0000
- redirect xt A:  2
- redirect xt B:  2
- redirect xt C:  2
- partial boundary fitting:  smin: 0.00 smax: 100.00
- use for sigma of MC sims: 0.0100  use local rmsd:

Two dialog boxes are open to set the range for meniscus fitting:

- "DATA #2: set range for meniscus var..." dialog: enter upper limit: 6.101200
- "DATA #2: set range for meniscus var..." dialog: enter lower limit: 6.041200

# Change the Fitting Algorithm



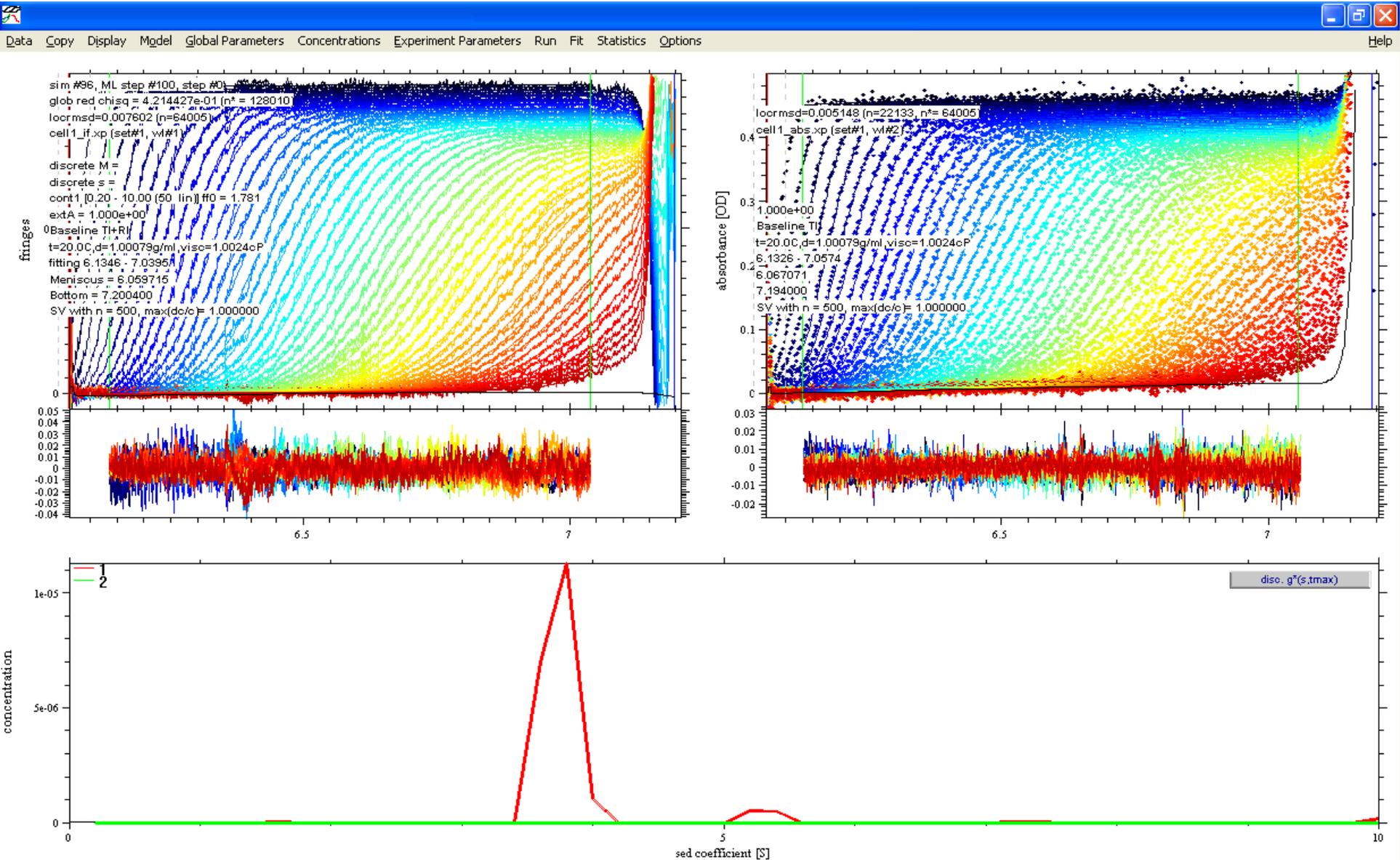
# Step 12

Another Global Fit was performed. After about three minutes, the fit converged.

# Step 13

The quality of the fit was inspected (see the next two pages). The rmsd's were markedly improved to 0.008 fringes for the IF data and 0.005 OD for the  $A_{280}$  data. Additionally, the residuals exhibit much less systematicity. Now, the  $c_{\text{GST-VCA}}(s)$  distribution exhibited two peaks: one at 3.7 S, and the other at 5.3 S. Examination of the Global Parameters yielded two significant facts: the  $\epsilon_{\text{ABS280}}^{\text{GST-VCA}}$  had refined to  $92,416.2 \text{ M}^{-1}\cdot\text{cm}^{-1}$ , and the  $f_r$  had refined to 1.781. The latter value indicated that GST-VCA has a significantly elongated shape in solution. Of course, the refinement of  $f_r$  is sensitive to the value of  $v_{\text{bar}}$ . Thus, the 1.781 value reported here is not the correct value for GST-VCA, because the default value of  $v_{\text{bar}}$  was accepted (Step 2). However, inputting the correct value of  $v_{\text{bar}}$  ( $0.7355\text{cm}^3/\text{g}$ ) does not significantly change the refined value for  $f_r$  (1.743). The next page shows a screenshot of the fitting session after this Global Fit, and the page after shows the resulting parameters in the Global Parameter box.

# After another Global Fit



# The Resulting Parameters

continuous segment 1

resolution	<input type="text" value="50"/>	<input checked="" type="radio"/> linear	<input type="text" value="0.200"/>	<input type="text" value="10.000"/>	frictional ratio	<input type="text" value="1.781"/>
		<input type="radio"/> log			<input checked="" type="checkbox"/> fit f0	
<input checked="" type="checkbox"/> spectrum 1	xt1/chr' <input type="text" value="1.000"/>	xt2/chr' <input type="text" value="0.000"/>	<input type="text" value="PP"/>			
<input type="checkbox"/> spectrum 2	<input type="text" value="0.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>			

segment 2

resolution	<input type="text" value="0"/>	<input checked="" type="radio"/> linear	<input type="text" value="17.000"/>	<input type="text" value="60.000"/>	frictional ratio	<input type="text" value="1.200"/>
		<input type="radio"/> log			<input type="checkbox"/> fit f0	
<input checked="" type="checkbox"/> spectrum 1	xt1/chr' <input type="text" value="0.000"/>	xt2/chr' <input type="text" value="0.000"/>	<input type="text" value="PP"/>			
<input checked="" type="checkbox"/> spectrum 2	<input type="text" value="0.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>			

segment 3

resolution	<input type="text" value="0"/>	<input checked="" type="radio"/> linear	<input type="text" value="17.000"/>	<input type="text" value="60.000"/>	frictional ratio	<input type="text" value="1.200"/>
		<input type="radio"/> log			<input type="checkbox"/> fit f0	
<input checked="" type="checkbox"/> spectrum 1	xt1/chr' <input type="text" value="0.000"/>	xt2/chr' <input type="text" value="0.000"/>	<input type="text" value="PP"/>			
<input checked="" type="checkbox"/> spectrum 2	<input type="text" value="0.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>			

discrete spectra in multiples of chromophore xt  
 contin. spectra in multiples of chromophore xt

	xt wl 1	xt wl 2
chromophore #1	<input type="checkbox"/> <input type="text" value="193089."/>	<input checked="" type="checkbox"/> <input type="text" value="92416.2"/>
chromophore #2	<input type="checkbox"/> <input type="text" value="0.000"/>	<input type="checkbox"/> <input type="text" value="0.000"/>

with Tikhonov Regularization P=

normalize distributions

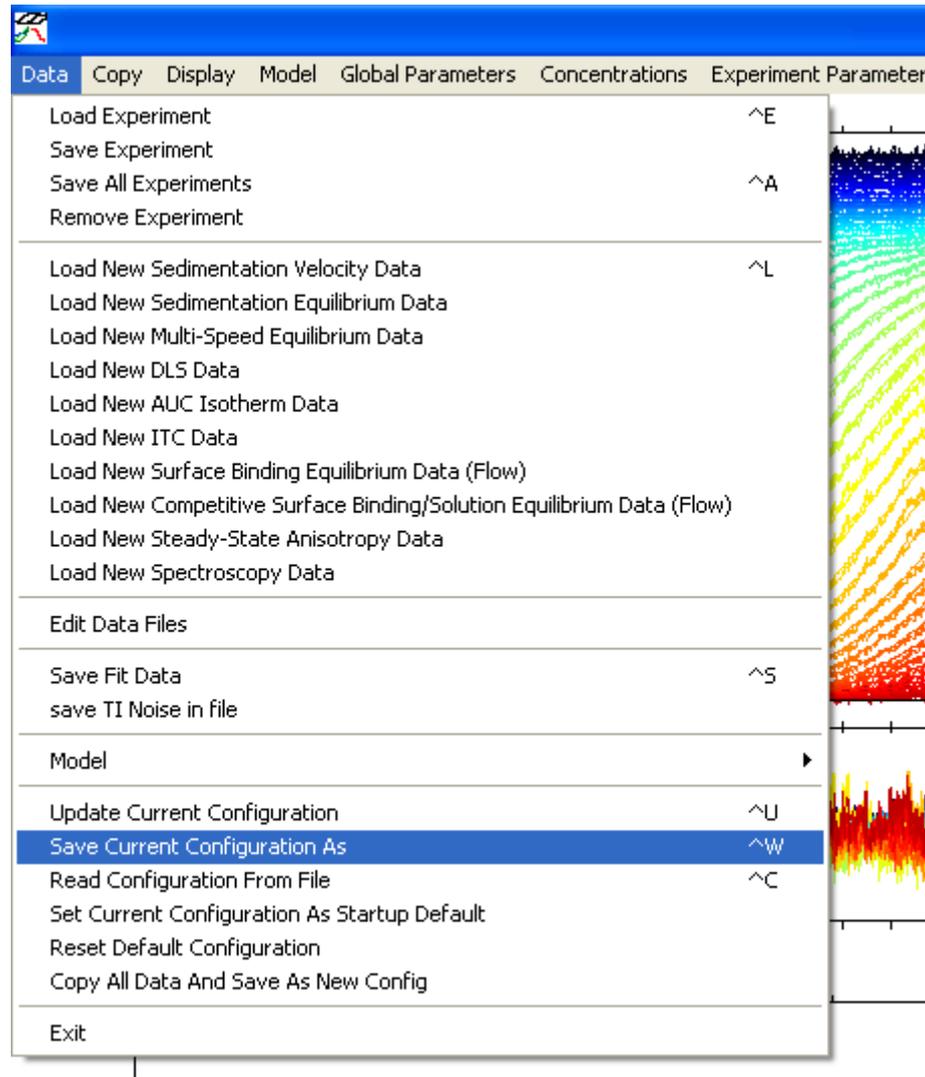
Cancel

OK

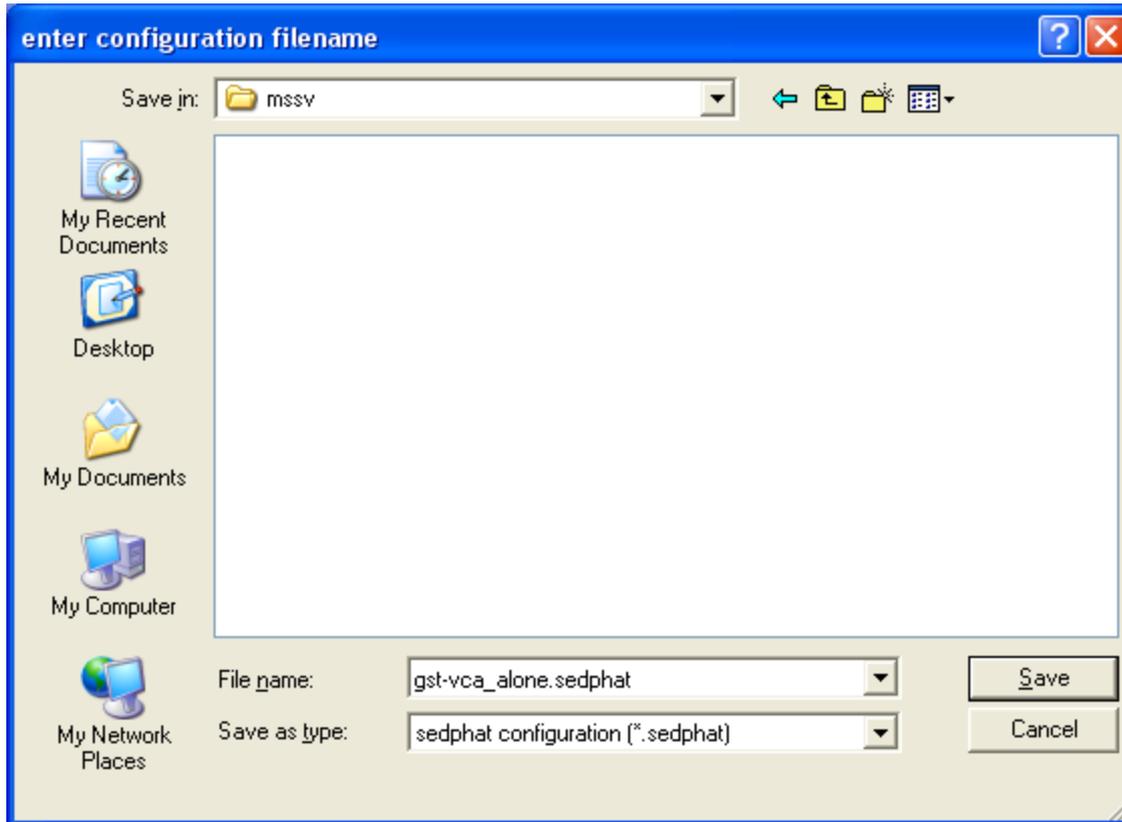
# Step 14

The fitted parameters were saved by clicking on the menu item “Data” and selecting “Save Current Configuration As.” The user was prompted to save new copies of the experimental parameter files with updated parameters. This option was accepted. See the next two pages. It is a good idea at this point to note down the refined  $\epsilon_{\text{ABS280}}^{\text{GST-VCA}}$  so that it can be easily recalled for input later (Step 21). Of course this value can be recalled at a later time by loading the saved configuration into SEDPHAT.

# Save the Configuration



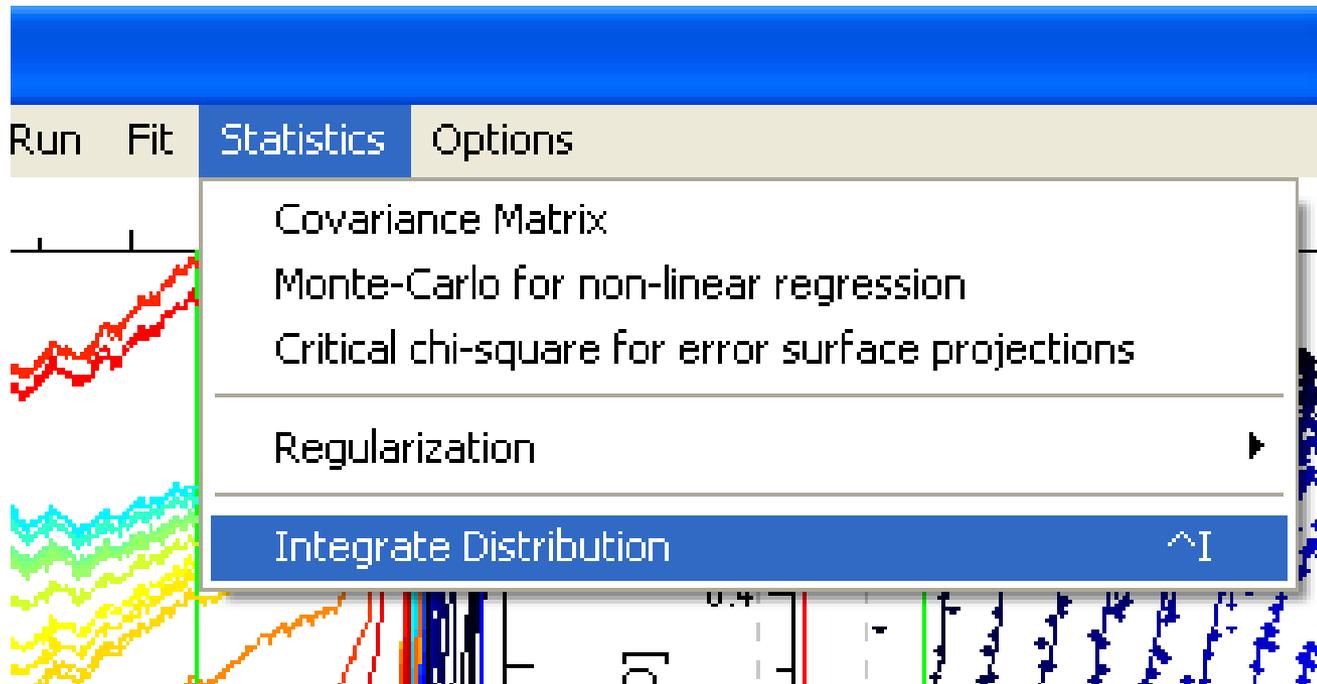
# Saving...



# Step 15

Under Statistics, the “Integrate Distribution” option was selected. The user was prompted to “determine the range by drawing a rectangle while the right mouse button is down.” The entire distribution was highlighted by dragging over it with the right mouse button depressed. The resultant [GST-VCA] was 4.09  $\mu\text{M}$ . This process is illustrated in the next 3 pages.

# Integrate the Distribution



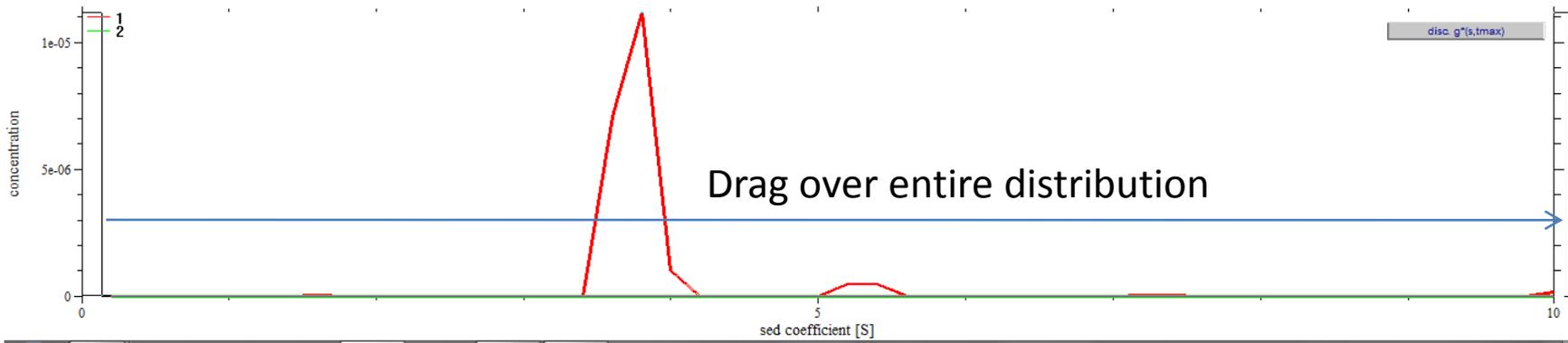
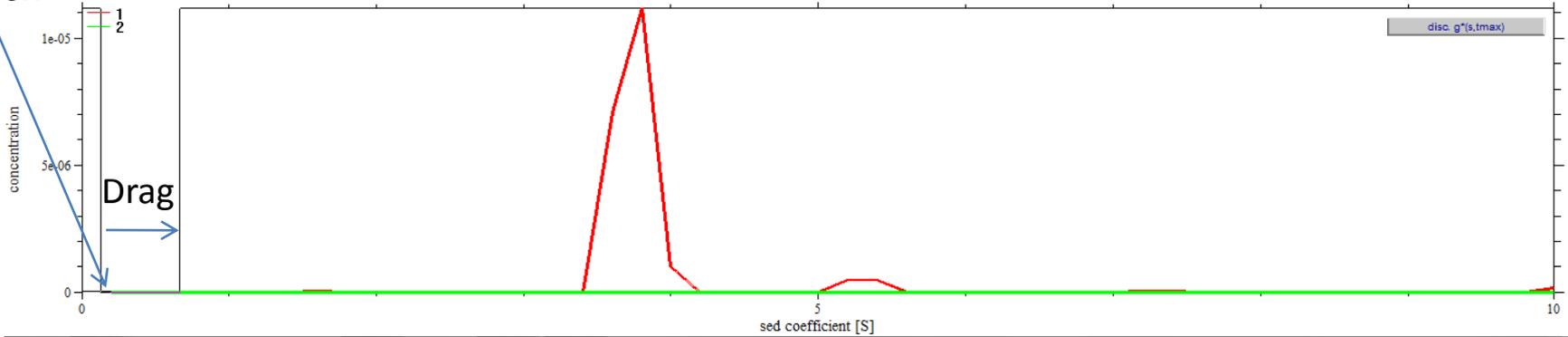
## SELECT INTEGRATION RANGE

determine the range by drawing a rectangle while the right mouse button is down

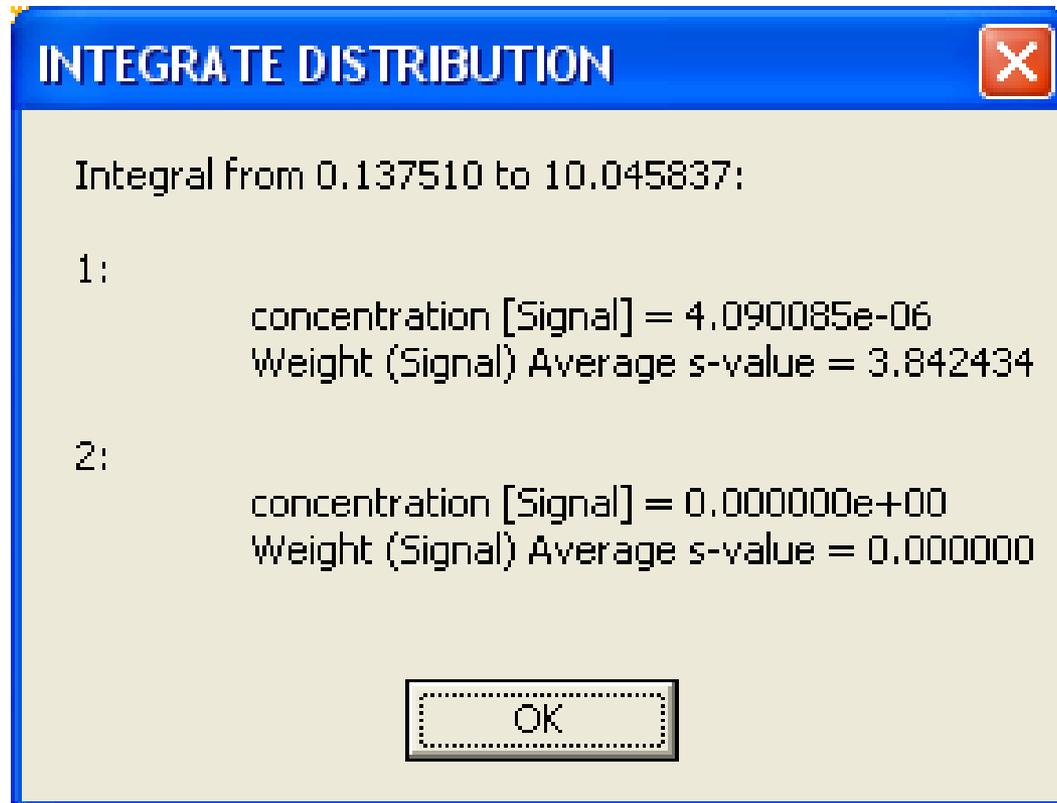
OK

# Right Click and Drag

Right click



# Integration



Incidentally, SEDPHAT automatically copies these values into the clipboard for you!

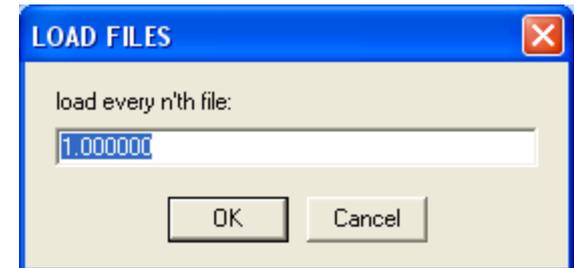
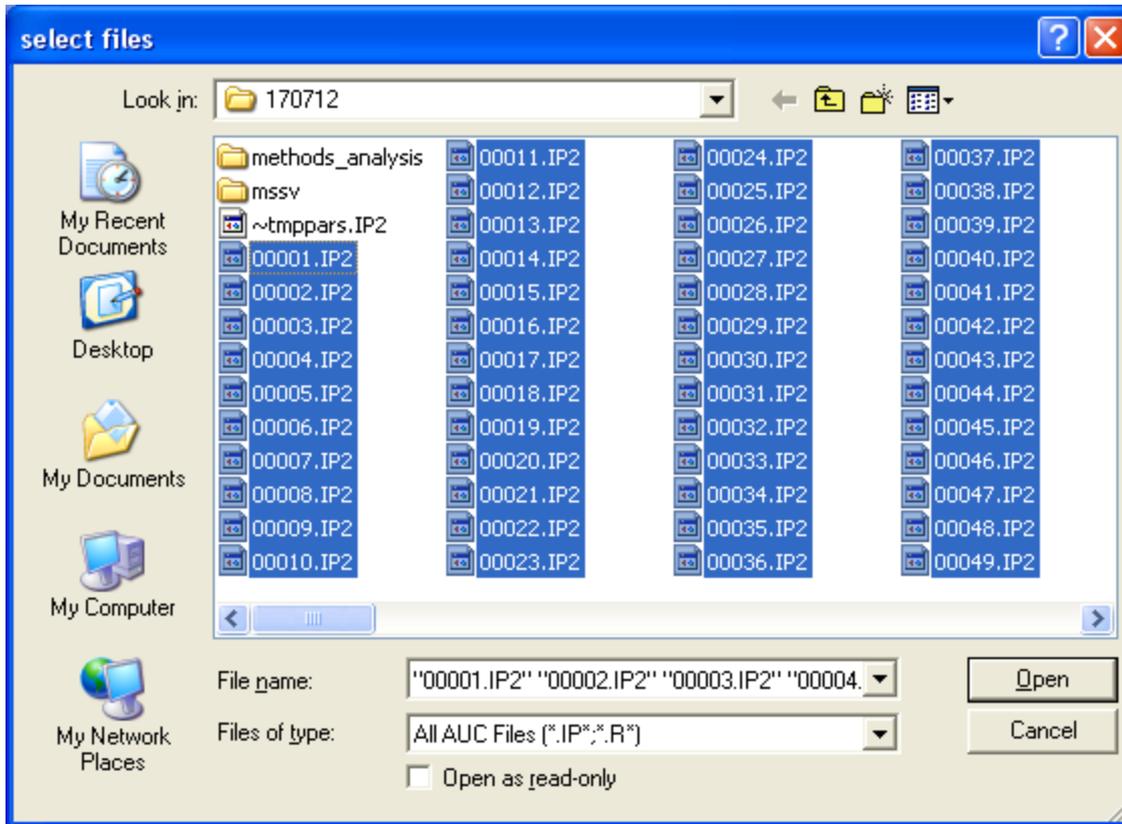
# Step 16

At this point, more fitting could have occurred. For example, the fitting algorithm could have been changed back to Simplex, and the fitting session repeated. However, experience has shown that continued fitting at this point will not significantly change the fitted parameters nor significantly improve the quality of the fit.

# Step 17

A new SEDPHAT session was started, and the data from Cell 2 were loaded into the program as in Step 1 above (with the IF data as Experiment 1 and the  $A_{280}$  data as Experiment 2; the data range was scans 1-50). These data were obtained from the sample with Arp2/3 alone. Steps 2-7 were performed identically for these data. See Table 4 for the pertinent parameters. This part of the analysis is detailed in the next 8 pages.

# A new SEDPHAT session- Load interference data from cell 2 this time



...and every file was loaded.

Scans 1-50 selected...

# Set Experimental Parameters & Save

**Experimental Parameters**

(1) INTERFERENCE data for SEDVELOCITY

C:\AUC Data\Rosen\120106\170712\mssv\cell2\_if.xp (00001.IP2 ...)

Comment

active

noise 0.0100  \*sqrt (N1/Nx)

Centerpiece 2

Pathlength 1.200000

Rotor type 0

no backdiffusion neces

v-bar (ml/g) 0.7300

buffer density (g/ml) 1.00079

buffer viscosity (P) 0.010024

Temperature 20.0

fit baseline

fit RI Noise

fit TI Noise

Meniscus 5.9999

Bottom 7.2004

For Associating Systems:

extinction coefficient A 1.0000

extinction coefficient B 1.0000

extinction coefficient C 0.0000

partial boundary fitting

smin 0.00 smax 100.00

use for sigma of MC sims: 0.0100

use local rmsd

**MACROMOLECULAR PARAMETERS at ...**

global partial spec. volume at 20C (ml/g)

0.730000

OK Cancel

**save experiment xp file as**

Save in: mssv

cell1\_abs.xp

cell1\_if.xp

gst-vca\_alone\_cell1\_abs.xp

gst-vca\_alone\_cell1\_if.xp

My Recent Documents

Desktop

My Documents

My Computer

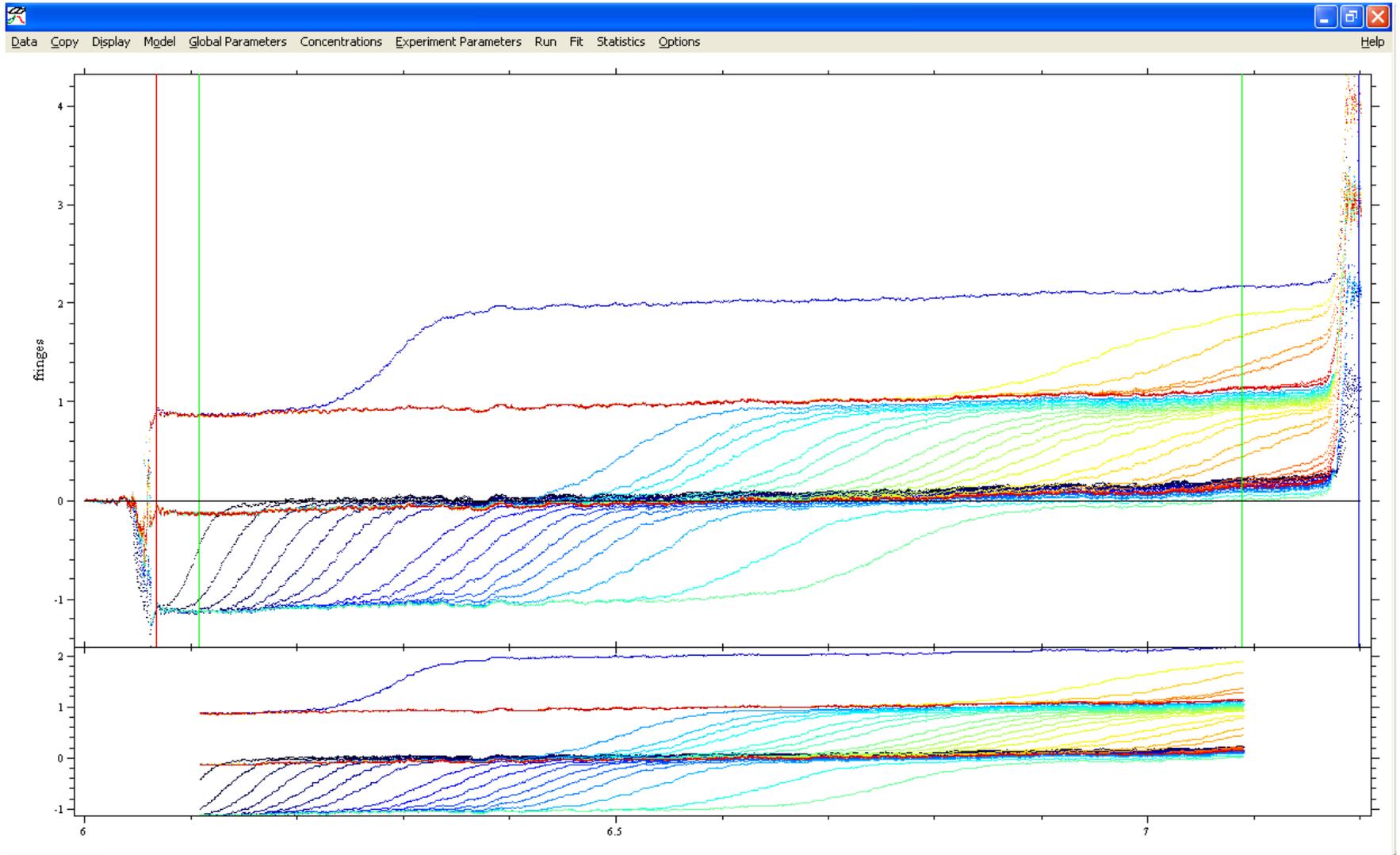
My Network Places

File name: cell2\_if

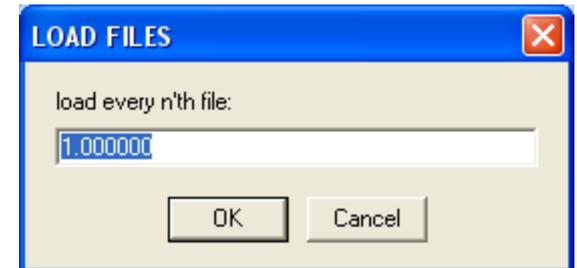
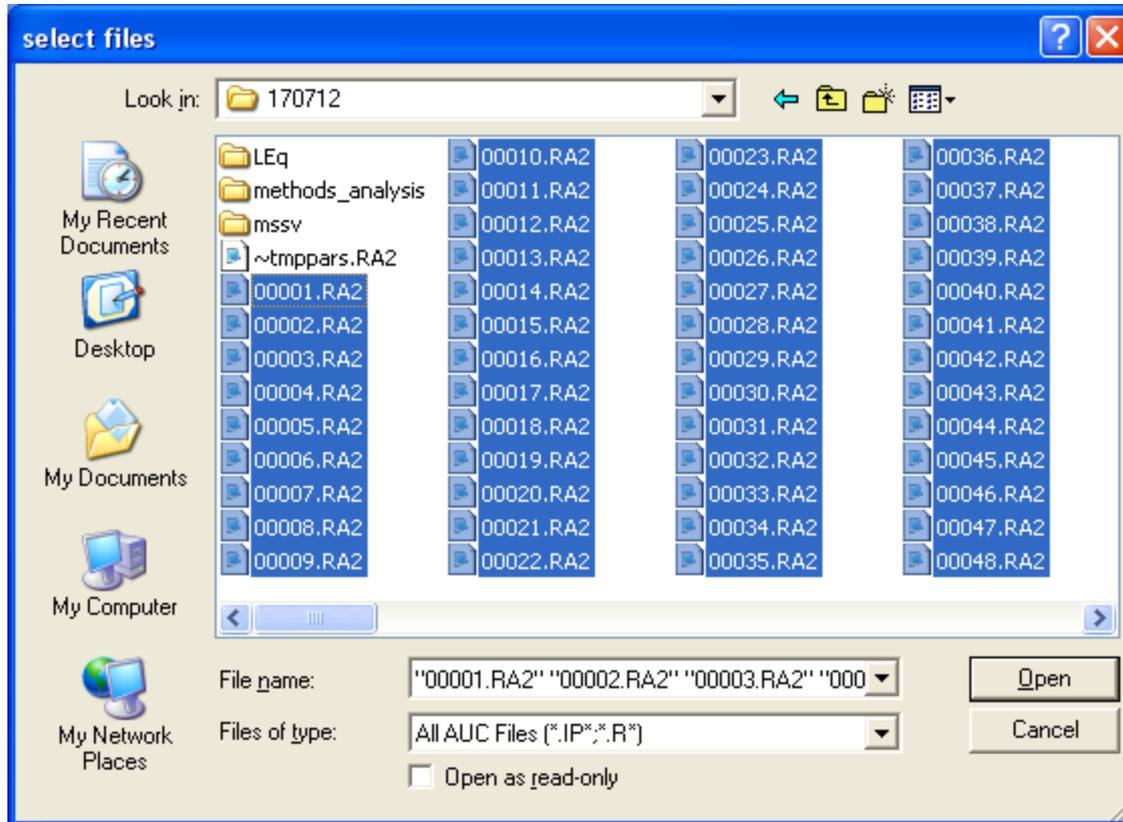
Save as type: Experiment Files (\*.xp)

Save Cancel

# Set the Meniscus, Bottom, Fitting Limits



# Load the ABS data



...and every file was loaded.

Files 1-50 were selected...

# Set the ABS Experimental Parameters

**Experimental Parameters** [X]

[2] ABSORBANCE data for SEDVELOCITY

C:\AUC Data\Rosen\120106\170712\... (00001.RA2 ...)

Comment

active

noise 0.0100   $\sqrt{|N1/Nx|}$

Centerpiece 2

Pathlength 1.200000

Rotor type 0

no backdiffusion neces

v-bar (ml/g) 0.7300

buffer density (g/ml) 1.00079

buffer viscosity (P) 0.010024

Temperature 20.0

fit baseline

fit RI Noise

fit TI Noise

Meniscus 6.0010

Bottom 7.1980

redirect men./bot. 2

For Associating Systems:

extinction coefficient A 1.0000

extinction coefficient B 1.0000

extinction coefficient C 0.0000

redirect xt A 2

redirect xt B 2

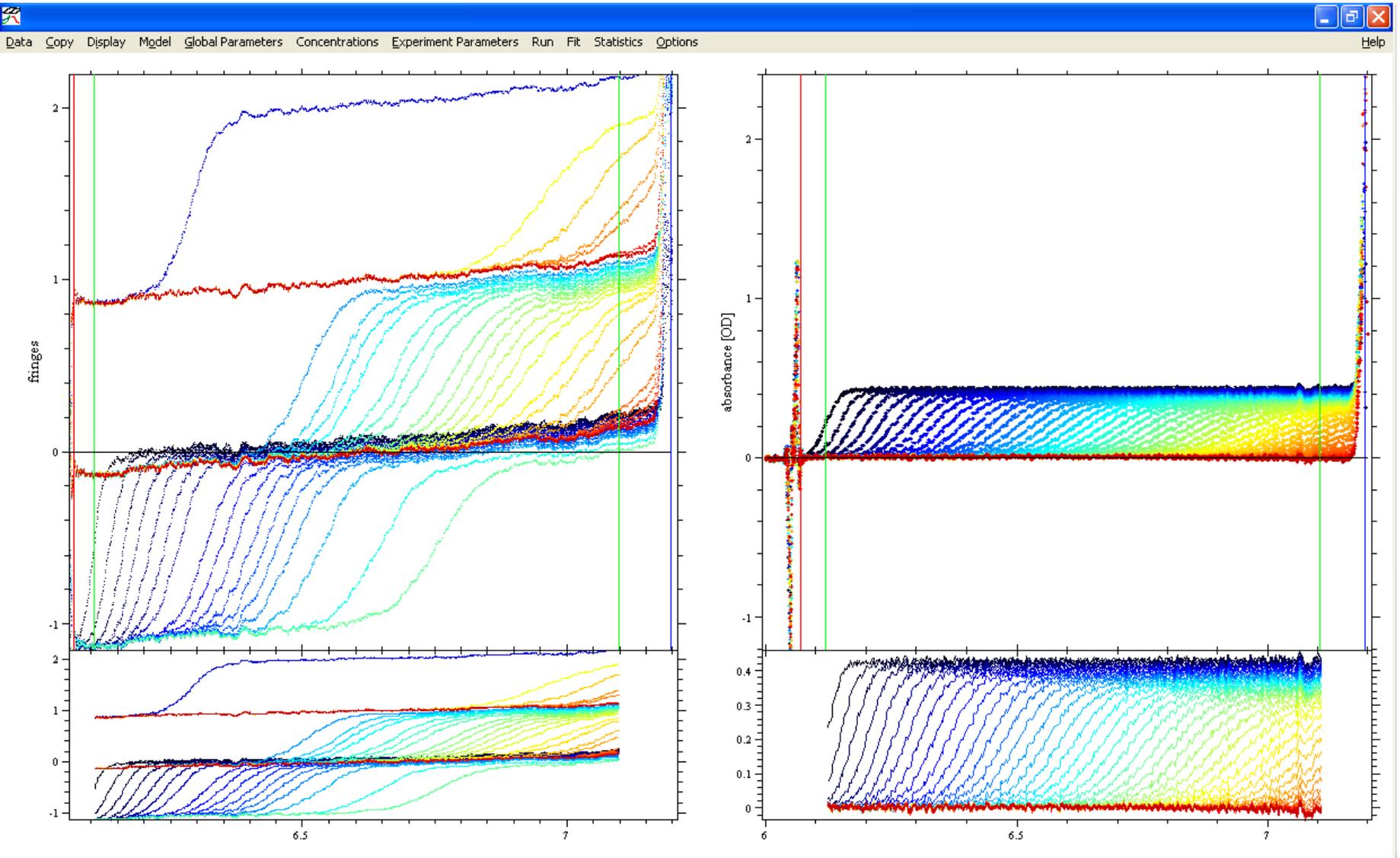
redirect xt C 2

partial boundary fitting smin 0.00 smax 100.00

use for sigma of MC sims: 0.0100  use local rmsd

Cancel OK

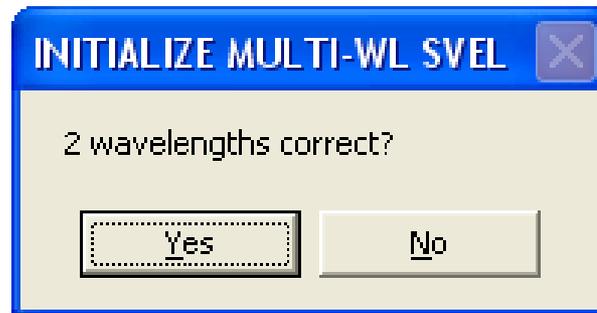
# Set the Meniscus, Bottom, Fitting Limits for the ABS Data



# Select the Model

Model	Global Parameters	Concentrations	Experiment Parameters	Run	Fit	Statistics	Options
<input checked="" type="checkbox"/> Species Analysis							
Linear Fractional Boundary Model							
Species Analysis with Mass Conservation Constraints							
Global Discrete Distribution							
Hybrid Local Continuous Distribution and Global Discrete Species							
Hybrid Global Continuous Distribution and Global Discrete Species							
Multi-Wavelength Discrete/Continuous Distribution Analysis							
Single Nonideal Species							
A (Single Species of Interacting System)							
A <-> A* (Single Species in Two Conformations)							
Monomer-Dimer Self-Association							
Monomer-Trimer Self-Association							
Monomer-n-mer Self-Association							
Monomer-Dimer-Tetramer Self-Association							
Monomer-Tetramer-Octamer Self-Association							
Monomer-'m-mer'-'n-mer' Self-Association							
A+B <-> AB Hetero-Association							
A+B+B <-> {AB}+B <-> ABB; with 2 symmetric sites, macroscop K							
A+B+B <-> AB+B <-> BA + B <-> BAB; with 2 non-symmetric sites, microscop K							
A+B+B+B <-> AB+B+B <-> ABB+B <-> ABBB; with 3 symmetric sites, macroscop K							
A+B+C <-> AB + C <-> AC + B; competing B and C for A							
A+B+B+C forming complexes AB, BA, BAB, BC, CB, BCB; competing A and C for B, microscop K							
A+B+B+C+C forming complexes AB, BA, BAB, AC, CA, CAC, BAC, CAB; competing B and C for 2 Sites on A, microscop K							
A+B+C <-> AB + C <-> AC + B <-> ABC; triple complex							
A+B+B+C forming complexes AB, BA, BAB, AC, ABC, BAC, BABC; quadruple complex, microscop K							
A+B+B+C+C forming complexes AB, BA, BAB, CB, CBA, ABC, CBAB, BABC, CBABC, quintuple complex, microsc. K							
(A+A)+B+B forming complexes (AA), AB, (AA)B, (AA)BB; self-asso. A with 2 symmetric sites, macroscop K							
(A+A)+(B+B) <-> A+AB+B <-> (AA)B+B <-> A+A(BB) <-> (AA)(BB); self-assoc A and B, macroscop K							
(A+A)+(B+B) forming complexes (AA), (BB), AB, (AB)(AB); self-assoc w heterodimer of homodimers							

# Answer Yes in Both Dialogs



# Step 18

See the next page for the values input into the Global Parameter box. Notably, the “chromophore 2” row was used, for consistency with what will follow. The  $\epsilon_{\text{IF}}^{\text{Arp2/3}}$  was fixed at  $615,516 \text{ fringes} \cdot \text{M}^{-1} \cdot \text{cm}^{-1}$ , a value based on the estimated molar mass of the protein complex. The  $\epsilon_{\text{ABS280}}^{\text{Arp2/3}}$  was set to  $230,000 \text{ OD} \cdot \text{M}^{-1} \cdot \text{cm}^{-1}$ , which was based on a calculation performed by SEDNTERP. This value was allowed to refine in the analysis; indeed, the purpose of this portion of the analysis is to obtain  $\epsilon_{\text{ABS280}}^{\text{Arp2/3}}$ .

# Setup the Global Parameters

<input checked="" type="checkbox"/> continuous segment 1		resolution	s min	s max	frictional ratio
<input type="checkbox"/> spectrum 1	<input checked="" type="checkbox"/> spectrum 2	50	0.200	15	1.500
		<input checked="" type="radio"/> linear <input type="radio"/> log			<input type="checkbox"/> fit ffo
		xt1/chr:	xt2/chr:	PP	
		0.000	0.000	PP	
		0.000	1	PP	

<input type="checkbox"/> segment 2		resolution	s min	s max	frictional ratio
<input checked="" type="checkbox"/> spectrum 1	<input checked="" type="checkbox"/> spectrum 2	0	17.000	60.000	1.200
		<input checked="" type="radio"/> linear <input type="radio"/> log			<input checked="" type="checkbox"/> fit ffo
		xt1/chr:	xt2/chr:	PP	
		0.000	0.000	PP	
		0.000	0.000	PP	

<input type="checkbox"/> segment 3		resolution	s min	s max	frictional ratio
<input checked="" type="checkbox"/> spectrum 1	<input checked="" type="checkbox"/> spectrum 2	0	17.000	60.000	1.200
		<input checked="" type="radio"/> linear <input type="radio"/> log			<input checked="" type="checkbox"/> fit ffo
		xt1/chr:	xt2/chr:	PP	
		0.000	0.000	PP	
		0.000	0.000	PP	

<input checked="" type="checkbox"/> discrete spectra in multiples of chromophore xt			
<input checked="" type="checkbox"/> contin. spectra in multiples of chromophore xt			
	xt wl 1	xt wl 2	
chromophore #1	<input type="checkbox"/> 0.000	<input type="checkbox"/> 0.000	
chromophore #2	<input type="checkbox"/> 615516	<input checked="" type="checkbox"/> 230000	

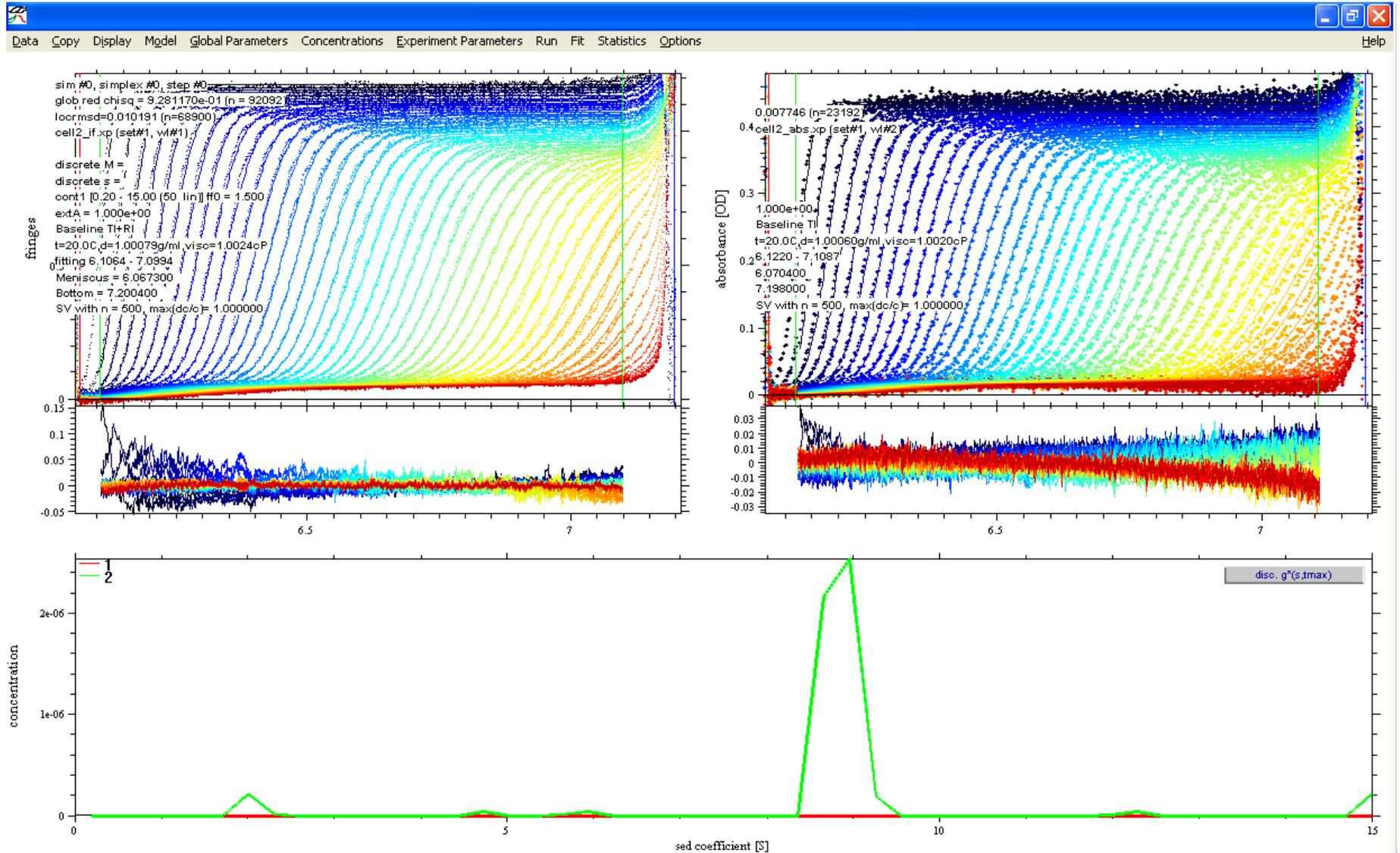
<input checked="" type="checkbox"/> with Tikhonov Regularization P=	0.700
<input checked="" type="checkbox"/> normalize distributions	

Cancel  
OK

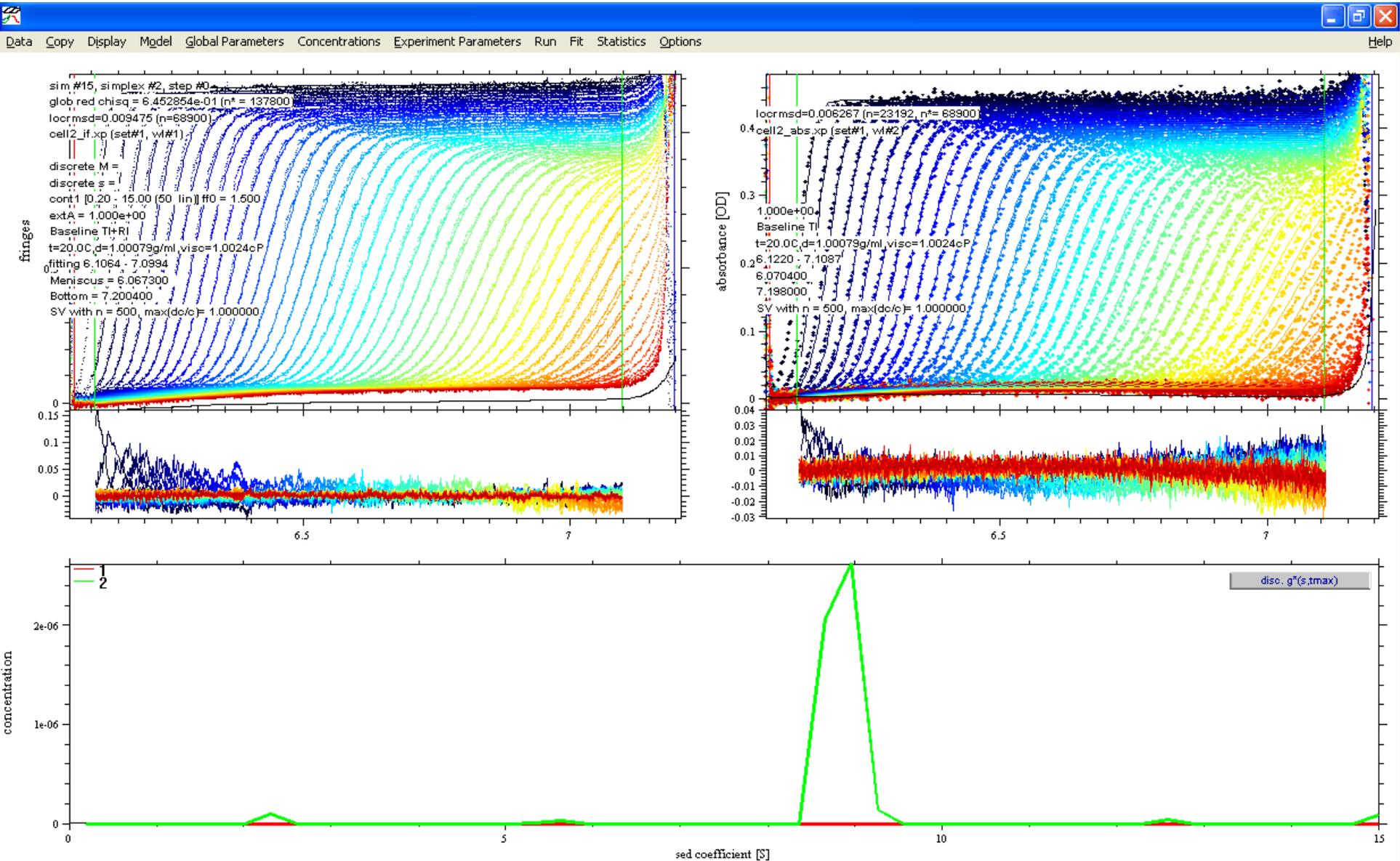
# Step 19

A Global Run was performed. The parameters were deemed close enough to initiate a Global Fit, and that was done. After that, other nonlinear parameters were allowed to refine, as in Step 11. The final value of  $\varepsilon_{\text{ABS280}}^{\text{Arp2/3}}$  was 244,420 M<sup>-1</sup>·cm<sup>-1</sup>, and the  $f_r$  of Arp2/3 was refined to 1.618. The fit was saved. The [Arp2/3] was determined as in step 15. It was 1.49 μM. These substeps are detailed on the next twelve pages.

# After Global Run



# After Global Fit



# Fit ff0 Now

continuous  
 segment 1

resolution:   linear  log

s min:  s max:  frictional ratio:

xt1/chr:  xt2/chr:

spectrum 1  spectrum 2

spectrum 2

fit ff0

discrete spectra in multiples of chromophore xt  
 contin. spectra in multiples of chromophore xt

xt wl 1:  xt wl 2:

chromophore #1

chromophore #2

segment 2

resolution:   linear  log

s min:  s max:  frictional ratio:

xt1/chr:  xt2/chr:

spectrum 1  spectrum 2

spectrum 2

fit ff0

with Tikhonov Regularization P=

normalize distributions

segment 3

resolution:   linear  log

s min:  s max:  frictional ratio:

xt1/chr:  xt2/chr:

spectrum 1  spectrum 2

spectrum 2

fit ff0

# After clicking “Experiment Parameters”, Fit Meniscus #1

**Experimental Parameters**

(1) INTERFERENCE data for SEDVELOCITY  
C:\AUC Data\Rosen\120106\170712\mssv\cell2\_if.xp (00001.IP2 ...)

Comment

active

noise 0.0100  \*sqrt (N1/Nx)

Centerpiece 2  
Pathlength 1.200000  
Rotor type 0

no backdiffusion neces

v-bar (ml/g) 0.7300  
buffer density (g/ml) 1.000790  
buffer viscosity (P) 0.010024  
Temperature 20.0

fit baseline  
 fit RI Noise  
 fit TI Noise

Meniscus 6.0673  
 Bottom 7.2004

redirect men./bot. 1

For Associating Systems:  
 extinction coefficient A 1.0000  
 extinction coefficient B 1.0000  
 extinction coefficient C 0.0000

redirect xt A 1  
 redirect xt B 1  
 redirect xt C 1

partial boundary fitting smin 0.00 smax 100.00

use for sigma of MC sims: 0.0100  use local rmsd

**DATA #1: set range for meniscus var...**

enter upper limit

6.097300

OK Cancel

**DATA #1: set range for meniscus var...**

enter lower limit

6.037300

OK Cancel

# Fit Meniscus #2

**Experimental Parameters**

(2) ABSORBANCE data for SEDVELOCITY  
C:\AUC Data\Rosen\120106\170712\mssv\cell2\_abs.xp (00001.RA2 ...)

Comment

active

noise   \*sqrt (N1/Nx)

Centerpiece   
Pathlength   
Rotor type

no backdiffusion neces

v-bar (ml/g)   
buffer density (g/ml)   
buffer viscosity (P)   
Temperature

fit baseline  
 fit RI Noise  
 fit TI Noise

Meniscus   
 Bottom

redirect men./bot.

For Associating Systems:  
 extinction coefficient A   
 extinction coefficient B   
 extinction coefficient C

redirect xt A   
 redirect xt B   
 redirect xt C

partial boundary fitting smin  smax

use for sigma of MC sims:   use local rmsd

**DATA #2: set range for meniscus var...**

enter upper limit

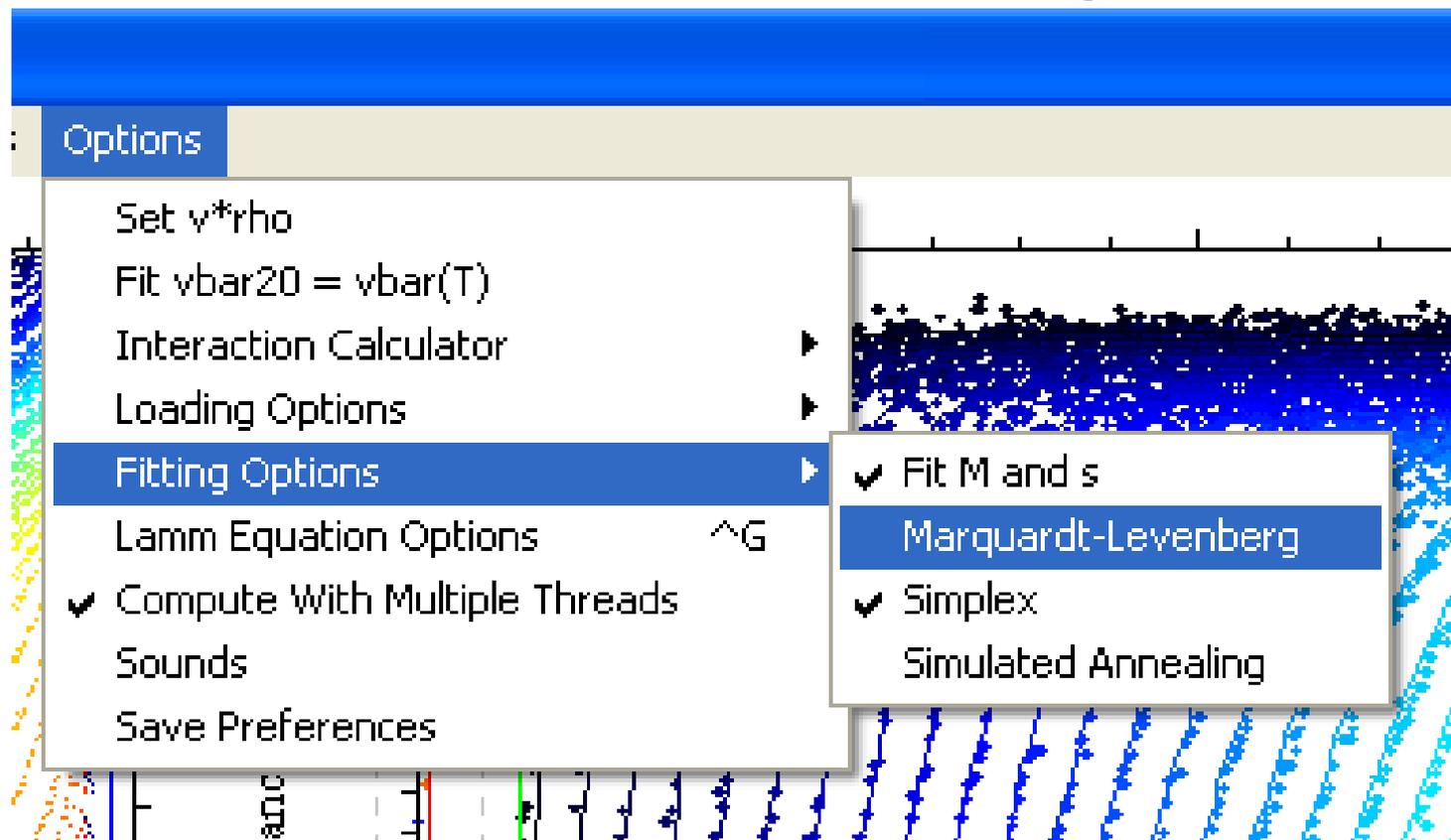
OK Cancel

**DATA #2: set range for meniscus var...**

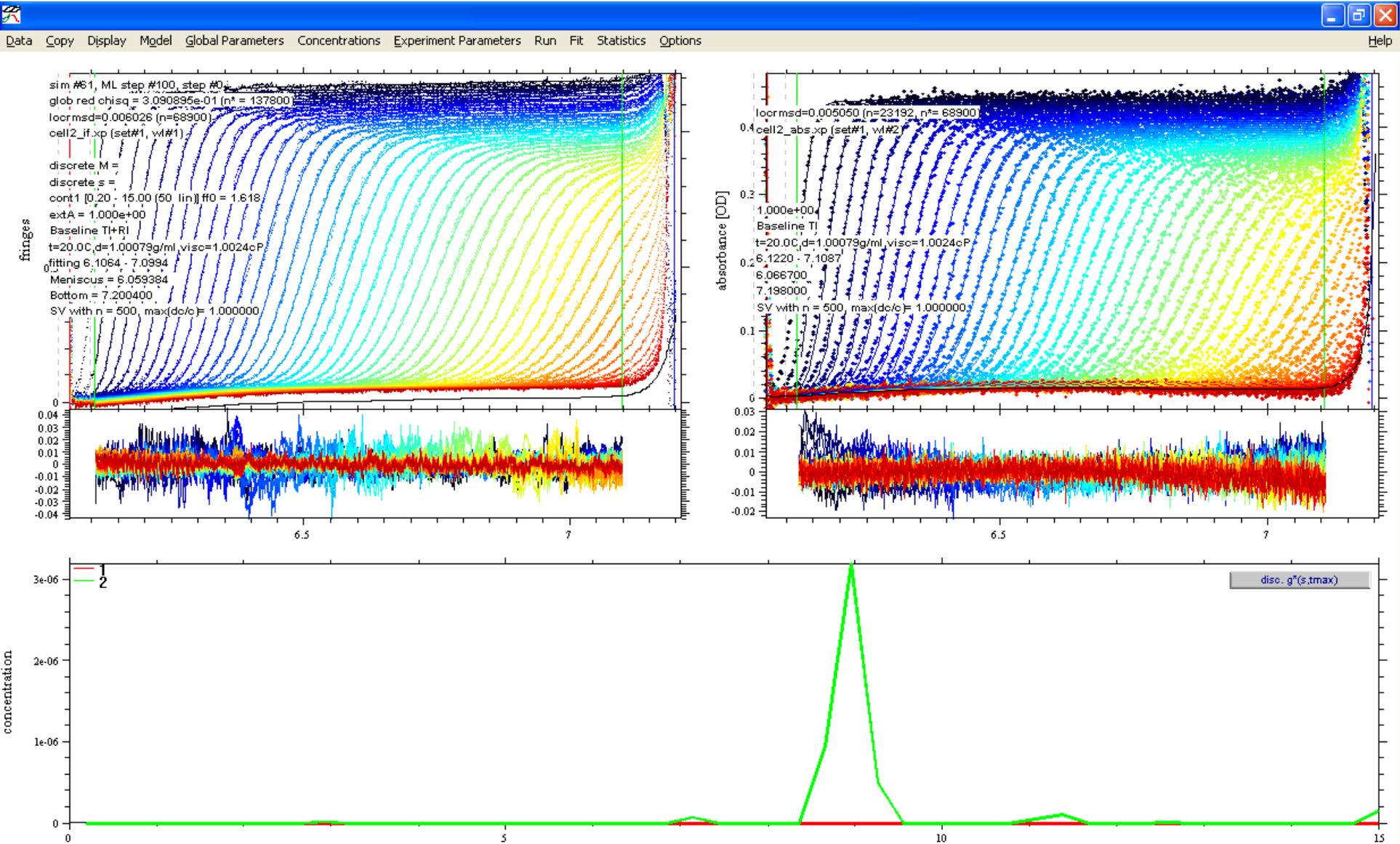
enter lower limit

OK Cancel

# Change Fitting Algorithm to Marquardt Levenberg



# After Global Fitting



# Resultant Parameters

continuous	resolution	s min	s max	frictional ratio	
<input checked="" type="checkbox"/> segment 1	50	<input type="radio"/> linear <input checked="" type="radio"/> log	0.200	15.000	1.618
<input type="checkbox"/> spectrum 1	xt1/chr: 0.000	xt2/chr: 0.000	PP		<input checked="" type="checkbox"/> fit f0
<input checked="" type="checkbox"/> spectrum 2	0.000	1.000	PP		

segment 2	resolution	s min	s max	frictional ratio	
<input type="checkbox"/> segment 2	0	<input type="radio"/> linear <input checked="" type="radio"/> log	17.000	60.000	1.200
<input checked="" type="checkbox"/> spectrum 1	xt1/chr: 0.000	xt2/chr: 0.000	PP		<input type="checkbox"/> fit f0
<input checked="" type="checkbox"/> spectrum 2	0.000	0.000	PP		

segment 3	resolution	s min	s max	frictional ratio	
<input type="checkbox"/> segment 3	0	<input type="radio"/> linear <input checked="" type="radio"/> log	17.000	60.000	1.200
<input checked="" type="checkbox"/> spectrum 1	xt1/chr: 0.000	xt2/chr: 0.000	PP		<input type="checkbox"/> fit f0
<input checked="" type="checkbox"/> spectrum 2	0.000	0.000	PP		

<input checked="" type="checkbox"/> discrete spectra in multiples of chromophore xt
<input checked="" type="checkbox"/> contin. spectra in multiples of chromophore xt
chromophore #1 <input type="checkbox"/> xt wl 1: 0.000 <input type="checkbox"/> xt wl 2: 0.000
chromophore #2 <input type="checkbox"/> 615516. <input checked="" type="checkbox"/> 244420.

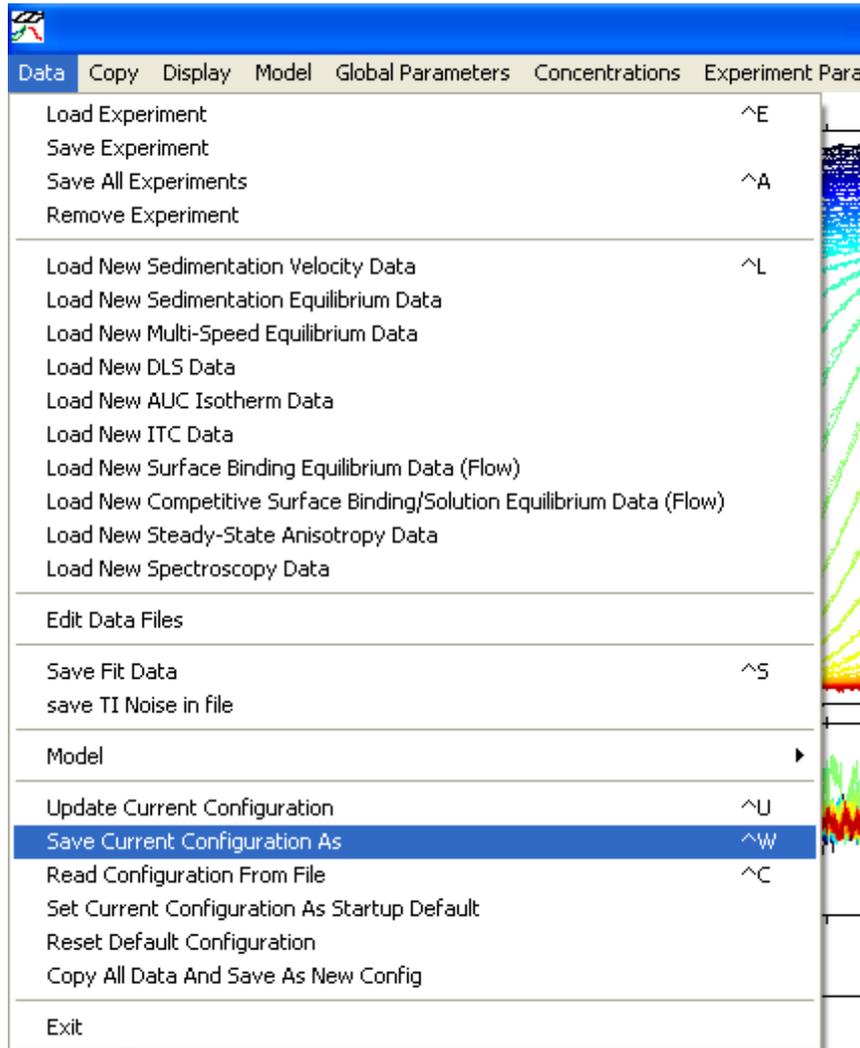
  

<input checked="" type="checkbox"/> with Tikhonov Regularization P= 0.700
<input checked="" type="checkbox"/> normalize distributions

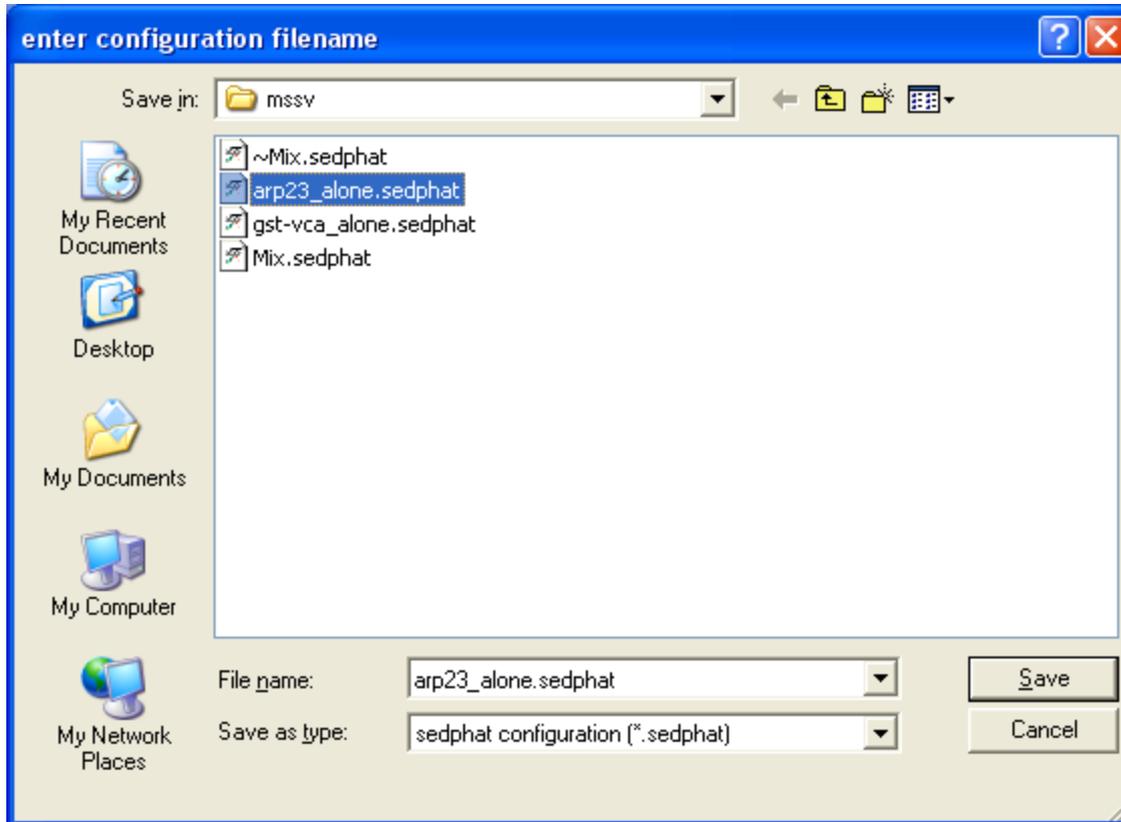
Cancel  
OK

It is a good idea at this point to note down the refined  $\epsilon_{\text{ABS280}}^{\text{Arp2/3}}$  so that it can be easily recalled for input later (Step 21).

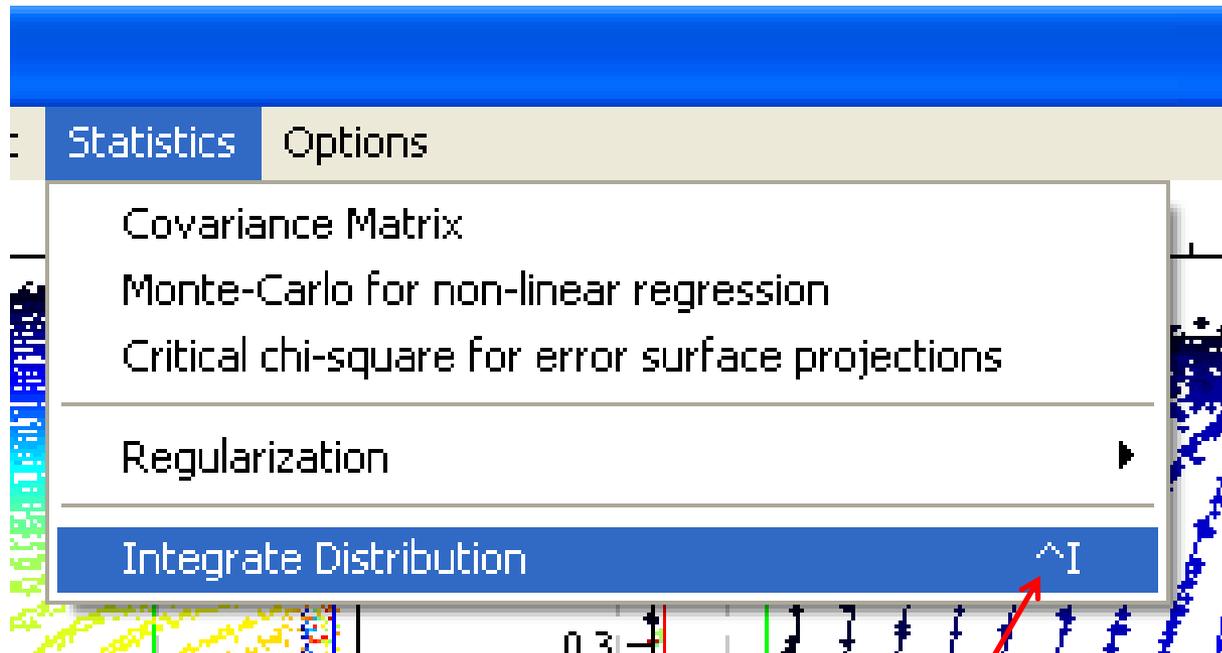
# Save the Configuration



# Save Configuration Dialogs

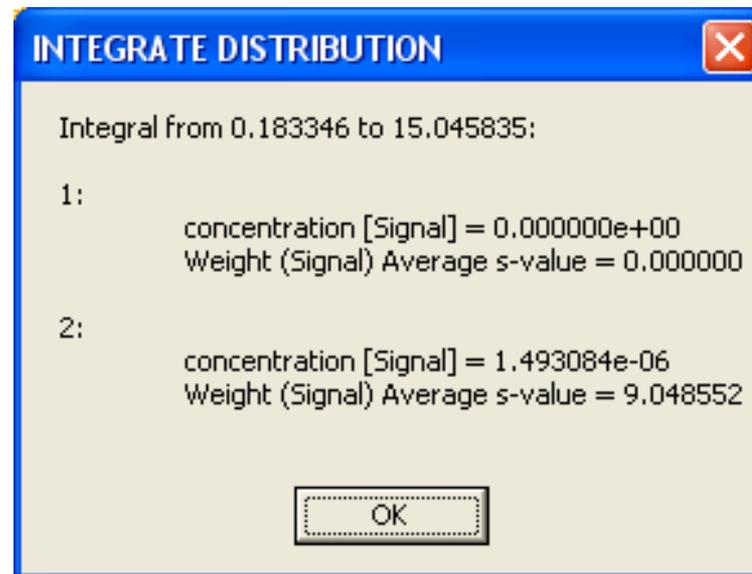
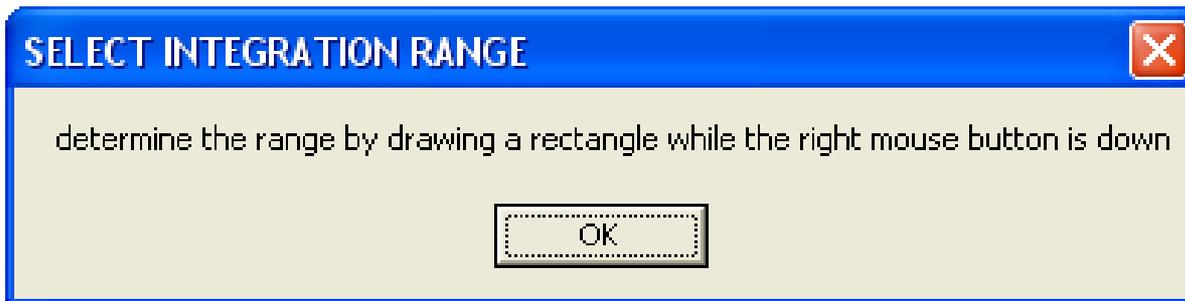


# Integrate the distribution



Note that SEDPHAT tells you if a keyboard shortcut is available. Here, instead of choosing the menu item, I could have pressed “Ctrl-I.”

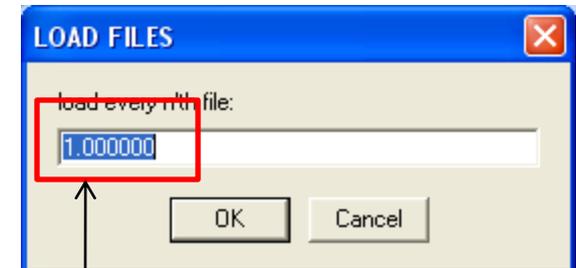
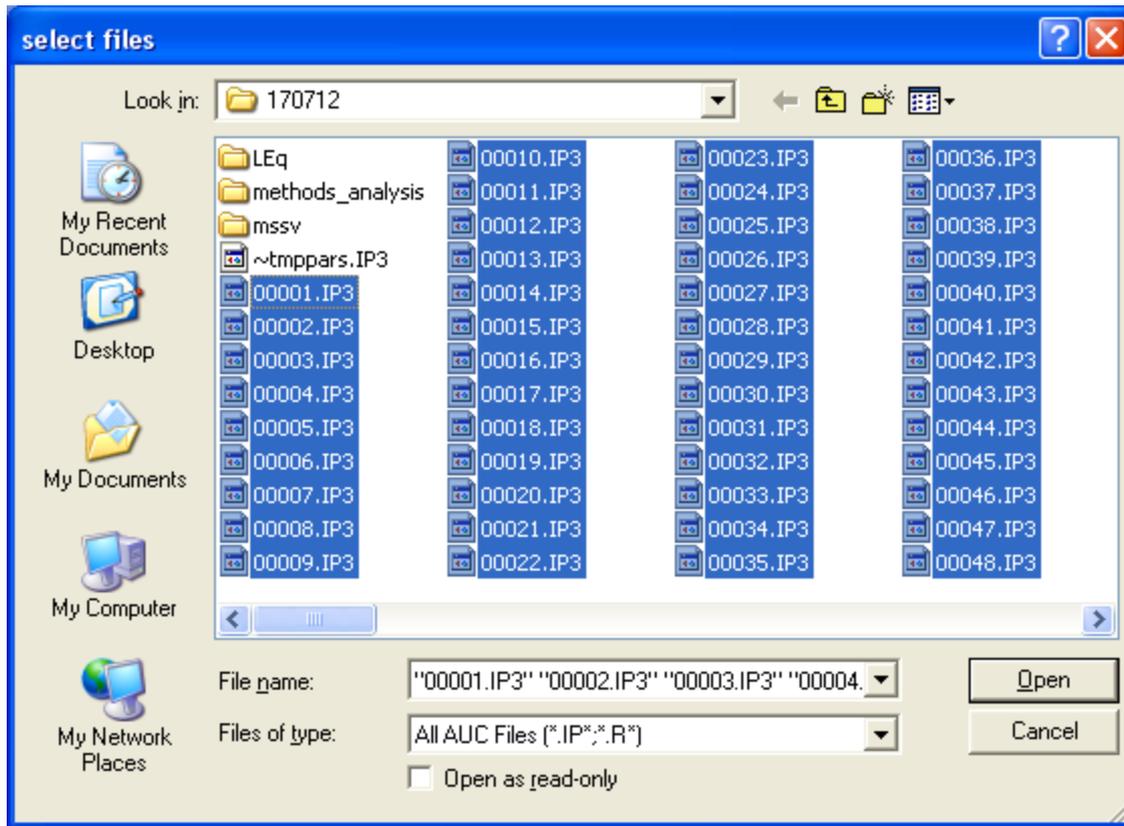
# Integration Results



# Step 20

The next task was to analyze the mixture. The data from the mixture were acquired from Cell 3. Based on the analyses above and the volumes of the protein stocks pipetted into the mixture, roughly 3.0  $\mu\text{M}$  GST-VCA and 0.4  $\mu\text{M}$  Arp2/3 were expected to be detected. The data were loaded into a new SEDPHAT session as detailed in Steps 1-6 above. These substeps are pictorially described on the next eight pages.

# Start a New SEDPHAT Session. Load the Mixture IF data.



Important!

...and every file is loaded.

Files 1-101 from Cell 3 are selected...

# Input the Experimental Parameters for the Interference Data

**Experimental Parameters**

(1) INTERFERENCE data for SEDVELOCITY

C:\AUC Data\Rosen\120106\170712\... (00001.IP3 ...)

Comment

active

noise 0.0100  \*sqrt (N1/Nx)

Centerpiece 2

Pathlength 1.200000

Rotor type 0

no backdiffusion neces

v-bar (ml/g) 0.7300

buffer density (g/ml) 1.00079

buffer viscosity (P) 0.010024

Temperature 20.0

fit baseline

fit RI Noise

fit TI Noise

Meniscus 5.9999

Bottom 7.2004

redirect men./bot. 1

For Associating Systems:

extinction coefficient A 1.0000

extinction coefficient B 1.0000

extinction coefficient C 0.0000

redirect xt A 1

redirect xt B 1

redirect xt C 1

partial boundary fitting smin 0.00 smax 100.00

use for sigma of MC sims: 0.0100  use local rmsd

Cancel OK

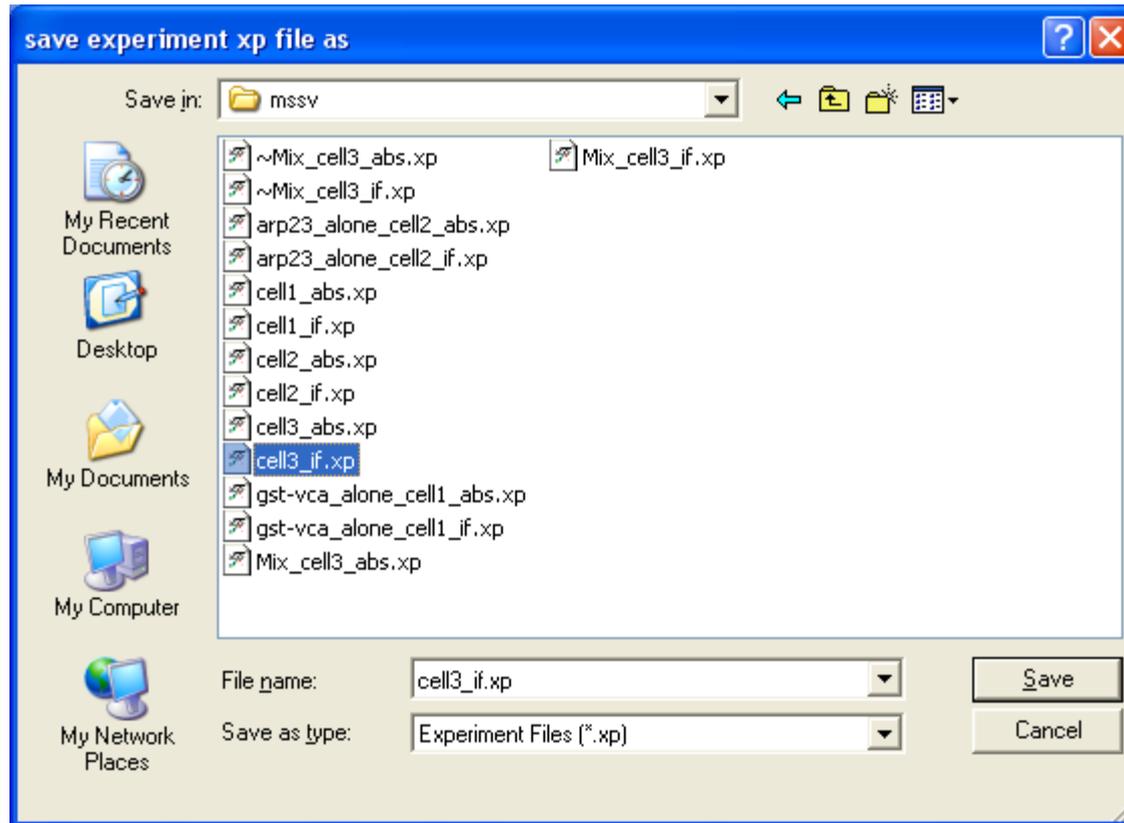
**MACROMOLECULAR PARAMETERS at ...**

global partial spec. volume at 20C (ml/g)

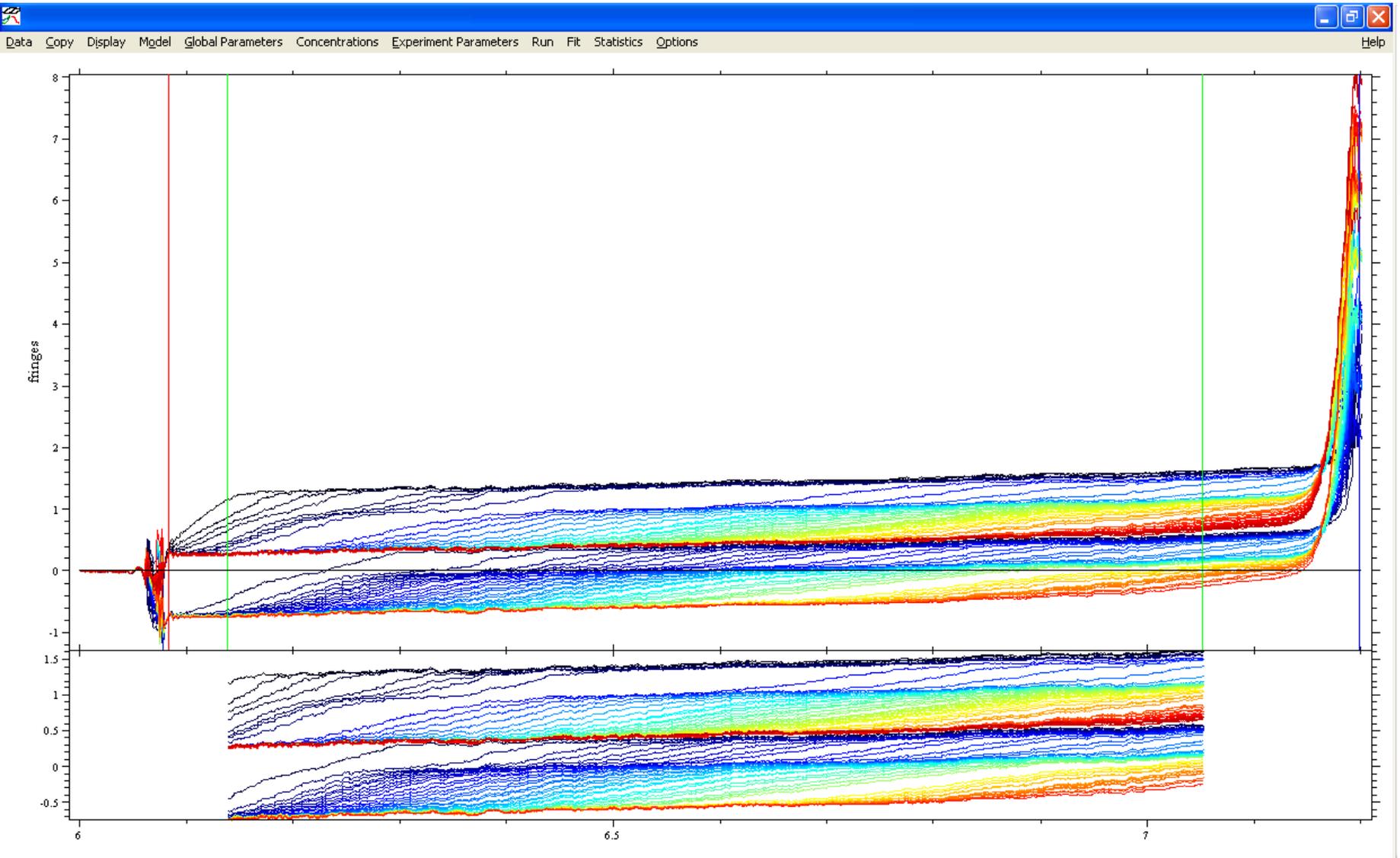
0.730000

OK Cancel

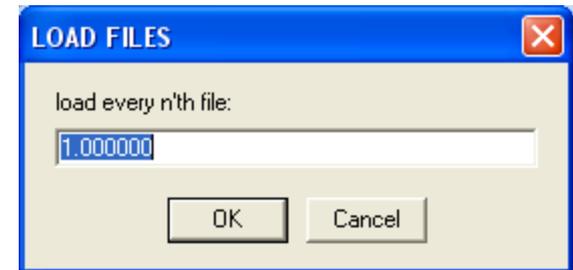
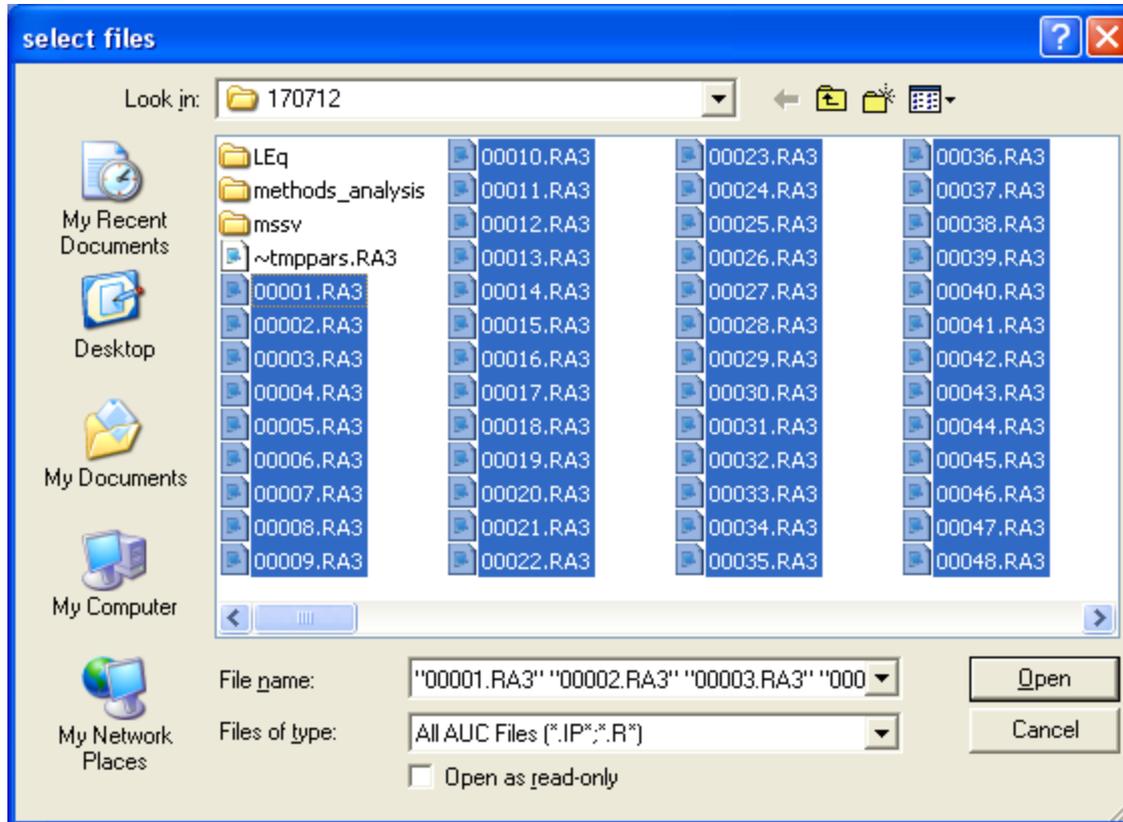
# Save the Experiment



# Set the Meniscus, Bottom, Fitting Limits



# Load ABS280 for the Mixture



...and every file is loaded.

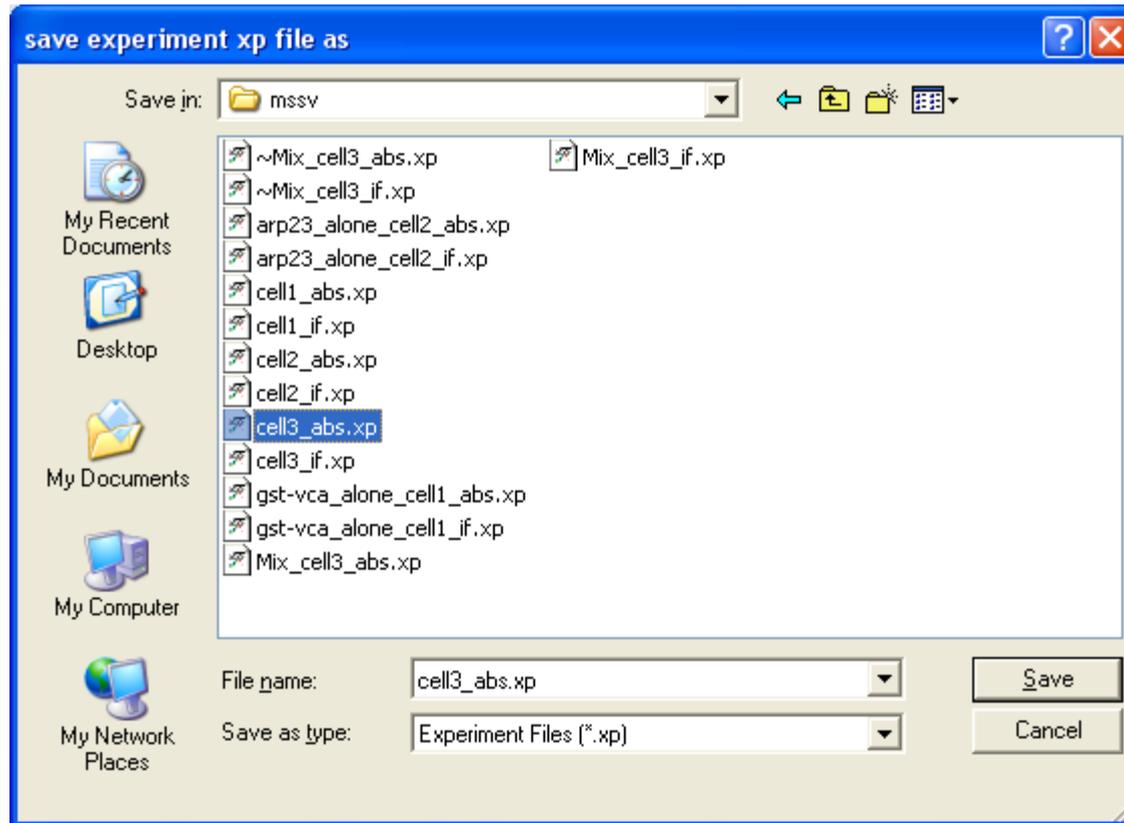
Files 1-101 from Cell 3 are selected...

# Change the Appropriate Experimental Parameters

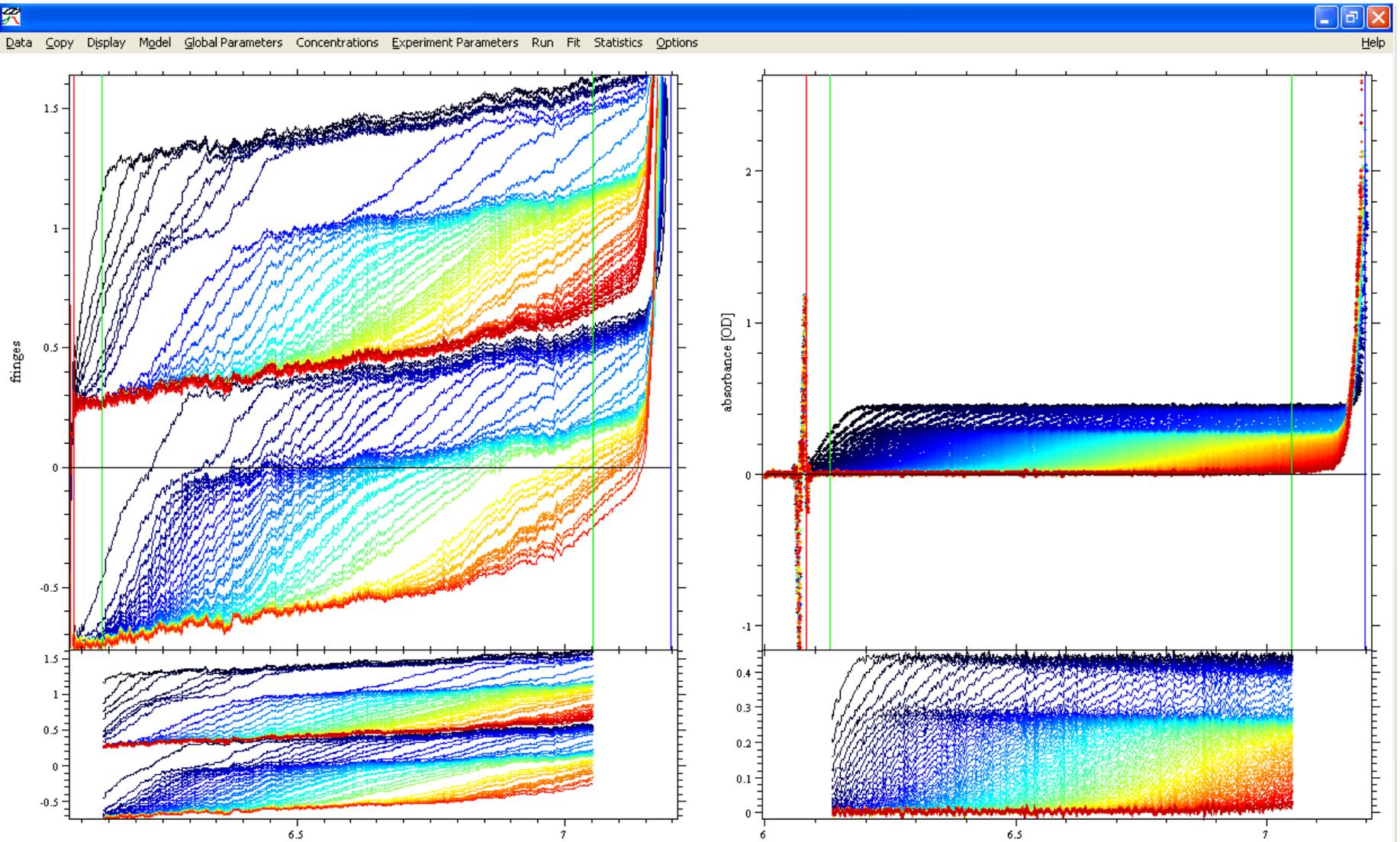
The screenshot shows the 'Experimental Parameters' dialog box with the following fields and values:

- File name: (2) ABSORBANCE data for SEDVELOCITY
- Path: C:\AUC Data\Rosen\120106\170712\... (00001.RA3 ...)
- Comment: (empty)
- active:
- noise: 0.0100
- Model:  sqrt(N1/Nx) (highlighted in red)
- Centerpiece: 2
- Pathlength: 1.200000
- Rotor type: 0
- no backdiffusion neces:
- v-bar (ml/g): 0.7300
- buffer density (g/ml): 1.000790 (highlighted in red)
- buffer viscosity (P): 0.010024 (highlighted in red)
- Temperature: 20.0
- fit baseline:
- fit RI Noise:
- fit TI Noise:  (highlighted in red)
- Meniscus:  6.0030
- Bottom:  7.2000
- redirect men./bot:  2
- For Associating Systems:
  - extinction coefficient A:  1.0000
  - extinction coefficient B:  1.0000
  - extinction coefficient C:  0.0000
- redirect xt A:  2
- redirect xt B:  2
- redirect xt C:  2
- partial boundary fitting:  smin: 0.00 smax: 100.00
- use for sigma of MC sims:  0.0100  use local rmsd

# Save the Experiment

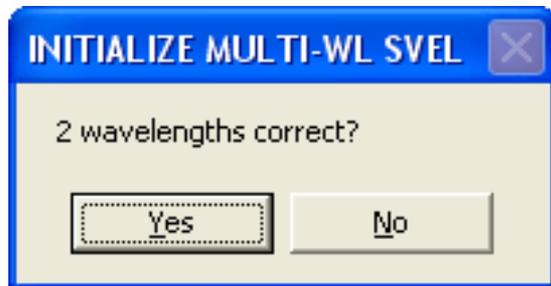
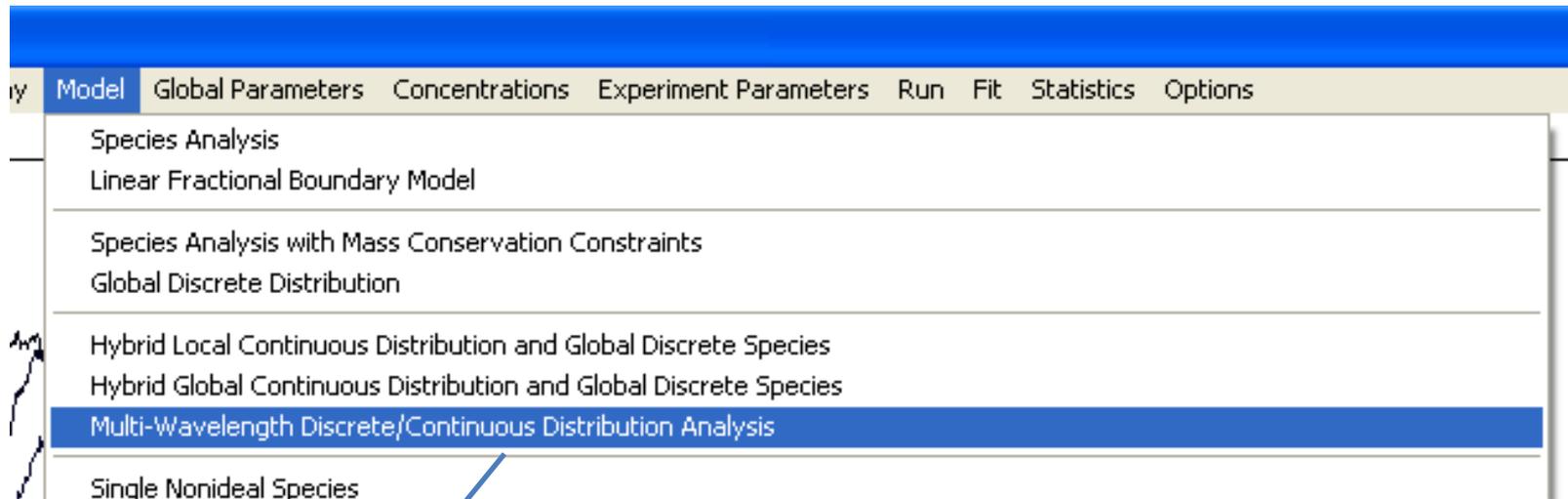


# Set the Meniscus, Bottom, Fitting Limits



# Step 21

The Multi-Wavelength model was chosen, as before.



# Step 21, continued

The Global Parameters Box was opened. Again, the upper part of the parameter box, which pertains to discrete species, was not used. In this case, two “segments” were activated by checking their respective checkboxes. The reason for this expedient is twofold. First, the  $f_r$  of free GST-VCA under these conditions was expected to be substantially different from that of the GST-VCA:Arp2/3 complex. The use of two segments allows two different  $f_r$ 's to be refined during the analysis. Further, the use of separate segments to describe the two boundaries present in the data allows for stoichiometric constraints to be placed on the data for certain  $s$ -ranges (see Step 27). Segment 1 was given a resolution of 25, and  $s$  limits of 0.2 to 5.3 S. The  $f_r$  was set to 1.8, and allowed to refine. Both “spectra” were activated: the left-hand and right-hand boxes for spectrum 1 were set to 1 and 0, respectively. Thus spectrum 1 will report on the presence of chromophore 1 (GST-VCA) only; it will be a  $c_{\text{GST-VCA}}(s)$  distribution. See the next page.

# Setup Segment 1

continuous  
 segment 1

resolution   linear  log

s min  s max  frictional ratio

spectrum 1

spectrum 2

fit ffo

discrete spectra in multiples of chromophore xt  
 contin. spectra in multiples of chromophore xt

xt wl 1 xt wl 2

chromophore #1

chromophore #2

segment 2

resolution   linear  log

s min  s max  frictional ratio

spectrum 1

spectrum 2

fit ffo

with Tikhonov Regularization P=

normalize distributions

segment 3

resolution   linear  log

s min  s max  frictional ratio

spectrum 1

spectrum 2

fit ffo

Cancel

OK

# Step 21 (continued)

The boxes for Spectrum 2 were set to 0 and 1, respectively; it will be a  $c_{\text{Arp}2/3}(s)$  distribution. In Segment 2, a resolution of 25 was entered, and the  $s$ -range was set to 6.8-15 S. Experience has shown that it is best not to have substantial gaps or overlaps in molar mass- ( $M$ -) space when calculating segmented distributions. Given the large difference  $f_r$ 's for the two segments, a 1.5-S gap in  $s$ -space was needed to ensure the proper coverage of  $M$ -space. As the analysis below demonstrates, there is no deleterious effect consequent to this gap. The  $f_r$  of segment 2 was set to 1.6, and its refinement was enabled. Both spectra were set up exactly as in segment 1. See the next page.

# Setup Segment 2

continuous  
 segment 1

resolution   linear  log

s min  s max  frictional ratio

spectrum 1

spectrum 2

fit ffo

segment 2

resolution   linear  log

s min  s max  frictional ratio

spectrum 1

spectrum 2

fit ffo

segment 3

resolution   linear  log

s min  s max  frictional ratio

spectrum 1

spectrum 2

fit ffo

discrete spectra in multiples of chromophore xt  
 contin. spectra in multiples of chromophore xt

xt wl 1 xt wl 2

chromophore #1

chromophore #2

with Tikhonov Regularization P=

normalize distributions

Cancel

OK

# Step 21 continued

In the extinction section, GST-VCA was designated “chromophore #1,” and its extinction properties were entered in this row: 193,089 and 92,420.2 (remembering that Experiment 1 was the IF data and Experiment 2 was the absorbance data). The extinction properties of “chromophore #2,” Arp2/3, were input in the proper entry fields: 615,516 and 244,420. It is very important to disallow the refinement of the extinction parameters in this final phase of the MSSV analysis. That is, none of the checkboxes next to the extinction parameters should be checked. Tikhonov Regularization was activated ( $P=0.70$ ), as was normalization. See the next page.

# Enter Extinction, Regularization, and Normalization Info

<input checked="" type="checkbox"/> continuous <input checked="" type="checkbox"/> segment 1	resolution <input type="text" value="25"/>	<input checked="" type="radio"/> linear <input type="radio"/> log	s min <input type="text" value="0.200"/>	s max <input type="text" value="5.3"/>	frictional ratio <input type="text" value="1.8"/>	<input checked="" type="checkbox"/> discrete spectra in multiples of chromophore xt <input checked="" type="checkbox"/> contin. spectra in multiples of chromophore xt
<input checked="" type="checkbox"/> spectrum 1	xt1/chr <input type="text" value="1"/>	xt2/chr <input type="text" value="0.000"/>	<input type="text" value="PP"/>	<input checked="" type="checkbox"/> fit ffo	xt wl 1 <input type="text" value="193089"/>	xt wl 2 <input type="text" value="92416.2"/>
<input checked="" type="checkbox"/> spectrum 2	<input type="text" value="0.000"/>	<input type="text" value="1"/>	<input type="text" value="PP"/>		chromophore #1 <input type="text" value="615516"/>	chromophore #2 <input type="text" value="244420"/>
<input checked="" type="checkbox"/> segment 2	resolution <input type="text" value="25"/>	<input checked="" type="radio"/> linear <input type="radio"/> log	s min <input type="text" value="6.8"/>	s max <input type="text" value="15"/>	frictional ratio <input type="text" value="1.6"/>	<input checked="" type="checkbox"/> with Tikhonov Regularization P= <input type="text" value="0.700"/>
<input checked="" type="checkbox"/> spectrum 1	xt1/chr <input type="text" value="1"/>	xt2/chr <input type="text" value="0.000"/>	<input type="text" value="PP"/>	<input checked="" type="checkbox"/> fit ffo	<input checked="" type="checkbox"/> normalize distributions	
<input checked="" type="checkbox"/> spectrum 2	<input type="text" value="0.000"/>	<input type="text" value="1"/>	<input type="text" value="PP"/>			
<input type="checkbox"/> segment 3	resolution <input type="text" value="0"/>	<input checked="" type="radio"/> linear <input type="radio"/> log	s min <input type="text" value="17.000"/>	s max <input type="text" value="60.000"/>	frictional ratio <input type="text" value="1.200"/>	
<input checked="" type="checkbox"/> spectrum 1	xt1/chr <input type="text" value="0.000"/>	xt2/chr <input type="text" value="0.000"/>	<input type="text" value="PP"/>	<input checked="" type="checkbox"/> fit ffo		
<input checked="" type="checkbox"/> spectrum 2	<input type="text" value="0.000"/>	<input type="text" value="0.000"/>	<input type="text" value="PP"/>			

Cancel  
OK

# Step 22

The Experimental Parameters boxes were recalled, and the refinements of the respective menisci were enabled. The default values for the refinement limits of the menisci were accepted. See the next two pages.

# Activate Meniscus Fitting, Experiment #1

**Experimental Parameters**

(1) INTERFERENCE data for SEDVELOCITY

C:\AUC Data\Rosen\120106\170712\mssv\cell3\_if.xp (00001.IP3 ...)

Comment

active

noise 0.0100  \*sqrt (N1/Nx)

Centerpiece 2

Pathlength 1.200000

Rotor type 0

no backdiffusion neces

v-bar (ml/g) 0.7300

buffer density (g/ml) 1.000790

buffer viscosity (P) 0.010024

Temperature 20.0

fit baseline

fit RI Noise

fit TI Noise

Meniscus 6.0850

Bottom 7.2004

redirect men./bot 1

For Associating Systems:

extinction coefficient A 1.0000

extinction coefficient B 1.0000

extinction coefficient C 0.0000

redirect xt A 1

redirect xt B 1

redirect xt C 1

partial boundary fitting smin 0.00 smax 100.00

use for sigma of MC sims: 0.0100  use local rmsd

**DATA #1: set range for meniscus var...**

enter upper limit

6.115004

OK Cancel

**DATA #1: set range for meniscus var...**

enter lower limit

6.055003

OK Cancel

# Activate Meniscus Fitting, Experiment

## #2

**Experimental Parameters**

[2] ABSORBANCE data for SEDVELOCITY  
C:\AUC Data\Rosen\120106\170712\mssv\cell3\_abs.xp (00001.RA3 ...)

Comment

active

noise 0.0100  \*sqrt (N1/Nx)

Centerpiece 2  
Pathlength 1.200000  
Rotor type 0

no backdiffusion neces

v-bar (ml/g) 0.7300  
buffer density (g/ml) 1.000790  
buffer viscosity (P) 0.010024  
Temperature 20.0

fit baseline  
 fit RI Noise  
 fit TI Noise

Meniscus 6.0863  
 Bottom 7.2000

redirect men./bot. 2

For Associating Systems:  
 extinction coefficient A 1.0000  
 extinction coefficient B 1.0000  
 extinction coefficient C 0.0000

redirect xt A 2  
 redirect xt B 2  
 redirect xt C 2

partial boundary fitting smin 0.00 smax 100.00

use for sigma of MC sims: 0.0100  use local rmsd

**DATA #2: set range for meniscus var...**

enter upper limit  
6.116300

OK Cancel

**DATA #2: set range for meniscus var...**

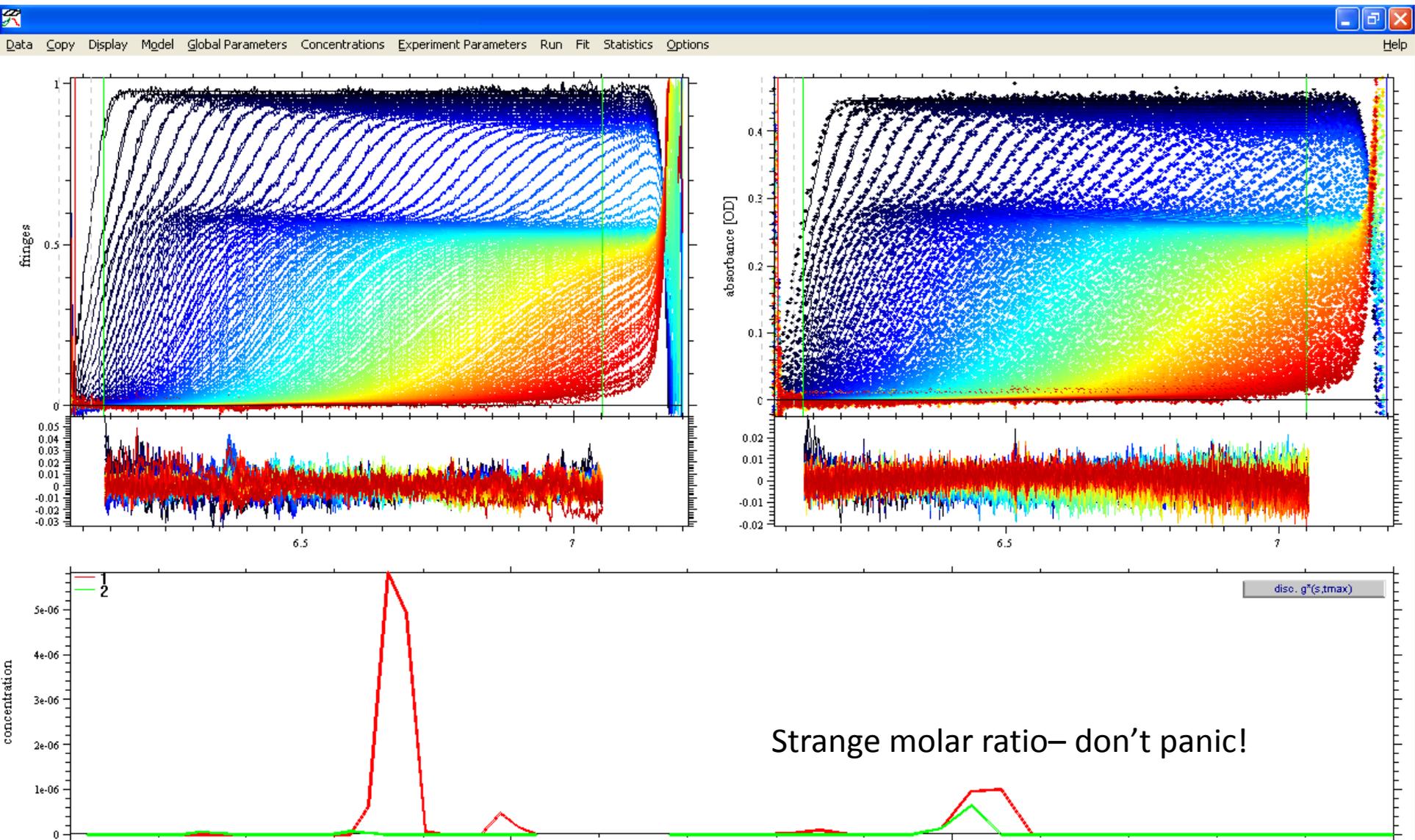
enter lower limit  
6.056300

OK Cancel

# Step 23

A Global Run was initiated, followed by the subtraction of TI and RI noise (where appropriate). The fit, especially in the absorbance data, clearly has systematic features. The distributions (“spectra”) in segment 2 show a significant molar excess of GST-VCA complexed to Arp2/3. The reader is cautioned not to attempt an analysis of molar ratio at this point. Experience has demonstrated that, until the fit is optimized, erroneous values for molar ratio can be arrived at. Despite the systematic errors present in the fit residuals, it was deemed close enough to begin an optimization of the non-linear parameters. See the result on the next page.

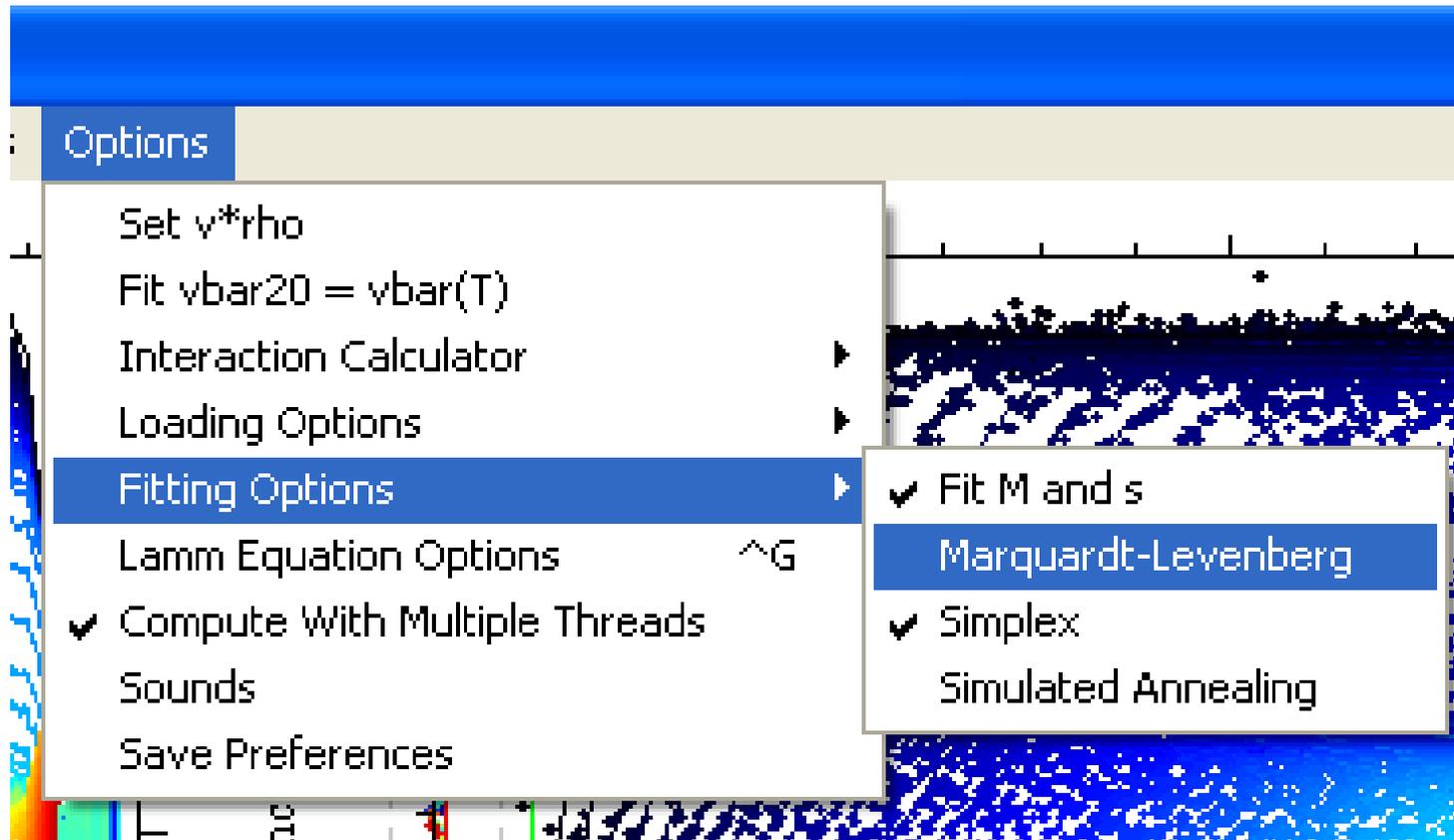
# A Global Run



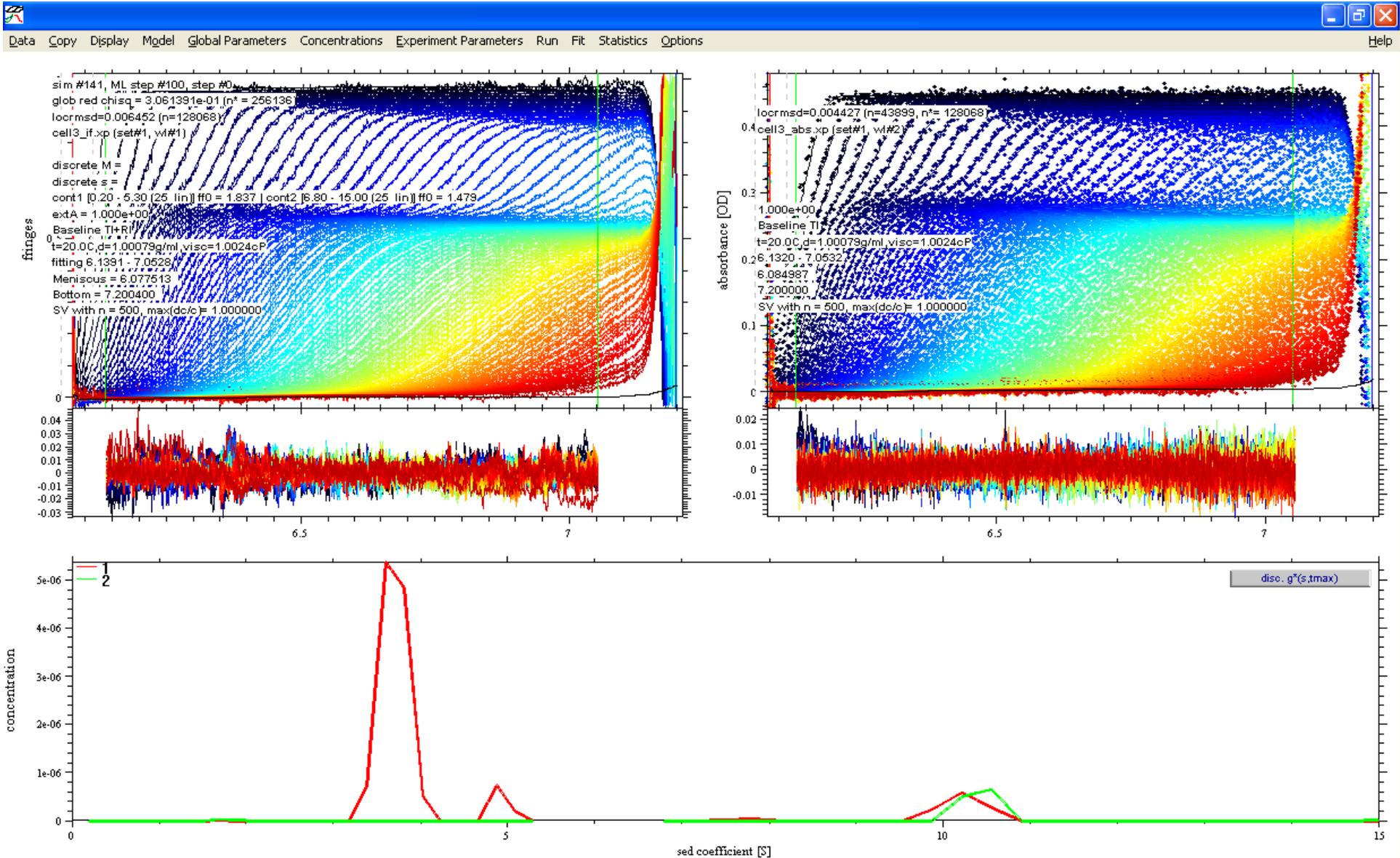
# Step 24

A Global Fit was started using the Marquardt-Levenberg minimization algorithm. After about 15 minutes of minimization, convergence was achieved. The configuration was saved. The quality of the fit was judged to be good. The local rmsd for the IF data was 0.006452 and that for the  $A_{280}$  data was 0.004427. The residuals were non-systematic. At this point, the fitted  $\chi^2$ , or  $\chi_b^2$ , was noted to be 0.3061391. The fit was saved. See the next four pages.

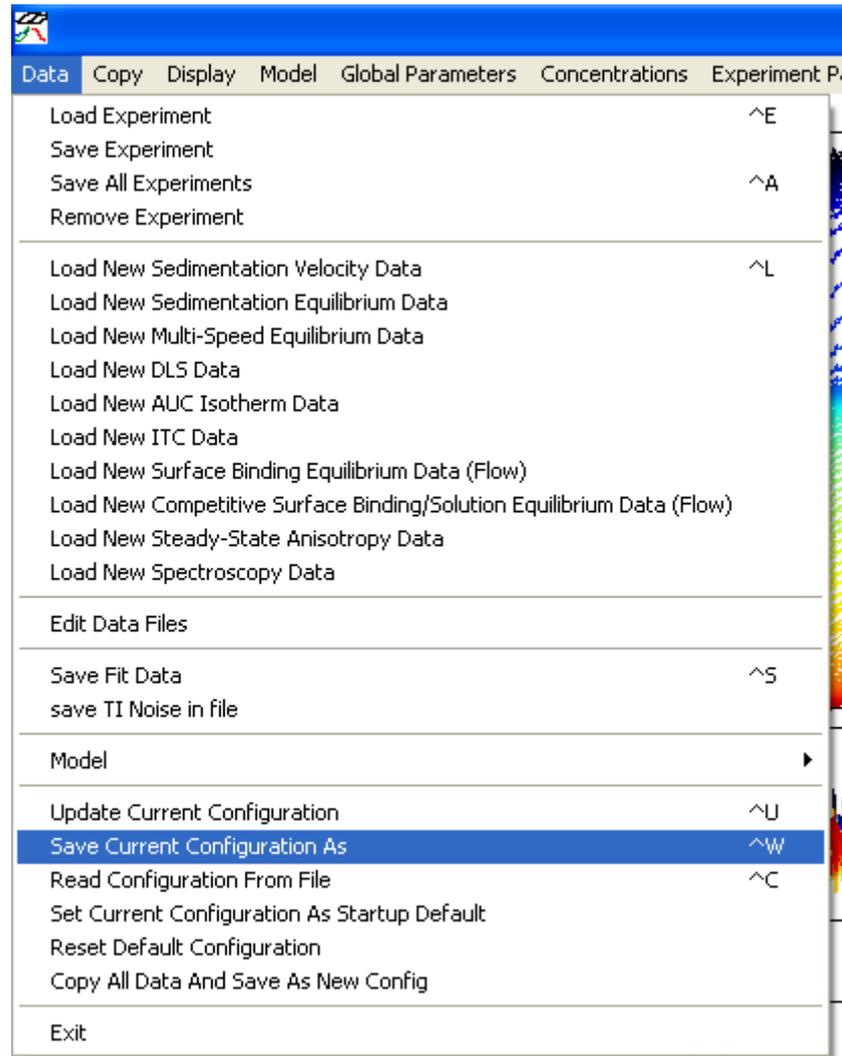
# Set the Minimization Algorithm to ML



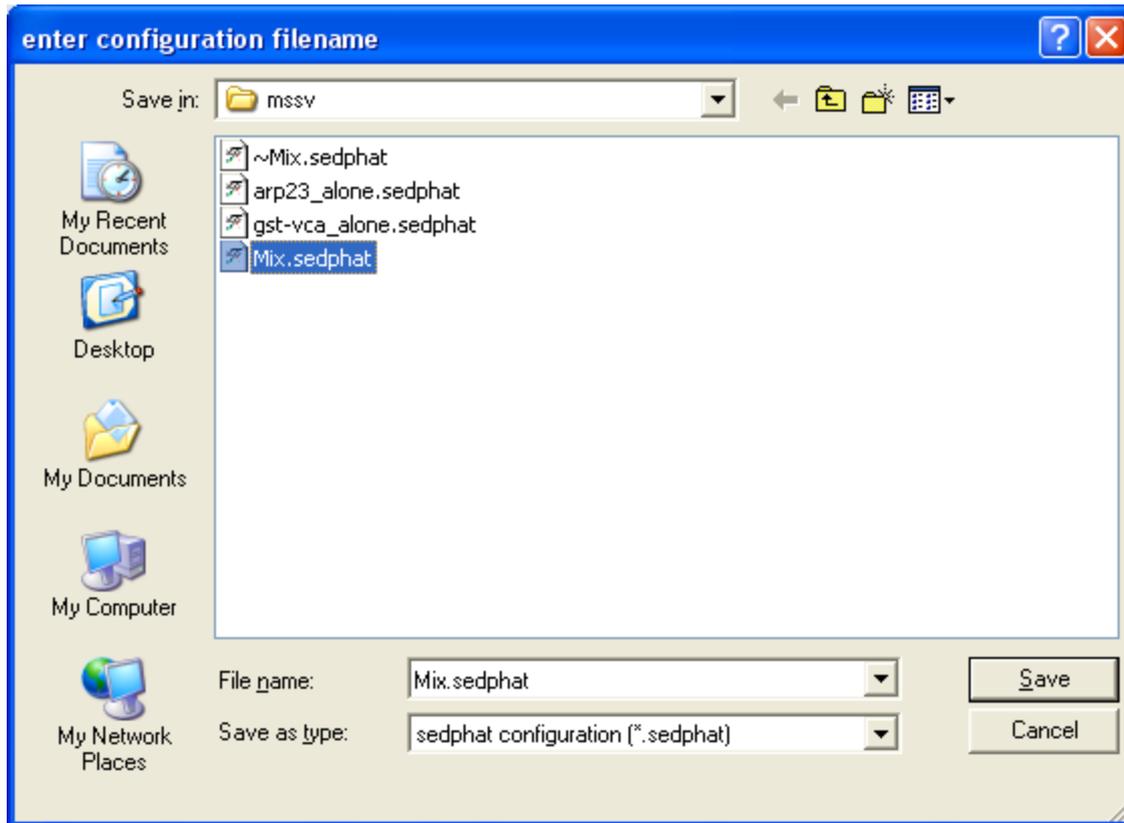
# After Global Fit



# Save the Configuration



# Save Configuration Dialogs



# Step 25

At this point, we turned to an initial assessment of the fit's reliability. As mentioned in section 4.1 of the paper,  $\chi_b^2$  alone is not a sufficient criterion to assess the outcome. The most important criterion to assess the success of the spectral discrimination is mass conservation; in other words, are the concentrations of components detected in the distributions close to our expectations based on the concentrations obtained from the analyses of the individual components? For example, in the GST-VCA alone experiment, the concentration of the protein was found to be 4.09  $\mu\text{M}$ . Because 87  $\mu\text{L}$  of the stock solution was included in 410  $\mu\text{L}$  of total sample, the stock concentration of GST-VCA was calculated to be 19.1  $\mu\text{M}$ . Because 66  $\mu\text{L}$  of this stock was used to make the 410  $\mu\text{L}$  of the mixture sample, the expected [GST-VCA] is 3.08  $\mu\text{M}$ . By integrating both  $c_{\text{GST-VCA}}(s)$  distributions as in Step 15 above (see page 105 of this protocol for this result), it was found that [GST-VCA] = 3.01  $\mu\text{M}$ , in excellent agreement with the expectation. Similarly, the expected [Arp2/3] was calculated to be 0.38  $\mu\text{M}$ , and the actual detected [Arp2/3] = 0.40  $\mu\text{M}$ . Again, the agreement is excellent, and thus the most important criterion for success is easily met.

# Step 25 continued

Another important criterion is the absence of compositional contamination. In this case, it is important that little to no Arp2/3, a large protein with a sedimentation coefficient of about 8.9 S, be detected at low  $s$ -values where the excess GST-VCA was detected. Examination of Page 99 demonstrates that this criterion is also met; there is no signal for Arp2/3 in the peaks that describe the sedimentation of GST-VCA at 3.7 and 4.9 S. Also, as expected, GST-VCA and Arp2/3 appear to be sedimenting in a complex of the two components. Two of the most important criteria for spectral discrimination are therefore met, and the expectation is that this analysis was successful. According to the refined signal increment/extinction information for this system,  $D_{norm} = 0.068$ ; in retrospect, good spectral discrimination was therefore to be expected for these two proteins, according to the simulations carried out in section 4.1 of the paper.

# Step 26

Another integration was performed. This time, only the range 9.3-11.2 S was considered. This was the region of the cosedimenting complex. Here, [GST-VCA] = 0.36  $\mu$ M and [Arp2/3] = 0.39  $\mu$ M. Thus, the molar ratio of GST-VCA to Arp2/3 in the complex was calculated to be 0.92 to 1. The molar ratio appears to be close to 1:1. See the next page.

# Some more integration

**INTEGRATE DISTRIBUTION** 

Integral from 0.188694 to 15.033919:

1:  
concentration [Signal] =  $3.013590 \times 10^{-6}$   
Weight (Signal) Average s-value = 4.590147

2:  
concentration [Signal] =  $3.975290 \times 10^{-7}$   
Weight (Signal) Average s-value = 10.255669

**INTEGRATE DISTRIBUTION** 

Integral from 9.301604 to 11.212376:

1:  
concentration [Signal] =  $3.616159 \times 10^{-7}$   
Weight (Signal) Average s-value = 10.226027

2:  
concentration [Signal] =  $3.891436 \times 10^{-7}$   
Weight (Signal) Average s-value = 10.411008

These conform to expected values.

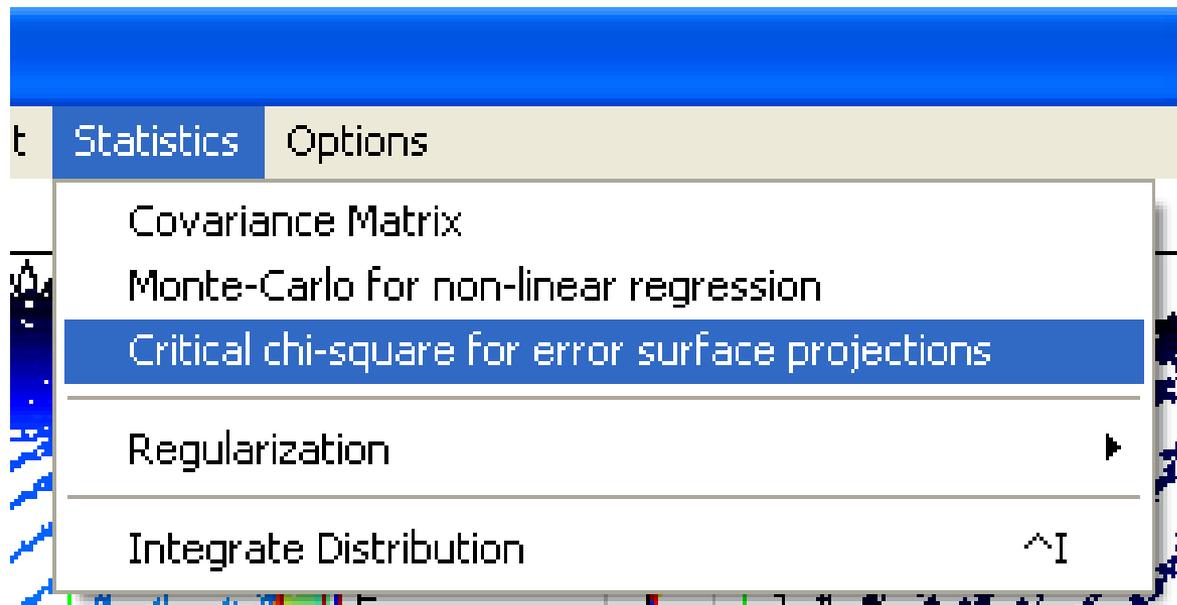
# Step 27

Given the data, it seemed reasonable to conclude that all of the material in segment 2 could be represented by 1:1 molar ratios of GST-VCA and Arp2/3. To examine this possibility, a statistical criterion for acceptability must be defined. If the material between 6.8 and 15 S could be modeled using 1:1 complexes with the fit becoming less than  $1(\sigma)$  worse than the best fit, the 1:1 model could be deemed acceptable. The change in the quality of the fit was to be judged using the  $\chi^2$  statistic. The  $\chi^2$  of the converged fit ( $\chi_b^2$ ) was 0.3061391. To determine whether any alternative fits to the data are worse by the criterion mentioned above, the  $\chi^2$  of a  $1(\sigma)$  worse fit must be established. To do this, the “Statistics” menu item was clicked, and “Critical chi-square for error surface projections” was selected. A dialog appeared, prompting the user to enter the desired confidence level. Because  $1(\sigma)$  was desired, the default value of “0.683” was accepted. Another dialog then appeared, telling the user that the critical  $\chi^2$  ( $\chi_{c,1\sigma}^2$ ) was 0.306843. Thus, if the  $\chi^2$  of any alternative fit (the “test  $\chi^2$ ”, or  $\chi_t^2$ ) exceeds this value, the quality of the fit will be deemed statistically worse than that of the best fit.

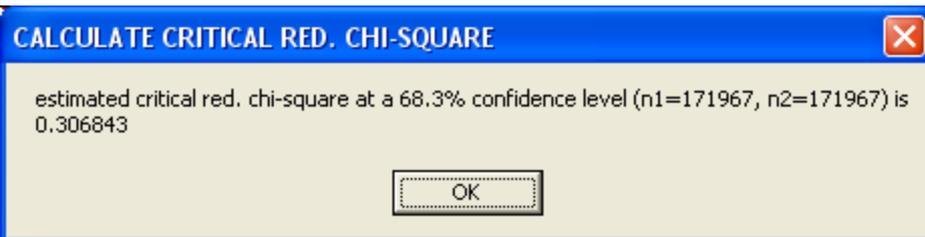
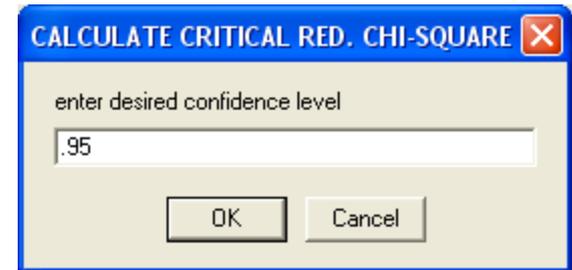
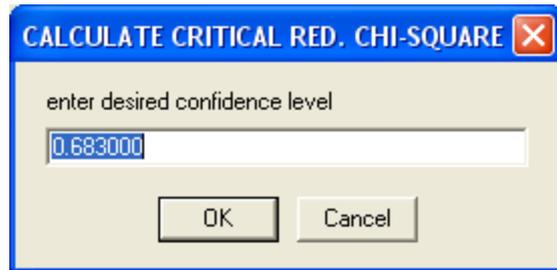
# Step 27 continued

Also, a rejection criterion was established. The same statistical calculation was performed, but the confidence level was set to 0.95. This value was termed " $\chi_{c,2\sigma}^2$ " and found to be 0.308574; if  $\chi_t^2$  for a constrained fit exceeded this value, it could be safely rejected as likely to be incorrect. See the next two pages for a pictorial description of these calculations.

# Initiate Statistics Calculations

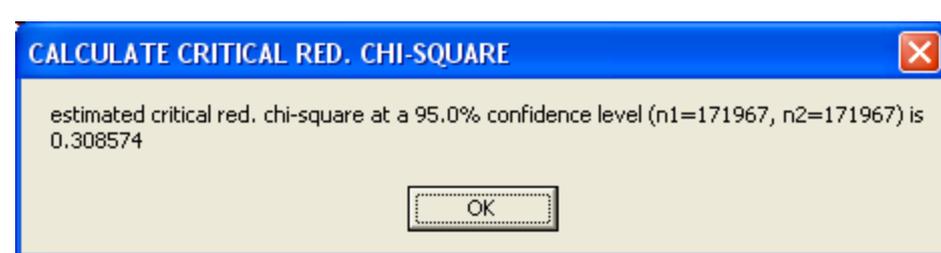


# Calculating the critical $\chi^2$ 's



$$\chi_{c,1\sigma}^2$$

The criterion for "statistical distinguishability"



$$\chi_{c,2\sigma}^2$$

The criterion for remorseless rejection

# Step 28

The Global Parameter Box was recalled. In the “segment 2” section, “spectrum 2” was deactivated, and the boxes for “spectrum 1” were altered such that they both had “1” in them. Thus, the program will attempt to model the material sedimenting between 6.8 and 15 S as 1:1 complexes of GST-VCA and Arp2/3. In other words, a single  $c_{\text{GST-VCA:Arp2/3}}(s)$  distribution will be used to model these data in the given  $s$ -range. The parameter box was dismissed by pressing “OK.” See the next page.

# First, let's try a 1:1 molar ratio

The screenshot shows a software interface for fitting spectra. It is organized into three segments, each with its own set of parameters. Segment 2 is highlighted with a red box, and its 'xt1/chr.' and 'xt2/chr.' fields are both set to 1.000, indicating a 1:1 molar ratio. An arrow points from the text below to this red box.

segment	continuous	resolution	linea log	s min	s max	frictional ratio	xt1/chr.	xt2/chr.	fit ff0
segment 1	<input checked="" type="checkbox"/>	25	<input checked="" type="radio"/>	0.200	5.300	1.837	1.000	0.000	<input checked="" type="checkbox"/>
segment 2	<input checked="" type="checkbox"/>	25	<input checked="" type="radio"/>	6.800	15.000	1.479	1.000	1.000	<input checked="" type="checkbox"/>
segment 3	<input type="checkbox"/>	0	<input checked="" type="radio"/>	17.000	60.000	1.200	0.000	0.000	<input type="checkbox"/>

Additional parameters for the fit:

- discrete spectra in multiples of chromophore xt
- contin. spectra in multiples of chromophore xt
- chromophore #1:  193089.  92416.2
- chromophore #2:  615516.  244420.
- with Tikhonov Regularization P= 0.700
- normalize distributions

Buttons: Cancel, OK

This constrains segment 2 to fit the data with a 1:1 ratio of the two “chromophores”

# Step 29

A Global Run was performed. The value of  $\chi^2$  was 0.3063701. Global fitting at this point would only make the fit better, yet the new fit already meets the established criterion for acceptability. According to the statistical criteria that we have defined, we can accept the 1:1 constraint as consistent with the MSSV data.

# Global Run

C:\AUC Data\Rosen\120106\170712\mssv\Mi

a Copy Display Model Global Parameters Concer

sim #141, ML step #100, step #0  
glob red chisq = 3.063701e-01 (n\* = 256136)  
locrmsd=0.006453 (n=128068)  
Mix\_cell3\_if.xp (set#1, w1#1)  
discrete M =  
discrete s =  
cont1 [0.20 - 5.30 (25 lin)] ff0 = 1.837 | cont2 [6.80 -  
setA = 1.000e+00

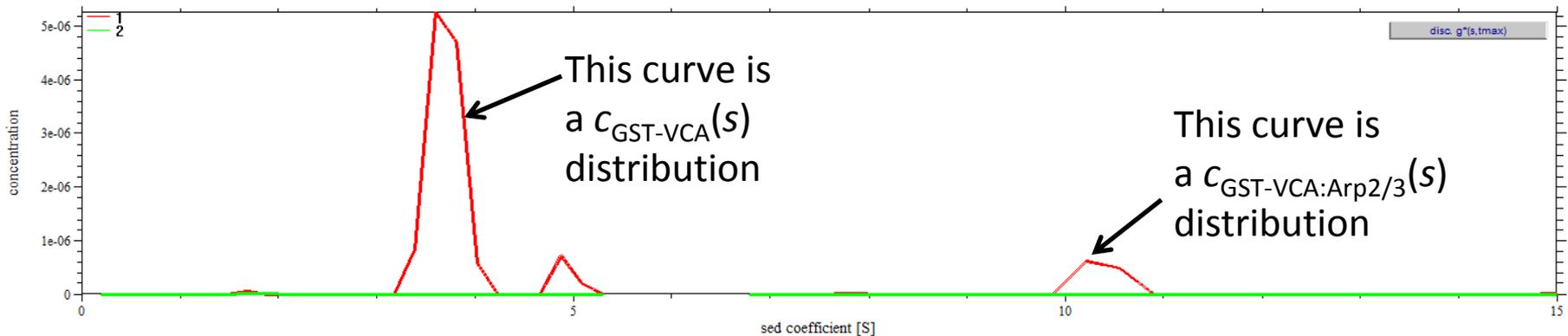
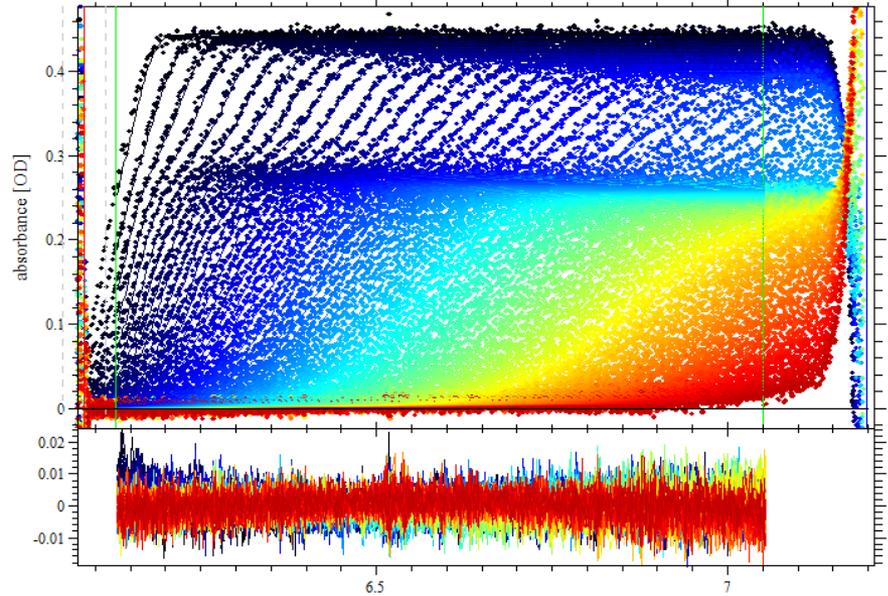
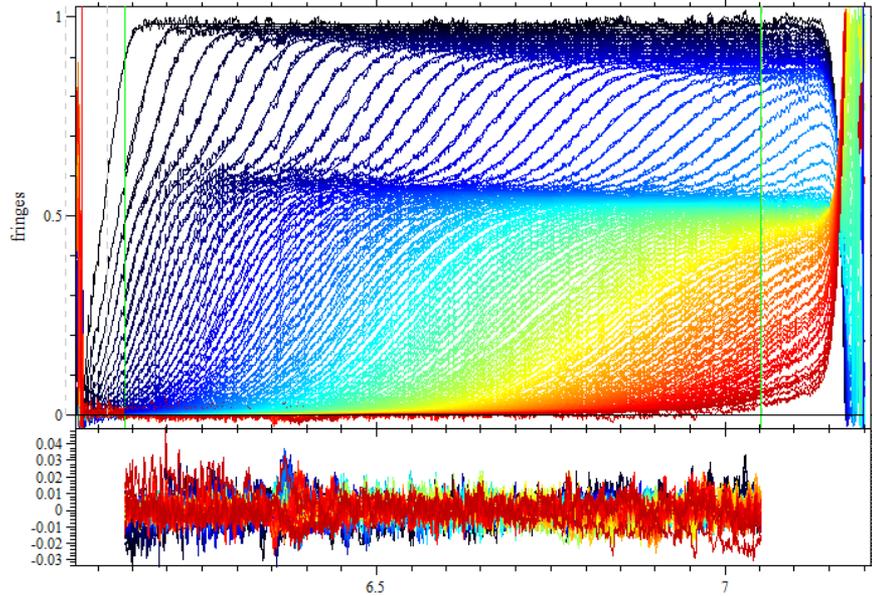
The value isn't above either critical chi-squared, so this molar ratio is consistent with the data.

# Step 30

For illustrative purposes, a Global Fit was initiated. After about 10 min., the fit converged. The  $\chi^2$  was 0.3063701, unchanged from the value obtained from the Global Run in Step 29. As a check on the internal consistency of the result, the 10.3-S peak in the  $c_{\text{GST-VCA:Arp2/3}}(s)$  distribution was integrated. Our calculation above indicated that 0.38  $\mu\text{M}$  Arp2/3 was expected, so 0.38  $\mu\text{M}$  of the complex should also be expected. Indeed, the integrated  $[\text{GST-VCA:Arp2/3}] = 0.38 \mu\text{M}$ . The 1:1 model was therefore accepted as consistent with our MSSV data. See the next two pages.

# After a Global Fit

Data Copy Display Model Global Parameters Concentrations Experiment Parameters Run Fit Statistics Options Help



# The Integration

---

Integral from 9.545454 to 11.126814:

1:

concentration [Signal] = 3.818431e-07  
Weight (Signal) Average s-value = 10.367032

2:

concentration [Signal] = 0.000000e+00  
Weight (Signal) Average s-value = 0.000000

---

# Step 31

What about other possible stoichiometries? For the 10.4-S species, the only other stoichiometries that merit consideration are two GST-VCA's to one Arp2/3 complex and two Arp2/3 complexes bound to one GST-VCA dimer (although stoichiometries involving more than one Arp2/3 complex should have greater  $s$ -values than 10.4 S). To explore these possibilities, the saved, best-fit configuration of unconstrained fit was opened. To do this, the Data menu item was selected, and "Read Configuration from File" was selected. The defaults were accepted, and the Global Parameter box automatically appeared. All of these parameters were correct except for those in the segment 2 portion of the box. First, the possibility of two GST-VCA's binding to one Arp2/3 was examined. As in Step 28, "spectrum 2" was turned off (unchecked). The left and right boxes for spectrum 1 were altered to read "2" and "1", respectively. This fits the 6.8 to 15 S region with a two GST-VCA dimer to one Arp2/3 complex. The Global Parameters box was dismissed by pressing "OK." The next page shows the Global Parameters box for this trial.

# Trying 2:1 Now

continuous	resolution	s min	s max	frictional ratio	
<input checked="" type="checkbox"/> segment 1	25	<input type="radio"/> linear <input type="radio"/> log	0.200	5.300	1.837
<input checked="" type="checkbox"/> spectrum 1	xt1/chr: 1.000	xt2/chr: 0.000	PP	<input checked="" type="checkbox"/> fit f0	
<input checked="" type="checkbox"/> spectrum 2	0.000	1.000	PP		

segment 2	resolution	s min	s max	frictional ratio	
<input checked="" type="checkbox"/> segment 2	25	<input type="radio"/> linear <input type="radio"/> log	6.800	15.000	1.479
<input checked="" type="checkbox"/> spectrum 1	xt1/chr: 2	xt2/chr: 1	PP	<input checked="" type="checkbox"/> fit f0	
<input type="checkbox"/> spectrum 2	0.000	1.000	PP		

segment 3	resolution	s min	s max	frictional ratio	
<input type="checkbox"/> segment 3	0	<input type="radio"/> linear <input type="radio"/> log	17.000	60.000	1.200
<input checked="" type="checkbox"/> spectrum 1	xt1/chr: 0.000	xt2/chr: 0.000	PP	<input type="checkbox"/> fit f0	
<input checked="" type="checkbox"/> spectrum 2	0.000	0.000	PP		

<input checked="" type="checkbox"/> discrete spectra in multiples of chromophore xt
<input checked="" type="checkbox"/> contin. spectra in multiples of chromophore xt
chromophore #1 <input type="checkbox"/> 193089. <input type="checkbox"/> 92416.2
chromophore #2 <input type="checkbox"/> 615516. <input type="checkbox"/> 244420.

<input checked="" type="checkbox"/> with Tikhonov Regularization P= 0.700
<input checked="" type="checkbox"/> normalize distributions

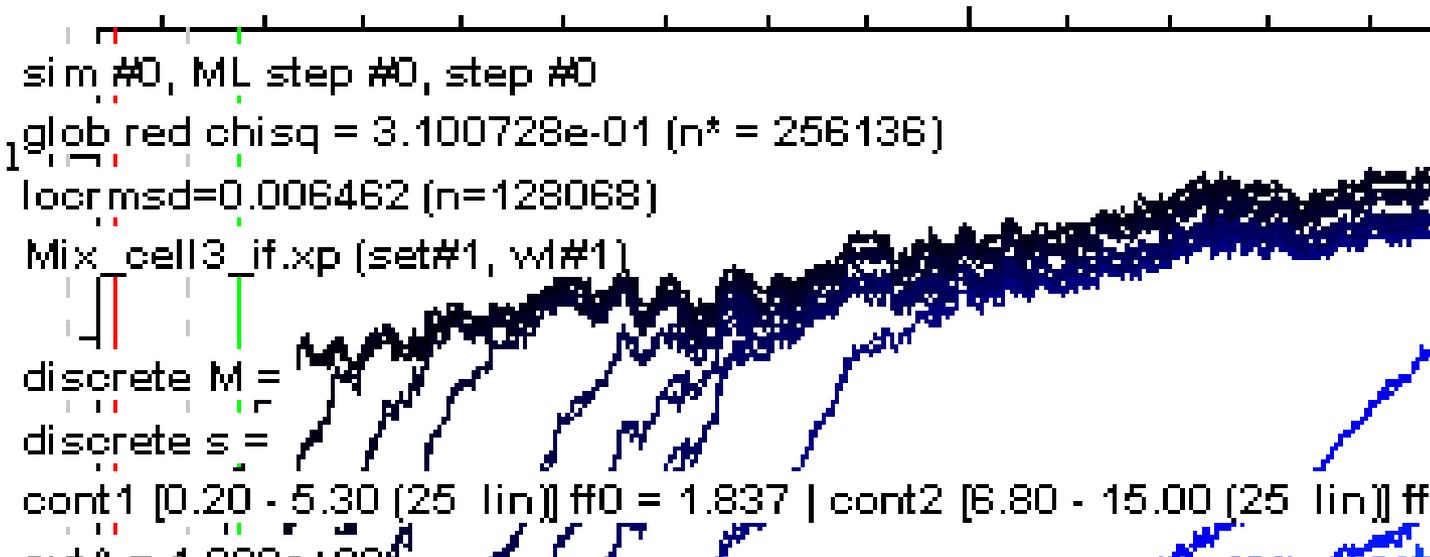
Cancel  
OK

# Step 32

A Global Run was initiated. The was 0.3100728, well above both  $\chi_{c,1\sigma}^2$  and  $\chi_{c,2\sigma}^2$ . A Global Fit was then performed. The resulting  $\chi_t^2$  was 0.3096959, which was greater than  $\chi_{c,2\sigma}^2$ . Thus, the 2:1 stoichiometric constraint was safely rejected. These statistics are documented on the next two slides.

It is worth noting that, in an initial, unshown analysis of these data was carried out with half of the scans shown here. When applying the 2:1 stoichiometric constraint, the situation arose of  $\chi_{c,1\sigma}^2 < \chi_t^2 < \chi_{c,2\sigma}^2$ , as in Step 35. By including *all* of the data (above), the 2:1 constraint was easily rejected. This is an example of the fact that including more data results in superior spectral resolution, as mentioned in section 4.1 of the text of the parent paper of this protocol, Padrick & Brautigam, Methods, 2011.

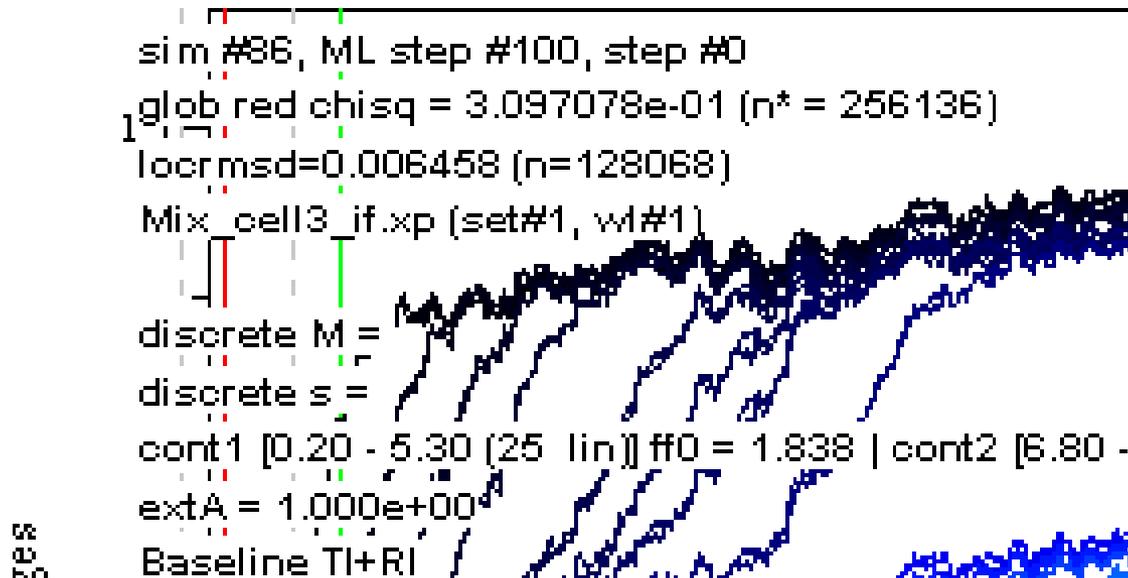
# After a Global Run



```
sim #0, ML step #0, step #0
glob red chisq = 3.100728e-01 (n* = 256136)
locrmsd=0.006462 (n=128068)
Mix_cell3_if.xp (set#1, wl#1)
discrete M =
discrete s =
cont1 [0.20 - 5.30 (25 lin)] ff0 = 1.837 | cont2 [6.80 - 15.00 (25 lin)] ff1
```

This  $\chi^2$  is above both critical values. We'd better fit to see if the ML algorithm can improve the stat.

# After Global Fit



The fit has converged, but the chi-squared is still above both critical values. We may remorselessly reject the 2:1 molar ratio.

# Step 33

To examine the possibility of 1 GST-VCA and 2 Arp2/3's in the complex, a new SEDPHAT session was started, and the best configuration was loaded into the program by selecting "Read Configuration from File" from the Data menu item.

# Step 34

The Global Parameter box was summoned, and Step 31 was repeated, but this time, the left and right spectrum 1 boxes were altered to read “1” and “2”, respectively. See the next page.

# Trying a 1:2 Molar Ratio

continuous	resolution	s min	s max	frictional ratio	
<input checked="" type="checkbox"/> segment 1	25	<input type="radio"/> linear <input type="radio"/> log	0.200	5.300	1.837
<input checked="" type="checkbox"/> spectrum 1	xt1/chr: 1.000	xt2/chr: 0.000	<input type="button" value="PP"/>	<input checked="" type="checkbox"/> fit f0	
<input checked="" type="checkbox"/> spectrum 2	0.000	1.000	<input type="button" value="PP"/>		

segment 2	resolution	s min	s max	frictional ratio	
<input checked="" type="checkbox"/> segment 2	25	<input type="radio"/> linear <input type="radio"/> log	6.800	15.000	1.479
<input checked="" type="checkbox"/> spectrum 1	xt1/chr: 1.000	xt2/chr: 2.000	<input type="button" value="PP"/>	<input checked="" type="checkbox"/> fit f0	
<input type="checkbox"/> spectrum 2	0.000	1.000	<input type="button" value="PP"/>		

segment 3	resolution	s min	s max	frictional ratio	
<input type="checkbox"/> segment 3	0	<input type="radio"/> linear <input type="radio"/> log	17.000	60.000	1.200
<input checked="" type="checkbox"/> spectrum 1	xt1/chr: 0.000	xt2/chr: 0.000	<input type="button" value="PP"/>	<input type="checkbox"/> fit f0	
<input checked="" type="checkbox"/> spectrum 2	0.000	0.000	<input type="button" value="PP"/>		

<input checked="" type="checkbox"/> discrete spectra in multiples of chromophore xt
<input checked="" type="checkbox"/> contin. spectra in multiples of chromophore xt
chromophore #1 <input type="checkbox"/> xt wl 1: 193089. <input type="checkbox"/> xt wl 2: 92416.2
chromophore #2 <input type="checkbox"/> 615516. <input type="checkbox"/> 244420.

with Tikhonov Regularization P= 0.700

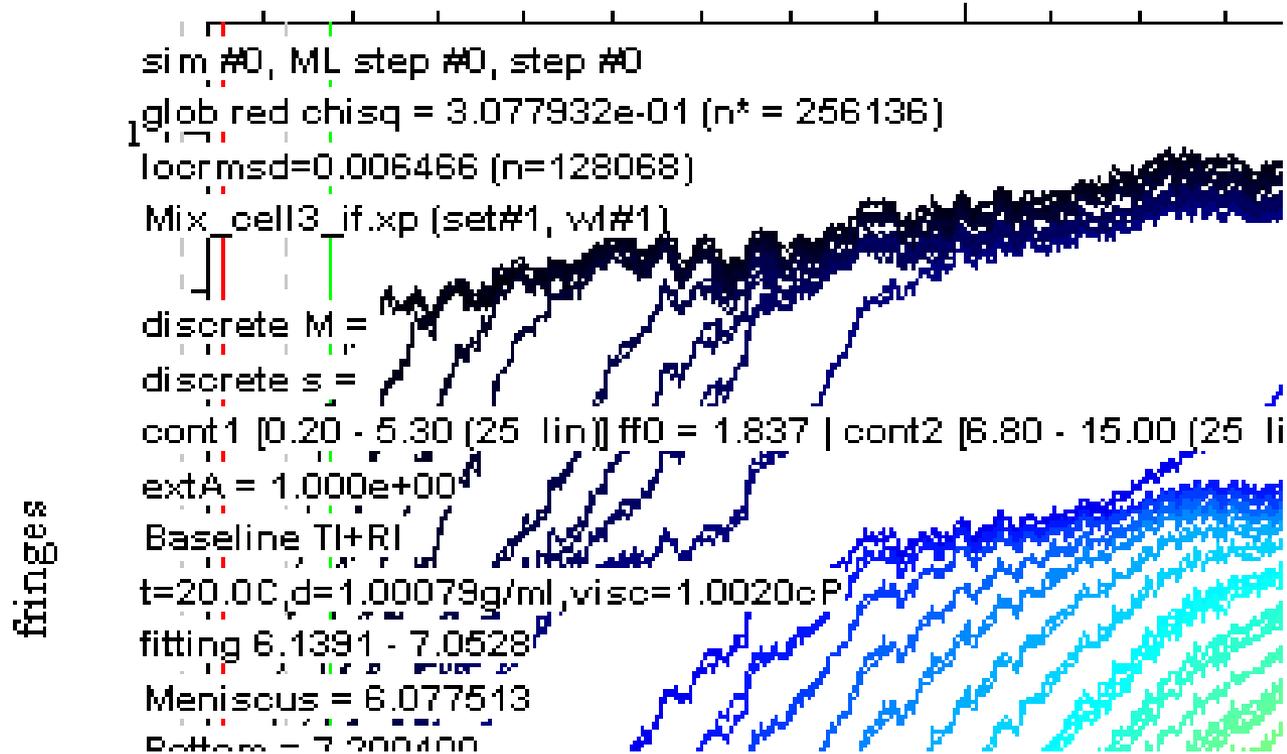
normalize distributions

**IMPORTANT:** This is a new SEDPHAT session– we reloaded the saved fitting parameters, then summoned this box.

# Step 35

A Global Run was performed. The  $\chi_t^2$  value was 0.3077832. A Global Fit was performed using the Marquardt-Levenberg fitting algorithm. The final converged value was 0.3077230. This value is above  $\chi_{c,1\sigma}^2$ , but below  $\chi_{c,2\sigma}^2$ . Thus, the stoichiometric constraint has made the fit significantly worse, but the constraint is not rejected by our  $2(\sigma)$  criterion. However, consideration of conservation of mass casts significant suspicion on this stoichiometry. The Arp2/3 concentration in the complex with the 1:2 stoichiometry was found to be 0.434  $\mu\text{M}$ , 14% above the input concentration of 0.38  $\mu\text{M}$ . Additionally, other factors, such as the hydrodynamic behavior of the complex, make the 1:2 complex of GST-VCA and Arp2/3 very unlikely. Salient points concerning this step are found on the next three pages.

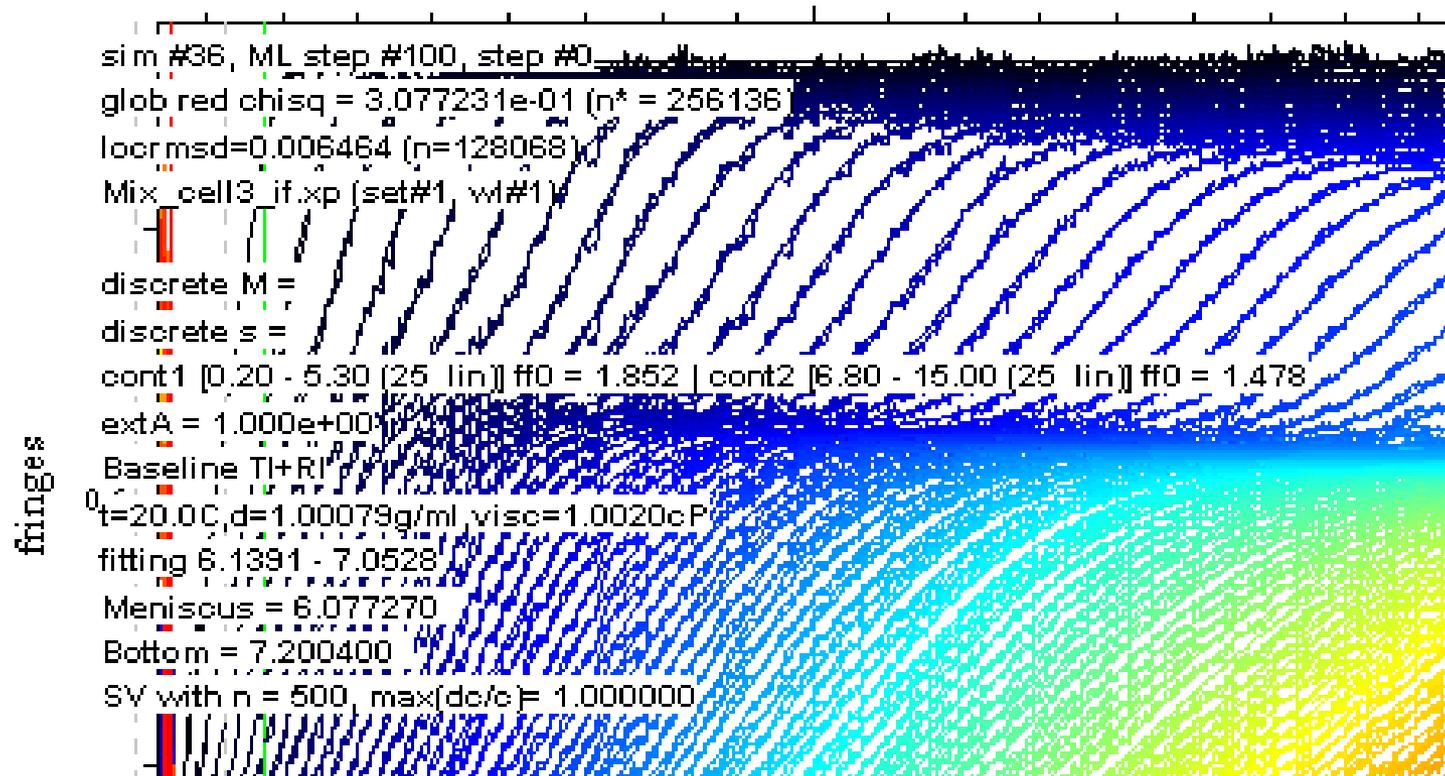
# After a Global Run



We are already below the  $2(\sigma)$  cutoff. Can the ML algorithm get us to statistical indistinguishability?

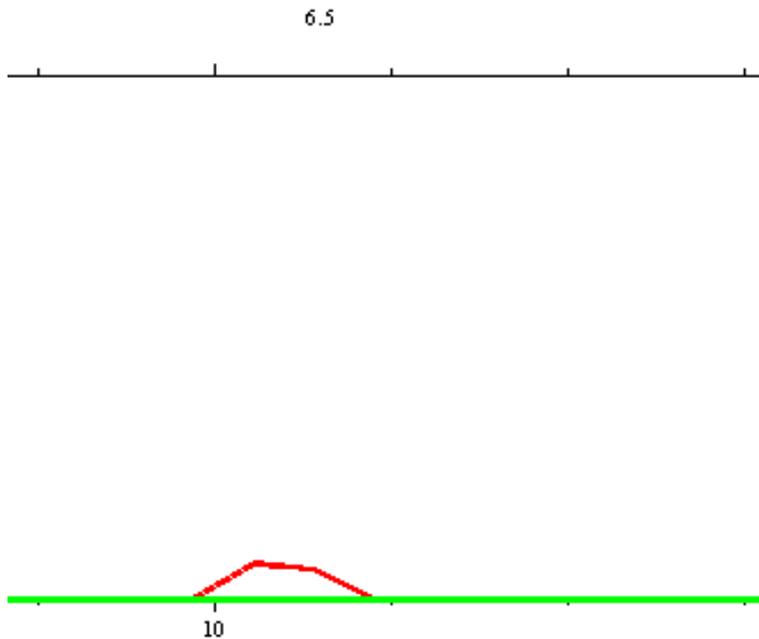
# After a Global Fit

Data Copy Display Model Global Parameters Concentrations Experiment Parameters



No, we didn't get there. This fit is statistically worse than the best, unconstrained fit. But it isn't instantly rejectable, either.

# Concentration



**INTEGRATE DISTRIBUTION** ✕

Integral from 9.602750 to 11.069518:

1: concentration [Signal] = 2.173753e-07  
Weight (Signal) Average s-value = 10.370193

2: concentration [Signal] = 0.000000e+00  
Weight (Signal) Average s-value = 0.000000

But, let's integrate the peak in the second segment. The concentration of the complex is 0.217  $\mu\text{M}$ . That means that  $[\text{Arp2/3}] = 0.434 \mu\text{M}$ . But we expected 0.38  $\mu\text{M}$ . Our integrated value is thus 14% greater than our expected value.

# References

[1] P. Schuck, Size distribution analysis of macromolecules by sedimentation velocity ultracentrifugation and Lamm equation modeling. *Biophysical J.* 78 (2000) 1606-1619.

[2] J.L. Cole, J.W. Lary, T.P. Moody, and T.M. Laue, Analytical ultracentrifugation: sedimentation velocity and sedimentation equilibrium. in: J.J. Correia, and H.W.I. Detrich, (Eds.), *Biophysical Tools for Biologists. Volume One: In Vitro Techniques.*, Academic Press, 2008, pp. 143-179.

[3] S.B. Padrick, R.K. Deka, J.L. Chuang, R.M. Wynn, D.T. Chuang, M.V. Norgard, M.K. Rosen, and C.A. Brautigam, Determination of protein complex stoichiometry through multisignal sedimentation velocity experiments. *Anal. Biochem.* 407 (2010) 89-103.

**FINIS**