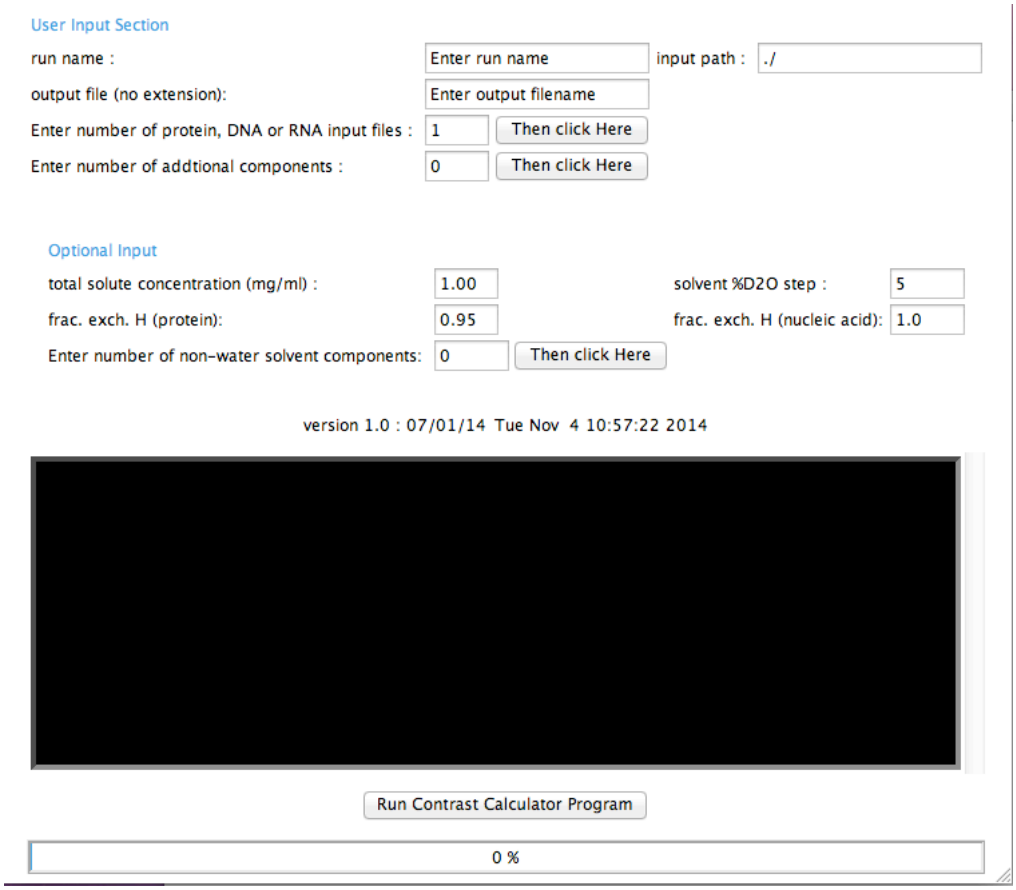
Contrast Calculator Description

Contrast Calculator is a SASSIE module that calculates the neutron and x-ray scattering length density (SLD), contrast and I(0) values for a single molecule or complex consisting of proteins and/or nucleic acids, as well as other compounds such as polymers, lipids and carbohydrates, based only on sequence or chemical formula information. The input files for proteins and nucleic acids can be in either FASTA or PDB format. Chemical formulas are entered manually. The neutron values are calculated as a function of the $\%D_2O$ in the solvent. The fraction of deuteration for the proteins and/or nucleic acids is considered in the calculation. D atoms are inserted into chemical formulas manually. Non-water solvent components can also be considered if desired. There is no limit to the number of proteins, nucleic acids or other components that comprise the complex. However, plots of the neutron SLD, contrast and I(0) as a function of $\%D_2O$ are only provided if the total number of components is less than 3. The SLD, contrast and I(0) information are output in 3 separate files. The output files contain the input information as well as the output parameters for both x-rays and neutrons.

Contrast Calculator Tutorial

The standalone http://www.smallangles.net/sassie/SASSIE/SASSIE_HOME.html and web http://sassie-web.chem.utk.edu/sassie2 versions of SASSIE are both covered in this tutorial. Screenshots are from the standalone version, but the layout for the web version is similar. Differences will be noted in the tutorial. The Contrast Calculator is started from the Tools menu. The Contrast Calculator screen is divided into 3 parts. The top of the screen contains the User Input Section, which contains the run directory and output file names as well as the number of input files (FASTA sequences, PDB files or chemical formulas). The middle of the screen contains Optional Input, which includes the total solute concentration (default = 1 mg/ml), the solvent $\%D_2O$ step (default = 5% increments in $\%D_2O$), the fraction of exchangeable hydrogen atoms that actually do exchange for both protein and nucleic acid components (defaults = 0.95 and 1.0 for proteins and nucleic acids, respectively) and the number of non-water solvent components (optional; default = 0). Note that the fraction of exchangeable hydrogen atoms for chemical formulas is entered differently, as will be shown examples 6 & 7 below. The bottom of the screen contains a box where status messages and other information are provided. The "Run Contrast Calculator Program" button ("Submit" in the web version) is used to start the calculations once all necessary parameters have been entered. Finally, a slider at the bottom of the screen tracks the progress of the contrast calculations.

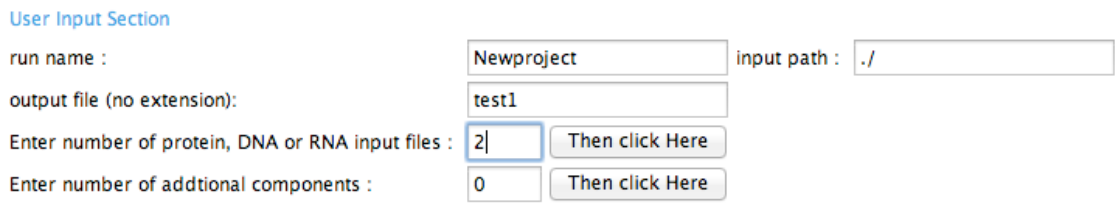


User Input Section

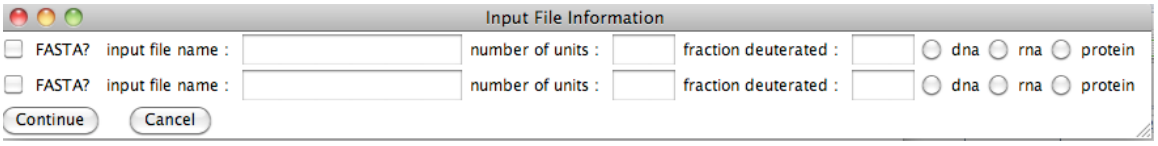
First, enter the run name and output file names. The run name will be the name of the directory that will contain the output files, and may be a run name used in other SASSIE modules as well. This directory will be created under the current directory in the standalone version and under the project name directory in the web version. The output files will be in a subdirectory called "contrast_calc" under the run name directory and they will be named as designated in the output filename box. Do not include extensions in the output file names. The output files created will have the names: filename_sld.txt, filename_izero.txt and filename_contrast.txt and they will contain the calculated scattering length densities, I(0) values and scattering contrasts, respectively.

Next, enter the number of protein, DNA or RNA input (FASTA or PDB) files as well as the number of additional components, which will be input in the form of chemical formulas. Either of these values can be 0, but not both. The input path is the location of these input files and will typically be the current directory. However, this can be changed by entering a different input path. (In the web version, the files will be uploaded from a directory of your choosing.)

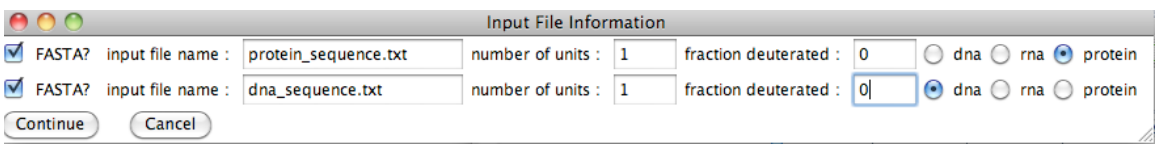
Example 1: Protein/DNA Complex (FASTA files)



Once all of the information has been input, click the button to the right of the first input box that says, "Then click Here". This will open the first input file dialog box. (Press the Tab key after entering the number of files in the web version and the appropriate input boxes will appear.)

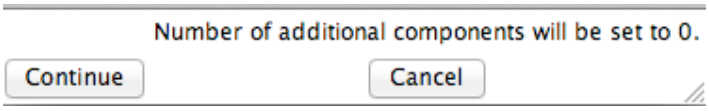


Note that the number of input lines corresponds to the number of input files in the User Input Section. This can be changed by clicking the "Cancel" button and entering a different number of input files.



Enter the appropriate information in the boxes and check the appropriate radio buttons. The FASTA box must be checked if your input file contains sequence information. (Choose "on" from the menu on the web version.) Do not check the FASTA box if your input file is a PDB file. (Choose "off" from the menu in the web version.) The "number of units" boxes should be filled out to indicate the stoichiometry of the complex in terms of the sequence information that is in the FASTA or PBD files. The "fraction deuterated" should indicate the fractional amount (0-1) of H atoms that are replaced by D atoms in that portion of the complex. The type of molecule, i.e., dna, rna and protein, must be chosen by clicking the appropriate radio button. Once all parameters are entered, click the "Continue" button.

Then click the button to the right of the second input box that says, "Then click Here". This will open the second input file dialog box. (Press the Tab key after entering the number of additional components in the web version and the appropriate input boxes will appear.)

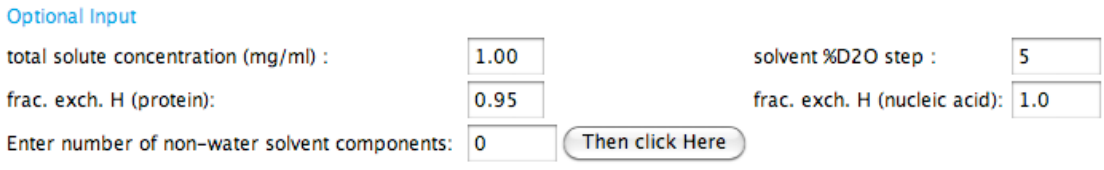


In this example, there is nothing to fill out since there are no additional components that will be entered as chemical formulas. Click "Continue" to accept the value. (The dialog box doesn't appear in web version.)

At this point, the contrast calculator can be run by clicking on the "Run Contrast Calculator Program" button ("Submit" in the web version) at the bottom of the screen. In this case, the program will be run with the default Optional Input parameters.

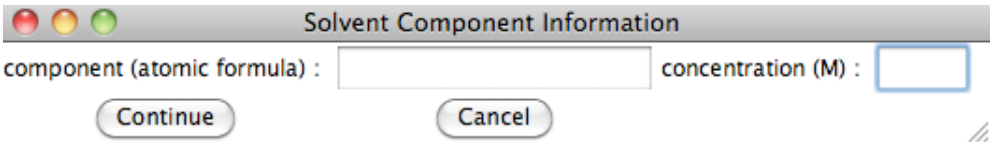
Optional Input Section

If the Optional Input parameters need to be changed from their default values, this is done in the Optional Input part of the screen.



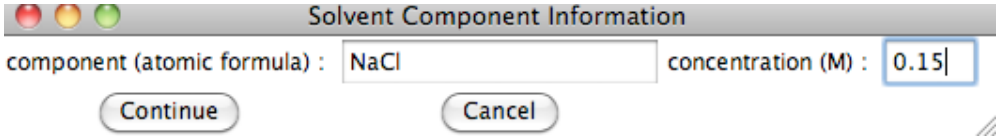
To change the number of non-water solvent components, click the button to the right of the input box that says, "Then click Here". This will open the input file

dialog box. (Press the Tab key after entering the number of non-water solvent components in the web version and the appropriate input boxes will appear).



Note that the number of input lines corresponds to the number of non-water solvent components in the Optional Input section. This can be changed by clicking the "Cancel" button and entering a different number of components.

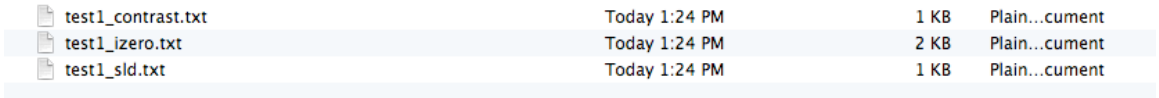
Enter the atomic formula and molar concentration (mol/L) of the components and then click the "Continue" button.



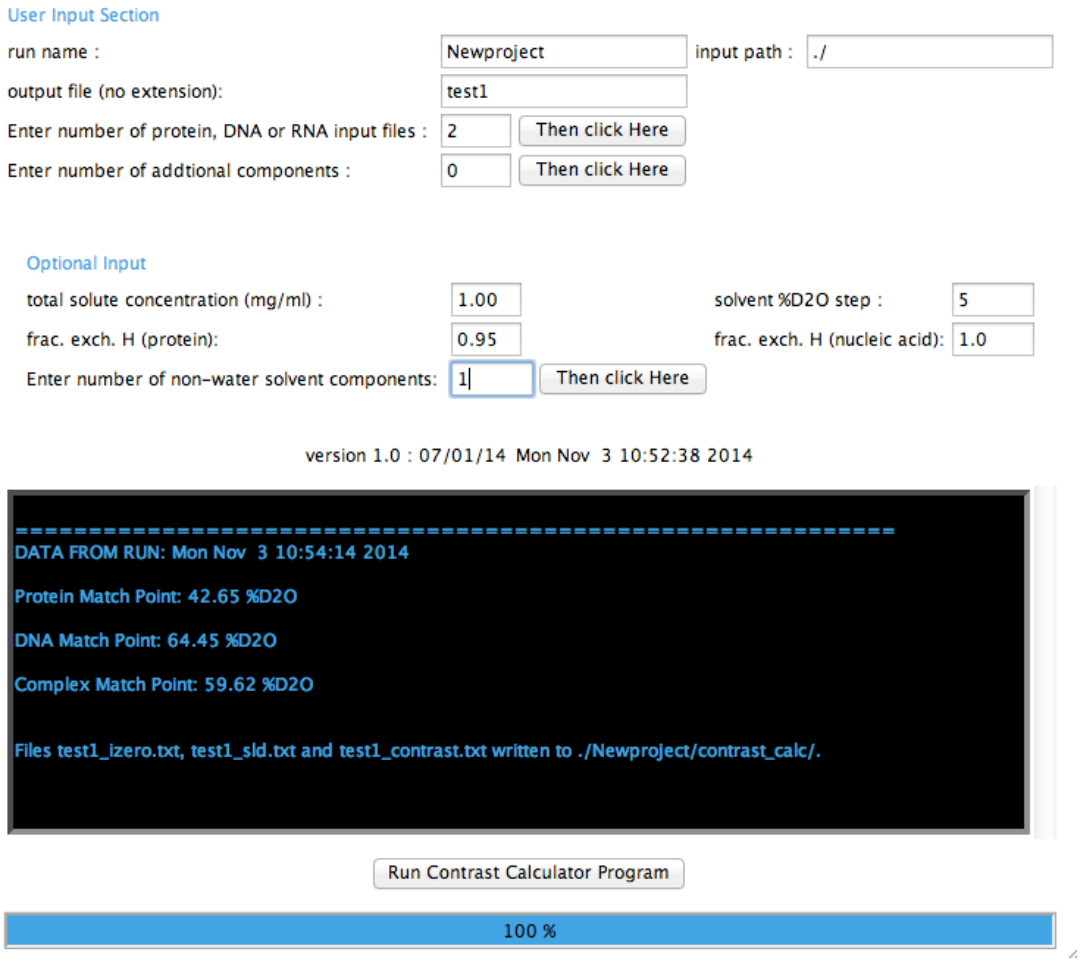
Now, run the program by clicking on the "Run Contrast Calculator Program" button ("Submit" in the web version) at the bottom of the screen. It will now use the entered Optional Input parameters.

After the program runs, status and other information can be found in the box under the Optional Input section of the screen. If the number of components is less than three, plots of the SLD, contrast, I(0) and sqrt(I(0)) values as a function of the $\%D_2O$ will be output as well. Note that the plots may have to be cleared manually depending on the operating system used to run SASSIE. (Plots can't be cleared in the web version.)

Note that the last line in the output box states the location of the output files in your current directory (in this case ./Newproject/contrast_calc/). A look at that directory will show files that start with the user-inputted file name. (In the web version, the "contrast_calc" directory will be located under your project name – which is set by clicking on the "person" icon in the upper right corner of the page. The directory must be downloaded to your local machine in order to look at the output files. Click on the file cabinet icon on the upper right of the screen to choose the files to download. Then, click on the created links to view and/or save the files.)



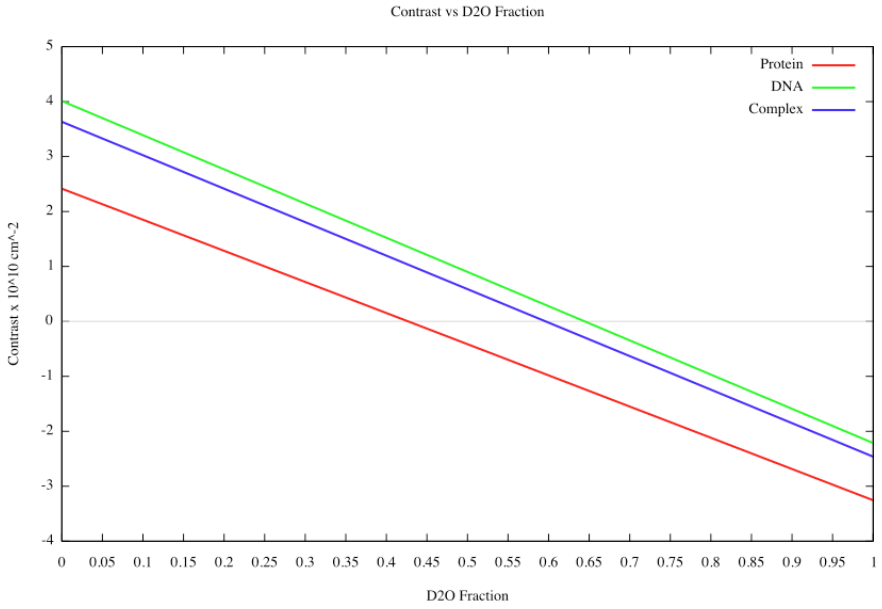
The files contain a summary of the input information as well as the x-ray and neutron parameters of interest. These files can be used to re-create the plots generated by the Contrast Calculator program.



Experiment Planning Note: The match points for the protein and DNA components are well separated, which means that the scattering from the two components can theoretically be separated by performing a contrast variation series of measurements. Successfully doing this in practice depends on a number of factors including the relative mass fractions of the two components, the total concentration of the complex, the H_2O:D_2O conditions chosen, etc. In this particular case, the mass fraction of DNA is much higher than that of the protein. Therefore, the match point of the complex is very close to that of the DNA component. Thus, it will be difficult to measure near the match point of the DNA component. The I(0) values in the test1_izero.txt output file will help determine how close to the two match points it will be possible to measure.

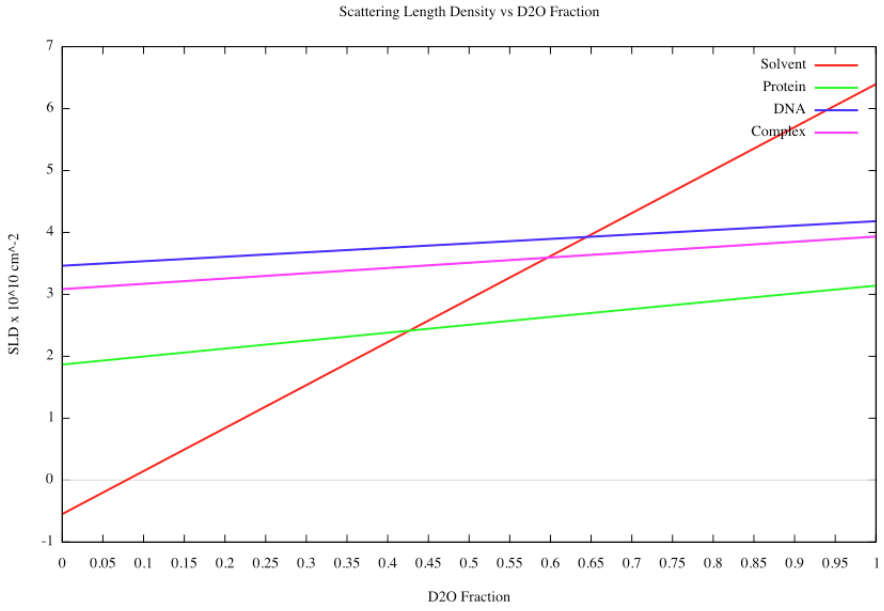
test1_contrast.txt:

```
#Date: Fri Oct 31 14:18:22 2014

#Files used: protein_sequence.txt (1), dna_sequence.txt (1)

#Solvent Components:
#Component, molar conc, volume (A^3), Mw (kDA), x-ray SL, neutron SL (10^-12 cm), x-ray SLD, neutron SLD (10^10 cm^-2)
#       H2O      55.51   29.9    0.018    2.820  -0.168   9.428  -0.561
#       Na1Cl1    0.15   29.3    0.058    7.896   1.321  26.958   4.509
#Totals: x-ray SL, neutron SL (10^-12 cm), x-ray SLD, neutron SLD (10^10 cm^-2)
#       157.298  -9.097   9.474  -0.548

#XRAY Contrast (10^10 cm^-2):
#Protein, NA, Complex
#  2.987          6.109   5.370

#NEUTRON Contrast:
#Protein Match Point: 42.65 %D2O
#DNA Match Point: 64.45 %D2O
#Complex Match Point: 59.62 %D2O

#Fraction of exchanged protein hydrogens: 0.95
#Fraction of exchanged nucleic acid hydrogens: 1.00

#NEUTRON Contrast (10^10 cm^-2):
# frac D2O, Protein, DNA, Complex
 0.00     2.415   4.011   3.633
 0.05     2.132   3.700   3.328
 0.10     1.850   3.389   3.024
 0.15     1.567   3.078   2.719
 0.20     1.284   2.767   2.415
 0.25     1.001   2.456   2.110
 0.30     0.718   2.145   1.806
 0.35     0.435   1.833   1.501
 0.40     0.152   1.522   1.197
 0.45    -0.131   1.211   0.892
 0.50    -0.415   0.900   0.587
 0.55    -0.698   0.589   0.283
 0.60    -0.982   0.277  -0.022
 0.65    -1.266  -0.034  -0.327
 0.70    -1.550  -0.345  -0.632
 0.75    -1.833  -0.657  -0.937
 0.80    -2.117  -0.968  -1.242
 0.85    -2.402  -1.279  -1.547
 0.90    -2.686  -1.591  -1.852
 0.95    -2.970  -1.902  -2.157
 1.00    -3.254  -2.214  -2.462
```



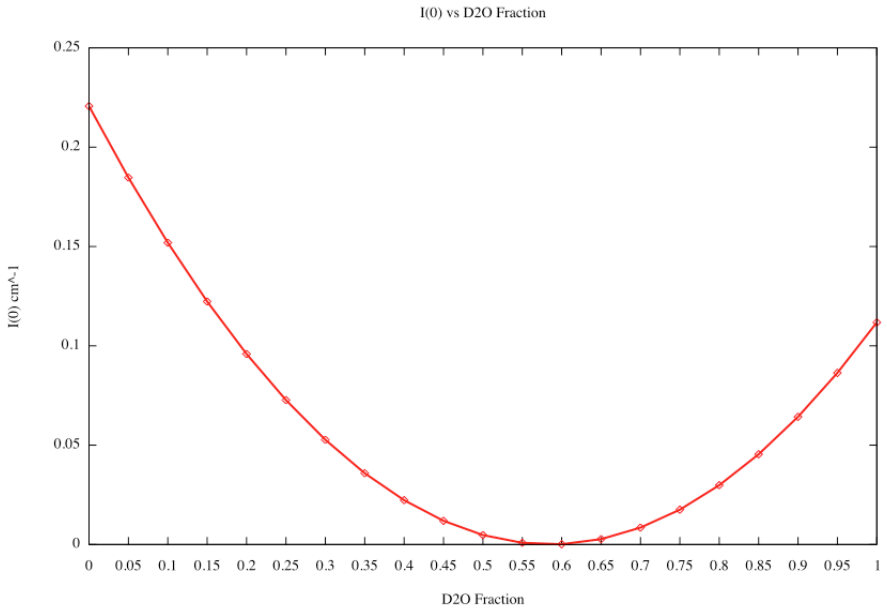
test1_sld.txt:

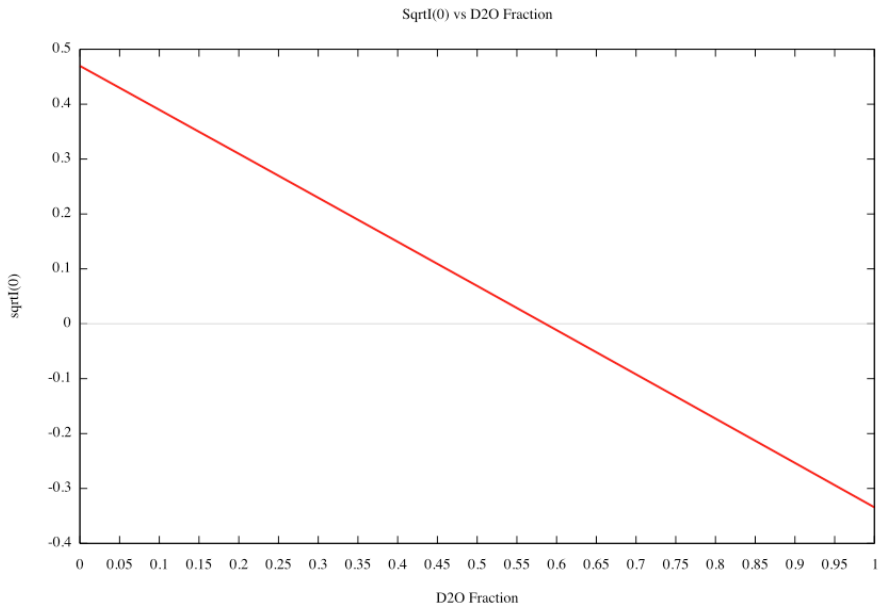
```
#Date: Fri Oct 31 14:18:22 2014

#Files used: protein_sequence.txt (1), dna_sequence.txt (1)

#Solvent Components:
#Component, molar conc, volume (A^3), Mw (kDA), x-ray SL, neutron SL (10^-12 cm), x-ray SLD, neutron SLD (10^10 cm^-2)
#       H2O      55.51   29.9    0.018    2.820  -0.168   9.428  -0.561
#       Na1Cl1    0.15   29.3    0.058    7.896   1.321  26.958   4.509
#Totals: x-ray SL, neutron SL (10^-12 cm), x-ray SLD, neutron SLD (10^10 cm^-2)
#       157.298  -9.097   9.474  -0.548

#XRAY SLD (10^10 cm^-2):
#Protein, NA, Complex, Solvent
# 12.461         15.583  14.844   9.474

#NEUTRON SLDs:
#Protein Match Point: 42.65 %D2O
#DNA Match Point: 64.45 %D2O
#Complex Match Point: 59.62 %D2O

#Fraction of exchanged protein hydrogens: 0.95
#Fraction of exchanged nucleic acid hydrogens: 1.00

#NEUTRON SLDs (10^10 cm^-2):
# frac D2O, Protein, DNA, Complex, Solvent
 0.00     1.867   3.463   3.085  -0.548
 0.05     1.932   3.499   3.128  -0.201
 0.10     1.996   3.535   3.170   0.146
 0.15     2.061   3.571   3.213   0.494
 0.20     2.125   3.608   3.256   0.841
 0.25     2.189   3.644   3.298   1.188
 0.30     2.253   3.680   3.341   1.535
 0.35     2.317   3.716   3.384   1.882
 0.40     2.381   3.752   3.426   2.229
 0.45     2.445   3.788   3.469   2.577
 0.50     2.509   3.824   3.511   2.924
 0.55     2.573   3.860   3.553   3.271
 0.60     2.636   3.895   3.596   3.618
 0.65     2.700   3.931   3.638   3.965
 0.70     2.763   3.967   3.680   4.312
 0.75     2.826   4.003   3.723   4.660
 0.80     2.889   4.039   3.765   5.007
 0.85     2.952   4.075   3.807   5.354
 0.90     3.015   4.110   3.849   5.701
 0.95     3.078   4.146   3.891   6.048
 1.00     3.141   4.182   3.934   6.395
```



test1_izero.txt:

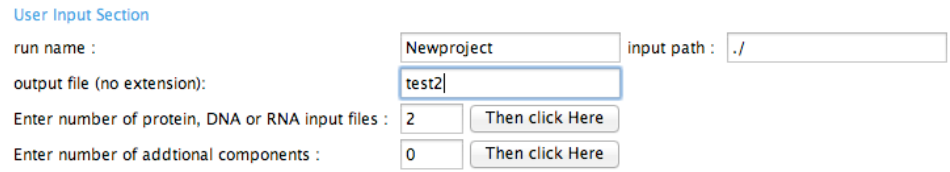
```
#Date: Fri Oct 31 14:18:22 2014

#Files used: protein_sequence.txt (1), dna_sequence.txt (1)

#Solvent Components:
#Component, molar conc, volume (A^3), Mw (kDA), x-ray SL, neutron SL (10^-12 cm), x-ray SLD, neutron SLD (10^10 cm^-2)
#       H2O      55.51   29.9    0.018    2.820  -0.168   9.428  -0.561
#       Na1Cl1    0.15   29.3    0.058    7.896   1.321  26.958   4.509
#Totals: x-ray SL, neutron SL (10^-12 cm), x-ray SLD, neutron SLD (10^10 cm^-2)
#       157.298  -9.097   9.474  -0.548

#Complex concentration:  1.0 mg/ml

#XRAY I(0):
#Protein Mw, NA Mw, Complex Mw (kDa), I(0) (cm^-1)
#  69.307        223.165         292.472          0.475

#NEUTRONS:
#Protein Match Point: 42.65 %D2O
#DNA Match Point: 64.45 %D2O
#Complex Match Point: 59.62 %D2O

#Fraction of exchanged protein hydrogens: 0.95
#Fraction of exchanged nucleic acid hydrogens: 1.00

# frac D2O, Protein Mw, DNA Mw, Complex Mw (kDa), I(0) (cm^-1), sartI(0)
 0.00     69.307         223.165         292.472          0.221   0.470
 0.05     69.360         223.239         292.600          0.185   0.430
 0.10     69.414         223.314         292.727          0.152   0.390
 0.15     69.467         223.388         292.855          0.122   0.350
 0.20     69.520         223.463         292.983          0.096   0.310
 0.25     69.574         223.537         293.111          0.073   0.270
 0.30     69.627         223.611         293.238          0.053   0.230
 0.35     69.680         223.686         293.366          0.036   0.189
 0.40     69.734         223.760         293.494          0.022   0.149
 0.45     69.787         223.835         293.622          0.012   0.109
 0.50     69.840         223.909         293.749          0.005   0.069
 0.55     69.894         223.983         293.877          0.001   0.029
 0.60     69.947         224.058         294.005          0.000  -0.012
 0.65     70.000         224.132         294.133          0.003  -0.052
 0.70     70.054         224.207         294.260          0.008  -0.092
 0.75     70.107         224.281         294.388          0.018  -0.132
 0.80     70.160         224.355         294.516          0.030  -0.173
 0.85     70.214         224.430         294.644          0.045  -0.213
 0.90     70.267         224.504         294.771          0.064  -0.253
 0.95     70.320         224.579         294.899          0.086  -0.294
 1.00     70.374         224.653         295.027          0.112  -0.334
```



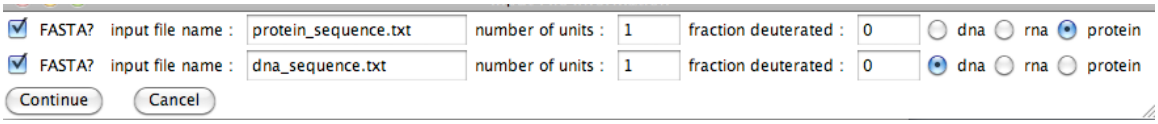


Example 2: Protein/Deuterated Protein Complex (FASTA files)

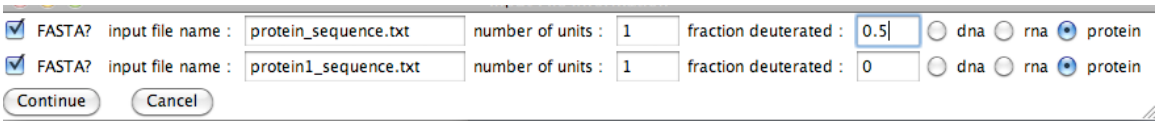
Enter a new run and/or output file name as well as new values for the number of input files and additional components. Then click the "Then click Here" button to the right of the first input box. (Press the Tab key after entering the number of additional components in the web version and the appropriate input boxes will appear.)



If the number of input files hasn't changed from the previous value, the previous parameters will be remembered.



Change them as necessary for the new complex. The following is an example of a two-protein complex with one of the proteins 50% deuterated.

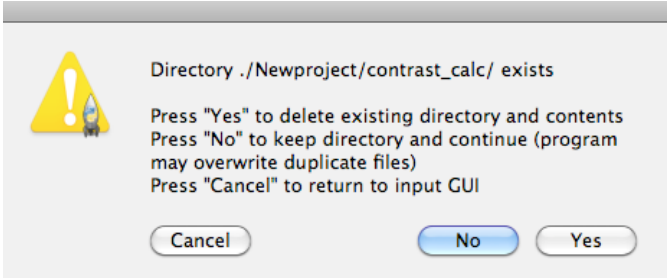


Click the “Continue” button to accept the new values.

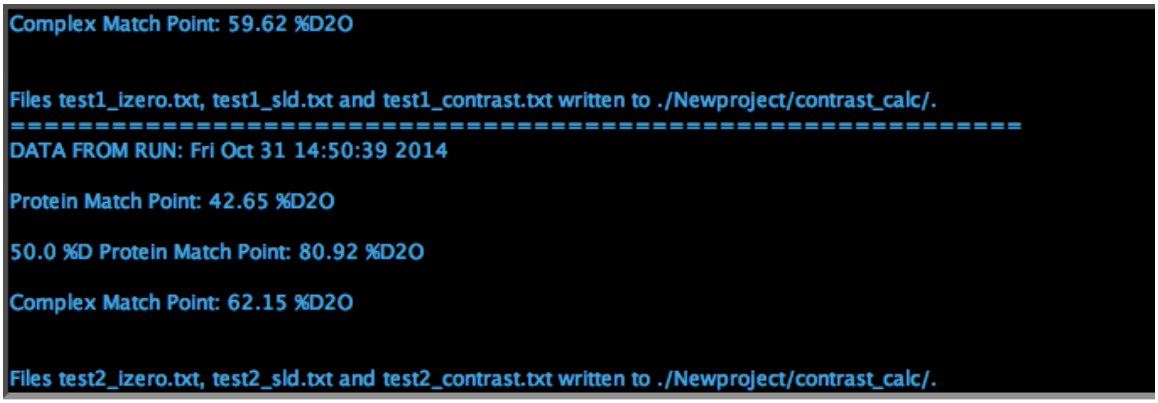
Since no changes have been made to the number of additional components, nothing further needs to be with the input boxes.

Make changes to the Optional Input values if necessary. Otherwise, the previously saved information will be used. To check the current non-water solvent component information, click on the “Then click Here” button next to the number of non-water solvent components. If no changes are needed, click “Cancel”. Otherwise, make the needed changes and then click “Continue”.

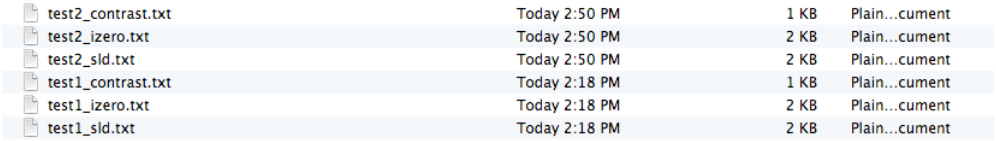
Click the “Run Contrast Calculator Program” button (“Submit in the web version”). Since the run name directory already exists, the follow dialog box will appear:

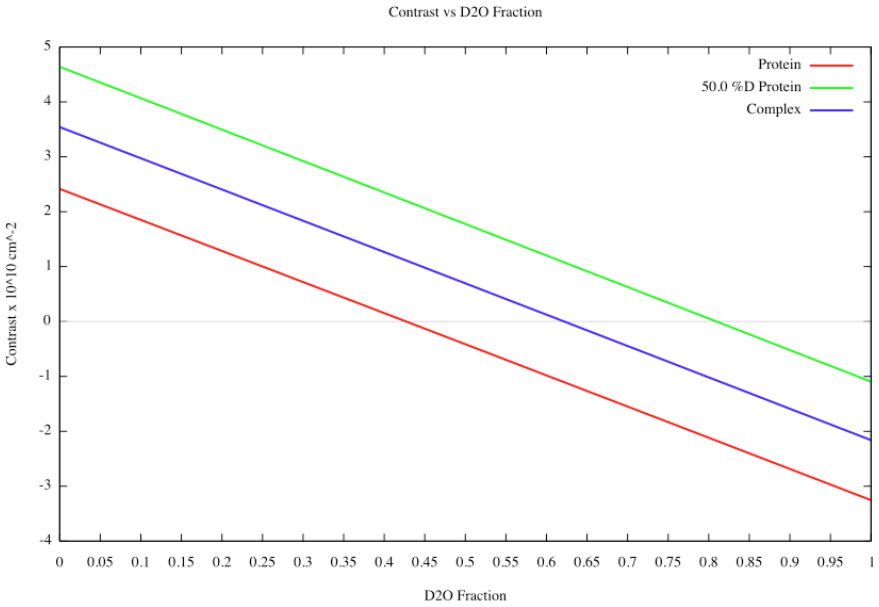


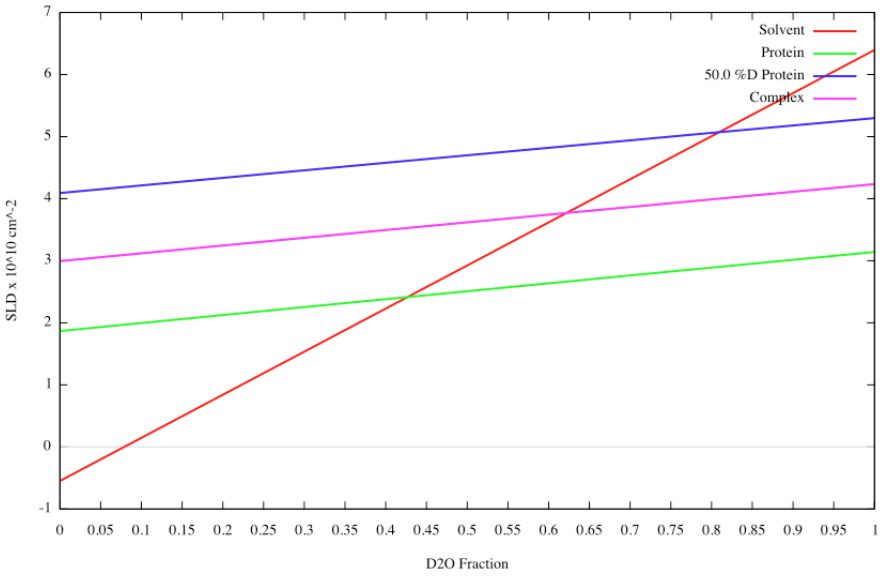
Click “No” to keep the directory. (This dialog box does not appear in the web version.) The program will then run and the status information will be output to the window. The latest information will be at the bottom of the screen. Scroll up or down to look at all of the output from all runs in the session (standalone only).

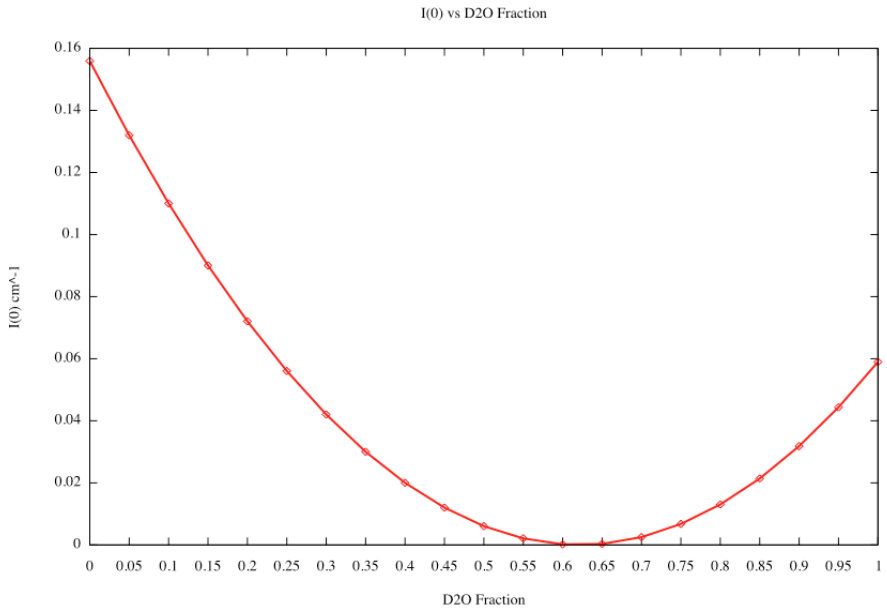


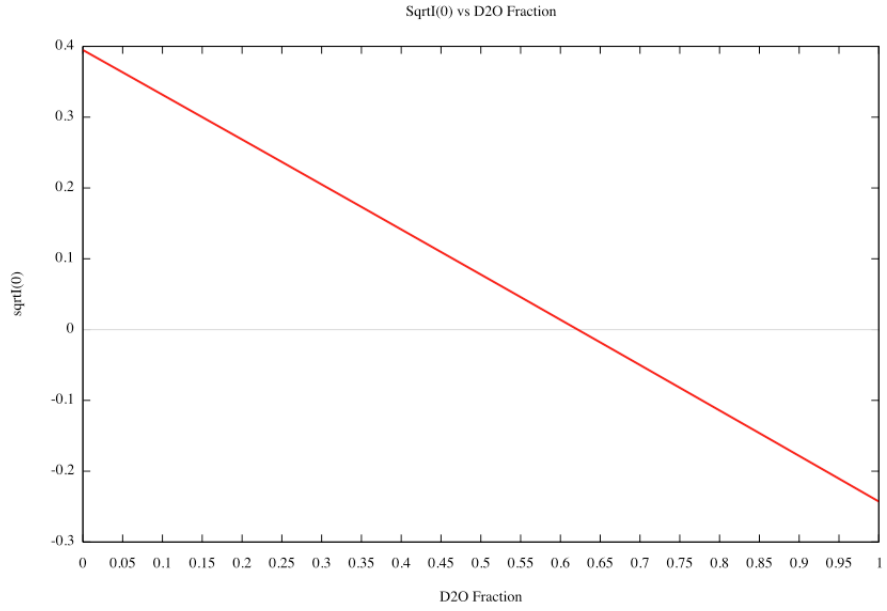
The run directory now contains the new files and the new plots will pop up since the number of components is less than 3. Clear the plots manually if necessary.




Contrast vs D2O Fraction
Protein
50.0 %D Protein
Complex
Contrast x 10^10 cm^-2
D2O Fraction
5
4
3
2
1
0
-1
-2
-3
-4
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
0.65
0.7
0.75
0.8
0.85
0.9
0.95
1

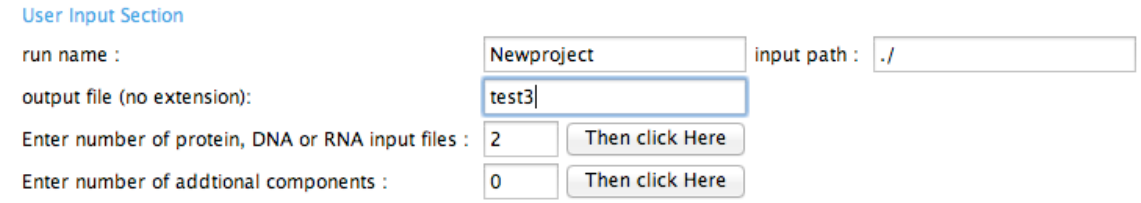

Scattering Length Density vs D2O Fraction
Solvent
Protein
50.0 %D Protein
Complex
SLD x 10^10 cm^-2
D2O Fraction
7
6
5
4
3
2
1
0
-1
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
0.65
0.7
0.75
0.8
0.85
0.9
0.95
1


I(0) vs D2O Fraction
I(0) cm^-1
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5 0.55 0.6 0.65 0.7 0.75 0.8 0.85 0.9 0.95 1
D2O Fraction

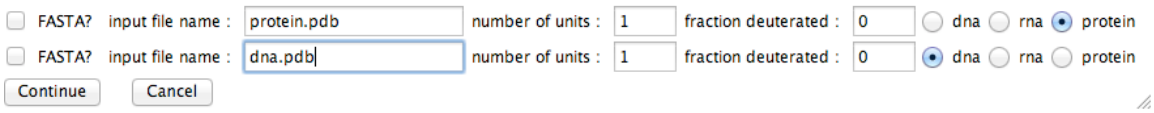

SqrtI(0) vs D2O Fraction
sqrtI(0)
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5 0.55 0.6 0.65 0.7 0.75 0.8 0.85 0.9 0.95 1
D2O Fraction

Example 3: Protein/DNA Complex (PDB files)

Enter a new run and/or output file name as well as new values for the number of input files and additional components. Then click the "Then click Here" button to the right of the first input box. (Press the Tab key after entering the number of additional components in the web version and the appropriate input boxes will appear.)



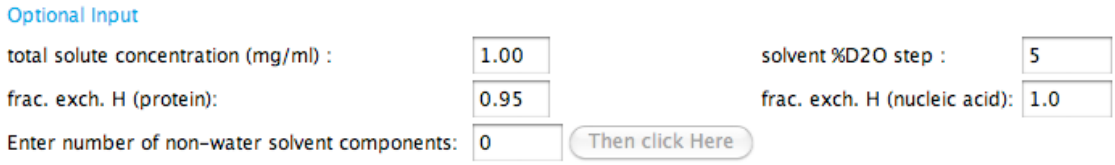
Change the input file names as necessary for the new complex. Do not check the FASTA boxes. (Select "off" in the web version.)



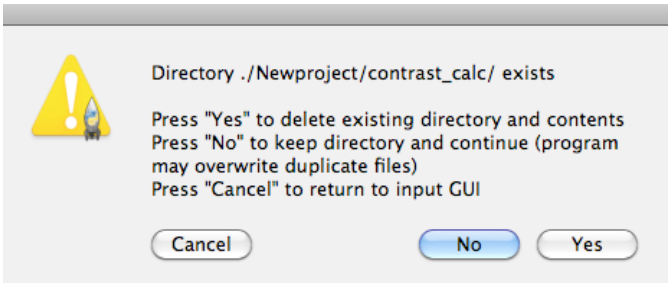
Click the "Continue" button to accept the new values.

Since no changes have been made to the number of additional components, nothing further needs to be with the input boxes.

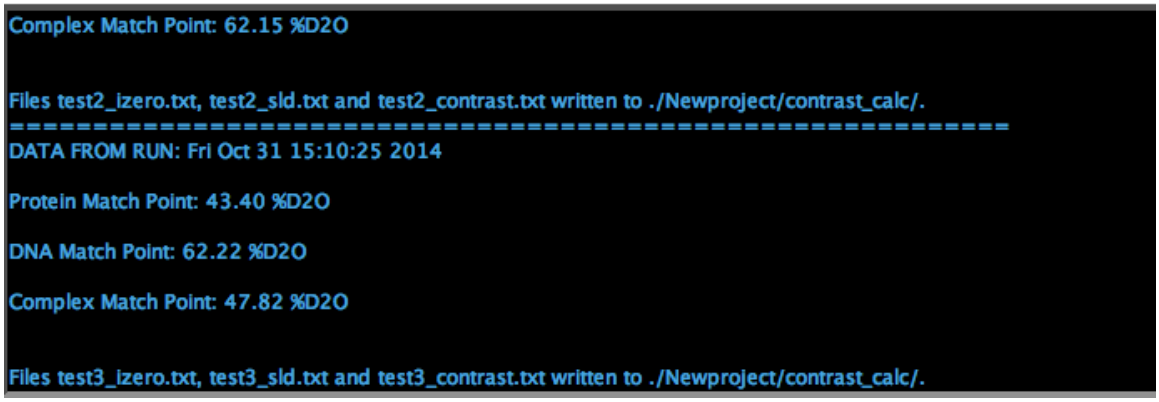
Make changes to the Optional Input values if necessary. Otherwise, the previously saved information will be used. To check the current non-water solvent component information, click on the "Then click Here" button next to the number of non-water solvent components. If no changes are needed, click "Cancel". Otherwise, make the needed changes and then click "Continue". Here, the number of non-water solvent components has been changed to 0.



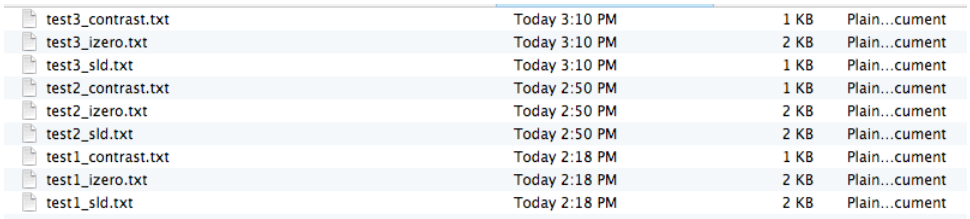
Click the "Run Contrast Calculator Program" button ("Submit" in the web version). Since the run name directory already exists, the follow dialog box will appear:



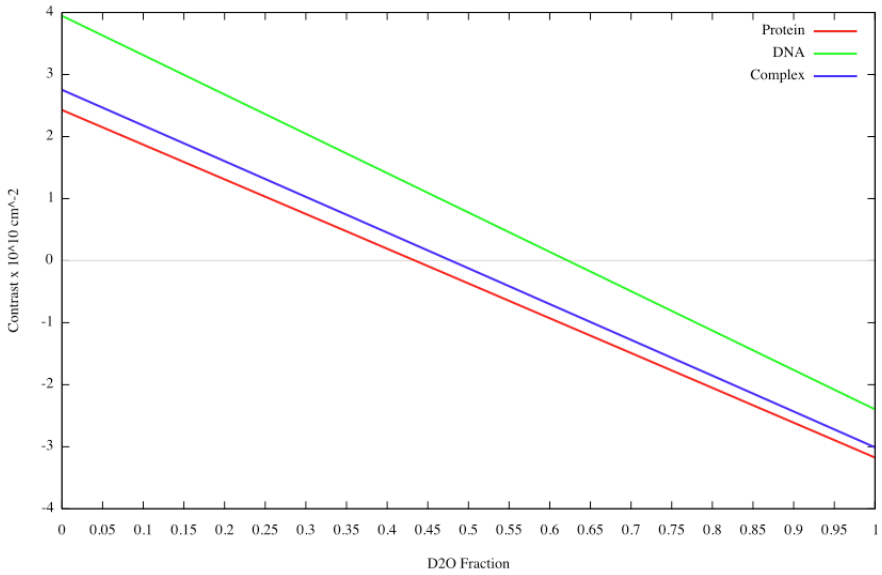
Click “No” to keep the directory. (This dialog box doesn’t appear in the web version.) The program will then run and the status information will be output to the window. The latest information will be at the bottom of the screen. Scroll up or down to look at all of the output from all runs in the session (standalone only).



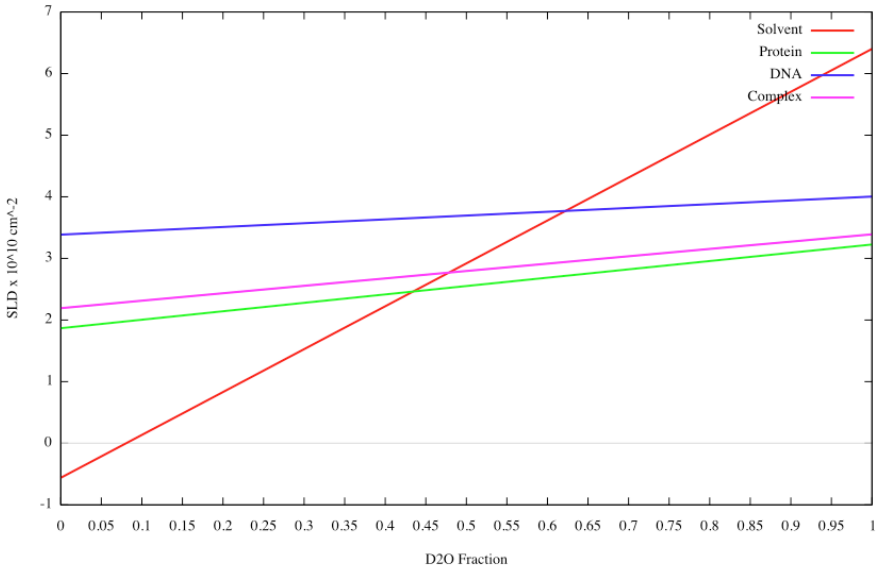
The run directory now contains the new files and the new plots will pop up since the number of components is less than 3. Clear the plots manually if necessary.

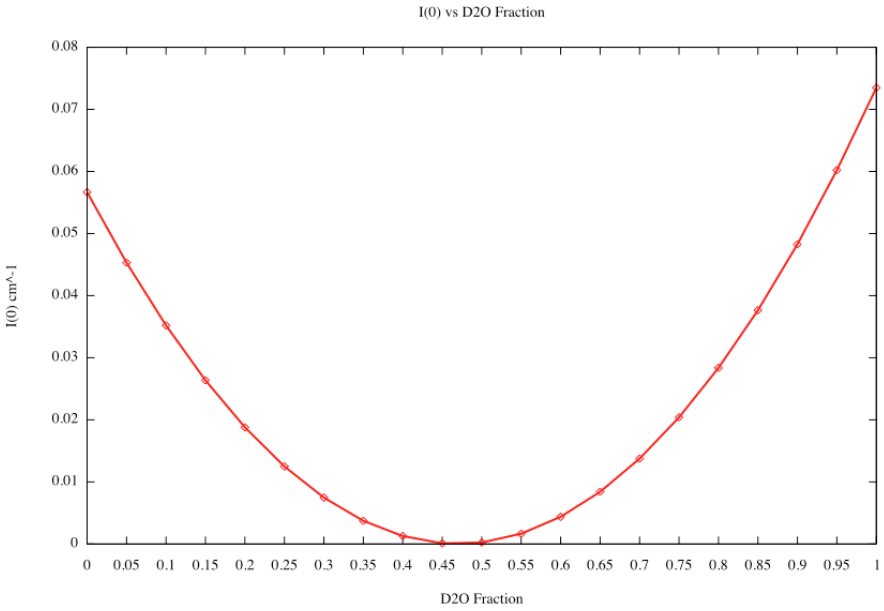


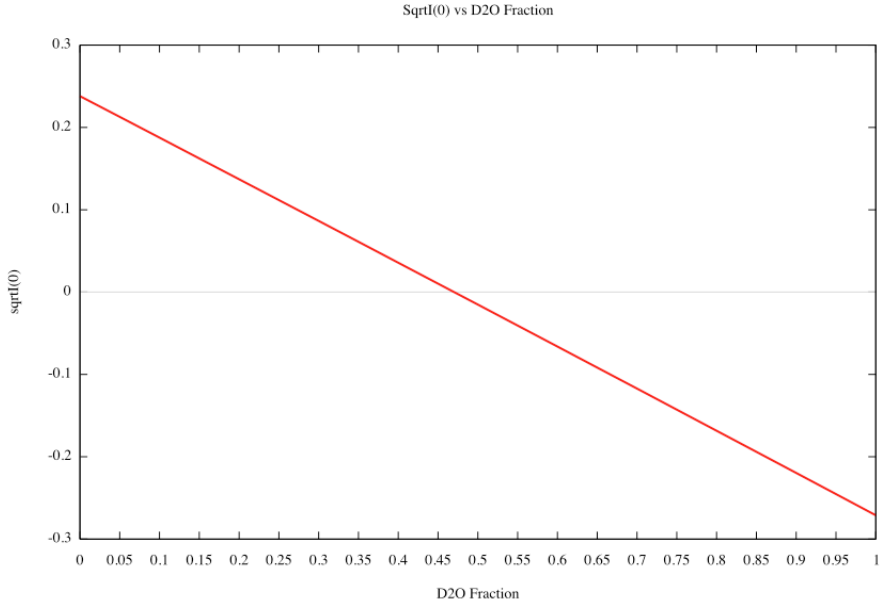
Contrast vs D2O Fraction



Scattering Length Density vs D2O Fraction

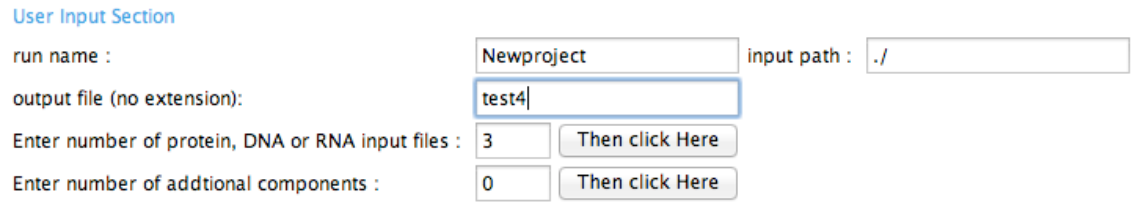



I(0) vs D2O Fraction
I(0) cm^-1
D2O Fraction
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5 0.55 0.6 0.65 0.7 0.75 0.8 0.85 0.9 0.95 1

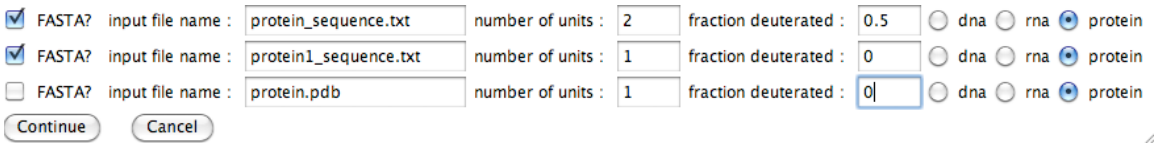

SqrtI(0) vs D2O Fraction
sqrtI(0)
D2O Fraction
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5 0.55 0.6 0.65 0.7 0.75 0.8 0.85 0.9 0.95 1

Example 4: Multisubunit Protein with Deuterated Subunit (FASTA and PDB files)

Enter a new run and/or output file name as well as new values for the number of input files and additional components. Then click the "Then click Here" button to the right of the first input box. (Press the Tab key after entering the number of additional components in the web version and the appropriate input boxes will appear.)



Change the input file names as necessary for the new complex. Note that there are 2 units of the protein_sequence.txt. Also note that this subunit is 50% deuterated. Although there are 3 different proteins in this complex, only one of them is deuterated. Therefore, for contrast calculation purposes, this is a two-component complex consisting of 50% deuterated protein and non-deuterated protein. The information for the non-deuterated subunits will be combined before the contrast parameters are calculated.

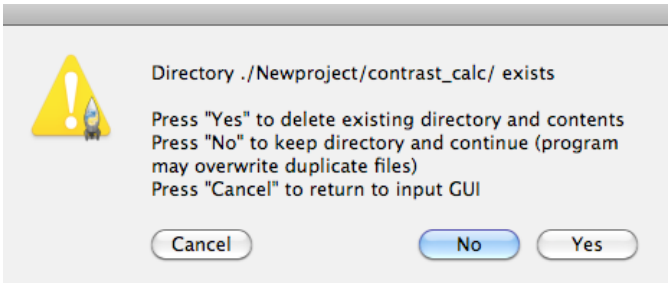


Click the "Continue" button to accept the new values.

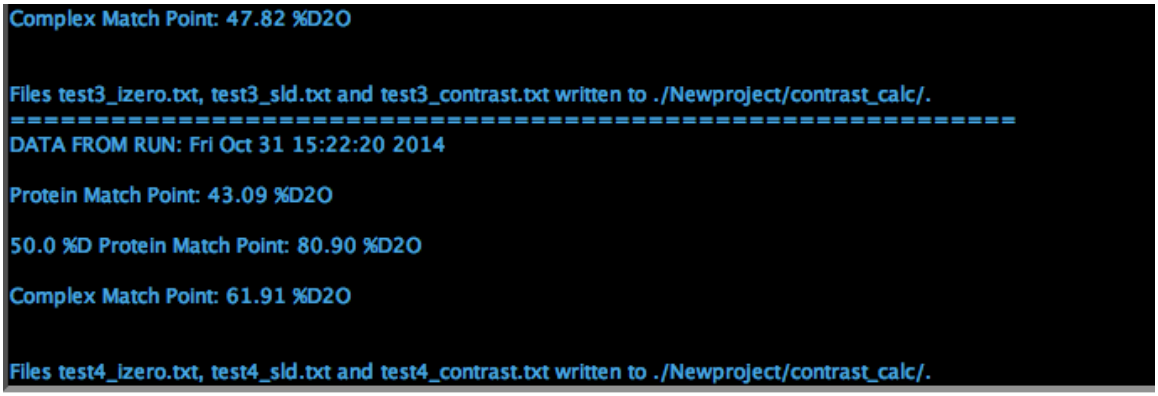
Since no changes have been made to the number of additional components, nothing further needs to be with the input boxes.

Make changes to the Optional Input values if necessary. Otherwise, the previously saved information will be used. To check the current non-water solvent component information, click on the "Then click Here" button next to the number of non-water solvent components. If no changes are needed, click "Cancel". Otherwise, make the needed changes and then click "Continue".

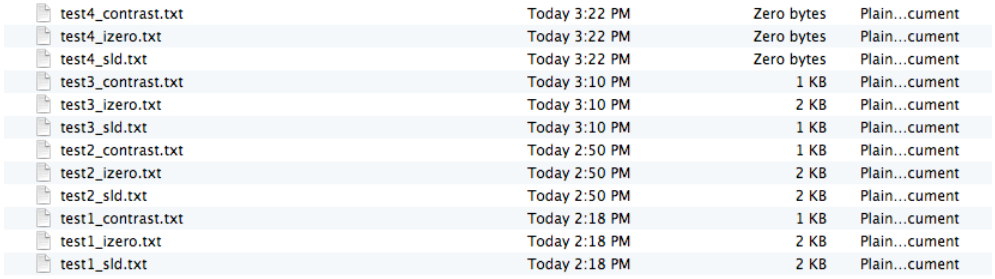
Click the "Run Contrast Calculator Program" ("Submit" in the web version) button near the bottom of the screen. Since the run name directory already exists, the follow dialog box will appear:

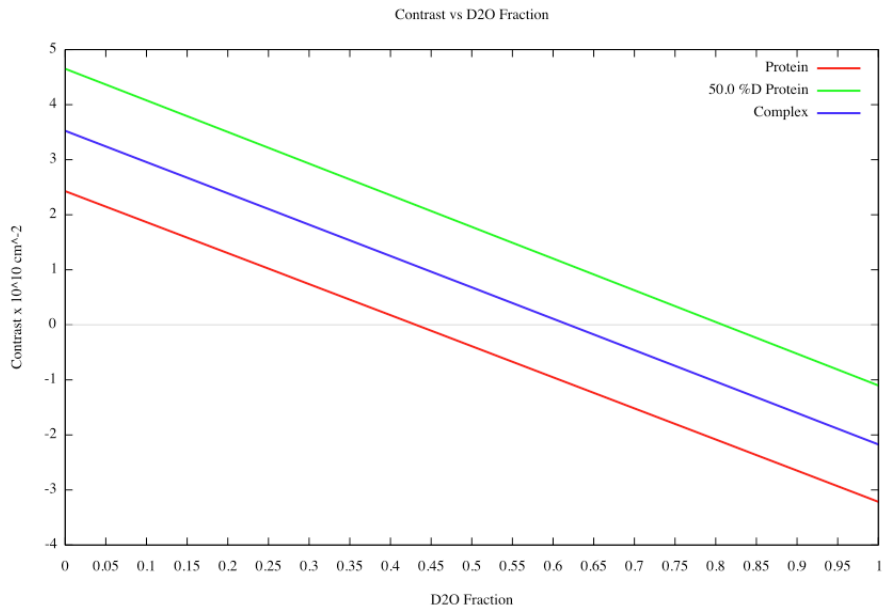


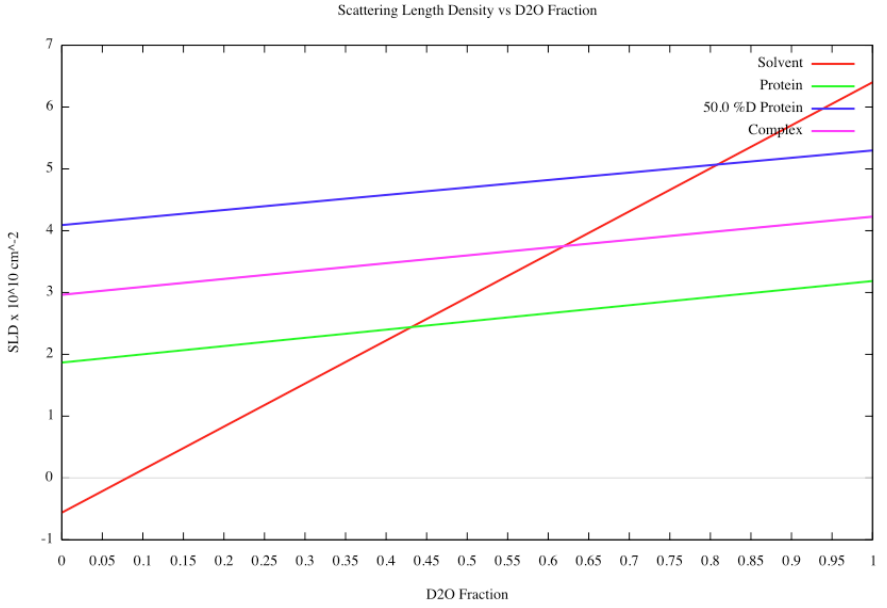
Click “No” to keep the directory. (This dialog box doesn’t appear in the web version.) The program will then run and the status information will be output to the window. The latest information will be at the bottom of the screen. Scroll up or down to look at all of the output from all runs in the session.

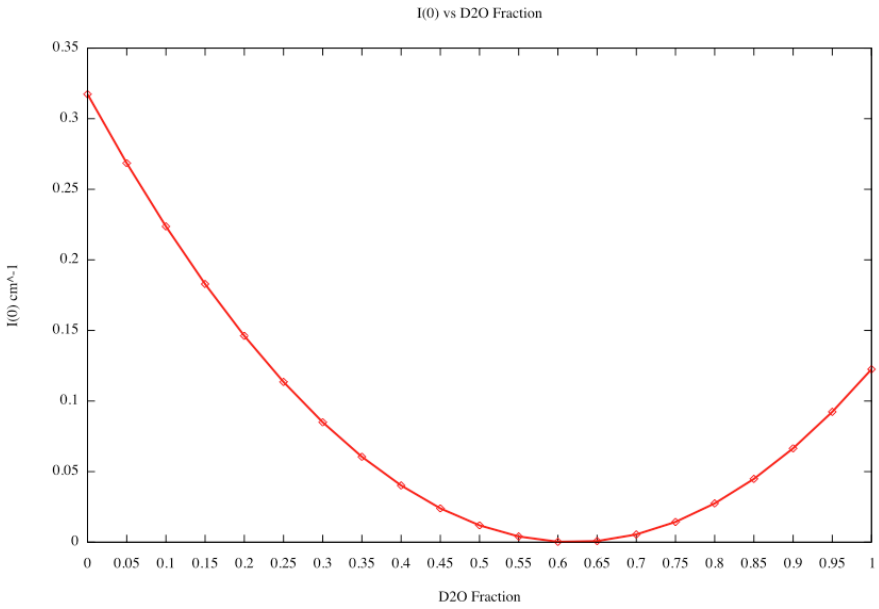


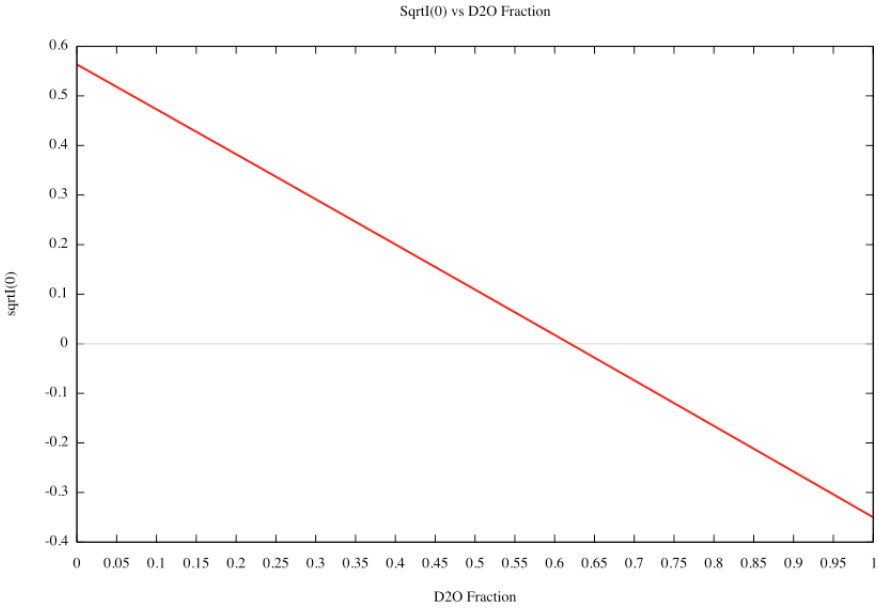
The run directory now contains the new files and the new plots will pop up since the number of **components** is less than 3. Clear the plots manually if necessary. (Plots may not appear in web version.)




Contrast vs D2O Fraction
Protein
50.0 %D Protein
Complex
Contrast x 10^10 cm^-2
D2O Fraction

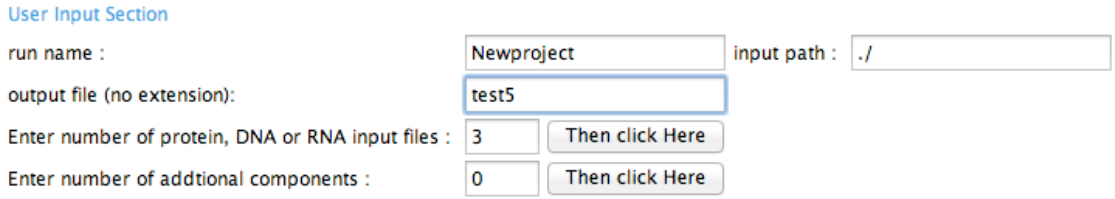

Scattering Length Density vs D2O Fraction
Solvent
Protein
50.0 %D Protein
Complex
SLD x 10^10 cm^-2
D2O Fraction


I(0) vs D2O Fraction
I(0) cm^-1
D2O Fraction
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5 0.55 0.6 0.65 0.7 0.75 0.8 0.85 0.9 0.95 1

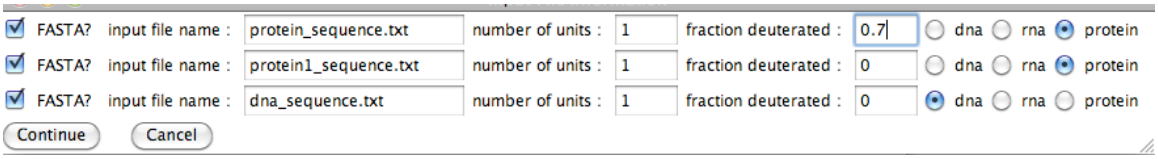

SqrtI(0) vs D2O Fraction
sqrtI(0)
D2O Fraction
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5 0.55 0.6 0.65 0.7 0.75 0.8 0.85 0.9 0.95 1

Example 5: Multisubunit Protein/DNA complex

Enter a new run and/or output file name as well as new values for the number of input files and additional components. Then click the "Then click Here" button to the right of the first input box. (Press the Tab key after entering the number of additional components in the web version and the appropriate input boxes will appear.)



Change the input file names as necessary for the new complex. Note that there are now two protein subunits and one DNA subunit. The protein_sequence.txt subunit is 70% deuterated. Therefore, for contrast calculation purposes, this is a three-component complex consisting of 70% deuterated protein, non-deuterated protein and DNA.

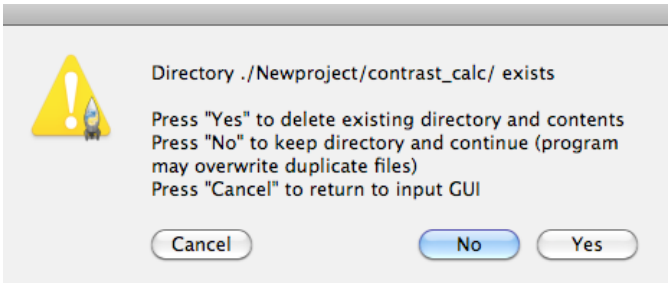


Click the "Continue" button to accept the new values.

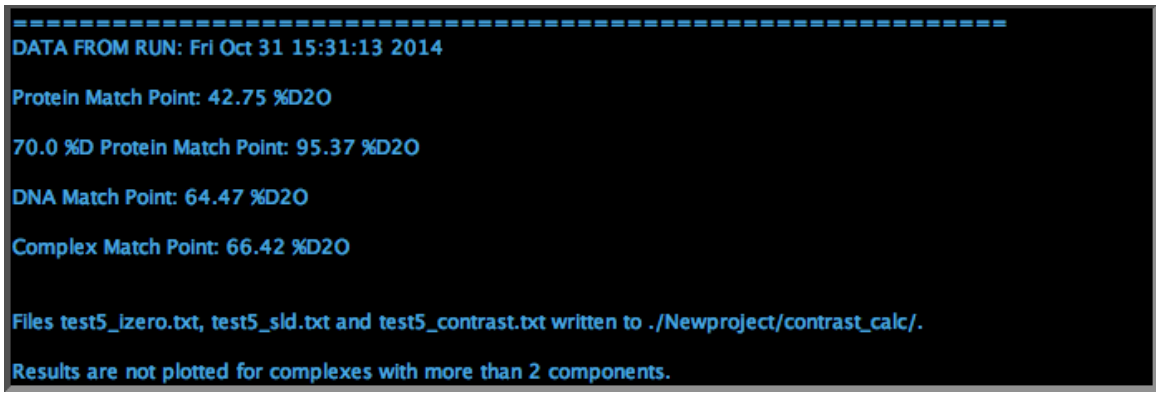
Since no changes have been made to the number of additional components, nothing further needs to be with the input boxes.

Make changes to the Optional Input values if necessary. Otherwise, the previously saved information will be used. To check the current non-water solvent component information, click on the "Then click Here" button next to the number of non-water solvent components. If no changes are needed, click "Cancel". Otherwise, make the needed changes and then click "Continue".

Click the "Run Contrast Calculator Program" button ("Submit in the web version). Since the run name directory already exists, the follow dialog box will appear:

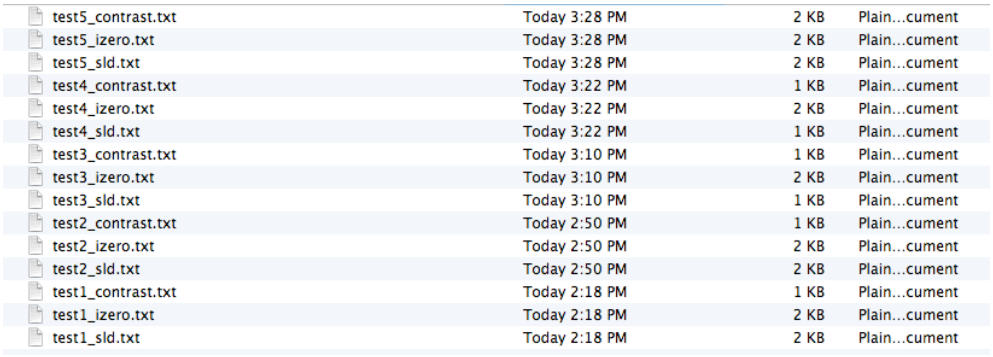


Click "No" to keep the directory. (This dialog box doesn't appear in the web version.) The program will then run and the status information will be output to the window. The latest information will be at the bottom of the screen. Scroll up or down to look at all of the output from all runs in the session. Note that there is a message indicating that the results are not plotted since the complex contains more than 2 components.



Experiment Planning Note: The match points for each component are indicated in the output screen. Note that the DNA match point is very close to that for a 50% deuterated protein. In order to have more widely-separated match points for all 3 of the components in this complex, the amount of deuteration in the protein was increased to 70%, which gives a match point of 95.37% D_2O. However, under these conditions, the DNA match point of 64.47% is almost the same as that for the total complex (66.42%). Thus, measurements near the DNA match point will not be possible for this particular complex. As the number of components in the complex increases, it becomes more difficult to find conditions that allow measurements near all of the match points. Thus, several contrast variation experiments may have to be performed on complexes with differing amounts of deuteration in the deuterated component. The contrast calculator can be used to determine the match points for each of the components as well as the total complex. This information combined with the calculated I(0) values at a given concentration will help to determine which measurements will be feasible.

The run directory now contains the new files.



test5_contrast.txt	Today 3:28 PM	2 KB	Plain…cument
test5_izero.txt	Today 3:28 PM	2 KB	Plain…cument
test5_sld.txt	Today 3:28 PM	2 KB	Plain…cument
test4_contrast.txt	Today 3:22 PM	1 KB	Plain…cument
test4_izero.txt	Today 3:22 PM	2 KB	Plain…cument
test4_sld.txt	Today 3:22 PM	1 KB	Plain…cument
test3_contrast.txt	Today 3:10 PM	1 KB	Plain…cument
test3_izero.txt	Today 3:10 PM	2 KB	Plain…cument
test3_sld.txt	Today 3:10 PM	1 KB	Plain…cument
test2_contrast.txt	Today 2:50 PM	1 KB	Plain…cument
test2_izero.txt	Today 2:50 PM	2 KB	Plain…cument
test2_sld.txt	Today 2:50 PM	2 KB	Plain…cument
test1_contrast.txt	Today 2:18 PM	1 KB	Plain…cument
test1_izero.txt	Today 2:18 PM	2 KB	Plain…cument
test1_sld.txt	Today 2:18 PM	2 KB	Plain…cument

test5_contrast.txt

```
#Date: Fri Oct 31 15:31:13 2014

#Files used: protein1_sequence.txt (1), protein_sequence.txt (1), dna_sequence.txt (1)

#Solvent Components:
#Component, molar conc, volume (A^3), Mw (kDA), x-ray SL, neutron SL (10^-12 cm), x-ray SLD, neutron SLD (10^10 cm^-2)
#       H2O      55.51   29.9    0.018    2.820  -0.168   9.428  -0.561

#XRAY Contrast (10^10 cm^-2):
#Protein, NA, Complex
#  2.801          6.156   4.855

#NEUTRON Contrast:
#Protein Match Point: 42.75 %D2O
#70.0 %D Protein Match Point: 95.37 %D2O
#DNA Match Point: 64.47 %D2O
#Complex Match Point: 66.42 %D2O

#Fraction of exchanged protein hydrogens: 0.95
#Fraction of exchanged nucleic acid hydrogens: 1.00

#NEUTRON Contrast (10^10 cm^-2):
# frac D2O, Protein, 70.0 %D Protein, DNA, Complex
 0.00     2.428   5.509   4.024   4.014
 0.05     2.145   5.220   3.712   3.712
 0.10     1.861   4.932   3.400   3.410
 0.15     1.578   4.644   3.088   3.108
 0.20     1.294   4.356   2.776   2.806
 0.25     1.010   4.067   2.464   2.504
 0.30     0.726   3.779   2.152   2.202
 0.35     0.442   3.490   1.840   1.900
 0.40     0.158   3.201   1.528   1.598
 0.45    -0.126   2.912   1.216   1.296
 0.50    -0.411   2.624   0.904   0.994
 0.55    -0.695   2.335   0.592   0.691
 0.60    -0.980   2.045   0.280   0.389
 0.65    -1.264   1.756  -0.033   0.087
 0.70    -1.549   1.467  -0.345  -0.216
 0.75    -1.834   1.178  -0.657  -0.518
 0.80    -2.119   0.888  -0.969  -0.821
 0.85    -2.404   0.599  -1.282  -1.123
 0.90    -2.689   0.309  -1.594  -1.426
 0.95    -2.974   0.020  -1.906  -1.728
 1.00    -3.259  -0.270  -2.219  -2.031
```

test5_sld.txt

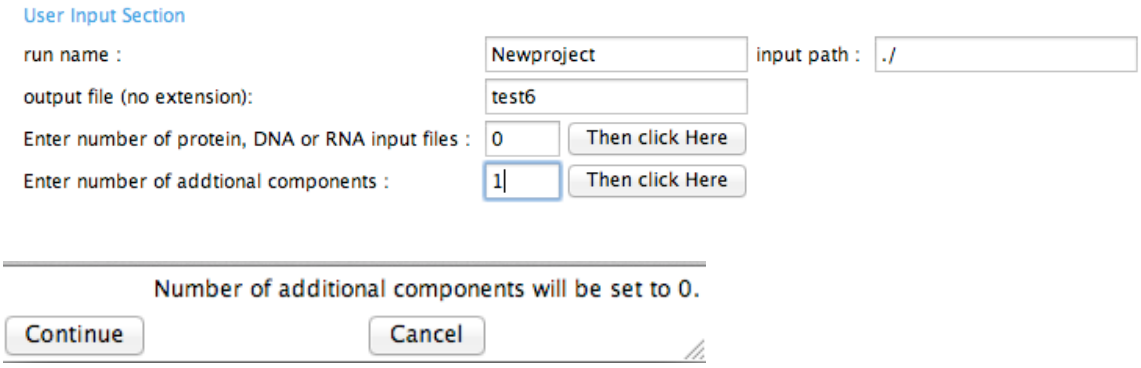
```
#Date: Fri Oct 31 15:31:13 2014

#Files used: protein1_sequence.txt (1), protein_sequence.txt (1), dna_sequence.txt (1)

#Solvent Components:
#Component, molar conc, volume (A^3), Mw (kDA), x-ray SL, neutron SL (10^-12 cm), x-ray SLD, neutron SLD (10^10 cm^-2)
#       H2O      55.51   29.9    0.018    2.820  -0.168   9.428  -0.561

#XRAY SLD (10^10 cm^-2):
#Protein, NA, Complex, Solvent
# 12.229         15.583  14.283   9.428

#NEUTRON SLDs:
#Protein Match Point: 42.75 %D2O
#70.0 %D Protein Match Point: 95.37 %D2O
#DNA Match Point: 64.47 %D2O
#Complex Match Point: 66.42 %D2O

#Fraction of exchanged protein hydrogens: 0.95
#Fraction of exchanged nucleic acid hydrogens: 1.00

#NEUTRON SLDs (10^10 cm^-2):
# frac D2O, Protein, 70.0 %D Protein, DNA, Complex, Solvent
 0.00     1.867   4.947   3.463   3.452  -0.561
 0.05     1.932   5.007   3.499   3.499  -0.213
 0.10     1.996   5.067   3.535   3.545   0.135
 0.15     2.061   5.127   3.571   3.591   0.483
 0.20     2.125   5.187   3.608   3.637   0.831
 0.25     2.189   5.246   3.644   3.683   1.179
 0.30     2.253   5.306   3.680   3.729   1.527
 0.35     2.317   5.365   3.716   3.775   1.875
 0.40     2.381   5.425   3.752   3.821   2.223
 0.45     2.445   5.484   3.788   3.867   2.571
 0.50     2.509   5.543   3.824   3.913   2.920
 0.55     2.573   5.602   3.860   3.959   3.268
 0.60     2.636   5.661   3.895   4.005   3.616
 0.65     2.700   5.720   3.931   4.050   3.964
 0.70     2.763   5.779   3.967   4.096   4.312
 0.75     2.826   5.838   4.003   4.142   4.660
 0.80     2.889   5.896   4.039   4.187   5.008
 0.85     2.952   5.955   4.075   4.233   5.356
 0.90     3.015   6.014   4.110   4.278   5.704
 0.95     3.078   6.072   4.146   4.324   6.052
 1.00     3.141   6.130   4.182   4.369   6.400
```

test5_izero.txt

```
#Date: Fri Oct 31 15:31:13 2014

#Files used: protein1_sequence.txt (1), protein_sequence.txt (1), dna_sequence.txt (1)

#Solvent Components:
#Component, molar conc, volume (A^3), Mw (kDA), x-ray SL, neutron SL (10^-12 cm), x-ray SLD, neutron SLD (10^10 cm^-2)
#       H2O      55.51   29.9    0.018    2.820  -0.168   9.428  -0.561

#Complex concentration:  1.0 mg/ml

#XRAY I(0):
#Protein Mw, NA Mw, Complex Mw (kDa), I(0) (cm^-1)
# 141.251        223.165         364.416          0.510

#NEUTRONS:
#Protein Match Point: 42.75 %D2O
#70.0 %D Protein Match Point: 95.37 %D2O
#DNA Match Point: 64.47 %D2O
#Complex Match Point: 66.42 %D2O

#Fraction of exchanged protein hydrogens: 0.95
#Fraction of exchanged nucleic acid hydrogens: 1.00

# frac D2O, Protein Mw, 70.0 %D Protein Mw, DNA Mw, Complex Mw (kDa), I(0) (cm^-1), sqrtI(0)
 0.00     69.307          71.944          223.165          364.416          0.382   0.618
 0.05     69.360          71.997          223.239          364.597          0.327   0.572
 0.10     69.414          72.050          223.314          364.778          0.276   0.526
 0.15     69.467          72.104          223.388          364.959          0.230   0.479
 0.20     69.520          72.157          223.463          365.140          0.188   0.433
 0.25     69.574          72.211          223.537          365.321          0.150   0.387
 0.30     69.627          72.264          223.611          365.502          0.116   0.341
 0.35     69.680          72.317          223.686          365.683          0.087   0.295
 0.40     69.734          72.371          223.760          365.864          0.062   0.248
 0.45     69.787          72.424          223.835          366.045          0.041   0.202
 0.50     69.840          72.477          223.909          366.227          0.024   0.156
 0.55     69.894          72.531          223.983          366.408          0.012   0.109
 0.60     69.947          72.584          224.058          366.589          0.004   0.063
 0.65     70.000          72.637          224.132          366.770          0.000   0.016
 0.70     70.054          72.691          224.207          366.951          0.001  -0.030
 0.75     70.107          72.744          224.281          367.132          0.006  -0.077
 0.80     70.160          72.797          224.355          367.313          0.015  -0.123
 0.85     70.214          72.851          224.430          367.494          0.029  -0.170
 0.90     70.267          72.904          224.504          367.675          0.047  -0.216
 0.95     70.320          72.957          224.579          367.856          0.069  -0.263
 1.00     70.374          73.011          224.653          368.037          0.096  -0.310
```

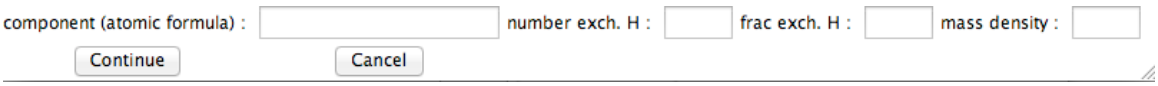
Example 6: Polymer (chemical formula)

Enter a new run and/or output file name as well as new values for the number of input files and additional components. Then click the "Then click Here" button to the right of the first input box. (Press the Tab key after entering the number of additional components in the web version and the appropriate input boxes will appear.)

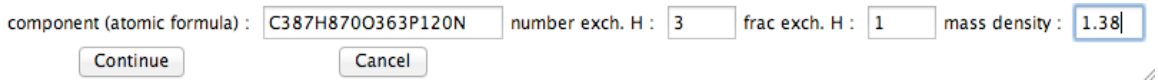


In this example, there is nothing to fill out since there are no protein, DNA or RNA components that will be entered as file names. Click "Continue" to accept the value. (The dialog box doesn't appear in web version.)

Then click the button to the right of the second input box that says, "Then click Here". This will open the second input file dialog box. (Press the Tab key after entering the number of additional components in the web version and the appropriate input boxes will appear.)



Note that the number of input lines corresponds to the number of input files in the User Input Section. This can be changed by clicking the "Cancel" button and entering a different number of input files.



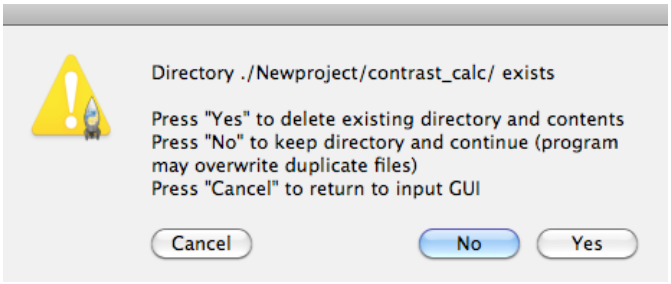
Enter the chemical formula. Parentheses can be used, i.e., (NH4)2SO4. Also, enter the number of exchangeable hydrogen atoms based on the chemical structure, the fraction of exchangeable hydrogen atoms that actually do exchange and the mass density. Once all parameters are entered, click the "Continue" button.

At this point, the contrast calculator can be run by clicking on the "Run Contrast Calculator Program" button ("Submit" in the web version) at the bottom of the

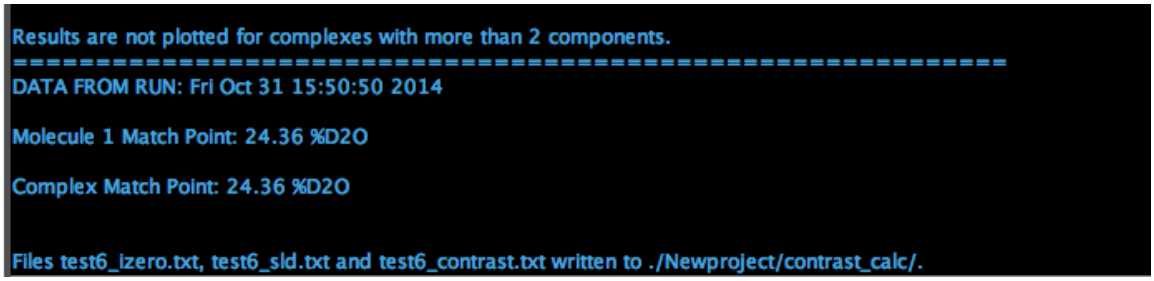
screen. In this case, the program will be run with the default Optional Input parameters.

Make changes to the Optional Input values if necessary. Otherwise, the previously saved information will be used. To check the current non-water solvent component information, click on the "Then click Here" button next to the number of non-water solvent components. If no changes are needed, click "Cancel". Otherwise, make the needed changes and then click "Continue".

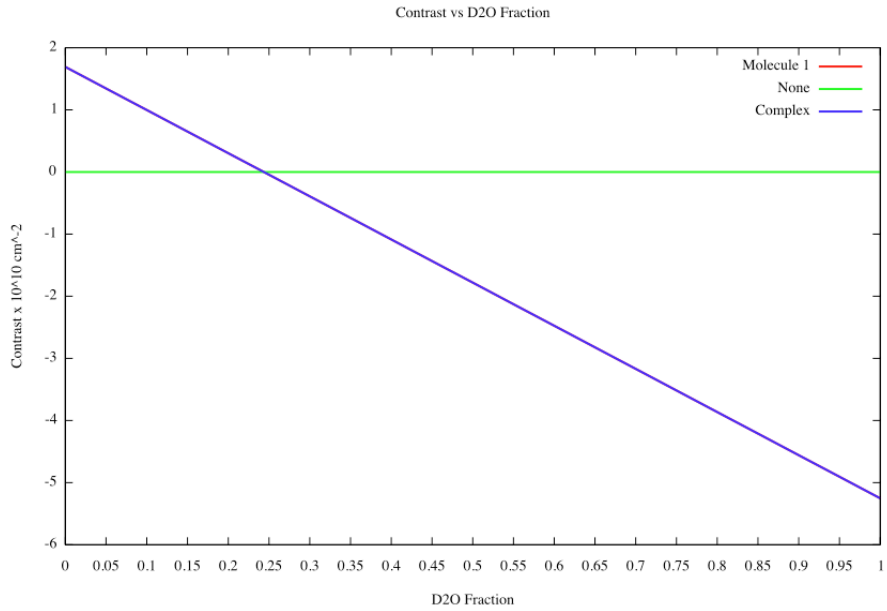
Click the "Run Contrast Calculator Program" ("Submit" in the web version) button near the bottom of the screen. Since the run name directory already exists, the follow dialog box will appear:

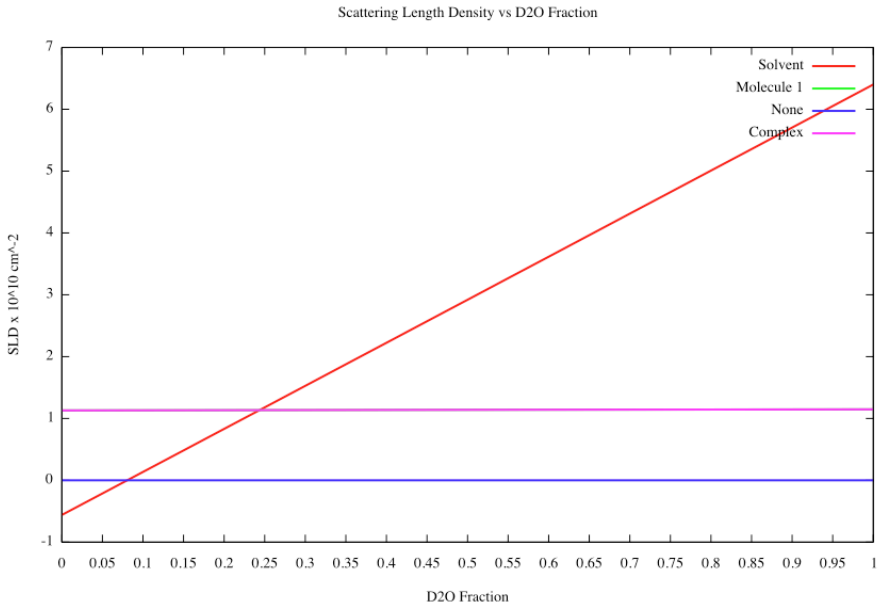


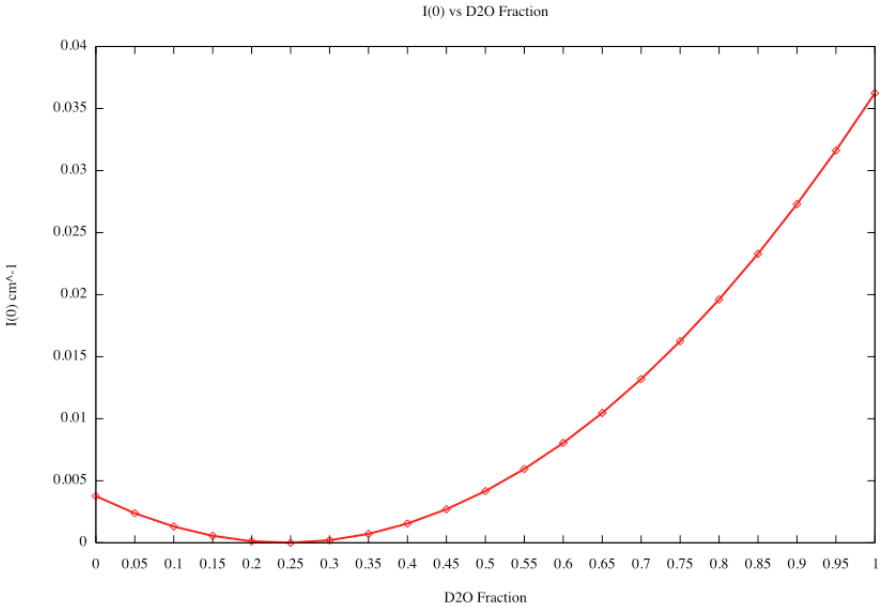
Click "No" to keep the directory. (This dialog box doesn't appear in the web version.) The program will then run and the status information will be output to the window. The latest information will be at the bottom of the screen. Scroll up or down to look at all of the output from all runs in the session.

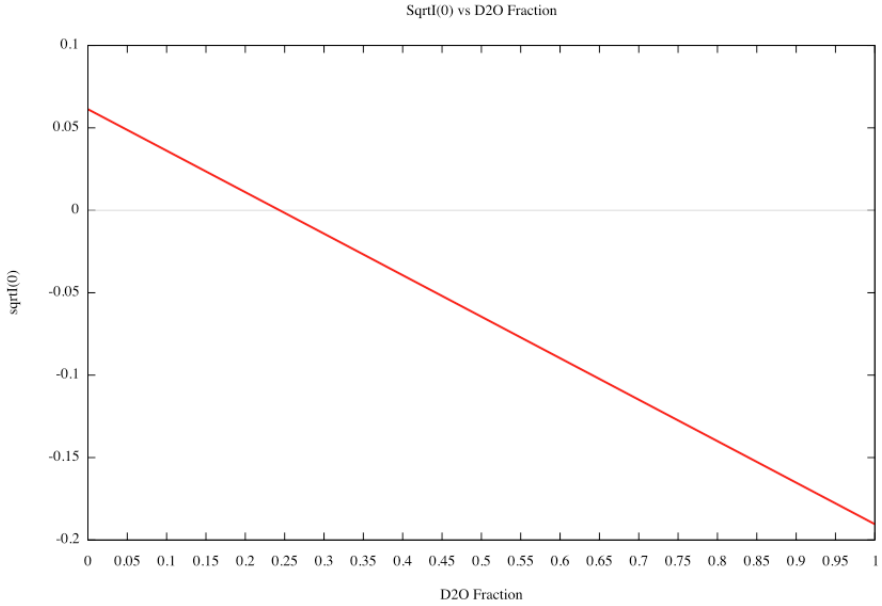


The run directory now contains the new files and the new plots will pop up since the number of components is less than 3. Clear the plots manually if necessary.


Contrast vs D2O Fraction
Molecule 1
None
Complex
Contrast x 10^10 cm^-2
D2O Fraction
2
1
0
-1
-2
-3
-4
-5
-6
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
0.65
0.7
0.75
0.8
0.85
0.9
0.95
1

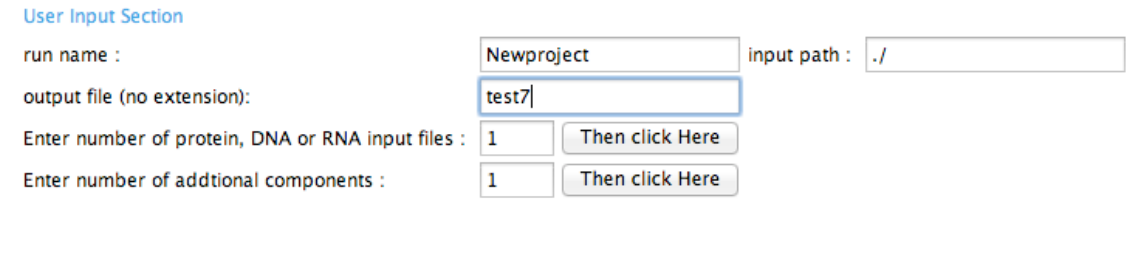

Scattering Length Density vs D2O Fraction
Solvent
Molecule 1
None
Complex
SLD x 10^10 cm^-2
D2O Fraction
7
6
5
4
3
2
1
0
-1
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
0.65
0.7
0.75
0.8
0.85
0.9
0.95
1


I(0) vs D2O Fraction
I(0) cm^-1
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5 0.55 0.6 0.65 0.7 0.75 0.8 0.85 0.9 0.95 1
D2O Fraction

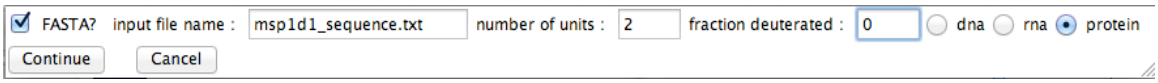

SqrtI(0) vs D2O Fraction
sqrtI(0)
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
0 0.05 0.1 0.15 0.2 0.25 0.3 0.35 0.4 0.45 0.5 0.55 0.6 0.65 0.7 0.75 0.8 0.85 0.9 0.95 1
D2O Fraction

Example 7: Protein/Lipid Complex (Nanodisc)

Enter a new run and/or output file name as well as new values for the number of input files and additional components. Then click the "Then click Here" button to the right of the first input box. (Press the Tab key after entering the number of additional components in the web version and the appropriate input boxes will appear.)



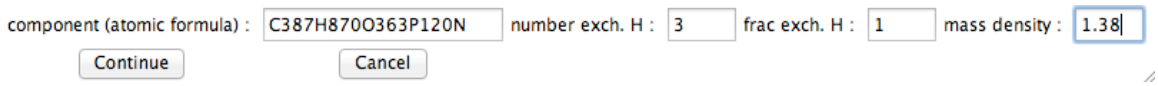
Fill in the appropriate file name for the protein component. In this case the protein component consists of two MSP1D1 proteins.



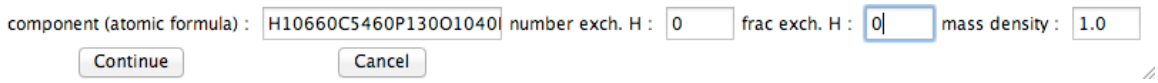
Click the "Continue" button to accept the new values.

Then click the button to the right of the second input box that says, "Then click Here". This will open the second input file dialog box. (Press the Tab key after entering the number of additional components in the web version and the appropriate input boxes will appear.)

If the number of input files hasn't changed from the previous value, the previous parameters will be remembered.

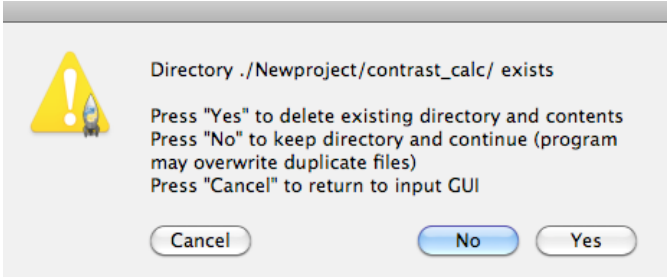


Change them as necessary for the new complex and then click "Continue". In this case, the lipid component of the nanodisc consists of 130 POPC molcules with chemical formula, C42H82NO8P. Thus the formula for the lipid component can be entered as either (C42H82NO8P)130 or H10660C5460P130O1040N130.



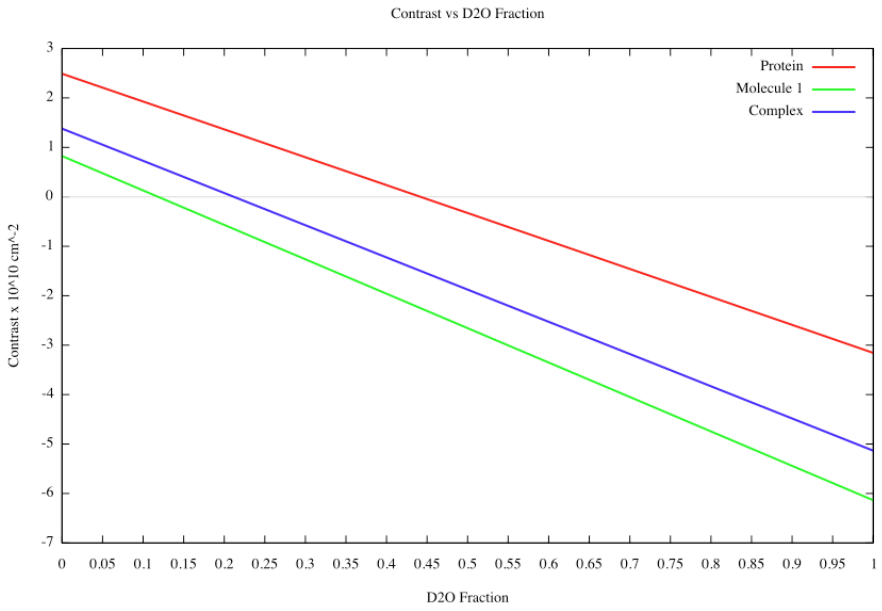
Make changes to the Optional Input values if necessary. Otherwise, the previously saved information will be used. To check the current non-water solvent component information, click on the "Then click Here" button next to the number of non-water solvent components. If no changes are needed, click "Cancel". Otherwise, make the needed changes and then click "Continue".

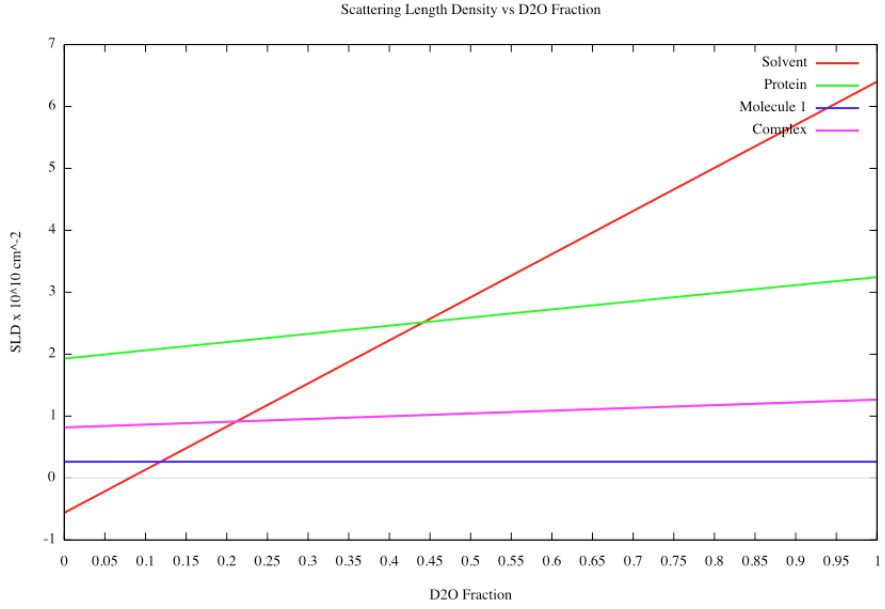
Click the "Run Contrast Calculator Program" button ("Submit in the web version"). Since the run name directory already exists, the follow dialog box will appear:

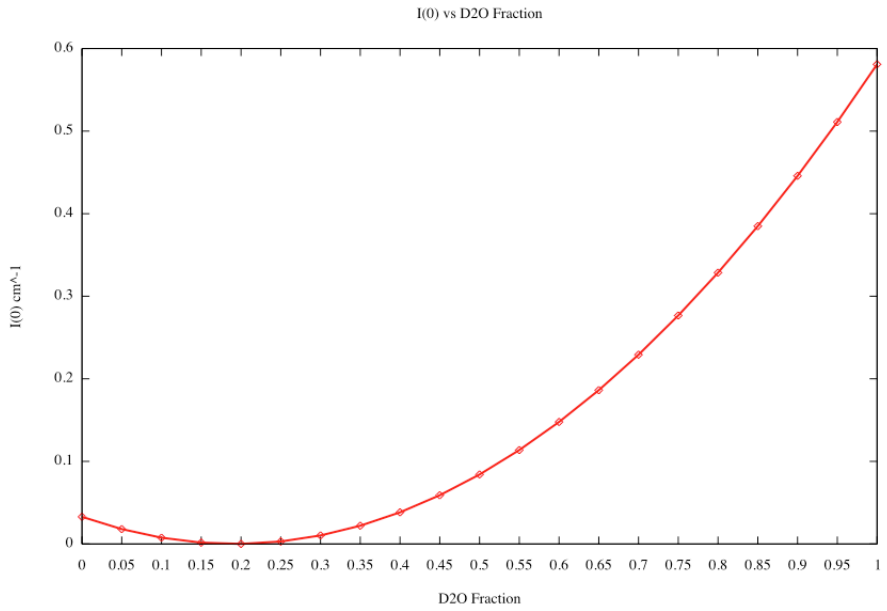


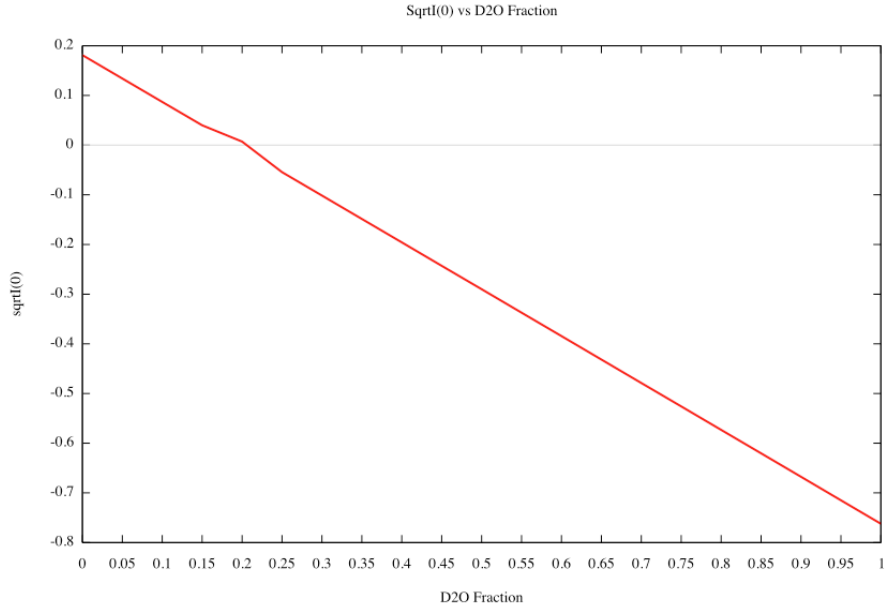
Click "No" to keep the directory. (This dialog box does not appear in the web version.) The program will then run and the status information will be output to the window. The latest information will be at the bottom of the screen. Scroll up or down to look at all of the output from all runs in the session.

The run directory now contains the new files and the new plots will pop up since the number of components is less than 3. Clear the plots manually if necessary.


Contrast vs D2O Fraction
Protein
Molecule 1
Complex
Contrast x 10^10 cm^-2
D2O Fraction
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
0.65
0.7
0.75
0.8
0.85
0.9
0.95
1


Scattering Length Density vs D2O Fraction
Solvent
Protein
Molecule 1
Complex
SLD x 10^10 cm^-2
D2O Fraction
7
6
5
4
3
2
1
0
-1
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0.55
0.6
0.65
0.7
0.75
0.8
0.85
0.9
0.95
1


I(0) vs D2O Fraction
I(0) cm^-1
D2O Fraction
0
0.1
0.2
0.3
0.4
0.5
0.6
0.05
0.15
0.25
0.35
0.45
0.55
0.65
0.75
0.8
0.85
0.9
0.95
1


SqrtI(0) vs D2O Fraction
sqrtI(0)
D2O Fraction
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
-0.8
0.05
0.15
0.25
0.35
0.45
0.55
0.65
0.75
0.8
0.85
0.9
0.95
1