Small-Angle Scattering Contrast Calculator for Protein and Nucleic Acid Complexes in Solution

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Supplemental Material

1. Examples

Two examples of the SASSIE Contrast Calculator (SCC) are provided. The first is a two-component protein complex comprising the trimeric chaperone skp bound to the outer membrane beta-barrel OmpA (Walton & Sousa, 2004), in which the latter is 50% deuterated. Non-water solvent components are not considered in the calculations. The second is a two-component protein-DNA complex consisting of two synapsis-deficient Cre(A36V) recombinases bound to a single *loxP* site (Ghosh et al., 2007). In this example, non-water solvent components are considered in the calculations. Screenshots of the outputs and plots are provided. The resultant SLD and contrast values are in agreement with those calculated using the MULCh program (Whitten et al., 2008).

1.1. Protein/Deuterated Protein Complex

Contrast parameters were calculated for a complex containing the chaperone protein skp, undeuterated, bound to the 50% deuterated outer membrane beta-barrel OmpA. Therefore, this is a two-component system even though both subunits belong to the protein molecule type. The non-deuterated skp component consists of three identical chains. The input files were in FASTA format. The total concentration of the complex was kept at the default value of 1 mg/mL. The $\%D_2O$ step for the calculations was kept at the default value of 5% and the fraction of exchanged hydrogens for the protein components was set at the protein default value of 0.95. The number of non-water solvent components was kept at the default value of 0.

The resultant neutron contrast match points for the skp and OmpA components are 44.05% D_2O and 80.41% D_2O, respectively. The match point for the entire complex is 59.75% D_2O. This information appears in the output screen in Figure S1. This result can be seen graphically in Figure S2, which is a plot of the scattering length density (SLD) vs. D_2O fraction in the solvent (f_{D2O}) for both components, the solvent and the entire complex. Figure S3 shows the contrast vs. f_{D2O} for both components and the entire complex. The match points are indicated at the points where the contrast equals 0. The match point of the entire complex can also be found from Figure S4, which shows $\sqrt{I(0)}$ vs. f_{D2O}, at the point where $\sqrt{I(0)}$ equals 0. Figure S5 shows $I(0)$ vs. f_{D2O} for the complex, which can be helpful in determining which contrast values will result in measurable SANS signals.

1.2. Protein/DNA Complex

Contrast parameters were calculated for a complex containing two molecules of Cre(A36V) recombinase bound to a *loxP* site. This is also a two-component system even though neither component is deuterated since the complex consists of two different molecule types. The input files were in PDB format. If PDB files are used as input, the M_w, and thus the calculated *I(0)*, will not be correct if there are missing residues in the PDB file; it is the responsibility of the user to ensure that the PDB files are complete. The total concentration of the complex is kept at the default value of 1 mg/mL. The fractions of exchanged hydrogens for the protein and nucleic acid components were set at the default values of 0.95 and 1.0, respectively. The $\%D_2O$ step for the calculations was kept at the default value of 5%. For this example, the solvent contains 0.5 M NaCl.

The resultant neutron contrast match points for the protein and DNA components are 43.07% D_2O and 62.12% D_2O, respectively. The match point for the entire complex is 47.55% D_2O. This information appears in the output screen in Figure S6. This result can be seen graphically in Figure S7, which is a plot of the SLD vs. f_{D2O} for both components, the solvent and the entire complex. Figure S8 shows the contrast vs. f_{D2O} for both components and the entire complex. The match points are indicated at the points where the contrast equals 0. The match point of the entire complex can also be found from Figure S9, which shows $\sqrt{I(0)}$ vs. f_{D2O}, at the point where $\sqrt{I(0)}$ equals 0. Figure S10 shows *I(0)* vs. f_{D2O} for the complex.

1.3. Output Files

Screenshots of the output files for the skp-OmpA protein complex are shown in Figures S11, S12 and S13. Figure S11 is the SLD output file. The first line contains the date and time the file was written. Then a list of the files used follows. The number in parentheses after the file name is the number of units indicating how many times the information in the file was used for the calculations. Next, the solvent components used in the calculations are described. In this case, the solvent is composed of only water; thus, the molar concentration, volume, M_w, x-ray and neutron scattering lengths and the corresponding x-ray and neutron SLDs for H_2O are listed in the file. The x-ray SLDs of the components and the entire complex follow, along with the neutron match points of the components and the complex. Finally, the fraction of exchanged hydrogens for the protein and nucleic acid components are given, followed by a table of SLD values for the components, complex and solvent as a function of the D_2O fraction in the solvent. Figure S12 is the contrast output file. Its format is similar to the SLD file except that x-ray and neutron contrasts are given instead of SLDs. Similarly, Figure S13 shows the *I(0)* output file containing similar information as the other two. However, in this case, the concentration of the complex is given along with the x-ray and neutron *I(0)* values in cm^{-1}. The table of neutron values also contains the M_w of the components and the complex, as well as *I(0)* and $\sqrt{I(0)}$, all as a function f_{D2O}.

Screenshots of the output files for the Cre-*loxP* complex are shown in Figures S14, S15 and S16. Since the solvent in this example contained 0.5 M NaCl, the output files contain the relevant information pertaining to both the water and the salt. The total x-ray and neutron scattering lengths of the two-component solvent, along with the corresponding x-ray and neutron SLDs are listed in the file. The other outputs are the same as for the previous example, with the relevant parameters listed for the protein and DNA components as well as the complex.

References

Ghosh, K., Guo, F., & Van Duyne, G. D. (2007). *J. Biol. Chem.* **282**, 24004–24016.

Walton, T. A. & Sousa, M. C. (2004). *Mol. Cell*. **15**, 367–374.

Whitten, A. E., Cai, S., & Trewhella, J. (2008). *J. Appl. Crystallogr.* **41**, 222–226.

2. Supplemental Figures

2.1. Protein/Deuterated Protein Complex

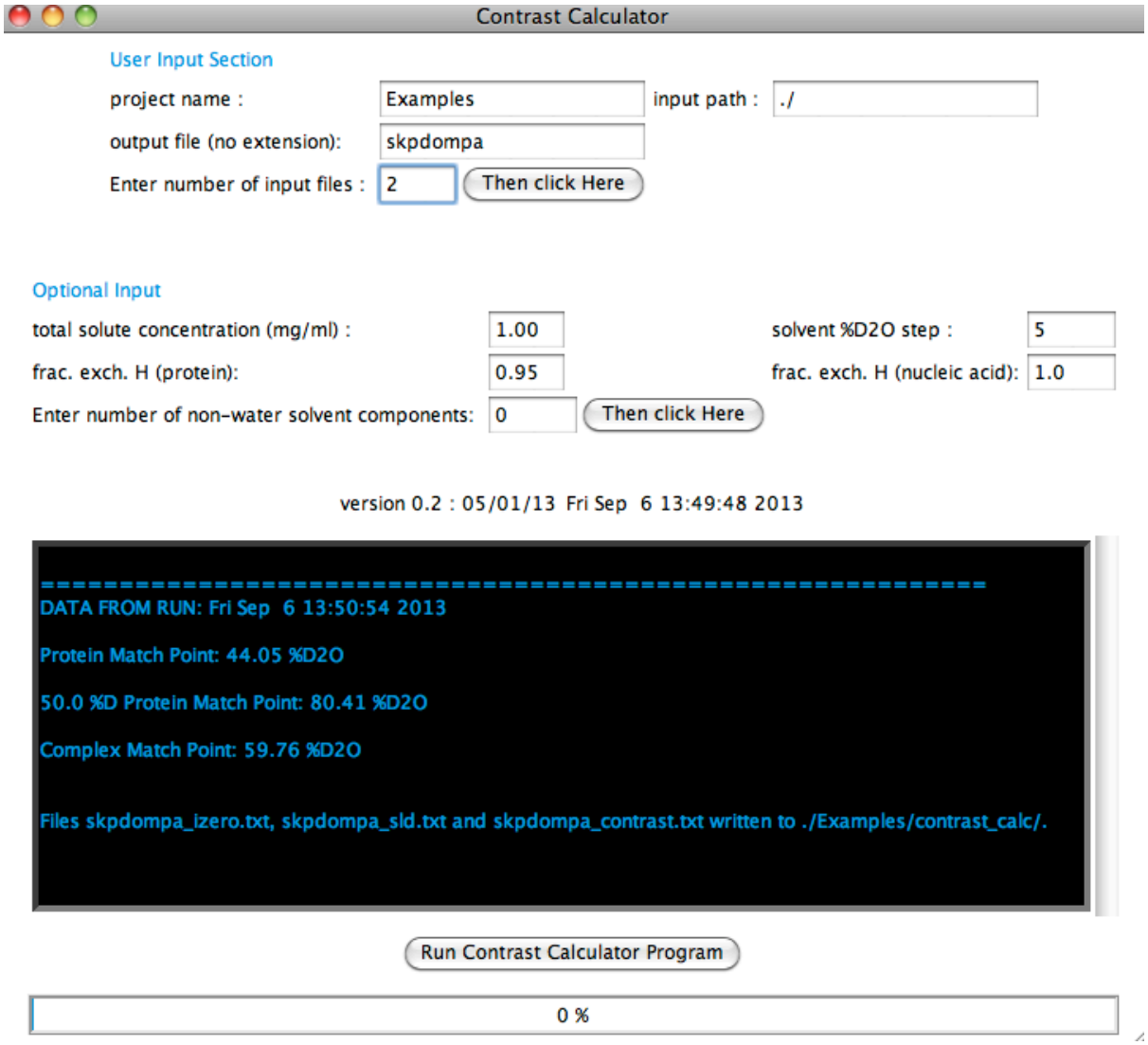

Figure S1 Screenshot of SASSIE Contrast Calculator screen for the skp-OmpA complex with no non-water solvent components after the calculation has been completed. The OmpA component is 50% deuterated.

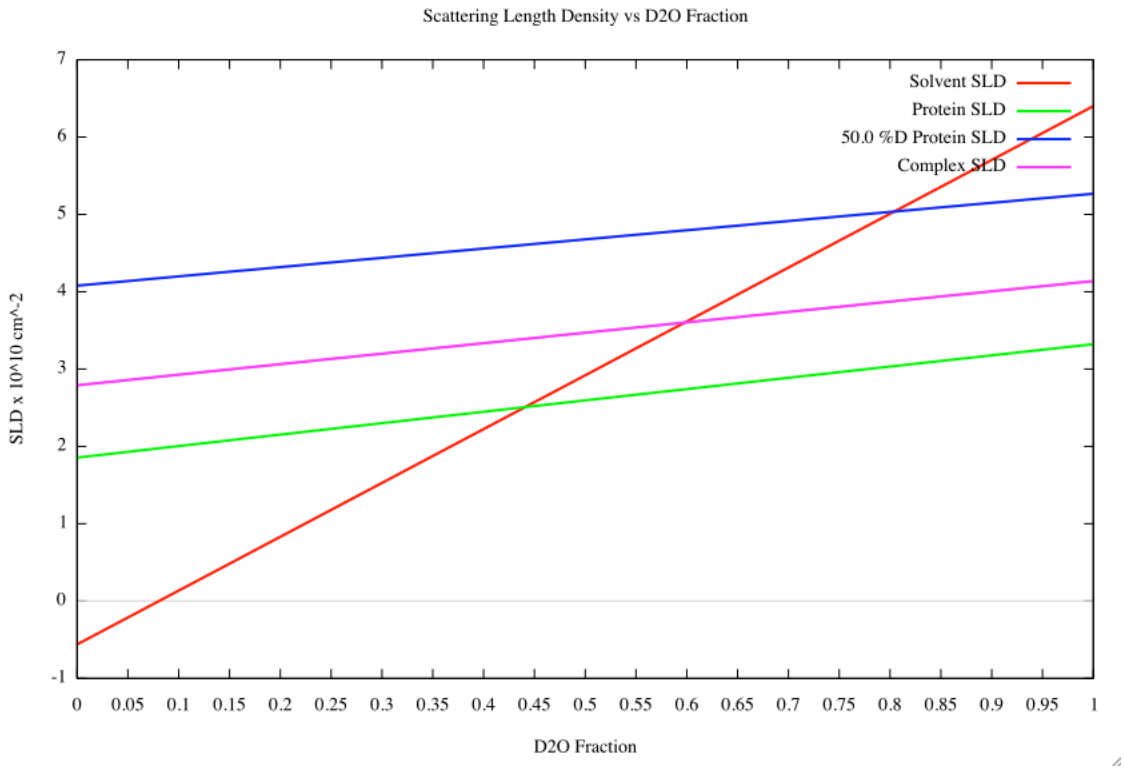

Figure S2 Plot of the neutron SLD vs. D_2O fraction in the solvent for the two protein components, the complex and the solvent. Match points are indicated where the curves intersect.

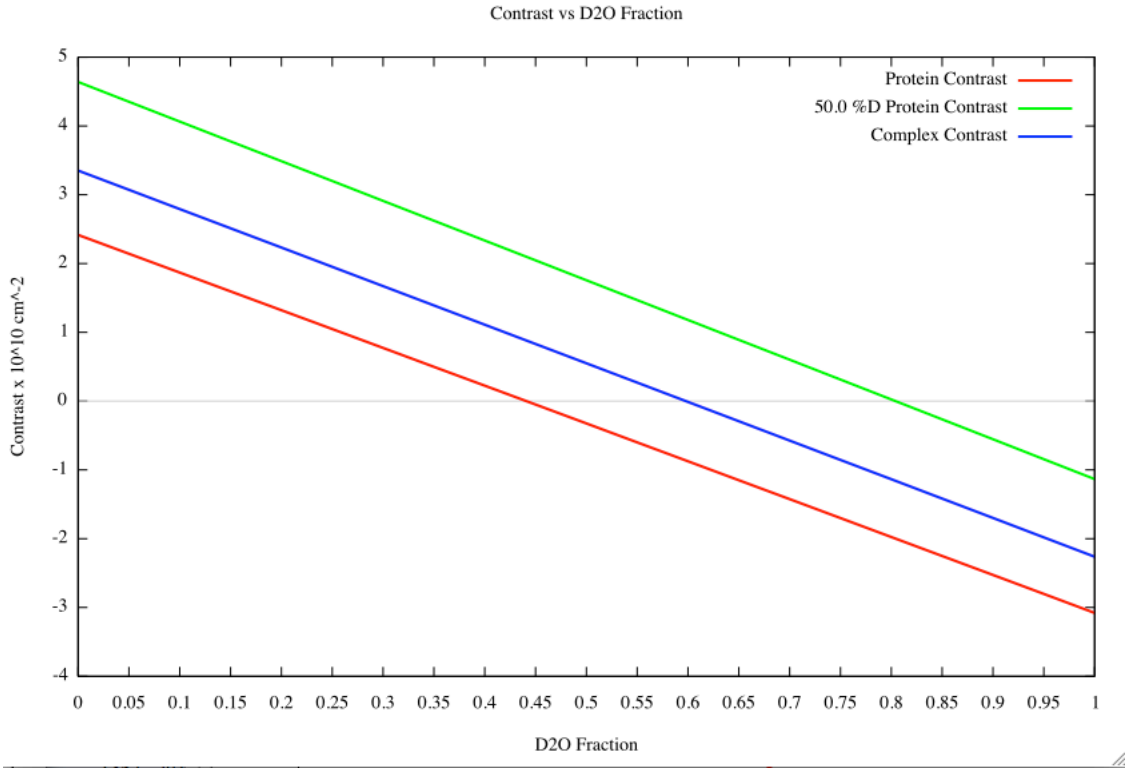

Figure S3 Plot of the neutron contrast vs. D_2O fraction in the solvent for the two protein components and the complex. Match points are indicated when the contrast equals zero.

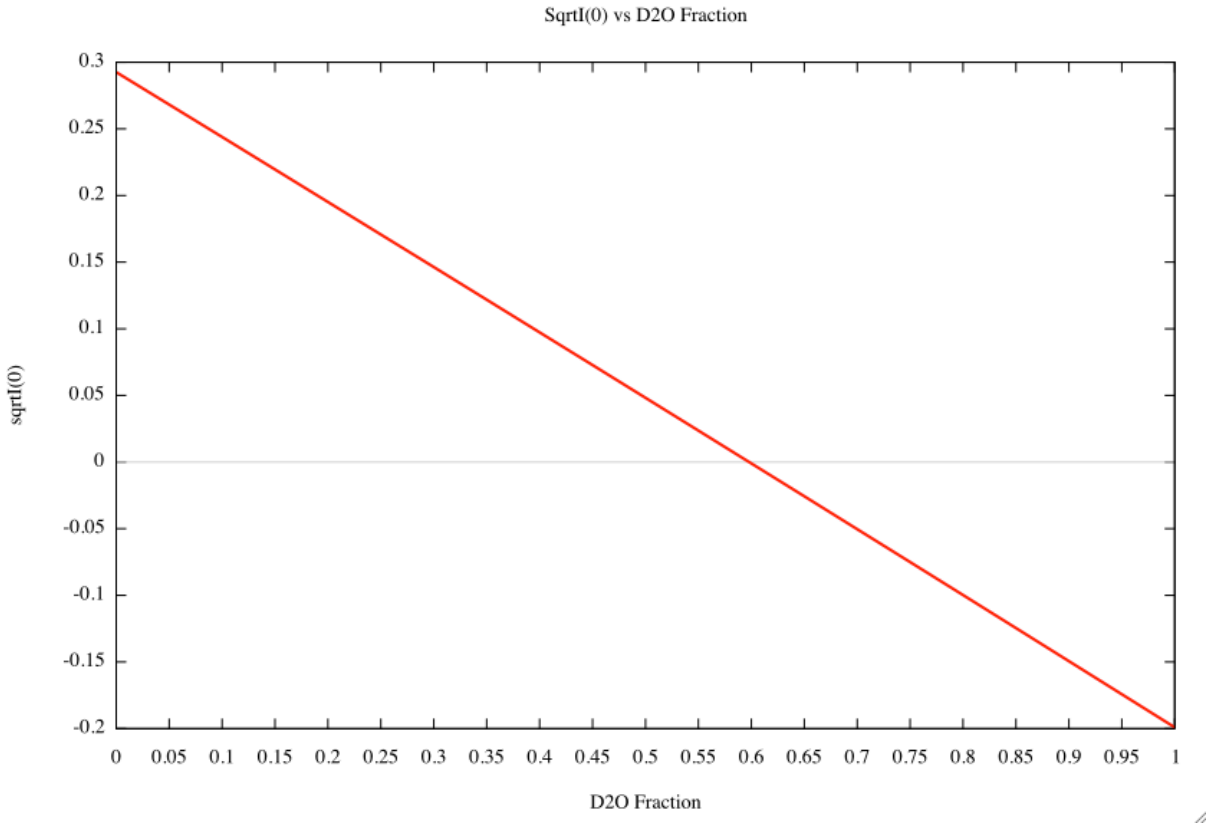

Figure S4 Plot of √I(0) vs. D_2O fraction in the solvent for the skp-OmpA complex. The match point of the complex is indicated when √I(0) equals zero.

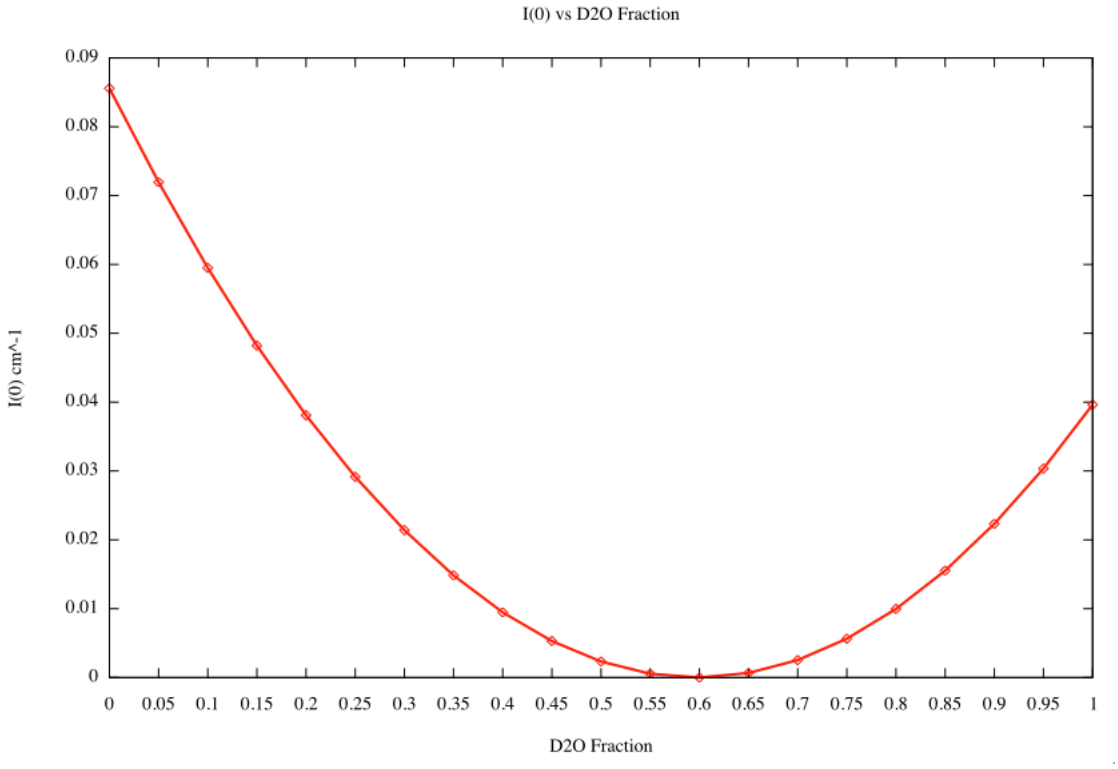

Figure S5 Plot of I(0), on an absolute scale in cm^{-1}, vs. D_2O fraction in the solvent for the skp-OmpA complex.

2.2. Protein/DNA Complex

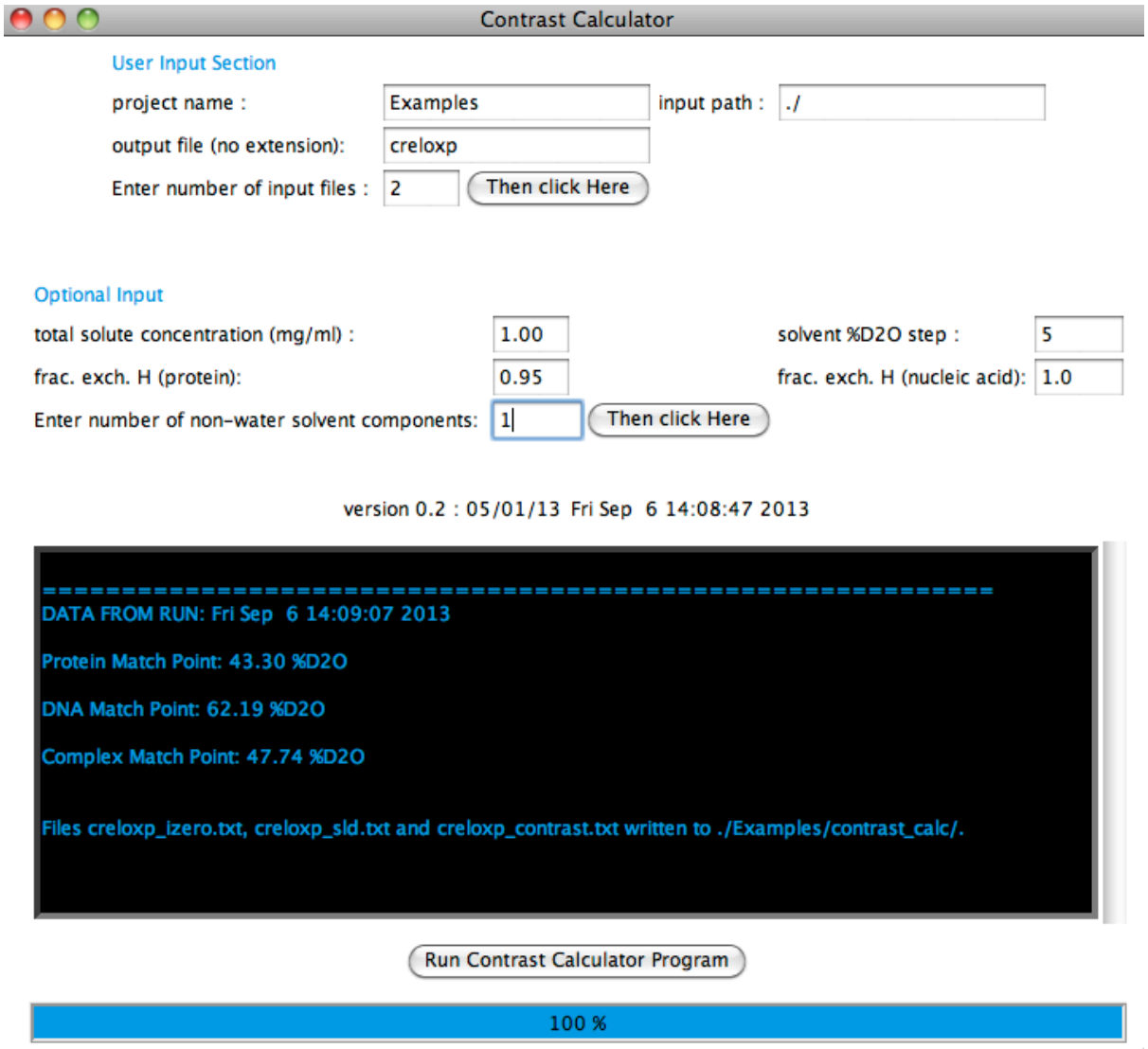

Figure S6 Screenshot of SASSIE Contrast Calculator screen for the Cre-*loxP* complex with one non-water solvent component after the calculation has been completed.

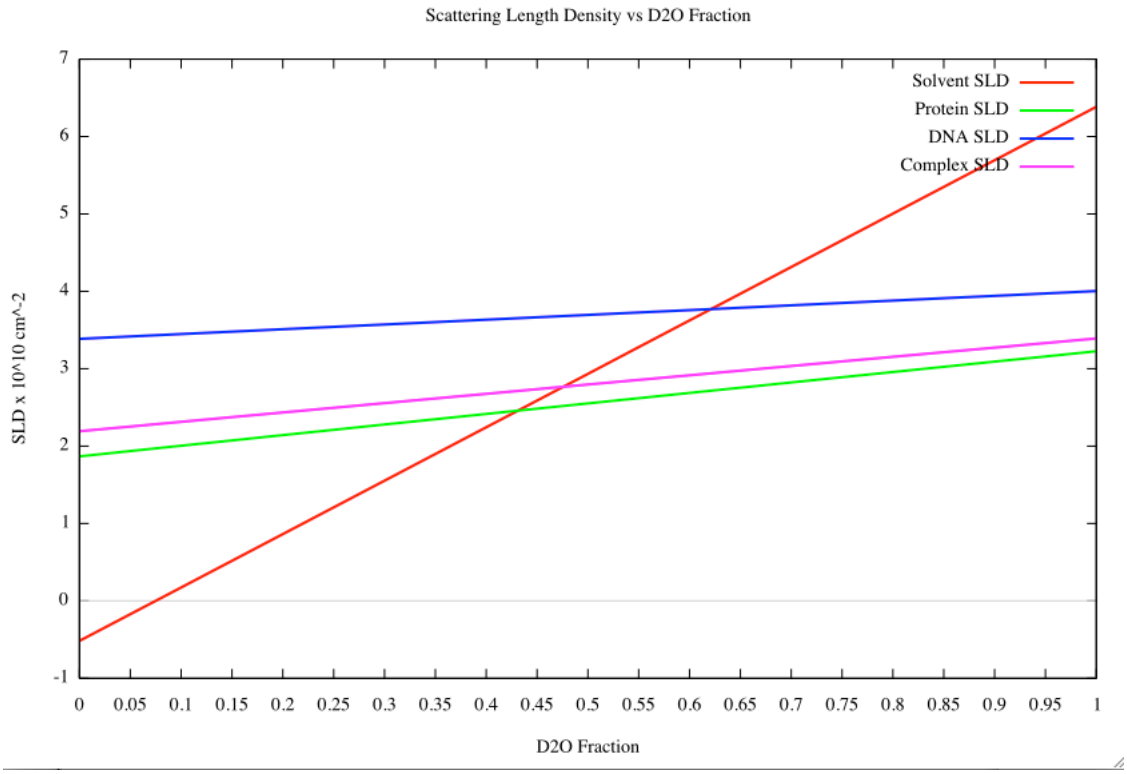

Figure S7 Plot of the neutron SLD vs. D_2O fraction in the solvent for the protein and DNA components, the complex and the solvent. Match points are indicated where the curves intersect.

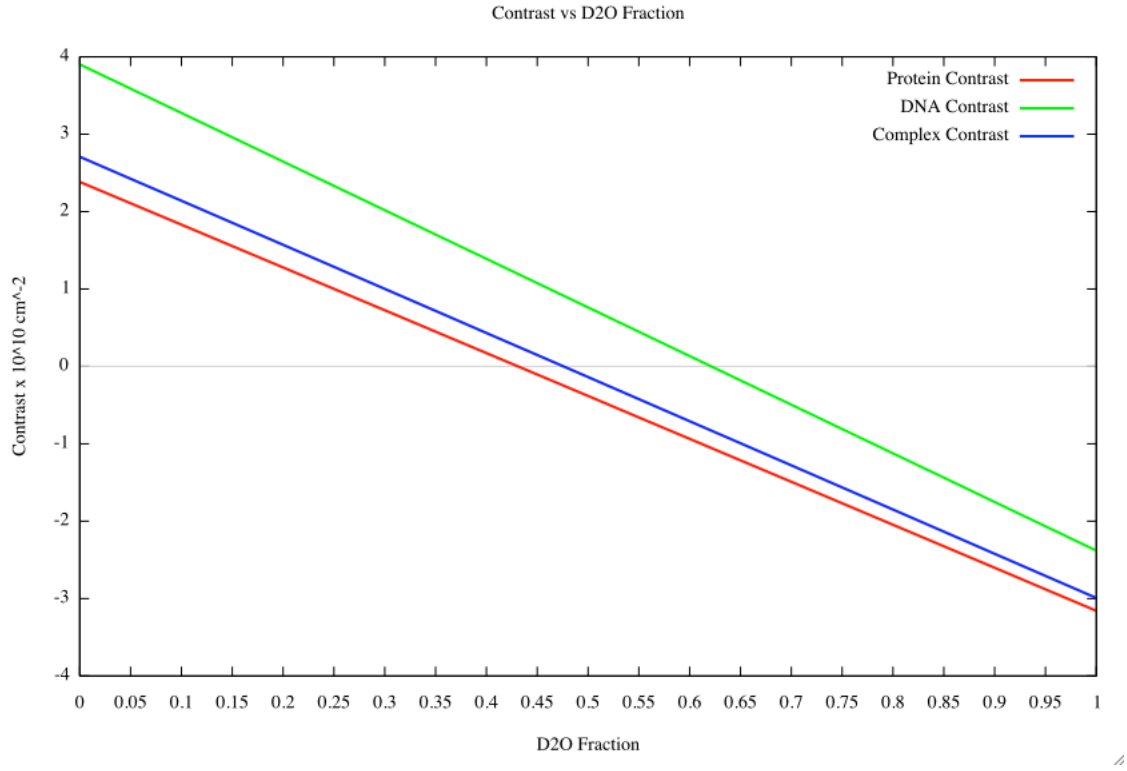

Figure S8 Plot of the neutron contrast vs. D_2O fraction in the solvent for the protein and DNA components and the complex. Match points are indicated when the contrast equals zero.

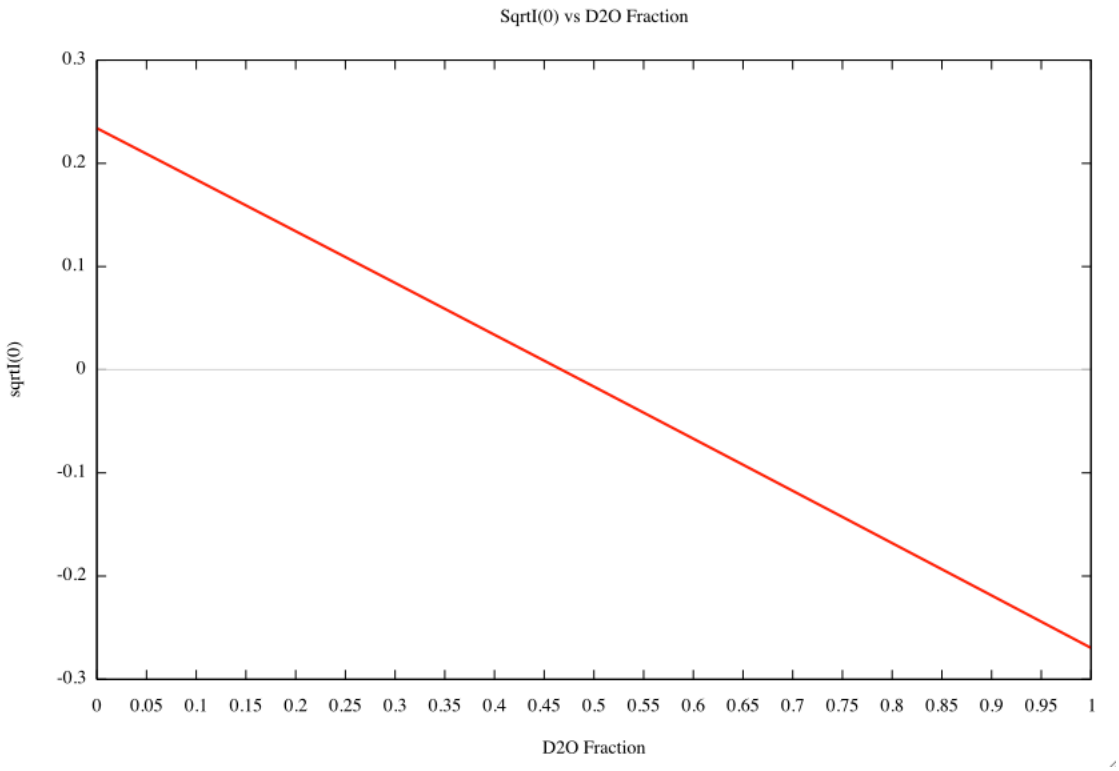

Figure S9 Plot of √I(0) vs. D_2O fraction in the solvent for the Cre-*loxP* complex. The match point of the complex is indicated when √I(0) equals zero.

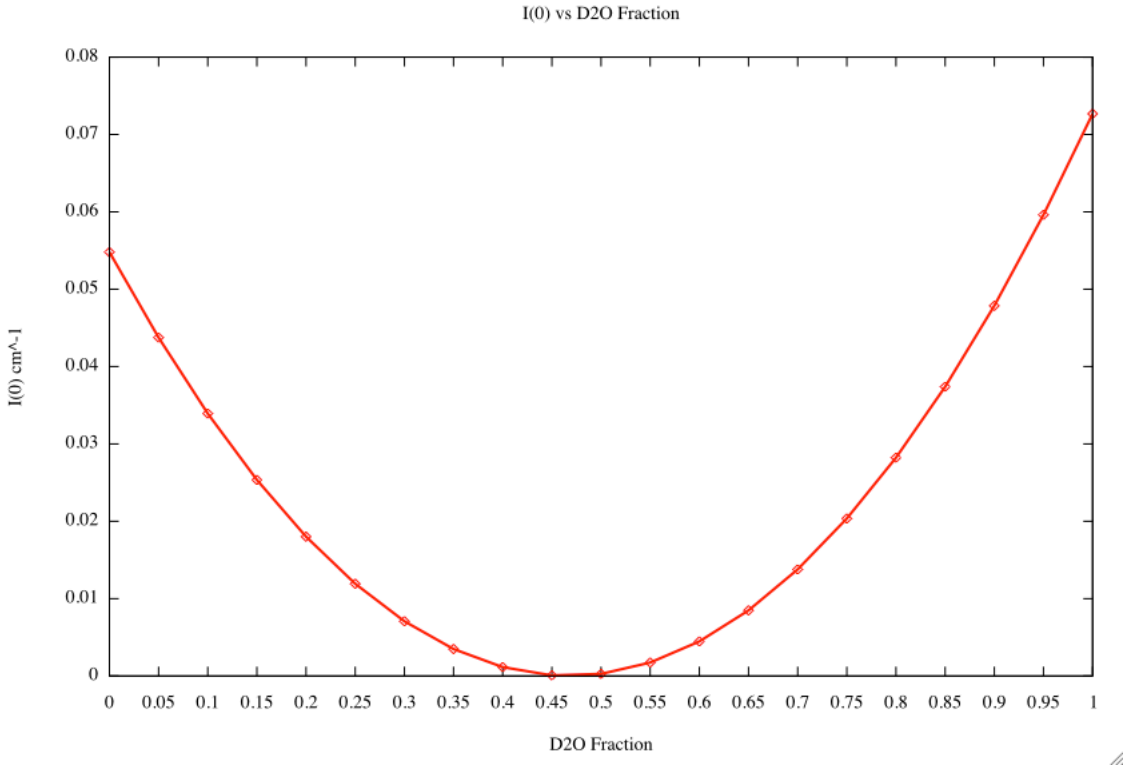

Figure S10 Plot of I(0), on an absolute scale in cm^{-1}, vs. D_2O fraction in the solvent for the Cre-*loxP* complex.

3. Sample Output Files

3.1. Protein/Deuterated Protein Complex

```
#Date: Fri Sep  6 13:50:54 2013

#Files used: skp_sequence.txt (3), ompa_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 SLDs (10^10 cm^-2):
#Protein SLD: 12.316; complex SLD: 12.316; solvent SLD:  9.428

#NEUTRON SLDs:
#Protein Match Point: 44.05 %D2O
#50.0 %D Protein Match Point: 80.41 %D2O
#Complex Match Point: 59.76 %D2O

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

# frac D2O, Protein SLD, 50.0 %D Protein SLD, Complex SLD, Solvent SLD (10^10 cm^-2)
 0.00     1.855   4.078   2.790  -0.561
 0.05     1.930   4.138   2.858  -0.213
 0.10     2.004   4.199   2.927   0.135
 0.15     2.078   4.259   2.995   0.483
 0.20     2.152   4.319   3.063   0.831
 0.25     2.227   4.379   3.131   1.179
 0.30     2.300   4.439   3.199   1.527
 0.35     2.374   4.498   3.267   1.875
 0.40     2.448   4.558   3.335   2.223
 0.45     2.521   4.617   3.402   2.571
 0.50     2.595   4.677   3.470   2.920
 0.55     2.668   4.736   3.537   3.268
 0.60     2.741   4.796   3.604   3.616
 0.65     2.814   4.855   3.671   3.964
 0.70     2.887   4.914   3.738   4.312
 0.75     2.960   4.973   3.805   4.660
 0.80     3.032   5.032   3.872   5.008
 0.85     3.105   5.091   3.939   5.356
 0.90     3.177   5.150   4.005   5.704
 0.95     3.250   5.208   4.072   6.052
 1.00     3.322   5.267   4.138   6.400
```

Figure S11 Scattering length density output file for the skp-OmpA complex.

```
#Date: Fri Sep  6 13:50:54 2013

#Files used: skp_sequence.txt (3), ompa_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 contrast:  2.889; complex contrast:  2.889

#NEUTRON contrast:
#Protein Match Point: 44.05 %D2O
#50.0 %D Protein Match Point: 80.41 %D2O
#Complex Match Point: 59.76 %D2O

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

# frac D2O, Protein Contrast, 50.0 %D Protein Contrast, Complex CONTRAST (10^10 cm^-2)
 0.00     2.416   4.640   3.351
 0.05     2.143   4.352   3.072
 0.10     1.869   4.064   2.792
 0.15     1.595   3.776   2.512
 0.20     1.321   3.488   2.232
 0.25     1.047   3.200   1.952
 0.30     0.773   2.911   1.672
 0.35     0.499   2.623   1.392
 0.40     0.224   2.335   1.111
 0.45    -0.050   2.046   0.831
 0.50    -0.325   1.757   0.550
 0.55    -0.600   1.469   0.269
 0.60    -0.875   1.180  -0.011
 0.65    -1.150   0.891  -0.292
 0.70    -1.425   0.602  -0.573
 0.75    -1.700   0.313  -0.855
 0.80    -1.976   0.024  -1.136
 0.85    -2.251  -0.265  -1.417
 0.90    -2.527  -0.555  -1.699
 0.95    -2.803  -0.844  -1.980
 1.00    -3.079  -1.133  -2.262
```

Figure S12 Contrast output file for the skp-OmpA complex.

```
#Date: Fri Sep  6 13:50:54 2013

#Files used: skp_sequence.txt (3), ompa_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) = 0.0636 (cm^-1)

#NEUTRONS:
#Protein Match Point: 44.05 %D2O
#50.0 %D Protein Match Point: 80.41 %D2O
#Complex Match Point: 59.76 %D2O

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

# frac D2O, Protein Mw, 50.0 %D Protein Mw, Complex Mw (kDa), I(0) (cm^-1), sqrtI(0)
 0.00     49.906          36.218          86.124          0.086   0.293
 0.05     49.950          36.244          86.195          0.072   0.268
 0.10     49.995          36.271          86.266          0.059   0.244
 0.15     50.039          36.298          86.337          0.048   0.220
 0.20     50.083          36.325          86.408          0.038   0.195
 0.25     50.127          36.351          86.479          0.029   0.171
 0.30     50.172          36.378          86.550          0.021   0.146
 0.35     50.216          36.405          86.621          0.015   0.122
 0.40     50.260          36.431          86.692          0.009   0.097
 0.45     50.305          36.458          86.763          0.005   0.073
 0.50     50.349          36.485          86.834          0.002   0.048
 0.55     50.393          36.511          86.905          0.001   0.024
 0.60     50.438          36.538          86.976          0.000  -0.001
 0.65     50.482          36.565          87.047          0.001  -0.026
 0.70     50.526          36.592          87.118          0.003  -0.050
 0.75     50.571          36.618          87.189          0.006  -0.075
 0.80     50.615          36.645          87.260          0.010  -0.100
 0.85     50.659          36.672          87.331          0.016  -0.125
 0.90     50.704          36.698          87.402          0.022  -0.149
 0.95     50.748          36.725          87.473          0.030  -0.174
 1.00     50.792          36.752          87.544          0.040  -0.199
```

Figure S13 I(0) output file for the skp-OmpA complex.

3.2. Protein/DNA Complex

```
#Date: Fri Sep  6 14:09:07 2013

#Files used: crelox_protein.pdb (1), crelox_dna.pdb (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
#       NaCl      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 SLDs (10^10 cm^-2):
#Protein SLD: 12.463;  NA SLD: 15.595;  complex SLD: 13.134; solvent SLD:  9.474

#NEUTRON SLDs:
#Protein Match Point: 43.30 %D2O
#DNA Match Point: 62.19 %D2O
#Complex Match Point: 47.74 %D2O

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

# frac D2O, Protein SLD, DNA SLD, Complex SLD, Solvent SLD (10^10 cm^-2)
 0.00     1.867   3.386   2.192  -0.548
 0.05     1.936   3.417   2.253  -0.201
 0.10     2.005   3.448   2.314   0.146
 0.15     2.073   3.479   2.374   0.494
 0.20     2.142   3.510   2.435   0.841
 0.25     2.211   3.541   2.495   1.188
 0.30     2.279   3.572   2.555   1.535
 0.35     2.347   3.603   2.616   1.882
 0.40     2.416   3.634   2.676   2.229
 0.45     2.484   3.665   2.736   2.577
 0.50     2.552   3.695   2.796   2.924
 0.55     2.620   3.726   2.856   3.271
 0.60     2.687   3.757   2.915   3.618
 0.65     2.755   3.788   2.975   3.965
 0.70     2.823   3.819   3.035   4.312
 0.75     2.890   3.850   3.094   4.660
 0.80     2.957   3.880   3.154   5.007
 0.85     3.025   3.911   3.213   5.354
 0.90     3.092   3.942   3.272   5.701
 0.95     3.159   3.973   3.332   6.048
 1.00     3.226   4.003   3.391   6.395
```

Figure S14 Scattering length density output file for the Cre-*loxP* complex.

```
#Date: Fri Sep  6 14:09:07 2013

#Files used: crelox_protein.pdb (1), crelox_dna.pdb (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
#       NaCl      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 contrast:  2.989;  NA contrast:  6.121;  complex contrast:  3.660

#NEUTRON contrast:
#Protein Match Point: 43.30 %D2O
#DNA Match Point: 62.19 %D2O
#Complex Match Point: 47.74 %D2O

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

# frac D2O, Protein Contrast, DNA Contrast, Complex CONTRAST (10^10 cm^-2)
 0.00     2.415   3.934   2.740
 0.05     2.136   3.618   2.454
 0.10     1.858   3.302   2.167
 0.15     1.580   2.985   1.880
 0.20     1.301   2.669   1.594
 0.25     1.023   2.353   1.307
 0.30     0.744   2.037   1.020
 0.35     0.465   1.721   0.733
 0.40     0.186   1.404   0.446
 0.45    -0.093   1.088   0.159
 0.50    -0.372   0.772  -0.128
 0.55    -0.651   0.455  -0.415
 0.60    -0.931   0.139  -0.703
 0.65    -1.210  -0.177  -0.990
 0.70    -1.490  -0.494  -1.278
 0.75    -1.770  -0.810  -1.565
 0.80    -2.049  -1.126  -1.853
 0.85    -2.329  -1.443  -2.141
 0.90    -2.609  -1.759  -2.429
 0.95    -2.890  -2.076  -2.717
 1.00    -3.170  -2.392  -3.005
```

Figure S15 Contrast output file for the Cre-*loxP* complex.

```
#Date: Fri Sep  6 14:09:07 2013

#Files used: crelox_protein.pdb (1), crelox_dna.pdb (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
#       NaCl      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) = 0.0976 (cm^-1)

#NEUTRONS:
#Protein Match Point: 43.30 %D2O
#DNA Match Point: 62.19 %D2O
#Complex Match Point: 47.74 %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), sqrtI(0)
 0.00     76.995          20.987          97.982          0.056   0.237
 0.05     77.058          20.993          98.051          0.045   0.212
 0.10     77.121          20.999          98.120          0.035   0.187
 0.15     77.185          21.005          98.190          0.026   0.161
 0.20     77.248          21.011          98.259          0.019   0.136
 0.25     77.311          21.017          98.328          0.012   0.111
 0.30     77.374          21.023          98.397          0.007   0.086
 0.35     77.438          21.029          98.467          0.004   0.060
 0.40     77.501          21.035          98.536          0.001   0.035
 0.45     77.564          21.041          98.605          0.000   0.010
 0.50     77.627          21.047          98.674          0.000  -0.016
 0.55     77.691          21.053          98.744          0.002  -0.041
 0.60     77.754          21.059          98.813          0.004  -0.066
 0.65     77.817          21.065          98.882          0.008  -0.092
 0.70     77.881          21.071          98.952          0.014  -0.117
 0.75     77.944          21.077          99.021          0.020  -0.143
 0.80     78.007          21.083          99.090          0.028  -0.168
 0.85     78.070          21.089          99.159          0.038  -0.194
 0.90     78.134          21.095          99.229          0.048  -0.219
 0.95     78.197          21.101          99.298          0.060  -0.245
 1.00     78.260          21.107          99.367          0.073  -0.271
```

Figure S16 I(0) output file for the Cre-*loxP* complex.