ROTDIF-Web: Quick Start Guide

Table of Contents (clickable)

1. Introduction:

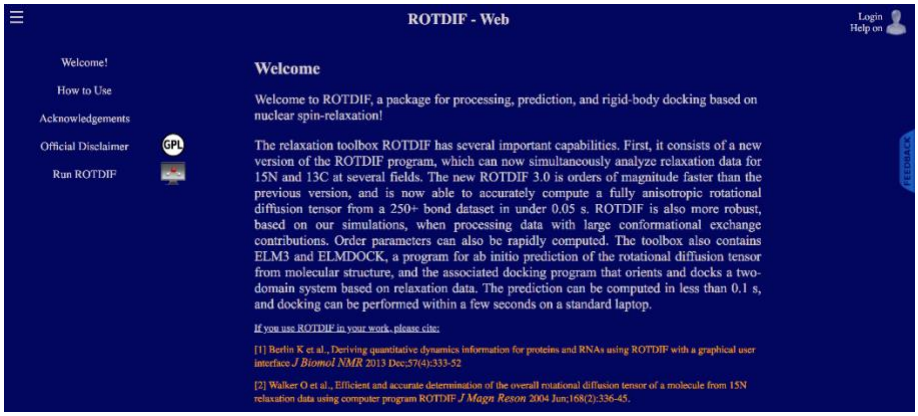
ROTDIF-Web is an online tool that enables researchers to perform accurate and comprehensive analysis of NMR spin-relaxation data. It is developed based on the original programs and software packages ROTDIF, ELM, ELMDOCK, and DYNAMICS developed by David Fushman's group at the University of Maryland (see References [1-8]). Besides retaining the core features of these programs, data visualization is made available in ROTDIF-Web. ROTDIF-Web is based on the ROTDIF 3 Java version written by Konstantin Berlin -- see publication [2]. The stand-alone Java code is available for download from Fushman Lab or Armor package. The GenApp adaptation of the Java version was performed by Yuexi Chen with the help from Alexey Savelyev and Emre Brookes. For funding information, please check the "Acknowledgements" of ROTDIF-Web.

ROTDIF-Web includes the following main modules and features:

- ROTDIF & DYNAMICS: Determine the overall rotational diffusion tensor and characterize local dynamics in proteins and nucleic acids [1-5].
- ELM predictor: *Ab initio* prediction of the rotational diffusion tensor of a macromolecule directly from the atom coordinates using an ellipsoid model representation [6].
- ELMDOCK: Build macromolecular complexes using rigid-body docking guided by experimental rotational diffusion tensors [7,8].

2. Preparation:

ROTDIF-Web Interface



Register: Before you start this tutorial, you will need to create an account and log in to ROTDIF-Web. To register, please click the "human-head" icon  in the top right corner. If you already have an account, please click Login.

Tutorial Data: We have provided the following data to help you work through this tutorial. To download these data to your computer, please click the "How to use" option on the left-side bar. For the monomer, these include ^{15}N relaxation data for the B3 domain of protein G (GB3) [4,5]; for the dimer we use artificial ^{15}N relaxation data for K48-linked di-Ubiquitin [9]. Here are the data files. You can download download all sample data as a zip file, or download them separately.

monomer:
A PDB coordinate file of the B3 domain of protein G (GB3): 1P7F.pdb

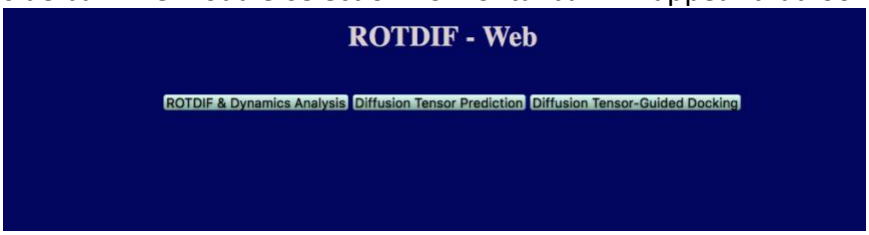
A Relaxation Data file containing ^{15}N relaxation data measured at a single magnetic field (^{1}H frequency 600 MHz): GB3_600_MHz.txt

A Relaxation Data file containing ^{15}N relaxation data measured at three magnetic fields: GB3_3Fields.txt

dimer:
A coordinate file containing two arbitrarily positioned ubiquitin molecules: diUb_AB.pdb
the coordinates for each ubiquitin molecule are from PDB ID 1D3Z.

A Relaxation Data file for ubiquitin dimer: diUb_AB_relax.txt

After downloading these files, you can click the  icon (of the Menu help icon) in the top left corner to show or hide the left-side bar.
To get access to various modules/tools of the ROTDIF package, click "Run ROTDIF" on the left-side bar. The module-selection horizontal bar will appear that looks like this:

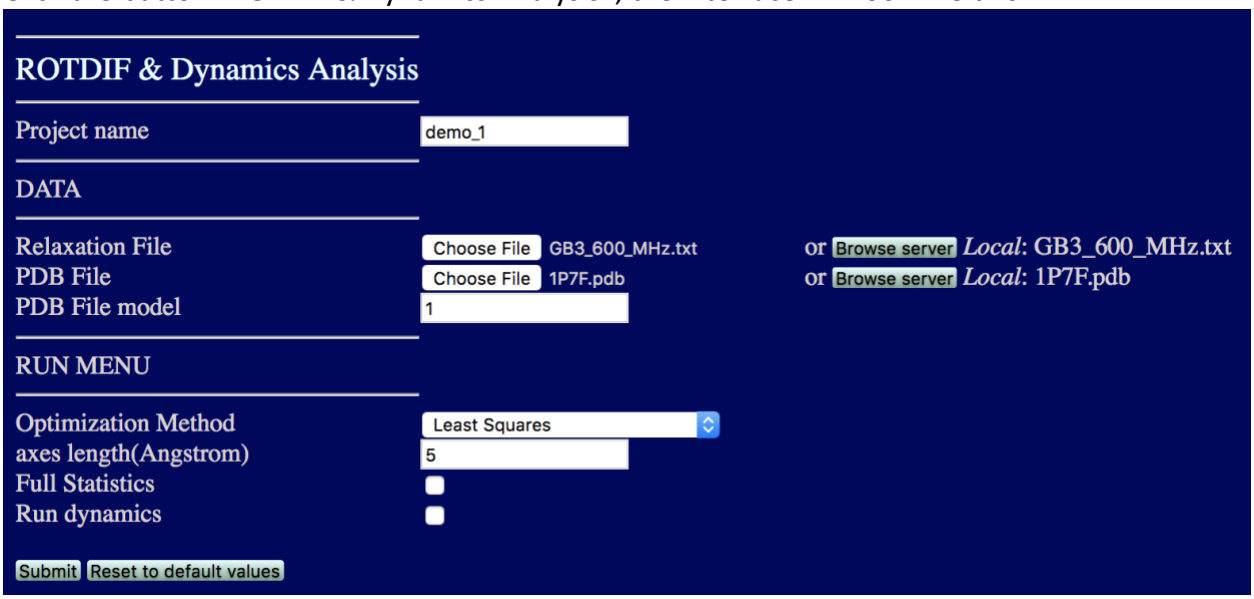


3. Modules

3.1 ROTDIF & Dynamics Analysis

This module allows you to determine the overall rotational diffusion tensor from the experimental spin-relaxation data and to characterize local dynamics in proteins and nucleic acids.

Click the button “ROTDIF & Dynamics Analysis”; the interface will look like this:



- **Project name:** The name of the folder to store all the results of the current session
- **Relaxation File:** Upload the file (e.g., GB3_600_MHz.txt) containing experimental relaxation data. The data file is a text (ascii) file with the following format.

  Format:

```
%<residue><chainID><atom 1><atom 2><frequency><R1><R1 error><R2><R2 error><NOE><NOE error>
2 A N H 500.00 2.667483 0.037891 4.905820 0.035742 0.591190 0.010283
3 A N H 500.00 2.672639 0.033890 4.726413 0.051759 0.619165 0.008180
4 A N H 500.00 2.735406 0.020873 4.987897 0.081378 0.641458 0.007477
5 A N H 500.00 2.692827 0.020037 4.916255 0.102146 0.621036 0.007433
```

  If the input file includes relaxation data measured at multiple frequencies, please leave a blank line between the data from different fields, as shown below:

```
53 A N H 500.00 2.669148 0.035118 4.969276 0.070063 0.650269 0.007676
54 A N H 500.00 2.664422 0.019579 4.855702 0.080907 0.622826 0.007711
55 A N H 500.00 2.616434 0.048870 4.851592 0.059640 0.627973 0.007723
56 A N H 500.00 2.624163 0.006349 4.952994 0.013155 0.613304 0.006629

2 A N H 600.00 2.300308 0.038523 5.056525 0.039846 0.639089 0.009900
3 A N H 600.00 2.276911 0.024362 4.778002 0.042812 0.695735 0.009900
4 A N H 600.00 2.356777 0.023803 5.099474 0.031392 0.693268 0.008500
5 A N H 600.00 2.387973 0.019532 5.203519 0.105184 0.703889 0.008400
```

  Here “A” is the “chainID” in the coordinate file, “frequency” is the ^{1}H frequency of the spectrometer, “N” and “H” indicate the atom pair in which the relaxation of the first one (^{15}N) was measured; R1 and R2 are the longitudinal and transverse, respectively, ^{15}N

autorelaxation rates, and NOE is the steady-state heteronuclear $^{15}N\{^{1}H\}$ NOE, the columns marked "R1 error" etc. correspond to the errors in the respective experimental data.
If there are unwanted residues in the data file, they can be either removed or commented out by placing % at the very beginning of the corresponding line. Two types of comments can be used:
% designates residues that should be completely ignored (the same as deleting the line)
* designates residues that should be excluded from the determination of the diffusion tensor but will be used when determining parameters for the local dynamics.

These are typically residues affected by conformational/chemical exchange (resulting in elevated R2 values) or those residues where the molecular structure is not well defined (e.g., due to increased flexibility or other issues), as well as other outliers.
An example of comments:

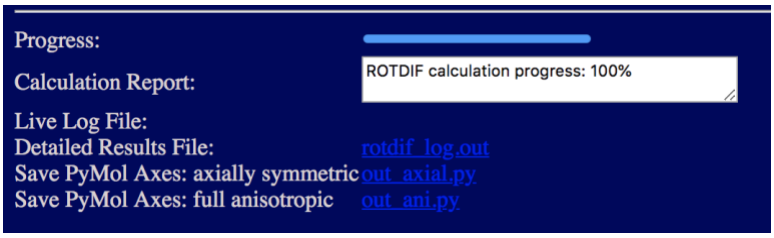
```
*12 A N H 600.00 1.869316 0.013644 3.780496 0.018387 0.589100 0.006100
%13 A N H 600.00 2.129328 0.018976 4.658710 0.039196 0.633860 0.007000
```

- **PDB File:** Upload the coordinate file (e.g., 1P7F.pdb).
- **PDB File model:** If the coordinate file contains more than one structural model, please specify the model you want to analyze. The default model number is 1.
- **Optimization Method:** Choose the optimization method: "Least Squares" or "Robust Least Squares (3.0sig)". Least Squares is the conventional minimization method, while the Robust Least Squares, introduced in ROTDIF 3, dampens the contributions of residuals that are greater than 3σ (above 99.7 percentile)(see our publication [2] for more details).
- **Axes length:** Please specify according to the size of proteins. The default is 5 Angstrom. This parameter sets the desired length of the diffusion tensor axes, to be stored through the *.py files and visualized in PyMol. If the axis length is too short (shorter than the size of the protein) it might not be clearly visible when shown together with the protein.
- **Full Statistics:** Check this box if you want to perform error analysis of the results. The program will generate synthetic (Monte-Carlo) data sets based on the experimental errors in the relaxation rates, for each set perform the same analysis, and produce a full statistics report.
- **Run Dynamics:** Check this box if you want to perform model-free analysis of internal motions in the protein.

Let us fill all the fields with 1P7F.pdb and GB3_600_MHz.txt. and click 

Caution: it may take a long time to run the calculations with "Full Statistics" checked. If this is not necessary, we suggest to leave this box unchecked.

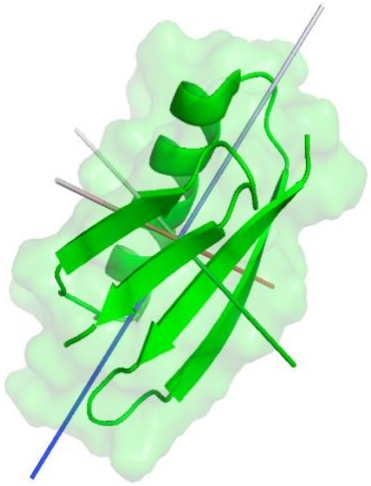
After the progress is 100%, you should be able to see all the output files and figures.



You can download the following files:

- Detailed Results File: the .out file records all the data used to generate the plots shown below
- Saved PyMol Axes: axially symmetric model: the out_axial.py file (when run in PyMol) builds axes based on the results of the axially symmetric rotational diffusion model
- Saved PyMol Axes: fully anisotropic model: the out_ani.py file (when run in PyMol) builds axes based on fully anisotropic rotational diffusion model

You can visualize the axes encoded in the *.py files in PyMol together with your protein (outside ROTDIF-Web). The model below is visualized by PyMol.



Once the calculation is finished, the program will output the following plots:

Figure 1. Experimental Data.
This plot shows input experimental data (with error bars) as a function of residue number. Shown are data for all residues that are not marked with %. The parameter ρ (Rho) represents the ratio of the modified relaxation rates – see [2]. You can use the mouse/cursor to read the values for individual residues.

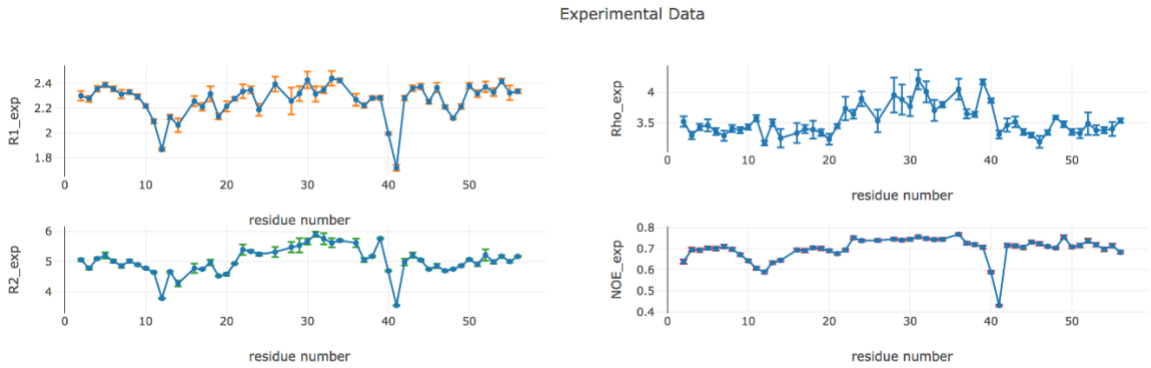


Figure 2. Bond Orientations Plot

This 3D plot shows the distribution of bond orientations (represented by unit vectors) for the residues included in the analysis, to allow visual inspection of how well these vectors sample the orientational space. The sampling tensor quantifying the degree of orientational sampling will be included in the Detailed Results output file. Uniform sampling of the orientations could be important for accurate analysis of the diffusion tensor – see publication [10] for more details. Use the mouse to rotate the plot or zoom.

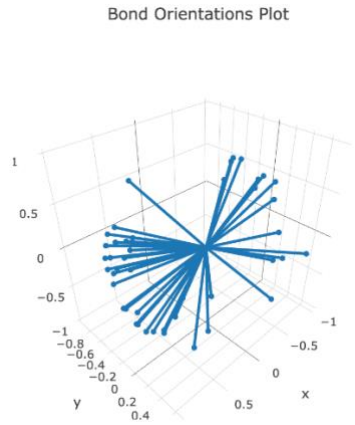


Figure 3. Chi-square Plot

This 3D plot shows the resulting χ^2 value as the function of the Euler angles alpha and beta that determine the orientation of the principal axes of the overall rotational diffusion tensor (axially symmetric model) in the coordinate frame of the protein. Use the mouse to rotate the plot or zoom.

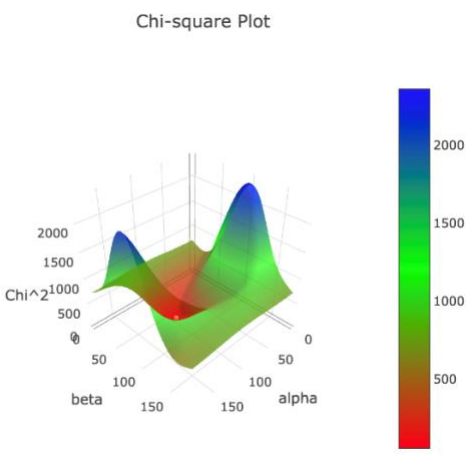


Figure 4. 2D Model Fit

This 2D plot shows the ρ values for the individual residues as the function of the angle θ between the corresponding bond and the z-axis of the rotational diffusion tensor (axially symmetric model). The curves represent the upper and lower bounds of the theoretical dependence of ρ as a function θ for the derived fully anisotropic diffusion tensor (see Figure 5 and publication [1] for details). You can use the mouse/cursor to read the values for individual residues.

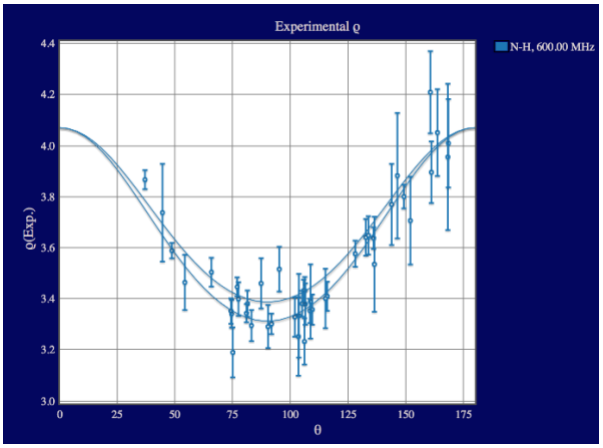


Figure 5. 3D Model Fit

This 3D plot shows the ρ values for the individual residues as the function of the angles θ (theta) and ϕ (phi) that determine the orientation of each bond with respect to the principal axes of the derived rotational diffusion tensor (fully anisotropic model). The surface represents the theoretical dependence of ρ as a function of θ and ϕ for the resulting diffusion tensor; the dots represent experimental data points. Use the mouse to rotate the plot or zoom. You can use the mouse/cursor to read the values for individual data points.

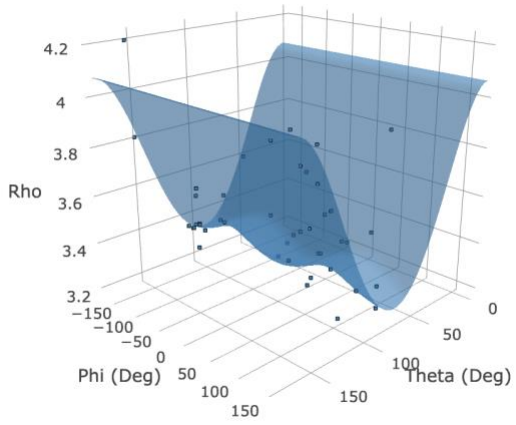


Figure 6. Isotropic Model Fit

These plots illustrate how well or not the experimental data agree with the isotropic rotational diffusion tensor model. Shown on the left is the agreement between the experimental and back-calculated/predicted values of ρ; the line corresponds to absolute agreement. Shown on the right are the normalized residuals of fit for each residue. You can use the mouse/cursor to read the values for individual residues.

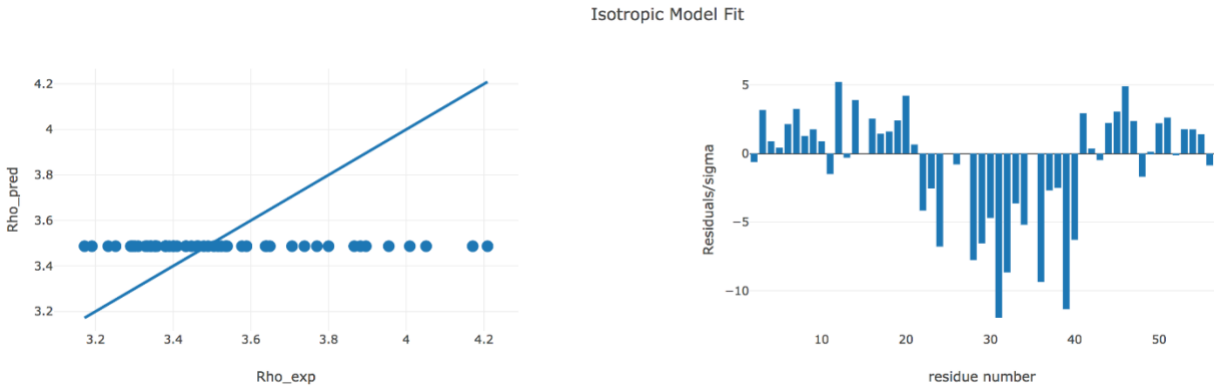


Figure 7. Axially Symmetric Model Fit

These plots illustrate how well or not the experimental data agree with the axially symmetric rotational diffusion tensor model. Shown on the left is the agreement between the experimental and back-calculated/predicted values of ρ; the line corresponds to absolute agreement. Shown on the right are the normalized residuals of fit for each residue. You can use the mouse/cursor to read the values for individual residues.

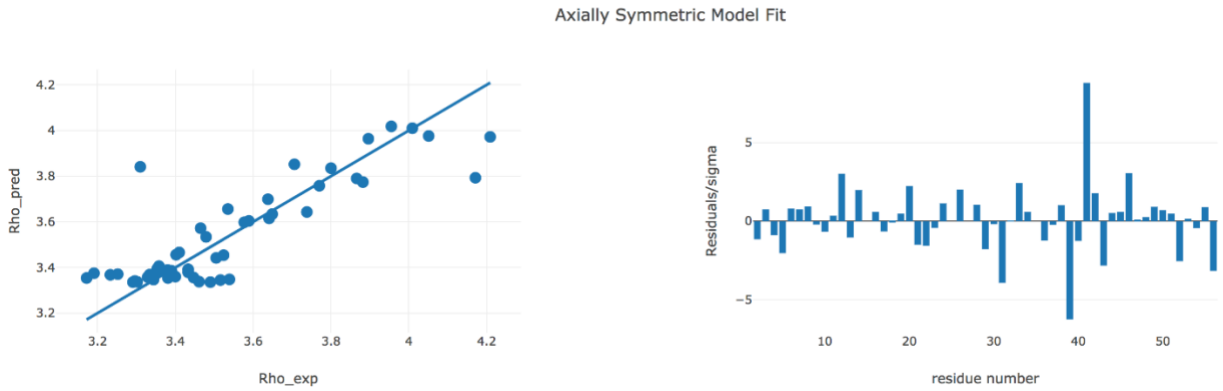


Figure 8. Fully Anisotropic Model Fit

These plots illustrate how well or not the experimental data agree with the fully anisotropic rotational diffusion tensor model. Shown on the left is the agreement between the experimental and back-calculated/predicted values of ρ; the line corresponds to absolute agreement. Shown on the right are the normalized residuals of fit for each residue. You can use the mouse/cursor to read the values for individual residues.

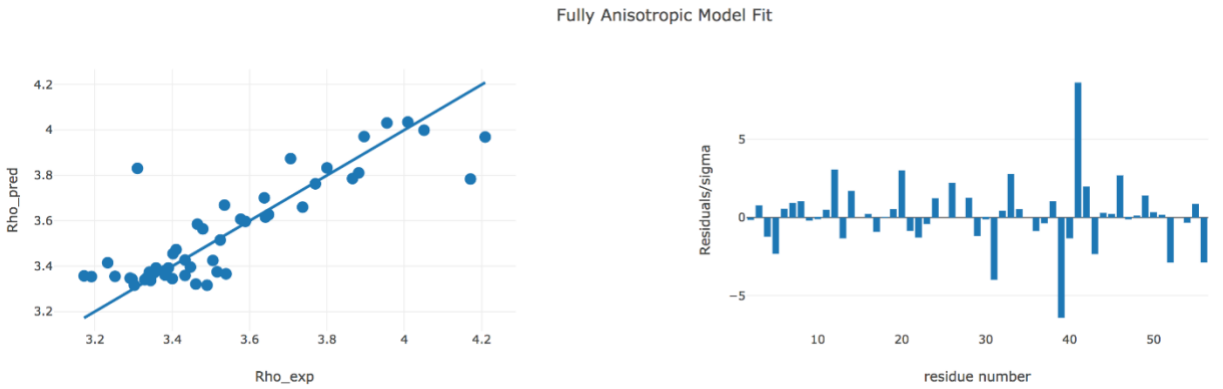


Figure 9. Dynamics: Isotropic Model

These plots depict the model-free parameters of local motions derived from the experimental data assuming isotropic rotational diffusion tensor model: the squared order parameter (S^2) and the related local correlation time (tau_loc), as well as, when applicable, the squared order parameter for fast motion (S^2_fast) and the conformational exchange contribution (Rex) to R_2. See publications [3, 11] for a detailed description of these parameters. You can use the mouse/cursor to read the values for individual residues.

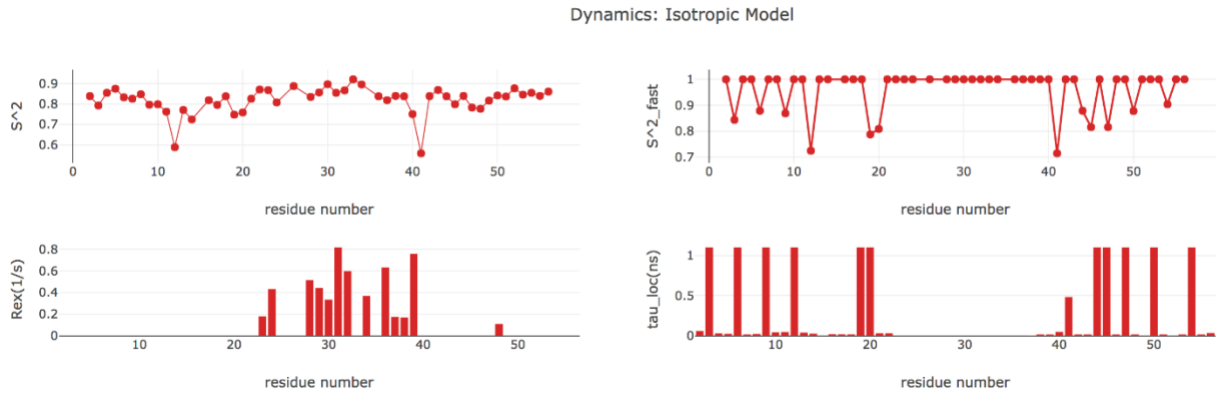


Figure 10. Dynamics: Axially Symmetric Model
These plots depict the model-free parameters of local motions derived from the experimental data assuming axially symmetric rotational diffusion tensor model: the squared order parameter (S^2) and the related local correlation time (tau_loc), as well as, when applicable, the squared order parameter for fast motion (S^2_fast) and the conformational exchange contribution (Rex) to R_2. See publications [3, 11] for the details of these parameters. You can use the mouse/cursor to read the values for individual residues.

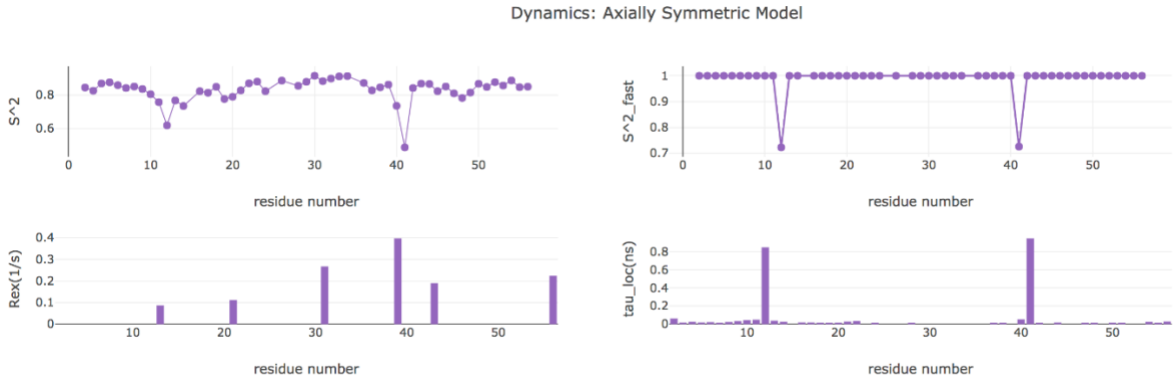
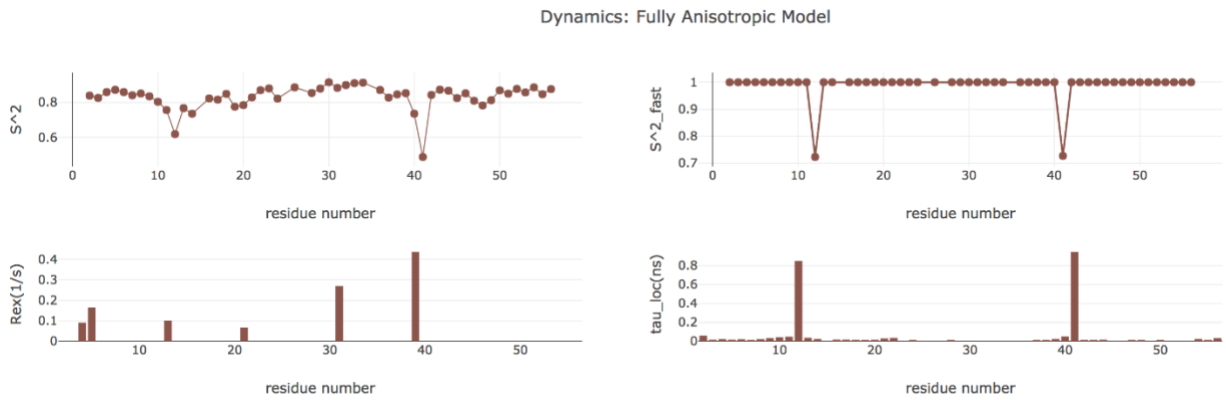
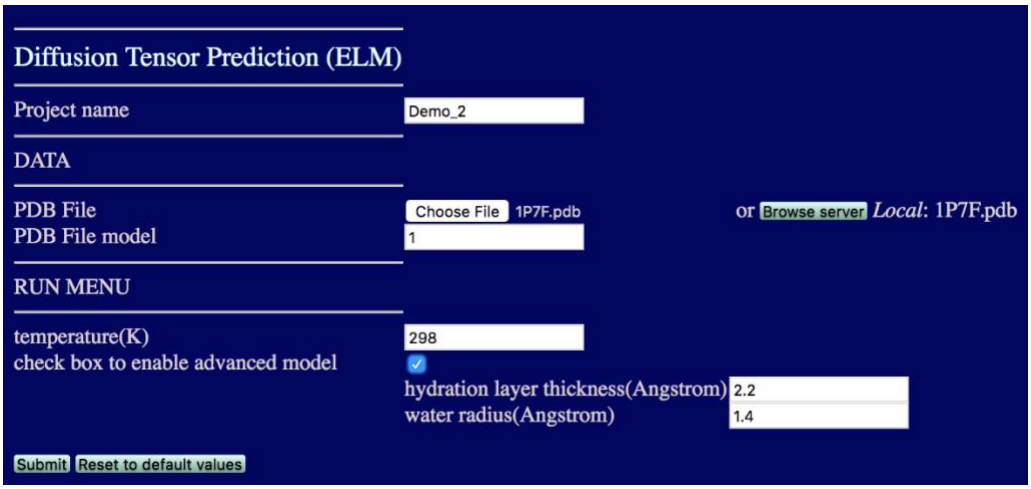


Figure 11. Dynamics: Axially Symmetric Model
These plots depict the model-free parameters of local motions derived from the experimental data assuming fully anisotropic rotational diffusion tensor model: the squared order parameter (S^2) and the related local correlation time (tau_loc), as well as, when applicable, the squared order parameter for fast motion (S^2_fast) and the conformational exchange contribution (Rex) to R_2. See publications [3, 11] for the details of these parameters. You can use the mouse/cursor to read the values for individual residues.



3.2 Diffusion Tensor Prediction

This module performs *ab initio* prediction of the rotational diffusion tensor of a macromolecule directly from the atom coordinates using an ellipsoid model (ELM) representation [6].



- **Project name:** The name of the folder to store all the results
- **PDB File:** Upload the coordinate file to be used for diffusion tensor prediction
- **PDB File model:** If the coordinate file contains more than one structural model, please specify the model you want to analyze. The default model number is 1.
- **temperature:** The desired temperature (in K) of the aqueous medium. Please note that the program assumes that molecule of interest is tumbling in water, and the empirical formula for the water viscosity used here [6, 12] is valid in the range from 273K to 373 K.
- **check box to enable advanced model:** These options are designed for advanced adjustment of the hydration layer thickness and the water radius. It is recommended that you use the default values.

Click 

. After the progress has completed, you should be able to see and download a file "ELM prediction" containing the output of the diffusion tensor prediction.

It looks like:

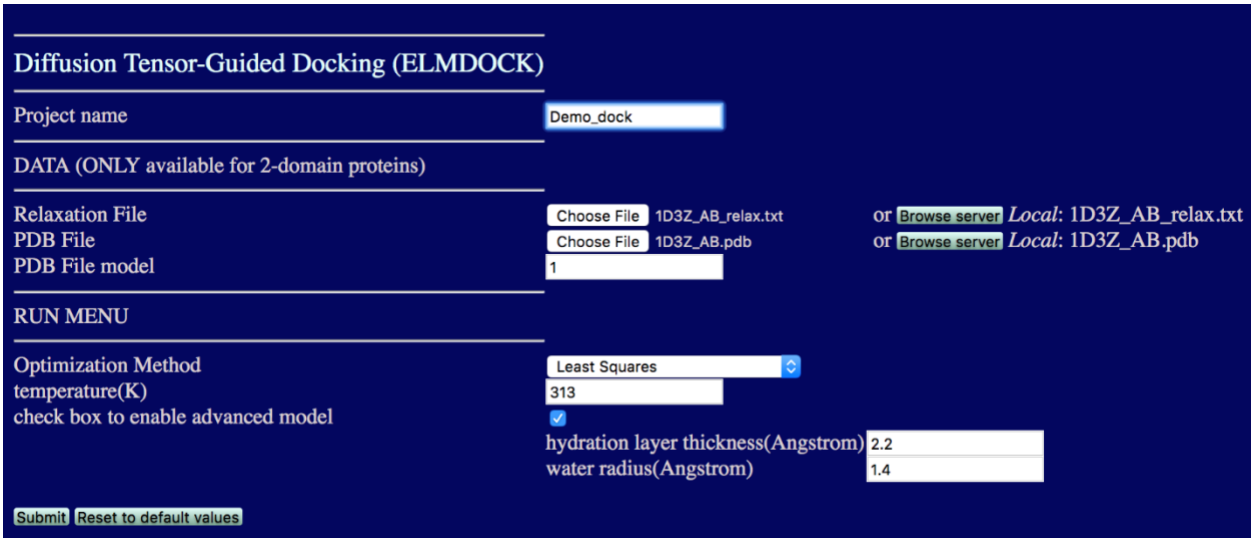
```
Options:
temp = 298.0
robust = false
relax =
elm = true
axes = false
axesl = 5.0
pdb = 1P7F.pdb
out = out
help = false
nostat = false
nodynamics = false
nogui = true
model = 1
wr = 1.4
dock = false
sr = 2.2

Parsing pdb file....done.
Predicting tensor...done.

Dx = 3.94224 *(10^7) 1/s
Dy = 4.05543 *(10^7) 1/s
Dz = 5.24879 *(10^7) 1/s
alpha = 97.11 deg
beta = 70.15 deg
gamma = 104.20 deg
TAUc = 3.77459 ns
anisotropy = 1.31258
rhombicity = 0.13583

====Diffusion Tensor====
    [ 3.96910 -0.15156 -0.02235]
D = [-0.15156  5.09034  0.38352] *(10^7) 1/s
    [-0.02235  0.38352  4.18702]

====Diffusion Tensor Sorted Eigendecomposition====
    [ -0.9517    0.2841   -0.1165][  3.9422    0.0000    0.0000][ -0.9517   -0.2027    0.2307]
D = [ -0.2027   -0.2963    0.9333][  0.0000    4.0554    0.0000][  0.2841   -0.2963    0.9118] *(10^7) 1/s
    [  0.2307    0.9118    0.3396][  0.0000    0.0000    5.2488][ -0.1165    0.9333    0.3396]
```

3.3 Diffusion Tensor-Guided Docking

This module performs a rigid-body docking of macromolecular complexes guided by experimental rotational diffusion tensors using the program ELMDOCK [7,8].

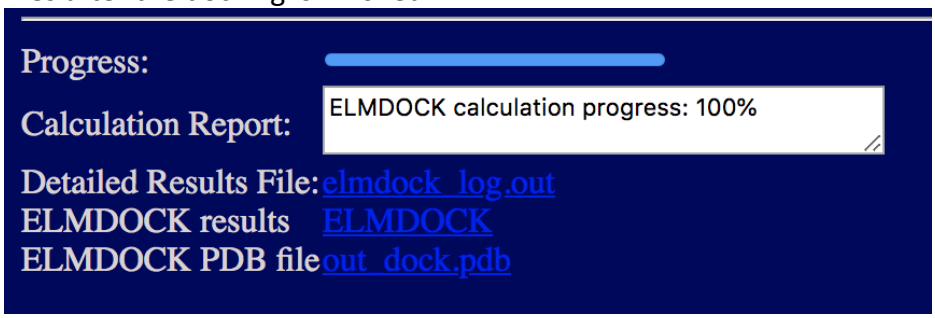
Caution: this part is currently designed only for 2-domain/component systems.

Please use the examples “diUb_AB_relax.txt” and “diUb_AB.pdb” to go through the process.



- **Project name:** The name of the folder to store all the results
- **Relaxation File:** Upload a text file containing spin relaxation data. The format of the data file is the same as spelled above in the ROTDIF & Dynamics Analysis, except that it has to contain data for two molecules (chainID), and the chainID letters must match those in the PDB file.
- **PDB File:** Upload the starting coordinate file to be used for the docking. The file must contain coordinates for both molecules (chainID) listed in the relaxation data file.
- **PDB File model:** If the coordinate file contains more than one structural model, please specify the model you want to analyze. The default model number is 1.
- **Temperature:** The desired temperature (in K) of the aqueous medium. The value of the temperature could vary from complex to complex and might need to be adjusted to avoid steric clashes. For the diUb_AB example provided we suggest 301 K
- **Advanced model:** As in the ELM Predictor, these options are designed for advanced adjustment of the hydration layer thickness and the water radius. It is recommended that you use the default values.

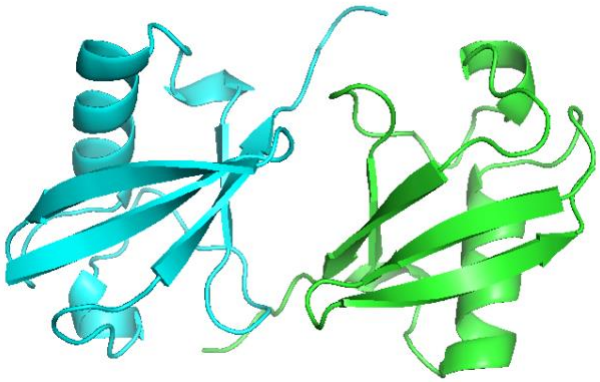
After you uploaded all the files, click the Submit button. You can download the following 3 files after the docking is finished.



- **Detailed Results File:** elmdock_log.out. This file contains the records of all the details.
- **ELMDOCK results:** ELMDOCK. This file contains the following information on the found solutions:

```
====Docking Results====
Docking transformations of second domain ([x_t;y_t;z_t] = R*[x;y;z]+t):
Solution 1:
Chi^2 = 1827.7349679509214
R = [0.3366     -0.4478 0.8284
-0.8965 -0.4215 0.1364
0.2881  -0.7886 -0.5433 ]
t = [-27.416 13.139 -0.228]

Solution 2:
Chi^2 = 1904.196393823229
R = [-0.6257    0.3753  -0.6838
-0.7342 0.0127  0.6788
0.2634  0.9268  0.2675  ]
t = [-14.688 12.880 -0.587]

Solution 3:
Chi^2 = 2304.438731551524
R = [-0.6257    0.3753  -0.6838
-0.7342 0.0127  0.6788
0.2634  0.9268  0.2675  ]
t = [7.507 4.121 -5.723]
```

- **ELMDOCK PDB file:** out_dock.pdb. You need to download this file and view it in a molecular viewing program. Here is the view from PyMol. The temperature is set to be 301K.



4. Exercise

This exercise includes analysis of 15N relaxation data at three different fields (1H resonance frequencies). Please use files “1P7F.pdb” and “GB3_3Fields.txt” to run ROTDIF-Web in all applicable modules. (Note that 1P7F only has one domain and ELMDOCK module requires the protein have 2-domains.) You should be able to see plots like

5. References:

1. O. Walker, R. Varadan, D. Fushman,"Efficient and accurate determination of the overall rotational diffusion tensor of a molecule from 15N relaxation data using computer program ROTDIF," J. Magn. Reson. (2004) 168, 336-345.
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