

# Practical Manual

## General outline to use the structural information obtained from molecular alignment

1. In order to use the information one needs to know the direction and the size of the tensor (susceptibility, alignment, etc).
2. Minimization of the deviation between the measured quantity and its calculated value from a given structure and set of tensor parameters.
3. One has to be able to evaluate the outcome of the minimization.

## General outline for the minimization procedure

1. Get a good estimate on the tensor parameters (anisotropy and rhombicity/asymmetry).
2. Define structural constraints with respect to the initially arbitrary tensor coordinate system.
3. Turn on the force constant for a particular alignment potential such that the final RMS between the measured and calculated values reflect the experimental error.
4. During the calculation the tensor orientation will be automatically determined through global minimization with respect to the current structure.
5. Refine the initial estimate of the tensor parameters. This can be achieved through either grid search method or built into the minimization it self.

## Dipolar coupling refinement using Xplor-NIH

The measured dipolar couplings depend on the relative orientation of the dipolar vectors with respect to the alignment coordinate frame ( $\theta$  and  $\phi$ ), the magnitude of alignment tensor ( $A_a$ ,  $A_r$ ; or  $D_a$ ,  $R$ ) the gyromagnetic ratios of the interacting nuclei ( $\gamma_p$  and  $\gamma_q$ ), the distance between them ( $r_{pq}$ ), and the generalized order parameter of the dipolar vector ( $S$ ).

$$D^{pq} \propto -S \gamma_p \gamma_q [A_a (3\cos^2\theta - 1) + 3/2 A_r (\sin^2\theta \cos 2\phi)] / r_{pq}^3$$

In practice the above equation is usually reduced to:

$$D^{pq} = D_a^{pq} [ (3\cos^2\theta - 1) + 3/2 R (\sin^2\theta \cos 2\phi) ].$$

In Xplor-NIH the function that is being minimized is the difference between calculated and measured dipolar couplings.

$$E = k (D_{\text{meas}} - D_{\text{calc}})^2$$

The variable k is a force constant that can be adjusted during the calculation.

### I. Obtain a good estimate on the magnitude of the alignment tensor: Da and R

In order to get a good estimate for Da and R one would need to create a histogram that reflects the distribution of the measured dipolar couplings. The extremes of the histogram correspond to the alignment tensor components  $A_{xx}$ ,  $A_{yy}$ , and  $A_{zz}$  ( $|A_{zz}| \geq |A_{yy}| \geq |A_{xx}|$ ). Also note that the alignment tensor is traceless, that is  $A_{xx} + A_{yy} + A_{zz} = 0$ . Da and R can be computed with the following equations:

$$A_{zz} = 2 Da$$

$$A_{yy} = - Da (1 + 3/2 R)$$

$$A_{xx} = - Da (1 - 3/2 R)$$

The larger the number of dipolar couplings used in creating the histogram the better the estimate is going to be. In order to use different types of dipolar coupling data (CH, NH, CaC', NC', etc) to create one histogram, one needs to first scale them by their corresponding  $\gamma$  and r values so that they are all normalized with respect to one type of dipolar couplings. Residues with substantially lower order parameter than average should be excluded from the histogram.

#### Exercise:

1. Go to the subdirectory **dipolar\_csa/tables** and find the files: **NH.data** and **CH.data**
2. Residues after L71 has order parameters that are lower than average and they must be excluded from the histogram. Type: "*mstat NH.data 1*" and take a look at the resulting histogram. The histogram is rotated by 90°. Do the same with the **CH.data** file and note the extremes on these histograms. Note "mstat" is a program that is a component of nmrPipe package.
3. Go ahead and scale the **CH.data** so that it is normalized with respect to NH dipolar couplings.

Type "*scale.awk CH.data > CH\_scaled.data*". The new normalized values are in

the file **CH\_scaled.data**.

4. Combine the NH and CH dipolar couplings into one file.

Type “*cat NH.data CH\_scaled.data > all.data*”

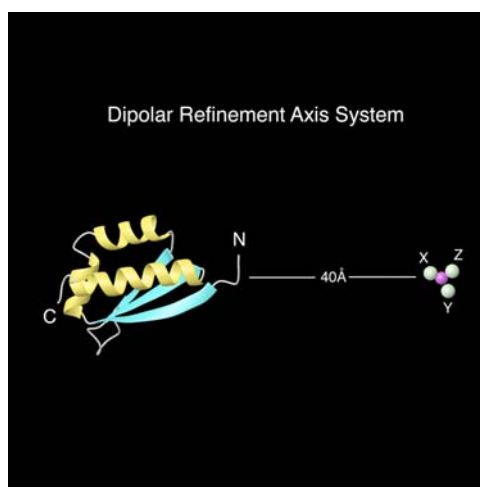
5. Type “*mstat all.data 1*” to get the final histogram.
6. Get the values for  $A_{xx}$ ,  $A_{yy}$ , and  $A_{zz}$  from the histogram. Go ahead and calculate  $D_a$  and  $R$ .

## II. Define axis representing the alignment tensor coordinate system

In order to calculate the dipolar coupling for a pair of nuclei from a given structure one needs to be able to calculate the angles  $\theta$  and  $\phi$ . These angles are measured between the dipolar vector and the alignment coordinate system. In Xplor-NIH the coordinate system is represented by four pseudo atoms: OO, X, Y, and Z. OO represents the origin for the coordinate system, while X, Y, and Z represent the Cartesian axis system. The projection of the dipolar vector onto the OO-Z axis results in  $\cos \theta$ , while projection onto OO-X and OO-Y will result in  $\sin \theta \cos \phi$  and  $\sin \theta \sin \phi$ , respectively. Different alignment tensor will have its own axis system.

Considerations in setting up the axis system:

1. It has to be orthogonal.
2. It has to be positioned far enough away from the molecule to be calculated such that non-bonded interactions (steric, electrostatic, etc) are not present between them.
3. It has to be able to rotate freely without translation.



Exercise:

1. Go to the subdirectory **dipolar\_csa** and find the file **axis.psf**.
2. In this file the axis system is defined. Take a look at **axis.psf** file.
3. The file **par\_axis\_3.pro** defines the geometry and energetic of the axis system.
4. Find the file **axis.pdb**. Open it with a text editor and examine the coordinates of the atoms as well as the numbering of the atoms. This axis file has to be combined with the protein coordinate. Find and open the file **ubq.pdb** and go to the end of the file and notice the axis

coordinates.

5. Create the second axis system:

Type “cp axis.pdb axis\_501.pdb”.

Edit **axis\_501.pdb** and change the residue numbers from 500 to 501.

Also move the axis system by 100 Å along the z direction in the pdb frame, save the file and exit from the editor. Edit the file **ubq.pdb** and copy and paste **axis\_501.pdb** entries into the end of **ubq.pdb**.

Edit the atom numbering to correspond to the new addition of axis system, save the new **ubq.pdb** file and exit the text editor.

Type “cp axis.psf axis\_501.psf”

Edit the **axis\_501.psf** file. Look for the line containing “!NATOM”. Changed the number 500 to 501 in the next following lines, save the file and exit the editor.

6. If possible open the **ubq.pdb** file with a molecular graphic program and take a look at where the axis are relative to the protein.

### III. Creating dipolar constraint file

The constraint file for dipolar coupling in XPLOR-NIH should contain the axis system representing the alignment tensor, the dipolar interacting nuclei, measured dipolar coupling, and experimental error of the dipolar coupling. An example of a constraint entry:

```
assign ( resid 500 and name OO )  
      ( resid 500 and name Z )  
      ( resid 500 and name X )  
      ( resid 500 and name Y )  
      ( resid 2 and name HN )  
      ( resid 2 and name N ) -8.182 0.6 0.5
```

This constraint is to the alignment tensor associated with axis 500 and for residue 2. The measured NH dipolar coupling is  $-8.182$  and the estimated experimental errors are  $-0.6$  and  $+0.5$ . The dipolar couplings of mobile residues have to be treated differently. The use of square open potential would be more appropriate in this case. This can be achieved by extending the positive error to very large value when the measured dipolar coupling is positive, and by extending the negative error when the dipolar coupling is negative.

#### Exercise:

1. Go to the subdirectory **dipolar\_csa/tables**.
2. Used the first three entries in **CH.data** file to create a constraint table with axis system 500 and experimental error of  $\pm 0.2$  Hz.
3. Compare your file to the **CH.tbl** file. Note that the order for the atoms OO, Z, X, and Y are important, while order for the dipolar interacting atoms are not.
4. Create NH dipolar constraints for the following mobile residues ( $S^2 < 0.5$ ):

R74            5.459 Hz

G75            2.114 Hz

G76            1.137 Hz.

The estimated experimental error was  $\pm 0.5$ Hz.

5. Compare your table to **nh\_mob.tbl** file.
6. Create  $C\alpha H\alpha$  dipolar constraints for the following residues:

Q2	4.727 Hz
I3	8.186 Hz
F4	16.414 Hz.

These were measured in a different alignment medium and the experimental errors were  $\pm 0.5$  Hz. Use the axis system created in Part **II** step 5.

7. Compare your constraints to **CH2.tbl** file.

#### IV. Setting up XPLOR-NIH input file for dipolar coupling refinement

At this point you should already have the estimate for  $D_a$  and  $R$ ; constraint tables, and the axis system(s) set up. In the input file you have to define the potential to be used for refinement, restraining the axis system from translation, force constant to be used during the simulated annealing protocol.

##### Exercise:

1. Go to the subdirectory **dipolar\_csa** and find the file **refine.inp**.
2. Locate where the **axis.psf**, **par\_axis\_3.pro**, and **ubq.pdb** files are read in.
3. Locate where  $D_a$  and  $R$  are defined. Check with the values that you estimated in Part I step 6.
4. Locate where the dipolar potentials are being setup. Check for the potential type, force constant variable, and averaging being used in the refinement. What are the differences between the dipolar classes “jch” and “jch2”. What are the similarities and differences between dipolar classes “side” and “jnh2”.
5. Locate the line containing “constraints fix” statement. Identify which atoms are being fixed and explain why?
6. Locate the variables: “ini\_sani”, “fin\_sani”, and “sani\_fac” they define the force constant initial, final, and how fast it is being ramped up during the calculation. Find out the assigned values for these variables.
7. Find out how these variables are related to the dipolar force constants ( $k_{sani}$ ).

## V. Running dipolar coupling refinement with XPLOR-NIH.

At this point you should be ready to run the refinement.

1. Type “*xplor < refine.inp > refine.log*”.
2. Check the log file as the calculation is running to make sure that there is no error during the refinement.
3. Once the calculation is finished, check the log file. Towards the end the agreement between measured and calculated dipolar couplings will be printed out.
4. Make sure that the RMSD is close to the estimated experimental error.
5. Check the gradient energy during the calculation by typing “*grep “grad(E)” refine.log > grad.out*”.
6. Open the file **grad.out** with a text editor and examine the gradient energy as the calculation progressed.
7. Check the resulting structure **ubq\_dipo1.pdb** using a molecular graphics package.
8. Make sure that the axis systems are not distorted.
9. Make sure that other potential energies are not increased substantially.

## How to evaluate structures produced by minimizing against alignment data

1. The final dipolar RMS between measured .vs. calculated values
2. Consistency between dipolar coupling data and NOE data
3. If one has more than one class of dipolar couplings then one way to check for consistency for the data is to calculate the quality (Q) factor which can be shortly be described as follows:

- a. Calculate RMS from zero of the dipolar coupling class of interest for instance: C $\alpha$ -H $\alpha$  dipolar class.

$$[\sum_i (D^i_{C\alpha H\alpha})^2 / N ]^{1/2}$$

- b. Minimize structures against all other classes of dipolar couplings except the C $\alpha$ -H $\alpha$  class in addition to the usual NOE, dihedral, etc. constraints.
- c. Calculate the RMS between the measured .vs. calculated C $\alpha$ -H $\alpha$  dipolar coupling values using the above structures.

$$[\sum (D^m_{C\alpha H\alpha} - D^c_{C\alpha H\alpha})^2 / N ]^{1/2}$$

- d. The Q factor is the ratio between the values than one obtains in step (c) over the value in step (a).

This Q factor is a measure of how consistent the dipolar couplings are with respect to each other as well as against the other typical constraints such as: NOE, dihedral, chemical shifts, etc.

4. Use programs such as Procheck to look at the overall quality of the structure
5. In most cases where one obtains a high degree of consistency one would also gain in the overall RMSD of the family of calculated structures.

Exercise:

1. Go to the directory **dipolar\_csa/tables**.
2. Type “*Q\_rms.awk CH2.data*”. This will result in the RMS value defined in 3a above.
3. One now needs to run a calculation where dipolar restraints corresponding to **CH2.tbl** are excluded.
4. Edit the **check.inp** file and make sure that the resulting coordinate from step 3 is used as input.
5. Type “xplor<check.inp>check.log”.
6. Examine the check.log file and look for the RMS between calculated and measured values for dipolar couplings corresponding to **CH2.tbl**.
7. Calculate the Q factor.

## Hydrogen Bond Angle and Distance Correlation refinement using Xplor-NIH

We found a correlation between distance and angle of hydrogen bonds in protein through a sampling of high-resolution Xray structures (resolution < 1.0 Å). This was confirmed through *ab initio* calculation. An empirical potential was derived to limit sampling of hydrogen bond geometry during an xplor calculation to increase efficiency and more accurate representation of hydrogen bond in protein. The empirical formula is:

$$1/R^3 = A + [B / \{2.07 + \cos \theta_{\text{NHO}}\}^3]$$

where A equals 0.019 and B is 0.21 Å<sup>-3</sup>. In Xplor-NIH the target function for hydrogen bond angle and distance correlation is:

$$E = k ( 1/R^3 - A - [B / \{2.07 + \cos \theta_{\text{NHO}}\}^3] )^2$$

when the term inside the parenthesis is larger than zero, otherwise E is set to zero. This drives the correlation between the hydrogen bond distance and angle to be under the correlation curve.

The use of hydrogen bond angle and distance correlation in XPLOR-NIH is quite straightforward. The hydrogen bond is defined in the constraint table as:

```
assign ( resid 4 and name N )  
      ( resid 4 and name HN )  
      ( resid 65 and name O ).
```

During the xplor refinement a force constant associated with the hydrogen bond potential is increased gradually during the calculation.

### Exercise:

1. Go to the subdirectory **dipolar\_csa**.
2. Identify some hydrogen bonds in **ubq.pdb** file using a molecular graphics package.
3. Create a constraint table reflecting those hydrogen bonds found. No bifurcated hydrogen bonds should be included at this time. Compare your table to the file **hbda.tbl** in the **dipolar\_csa/tables** directory.
4. Open the **refine\_hbda.inp** file using a text editor.

5. Locate where the hbda potential is defined.
6. Locate the variables “ini\_hbda”, “fin\_hbda”, and “hbda\_fac” and find their values.
7. Notice again how they are related to the force constant used in the refinement (k<sub>hbda</sub>).
8. Run xplor calculation by typing: “*xplor < refine\_hbda.inp > refine\_hbda.log*”
9. When the calculation is done, check the log file for any errors.
10. Check the log file for the RMSD of the HBDA term.
11. Make sure that no other potential energies are disturbed.
12. Finally check for the gradient energy of the refinement as in dipolar coupling refinement part **V** step 5 and 6.
13. Examine the resulting structure using a molecular graphics package.
14. Look at the header of the structure calculated in this part and note the final energies. Compare these energy values to the one obtained for the structure calculated in the dipolar coupling section.

## Chemical shift anisotropy refinement using Xplor-NIH

In the event that a small degree of alignment can be introduced to the molecule under study any second rank tensor of the Hamiltonian will not be averaged to zero. For instance the dipolar coupling is no longer averaged to zero and results in small but measurable residual dipolar coupling. Similarly chemical shift anisotropy tensor will also not be averaged to zero. In this case a small difference in chemical shift can be observed between the isotropic and anisotropic samples. The difference corresponds to the projection of the CSA tensor onto the alignment tensor, and it can be expressed as:

$$\Delta\delta = \sum_{i=x,y,z} \sum_{j=x,y,z} A_{ij} \cos^2 \theta_{ij} \delta_{ii}$$

where  $\theta_{ij}$  is the angle between  $A_{ij}$  principal axis of the alignment tensor and the  $\delta_{ii}$  principal axis of the CSA tensor. This residual CSA contains useful structural information that can easily be used in a structure refinement.

In Xplor-NIH the target function for the CSA refinement is:

$$E = k (\Delta\delta_{\text{meas}} - \Delta\delta_{\text{calc}})^2$$

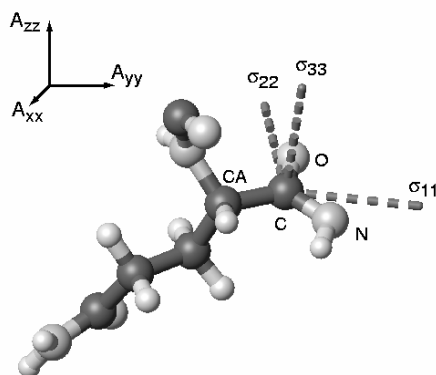
where  $k$  is the adjustable force constant, while  $\Delta\delta_{\text{meas}}$  and  $\Delta\delta_{\text{calc}}$  are the measured and calculated  $\Delta\delta$ , respectively.

### I. Creating CSA constraint file

The constraint file follows the same convention as in the dipolar coupling case. The axis system representing the alignment tensor has to be set up, and  $D_a$  and  $R$  have to be estimated. The format of the constraint for carbonyl CSA is as follows:

```
Assign ( resid 500 and name OO )  
      ( resid 500 and name Z )  
      ( resid 500 and name X )  
      ( resid 500 and name Y )  
      ( resid 2 and name C )  
      ( resid 2 and name O )  
      ( resid 3 and name N ) -38.36 5.00 5.00
```

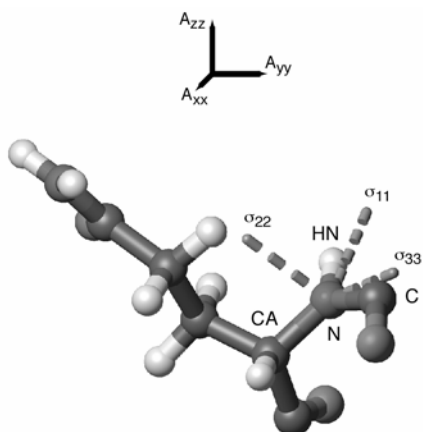
in this case the atoms C, O, and N defines the local CSA coordinate system. This particular entry for carbonyl CSA of residue 2 with respect to alignment tensor represented by residue 500. The local CSA coordinate system is given below.



In contrast to dipolar coupling constraint that deals with a vector connecting the two dipolar interacting nuclei, the CSA value is a function of tensor projection, thus it has to be defined by the three atoms to reference the local CSA geometry. The format for nitrogen CSA is:

```
assign ( resid 500 and name OO )
        ( resid 500 and name Z )
        ( resid 500 and name X )
        ( resid 500 and name Y )
        ( resid 2 and name C )
        ( resid 3 and name N )
        ( resid 3 and name HN ) -73.28 5.00 5.00
```

that restraints nitrogen CSA of residue 3 due to alignment represented by residue 500. The local CSA coordinate system is given below:



### Exercise:

1. Go to the subdirectory **csa**.
2. Create constraints for the first three entries in the **nca.data** file. This file contains nitrogen CSA values. Use residue 500 to represent the axis system. Use  $\pm 5$  ppb as the experimental error.
3. Create constraints for the first three entries in the **ccsa.data** file. Use the same axis system as the nitrogen csa. Use  $\pm 5$  ppb as the experimental error.
4. Go to the subdirectory **dipolar\_csa/tables** and compare your files with **nca.tbl** and **ccsa.tbl** files.

## **II. Setting up XPLOR-NIH input file for CSA refinement**

The steps needed here are the same as in the dipolar coupling refinement. The axis system(s), constraint files, as well as estimated Da and R should already be available. In the input file one has to set up the potentials and force constants for various CSA restraints to be used in the refinement as well as ensuring that there is no translation of the axis system. The only difference between the CSA and dipolar coupling set up in XPLOR-NIH is that one needs to define the chemical shift tensor components ( $\sigma_{11}$ ,  $\sigma_{22}$ , and  $\sigma_{33}$ ).

### Exercise:

1. Go to the subdirectory **dipolar\_csa**.
2. Find and open **refine\_csa.inp** file in a text editor.
3. Locate the section in which the CSA potential is defined (DCSA).
4. Evaluate the differences between the nitrogen and carbonyl CSA setup.
5. Find out how the force constants are being ramped up (kcsa).
6. At this point you should be able to start the CSA refinement by typing “*xplor < refine\_csa.inp > refine\_csa.log*”.
7. When the calculation has finished, take a look at the **refine\_csa.log** file and check for energies as well as final RMSD for the CSA restraints. Also check for the energy gradient (see dipolar

coupling refinement part **V** steps 5 & 6). Note that one must take into account that the tensor values used are average values with a given standard of deviation. Thus the true target RMSD would be the sum of the experimental error and this standard of deviation.

8. Take a look at the resulting structure (ubq\_csa1.pdb) using a molecular graphics package and make sure that the axis and the molecule are not distorted.

## Diffusion Anisotropy refinement using Xplor-NIH

A non-spherical molecule will have an anisotropic rotational diffusion tensor. The dipolar relaxation rates of two interacting nuclei will depend on how the vector connecting them is oriented relative to the diffusion tensor. This orientation dependence can structurally be useful. In practice one needs an anisotropy in rotational diffusion that is substantially large to make it useful. This is limited by the error in the measurement. As a rule of thumb, one would need at least one order of magnitude between the measured quantity to be used in refinement and its experimental error.

Dipolar relaxation rates for two spins 1/2 (such as N-H) are given as:

$$1/T_1 = d^2 [ J(\omega_H - \omega_N) + 3 J(\omega_N) + 6 J(\omega_H + \omega_N) ] + c^2 J(\omega_N)$$

$$1/T_2 = 0.5 d^2 [ 4 J(0) + J(\omega_H - \omega_N) + 3 J(\omega_N) + 6 J(\omega_H) + 6 J(\omega_H + \omega_N) ] + (1/6) c^2 [ 3 J(\omega_N) + 4 J(0) ]$$

$$NOE = 1 + ( \gamma_H / \gamma_N ) d^2 ( 6 J(\omega_H + \omega_N) - J(\omega_H - \omega_N) ) T_1$$

where

$$d^2 = 0.1 [ (\gamma_H \gamma_N h) / ( 2 \pi r_{NH}^3 ) ]^2$$

$$c^2 = (2/15) [ \omega_N^2 ( \sigma_{//} - \sigma_{\perp} )^2 ]$$

Spectral density is given by the fourier transform of the correlation function.

$$J(\omega) = \int_{-\infty}^{\infty} C(t) e^{-i\omega t} dt$$

For an isotropic diffusion:

$$J(\omega) = [ S_f^2 \tau_c / ( 1 + (\omega \tau_c)^2 ) + ( 1 - S_f^2 ) \tau' / ( 1 + (\omega \tau')^2 ) ]$$

$$1/\tau' = 1/\tau_c + 1/\tau_e$$

For an anisotropic diffusion (axial symmetry):

$$J(\omega) = S^2 ( A_1 \tau_1 / [ 1 + (\omega \tau_1)^2 ] + A_2 \tau_2 / [ 1 + (\omega \tau_2)^2 ] + A_3 \tau^3 / [ 1 + (\omega \tau_3)^2 ] ) + ( 1 - S^2 ) \tau' / [ 1 + (\omega \tau')^2 ]$$

$$A_1 = 0.75 \sin^4 \alpha$$

$$A_2 = 3 \sin^2 \alpha \cos^2 \alpha$$

$$A_3 = ( 1.5 \cos^2 \alpha - 1 )$$

$$1/\tau_1 = ( 4 D_{//} + 5 D_{\perp} )$$

$$1/\tau_2 = ( 5 D_{//} + D_{\perp} )$$

$$1/\tau_3 = ( 6 D_{\perp} )$$

where  $\alpha$  is the angle between the dipolar vector and the principal axis of diffusion tensor. To a first approximation the ratio of  $T_1$  and  $T_2$  is not sensitive to fast motion ( $S^2$  and  $\tau'$ ) and variation in CSA values. From the above equation variations in  $T_1/T_2$  will be mostly due to the angle  $\alpha$ . When the rotational diffusion tensor is fully asymmetric the rates will depend on two angles describing the

orientation of the dipolar vector in the diffusion tensor frame and three diffusion rates. Xplor-NIH treats the diffusion anisotropy as fully asymmetric. You have to set asymmetry parameter ( $\eta$ ) to one to reduce the diffusion tensor to an axially symmetric one.

The target function for diffusion anisotropy refinement in Xplor-NIH is:

$$E = k [(T_1/T_2)_{\text{meas}} - (T_1/T_2)_{\text{calc}}]^2$$

where  $k$  is the adjustable force constant.

### I. Creating diffusion anisotropy constraint file

The constraint file follows the same convention as in the dipolar coupling case. While in the dipolar coupling case one refines an orientation of a vector in an alignment tensor frame, in the diffusion anisotropy case it is a refinement of a vector in the diffusion tensor frame. One would need an axis system. In this case the axis will represent the diffusion tensor. The format of the constraint file is exactly the same as in the dipolar coupling case, except the last two atoms assigned belong to the dipolar relaxing ones,

```
Assign ( resid 502 and name OO )
      ( resid 502 and name Z )
      ( resid 502 and name X )
      ( resid 502 and name Y )
      ( resid 3 and name N )
      ( resid 3 and name HN ) 6.8860 0.2500
```

the error in the constraint file is taken as  $\pm$  error. It is important to make sure that residues experiencing large amplitude fast motion or exchange be eliminated from the restraint table. These residues will typically be over estimating the  $T_1/T_2$  ratio.

#### Exercise:

1. Go to the subdirectory **dipolar\_csa/tables** and take a look at the file **t1\_t2.data**. It contains the  $T_1/T_2$  ratios for “LARGE” ubiquitin acquired on a 600 MHz NMR spectrometer.
2. The relaxation rates do depend on second order polynomial of the directional cosines ( $\cos \alpha$ ), thus one would expect to see similar type of histograms as in the dipolar case (dipolar, part I step 2). Try to create the histogram of the  $T_1/T_2$  ratio and describe the diffusion tensor.
3. From the histogram determine the minimum, maximum, and average  $T_1/T_2$  values.
4. Run the program “diff\_estimate” to try to estimate the diffusion parameters:  $\tau_c$ ,  $D_{//} / D_{\perp}$ , and  $\eta$

(asymmetry).

5. Find and open the file **diff\_anis.tbl** with a text editor and examine the restraint table. Note the residue used to represent the diffusion tensor axis system.
6. Go ahead and create a new axis system represented by residue 502. See dipolar coupling refinement part **II** step 5.

## **II. Setting up XPLOR-NIH input file for diffusion anisotropy refinement**

At this point you should have everything that you would need to create an input file for Xplor-NIH. The rest is the same routine that you have gone through to set up the input file for dipolar coupling as well as the CSA refinement.

### Exercise:

1. Go to the subdirectory `dipolar_csa`.
2. Find and open the input file **refine\_dani.inp** in a text editor.
3. Locate the section where the diffusion anisotropy potential is defined (DANI).
4. Examine the coefficients used and compare to what you found in part I.
5. Check the force constant being used and make sure that they are being incremented properly (kdani).
6. At this point you can start the refinement by typing “*xplor < refine\_dani.inp > refine\_dani.log*”.
7. If possible check for the energy gradient, other potential energies, as well as the resulting structure “`ubq_dani1.pdb`” for any irregularities.

### **Notes on diffusion refinement:**

1. A diffusion anisotropy minimum of 1.3 – 1.4 will be needed in order to make this approach practical. This assumes a maximum error in  $T_1/T_2$  of 0.2-0.4.

2. Residues with large amplitude fast motion ( $S^2 < 0.6$ ) must be excluded from any consideration.
3. Elimination of residues which contain exchange contribution to  $T_2$  is essential in order to get an unbiased estimate of the diffusion parameters.
4. Use the following criteria to eliminate anomalous  $T_2$ :

$$\langle T_2 \rangle - T_{2,n} > SD$$

$$(T_{2,n} - \langle T_2 \rangle) / T_{2,n} > 3 \times (\langle T_1 \rangle - T_{1,n}) / T_{1,n},$$

where  $\langle T_1 \rangle$  and  $\langle T_2 \rangle$  are the averages over the residues that have  $\text{NOE} > 0.65$  and  $SD$  is the standard deviation of the  $T_2$ .