

TENSOR2

Volume II - Practical manual

Patrice Dosset, Dominique Marion & Martin Blackledge

Institut de Biologie Structurale J.P. EBEL CEA-CNRS

[Introduction](#)

[Theory](#)

[Whats New in TENSOR2](#)

[How to use TENSOR2.](#)

[Material Required](#)

[Running the Program](#)

[Format of Input Files](#)

[Visualisation Menu](#)

[Selection of residues to be taken into account for determination of **D** or **tau-c**](#)

[Inertia Tensor](#)

[Selection of the spectrometer frequency](#)

[Selection of the angle between csa and dipolar interactions](#)

Fit of relaxation data using isotropic tensor.

[Simple Fit](#)

[Error Calculation and Confidence limit testing](#)

Fit of relaxation data using Anisotropic tensor.

[Simple Fit](#)

[Error Calculation and Confidence limit testing](#)

Visualisation

[Diffusion Axes Display](#)

[Axis Orientation](#)

[Comparison of Inertia and Diffusion Tensors.](#)

[Angular Dispersion](#)

[Fully Asymmetric Model.](#)

[Viewing Dispersion of Diffusion Tensor Axes.](#)

[Viewing results of Monte-Carlo Simulations](#)

[Specific Rotation](#)

Internal Mobility

[Spectral density functions.](#)

[Select Internal Mobility Mode](#)

[Selection of sites to be fitted.](#)

[Available models.](#)

[Selection of Internuclear Distance NH.](#)

[Selection of Specific Tau-c.](#)

[Number of simulations to be performed for the Monte-Carlo analysis of Internal Mobility.](#)

[Starting the Calculation.](#)

[Graphical Representation of the Internal mobility Parameters.](#)

[Inspection of \$\{S^2, \tau_i\}\$ parametric space.](#)

[Superposition of Internal Mobility Parameters onto the Protein Ribbon/Backbone.](#)

Output Coordinate Files

[Write coordinate file in Inertial Frame](#)

[Write coordinate file in Diffusion Frame](#)

Postscript Output

Output Files

[resaniso.0 \(results of the fits, error calculations and validity tests \) :](#)

[resaniso.1 \(Resultats of Monte-Carlo simulations \) :](#)

Introduction

We present the program TENSOR2, which allows the determination of rotational diffusion from three-dimensional structure coordinates and experimental ^{15}N relaxation data, and now, as mentioned below, allows a rigorous model-free analysis of local internal mobility affecting backbone amides, from ^{15}N $\{R_1, R_2, nOe\}$ relaxation rates in the presence of an isotropic of anisotropic rotational diffusion tensor. Confidence in the various models available to describe the tensors and the internal mobility parameters are estimated using Monte-Carlo sampling methods in combination with appropriate χ^2 and F-tests, made possible by a highly efficient simulated annealing algorithm.

A graphical interface allows the visualisation of the orientation of tensorial components with respect to the three-dimensional coordinates of the molecule, and provides a presentation of the statistical behaviour of the system.

What's New in Tensor Version 2.0?

Firstly and most importantly, Tensor2 now carries out an analysis of internal motion using the Lipari-Szabo or extended Lipari-Szabo type analysis. It's about time. This can either be performed using an isotropic rotor to describe rotational diffusion, or using the anisotropic tensor determined using a subset of spins, themselves assumed to have a negligible component to their internal motion. The internal mobility analysis is performed using the logic introduced by the NIH lab (Clare et al 1990), and reproduced by Professor Art Palmer's lab

(Mandel et al 1995), sequentially fitting with more complicated models until the data are adequately reproduced. The confidence limits and uncertainty in the parameters are determined using a rapid monte-carlo based statistical analysis. As has been noted by numerous authors, the error function defined by the fitted internal mobility parameters $\{S^2, \tau_i\}$ is highly non-linear when fitting R_1, R_2 and heteronuclear nOe . It is now possible to rapidly scan the monte-carlo simulations from the fit of each ^{15}N site to check the definition of the optimised internal mobility parameters.

The program also has undergone a large number of cosmetic improvements (ribbon and ball-and-stick display options) and the local motion can now be indicated using colour scales on the molecule. Interactive residue-identification using the cursor aids in recognising more or less mobile parts of the molecule.

How to use TENSOR.

Material Required.

The program has been compiled for the following platforms -

1. Silicon Graphics running Unix 6.5 or higher.
 2. Sun Sparc
 3. PC Linux : for redhat 9.0 you will need [openmotif-2.2.2-14](#)
 4. Mac OS X 10.2 or 10.3: you will need Apple's free X11 server and [openmotif-2.2.0-OSX](#)
-

Running the Program

- tensor2 [*Relaxation-Data-File* *Coordinate-File*]

The name of the output files are

For the Diffusion tensor analysis :anisores.0 for the results of the fit and anisores.1 for the results of the Monte-Carlo error analysis.
For the Analysis of Internal Mobility : ?????

Format of Input Files.

Relaxation Data :**Example :**

| | | | | | | |
|----------|---|--------|----------|---------|---------|----------|
| 0 | 2 | 1.1407 | 0.043841 | 12.6434 | 0.28419 | 0.768764 |
| 0.014567 | | | | | | |
| 1 | 3 | 1.2425 | 0.033048 | 13.3264 | 0.20920 | 0.80534 |
| 0.023311 | | | | | | |
| 1 | 4 | 1.2274 | 0.029092 | 13.0570 | 0.16854 | 0.57453 |
| 0.045632 | | | | | | |
| 1 | 5 | 1.2125 | 0.055488 | 12.8067 | 0.20193 | 0.764352 |
| 0.067832 | | | | | | |
| | | | | | | |

The 1st column (optional) is a flag describing whether the residue should be taken into account in the fit or not. (if 0 the residue is not taken included in the error function).

The 2nd column specifies the number of the resiude in the primary sequence.

Columns 3 and 4 represent R_1 and associated uncertainty (in s^{-1}).

Columns 5 and 6 represent R_2 and associated uncertainty (in s^{-1}).

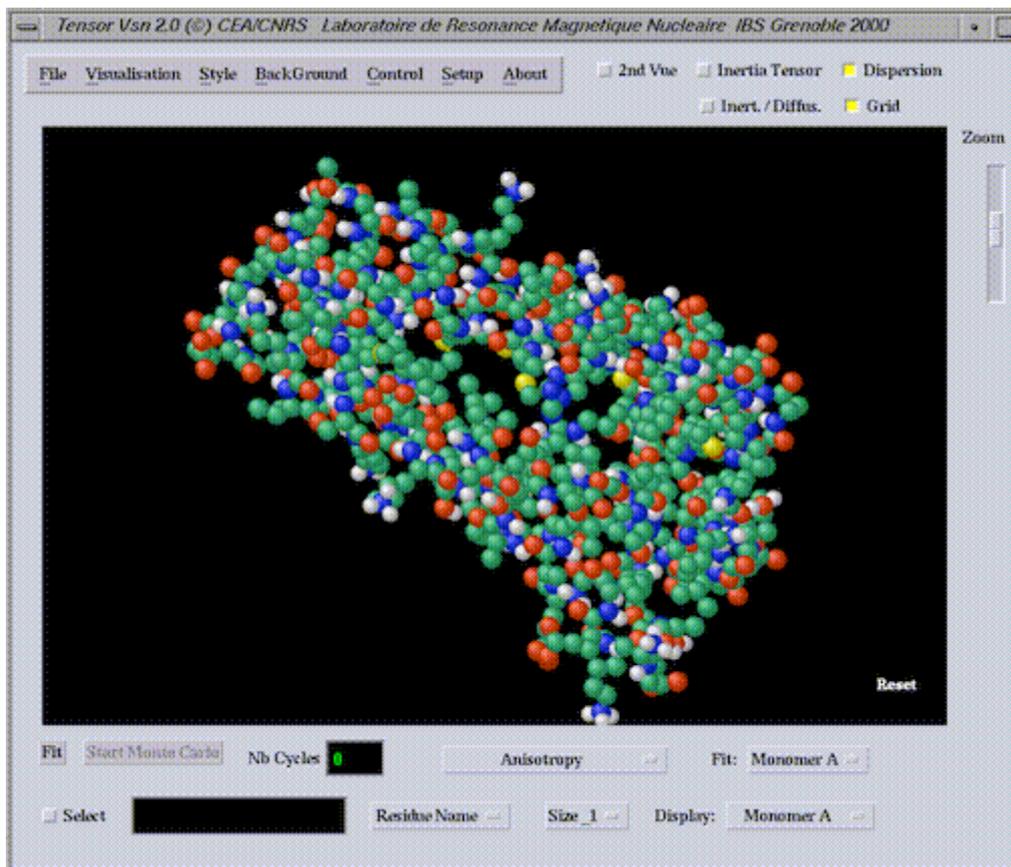
Columns 7 and 8 represent the heteronulcear nOe and associated uncertainty (no units) - these are not necessary (and can be left out) if no analysis of internal mobility is to be performed.

Coordinate File :

The coordinate file is a standard .pdb file

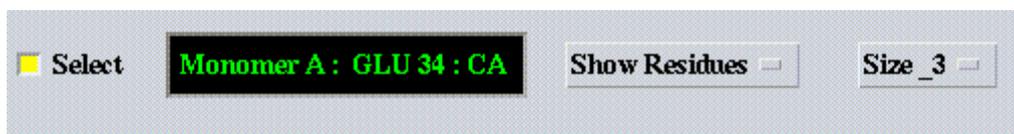
Visualisation Menu.

When the prgram is opened the molecule hree dimensional coordinates of the molecule are displayed, using one of diverse ribbon-type formats, backbone trace, ball-and-stick or stick representations chosen in the **Style** menu Atoms are coloured according to atom type.



The display shows the three-dimensional coordinates of the N atoms from the input file. Note that the molecule can be rotated about x, y and z using the left and (right mouse button or click with the left mouse button while holding down the Shift key). If the molecule is dimeric, either or both monomers can be selected for display using the button on the right.

The individual atoms or residues of the molecule can be identified using the **Select** button bottom left of the display, and the identification mode button (Residue-name, atom-name ...).



In ribbon mode, individual residues can be displayed by clicking on the appropriate part of the ribbon.

Selection of residues to be taken into consideration for fitting overall diffusion/correlation time.

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|
| E | T | N | G | M | G | A | W | N | S | M | I | S | P | N | A | P | I | Y | A | N | A | Q | E | A | G | A | A | N | Y | G | A | A | N | M | G | N | M | A | | | | | | | | | | | | | |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | | | | |
| T | E | Y | A | M | A | I | T | S | T | E | I | H | T | N | A | L | V | A | D | P | E | I | A | N | I | N | A | P | K | G | A | I | I | A | L | A | A | D | | | | | | | | | | | | | |
| 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 |
| A | I | E | I | A | A | I | A | A | M | H | N | I | I | T | N | A | F | K | E | E | W | C | G | G | E | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 101 | 102 | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | | | | | | | | | | | | | | | | | | | | | | |

Using the menu **Visualisation-Definition** the primary sequence will be displayed - from this the user can select the residues to be used in the fitting of the molecular diffusion.

If the user had included a first column in the input-data file, the residues chosen were already highlighted and are those which will be used.

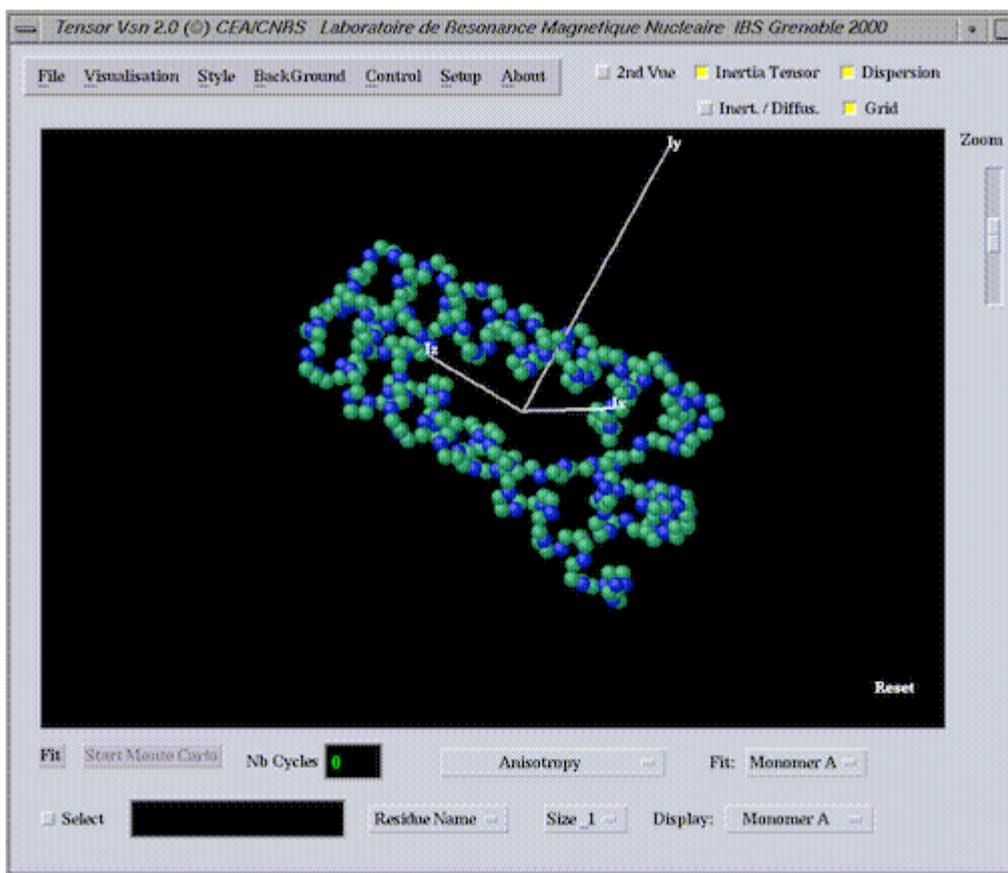
Otherwise to select residues, simply use the cursor and click **Select** or **Unselect**.

The points above the letters signify that there are R1,R2 data available for these residues.

While selecting data to be incorporated into the fit, the user can visualise the molecule by clicking on the button 2nd Display.

Inertia Tensor.

To calculate the inertia tensor, just click on Inertia Tensor :



The principal axes of the inertia tensor are shown in white.

The inertia calculation is performed using the displayed molecular coordinates.

In the case of a dimer this can be either monomer or the complete molecule.

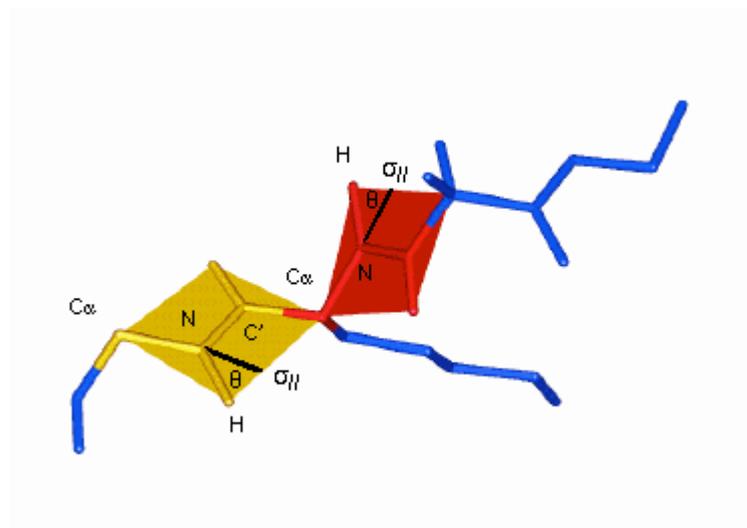
The relative moments are shown in the text window.

Selection of the spectrometer frequency:



The spectrometer frequency can be selected **Spectro** from the menu **Setup**. Select the ^1H frequency using the list. The exact frequency of ^1H et ^{15}N can also be modified manually.

Selection of the angle between csa and dipolar interactions



A common angle θ between the csa and dipolar interactions can be set using the option **Csa** from the menu **Setup**. The default value is 0° , that is the interactions are assumed to be coaxial as in the standard treatment.

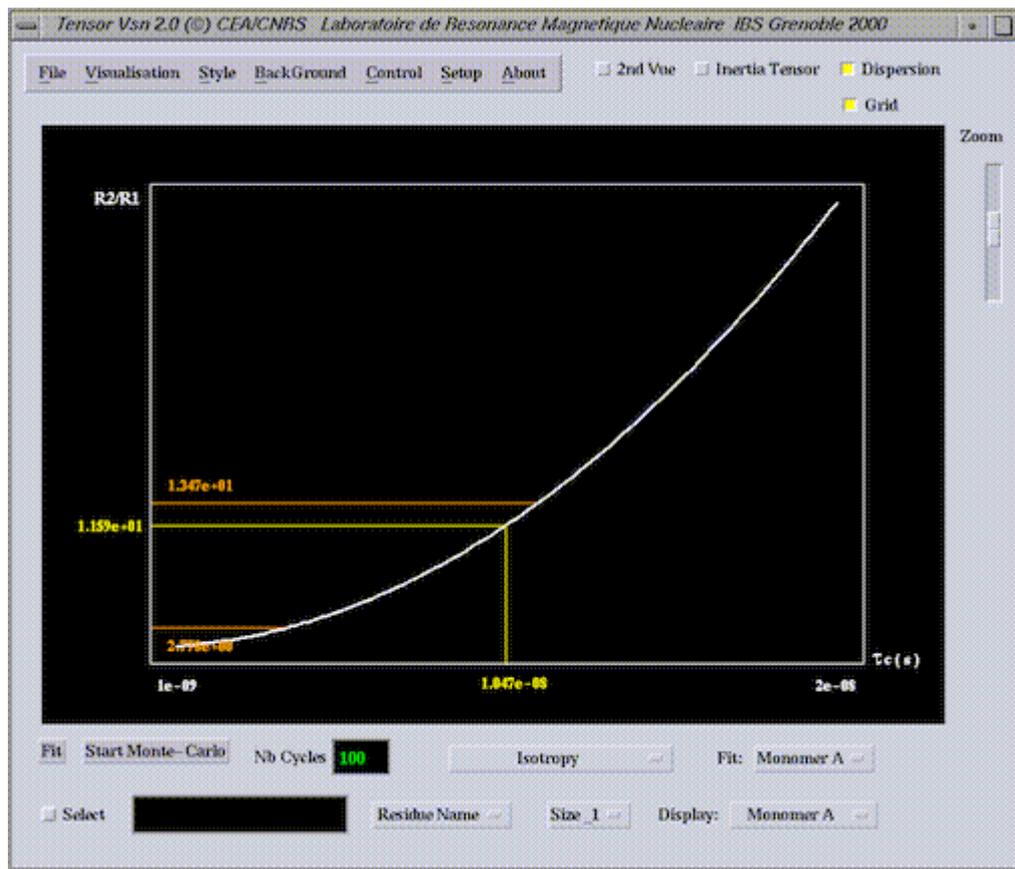
Fit of relaxation data using isotropic tensor.

In this case all directions are assumed equal and the contribution to rotational diffusion is independent of vector orientation. This is simply a fit of the average R_2/R_1 ratio to τ_c for those residues chosen in the [selection procedure](#) performed above.

Simple Fit:

Select **Isotropy** in the menu immediately underneath the main window (options -Anisotropy, Isotropy, Internal Mobility D-Iso, Internal Mobility D-Aniso).
Click on **Fit**.

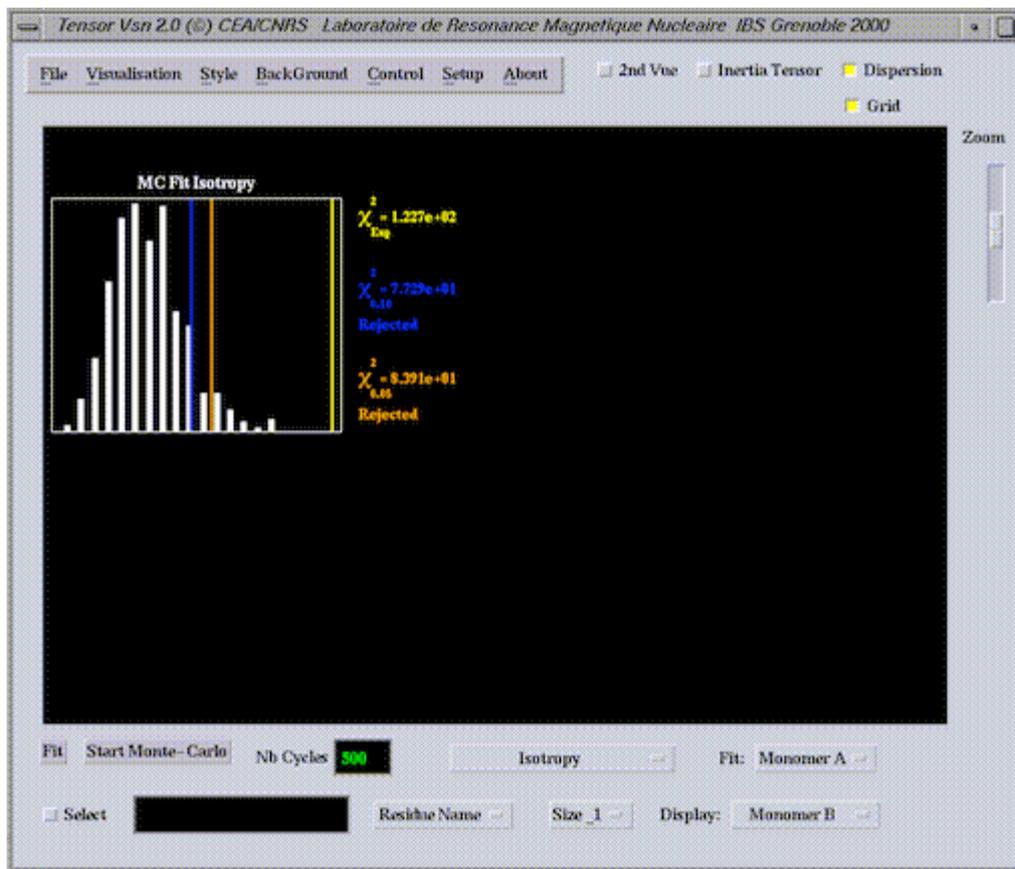
This gives the following display of the average R_2/R_1 ratio with respect to overall correlation time, and the limits of the R_2/R_1 range.



Error Calculation and Confidence limit testing :

Specify the number of simulations for the Monte-Carlo procedure and click on **Monte-Carlo**. This calculation not only estimates the uncertainty in the overall correlation time determined from the selected residues, but also provides a measure of the confidence in the predicted value. The comparison with the simulated χ^2 distribution allows you to decide whether isotropic tumbling is in fact applicable to your molecular system.

In the case shown below such a model is rejected using 95% confidence limits, as the experimental χ^2 (whose value is shown as the yellow line) is much higher than that simulated from the optimal correlation time (95% of simulated χ^2 values are lower than the orange line), assuming the experimental errors describe the random fluctuation of the measured relaxation rates.



If this model is satisfactory, you can now go straight to the **Internal Mobility** analysis, using this correlation time to describe the overall tumbling in the Lipari-Szabo-type model-free analysis;

$$J(\omega) = S_2^2 \left\{ S_1^2 \frac{\tau_c}{1 + (\omega\tau_c)^2} + (1 - S_1^2) \frac{\tau'}{1 + (\omega\tau')^2} \right\}$$

To do this simply click on the button currently displaying **Isotropy**, (immediately underneath the main window (options -Anisotropy, Isotropy, Internal Mobility D-Iso, Internal Mobility D-Aniso)) and select **Internal-Mobility-D-Iso**.

Fit of relaxation data using Anisotropic tensor.

Simple Fit:

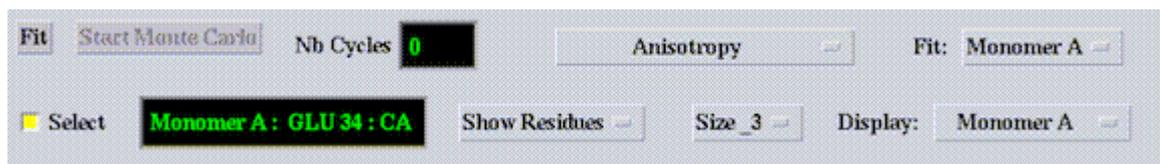
To choose the anisotropic diffusion tensor click on the button immediately underneath the main window (options -Anisotropy, Isotropy, Internal Mobility D-Iso, Internal Mobility D-Aniso)) and select **Anisotropy**.

```

ETNGMGAWNSMIISPNAPIYANAQEAQAANYGAANMGNMA
 2  3  4  5  7  8  9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48
TEYAMAITSTETHTNAALVADPEIANINAPKGAIIALAAD
 49 50 51 52 53 54 55 56 57 58 59 61 62 63 64 65 66 67 68 69 71 72 73 74 75 76 77 78 79 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
AIEIAAIAAMHNIITNAFKEEWCGGE
101 102 103 104 105 106 107 108 109 111 112 113 115 116 117 119 120 121 122 123 124 125 126 127 128 129

```

Again the residues to be taken into consideration for the fit to the overall diffusion tensor can be selected using the interactive graphical selection in **Visualisation-Definition**.



To perform the fit, using the selected data and molecular coordinates, click on **Fit**.

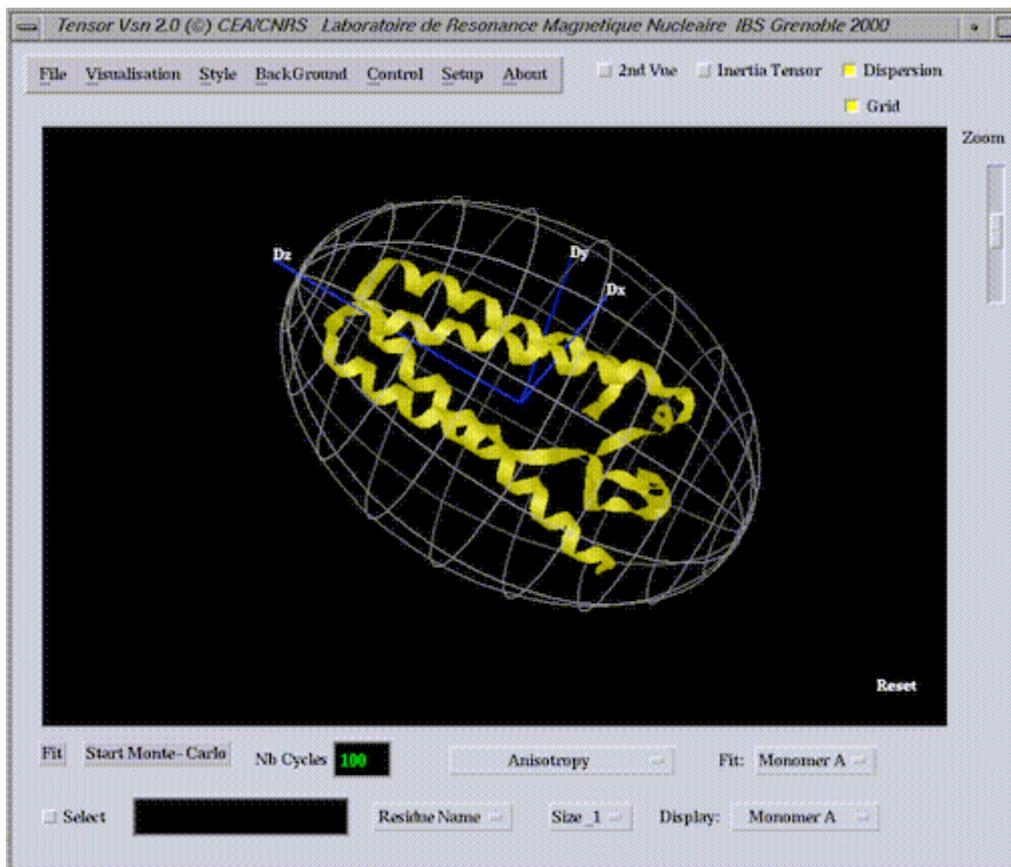
Error Calculation and Confidence limit testing :

Specify the number of simulations for the Monte-Carlo procedure and click on Monte-Carlo.

Visualisation of Result

Diffusion Axis Display

The results of the fit can be visualised by selecting **Diffusion Axis** in the menu **Visualisation**. An example is shown below - Note that the user can again move the molecular orientation using the mouse to inspect the axis orientation. A grid representation can be added to facilitate visualisation of the tensor.

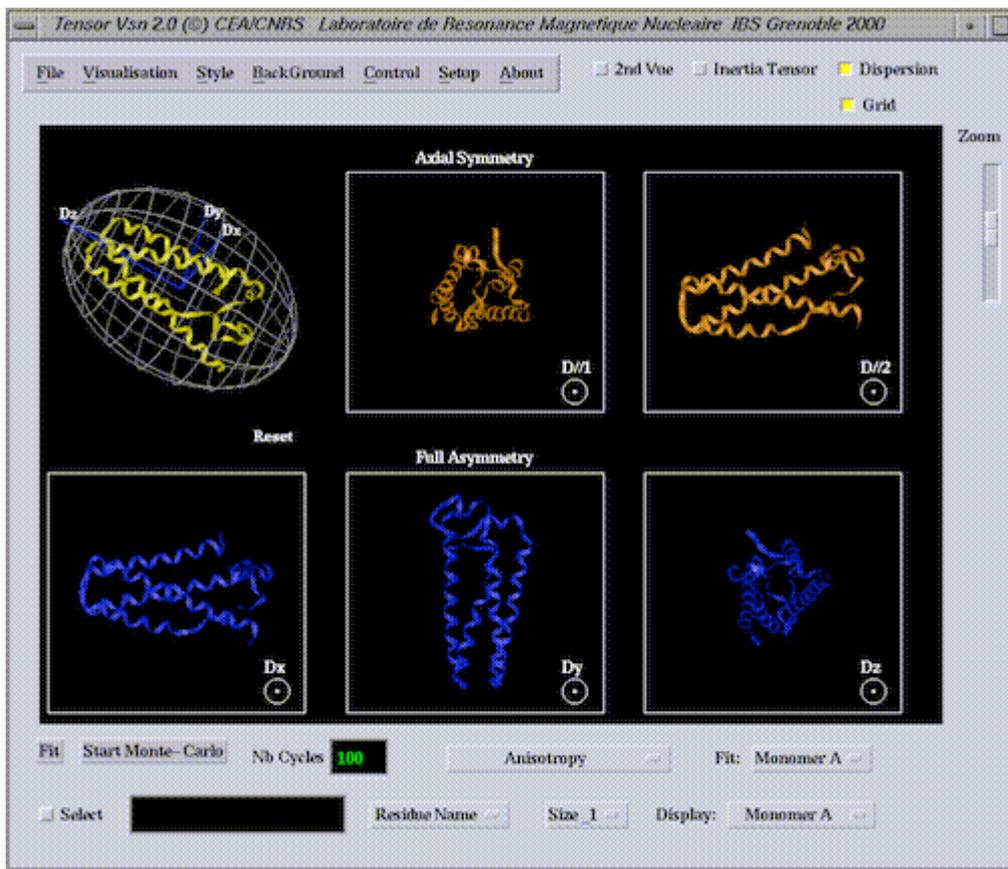


Axis Orientation.

The different orientations can also be visualised using the **Orientation** option in the menu **Visualisation**.

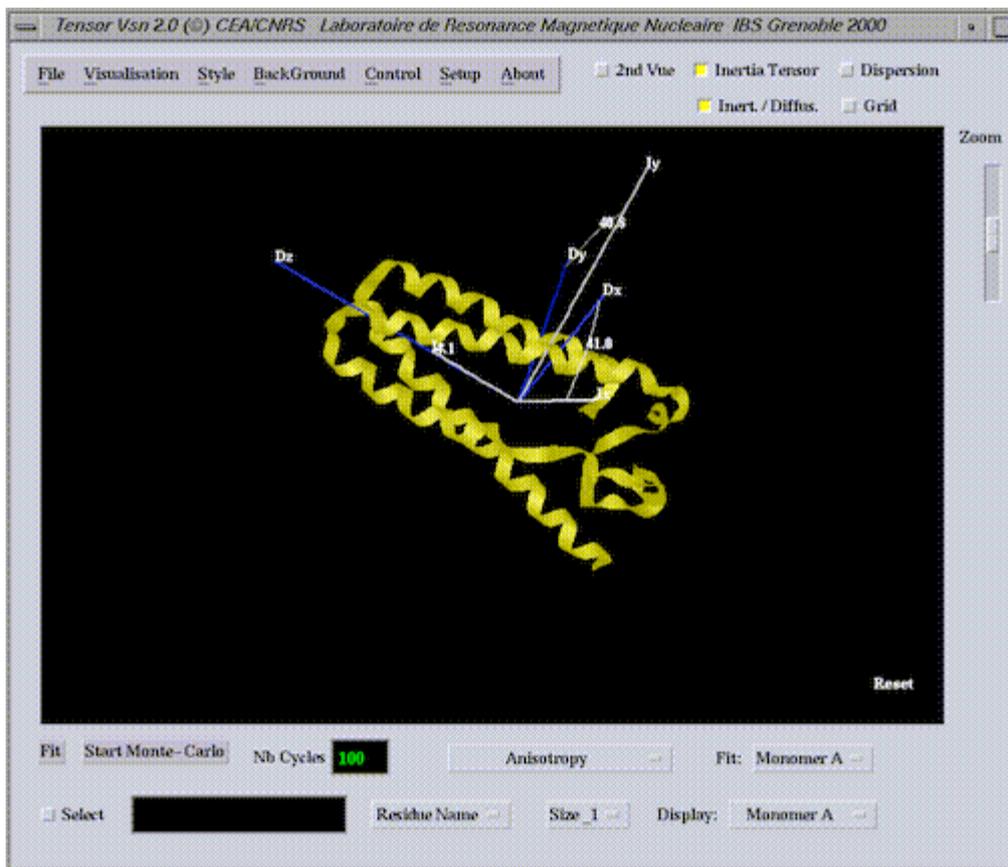
D_{xx} , D_{yy} and D_{zz} are shown in the same representation as above (blue axes).

The unique axes of the prolate and oblate axially symmetric tensors are shown as the z-axis (coming out of the screen) in the centre and right hand panels of the top half of this representation and the molecule is oriented in the bottom panels such that the three axes D_{xx} , D_{yy} and D_{zz} are aligned perpendicular to the screen plane. Not surprisingly in the example shown, two of the axes of the fully anisotropic tensor coincide with the oblate and prolate axially symmetric tensors ([Blackledge et al 1998](#), [Cordier et al 1998](#)).



Comparison of Inertia and Diffusion Tensors.

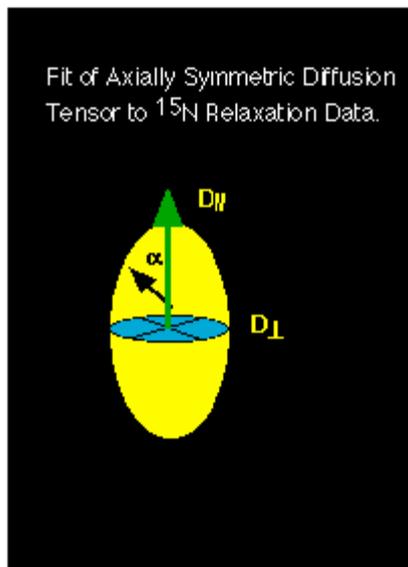
To compare the inertia tensor orientation with the diffusion tensor orientation, click on inert/diff :



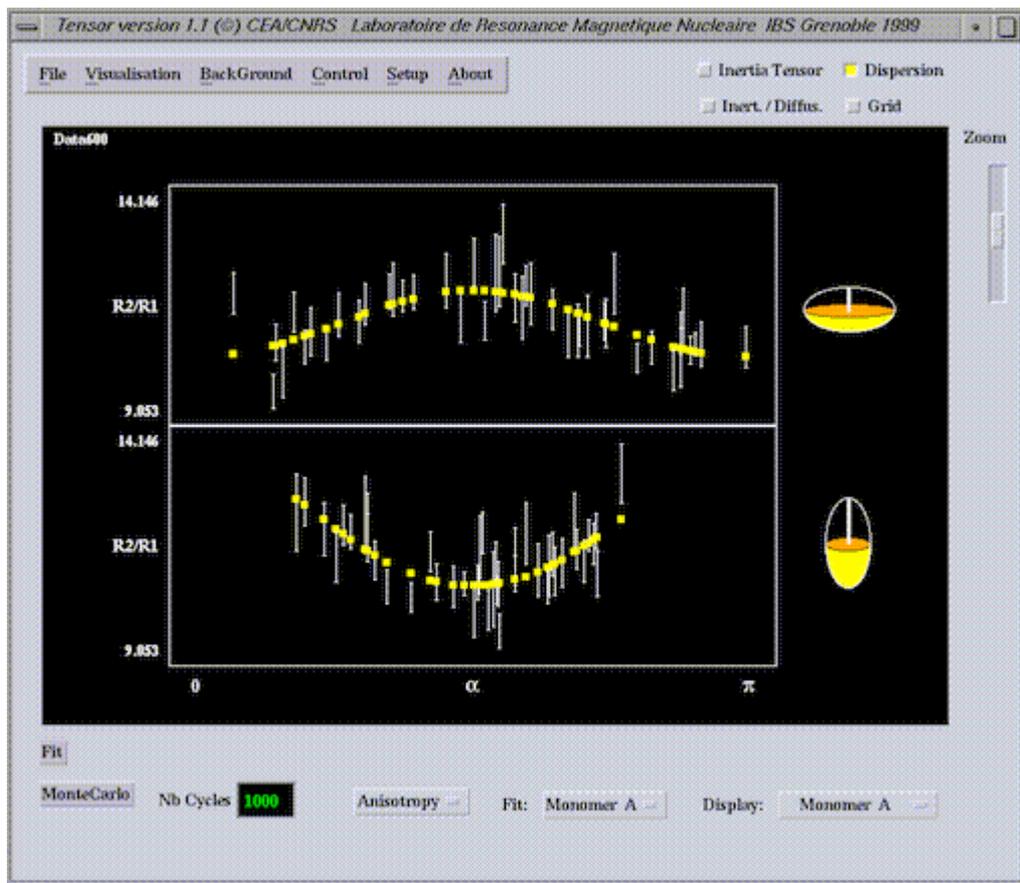
The angles between the respective axes are shown as white arcs, and their values are given in degrees.

Angular Dispersion.

The dispersion of the experimental and calculated relaxation rate ratios can be visualised by selecting **R1/R2Alpha** in the menu **Visualisation**. As shown for the example below, the data (centred on the error bars) can be compared to the optimal dispersion calculated from the best-fit tensor for this model (yellow points). A graphical logo is used to remind the user which of the dispersions is characteristic of an oblate and which of a prolate tensor.



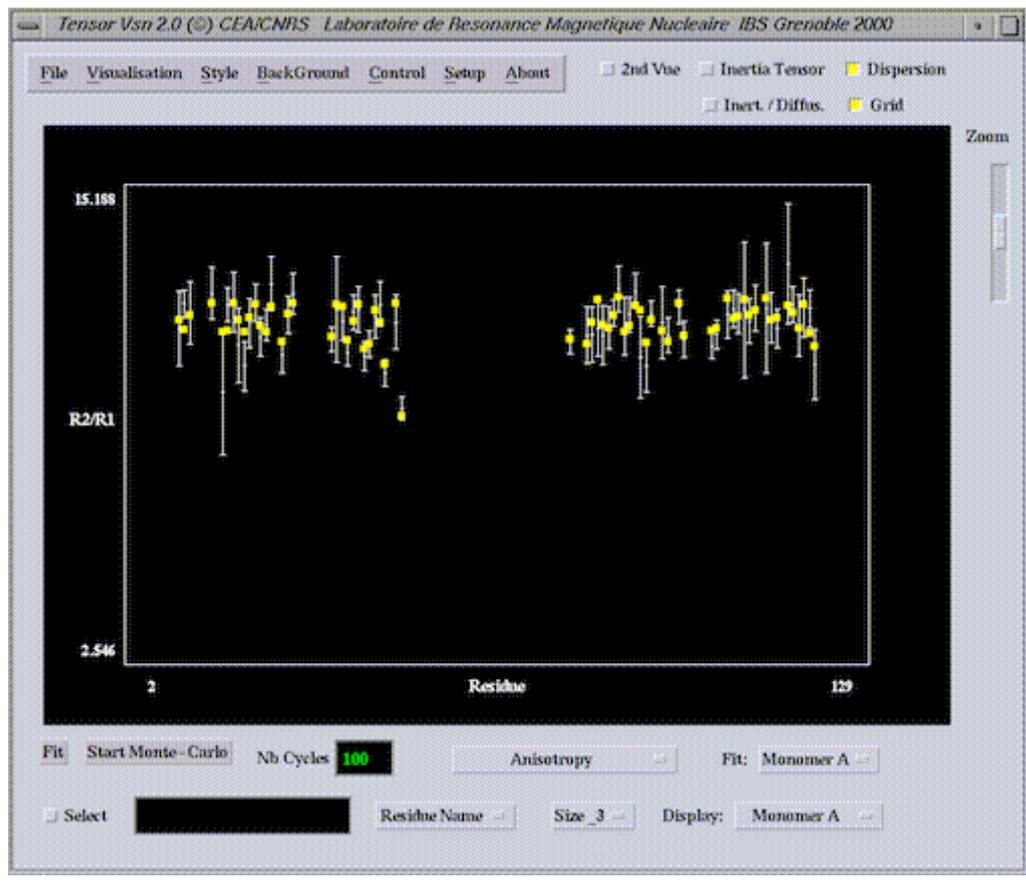
The range of α is shown from 0° to 180° , although orientations of α and $(\pi - \alpha)$ relative to the unique axis of the tensor are of course equivalent.



Fully Asymmetric Model.

The experimental relaxation rate ratios and those expected from a fully asymmetric diffusion tensor can be visualised with respect to the protein sequence by selecting **R1/R2Back** in the menu **Visualisation**.

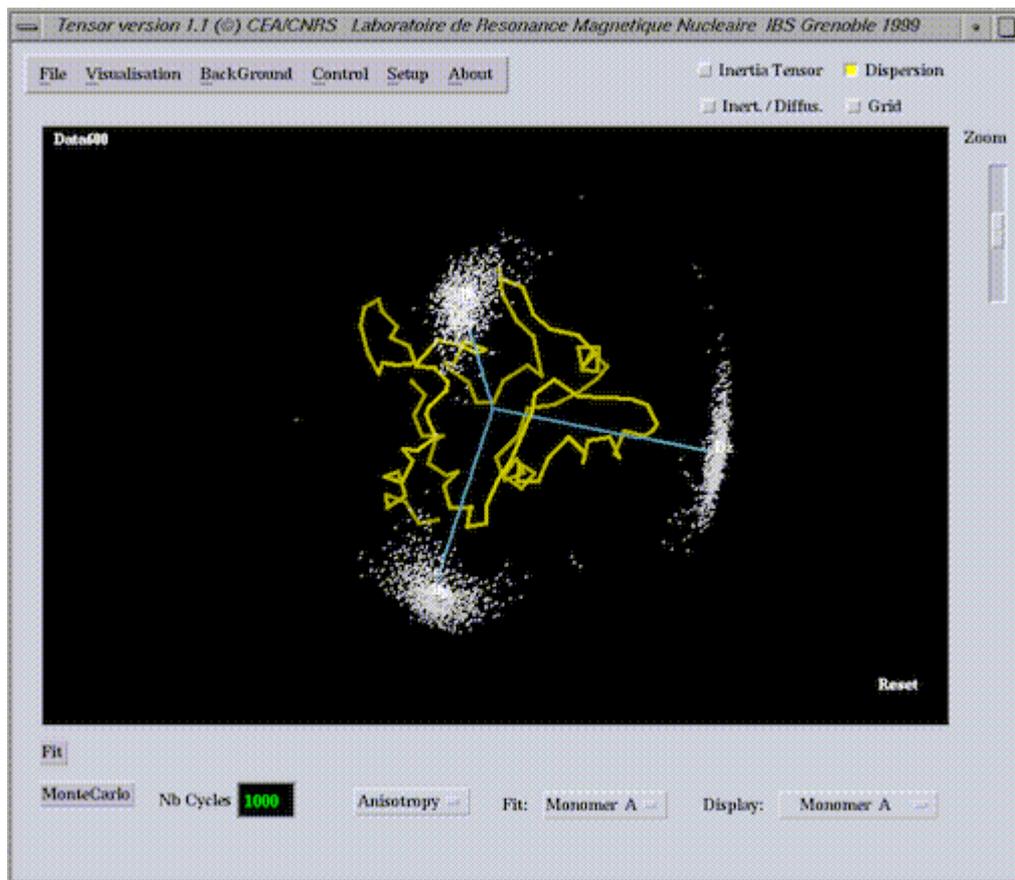
Again the data (centred on the error bars) can be compared to the optimal values calculated from the best-fit tensor for this model (yellow points).



Viewing Dispersion of Diffusion Tensor Axes.

The dispersion in the directions of the axes of the diffusion tensor, due to experimental uncertainty, is calculated directly from Monte Carlo simulations and can be viewed by selecting the **Dispersion** button. This gives an idea of the accuracy available from the relaxation measurements.

The three axis orientations are shown as points around the three optimal axes which are shown as solid lines.

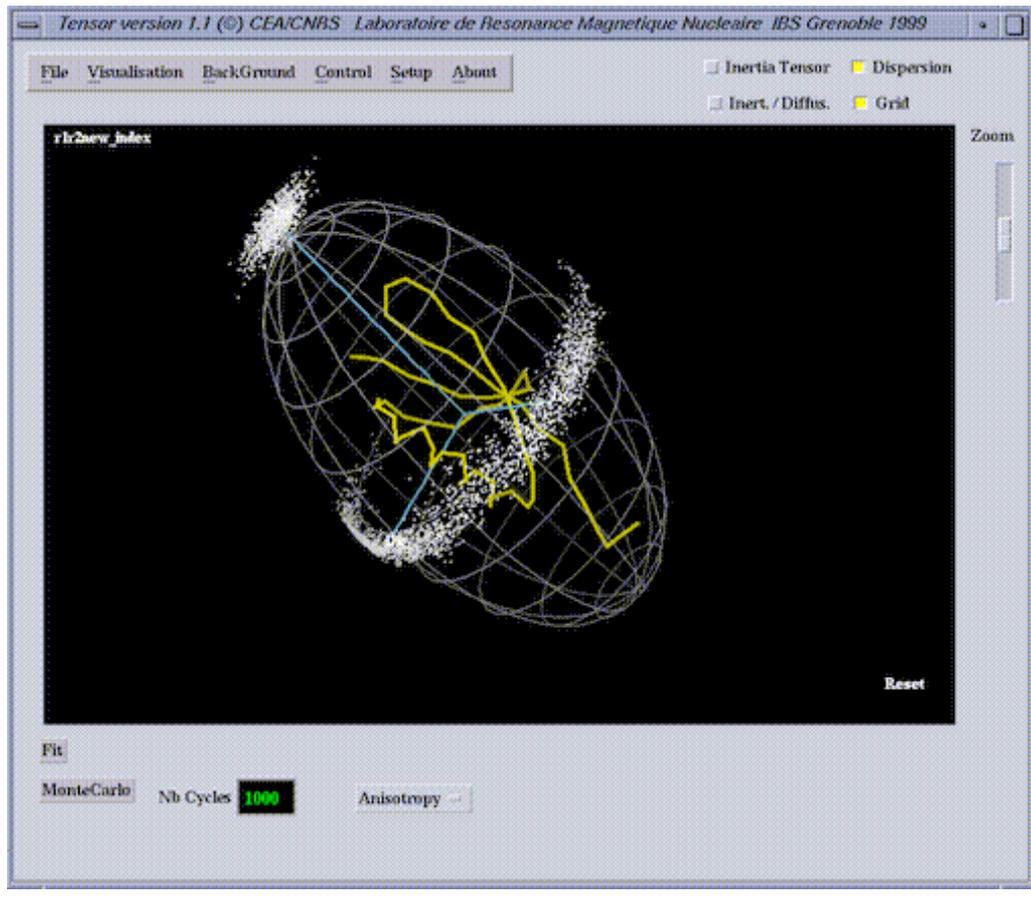


Below is a second example where the tensor is an axially symmetric prolate rotor - note that the perpendicular components are degenerate and their orientation ill-defined, while the direction of the unique axis (D_{zz}) is well defined.

This example shows quite clearly the redundancy of axially symmetric tensorial models - if the tensor is axially symmetric, and there is absolutely no reason why it should be - this will be obvious from the analysis of the dispersion of the tensor components in the plane perpendicular to the unique axes.

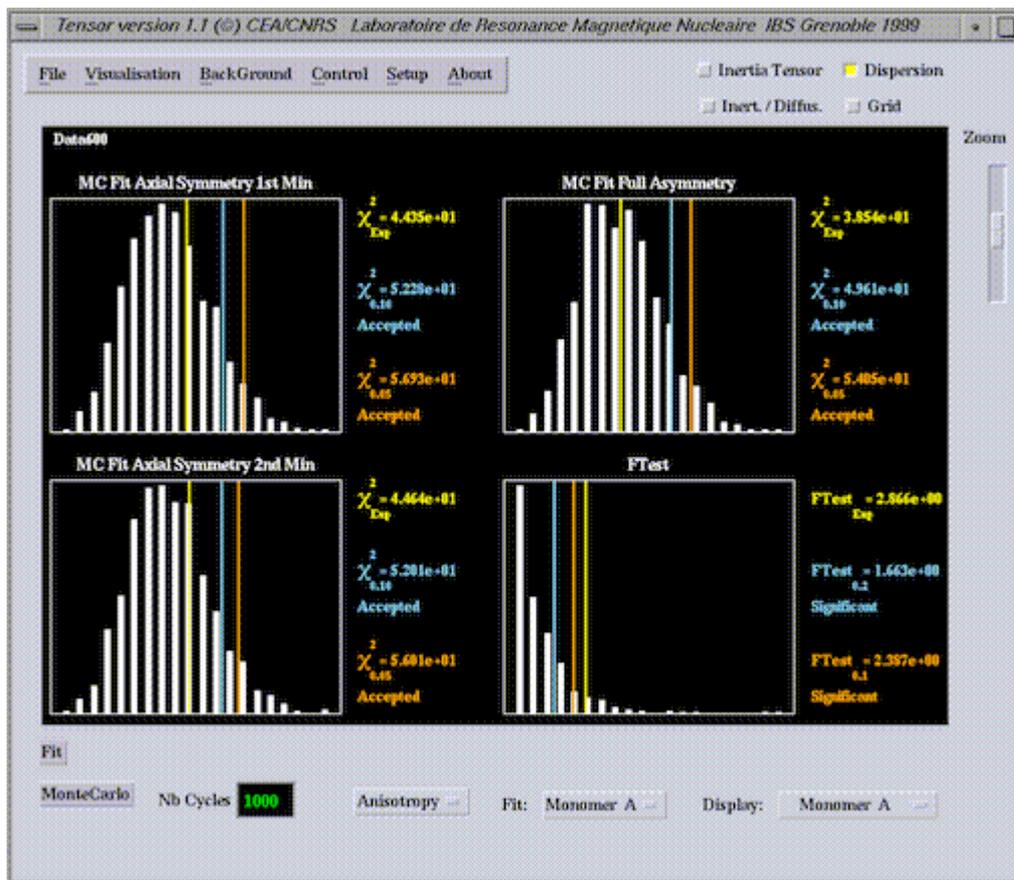
As mentioned in the Theory part of this documentation, we feel that the axially symmetric model is a meaningless approximation, which, due to its dual-definition with two minima for the prolate and oblate approximations, is very difficult to test statistically, and brings no significant improvement to the data analysis.

If in doubt, ask a solid state nmr spectroscopist why she/he doesn't assume CSA tensors to be axially symmetric to simplify their analysis.



Viewing results of Monte-Carlo Simulations :

Nevertheless, to satisfy demand, we retain the analysis using axially symmetric models, for the diffusion tensors. The statistical analysis performed using the Monte-Carlo function can be viewed by selecting **Monte_Carlo** in the menu **Visualisation**.



The results of four calculations are summarised in these plots.

1. The top left-hand figure shows the χ^2 distribution for the Monte-Carlo simulations for the first axially symmetric tensorial model (that with the lowest experimental χ^2).
2. The bottom left-hand figure shows the χ^2 distribution for the Monte-Carlo simulations for the second axially symmetric tensorial model (that with the higher experimental χ^2).
3. The top right-hand figure shows the χ^2 distribution for the Monte-Carlo simulations for the fully anisotropic tensorial model.

The explicitly simulated χ^2 distributions are compared to the experimental fit and summarised using the following code :

yellow : experimental χ^2

blue : χ^2 (0.10)

orange : χ^2 (0.05)

If the experimental χ^2 is higher than the simulated limits then the model cannot be used to

explain the experimental data with acceptable confidence.

- The bottom right-hand figure shows the comparison of simulated [F-values](#) (datasets from the best four-parameter fit fitted with 4 and 6 parameters to calculate the random statistical improvement due to the more complex fit) and the experimental improvement.

In this case -

yellow : experimental F

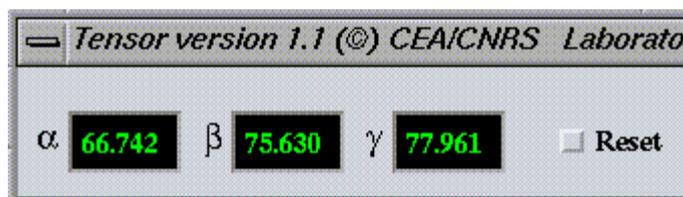
blue : F (0.20)

orange : F (0.10)

If the experimental F is higher than the simulated limits then the improved fit found when using the more complex fit is above the random expected level and can be used with acceptable confidence.

Specific Rotation.

Any Euler rotation of the diffusion tensor can be applied by entering specific euler angles using the menu Control - Rotation as shown -



Internal Mobility

Spectral density functions

Once the overall diffusion has been determined, the program will perform a rigorous [Lipari-Szabo](#) type analysis of the internal mobility of the molecule using either the

isotropic tensor as determined from the average R_2/R_1 ratio with the spectral density function of the form -

$$J(\omega) = S_1^2 \left\{ S_1^2 \frac{\tau_c}{1 + (\omega\tau_c)^2} + (1 - S_1^2) \frac{\tau'}{1 + (\omega\tau')^2} \right\}$$

or using the anisotropic diffusion tensor resulting from the above analysis, with the analogous spectral density function -

$$J(\omega) \approx S^2 \sum_j \frac{A_j \tau_j}{1 + (\omega\tau_j)^2} + (1 - S^2) \frac{\tau'}{1 + (\omega\tau')^2}$$

where A_j are functions of the orientation of the vector to be analysed and τ_j are defined by D_{xx}, D_{yy} and D_{zz} .

Select Internal Mobility Mode

To select the Internal Mobility mode of TENSOR2 click on the menu immediately underneath the main window (options -Anisotropy, Isotropy, Internal Mobility D-Iso, Internal Mobility D-Aniso) and select **Internal Mobility D-Iso** or **Internal Mobility D-Aniso**.

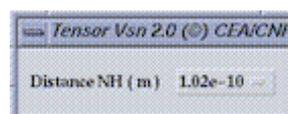
Selection of sites to be fitted.



Using the menu **Definition** the user can again select residues to be treated (this will often be all the molecule) by using the cursor to define the regions of the primary sequence to be included in the analysis.

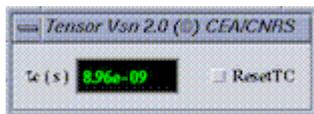
Selection of Internuclear Distance NH.

Our analysis of internal mobility assumes co-axial NH and axially symmetric csa tensor. the user can choose the internuclear distance from the menu **Control - DistanceNH**, from 1.01 to 1.05Å.



Selection of Specific Tau-c.

The user can define a tau-c, different from the one fitted using the average R2/R1, from the menu **Control - Tc** (in units of seconds).



Available models.

| Dynamic Parameters | |
|--------------------|--------------------------------|
| Model 1 | s^2 |
| Model 2 | s^2, τ_i |
| Model 3 | s^2, R_{exch} |
| Model 4 | $s^2, \tau_i, R_{\text{exch}}$ |
| Model 5 | s^2, τ_i, τ_s |

The program will then test the models (1-5) shown above, (see [theory](#)) against the experimental $\{R1, R2, nOe\}$ for each residue selected. Once the data can be reproduced satisfactorily from one of the models (as determined from Monte-Carlo simulations) the program selects this model and then goes onto the next residue. If one of the models (2 or 3) does not improve the fit enough to satisfy the F-test ($\alpha=0.2$), then the previously failed, but marginal, model 1 is accepted.

If none of the models can reproduce the data satisfactorily the program will assign model 6 - this means that neither models 4 or 5 {3 parameters for 3 measureables} was sufficiently close to a 0 χ^2 for the model to be acceptable. The mobility parameters for the best of these models are nevertheless given in the final table.

Number of simulations to be performed for the Monte-Carlo analysis of Internal Mobility.

This is controlled by entering the required number (per model per residue, so this governs how long the calculation will take) in the window **Nb Cycles**.

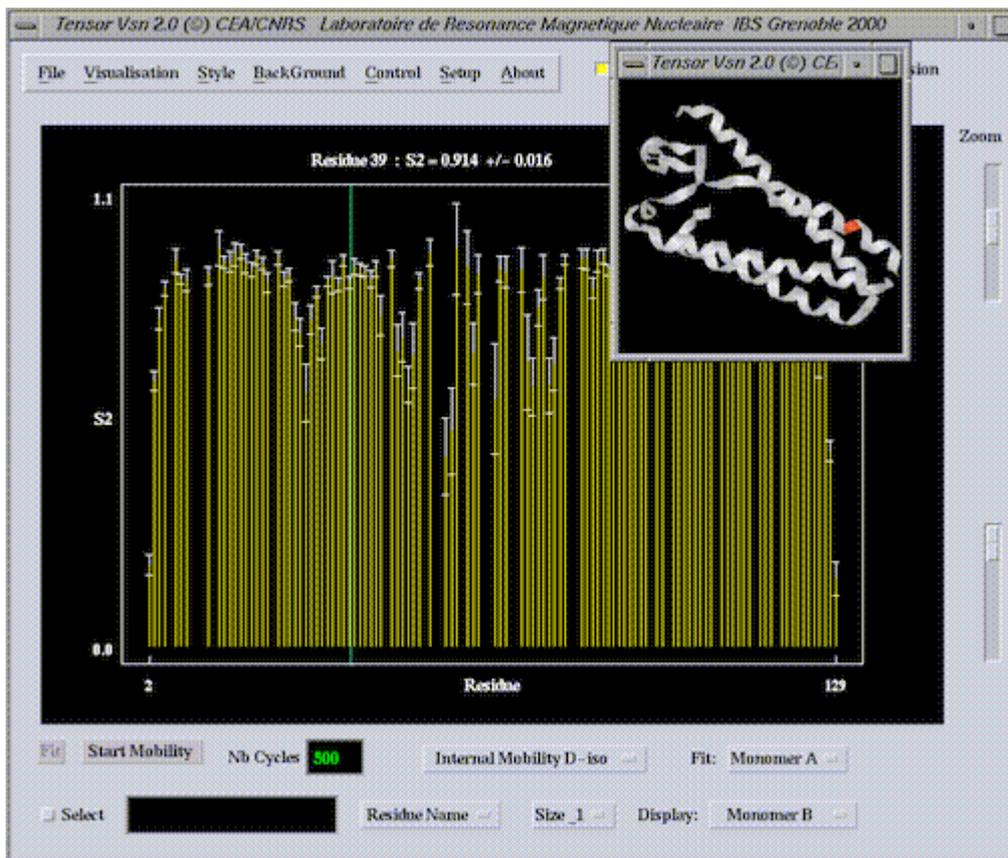
Starting the Calculation.

Once these parameters have been set the calculation can be started using the button **Start-Mobility**.

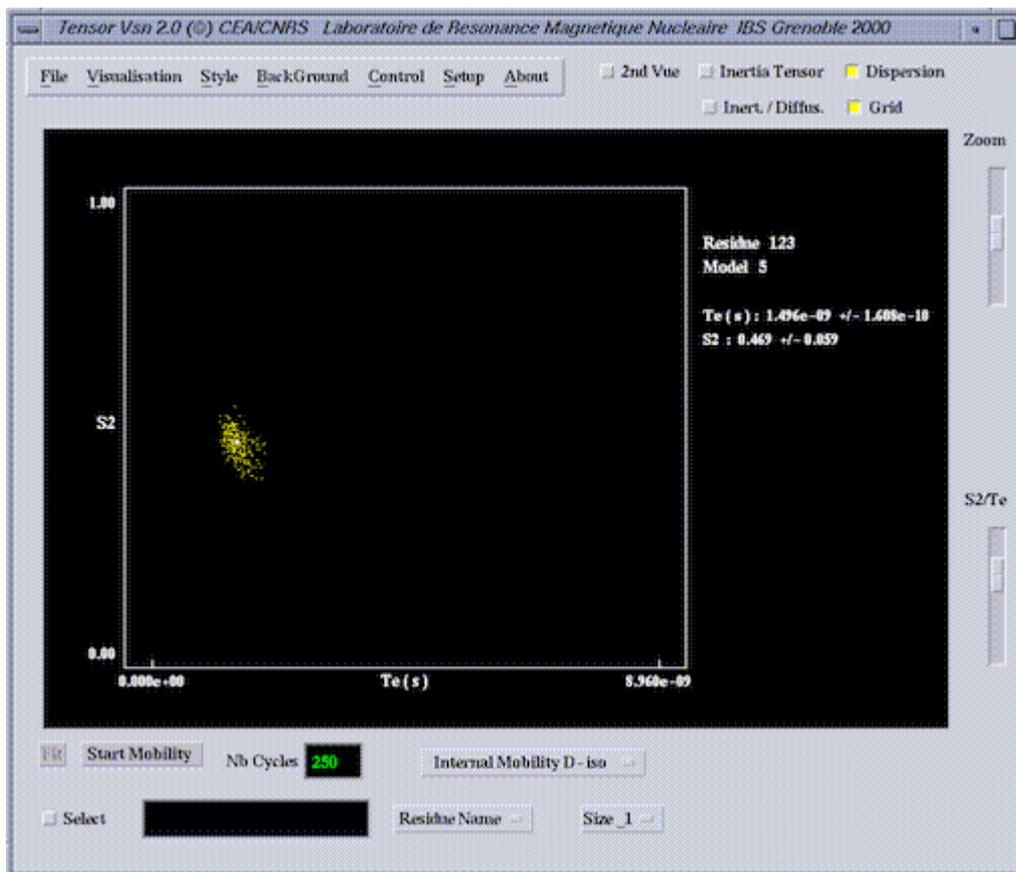
Graphical Representation of the Internal mobility Parameters.



Once the calculation is finished, the user can inspect the fits graphically from the menu **Visualisation - Mobility**. This gives bar charts of all of the internal mobility parameters and the optimal models for each dataset.

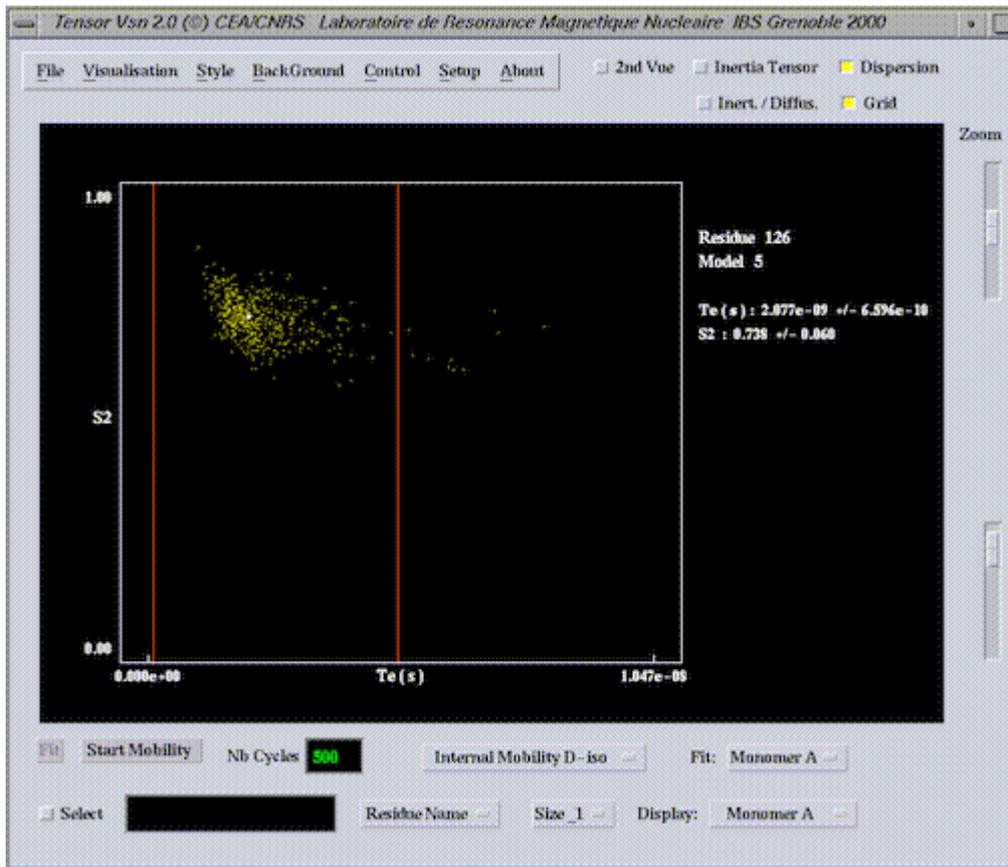


Each of these can be inspected in detail using the **Visualisation-Mobility** menu, and the position of the cursor in the primary sequence can be highlighted using the **2nd View** option.

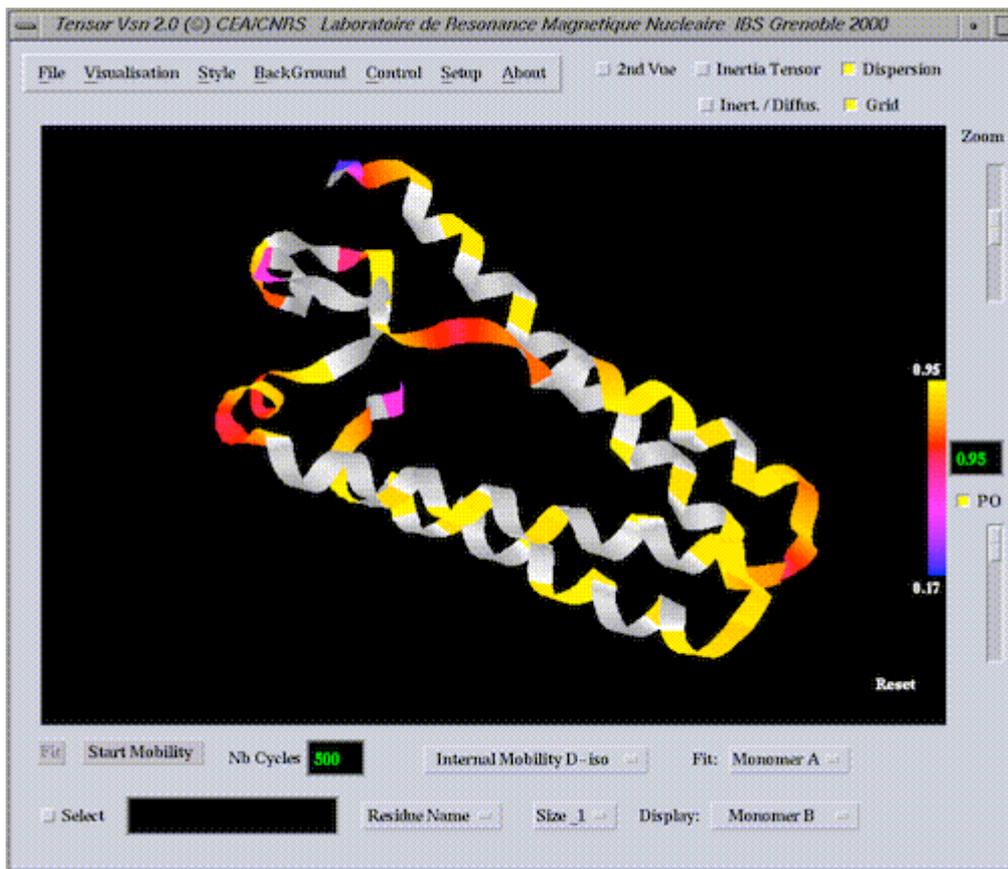


Inspection of $\{S^2, \tau_i\}$ parametric space.

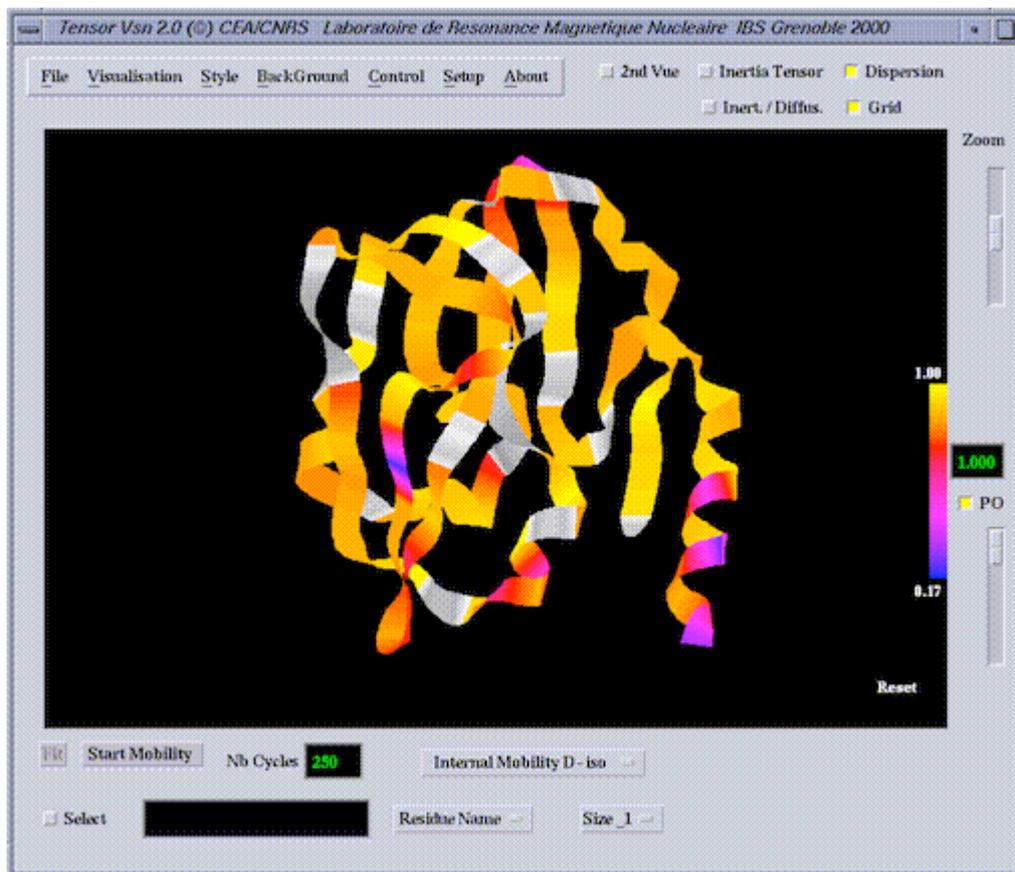
For those sites fitted using a model $\{2,4,5\}$ that is with $\{S^2, \tau_i\}$, the error function samples by the Monte-Carlo simulations can be inspected to check for anomalies, out-liers or double mimima (can be a problem for shorter τ_c). This is again available in the **Visualisation-Mobility** menu (S^2/τ_i). Note that in the case of outliers, the user can cheat, by changing the limits for the calculation of the uncertainty in τ_i by sliding the red-error limits from each side of the window - use with care!



Superposition of Internal Mobility Parameters onto the Protein Ribbon/Backbone.



Using the **Visualisation View-Molecule** menu, the user can colour the different ball-and-stick, stick, backbone or ribbon representations of the molecule using the order parameter as a scale to visualise differential mobility.



Output Coordinate Files

Write coordinate file in Inertial Frame (menu File -> Save PDB file in Inertial Frame)

The coordinates of the displayed molecule can be written in the inertial frame (Cartesian x,y and z are aligned along Ix, Iy and Iz) using the File menu .

Write coordinate file in Diffusion Frame (menu File -> Save PDB file in Diffusion Frame)

The coordinates of the displayed molecule can also be written in the diffusion frame (Cartesian x,y and z are aligned along Dxx, Dyy and Dzz) using the File menu .

Postscript Output

Postscript files of each of the available displays can be saved or printed using the **Menu : Print-Graph** option.

Output Files - Results

Text files iso.0 and iso.1 and [resaniso.0](#) and [resaniso.1](#) containing details of the different fits and

Monte Carlo simulations can be saved or printed using the **Menu : Print-Result** option.

resaniso.0 (results of the fits, error calculations and validity tests):

Full Asymmetry :

| Chi2 | Alpha | Beta | Gamma | Dx | Dy | Dz |
|-------------|---------------|-------------|--------------|------------|------------|-----------|
| 3.8545e+01 | -> 6.6733e+01 | 7.5621e+01 | 7.7959e+01 | 1.4079e-01 | 1.5660e-01 | |
| 1.8301e-01 | | | | | | |

Axial Symmetry (1st min) :

| Chi2 | Phi | Teta | DPer | Dpar |
|-------------|----------------|-------------|-------------|-------------|
| 4.4357e+01 | -> -2.9704e+01 | 2.0094e+01 | 1.6992e-01 | 1.4323e-01 |

Axial Symmetry (2nd min) :

| Chi2 | Phi | Teta | DPer | Dpar |
|-------------|---------------|-------------|-------------|-------------|
| 4.4646e+01 | -> 7.8328e+01 | 7.2317e+01 | 1.4410e-01 | 1.9258e-01 |

Number of simulations : 500

Axial Symmetry 1st Minimum

Phi(deg.) : -29.704 +- 4.770
Teta(deg.) : 20.094 +- 10.735
Dper : 1.699e-01 +- 1.101e-03
Dpar : 1.432e-01 +- 2.292e-03

Chi2_Exp : 4.4357e+01
Chi2_Mc (0.10) : 5.0664e+01
Chi2_Mc (0.05) : 5.3855e+01

0.10 Chi2 Montecarlo Aniso. Ax. (5.0664e+01) > Chi2exp (4.4357e+01) :
--> **Accepted Model**

0.05 Chi2 Montecarlo Aniso. Ax. (5.3855e+01) > Chi2exp (4.4357e+01) :
--> **Accepted Model**

Axial Symmetry 2nd Minimum

Phi(deg.) : 78.329 +- 3.001
Teta(deg.) : 72.316 +- 3.893
Dper : 1.441e-01 +- 2.309e-03
Dpar : 1.926e-01 +- 3.808e-03

Chi2_Exp : 4.4646e+01
Chi2_Mc (0.10) : 5.0941e+01
Chi2_Mc (0.05) : 5.3802e+01

0.10 Chi2 Montecarlo Aniso. Ax. (5.0941e+01) > Chi2exp (4.4646e+01) :
--> **Accepted Model**

0.05 Chi2 Montecarlo Aniso. Ax. (5.3802e+01) > Chi2exp (4.4646e+01) :
--> **Accepted Model**

Full Asymmetry

Alpha(deg.) : 66.733 +- 8.861
Beta(deg.) : 75.621 +- 3.526
Gamma(deg.) : 77.959 +- 9.020
Dx : 1.408e-01 +- 2.698e-03
Dy : 1.566e-01 +- 4.878e-03
Dz : 1.830e-01 +- 4.947e-03

Chi2_Exp : 3.8545e+01
Chi2_Mc (0.10) : 4.8427e+01
Chi2_Mc (0.05) : 5.2107e+01

0.10 Chi2 Montecarlo Aniso. Tot. (4.8427e+01) > Chi2exp (3.8545e+01) :
--> **Accepted Model**

0.05 Chi2 Montecarlo Aniso. Tot. (5.2107e+01) > Chi2exp (3.8545e+01) :
--> **Accepted Model**

Fexp = 2.8646e+00 (**P** = 6.9377e-02)

Fexp > 0.2 FTest (1.7549e+00) : **Significant Improvement**

Fexp > 0.1 FTest (2.5394e+00) : **Significant Improvement**

resaniso.1 (Resultats of Monte-Carlo simulations) :

Monte_carlo Axial Symmetry 1st min ...

| | Chi2 | Phi | Teta | DPer | Dpar |
|-------|-------------------------|----------------|-------------|-------------|-------------|
| 0 | McTotal4_1 : 3.0864e+01 | -> -3.8505e+01 | 4.7903e+01 | 1.7136e-01 | 1.3968e-01 |
| 1 | McTotal4_1 : 3.4538e+01 | -> -2.3209e+01 | 8.1993e+00 | 1.7078e-01 | 1.4047e-01 |
| 2 | McTotal4_1 : 3.4669e+01 | -> -2.9521e+01 | 1.2921e+01 | 1.6937e-01 | 1.4394e-01 |
| 3 | McTotal4_1 : 3.5939e+01 | -> -3.4292e+01 | 2.5437e+01 | 1.7108e-01 | 1.4188e-01 |
| | | | | | |

Monte_carlo Axial Symmetry 2nd min ...

| | Chi2 | Phi | Teta | DPer | Dpar |
|-------|-------------------------|---------------|-------------|-------------|-------------|
| 0 | McTotal4_2 : 3.3527e+01 | -> 6.8408e+01 | 7.2778e+01 | 1.4723e-01 | 1.8727e-01 |
| 1 | McTotal4_2 : 3.3805e+01 | -> 7.9317e+01 | 7.2583e+01 | 1.4144e-01 | 1.9637e-01 |
| 2 | McTotal4_2 : 3.4567e+01 | -> 8.2116e+01 | 7.1891e+01 | 1.4027e-01 | 1.9930e-01 |
| 3 | McTotal4_2 : 3.5743e+01 | -> 7.5627e+01 | 7.1596e+01 | 1.4403e-01 | 1.9361e-01 |
| | | | | | |

Monte_carlo Full Asymmetry ...

| Dyy | Chi2 | Alpha | Beta | Gamma | Dxx | |
|------------|-----------------------|---------------|-------------|--------------|------------|------------|
| | Dzz | | | | | |
| 0 | McTotal6 : 2.9449e+01 | -> 6.8753e+01 | 6.3743e+01 | 8.7595e+01 | 1.3859e-01 | 1.6400e-01 |
| 1 | McTotal6 : 3.1338e+01 | -> 6.4532e+01 | 7.8114e+01 | 7.4422e+01 | 1.4017e-01 | 1.4735e-01 |
| 2 | McTotal6 : 3.3729e+01 | -> 7.2315e+01 | 8.0004e+01 | 7.7963e+01 | 1.3652e-01 | 1.5369e-01 |
| 3 | McTotal6 : 3.5382e+01 | -> 6.3496e+01 | 7.2177e+01 | 7.5137e+01 | 1.4078e-01 | 1.5703e-01 |
| 1.8393e-01 | | | | | | |

.....



References.

Woessner, D. E. *J.Chem.Phys.* **1962**, *37*, 647-654.

Blackledge, M.; Cordier, F.; Dosset, P.; Marion, D. *J.Am.Chem.Soc.* **1998**, *120*, 4538-4539.

Cordier, F.; Caffrey, M.; Brutscher, B; Cusanovich, M.; Marion, D.; Blackledge, M. *J.Mol.Biol.* **1998**, *281*, 341-361.

Efficient analysis of macromolecular rotational diffusion from heteronuclear relaxation data. Dosset, P.; Hus, J-C; Blackledge, M.; Marion, D. *J.Biomol.NMR.* **2000**, *16*: 23-28.

Rotational diffusion anisotropy and local backbone dynamics of carbon monoxide bound *Rhodobacter capsulatus* cytochrome c'. Tsan, P.; Hus, J-C; Caffrey, M. ; Marion. D. & Blackledge, M. *J.Am.Chem.Soc.* **2000**, *121*,2311-2312.