Travaux Dirigés : Relaxation

Necessary files :

Blackledge2.pdf
Relaxation_TD1.pdf
Relaxation_TD2.pdf
Relaxation _data.zip

1: We will look at the local dynamics of Calbindin D9k both in the absence and presence of calcium ions.

The file contains R1, R2 and heteronuclear nOe data measured using a 500MHz spectrometer, at 300K on the Calcium bound form of the protein. Look at the data.
For some of the amino acids there is no data. Why might this be?
Do they vary along the sequence?

The physical parameters of motions are the spectral densities of motions at the five frequencies $\omega=0$, ω_N, ω_H, $\omega_H+\omega_N$, $\omega_H-\omega_N$. How can these spectral densities $J(\omega)$ be obtained from the relaxation parameters ? What are the approximations that have to be made ? The three spectral densities $J(0)$, $J(\omega_N)$ and $\langle J(\omega_H)\rangle$ have been estimated from the data at 500MHz, and are given in the file calb_J_500.tbl. From the variations of the three spectral densities along the sequence, give a rough estimation of the time scales of NH bond fluctuations along the protein.

Open the program Tensor2, and the manual (Blackledge2.pdf and Relaxation_TD2.pdf).

Read the data from Calbindin, in the Calcium bound form.
This is called :
calb_ca_500.tbl
Read in the coordinates of the molecule
These are in the file :
1clb_1.pdb
The primary (amino acid) sequence and three dimensional atomic coordinates will be displayed.

Figure 1 can be used to estimate the overall correlation time from the average R2/R1 values in the list (take the values that are preceded by the number 1, rather than 0, these are the values that have least internal motion). Figurie 1 shows the molecular correlation time for rigidly placed relaxation mechanisms present in an isotropically tumbling rotor at 500MHz proton spectrometer frequency. Comparison of the experimentally determined average R2/R1 values will allow an estimate of the overall correlation time. For the tau-c value that appears to be optimal, how do the values of R2 and R1 compare to predicted values for a rigid rotor? Why might they be different?

Tensor2 can also be used to determine an anisotropic overall rotational diffusion tensor, or simply assume an isotropic global rotational diffusion model. In this case we will use the simple model, as Calbindin is not expected to tumble anisotropically.

Determine the overall correlation time using sites present in secondary structural elements, and with a heteronuclear nOe that is not lower than 0.65. Think about why we might select only these sites.

Once the overall rotational correlation time has been estimated, apply a Lipari-Szabo type analysis to the relaxation data.

Look at the average value of the S2 order parameter in the alpha helices. Assuming a model of diffusion in a cone, what is the effective opening angle of a motion of this amplitude?

Identify the most flexible regions.

2. Look at the experimental data for Calbindin in the absence of Calcium (calb_apo_500.tbl). Compare them to the Calcium bound form. Are there any differences?

Perform a first analysis of the data using the reduced density mapping approach as for the calcium bound form. The file containing the spectral densities is calb_J_500.tbl.

Open a second Tensor2 program. Read in the data from Calbindin in the absence of Calcium.

Read in the 3D atomic coordinates again.

Determine the rotational diffusion correlation time for this molecule. Compare this to the Calcium bound form.

Determine the Lipari-Szabo parameters for this form of the protein and compare them to the parameters from the Ca-bound form.

Are there any differences? If so where are they. Do they have any relevance for the differences between the two forms of the protein? (The ion binding loops are 16-24 and 55-62).

Look at the local motional parameters as determined from the Lipari Szabo type approach. Compare the precision of the different parameters. Compare the precision of the different Tau-internal parameters.

Look at the data from amino acids 6, 14 and 40. Use the graphs at the end of this file, showing isocontours of R1, R2 and nOe values plotted onto the S2/tau-e graphs to propose the best fitting solution to the Lipari Szabo analysis. Does this indicate why some parameterisations are well defined, and others less well defined? You one can analyze the error function of the S2/tau-internal analysis by looking in the menu S2/Te. This should confirm the graphical analysis. If it is not clear select residue 14 alone for the internal mobility analysis and use a larger number of Monto Carlo simulations for this residue.

3. Apply the same analyses as above to the Cadmium bound form of the protein.

Calb_Cd_500.tbl / Calb_Cd_J_500.tbl

How do these data differ from the Calcium bound form? How do the Lipari-Szabo parameters differ? Where in the molecule are slower motions now implicated?

4. Read in the data, and molecular coordinates from protein G.
Files : 1P7E.pdb; data_gb_df_500a;
Select the secondary structural elements (helices and sheet) and determine the best fitting isotropic overall correlation time.
Use Tensor2 to determine the local dynamic parameters.
What does this distribution look like?
What appears to be happening in the helix?

Now take the same data points and determine the overall rotational diffusion tensor. You can inspect the quality of the fit to the data by looking at the shape of the tensor compared to the molecule. Compare the orientations of these axes with the orientation of the axes of the inertia tensor of the molecule. Why might these be compared?
Use the Monte-Carlo analysis tool in Tensor2 to determine the uncertainty in the description of the rotational diffusion tensor. What does this tell you about the tensor?

Now use this description of the overall tumbling to perform a hybrid Lipari-Szabo type analysis, where the overall diffusion is accoutned for explicitly in the spectral density function. The internal motion is of course assumed to completely uncoupled to this diffusion.

What do we now see in terms of internal mobility in the helix?

Repeat this analysis using a second data set measured at another of the fields (400,500,600,700 and 800 are available).

How do the results at 800MHz compare with those measured at 500MHz? Are there any differences? Can you think of any possible causes of these differences?

5. Read in the data from low temperature measurements of spin relaxation of protein G (data_GB_273K). These data were also measured at 500MHz. Determine the rotational diffusion tensor using the program Tensor2 (you can also compare the average tau-c using figure 1). Compare with the diffusion tensor determined at 300K. Is this expected? Compare the internal dynamic parameters.

References

Data for this TD were taken from the following articles :

Biochemistry 1993, 32, 9832-9844
Effects of Ion Binding on the Backbone Dynamics of Calbindin D9k Determined by15N NMR Relaxation
Mikael Akke, Nicholas J. Skelton, Johan Kordel, Arthur G. Palmer and Walter J. Chazin

Protein Sci. 2000 9: 1177-1193
The role of backbone conformational heat capacity in protein stability: temperature dependent dynamics of the B1 domain of Streptococcal protein G
MJ Seewald, K Pichumani, C Stowell, BV Tibbals, L Regan and MJ Stone

J. Am. Chem. Soc. 2006, 128, 7855-7870
Variability of the 15N Chemical Shielding Tensors in the B3 Domain of Protein G from 15N Relaxation Measurements at Several Fields. Implications for Backbone Order Parameters
Jennifer B. Hall and David Fushman

Figure 1

Dependence of R1 and R2 and R2/R1 values on molecular correlation time for rigidly placed relaxation mechanisms present in an isotropically tumbling rotor. 500MHz proton spectrometer frequency. Comparison of the experimentally determined average R2/R1 values will allow an estimate of the overall correlation time.

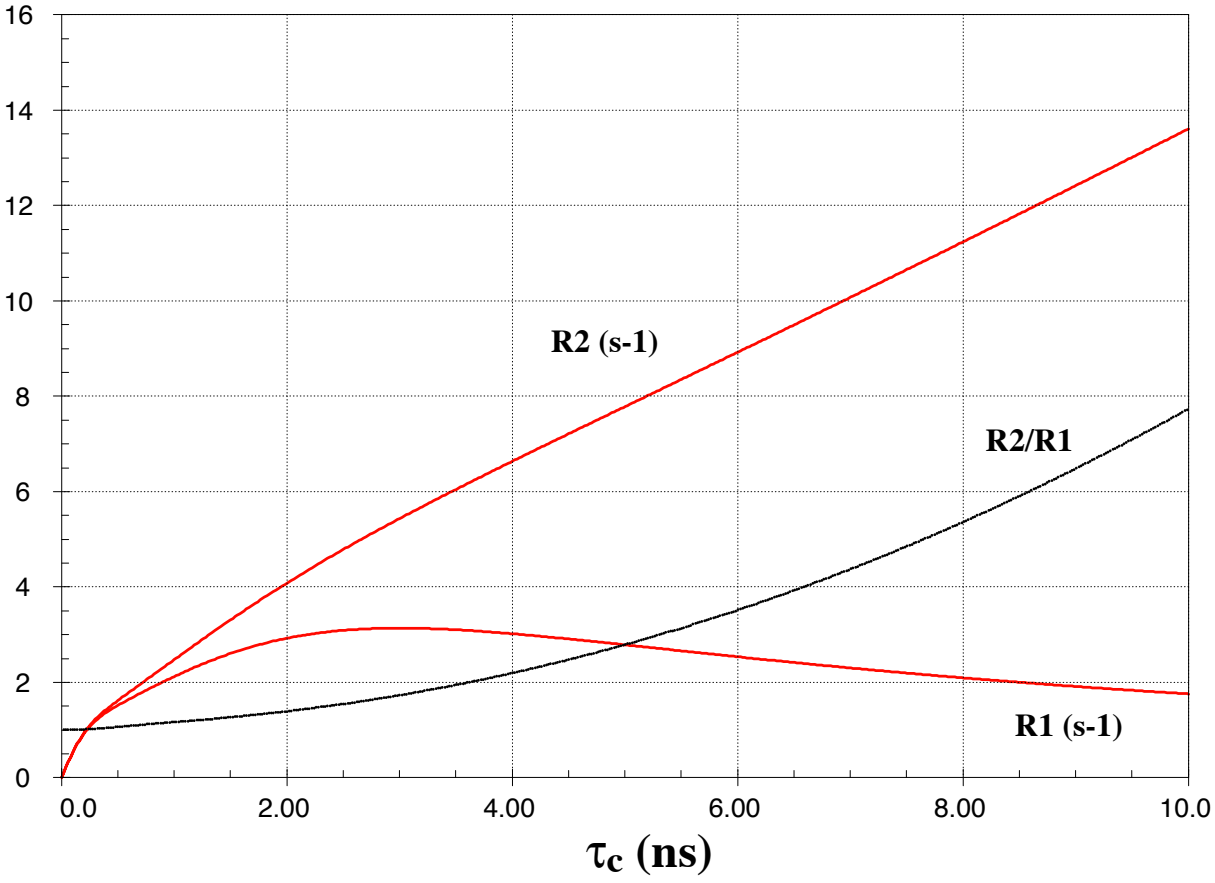

Figure 2

Isocontours of R1, R2 and 15N-1H nOe values for different Lipari-Szabo type motional regimes for a molecule with isotorpic rotational correlation time of 4.22ns, and measurements made at 500MHz proton spectrometer frequency. A relaxation rate can adopt any of the points on a given contour.

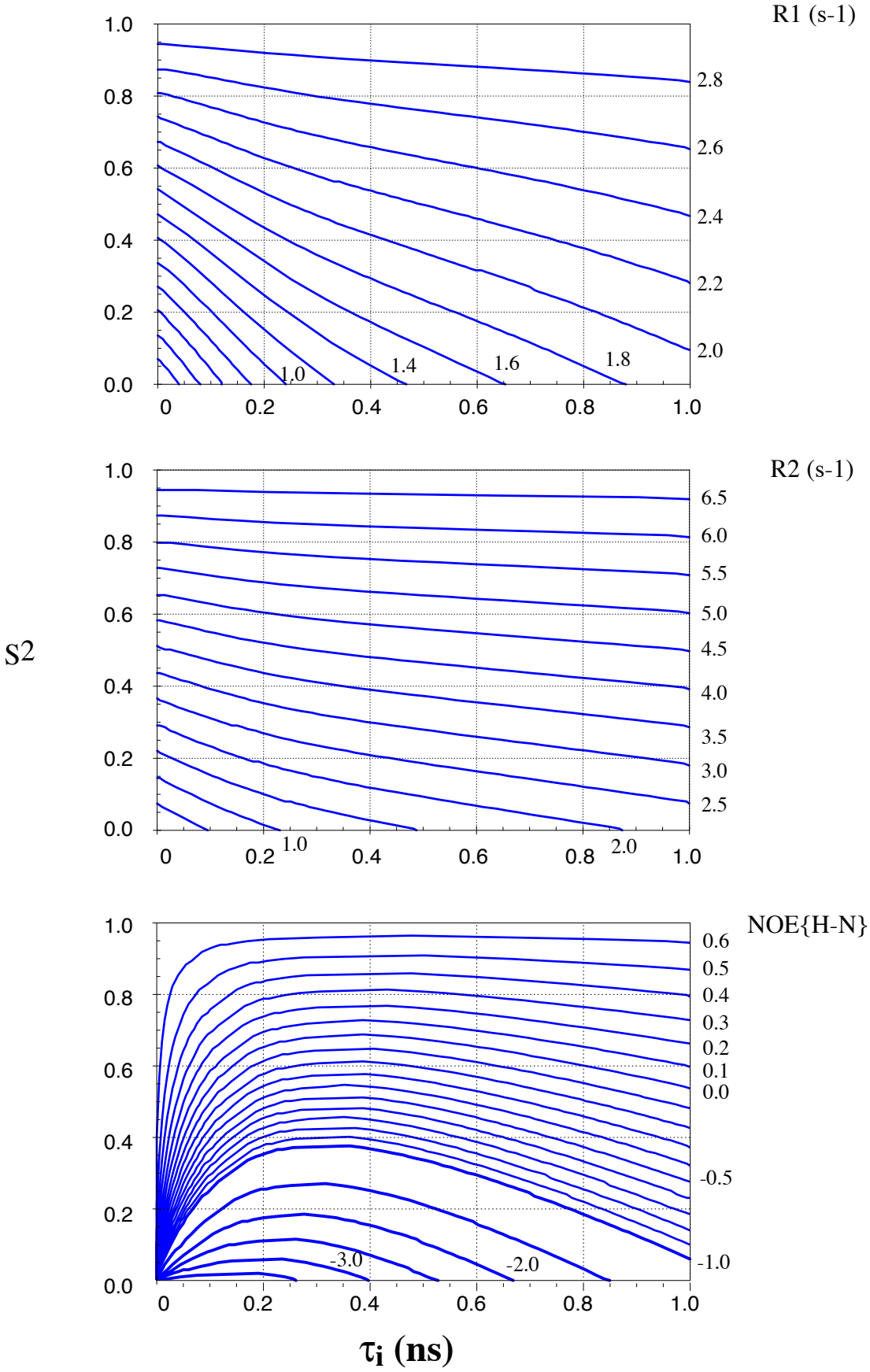

Figure 3

Isocontours of R1, R2 and 15N-1H nOe values for different Lipari-Szabo type motional regimes for a molecule with isotorpic rotational correlation time of 10ns, and measurements made at 500MHz proton spectrometer frequency. A relaxation rate can adopt any of the points on a given contour.

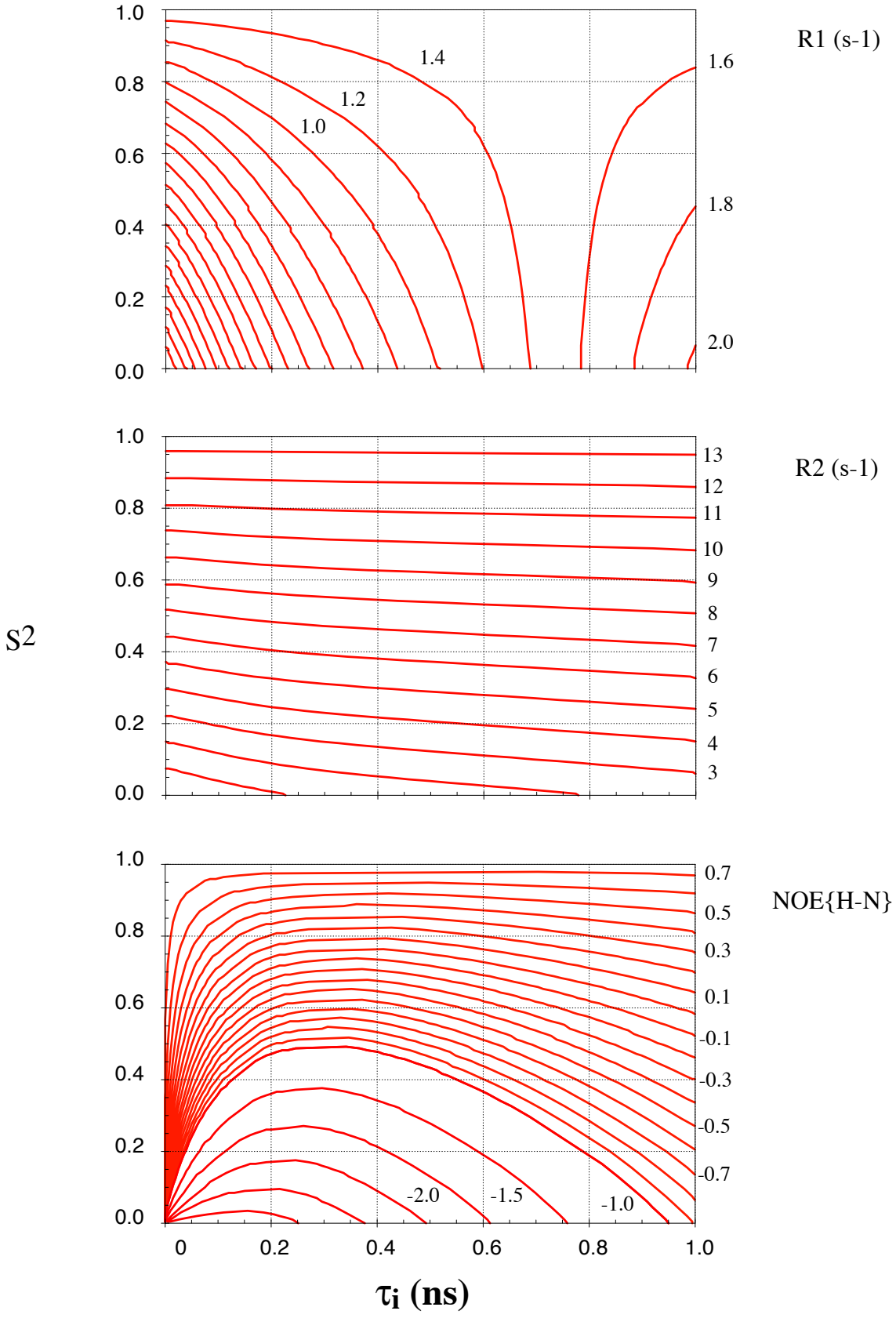

Figure 4

Dependance of R1, R2 and 15N-1H nOe values for different Lipari-Szabo type motional regimes for a molecule with isotropic rotational correlation time of 10ns, and measurements made at 500MHz proton spectrometer frequency.