

Spin Relaxation

BCMB/CHEM 8190

Feb 2, 2005

T_1 , T_2 , NOE (reminder)

T_1 is the time constant for longitudinal relaxation - the process of re-establishing the Boltzmann distribution of the energy level populations of the system following perturbation

T_2 is the time constant for transverse relaxation - loss of phase coherences of the nuclear dipoles in the transverse plane

The Nuclear Overhauser Effect is the change in intensity for a signal (resonance) when the equilibrium spin populations of a different spin are perturbed

What are the origins of T_1 and T_2 relaxation and the Nuclear Overhauser Effect (NOE)?

Key: A fluctuating interaction is capable of causing a transition
-just like an rf pulse.

$$\mathbf{H}(t) = -B_1(t) \gamma \mathbf{I}_x$$

But, $B_1(t)$ is natural in origin (tumbling of molecules)

Some sources of interaction:

- chemical shift anisotropy
- dipole-dipole (nucleus-nucleus or nucleus-electron)
- nuclear quadrupole - electric field gradient
- others....

Chemical Shift Anisotropy (CSA)

Chemical shifts arise from electronic shielding of the nucleus

- shielding depends on orientation of the molecule with respect to B_0
- the orientation dependent chemical shift differences or range is called the CSA
- in solution, rapid reorientation results in averaging of the chemical shift

Rapid molecular reorientation results in local, fluctuating magnetic fields (magnitude and direction)

- these local fluctuating fields lead to energy level transitions, just like applied fields

An Example for CSA Relaxation

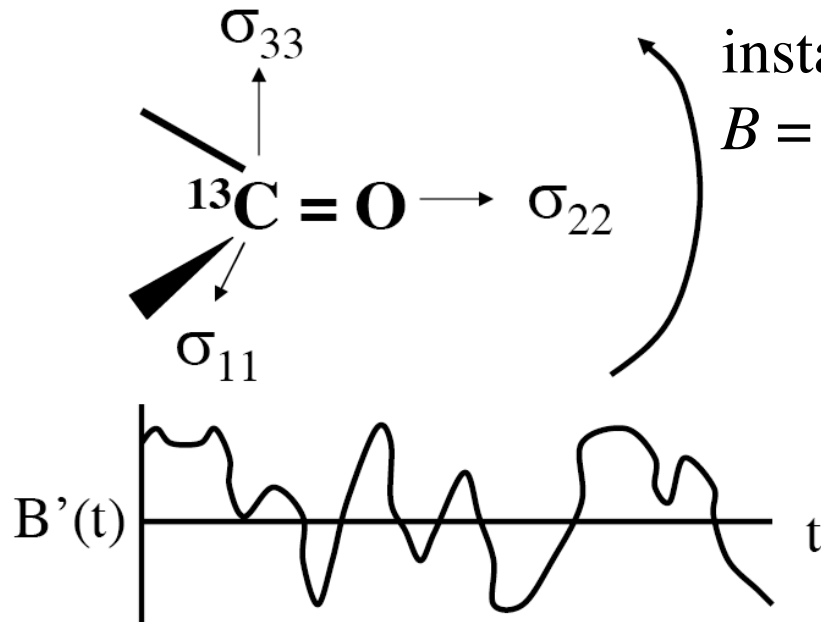
The nuclear shielding can be described by a tensor, σ , relating the induced field to the applied field

-the average (isotropic) shielding

is defined as $\sigma_{\text{iso}} = (\sigma_{11} + \sigma_{22} + \sigma_{33})/3$

$$\begin{bmatrix} \sigma_{11} & \sigma_{12} & \sigma_{13} \\ \sigma_{21} & \sigma_{22} & \sigma_{23} \\ \sigma_{31} & \sigma_{32} & \sigma_{33} \end{bmatrix}$$

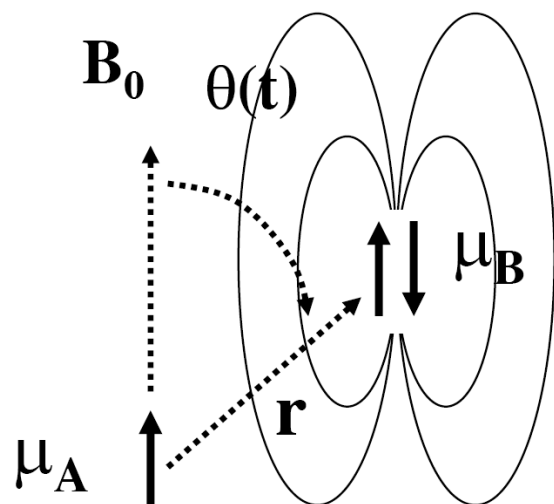
Orientation determines effective field: for instance, if σ_{33} is aligned with B_0 , then $B = (1 - \sigma_{33}) B_0$



As a molecule rapidly reorients in solution, the effective field at a given nucleus fluctuates rapidly with time

CSA can cause zero (W_0) and one (W_1) quantum transitions

The Dipole-Dipole Interaction



The dipolar interaction depends on distance ($1/r^3$) and orientation (θ)

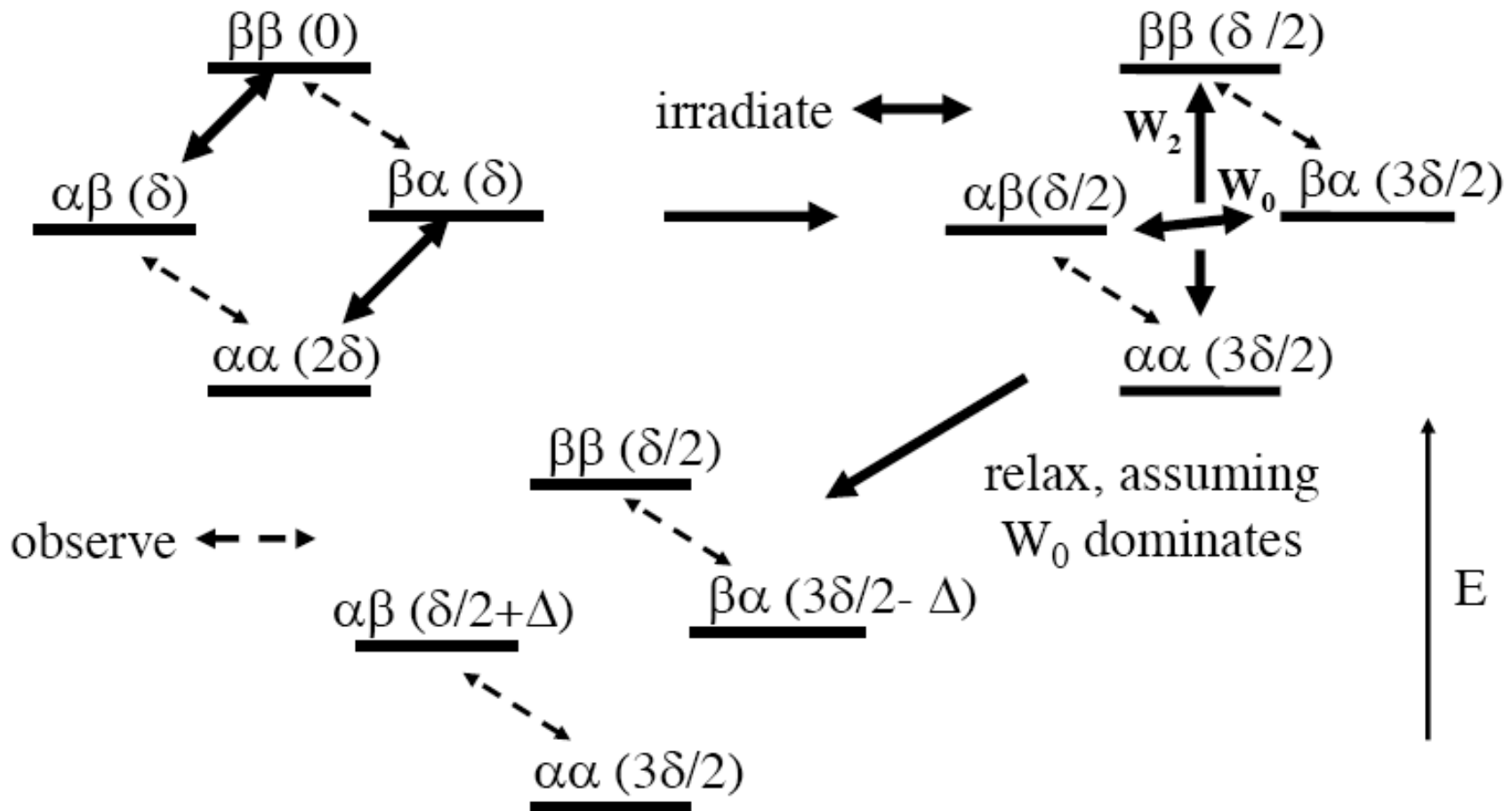
A local fluctuating magnetic field is experienced at nucleus A as molecule tumbles and θ changes

The fluctuating fields can cause zero (W_0), single (W_1), and two (W_2) quantum transitions

The magnitude of μ_B is important - an unpaired electron is about $(2000)^2$ more efficient than a proton at the same distance

The Nuclear Overhauser Effect (NOE)

-depends on competition between W_0 and W_2 processes:

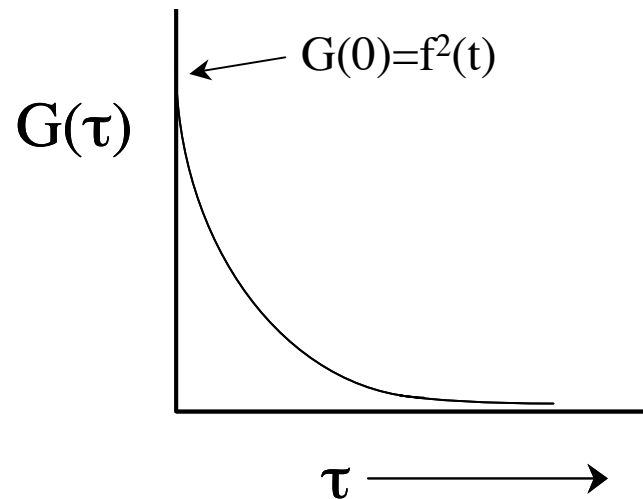
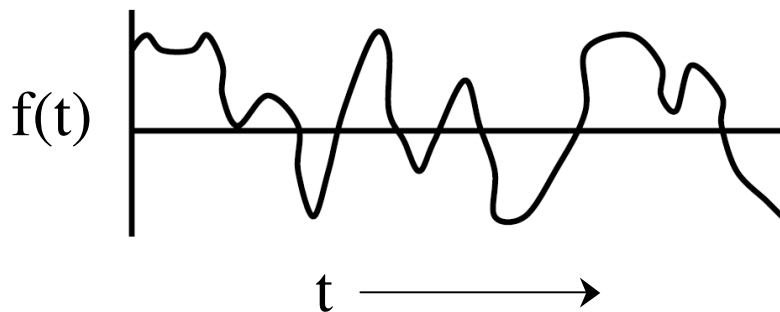


Correlation Functions

The fluctuating local magnetic fields are time dependent and average to zero for long times.

Correlation / Autocorrelation Function, $G(\tau)$: defines the rate at which these fields fluctuate.

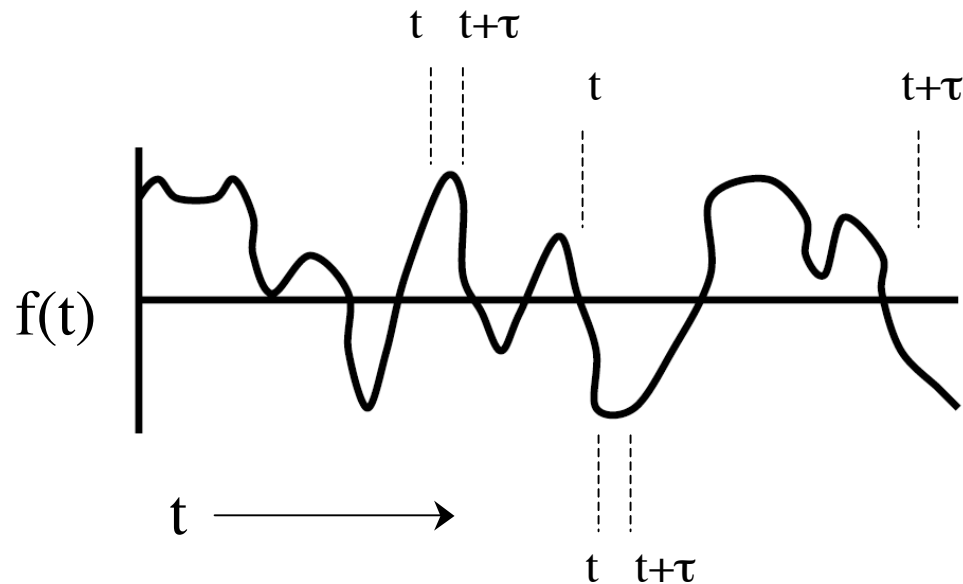
time average of $f(t)$ and $f(t + \tau)$: $G(\tau) = \overline{f(t + \tau)f(t)}$



Correlation Functions

Correlation function averages two points at increasing separation, τ .

- for small τ , t and $t+\tau$ tend to be similar (and same sign), so for the ensemble, the average of $f(t)$ and $f(t+\tau)$ is high
- for large τ , t and $t+\tau$ are unrelated, and the ensemble average tends toward zero



Correlation Functions

Random processes give rise to exponential correlation functions:

$G(\tau) = G(0) \exp(-|\tau| / \tau_c)$, where τ_c is a “correlation time”, the time constant for decay of $G(\tau)$

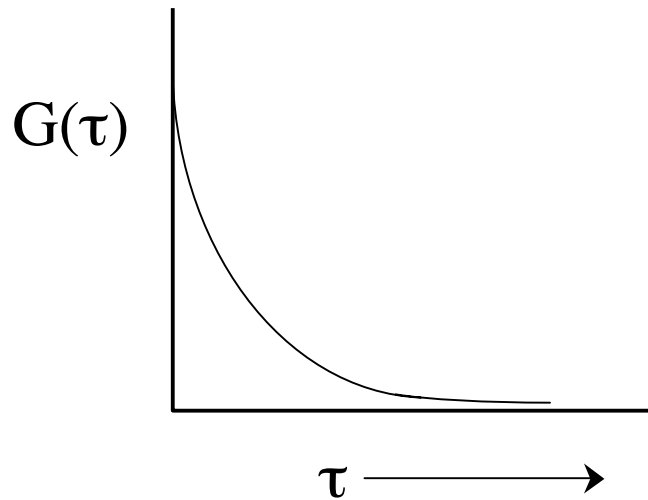
τ_c is a measure of the rotational correlation time of molecules in solution

Stoke's Law relates τ_c to molecular size, solvent viscosity and temp.:

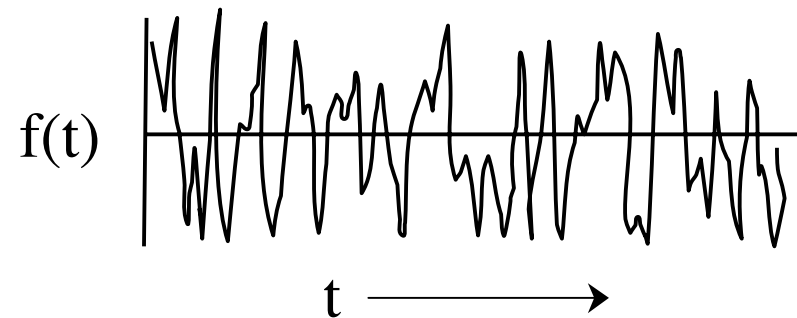
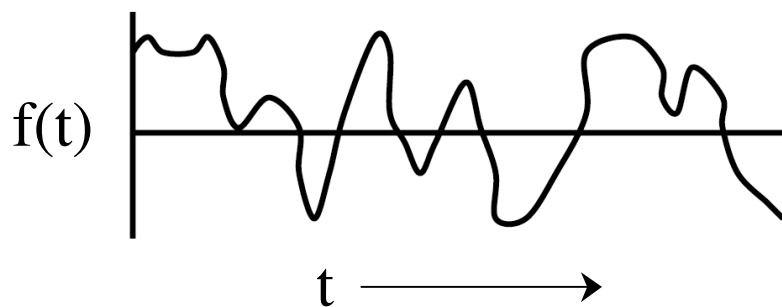
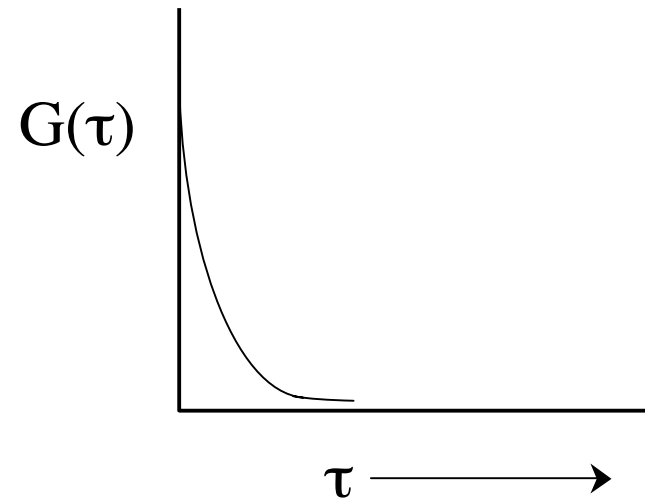
$\tau_c = 4\pi\eta a^3 / (3 k_b T)$: small molecule, high T, low η means small τ_c

Correlation Functions

- slow fluctuations
- large molecules
- long τ_c



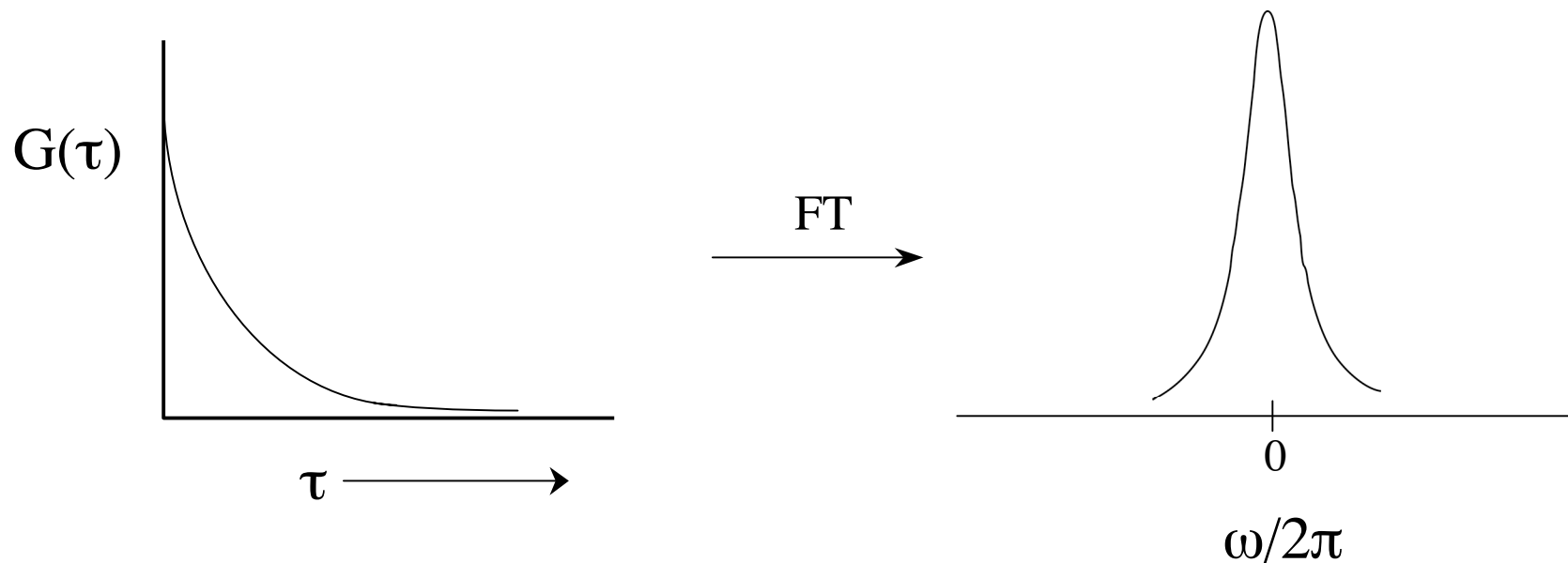
- fast fluctuations
- small molecules
- short τ_c



Power Spectral Densities

The Fourier transform of an exponential is a Lorentzian line.
The Fourier transform of the correlation function exponential is called the spectral density, $J(\omega)$

$$\exp(-|\tau| / \tau_c) \xrightarrow{\text{FT}} 2\tau_c / (1 + \omega^2\tau_c^2) = J(\omega)$$

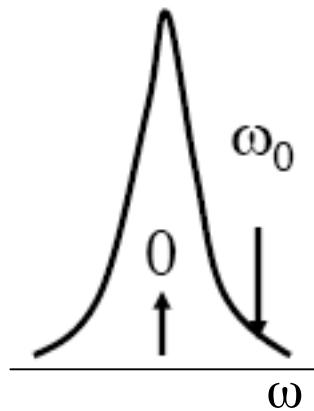


Power Spectral Densities

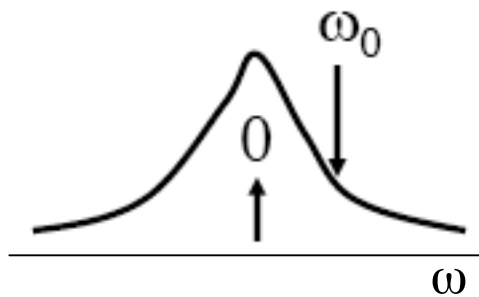
The random fluctuating fields produce a function composed of a range of frequencies (not discrete frequencies)

-spectral density curve represents power versus frequency, or the concentration of fluctuating fields present at a given frequency, or probability that motion of a given frequency exists, etc.

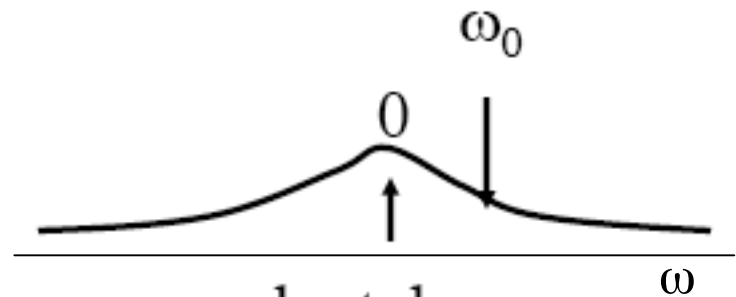
-the area under the curve is conserved



τ_c long, low power, long T_1



τ_c moderate, higher power, short T_1



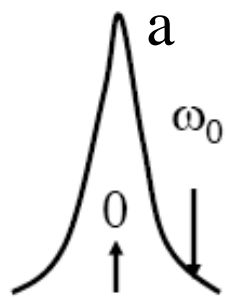
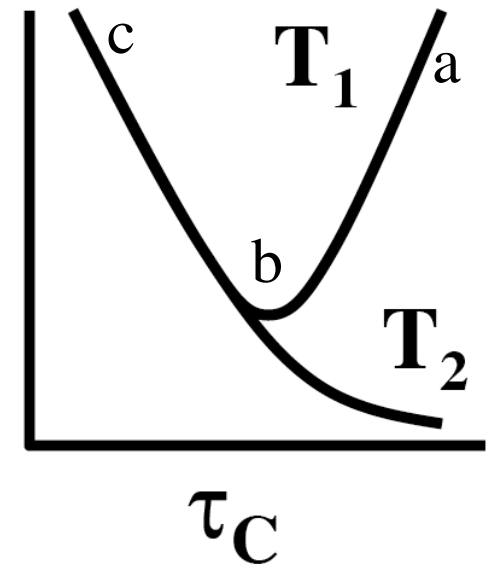
τ_c short, low power, long T_1

Spectral Density and Relaxation

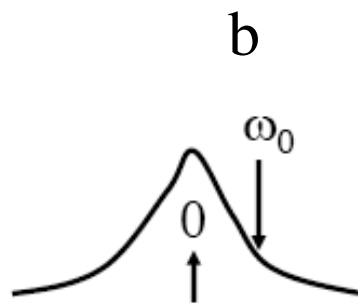
In order to cause the transitions necessary to promote relaxation, the spectral density must have frequency components at the Larmor frequency

T_1 has a complex τ_c dependence

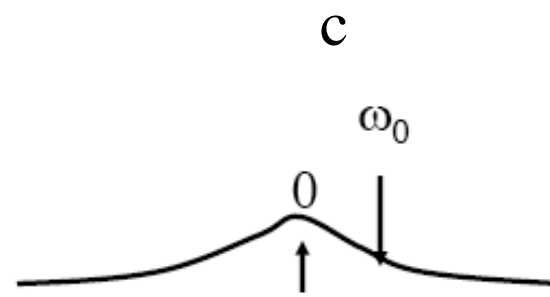
T_2 depends on $J(\omega)$ at $\omega = 0$, and it decreases monotonically with τ_c



τ_c long, low power, long T_1



τ_c moderate, higher power, short T_1



τ_c short, low power, long T_1

Heteronuclear dipolar relaxation

$^{13}\text{C}-^1\text{H}$ – for example

$$R_{1S} = 1/T_1 = (\mu_0 h^2 \gamma_I^2 \gamma_S^2 r_{IS}^{-6} / 64\pi^3)(J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I + \omega_S))$$

$$R_{2S} = 1/T_2 =$$
$$(\mu_0 h^2 \gamma_I^2 \gamma_S^2 r_{IS}^{-6} / 128\pi^3)(4J(0) + J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I) + 6J(\omega_I + \omega_S))$$

$$J(\omega) = (2/5)\tau_c / (1 + \omega^2 \tau_c^2)$$

Note: if τ_c is small (small molecule), $R_{1S} = R_{2S}$.
if τ_c is large, $R_{2S} \gg R_{1S}$

Note: if given R_{1S} and R_{2S} for a molecule with a spin pair at a known distance (0.11 nm for C-H) can use Stoke's formula to calculate molecule size

TROSY

Transverse Relaxation Optimized Spectroscopy

Pervushin, Riek, Wider and Wuthrich (1997) *Proc. Natl. Acad. Sci. USA* 94, 12366-12371.

Relaxation by T_2 limits the size of macromolecules that can be studied by NMR.

- large molecule, long τ_c , large $J(\omega)$ \rightarrow large R_2 /short T_2 \rightarrow very broad line widths and poor S/N
- the mechanism for T_2 relaxation includes contributions from both dipole-dipole coupling and chemical shift anisotropy
- sometimes, constructive interference of the dipole-dipole coupling and CSA contributions can be effected, thus increasing T_2 and dramatically improving S/N

TROSY, Example

In a decoupled ^1H , ^{15}N HSQC spectrum, each peak is an average of the four multiplet components

The S/N and line widths of the individual multiplet components are very different: each has different contributions from CSA and dipole-dipole coupling to T_2

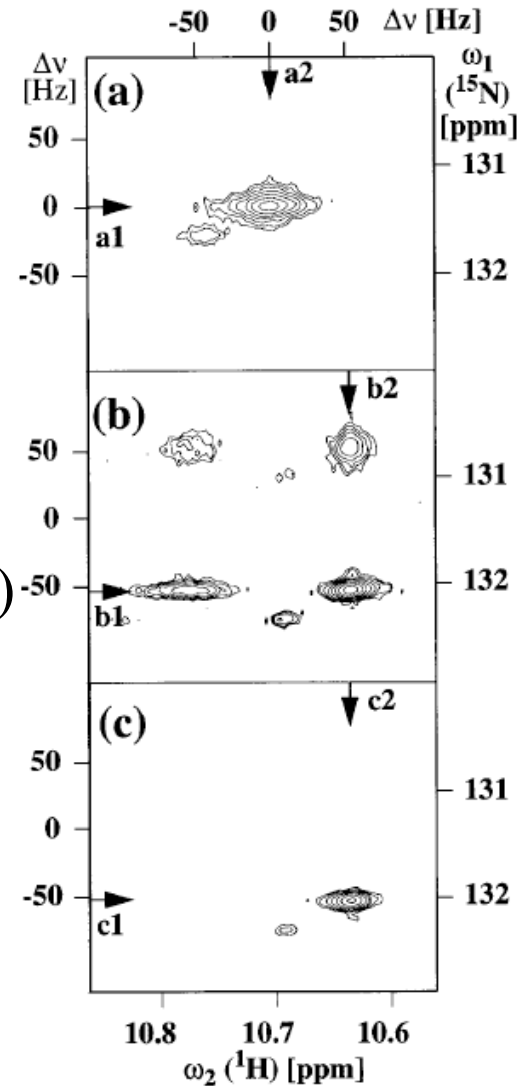
TROSY selects for one of the components

-for this component, the the CSA and dipole-dipole contributions to T_2 cancel one another (highest S/N)

decoupled
HSQC

HSQC
(no decoupling)

TROSY



TROSY

$R_{2\alpha}$ and $R_{2\beta}$ are the transverse relaxation rates of the narrow and broad components of the ^{15}N doublet, respectively

$$R_{2\alpha} = (p - \delta_{\text{N}})^2 (4J(0) + 3J(\omega_{\text{N}})) + p^2 (J(\omega_{\text{H}} - \omega_{\text{N}}) + 3J(\omega_{\text{H}}) + 6J(\omega_{\text{H}} + \omega_{\text{N}})) + 3\delta_{\text{H}}^2 J(\omega_{\text{H}})$$

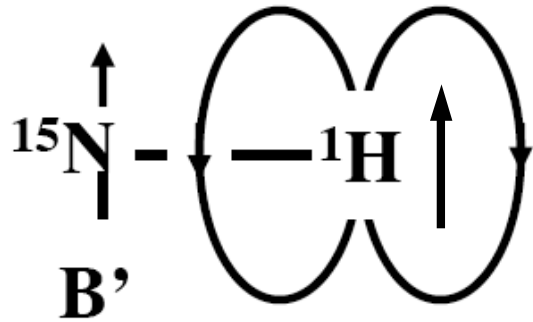
$$R_{2\beta} = (p + \delta_{\text{N}})^2 (4J(0) + 3J(\omega_{\text{N}})) + p^2 (J(\omega_{\text{H}} - \omega_{\text{N}}) + 3J(\omega_{\text{H}}) + 6J(\omega_{\text{H}} + \omega_{\text{N}})) + 3\delta_{\text{H}}^2 J(\omega_{\text{H}})$$

p = dipole-dipole contribution

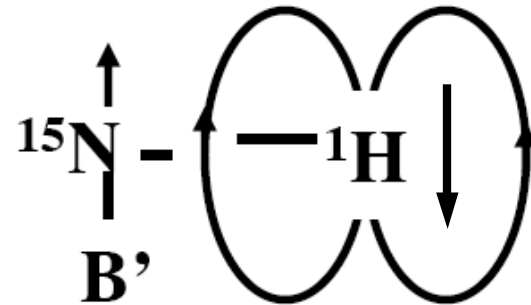
δ_{N} = CSA contribution $\propto B_0$

-as $\delta_{\text{N}} \rightarrow p$, the dipole-dipole and CSA contributions to $R_{2\alpha}$ cancel, T_2 increases, and the line width decreases

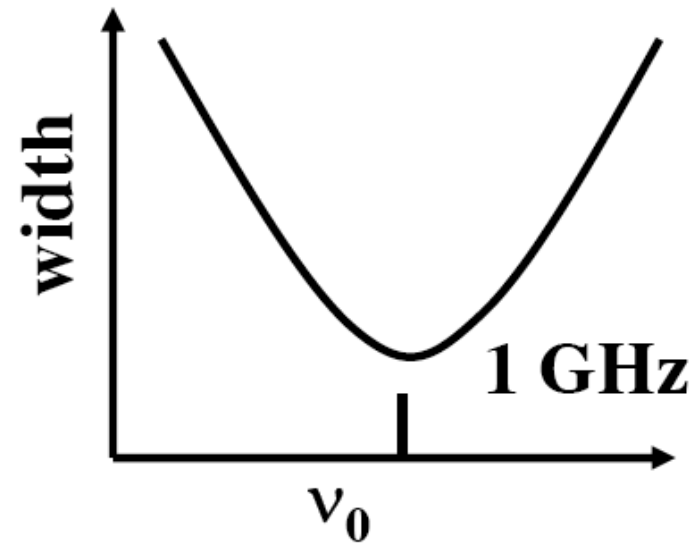
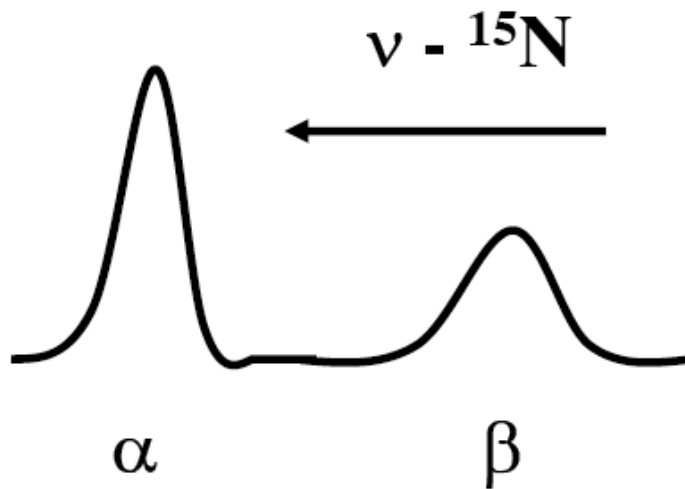
^{15}N CSA and ^1H - ^{15}N Dipole Interactions Interfere



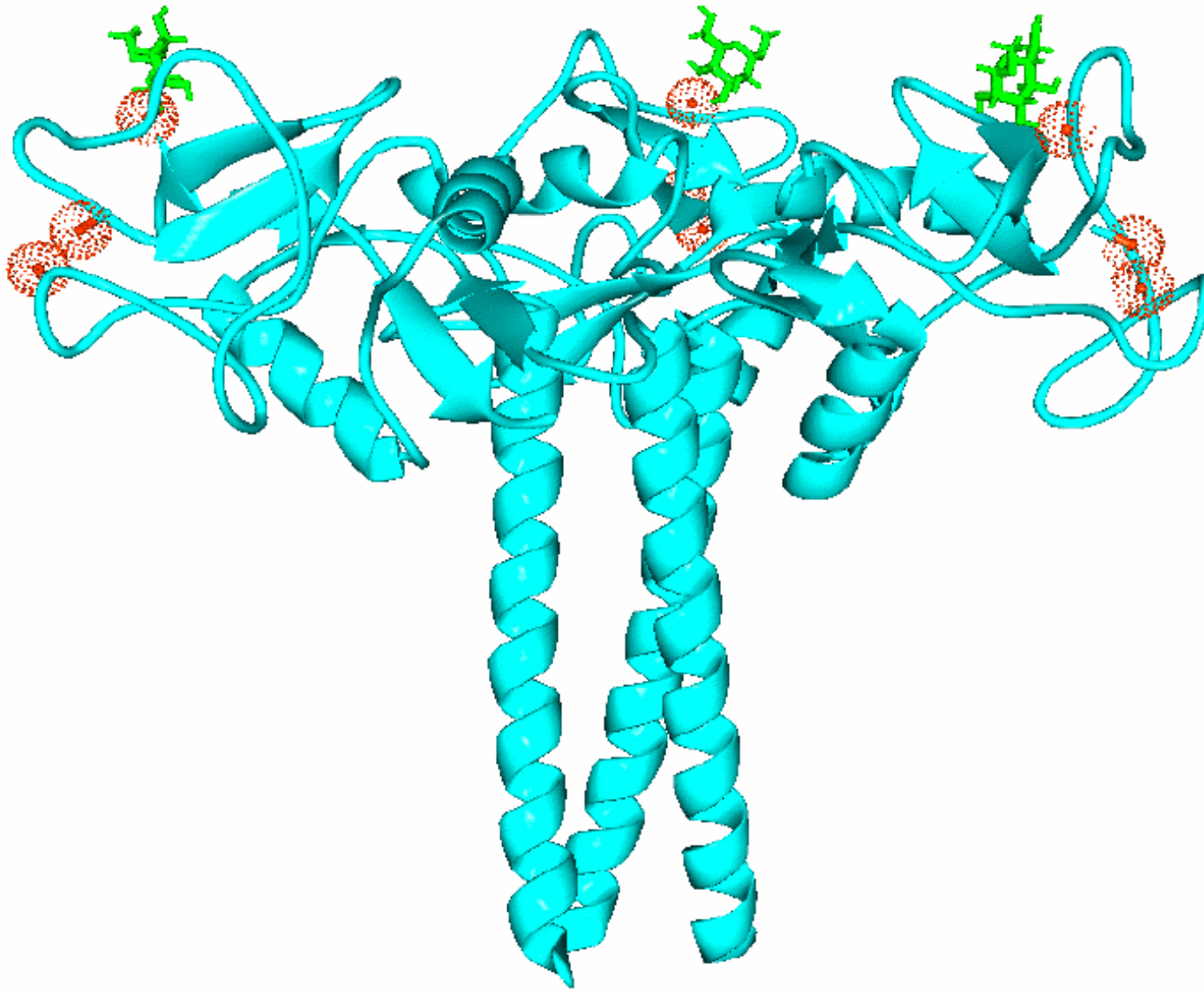
α - fields interfere



β - fields reinforce



α -Methyl Mannose Bound to Mannose Binding Protein



HSQC and TROSY of ^2H , ^{15}N -labeled mannose binding protein

