

# Spin Relaxation

BCMB/CHEM 8190

Feb 1, 2006

# $T_1$ , $T_2$ , 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

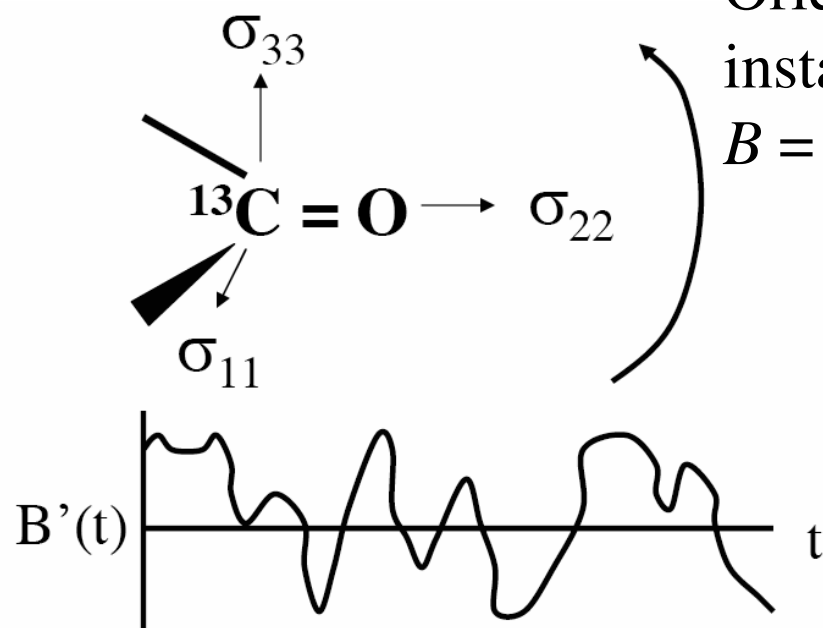
The nuclear shielding can be described by a tensor,  $\sigma$ , relating the induced field to the applied field

-the average (isotropic) shielding

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

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

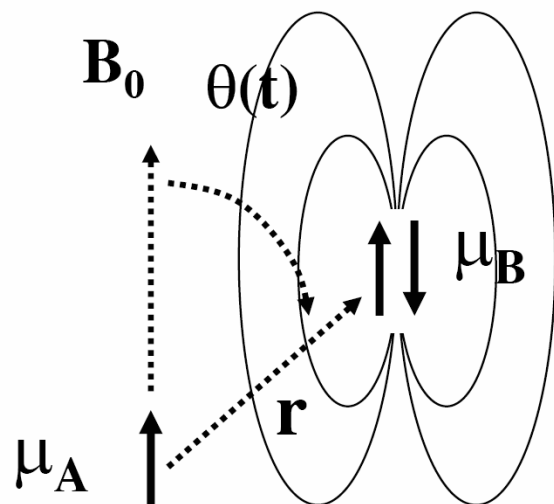
Orientation determines effective field: for instance, if  $\sigma_{33}$  is aligned with  $B_0$ , then  $B = (1 - \sigma_{33}) B_0$



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 ( $\theta$ )

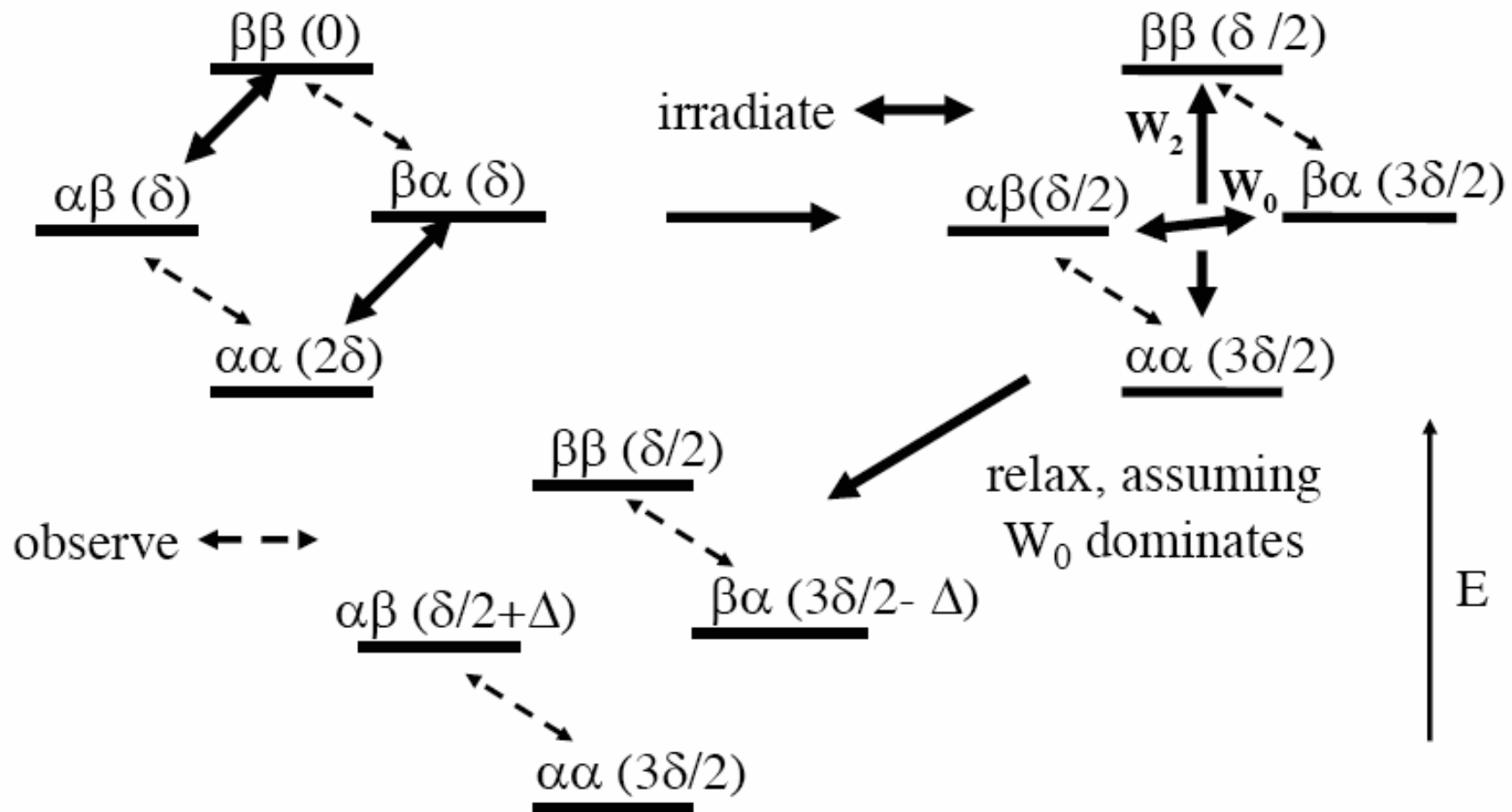
A local fluctuating magnetic field is experienced at nucleus A as molecule tumbles and  $\theta$  changes

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

The magnitude of  $\mu_B$  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:

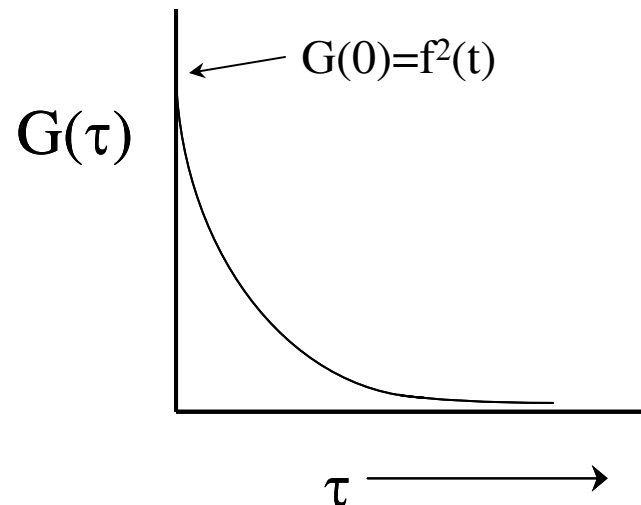
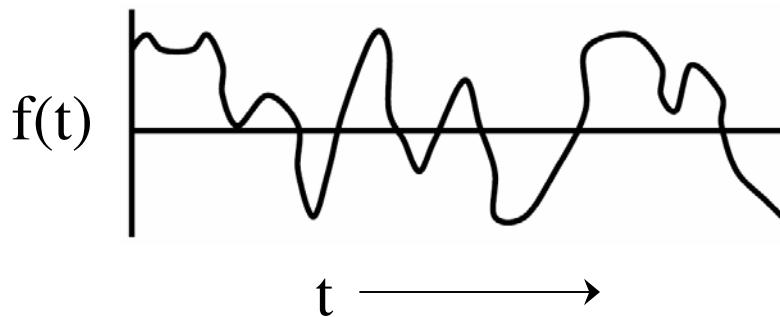


# Correlation Functions

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.

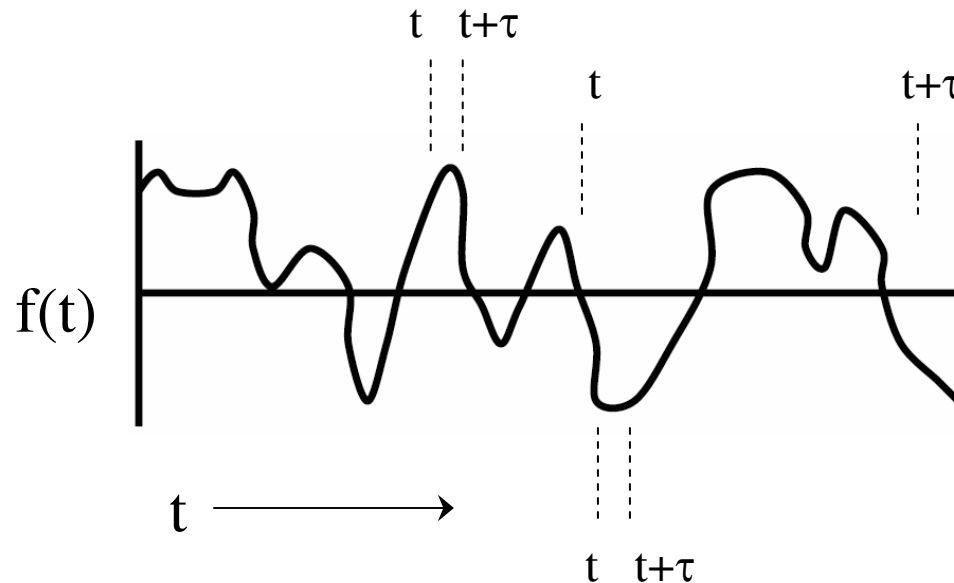
time average of  $f(t)$  and  $f(t + \tau)$ :  $G(\tau) = \overline{f(t + \tau)f(t)}$



# Correlation Functions

Correlation function averages two points at increasing separation,  $\tau$ .

- for small  $\tau$ ,  $t$  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  $\tau$ ,  $t$  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  $\tau_c$  is a “correlation time”, the time constant for decay of  $G(\tau)$

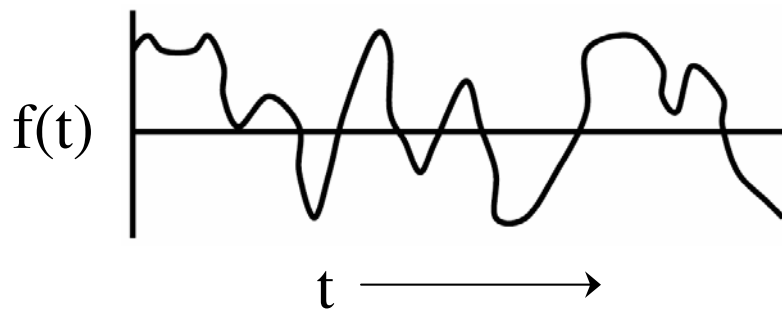
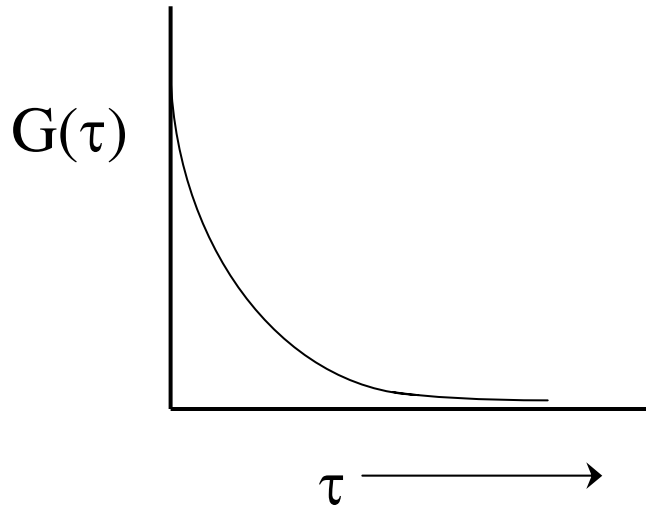
$\tau_c$  is a measure of the rotational correlation time of molecules in solution

Stoke's Law relates  $\tau_c$  to molecular size, solvent viscosity and temp.:

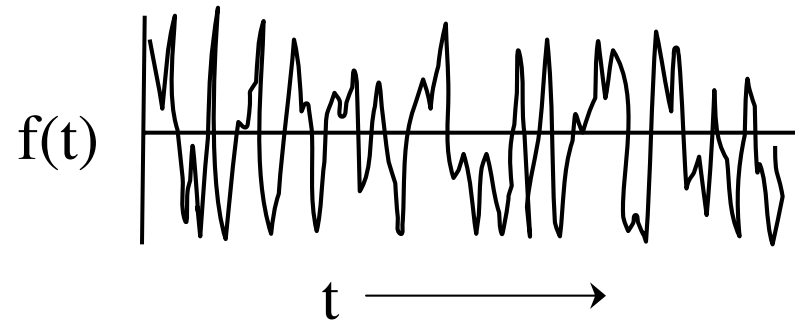
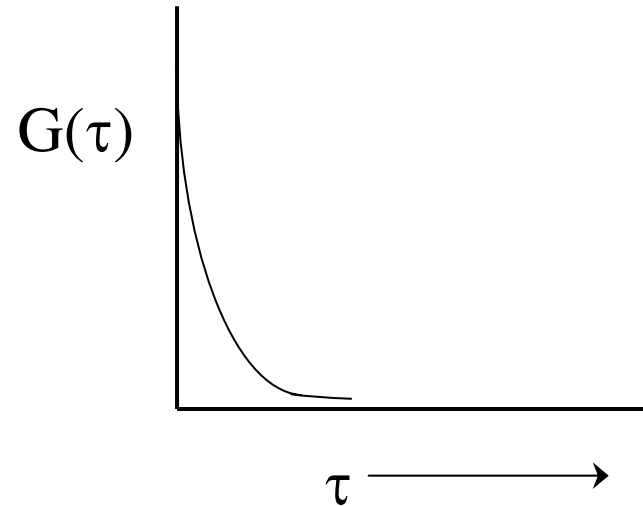
$\tau_c = 4\pi\eta a^3 / (3 k_b T)$ : small molecule, high T, low  $\eta$  means small  $\tau_c$

# Correlation Functions

- slow fluctuations
- large molecules
- long  $\tau_c$



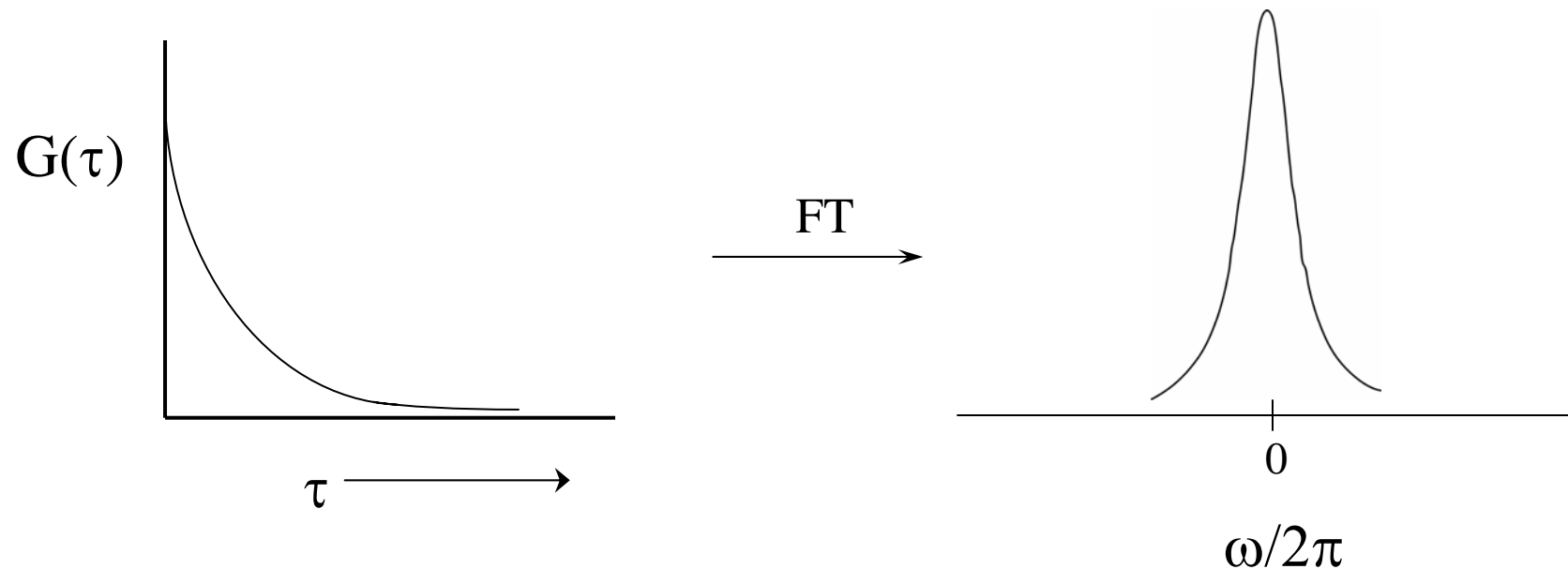
- fast fluctuations
- small molecules
- short  $\tau_c$



# 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,  $J(\omega)$

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

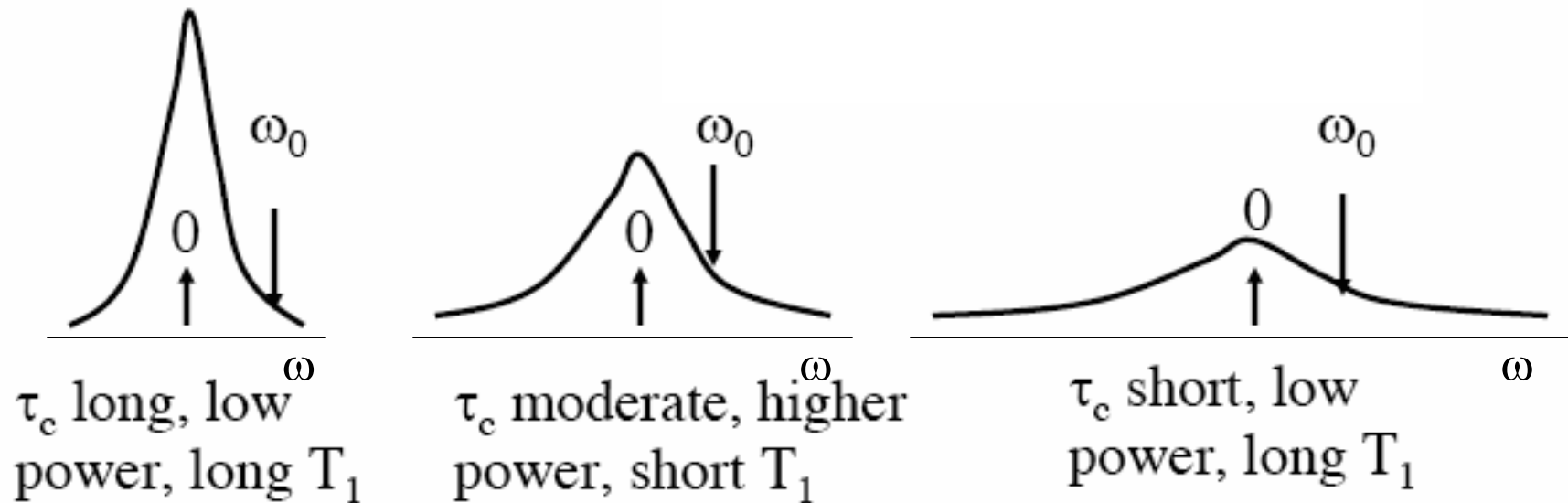


# Power Spectral Densities

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

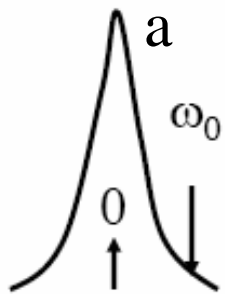
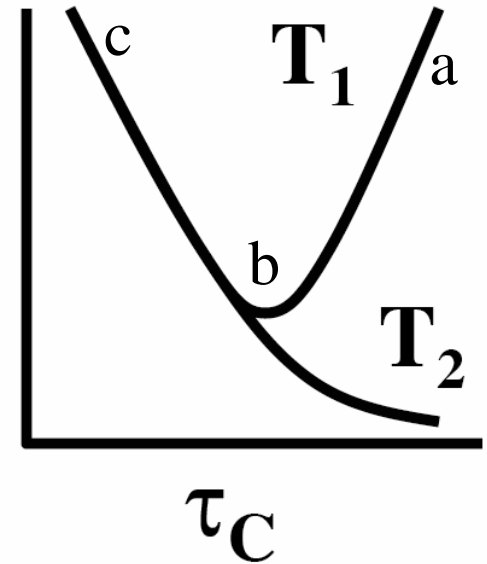


# Spectral Density and Relaxation

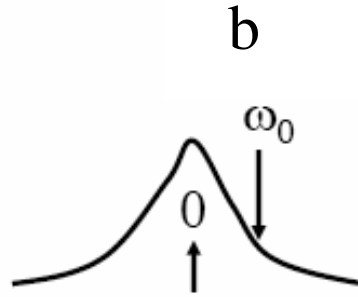
In order to cause the transitions necessary to promote relaxation, the spectral density must have frequency components at the Larmor frequency

$T_1$  has a complex  $\tau_c$  dependence

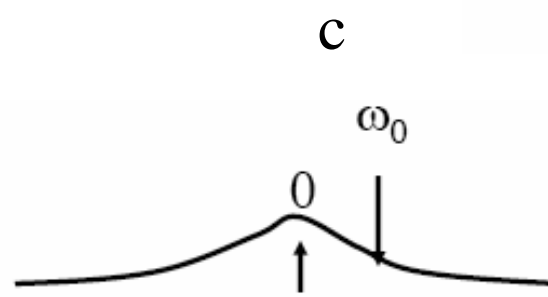
$T_2$  depends on  $J(\omega)$  at  $\omega = 0$ , and it decreases monotonically with  $\tau_c$



$\tau_c$  long, low power, long  $T_1$



$\tau_c$  moderate, higher power, short  $T_1$



$\tau_c$  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  $\tau_c$  is small (small molecule),  $R_{1S} = R_{2S}$ .  
if  $\tau_c$  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  $\tau_c$ , 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  $^1\text{H}$ ,  $^{15}\text{N}$  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  $T_2$

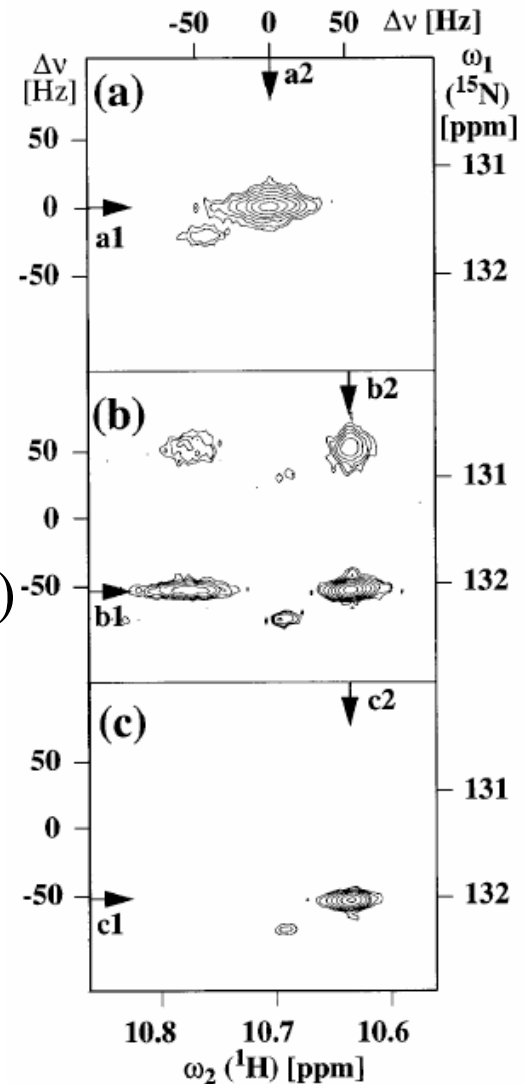
TROSY selects for one of the components

-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  $^{15}\text{N}$  doublet, respectively

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

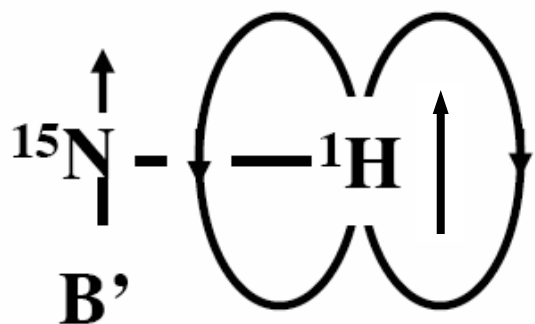
$$R_{2\beta} = (p + \delta_{\text{N}})^2 (4J(0) + 3J(\omega_{\text{N}})) + p^2 (J(\omega_{\text{H}} - \omega_{\text{N}}) + 3J(\omega_{\text{H}}) + 6J(\omega_{\text{H}} + \omega_{\text{N}})) + 3\delta_{\text{H}}^2 J(\omega_{\text{H}})$$

$p$  = dipole-dipole contribution

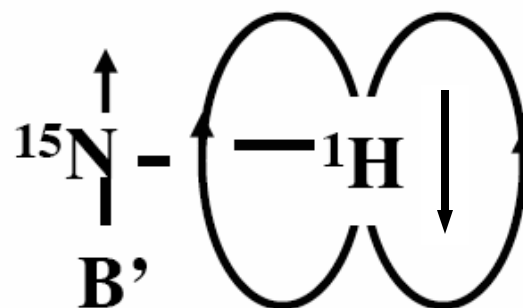
$\delta_{\text{N}}$  = CSA contribution  $\propto B_0$

- - 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

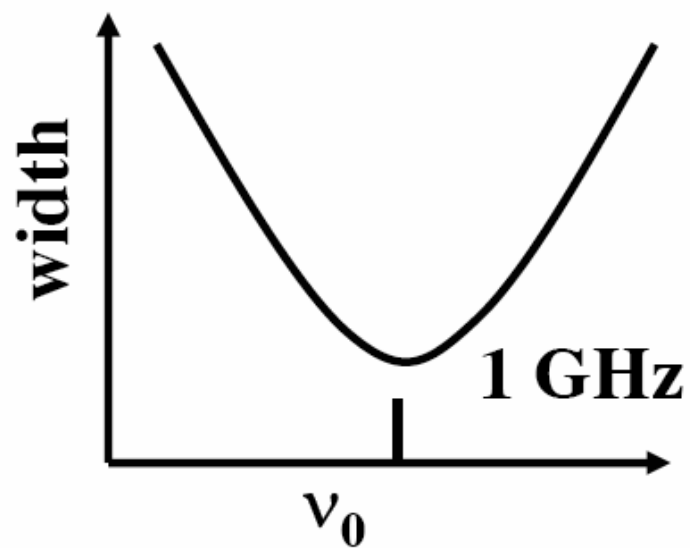
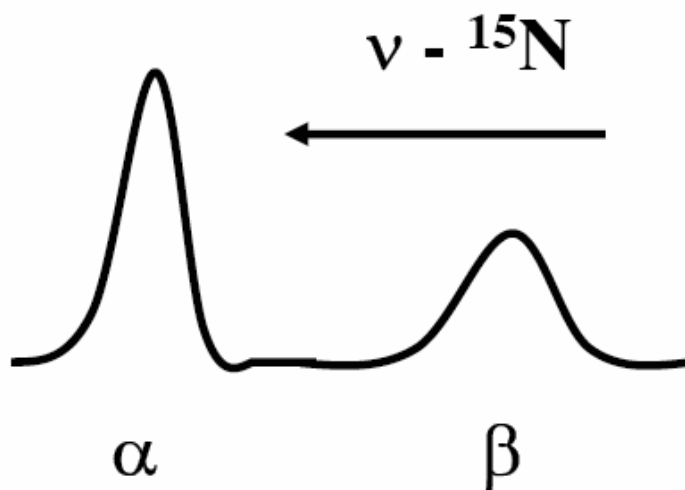
# $^{15}\text{N}$ CSA and $^1\text{H}$ - $^{15}\text{N}$ Dipole Interactions Interfere



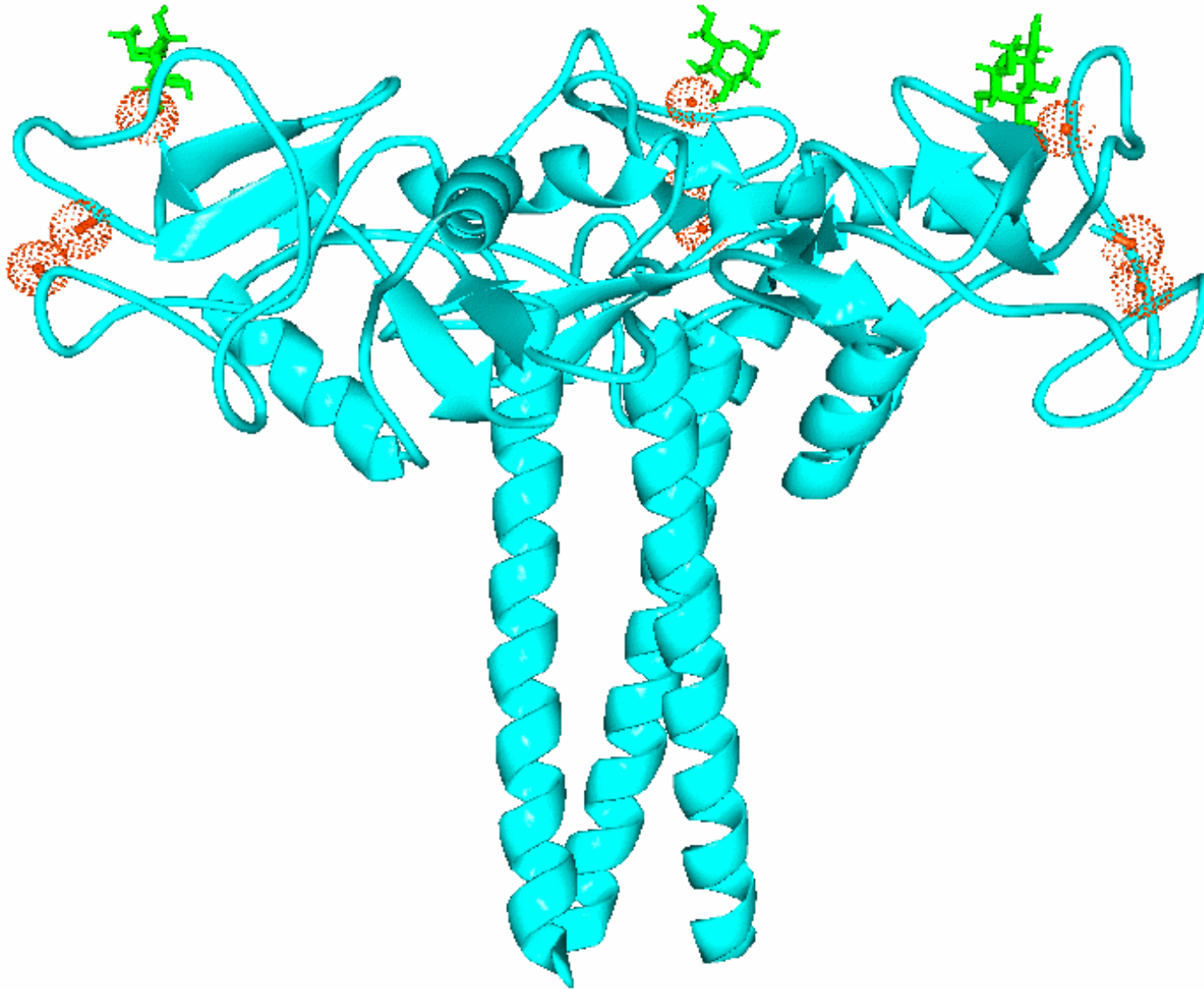
$\alpha$  - fields interfere



$\beta$  - fields reinforce



# $\alpha$ -Methyl Mannose Bound to Mannose Binding Protein



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

