

SPIN RELAXATION AND THE NUCLEAR OVERHAUSER EFFECT

BCMB/CHEM 8190

T_1 , T_2 (reminder), NOE

- 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 (NOE) is the change in intensity of a signal (resonance) when the equilibrium spin populations of a neighboring spin are perturbed

Practical Importance of Relaxation and the NOE

- Relaxation and the NOE are excellent probes of dynamics of molecules
 - methods based on relaxation and the NOE can probe motions in molecules on timescales of the order of picoseconds/nanoseconds (bond vector reorientations), nanoseconds to milliseconds (side chain reorientations), microseconds to seconds (collective group motions, domain motions)
 - example: interestingly, globular protein interiors are packed as tightly as crystalline materials, yet there are significant side chain motions on many timescales
 - 90% of phenylalanine rings in protein interiors flip 180° on ms timescale
 - protein functions depend on dynamic motions
- The NOE is the principle means for distance measurements in proteins for determining high resolution structures
 - a relaxation-dependent population transfer
 - transfer depends on $1/r^6$, which provides route for distance measurements

Origins of T_1 and T_2 Relaxation and the Nuclear Overhauser Effect (NOE)

- Key: a fluctuating interaction is capable of causing a transition, analogous to an RF pulse, between two states with a given probability (transition probabilities)
 - we can write a (perturbation) Hamiltonian describing some (time dependent) perturbation of an initial spin state
 - here, the perturbation is an RF pulse (B_1 field) along the x-axis

$$\hat{H}'(t) = -\gamma B_1(t) \hat{I}_x = -\gamma B_1(t) (\hat{I}_{x1} + \hat{I}_{x2})$$

- the transition probability between two states (here, $\beta\beta$ to $\alpha\beta$, a single quantum transition) is proportional to the square of the perturbation Hamiltonian operating on one state, multiplied on the left by the complex conjugate of the other (integrated over all space)

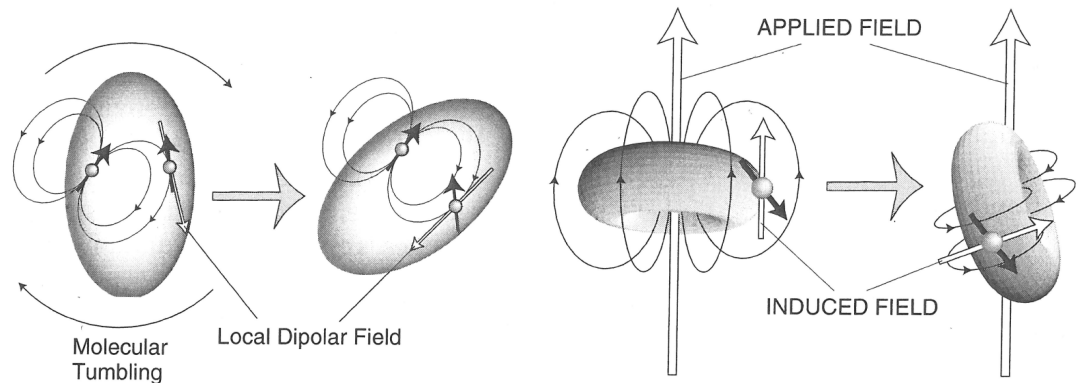
$$\rho_{k \rightarrow l} \propto \left| \langle \phi_l | \hat{H}' | \phi_k \rangle \right|^2 \quad \rho_{\beta\beta \rightarrow \alpha\beta} \propto \left| \langle \beta\beta | \gamma B_1 (\hat{I}_{x1} + \hat{I}_{x2}) | \alpha\beta \rangle \right|^2 \quad \hat{I}_x = (\hat{I}_+ + \hat{I}_-)/2$$

- For relaxation, the " $B_1(t)$ " is due to the natural motions of molecules and nuclei and the local fluctuating magnetic fields they produce, and these can induce transitions between states

Origins of T_1 and T_2 Relaxation and the Nuclear Overhauser Effect (NOE)

$$\hat{H}' = \gamma B_1 (\hat{I}_{x1} + \hat{I}_{x2}) \quad \rho_{k \rightarrow l} \propto \left| \langle \phi_l | \hat{H}' | \phi_k \rangle \right|^2 \quad \rho_{\beta\beta \rightarrow \alpha\alpha} \propto \left| \langle \beta\beta | \gamma B_1 (\hat{I}_{x1} + \hat{I}_{x2}) | \alpha\alpha \rangle \right|^2$$

- Are two requirements for the fluctuating local fields to be able to produce transitions
 - the Hamiltonian must have the appropriate spin operator(s) (i.e. for an RF pulse, a pulse along the z-axis does nothing to z-magnetization) in order for the transition probability to be finite
 - the second (obvious) requirement is that $\Delta E = h\nu$
 - important to keep in mind that the local fluctuating fields are not of a single frequency but a distribution of frequencies, that must include the necessary frequency/frequencies for transitions to occur
- Sources of local fields
 - dipole-dipole interactions
 - chemical shift anisotropy
 - electric field gradients (quadrupolar nuclei only)
 - others



From Levitt, 2001

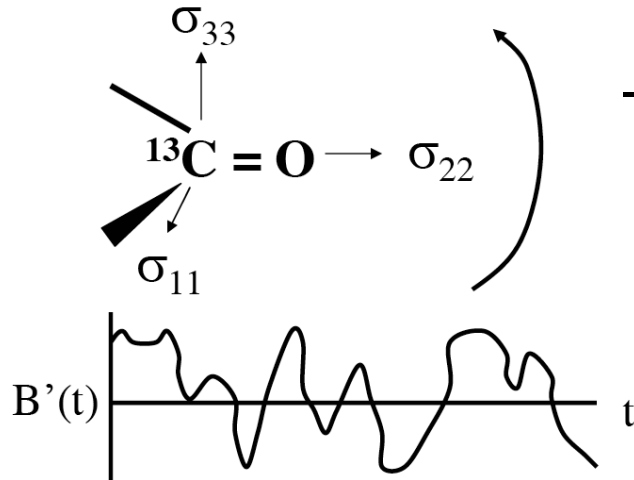
Chemical Shift Anisotropy (CSA)

- Chemical shifts arise from electronic shielding of the nucleus
 - shielding depends on orientation of the molecule with respect to B_0
 - the shielding modifies the B_0 differently for different orientations of the molecule with respect to B_0
- Rapid molecular reorientation results in local, fluctuating magnetic fields (magnitude and direction)
 - these local fluctuating fields lead to energy level transitions, just like applied rf fields
- CSA plays an important role in a resolution enhancement phenomenon called 'TROSY'

An Example for CSA Relaxation

- The nuclear shielding can be described by a tensor, σ , relating the induced field to the applied field
 - when the tensor is rotated such that the induced field is parallel to the applied field (diagonalizing the matrix) the average (isotropic) shielding, σ_{iso} , is $(\sigma_{11} + \sigma_{22} + \sigma_{33})/3$

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



- orientation determines effective field: if σ_{33} is aligned with B_0 , then $B_{eff} = (1 - \sigma_{33}) B_0$

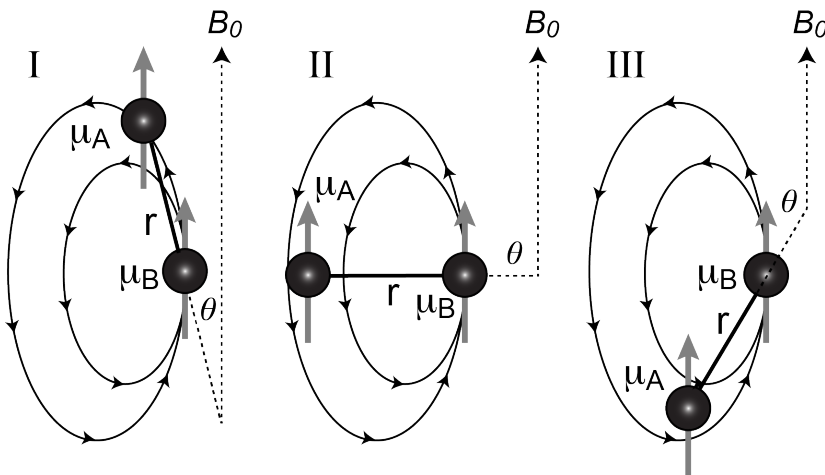
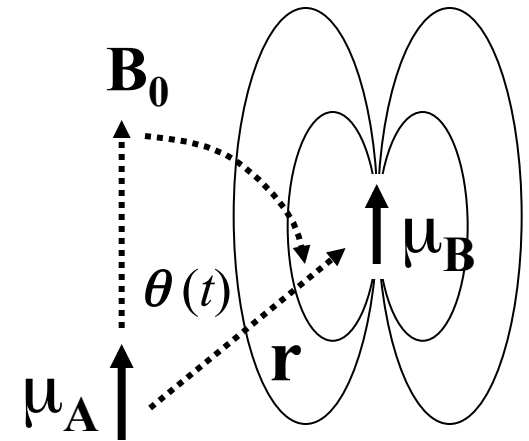
- as a molecule rapidly reorients in solution, the B' (shielding) component fluctuates rapidly with time, and then so does the effective field (nsec timescale)

- CSA can cause one (W_1) and zero (W_0) quantum transitions
 - in solution, σ_{11} , σ_{22} , and σ_{33} (i.e. x, y, and z) components are present, for any particular orientation, thus operators are \hat{I}_x , \hat{I}_y , and \hat{I}_z
 - \hat{I}_x and \hat{I}_y can promote $\alpha \rightarrow \beta$ transitions (i.e., one quantum), \hat{I}_z cannot
 - \hat{I}_z components fluctuate, indicating precession (chemical shift) fluctuations, or dephasing in transverse plane (W_0), T_2

$$\hat{I}_x|\alpha\rangle = 1/2\beta \quad \hat{I}_x|\beta\rangle = 1/2\alpha \quad \hat{I}_y|\alpha\rangle = 1/2i\beta \quad \hat{I}_y|\beta\rangle = -1/2i\alpha \quad \hat{I}_z|\alpha\rangle = 1/2\alpha \quad \hat{I}_z|\beta\rangle = -1/2\beta$$

The Dipole-Dipole Interaction

- Dipolar interaction between H nuclei leads to the NOE
- Dipolar interaction between ^{13}C and directly bonded H is principle mechanism for relaxation of such ^{13}C nuclei
- Dipolar interaction depend on distance ($1/r^3$) and orientation (θ)
- A local fluctuating magnetic field is experienced at nucleus A as molecule tumbles and θ changes



Here spin A and spin B are directly bonded (bond length r). As the molecule rotates:

I - magnetic field lines from spin B at the position of spin A augment B_0

II - magnetic field lines from spin B at the position of spin A oppose B_0

III - magnetic field lines from spin B at the position of spin A do not significantly augment or oppose B_0 much

The Dipole-Dipole Interaction

- Unlike CSA, here *pairs* of spins are interacting
 - the Hamiltonian is complex, and includes dot products of dipole moments
 - $\mu \cdot \mu \propto \hat{\mathbf{I}} \cdot \hat{\mathbf{I}} = \hat{I}_x \cdot \hat{I}_x + \hat{I}_y \cdot \hat{I}_y + \hat{I}_z \cdot \hat{I}_z$, for ease write as raising/lowering operators

$$\hat{H}_D = \frac{\mu_0}{4\pi} \frac{(\vec{\mu}_I \cdot \vec{\mu}_S)}{r^3} - \frac{3(\vec{\mu}_I \cdot \mathbf{r})(\vec{\mu}_S \cdot \mathbf{r})}{r^5} = \frac{\mu_0 \gamma_I \gamma_S \hbar^2}{(16\pi^3 r^3)} (A + B + C + D + E + F)$$

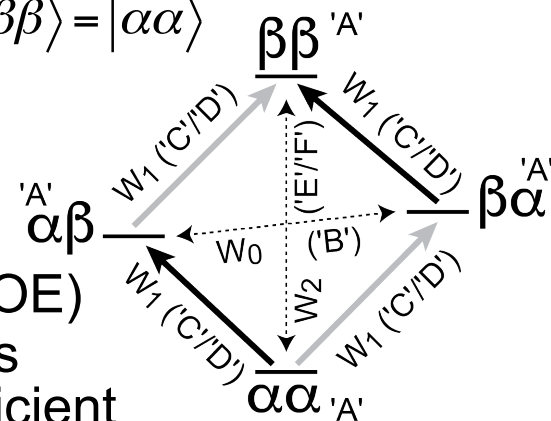
$$A = -\hat{I}_z \hat{S}_z (3\cos^2 \theta - 1), B = (1/4)(\hat{I}^+ \hat{S}^- + \hat{I}^- \hat{S}^+) (3\cos^2 \theta - 1), C = -(3/2)(\hat{I}^+ \hat{S}_z + \hat{I}_z \hat{S}^+) (\sin \theta \cos \theta e^{-i\varphi})$$

$$D = -(3/2)(\hat{I}^- \hat{S}_z + \hat{I}_z \hat{S}^-) (\sin \theta \cos \theta e^{i\varphi}), E = -(3/4) \hat{I}^+ \hat{S}^+ \sin 2\theta e^{-2i\varphi}, F = -(3/4) \hat{I}^- \hat{S}^- \sin 2\theta e^{2i\varphi}$$

- Zero (W_0), single (W_1) and two (W_2) relaxation mechanisms all operate for fluctuating fields from dipole-dipole interactions

- recall $\hat{I}^+ |\alpha\rangle = 0$ $\hat{I}^- |\alpha\rangle = \beta$ $\hat{I}^+ |\beta\rangle = \alpha$ $\hat{I}^- |\beta\rangle = 0$ $\hat{I}_z |\alpha\rangle = 1/2 \alpha$ $\hat{I}_z |\beta\rangle = -1/2 \beta$
- 'B' term, zero quantum (W_0), example: $\hat{I}^+ \hat{S}^- |\beta\alpha\rangle = |\alpha\beta\rangle$
- 'C' and 'D' terms, one quantum (W_1), example: $\hat{I}^+ \hat{S}_z |\beta\alpha\rangle = 1/2 |\alpha\alpha\rangle$
- 'E' and 'F' terms, two quantum (W_2), example: $\hat{I}^+ \hat{S}^+ |\beta\beta\rangle = |\alpha\alpha\rangle$
- 'A' term, precession (chemical shift) fluctuations, or dephasing in transverse plane (W_0), T_2

- Remember $\rho_{\beta\beta \rightarrow \alpha\alpha} \propto |\langle \beta\beta | \gamma B_1 (I_{x1} + I_{x2}) | \alpha\beta \rangle|^2$
 - so, probability depends on $(\mu \cdot \mu)^2$ and $(1/r^3)^2 = 1/r^6$ (NOE)
 - magnetic dipole moment of an electron is ~650 times larger than the proton moment, so is $(650)^2$ more efficient

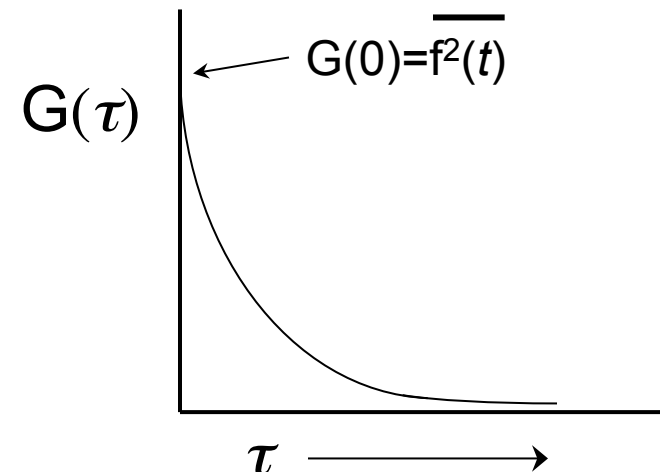
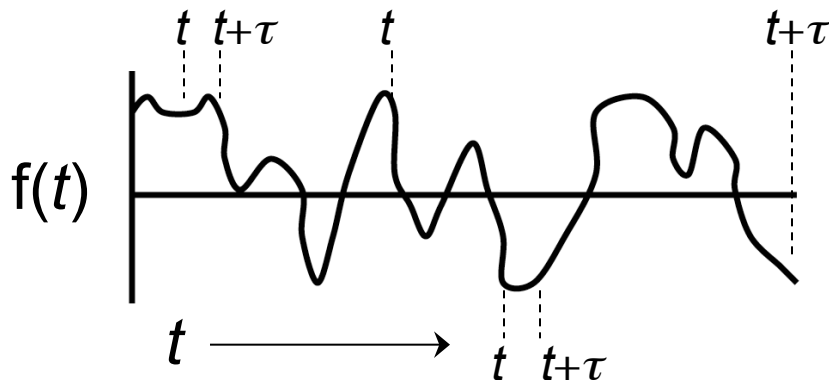


Correlation Functions

- We need to be able to analyze the magnetic field fluctuations
 - need to determine what frequency components are present, their intensities, and if these are appropriate ($\Delta E = h\nu$) for exciting zero, one or two quantum transitions
- The correlation function is a means to quantify the time dependence of the fluctuating local magnetic fields
 - the fluctuating local magnetic fields are time dependent and average to zero over long times
 - the correlation (autocorrelation) function, $G(\tau)$, defines the rate at which these fields fluctuate (averages two points at increasing separation, τ)

$$G(\tau) = \overline{f(t+\tau) \times f(t)} \quad \text{i.e. time average of } f(t) \text{ and } f(t+\tau)$$

- 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 produce exponential correlation functions
 - these decay with a time constant, τ_c , called a *correlation time*
 - for our random local magnetic field fluctuations we can write

$$G(\tau) = G(0)\exp(-|\tau|/\tau_c)$$

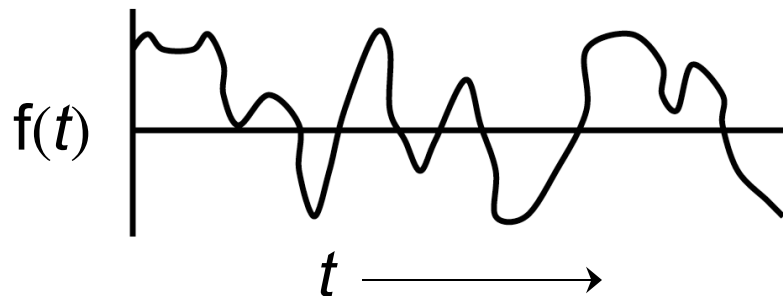
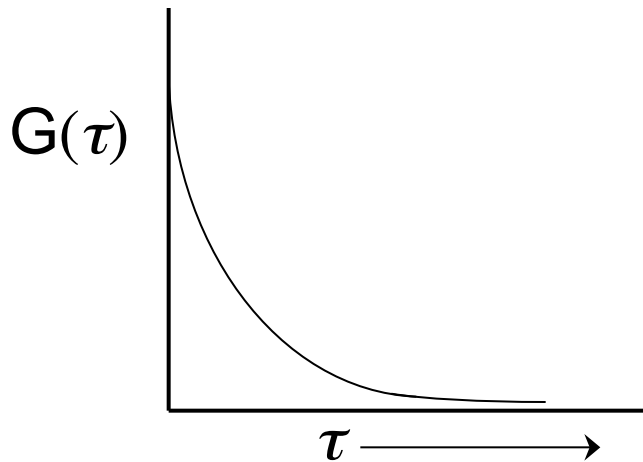
- The time constant τ_c is a measure of how fast $f(t)$ and $f(t+\tau)$ become uncorrelated
 - for small molecules (rapid tumbling) the fluctuations are fast, so τ_c is short
 - for large molecules (slower tumbling) the fluctuations are slow, τ_c is longer
- Thus, the time constant τ_c is a measure of the rotational correlation time (tumbling time), and, therefore, molecular size
 - Stokes Law relates τ_c to molecular size

$$\tau_c = 4\pi\eta a^3 / (3k_b T)$$

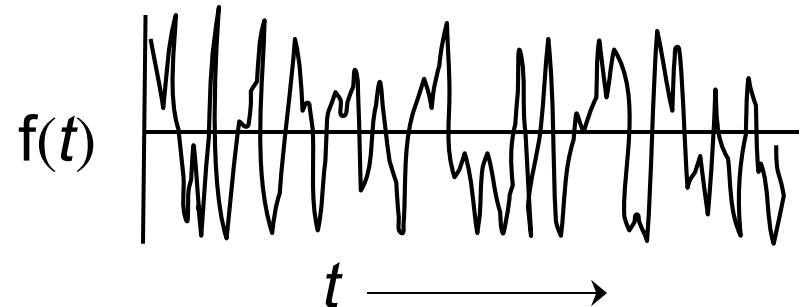
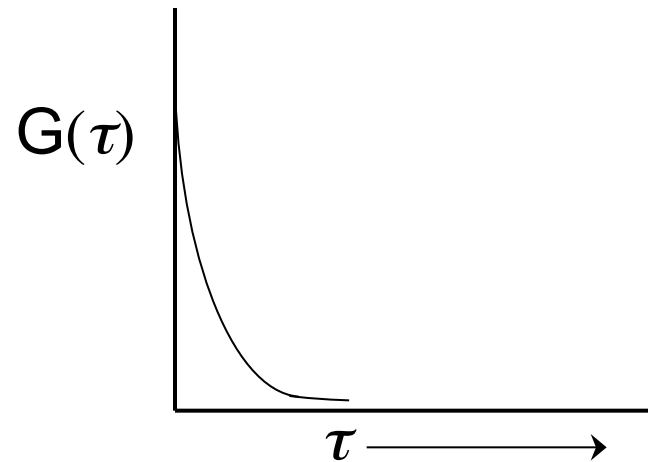
- a is the radius of a sphere that approximates the size of the molecule
- η is the viscosity of the solution
- small molecule, low η , high T , means small τ_c
- large molecule, high η , low T , means larger τ_c
- because MW directly proportional to a^3 (volume), τ_c is directly proportional to MW (70 kDa protein at 20 °C, $\tau_c \sim 35$ ns.....rule of thumb, $\tau_c \sim 0.5$ MW in kDa at room temperature)

Summary

- slow fluctuations
- large molecules
- low temperature
- high viscosity
- longer τ_c



- fast fluctuations
- small molecules
- high temperature
- low viscosity
- shorter τ_c

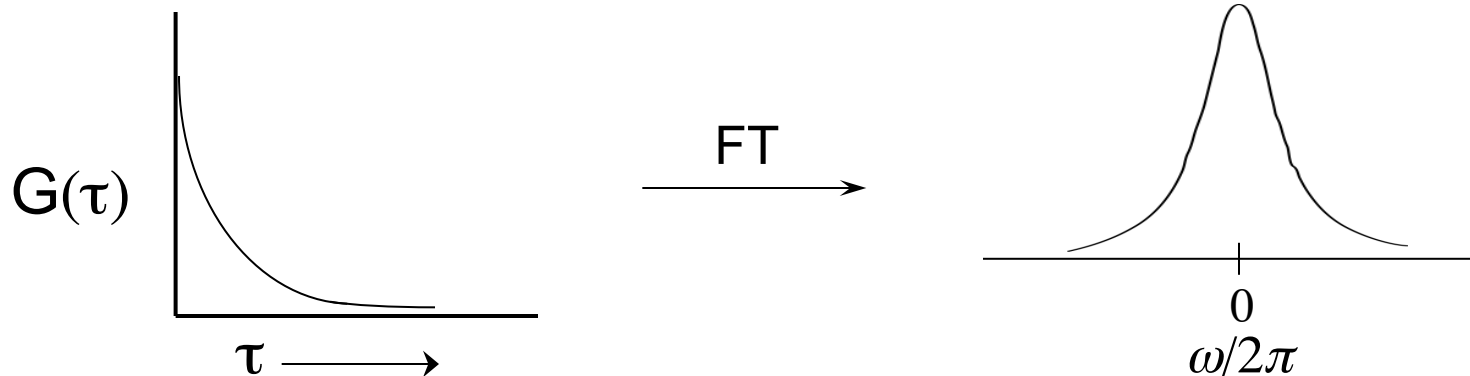


Power Spectral Densities

- We need to be able to analyze the magnetic field fluctuations
 - need to determine what frequency components are present, their intensities, and if these are appropriate ($\Delta E = h\nu$) for exciting zero, one or two quantum transitions
- We can convert the exponentially decaying, time-domain correlation function to a frequency domain via Fourier transform
 - the Fourier transform of an exponential is a Lorentzian shaped distribution
 - the Fourier transform of the correlation function is called the power spectral density, $J(\omega)$

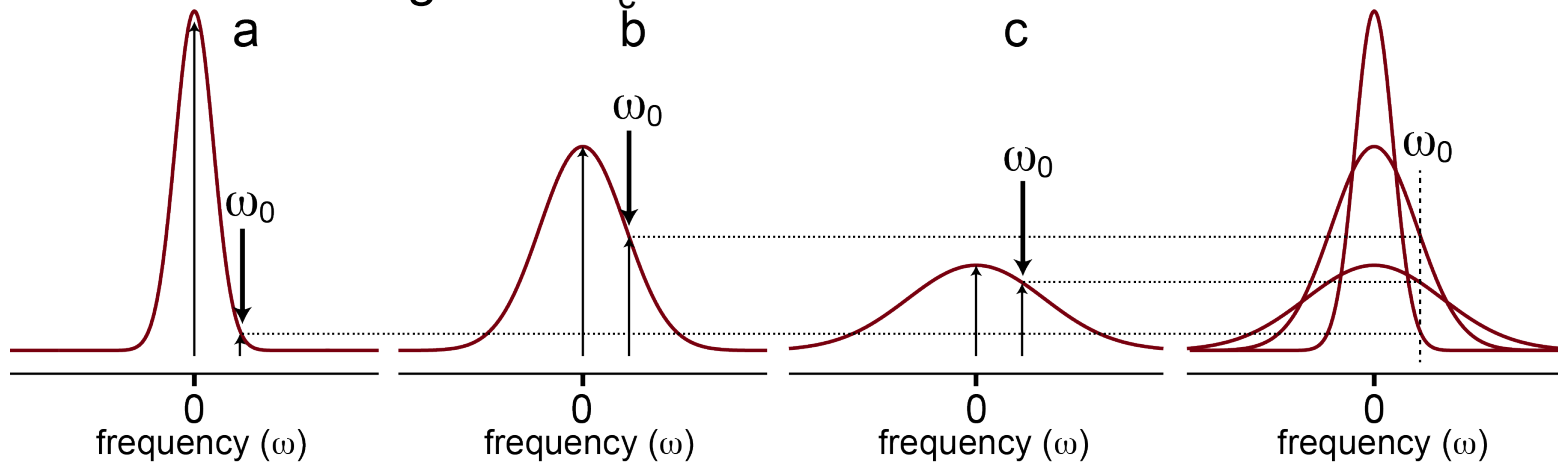
$$\exp(-|\tau|/\tau_c) \xrightarrow{\text{FT}} \tau_c / (1 + \omega^2 \tau_c^2) = 1/2 J(\omega)$$

- the spectral density gives information about intensity/density/power of fluctuations at at given frequencies
- we are interested in the density, or 'power' of frequencies at zero frequency (zero quantum, W_0), at the Larmor frequency (one quantum, W_1), and twice the Larmor frequency (two quantum, W_2)



Power Spectral Densities

- The random fields fluctuate at a continuum of frequencies
- The power spectral density function gives the power or intensity at a frequency of interest
 - long τ_c (slowly decaying exponential, $G(\tau)$), FT give sharp line/distribution
 - short τ_c (quickly decaying exponential, $G(\tau)$), FT give broad line/distribution
 - areas under curves conserved
- The variation of power with τ_c depends on the frequency
 - at zero frequency (W_0), power decreases monotonically as τ_c decreases
 - at the Larmor frequency (W_1), power increases initially as τ_c decreases, then it decreases again as τ_c continues to decrease



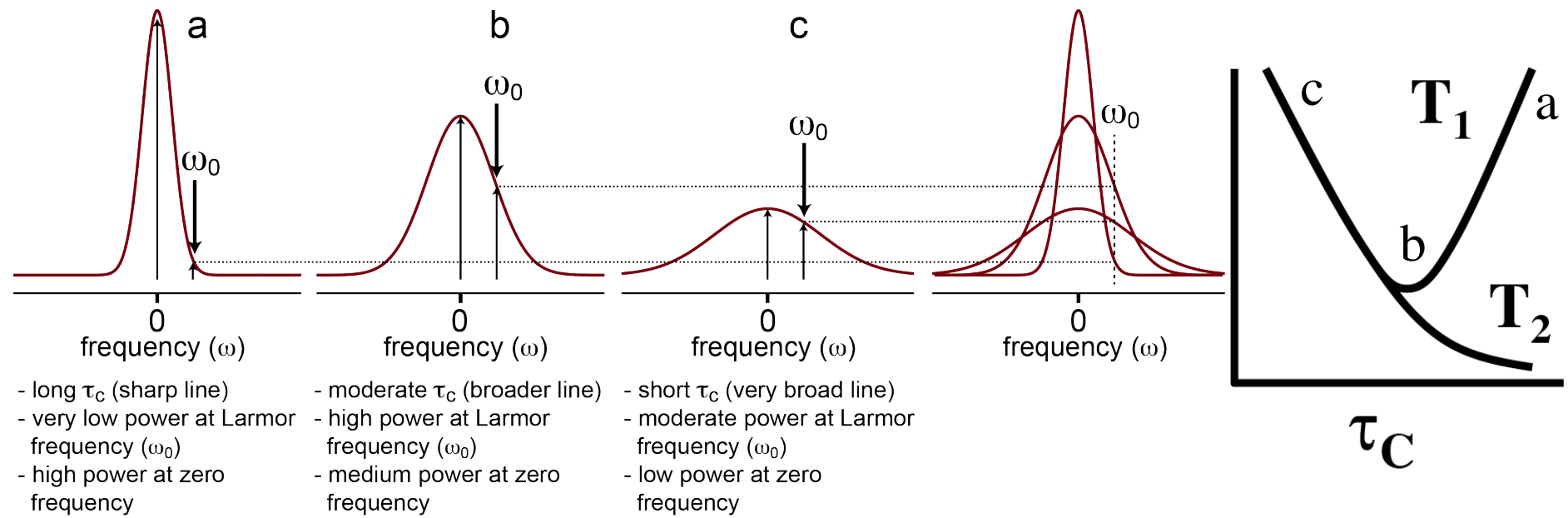
- long τ_c (sharp line)
 - very low power at Larmor frequency (ω_0)
 - high power at zero frequency

- moderate τ_c (broader line)
 - high power at Larmor frequency (ω_0)
 - medium power at zero frequency

- short τ_c (very broad line)
 - moderate power at Larmor frequency (ω_0)
 - low power at zero frequency

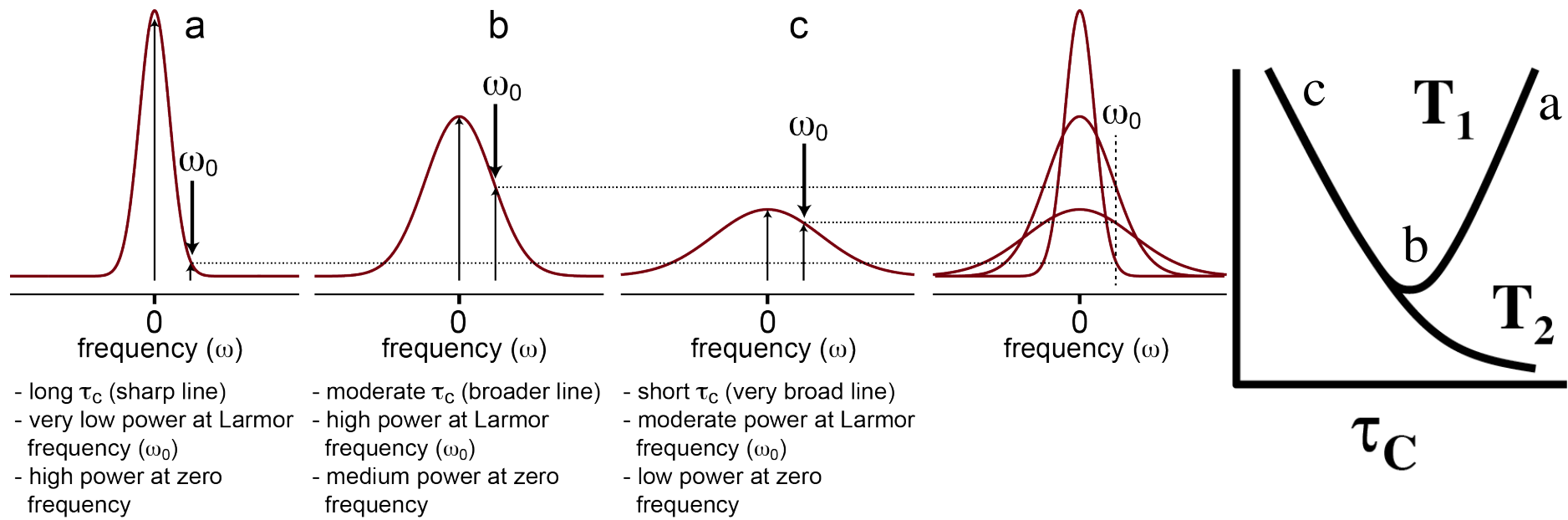
Spectral Density and Relaxation

- To cause transitions needed to promote relaxation, the spectral density must have the requisite power at the frequency of interest
- Consider T_1 relaxation: T_1 depends on the spectral density ($J(\omega)$) at the Larmor frequency (ω_0 , a one quantum, W_1 , process) and has a complex τ_c dependence
 - for large molecules (long τ_c), the FT of $G(\tau)$ gives a sharp distribution, with very little power at ω_0 , so, inefficient W_1 relaxation and long T_1 ('a' below)
 - for mid-sized molecules (moderate τ_c), the FT of $G(\tau)$ gives a broader distribution, with high power at ω_0 , so, efficient relaxation and short T_1 ('b')
 - for small molecules (short τ_c), the FT of $G(\tau)$ gives a very broad distribution with very low power at ω_0 , so, inefficient relaxation and long T_1 ('c')



Spectral Density and Relaxation

- To cause transitions needed to promote relaxation, the spectral density must have the requisite power at the frequency of interest
- Consider T_2 relaxation: T_2 depends on the spectral density ($J(\omega)$) at zero frequency (a zero quantum, W_0 , process) and has a straightforward τ_c dependence
 - for large molecules (long τ_c), the FT of $G(\tau)$ gives a sharp distribution, with very high power zero frequency (at $J(\omega)=0$), so, very efficient W_0 relaxation and very short T_2 ('a' below)
 - as τ_c decreases (smaller molecules), the FT of $G(\tau)$ gives increasingly broader distributions, with monotonically decreasing power (at $J(\omega)=0$), so, less efficient relaxation and longer T_2 ('b' then 'c')



Heteronuclear Dipolar Relaxation: ^{13}C - ^1H for Example

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

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

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

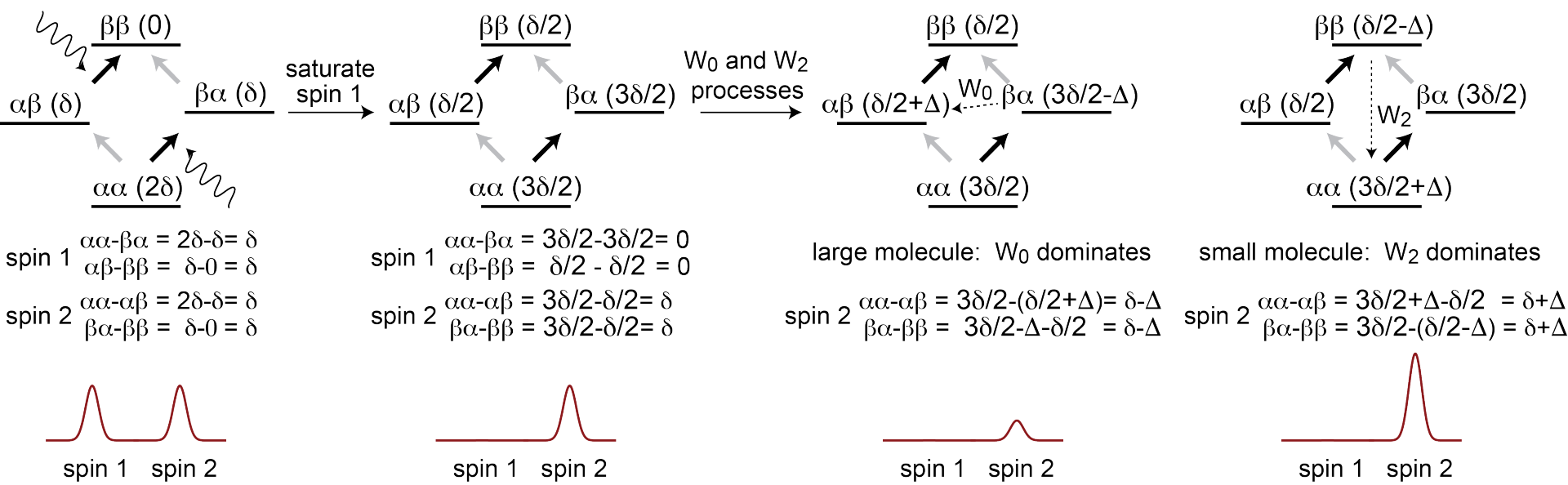
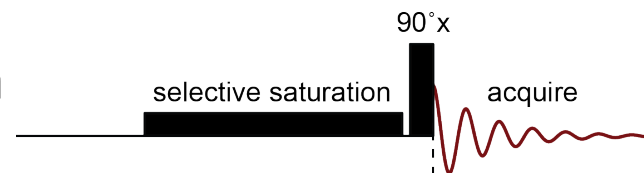
μ_0 is the permeability of free space (vacuum permeability) = $4\pi \times 10^{-7} \text{ kg m s}^{-2} \text{ A}^{-2}$ (A is Amperes)

$$\frac{R_{2S}}{R_{1S}} = \frac{T_1}{T_2} = \frac{1}{2} \frac{(4J(0) + J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I) + 6J(\omega_I + \omega_S))}{(J(\omega_I - \omega_S) + 3J(\omega_S) + 6J(\omega_I + \omega_S))}$$

- If τ_C is large (large molecule), $R_{2S} \gg R_{1S}$
- If τ_C is small (small molecule), $R_{2S} \rightarrow R_{1S}$
- If given R_{2S} and R_{1S} for a molecule with a spin pair at a known distance (1.1 Å for C-H), can use Stoke's formula to calculate the size of the molecule

The Nuclear Overhauser Effect (NOE)

- The nuclear Overhauser effect (NOE) is the change in intensity of a signal from one spin when the equilibrium spin populations of a neighboring spin are perturbed
- The *steady state* NOE is observed for a given spin when a neighboring spin is *selectively saturated*
- The result depends on relative rates of W_0 and W_2 (relaxation) processes
- Example (below): ^1H - ^1H NOE (very important for structural studies)
 - ^1H - ^1H NOE is large (remember, relaxation processes depend on $\gamma^2\gamma^2$)
 - here (below) δ is the population excess of α states over β states for ^1H
 - here Δ is the spin population transferred via W_0 or W_2 processes
 - saturate spin 1, see what happens to signal from spin 2



NOEs are Positive for Small Molecules, Negative for Large

- For the steady-state NOE experiment, the NOE enhancement, η , is:

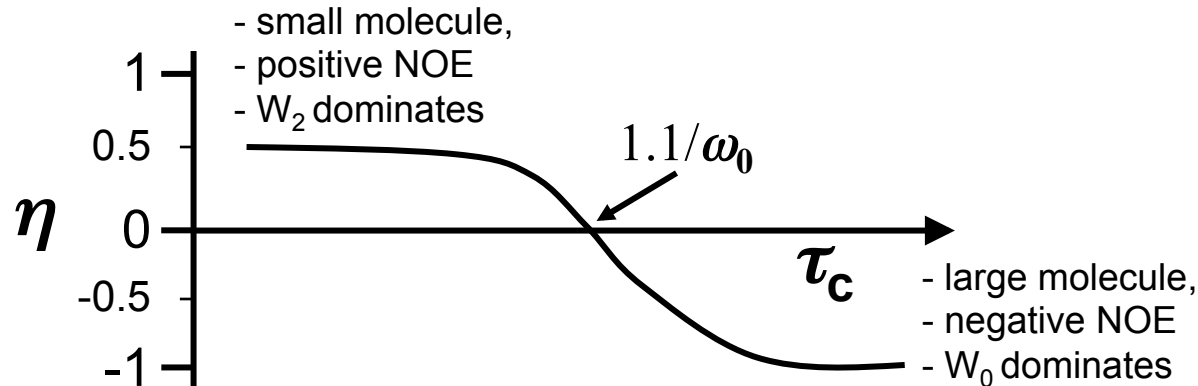
$$\eta = \left[-1 + \frac{6}{(1 + 4\omega_0^2\tau_c^2)} \right] / \left[1 + \frac{3}{(1 + \omega_0^2\tau_c^2)} + \frac{6}{(1 + 4\omega_0^2\tau_c^2)} \right]$$

- for small molecules ("extreme narrowing limit", $\omega_0\tau_c \ll 1$):

$$\eta = \left[-1 + \frac{6}{(1+0)} \right] / \left[1 + \frac{3}{(1+0)} + \frac{6}{(1+0)} \right] = \frac{5}{10} = 0.5$$

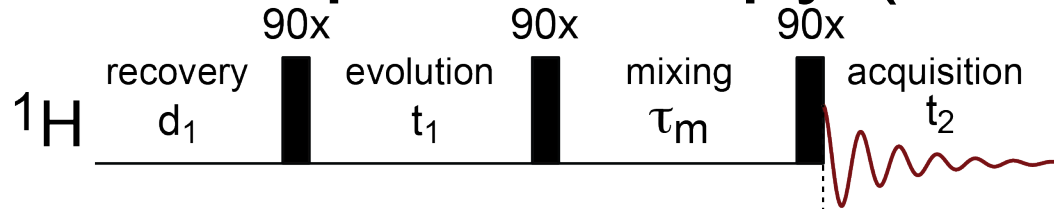
- for large molecules ("spin diffusion limit", $\omega_0\tau_c \gg 1$):

$$\eta = [-1 + 0] / [1 + 0 + 0] = -\frac{1}{1} = -1$$

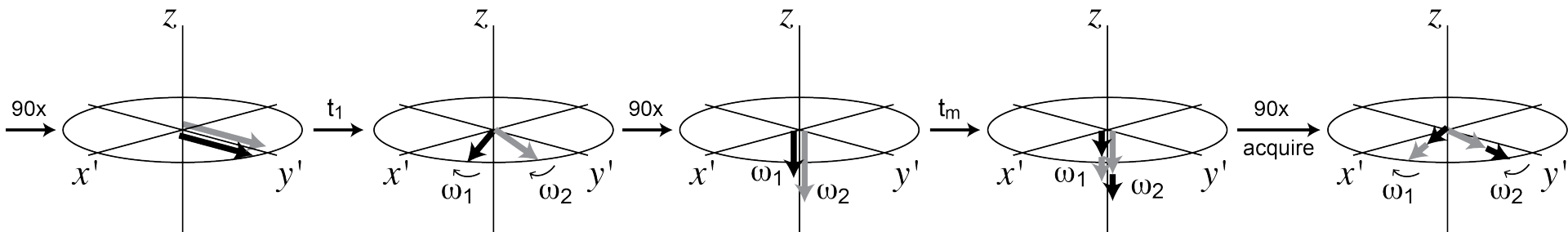


- Note that η can be 0; this is dependent on field strength
 - for example, at 800 MHz, for ~2000 Da, η is 0
 - rotating frame experiments ('ROESY') can be used to circumvent this (rotating frame mimics very low apparent field, like TOCSY, and all enhancements have the same sign)

2D NOE Spectroscopy (NOESY)



- 2D (3D) NOE experiments give crosspeaks that identify pairs of nuclei (${}^1\text{H}$) that are close in space, and whose intensities provide a measure of the distance between the nuclei
 - rather than saturating a single spin, all spins are inverted (90° - t_1 - 90°) to perturb the populations and allow for W_0 and W_2 processes
 - during t_1 , spin 1 precesses at ω_1 , spin 2 precesses at ω_2
 - the second 90° pulse puts y components along $-z$
 - during τ_m , population/magnetization transfer between these components can then occur via W_0 and W_2
 - the last 90° pulse gives transverse magnetization that evolves during t_2
 - diagonal peaks: during t_2 , some spin 1 magnetization that precessed at ω_1 during t_1 continues to precess at ω_1 (similar for spin 2)
 - crosspeaks: during t_2 , some spin 1 magnetization that precessed at ω_1 during t_1 now precess at ω_2 (similar for spin 2)



In Practice Data May be Collected from Cross Peaks at a Series of Mixing Times

- Intensities of NOE crosspeaks give distance information

$$I_{cp} = C\{\exp(-\rho T) \cdot (1 - \exp(-2\sigma T))\}$$

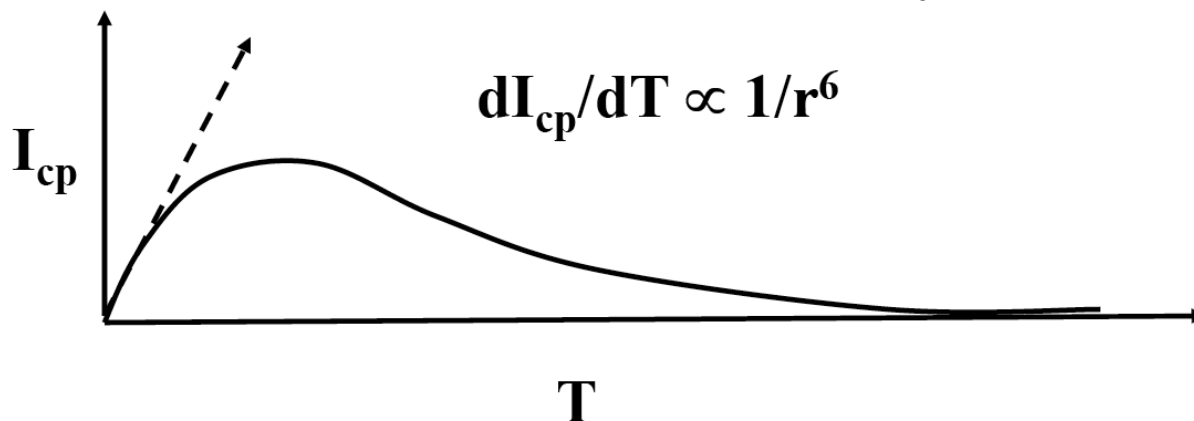
$$\rho = 2W_1 + W_2 + W_0$$

$$\sigma = (W_2 - W_0)$$

ρ : direct spin-lattice relaxation rate

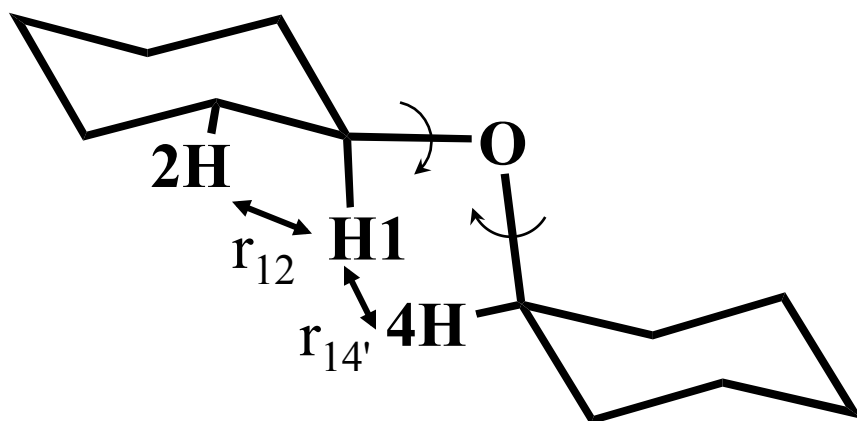
σ : cross-relaxation rate

- are dependent on mixing time (here 'T' rather than τ_m)
 - at very short T, $(1 - \exp(-2\sigma T)) \rightarrow 0$, so $I_{cp} \rightarrow 0$
 - if T is too long, magnetization is lost due to spin-lattice relaxation ($\exp(-\rho T)$)
- Crosspeak intensities are proportional to $1/r^6$ for short T
 - at shorter mixing times, intensities are approximately linear with T
 - if highly accurate measurements are needed, can vary T



NOEs Give Structural Information

- Intensities of NOE crosspeaks give distance information
- Example: disaccharide conformation
 - here a known distance (H2-H1, 2.5 Å) is used as an internal calibration standard
 - rotation about indicated bonds suggests many possible conformations: how close is H1 to H4 ?

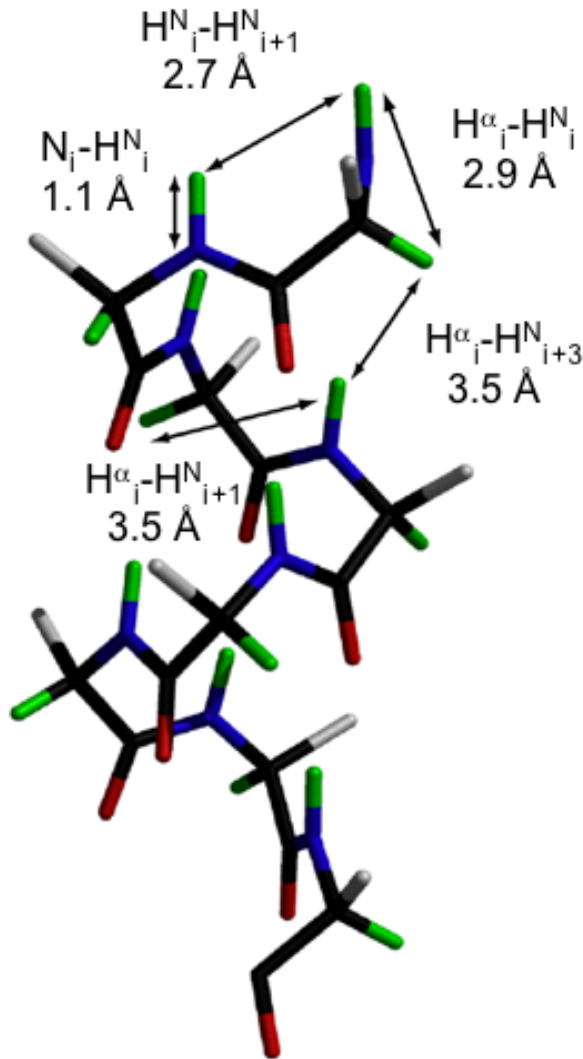


$$I_{14'} / I_{12} = r_{12}^6 / r_{14'}^6$$

$$r_{12} = 2.5 \text{ \AA}, \quad I_{14'} / I_{12} = 0.25, \quad \text{so: } r_{14'} = 3.15 \text{ \AA}$$

- Keep in mind that dynamics (conformational changes) during the mixing time can complicate the interpretation
 - for most structural studies NOE intensities are used in a more qualitative way

Potential NOE Interactions in an α -Helix



NOE interactions in
an idealized α -helix

- Sequential NOEs (NOEs between neighboring residues) define secondary structure
 - Short, well-defined ^1H - ^1H distances can be used to calibrate NOE intensities
 - In proteins, NOE intensities are usually converted to approximate distance ranges
 - “strong” 1.8-2.7 Å
 - “medium” 1.8-3.3 Å
 - “weak” 1.8-5.0 Å
 - “very weak” 1.8-6.0 Å
- (lower bound is sum of van der Waals radii for two protons)

NOESY Spectrum of Acyl Carrier Protein (ACP)

- Crosspeaks in NOE spectra give ^1H - ^1H distances
 - crosspeak intensities are approximately proportional to $1/r^6$
 - big crosspeaks, short distances, small crosspeaks, larger distances (up to approximately 5 or 6 Å or so)

