

# Rotational motion and diffusion

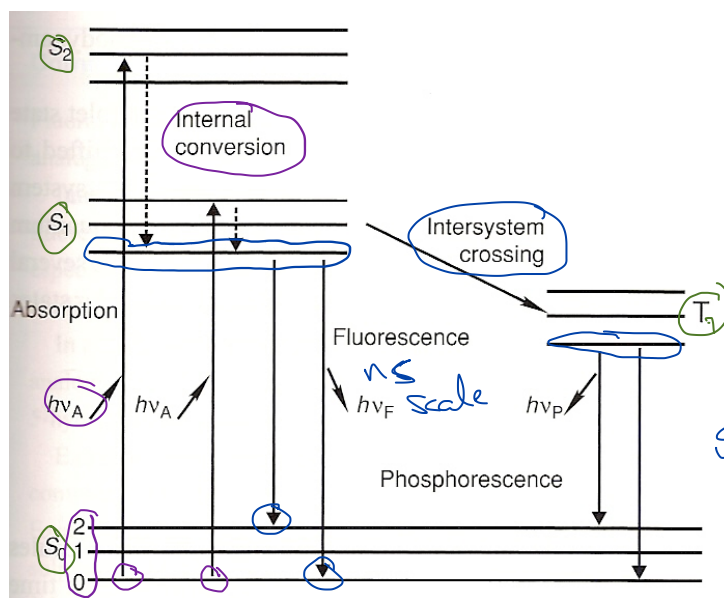
CCB 422/522, Spring, 2021

## 1 Rotational motion

What we studied last time probed translational diffusion. There is another set of experiments that see size and shape through the lens of rotational motion. We can still use the Einstein-Sutherland equation  $D = k_B T / f$ , where now  $D$  and  $f$  are rotational diffusion and friction coefficients. A big difference here, though, is that there are no concentration gradients for rotational motion.

In isotropic solution, all orientations are equally likely, and something must be done to observe rotational diffusion. As with translation, there are two broad categories of experiments. In one, the initial distribution is perturbed, creating a non-isotropic distribution of molecular orientations. Then the decay back to equilibrium is analyzed. In the second set of experiments, a force is applied to achieve partial orientation, and we solve an equilibrium problem where the external torque (which creates an oriented sample) is balanced by rotational diffusion (which tends to destroy such orientation). The first category includes **fluorescence depolarization** and **NMR relaxation**; the second includes **electric birefringence** and **flow birefringence**.

Starting with fluorescence, let's remember some basics:



Consider a rigid, isotropic sample. The probability of finding a molecule with its transition dipole pointing the  $\theta, \phi$  direction is proportional to  $\sin \theta d\theta d\phi$ . Now excite this with light polarized along the  $z$  direction: the excitation probability is proportional to  $|\mu \cdot \mathbf{E}|^2$ , or to  $\cos^2 \theta$ . Thus, the distribution of excited molecules will go like  $\cos^2 \theta \sin \theta d\theta d\phi$ . Normalizing this, we can write the distribution of excited states as

$$W(\theta, \phi, t) = [(3/4\pi) \cos^2 \theta \sin \theta d\theta d\phi] \exp(-t/\tau_F)$$

fluorescence time scale

At a subsequent time, the molecule may emit (fluoresce). Because the excited molecules are anisotropic, the emitted radiation will also be anisotropic. The emitted radiation polarized along  $z$  will be

$$I_{\parallel} \propto \iint \cos^2 \theta W(\theta, \phi, t) d\theta d\phi = (3/4\pi) \exp(-t/\tau_F) \iint \cos^4 \theta \sin \theta d\theta d\phi = (3/5) \exp(-t/\tau_F)$$

Radiation polarized on  $x$  (or along  $y$ ) will be

$$I_{\perp} \propto \iint \sin^2 \theta \cos^2 \phi W(\theta, \phi, t) d\theta d\phi = (3/4\pi) \exp(-t/\tau_F) \iint \cos^2 \phi \cos^2 \theta \sin^3 \theta d\theta d\phi = (1/5) \exp(-t/\tau_F)$$

There are two conventional ways to express this, as **polarization** or as **anisotropy**:

$$P = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + I_{\perp}); \quad A = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + 2I_{\perp}) \quad A(0) = \frac{3/5 - 1/5}{3/5 + 2/5} = 2/5$$

If there is no motion, then  $P(0) = 1/2$  and  $A(0) = 2/5$ . If the emission dipole moment is aligned at an angle  $\xi$  relative to the absorption dipole, so that  $\cos \xi = \mu \cdot \mu'$ , then

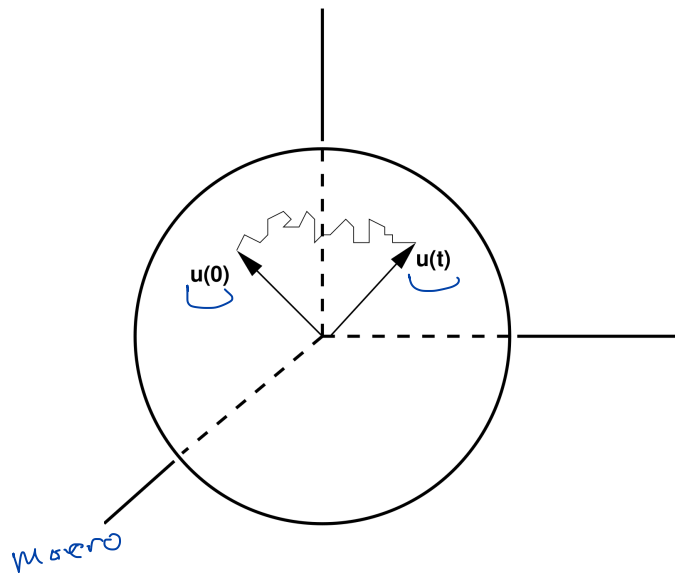
$$P = (3 \cos^2 \xi - 1) / (\cos^2 \xi + 3); \quad A = (3 \cos^2 \xi - 1) / 5 = (2/5) P_2(\cos \xi)$$

Now, for a **non-rigid** system, if we measure the time dependence of polarization of fluorescence, it will look like a time correlation function:

$$A(t) = (2/5) \langle P_2(\mu(0) \cdot \mu(t)) \rangle \exp(-t/\tau_F) = (2/5) \langle P_2(\cos \xi(t)) \rangle \exp(-t/\tau_F)$$

$P_2 = 2^{\text{nd}}$  order Legendre polynomial

## 2 Rotational diffusion



In a liquid, the molecule will suffer many small reorienting collisions with the solvent molecules, and hence  $\mathbf{u}$  should execute a "random walk" on the surface of the unit sphere. Let  $f(\mathbf{u}, t)$  be the probability density of having a molecule with orientation  $\mathbf{u}$  at time  $t$ ; hence  $f(\mathbf{u}, t) \sin \theta d\theta d\phi$  is the fraction of molecules in the solid angle  $\sin \theta d\theta d\phi$ . Debye proposed a diffusion equation:

cf. Fick's second law

$$\frac{\partial}{\partial t} f(\mathbf{u}, t) = D \nabla^2 f(\mathbf{u}, t) \quad \text{caveat: } D_{\text{rot}} \neq D_{\text{trans}} \quad (1)$$

Let's use spherical polar coordinates  $(r, \theta, \phi)$ , where  $r = 1$ . The Laplacian operator is then

$$\nabla^2 = \frac{1}{\sin^2 \theta} \left[ \sin \theta \frac{\partial}{\partial \theta} \left( \sin \theta \frac{\partial}{\partial \theta} \right) + \frac{\partial^2}{\partial \theta^2} \right] \equiv -\hat{I}^2 \quad (2)$$

$\hat{I} = \text{integral tensor}$

$$\frac{\partial}{\partial t} f(\mathbf{u}, t) = -D \hat{I}^2 f(\mathbf{u}, t) \quad (3)$$

The formal solution of this differential equation is easily written down:

matrix exponential solution  $\Rightarrow$  finding eigenvalues + eigenvectors

$$f(\mathbf{u}, t) = \exp(-Dt\hat{I}^2)f(\mathbf{u}, 0) \quad (4)$$

They can be written in terms of the eigenfunctions of  $\hat{I}^2$ , which are the spherical harmonics:

$$\hat{I}^2 Y_{lm}(\mathbf{u}) = l(l+1) Y_{lm}(\mathbf{u}) \quad (5)$$

$$\exp(-Dt\hat{I}^2) Y_{lm}(\mathbf{u}) = \exp(-l(l+1)Dt) Y_{lm}(\mathbf{u}) \quad \leftarrow$$

The particular solution to Eq. 3 we want here corresponds to an initial condition where  $\mathbf{u}$  is sharply peaked at  $\mathbf{u}_0$ :

$$f(\mathbf{u}, 0) = \left(\frac{1}{4\pi}\right) \delta(\mathbf{u} - \mathbf{u}_0) = \left(\frac{1}{4\pi}\right) \sum_{lm} Y_{lm}(\mathbf{u}_0) Y_{lm}^*(\mathbf{u}) \quad (6)$$

summation formula for spherical harmonics

where the final equality exploits the closure relation of the spherical harmonics. Combining this with Eq. 4 gives:

$$f(\mathbf{u}, t) = \sum_{lm} \exp(-l(l+1)Dt) Y_{lm}(\mathbf{u}_0) Y_{lm}^*(\mathbf{u}) \quad (7)$$

$\rightarrow$  The time-correlation functions needed for NMR relaxation only require the second moments of this distribution. If  $F_n(\mathbf{u}) \sim (4\pi/5)^{1/2} Y_{2n}(\mathbf{u})$ :

$$\begin{aligned} \langle F_n(\mathbf{u}_0) F_n^*(\mathbf{u}) \rangle &\sim \left(\frac{1}{4\pi}\right) \int d^2u_0 \int d^2u \left[\frac{4\pi}{5}\right] Y_{2n}(\mathbf{u}_0) Y_{2n}^*(\mathbf{u}) \\ &= \left(\frac{1}{5}\right) \exp(-6D\tau) \rightarrow l(l+1) \text{ when } l=2 \end{aligned}$$

Hence, for a rigid molecule undergoing such isotropic Brownian rotational motion, the normalized  $F_n$  time-correlation functions for all internuclear vectors all decay in the same way, as

measure  $A(t)$  yields

$$C^{rot}(\tau) = \langle P_2(\mathbf{u}_0 \cdot \mathbf{u}) \rangle = \sum_{n=-2}^{n=2} \langle F_n(\mathbf{u}_0) F_n^*(\mathbf{u}) \rangle = \exp(-\tau/\tau_c) \quad (8)$$

where the time constant  $\tau_c$  is  $1/6D = V_h\eta/k_B T$ . (For later): its Fourier transform is then a Lorentzian:

microscopic result

$$j^{rot}(\omega) \equiv \frac{2}{5} \int_0^\infty (\cos \omega t) C(t) dt = \left(\frac{2}{5}\right) \frac{\tau_c}{1 + \omega^2 \tau_c^2} \quad (9)$$

Lorentzian function (Aside for now)

### 3 Example: fluorescence depolarization in DNA

#### Dynamics of Mismatched Base Pairs in DNA<sup>†</sup>

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Received July 10, 1990; Revised Manuscript Received December 12, 1990

AP = modified adenine

**ABSTRACT:** The structural dynamics of mismatched base pairs in duplex DNA have been studied by time-resolved fluorescence anisotropy decay measurements on a series of duplex oligodeoxynucleotides of the general type d[CGG(AP)GGC]-d[GCCXCCG], where AP is the fluorescent adenine analogue 2-aminopurine and X = T, A, G, or C. The anisotropy decay is caused by internal rotations of AP within the duplex, which occur on the picosecond time scale, and by overall rotational diffusion of the duplex. The correlation time and angular range of internal rotation of AP vary among the series of AP-X mismatches, showing that the native DNA bases differ in their ability to influence the motion of AP. These differences are correlated with the strength of base-pairing interactions in the various AP-X mismatches. The interactions are strongest with X = T or C. The ability to discern differences in the strength of base-pairing interactions at a specific site in DNA by observing their effect on the dynamics of base motion is a novel aspect of the present study. The extent of AP stacking within the duplex is also determined in this study since it influences the excited-state quenching of AP. AP is thus shown to be extrahelical in the AP-G mismatch. The association state of the AP-containing oligodeoxynucleotide strand is determined from the temperature-dependent tumbling correlation time. An oligodeoxynucleotide triplex is formed with a particular base sequence in a pH-dependent manner.

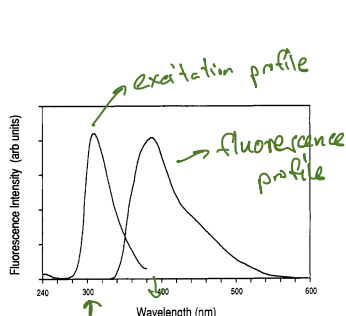


FIGURE 3: Steady-state fluorescence excitation (at left) and emission (at right) spectra of 50 μM d[CGG(AP)GGC]-d[GCC(T)CCG] in 50 mM Tris-HCl, pH 7.4, and 0.15 M NaCl at 20 °C. In the excitation spectrum, the emission is observed at 380 nm, while in the emission spectrum the excitation wavelength is 320 nm. The band-pass is 5 nm for both excitation and emission.

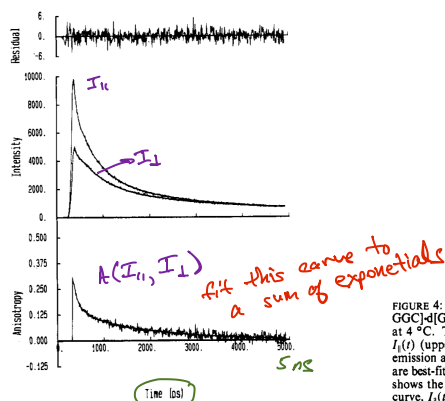


FIGURE 4: Time-resolved emission depolarization of d[CGG(AP)GGC]-d[GCCGCCG] in 50 mM Tris-HCl, pH 7.4, and 0.15 M NaCl at 4 °C. The center panel shows the polarized emission intensities  $I_{\parallel}(t)$  (upper curve) and  $I_{\perp}(t)$  (lower curve). The time-dependent emission anisotropy is shown in the lower panel. The smooth lines are best-fit curves generated as described in the text. The upper panel shows the weighted deviation between the experimental difference curve,  $I_{\parallel}(t) - I_{\perp}(t)$ , and the best-fit difference curve.

internal motion overall rotation

Table II: Anisotropy Decay Parameters<sup>a</sup>

X <sup>b</sup>	T (°C) (±0.5)	$\tau_{r1}$ (ps) (±15)	$\tau_{r2}$ (ns) (±0.07)	$r_{01}$ (±0.015)	$r_{02}$ (±0.015)	$r_0$ (±0.030)
T WC duplex	40	60	0.50	0.210	0.127	0.337
	30	73	0.65	0.229	0.127	0.356
	20	85	0.80	0.227	0.117	0.344
	10	100	1.10	0.235	0.106	0.341
	4	124	1.70	0.249	0.099	0.348

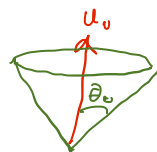
$$A(t) = (2/5) [S^2 + (1 - S^2) \exp(-t/\tau_i)] \exp(-t/\tau_c) \exp(-t/\tau_F)$$

$S^2 \equiv$  order parameter

$S^2 = 1$  no extra time dependence = rigid

$S^2 < 1$  more + more ps time scale motion

"model-free" analysis : no assumed type of internal motion  
 $\Rightarrow$  intermediate stage



diffusion in a cone model

**Table III: Correlation Time, Order Parameter, and Cone Angle for Restricted Motion of 2-Aminopurine<sup>a</sup>**

$X^b$	$T$ (°C) (±0.5)	$\tau_i$ (ps) (±15)	$S$ (±0.070)	$\theta_0$ (deg) (±3)
T	40	68	0.614	44
	30	82	0.597	45
	20	95	0.583	46
	10	110	0.558	48
	4	135	0.533	50

model-free

model-specific

→ interpreting this in semantic terms

## 4 Steady-state fluorescence polarization

Instead of measuring the time-dependence of polarization, it is usually easier to look at the averages, or steady-state values under constant illumination:

$$\langle I_{\parallel} \rangle = \tau_F^{-1} \int_0^{\infty} I_{\parallel}(t) dt; \quad \langle I_{\perp} \rangle = \tau_F^{-1} \int_0^{\infty} I_{\perp}(t) dt$$

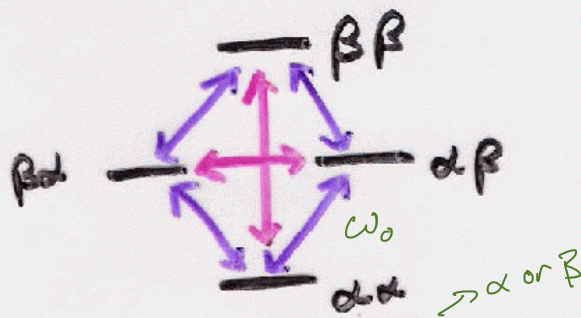
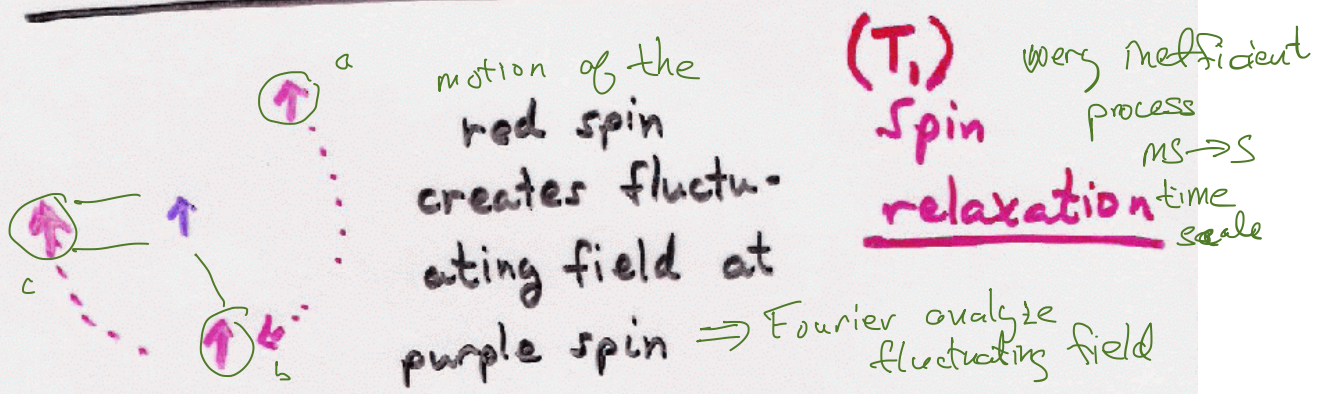
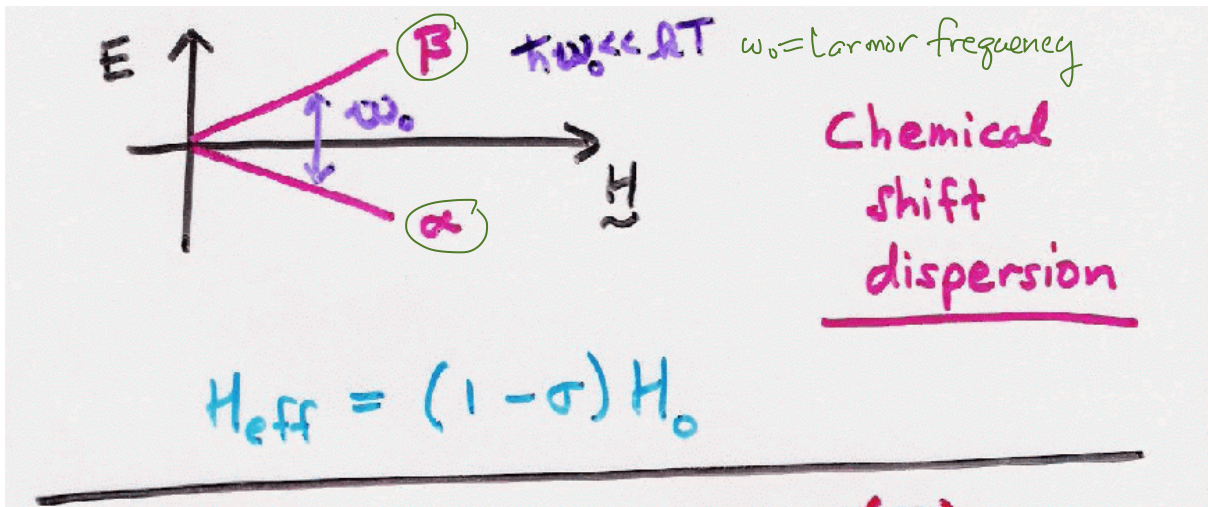
→ averaging over all time delays

Note that the amount of depolarization will depend on the ratio of  $\tau_c$  (how fast the molecule tumbles) to  $\tau_F$  (how long it takes on average to fluoresce). Plugging our previous values in (ignoring internal motion for simplicity for now):

$$\langle A \rangle^{-1} = (5/2)(1 + \tau_F/\tau_c) = (5/2)(1 + \tau_F k_B T / V_h \eta)$$

This is called the Perrin equation.

# 5 NMR relaxation



Fourier components at  $\sim 0, \omega_0, 2\omega_0$  will cause spin transitions

$M_{zi} = \text{magnetization} = \langle \hat{\mu}_{zi} \rangle$

continuous variable

Spectral density  $J(\omega)$

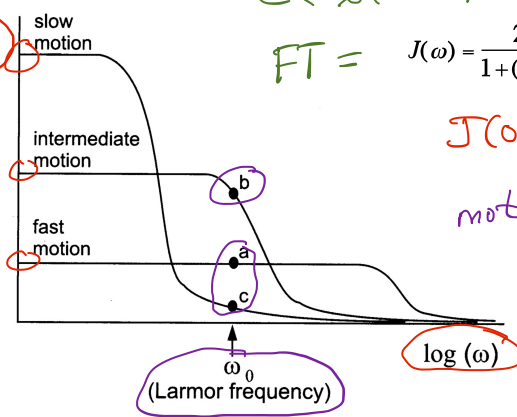
slow motion

intermediate motion

fast motion

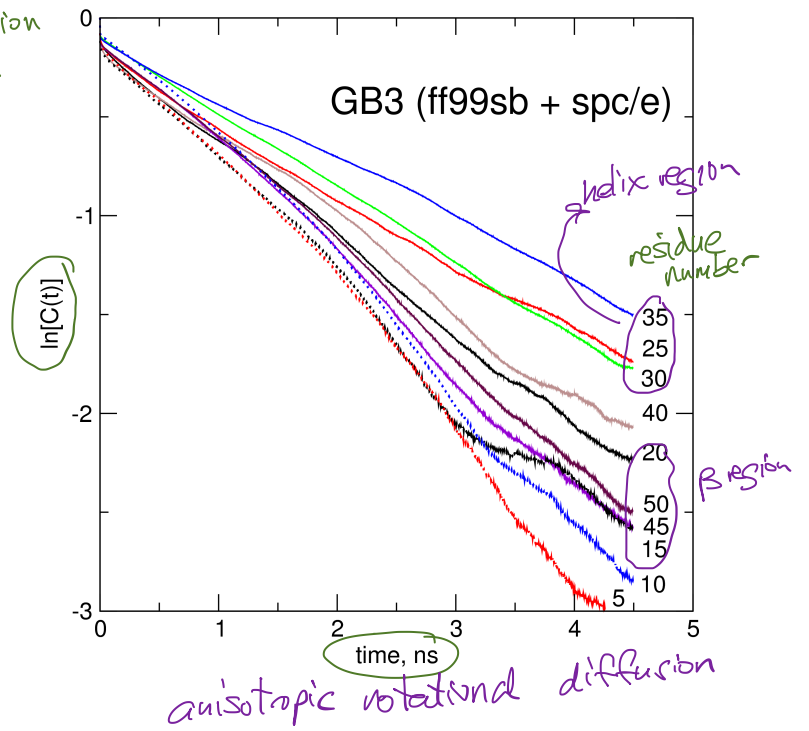
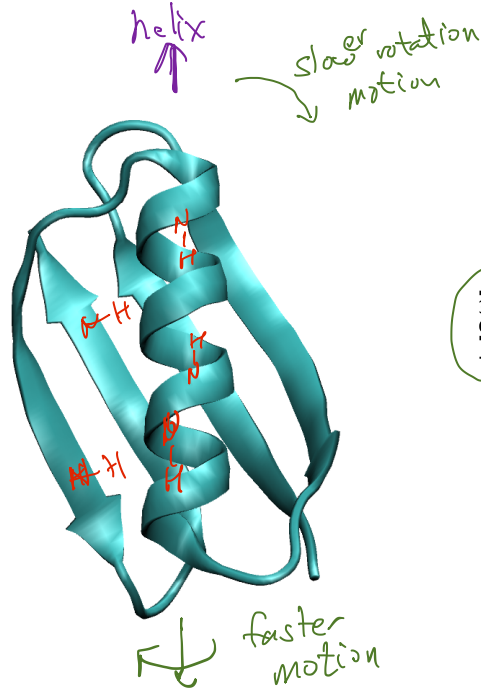
FT =  $J(\omega) = \frac{2\tau_c}{1 + (\omega\tau_c)^2} = \text{Lorentzian function}$

$J(0) = 2\tau_c$

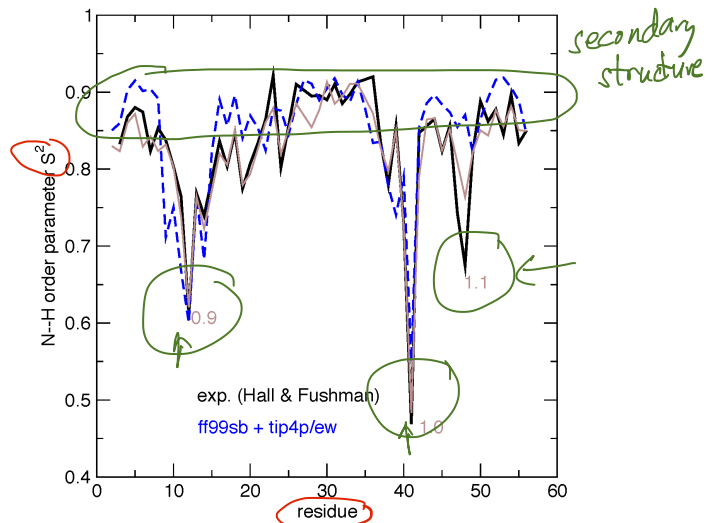
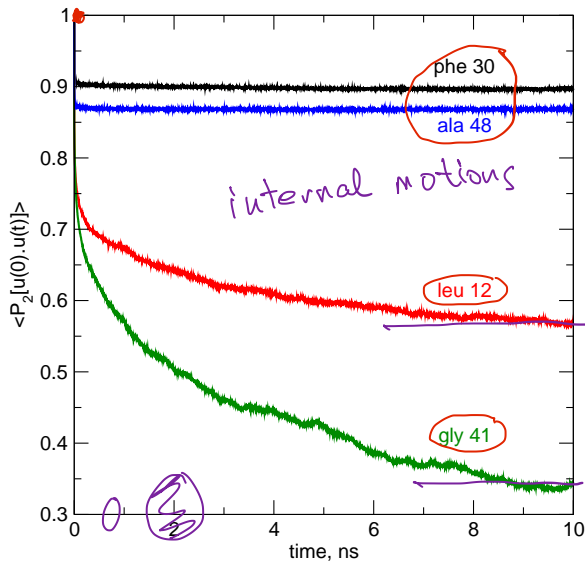


motion on the time scale of the Larmor frequency has the greatest chance of causing transitions

### 5.1 Example for protein GB1: overall tumbling



### 5.2 Consider internal motions



$$c(\tau) = \sum_i [S^2 + (1-S^2)e^{-\tau/\tau_i}] e^{-\tau/\tau_R}$$

always: very fast vibrational motion sub-ps time scale

residues 30, 41:  $S^2 = 1$   
 12, 41:  $S^2 \sim .4 \rightarrow .6$   $\tau_i \approx 1$  ns