Overview of Protein Dynamics Studies Using NMR

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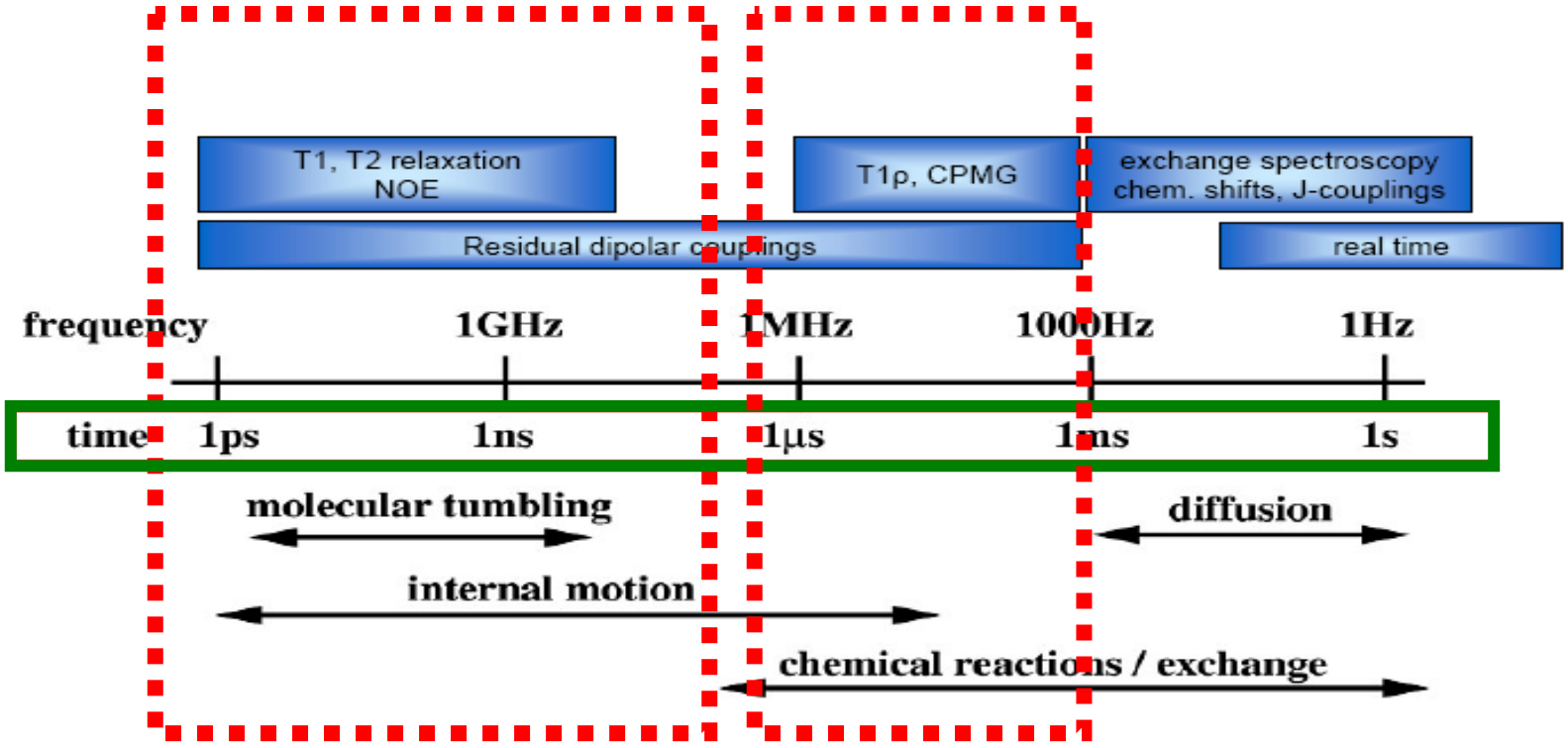
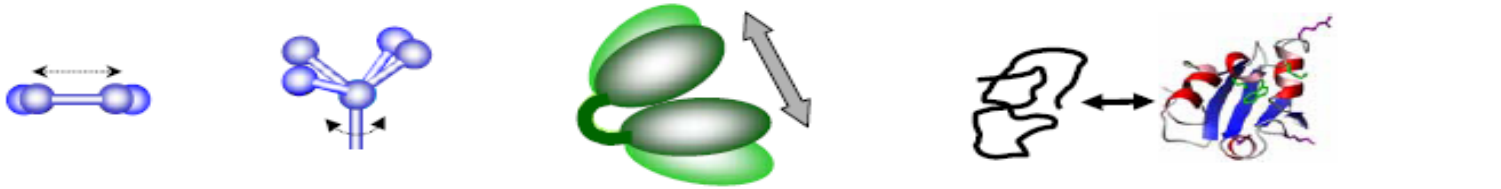
NMR Time Scales for Protein Dynamics Studies

(From http://www.embl.de/nmr/sattler/teaching)





bond vibrations | *side chain rotations* | *domain movements* | *enzyme kinetics* | *folding, H/D exchange*

Part I. Study pico-sec to nano-sec motion

Step 1: Set up T1, T2, NOE Experiments

(1-1) T1 Experiment

<Option 1: Collect a series of 2D expts with different delay time>

- Experiment Type: NH T1
- Standard Parameter Set: std1_2D_15N-T1_hsqct1etf3gpsi
- Pulse Program: hsqct1etf3gpsi

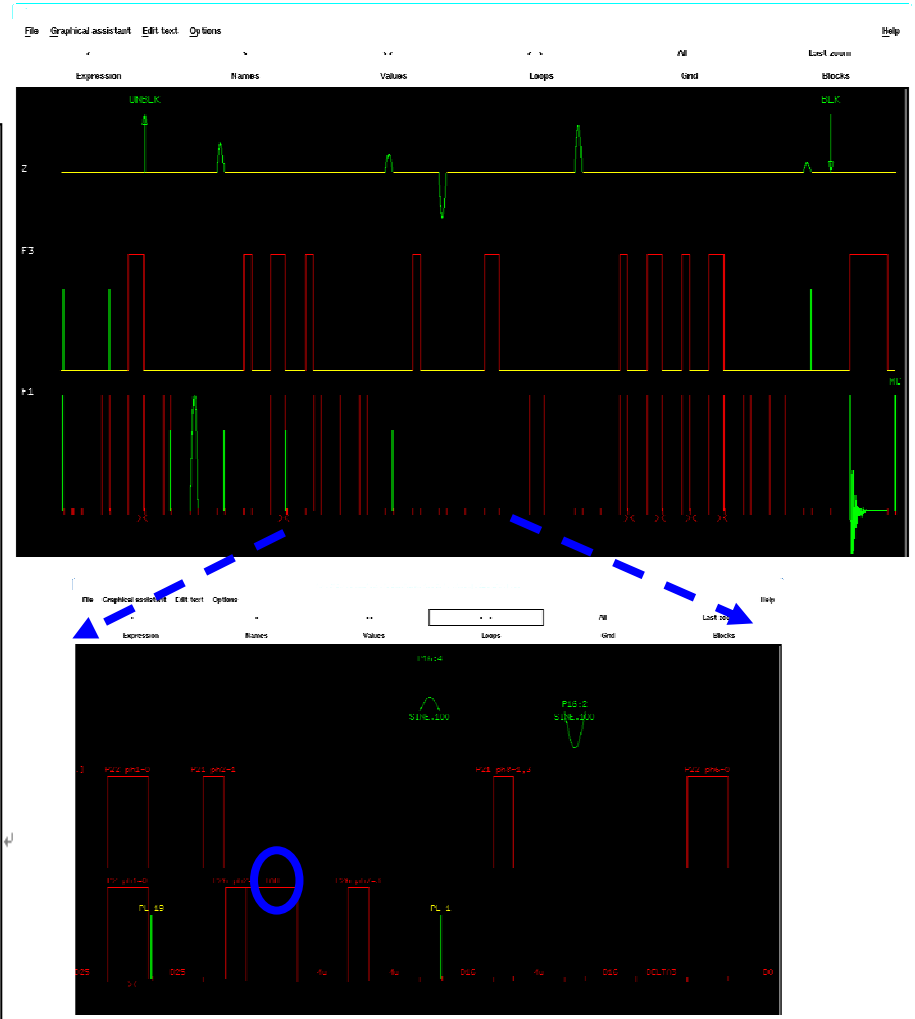
- Easy Set Up Steps:

(1)rpar std1_2D_15N-T1_hsqct1etf3gpsi

(2)getprosol 1H (us) (db)

(3)d7 : delay for inversion recovery in sec (different d7 for different expts)

(4)13C/15N sample: ZGOPTINS -DLABEL_CN



(T1 delay → TAU value→ d7)

(1-1) T1 Experiment

<Option 2: Collect a set of pseudo 3D expt using vd-list for different delay time>

- Experiment Type: NH T1 pseudo3D
- Standard Parameter Set: std1_3D_15N-T1_hsqct1etf3gpsi3d.2
- Pulse Program: hsqct1etf3gpsi3d.2

- Easy Set Up Steps:

(1) rpar std1_3D_15N-T1_hsqct1etf3gpsi3d.2

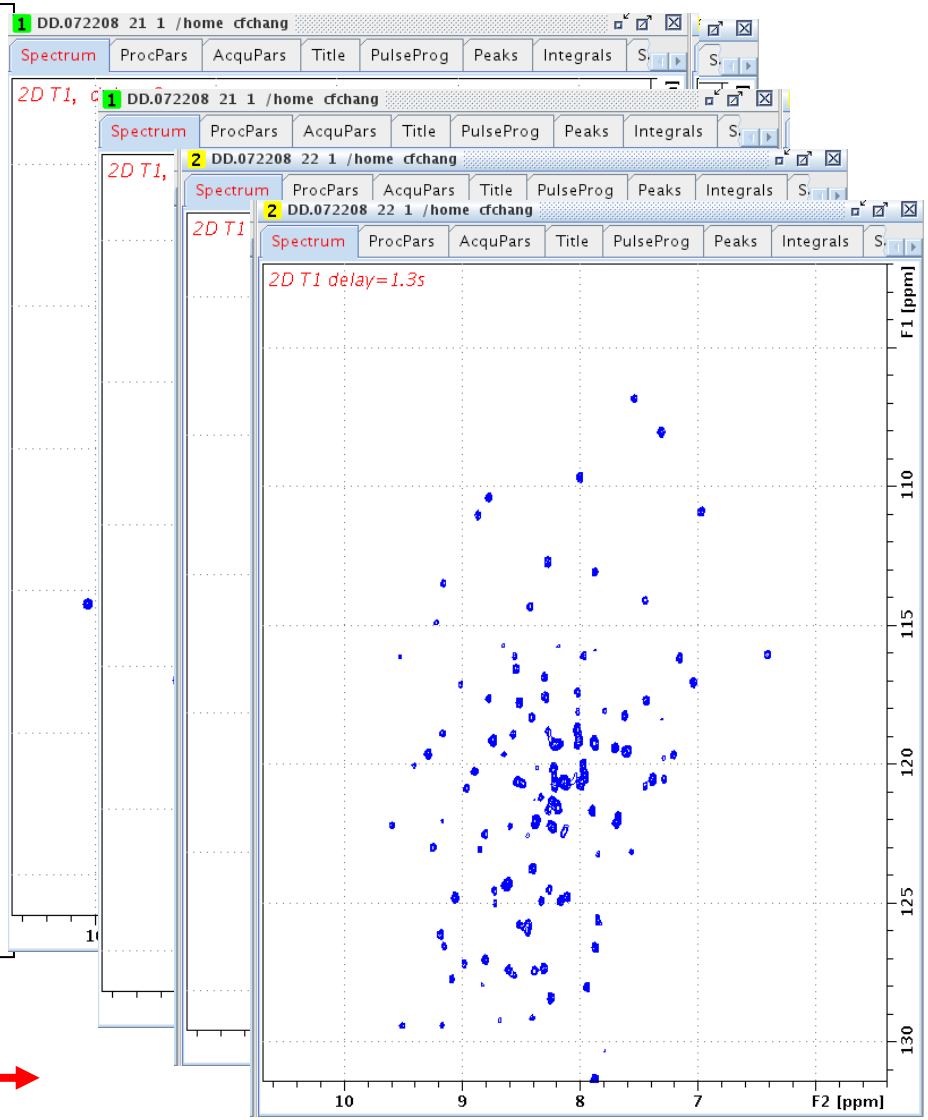
(2) getprosol 1H (us) (db)

(3) edit vd-list : delay in sec, ex: std_t1 (ex: d7 in 2D version)

(4) NBL: number of delays in vd-list

(5) td1: number of delays in vd-list (QF)

(6) 13C/15N sample: ZGOPTINS -DLABEL_CN



Increasing delays

(1-2) T2 Experiment

<Option 1: Collect a series of 2D expts with different delay time>

- Experiment Type: NH T2
- Standard Parameter Set: std1_2D_15N-T2_hsqct2etf3gpsi
- Pulse Program: hsqct2etf3gpsi

- Easy Set Up Steps:

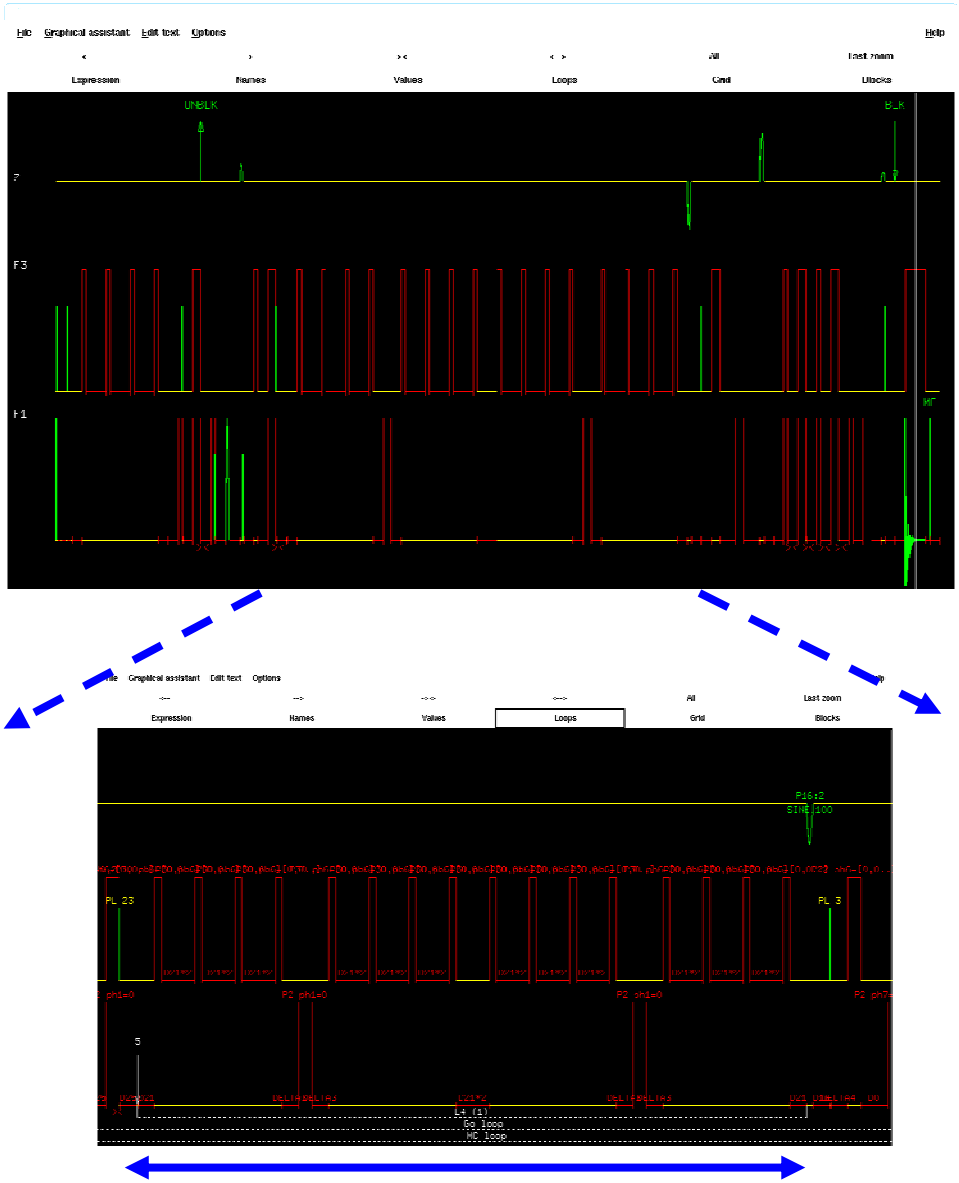
(1)par std1_2D_15N-T2_hsqct2etf3gpsi

(2)getprosol 1H (us) (db)

(3)d20 : T2 delay sec (different d20 for different expts)

(4)L5: loop for compensation for heating effects

(5)13C/15N sample: ZGOPTINS -DLABEL_CN



(T2 delay → loop number → d20)

(1-2) T2 Experiment

<Option 2: Collect a set of pseudo 3D expt using vc-list for different delay time>

- Experiment Type: NH T2 pseudo3D
- Standard Parameter Set: std1_3D_15N-T2_hsqct2etf3gpsi3d
- Pulse Program: hsqct2etf3gpsi3d

- Easy Set Up Steps:

(1) rpar std1_3D_15N-T2_hsqct2etf3gpsi3d

(2)getprosol 1H (us) (db)

(3)edit vc-list : loop counter for T2 delay

(ex: c =d20/(p30*16+d21*32) in 2D version)

(4)NBL: number of delays in vc-list

(5)td1: number of delays in vd-list (QF)

(6)13C/15N sample: ZGOPTINS -DLABEL_CN

Increasing delays

(1-3) heteronuclear NOE Experiment

<Option : Collect an “interleave” expt for non-saturated and saturated spectra

- Experiment Type: NH NOE
- Standard Parameter Set: std1_2D_15N-NOE_hsqcnoef3gpsi
- Pulse Program: hsqcnoef3gpsi

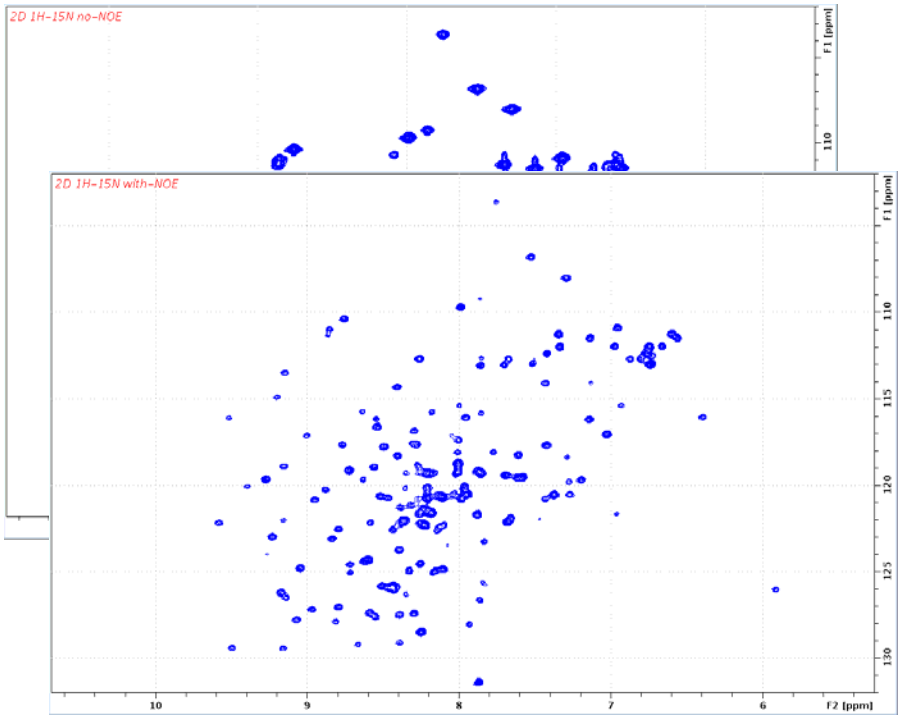
- Easy Set Up Steps:

(1) rpar std1_2D_15N-NOE_hsqcnoef3gpsi

(2) getprosol 1H (us) (db)

(3) D1 (ex: 5 sec)

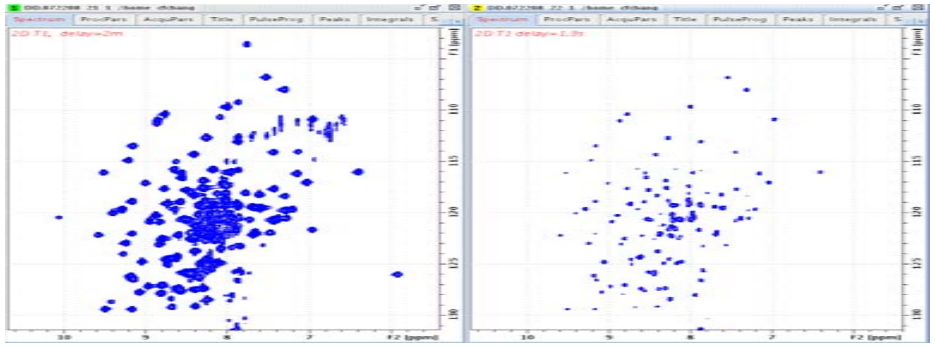
(4) Note for process: xau splitinvnoe



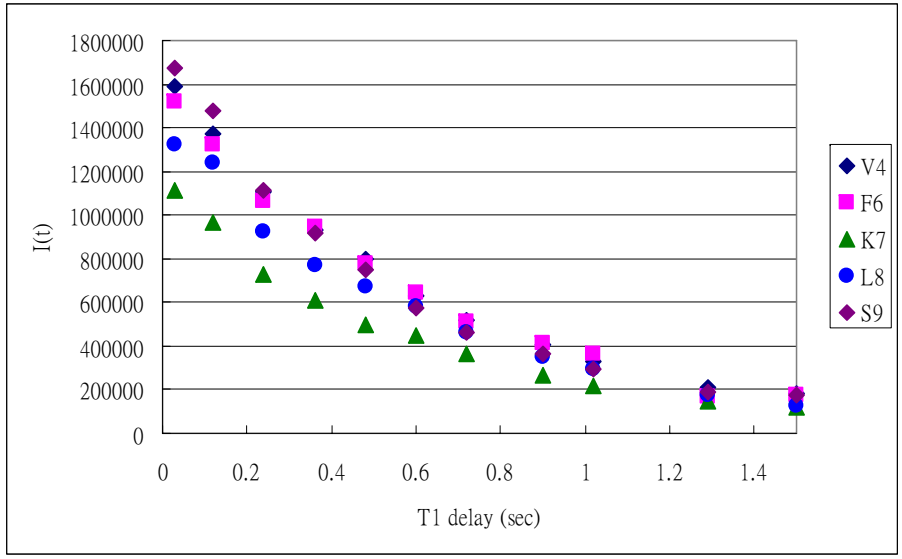
Part I. Study pico-sec to nano-sec motion

Step 2: Determine T1, T2, NOE Values for each residue

(2-1) T1 value from Curve Fitting

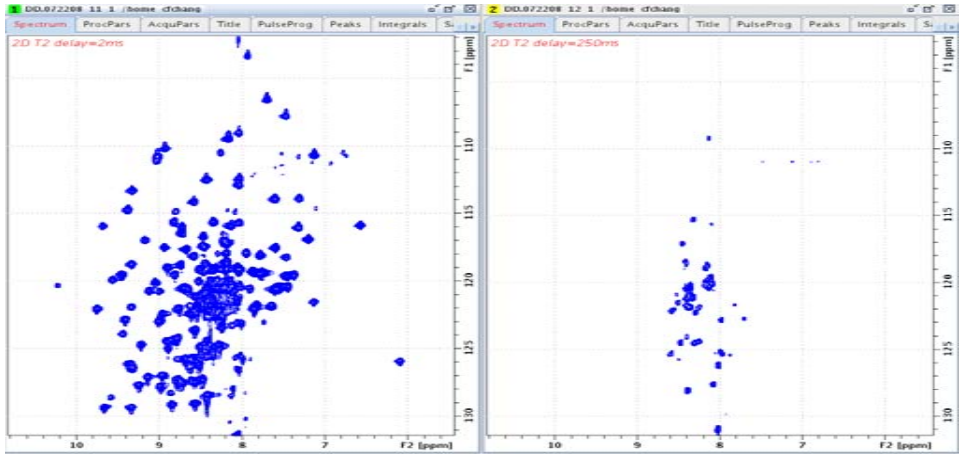


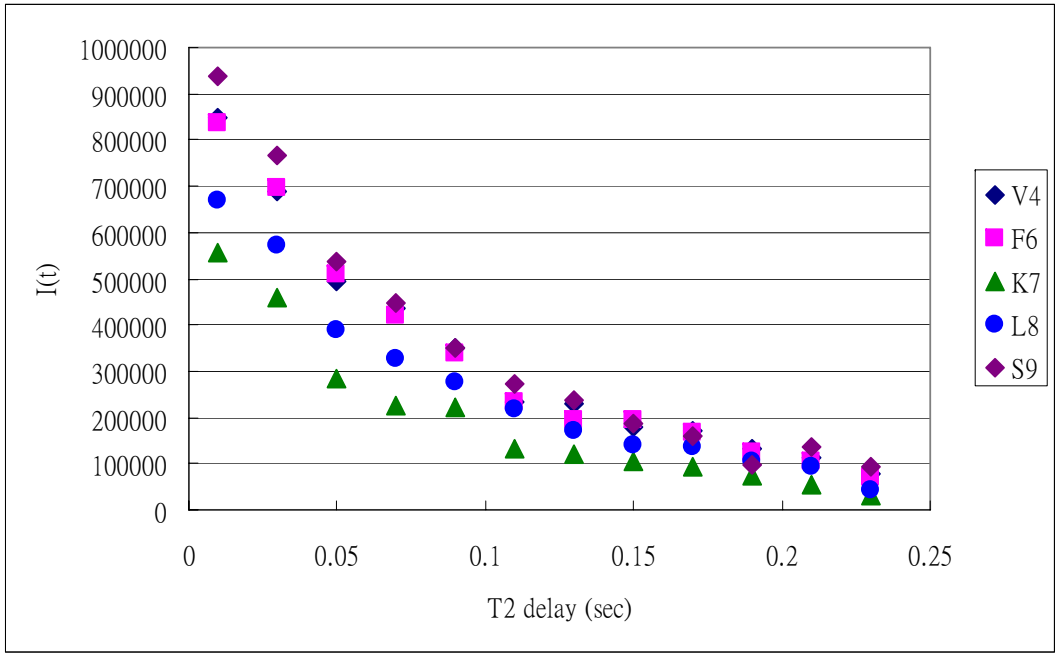
$$I(t)=I_0\exp(-t/T_1)$$
$$R_1=1/T_1$$



	R1	dR1	Goodness of Fit
V4	1.5876	0.022816	0.99891
F6	1.5273	0.038721	0.99658
K7	1.651	0.051077	0.99498
L8	1.5583	0.05192	0.99416
S9	1.8034	0.045471	0.99682

(2-2) T2 Value from Curve Fitting



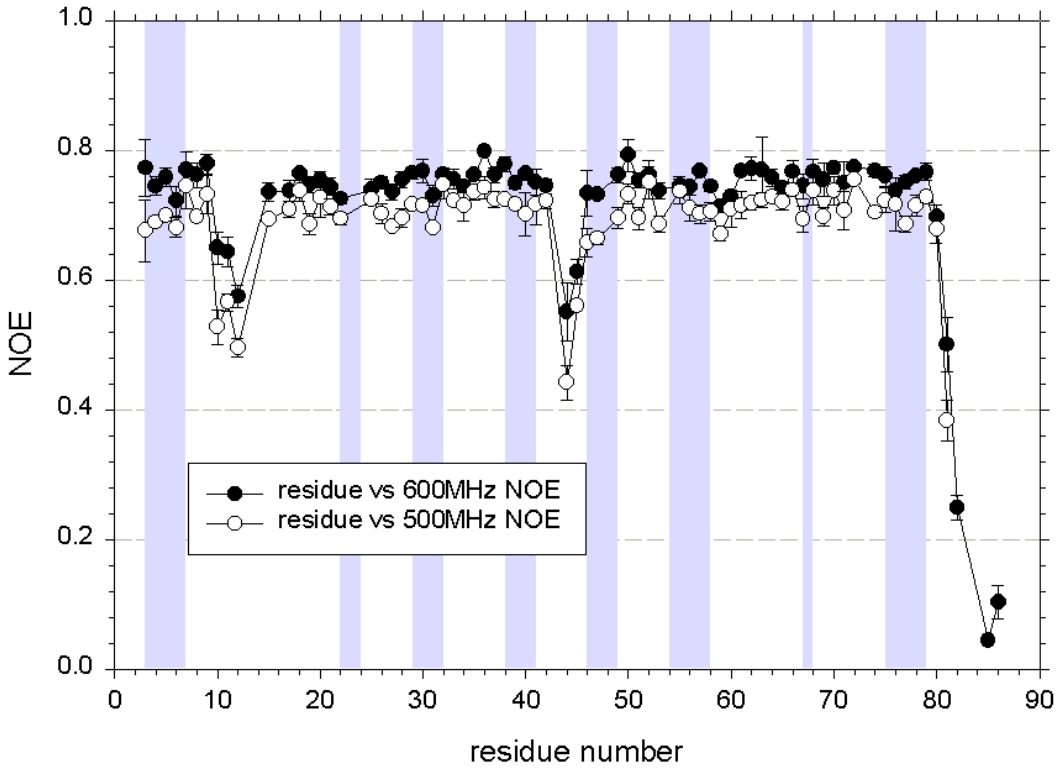


$$I(t)=I_0\exp(-t/T_2)$$
$$R_2=1/T_2$$

	R2	dR2	Goodness of Fit
V4	11.015	0.4209	0.99082
F6	11.274	0.39935	0.99225
K7	12.834	0.77788	0.97916
L8	11.122	0.4692	0.98897
S9	11.751	0.44541	0.99127

(2-3) heteronuclear NOE Values

NOE : intensity ratio ($I_{H,sat}/I_{un-sat}$)



	{noe}	STDEV
V4	0.743406	0.015822
F6	0.755803	0.027293
K7	0.768606	0.012184
L8	0.738638	0.033837
S9	0.761837	0.026417

Part I. Study pico-sec to nano-sec motion

Step 3: Dynamics Parameters from T1 (1/R1) , T2 (1/R2), NOE

(3-1) Reduced Spectral Density Mapping : $J(0.87\omega_H)$, $J(\omega_N)$, $J(0)$

From R1, R2, NOE values

$\sigma_{NH} = R_1(NOE-1)(\gamma_H/\gamma_N)$

$J(0.87\omega_H) = 4\sigma_{NH}/(5d^2)$

$J(\omega_N) = (4R_1 - 5\sigma_{NH})/(3d^2+4c^2)$

$J(0) = (6R_2 - 3R_1 - 2.72\sigma_{NH})/(3d^2+4c^2)$

(Farrow et al, 1995)

Reduced Spectral density function mapping

Obtain parameter $J(0)$, $J(\omega_N)$, $J(0.87\omega_H)$, S^2, τ_μ and R_{ex} for each residue

- $J(0)$, $J(\omega_N)$ decrease: Greater internal motion
- $J(0.87\omega_H)$ increase: Greater internal motion
- Significantly increased $J(0)$: Chemical exchange

(3-2) Model Free Analysis: S^2, τ, Rex

From R1, R2, NOE values to Spectral Density Function

$$R_1=(d^2/4)[J(\omega_H-\omega_N)+3J(\omega_N)+6J(\omega_H+\omega_N)]+c^2J(\omega_N)$$

$$R_2=(d^2/8)[4J(0)+J(\omega_H-\omega_N)+3J(\omega_N)+6J(\omega_H)+6J(\omega_H+\omega_N)]+(c^2/6)[4J(0)+3J(\omega_N)]+R_{ex}$$

$$NOE=1+(d^2/4R_1)(\gamma_H/\gamma_N)+[6J(\omega_H+\omega_N)-J(\omega_H-\omega_N)]$$

in which $d=\mu_0 h\gamma_X\gamma_H\left\langle r_{XH}^{-3}\right\rangle/(8\pi^2)$, $c=\omega_X\Delta\sigma/\sqrt{3}$, μ_0 is the permeability of free space; h is Planck's constant; γ_H and γ_X are the gyromagnetic ratios of ^{1}H and the X spin (X=^{13}C or ^{15}N), respectively; r_{XH} is the X-H bond length; ω_H and ω_X are the Larmor frequencies of ^{1}H and X spins, respectively; and $\Delta\sigma$ = is the chemical shift anisotropy of the X spin (assuming an axially

(Clore et al., 1990)

From Spectral Density Function J(ω) to dynamics parameters

$$J(\omega)=\frac{2}{5}\left[\frac{S^2\tau_m}{1+(\omega\tau_m)^2}+\frac{\left(S_f^2-S^2\right)\tau}{1+(\omega\tau)^2}\right]$$

$$=\frac{2}{5}S_f^2\left[\frac{S_s^2\tau_m}{1+(\omega\tau_m)^2}+\frac{\left(1-S_s^2\right)\tau}{1+(\omega\tau)^2}\right]$$

$$S^2=S_f^2S_s^2$$

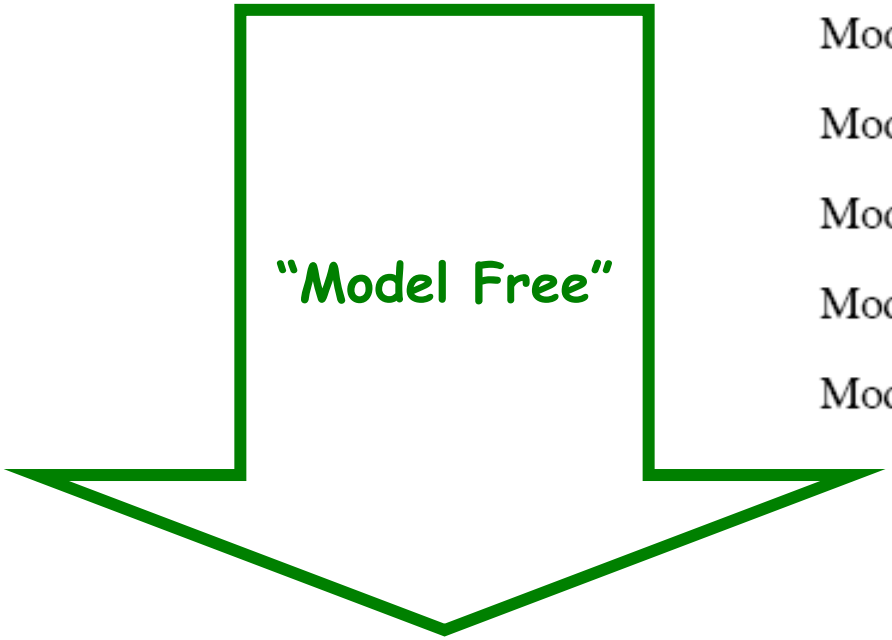
$$\tau=\tau_s\tau_m/(\tau_s+\tau_m)$$

in which $\tau=\tau_s\tau_m/(\tau_s+\tau_m)$, τ_m is the isotropic rotational correlation time of the molecule, τ_s is the effective correlation time for internal motions, $S^2=S_f^2S_s^2$ is the square of the generalized order parameter characterizing the amplitude of the internal motions, and S_f^2 and S_s^2 are the squares of the order parameters for the internal motions on the fast and slow time scales, respectively. Generalized

(Model-Free Manual --Palmer)

(3-2) Model Free Analysis: S^2, τ, Rex

From R1, R2, NOE values , and based on Spectral Density Function J(ω)



Model 1: S2s

Model 2: S2s and te

Model 3: S2s and Rex

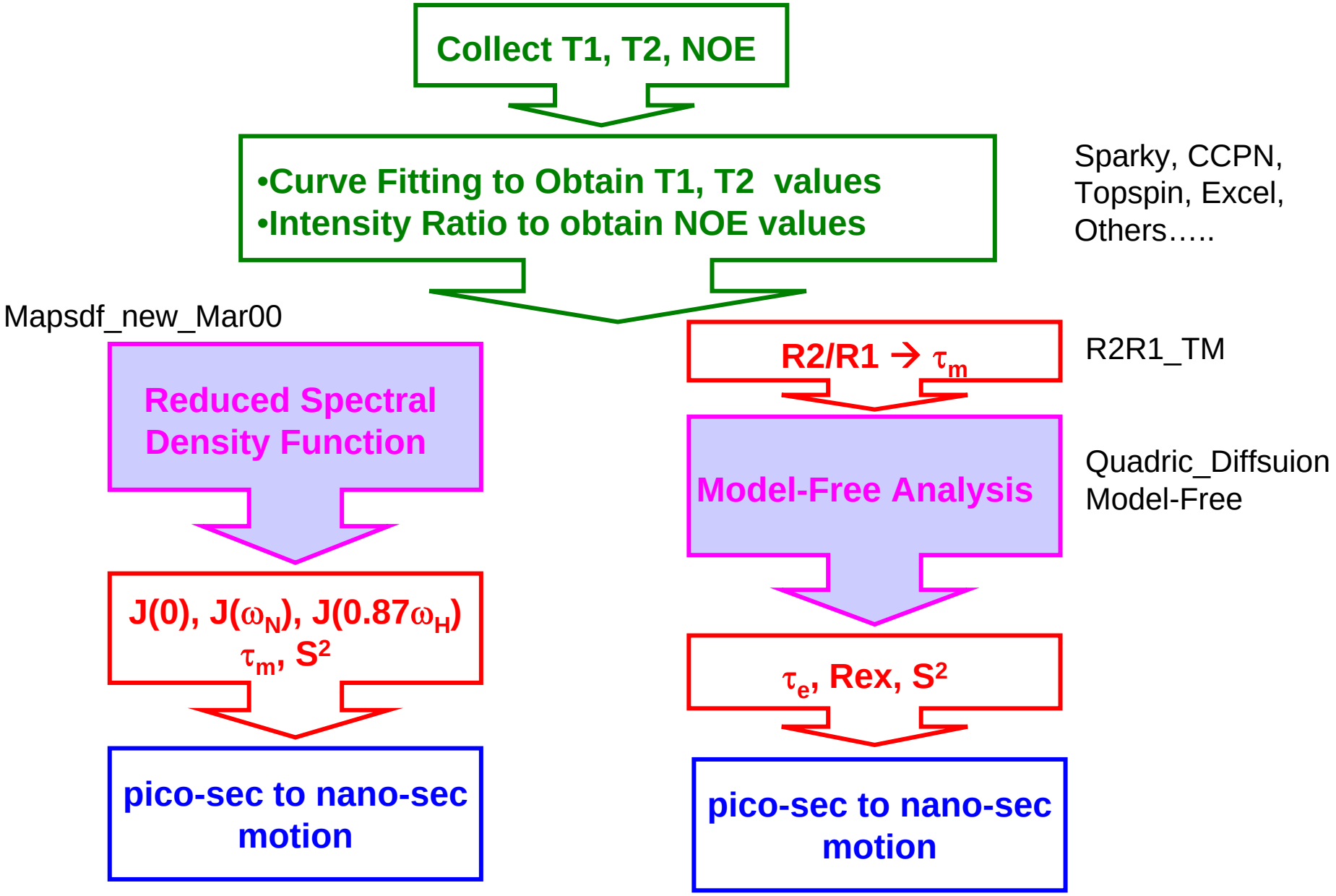
Model 4: S2s, te and Rex

Model 5 Sf2, Ss2, and te

Obtain parameter S^2, τ and R_{ex} for each residue

- Order parameter, S^2: Degree of internal motion
- Correlation time, τ: Rate of molecule motion
- Exchange rate, R_{ex}: Conformational exchange

Part I. Study pico-sec to nano-sec motion



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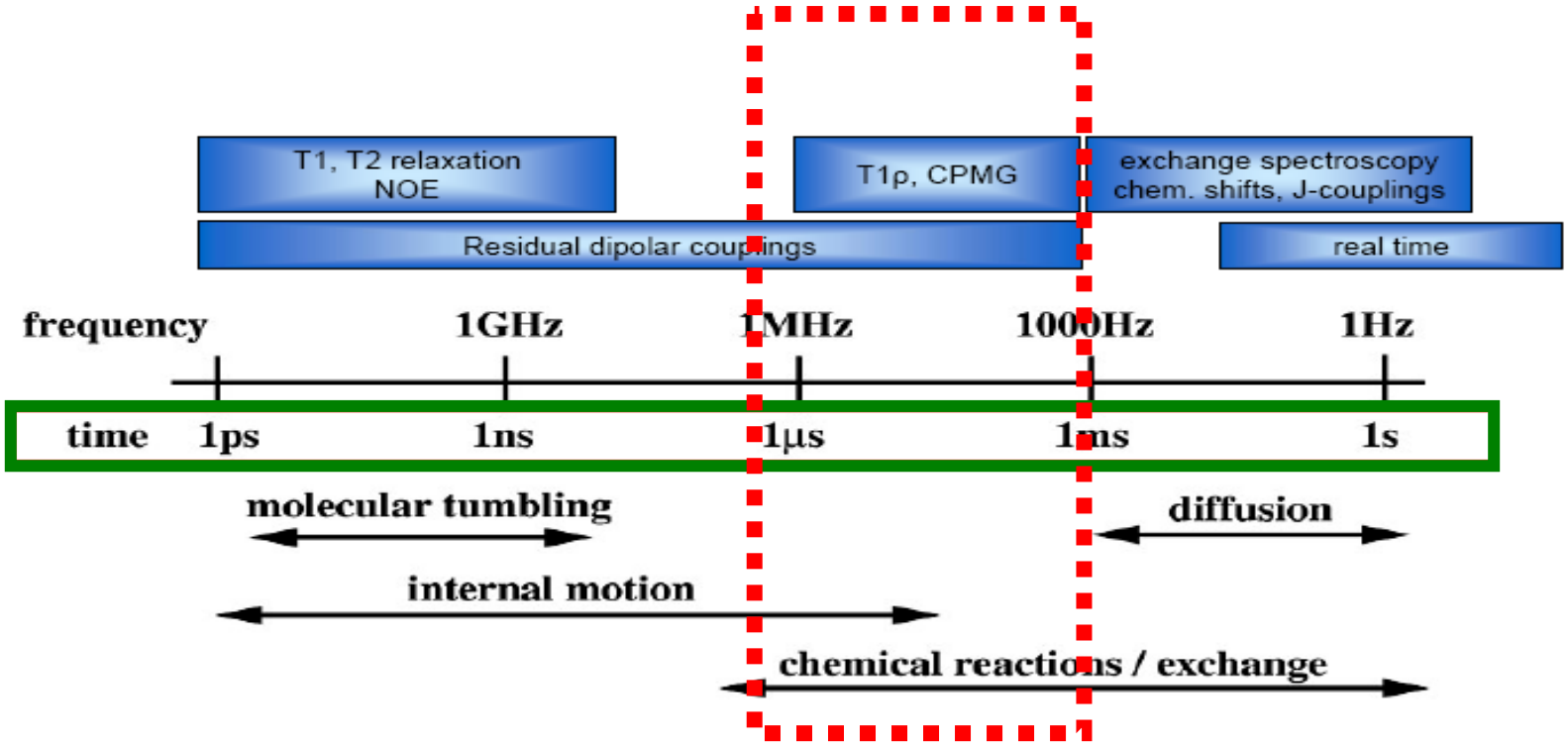
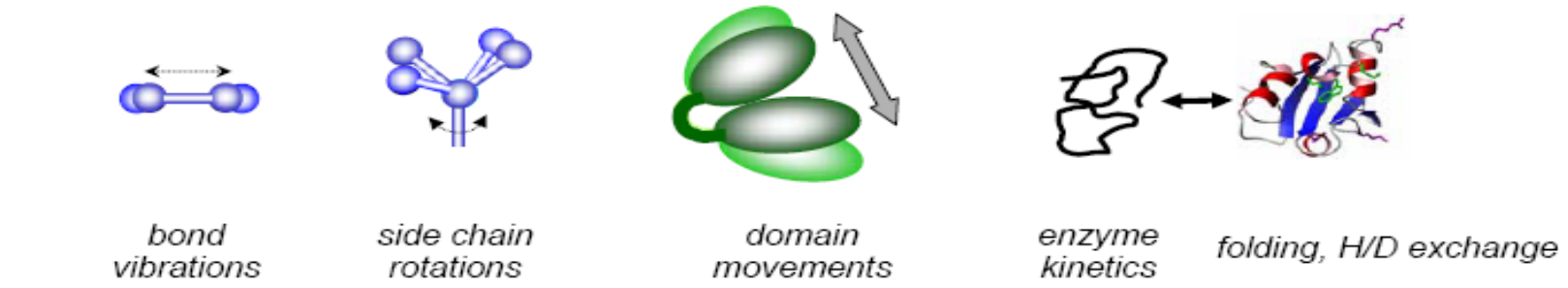




Note on relaxation dispersion

- Relaxation dispersion monitor dynamics on the range or **us to ms (10^{-6} to 10^{-3})**.

- Relaxation dispersion can separate the contribution from exchange between different conformations (**Rex**) from total R2 relaxation

- **Rex** is a function of **exchange rates, populations and chemical shifts** of the different conformations. Thus, give information on the kinetics, thermodynamics, as well as structure of protein substrates. Folding intermediates can be detected as well.

- Parameters that characterize the kinetics of the chemical exchange process are obtained from the variation of **R1rho as a function of ω_e** (effective field in the rotation frame), called **relaxation dispersion**.

- ***CMPG*** relaxation experiments monitor the decay of transverse magnetization in a series of spin-echo pulse sequence elements. Chemical exchange is characterized from the variation in the transverse relaxation rate constant , **R2, as a function of the time delay τ_{cp}.** Where an effective field strength for the CPMG experiment can be defined as $\omega_{cpmg}=12^{1/2}/\tau_{cp}$

Part II. Study micro-sec to milli-sec motion

Method 1: R1rho relaxation Dispersion Experiments (μs)

(1-1) R1rho Experiment

<Collect a set of pseudo 3D expt using vd-list for different field>

- Experiment Type: NH HSQC type pseudo3D
- Standard Parameter Set: std1_3D_15N-R1rho_hsqctretf3gpsi3d.2
- Pulse Program: hsqctretf3gpsi3d.2
- Reference: JACS, 124,10743 (2002)

- Easy Set Up Steps:

(1) rpar std1_3D_15N-R1rho_hsqctretf3gpsi3d.2

(2) getprosol 1H (us) (db)

(3) edit vd-list : delay in sec, ex: std_t1rho

(4) NBL: number of delays in vd-list

(5) td1: number of delays in vd-list (QF)

(6) 13C/15N sample: ZGOPTINS -DLABEL_CN

(1-2) Determine Kex from R1rho experiment

$$R_{ex} = k_{ex}\Phi_{ex}/(k_{ex}^2 + \omega_e^2)$$

$$R_{1\rho} = R_1 \cos^2\theta + R_2^0 \sin^2\theta + \frac{\varphi_{ex} k_{ex}}{k_{ex}^2 + \omega_e^2} \sin^2\theta$$

$$\theta = \arctan(\omega_1/\Omega)$$

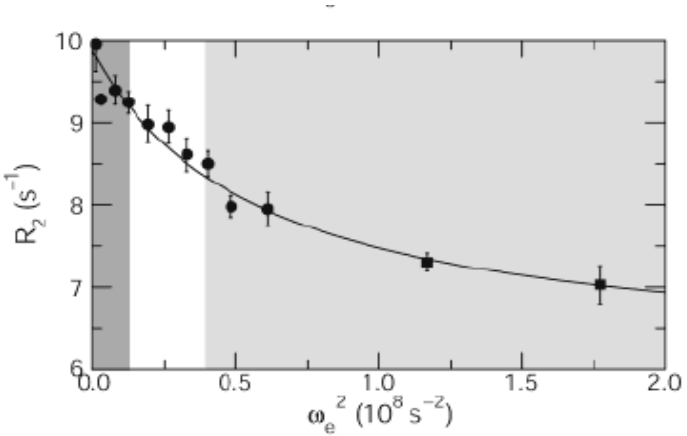
Ω : Resonance offset from the spin-lock frequency

ω_1 : amplitude of the spin-lock field

$\omega_e = (\omega_1^2 + \Omega^2)^{1/2}$: effective spin-lock field

$\varphi_{ex} : p_A p_B \Delta\omega^2$

$$R_2 = \frac{\varphi_{ex} k_{ex}}{k_{ex}^2 + \omega_e^2} + R_2^0$$



(Loria et al., Accounts of Chemical Research, 214-221, 2008)

Method 2: CPMG relaxation Dispersion Experiments (ms)

(2-1) CPMG Experiment

<Collect a set of pseudo 3D expt using vd-list for τ_{cp}>

- Experiment Type: NH TROSY type pseudo3D
- Standard Parameter Set: std2_3D_15N-T2Rex_ trrexetf3gpsi3d_3.cf
- Pulse Program: trrexetf3gpsi3d_3.cf
- Reference: JACS 130, 2432-3 (2008)

- Easy Set Up Steps:

(1) rpar std2_3D_15N-T2Rex_ trrexetf3gpsi3d_3.cf

(2) getprosol 1H (us) (db)

(3) edit vd-list : field strength in Hz, ex: std_Rex

(4) NBL: number of delays in vd-list

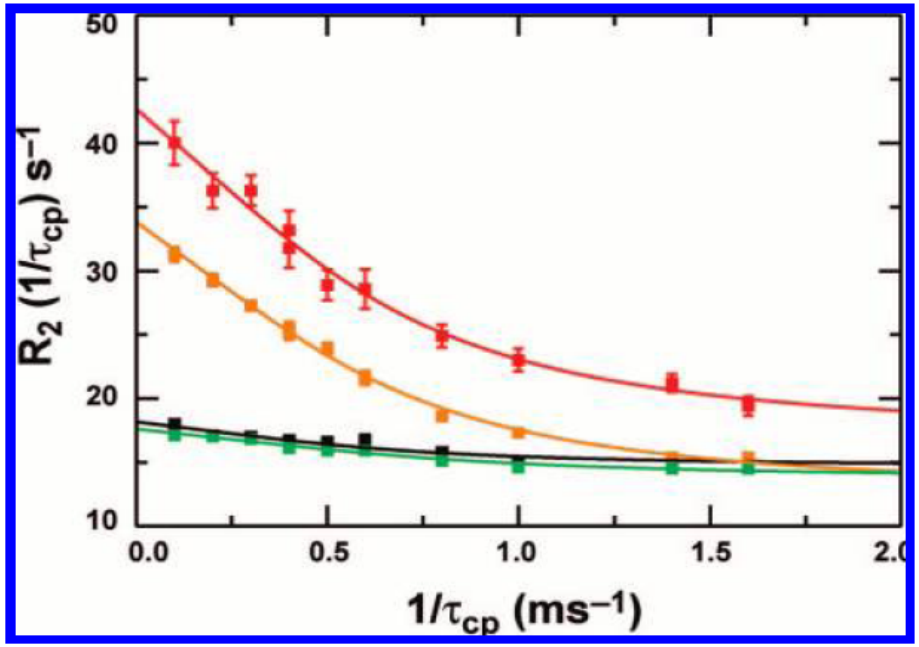
(5) td1: number of delays in vd-list (QF)

(6) decide d21 value: length of mixing time , ex: 25ms

(7) 13C/15N sample: ZGOPTINS –DLABEL_CN

(8) d1=2.5sec

(2-2) Determine Kex from CPMG experiment

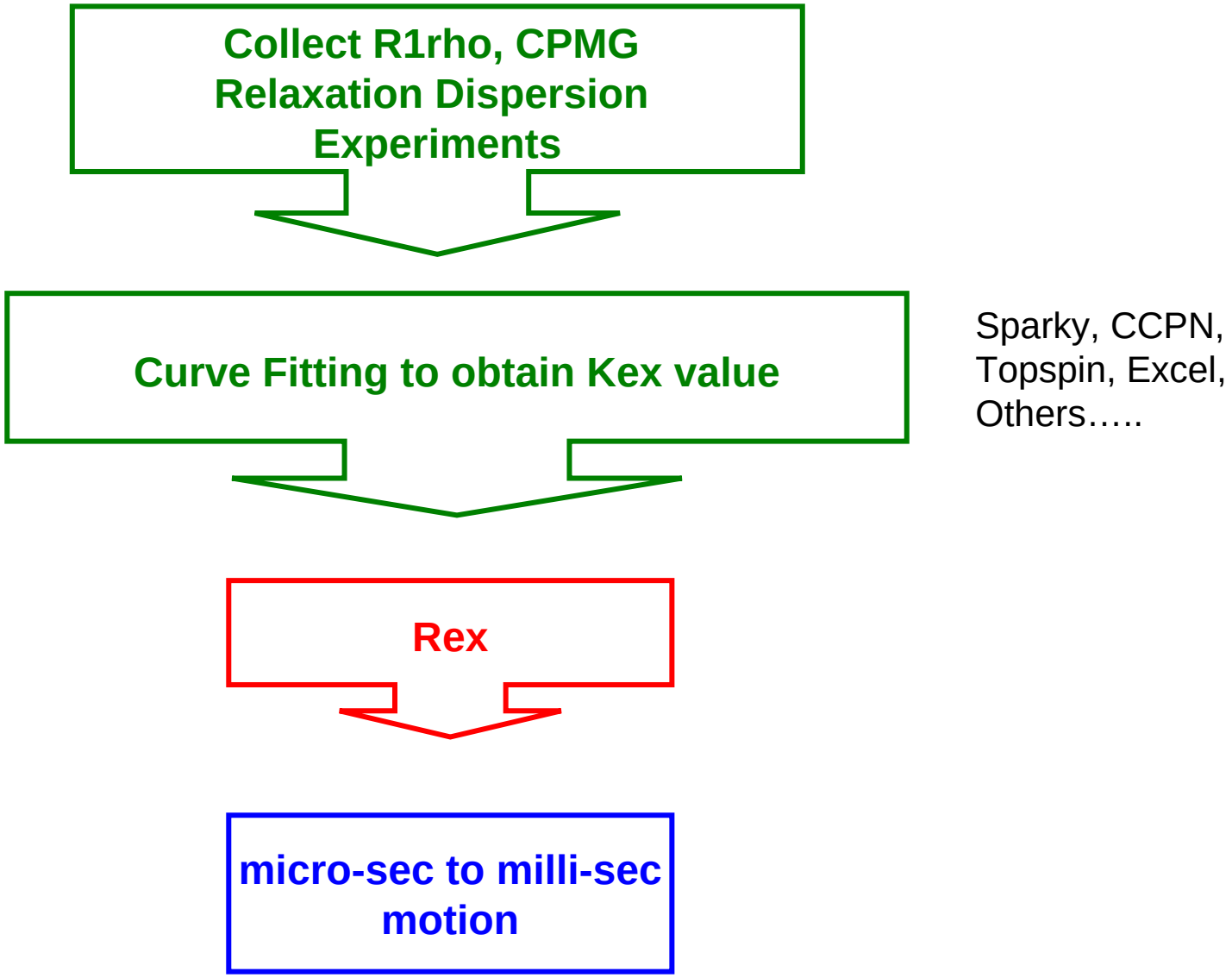


$$R_2(1/\tau_{cp}) = R_2^0 + \varphi_{ex}[1 - 2\tanh(k_{ex}\tau_{cp}/2)/(k_{ex}\tau_{cp})]$$

$$\omega_e = 12^{1/2}/\tau_{cp}$$

(Loria et al., Accounts of Chemical Research, 214-221, 2008)

Part II. Study micro-sec to milli-sec motion



Now,

Let's See how

"Protein Dynamics Center"

Could help?