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NMR for Drug Design

Protein – Ligand binding

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Binding detection

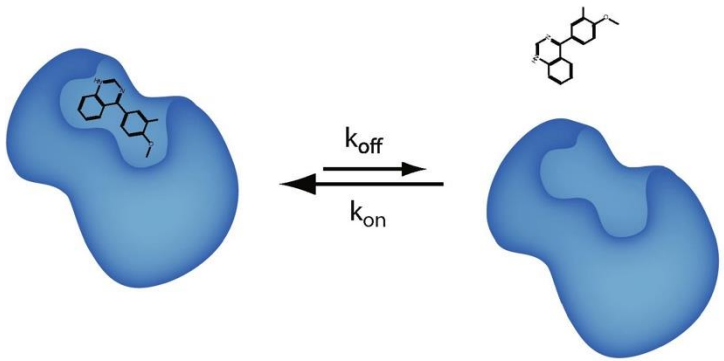
$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PL$$

Proteins and ligands constantly associate and dissociate with rate constants k_{on} and k_{off}, respectively, and establish equilibrium populations of free and bound states

Binding detection

Ligand properties

bound		free
protein	chemical environment	solvent
ω_{bound}	chemical shift	ω_{free}
slow	rotational tumbling	fast
fast strong positive	transverse relaxation NOE cross-peaks	slow weak negative
slow	translational diffusion	fast



Protein properties

bound		free
ligand	chemical environment	solvent/protein
ω_{bound}	chemical shift	ω_{free}

$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PL$$

k_{on} is diffusion limited to ~10^8 $M^{-1}s^{-1}$

k_{on} on the order of 10^3 $M^{-1}s^{-1}$ means that large conformational rearrangement occur

Dahl, Akerud, Drug Discov. Today(2013)
Gossert, Jahnke. Prog Nucl Mag Res Sp (2016)

Binding detection

Ligand properties

bound		free
protein	chemical environment	solvent
ω_{bound}	chemical shift	ω_{free}
slow	rotational tumbling	fast
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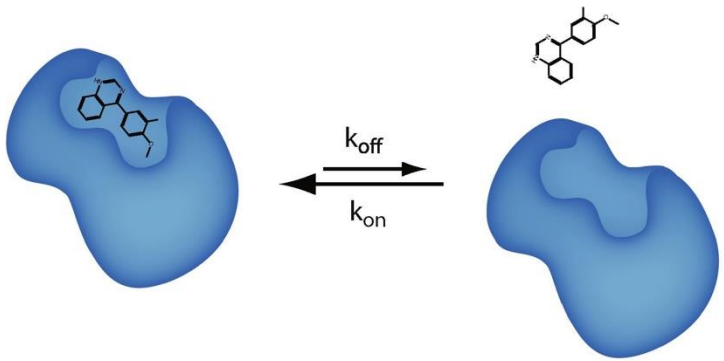


Protein properties

bound		free
ligand	chemical environment	solvent/protein
ω_{bound}	chemical shift	ω_{free}

$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PL$$

Chemical shift (CS) -> CS Perturbation (CSP)

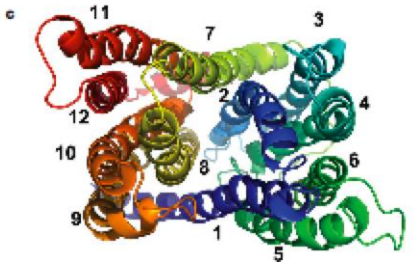
Correlation time -> relaxation properties

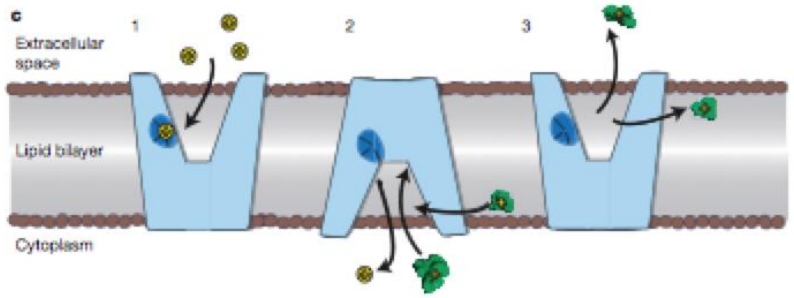
Translation Diffusion-> DOSY type of experiments

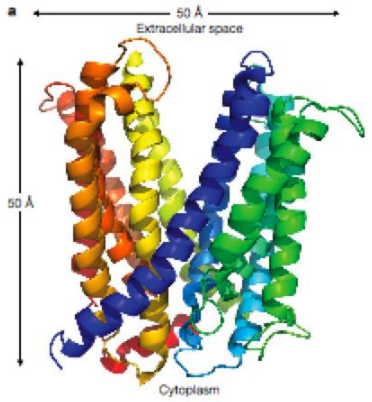
MATE (multidrug and toxic compound extrusion)

- MATE family
  - multiple-drug resistance (MDR)
    - modulate the efficacy of many pharmaceutical drugs
- MATE transporters: electrochemical gradients

NorM MATE transporter (3.65Å)
12 transmembrane helices







Chang, Nature 2010

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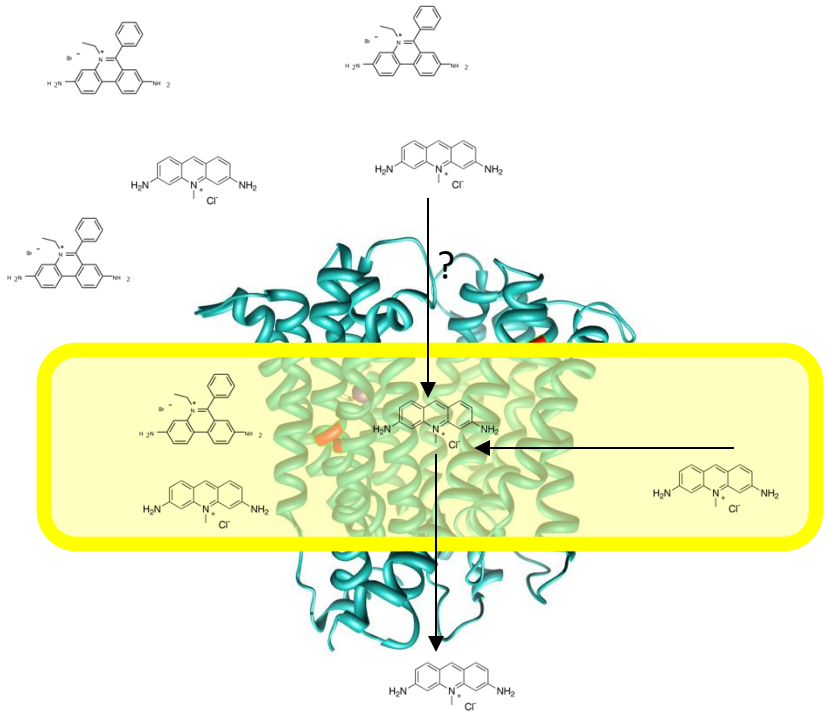
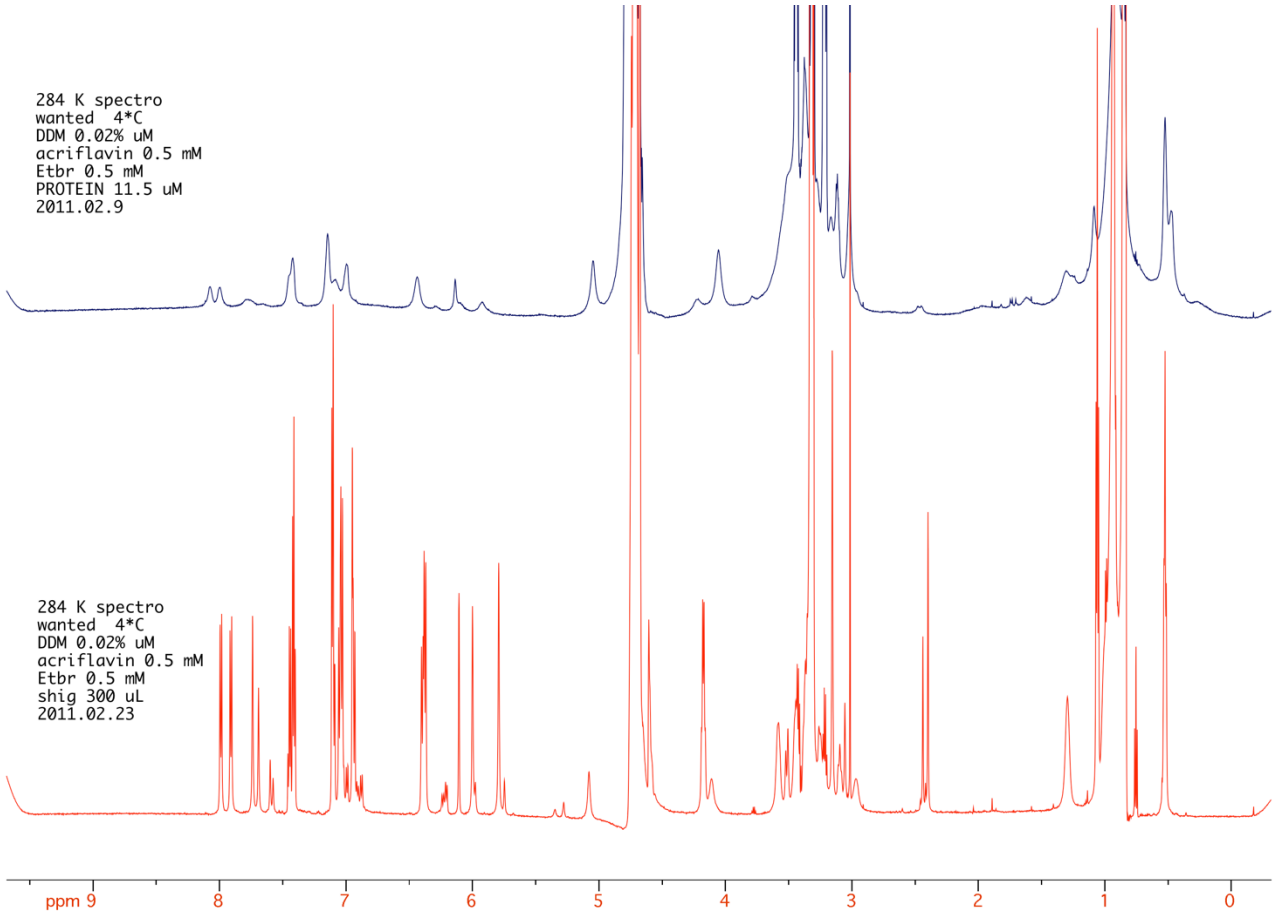
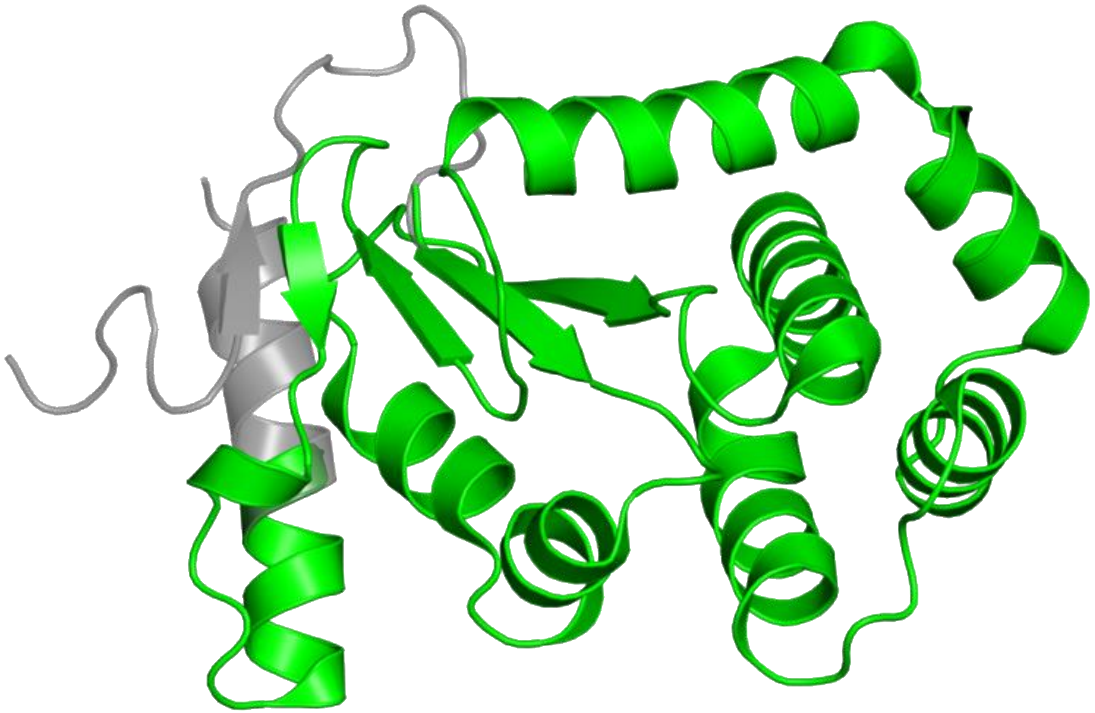
Acri. And EtBr. bind to the proteins micelles complexes





DsBA

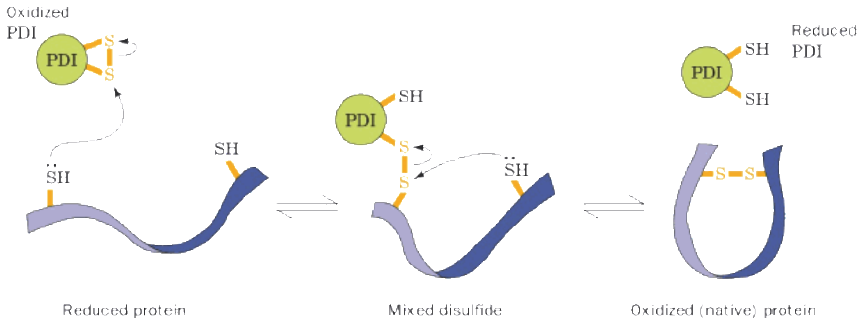


1a2j X-ray diffraction
2Å resolution

21.16 kDa
189 amino acids
9400 Å^2

protein disulfide isomerase activity

catalyzes intra-chain disulfide bond formation as peptides emerge into the cell's periplasm



Guddat, Structure 6 757-67 (1998)

www3.nd.edu

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Ligand properties

bound		free
protein	chemical environment	solvent
ω_{bound}	chemical shift	ω_{free}
slow	rotational tumbling	fast
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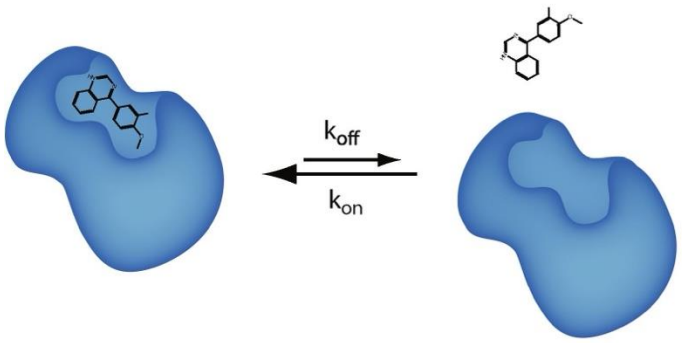
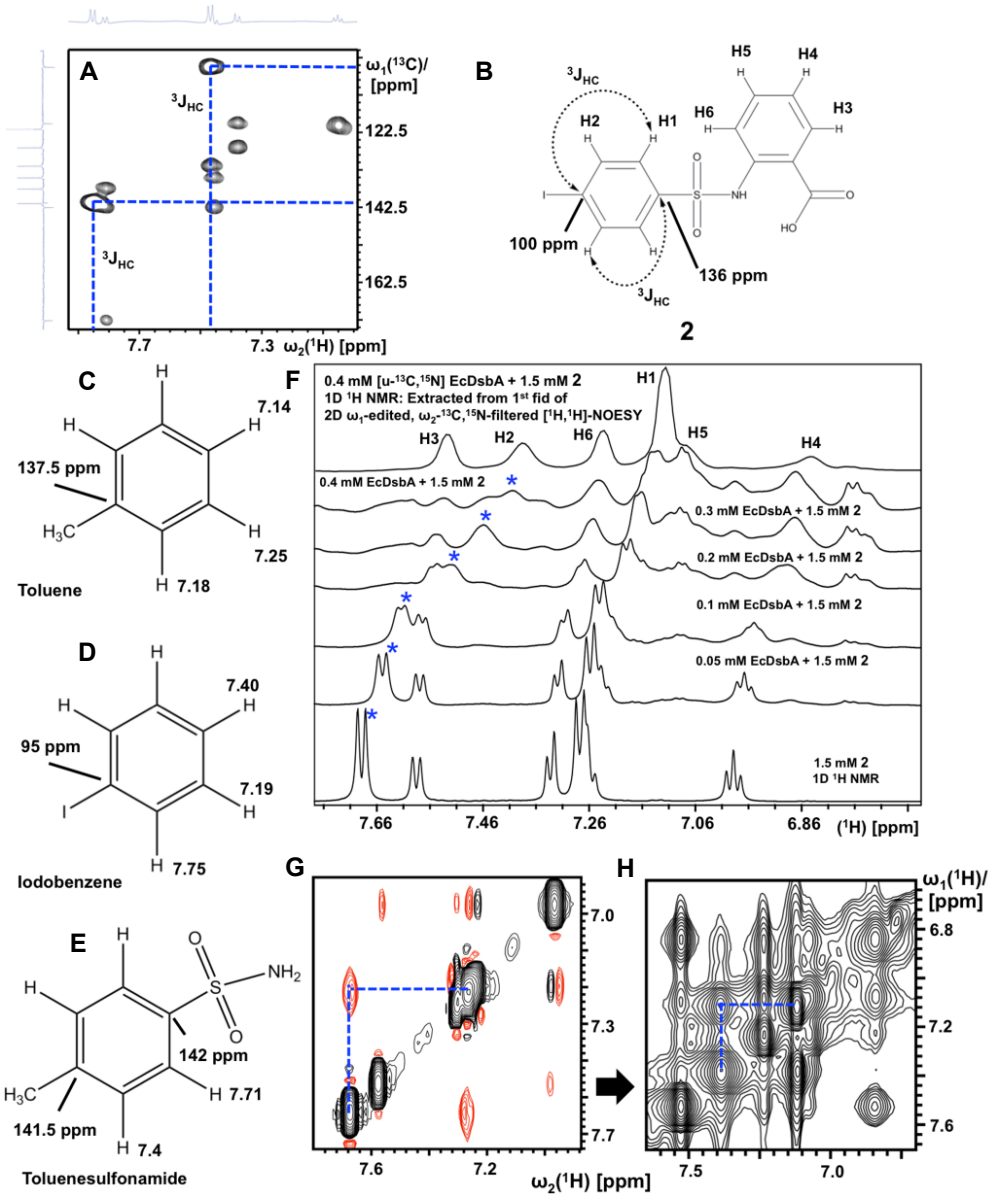


Protein properties

bound		free
ligand	chemical environment	solvent/protein
ω_{bound}	chemical shift	ω_{free}



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^{15}N-HSQC NMR screening

Ligand properties

bound		free
protein	chemical environment	solvent
ω_{bound}	chemical shift	ω_{free}
slow	rotational tumbling	fast
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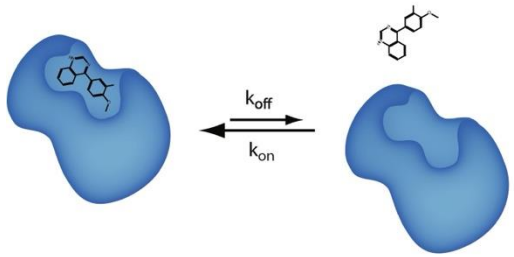
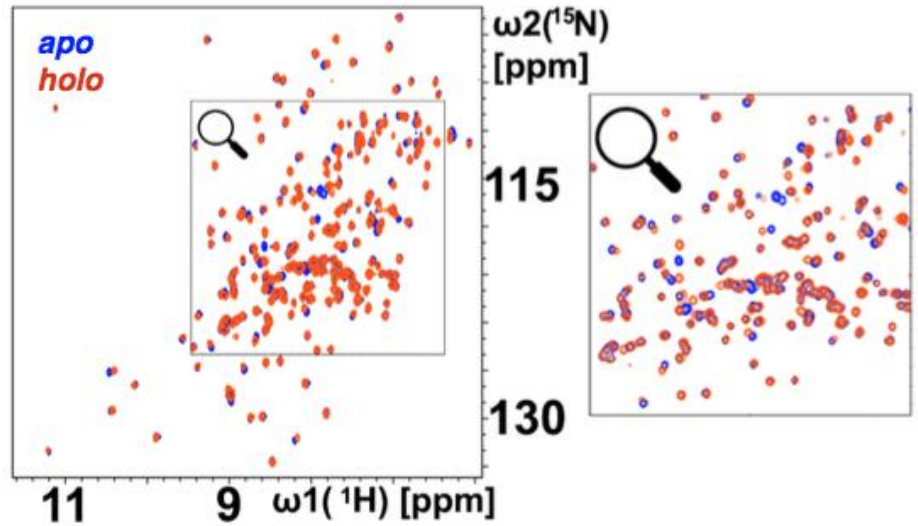
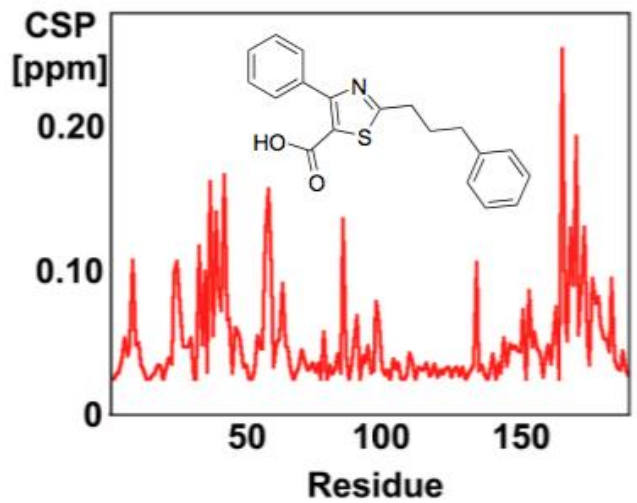
slow	translational diffusion	fast

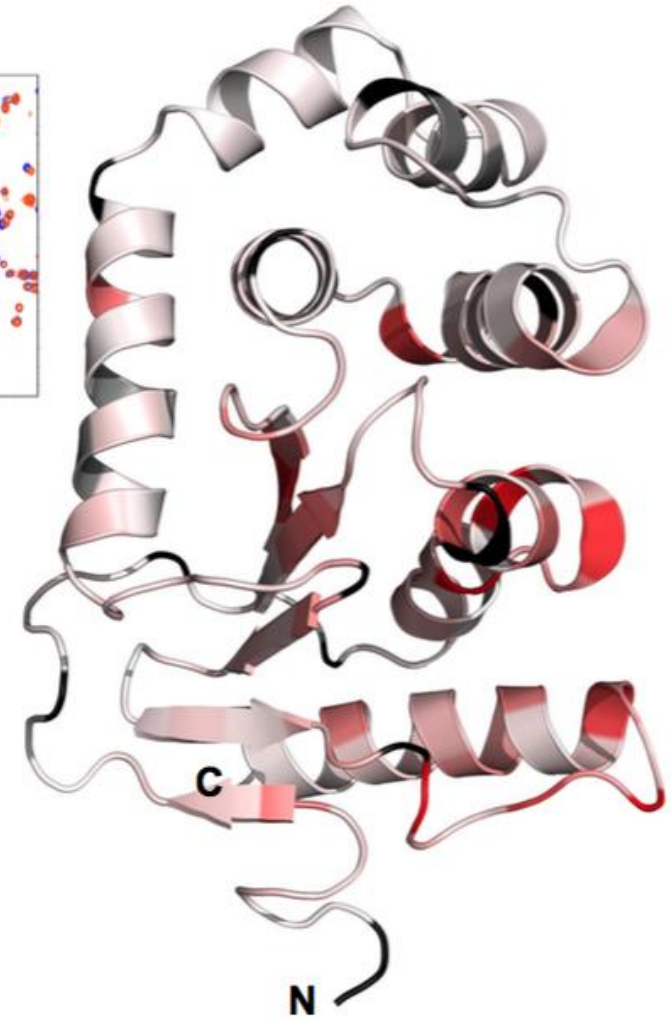


Protein properties

bound		free
ligand	chemical environment	solvent/protein
ω_{bound}	chemical shift	ω_{free}







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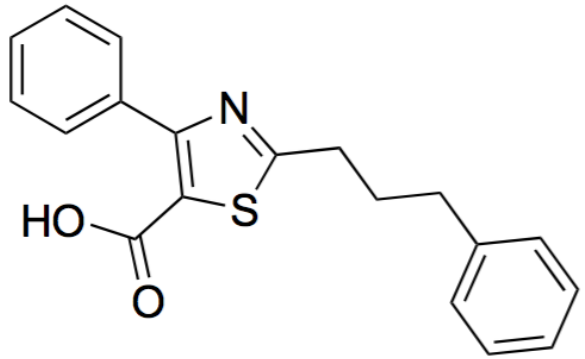
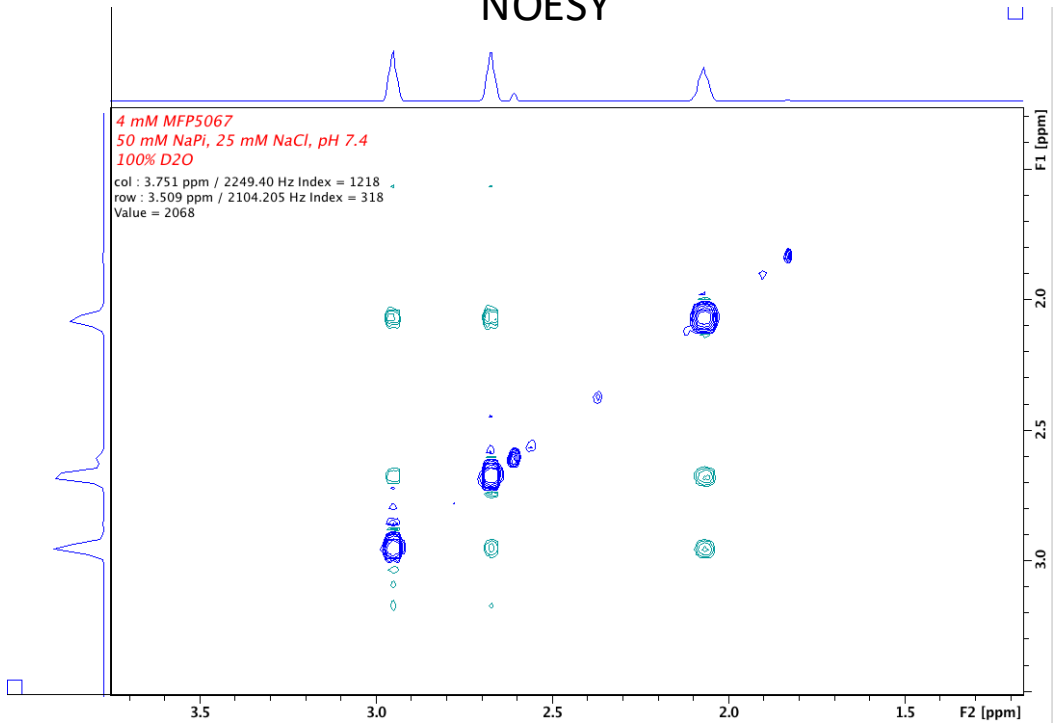
DsBA

Phenylthiazole



MFP0005067

NOESY



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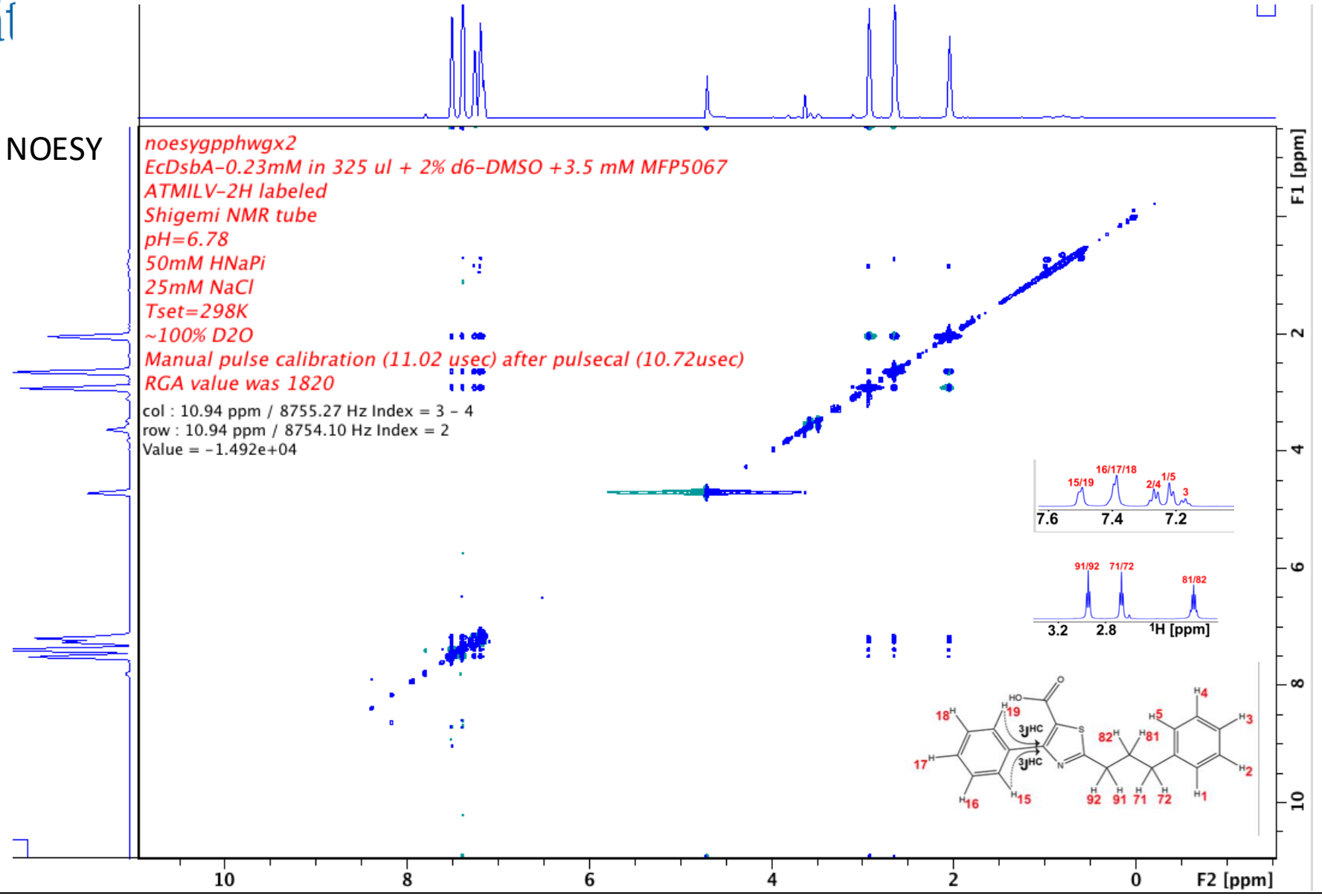
NOESY



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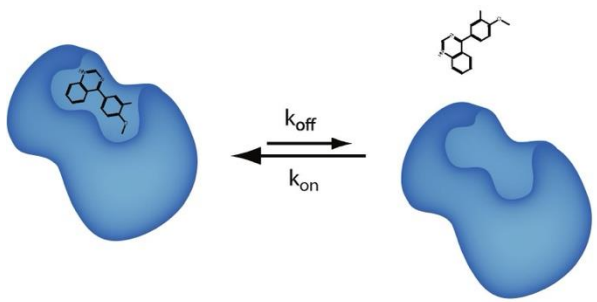
Overlay of 2D [^{13}C,^{1}H] - HSQCs

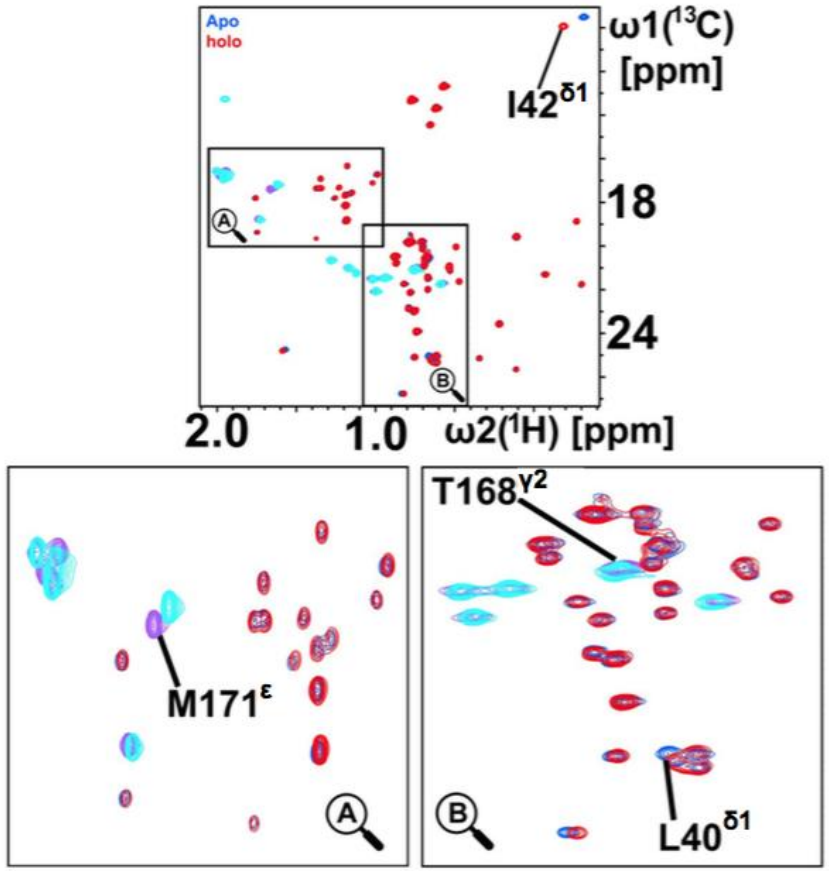
Ligand properties

bound		free
protein	chemical environment	solvent
ω_{bound}	chemical shift	ω_{free}
slow	rotational tumbling	fast
fast strong positive	transverse relaxation NOE cross-peaks	slow weak negative
slow	translational diffusion	fast



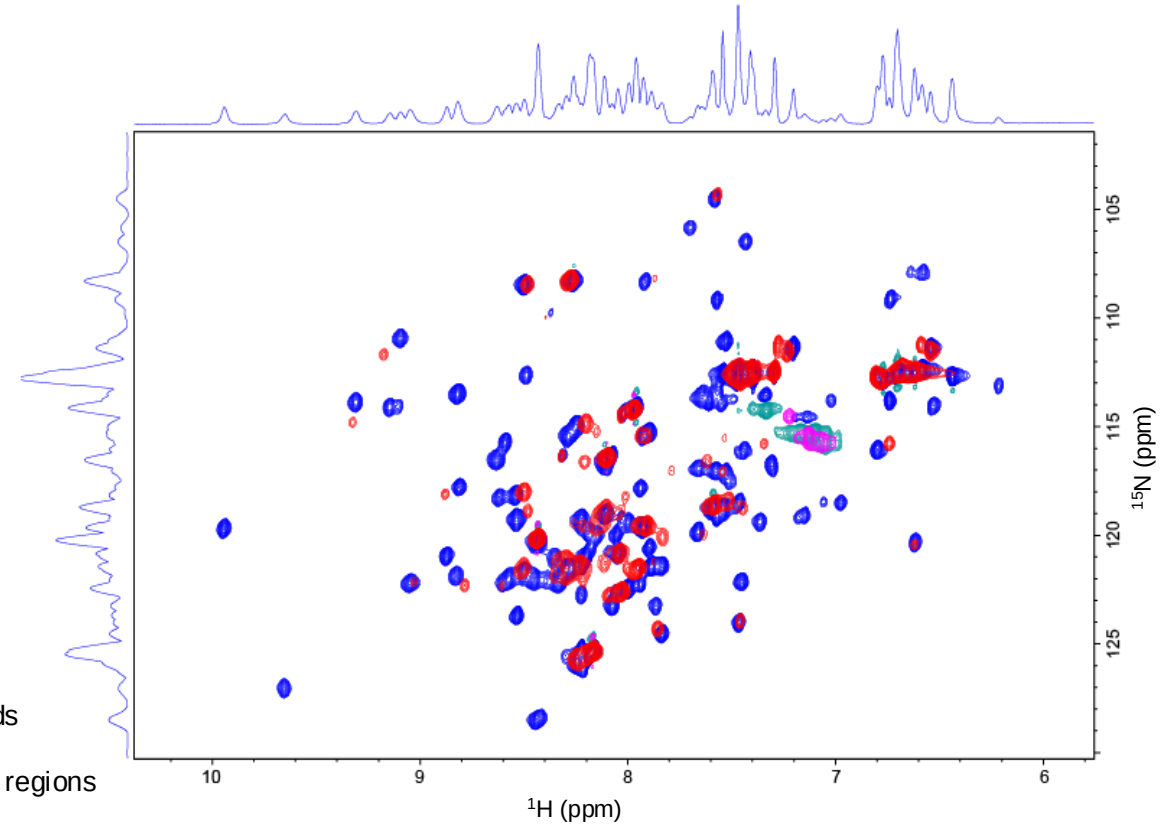
Protein properties

bound		free
ligand	chemical environment	solvent/protein
ω_{bound}	chemical shift	ω_{free}



HDMX-ligand

With/without
peptide inhibitor



"MDM4 binds ligands
via a mechanism
in which disordered regions
become structured"

Sanchez, FEBS Letters 584, 3035–3041 (2010)

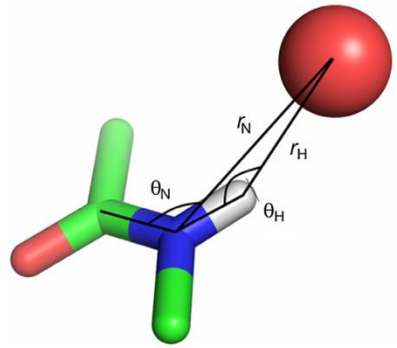


Fig. 5. The electric field effect caused by a charged atom (shown as a sphere) is proportional to $q\cos\theta/r^2$, where q is the charge on the atom [107]. The angle θ is the angle made to the bond along which electron density is pushed or pulled: where there is a choice, this will be the most polarisable bond. For ^1H_N, this is the H–N bond. For N, this is the N–C′ bond. Because the relevant angles for H and N can be completely different, the effect on the chemical shifts of H and N can also be very different.

($^1H^{15}N$, 290 K, 700 MHz), [P]=0.5 mM
pH 7.5, 25 mM Phos., 25 mM NaCl,
2 mM TCEP, 0.1 mM EDTA

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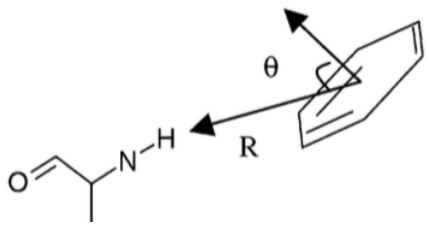
Molecular structure and environment → **Chemical shift**

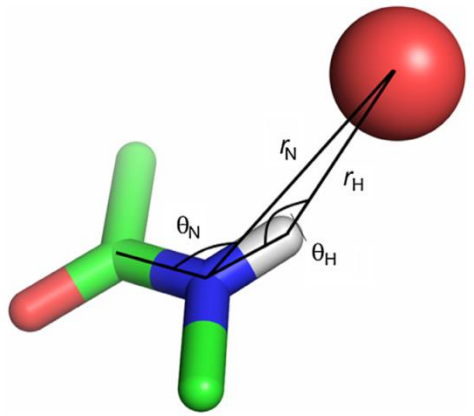
$$\sigma_{iso} = \sigma_{dia} + \sigma_{para} + \sigma_{rc} \ldots \quad (4)$$

$$\omega = -\gamma B_0 (1 - \sigma_{iso}) \quad (12)$$

A



$$\Delta CS(i) = CS(i)_{P+L} - CS(i)_P \approx \left(\frac{B_{dip}}{R_i^3}\right)(1 - 3\cos^2\theta_i)$$



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M. P. Williamson, *Prog Nucl Mag Res Sp* 2013, *73*, 1-16

Population averaged NMR parameters

$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PL$$

slow exchange regime: if $|A_{bound} - A_{free}| \ll k_{ex}$ (Eq. 7a)

intermediate exchange regime: if $|A_{bound} - A_{free}| \approx k_{ex}$ (Eq. 7b)

fast exchange regime: if $|A_{bound} - A_{free}| \gg k_{ex}$ (Eq. 7c)

$$k_{ex}^{L} = k_{on}[P]_{free} + k_{off} \quad \text{(Eq. 6a)}$$

$$k_{ex}^{P} = k_{on}[L]_{free} + k_{off} \quad \text{(Eq. 6b)}$$

$$k_{ex}^{L} = k_{on}([P]_{free} + K_d)$$

$$A_{avg} = A_{free} \cdot p_{free} + A_{bound} \cdot p_{bound} \quad \text{(Eq. 8)}$$

A = Chemical Shift, Relaxation, etc.

Orts, Methods (2018)

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Population averaged NMR parameters

$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PL$$

$$A_{avg} = A_{free} \cdot p_{free} + A_{bound} \cdot p_{bound} \text{ (Eq. 8)}$$

$$p^L_{\text{bound}} = \frac{[PL]}{[L]_{\text{tot}}} = 1 - p^L_{\text{free}} \text{ (Eq. 3a)}$$

$$p^P_{\text{bound}} = \frac{[PL]}{[P]_{\text{tot}}} = 1 - p^P_{\text{free}} \text{ (Eq. 3b)}$$

$$[PL] = \frac{[L]_{\text{tot}} + [P]_{\text{tot}} + K_{\text{D}} - \sqrt{([L]_{\text{tot}} + [P]_{\text{tot}} + K_{\text{D}})^2 - 4[L]_{\text{tot}} \cdot [P]_{\text{tot}}}}{2} \qquad K_{\text{D}} = \frac{[P]_{\text{free}}[L]_{\text{free}}}{[PL]} = \frac{k_{\text{off}}}{k_{\text{on}}}$$

Orts, Methods (2018)

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Rotational diffusion

$$\tau_c = \frac{4\pi\eta r_H^3}{3k_b T} \qquad (5)$$

Relaxation

Transverse relaxation through dipole-dipole interactions

$$R_{2,DD} = \frac{1}{20}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(5\tau_c + \frac{9\tau_c}{1+\omega_0^2\tau_c^2} + \frac{6\tau_c}{1+4\omega_0^2\tau_c^2}\right) \qquad (13)$$

Transverse relaxation through chemical shift anisotropy

$$R_{2,CSA} = \frac{1}{24}\left(\sigma_{\parallel-\perp}^2 B_0^2\gamma^2\right)\left(4\tau_c + \frac{3\tau_c}{1+\omega_0^2\tau_c^2}\right) \qquad (14)$$

Nuclear Overhauser effect (NOE)

$$R_C^L = \frac{1}{10}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(\tau_c - \frac{6\tau_c}{1+4\omega_0^2\tau_c^2}\right) \qquad (15)$$

Transverse nuclear Overhauser effect (ROE)

$$R_C^T = -\frac{1}{10}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(2\tau_c + \frac{3\tau_c}{1+\omega_0^2\tau_c^2}\right) \qquad (16)$$

Longitudinal relaxation

$$R_{1,DD} = \frac{1}{10}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(\frac{3\tau_c}{1+\omega_0^2\tau_c^2} + \frac{12\tau_c}{1+4\omega_0^2\tau_c^2}\right) \qquad (17)$$

Paramagnetic relaxation

$$R_{2,para} = \frac{1}{20}\frac{\hbar^2\mu_0^2\gamma_n^2\gamma_e^2}{r^6}\left(4\tau_c + \frac{3\tau_c}{1+\omega_0^2\tau_c^2}\right) \qquad (18)$$

Gossert, Jahnke. Prog Nucl Mag Res Sp (2016)

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Relaxation

Transverse relaxation through dipole-dipole interactions

$$R_{2,DD} = \frac{1}{20}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(5\tau_c + \frac{9\tau_c}{1+\omega_0^2\tau_c^2} + \frac{6\tau_c}{1+4\omega_0^2\tau_c^2}\right) \quad (13)$$

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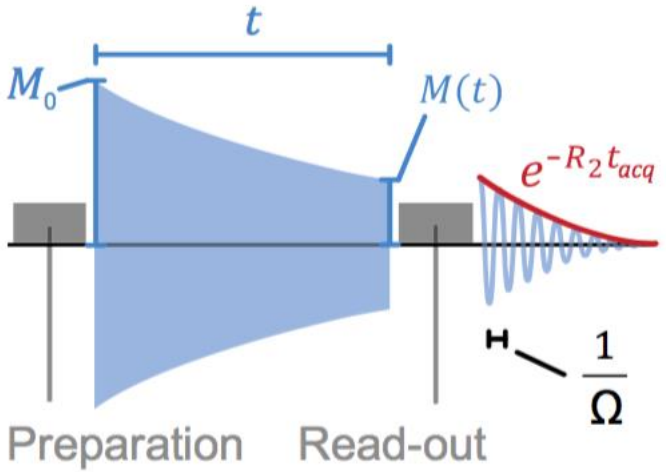
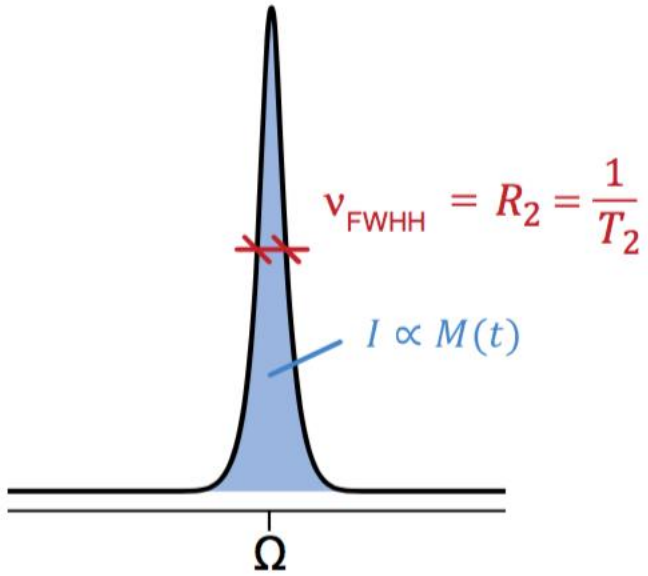
Longitudinal relaxation

$$R_{1,DD} = \frac{1}{10}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(\frac{3\tau_c}{1+\omega_0^2\tau_c^2} + \frac{12\tau_c}{1+4\omega_0^2\tau_c^2}\right) \quad (17)$$

Paramagnetic relaxation

$$R_{2,para} = \frac{1}{20}\frac{\hbar^2\mu_0^2\gamma_n^2\gamma_e^2}{r^6}\left(4\tau_c + \frac{3\tau_c}{1+\omega_0^2\tau_c^2}\right) \quad (18)$$





Gossert, Jahnke. Prog Nucl Mag Res Sp (2016)

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Relaxation

Transverse relaxation through dipole-dipole interactions

$$R_{2,DD} = \frac{1}{20}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(5\tau_c + \frac{9\tau_c}{1+\omega_0^2\tau_c^2} + \frac{6\tau_c}{1+4\omega_0^2\tau_c^2}\right) \quad (13)$$

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Nuclear Overhauser effect (NOE)

$$R_C^L = \frac{1}{10}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(\tau_c - \frac{6\tau_c}{1+4\omega_0^2\tau_c^2}\right) \quad (15)$$

Transverse nuclear Overhauser effect (ROE)

$$R_C^T = -\frac{1}{10}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(2\tau_c + \frac{3\tau_c}{1+\omega_0^2\tau_c^2}\right) \quad (16)$$

Longitudinal relaxation

$$R_{1,DD} = \frac{1}{10}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(\frac{3\tau_c}{1+\omega_0^2\tau_c^2} + \frac{12\tau_c}{1+4\omega_0^2\tau_c^2}\right) \quad (17)$$

Paramagnetic relaxation

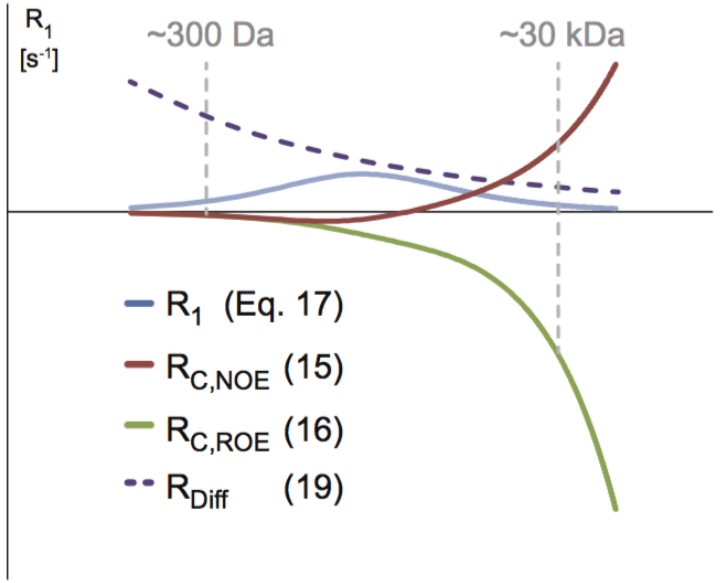
$$R_{2,para} = \frac{1}{20}\frac{\hbar^2\mu_0^2\gamma_n^2\gamma_e^2}{r^6}\left(4\tau_c + \frac{3\tau_c}{1+\omega_0^2\tau_c^2}\right) \quad (18)$$

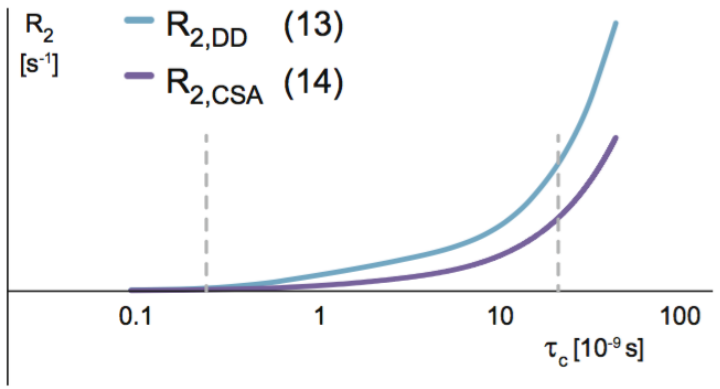
Translational diffusion

$$D = \frac{k_B T}{6\pi\eta r_H} \quad (6)$$

Diffusion in gradient echoes

$$R_{Diff}^* = -D\gamma^2 g^2 \delta^2 \quad (19)$$





	R_1	R_{NOE}	R_{ROE}	$R_{2,DD}$	$R_{2,CSA}$	R_{Diff}
$\frac{R(\tau_c=0.2\ ns)}{R(\tau_c=20\ ns)}$	0.3	-21	41	26	58	0.2

Gossert, Jahnke. Prog Nucl Mag Res Sp (2016)

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Kinetics of binding

$$k_{on} = Z\rho\, e^{\frac{-Ea}{N_A k_B T}} \qquad (2a)$$

$$= e^{-\frac{\Delta \ddagger G}{N_A k_B T}} \qquad (2b)$$

$$k_{off} = \frac{1}{\tau_R} = K_D k_{on} \qquad (3)$$

Exchange phenomena

Exchange rate

$$k_{ex}^{L} = k_{off} + k_{on}[P], \qquad k_{ex}^{P} = k_{off} + k_{on}[L] \qquad (8a, b)$$

Fast exchange approximation $(|\omega_F - \omega_B| \ll k_{ex}, |R_F - R_B| \ll k_{ex})$

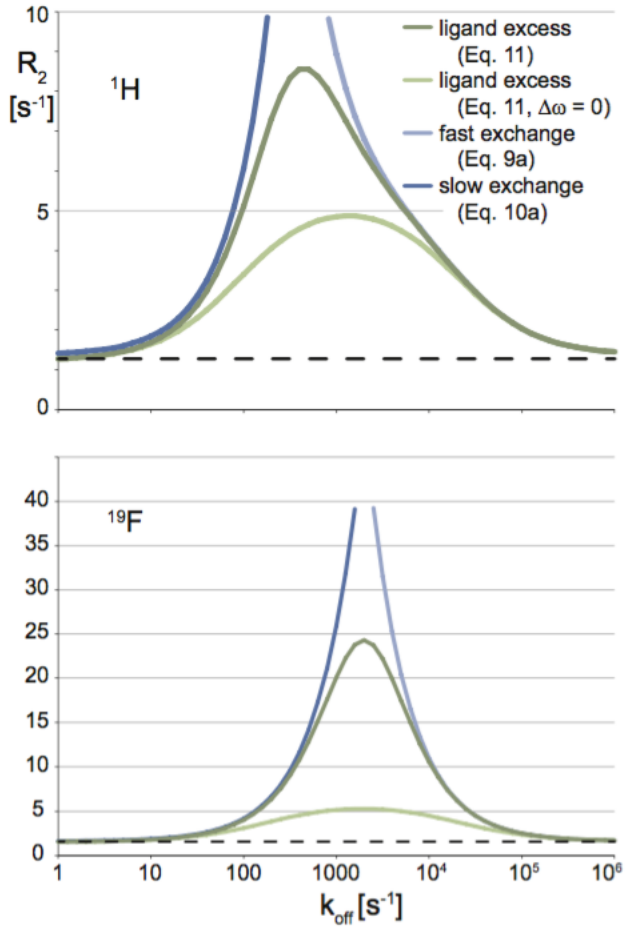
$$R_{2,fast} = p_F R_{2,F} + p_B R_{2,B} + \frac{(\omega_F - \omega_B)^2 p_F p_B}{k_{ex}}; \quad \omega_{fast} = p_F \omega_F + p_B \omega_B \qquad (9a; b)$$

Slow exchange approximation $(|\omega_F - \omega_B| \gg k_{ex}, |R_F - R_B| \gg k_{ex})$

$$R_{2,F,slow}^{L} = R_{2,F} + k_{on}[P] = R_{2,F} + p_B k_{ex}\,; \quad \omega_{F,slow} = \omega_F \qquad (10a; b)$$
$$R_{2,B,slow} = R_{2,B} + k_{off} = R_{2,B} + p_F k_{ex}\,; \quad \omega_{B,slow} = \omega_B \qquad (10c; d)$$

Approximation for ligand excess for all time scales $(p_F^L \gg p_B^L)$

$$R_{2,p_F \gg p_B} = p_F R_{2,F} + p_F p_B k_{ex}\left(\frac{R_{2B}(R_{2B} + p_F k_{ex}) + (\omega_F - \omega_B)^2}{(R_{2B} + p_F k_{ex})^2 + (\omega_F - \omega_B)^2}\right) \qquad (11)$$



[P] = 5 uM
[L] = 200 uM
kon = 10^8 $M^{-1}s^{-1}$
Δ**ω**(H) = 300 Hz
Δ**ω**(F) = 2000 Hz
R_2 = 1.2 s^{-1} free and 30 s^{-1} bound for H
R_2 = 1.3 s^{-1} free and 80 s^{-1} bound for H

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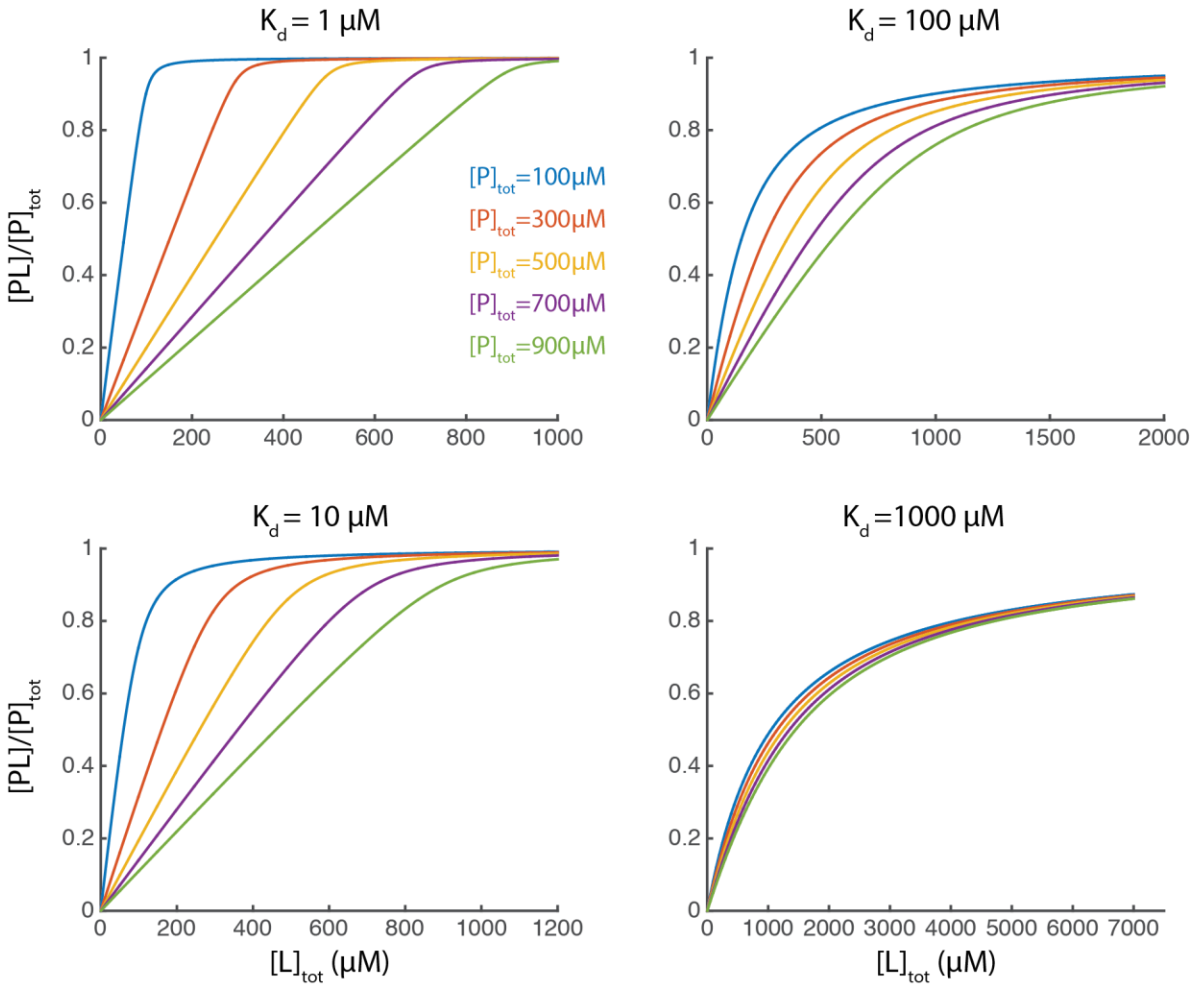
$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PL$$



$$[PL] = \frac{[L]_{\mathrm{tot}} + [P]_{\mathrm{tot}} + K_{\mathrm{D}} - \sqrt{([L]_{\mathrm{tot}} + [P]_{\mathrm{tot}} + K_{\mathrm{D}})^2 - 4[L]_{\mathrm{tot}} \cdot [P]_{\mathrm{tot}}}}{2}$$

Bound population of protein with respect to the total ligand concentration at different complex affinities, K_D.

$$p^{P}_{\mathrm{bound}} = \frac{[PL]}{[P]_{\mathrm{tot}}} = 1 - p^{L}_{\mathrm{free}}$$

Orts Methods (2018)

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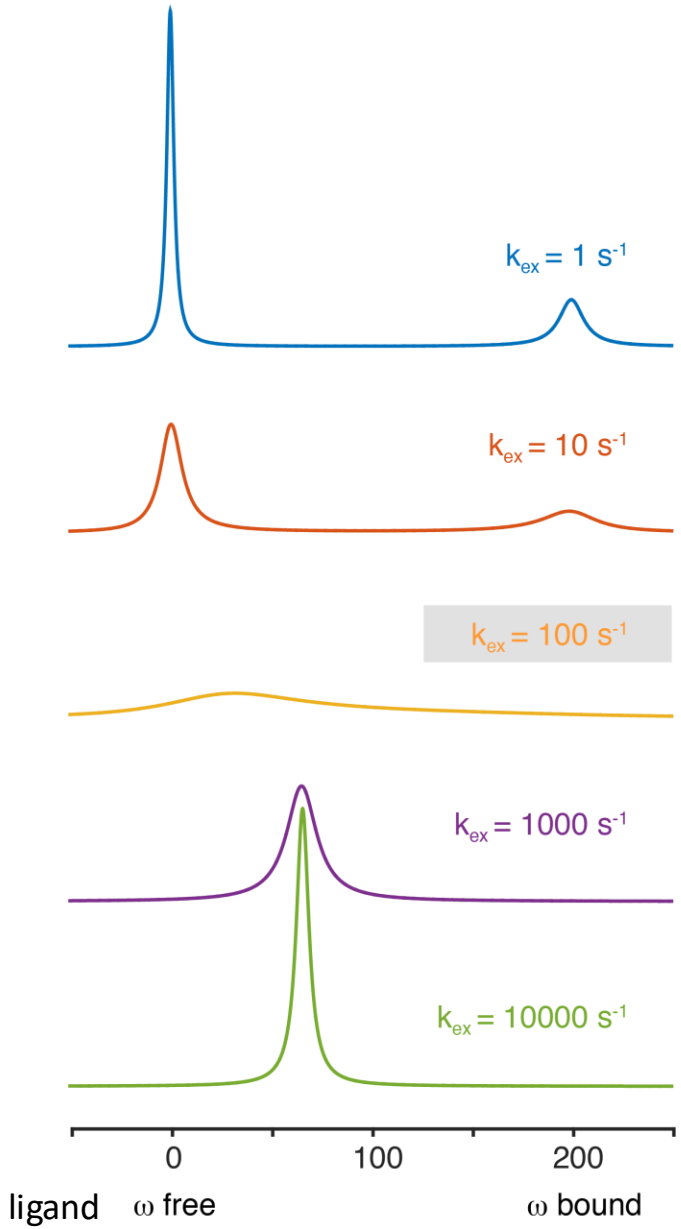
$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} PL$$



Line broadening effect on ligand resonance induced by protein binding with different exchange kinetics.

$[L]_{tot}$ = 1500 µM,
$[P]_{tot}$ = 500 µM
K_D = 10 µM

$$k_{ex}^{L} = k_{on}([P]_{free} + K_d)$$

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Fast exchange regime

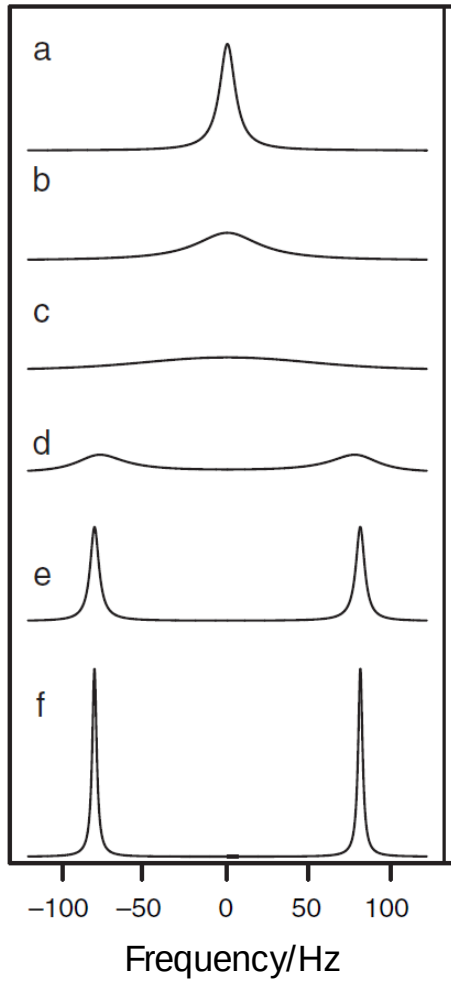
$$\frac{1}{\tau_1} \gg \Delta\omega$$

Intermediate exchange regime

$$\frac{1}{\tau_1} \approx \Delta\omega$$

Slow exchange regime

$$\frac{1}{\tau_1} \ll \Delta\omega$$



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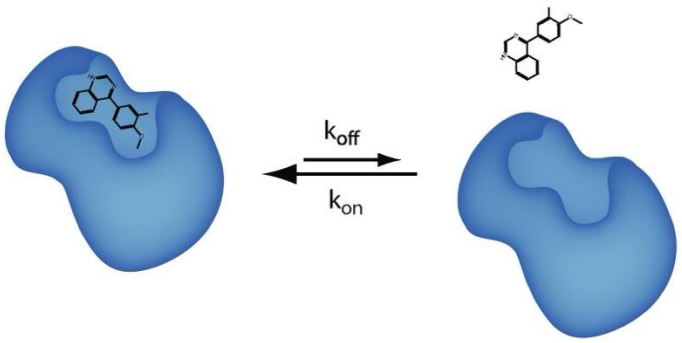
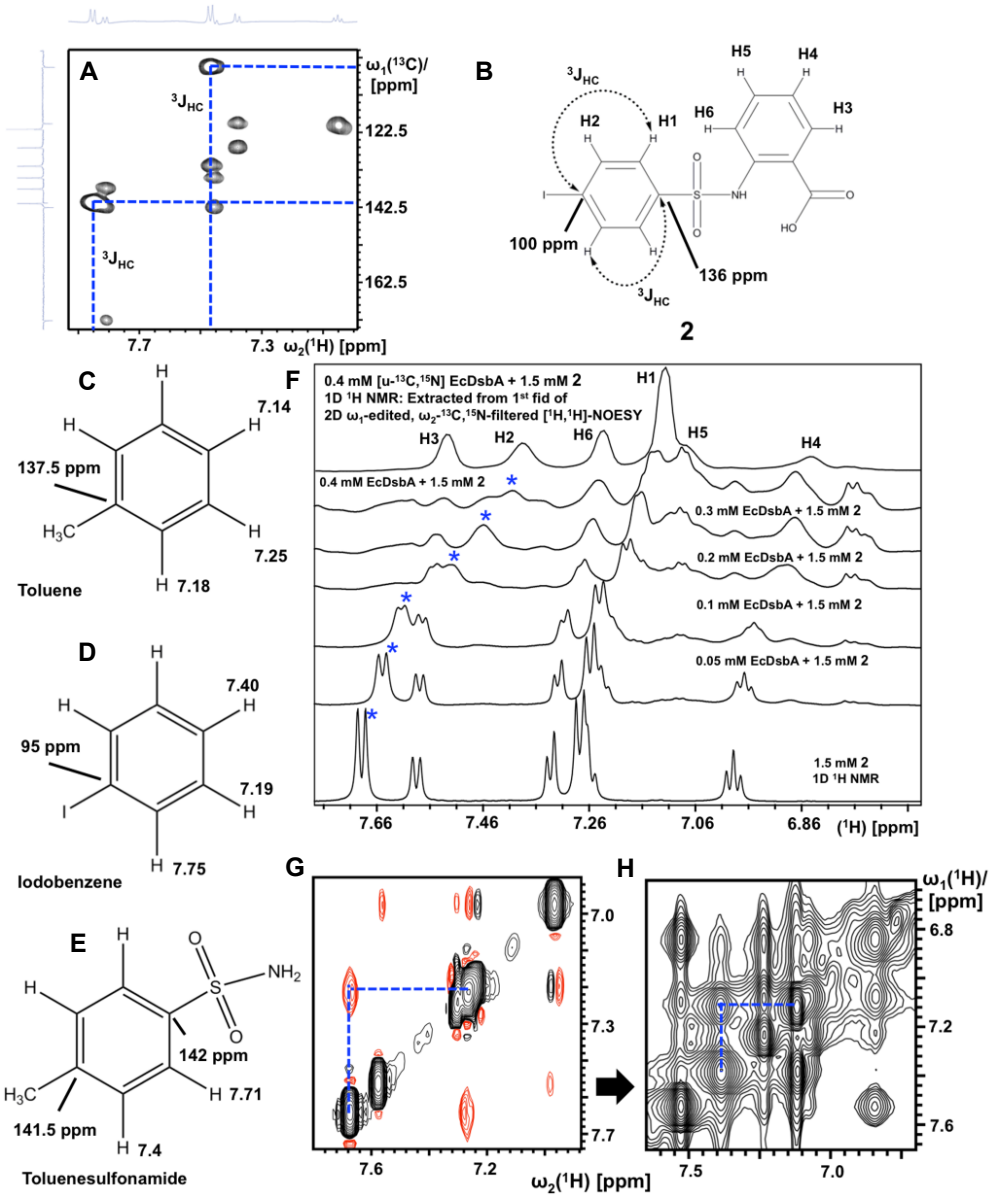
Ligand properties

bound		free
protein	chemical environment	solvent
ω_{bound}	chemical shift	ω_{free}
slow	rotational tumbling	fast
fast strong positive	transverse relaxation NOE cross-peaks	slow weak negative
slow	translational diffusion	fast



Protein properties

bound		free
ligand	chemical environment	solvent/protein
ω_{bound}	chemical shift	ω_{free}



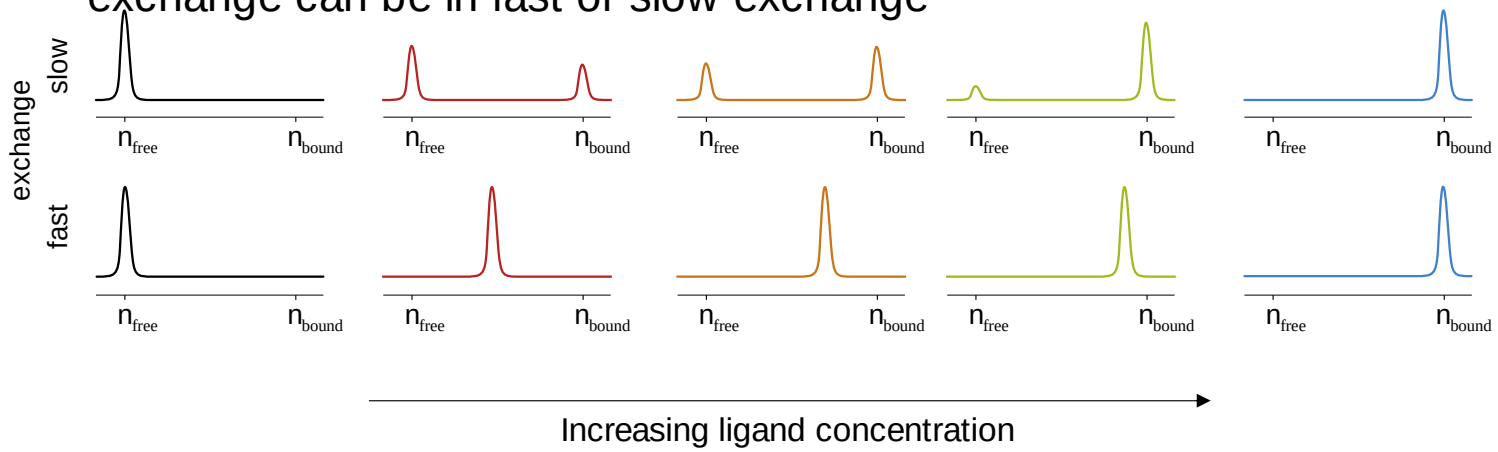
Exchange regimes in ligand binding

Populations affect intensity or position of the signals in titrations

- Two-state exchange is also observed in ligand binding, between bound and free states.

$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} PL$$

- Depending on the association (k_{on} [L]) and dissociation (k_{off}) rates, this exchange can be in fast or slow exchange



Orts Methods (2018)

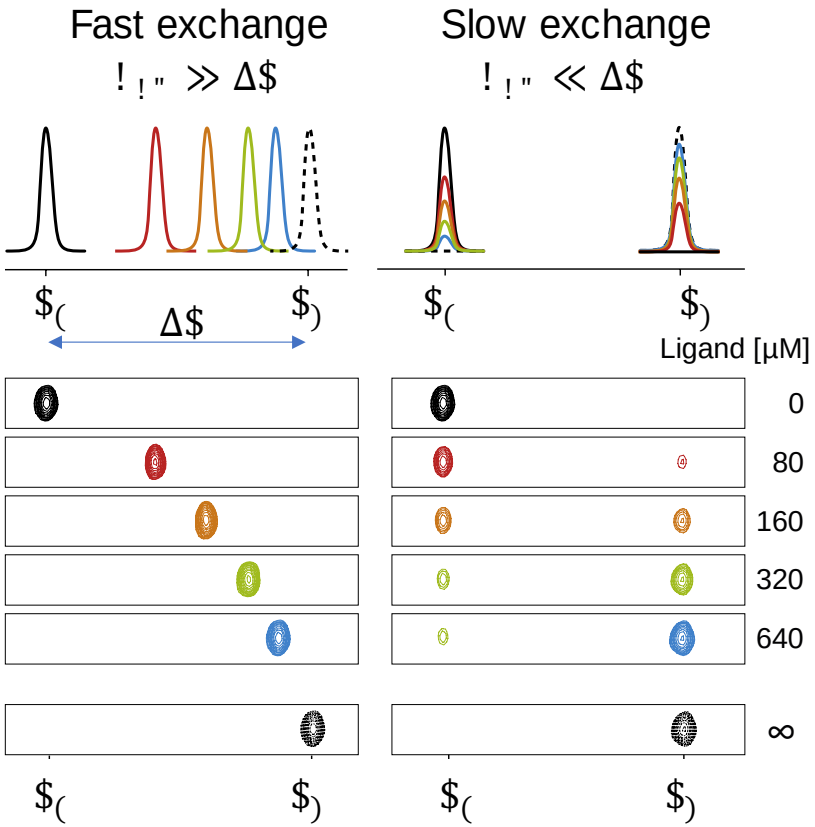
Exchange regimes in ligand binding

Examples of fast or slow exchange and 1D or 2D spectra

- Example:
  Protein signal at increasing concentrations of ligand

$$P + L \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} PL$$

$$!_{!"} = !_{\%\&}[)] + !_{\%'}$$

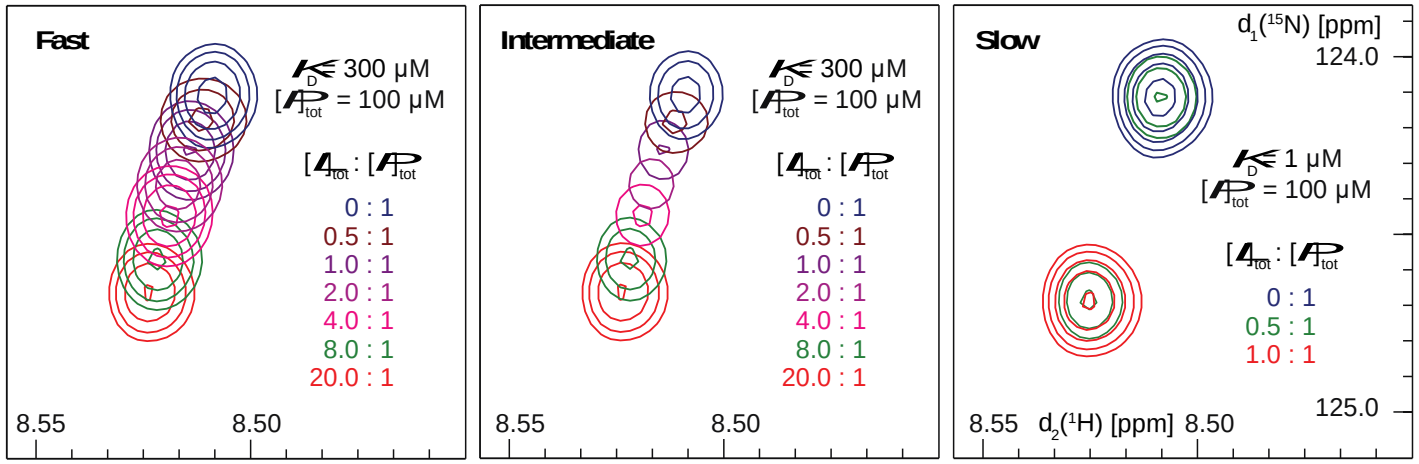


Orts Methods (2018)

Exchange regimes in ligand binding

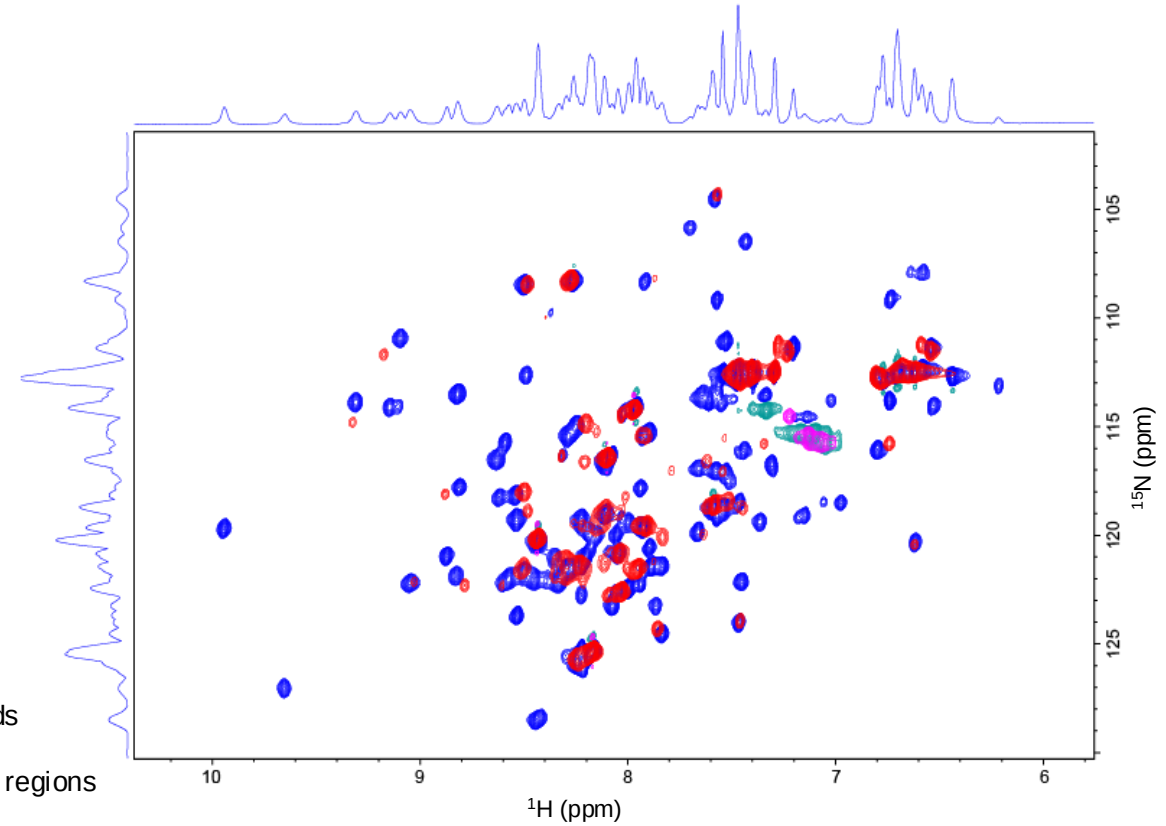
Examples for titrations

- Depending on the exchange kinetics, protein signals have different signal positions and line-width at different concentrations of the binding partner.
- This is well-observable in titrations.



HDMX-ligand

With/without
peptide inhibitor



"MDM4 binds ligands
via a mechanism
in which disordered regions
become structured"

Sanchez, FEBS Letters 584, 3035–3041 (2010)

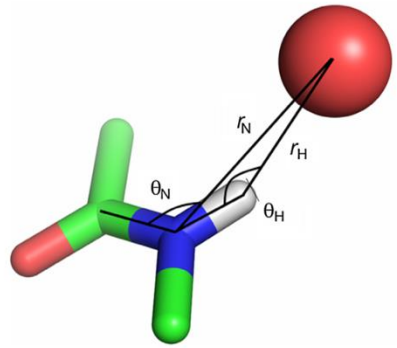


Fig. 5. The electric field effect caused by a charged atom (shown as a sphere) is proportional to $q\cos\theta/r^2$, where q is the charge on the atom [107]. The angle θ is the angle made to the bond along which electron density is pushed or pulled: where there is a choice, this will be the most polarisable bond. For ^1H_N, this is the H–N bond. For N, this is the N–C′ bond. Because the relevant angles for H and N can be completely different, the effect on the chemical shifts of H and N can also be very different.

($^1H^{15}N$, 290 K, 700 MHz), [P]=0.5 mM
pH 7.5, 25 mM Phos., 25 mM NaCl,
2 mM TCEP, 0.1 mM EDTA

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Parameters for characterization of a protein-ligand complex

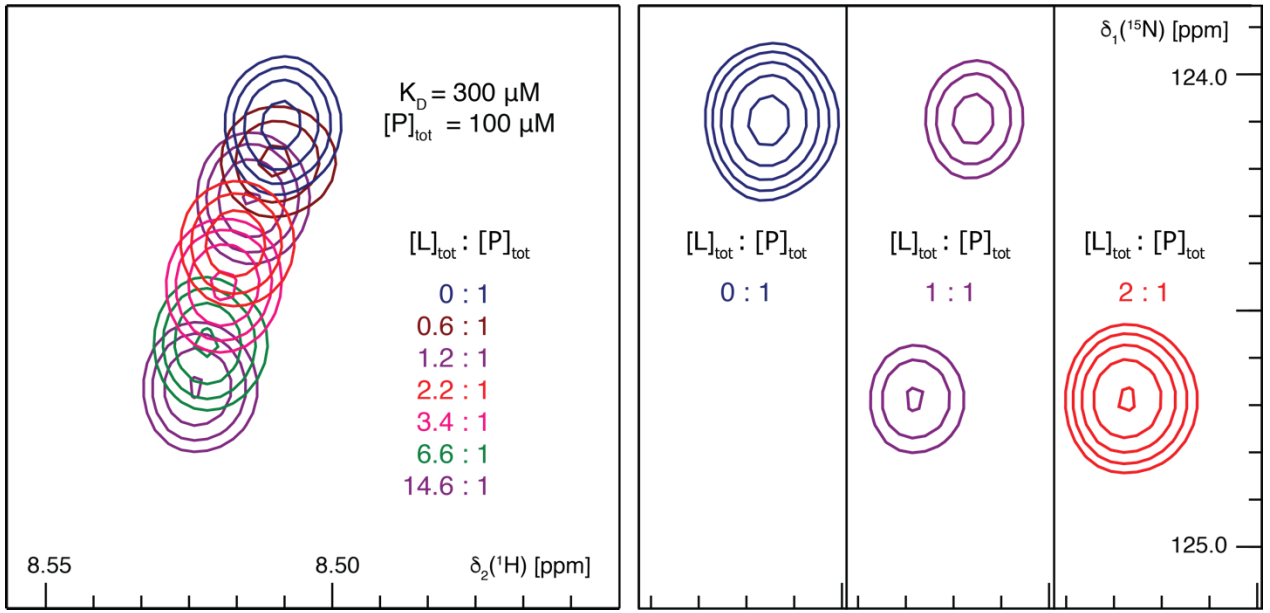
- Protein concentration and stability in sample
- Ligand concentration and stability in sample
- Dissociation constant (K_D) of complex
- Exchange kinetics of complex (k_{ex}: slow, intermediate, fast)

$$P + L \underset{}{\overset{k_{on}}{\rightleftharpoons}} PL$$

Knowing the K_D is crucial

$$A_{avg} = A_{free} \cdot p_{free} + A_{bound} \cdot p_{bound} \text{ (Eq. 8)}$$

$$K_D = \frac{[P]_{free}[L]_{free}}{[PL]} = \frac{k_{off}}{k_{on}}$$



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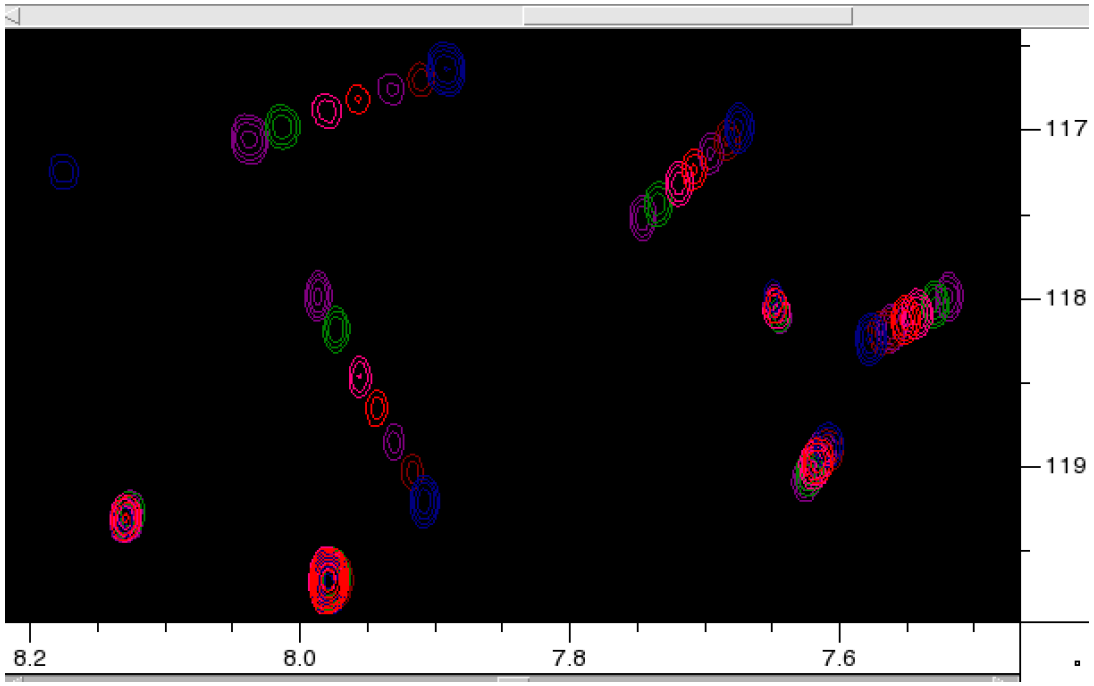
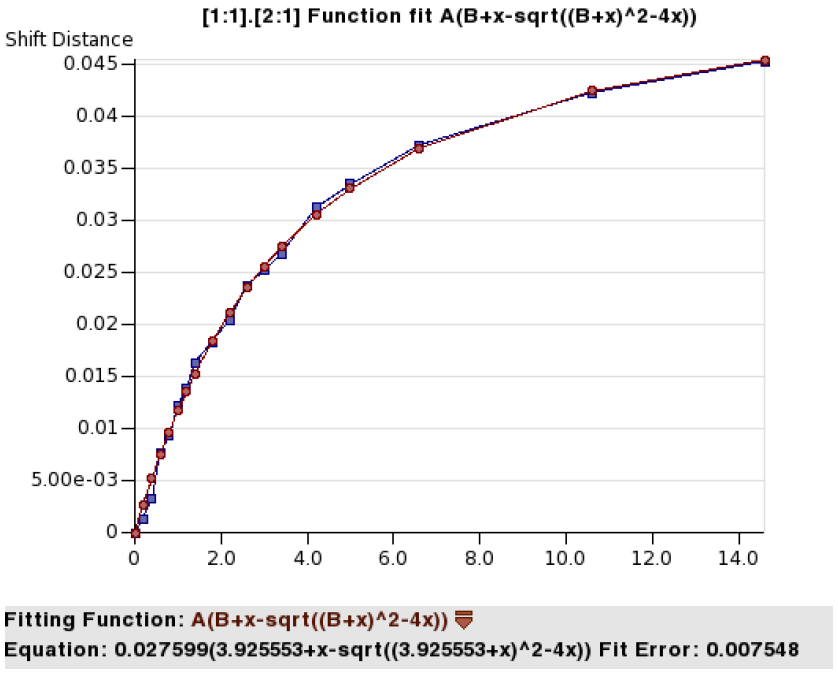
Example:
PDZ + phosphorylated peptide

$$\Delta\delta_{obs} = \Delta\delta_{max}\left\{([P]_t + [L]_t + K_d) - [([P]_t + [L]_t + K_d)^2 - 4[P]_t[L]_t]^{1/2}\right\}/2[P]_t \quad (6)$$



$[P]_t$=100uM

Kd=339 uM



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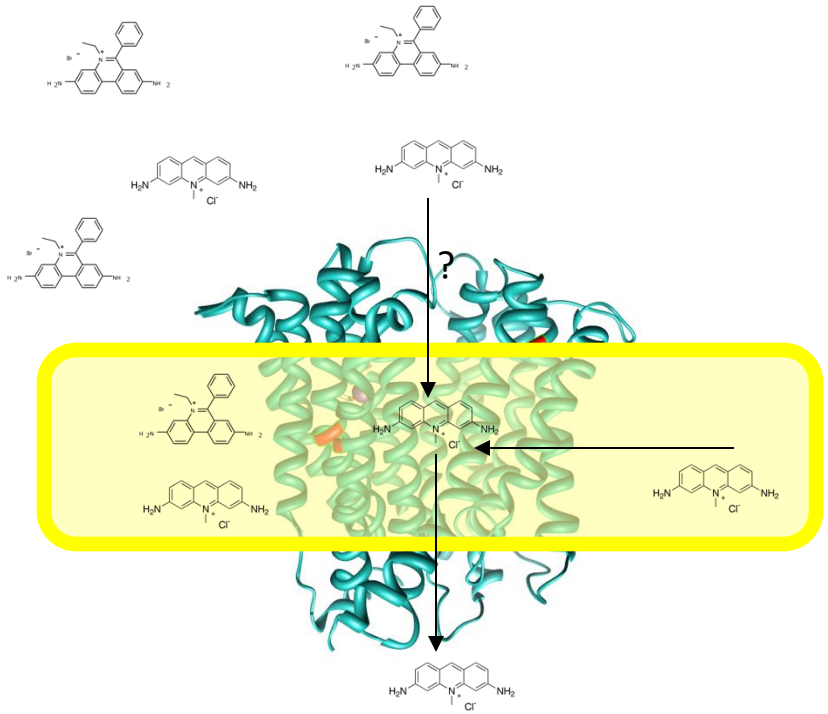
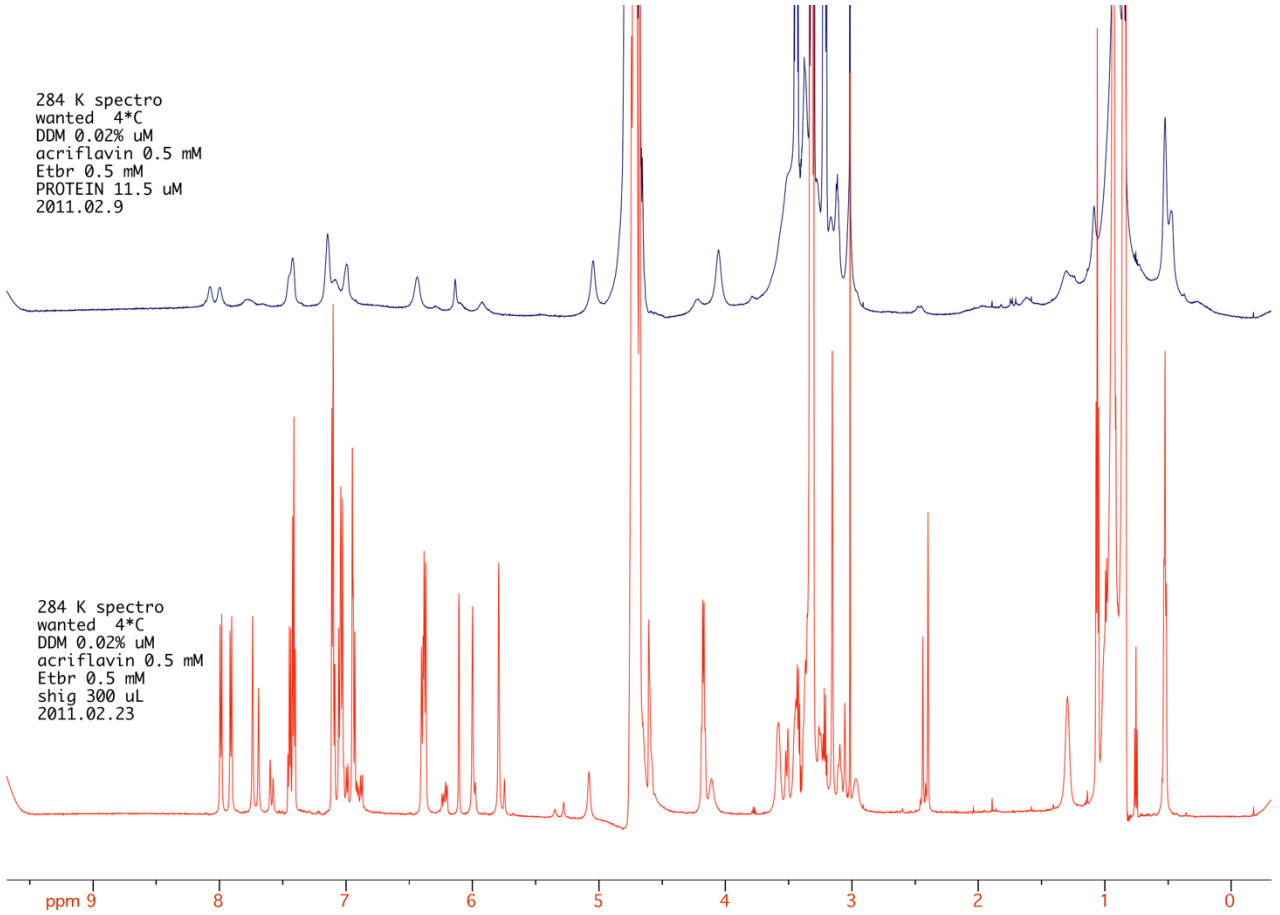
Acri. And EtBr. bind to the proteins micelles complexes





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Relaxation

Transverse relaxation through dipole-dipole interactions

$$R_{2,DD} = \frac{1}{20}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(5\tau_c + \frac{9\tau_c}{1+\omega_0^2\tau_c^2} + \frac{6\tau_c}{1+4\omega_0^2\tau_c^2}\right) \quad (13)$$

Transverse relaxation through chemical shift anisotropy

$$R_{2,CSA} = \frac{1}{24}\left(\sigma_{\parallel-\perp}^2 B_0^2\gamma^2\right)\left(4\tau_c + \frac{3\tau_c}{1+\omega_0^2\tau_c^2}\right) \quad (14)$$

Nuclear Overhauser effect (NOE)

$$R_C^L = \frac{1}{10}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(\tau_c - \frac{6\tau_c}{1+4\omega_0^2\tau_c^2}\right) \quad (15)$$

Transverse nuclear Overhauser effect (ROE)

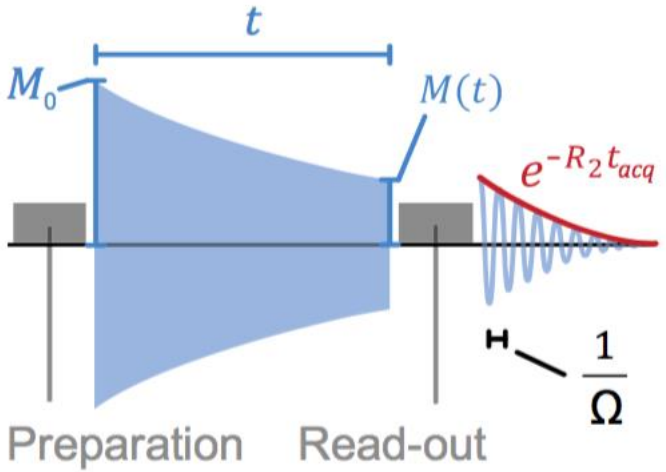
$$R_C^T = -\frac{1}{10}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(2\tau_c + \frac{3\tau_c}{1+\omega_0^2\tau_c^2}\right) \quad (16)$$

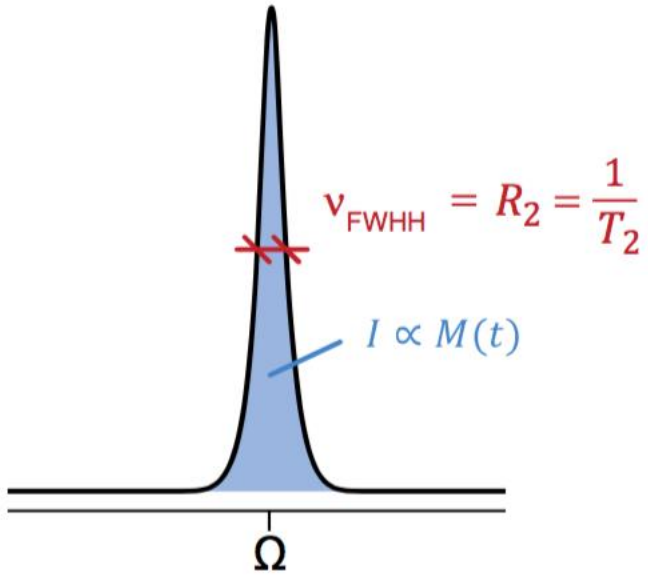
Longitudinal relaxation

$$R_{1,DD} = \frac{1}{10}\frac{\hbar^2\mu_0^2\gamma^4}{r^6}\left(\frac{3\tau_c}{1+\omega_0^2\tau_c^2} + \frac{12\tau_c}{1+4\omega_0^2\tau_c^2}\right) \quad (17)$$

Paramagnetic relaxation

$$R_{2,para} = \frac{1}{20}\frac{\hbar^2\mu_0^2\gamma_n^2\gamma_e^2}{r^6}\left(4\tau_c + \frac{3\tau_c}{1+\omega_0^2\tau_c^2}\right) \quad (18)$$





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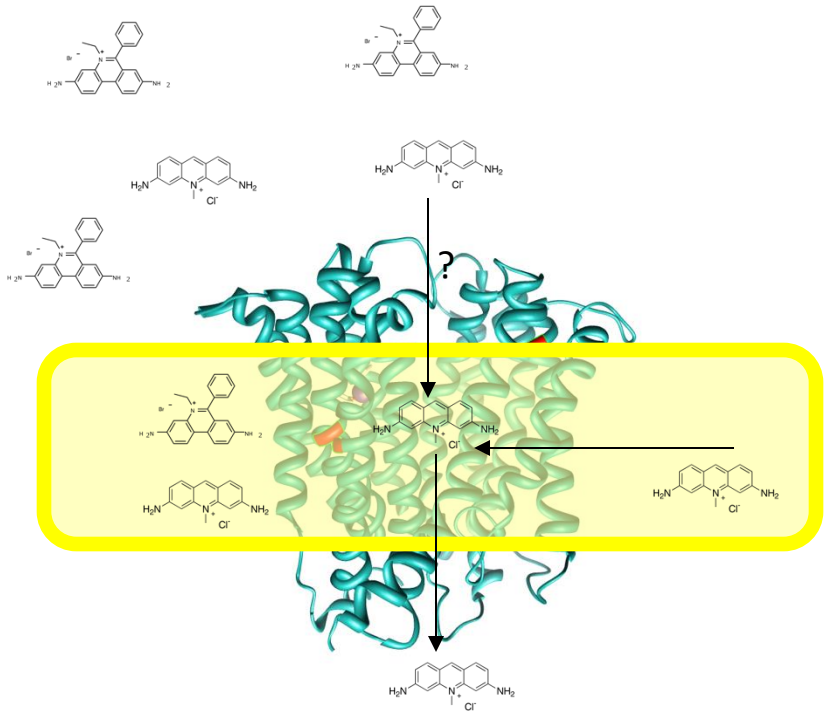
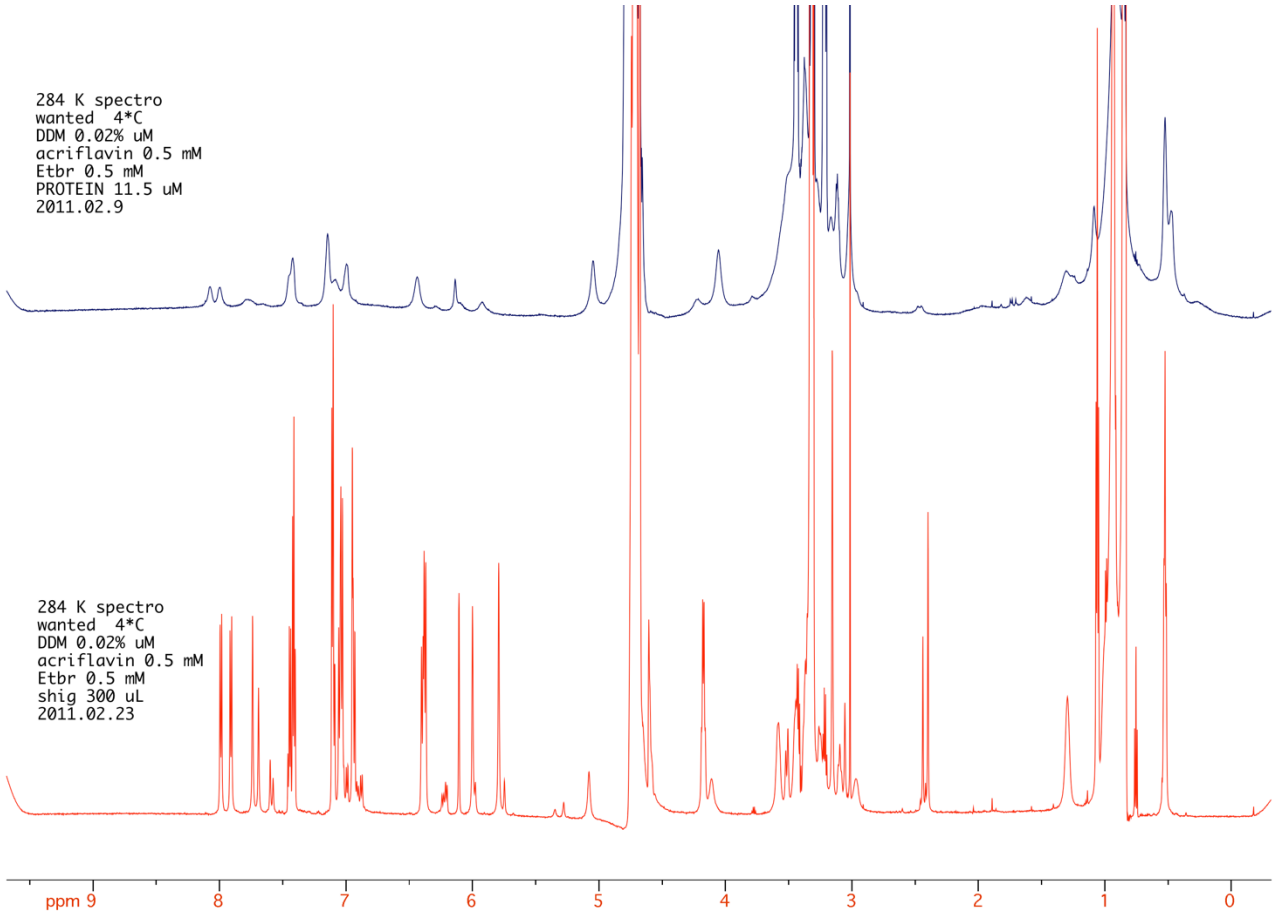
Acri. And EtBr. bind to the proteins micelles complexes





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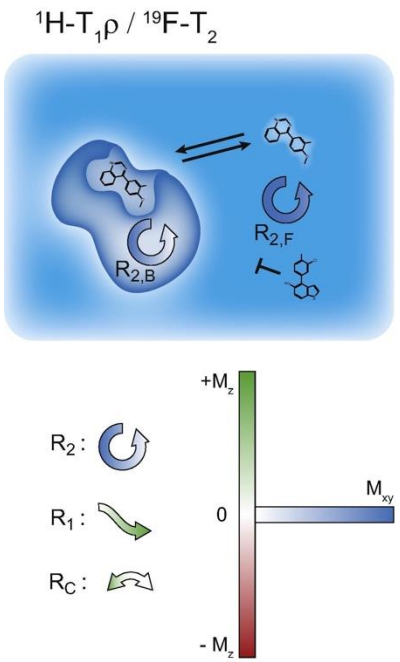

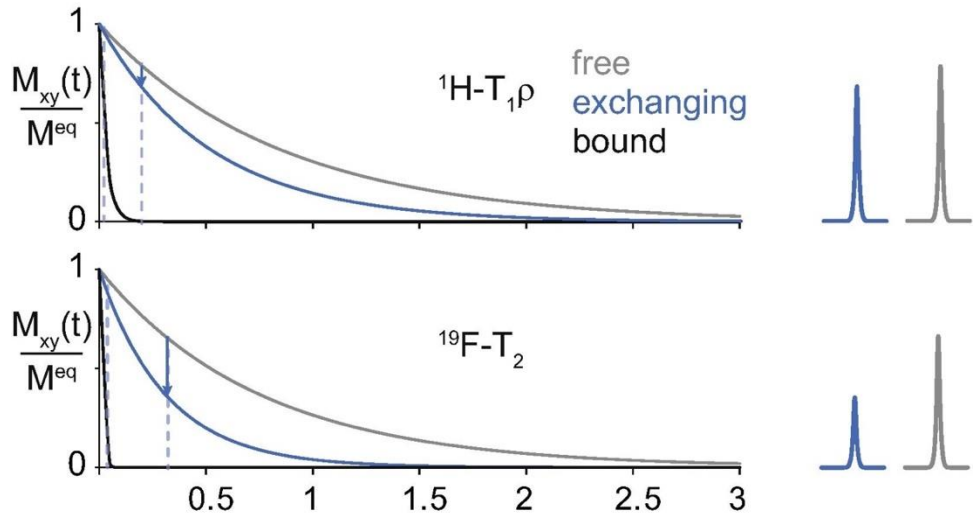

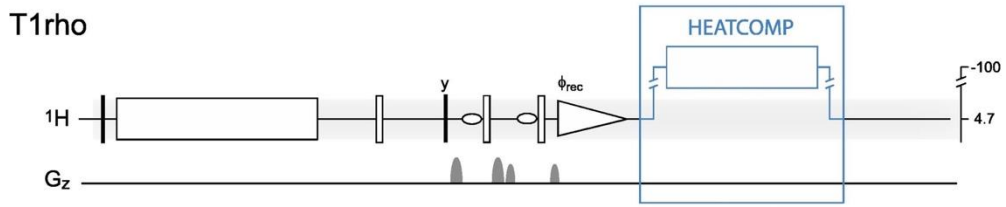

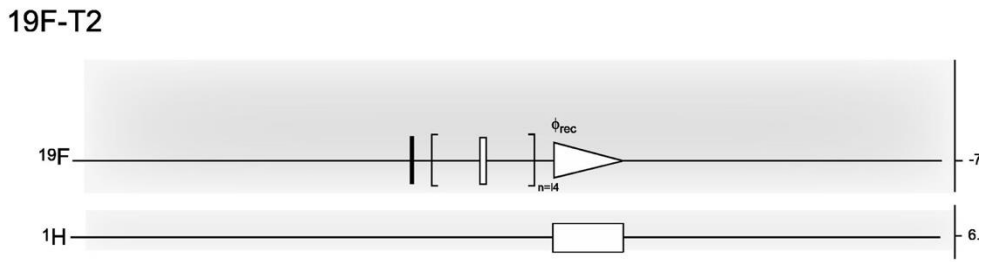

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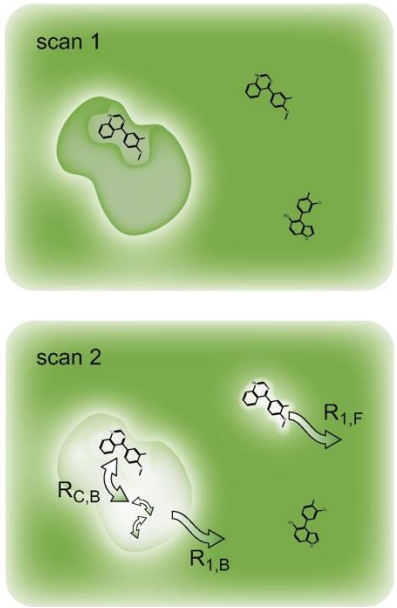

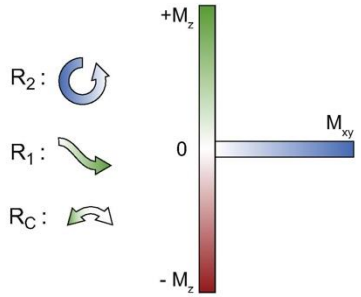

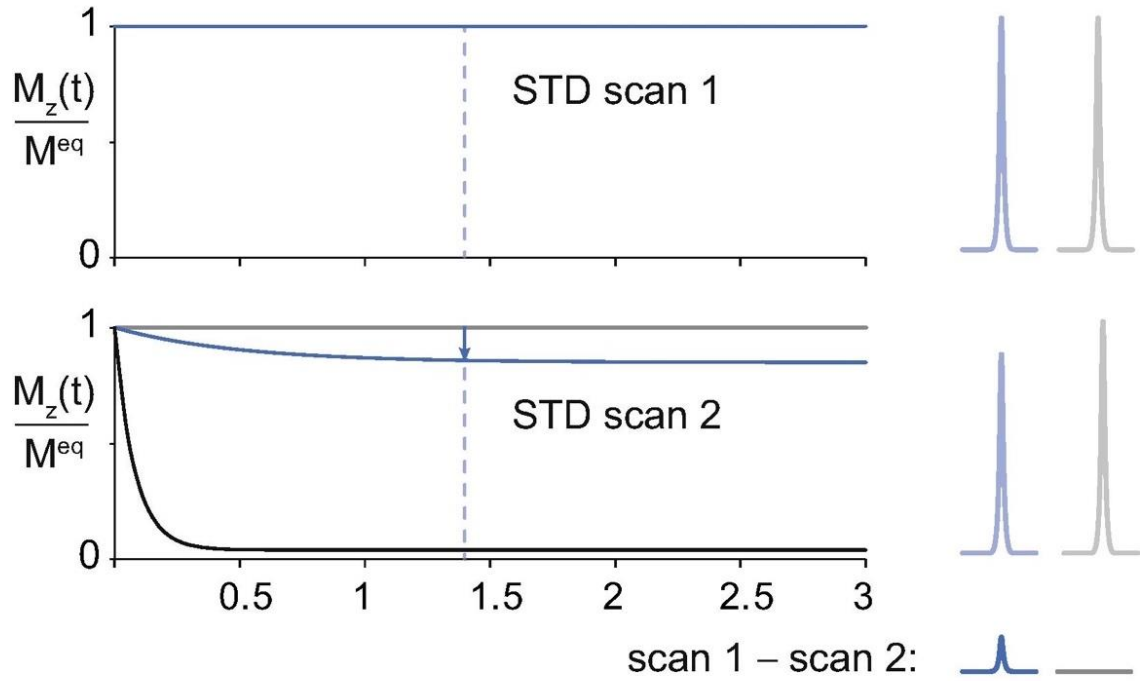

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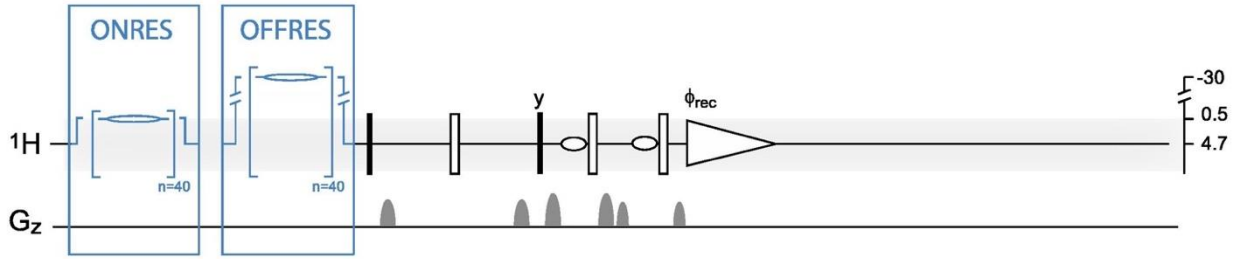

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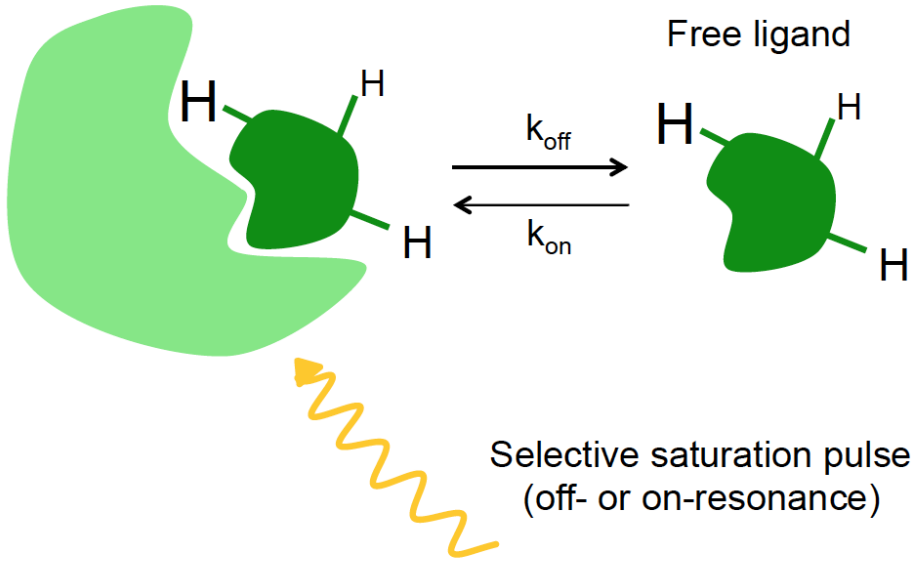
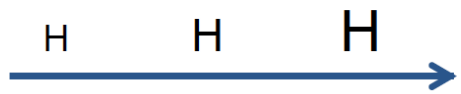
Protein-ligand complex



Two spectra recorded:

1. Off-resonance irradiation: 60 ppm
2. On-resonance irradiation: -1 ppm

Difference off- minus on-resonance:

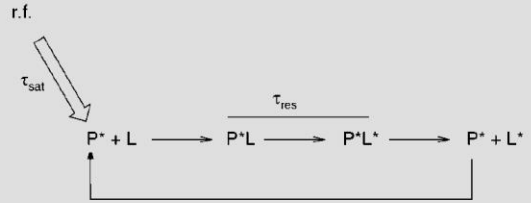


Increasing saturation/ intensity

What Experimental setup?
Pulses, delays, recovery, filter, etc.

Box 2: Parameters affecting STD effects

The Scheme shows the situation of a solution containing ligand and receptor after the initial saturation time of typically 50 to 200 ms. Assuming a large excess of ligand over protein, the rebinding of already saturated ligands can be neglected. (r.f.: radio frequency for saturation; τ_{sat}: saturation time; τ_{res}: residence time of ligand in the binding site; P*: saturated protein; L: unsaturated ligand; L*: saturated



ligand.) During the saturation time τ_{sat} the binding site of the protein is consecutively occupied by n ligand molecules with $n = f_{PB} * \tau_{sat}/\tau_{res}$, where f_{PB} is the fraction of occupied binding sites (see Box 1). This turnover is responsible for the amplification of the information resulting from the saturated protein. A large excess of ligands allows for the maximum effect to be observed.

A recent paper describes the application of full-relaxation matrix theory to the calculation of theoretical STD effects taking into account the binding kinetics and thermodynamics as well as all protons of the binding site.[128]

Mayer and Meyer JACS (2001)

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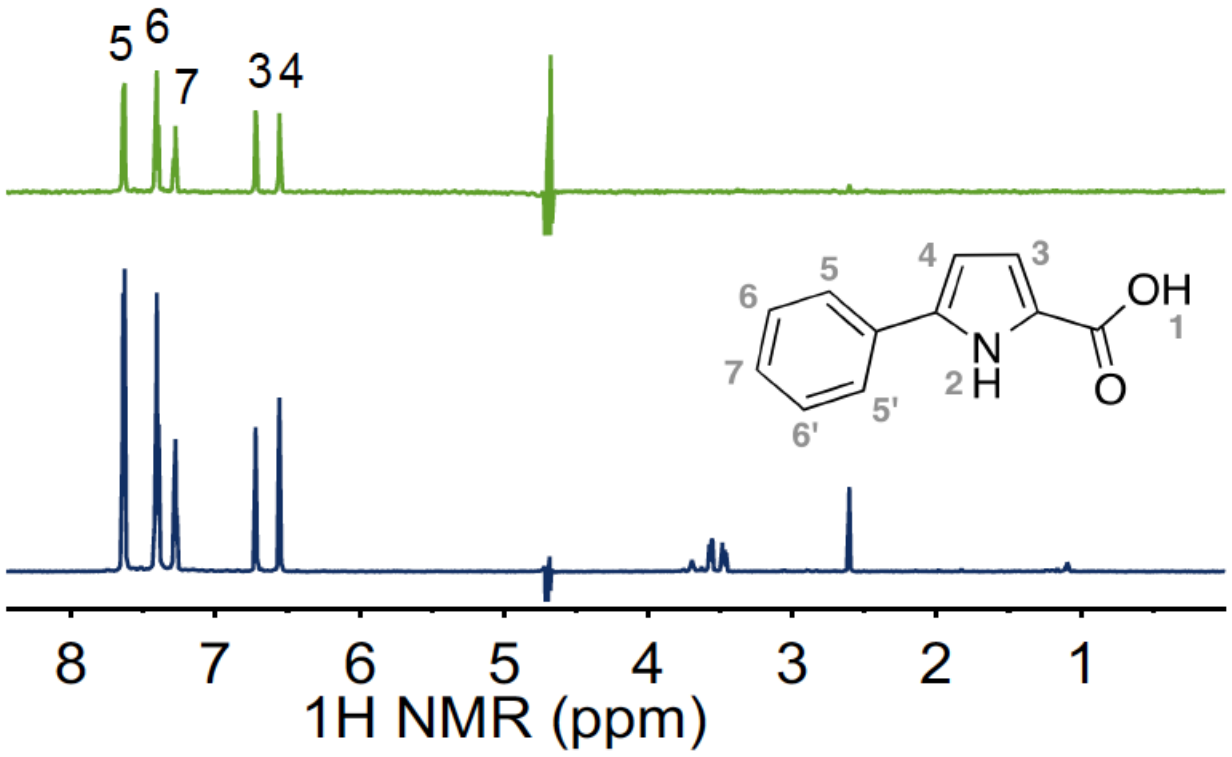
Sample solution – Aβ/ligand 1:100

- 150 µL Aβ fibrils in phosphate buffer (~25 µM)
- 3.75 µL Ligand d_6-DMSO/D_2O 95:5 (100 mM)*
- 17 µL D_2O **



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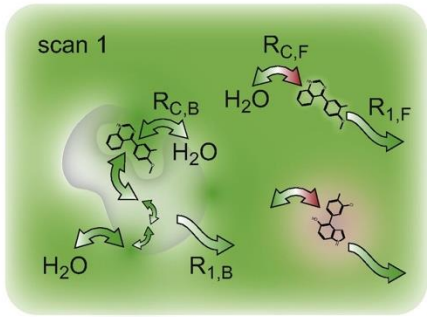

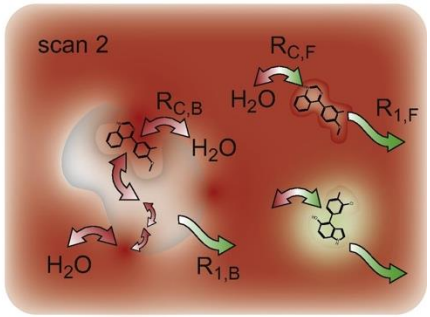

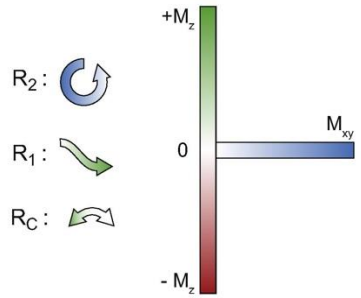

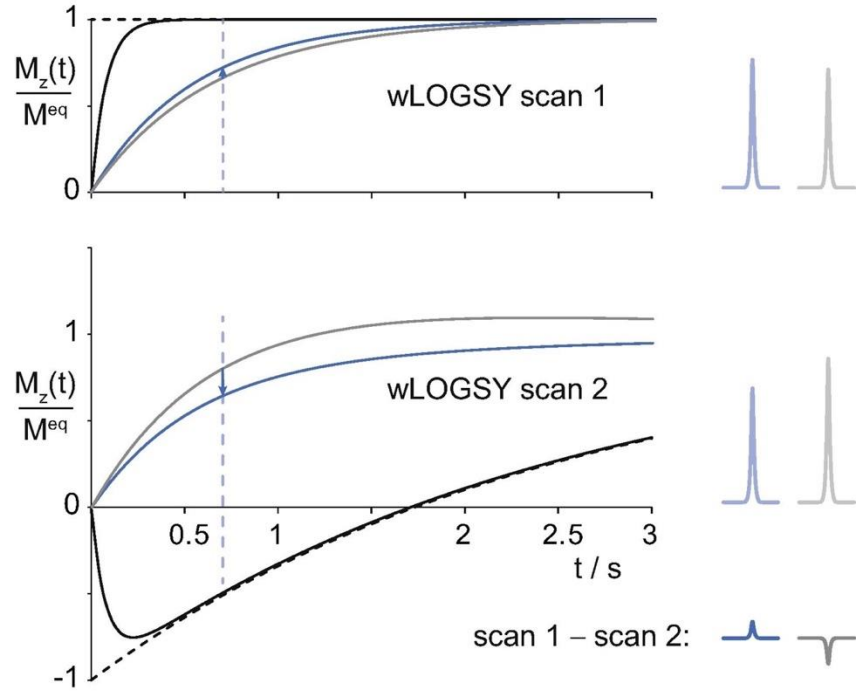

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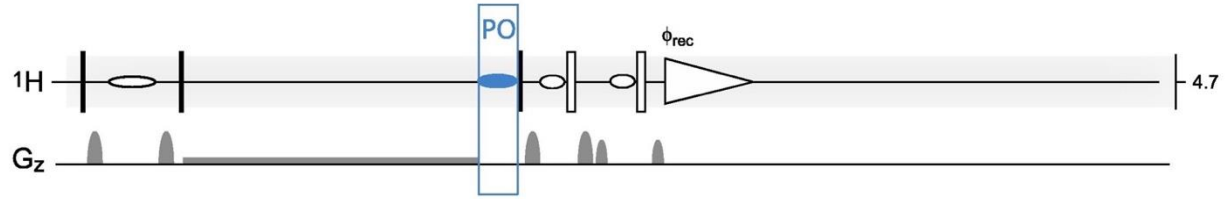

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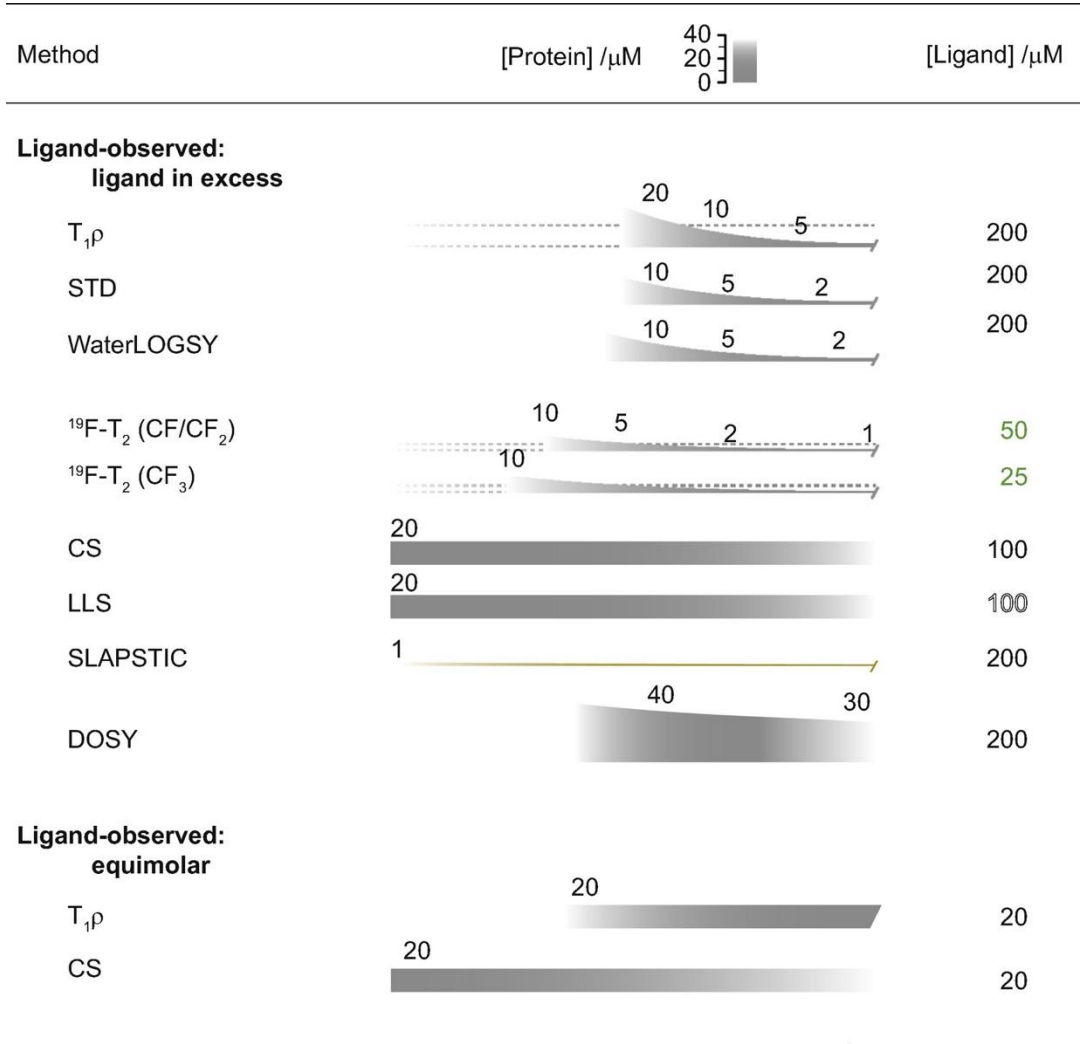

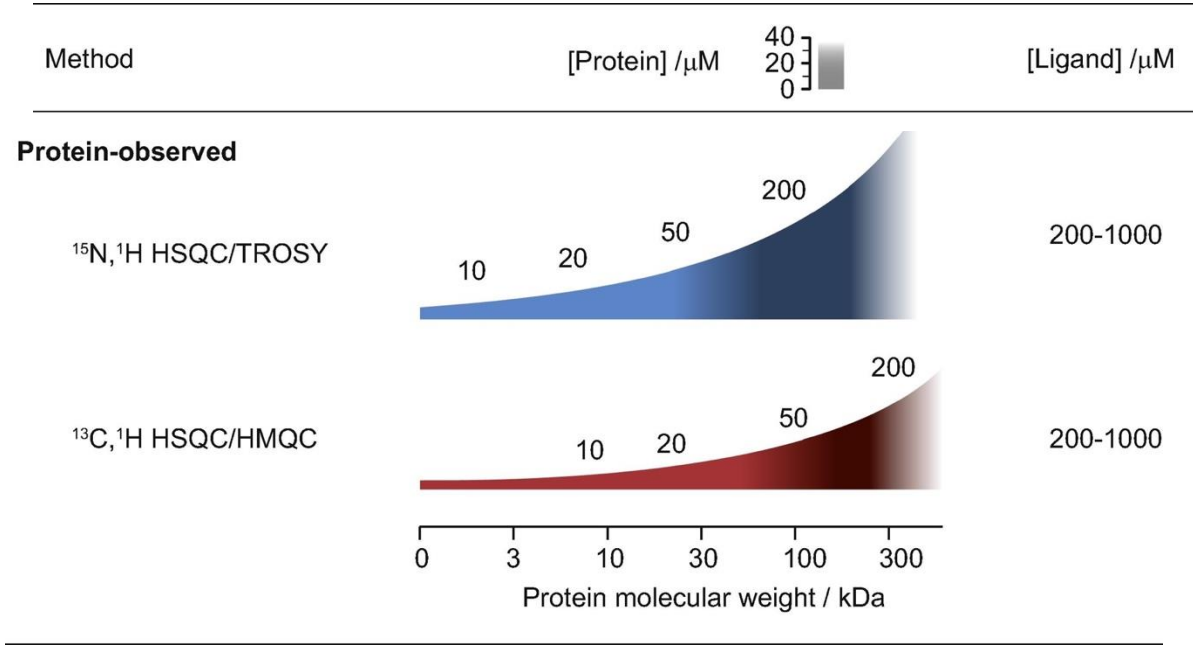

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Parameters determining feasibility of a structure determination of a protein-ligand complex

- Protein solubility (> 300 µM)
- Ligand solubility (> K_d)
- Minimal population of bound protein (> 0.8 achievable) *(not for trNOE experiments)
- Exchange kinetics (slow or fast for signals to be visible, *e.g.* $k_{ex} < 1\ s^{-1}$ or $k_{ex} > 1000\ s^{-1}$)
- Quality of protein spectrum (dispersion, resonance assignments available)
- Quality of ligand and its spectrum (number and distribution of hydrogens on ligand, dispersion of NMR signals, resonance assignment)

		Part of complex to be studied			
		Bound ligand	Intermolecular	Bound protein	Full complex
Exchange rate	k_{ex} fast	$[P]_{tot} \ll [L]_{tot}$ 1:5–50 [PL]/[L]= 0.2–0.02	$[P]_{tot} < [L]_{tot}$ (max [PL])	$[P]_{tot} < [L]_{tot}$ (max [PL] and min $[P]_{free}$)	$[P]_{tot} < [L]_{tot}$ (max [PL] and min $[P]_{free}$)
	k_{ex} slow	$[P]_{tot} \geq [L]_{tot}$ (min $[L]_{free}$)	$[P]_{tot} \approx [L]_{tot}$ (max [PL])	$[P]_{tot} \leq [L]_{tot}$ (min $[P]_{free}$)	$[P]_{tot} \leq [L]_{tot}$ (min $[P]_{free}$)

Ligand solubility

$[L]_{free}=[L]_{sol}$ $\qquad K_D = \frac{[P]_{free}[L]_{free}}{[PL]}$

$$p_B^P = \frac{[PL]}{[P]_{tot}} = \frac{[L]_{sol}}{[L]_{sol}+K_d} \text{ (Eq. 9a)}$$

$$[L]_{tot} = [L]_{sol} + [PL] = [L]_{sol}\left(1 + \frac{[P]_{tot}}{[L]_{sol}+K_d}\right) \text{(Eq. 9b)}$$

Preparation of protein-ligand samples

- Use Table for suggested protein to ligand ratios, try to reach p_B of > 0.8
- Avoid the intermediate exchange regime (change magnetic field, temperature, viscosity and concentrations of protein and ligand)
- If possible, avoid large excess of free ligand to minimize spectral artefacts

In cases of limited solubility of the ligand

- Use Eq. 9b to calculate maximal possible ligand concentration
- Add ligand to protein solution, not vice versa, in order to exploit the full concentration of bound ligand

$[P]_{tot}$ = 1mM