Experiment and Modelling in Structural NMR

November 28th – December 2nd 2011
INSTN – CEA Saclay, France

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Molecular interactions
by NMR spectroscopy

[03001]

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Article available at <http://www.epj-conferences.org> or <http://dx.doi.org/10.1051/epjconf/20123003001>

Molecular interactions by NMR spectroscopy

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Interaction by NMR

$$\text{Thermodynamic view : } K_D = \frac{[A][B]}{[AB]} = \exp(-\Delta G/RT)$$

- Hypotheses : 1) We can define concentrations
2) Equilibrium
3) $\Delta G = G(A, B, \text{ solvent with } A \& B) - G(AB, \text{ solvent with } AB)$

Interaction by NMR

Kinetic view

$$\frac{d}{dt}[AB] = -k_{\text{off}}[AB] + k_{\text{on}}[A][B]$$

$$\text{At equilibrium : } \frac{d}{dt}[AB] = -k_{\text{off}}[AB] + k_{\text{on}}[A][B] = 0$$

$$K_D = \frac{[A][B]}{[AB]} = \frac{k_{\text{off}}}{k_{\text{on}}}$$

McConnell – Bloch equations

Different frequency for A when alone (A) or in complex (A')

$$\frac{d}{dt}M_{Ax} = -\omega_A M_{Ay} + k_{-1} M_{A'x} - k_1 M_{Ax}$$

$$\frac{d}{dt}M_{A'x} = -\omega_{A'} M_{A'y} - k_{-1} M_{A'x} + k_1 M_{Ax}$$

McConnell – Bloch equations

A

B

k₁

k₋₁

$$\frac{d}{dt} \begin{bmatrix} [A] \\ [B] \end{bmatrix} = \begin{bmatrix} -k_1 & k_{-1} \\ k_1 & -k_{-1} \end{bmatrix} \begin{bmatrix} [A] \\ [B] \end{bmatrix}$$

$$\frac{d}{dt} \begin{bmatrix} M_A^+ \\ M_B^+ \end{bmatrix} = \begin{bmatrix} -R_{2,A} + i\omega_A & \\ & -R_{2,B} + i\omega_B \end{bmatrix} \begin{bmatrix} M^+ \\ M^- \end{bmatrix}$$

$$\frac{d}{dt} \begin{bmatrix} M_A^+ \\ M_B^+ \end{bmatrix} = \left\{ \begin{bmatrix} -R_{2,A} + i\omega_A & \\ & -R_{2,B} + i\omega_B \end{bmatrix} + \begin{bmatrix} -k_1 & k_{-1} \\ k_1 & -k_{-1} \end{bmatrix} \right\} \begin{bmatrix} M_A^+ \\ M_B^+ \end{bmatrix}$$

McConnell – Bloch equations

$$\begin{bmatrix} M_A^+(t) \\ M_B^+(t) \end{bmatrix} = \begin{bmatrix} a_{11}(t) & a_{12}(t) \\ a_{21}(t) & a_{22}(t) \end{bmatrix} \begin{bmatrix} M_A^+(0) \\ M_B^+(0) \end{bmatrix} \quad (15)$$

with

$$a_{11} = \frac{1}{2} \left[\left(1 - \frac{i\Delta\omega - \Delta R_2 + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)} \right) e^{-\lambda_- t} + \left(1 + \frac{i\Delta\omega - \Delta R_2 + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)} \right) e^{-\lambda_+ t} \right]$$

$$a_{22} = \frac{1}{2} \left[\left(1 + \frac{i\Delta\omega - \Delta R_2 + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)} \right) e^{-\lambda_- t} + \left(1 - \frac{i\Delta\omega - \Delta R_2 + k_1 - k_{-1}}{(\lambda_+ - \lambda_-)} \right) e^{-\lambda_+ t} \right]$$

$$a_{12} = \frac{k_{-1}}{(\lambda_+ - \lambda_-)} [e^{-\lambda_- t} - e^{-\lambda_+ t}]$$

$$a_{21} = \frac{k_1}{(\lambda_+ - \lambda_-)} [e^{-\lambda_- t} - e^{-\lambda_+ t}]$$

$$\Delta\omega = \omega_B - \omega_A \text{ and } \Delta R_2 = R_{2,B} - R_{2,A}$$

$$\lambda_{\pm} = \frac{1}{2} \left[(-i\omega_A - i\omega_B + R_{2,A} + R_{2,B} + k_{ex}) \pm \{ (i\Delta\omega - \Delta R_2 + k_1 - k_{-1})^2 + 4k_1 k_{-1} \}^{1/2} \right]$$

McConnell – Bloch equations for 2-site exchange

Lifetime of state A:
 $\tau_A = 1/k_{-1}$

Lifetime of state B:
 $\tau_B = 1/k_1$

Use a single lifetime
 $1/\tau = 1/\tau_A + 1/\tau_B$
 $= k_{+1} + k_{-1}$

Case dependent

Labeled protein available – Only unlabeled protein available

Strong binding ligands – weak binding ligands

Binary answer (yes/no) – detailed answer (affinity/where?)

Single ligands – mixtures

2

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Role for NMR?
<ul style="list-style-type: none"> -Binary response (does/does not interact) - Thermodynamic parameters -Kinetic parameters -Characterization of the complex

A first case of high affinity interaction

Slow exchange

Cyclophilin + Cyclosporin A

10

CypA becomes « charged » by Cyclosporin A

Dry CsA in NMR tube –
 When comes in solution, captured by CypA

12

Monte Carlo simulation of chemical exchange

Consider m molecules, f bound
(1), $(m-f)$ unbound (0)

Draw random number $0.0 < r < 1.0$

Compare r to a predefined probability p

$r < p$: Change state from bound (1) to unbound (0)
 $r > p$: Leave molecules in the bound (1) state

Ensure micro-equilibrium

37

Probability $p \sim$ off-rate ($1/p$)

2000 molecules

1000 time steps

*Relax the requirement
for micro-equilibrium*

40

Ensemble behaviour : 500 molecules, 191 bound

$K_D = 300\mu\text{M}$

$[\text{WW}] = 0.2\text{mM}$

$[\text{Pep}] = 0.2\text{mM}$

$P=0.01/\text{step}$

41

Time behaviour : individual trajectories

$K_D = 300\mu\text{M}$

$[\text{WW}] = 0.2\text{mM}$

$[\text{Pep}] = 0.2\text{mM}$

$P=0.01/\text{step}$

5120 steps

42

2 sample experiments

Take a spectrum of a mixture without/with the protein target

Look at a NMR parameter that changes upon interaction

Line width / Diffusion coefficient / relaxation rate (transfer NOE)

Use difference spectrum to isolate the compound

Advantage : little protein needed / mixtures possible

Problem : Comparing spectra of two samples!!!

57

2 sample experiments

Figure 1. Expanded region of the 1D ^1H spectra recorded for 40 μM P13 285283 in the presence (a) and in the absence (b) of the kinase (2 μM in PBS). The data were multiplied with a cosine window function prior to Fourier transformation. An arrow indicates the resonance undergoing significant broadening in the presence of the protein.

Figure 2. Expanded region of the 1D ^1H spectra recorded at different 285283 concentrations in the presence of the kinase (1.5 μM in PBS). Spectra were acquired with 128 scans and 2.82 s repetition time. The concentration was 40 (a), 80 (b) and 140 μM (c).

58

Transfer NOE : $\text{NOE} = f(\tau_c)$

Positive NOE for small molecules leads to weak negative cross peaks

Negative NOE for big molecules leads to strong positive cross peaks

60