Intermolecular interactions and NMR

The study of molecular interactions between biological macromolecules and their interaction partners (other macromoleules or small ligands) at an atomic resolution is the next step after determining structures of individual molecules.
Studying interactions results in describing action in living systems.

structural and dynamic characterization at an atomic resolution of already known interactions
detection of unknown interaction between

- biological molecules for describing cellular processes or
- between biological and synthetical molecules for pharmaceutical purposes

Impact of NMR spectroscopy for characterizing molecular interactions

spectroscopy with atomic resolution

individual observation of interacting molecules by

- isotope labeling, if MW(Protein) < 100 * MW(ligand)
- observing ligand, if MW(Protein) > 100 * MW(ligand) and MW(ligand < 1000 Da)

direct observation of pairwise spin-interactions between protein and ligand

structure determination of complexes

screening techniques for pharmaceutical research

NMR parameters and their impact for studying molecular interactions

chemical shifts	structural changes
scalar couplings, residual dipolar couplings	structural changes
Relaxation	dynamics, conformational flexibility
translational diffusion	size of complexes
intermolecular cross relaxation	intermolecular distance information

Bimolecular reaction

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

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

$$[P] + [PL] = [P]_0$$

$$K_D = \frac{([P]_0 - x_{PL}[P]_0)([L]_0 - x_{PL}[P]_0)}{x_{PL}[P]_0}$$

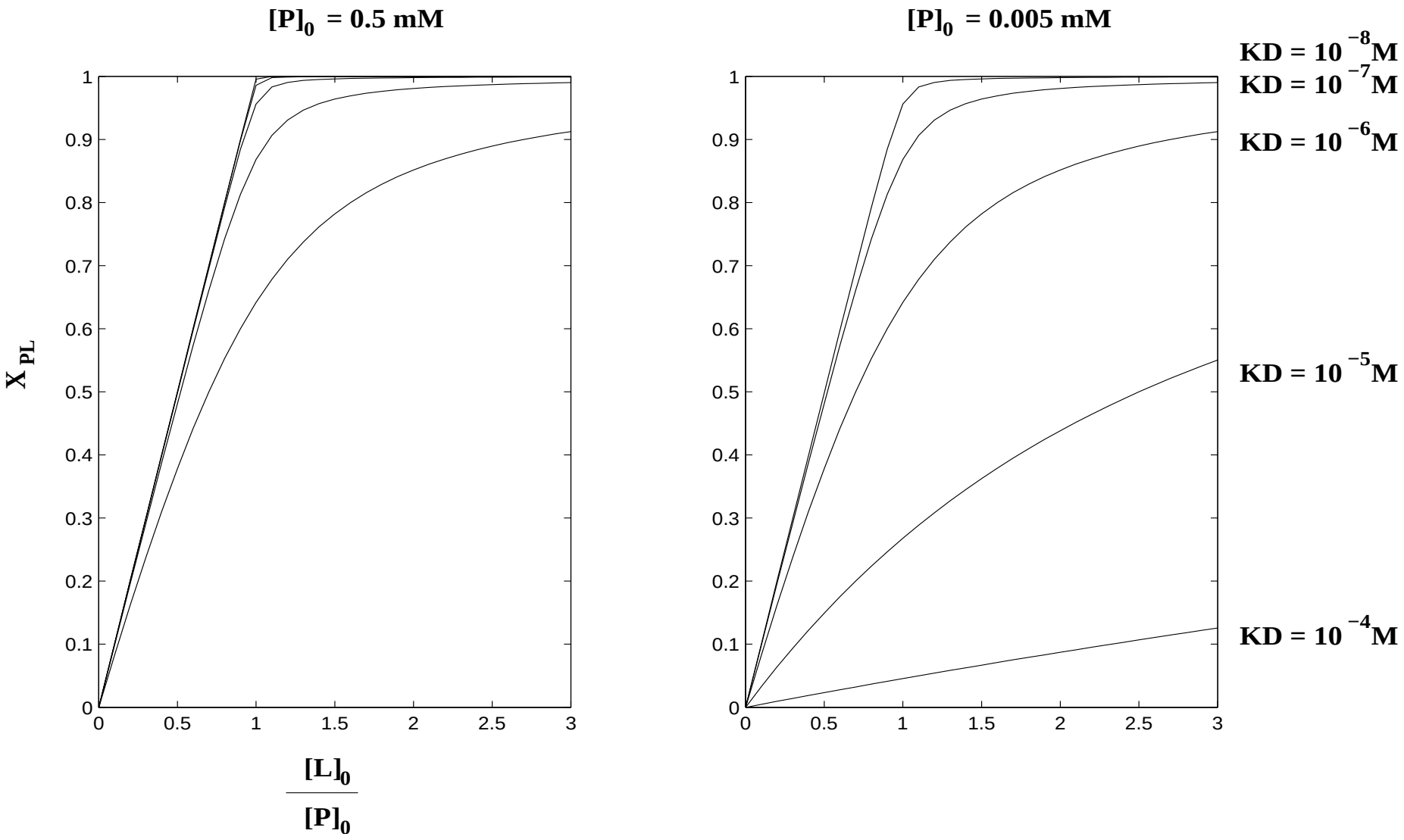
$$x_{PL} + x_P = 1$$

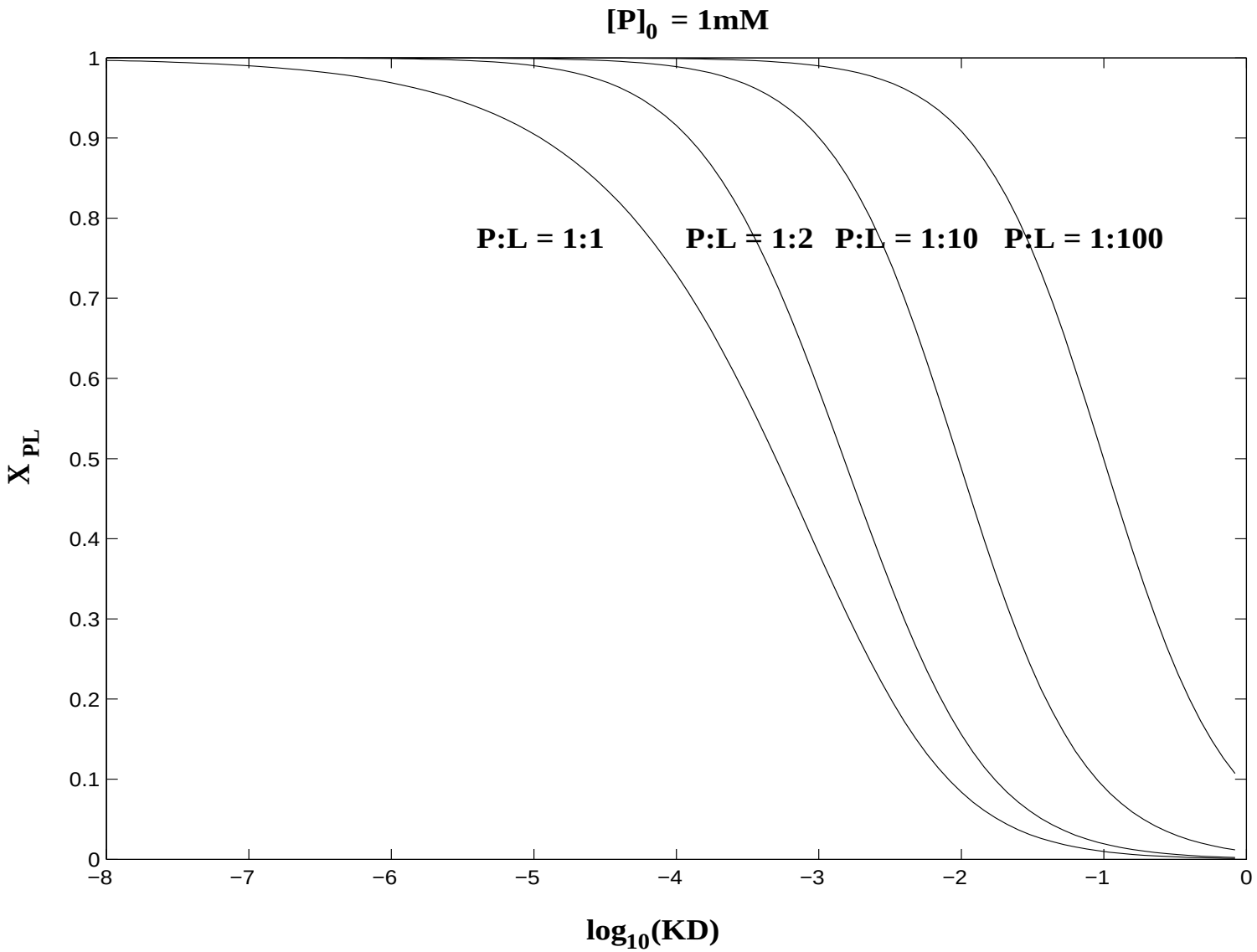
$$r = \frac{[L]_0}{[P]_0}$$

$$0 = x_{PL}^2 - x_{PL}\frac{1}{[P]_0}\{K_D + (1+r)[P]_0\} + r$$

$$x_{PL} = \frac{\{K_D + (1+r)[P]_0\}}{2[P]_0} - \frac{\sqrt{(K_D + (1+r)[P]_0)^2 - 4[P]_0^2 r}}{2[P]_0}$$

Amount of complex for different dissociation constants and L/P ratios




[P]0 = 1mM
P:L = 1:1
P:L = 1:2
P:L = 1:10
P:L = 1:100
X PL
log10(KD)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
−8
−7
−6
−5
−4
−3
−2
−1
0

Summary: dissociation constants and concentrations

At usual concentrations required for NMR spectroscopy (0.3 - 1 mM) is

the determination of dissociation constants smaller than $10^{-2} * [P]_0$ not possible

NMR spectroscopy allows the determination of dissociation constants in the µM - mM range

dissociation constants larger than 10 µM require at least a twofold excess of ligand for observing more the 90% bound protein.

Influence of chemical exchange to the NMR spectrum

chemical exchange : change of of conformational or chemical state A with resonance frequency ω_A

into a state B with resonance frequency ω_B

by complex formation

conformational change

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$$

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

Chemical exchange and relaxation

longitudinal relaxation

$$\frac{d}{dt}\begin{pmatrix} A_z \\ B_z \end{pmatrix}(t) = -\begin{bmatrix} \rho_A & \sigma_{AB} \\ \sigma_{AB} & \rho_B \end{bmatrix}\begin{pmatrix} (A_z - A_z^0) \\ (S_z - S_z^0) \end{pmatrix}(t) = -R\begin{pmatrix} (A_z - A_z^0) \\ (B_z - B_z^0) \end{pmatrix}(t)$$

chemical exchange

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$$

$$\frac{d}{dt}[A] = -k_1[A] + k_{-1}[B]$$

$$\frac{d}{dt}[B] = -k_1[B] + k_{-1}[A]$$

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

general relations

$$\frac{d}{dt} M_{jz} = -R_{1j}\{M_{jz}(t) - M_{jz}(0)\} + \sum K_{jk} M_{kz}(t)$$

$$M_z(t) = \begin{bmatrix} M_{1z}(t) \\ M_{2z}(t) \\ \dots \\ \dots \\ M_{Nz}(t) \end{bmatrix}$$

$$\frac{d}{dt} M_z(t) = -R\,(M_z(t) - M_{(0)}) + K\,M_z(t) = (-R + K)(M_z(t) - M_z(0)) - K\,M_z(0)$$

$K\,M_z(0) = 0$ equilibrium condition

$\frac{d}{dt} \Delta M_z(t) = (-R + K)(\Delta M_z(t))$ longitudinal relaxation and chemical exchange ($R_{ii} = R_{1i}$, $R_{ij} = \sigma_{ij}$)

$\frac{d}{dt} M^+(t) = (i\,\Omega - R + K)\,M^+(t)$ transversal realxation and chemical exchange ($R_{ij} = \delta_{ij}\,R_{2i}$), δ_{ij} = Kronecker delta

Chemical exchange and longitudinal relaxation

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$$

$$\frac{d}{dt}\Delta M_z(t) = (-R+K)\Delta M_z(t) = -\begin{bmatrix} R_{1A}+k_1 & -k_{-1} \\ -k_{-1} & R_{1B}+k_1 \end{bmatrix}\Delta M_z(t)$$ assumption: no cross relaxation

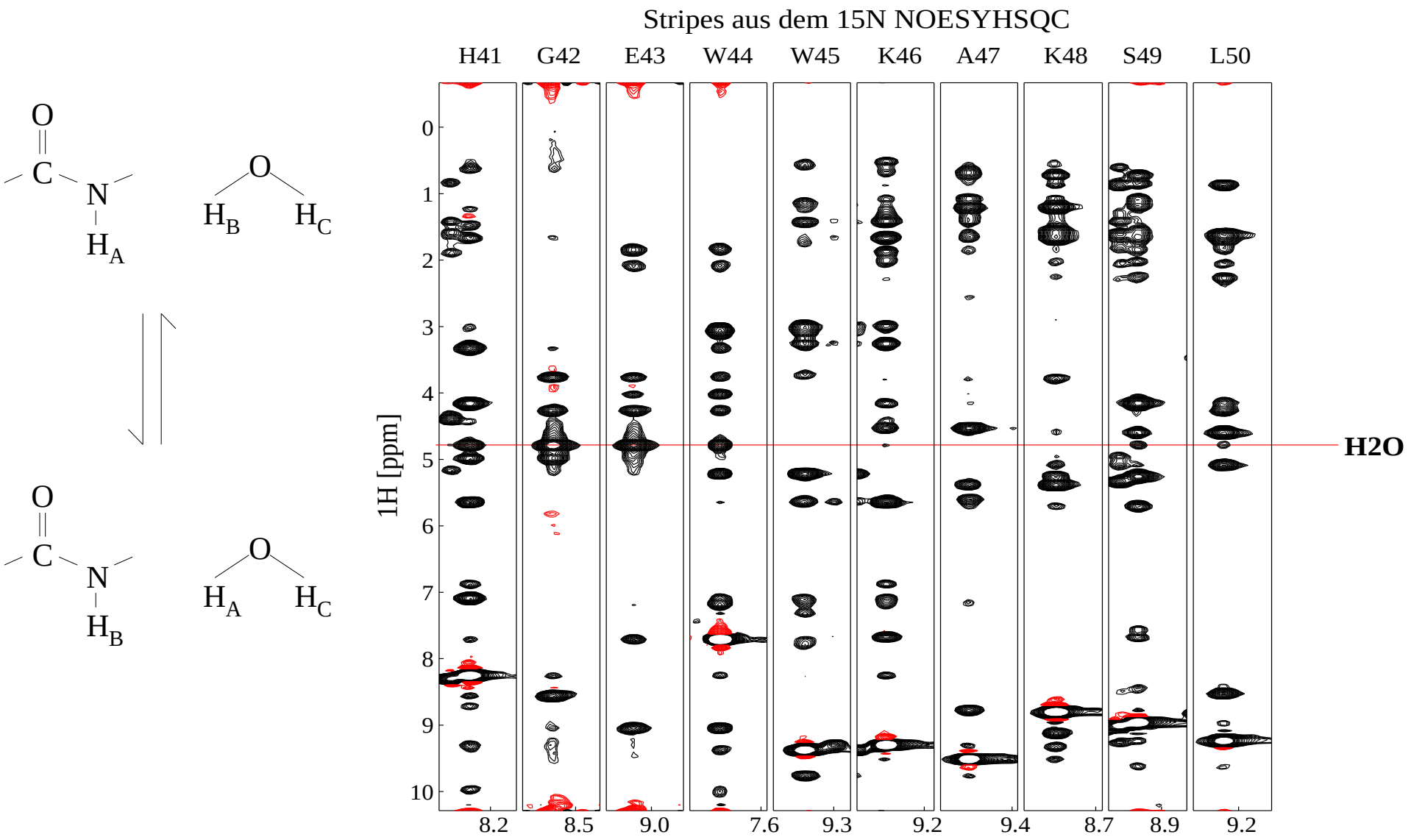
Chemical exchange: enhances effective the autorelaxation rate

allows magnetization transfer by chemical exchange

k_1 and k_{-1} are always positive: The behaviour in a NOESY experimentis analogue to the case of negative cross relaxation rates(large molecules)

in a NOESY experiments chemical exchange results in cross peaks at (ω_A, ω_B), if k_1 and $k_{-1} > R_{1A,B}$

H_2O exchange of amide protons in a NOESY experiment



Exchange between the folded and unfolded drk SH3 domain

(Zhang et al. (1994), J. Biomol. NMR 4, 845-858)

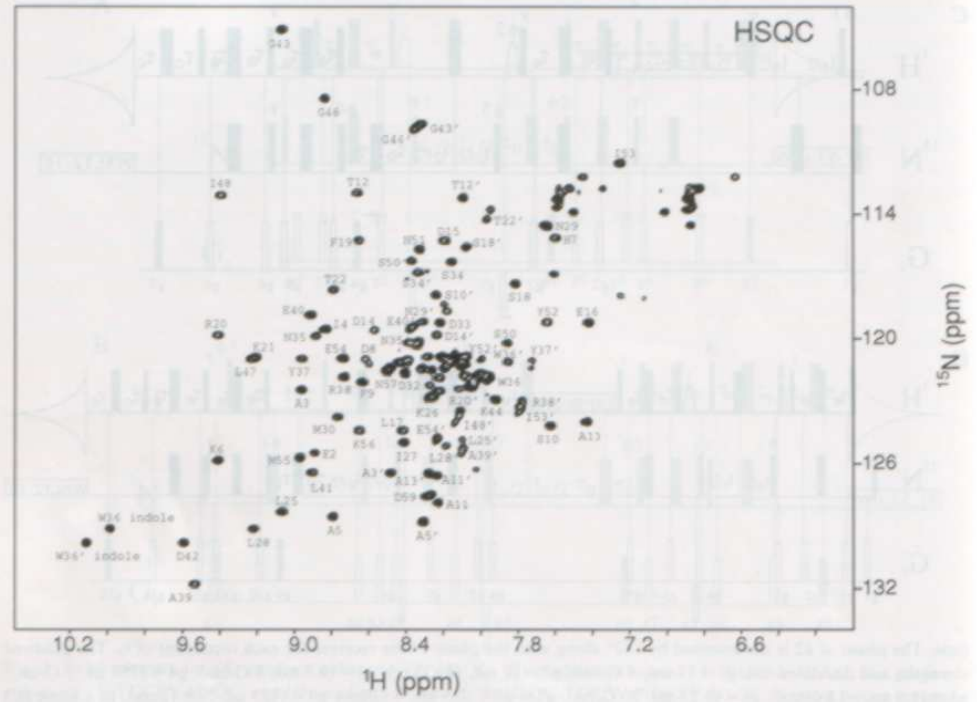


Fig. 2. ^{15}N-^{1}H HSQC spectrum of 1 mM drkN SH3 in 50 mM sodium phosphate, pH 6.0, 30 °C. Most backbone amide cross peaks have been labeled with the amino acid name (one-letter code); resonances of the unfolded form are also marked with a prime (′). Resonances of the side-chain glutamine and asparagine amide groups have not been labeled.

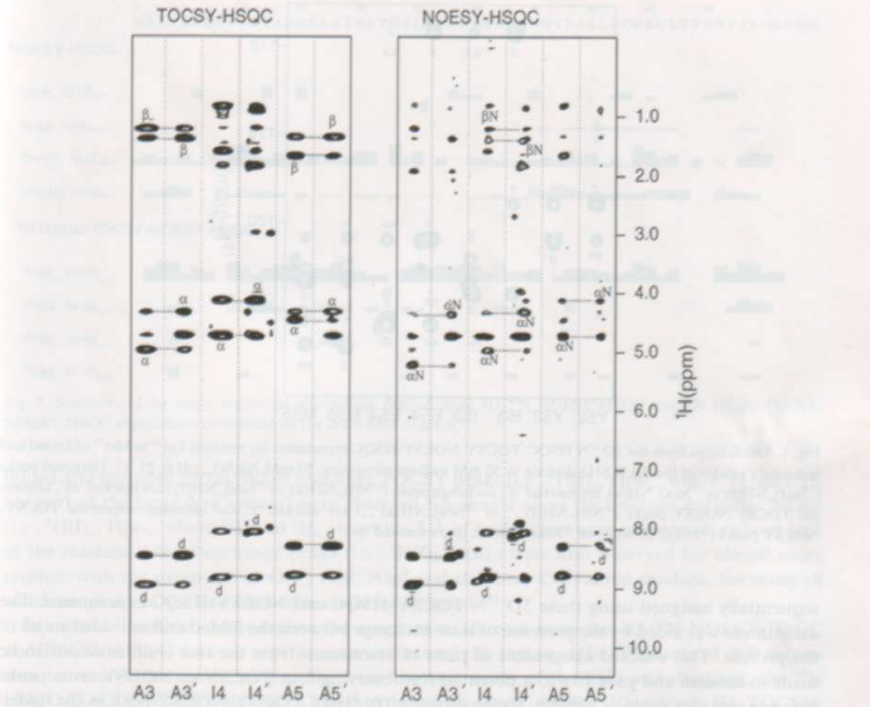


Fig. 3. Amide strips from the 3D ^{15}N TOCSY-HSQC and NOESY-HSQC experiments for resonances of Ala^{3} to Ala^{5} of folded and unfolded (′) states of the drkN SH3 domain in 50 mM sodium phosphate, pH 6.0, 30 °C. Intraresidue $C^{\alpha}H_{\alpha}NH$ and $C^{\beta}H_{\alpha}NH$ TOCSY peaks are labeled α and β, respectively, while sequential $C^{\alpha}H_{\alpha}NH_{i+1}$ and $C^{\beta}H_{\alpha}NH_{i+1}$ NOESY peaks are labeled αN and βN, respectively. Diagonal peaks are labeled d and dotted lines connect the direct peaks with exchange peaks, including diagonal, TOCSY and NOESY exchange peaks.

Chemical exchange and free precession

$$\frac{d}{dt} M^{+}(t) = (i\,\Omega - R + K)\, M^{+}(t)$$

transversal relaxation and chemical exchange
($R_{ij} = \delta_{ij}\, R_{2i}$) δ_{ij} = Kronecker delta

$$M^{+}(t) = M^{+}(0) \exp\{-(-i\,\Omega + R + K)\, t\}$$

general solution
(see lecture Biomolecular NMR I, NOESY)

$$\begin{bmatrix} M_1^{+}(t) \\ M_2^{+}(t) \end{bmatrix} = \begin{bmatrix} a_{11}(t) & a_{12}(t) \\ a_{21}(t) & a_{22}(t) \end{bmatrix} \begin{bmatrix} M_1^{+}(0) \\ M_2^{+}(0) \end{bmatrix}$$

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$$

special solution for $\Omega_A = -\Omega_B = \Omega$, $R_{2A} = R_{2B} = R_2$, $k_{-1} = k_1 = k$

$$a_{11}(t) = \frac{1}{2}\left[(1 + \frac{i\,\Omega}{\Delta}) \exp\{-(R_2 + k - \Delta)t\} + (1 - \frac{i\,\Omega}{\Delta}) \exp\{-(R_2 + k - \Delta)t\} \right]$$

$$a_{22}(t) = \frac{1}{2}\left[(1 - \frac{i\,\Omega}{\Delta}) \exp\{(-R_2 + k - \Delta)t\} + (1 + \frac{i\,\Omega}{\Delta}) \exp\{(-R_2 + k - \Delta)t\} \right]$$

$$a_{21}(t) = a_{12}(t) = \frac{k}{2\,\Delta}\left[\exp\{-(R_2 + k - \Delta)t\} - \exp\{-(R_2 + k + \Delta)t\} \right]$$

$$\Delta = \sqrt{k^2 - \Omega^2}$$

The two special cases: slow and fast exchange limit

slow exchange limit: $\Omega >> k$

$$a_{11}(t) = \exp\{-(R_2 + k - i\,\Omega)t\}$$

$$a_{22}(t) = \exp\{-(R_2 + k + i\,\Omega)t\}$$

$$a_{12}(t) = a_{21}(t) = 0$$

$$\begin{bmatrix} M_1^+(t) \\ M_2^+(t) \end{bmatrix} = \begin{bmatrix} a_{11}(t) & 0 \\ 0 & a_{22}(t) \end{bmatrix} \begin{bmatrix} M_1^+(0) \\ M_2^+(0) \end{bmatrix} = \begin{bmatrix} \exp\{-(R_2 + k - i\,\Omega)t\}\, M_A^+(0) \\ \exp\{-(R_2 + k + i\,\Omega)t\}\, M_B^+(0) \end{bmatrix}$$

→ Two separated resonance lines, one at $+\Omega$ and one at $-\Omega$

half width : $(R_2 + k) / \pi$

fast exchange limit : $\Omega \ll k$

$$a_{11}(t) = a_{22}(t) = \frac{1}{2}[1 + \exp\{-2kt\}]\exp\{-R_2 t\}$$

$$a_{21}(t) = a_{12}(t) = \frac{1}{2}[1 - \exp\{-2kt\}]\exp\{-R_2 t\}$$

$$M_A^+(t) + M_B^+(t) = a_{11} M_A^+(0) + a_{12} M_B^+(0) + a_{22} M_B^+(0) + a_{21} M_A^+(0)$$

$$M_A^+(t) + M_B^+(t) = [a_{11}(t) + a_{12}(t) + a_{22}(t) + a_{21}(t)] M^+(0)/2 = M^+(0)\exp\{-R_2 t\}$$

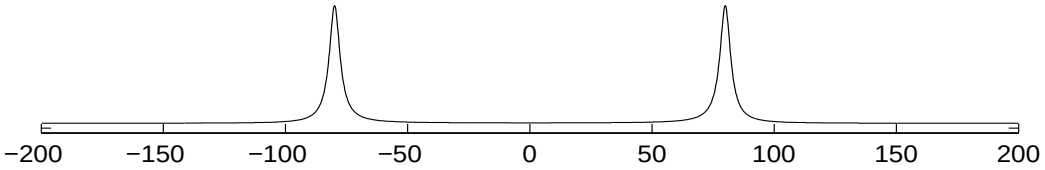
⟶ One resonance line at $0.5 * (\Omega_A + \Omega_B) = 0$
half width : R_2 / π

frequency separation between the two interchanging states determines the NMR time scale for observing the individual states

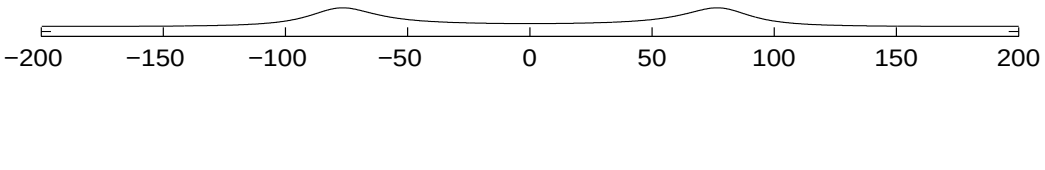
Chemical exchange in a two state system

Ω_A = 80 Hz, Ω_B = -80 Hz, R_2 = 10 s^{-1}

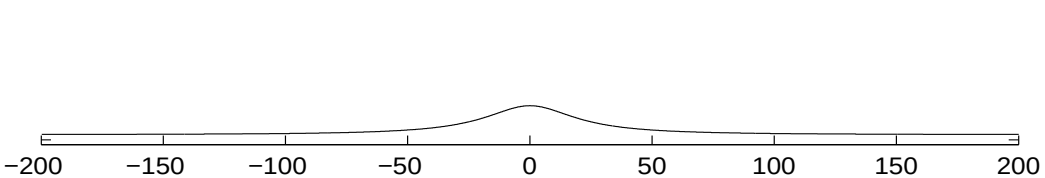
k = 10 s^{-1}



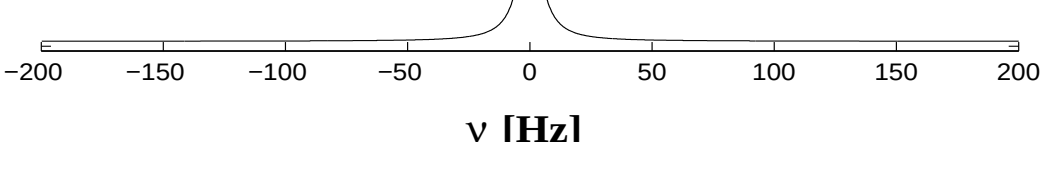
k = 100 s^{-1}



k = 450 s^{-1}



k = 1000 s^{-1}



k = 5000 s^{-1}

ν [Hz]

Summary : Chemical Exchange

Chemical exchange allows magnetization transfer

for $k > R_1$ and $k < \Delta\Omega$ chemical exchange results in cross signals in NOESY (EXSY) and TOCSY experiments

for $k < R_1$ the exchange cannot be determined by NMR

Chemical exchanges interferes with free precession

for $k << \Delta\Omega$ two sperated resonances can be observed for the individual states ($\Delta\Omega \neq 0$),
that means that the species are distinguished by NMR spectroscopy
$\Delta\Omega$ defines the NMR (chemical shift) time scale

for $k >> \Delta\Omega$ are population averaged signal can be observed (fast exchange)

for $k \approx \Delta\Omega$ the chemical exchange results in a line broadening (coalescence),
in the cases of extreme line broadening the signal can dissapear !

Chemical exchange and a bimolecular reaction, e.g. complex formation

$$P + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PL$$

$$\frac{d}{dt}[PL] = -k_{-1}[PL] + k_1[L][P]$$

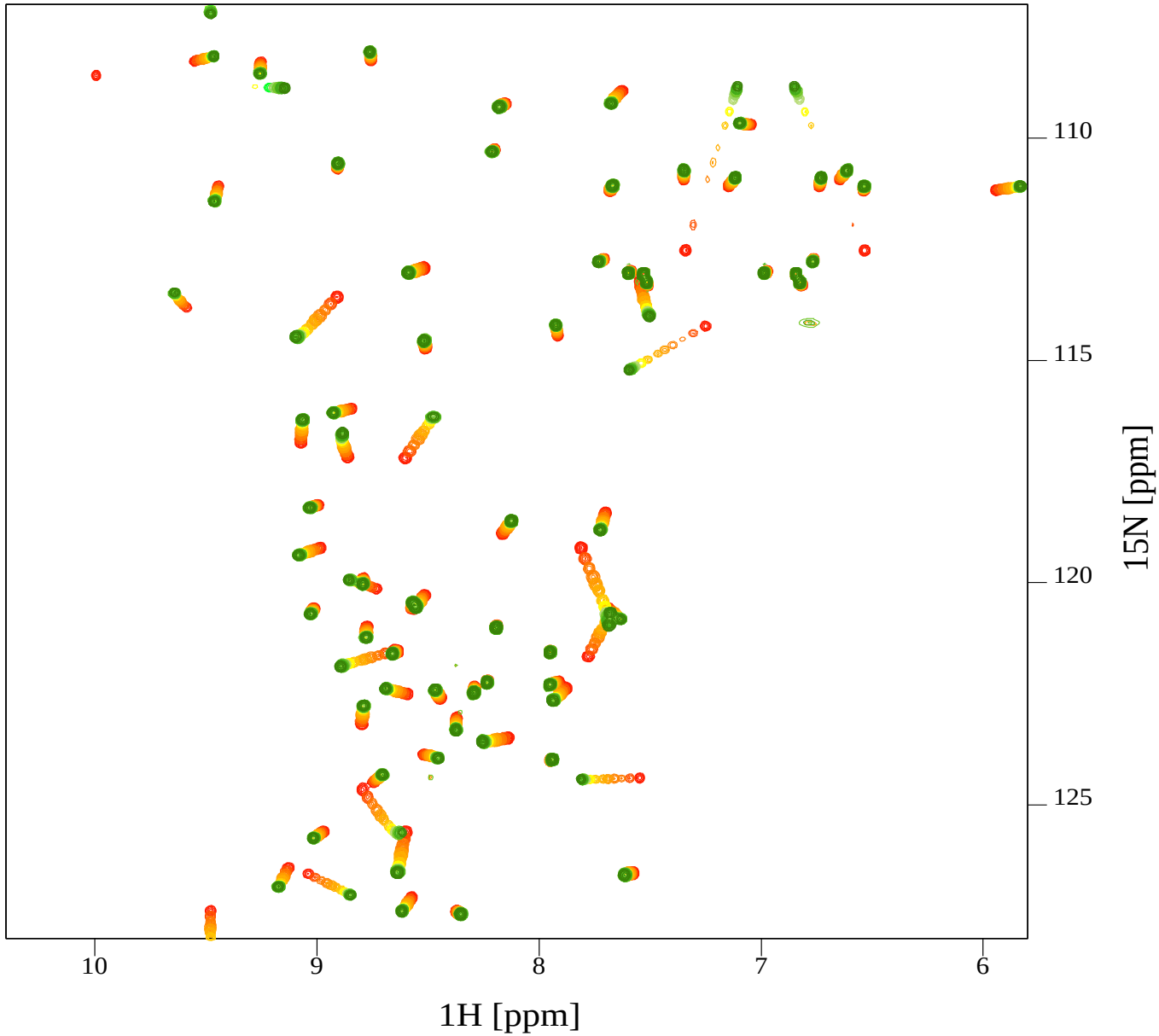
$$\frac{d}{dt}[P] = -k_{-1}[PL] - k_1[L][P]$$

$$\frac{d}{dt}\begin{pmatrix}[P]\\[PL]\end{pmatrix}(t) = \begin{bmatrix}-k_1[L] & k_{-1}\\ k_1[L] & -k_{-1}\end{bmatrix}\begin{pmatrix}[P]\\[PL]\end{pmatrix}(t)$$

→ the concentration of the ligand L affects the reaction kinetics
and therefore the effect of chemical exchange onto the NMR experiments

Fast exchange and a HSQC titration

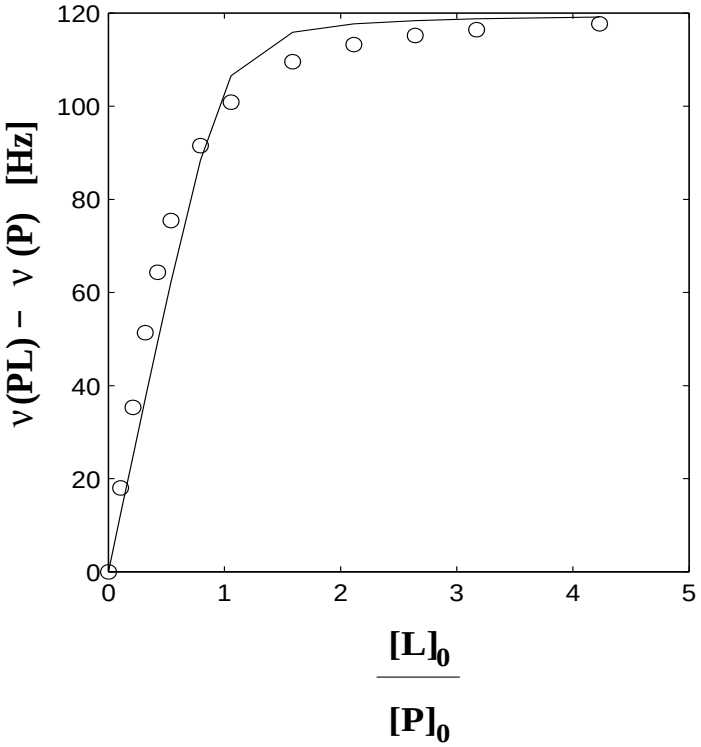
LckSH3 domain + 13mer peptide
$[P]_0$ = 0.83 mM
[L] stock solution ca. 3 mM

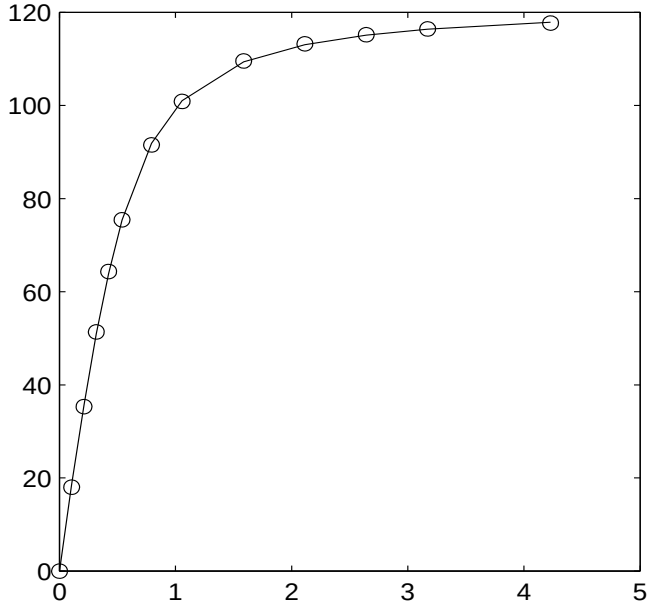


Determining the dissociation constant from chemical shift changes (fitting the titration curves)

$$\delta_{obs} = x_P \delta_P + x_{PL} \delta_{PL} = \delta_P + (\delta_{PL} - \delta_P) x_{PL}$$

$$\delta_{obs} = \delta_P + (\delta_{PL} - \delta_P) \left[\frac{\{K_D + (1+r)[P]_0\}}{2[P]_0} - \frac{\sqrt{(K_D + (1+r)[P]_0)^2 - 4[P]_0^2 r}}{2[P]_0} \right]$$





Fit with given r = $[L]_0$ / $[P]_0$

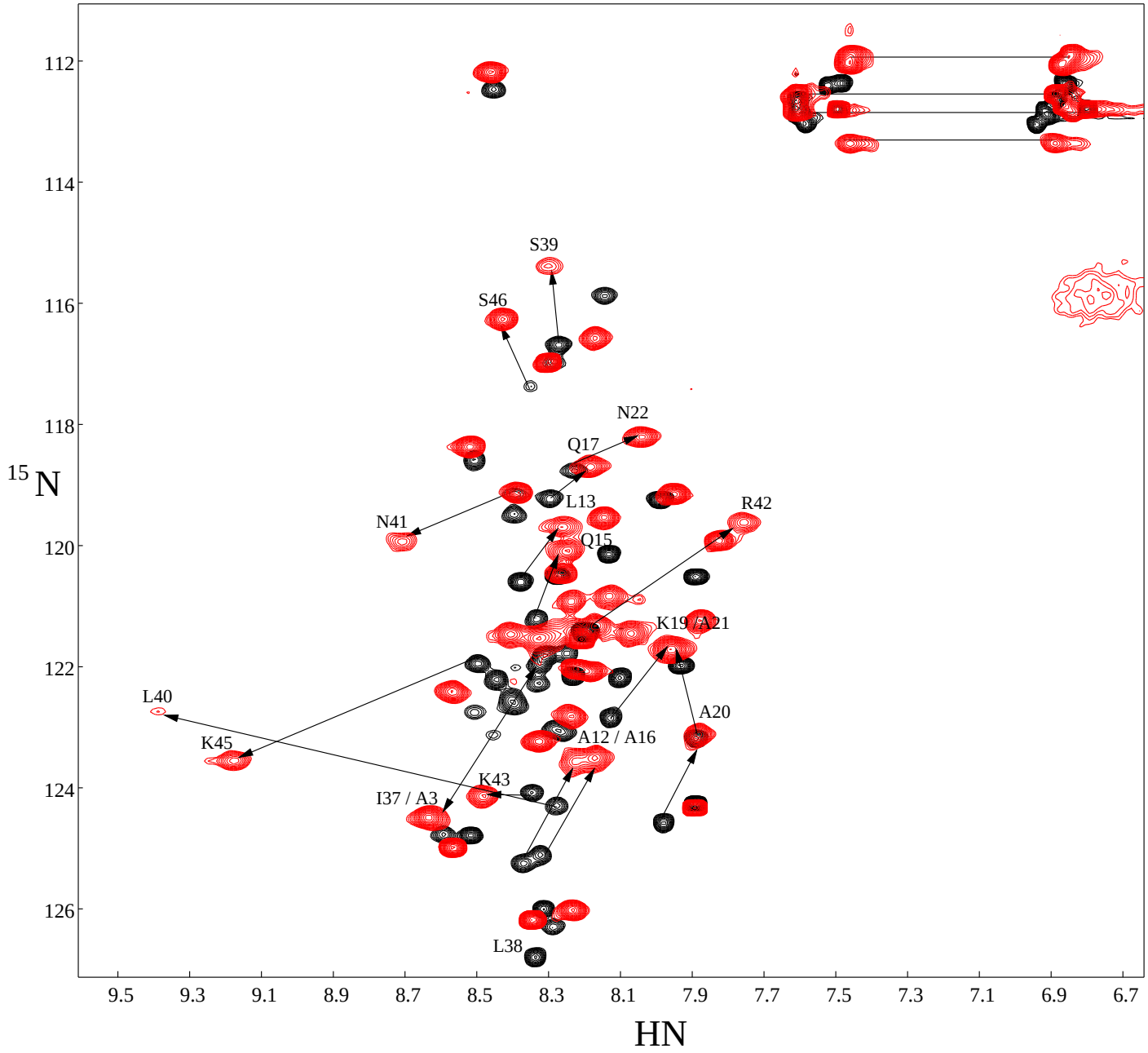
K_D = 15 µM

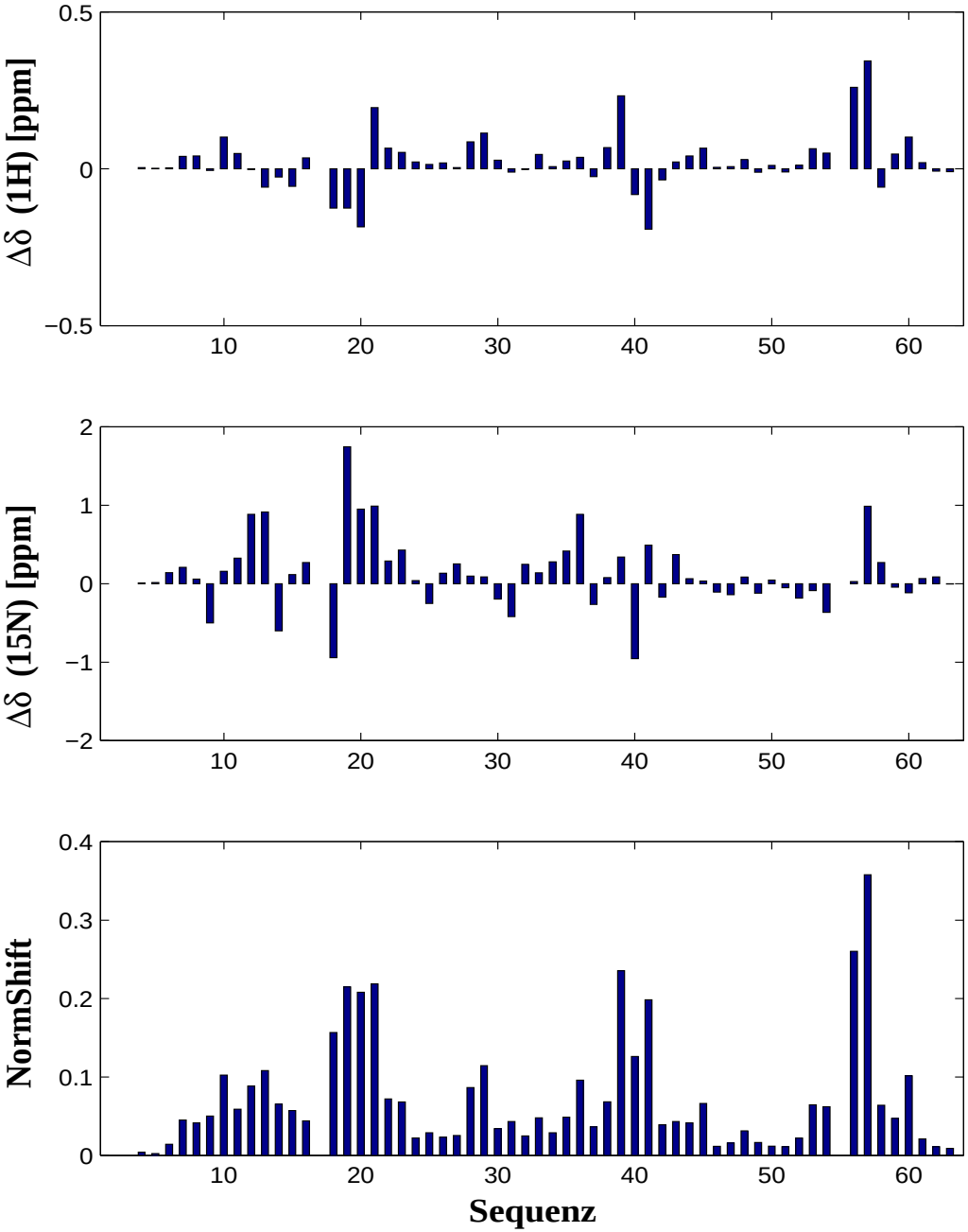
Fit with correction factor for r = $[L]_0$ / $[P]_0$

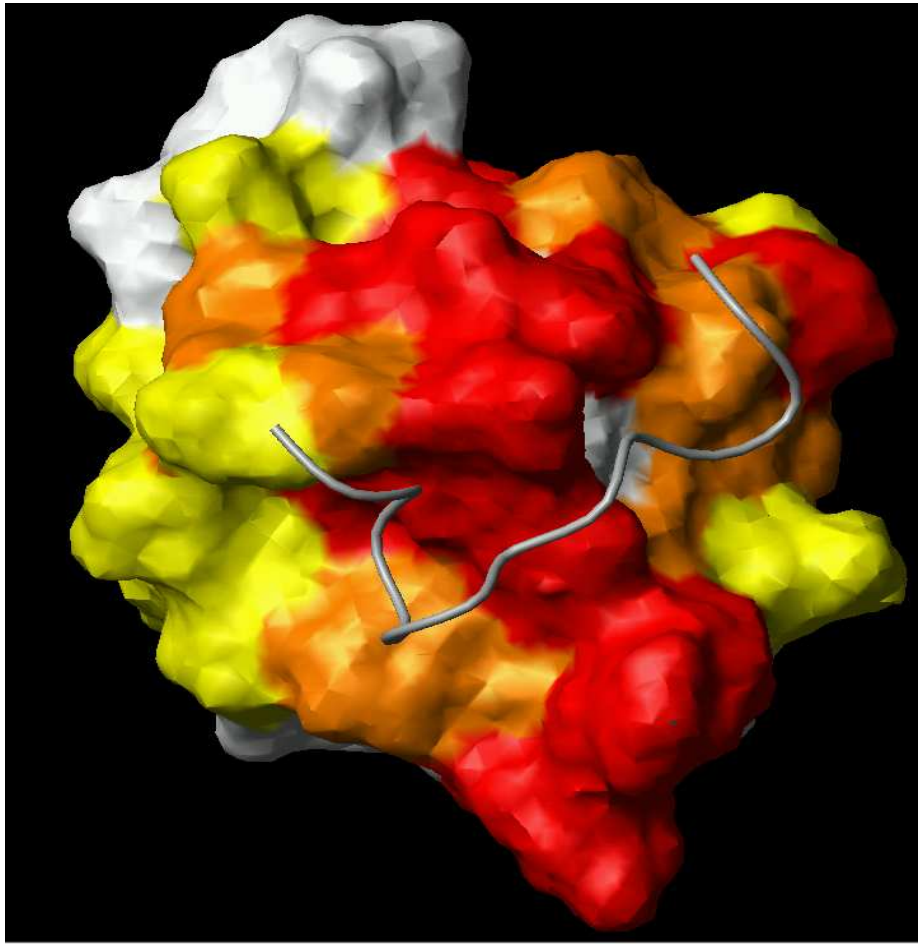
corr * $r_{initial}$ = $r_{optimal}$

K_D = 165 µM, corr = 1.7

example for slow exchange: λN (1-53) / NusA(339-495)




Δδ (1H) [ppm]
0.5
0
−0.5
10
20
30
40
50
60
Δδ (15N) [ppm]
2
1
0
−1
−2
NormShift
0.4
0.3
0.2
0.1
0
Sequenz



Summary : NMR titrations

Isotope labeling allows isolated observation of the labeled complex component

Chemical shifts changes allow the determination of the interaction contact surface

Exchange behaviour in the fast exchange regime of the NMR time scale:

- Simple assignment of the resonances by following the resonance changes during the titration
- determination of the dissociation constants from the chemical shift changes

Exchange behaviour in the slow exchange regime

- Assignment by following the chemical shift changes during the titration often not possible requires often a de novo assignment