Chemical exchange

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Chemical exchange

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

- Reversible unimolecular reaction, A <=> B, e.g.:
  - folded <=> unfolded
  - open <=> closed
  - trans <=> gauche
  - aromatic ring flips
- Binding reactions will be considered later

Characterising two-site exchange

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

$$\tau_A = 1/k_1$$

$$\tau_B = 1/k_{-1}$$

$$k_{ex} = k_1 + k_{-1}$$

$$\tau_{ex} = \frac{1}{k_1 + k_{-1}} = 1/k_{ex}$$

$$p_A = \frac{k_{-1}}{k_1 + k_{-1}}$$

$$p_B = \frac{k_1}{k_1 + k_{-1}}$$

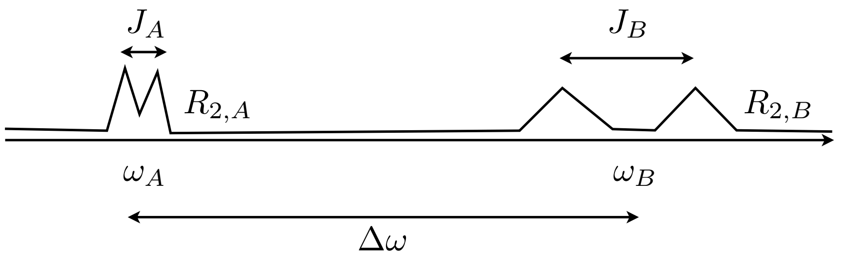
$$p_A + p_B = 1$$

- Two-site exchange is fully characterised by two parameters:
  - 1 x thermodynamic (i.e. position of equilibrium)
  - 1 x kinetic (i.e. timescale or rate constant)

Exchange vs the ‘NMR timescale’

- Central concept in understanding all exchange phenomena:
- How fast is exchange ***RELATIVE*** to some NMR process?

NMR timescales

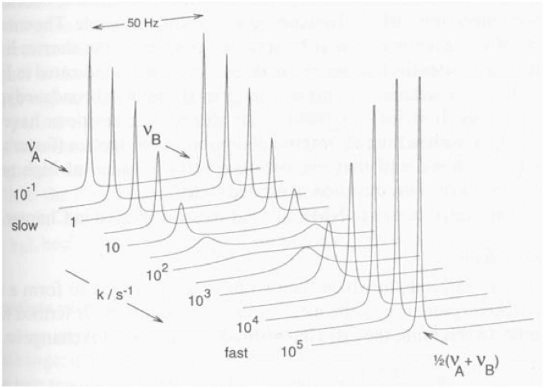


- Chemical shift: $\Delta\omega$
- Transverse relaxation (linewidth): ΔR_2
- Scalar coupling: ΔJ
- Note units – all in Hz
  – chemical shifts must be converted to frequencies to calculate $\Delta\omega$

Exchange regimes

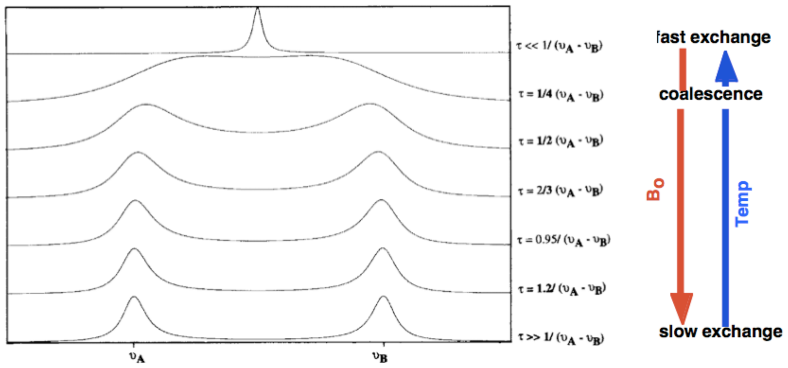
	slow	intermediate	fast
chemical shift	$k_{ex} \ll \Delta\omega$	$k_{ex} \sim \Delta\omega$	$k_{ex} \gg \Delta\omega$
tranverse relaxation	$k_{ex} \ll \Delta R_2$	$k_{ex} \sim \Delta R_2$	$k_{ex} \gg \Delta R_2$
scalar coupling	$k_{ex} \ll \Delta J$	$k_{ex} \sim \Delta J$	$k_{ex} \gg \Delta J$

Effect of exchange rate



- Exchange between ω_A and ω_B
- Coalescence when $k_{ex} = \pi\, \Delta v / \sqrt{2}$

Modulating the exchange regime



- Increasing temperature accelerates reaction, shifting towards fast EX
  – assuming populations don't change significantly
- Increasing B_0 increases $\Delta\omega$ and shifts towards slower exchange

Fast vs slow exchange

- Fast exchange:
  - single resonance at shift $\delta = p_A\delta_A + p_B\delta_B$ (population weighted average)
  - average shift / linewidth / coupling etc.
- Slow exchange:
  - two resonances
  - separate shifts / linewidths / couplings etc.
- Intermediate exchange:
  - Usually broadened to point of unobservability

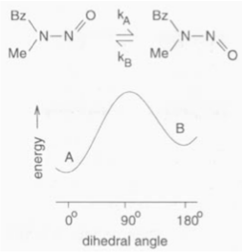
Fast intermediate exchange

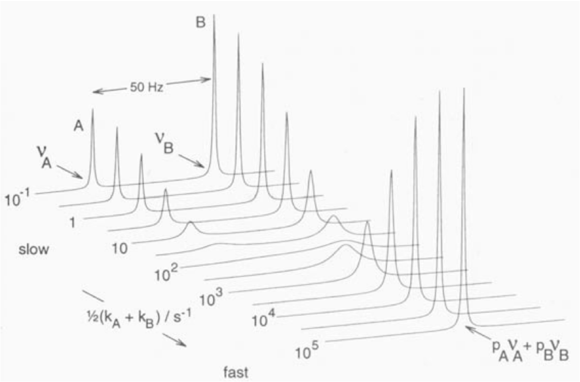
- Fast-intermediate exchange:
  - $k_{ex} > \Delta\omega$ (but not much greater)
  - single resonance at shift $\delta = p_A\delta_A + p_B\delta_B$ (population weighted average)
  - some additional line broadening
- 'Exchange contribution' to R_2:
  - $R_{ex} = p_A p_B \Delta\omega^2 / k_{ex} = (p_A p_B \Delta\omega^2)\tau_{ex}$
  - cf. adiabatic local field relaxation, $R_2 = \langle B_{loc}^2 \rangle J(0)$
  - $R_{ex} \propto B_0^2$

Slow intermediate exchange

- Slow-intermediate exchange:
  - $k_{ex} < \Delta\omega$ (but not much less)
  - two resonances close to original positions
  - some additional line broadening
- 'Lifetime broadening' contribution to R_2:
  - $\Delta R_{2,A} = k_1$
  - $\Delta R_{2,B} = k_{-1}$
- Important consequence: hard to observe minor species
- ΔR_2 not dependent on B_0

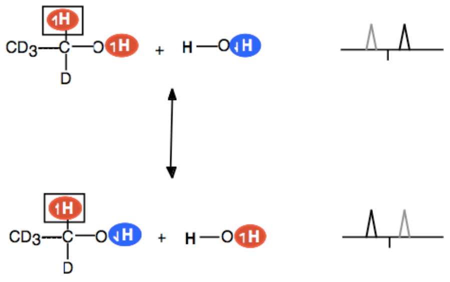
Asymmetric exchange



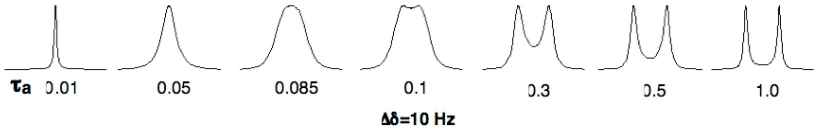


- Slow exchange: minor peak broadening much more significant
- Fast exchange: population weighted peak position heavily biased towards major state chemical shift

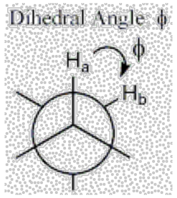
Scalar coupling exchange

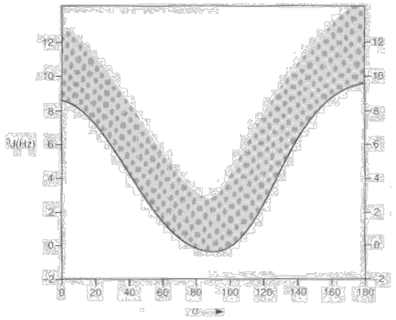


- Intermolecular exchange: J_B=0
- Collapse of multiplet to singlet in fast EX



Scalar coupling exchange



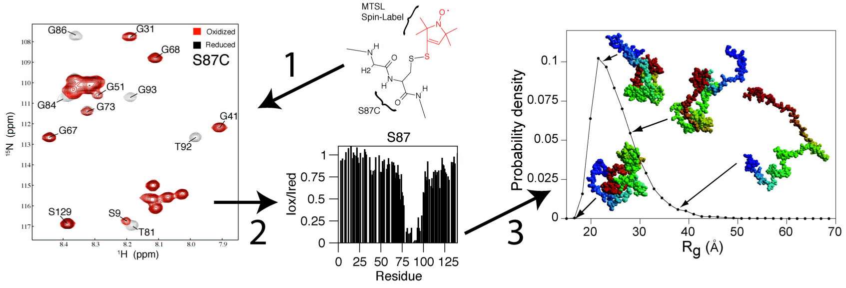


- Intramolecular exchange e.g. sidechain rotamers
- $^3J_{HAHB}$ dependence on χ_1 angle described by Karplus equation
- Slow exchange: $J \approx$ 3 and 12 Hz
- Fast exchange: J ≈ (3 + 3 + 12) / 3 ≈ 6 Hz

Transverse relaxation exchange

$$R_{2,obs} = p_A R_{2,A} + p_B R_{2,B} \qquad (\Delta R_2 \ll k_{ex})$$

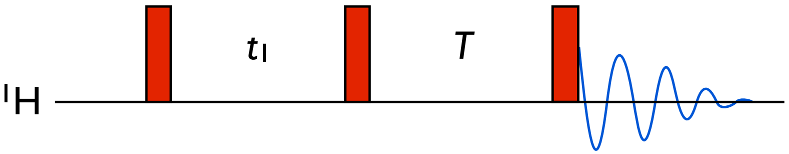
- In fast EX, observed relaxation rate is population weighted average of individual relaxation rates
- e.g. PREs to probe residual structure in disordered proteins



Bloch-McConnell equations

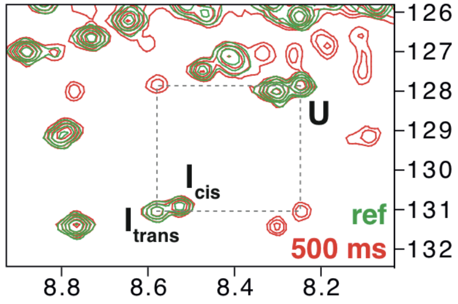
- Classical analysis of exchanging systems
- Not strictly appropriate for coupled systems
- Sufficient for analysis in many (most?) cases

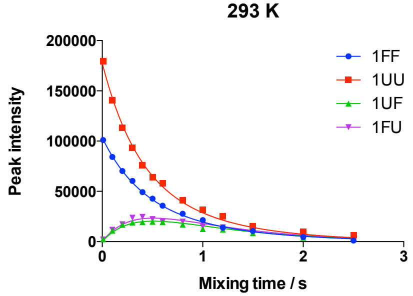
ZZ exchange for analysis of slow exchanging systems (EXSY)



- Introduce a variable delay between measurement of indirect and direct chemical shift dimensions
- Store magnetization longitudinally during delay – sensitive to $\tau_{ex} \lesssim T_1$
- Reactions occuring (in dynamic equilibrium) during this time give rise to cross-peaks in characteristic square pattern

ZZ exchange for analysis of slow exchanging systems (EXSY)





- Fitting to integrated Bloch-McConnell equations
- Compensation for differential transverse relaxation?

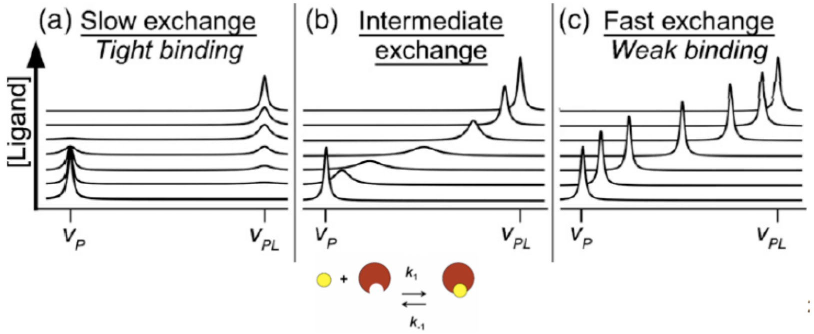
Binding equilibria (bimolecular reactions)

$$P_{\text{free}} + L \underset{k_{\text{off}}}{\overset{k_{\text{on}}}{\rightleftharpoons}} P_{\text{bound}} \longrightarrow P_{\text{free}} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P_{\text{bound}}$$

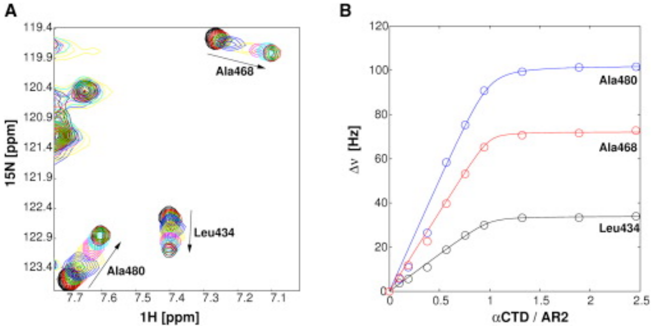
$$k_1 = k_{\text{on}} L_{\text{free}}$$

- Exchange involving a bimolecular event (2nd order rate constant) cannot be analysed directly
- From the perspective of the observed nucleus, system is still just in two site exchange between free and bound states
- Calculate pseudo-first order rate constant and equilibrium populations as function of k_{on}, k_{off} and L_0

Binding equilibria (bimolecular reactions)



Determination of binding constants from HSQC chemical shift perturbations



- Chemical shift perturbations give %bound as function of ligand concentration
- Fitting to determine K_d
- Caution – only in very fast EX! Can result in substantial errors!

Three state exchange