Effects of Chemical Exchange on NMR Spectra

• Chemical exchange refers to any process in which a nucleus exchanges between two or more environments in which its NMR parameters (e.g. chemical shift, scalar coupling, or relaxation) differ.
• DNMR deals with the effects in a broad sense of chemical exchange processes on NMR spectra; and conversely with the information about the changes in the environment of magnetic nuclei that can be derived from observation of NMR spectra.
Types of Chemical Exchange

**Intramolecular exchange**
- Motions of sidechains in proteins
- Helix-coil transitions of nucleic acids
- Unfolding of proteins
- Conformational equilibria

**Intermolecular exchange**
- Binding of small molecules to macromolecules
- Protonation/deprotonation equilibria
- Isotope exchange processes
- Enzyme catalyzed reactions

Because NMR detects the molecular motion itself, rather the numbers of molecules in different states, NMR is able to detect chemical exchange even when the system is in equilibrium.
2-state First Order Exchange

Lifetime of state A:
\[ \tau_A = \frac{1}{k_+} \]

Lifetime of state B:
\[ \tau_B = \frac{1}{k_-} \]

Use a single lifetime
\[ \frac{1}{\tau} = \frac{1}{\tau_A} + \frac{1}{\tau_B} = k_+ + k_- \]
Rationale for Chemical Exchange

For slow exchange

\[ \frac{dM_{AX}}{dt} = -(\Delta \omega_A)M_{AY} - \frac{M_{AX}}{\tau_A} + \frac{M_{BX}}{\tau_B} \]

For fast exchange

\[ \frac{dM_{AX}}{dt} = -(\Delta \omega_A)M_{AY} - \frac{M_{AX}}{\tau_A} + \frac{M_{BX}}{\tau_B} \]

Bloch equation approach:

\[ \frac{dM_{BX}}{dt} = -(\Delta \omega_B)M_{BY} - \frac{M_{BX}}{\tau_B} + \frac{M_{AX}}{\tau_A} \]

\[ \cdots \]

\[ \cdots \]
2-state 2nd Order Exchange

\[ K_d = \frac{[M][L]}{[ML]} = \frac{k_{-1}}{k_{+1}} \]

\[ K_d = 10^{-3} - 10^{-9} \text{ M} \]
\[ k_{on} = k_{+1} \sim 10^8 \text{ M}^{-1} \text{ s}^{-1} \text{ (diffusion-limited)} \]
\[ k_{-1} \sim 10^{-1} - 10^{-5} \text{ s}^{-1} \]

Lifetime \( \frac{1}{\tau} = \frac{1}{\tau_{ML}} + \frac{1}{\tau_1} = k_{-1} (1 + f_{ML}/f_L) \)

\( f_{ML} \) and \( f_L \) are the mole fractions of bound and free ligand, respectively.
 Typical Motion Time Scale for Physical Processes

SLOW → very slow → slow → fast → very fast → ultrafast → FAST

MACROSCOPIC DIFFUSION, FLOW

CHEMICAL EXCHANGE

MOLECULAR ROTATIONS

MOLECULAR VIBRATIONS

RELAXATION TIMESCALE
SPECTRAL TIMESCALE
LARMOR TIMESCALE
# NMR Time Scale

<table>
<thead>
<tr>
<th>Time Scale</th>
<th>Chem. Shift, $\delta$</th>
<th>Coupling Const., $J$</th>
<th>T2 relaxation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td>$k &lt;&lt; \delta_A - \delta_B$</td>
<td>$k &lt;&lt; J_A - J_B$</td>
<td>$k &lt;&lt; 1/ T_{2,A} - 1/ T_{2,B}$</td>
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<tr>
<td>Intermediate</td>
<td>$k = \delta_A - \delta_B$</td>
<td>$k = J_A - J_B$</td>
<td>$k = 1/ T_{2,A} - 1/ T_{2,B}$</td>
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<tr>
<td>Fast</td>
<td>$k &gt;&gt; \delta_A - \delta_B$</td>
<td>$k &gt;&gt; J_A - J_B$</td>
<td>$k &gt;&gt; 1/ T_{2,A} - 1/ T_{2,B}$</td>
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<tr>
<td>Sec$^{-1}$</td>
<td>0 – 1000</td>
<td>0 – 12</td>
<td>1 - 20</td>
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- NMR time-scale refers to the chemical shift timescale.
- The range of the rate can be studied 0.05-5000 s$^{-1}$ for H can be extended to faster rate using $^{19}$F, $^{13}$C and etc.
Slow Exchange $k \ll \delta_A - \delta_B$

- Separate lines are observed for each state.
- The exchange rate can be readily measured from the line widths of the resonances.
- Like the apparent spin-spin relaxation rates, $1/T_{2i,obs}$
  
  $1/T_{2A,obs} = 1/T_{2A} + 1/\tau_A = 1/T_{2A} + 1/k_1$
  
  $1/T_{2B,obs} = 1/T_{2B} + 1/\tau_B = 1/T_{2B} + 1/k_{-1}$

  line width $L_w = 1/\pi T_2 = 1/\pi T_2 + k_1/\pi$

  Each resonance is broadened by $\Delta L_w = k/\pi$

  Increasing temperature $k$ increases, line width increases.
Slow Exchange for $M+L \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ML$

<table>
<thead>
<tr>
<th>M_F</th>
<th>M_B</th>
<th>L_F</th>
<th>L_B</th>
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- Separate resonances potentially are observable for both the free and bound states $M_F$, $M_B$, $L_F$, and $L_B$.
- The addition of a ligand to a solution of a protein can be used to determine the stoichiometry of the complex.
- Once a stoichiometric mole ratio is achieved, peaks from free ligand appear with increasing intensity as the excess of free ligand increases.
- Obtain spectra over a range of $[L]/[M]$ ratios from 1 to 10.
Slow Exchange for $M+L \xrightleftharpoons[k_{-1}]{k_1} ML$

- For free form
  
  \[
  \frac{1}{T_{2L,\text{obs}}} = \frac{1}{T_{2L}} + \frac{1}{\tau_L} = \frac{1}{T_{2L}} + k_{-1} \frac{f_{ML}}{f_L}
  \]
  
  \[
  \frac{1}{T_{2M,\text{obs}}} = \frac{1}{T_{2M}} + \frac{1}{\tau_M} = \frac{1}{T_{2M}} + k_{-1} \frac{f_{ML}}{f_M}
  \]

  For complex form

  \[
  \frac{1}{T_{2ML,\text{obs}}} = \frac{1}{T_{2ML}} + \frac{1}{\tau_{ML}} = \frac{1}{T_{2ML}} + k_{-1}
  \]

  Measurements of line width during a titration can be used to derive $k_{-1}$ ($k_{\text{off}}$).
Coalescence Rate

- For $A \Leftrightarrow B$ equal concentrations, there will be a rate of interchange where the separate lines for two species are no longer discernible.
- The coalescence rate

$$k_c = \pi \frac{\Delta \delta}{\sqrt{2}} = 2.22 \Delta \delta$$

$\Delta \delta$ is the chemical shift difference between the two signals in the unit of Hz.

$\Delta \delta$ depends on the magnetic field.
Coalescence Temperature

- Since the rate depends on the $\Delta G$ of the inversion, and the $\Delta G$ is affected by $T$, higher temperature will make things go faster.
- $T_c$ is the temperature at which fast and slow exchange meet.
- $T>T_c$, fast exchange
- $T<T_c$, slow exchange

we can calculate the $\Delta G^\ddagger$ of the process

$$\Delta G^\ddagger = R \times T_c \times [22.96 + \ln \left( \frac{T_c}{\Delta \delta} \right)]$$
A single resonance is observed, whose chemical shift is the weight average of the chemical shifts of the two individual states:

$$\delta_{\text{obs}} = f_A \delta_A + f_B \delta_B, \quad f_A + f_B = 1$$

**For very fast limit**

$$\frac{1}{T_{2,\text{obs}}} = \frac{f_A}{T_{2A}} + \frac{f_B}{T_{2B}}$$

**For moderately fast**

$$\frac{1}{T_{2,\text{obs}}} = \frac{f_A}{T_{2A}} + \frac{f_B}{T_{2B}} + f_A f_B \frac{4\pi (\Delta \delta_{AB})^2}{k_1}$$

Maximal line broadening is observed when

$$f_A = f_B = 0.5$$
Fast Exchange $k >> \delta_A - \delta_B$

$$\text{M} + \text{L} \underset{k^{-1}}{\overset{k^+}{\rightleftharpoons}} \text{ML}$$

For M
$$\delta_{M,\text{obs}} = f_M \delta_M + f_{ML} \delta_{ML}$$

For L
$$\delta_{L,\text{obs}} = f_L \delta_L + f_{ML} \delta_{ML}$$

$$\frac{1}{T_{2,\text{obs}}} = f_{ML}/T_{2,ML} + f_L/T_{2,L} + f_{ML} f_L^2 4\pi \left(\delta_{ML} - \delta_L\right)^2/k_{-1}$$

- A maximum in the line broadening of ligand or protein resonances occurs during the titration at a mole ratio of approx. ligand:protein 1:3
- The dissociation constant for the complex can be obtained by measuring the chemical shift of the ligand resonance at a series of $[L]$. 
Measuring Binding Constant

\[ \delta_{M,\text{obs}} = f_M \delta_M + f_{ML} \delta_{ML}, \]

The total change in \( \delta \) of \( M \)

\[ \Delta \delta_{Mo} = \delta_{ML} - \delta_M \]

At \( [L] \), \( \Delta \delta_M = \delta_{M,\text{obs}} - \delta_M \)

If \([M]\) is fixed,

\[ \Delta \delta_M = \Delta \delta_{Mo}[L]/([L]+K_d) \]

At 0.5 \( \Delta \delta_{Mo} \), \( K_d = [L] \)

Similar for

\[ A^-+H^+ \xrightarrow{k+1} AH \]

\[ H=L, \ K_d = K_a \]

\[ \Delta \delta_H = \Delta \delta_{Ho}[H]/([H]+K_a) \]

Inhibitor G-3-P binds to Triosephapte Isomerase

- JMB, 1976 100:319
The Ionization of His in Oxy & DeOxyhemoglobin

\[
\begin{align*}
\text{H}_4\text{R} \quad \text{H}_4\text{R} \\
\text{NH}_2 \quad \text{NH}_2
\end{align*}
\]

\[
\text{H}_4\text{R} + \text{H}^+ \rightleftharpoons \text{H}_4\text{R}^+ \\
\text{NH}_2 \quad \text{NH}_2
\]

- Bohr effect: Hb takes up H\(^+\) on releasing O2
- The pKa of His-\(\beta\)-146 changes by more than 1pH unit upon ionization due to the stabilization of the charged form by Asp94 in the deoxy structure.

C2H 7.7 ppm 8.7 ppm
C4H 7.0 ppm 7.4 ppm
Identifying & Designing Ca(II)-binding Proteins

The Average Structure of CD2-6D15
Calcium Titration of CD2-6D15 Using HSQC

\[ \Delta S = \frac{\Delta S_1 \times [\text{Ca}]}{K_{d1} + [\text{Ca}]} + \frac{\Delta S_2 \times [\text{Ca}]}{K_{d2} + [\text{Ca}]} \]

Yang et al. (2005) JACS, 127, 2085-93
Manganese Relaxation of CD2-6D15

Yang et al. (2005) JACS, 127, 2085-93

Dynamic Properties of Ca.CD2

Yang et al., Biochemistry, 2005 In Press