NMR VI: LIGAND-PROTEIN INTERACTIONS

A. Chemical shift timescales for ligand-protein interactions
   fast, intermediate and slow exchange regimes

B. Probing ligand-protein contacts by NMR
   1. chemical shift mapping
      application: PH domain from transcription factor TFIIH;
      interactions with PI(5)P and VP16
   2. inter-molecular NOEs
      isotope-editing vs. -filtering
      application: Human ileal bile acid binding protein
      site-selective steroid-protein interactions
Exchange on the Chemical Shift Time Scale

e.g. ligand on/off binding site

\[ L_{\text{unbound}} \rightleftharpoons L_{\text{bound}} \]

\[ k_{\text{ex}} \], exchange rate in Hz:

\[ (k_1 + k_{-1})/2 \]

\( \Delta \nu \), chemical shift difference in Hz

slow exchange: \( k_{\text{ex}} \ll \Delta \nu \)

intermediate exchange: \( k_{\text{ex}} \approx \Delta \nu \)

fast exchange: \( k_{\text{ex}} \gg \Delta \nu \)

from Evans, Ch. 1, p. 45
CHEMICAL SHIFT TIME SCALE VARIES WITH NUCLEUS AND $B_0$

<table>
<thead>
<tr>
<th>nucleus</th>
<th>$B_0$ (T)</th>
<th>$\nu_0$ (MHz)</th>
<th>timescale in Hz: (e.g. for $\Delta \nu = 0.5$ ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1H$</td>
<td>11.75 T</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>14.10 T</td>
<td>600</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>16.45 T</td>
<td>700</td>
<td>350</td>
</tr>
<tr>
<td>$^{13}C$</td>
<td>11.75 T</td>
<td>125</td>
<td>62.5</td>
</tr>
<tr>
<td></td>
<td>14.10 T</td>
<td>150</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>16.45 T</td>
<td>175</td>
<td>87.5</td>
</tr>
<tr>
<td>$^{15}N$</td>
<td>11.75 T</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>14.10 T</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>16.45 T</td>
<td>70</td>
<td>35</td>
</tr>
</tbody>
</table>
CHEMICAL SHIFT MAPPING

Goal:
Map the location of ligand binding site(s) in a protein

Basis:
Ligand binding perturbs the local environment (chemical shifts) of protein atoms in the binding site

Approach:
1. Establish resonance assignments for apo-protein (no ligand)
2. If possible, establish assignments for protein with bound ligand OR
   titrate protein with ligand and monitor changes
3. Calculate and map the chemical shift differences onto apo structure

Caveats:
works best with “rigid-body interactions”
if ligand binding is coupled to conformational changes, mapping may not be possible
PLECKSTRIN HOMOLOGY (PH) DOMAINS

adaptor modules of 100-120 residues
function in signal-dependent membrane recruitment
bind to PIPs and proteins

model of a PH-PIP interaction at membrane surface

Individual PH domains recognize distinct PIP species

TRANSCRIPTION FACTOR TFIIH CONTAINS A PH DOMAIN IN ITS Tfb1 SUBUNIT

Tfb1 specifically recognizes PI(3)P and PI(5)P (levels of individual PIP species vary in nucleus during cell cycle)
also interacts with transcriptional activation domains from VP16, p53, E2F-1, HIV-1 Tat protein

Di Lello et al. (2005) solved NMR structure of Tfb1 PH domain (apo) mapped interactions with PI(5)P and VP16 using chemical shifts
Chemical Shift Mapping: PH Domain from TFIIH Interactions with PI(5)P

overlayed HSQC spectra at different ligand/protein ratios

weighted amide chemical shift differences

mapped interaction surfaces (red)

DiLello et al. (2005) Biochemistry 44, 7678
Chemical Shift Mapping: PH Domain from Tf2b Interactions with VP16

overlaid HSQC spectra at different ligand/protein ratios

weighted amide chemical shift differences

mapped interaction surfaces (yellow)

DiLello et al. (2005) Biochemistry 44, 7678
CHEMICAL SHIFT MAPPING SHOWS OVERLAP OF INTERACTION SURFACES

Phospholipid and protein ligands appear to interact with transcription factor at the same location

Competitive binding?
INTER-MOLECULAR NOEs FOR PROBING LIGAND-PROTEIN INTERACTIONS

**Isotope-editing:**
follows $^1$H’s attached to X nuclei ($^{13}$C or $^{15}$N)
suppresses all other $^1$H’s

e.g., $^{15}$N- or $^{13}$C-edited NOESY

**Isotope-filtering:**
suppresses $^1$H’s attached to X nucleus ($^{13}$C or $^{15}$N)
follows all other $^1$H’s

e.g., $^{15}$N- or $^{13}$C-filtered NOESY
ISOTOPE-EDITED NOESY

e.g., $^{13}$C-enriched ligand bound to unenriched protein

$^{13}$C-1H

$^{13}$C-ligand

Protein (unenriched)

$^{13}$C-1H

3D $^{13}$C-edited NOESY (NOESY-HSQC):

\[
\text{NOE} \quad \overset{^{1}J_{CH}}{\longrightarrow} \quad \text{NOE} \quad \overset{^{1}J_{CH}}{\longrightarrow}
\]

$^{1}$H (protein) $\cdots$ $^{1}$H (ligand) - $^{13}$C (ligand)

F1 F3 F2
**Ileal Bile Acid Binding Protein (I-BABP)**

- member of intracellular lipid binding protein family
- adopts \( \beta \)-clam topology
- abundant in absorptive cells of distal small intestine (ileum)
- functions in bile acid recycling between ileum and liver
Bile Acids

- steroid ring structure
- synthesized in the liver from cholesterol
- conjugated to glycine or taurine in vivo
- variable OH groups at 3, 7 and 12 positions
- amphipathic: “the detergents of the GI tract”
To solve structure of complex:

1. Determined protein structure: [U-\(^{13}\)C/\(^{15}\)N]-protein, unenriched ligand

2. Obtained ligand-protein NOEs: unenriched protein, \(^{13}\)C- or \(^{15}\)N-ligand

3. Docked ligands into binding sites using NOE restraints
I-BABP COMPLEX:
DETERMINATION OF PROTEIN STRUCTURE
(ligands present, but not shown)

~3800 NOE restraints
~30 restraints/residue

$^{15}$N$^{15}$N NOESY
$^{15}$NH NOESY
$^{13}$C-$^{13}$C Methyl NOESY
$^{13}$CH Aromatic NOESY
$^{13}$CH NOESY

TALOS $\alpha$ & $\beta$ dihedrals

CSI H-bonds for $\alpha$-helix
Ligand Isotopic Incorporation Strategy

Via Labeled Glycine Conjugation

Via Synthetic Isotopic Incorporation
$^{13}$C HSQC: 3/1 GCDA/I-BABP
$^{13}$C HSQC: 3/1 GCDA/I-BABP

$U \equiv$ unbound ligand
$^{13}$C HSQC: 3/1 GCDA/I-BABP

$U \equiv$ unbound ligand
$^{13}$C HSQC: 3/1 GCDA/I-BABP

U ≡ unbound ligand
$^{13}$C HSQC: 3/1 GCDA/I-BABP

Binding site 1
$^{13}$C HSCQ:
3/1 GCDA/I-BABP

Binding site 2
ISOTOPE-EDITED NOESY

e.g., $^{13}\text{C}$-enriched ligand bound to unenriched protein

3D $^{13}\text{C}$-edited NOESY (3D NOESY-HSQC):

$^{1}\text{H}$ (protein) $\cdots\cdots\cdots$ $^{1}\text{H}$ (ligand) - $^{13}\text{C}$ (ligand)

$^{1}\text{H}$ (protein) $\cdots\cdots\cdots$ $^{1}\text{H}$ (ligand) - $^{13}\text{C}$ (ligand)

$F1$ $F3$ $F2$
$^{13}$C-Edited NOESY: \([3,4-^{13}\text{C}]-\text{Bile Salts Bound to I-BABP}\)
NOE-detected contacts between Bile Salts and I-BABP

1' Position

<table>
<thead>
<tr>
<th>Site 1: GCDA</th>
<th>Site 2: GCA</th>
</tr>
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<tbody>
<tr>
<td>T73 $\alpha$H, $\beta$H, $\gamma$H</td>
<td>Y53 $\delta$H, $\varepsilon$H</td>
</tr>
<tr>
<td>M74 NH</td>
<td></td>
</tr>
<tr>
<td>G75 NH</td>
<td></td>
</tr>
<tr>
<td>I23 NH, $\gamma$H, $\delta$H, $\varepsilon$H</td>
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15N Position

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<tr>
<th>Site 1: GCDA</th>
<th>Site 2: GCA</th>
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<tbody>
<tr>
<td>Y53 $\varepsilon$H</td>
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23 Position

<table>
<thead>
<tr>
<th>Site 1: GCDA</th>
<th>Site 2: GCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>T73 $\alpha$H, $\gamma$H</td>
<td>Y53 $\varepsilon$H</td>
</tr>
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</table>

3,4 Position

<table>
<thead>
<tr>
<th>Site 1: GCDA (3)</th>
<th>GCDA (4)</th>
<th>Site 2: GCA (3)</th>
<th>GCA (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F63 $\delta$H, $\varepsilon$H, $\zeta$H</td>
<td>F63 $\delta$H</td>
<td>F6 $\varepsilon$H</td>
<td>F2 $\varepsilon$H</td>
</tr>
<tr>
<td>W49 $\alpha$H, $\beta$H</td>
<td>L90 $\gamma$H, $\delta$H</td>
<td>W49 5H, 6H</td>
<td>L108 $\beta$H</td>
</tr>
<tr>
<td>L90 $\beta$H, $\gamma$H, $\delta$H</td>
<td>F63 $\delta$H</td>
<td>L108 $\beta$H</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L90 $\gamma$H</td>
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NOE-observable Ligand-Protein Contacts
Residues that may mediate cooperativity and selectivity
DETECTING LIGAND-PROTEIN NOEs WITHOUT ISOTOPE ENRICHMENT OF LIGAND

Sample: [U-$^{13}$C/$^{15}$N]-protein, unenriched ligand

**Isotope-editing:**
selects $^1$H’s attached to X nuclei ($^{13}$C or $^{15}$N)

**Isotope-filtering:**
selects $^1$H’s attached to $^{12}$C or $^{14}$N

These two approaches can be combined into one experiment

e.g., $^{12}$C,$^{14}$N-filtered (F1) – $^{15}$N-edited (F2) NOESY
(ligand) (protein)
X-filter (refocused half-filter)

Where:
\( I = ^1H \) attached to X nucleus
\( H = \) all other \(^1H\) (not attached)

And: \( \Delta_1 = \frac{1}{2} J_{IS} \)
(X nucleus coupling constant)

\[
\begin{align*}
\text{Hz} + \text{Iz} & \xrightarrow{90^\circ(^1H)} -\text{Hy} - \text{Iy} \\
& \xrightarrow{\Delta_1} -\text{Hy} - \text{Iy}\cos\pi J_{IS}\Delta_1 + 2\text{IzSz}\sin\pi J_{IS}\Delta_1 \quad (= -\text{Hy} + 2\text{IzSz}) \\
\end{align*}
\]

\[
\begin{align*}
\text{180}^\circ(^1H,X) & \quad \begin{cases} 
\text{Hy} - 2\text{IzSz} \xrightarrow{\Delta_1} \text{Hy} - \text{Iy} & \text{A} \\
\text{Hy} + 2\text{IzSz} \xrightarrow{\Delta_1} \text{Hy} + \text{Iy} & \text{B} 
\end{cases} 
\end{align*}
\]

\[
\begin{align*}
\text{A} + \text{B} &= \text{Hy} \text{ (filtered)} \quad \text{OR} \quad \text{A} - \text{B} = \text{Iy} \text{ (edited)}
\end{align*}
\]
2D $^{13}$C, $^{15}$N Double-half-filtered NOESY
(Slijper et al. 1996, J. Magnetic Resonance B 111, 119)

Sample: U-$^{13}$C,$^{15}$N-enriched protein, unenriched ligand

Adjustment of 180 pulses leads to selection of:
$^{13}$C-attached protons
$^{15}$N-attached protons
$^{12}$C- or $^{14}$N-attached protons
I-BABP Complex: $^{12}$C-filtered ($F_1$), $^{13}$C-edited ($F_2$) NOESY-HSQC

- Ligand $^1$H's
- Protein Aromatic $^1$H's

$^1$H from Unenriched Bile Salts

$^1$H from $^{13}$C/$^{15}$N I-BABP