Drug Discovery – Small Molecule Binding by NMR

BCMB/CHEM 8190
SAR by NMR

\[ \Delta G_{AB} = \Delta G_A + \Delta G_B, \quad RT \ln(K) = -\Delta G_{AB}, \quad K_{AB} = K_A \times K_B \]

\[ K_A = 2 \times 10^3, \quad K_B = 5 \times 10^3, \quad K_{AB} = 1 \times 10^7 \]
α-Me Mannose Bound to MBP
Addition of $\alpha$-methyl mannose to MBP
900 MHz TROSY of $^{15}$N, $^2$H Labeled Mannose Binding Protein, 0.1mM
Binding Site of Mannose Binding Protein
SAR by NMR Examples

A

\[ \begin{align*}
&\text{2 \text{ \(\mu\)M}} \\
\rightarrow &\text{100 \text{ \(\mu\)M}} \\
\rightarrow &\text{19 nM}}
\end{align*} \]

FKBP

B

\[ \begin{align*}
&\text{17 mM}} \\
\rightarrow &\text{20 \text{ \(\mu\)M}} \\
\rightarrow &\text{15 nM}}
\end{align*} \]

Stromelysin
Transferred NOEs in Drug Discovery

• Determine the geometry of a bound ligand
  (Sayers and Prestegard, Biophysical J, 81, 0000 (2001))

• Screening of a library of potential ligands

• NMR as a tool for structure-based drug design
  (B. Stockman, Prog. NMR Spec., 33, 109 (1998))
Transfer NOE

*Cross-relaxation rate $\alpha \tau_c, r, \omega_0$

*Chemical exchange fast w.r.t cross-relaxation rate and chemical shift scale

*Observed NOE is weighted average of free and bound states

*Change in sign of NOE upon change in molecular weight

*Spin Diffusion and on, off rates can complicate interpretation
NOEs from large and small molecules have opposite signs

\[ \tau_c = 1.1 \left( \frac{1}{\omega_0} \right) \]

Rates of transfer (\( \sigma \)) are proportional to \( \tau_c \) for large molecules.

Observe: \( \text{NOE}(\text{obs}) = p(\text{bound}) \cdot \sigma(\text{bound}) + p(\text{free}) \cdot \sigma(\text{free}) \)

NOE from bound state dominates for \( p(\text{bound})/p(\text{free}) > 0.05 \)
Transfer NOE studies of Trimannoside with MBP

*Methyl 3,6-di-O-(α-D-mannopyranosyl)-(α-D-mannopyranoside)*

Schematic representation of trimannoside core structure depicting some of the intra and inter-residue NOEs observed
Pulse Sequence for Selective 1D NOE

90x 180y 180y 90+-x 90x

Mix

Gradient

Behavior for selected resonance from two different volume elements on +x 90° pulse. Vectors return to +z with –x 90° pulse.
Selective saturation NOE difference spectra for trimannoside

0.1 mM MBP, 1 mM Trimannoside

Selective saturation

H1 H1' H1'' H2 H3' H4'

Tris

trNOE

NOE

-OCH3

ppm

5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4
Epitope Mapping in Drug Discovery

• Mayer, M & Meyer, B. JACS, 123, 6108-6117 (2001)
Spin Diffusion is Efficient in Macromolecules – Saturation of Protein Spins transfers to Ligands

\[ \frac{1}{T_{1,2}} = \sum_{ij} J_i (\omega_i) |D_{ij}|^2, \quad J_i (\omega_i) = \frac{2\tau_c}{(\omega_i^2 \tau_c^2 + 1)} \]

\[ \frac{1}{T_2} \approx (\text{const}) J_i (\omega_i) |D_i(I_+I_-, I_+I_+)|^2 \text{ for large } \tau_c \]
Saturation Transfer Difference Method (STD) for Ligand Screening

Figure 1 On the right, a heterodimeric integrin $\alpha_{IIb}\beta_3$ molecule is shown schematically reconstituted in a liposome also called large unilamellar vesicle (LUV). The protein molecules are randomly inserted into the lipid bilayer so that only half of the binding sites are pointing outside and are accessible to ligands in the buffer solution. Ligand binding is detected with STD NMR spectroscopy. Saturation of the receptor is achieved by selective irradiation within 100 ms. The saturation is transferred to bound ligands via intermolecular spin diffusion. The effect of the saturation is made visible by subtracting the spectrum with protein saturation from one where the receptor has not been saturated. Signals of nonbinding molecules are canceled out by subtraction. Ligand protons in close proximity to the protein receive larger saturation and thereby reveal the ligand’s binding epitope.
Peptides Used in STD Experiments

Figure 2 RGD-peptides with binding affinity to integrin $\alpha_{IIb}\beta_3$, i.e., RGD, RGDS, and cyclo(RGDfV), as well as peptides without binding affinity, i.e., AGSE and TPL-NH$_2$. The inhibitory effects of RGD and RGDS on fibrinogen binding to activated platelets that present the integrin were investigated by Tranqui et al.$^{29}$ The IC$_{50}$ values (ELISA) and the dissociation constants of cyclo(RGDfV) were determined by Aumailley et al.$^{17}$ Pfaff et al.$^{19}$ and Keenan et al.$^{20}$
Figure 3 (a) Normal $^1$H NMR spectrum of 29 μM cyclo(RGDfV) and integrin $\alpha_{IIb}\beta_3$ liposomes showing signals of the ligand and residual protein resonances. (§ TRIS buffer; * impurities). (b) STD NMR spectrum of 29 μM cyclo(RGDfV) and integrin $\alpha_{IIb}\beta_3$ containing liposomes showing STD effects of the ligand. (c) Same as b but 275 μM cyclo(RGDfV) showing the increase of the STD signals with ligand excess. (d) STD NMR spectrum of 260 μM cyclo(RGDfV) and 260 μM RGD with liposomes devoid the integrin receptor showing no unspecific binding to the liposomes. (e) STD NMR spectrum of 160 μM AGSE and integrin $\alpha_{IIb}\beta_3$ containing liposomes. The nonbinding peptide shows no STD effects.
Relaxation Enhancement can also Identify Interaction Sites.
Example: Galectin Interacting with LacNAc
Synthesis of a Spin-Labeled N-acetyllactosamine
Change in 15N HSQC spectrum (800 MHz) of Galectin-3 upon addition of LacNac-TEMPO

0 mM

10 mM
X-Ray crystal structure of Galectin-3 (Seetharamana et al. 1998)