Introduction solution NMR

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EMBO Global Exchange course, IHEP, Beijing
April 28 - May 5, 2011

Why use NMR for structural biology...?
The very basics
Multidimensional NMR
Resonance assignment
Structural parameters
NMR relaxation & dynamics

Topics

Why use NMR.... ?
NMR & Structural biology


...allows to study the dynamics of biomolecular systems...

Pros & cons of solution NMR in structural biology

Pros...
- no need for crystal:
  - no crystal packing artefacts, solution more native-like
- potential to study dynamics:
  - picosecond to seconds time scales, conformational averaging, chemical reactions, folding...
- easy study of protein-protein, protein-DNA, protein-ligand interactions

Cons...
- NMR structure determination is a bit slow...
- Need isotope labeling ($^{13}$C, $^{15}$N)
- solution NMR works best for MW < 50 kDa
The very basics of NMR

**Nuclear spin**

\[ E = -\vec{\mu} \cdot \vec{B} = -\mu_z B_z \]

\[ |\mu| = \gamma \hbar \sqrt{I(I+1)} \quad I = \text{quantum number} \]

\[ \mu_z = \gamma \hbar m \quad m = I, I-1, I-2... -I = \text{allowed states} \]

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**Table 1.1**

Properties of Selected Nuclei

<table>
<thead>
<tr>
<th>Nucleus</th>
<th>I</th>
<th>( \gamma ) (rad. T(^{-1}), s(^{-1}))</th>
<th>Natural abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^1\text{H})</td>
<td>½</td>
<td>(2.6752 \times 10^8)</td>
<td>99.98</td>
</tr>
<tr>
<td>(^2\text{H})</td>
<td>½</td>
<td>(4.107 \times 10^7)</td>
<td>0.02</td>
</tr>
<tr>
<td>(^13\text{C})</td>
<td>½</td>
<td>(6.728 \times 10^7)</td>
<td>1.11</td>
</tr>
<tr>
<td>(^14\text{N})</td>
<td>½</td>
<td>(1.934 \times 10^7)</td>
<td>99.64</td>
</tr>
<tr>
<td>(^15\text{N})</td>
<td>½</td>
<td>(2.712 \times 10^7)</td>
<td>0.36</td>
</tr>
<tr>
<td>(^16\text{O})</td>
<td>½</td>
<td>(-3.628 \times 10^7)</td>
<td>0.04</td>
</tr>
<tr>
<td>(^19\text{F})</td>
<td>½</td>
<td>(2.5181 \times 10^8)</td>
<td>100.00</td>
</tr>
<tr>
<td>(^23\text{Na})</td>
<td>½</td>
<td>(7.080 \times 10^7)</td>
<td>100.00</td>
</tr>
<tr>
<td>(^31\text{P})</td>
<td>½</td>
<td>(1.0841 \times 10^8)</td>
<td>100.00</td>
</tr>
<tr>
<td>(^113\text{Cd})</td>
<td>½</td>
<td>(-3.934 \times 10^7)</td>
<td>12.26</td>
</tr>
</tbody>
</table>

\(^*\) The angular momentum quantum number, I, and the gyromagnetic ratio, \(\gamma\), and natural isotopic abundance for nuclei of particular importance in biological NMR spectroscopy are shown.
**Larmor frequency**

\[ \omega_m = \gamma \hbar B_0 = 2\pi v_H \]

**Boltzmann distribution**

\[ \frac{n_\beta}{n_\alpha} = \exp\left(-\frac{\Delta E}{k_B T}\right) = \exp\left(-\frac{\gamma \hbar B_0}{k_B T}\right) = 0.9999 \]

**Net magnetization**

\[ \frac{n_\beta}{n_\alpha} = \exp\left(-\frac{\Delta E}{k_B T}\right) = \exp\left(-\frac{\gamma \hbar B_0}{k_B T}\right) = 0.9999 \]

**Chemical shielding**

Local magnetic field is influenced by electronic environment
Chemical shielding

\[ \nu = \frac{\gamma B_0}{2\pi} \left(1 - \sigma \right) \]

Chemical shift:
- \( \delta \sigma_{\text{iso}} \, [\text{Hz}] = \nu_{\text{obs}} - \nu_0 \)
- \( \delta \sigma_{\text{iso}} \, [\text{ppm}] = \frac{(\nu_{\text{obs}} - \nu_0)}{\nu_0 \times 10^{-6}} \)
  - ppm: parts per million
  - ppm value is not field dependent

14 Tesla: \( \nu = 600 \, \text{MHz} \)
1 ppm = 600 Hz

21 Tesla: \( \nu = 900 \, \text{MHz} \)
1 ppm = 900 Hz

Pulse

Observe with the Lamor frequency "rotating frame"

FID: analogue vs digital

Free Induction Decay (FID)
**Fourier Transform**

- Signal vs. time (ms)
- Signal vs. freq. (Hz)

**Relaxation**

- **NMR Relaxation**
  - Restoring Boltzmann equilibrium
- **T2-relaxation**
  - Disappearance of transverse (x,y) magnetization
  - \(1/T_2 \approx \) signal line-width
- **T1-relaxation**
  - Build-up of longitudinal (z) magnetization
  - Determines how long you should wait for the next experiment

**NMR spectral quality**

- **Sensitivity**
  - Signal to noise ratio (S/N)
    - Sample concentration
    - Field strength
  - **Resolution**
    - Peak separation
      - Line-width (T2)
      - Field strength

**Scalar coupling / J-coupling**

- \(^{15}\text{N}-^1\text{H}\)
  - \(^3J_{HH}\)
- \(\text{H}_3\text{C} - \text{CH}_2 - \text{Br}\)
Multidimensional NMR

- multidimensional NMR experiments
  - resolve overlapping signals
  - enables assignment of all signals
- encode structural and/or dynamical information
  - enables structure determination
  - enables study of dynamics

Why multidimensional NMR

2D NMR

3D NMR

Figure 1. Illustration of the increase in resolution afforded by the increase in dimensionality. In the 2D spectrum, four cross peaks overlap. By correlating with a third resonance frequency, each cross peak obtains a different position along a line in the 3D spectrum, thus resolving the overlap problem.
Magnetization transfer

- magnetic dipole interaction (NOE)
  - Nuclear Overhauser Effect
  - through space
  - distance dependent (1/r^6)
  - NOESY -> distance restraints

- J-coupling interaction
  - through 3-4 bonds max.
  - chemical connectivities
  - assignment
  - also conformation dependent

homonuclear NMR

- NOESY
  - t1, t2
  - crosspeak intensity ~1/r^6 up to 5 Å

- COSY
  - t1, t2
  - J-coupling interaction
    - transfer over one J-coupling, i.e. max. 3-4 bonds

- TOCSY
  - t1, t2
  - J-coupling interaction
    - transfer over several J-couplings, i.e. multiple steps over max. 3-4 bonds
**homonuclear NMR**

**NOESY**

- Measure frequencies of different nuclei; e.g. $^1$H, $^{15}$N, $^{13}$C
- No diagonal peaks
- Mixing not possible using NOE, only via J

**2D COSY & TOCSY**

**2D COSY**

**2D TOCSY**

**J coupling constants**

- $^{13}C_\beta$ = 35 Hz
- $^{13}C_\alpha$ = 55 Hz
- $^{13}C_\gamma$ = 35 Hz
- $^{13}C_\alpha$ = 55 Hz
- $^{13}C_\beta$ = 130 Hz
- $^{15}N$ = -92 Hz
- $^{15}N$ = 7 Hz
- $^{13}C_\alpha$ = 140 Hz
- $^{13}C_\beta$ = -1 Hz
- $^{13}C_\alpha$ = 55 Hz
- $^{13}C_\beta$ = -75 Hz
- $^{13}C_\gamma$ = -9 Hz
- $^{13}C_\gamma$ = -92 Hz
- $^{13}C_\gamma$ = -9 Hz
J coupling constants

$^{1}J_{HN} = -92$ Hz

Heteronuclear NMR

$^{1}H^{15}N$ HSQC: ‘protein fingerprint’

Note that spectrum is decoupled: no NH J-coupling
**J coupling constants**

- $J_{1JHN} = -92 \text{ Hz}$
- $J_{2JCaN} = 7 \text{ Hz}$
- $J_{1JCaN} = -11 \text{ Hz}$

**Triple resonance NMR**

- $1^H$-mix block
- $1^N$-mix block
- $1^C$-mix block

- $J_{1JNH}$
- $J_{2JNCa(i)}$
- $J_{1JNCa(i-1)}$

**Resonance assignment**

- $\omega_{13Ca(i)}$, $\omega_{15N(i)}$, $\omega_{1H(i)}$
- $\omega_{13Ca(i-1)}$, $\omega_{15N(i)}$, $\omega_{1H(i)}$

**Structural parameters**

- $(F_1, F_2, F_3) = (\omega_{13Ca(i)}, \omega_{15N(i)}, \omega_{1H(i)})$ & $(\omega_{13Ca(i-1)}, \omega_{15N(i)}, \omega_{1H(i)})$
Structural study by NMR

- Sample preparation (months)
- Acquisition of NMR spectra (~1 month)
- Chemical shift assignments
  - Backbone (days)
  - Side-chains (days)
- Analysis of NOESY spectra (weeks)
- Structure calculations (days)
- Functional studies with NMR
  - Interaction with partner

Sources of structural information

- OBSERVABLES
  - Chemical shifts ($^1$H, $^{15}$N, $^{13}$C, $^{31}$P)
  - J-couplings, e.g. J($H_N$,$H_\alpha$)
  - Long-range NOEs
  - Residual dipolar couplings
  - $H / D$ exchange
  - Effects of $pH / T$
  - Effects of interacting partners
  - Relaxation rates
  - ....

Secondary structure
- Antiparallel $\beta$-strand
- $\alpha$-helix

Tertiary structure
- Restraints: dihedral angles
- Distances between atoms
- Orientation between bond vectors
Ramachandran plot

\[ \phi \quad -130 \quad -60 \]
\[ \psi \quad 125 \quad -45 \]

\[ \beta\text{-strand} \quad \alpha\text{-helix} \]

OBSERVABLE: chemical shift

- $^{13}$C$\alpha$ and $^{13}$C$\beta$ chemical shifts
  - sensitive to dihedral angles
  - report on secondary structure elements

OBSERVABLE: homonuclear J-couplings

Karplus

\[ J = A \cos^2(\phi) + B \cos(\phi) + C \]

measured $J(H_NH_\alpha)$ reports on $\phi$

RESTRAINT: distances
**OBSERVABLE: NOE**

- **1H-1H NOEs (2D NOESY, 3D NOESY-HSQC)**
  - signal intensity proportional to \(1/r^6\)
  - reports on distance between protons
  - distance restraints

**NOEs in secondary structure elements**

<table>
<thead>
<tr>
<th>(\beta_{\beta}p)</th>
<th>(\alpha)-helix</th>
<th>31P-helix</th>
<th>turn I</th>
<th>turn II</th>
<th>turn II'</th>
<th>half-turn</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\rho_{N}(i+4))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\rho_{N}(i+3))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\rho_{N}(i+2))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\rho_{N}(i+1))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\rho_{N}(i))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\rho_{N}(i-1))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sequential & medium range NOEs - **SECONDARY STRUCTURE**

- **1H-1H NOEs**
  - signal intensity proportional to \(1/r^6\)
  - reports on distance between protons
  - distance restraints

**OBSERVABLE: NOE**

- **1H-1H NOEs**
  - signal intensity proportional to \(1/r^6\)
  - reports on distance between protons
  - distance restraints

 Sequential

 Intra-residue
  (used for identifying spin-systems)

 Medium range

 Sequential & medium range NOEs - **SECONDARY STRUCTURE**

- **Long range NOEs**
  - signal intensity proportional to \(1/r^6\)
  - reports on distance between protons
  - distance restraints

 Intra-residue
  (used for identifying spin-systems)

 Medium range

 Long range NOEs - **TERTIARY STRUCTURE**
Long-range NOEs

- Tertiary information
  - distances < 5 Å
  - Important structural information

OBSERVABLE: Residual dipolar couplings

Dipolar coupling

\[ D_0 = \frac{\gamma_i \gamma_j \mu_0}{4 \pi r^3} \left( \frac{3 \cos^2 \Omega(t) - 1}{2} \right) \]

No protein alignment

ISOTROPIC SYSTEM

D = 0

RESTRAINT: Orientation

OBSERVABLE: Residual dipolar couplings

Dipolar coupling

\[ D_0 = -\frac{\gamma_i \gamma_j \mu_0}{4 \pi r^3} \left( \frac{3 \cos^2 \Omega(t) - 1}{2} \right) \]

No protein alignment

ISOTROPIC SYSTEM

D = 0

Protein alignment

ANISOTROPIC SYSTEM

D ≠ 0
OBSERVABLE: Residual dipolar couplings

- **ISOTROPIC**
  \[ J_{NH} \]

- **ANISOTROPIC**
  \[ J_{NH} + D_{NH} \]

⇒ Difference gives RDC \( D_{NH} \)

Residual dipolar coupling

- B

- RDC reports on orientation of bond-vector
  - orientation of bond-vector within an alignment tensor (defined by \( A_a \) and \( A_r \)) with respect to the magnetic field
  - \( i.e. \) orientation of bond vector with respect to other bonds

\[ D_v = \frac{\gamma^2 \hbar \mu_0}{8 \pi^2} \left[ A_y \left( 3 \cos^2 \theta - 1 \right) + \frac{3}{2} A_z \sin^2 \theta \cos(2\varphi) \right] \]

Long-range orientational restraint - TERTIARY STRUCTURE

OBSERVABLE: H/D exchange rates

- Exchange \(^1\text{H} \) by \(^2\text{H} \)
  - peaks disappear in time
  - accessibility of sites
  - stability of secondary structure
- H-bonds

\[ N - H \cdots O = C \stackrel{k_{open}}{\rightleftharpoons} N - H \cdots O \rightarrow N - D \]

Increasing \(^1\text{H} \) protection

OBSERVABLE: chemical shift changes

- Titration
  - add in steps an interacting molecule (ligand / protein / DNA)
  - observe changes in chemical shift
- map interaction site
**OBSERVABLE: chemical shift changes**

- **Titration**
  - add in steps an interacting molecule (ligand / protein / DNA)
  - observe changes in chemical shift
- map interaction site

**Key concepts structural parameters**

- **OBSERVABLES**
  - chemical shifts (\(^1\)H, \(^{15}\)N, \(^{13}\)C, \(^{31}\)P)
  - J-couplings, e.g. \(^3\)J(H\(^N\), H\(^\alpha\))
  - medium-range NOEs
  - long-range NOEs
  - residual dipolar couplings (RDCs)
  - H / D exchange
  - effects of interacting partners
- ...

- **RESTRAINTS**
  - dihedral angles
  - dihedral angles
  - medium range distances
  - long-range distances
  - orientations bond-vectors
  - accessibility / H-bonds
  - interaction surface
- ...

**Relaxation & dynamics**

**NMR time scales**

- protein folding
- domain motions
- side chain motions
- loop motions
- bond vibrations
- overall tumbling
- enzyme catalysis; allosterics
- NMR relaxation dispersion
- real time NMR
- J-couplings
- H/D exchange
- RDC
- \(R_1, R_2\) NOE
- fs ps ns \(\mu\)s ms s
Local fluctuating magnetic fields

- Fast motion
  - Locally induced magnetic field changes
  - Causes relaxation

Relaxation

- Return to equilibrium
  - Longitudinal relaxation $\rightarrow T_1$ relaxation
  - Return to z-axis

- Transversal relaxation $\rightarrow T_2$ relaxation
  - Dephasing of magnetization in the x/y plane

Relaxation time is related to rate of motion

$R_1 = 1/T_1$
$R_2 = 1/T_2$

FAST (ps-ns): rotation correlation time
**FAST (ps-ns): protein flexibility**

- Protein flexibility can be assessed through NMR time scales.
- The figure illustrates the distribution of residue numbers with respect to R1/R2 values, highlighting the dynamic range of protein flexibility.

**SLOW (µs-ms): conformational exchange**

- Conformational exchange in proteins can be due to slow processes.
- The exchange rates can cause line-broadening and affect relaxation times (T1, T2).
- The diagrams show the exchange between two states A and B with rate constants kA and kB.

**NMR time scales**

- The NMR time scales diagram categorizes dynamic processes into different time scales:
  - **fs** (femtoseconds) for bond vibrations
  - **ps** (picoseconds) for side chain motions
  - **ns** (nanoseconds) for overall tumbling

- Processes such as protein folding, domain motions, and loop motions contribute to the overall dynamics of proteins.

**SLOW (µs-ms): conformational exchange**

- Causes line-broadening, making T2 relaxation faster when populations are skewed (pA >> pB).
- The diagrams show the spectra with and without exchange, illustrating the broadening effect.
Key concepts relaxation

- wide range of time scales
- fluctuating magnetic fields
- rotational correlation time (ns)
- fast time scale flexibility (ps-ns)
- slow time scale (μs-ms): conformational exchange