Introduction to solution NMR

Structural and biophysical methods for biological macromolecules in solution

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with thanks to Dr. Klaartje Houben

Solution NMR: 950, 900-cryo, 750, 600-cryo, 600US, 2x500 MHz  2017?: 1.2 GHz
Solid-state NMR: 800WB-DNP, 400WB-DNP, 700US, 500WB MHz
E-infrastructure: >1900 CPU cores + EGI grid (>110’000 CPU cores)

The NMR research group

Prof. Marc Baldus
Prof. Rolf Boelens

Prof. Alexandre Bonvin

http://www.uu.nl/nmr
Why use NMR for structural biology...

The very basics

Multidimensional NMR (intro)

Resonance assignment (lecture Banci)

Structure parameters & calculations (lecture Banci)

NMR relaxation & dynamics

NMR & Structural biology

Dynamic activation of an allosteric regulatory protein Tzeng
**NMR & Structural biology**

- **Allosteric regulation**
  - Dynamic interaction between ligand-binding & DNA binding site


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**Biomolecular interactions**

- Even weak and transient complexes can be studied

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**Excited States**

![Diagram showing excited state (E) and ground state (G)]

Shekhar & Kay PNAS 2013

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**Membrane Proteins**

- Native like environment
  - Structural changes due to lipid environment

van der Cruysen, ... & Baldus PNAS 2013

**The very basics of NMR**

• isotope labeling
  – $^{15}$N, $^{13}$C, $^2$H
  – selective labeling (e.g. only methyl groups)
  – recombinant expression in E.coli

• sample
  – pure, stable and high concentration
    – 500 ul of 0.5 mM solution $\sim$ 5 mg per sample
  – preferably low salt, low pH
  – no additives

**Study proteins in their native cellular environment**

• Outermembrane protein in bacterial cell envelop

Renault M, ..... & Baldus PNAS 2012

**The NMR sample**
Nuclear spin

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

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

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

Nuclear spin & Radiowaves

- Nuclear magnetic resonance
  - Only nuclei with non-zero spin quantum number are "magnets"
  - Commonly used spins are spin ½ nuclei: \(^1\text{H}, \(^{13}\text{C}, ^{15}\text{N}, ^{31}\text{P}\) etc.

Larmor frequency

\[ \nu = (\gamma B_0)/2\pi \]

Nuclear Spin & Radiowaves

\[ \Delta E = \gamma \hbar B_0 \]

\[ \beta \]

Quantum number \( I = \frac{1}{2} \)

Magnetic field strength

Gyromagnetic ratio (different for each type of nucleus)

\[ \nu = (\gamma B_0)/2\pi \]

Nuclear spin

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>( \frac{1}{2} )</td>
<td>( 2.6752 \times 10^6 )</td>
<td>99.98</td>
</tr>
<tr>
<td>(^2\text{H})</td>
<td>( \frac{1}{2} )</td>
<td>( 4.107 \times 10^7 )</td>
<td>0.02</td>
</tr>
<tr>
<td>(^{13}\text{C})</td>
<td>( \frac{1}{2} )</td>
<td>( 6.728 \times 10^7 )</td>
<td>1.11</td>
</tr>
<tr>
<td>(^{19}\text{F})</td>
<td>( \frac{1}{2} )</td>
<td>( 6.786 \times 10^7 )</td>
<td>99.54</td>
</tr>
<tr>
<td>(^{14}\text{N})</td>
<td>( \frac{1}{2} )</td>
<td>( 2.712 \times 10^7 )</td>
<td>0.36</td>
</tr>
<tr>
<td>(^{15}\text{N})</td>
<td>( \frac{1}{2} )</td>
<td>( 3.028 \times 10^7 )</td>
<td>0.04</td>
</tr>
<tr>
<td>(^{31}\text{P})</td>
<td>( \frac{1}{2} )</td>
<td>( 2.5181 \times 10^7 )</td>
<td>100.00</td>
</tr>
<tr>
<td>(^{20}\text{Na})</td>
<td>( \frac{1}{2} )</td>
<td>( 7.080 \times 10^7 )</td>
<td>100.00</td>
</tr>
<tr>
<td>(^{31}\text{P})</td>
<td>( \frac{1}{2} )</td>
<td>( 1.0841 \times 10^8 )</td>
<td>100.00</td>
</tr>
<tr>
<td>(^{13}\text{C})</td>
<td>( \frac{1}{2} )</td>
<td>( 5.934 \times 10^7 )</td>
<td>12.26</td>
</tr>
</tbody>
</table>

\( ^{\text{o}} \) 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.

Nuclear Spin & Radiowaves

- NMR a non invasive technique
  - Low energy radiowaves

\[ \text{Frequency:} \quad \text{GHz} \quad \text{MHz} \quad \text{kHz} \quad \text{Hz} \]

- Wavelength: 1 mm 1 mm 1 mm 1 mm 1 mm

\[ \gamma \text{-rays} \quad \text{X-rays} \quad \text{UV} \quad \text{IR} \quad \text{FIR} \quad \text{microwaves} \quad \text{radiowaves} \]
Boltzmann distribution

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

Example
- 20.001 spins
- Only 1 more spin in lower energy state

Net magnetization

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

Pulse
- Radio frequency pulses
- Turn on an amplifier for a certain amount of time & certain amount of power (\(B_1\) field)

\[ \nu = \frac{\gamma B_0}{2\pi} \]

\[ \nu' = \frac{\gamma B_1}{2\pi} \]

Rotating frame: observe with frequency \(\nu_0\)

Chemical shielding

Local magnetic field is influenced by electronic environment
\[ \implies \text{frequencies of nuclei will differ} \]
**Chemical shift**

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

More conveniently expressed as part per million by comparison to a reference frequency:

\[ \delta = 10^6 \frac{\nu - \nu_{\text{ref}}}{\nu_{\text{ref}}} \]

**Free induction decay (FID)**

**The spectrometer**

**FID: analogue vs digital**
Relaxation

• NMR Relaxation
  – Restores Boltzmann equilibrium

• T2-relaxation (transverse relaxation/spin-spin)
  – disappearance of transverse (x,y) magnetization
  – contributions from spin-spin and T1 relaxation
  – 1/T2 ~ signal line-width

• T1-relaxation (longitudinal relaxation / spin-lattice)
  – build-up of longitudinal (z) magnetization
  – determines how long you should wait for the next experiment

!! 1/T2 ~ signal line-width !!

!! T1 determines when to start 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

- H\textsubscript{3}C - CH\textsubscript{2} - Br
- \textsuperscript{3}J\textsubscript{HH}
- \textsuperscript{1}J\textsubscript{NH}

Key concepts NMR

- **Nuclear magnetic resonance**
  - In a magnetic field magnetic nuclei will resonate with a specific frequency
- **FT-NMR**
  - Pulse, rotating frame, FID
- **Chemical shift**
  - Electronic environment influences local magnetic field -> frequency
- **NMR relaxation**
  - T\textsubscript{1} & T\textsubscript{2}
- **J-coupling**

Multidimensional NMR
Why multidimensional NMR

* Resolve overlapping signals
  * observe signals from different nuclei separately

* Correlate chemical shifts of different nuclei
  * needed for assignment of the chemical shifts

* Encoding structural and/or dynamical information
  * enables structure determination
  * enables study of dynamics

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2D NMR

3D NMR

nD experiment

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

- mixing/magnetization transfer

\[ E = \mu B \]

proton A

spin-spin interactions

proton B

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

<table>
<thead>
<tr>
<th>Experiment</th>
<th>t1</th>
<th>t2</th>
<th>FID</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOESY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COSY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOCSY</td>
<td>mlev</td>
<td></td>
<td>FID</td>
</tr>
</tbody>
</table>

**2D NOESY**

- Uses dipolar interaction (NOE) to transfer magnetization between protons
  - cross-peak intensity \(~1/r^6\)
  - distances \(r < 5\AA\)

[Diagram of 2D NOESY spectrum with cross-peaks and diagonal lines]
Homonuclear scalar coupling

\[ J_{\text{HNH}} \approx 2-10 \text{ Hz} \]
\[ J_{\text{HNN}} \approx 3-12 \text{ Hz} \]

2D COSY & TOCSY

Homonuclear NMR

Diagonal
Cross-peak

heteronuclear NMR

- measure frequencies of different nuclei; e.g. \(^1\text{H}, ^{15}\text{N}, ^{13}\text{C}\)
- no diagonal peaks
- mixing not possible using NOE, only via \(J\)
**J coupling constants**

- $\nu_{1J}^{13}C_{\alpha}^{13}C' = 55 \text{ Hz}$
- $\nu_{1J}^{13}C_{\alpha} = 15 \text{ Hz}$
- $\nu_{1J}^{13}C_{\alpha} = -11 \text{ Hz}$
- $\nu_{1J}^{13}C_{\alpha} = -92 \text{ Hz}$
- $\nu_{1J}^{13}C_{\beta} = 35 \text{ Hz}$
- $\nu_{1J}^{13}C_{\beta} = 55 \text{ Hz}$
- $\nu_{1J}^{13}C_{\beta} = 7 \text{ Hz}$
- $\nu_{1J}^{13}C_{\beta} = 92 \text{ Hz}$
- $\nu_{1J}^{13}C_{\beta} = 35 \text{ Hz}$
- $\nu_{2J}^{13}C_{\beta}^{15}N_{C'} < 1 \text{ Hz}$

**$^{15}N$ HSQC**

- Backbone HN
- Side-chain NH and NH$_2$

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

- Structured protein
- Unstructured protein
Key concepts multidimensional NMR

- Resolve overlapping signals
- Mixing/magnetization transfer
- NOESY, TOCSY, COSY
- HSQC
- 3D NOESY-HSQC, 3D TOCSY-HSQC
- Triple resonance

NMR relaxation

- Return to equilibrium
  - Spin-lattice relaxation
  - Longitudinal relaxation → T1 relaxation
    - Return to z-axis
  - Spin-spin relaxation
  - Transversal relaxation → T2 relaxation
    - Dephasing of magnetization in the x/y plane + return to z-axis

Relaxation & dynamics

Relaxation is caused by dynamics

- Fluctuating magnetic fields
  - Overall tumbling and local motions cause the local magnetic fields to fluctuate in time
**Relaxation is caused by dynamics**

- Fluctuating magnetic fields
  - Overall tumbling and local motions cause the local magnetic fields to fluctuate in time
  - $B_{\text{loc}}(t)$ is thus time dependent
  - If $B_{\text{loc}}(t)$ is fluctuating with frequency components near $\omega_0$, then transitions may be induced that bring the spins back to equilibrium
  - The efficiency of relaxation also depends on the amplitude of $B_{\text{loc}}(t)$

**Local fluctuating magnetic fields**

- $B_{\text{loc}}(t) = B_{\text{loc}}[\text{iso}] + B_{\text{loc}}(t)[\text{aniso}]$
  - Isotropic part is not time dependent
    - chemical shift
    - J-coupling
  - Only the anisotropic part is time dependent
    - chemical shift anisotropy (CSA)
    - dipolar interaction (DD)

**Components of the local field**

- $B_{\text{loc}}(t)\cdot e_x$
  - Transverse fluctuating fields
    - Non-adiabatic: exchange of energy between the spin-system and the lattice [environment]

- $B_{\text{loc}}(t)\cdot e_y$
  - Longitudinal fluctuating fields

**T_1 relaxation**
Correlation function

- Describes the fluctuating magnetic fields
  - correlation function $C(t)$ decays exponentially with a characteristic time $T_c$
  - Time correlation function, $C(t)$
    $C(t) = \langle B_{\text{loc}}(t) B_{\text{loc}}(t+\tau) \rangle = \langle B_{\text{loc}}(0) B_{\text{loc}}(\tau) \rangle$
    $C(\infty) = \langle B_{\text{loc}}(t)^2 \rangle = 0$
    $C(t) = \exp(-t/T_c)$

Stationary random function, $B_{\text{loc}}(t)$

- $\langle B_{\text{loc}}(t) \rangle = 0$
- $\langle B_{\text{loc}}(t)^2 \rangle = 0$

Components of the local field

- $B_{\text{loc}}(t) \cdot e_{xy}$
  - Transverse fluctuating fields
  - Non-adiabatic: exchange of energy between the spin-system and the lattice [environment]
  - Heisenberg's uncertainty relationship:
    - shorter lifetimes $\leftrightarrow$ broadening of energy levels

Components of the local field

- $B_{\text{loc}}(t) \cdot e_z$
  - Longitudinal fluctuating fields
  - Adiabatic: NO exchange of energy between the spin-system and the lattice
  - Effective field along z-axis varies
    - frequency $\omega_0$ varies
      - $\approx$ variations of $\omega_0$

- $B_{\text{loc}}(t) \cdot e_{xy}$: transitions between states reduce phase coherence
- $B_{\text{loc}}(t) \cdot e_x$: frequency $\omega_0$ varies due to local changes in $B_0$
- $B_{\text{loc}}(t) \cdot e_y$: frequency $\omega_0$ varies

Spectral density function

- Frequencies of the random fluctuating fields
  - Spectral density function $J(\omega)$ is the Fourier transform of the correlation function $C(t)$
  - $J(\omega)$ describes if a certain frequency can induce relaxation and whether it is efficient

$$J(\omega) = \frac{T_c}{1 + \omega^2 T_c^2}$$
Link to rotational motions in liquids

- Molecules in solution
  “tumble” (rotational diffusion combining rotations and collisions with other molecules)
- Can be characterized by a rotational correlation time $\tau_c$
  - $\tau_c$ is the time needed for the rms deflection of the molecules to be $\sim 1$ radian ($60^\circ$)

Relaxation

- relaxation time is related to rate of motion

$T_1, T_2$

$T_1 = 1/T_1$

$R_2 = 1/T_2$

NMR time scales

- protein folding
- domain motions
- side chain motions
- enzyme catalysis; allosteries
- overall tumbling
- bond vibrations
- relaxation dispersion
- real time NMR
- J-couplings
- H/D exchange

Small molecules (or high temperature):
- smaller (shorter) correlation times (fast tumbling),
- $J(\omega)$ extends to higher frequencies - spectrum is flatter

Large molecules (or low temperature):
- larger (longer) correlation times (slow tumbling)
- $J(\omega)$ larger close to 0

$$J(\omega) = \tau_c/(1 + \omega^2 \tau_c^2)$$

$\omega$

$\tau_c$

5 ns

10 ns

20 ns

$T_c$

The Spectral Density Function

- Time correlation function, $C(t)$
- Spectral density function, $J(\omega)$

$J(\omega)$ is the Fourier transform of $C(t)$

$J(0) = \langle 0 \rangle$

$J(\infty) = \langle \rangle$

$J(\omega) \sim \frac{1}{\tau_c}$

$\tau_c$ is the correlation time or the rotational diffusion coefficient

$\tau_c$ is related to the rotational diffusion coefficient $D$ by $\tau_c = \frac{0.41 \mu}{D}$
Protein backbone dynamics

- $^{15}$N relaxation to describe ps-ns dynamics
  - $R_1$: longitudinal relaxation rate
  - $R_2$: transversal relaxation rate
  - hetero-nuclear NOE: $\{^{1}H\}^{15}N$

\[
R_1 = \frac{d^2}{2} [2 (\cos \theta \sin \theta + 3 \cos \phi \sin \phi) + 6 (\cos \phi \sin \phi)] + c^2 \ln (\sin \theta) \\
R_2 = \frac{d^2}{2} [4 \sin \phi \cos \phi] + 6 (\cos \phi \sin \phi) + 6 (\cos \phi \sin \phi)] + (c^2/3) [4 \sin \phi \cos \phi] + \ldots
\]

\[\text{NOE} = 1 + \left[\frac{(c/3)x(3y^2)}{(6x^2 + 6y^2 + 6xy)R_1}\right]\]

where $d^2 = (1/10) y_0^2 x_0^2 \sin^2 \theta$, $c^2 = (2/15) x_0^2$, $\alpha_0^2$, $\gamma^2$

Relaxation rates

- $^{15}$N relaxation to describe ps-ns dynamics
  - $R_1$: longitudinal relaxation rate
  - $R_2$: transversal relaxation rate
  - hetero-nuclear NOE: $\{^{1}H\}^{15}N$

- Measured as a 2D $^{1}H^{15}N$ spectrum
  - $R_1, R_2$: Repeat experiment several times with increasing relaxation-delay
  - Fit the signal intensity as a function of the relaxation delay
    - $I_0 \exp(-Rt)$
  - $\{^{1}H\}^{15}N$ NOE: Intensity ratio between saturated and non-saturated experiment

Lipari-Szabo MODELFREE

- Overall and local motion are considered to be uncorrelated
- $S^2 =$ order-parameter

\[
\begin{align*}
C(t) &= \frac{1}{S_2} \\
C(t) &= S^2 \int_{0}^{t} C(\tau) = \tau \\
J(\omega) &= \frac{2}{S_2} \frac{S_2^2 \tau_0^2}{1 + \omega^2 \tau_0^2} + \frac{(1 - S^2) \tau^2}{1 + \omega^2 \tau^2} \\
\tau^{-1} &= \tau_0^{-1} + \tau_{c}^{-1}
\end{align*}
\]
Modelfree analysis

Dynamics of Partly Unstructured Protein that has positive hetNOE values lacks both Ala and Gly ubiquitin (52)). This indicates that the positive hetNOEs

Several residues in the structured part of PX have high

$\tau_c$). This is confirmed by the

fact that lower transverse relaxation rates were found for these

sequence during the relaxation period (see Fig. 5, ) instead of a CPMG diffusion of the structured part can be accurately described

not a priori known, it is not necessarily true that the rotational

interconvert rapidly. But since the timescale of this process is

of the unstructured part would therefore result in completely

expected for a 12.7 kDa globular protein. This might be due

aggregation (26), which is not taken into account by the LS 

part. These features are clearly related to the observed

Fitting of the relaxation data at a high protein concentra-

0.2 mM. ( 79) and N-568

extended model including

used for T-567 ( ¼

$S_c$ was only

was only

rotational correlation time

Local correlation time

side chain motions

bond vibrations

overall tumbling

enzyme catalysis; allostERIC

protein folding

domain motions

loop motions

NMR

R$_c$, R$_n$, NOE

relaxation dispersion

real time NMR

J-couplings

H/D exchange

RDC

Conformational exchange

equal populations: $p_A = p_B$

skewed populations: $p_A >> p_B$

Conformational exchange

• Causes line-broadening of the signals

$-R_{2,eff} = R_2 + R_{ex}$
H/D exchange

Lac headpiece
Kalodimos et al. Science

- protected in the free state
- protected only in the DNA-bound state

Key concepts relaxation

- time scales
- fluctuating magnetic fields
- correlation function, spectral density function
- molecular motions
- rotational correlation time (ns)
- fast time scale flexibility (ps-ns)
- slow time scale (μs-ms): conformational exchange

The End

Thank you for your attention!