Introduction to NMR Spectroscopy: Structure, Dynamics and Molecular Interactions

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NMR spectroscopy is a powerful method for resolving the structure, dynamics and interactions of proteins and nucleic acids in solution. The LMB has a state-of-the-art NMR facility with several ongoing programmes of research into the structure of proteins, characterisation of protein complexes, membrane and intrinsically disordered proteins and proteins of the ubiquitination, Wnt signalling and ribosome maturation pathways to name but a few.

The LMB NMR facility was relocated to a purpose-built Magnetic Resonance Spectroscopy (MRS) building in 2001 (see: [LMB Intranet](https://www.mrc-lmb.cam.ac.uk)). The NMR facility contributes to projects across the full range of research activities at the LMB and is complementary to other structural and biophysical techniques. It is a biology-driven facility that makes NMR accessible to the non-specialist user. All relevant advanced NMR methods have been implemented and our support staff are able to adapt them to the specific needs of the LMB scientists.

Our website contains many useful resources including protein expression protocols, software plugins and in-house scripts for use in your NMR project. The contact for any enquiries about the use of the NMR facility and NMR support is Stefan Armstead, ext. 8074 or 8077.

Click on an image below to learn a little more about NMR.
Molecular Interactions

Wnt enhanceosome multiprotein complex

Characterisation of mechanistic principles:
- direct binding interactions
- contact surfaces
- stable or transient complexes

Overview

Where NMR is good:

STRUCTURE composition, conformation, complexation

DYNAMICS internal mobility, diffusion (MW), exchange

SITE SPECIFICITY define the locus of changes & binding interfaces

QUANTIFICATION relative concentrations of materials in the same sample (kinetics, titration, $k_{eq}$)

Where NMR is ‘less good’:

SYMMETRY cannot resolve planes of symmetry (can the symmetry be broken ?)

HETEROGENEITY requires *pure*, monodisperse, single conformers (?)

SAMPLE QUANTITY requires minimum 0.28 ml of >20 µM fixed volume, therefore there is a minimum concentration

HIGH MW struggles with slowly rotating rigid molecules

LOW SPIN DENSITY requires many ‘coupled’ NMR active atomic nuclei

SPEED often time-consuming — data acquisition is getting faster but data analysis not yet reliably automated
**DYNAMICS — NMR Sees All States in the Ensemble**

Solid State methods report on individual frames; multiple states can be resolvable.

Solution State methods report all states in the ensemble — but structural data (NOEs) may be missing from flexible regions.

DYNAMICS —
- NMR in combination with computational methods
- Quantify both the extent and rates of motion
- Interpreting NMR data in terms of a single structure can result in an erroneous virtual conformation.

Adapted from slide by: Robert Rambo, Diamond Light Source, Oxford

**NMR Spectra are Sensitive to Motion Across a Wide Span of Timescales**

- Relaxation ($T_1$, $T_2$, Het-NOE)
- ZZ exchange
- H-D exchange
- Residual dipolar coupling
- Relaxation dispersion (CPMG)
- EXSY
- Allometric regulation
- Side-chain rotation
- Segmental motion
- Ligand binding
- Protein folding
- Vibration

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\[ \Delta E = h \nu = h\gamma B_0 / 2\pi \]

**NMR Spectrometer Field Strength Dependence**

**Advantages of increased \( B_0 \) field strengths:**
- increased sensitivity \( I \propto B_0^{3/2} \)
- increased signal dispersion (resolution)
- altered relaxation (TROSY, NOESY)

**Disadvantages:**
- cost & housing
- radiofrequency power handling
- some lines broaden at high field (particularly carbonyl \( ^{13}\text{C} \))

Put in the most simple terms, the stronger the magnet the better
Linewidth, \( \lambda \), is inversely proportional to \( T_2 \) (where \( \lambda \) = linewidth at half height; \( T_2 \) = exponential time constant for signal decay; \( A \) = signal amplitude; \( \omega \) = frequency in rad/s)
Relationship Between Relaxation Rates and Linewidth

\[ \lambda \approx \frac{1}{\pi T_2} \]

FID for macromolecule, \( T_2 = 20 \text{ ms} \)

FID for small molecule, \( T_2 = 1.6 \text{ s} \)

\( \lambda \approx 0.2 \text{ Hz} \)

\( \lambda \approx 16 \text{ Hz} \)

\( \lambda \approx \frac{1}{\pi} \)

FT

Fourier Transformation

"free induction decay" (FID)

time domain

frequency domain

frequency
Multi-Dimensional NMR: Reducing Peak Overlap

Fourier Transformation

- Time domain
- Frequency domain

"Free induction decay" (FID)
Magnetic Interactions Between a Nucleus and its Environment

\[ H = \sum \gamma B_0 (1 - \sigma_j) I_z^j + \sum_{jk} 2\pi J_{jk} I_z^j I_z^k + \sum_{jk} 2\pi D_{jk} I_z^j I_z^k \]

1. Shielding

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Magnetic Interactions Between a Nucleus and its Environment

\[ H = \sum \gamma B_0 (1 - \sigma_j) I_z^j + \sum_{jk} 2\pi J_{jk} I_z^j I_z^k + \sum_{jk} 2\pi D_{jk} I_z^j I_z^k \]

1. Shielding

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Chemical shift axis
2. **J-Coupling** (scalar coupling through covalent bonds)

\[ H = \sum_j \gamma_H B_0 (1 - \sigma_j) I^j_z + \sum_{j \neq k} 2 \pi J_{jk} I^j_z I^k_z + \sum_{j \neq k} 2 \pi D_{jk} I^j_z I^k_z \]
Magnetic Interactions Between a Nucleus and its Environment

\[ H = \sum_{i} \gamma_i B_0 (1 - \sigma_i) I_i + \sum_{j \neq k} 2 \pi J_{jk} I_j^z I_k^z + \sum_{j \neq k} 2 \pi D_{jk} I_j^z I_k^z \]

3. Dipole-Dipole Coupling (through space, up to 7 Å)

Dipolar Coupling in Structure Determination

\[ D = D_{\text{max}} \langle 1/2 (3 \cos^2 \theta - 1) \rangle \]

no averaging over time:

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rapid averaging: nuclear Overhauser effect — measure of inter-nuclear distance

NOE \( \propto 1/r^6 \)

incomplete averaging: ‘residual’ dipolar coupling — measure of bond orientation
NOE-based Structural Analysis of Proteins
(Kurt Wüthrich, 1980's; Nobel Prize 2002)

- structure determined from short-range H-H interactions (<6Å — i.e. within 4 carbon-carbon bond lengths)
- apply typically 25 restraints per residue in combined distance-geometry & simulated annealing

NOESY: a complex jigsaw puzzle

- Main structural information
  Intensity $\propto 1/r^6$ (< 6.0 Å)
- Very complex to analyse
  Requires proton assignments
  Highly overlapped
  (requires 3D or 4D NMR)
  Spin diffusion
- Quality degrades as MW increases

Realistic MW limit for high resolution structure ≈ 40 kDa

Residual Dipolar Coupling
(N.Tjandra & A.Bax, Science, 278, 1111, 1997)

Recorded for isotropically tumbling protein
\( \langle D \rangle = 0 \) Hz

Recorded in the presence of weak alignment medium
\( J_{NH} = -93 \) Hz
\( D_{NH} = -20 \) Hz

Sample with 1 part in \( \sim 10^3 \) excess population of its major diffusion axis aligned with \( B_0 \) recovers a few Hertz of ‘residual’ dipolar coupling; the magnitude is sensitive to bond orientation.

RDC for Intrinsically Disordered Protein Segments

\( p53 \) (1-355)

\( p53 \) N-terminal domain (residues 1-93)
\[ E = \frac{1}{2} k (x - x_0)^2 \]

Add experimental restraints from NMR data

**Experimentally Derived Structural Restraints**

- **NOE (Nuclear Overhauser effect)**
  - structure determined from short-range H-H interactions (<6 Å)

- **Residual Dipolar Coupling**
  - bond orientation (WRT global coordinate frame)

- **Chemical Shift Index (TALOS)**
  - bond dihedral angles

- **J-coupling constants**
  - bond dihedral angles

- **Hydrogen bonds**
  - from hydrogen exchange protection factors

Energy Coordinate

Simulated Annealing

NMR Structure PDB Submissions
superposition of 20-100 models
depicting the consensus structure

Characteristics of the $^1$H-NMR Spectrum of Proteins

$^1$H chemical shift (ppm)
HSQC: the Protein ‘Fingerprint’


Heteronuclear Single Quantum Correlation

probe the structural environment at >200 individual sites

$^{15}$N-labelled wild-type p53 core domain, 219 residues

Mapping Protein Binding Interfaces

High Ambiguity Driven Docking “HADDOCK”

Quantifying Binding Affinities

\[ \Delta_{\text{obs}} = \Delta_{\text{max}} \frac{[L] + [E] + K_d}{2[E]} - \frac{\sqrt{([L] + [E] + K_d)^2 - 4[L][E]}}{2[L]} \]

\( \Delta \) is the chemical shift perturbation, [L] & [E] are the total concentration of ligand & protein.

Molecular Weight Limit for NMR?

Molecular Weight Discrimination Using Relaxation Filters

(I.D. Campbell, C.M. Dobson, R.J.P. Williams & P.E. Wright, FEBS Lett., 57, 96, 1975)

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FID for small molecule, $T_2 = 1.6$ s

John O’Neill, LMB Cell Biology Division
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$^1$H NMR of intact human erythrocytes

relaxation filtered (CPMG)

John O'Neill, LMB Cell Biology Division

Effective Molecular Weight

(J.Anglister, S.Grzesiek, H.Rein, C.B.Klee & A.Box, J. Biomol. NMR, 3, 121, 1993)

$T_2$ is obtained from the NH region peak intensity ratio between two spectra acquired with different values of the delay time $\Delta$.

$$T_2 = \frac{2(\Delta A - \Delta B)}{\ln(I_A/I_B)}$$

$T_2$ correlates with MW:

$$\tau \approx \frac{1}{5T_2} = \frac{1}{2} \text{MW @ } 20^\circ\text{C}$$

for rotational correlation time $\tau$, in ns, relaxation time constant $T_2$ in s.

NH region

1H chemical shift (ppm)

One-Dimensional spin-echo

A. recorded with short relaxation delay, $\Delta$

B. recorded with longer $\Delta$

Peak Intensity, $I$

$I_A/I_B$

$\Delta A - \Delta B$

$\ln(I_A/I_B)$

$2T$

90˚

$T$

$\Delta$

$I$ recorded with short relaxation delay, $\Delta$

$I$ recorded with longer $\Delta$
Is My Protein Folded or Unfolded?

![Diagram showing 1H chemical shift and N chemical shift plots for folded and unfolded proteins.](image)
Optimising Domain Boundaries for Crystallisation or NMR

Quantifying Individual pKₐ Values

**High-Throughput NMR Applications**

Design of biochemical probes & measurement of binding affinities for:
- dissecting function of proteins
- target validation of protein drug targets
- (hit-to-lead development)

- chemical fragment libraries (1° screening)
- hits from HTS / in silico (2° screening)
- systematic exploration of conditions for synthesis

**NMR as a Screening Tool in Drug Discovery & Design**

**Ligand-Observed NMR Assays**
- Yes/No binding
- can assay as competition binding to a known site
- requires ~1 - 10 µM unlabelled protein
- no MW restriction for the protein

  **basis**
  - change in diffusion rate (CPMG)
  - transfer of energy from an irradiated protein (STD)
  - nuclear Overhauser effect (waterLOGSY)
  - chemical shift perturbation of 19F resonances

**Protein-Observed NMR Assays**
- Yes/No & characterise the binding site
- "gold standard" proof of binding
- requires 15N or 13C-labelled protein, >50 µM
- MW <50 kDa

  **basis**
  - chemical shift perturbation (HSQC, methyl-TROSY)
Ligand Observed Methods for Fragment Based Screening

Elizabetta Chiarparin (Astex)

Summary

- **STRUCTURE**: NOE, RDC, chemical shifts
- **DYNAMICS**: internal mobility, diffusion & exchange
- **INTERACTIONS**: protein-protein, protein-DNA, protein-small molecule
- **QUANTIFICATION**: of reaction kinetics and $k_{eq}$, approx. MW, exchange rates
# Recommended NMR Texts

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