Solid-State NMR: Principles

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Solid-State NMR

Matter

Gas

Liquid

Solid

Isotropic

Anisotropic

Ordered

Disordered

Membrane

Crystals

Fibrils

Glasses

Biological materials
Membrane proteins

Rhodopsin, Gramicidin, .......
Targets for SSNMR

$\beta$-Amyloid fibrils
• Lipid bilayers

• Membranes reconstituted with different additives such as cholesterol, drugs or peptides

• Structure analysis of membrane-active peptides, ion channels, and receptors

• Amyloid fibrils, silk, and elastic proteins
Difficulties

• Restricted or no internal motion, unlike solution-state

• All interactions present in toto

• Interactions are anisotropic leading to broadening of spectral lines

• Plethora of information present, leading to a complete characterisation of materials
Simple 1D solution-state spectrum
$^1$H spectrum of a protein

Simple 1D solid-state spectrum
$^{13}$C spectrum of glycine
• Mimick the inherent averaging processes in solution-state to obtain high-resolution, isotropic information

• Goal #1: (Resolution and Sensitivity): Remove anisotropic parts and retain only isotropic parts: Decoupling

• Goal #2: (Let us have the cake and eat it as well) Get back the anisotropic parts for elucidation of geometry parameters: Recoupling
Remedies

- Anisotropic Part
- Spatial Part
- Spin Part

**Independent:** Can be individually manipulated

- Mechanical manipulation
- RF manipulation

Anisotropic Part → Spatial Part → Spin Part → Independent: Can be individually manipulated → RF manipulation → Mechanical manipulation
Hamiltonians and their Manipulation

$$H_{TOTAL} = \left[ H_{SPACE} \otimes H_{SPIN} \right]_{anisotropic} + H_{isotropic}$$

**Spatial Part: Manipulation**
- Rotating the crystallites in a given powder
- Sample spinning: Mechanical manipulation
- Easier to visualise
- Difficult to implement

**Spin Part: Manipulation**
- Rotating the spins in a given powder
- Spins rotation: Manipulation by RF pulses
- Easier to Implement
- Difficult to visualise
Which Angle to Rotate at?

Spatial part of the anisotropic Hamiltonian

\[ P_2(\cos \theta) = \frac{1}{2}(3\cos^2 \theta - 1) \]

\[ P_2(\cos \theta) = 0 \text{ for } \theta = 54.7^0 \]
Average out the chemical shift anisotropy, to achieve good sensitivity and resolution
Averages out the chemical shift anisotropy, to achieve good sensitivity and resolution.
Resolution and Sensitivity Enhancement by MAS

$^{13}$C spectra of $[^{13}$C$_2]$-glycine

- no spinning
- with MAS at 12 kHz
Magic-Angle Spinning Spectra: Resolution Enhancement

The powder pattern breaks up into a centreband and sidebands spaced at integer multiples of the rotor frequency.

Glycine

The powder pattern breaks up into a centreband and sidebands spaced at integer multiples of the rotor frequency.
MAS Rotor Types

- 2.5 mm
- 4 mm
- 7 mm
- 10 mm
Rotor caps

- ZrO₂
- Macor
- BN
- Kel-F
- Vespel
Standard Bore MAS Probe

- proton trap
- BN stator
- RF coil
- stator flip mechanism
- bearing gas inlet
- RF electronics
Rare spins experience weaker homonuclear dipolar couplings, hence, the resolution limiting aspect is the heteronuclear dipolar coupling to the abundant $^1$H.
Heteronuclear Dipolar Decoupling

- Homonuclear dipolar coupling
- Heteronuclear dipolar coupling

- Abundant spins: $^1\text{H}$
- Rare spins: $^{13}\text{C}$, $^{15}\text{N}$

Typical $^1\text{H}-^{13}\text{C}$ coupling = -25 kHz
Heteronuclear Dipolar Decoupling

\[ \frac{\pi}{2}_y \]

\[ ^1H \]

\[ ^1H \]

\[ ^{13}C \]

S spin detection

MAS

Decoupling

RF
MAS + Heteronuclear Dipolar Decoupling

$^{13}$C spectra of adamantane
MAS + Heteronuclear Dipolar Decoupling

(a) 5 kHz broadening
(b) Only decoupling
(c) Only MAS
(d) MAS+Decoupling

2-\(^{13}\)C Glycine

CW decoupling at 150 kHz
MAS at 30 kHz

334 Hz broadening
80 Hz broadening
Energy levels of both nuclei are matched in the doubly rotating frame. A spin-lock RF field is equivalent to producing a rotating-frame transformation. Hence, we need a continuous spin-lock RF field on both the nuclei for CP.

A match of the energy levels is produced when the nutation frequencies of both the spins along the effective RF field direction are the same: \( B_{1I} = B_{1S} \) or in other words \( \gamma_I \omega_I = \gamma_S \omega_S \).

**Hartman-Hahn condition**
MAS and heteronuclear decoupling lead to resolution
CP leads to sensitivity

CPMAS, basic pulse block in solid-state NMR for both sensitivity and resolution
*Enhanced signal, $\sim \gamma_1/\gamma_S$

*T$_1$ of abundant high-$\gamma$ nuclei shorter than that of the rare low-$\gamma$ nuclei

*Spatial proximity
Decoupling

$IH_{13C}$

The routine way towards high-resolution and sensitivity in solid-state NMR experiments

$\theta = 54.7$

Stejskal, Schaefer, Waugh, JMR, 18,560,1975
Stejskal, Schaefer, Waugh, JMR, 28,105,1977
SOLID STATE NMR

Electromagnetic irradiation >> internal coupling strengths
Selection rules may be generated at will
Recoupling under Magic-Angle Spinning: Retrieving Lost Interactions
What is Recoupling and Why Recoupling

Solid-state NMR

Anisotropic interactions with geometry information

CSA: Local chemical environment
DD: Distances and angles
Quad: Local environment, asymmetry, distribution

Solution-state NMR

Only isotropic information are inherently present

Geometry information available indirectly via relaxation experiments

Direct manifestation of geometry parameters

Problem: High-resolution schemes kill the anisotropy and geometry information

Question: Can the lost anisotropic interactions retrieved whilst retaining the isotropic resolution?

Having the cake and eat it too!
Recoupling is done in solution-state NMR, NOE for example

Rapid molecular motions modulate the dipole-dipole interactions and the fluctuating dipolar fields can drive magnetisation exchange (or cross relaxation) between spins over a wide range of chemical shifts

This mechanism fails in solid state due to the restricted molecular motions that cannot supply the energy differences necessary for molecular motions (also resolution and sensitivity considerations play a role)
Recoupling of Interactions

Rigid Solid

MAS

RF Irradiation: Recoupling interactions

Resolution, but Information sacrificed!

Both resolution and geometry information
Hetero/Homonuclear Recoupling

Pay a price: A scale factor!

Couplings may be reintroduced for certain periods of the experiment

Pay a price: A scale factor!
Rotational Resonance

Condition:
\[ \Delta \omega_{\text{iso}} = n \omega_r \]

The splitting indicates the strength of the dipolar coupling between the two spins.

Raleigh et al., Chem. Phys. Lett. 146, 71, 1988
[U-\textsuperscript{13}C-\textsuperscript{15}N]achatin-II
16 \( \pi \) pulses
MAS 10 kHz

Being used currently in biomolecules for \textsuperscript{13}C-\textsuperscript{13}C correlation towards assignments
RFDR: SH3 Domain Protein

Secondary Structure Elements

Backbone conformation by correlating two anisotropic interactions such as CSA or DD

**FIGURE 6.** Alternative approaches to directly determine secondary structure elements: correlating dipolar tensors such as \((C_\alpha,N)-(CO,N)\) (blue) or carbonyl CSA tensors (red) and measuring \(H_\alpha(i)-H_N(i+1)\) distances (green).

*Interactions measured from 2D experiments: Recoupling, double-quantum methods*
Structure by Solid-State NMR: Schematic

1. SAMPLE PREPARATION
2. CHEMICAL SHIFT ASSIGNMENT
3. STRUCTURAL PARAMETERS

EXPERIMENTS

1D

2D

2D, 3D

STRUCTURE

SECONDARY STRUCTURE

TERTIARY STRUCTURE
Assignments in Solid-State NMR: Example

α-spectrin SH3 domain

NCACB
Correlation Experiments: HNCA in Solid-State

$^{15}$N-α-Spectrin SH3 domain

$^1$H-$^1$5N-HSQC (dipolar). 17.6 T, MAS 8 kHz

$^1$H-$^1$5N-$^1$3C HNCA 9.4 T, MAS 8 kHz

Figure 4. Assignment of the amides of T24, G28 and Q50. (A) shows a section of the 2D $^1$H-$^1$5N experiment of Figure 2A, centred around the $^1$5N chemical shift of the three residues ($\approx 116.6$ ppm). In (B), a plane from the 3D dataset is shown, extracted at the same $^1$5N chemical shift. Finally, (C) shows a strip from a 2D NCA experiment, recorded from ($^1$H-$^1$5N-$^1$3C) α-spectrin SH3 domains at a field of 9.4 T and using a spinning frequency $\omega_0/2\pi = 8.0$ kHz.

J. Biomol. NMR, 25, 217, 2003
Sample Preparation Issues

Ubiquitin

crystalline

lyophilised

Sample Preparation Issues

SH3 domain protein

- Lyophilised from aqueous low-salt buffer
- Lyophilised from aqueous low-salt buffer + adding water
- Lyophilised from a solution also having PEG and sucrose
- Precipitated from a rich ammonium sulphate solution

Pauli, ...., Oschkinat, J. Biomol. NMR, 25, 217, 2003
Membrane proteins
Amyloid fibrils
SH3 domain protein
Ubiquitin
Bacillus Subtilis protein Crh
$\alpha$-Synuclein
Sequential Assignment and Conformational Analysis

2*10.4 kDa dimeric form of The Bacillus Subtilis protein Crh

Bockmann,....., Baldus, J. Biomol. NMR, 27, 323, 2003
Sequential Assignment and Conformational Analysis

Bockmann,....., Baldus, J. Biomol. NMR, 27, 323, 2003
Conclusions

• Solid-state NMR has come of age

• A rich pasture for spin gymnastics and choreography

• A judicious combination of MAS and RF very vital for most experiments

• Selective manipulation of each spin interaction possible

• The methods in vogue now are being used to develop tools to do solution-state kind of experiments for assignments in biomolecules

• Remarkable progress already made in various biomolecular systems