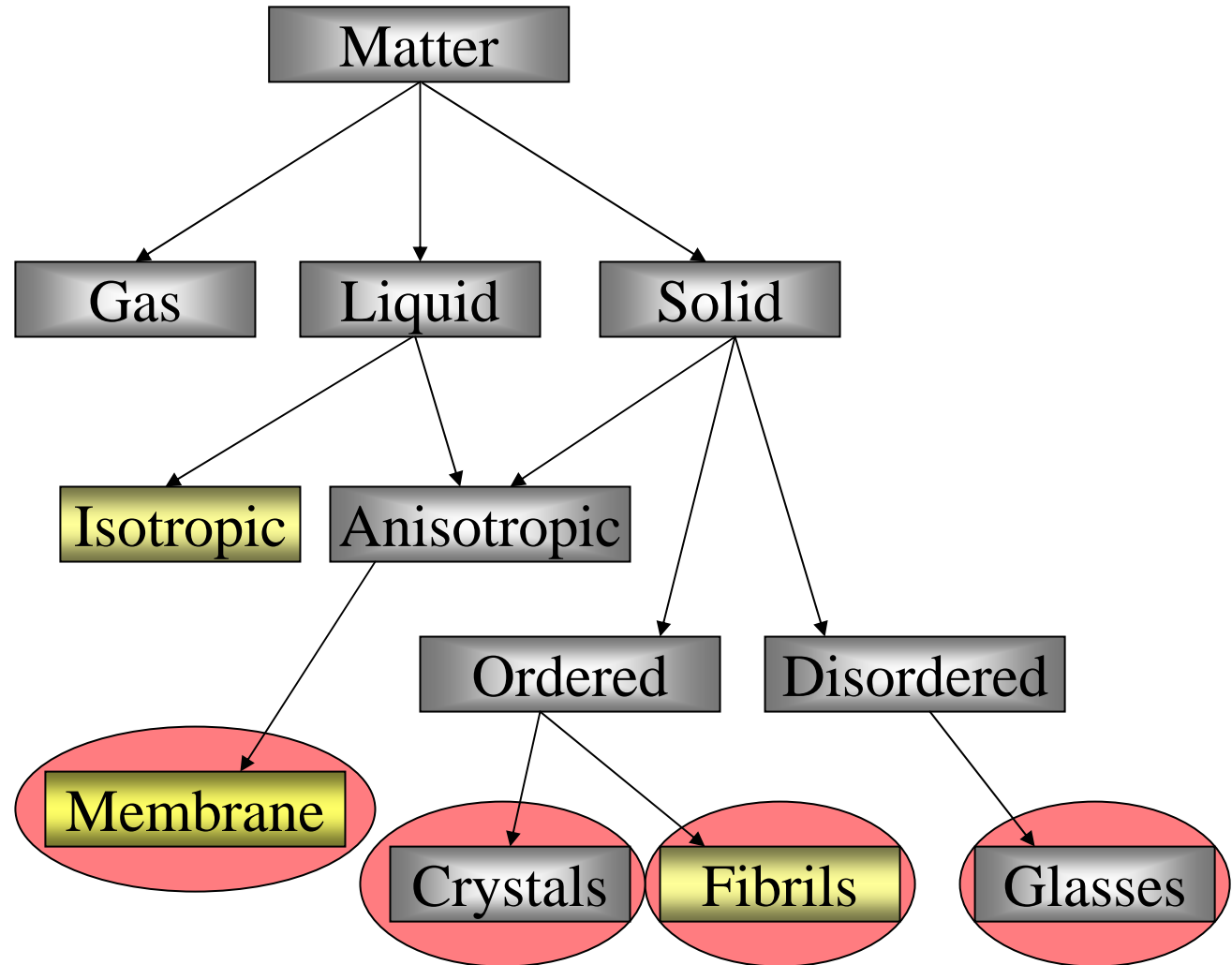


Solid-State NMR: Principles

P. K. Madhu
Department of Chemical Sciences
Tata Institute of Fundamental Research
Homi Bhabha Road
Colaba
Mumbai 400 005, India

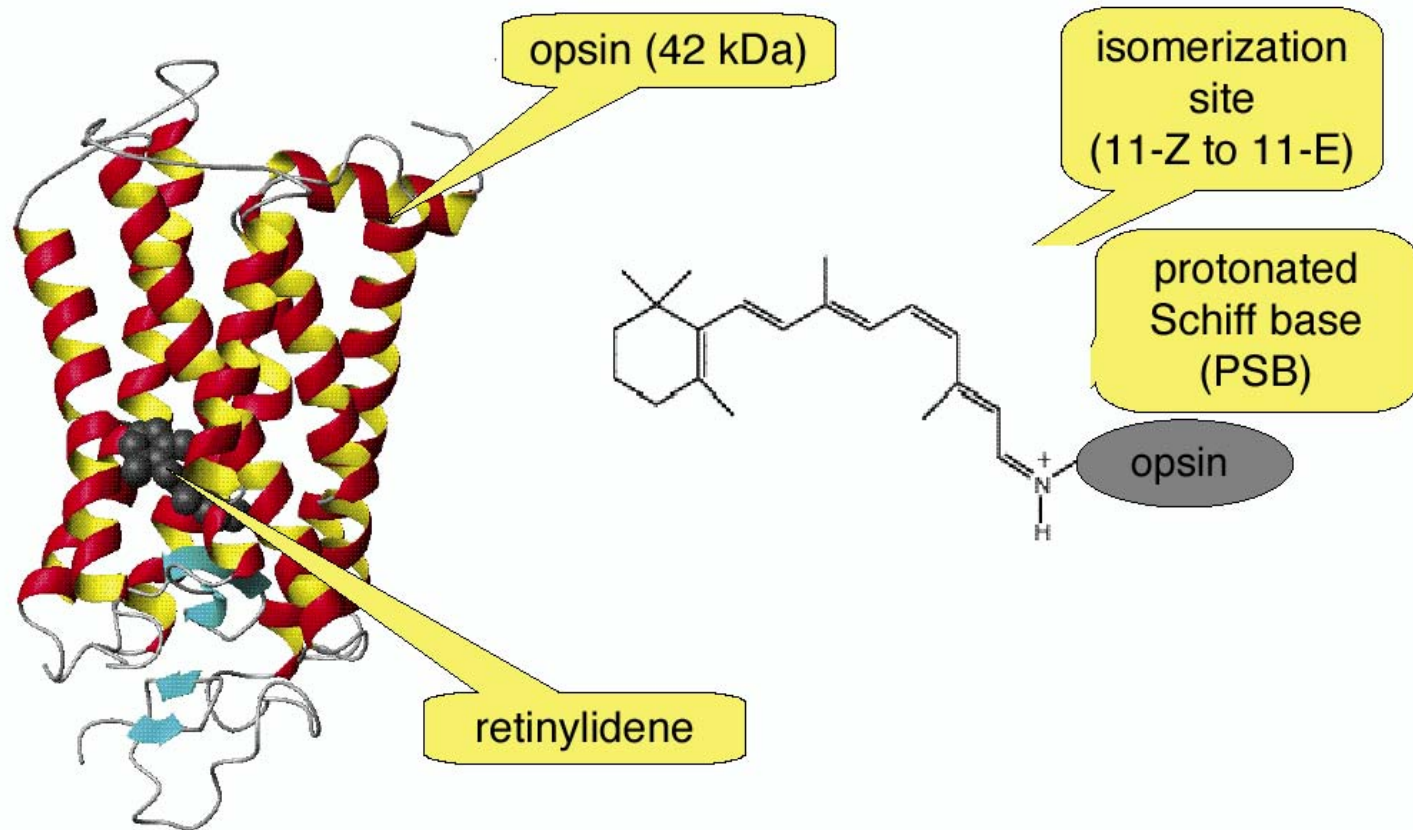
Solid-State NMR



Biological materials

Targets for SSNMR

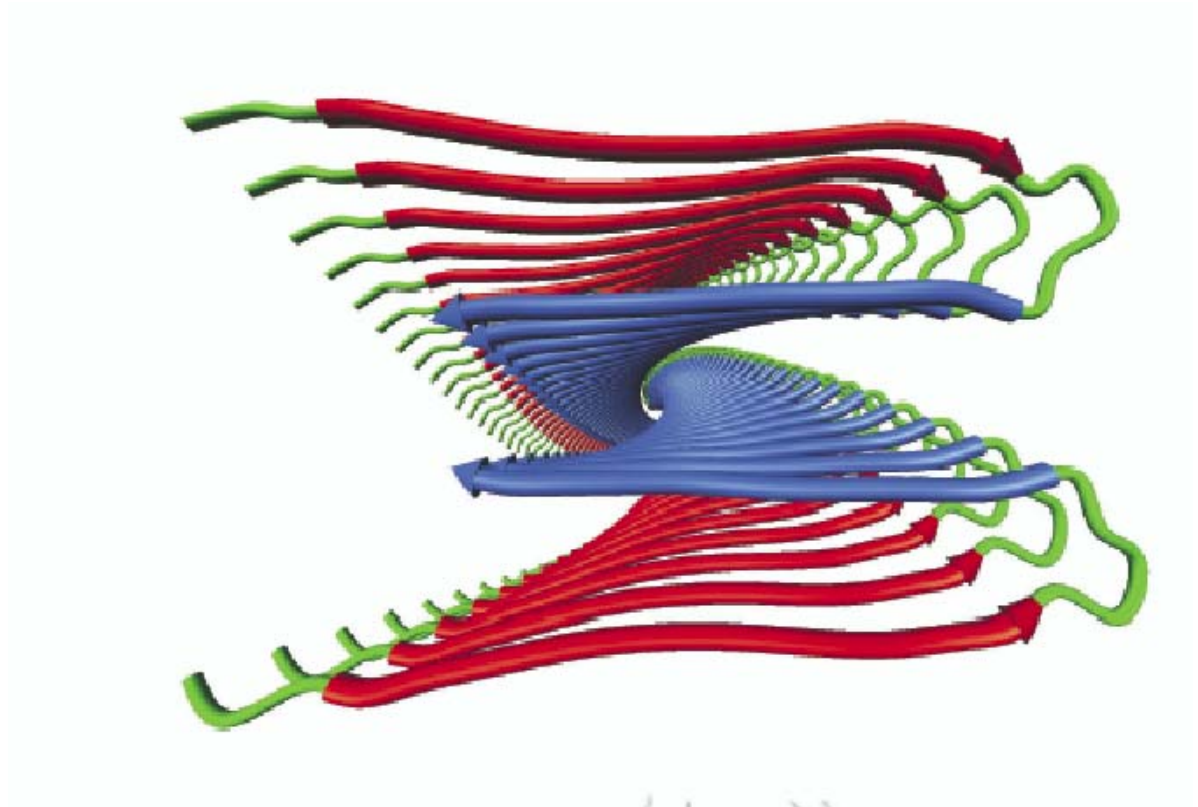
Membrane proteins



Rhodopsin, Gramicidin,

Targets for SSNMR

β -Amyloid fibrils



Targets for SSNMR: Biology

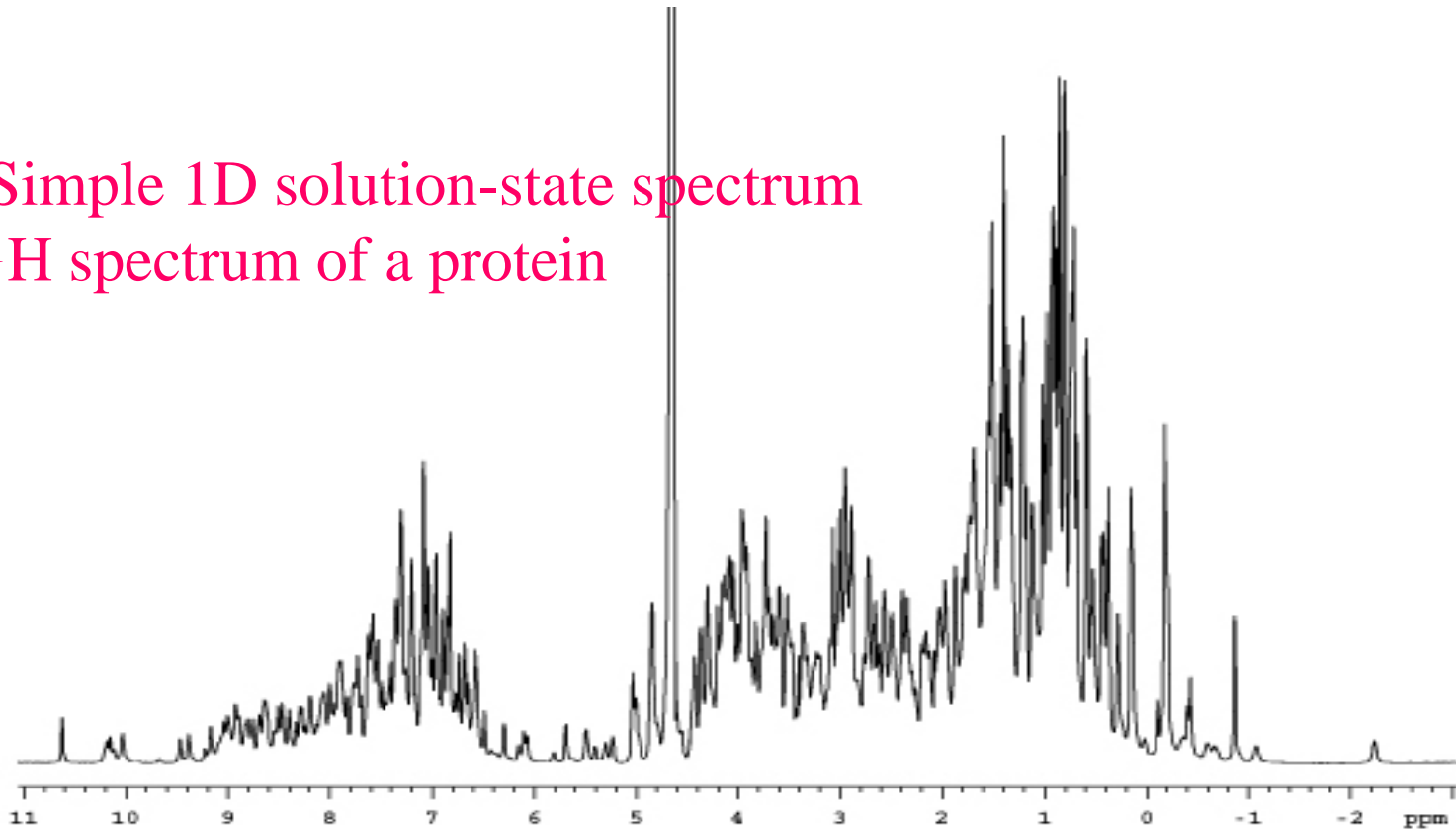
- Lipid bilayers
- Membranes reconstituted with different additives such as cholesterol, drugs or peptides
- Structure analysis of membrane-active peptides, ion channels, and receptors
- Amyloid fibrils, silk, and elastic proteins

Difficulties

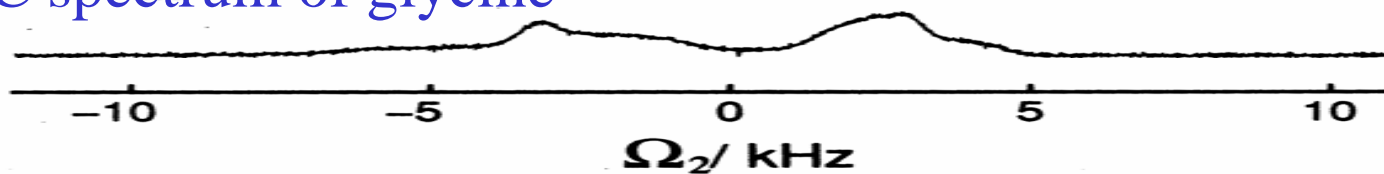
- Restricted or no internal motion, unlike solution-state
- All interactions present in toto
- Interactions are anisotropic leading to broadening of spectral lines
- Plethora of information present, leading to a complete characterisation of materials

Reality

Simple 1D solution-state spectrum
 ^1H spectrum of a protein



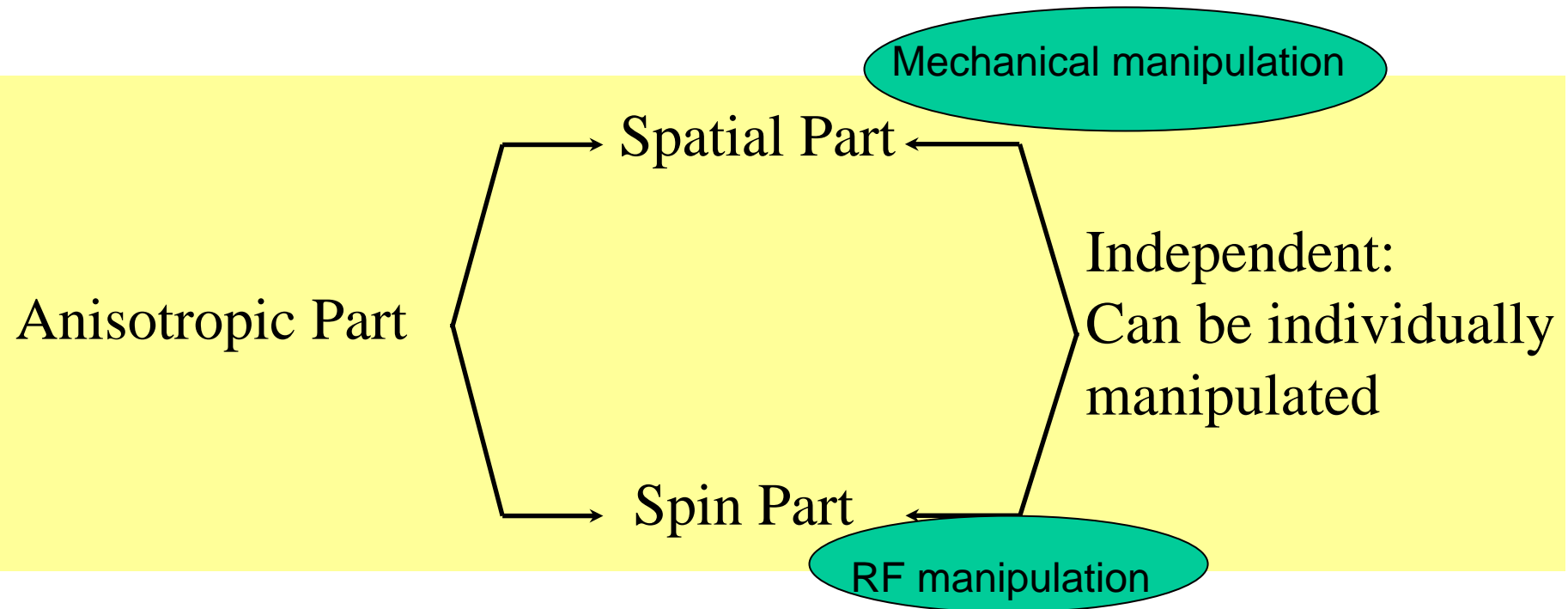
Simple 1D solid-state spectrum
 ^{13}C spectrum of glycine



Remedies

- **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



Hamiltonians and their Manipulation

$$H_{TOTAL} = [H_{SPACE} \otimes H_{SPIN}]^{anisotropic} + H^{isotropic}$$

Spatial Part: Manipulation

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

Spin Part: Manipulation

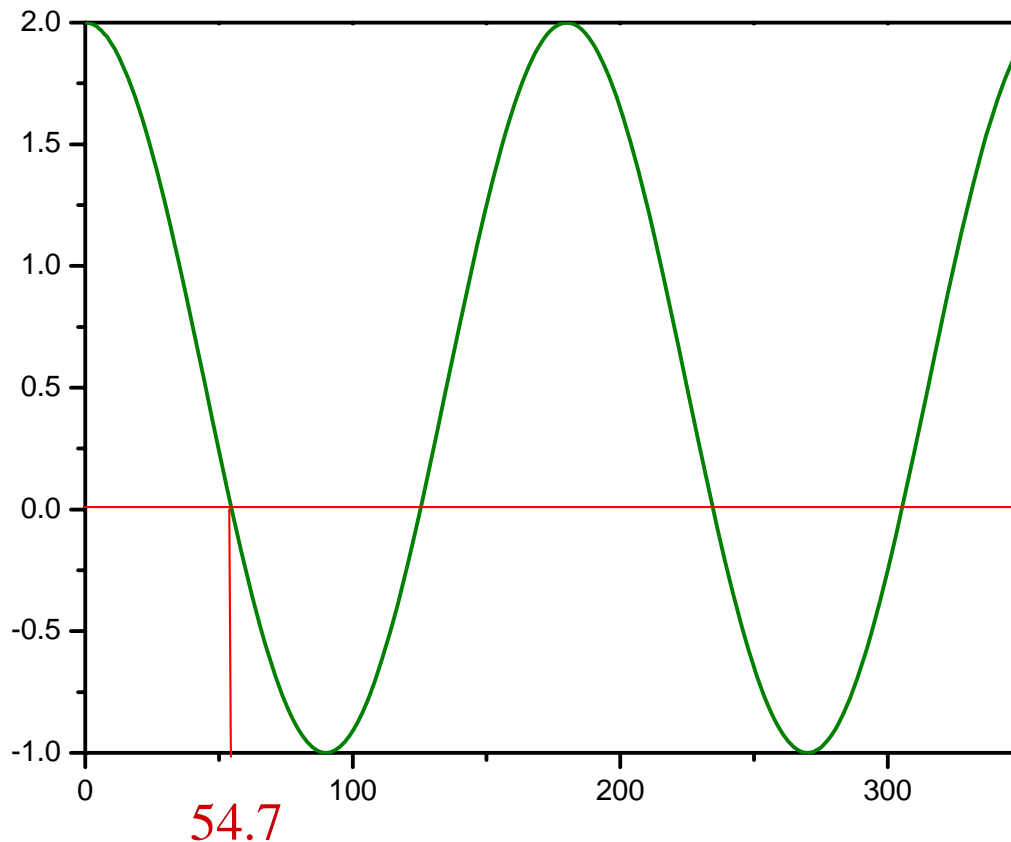
- Rotating the spins in a given powder
- Spins rotation: Manipulation by RF pulses
- Easier to Implement
- Difficult to visualise

Which Angle to Rotate at?

Spatial part of the anisotropic Hamiltonian

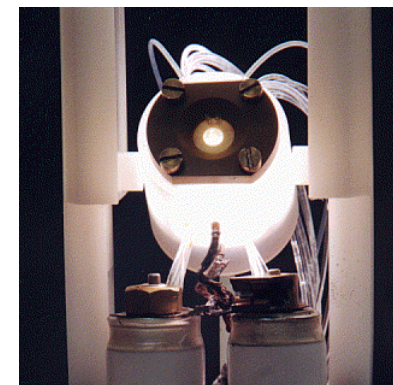
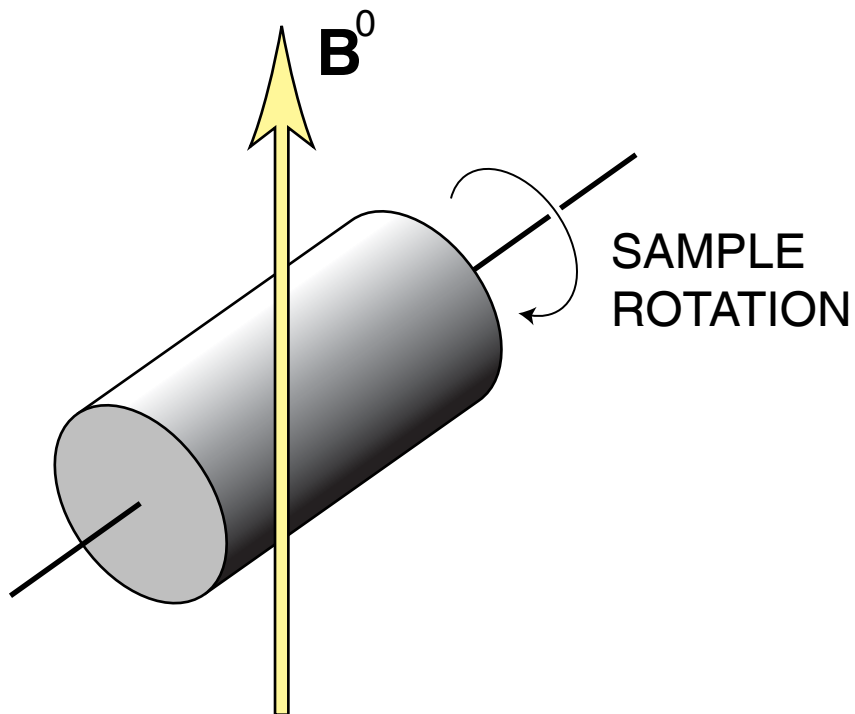
$$P_2(\cos \theta)$$

$$1/2(3 \cos^2 \theta - 1)$$



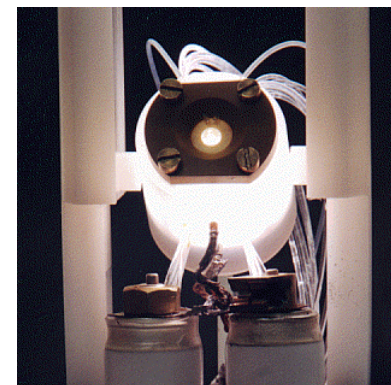
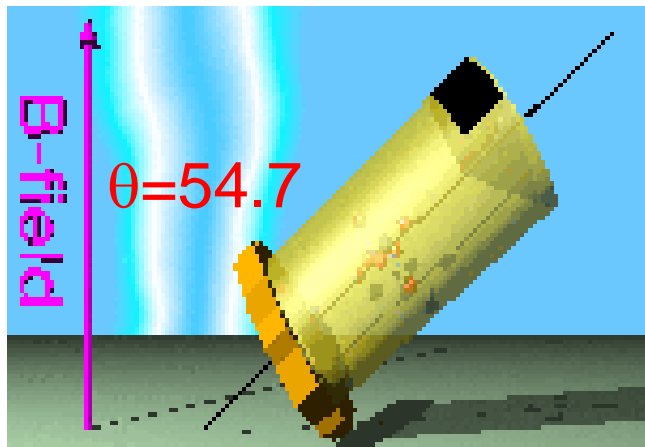
$P_2(\cos \theta) = 0$ for $\theta = 54.7^\circ$

Magic-Angle Spinning (MAS)



Average out the chemical shift anisotropy, to achieve good sensitivity and resolution

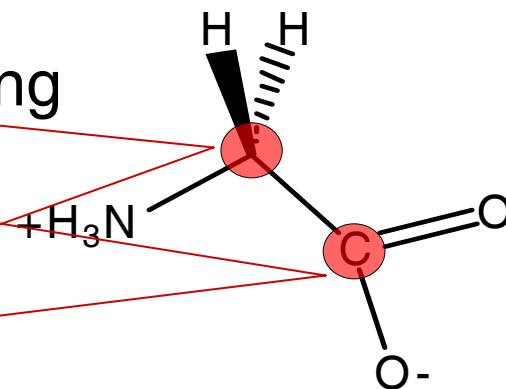
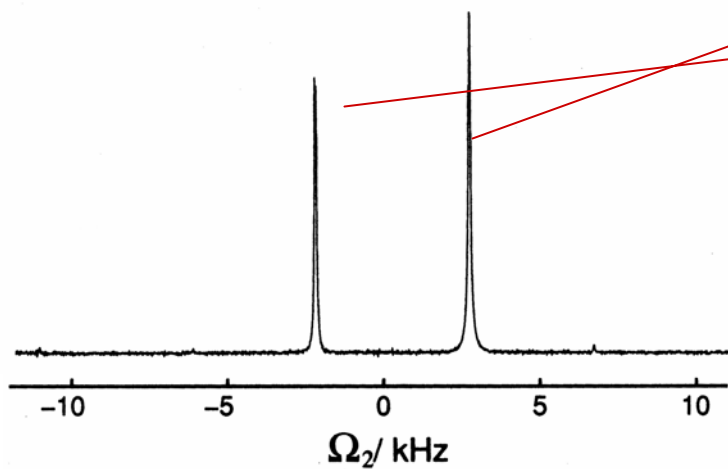
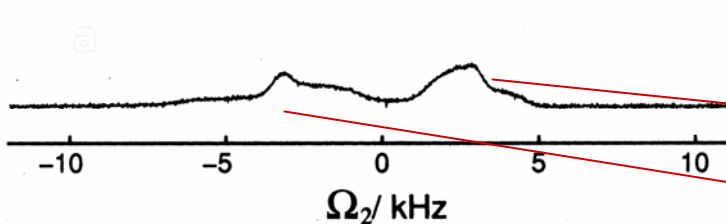
Magic-Angle Spinning (MAS)



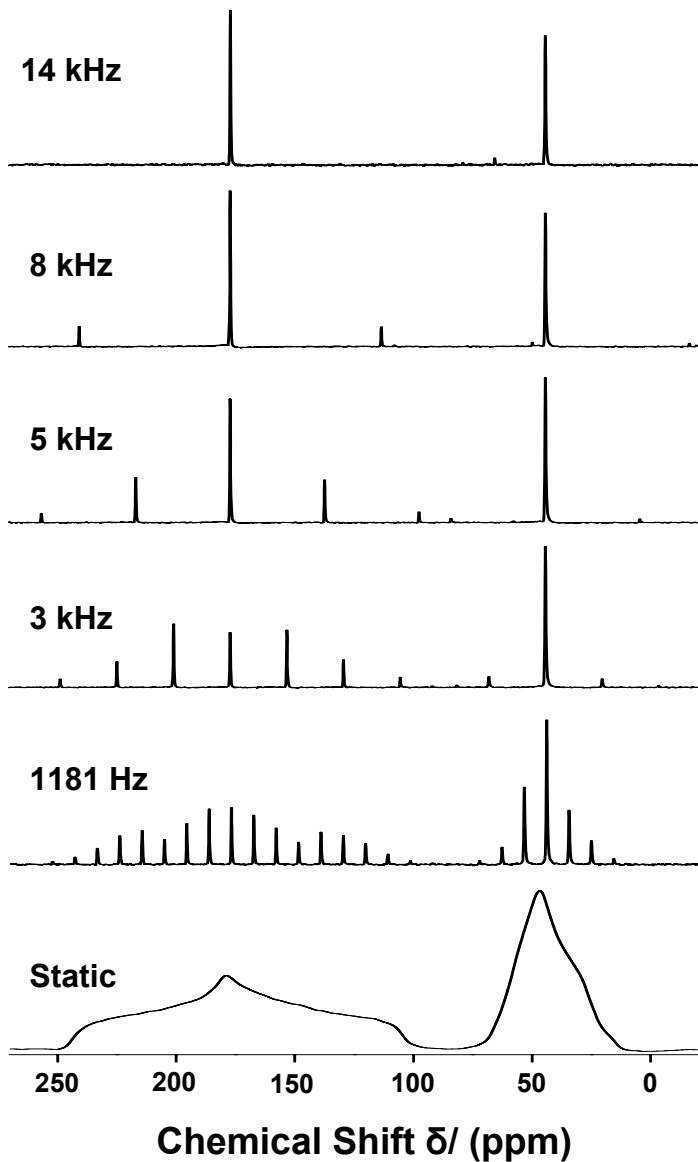
Averages out the chemical shift anisotropy, to achieve good sensitivity and resolution

Resolution and Sensitivity Enhancement by MAS

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



Magic-Angle Spinning Spectra: Resolution Enhancement



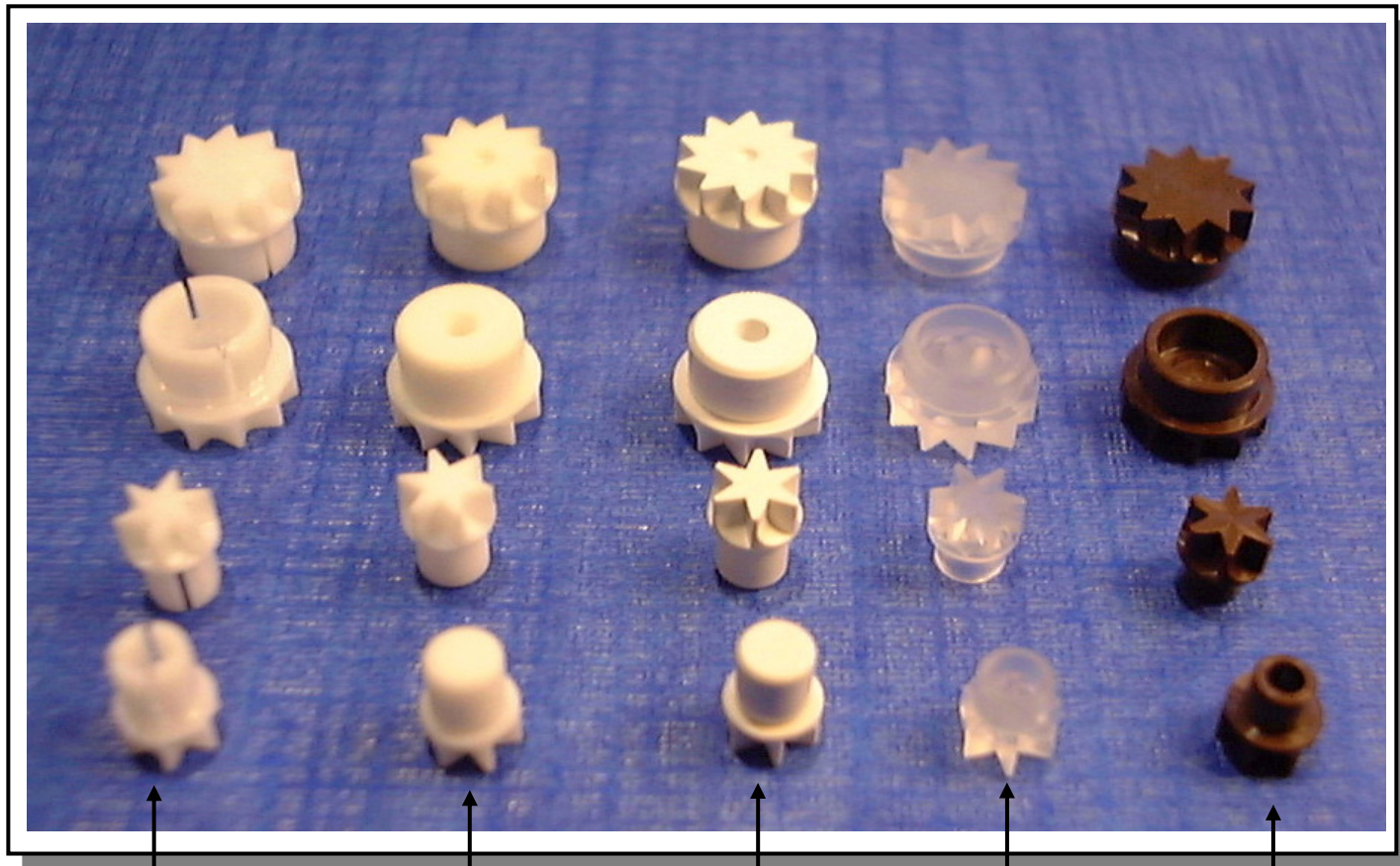
Glycine

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

MAS Rotor Types



Rotor caps



ZrO₂

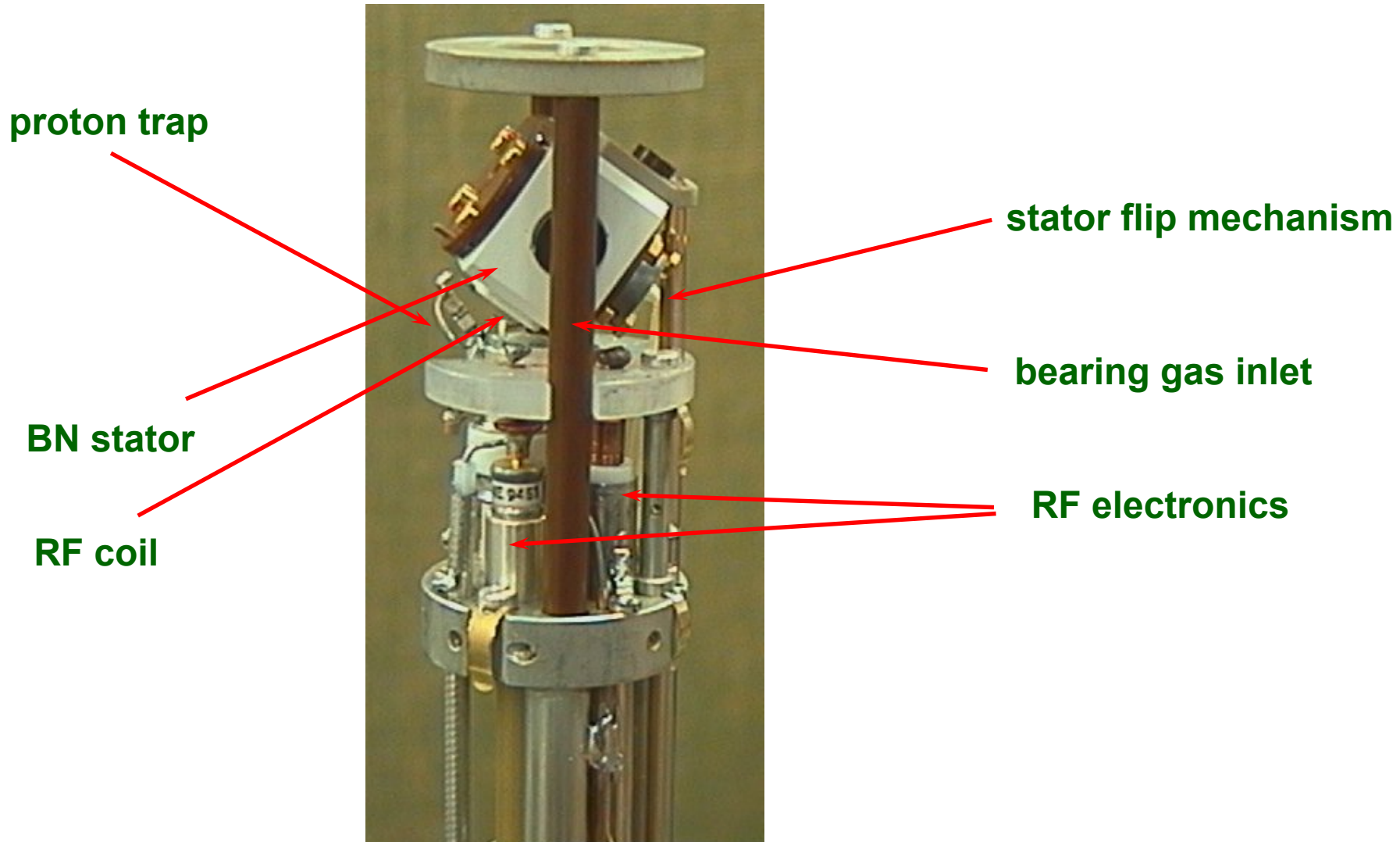
Macor

BN

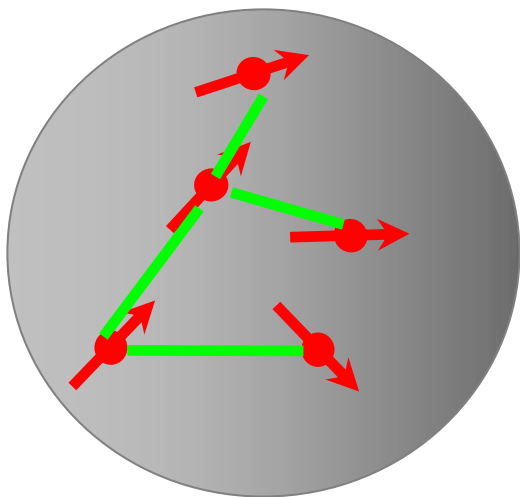
Kel-F

Vespel

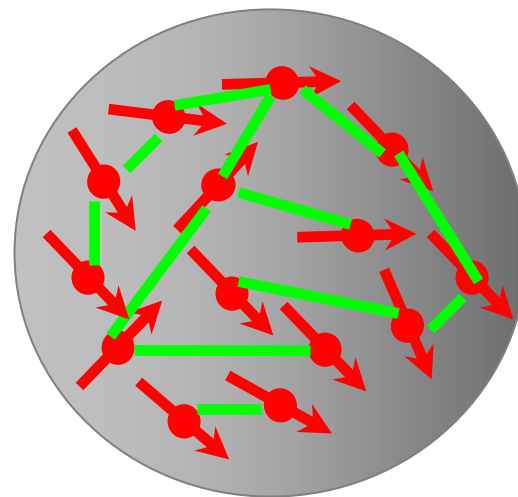
Standard Bore MAS Probe



Abundant and Rare Nuclear Spins



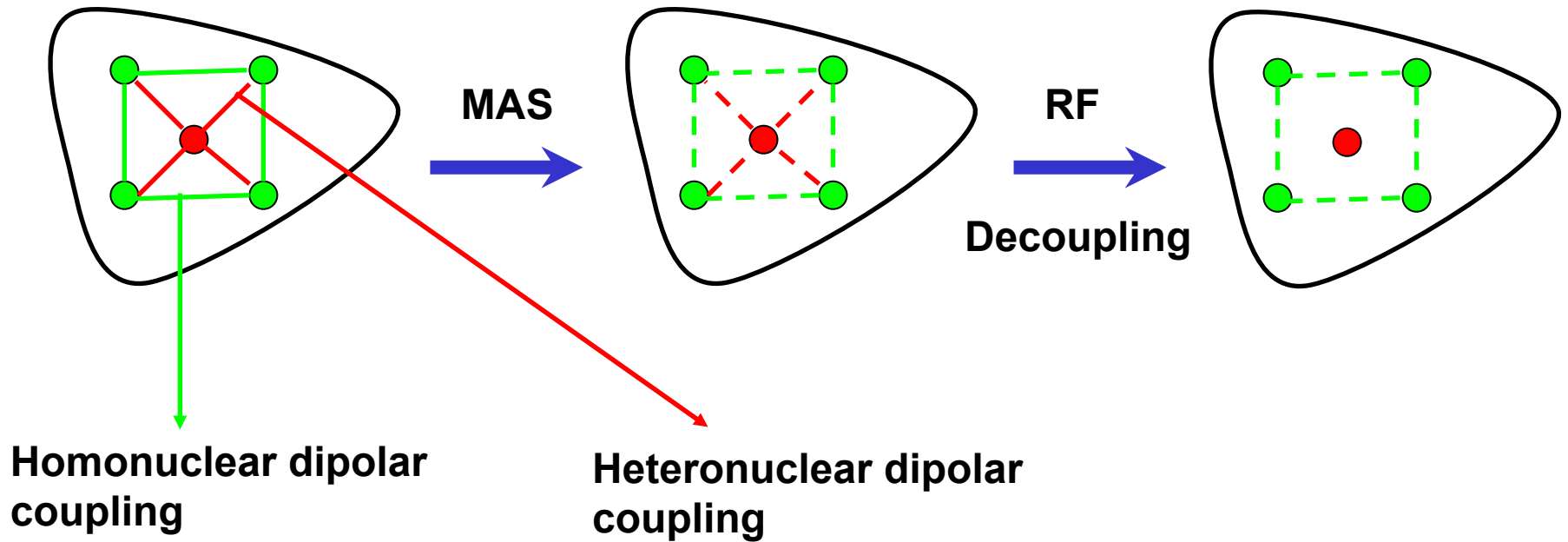
^{13}C , ^{15}N



^1H , ^{19}F

Rare spins experience weaker homonuclear dipolar couplings, hence, the resolution limiting aspect is the heteronuclear dipolar coupling to the abundant ^1H

Heteronuclear Dipolar Decoupling

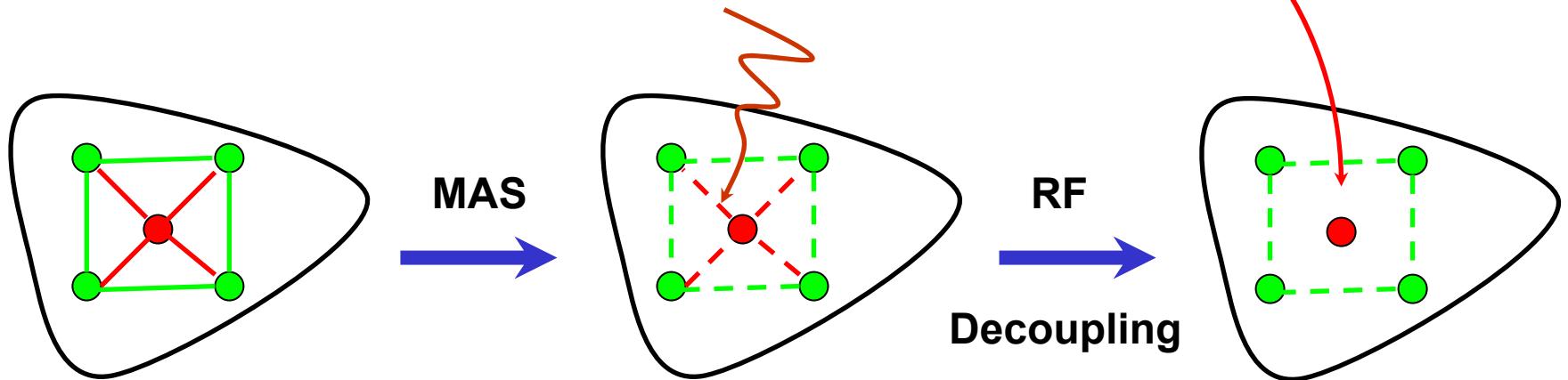
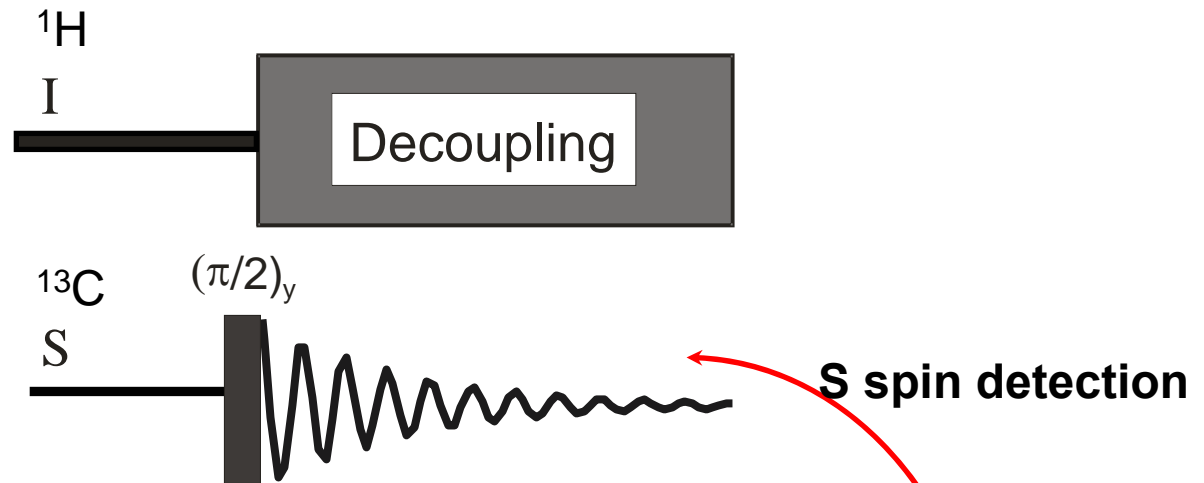


● Abundant spins
 ^1H

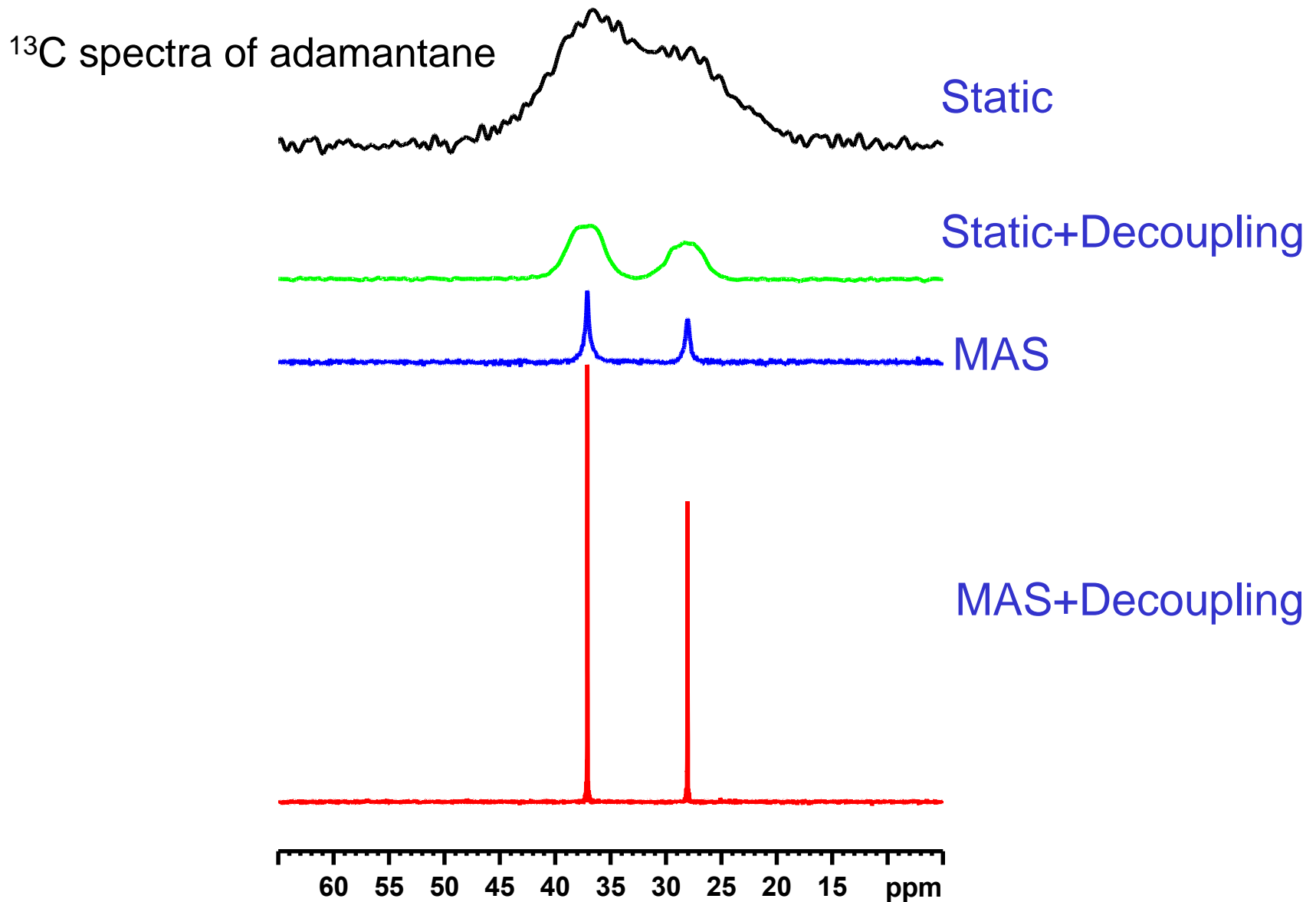
● Rare spins
 ^{13}C , ^{15}N

Typical ^1H - ^{13}C coupling = -25 kHz

Heteronuclear Dipolar Decoupling

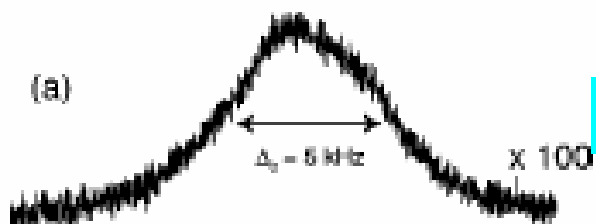


MAS + Heteronuclear Dipolar Decoupling



MAS + Heteronuclear Dipolar Decoupling

2-¹³C Glycine



5 kHz broadening



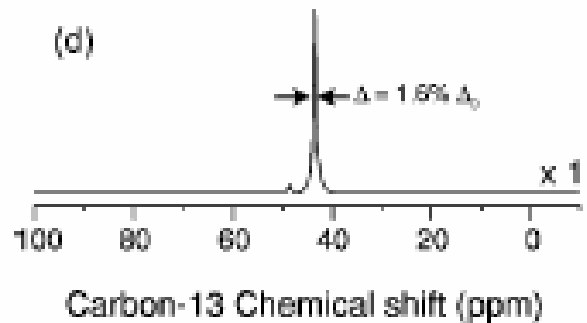
Only decoupling

CW decoupling at 150 kHz
MAS at 30 kHz



Only MAS

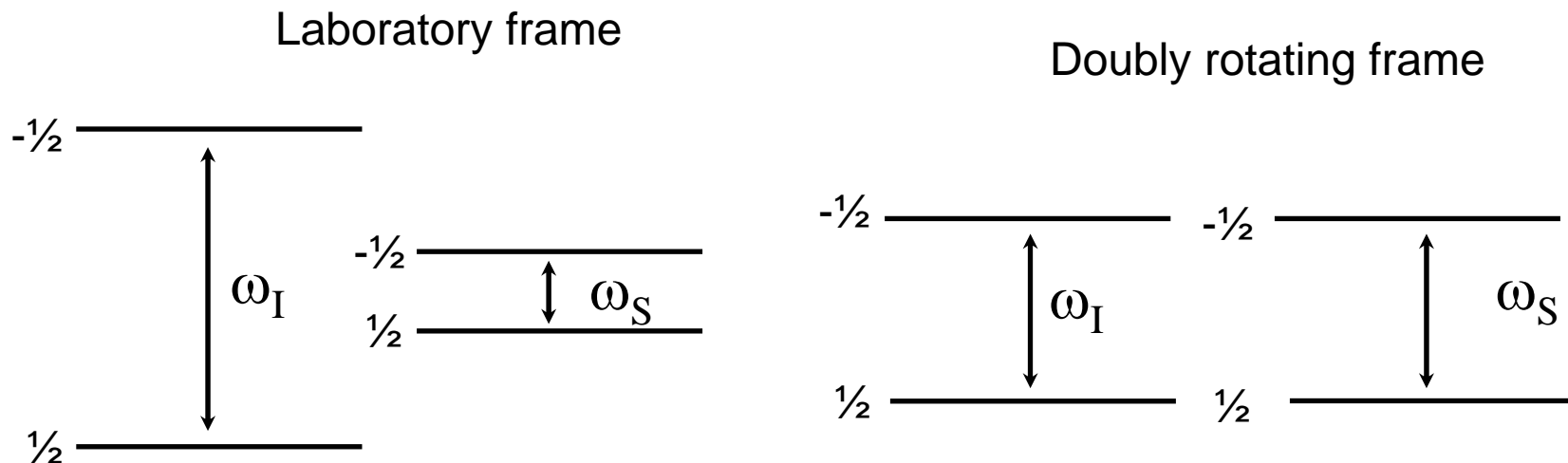
334 Hz broadening



MAS+Decoupling

80 Hz broadening

Cross Polarisation, CP



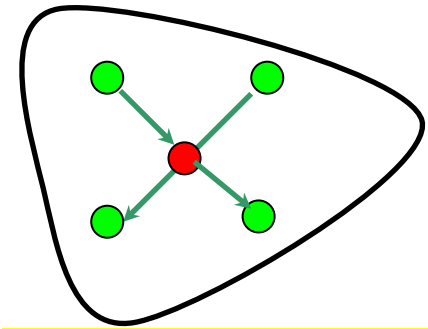
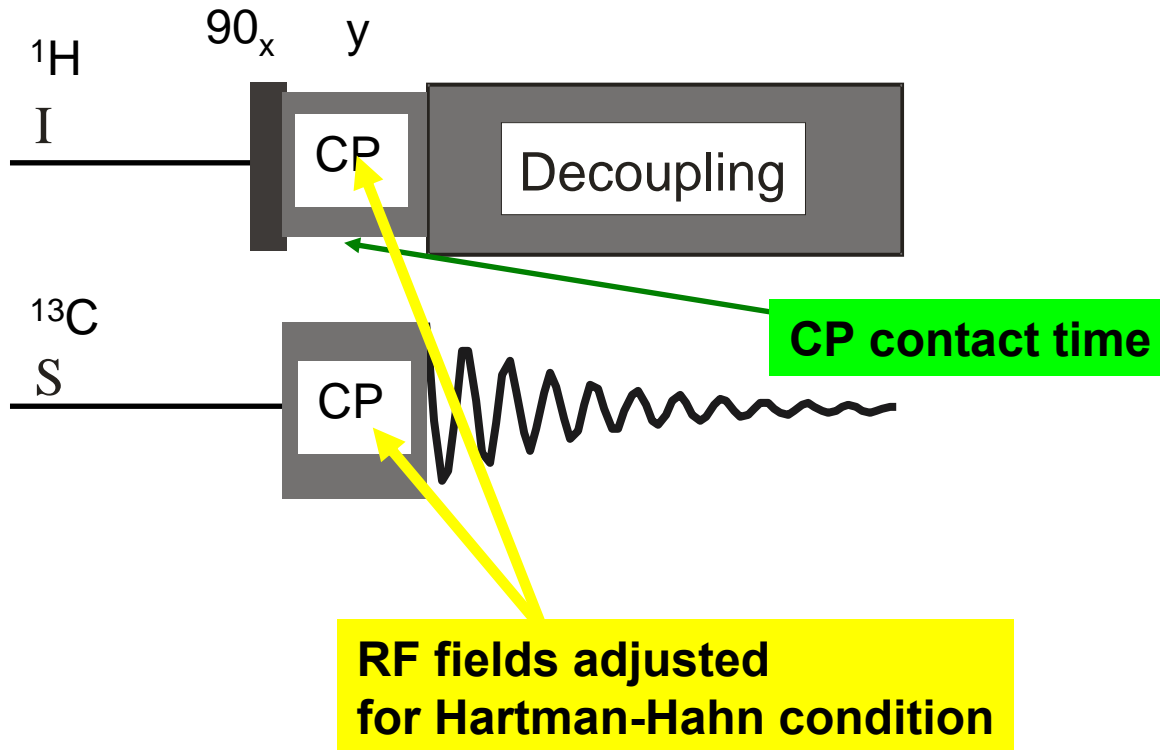
Energy levels of both nuclei are matched in the doubly rotating frame. A spin-lock RF field is equivalent to producing a rotating-frame transformation. Hence, we need a continuous spin-lock RF field on both the nuclei for CP.

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

CP Pulse Sequence+Decoupling

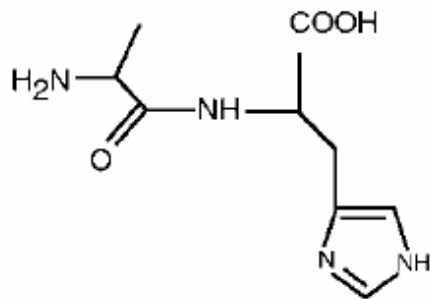
MAS and heteronuclear decoupling lead to resolution
CP leads to sensitivity



Magnetisation transfer

CPMAS, basic pulse block in solid-state NMR for both sensitivity and resolution

CPMAS Spectrum

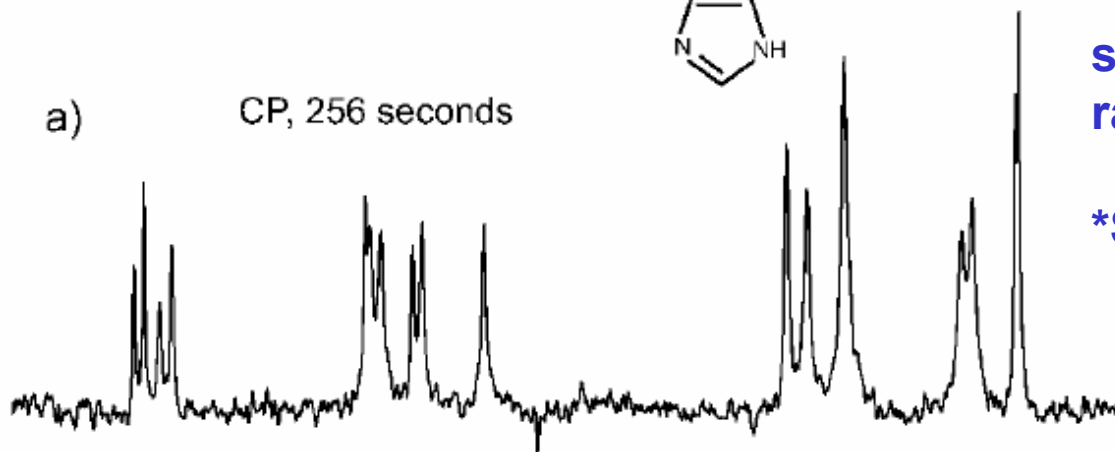


*Enhanced signal, $\sim \gamma_I / \gamma_S$

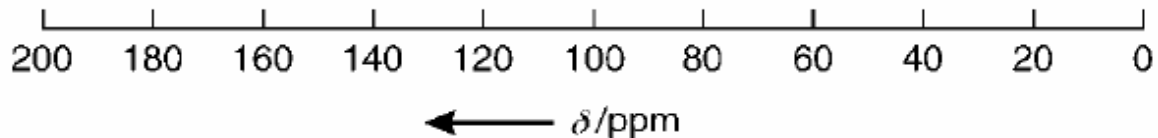
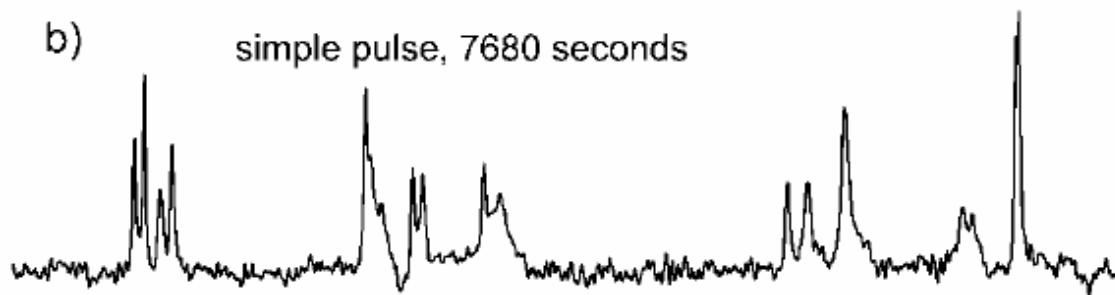
* T_1 of abundant high- γ nuclei shorter than that of the rare low- γ nuclei

*Spatial proximity

a) CP, 256 seconds

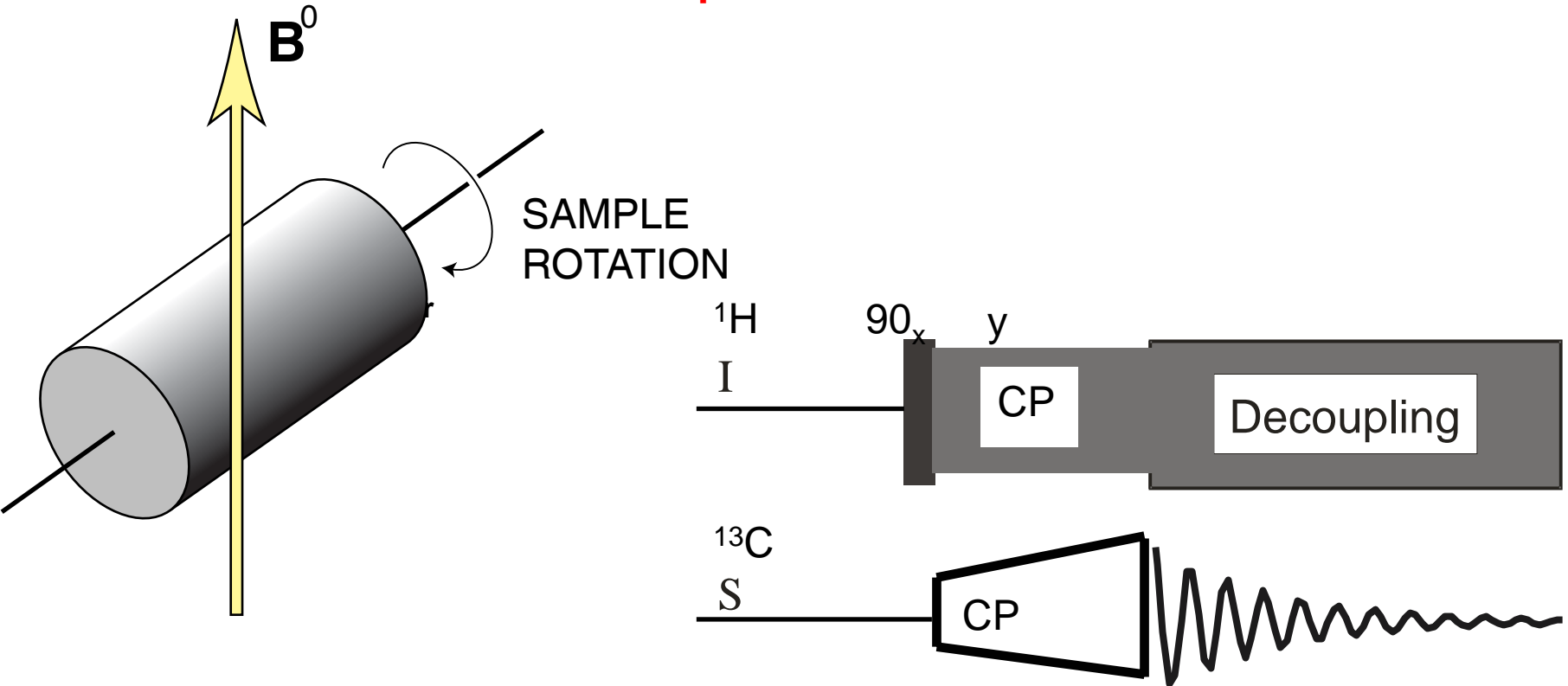


b) simple pulse, 7680 seconds



CPMAS

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

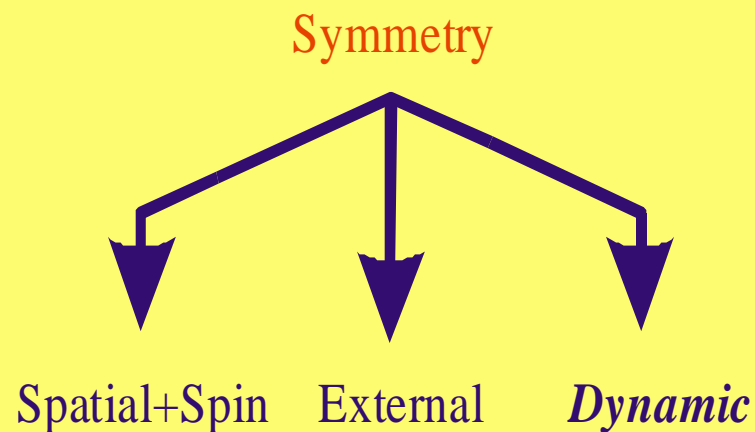
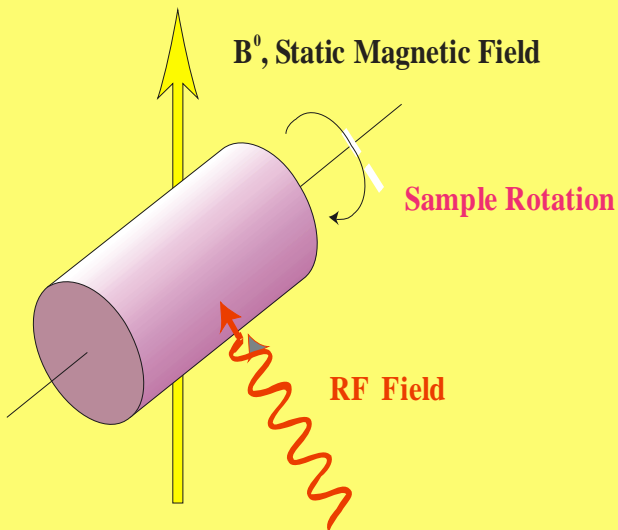


$$\theta = 54.7$$

Stejskal, Schaefer, Waugh, JMR, 18,560,1975

Stejskal, Schaefer, Waugh, JMR, 28,105,1977

SOLID STATE NMR



Electromagnetic irradiation \gg 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

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?

Solution-state NMR

Only isotropic information are
inherently present

Geometry information available
indirectly via relaxation experiments

Having the cake and eat it too!

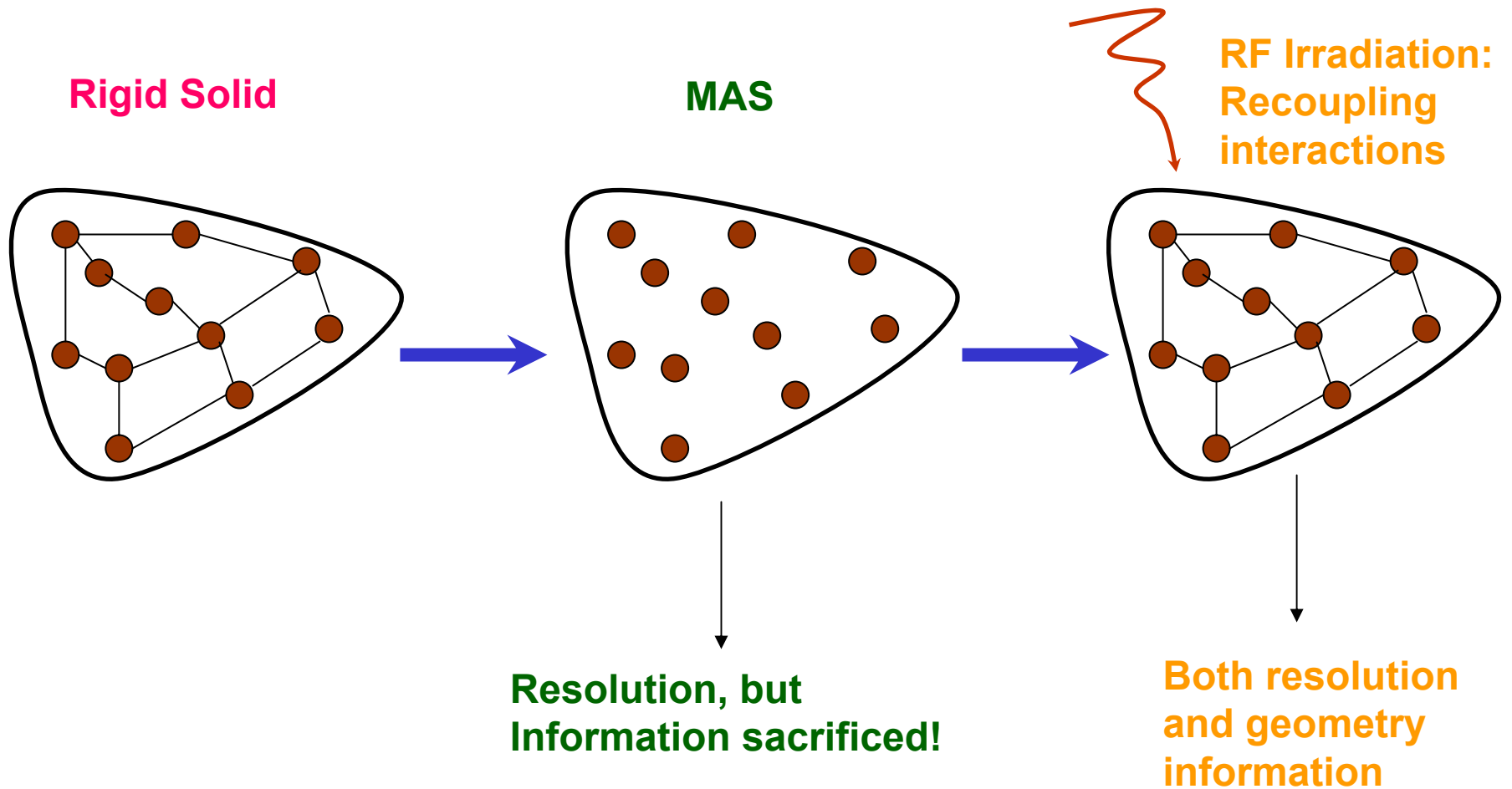
Recoupling in Solution State

Recoupling is done in solution-state NMR, NOE for example

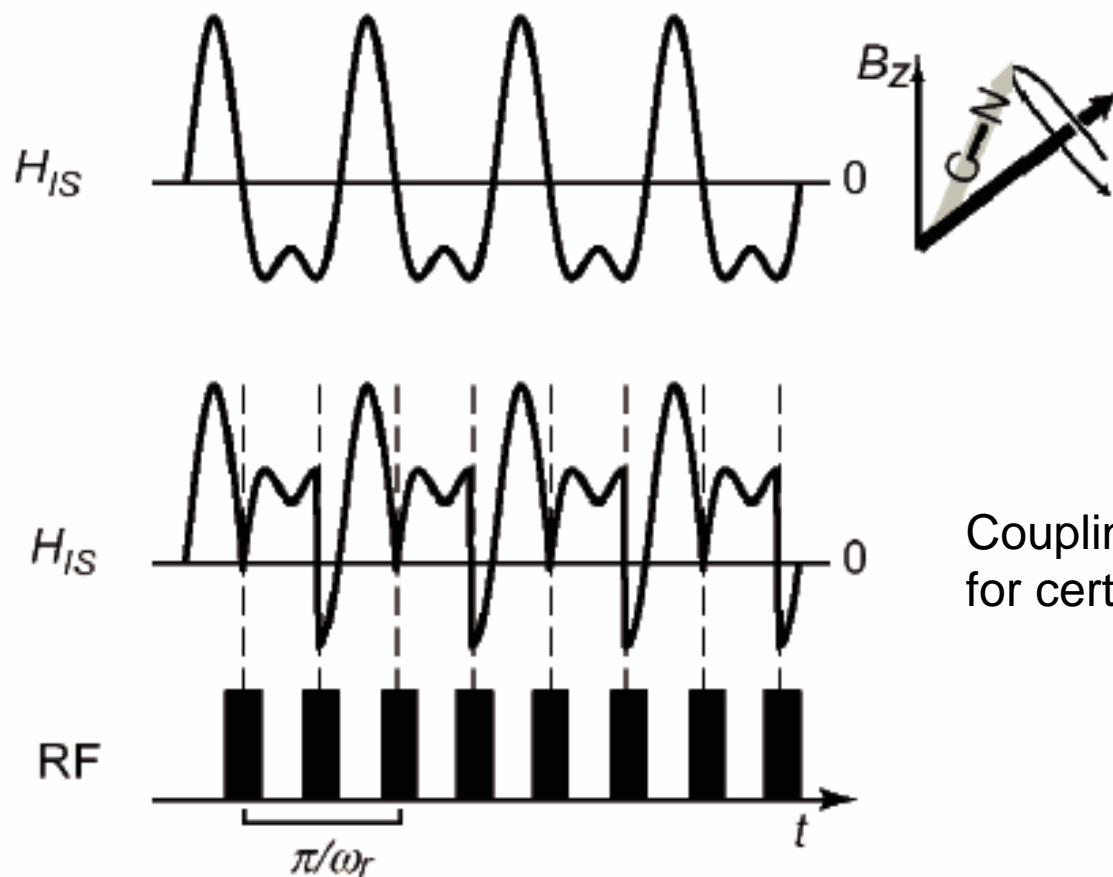
Rapid molecular motions modulate the dipole-dipole interactions and the fluctuating dipolar fields can drive magnetisation exchange (or cross relaxation) between spins over a wide range of chemical shifts

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



Hetero/Homonuclear Recoupling



Couplings may be reintroduced for certain periods of the experiment

Pay a price: A scale factor!

Sequence Zoo

CN_n^v

DARR

RIL

SEDOR

MELODRAMA

RN_n^v

USEME

BABA

HORROR

R^3

REDOR

DRAMA

DRAWS

SEDRA

R^2

RFDR

Rotational Resonance

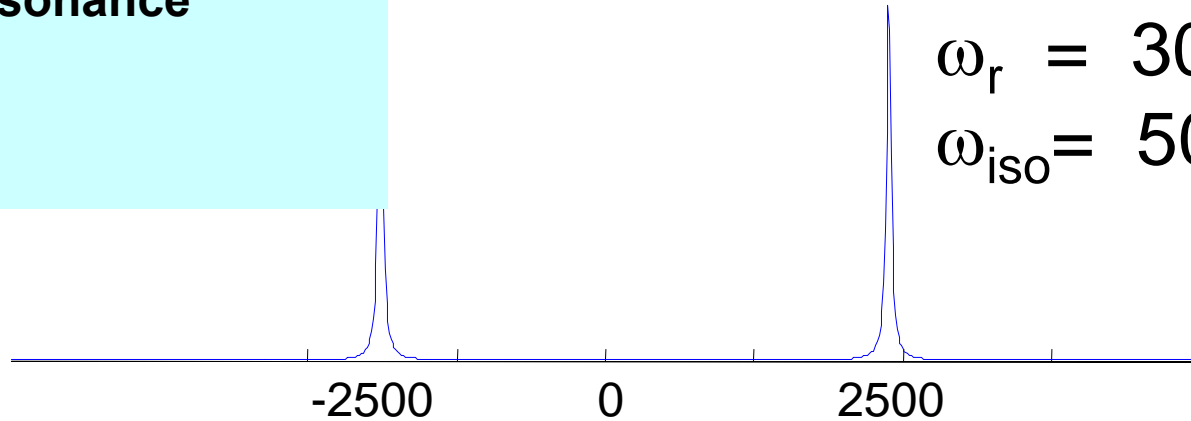
Rotational Resonance

Condition:

$$\Delta\omega_{\text{iso}} = n \omega_r$$

$$\omega_r = 3000 \text{ Hz}$$

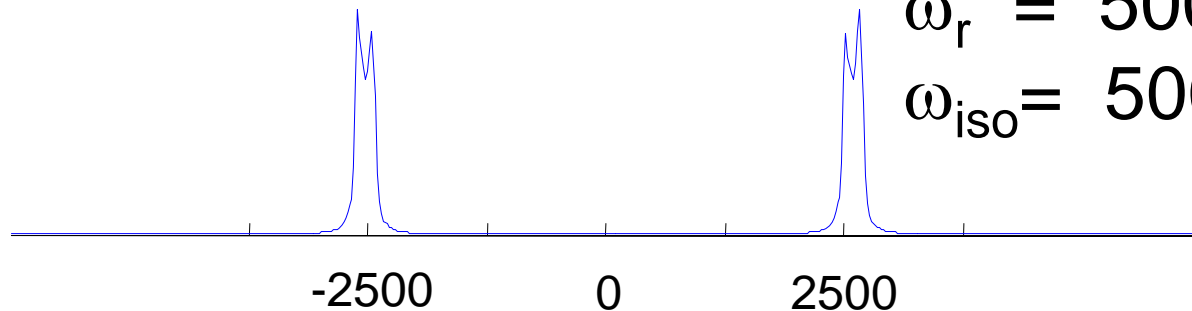
$$\omega_{\text{iso}} = 5000 \text{ Hz}$$



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

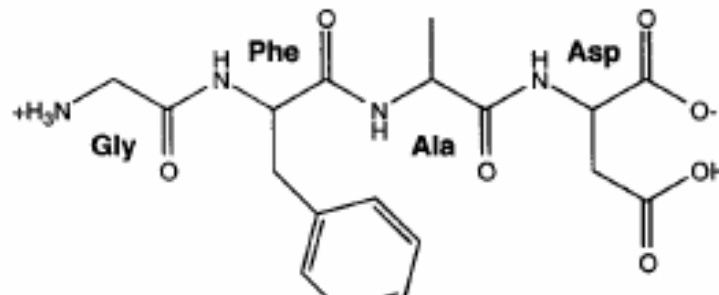
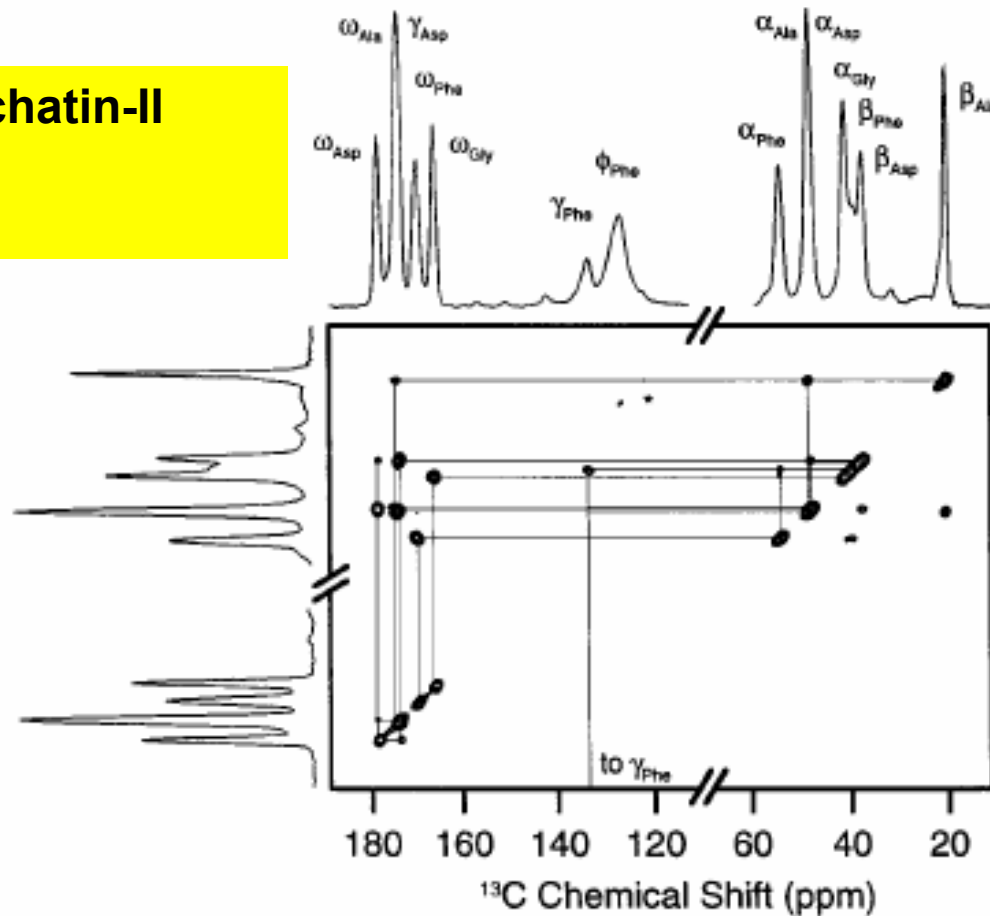
$$\omega_r = 5000 \text{ Hz}$$

$$\omega_{\text{iso}} = 5000 \text{ Hz}$$



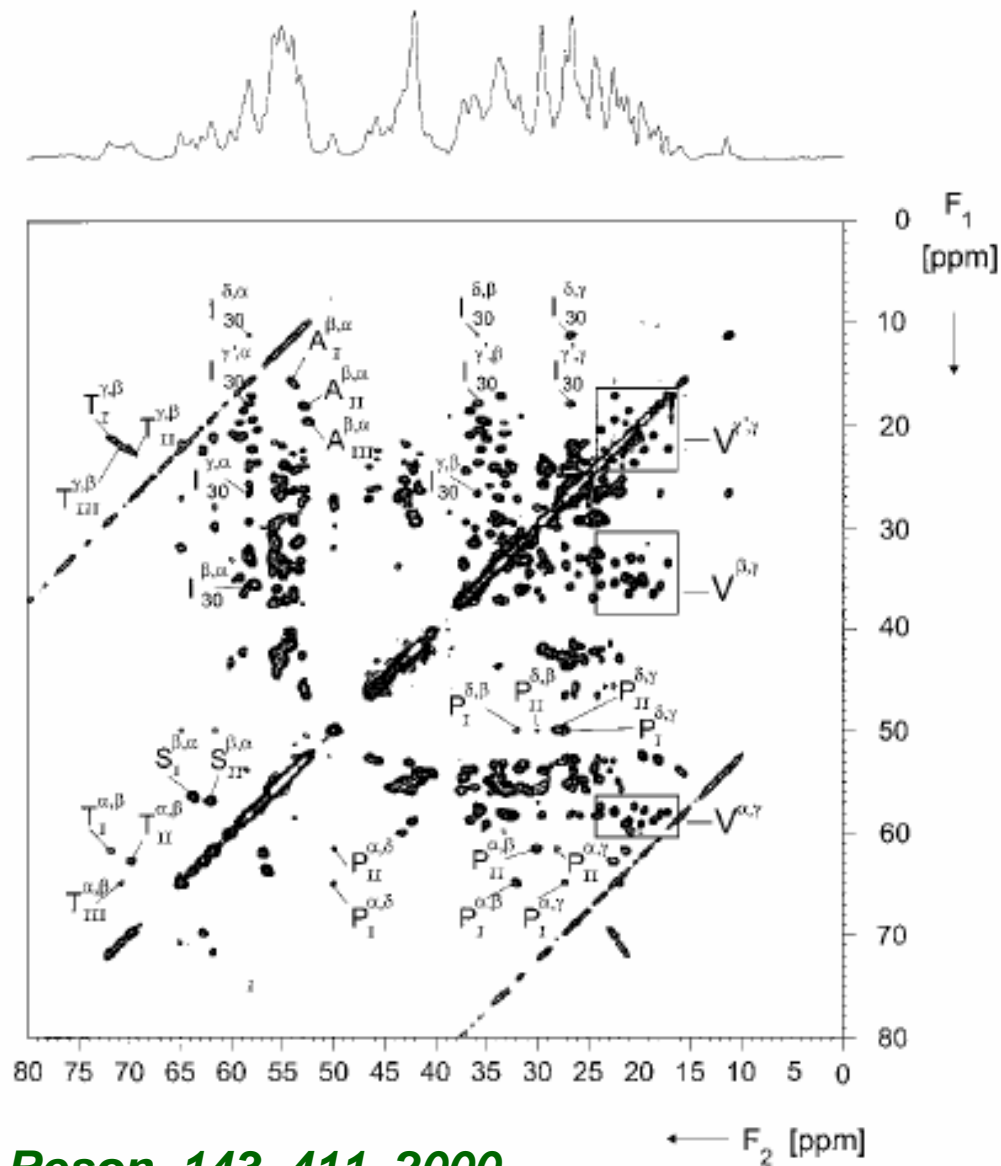
Correlation Spectrum :RFDR

[U-¹³C-¹⁵N]achatin-II
16 π pulses
MAS 10 kHz



Being used currently in biomolecules for ¹³C-¹³C correlation towards assignments

RFDR: SH3 Domain Protein



Secondary Structure Elements

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

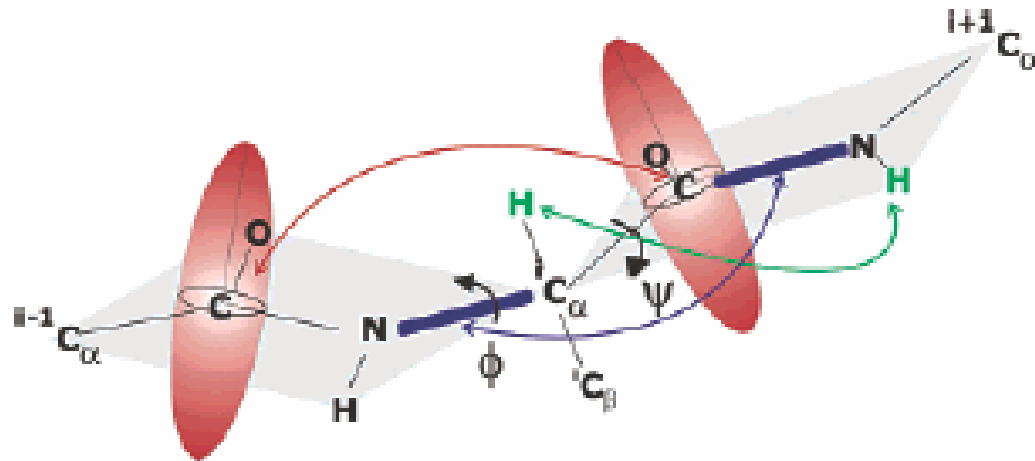
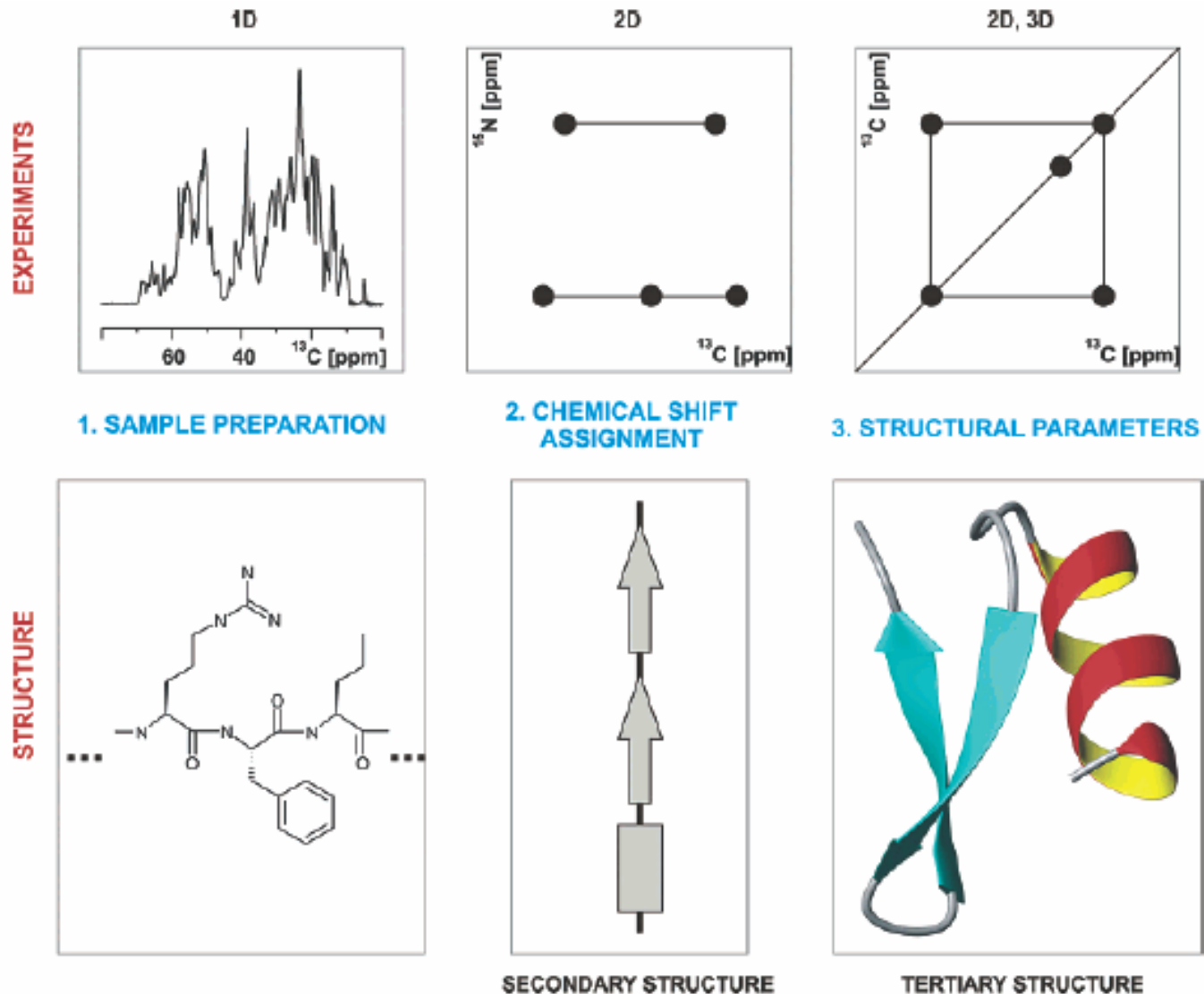


FIGURE 6. Alternative approaches to directly determine secondary structure elements: correlating dipolar tensors such as $(C_{\alpha},N)-(CO,N)$ (blue) or carbonyl CSA tensors (red) and measuring $H_{\alpha}(i)-H_N(i+1)$ distances (green).

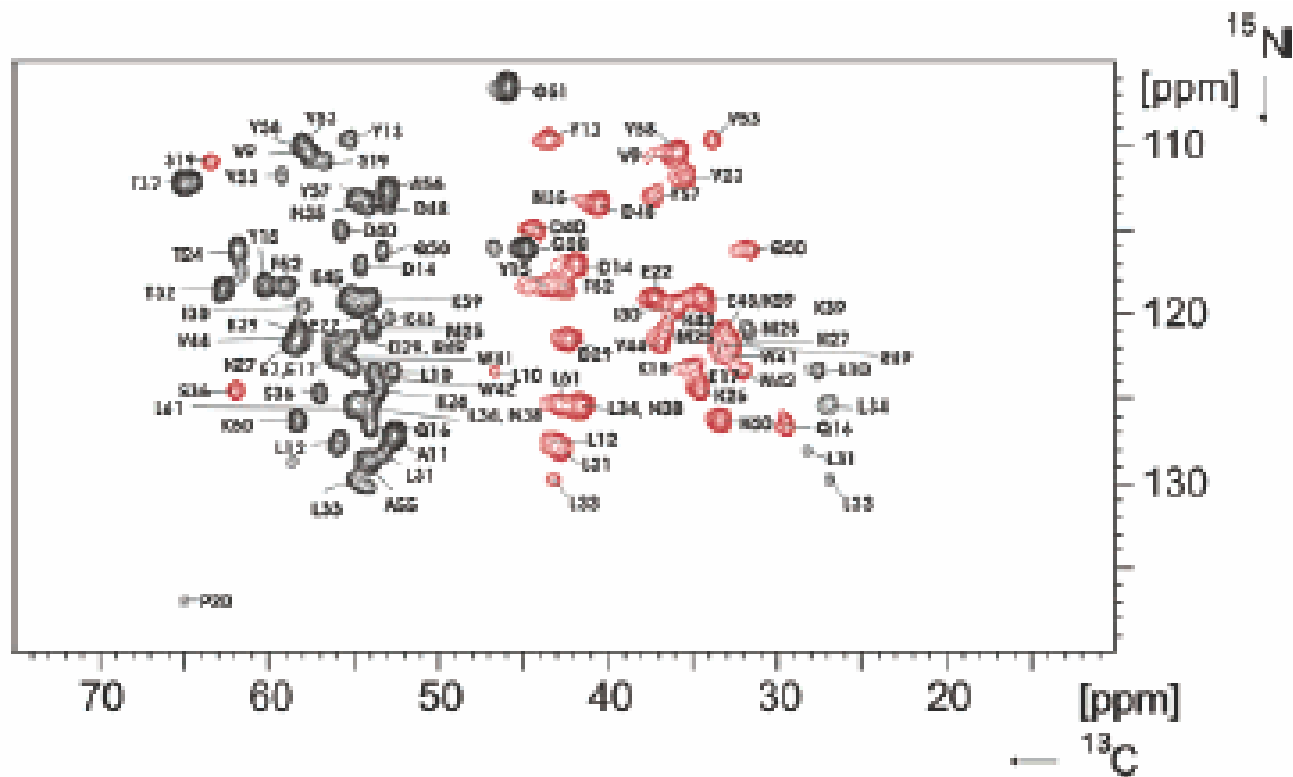
*Interactions measured from 2D experiments:
Recoupling, double-quantum methods*

Structure by Solid-State NMR: Schematic



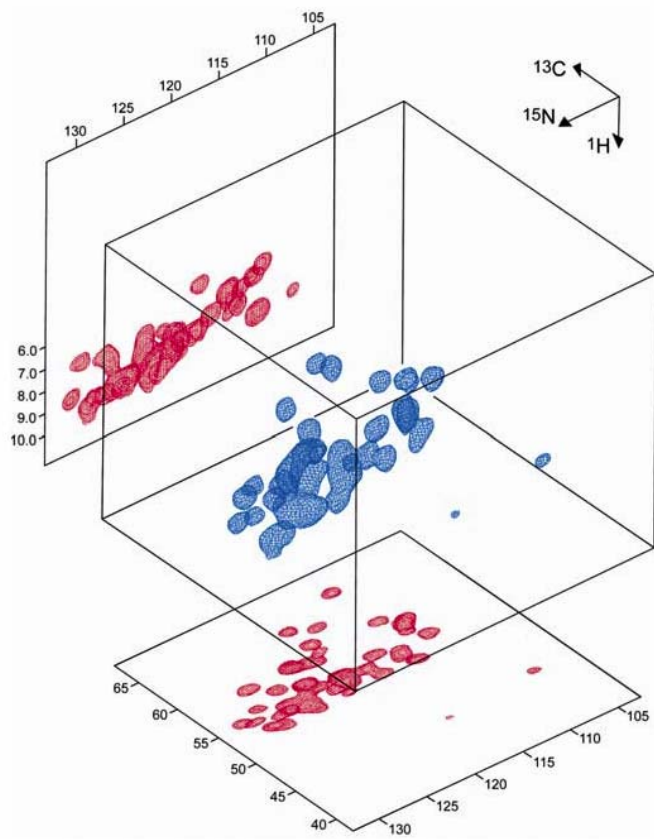
Assignments in Solid-State NMR: Example

α -spectrin SH3 domain



Correlation Experiments: HNCA in Solid-State

^{15}N - α -Spectrin SH3 domain



^1H - ^{15}N HSQC (dipolar). 17.6 T, MAS 8 kHz

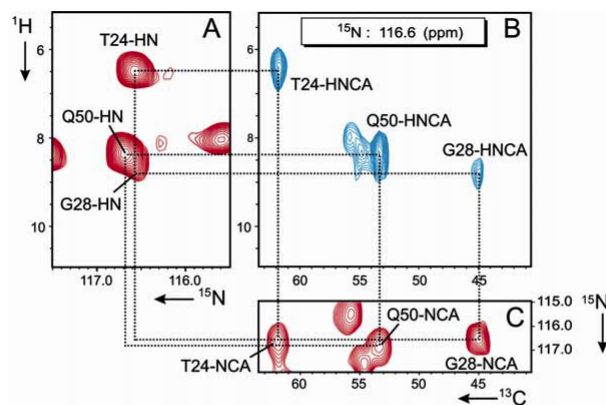
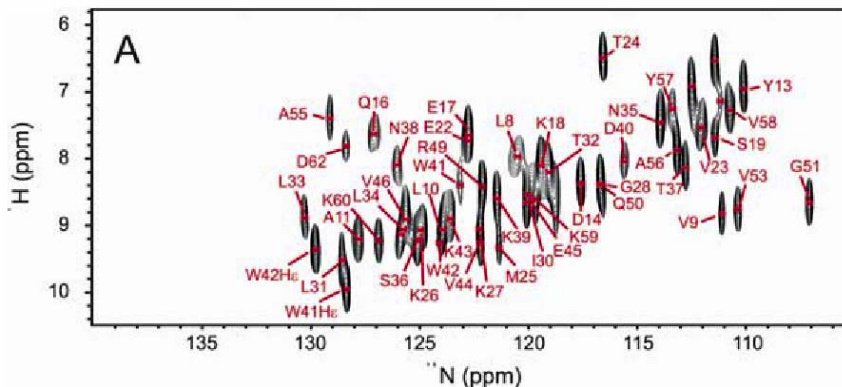


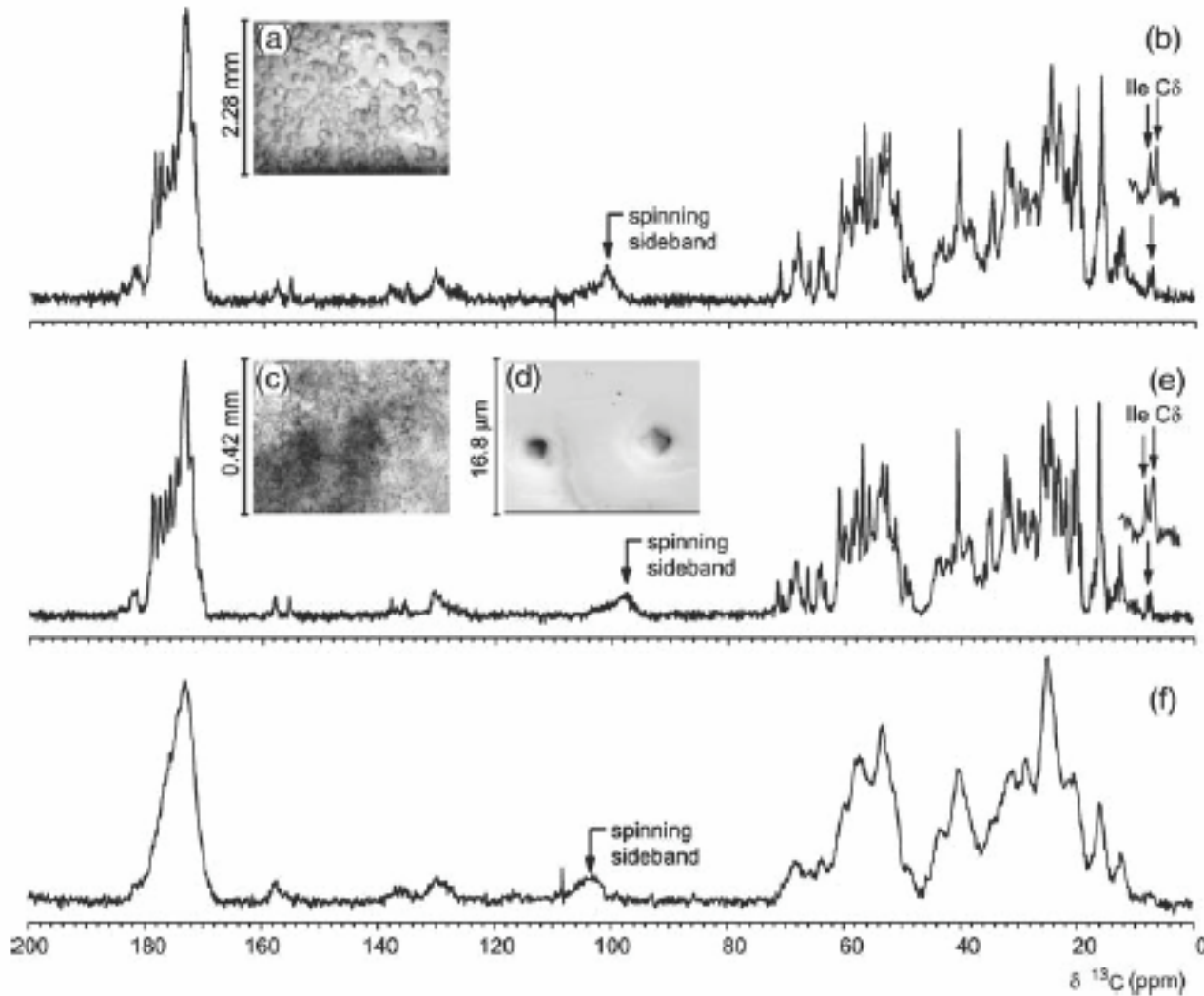
Figure 4. Assignment of the amides of T24, G28 and Q50. (A) shows a section of the 2D ^1H - ^{15}N experiment of Figure 2A, centred around the ^{15}N chemical shift of the three residues (~ 116.6 ppm). In (B), a plane from the 3D dataset is shown, extracted at the same ^{15}N chemical shift. Finally, (C) shows a strip from a 2D NCA experiment, recorded from (^{15}N , ^{13}C) α -spectrin SH3 domains at a field of 9.4 T and using a spinning frequency $\omega_{\text{R}}/2\pi = 8.0$ kHz.

^1H - ^{15}N - ^{13}C HNCA 9.4 T, MAS 8 kHz

J. Biomol. NMR, 25, 217, 2003

Sample Preparation Issues

Ubiquitin



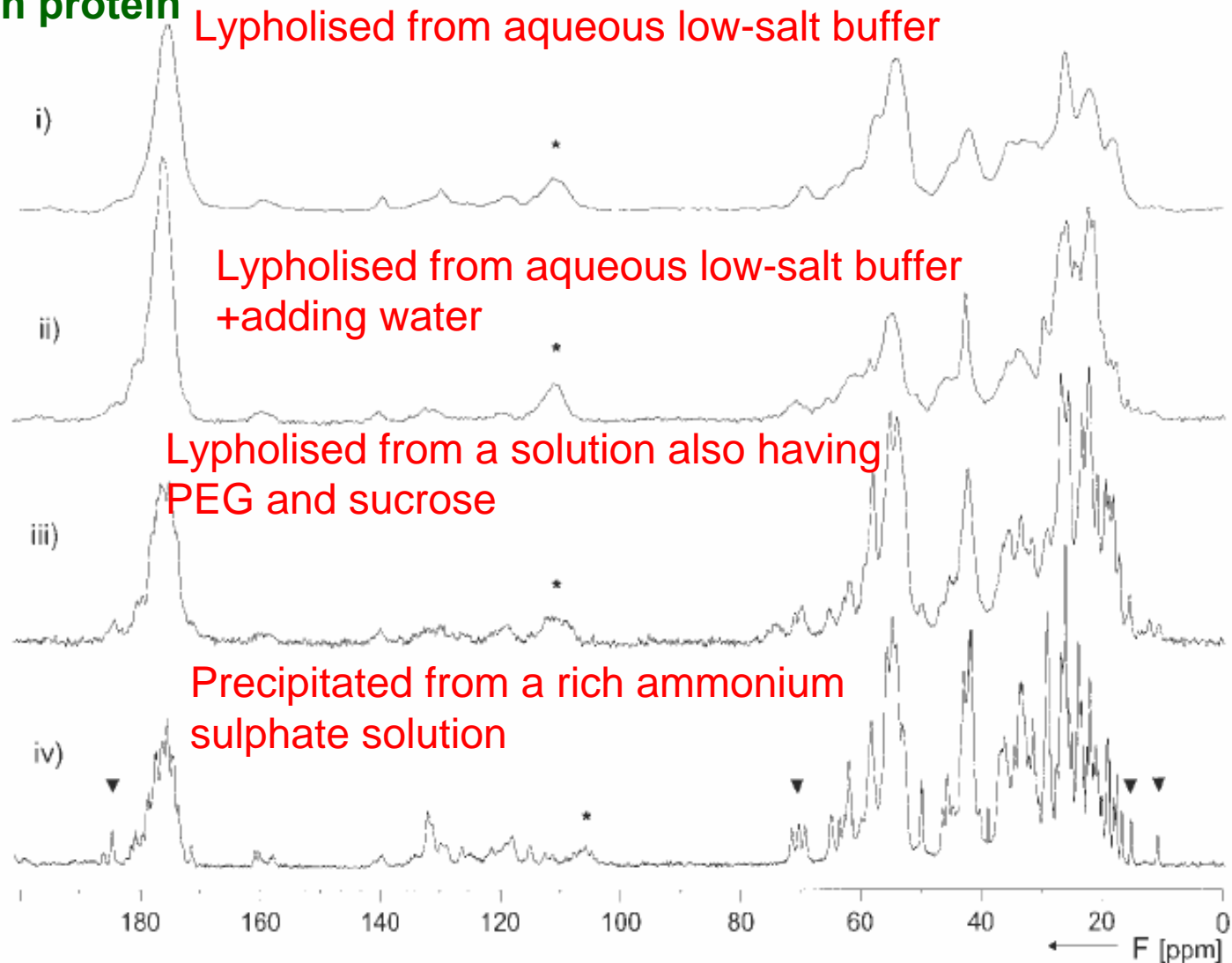
polycrystalline

nanocrystalline

lyophilised

Sample Preparation Issues

SH3 domain protein



Proteins in Solid-State NMR

Membrane proteins

Amyloid fibrils

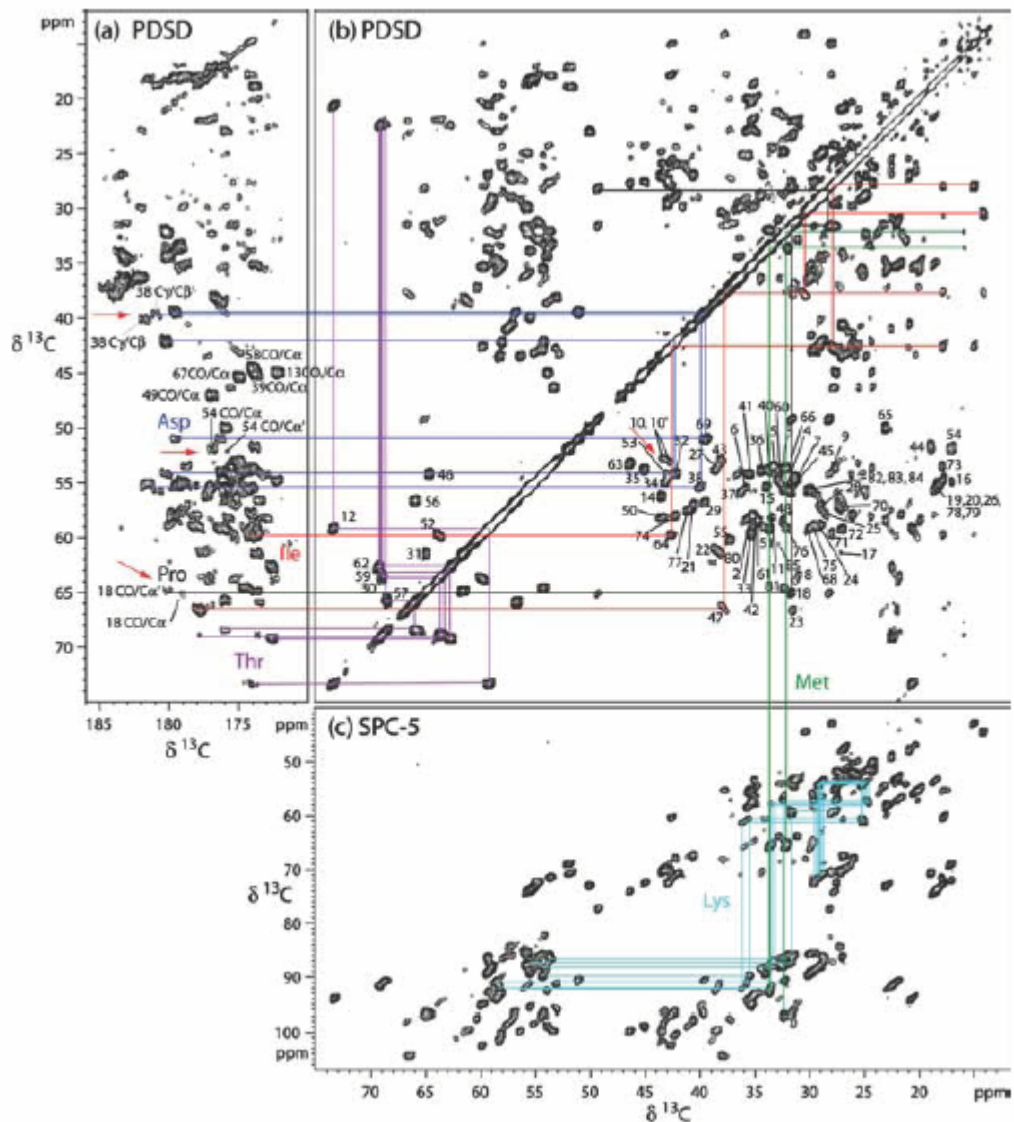
SH3 domain protein

Ubiquitin

Bacillus Subtilis protein Crh

α -Synuclein

Sequential Assignment and Conformational Analysis



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

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**