Experiment and Modelling in Structural NMR

November 28th – December 2nd 2011
INSTN – CEA Saclay, France

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3D modern techniques

[03002]

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Lectures

History, General principles	M. Goldman, CEA Saclay
Formalism and Interactions	H. Desvaux, CEA Saclay
Molecular Modelling	M. Nilges, Institut Pasteur
Anisotropy for Conformational studies	M. Blackledge, CEA Grenoble
Oriented media	D. Merlet, Univ. Paris-Sud 11
Techniques of solid-state NMR	D. Massiot, CNRS Orléans
NMR crystallography	F. Taulelle, UVSQ
3D Modern Techniques	N. Birlirakis ICSN - CNRS
Interactions ligand-macromolecules	G. Lippens, University of Lille
Bio-Solid State NMR	H. Oshikata, University of Berlin
Bioinformatics-protein assembly	R. Guérois, CEA Saclay
Materials Modelling & Simulations	C. Meis, INSTN - CEA, Saclay
DFT calculations of NMR parameters.	J. Yates, Oxford University
Inorganic Materials-Ceramics	S. Ashbrook, University of St Andrews
Amorphous materials	T. Charpentier, CEA Saclay
Hyper-polarization	G. De Paepe, CEA Grenoble
Fast NMR	N. Giraud, Univ. Paris-Sud 11
NMR Diffusometry : Dynamics in Complex Systems over many decades	R. Kimmich, University of Ulm

ORGANISATION

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A limited number of scholarships can be obtained for PhD students

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Winter-School

"Experiment and Modelling in Structural NMR"

The aim of the school is to provide basic understanding on the coupling of theoretical Modelling and Simulation methods to the Experiments in structural NMR, for biology and material science. It is composed of Seminars in the morning and Practical Training in the afternoon involving both the theoretical and experimental aspects.

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INSTN - CEA, Saclay

<http://www-instn.cea.fr> (events 2011)

3D NMR experiments applied to proteins

amino acid

peptide < 50aa < protein

Essential constituents of all living organisms

Linear chains consisting of 20 L-amino acids

Their folding to particular 3D structures triggers their biological function

Attractive targets for therapeutic applications once their structure-activity relationship is understood

General considerations

- Chemically synthesized molecules (SPPS)
- Isotopic labelling expensive → homonuclear ^1H - ^1H 2D experiments

Spectral assignment

- Starting point for magnetisation transfer: backbone NH protons (experiments in H_2O)
- Spin system identification via scalar couplings: **2D COSY**, **RELAYS**, **TOCSY**
- Sequential assignment: **2D NOESY**

Extraction of geometrical informations

- Dihedral angle ϕ determination from $^3J_{\text{HN-H}\alpha}$ couplings: 2D-COSY-DQF (Karplus equations)
- Interproton distance determination: 2D NOESY (build up with several τ_m)

General considerations

- Favorable T_2 relaxation
- Increased ^1H signal density → increased spectral complexity and peak overlapping (structuration dependent)

Spectral assignment and structure elucidation

- Same strategy as previously
- Addition of one more dimension to increase resolution: first 3D experiment **NOESY-TOCSY**, Nature **332**, 374 (1988)

Breakthrough

- Recombinant protein expression → affordable ^{15}N labelling (natural abundance 0.366%)

- The gene encoding the protein of interest is inserted to the plasmids
- Plasmids contain genes conferring resistance to antibiotics (selection)
- Plasmids are inserted to the bacteria (transformation)
- Use of rich media (LB) for cell replication
- Induction in poor media (M9) containing $^{15}\text{NH}_4\text{Cl}$
- Rare codons
- Protein folding and inclusion bodies
- Post-translational modifications

Heteronuclear 3D experiments

- Resolve the $^1\text{H-N}$ overlapping by the use of ^{15}N frequency : 4ppm versus 30-35ppm

Sensitivity considerations (S/N)

$$S \sim n A (1/T) B_0^{3/2} \gamma_{\text{obs}}^{3/2} \gamma_{\text{exc}} T_2 \sqrt{N_{\text{scan}}}$$

Cryoprobe technology: N_{d}

n: sample quantity (v*c)

A: active nuclei abundance

NOESY(TOCSY) - fHSQC

TOCSY mixing: use clean CSL schemes to suppress dipolar cross relaxation and NOESY/ROESY artifacts

Going from 2D to 3D

2D NOESY ($\tau_m = 150\text{ms}$)
4 scans

3D NOESY-HSQC H-H-N ($\tau_m = 150\text{ms}$)
8 scans

- Due to increased spectral complexity, peak assignment is difficult through 3D H-H-N TOCSY/NOESY-HSQC experiments
- Slow molecular tumbling results in rapid T_2 proton relaxation: COSY and TOCSY HN $\rightarrow$ H α magnetisation transfer fails especially in helical regions ($^3J_{\text{HN-H}\alpha} \sim 3\text{Hz}$)

- Recombinant expression of ^{13}C , ^{15}N labelled proteins using $^{15}\text{NH}_4\text{Cl}$ and $^{13}\text{C}_6\text{-glucose}$
→ access to triple-resonance experiments

- Resolve the $^1\text{H}\text{C}$ overlapping by using ^{13}CH frequency: 5ppm versus 65-70ppm for the aliphatics
- Use of heteronuclear couplings $^1\text{H}-^{15}\text{N}$, $^1\text{H}-^{13}\text{C}$, $^{13}\text{C}-^{13}\text{C}$, $^{15}\text{N}-^{13}\text{C}$ (^1J and ^2J) for unambiguous intra- and inter-residual assignment
- Magnetisation transfers implicating slowly relaxing nuclei

Water magnetization at z prior to WATERGATE module

Proton decoupled version (longer T_2 relaxation for ^{15}N , ^{13}C nuclei)

Constant time at ^{15}N dimension

$^1J_{\text{C}\alpha\text{C}\beta}$ couplings not refocused during t_2

$\Delta_1 = 1/4^1J_{\text{HN}} = 2.7\text{m}$

$\Delta_2 = 12\text{m}$ (relaxation optimized, $1/4 J_{\text{NC}\alpha} \sim 22\text{m}$)

Same sequence for HNCO experiment (via $\text{C}\alpha\text{-CO}$ channel interconversion)

- Shaped pulses
- Pulsed field gradients
- Water suppression

Rectangular hard pulses (polychromatic): $\gamma B_1/2\pi \gg \Delta F$
 Typical hard pulse lengths @ 800MHz, max admitted power:
 $^1\text{H} \rightarrow 7\text{--}10\mu\text{s}$
 $^{15}\text{N} \rightarrow 39.5\mu\text{s}$
 $^{13}\text{C} \rightarrow 14\mu\text{s}$

Amplitude modulation: first trials

Amplitude modulation: peak selection

90° Gaussian δ and J evolution during pulse:

Amplitude/phase modulation: band selection (top-hat profile)
Gaussian Cascade and BURP families

Amplitude/phase modulation: band selection (top-hat profile)

Gaussian Cascade family

- Universal pulses: Q5 (90°, 6.18) and Q3 (180°, 3.41/3.448)
- Excitation pulse: G4 (7.82)
- Inversion pulse: G3 (3.556)

BURP families

- Universal pulses: U-BURP (90°, 4.666) and RE-BURP (180°, 4.64/5.814)
- Excitation pulses: E-BURP1 (4.514) and E-BURP2 (4.952)
- Inversion pulses: I-BURP1 (4.498) and I-BURP2 (4.53)

Figure of merit (depends on the shape): $FM = \tau_p \Delta \Omega$

Frequency shifted (laminar) pulses: $\Delta \phi = 2\pi \tau_p \Delta F / k$

Advantages

- zoom into a precise spectral region
- suppression of undesired signals (solvent)
- speed-up experiments
- resolution increase
 - precise measurement of δ et J
 - refocusing of homonuclear couplings
- possibility to direct transfers
- possibility to treat an homonuclear system as heteronuclear

- Differentiation between $^{13}\text{C}_\alpha$ (or $^{13}\text{Caliph}$) and ^{13}CO regions

Use of rectangular pulses: $\tau_{180^\circ} = \sqrt{3} / 2\Delta\Omega$ and $\tau_{90^\circ} = \sqrt{15} / 4\Delta\Omega$

where $\Delta\Omega$ is the difference of pulse carrier and the first null point

Problems: inhomogeneous excitation/inversion and cryoprobe arcing if executed simultaneously high power ^{13}C and ^{15}N pulses

Use of universal top-hat pulses (or interleaved G4 and G4tr)

90° pulse comparison: 36μs square and 300μs G4

180° pulse comparison: 33μs square and 200μs Q3

ATTENTION
Offset-dependent
transient Bloch-Siegert
phase shifts in
homorefocusing

- Selective perturbation of ^1HN region (BEST experiments)
- Solvent selection (flipback or suppression)

- High field spectrometers → large ^{13}C spectral width in Hz → imperfect inversion/decoupling
→ use of adiabatic pulses: smoothed CHIRP and WURST families

180° pulse comparison: 28μs square and 500μs CA-WURST-4

Adiabatic decoupling:

Use P4M5 supercycle

Use bi-level decoupling for reduction of sidebands in ^1H - ^1H - ^{13}C and ^1H - ^1H - ^{15}N experiments

Homonuclear decoupling → offset dependent Bloch-Siegert frequency shifts

- Adiabatic ^{15}N inversion and decoupling is used to reduce power and thus probe heating, important in rapid scan conditions
- Adiabatic pulses are tolerant to Rf inhomogeneity
- Interesting pseudoadiabatic pulses: Bip and power-BIBOP for inversion and power-BEBOP for excitation

Linear variation of B_0 at a certain direction

- Coherence transfer pathway selection: $\sum \gamma_i p_i G_i = 0$
- Artifact suppression per scan ie $^1\text{H}^{12}\text{C}$ artifacts with short phase cycling (...subtraction)
- Solvent suppression and control of water magnetisation (radiation damping)
- *Translational diffusion measurements*
- *Magnetic Resonance Imaging*

gradient sensitivity enhanced HSQC (J_{SS} , T_{11} , T_{2S})

Strong water (~50M) signal deteriorates spectral dynamic range

High fields + cryoprobes → strong radiation damping → large water signal

Strategies

- Observe an heteroatom
- Presaturation: forbidden in the case of fastly exchangeable protons
- Coherence selection with gradients
- Return of water at z axis: polynomial sequences (jump and return...), water selective flip-back pulses, manipulation of radiation dumping
- Dynamic filters: based on the differences in relaxation rates or diffusion coefficients
- Selective defocusing of water via a gradient echo (WATERGATE, excitation sculpting)
- Purging pulses: B_0 or R_f gradient

INEPT version

$$\Delta_1 = 1/4 J_{HN} = 2.7\text{m}$$

$$\Delta_2 = 12\text{m (relaxation optimized, } 1/4 J_{NC\alpha} \sim 22\text{m)}$$

$$\Delta_3 = 3.5\text{-}4\text{m (relaxation optimized, } 1/4 J_{COC\alpha} = 4.5\text{m)}$$

mixed INEPT / DQ version

$$\Delta_1 = 1/4 J_{HN} = 2.7\text{m}$$

$$\Delta_2 = 13.5\text{m (relaxation optimized, } 1/4 J_{NCO} = 33\text{m)}$$

$$\Delta_3 = 8\text{m (relaxation optimized, } 1/2 J_{COC\alpha} = 9\text{m)}$$

mixed INEPT / RELAY sequence

$$\Delta_1 = 1/4 J_{HN} = 2.7\text{m}$$

$$\Delta_2 = 12\text{m (relaxation optimized, } 1/4 J_{NC\alpha} \sim 22\text{m)}$$

$$\Delta_3 = 3.6\text{m (relaxation optimized, } 1/4 J_{C\alpha C\beta} = 7.1\text{m)}$$

$^1J_{C\alpha C\beta}$ and $^1J_{C\beta C\gamma}$ couplings not refocused during t_2

$C\alpha$, $C\beta$ peaks of opposite sign

- In ^1H - ^{15}N correlation experiments ^1HN longitudinal dipolar relaxation is accelerated if the rest of the protein protons (aliphatics) and $^1\text{H}_2\text{O}$ are unperturbed (at z axis)
- Substitution of ^1H hard pulses with shaped ones (top-hat).

: PC9 pulse (flip-angle 120°, Ernst angle)
band-selective excitation pulse

: PC9 pulse (flip-angle 90°)
band-selective 90° pulse

: band-selective universal 180° pulse

: band-selective universal 180° pulse

SOFAST-HMQC

BEST-HSQC

90° pulse → 1770μs Q5.1000

¹H carrier frequency @ 7.999ppm

180° pulse → 1260μs Q3.1000

P5M4 ¹⁵N decoupling using 2ms CA-WURST-2 adiabatic pulse

1scan, 128 transients, AQ=70ms, RD=300ms, Total Experimental Time: 55s

█: 180° broadband inversion pulse → 155μs power-BIBOP pulse
10kHz inversion, tolerant to 20% inhomogeneity

BEST-HNCA: 4scans, 1h 53min

BEST-HNCO: 4scans, 58min

BEST-HNCACB: 4scans, 3h 54min

BEST-HN(CO)CA: 4scans, 2h

BEST-HN(CA)CO: 4scans, 59min

BEST-HN(CO)CACB: 4scans, 4h 9min

- Usefull for protonated proteins < 150aa (above, fractional deuteration is required)

- Other fast methods are based on reduced sampling at the indirect dimensions:

- Spectrum compression through folding

- Exponential sampling and maximum entropy

- Projection reconstruction (radial sampling)

- Hadamard transform

- Filter Diagonalisation Method

- Sharc NMR

- ...

Refocused INEPT / z-TOCSY

Constant time at both indirect dimensions

$\Delta 1 = 1/2^1J_{HC} = 37m$ (optimized for CH₂)

Purging of residual water magnetization with B₀ and RF gradients

- Decrease transverse ^{13}CH relaxation rates through deuteration:
 Expression in D_2O → up to 70% deuteration (isotopic effect)
 Expression in $\text{D}_2\text{O} + \text{U}[^{13}\text{C}, \text{D}]\text{-glucose}$ → up to ~100% deuteration
 Use of pyruvate or α -ketobutyrate/ α -ketoisovalerate for production of methyl protonated perdeuterated proteins.
 (*back-exchange of ND to NH, introduction of D decoupling*)
- Decrease transverse $^{15}\text{N}^1\text{H}$ relaxation rates through TROSY effect at high field:
 Selection of the slower relaxing component due to the destructive interference of cross-correlated relaxation (DD - CSA)

- At high fields transverse ^{13}CO relaxation rates increase due to CSA
 Use i-HNCA instead of HN(CO)CA