Experiment and Modelling in Structural NMR

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Biosolid state NMR

[03004]

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Bio-SolidState

Saclay, 30th of November 2011

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Solid-state NMR as a tool for 'difficult' areas of structural biology

Heterogeneous, filamentous systems:

- cytoskeleton-associated proteins
- protofibrils

'Dynamical complexes'

- f-actin and associated proteins
- focal adhesions
- full-length α B-crystallin

Membrane protein complexes in native lipid environments

- all states of one ABC-transporter

NMR on membrane proteins

Bilayers and liposomes:

Solid-state NMR

Micelles:

Solution NMR

Principles of solid state NMR to study biological membranes

Spin Hamiltonian given as:

$$H_{\text{total}} = H_Z + H_{\text{CS}} + H_J + H_D + H_Q$$

Zeeman interaction (H_Z)
 chemical shielding (H_{CS}),
 J-coupling (H_J)
 dipolar coupling (H_D)
 quadrupolar coupling (H_Q)

In solids: anisotropic contributions ($\langle 3\cos^2q-1 \rangle$ dependence); q is angle between the angle of the tensor element with respect to the applied field, B_0 .

For large ($M_r \gg 30k$) and slowly tumbling molecules ($t_r > \sim 10$'s kHz), with all orientations of the nucleus present at any one time, give rise to broad, powder-like NMR spectra which are an envelope of the individual orientational frequency dependent lines.

Anisotropy is often exploited in a biomembrane system

Experimental Observation

The Hamilton Operator of the Dipolar Interaction and the Dipolar Alphabet

The Hamilton Operator of the dipolar interaction for 2 particles with spin 1/2 is given by

$$\hat{H}_{IS}^{DD} = b_{IS} \left\{ \frac{3}{r_{IS}^2} (\vec{I} \cdot \vec{r}_{IS})(\vec{S} \cdot \vec{r}_{IS}) - \vec{I} \cdot \vec{S} \right\}$$

with $b_{IS} = -\mu_0 \frac{\gamma_I \gamma_S \hbar}{4\pi r_{IS}^3}$

$$\hat{H}_{IS}^{DD} = \mu_0 \frac{\gamma_I \gamma_S \hbar}{4\pi r_{IS}^3} \frac{3 \cos^2 \beta - 1}{2} (3I_z S_z - \vec{I} \cdot \vec{S})$$

$\gamma \equiv$ gyromagnetic ratio of the nuclei

$\hbar \equiv$ Planck's constant

$\mu_0 \equiv$ magnetic permeability

$r_{IS} \equiv$ distance between I and S.

Effects of magic angle spinning on solids

NMR Probes for solid state NMR

7mm i.d. sample rotor; 400MHz HXY probe

Practicalities of magic angle sample spinning

Sample contained in rotor 1.2 - 9mm diameter;
Usually fully hydrated; ~ 10 - 300nmols of sample of interest; Usually
labeled with ^{13}C , ^{15}N , ^2H , ^{19}F , etc

Physics of Dipolar Recoupling

$$H_D^{IS}(t) = \sum_{m=-2}^{+2} \omega^{(m)}(\Omega_{\text{CR}}) e^{im\omega_r t} T_{20}^{AB}$$

$$\tilde{H}_D^{IS}(t) = \sum_{m=-2}^{+2} \sum_{\mu=-2}^{+2} \omega^{(m)}(\Omega_{\text{CR}}) e^{im\omega_r t} \times d_{\mu 0}^{(2)}(\omega_{\text{rf}} t) T_{2\mu}^{AB} e^{-i\mu\phi}$$

M. H. Levitt et al. *J. Chem. Phys.* **92**, 6347 (1990).
X. Feng et al. *Chem. Phys. Lett.* **257**, 314 (1996).

Typical magnitude in ppm of magnetic interactions for various nuclei

Tab. 11.1 Typical magnitude of important interactions

Interaction	Nuclei	Typical magnitude
Chemical shift range	^1H	~ 15 ppm
	^{13}C	~ 200 ppm
	^{15}N	~ 200 ppm
Anisotropy of CSA	^1H	< 10 ppm
	^{13}C	< 140 ppm
	^{15}N	< 200 ppm
Anisotropy of one-bond dipolar coupling	^1H - ^{13}C	~ 22 kHz
	^1H - ^{15}N	~ 20 kHz
	^{13}C - ^{13}C	4.5 kHz
	^{13}C - ^{15}N	2 kHz
	^{15}N - ^{15}N	< 1 kHz
^1J coupling constant	^1H - ^{13}C	120–250 Hz
	^1H - ^{15}N	~ 90 Hz
	^{13}C - ^{13}C	30–60 Hz
	^{13}C - ^{15}N	~ 15 Hz

Magic angle spinning solid state NMR narrows anisotropically broadened spectra

^{13}C NMR

magic angle 54.7°

Static NMR spectrum

Anisotropy broadened
(in chemical shift/
dipolar couplings)

MAS NMR spectrum

Anisotropy averaged
by spinning
($\omega_r \sim 1 - 15\text{kHz}$)

Small ($M_r \sim 3\text{k}$) peptide in fluid lipid bilayers

Cross Polarization

Hartmann – Hahn matching: $\gamma_H B_{1H} = \gamma_P B_{1P}$

$$\frac{M_P}{M_{0P}} = f [\text{correlation time, dipolar coupling, contact time}]$$

Membrane protein structures by solid-state NMR

Samples and Strategy

Systems with short-range order can be investigated by solid-state NMR

Proteins in a
micro-crystalline state
e.g. SH3 domain

2D crystals of
membrane proteins
e.g. OmpG

Ligand /cofactor bound
to a receptor
e.g. NT II + nAChR

Amyloid fibrils

Reconstitution into lipid bilayers and two dimensional crystallisation by dialysis

LPR

1.0

3 k

- dialysis 6 days

'298 K'
400MHz

1.0

45 k

- dialysis 8weeks

LPR

0.75

45 k

- dialysis 8weeks

'298 K'
400MHz

0.5

45 k

- dialysis 8weeks

α -spectrin SH3 Domain

Barely soluble at pH 7

Precipitates in microcrystalline form by changing from 3.5 to 7

Protein structures by solid-state NMR

ASSIGNMENTS

**Proton-driven spin diffusion (PDSD) experiment
to detect ^{13}C - ^{13}C and ^{15}N - ^{15}N long-range
correlations**

t_{mix} : from 15 to 500
ms

Identification of the single amino acids

Chemical shift assignment

- Identification of the single amino acids

- Sequential assignment

- Proton assignment
Based on the ^{13}C and ^{15}N resonance assignment

Chemical shift assignment

- Identification of the single amino acids

- Sequential assignment

- → Protonless MAS NMR requires thus a better definition of the amino acid type prior to the sequential assignment

Resonance assignments using $u\text{-}^{13}\text{C}$, ^{15}N -labelled protein

^{13}C , ^{15}N Backbone assignments:

NCA	NCACX	(specific CP!)
NCO	NCOCX	
^{13}C -PDSD	^{15}N -PDSD

^{13}C Side chain assignments:

^{13}C -PDSD ^{13}C -RFDR

^1H (^{13}C) assignments:

^1H - ^{13}C FSLG/PMLG
3D ^1H - ^{13}C - ^{13}C FSLG-RFDR

^1H (^{15}N) assignments:

^1H - ^{15}N FSLG/PMLG
3D ^1H - ^{15}N - ^{13}C HNCA

NCO and NCA spectra of (U-¹³C, ¹⁵N) α-spectrin SH3 domains

17.6 T, 278 K,
12 kHz MAS

PDSD, NCOCX and NCACX spectra of (U-¹³C, ¹⁵N) SH3 domains

17.6 T, 278 K

17.6 T, 278 K
8 kHz MAS

[illegible]

2D ^1H - ^{13}C FSLG of ^{13}C - ^{15}N SH3 domain (11.5 kHz MAS)

Assignment of amide protons from 3D HNCA spectrum

400 MHz (9.4 T)

MAS : 8 kHz

T = 280 K

Solid-state NMR of full-length α B-crystallin

α -crystallins (α A-crystallin and α B-crystallin)
where discovered in 1896 in the eye lens

They are together a chaperone system for
 β - and γ -crystallin.

α B-crystallin is distributed ubiquitously, acts as a
sHSP and mutants are involved in many diseases.

(e.g., multiple sclerosis, Alzheimers disease, cardiomyopathie)

α B crystallin forms dynamic, polydisperse oligomers
of 24 – 32 subunits, ~600kDa, with dimers as basic building blocks

alpha-B crystallin is a small heat shock protein

Dataset/approach

3 Samples:

uniformly ^{13}C , ^{15}N

made from 1,3 - ^{13}C - glycerol

made from 2 - ^{13}C - glycerol

With help from Ponni Rajagopal,
Klevit Lab in Seattle!

- 3D NCACX (N_iC_i), NCOCX (N_iC_{i-1})
- 3D NCACB (N_iC_i), NCACBCX (N_iC_i)
- 2D ^{13}C - ^{13}C correlations (PDSD)
- J-decoupling
- Methyl-Filtering

Structure of alpha-B crystallin in functional oligomers

Intermolecular N- and C-terminal interactions are responsible for oligomerisation

Summary of structural constraints and structure statistics	
Number of restraints (per monomer)	
Distance restraints	
Intra-monomer	517
Intra-residue ($ i-j = 0$)	3
Sequential ($ i-j = 1$)	129
Medium-range ($2 \leq i-j \leq 5$)	88
Long-range ($ i-j > 5$)	168
Ambiguous	129
Inter-monomer	26
Total	543
Dihedral angle restraints (ϕ/ψ)	135 (68/67)
Hydrogen bonds restraints (intra/inter)	42 (38/4)
Restraints statistics [†]	
Number of distance violation > 0.5 Å	0 $\pm$ 0
Number of distance violation > 0.3 Å	0.2 $\pm$ 0.6
RMS of distance violations	0.021 $\pm$ 0.002 Å
Number of dihedral violation $> 5^\circ$	0 $\pm$ 0
RMS of dihedral violations	0.960 $\pm$ 0.112°
Structural quality [†]	
Ramachandran statistics [†]	
Most favoured regions	89.4 $\pm$ 2.7 %
Allowed regions	9.4 $\pm$ 2.8 %
Generously allowed regions	0.7 $\pm$ 1.2 %
Disallowed regions	0.4 $\pm$ 0.7 %
WHAT-IF Z-scores	
Backbone conformation	-2.19 $\pm$ 0.73
2 nd generation packing quality	-0.90 $\pm$ 0.37
Ramachandran plot appearance	-0.09 $\pm$ 0.25
χ^1/χ^2 rotamer normality	-2.83 $\pm$ 0.91

SAXS investigations

An oligomer model can be reconstructed, assuming tetrahedral symmetry

pH modulation of the chaperone binding site

Solution NMR of flexible residues

SAXS measurements at different pH

Conclusions

The structure suggests that the activity of α B-crystallin is tightly connected to its oligomeric state. An intact oligomer should not be active.

structural changes at pH 6.5, 9.0 and 4.5 suggest regulation by pH

Protein structures determined by MAS solid-state NMR

1M8M, 62 aa SH3 domain <i>Oschkinat</i>		2K0P, 56 aa GB1 (CS) Robustelli et al. 2008		2KRJ, 152 aa MMP-12 Bertini et al. 2010
1RVS, 10 aa Transthyretin Jaroniec et al. 2004		2K9C, 152 aa MMP-12 (PCS) Balayssac et al. 2008		2NNT, 4x 31 aa ww2 domain Ferguson et al. 2006
1XSW, 38 aa Kaliotoxin Lange et al. 2005		2KHT, 30 aa alpha defensin HNP-1 Li et al. 2010		2RLZ, 2x 85 aa Crh dimer Loquet et al. 2007
2E8D, 4x 22 aa beta2-microglobulin Iwata et al. 2006		2KIB, 8x 7 aa hIAPP Nielsen et al. 2009		2RNM, 5x 71 aa HET-s prion Wasmer et al. 2008
2JSV, 56 aa GB1 (VEAN) Franks et al. 2007		2KLR, 2x 82 aa aB crystallin Jehle et al. 2010		2UVS, 38 aa Kaliotoxin Korukottu et al. 2008
2JU6, 56 aa GB1 (3D protons) Zhou et al. 2006		2KQ4, 56 aa GB1 (3D TEDOR) Nieuwkoop et al. 2009		2KWD GB1 (TEDOR Intermolecular) Nieuwkoop et al. 2010
2JZZ, 76 aa Ubiquitin Manolikas et al. 2008				2KJ3, 3x 71 aa HET-s prion Van Melckebeke et al. 2010

Quality of MAS solid-state NMR structures

Whatif quality Z-scores

- Crystalline proteins
- △ Fibrils, aggregates

■ solution NMR (inter-quartile range)
----- best expected value

Ramachandran plot appearance

Packing quality

Backbone Normality

χ_1/χ_2 rotamers normality

Solid-state NMR vs. X-ray vs. solution NMR

Molprobit Score percentile

□ vs. X-ray structures (1700+) ■ vs. solution NMR structures (3600+)

2K0P = GB1 from ssNMR chemical shifts only

2KLR = aB crystallin dimer

2KRJ = MMP-12 (CHHC + PDSD/DARR + PAR + PAIN + PCS)

Molprobit score = f(clashes, rotamer outliers, Ramachandran non-favored)

NMR on membrane proteins

ArtMP, an ABC transporter

Arginine import system of *Geobacillus stearothermophilus*

The holy grail:

All functional states of one system in native lipid bilayers

Preparation of ArtMP

NMR-Spectra of ArtMP-2D-Crystals

ArtMP-PY
without ATP

^{13}C - ^{13}C -PDSD: 600 MHz, 285 K, Mischzeit 20 ms

ArtMP-TYP
with ATP

Vivien Lange

Ergebnisse

^1H detection of perdeuterated samples, recrystallized from $\text{H}_2\text{O}/\text{D}_2\text{O} = 1:9$: SH3 with ^1H , ^2H , ^{13}C and ^{15}N

By Rasmus Linser, Vipin Agarwal, Veniamin Chevelkov, Bernd Reif

Use of standard pulse solution programs

R. Linser et al., Sensitivity Enhancement using paramagnetic relaxation in MAS solid state NMR of perdeuterated proteins, J. Magn. Res. subm.

HNCa of SH3 α -spectrin, 75mM Cu-edta doped, $\omega_r = 24\text{kHz}$ (R. Linser, AG Reif)

Reconstitution of ArtMP

ArtM(P) ^{15}N

CP

INEPT

¹H detection of perdeuterated samples, recrystallized from
 $\text{H}_2\text{O}/\text{D}_2\text{O} = 9:1$
 By Rasmus Linser, Vipin Agarwal, Veniamin Chevelkov,
 Bernd Reif

Optimum Levels of Exchangeable Protons in Perdeuterated Proteins for Proton Detection in MAS Solid-State NMR Spectroscopy: A study at 24 kHz spinning

By the way: there are as many exchangeable sites in the side chains of the 20 aa as in the backbone!

Effect of $^1\text{H}\%$ on ^1H -Linewidths @ 24kHz-MAS

$^1\text{H}\%$	Average* ^1H FWHM (Hz)	Average ^{15}N FWHM (Hz)
10	$19.0 \pm 3.3^{**}$	11.3 ± 5.4
20	20.2 ± 5.7	12.4 ± 7.2
30	22.5 ± 6.5	15.4 ± 7.5
40	27.5 ± 6.1	16.9 ± 7.3
60	32.5 ± 10.3	25.1 ± 13.5
80	37.5 ± 9.9	32.3 ± 14.3
100	58.3 ± 20.5	41.2 ± 14.4

Another Issue !!

Remaining & Disappearing Signals

2D ^1H - ^{15}N MAS NMR: Double-CP, 24 kHz, 275K, 400MHz

Red: 20%
Black: 60%

Red: 20%
Black: 100%

Remaining & Disappearing Signals

Remaining: From the mobile parts, (L61, D62).

Disappearing: From the more rigid parts, (V44, A55).

S/N: CP

S/N per unit-time

$$S/N \text{ (per unit time)} = S/N \sqrt{\frac{7}{T_1 * 1.3}}$$

¹ H %	¹ H T1 (s)	Average S/N Ratio (CP)	S/N Ratio (CP) per unit-time
10	4.28	18.9 ± 4.7	21.1
20	2.58	33.5 ± 11.4	48.4
30	2.13	41.1 ± 16.4	65.3
40	1.67	44.6 ± 22.6	80.1
60	0.85	26.4 ± 15.7	66.5
80	0.76	18.3 ± 13.9	48.7
100	0.68	11.1 ± 6.3	30.9

Relaxation Delay: 7s.

Dynamic Nuclear Polarization

Trent Franks, Sascha Lange, Arne Linden, Barth van Rossum

Electron and Nuclear Polarization Temperature and Field Dependence

POLARIZATION

$$P = \frac{n^+ - n^-}{n^+ + n^-} = \tanh\left(\frac{\gamma\hbar B_0}{2kT}\right)$$

$$P = \frac{\gamma\hbar B_0}{2kT}$$

$$(\gamma_e/\gamma_{1H}) \sim 660$$

- Much larger spin polarization is present in the electron spin reservoir
- Transfer the electron polarization to the nuclear spins by irradiating the electrons with *high frequency microwaves* !

DNP Hardware

FEM

• DNP OF PROTEINS

technique – nACh-Receptor

Technicalities

Different sources of line broadening: radicals, freezing of sample

Which labelling pattern gives the best enhancement?

Which nuclei should be excited first?

Effect of the biradical concentration

FMP

Which nuclei to excite best?

FMP

For Proton:

For Heteronuclei:

Polarisation build-up in [s]

- Buildup behavior is different for diff. Nuclei
- $^1\text{H} < ^{13}\text{C} \ll ^{15}\text{N}$
- Deuteration increases the τ_B significantly
- Dipolar coupling & spin diffusion is important.

DNP buildup is also a function of spin diffusion

FMP

Systems and themes investigated in Berlin:

Membrane proteins without purification

→ Neurotoxin bound to nAChR in native membranes

→ Mystic expressed in E.coli membranes

The nascent chain in ribosomes

Retinal in Drosophila eyes

The system

<http://www.dkimages.com/discover/previews/624/620072.jpg> http://www.sevokphoto.com/images/naja_naja_2.jpg

AChR:
muscarinic: metabotropic (G-Protein coupled; brain, heart, vessels)
nicotinic: ionotropic (ligand gated ion-channels)
parasympathetic autonomic nervous system, neuromuscular junction
similar: GABA_A-, Glutamat-Receptor; five different subunits

room temperature

DNP

Resolved spectra

NTII at nAChR compared to room temperature

FMP

The nascent chain in the tunnel of the ribosome

FMP

Do proteins fold within the ribosome exit tunnel?

- the tunnel is ~100Å long
- with a diameter of ~10Å at a constriction point
- and ~20Å at the widest region

Voss et al., 2006 *J. Mol. Biol.* **360**, 893-906

DsbA-SecM:

MKKIW LALAG LVLA F SASAA FSTPV WISQA QGIRA GP

Enolase-SecM:

MSKIV KIIGR REIID SRGNP T FSTPV WISQA QGIRA GP

FMP

C7

DsbA-SecM

VS.

Enolase-SecM

CP 750 us
Mix 1ms
Exp.Time 3.8d

CP 750 us
Mix 1ms
Exp.Time 4d

FMP

Ser - C α :

G(B) = 5,5%

G(H) = 48,2%

G(C) = 46,3%

Ser - C β :

G(B) = 6,8%

G(H) = 65,1%

G(C) = 28,1%

joint-probability:

Ser (C α ,C β) :

P(B) = 0,83%

P(H) = 70,13%

P(C) = 29,04%

Ser - C α :

G(B) = 64,8%

G(H) = 0,1%

G(C) = 35,1%

Ser - C β :

G(B) = 54,8%

G(H) = 0,14%

G(C) = 54,1%

joint-probability:

Ser (C α ,C β) :

P(B) = 69,2%

P(H) = 0,0%

P(C) = 30,83%

Ser - C α :

G(B) = 14,9%

G(H) = 29,7%

G(C) = 55,4%

Ser - C β :

G(B) = 26,1%

G(H) = 21,4%

G(C) = 26,5%

joint-probability:

Ser (C α ,C β) :

P(B) = 9,8%

P(H) = 16,1%

P(C) = 74,1%

Samples	SH3	Ribosomes	Eyes	NT II	Kinesin	Mystic	Proline
sample amount [nmol/rotor]	2000	16	2	40	50	30	99
sample appearance	solid	wet pellet	wet pellet	solid	wet pellet	wet pellet	solid
added glycerol [μl]	15	5	5	6	15	5	15
S/N (carbonyls)	194	33	23	86	73	62	67
enhancement	39	16	17	10	11	30	38.5
T1 H [s]	3,00	2,30	1,40	1,90	0,60	1,20	1,90

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 - Venjamin
- .

DNPers (Dashing Nitrogen Pushers)

Protein structure determination by magic-angle spinning solid-state NMR

NMR/structure calculation

B. van Rossum
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S. Lange
A. Linden
V. Lange
R. Kühne
J. Pauli
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N. Schröder
P. Schenieder

Preparation SH3 and aB-c:

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A. Diehl

ABC-Transporter:

M. Schneider, HUB

OmpG:

M. Hiller
W. Kühlbrandt (Fra)
Ö. Yildiz (Fra)
K. Vinothkumar

NeurotoxinII/nAChR:

L. Krabben
C. Weise, FUB
F. Hucho, FUB

WW-Fibrils:

A. Fersht, Cam.
N. Ferguson
T.D. Sharpe
M. Petrovich

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