Etats des lieux et développements en RMN

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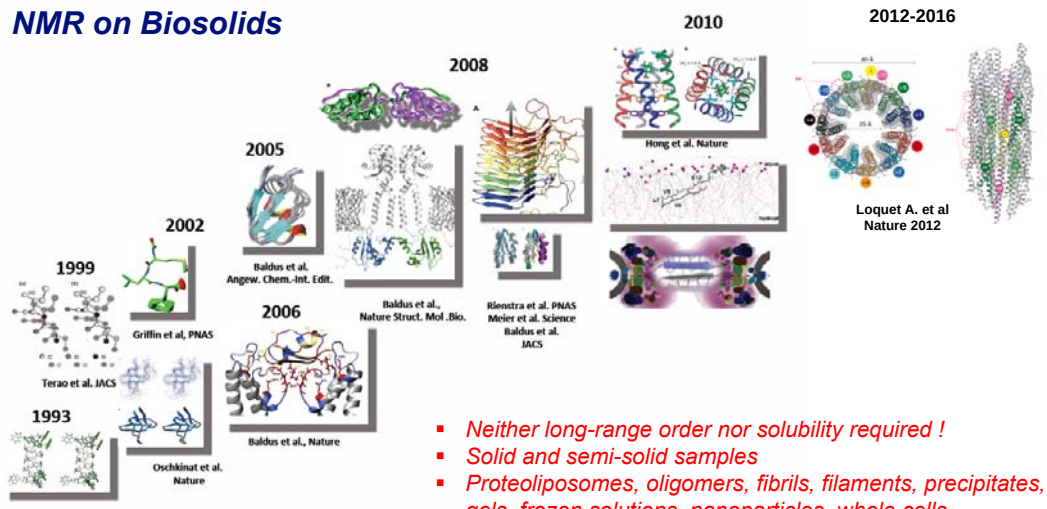
ReNaFobis 2016

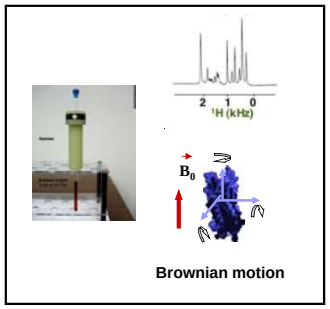
NMR on Biosolids



- *Neither long-range order nor solubility required !*
- *Solid and semi-solid samples*
- *Proteoliposomes, oligomers, fibrils, filaments, precipitates, gels, frozen solutions, nanoparticles, whole cells …*

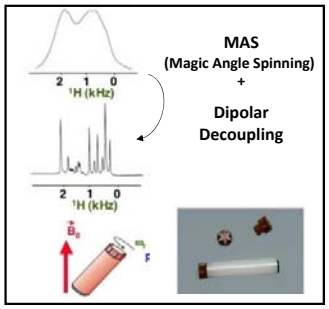
Liquid VS solid NMR probe

Liquid-state NMR



~ 400 µl of soluble sample

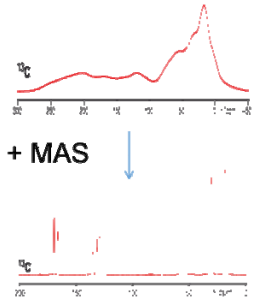
Solid State NMR



~ 20 µl of hydrated insoluble sample

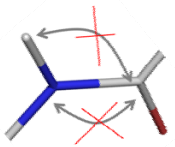
High-resolution ssNMR methods overcome broadening due to large anisotropic interactions in biosolids

Can we turn on and off the anisotropic interactions as we want ?



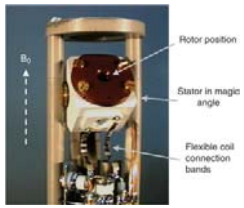
Through **MAS** and **decoupling pulse sequences**, we have turned off the anisotropic interactions (CSA, dipolar coupling).
→ High spectral resolution

But these interactions could be useful, e.g. to transfer via the dipolar couplings, to collect structural restraints.
→ Can we "switch them on" again ??
→ **Recoupling pulse sequences**

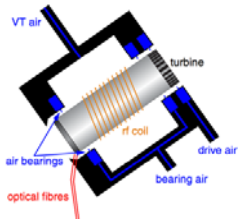


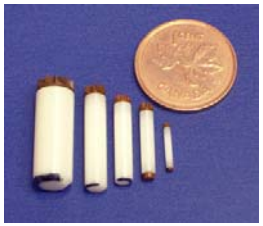
Instrumentation for high-resolution solid-state NMR

1200 MHz soon !

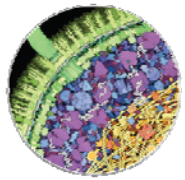


MAS probe



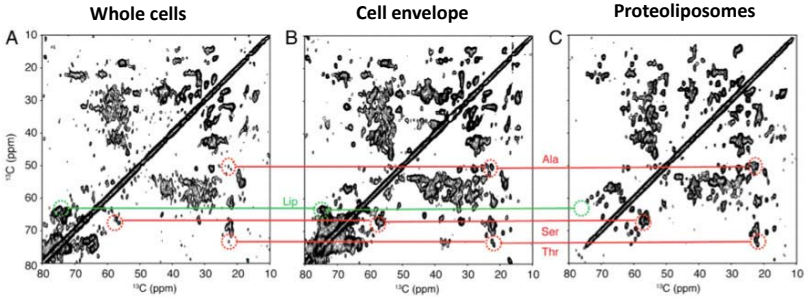


ssNMR at the interface of structural and cellular biology



- *Crystallinity not required, no size limitation*
- *NMR measurement close to physiological conditions*
- *Selectivity for certain rare isotopes (^{15}N, ^{13}C)*
- *Sensitivity of the chemical shifts to changes in the environment*
- *Advanced ssNMR methods to deal with large spectral complexity and critical sensitivity*

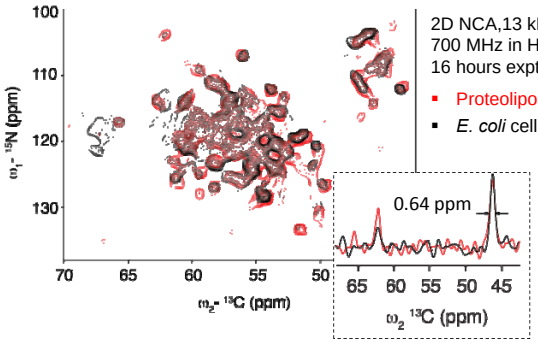
Recombinant MP
Endogenous lipids



Renault *et al.* **Angew. Chem. Int. Ed.** (2012), **PNAS** (2012) & **PNAS commentary** (2012)

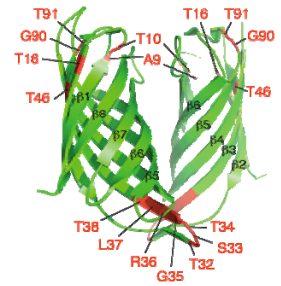
Membrane protein associated with native membrane

- Overexpression of the protein target
- Modification of the endogenous protein background (genome mutation, rifampicin treatment, RNA interferase)
- Use of « targeted » isotope labelling schemes
- Cellular preparations of various levels of molecular complexity : Whole cells > cell envelope > outer membrane
- ^{15}N-edited, ^{13}C-edited NMR, DQ-SQ 2D/3D pulse sequences



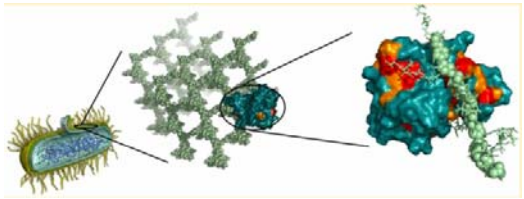
2D NCA,13 kHz MAS, 271 K, 700 MHz in H_2O-based buffer, 16 hours exptl time

- Proteoliposomes
- *E. coli* cell envelope



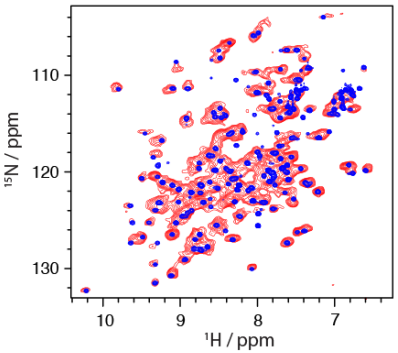
Renault, M. et al. PNAS 109, 4863–8 (2012)

Soluble protein immobilized on peptidoglycan



- Purified [U-HN, ^{2}H,^{13}C,^{15}N]-protein
- Mix with purified unlabeled PG
- Atomic model of the Protein/PG complex from ^{1}H-detected ssNMR experiments at fast MAS frequency
- Protein / PG flexibility from the measurement of NH-dipolar couplings derived order parameters of bond motions

Protein-peptidoglycan spectrum
39 kHz MAS, 600 MHz
deuterated protein + deuterated PG
in H_2O-based buffer
3D in about 3 days exptl time.



Schanda, P. et al. JACS ***136,*** *17852–60 (2014)*

Proton-detected solid-state NMR at ultra-fast MAS

Advantages of ^{1}H detection :

- Natural abundance of more than 99.9 %
- High gyromagnetic ratio
- Greater detection sensitivity than ^{13}C and ^{15}N

$$S/N = \frac{\mathrm{n}\,\gamma_2^5 B0_2^3}{\mathrm{T}}$$

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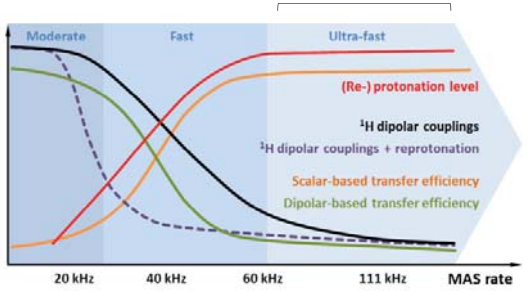
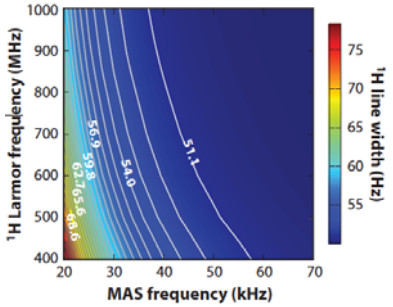
Challenges to reach high resolution :

- Averaging intense dipolar ^{1}H-^{1}H couplings
- ^{1}H dilution : deuteration
- Increase MAS frequency (up to 111 kHz)

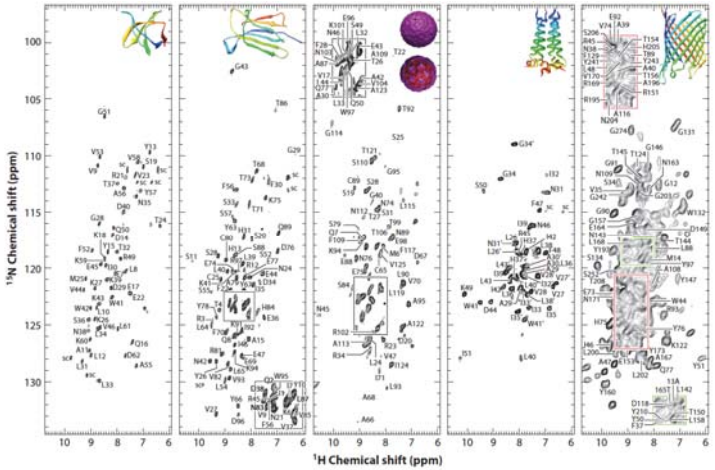


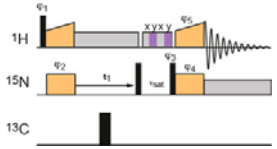
From Venita Daebel, Bruker BioSpin GmbH, Rheinstetten, Germany



Su Y., et al. Annu. Rev. Biochem. 2015. 84:465–97

Proton-detected solid-state NMR at ultra-fast MAS



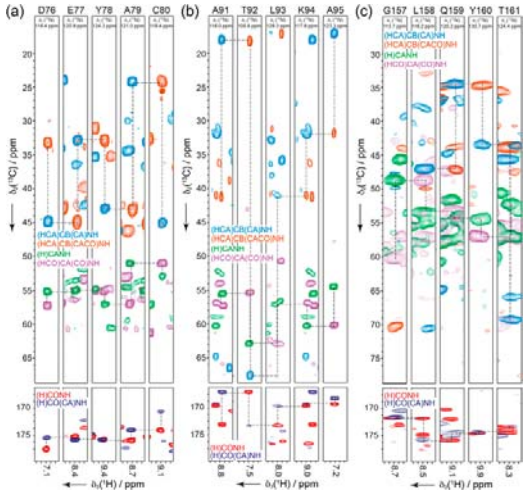


1 GHz spectrometer
60 kHz MAS
[U-HN, ^{2}H,^{13}C,^{15}N]-labeled samples :

Microcrystalline SH3
Microcrystalline β2m
Sedimented nucleocapsids
M2 channel
OmpG

Barbet-Massin et al., J. Am. Chem. Soc. 2014, 136, 12489−12497

Proton-detected 3D experiments for ssNMR assignments



Intraresidue correlations :

- *(H)CO(CA)NH* => (CO_i N_i H_i)
- *(H)CANH* => (CA_i N_i H_i)
- *(H)(CA)CB(CA)NH* => (CB_i N_i H_i)

Sequential correlations :

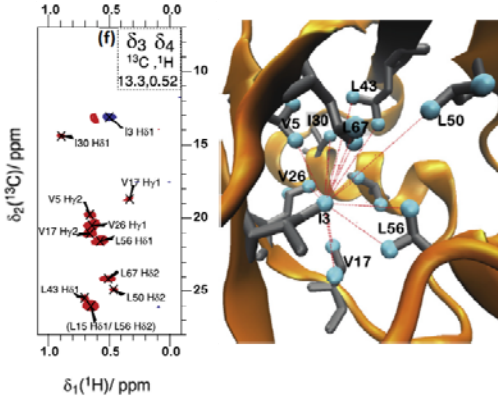
- *(H)CONH* => ($CO_{i-1}N_i$ H_i)
- *(HCO)CA(CO)NH* => ($CA_{i-1}N_i$ H_i)
- *(H)(CA)CB(CACO)NH* => ($CB_{i-1}N_i$ H_i)

Magnetization transfers

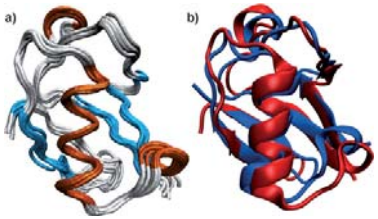
- ***Dipolar-based CP*** *^{1}H-^{13}C; CP ^{15}N-^{13}C; CP ^{1}H-^{15}N*
- ***J-based transfers*** *for ^{13}C-^{13}C due to long ^{13}C coherence lifetimes at 60 kHz MAS*

Barbet-Massin et al., J. Am. Chem. Soc. 2014, 136, 12489−12497

De Novo 3D Structure Determination from Sub-milligram Protein Samples by Solid-State 100 kHz MAS NMR Spectroscopy



- Spectra of ILV-labelled ubiquitin
- 4D HSQC-DREAM-HSQC experiment
- Methyl–Methyl correlations
- 82 HMet-Hmet distance restraints
- 130 HN-HN distance restraints
- Lowest Energy NMR structures VS X-ray structure

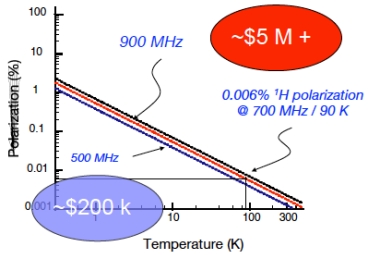


Samoson A., Ernst M., Bockman A., Meier B. et al. Angew. Chem. Int. Ed. 2014, 53, 12253 –12256

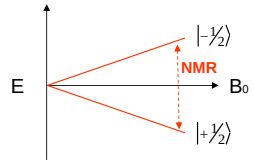
Sensitivity boost for NMR ?

- Observe nuclear spins in magnetic field (7-23.5 T)
- Interaction with RF field (300-1000 MHz for ^{1}H spins)
- Relatively low sensitivity due to small polarization at thermal equilibrium

$$P = \frac{\gamma\hbar B0}{2\,k\,T} \qquad S/N = \frac{n\,\gamma_2^5 B0_2^3}{T}$$



Spin 1/2 nucleus in magnetic field (B_0):



Only 32 excess ^{1}H spins in lower energy level for 1,000,000 ^{1}H's at 9.4 T, 300 K !

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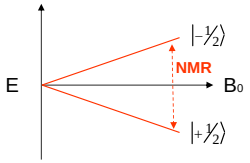
- Increase Sensitivity by pushing spins away from thermal equilibrium = **Hyperpolarization**:
  - Ultra-low temperature
  - Dynamic Nuclear Polarisation (DNP)
  - Optical pumping (e.g. Xenon)
  - Para Hydrogen

Spin 1/2 nucleus in magnetic field (B_0):



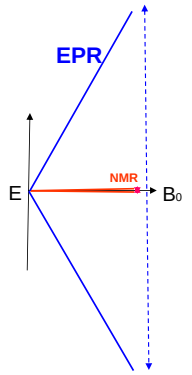
Only 32 excess ^{1}H spins in lower energy level for 1,000,000 ^{1}H's at 9.4 T, 300 K !

Dynamic Nuclear Polarization

- Transfer high electron polarization to nuclei:
  - Irradiate at EPR frequency (GHz)
  - Detect at NMR frequency (MHz)

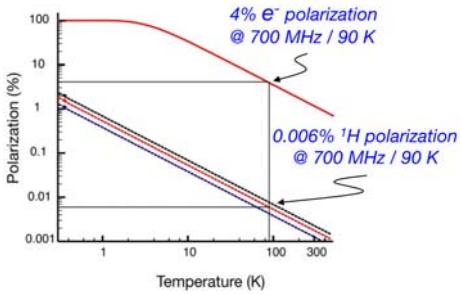
$$P = \frac{\gamma\hbar B0}{2\,k\,T} \qquad \left(\frac{\gamma_{electron}}{\gamma_{proton}}\right) = 660$$

Polarization of electron spins in magnetic field (B_0):

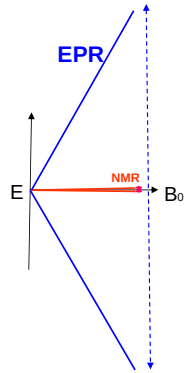


Dynamic Nuclear Polarization

- Much larger spin polarization is present in the electron spin reservoir
- High potential gain in sensitivity (10-100's+)
- Dramatic reduction in signal averaging time

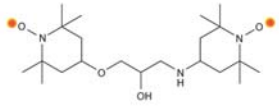


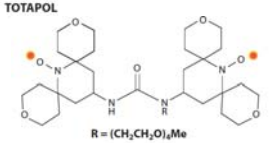
Polarization of electron spins in magnetic field (B_0):



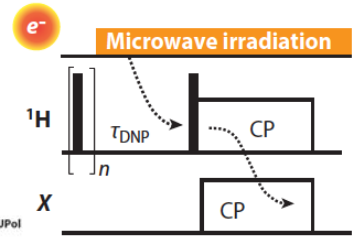
Solid-state DNP-NMR : what it is

- Pioneered by Bob Griffin and Rick Temkin at MIT
- DNP samples are prepared by adding a polarizing agent to a shared solvent or exploiting a native radical on the sample of interest.
- Experiments are performed under MAS conditions at low temperature, 100-170 K, and with continuous microwave irradiation
- Transfer the electron polarization to the nuclear spins by irradiating the electrons with high frequency microwaves !
- Polarize ^{1}H spins followed by cross polarization to ^{13}C, ^{15}N, ^{31}F
- In-situ polarization enhancement each and every scan!
- Compatible with biological solids





AMUPol

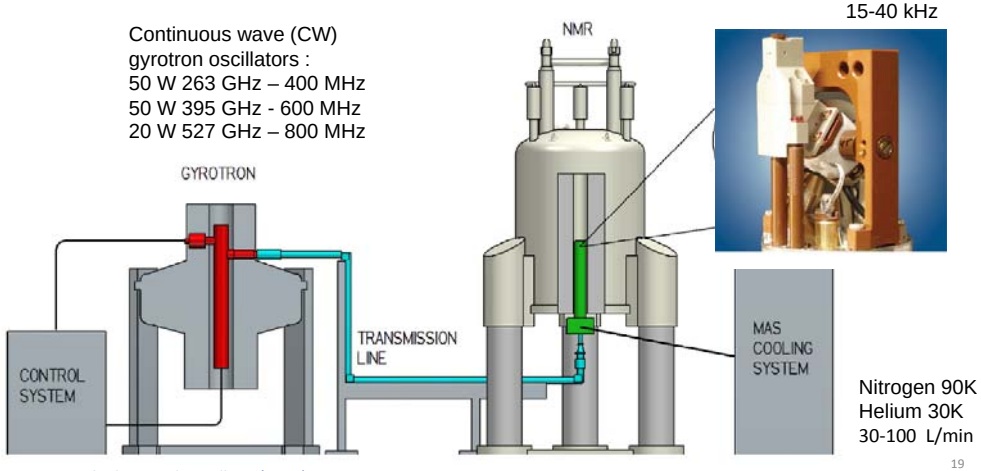


Solid-state NMR DNP system

DNP NMR magnet
With sweep coils
Bruker Ascend DNP

LT-MAS DNP probe
HCN channels
1.3 mm - 3.2 mm
15-40 kHz

Continuous wave (CW)
gyrotron oscillators :
50 W 263 GHz – 400 MHz
50 W 395 GHz - 600 MHz
20 W 527 GHz – 800 MHz



Nitrogen 90K
Helium 30K
30-100 L/min

Rosay M, Blank M. and Engelke F. (2016), JMR 264, 88-98

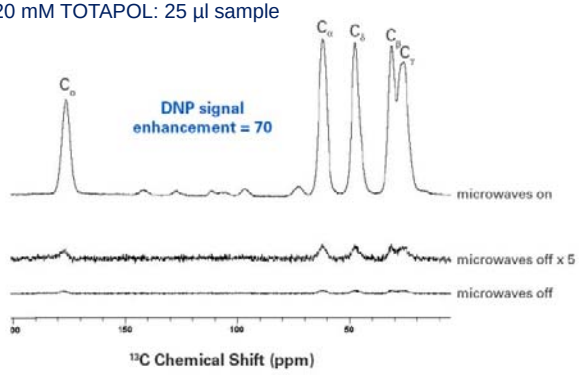
Photograph of 527 GHz DNP Spectrometer at the University of Utrecht, The Netherlands.
Left to right: 800 WB NMR magnet, microwave transmission line, second-harmonic cryogen-free gyrotron tube and magnet, control system.

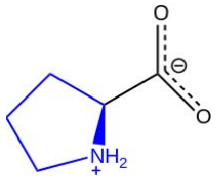
DNP-enhanced CP MAS on (U-^{13}C)-Proline

MAS : 8 kHz
Sample temperature : 100K
DNP matrix : Glycerol/Water with
20 mM TOTAPOL: 25 µl sample

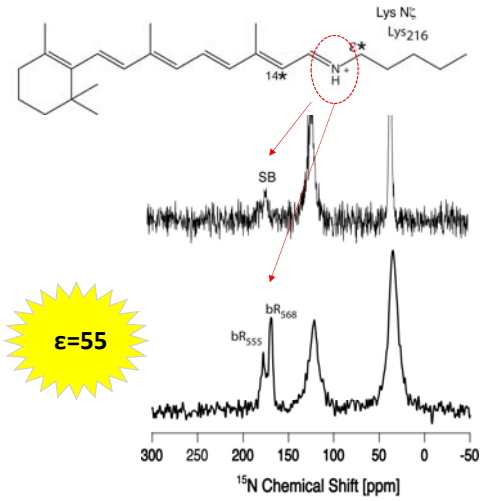
DNP signal enhancement :
$\varepsilon = I_{ON} / I_{OFF}$

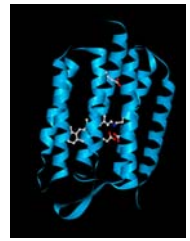
Enhanced Absolute Sensitivity





DNP-Enhanced ^{15}N MAS Spectra ε-^{15}N -Lys labeled bR





SANS DNP, 317 MHz
5 mm rotor, 160 uL
25856 scans, 14.4 hours

With DNP, 211 MHz
4 mm rotor, 40 uL, T=90 K
1280 scans, 1 hour

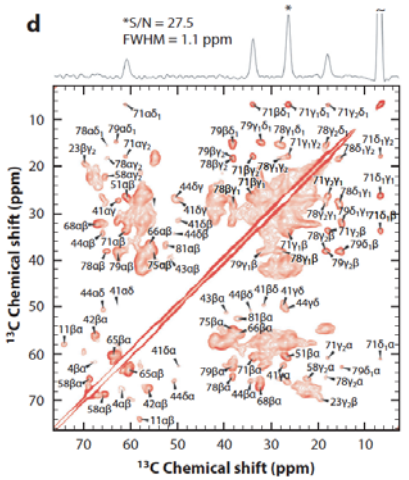
M. Rosay, et. al. JACS (2003)

Current applications on biological samples

Biosystem	ε	Temperature (K)	Radical (mM)	B_0 (^{1}H frequency)
GNNQQNY	20	100	TOTAPOL (35)	400 MHz
TTR105–115	12	100	TOTAPOL (10)	400 MHz
PI3-SH3	30	100	TOTAPOL (10)	400 MHz
Aβ1–40	20	96	TOTAPOL (30)	400 MHz
Peptidoglycan	8.8	100	TOTAPOL (~80)	400 MHz
hΦ17W	18	100	bTbK[a]	400 MHz
M218–60	2.5	100	TOTAPOL (4)	600 MHz
Arabidopsis cell wall	27	100	TOTAPOL (35)	600 MHz
Mistic	20–30	100	TOTAPOL (30–40)	400 MHz
Whole cells	10	100	TOTAPOL (60)	400 MHz
Cell envelopes	26	100	TOTAPOL (60)	400 MHz
Ribosome	25	100	TOTAPOL (20)	400 MHz
Cell wall	20–40	100	TOTAPOL (1)	400 MHz
Escherichia coli SecYEG	32	100	TOTAPOL (20)	393 MHz
nAChR-bound NTII	26	100	TOTAPOL (50)	400 MHz
Apoferritin	20	90	TOTAPOL (1)	212 MHz
T2SS needles	30	104	TOTAPOL (28)	600 MHz
bR	75	83	AMUPol (40)	380 MHz

Limitations and future challenges of DNP-MAS

- ❑ Line broadening occasionally encountered :
  - Temperature regime from 80 to 110 K
  - Proximity of paramagnetic species
- ❑ Future directions
  - New methods for cryoprotection (rapid freezing)
  - Sparse labelling (glycerol, glucose, acetate)
  - DNP at > 800 MHz
  - Increased MAS frequencies
  - Pulsed DNP



2D ^{13}C-^{13}C correlation spectrum
MAS ~ 11 kHz, T= 100 K, 600 MHz
(U-^{13}C,^{15}N)-labeled MXIH needles
TOTAPOL 28 mM
DNP enhancement ~ 30
^{13}C linewidth ~ 1 ppm

Fricke P, Demers JP, Becker S, Lange A. 2014. ChemPhysChem 15:57–60

Thank you for your attention !