Integrative Structural Biology Summer School

5-12 June 2015 – Oléron, France

Inventory and developments in NMR

Bruno Kieffer, IGBMC, Strasbourg
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Biomolecular NMR : 35 years of methodological developments

K. Wüthrich
Protein Struct.

^{13}C, ^{15}N- labelling
3D Triple Reson.
(A. Bax)

TROSY
(A. Pervushin)

Relaxation
Dispersion (A. Palmer)

Longitudinal Relax.
Optimized spectr.
SOFAST (B. Brutscher)

1980 — 500 MHz

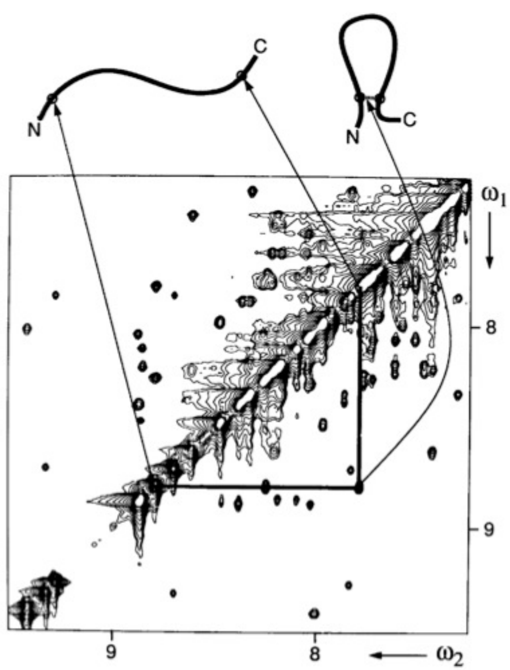
1990 — 800 MHz

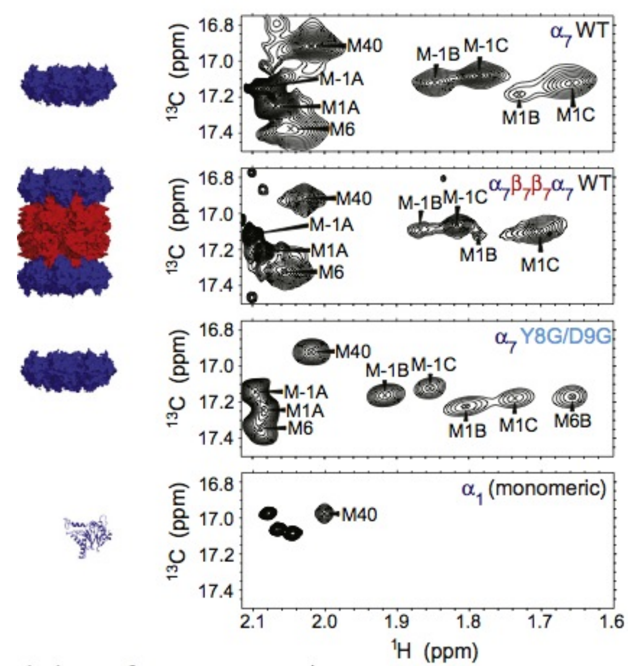
2000 — 900 MHz

2010 — 1 GHz

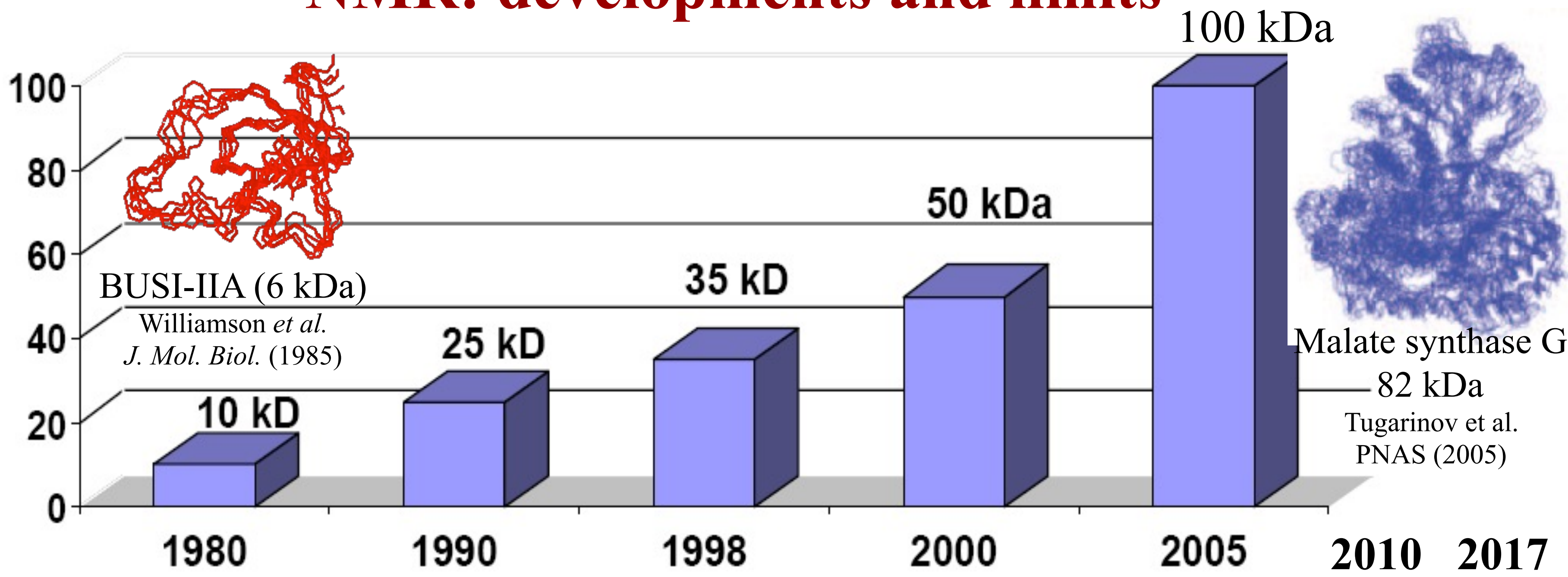
Methyl labelling
(L. Kay)

RDC
(J Prestegard,
A. Bax)





NMR: developments and limits



Magnets 500 MHz 600 MHz 800 MHz 900 MHz 950 MHz 1.0-1.2 GHz

Cryoprobes 500, 600 700,800 1000

Sequences 2D 3D, 4D TROSY
Triple resonance
Gradients

Constraints NOE RDC, CCR

Labeling none ^{13}C, ^{15}N ^{2}H, ^{13}C,^{15}N specific labeling

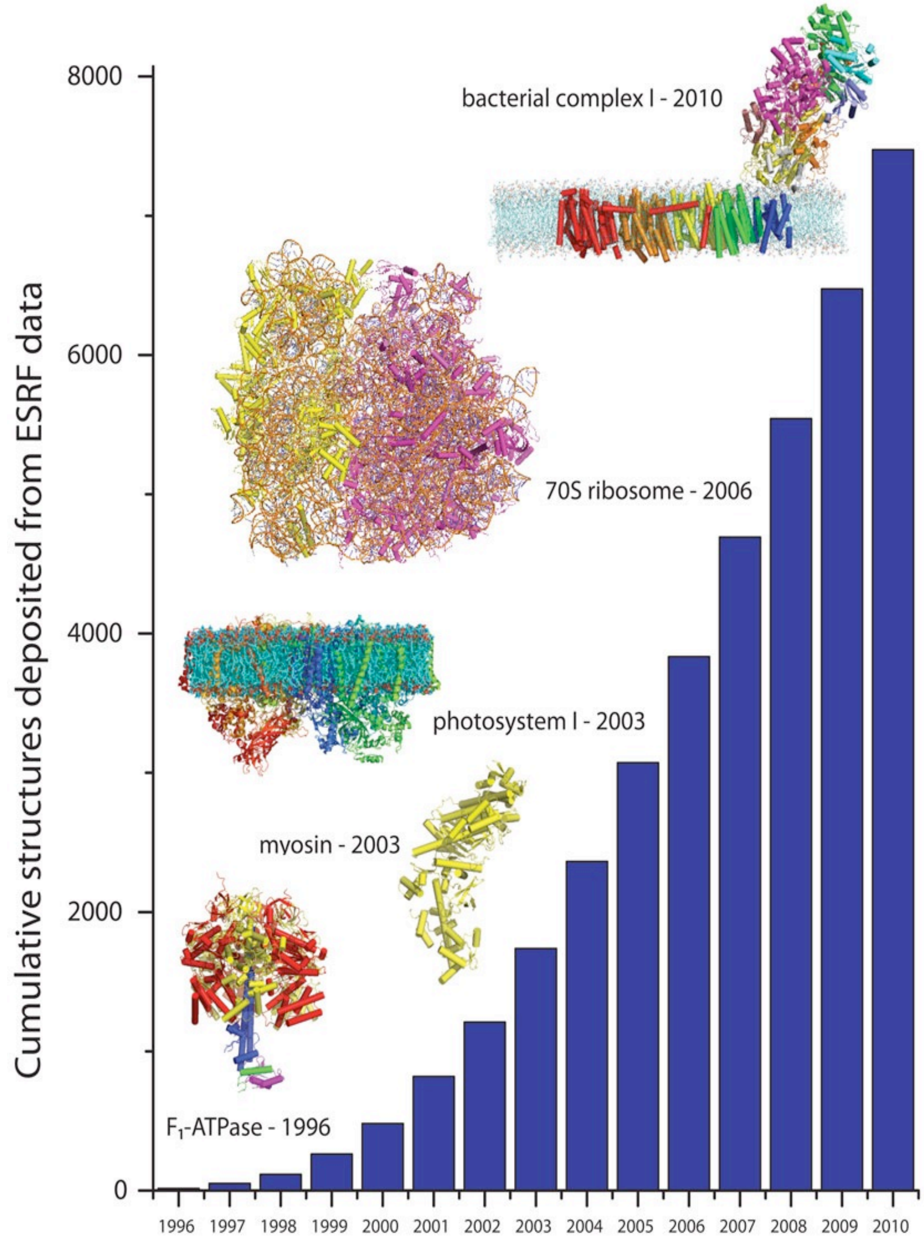
NMR : a structural biology tool among others

	Proteins	Nucleic Acids	Protein/ NA Complex	Other	Total
X-Ray	91091	1624	4534	4	97253
NMR	**9619**	**1121**	**225**	**8**	**10973**
Electron Microscopy	569	29	186	0	784
Other	165	4	6	13	188
Total	**101514**	**2781**	**4953**	**26**	**109274**

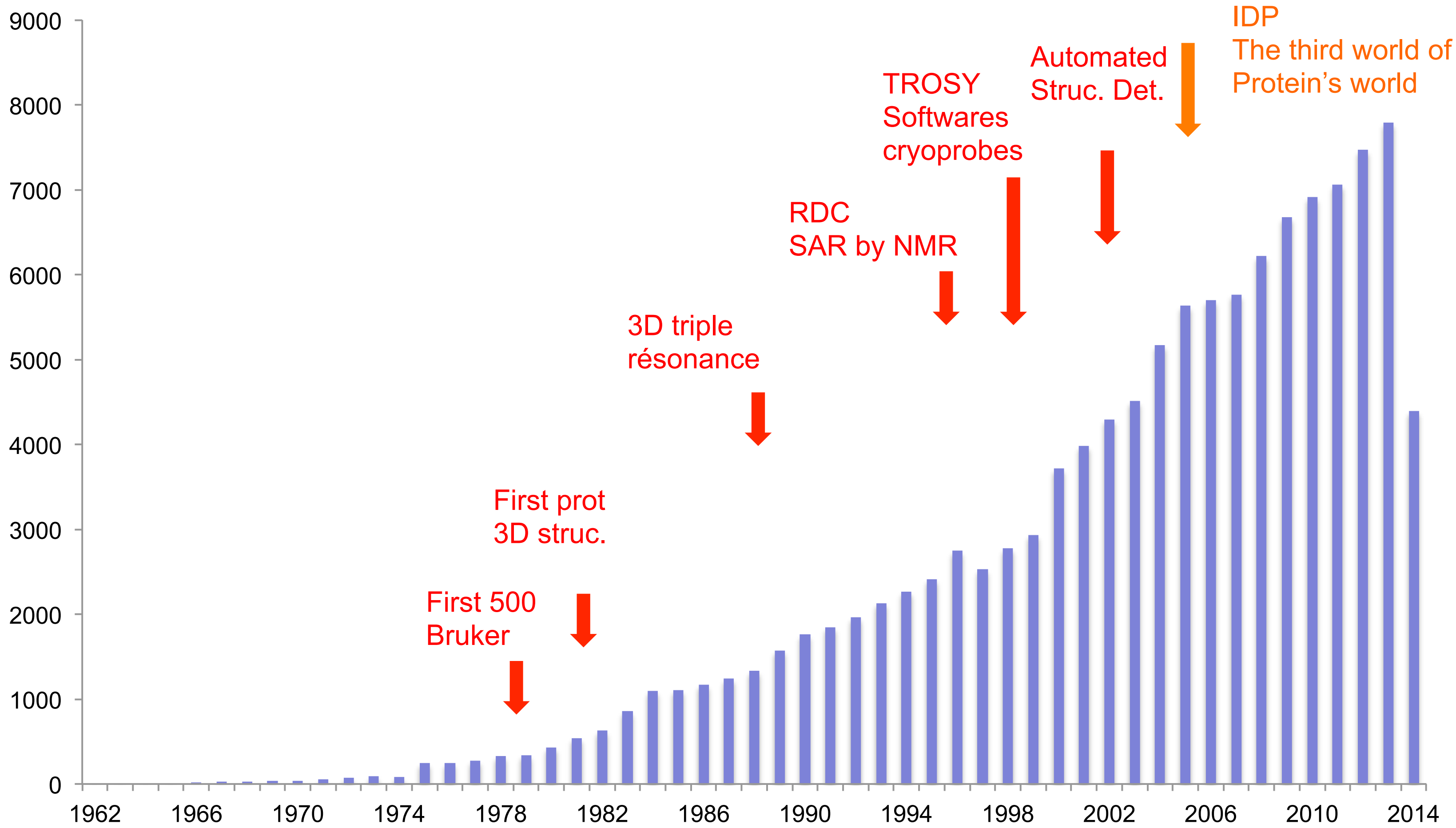
Protein Data Bank: June 6th, 2015 statistics

http://www.rcsb.org

PDB Statistics



Evolution of the number of articles containing the word NMR in the title/abstract in PubMed



BIOCHEMISTRY
including biophysical chemistry & molecular biology

Current Topic

pubs.acs.org/biochemistry

Jan 2013

The Quiet Renaissance of Protein Nuclear Magnetic Resonance

Paul J. Barrett,[†] Jiang Chen,[†] Min-Kyu Cho,[†] Ji-Hun Kim,[†] Zhenwei Lu,[†] Sijo Mathew,[†] Dungeng Peng,[†] Yuanli Song,[†] Wade D. Van Horn,[†,§] Tiandi Zhuang,[‡] Frank D. Sönnichsen,[∥] and Charles R. Sanders*[,†]

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[‡]Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, Virginia 22908, United States

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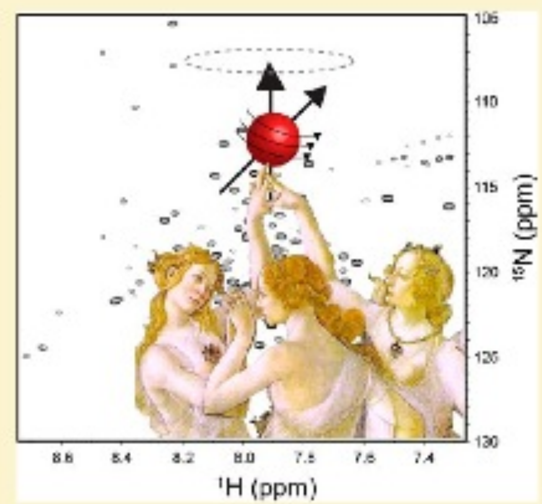
ABSTRACT: From roughly 1985 through the start of the new millennium, the cutting edge of solution protein nuclear magnetic resonance (NMR) spectroscopy was to a significant extent driven by the aspiration to determine structures. Here we survey recent advances in protein NMR that herald a renaissance in which a number of its most important applications reflect the broad problem-solving capability displayed by this method during its classical era during the 1970s and early 1980s.

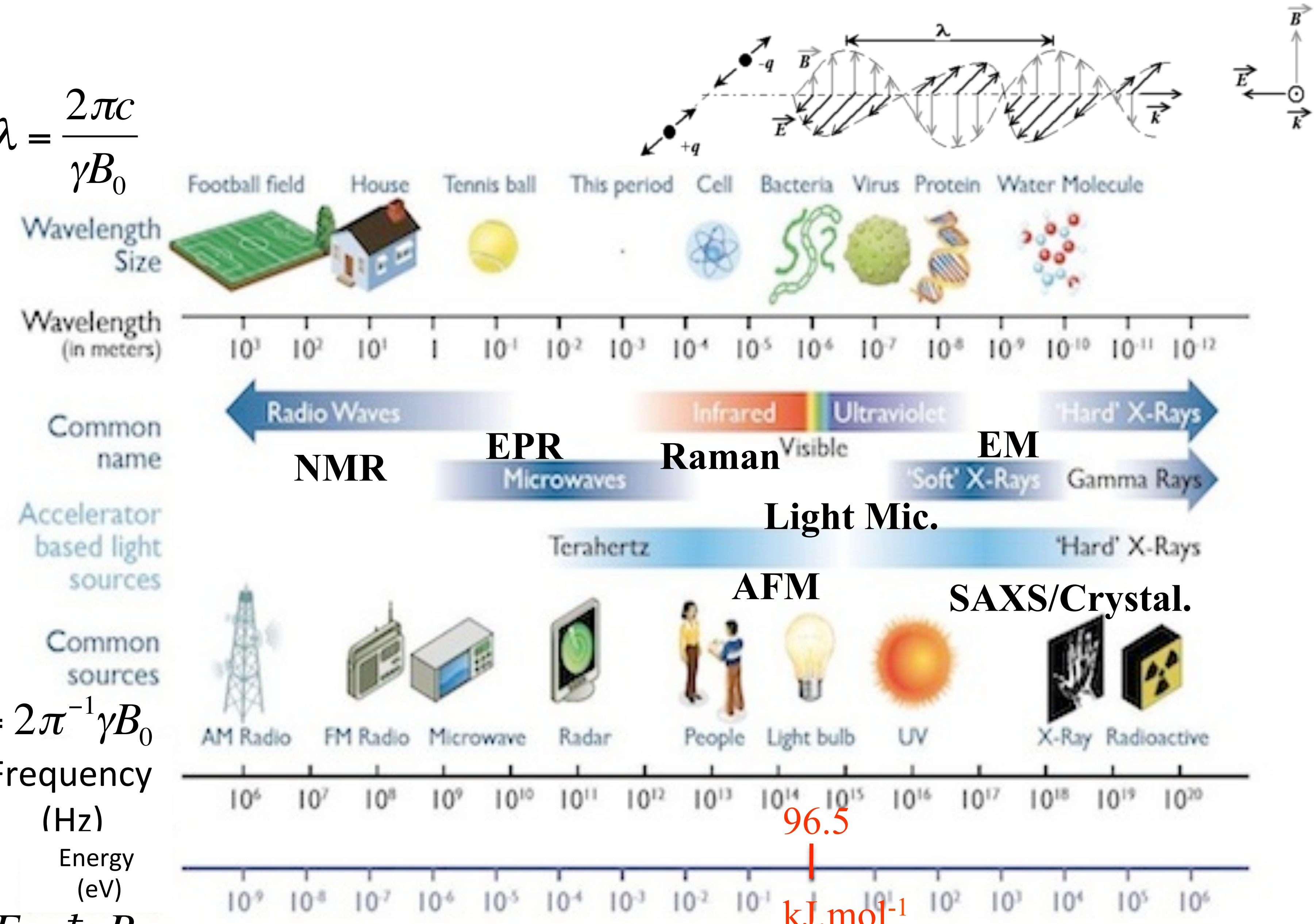


2005-2015
NMR: a tool for integrative structural biology

- ★ Study of intrinsically disordered proteins
- ★ Study of mechanisms of molecular recognition
- ★ Study of proteins and nucleic acid excited states
- ★ Study of the dynamics of very large complexes
- ★ In-cell NMR

Some basic NMR concepts

$$\lambda = \frac{2\pi c}{\gamma B_0}$$



$$\nu = 2\pi^{-1}\gamma B_0$$

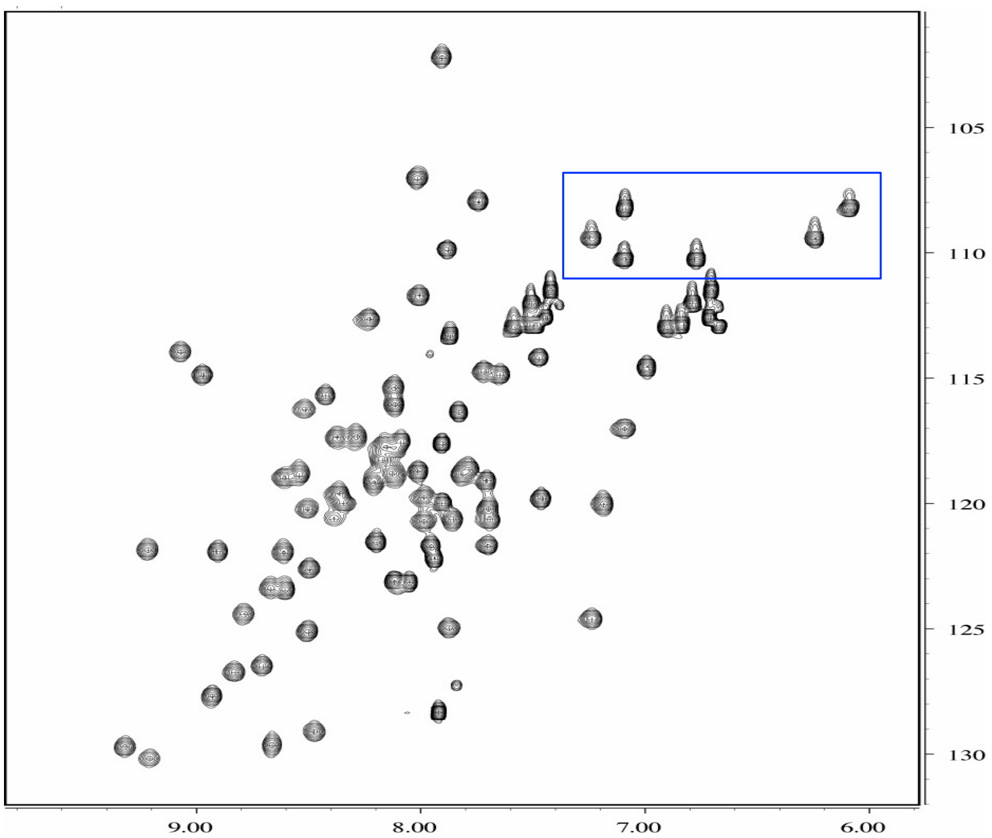
$$E = \hbar\gamma B_0$$

www.lightsources.org/what-light-source

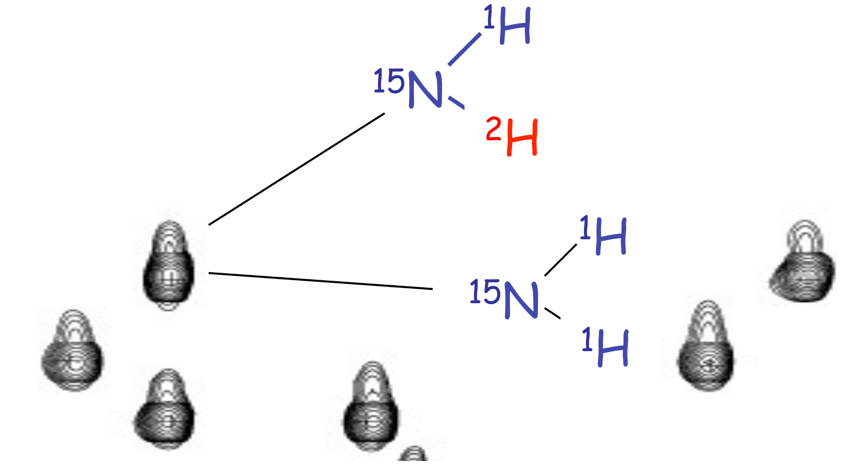
NMR, some advantages

A large number of probes

An incredible sensitivity to the environment



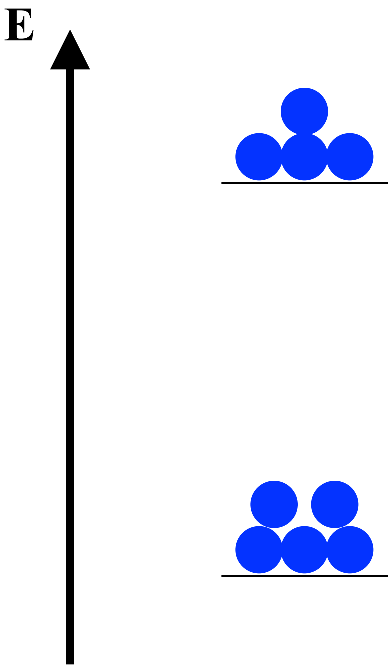
2D $^{1}H,^{15}N$-HSQC: the biomolecule fingerprint



Deuterium isotopic effects

NMR, some limitations

Sensitivity or signal-to-noise ratio



$$E_\beta = \frac{1}{2}\gamma\hbar B_0$$

$$E_\alpha = -\frac{1}{2}\gamma\hbar B_0$$

Particular case of spin 1/2

Boltzman

$$\frac{N_\alpha}{N_\beta} = e^{\frac{E_\beta - E_\alpha}{k_B T}}$$

$$\frac{N_\alpha}{N_\beta} \approx 1 + \frac{\gamma\hbar B_0}{kT}$$

$$\approx 1 + 9{,}66 \times 10^{-5}$$

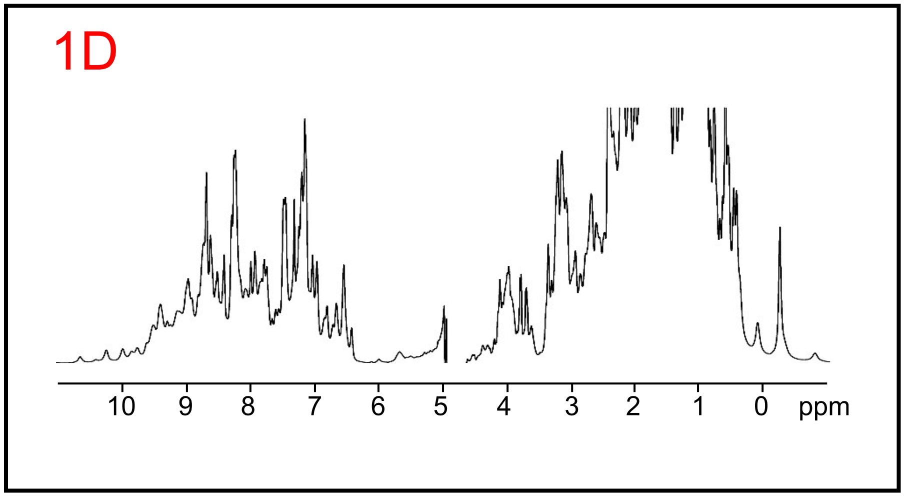
$$@ B_0 = 14.09T\,(600\,MHz)$$

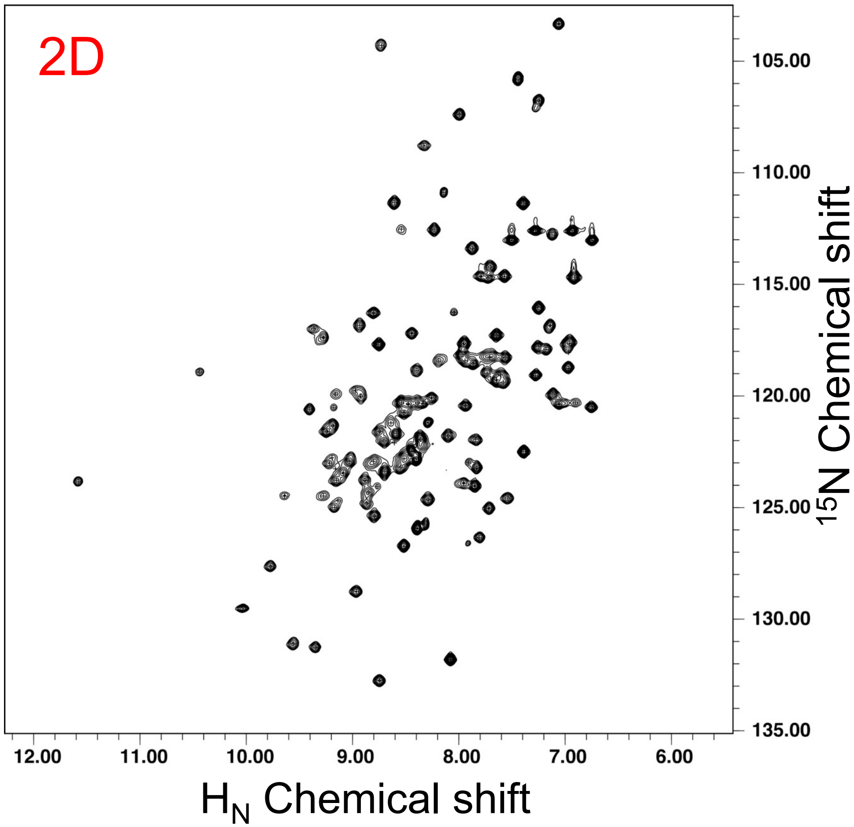
$$\vec{M} = \sum \vec{\mu} = \sum \gamma\hbar\vec{I}$$

$$\vec{M} = N\frac{\gamma\hbar B_0}{2kT}\gamma\hbar\frac{1}{2}\vec{z} = \frac{N(\gamma\hbar)^2 B_0}{4kT}\vec{z}$$

NMR, some limitations

Resolution and spectral hindrance





- Acquisition time: *few seconds*
- limited spectral resolution
- No necessary isotope labeling
- **Global characterization**

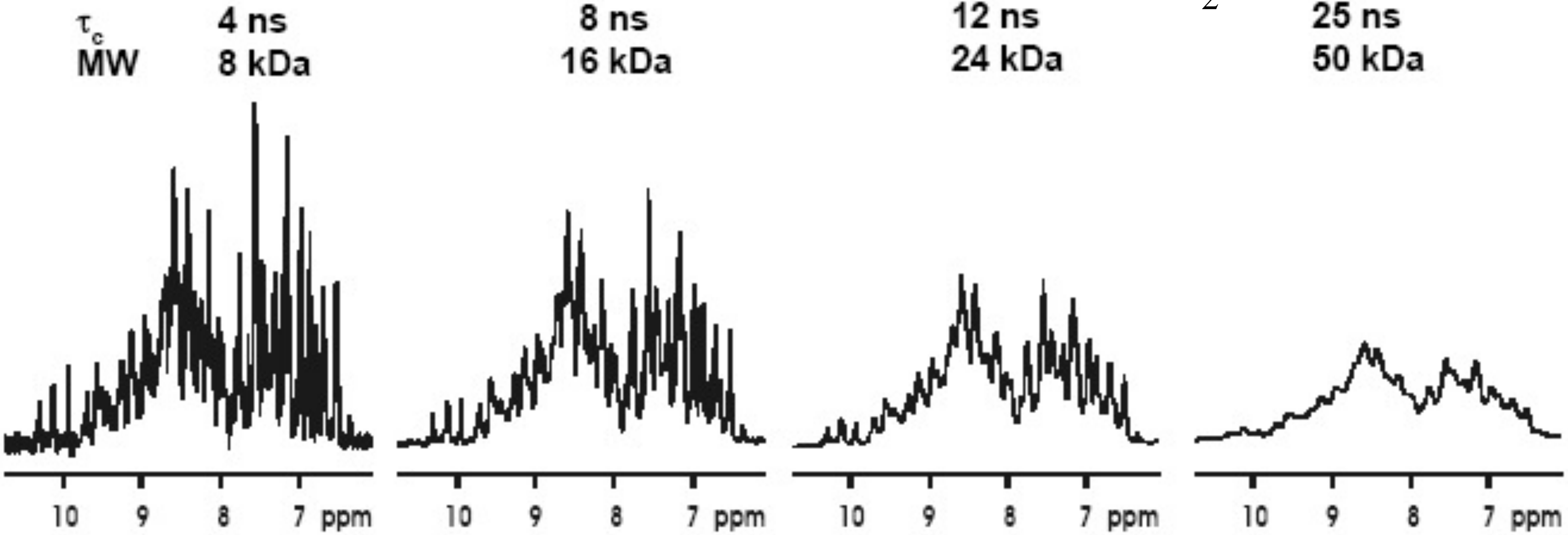
- Acquisition time: *few minutes*
- Increase in the spectral resolution
- Necessary isotope labeling (^{15}N)
- **More detailed information**

NMR, some limitations

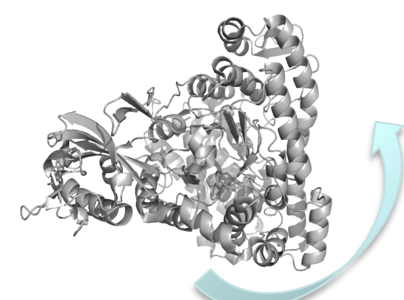
Liquid-state NMR a serious limit?

Linewidth

$$\Delta\nu_{1/2} = \frac{1}{\pi T_2}$$



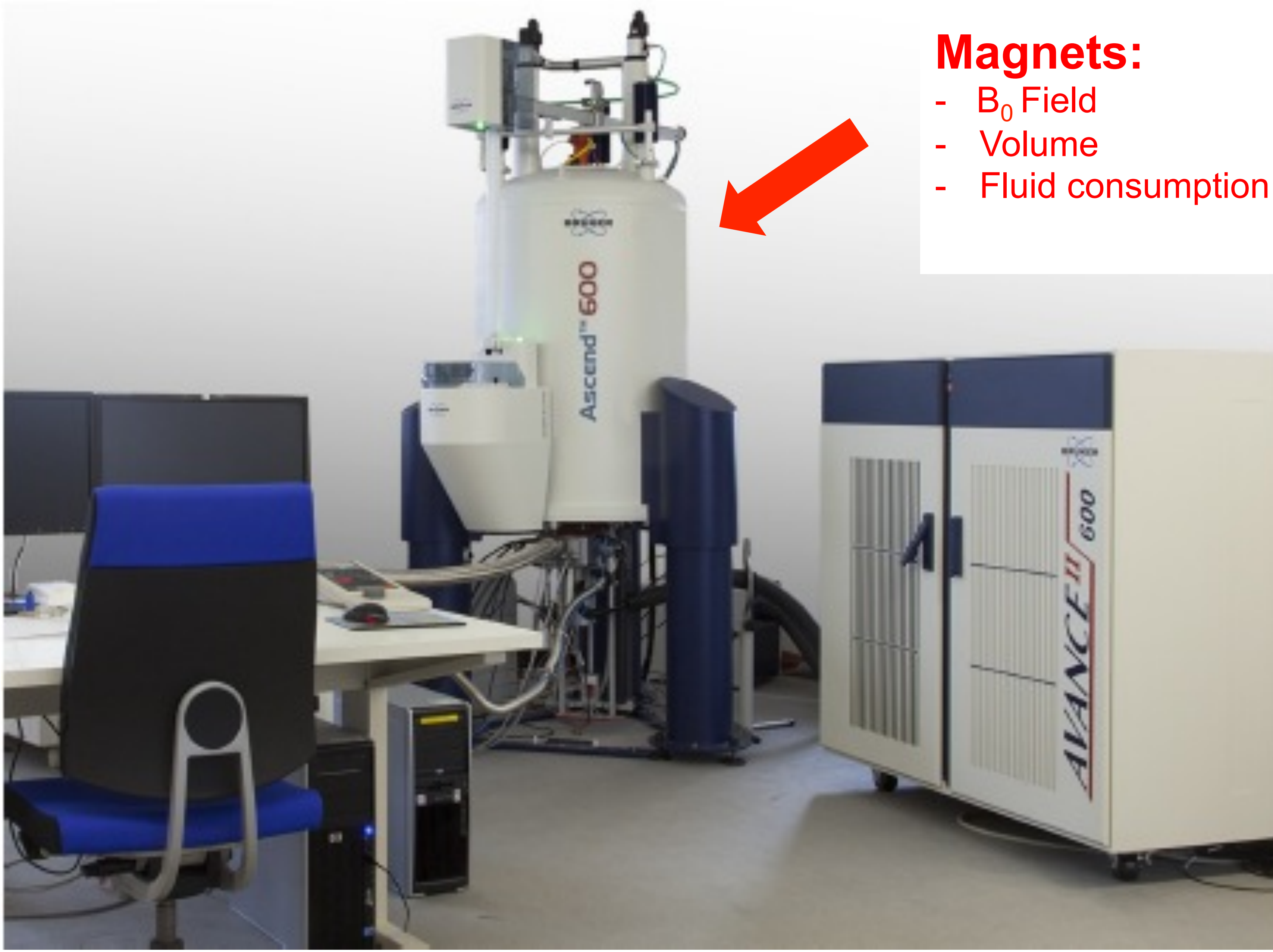
fast overall rotation



slow overall rotation

Technological innovations and developments

Technological innovations



Magnets:

- B_0 Field
- Volume
- Fluid consumption

Technological innovations

« The Hybrid » 45 T (1.9 GHz)
Talahassee
Florida, USA
Insert 11.4 T resistive magnet
External 33.7 T supraconducting
Or GHFML (35 T)

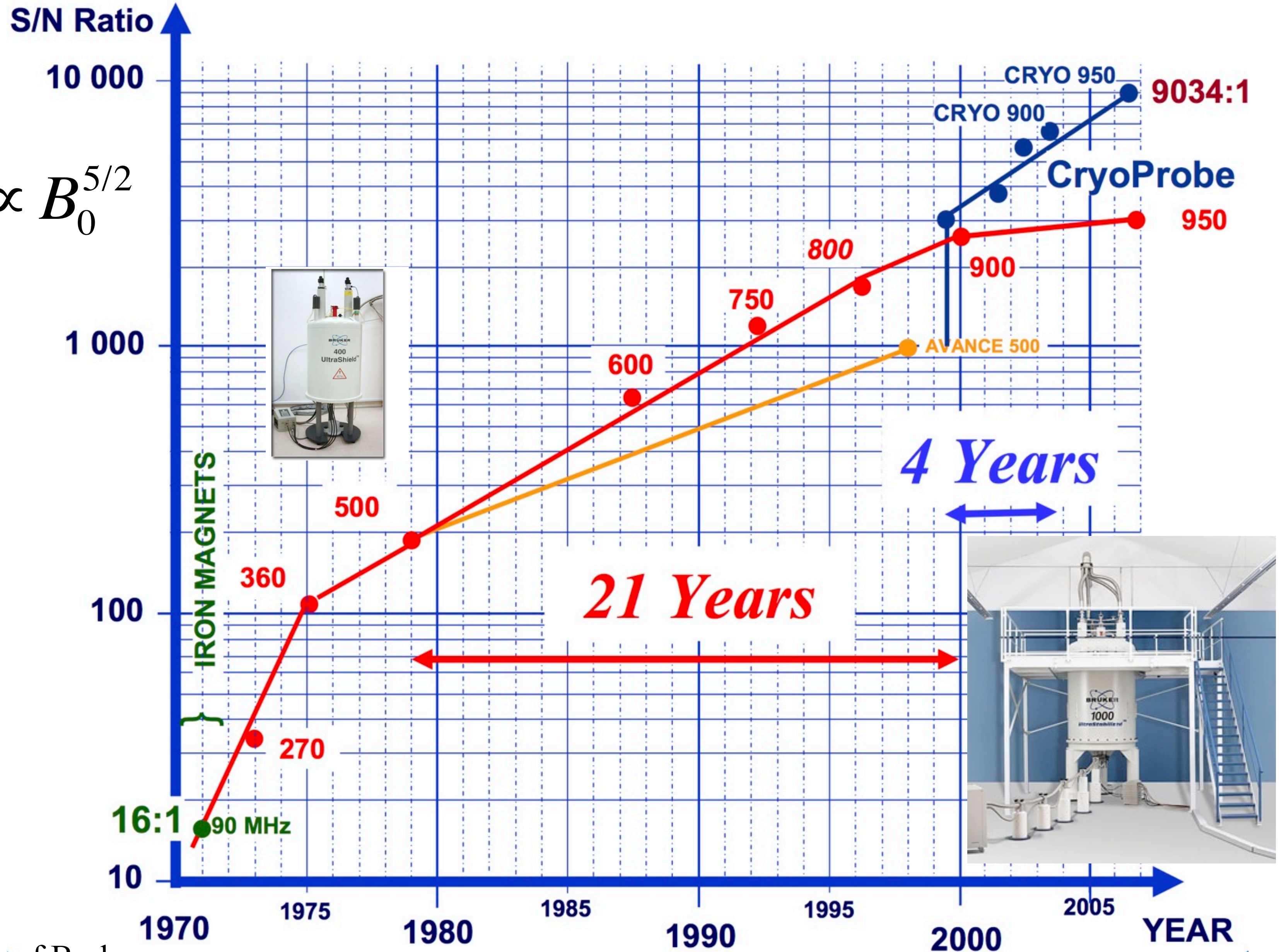
Low-resolution NMR
2-65 MHz
(1.5 T max) persistent magnet

High-resolution suprconducting magnet
1 GHz (Brüker)
23.5 T

Technological innovations

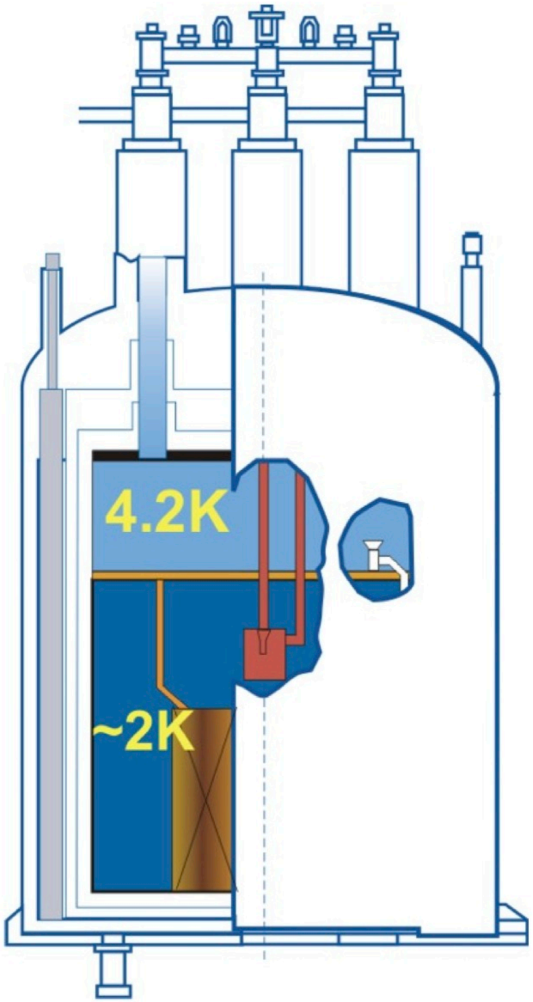
Signal-to-noise depends on the magnetic field

$$S / N \propto B_0^{5/2}$$



Data courtesy of Bruker

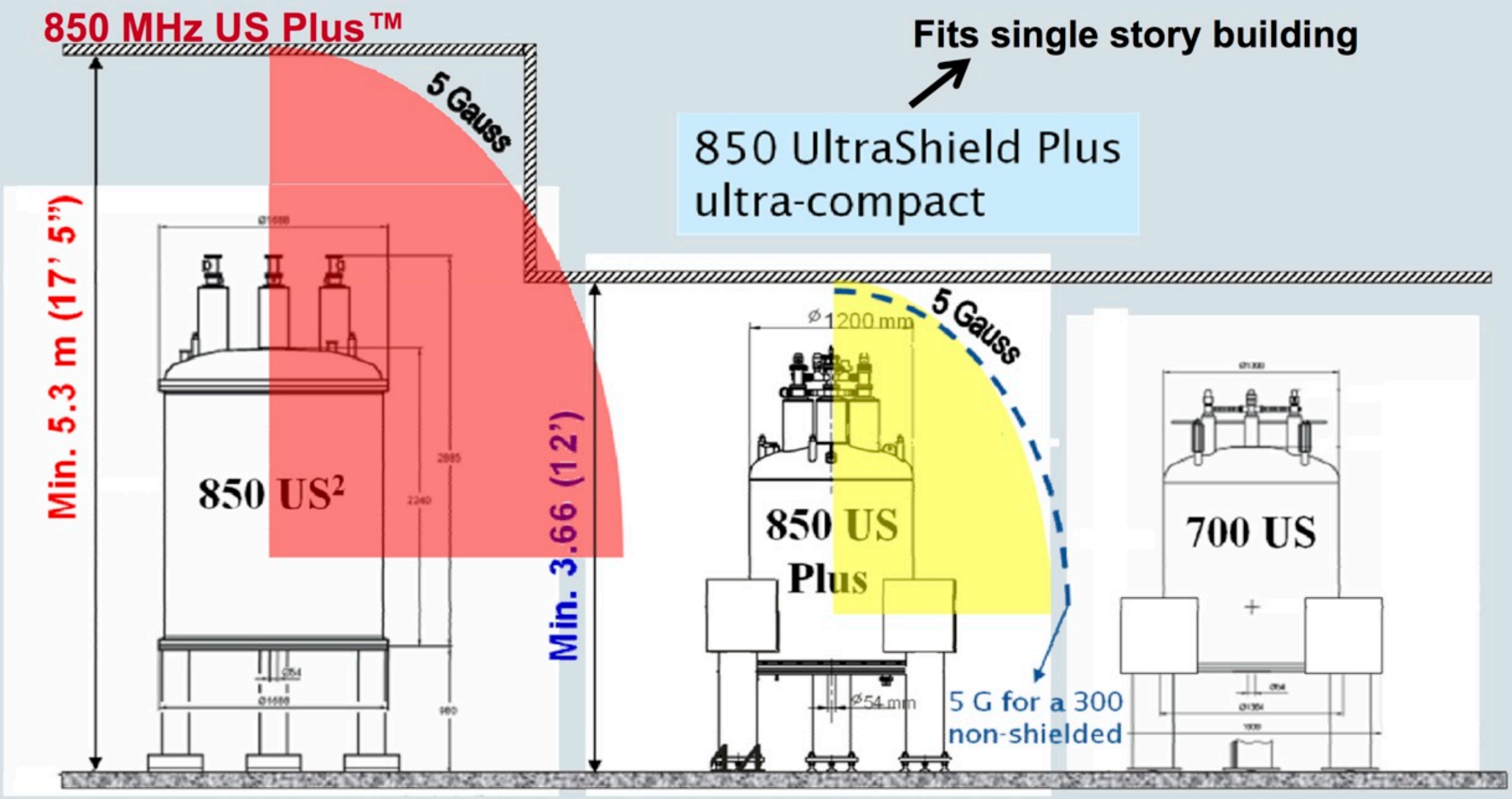
Technological innovations



UltraStabilized™ technology delivering unique performance, stability and safety

Technological innovations

Magnets become more compact



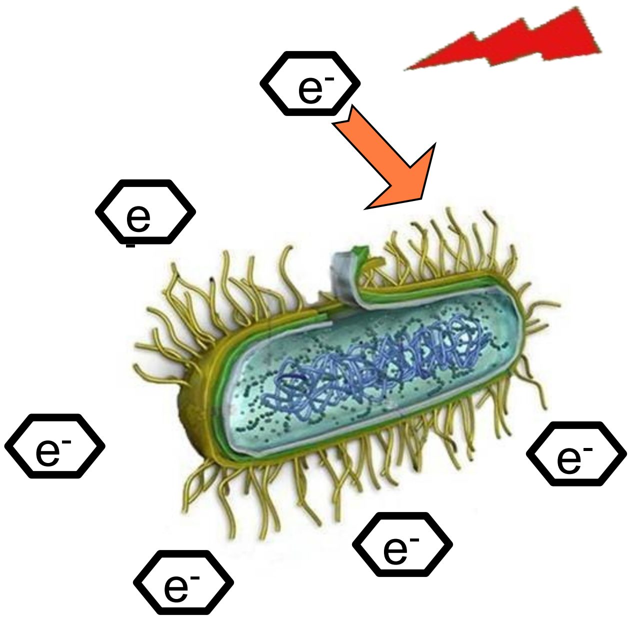
- Compact size and small stray field improve siting flexibility
- Outstanding stability and high-resolution NMR performance

Data courtesy of Bruker

Technological innovations: Dynamic Nuclear Polarization

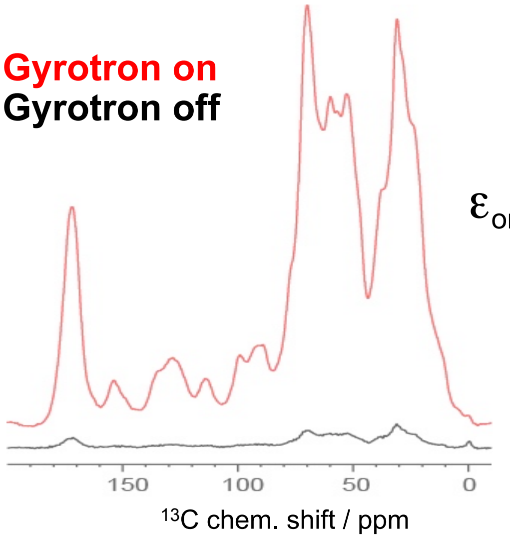
T ~ 100 K : compatible with cell survival

Gyrotron



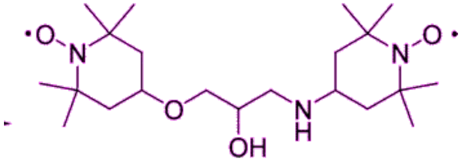
DNP @ CEA Grenoble

NMR signal of the cell surface nuclei ?



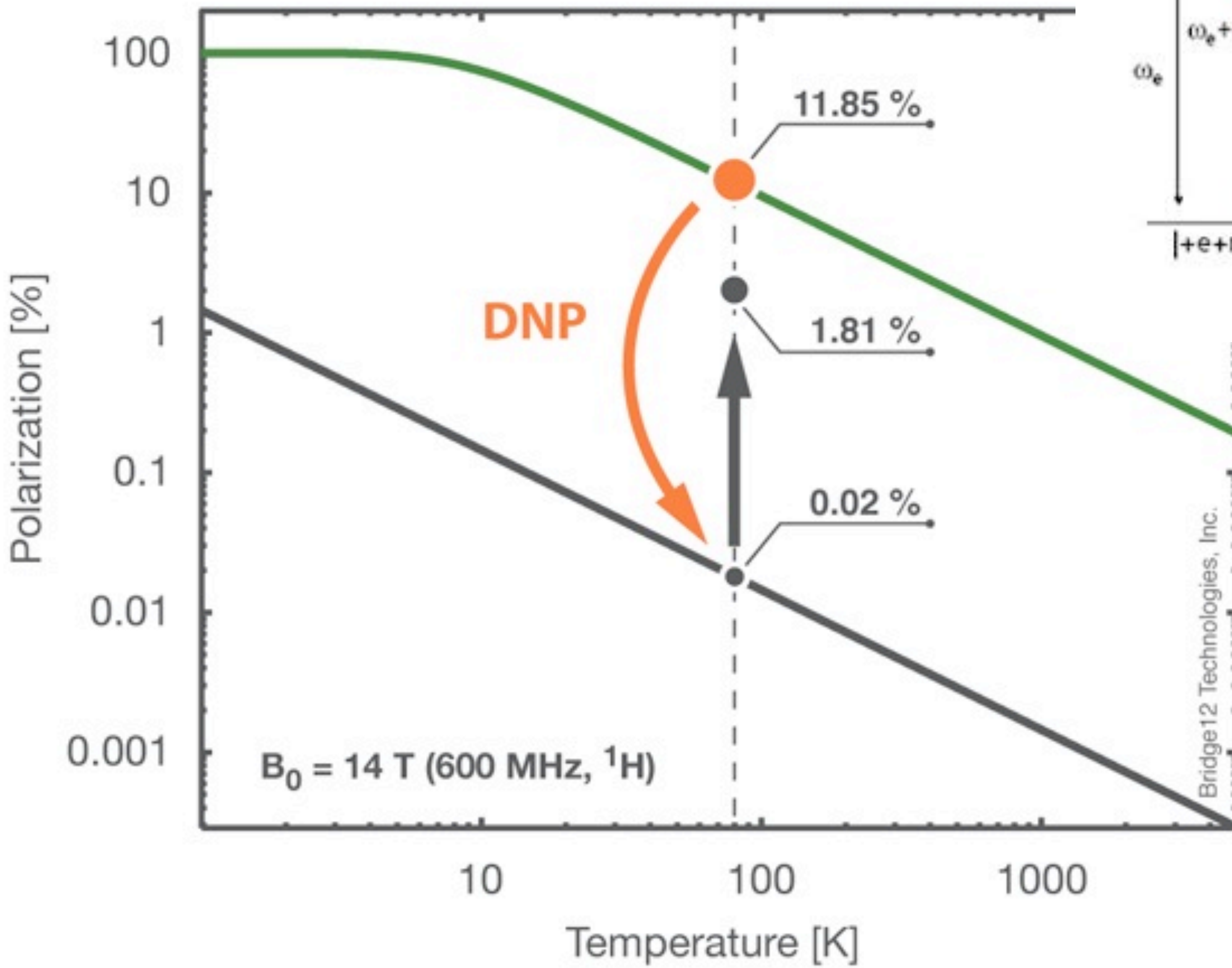
$\varepsilon_{on/off} \sim 25$

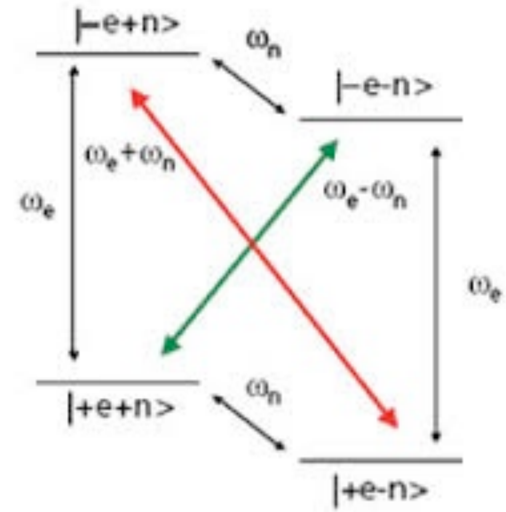
e- =



TOTAPOL

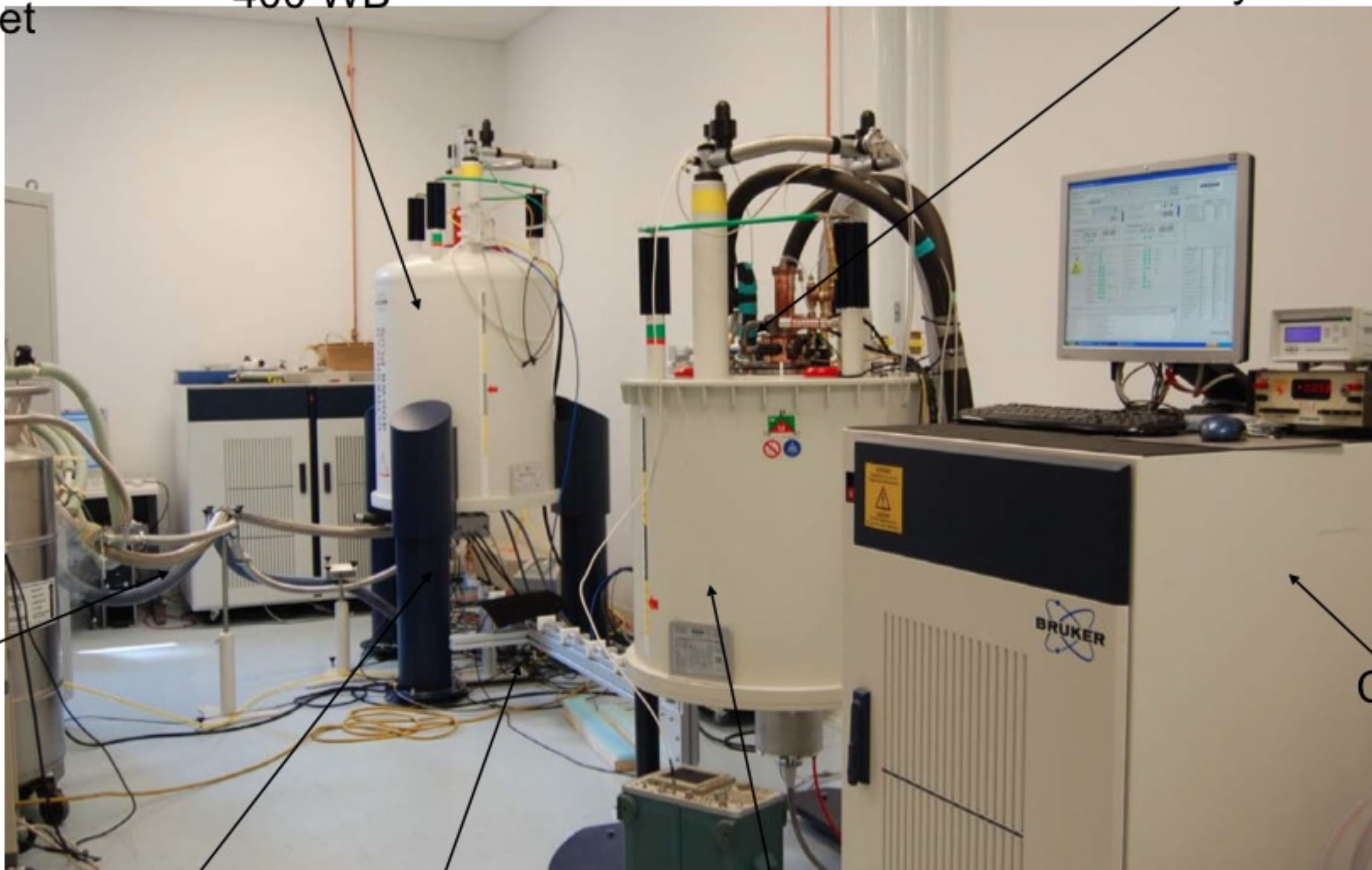
Technological innovations: Dynamic Nuclear Polarization





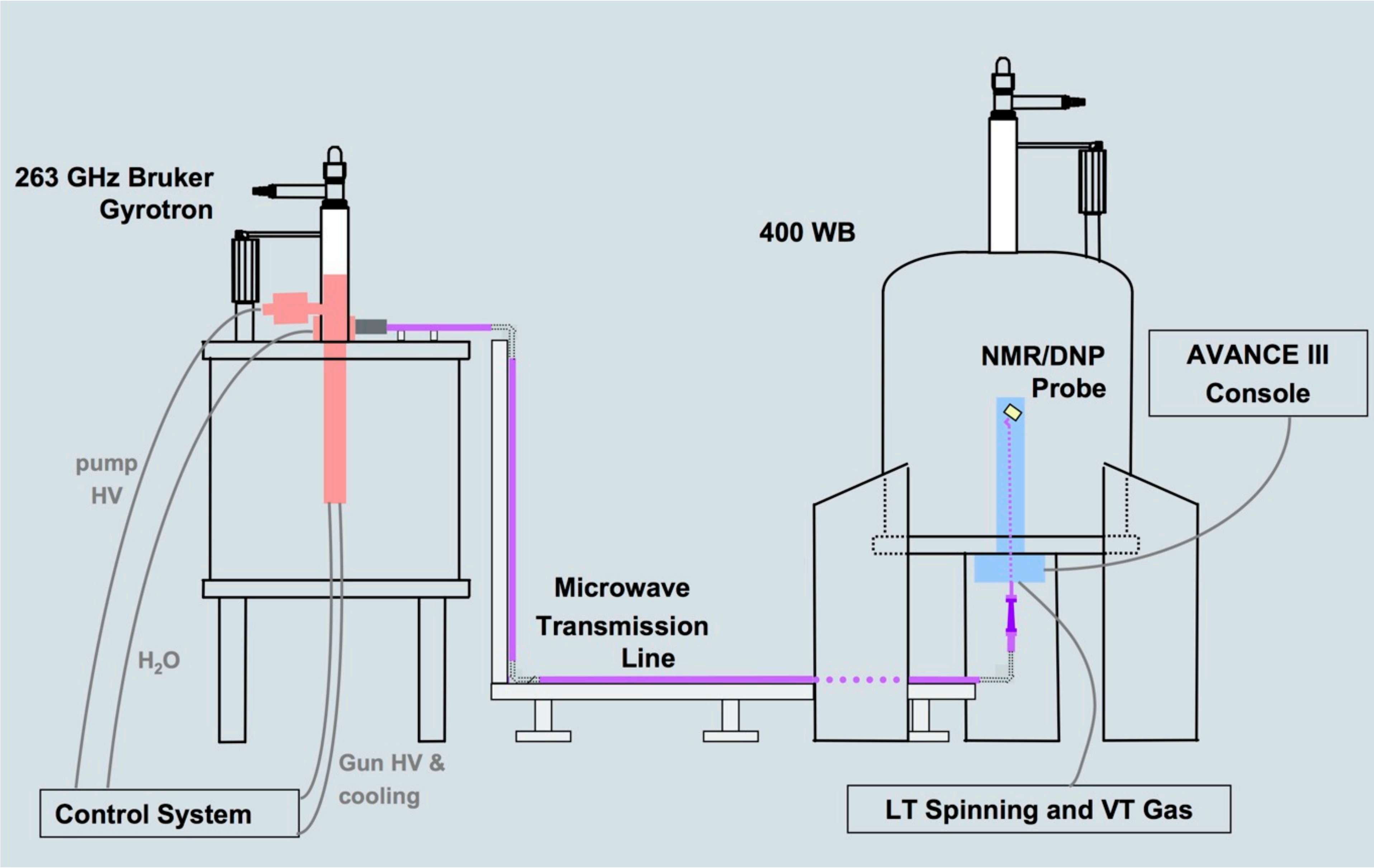
$$\frac{\gamma_{e^-}}{\gamma_H} = 660$$

263 GHz Gyrotron in Bruker-Billerica DNP Lab

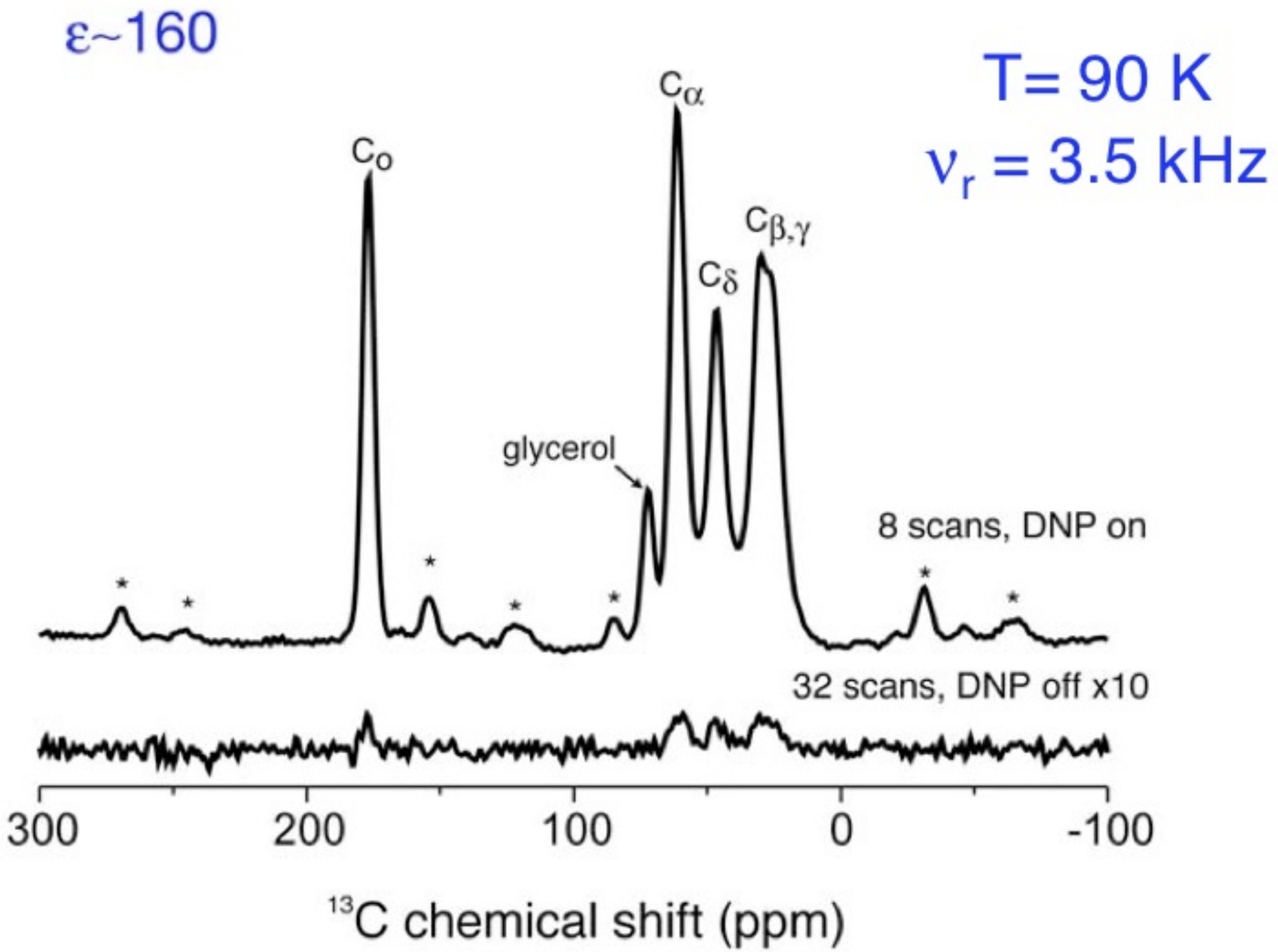


From B. Griffin

263 GHz solid-state DNP

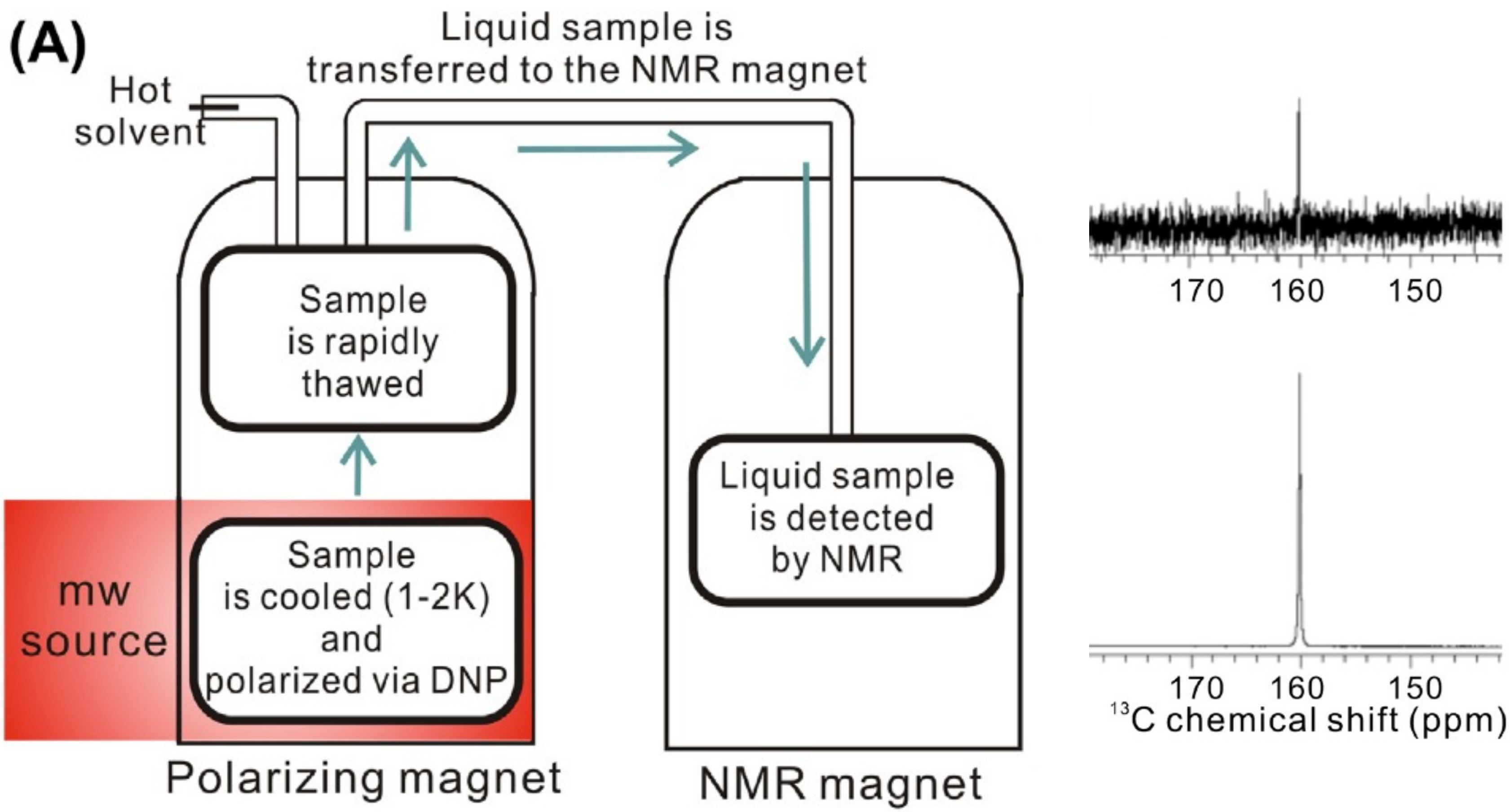


DNP-MAS spectrum of ^{13}C, ^{15}N-proline



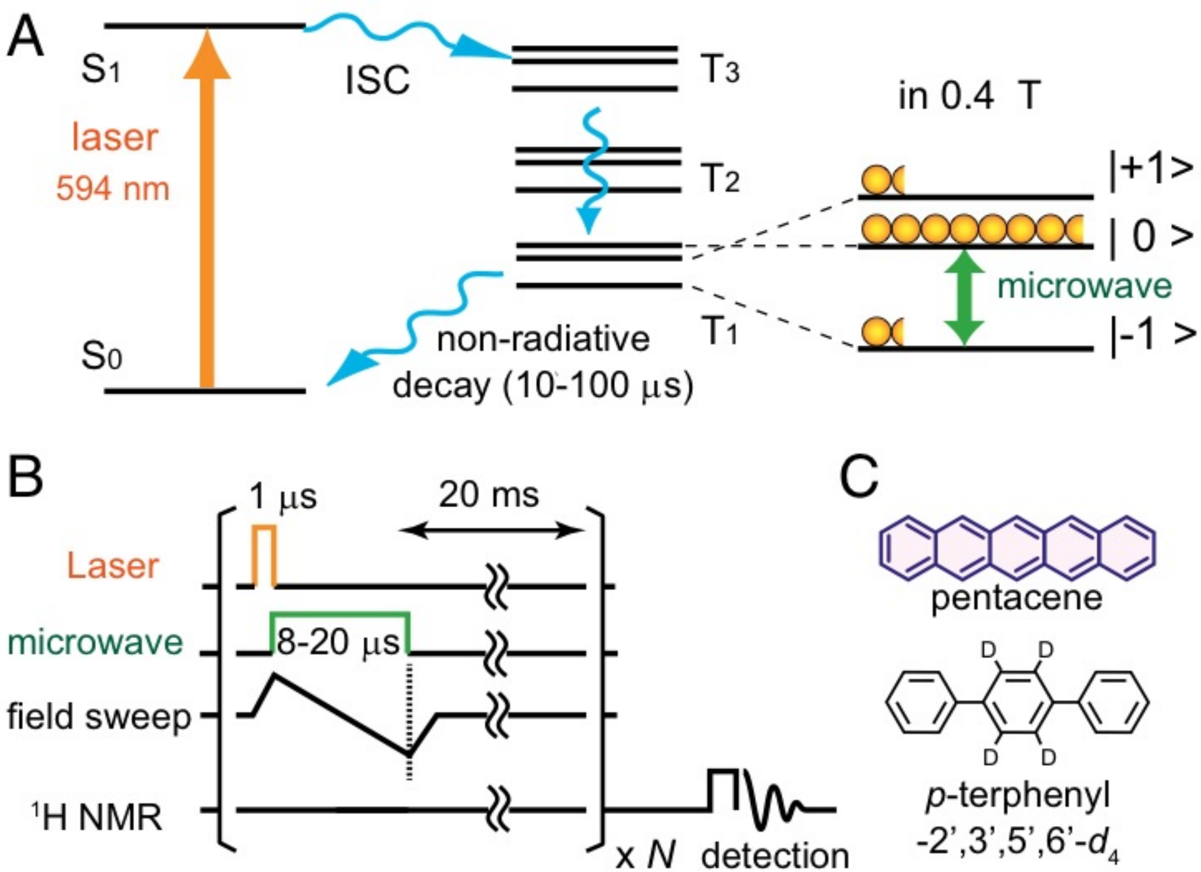
C. Song, T. Swager et. al., JACS (2006)

DNP in the liquid state at room temperature



From H. Ardenkjær-Larsen et al. Increase in signal-to-noise ratio of >10,000 times in liquid-state NMR, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 10158–10163.

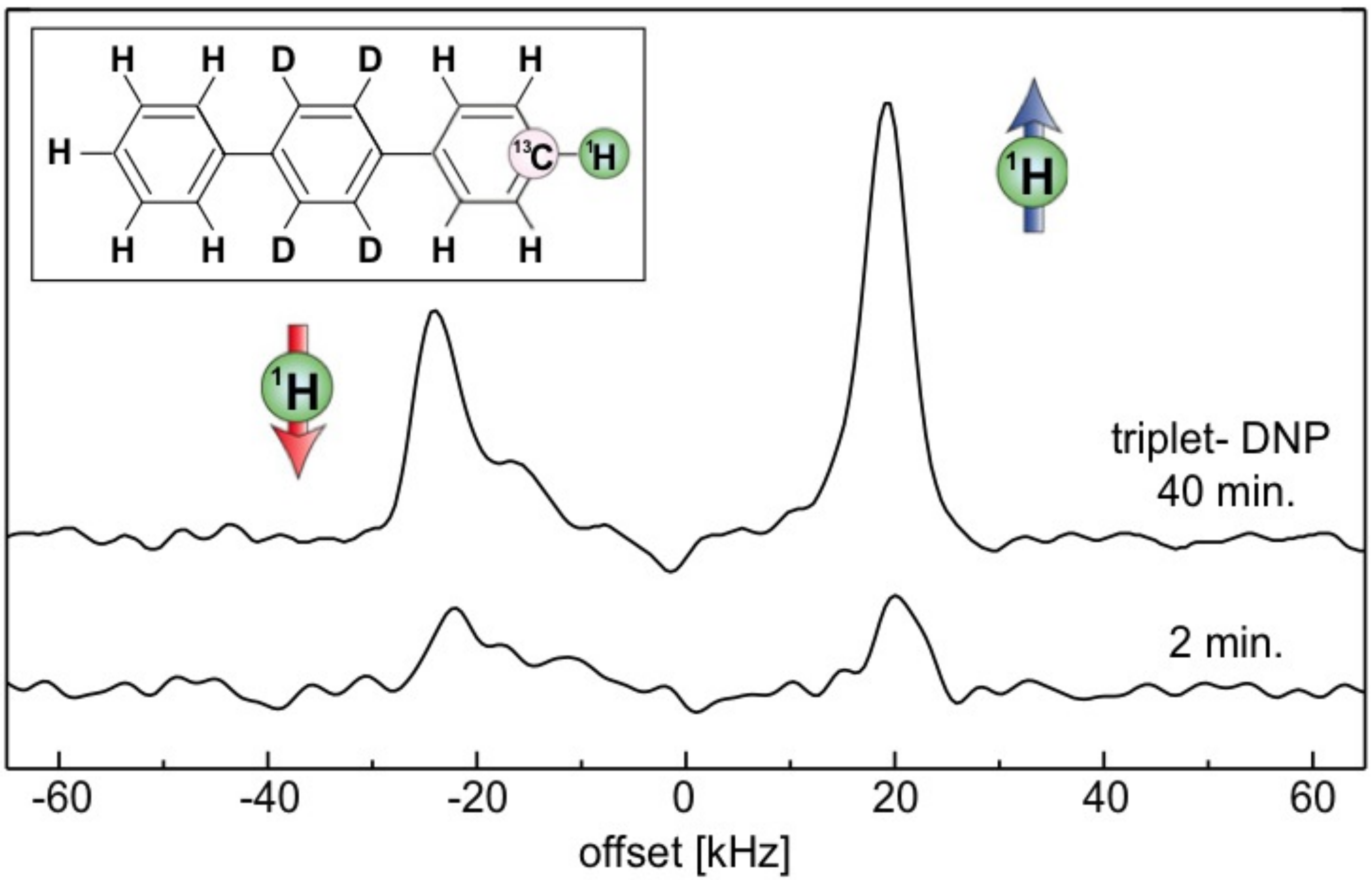
Photo-chemically induced DNP (Photo-CIDNP)



Kenichiro Tateishi et al, Room temperature hyperpolarization of nuclear spins in bulk, PNAS May 2014

Photo-chemically induced DNP (Photo-CIDNP)

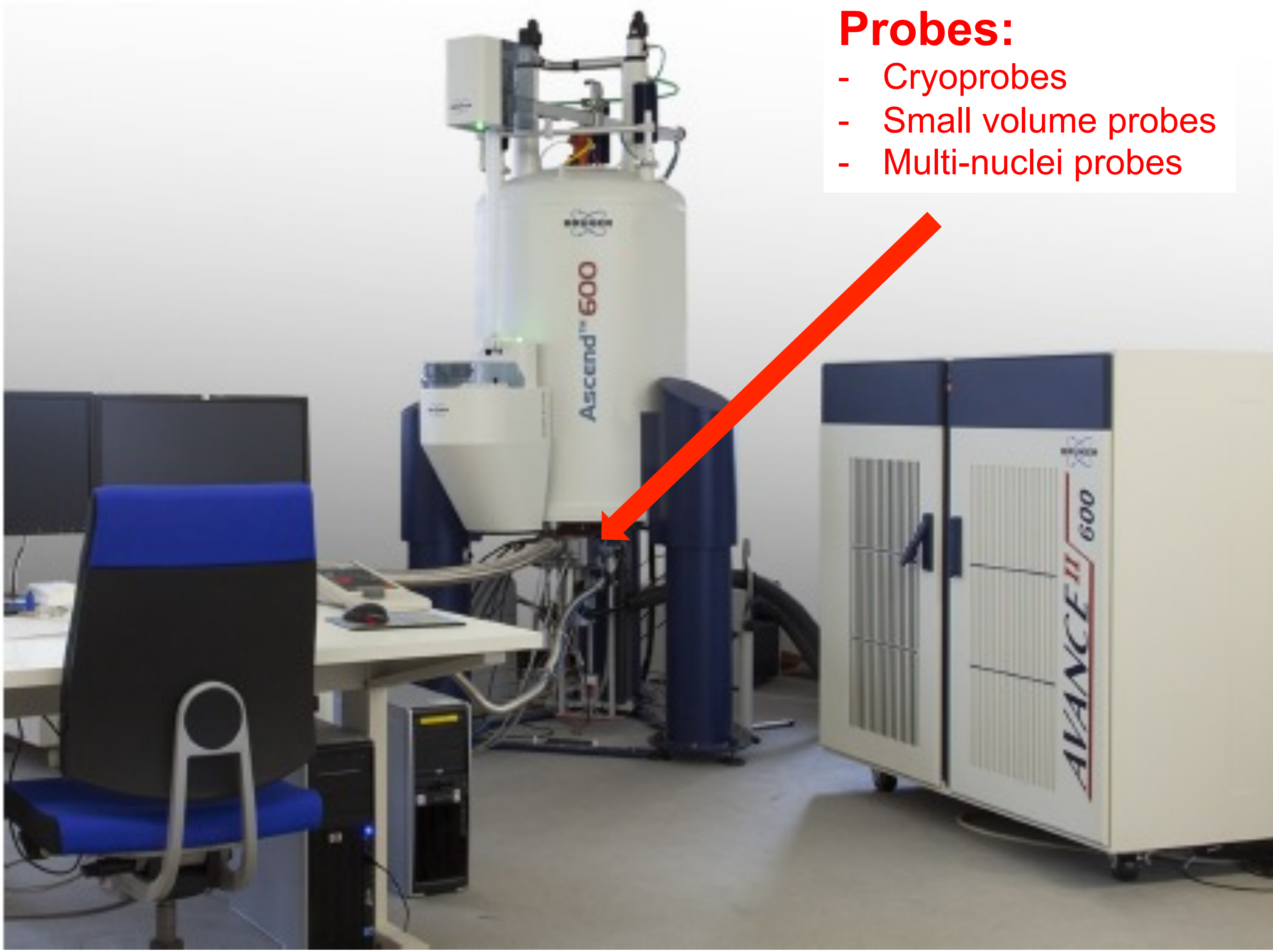
Proton polarization leads to lines with asymmetric coupling. 30% polarization at room temperature in this case (x 250 000)



Kenichiro Tateishi et al, Room temperature hyperpolarization of nuclear spins in bulk, PNAS May 2014

Technological innovations

Probes:
- Cryoprobes
- Small volume probes
- Multi-nuclei probes



Gain with a cryoprobe

Induced Signal Voltage to Noise Voltage

$$\frac{S}{N} \propto \frac{U_I}{U_N} \propto \frac{\omega \cdot M_0 \cdot V \cdot (B_1/I_{coil})}{\sqrt{4 \cdot k \cdot \Delta f \cdot R \cdot T}}$$

Coil Design

Coil Resistance

Temperature

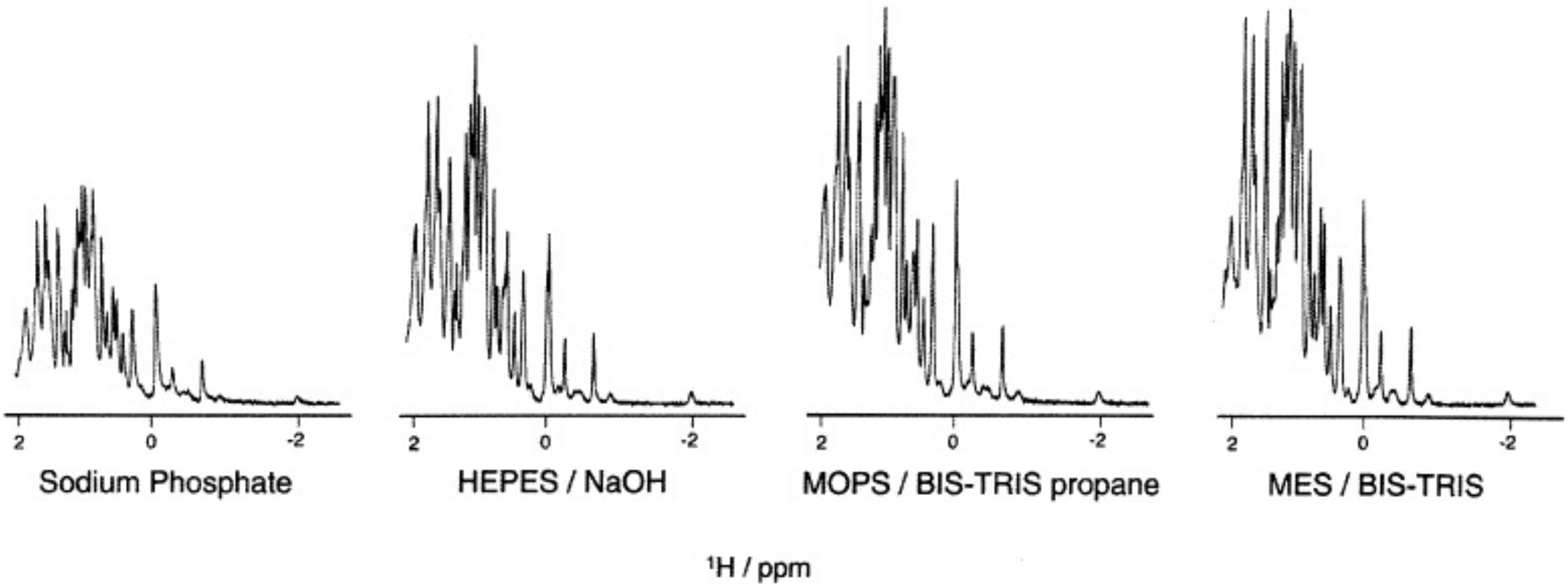
$$S/N \propto Q \eta M_0$$

Q quality factor, η filling factor

Limitations of cryoprobes

Low-Conductivity Buffers for High-Sensitivity NMR Measurements

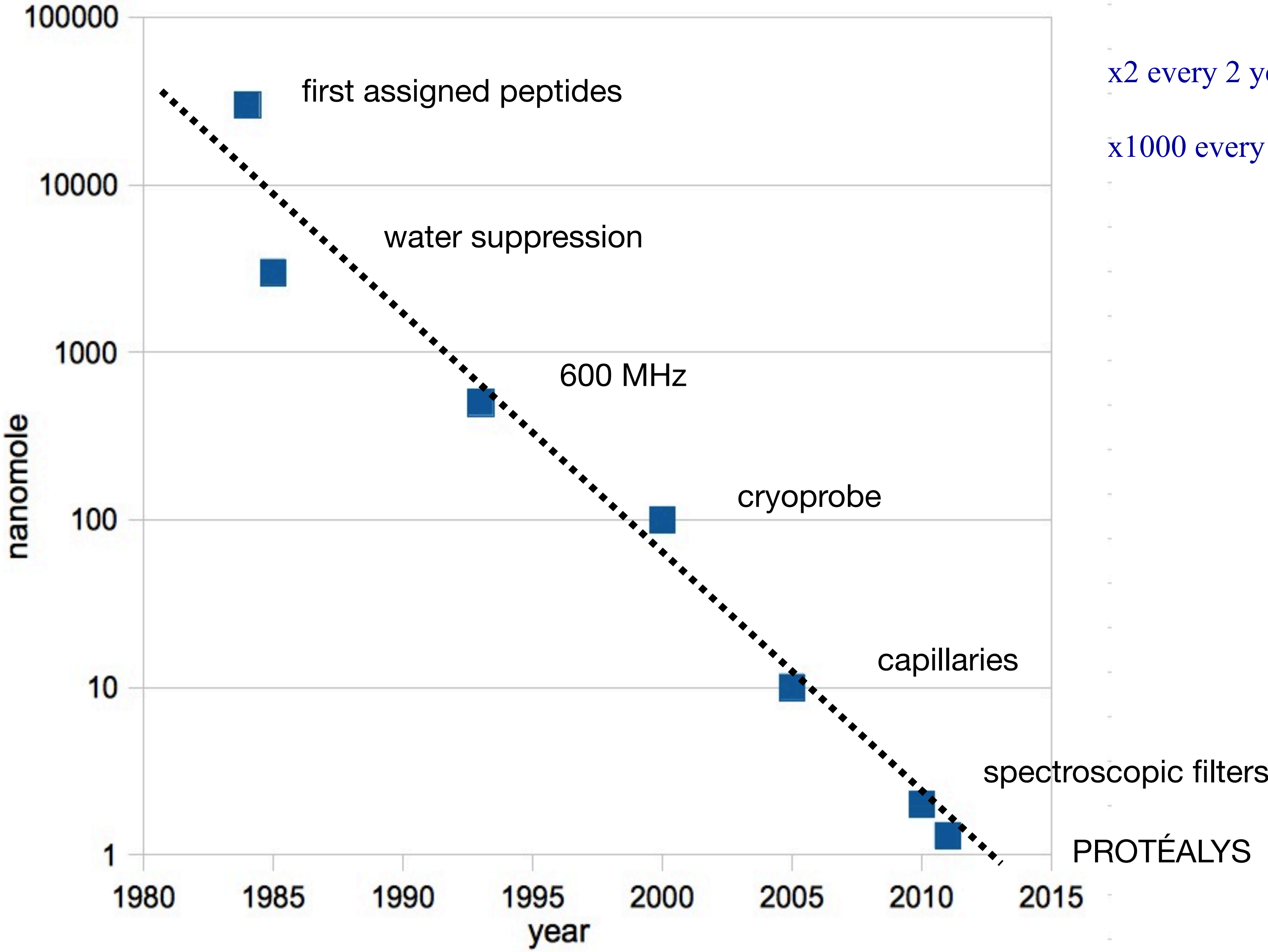
Alexander E. Kelly,† Horng D. Ou,† Richard Withers,‡ and Volker Dötsch*,§



JACS, 2002

Gain with a cryoprobe

Quantity of protein detected



x2 every 2 years

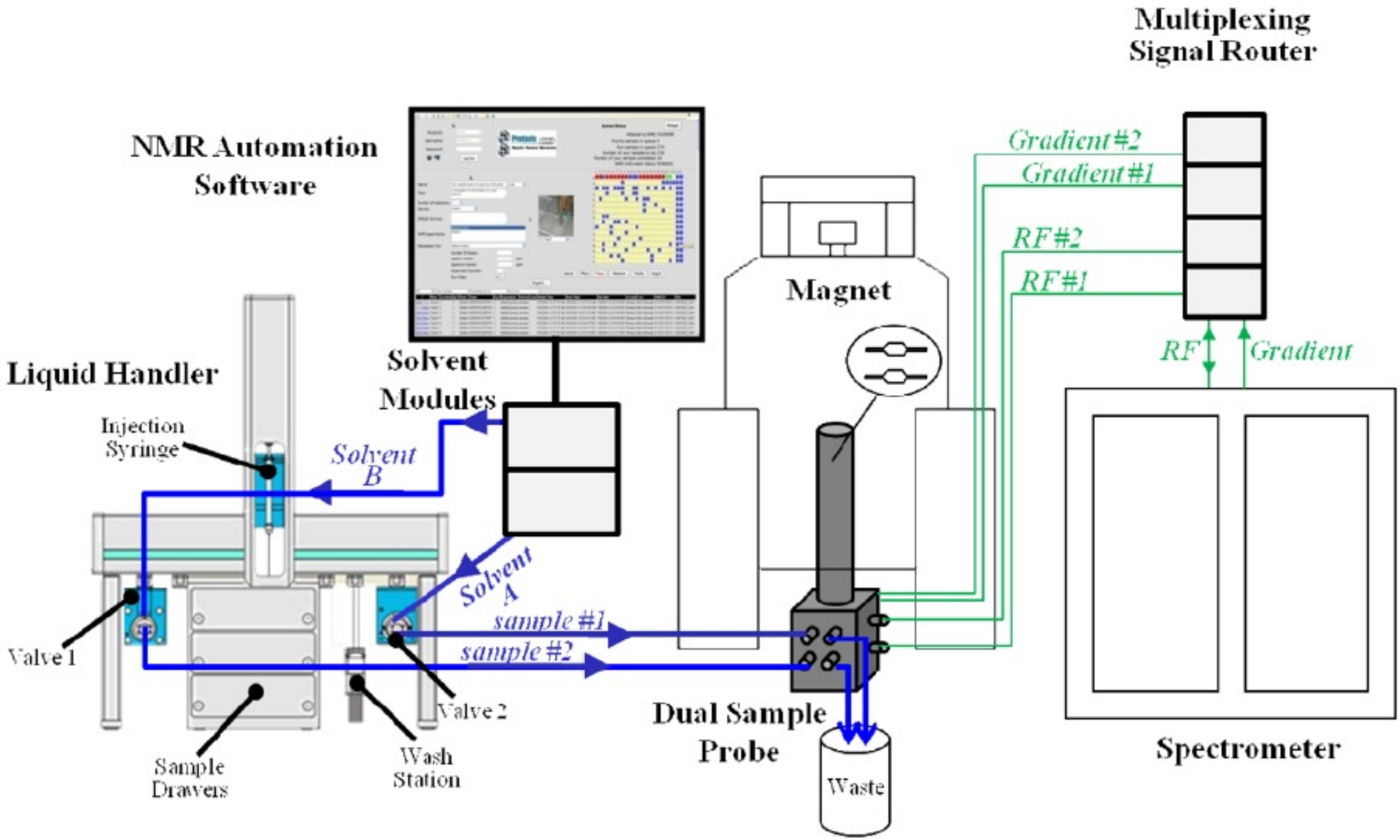
x1000 every 20 years

Data M.-A. Delsuc, IGBMC

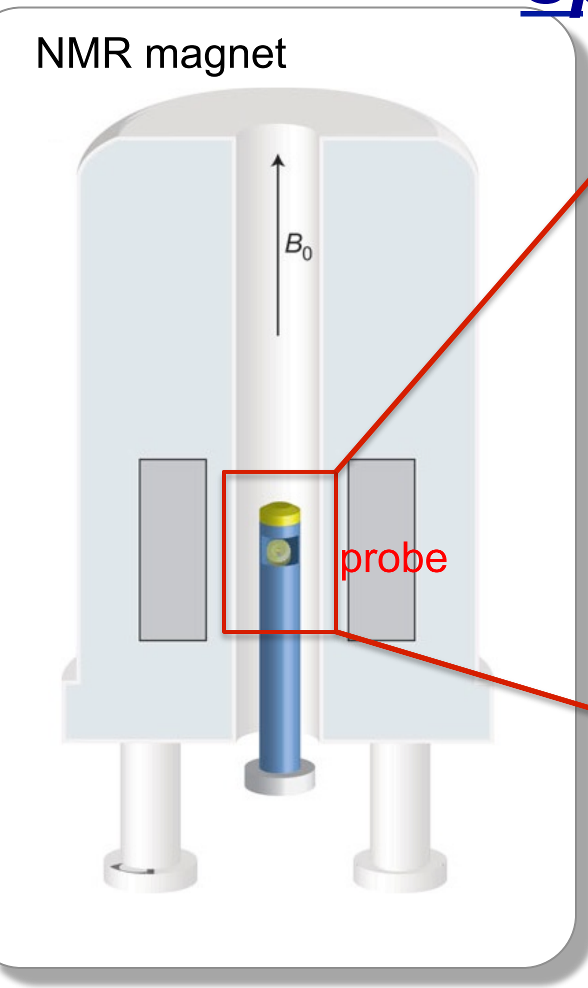
Anal Chem. 2010 September 1; 82(17): 7227–7236. doi:10.1021/ac101003f.

Multiplexed NMR: An Automated CapNMR Dual-Sample Probe

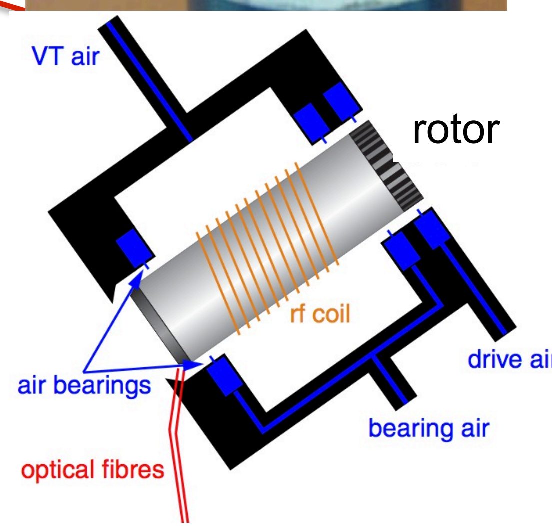
James A. Norcross[†], Craig T. Milling[†], Dean L. Olson[†], Duanxiang Xu[†], Anthony Audrieth[†], Robert Albrecht[†], Ke Ruan[§], John Likos[§], Claude Jones[§], and Timothy L. Peck[*,†]



Instrumentation for Magic-Angle-Spinning ssNMR

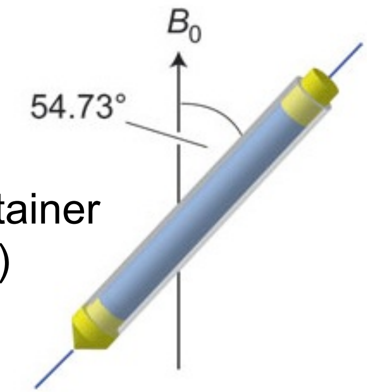


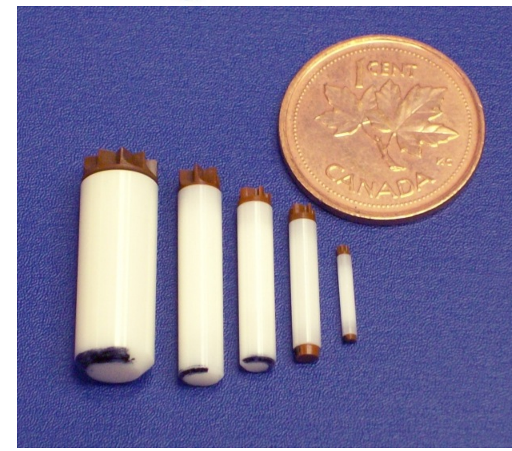
probe



rotation driven by gas flows

sample container
("rotor")



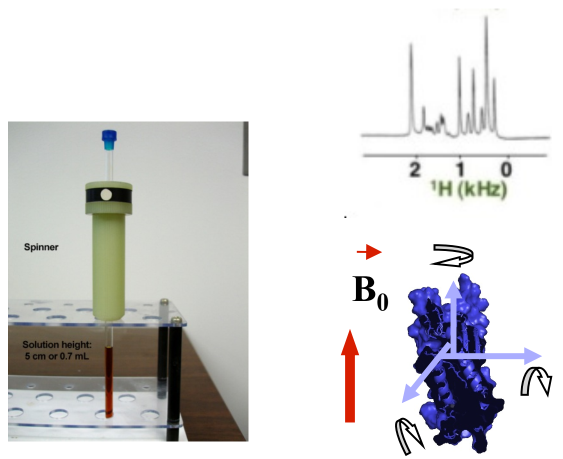


diameter	max. speed	sample volume
4 mm	15 kHz	70 μL
3.2 mm*	25 kHz	30 μL
1.6 mm*	40 kHz	8 μL
1.3 mm*	67 kHz	1.7 μL
0.9 mm	100 kHz	0.7 μL

*currently or soon at IBS

Liquid *vs* solid-state probe

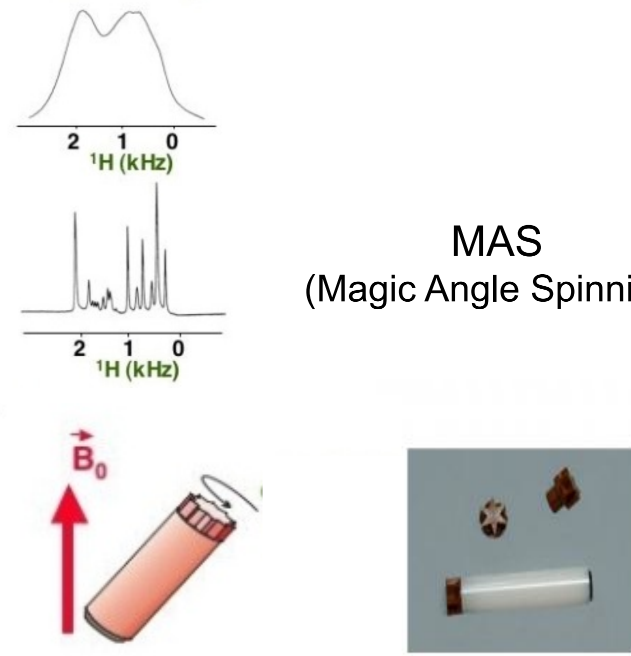
Liquid State NMR



Brownian motion

~ 400 µl of soluble sample

Solid State NMR

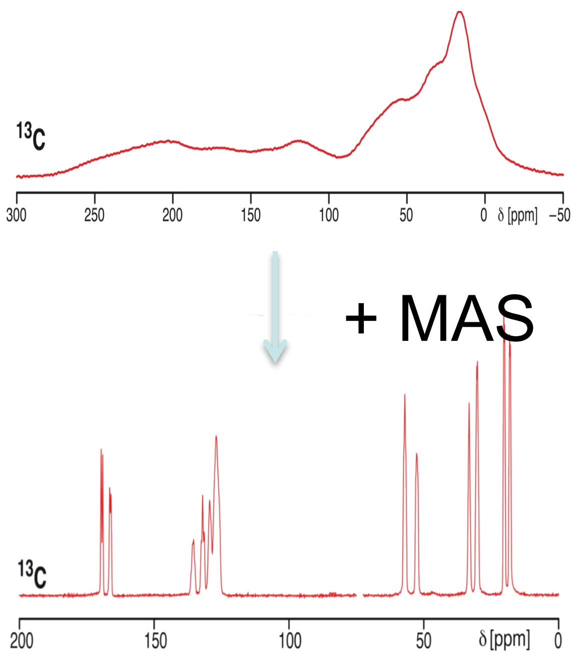


MAS
(Magic Angle Spinning)

~ 20 µl of hydrated insoluble sample

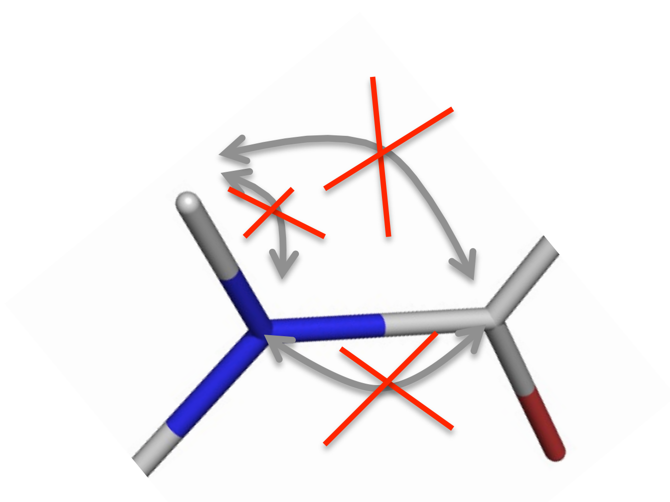
Solid-state NMR should allows to study large and insoluble proteins or biopolymers by NMR

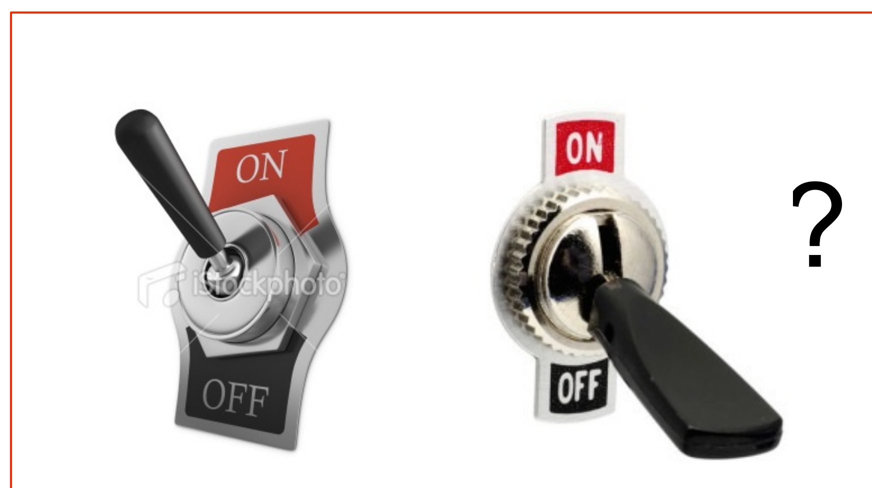
Can we turn on and off th anisotropic interactions as we want?



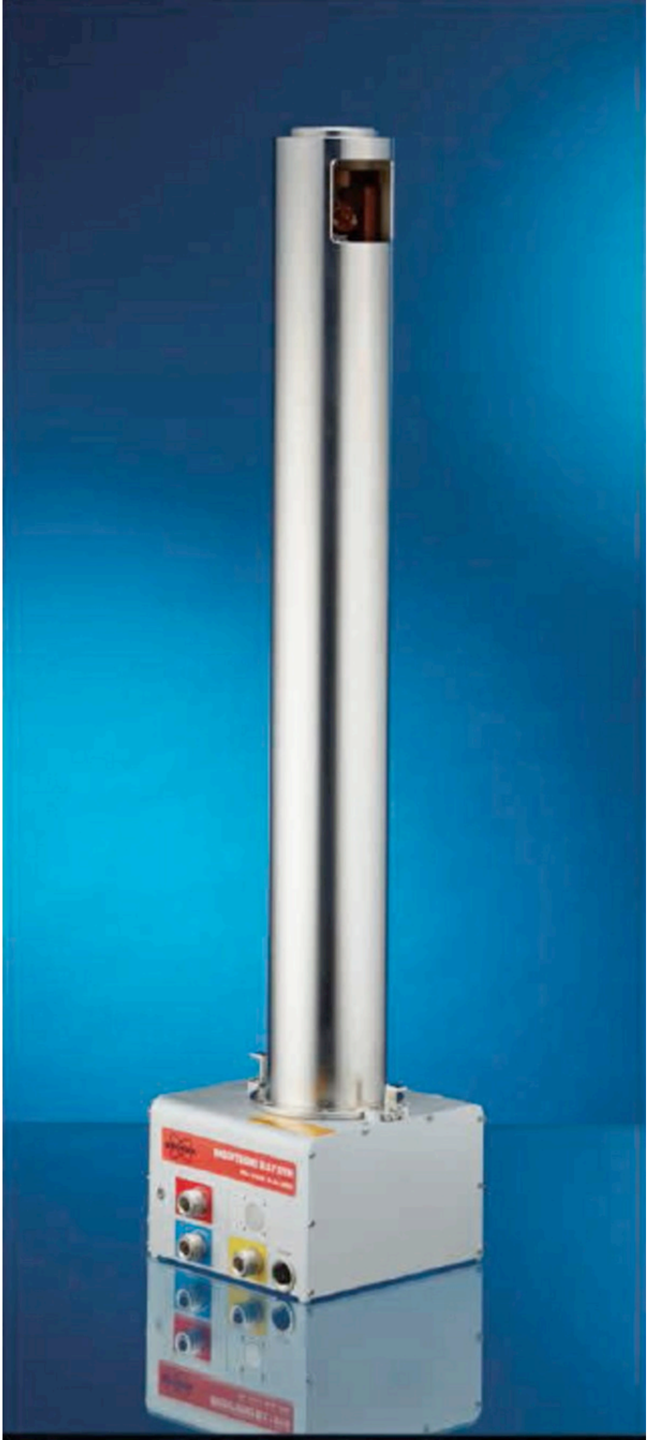
Through MAS we have turned off the anisotropic interactions (CSA, dipolar coupling).
→ nice resolution

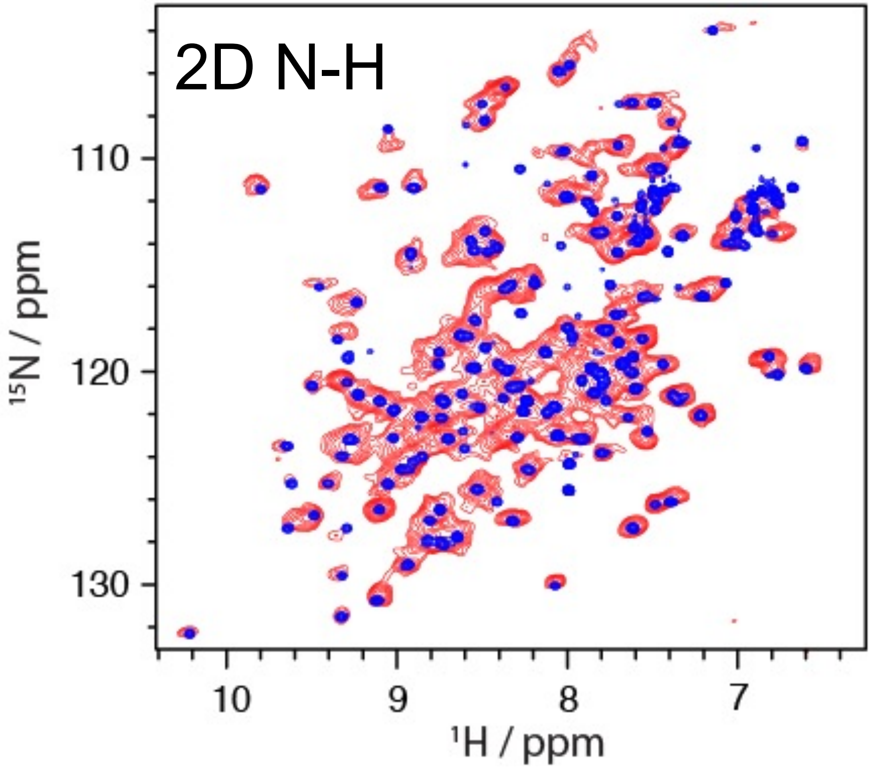
But these interactions could be useful, e.g. to transfer via the dipolar coupling.
→ Can we "switch them on" again ??





Solid-state fast rotation MAS (111 kHz)





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

Solid-state fast-rotation MAS

L. Emsley et al.
B. Meier et al.

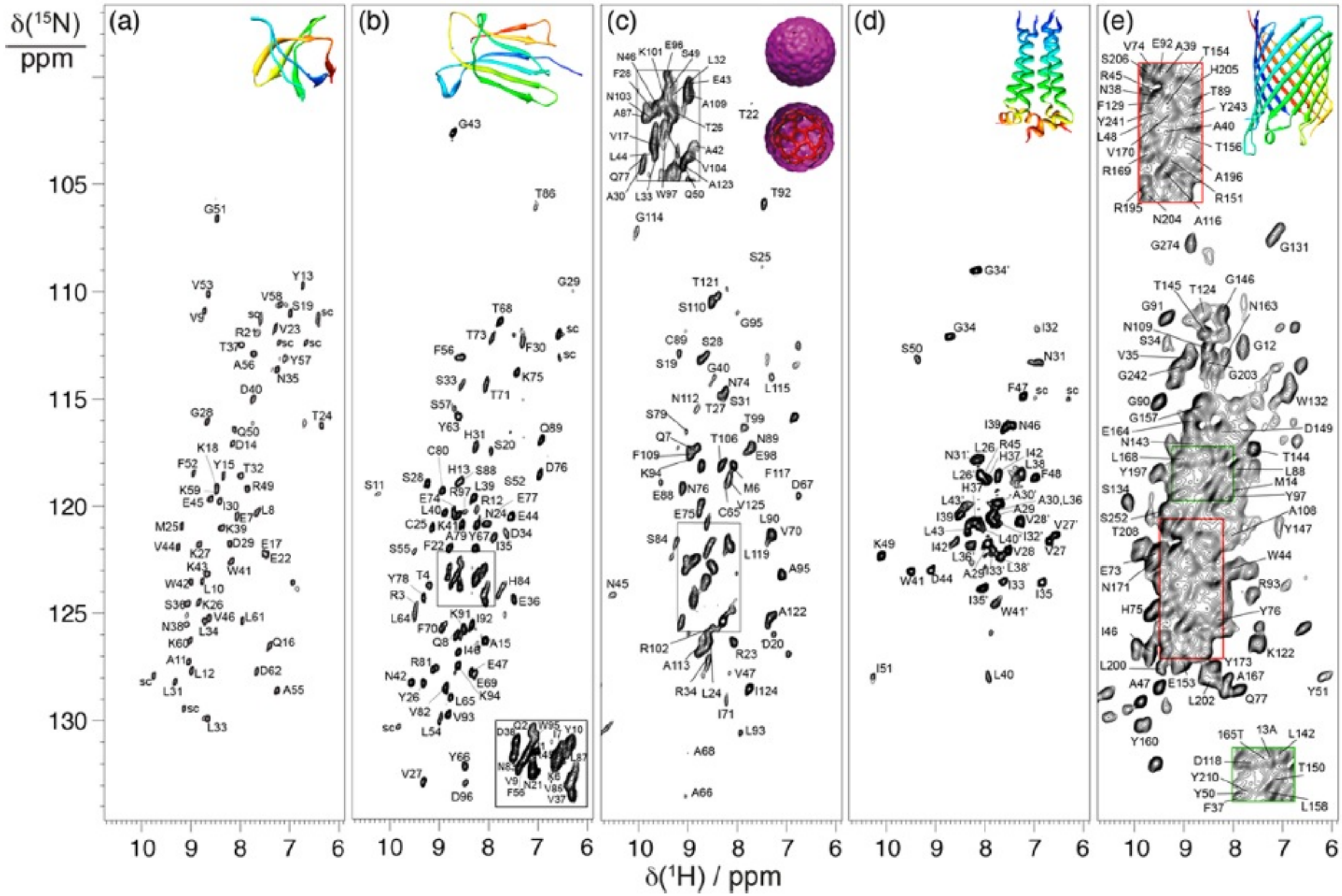
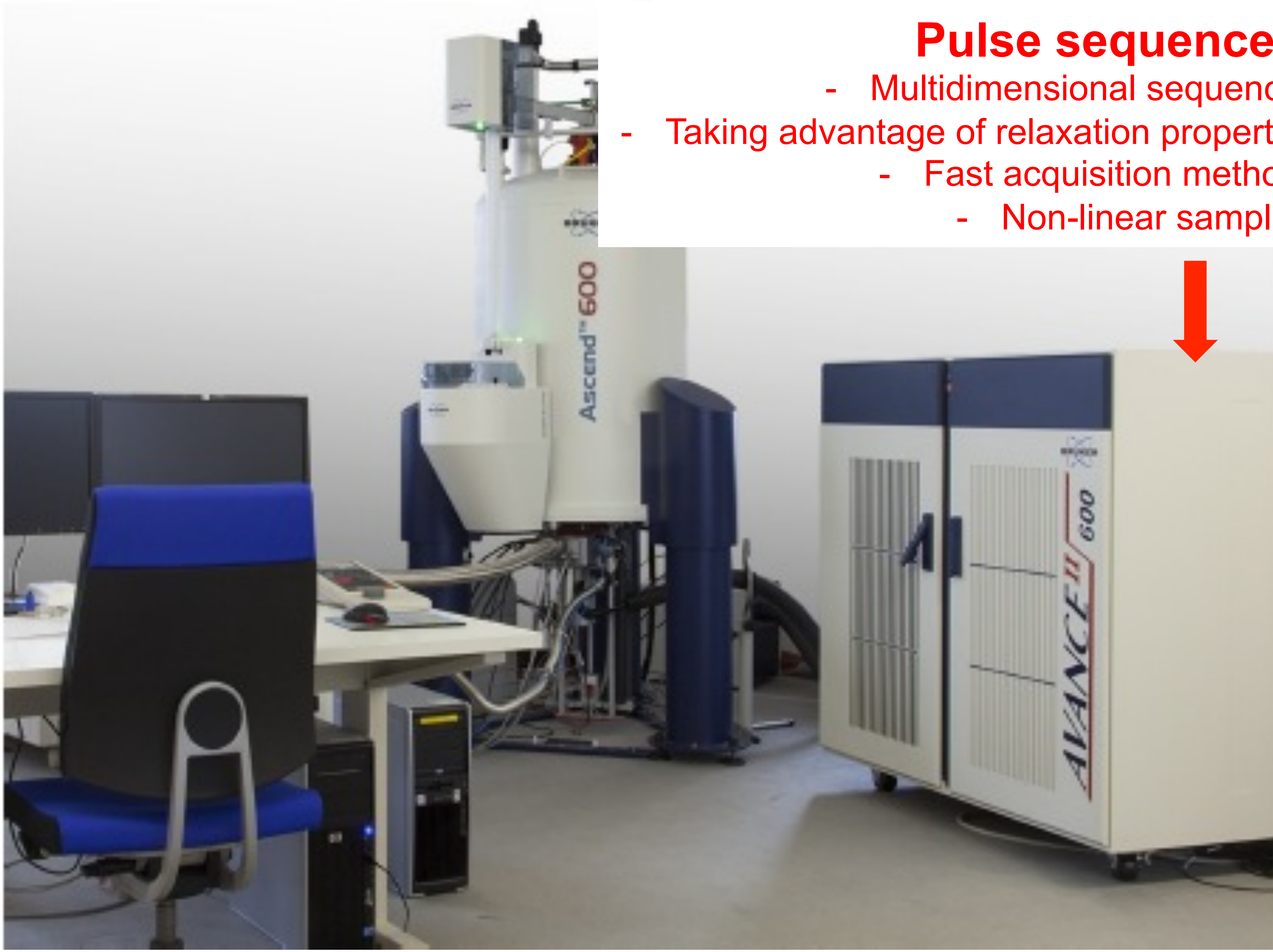


Figure 2. $^{15}N-^{1}H$ correlation spectra recorded on a 1 GHz spectrometer under 60 kHz MAS for [U-H^N,^2H,^{13}C,^{15}N]-labeled (a) microcrystalline SH3, (b) microcrystalline β2m, and (c) sedimented nucleocapsids of AP205, (d) M2 channel, and (e) OmpG.

Technological innovations

Pulse sequences:

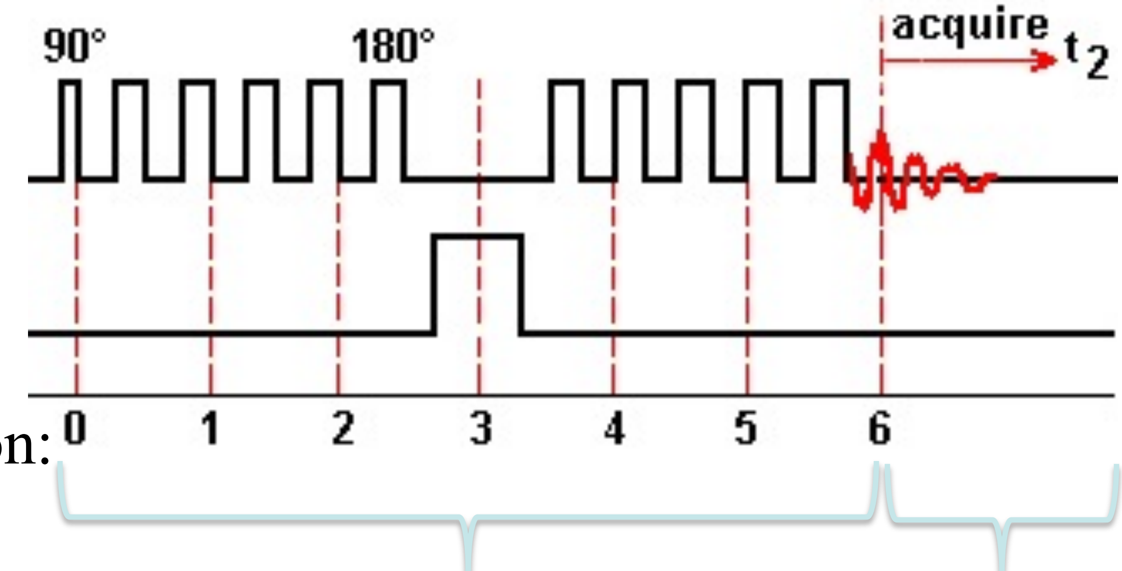
- Multidimensional sequences
- Taking advantage of relaxation properties
- Fast acquisition methods
- Non-linear sampling



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

Rotational Echo DOuble Resonance (REDOR)

sample rotation:



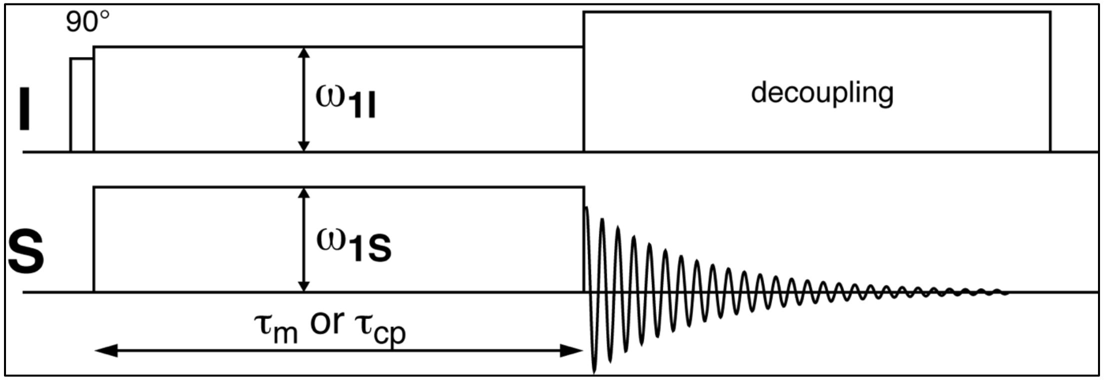
dipolar coupling "turned on"
i.e. we can transfer via D coupling

only MAS: high resolution
during detection period

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

More formally:
Interference between time-dependent processes:
- rotation of sample
- rotation of spins

Cross-polarization experiment



$$\omega_{1,I} \pm \omega_{1,S} = \omega_{MAS}$$

for example:
MAS = 100 kHz
RF field on 1H: 80 kHz
RF field on 13C: 20 kHz

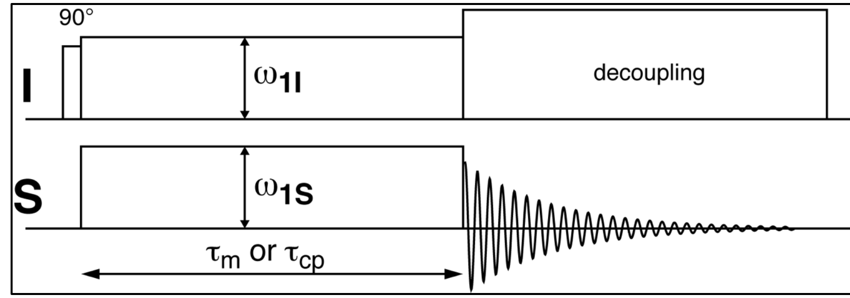
Dipolar transfer experiments in ssNMR

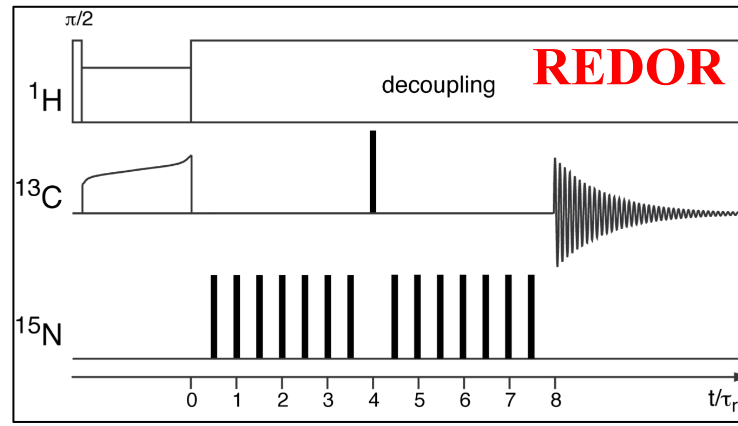
$$\widehat{\mathcal{H}} = \sum_i \sum_{l=0}^{2} A_l^{(i)} \cdot \hat{T}_l^{(i)}$$

space part

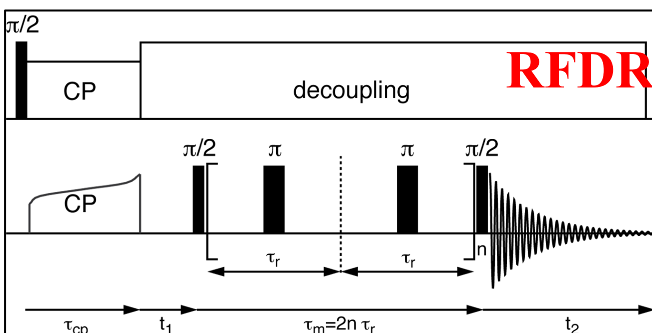
spin part

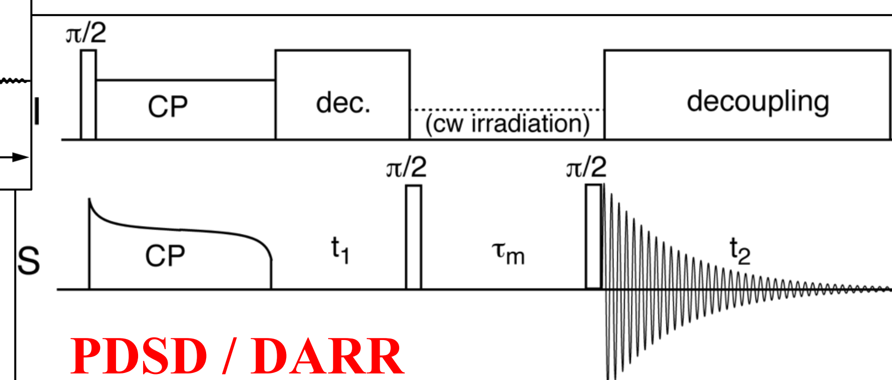
cross-polarization





General idea:
rotate sample (MAS)
+ rotate spins (RF irradiation)
→ create interference between these two processes



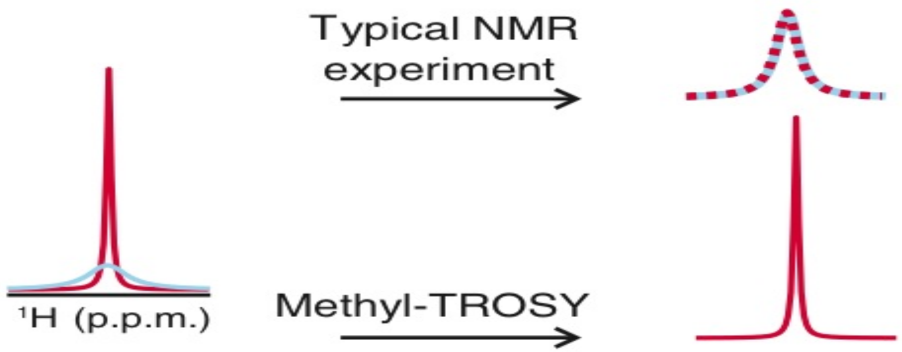


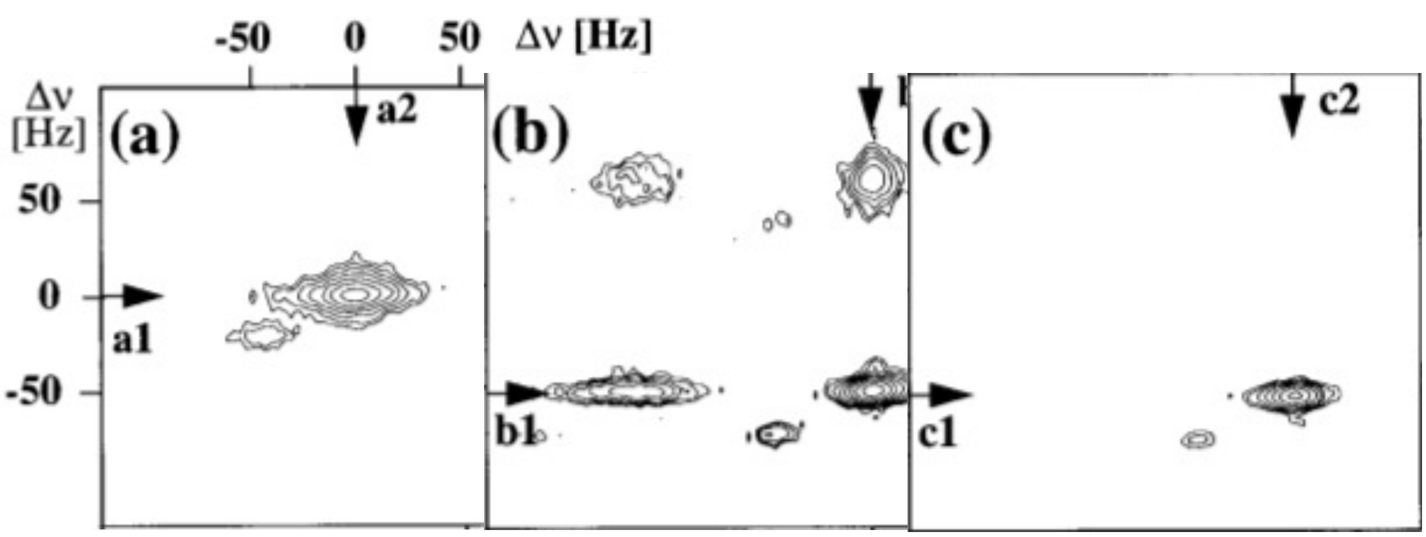
HORROR
DREAM
R-sequences
C-sequences ,….

Back to the liquid-state …
Exploitation of the relaxation properties

1. Transverse relaxation:

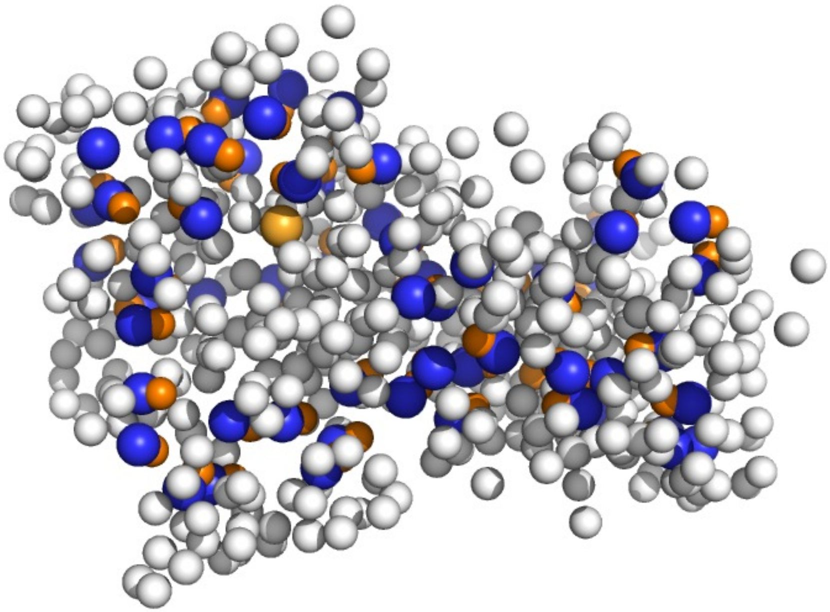
- Exploitation between different relaxation mechanisms (CSA-DD) => TROSY





Exploitation of the relaxation properties

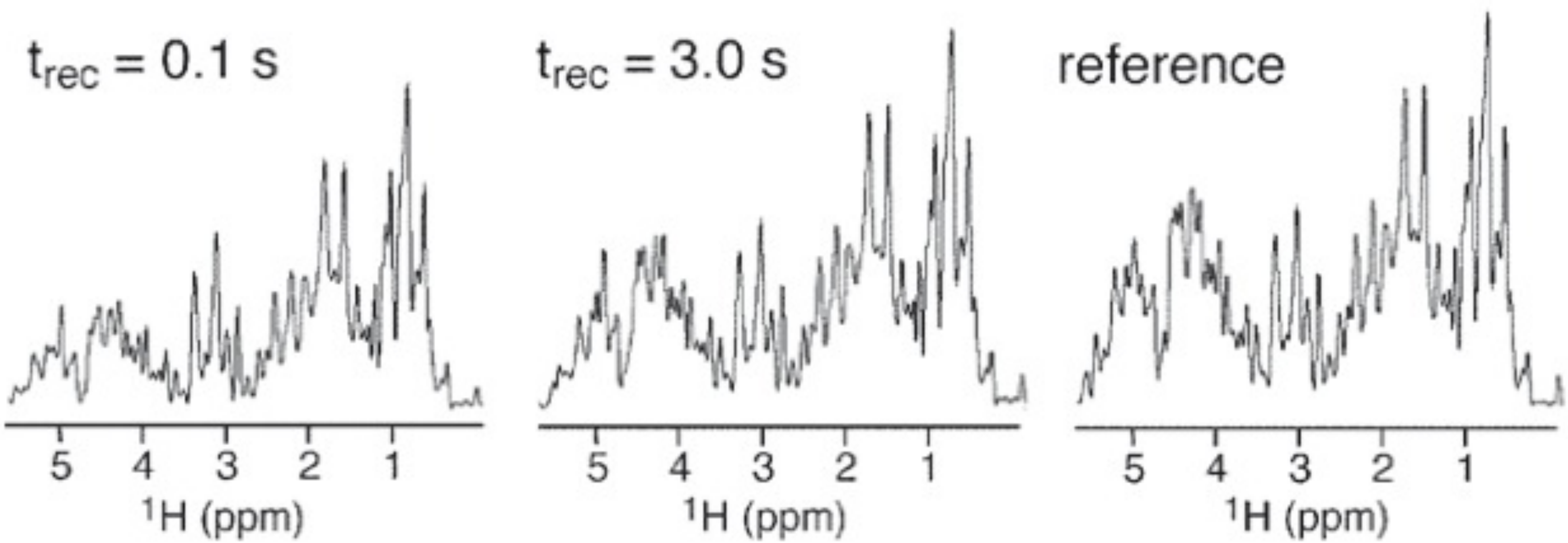
2. Longitudinal relaxation:
   - Accelerate the return to the thermodynamic equilibrium to speed-up the acquisition process => SOFAST, BST, BEST-TROSY



Schanda P, Brutscher B. JACS (2005), Lescop E, Schanda P, Brutscher B. J Magn Reson. (2007)
Schanda P, Van Melckebeke H, Brutscher B. JACS (2006)
Solyom Z, Schwarten M, Geist L, Konrat R, Willbold D, Brutscher B. J Biomol NMR. 2013 Apr;55(4):311-21.
Favier A, Brutscher B. J Biomol NMR. 2011 Jan;49(1):9-15.

Exploitation of the relaxation properties

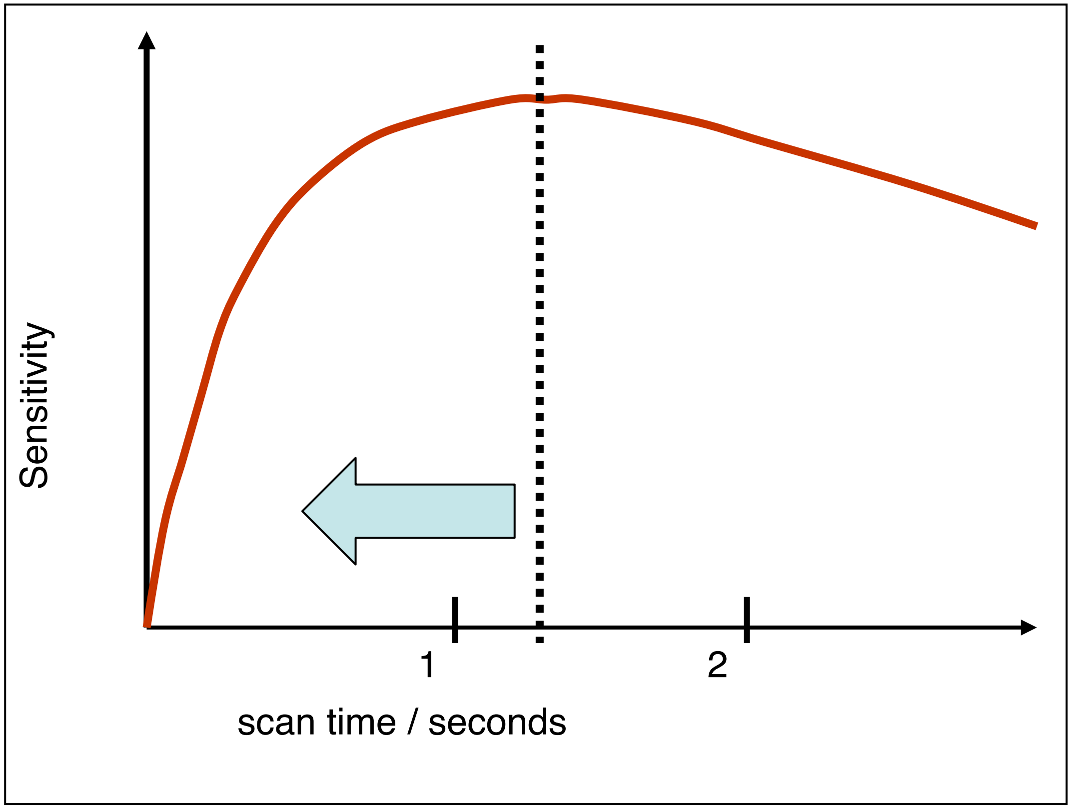
2. Longitudinal relaxation:
   – Accelerate the return to the thermodynamic equilibrium to speed-up the acquisition process => SOFAST, BST, BEST-TROSY



B. Brutscher et al. 2005

Exploitation of the relaxation properties

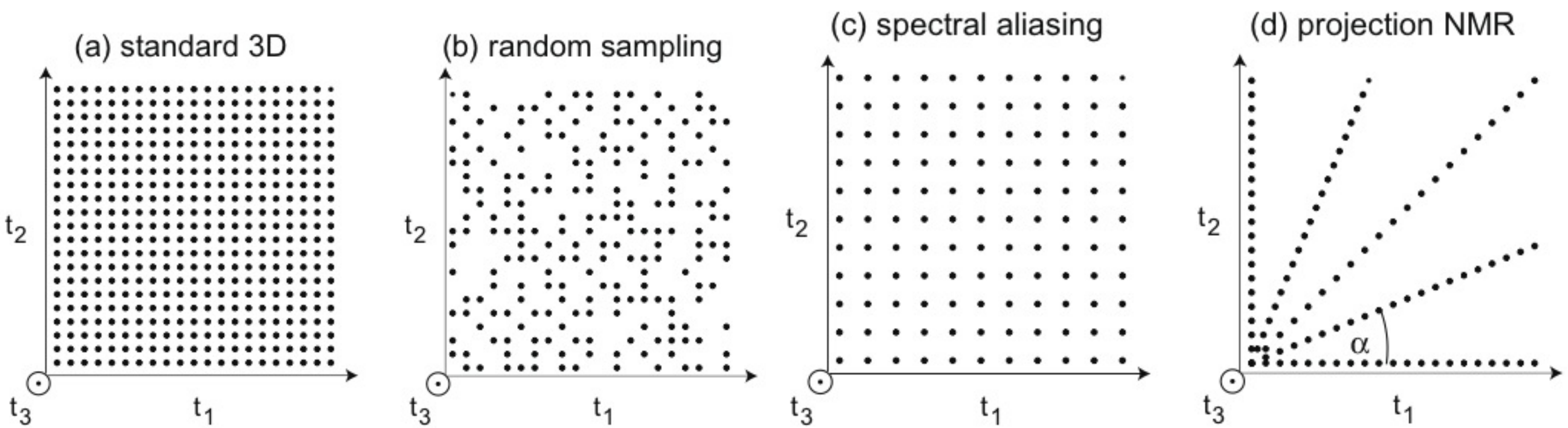
2. Longitudinal relaxation:
   - Accelerate the return to the thermodynamic equilibrium to speed-up the acquisition proces => SOFAST, BST, BEST-TROSY



B. Brutscher et al. 2005

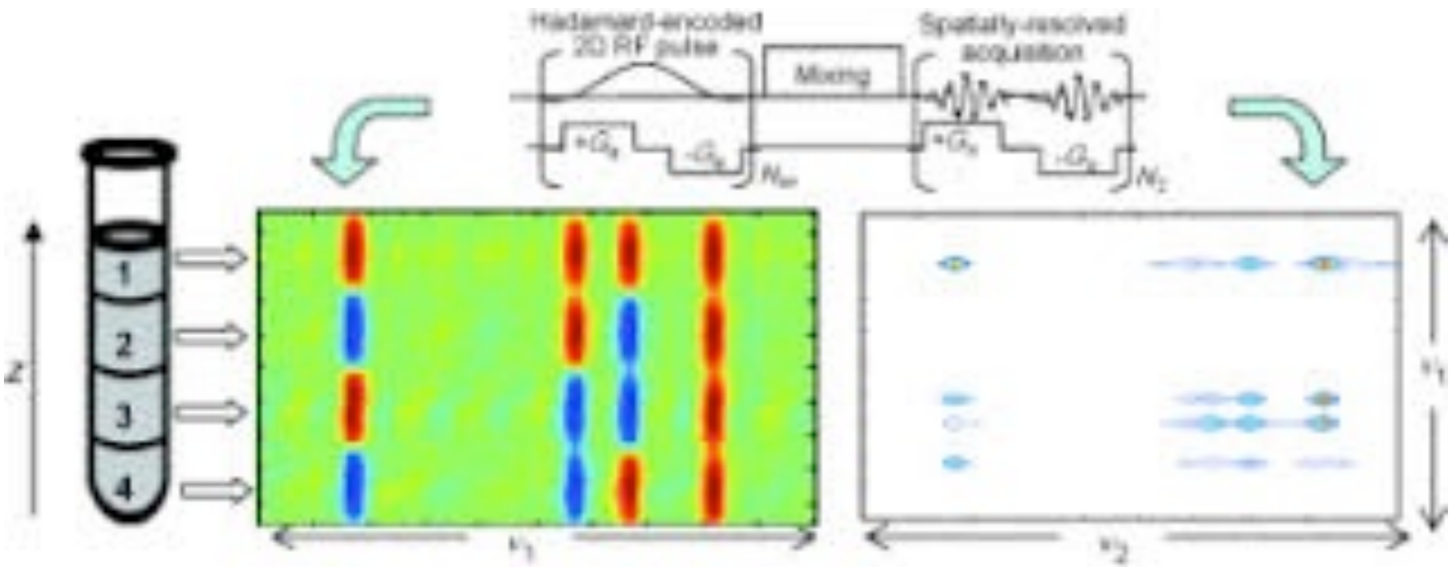
Alternative sampling methods

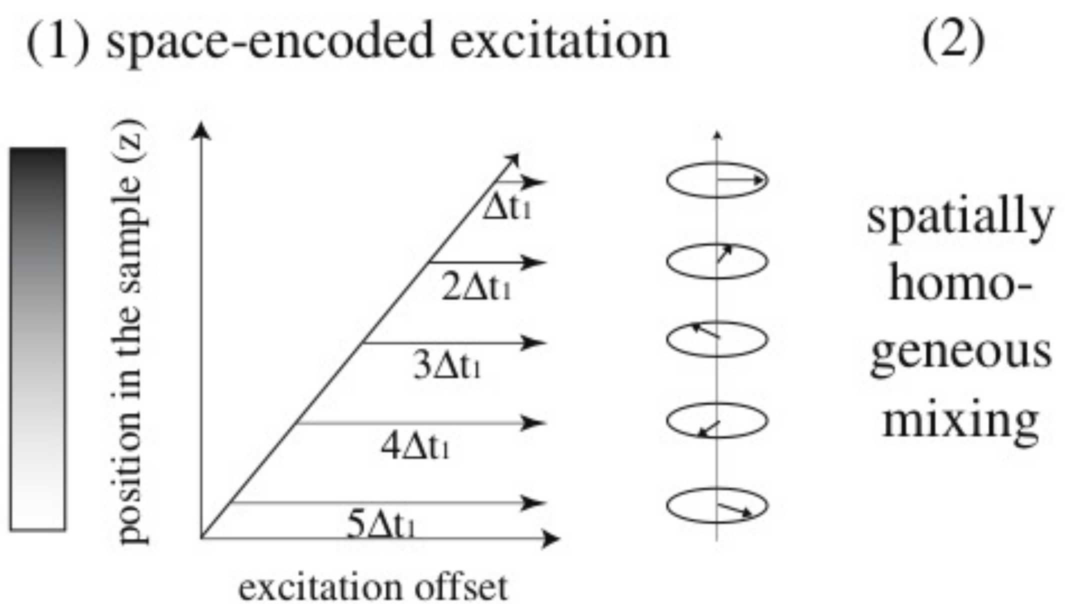
- The use of FFT implies a linear sampling
- Alternative methods (NUS) are now proposed

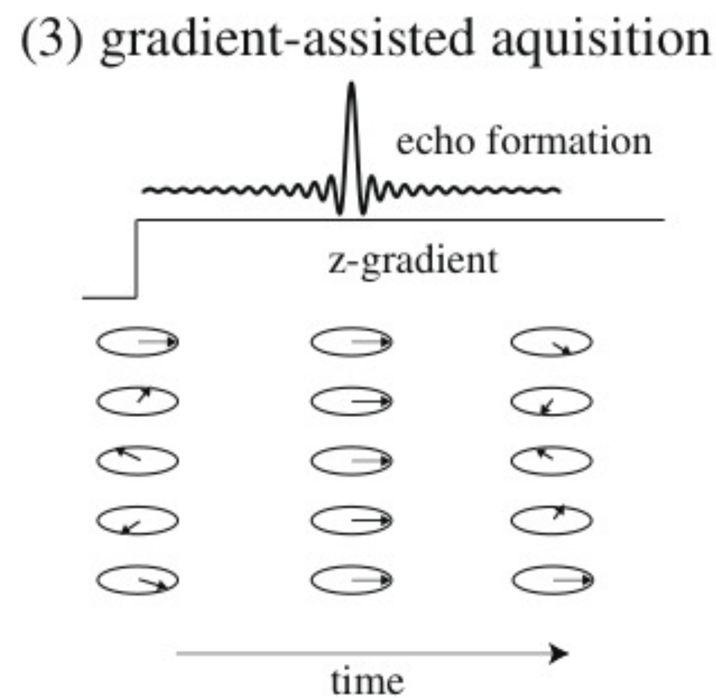


P Schanda Progress NMR Spect. 2009

Alternative sampling methods







Single-scan spectroscopy

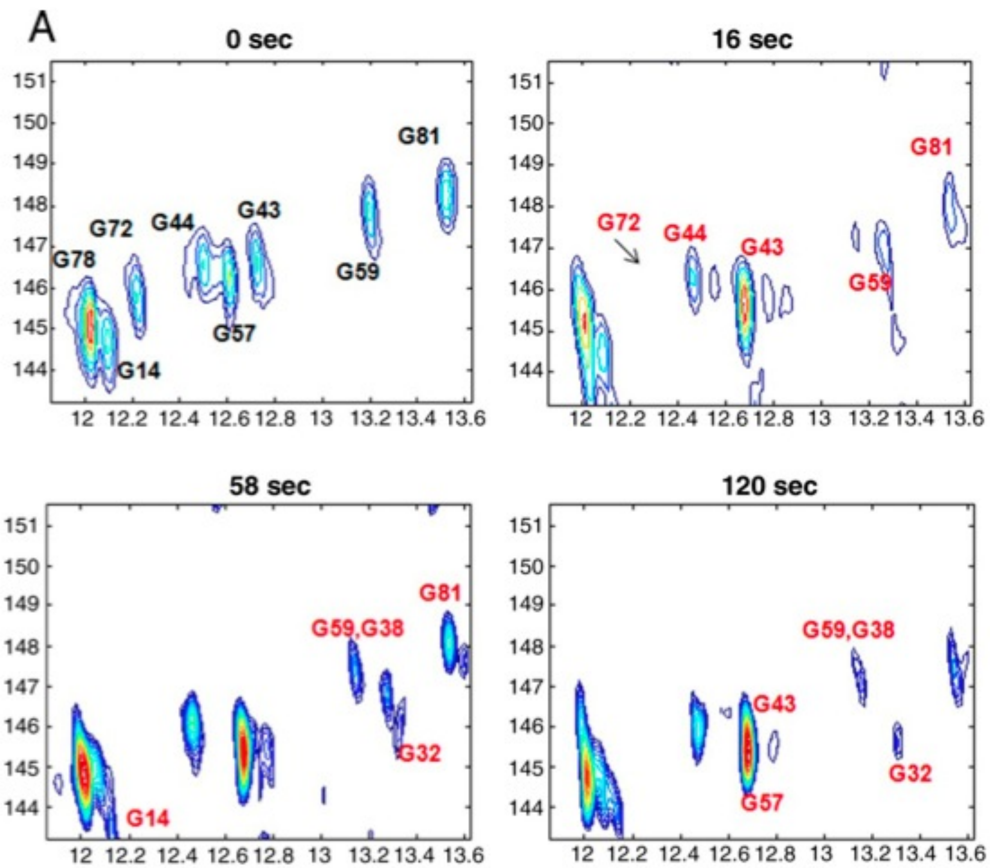
Frydman L, Scherf T, Lupulescu A. PNAS. 2002

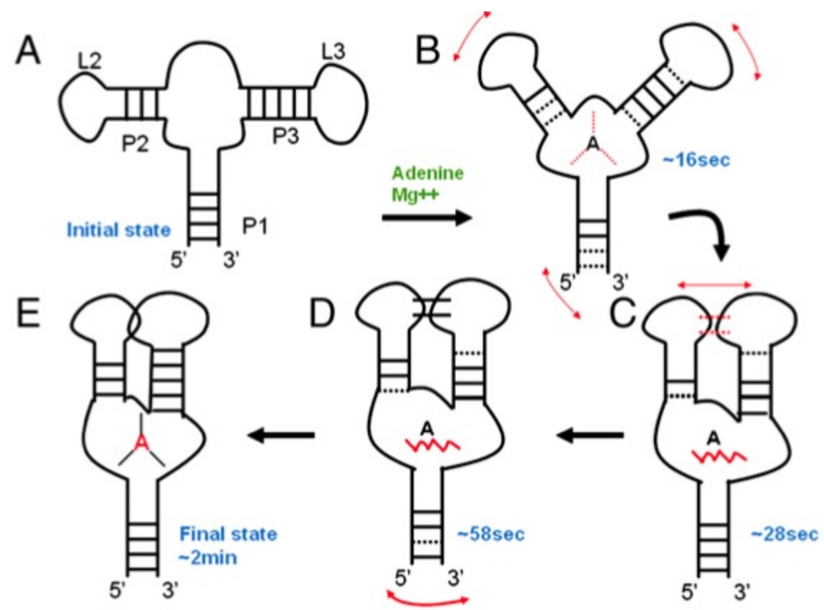
Application: following real-time folding of an RNA aptamer

Real-time multidimensional NMR follows RNA folding with second resolution

Mi-Kyung Lee[a,1], Maayan Gal[b,1], Lucio Frydman[b,2], and Gabriele Varani[a,c,2]

PNAS 2010





Technological innovations

Samples :

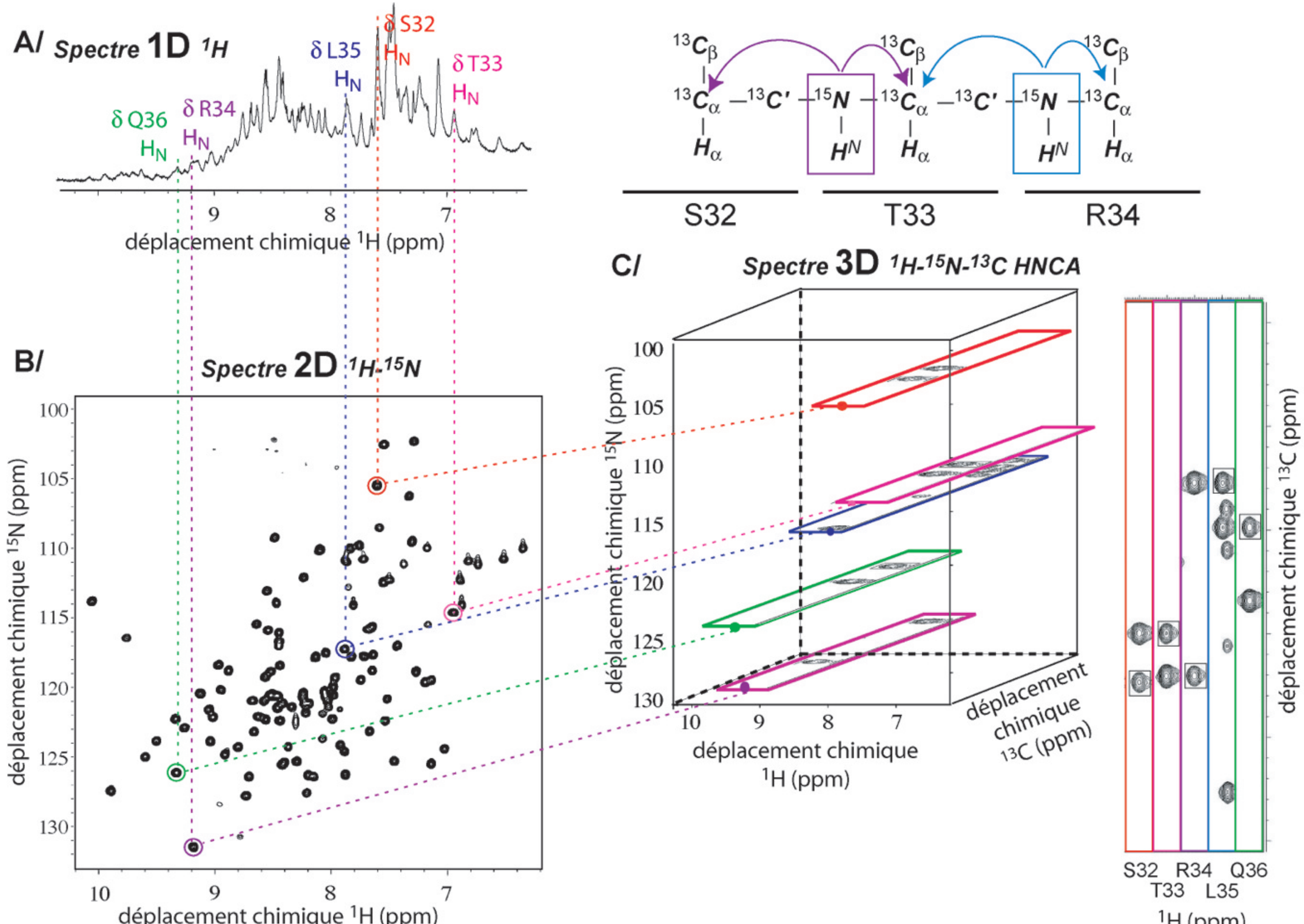
- Small volumes
- Isotopic labeling

Liquid-state small volume cryoprobe

- Proton-optimized triple resonance NMR probe
- Sensitivity increase by a factor of six on ^{1}H compared to a conventional 1.7 mm probe
- Four fully independent channels (plus lock channel)
- Simultaneous decoupling on multiple X-nuclei such as ^{13}C and ^{15}N
- Cooled ^{2}H preamplifier for excellent stability
- ATM (Automated Tuning and Matching) compatible
- 30 µL total sample volume (this volume perfectly matches the elution volume of HPLC-SPE and is well suited for automation)

BRUKER

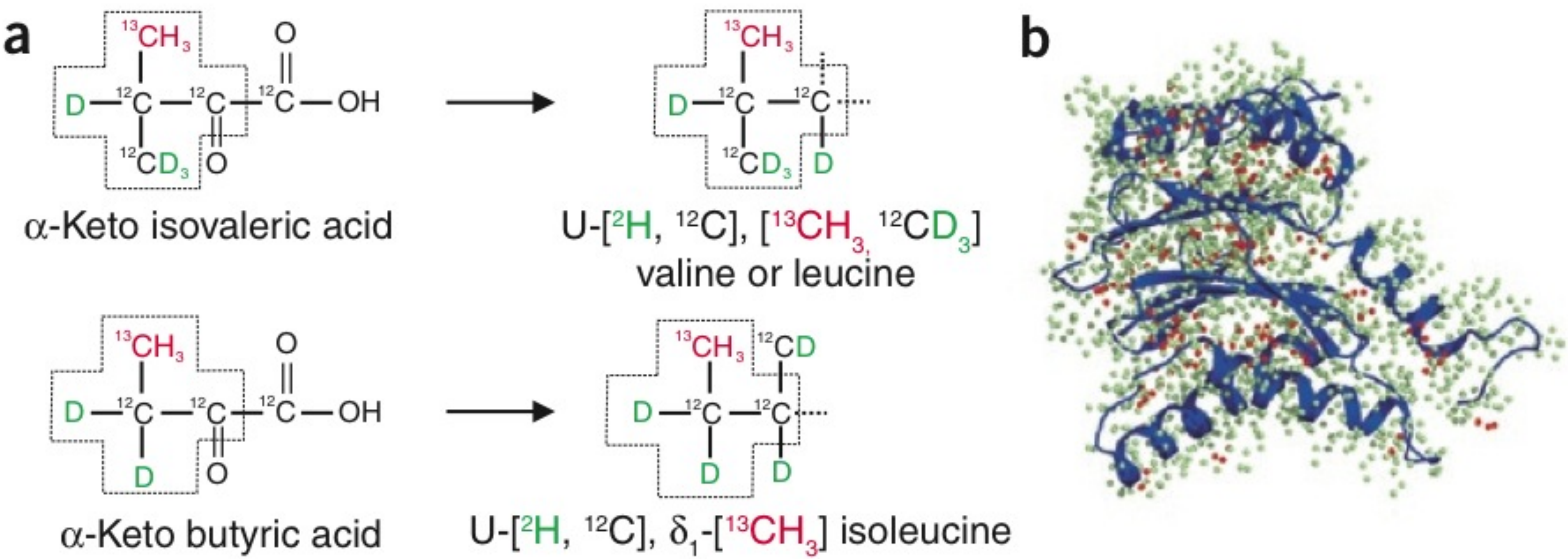
Standard methods: ^{13}C,^{15}N-labeling and 3D triple resonance spectroscopy



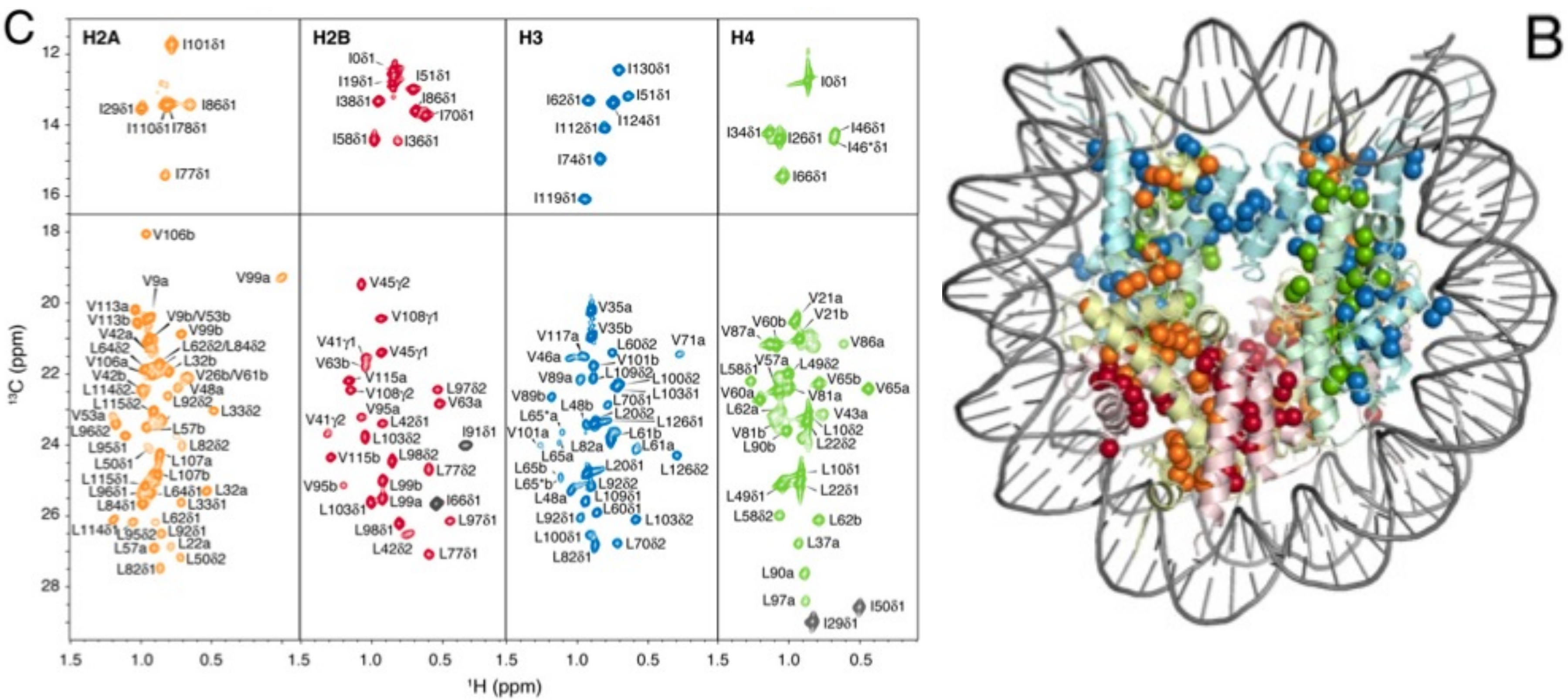
Solution NMR of supramolecular complexes: providing new insights into function

Remco Sprangers, Algirdas Velyvis & Lewis E Kay

Nature Methods 2007

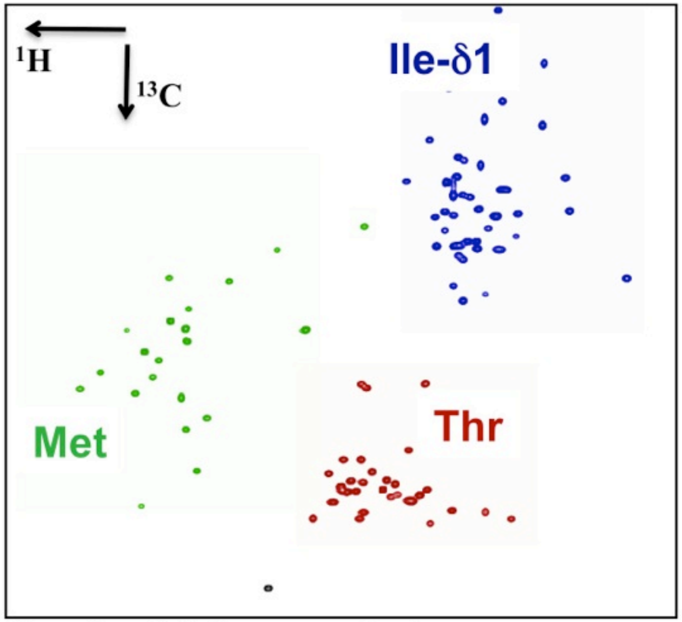


Exploring molecular mechanisms within the chromatin using NMR

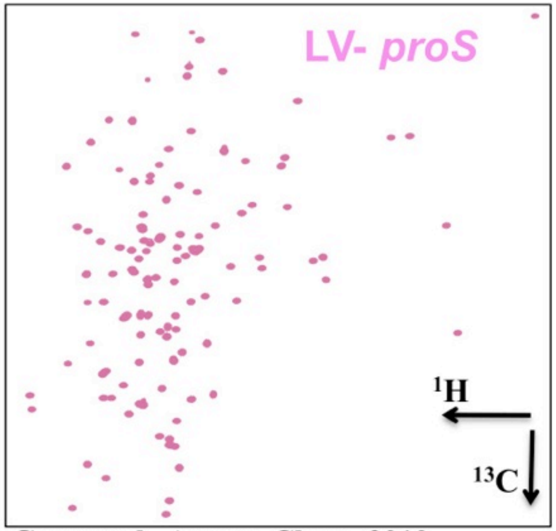


From Kato et al PNAS 2011

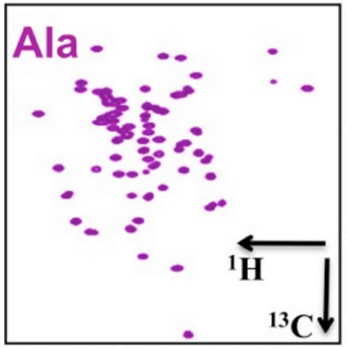
Methodologies developed in Grenoble



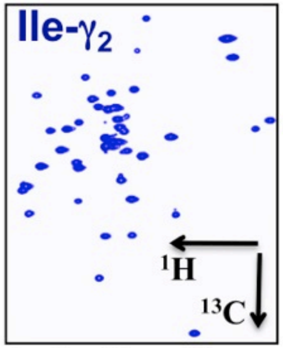
Hamelin et al. (in preparation)



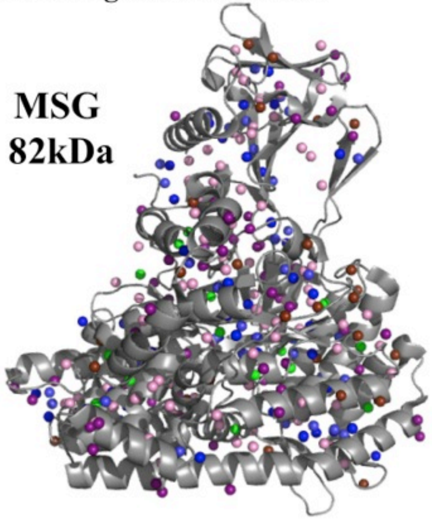
Gans et al. Angew. Chem. 2010



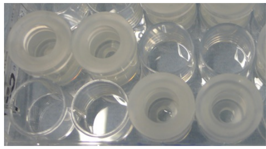
Ayala, J. Bio. NMR 2009

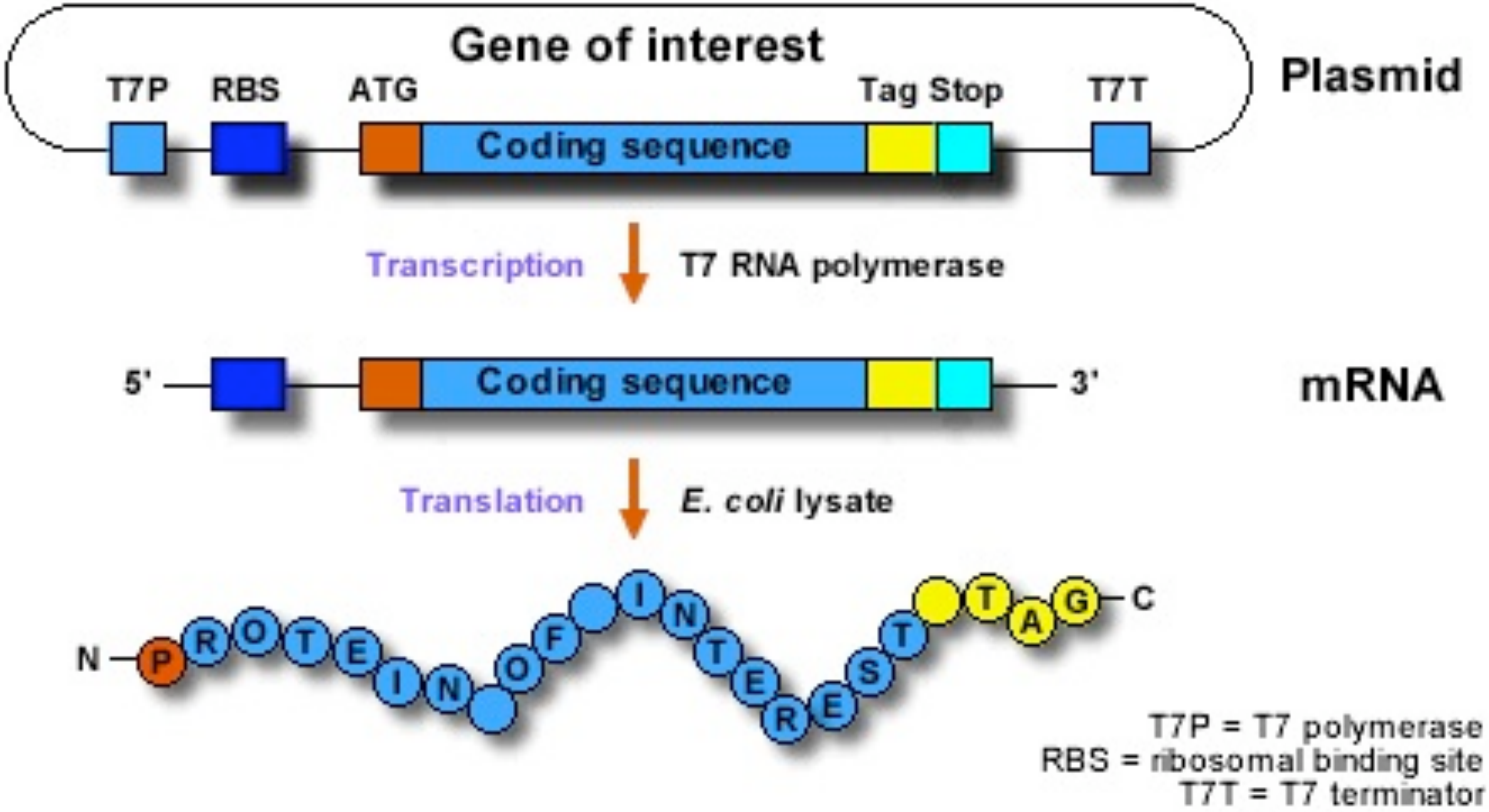


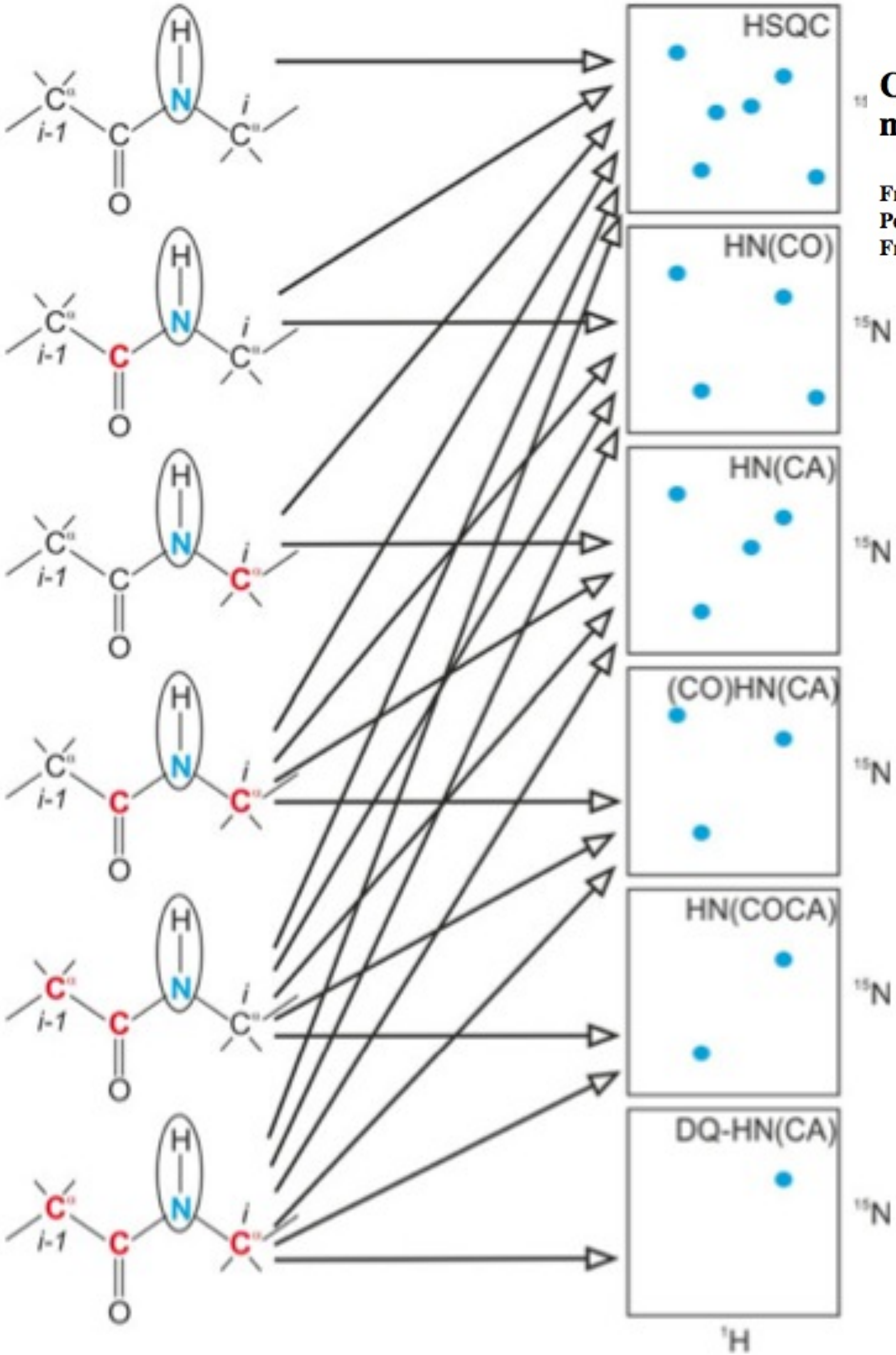
Ayala, Chem Comm 2012.



Cell free expression and combinatory isotopic labeling







Combinatorial triple-selective labeling as a tool to assist membrane protein backbone resonance assignment

Frank Löhr · Sina Reckel · Mikhail Karbyshev · Peter J. Connolly · Norzehan Abdul-Manan · Frank Bernhard · Jonathan M. Moore · Volker Dötsch

J Biomol NMR (2012)

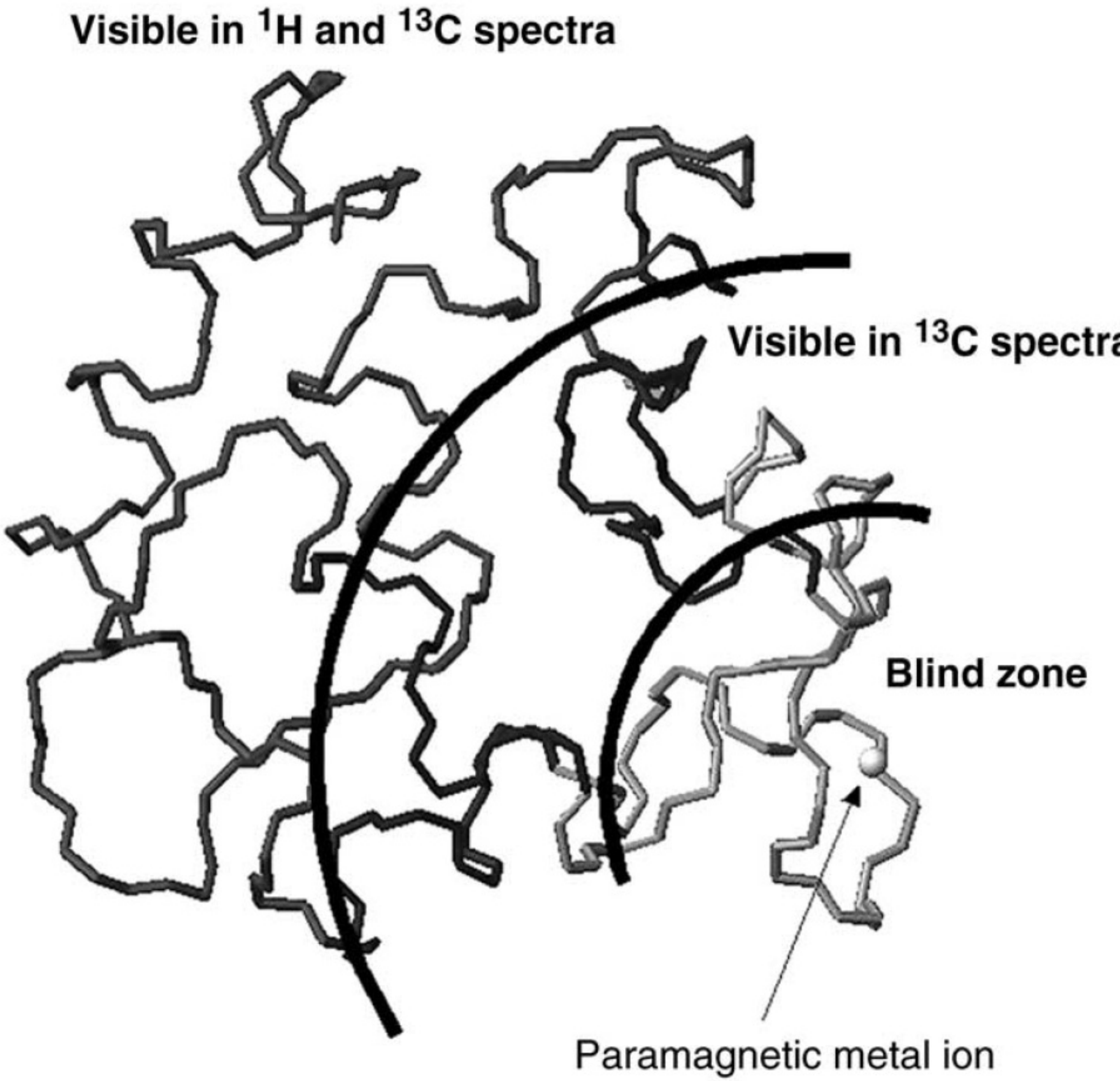
Amino acid type	Samples		
	1	2	3
Leucine	$^{13}C/^{15}N$	$1\text{-}^{13}C$	$1\text{-}^{13}C$
Valine	$1\text{-}^{13}C$	$^{13}C/^{15}N$	
Isoleucine			$^{13}C/^{15}N$
Methionine	^{15}N		
Lysine		^{15}N	
Phenylalanine			^{15}N
Arginine	^{15}N	^{15}N	
Tyrosine	^{15}N	$1\text{-}^{13}C$	^{15}N
Alanine		^{15}N	^{15}N
Threonine	^{15}N	^{15}N	^{15}N
Glycine	$1\text{-}^{13}C$		
Aspartate			$1\text{-}^{13}C$

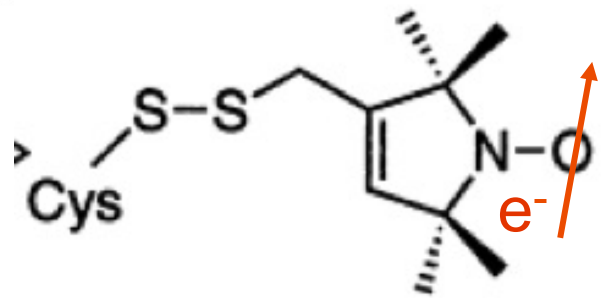
Technological innovations

Numerical processing:

- Filtering
- Data management and integration
- Structure calculation software

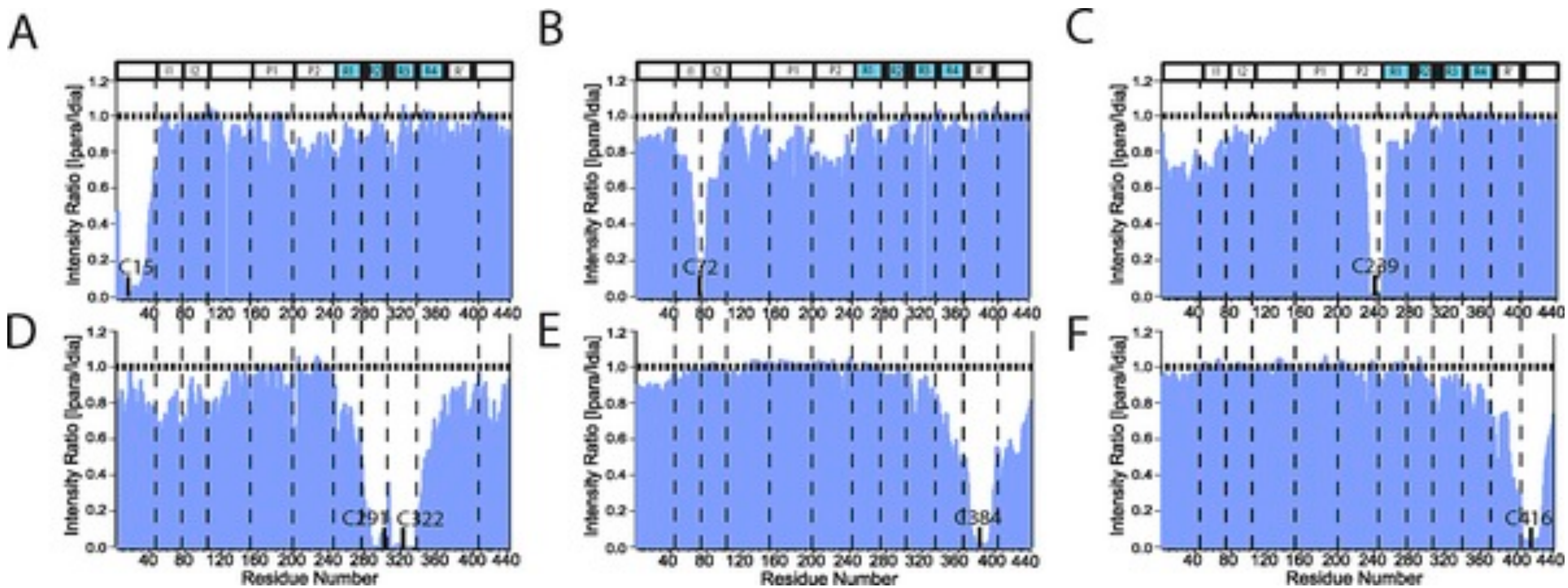
Effects of a paramagnetic centre on ^{1}H and ^{13}C spectra





I. Bertini *et al.*, ChemBioChem 2005, 6, 1536 – 1549

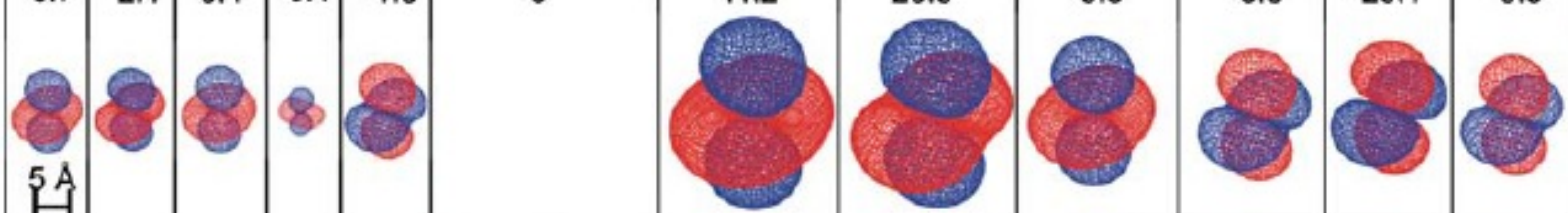
Application to the study of disordered proteins (Tau)



Mukrasch MD, Bibow S, Korukottu J, Jeganathan S, et al. (2009) Structural Polymorphism of 441-Residue Tau at Single Residue Resolution. PLoS Biol 7(2): e1000034. doi:10.1371/journal.pbio.1000034
http://www.plosbiology.org/article/info:doi/10.1371/journal.pbio.1000034

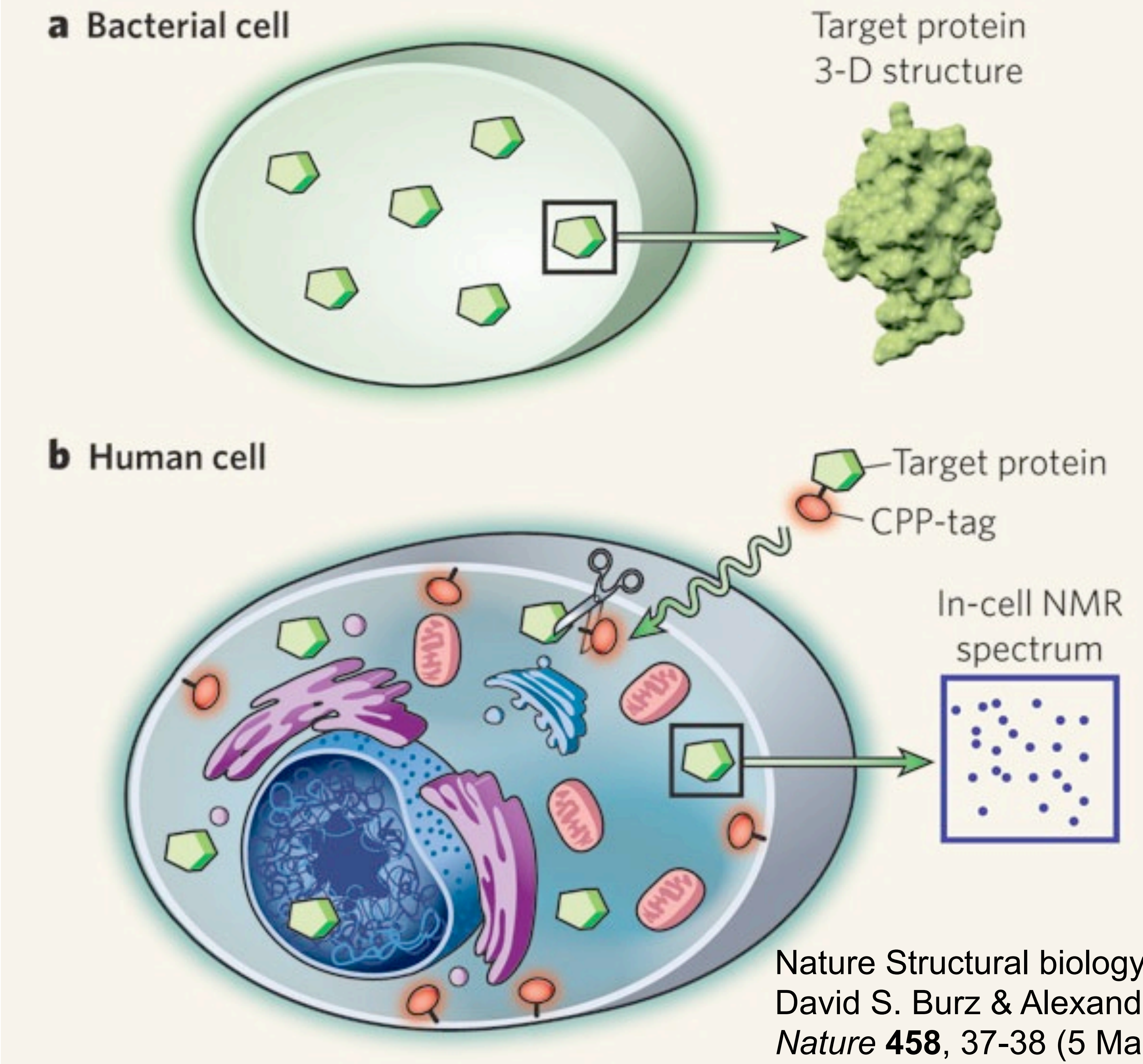
PLOS | BIOLOGY

Some other probes: paramagnetic lanthanide tag

	f1	f2	f3	f5	f6	f7	f8	f9	f10	f11	f12	f13
	Cerium	Praseodymium	Neodymium	Samarium	Europium	Gadolinium	Terbium	Dysprosium	Holmium	Erbium	Thulium	Ytterbium
	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb
J	5/2	4	9/2	5/2	0	7/2	6	15/2	8	15/2	6	7/2
$\chi\ /10^{-32}\ m^3$	5.6	11.2	11.4	0.6	~6	55.1	82.7	99.2	98.5	80.3	50.0	18.0
PRE												
$\Delta\chi_{ax}\ /10^{-32}\ m^3$	2.1	3.4	1.7	0.2	-2.3	0	42.1	34.7	18.5	-11.6	-21.9	-8.3
$\Delta\chi_{rh}\ /10^{-32}\ m^3$	0.7	2.1	0.4	-0.1	-1.6	0	11.2	20.3	5.8	-8.6	-20.1	-5.8
PCS												
τ_e /s	10^{-13}					10^{-7}	10^{-13}					

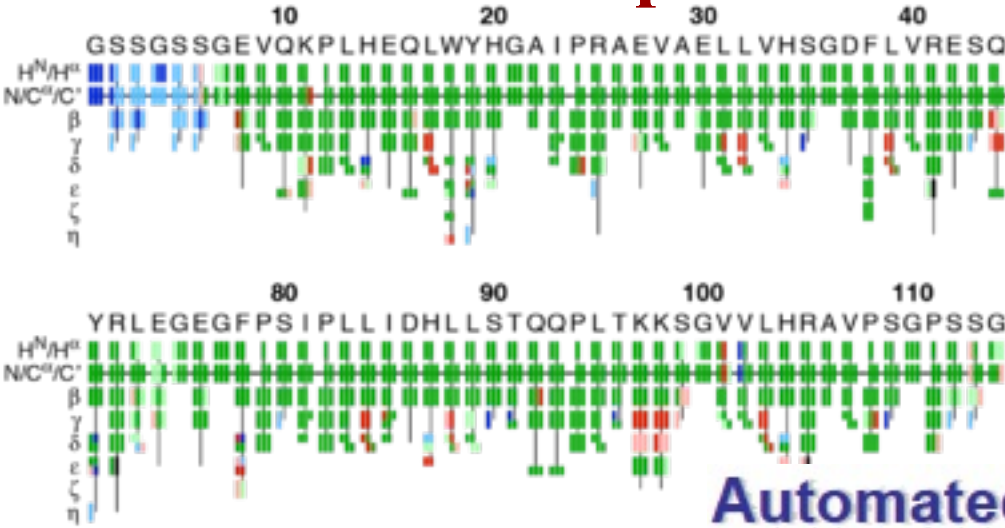
Otting, J. Biomol. NMR 2009

In-cell NMR



Nature Structural biology: Inside the living cell
David S. Burz & Alexander Shekhtman
Nature **458**, 37-38 (5 March 2009)

Software development for automatic assignment

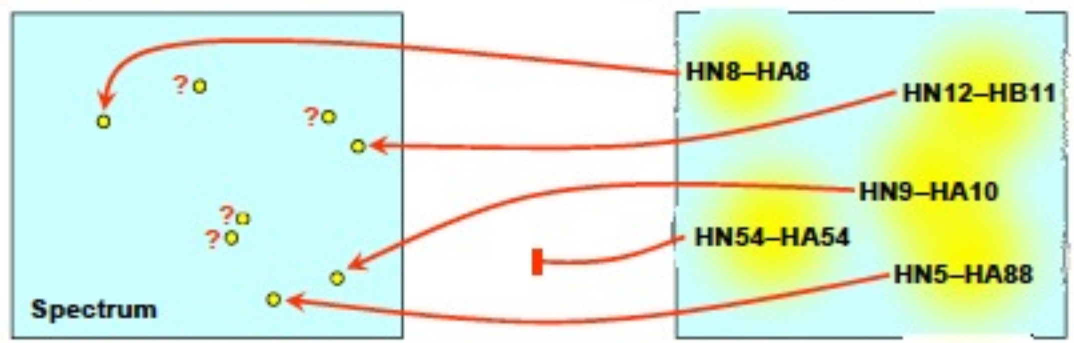


Fully automated structure calculation algorithm (FLYA)

Automated Chemical Shift Assignment

Observed peaks
Position known
Assignment unknown

Expected peaks
Assignment known
Position known only approximately



Assignment = Find **mapping** between expected and observed peaks.

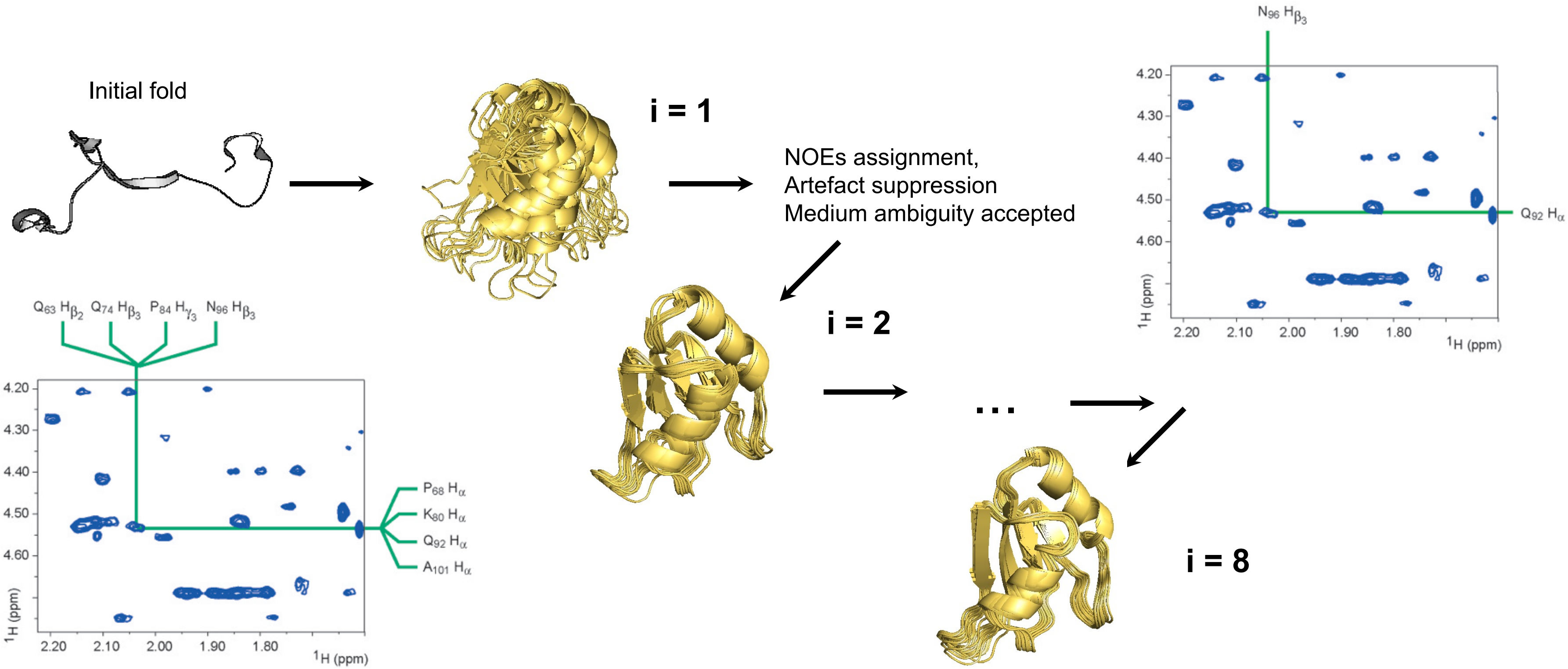
Score for assignment
Presence of expected peaks
Positional alignment of peaks assigned to the same atom
Normality of assigned resonance frequencies

Optimization of assignment
Genetic algorithm combined with local optimization

GARANT
Christian Bartels *et al.*
J. Comp. Chem. 18, 139–149 (1997)
J. Biomol. NMR 7, 207–213 (1996)

Development of structure calculation protocols

Incorporation of ambiguous distance restraints in iterative process protocols => M. Nilges, T. Herrmann

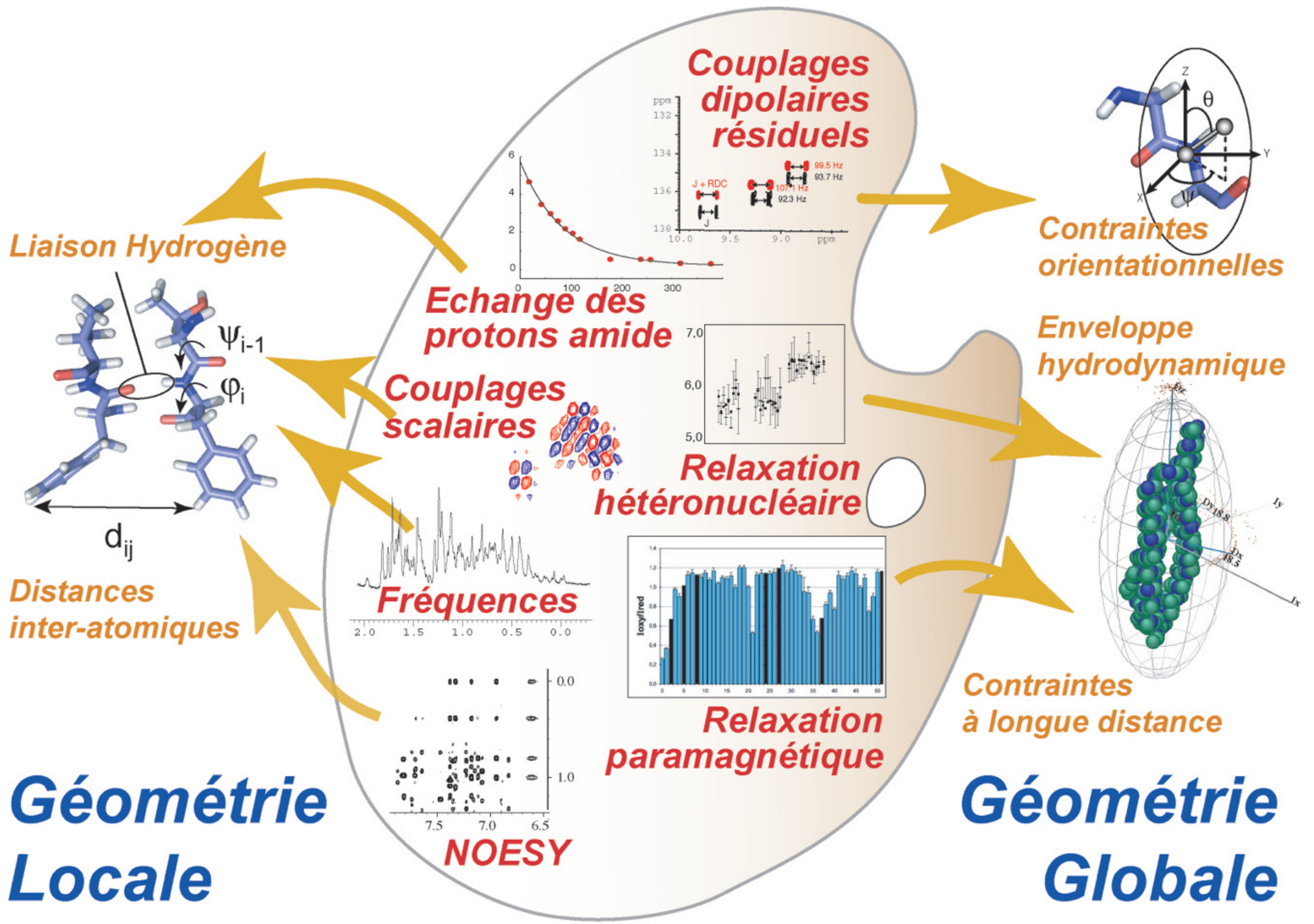


Software
ARIA, UNIO

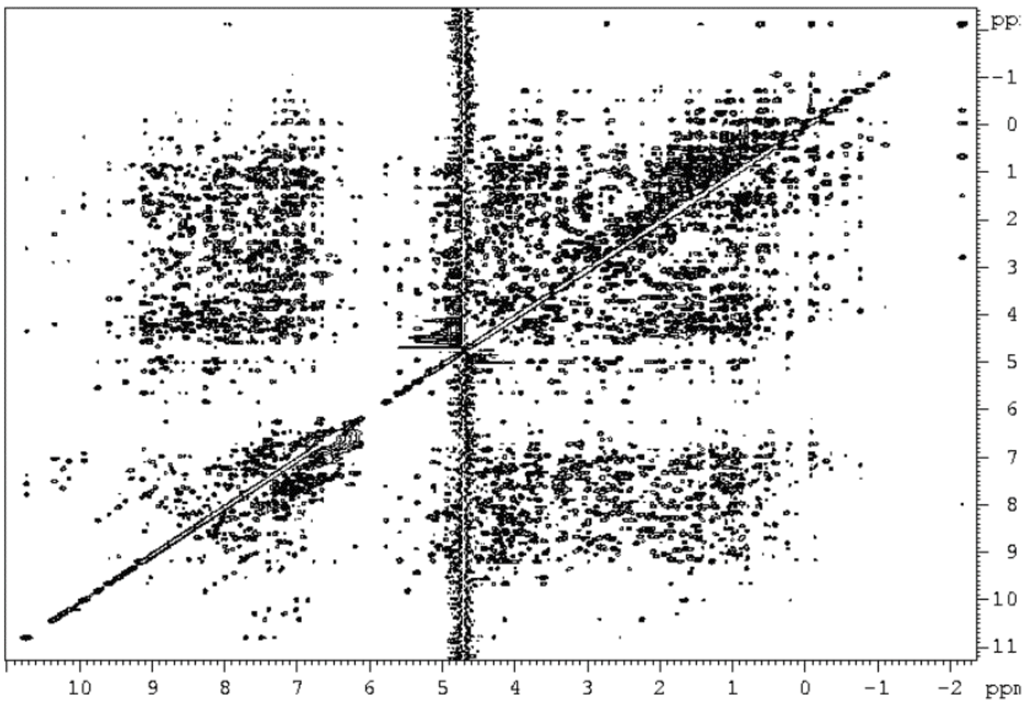
Rieping W., Habeck M., Bardiaux B., Bernard A., Malliavin T.E., Nilges M. (2007) ARIA2: automated NOE assignment and data integration in NMR structure calculation. Bioinformatics 23:381-382.

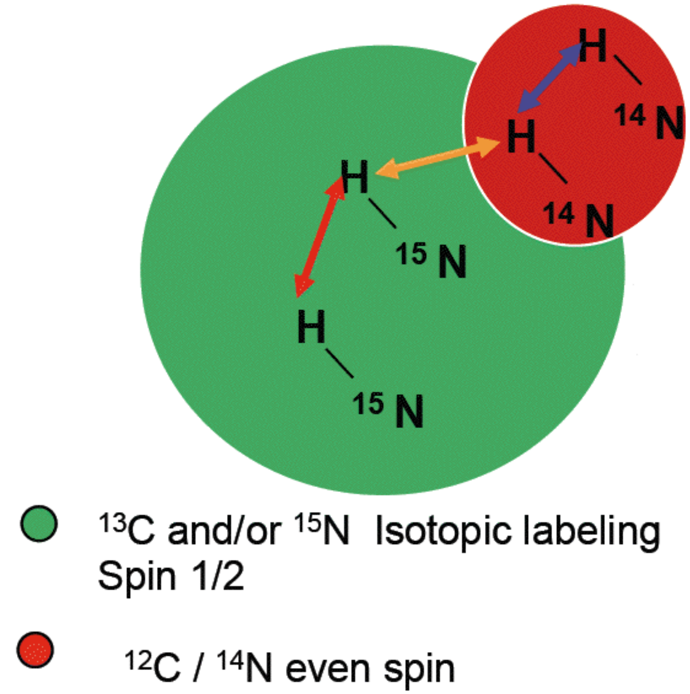
Volk, J.; Herrmann, T.; Wüthrich, K. J. Biomol.NMR. 2008, 41, 127-138..

Many structural parameters



Structural information from intra-molecular NOE

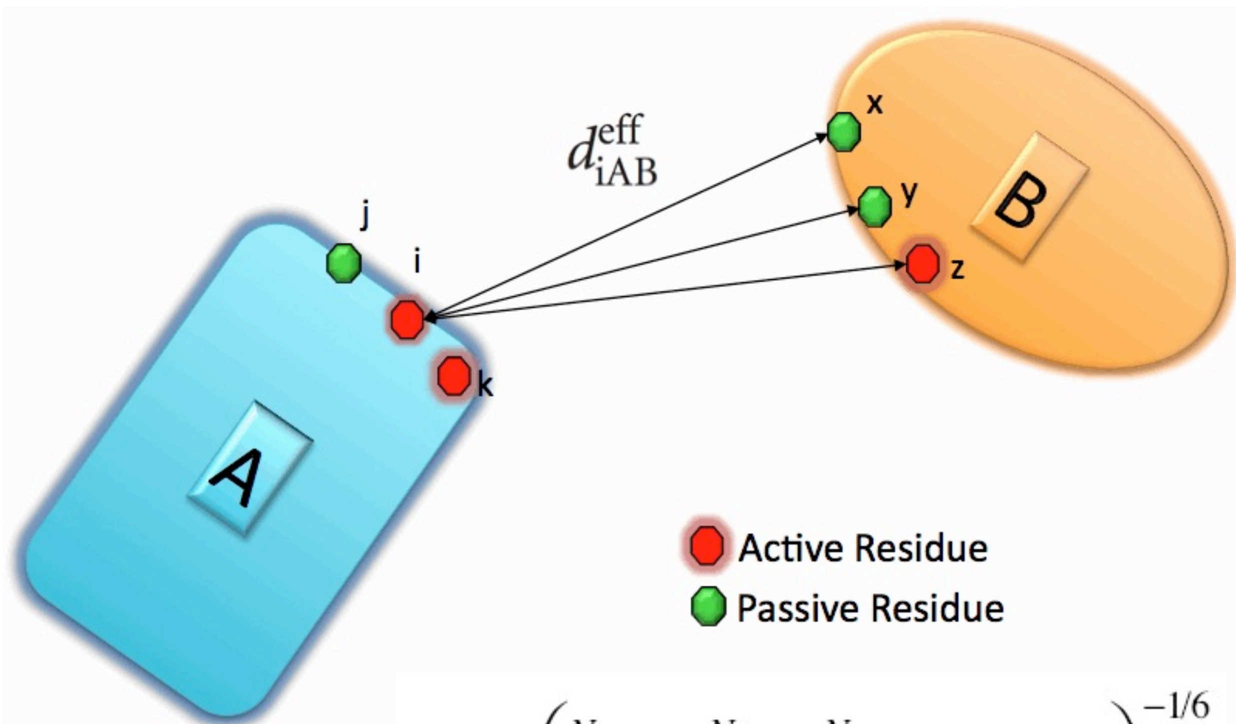




How to separate Intra
& inter molecular NOE ?

Filtered NOESY

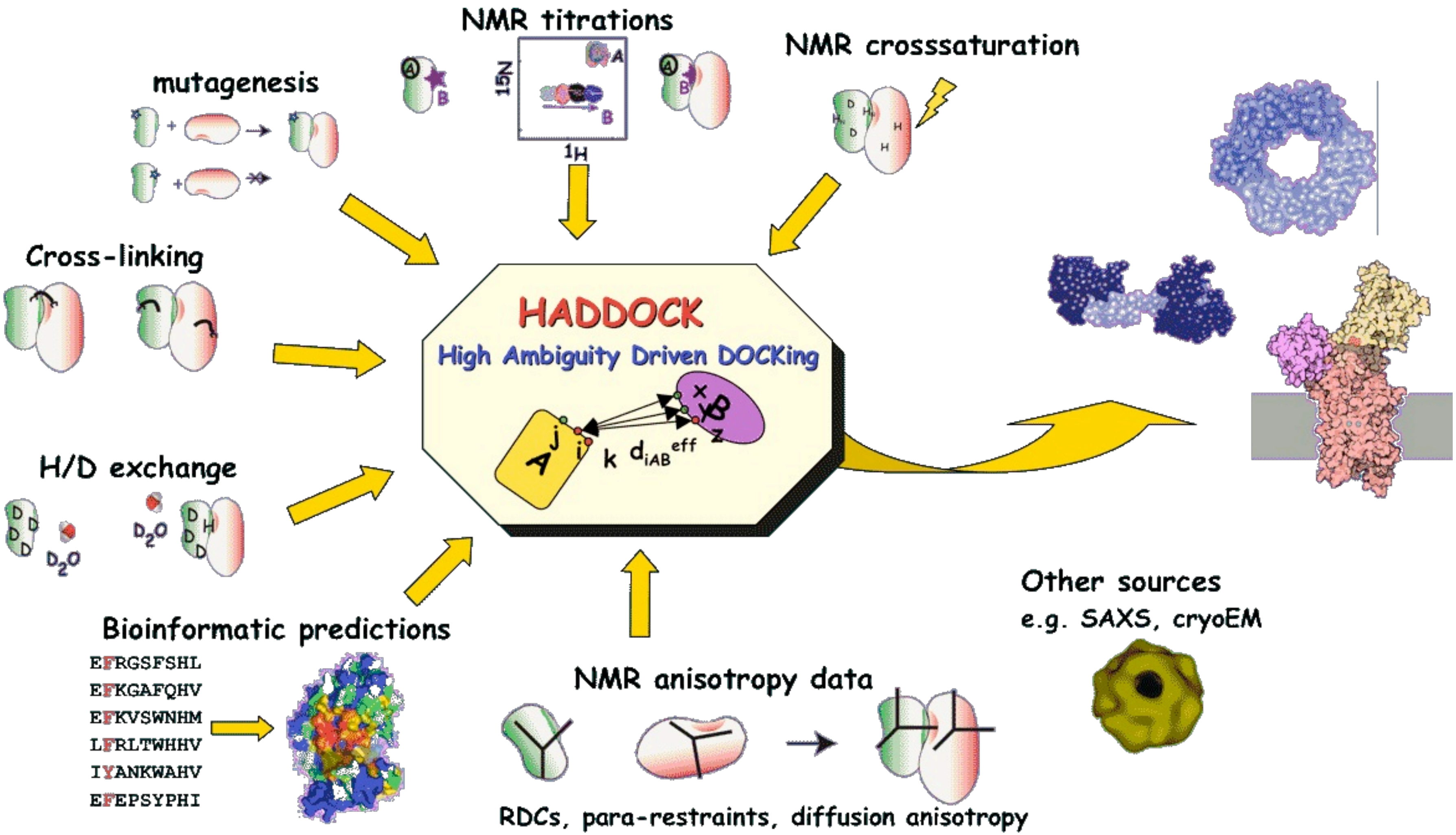
Use of Ambiguous Interaction Restraints for soft docking



$$d_{iAB}^{eff} = \left(\sum_{m_{iA}=1}^{N_{Aatom}} \sum_{k=1}^{N_{resB}} \sum_{n_{kB}=1}^{N_{Batom}} \frac{1}{d_{m_{iA} n_{kB}}^{6}} \right)^{-1/6}$$

Domingez C, Boelens R, Bonvin A, J. Am. Chem. Soc. 125, 1731-1737 (2003).

Use of Ambiguous Interaction Restraints for soft docking



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Users Meetings

15 Oct **7ème Réunion des Utilisateurs organisée par ICSN Gif/Yvette**
Gif-sur-Yvette

Evènements

22 May **Formation Atelier Pratique en RMN**
Grenoble

Acknowledgements :
« Financial support from the TGIR-RMN-THC Fr3050 CNRS for conducting the research is gratefully acknowledged. »

Since 2008 France has established a national network of flagship NMR facilities providing High-Resolution and High-Field capabilities, making available the latest cutting edge developments to a broad community of national and international users.

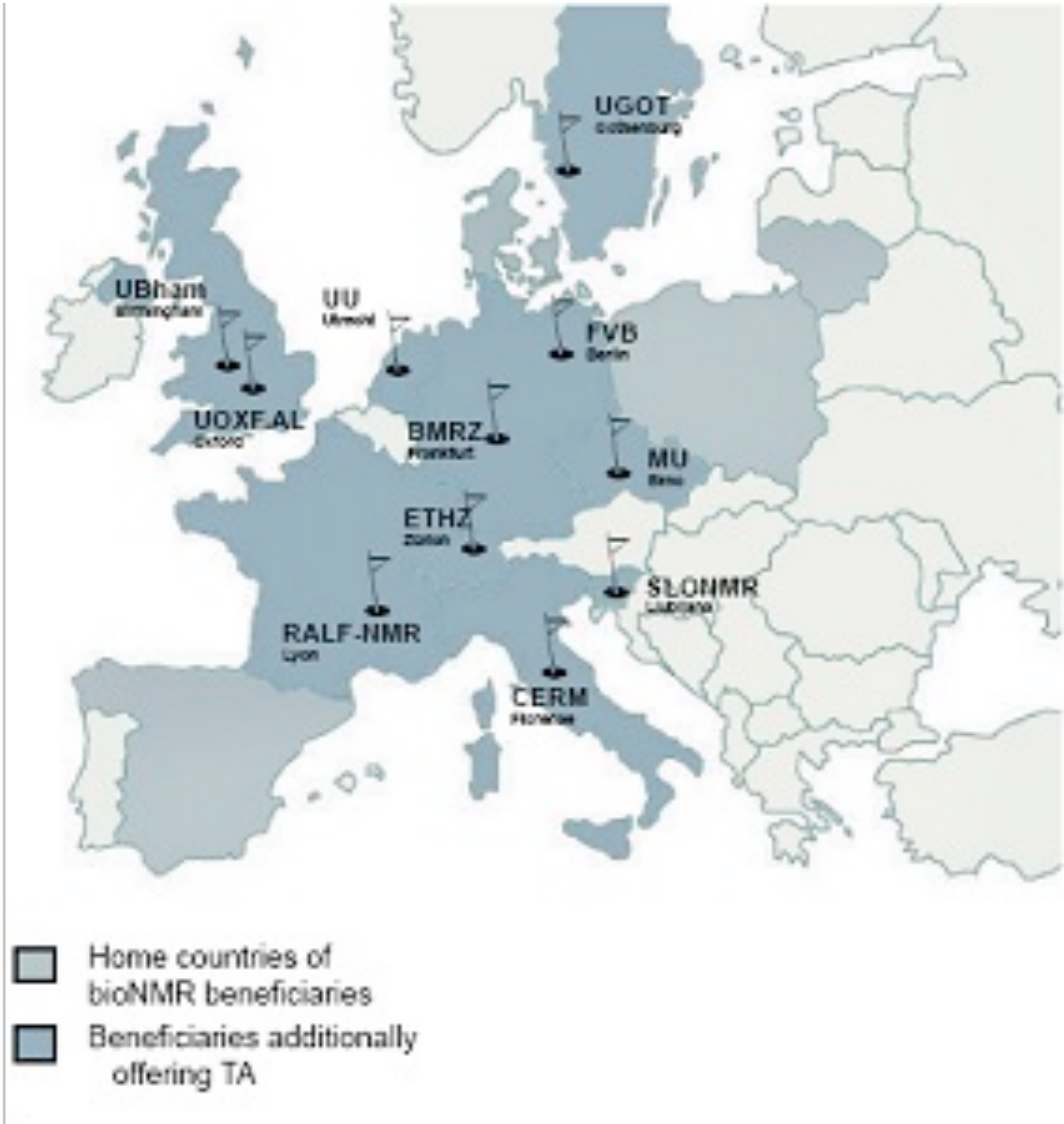
This multi-sited structure features research teams of international visibility providing a unique combinations of high-cost and high-performance instruments and associated skills in development of methods and application to biology, chemistry, physics, geology, or materials sciences.
Each of the sites is committed to provide one third of its experimental time and associated expertise [100 days per year] to the community, through a constantly open call for proposals.

News

27 April 2015
Commande du premier 1.2 GHz français
La première commande d'un spectromètre 1.2 GHz vient d'être passée auprès de la compagnie Buker. Il s'agit de la 7ème commande d'un appareil de...
Read more

users-meetings

BioNMR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BMRZ
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ETHZ
SLONMR
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Biology

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NMR

NMR relies on the detection of signals arising from nuclear spin transitions and thus provides information at the atomic level. It allows three-dimensional structural and dynamic information to be obtained in conditions as close as possible to physiological ones. It allows functional processes to be followed, even in living cells, and can investigate transient and weak protein-protein interactions.

Electron Paramagnetic Resonance

Fast field cycling relaxometry

Solid State NMR

Solution NMR