Integrative Structural Biology Summer School

21 June 2017 – Oléron, France

NMR spectroscopy: major advances and future developments

Catherine Bougault, IBS, Grenoble

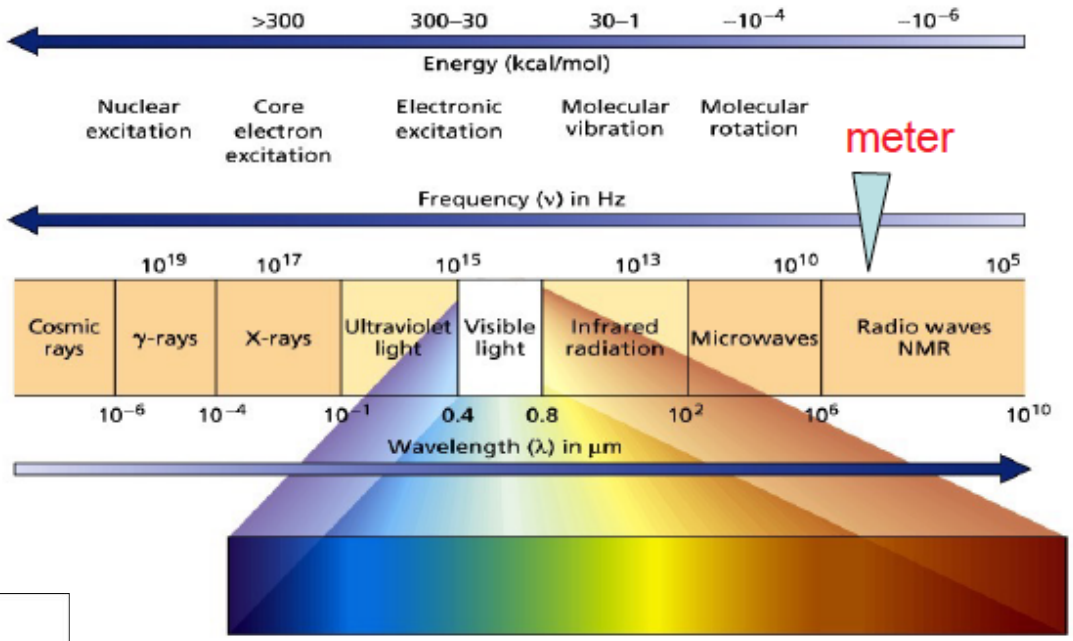
catherine.bougault@ibs.fr

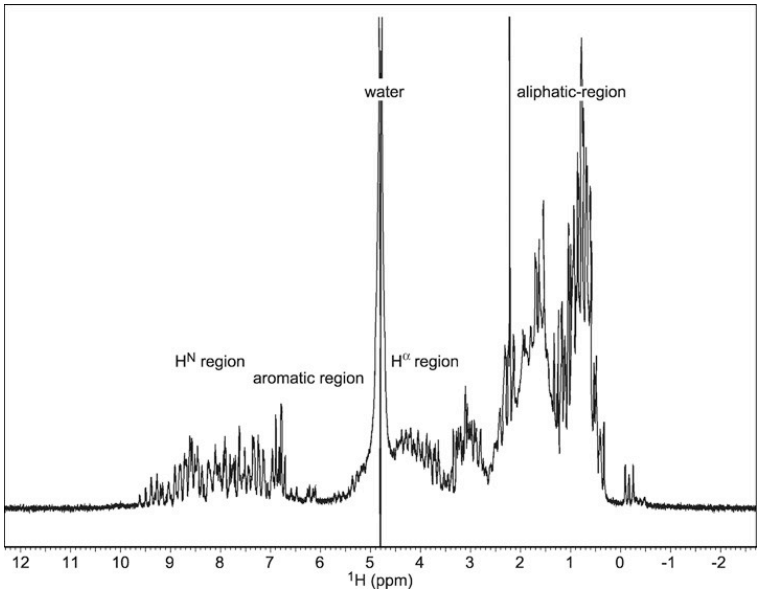
Biomolecular NMR : 35 years of methodological developments

K. Wüthrich
Protein Struct.

1980

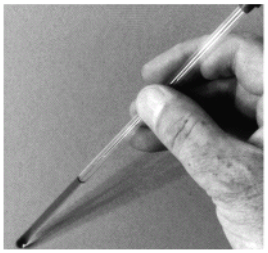
500 MHz



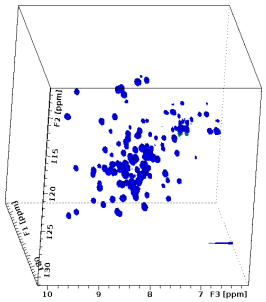


NMR: principles of structure determination

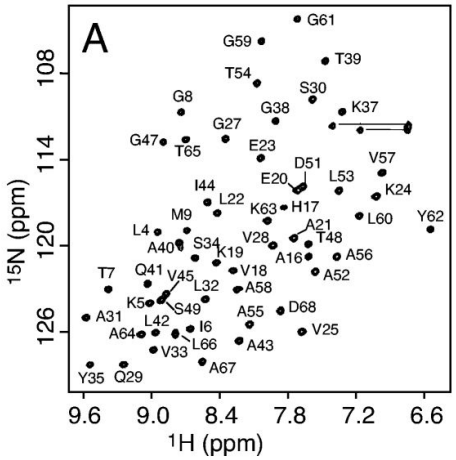
NMR sample



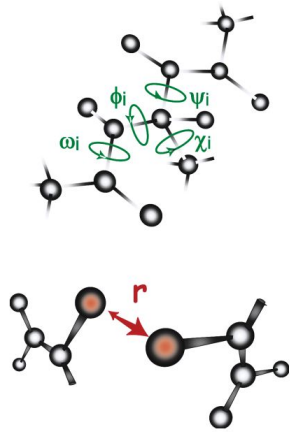
NMR data acquisition



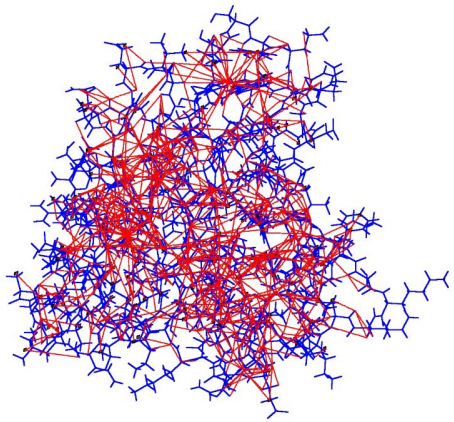
Resonance assignment



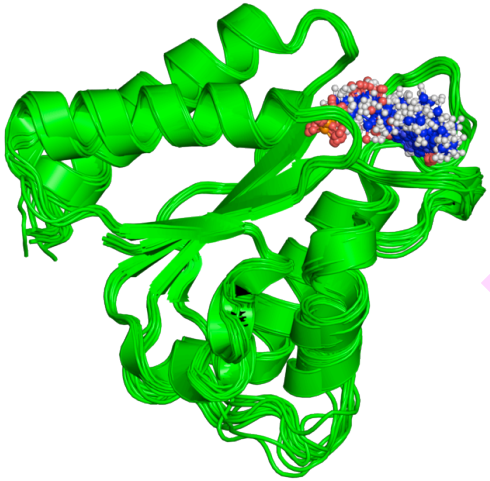
Structural parameters



Structure calculation



Structural ensemble



Biomolecular NMR : 35 years of methodological developments

K. Wüthrich
Protein Struct.

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

TROSY
(A. Pervushin)

Relaxation
Dispersion (A. Palmer)

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

IDP
Solids

Invisible
States (L. Kay)

1980 — **500 MHz**

1990 — **800 MHz**

2000 — **900 MHz**

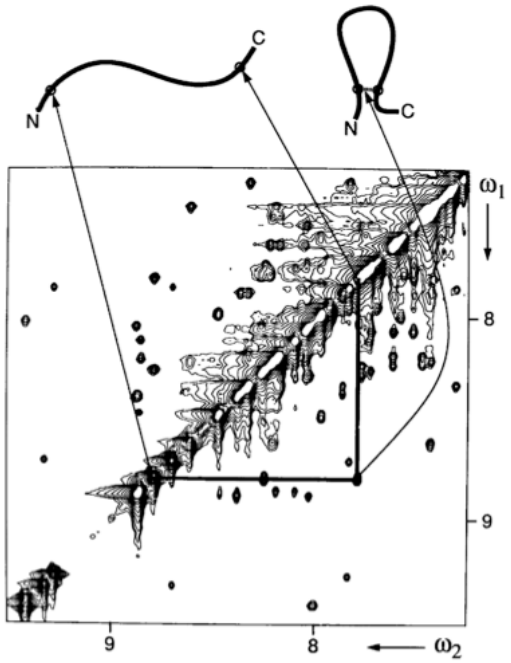
2010 — **1-1.2 GHz**

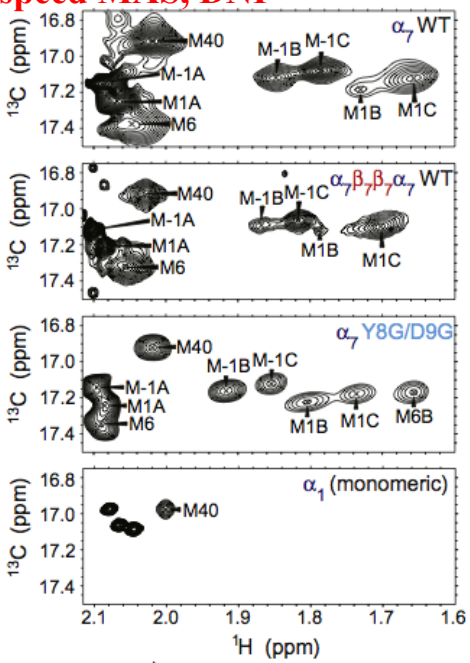
Cryo-probes

High-speed MAS, DNP

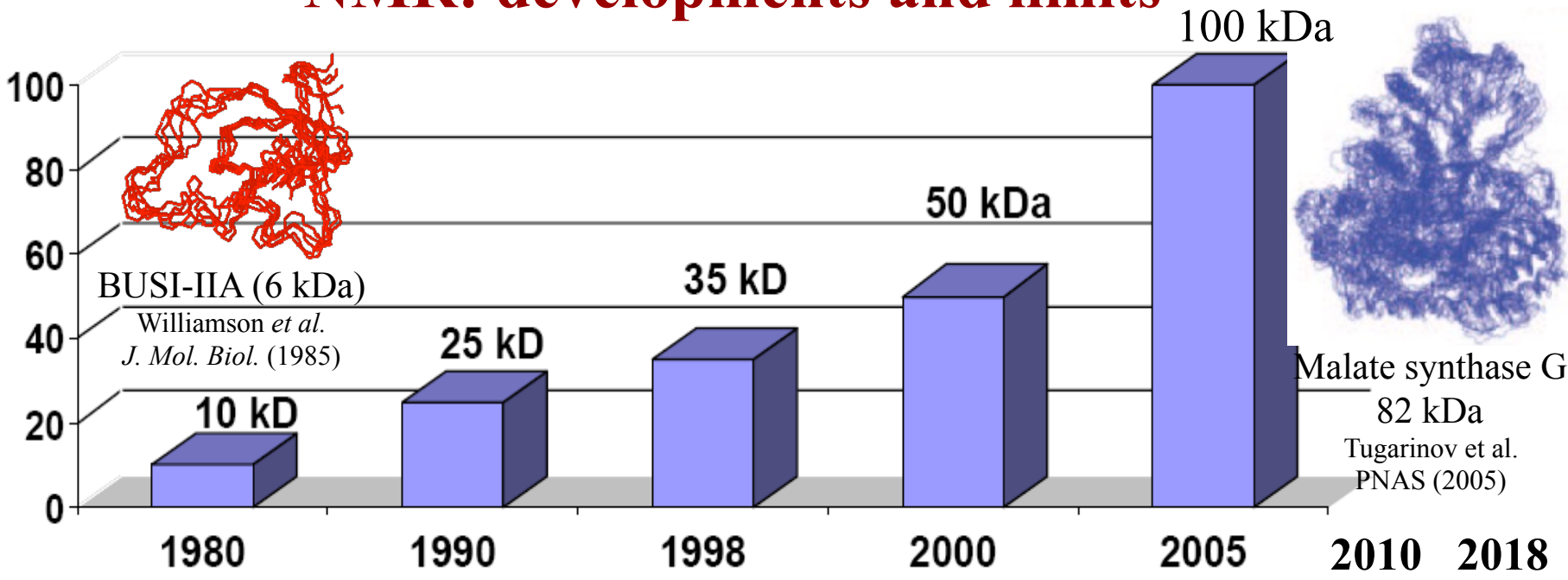
Methyl labeling
(L. Kay)

RDC
(J Prestegard,
A. Bax)
SAR (S. Fesik)



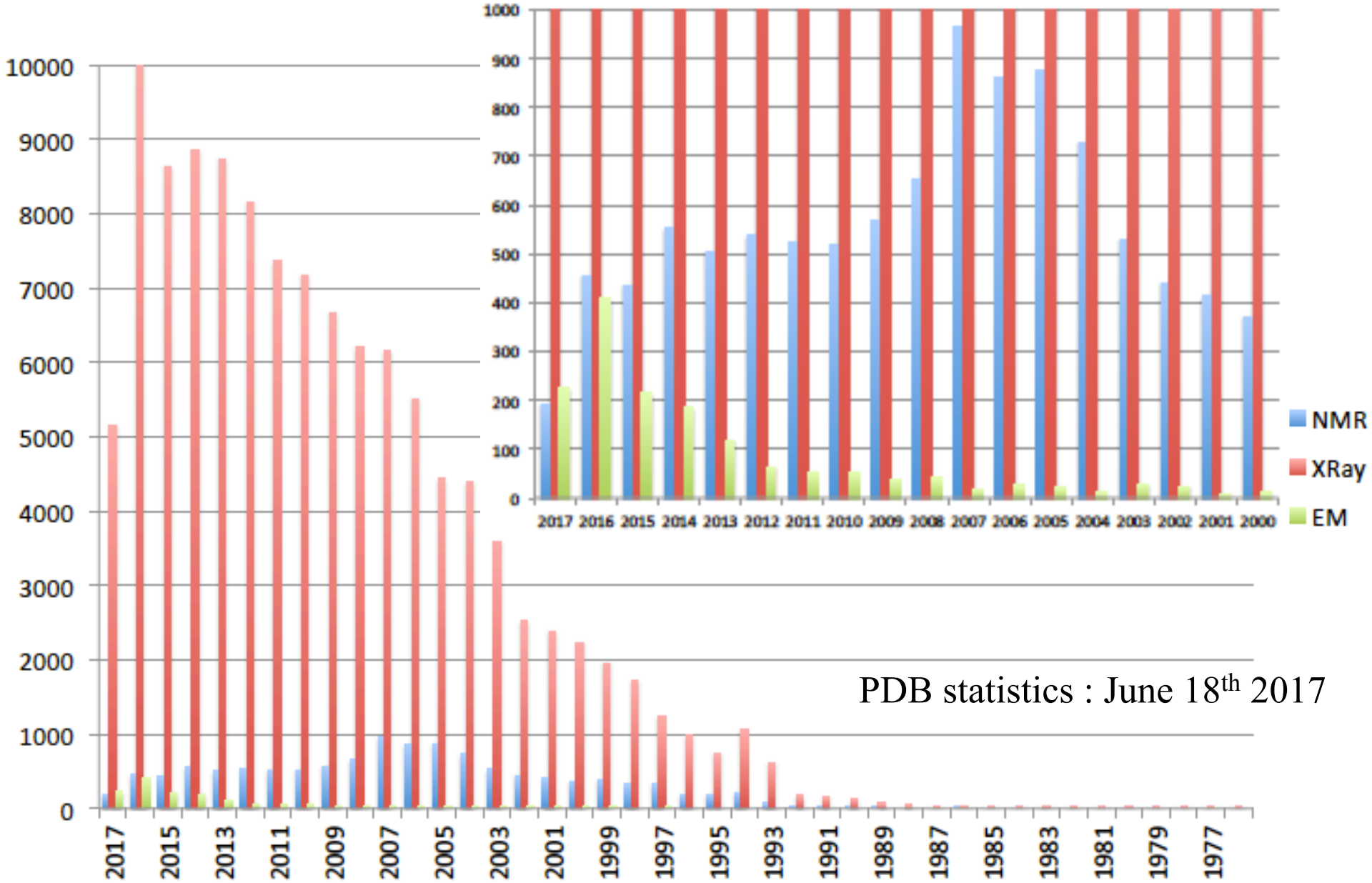


NMR: developments and limits



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	

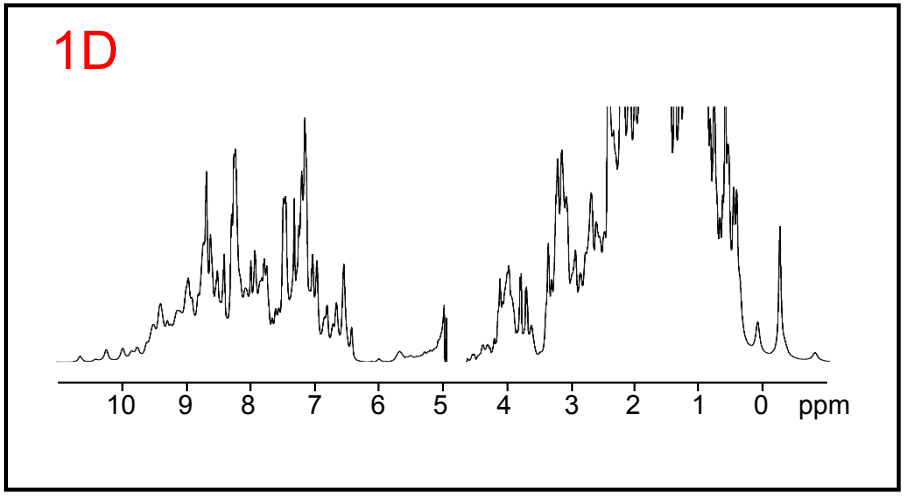
NMR: A limited competitiveness for structures

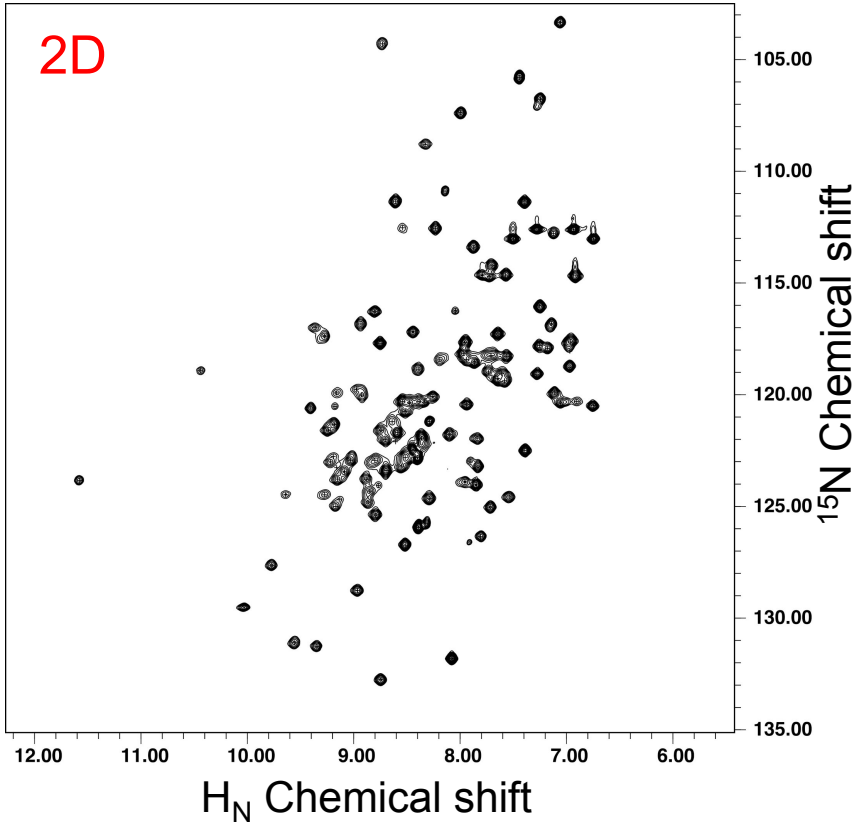


PDB statistics : June 18th 2017

NMR, some limitations

Resolution and spectral hindrance



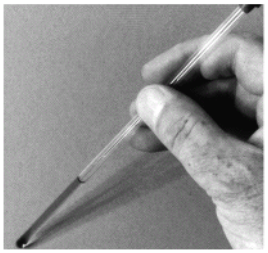


- Acquisition time: *few seconds*
- limited spectral resolution
- No necessary isotope labeling
- **Global characterization**

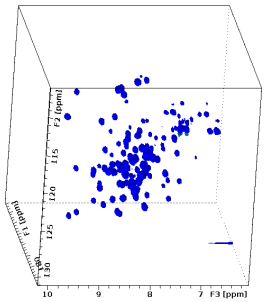
- Acquisition time: *few minutes*
- Increase in the spectral resolution
- Necessary isotope labeling (^{15}N)
- **More detailed information**

NMR, a limited competitiveness for structures: a lengthy process

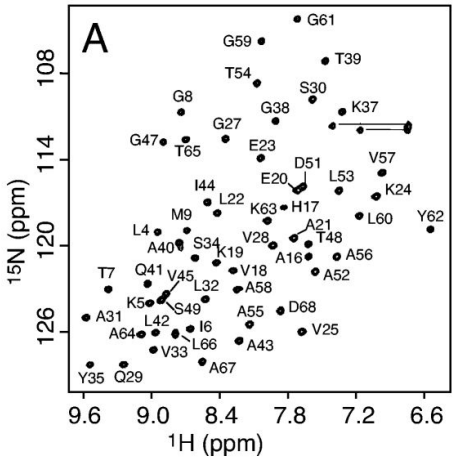
NMR sample



NMR data acquisition



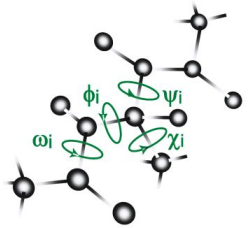
Resonance assignment

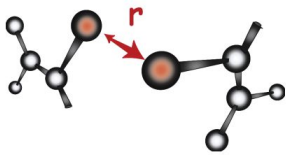


41.1 kDa

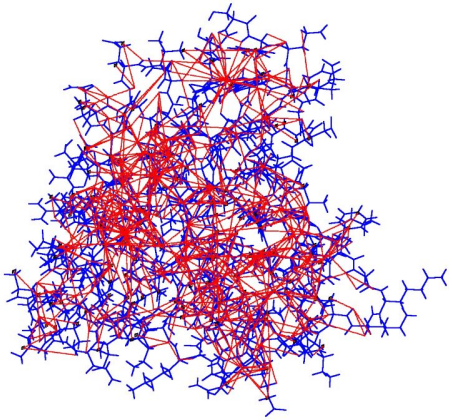
$C_{1827}H_{2869}N_{489}O_{570}S_{12}$

Structural parameters

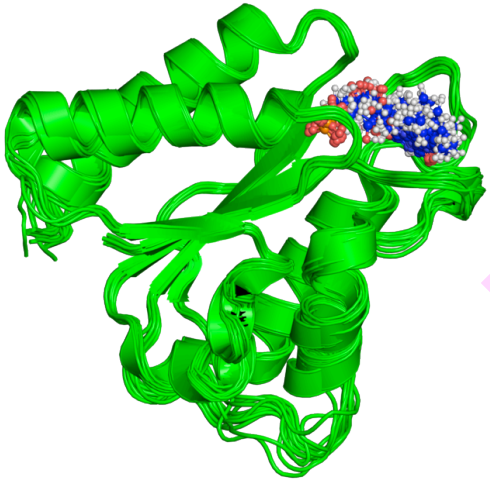




Structure calculation



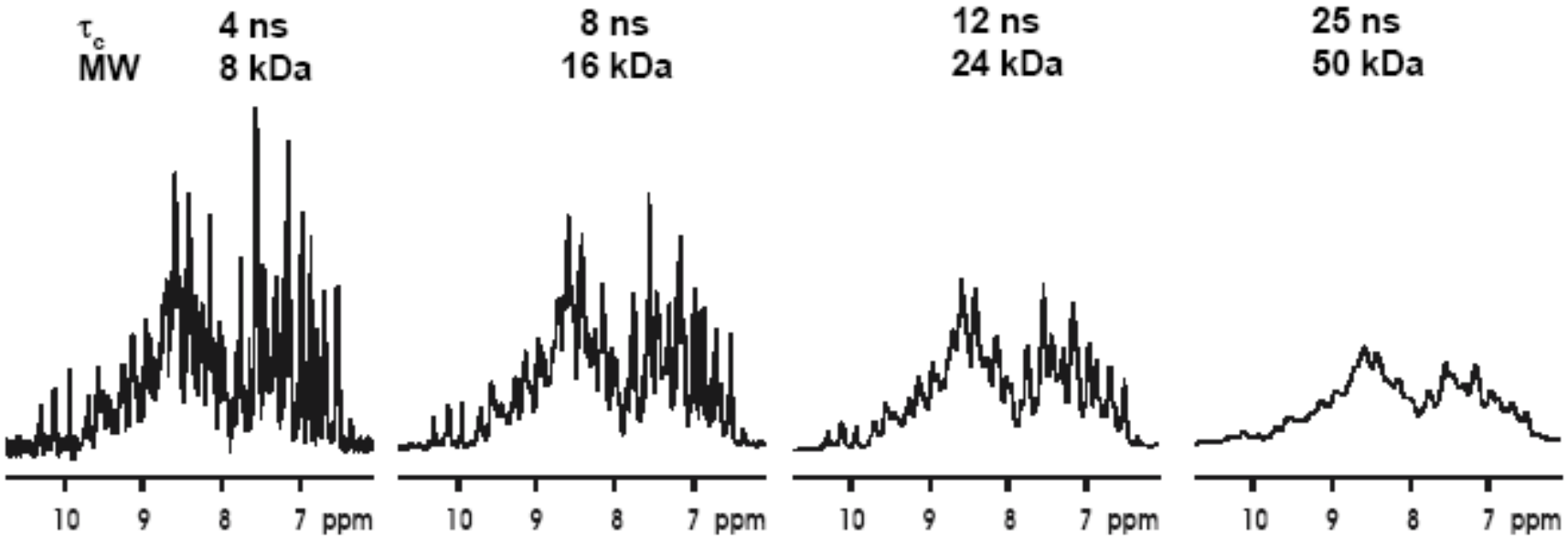
Structural ensemble

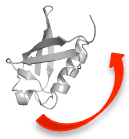


NMR, a limited competitiveness for structures: an intrinsic size limitation in solution

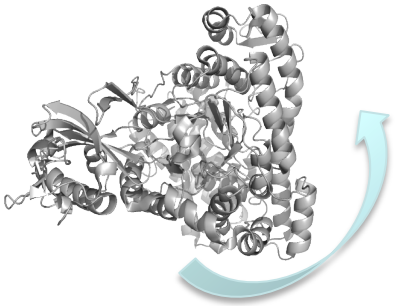
Liquid-state NMR a serious limit?

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





fast overall rotation



slow overall rotation

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*[,†]

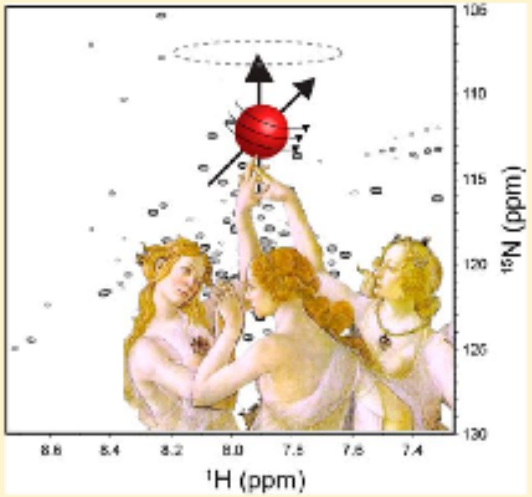
[†]Department of Biochemistry and Center for Structural Biology, Vanderbilt University, Nashville, Tennessee 37232-8725, United States

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

[§]Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287-1604, United States

[∥]Institute for Organic Chemistry, Christian-Albrechts University of Kiel, D-24118 Kiel, Germany

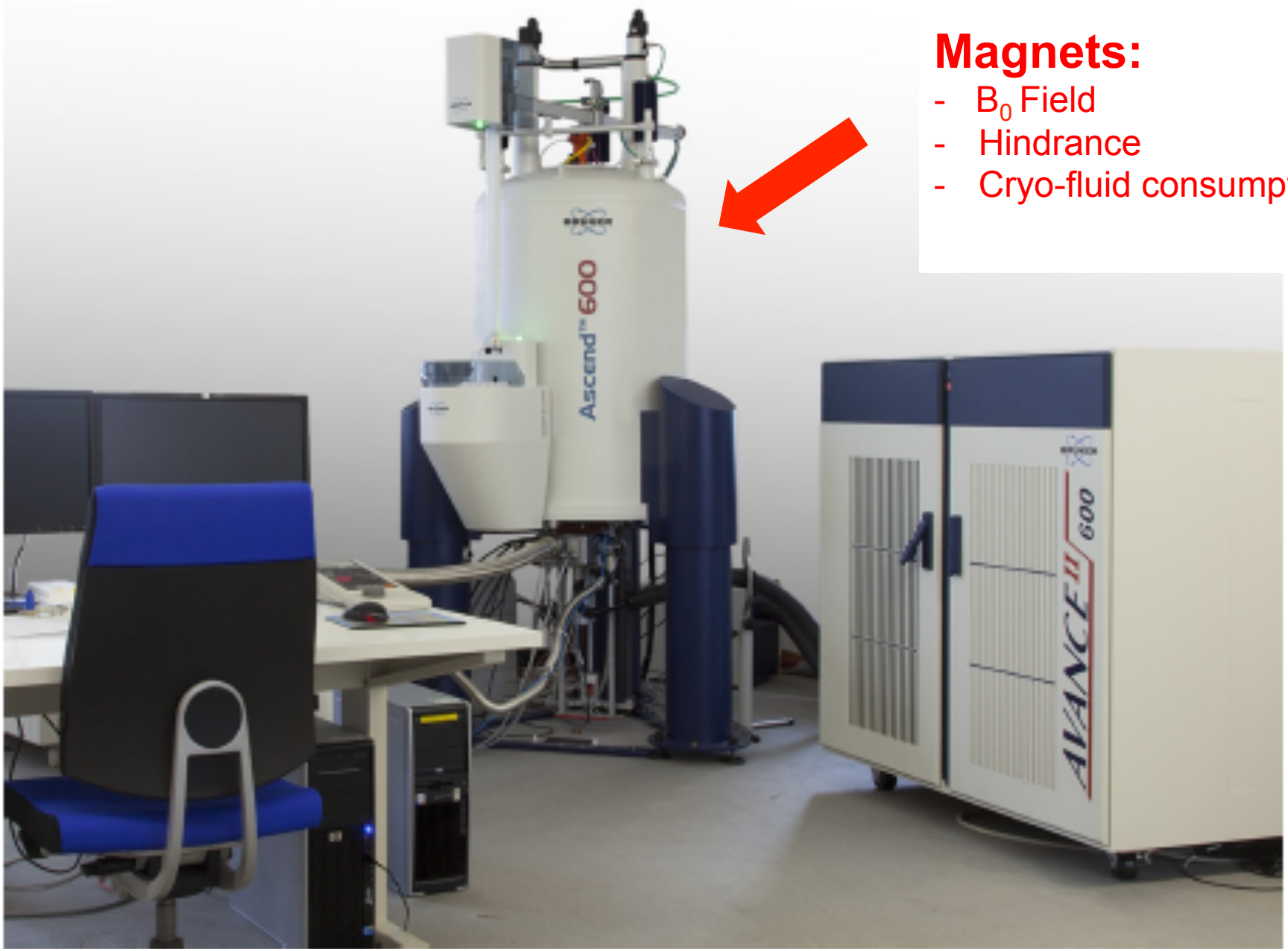
ABSTRACT: From roughly 1985 through the start of the new millennium, the cutting edge of solution protein nuclear magnetic resonance (NMR) spectroscopy was to a significant extent driven by the aspiration to determine structures. Here we survey recent advances in protein NMR that herald a renaissance 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.



2005-2017
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

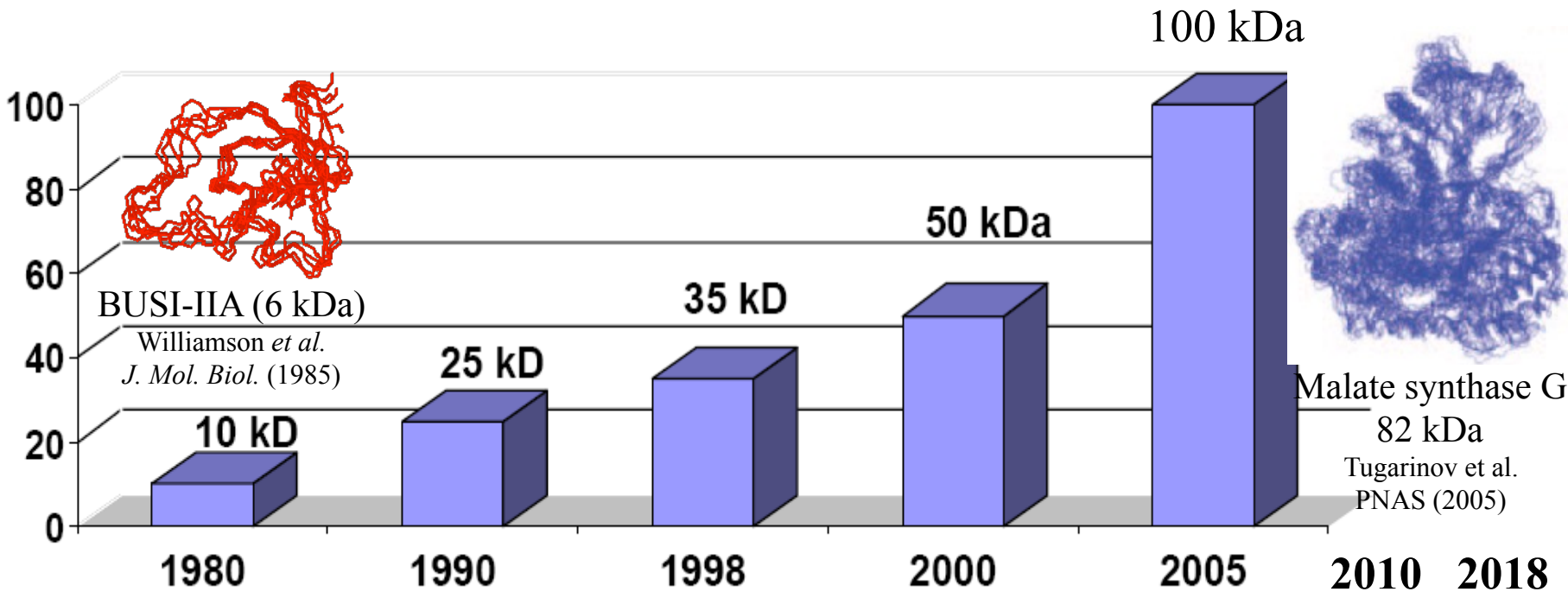
Technological innovations and developments



Magnets:

- B_0 Field
- Hindrance
- Cryo-fluid consumption

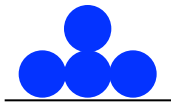
NMR: developments and limits



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

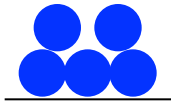
NMR, some limitations

Sensitivity or signal-to-noise ratio



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

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



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

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

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

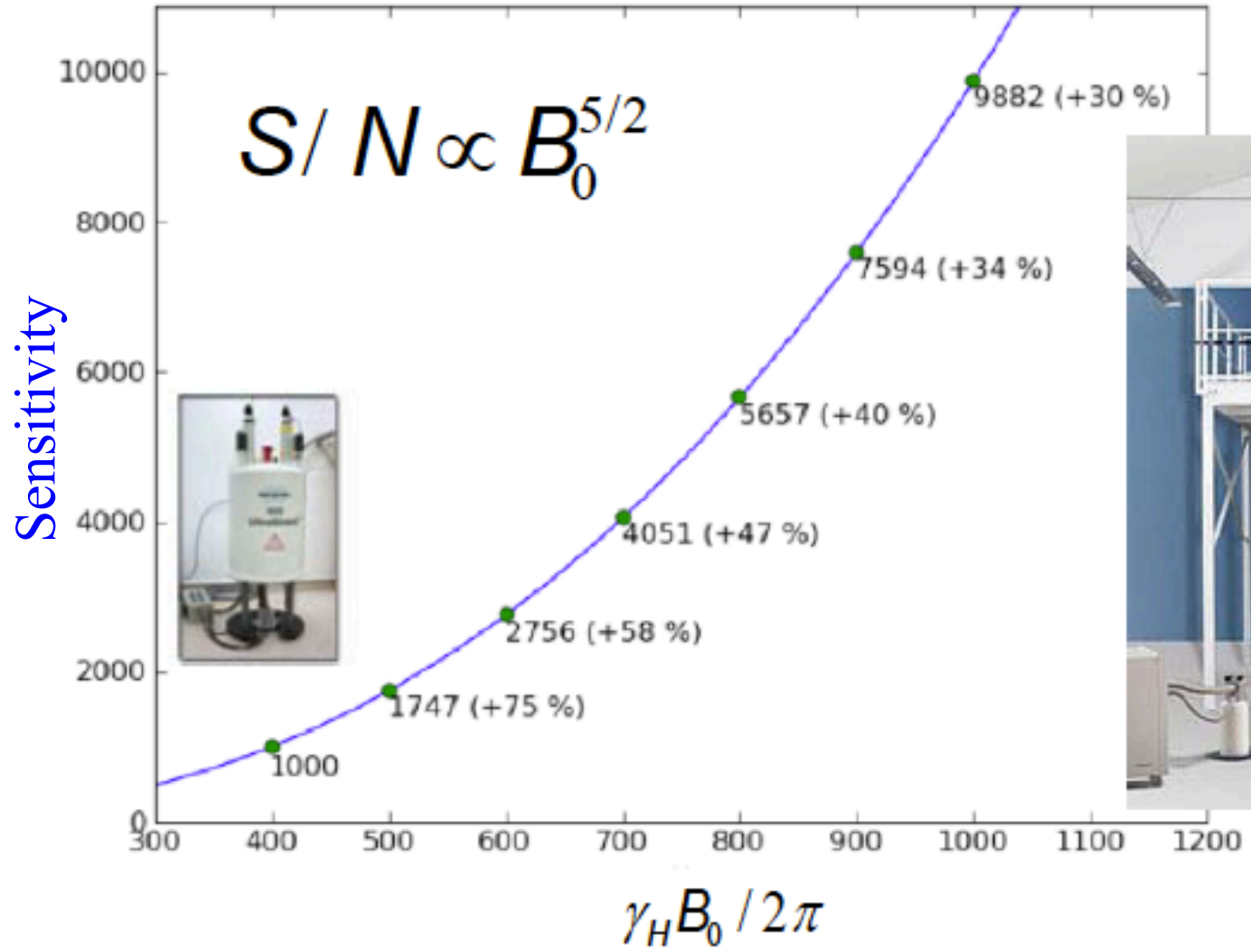
$$@\, B_0 = 14.09T\,(600MHz)$$

Particular case of spin 1/2

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

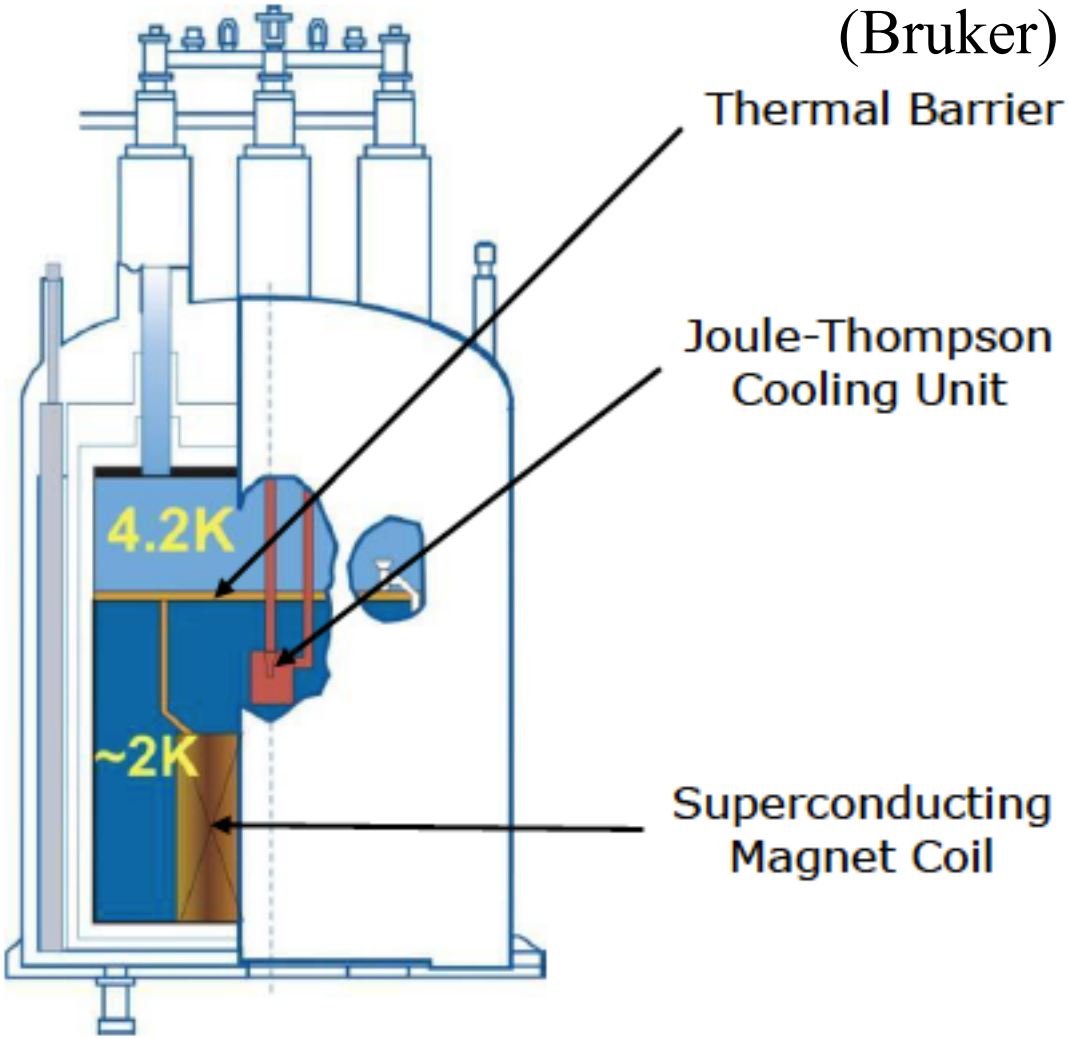
$$\vec{M} = N\frac{\gamma\hbar B_0}{2kT}\gamma\hbar\frac{1}{2}\vec{z} = \frac{N(\gamma\hbar)^2 B_0}{4kT}\vec{z}$$

Technological innovations



Technological innovations

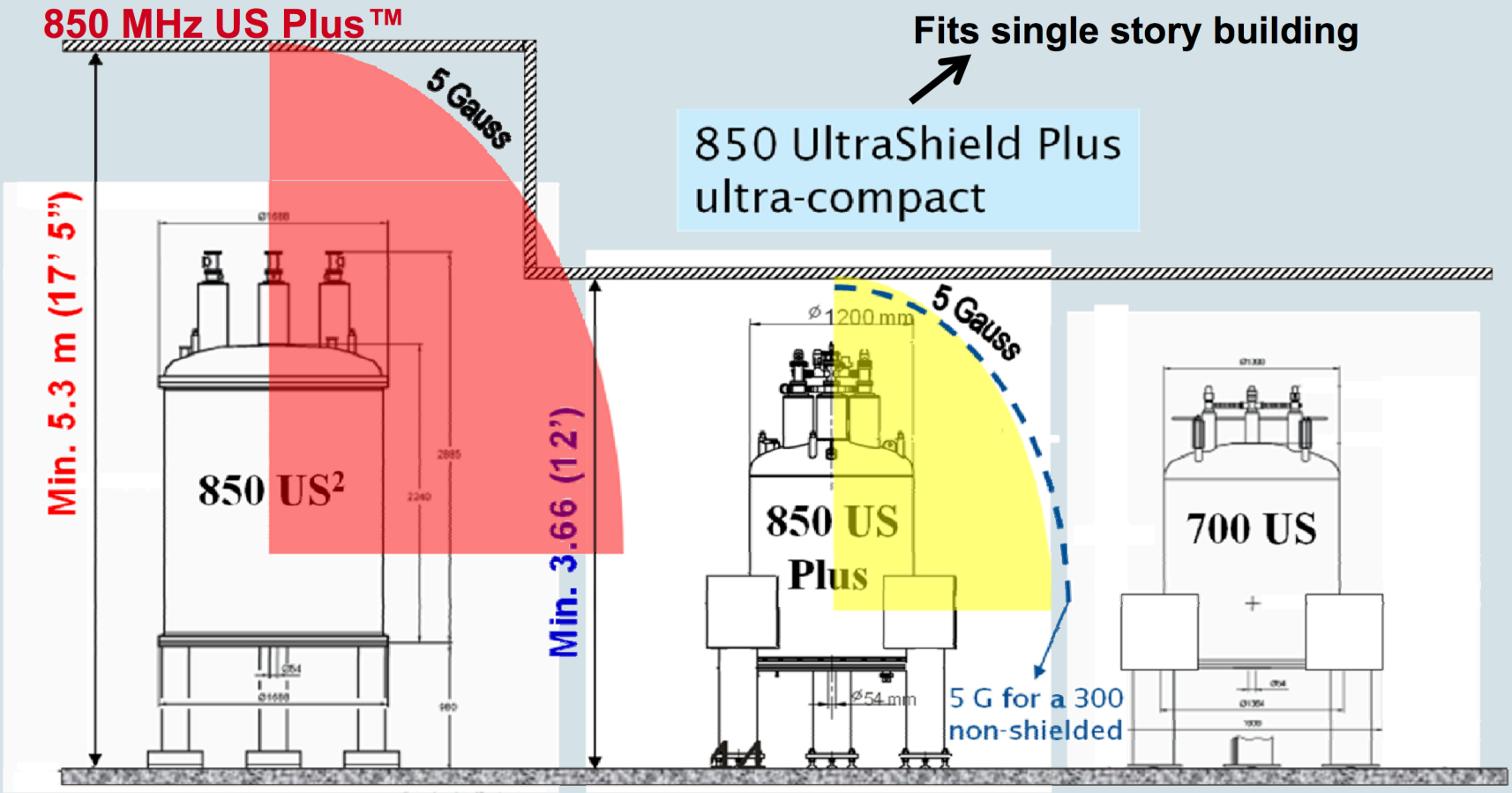
Aeon™ technology (Bruker)



UltraStabilized™ technology delivering unique performance, stability and safety

Technological innovations

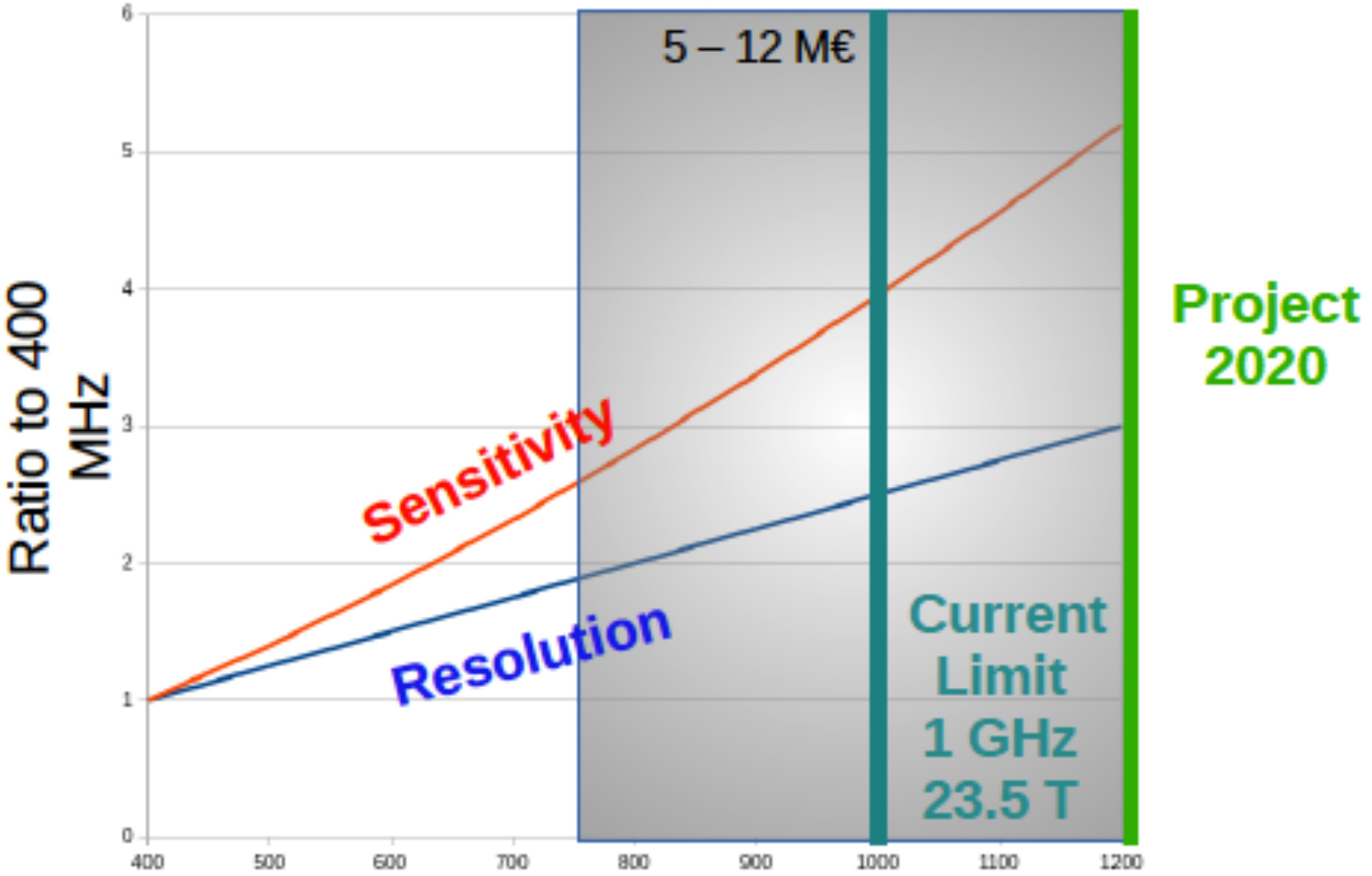
Magnets become more compact



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

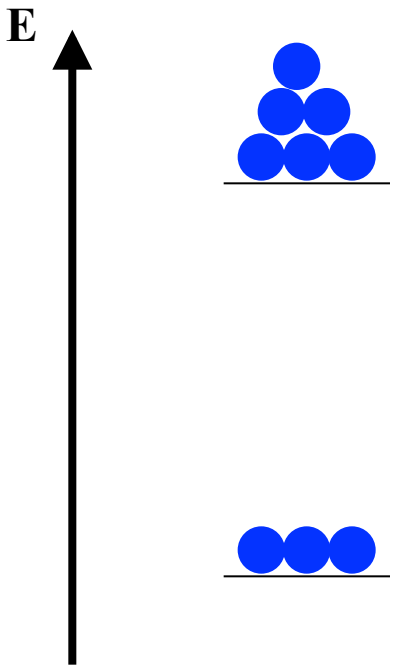
Data courtesy of Bruker

Technological innovations



NMR, overcoming some limitations

Sensitivity or signal-to-noise ratio



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

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

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

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

Spins 1/2

Alternatives to increase Boltzmann?

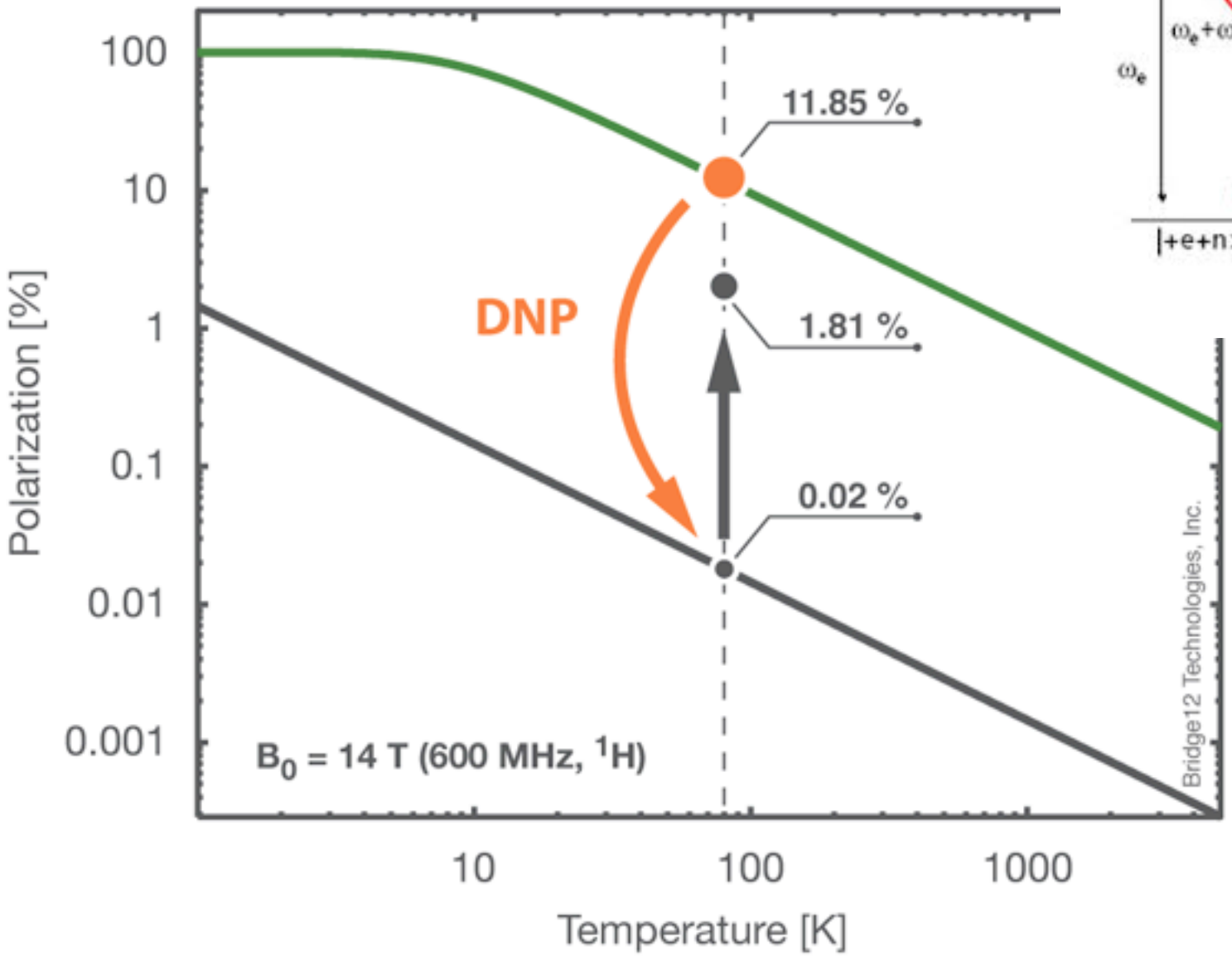
Optical pumping (Xe)

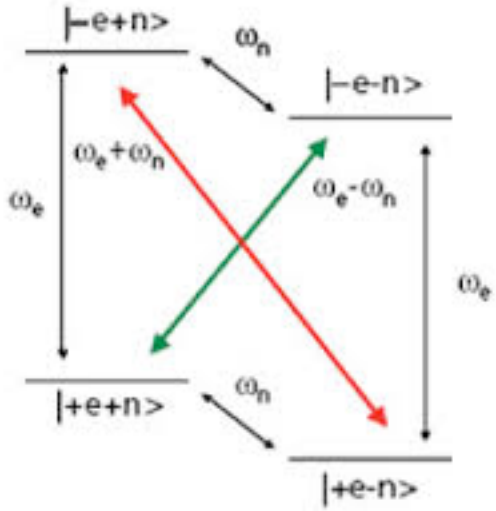
Parahydrogen

DNP

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

Technological innovations: Dynamic Nuclear Polarization



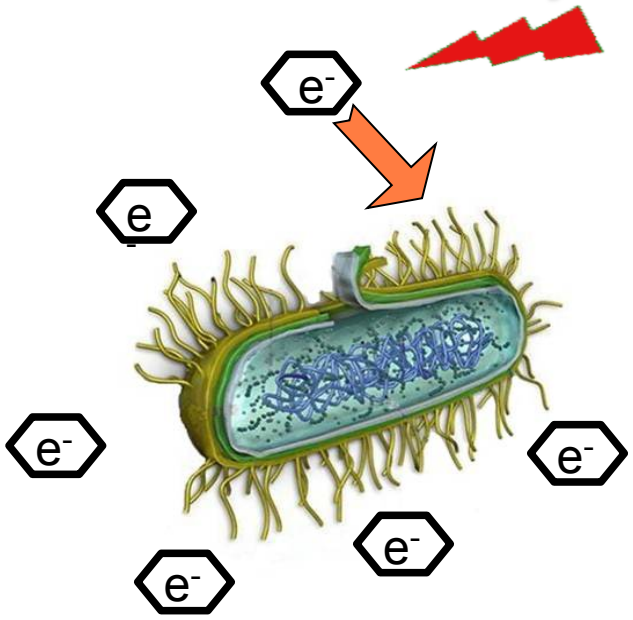


$$\frac{\gamma_{e^-}}{\gamma_H} = 660$$

Technological innovations: Dynamic Nuclear Polarization

T ~ 100 K : compatible with cell survival

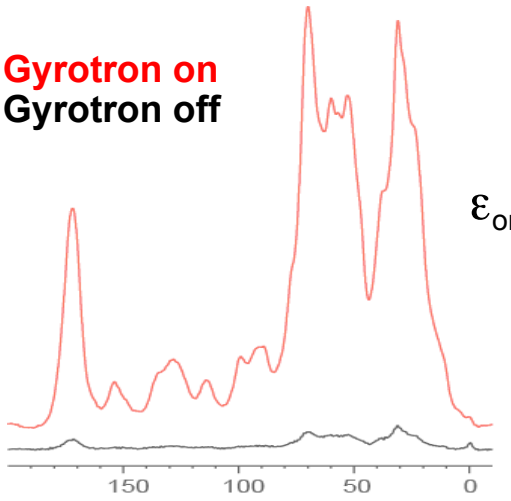
Gyrotron



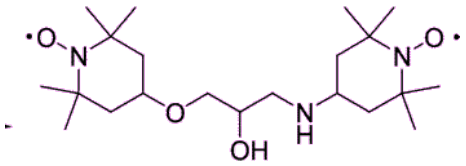
DNP @ CEA Grenoble

NMR signal of the cell surface nuclei ?

Gyrotron on
Gyrotron off

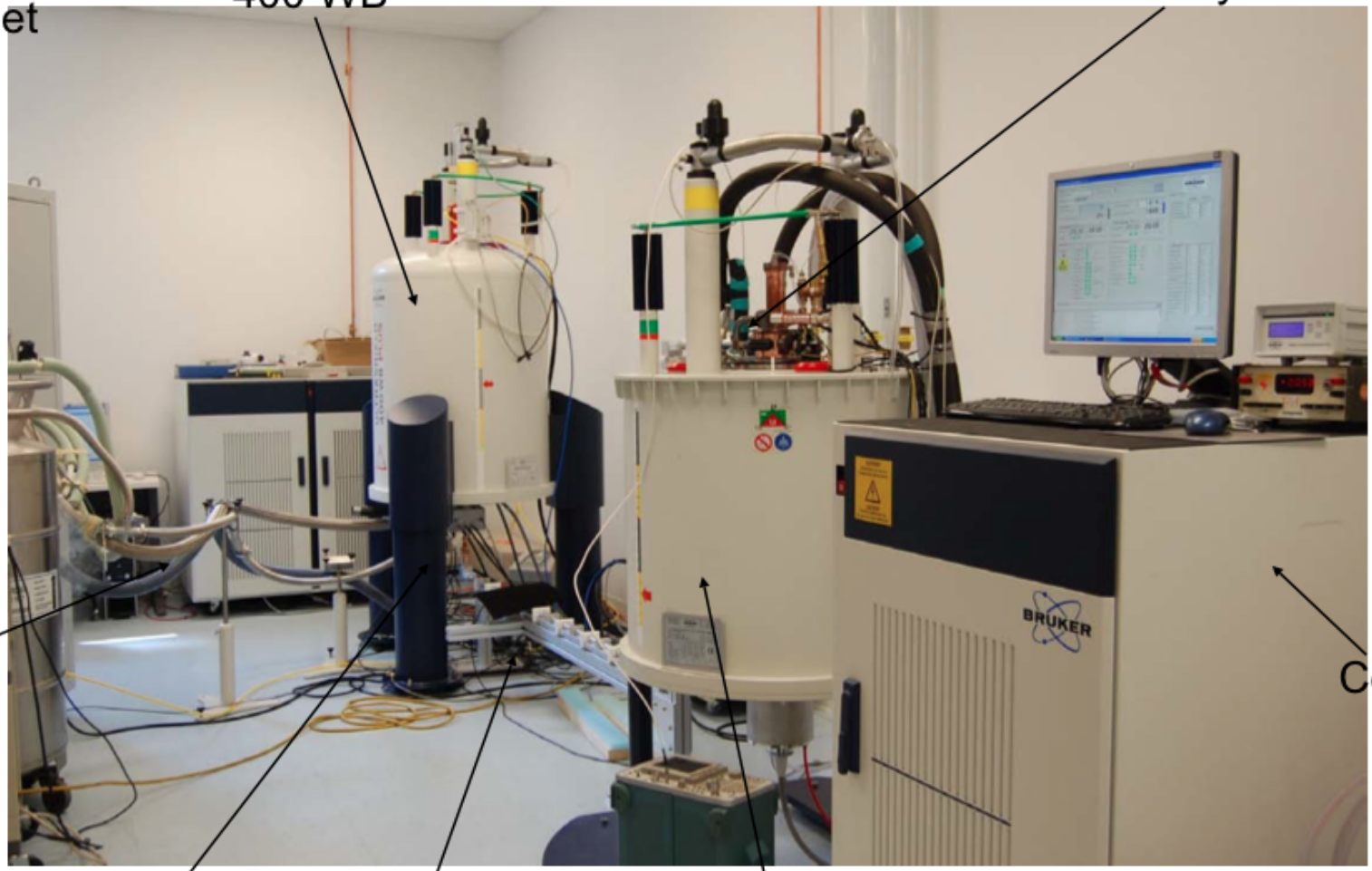


$\varepsilon_{on/off} \sim 25$

e- = 

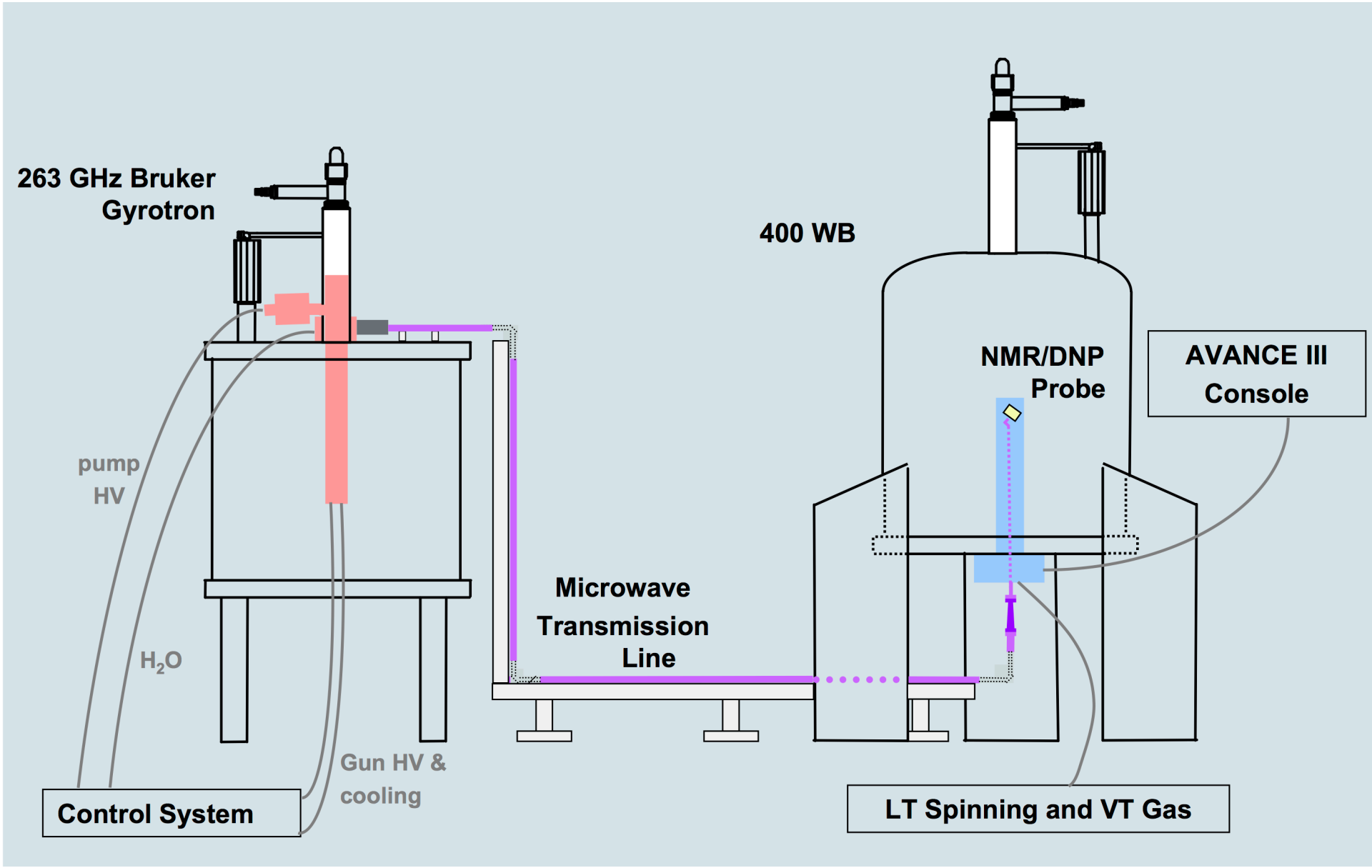
TOTAPOL

263 GHz Gyrotron in Bruker-Billerica DNP Lab

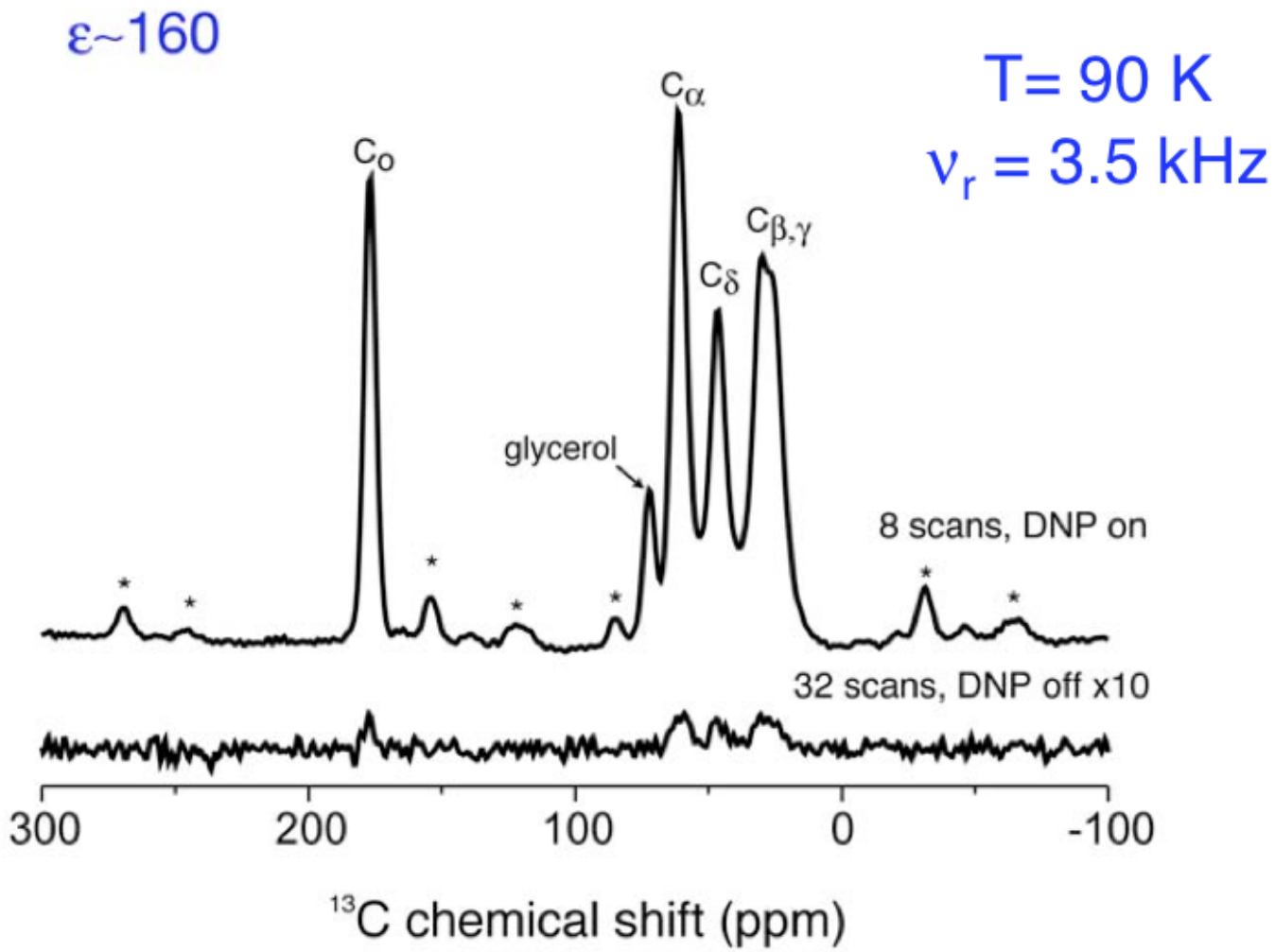


From B. Griffin

263 GHz solid-state DNP

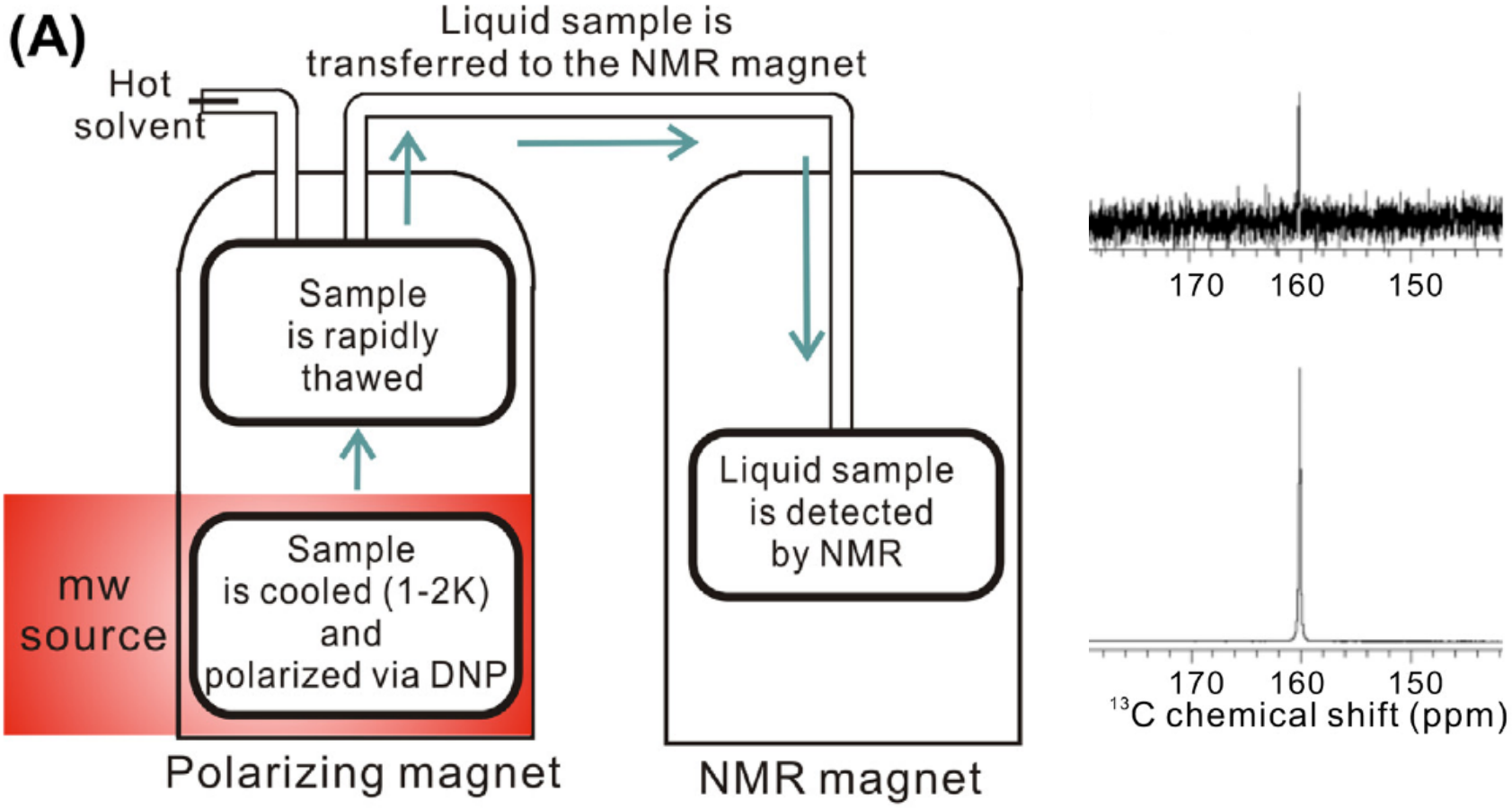


DNP-MAS spectrum of ^{13}C, ^{15}N-proline



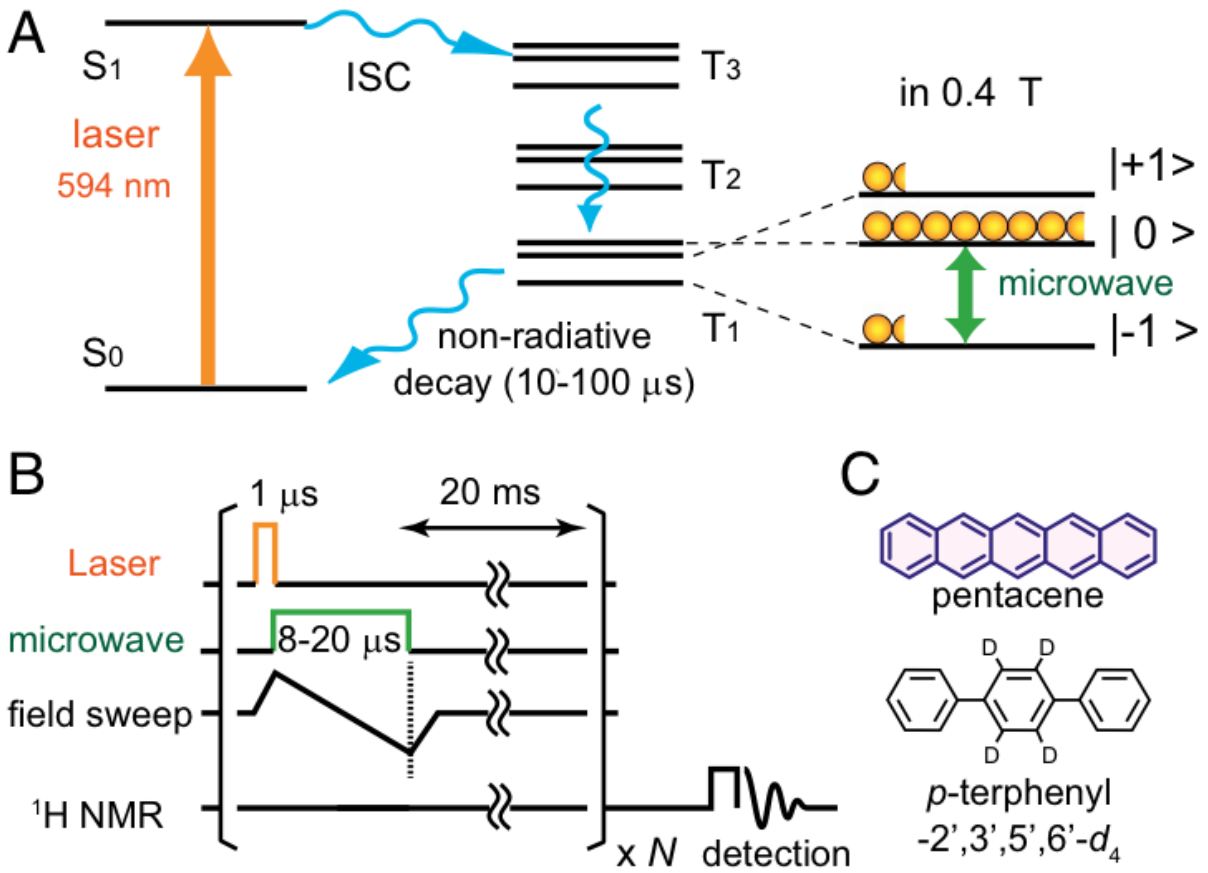
C. Song, T. Swager et. al., JACS (2006)

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.

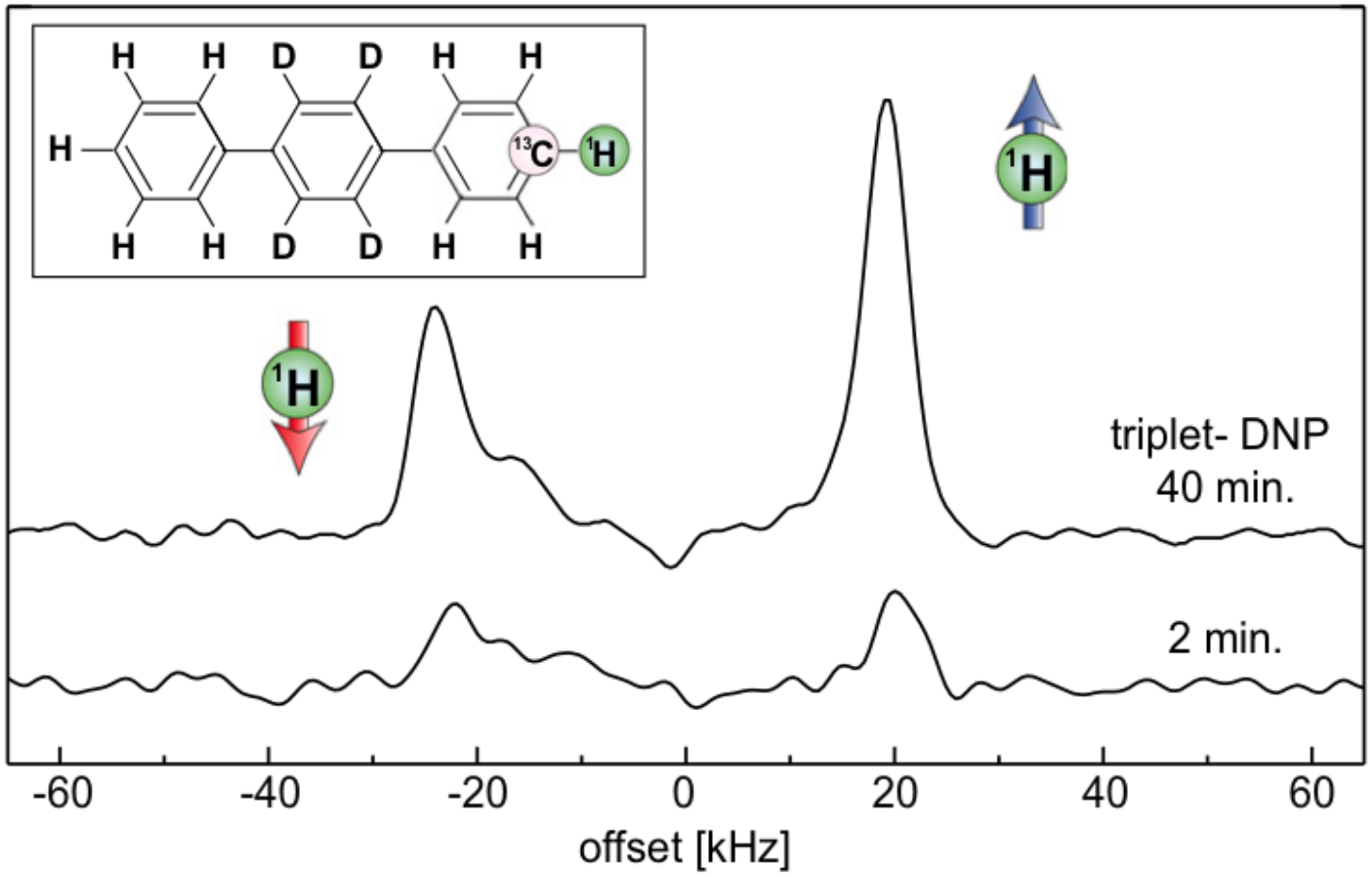
Photo-chemically induced DNP (Photo-CIDNP)



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

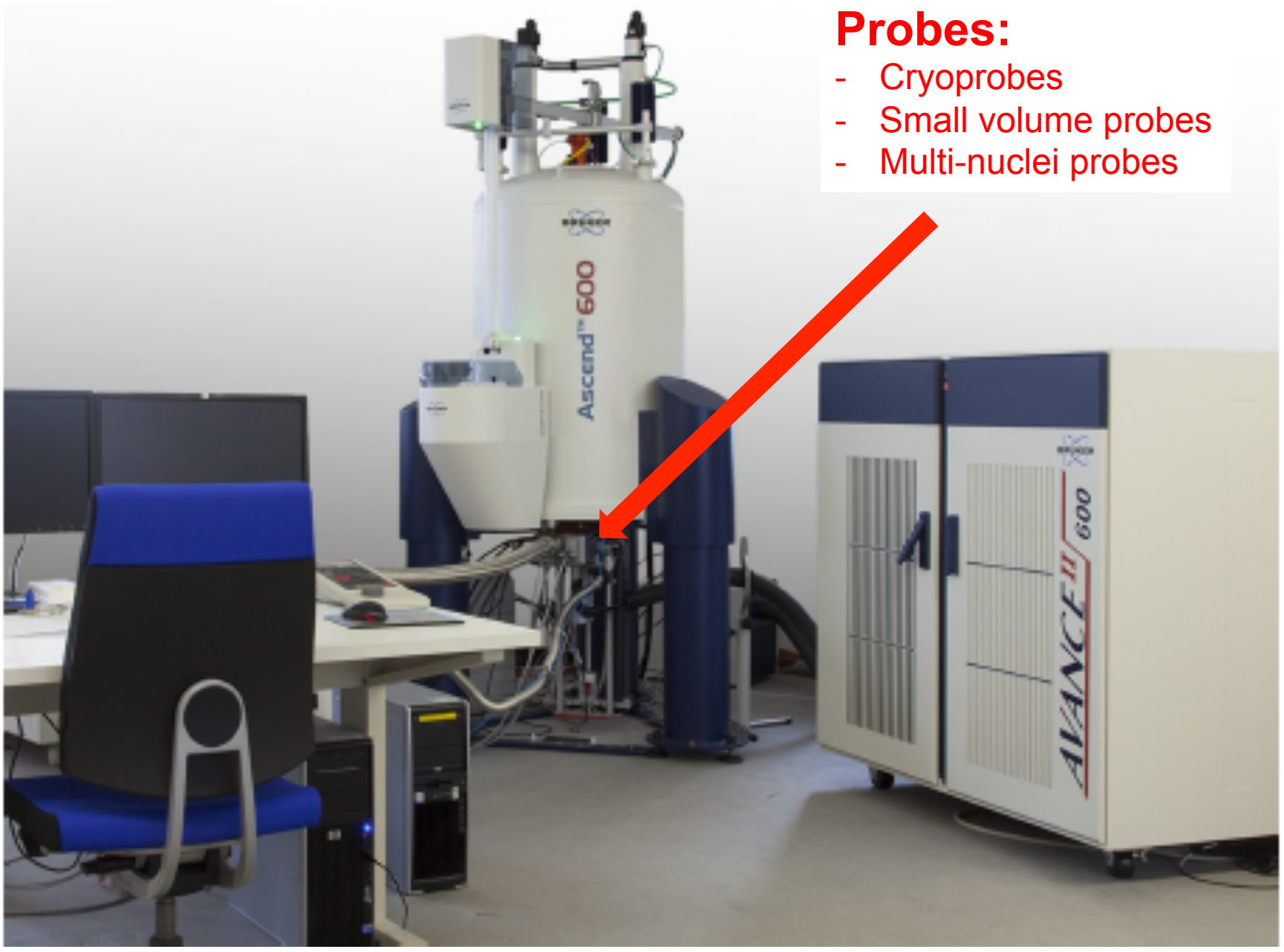
Photo-chemically induced DNP (Photo-CIDNP)

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

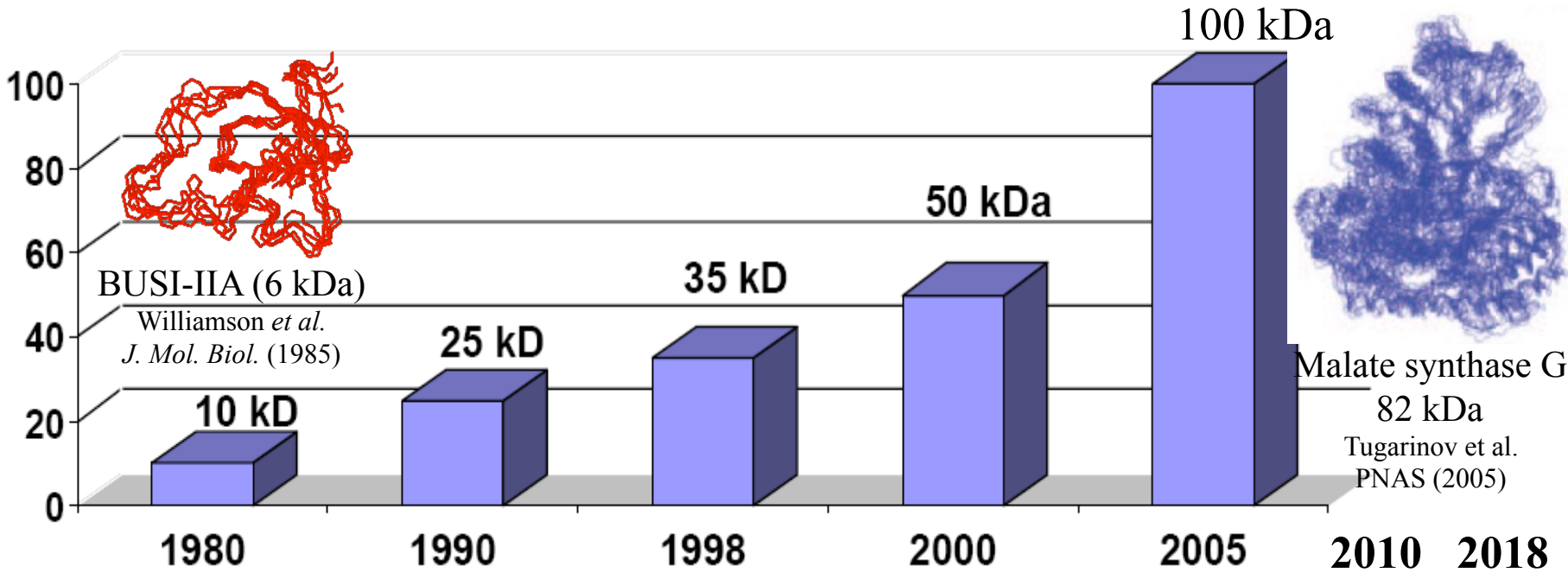


Kenichiro Tateishi et al, Room temperature hyperpolarization of nuclear spins in bulk, PNAS May 2014

Technological innovations

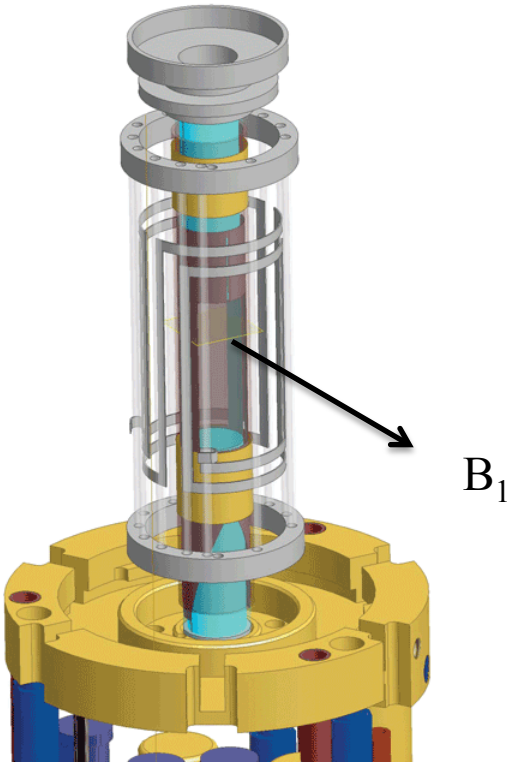


NMR: developments and limits



Cryoprobes		500, 600	700,800	1000

The probe



$$M_0 = \frac{N(\gamma\hbar)^2 B_0}{4kT}$$

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

Q quality factor, η filling factor

Gain with a cryoprobe

Induced Signal Voltage to Noise Voltage

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

Coil Design

Coil Resistance

Temperature

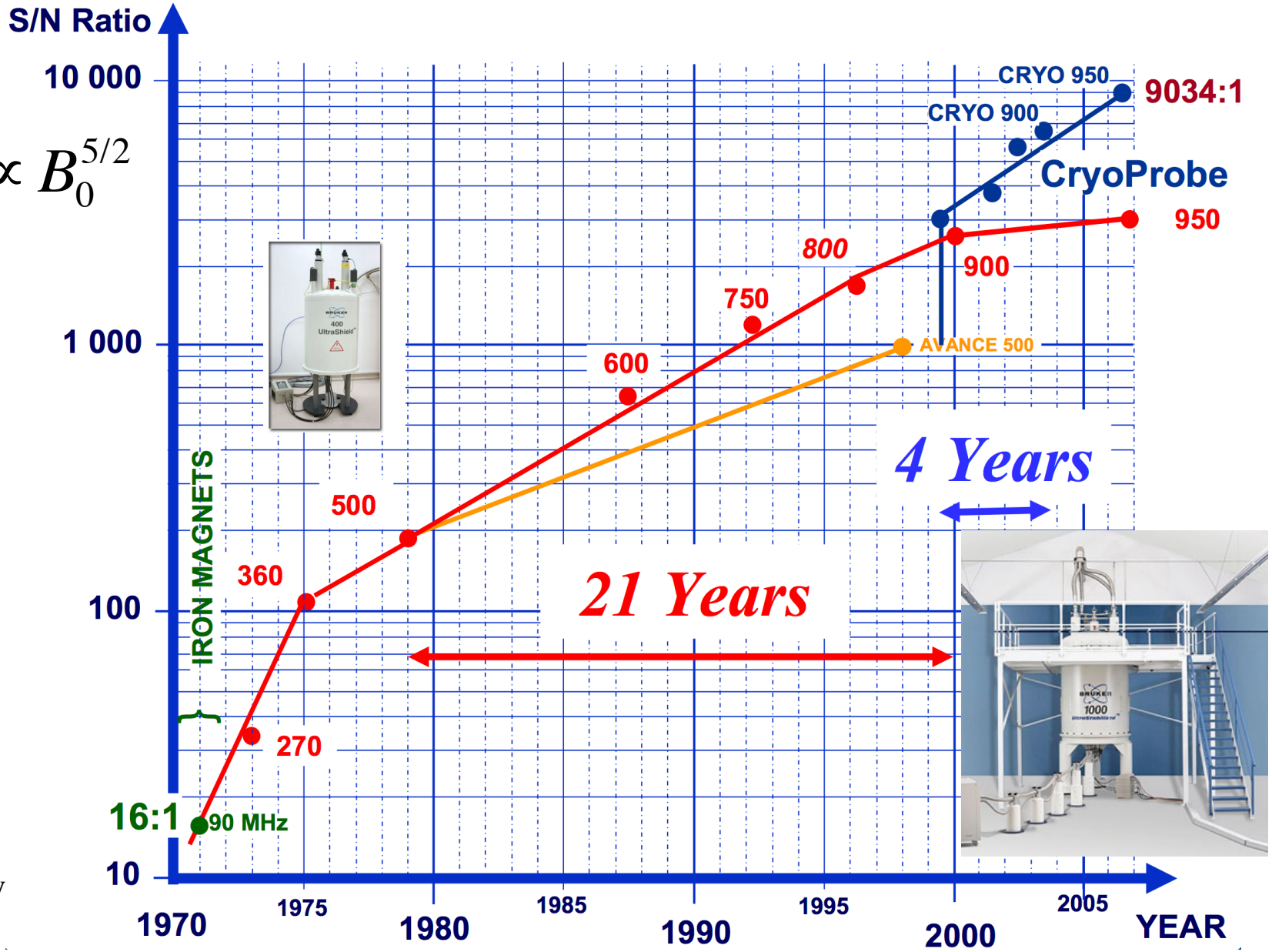
$$S/N \propto Q\eta M_o$$

Q quality factor, η filling factor

Technological innovations

Signal-to-noise depends on the magnetic field

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

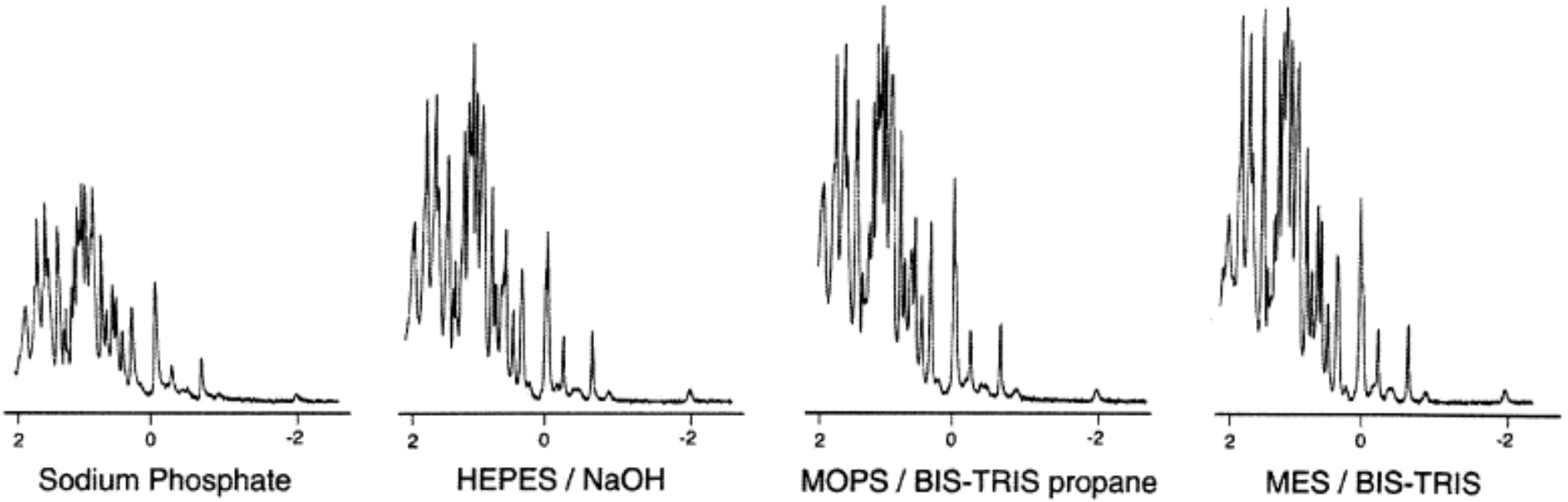


Data courtesy of Bruker

Limitations of cryoprobes

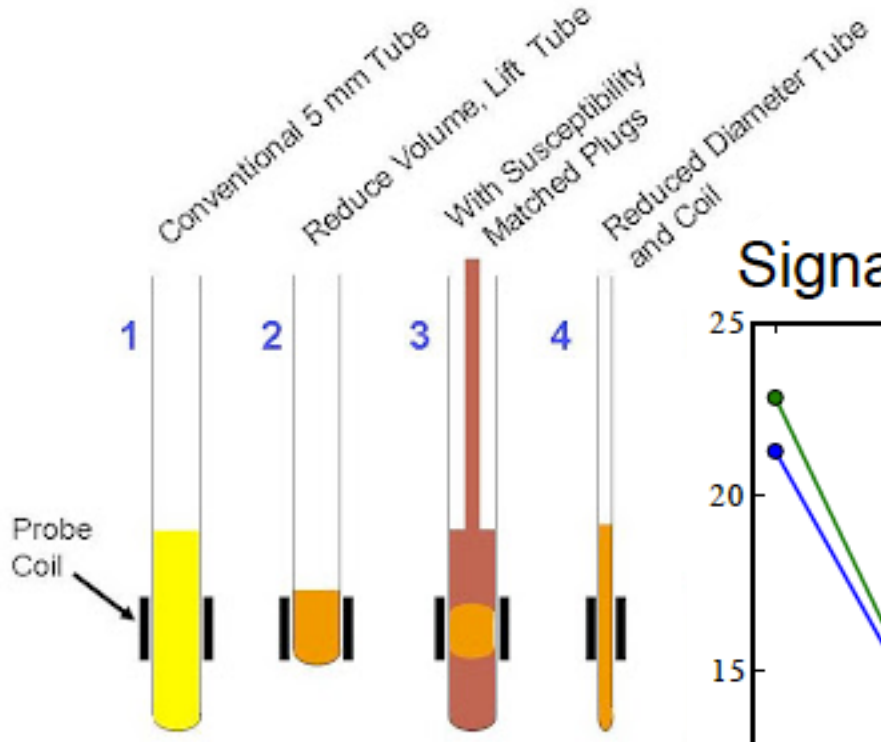
Low-Conductivity Buffers for High-Sensitivity NMR Measurements

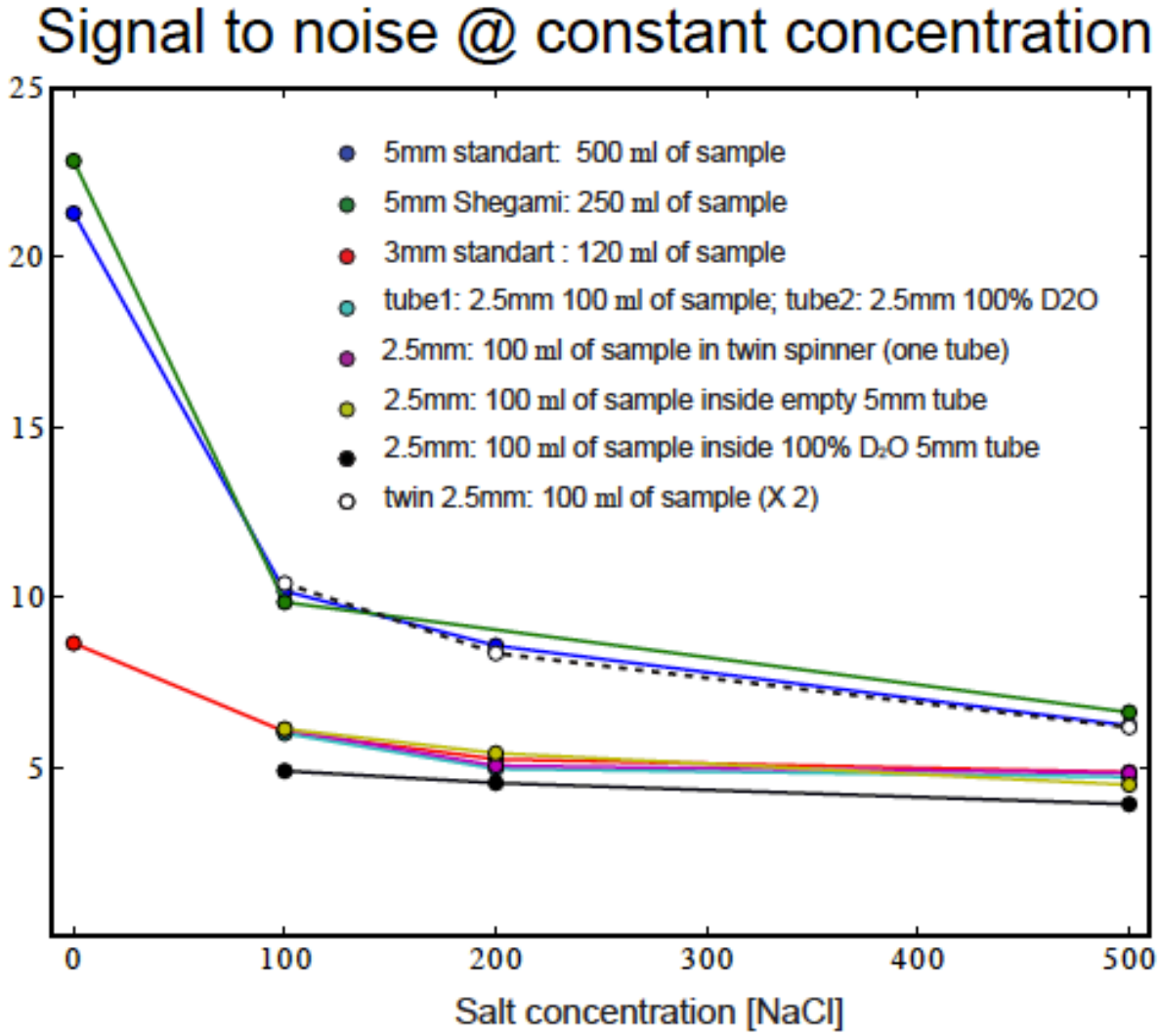
Alexander E. Kelly,† Horng D. Ou,† Richard Withers,‡ and Volker Dötsch*,§



JACS, 2002

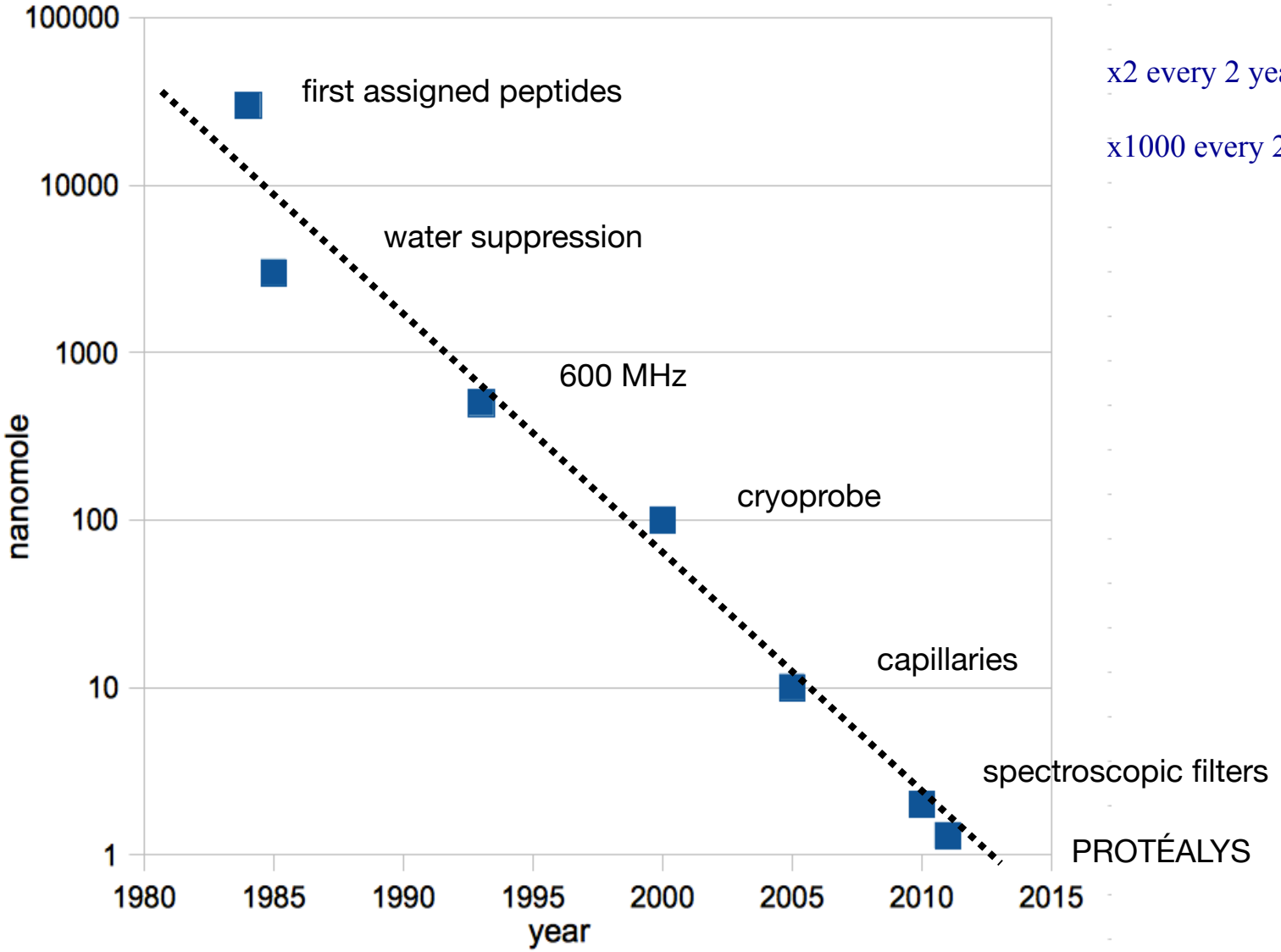
Limitations of cryoprobes





Gain with a cryoprobe

Quantity of protein detected



x2 every 2 years

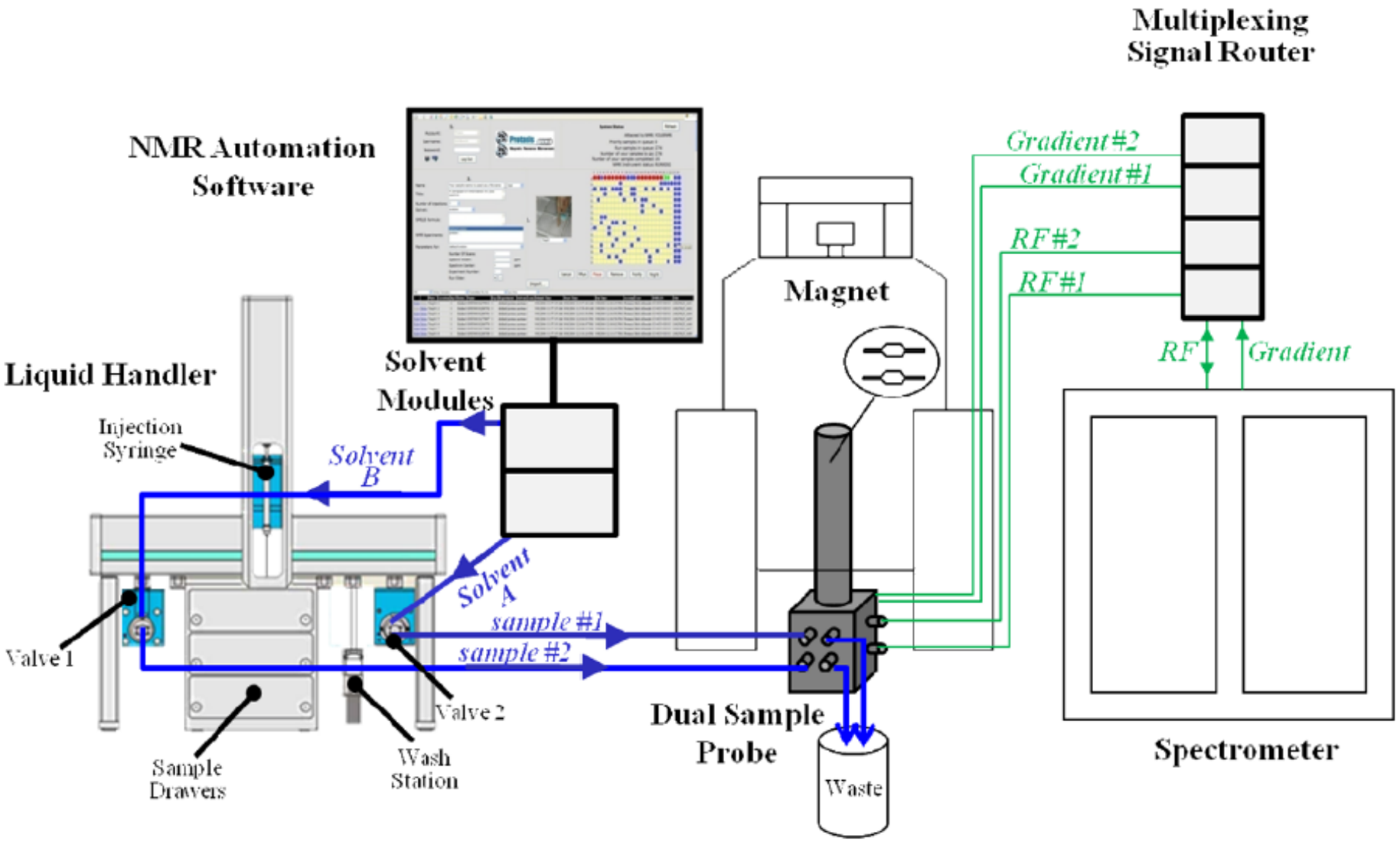
x1000 every 20 years

Data M.-A. Delsuc, IGBMC

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

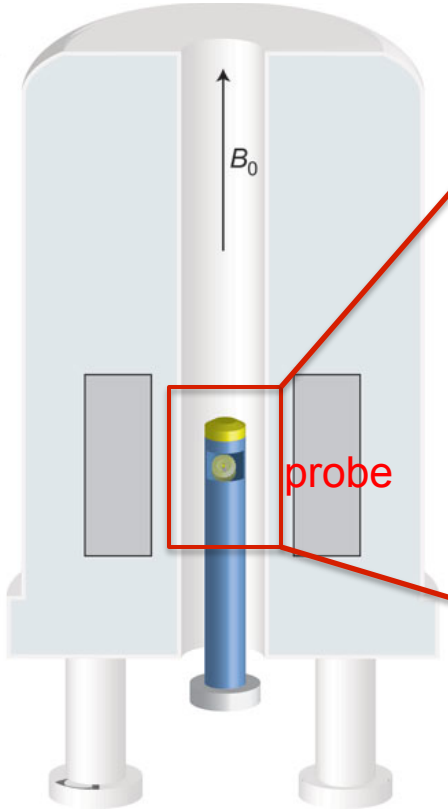
Multiplexed NMR: An Automated CapNMR Dual-Sample Probe

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*,†



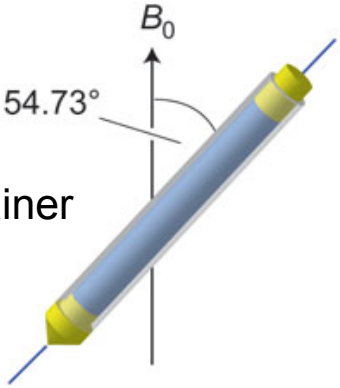
Instrumentation for Magic-Angle-Spinning ssNMR

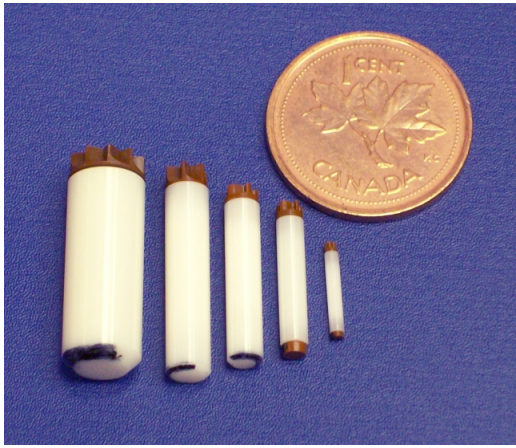
NMR magnet

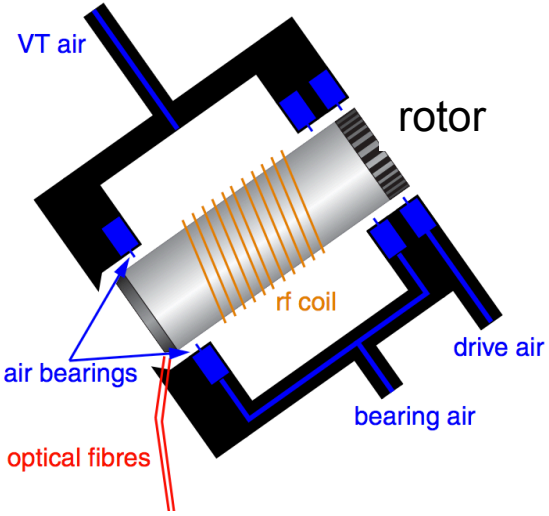


probe

sample container ("rotor")







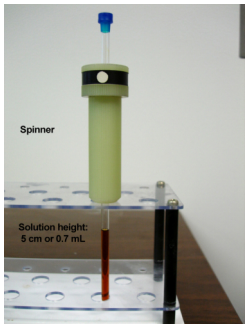
rotation driven by gas flows

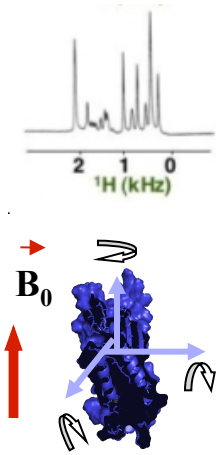
diameter	max. speed	sample volume
4 mm	15 kHz	70 µL
3.2 mm*	25 kHz	30 µL
1.6 mm*	40 kHz	8 µL
1.3 mm*	67 kHz	1.7 µL
0.9 mm	100 kHz	0.7 µL

*currently or soon at IBS

Liquid *vs* solid-state probe

Liquid State NMR

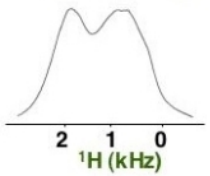


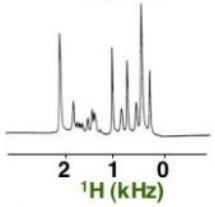


Brownian motion

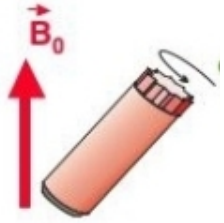
~ 400 µl of soluble sample

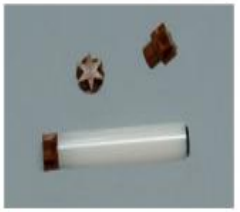
Solid State NMR





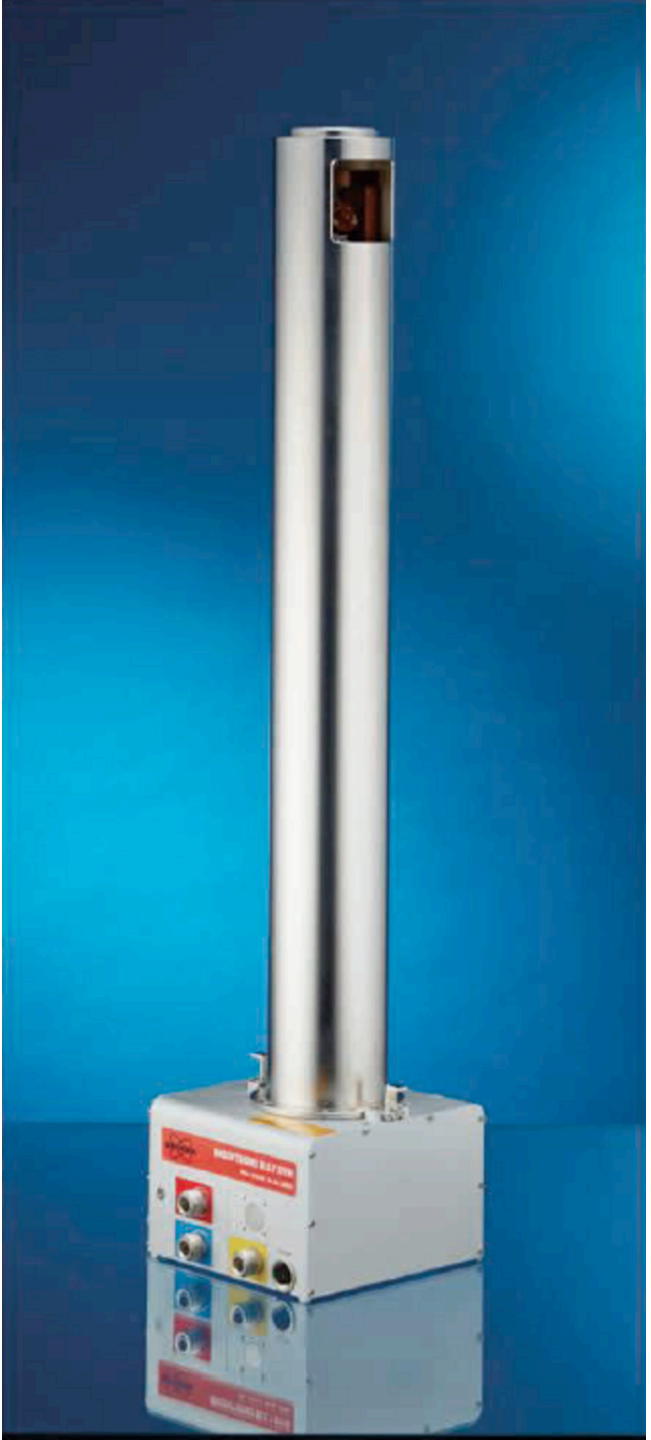
MAS
(Magic Angle Spinning)



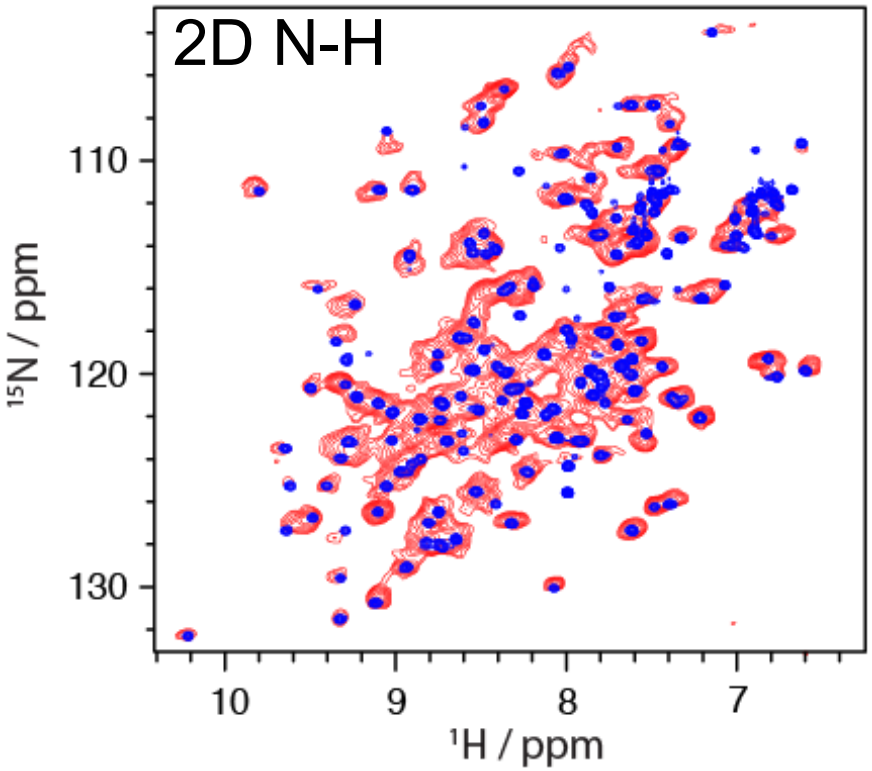


~ 20 µl of hydrated insoluble sample

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



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.

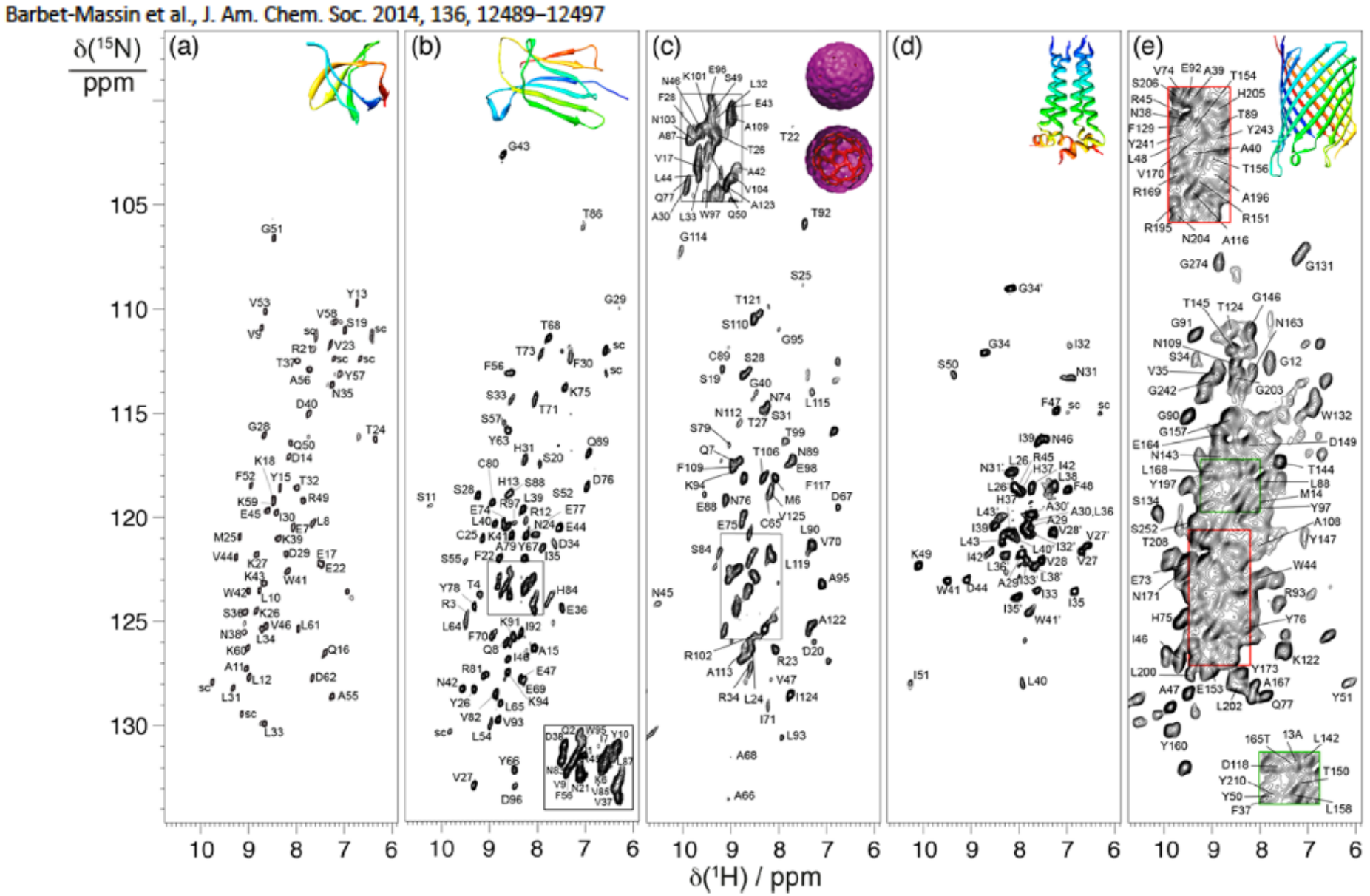
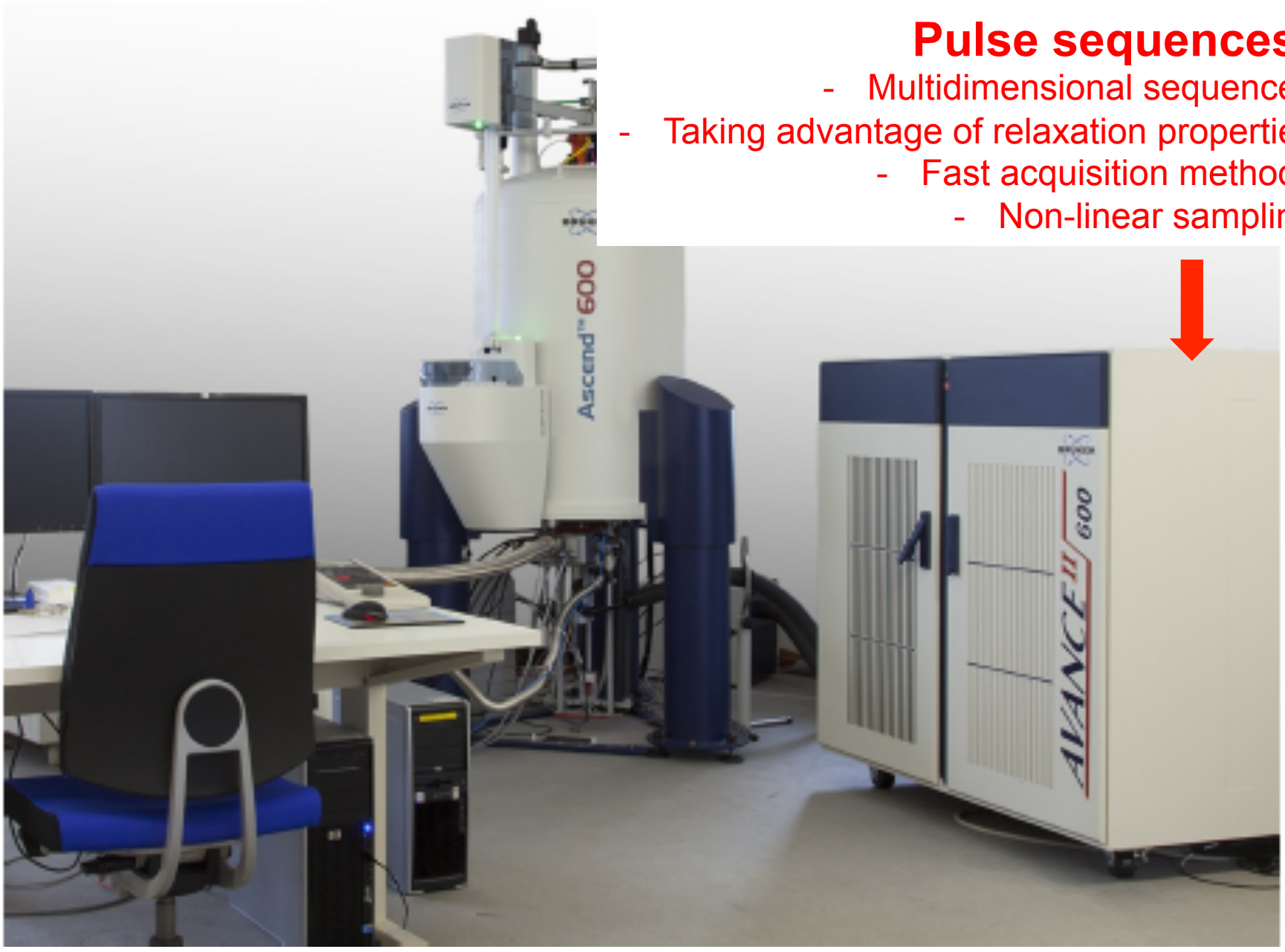


Figure 2. $^{15}N-^{1}H$ correlation spectra recorded on a 1 GHz spectrometer under 60 kHz MAS for [U-H^N,^2H,^{13}C,^{15}N]-labeled (a) microcrystalline SH3, (b) microcrystalline β2m, and (c) sedimented nucleocapsids of AP205, (d) M2 channel, and (e) OmpG.

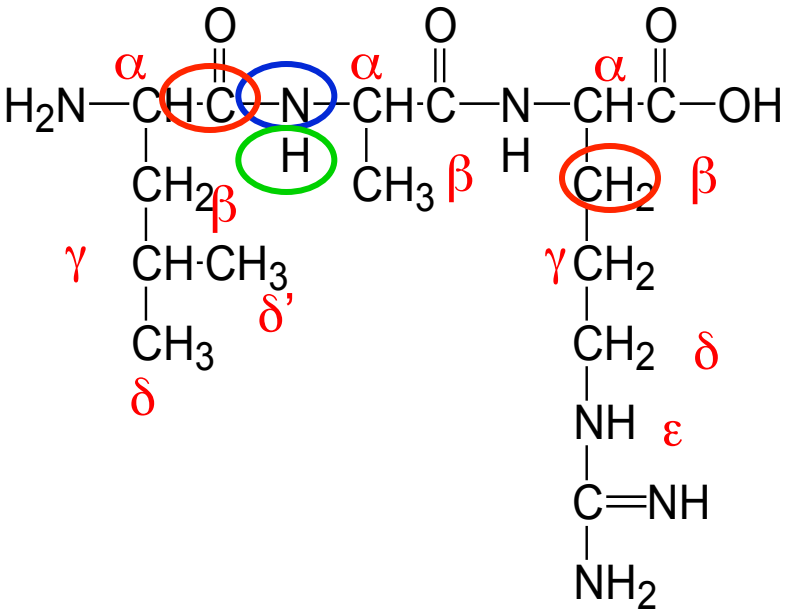
Technological innovations



Pulse sequences:

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

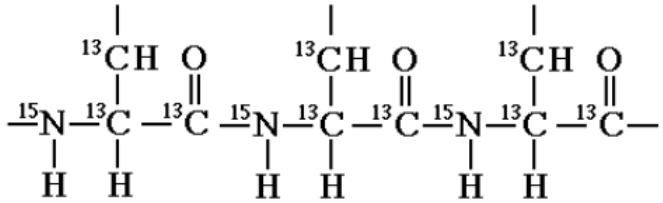
Coherence or magnetization transfer experiments

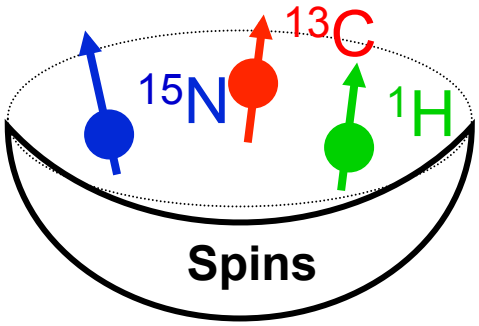


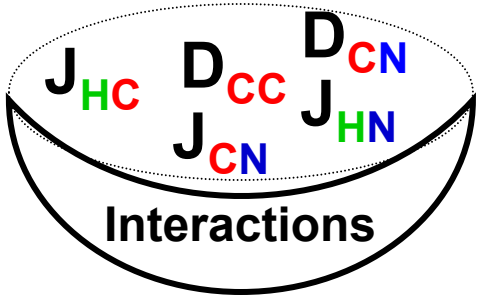
Doubly labeled sample:
^{13}C, ^{15}N

Recombinant protein in *E. coli*
$^{15}NH_4Cl$
^{13}C-glucose

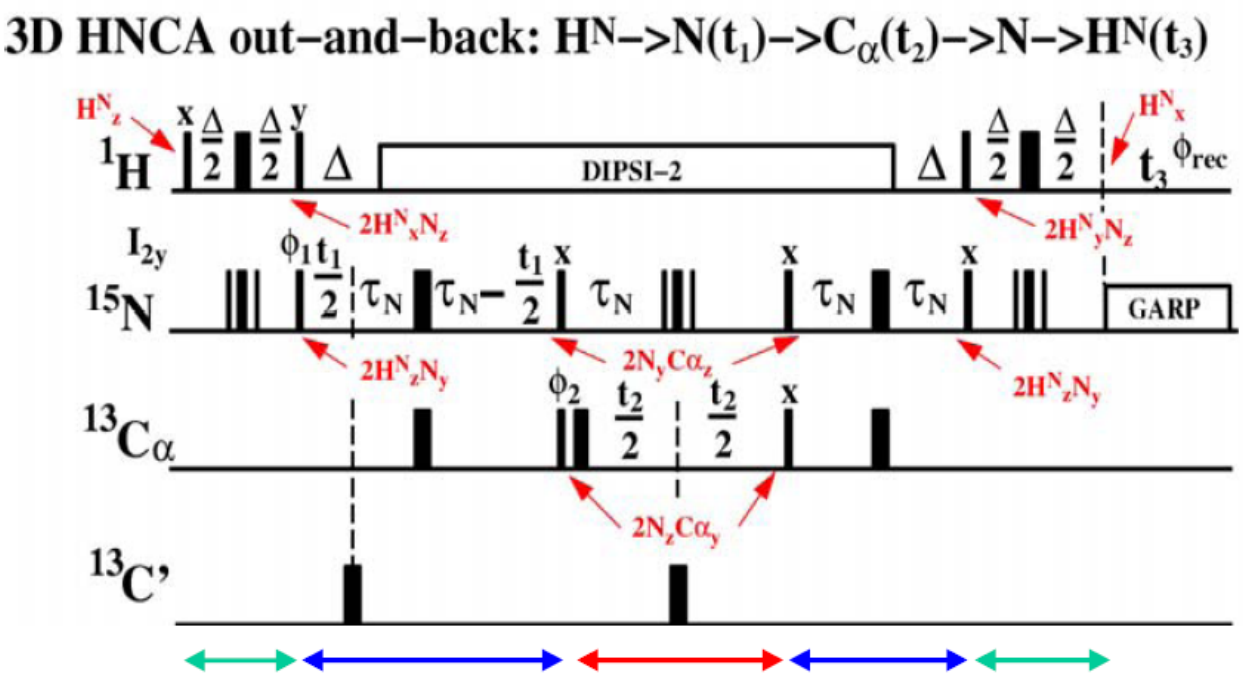
Recombinant DNA or RNA with labeled NTPs,
Enzymatic synthesis







Coherence or dipolar transfer experiments in liquids



$\sin^4(\pi\,{}^1J_{N,HN}\,\Delta)\,\exp(-2\Delta/T_{2HN})$

$* \sin^2(\pi\,{}^1J_{C\alpha,N}\,2\tau)\,\cos^2(\pi\,{}^2J_{C\alpha,N}\,2\tau)\,\exp(-4\tau/T_{2N})$

$* \cos(\pi\,{}^1J_{C\alpha,C\beta}\,t_2)\,\exp(-t_2/T_{2C\alpha})$

$H_i \xrightarrow{{}^1J_{HN}\ \text{INEPT}} N_i \xrightarrow[t_1]{{}^1J_{CN},\ {}^2J_{CN}} C_{\alpha i},\ C_{\alpha i-1} \xrightarrow[t_2]{{}^1J_{CN},\ {}^2J_{CN}} N_i \xrightarrow[t_3]{{}^1J_{HN}} H_i$

3D sequence allowing intra-amino acid assignment and sequential assignments

Bax *et al.*, 1990 Calmodulin

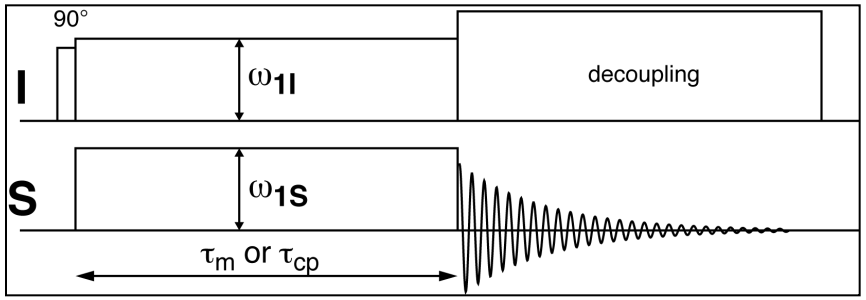
Dipolar transfer experiments in ssNMR

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

space part

spin part

cross-polarization

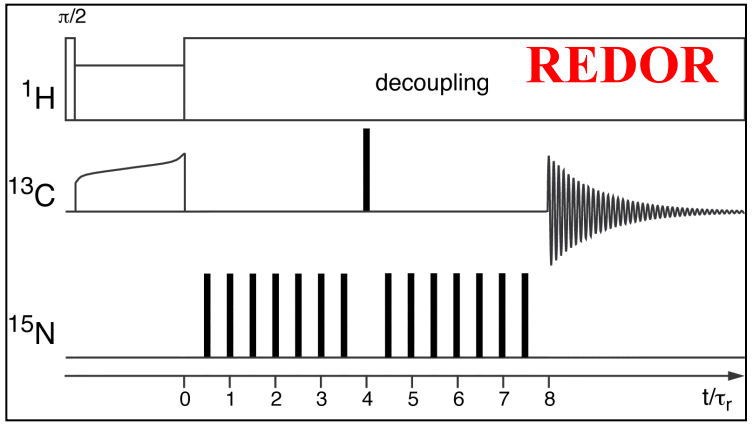


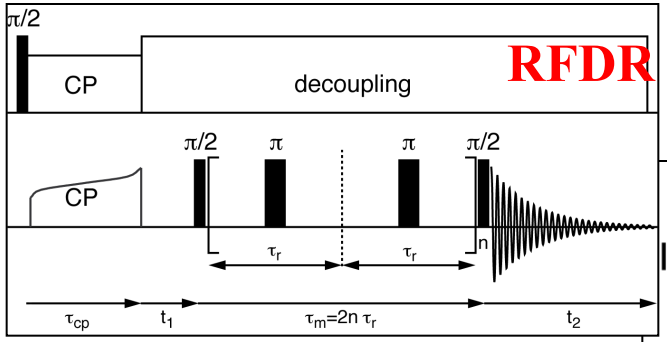
General idea:

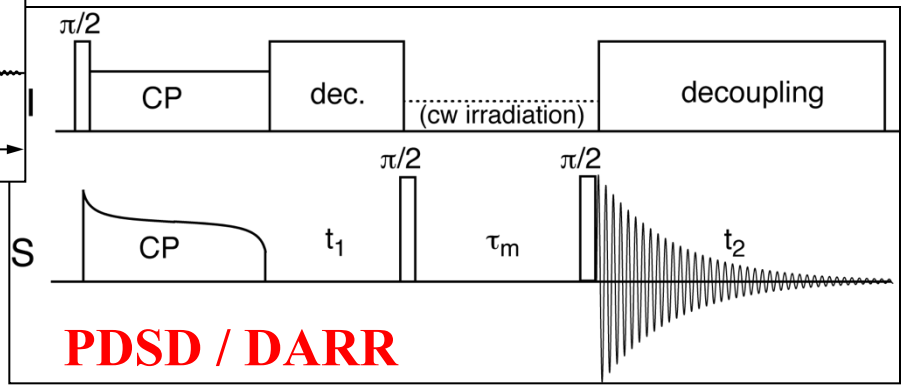
rotate sample (MAS)

+ rotate spins (RF irradiation)

→ create interference between these two processes







HORROR

DREAM

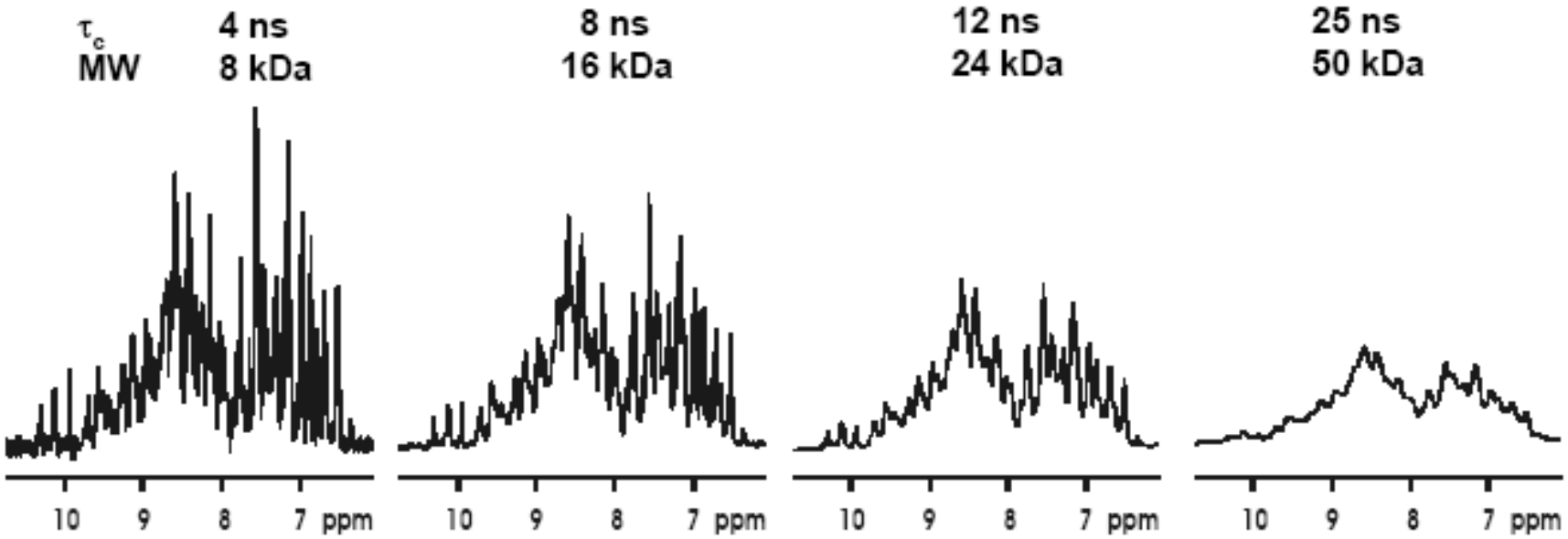
R-sequences

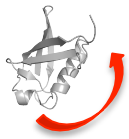
C-sequences ,….

NMR, a limited competitiveness for structures: an intrinsic size limitation in solution

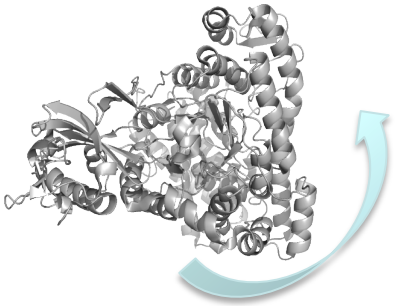
Liquid-state NMR a serious limit?

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





fast overall rotation

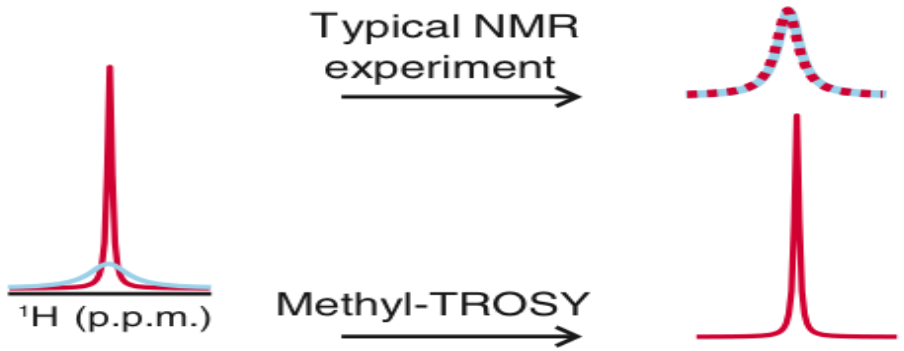


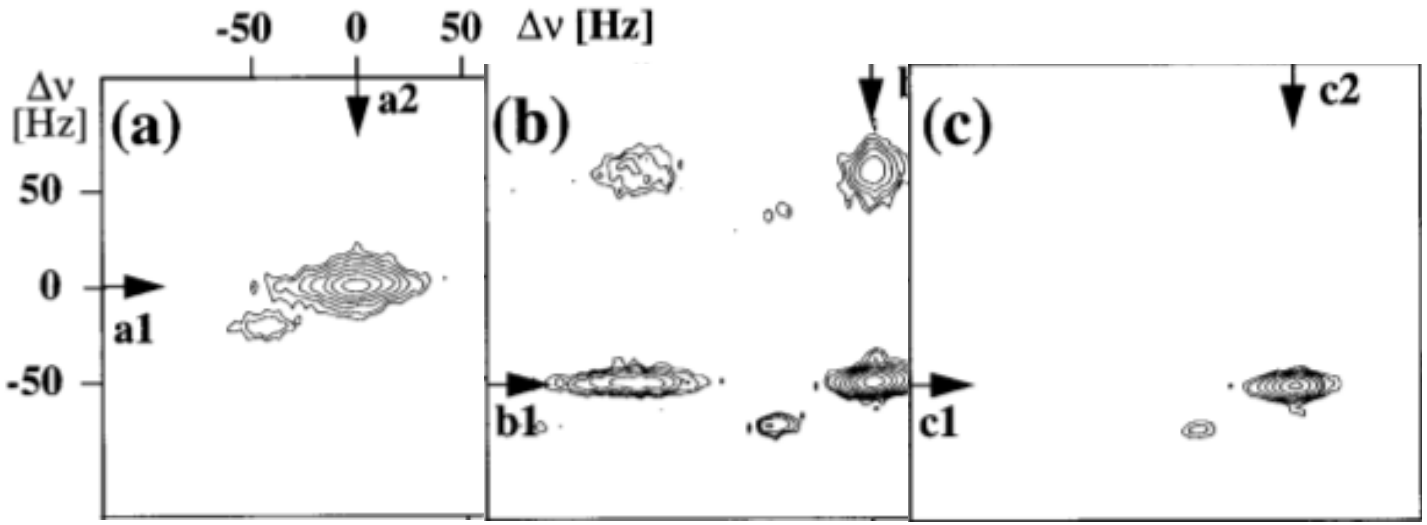
slow overall rotation

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

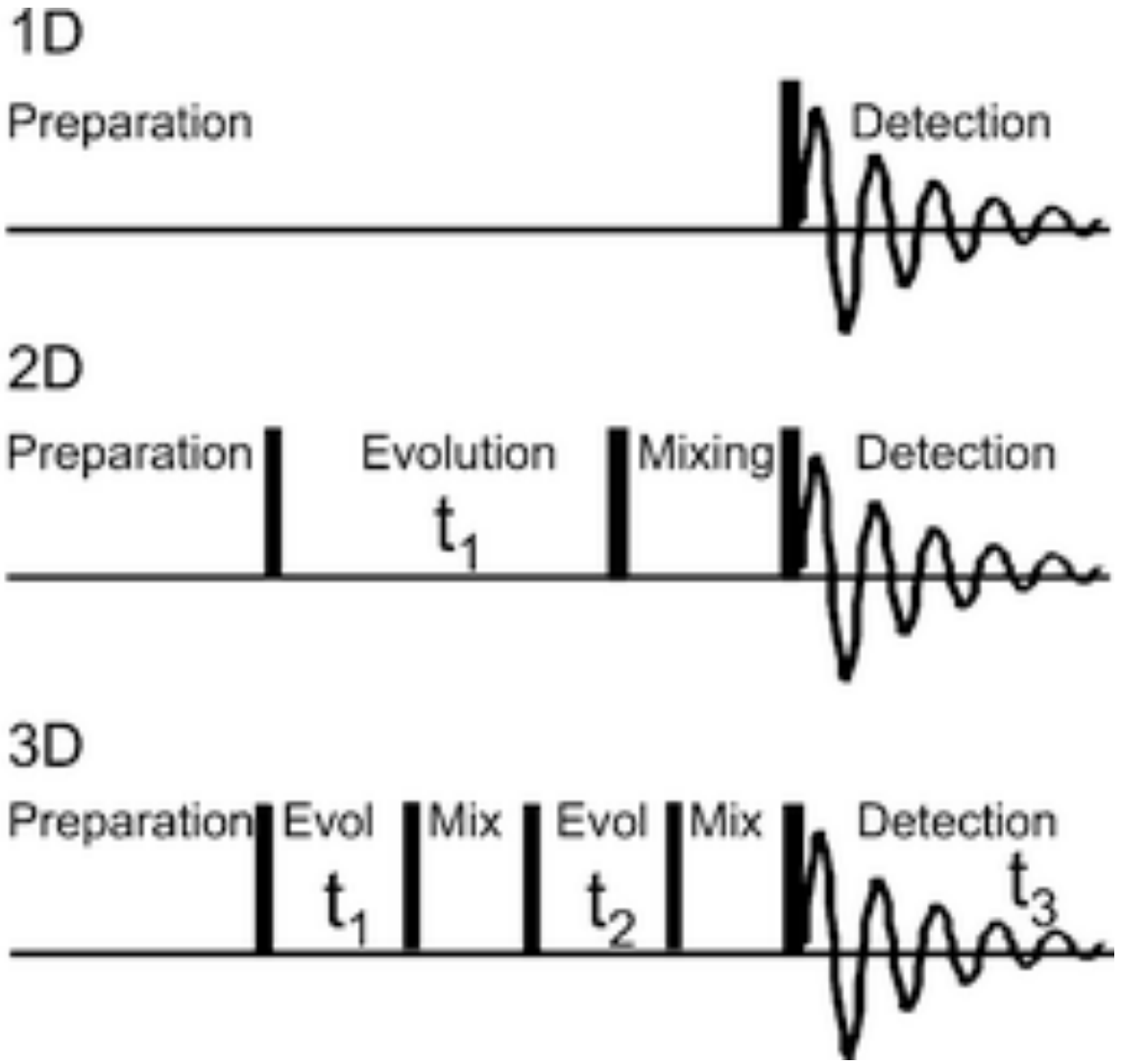
1. Transverse relaxation:

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





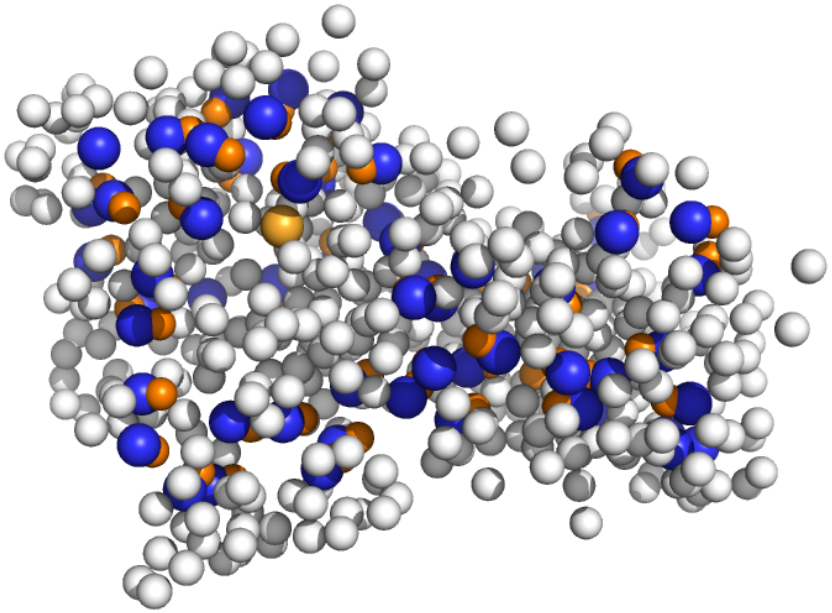
Data acquisition is full of dead times

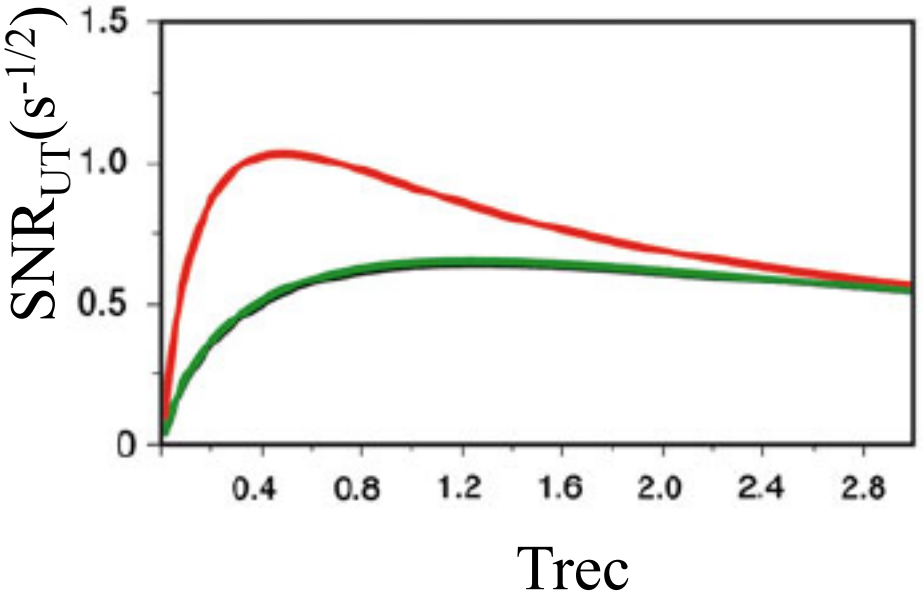


Exploitation of the relaxation properties

2. Longitudinal relaxation:

- Accelerate the return to the thermodynamic equilibrium to speed-up the acquisition process => SOFAST, BEST, BEST-TROSY

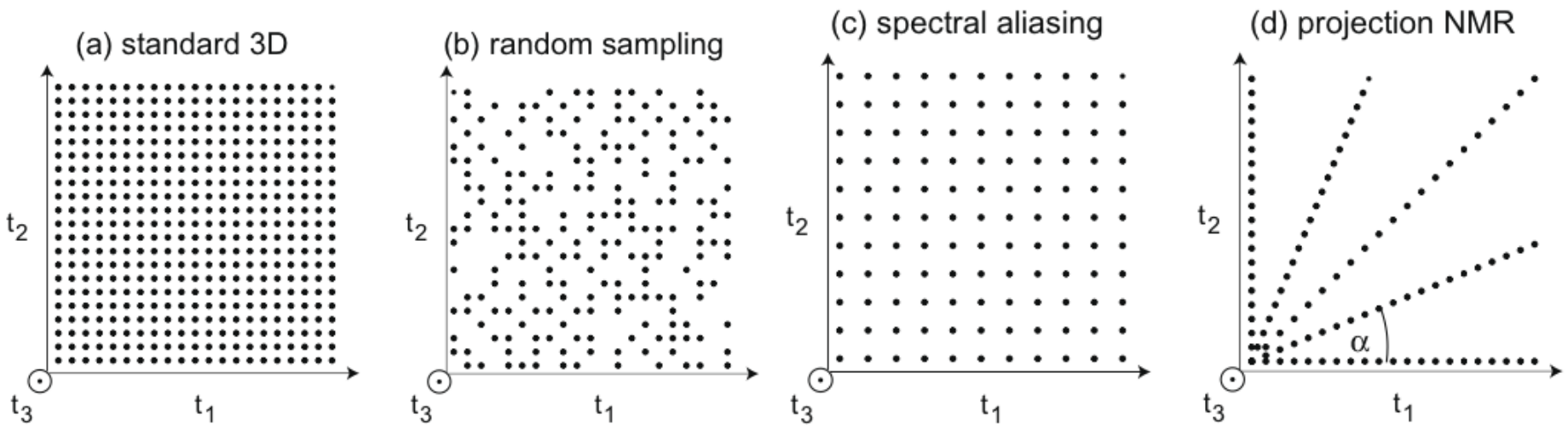




Solyom Z, Schwarten M, Geist L, Konrat R, Willbold D, Brutscher B. J Biomol NMR. 2013 Apr;55(4):311-21.

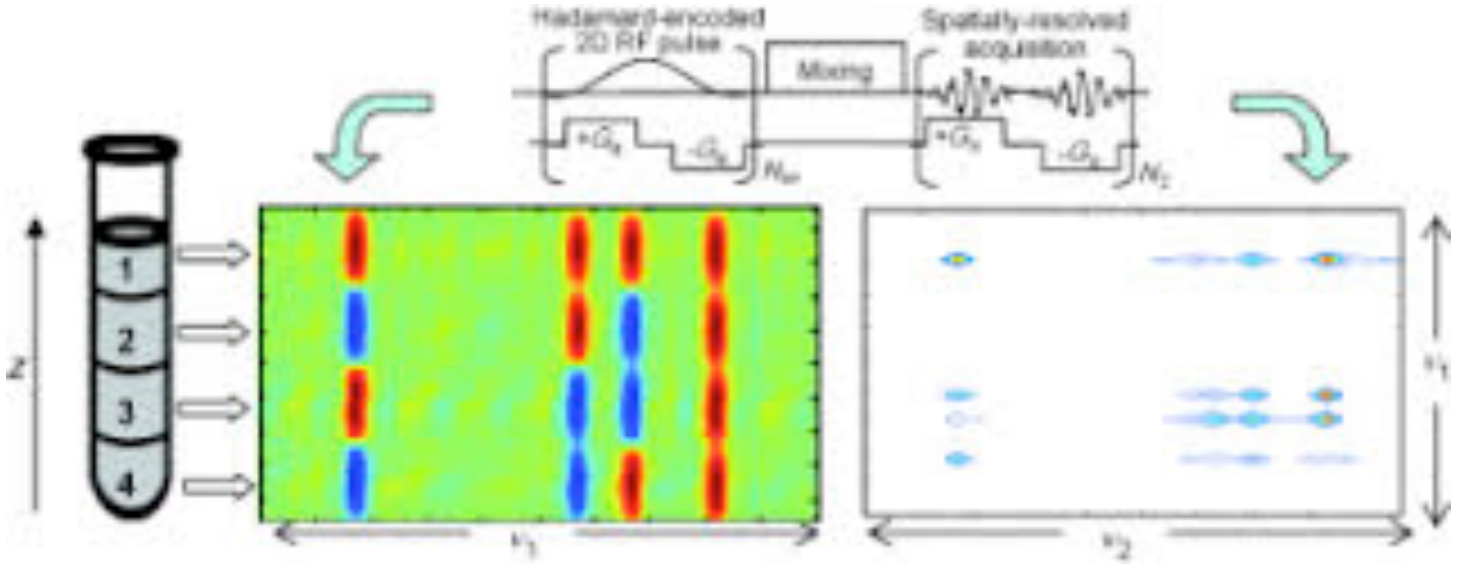
Alternative sampling methods

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

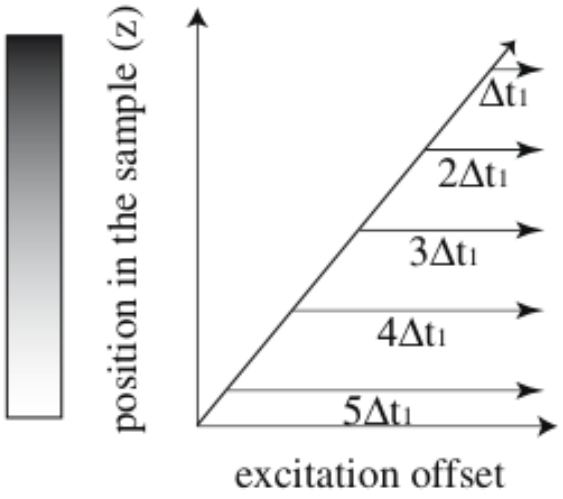


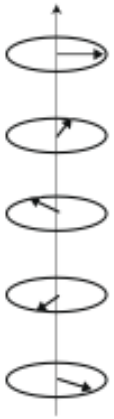
M. Mobli and J.C. Hoch Progress in Nuclear Magnetic Resonance Spectroscopy 83 (2014) 21–41

Alternative sampling methods

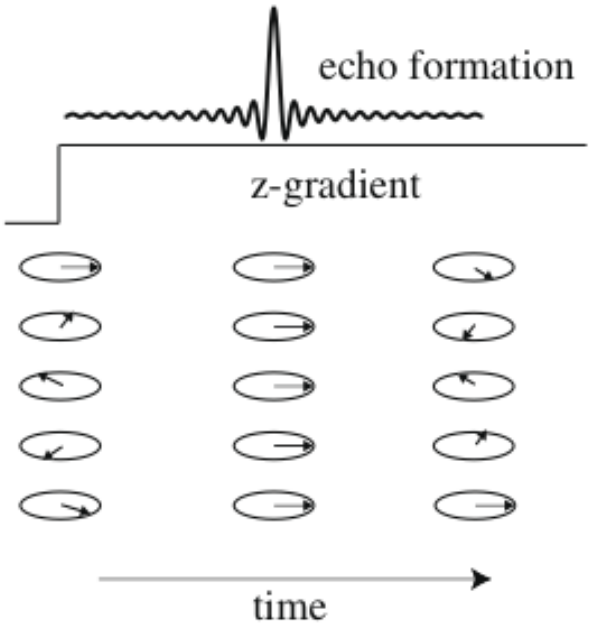


(1) space-encoded excitation (2) (3) gradient-assisted aquisition





spatially homo-geneous mixing



Single-scan spectroscopy

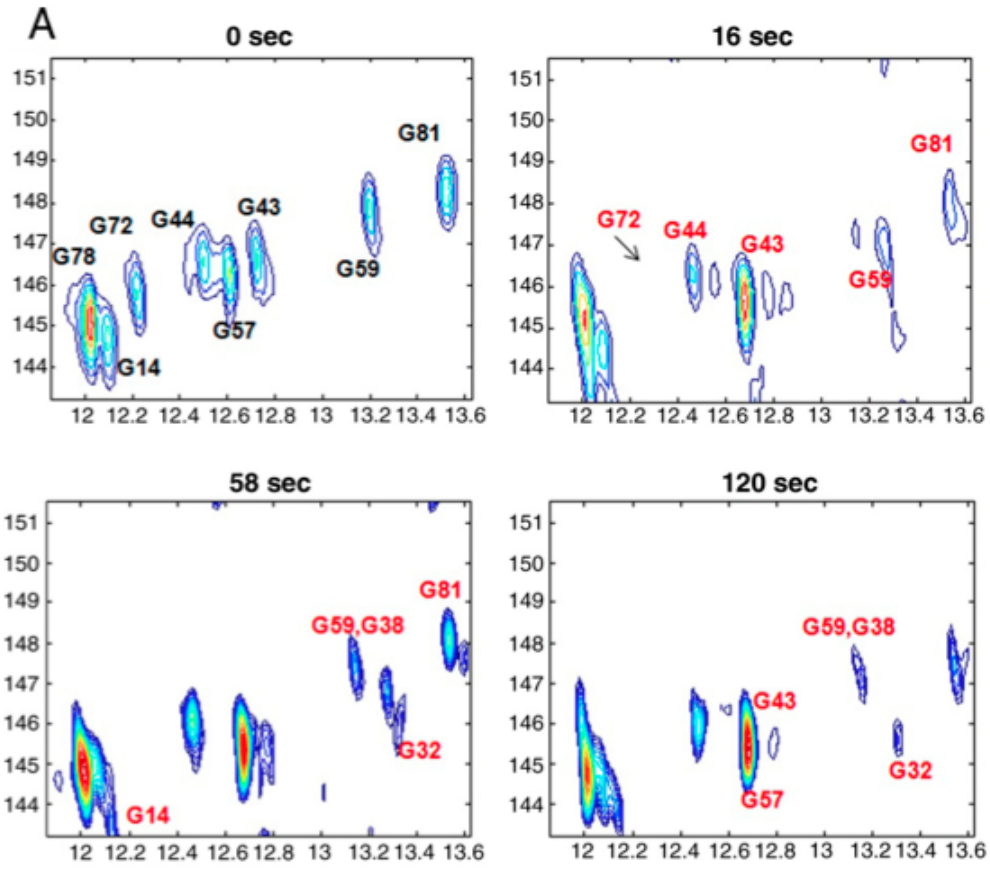
Frydman L, Scherf T, Lupulescu A. PNAS. 2002

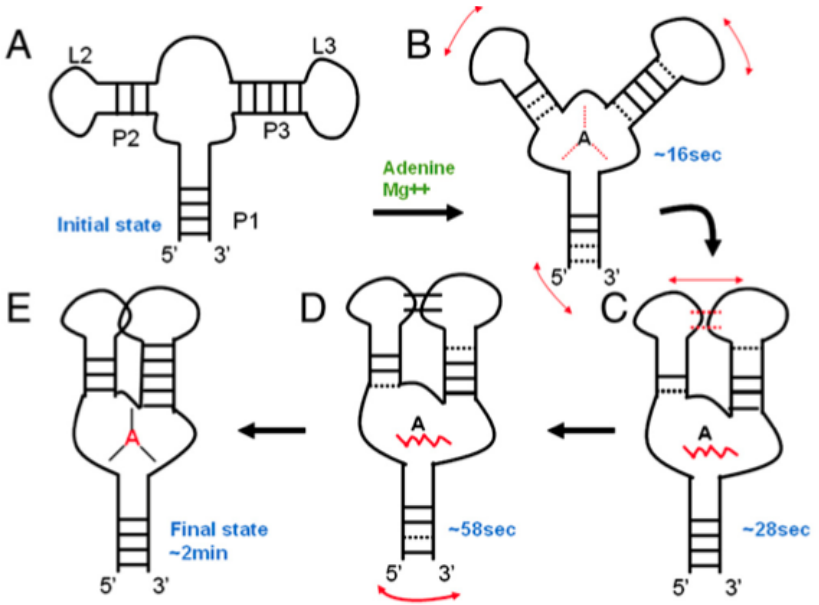
Application: following real-time folding of an RNA aptamer

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]

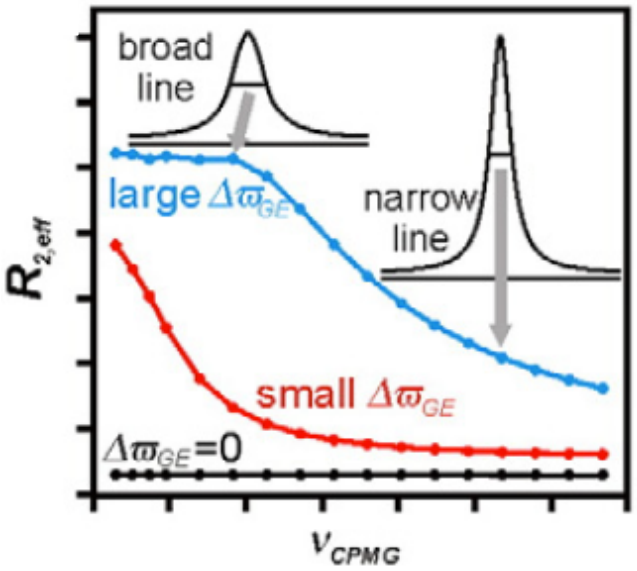
PNAS 2010

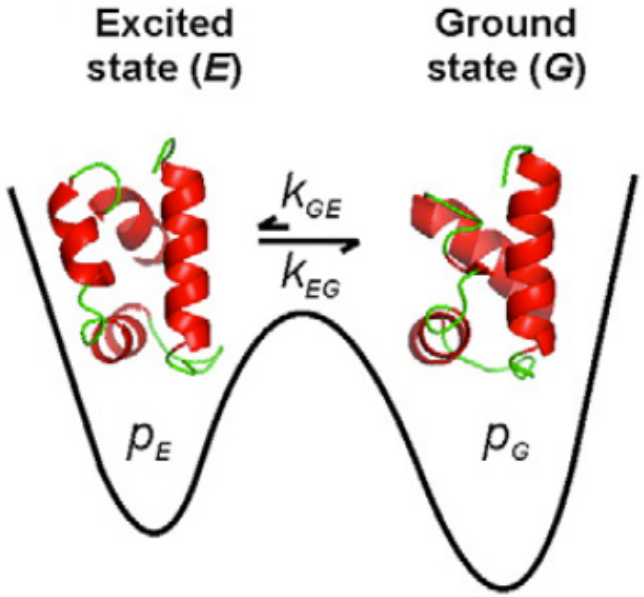




Assessing data on non-detectable states

CPMG $500 < k_{ex}\ (s^{-1}) < 2000$

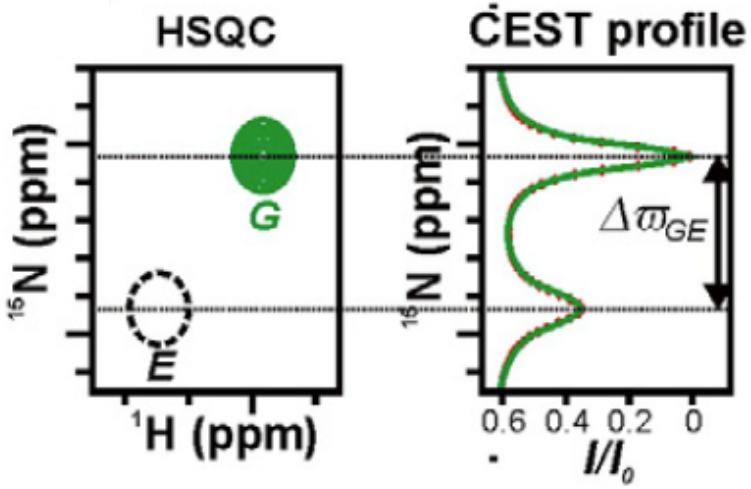




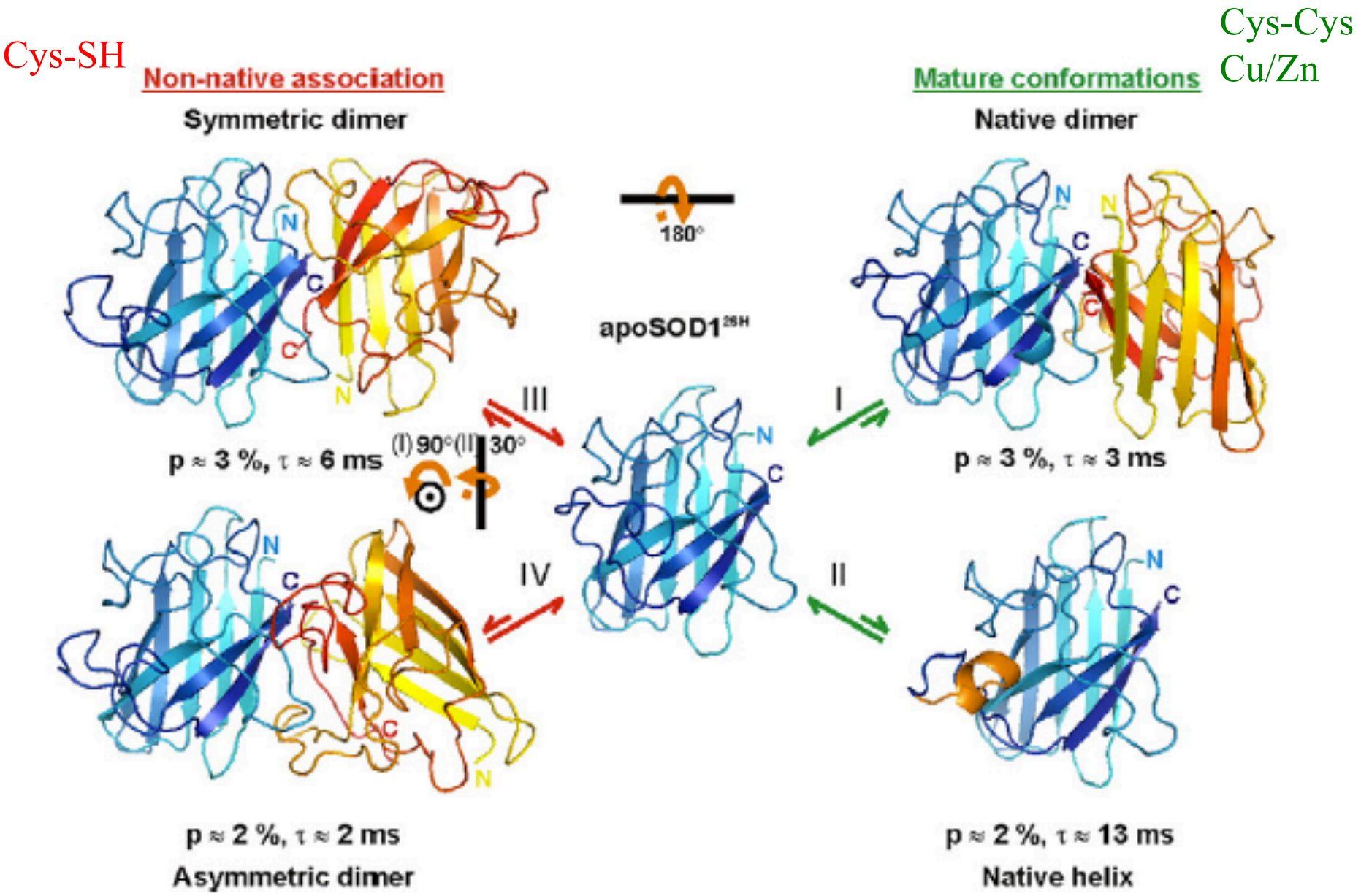
$p_G \gg p_E$

p_E 1% or more

CEST $50 < k_{ex}\ (s^{-1}) < 400$



Assessing data on non-detectable states



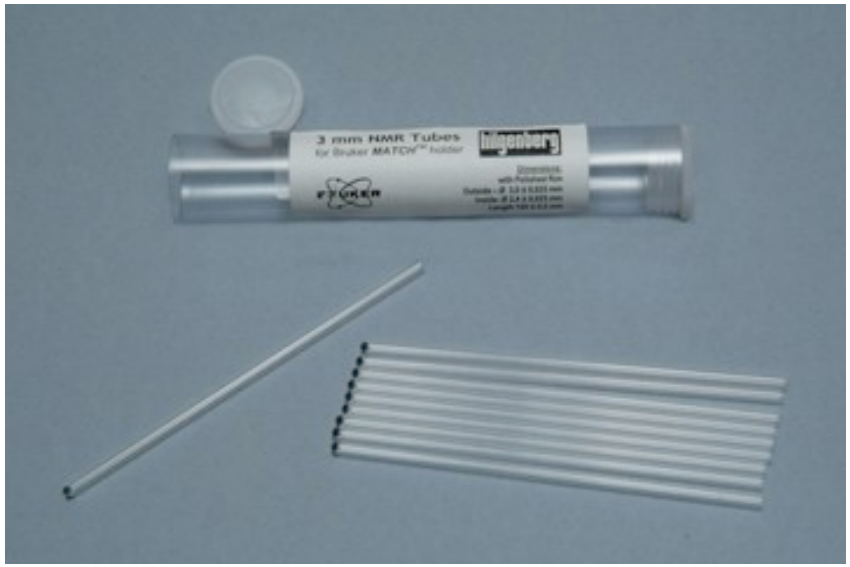
Sekhar and Kay, PNAS 2013, 12867-12874

Technological innovations

Samples :

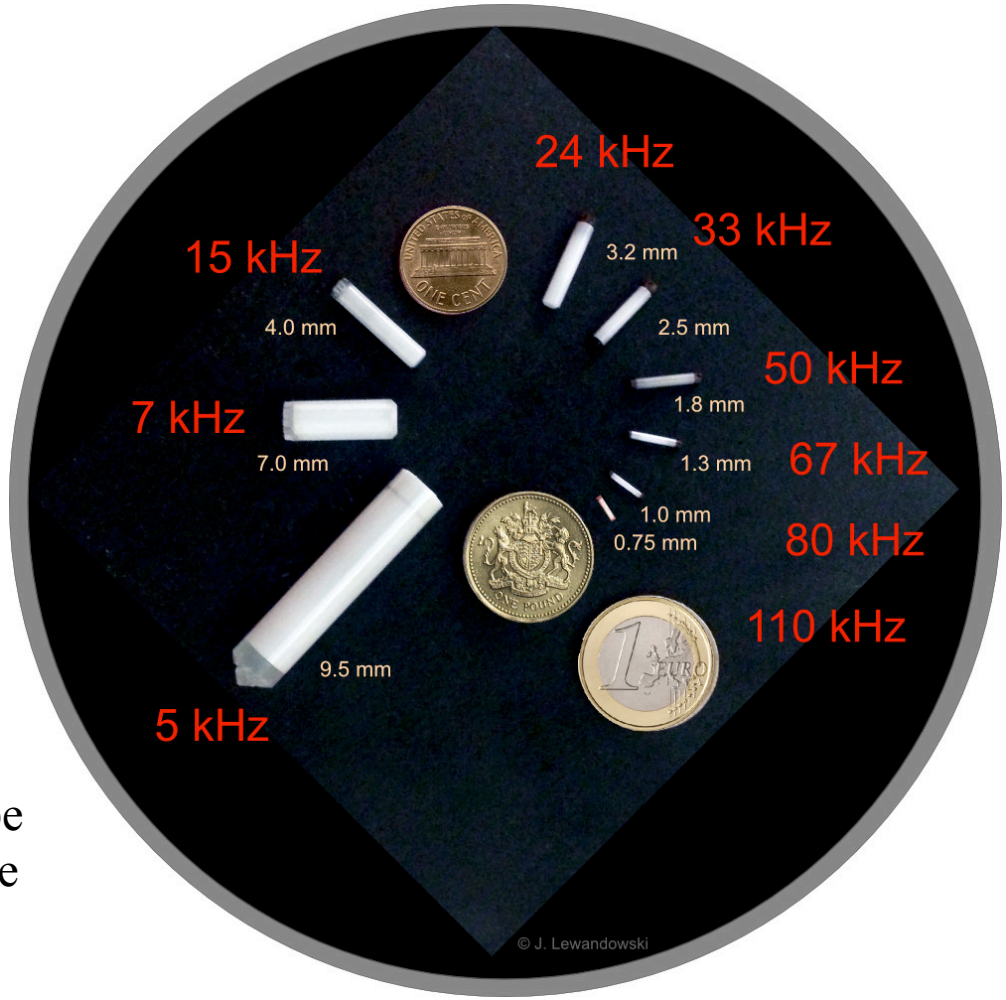
- Small volumes
- Isotopic labeling

Sample volume changes matches probe design



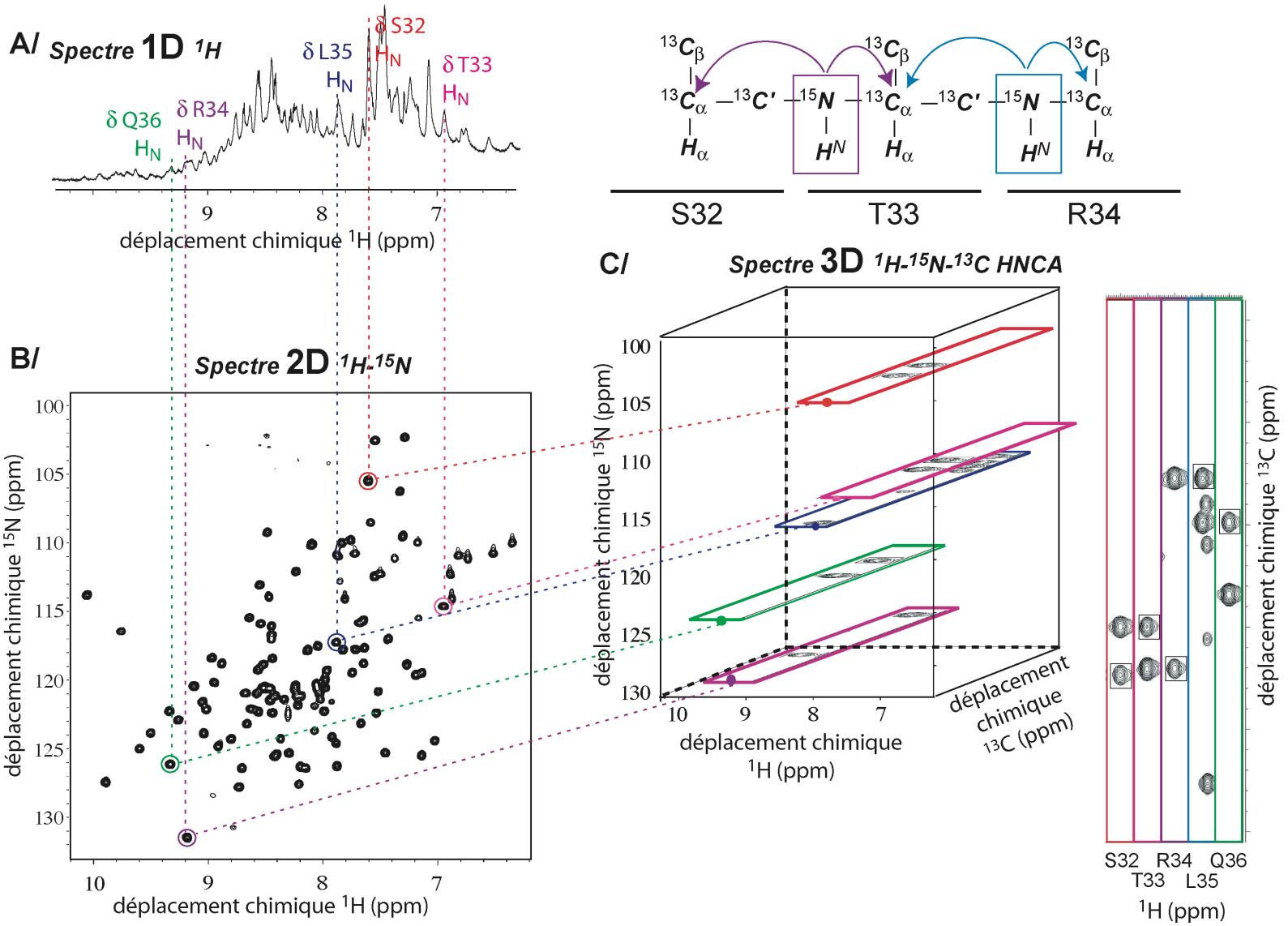
$$M_0 = \frac{N(\gamma\hbar)^2 B_0}{4kT} \qquad S/N \propto Q\eta M_O$$

1.7 mm cryoprobe
30 µL sample volume
Liquid-state NMR



111 kHz MAS probe
2 µL sample volume
Solid-state NMR

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



Is NMR limited to small molecules?

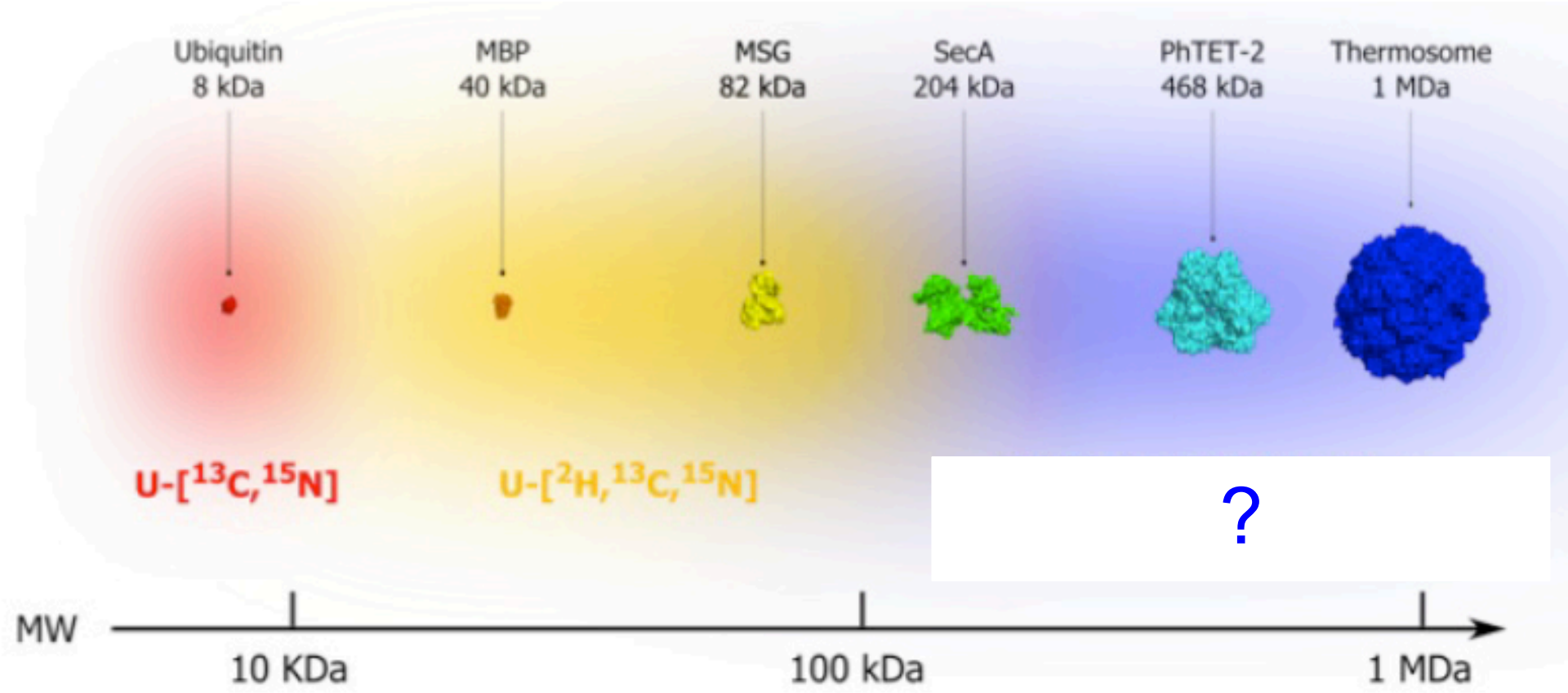
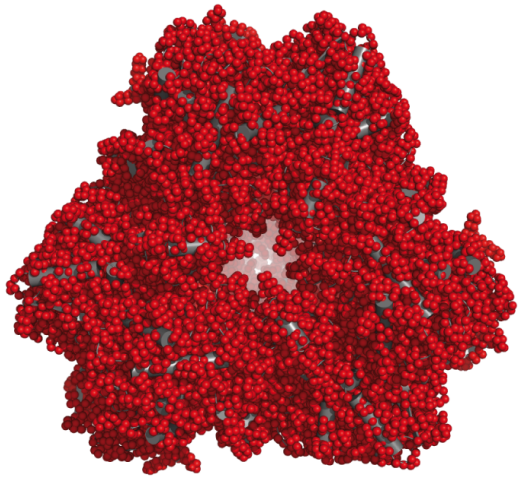


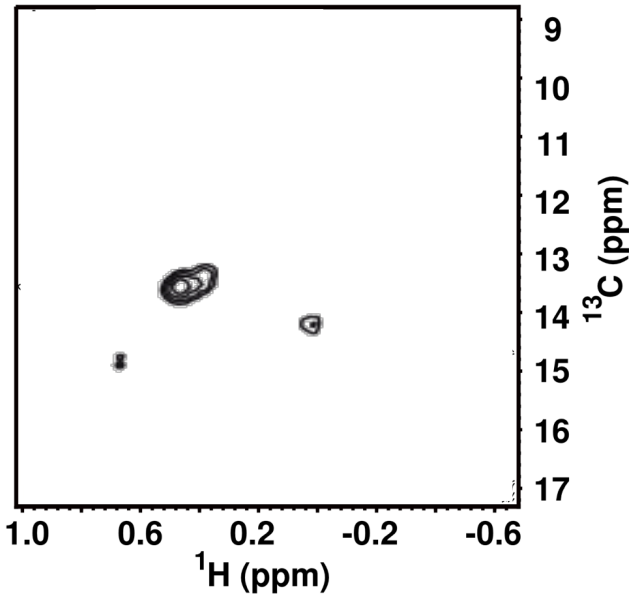
Figure courtesy of J. Boisbouvier

Can we investigate large functional machineries with NMR?

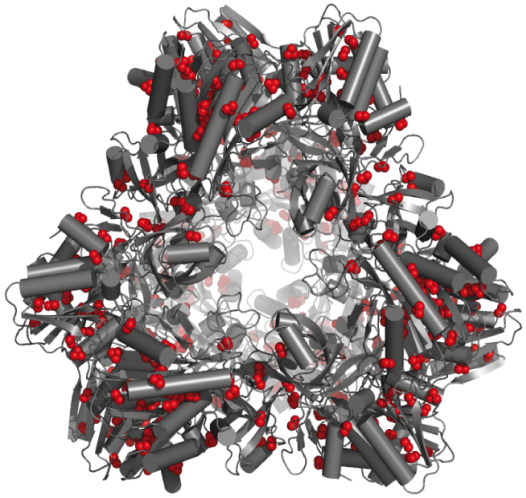


100% protonation

U-[^{1}H,^{13}C,^{15}N]

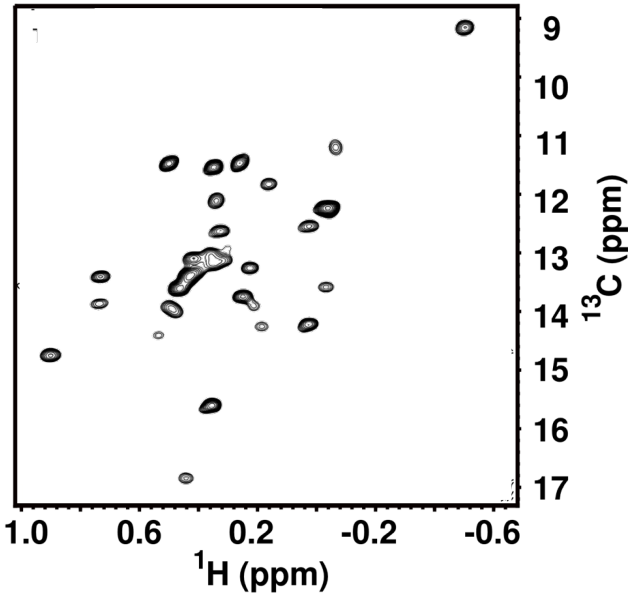


TET -468 kDa
12 x 39 kDa

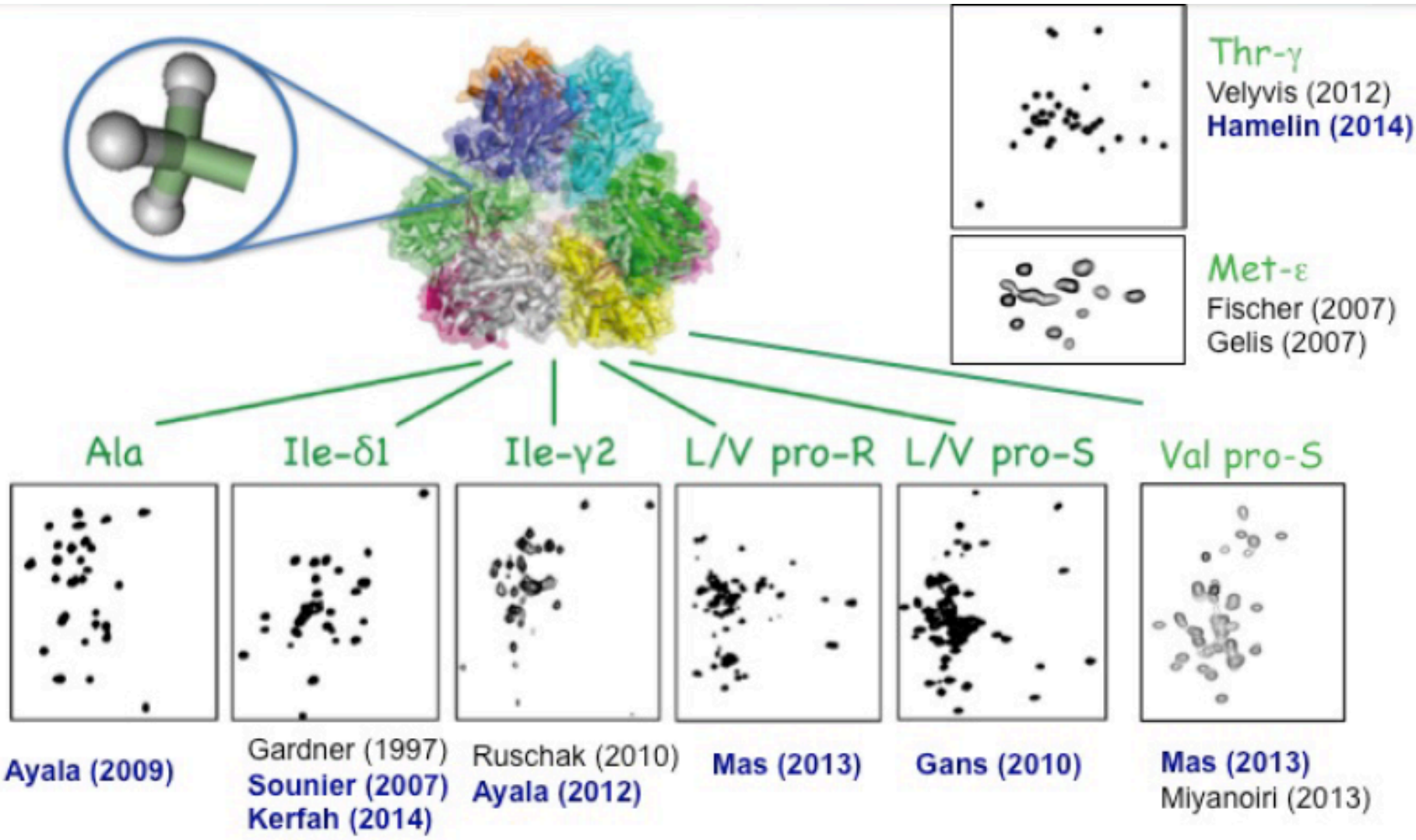


2% protonation

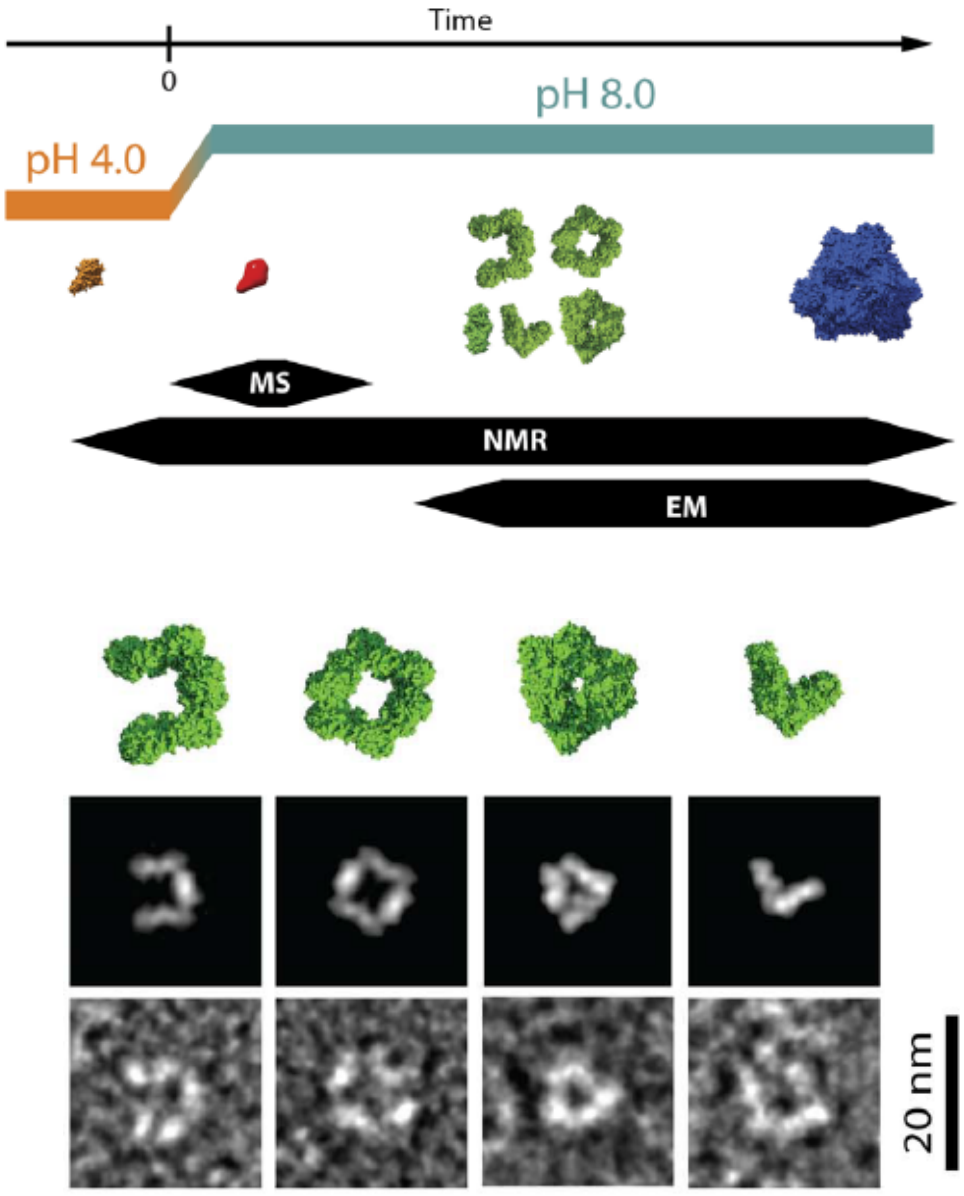
U-[^{2}H,^{12}C,^{15}N]
[δ_1-^{13}C**H$_3$**]-Ile

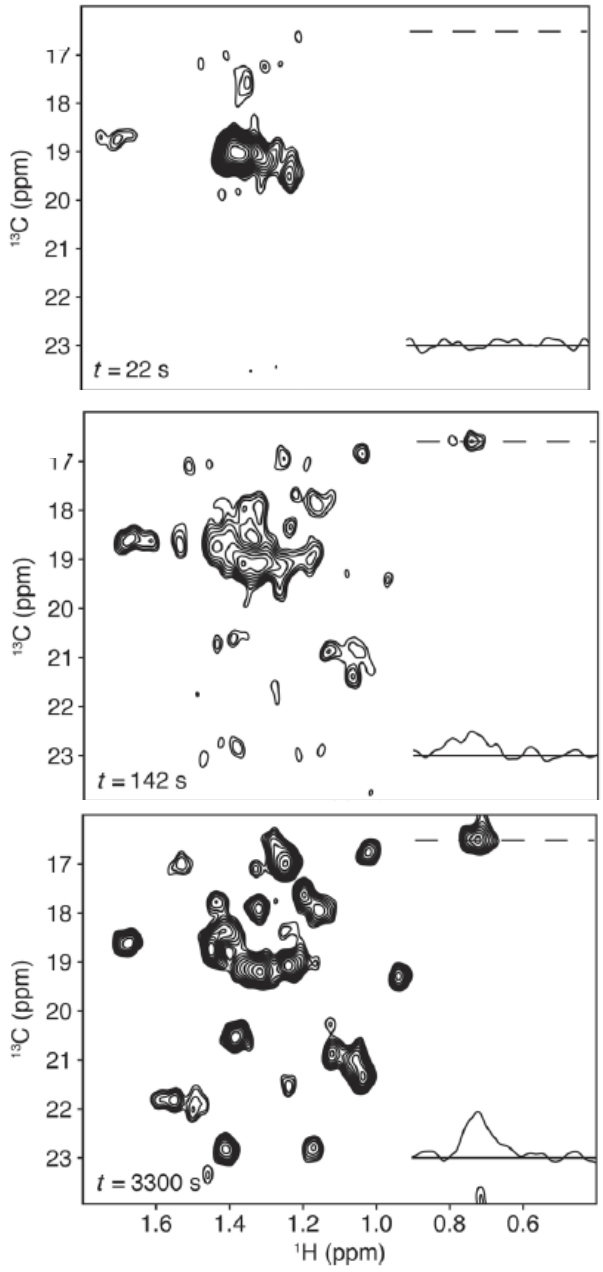


Me-labeling tool kits for NMR



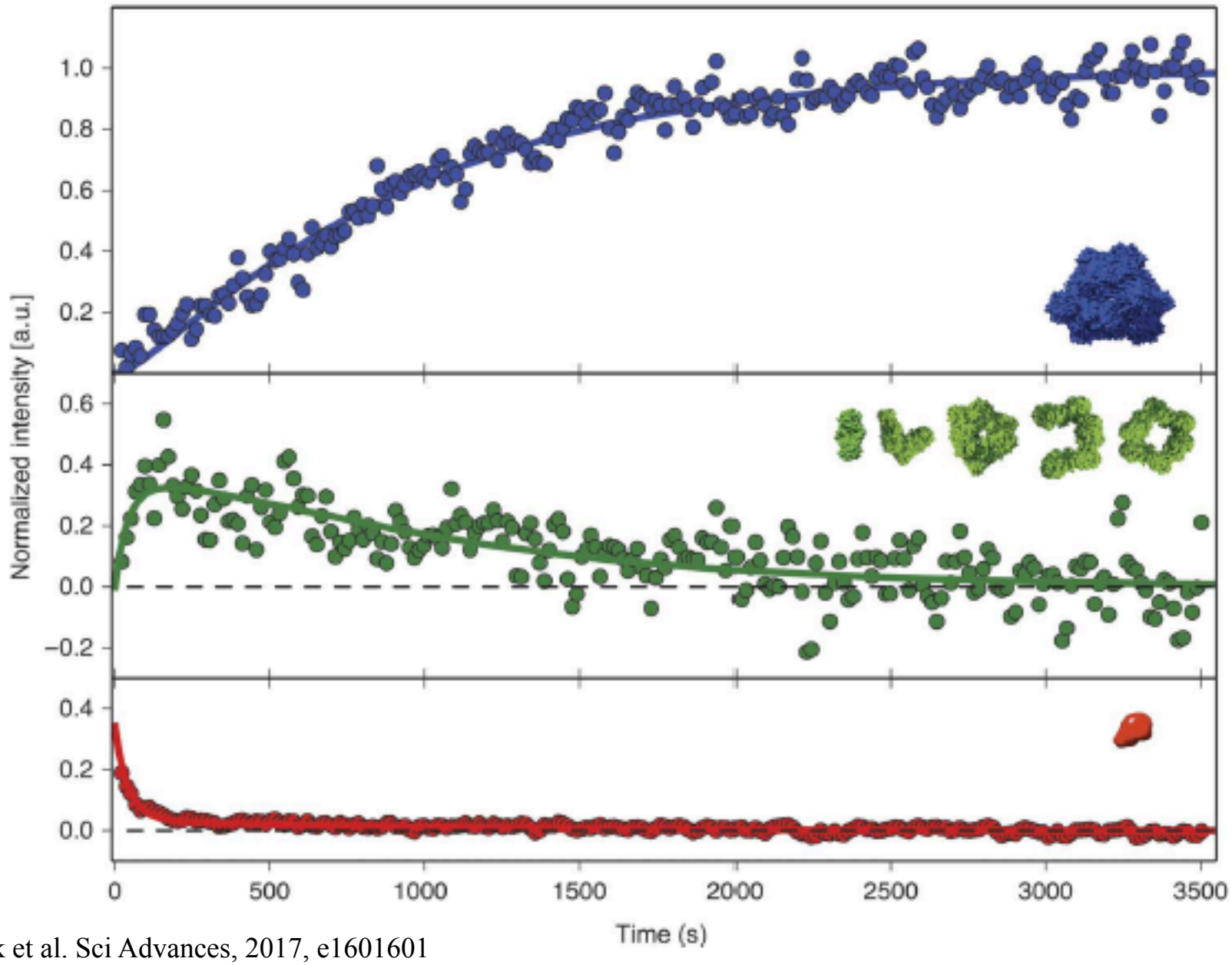
Monitoring of a molecular machine in action





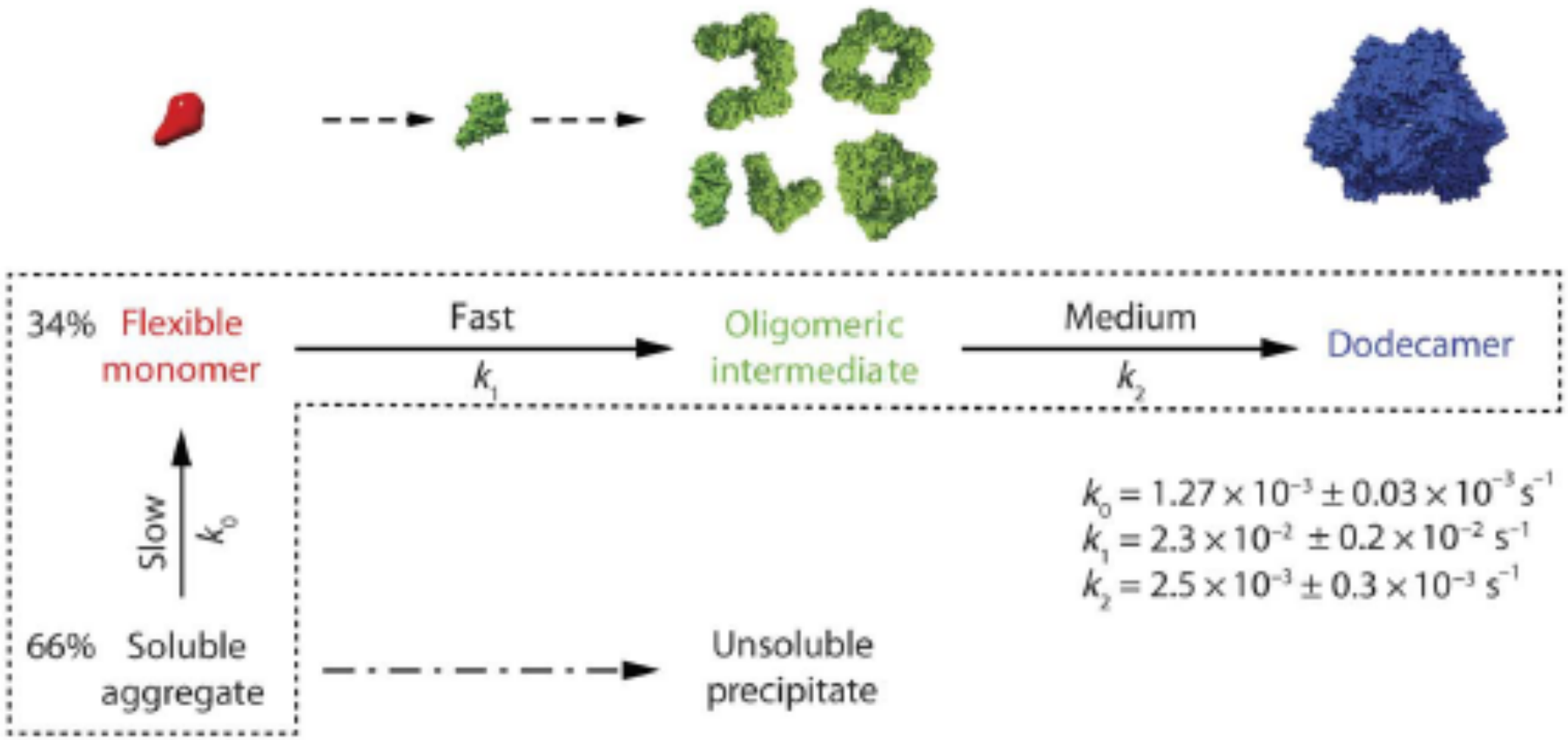
P. Macek et al. Sci Advances, 2017, e1601601

Monitoring of a molecular machine in action



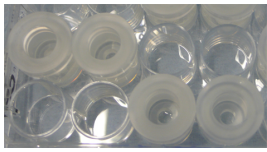
P. Macek et al. Sci Advances, 2017, e1601601

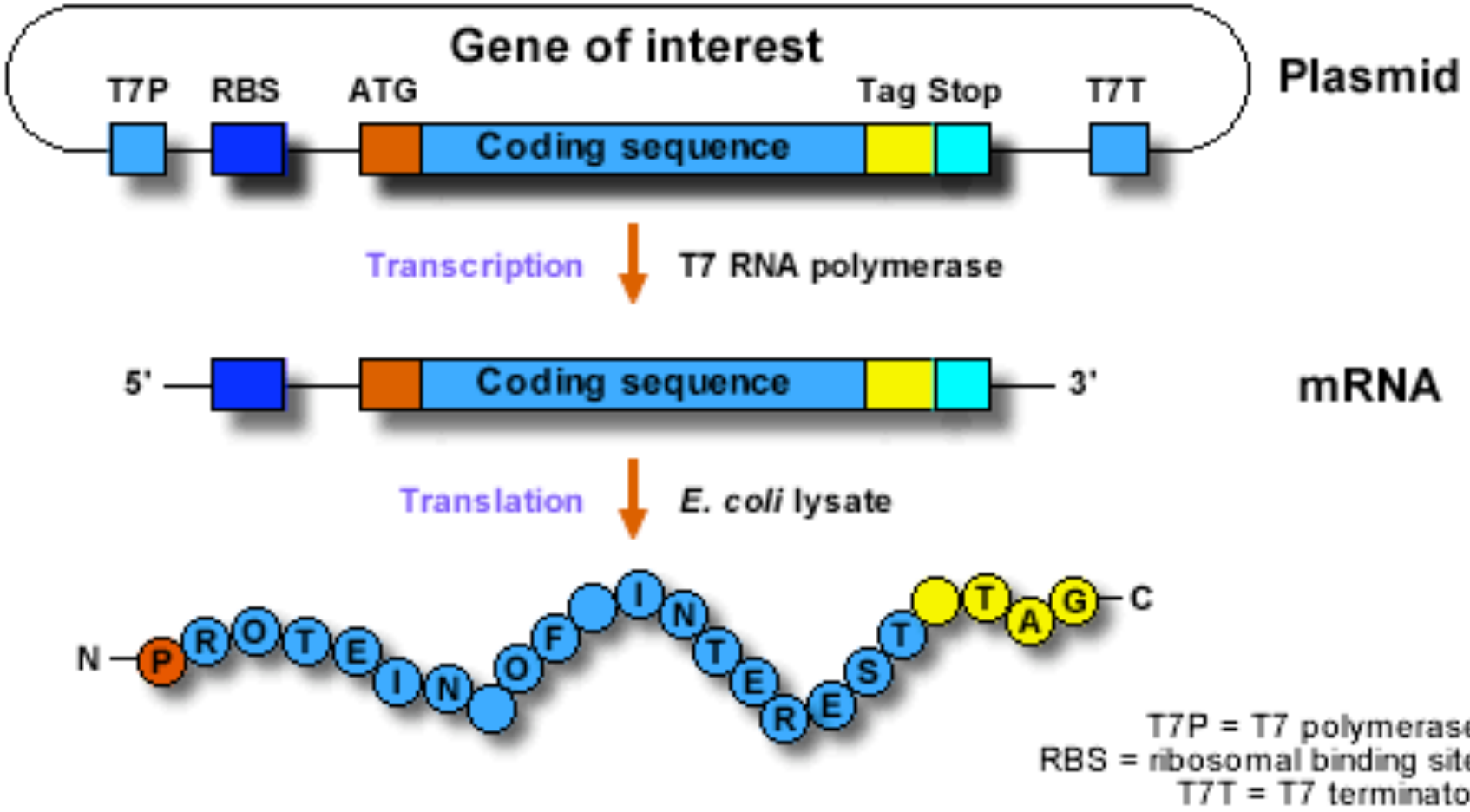
Monitoring of a molecular machine in action

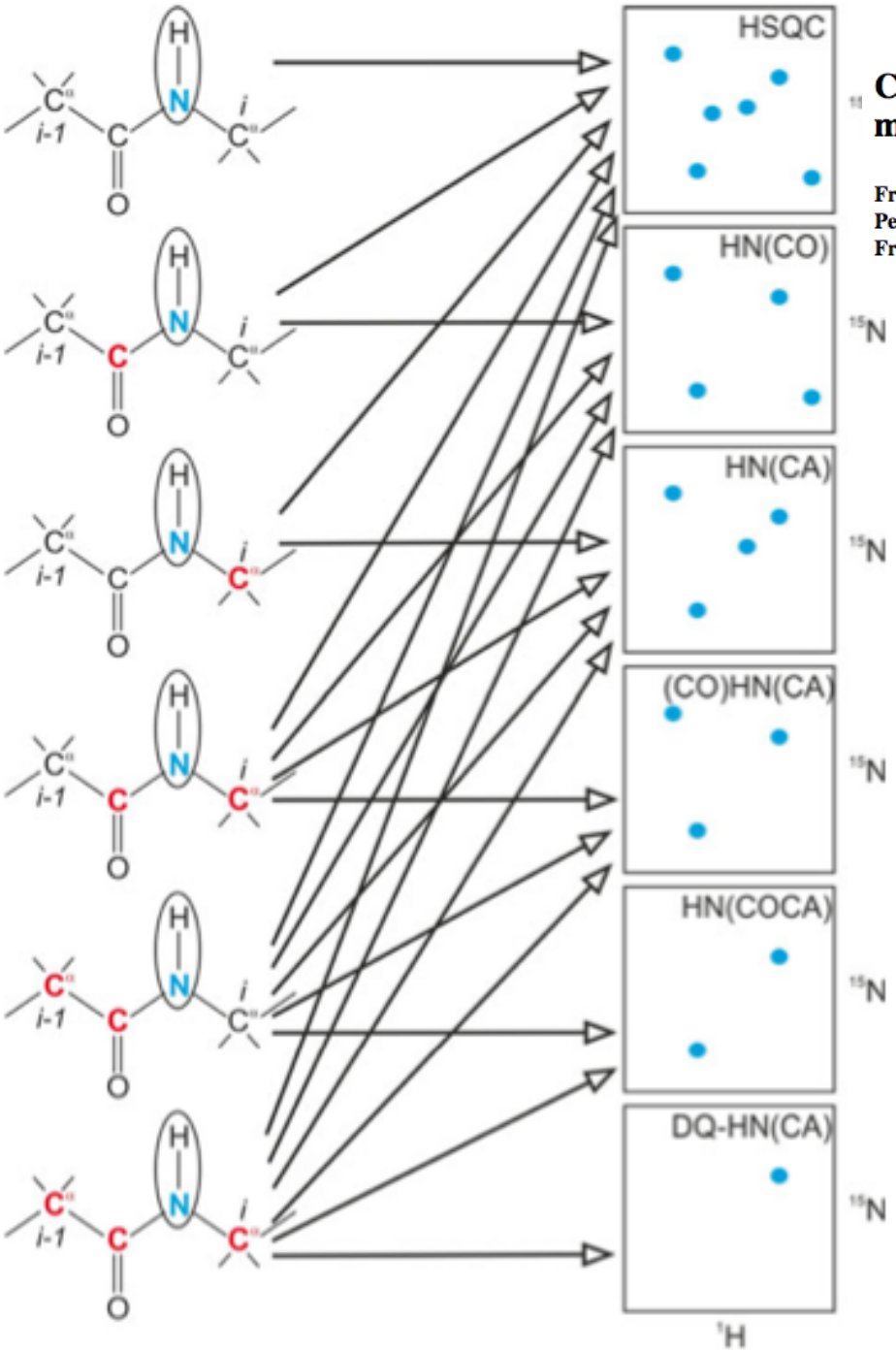


P. Macek et al. Sci Advances, 2017, e1601601

Cell free expression and combinatory isotopic labeling







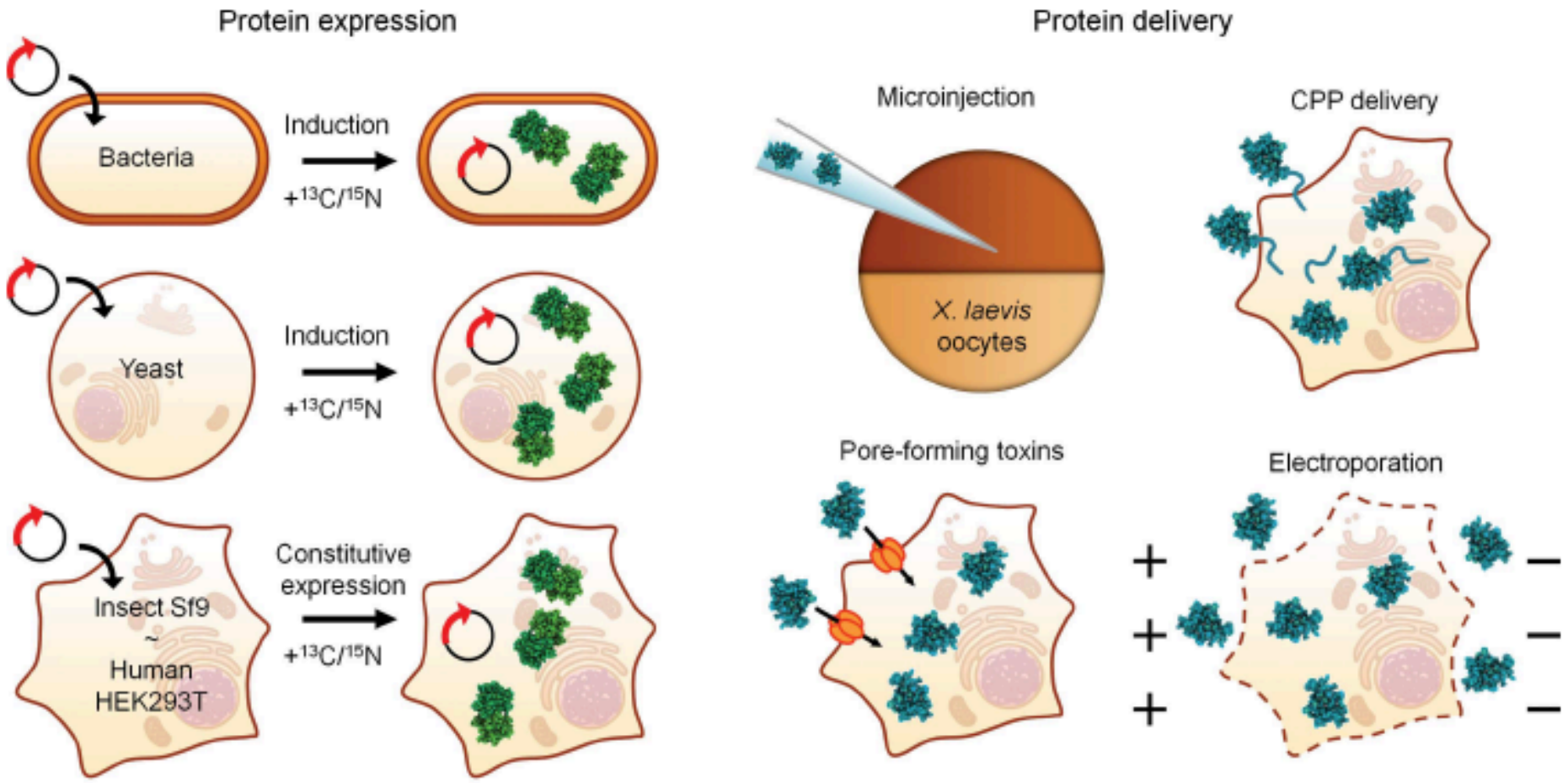
Combinatorial triple-selective labeling as a tool to assist membrane protein backbone resonance assignment

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-^{13}C	1-^{13}C
Valine	1-^{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-^{13}C	^{15}N
Alanine		^{15}N	^{15}N
Threonine	^{15}N	^{15}N	^{15}N
Glycine	1-^{13}C		
Aspartate			1-^{13}C

In-cell NMR: schematic overview of different approaches



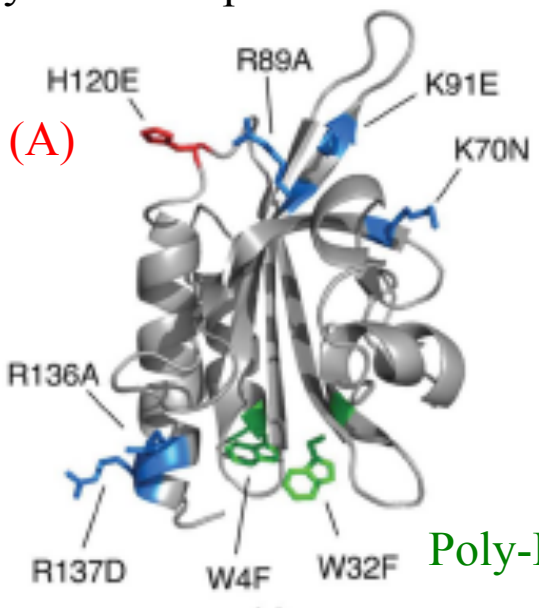
E. Luchinat and L. Banci, IUCrJ, 2017, 108-118

Deciphering interaction networks in cell

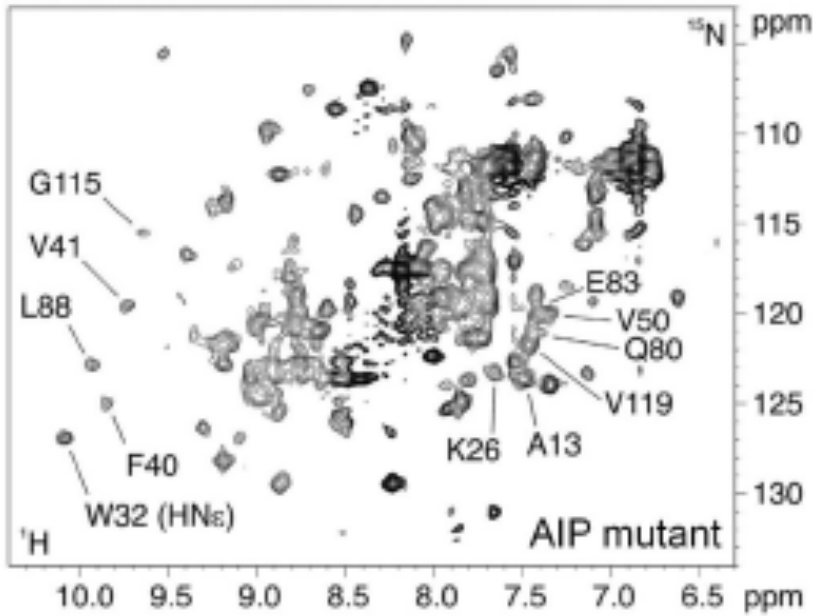
Barbieri et al., Sci. Report 2015, 14456

Human cytoskeletal profilin 1: Pfn1

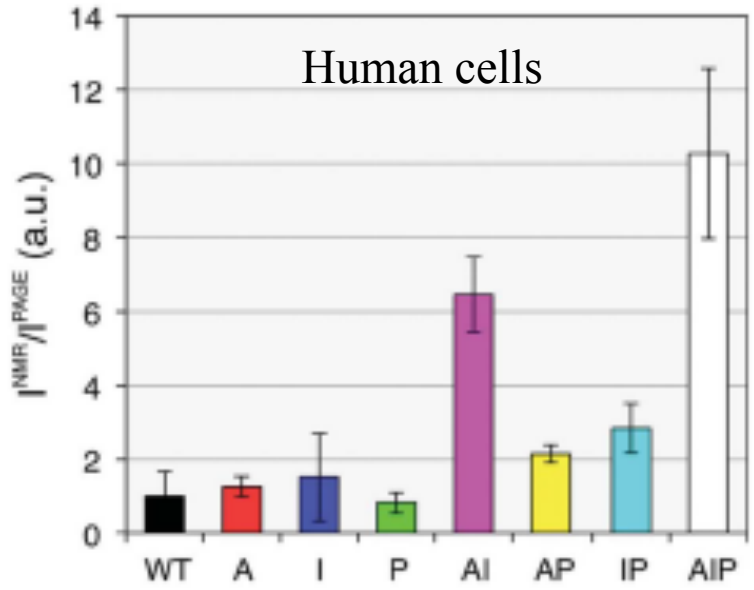
Actin (A)

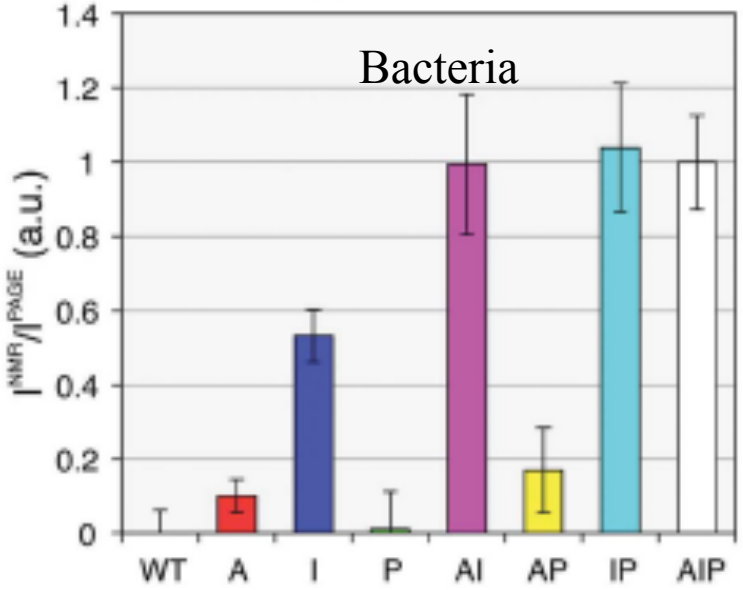


Phospho-Inositides (I)

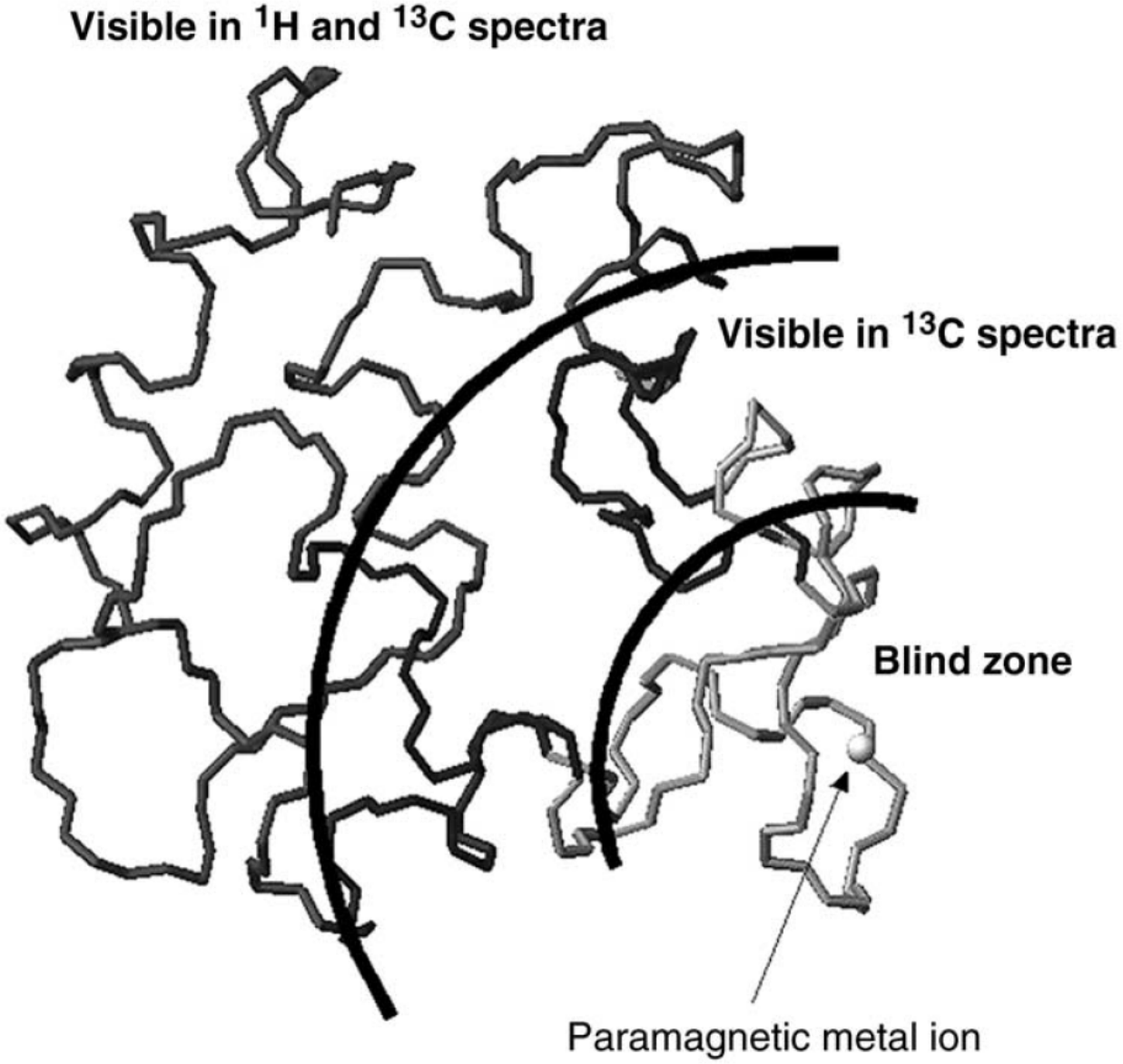


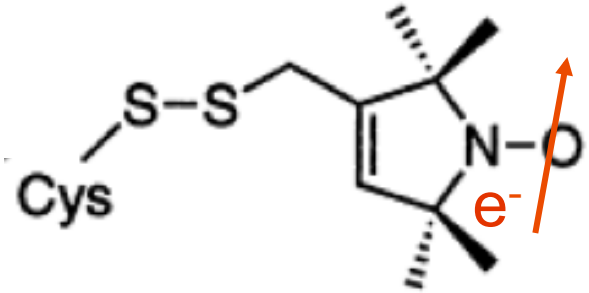
Poly-L-proline (P)





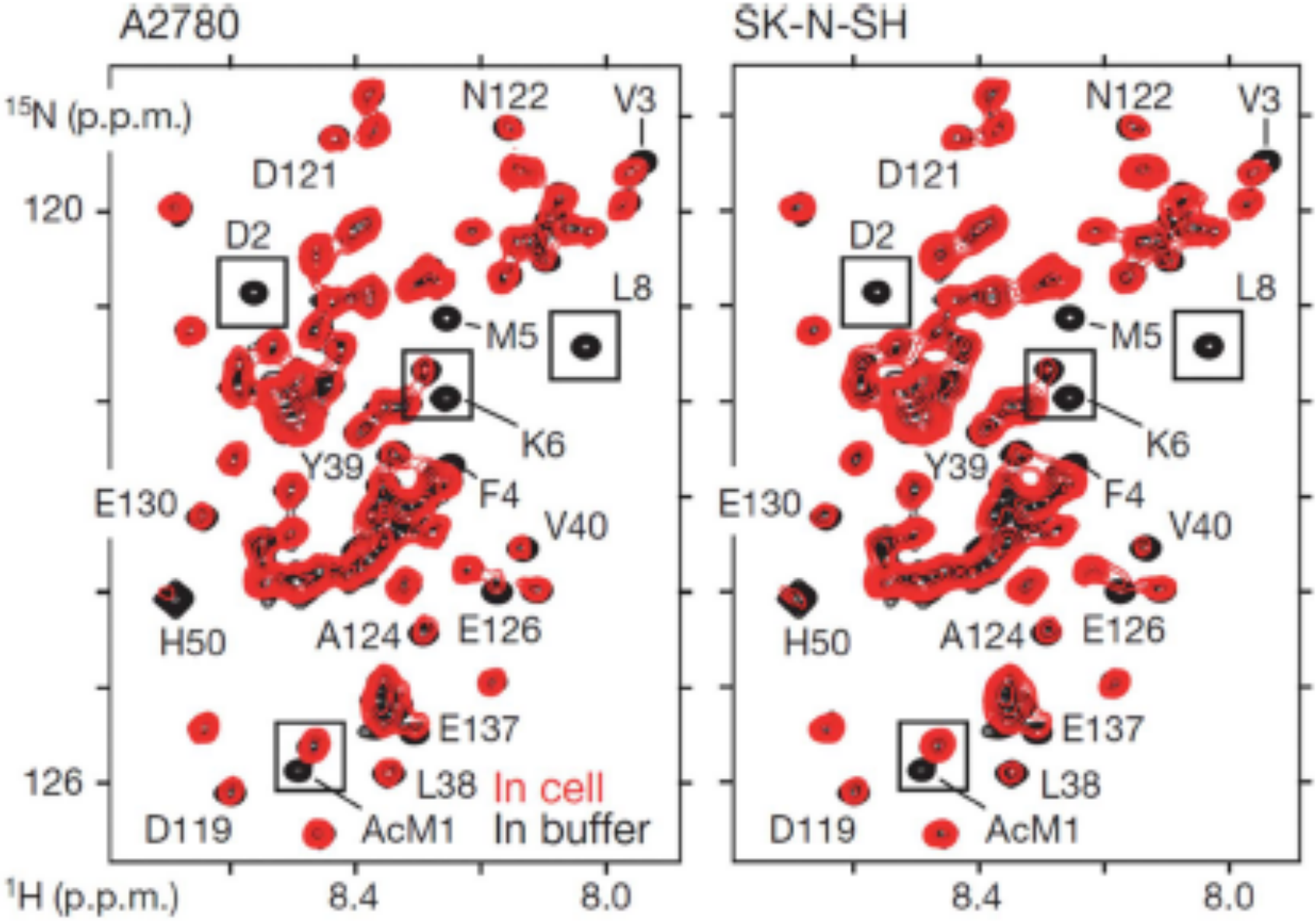
Effects of a paramagnetic tag on ^{1}H and ^{13}C spectra





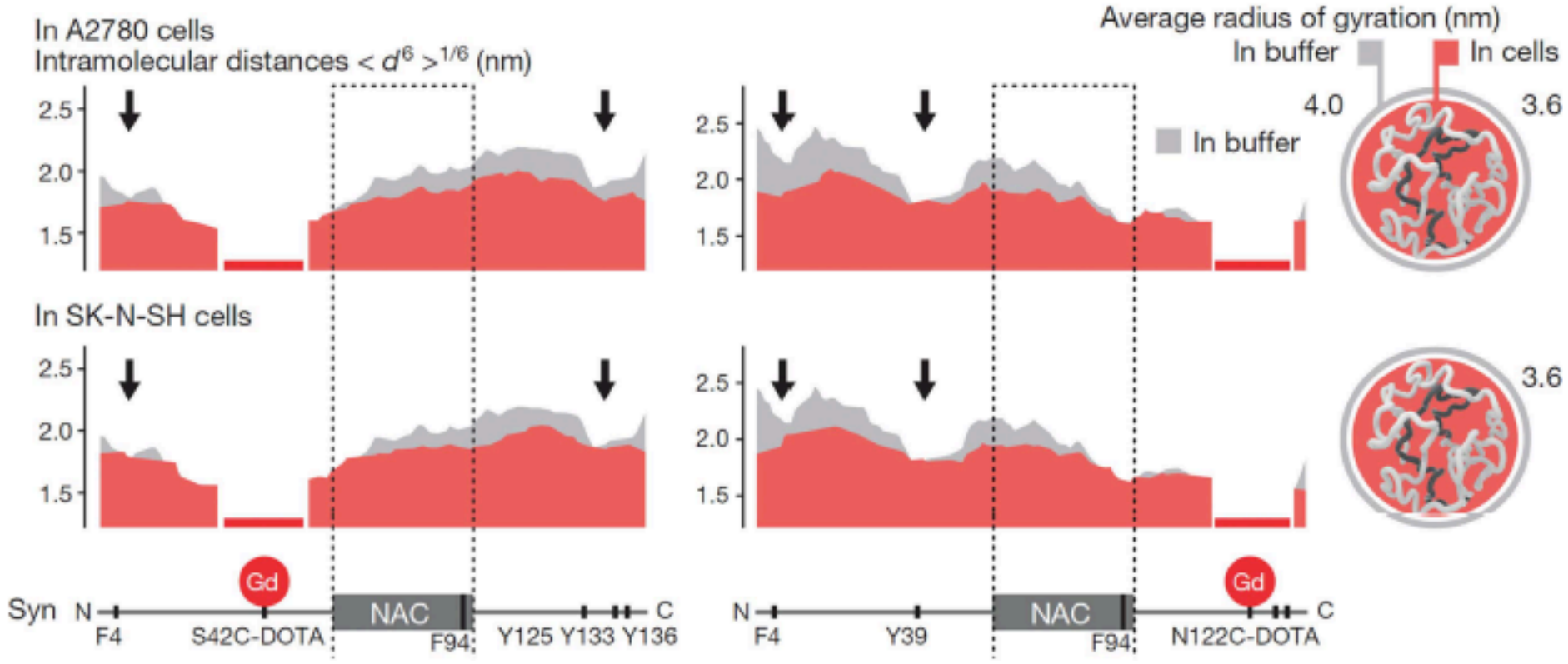
I. Bertini *et al.*, ChemBioChem 2005, 6, 1536 – 1549

Comparison of α-synuclein in different cell lines and *in vitro*



Theillet, Selenko et al.., Nature 2016, 45-50

Comparison of α-synuclein in different cell lines and *in vitro*



Theillet, Selenko et al.., Nature 2016, 45-50

Technological innovations

Numerical processing:

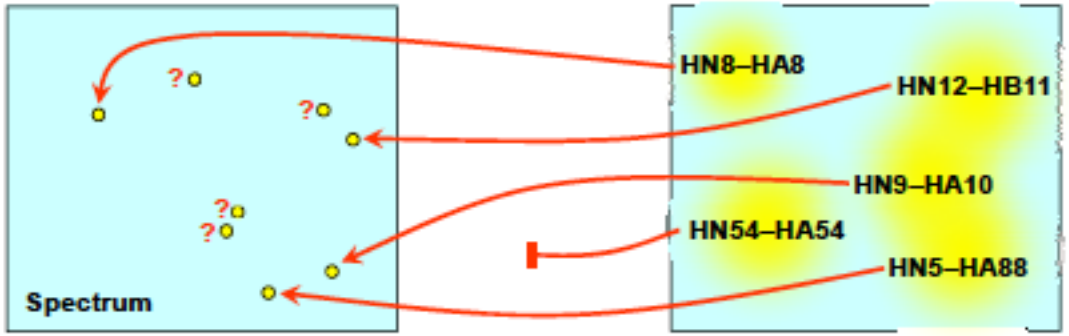
- Filtering
- Data management and integration
- Structure calculation software

Software development for automatic assignment

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

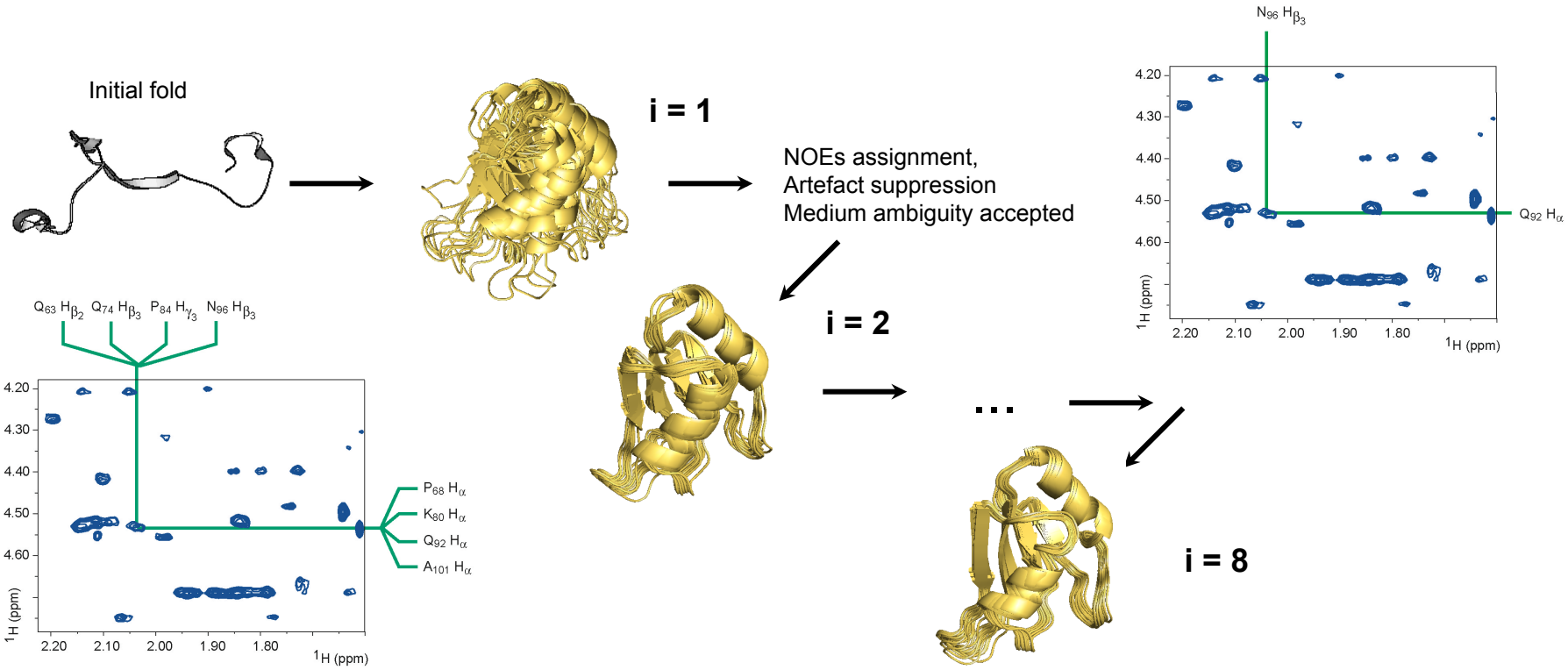
Optimization of assignment
Genetic algorithm combined with local optimization

Fully automated structure calculation algorithm (FLYA)

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



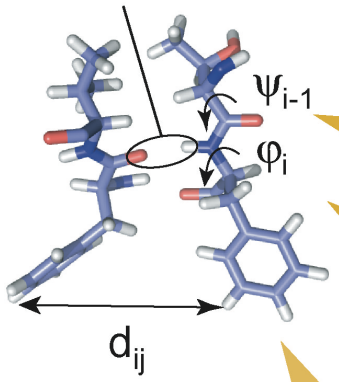
Software
ARIA, UNIO

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..

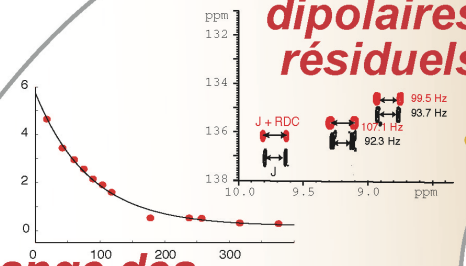
Many structural parameters

Liaison Hydrogène



Distances inter-atomiques

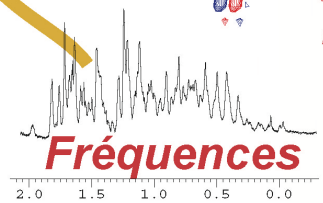
Couplages dipolaires résiduels



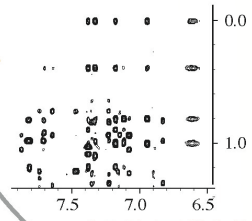
Echange des protons amide

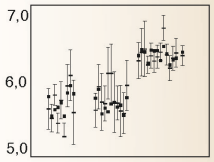
Couplages scalaires

Fréquences

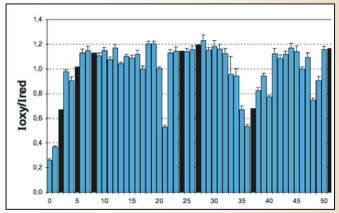


NOESY



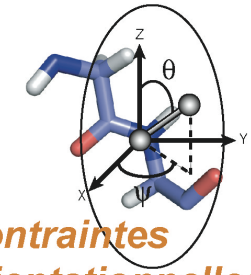


Relaxation hétéronucléaire

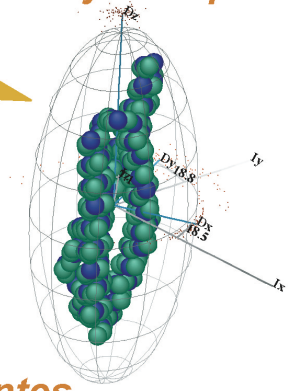


Relaxation paramagnétique

Contraintes orientationnelles



Enveloppe hydrodynamique

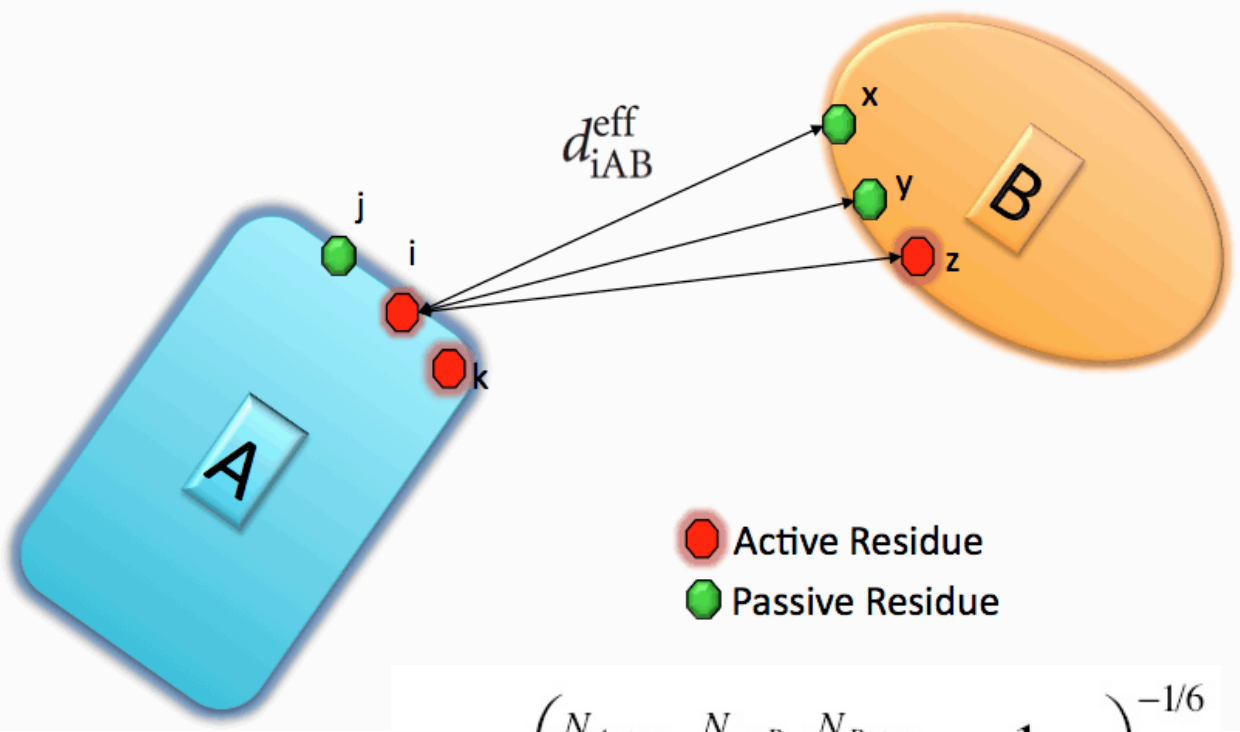


Contraintes à longue distance

Géométrie Locale

Géométrie Globale

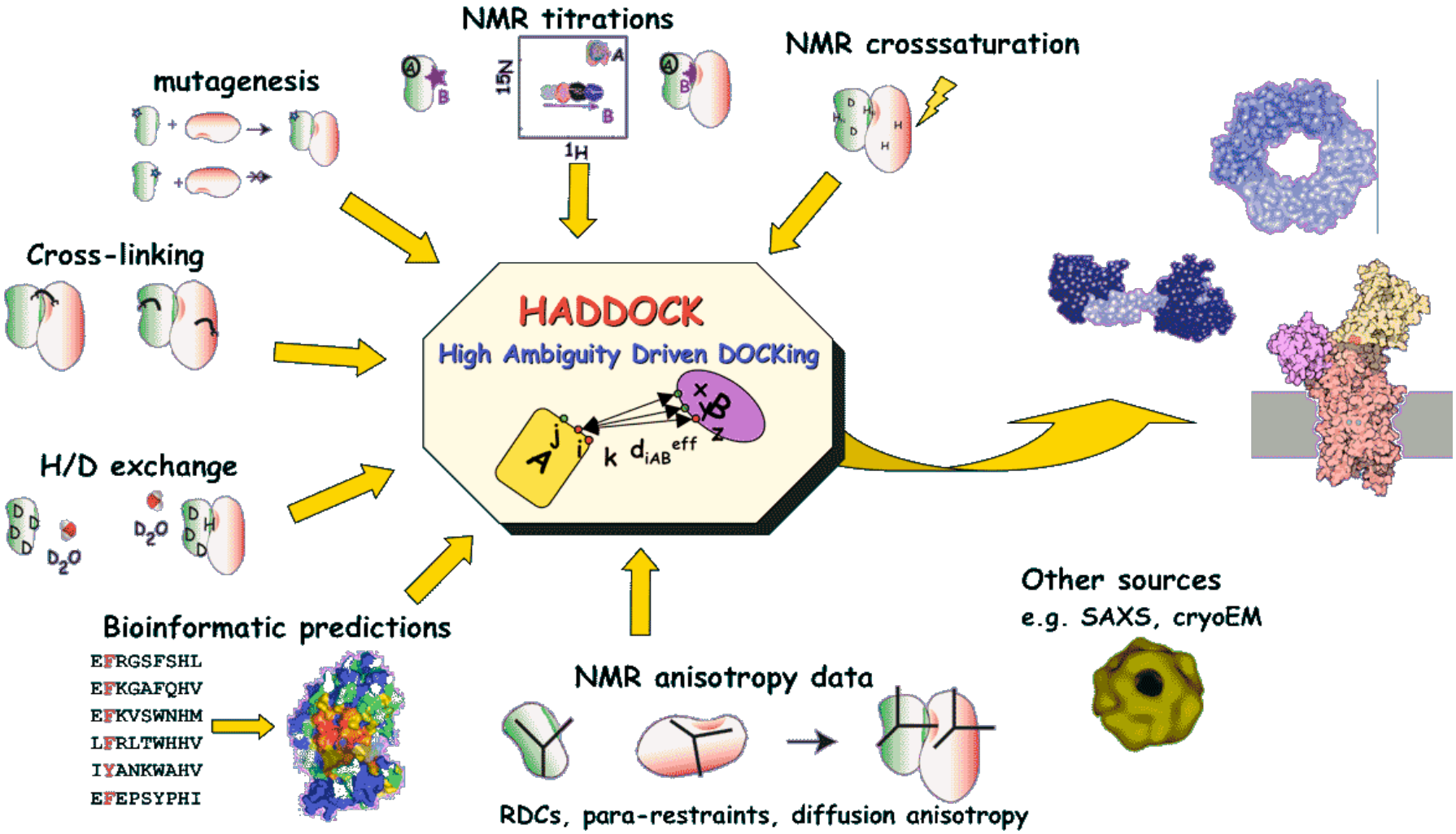
Use of Ambiguous Interaction Restraints for soft docking



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

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

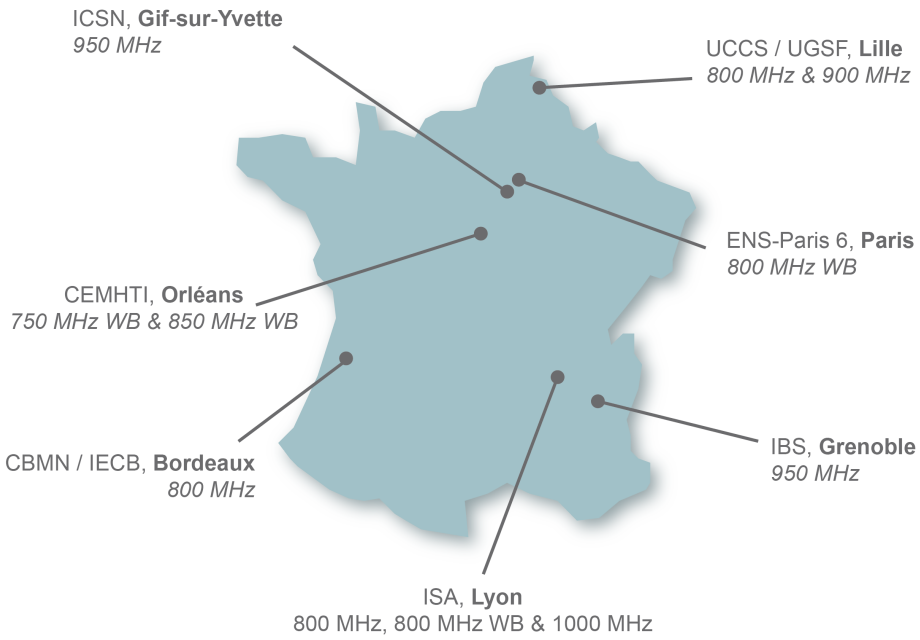
Use of Ambiguous Interaction Restraints for soft docking



High-field NMR facility @ IBS Grenoble

National users

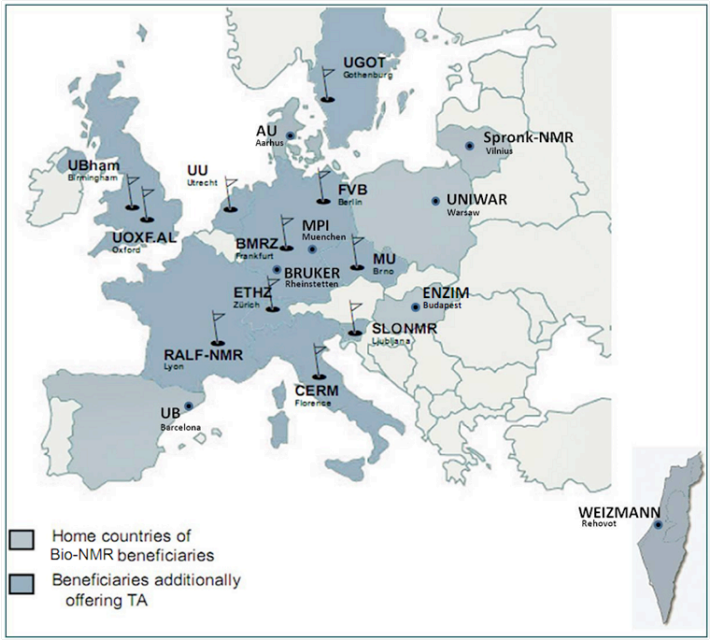
IR THC RMN



~ 100 access days/year

European users

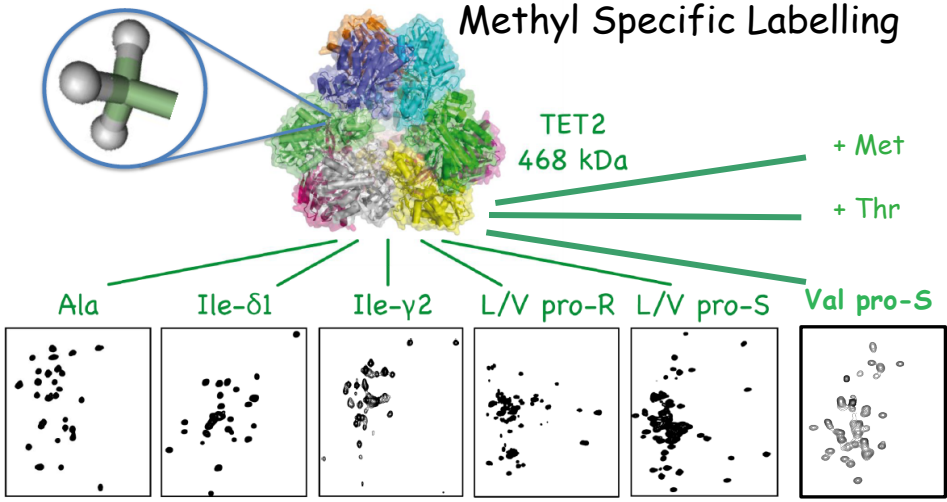
SEVENTH FRAMEWORK PROGRAMME



~ 25 access days/year

Isotopic Labelling Platform@ IBS Grenoble

- E. Coli Overexpression
- Optimisation in D_2O
- Uniform labelling ^{2}H, ^{13}C, ^{15}N
- Specific Labelling
- Users access program:

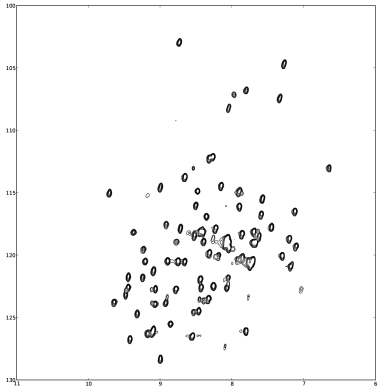


isabel.ayala@ibs.fr

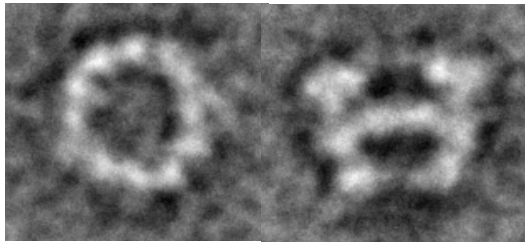
Cell-Free Platform @ IBS

- In vitro Expression
- Large scale production > 1 mg
- Soluble and membrane proteins
- RNAs Production
- Coexpression, large assemblies
- Isotopic Labelling ^{2}H, ^{13}C, ^{15}N
- Users access programs :

RNase free wetlab



Isotopic labelling



Large assembly

lionel.imbert@ibs.fr

Bridging Structural Biology with Biological Synthesis and Self Assembly to Reveal Key Processes in Living Systems

PROJECT AIM

To stimulate scientific excellence and innovation capacity of CEITEC MU through collaboration with three internationally-leading counterparts – University of Vienna, Université Grenoble Alpes, and University of East Anglia – that will foster practical relevance of research towards high value-added applications.

RESEARCH AREAS

- Cell and structural biology
- Biological chemistry and synthetic biology
- New generation of therapeutics

PROJECT ACTIVITIES

- Lectures and short courses of invited experts
- Short-term and mid-term secondments
- Joint supervision of young researchers
- Workshops, summer schools, and other events

www.twinning-bison.eu

CALL: H2020-TWINN-2015 / PROJECT NUMBER: 692068 / DURATION: 01. 01. 2016 – 31. 12. 2018 / EC CONTRIBUTION: 996 375 €

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 692068.

MASARYK UNIVERSITY CEITEC universität wien UNIVERSITÉ Grenoble Alpes UEA

Looking for new partnerships to raise structural biology PHD programs