Integrative Structural Biology Summer School

21 June 2017 – Oléron, France

NMR spectroscopy: major advances and future developments

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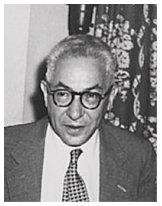
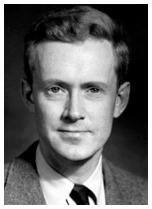
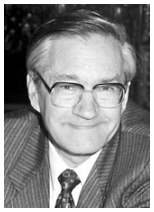
NMR : A young science





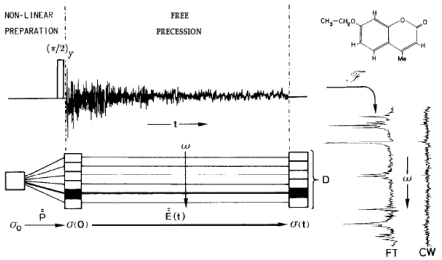
1936 : Nuclear spin (Rabi, Physics Nobel 1944)

1945 : First NMR signals (Bloch et Purcel, Physics Nobel 1952)



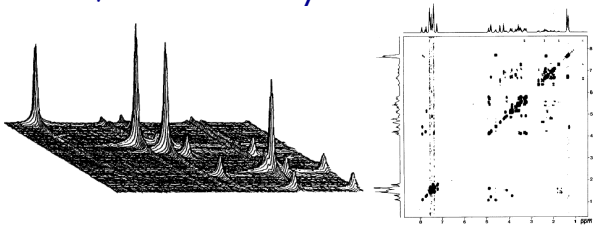
1949 : Chemical shift

1970 : Fourier transform spectrometers.

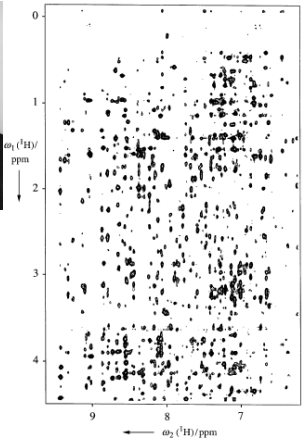


1972 : supra-conductor coils. 2D-NMR (Jeener)

1975 : 2D-NMR developments (Ernst, Chemistry Nobel 1991)

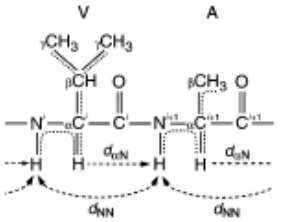


1983 : First MRI spectrometers

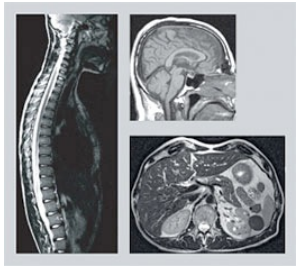



1990 : High resolution NMR,

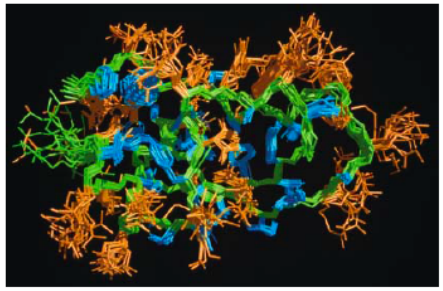
Structural biology (Wüthrich, Chemistry Nobel 2002)



2000 : Cryoprobes 500 et 600 MHz



Mauterbur and Mansfield Medicine Nobel (2003)

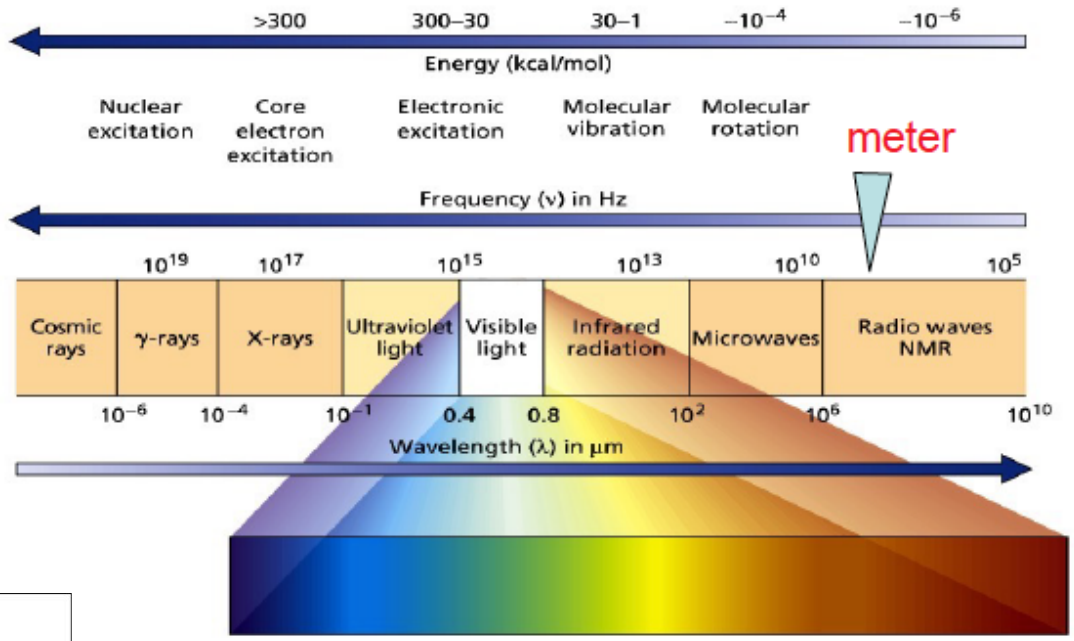


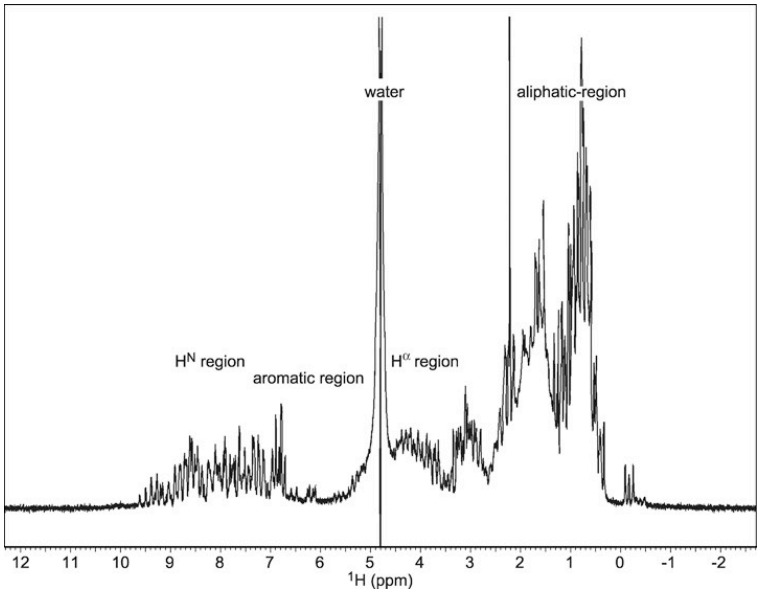
Biomolecular NMR : 35 years of methodological developments

K. Wüthrich
Protein Struct.

1980

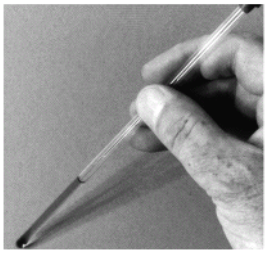
500 MHz



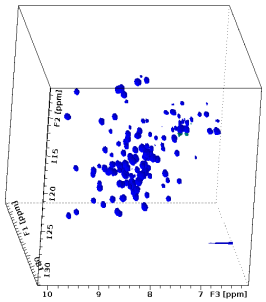


NMR: principles of structure determination

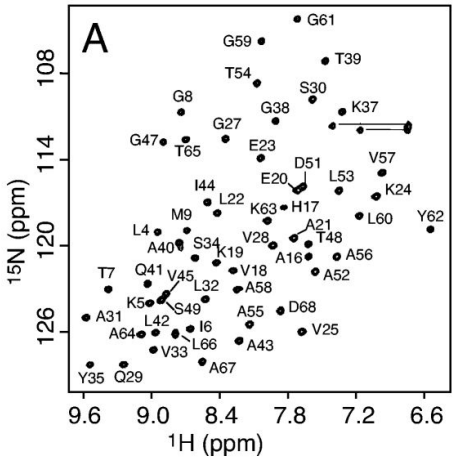
NMR sample



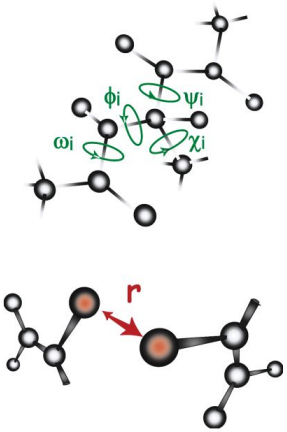
NMR data acquisition



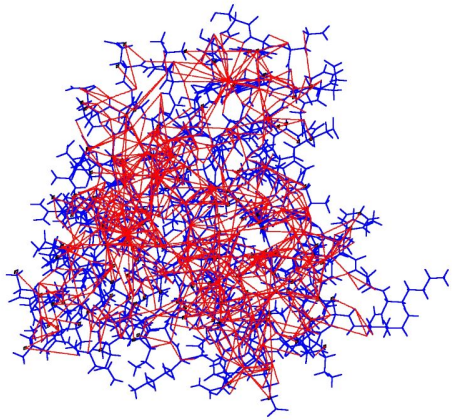
Resonance assignment



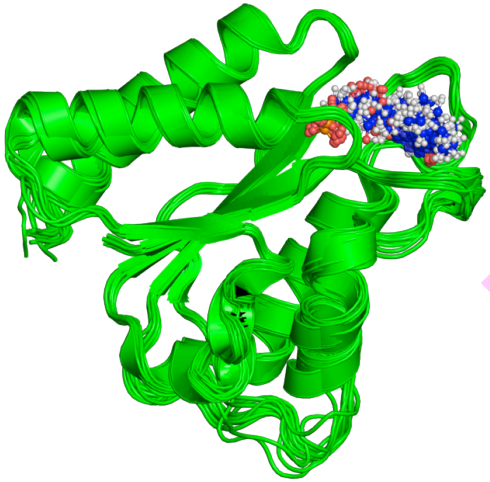
Structural parameters



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**

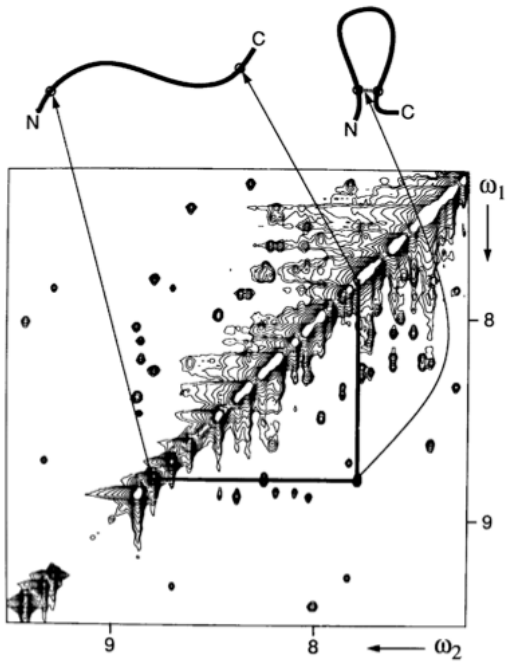
2010 — **1-1.2 GHz**

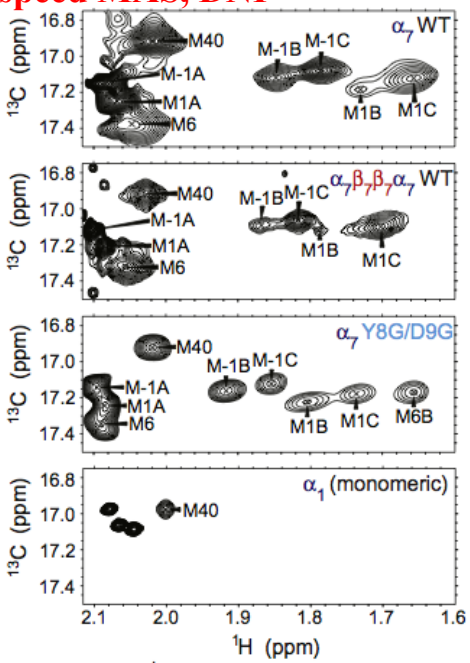
Cryo-probes

High-speed MAS, DNP

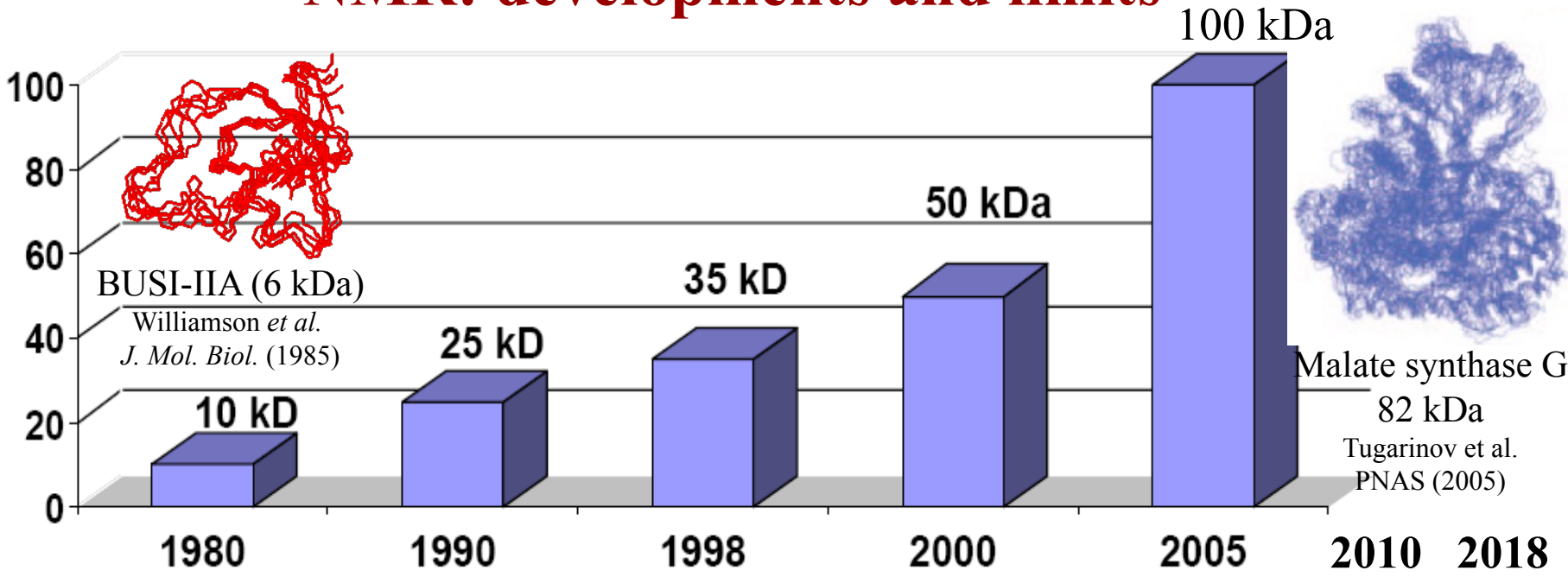
Methyl labeling
(L. Kay)

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



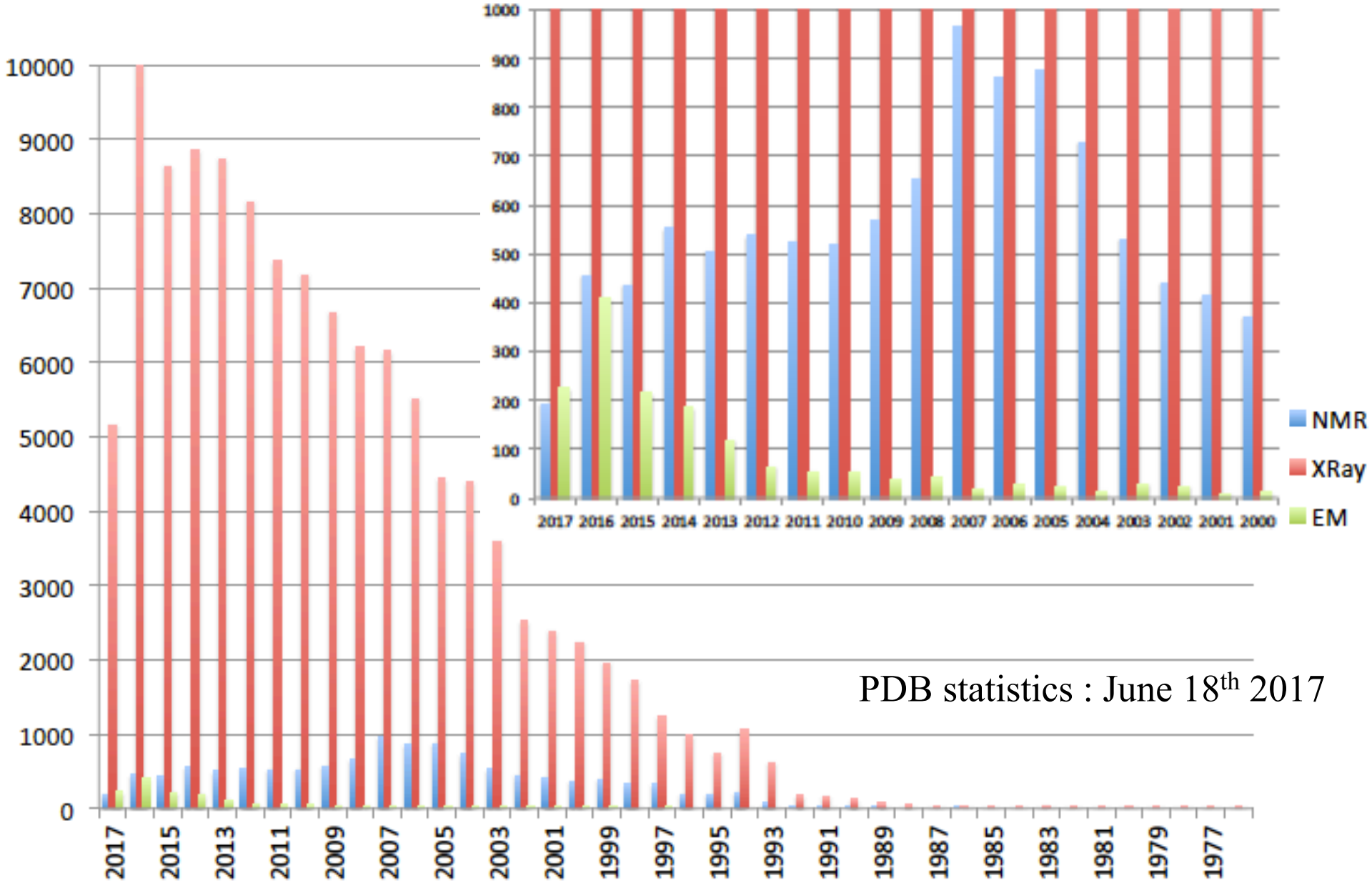


NMR: developments and limits



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, J				RDC, CCR, PRE		
Labeling	none	^{13}C, ^{15}N		^{2}H, ^{13}C,^{15}N		specific labeling	

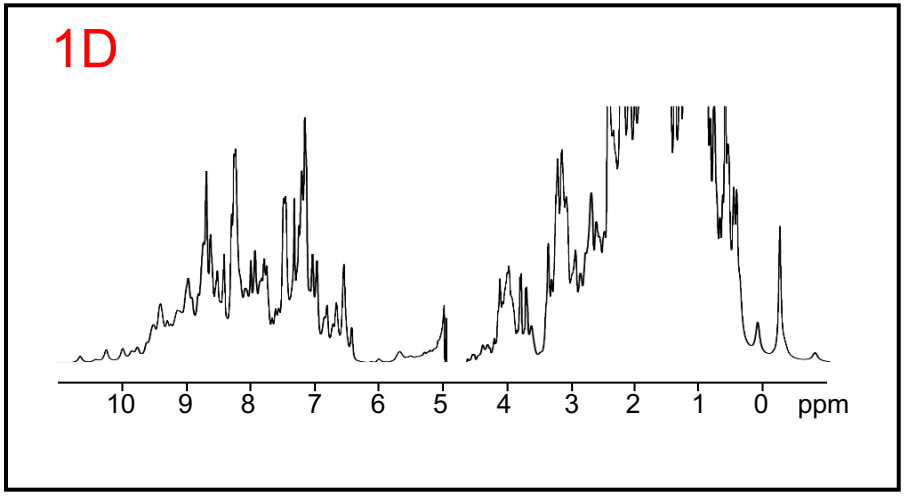
NMR: A limited competitiveness for structures

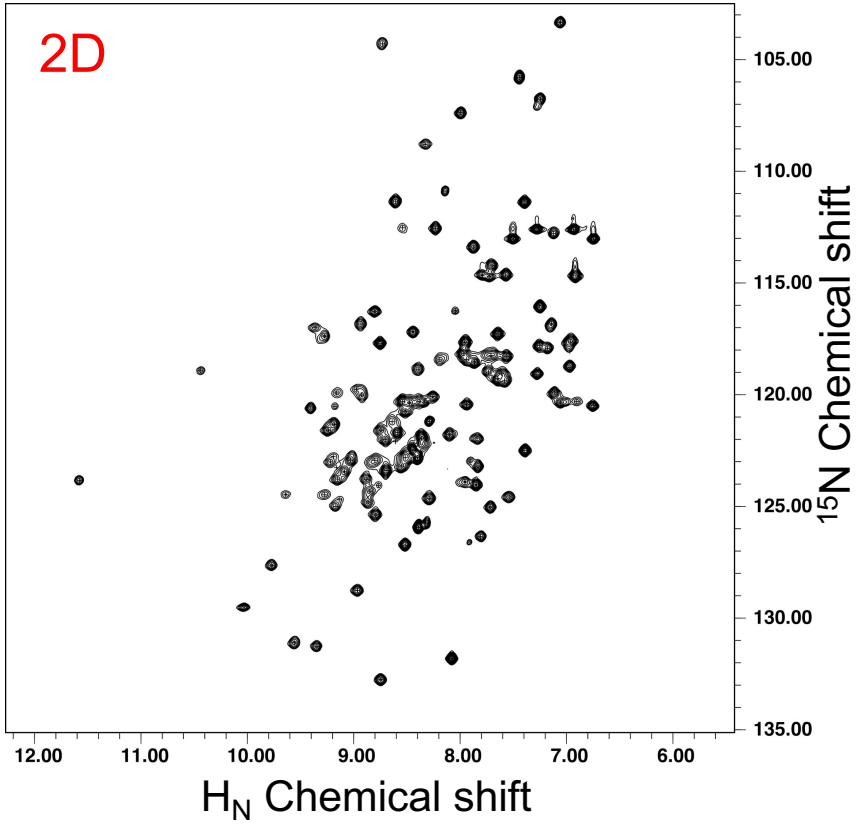


PDB statistics : June 18th 2017

NMR, some limitations

Resolution and spectral hindrance



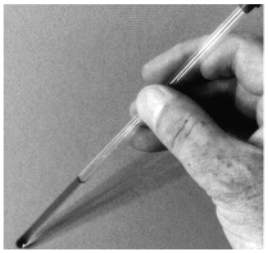


- Acquisition time: *few seconds*
- limited spectral resolution
- No necessary isotope labeling
- **Global characterization**

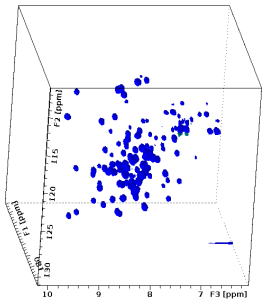
- Acquisition time: *few minutes*
- Increase in the spectral resolution
- Necessary isotope labeling (^{15}N)
- **More detailed information**

NMR, a limited competitiveness for structures: a lengthy process

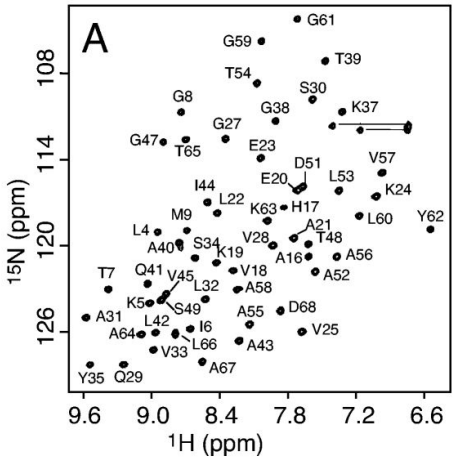
NMR sample



NMR data acquisition



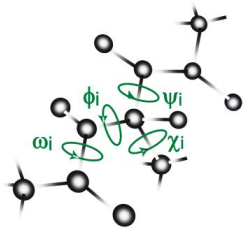
Resonance assignment

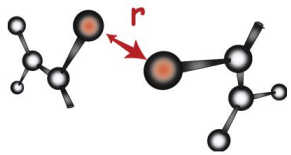


41.1 kDa

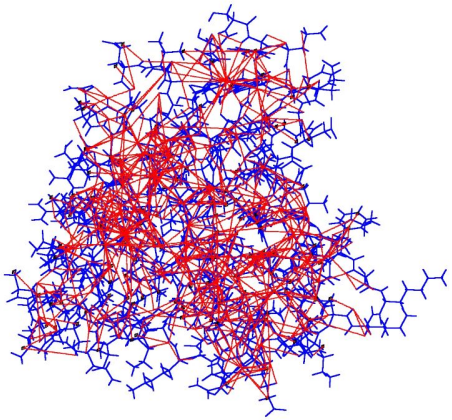
$C_{1827}H_{2869}N_{489}O_{570}S_{12}$

Structural parameters

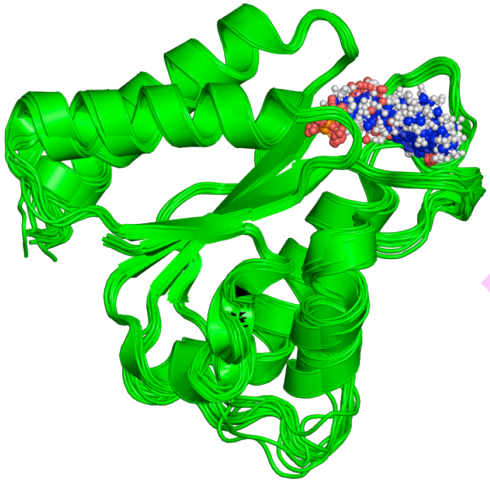




Structure calculation



Structural ensemble

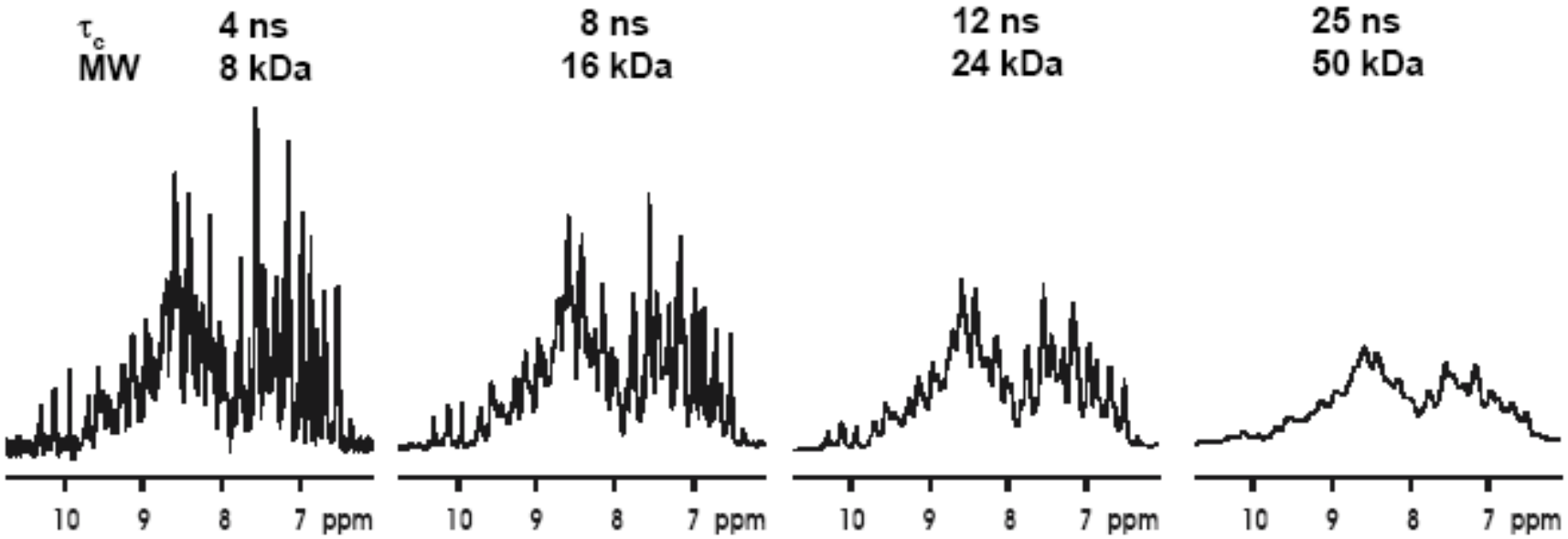


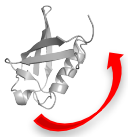
NMR, a limited competitiveness for structures: an intrinsic size limitation in solution

Liquid-state NMR a serious limit?

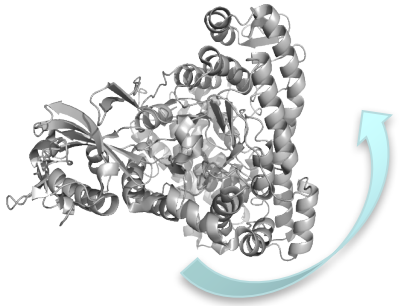
Linewidth

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





fast overall rotation



slow overall rotation

Jan 2013

BIOCHEMISTRY
including biophysical chemistry & molecular biology

Current Topic

pubs.acs.org/biochemistry

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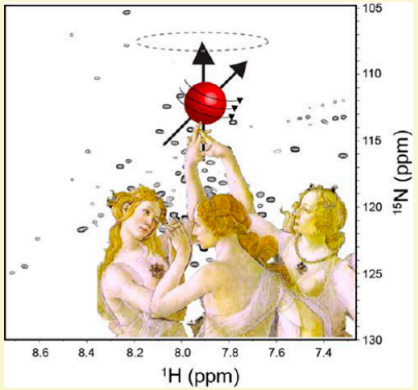
[∥]Institute for Organic Chemistry, Christian-Albrechts University of Kiel, D-24118 Kiel, Germany

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.

CONCLUSION: Other emerging methods not covered in this review, such as nonclassical ways of collecting and processing pulsed NMR data can also be expected to further expand the problem-solving capability of solution NMR, particularly if such approaches can be widely implemented in user-friendly form. While soothsaying is perilous, all indicators point to a bright future for NMR as a tool for studying protein structure, folding, dynamics, interactions, and function.



- THE ABILITY OF NMR TO ACCESS **DILUTE SAMPLES** HAS BEEN DRAMATICALLY ENHANCED
- THERE HAS BEEN A RESURGENCE IN THE USE OF SIMPLE ONE-DIMENSIONAL NMR EXPERIMENTS TO **TACKLE BIOLOGICAL PROBLEMS**, SUCH AS **SIGNALING BY GPCRS**
- THE USE OF NMR TO MONITOR **PROTEIN–LIGAND AND PROTEIN–PROTEIN INTERACTIONS** HAS BEEN EXTENDED
- NMR PARAMAGNETIC RELAXATION ENHANCEMENT (PRE) EXTENDS THE CAPABILITIES OF NMR TO **PROBE PROTEIN STRUCTURE AND DYNAMICS**
- METHYL TROSY NMR ALLOWS NMR TO **PROBE STRUCTURAL AND MECHANISTIC QUESTIONS FOR >200 kDa PROTEIN COMPLEXES**
- INCREASED USE OF **NMR IN DRUG DISCOVERY**
- NMR CAN NOW BE USED TO PROBE PROTEIN STRUCTURE AND INTERACTIONS **IN LIVING CELLS**
- RELAXATION DISPERSION PROVIDES UNPRECEDENTED ACCESS TO **CRYPTIC STRUCTURAL AND DYNAMIC STATES**
- NMR HAS PROVIDED SEMINAL INSIGHT INTO **INTRINSICALLY DISORDERED PROTEINS AND TETHERED MULTIDOMAIN PROTEINS**

2005-2017
NMR: a tool for integrative structural biology

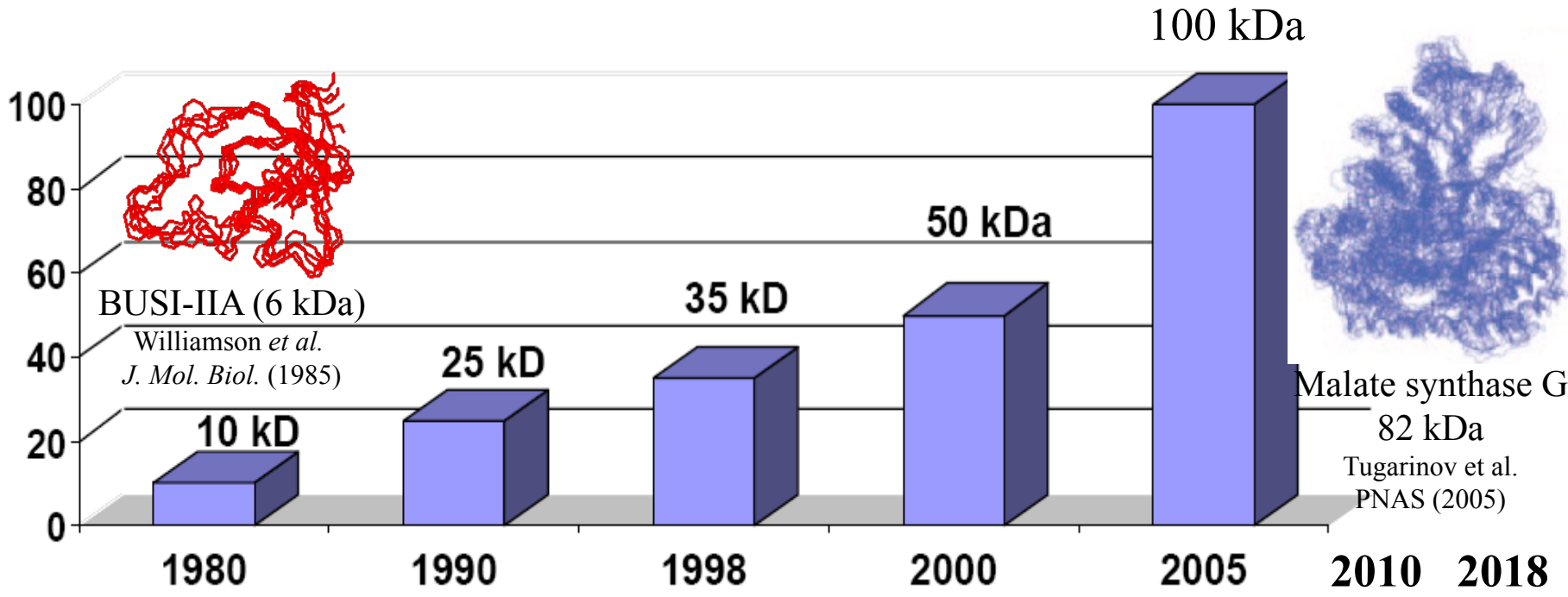
- ★ Study of intrinsically disordered proteins
- ★ Study of mechanisms of molecular recognition
- ★ Study of proteins and nucleic acid excited states
- ★ Study of the dynamics of very large complexes
- ★ In-cell NMR

Technological innovations and developments

Magnets:

- B_0 magnetic field
- Hindrance
- Cryo-fluid consumption

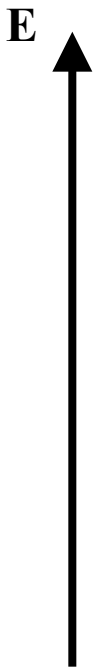
NMR: developments and limits

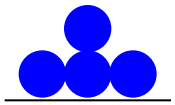


Magnets	500 MHz	600 MHz	800 MHz	900 MHz	950 MHz	1.0-1.2 GHz

NMR, some limitations

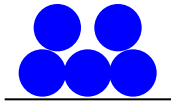
Sensitivity or signal-to-noise ratio





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

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



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

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

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

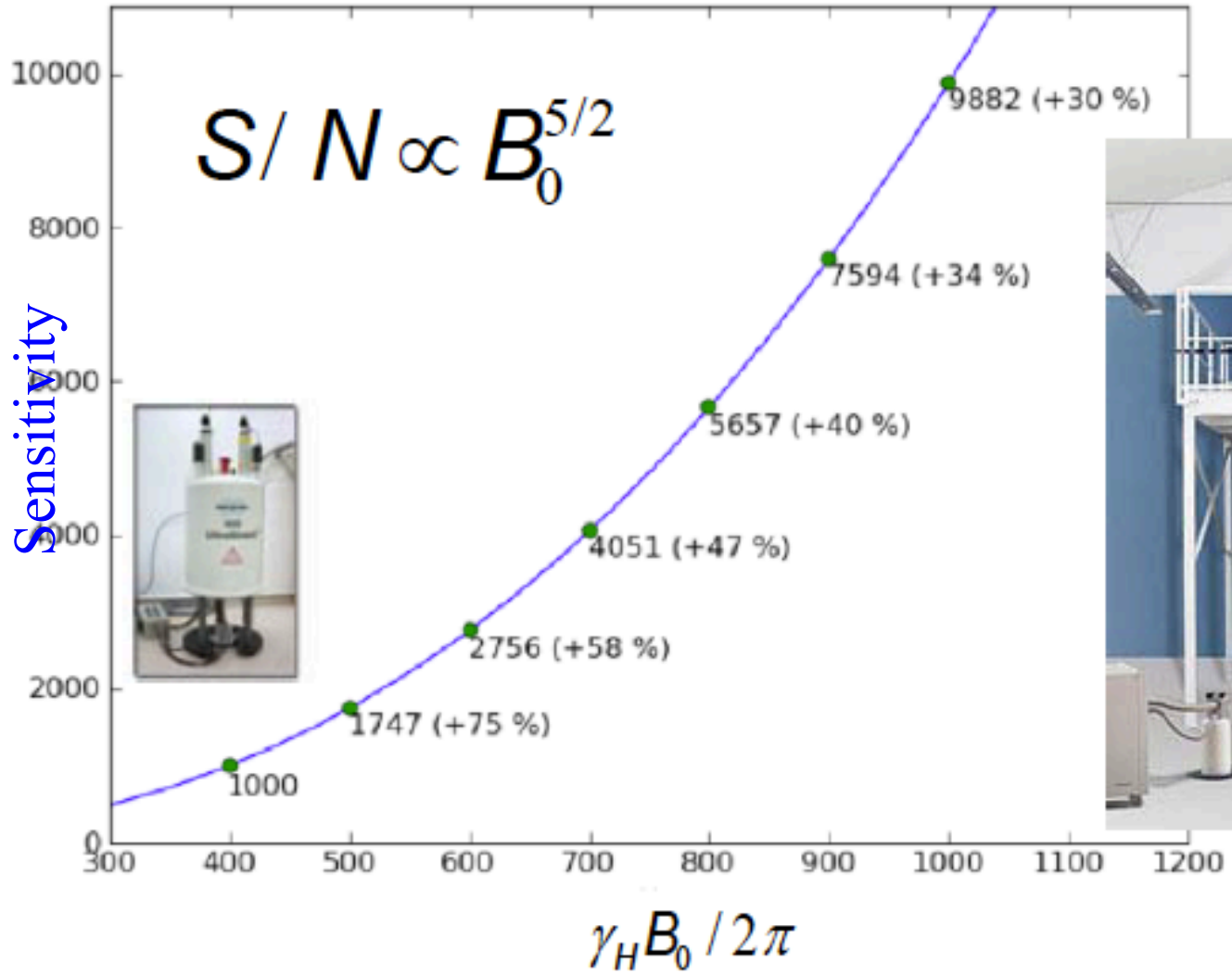
$$@\, B_0 = 14.09T\,(600MHz)$$

Particular case of spin 1/2

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

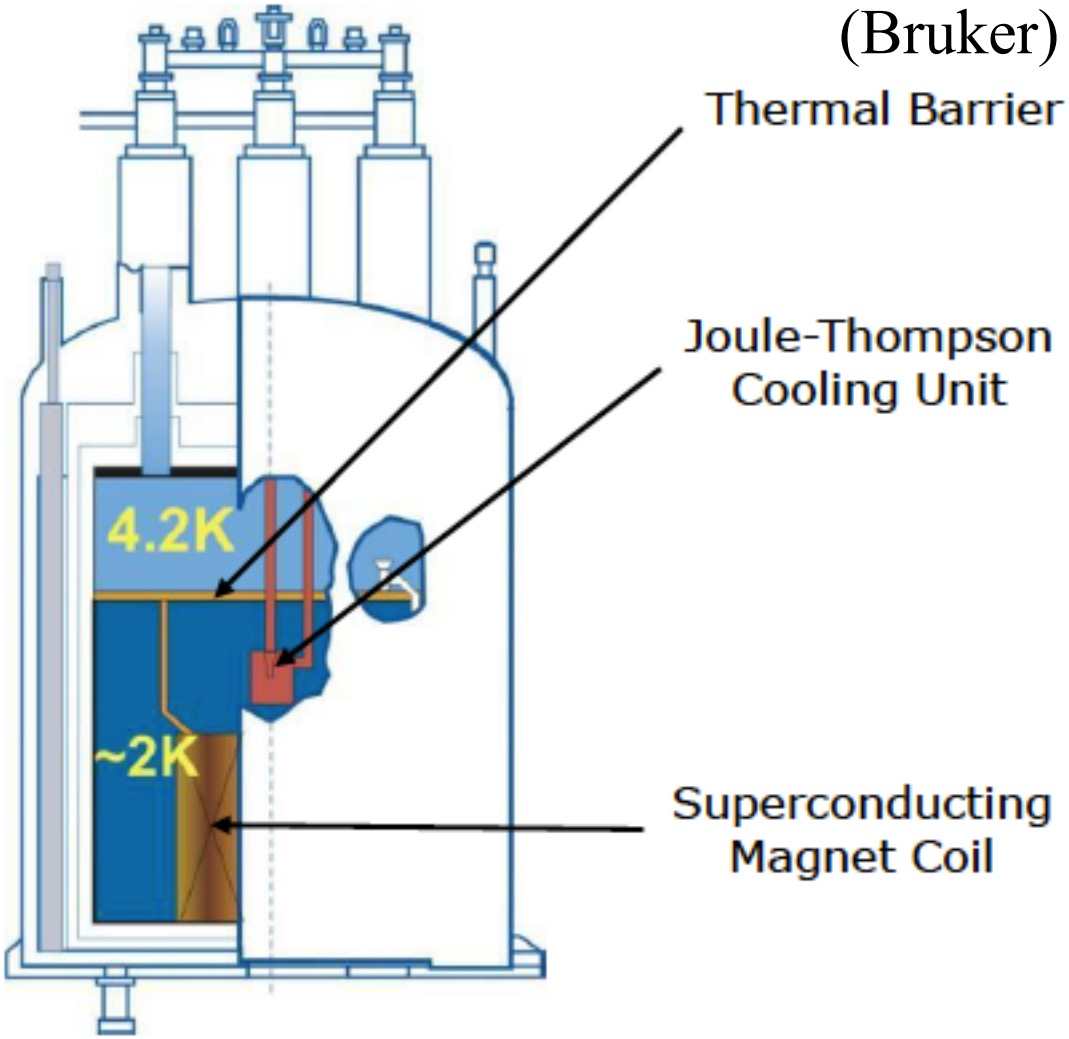
$$\vec{M} = N\frac{\gamma\hbar B_0}{2kT}\gamma\hbar\frac{1}{2}\vec{z} = \frac{N\left(\gamma\hbar\right)^2 B_0}{4kT}\vec{z}$$

Technological innovations



Technological innovations

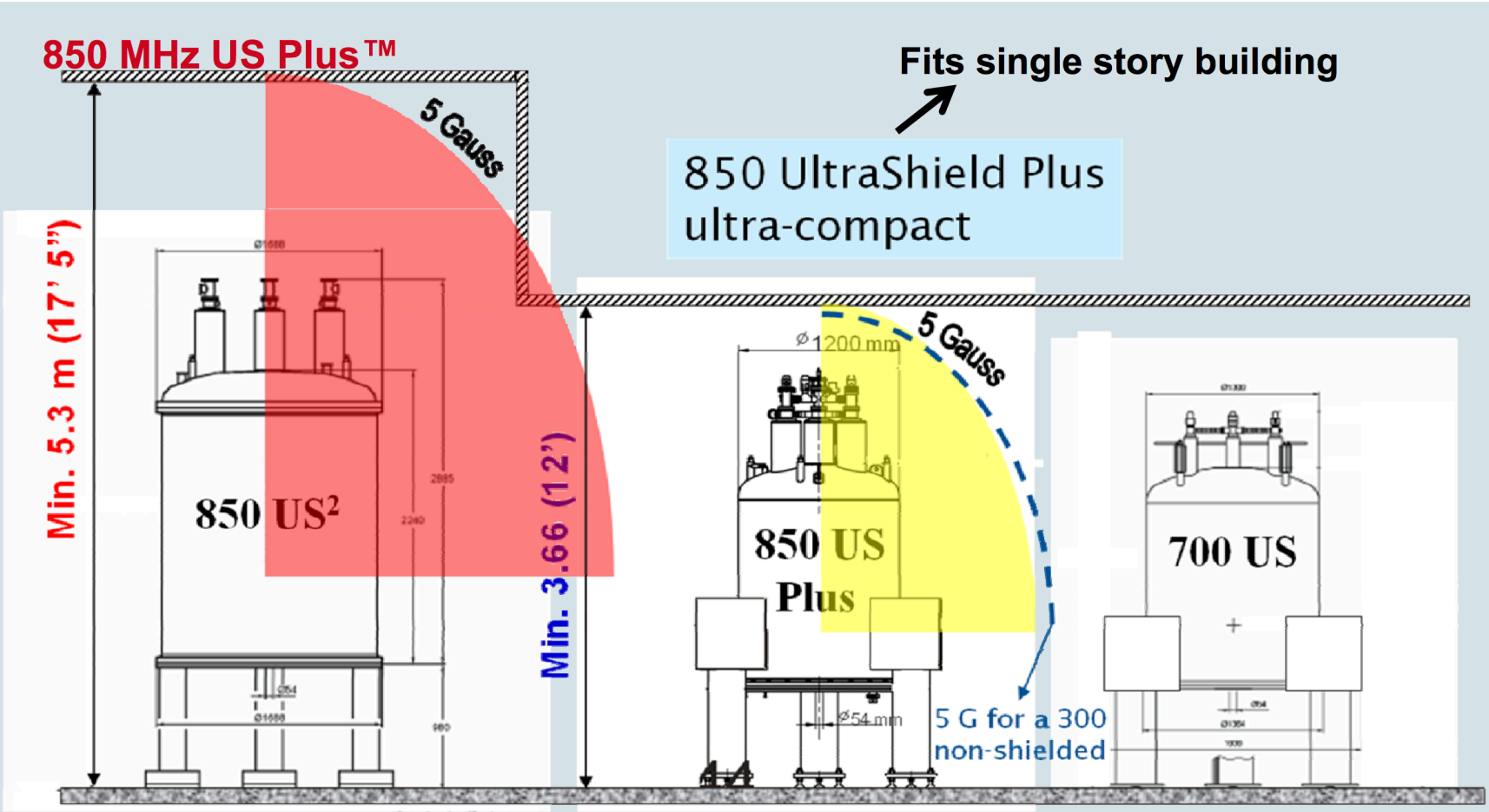
Aeon™ technology (Bruker)



UltraStabilized™ technology delivering unique performance, stability and safety

Technological innovations

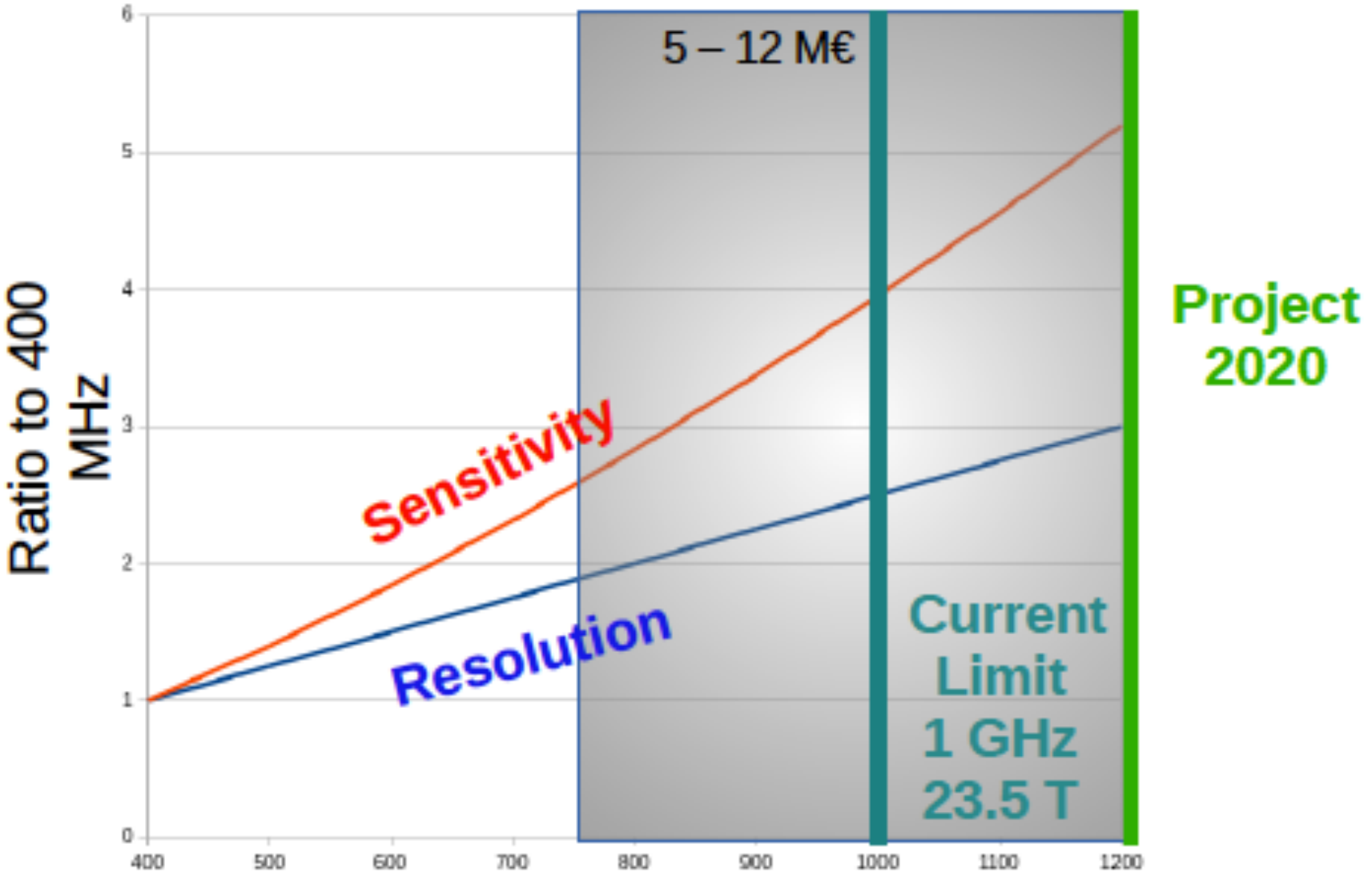
Magnets become more compact



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

Data courtesy of Bruker

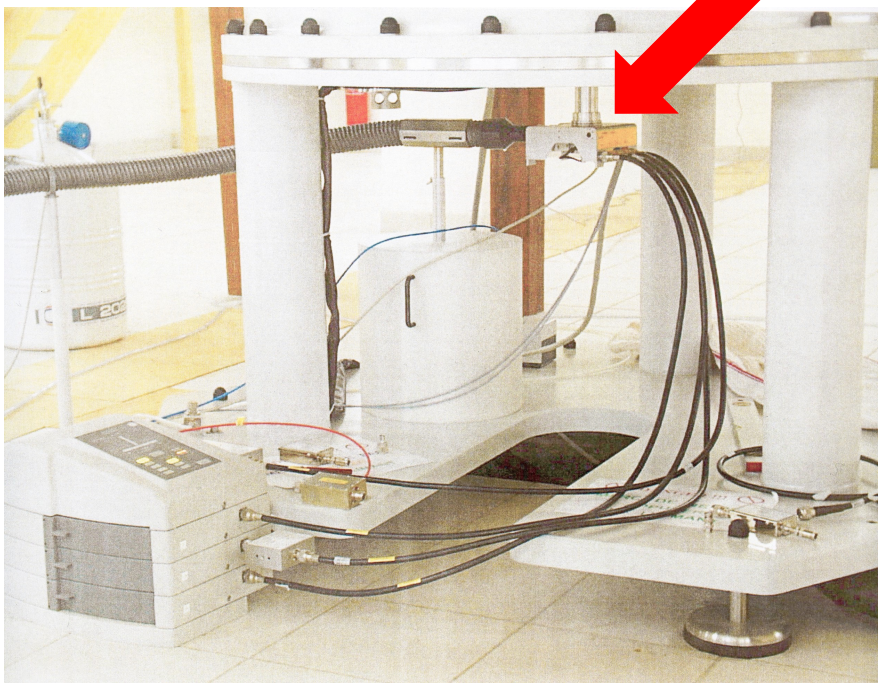
Technological innovations



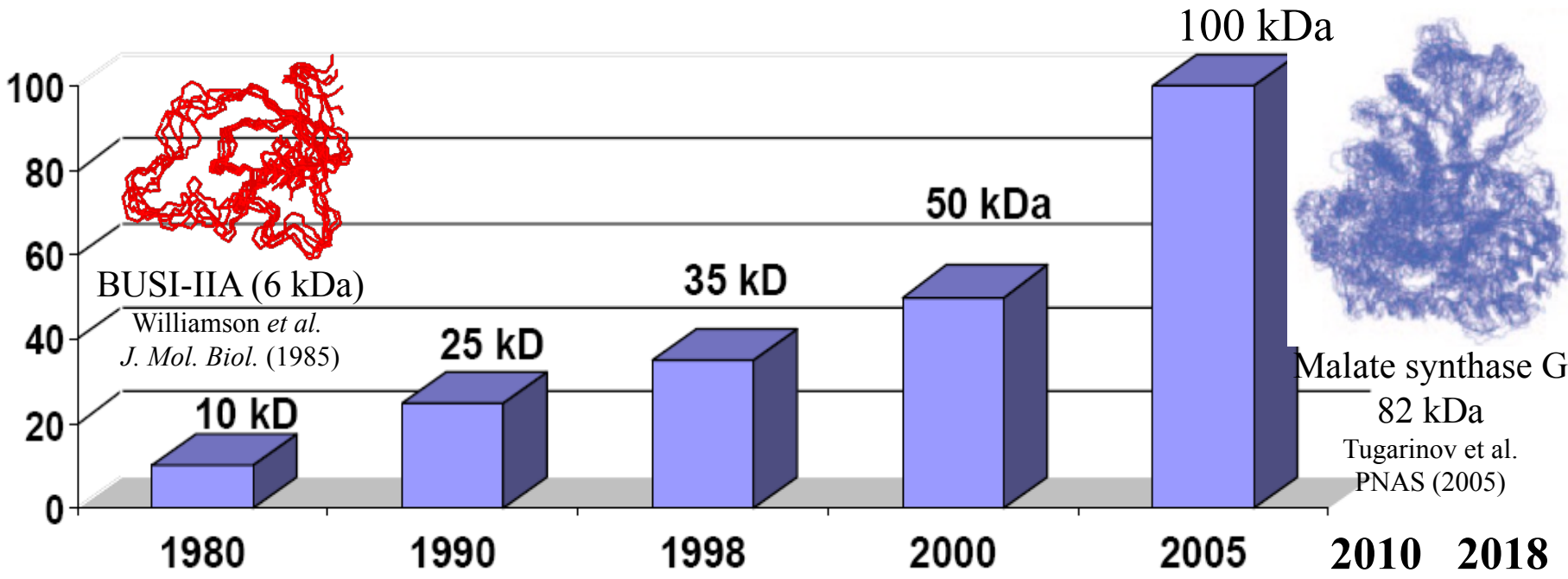
Technological innovations and developments

Probes:

- Cryoprobes
- Small volume probes
- Multi-nuclei probes

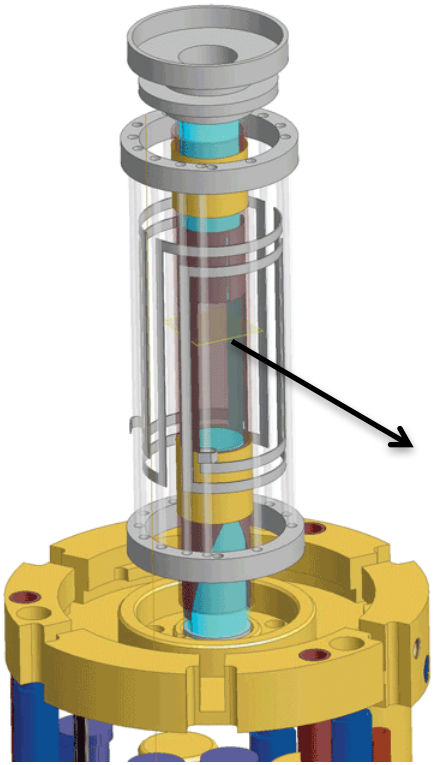


NMR: developments and limits



Cryoprobes		**500, 600 (Bruker/varian/~~Geol~~)**	**700,800**	**1000**

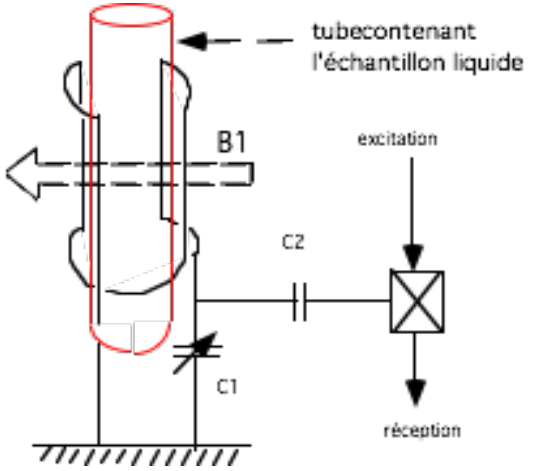
The probe



B_1

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

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



Q quality factor ($1/\omega_0 RC$)

η filling factor

Gain with a cryoprobe

Induced Signal Voltage to Noise Voltage

Coil Design

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

Coil Resistance

Temperature

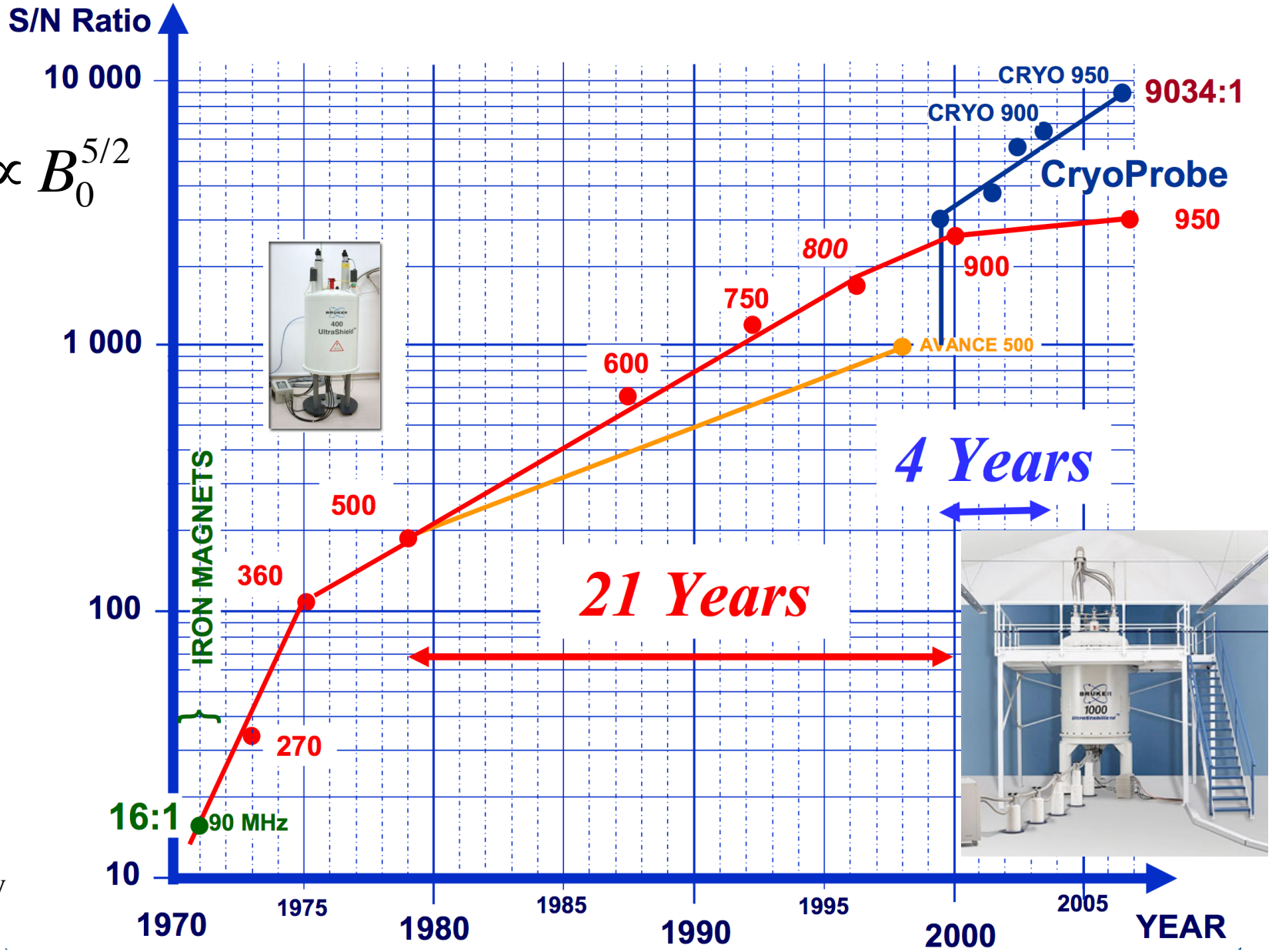
$$S/N \propto Q\eta M_o$$

Q quality factor, η filling factor

Technological innovations

Signal-to-noise depends on the magnetic field

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

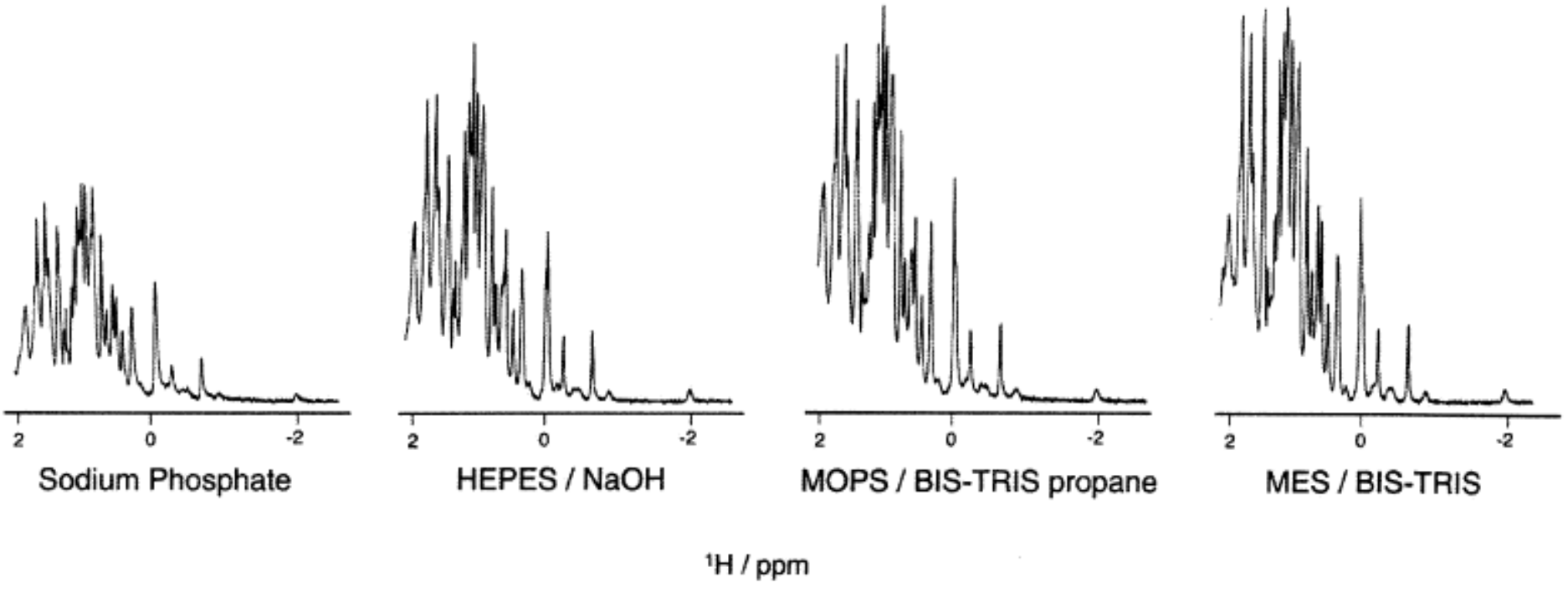


Data courtesy of Bruker

Limitations of cryoprobes

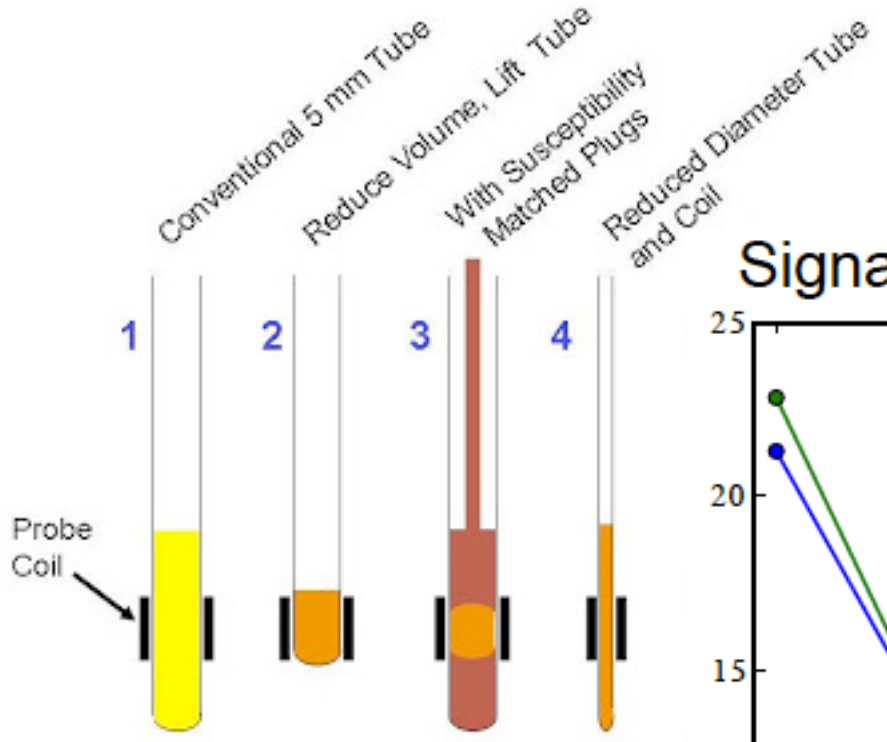
Low-Conductivity Buffers for High-Sensitivity NMR Measurements

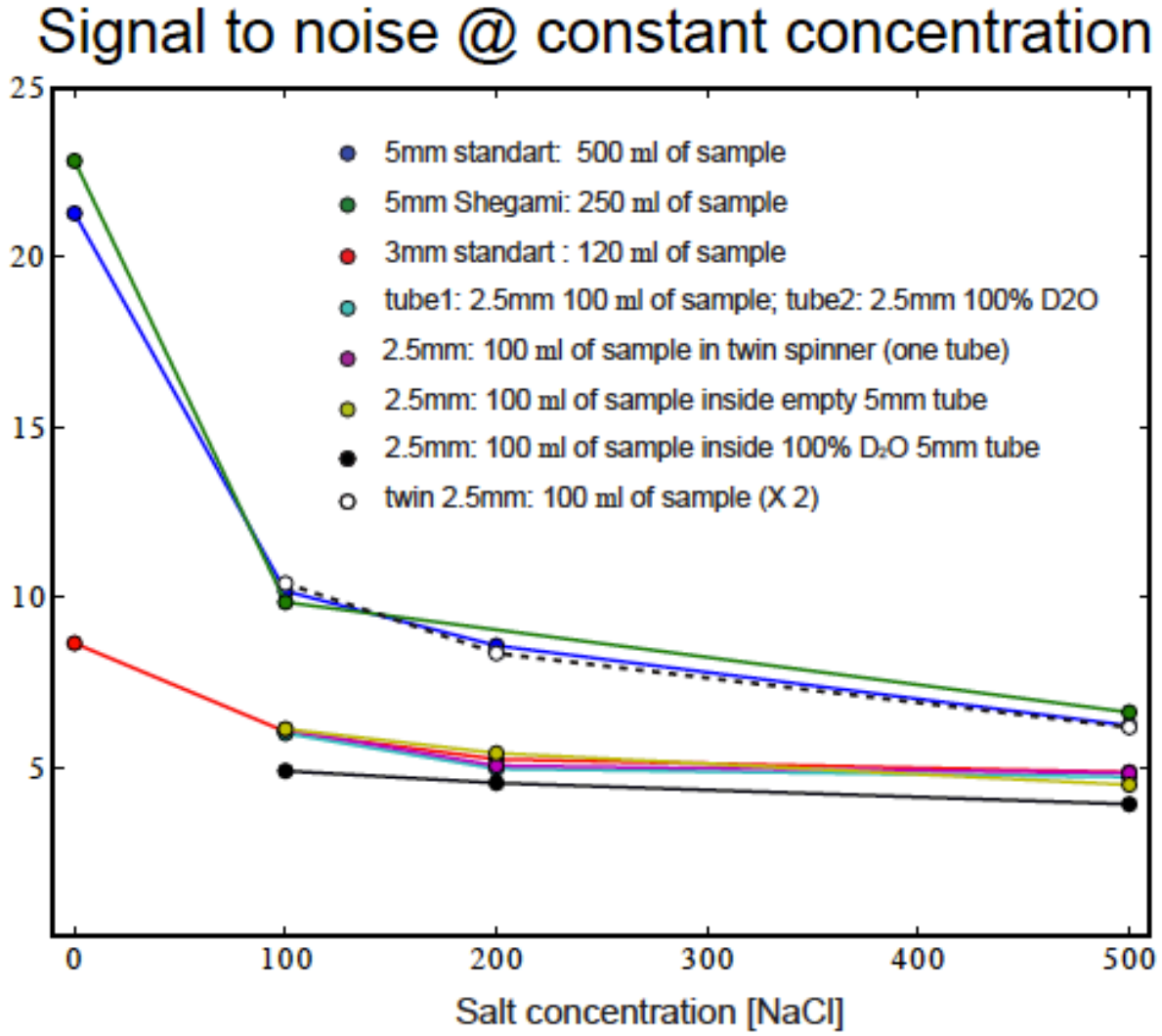
Alexander E. Kelly,† Horng D. Ou,† Richard Withers,‡ and Volker Dötsch*,§



JACS, 2002

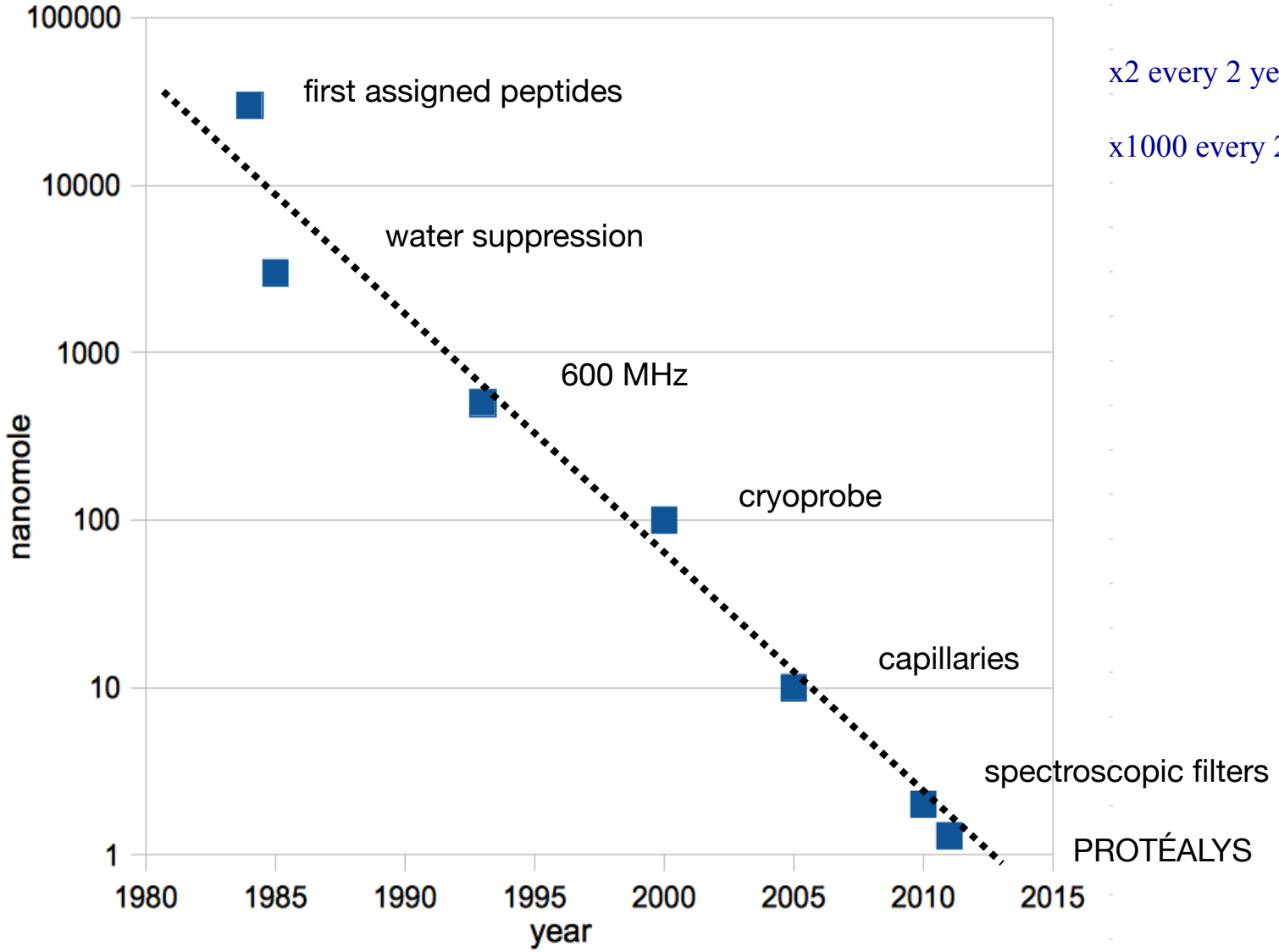
Limitations of cryoprobes





Gain with a cryoprobe

Quantity of protein detected



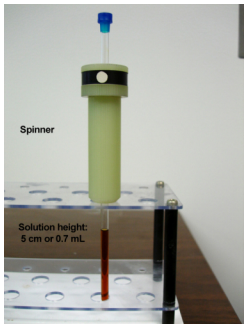
x2 every 2 years

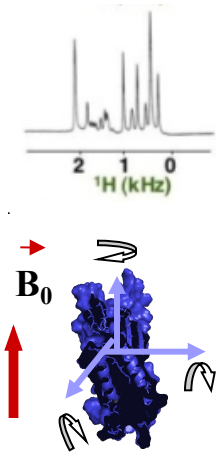
x1000 every 20 years

Data M.-A. Delsuc, IGBMC

Liquid *vs* solid-state probe

Liquid State NMR

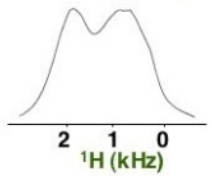


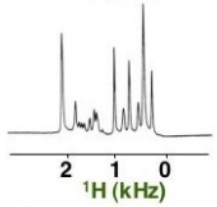


Brownian motion

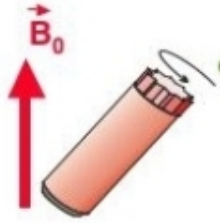
~ 400 µl of soluble sample

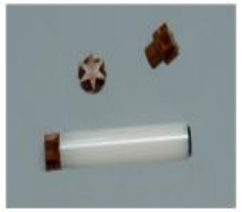
Solid State NMR





MAS
(Magic Angle Spinning)

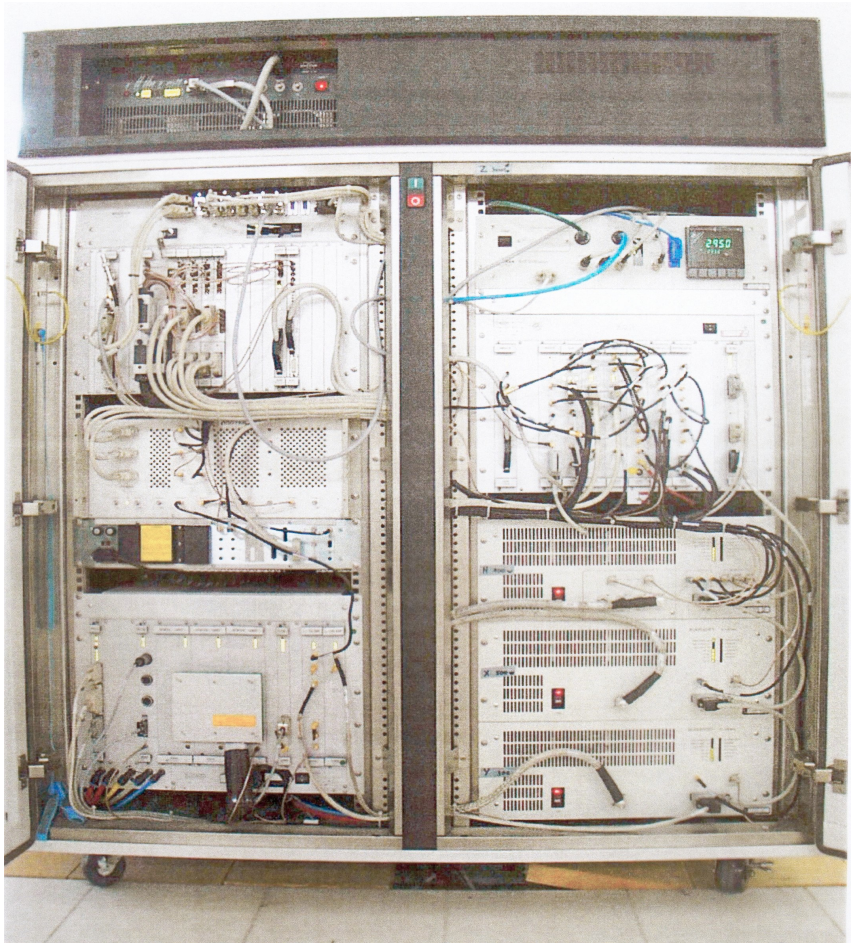




~ 20 µl of hydrated insoluble sample

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

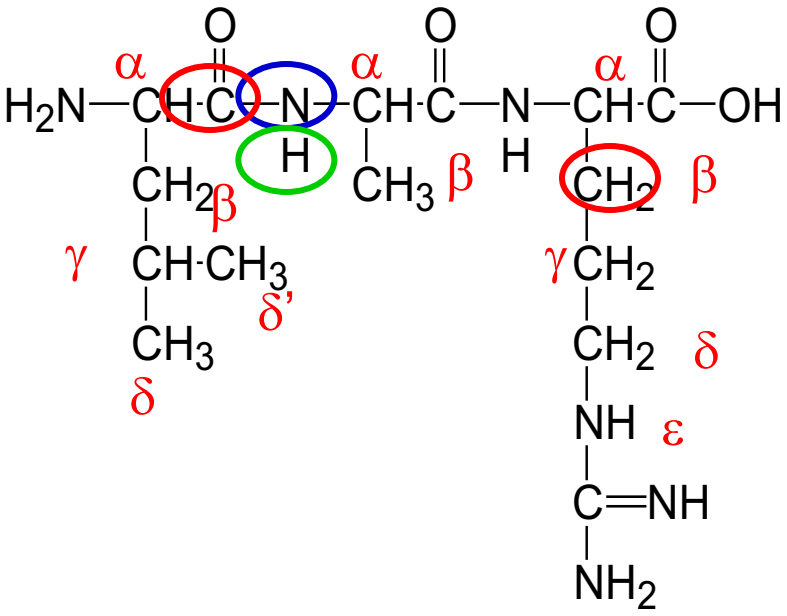
Technological innovations



Pulse sequences:

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

Coherence or magnetization transfer experiments

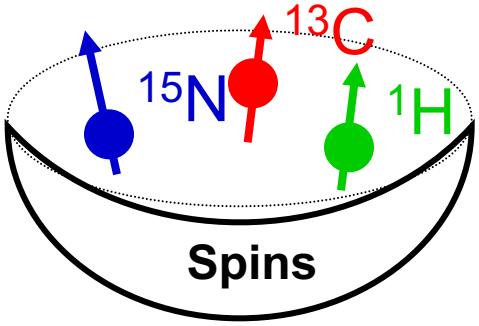


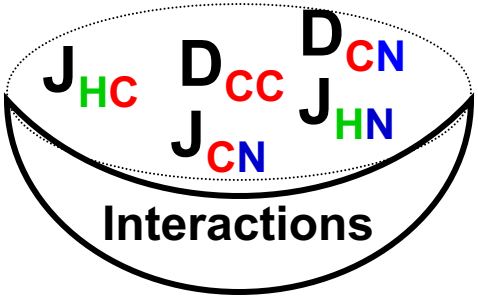
Doubly labeled sample:
^{13}C, ^{15}N

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

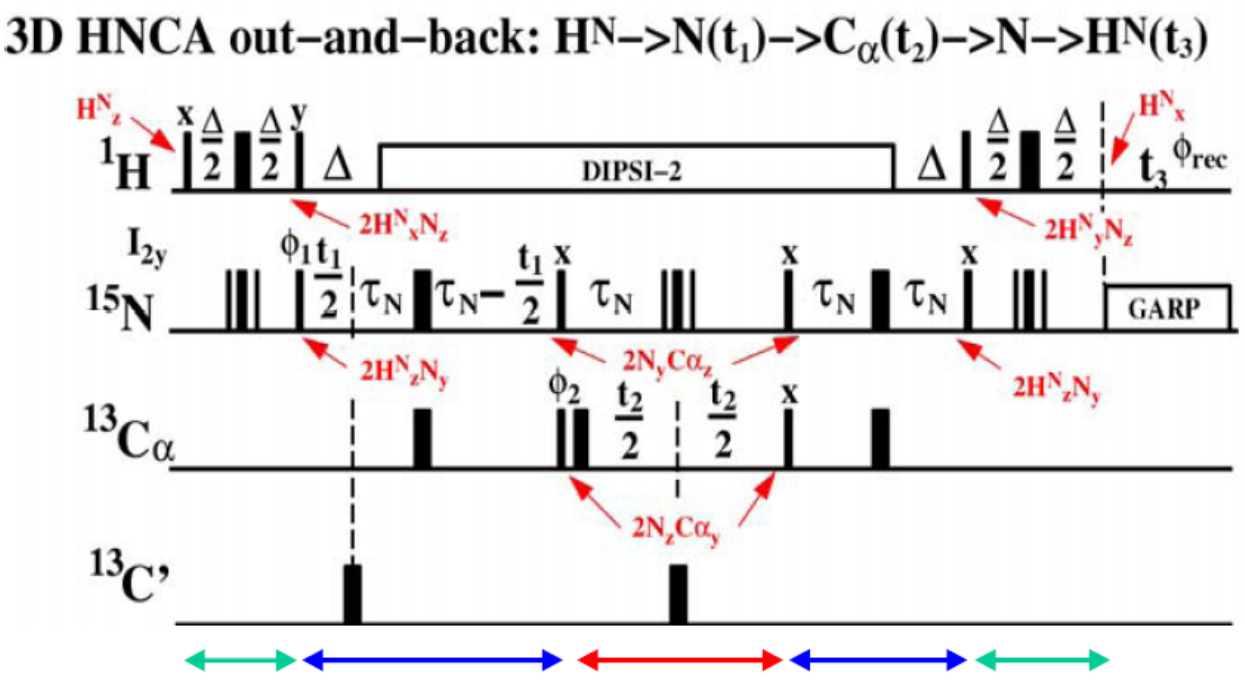
Recombinant DNA or RNA with labeled NTPs,
Enzymatic synthesis

Fluorinated ligands, amino-acids
^{19}F





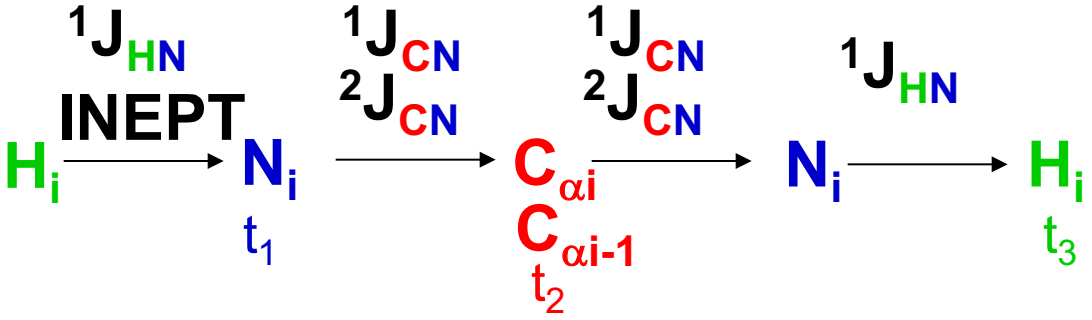
Coherence or dipolar transfer experiments in liquids



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

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

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



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

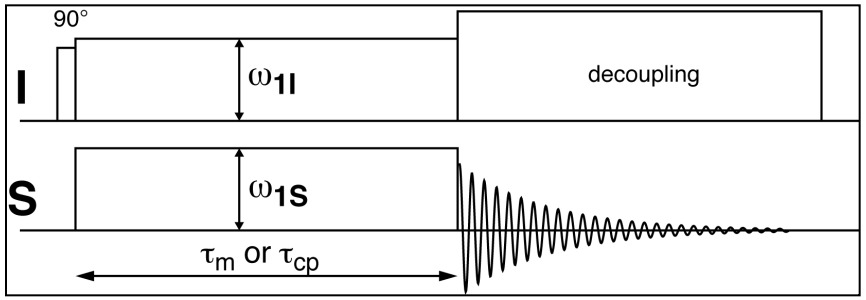
General idea:

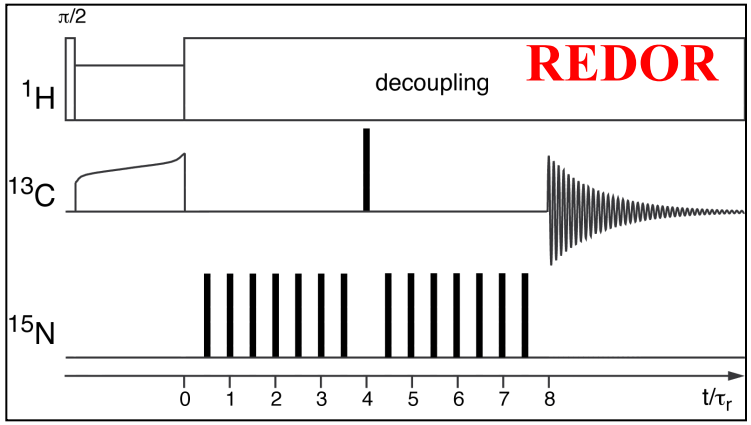
rotate sample (MAS)

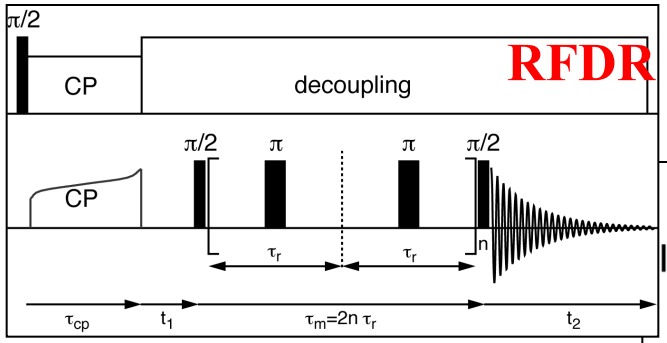
+ rotate spins (RF irradiation)

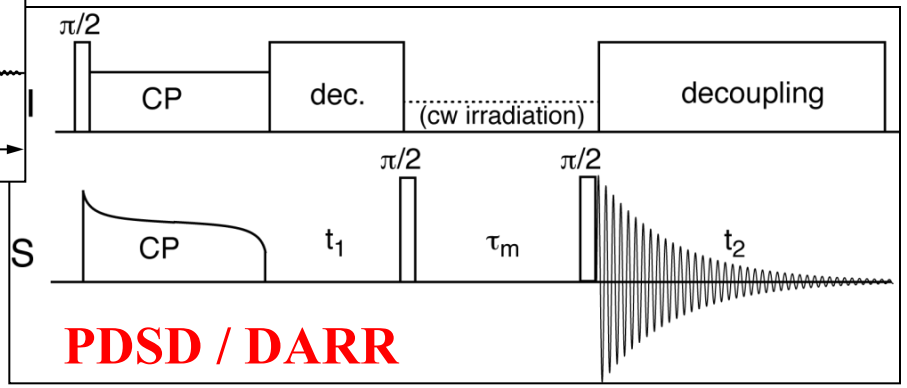
→ create interference between these two processes

cross-polarization







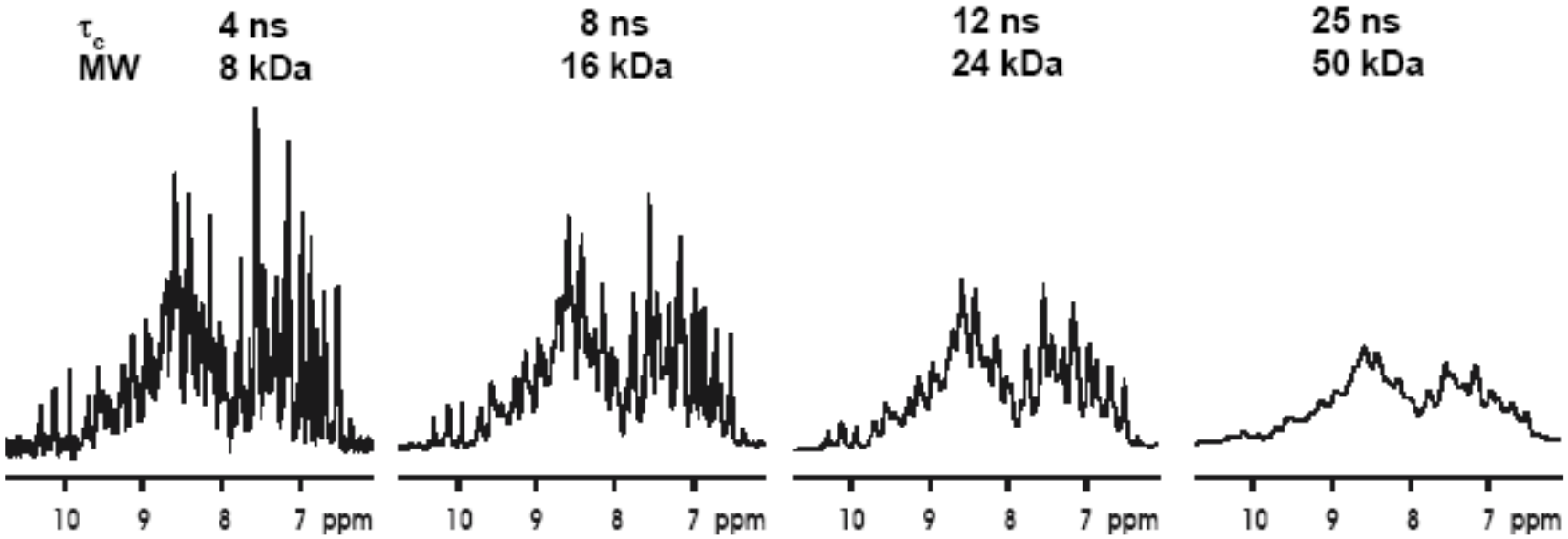


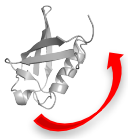
HORROR
DREAM
R-sequences
C-sequences ,….

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

Liquid-state NMR a serious limit?

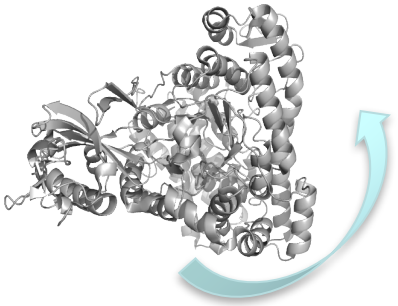
Linewidth





fast overall rotation

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

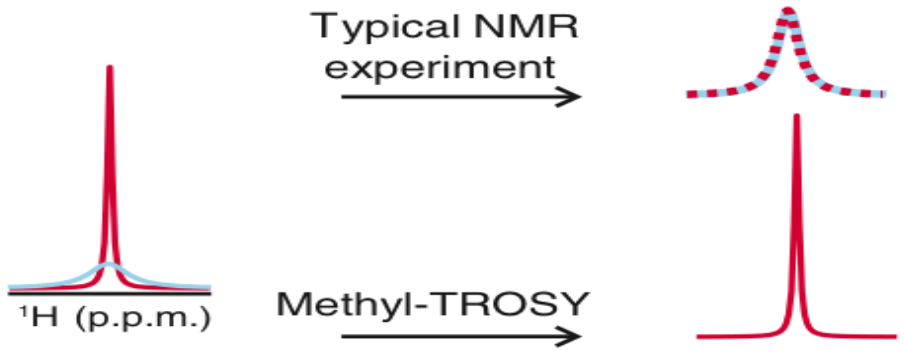


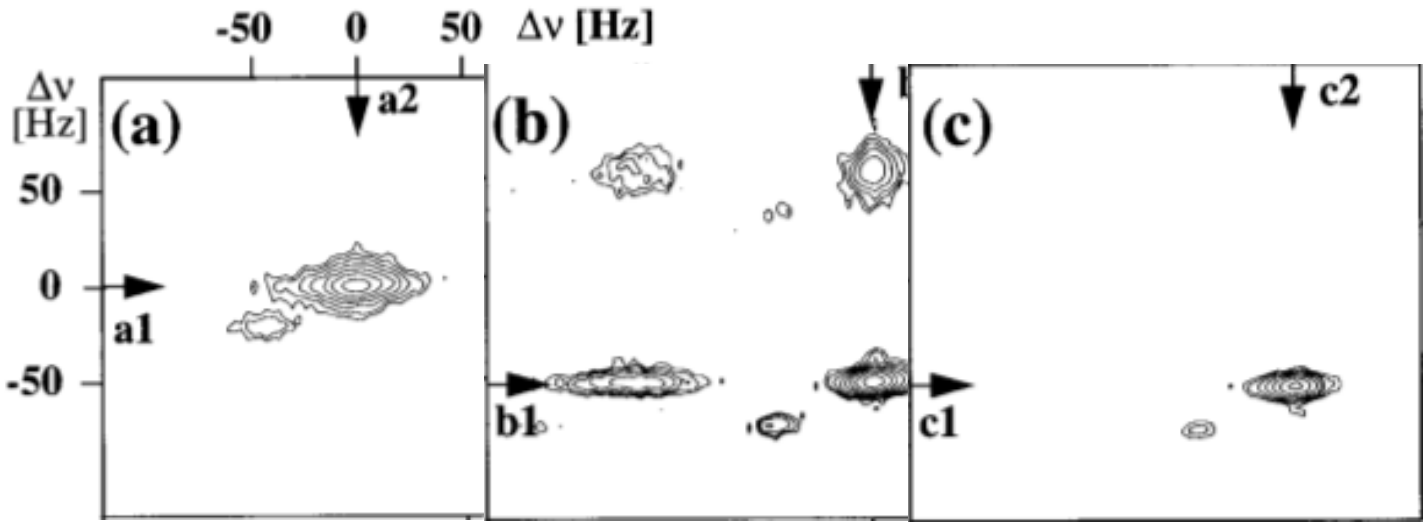
slow overall rotation

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

1. Transverse relaxation:

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





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

1. Transverse relaxation:

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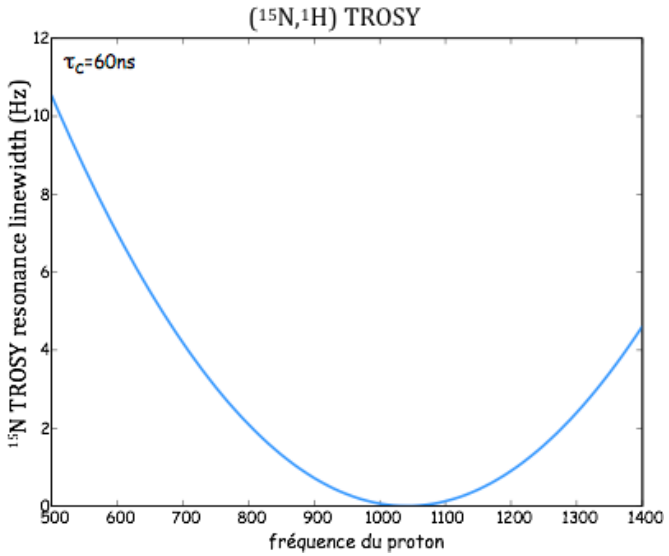
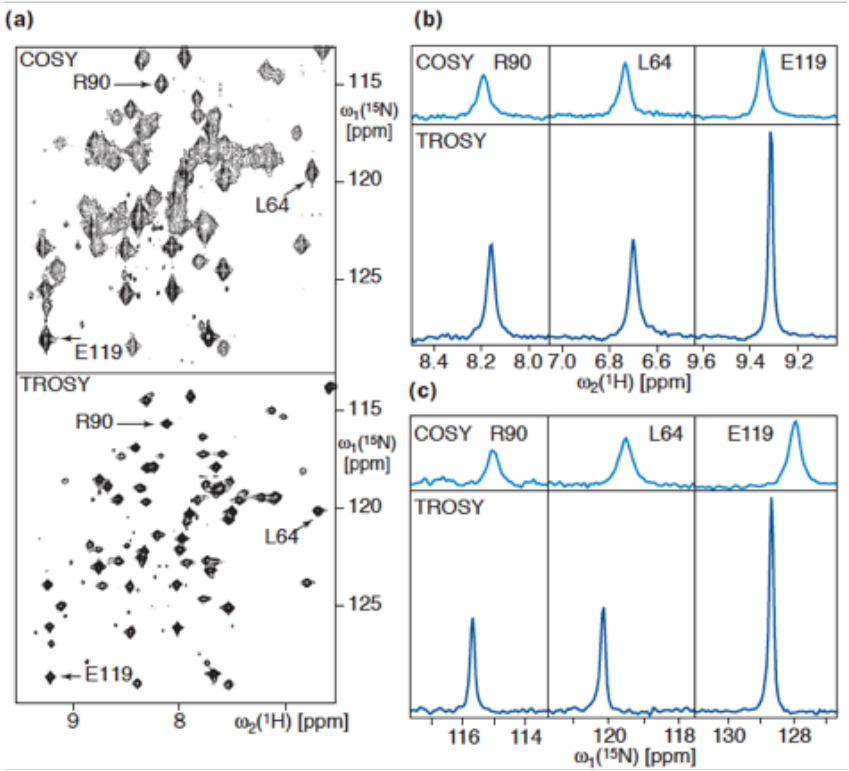
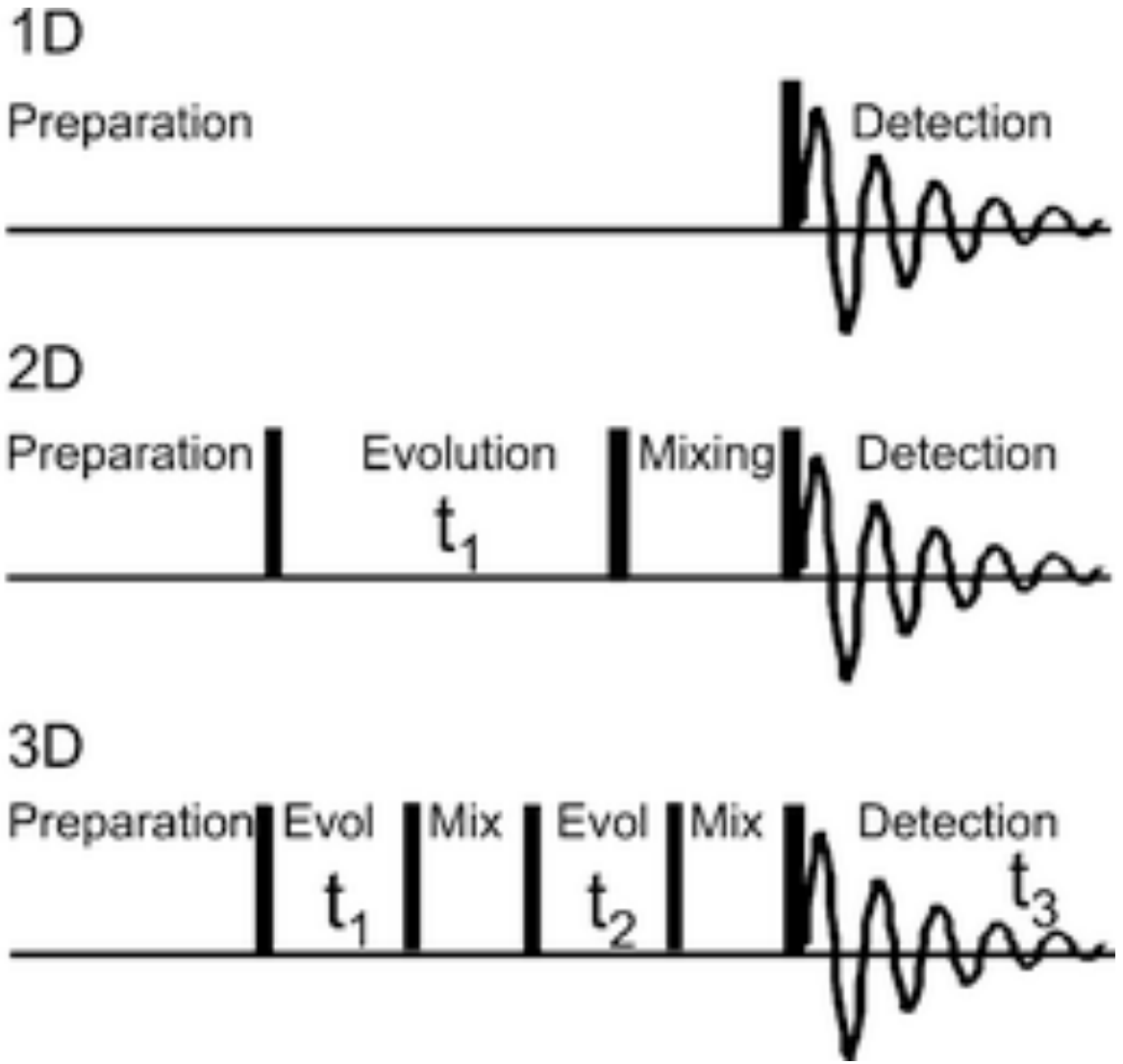


Pervushin, K. Riek, R., Wider, G. and Wütrich, K(1997) Attenuated T2 relaxation by mutual cancellation of dipole–dipole coupling and chemical shift anisotropy indicates an avenue to NMR structures of very large biological macromolecules in solution. Proc. Natl. Acad. Sci. U. S. A. 94, 12366–12371



2H,15N-labeled 110-kDa octameric protein 7,8-dihydroneopterin aldolase

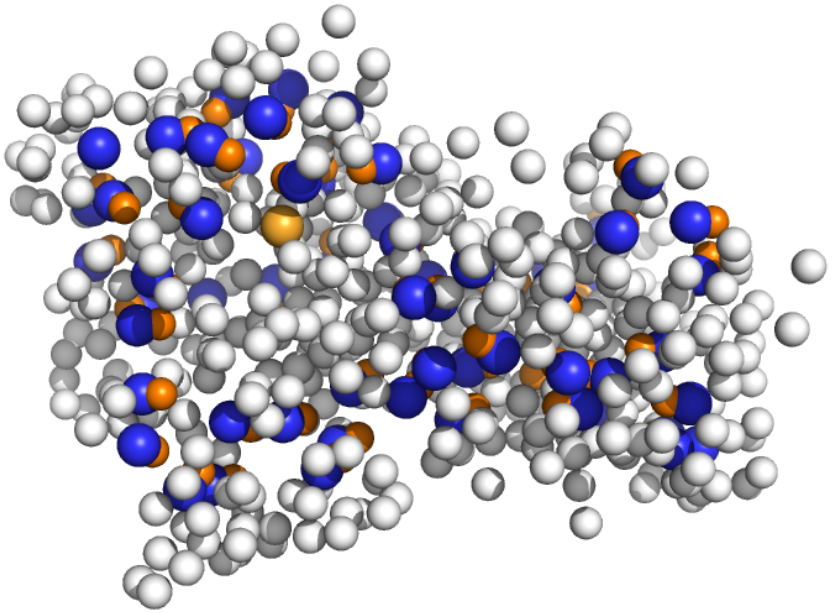
Data acquisition is full of dead times

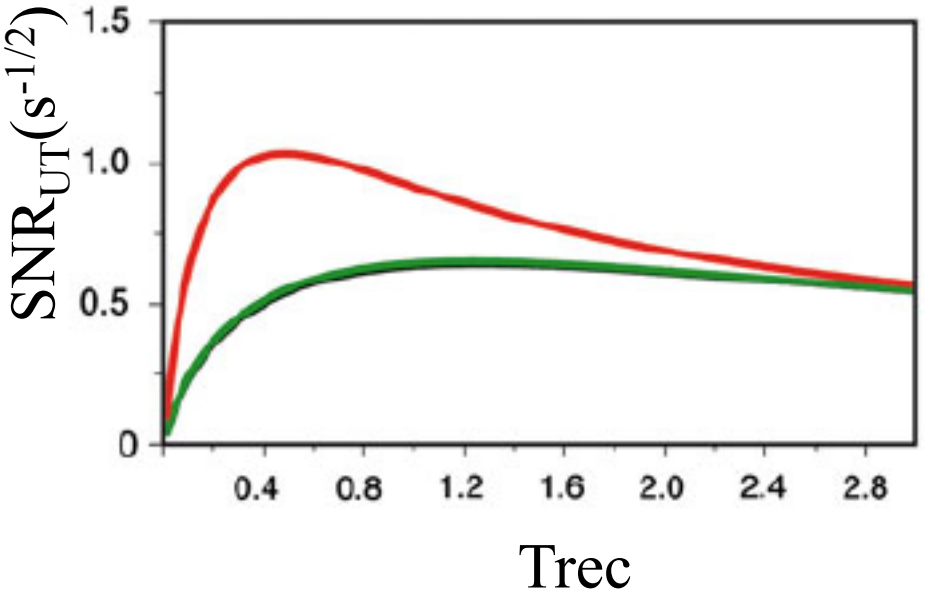


Exploitation of the relaxation properties

2. Longitudinal relaxation:

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

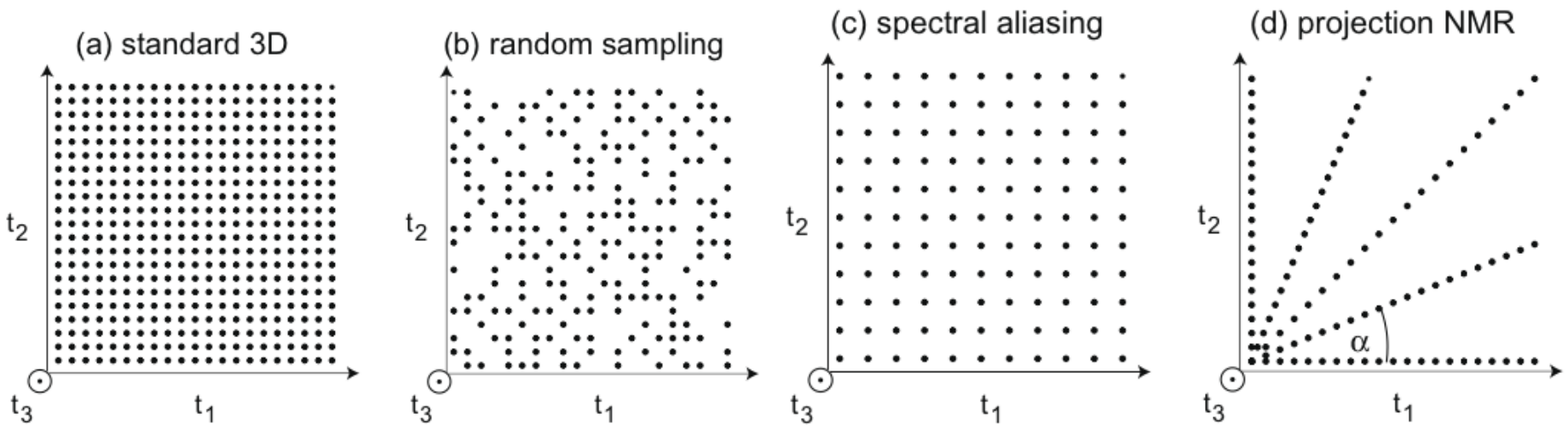




Solyom Z, Schwarten M, Geist L, Konrat R, Willbold D, Brutscher B. J Biomol NMR. 2013 Apr;55(4):311-21.

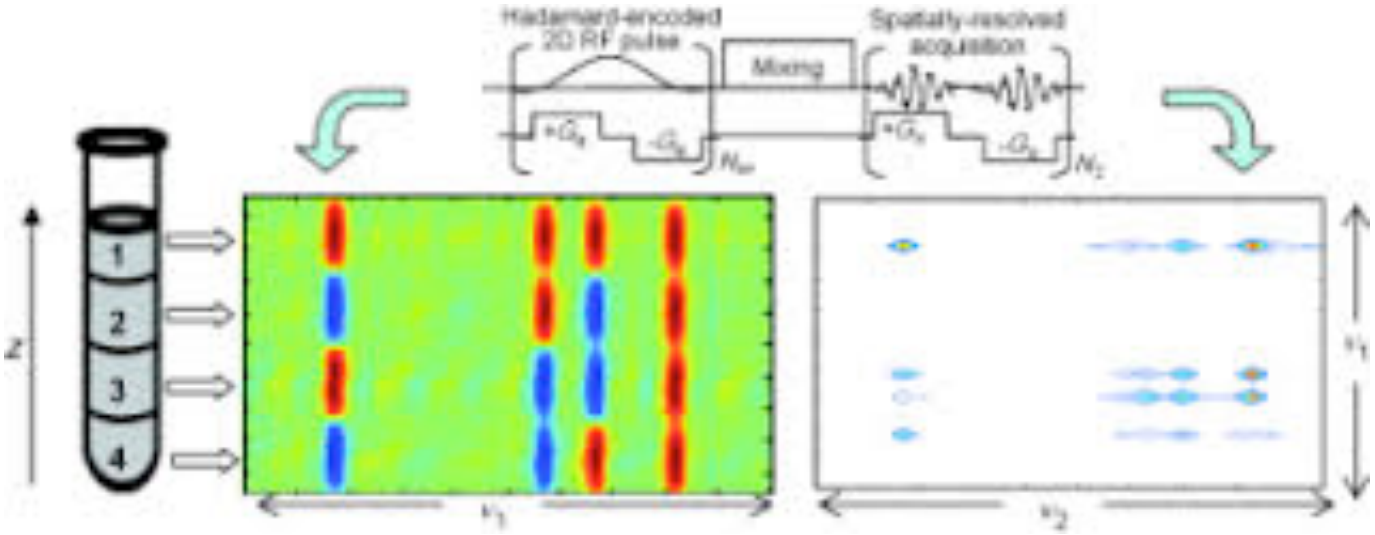
Alternative sampling methods

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



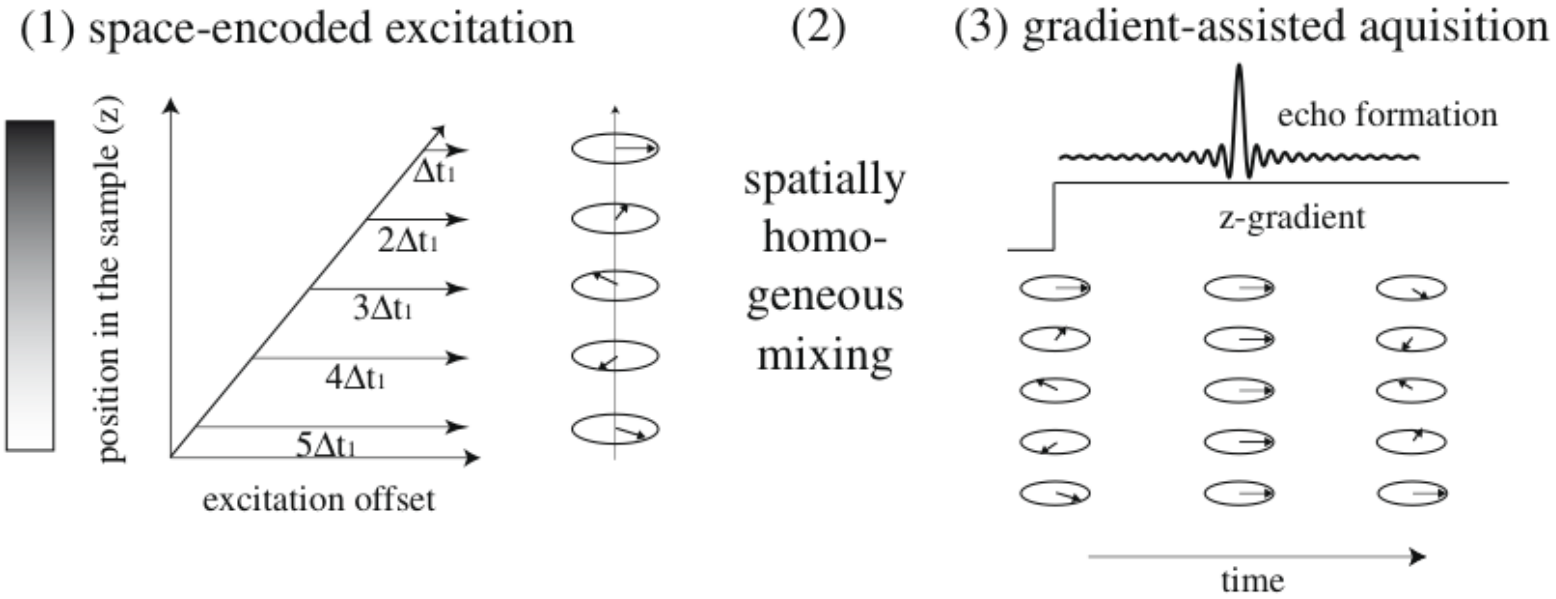
M. Mobli and J.C. Hoch Progress in Nuclear Magnetic Resonance Spectroscopy 83 (2014) 21–41

Alternative sampling methods



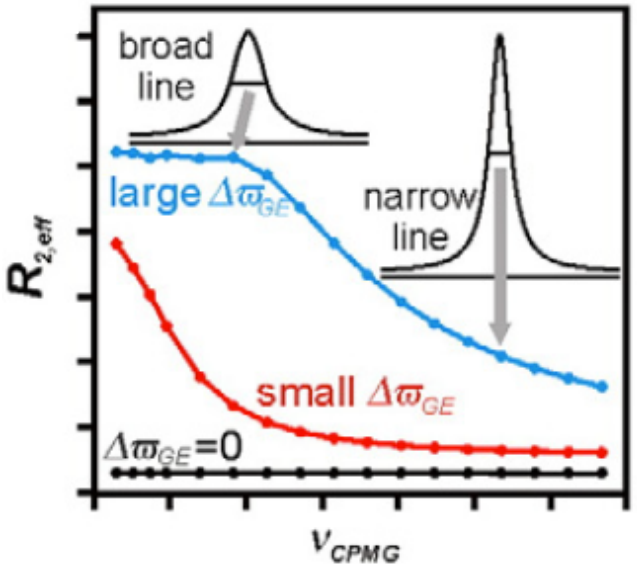
Single-scan spectroscopy

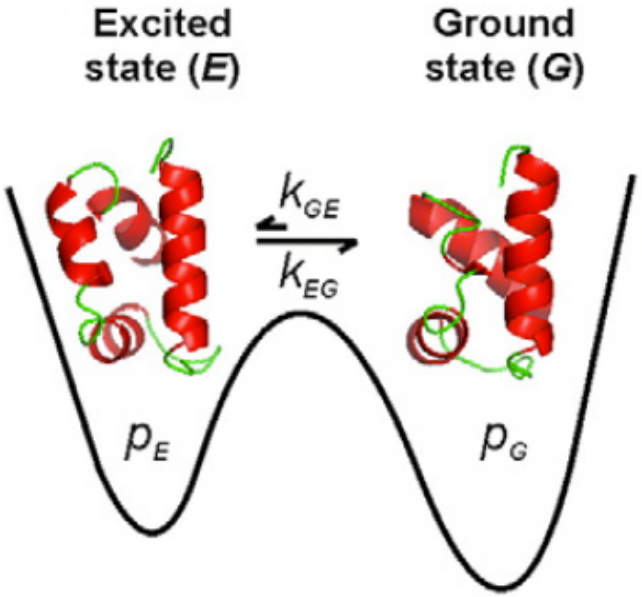
Lucio Frydman et al. PNAS 2002;99:25:15858-15862



Assessing data on non-detectable states

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

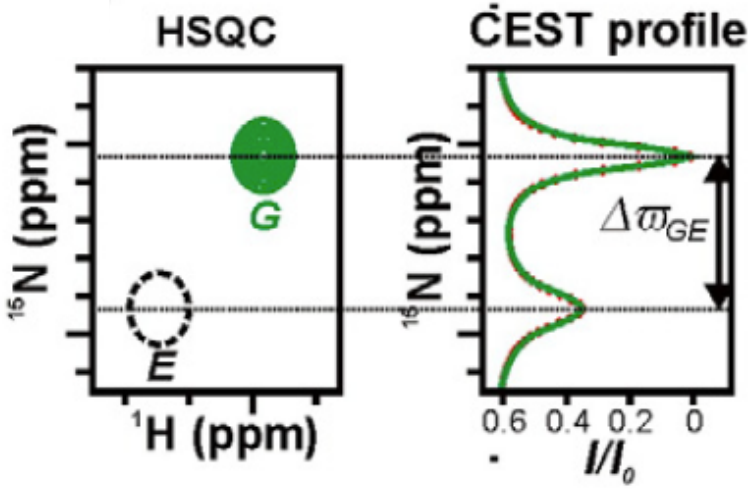




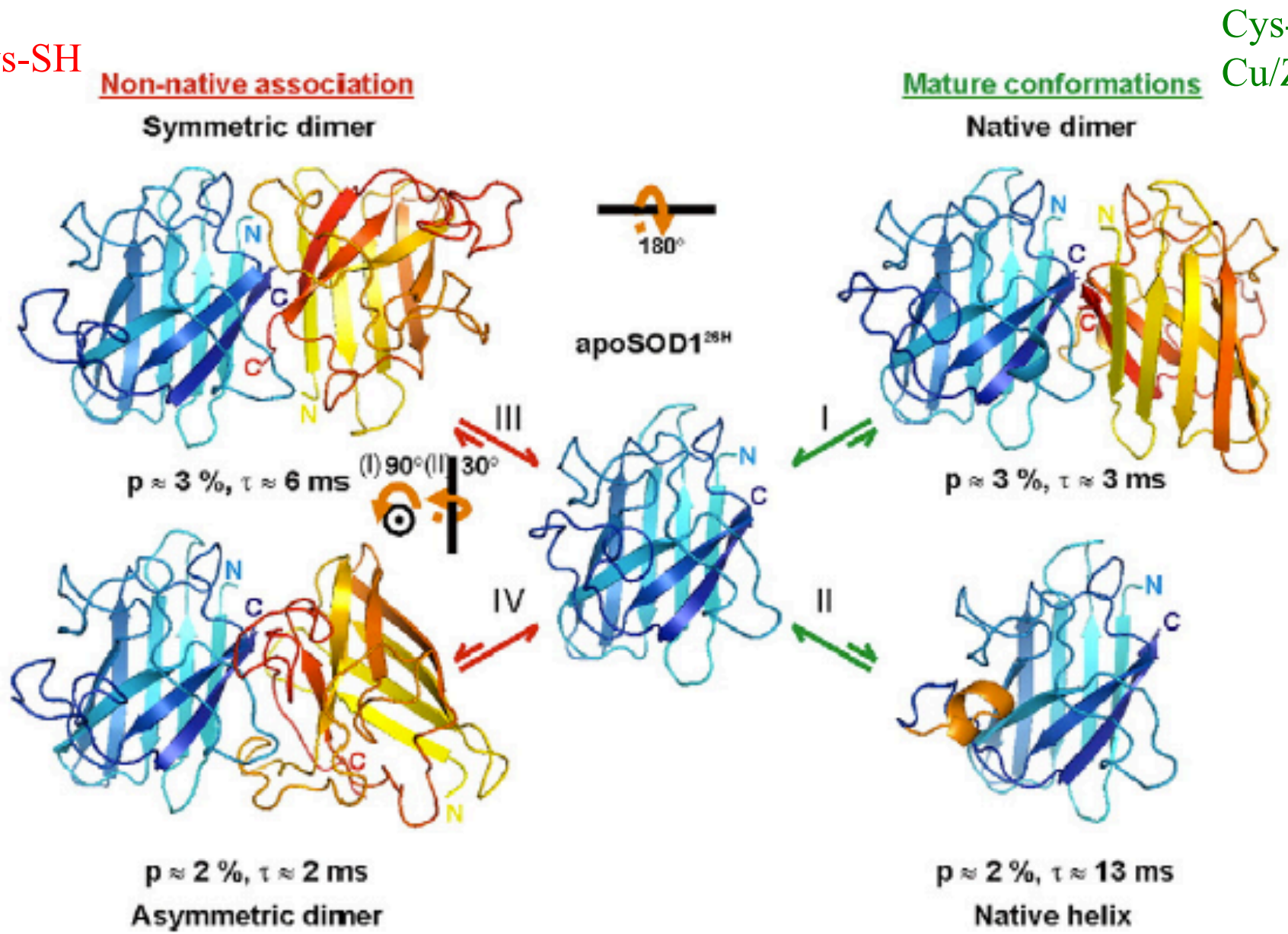
$p_G \gg p_E$

p_E 1% or more

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



Assessing data on non-detectable states



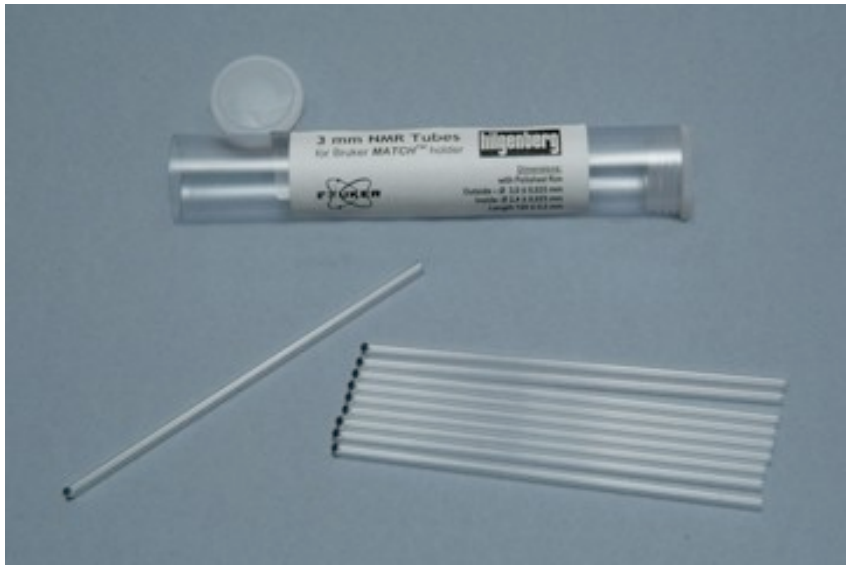
Sekhar and Kay, PNAS 2013, 12867-12874

Technological innovations

Samples :

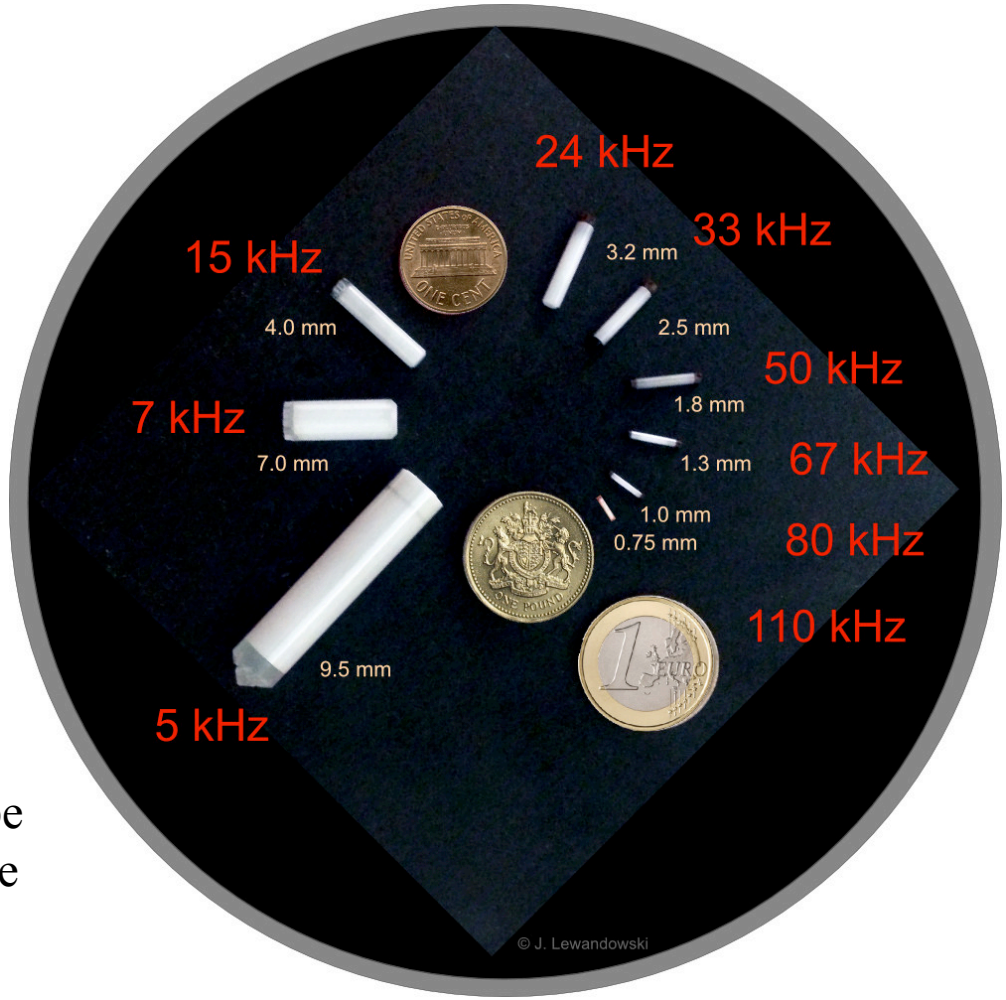
- Small volumes
- Isotopic labeling

Sample volume changes matches probe design



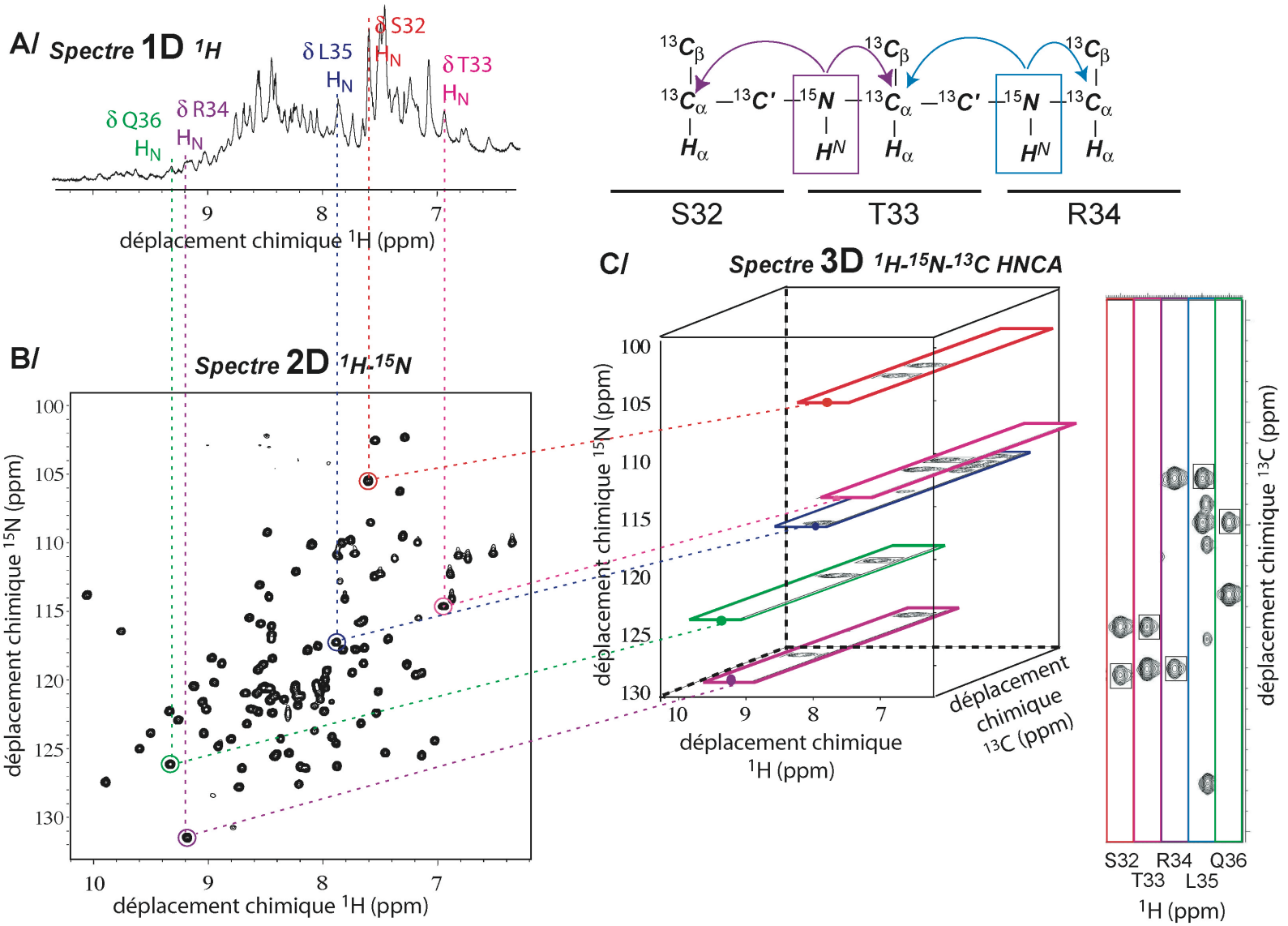
1.7 mm cryoprobe
30 µL sample volume
Liquid-state NMR

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



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?

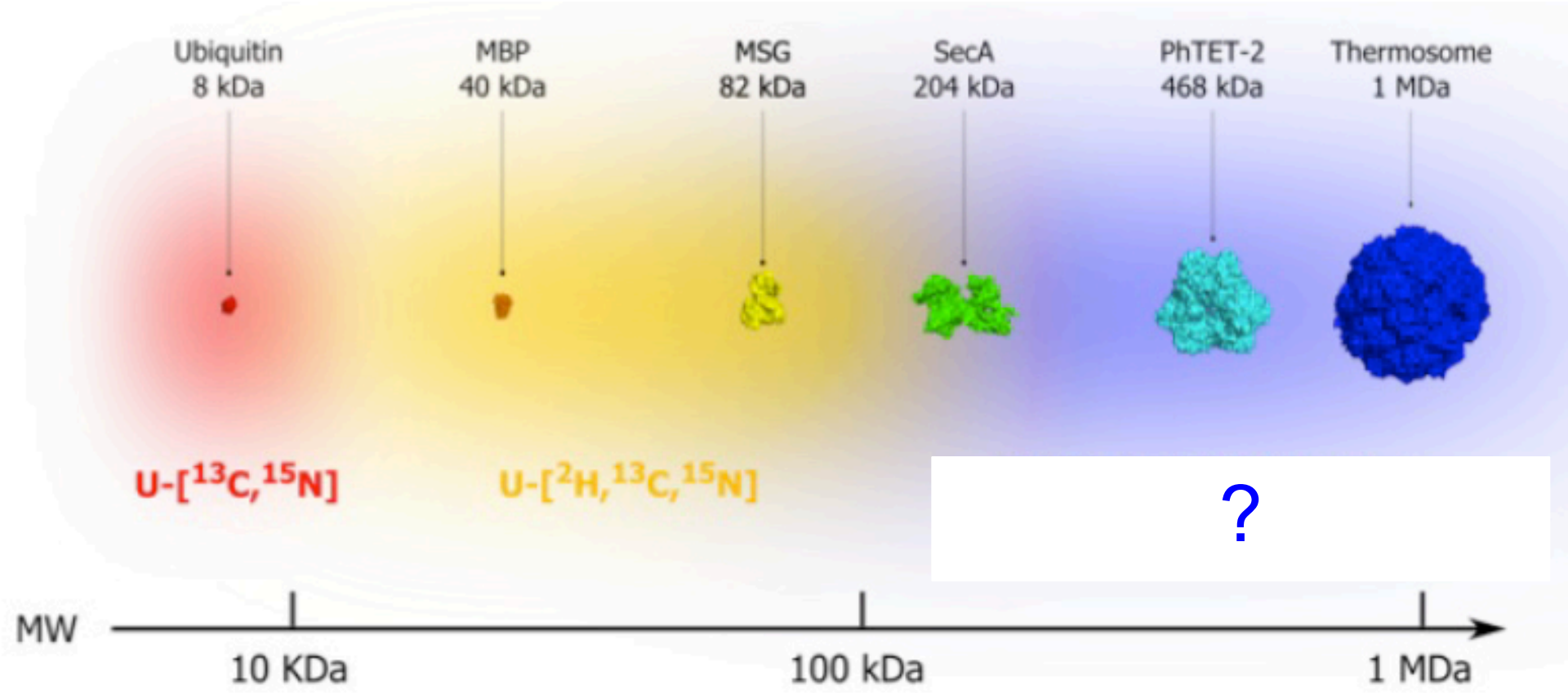
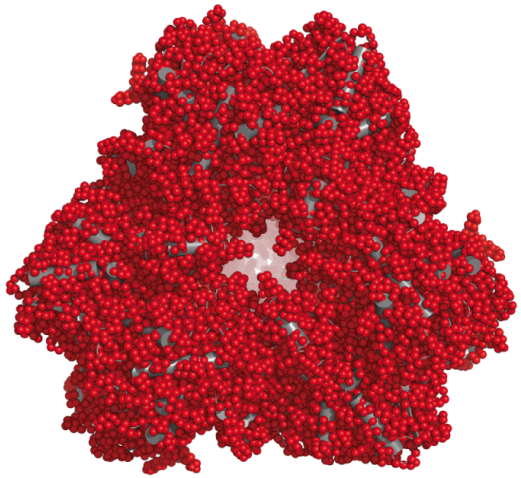


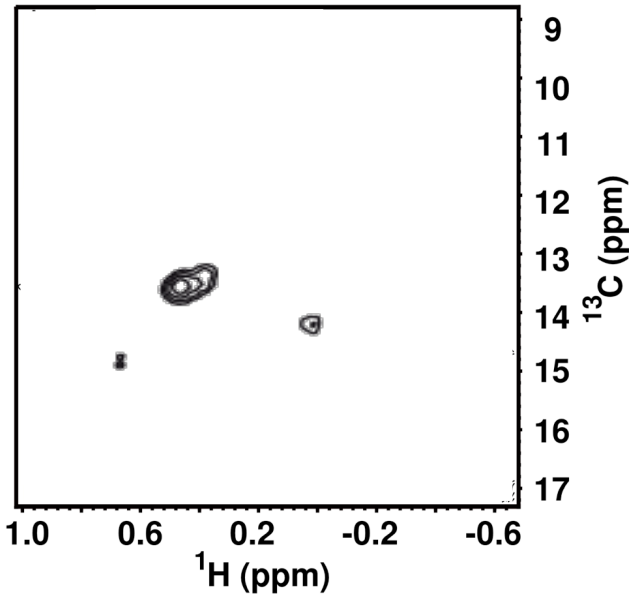
Figure courtesy of J. Boisbouvier

Can we investigate large functional machineries with NMR?

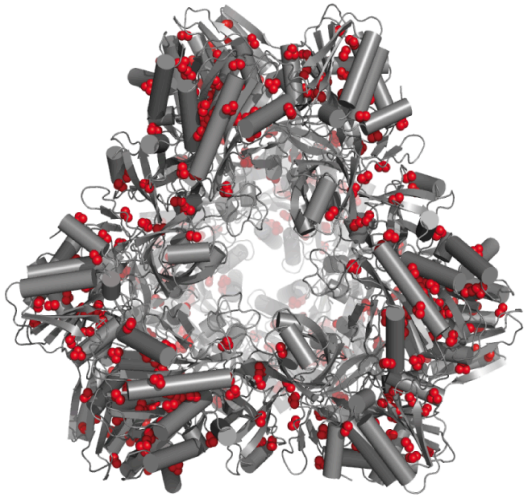


100% protonation

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

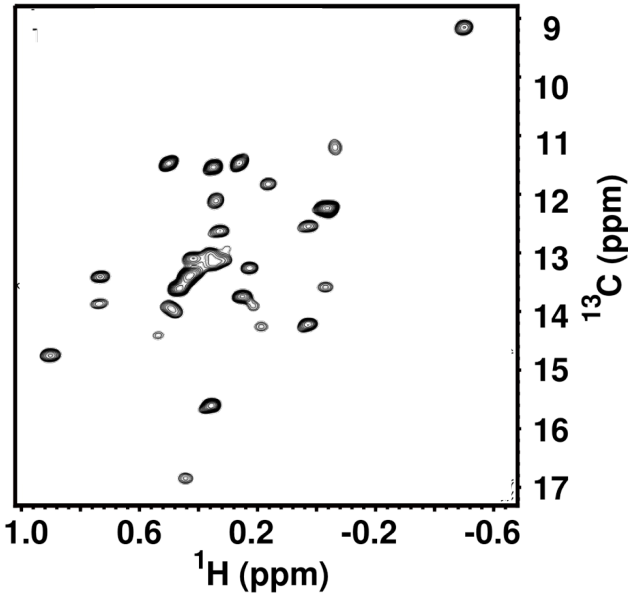


TET -468 kDa
12 x 39 kDa

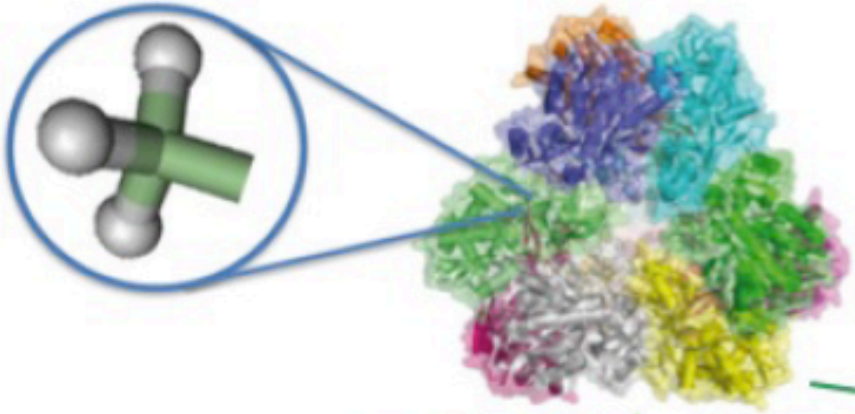


2% protonation

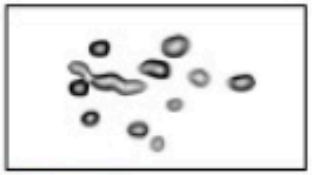
U-[^{2}H,^{12}C,^{15}N]
[δ_1-^{13}C**H$_3$**]-Ile



Me-labeling tool kits for NMR



Thr-γ
Velyvis (2012)
Hamelin (2014)



Met-ε
Fischer (2007)
Gelis (2007)

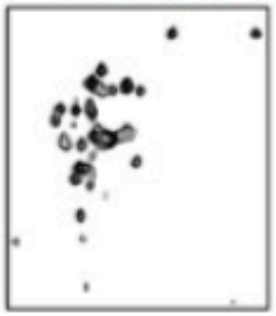
Ala

Ayala (2009)

Ile-δ1

Gardner (1997)
Sounier (2007)
Kerfah (2014)

Ile-γ2



Ruschak (2010)
Ayala (2012)

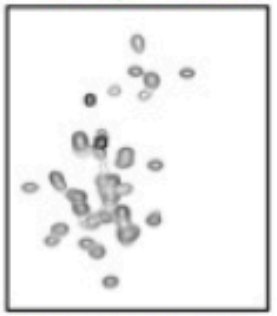
L/V pro-R

Mas (2013)

L/V pro-S

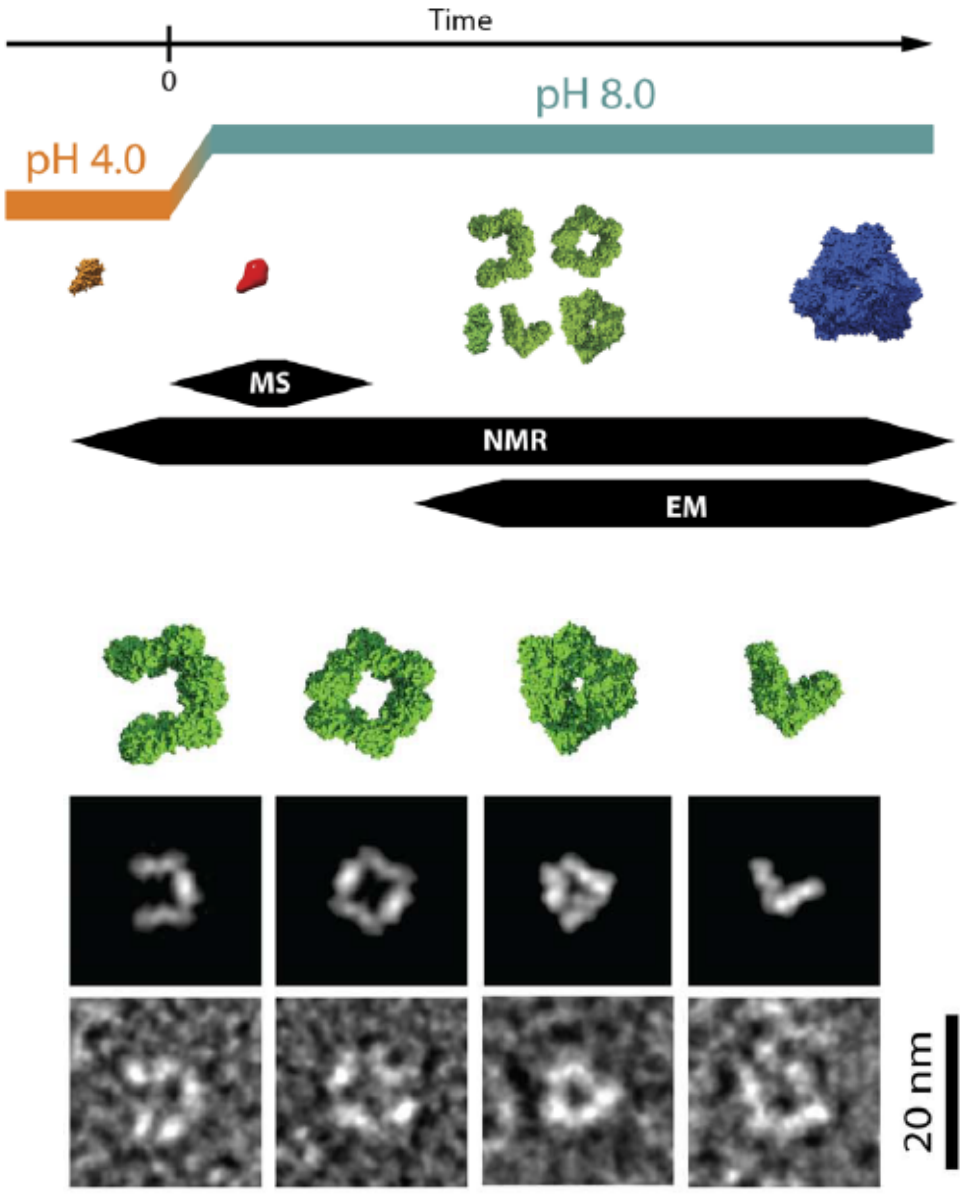
Gans (2010)

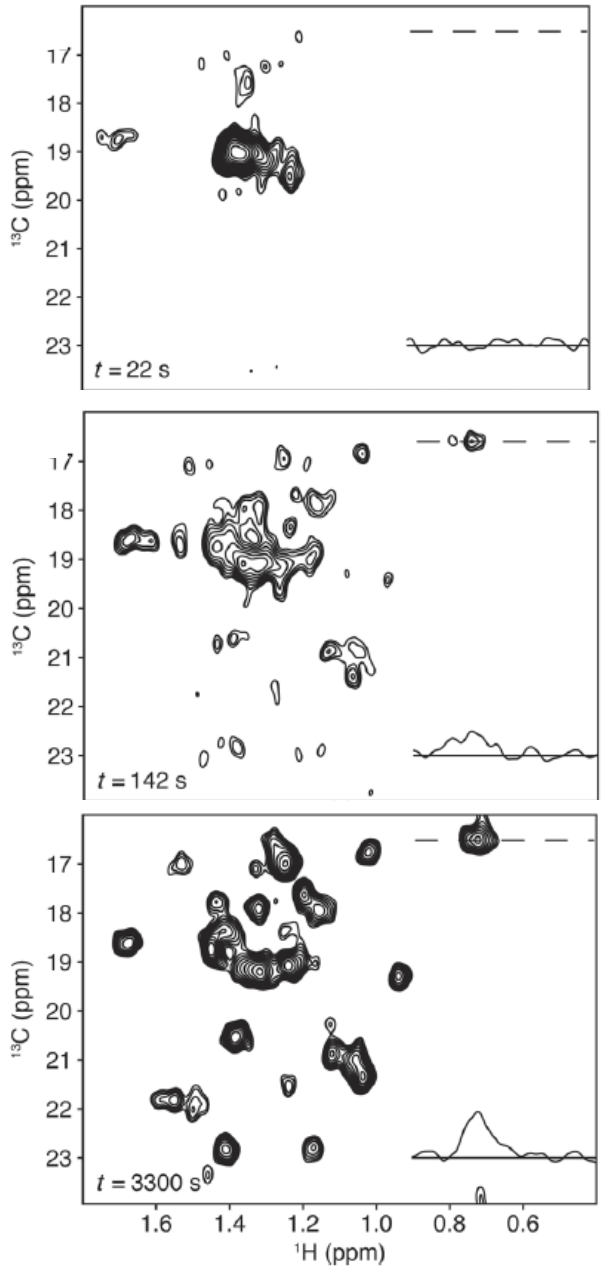
Val pro-S



Mas (2013)
Miyanoiri (2013)

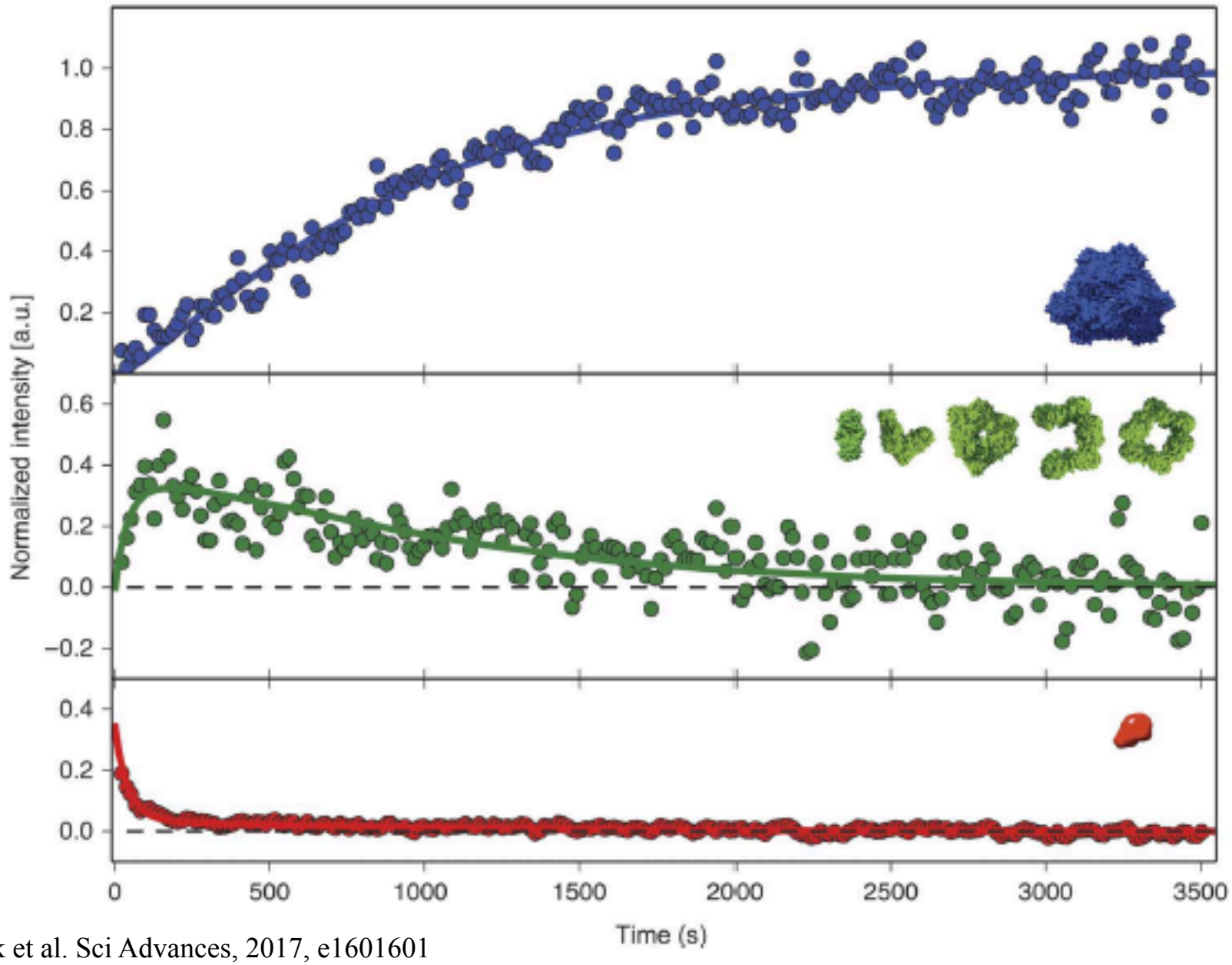
Monitoring of a molecular machine in action





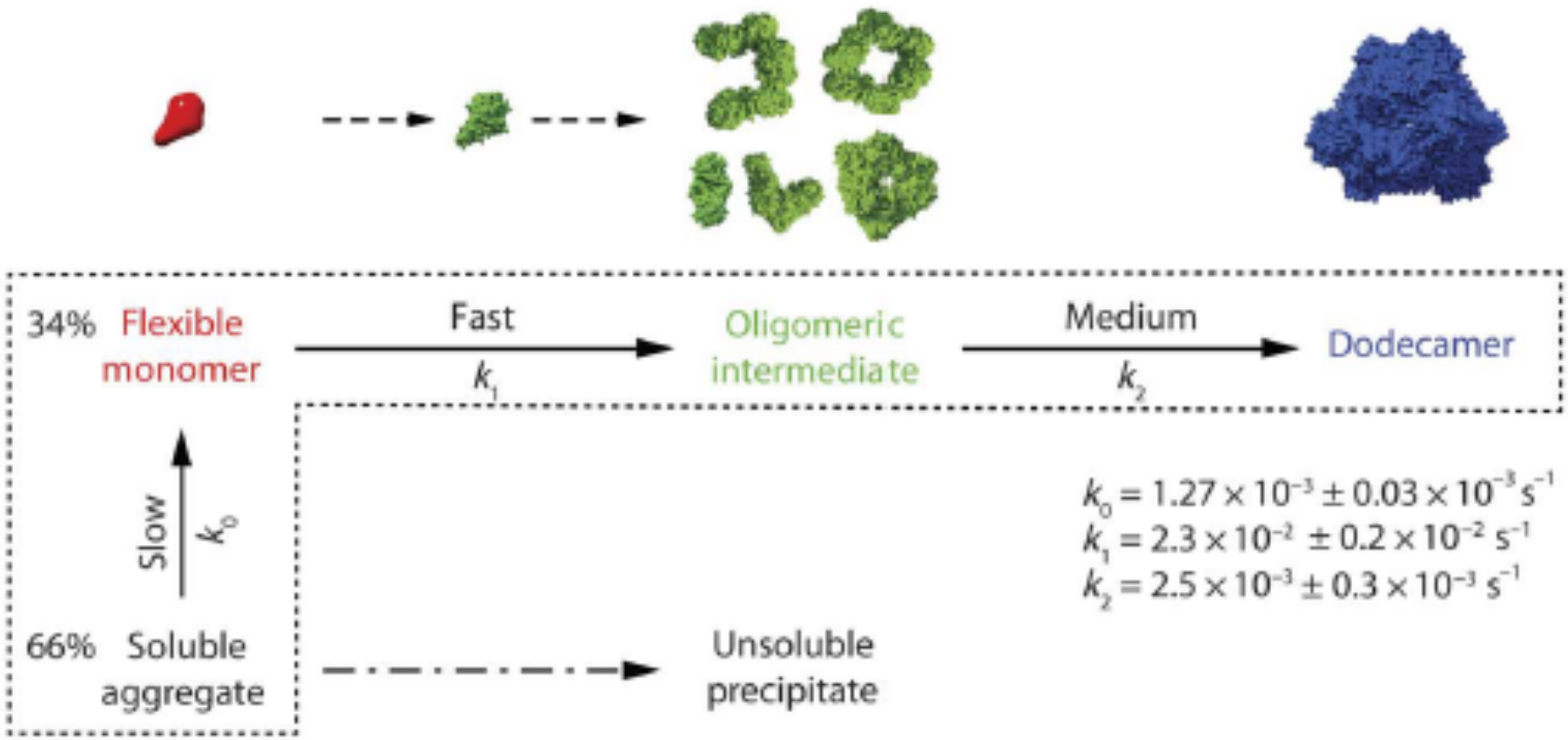
P. Macek et al. Sci Advances, 2017, e1601601

Monitoring of a molecular machine in action



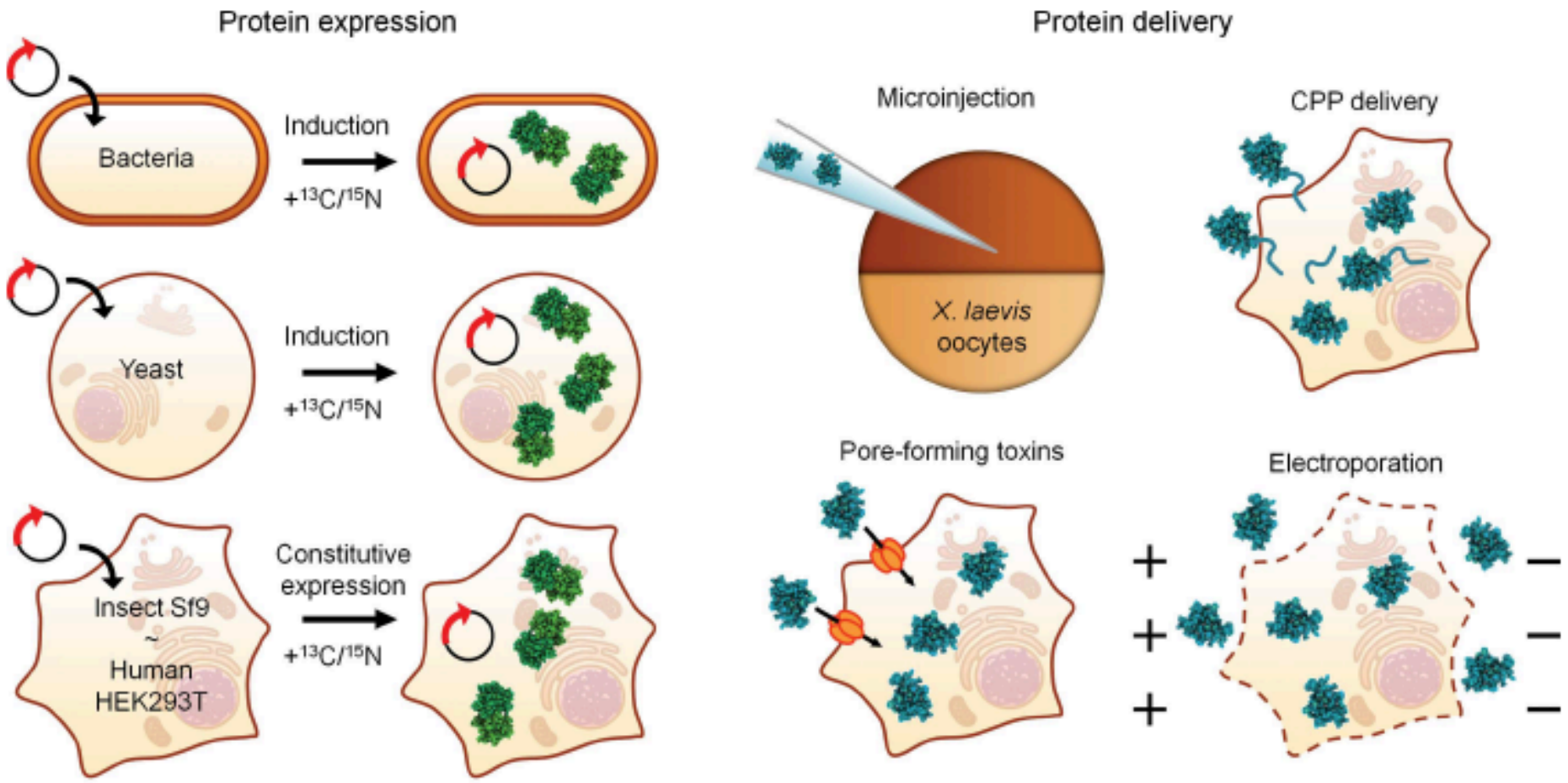
P. Macek et al. Sci Advances, 2017, e1601601

Monitoring of a molecular machine in action



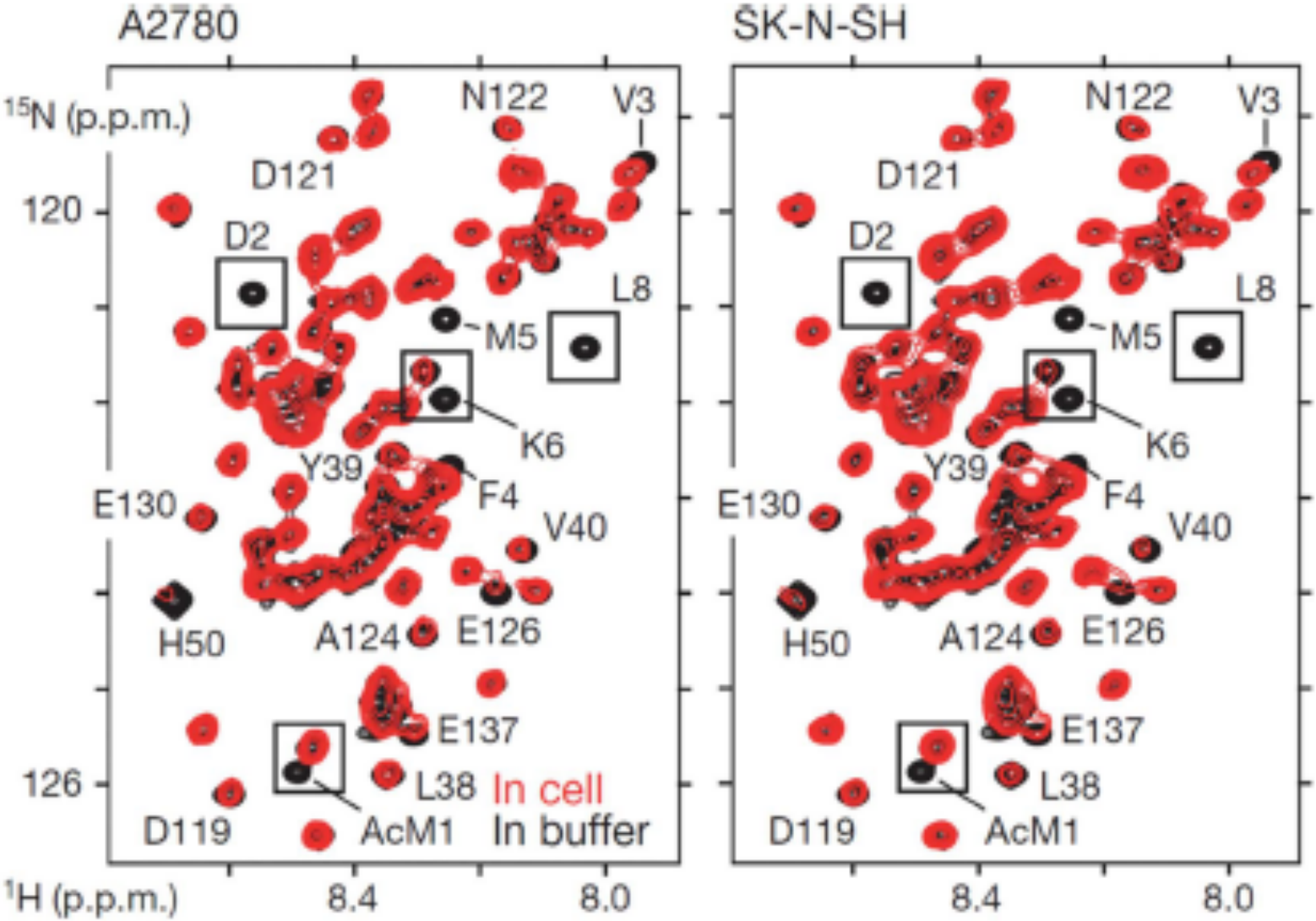
P. Macek et al. Sci Advances, 2017, e1601601

In-cell NMR: schematic overview of different approaches



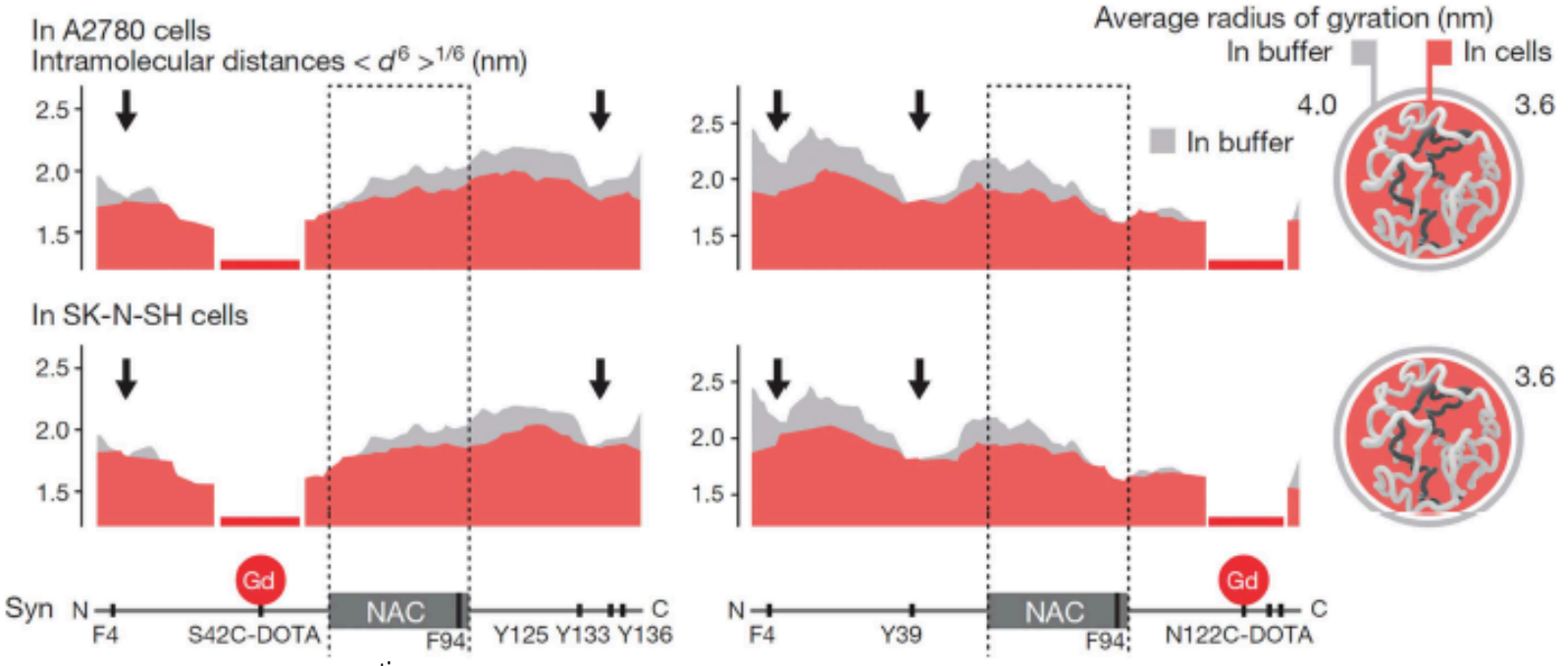
E. Luchinat and L. Banci, IUCrJ, 2017, 108-118

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

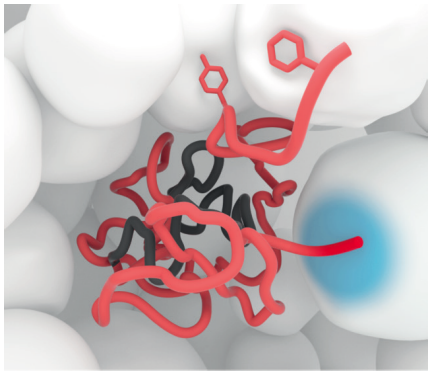


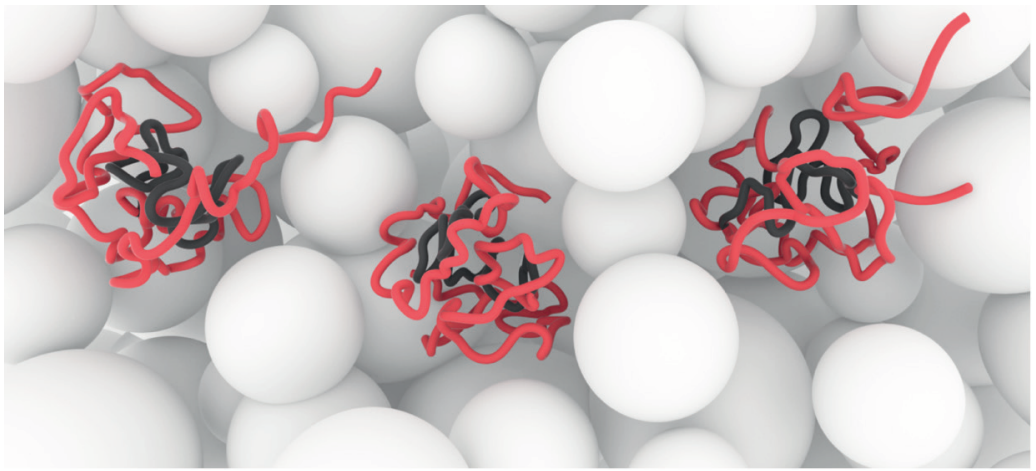
Theillet, Selenko et al.., Nature 2016, 45-50

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



aggregation-prone
non-amyloid-β component





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

Technological innovations

Numerical processing:

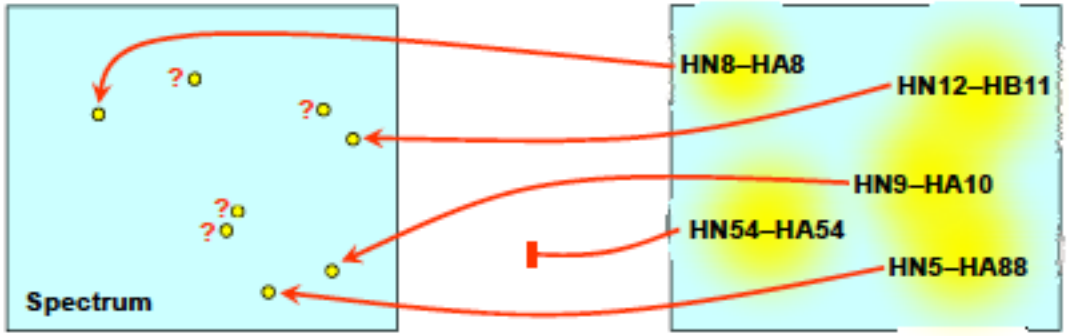
- Filtering
- Data management and integration
- Structure calculation software

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

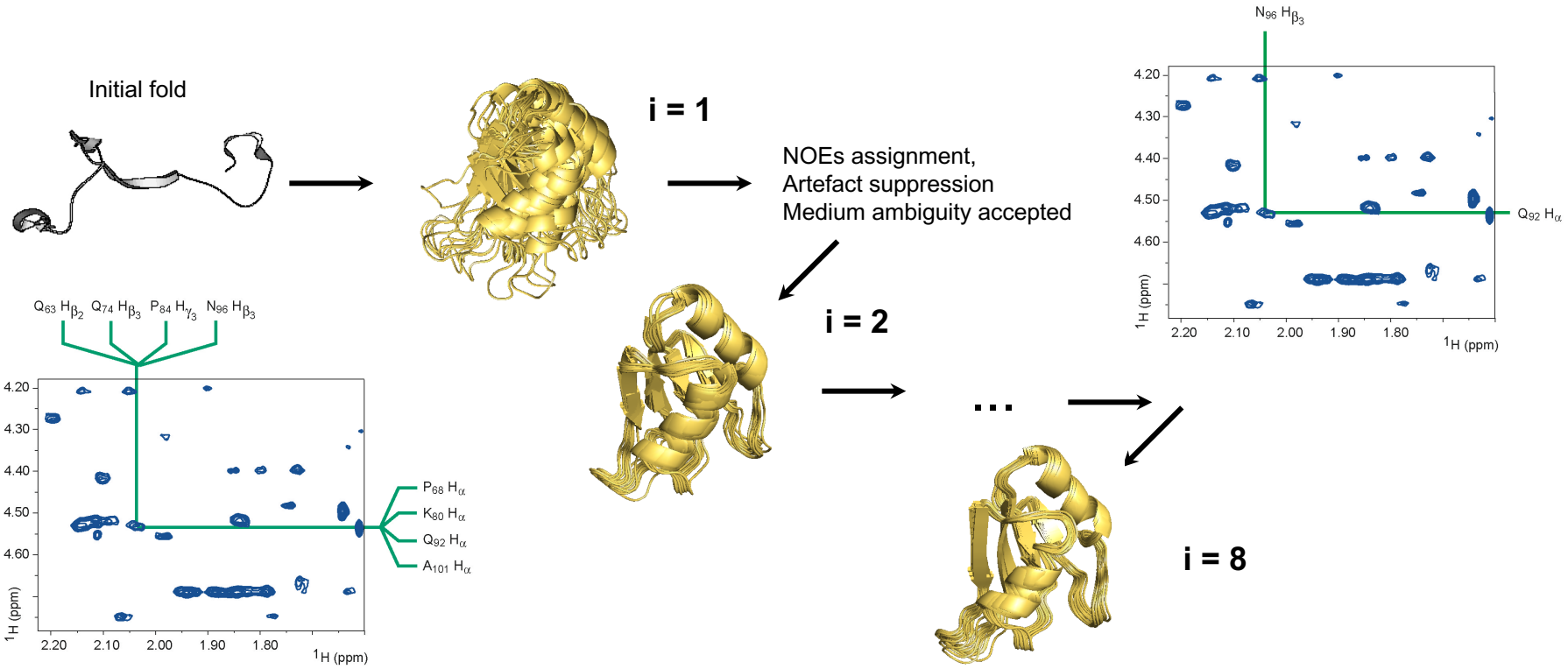
Optimization of assignment
Genetic algorithm combined with local optimization

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



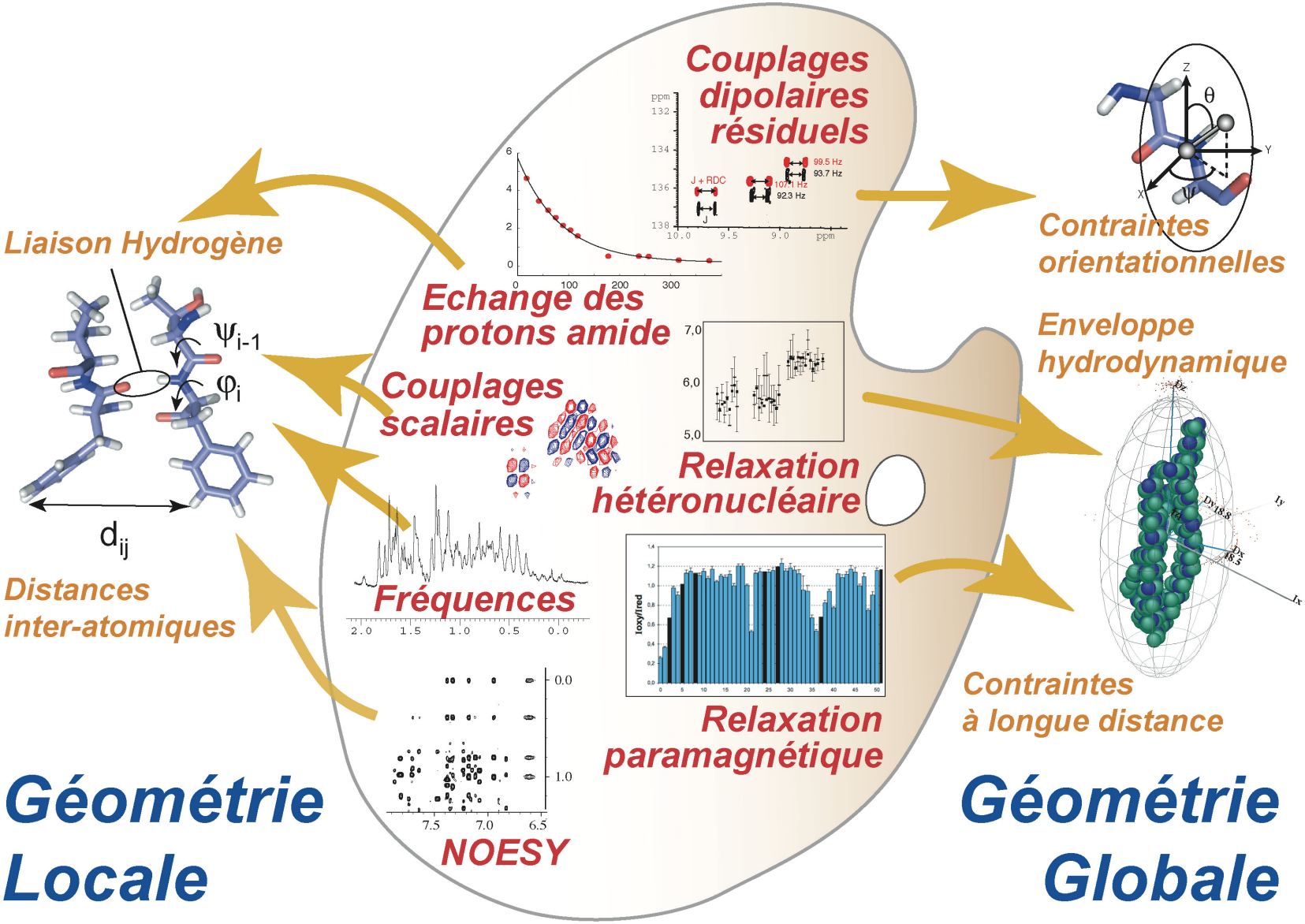
Software

ARIA, UNIO

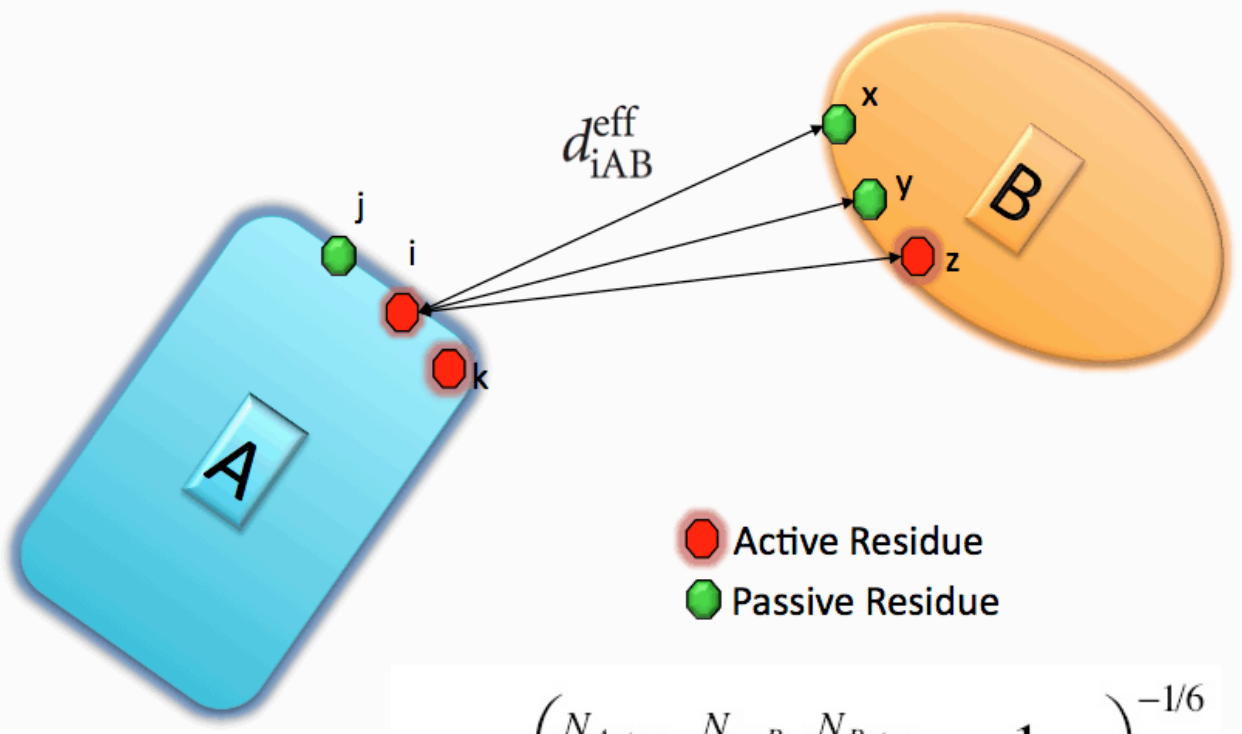
Rieping W., Habeck M., Bardiaux B., Bernard A., Malliavin T.E., Nilges M. (2007) ARIA2: automated NOE assignment and data integration in NMR structure calculation. Bioinformatics 23:381-382.

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

Many structural parameters



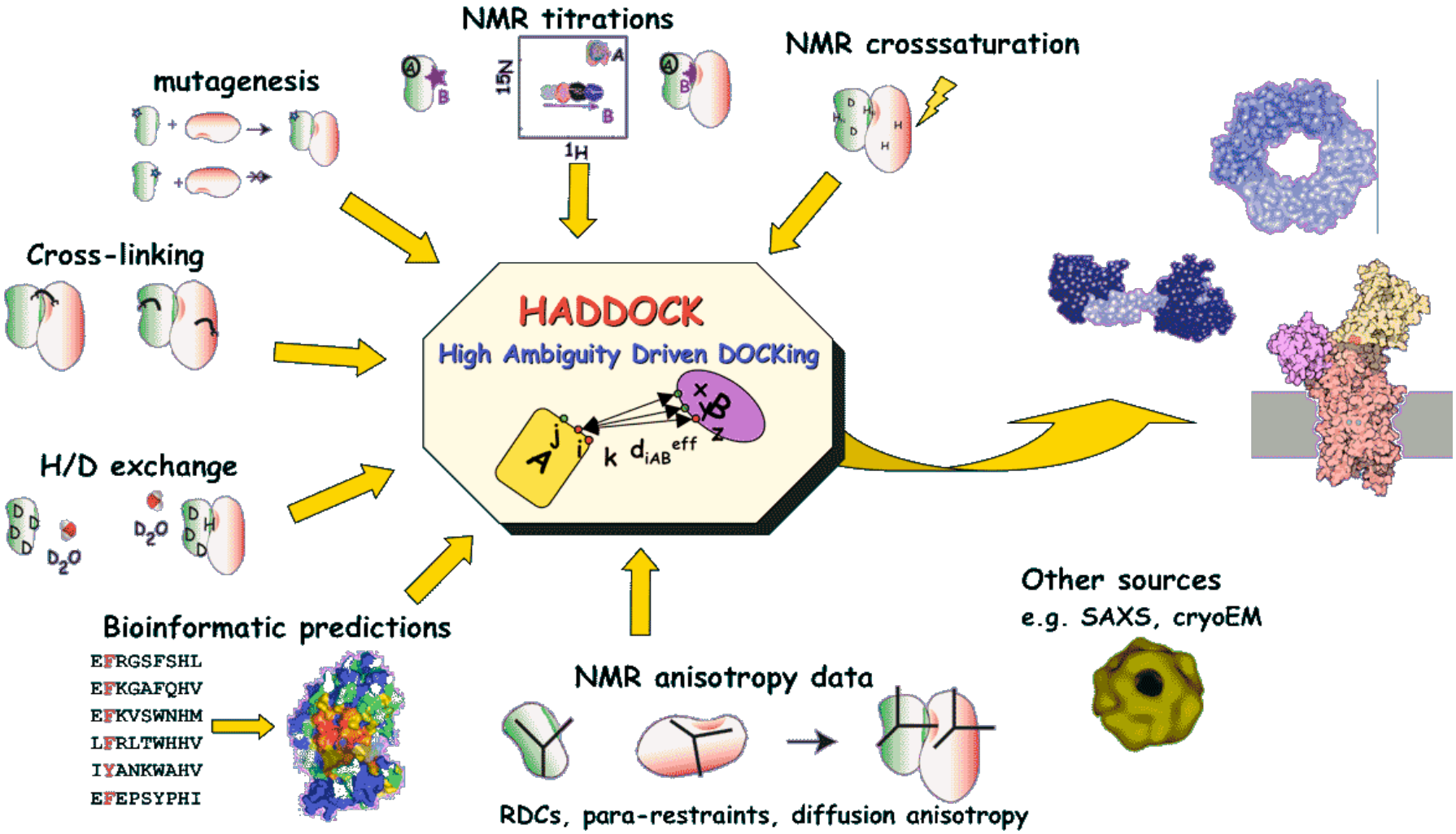
Use of Ambiguous Interaction Restraints for soft docking



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

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

Use of Ambiguous Interaction Restraints for soft docking

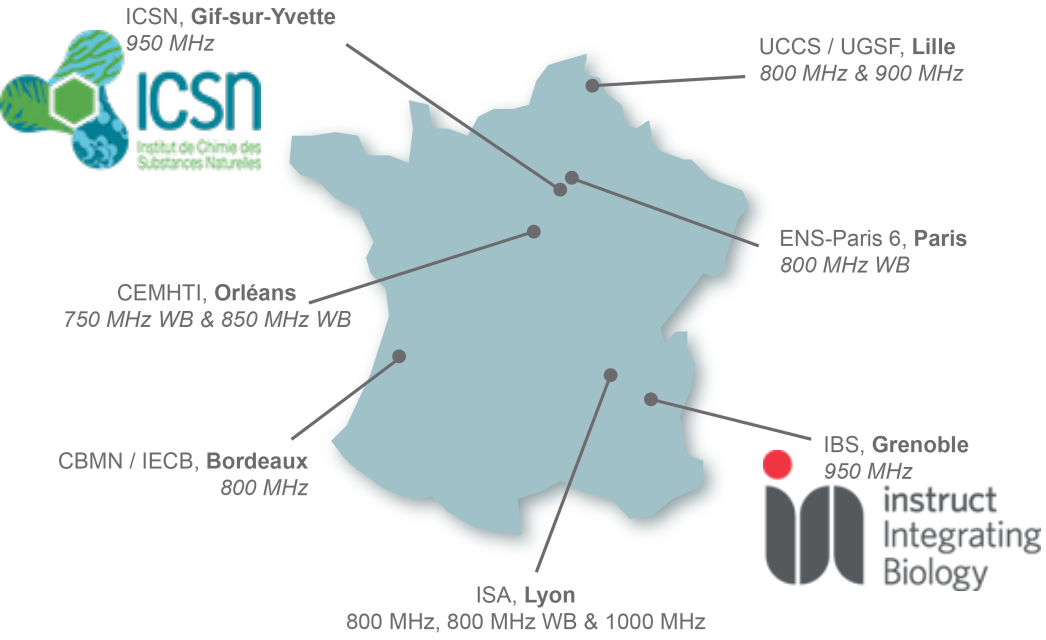


High-field NMR facility @ ICSN Gif sur Yvette

IR THC RMN

IBiSA

FRISBI Integrated Structural Biology



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