Integrative Structural Biology Summer School

21-28 June 2019 – Oléron, France

NMR spectroscopy: Major advances and future developments

Part 1: Liquid-state NMR

Catherine Bougault, IBS, Grenoble

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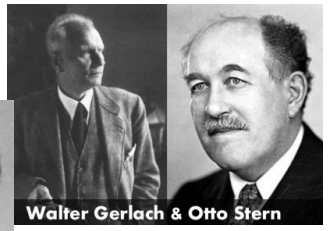
The early days of NMR

1922: Stern and Gerlach prove the existence of the spin of particles



1936: Rabi measures gyromagnetic ratios (Physics Nobel 1944)

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

1949: Chemical shift

1961: First commercial spectrometer, Varian A60

1965-1970: Fourier transform spectrometers
1972: Supraconducting coils

1971-1975: 2D-NMR idea (J. Jeener) + developments (Ernst, Chemistry Nobel 1991)

1971-1973: NMR of tissues (Damadian / Lauterbur and Mansfield, Medicine Nobel 2003) – MRI, tomography
1973-1977: MAS and CP developments for solid-state NMR

Biomolecular NMR : 35-40 years of 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/BEST (B. Brutscher)

IDP
Solids

Invisible
States (L. Kay)

1980 — **500 MHz**

1990 — **800 MHz**

2000 — **900 MHz**

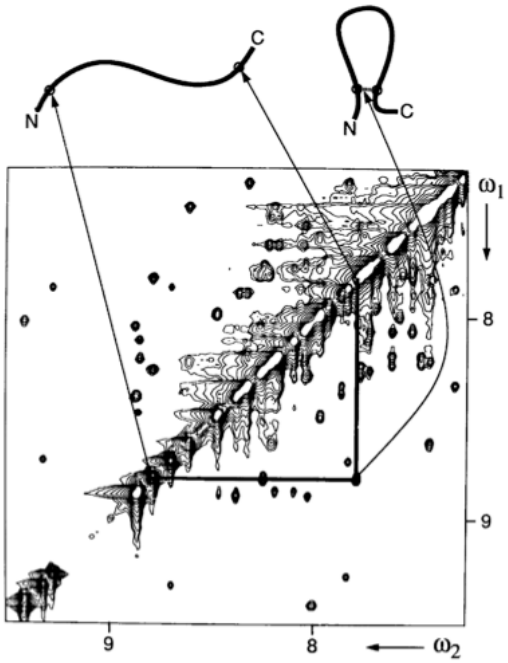
2010-20 — **1-1.2 GHz**

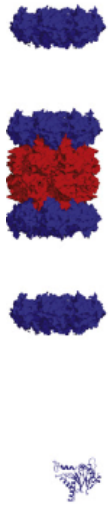
Cryo-probes

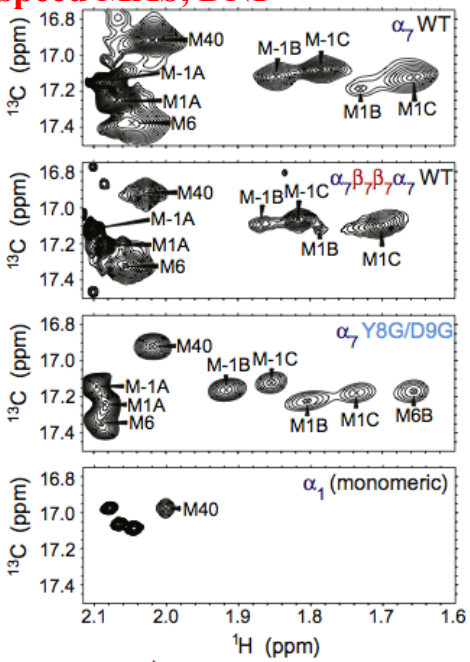
High-speed MAS, DNP

Methyl labeling
(L. Kay)

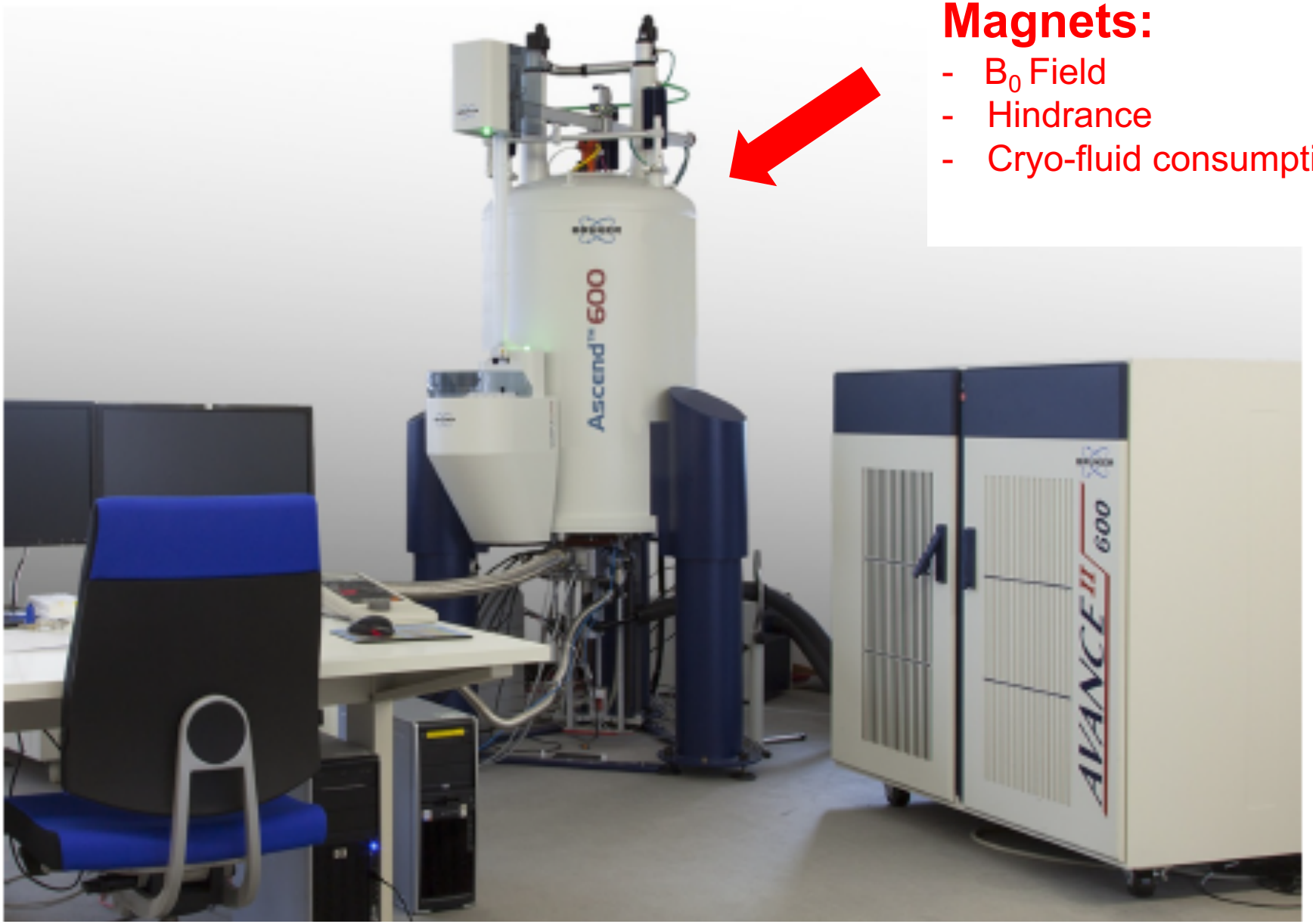
RDC
(J Prestegard,
A. Bax)
SAR (S. Fesik)







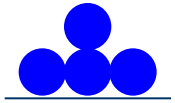
Technological innovations and developments



Magnets:

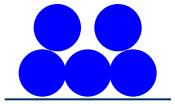
- B_0 Field
- Hindrance
- Cryo-fluid consumption

NMR, an intrinsically low sensitivity



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

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



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

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

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

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

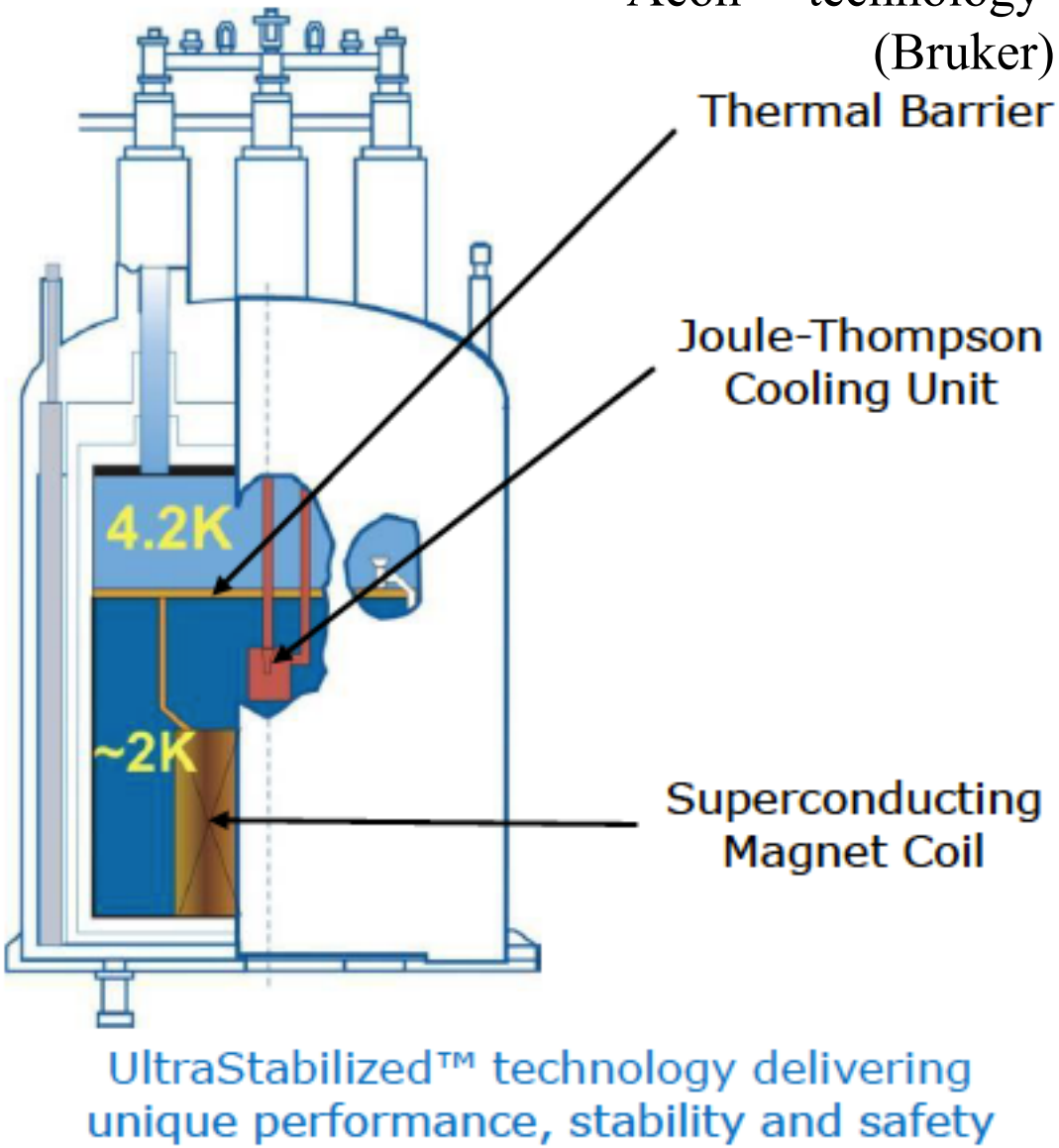
Particular case of spin 1/2

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

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

Technological innovations

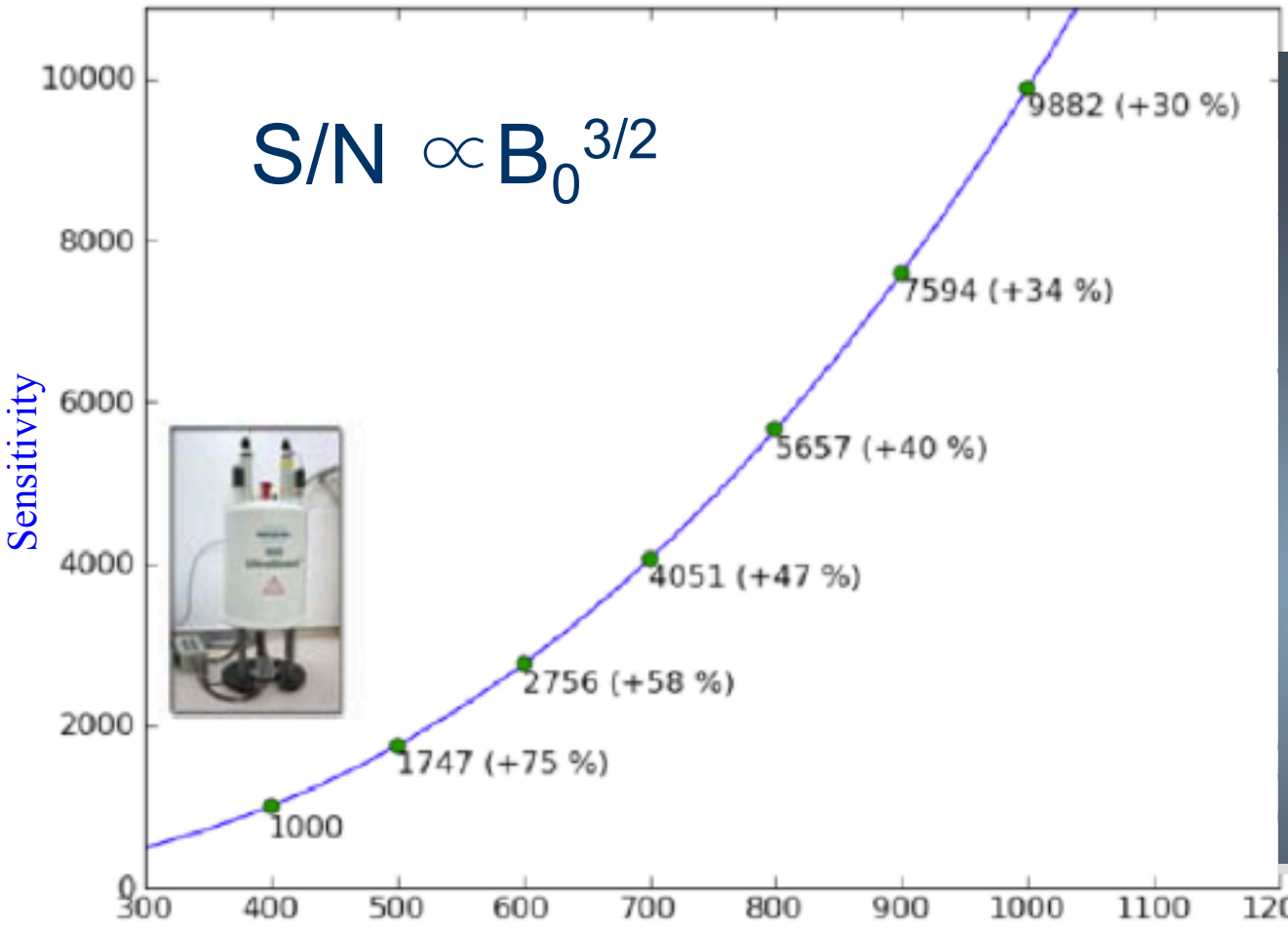
Aeon™ technology (Bruker)



UltraStabilized™ technology delivering unique performance, stability and safety

High and Low Temperature Superconductors

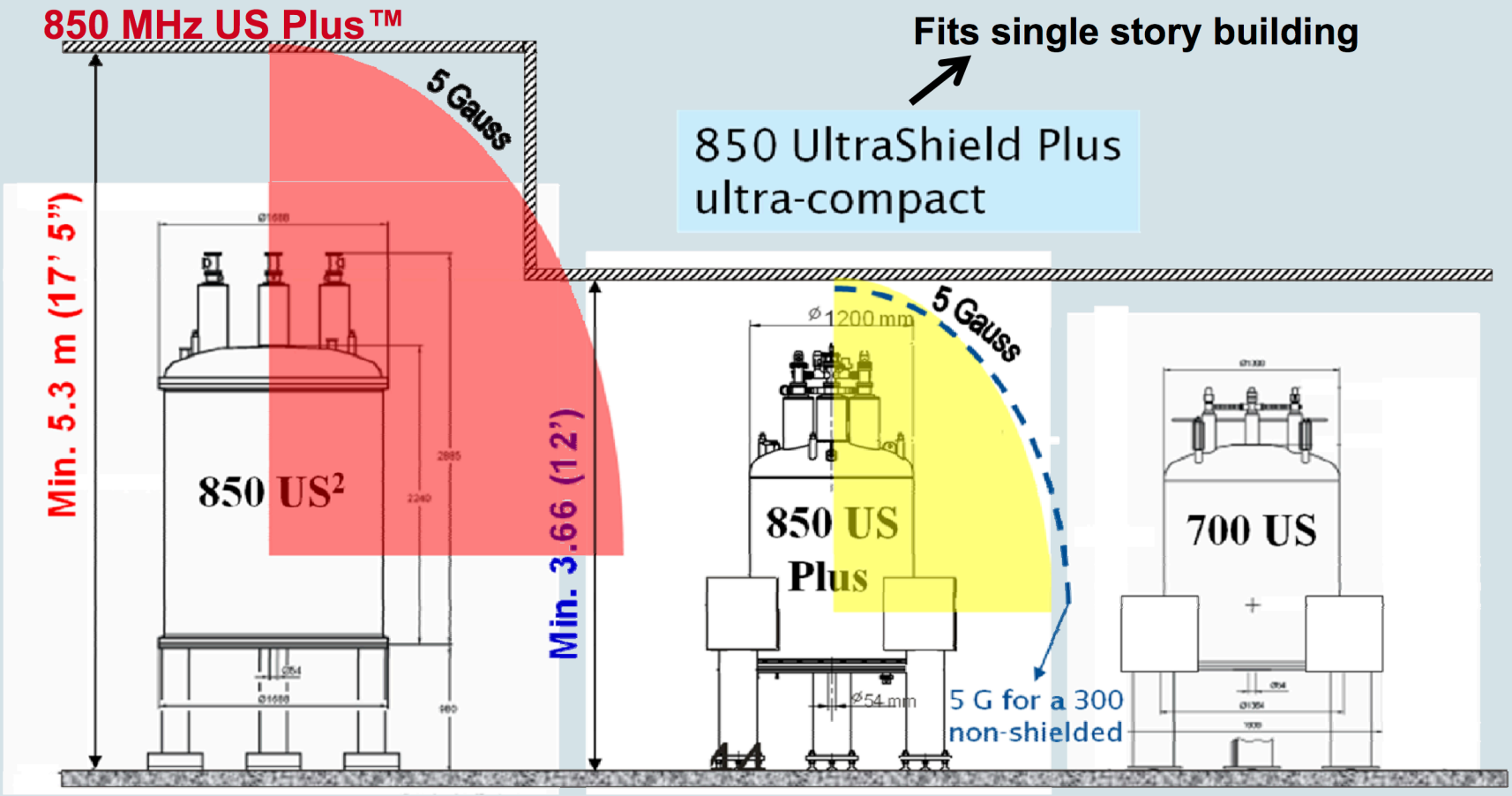
Technological innovation: spectrometer



	1970	1980	1990	2000	2006	2009	2020
Magnetic field	200-300	500	600-700	900	950	1000	1200
(MHz / T)	4.70-5.87	11.74	14.09-16.44	21.14	22.31	23.5	28.2

Technological innovations

Magnets become more compact

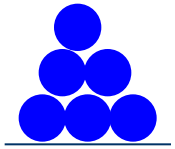


- **Compact size and small stray field improve siting flexibility**
- **Outstanding stability and high-resolution NMR performance**

Data courtesy of Bruker

NMR, overcoming Boltzman limitations

Sensitivity or signal-to-noise ratio



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

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

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

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

Spins 1/2

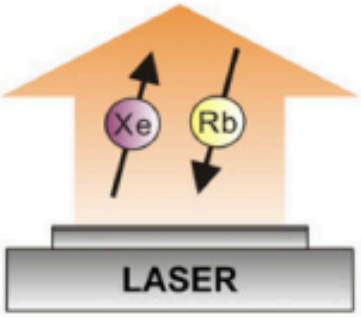
Alternatives to increase Boltzmann?

At a given field

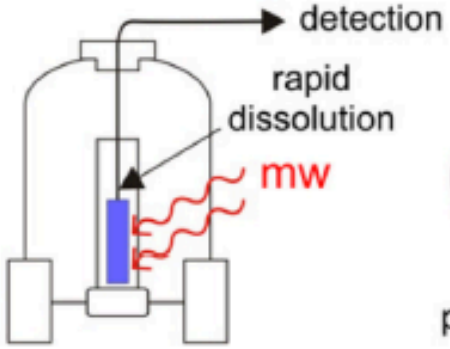
$$\frac{N_\alpha}{N_\beta} >> e^{\frac{E_\beta - E_\alpha}{k_B T}}$$

NMR, overcoming Boltzman limitations

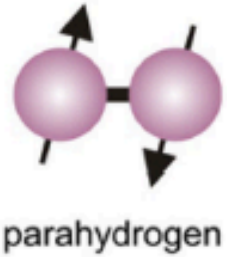
Optical Pumping



Dissolution DNP



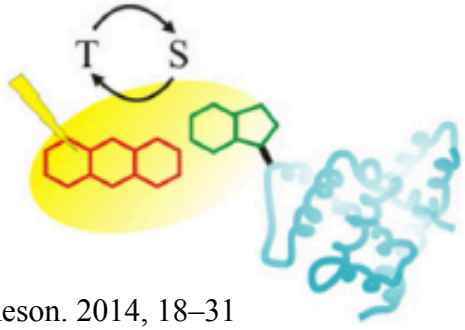
PHIP



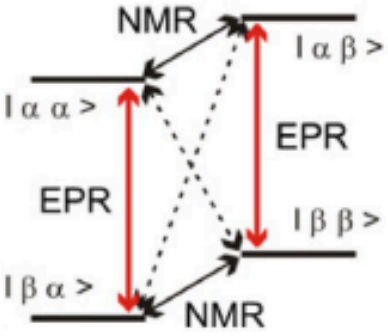
Not biomolecular

Methods that overcome the Boltzmann limit

Photo-CIDNP



Overhauser DNP

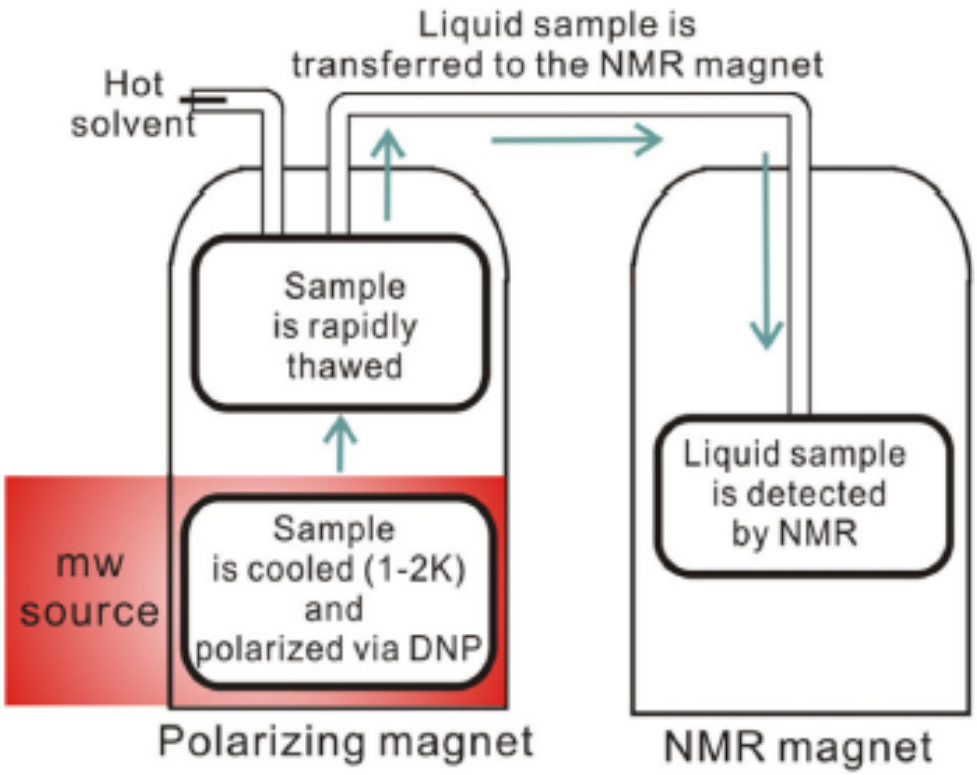


⇒ See talk by Robert on solid-state DNP

Jung Ho Lee et al., J Magn Reson. 2014, 18–31
doi:10.1016/j.jmr.2014.01.005.

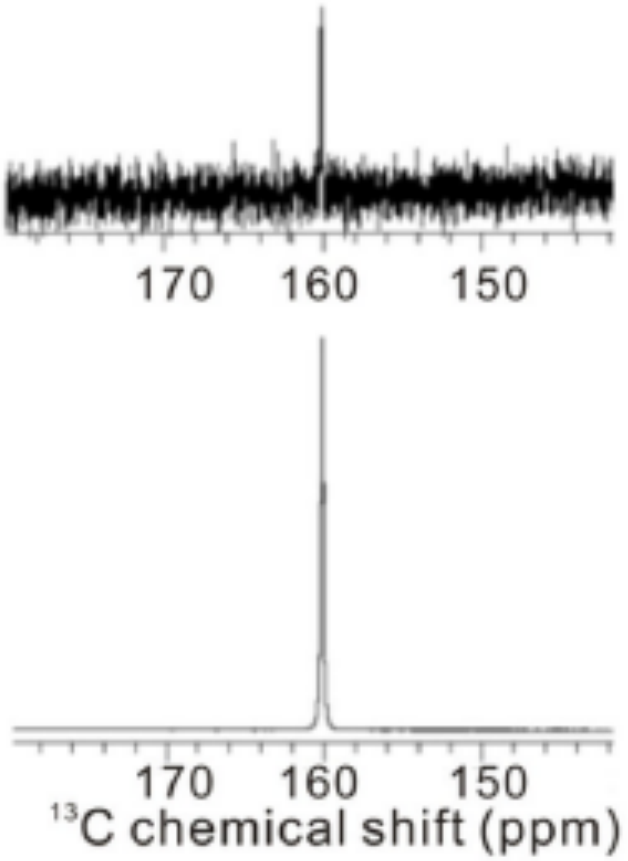
Dissolution DNP, as an alternative

Must be faster than T_1
Kept at low field



TEMPO
as polarizing agents

Usually limited to 1D NMR



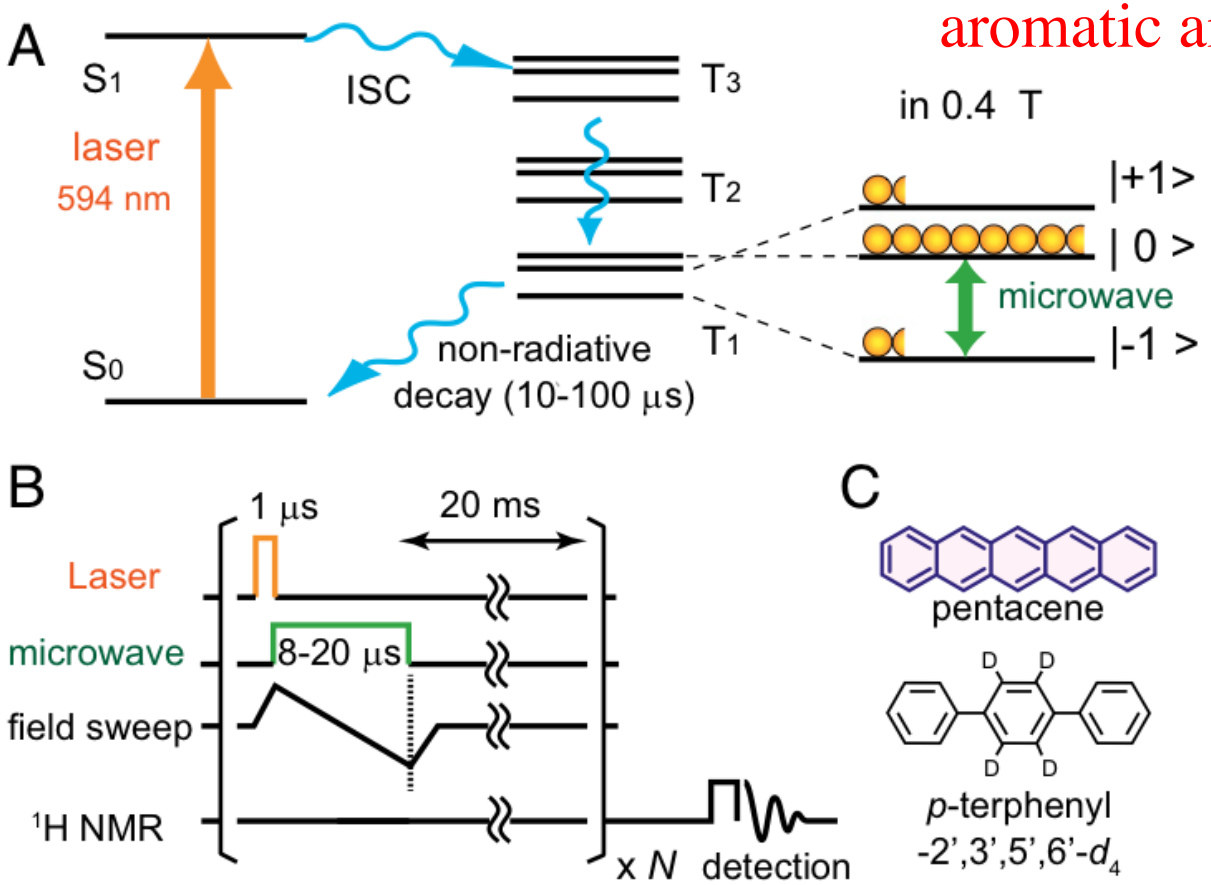
^{13}C S/N 44,400-fold larger

Kim Y et al., Methods Enzymol. 2019, 501-526

Photo-chemically induced DNP (Photo-CIDNP)

Enhances sensitivity of aromatic amino acids

Formation of radicals in-situ



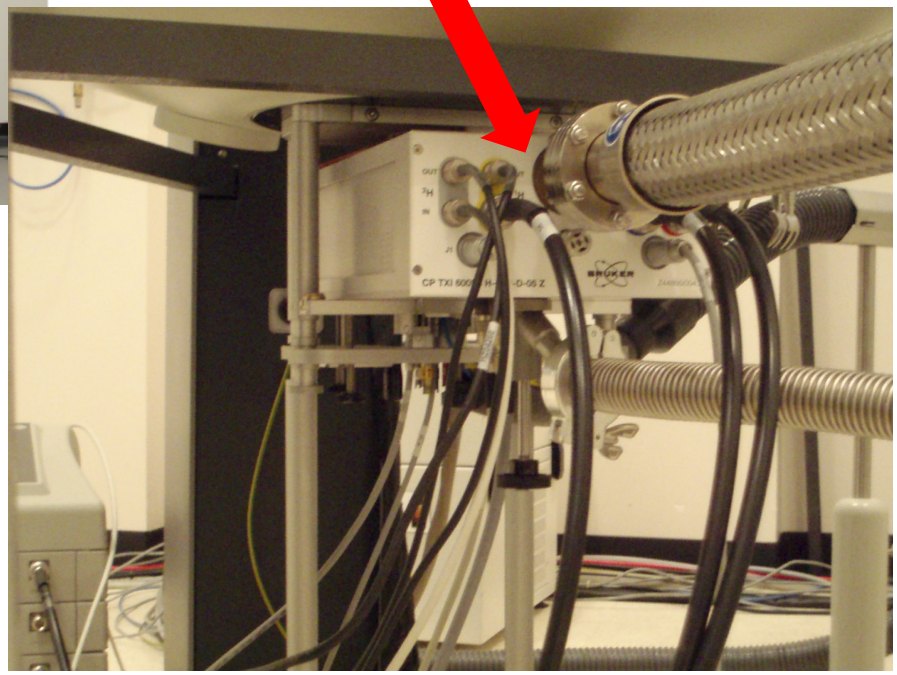
Applications in protein folding and biomolecular interaction

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

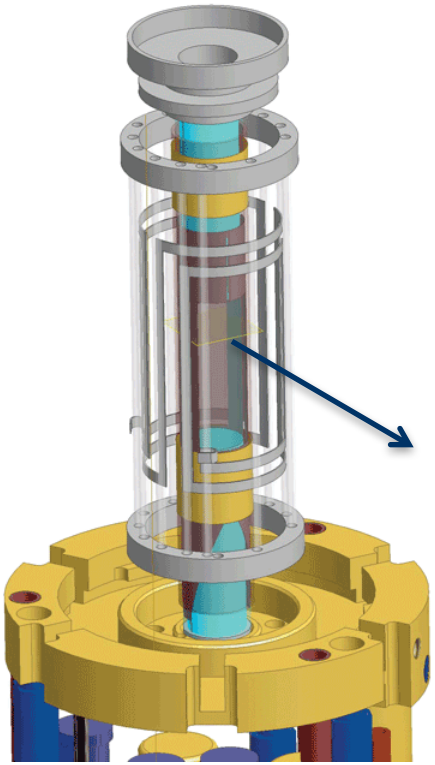
Technological innovations

Probes:

- Cryoprobes
- Small volume probes
- Multi-nuclei probes



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

Receiver coil at 80 K, pre-amplifier at 20 K

Coil Design

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

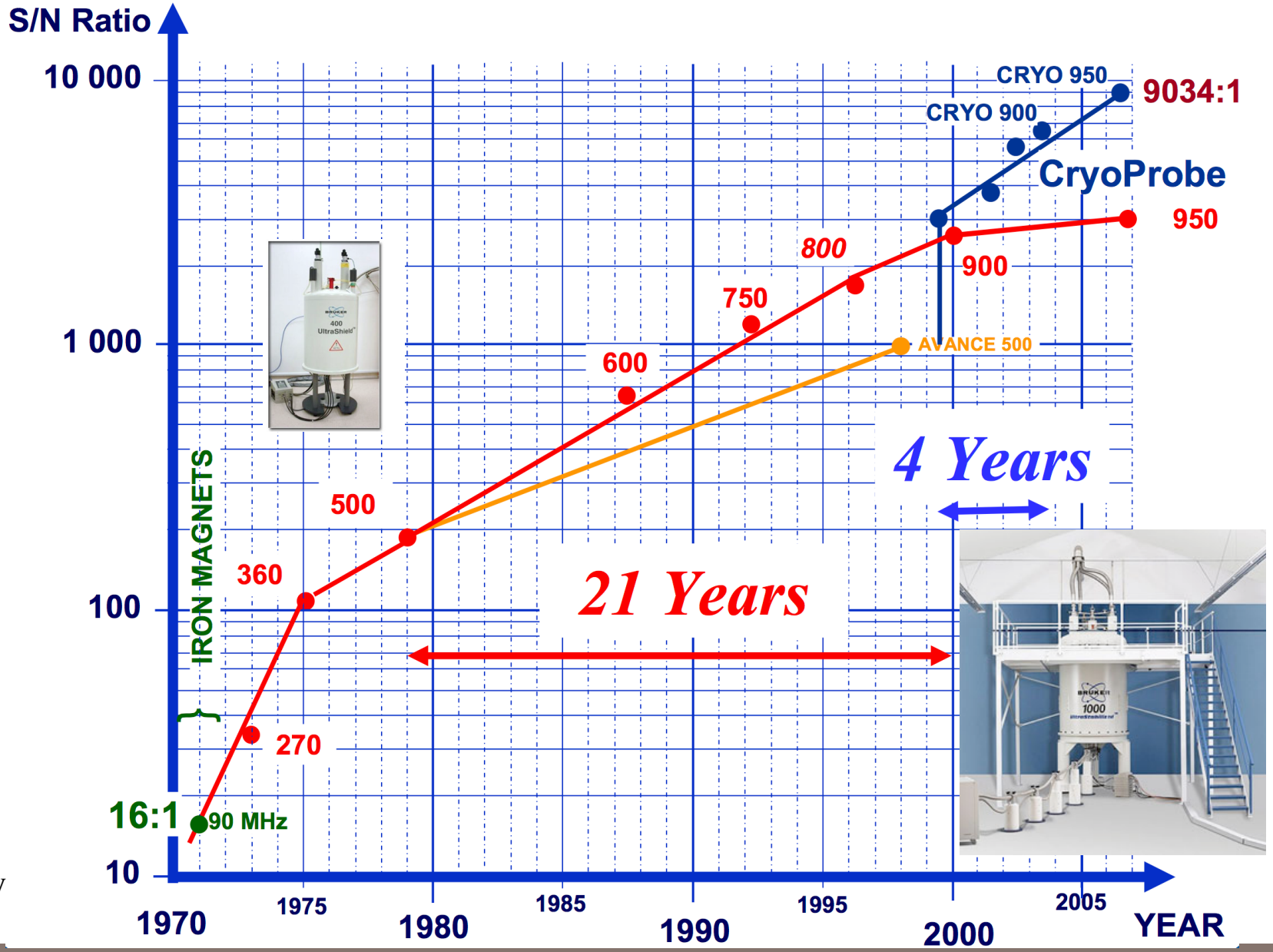
Coil Resistance

Temperature

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

Q quality factor, η filling factor

Technological innovation with cryoprobes

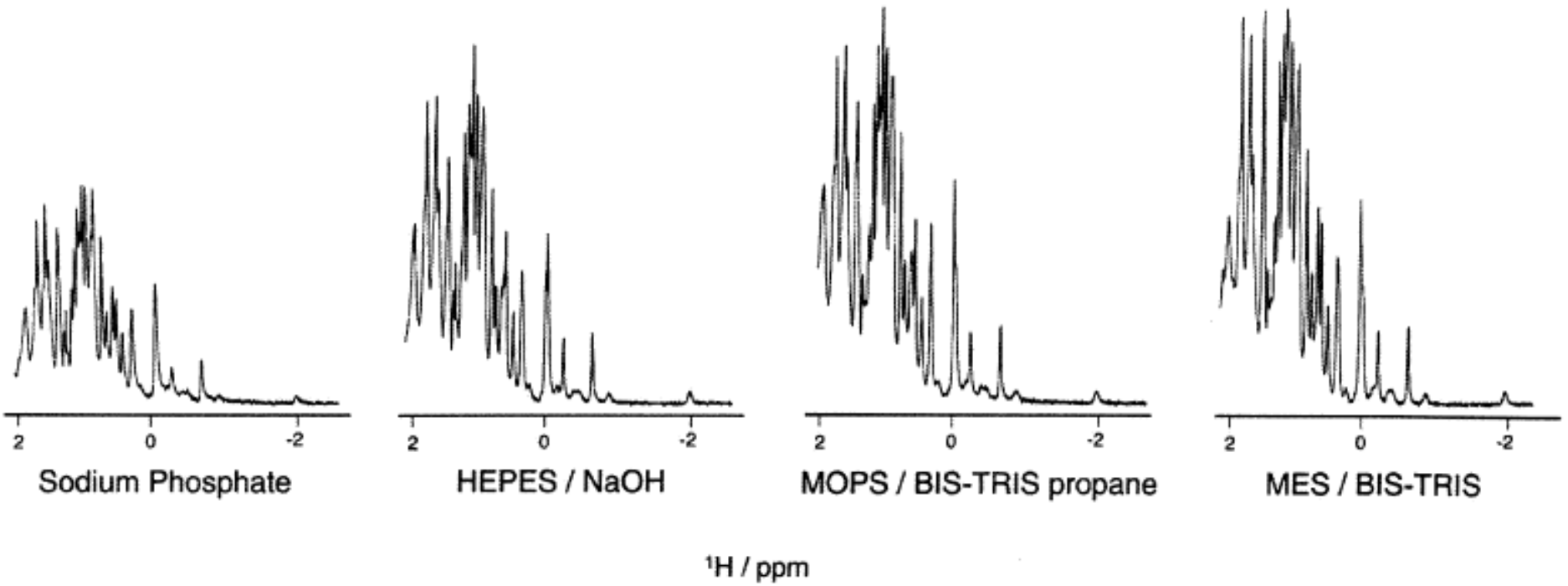


Data courtesy of Bruker

Limitation of cryoprobes

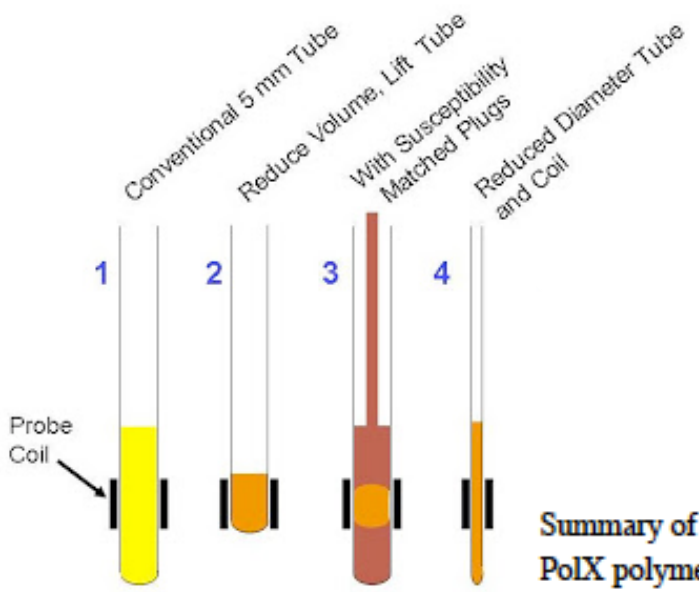
Low-Conductivity Buffers for High-Sensitivity NMR Measurements

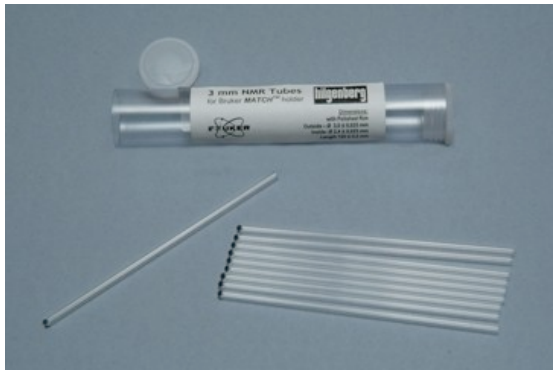
Alexander E. Kelly,† Horng D. Ou,† Richard Withers,‡ and Volker Dötsch*,§



JACS, 2002

Limitations of cryoprobes





1.7 mm cryoprobe
30 µL sample volume
Liquid-state NMR

At constant conc.

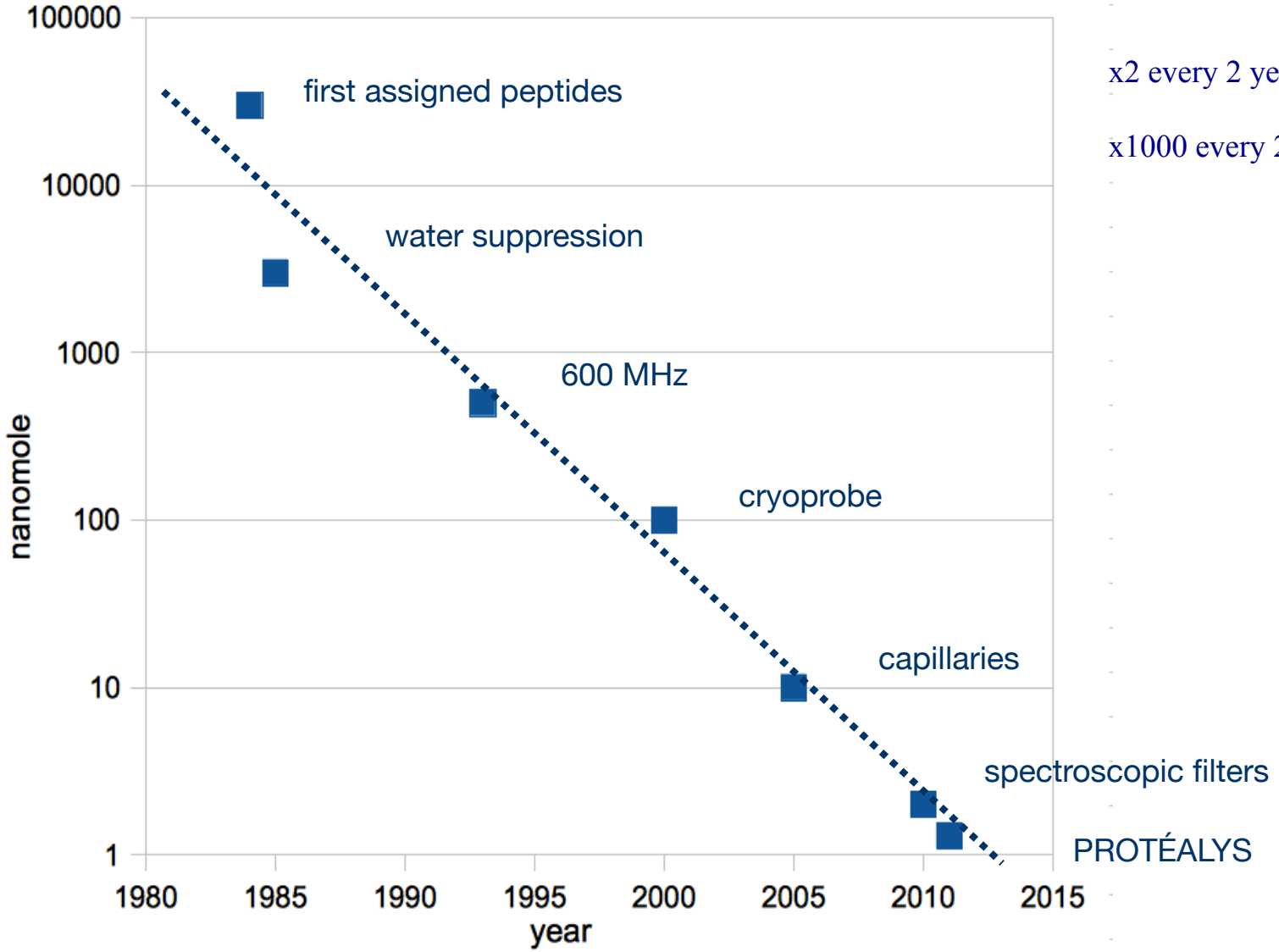
At constant quant. of matter

Summary of experimental conditions and results comparing ^{1}H–^{15}N HSQC spectra measured on a 22 kDa PolX polymerase in different NMR tubes

(a) Tube	5 mm	4 mm	3 mm	5 mm Shigemi
Total sample volume (µL)	500	300	160	250
Sample amount in active volume (nmol)	107.4	62.7	35.0	118.8
Estimated sensitivity gain/loss based on the sample amount in the active volume (%)	—	−41.6	−67.4	+10.6
π/2 ^{1}H pulse (µs)	16.32	12.65	10.10	16.74
Noise estimate	233,540	198,630	156,174	229,692
Median of sensitivity change (%)	—	−7.5	−37.0	+15.2
(b) Tube	**5 mm**	**4 mm**	**3 mm**	**5 mm Shigemi**
Total sample volume (µL)	500	300	160	250
Sample amount in active volume (nmol)	36.2	35.2	36.8	80
Estimated sensitivity gain/loss based on the sample amount in the active volume (%)	—	−2.7	1.6	+120
π/2 ^{1}H pulse (µs)	16.25	12.23	10.09	16.83
Noise estimate	207,687	176,990	151,863	214,687
Median of sensitivity change (%)	—	+52.3	+104.8	+114.1

Gain with a cryoprobe

Quantity of protein detected



x2 every 2 years

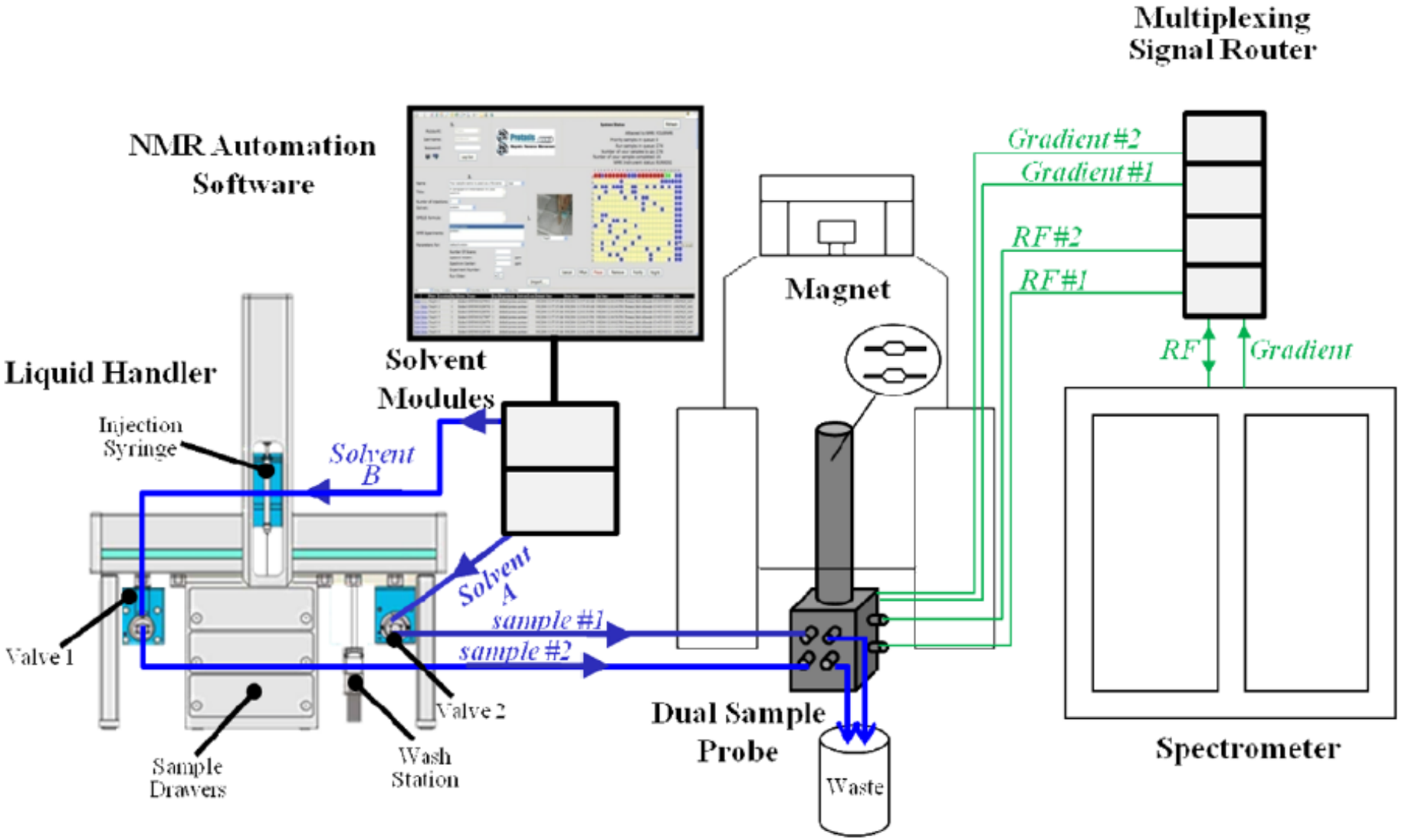
x1000 every 20 years

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

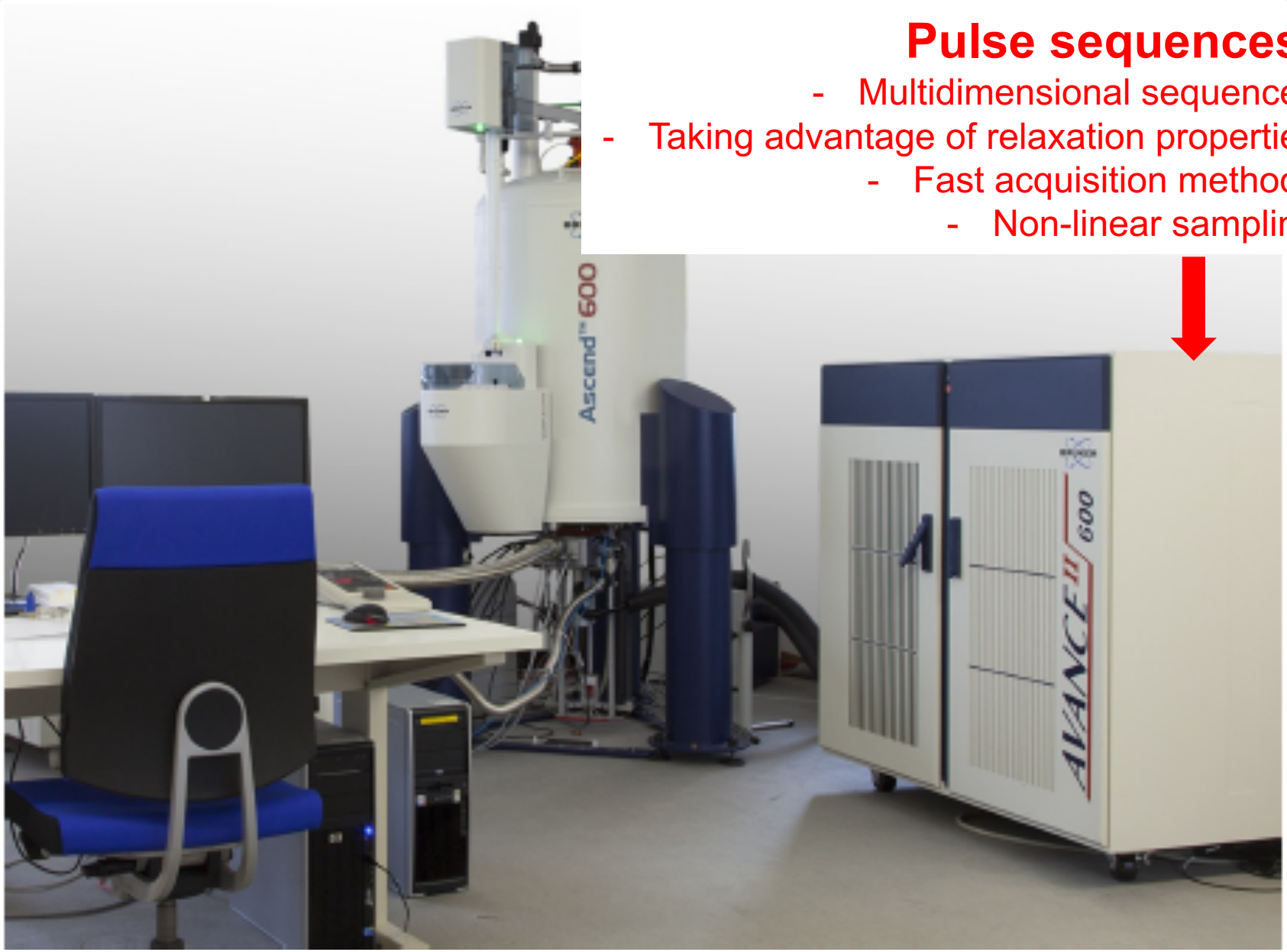
James A. Norcross†, Craig T. Milling†, Dean L. Olson†, Duanxiang Xu†, Anthony Audrieth†, Robert Albrecht†, Ke Ruan§, John Likos§, Claude Jones§, and Timothy L. Peck*,†



Methodological innovations

Pulse sequences:

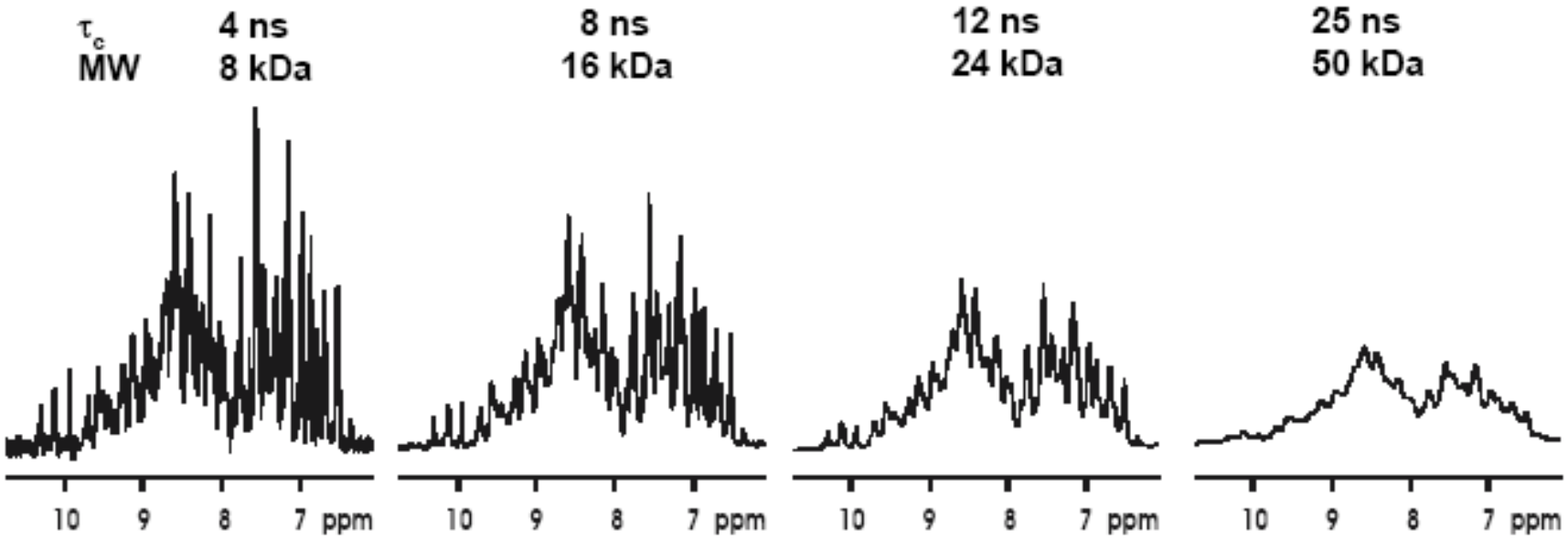
- Multidimensional sequences
- Taking advantage of relaxation properties
- Fast acquisition methods
- Non-linear sampling

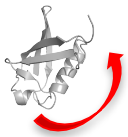


Sensitivity enhancement by attenuation of T_2 effects

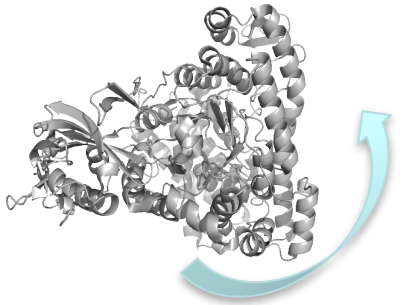
Liquid-state NMR a serious limit?

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





fast overall rotation

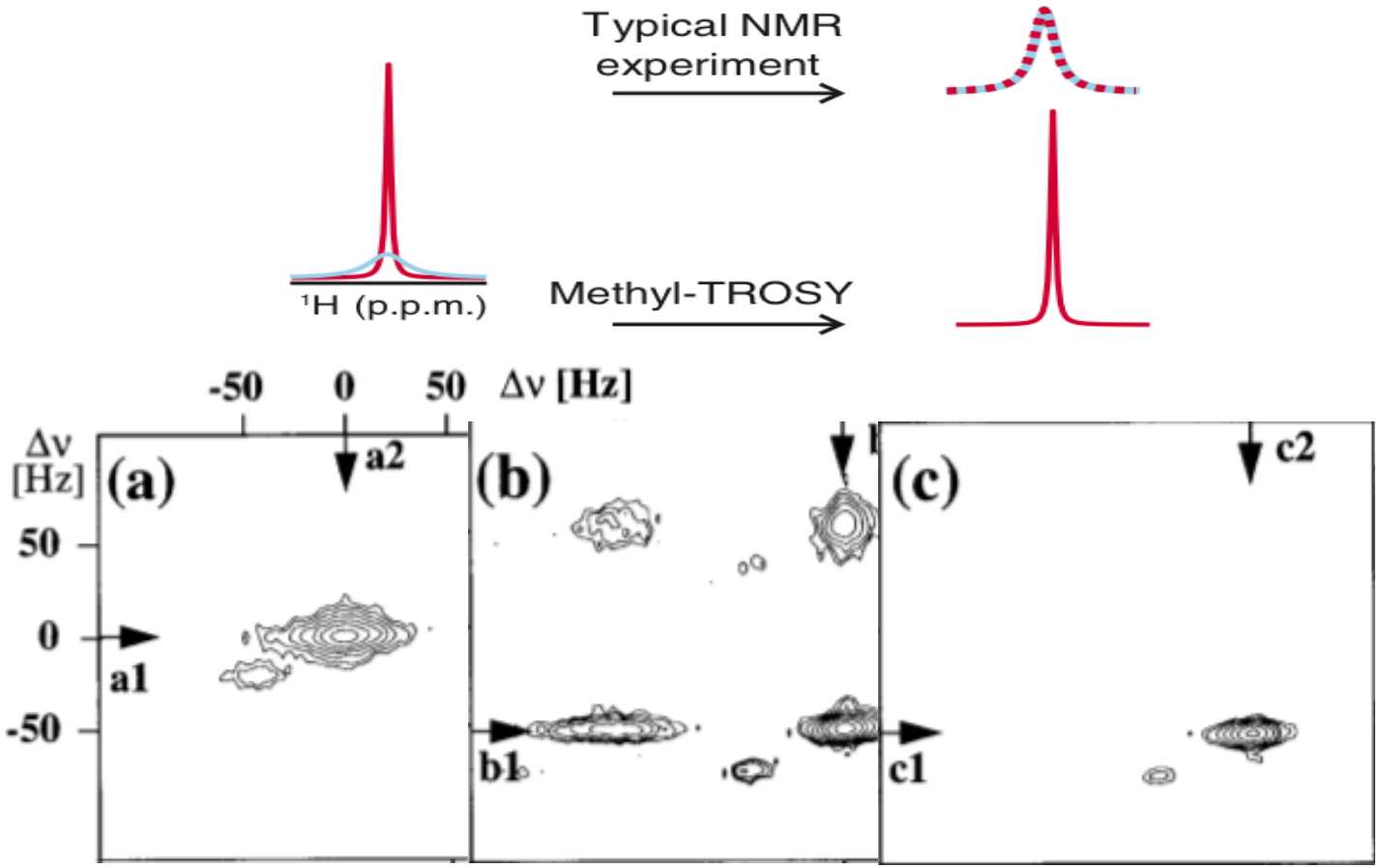


slow overall rotation

Sensitivity enhancement by attenuation of T_2 effects

1. Transverse relaxation:

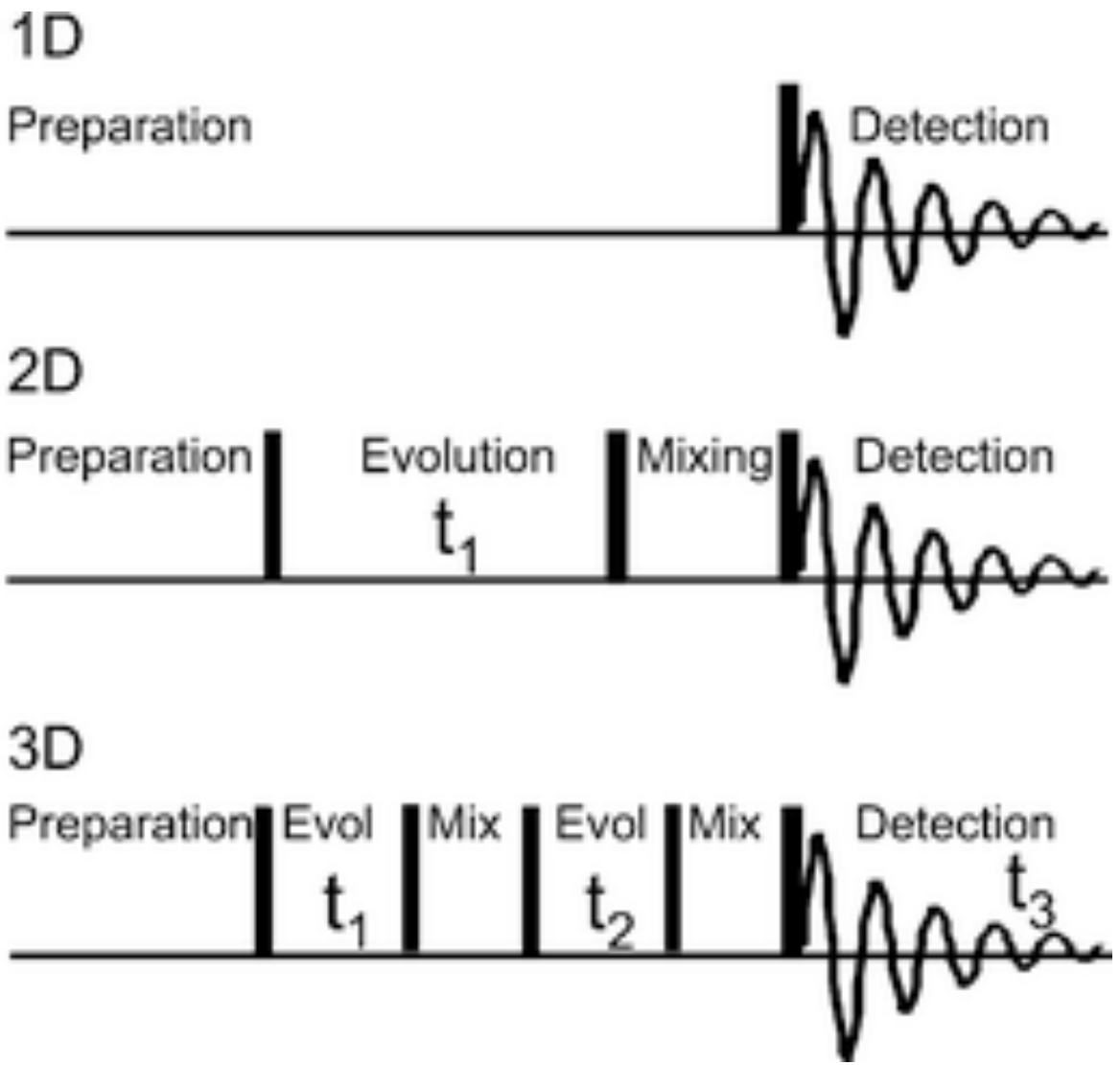
Canceling opposite relaxation mechanisms (CSA-DD) => TROSY



Salzmann et al., 110-kDa protein , J Am Chem Soc. 2000, 7543–7548

Sensitivity enhancement via fast-pulsing techniques

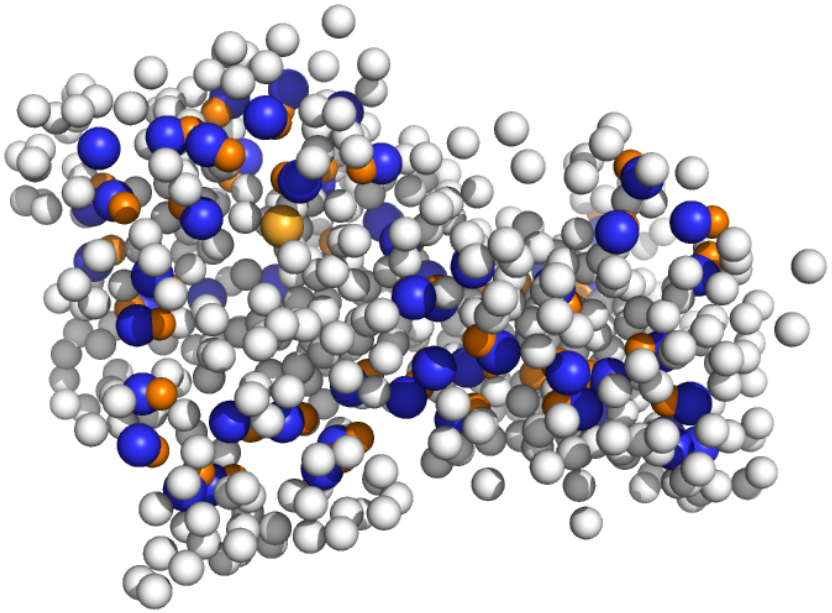
Data acquisition is full of dead times

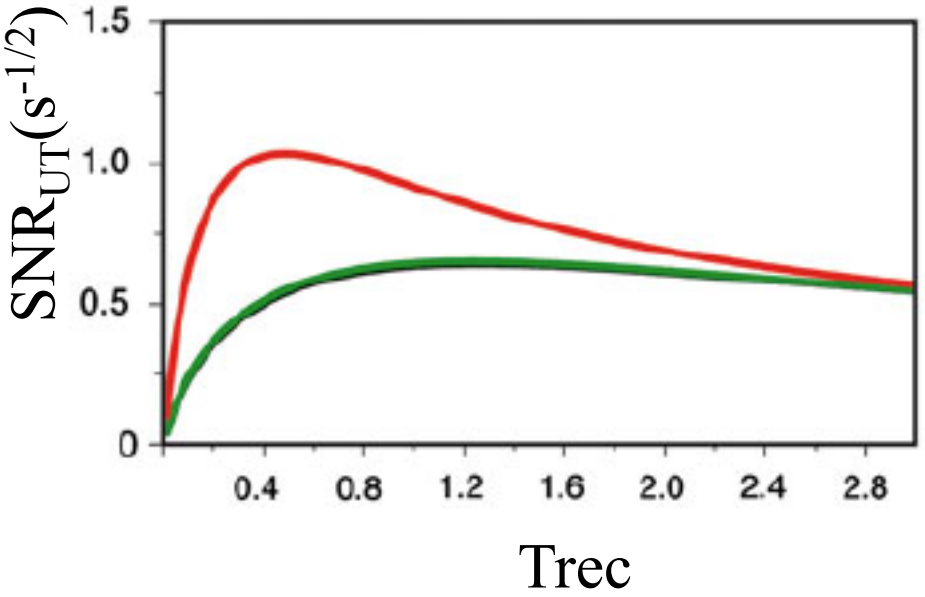


Sensitivity enhancement via fast-pulsing techniques

2. Longitudinal relaxation:

Only flip amideprotons and keep rest of protonsalong the z-axis to fasten T_1-relaxation, Ernst angle excitation => SOFAST, BEST, BEST-TROSY

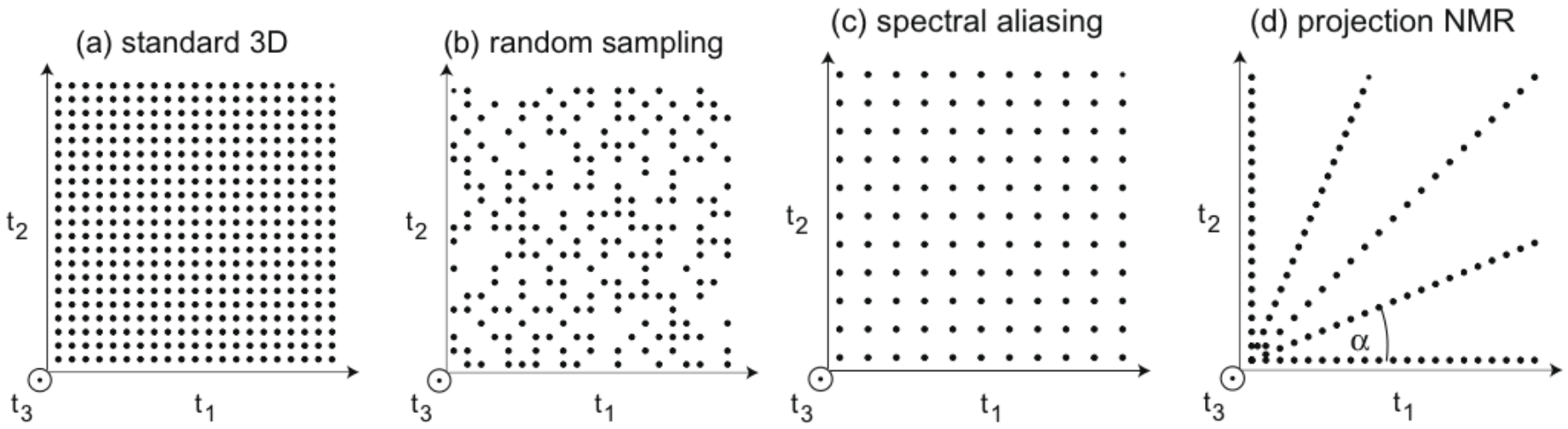




Solyom Z, Schwarten M, Geist L, Konrat R, Willbold D, Brutscher B. J Biomol NMR. 2013 Apr;55(4):311-21.

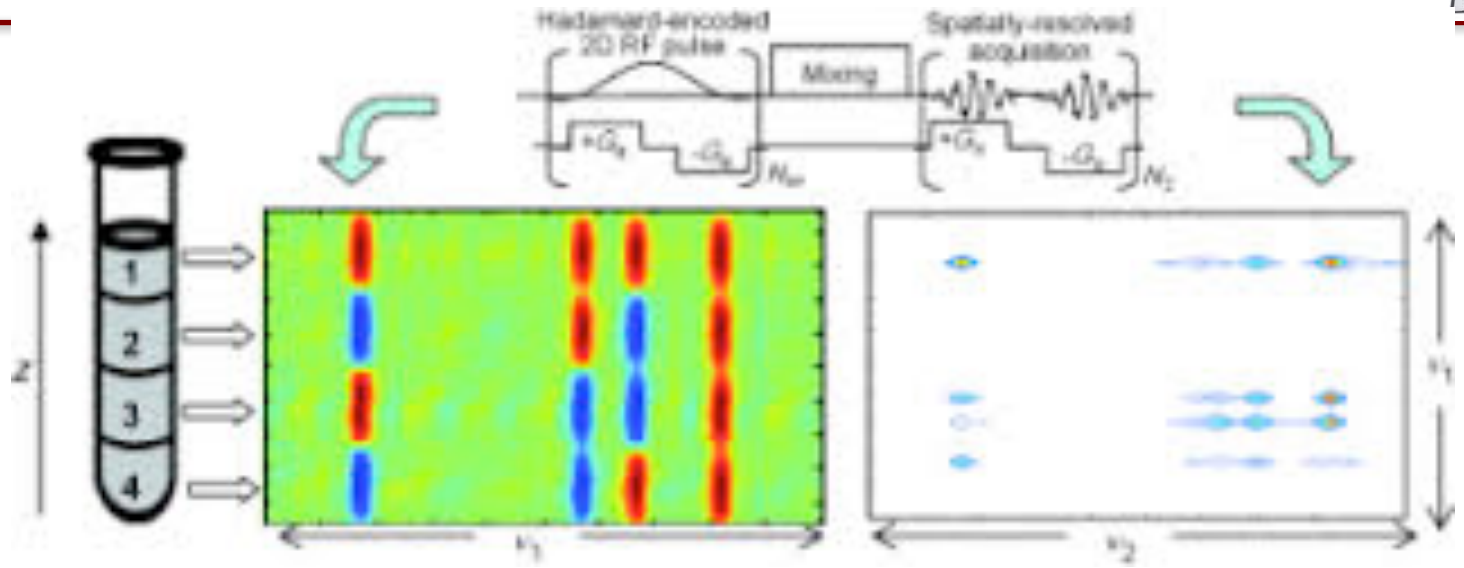
Alternative sampling methods

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

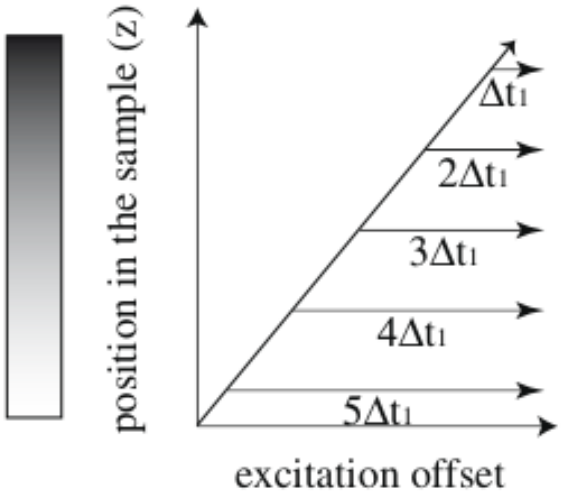


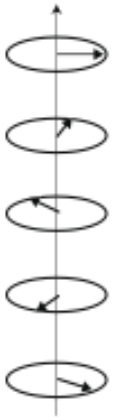
M. Mobli and J.C. Hoch Progress in Nuclear Magnetic Resonance Spectroscopy 83 (2014) 21–41

Alternative sampling methods

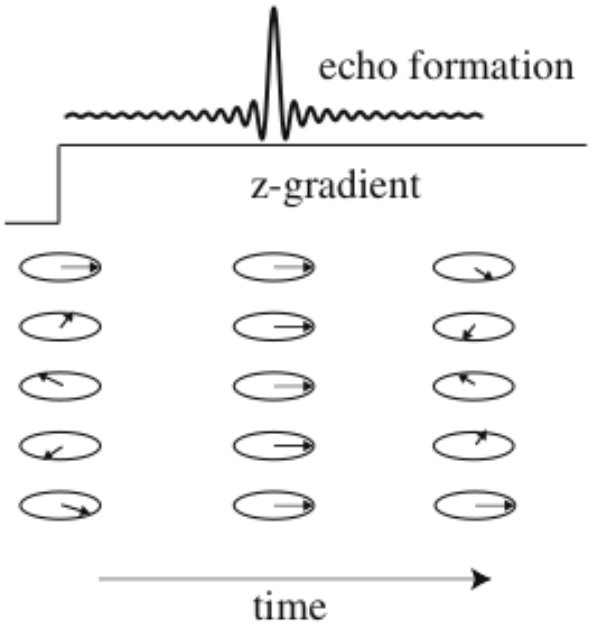


(1) space-encoded excitation (2) (3) gradient-assisted aquisition





spatially homo-geneous mixing



Single-scan spectroscopy

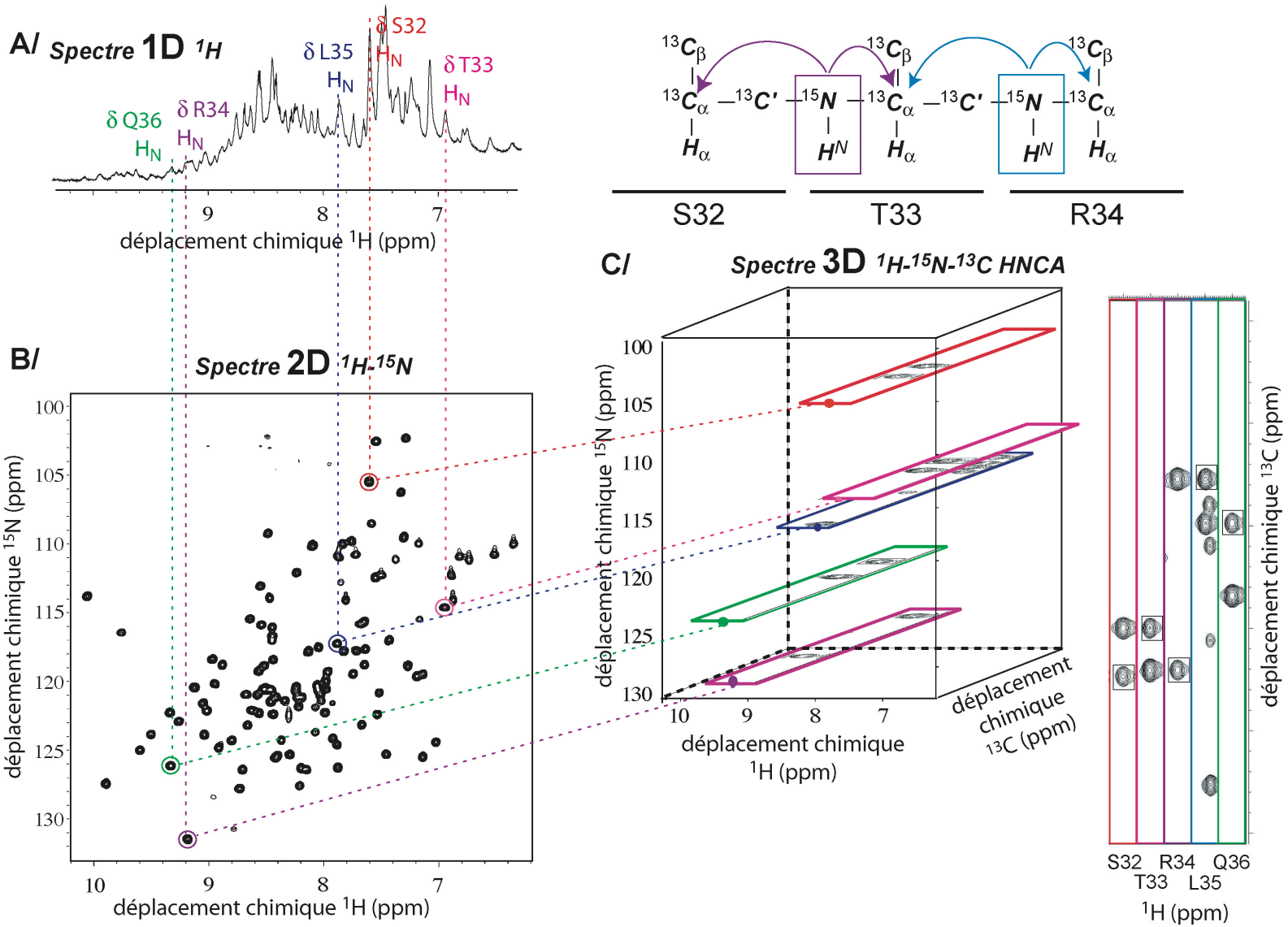
Frydman L, Scherf T, Lupulescu A. PNAS. 2002

Technological innovations

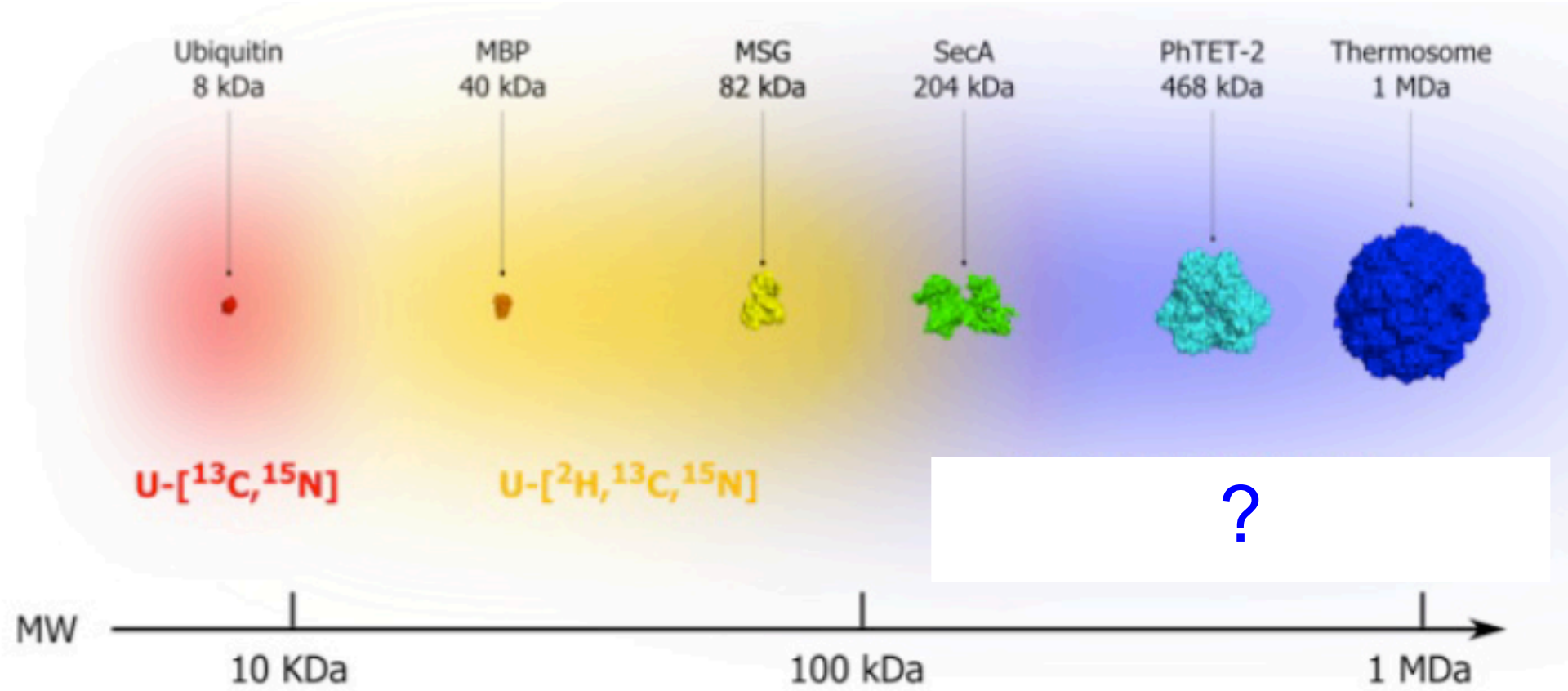
Samples :

- Small volumes
- Isotopic labeling

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



Is NMR limited to small biomolecules?

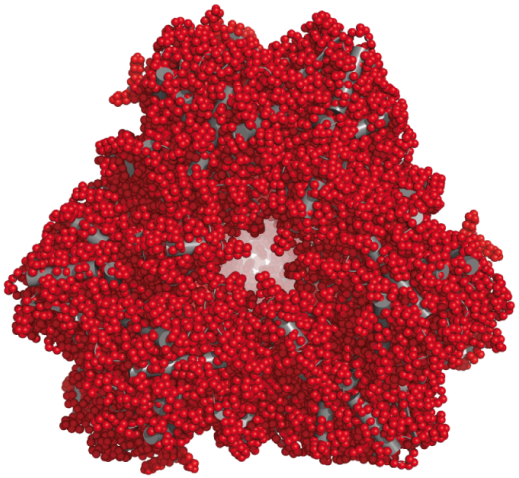


Structure of the Box CD enzyme, a 386-kDa complex solved by NMR (PDB 4BY9)

Lapinalte et al., Nature 2013

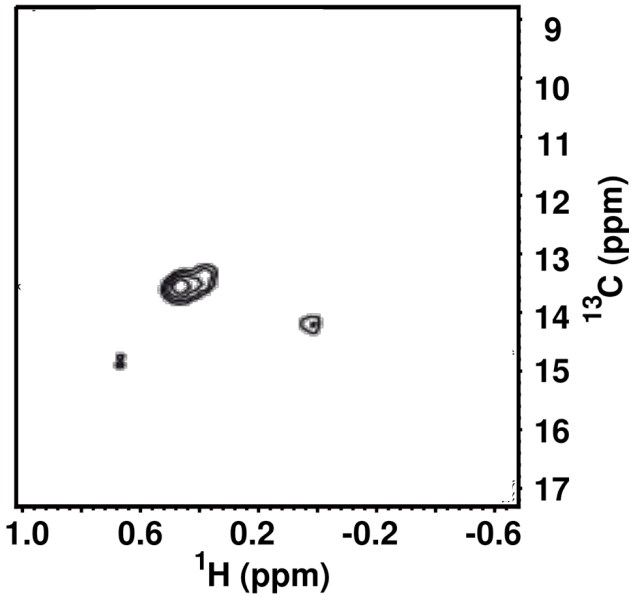
Figure courtesy of J. Boisbouvier

Can we investigate large machineries with NMR?

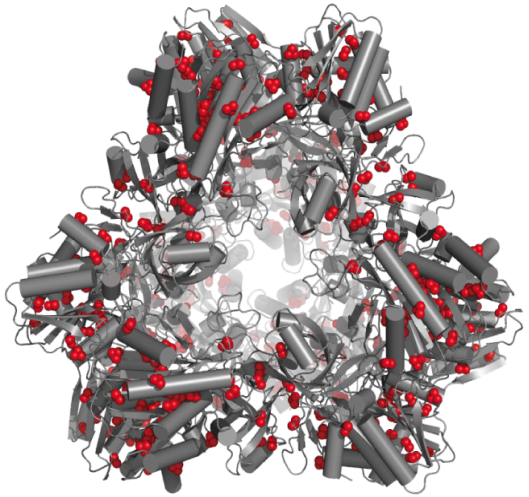


100% protonation

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

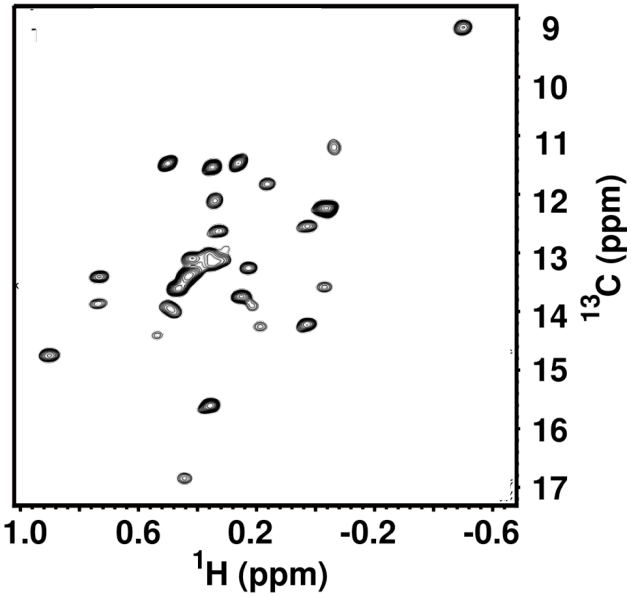


TET -468 kDa
12 x 39 kDa

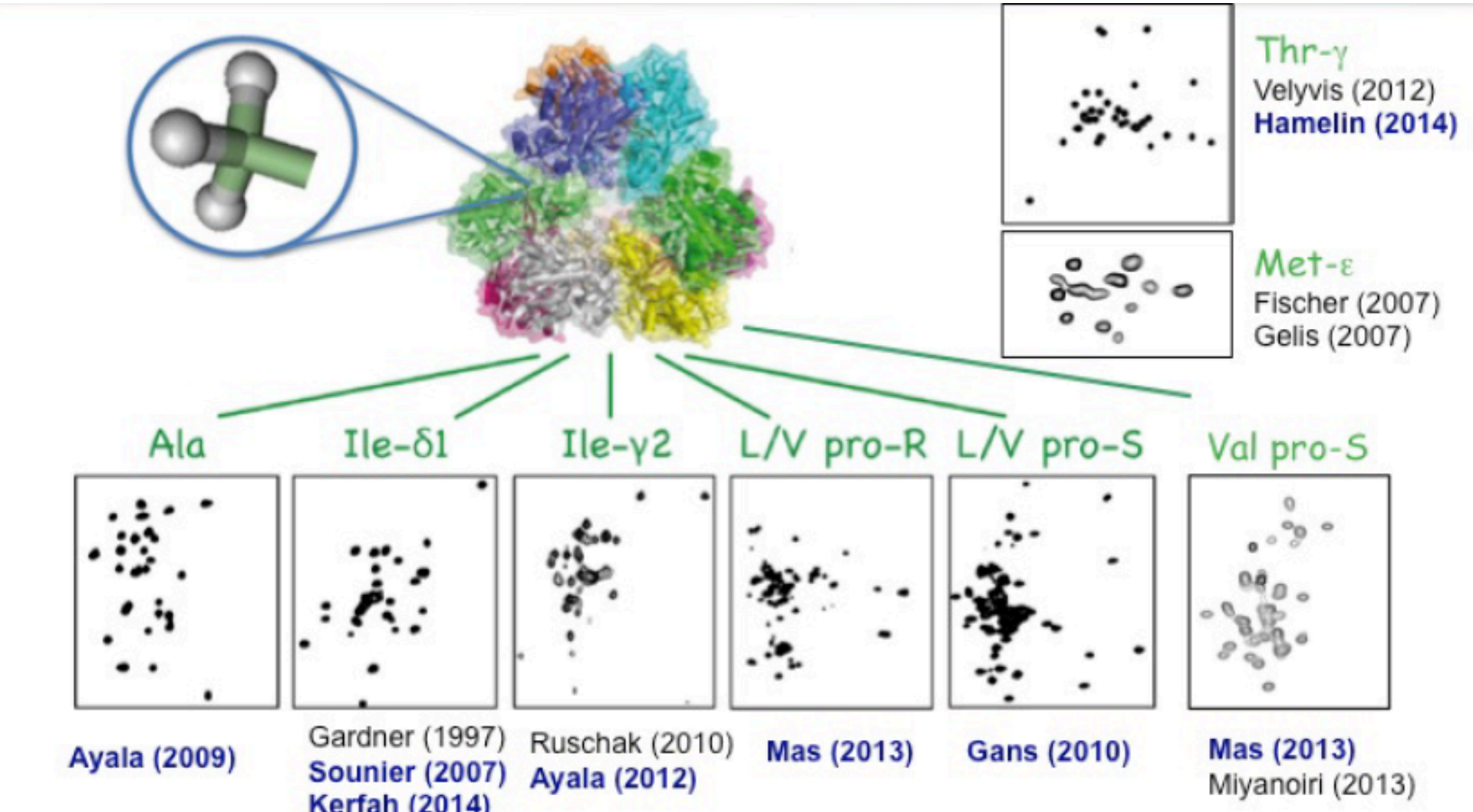


2% protonation

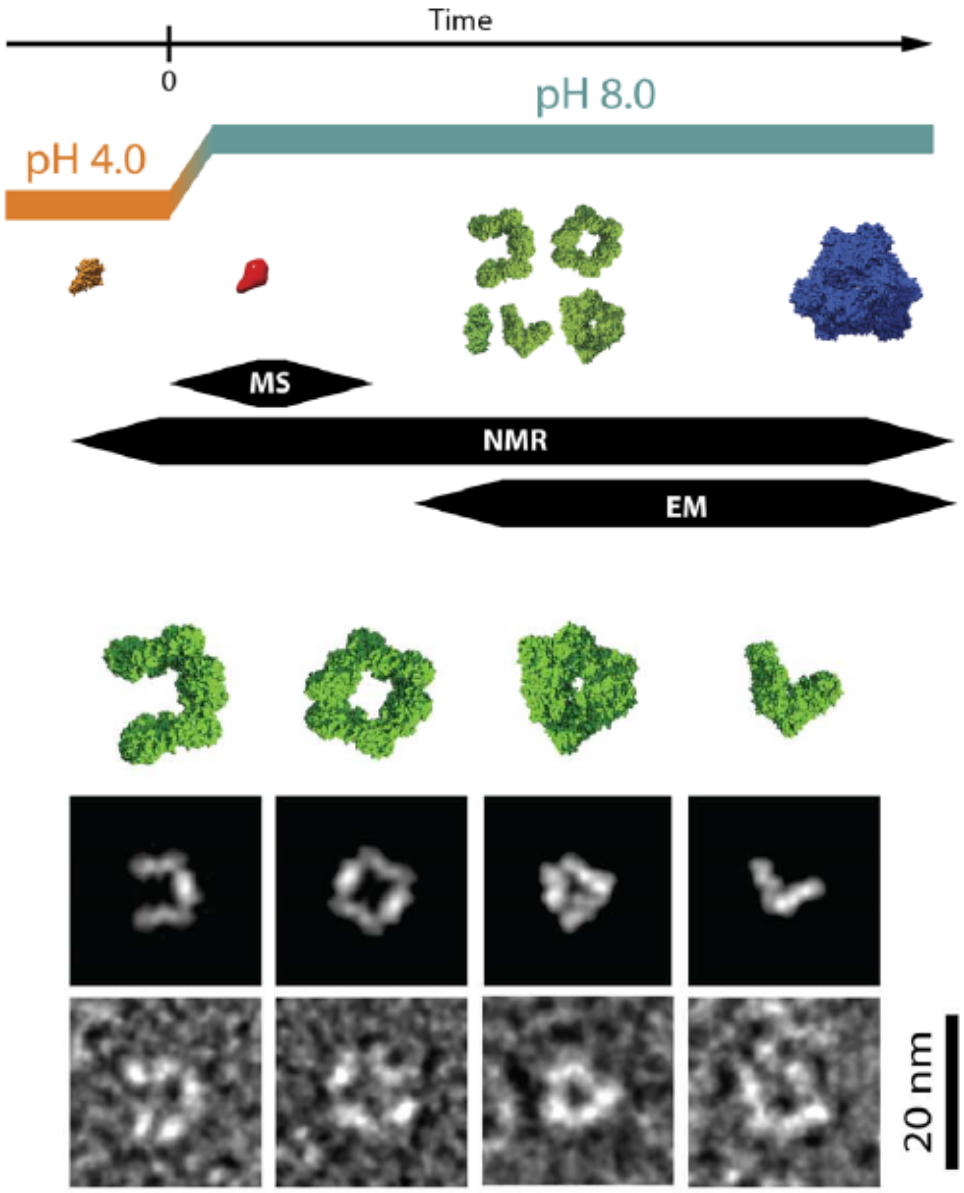
U-[^{2}H,^{12}C,^{15}N]
[δ_1-^{13}C**H$_3$**]-Ile

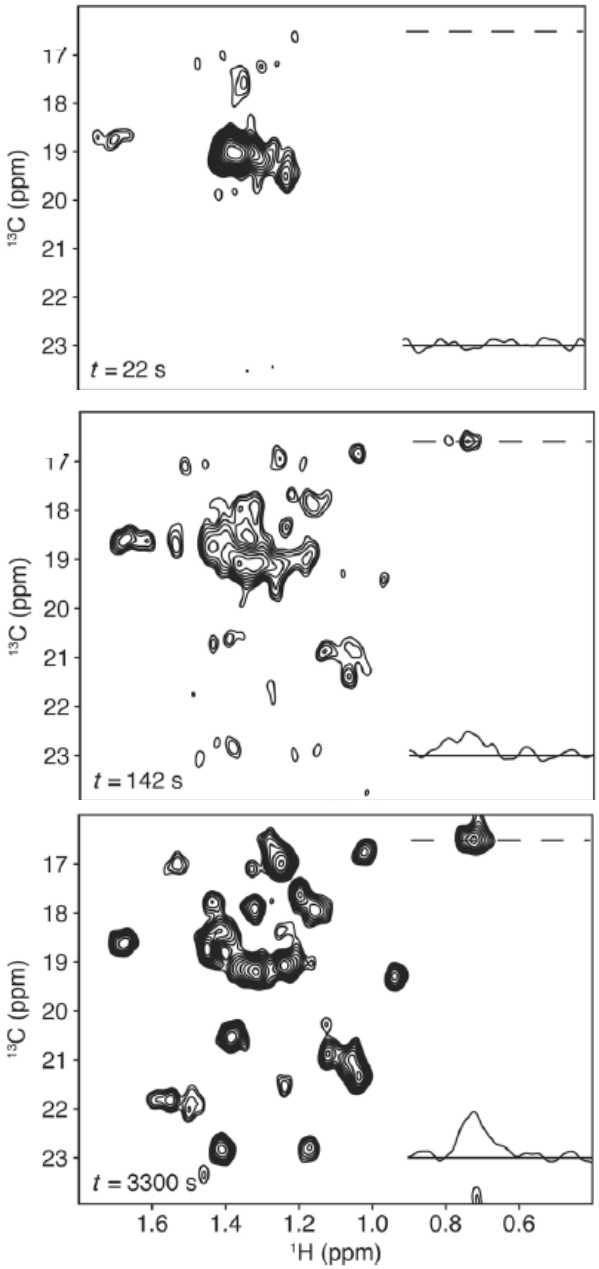


Me-labeling tool kits for NMR



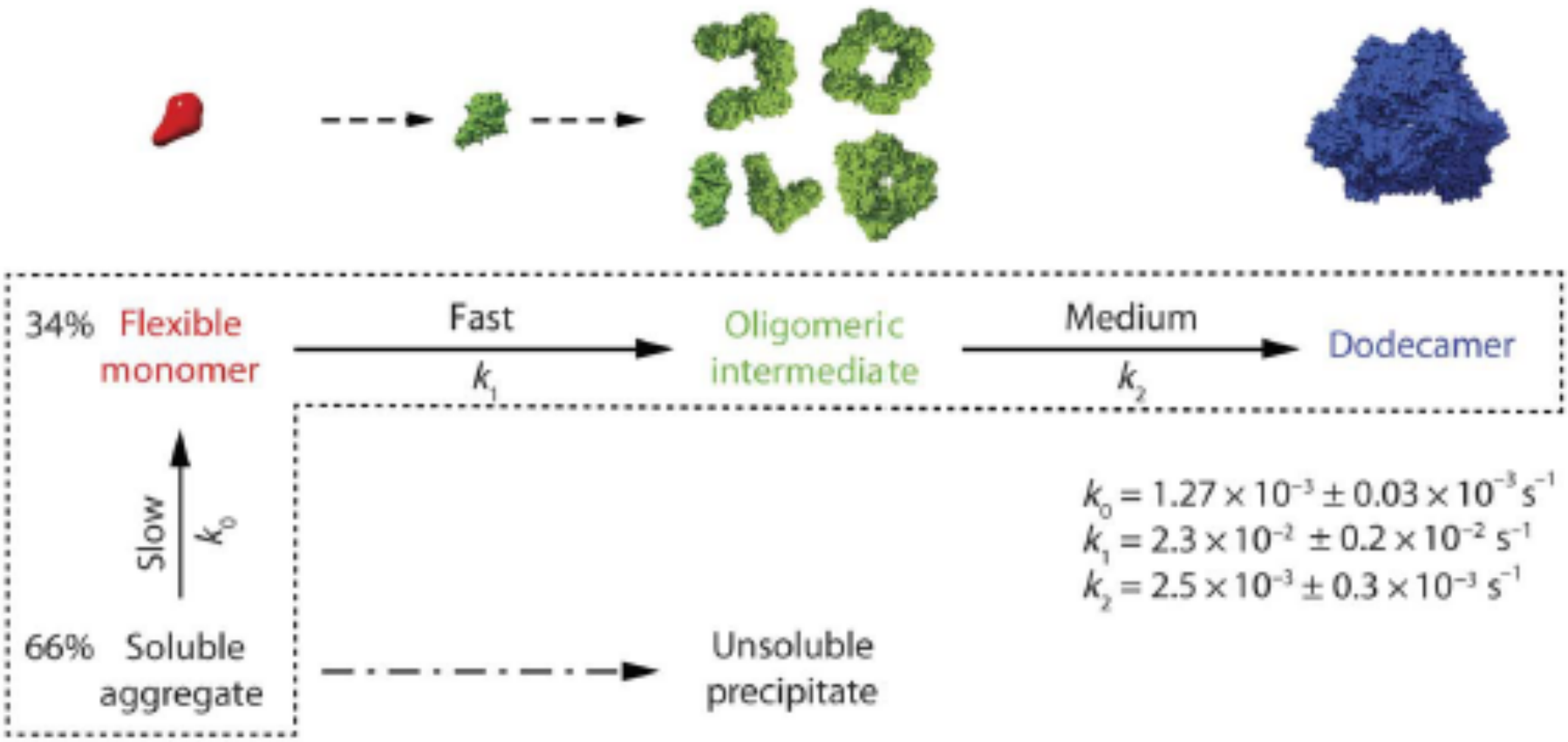
Monitoring of a molecular machine in action





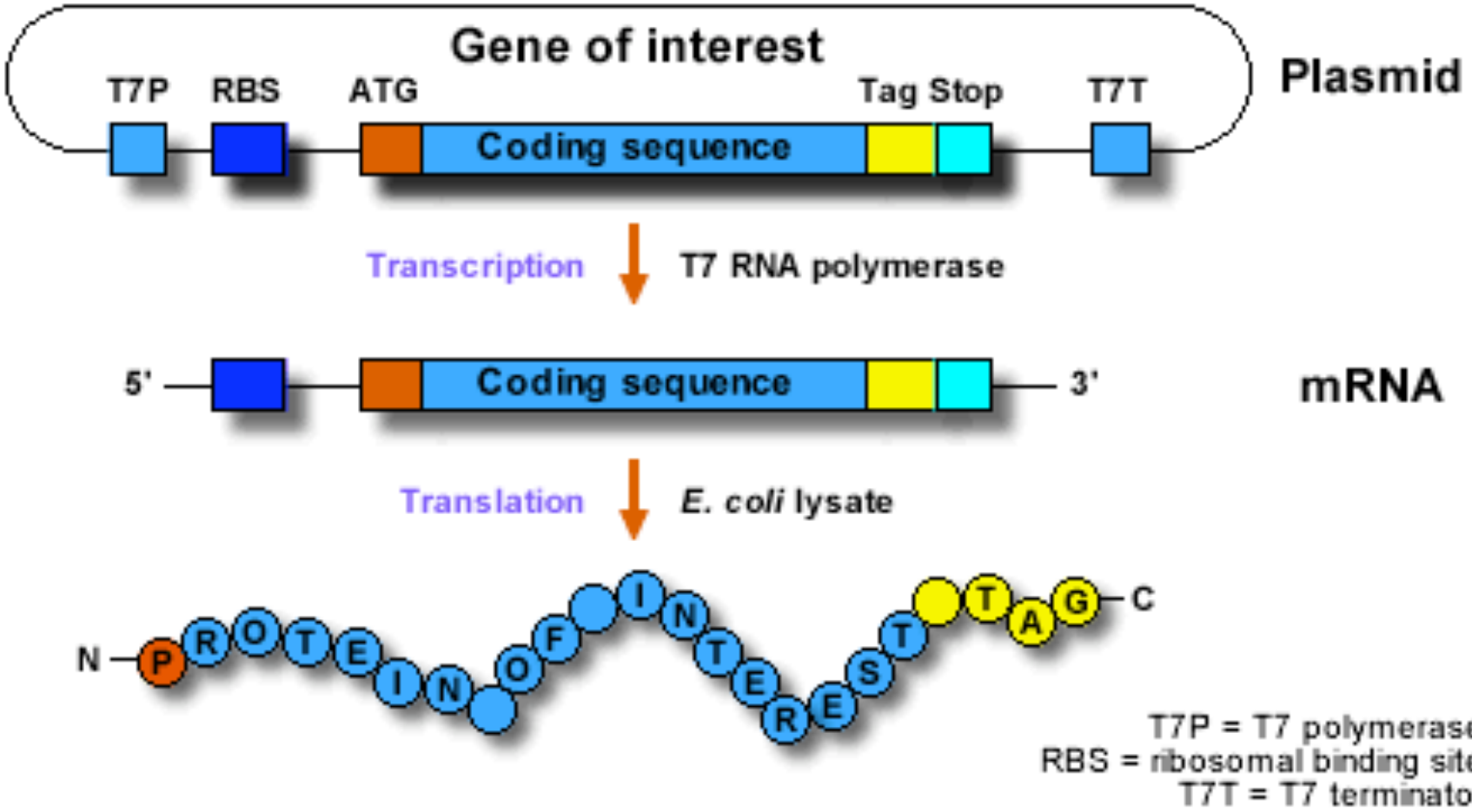
P. Macek et al. Sci Advances, 2017, e1601601

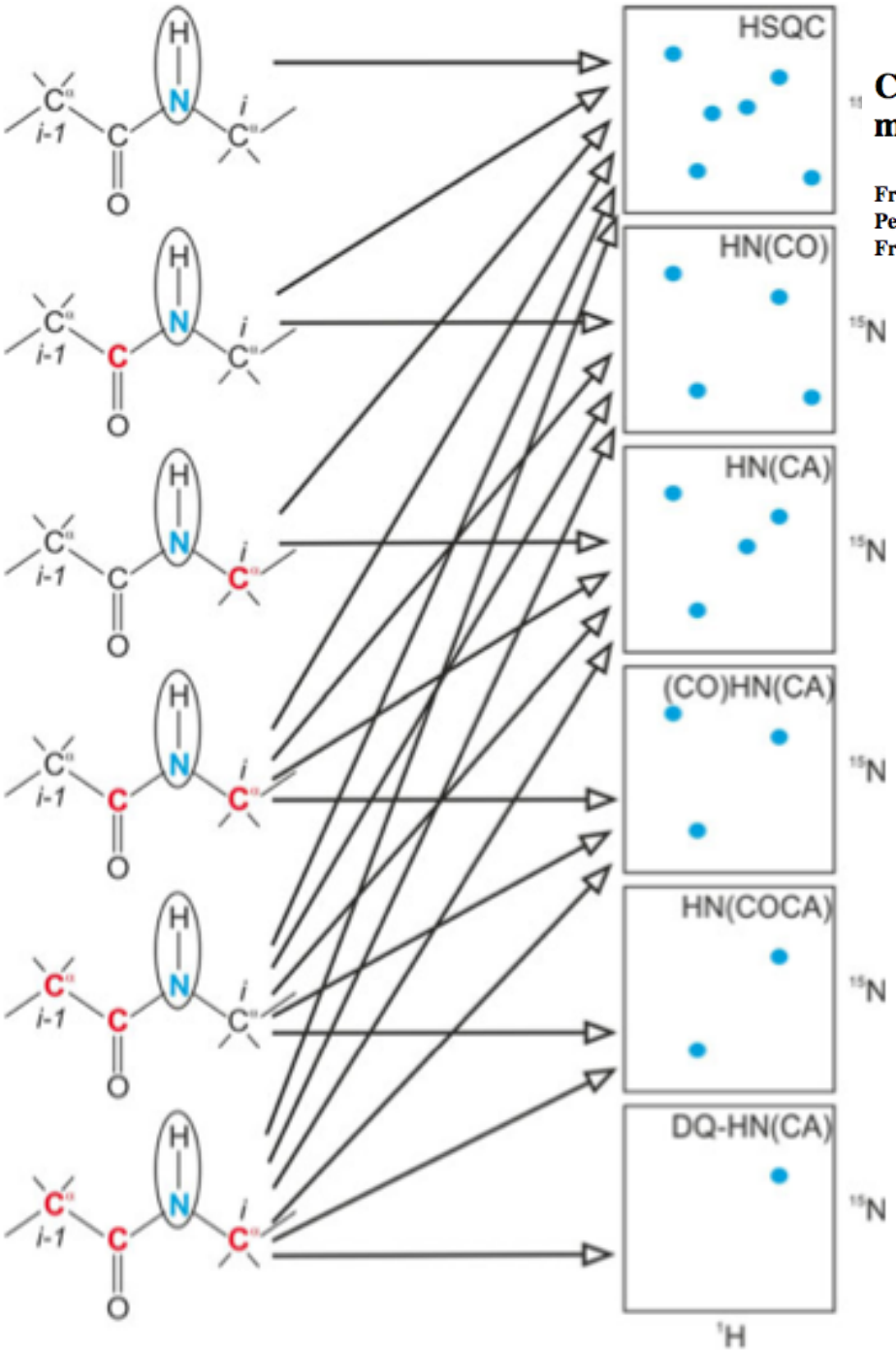
Monitoring of a molecular machine in action



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

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)

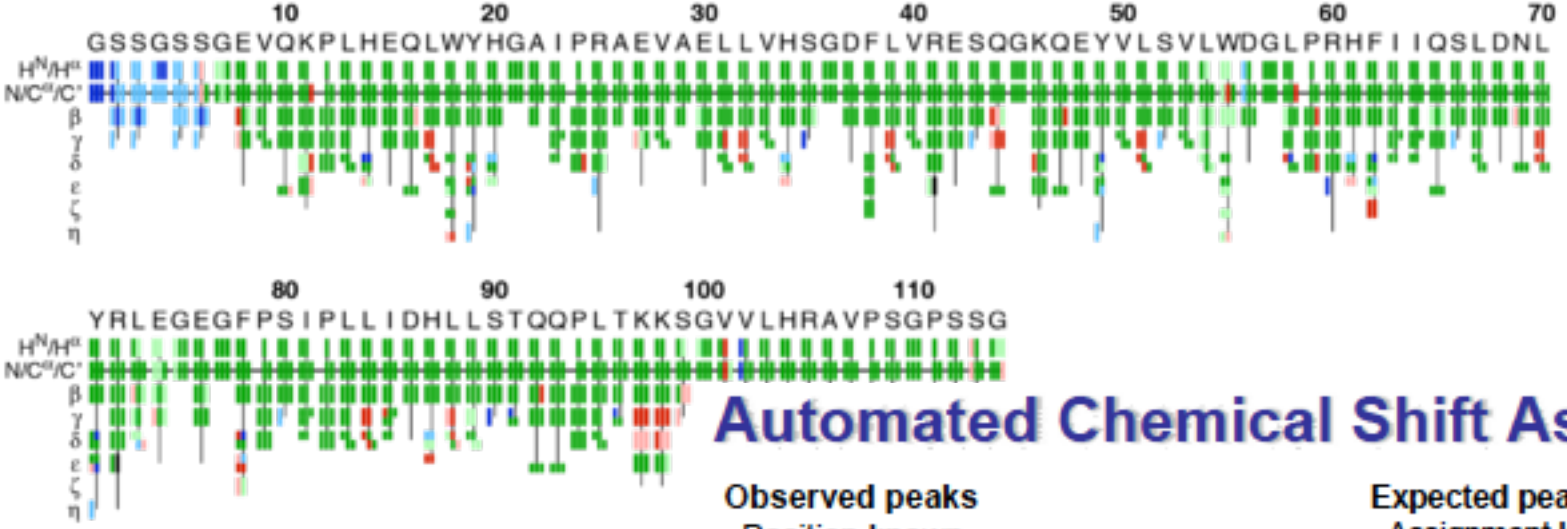
Amino acid type	Samples		
	1	2	3
Leucine	^{13}C/^{15}N	1-^{13}C	1-^{13}C
Valine	1-^{13}C	^{13}C/^{15}N	
Isoleucine			^{13}C/^{15}N
Methionine	^{15}N		
Lysine		^{15}N	
Phenylalanine			^{15}N
Arginine	^{15}N	^{15}N	
Tyrosine	^{15}N	1-^{13}C	^{15}N
Alanine		^{15}N	^{15}N
Threonine	^{15}N	^{15}N	^{15}N
Glycine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

Technological innovations

Numerical processing:

- Filtering
- Data management and integration
- Structure calculation software

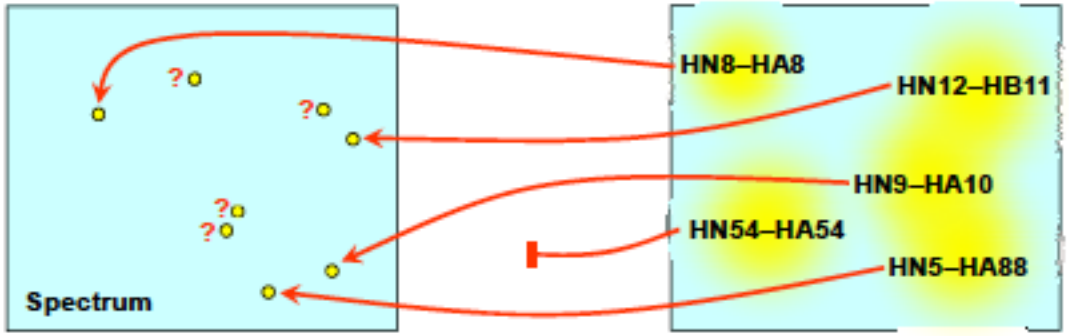
Software development for automatic assignment



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

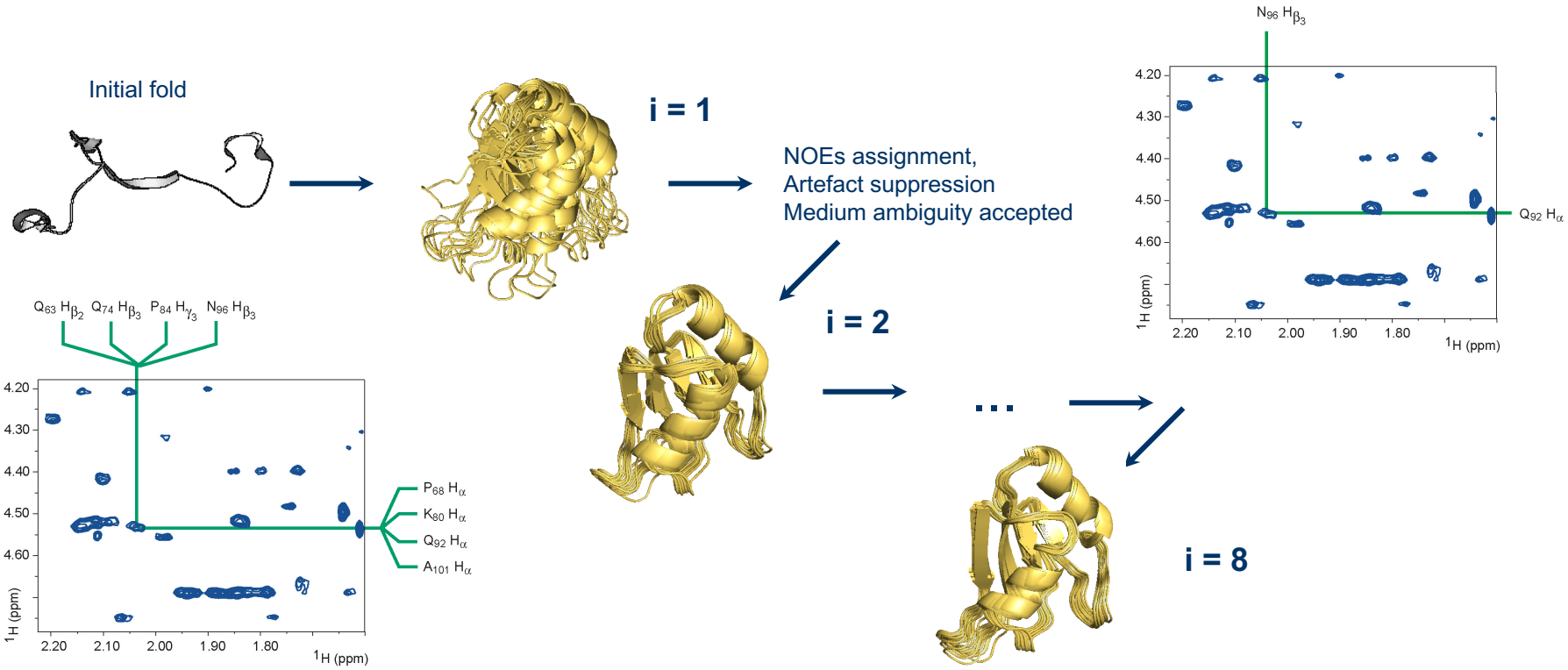
Optimization of assignment
Genetic algorithm combined with local optimization

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)

Software development for automatic structure calc.

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



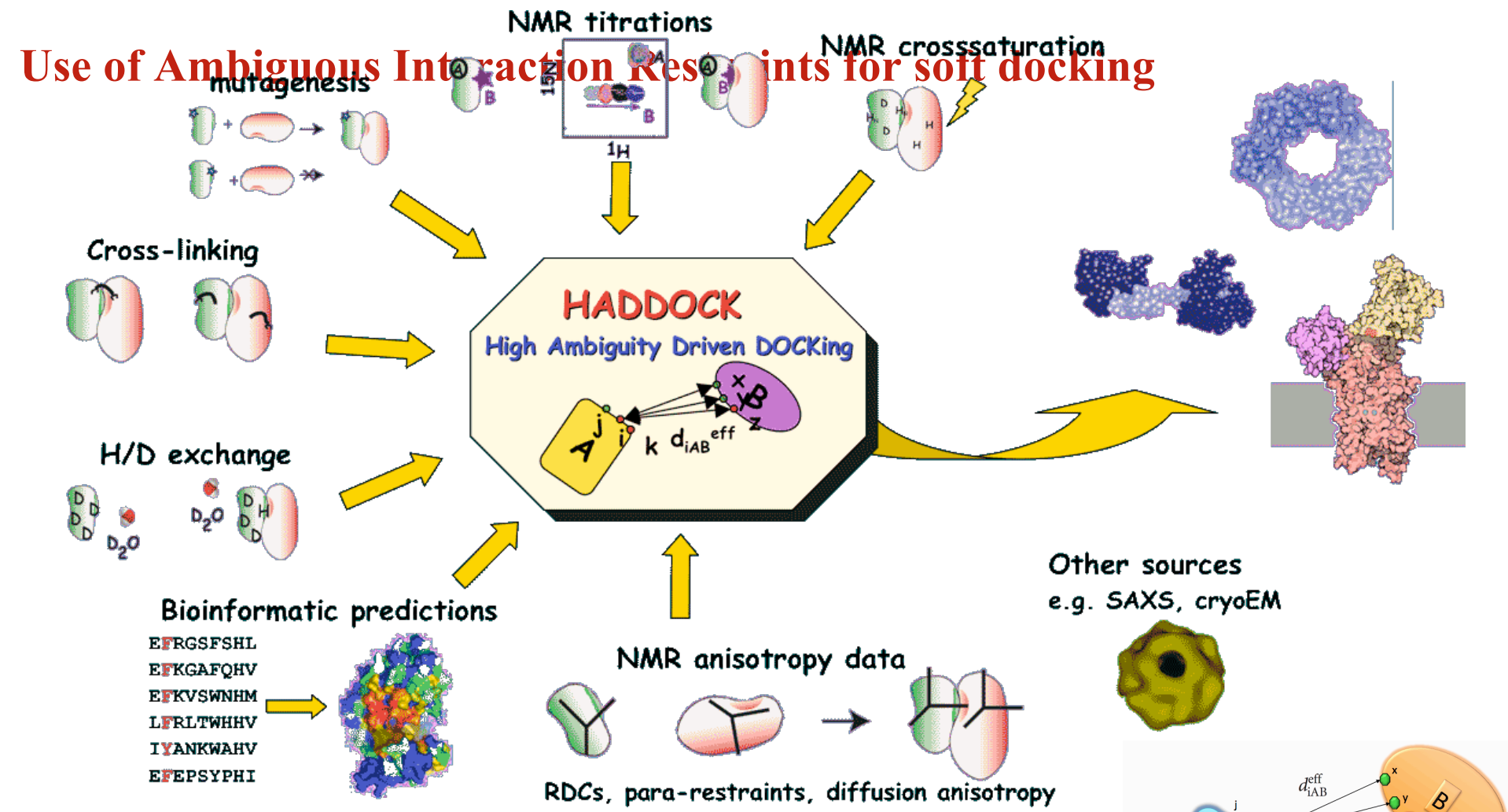
Software

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..

Ambiguous restraints for soft docking

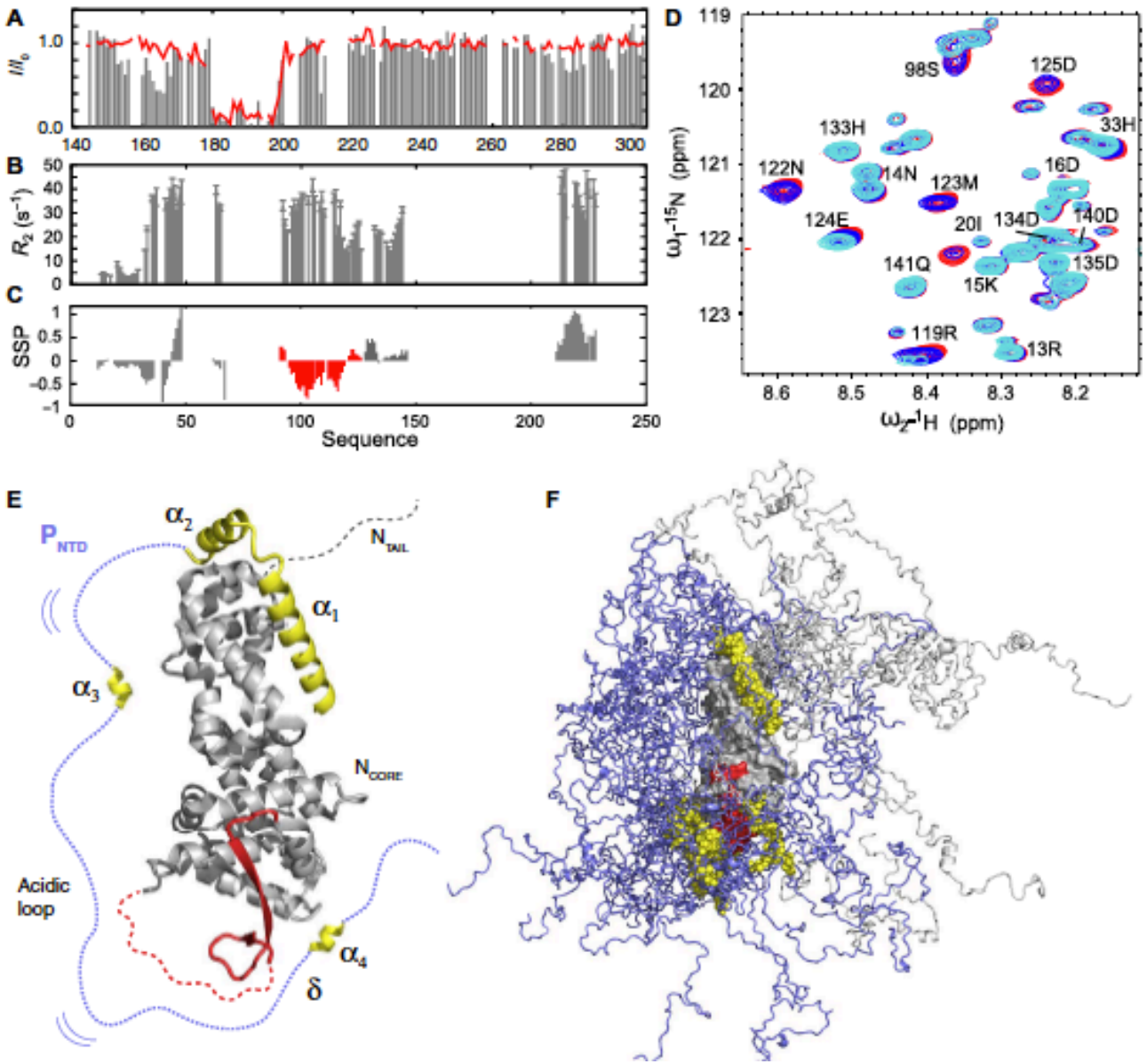


Domingez C, Boelens R, Bonvin A, J. Am. Chem. Soc. 125, 1731-1737 (2003).

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
- Integration of data from different methods

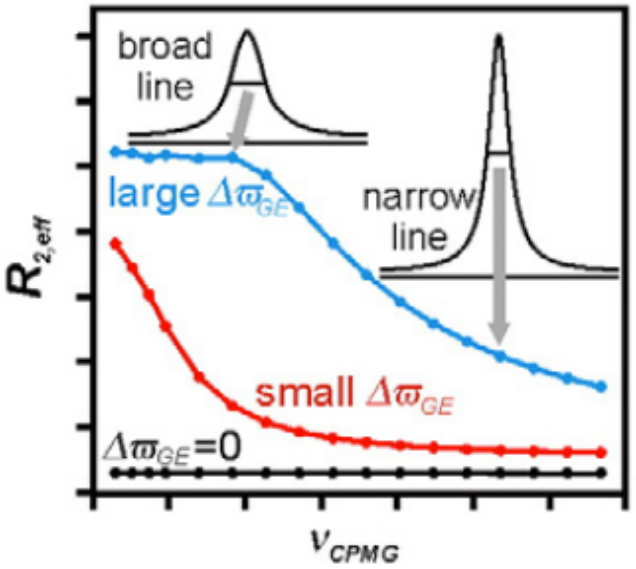
Study of intrinsically disordered proteins

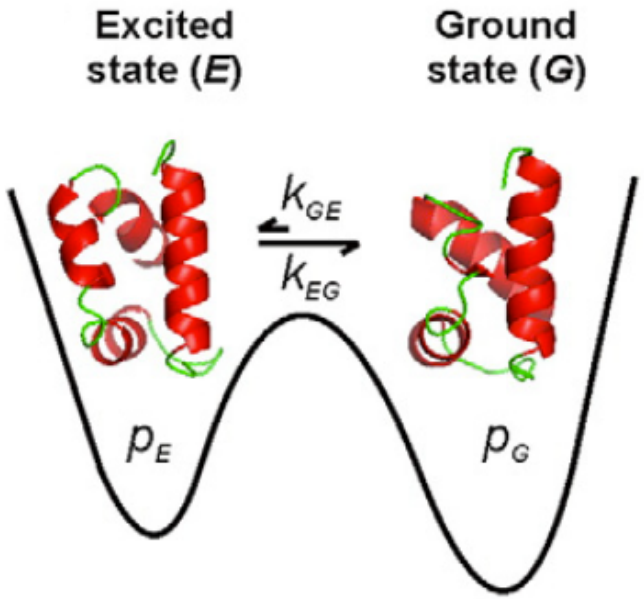


Miles et al., Sci. Adv. 2018, 4: eaat7778

Assessing data on non-detectable states

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

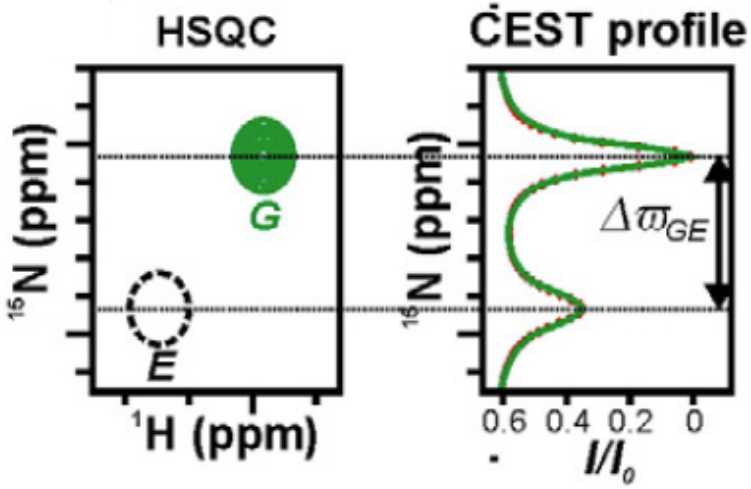




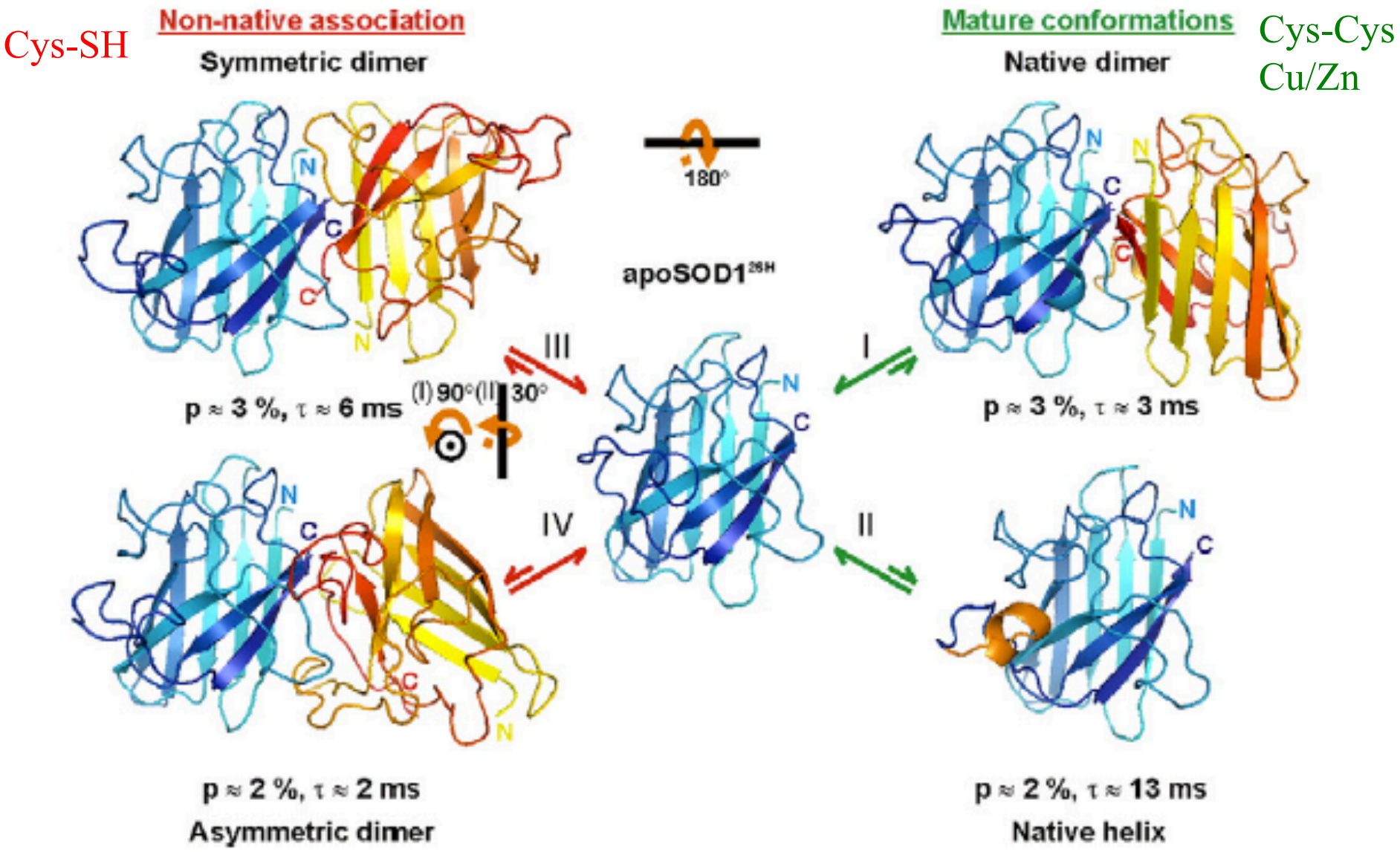
$p_G \gg p_E$

p_E 1% or more

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

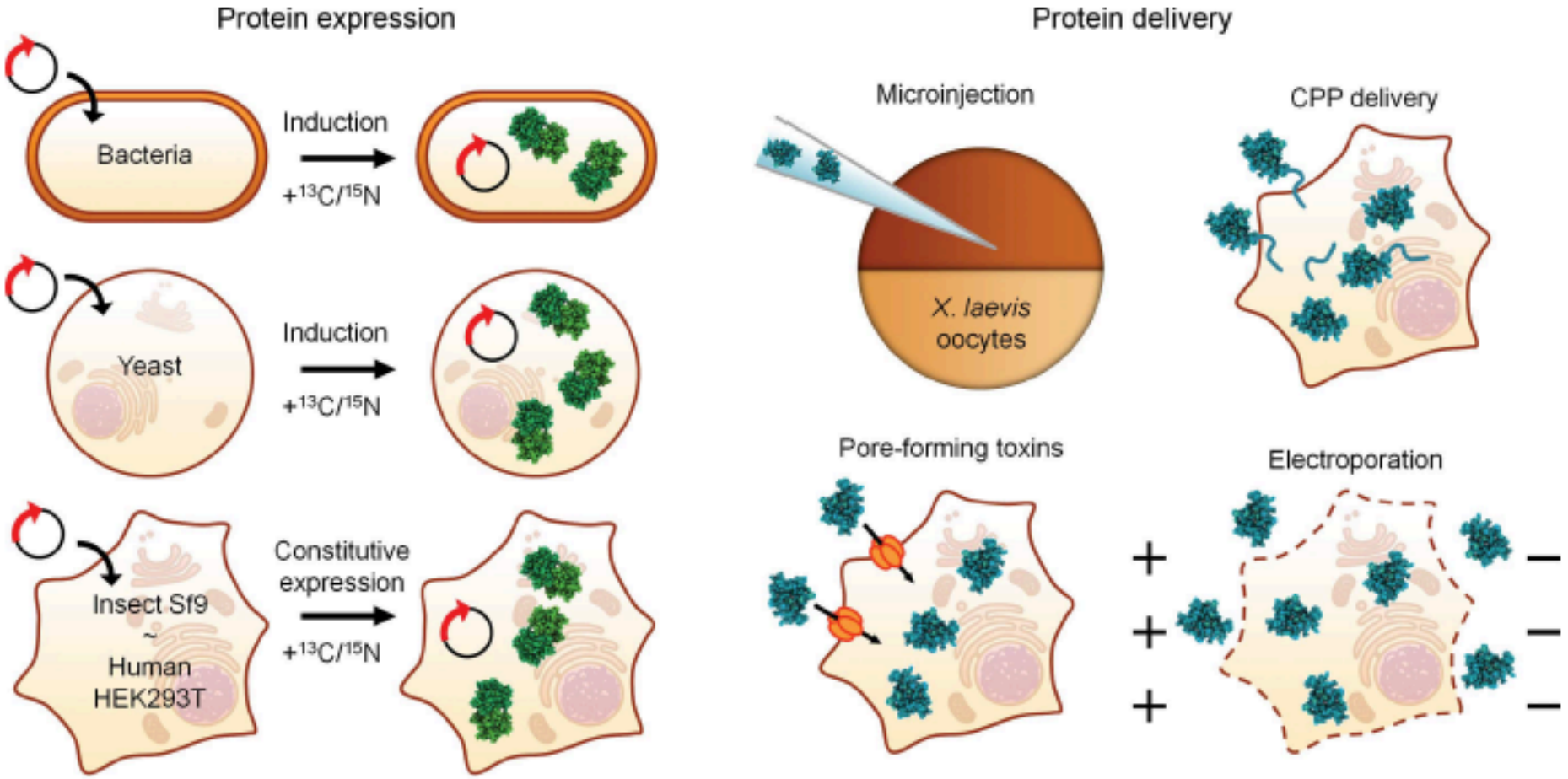


Assessing data on non-detectable states



Sekhar and Kay, PNAS 2013, 12867-12874

In-cell NMR: schematic overview



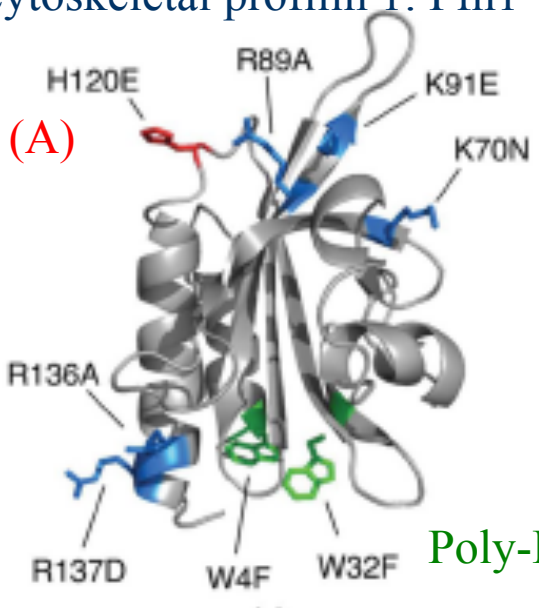
E. Luchinat and L. Banci, IUCrJ, 2017, 108-118

Deciphering interaction networks in cell

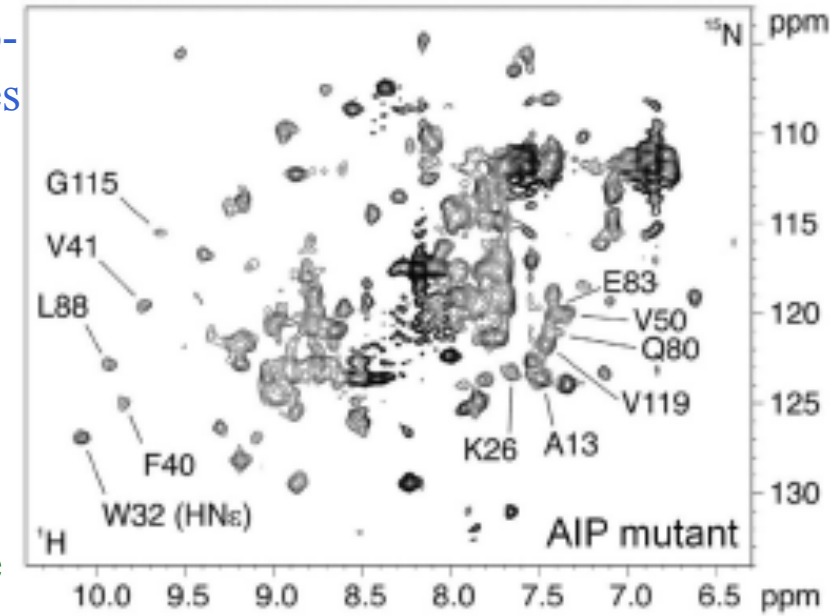
Barbieri et al., Sci. Report 2015, 14456

Human cytoskeletal profilin 1: Pfn1

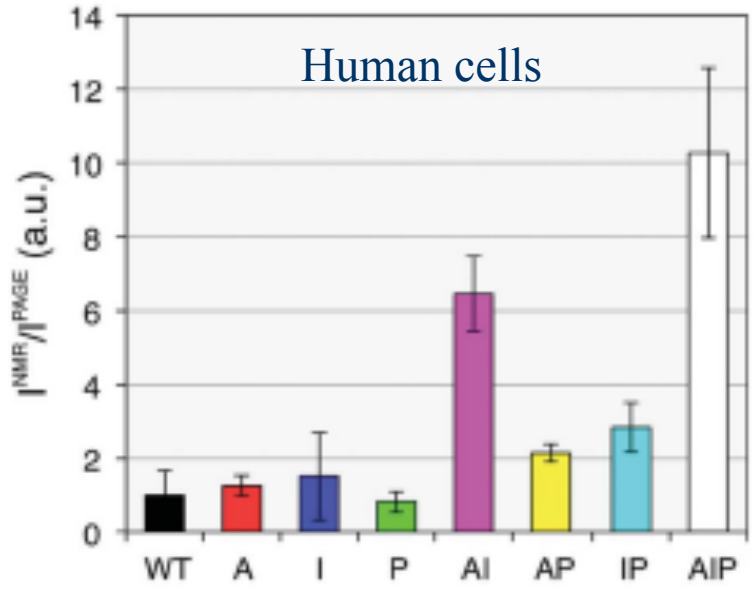
Actin (A)

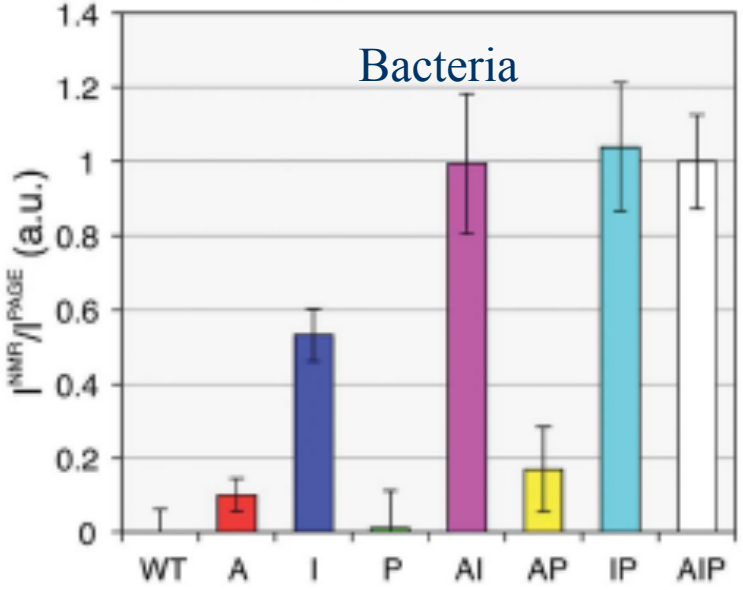


Phospho-Inositides (I)

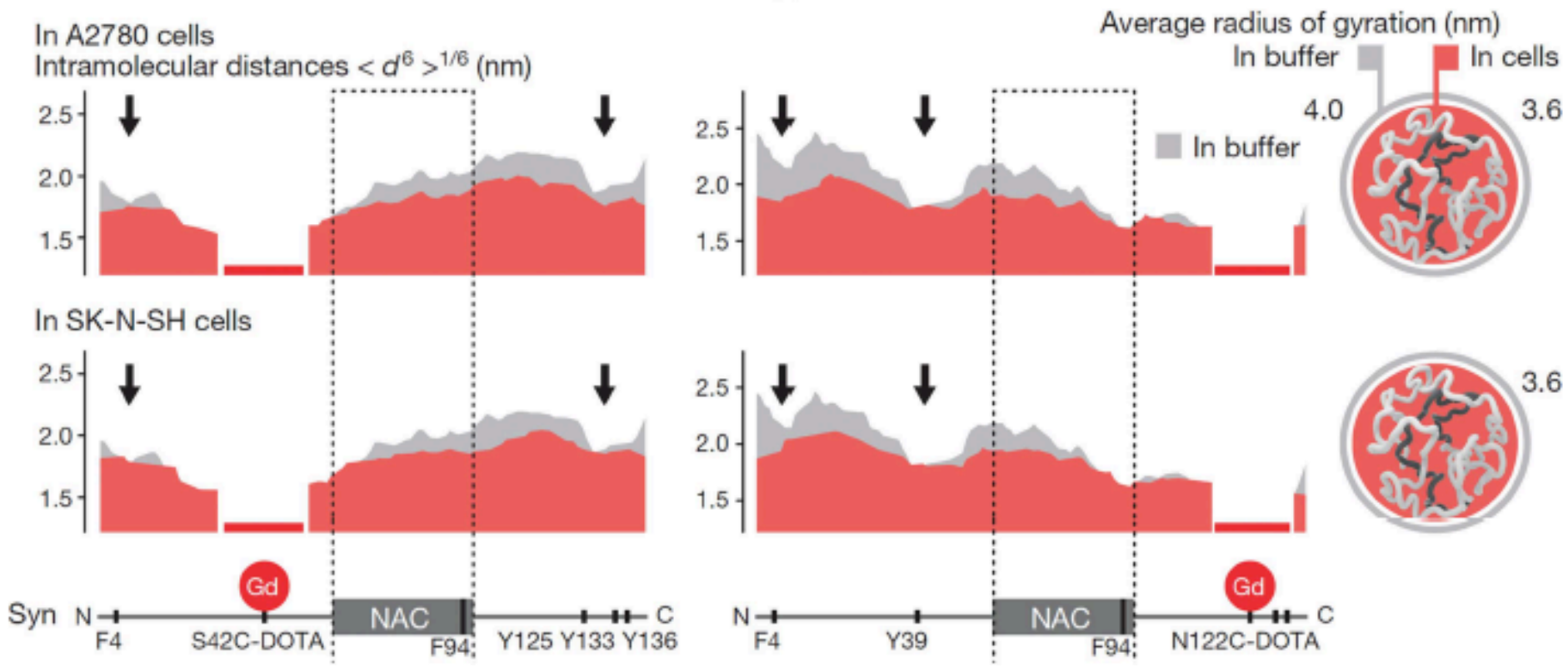


Poly-L-proline (P)





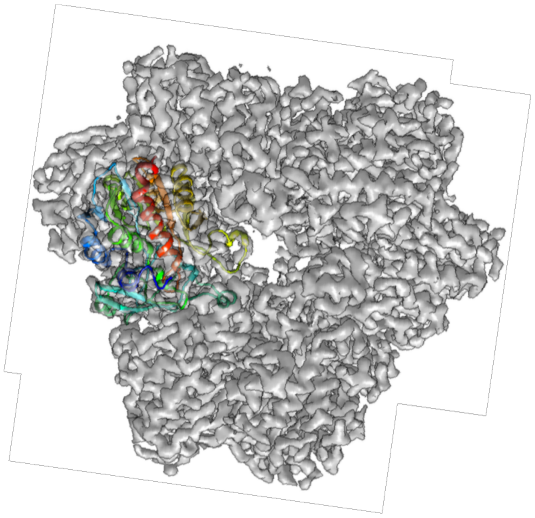
Comparison of α-synuclein in different cell lines and in vitro



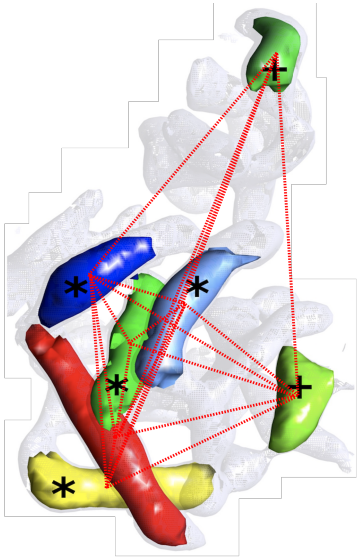
Theillet, Selenko et al.., Nature 2016, 45-50

Combination of NMR with other methods for structure determination: CryoEM

4.5 Å EM map



+



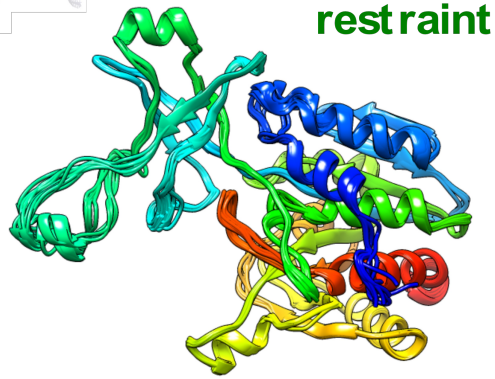
570 short distances

+

Talos dihedral angle restraints

+

21 inter-helices restraints



Gauto *et al.,* Nat. Com. (2019), in press