NMR developpments

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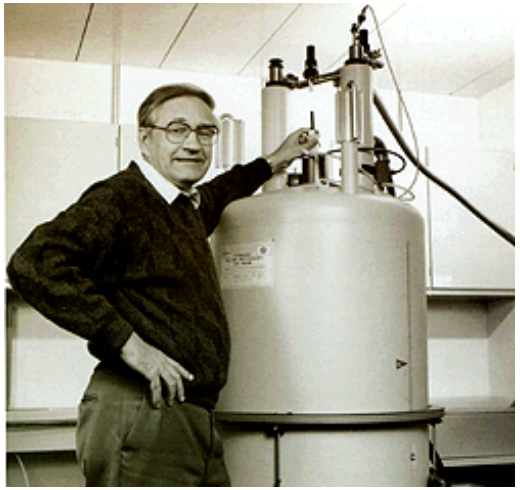
NMR developments: a tribute to R.R. ERNST

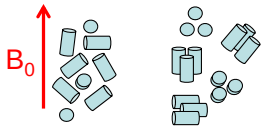


Why just NMR ?
- « Because there is hardly another technique that is so informative for so many different types of applications, and because there is no other technique that provides so much fun ».

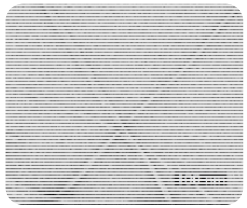
Richard Robert ERNST

Three States of living matter

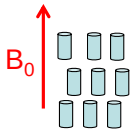
Solid state



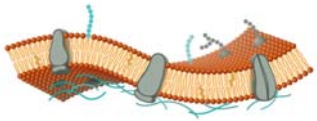
Static disorder



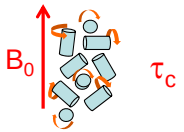
Solid state Oriented



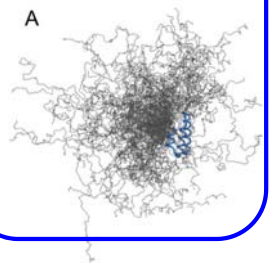
Static Order



Solution

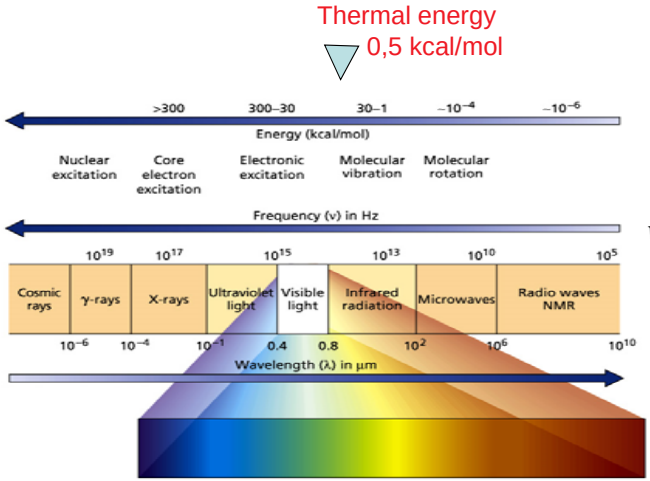


Dynamic disorder



Few introductory concepts about NMR

- NMR is a very very low energy spectroscopy



$$E = \hbar \gamma B_0$$

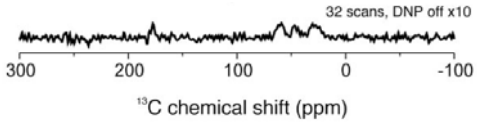
$$\nu = 2\pi^{-1}\gamma B_0$$

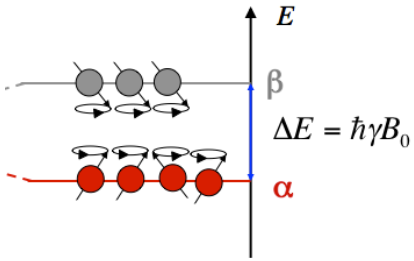
$$\lambda = \frac{2\pi c}{\gamma B_0}$$

Low energy means…

- **Intrinsically weak signal** due to low population difference
- Boltzman statistic:

$$\frac{P_\beta}{P_\alpha} = e^{\frac{-\Delta E}{kT}} = e^{\frac{-\gamma\hbar B_0}{kT}} \approx 1 - \underbrace{\frac{\gamma\hbar B_0}{kT}}_{10^{-4}}$$

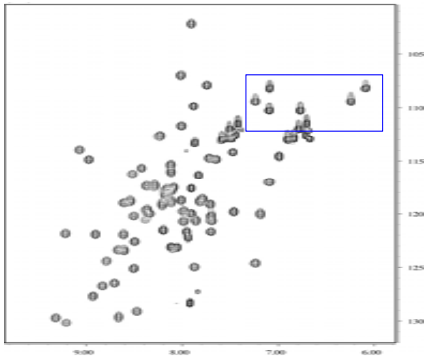


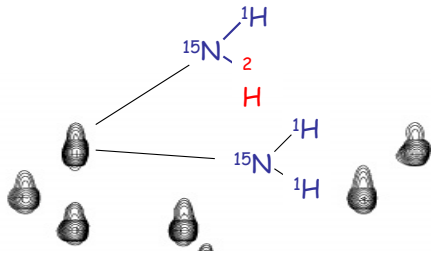


Low energy means…

- Very long live time of excited state…
- Subsequently an **extreme precision** on measurements

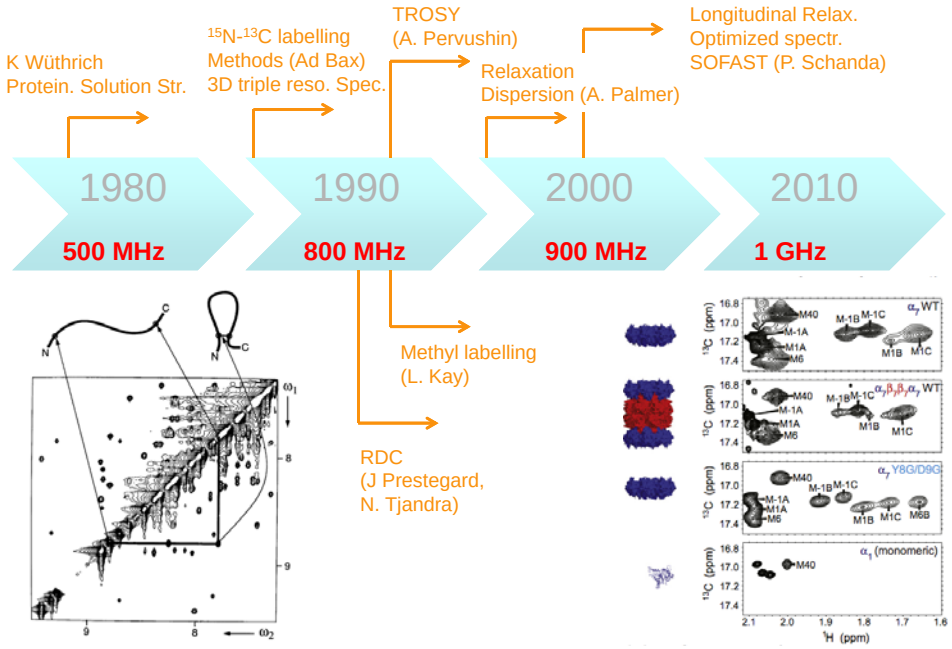
HSQC ^{1}H-^{15}N spectra



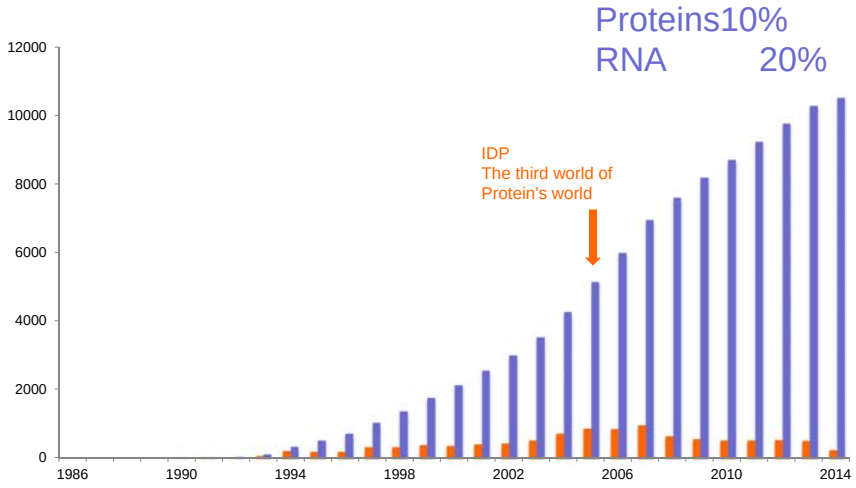


Example:
Deuterium isotopic shifts

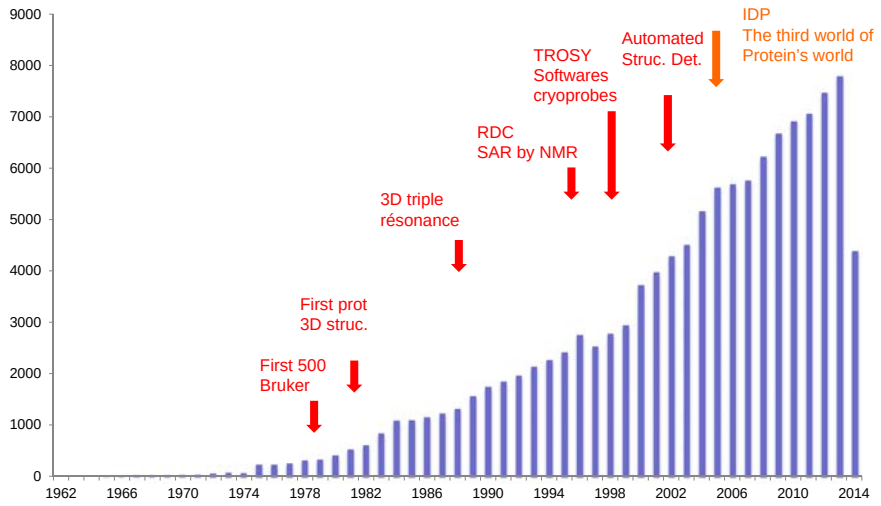
Biological NMR: 35 Years of innovations



Nb of structures determined by NMR in the PDB



PUBMED publications with NMR in the title



BIOCHEMISTRY
including biophysical chemistry & molecular biology
Current Topic
pubs.acs.org/biochemistry

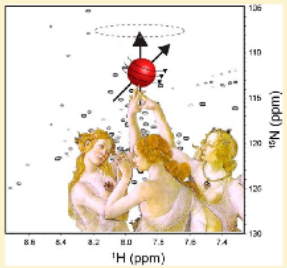
Jan 2013

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

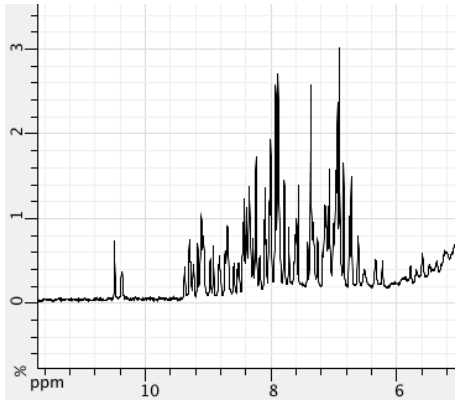
ABSTRACT: From roughly 1985 through the start of the new millennium, the cutting edge of solution protein nuclear magnetic resonance (NMR) spectroscopy was to a significant extent driven by the aspiration to determine structures. Here we survey recent advances in protein NMR that herald a renaissance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



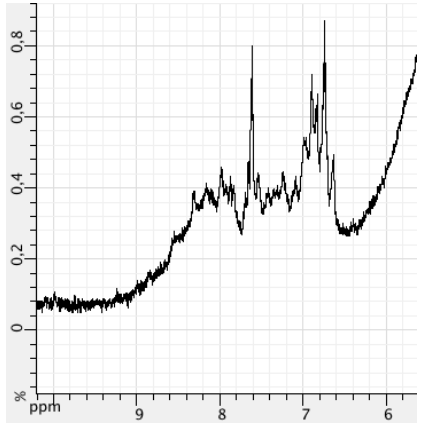
2005-2015
NMR: a tool for integrative structural biology

- Study of Intrinsically Disordered Proteins (IDP)
- Study of Molecular Recognition fundamental mechanisms
- Description of Protein and Nucleic Acid excited states
- Visualizing Large complexe's motions
- Monitoring protein's states within the cell

Recent methodological developments aim at:
- solving resolution problem (size limitation)
- sensitivity issues



GB1 (~6 kDa)



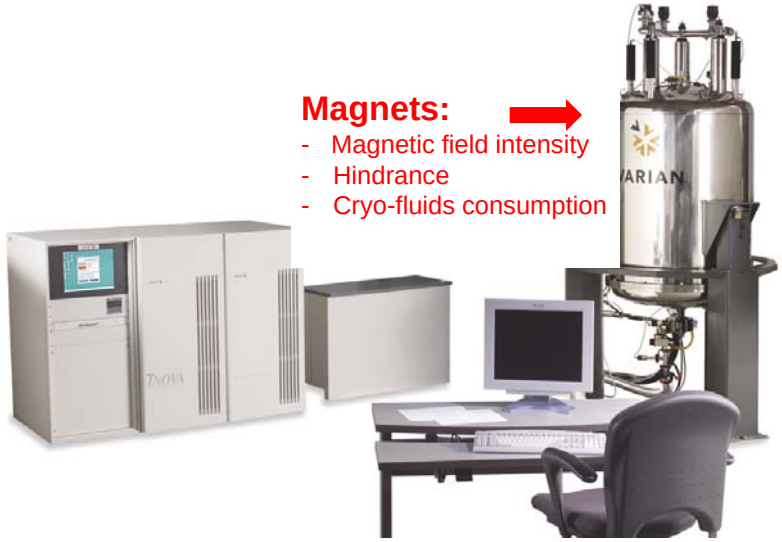
Dimère LBD RXR (~50 kDa)

NMR Methodological Innovations

Sample:
- Small volumes
- Isotopic labelling

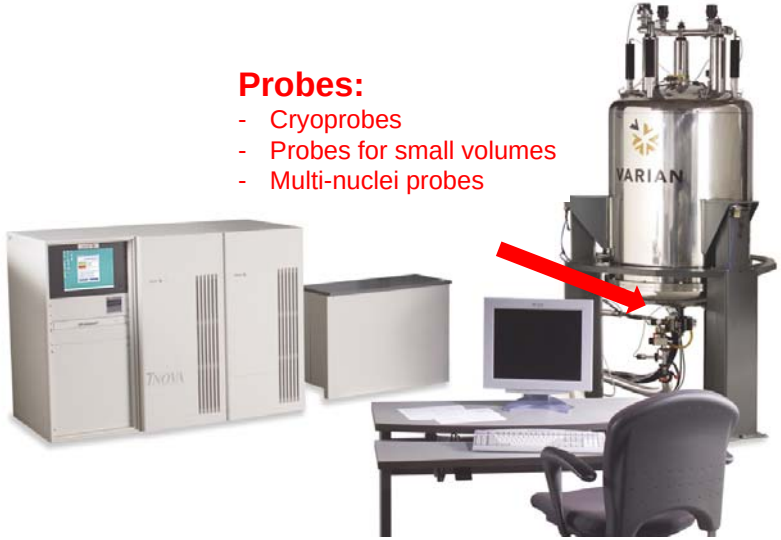
NMR Methodological Innovations

Magnets:
- Magnetic field intensity
- Hindrance
- Cryo-fluids consumption



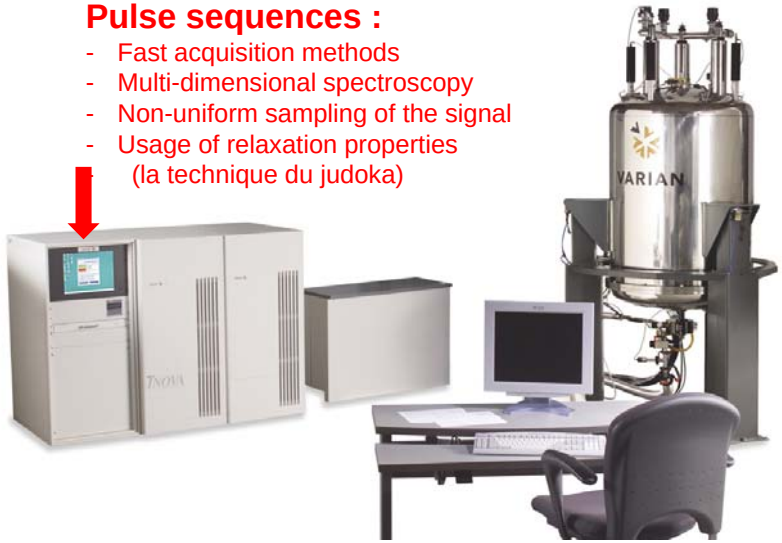
NMR Methodological Innovations

Probes:
- Cryoprobes
- Probes for small volumes
- Multi-nuclei probes



NMR Methodological Innovations

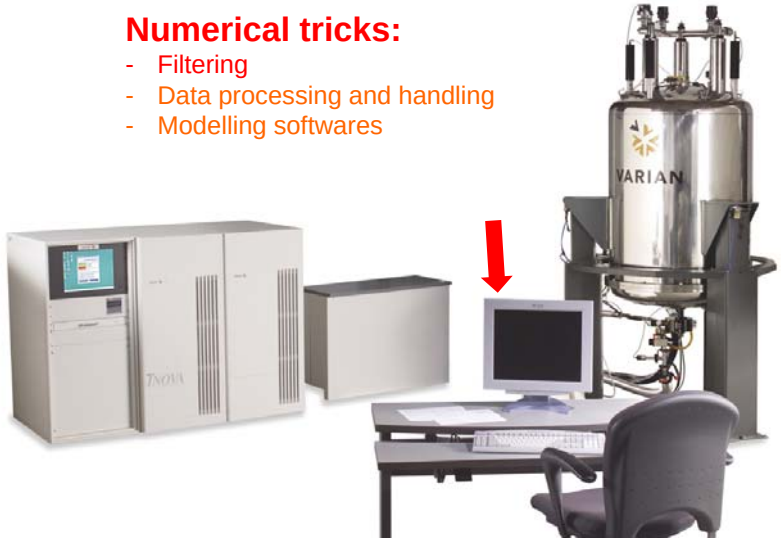
Pulse sequences :
- Fast acquisition methods
- Multi-dimensional spectroscopy
- Non-uniform sampling of the signal
- Usage of relaxation properties
  (la technique du judoka)



NMR Methodological Innovations

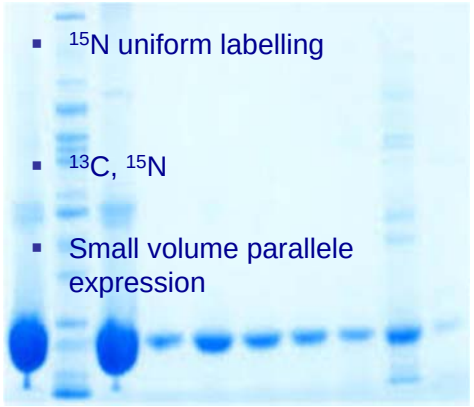
Numerical tricks:

- Filtering
- Data processing and handling
- Modelling softwares



NMR: co-evolution of biochemical and spectroscopic methods

- Unlabelled proteins
- ^{15}N uniform labelling
- ^{13}C, ^{15}N
- Small volume parallele expression

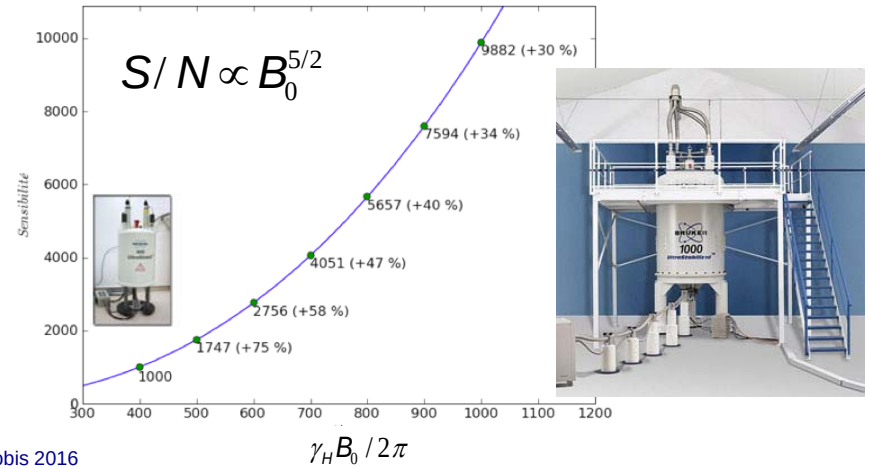


- 2D spectroscopy
- Heteronuclear Single Quantum Correlation (HSQC)
- HNCA, HNCO
- micro-cryoprobe

1. Recent advance in NMR instruments

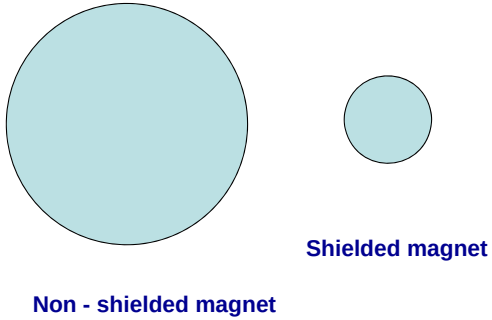
Recent advance in NMR instruments

- The Signal/Noise ratio depends on the applied magnetic field



Recent advance in NMR instruments

- Compactness of magnets



Shielded magnet

Non - shielded magnet

Cryoprobes

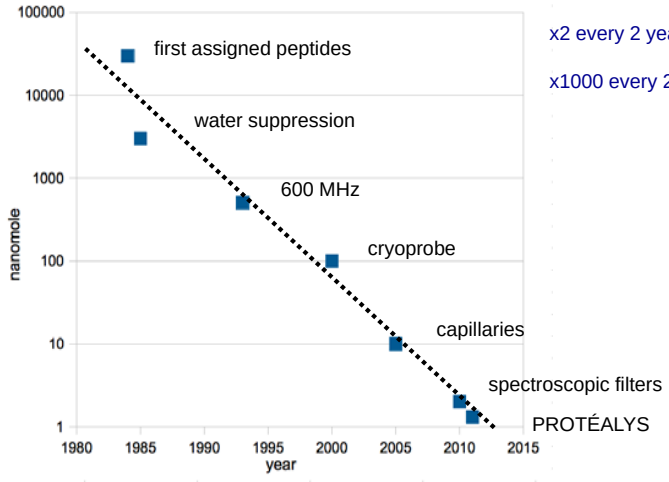
- Provided that the sample is properly conditionned (tubing) the sensitivity can be optimized (mass of matter needed for the experiment)
- Hoult law: $S/N \propto Q\eta M_O$

Q : facteur de qualité

η : facteur de remplissage

Evolution of Detection Sensitivity

Quantity of measured protein

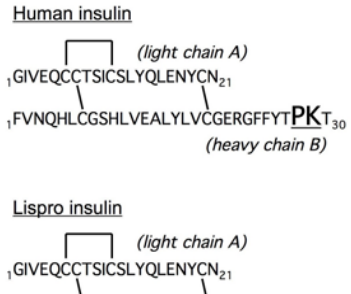


x2 every 2 years

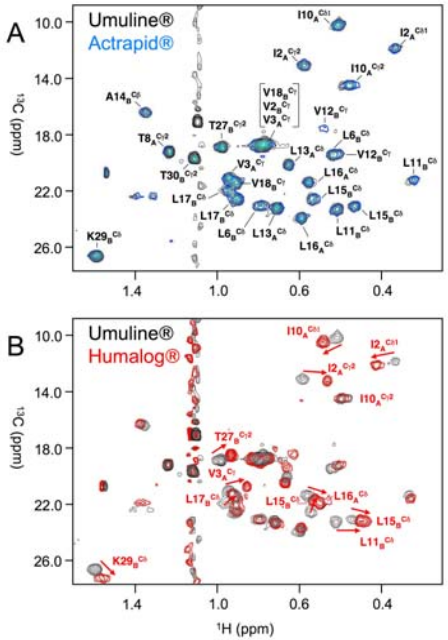
x1000 every 20 years

From MA Delsuc

Bio-drugs quality control by NMR



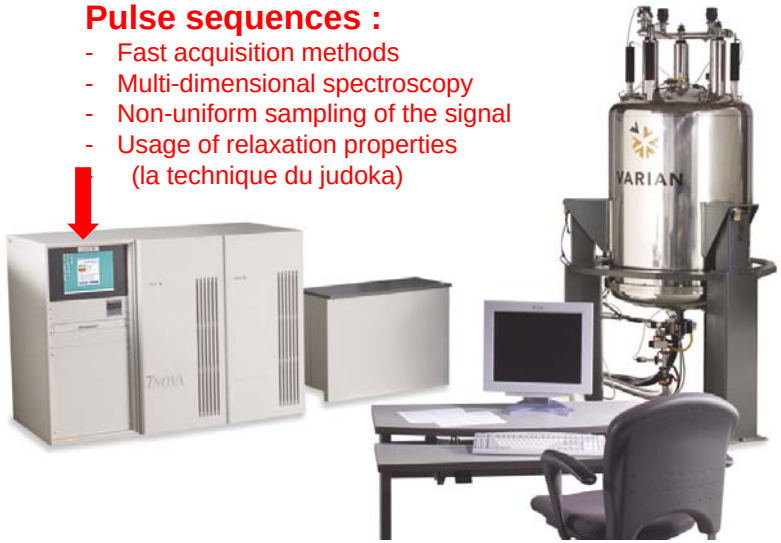
Brevet Kieffer B. (2011) WO 2011/080492



2. Recent advance in NMR methodologies

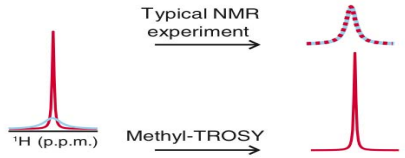
Pulse sequences :

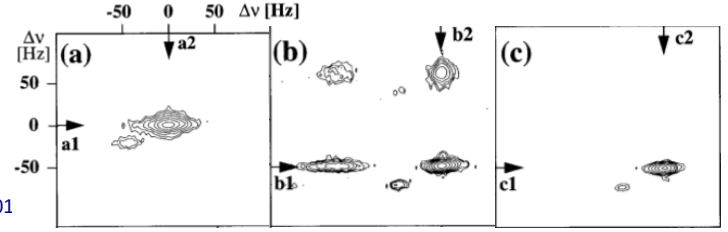
- Fast acquisition methods
- Multi-dimensional spectroscopy
- Non-uniform sampling of the signal
- Usage of relaxation properties
  (la technique du judoka)



Taking advantage of relaxation properties

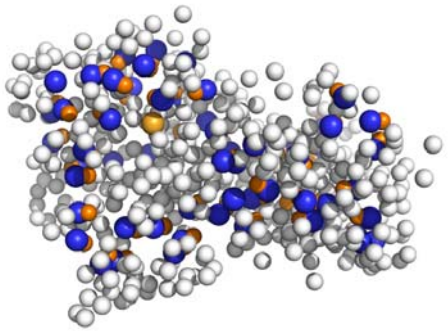
- 1. Transverse relaxation
  - Exploit cross-correlation phenomenons between several relaxation mechanisms (CSA-DD) => TROSY





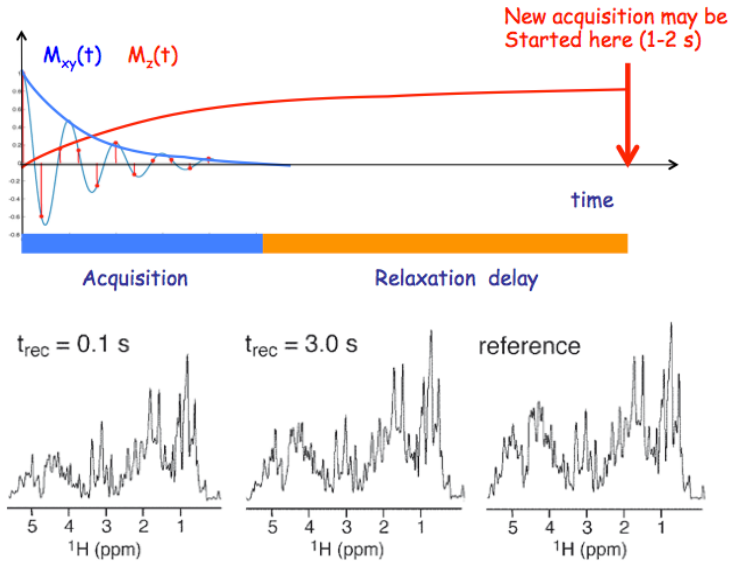
Taking advantage of relaxation properties

- 2. Longitudinal relaxation:
  - Accelerate the return to thermal equilibriumstate before the next acquisition => SOFAST



P Schanda et al. 2005

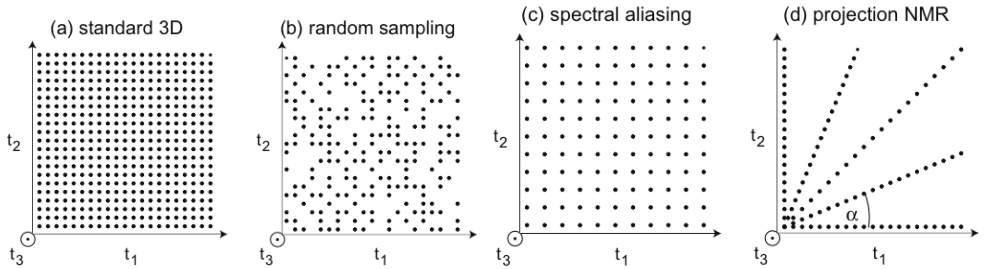
Taking advantage of relaxation properties



P Schanda et al. 2005

Working on sampling methods

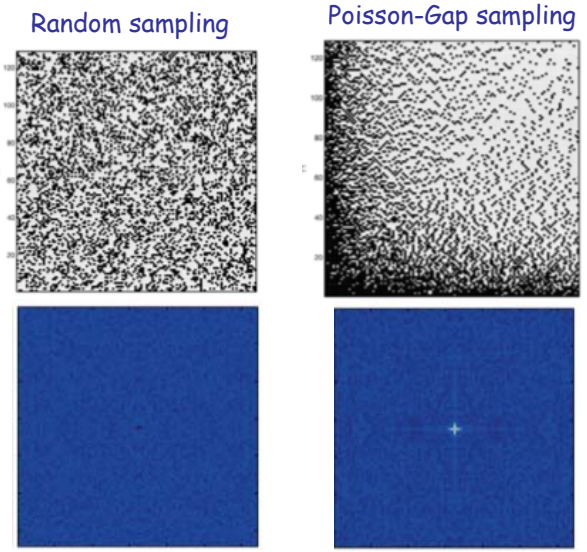
- The use of FFT imposes a linear sampling
- Alternate methods (NUS) are currently beeing developped allowing considerable gain of time



P Schanda Progress NMR Spect. 2009

Sampling 3D spaces

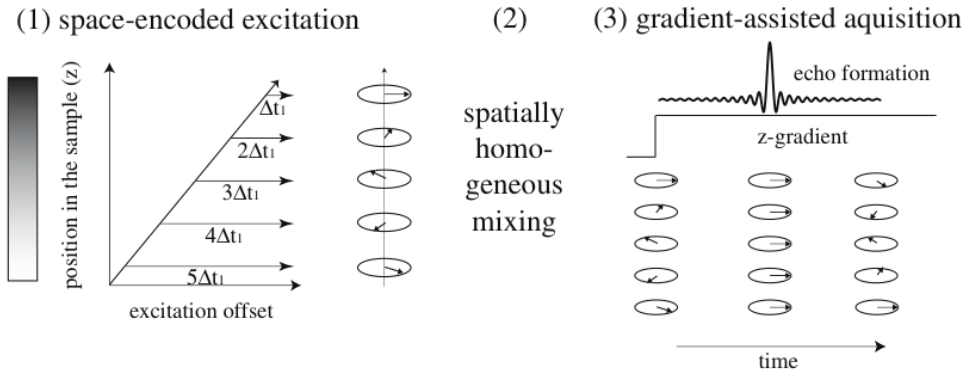
Exp : 30% of points sampled (Mobli, Hoch Prog NMR spect).



Ultra-Fast NMR

- Principle: Use field gradients to establish a relationship between space and time domain

  => A 2D can be recorded in few seconds (provided that you have enough S/N) (L. Friedman et al. PNAS 2002)



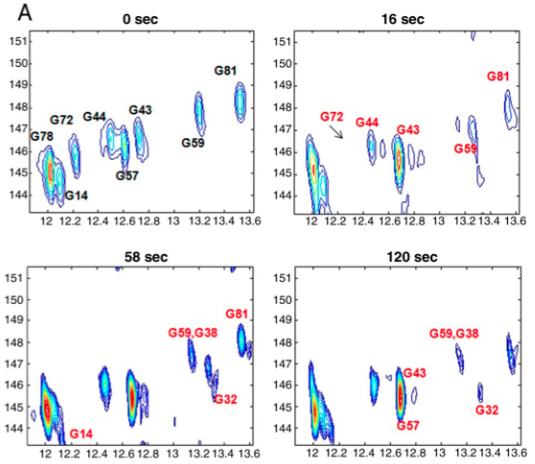
P Schanda Progress NMR Spect. 2009

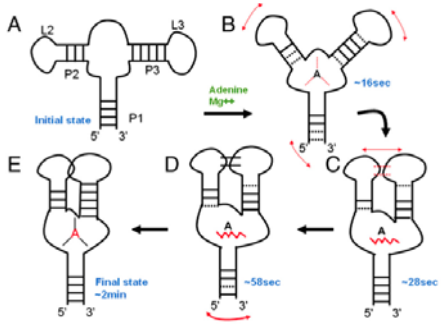
Application: Real-time follow-up of a folding process

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



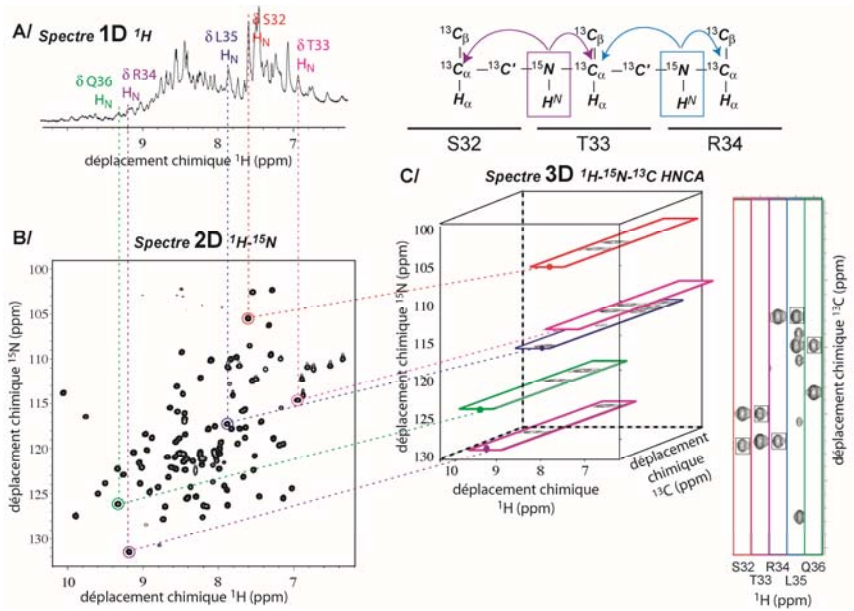


3. Sample preparation

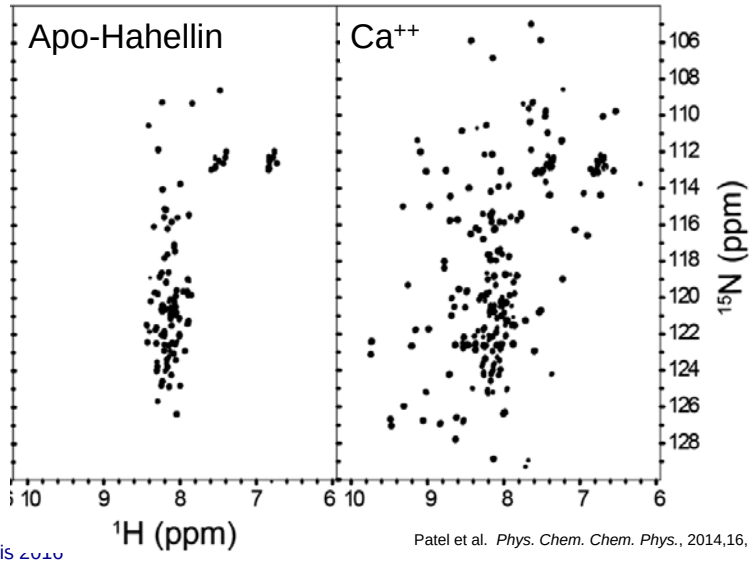
Sample preparation

- Isotopic Labelling:
  - Spectral Simplification (reduction of the number of frequencies present in the spectrum)
  - Reduce the relaxation sources (Partial deuteriation)
- Introduction of additional probes
  - Paramagnetic Relaxation
  - Fluorine NMR
- The complexity of observed medium
  - Use of anisotropic media (Residual Dipolar Couplings)
  - In-cell NMR

Standard approach: Uniform ^{15}N-^{13}C associated to multi-dimensional triple resonance spectroscopy

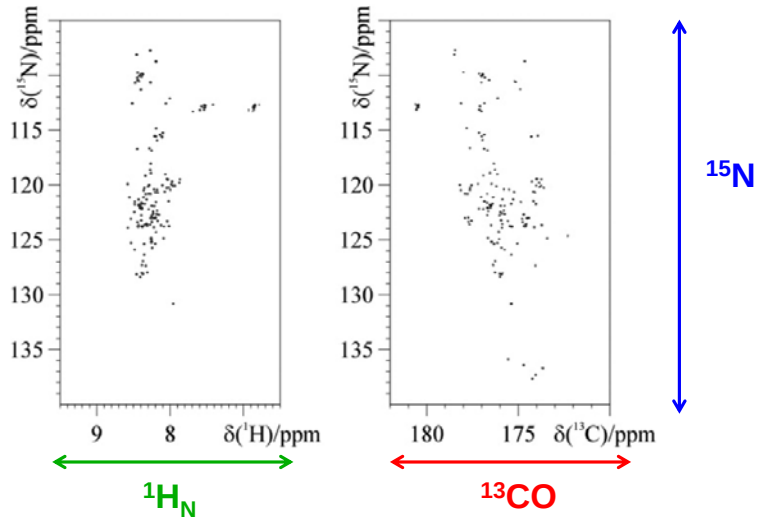


Development of specific methods for Intrinsically disordered proteins (IDP)



Patel et al. *Phys. Chem. Chem. Phys.*, 2014,16, 12703-12718

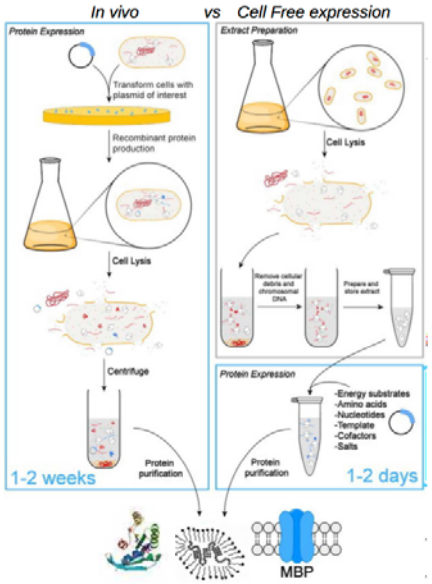
^{13}C, ^{15}N detection



I. Felli et al. IUBMB Life, 64(6): 473–481, June 2012

Cell free expression and combinatorial labelling

Lionel Imbert (IBS) Cell-Free expression platform
Adapted from Carlson *et al.* 2012 Kigawa *et al.* 2001 Schwartz *et al.* 2007



Overview of cell free isotopic labeling

ibs

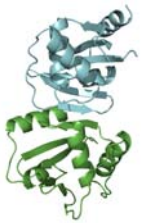
Specific'amino'acids'labeling

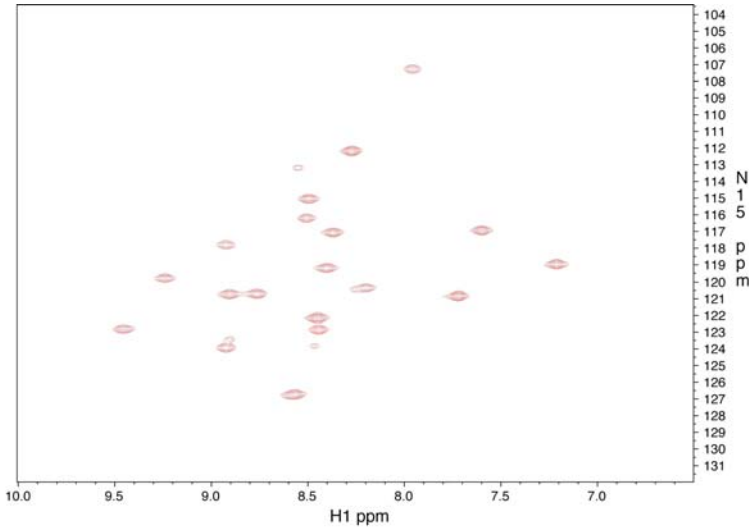
CECF
Val,'Thr'^{15}N,^{13}C
+'18'aa unlabeled

Expected'peaks:'21
13'Val
''8'Thr'

Observed':'22

Yield'4mg/ml

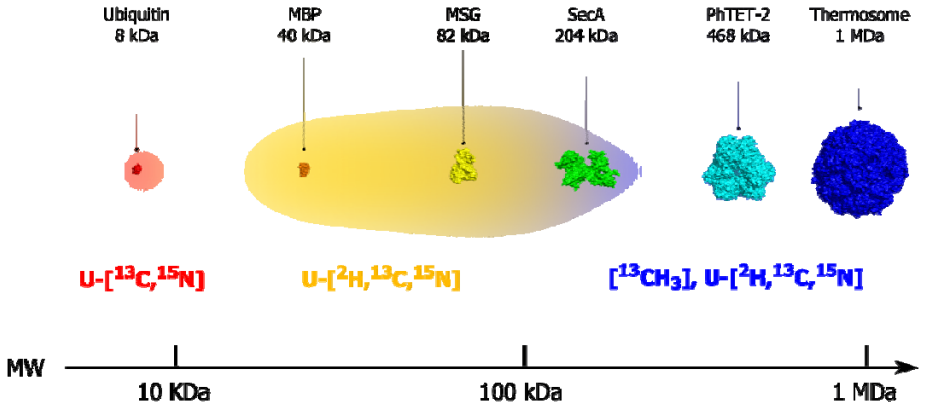




'HSQC''600MHz''H23'(16'kDa)'

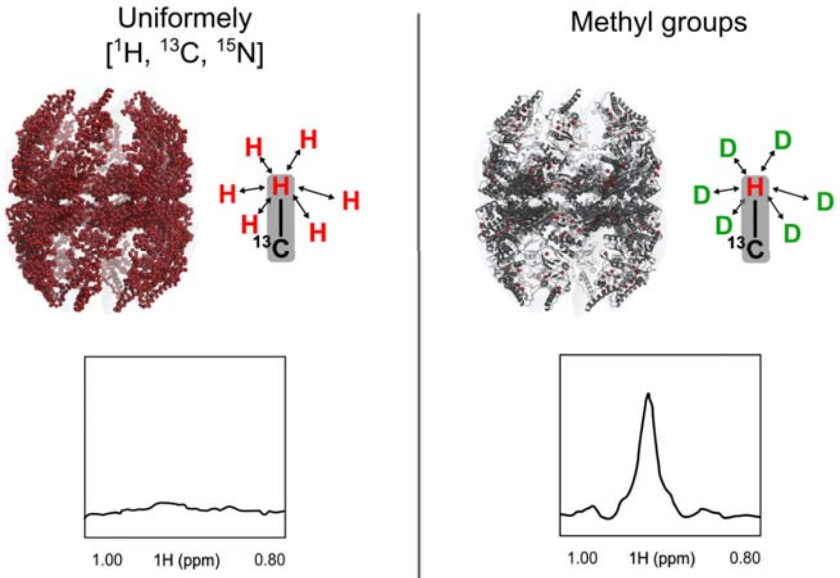
Lionel Imbert (IBS) Cell-Free expression platform

Is NMR spectroscopy only limited to small molecules?



Courtesy of J Boisbouvier et al.

Solution NMR Spectroscopy
Specific protonation of methyl groups

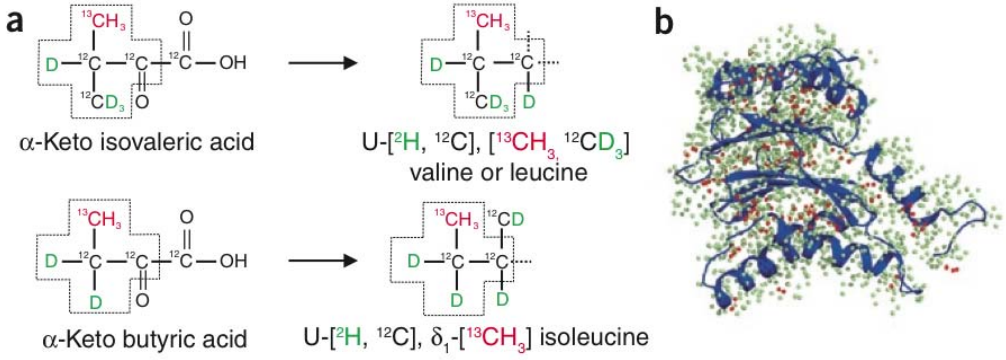


Gardner *et al*, J. Am. Chem. Soc. (1997)

Solution NMR of supramolecular complexes: providing new insights into function

Remco Sprangers, Algirdas Velyvis & Lewis E Kay

Nature Methods 2007



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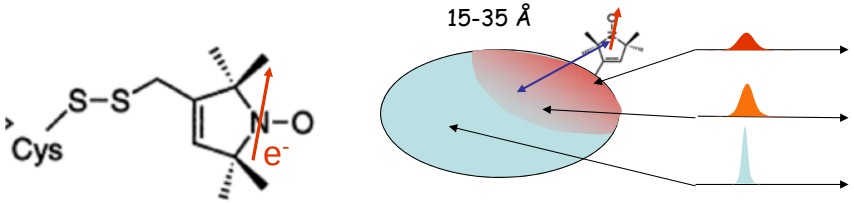
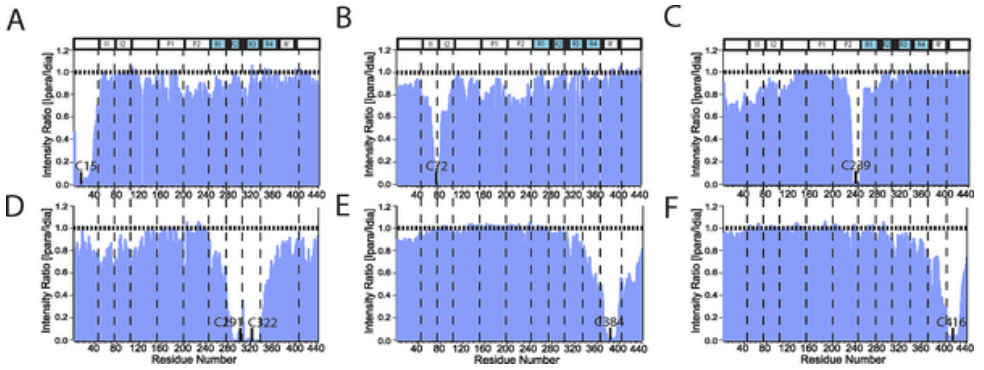
Introducing spin label (PRE)



- The electronic spin induces an efficient relaxation of nuclear spins at long range distances
- This effect can be used to get structural restraints in large complexes
- This method prove to be invaluable to detect transient molecular events (encounter complexes, excited states, protin sliding on DNA,…)

Application to disordered proteins (Tau)



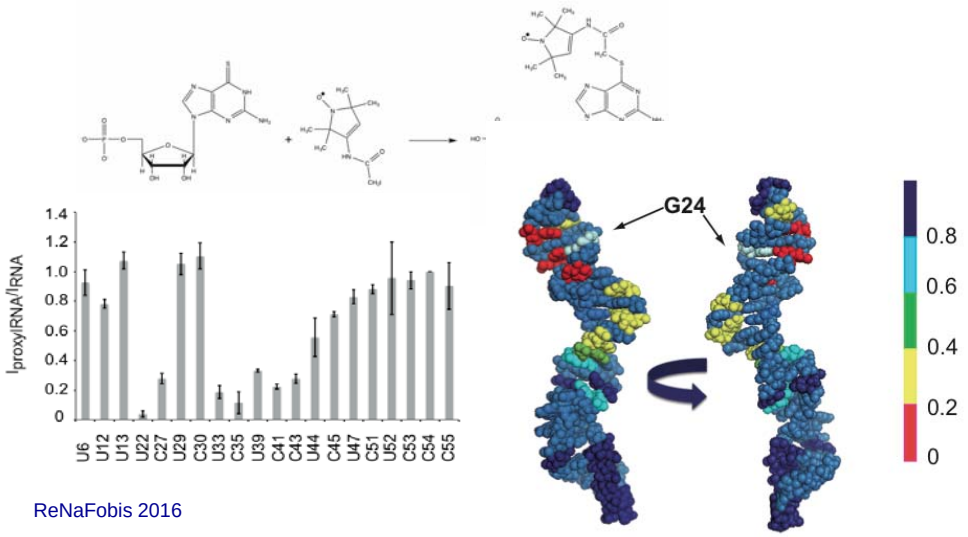
Mukrasch MD, Bibow S, Korukottu J, Jeganathan S, et al. (2009) Structural Polymorphism of 441-Residue Tau at Single Residue Resolution. PLoS Biol 7(2): e1000034. doi:10.1371/journal.pbio.1000034
http://www.plosbiology.org/article/info:doi/10.1371/journal.pbio.1000034

ReNaFobis 2016

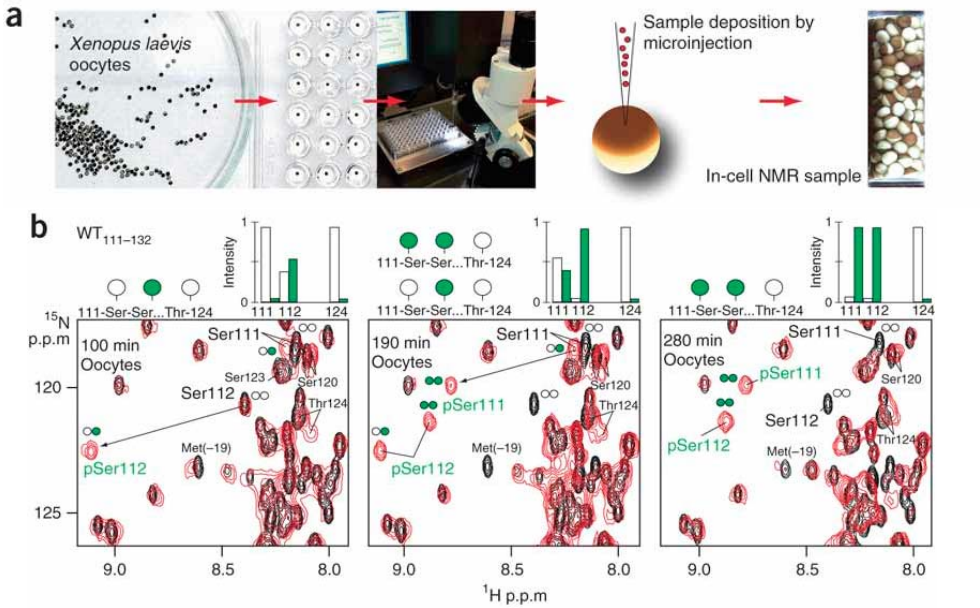
PLOS | BIOLOGY

RNA spin labelling

Lebars I.*, Vileno B., **Bourbigot S.**, Turek P., Wolff P. & **Kieffer B.** (2014)
"A fully enzymatic method for site-directed spin-labeling of long RNA", *Nucleic Acids Res*., 42(15), e117,



In-cell NMR



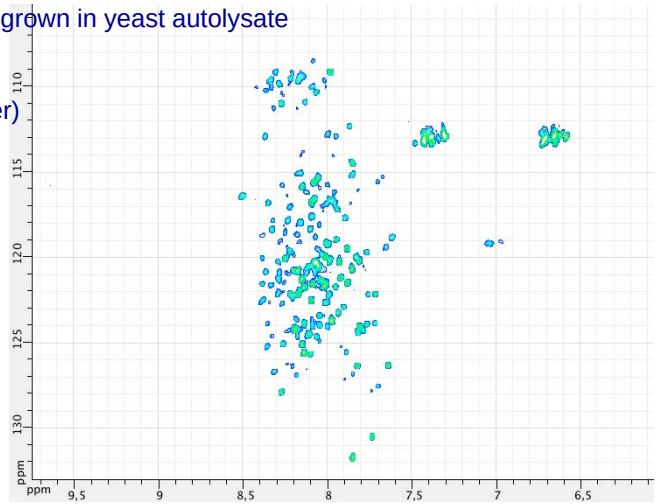
Selenko et al. Nature Structural & Molecular Biology 15, 321 - 329 (2008)

Expression of ^{13}C, ^{15}N labelling proteins in eukaryotic cells

Full length RXR nuclear receptor uniformly labelled with ^{15}N
The protein forms an homodimer with 100 kDa molecular weight.

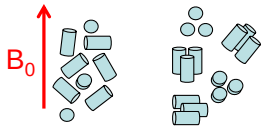
Expression in S2 insect cells grown in yeast autolysate

- Coll. François Bontems (Pasteur, ICSN, BioSpinger)

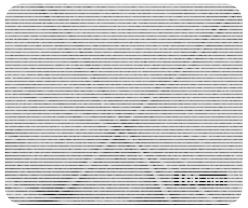


Three States of living matter

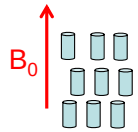
Solid state



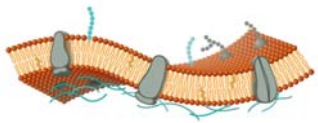
Static disorder



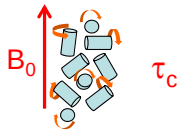
Solid state Oriented



Static Order



Solution



Dynamic disorder