Marc-André Delsuc

- Aujourd'hui à l'IGBMC de Strasbourg (8 ans)
  - après Gif-sur-Yvette (10 ans) et Montpellier (15 ans)
  - chimie-orga - peptides - protéines - IDP
- Intérets:
  - IDP - PolyProlines - Récepteur Androgène
- Méthodes
  - RMN

    méthodologie - processing
  - FT-MS *depuis peu*

    2D FT-ICR - processing - top-down
  - processing / analyse *depuis toujours*

    Analyse de Fourier

    Compressed Sensing

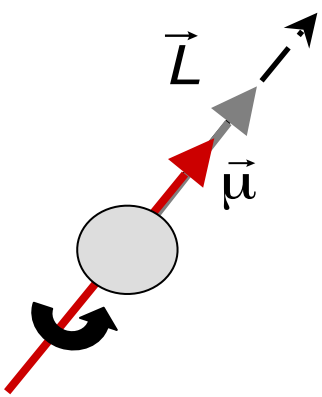
    Analyse statistique

NMR Principles
from observation to structural information

Marc-André Delsuc
Renafobis - 2017

Nuclear **M**agnetic **R**esonance

- Spectroscopy of the magnetic properties of the nuclei of atoms



Nuclear Magnetic Resonance

- Spectroscopy of the magnetic properties of the nuclei of atoms
  - Some atom nucleus have a spin≠0
    - ^{1}H spin ½ ≡ the proton
    - ^{2}H spin 1 low abund.
    - ^{13}C spin ½ low abund.
    - ^{15}N spin ½ low abund.
    - ^{19}F spin ½
    - ^{31}P spin ½
    - …

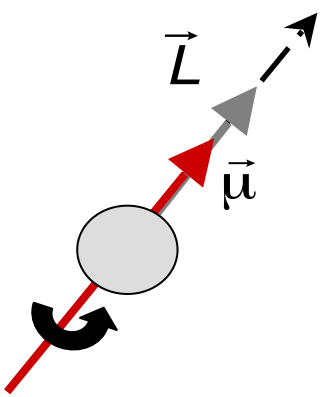
the most interesting to the biologist

- What is the spin ???

$$\left(\beta mc^2 + \sum_{k=1}^{3} \alpha_k p_k c\right) \psi(\mathbf{x},t) = i\hbar \frac{\partial \psi(\mathbf{x},t)}{\partial t}$$

  - appears as the solution of the Dirac equation
    - Schrödinger + Relativity
  - an intrinsic property of particules (*and black holes*)
    - as the mass or the charge
  - carries a momentum
    - magnetic momentum - angular momentum

$\vec{\mu}$ $\vec{L}$

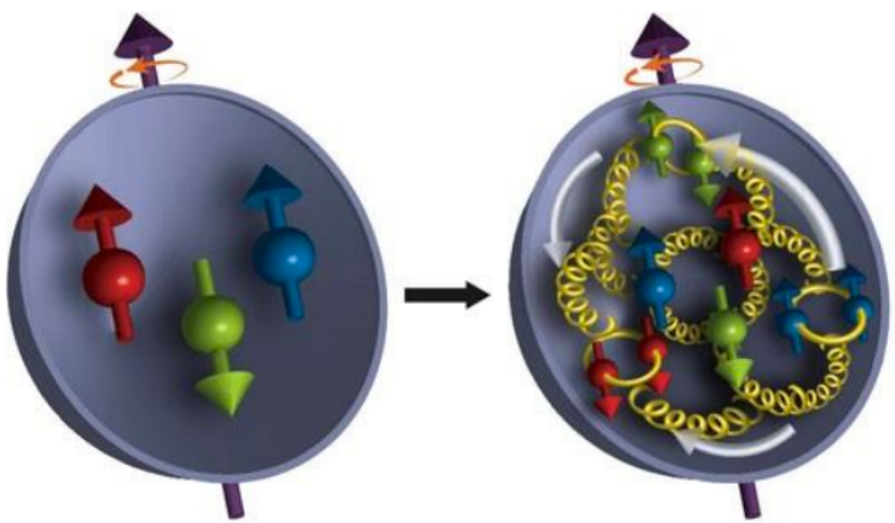


Nuclear Magnetic Resonance

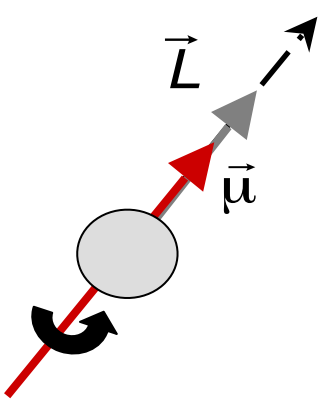
- Spectroscopy of the magnetic properties of the nuclei of atoms

How Did The Proton Get Its Spin?

Mon, 04/03/2017 - 11:01am Comments by Department of Energy, Office of Science



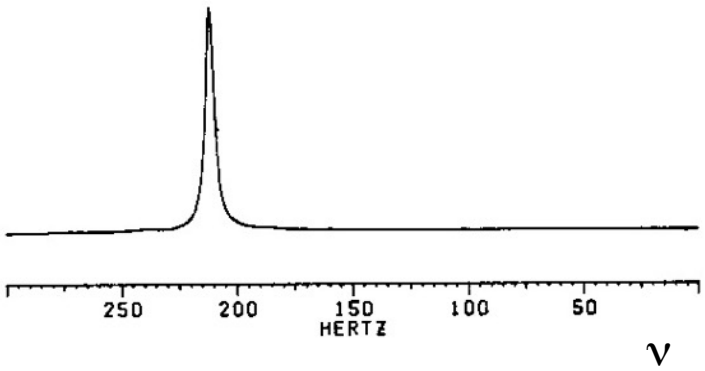
In the 1980s, scientists discovered that a proton's three valance quarks (red, green, blue) account for only a fraction of the proton's overall spin. More recent measurements have revealed that gluons (yellow corkscrews) contribute as much as or possibly more than the quarks. Photo courtesy of Brookhaven National Laboratory

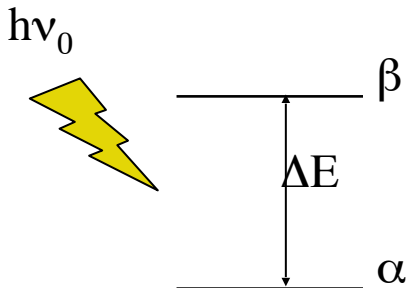


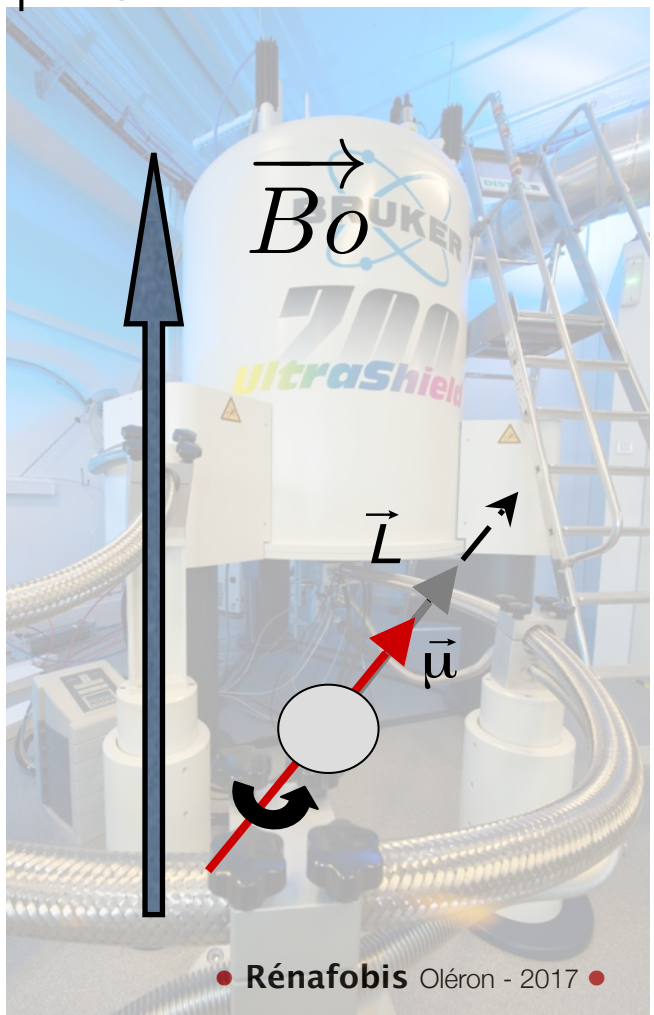
https://www.rdmag.com/news/2017/04/how-did-proton-get-its-spin

Nuclear **Magnetic** Resonance

- Spectroscopy of the magnetic properties of the nuclei of atoms
  - In presence of a strong magnetic field Bo, a spin *n* presents *2n+1* different energy states, so a spin ½ ⇒ 2 states
  - Energy difference ΔE
    - determines the transition frequency
    - is proportional to Bo





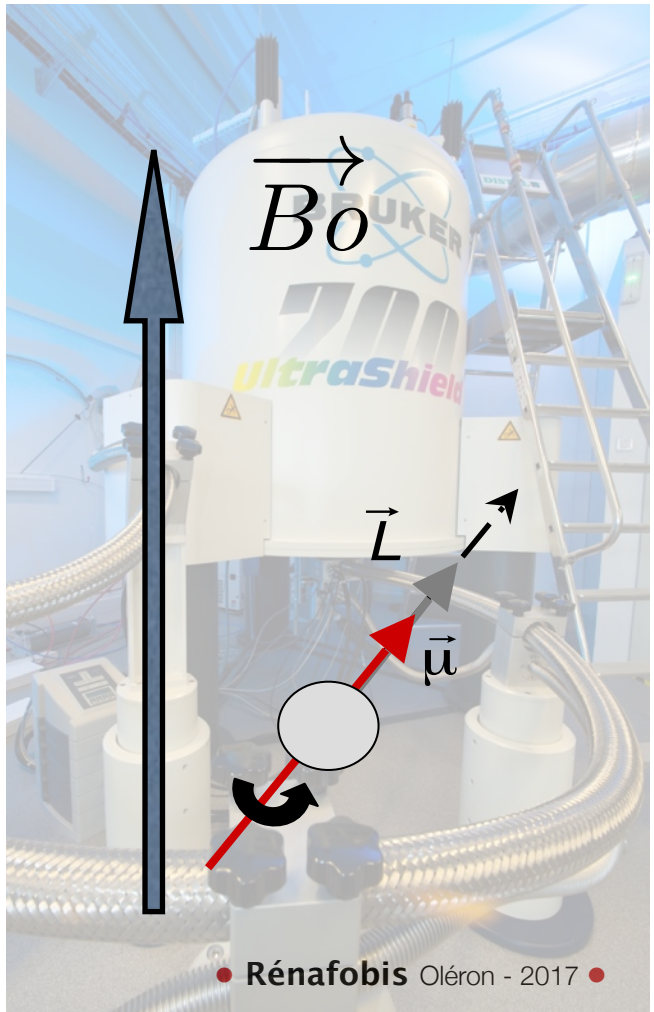


Nuclear **Magnetic** Resonance

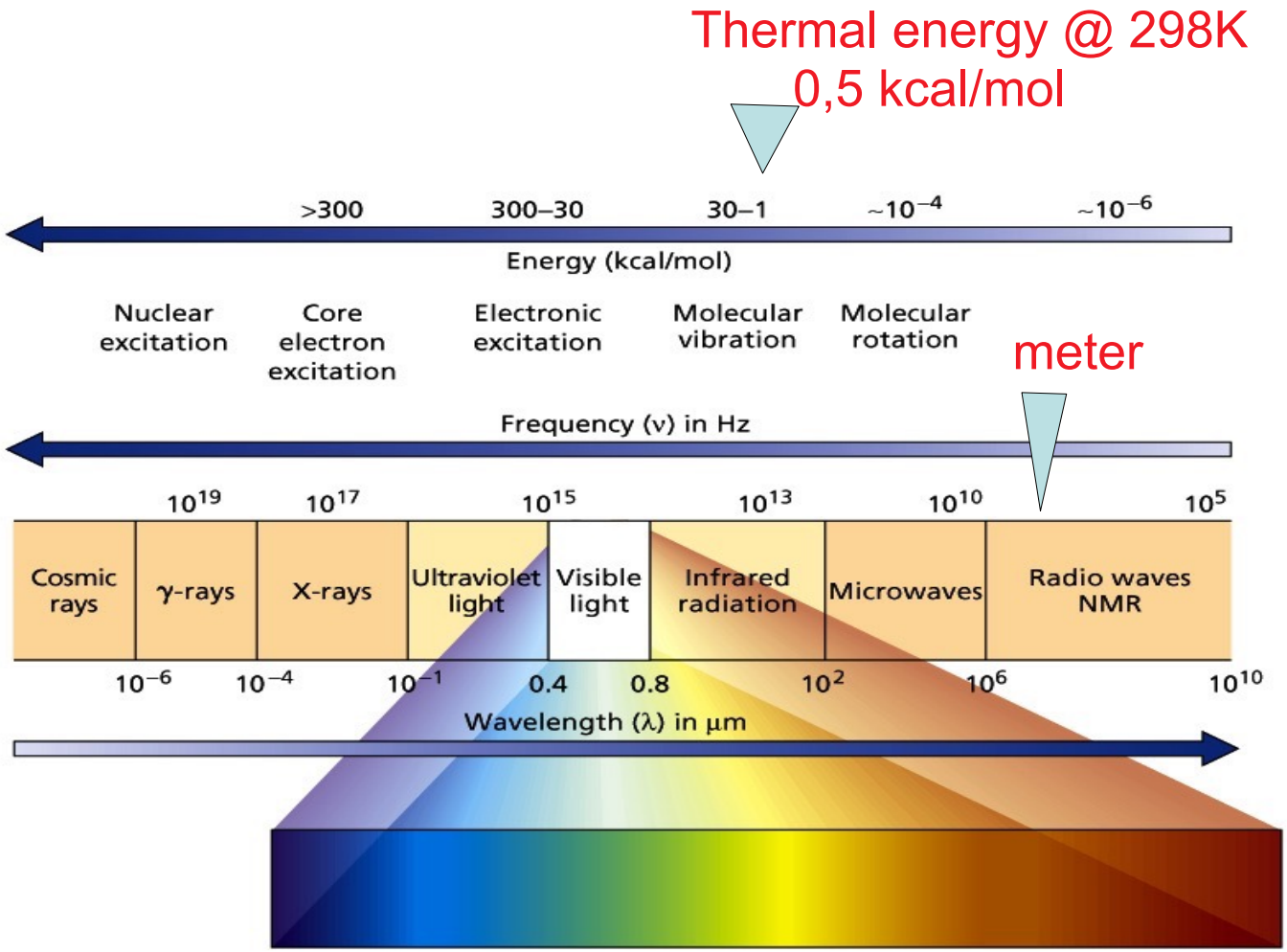
- Spectroscopy of the magnetic properties of the nuclei of atoms

$$E = \hbar\nu \qquad \nu = \gamma|Bo|$$

  - with γ depending on nucleus type
    - ^{1}H spin ½ 800 MHz
    - ^{2}H spin 1 low abund. 123 MHz
    - ^{13}C spin ½ low abund. 200 MHz
    - ^{15}N spin ½ low abund. 80 MHz
    - ….



NMR is a very low energy spectroscopy



Energy

$$E = \hbar \gamma B_0$$

Frequency

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

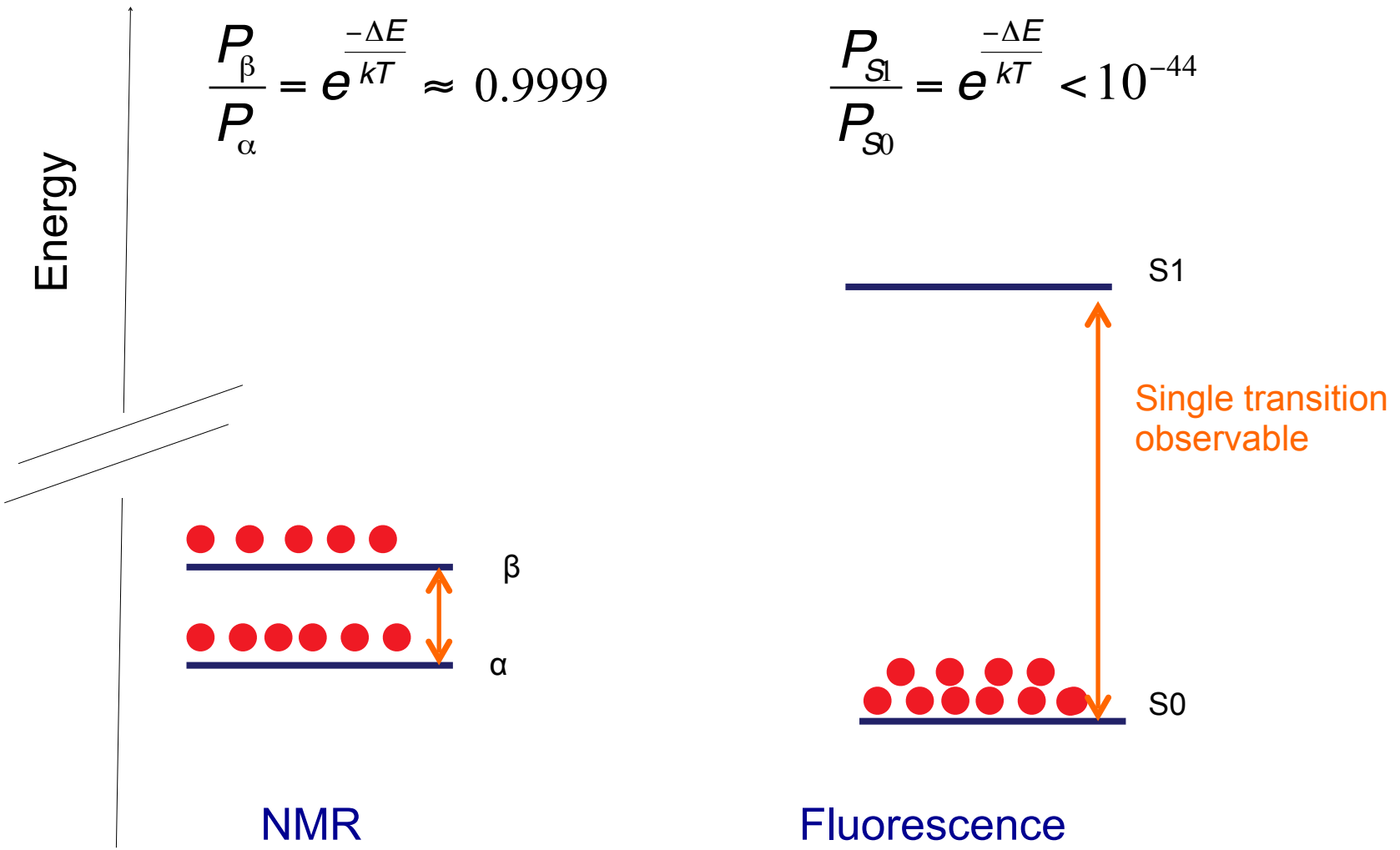
Wavelength

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

Low-energy = low sensitivity

$$\frac{P_\beta}{P_\alpha} = e^{\frac{-\Delta E}{kT}} \approx 0.9999$$

$$\frac{P_{S1}}{P_{S0}} = e^{\frac{-\Delta E}{kT}} < 10^{-44}$$

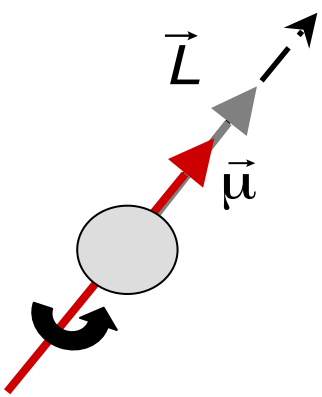


From B Kieffer

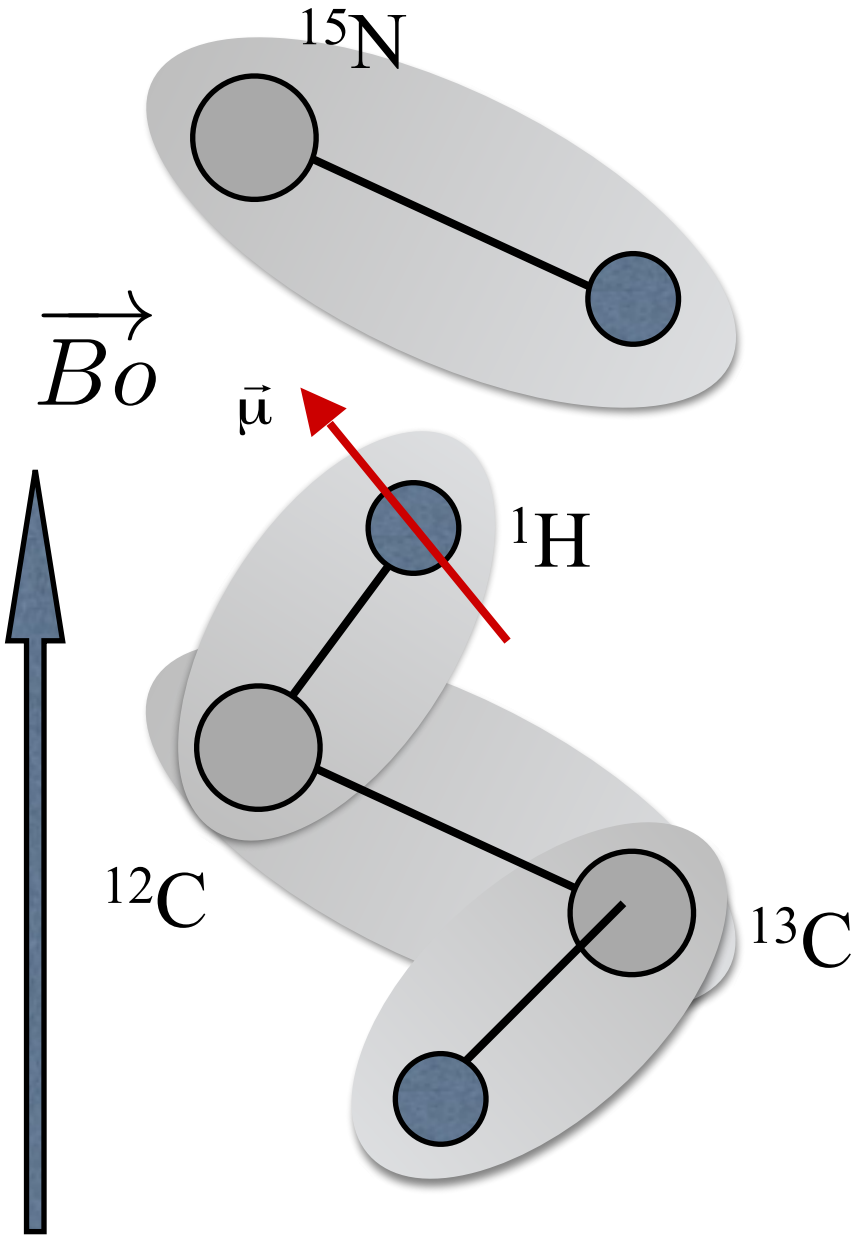
Nuclear Magnetic **Resonance**

- Spectroscopy of the magnetic properties of the nuclei of atoms
- The Quantum Resonance
  - phase coherence of the wave functions of the different particules
- observed / used in
  - LASERS
  - Quantum Computers
  - NMR
- characterized by
  - strange quantum effects
  - coherence transfers
  - decoherence limits life time
  - enhanced sensitivity

carry home messages:
NMR effect is QM
it is complicated!

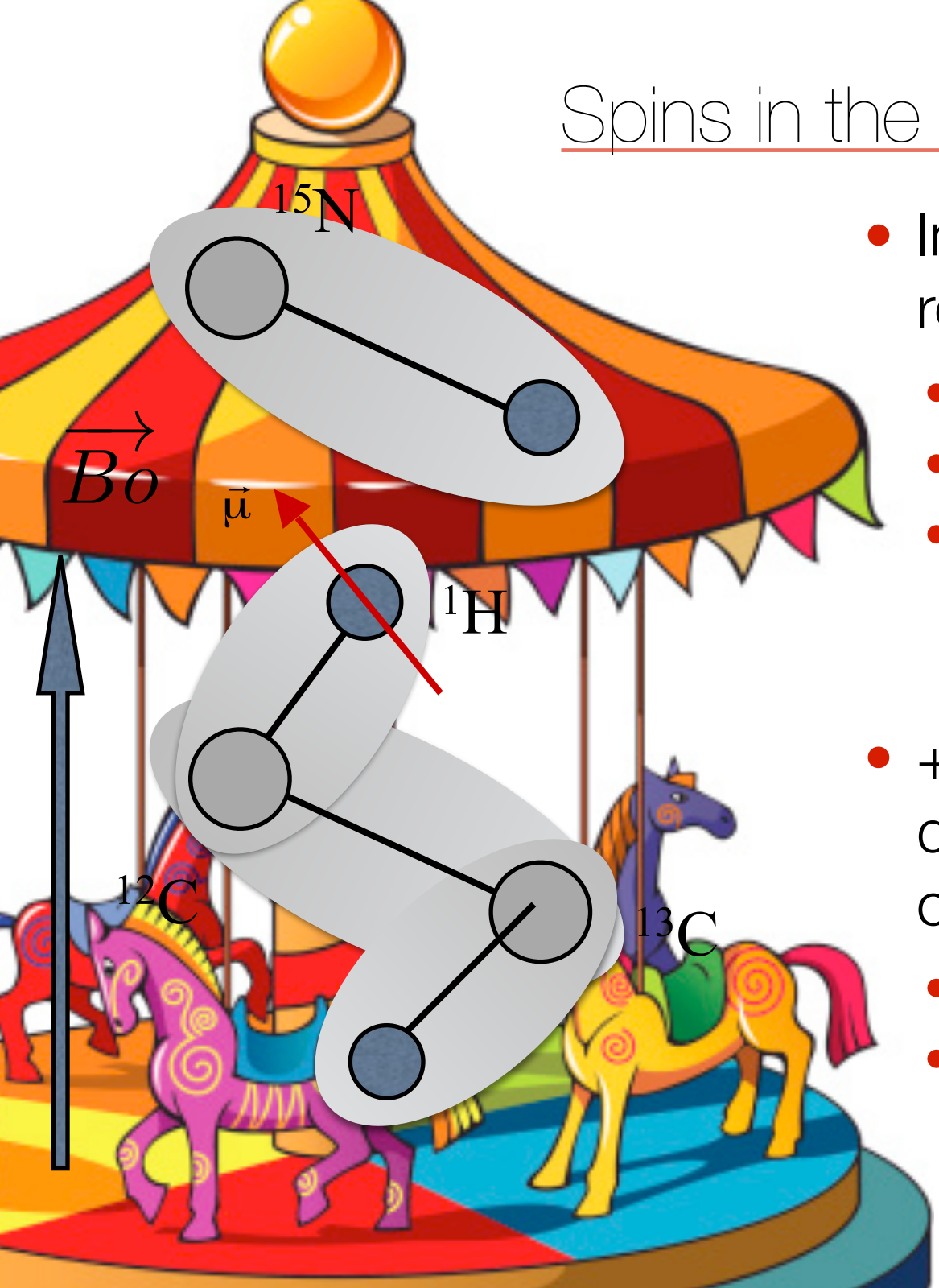


Spins in the Field…



- nuclear spins interact with
  - other nuclear spins
  - molecular orbitals
    - local
    - nearby
  - electronic spins
  - …
- act as perfect spies
  - no impact on molecular phenomenon
- and perfect reporters
  - will react to anything

Spins in the Field…



- In NMR EVERYTHING is rotating
  - in physical space
  - in quantum space
  - rotating in a rotating frame
    ⇒ Precession

- + ALL interactions are depending strongly on orientation
  - molecular axis vs Bo
  - spin-spin axis vs Bo
    ⇒ Tensor algebra

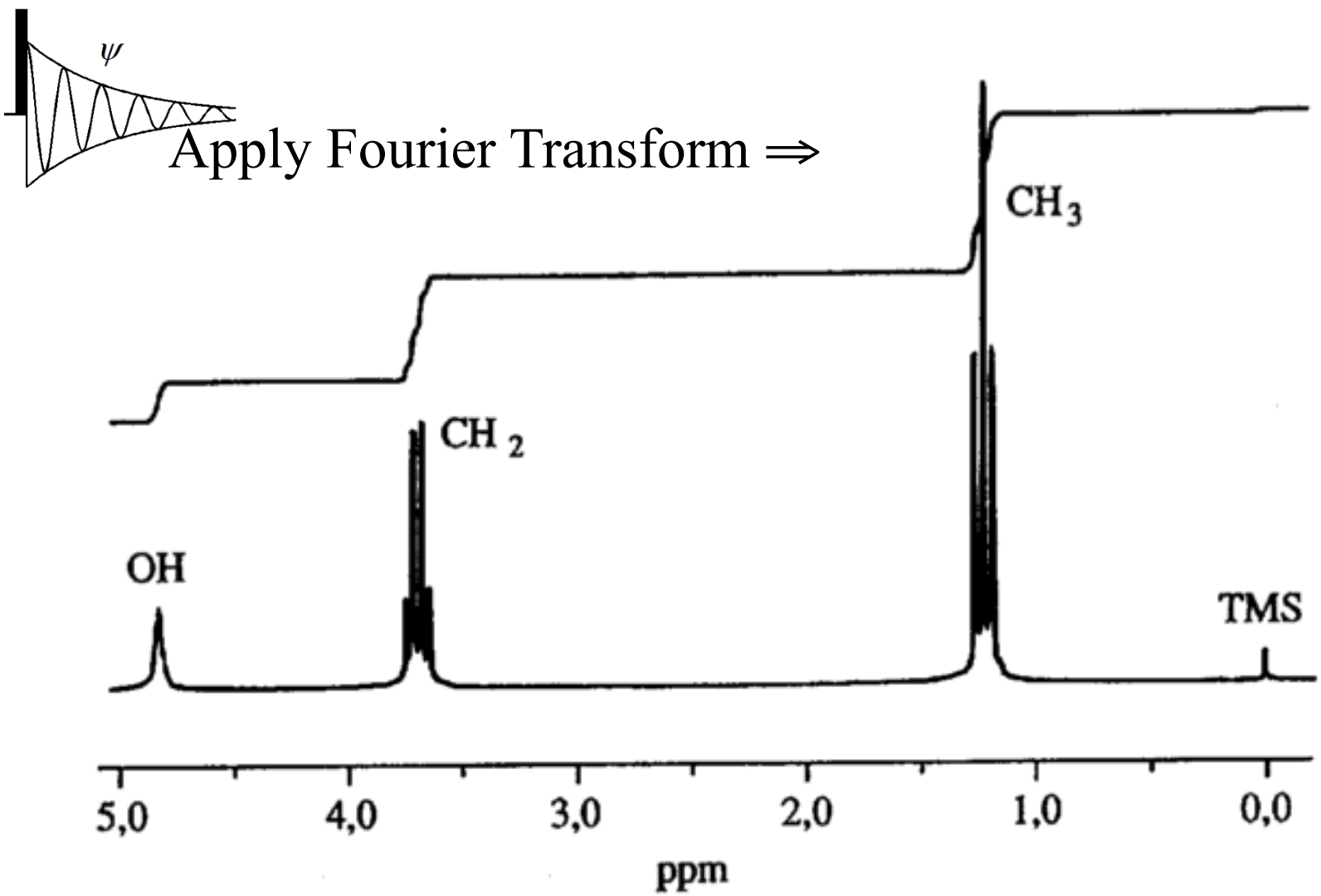
Phenomenon

- Chemical-Shift
  - resonance frequency
- Spin-Spin interaction
  - many effects J, D, RDC, NOE…
- Relaxation
  - *decoherence* of the quantum states
  - TWO main effects
    decoherence of resonance: loss of signal T_2
    return to initial steady state: recovery: T_1
  - …
- Measure by impulse response

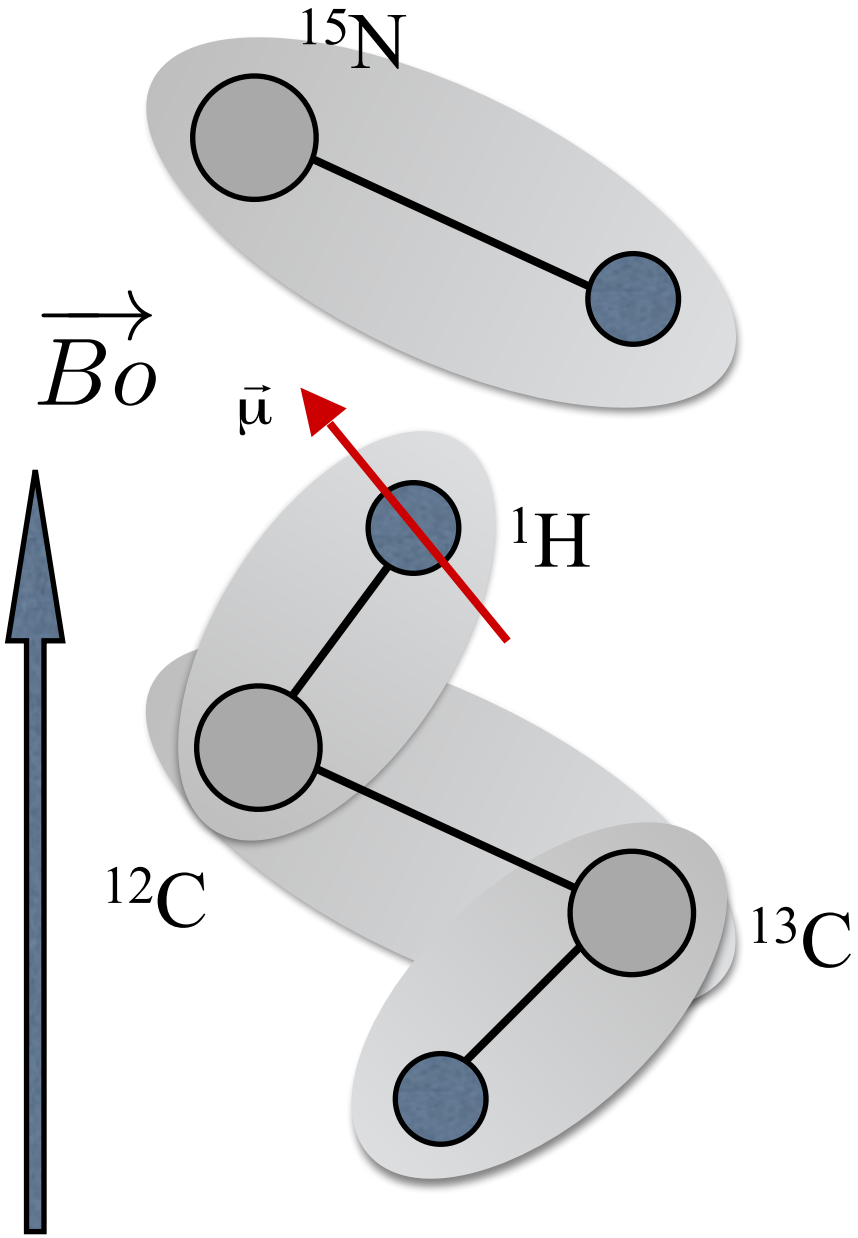
The NMR observables…on a simple molecule

- Ethanol ^1H spectrum

$HO\text{-}CH_2\text{-}CH_3$



Chemical Shift



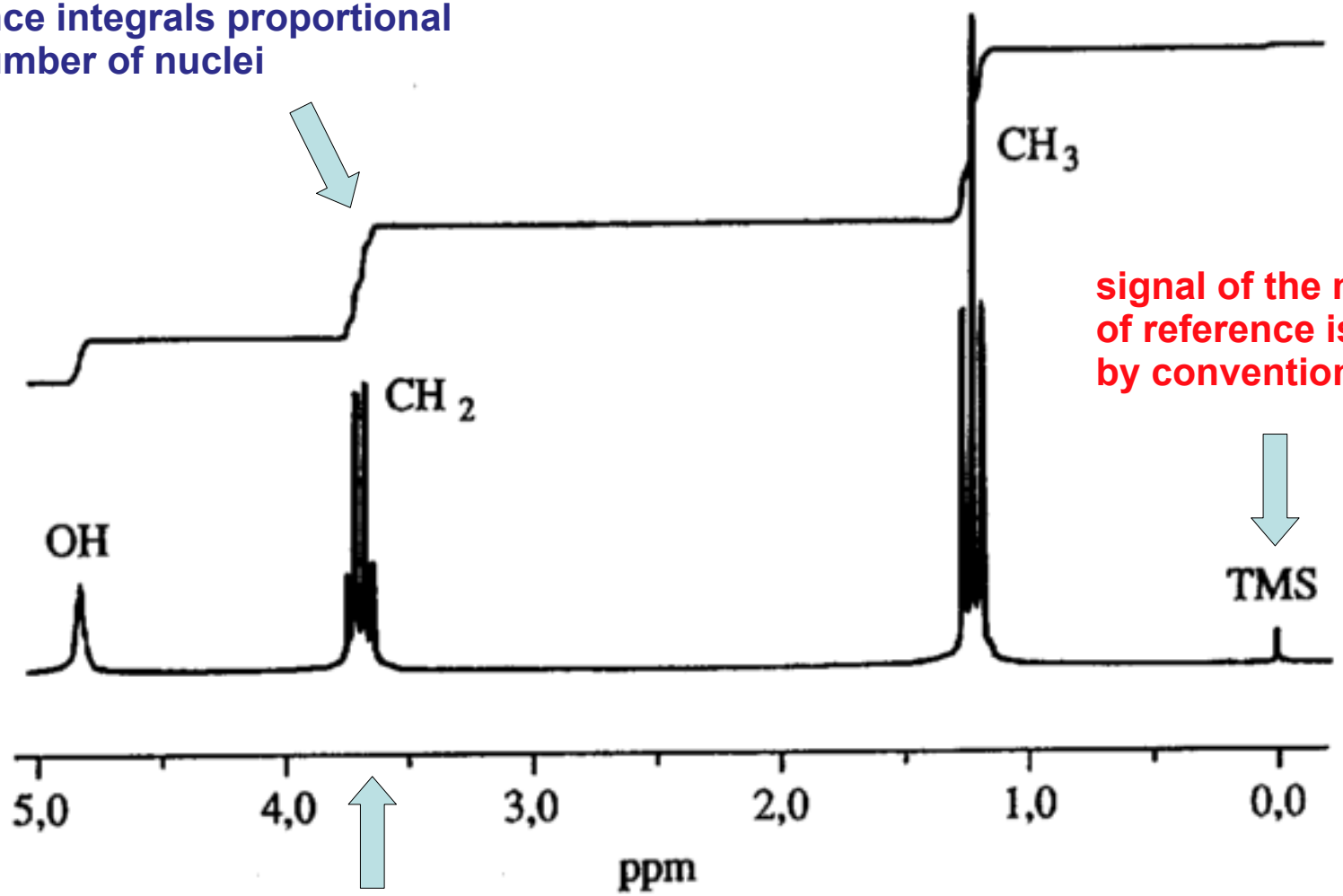
- CS depends strongly on molecular orientation vs Bo
- in liquids all values are averaged
  - only mean value is observed
  - in solids ⇒ wide lines
- isotropic CS is affected by shielding of the orbitals ⇒ *chemical* shift
- effect proportional to Bo ⇒ ratio (ppm) indep. of Bo

The NMR observables…on a simple molecule

- Ethanol ^1H spectrum

$HO\text{-}CH_2\text{-}CH_3$

Resonance integrals proportional to the number of nuclei



signal of the molecule of reference is set to 0 by convention

$Si\ (CH_3)_4$

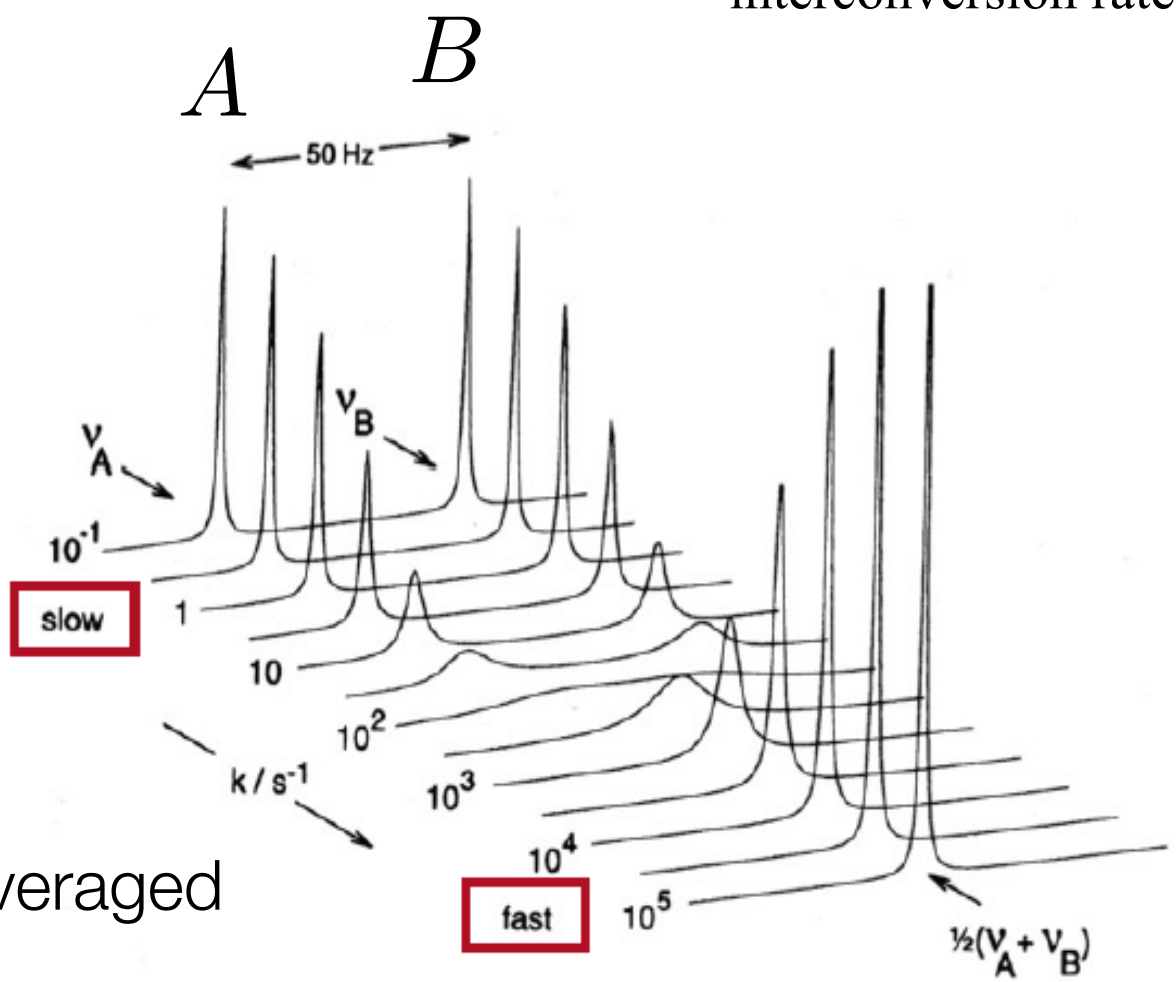
frequency depends on chemical environment => Chemical shifts

Averaging properties of NMR

$$A \overset{k}{\rightleftharpoons} B$$

k : characteristic interconversion rate

- True for *any* NMR observable
- coalescence depends *very much* on observable
  - ≠ life-time
  - ≠ frequency difference
  - ≠ interconversion rate

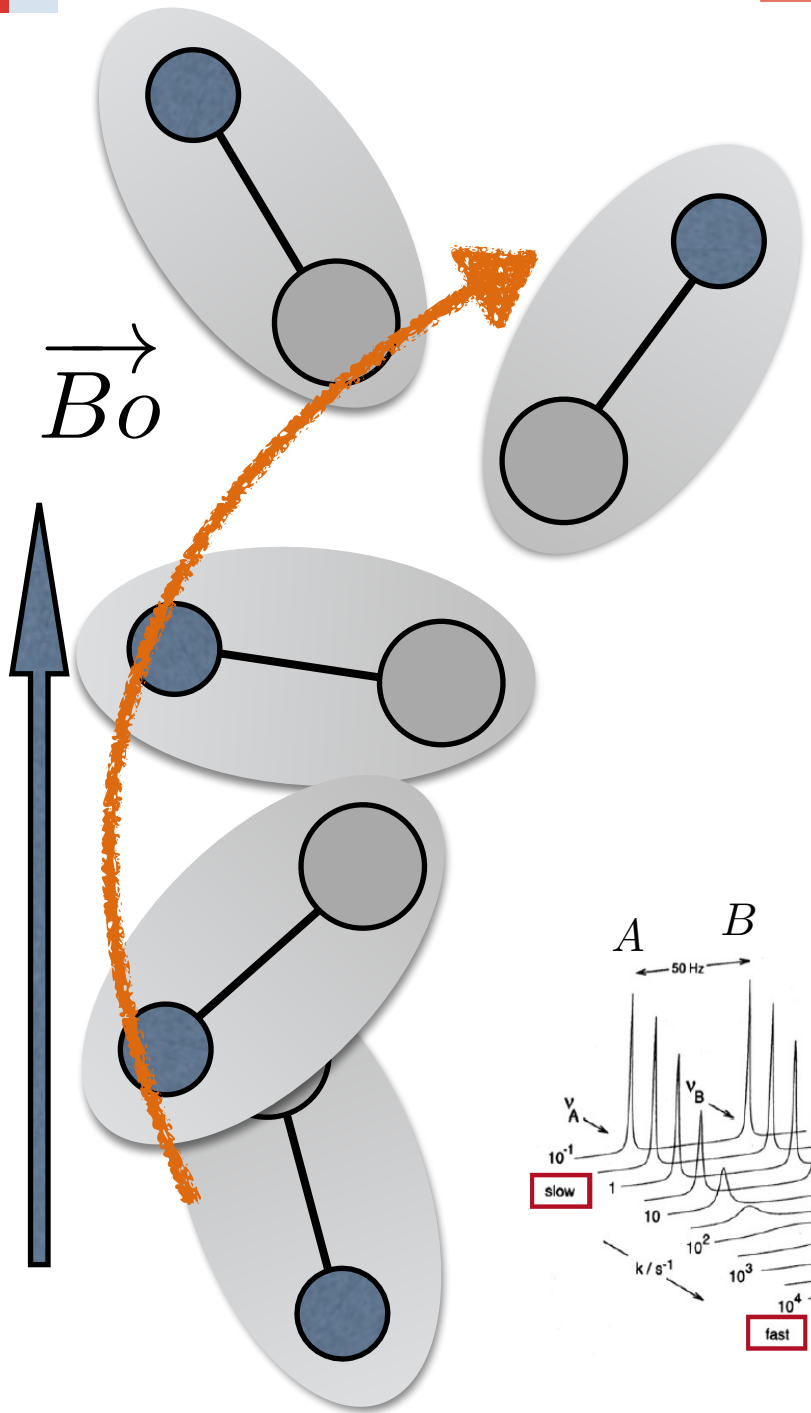


- in NMR EVERYTHING is averaged
  - over time
  - over molecules

double averaging

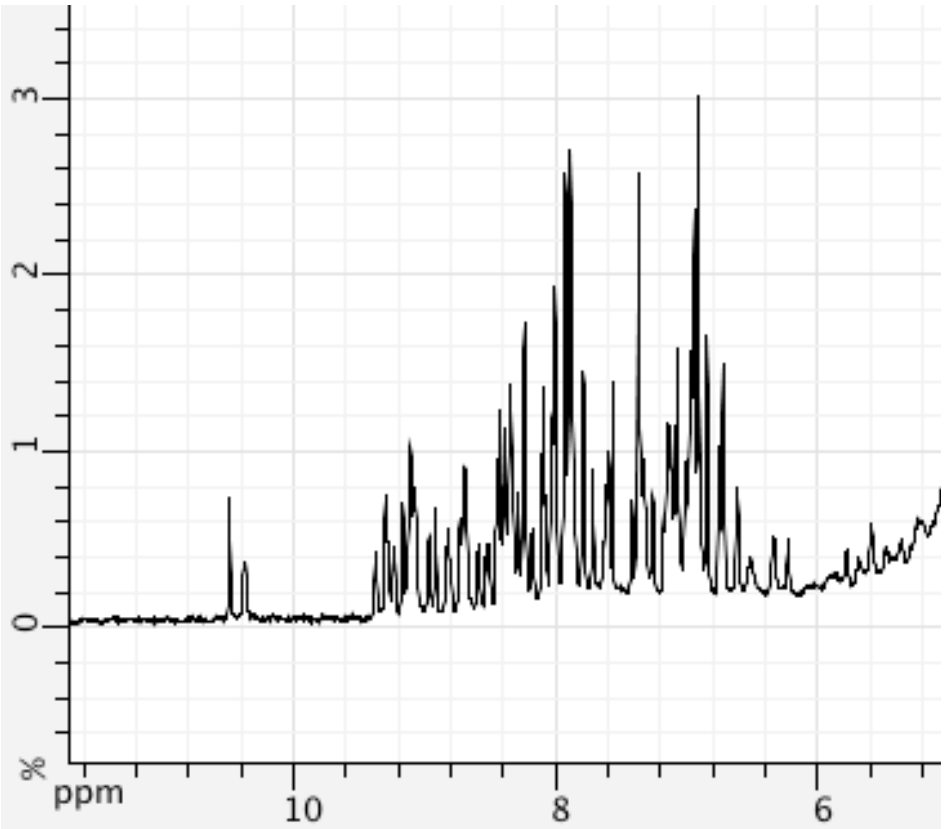
$$\frac{c_A\nu_A + c_B\nu_B}{c_A + c_B}$$

Chemical Shift

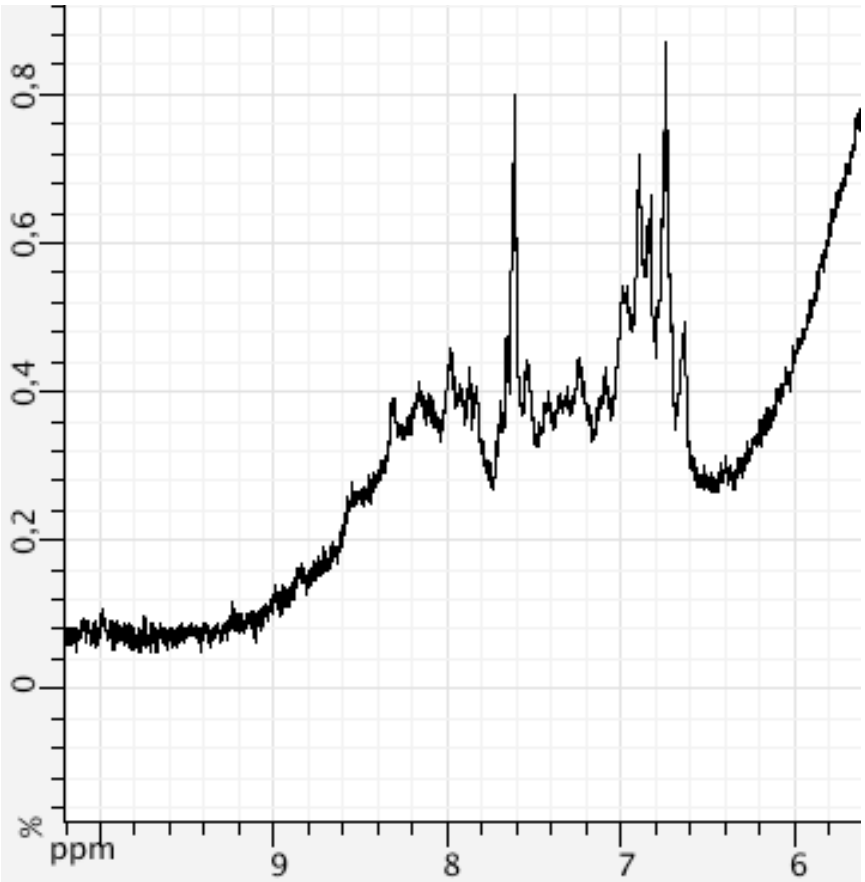


- In **liquids** during the measure, the molecules *tumble* and take successively the CS value of each orientation
- if *tumbling* is fast (small molecules) a sharp line is observed at mean CS value
- if *tumbling* is not so fast, the lines widen
- if in **solid** all CS appear ⇒ wide lines

effect of molecular size

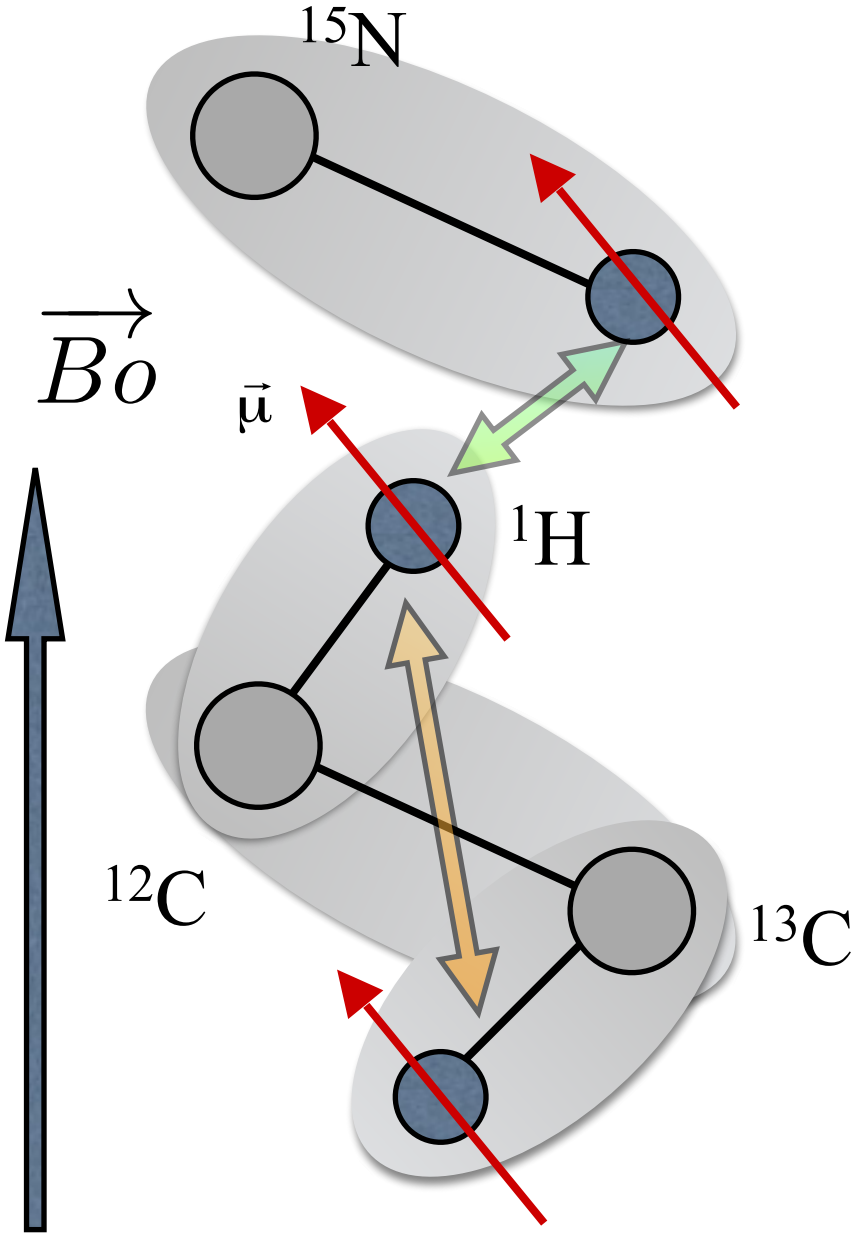


GB1 (~6 kDa)



LBD RXR Dimer (~50 kDa)

Spin-Spin interactions

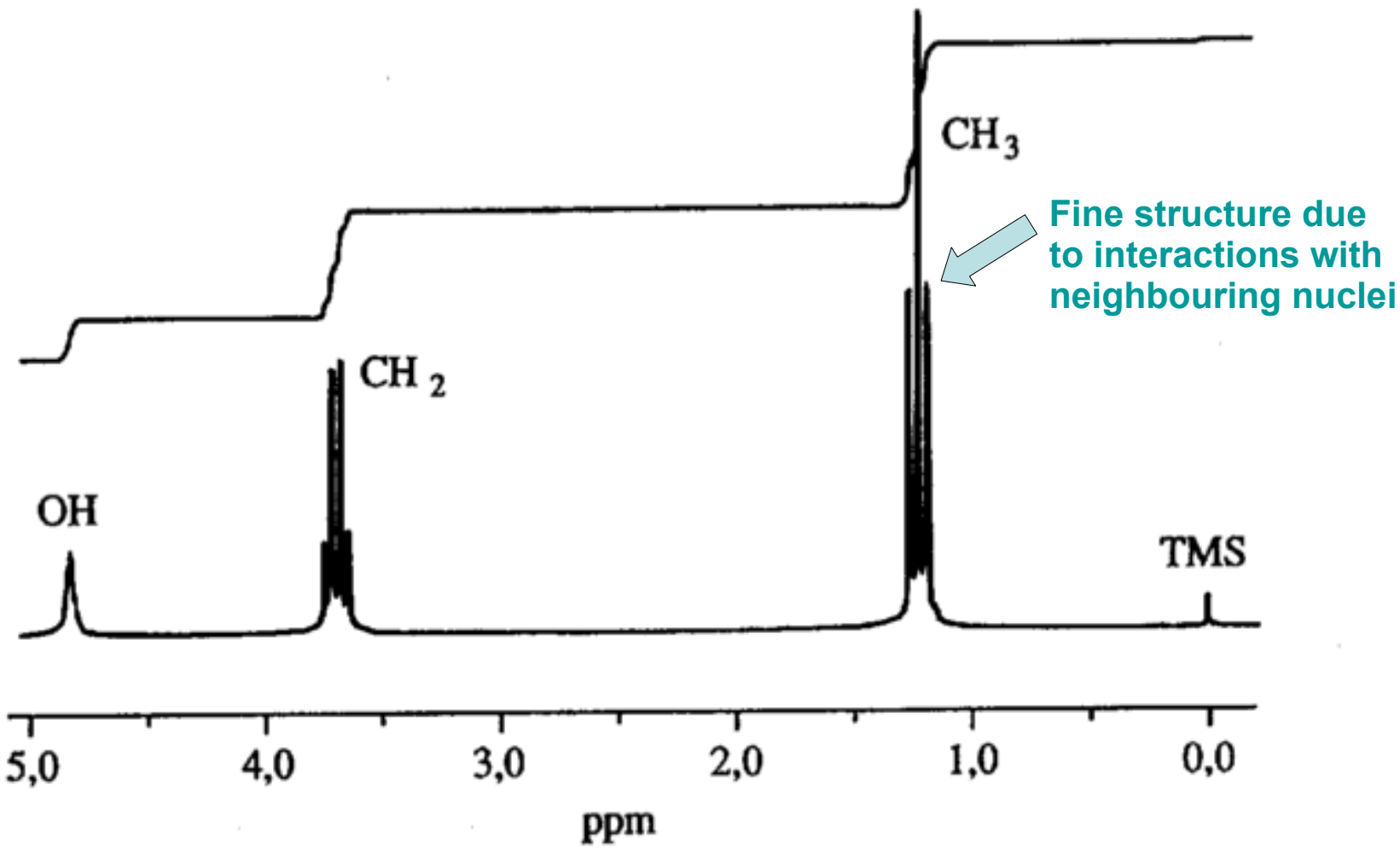


- Dipolar coupling **D**
  - Through space
  - depends on distance
    - and of course orientation
  - averaged to zero in isotropic liquids
  - relaxation effect

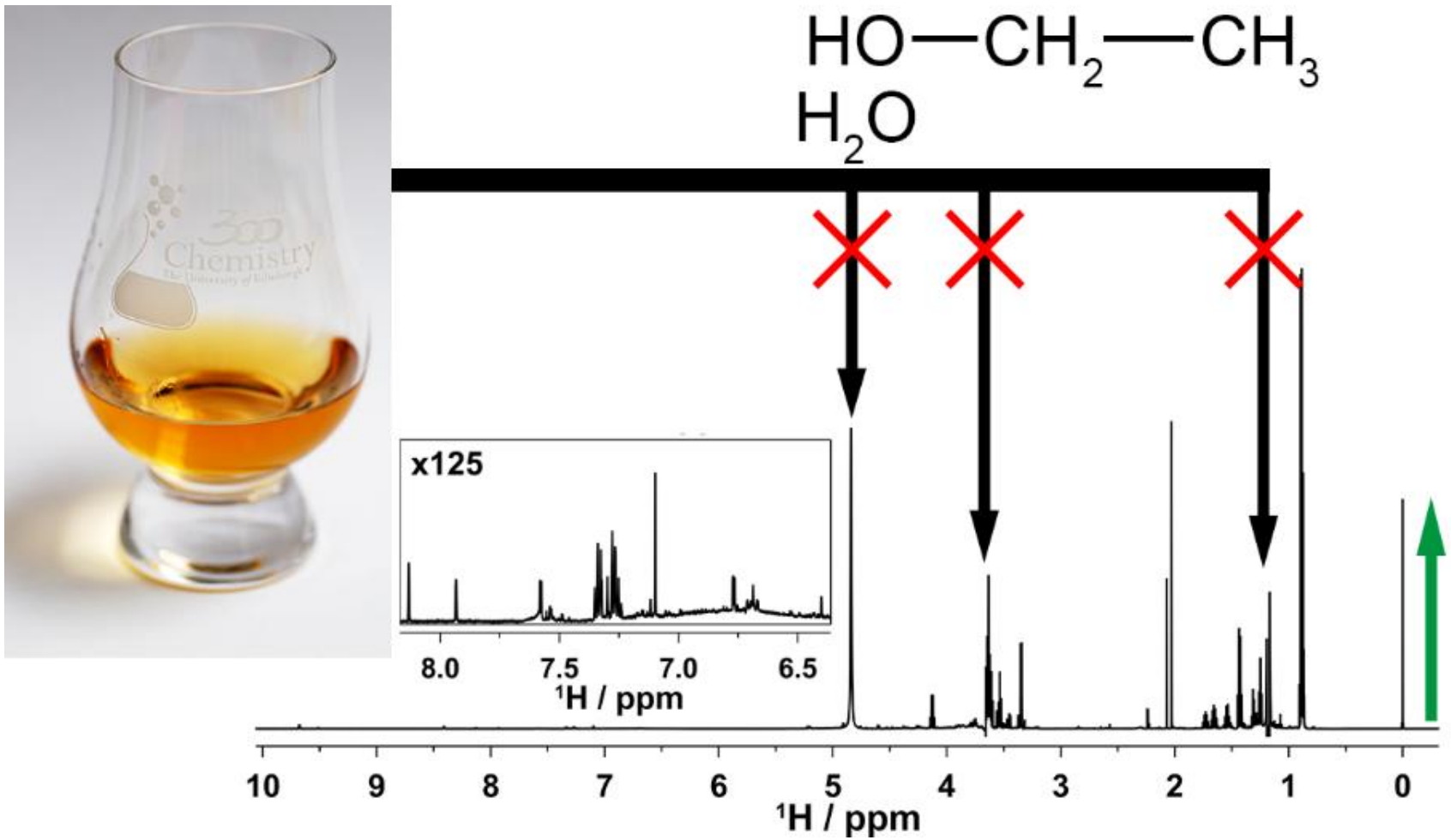
- Scalar coupling **J**
  - isotropic part of D
  - mediated by molecular orbitals
  - depends on molecular topology
    - and also on diedral angles

J-Coupling

- Ethanol ^{1}H spectrum

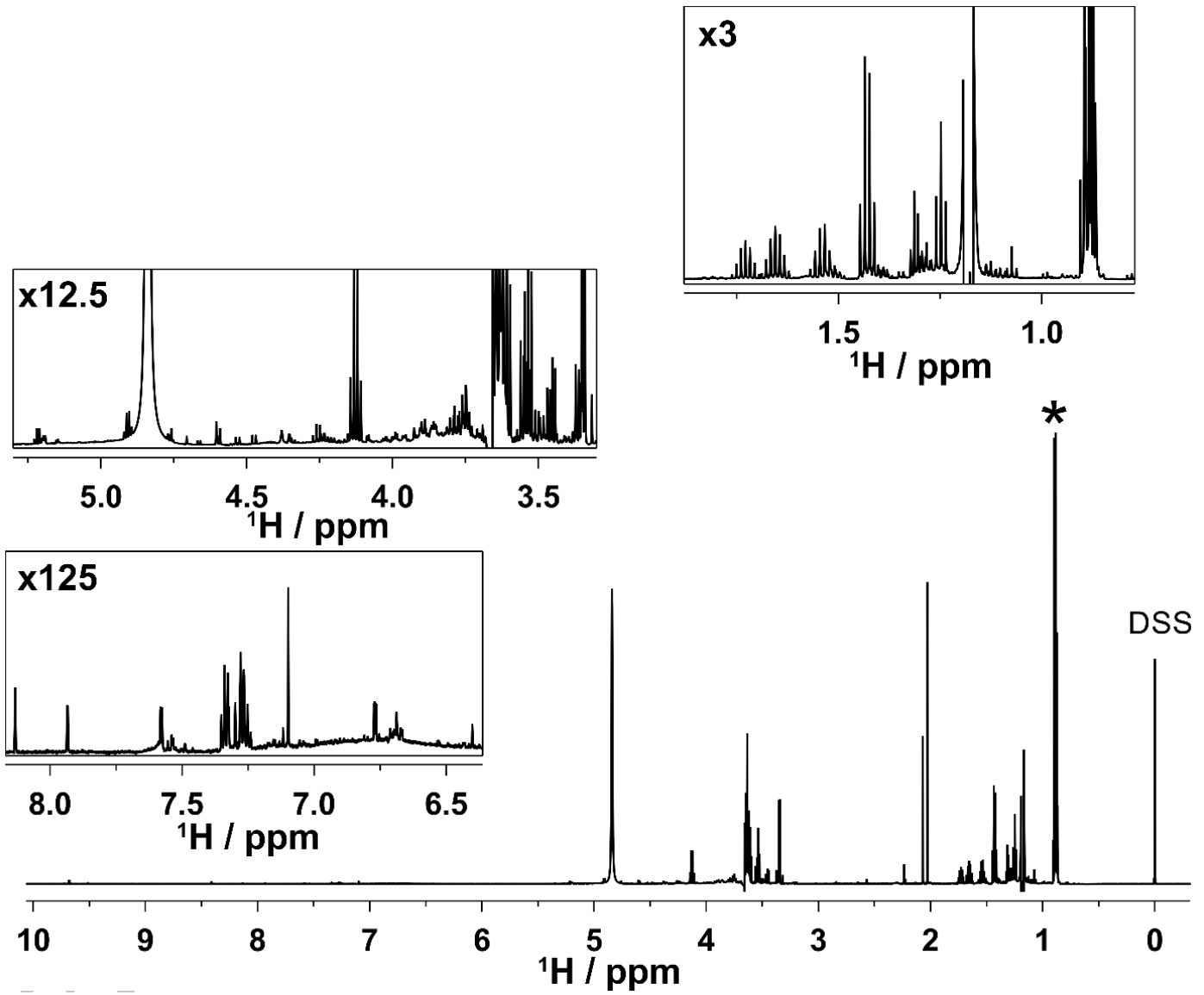


NMR Spectra of Scotch Whisky



Will Kew, Nicholle G. A. Bell, Ian Goodall, Dušan Uhrín
(2017) *Magn Reson Chem* DOI: 10.1002/mrc.4621

NMR Spectra of Scotch Whisky



Will Kew, Nicholle G. A. Bell, Ian Goodall, Dušan Uhrín
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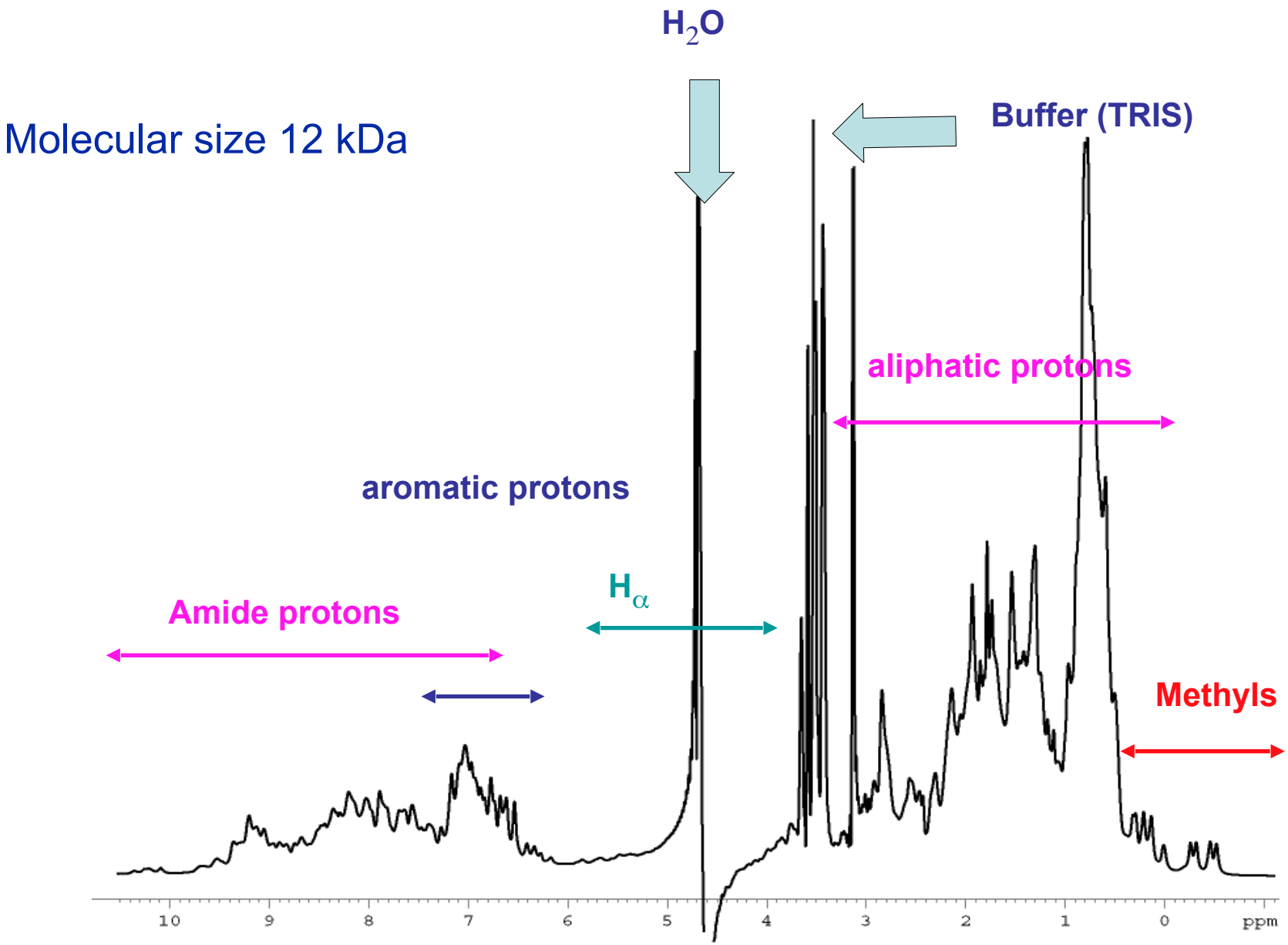
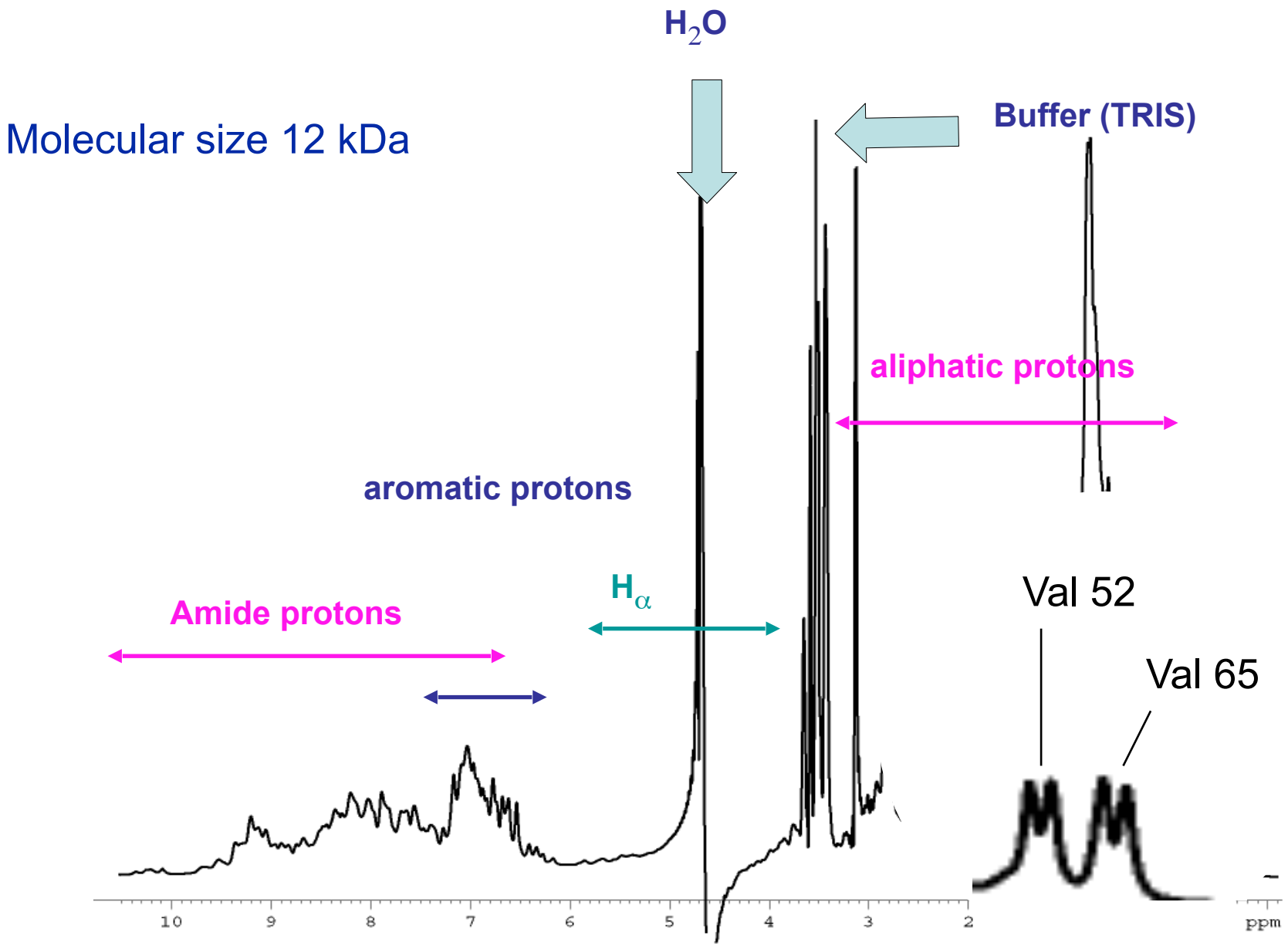
^{1}H NMR spectrum of a folded protein

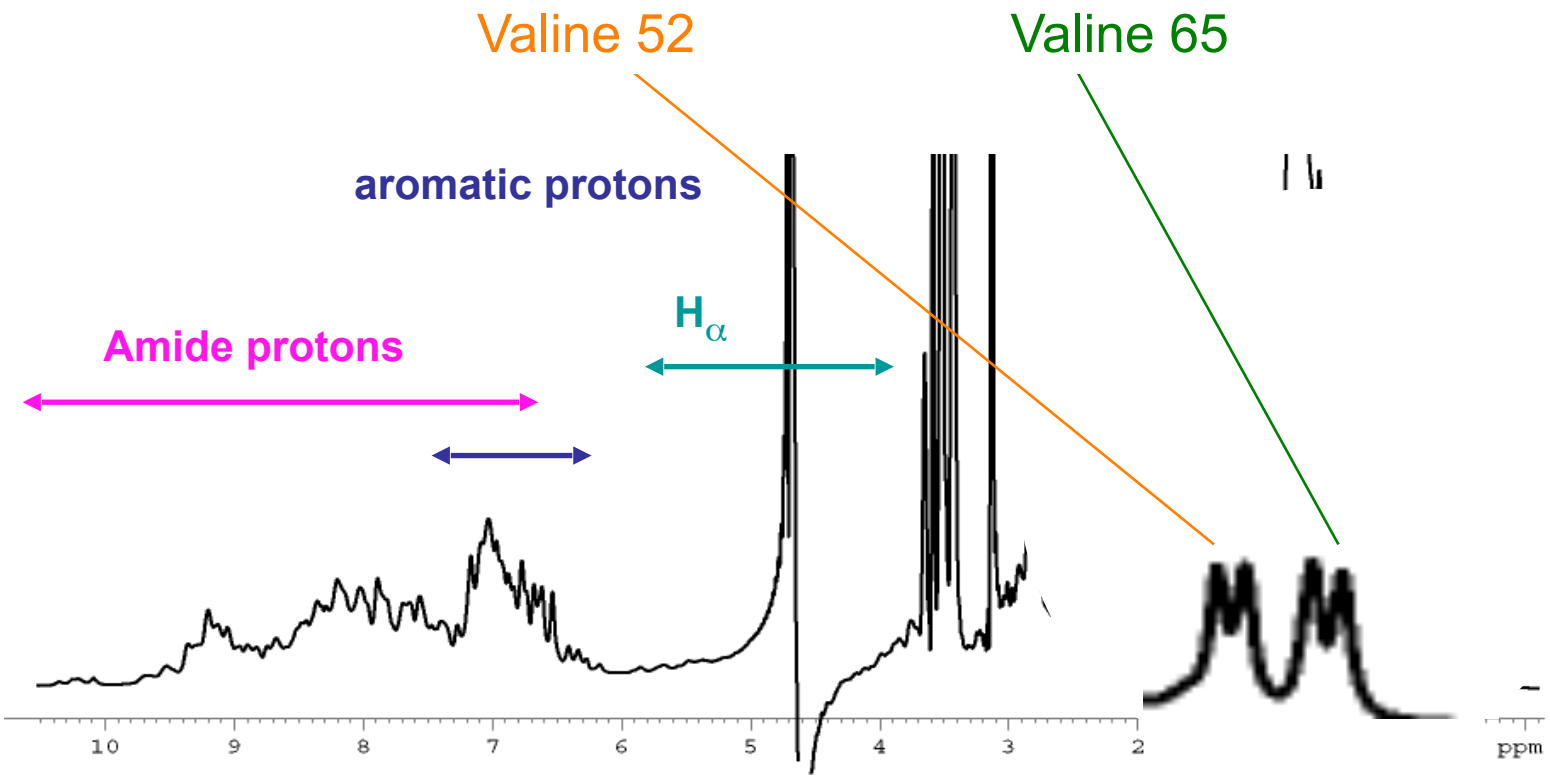


^{1}H NMR spectrum of a folded protein



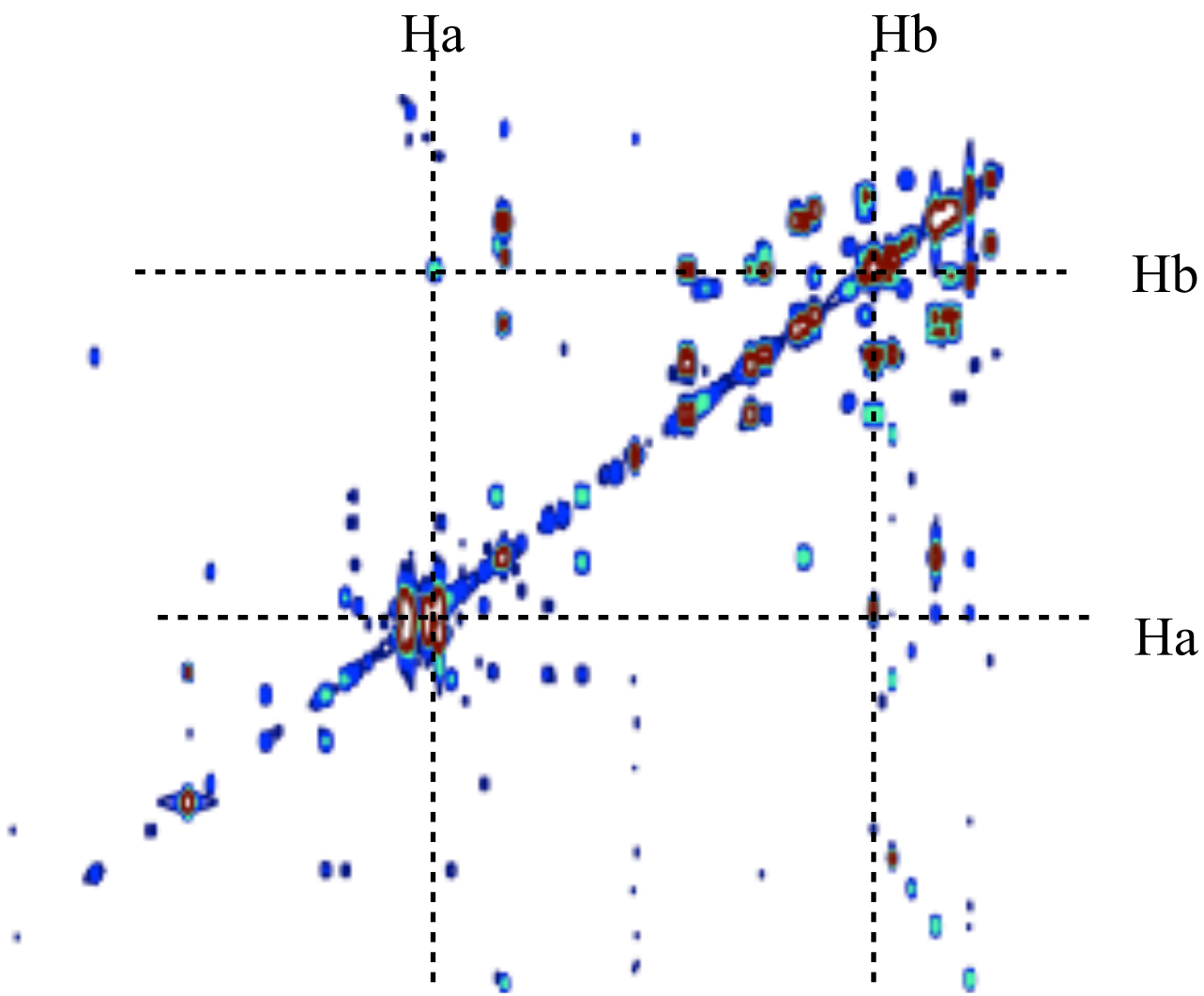
^{1}H NMR spectrum of a folded protein

```
MVKQIESKTA FQEALDAAGD KLVVVDFSAT WCGPCKMIKP
FFHSLSEKYS NVIFLEVDVD DCQDVASECE VKCMPTFQFF
KKGQKVGEFS GANKEKLEAT INELV
```

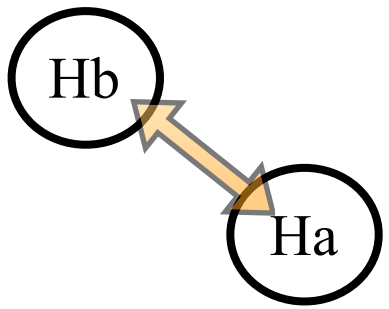


2D experiment

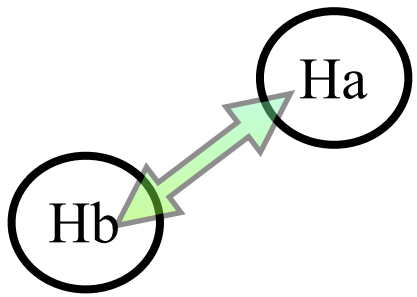
- using spin-spin interaction to transfer coherence from one spin to another



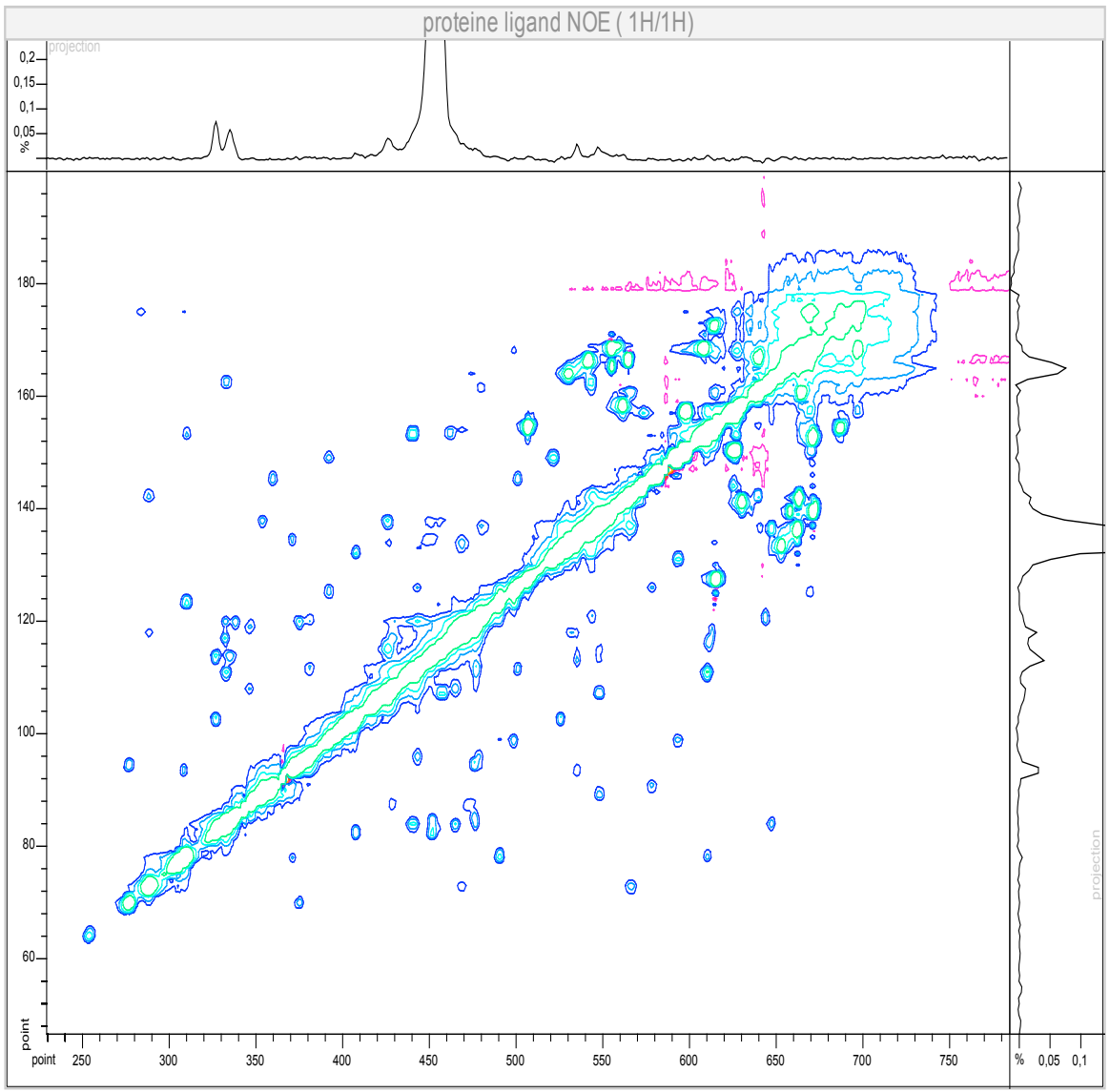
J: COSY / TOCSY



D: NOESY



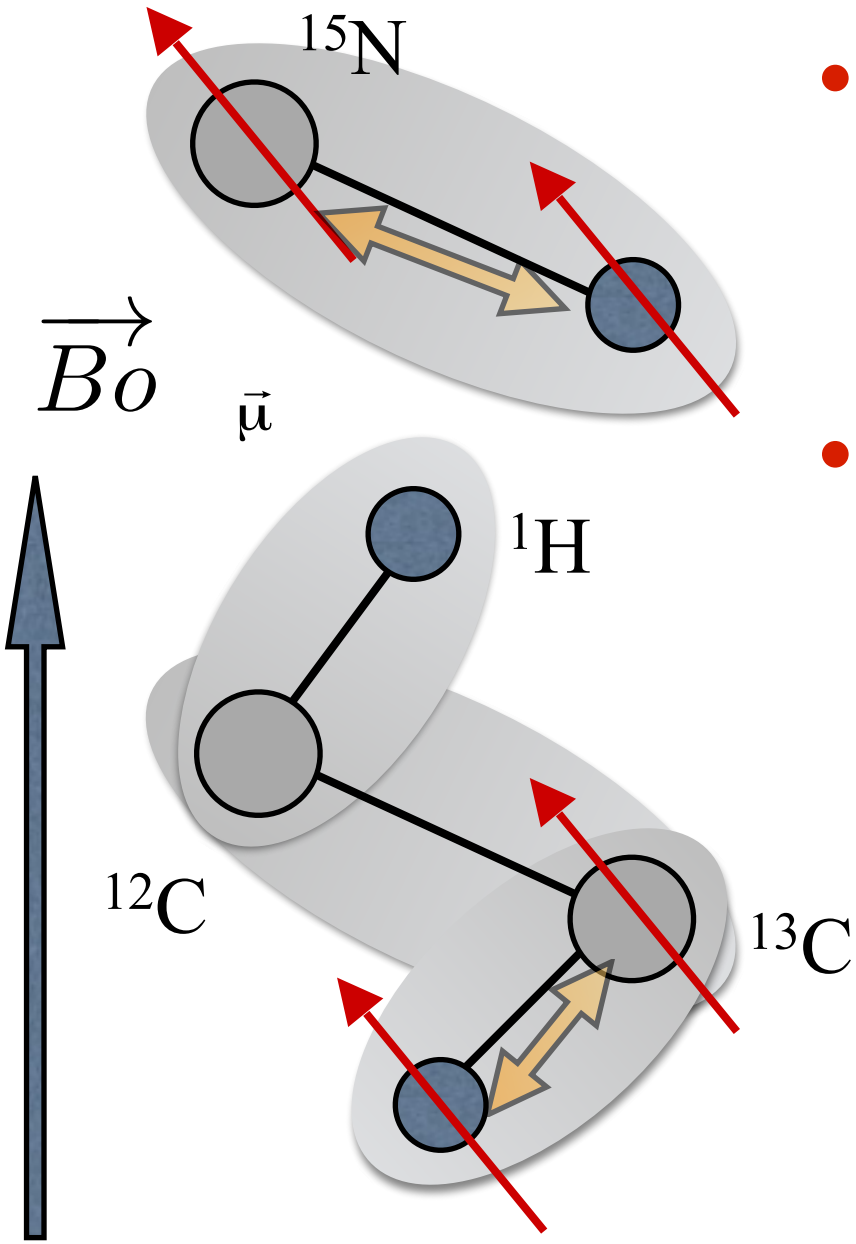
2D experiment - NOESY



Each correlation corresponds to a spatial proximity.

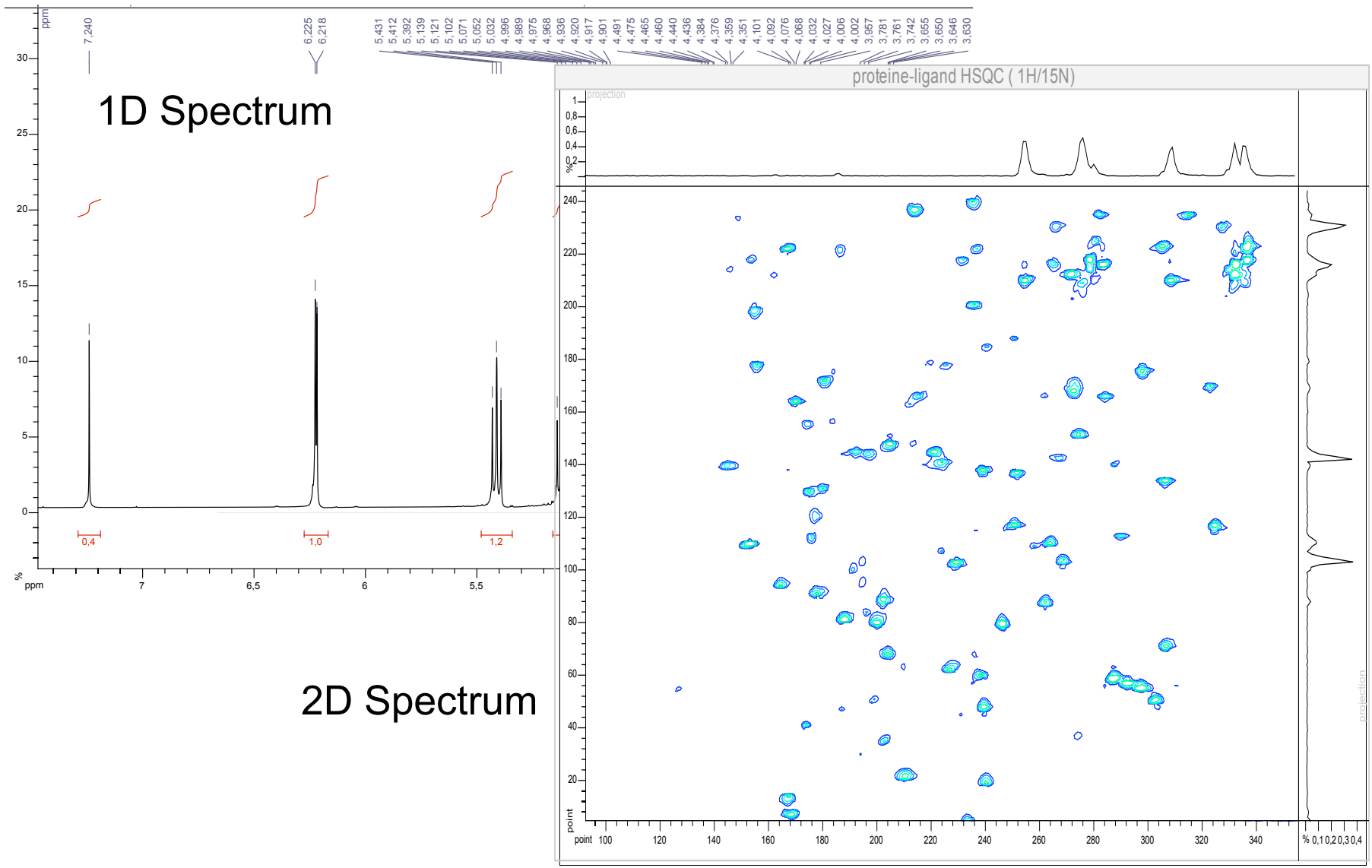
Here amide protons proximity
(for instance *i*-*i*+*3* and *i*-*i*+*4* in α-helices)

Heteronuclear interactions

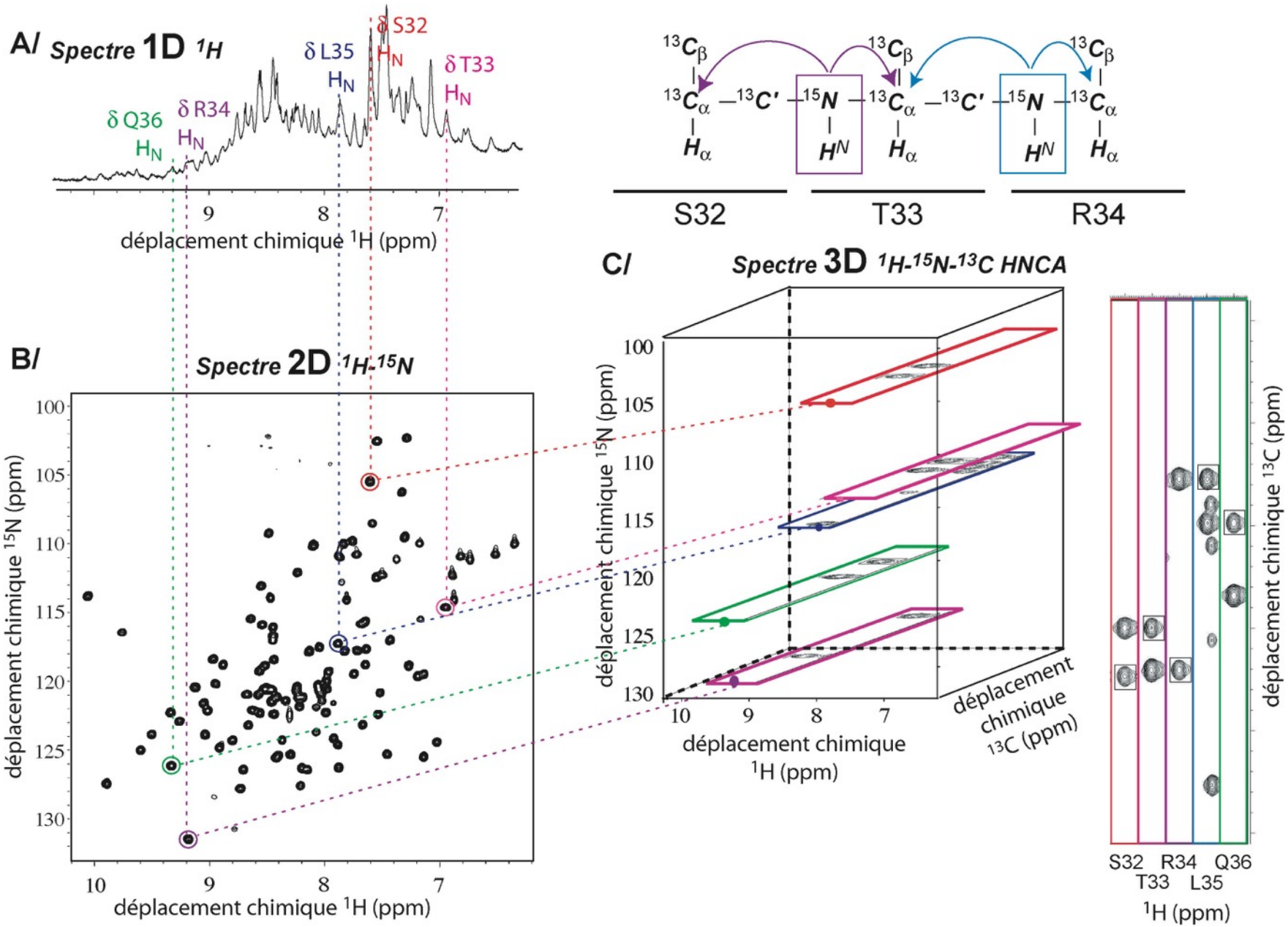


- HSQC
  - **H**eteronuclear **S**ingle **Q**uantum **C**orr.
  - 1 bound ^{13}C-^{1}H or ^{15}N-^{1}H
- Requires isotopic labelling (*usually*)
  - *E.coli* in minimum media
  - (^{15}N cheaper than ^{13}C)

2D HSQC


1D Spectrum
2D Spectrum
proteine-ligand HSQC (1H/15N)
projection
ppm
point

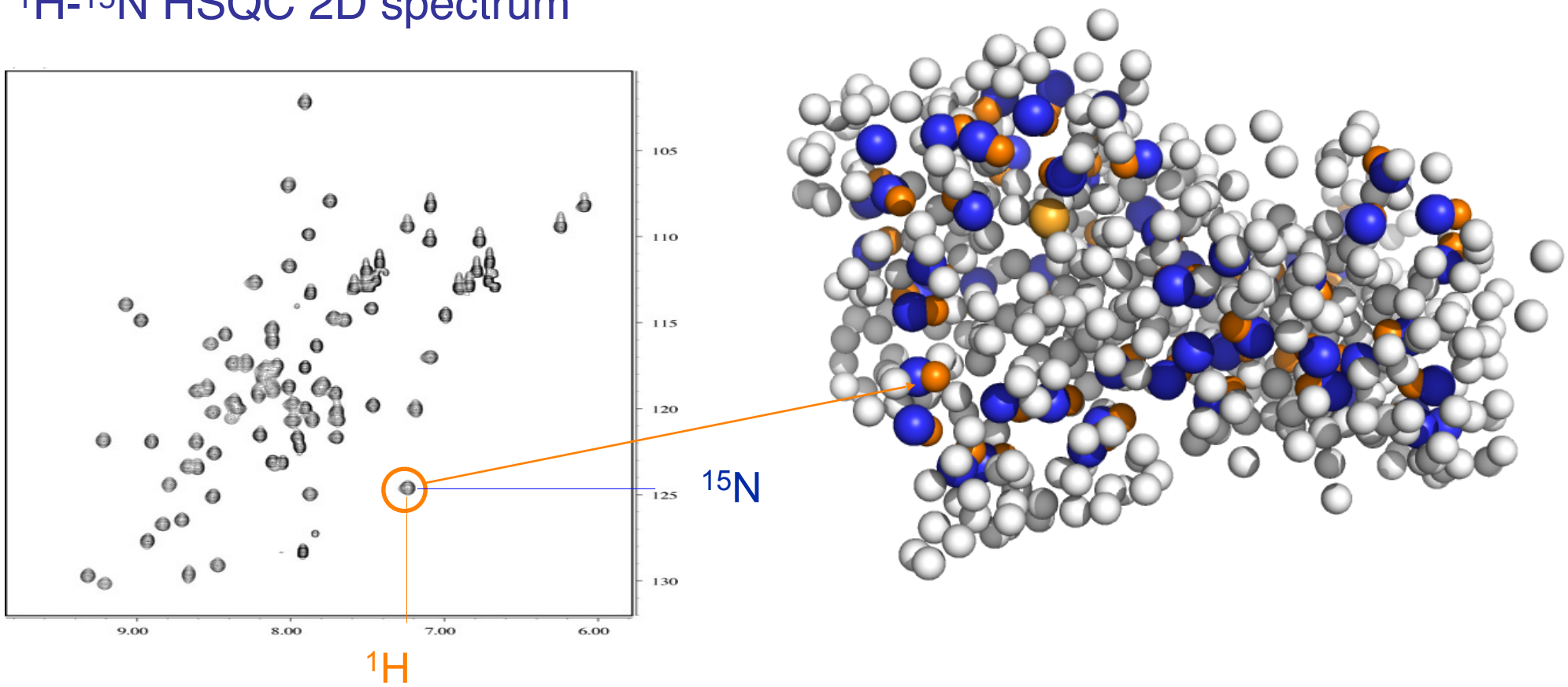
Study of large molecules requires ^{15}N-^{13}C labeling



Isotopic labeling

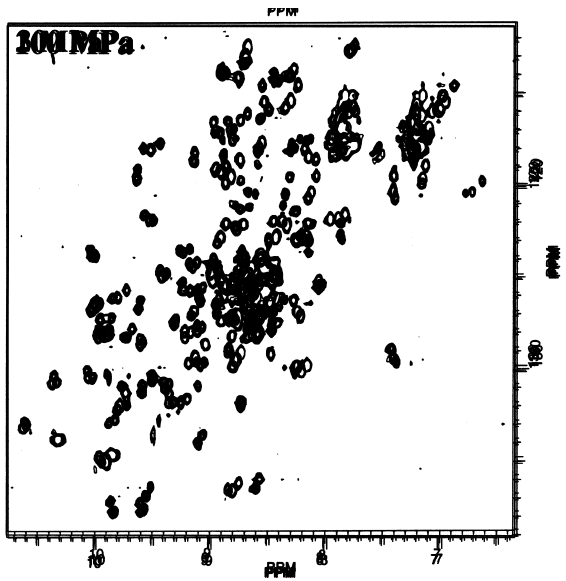
- Partial view =>
  a tool to address the complexity of protein NMR spectra

^{1}H-^{15}N HSQC 2D spectrum



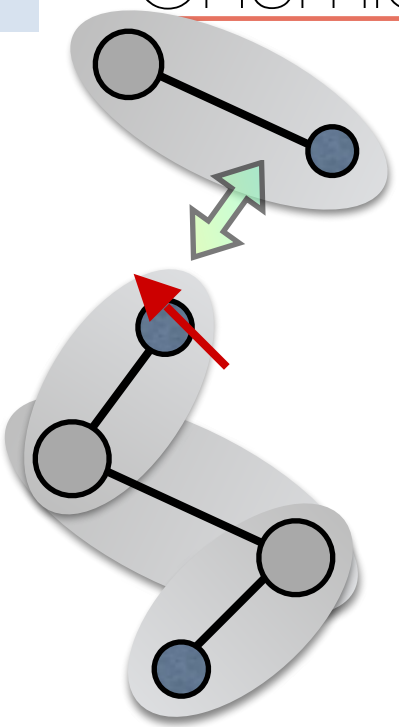
CS is sensitive to protein conformation

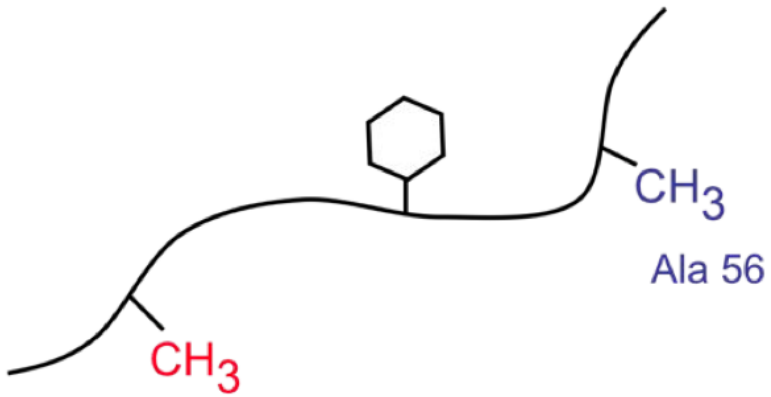
R. Kitahara, C. Royer, H. Yamada, M. Boyer, J-L. Saldana, K. Akasaka,C. Roumestand
Pressure-jump fluorescence and 15N/1H 2-D NMR studies of the unfolding of the beta-barrel protein, P13MTCP1.
J Mol Biol, 320, 3, (2002), pp 609-628

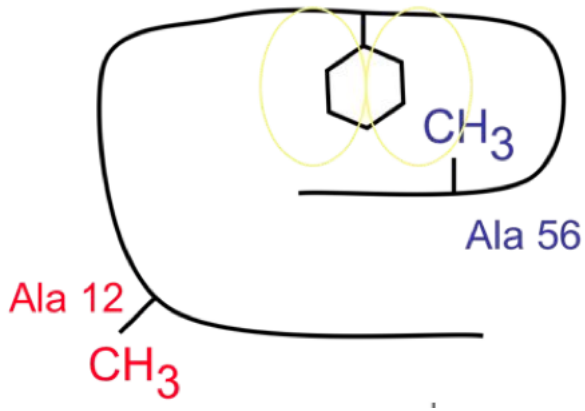


complete protein denaturation

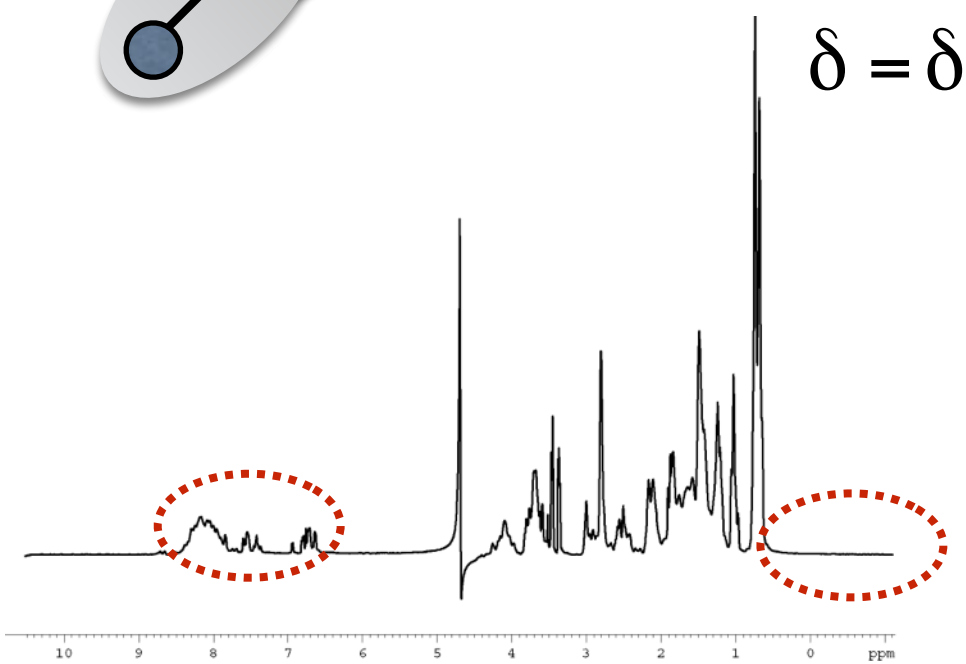
Chemical shifts contain a structural information







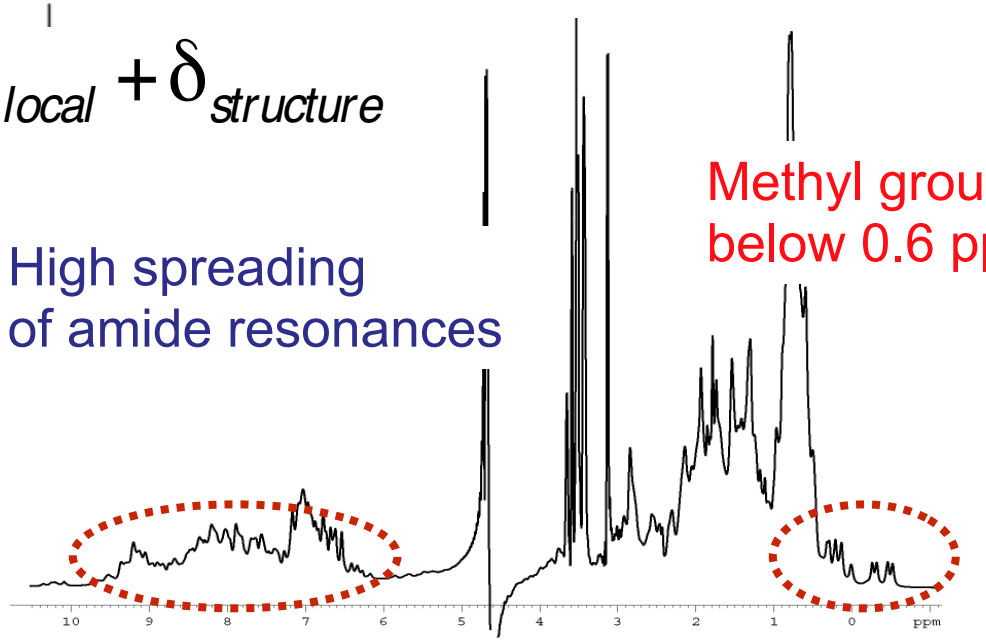
$$\delta = \delta_{local} + \delta_{structure}$$



Unfolded protein

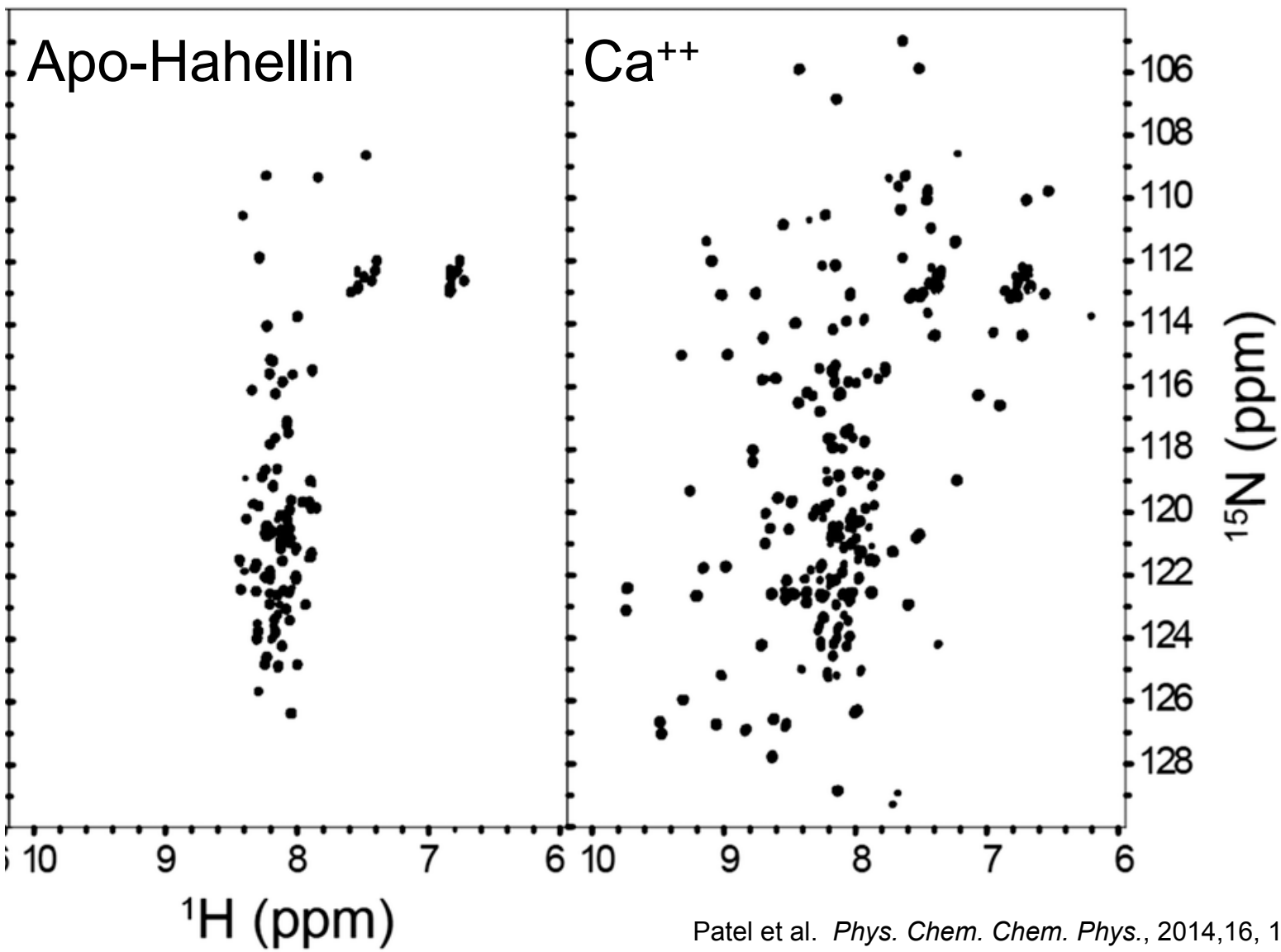
High spreading
of amide resonances

Methyl groups
below 0.6 ppm



Folded protein

Specific methods for IDP



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

Bio-drug control quality

Human insulin

(light chain A)

$_{1}$GIVEQCCTSICSLYQLENYCN$_{21}$

$_{1}$FVNQHLCGSHLVEALYLVCGERGFFYT**PK**T$_{30}$

(heavy chain B)

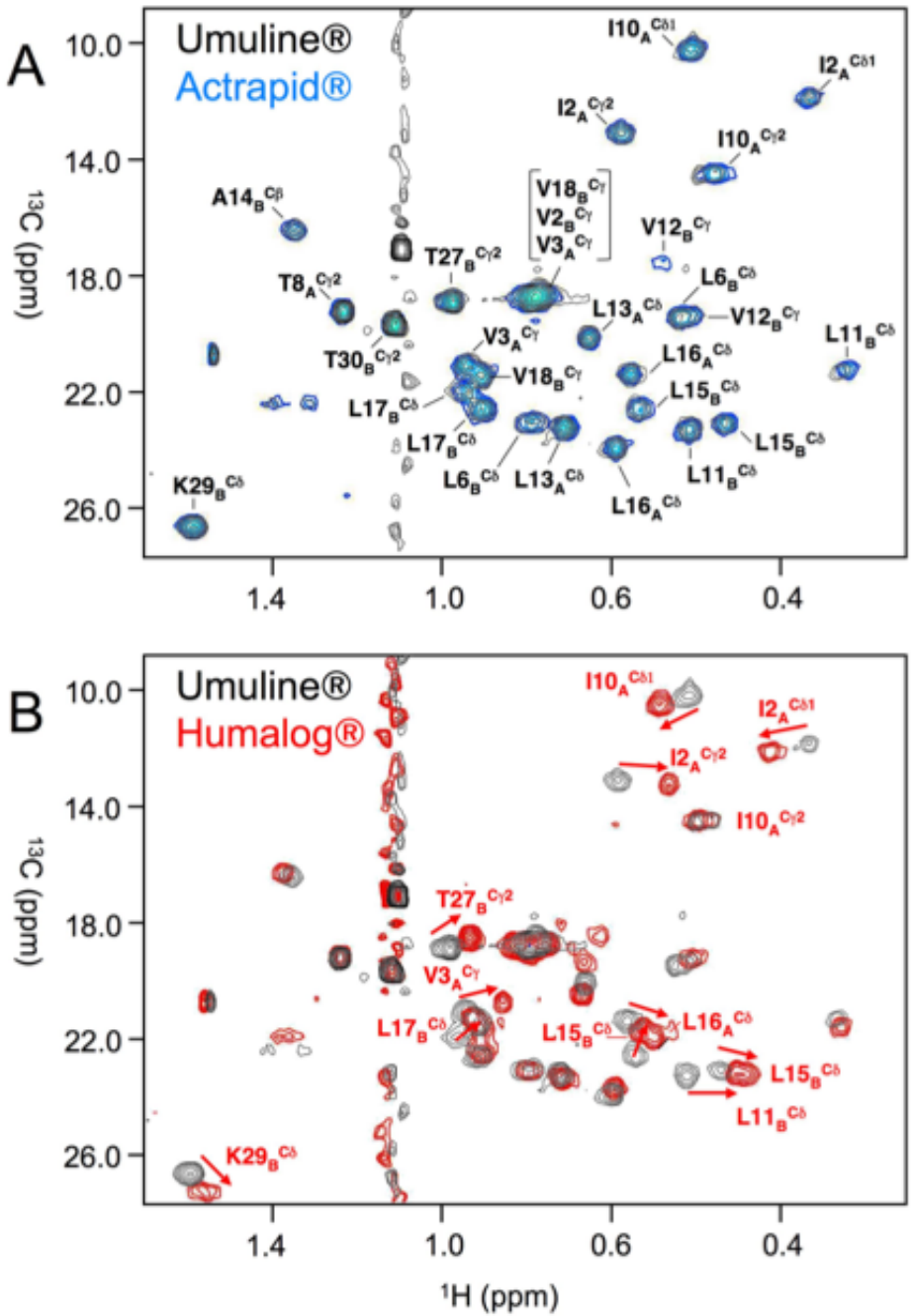
Lispro insulin

(light chain A)

$_{1}$GIVEQCCTSICSLYQLENYCN$_{21}$

$_{1}$FVNQHLCGSHLVEALYLVCGERGFFYT**KP**T$_{30}$

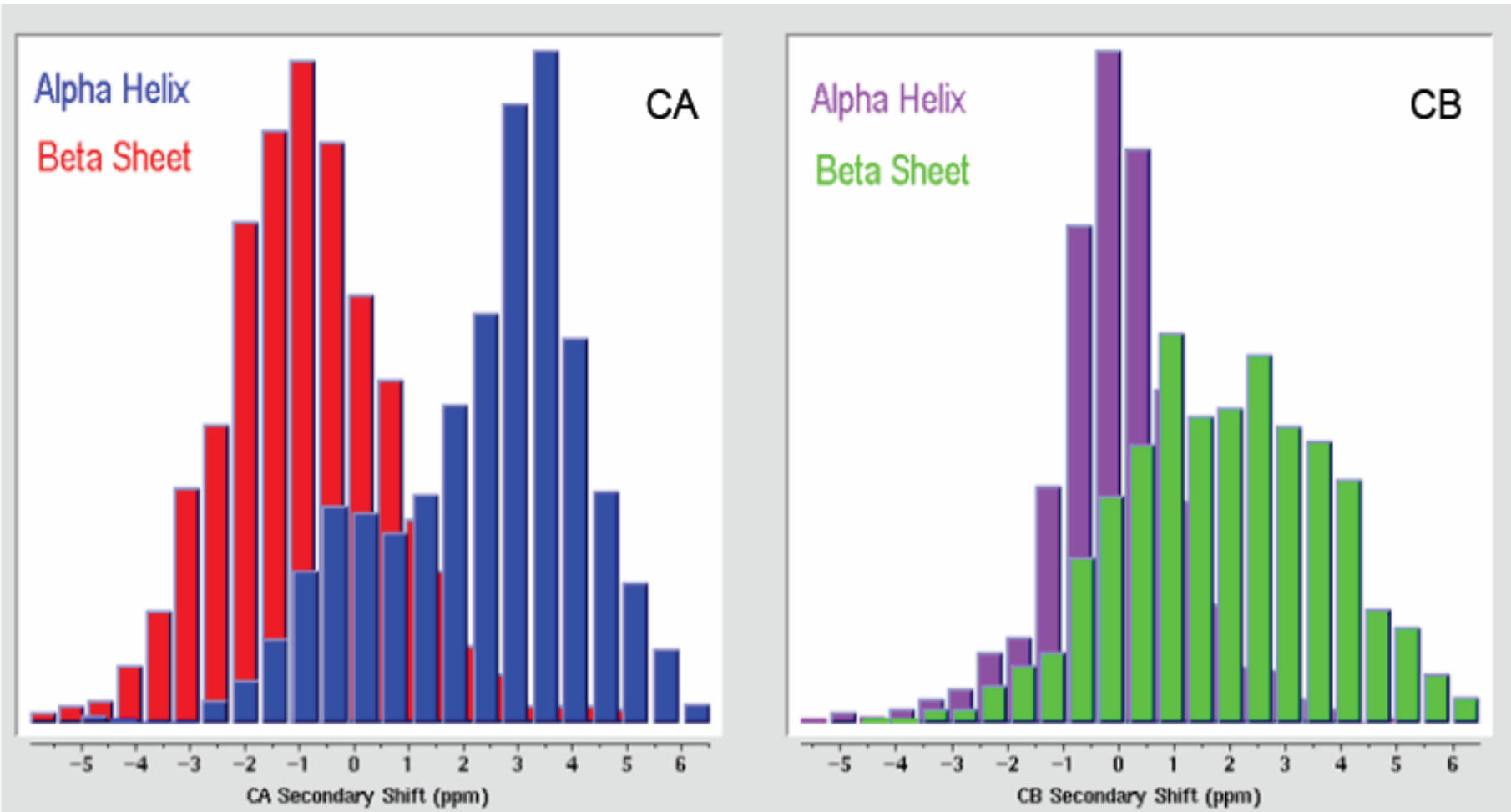
(heavy chain B)

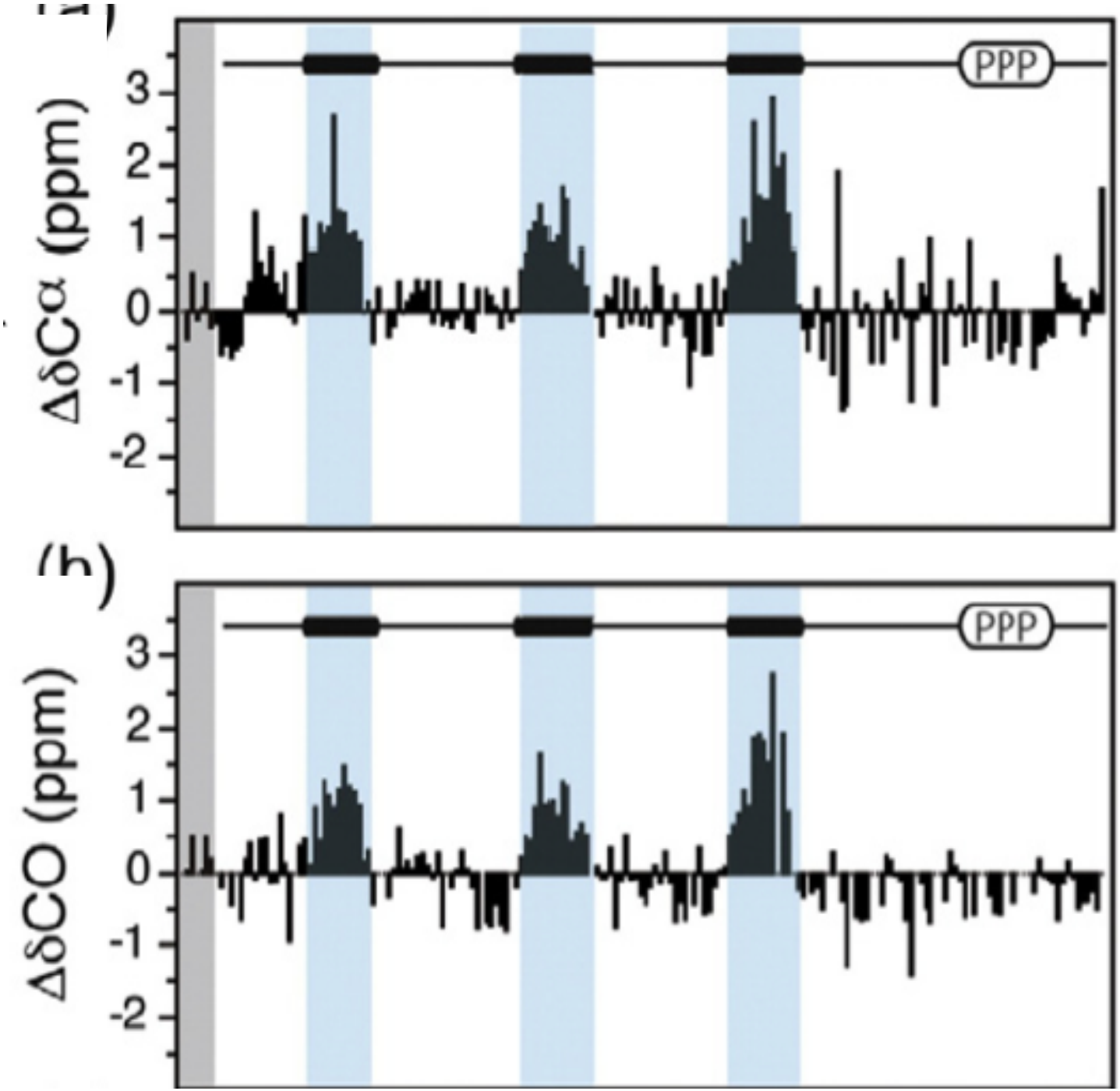


Quinternet, M., Starck, J.-P., Delsuc, M.-A., & Kieffer, B. (2013). J.Pharm. Biomed. Analysis, 78-79, 252–254. http://doi.org/10.1016/j.jpba.2013.02.016

CS of backbone provide structure information

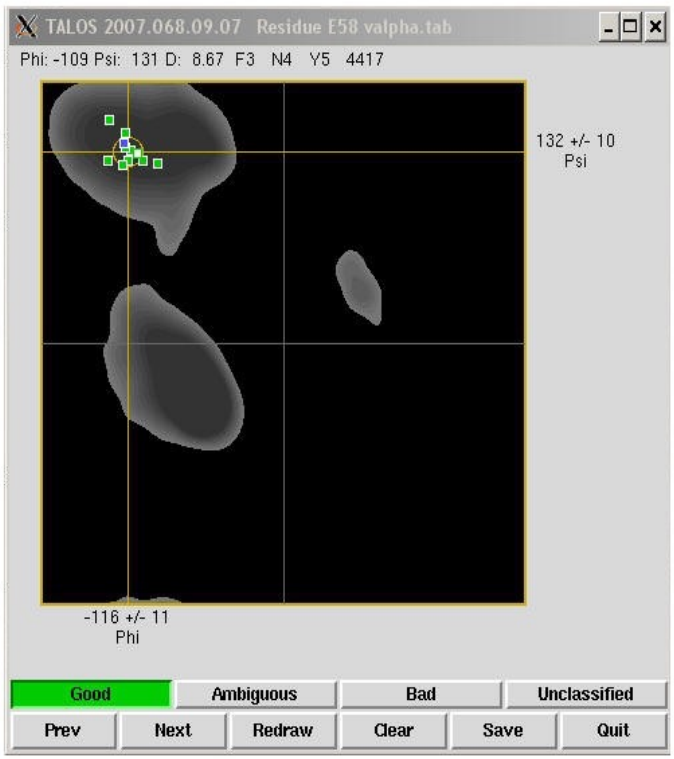
$$CSI = \delta_{measured} - \delta_{randomcoil}$$

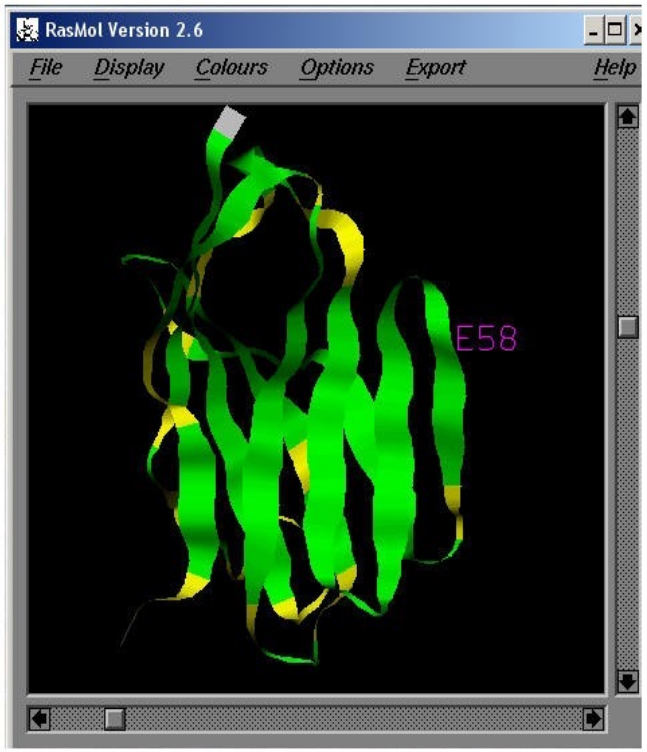


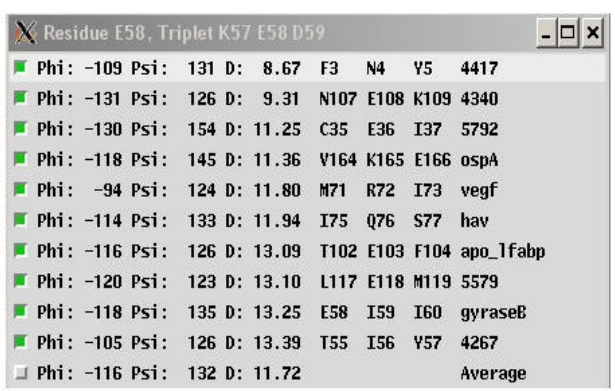

PPP
3
2
1
0
-1
-2
ΔδCα (ppm)
PPP
3
2
1
0
-1
-2
ΔδCO (ppm)

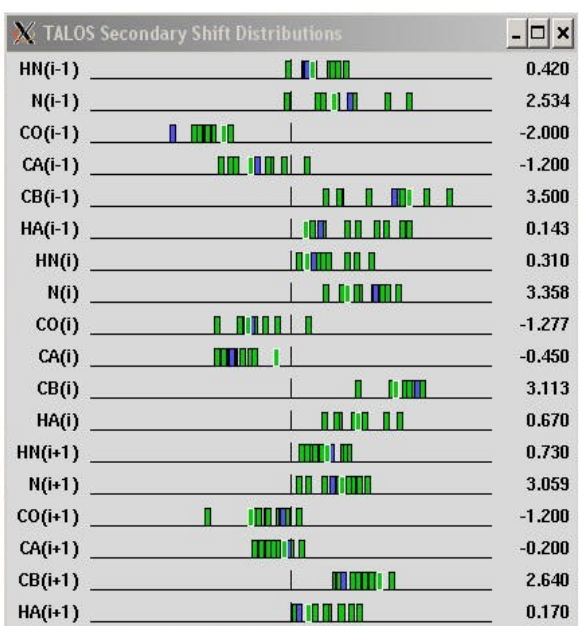
CS interpretation as ϕ-ψ angles

Talos+ : http//:spin.niddk.nih.gov/NMRPipe









Full structure calculations: CS ROSETTA

Consistent blind protein structure generation from NMR chemical shift data

Yang Shen*, Oliver Lange†, Frank Delaglio*, Paolo Rossi‡, James M. Aramini‡, Gaohua Liu‡, Alexander Eletsky§, Yibing Wu§, Kiran K. Singarapu§, Alexander Lemak¶, Alexandr Ignatchenko¶, Cheryl H. Arrowsmith¶, Thomas Szyperski§, Gaetano T. Montelione‡, David Baker†‖, and Ad Bax*‖

*Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892; †Department of Biochemistry and Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195; ‡Center for Advanced Biotechnology and Medicine, Department of Molecular Biology and Biochemistry, and Northeast Structural Genomics Consortium, Rutgers, The State University of New Jersey, and Robert Wood Johnson Medical School, Piscataway, NJ 08854; §Departments of Chemistry and Structural Biology and Northeast Structural Genomics Consortium, University at Buffalo, State University of New York, Buffalo, NY 14260; and ¶Ontario Cancer Institute, Department of Medical Biophysics, and Northeast Structural Genomics Consortium, University of Toronto, Toronto, ON, Canada M5G 1L5

www.pnas.org/cgi/doi/10.1073/pnas.0800256105 PNAS | March 25, 2008 | vol. 105 | no. 12 | 4685–4690

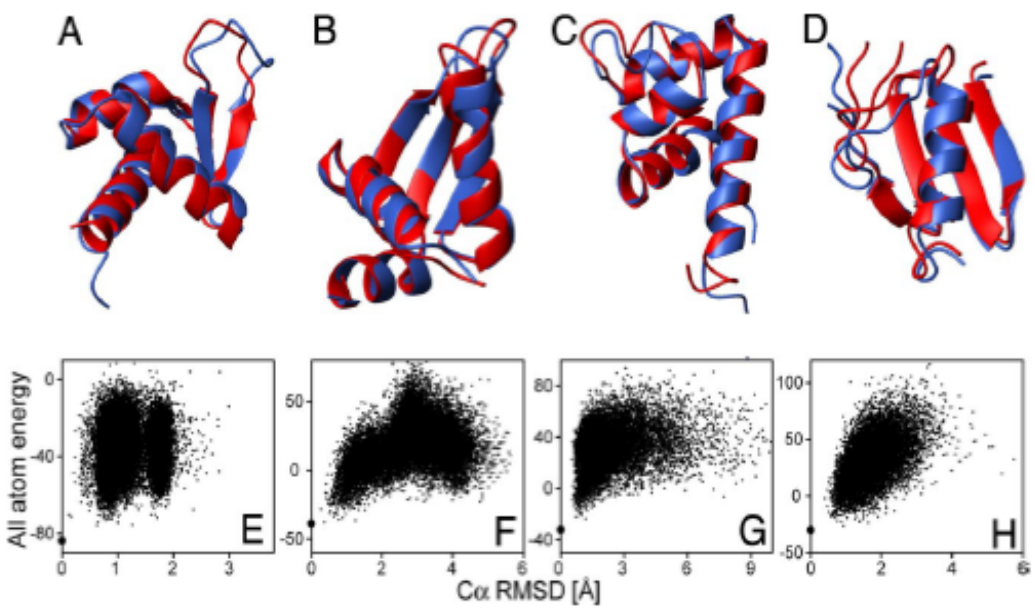
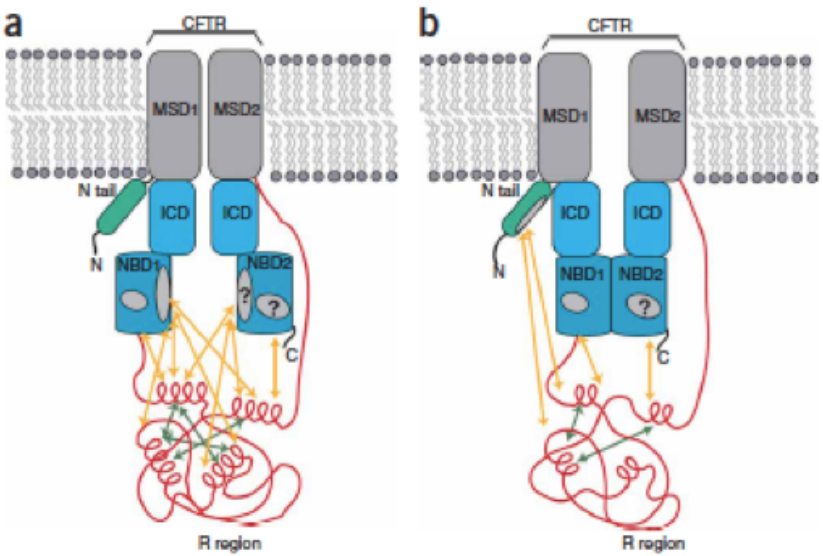
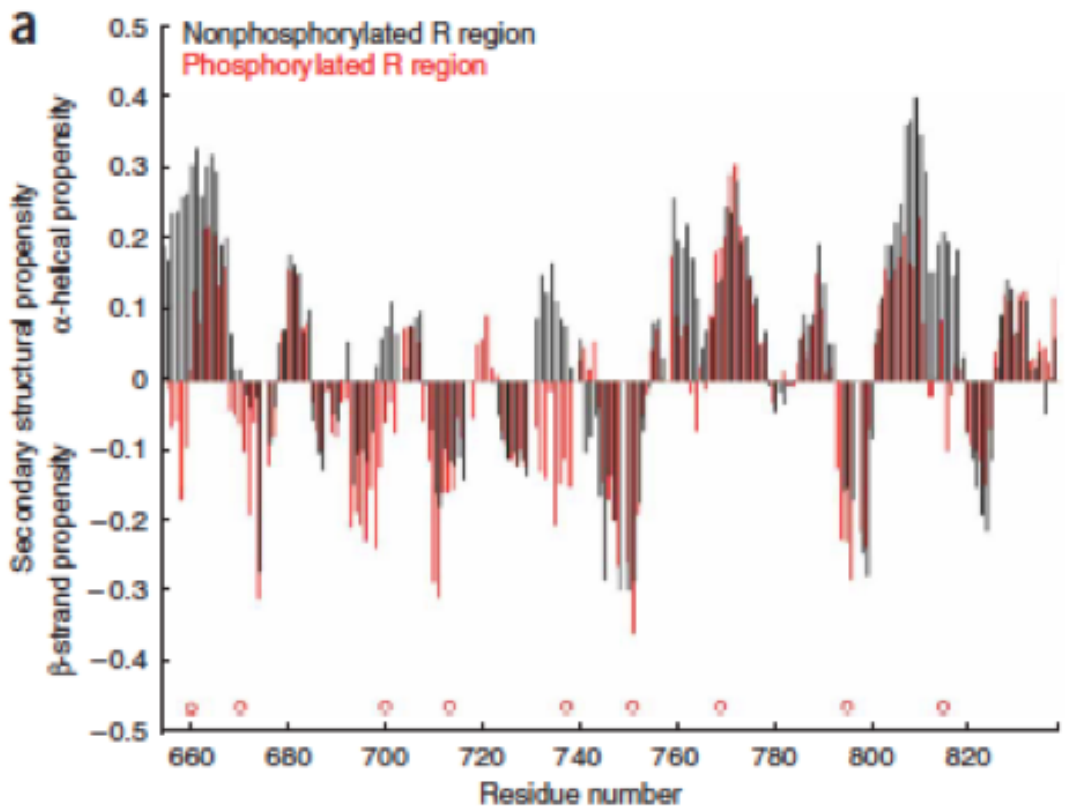


Fig. 4. Results from blind CS-ROSETTA structure generation for four structural genomics targets (Table 2). The remaining five are in SI Fig. 12. (*A–D*) Superposition of lowest-energy CS-ROSETTA models (red) with experimental NMR structures (blue), with superposition optimized for ordered residues, as defined in the footnote to SI Table 5. (*E–H*) Plots of rescored (Eq. 1) ROSETTA all-atom energy versus C^{α} rmsd relative to the lowest-energy model (bold dot on vertical axis). (*A* and *E*) StR82. (*B* and *F*) RpT7. (*C* and *G*) VfR117. (*D* and *H*) NeT4.

Local structure propensity from chemical shifts analysis

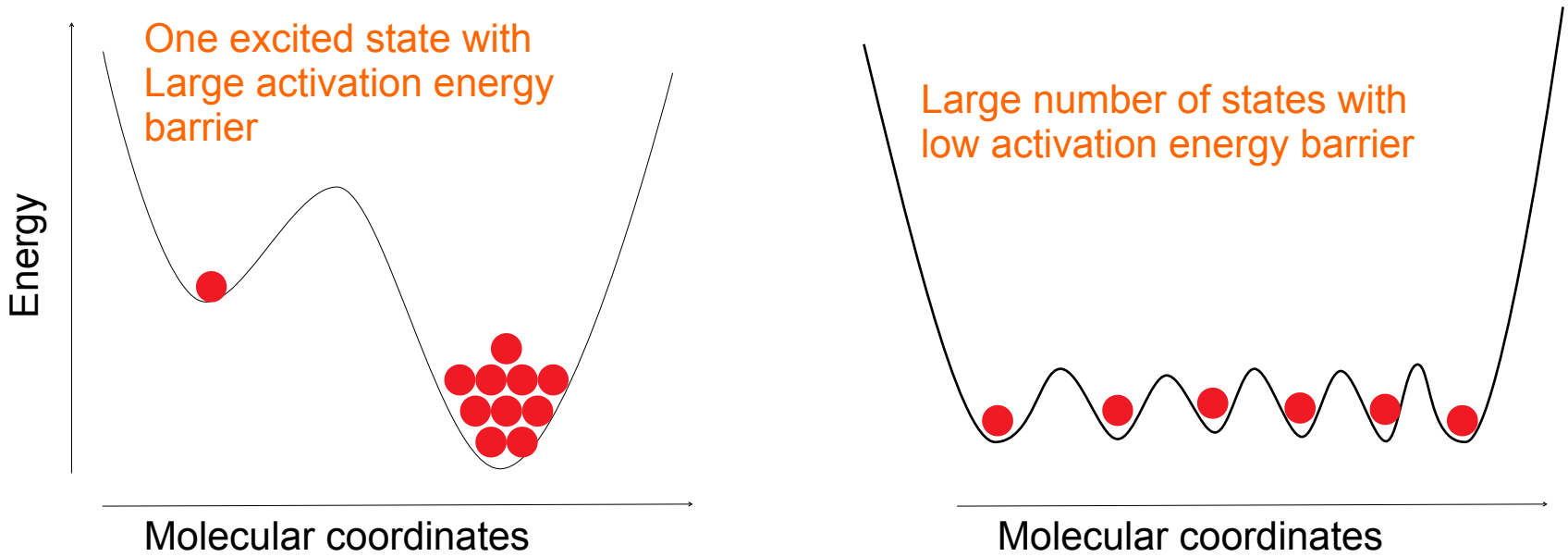




Baker et al 2007 *Nat. Struct. Mol. Biol.* **14**(8), 738

Large number of molecules

- Molecules will be distributed between the different states available for the observed molecular system



- NMR observables will result from an average over all these states

sensitive to all interactions

F. de Lamotte et al. / C. R. Acad. Sci. Paris, Chimie / Chemistry 4 (2001) 839–843

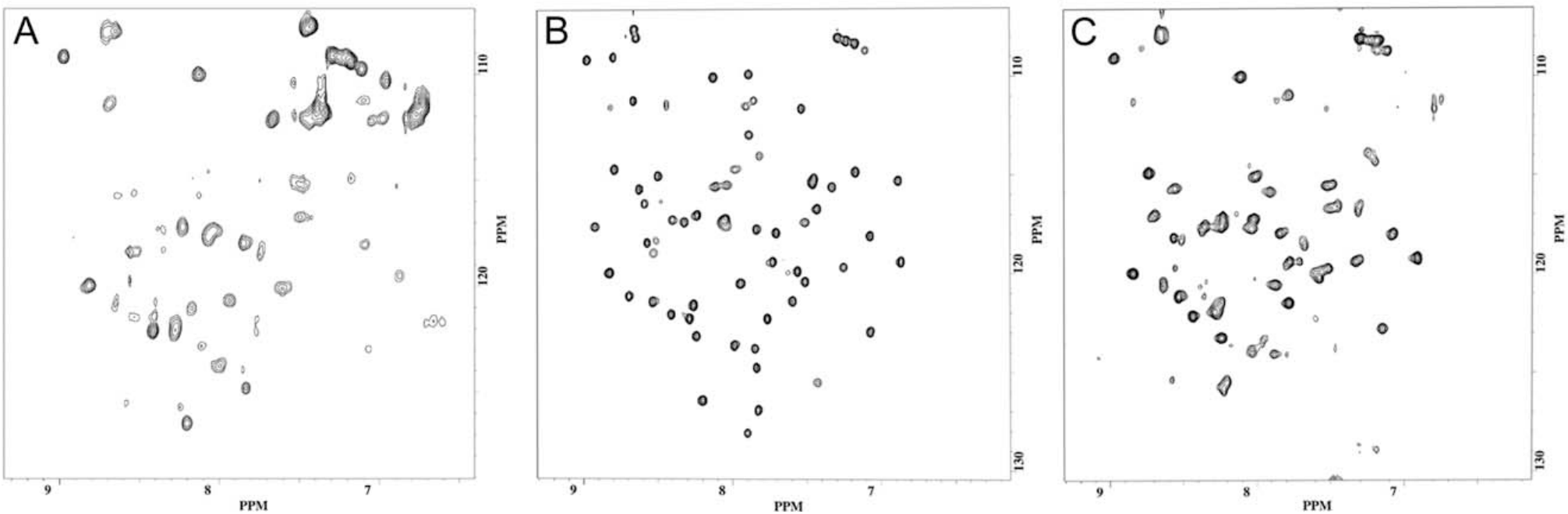
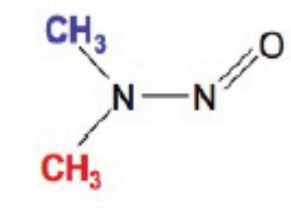
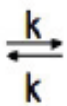


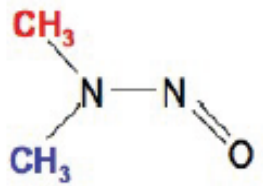
Figure 1. HSQC spectra of type 2 LTP. **A.** Unliganded. **B.** Liganded with 1.5 equiv of LPG. **C.** Liganded with 1.5 equiv of DPC.

Chemical shift averaging

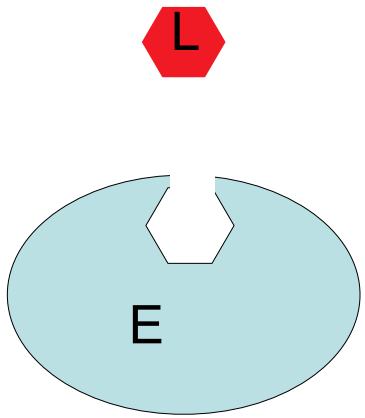


Conformation A



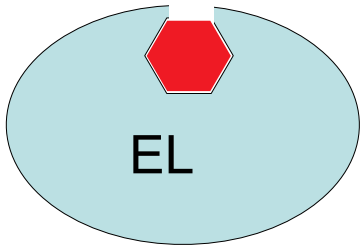


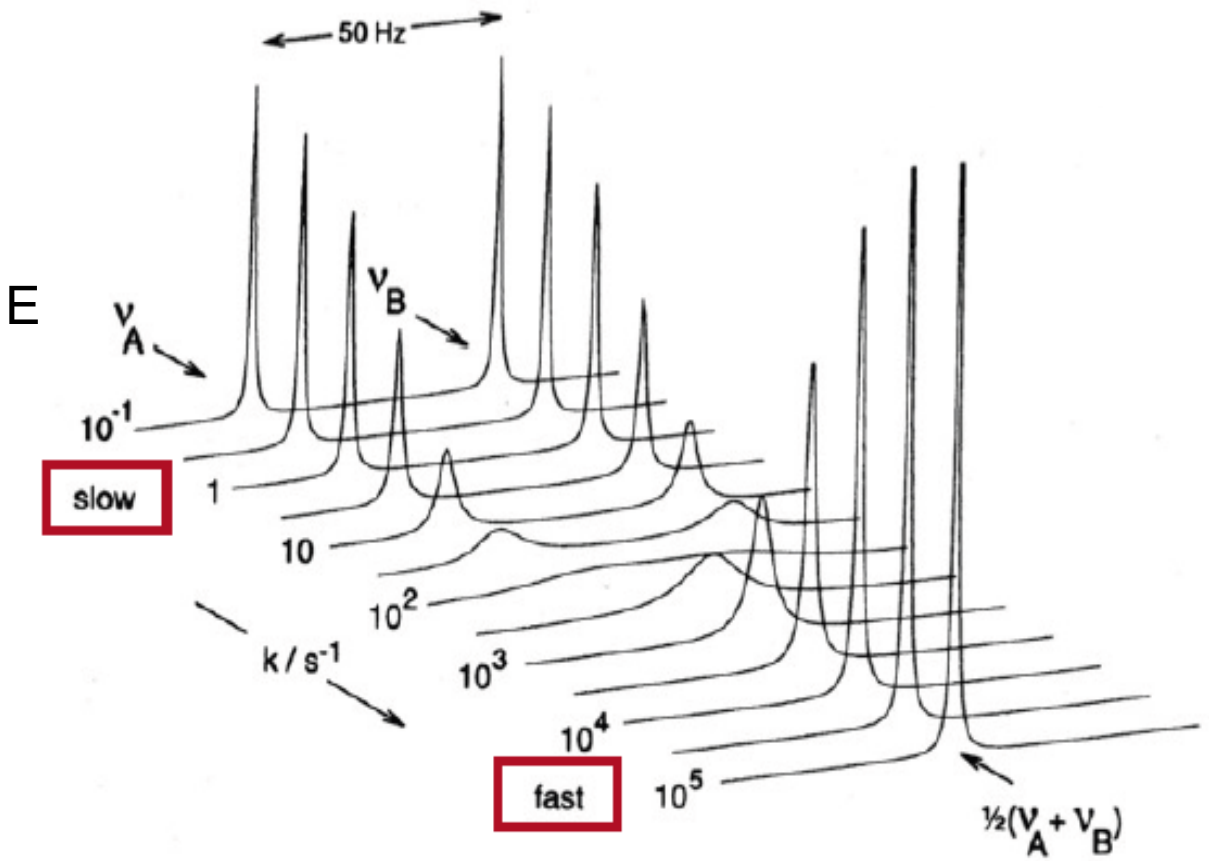
Conformation B



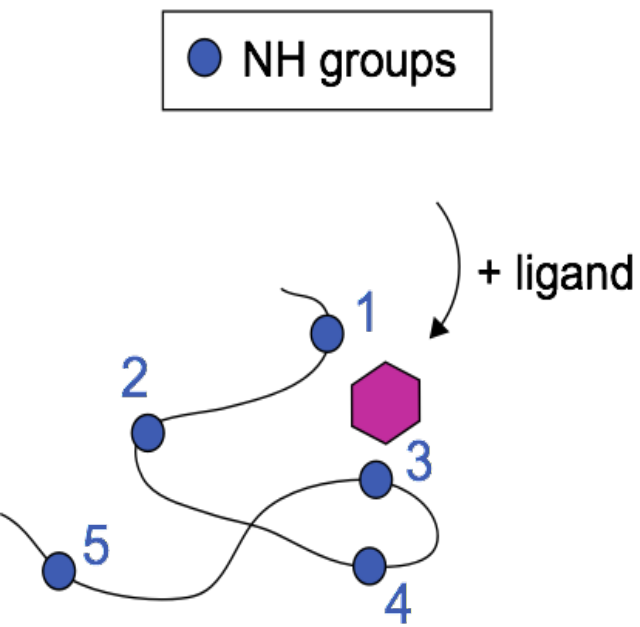
k_{on}

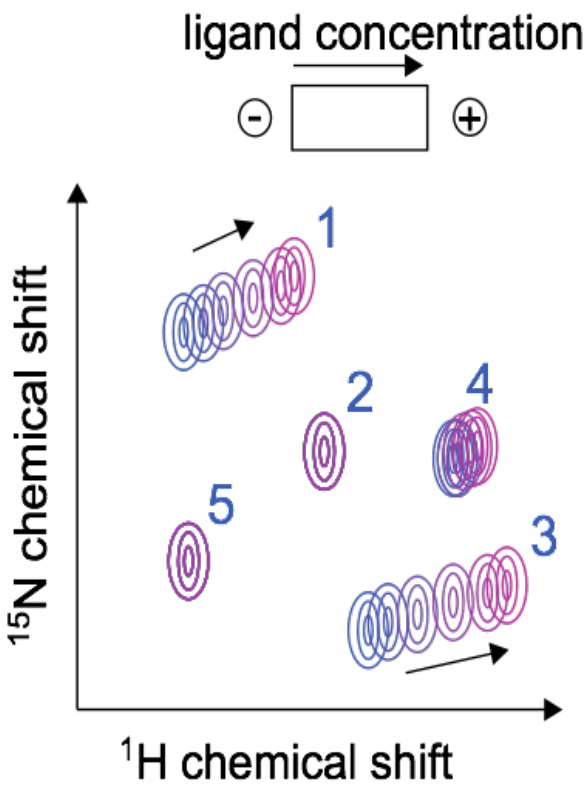
k_{off}

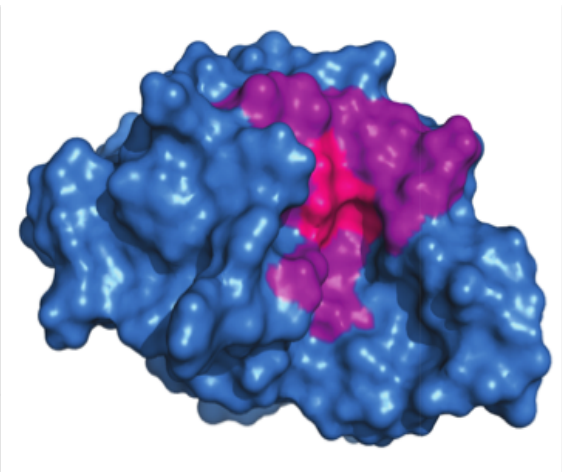


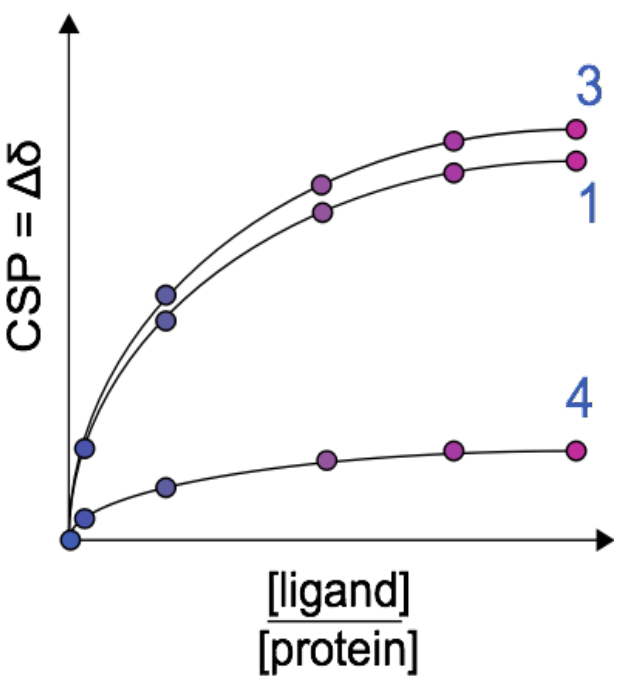


Using chemical shifts to study molecular interactions

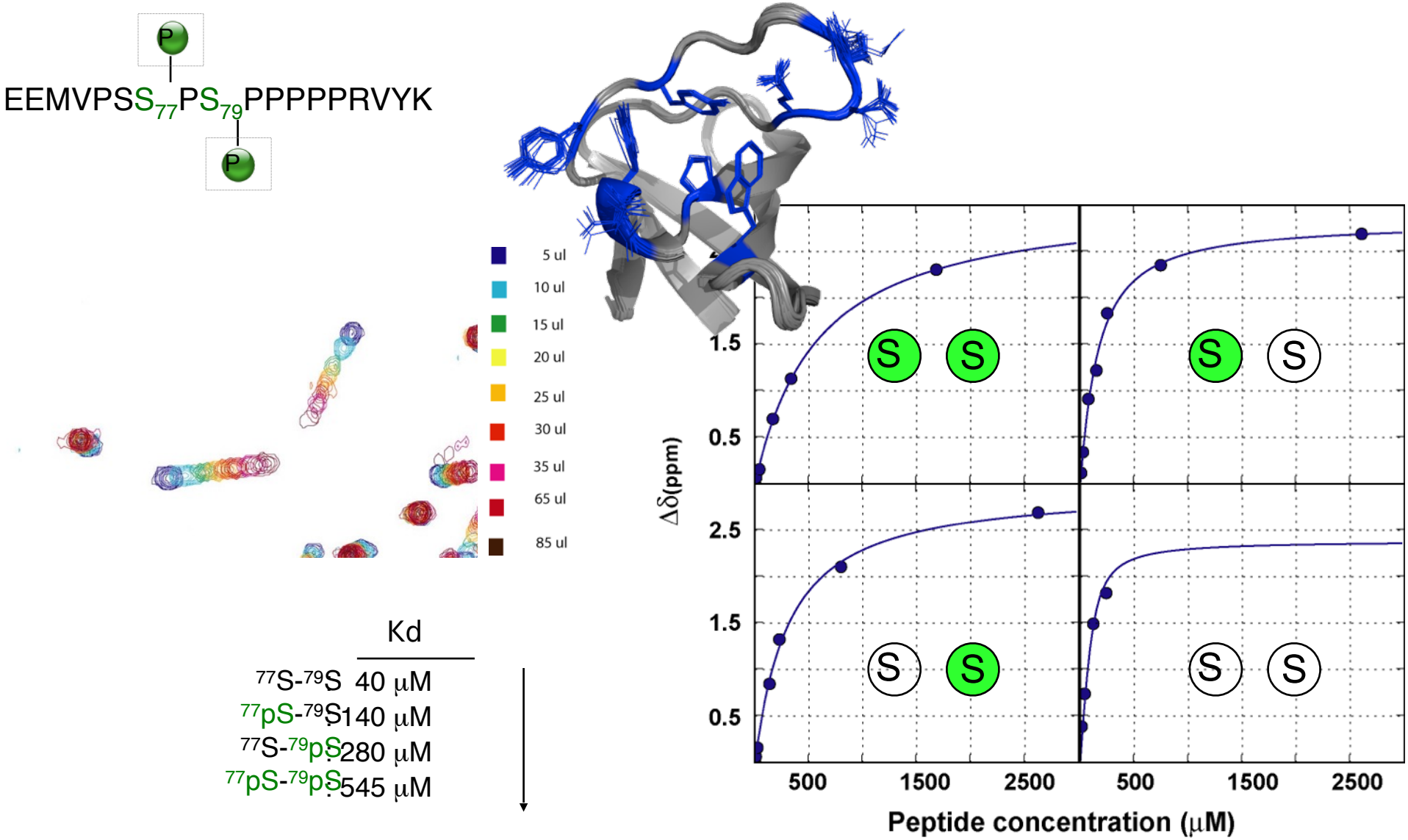








Application: modulation of binding affinity between RAR and vinexin by RAR phosphorylation



Lalevee et al. Vinexin , The FASEB Journal (2010) vol. 24 (11) pp. 4523-4534

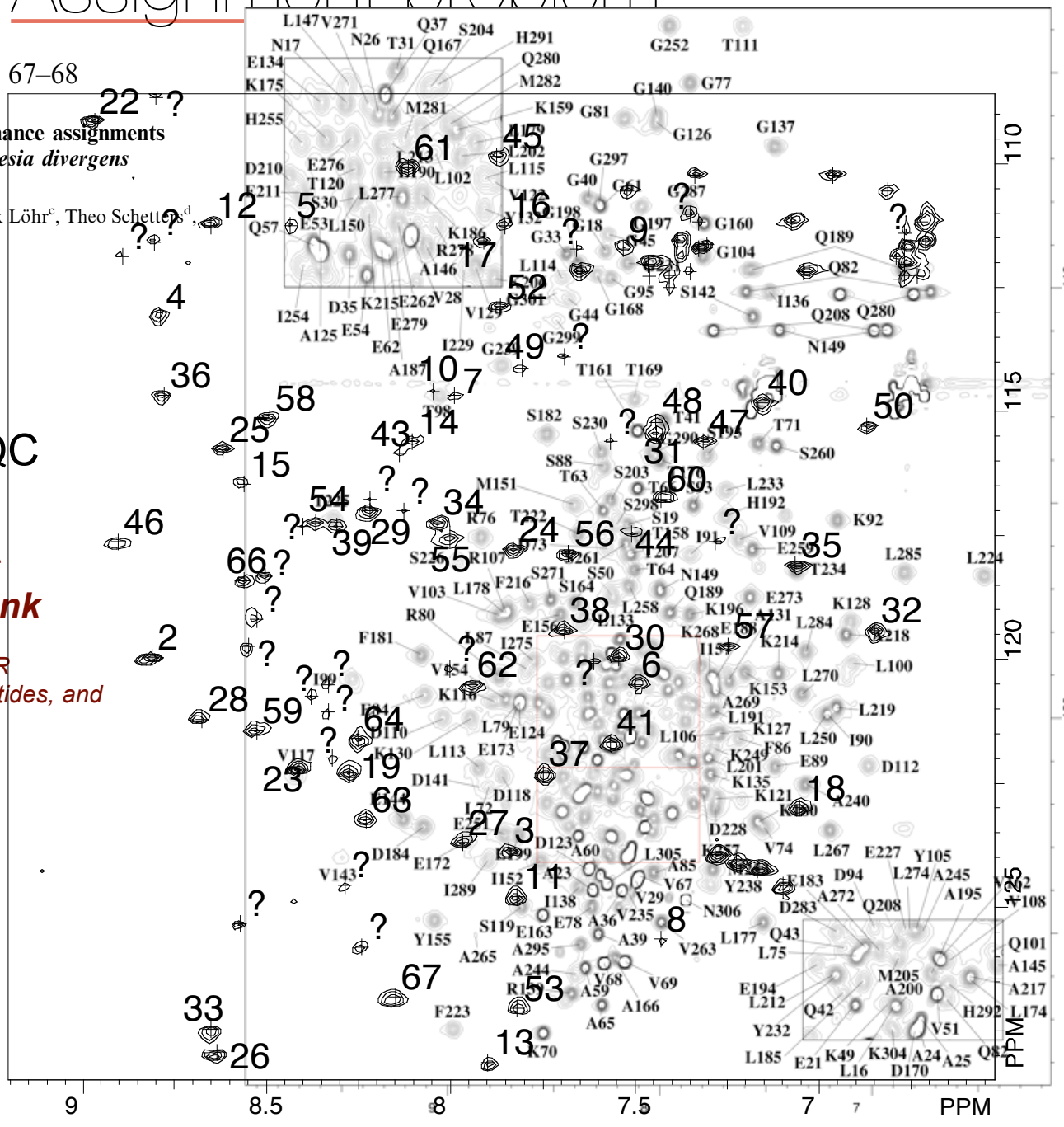
Assignment problem

Journal of Biomolecular NMR (2005) 31: 67–68

Letter to the Editor: ^{1}H, ^{15}N and ^{13}C Backbone resonance assignments of the 37 kDa surface antigen protein Bd37 from *Babesia divergens*

Yin-Shan Yang[a], Stephane Delbecq[b], Marie-Paule Strub[a], Frank Löhr[c], Theo Schetters[d], André Gorenflot[b], Eric Precigout[b] & Christian Roumestand[a,*]

294 residues
37 kDa
293 peaks in ^{15}N-HSQC



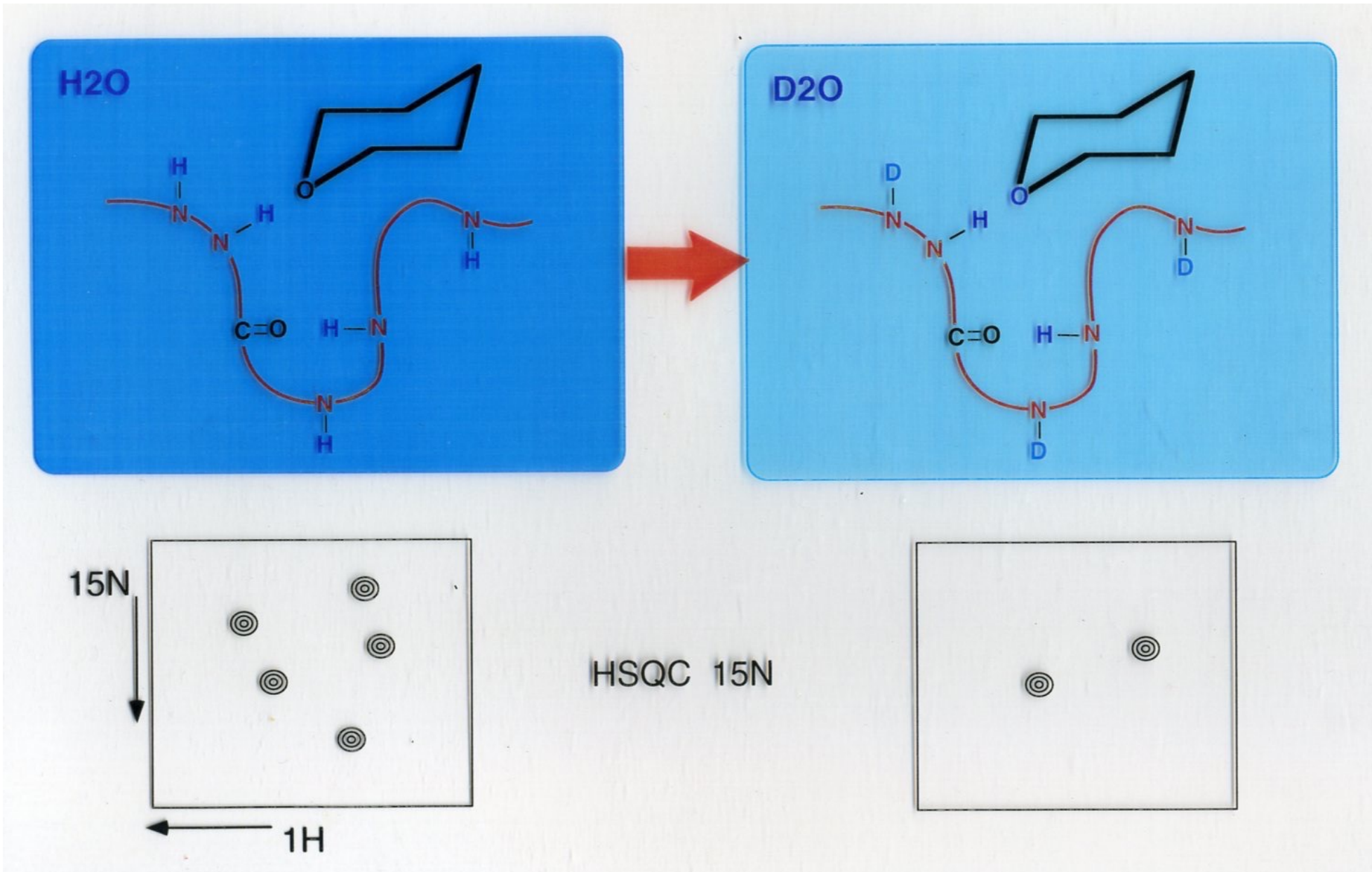
Biological Magnetic Resonance Data Bank

A Repository for Data from NMR Spectroscopy on Proteins, Peptides, and Nucleic Acids

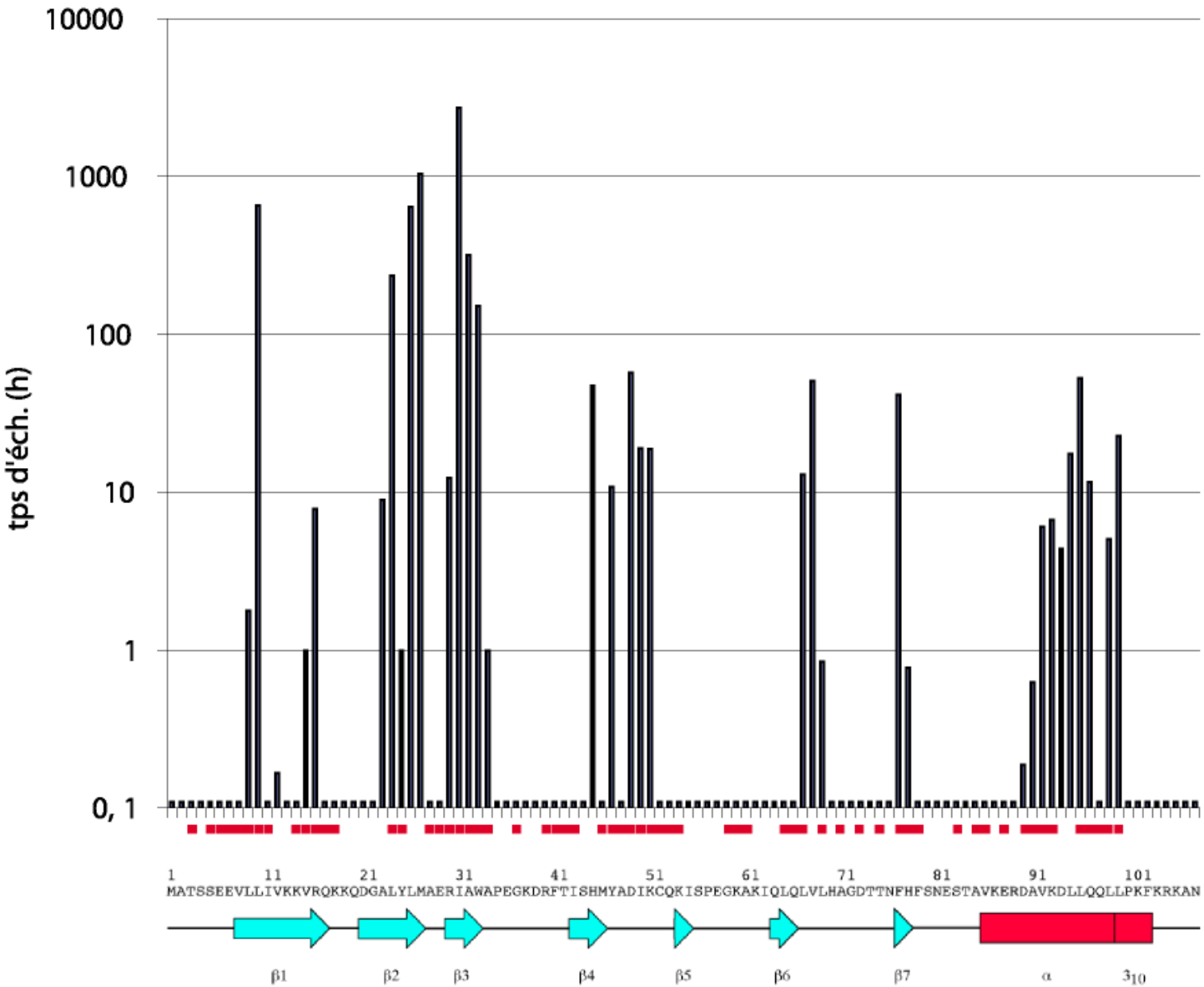
Experimental Data:

Type	Number of: Values	Sets
Assigned Chemical Shifts		1
- 1H Chemical Shifts	557	
- 15N Chemical Shifts	278	
- 13C Chemical Shifts	829	

Real-time solvent exchange kinetic experiment



The exchangeable protons



Measure of proton exchange rates of amide protons of the PH domain of TFIIH P62 subunit

Gervais et al.
Nat struct biol 2004

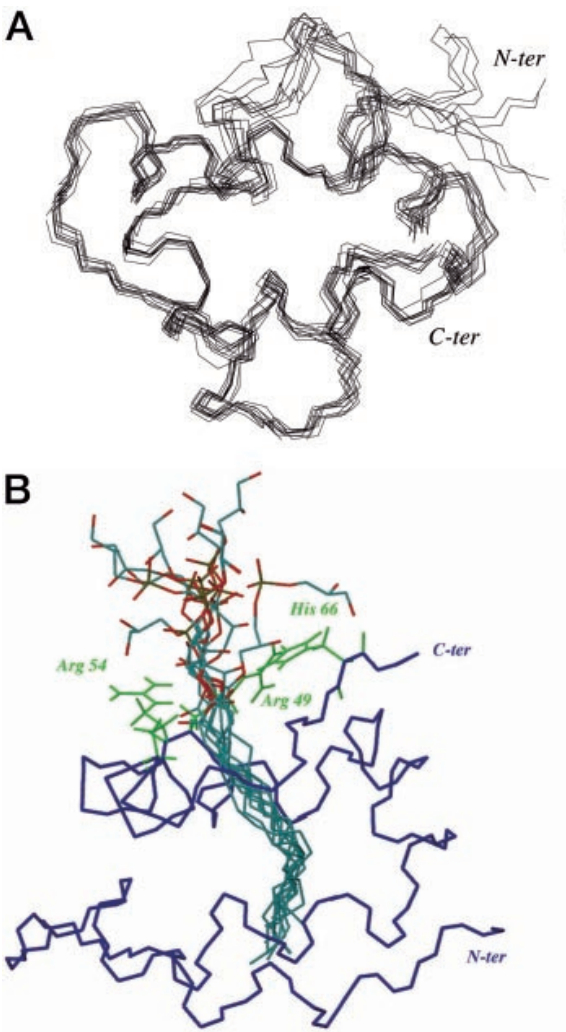
3D Structure Determination

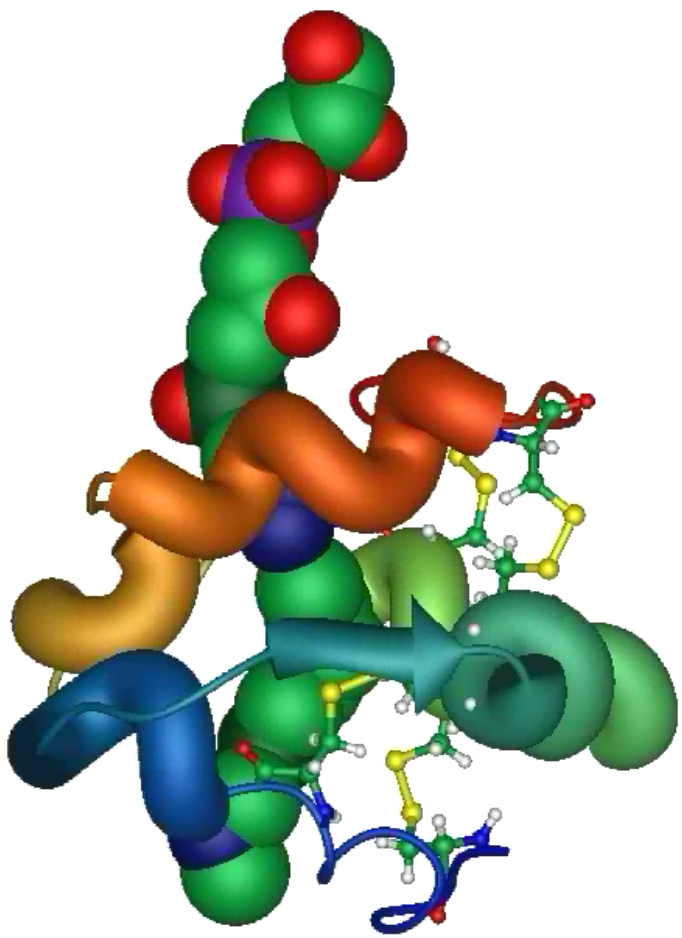
THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 16, Issue of April 18, pp. 14249–14256, 2003
Printed in U.S.A.

Refined Solution Structure of a Liganded Type 2 Wheat Nonspecific Lipid Transfer Protein*

Received for publication, November 15, 2002, and in revised form, January 10, 2003
Published, JBC Papers in Press, January 13, 2003, DOI 10.1074/jbc.M211683200

Jean-Luc Pons‡, Frédéric de Lamotte§, Marie-Françoise Gautier§, and Marc-André Delsuc‡¶





from NOE measures,
⇒ local proximities

between hydrogens
through space

obtained by MolMod

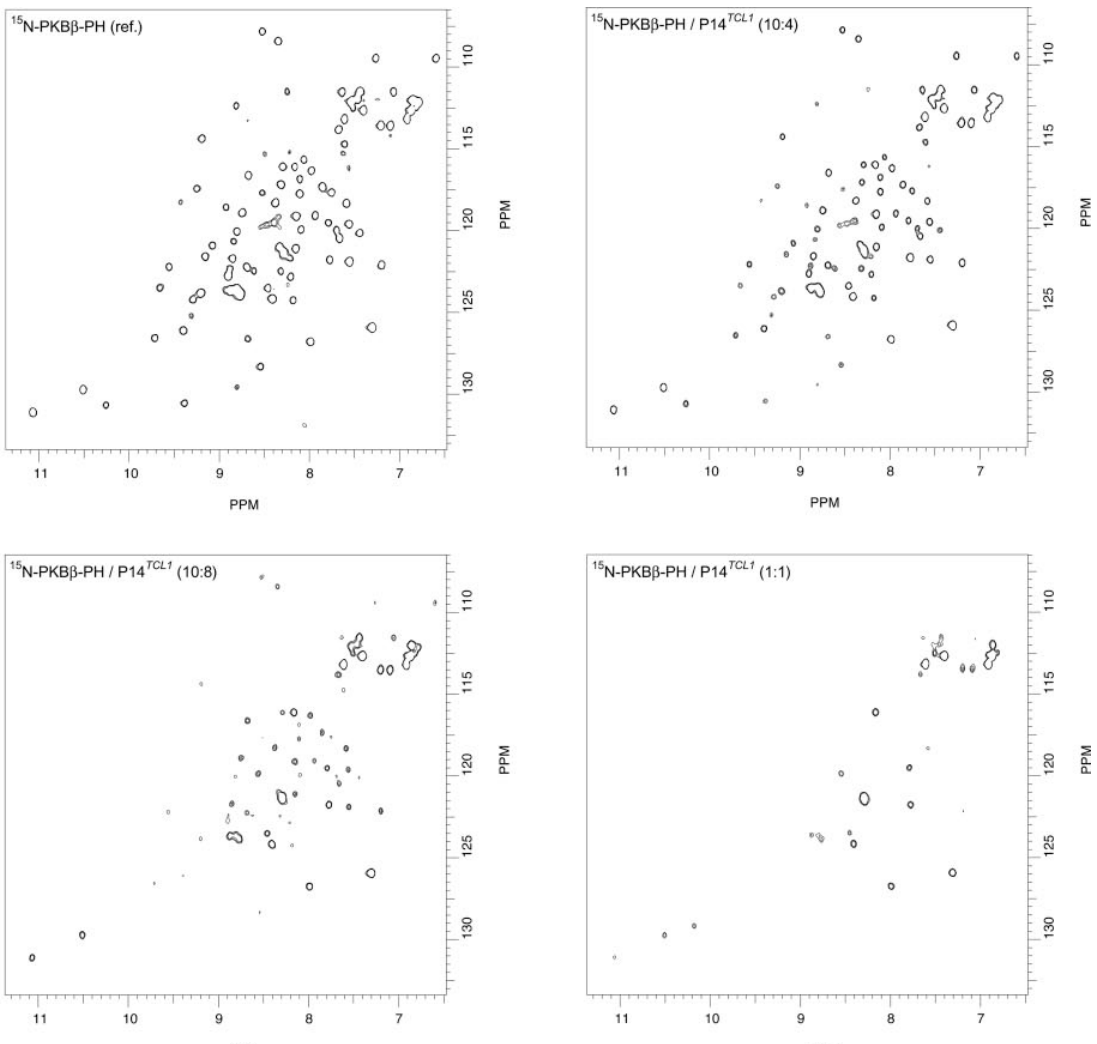
Protein-Protein interaction

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 34, Issue of August 20, pp. 35890–35902, 2004
Printed in U.S.A.

jbc

Structural Basis for the Co-activation of Protein Kinase B by T-cell Leukemia-1 (TCL1) Family Proto-oncoproteins*[S]

Daniel Auguin‡, Philippe Barthe‡, Catherine Royer‡, Marc-Henri Stern§, Masayuki Noguchi¶, Stefan T. Arold‡‖, and Christian Roumestand‡‖



titration de PKB (Akt) par l'oncoprotéine P14^{TCL1}

protein-protein interaction

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 34, Issue of August 20, pp. 35890–35902, 2004
Printed in U.S.A.

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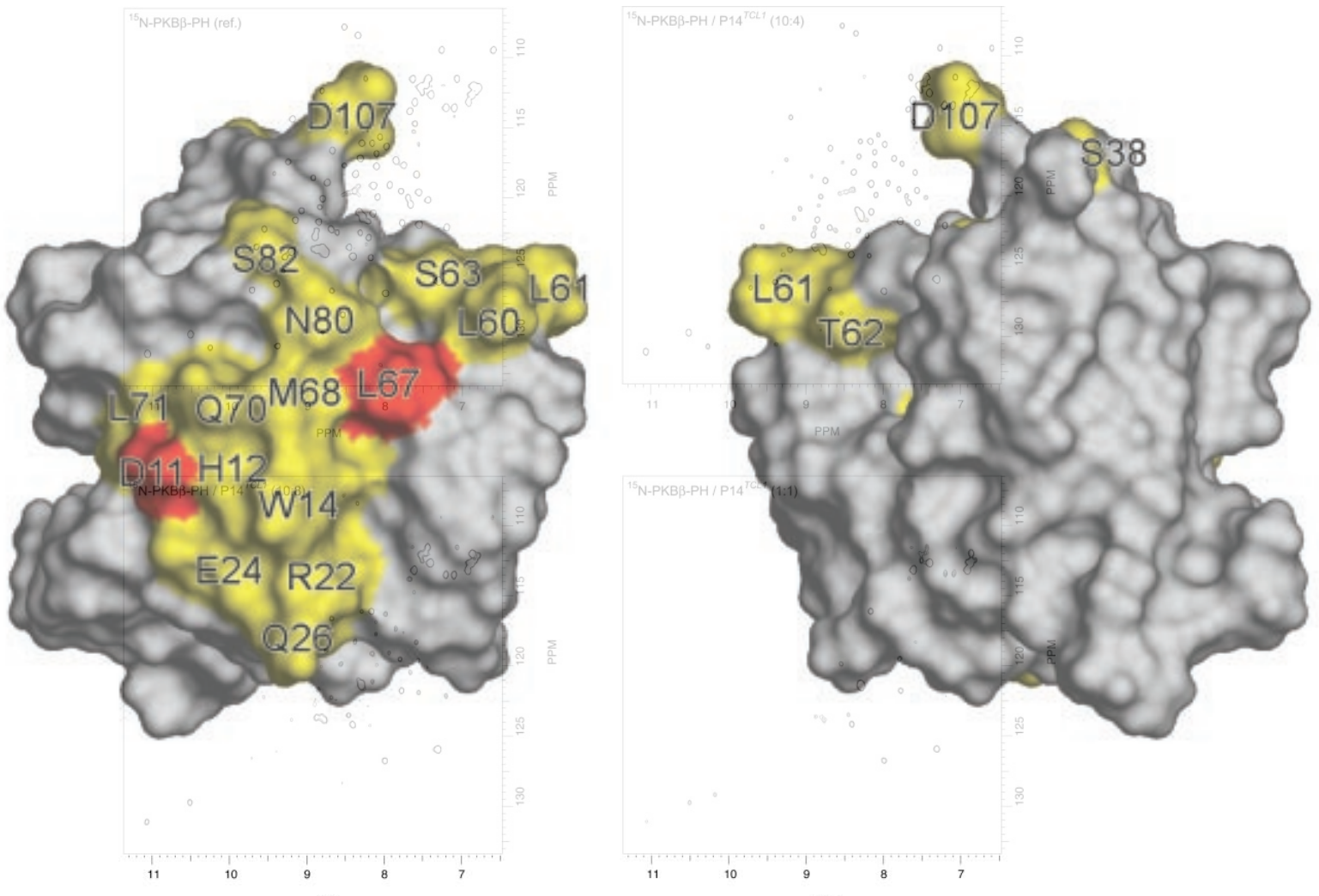

titration de PKB (Akt) par l'oncoprotéine $P14^{TCL1}$

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 34, Issue of August 20, pp. 35890–35902, 2004
Printed in U.S.A.

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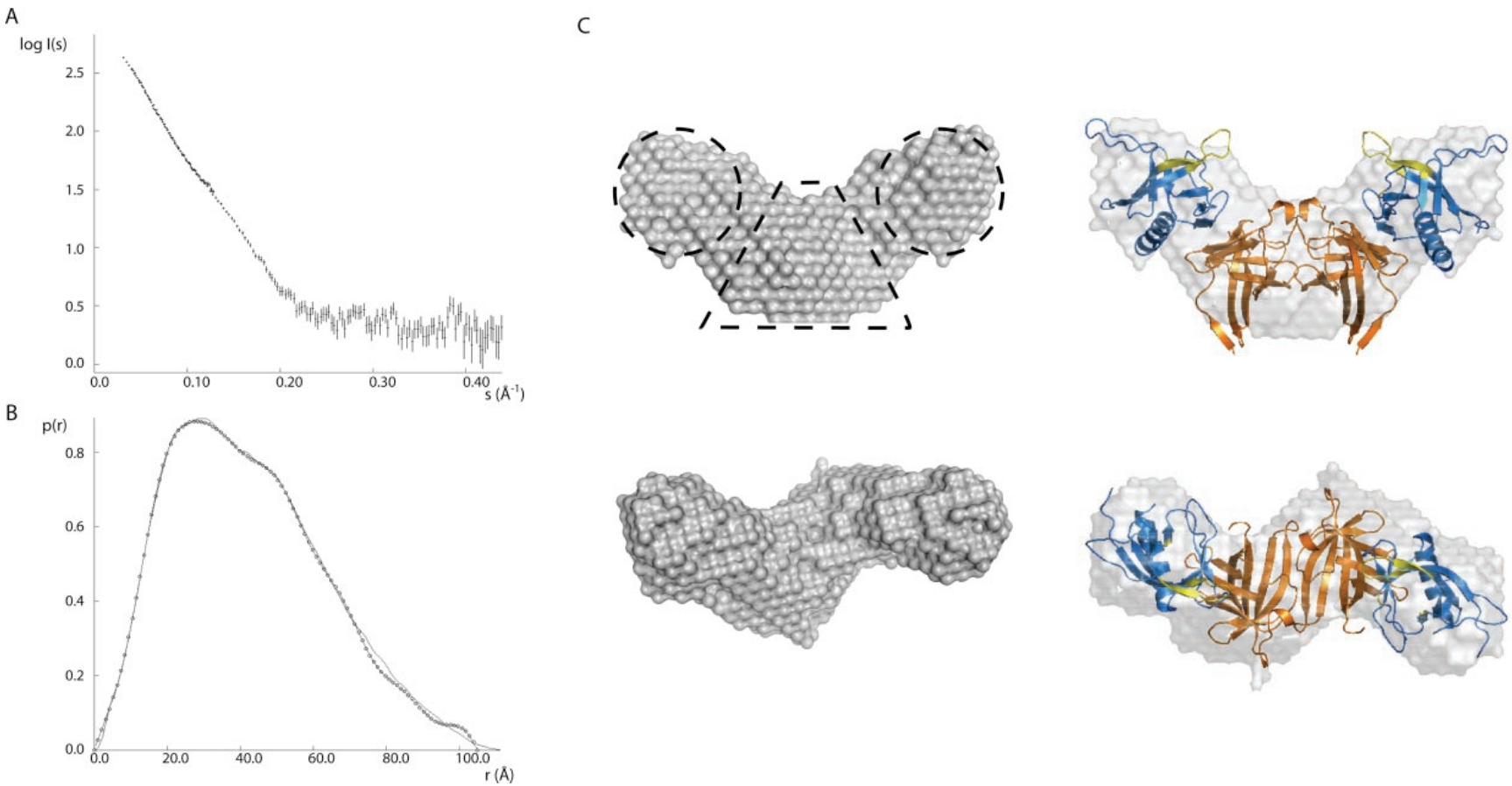
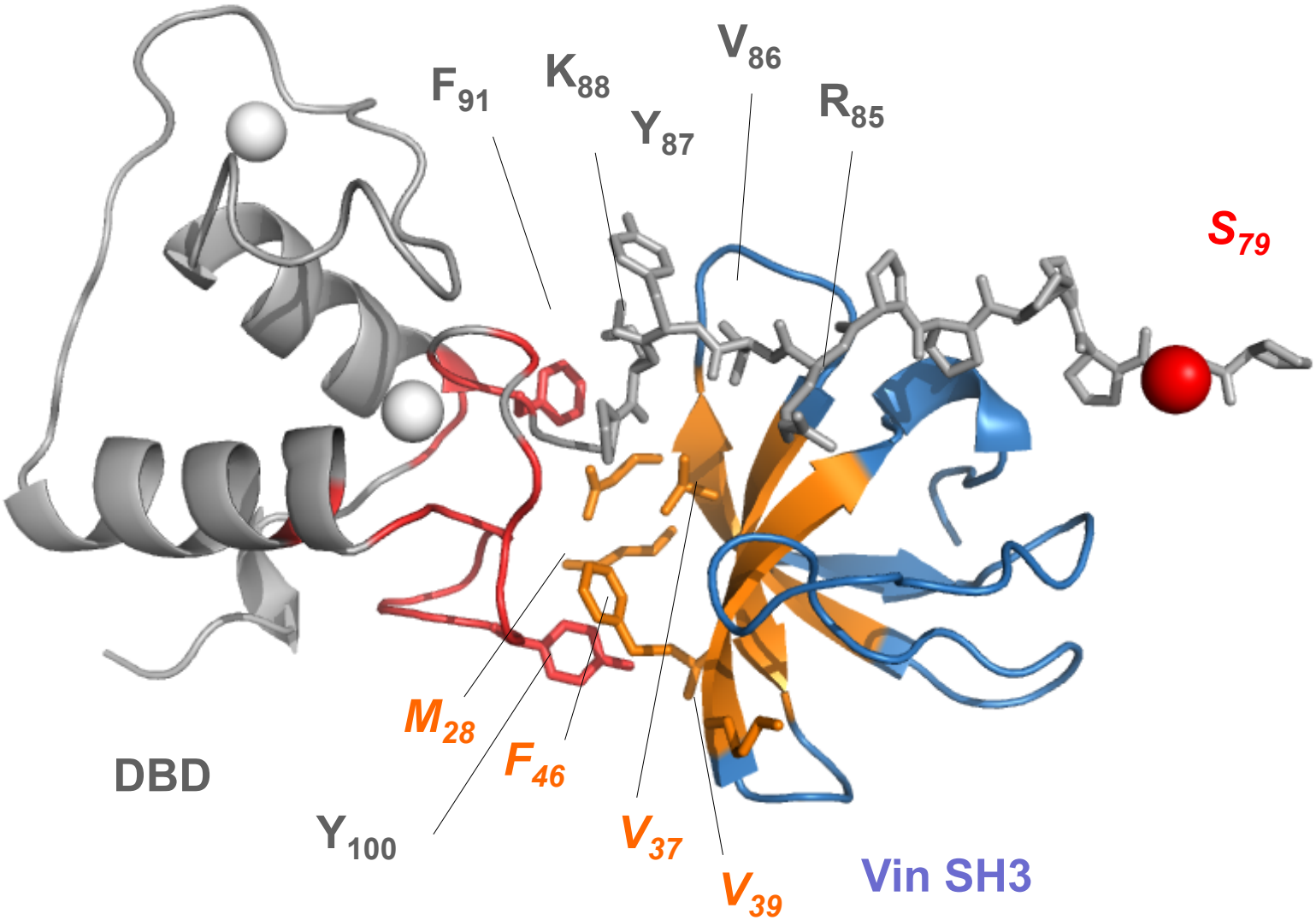


FIG. 6. **SAXS analysis of the PKBβ-PH·p14^{TCL1} complex.** *A*, experimental scattering data. *Error bars* are indicated by *vertical lines*. *B*, pair distribution function $p(r)$ for the PKBβ-PH·p14^{TCL1} complex. *Open circles*, experimental curve; *solid line*, calculated curve for a typical *ab initio* model. *C*, *side* and *top views* of the molecular envelope of the PKBβ-PH·p14^{TCL1} complex obtained by averaging 10 individual *ab initio* models. Proposed position of PKBβ-PH domains (*circles*) and the p14^{TCL1} dimer (*trapeze*) within the SAXS envelope are indicated in the *side view*.

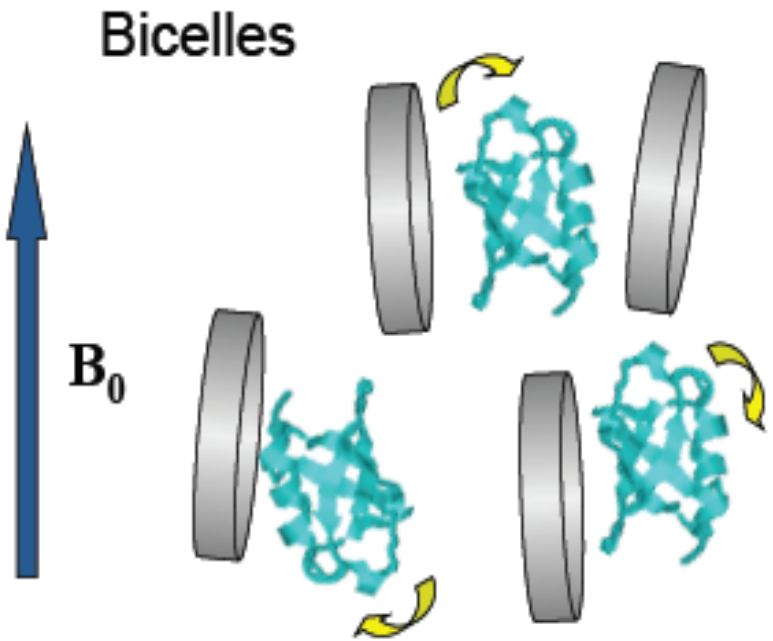
Protein-Protein interaction

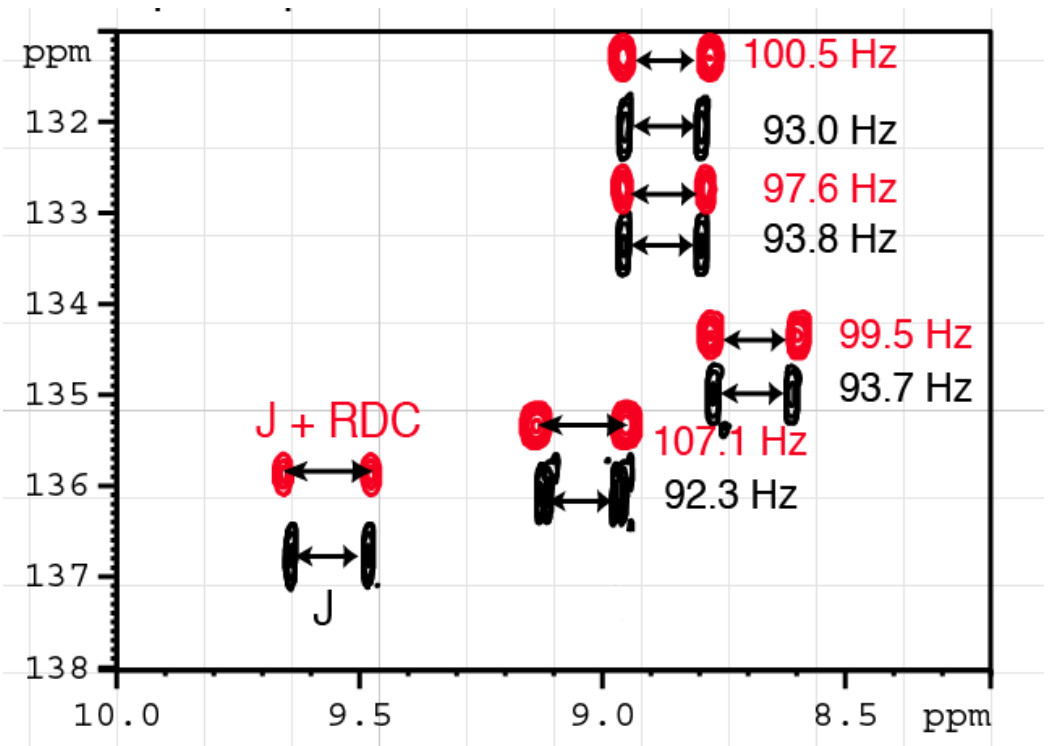
3D model of the Vin SH3.3 / RARγ DBD complex using HADDOCK



Residual dipolar couplings (RDC)

- Principle: the sample is diluted in an anisotropic medium
- This introduces a very small biais in the molecular orientations
- That leads to dipolar couplings that depends on the orientation of the internuclei vectors of the molecules

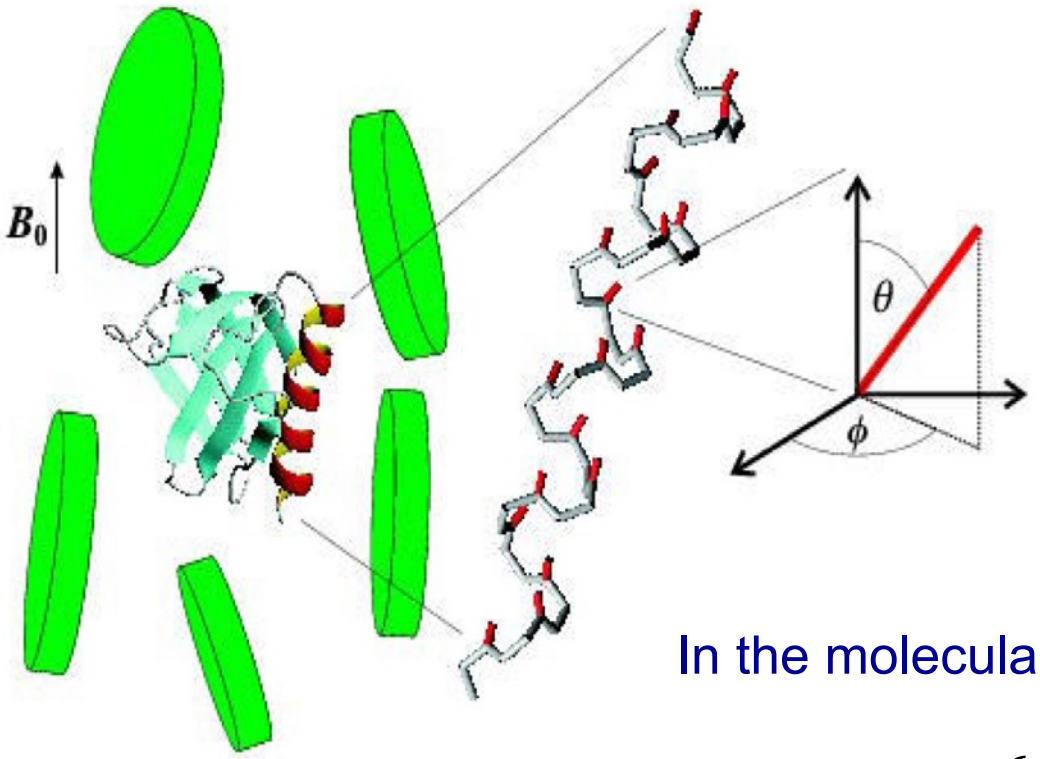




Partially oriented molecule

Isotropic phase

Modeling RDC data

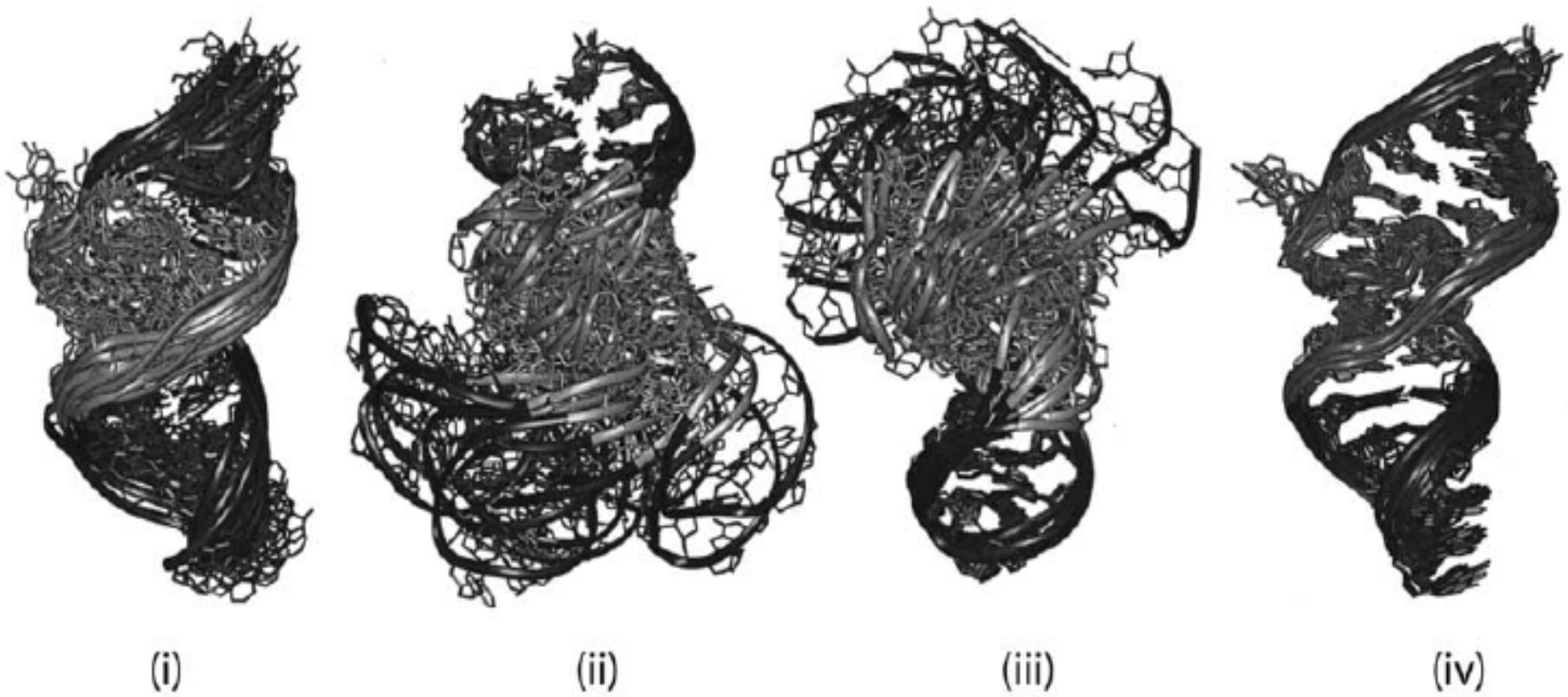


In the molecular frame:

$$RDC = D_a\left\{\left(3\cos^2\theta - 1\right) + \frac{3}{2}R\sin^2\theta\cos 2\phi\right\}$$

At least 5 RDC values are needed to define the molecular frame

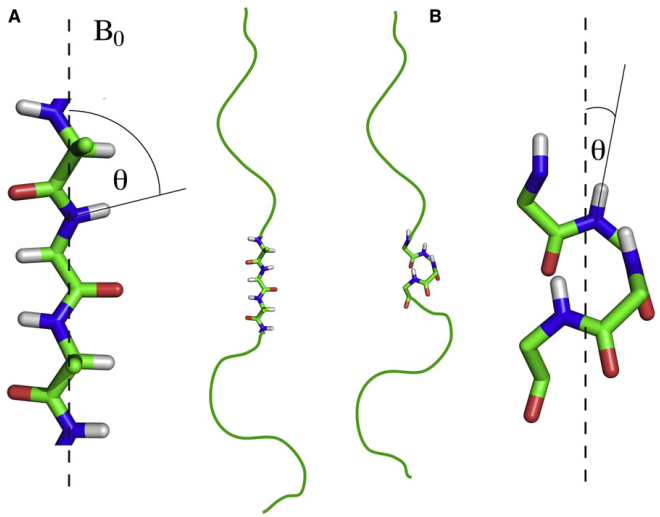
Application of RDC to RNA structure

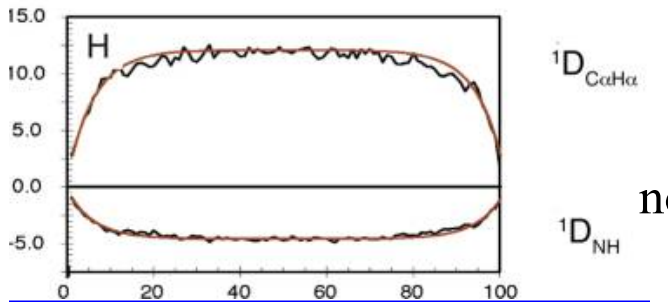


Calculation of the structure of the theophylline-binding RNA aptamer using ^{13}C–^{1}H residual dipolar couplings and restrained molecular dynamics.

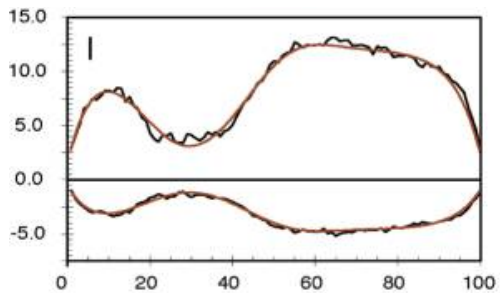
by adding the angular dependance : (non local)
to NOE constraints (local)

RDC of IDP

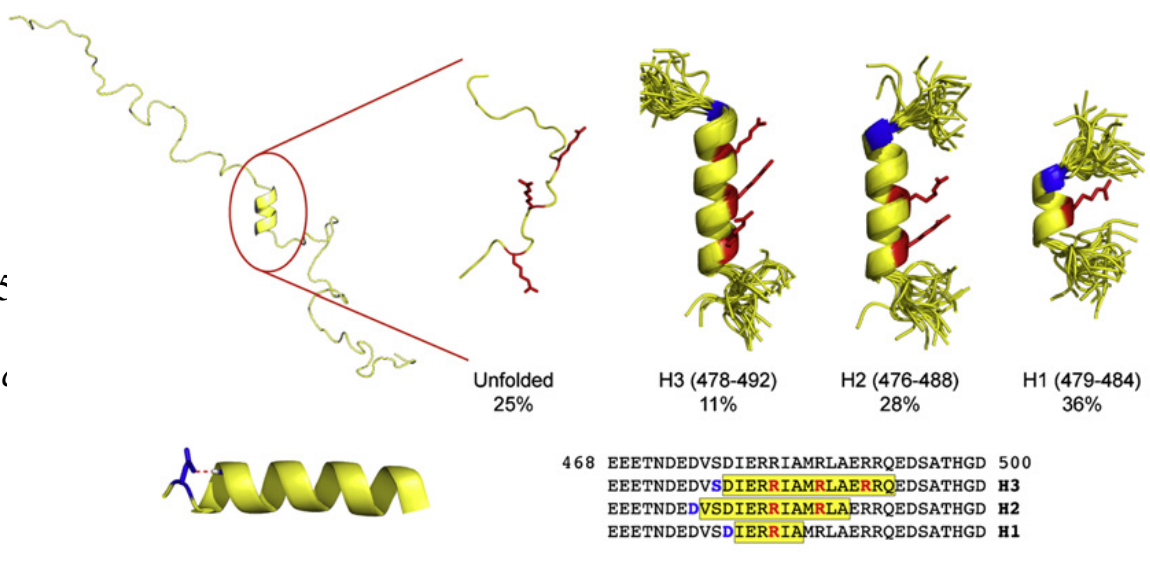


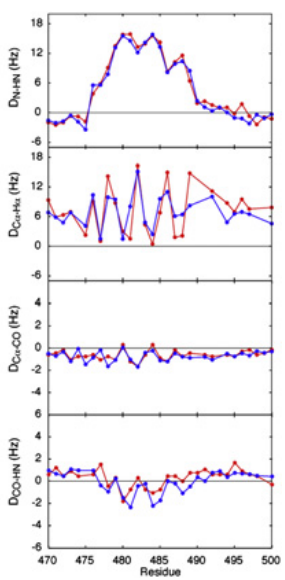


no LR contact



with LR contacts





Jensen, M. R., .. M. Blackledge (2009). *Structure* 17(9), 1169–118

Jensen, M. R.. Blackledge, M. *Proc Natl. Acad. Sci. U.*S.A. 2011, 108, 9839

Paramagnetic Relaxation Enhancement

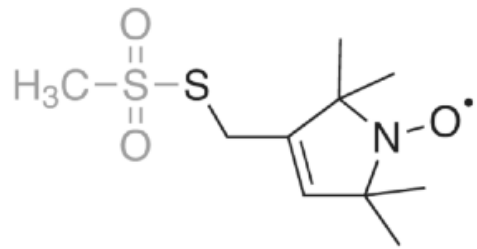
- The presence of an electronic spin in the vicinity of nuclear spins increases their relaxation rates

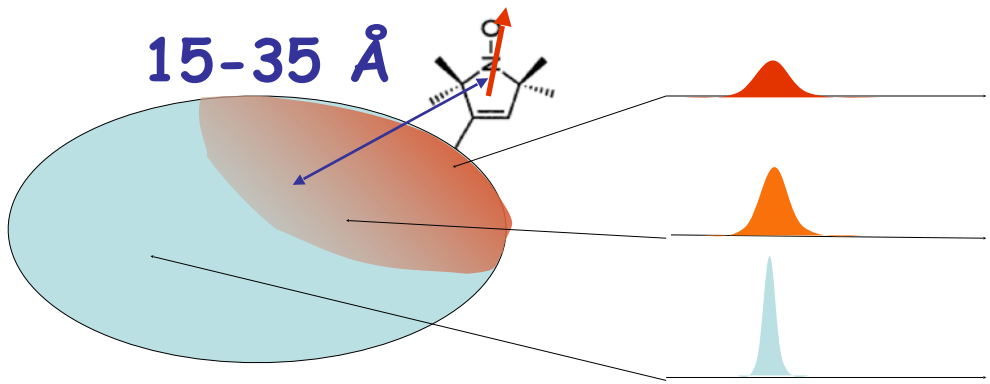
- If the electronic spin is characterized by an isotropic g tensor, the effect depends only on the distance between the nucleus and electronic spins

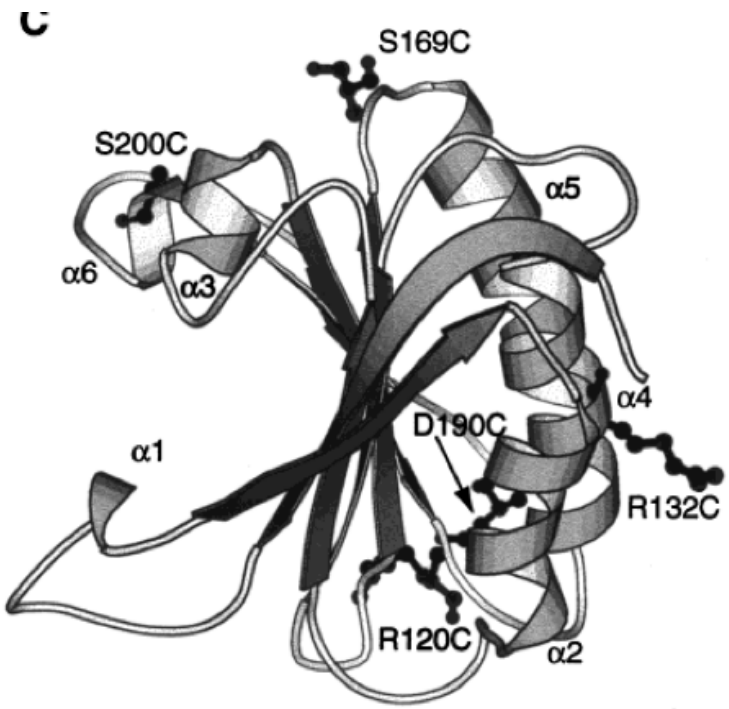
$$R_2^{PRE} = \frac{1}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \gamma_I^2 g^2 \mu_B^2 S(S+1)\left(\frac{1}{r_{IS}^6}\right)\left\{4\tau_c + \frac{3\tau_c}{1+(\omega_I\tau_c)^2}\right\}$$

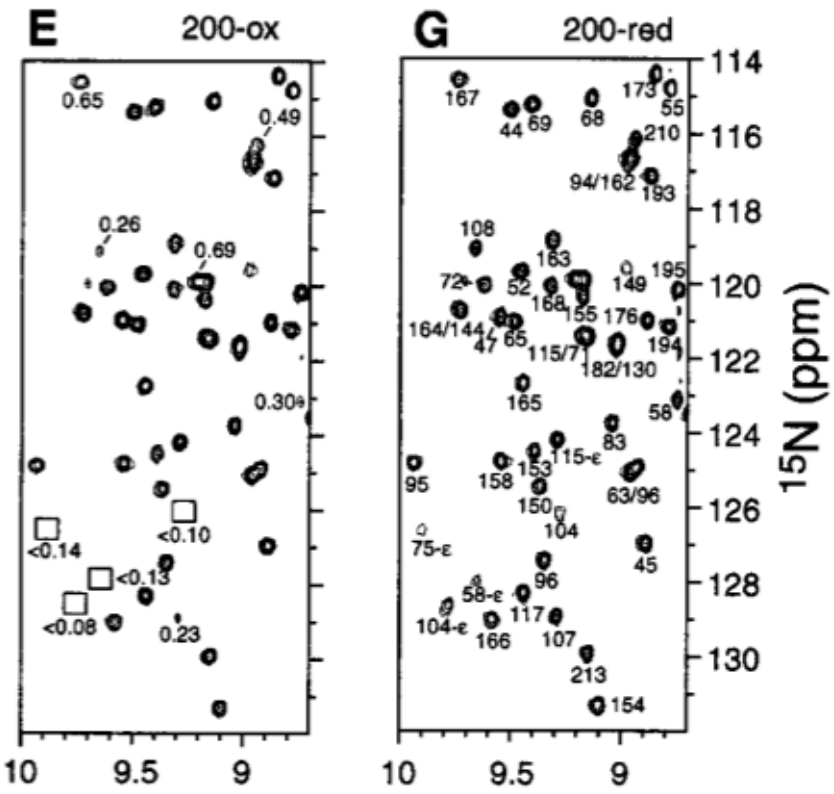
$$R_1^{PRE} = \frac{2}{5}\left(\frac{\mu_0}{4\pi}\right)^2 \gamma_I^2 g^2 \mu_B^2 S(S+1)\left(\frac{1}{r_{IS}^6}\right)\left\{\frac{\tau_c}{1+(\omega_I\tau_c)^2}\right\}$$

Introducing spin label to protein surface



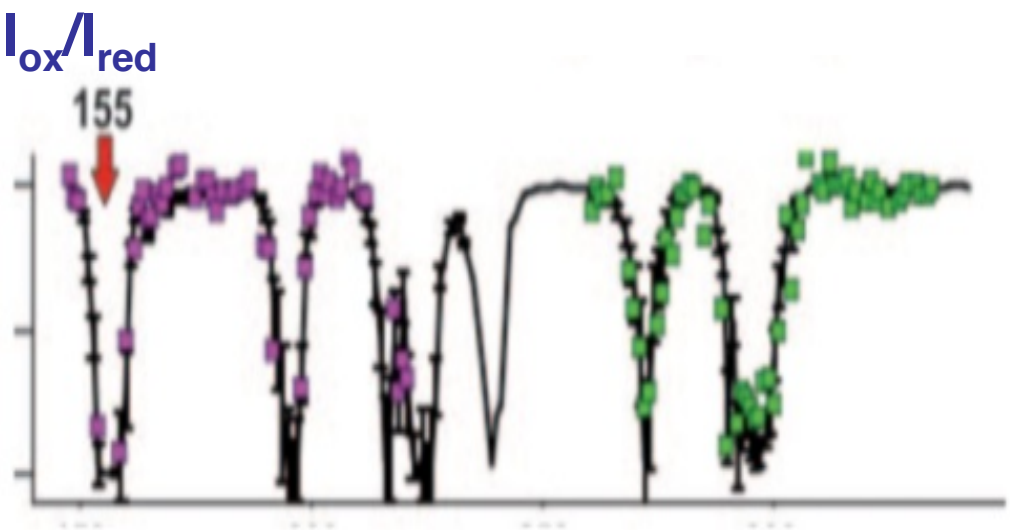


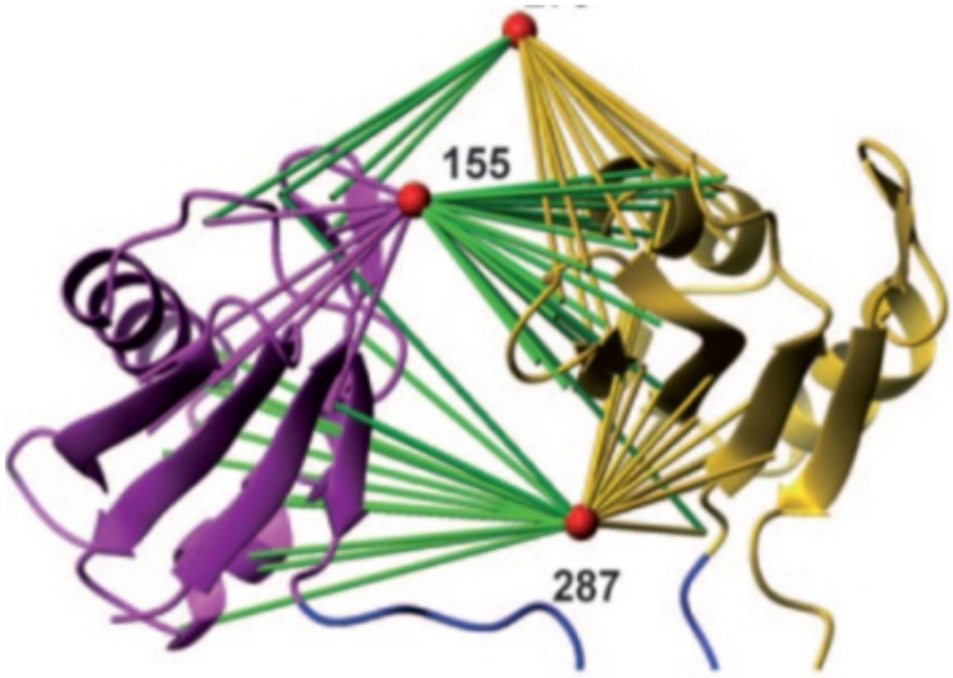




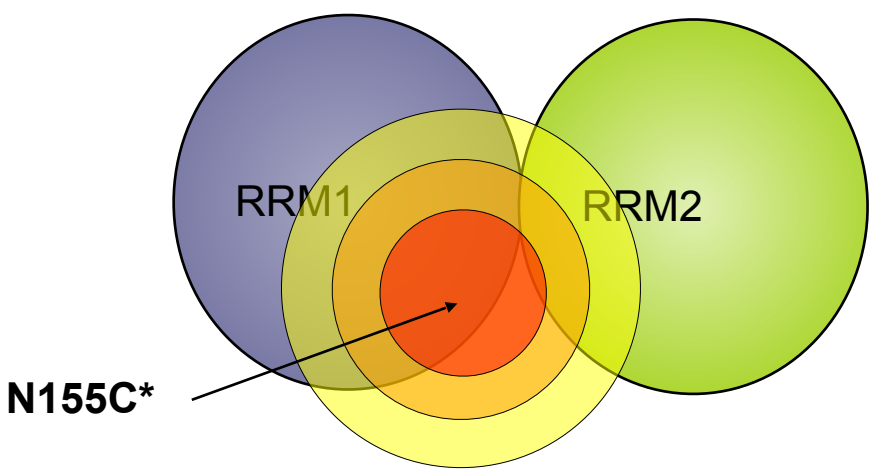
Battiste et Wagner Biochemistry V39, p5355 (2000)

Application to multiple domain proteins





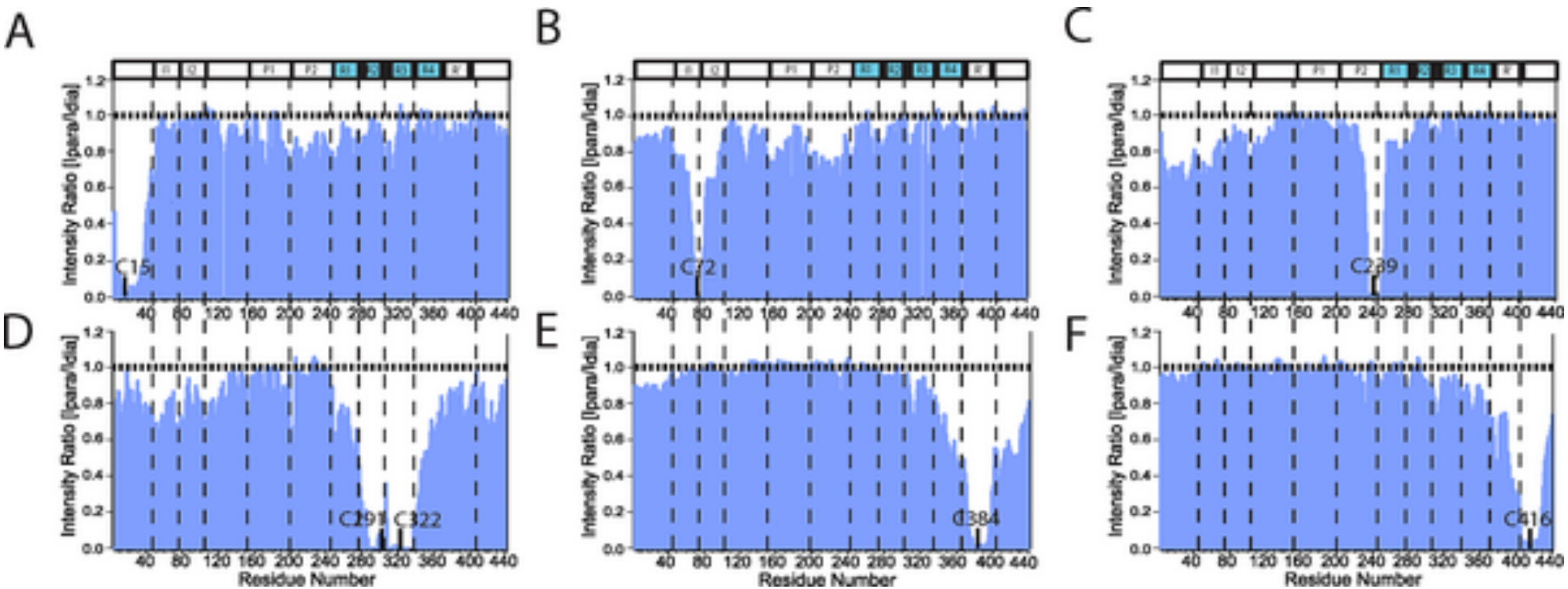
>12 Å (not bleached)
<20 Å (25% reduction)
<15 Å (50% reduction)
<10 Å (heavy bleaching)



from Bernt Simon M Sattler EMBL Heidelberg, *Angew. Chem.*, 2010, *122(11)*, 2011 - 2014

Applied to IDP (Tau)

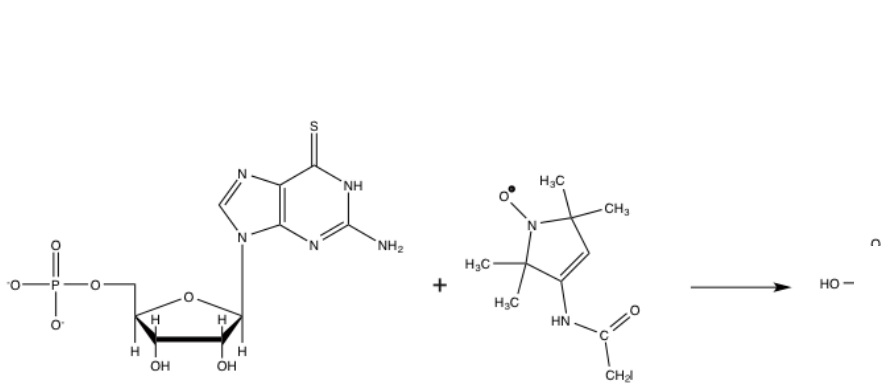
Figure 7. PRE of Amide Protons in Spin-Labelled Tau

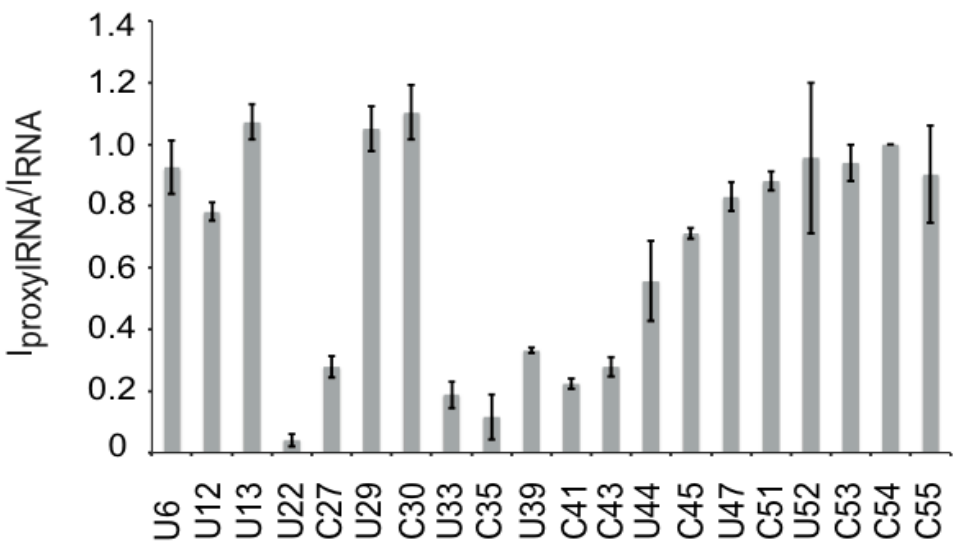


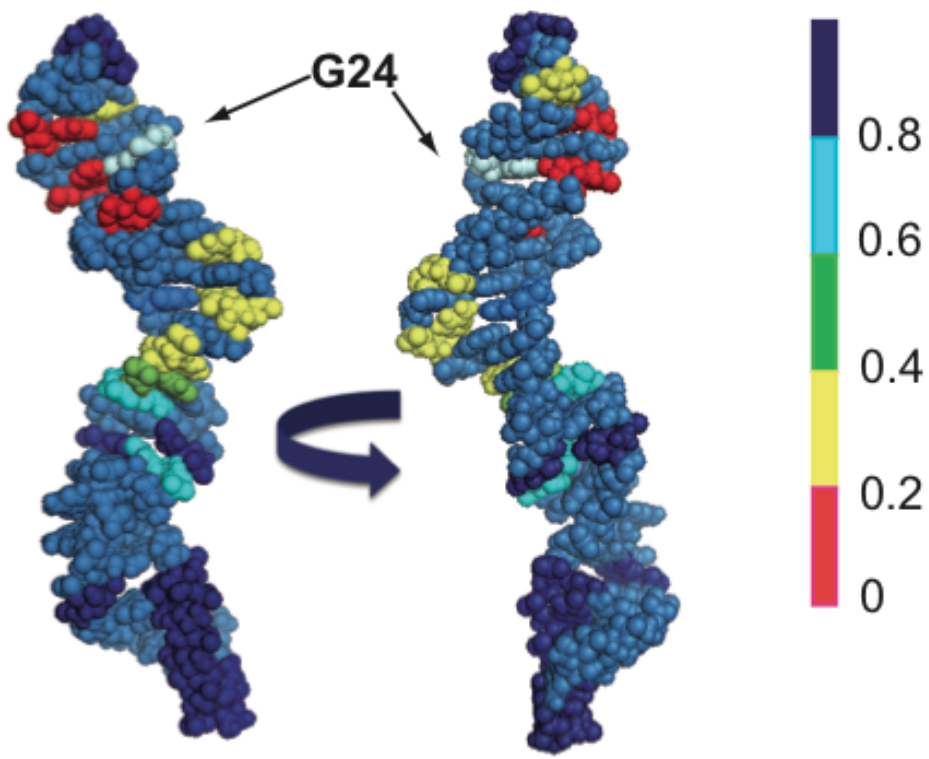
Mukrasch MD, Bibow S, Korukottu J, Jeganathan S, et al. (2009) Structural Polymorphism of 441-Residue Tau at Single Residue Resolution. PLoS Biol 7(2): e1000034. doi:10.1371/journal.pbio.1000034
http://www.plosbiology.org/article/info:doi/10.1371/journal.pbio.1000034

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,

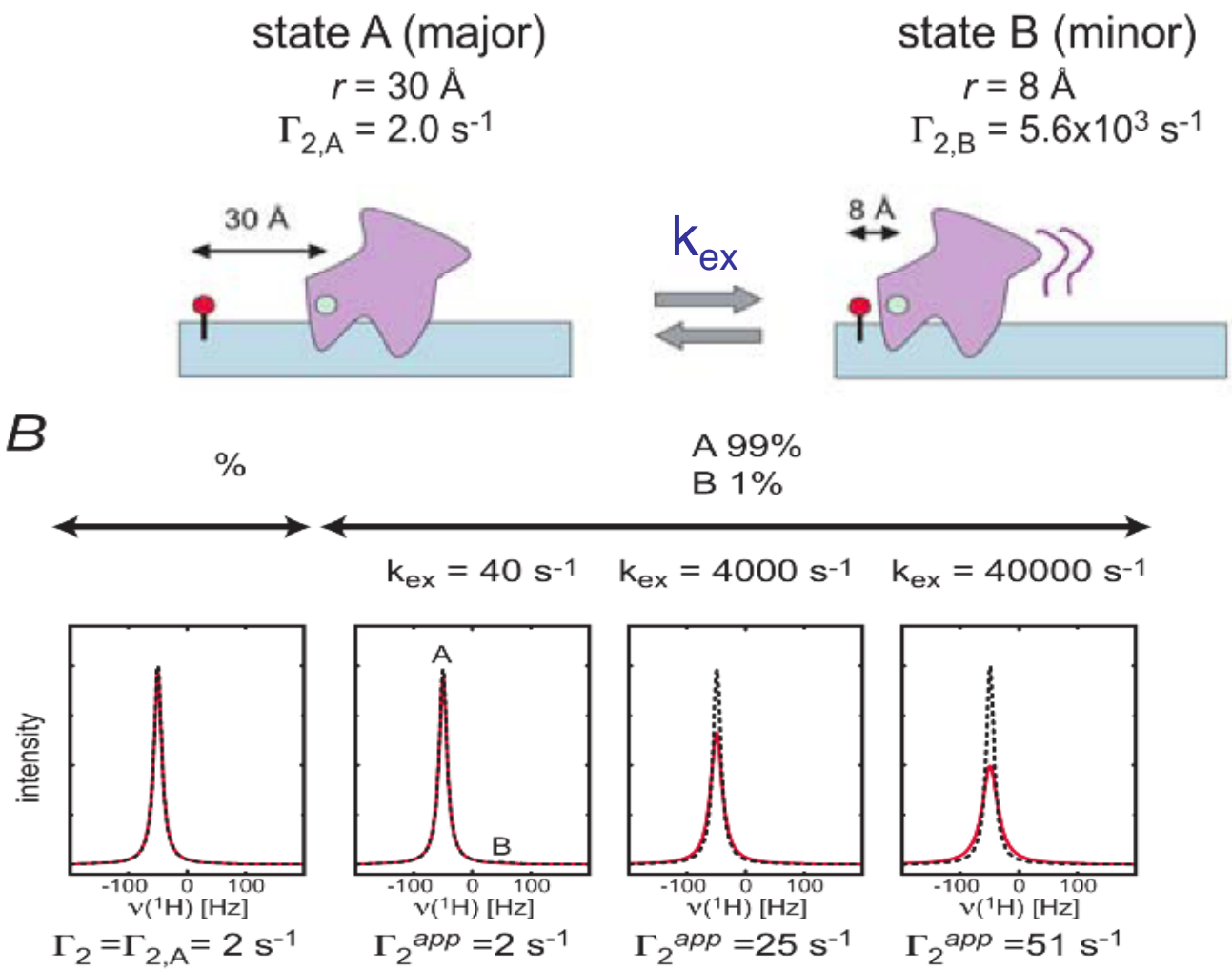






PRE based amplification of low population states

- Due to the r^{-6} distance dependance of the PRE, small distances in low populated states may be revealed



Iwahara & Clore (2006)
Nature 440, p 1227

Phenomenon

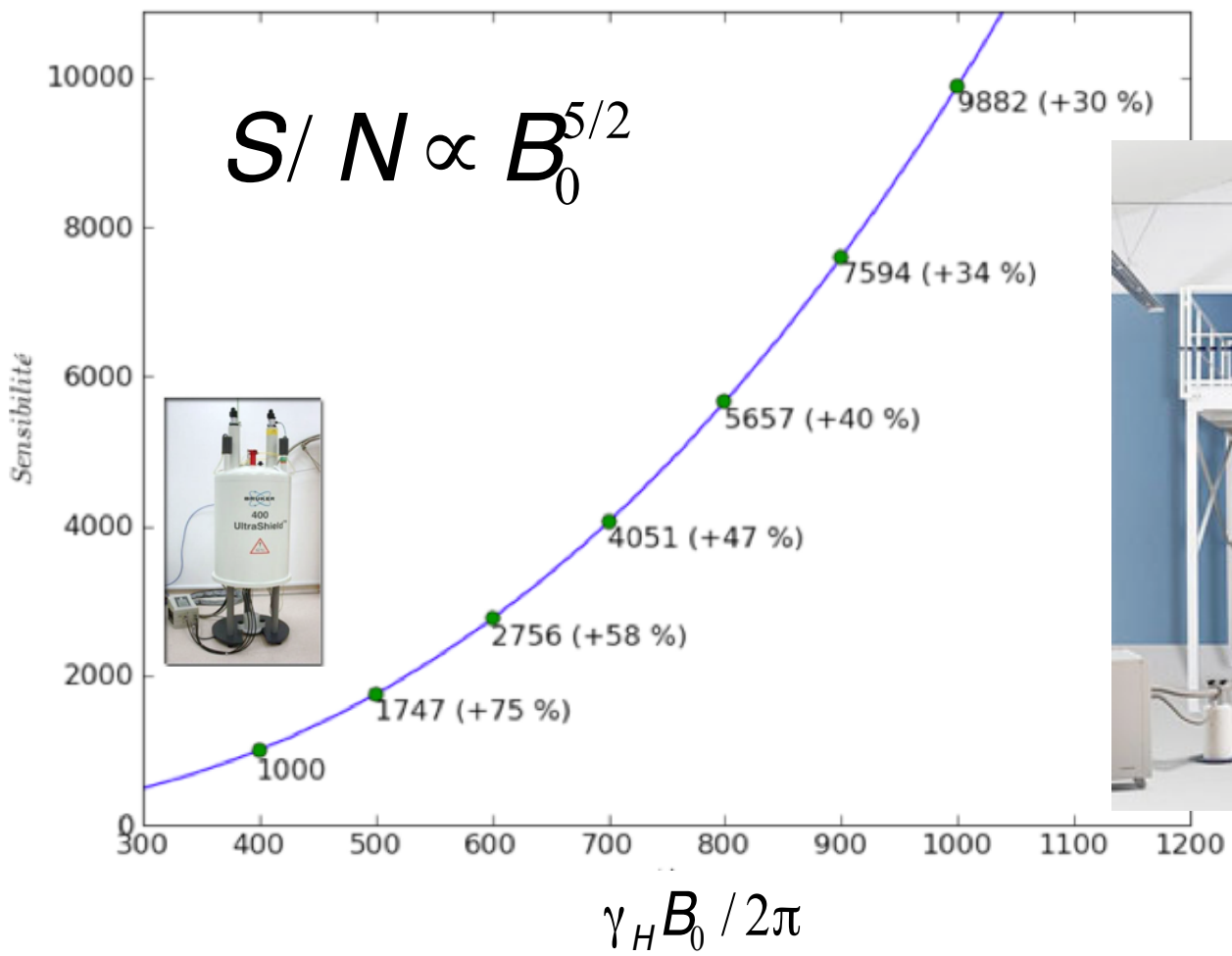
- Chemical-Shift ≡ position in the spectrum
- Spin-Spin coupling
  - “Dipolar” coupling - depends on spin-spin geometry
  - “Scalar” coupling - depends on electronic orbital
- Relaxation
  - *decoherence* of the quantum states
- NOE
  - relaxation due to spin-spin ⇒ information on atomic distances
- RDC: Reduced Dipolar Coupling
  - information on angular geometry
- PRE: Paramagnetic Relaxation Experiment
  - relaxation due to electronic spin-spin ⇒ information on molecular contacts

final remarks

- sensitivity aspects
- protein size aspects
- signal aspects
- more than structure

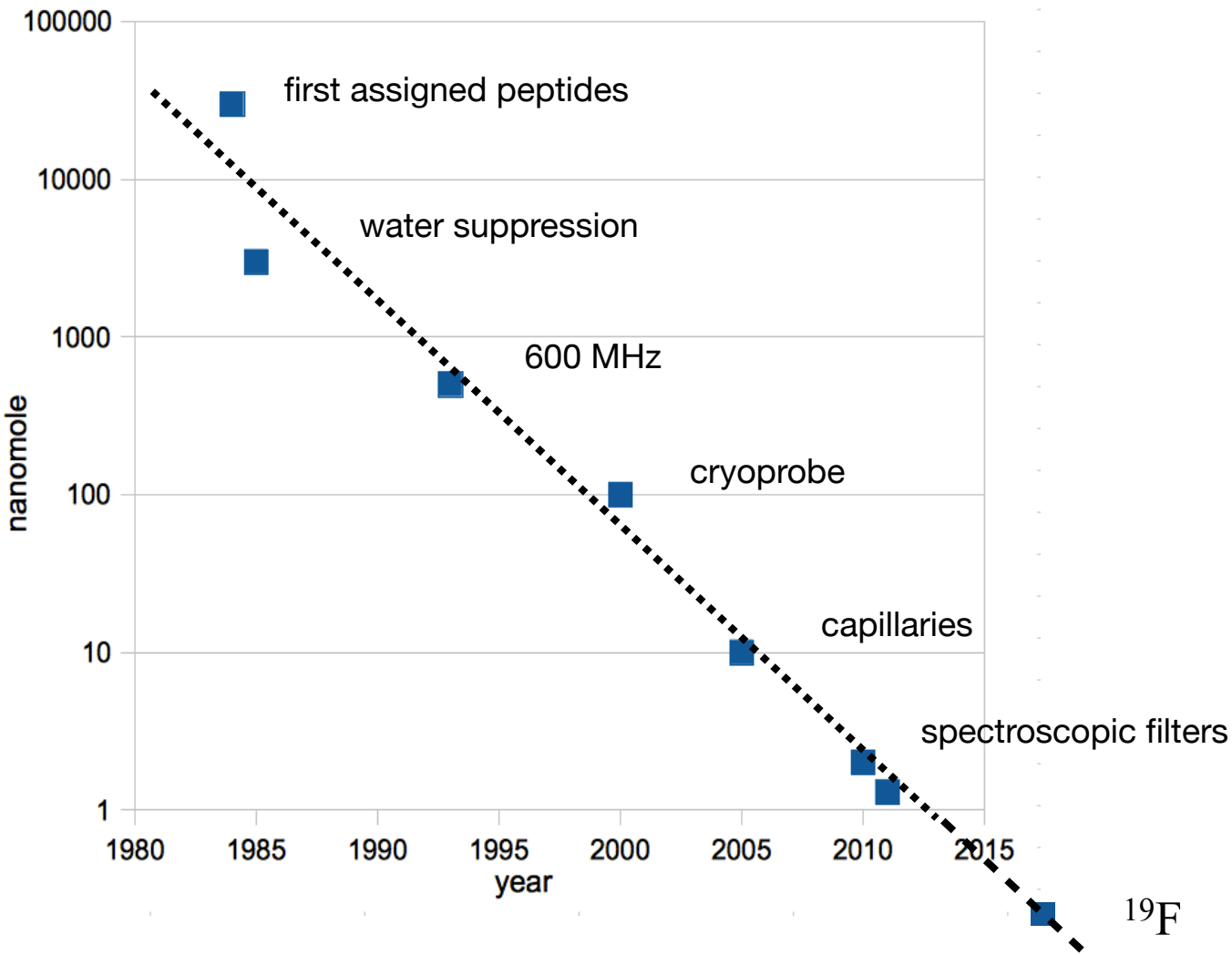
Recent advance in NMR instruments

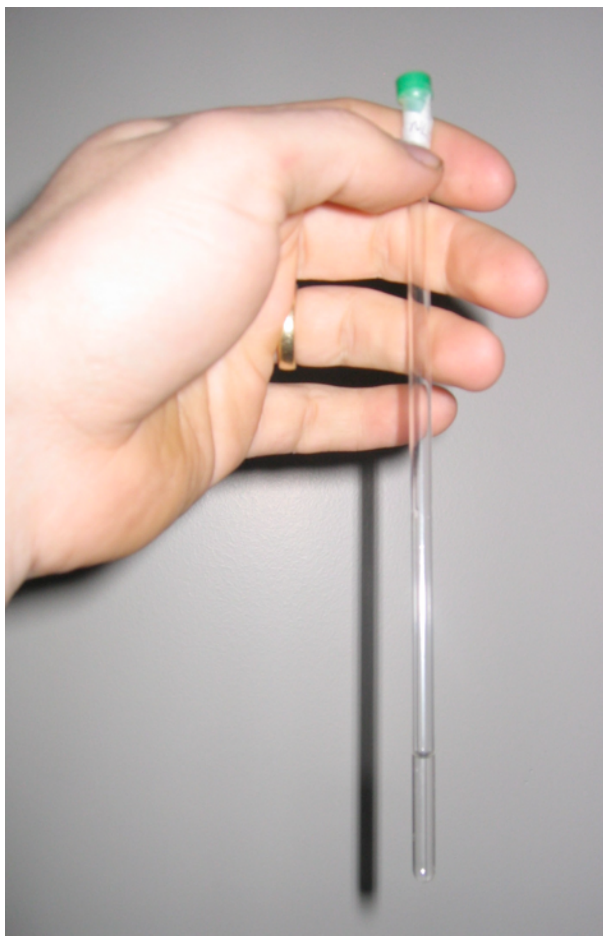
- The Signal/Noise ratio depends on the applied magnetic field



Sensiticity increase over my carrier

Protein quantity ~ Moore law
/2 every 2 years /1000 in 20 years





Strategy related to size

- tiny proteins M < 6 kD
  - very easy to study
  - peptides !
- small proteins M < 12-15 kD
  - easy -
  - ^{15}N labelling might be enough
- larger proteins M < 40 kD
  - ^{15}N and ^{13}C required - ^{2}H to be considered
- very large proteins M > 50 kD
  - ^{2}H labelling and ^{15}N / ^{13}C labelled specific incorporation
- beware of “hidden” oligomers

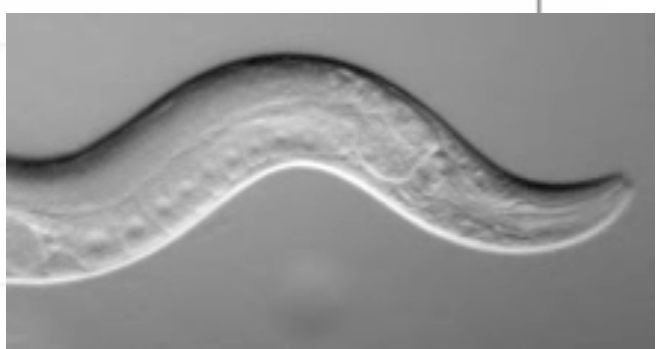
  Glutathion-S-Transferase 2x50kD

  Insuline : 6x6kD

Distribution de taille

protein size distribution in *C.Elegans* genome

from : *Genomic classification of protein-coding gene families* Erich M. Schwarz in *WormBook*



Overview of cell free isotopic labeling

Specific'amino'acids'labeling

CECF

Val,'Thr'^{15}N,^{13}C

+'18'aa unlabeled

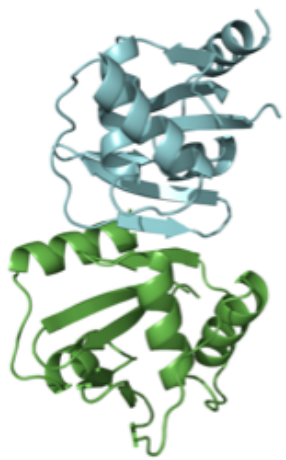
Expected'peaks:'21

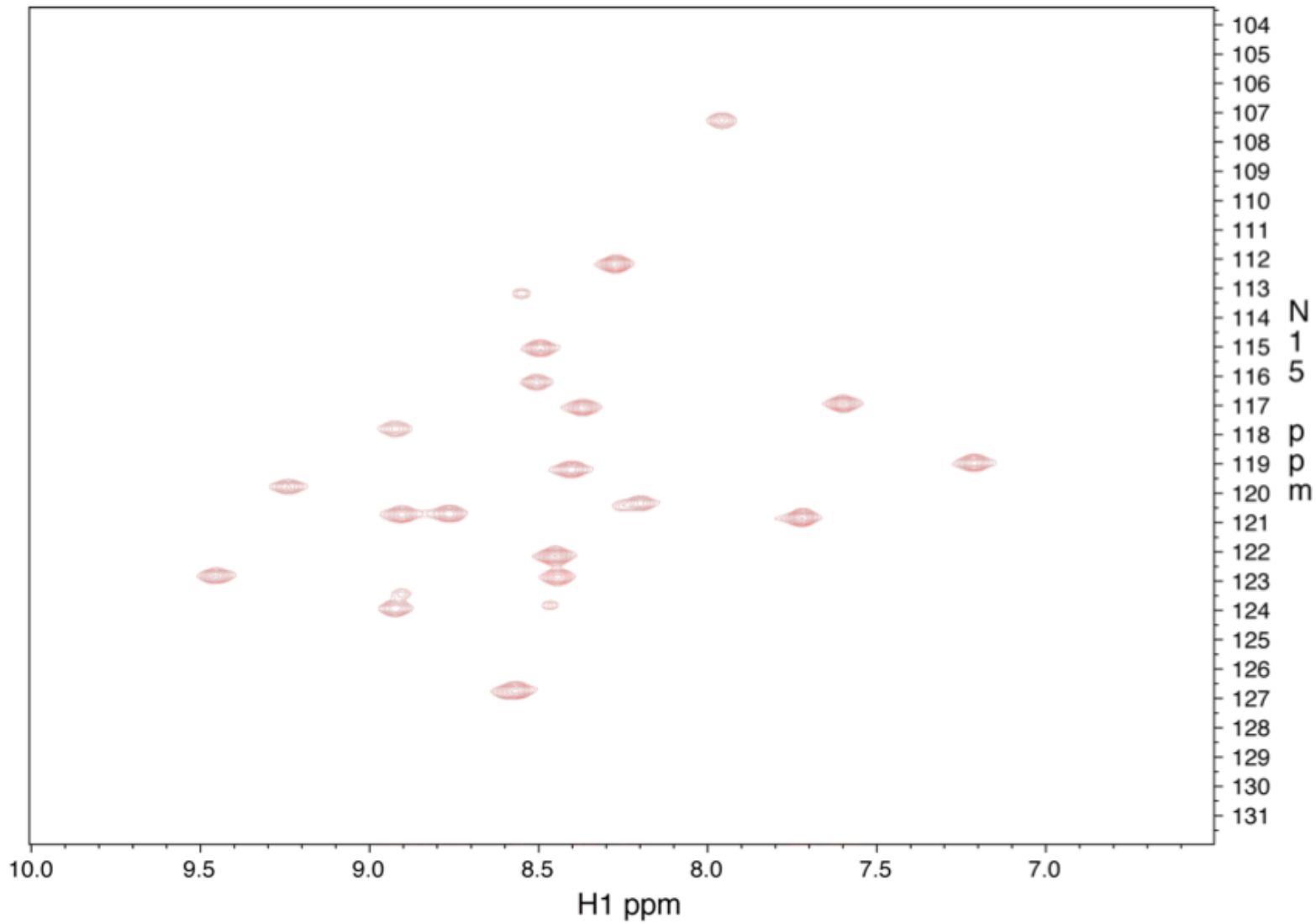
13'Val

''8'Thr'

Observed':'22

Yield'4mg/ml

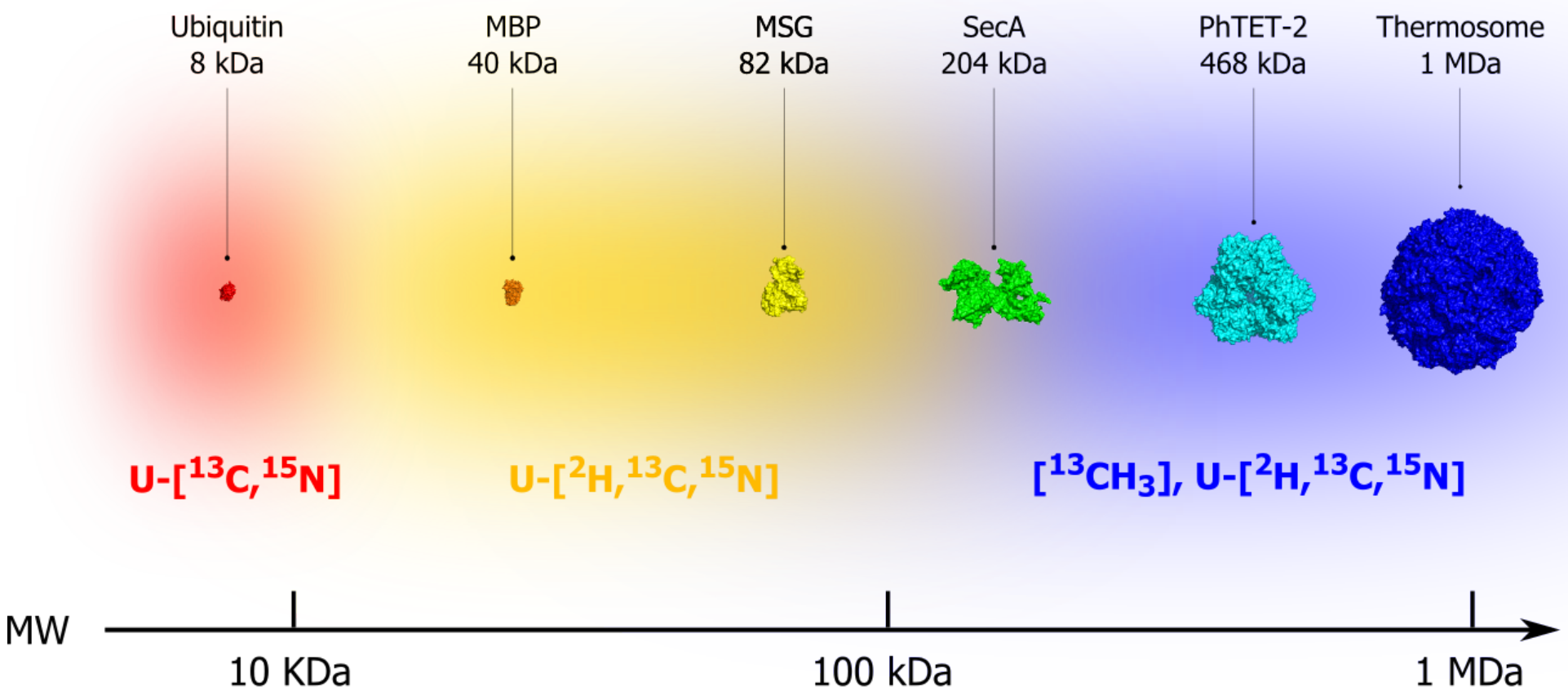




'HSQC''600MHz''H23'(16'kDa)'

Lionel Imbert (IBS) Cell-Free expression platform

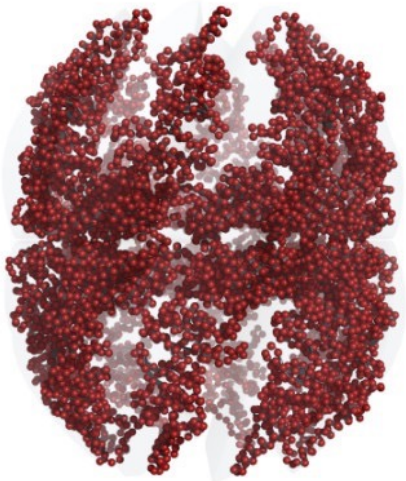
Is NMR spectroscopy only limited to small molecules?

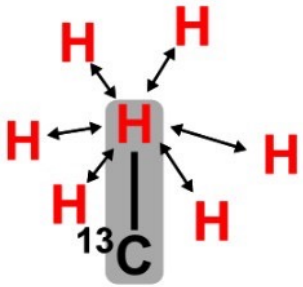


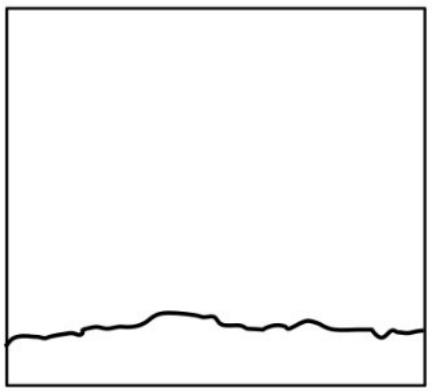
Courtesy of J Boisbouvier et al.

Solution NMR Spectroscopy
Specific protonation of methyl groups

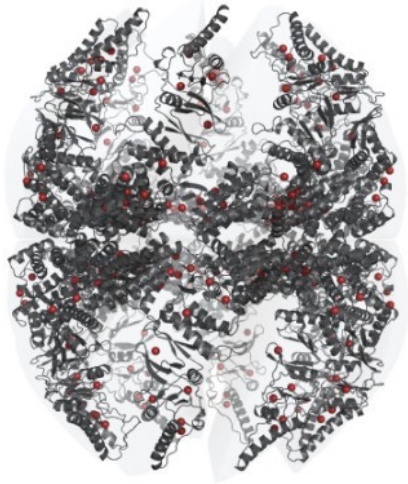
Uniformely
[^{1}H, ^{13}C, ^{15}N]

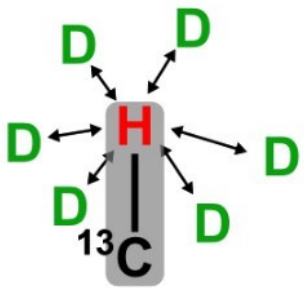


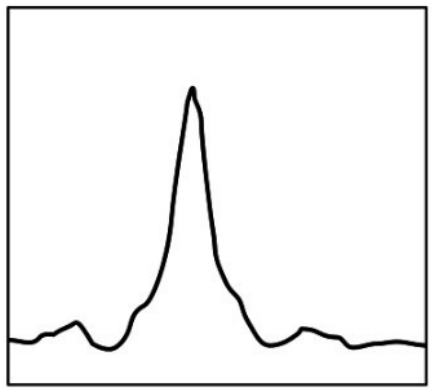




Methyl groups





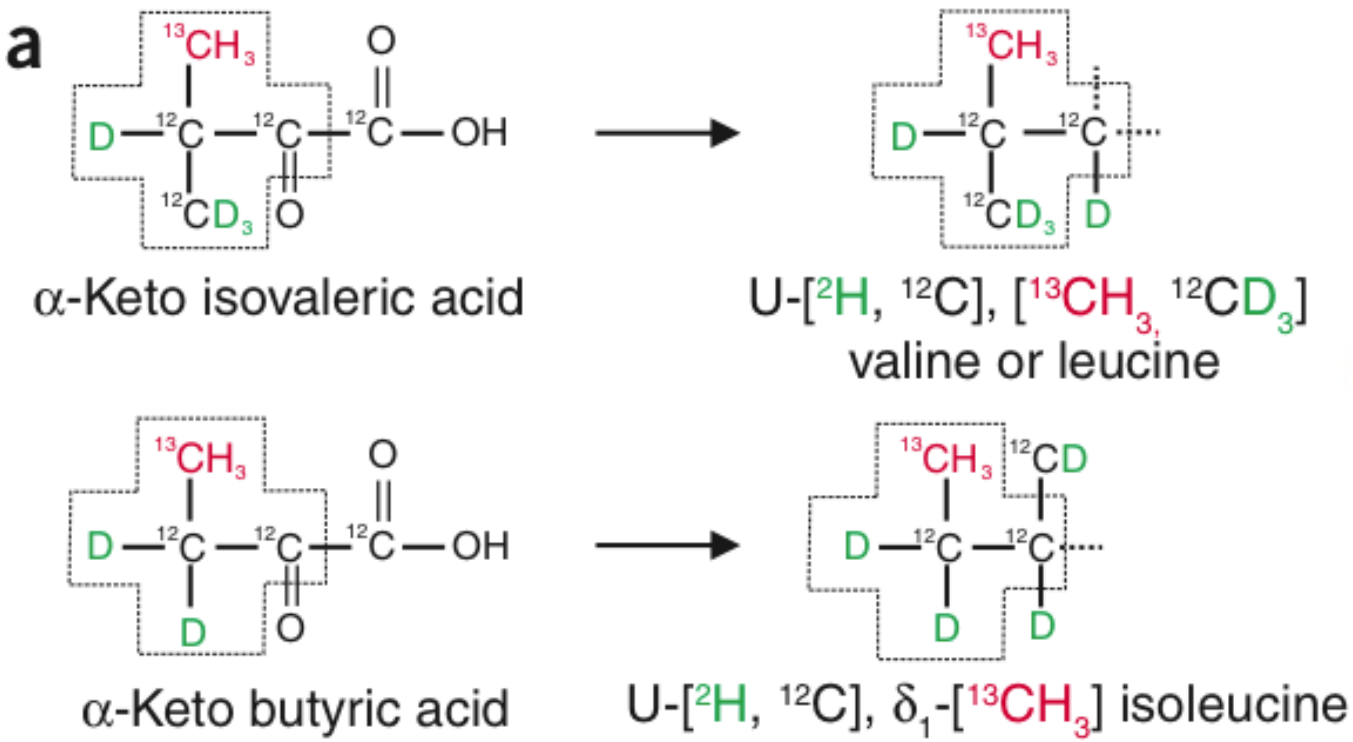


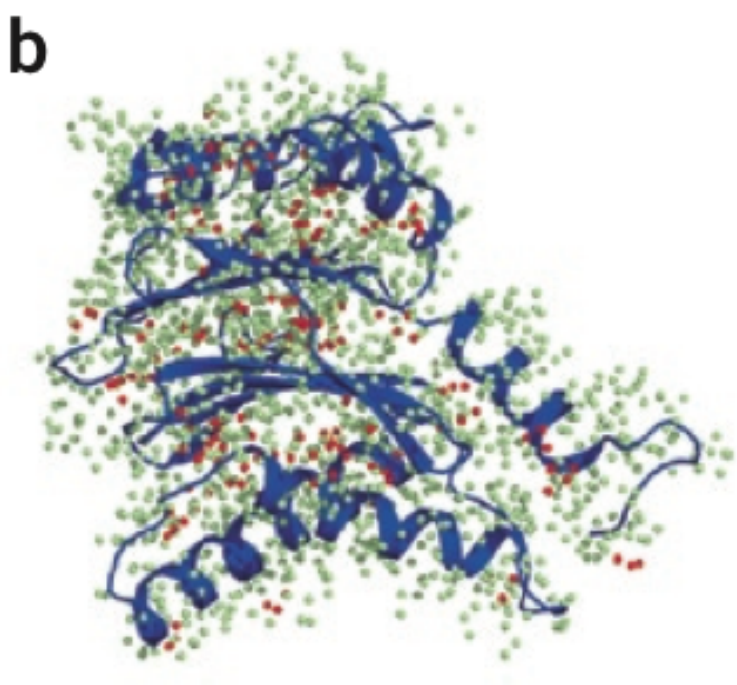
Gardner *et al*, J. Am. Chem. Soc. (1997)

Solution NMR of supramolecular complexes: providing new insights into function

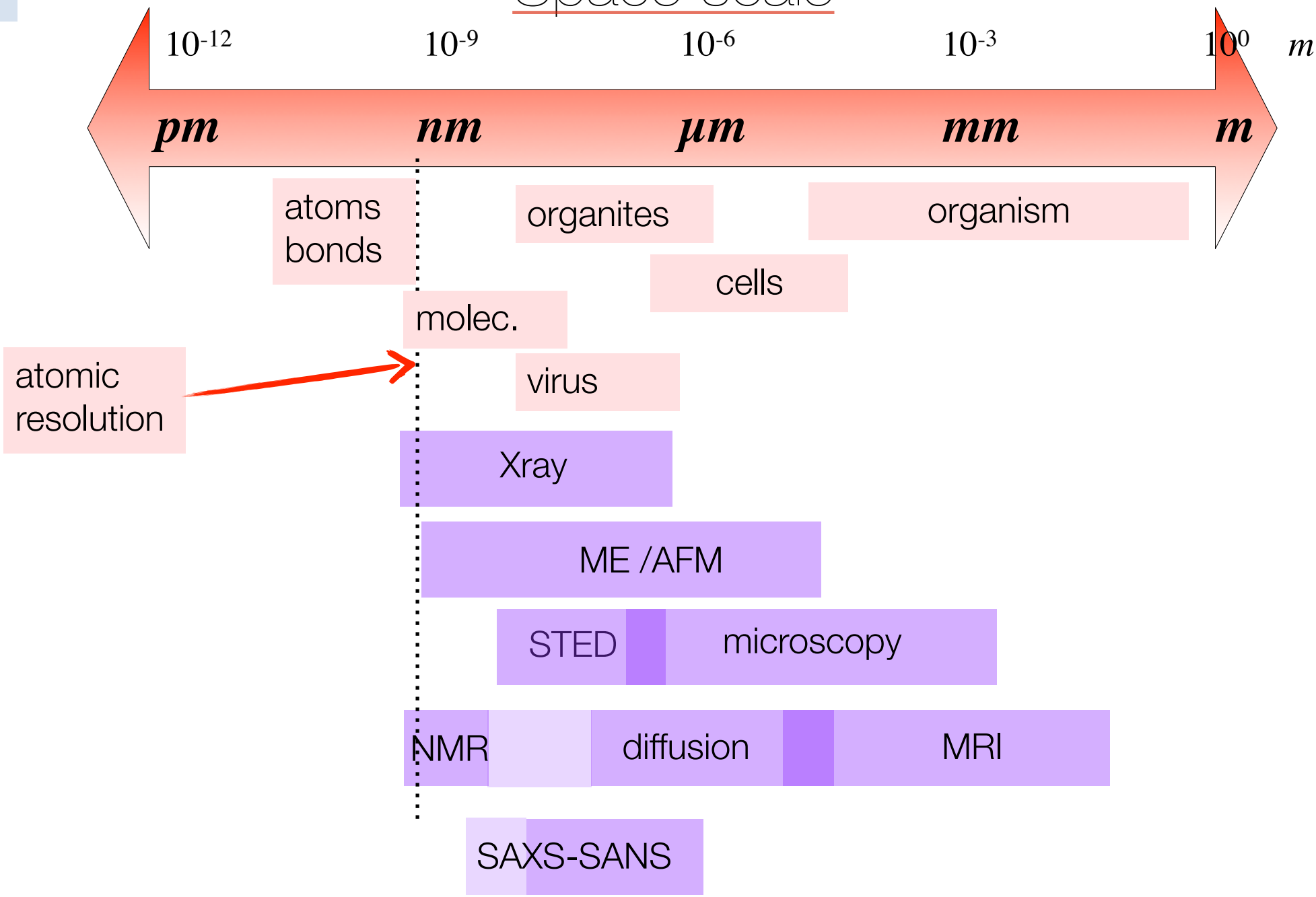
Remco Sprangers, Algirdas Velyvis & Lewis E Kay

Nature Methods 2007

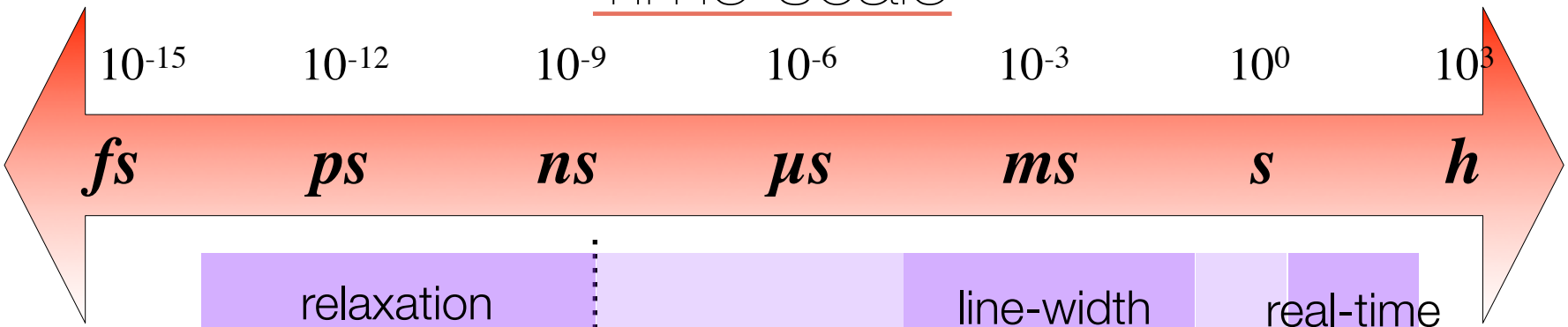




Space scale


10-12
10-9
10-6
10-3
100
m
pm
nm
µm
mm
m
atoms
bonds
organites
organism
cells
molec.
atomic
resolution
virus
Xray
ME /AFM
STED
microscopy
NMR
diffusion
MRI
SAXS-SANS

Time scale



relaxation

line-width

real-time

molecular tumbling time

molecular vibrations

rotations

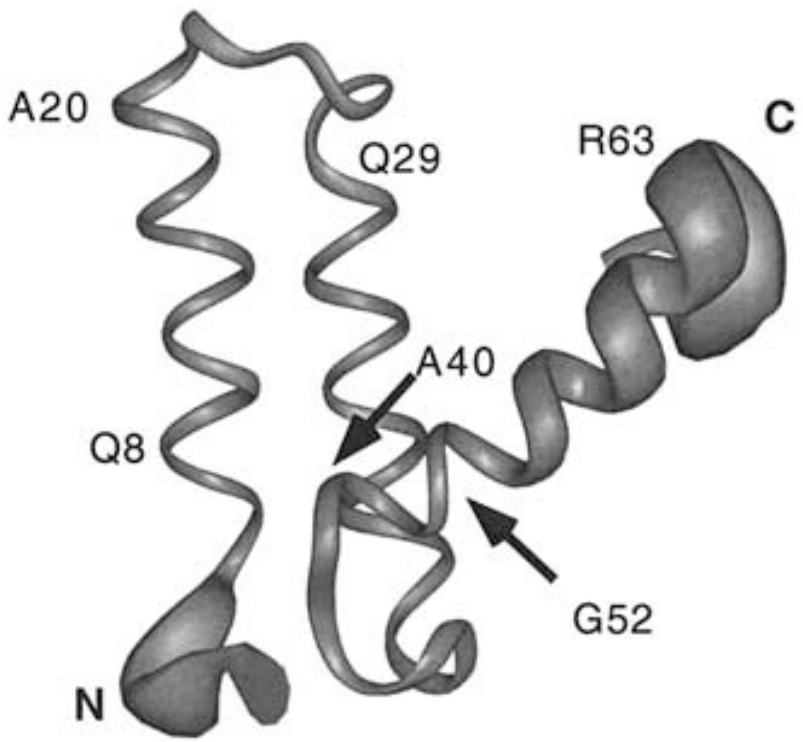
kon

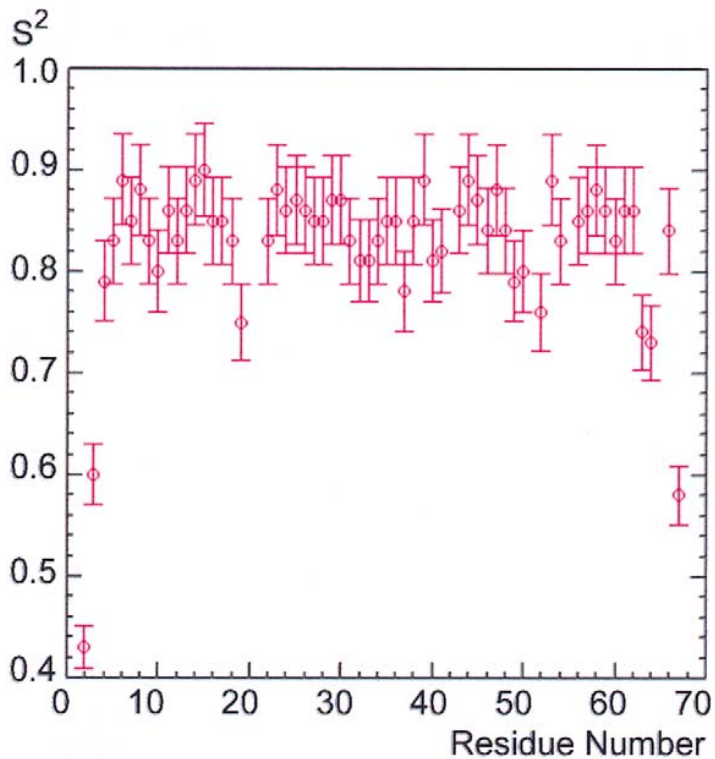
allostery

enzymatic reactions

folding unfolding

dynamic measurements





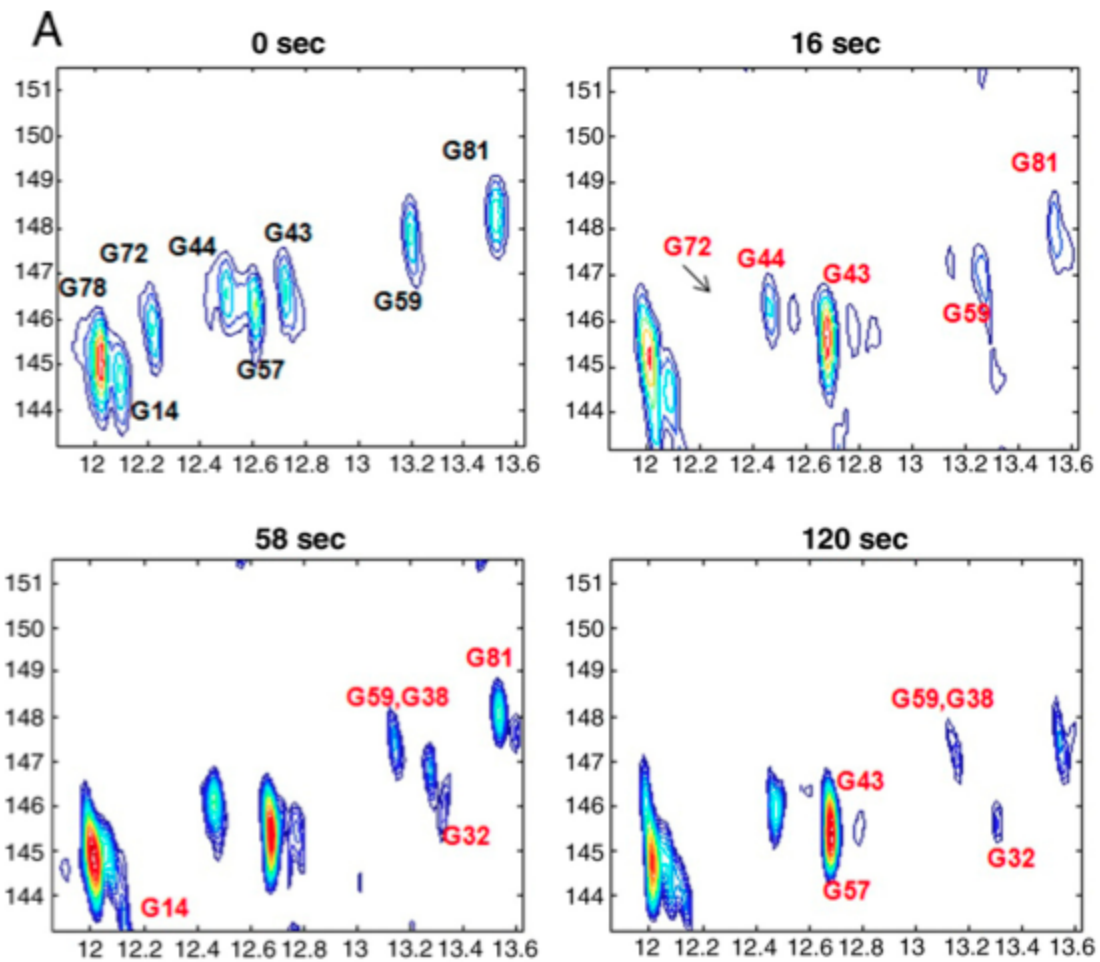
Déméné, H., Ducat, T., Barthe, P., Delsuc, M.-A., & Roumestand, C. (2002). Structure refinement of flexible proteins using dipolar couplings: application to the protein p8MTCP1. Journal of Biomolecular NMR, 22(1), 47–56.

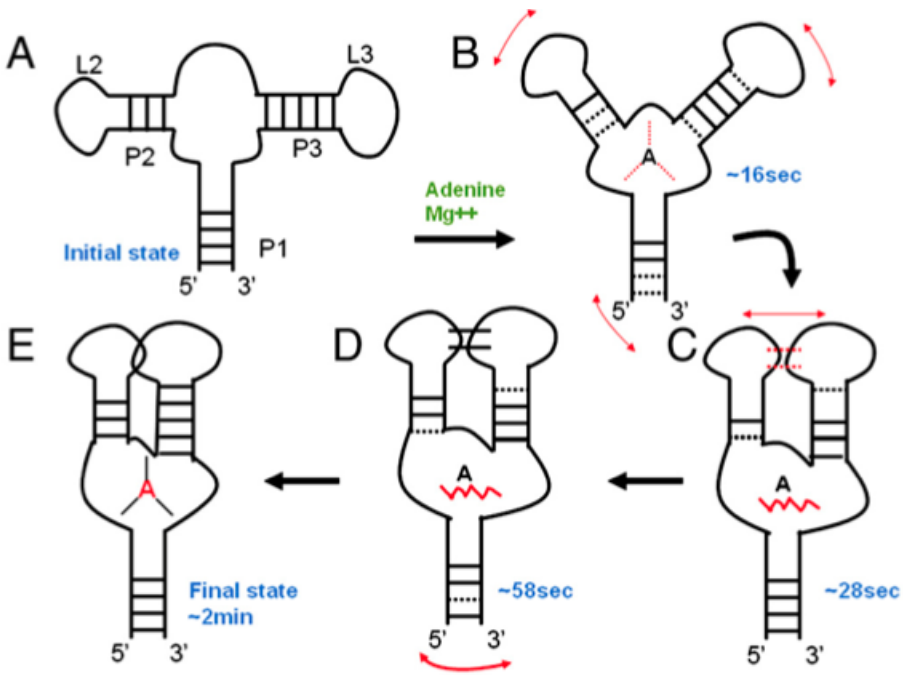
Application: Real-time follow-up of a folding

Real-time multidimensional NMR follows RNA folding with second resolution

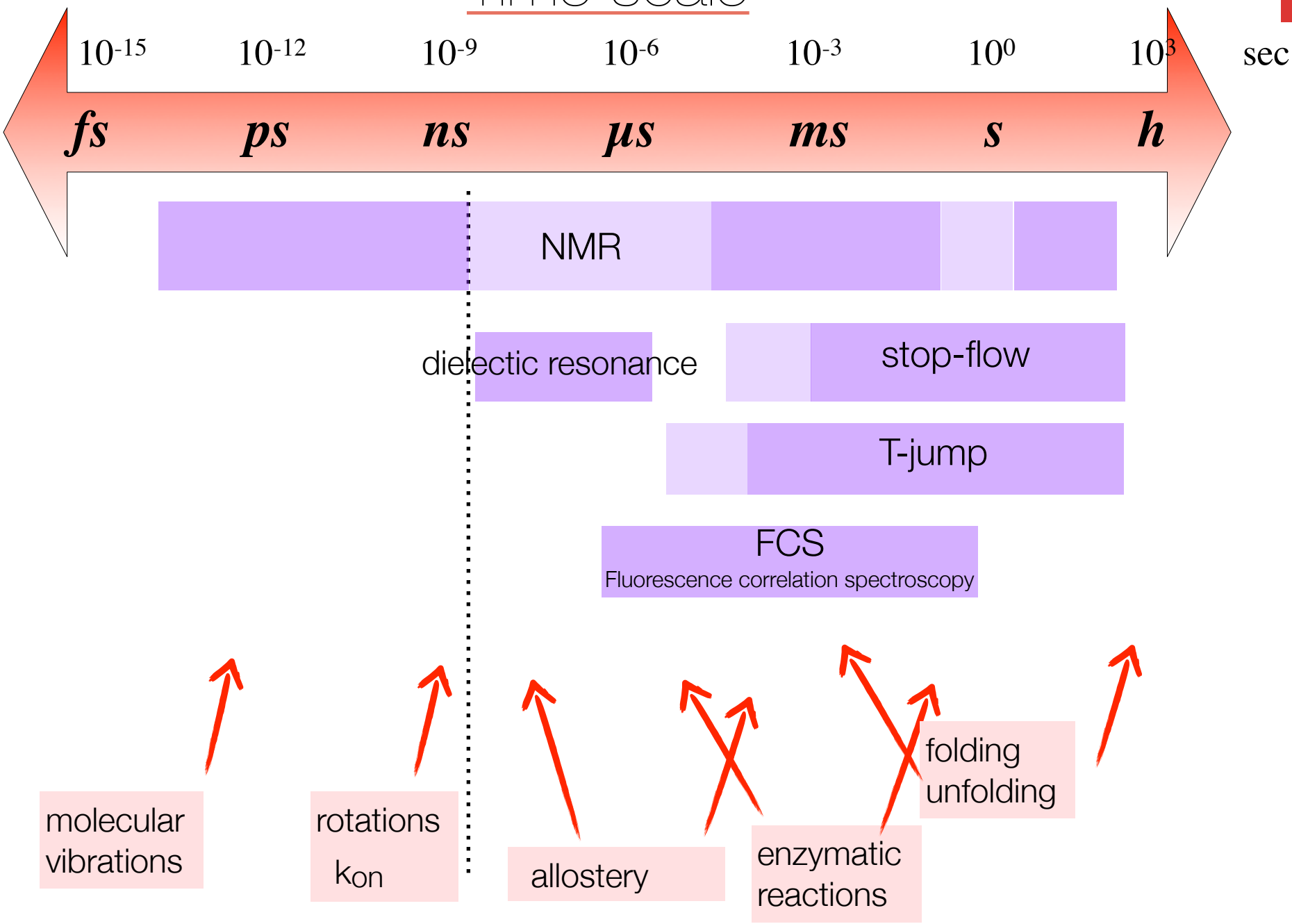
Mi-Kyung Lee[a,1], Maayan Gal[b,1], Lucio Frydman[b,2], and Gabriele Varani[a,c,2]

PNAS 2010

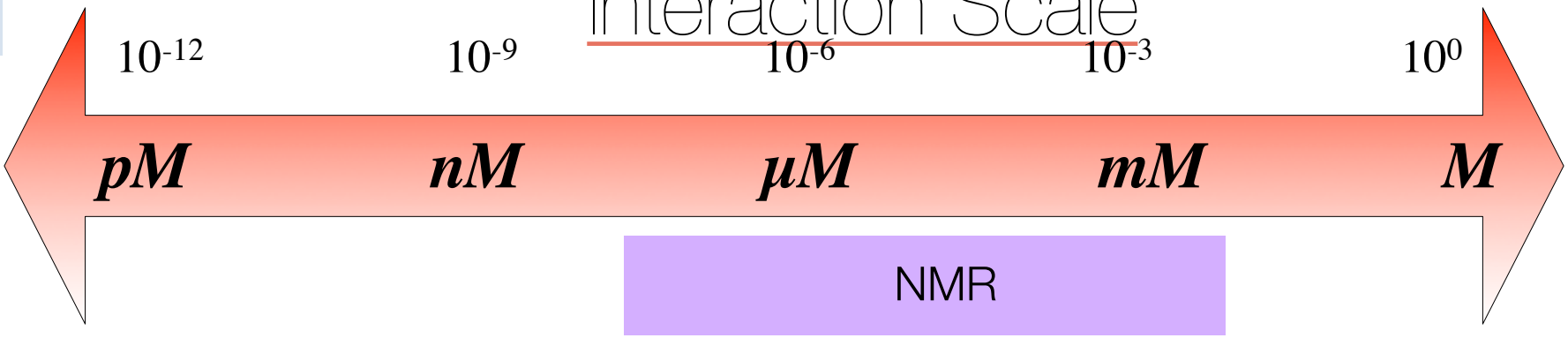




Time scale


10-15
10-12
10-9
10-6
10-3
100
103
sec
fs
ps
ns
µs
ms
s
h
NMR
dielectic resonance
stop-flow
T-jump
FCS
Fluorescence correlation spectroscopy
molecular vibrations
rotations
kon
allostery
enzymatic reactions
folding unfolding

Interaction Scale



NMR

SPR

MS

fluorescence

$$P + L \rightleftarrows PL$$

$$K_d = \frac{[P][L]}{[PL]}$$

10^{-14}

streptavidin
biotin

medication

strong
ligands

weak
ligands

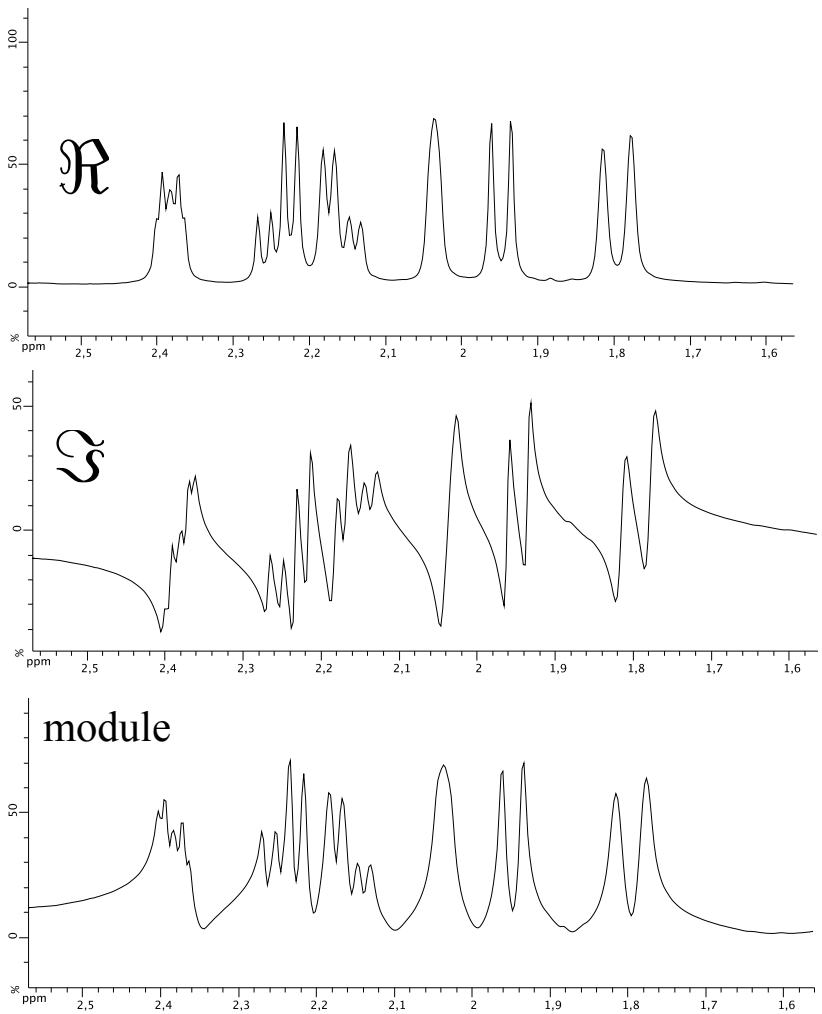
aspecific
interactions

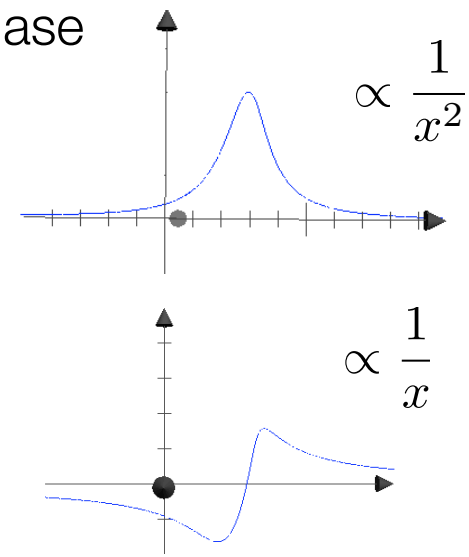
solvent
interactions

Mise en œuvre (1)

- calcul en module $\|S(\nu)\| = \sqrt{S(\nu)S^*(\nu)}$

  - phénomène cohérent = calcul sensible à la phase

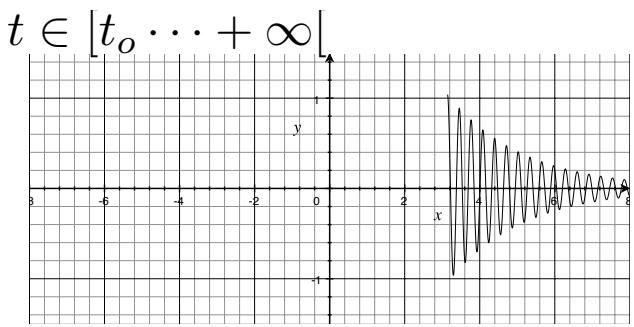


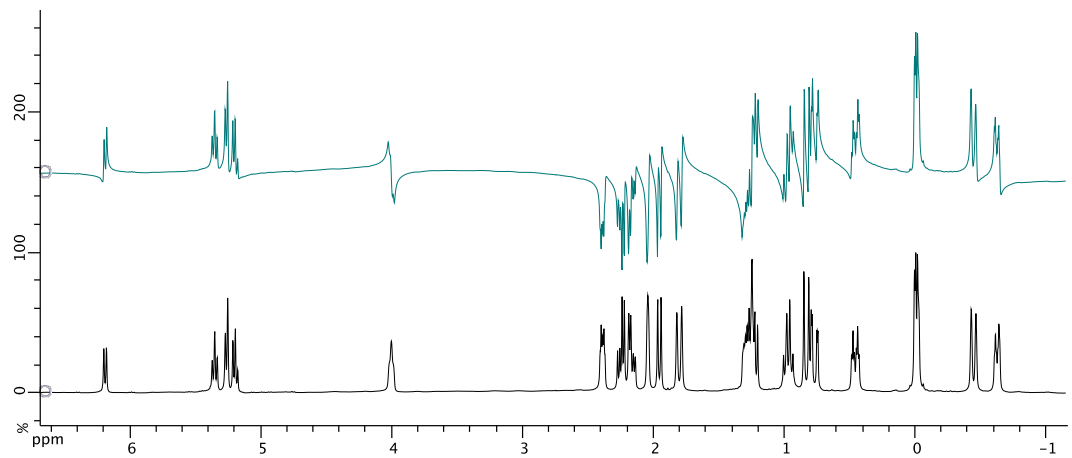


- calcul de la densité spectrale de puissance
- adapté aux signaux stationnaires
- mal adaptés aux FID

quelques remarques (2)

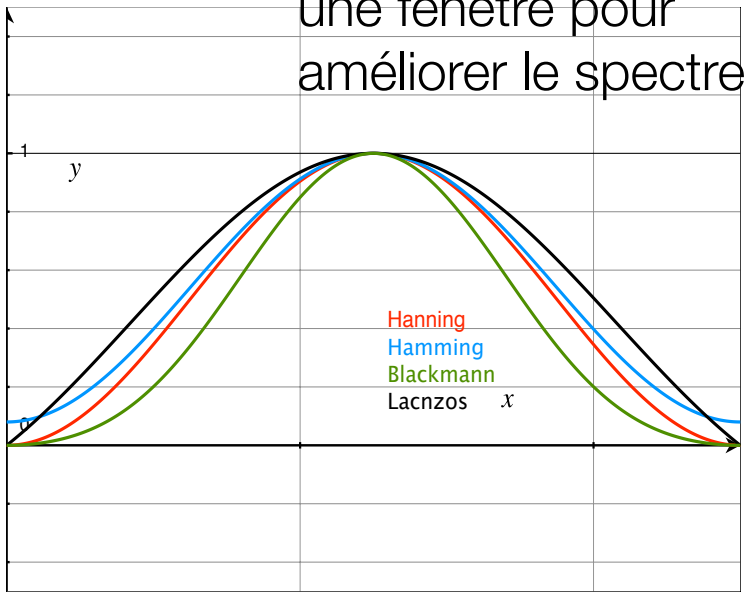
- Erreur sur t_o
  - => convolution du spectre par $e^{i\nu t_o}$
  - rotation de la phase proportionnel à la fréquence
  - correction de phase

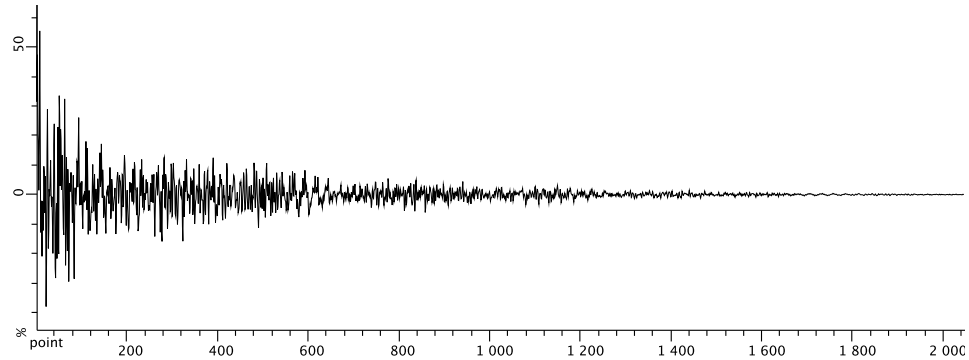


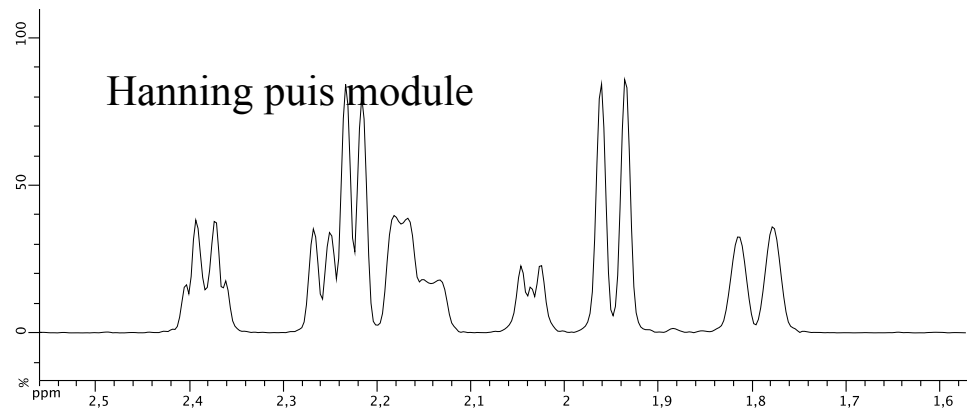


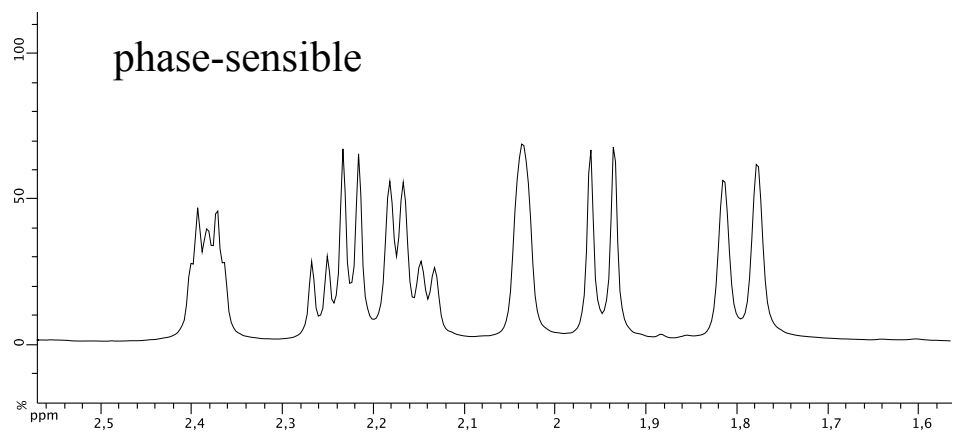
Mise en œuvre (2)

- Apodisation
  - multiplier les données par une fenêtre pour améliorer le spectre







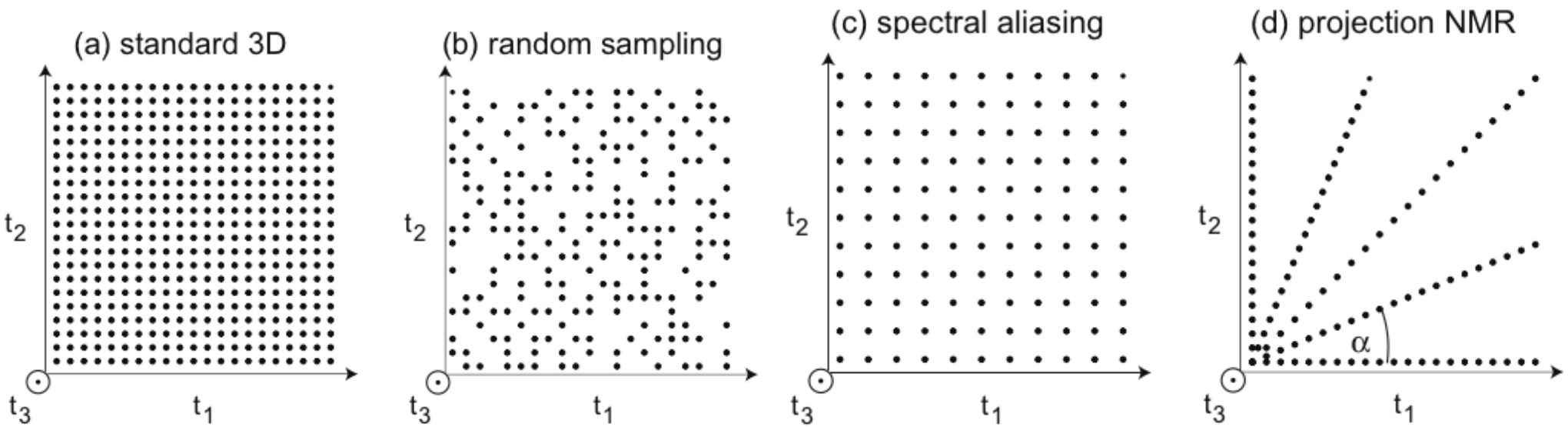


- artefacts
- forte de perte de signal dans certains cas
- modification du signal mesuré !

Working on sampling methods

- The use of FFT imposes a linear sampling

- Non Uniform Sampling (NUS) are currently being developed allowing considerable gain of time



P Schanda Progress NMR Spect. 2009

2005-2017

- 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

Protein Science (2000), *9*:1137–1148. Cambridge University Press. Printed in the USA.

Characterization and molecular basis of the oligomeric structure of HIV-1 Nef protein

STEFAN AROLD,[1,3] FRANÇOIS HOH,[1] STEPHANIE DOMERGUE,[1] CATHERINE BIRCK,[2] MARC-ANDRÉ DELSUC,[1] MAGALI JULLIEN,[1] AND CHRISTIAN DUMAS[1]

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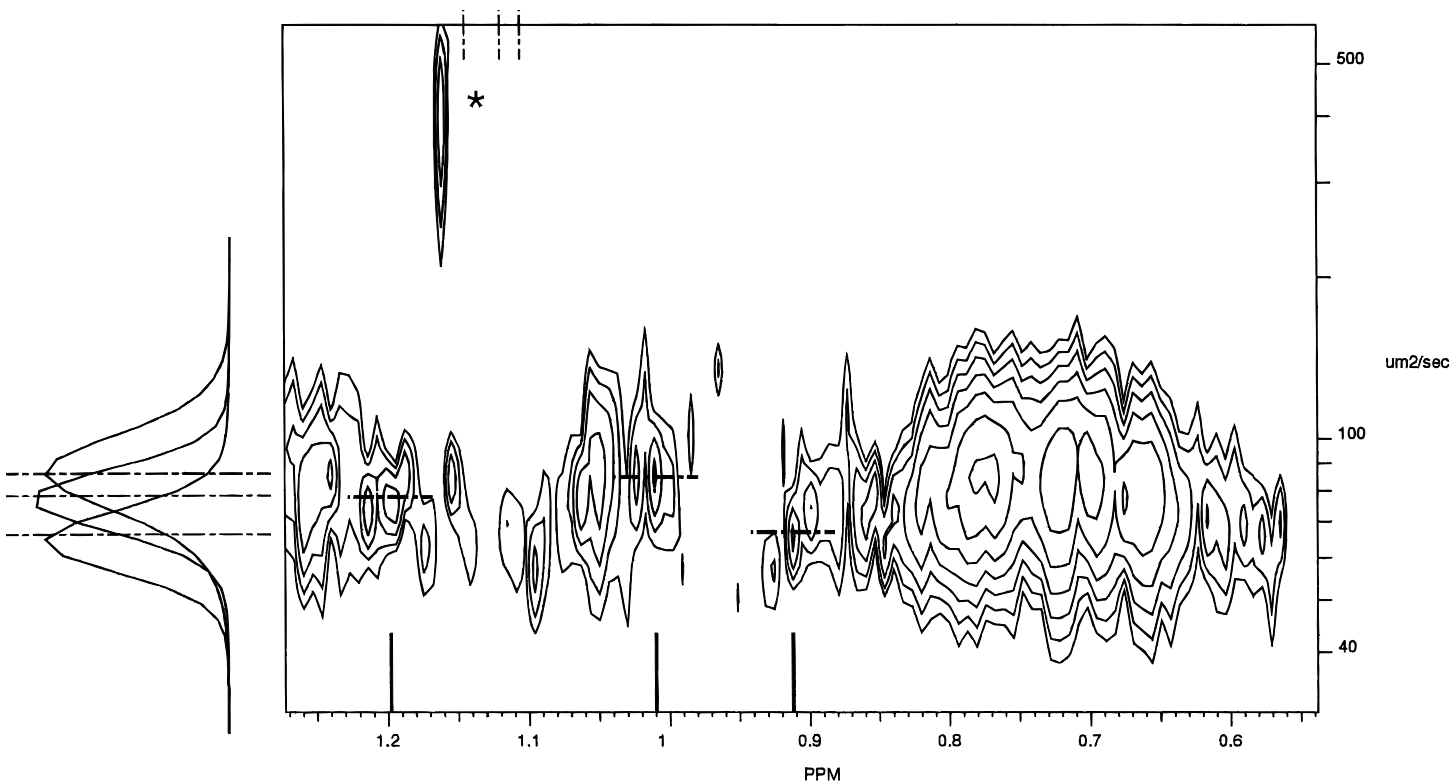

Fig. 4. Analysis of the oligomeric state based on NMR DOSY data: the methyl region of the DOSY spectrum of $Nef_{\Delta 1-56,\Delta 206}$ at 140 μM is shown. Three columns have been extracted at 0.91, 1.01, and 1.20 ppm and are reported to the left. The corresponding diffusion coefficients, materialized by hatched axes, are 66, 84, and 77 mm^2/s, respectively. The star indicates an impurity, with a much faster diffusion coefficient.