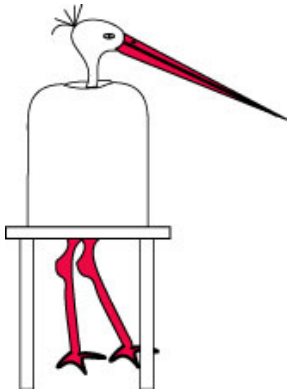
- Bruno Kieffer
  - Studies of Biochemistry-biophysics in Straasbourg
  - Thesis with JF Lefèvre 1992: studies of protein dynamics by NMR
  - étude de la dynamique des protéines par RMN
  - Post-doc ID Campbell (Oxford) structure of CD59
  - Maître de conférence at Ecole de Biotechnologie de Strasbourg
  - Currently professor at Ecole de Biotechnologie de Strasbourg

Nuclear Magnetic Resonance – Conceptual aspects

NMR observables:
A source of structural and dynamical information for the study of biomacromolecules



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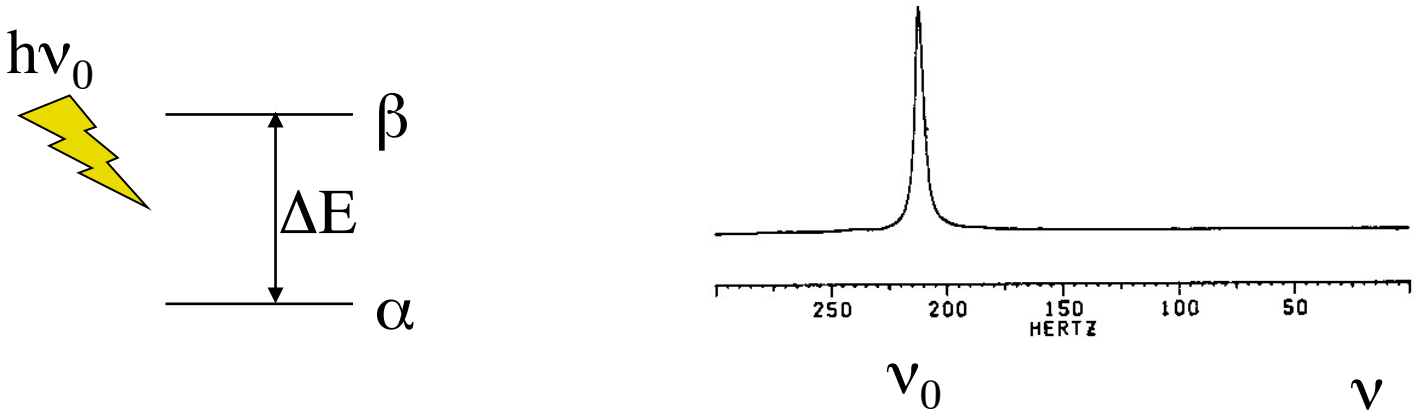
esbs
école supérieure de biotechnologie
STRASBOURG

Course outlines

- General principles of NMR spectroscopy
- The NMR observables
  - Chemical shifts
  - Resonance peak intensities
  - Relaxation rates

Basic concepts in RMN

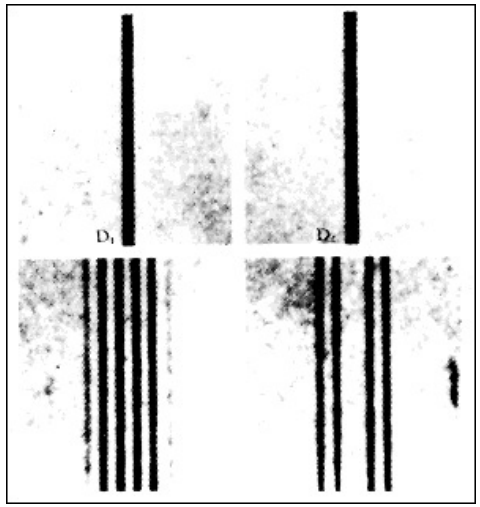
- It is a spectroscopic method



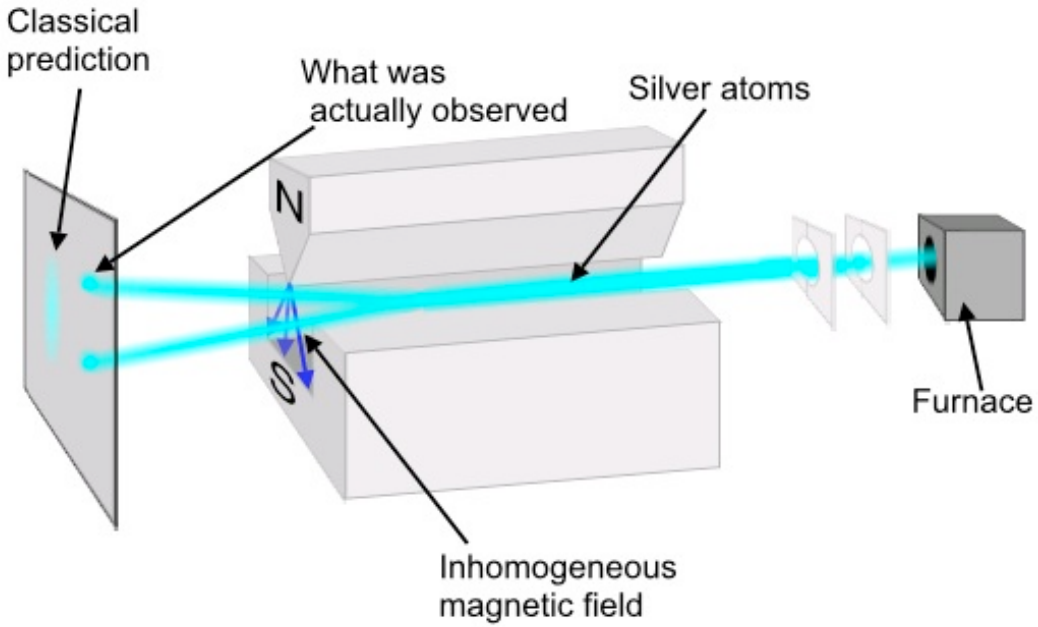
- Energy levels (Zeeman levels) shows up only in presence of a magnetic field B0 (RMN)

Magnetic fields reveals energy levels

- The zeeman experiment (Zeeman 1897): *The Effect of Magnetisation on the Nature of Light Emitted by a Substance*



- The Stern-Gerlach experiment (1922): *quantification of silver particles angular momentum*

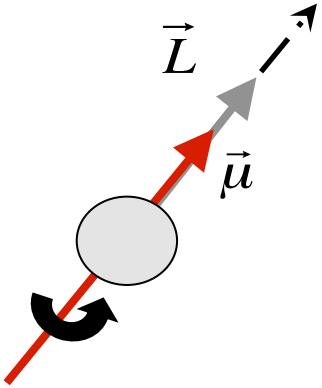


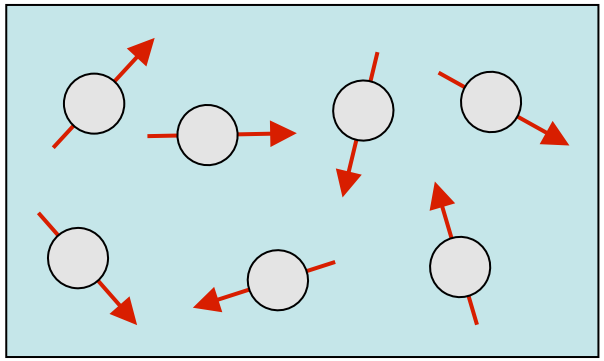
The spin

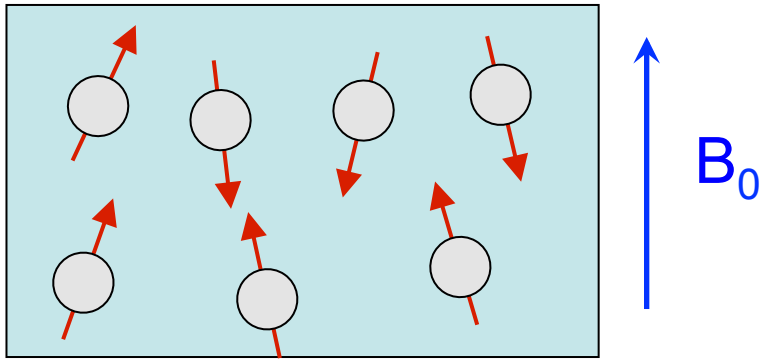
- Particles have an angular momentum and a colinear magnetic dipole

$$\vec{\mu} = \gamma \vec{L}$$

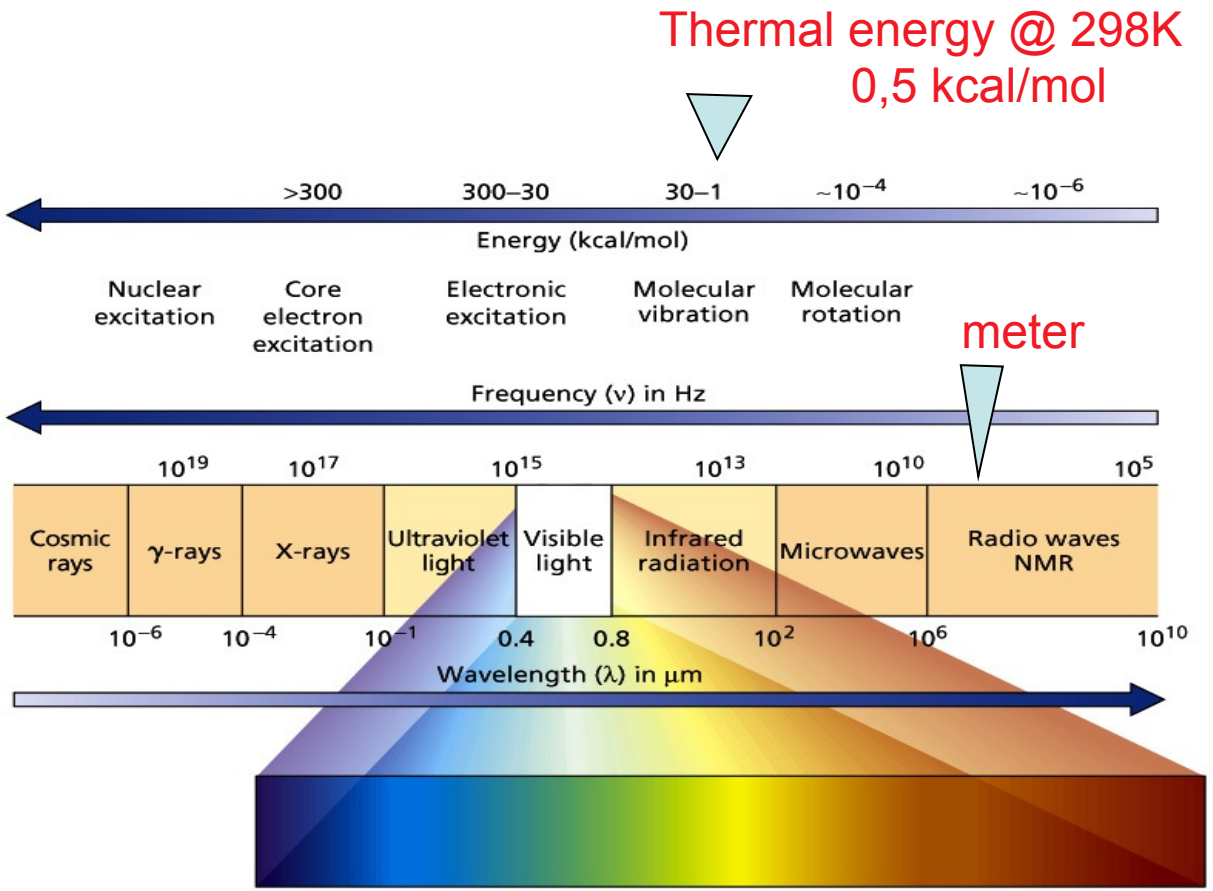
$\vec{\mu}$: *Magnetic dipole*
$\vec{L}$: *Angular momentum*
γ : *Magnetogyric ratio*







NMR is a very low energy spectroscopy



Energy

$$E = \hbar \gamma B_0$$

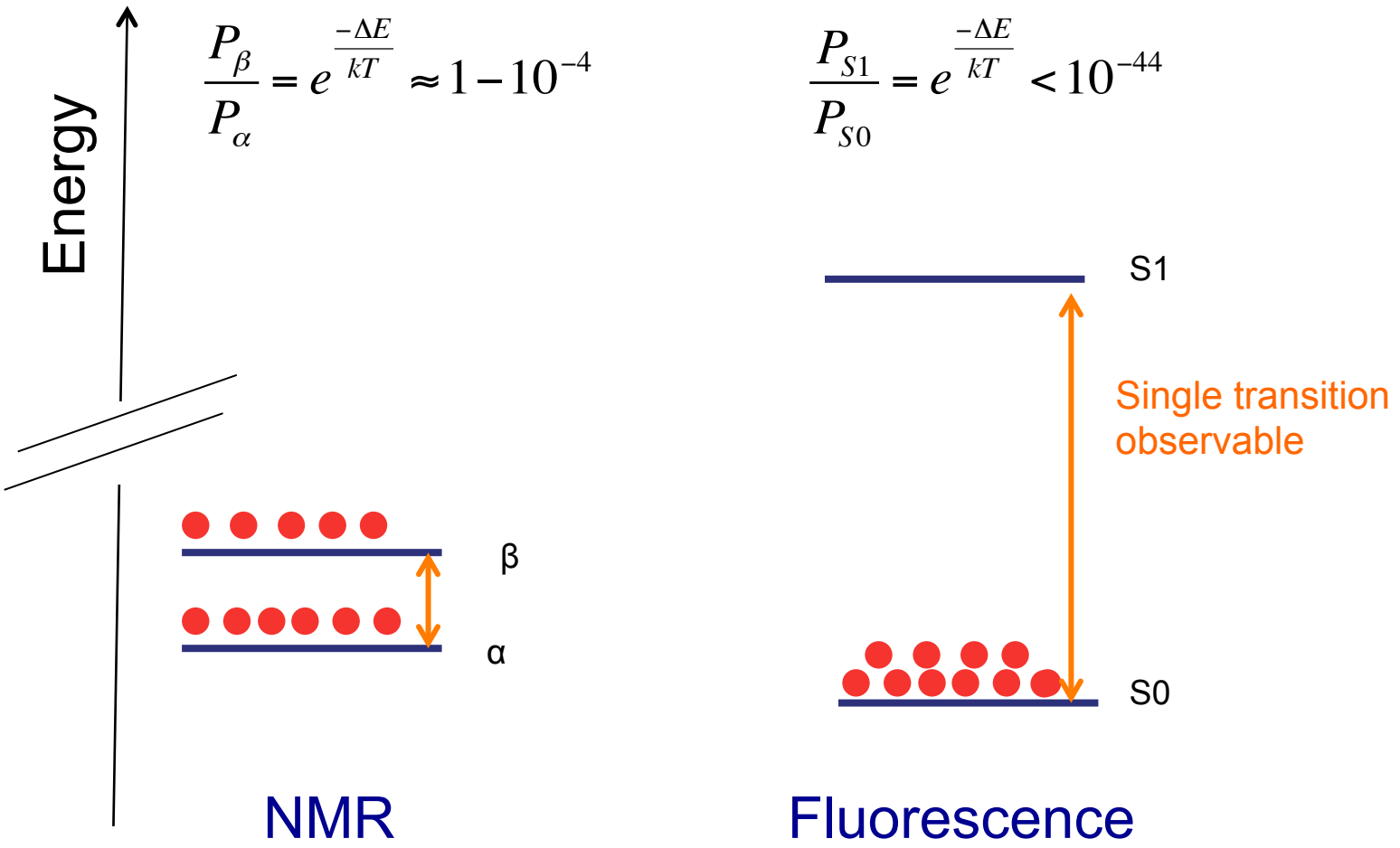
Frequency

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

Wavelength

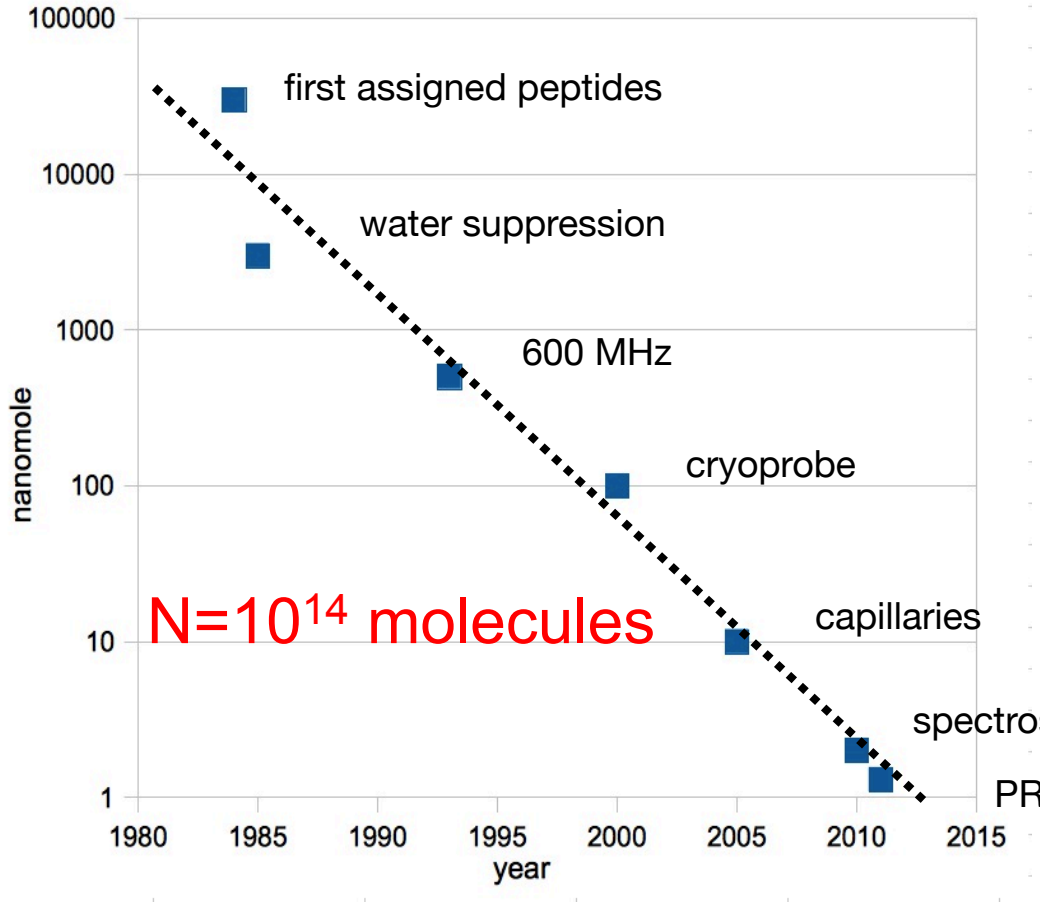
$$\lambda = \frac{2\pi c}{\gamma B_0}$$

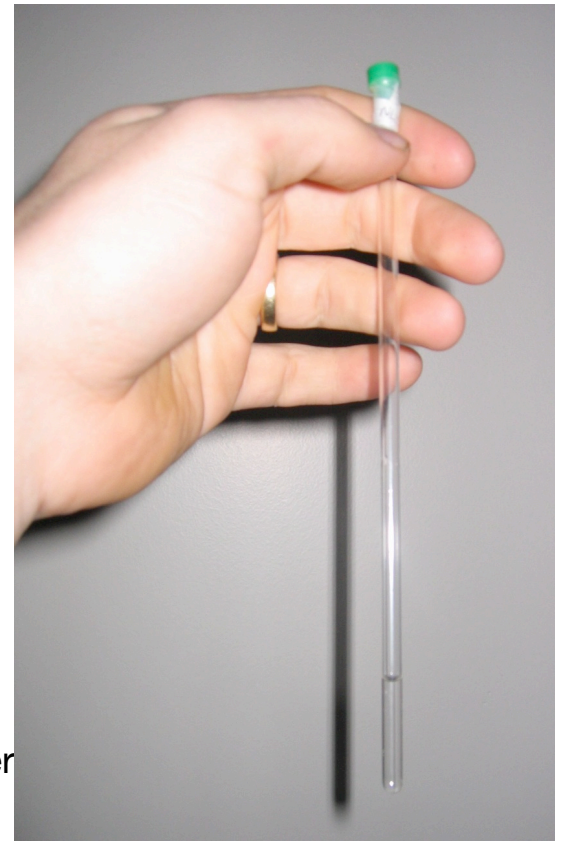
Low-energy = low sensitivity



The NMR sample: a very large number of molecules

Protein quantity

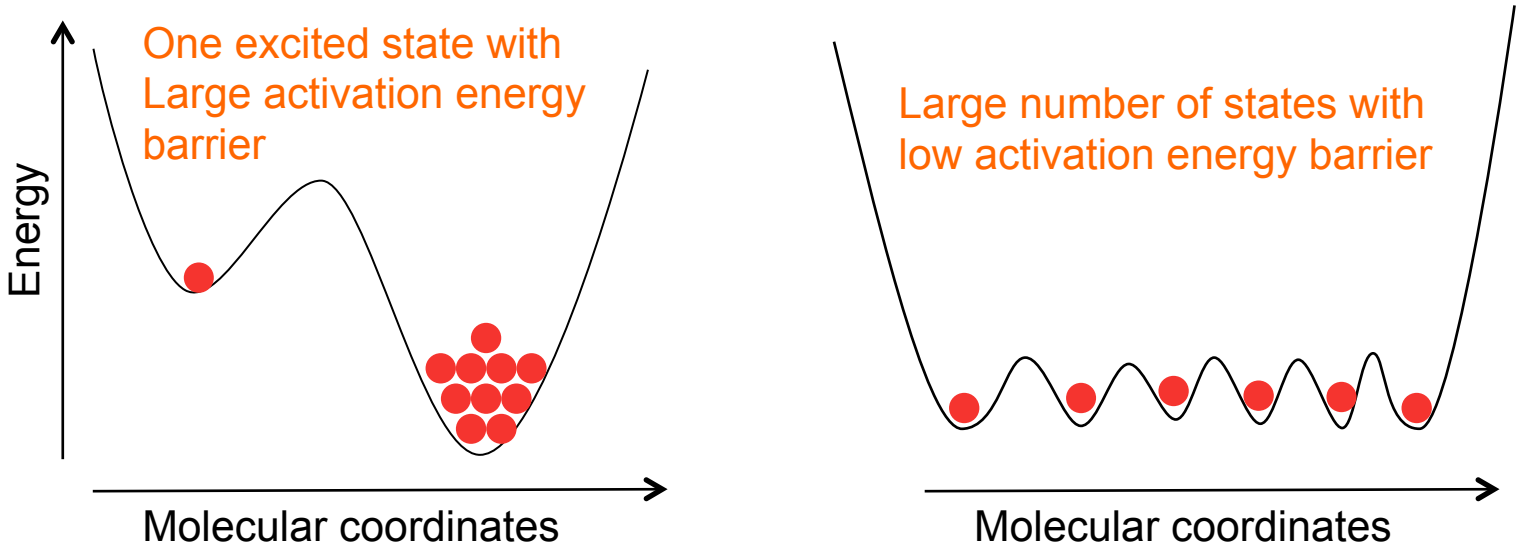




From MA Delsuc

Large number of molecules

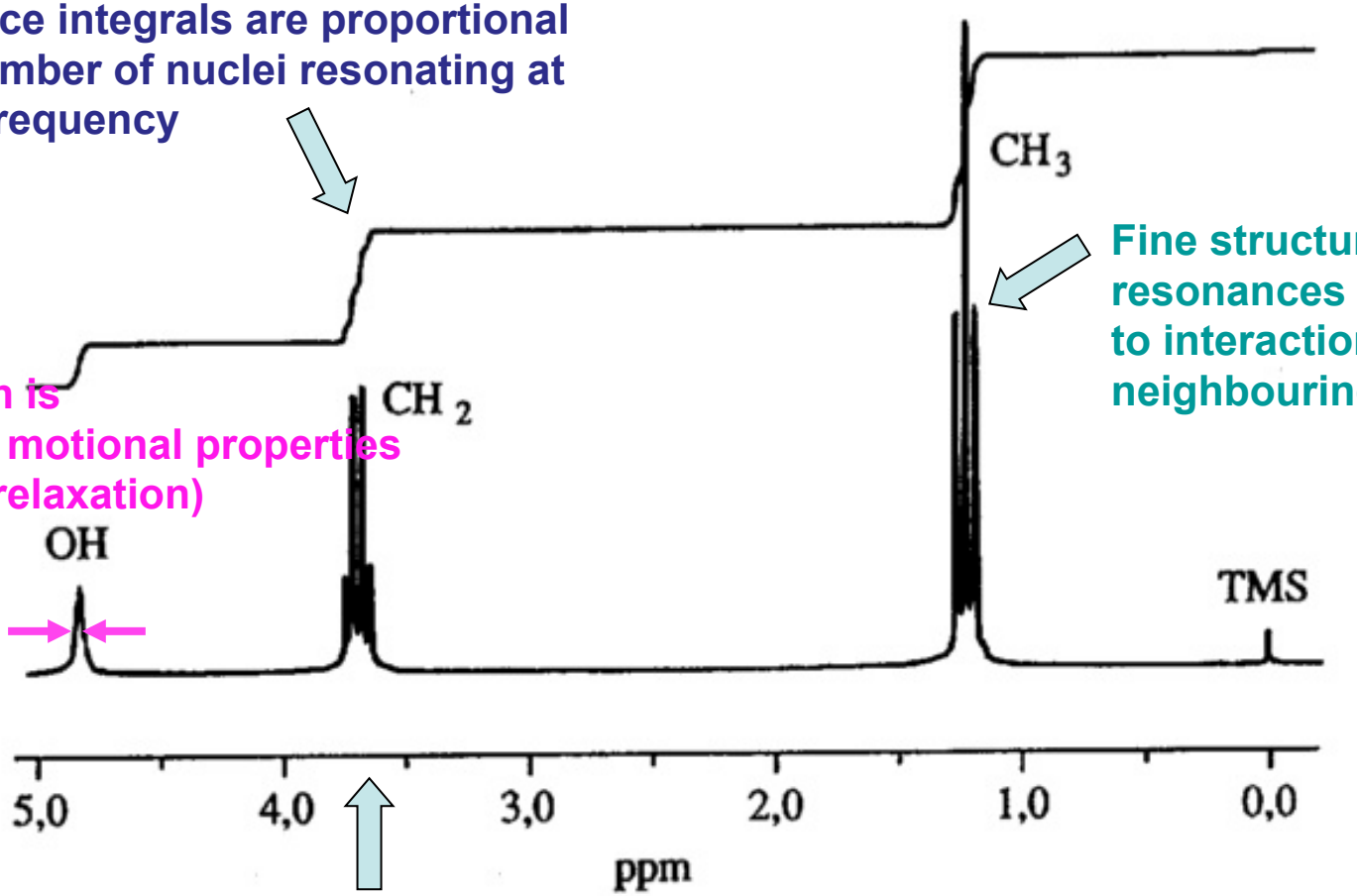
- Molecules will be distributed between the different states available for the observed molecular system



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

The NMR observables…on a simple molecule

Resonance integrals are proportional to the number of nuclei resonating at a given frequency

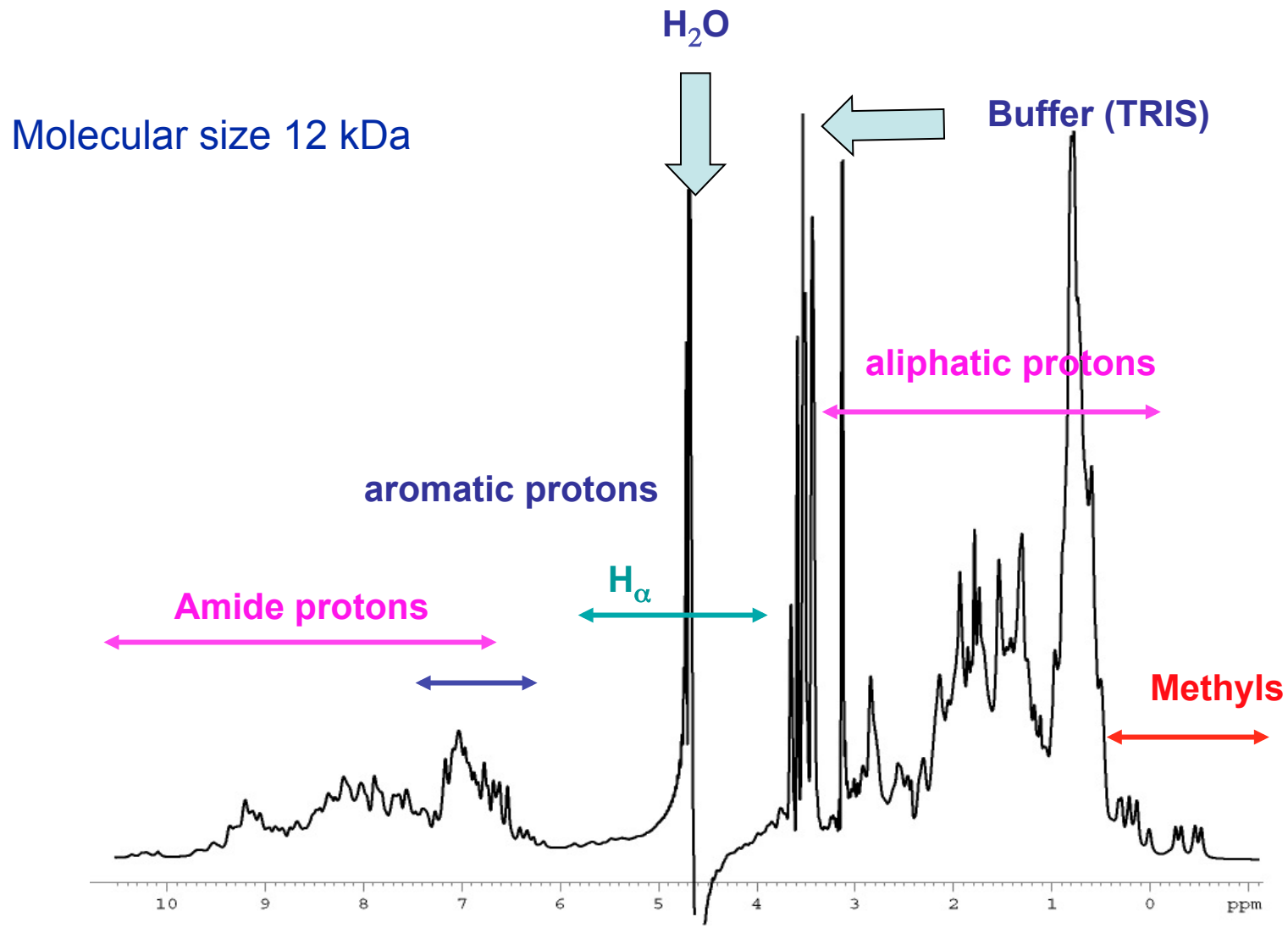


Fine structure of resonances is due to interactions with neighbouring nuclei

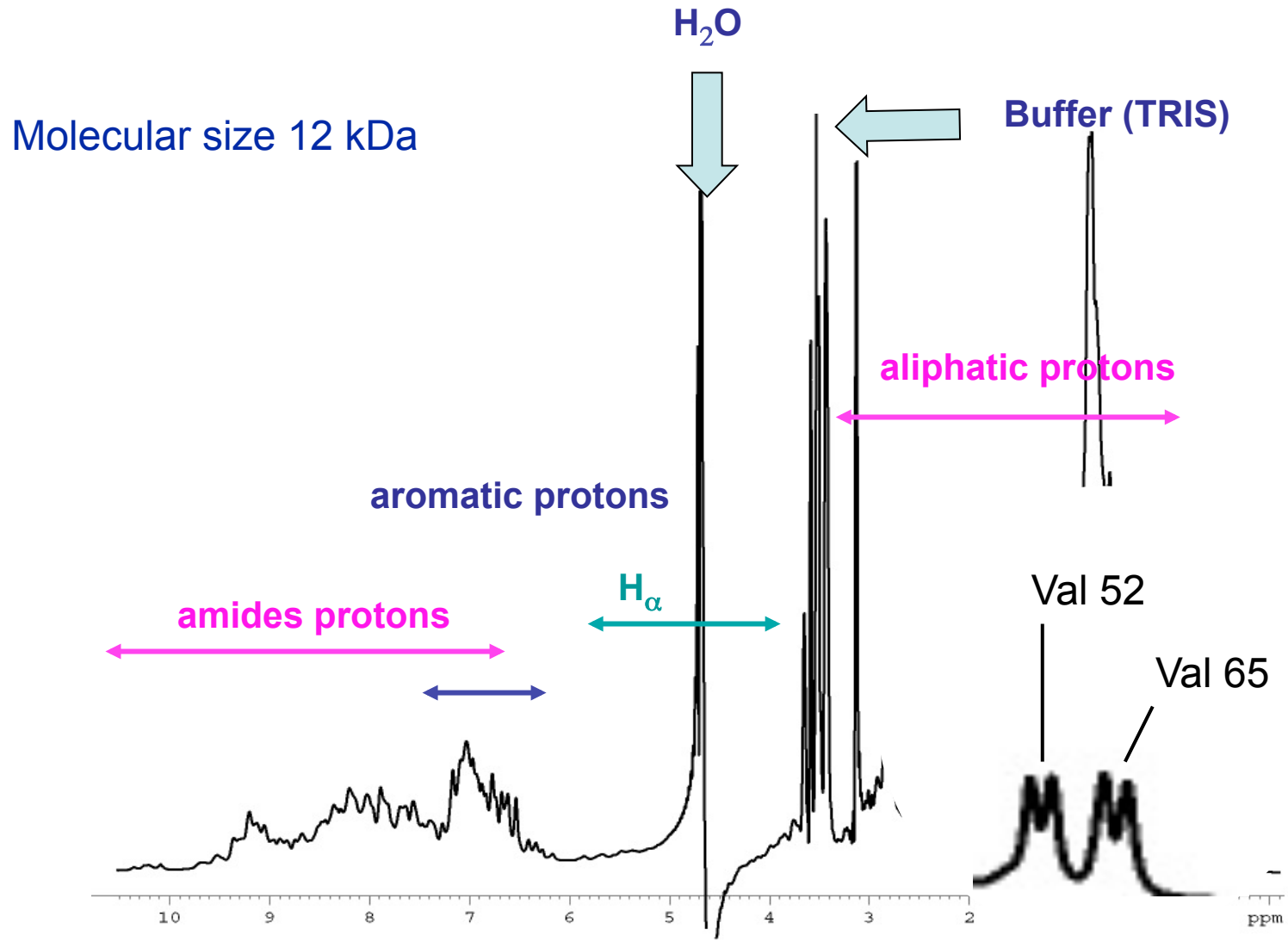
Line width is related to motional properties (through relaxation)

Resonance frequencies depends on the chemical environment
=> Chemical shifts

1H NMR spectrum of a folded protein

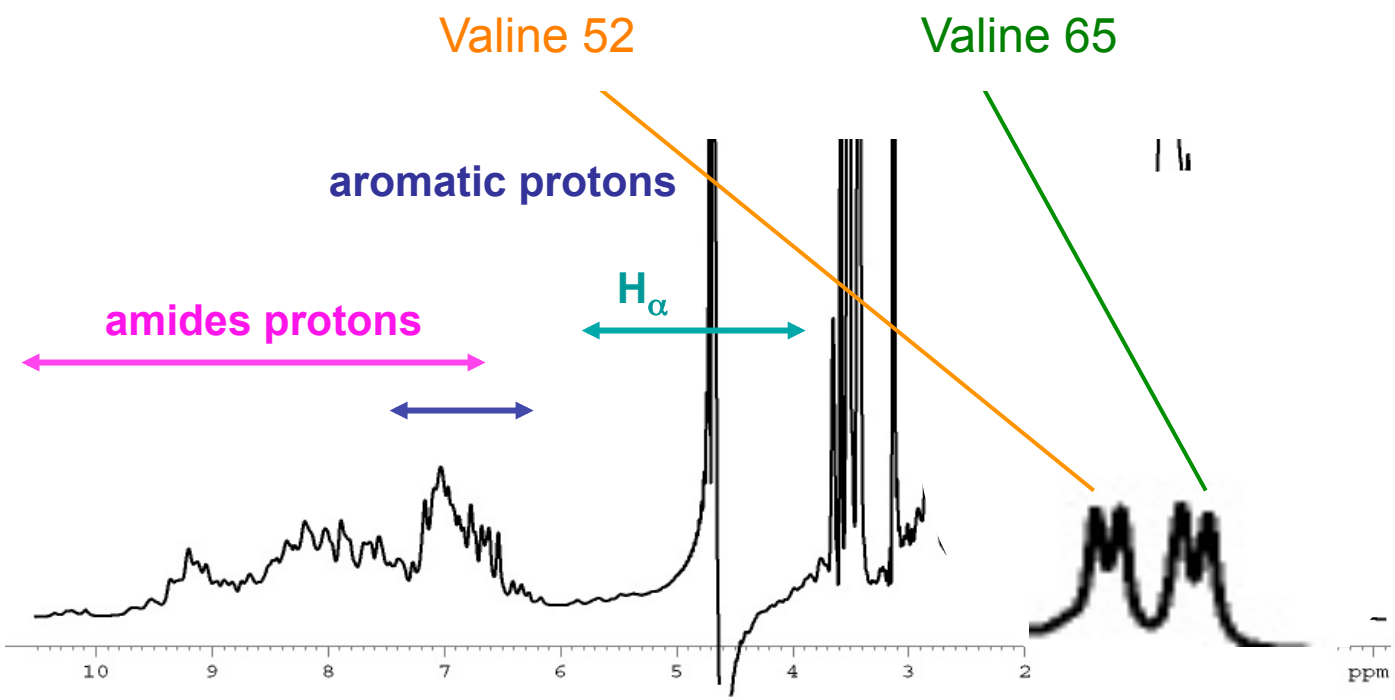


1H NMR spectrum of a folded protein

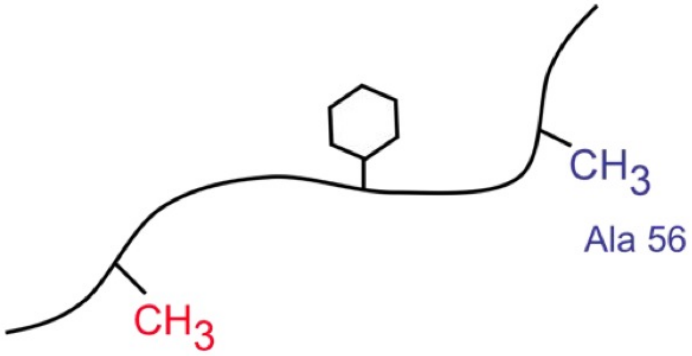


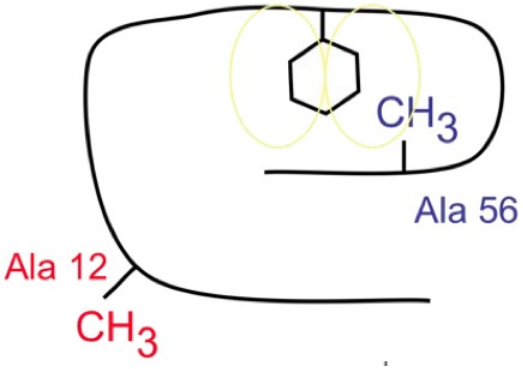
1H NMR spectrum of a folded protein

MVKQIESKTA FQEALDAAGD KLVVVDFSAT WCGPCKMIKP
FFHSLSEKYS NVIFLEVDVD DCQDVASECE VKCMPTFQFF
KKGQKVGEFS GANKEKLEAT INELV

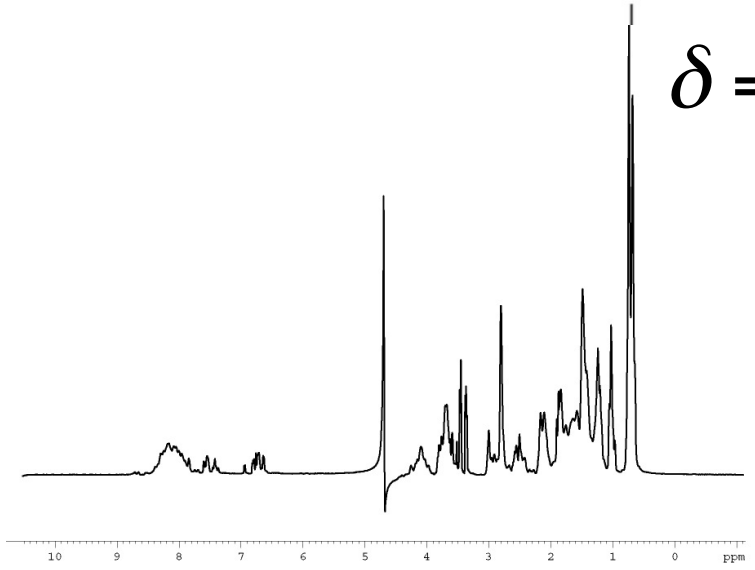


Chemical shifts contain a structural information





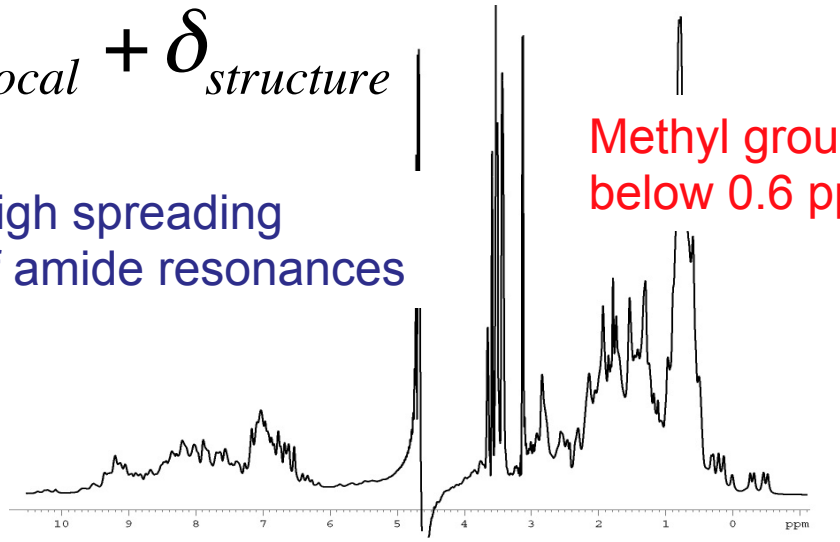
$$\delta = \delta_{local} + \delta_{structure}$$



Unfolded protein

High spreading of amide resonances

Methyl groups below 0.6 ppm



Folded protein

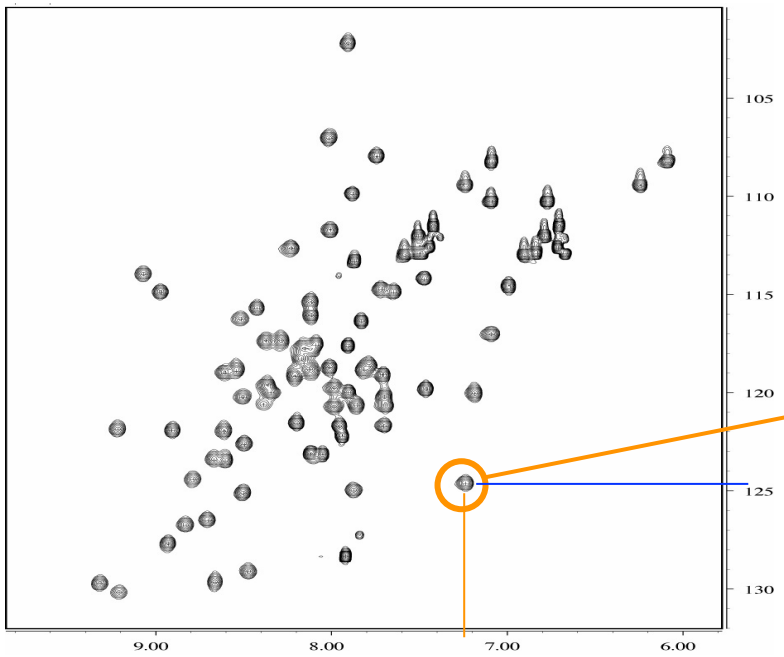
Chemical shifts

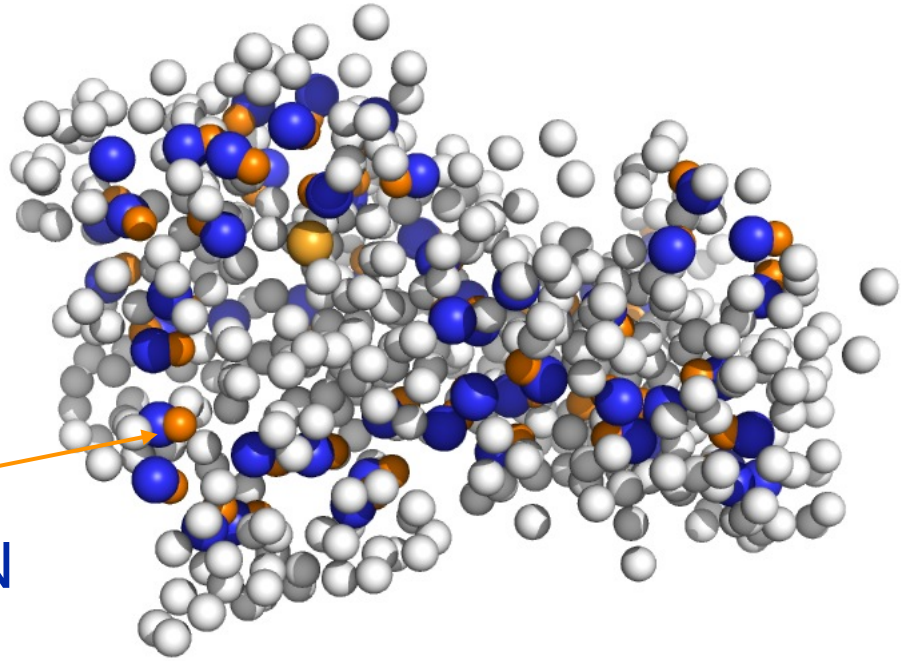
- Provides the spectral identity of all atoms of a biopolymer, or components of a complex mixture
- Contain a structural information on protein's
  - secondary structure
  - 3D structure
- They are very sensitive to any change of environment
- When several states are available to the system, the ability to distinguish these states will depends on the exchange kinetics between these states

Labeling with ^{15}N (and) ^{13}C stable isotopes is required to study larger objects

- Isotopic labeling provides a tool to address the complexity of protein NMR spectra

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

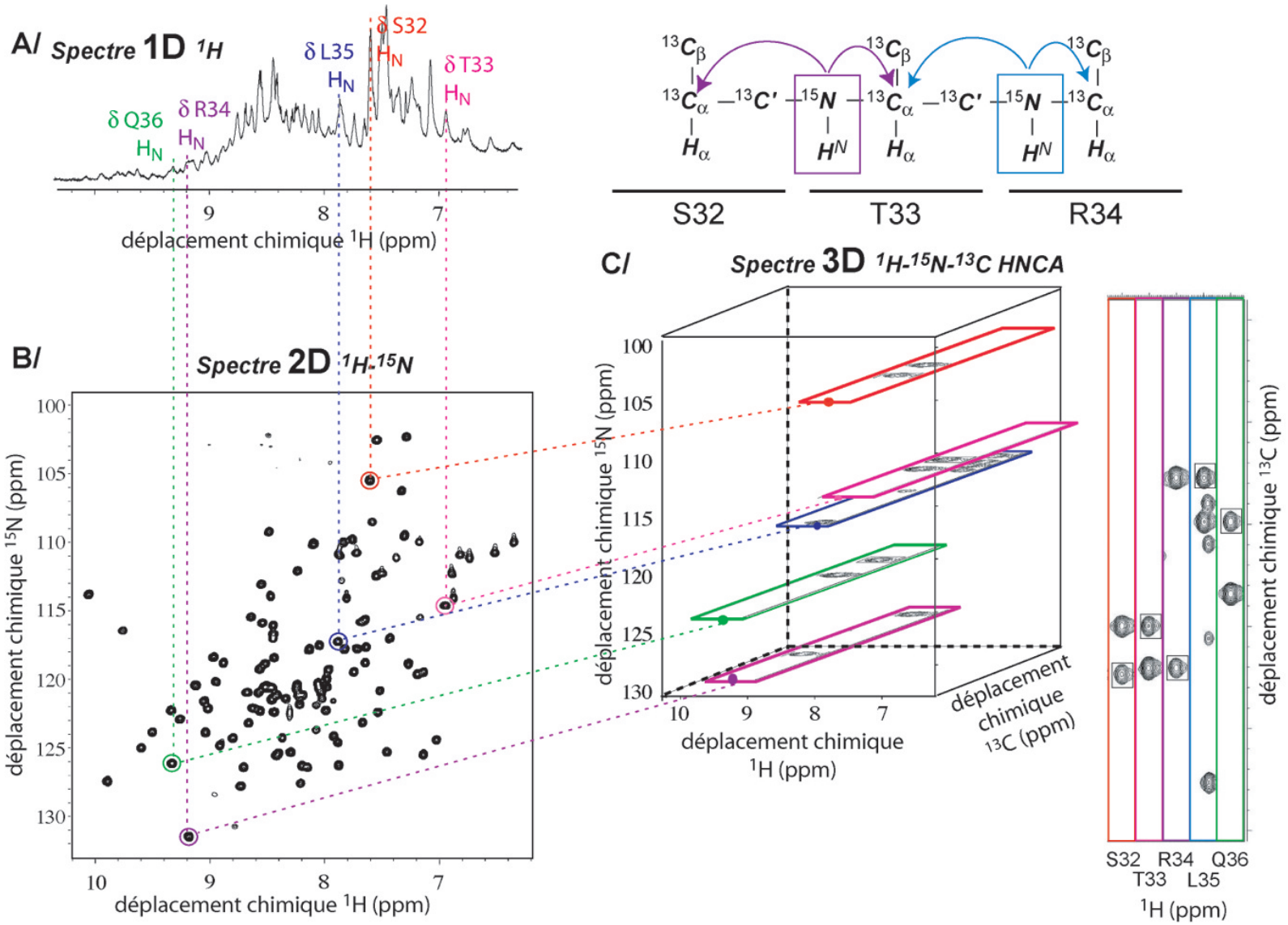




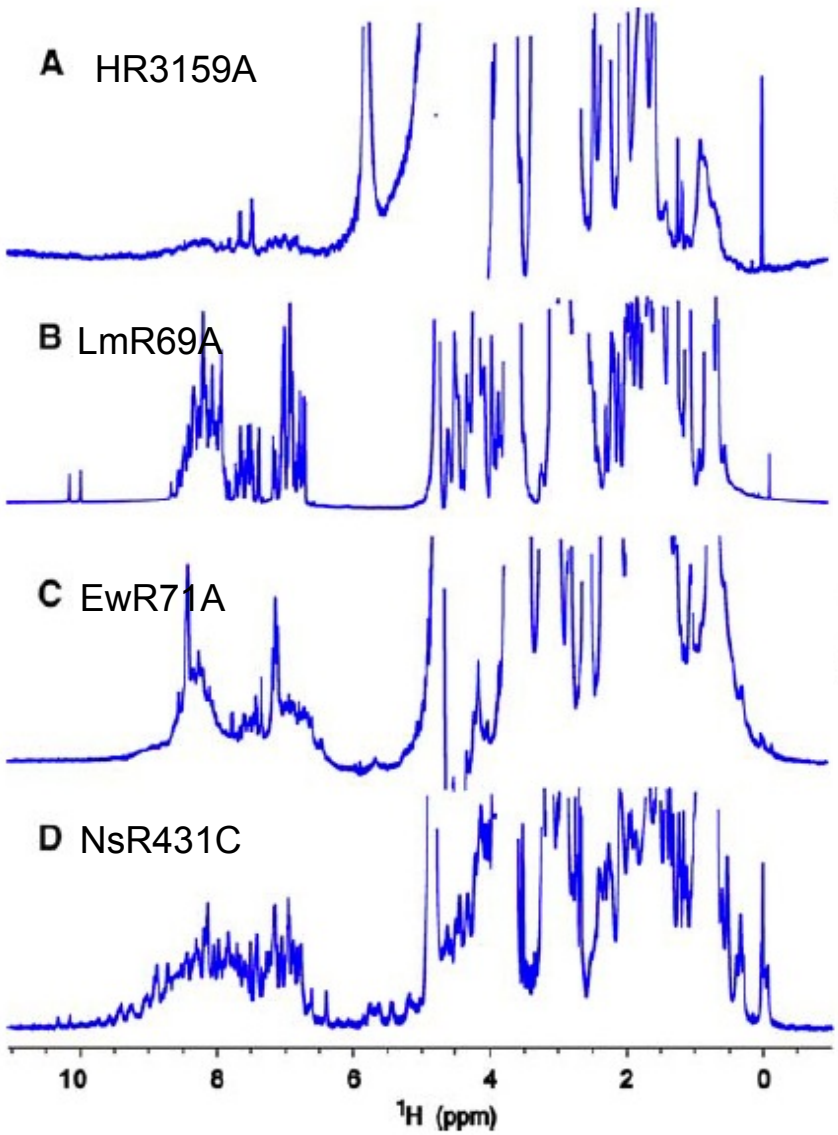
^{15}N

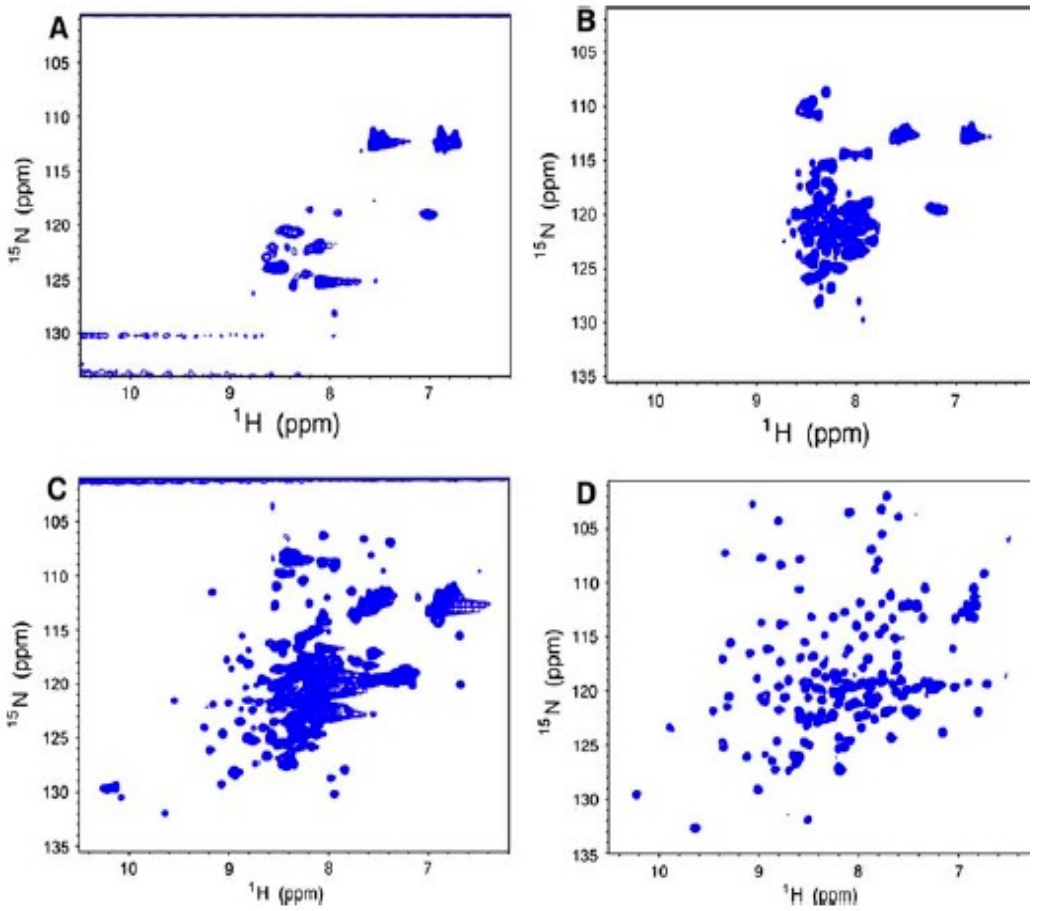
^{1}H

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



Application to sample quality screening

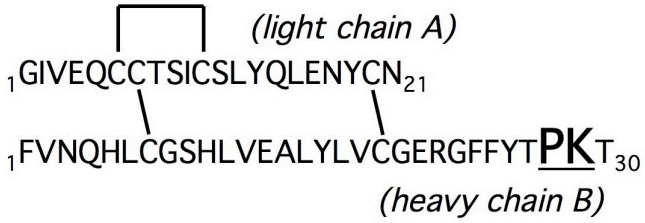




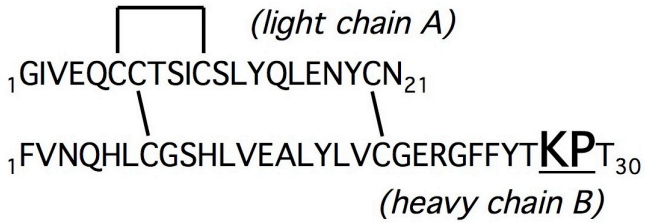
1.7 mm micro-probe at 20 °C
NESG, *J. Biomol NMR* 2010, 46, 11-22

Bio-drug control quality

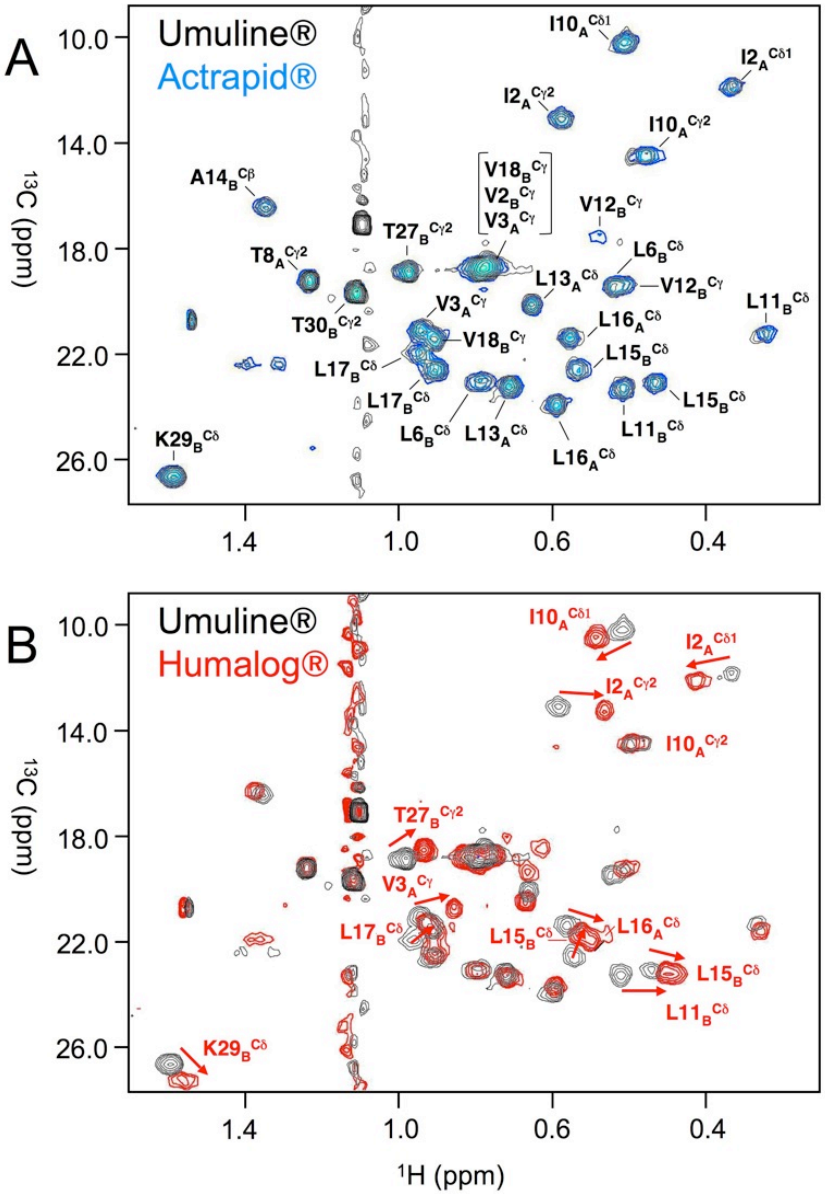
Human insulin



Lispro insulin

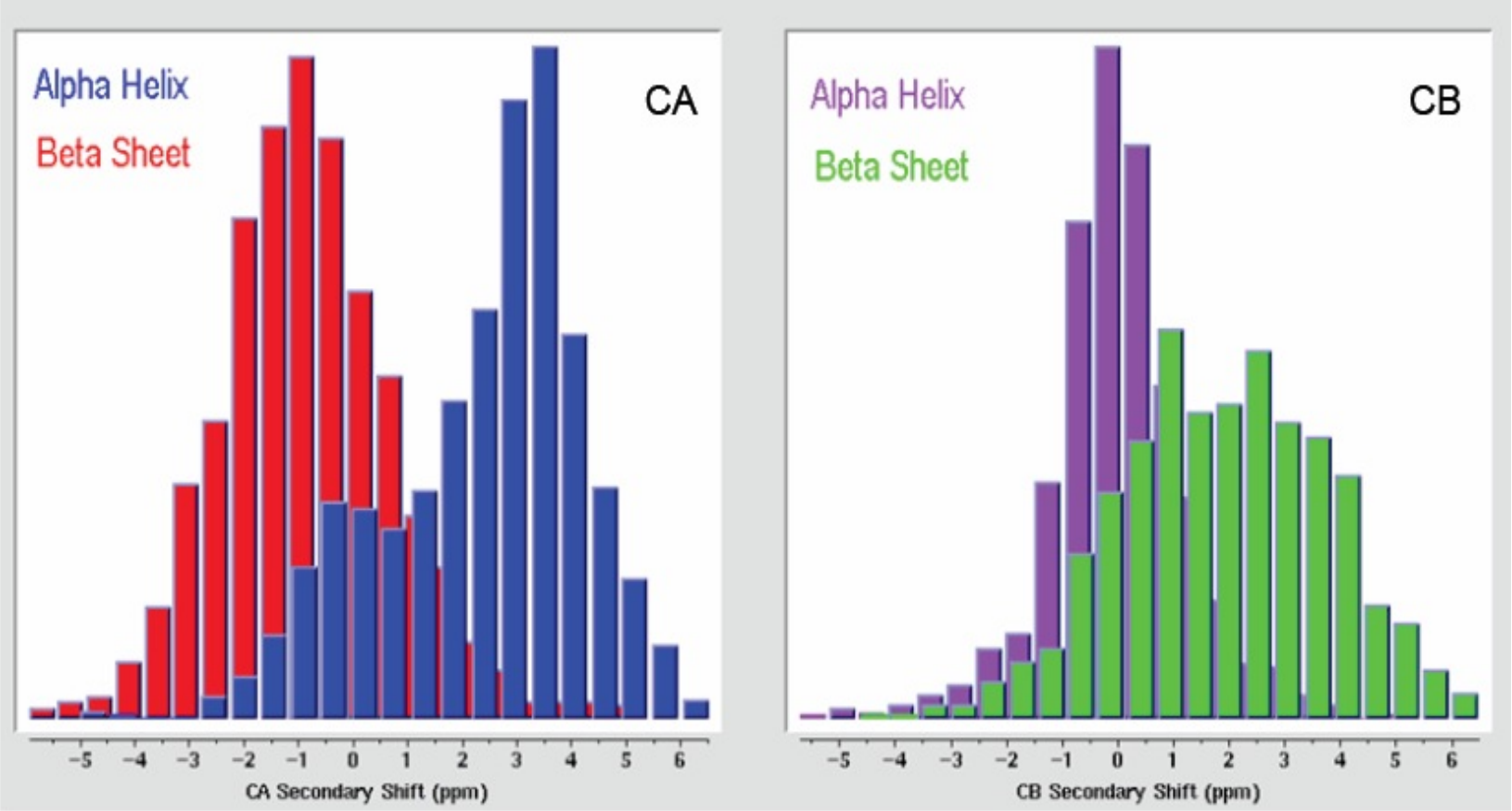


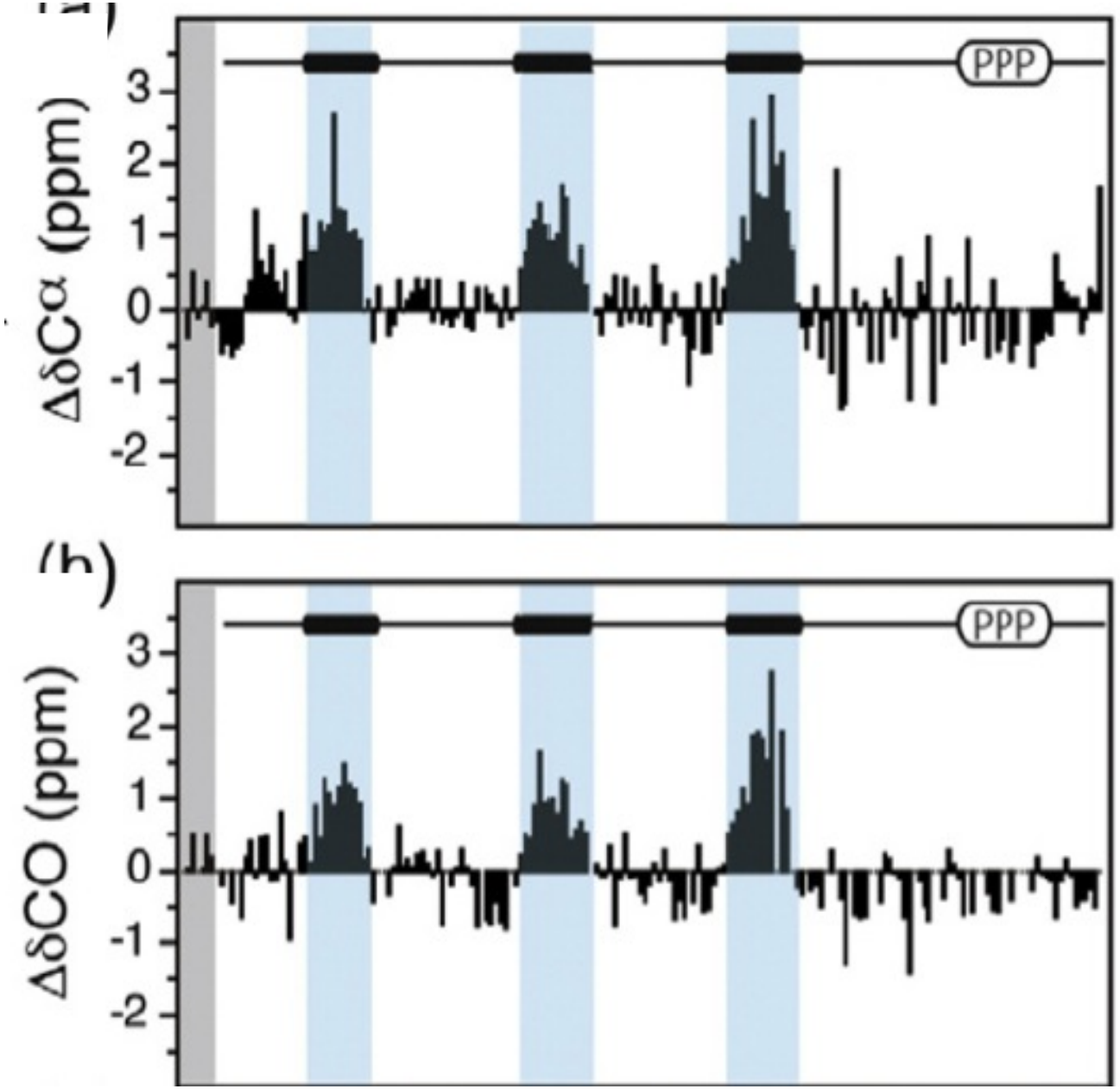
Brevet Kieffer B. (2011) WO 2011/080492



Chemical shift of backbone atoms provide information on local structure

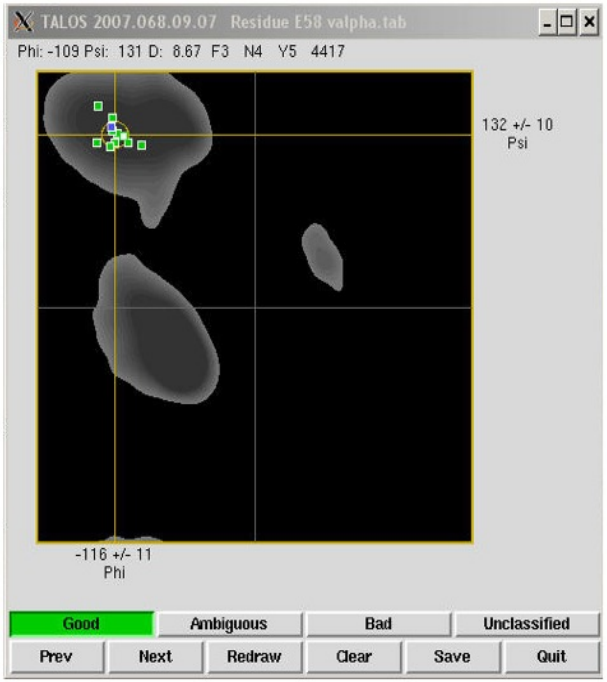
$$CSI = \delta_{measured} - \delta_{randomcoil}$$

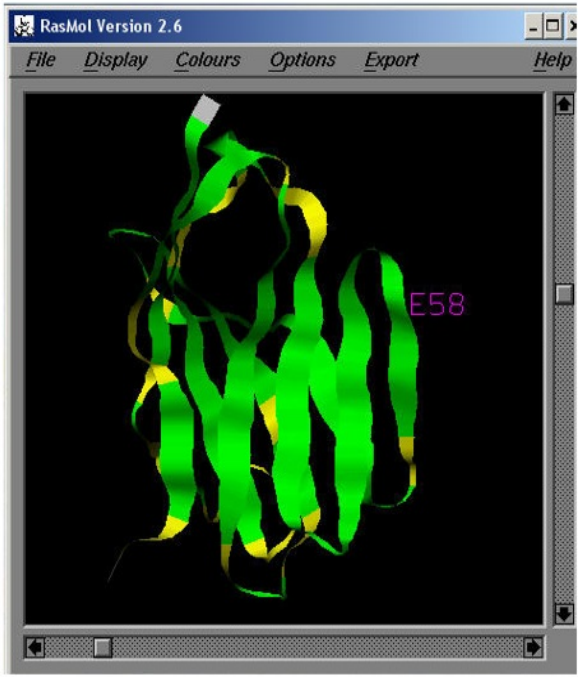


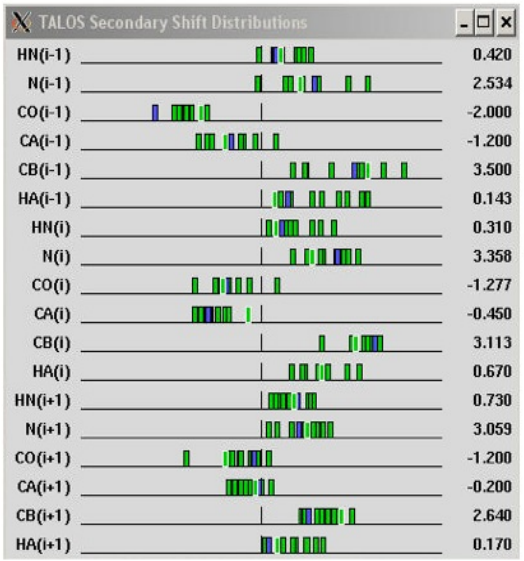

PPP
ΔδCα (ppm)
3
2
1
0
-1
-2
PPP
ΔδCO (ppm)
3
2
1
0
-1
-2

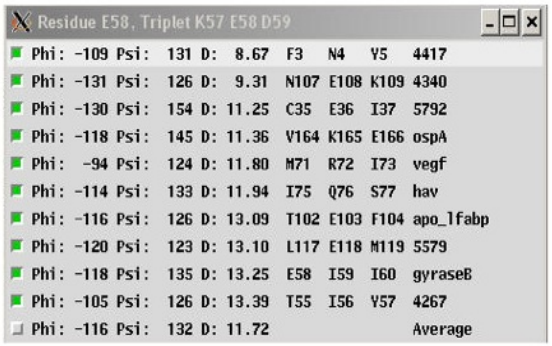
Interpretation of bb chemical shifts for protein phi,psi calculations

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









Full structure calculations: CS ROSETTA

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

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 IL5

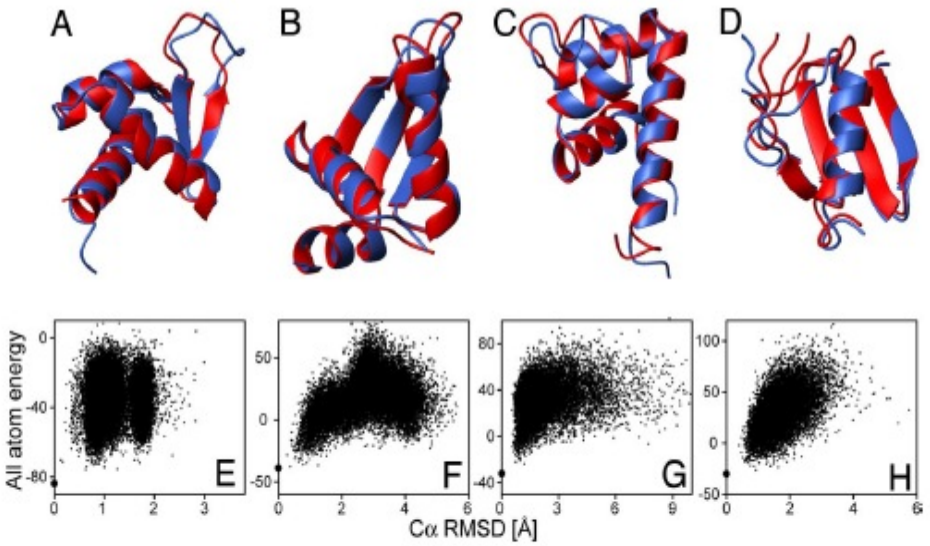
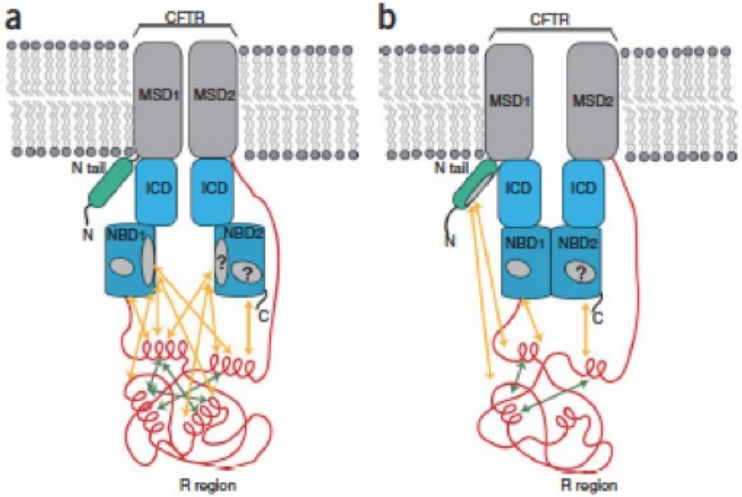
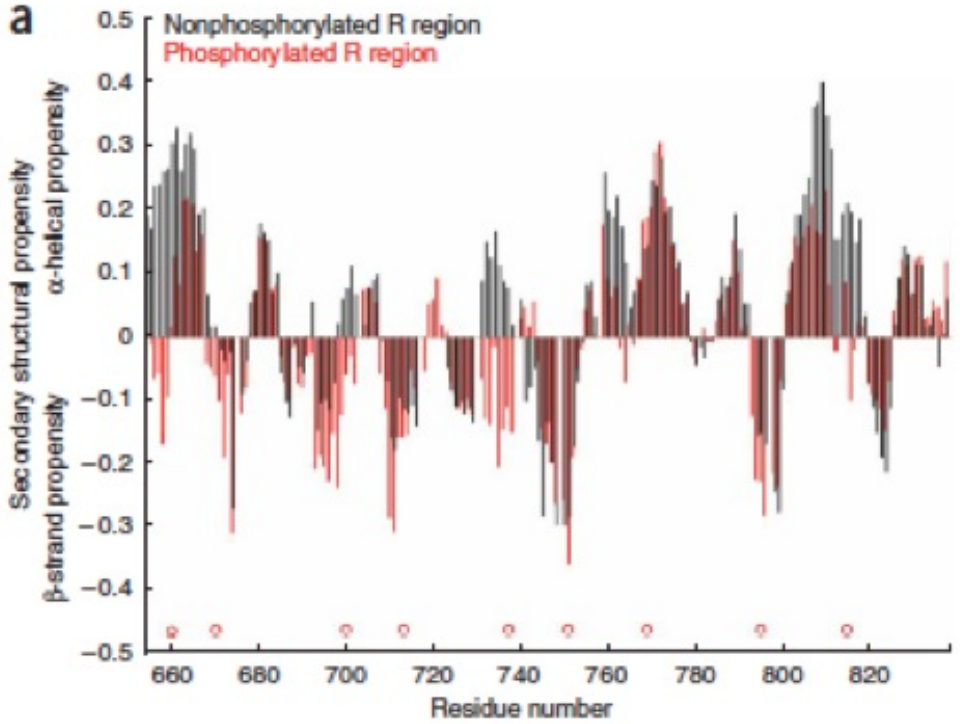


Fig. 4. Results from blind CS-ROSETTA structure generation for four structural genomics targets (Table 2). The remaining five are in SI Fig. 12. (*A–D*) Superposition of lowest-energy CS-ROSETTA models (red) with experimental NMR structures (blue), with superposition optimized for ordered residues, as 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.

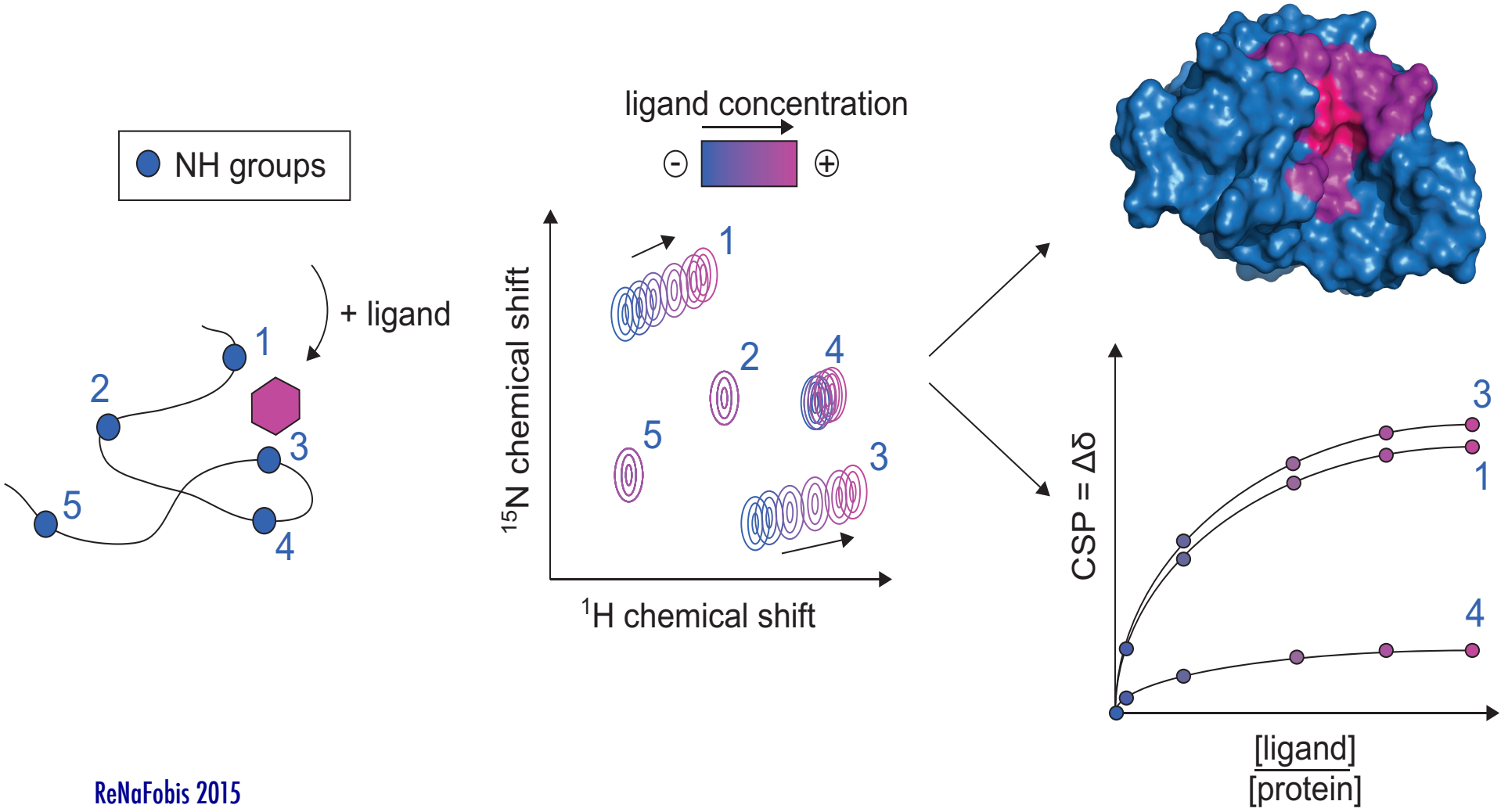
Local structure propensity from chemical shifts analysis



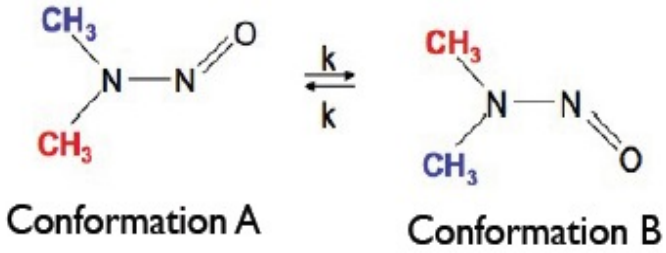


Baker et al 2007 *Nat. Struct. Mol. Biol.* **14**(8), 738

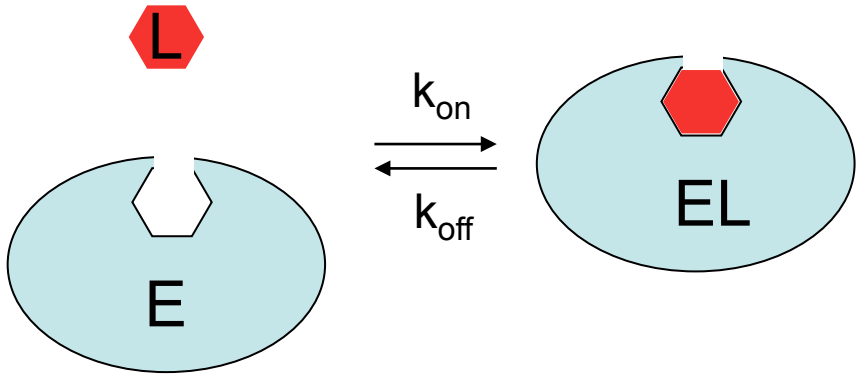
Using chemical shifts to study molecular interactions

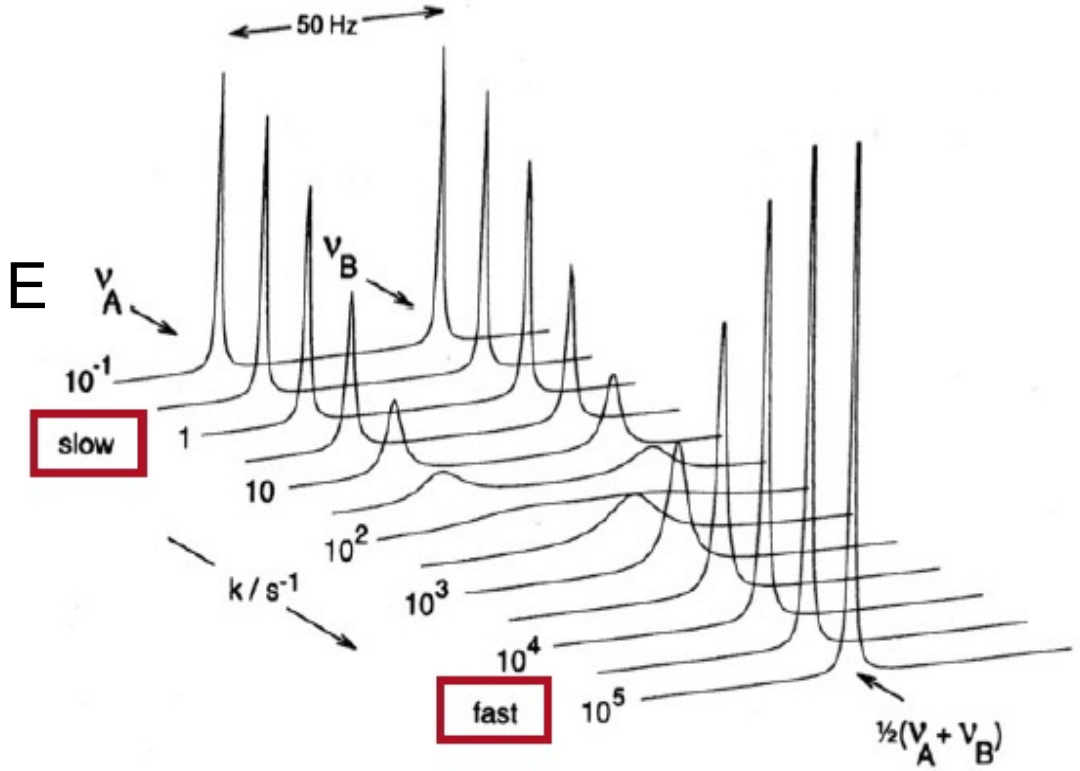


Chemical shift averaging



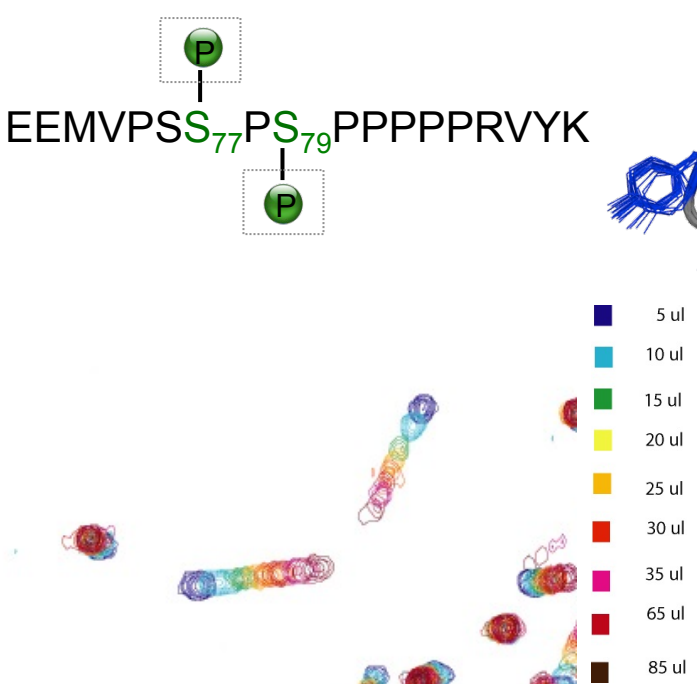
Conformation A Conformation B

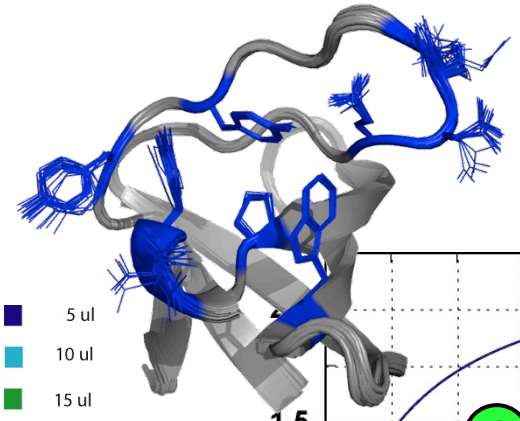




R

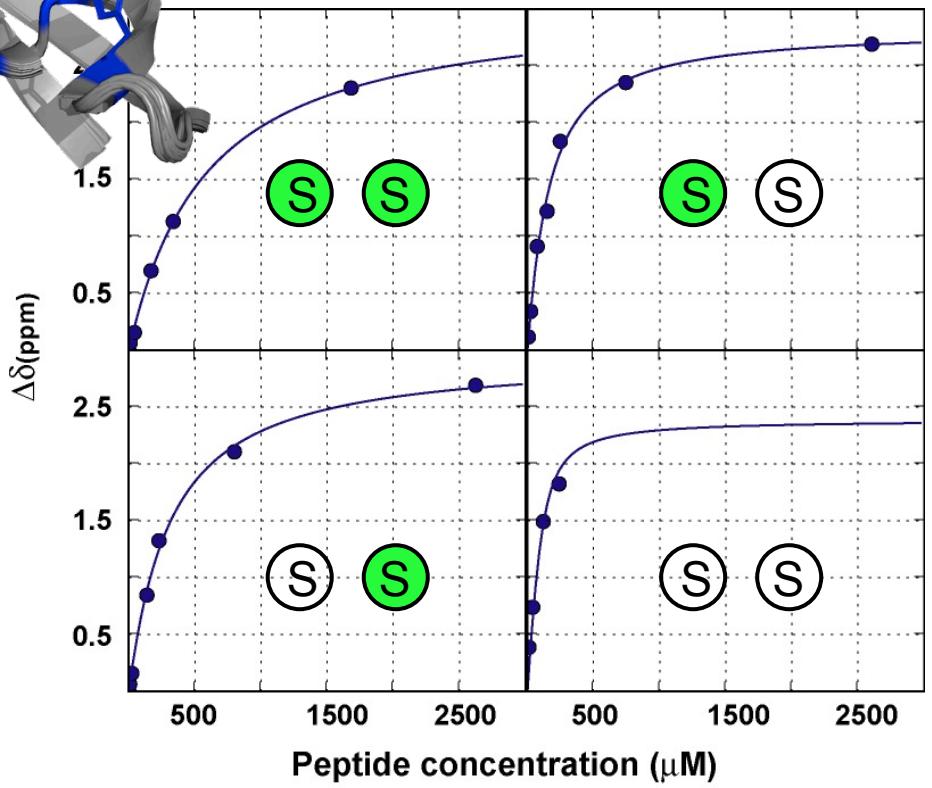
Application: modulation of binding affinity between RAR and vinexin by RAR phosphorylation





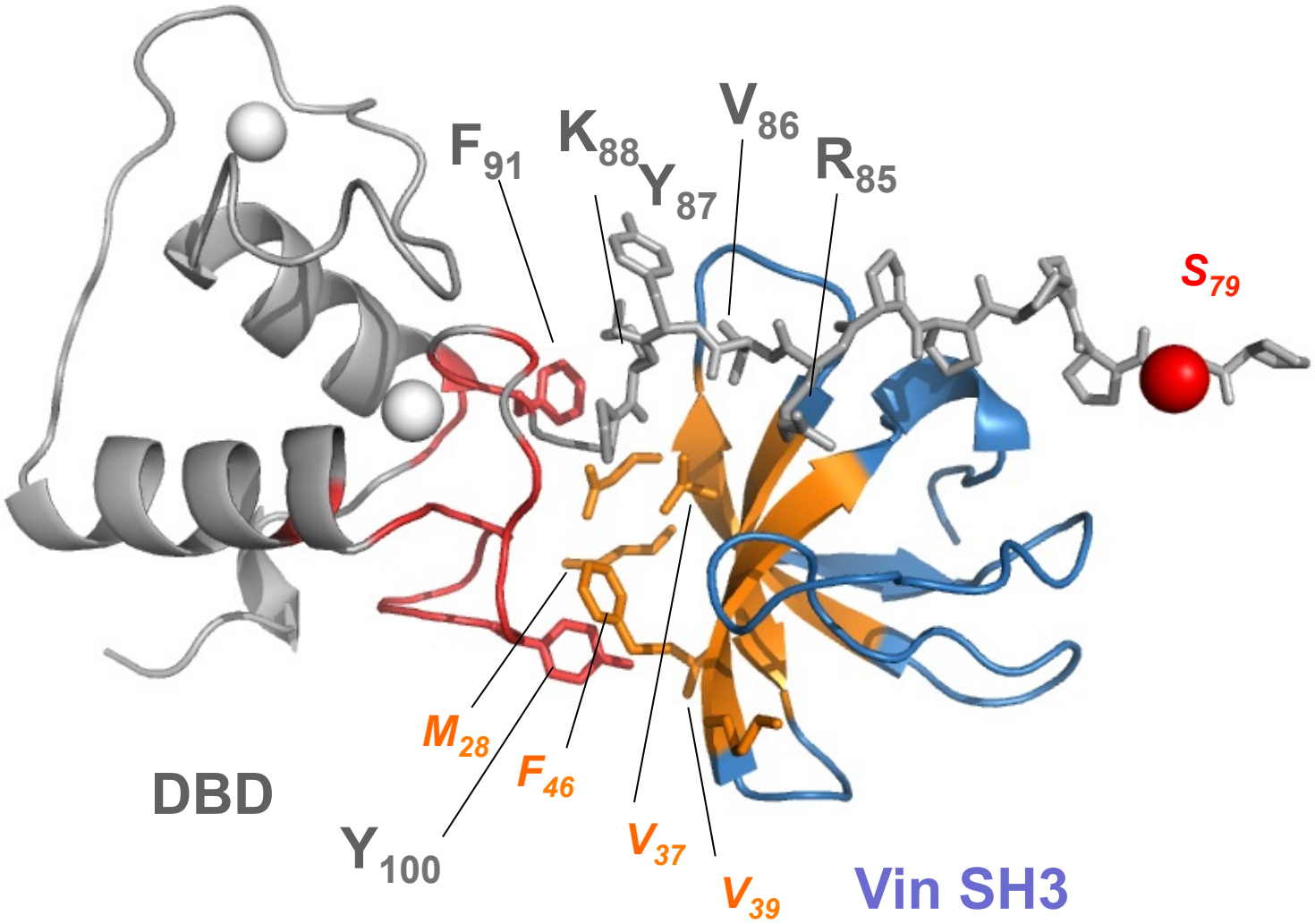
5 ul
10 ul
15 ul
20 ul
25 ul
30 ul
35 ul
65 ul
85 ul

	Kd
^{77}S-^{79}S:	40 μM
^{77}pS-^{79}S:	140 μM
^{77}S-^{79}pS:	280 μM
^{77}pS-^{79}pS:	545 μM



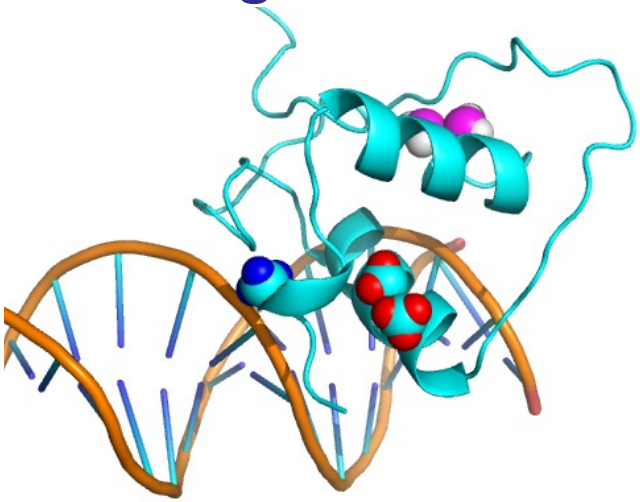
Lalevee et al. Vinexin , The FASEB Journal (2010) vol. 24 (11) pp. 4523-4534

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

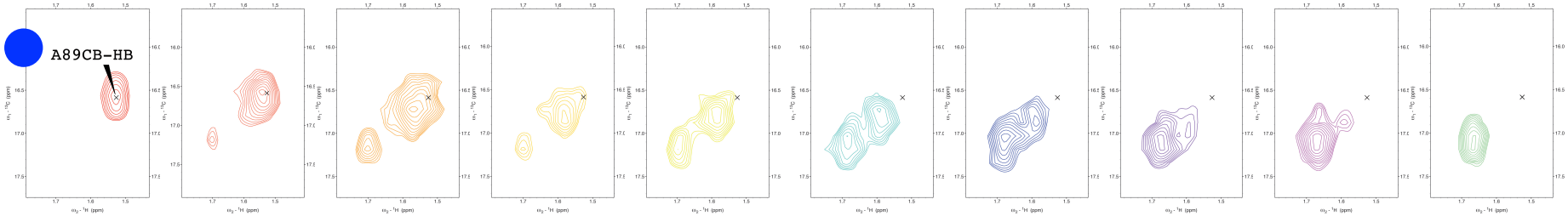


An example of an interaction in slow exchange

- *Assembly of RARγ DBD homo-dimer follows different paths according the topology of the Response Element*



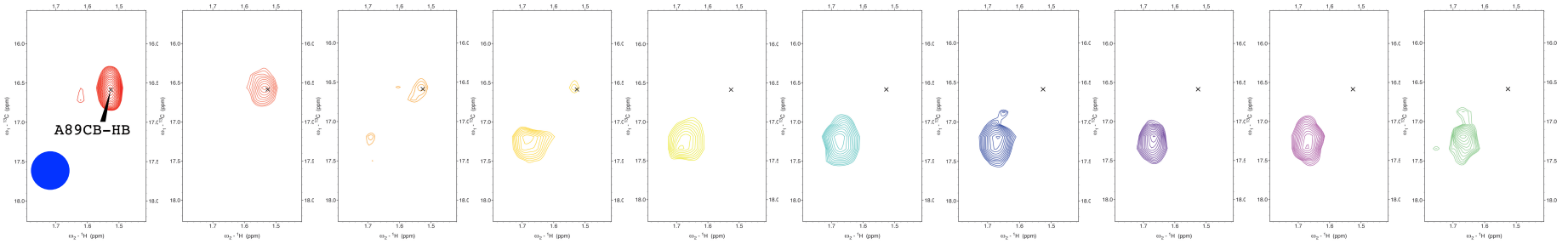
DR2



DR5

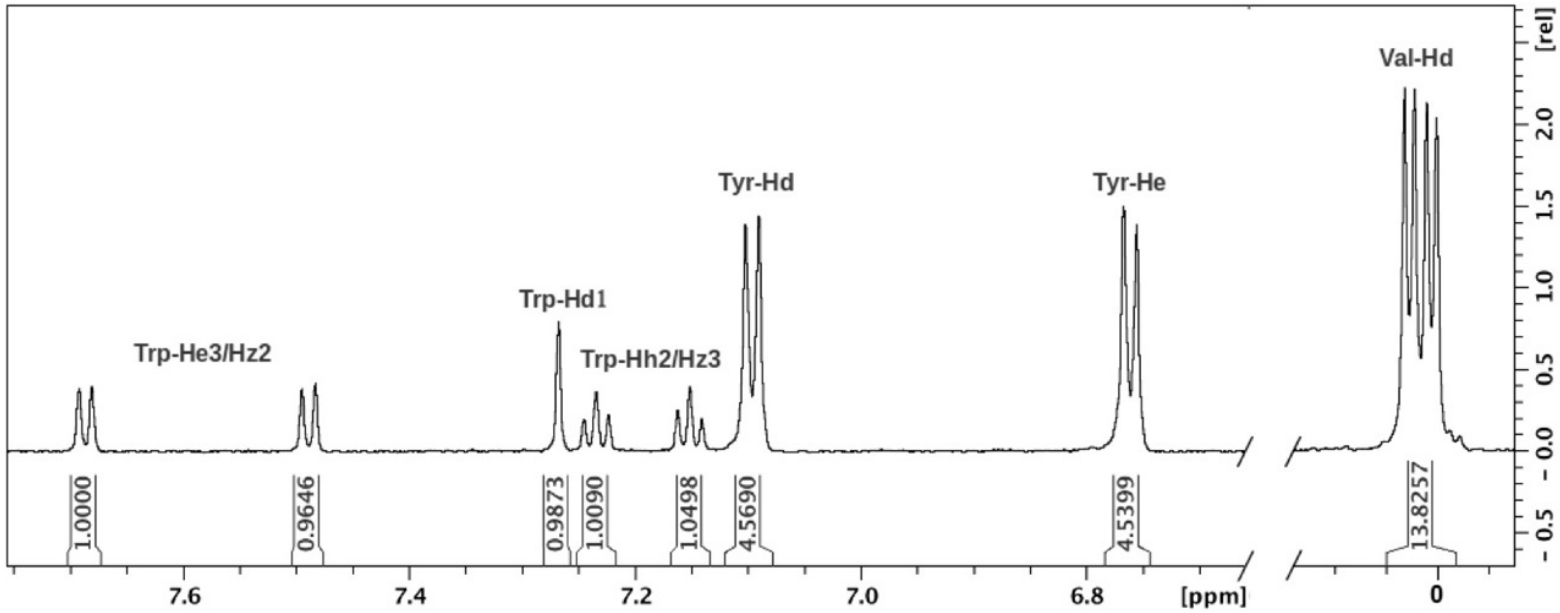
1:1

+ DNA



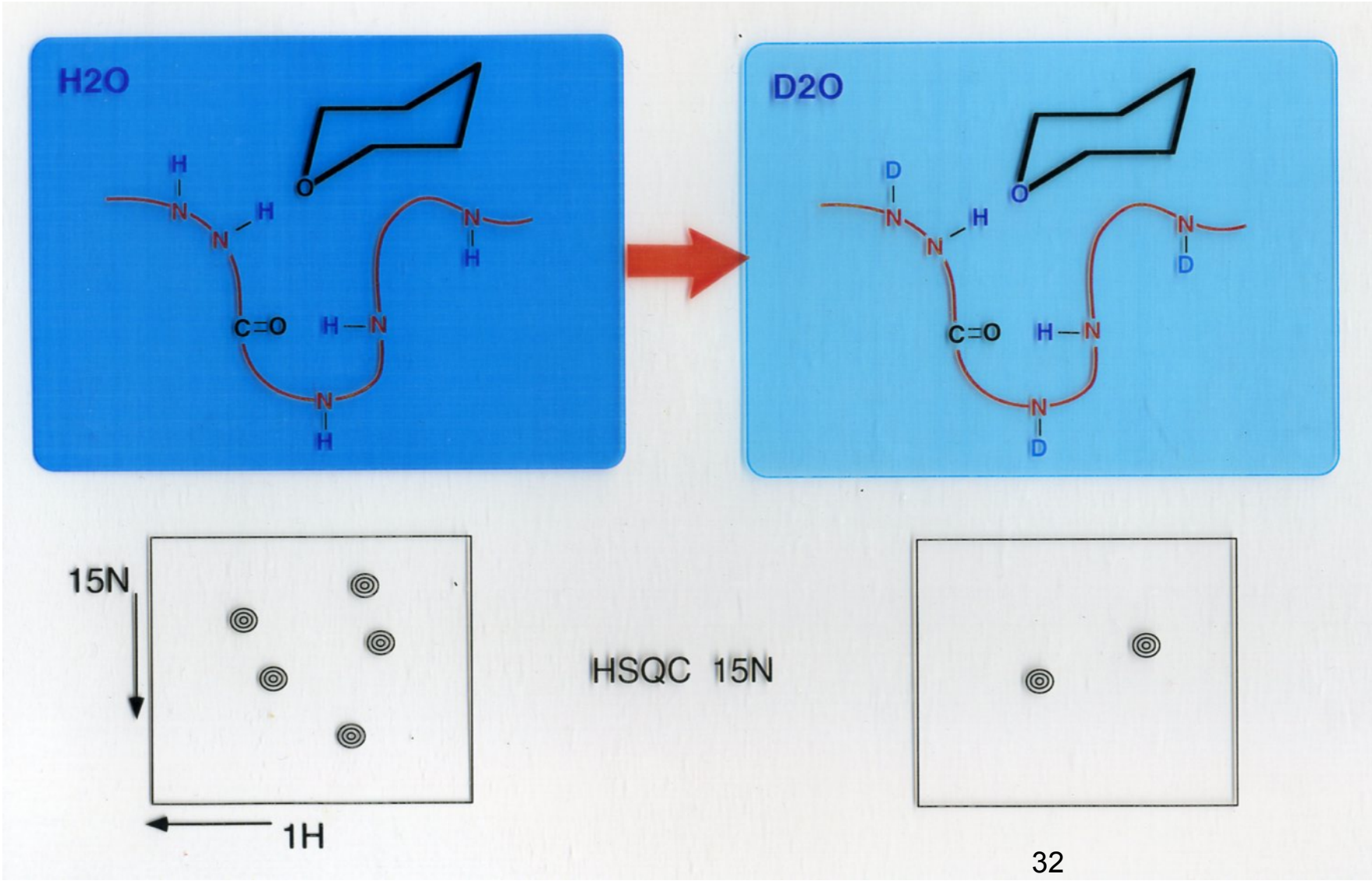
Observable: resonance line intensity
Use of NMR for peptide quantification

- Principle: mix a solution of Tryptophane with known concentration with a dilute peptide sample in D2O (cc 50 uM)

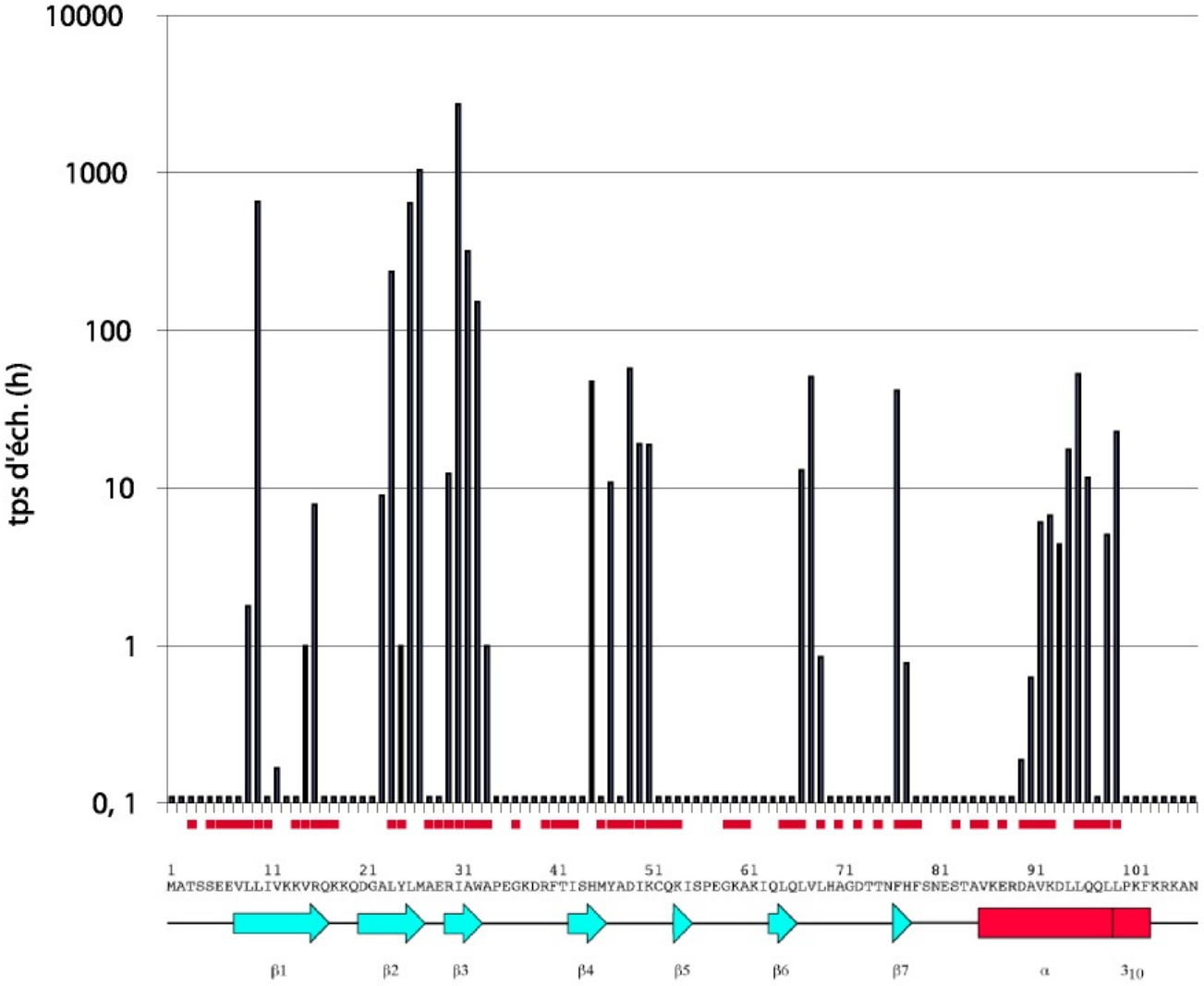


Köhler C et al. Methods Mol Biol. 2015;12;1286:279-96

Real-time solvent exchange kinetic experiment



The exchangeable protons

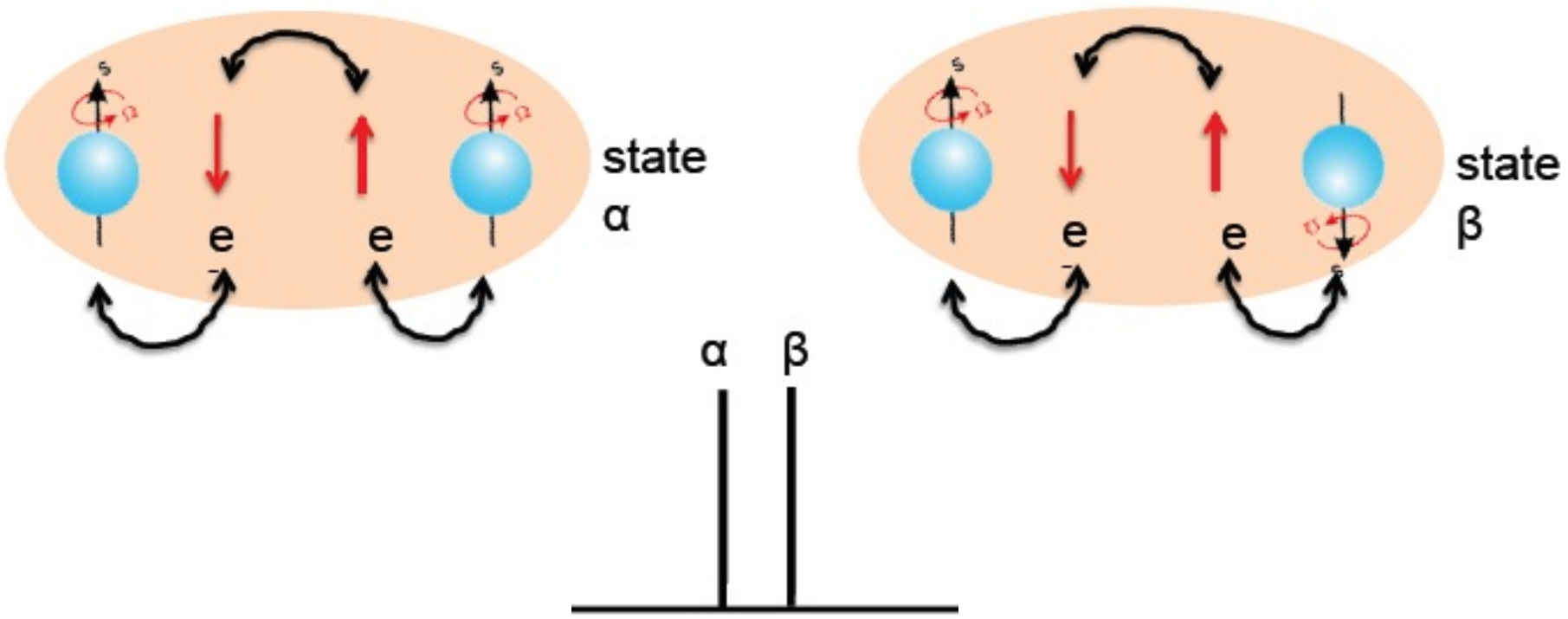


Measure of proton exchange rates of amide protons of the PH domain of TFIIH P62 subunit

Gervais et al.
Nat struct biol 2004

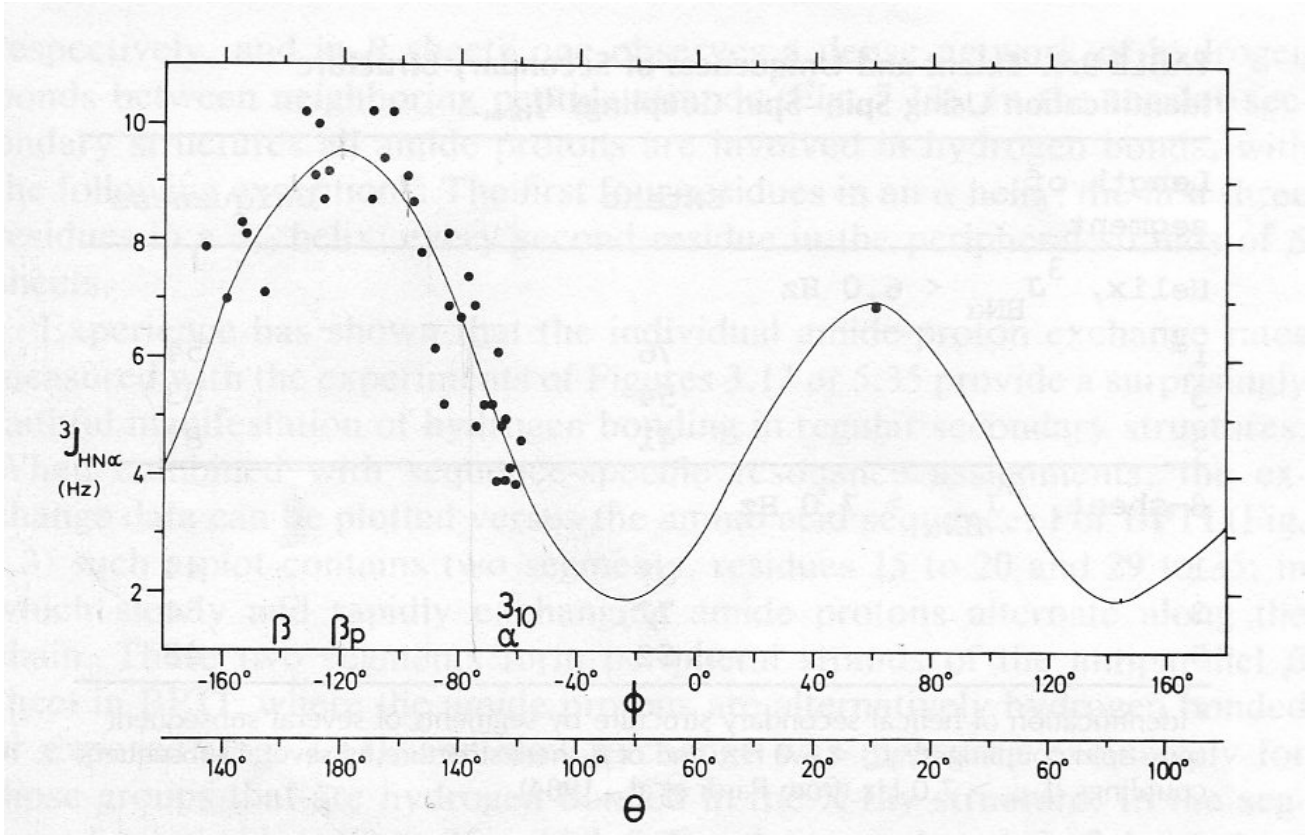
Observable: coupling constants

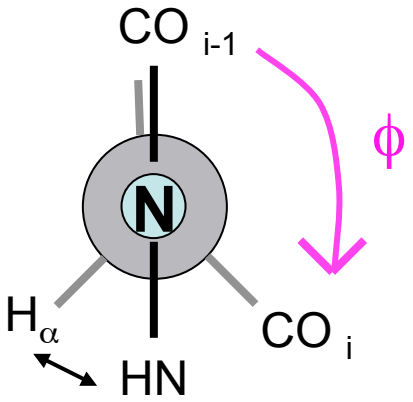
- Physical ground:
  - Nuclei are sensing the state of their neighbours spins through bond electrons



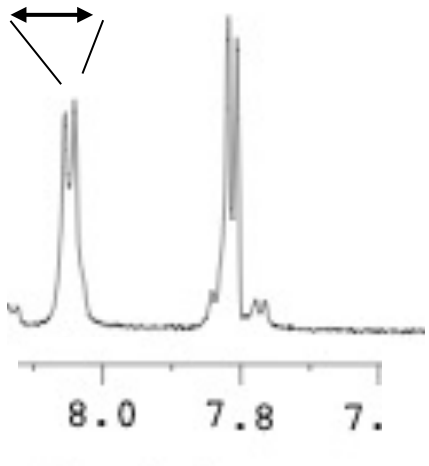
Observable: coupling constants

- Karplus equation provides a relationship between the dihedral angle and the coupling constants of two protons





$^3J_{HN-H\alpha}$



Oligosaccharide sugar-pucker

NMR Spectroscopy of RNA

B. Furtig, C. Richter, J. Wohnert and H. Schwalbe

ChemBioChem, **2003**, 4, 936 - 962

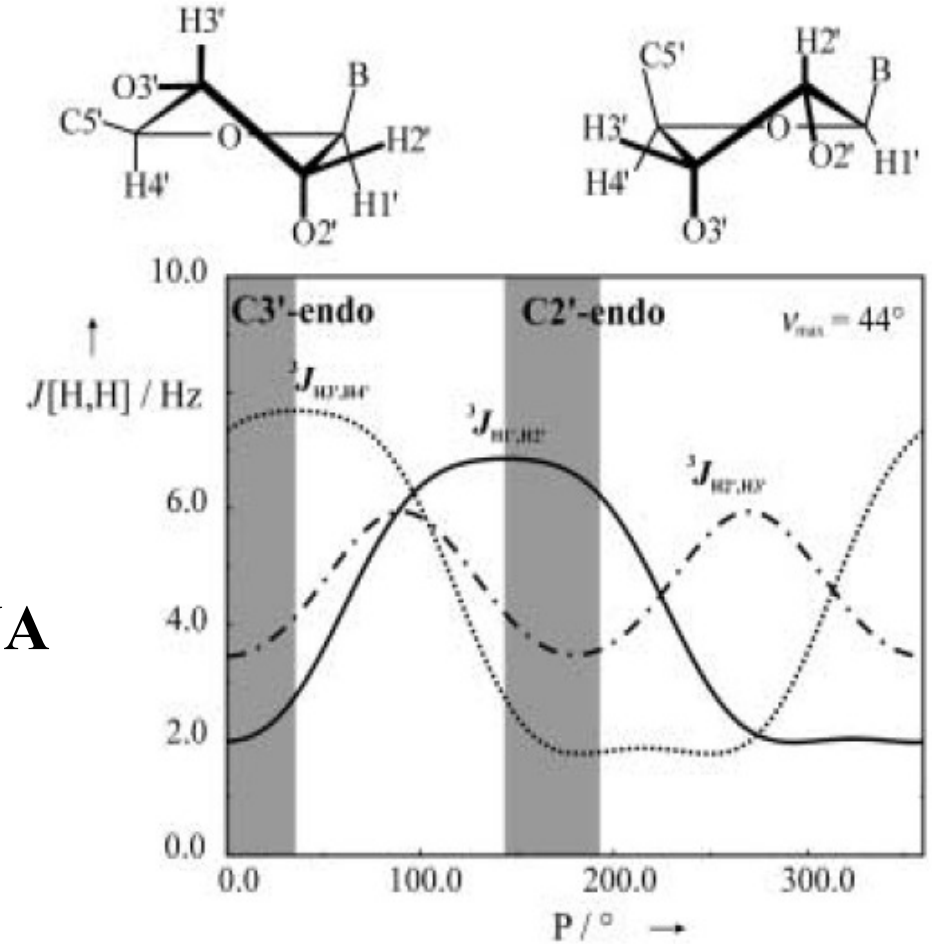
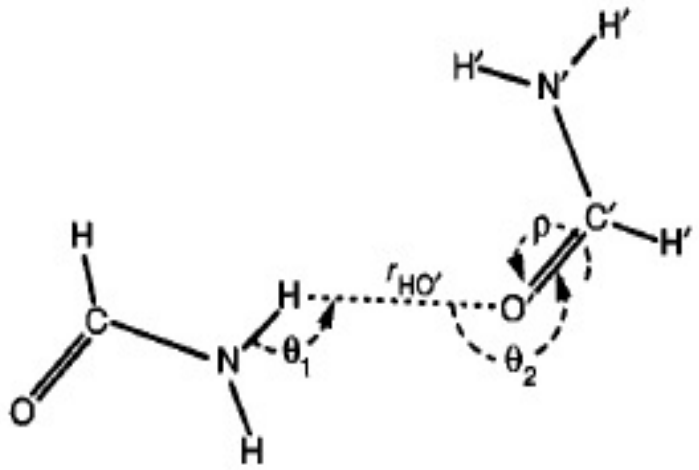
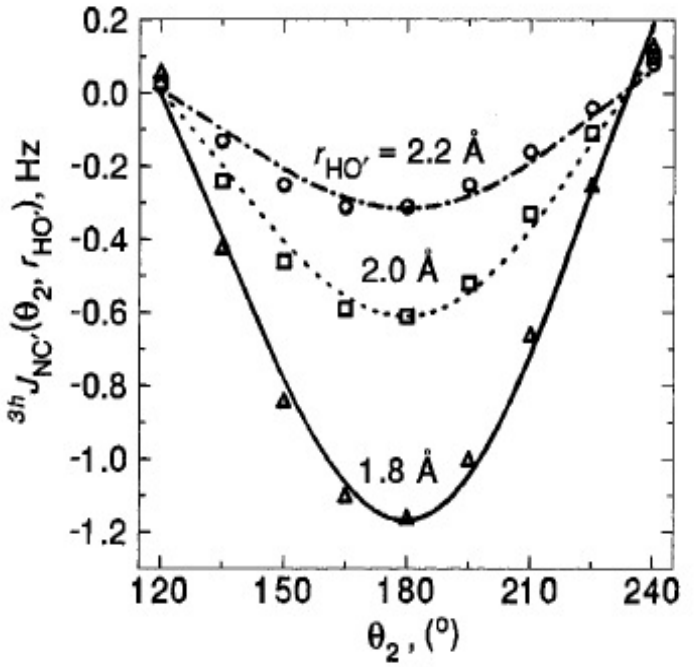


Figure 27. *Karplus relation of ^{3}J(H1′,H2′), ^{3}J(H2′,H3′), and ^{3}J(H3′,H4′) coupling constants depending on the pseudorotation phase P at a pseudorotation amplitude ν_{max} of 44°.*

Through H-bond coupling constants

Cordier, Grzesiek, *J. Am. Chem. Soc.*, **1999**, 1601-1602

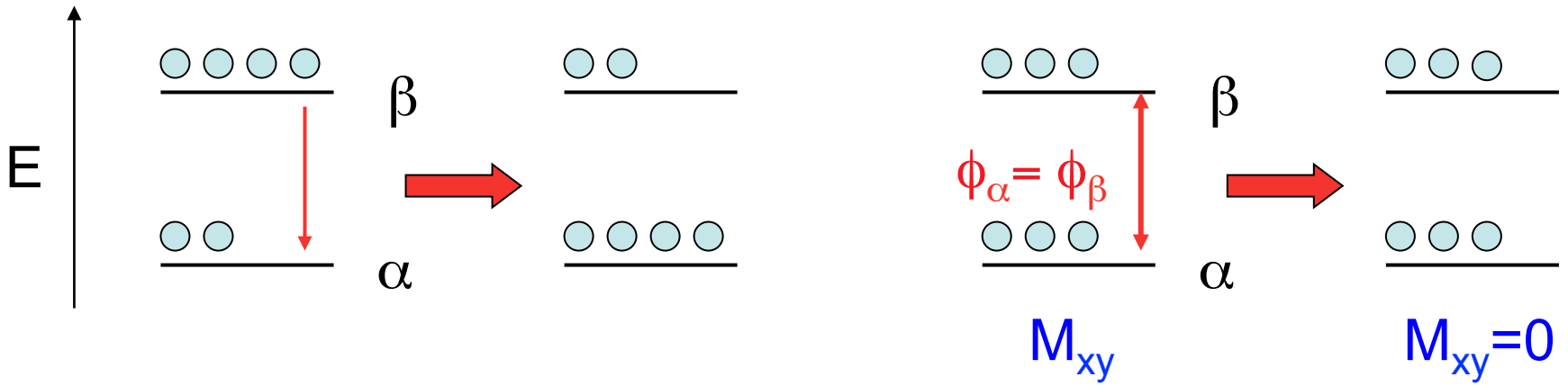




$$r_{NO} = 2.75 - 0.25\ln(-{}^{3h}J_{NC'}) \pm 0.06\,\text{Å}$$

Let's relax

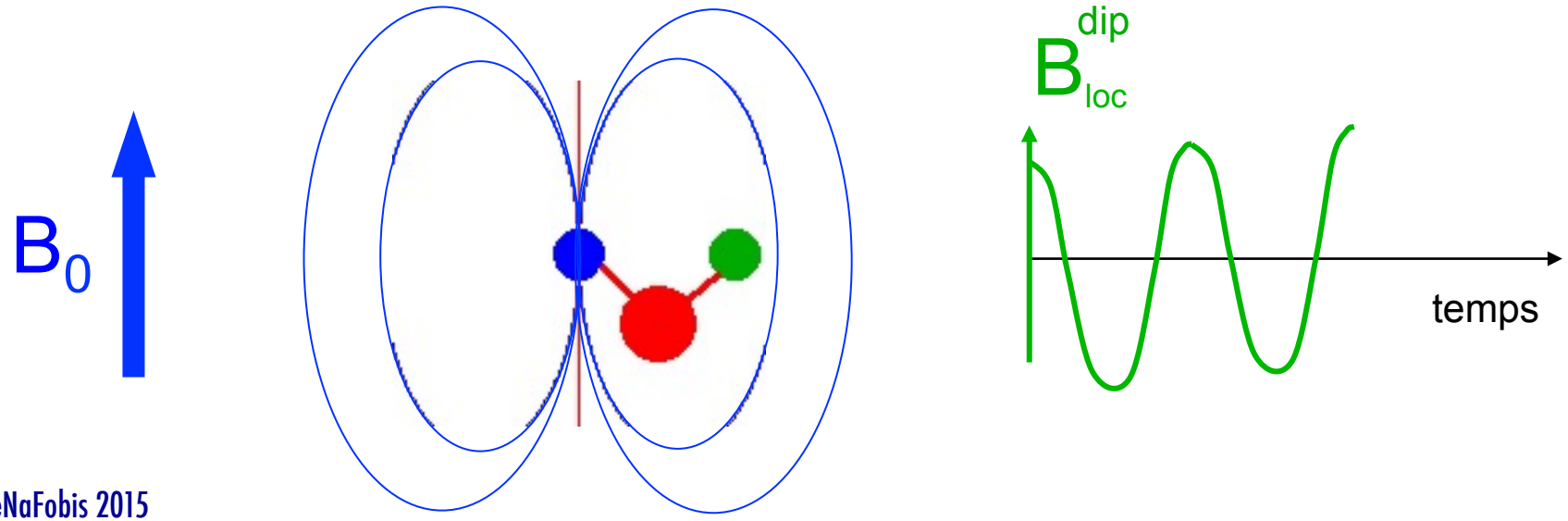
- Relaxation provides the most abundent source of information on a molecular system.
- Information is contained in the relaxation rates :
  - Longitudinal relaxation rates (R1, NOE) : how fast Boltzmann populations are re-established afer a perturbation
  - Transverse relaxation rates (R2) : the life-time of a coherent state



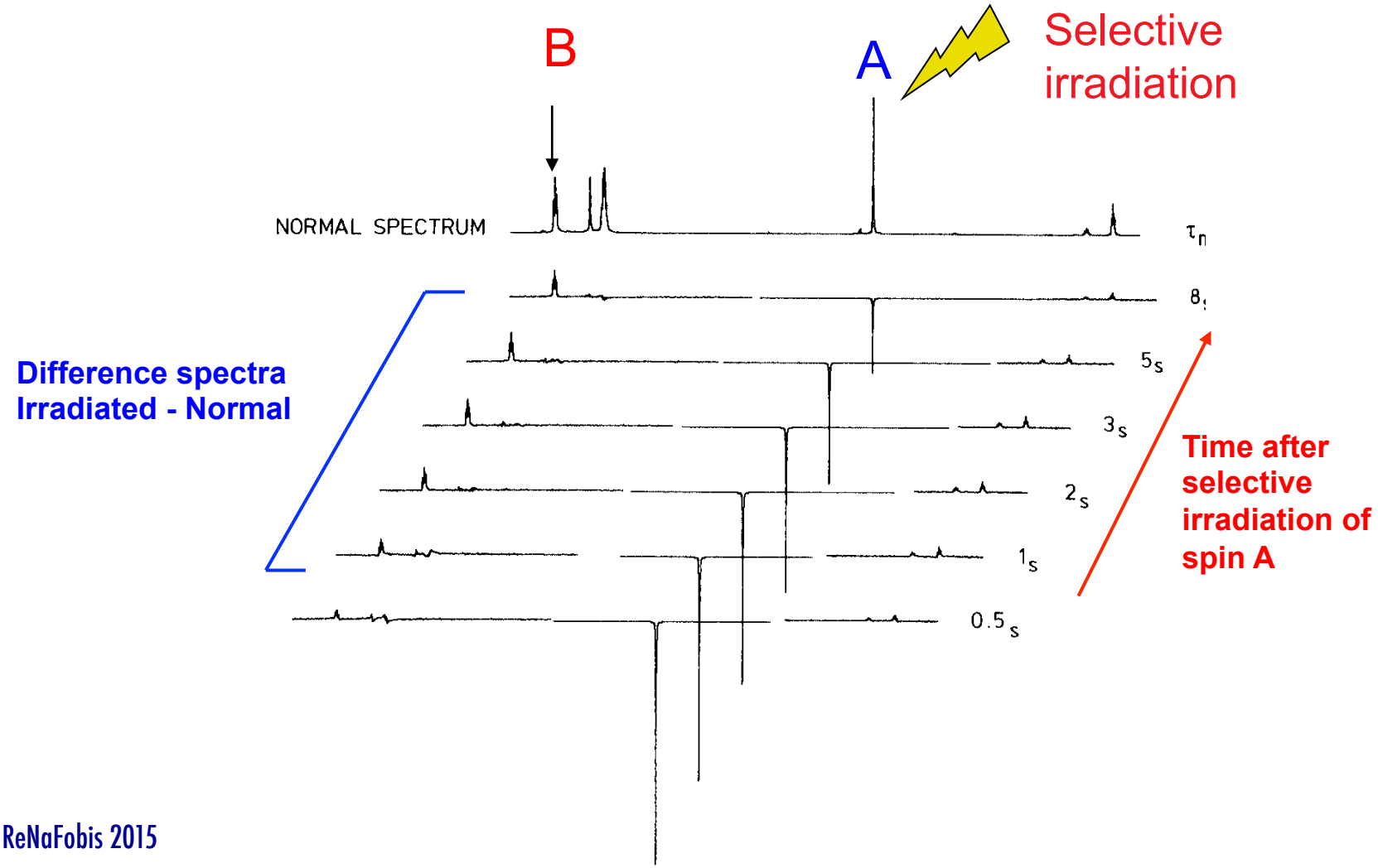
Relaxation and molecular motions

- Relaxation is related to molecular motions that produce random local magnetic fields fluctuations, leading to random transitions between spin states.

- The most important mechanisms are the dipole-dipole interactions and the chemical shift anisotropy



The Nuclear Overhauser Enhancement



Relaxation is described by first order kinetic equations

- Longitudinal relaxation

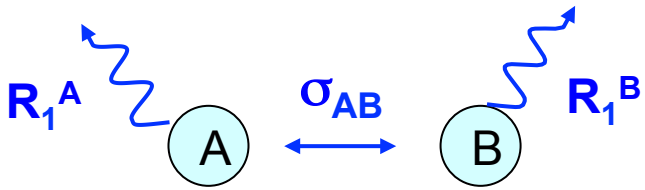
$$\frac{dM_z^B}{dt} = -R_1^B\left(M_z^B - M_z^{B0}\right) - \sigma_{AB}\left(M_z^A - M_z^{A0}\right)$$

- Transverse relaxation

$$\frac{dM_{XY}}{dt} = -R_2 M_{XY}$$

Protein structure from NOE data

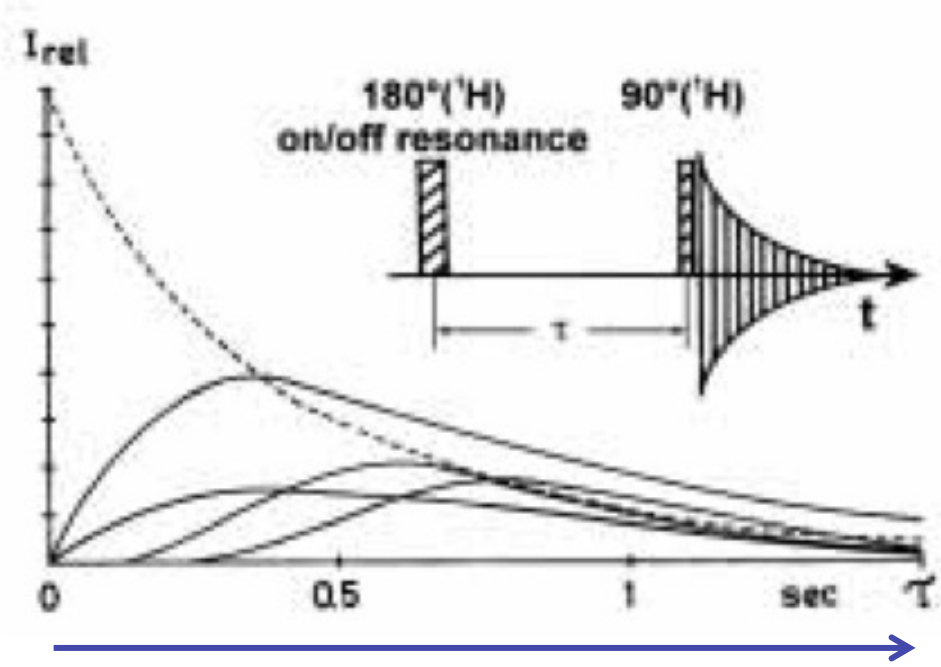
- K Wüthrich (1982) Inter-proton Nuclear Overhauser magnetization transfer may be used to build a model of protein's structure



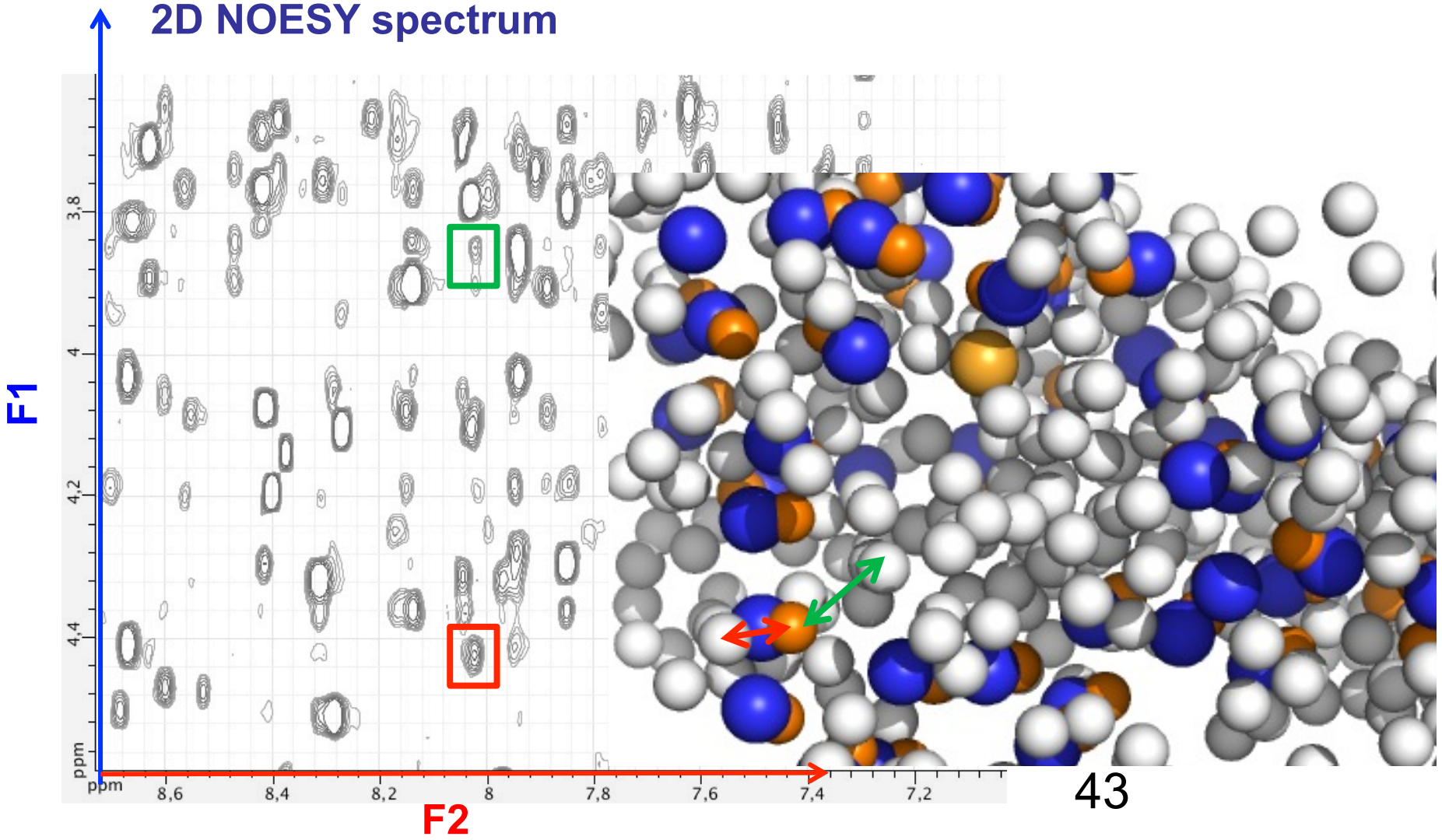
For macromolecules

$$\sigma_{AB} = -a\gamma^4 \tau_c \frac{1}{r_{AB}^6}$$

Dynamics Structure

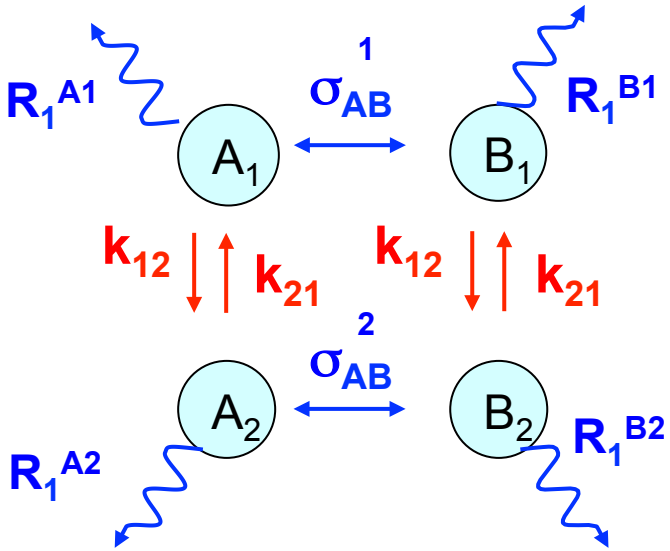


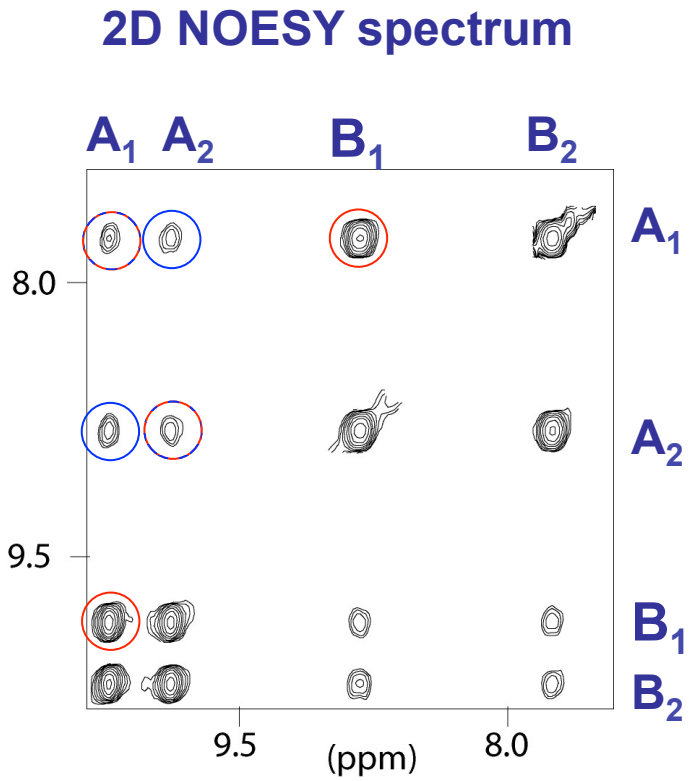
Measuring NOE in proteins using 2D NOESY spectra



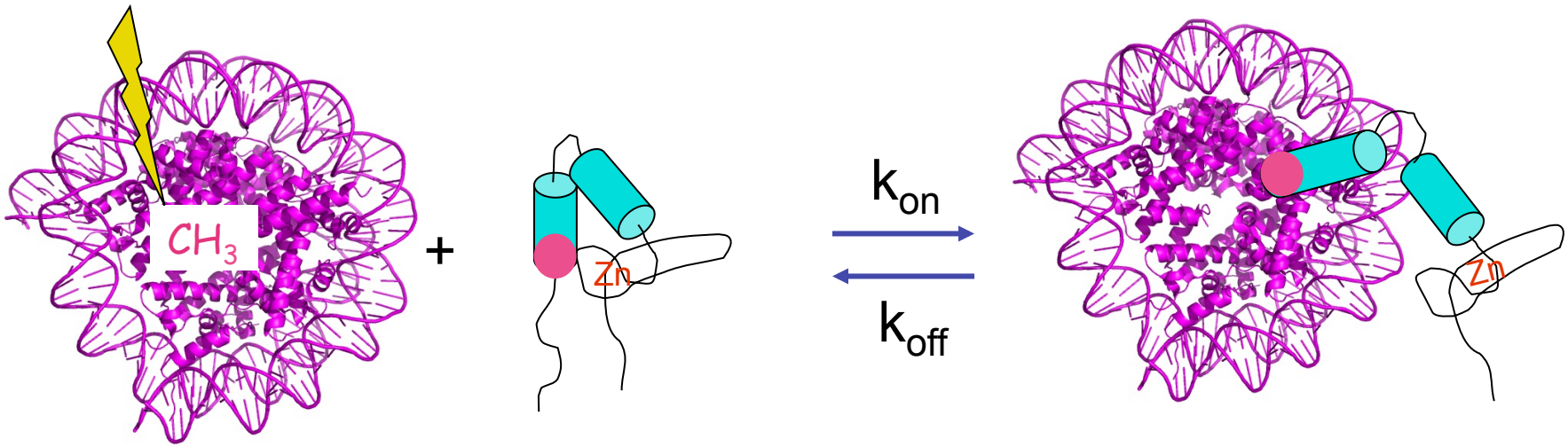
« Chemical exchange » may also lead to magnetization transfer

- If a system is exchanging between two states (1 and 2), magnetization is transported via both relaxation and chemical exchange

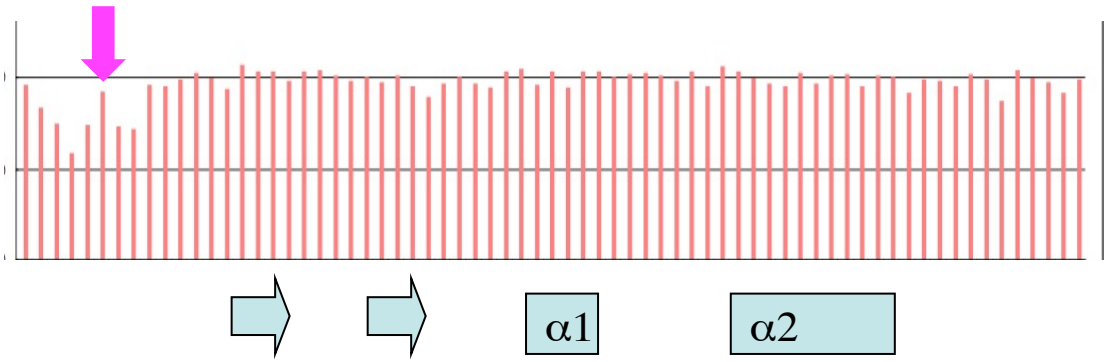




Interaction between the ATXN7 subunit of SAGA and the nucleosome



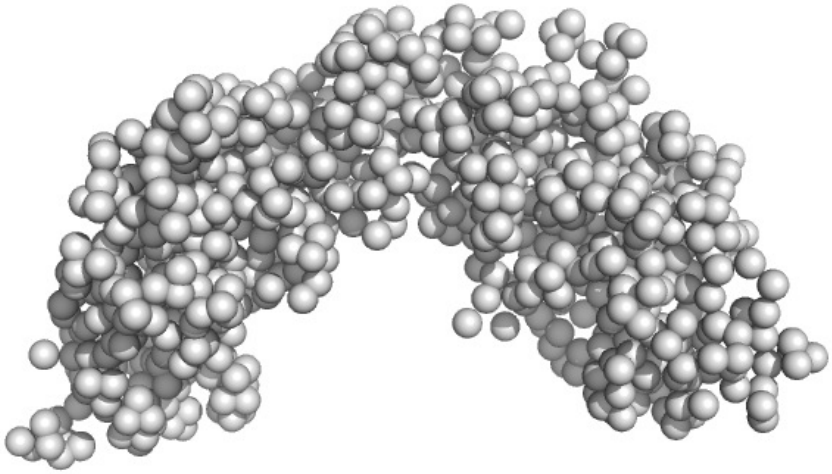
Changes of magnetization state is transfered in the free state with the k_{off} rate and leads to intensities changes In the spectrum of the free protein



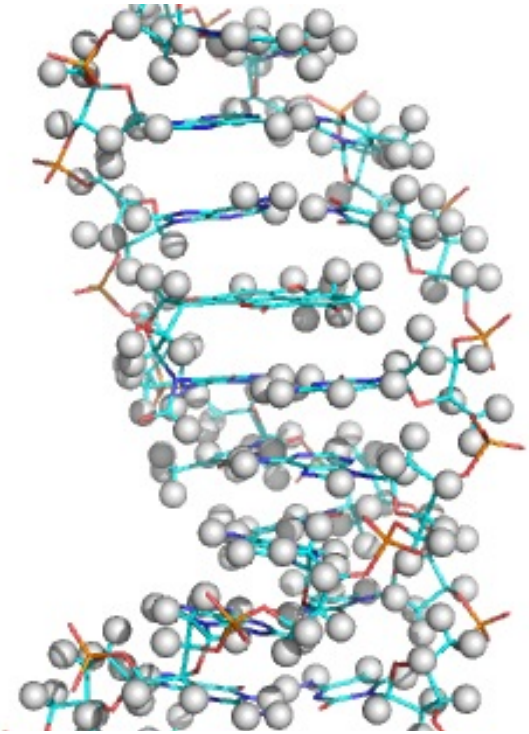
Bonnet J et al.
EMBO Rep. **11**(8),612-618

Drawbacks of NOE distance based structural data

- Low distance range (5-6 Å) : requires an homogenous spatial distribution of protons

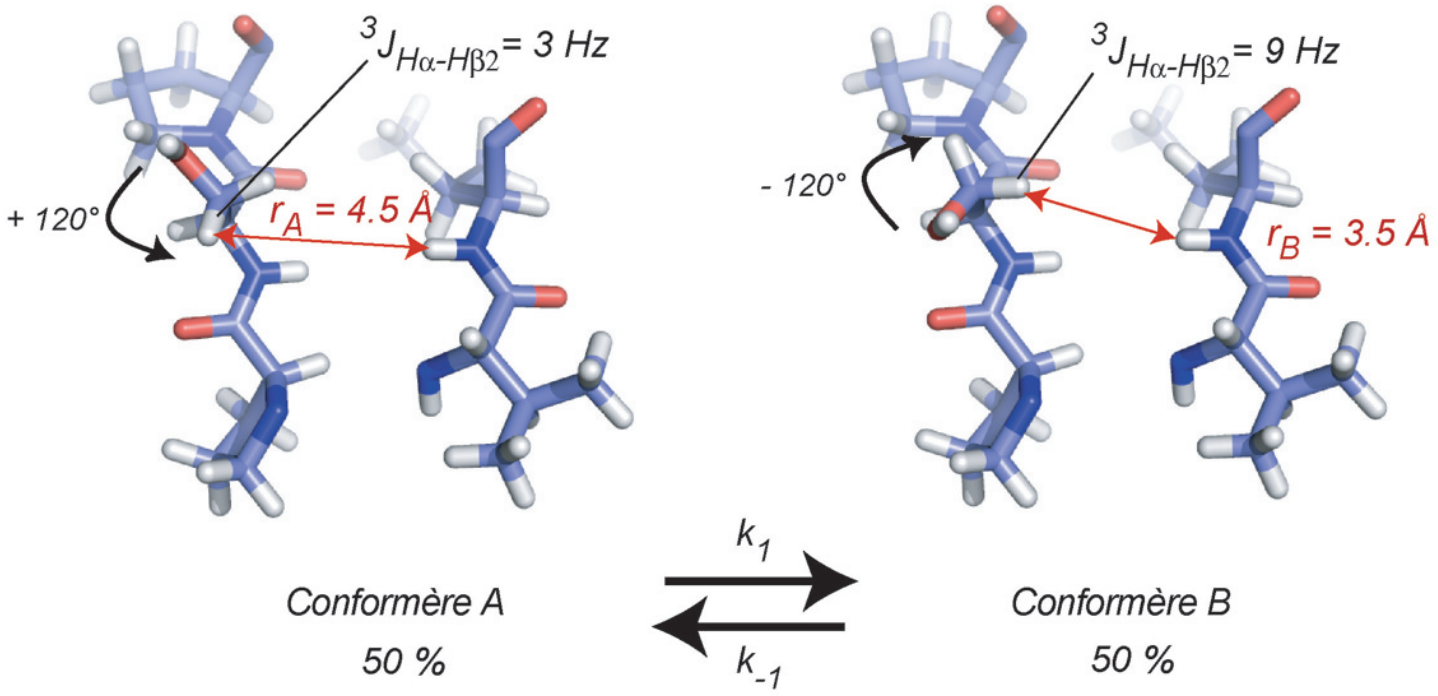


Multi-domains proteins



Nucleic acids

Effect of conformational averaging on NOE and coupling constants

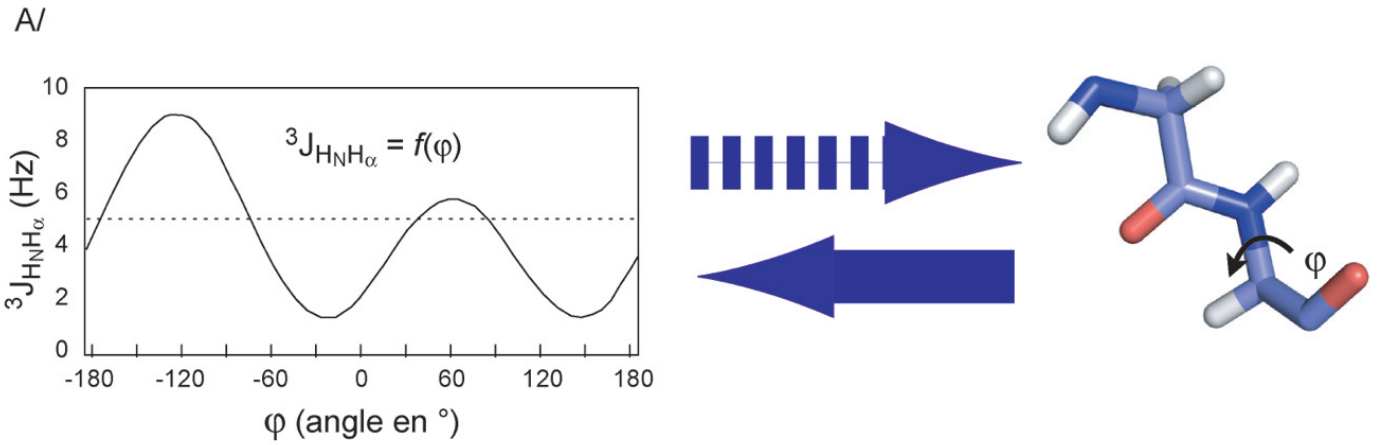


$$< r > = \frac{1}{\sqrt[6]{\frac{1}{2}\left(\frac{1}{r_A^6} + \frac{1}{r_B^6}\right)}} = 3.8 \text{ Å}$$

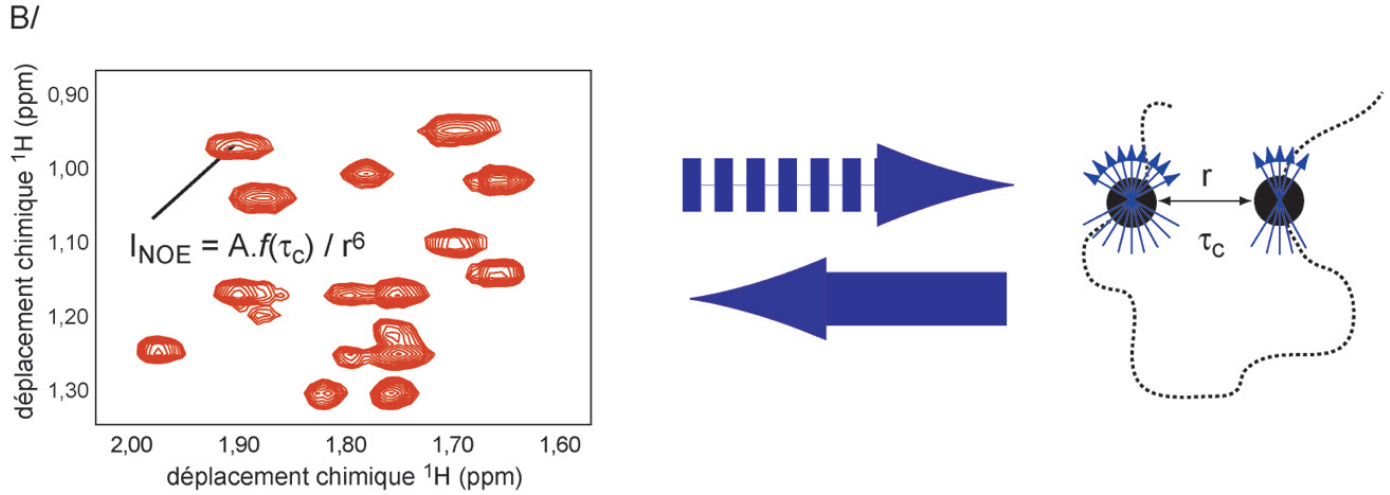
$$< {}^3J_{H_\alpha - H_{\beta 2}} > = 6 \text{ Hz}$$

Structure determination from NMR data is an non-linear inverse problem:

Dihedral angles



NOEs



NMR data modeling strategies

- NMR parameters are calculated as averages from large ensembles of possible strucures
- Subgroups of structures are selected to satisfy the average values of observables
- This strategy allows the description of disordered regions of proteins