Ecole Nationale de Biologie Structurale Intégrative

21-28 Juin 2019 – Oléron, France

Nuclear Magnetic Resonance

From basic principles to structural and dynamical information in biomacromolecules

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Some introductory principles of NMR

Biomolecular sample in solution
10 µM to 1 mM

Transfer to NMR tube

NMR facility

Some introductory principles of NUCLEAR Magn. Res.

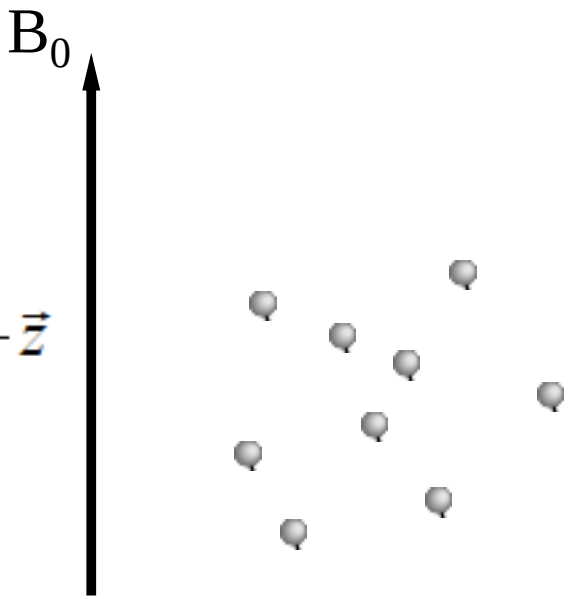
$$\vec{M} = \frac{N(\gamma\hbar)^2 B_0}{4kT}\vec{z}$$

Case 1

Nucleus of interest with no spin

^{12}C, I = 0

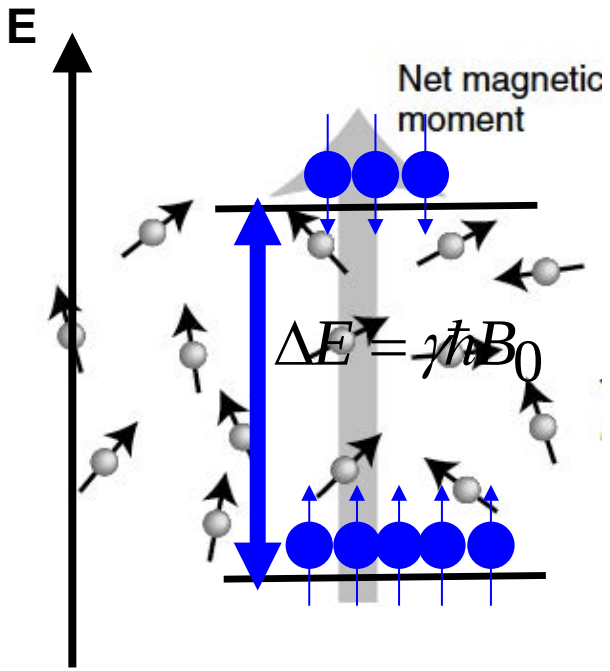
No NMR



Case 2

Nucleus of interest with spin

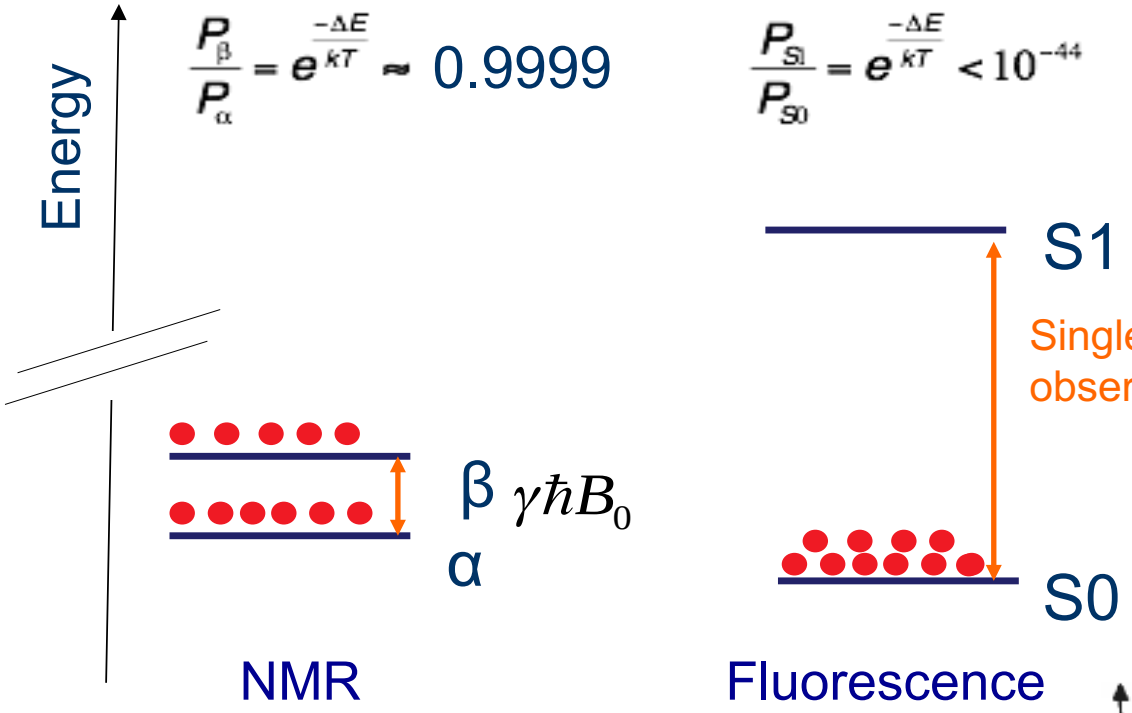
^{1}H, ^{13}C, ^{15}N, ^{19}F, ^{31}P
I = ½



Some remarks on the interaction of nuclei with B_0

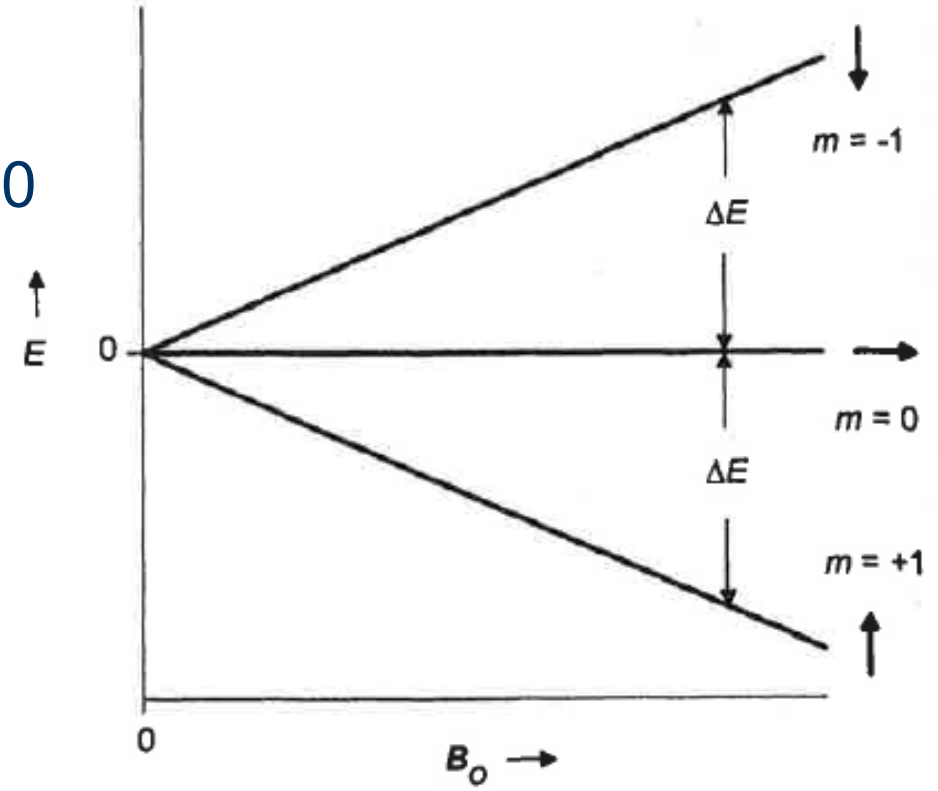
$$\frac{P_\beta}{P_\alpha} = e^{\frac{-\Delta E}{kT}} \approx 0.9999$$

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



^{2}H I = 1

2nI+1 spin states

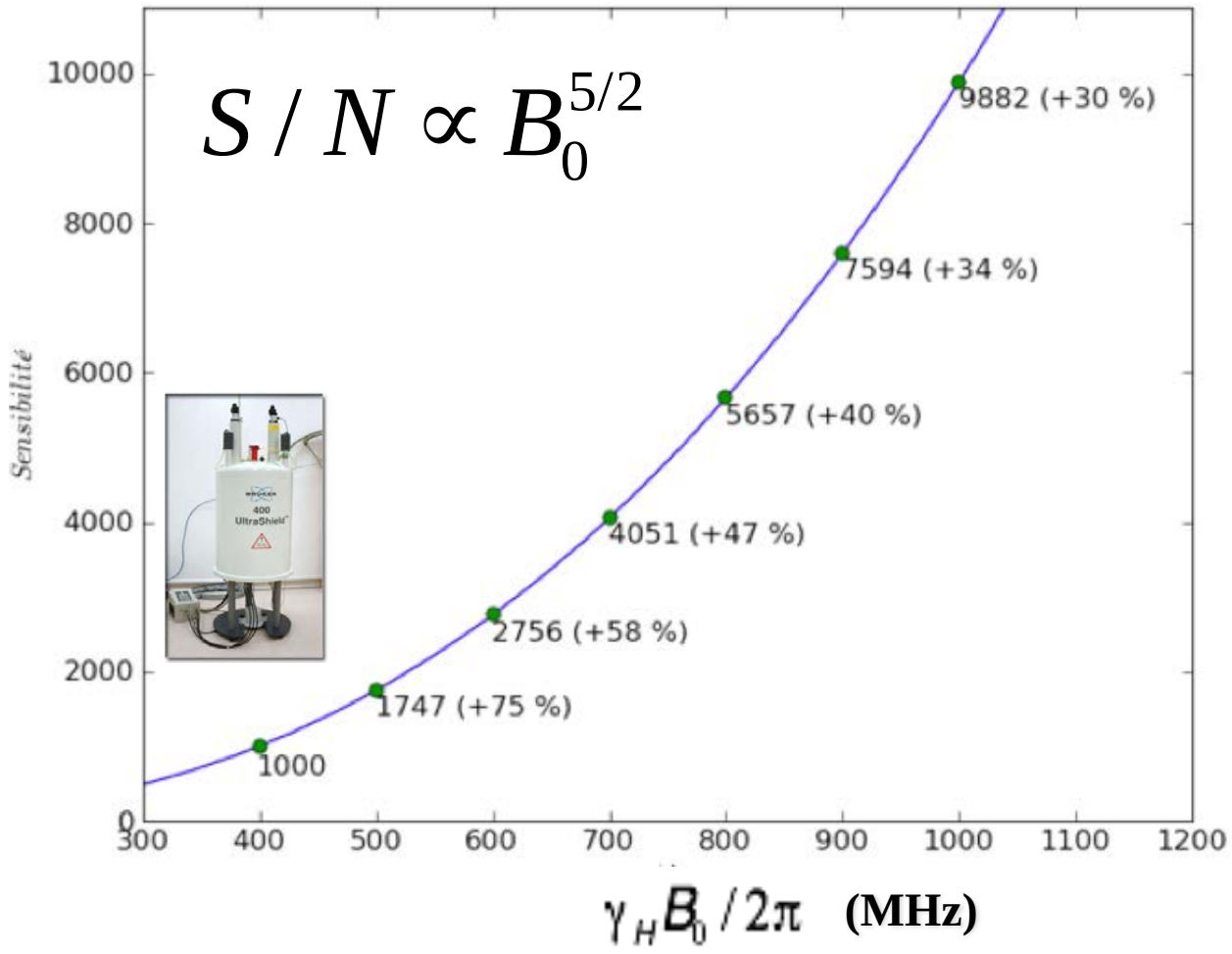


NMR signal

$$\vec{M} = \sum \vec{\mu}_N = \sum \gamma_N \hbar \vec{I}$$

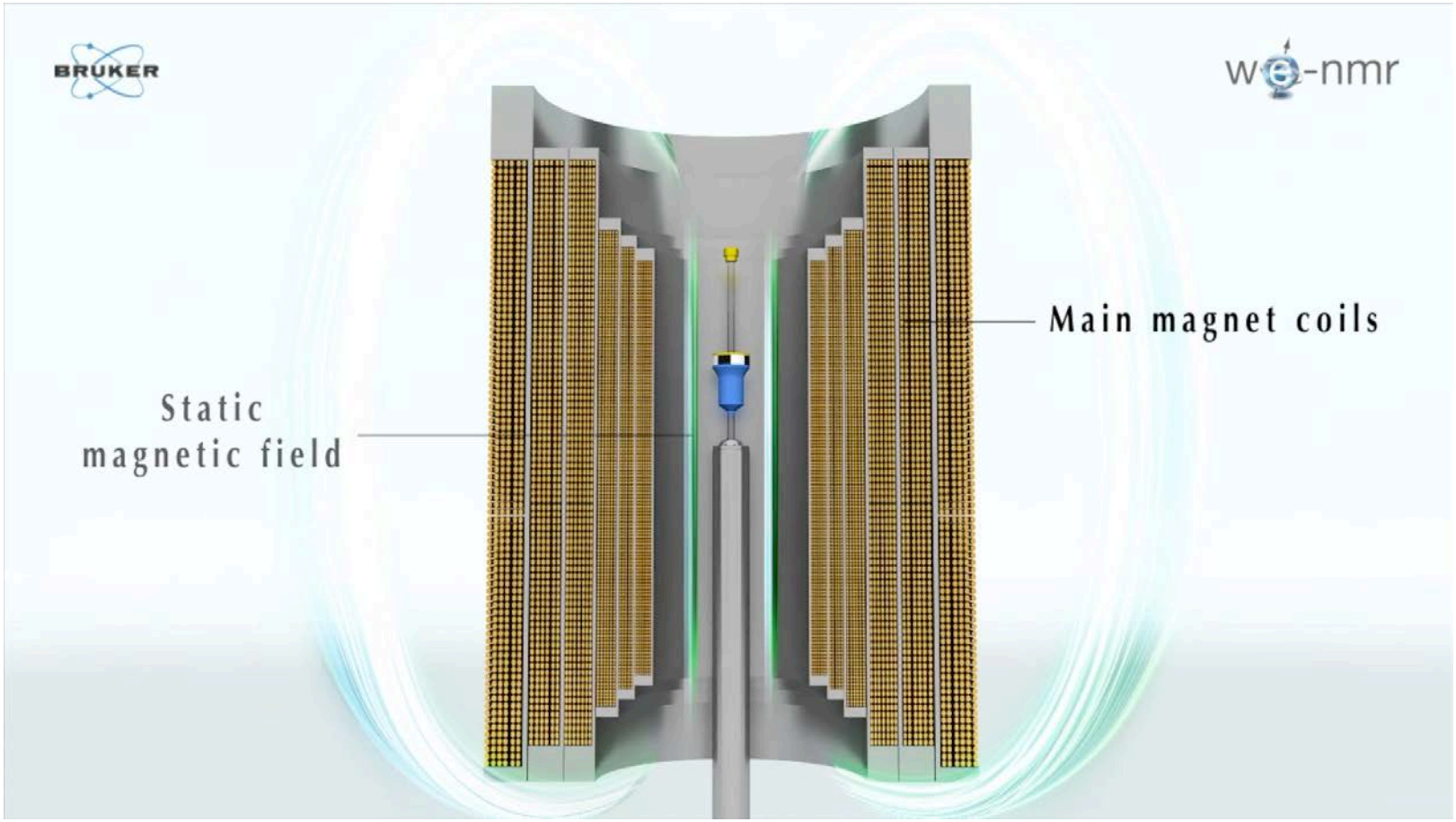
$$\vec{M} = \frac{N(\gamma\hbar)^2 I(I+1) B_0}{3kT} \vec{z}$$

The Nuclear MAGNETIC Res. incentive to go to high field



Some introductory principles

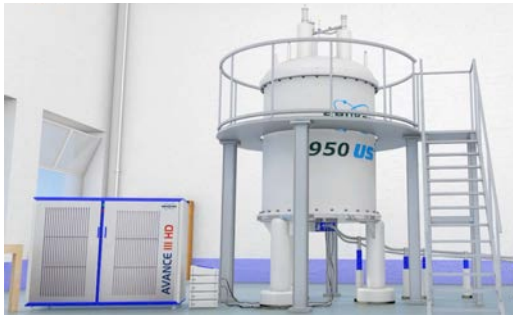
Sample in spectrometer and what next ?



Some introductory principles of Nucl. Magn. RESONANCE

Sample in spectrometer and what next ?

$h\upsilon = \gamma\hbar B_0$



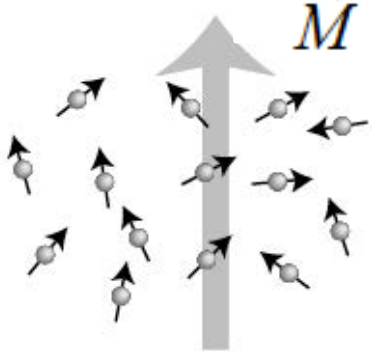
E

$\Delta E = \gamma\hbar B_0$

Thermal equilibrium

B_0

$\vec{M}$



Classical Mechanics

$$\frac{d\vec{M}}{dt} = \gamma\vec{M}\otimes\vec{B_0}$$

$\vec{\mu}_N = \gamma_N\hbar\vec{I}$

Quantum Mechanics

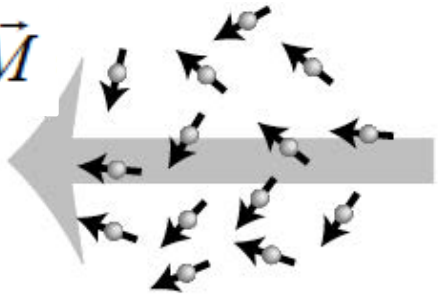
E

$\Delta E = \gamma\hbar B_0$

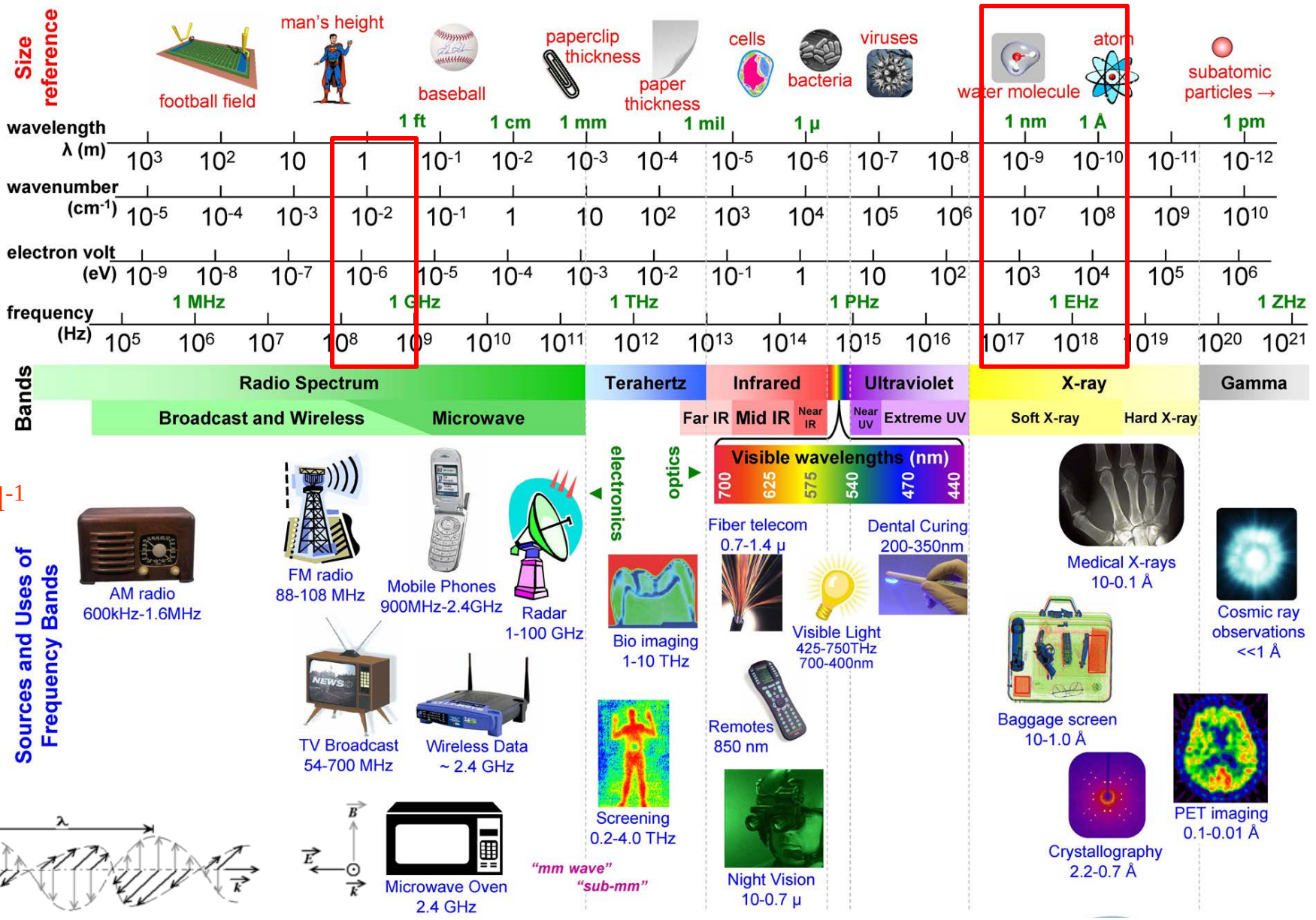
Out of equilibrium

B_0

$\vec{M}$



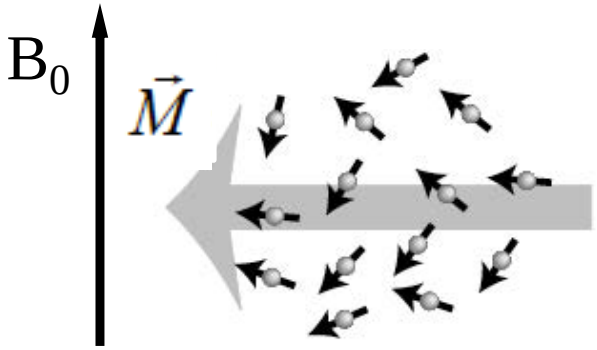
Energies involved in a typical NMR experiment

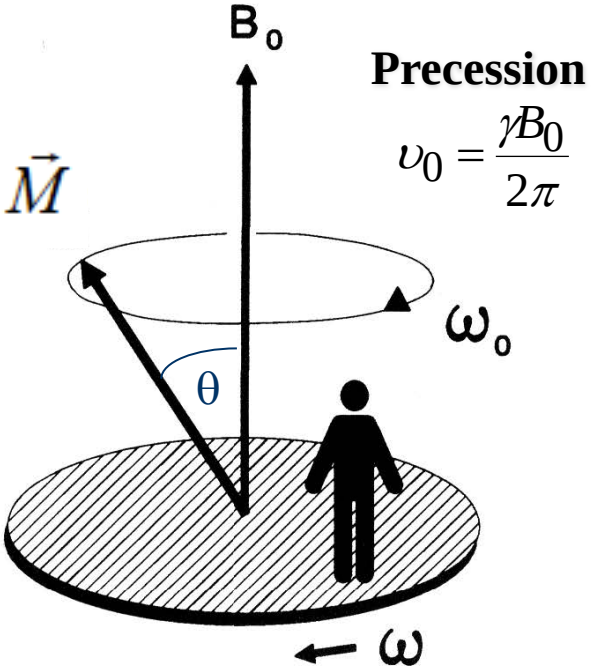


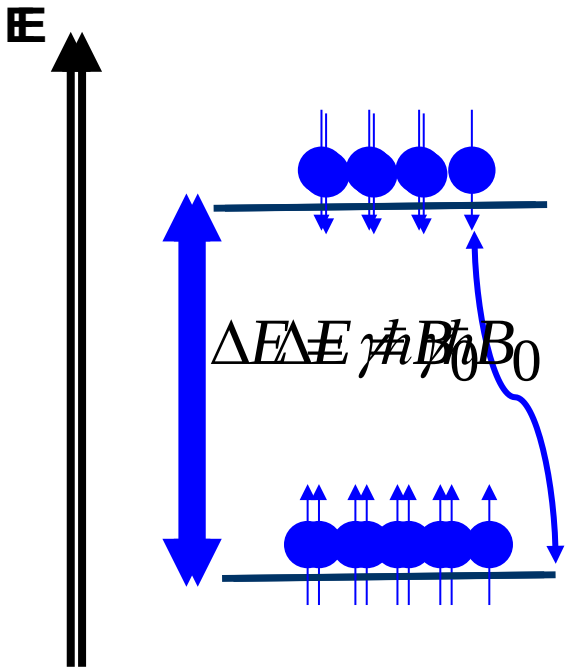
1 eV =
96.5 kJ.mol^{-1}

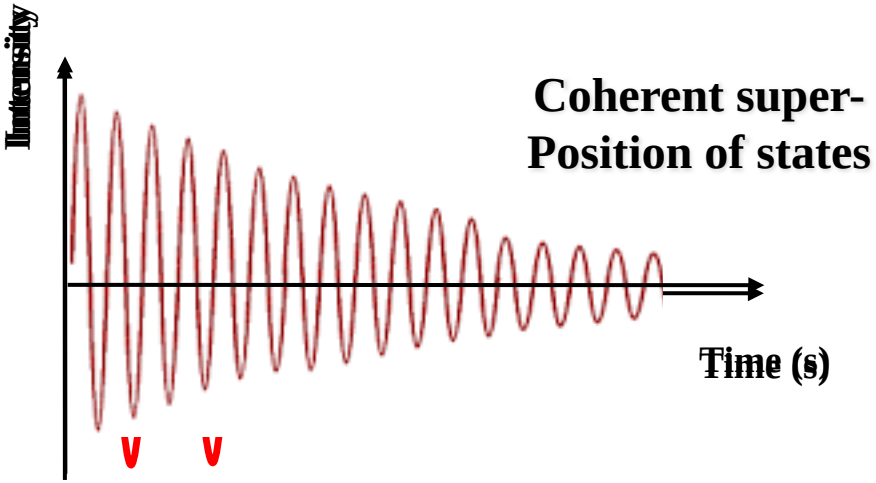
Some introductory principles of Nucl. Magn. RESONANCE

Situation after radiofrequency pulse

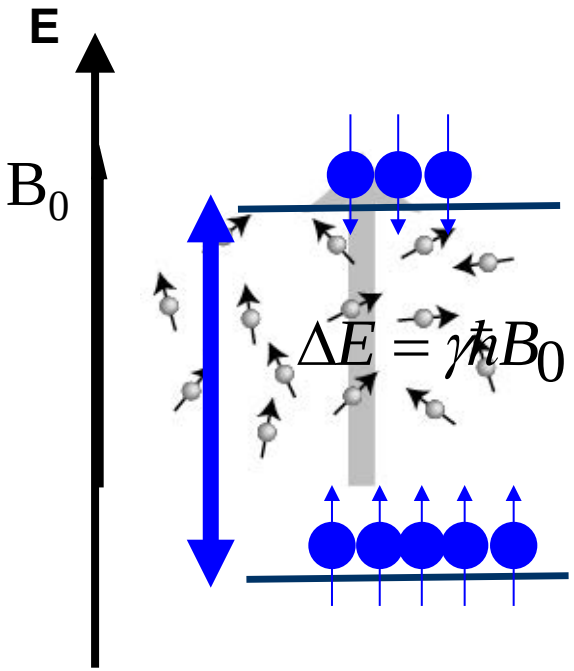




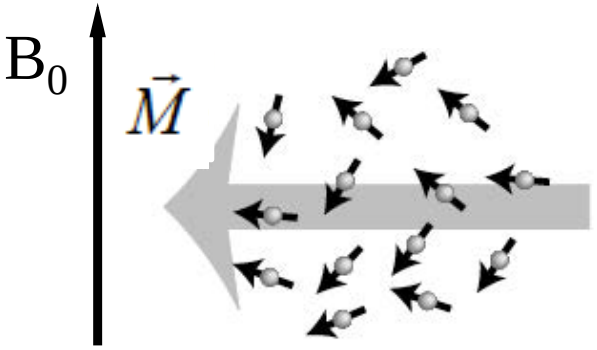


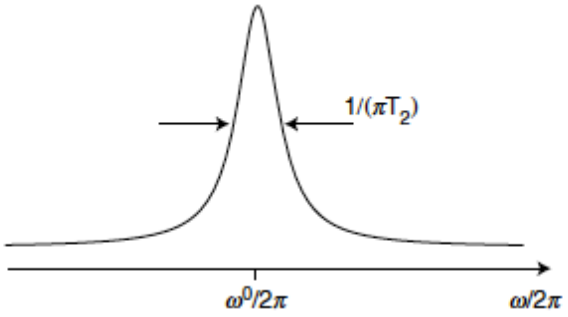


The typical 1D NMR experiment (summary of principles)



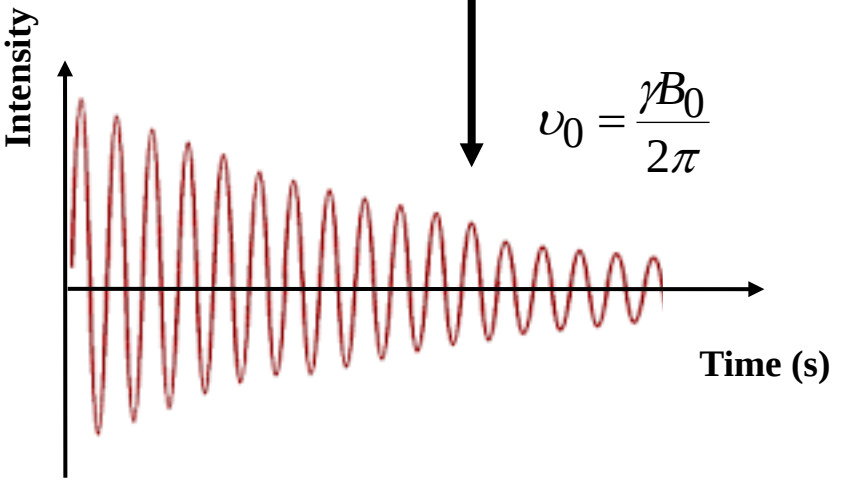
$h\upsilon = \gamma\hbar B_0$





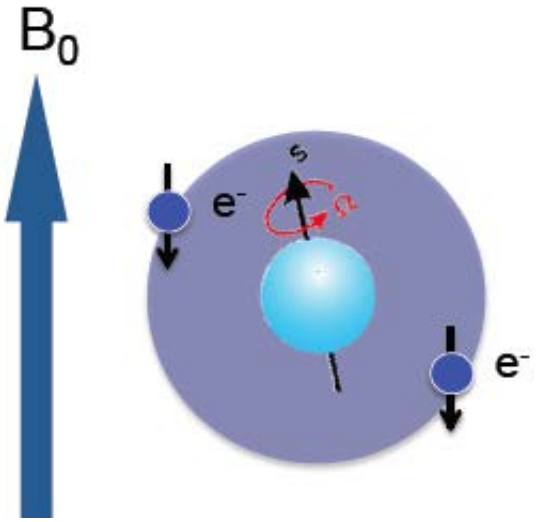
Fourier Transform

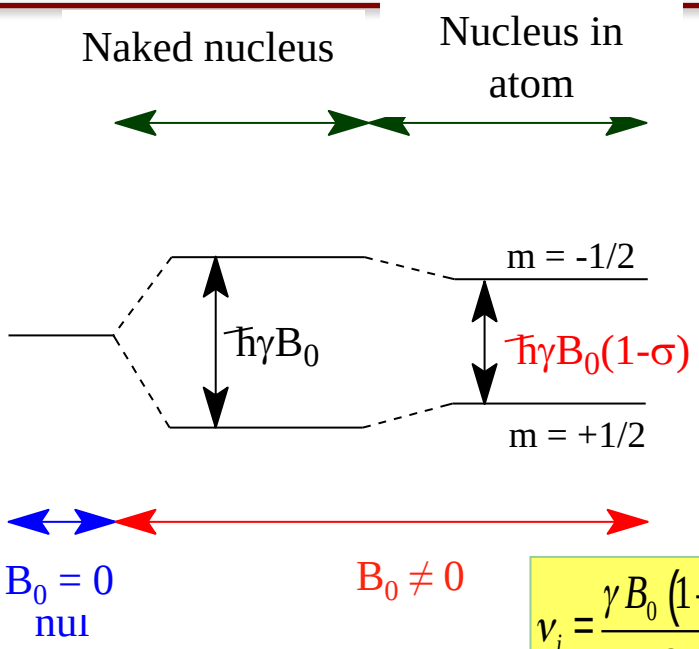
Data Processing



$\upsilon_0 = \frac{\gamma B_0}{2\pi}$

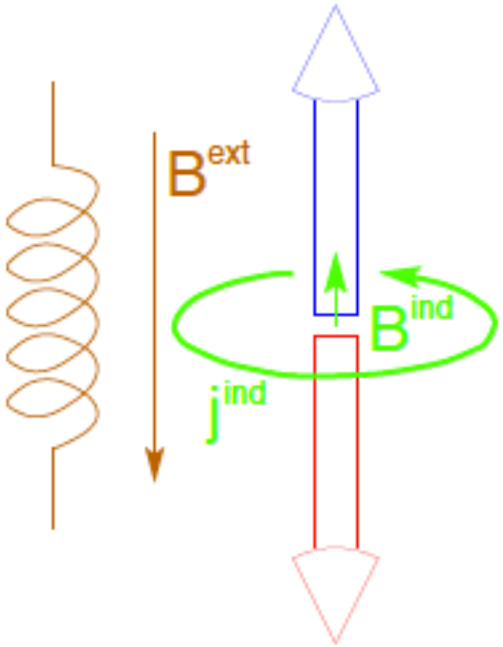
But the nucleus is not alone…

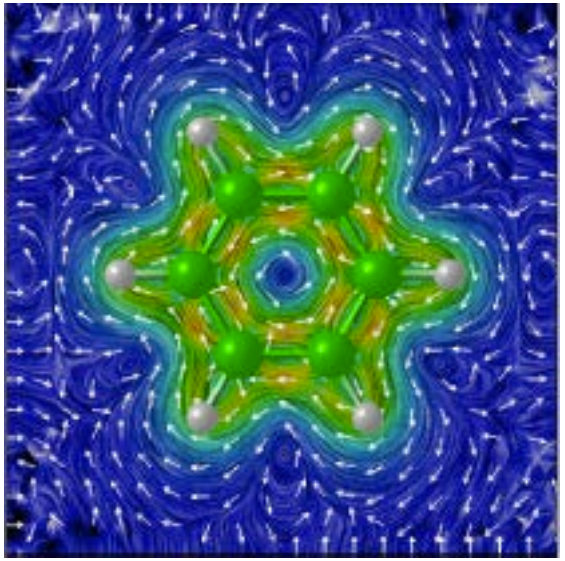




$$\nu_i = \frac{\gamma B_0 (1-\sigma_i)}{2\pi} \quad where \quad \sigma_i << 1$$

$$\delta_i = 10^6 \times \frac{(\nu_i - \nu_{réf\,(TMS)})}{\nu_0}$$

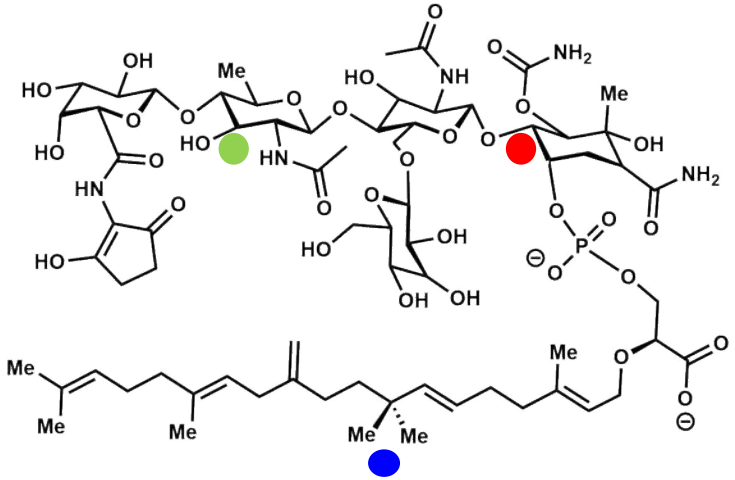


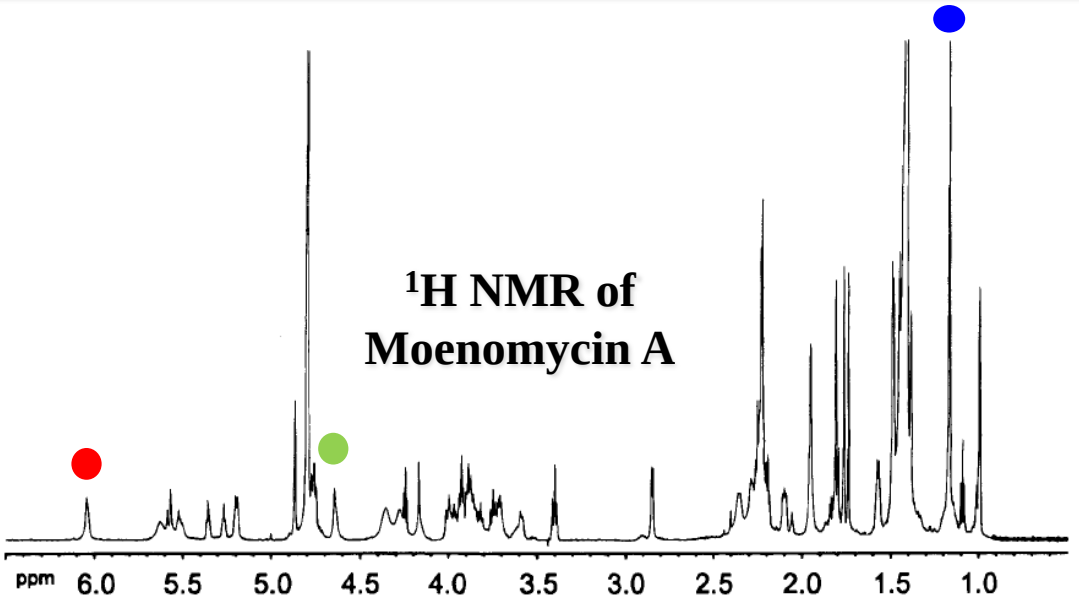


$$\sigma(\mathbf{R}) = -\frac{\partial \mathbf{B}^{ind}(\mathbf{R})}{\partial \mathbf{B}^{ext}} \ll 1$$

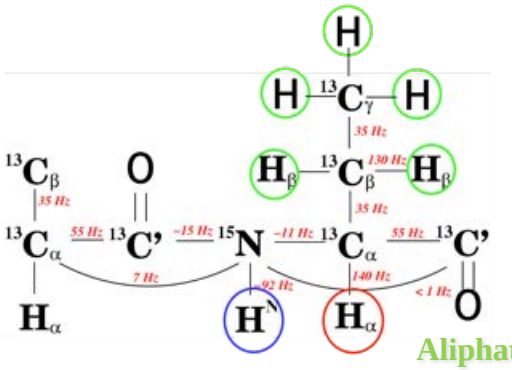
$$\delta(R) = \mathrm{Tr}\sigma^{TMS} - \mathrm{Tr}\sigma(R)$$

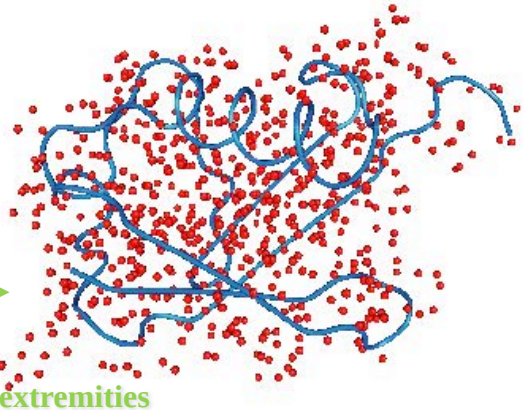
Chemical shift a good nucleus reporter

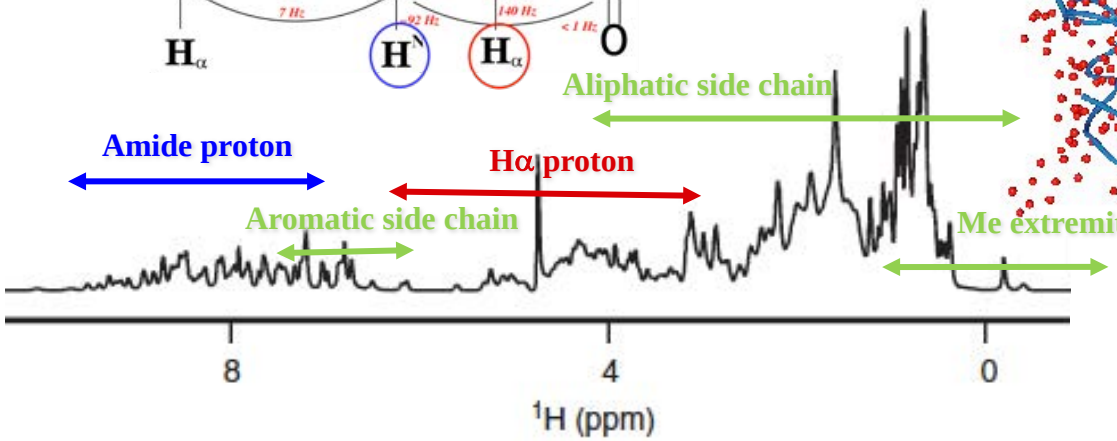




^{1}H NMR of Moenomycin A







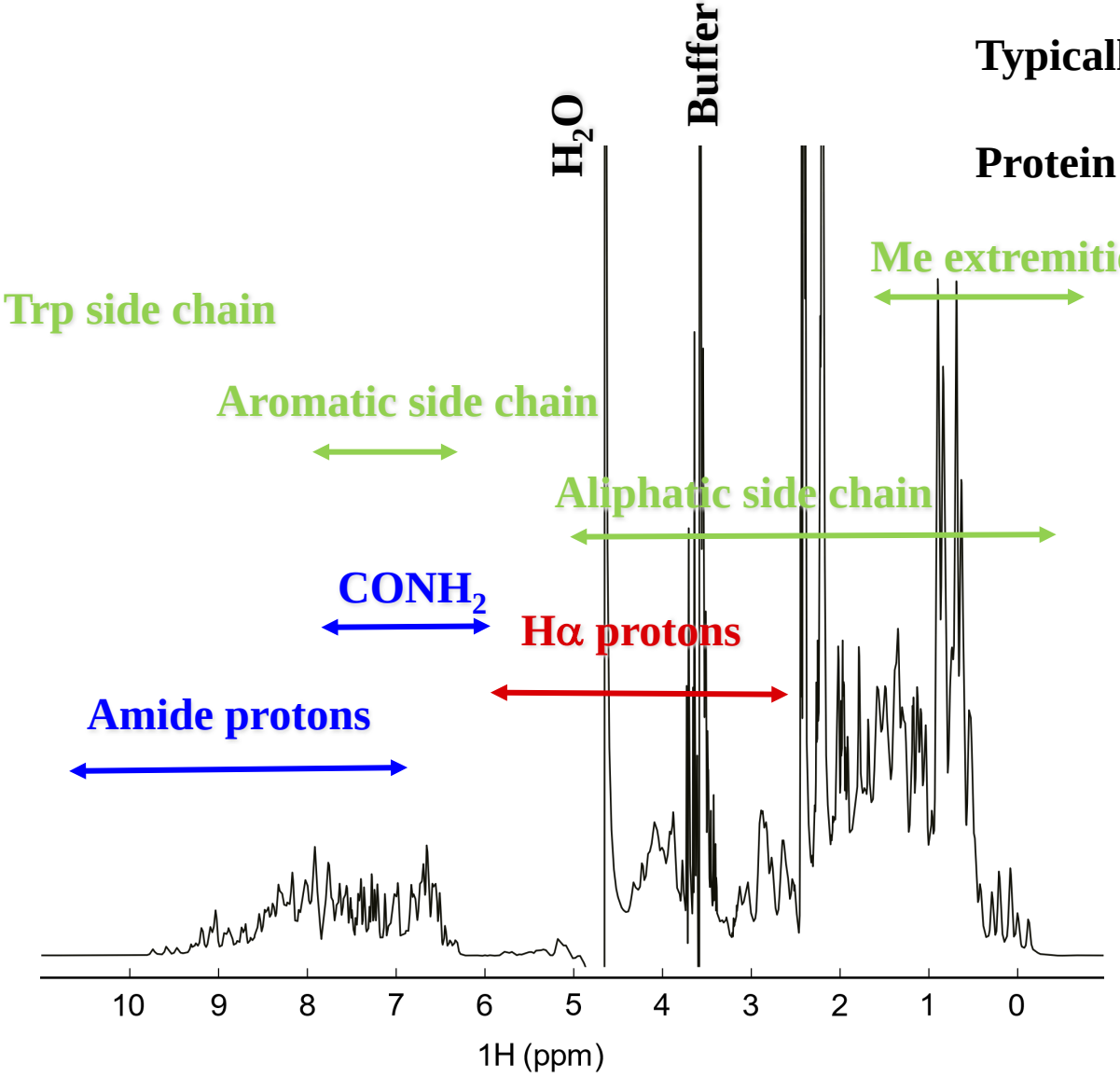
^{1}H NMR of protein

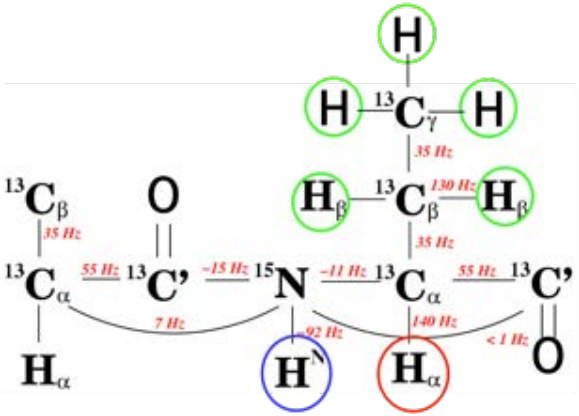
Take home message: Peak position reflects environment

Fingerprint of a protein samples (1D ^{1}H NMR)

Typically less than 10 minutes

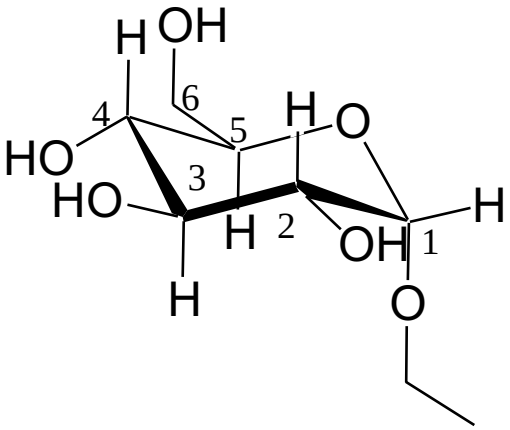
Protein concentration as low as few µM

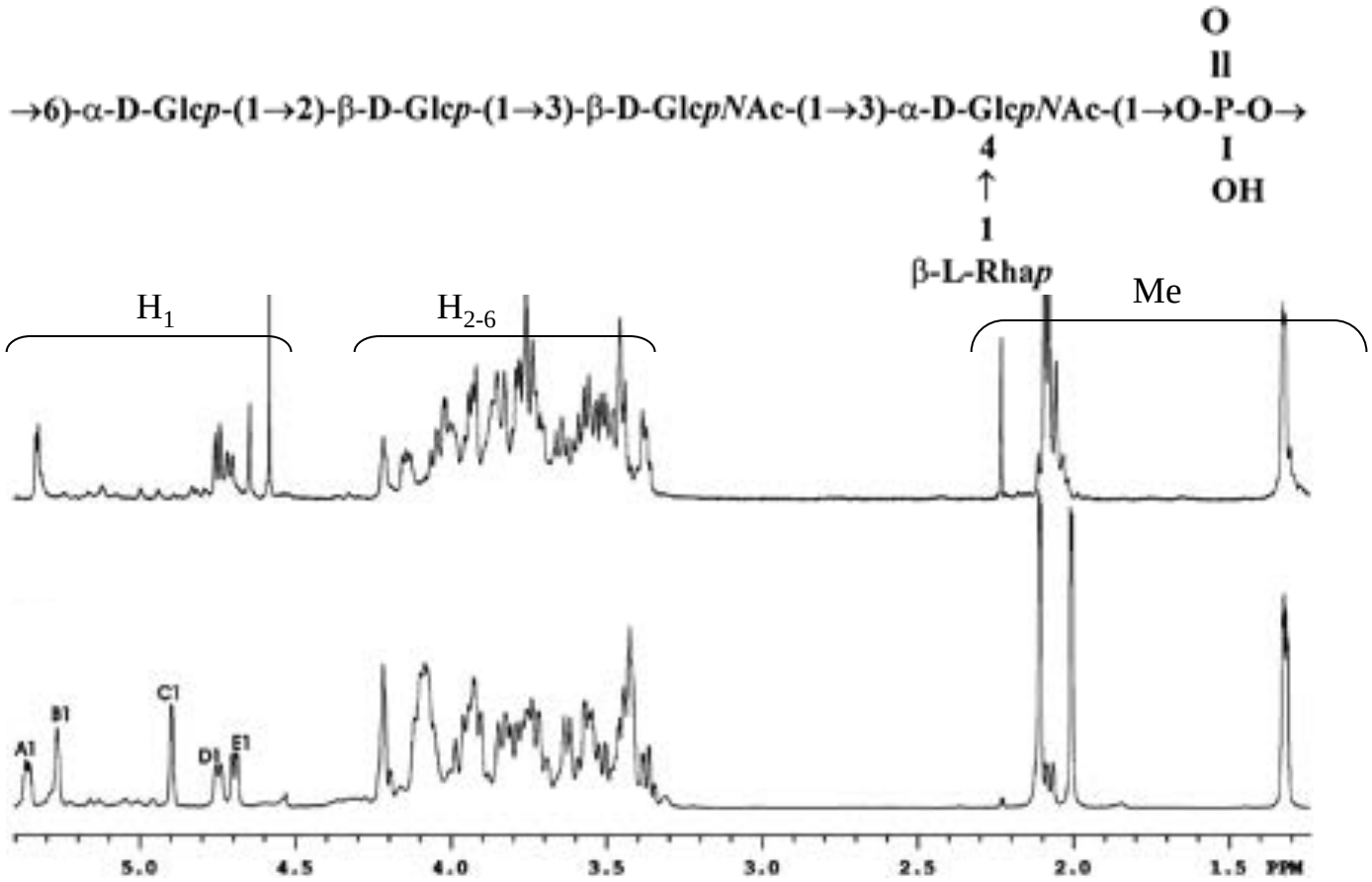




Credits A. Favier

Chemical shift: a finger print of the biomolecule

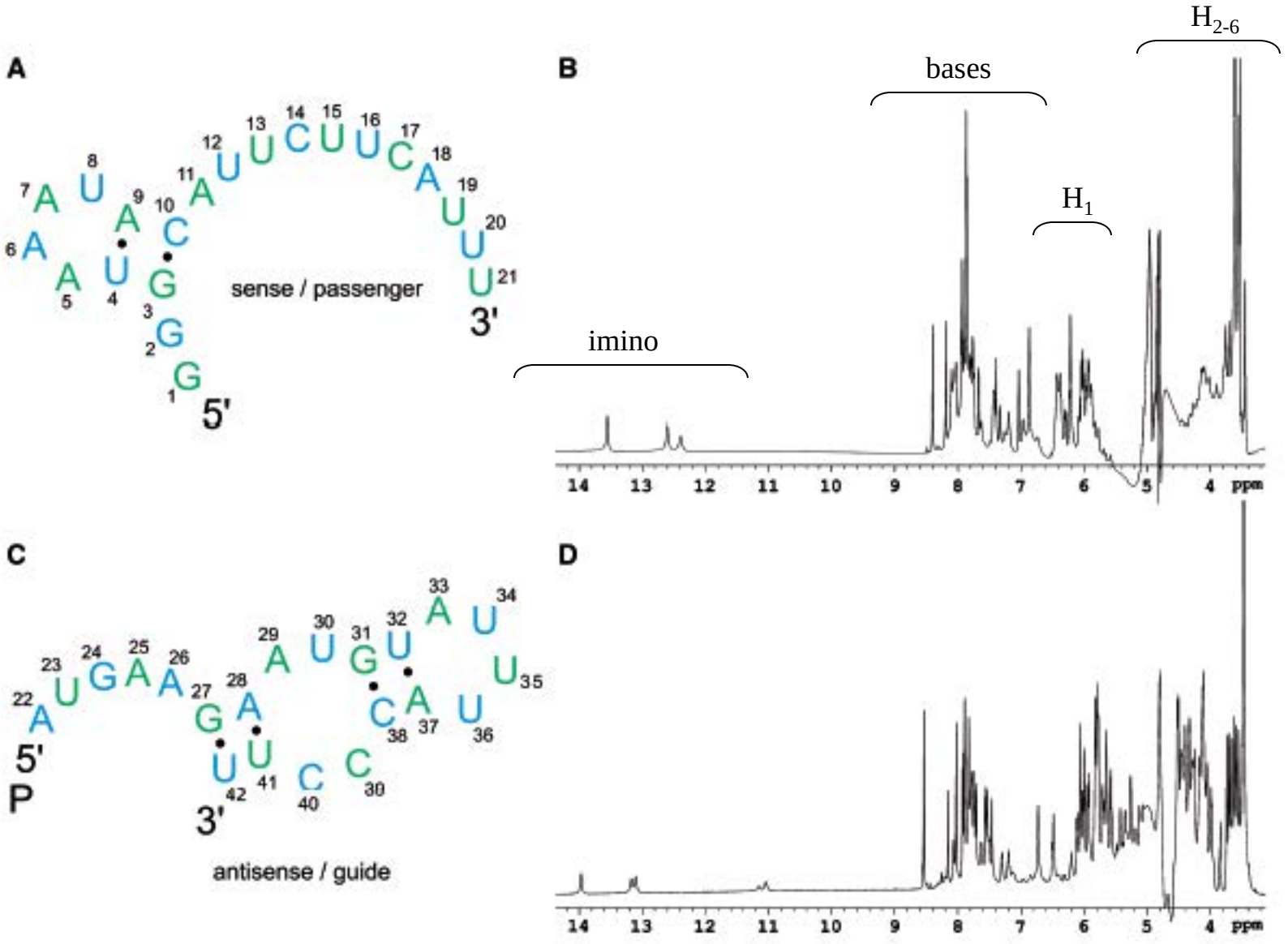




(A) Spectre ^{1}H-NMR (pD 7.8) de O-PS extrait de *C. rodentium*.
(B) Spectre ^{1}H-NMR du derivé de l'oligosaccharide **II** de *C. rodentium* (ATCC 51459)

Eur. J. Biochem. **268**, 5740-5746 (2001)

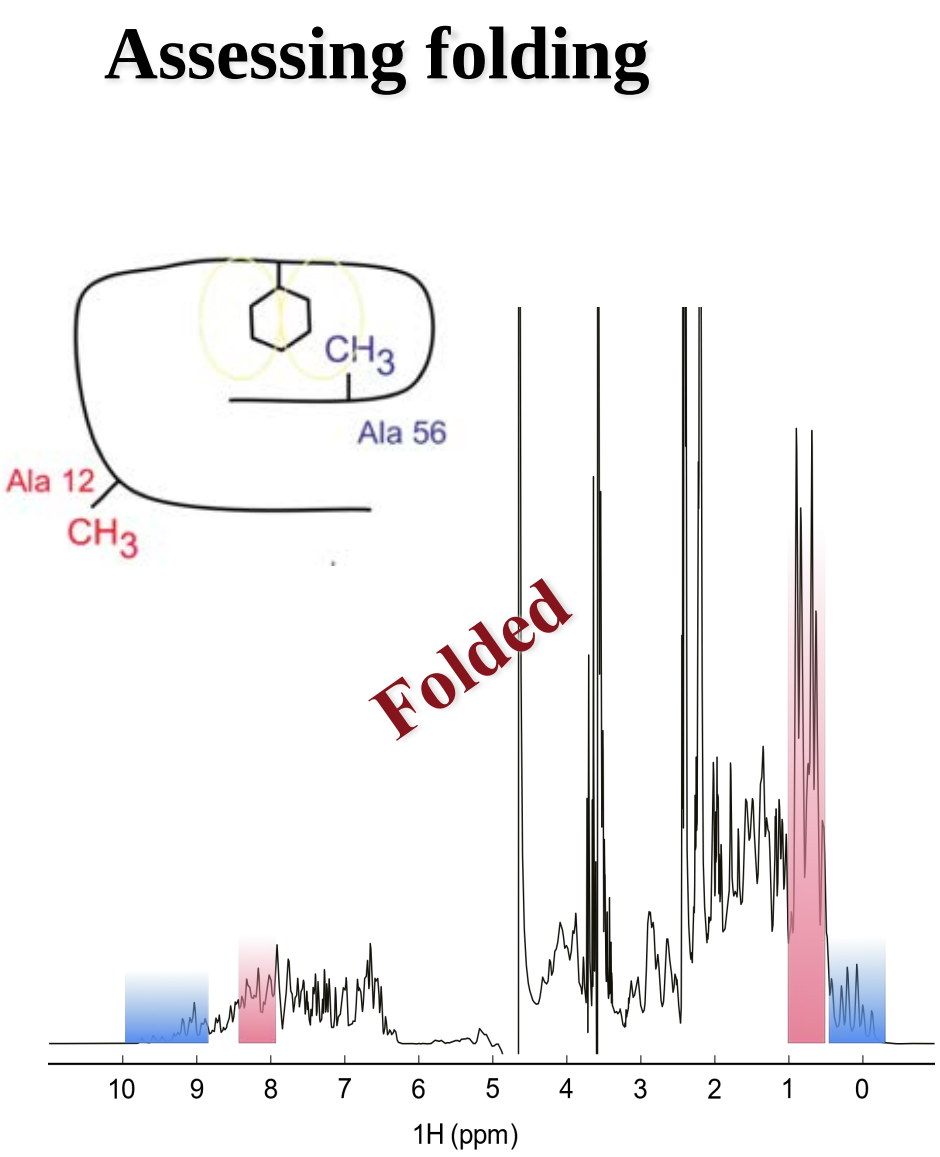
Chemical shift: a finger print of the biomolecule

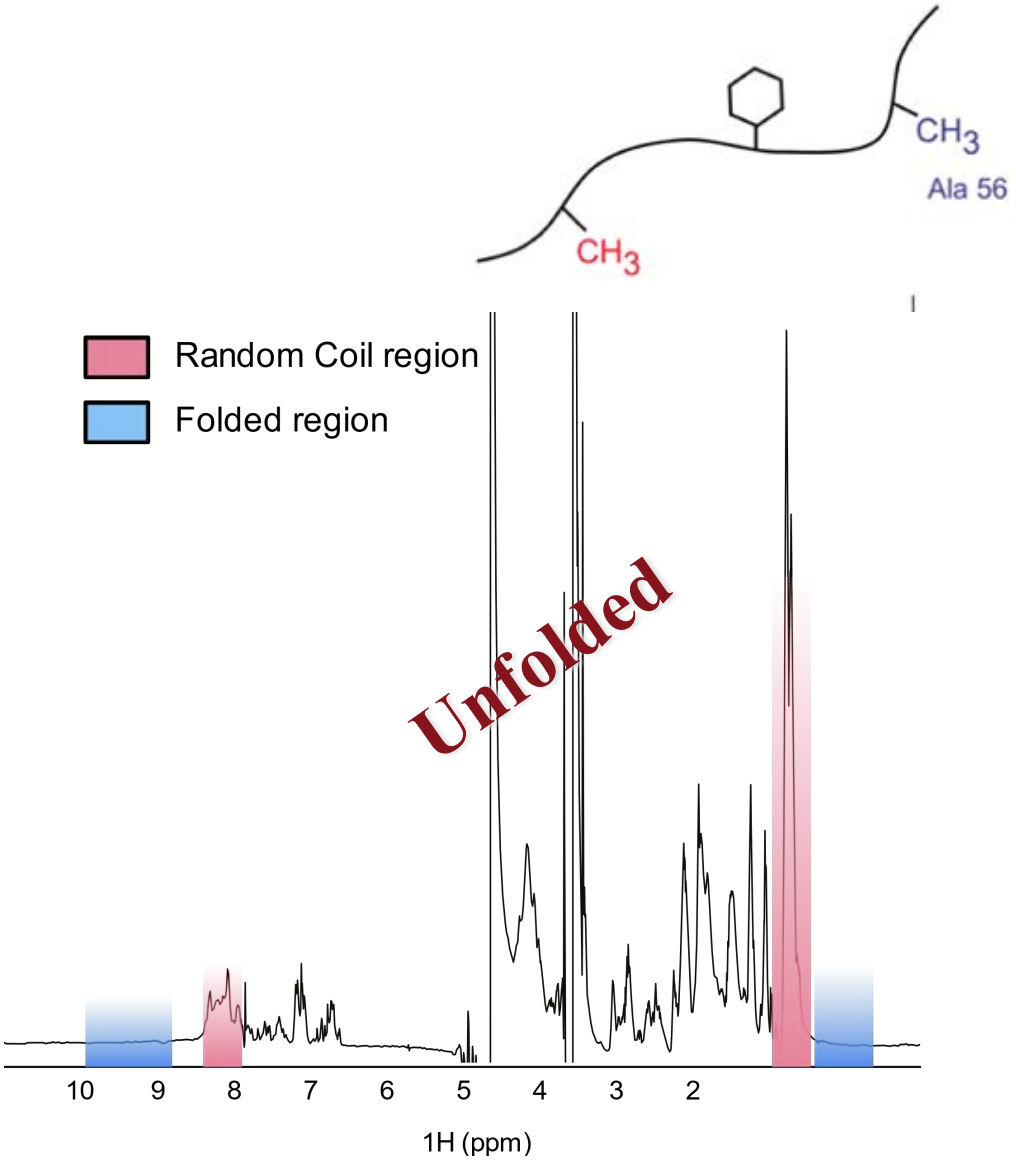


P. Podbevsek, C. R. Allerson, B. Bhat, J Plavec, Nucl. Acids Res., 2010, 7298-7307

Application: Quality control of protein samples using ^{1}H NMR

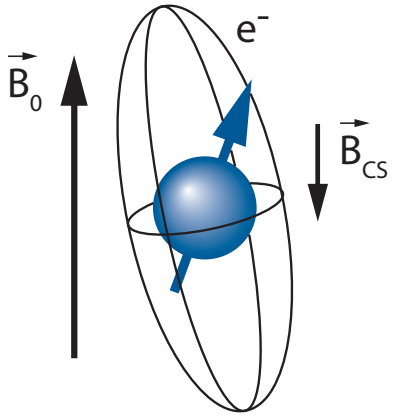
Assessing folding





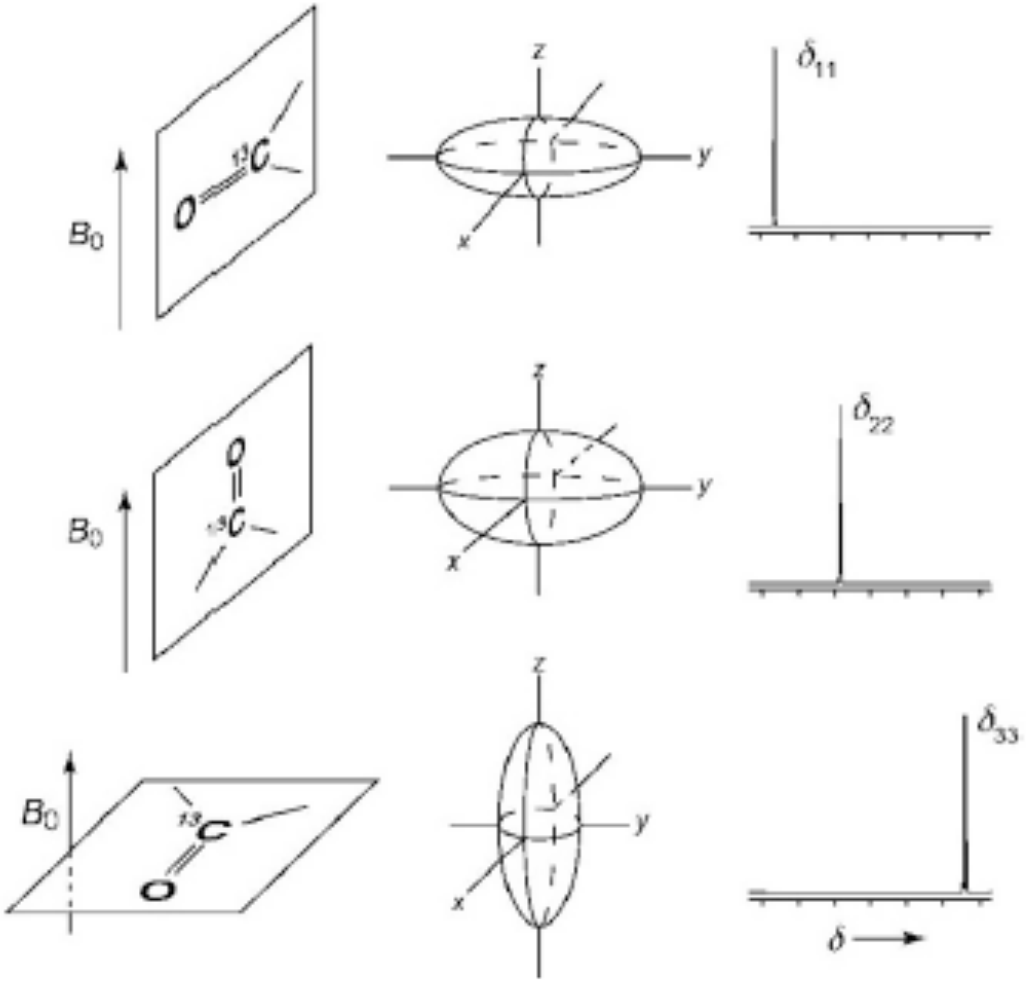
Credits A. Favier

Chemical shift is orientation dependent



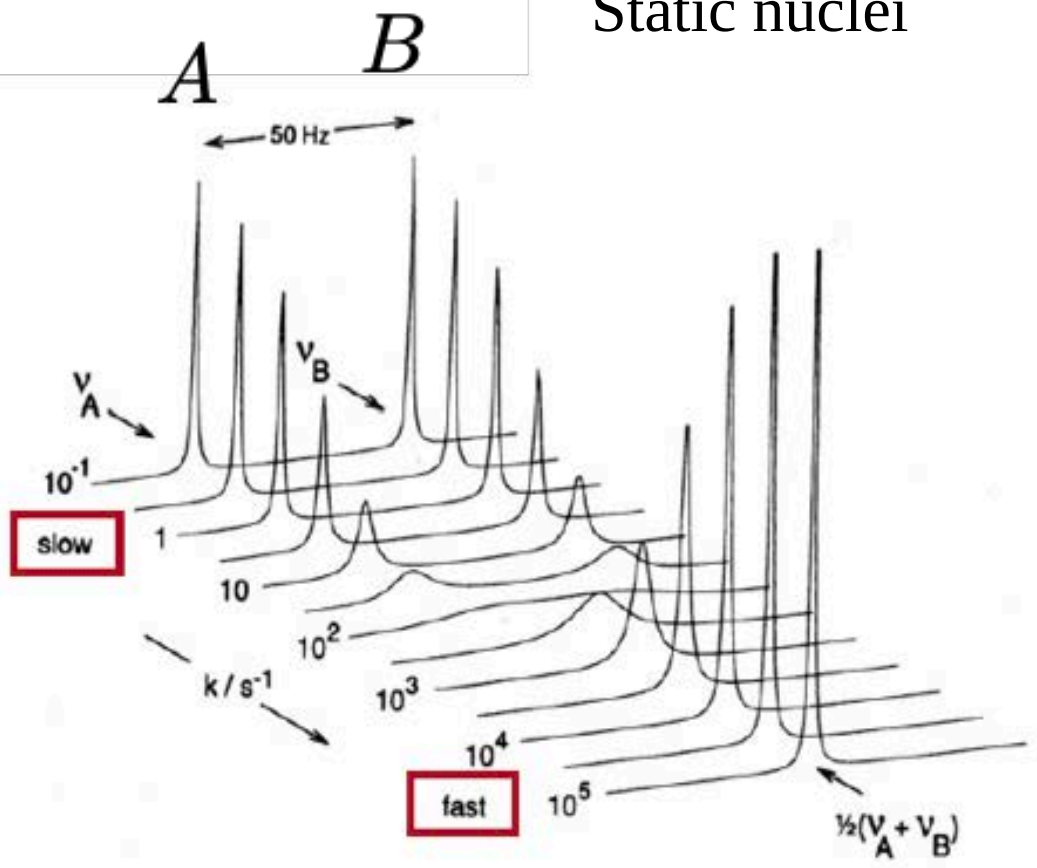
$$\hat{H}_{\mathrm{CS}} = -\gamma\, \hat{\boldsymbol{I}}\, \boldsymbol{\sigma}\, \boldsymbol{B}_0$$

Non symmetrical electronic distribution

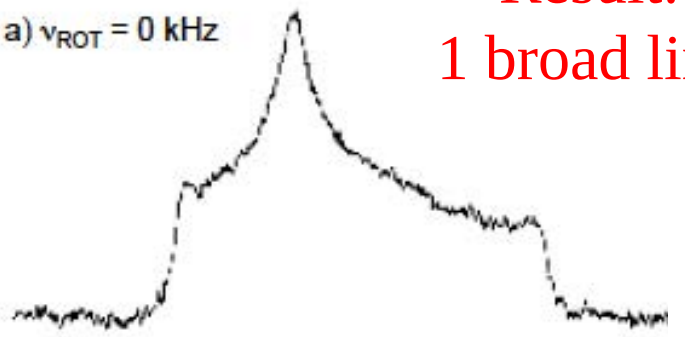


Chemical shift in liquids

In solids
Static nuclei

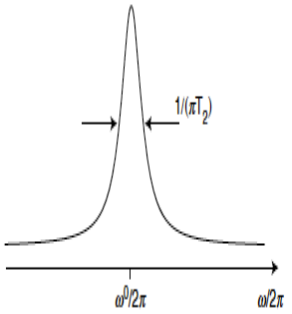


Trick to recover narrow lines in solids? => See Robert's talk



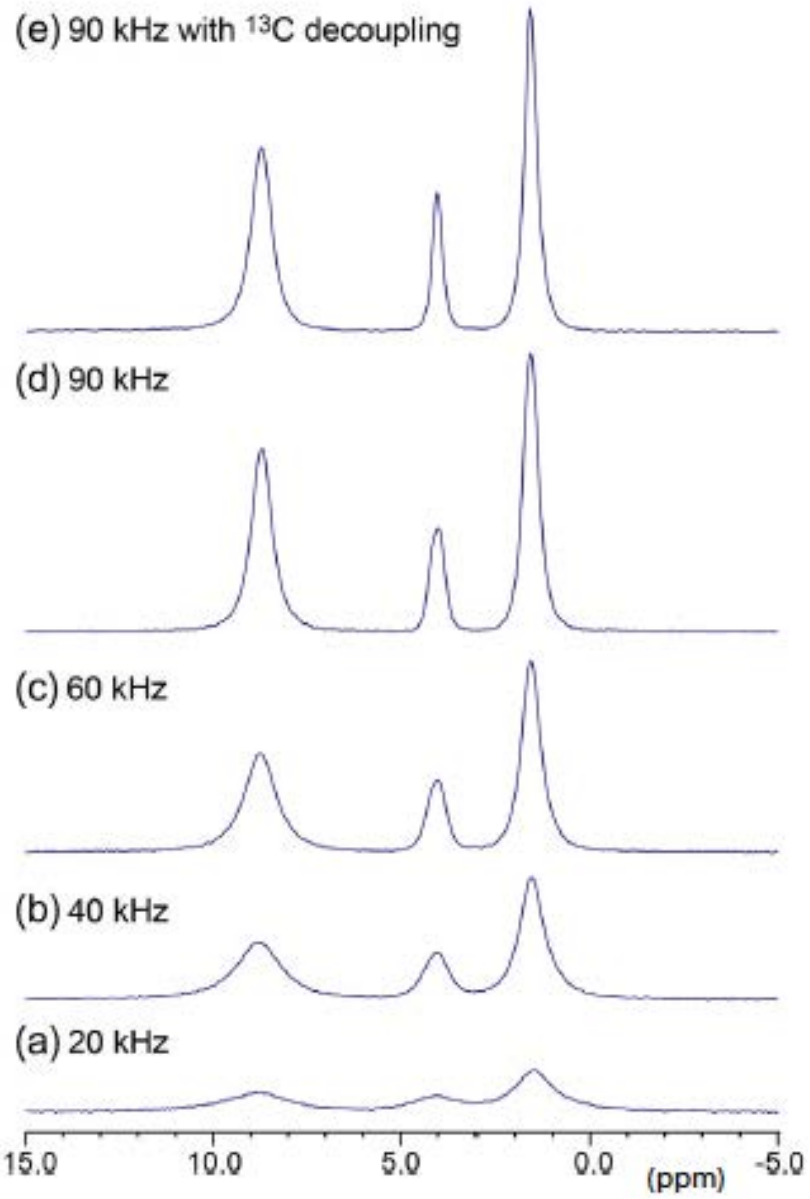
Result:
1 broad line

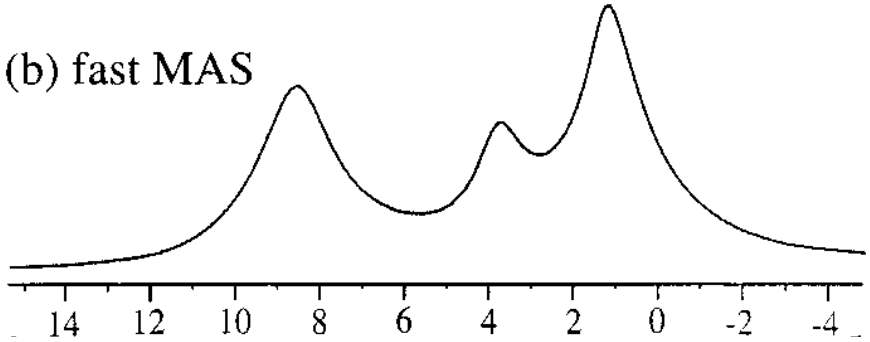
In solution
Fast molecular tumbling
Time and molecule averaging

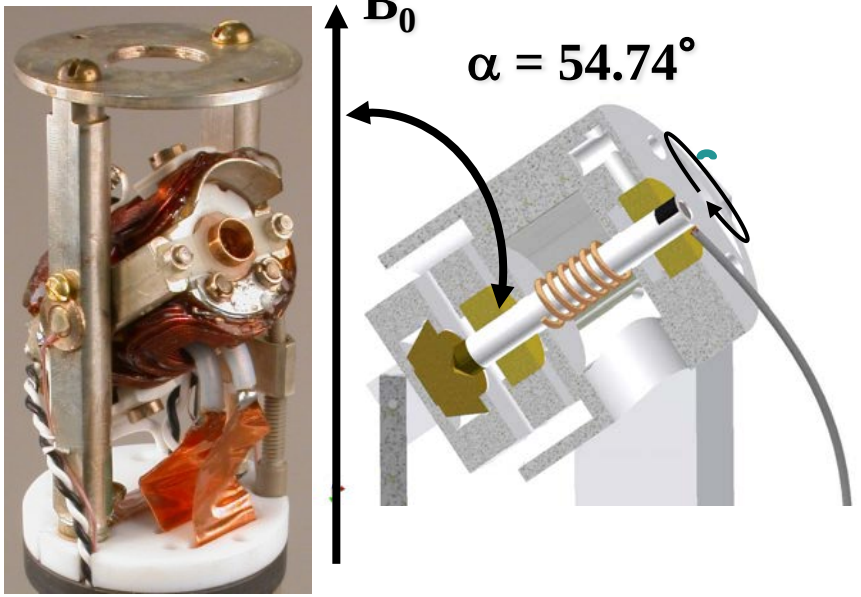


Result:
1 narrow line

Liquid-state *vs* solid-state NMR, some basic principles





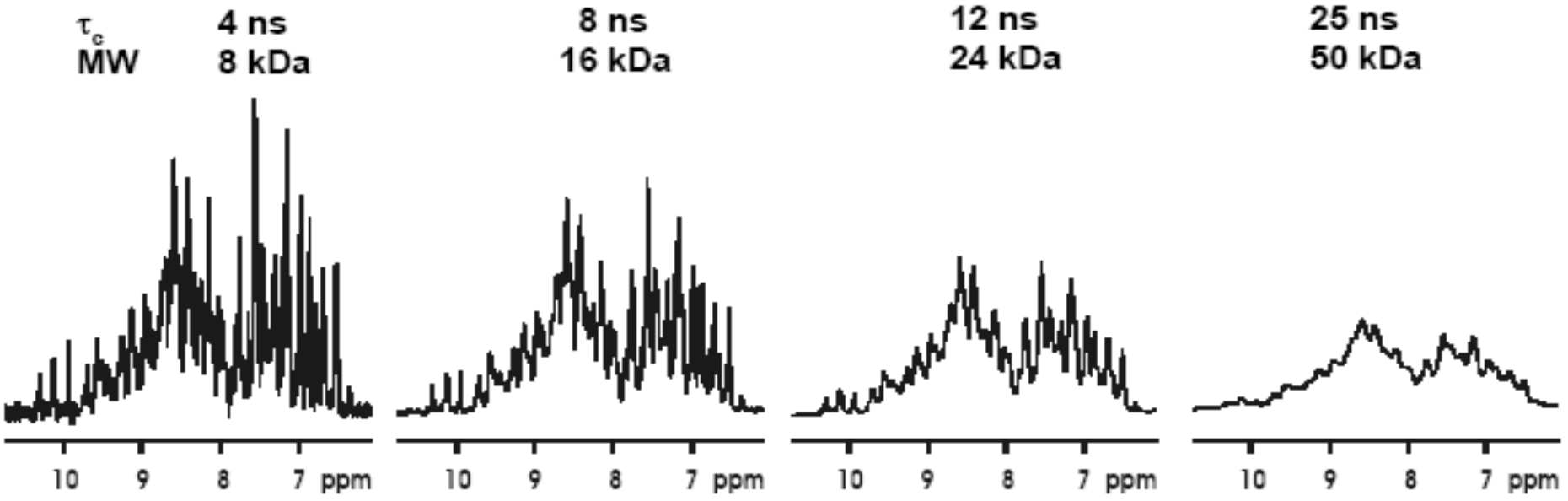


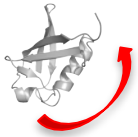
MAS solid-state NMR

The impact of molecular weight in liquids

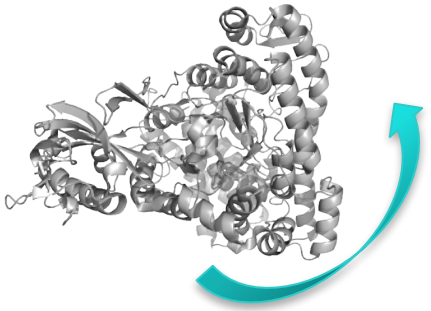
Liquid-state NMR a serious limit?

Linewidth





fast overall rotation



slow overall rotation

Chemical shift: a structural information content

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

Protein NMR chemical shifts are highly sensitive to local structure. A robust protocol is described that exploits this relation for *de novo* protein structure generation, using as input experimental parameters the $^{13}C^{\alpha}$, $^{13}C^{\beta}$, $^{13}C'$, ^{15}N, $^{1}H^{\alpha}$ and $^{1}H^{N}$ NMR chemical shifts. These shifts are generally available at the early stage of the traditional NMR structure determination process, before the collection and analysis of structural restraints. The chemical shift based structure determination protocol uses an empirically optimized procedure to select protein fragments from the Protein Data Bank, in conjunction with the standard ROSETTA Monte Carlo assembly and relaxation methods. Evaluation of 16 proteins, varying in size from 56 to 129 residues, yielded full-atom models that have 0.7–1.8 Å root mean square deviations for the backbone atoms relative to the experimentally determined x-ray or NMR structures. The strategy also has been successfully applied in a blind manner to nine protein targets with molecular masses up to 15.4 kDa, whose conventional NMR structure determination was conducted in parallel by the Northeast Structural Genomics Consortium. This protocol potentially provides a new direction for high-throughput NMR structure determination.

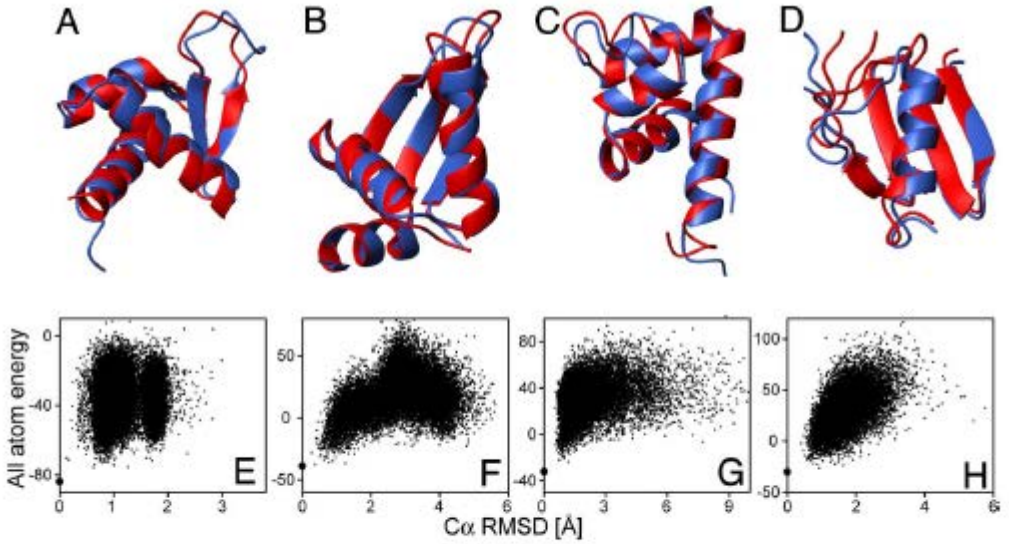
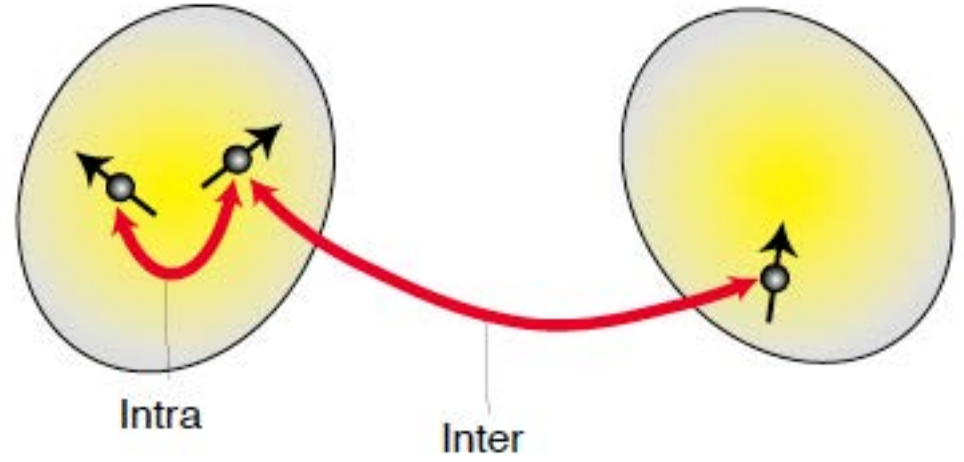
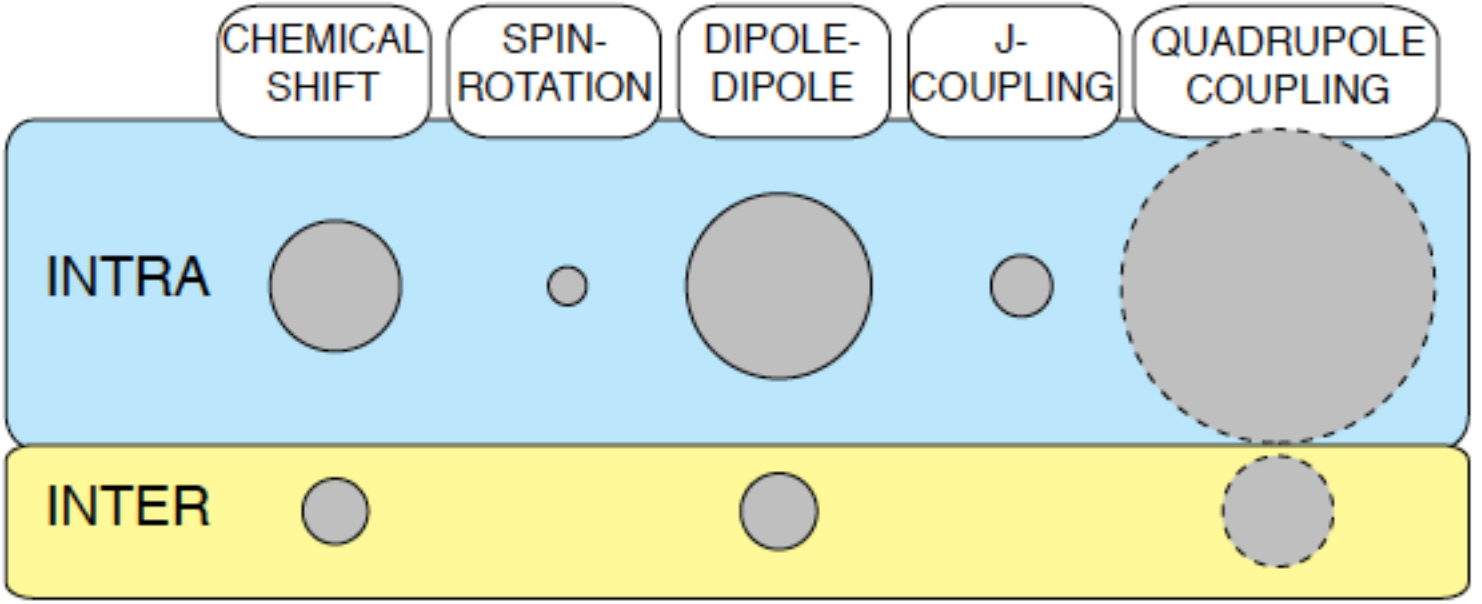


Fig. 4. Results from blind CS-ROSETTA structure generation for four structural genomics targets (Table 2). The remaining five are in SI Fig. 12. (*A–D*) Superposition of lowest-energy CS-ROSETTA models (red) with experimental NMR structures (blue), with superposition optimized for ordered residues, as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

No the end yet… many more interactions

$$H = H_z + H_{cs} + H_{rf} + H_J + H_D + H_Q$$

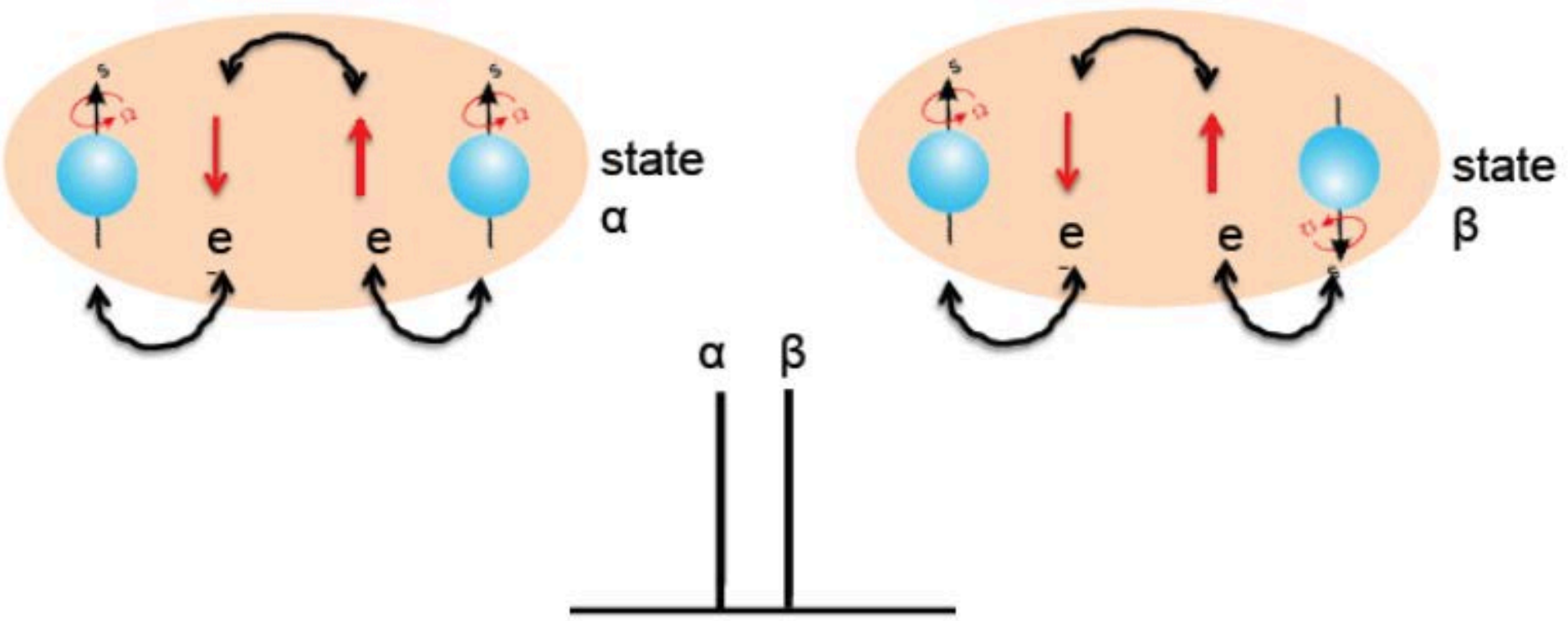




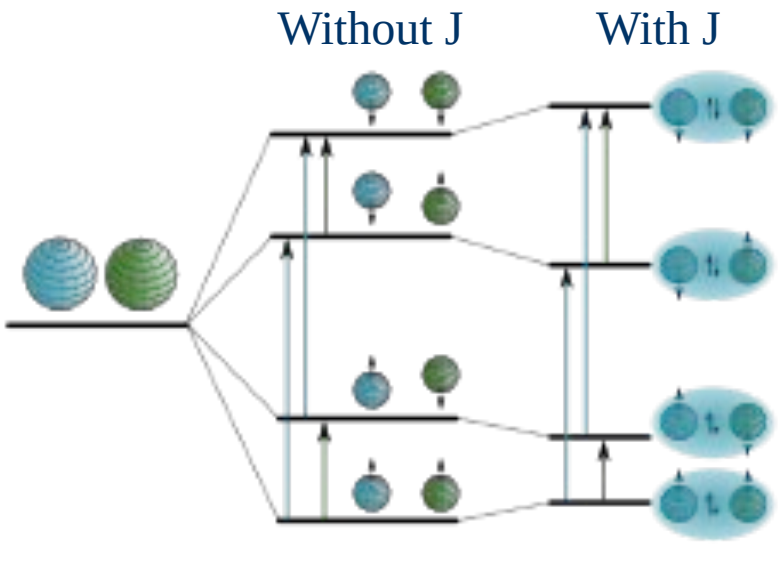
Scalar coupling, a spectral complication

$$\hat{\mathcal{H}}_{spin} = \hat{\mathcal{H}}_{J}$$

- Interaction mediated by electrons
- Depends on molecular orbital overlap
- Linked to molecular topology



Scalar coupling, a spectral complication

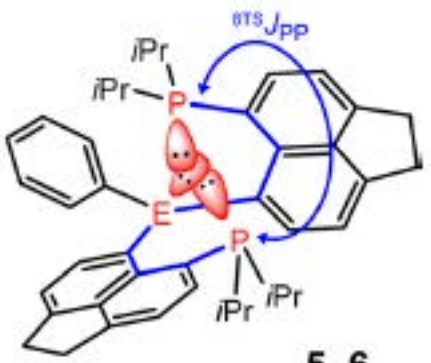


$$+\frac{\nu_I}{2}+\frac{\nu_S}{2}+\frac{J}{4}$$

$$+\frac{\nu_I}{2}-\frac{\nu_S}{2}-\frac{J}{4}$$

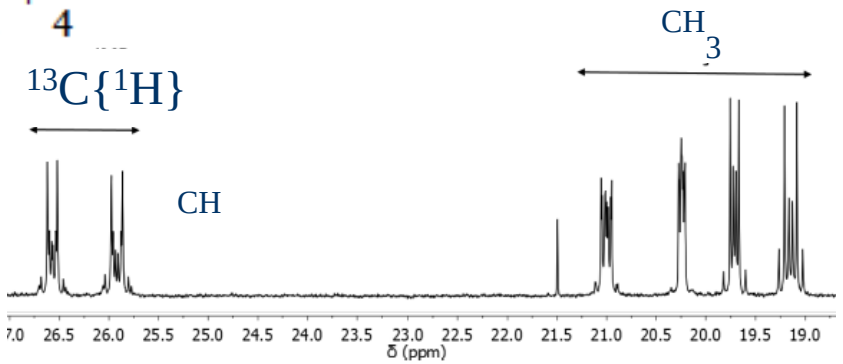
$$-\frac{\nu_I}{2}+\frac{\nu_S}{2}-\frac{J}{4}$$

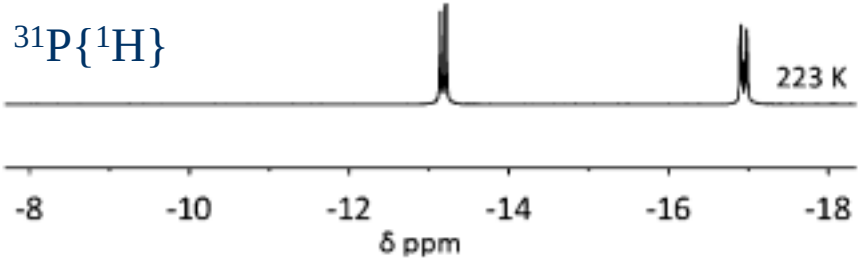
$$-\frac{\nu_I}{2}-\frac{\nu_S}{2}+\frac{J}{4}$$



5, 6

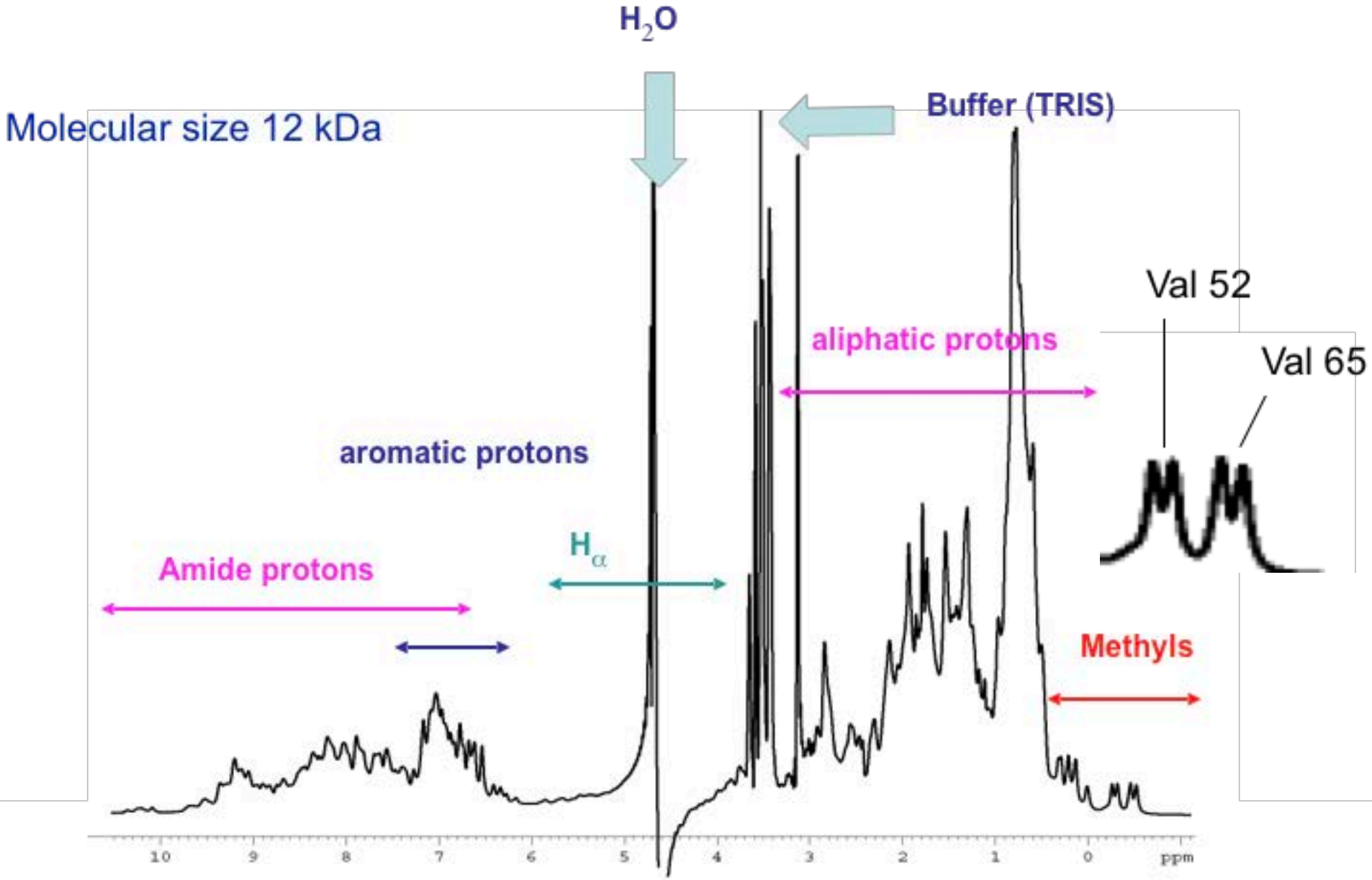
E= As, Sb





Inorg. Chem. 2016, 55, 7117−7125

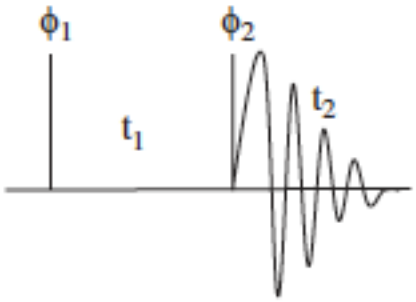
Scalar coupling, a spectral complication



Scalar coupling, a key benefit for coherence transfer

COSY experiment, a homonuclear coherence transfer and an entry to multidimensional NMR

J scalar coupling

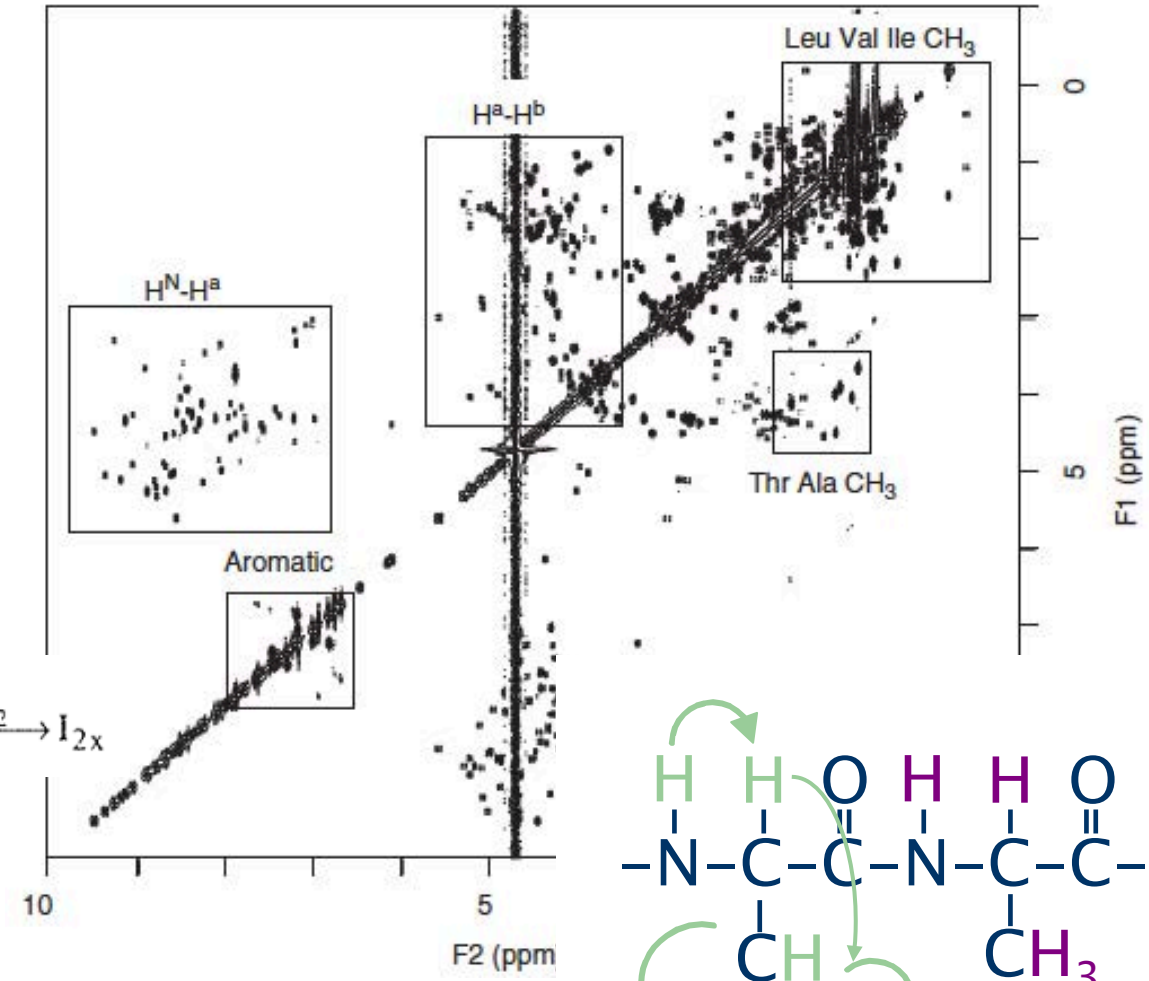


Coherence transfer – cross-peak

$$I_{1z} \xrightarrow{90_y} I_{1x} \xrightarrow[J]{t_1} 2I_{1x}I_{2z} \xrightarrow{90_y} -2I_{1z}I_{2x} \xrightarrow{t_2} I_{2x}$$

Coherence transfer – auto-peak

$$I_{1z} \xrightarrow{90_y} I_{1x} \xrightarrow[J]{t_1} I_{1y} \xrightarrow{90_y} I_{1y} \xrightarrow{t_2} I_{1x}$$

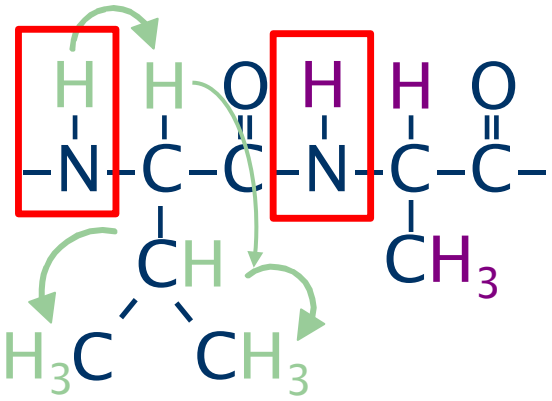


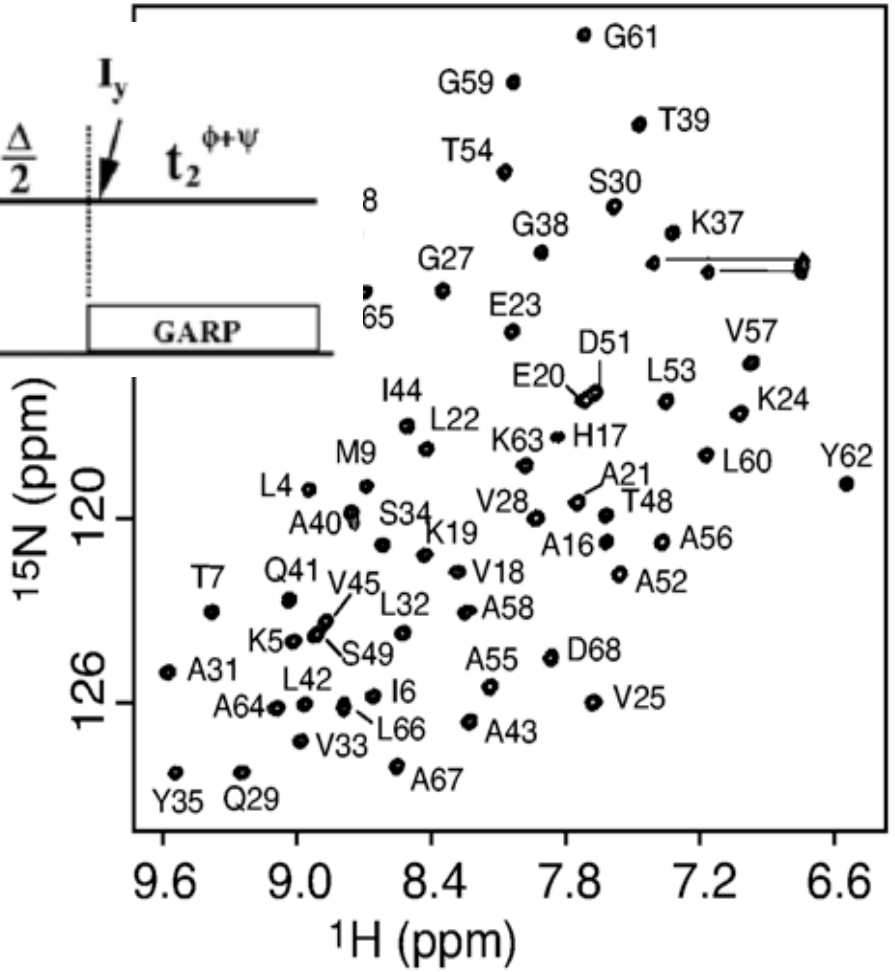
Scalar coupling, a key benefit for coherence transfer

Not limited to homonuclear,
A key experiment ^{15}N-HSQC (or ^{13}C-HSQC)

The sequence

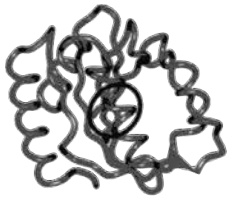
$\phi = x\ -x$
$\psi = x\ \ x\ -x\ -x$
$\phi_{rec} = x\ -x\ -x\ \ x$

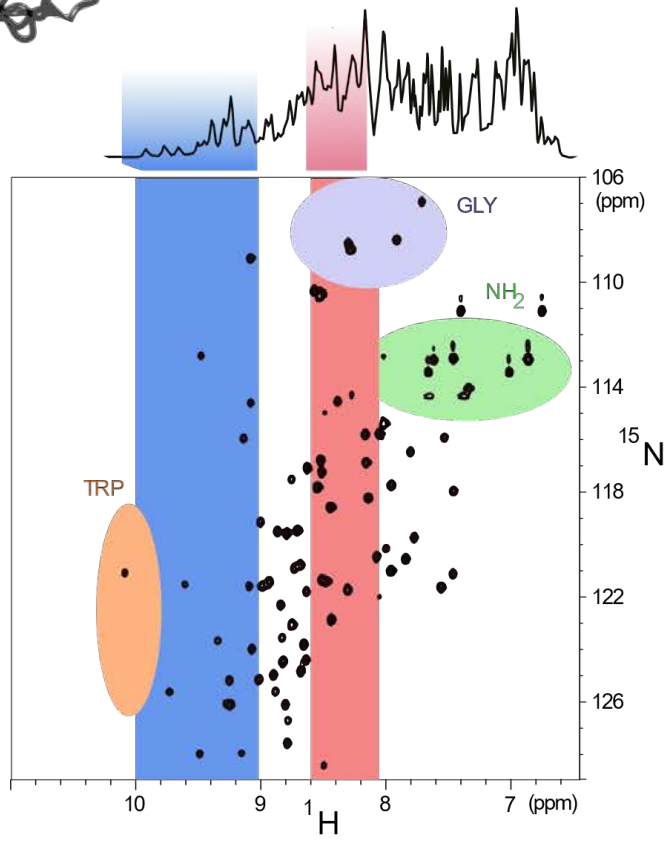


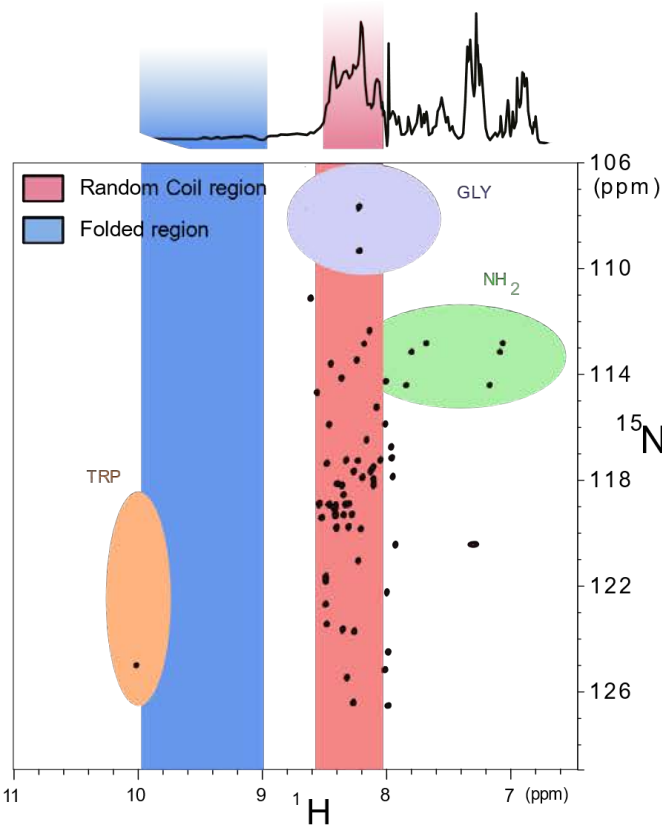


^{1}H-^{15}N correlation: 1 peak = 1 aminoacid

Assessing folding



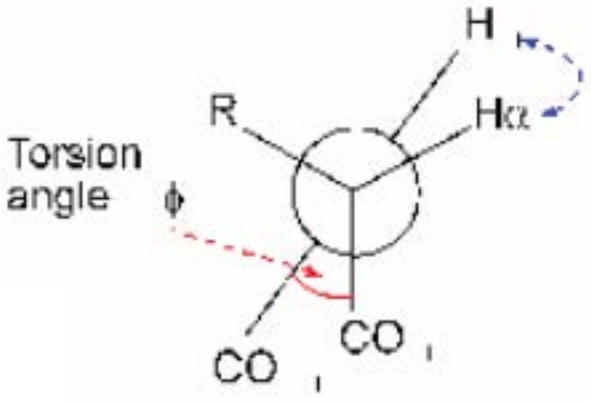


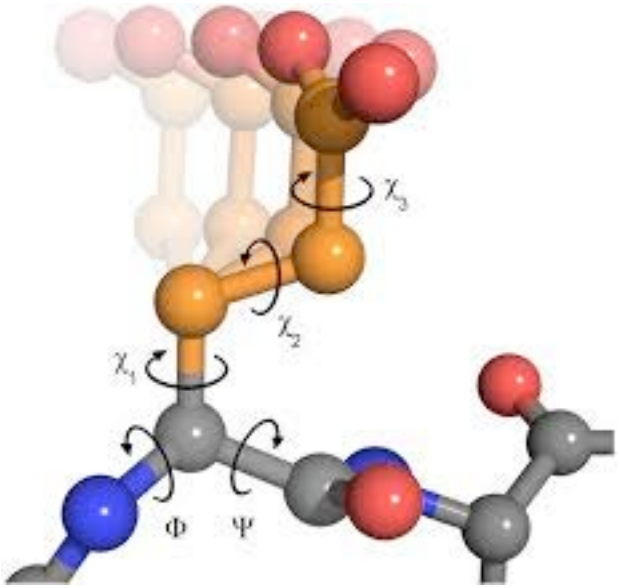


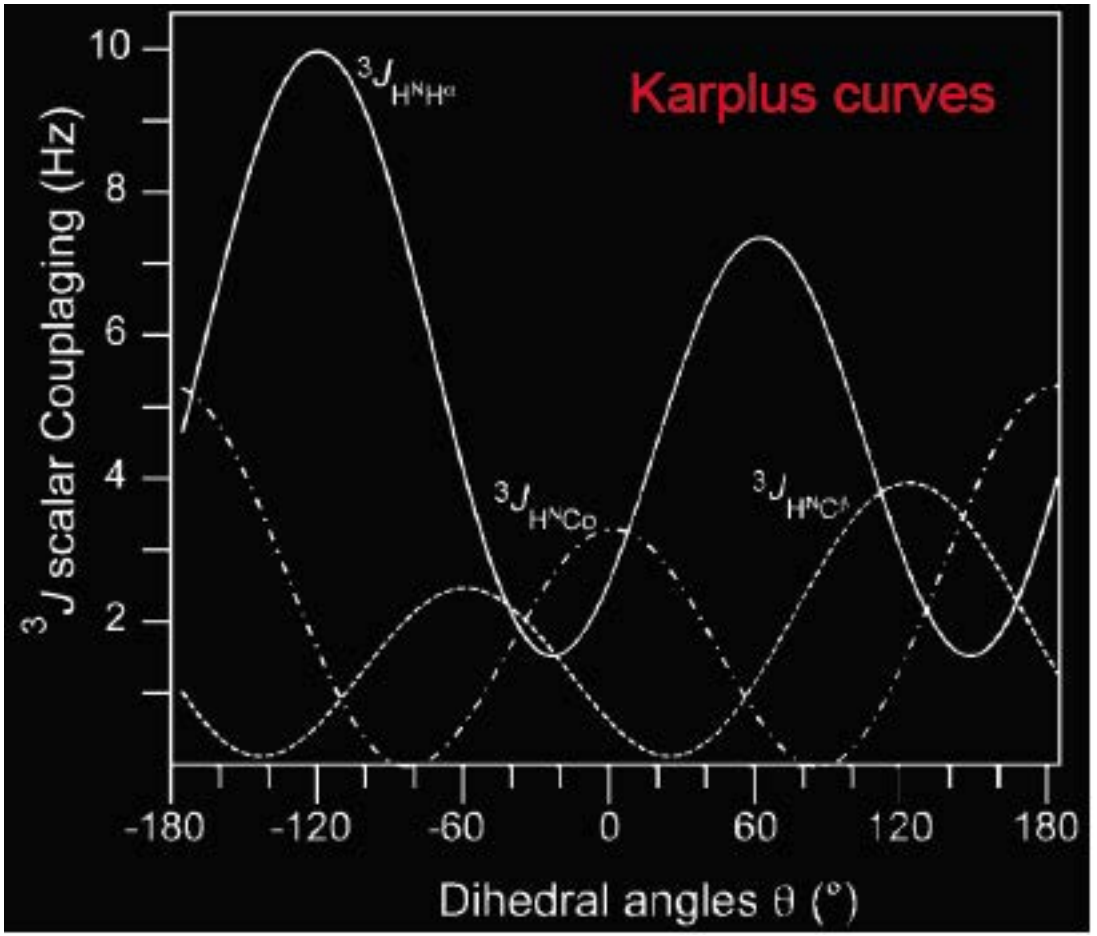
~ less than 1 hour
But requires isotopic labeling

Credits A. Favier

Scalar couplings: a structural information content







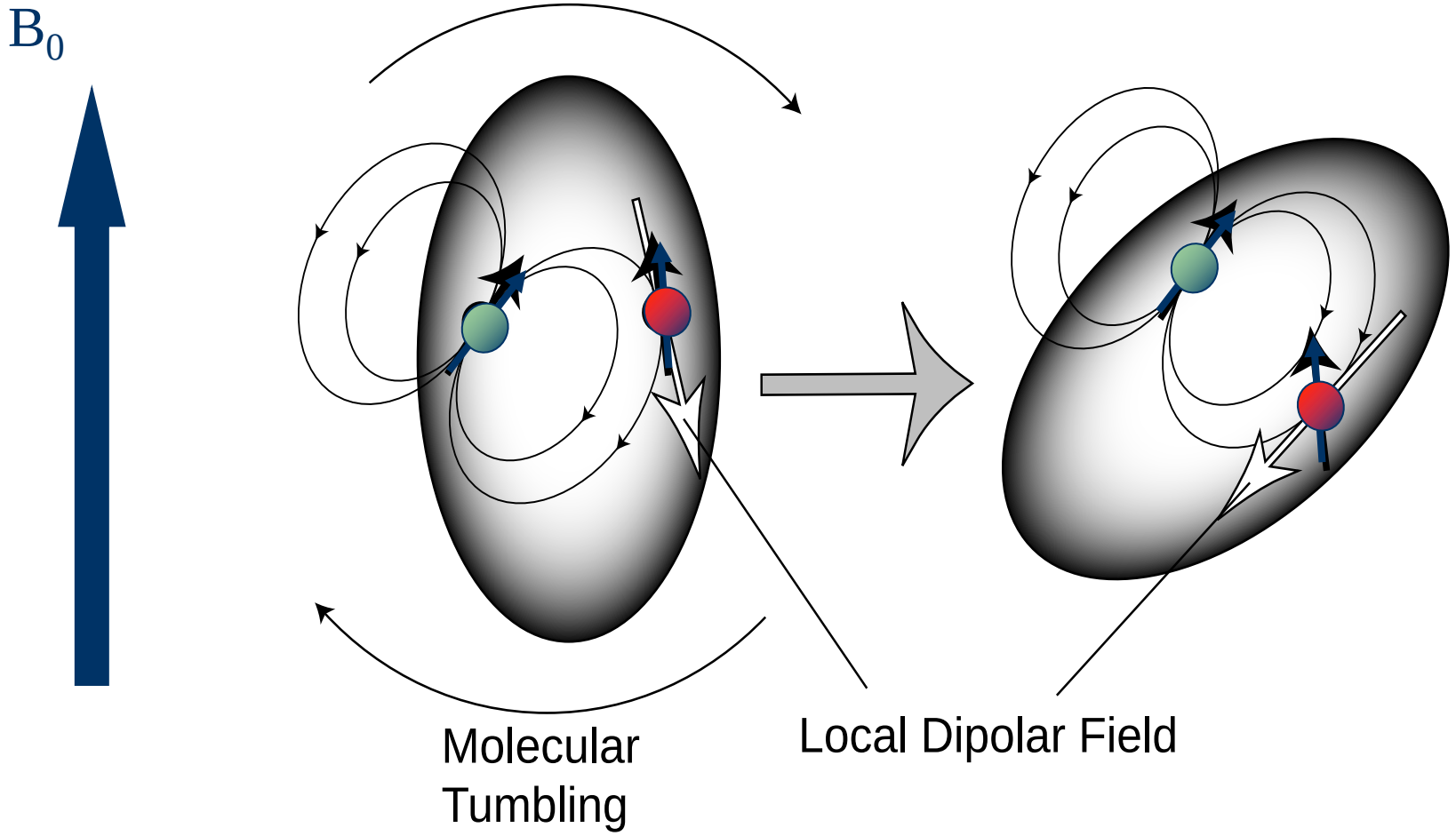
$$^3J_{Ha\text{-}Hb} = 6.98\cos^2(\phi\text{-}60) - 1.38\cos(\phi\text{-}60) + 1.72$$

J. Wang, A.Bax, J. Am. Chem. Soc., **1996**, 118, 2492

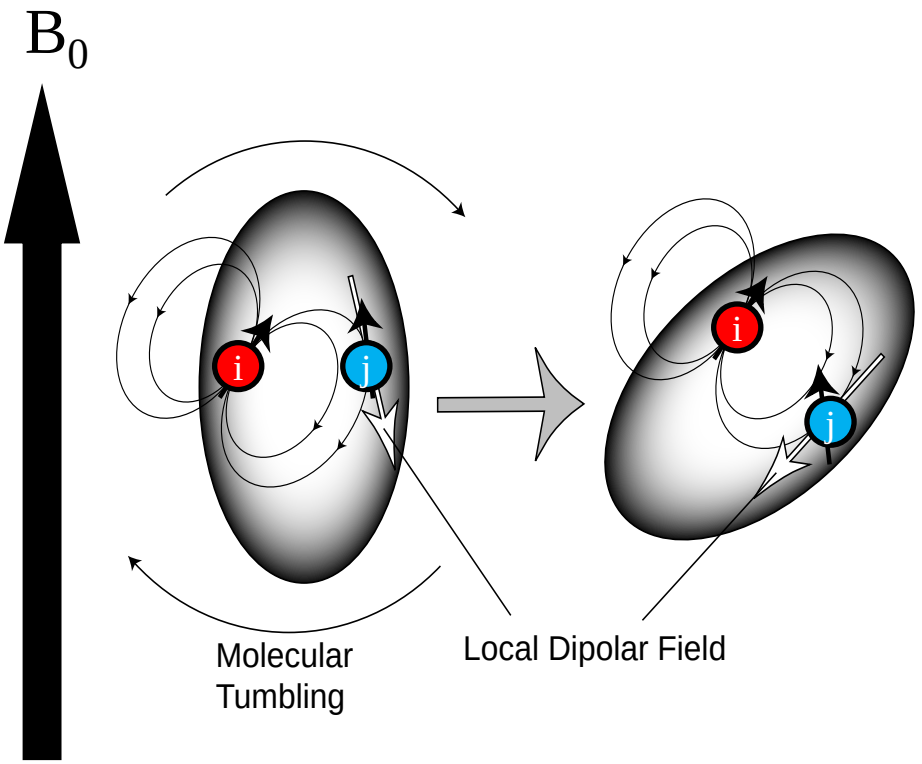
Dipolar interaction, a through-space interaction

$$\hat{\mathcal{H}}_{spin} = \hat{\mathcal{H}}_{DIP} = -\frac{\mu_0 \hbar^2}{4\pi} \sum_{i>j} \frac{\gamma_i \gamma_j}{r_{ij}^3} \left[3(\hat{I}_j . \vec{e}_{ij})(\hat{I}_i . \vec{e}_{ij}) - \hat{I}_j . \hat{I}_i \right]$$

$$\hat{H}_{\mathrm{D}},\ \hat{H}_{\mathrm{CSA}} \propto (3\cos^2\theta - 1)$$



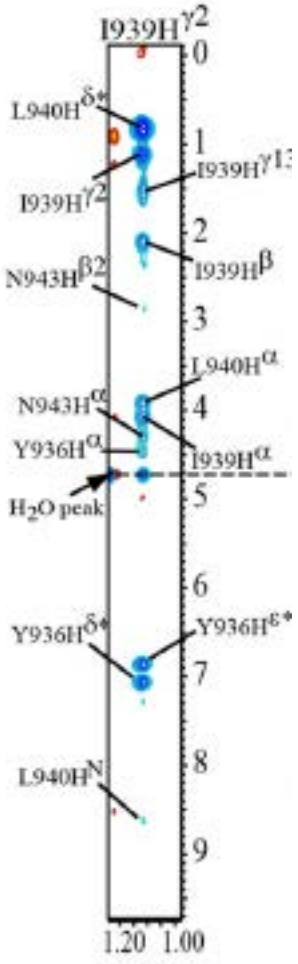
Dipolar interaction in liquids



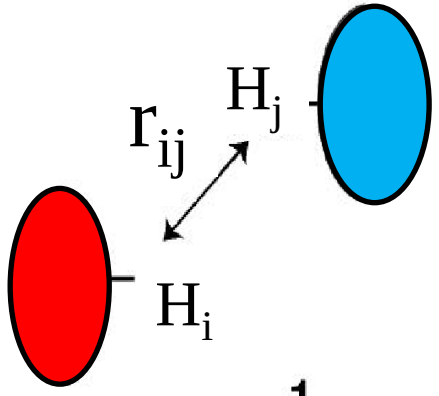
$$d_{ij} = -\frac{\mu_0 \gamma_i \gamma_j \hbar}{8\pi^2} \left\langle \frac{1}{2}\left(3\cos^2\theta_{ij} - 1\right) r_{ij}^{-3} \right\rangle_\theta$$

Dipolar interaction

Averaged to ZERO, not detected in spectra



Detection of dipolar interaction in NOESY spectra



$$NOE \propto \frac{1}{(r_{ij})^6}$$

Peak volume yield distance information

Dipolar interaction: a structural information content

RNA **Overhauser effect**

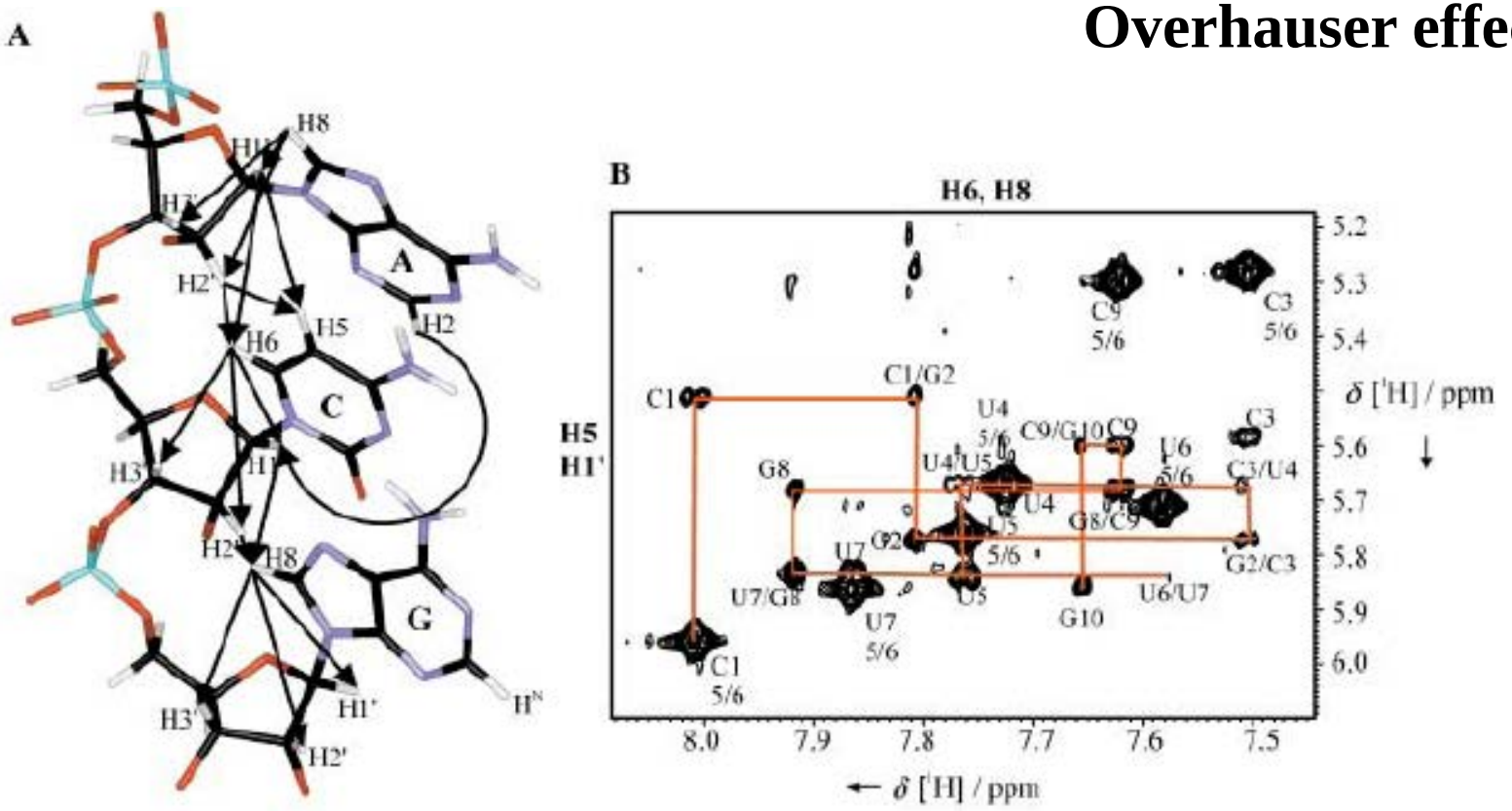
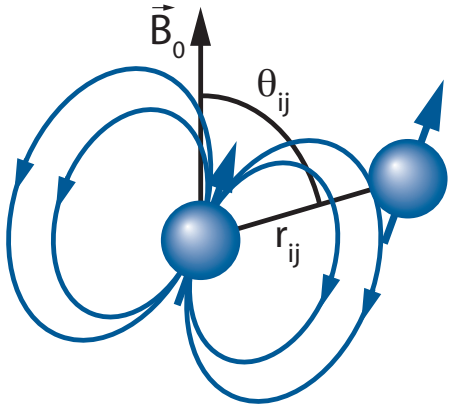
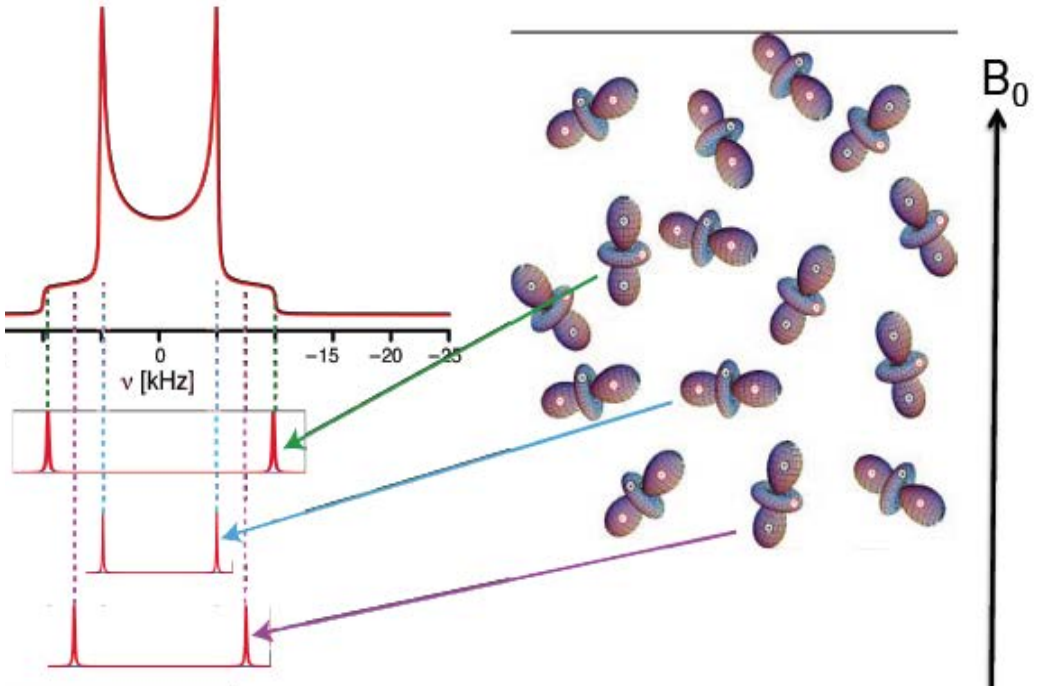


Figure 17. A) Schematic representation of the sequential assignment strategy in helical A-form RNA for nonexchangeable protons. The arrows show the intraresidual NOE connectivities between the aromatic and the sugar protons H1' – H3' and the sequential NOE correlation between the H3' – H6, H8 protons and the H5 – H1' protons. The sequential assignment of the helical A-form conformation is possible by determination of these NOE cross-peaks. In addition to the exchangeable protons, only the intercatenar NOE interactions between the adenine H2 and H1' of the corresponding RNA strand give information about the helical conformation. B) An example for the NOESY assignment procedure shown for the cUUUUg loop RNA. The NOESY spectrum was recorded in D_2O at 600 MHz and the mixing time was 300 ms. Annotation by using two residues indicates connectivities due to sequential NOE contacts and annotation with one nucleotide indicates intraresidual NOE interactions.

B. Furtig, C. Richter, J. Wohnert and H. Schwalbe
ChemBioChem, **2003**, 4, 936 - 962

Dipolar interaction in solids, a different perspective



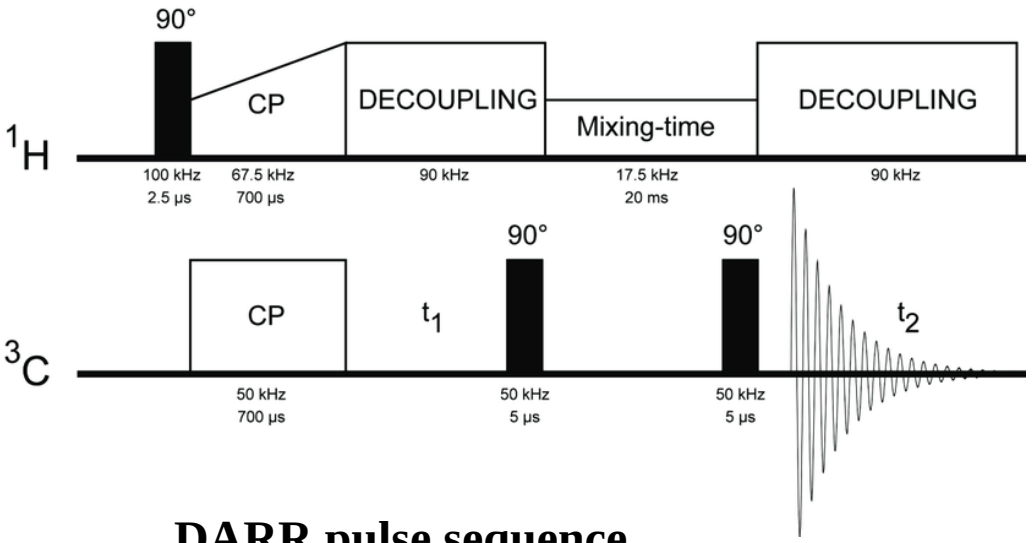


- Interaction between the **magnetic moments** of two spins
- Depends on internuclear **distance** (as $1/r^3$) and **orientation** of internuclear vector with respect to B_0
- Gives a **doublet** (similar as for J coupling) for a **single crystal** (where all internuclear vectors have the same orientation)

... a **Pake pattern** (superposition of two powder lineshapes) for random orientations

... and a **broad hump** for a network of coupled nuclei (such as the many ^{1}Hs in biomolecules!)

Dipolar interaction in solids, a different perspective

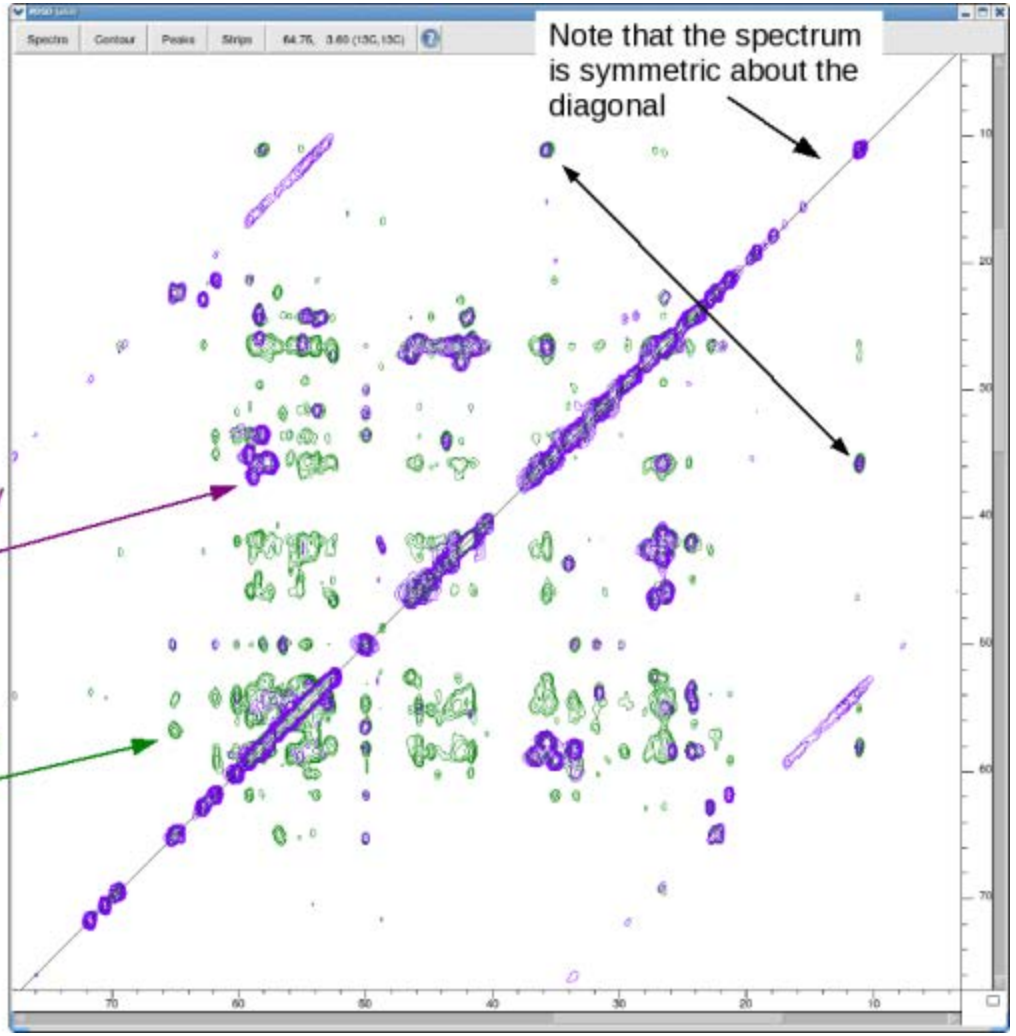


DARR pulse sequence

Example of ^{13}C-^{13}C correlations in solids

At short mixing times only the intra-residue peaks are visible (shown in purple).

At longer mixing times, many inter-residue peaks become visible (shown in green).

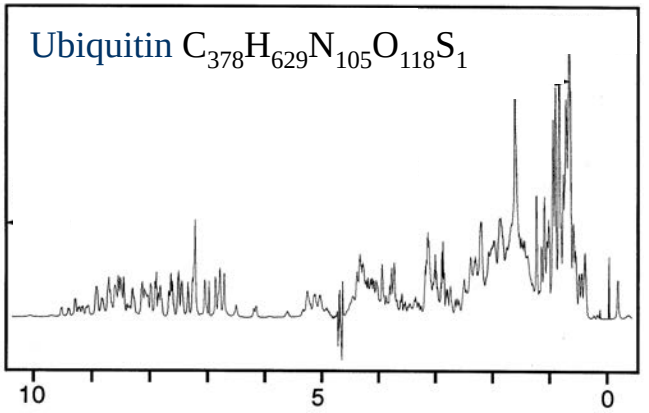


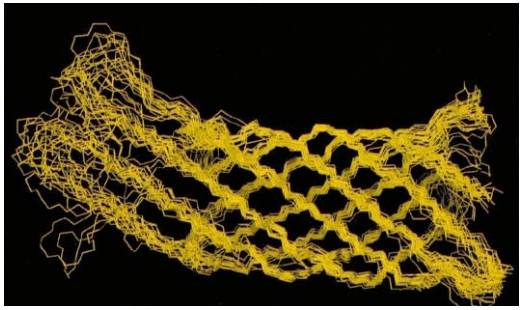
See Robert's talk

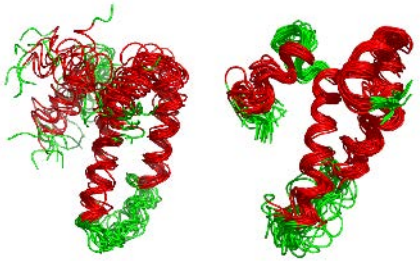
Putting structural information together for structure

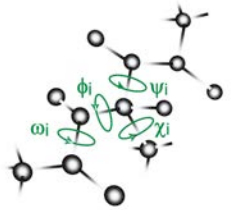
FID

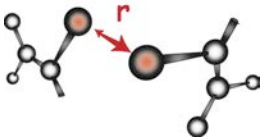
FT

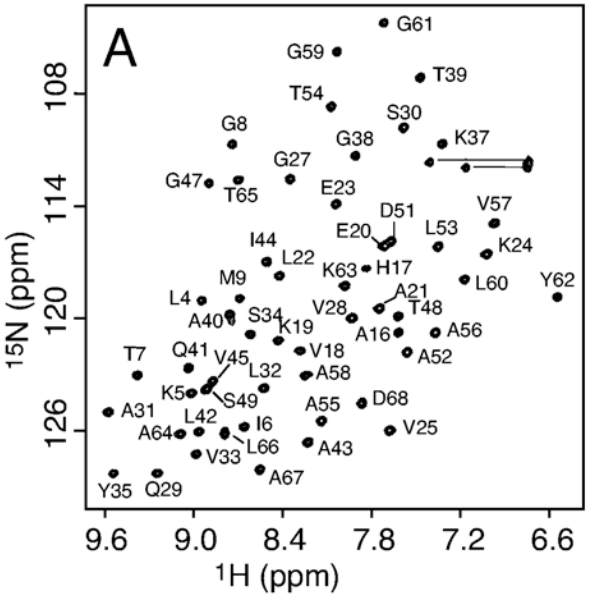






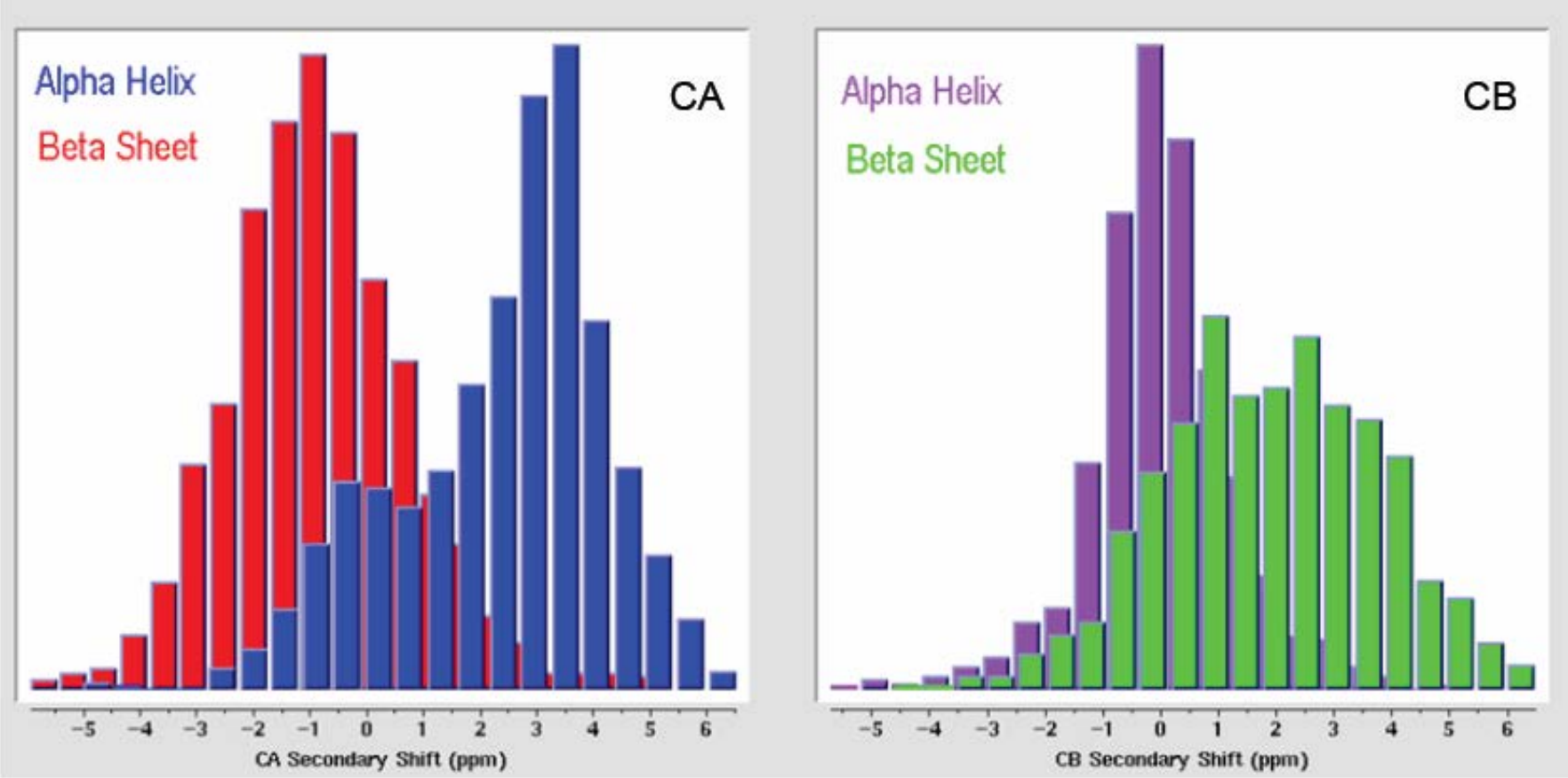






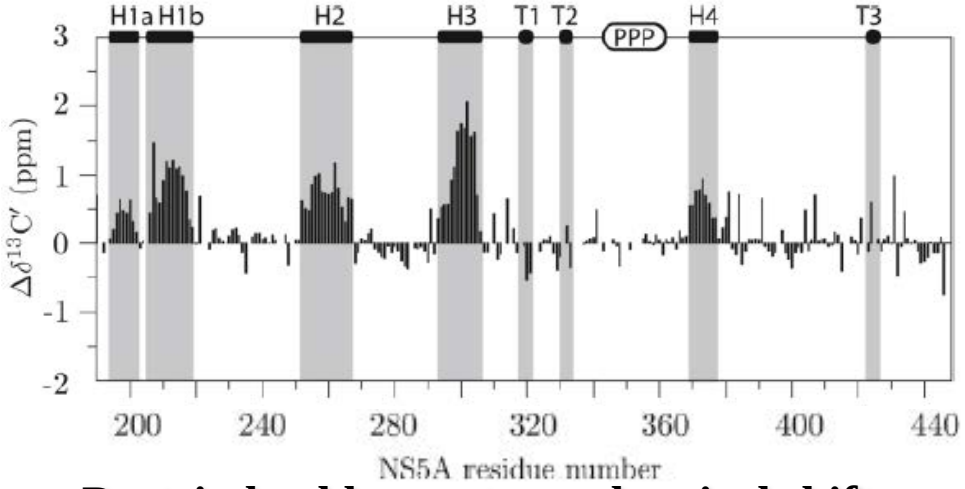
Chemical shift: a structural information content

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

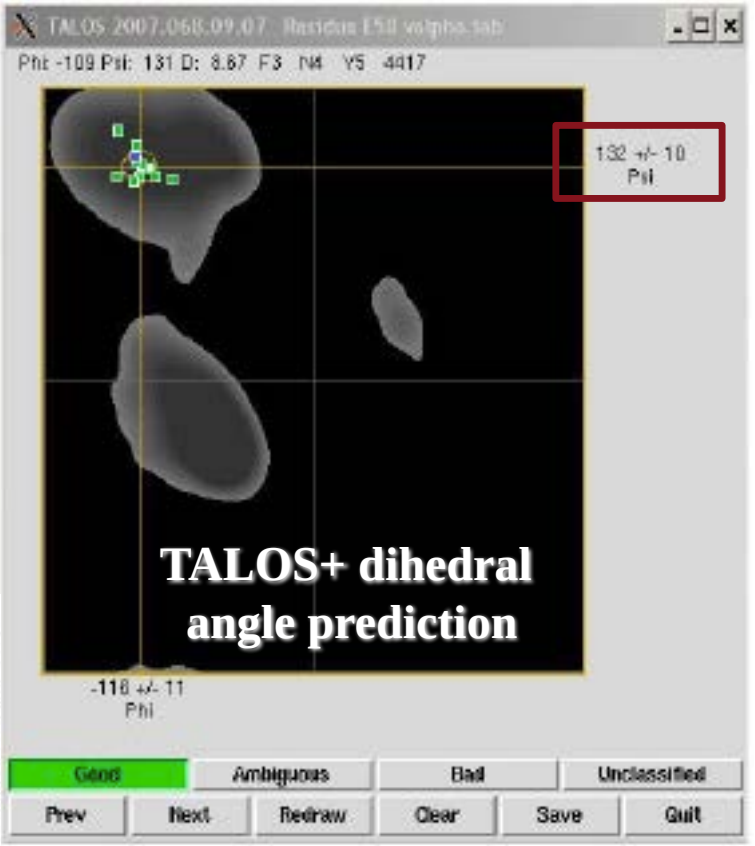


Chemical shift: a structural information content

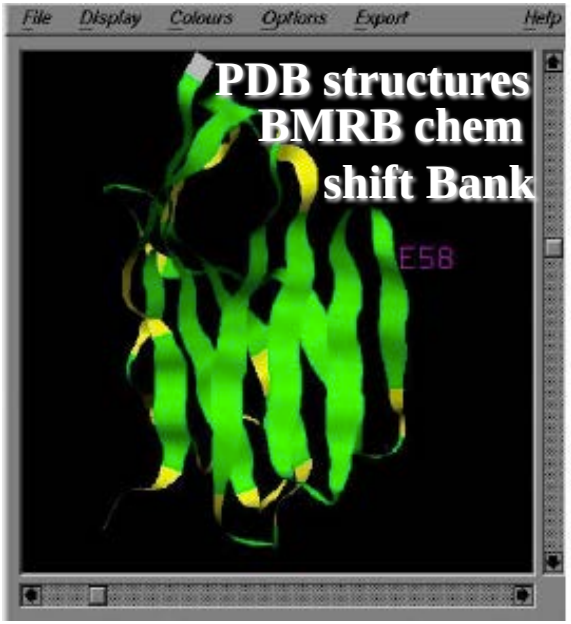
Solyom et al., *Biophys. J.* 2015

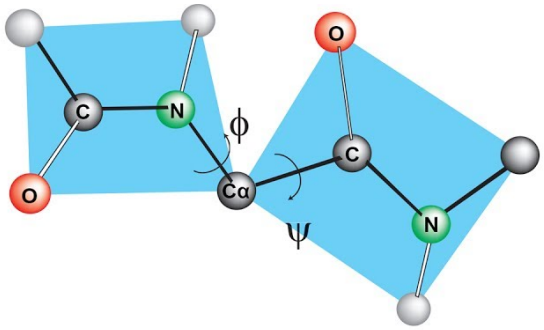


Protein backbone atoms chemical shifts

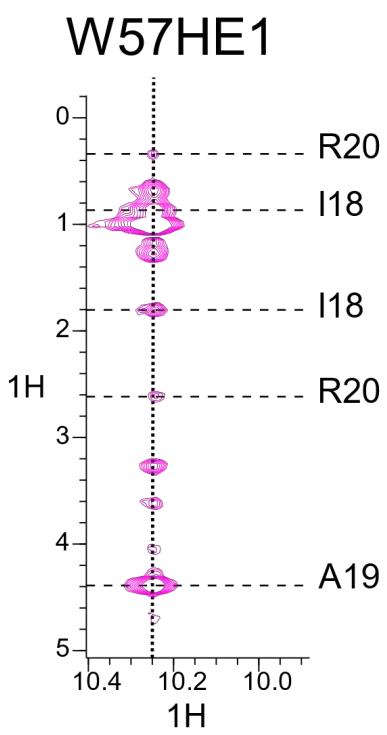


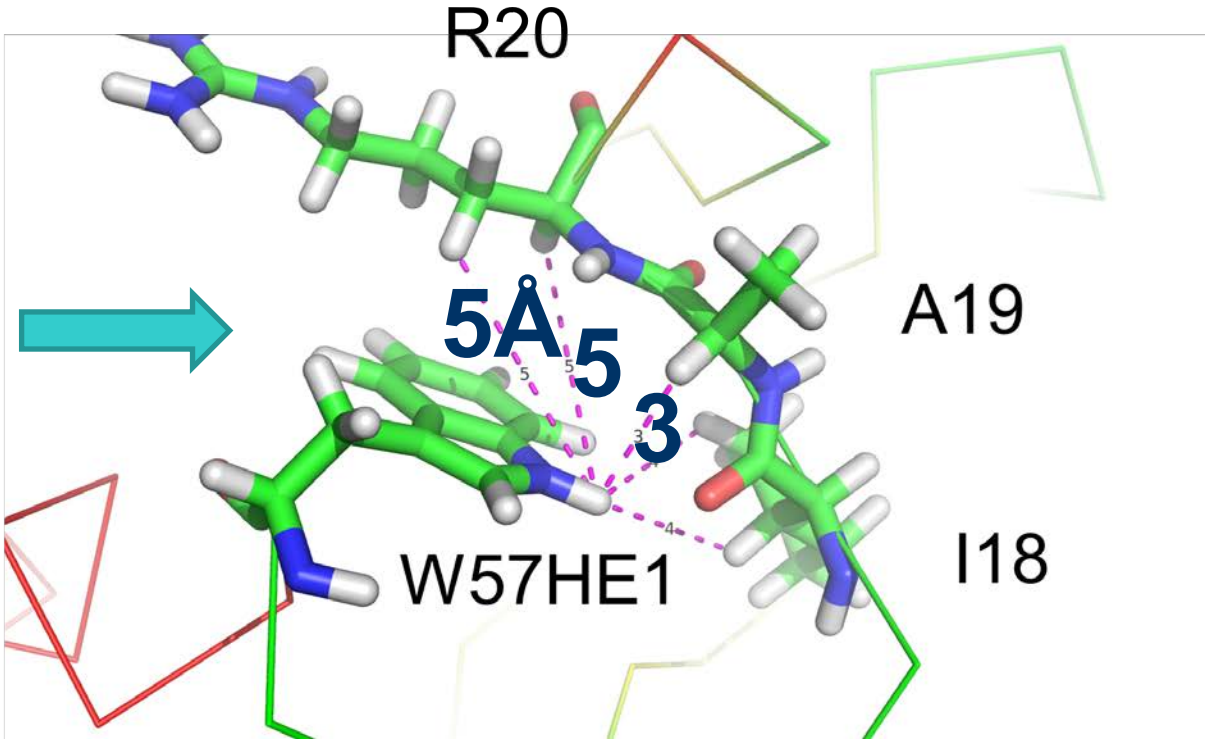
Protein sequence





Distance restraints from NOE

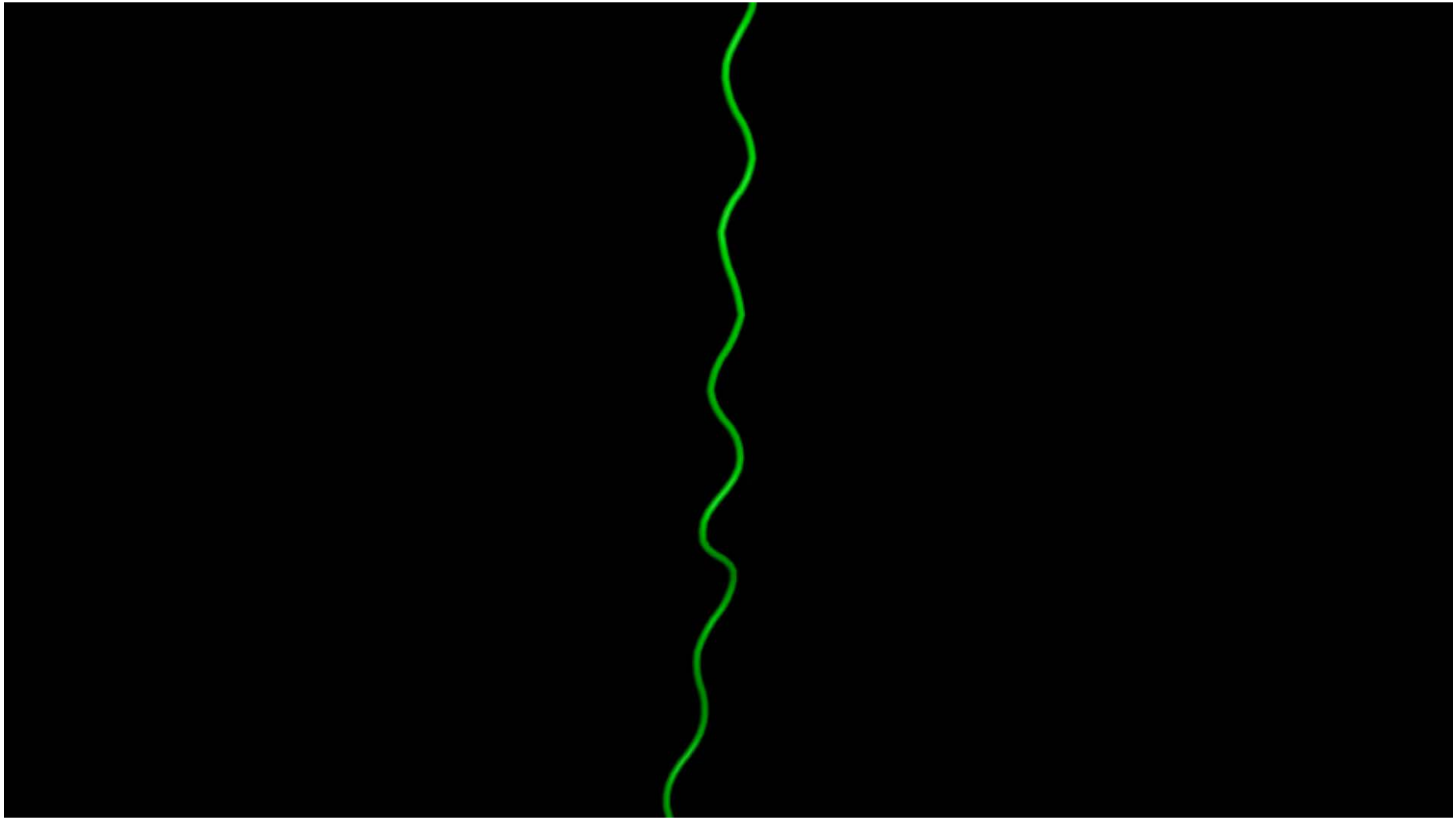




^{1}H-^{1}H connectivities

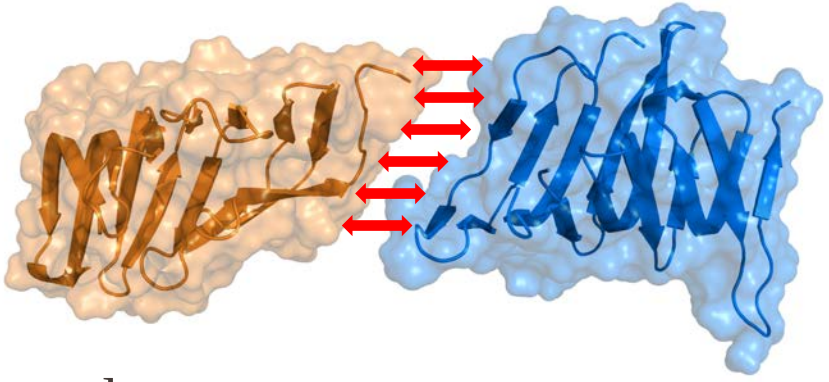
Collaboration H. Lortat-Jacob

Structure determination: iterative process backbone angles + distance restraints

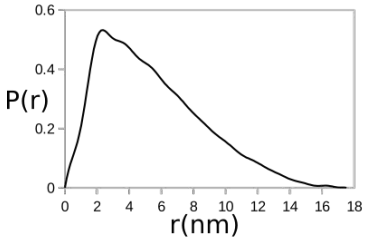


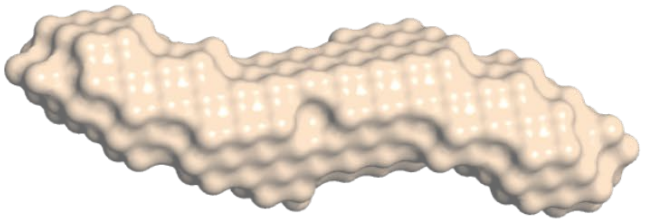
Combination of NMR with SAXS: elongated complex

NMR information: surfaces of contact

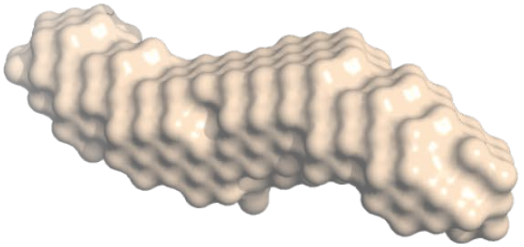


SAXS information global shape of the complex





+

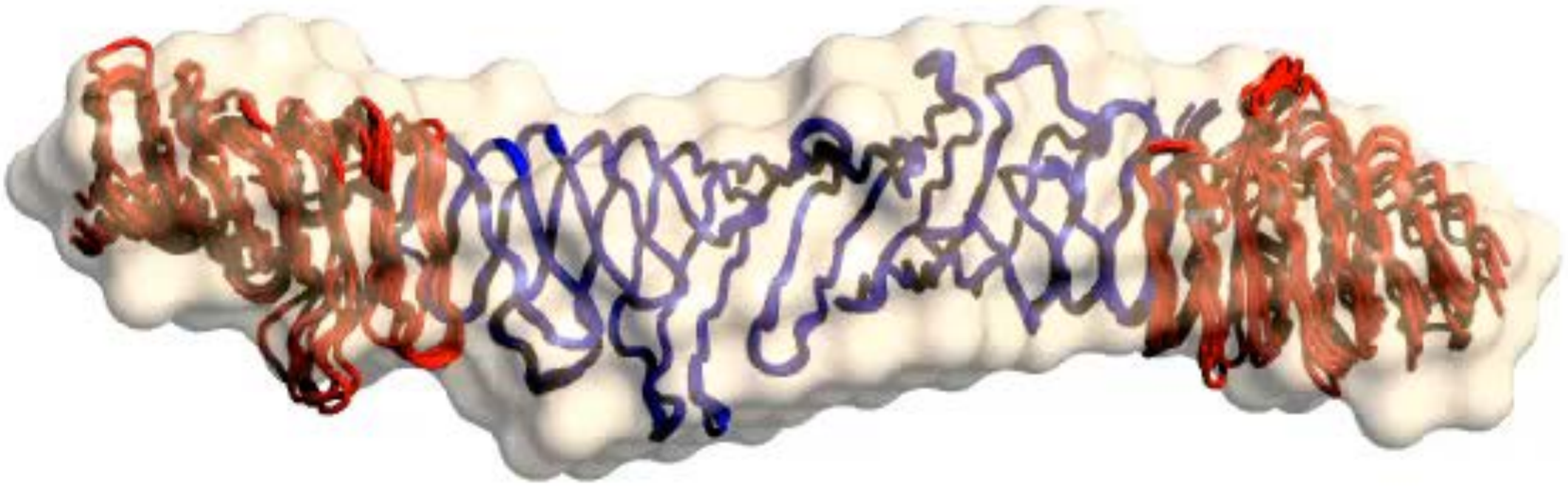


Docking from NMR data filtered by SAXS envelope

Team Simorre

Laguri *et al.*, Sci Reports (2017)

Combination of NMR with SAXS



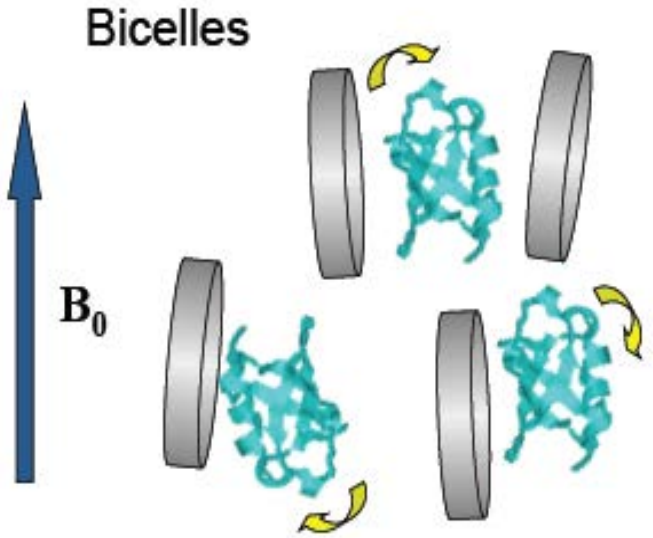
Team Simorre

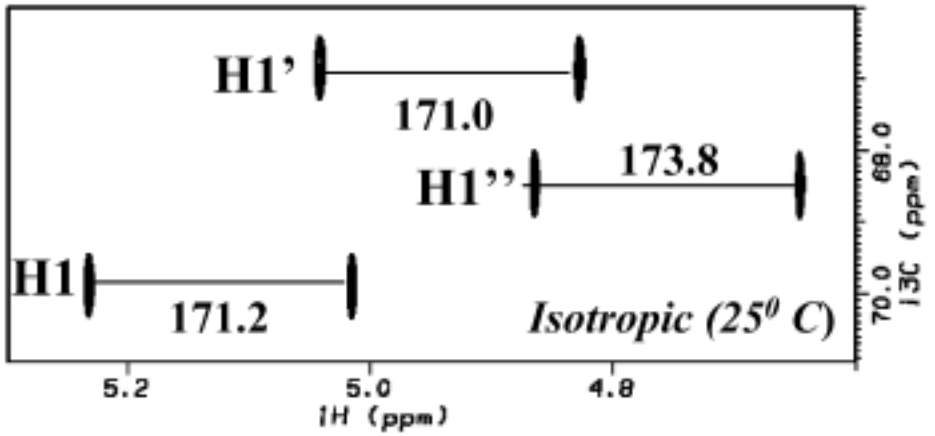
Laguri *et al.*, Sci Reports (2017)

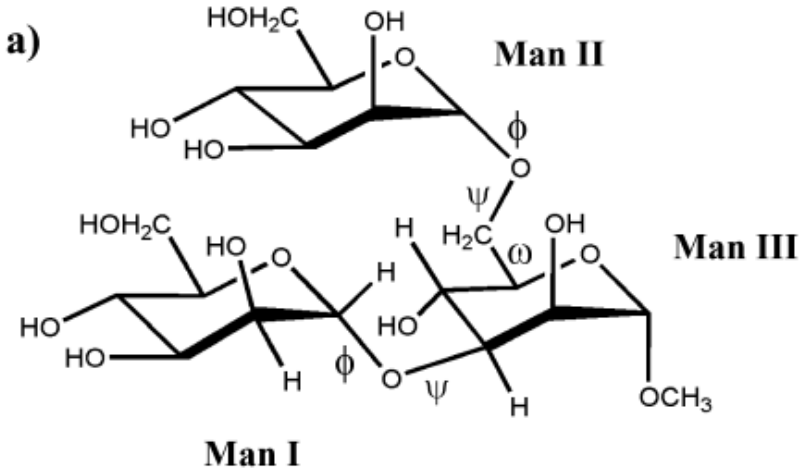
Additional structural information

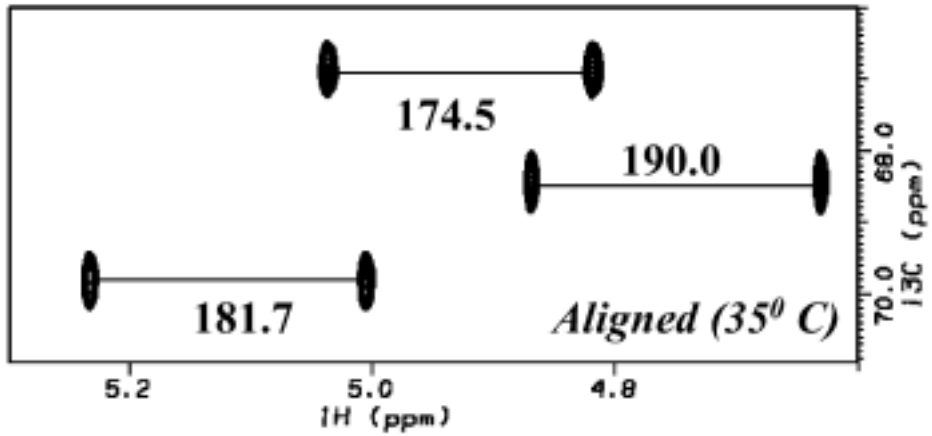
Residual Dipolar Couplings

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



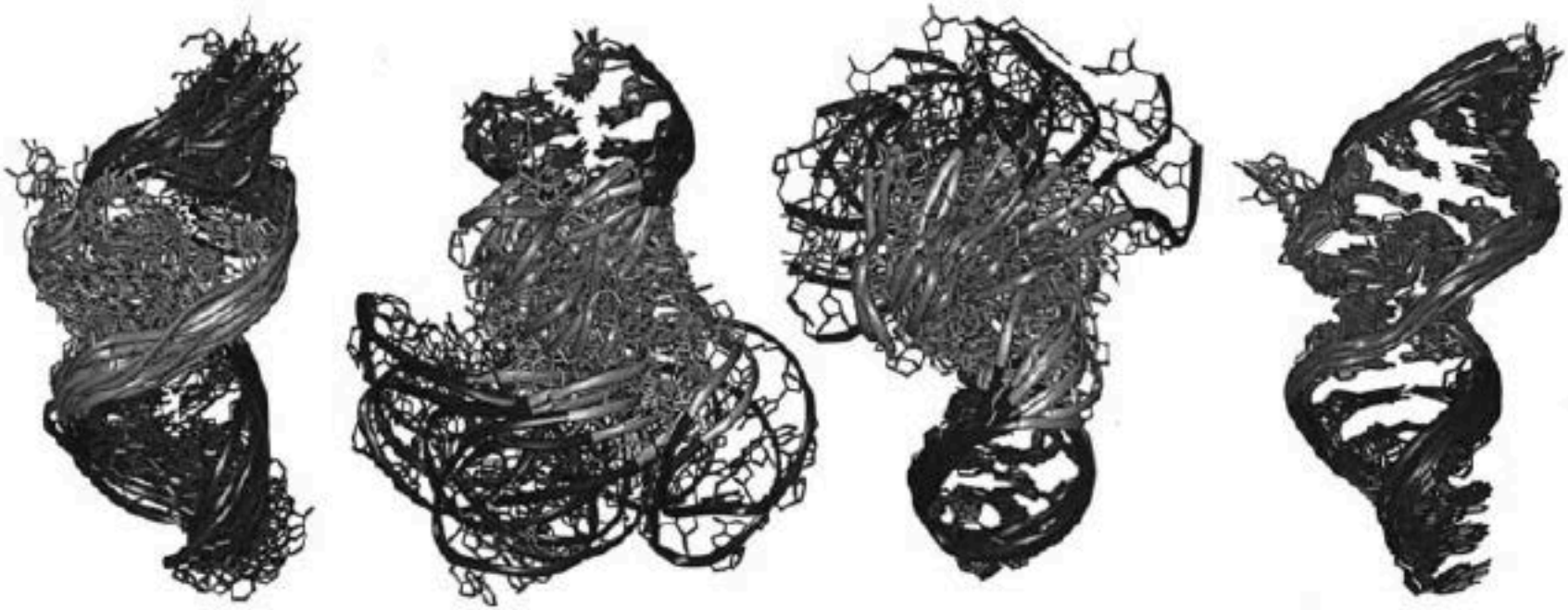






J. Mol. Biol. (2003) **328**, 451–462

Residual Dipolar Couplings

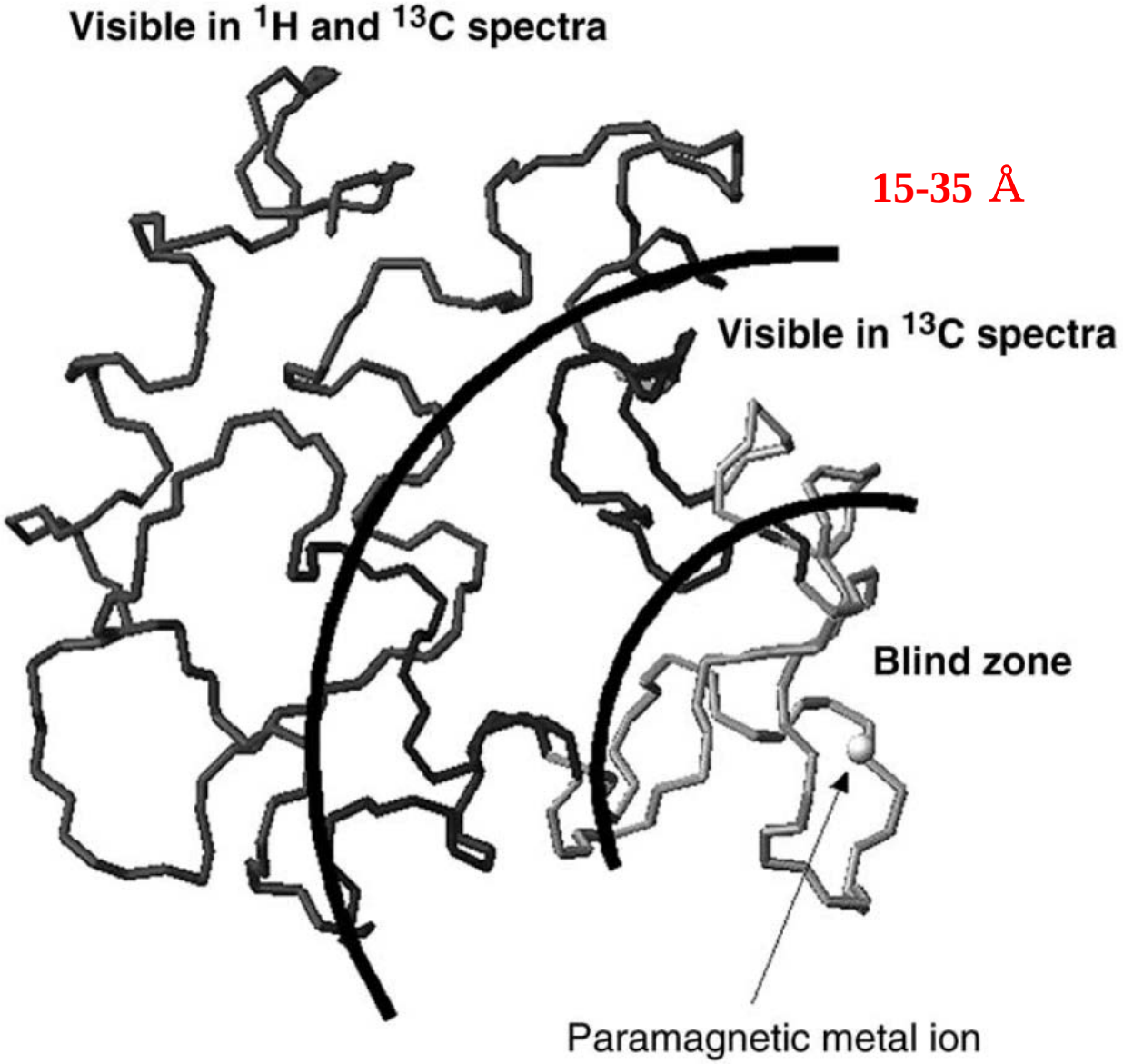


(i) (ii) (iii) (iv)

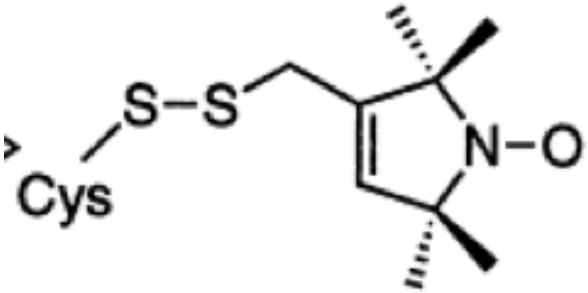
Calculation of the structure of the theophylline-binding RNA aptamer using ^{13}C–^{1}H residual dipolar couplings and restrained molecular dynamics.

Long-range angular restraints

Additional structural information



Paramagnetic Relaxation Enhancement



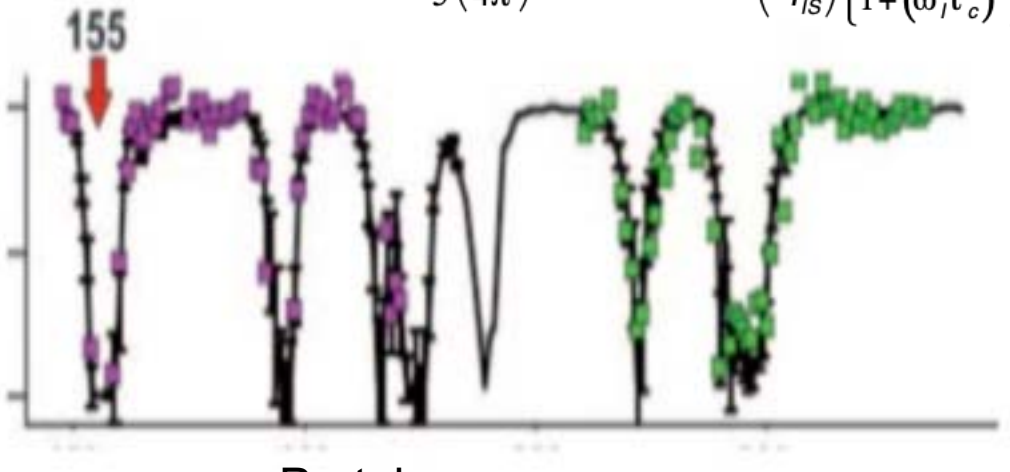
I. Bertini *et al.*, ChemBioChem 2005, 6, 1536 – 1549

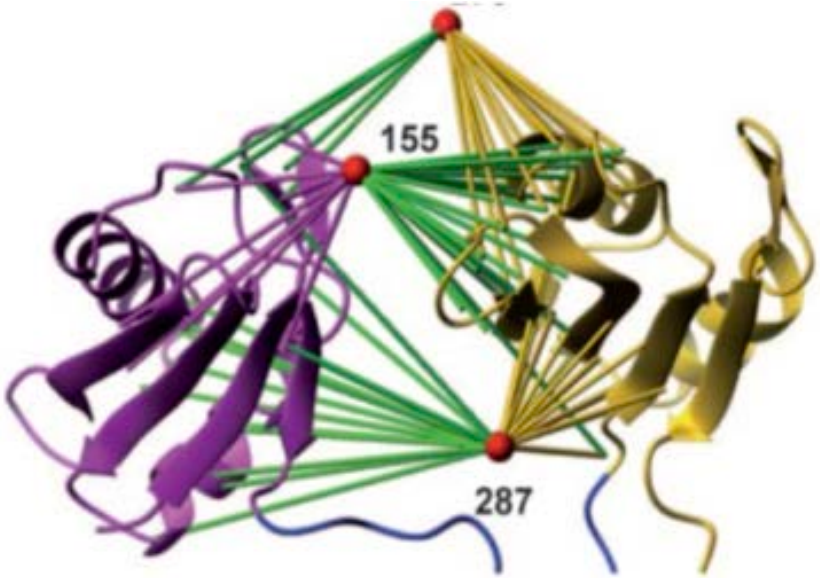
Additional structural information

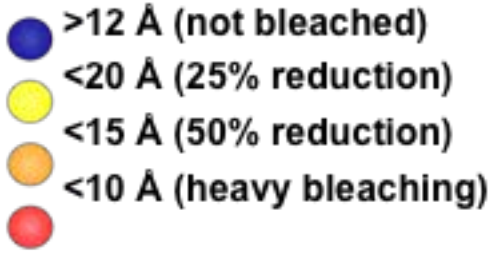
$$R_2^{PRE} = \frac{1}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \gamma_I^2 g^2 \mu_B^2 S(S+1)\left(\frac{1}{r_{IS}^6}\right)\left\{4\tau_c + \frac{3\tau_c}{1+(\omega_I\tau_c)^2}\right\}$$

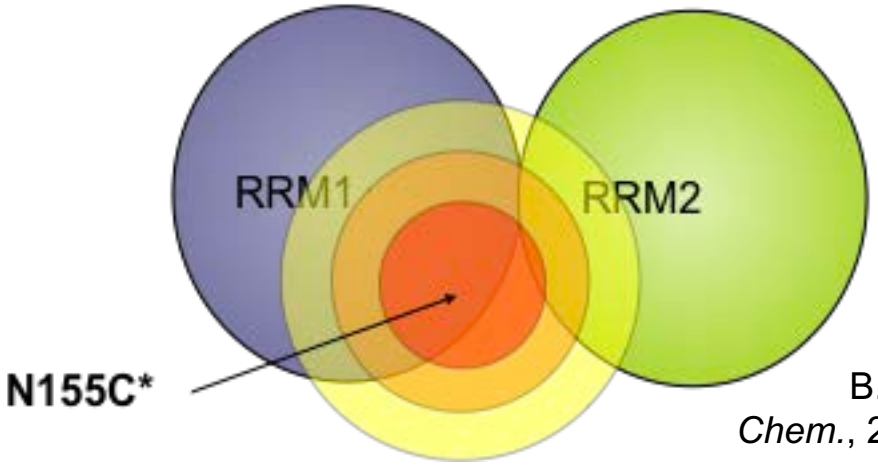
$$R_1^{PRE} = \frac{2}{5}\left(\frac{\mu_0}{4\pi}\right)^2 \gamma_I^2 g^2 \mu_B^2 S(S+1)\left(\frac{1}{r_{IS}^6}\right)\left\{\frac{\tau_c}{1+(\omega_I\tau_c)^2}\right\}$$

Iox/Ired





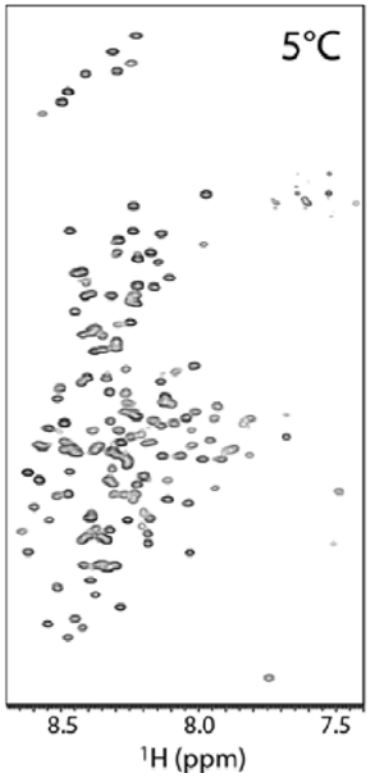




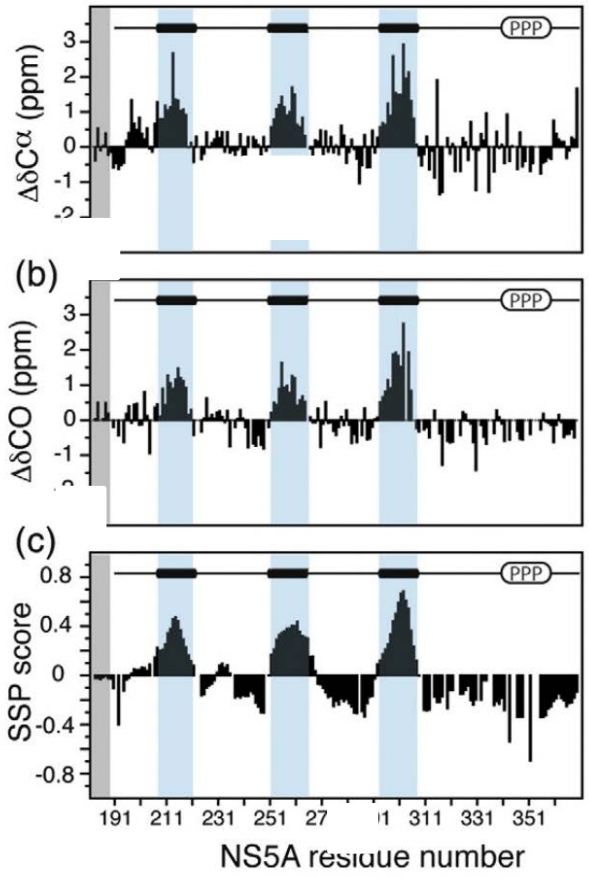
B.Simon, M. Sattler, *Angew. Chem.*, 2010, *122(11)*, 2011 - 2014

Intrinsically Disordered Protein
Lack of well defined secondary/tertiary structure

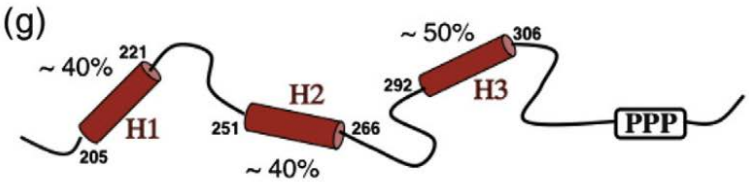
No distance restraints



^{15}N (ppm)



Backbone chemical shifts: Prediction of population in helical forms



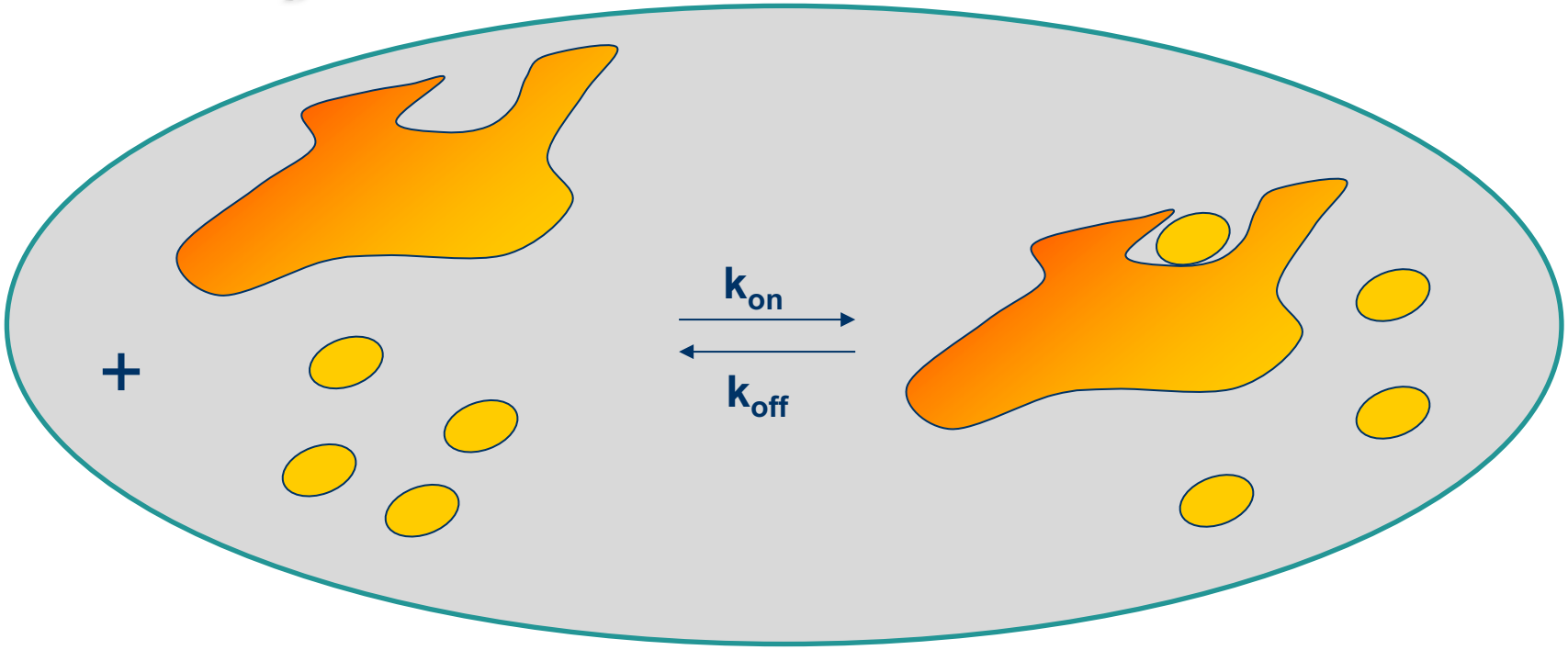
Feuerstein *et al.*, J. Mol. Biol. (2012)

Biomolecular interactions

Interaction Strength?

K_D

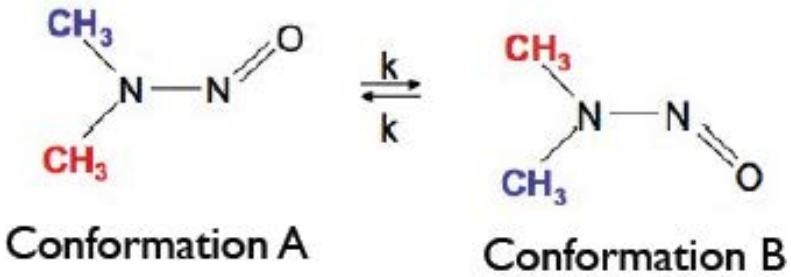
Interaction Site?



Complex structure?

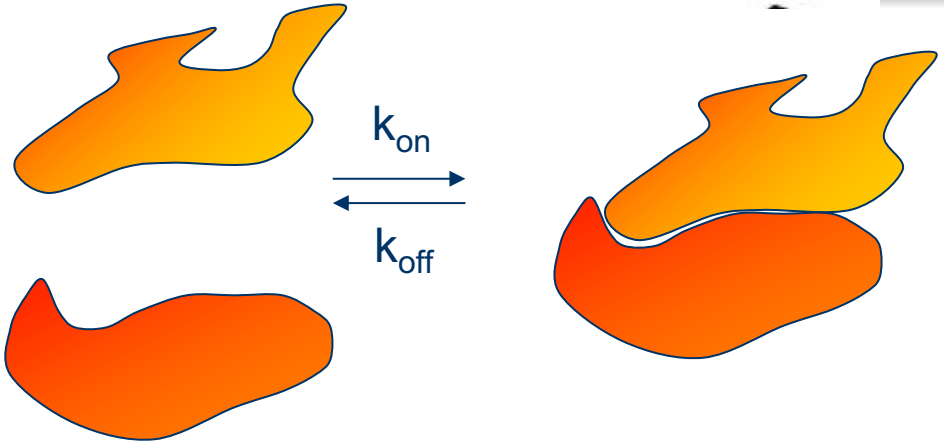
Interaction Dynamics?

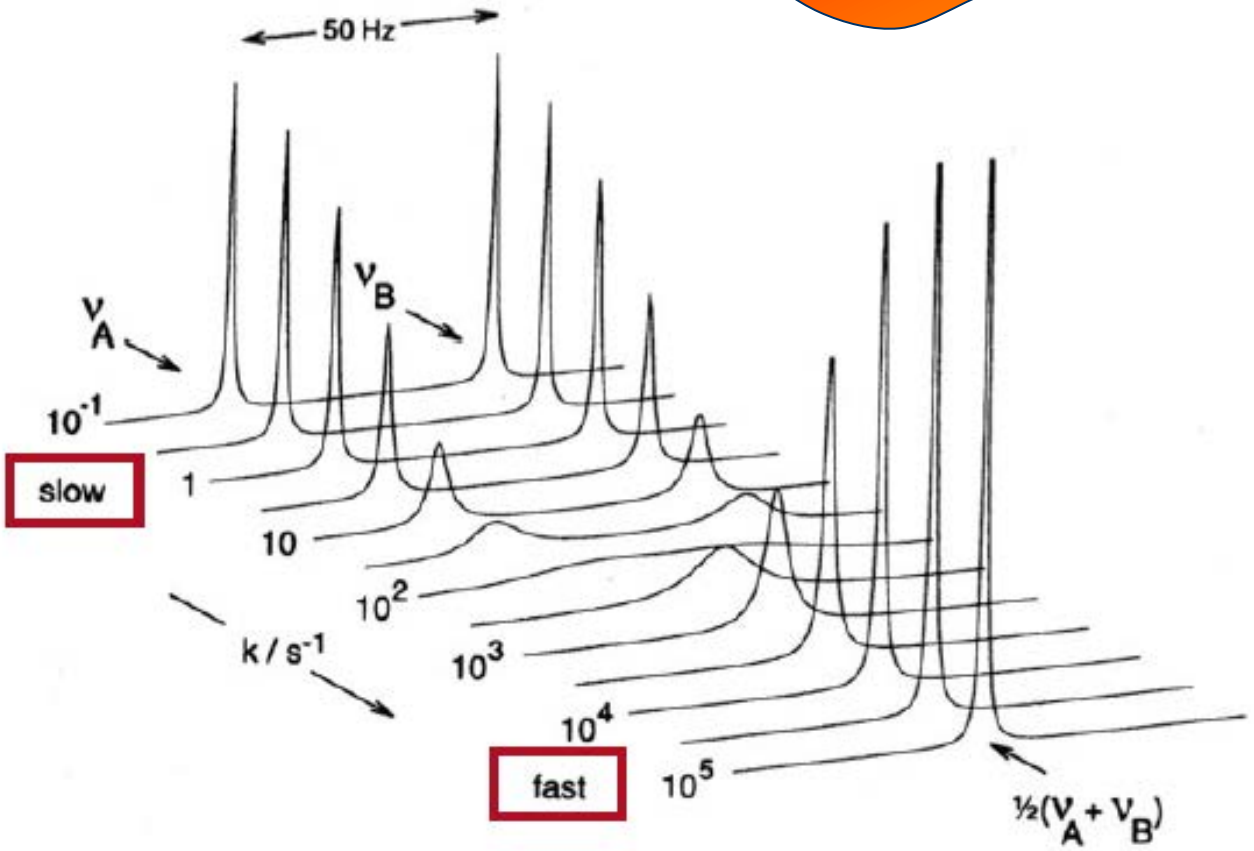
Chemical shift and chemical exchange (µs-ms)



Conformation A

Conformation B

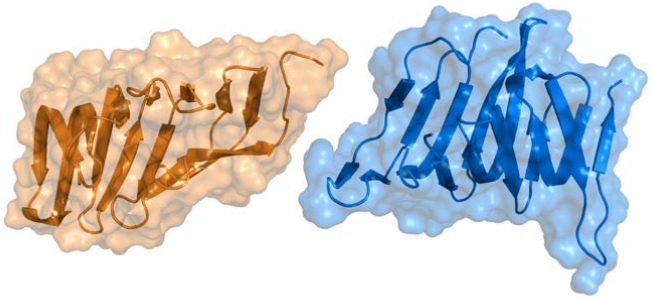


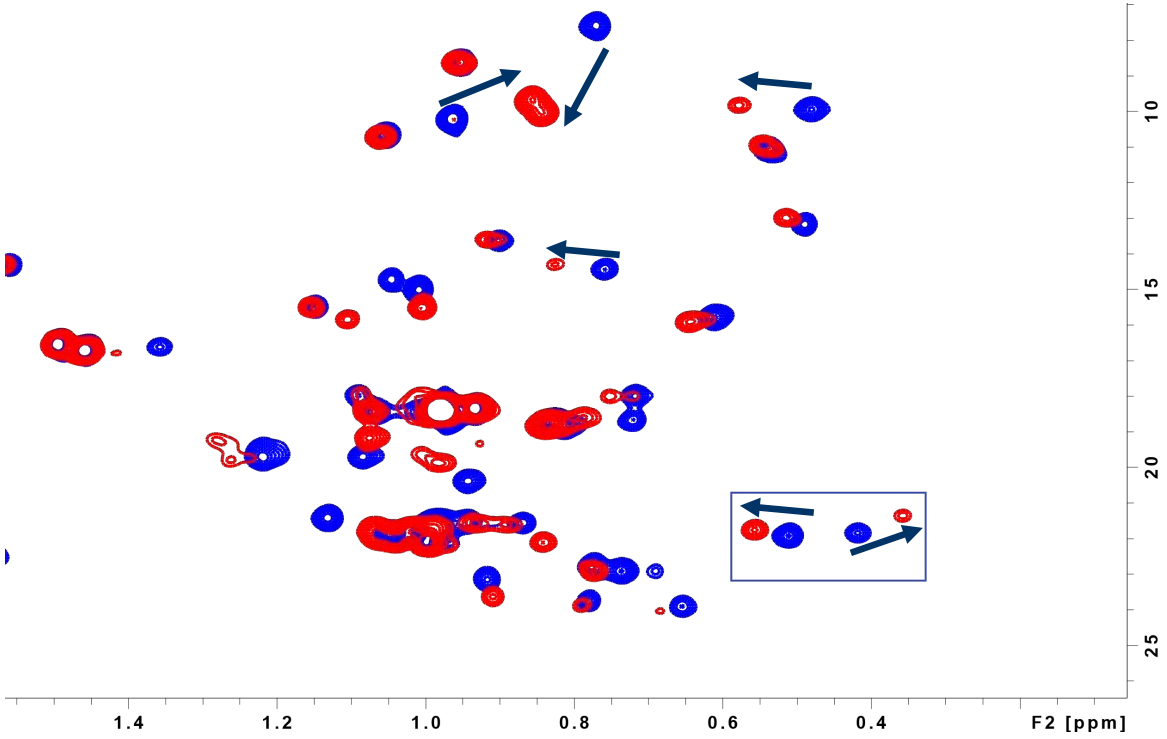


Strong protein-protein interaction

Free

Complex



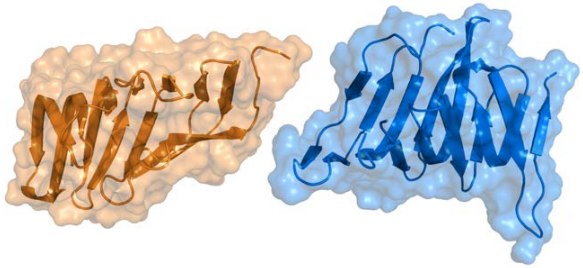


^{13}C (ppm)

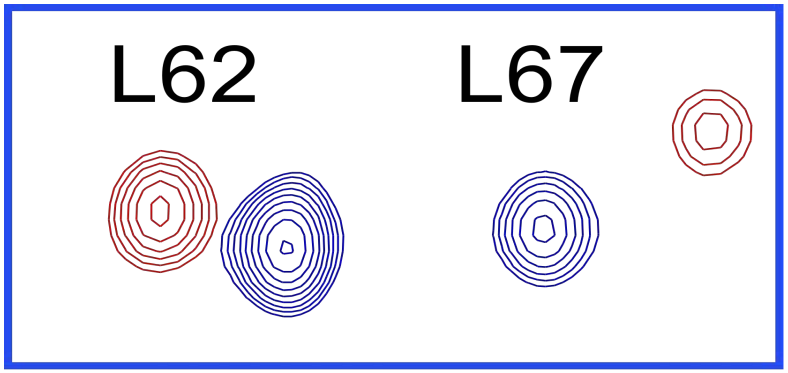
^{1}H (ppm)

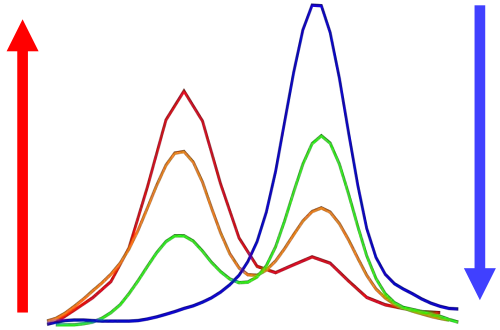
Laguri *et al.*, Sci Reports (2017)

Strong protein-protein interaction



Complex Free





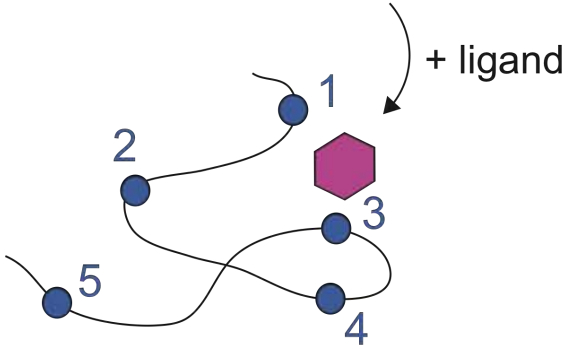
Ligand addition

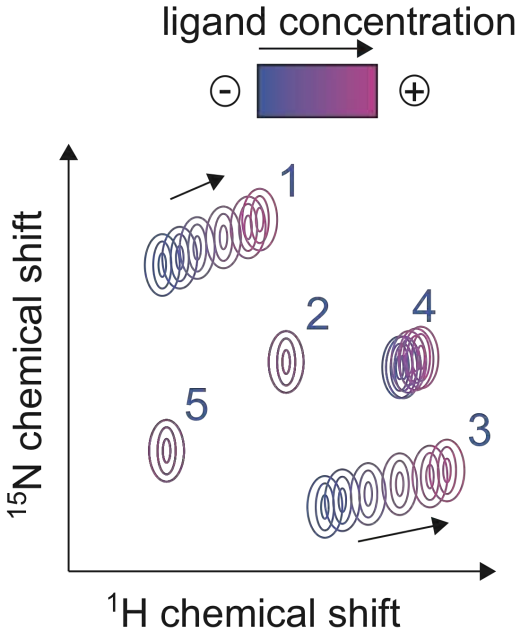
Complex Free

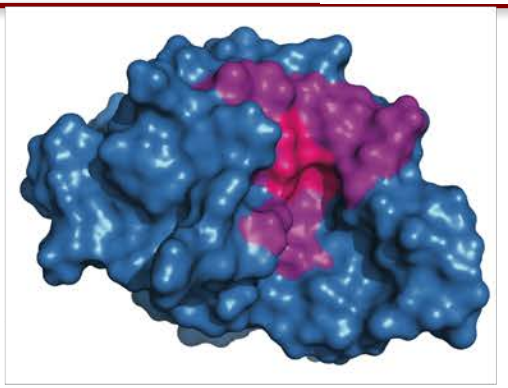
Problem: Need to reassign the peaks

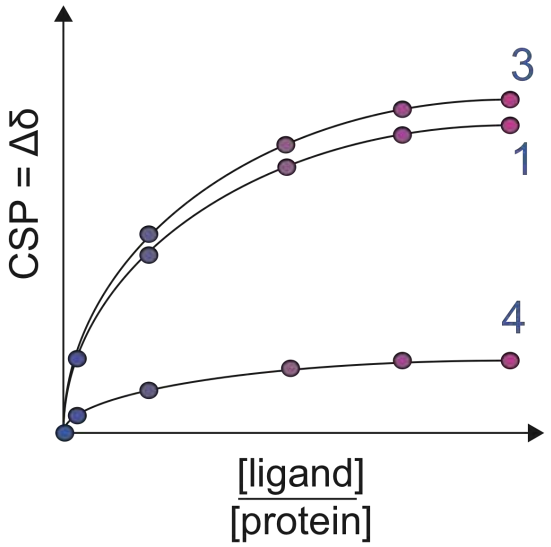
Laguri *et al.*, Sci Reports (2017)

Protein-small molecule weak interaction (CSP)

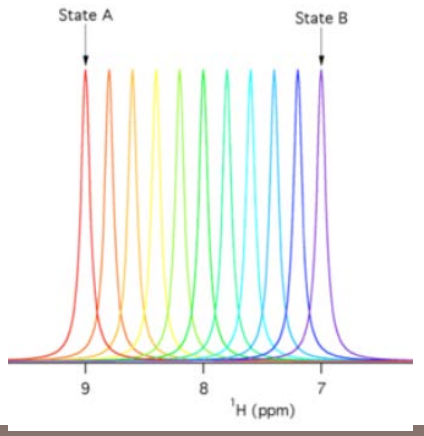






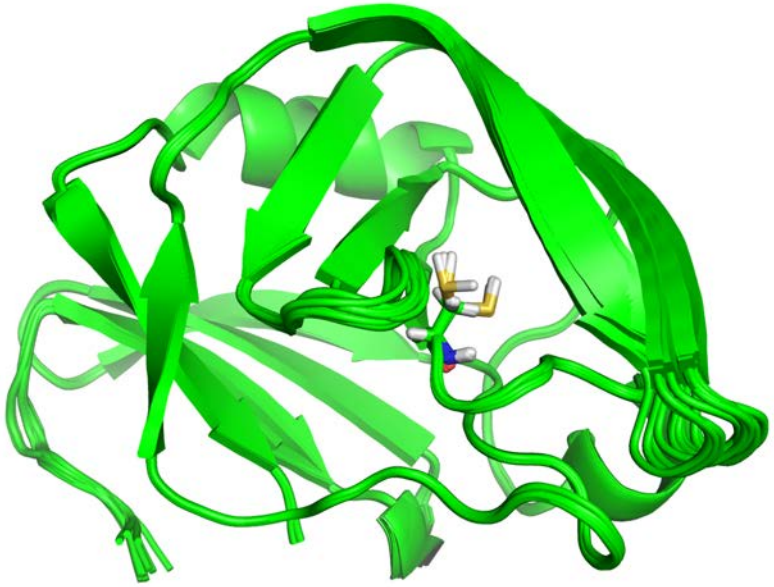


Fast exchange



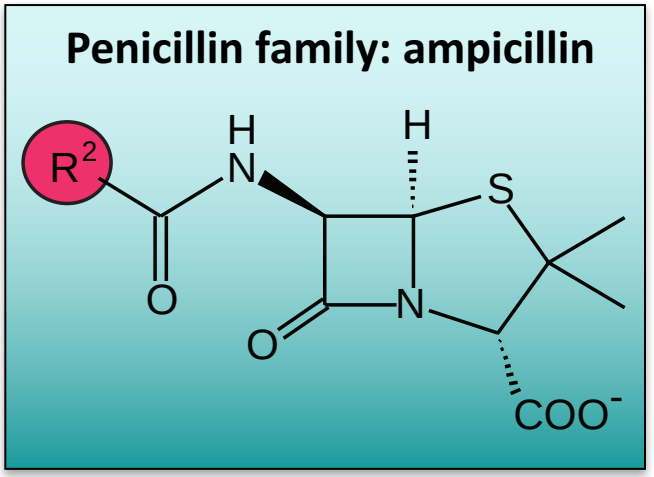
Protein Chemical Shift Perturbation (CSP)

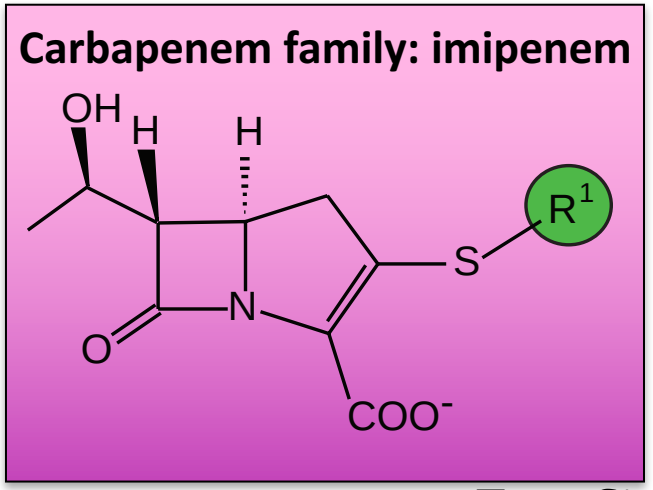
Protein-small molecule weak interaction (CSP)



Catalytic domain of L,D-transpeptidase from *Enterococcus faecium*

^{13}C, ^{15}N-labeled

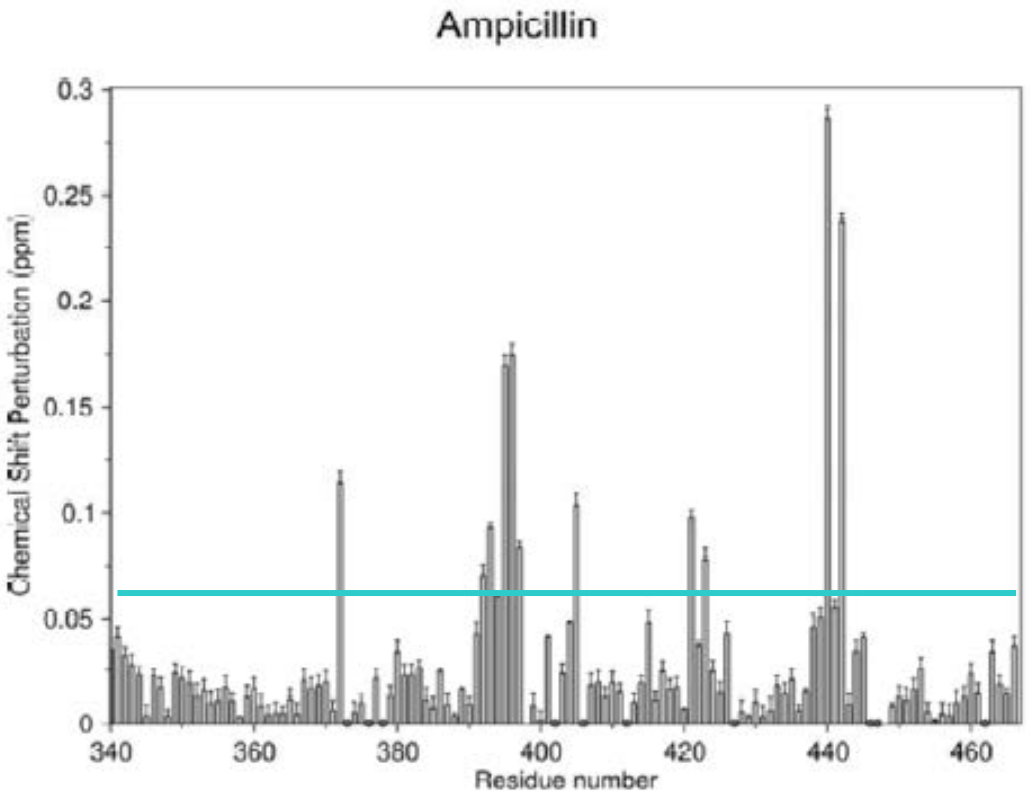




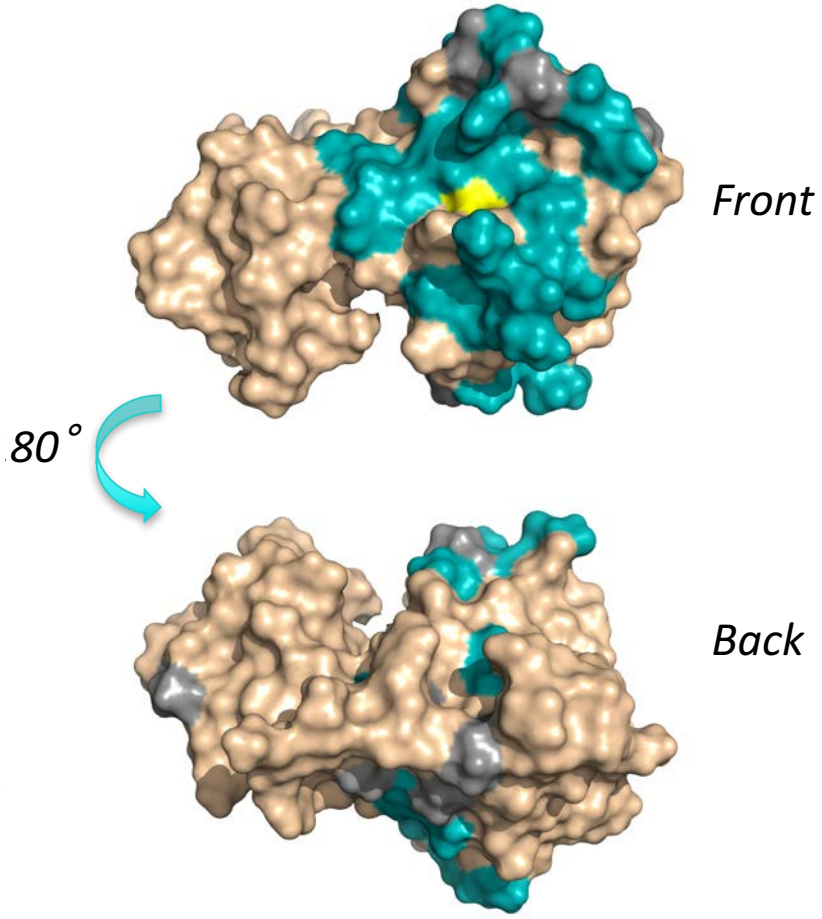
Team Simorre

Lecoq et al., *ACS Chem. Biol.*.2013

Protein-small molecule interaction (CSP)



Ampicillin



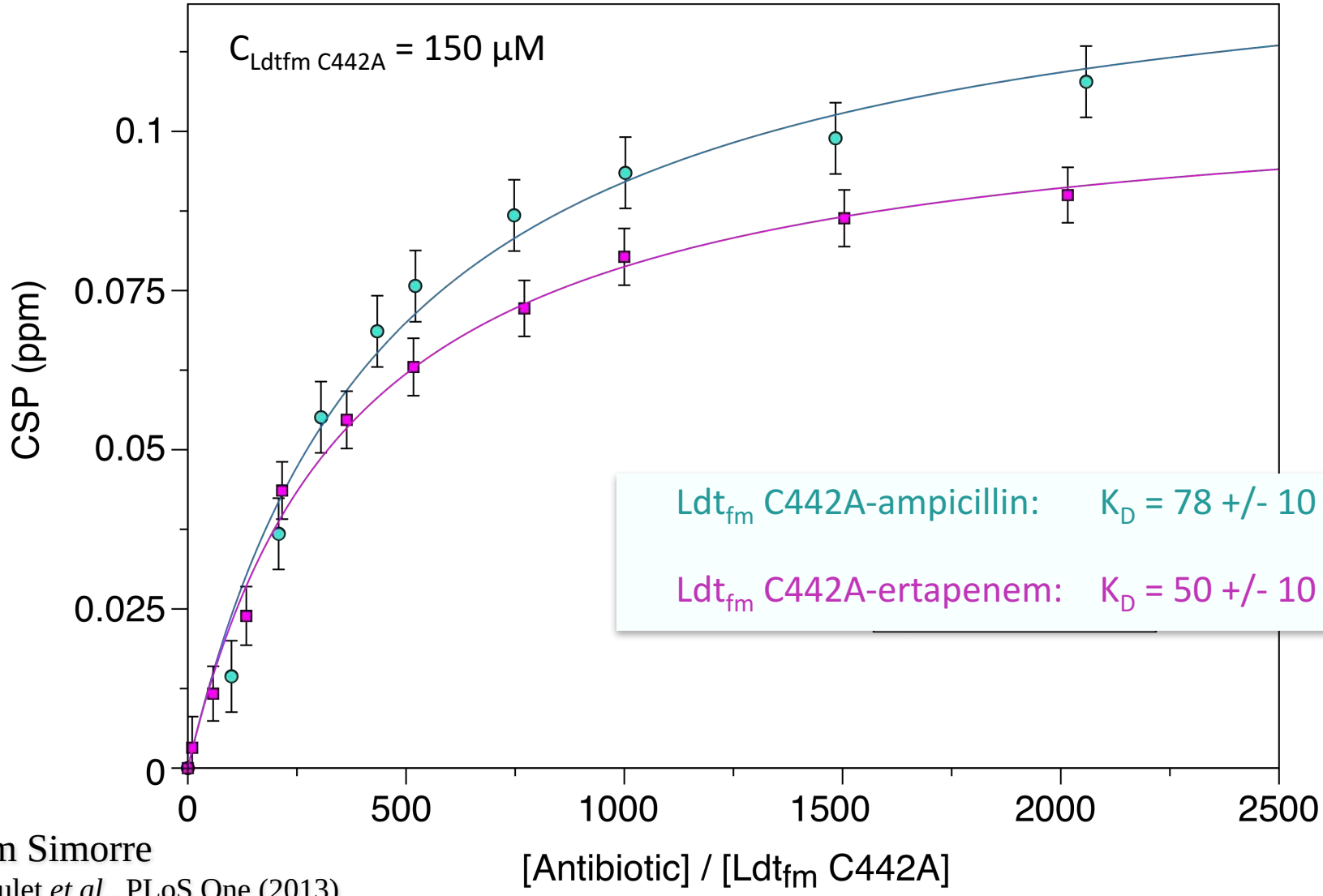
Ldt_{Bs} C142A-ampicillin

Team Simorre

Triboulet *et al.*, PLoS One (2013)

Protein-small molecule interaction (CSP)

K_D determination: fit all residues with CSP > 0.03 ppm with a single K_D value



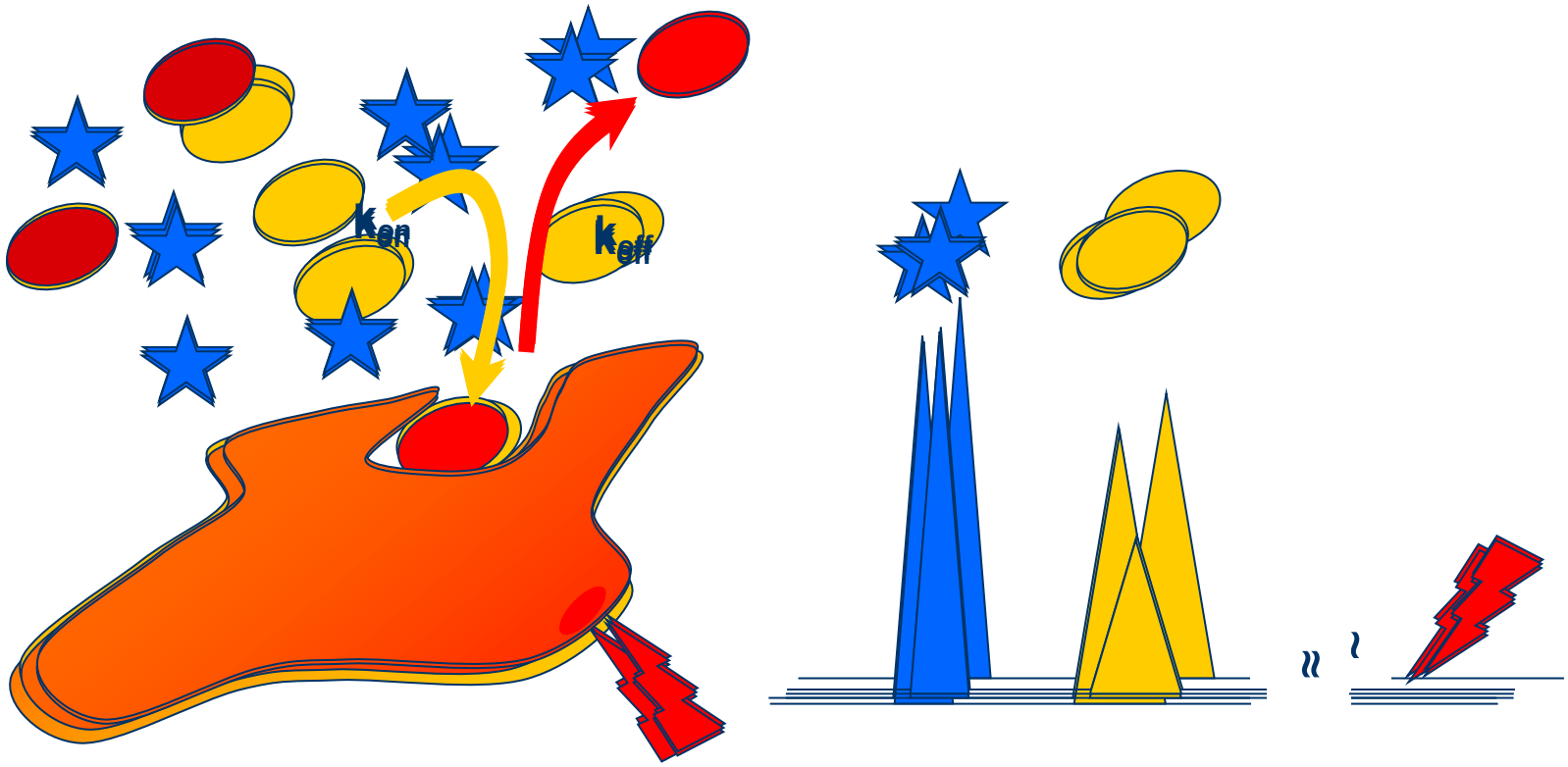
Ldt_{fm} C442A-ampicillin: K_D = 78 +/- 10 mM

Ldt_{fm} C442A-ertapenem: K_D = 50 +/- 10 mM

Team Simorre

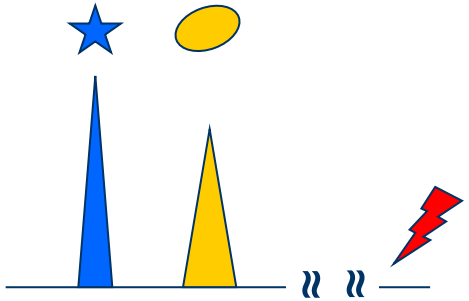
Triboulet *et al.*, PLoS One (2013)

Small molecule - protein interaction
Saturation transfer experiment for weak interaction



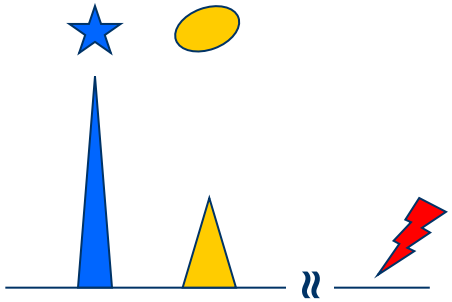
Small molecule - protein interaction (STD)

The reference experiment

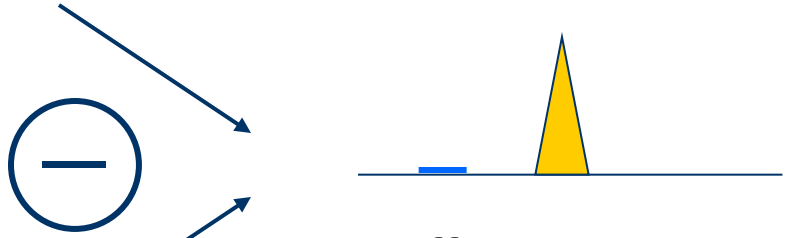


off-resonance saturation

Saturation experiment



on-resonance saturation



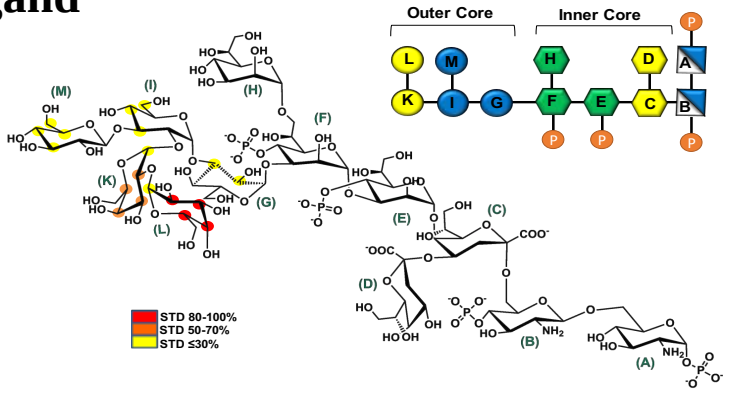
Difference spectrum

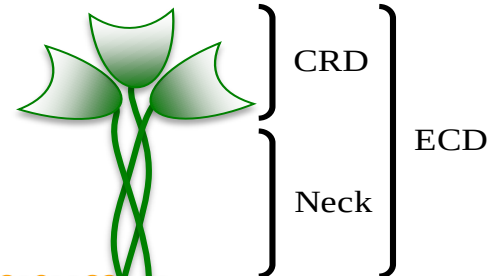
STD:

- **Possible in the case of low affinity**
- **Protein:ligand ratio<< 1**
- **No protein size limit**
- **Isotopic labeling of protein not requested**
- **Simplified ^{1}H NMR spectra**

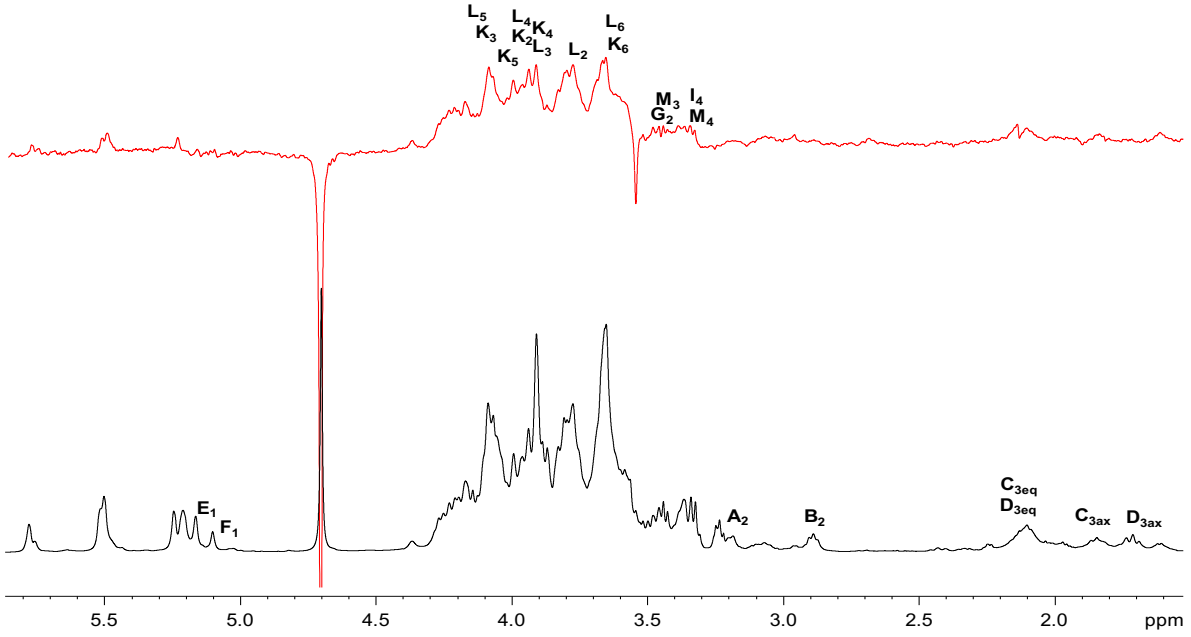
Detection of interaction on the small ligand

LPS ligand





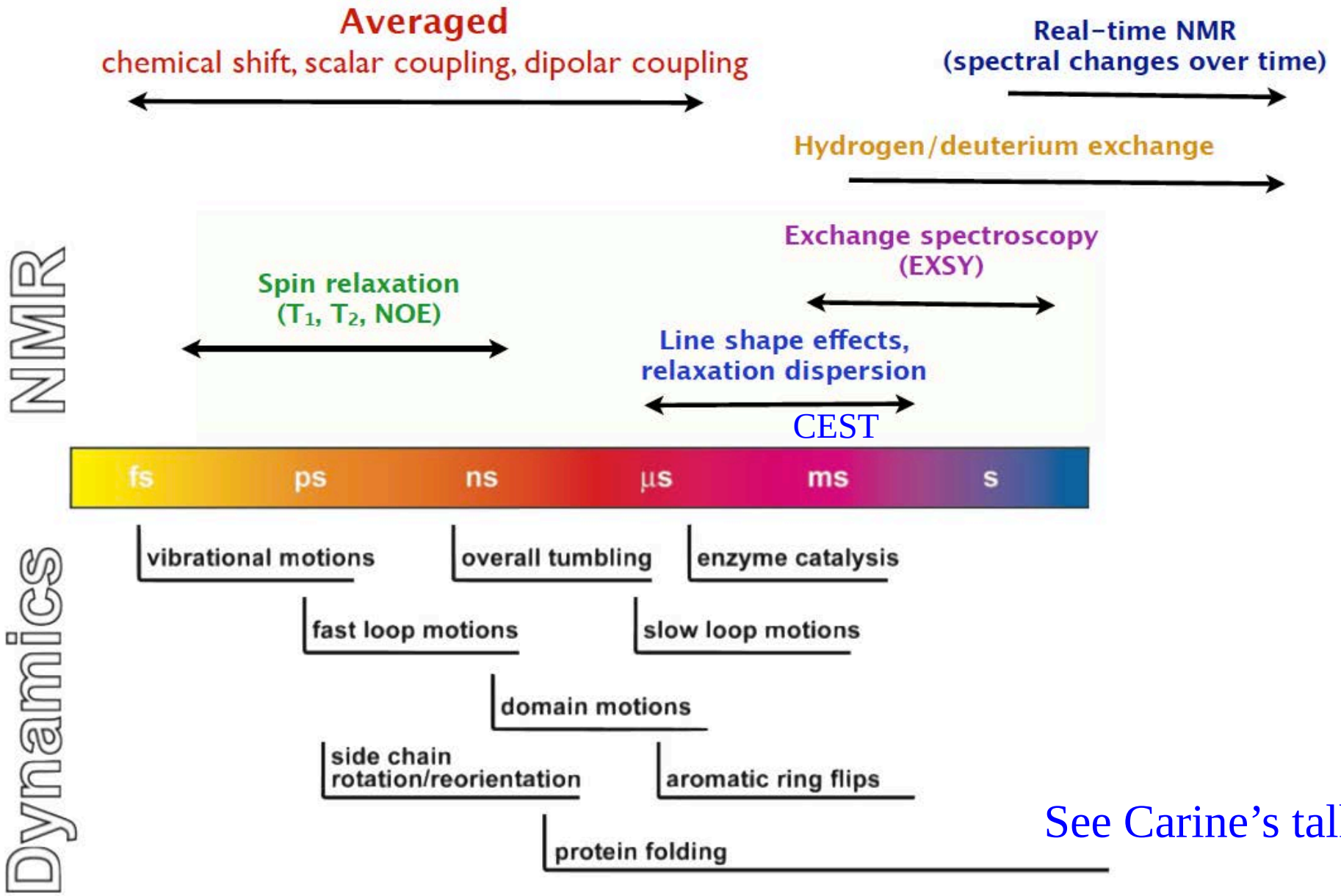
Protein



^{1}H (ppm)

Team Simorre
Collaboration F. Fieschi
Maalej *et al.*, ChemBioChem (2019)

Dynamics: what can NMR do?



See Carine's talk

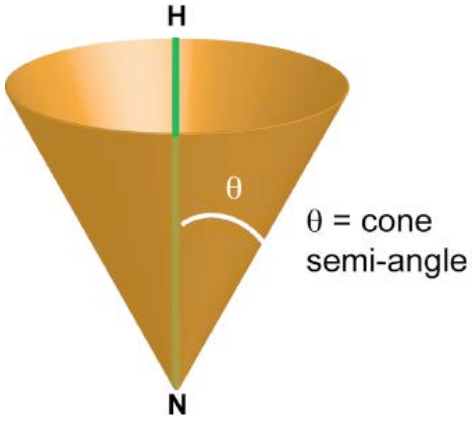
ps-ns time scales and T_1/T_2 relaxation
Rotational diffusion

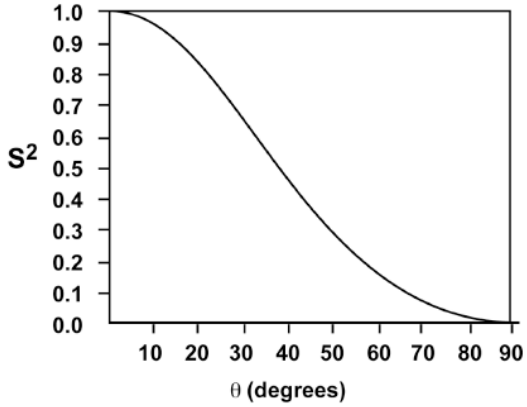
Extraction of global and local motion in macromolecules

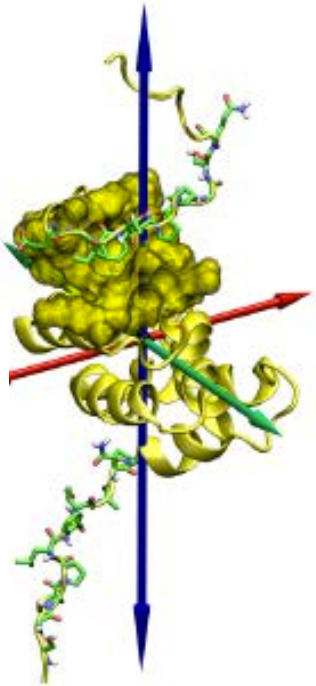
$$R_2/R_1 \approx (2/3)\omega_X^2\tau_c^2+7/6$$

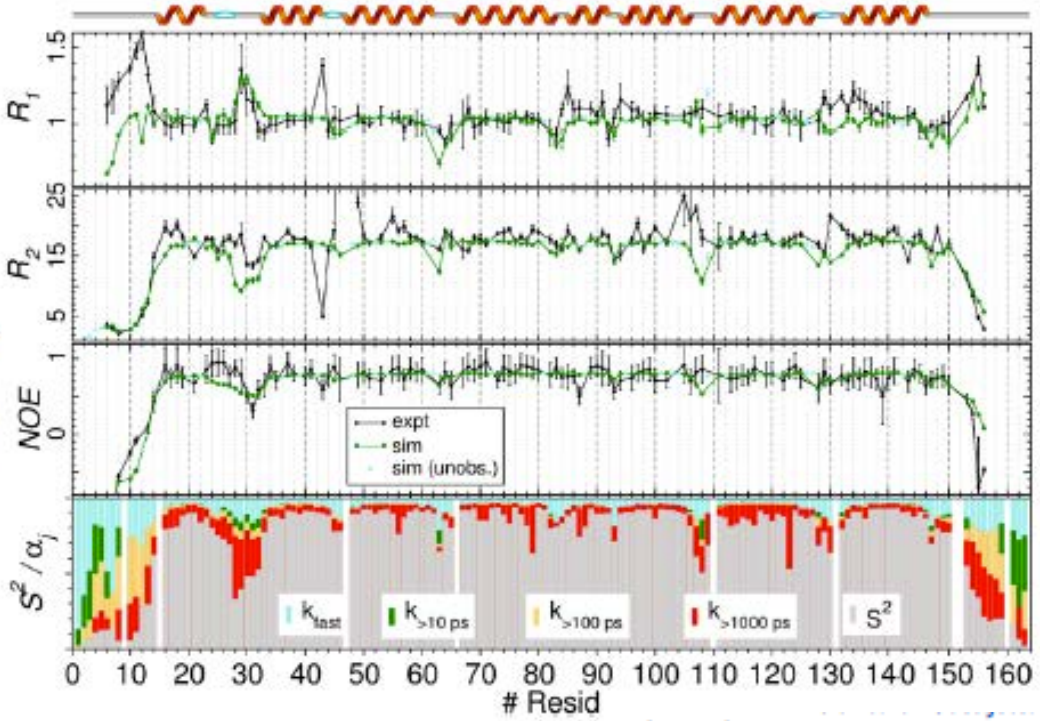
τ_c reflects molecular size

Lipari-Szabo S^2 reflects local motion amplitude



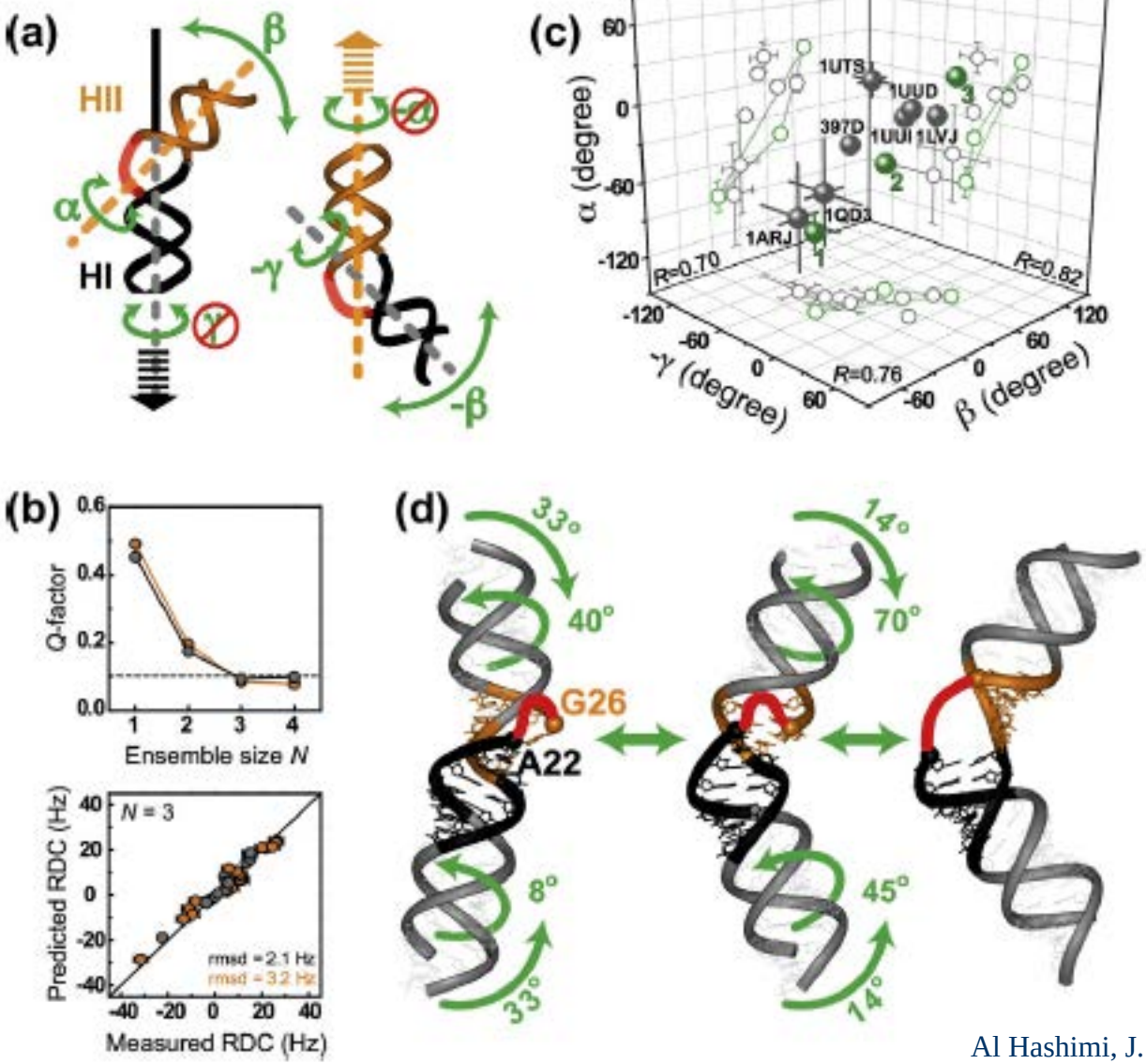






J. Chem. Theory Comput. 2018, 14, 1009−1019

Dipolar interactions: a dynamical information content



Al Hashimi, J. Magn. Res. **2013**, 191-204

µs-ms time scales and chemical exchange

$$A \underset{k}{\overset{k}{\leftrightarrow}} B$$

slow

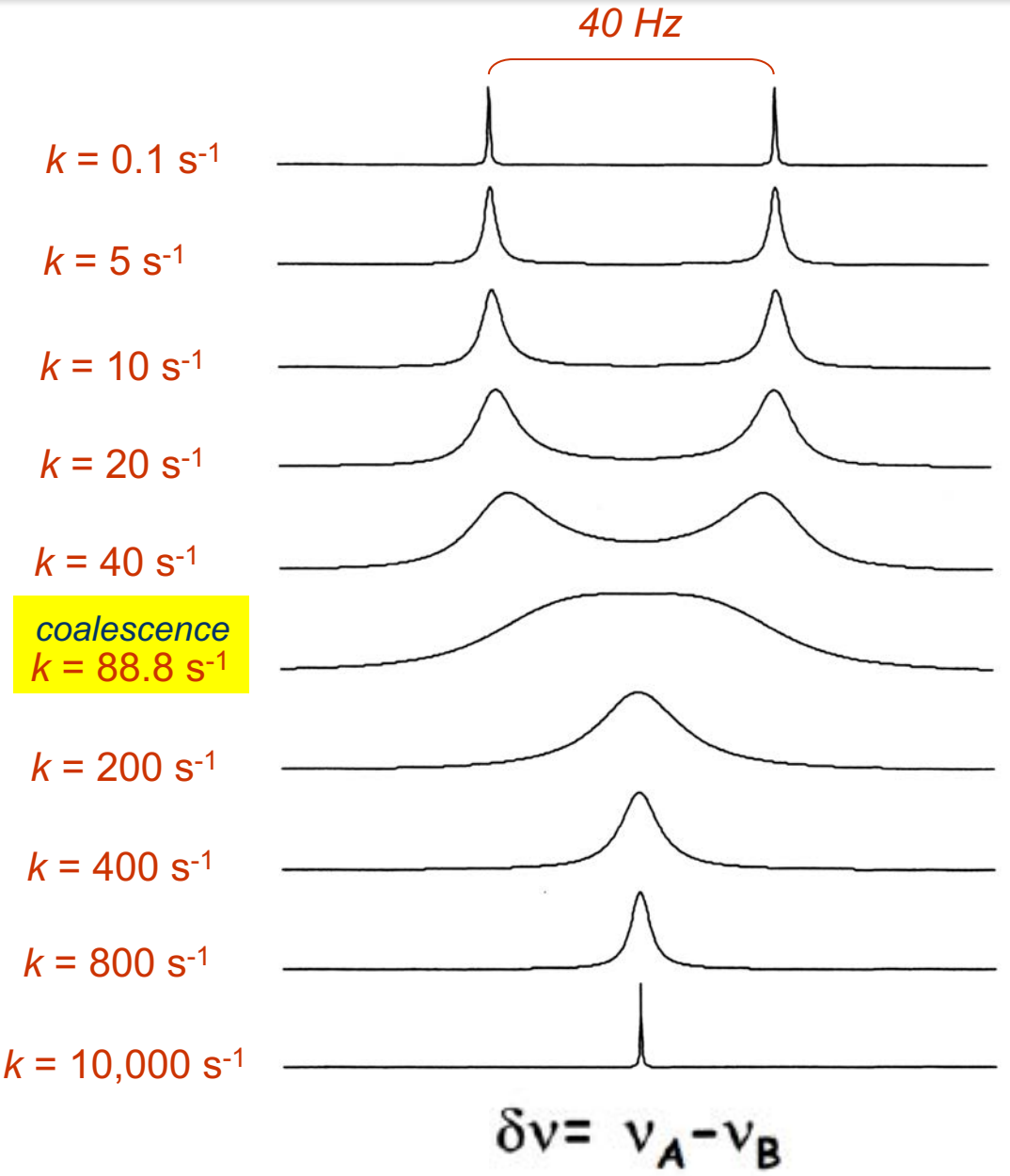
$$\Delta\nu = \frac{k}{\pi} = \frac{1}{\pi\tau}$$

$$\Delta\nu = \frac{\pi\,\delta\nu}{\sqrt{2}} = 2.2\,\delta\nu$$

$$\Delta\nu = \frac{\pi(\delta\nu)^2}{2k} = \frac{\pi(\delta\nu)^2\tau}{2}$$

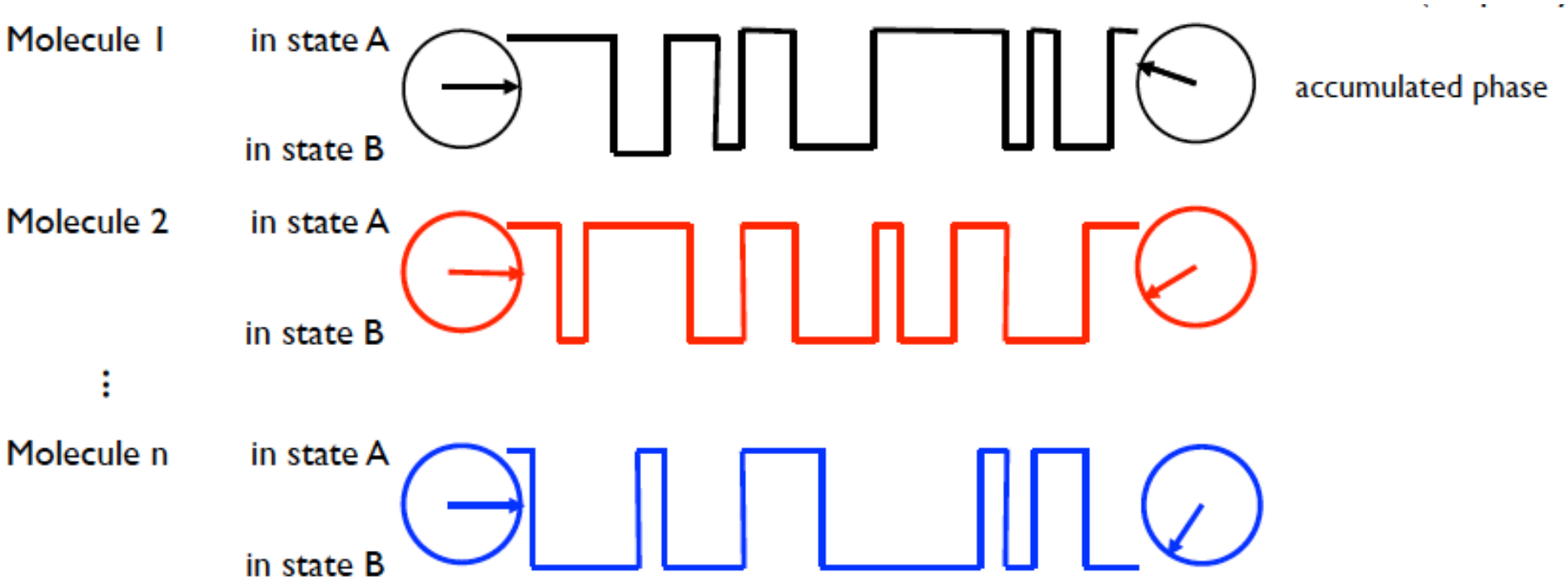
fast

Increasing Exchange Rate

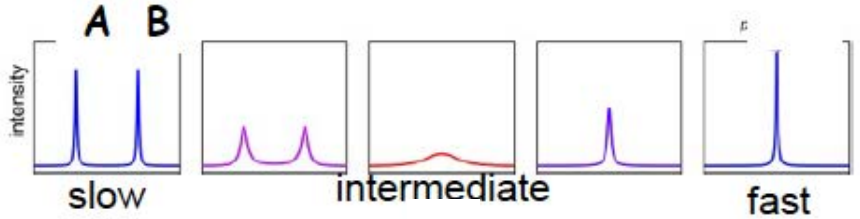


Probing conformational exchange by NMR

In the presence of a stochastic process



Conformational exchange leads to line broadening
– depending on the time scale



Probing conformational exchange by NMR: relaxation-dispersion experiment

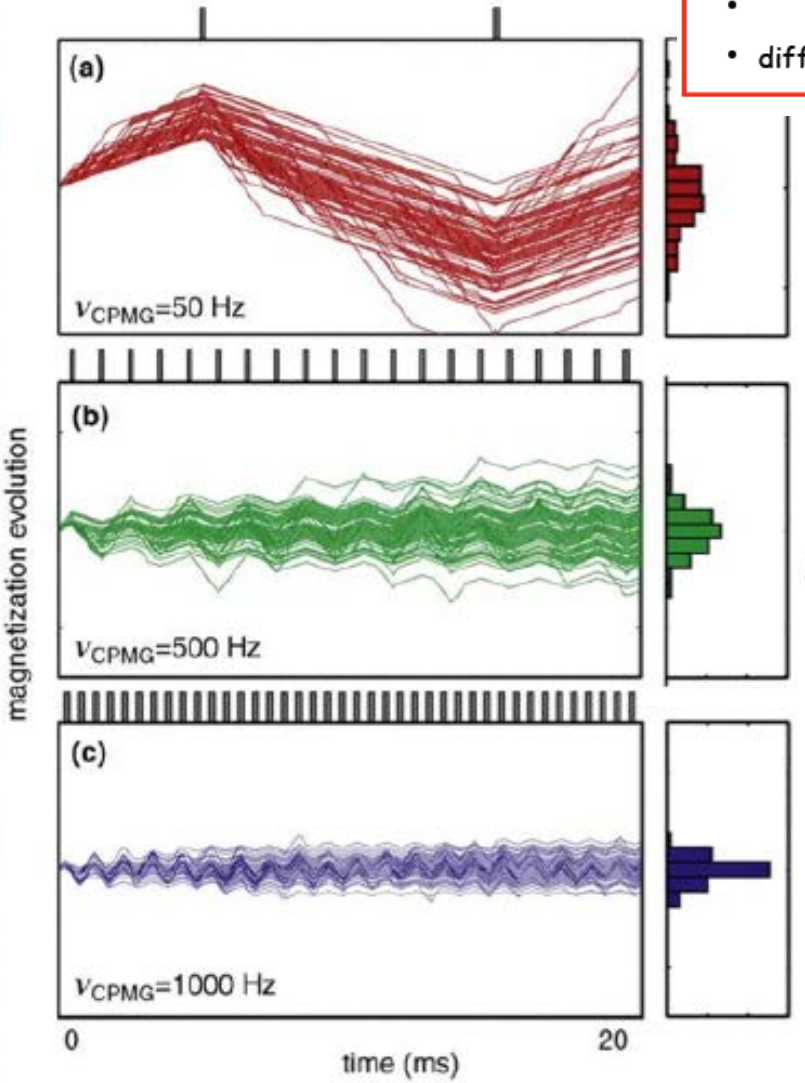
Fit of dispersion curves yields:

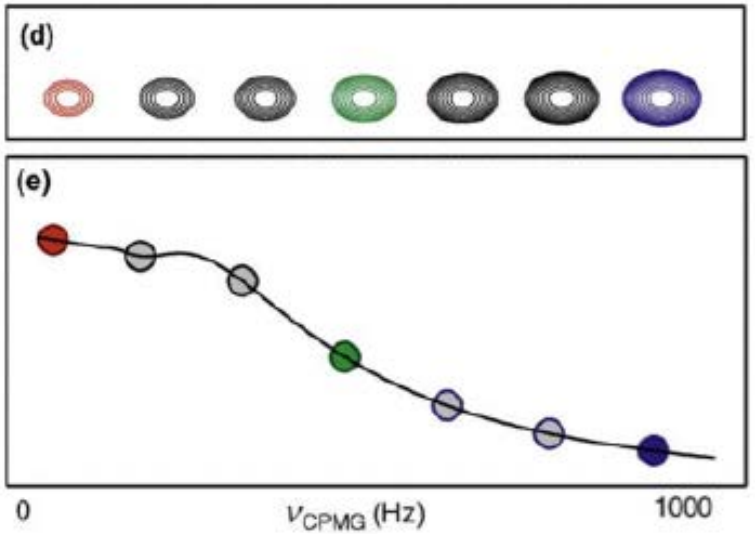
- kinetics of exchange process
- difference of chemical shifts of the involved conformations

Pi pulses applied every 20ms

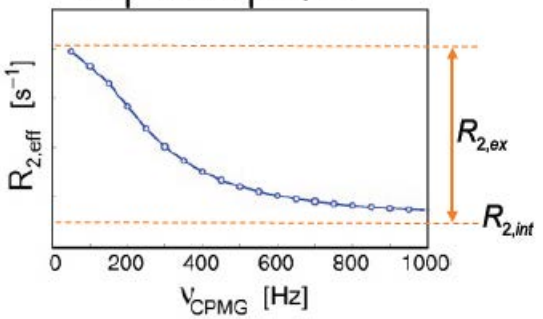
Pi pulses applied every 2ms

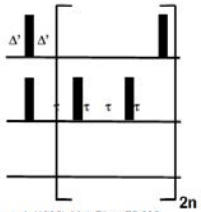
Pi pulses applied every 1ms





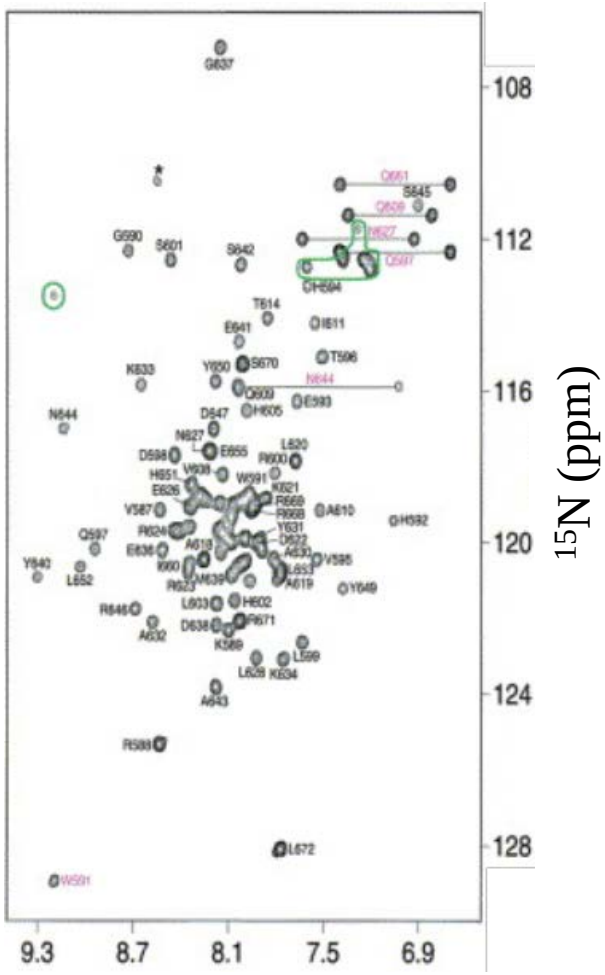
dispersion profile

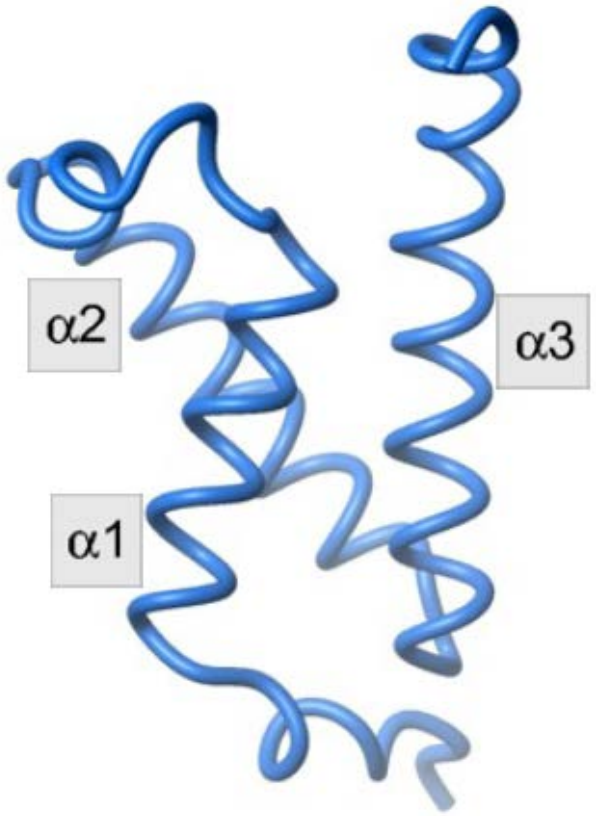




Palmer, et al. (1992) *Mol. Phys.* **75** 699

µs-ms time scales and chemical exchange

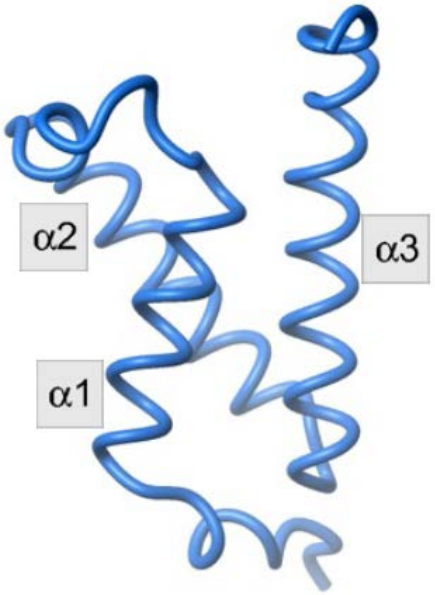




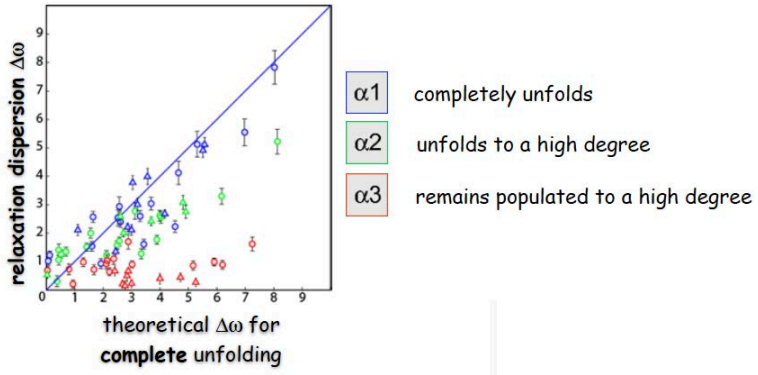
KIX a 3-helix bundle protein

Credits P. Schanda

µs-ms time scales and chemical exchange

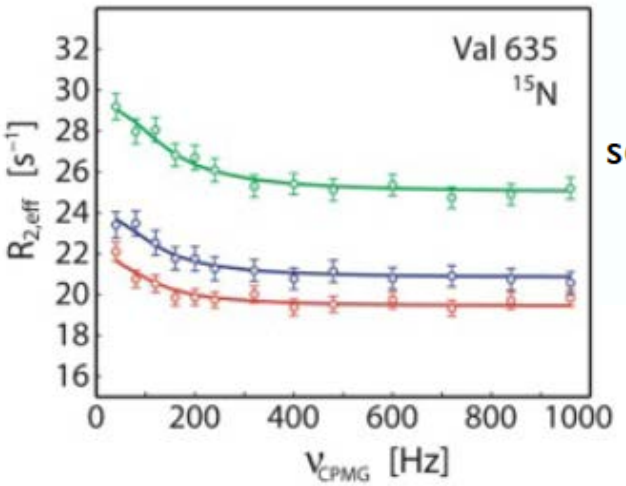


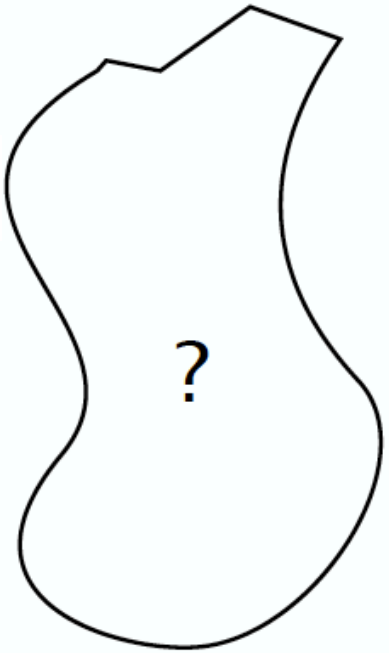
KIX a 3-helix bundle protein



$17\ s^{-1}$

$540\ s^{-1}$





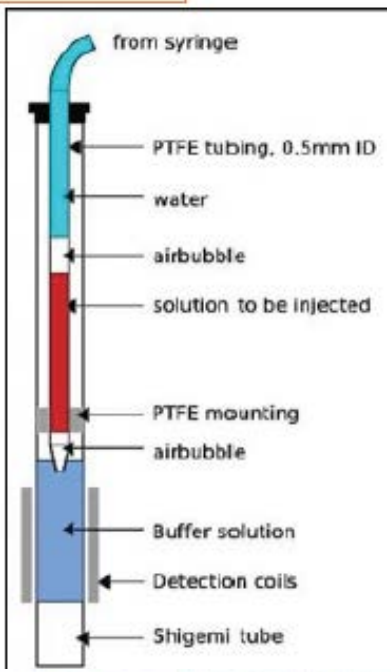
second conformation, in equilibrium with the main conformation. populated to 2.3%

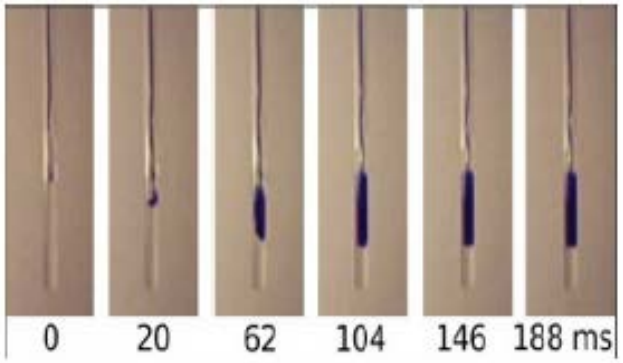
Team Schanda

Brüschweiler et al., *J. Am. Chem. Soc.* (2009)

Fast mixing and fast NMR methods allow to study rapid processes in real time

rapid mixing:

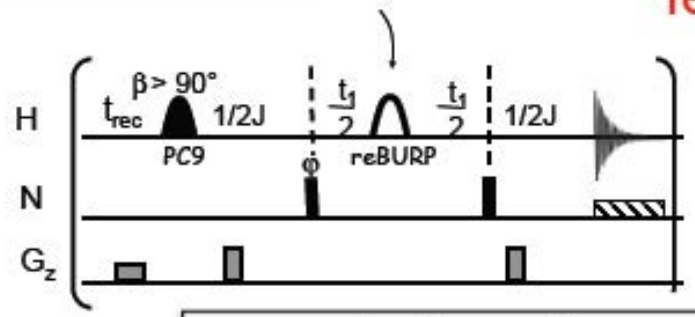


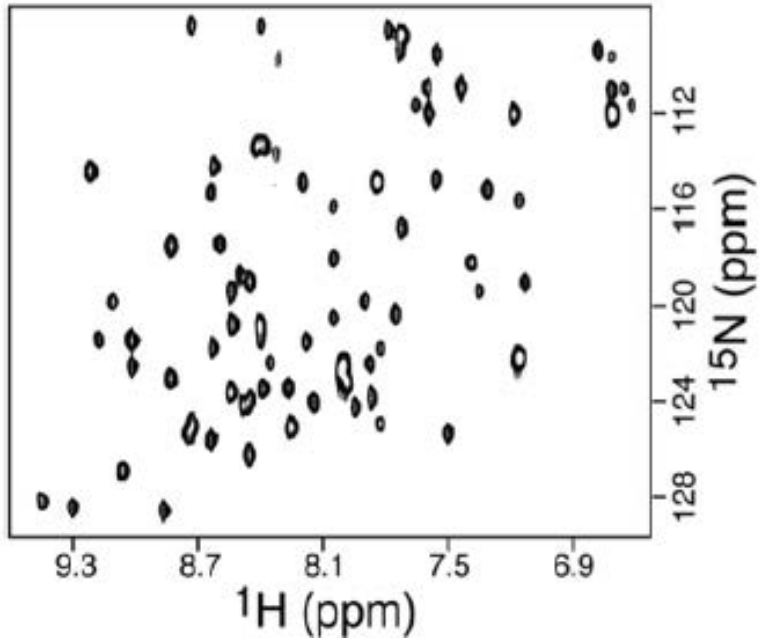


Mixing of solutions inside the NMR spectrometer

fast NMR methods:

2D spectrum in a few seconds





β2-microglobulin (8 kDa), c = 0.2 mM, experimental time: 4 s

β2-microglobulin forms intermediates with amyloid fibril character

Unfolded state ⇨ Intermediate(s)

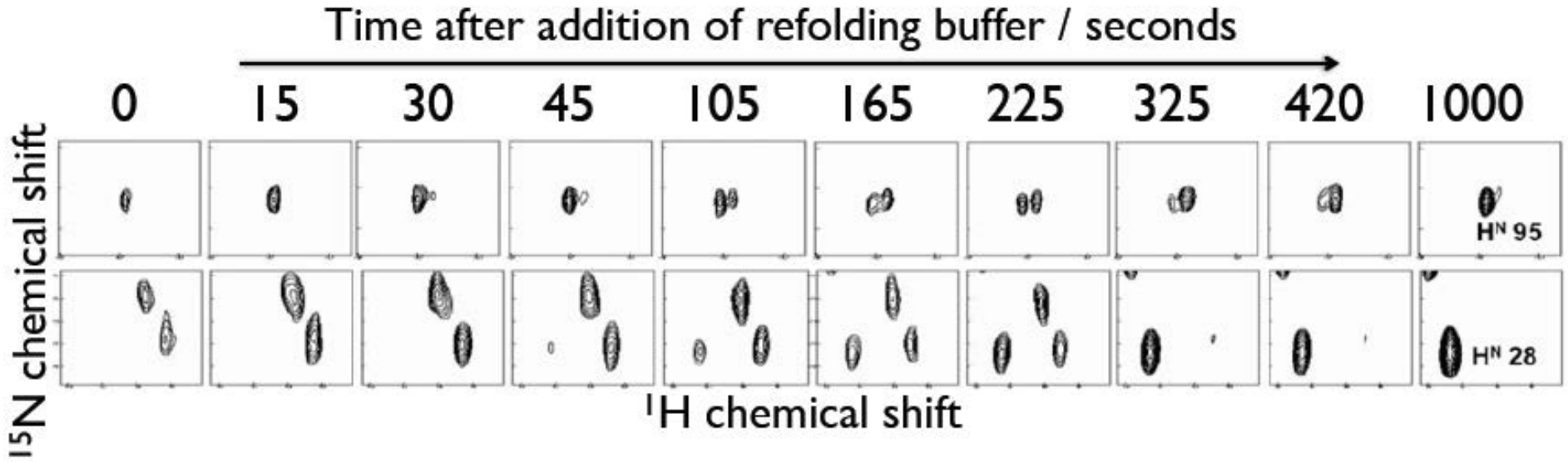
⇩

Amyloid fibrils

➔ Dialysis-related amyloidosis

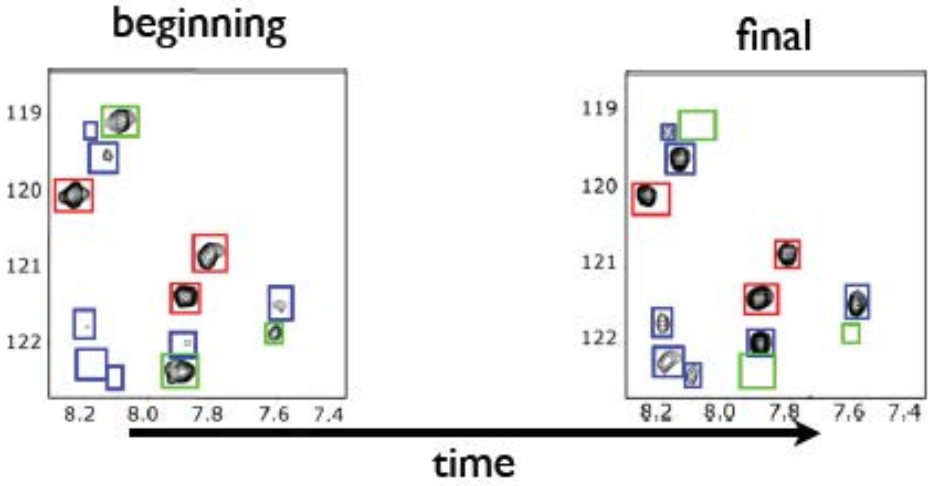
Native state

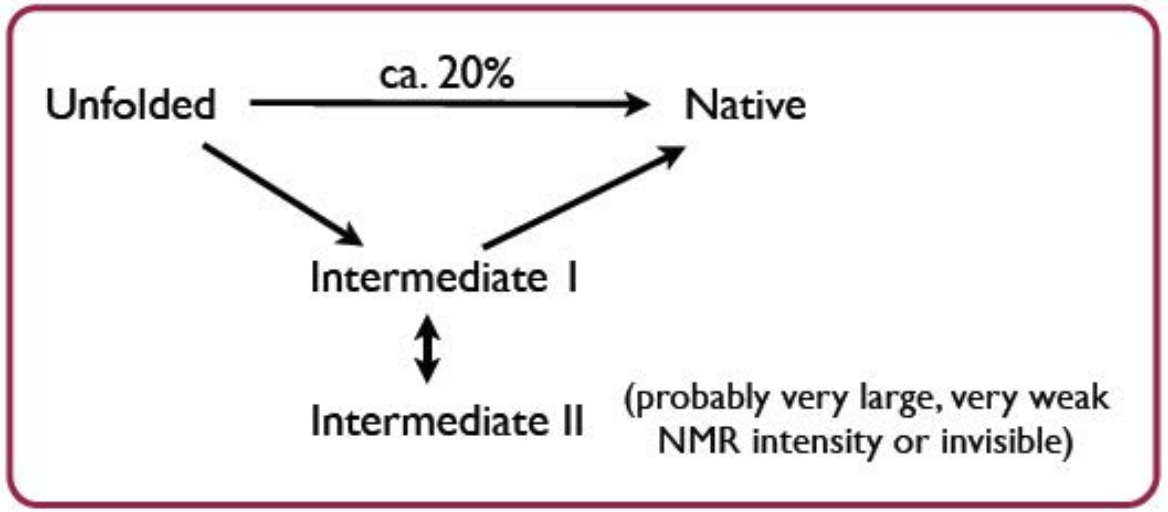
β2 microglobulin

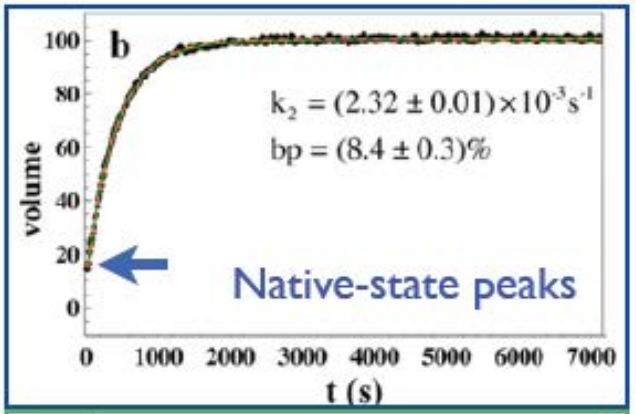


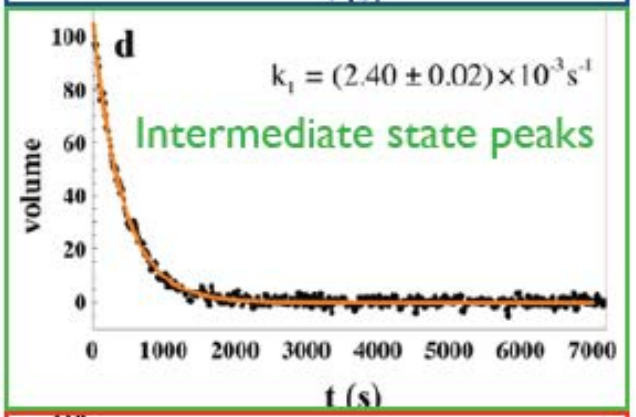
Corazza, Rennella, Schanda *et al., J. Biol. Chem.* (2010), 285: 5827-35

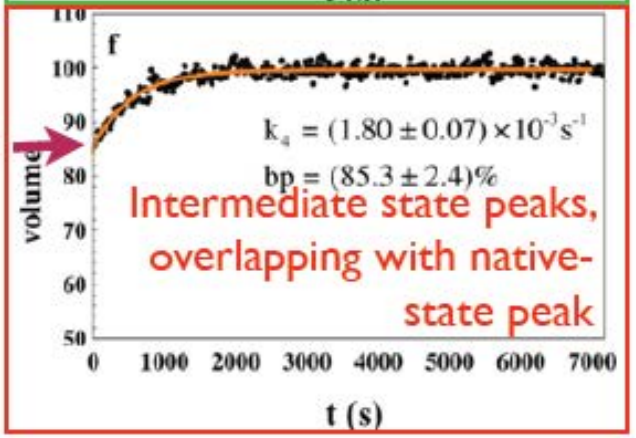
Model of the folding pathway of β2-microglobulin





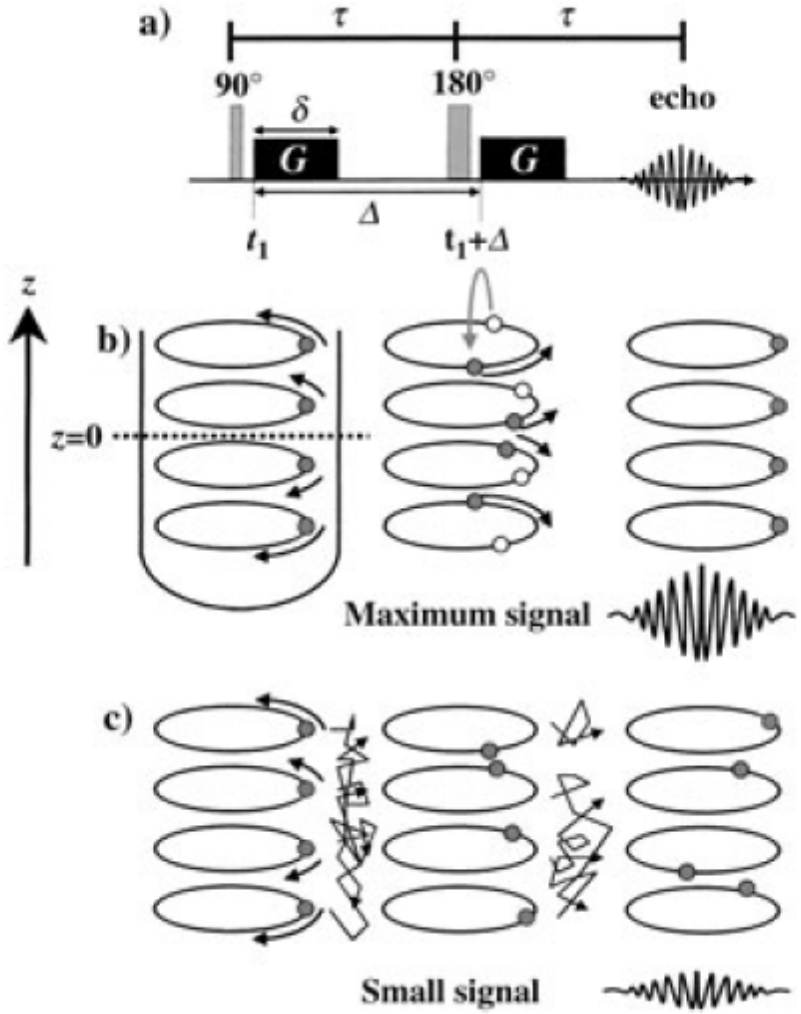






s-time scales and translational diffusion, size information: translational diffusion experiments

The modified Hahn-echo sequence : Stejskal and Tanner (1965)



$$\phi_i(\tau) = \gamma B_0 \tau + \gamma g_0 \int_{t_1}^{t_1+\delta} z_i(t)dt$$

no diffusion

with diffusion

Y. Cohen, L. Avram and L. Frish, *Angew. Chem. Int. Ed.*, **2005**, 44, 520-554

s-time scales and translational diffusion, size information: translational diffusion experiments

$$M_t(2\tau) = M_0 e^{-\frac{2\tau}{T_2}} e^{-\gamma^2 (G_z \delta)^2 (\Delta - \delta/3) D}$$

$$\ln \frac{M_t(2\tau, G_z)}{M_t(2\tau, 0)} = -\underbrace{\gamma^2 (G_z \delta)^2 \left(\Delta - \delta/3\right) D}_{b}$$

Large molecule

Small molecule

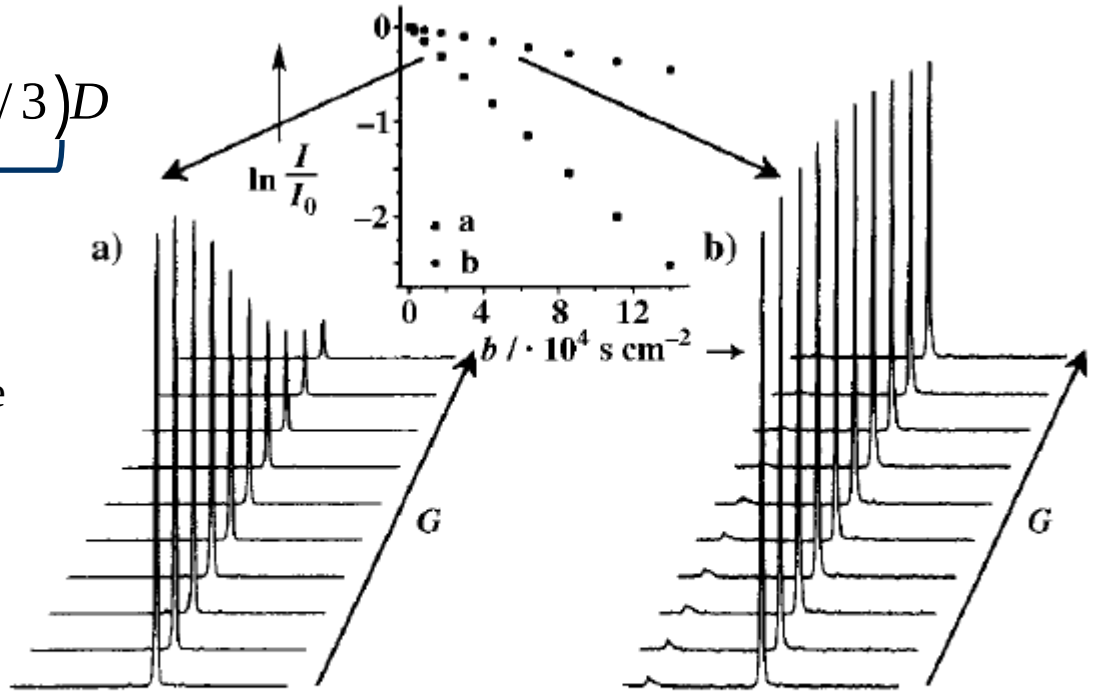


Figure 3. Signal decays as a function of G of the following diffusion coefficients: a) $D = 1.81 \times 10^{-5}\ cm^2 s^{-1}$ and b) $D = 0.33 \times 10^{-5}\ cm^2 s^{-1}$ together with the corresponding graphical analysis of the data; $\ln(I/I_0) \equiv \ln(I_{(2\tau,G)}/I_{(2\tau,0)})$.

Y. Cohen, L. Avram and L. Frish, *Angew. Chem. Int. Ed.*, **2005**, 44, 520-554

Interest of translational diffusion measurements

Distinguishing particles of different sizes in a mixture

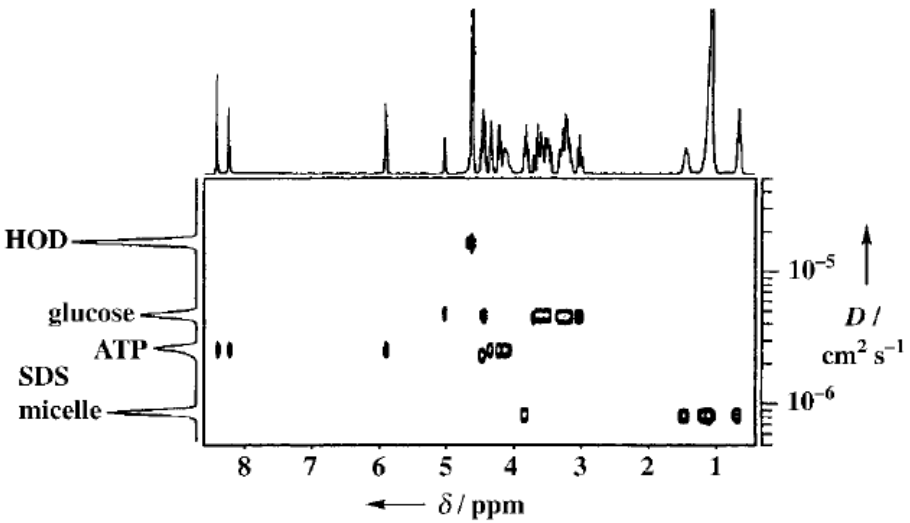
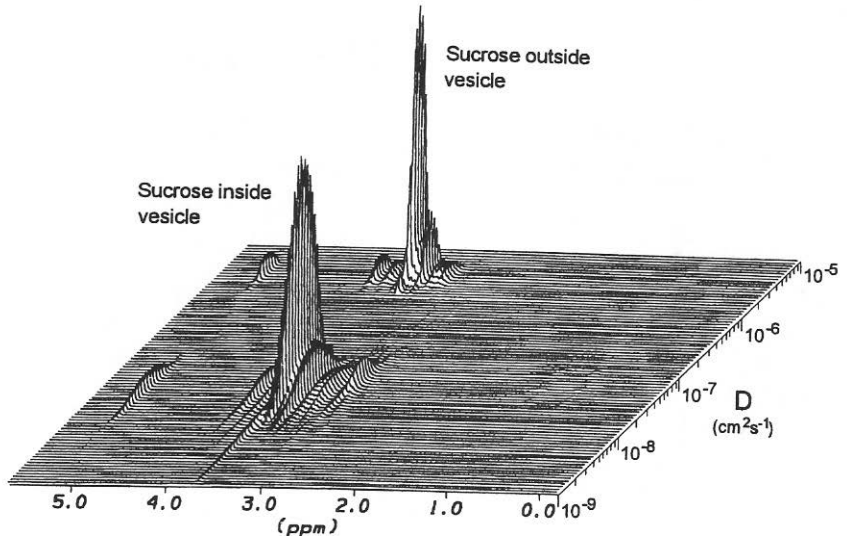


Figure 23. 2D DOSY spectrum of a mixture containing HOD, glucose, ATP, and SDS micelles. Reproduced with permission from Ref. [36a].



Unilamellar vesicle 30 mM lipid (POPC) with 100 mM sucrose

Angew. Chem. Int. Ed. **2005**, *44*, 520 – 554

Diffusion NMR Spectroscopy in Supramolecular and Combinatorial Chemistry: An Old Parameter—New Insights

Yoram Cohen, Liat Avram, and Limor Frish*

Conclusion

✪ **Chemical shift information:**

- a structural information content
- a powerful tool to follow local changes; specific interest in functional studies

✪ **Relaxation parameters:**

- a measure of the dynamics in the ps-ns time-scale; an access to motion
- a tool for interaction studies

✪ **Scalar couplings:**

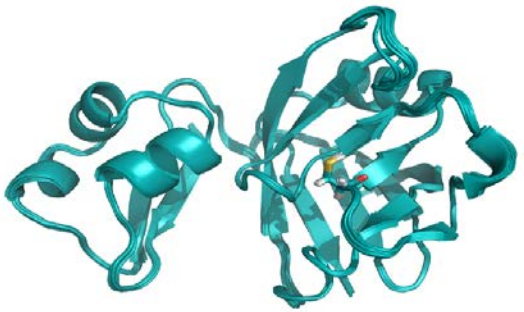
- a unique tool to transfer magnetization for the spectroscopist
- an angular information

✪ **Dipolar interactions:**

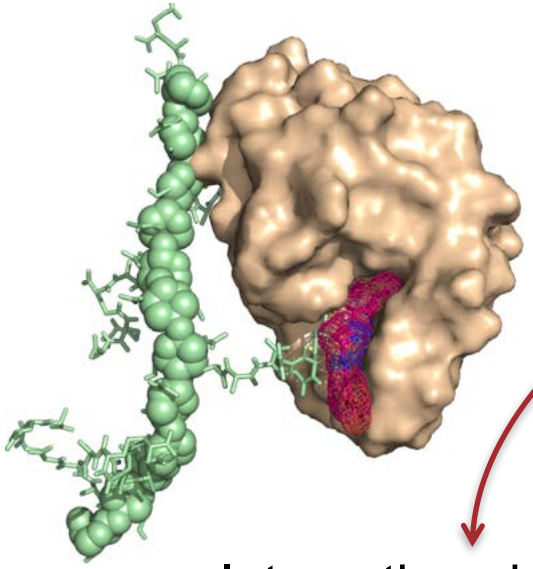
- an orientational and distance information
- a source of intermolecular contact information
- a source of dynamical information in the µs-ms time-scale

Conclusion

Structural information
Local probes

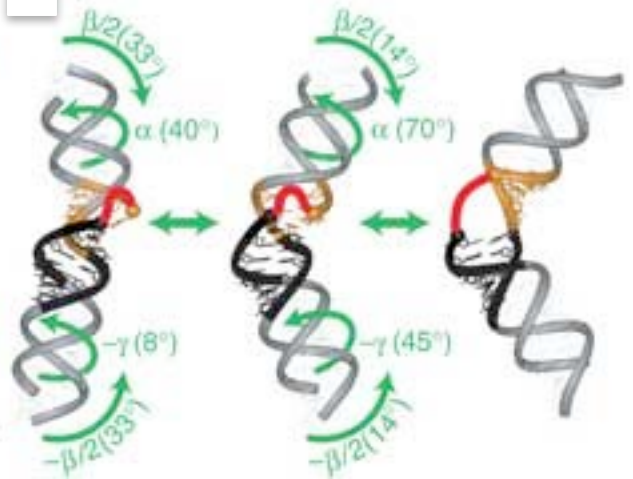


Biomolecular NMR



Interactions between different partner : interaction site, K_D

Dynamical information: amplitude and time-scale of motion




Thank
You