Basic protein characterisation

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Think before you start!

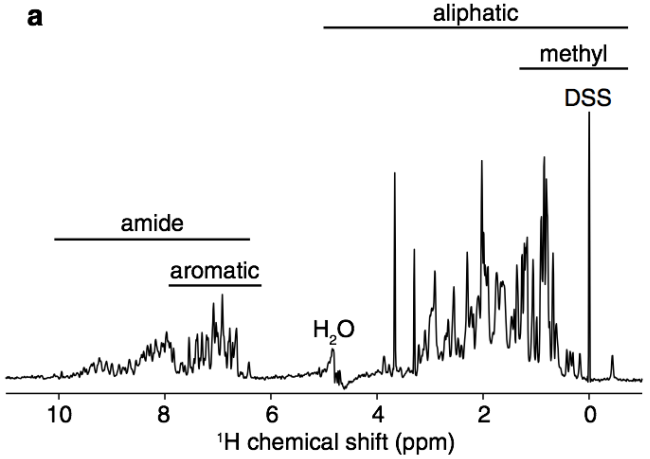
- Molecular weight
  - Folded / unfolded?
- Isotope labelling scheme
- Sample preparation
  - H_2O / D_2O
  - buffer
  - pH
  - ionic strength
- Stability (azide, protease inhibitors)
- Temperature
- Concentration
  - sensitivity
  - solubility
  - dimerisation?

Labelling schemes

^{1}H | ^{15}N ($+^{13}C$) | ^{15}N + partial deuteration | ^{2}H,^{15}N perdeuteration | ^{2}H, $^{13}CH_3$ M/I/LV labelling

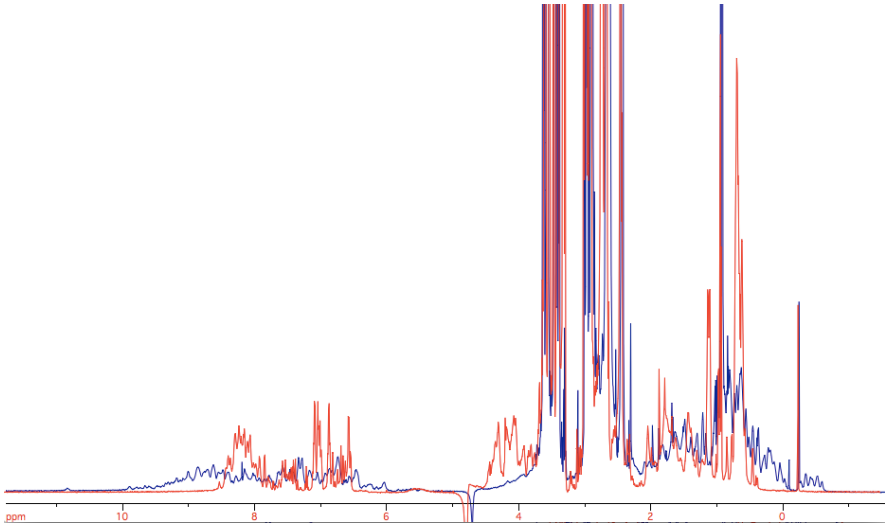
high MW
high sensitivity
high cost!

1D ^{1}H spectra

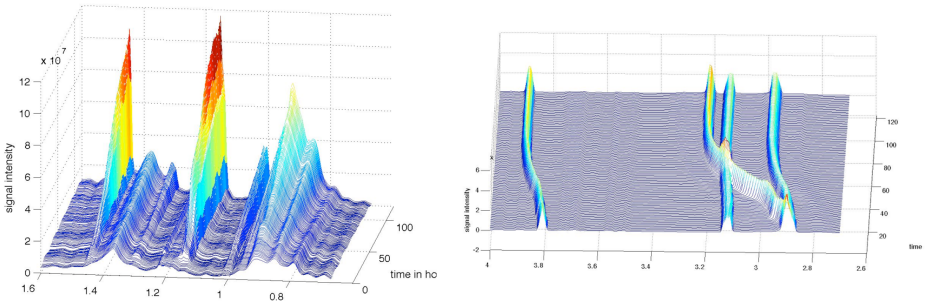


Cabrita et al. Methods Mol. Biol. 752, 97–120 (2011).

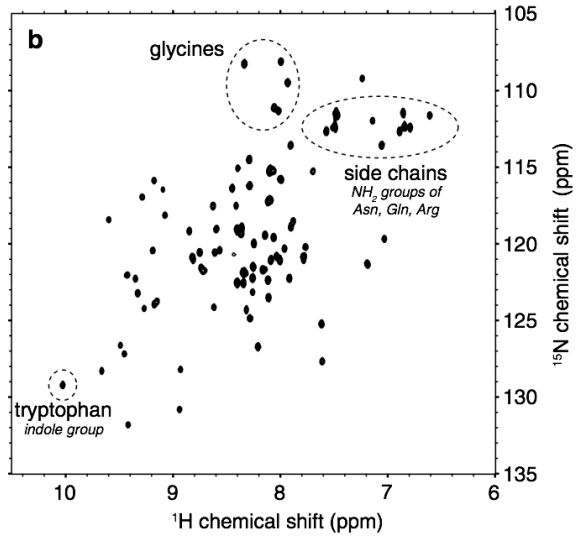
1D ^{1}H spectra: folded vs unfolded



1D ^{1}H spectra: stability over time



2D ^{1}H,^{15}N spectra: typical chemical shifts



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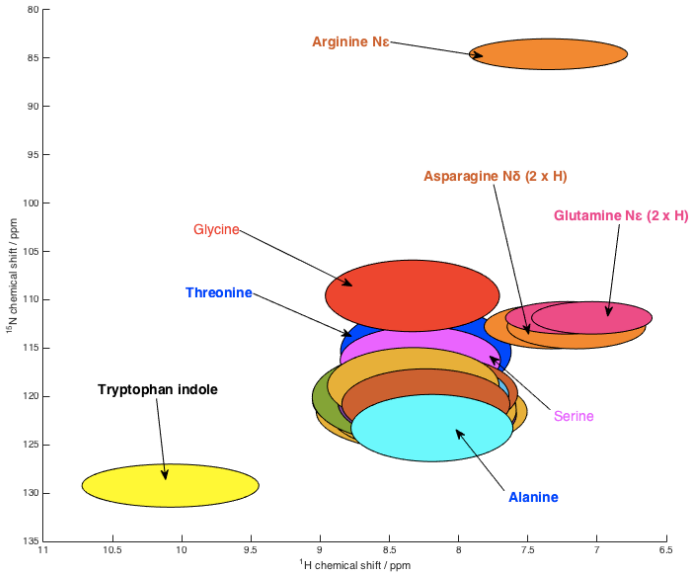
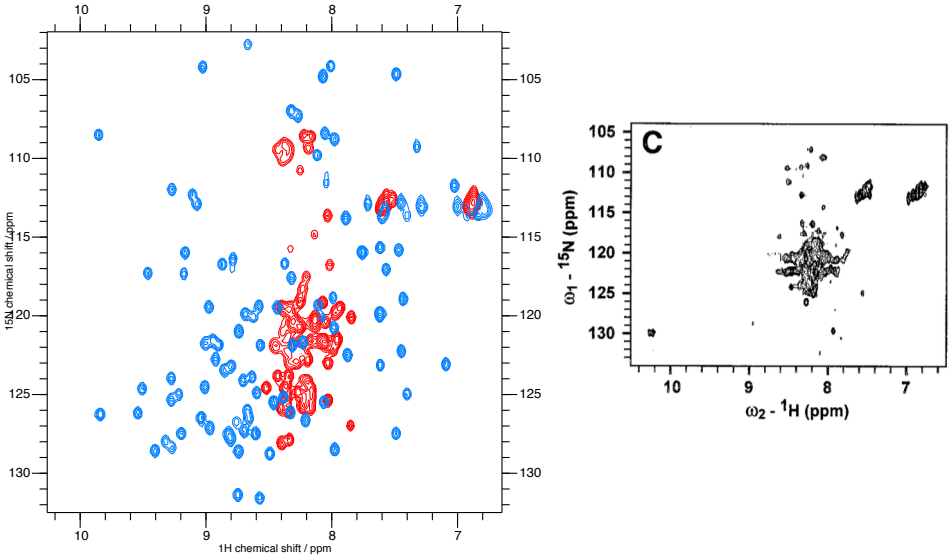
2D ^{1}H,^{15}N spectra: typical chemical shifts



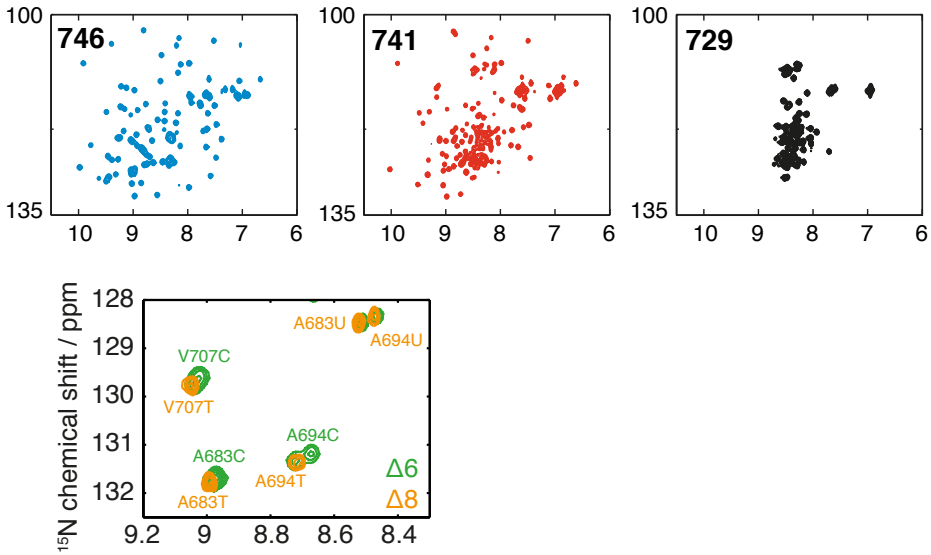
calculated from http://www.bmrb.wisc.edu

2D $^{1}H,^{15}N$ spectra: chemical shift dispersion

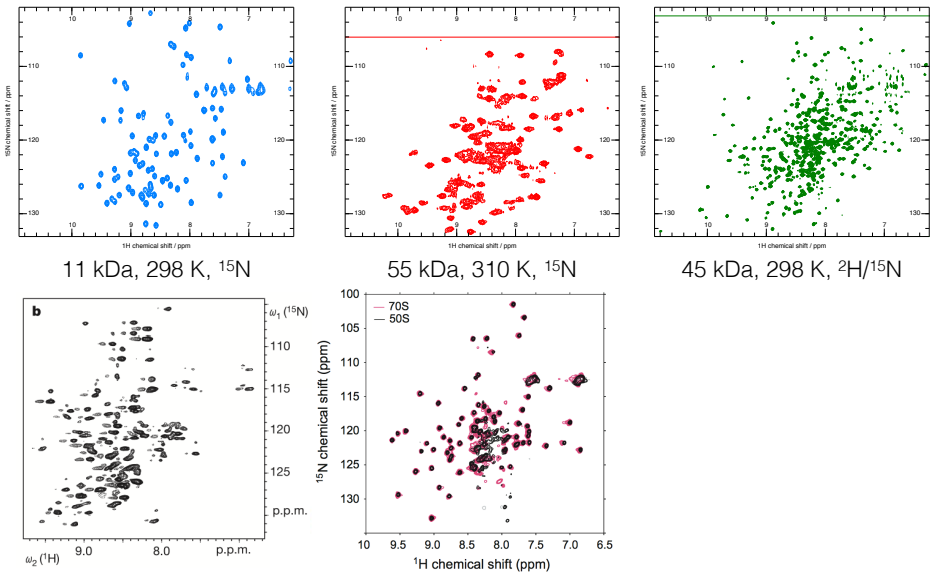


Song et al. Biochem J. 356, 151–158 (2001)

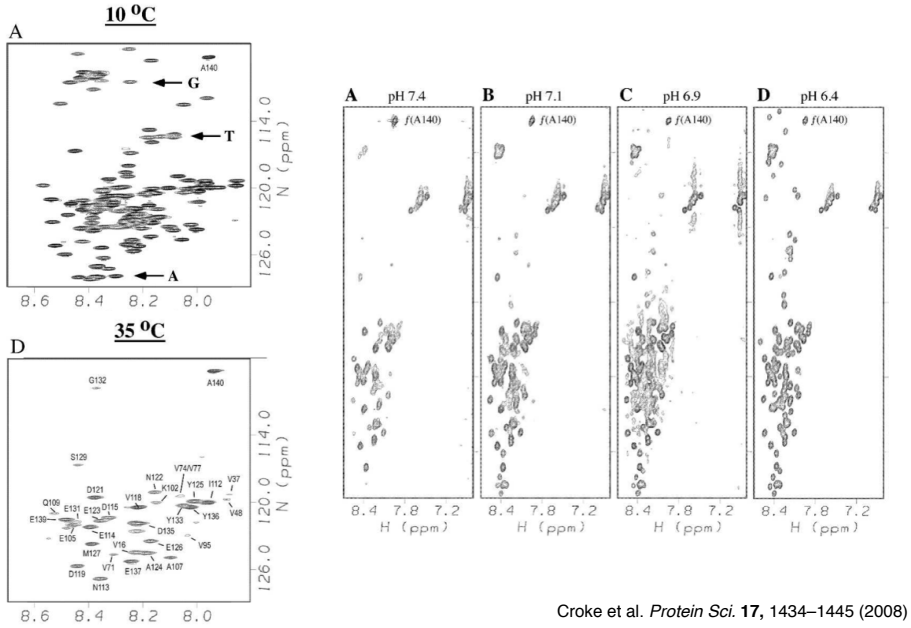
2D $^{1}H,^{15}N$ spectra: peak counts



2D $^{1}H,^{15}N$ spectra: molecular weight

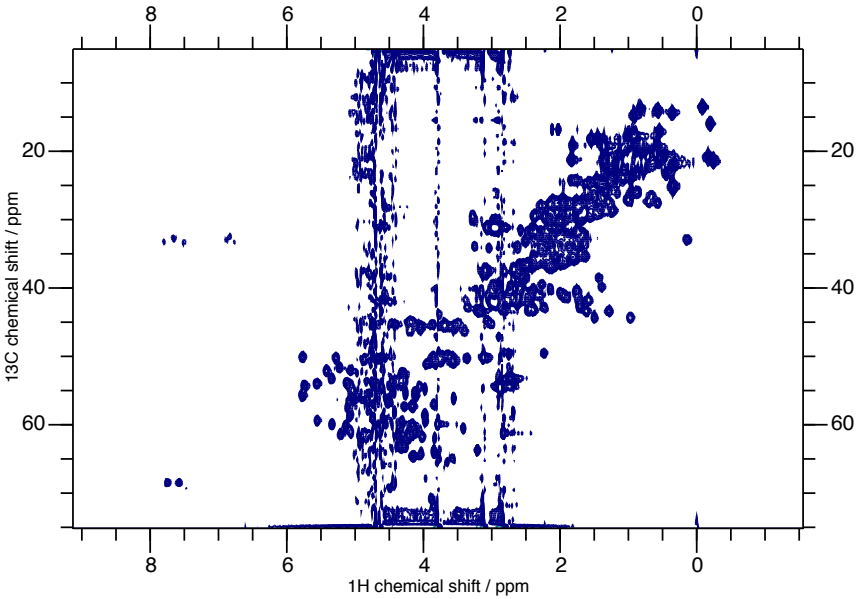


2D $^{1}H,^{15}N$ spectra: IDPs, temperature and pH

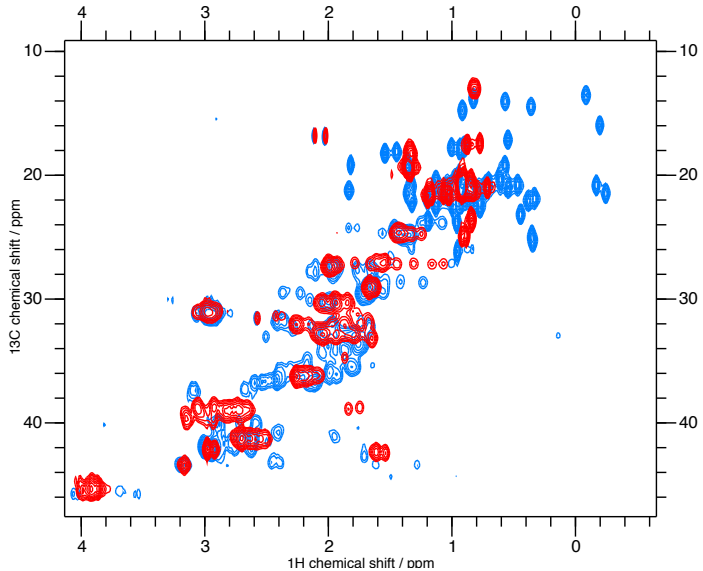


Croke et al. *Protein Sci.* **17,** 1434–1445 (2008)

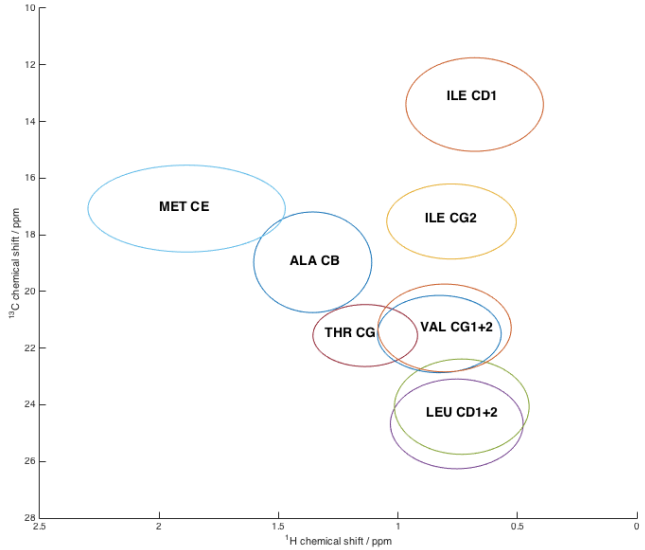
2D $^{1}H,^{13}C$ spectra: typical chemical shifts



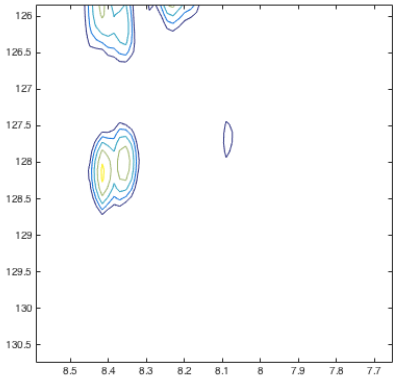
2D $^{1}H,^{13}C$ spectra: chemical shift dispersion

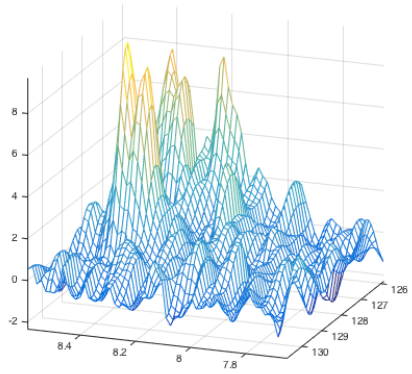


2D $^{1}H,^{13}C$ spectra: methyl chemical shifts



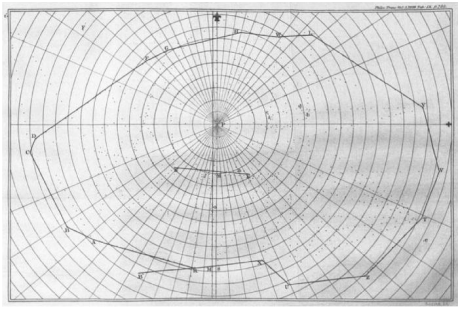
2D spectra: assessing sensitivity



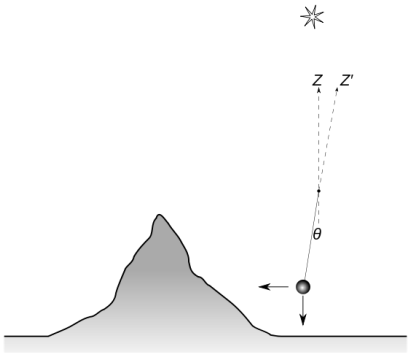


Contour plots can be deceptive!

The first contour plot: the Schiehallion experiment (1774)

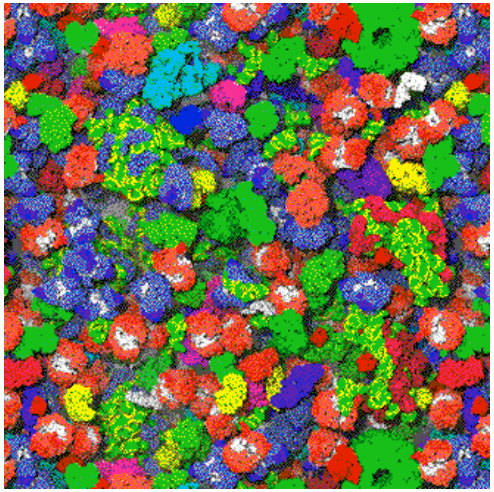


Charles Hutton (1778)



Map Of A Nation: A Biography Of The Ordnance Survey
By Rachel Hewitt

Rotational diffusion



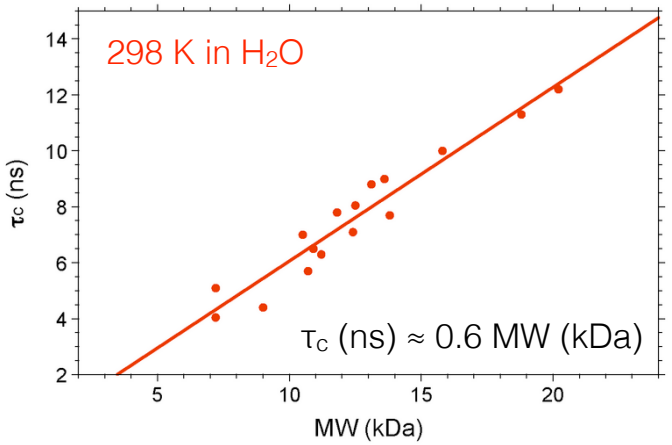
Rotational correlation time = average time to rotate by one radian (57°)

$$\tau_c = \frac{4\pi\eta r_h^3}{3k_BT}$$

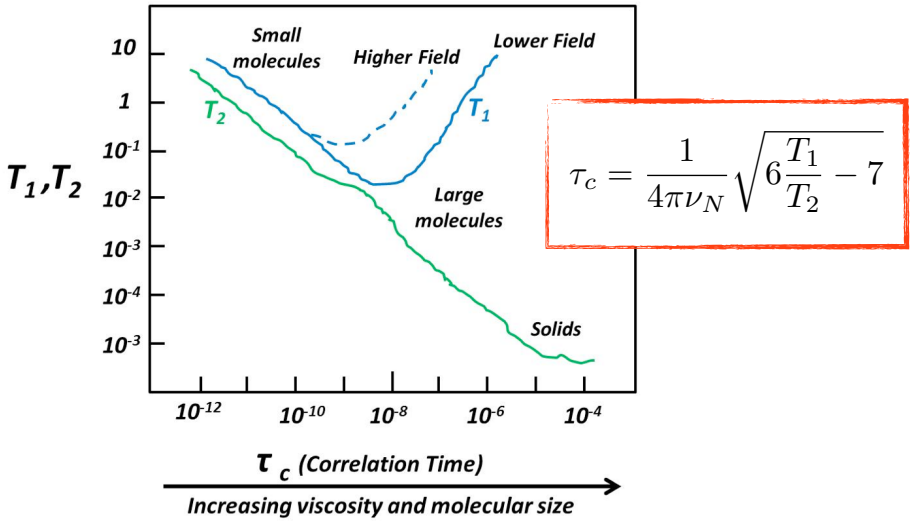
McGuffee, S. R. & Elcock, A. H. PLoS Comput. Biol. 6, e1000694 (2010).

Stokes–Einstein–Debye equation: correlation time *vs* molecular weight

$$\tau_c = \frac{4\pi\eta r_h^3}{3k_BT} = \frac{\eta V}{k_BT}$$



Estimating rotational correlation times from ^{15}N T_1 and T_2



$$\tau_c = \frac{1}{4\pi\nu_N}\sqrt{6\frac{T_1}{T_2} - 7}$$

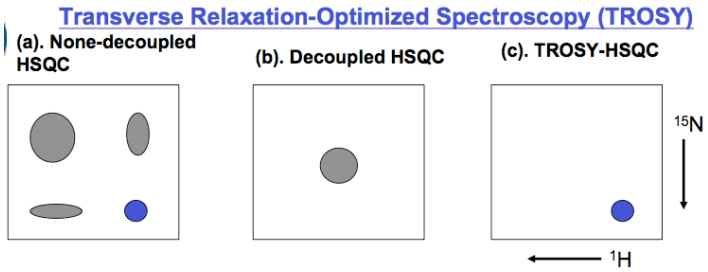
Rotational correlation times from ^{15}N T_1 and T_2 – assumptions!

- All peaks contribute equally

$$\tau_c = \frac{1}{4\pi\nu_N}\sqrt{6\frac{T_1}{T_2} - 7}$$

- Spherical molecule (isotropic tumbling)
- Rigid (S^2 order parameters = 1)
- Correlation time WILL ALWAYS BE UNDERESTIMATED!

TRACT: correlation times for big proteins



Effective rotational correlation times of proteins from NMR relaxation interference

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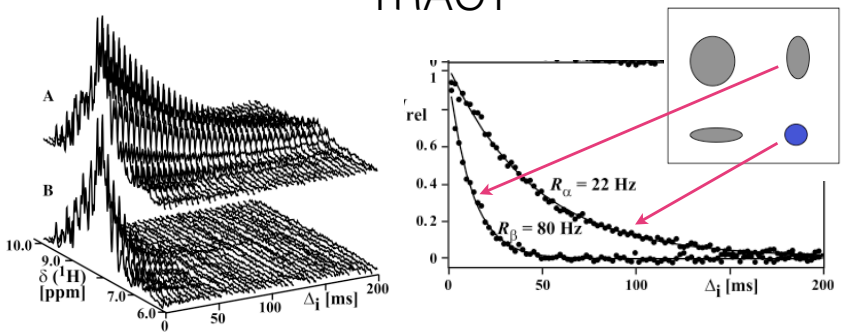
J. Magn. Reson. **178,** 72–76 (2006)

TRACT



$$R_\alpha = \lambda - \eta_{xy} + R_H + R_{CS}$$
$$R_\beta = \lambda + \eta_{xy} + R_H + R_{CS}$$

$$R_\beta - R_\alpha = 2\eta_{xy} = 2p\delta_N(4J(0) + 3J(\omega_N))(3\cos^2\theta - 1)$$

$$J(\omega) = 0.4\tau_c/[1 + (\tau_c\omega)^2]$$

TRACT analysis in Excel

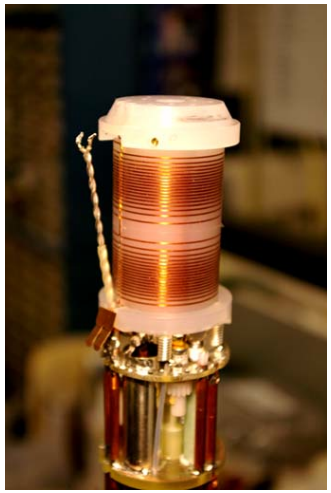
TRACT analysis						
Observed rates				**Constants**		
R_alpha	27	s-1		mu0	1.2566E-06	
R_beta	47	s-1		gammaH	2.68E+08	s-1 T-1
				gammaN	-2.71E+07	
Spectrometer				h	6.63E-34	J s
Field	700	MHz		rHN	1.02E-10	m
				ΔδN	1.60E-04	
Calculations				θ	17.00	degrees
ΔR	20	s-1				
η_{xy}	10	s-1		**Derived parameters**		
				B0	16.44	T
Result				p	-2.55E+04	
tau_c	8.35	ns		δN	-16813.707	
				pδN(3cos²θ-1)	7.47E+08	
				ω_N	2.80E+09	rad s-1

Translational diffusion

- Global property – all residues in a protein have the same diffusion coefficient
- Stokes–Einstein relation to hydrodynamic radius:

$$D = \frac{kT}{6\pi\eta R}$$

Pulsed-field gradients in NMR

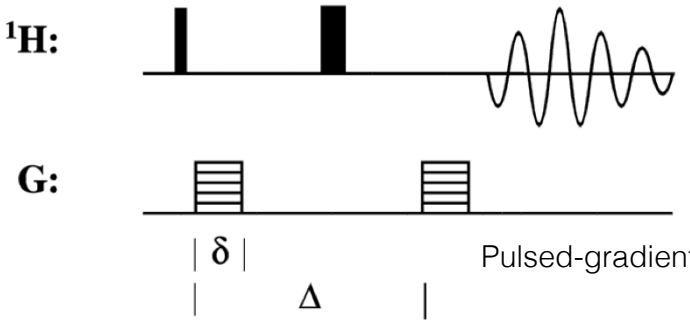


Disruption of field homogeneity

Magnetic field strength linearly proportional to position along *z*-axis:

$$B = B_0 + G \cdot z$$

NMR measurement of translational diffusion

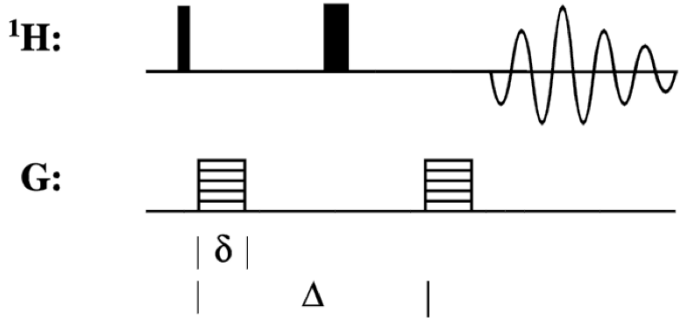


Pulsed-gradient spin-echo

Pairs of gradients encode & decode the z-position of spins.

Diffusion occurring during the delay Δ results in imperfect refocusing, and reduction in observed signal.

Data analysis: the Stejskal-Tanner equation



$$\frac{I}{I_0} = \exp\left[-(G\gamma\delta)^2(\Delta - \delta/3)D\right]$$

Hydrodynamic Radii of Native and Denatured Proteins Measured by Pulse Field Gradient NMR Techniques†

Deborah K. Wilkins, Shaun B. Grimshaw, Véronique Receveur,‡ Christopher M. Dobson, Jonathan A. Jones,§ and Lorna J. Smith*

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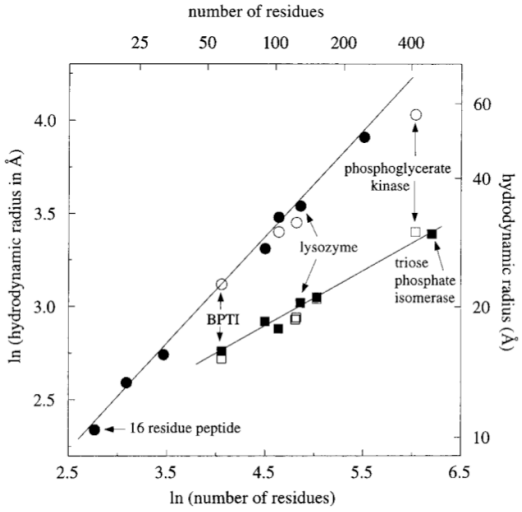

Summary: rotational *vs* translational diffusion

	Rotational	Translational
Experiment	T_1/T_2 ratio, TRACT	pulsed gradient echos
Labelling required	^{15}N (^{2}H)	none
Measurement type	local	global
Relation to structure	τ_c (ns) ≈ 0.6 MW (kDa)	$D = \frac{kT}{6\pi\eta R}$
Sensitivity to dimerisation	high (τ_c ~ MW)	low (D ~ MW$^{-1/3}$)