Introduction to biomolecular NMR spectroscopy

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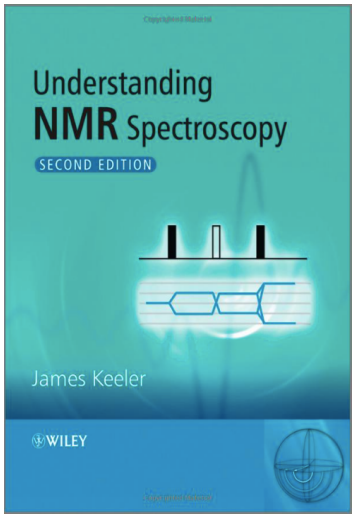
Course outline

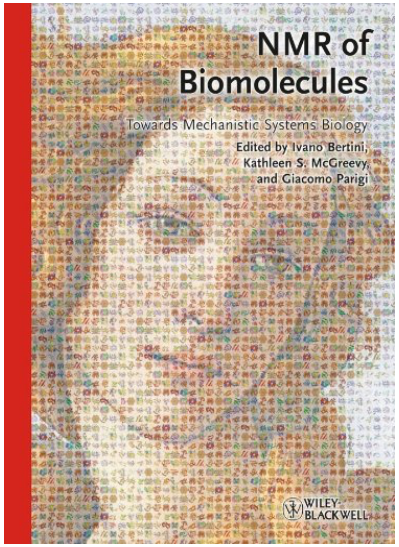
- Approx. 50:50 split:
  1 hr lectures,
  1 hr examples class
- Exercises from Keeler, Understanding NMR Spectroscopy, 2nd ed
- I will NOT be lecturing NMR theory directly – expect self-study during the week
- Excellent lectures from James Keeler are already available on YouTube:
  http://goo.gl/PdbkUQ



Further reading

- Online lectures for those who want a deeper understanding of quantum mechanics:

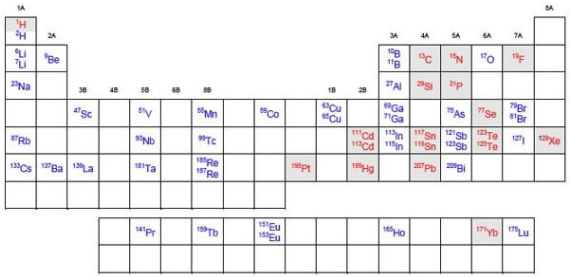
  http://theoreticalminimum.com



Nuclear spin and the Zeeman effect

- Nuclear spin $S = 0, 1/2, 1, 3/2, \ldots$ is a fundamental quantum mechanical property of a nucleus
- Zeeman effect: In the presence of a magnetic field, B, the system splits into $(2S - 1)$ energy levels

Magnetically-active nuclei



S = 1/2

NMR 'friendly'

S > 1/2

quadrupolar moment
=> broad lines

Common nuclei for biomolecular NMR

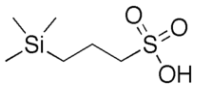
	Spin	Natural abundance	γ / 10^7 s^{-1} T^{-1}	Frequency / MHz
1H	1/2	99.985%	26.7522	700
2H	1	0.015%	4.1066	107.5
13C	1/2	1.108%	6.7283	176
15N	1/2	0.37%	–2.7126	71
19F	1/2	100%	25.18148	659
31P	1/2	100%	10.8394	283.6

frequencies calculated for $B_0 \approx 16.4$ T

The chemical shift

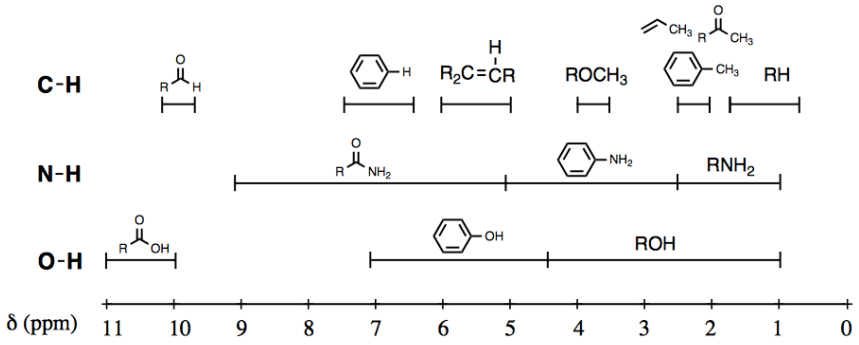
- Exact resonance frequencies are dependent on shielding by electrons at the nucleus:

- NMR absorption frequencies are dependent on the field strength B_0. Normalise using frequency of a reference compound to define the 'chemical shift', comparable between different NMR spectrometers:



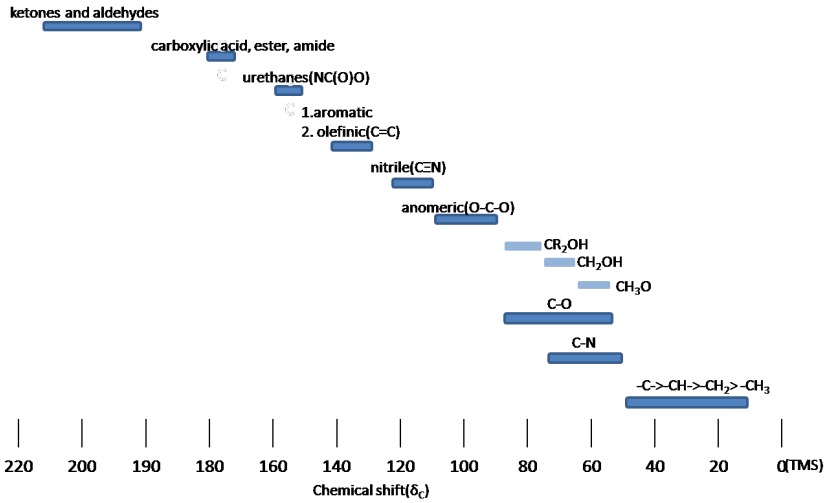
DSS

^{1}H chemical shifts



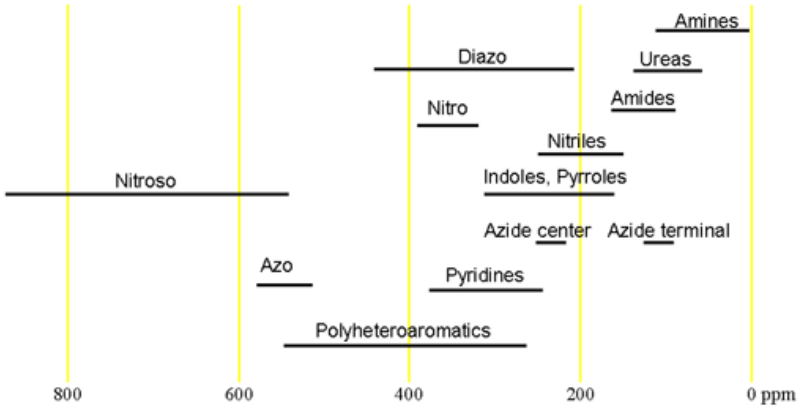
https://www.cpp.edu/~lsstarkey/courses/NMR/NMRshifts1H-general.pdf

^{13}C chemical shifts



http://chemwiki.ucdavis.edu/Physical_Chemistry/Spectroscopy/Magnetic_Resonance_Spectroscopies/Nuclear_Magnetic_Resonance/NMR%3A_Experimental/NMR%3A_Interpretation

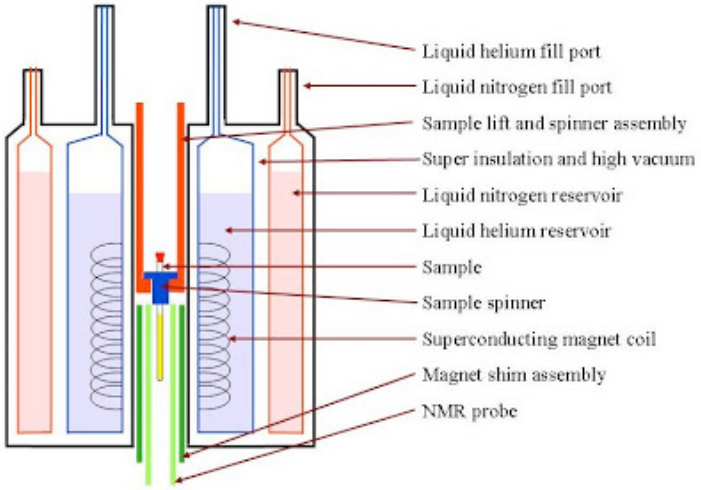
15N chemical shifts



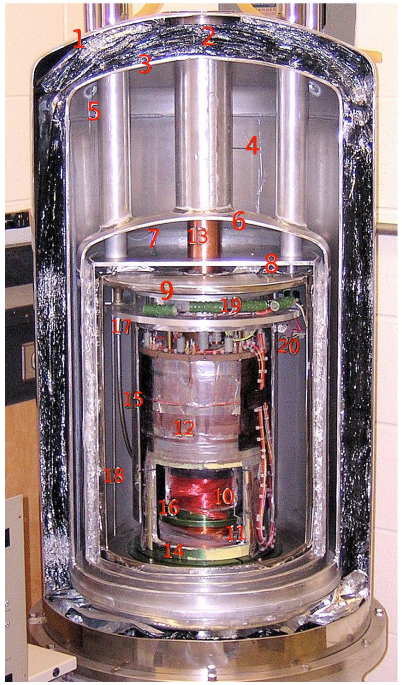
http://chem.ch.huji.ac.il/nmr/techniques/1d/row2/n.html

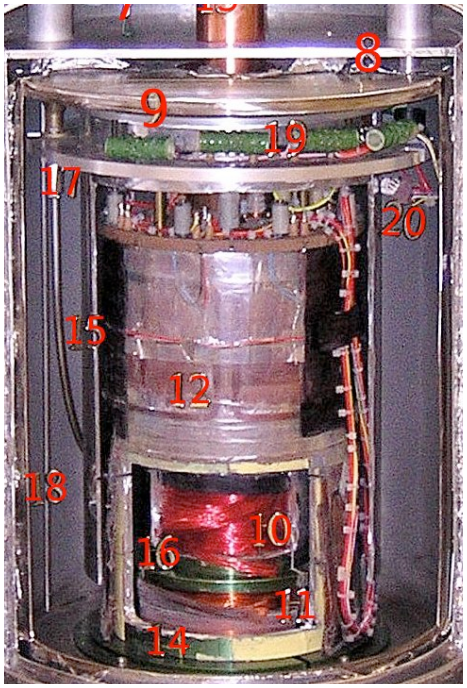
Introduction to NMR spectrometers

Cutaway of an NMR spectrometer

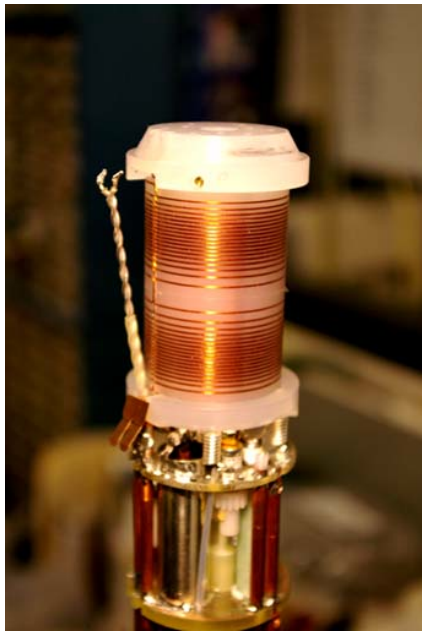


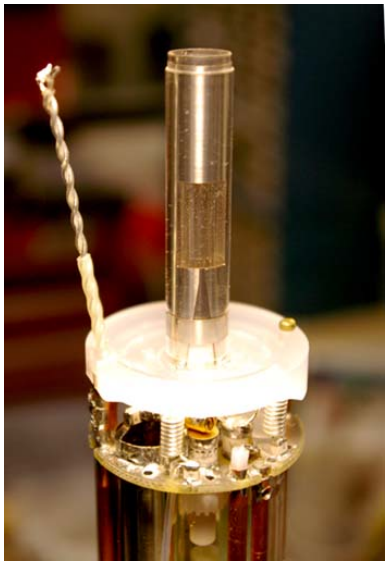
http://u-of-o-nmr-facility.blogspot.co.uk

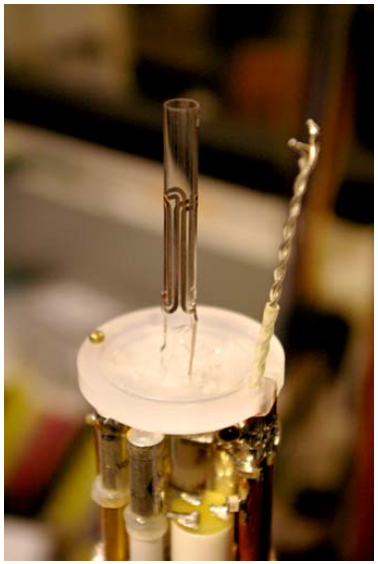




http://web.mit.edu/speclab/www/Facility/shim-probe-sample.html







Quenching

Sensitivity

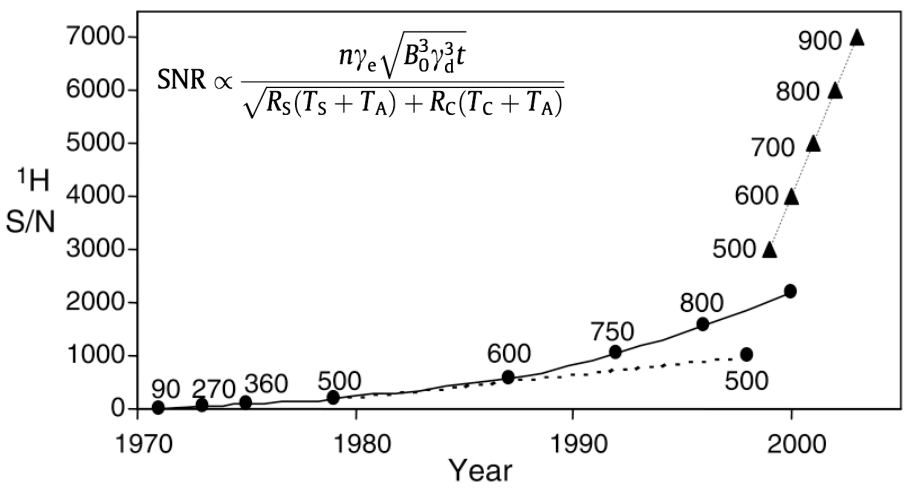
- NMR is not a sensitive technique – due mainly to the fact that the difference between energy levels is very small.
- The absolute sensitivity depends on many factors:

$$\mathrm{SNR} \propto \frac{n\gamma_e\sqrt{B_0^3\gamma_d^3 t}}{\sqrt{R_S(T_S+T_A)+R_C(T_C+T_A)}}$$

Cryoprobes

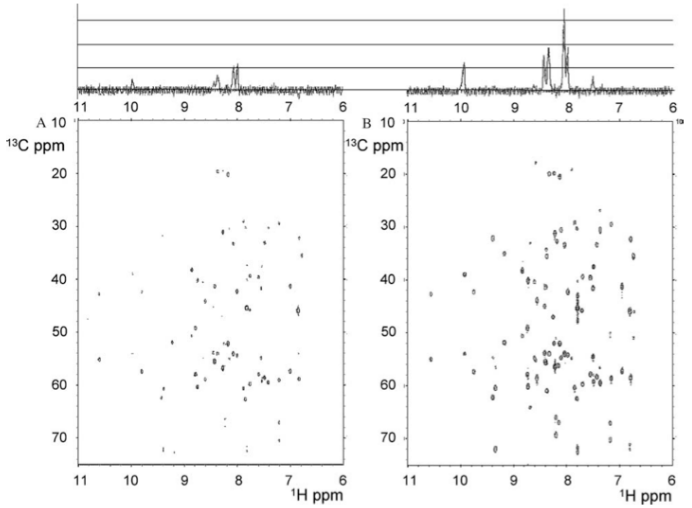
P. Styles et al. / JMR 213 (2011) 355–356
H. Kovacs et al. / Progress in Nuclear Magnetic Resonance Spectroscopy 46 (2005) 131–155

Cryoprobes



H. Kovacs et al. / Progress in Nuclear Magnetic Resonance Spectroscopy 46 (2005) 131–155

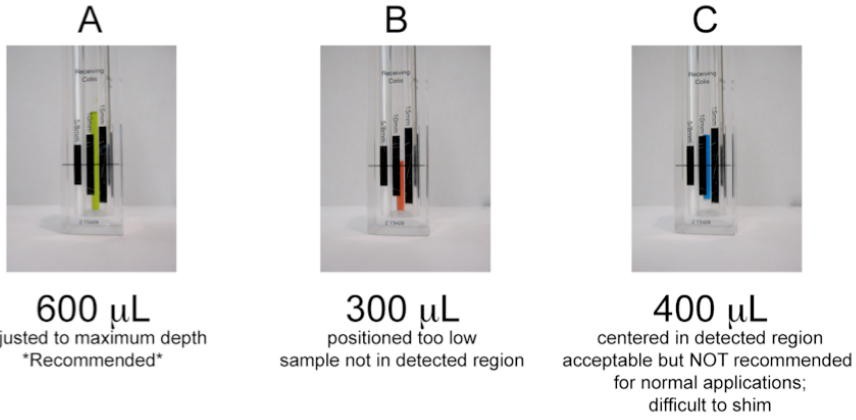
Cryoprobes



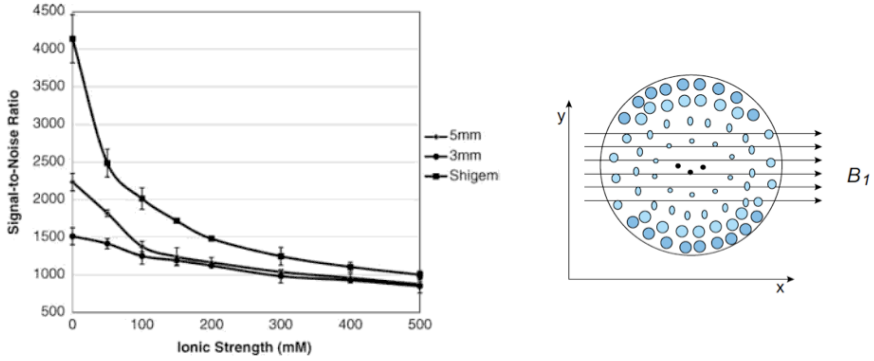
H. Kovacs et al. / Progress in Nuclear Magnetic Resonance Spectroscopy 46 (2005) 131–155

NMR tubes and sample volume

- Regular NMR tube: 550 – 600 µL
- Shigemi tube: 250 – 300 µL
- Shigemi without plunger: 400 µL
- 3 mm tubes: 200 – 250 µL

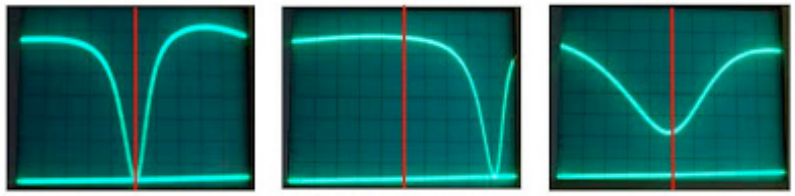


NMR tubes and sample volume



Tuning and matching

- Probe electronics act as a bandpass filter: for optimum sensitivity, must tune and match to let your signals through!



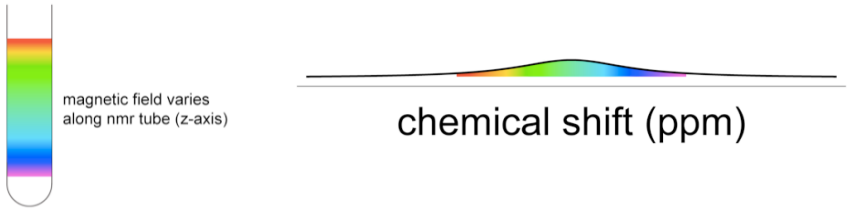
Locking

- Modern NMR demands highly homogeneous fields that do not vary over time
- Lock system: 'spectrometer within a spectrometer'
- Constantly monitors ^{2}H frequency in solvent (HDO resonance) and adjusts electromagnet to compensate for any drift

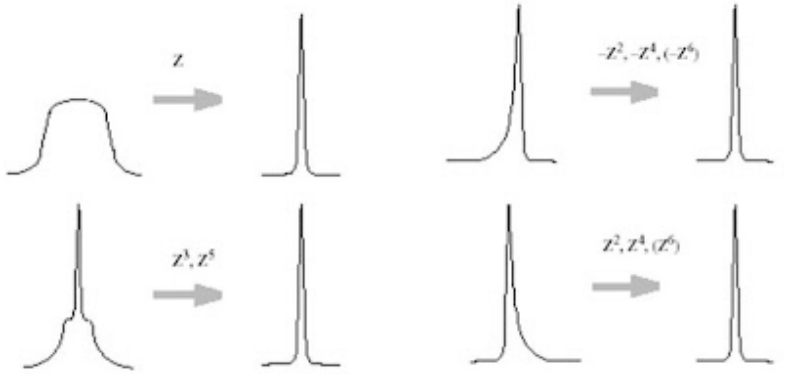
Shimming

- Process of optimising field homogeneity to ≤1 ppb (<1 Hz)
- But protein resonances are broad anyway – why bother?

poorly shimmed magnet

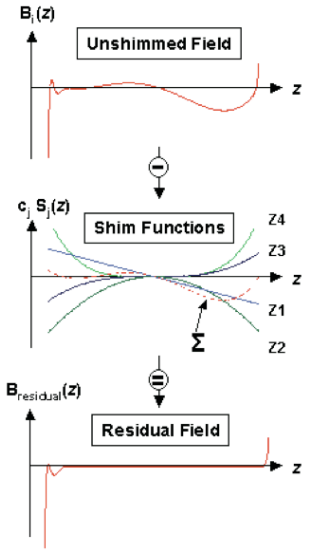


Effect on lineshapes

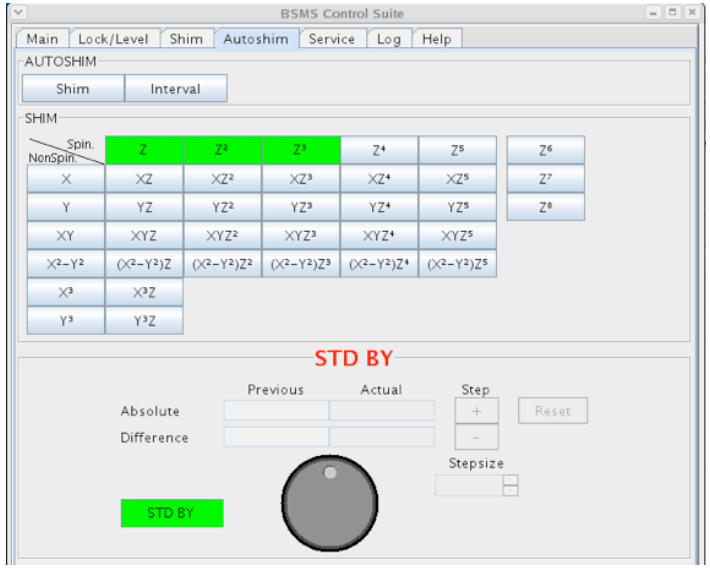


Gradient shimming

- Magnetic resonance imaging (MRI) experiment to map the water chemical shift across sample
- Shim coils then adjusted using their known profiles to obtain a homogenous field

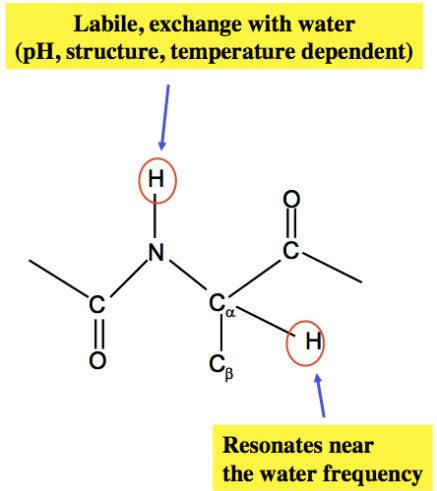


Autoshim



Sample preparation

- Solvent: H_2O / D_2O?



Sample preparation

- Solvent: H_2O / D_2O?
- Choice of buffer:
  - protonation / spectral overlap?
  - ionic strength
  - conductivity

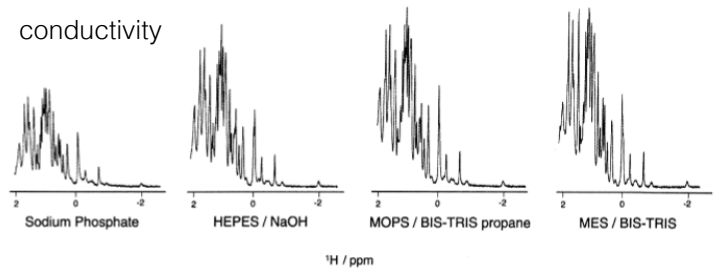


Figure 3. One-dimensional spectra of a 1 mM lysozyme sample measured in 50 mM sodium phosphate, 50 mM HEPES/NaOH, or 50 mM MOPS/BIS−TRIS propane buffer, all pH 7, and in 50 mM MES/BIS−TRIS, pH 6.0. Only the most high-field-shifted regions of the spectra are shown.

Kelly, A. E., Ou, H. D., Withers, R. & Dötsch, V. JACS 124, 12013–12019 (2002)

Effect of buffers on sensitivity

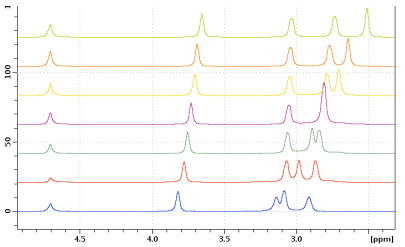
Table 1. R_0/R_c Values, Expected Sensitivity Factor *L*, and Dc Conductivity of Several Different Salts, All at 200 mM Concentration

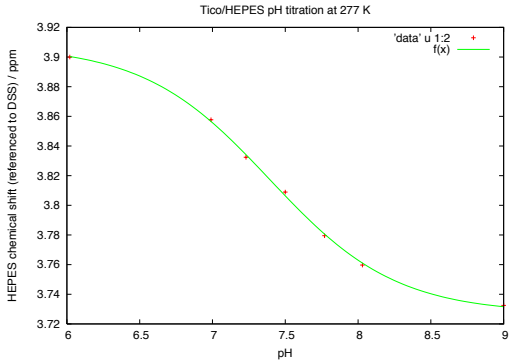
buffer	R_0/R_c	sensitivity factor *L*	conductivity (mS/cm)
pentasodium tripolyphosphate	2.71 ± 0.04	0.22	31.3
potassium chloride	1.93 ± 0.04	0.26	23.3
disodium phosphate (Na_2HPO_4)	1.89 ± 0.04	0.26	22.0
sodium pyrophosphate	1.70 ± 0.04	0.27	20.2
sodium chloride	1.64 ± 0.04	0.28	18.1
PIPES	1.33 ± 0.04	0.30	14.8
β-glycerophosphate	1.31 ± 0.04	0.30	14.9
potassium phosphate (KH_2PO_4)	1.25 ± 0.04	0.31	14.1
TRIS HCl	1.24 ± 0.04	0.31	14.1
BIS−TRIS HCl	1.12 ± 0.03	0.33	13.62
sodium acetate	1.11 ± 0.03	0.33	12.2
sodium phosphate (NaH_2PO_4)	0.95 ± 0.03	0.35	11.0
sodium TAPS	0.90 ± 0.03	0.36	9.55
sodium MES	0.88 ± 0.03	0.36	10.18
sodium MOPS	0.88 ± 0.03	0.36	9.86
sodium TES	0.84 ± 0.03	0.37	9.41
sodium HEPES	0.84 ± 0.03	0.37	9.25
tetrabutylammonium dihydrogen phosphate	0.69 ± 0.03	0.40	9.00
HEPES	0.22 ± 0.02	0.62	0.06
TAPS	0.14 ± 0.02	0.70	0.29
CAPS	0.14 ± 0.02	0.70	0.7
TES	0.12 ± 0.02	0.73	0.25
MOPS	0.10 ± 0.02	0.76	0.04
CHES	0.08 ± 0.02	0.79	0.06
MES	0.08 ± 0.02	0.80	0.15
bicine	0.05 ± 0.02	0.86	0.031
BIS−TRIS propane	0.05 ± 0.02	0.86	0.022
TRIS base	0.03 ± 0.02	0.91	0.1
BIS−TRIS	0.02 ± 0.02	0.93	0.0236
deionized−distilled H_2O	0.01 ± 0.02	0.98	0.0023

Kelly, A. E., Ou, H. D., Withers, R. & Dötsch, V. JACS 124, 12013–12019 (2002)

Buffer chemical shifts as internal pH reference

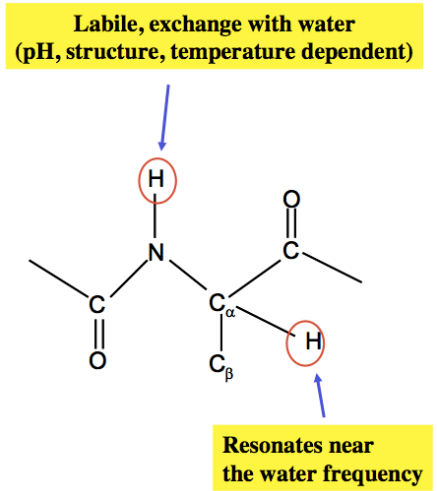
e.g. HEPES:



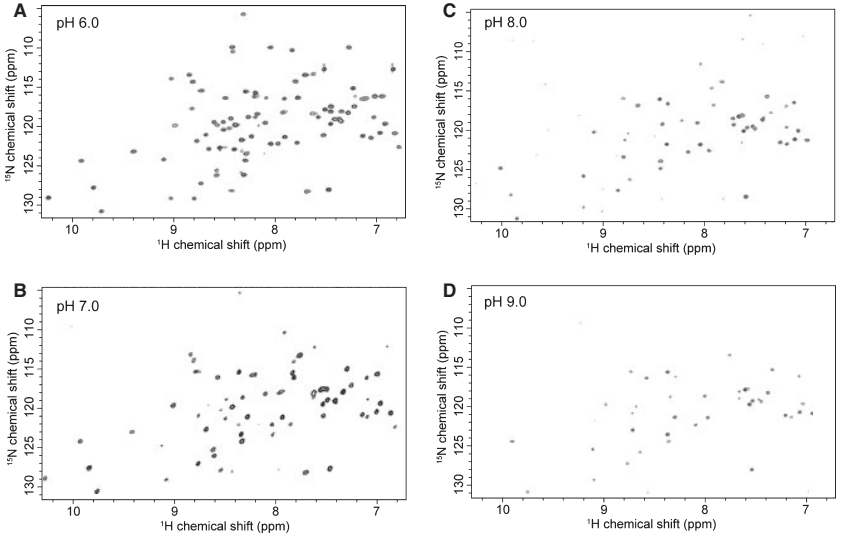


Sample preparation

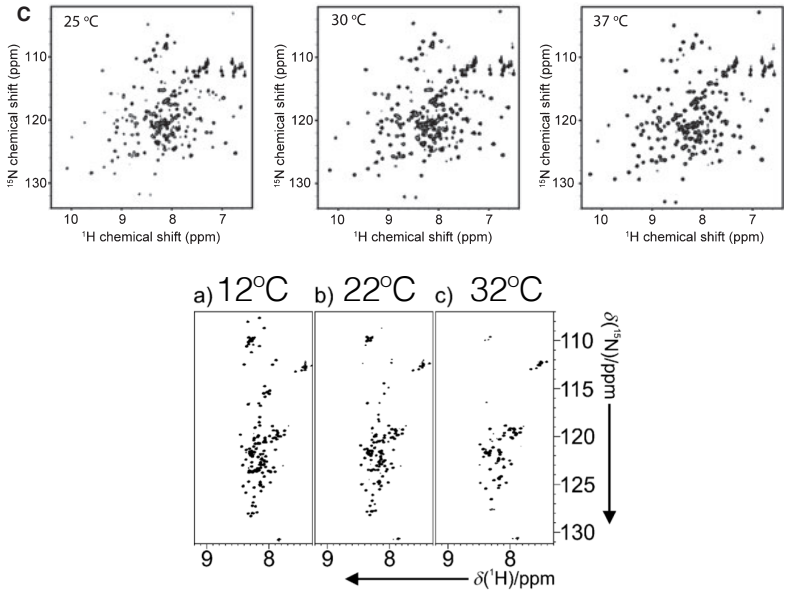
- Solvent: H_2O / D_2O?
- Choice of buffer
- pH/temperature



Effect of pH on ^1H,^{15}N HSQC spectra



Effect of temperature on ^{1}H,^{15}N HSQC spectra



Sample preparation

- Solvent: H_2O / D_2O?
- Choice of buffer
- pH/temperature
- DSS
- Protease inhibitors
- Filtration/centrifugation