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DEPARTMENT OF CHEMISTRY

Frans Mulder

PhD course Jyväskylä 2017

Where are the protons? Measuring and modelling proton equilibria in complex macromolecular systems.

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Lecture 2
NMR Spectroscopy of Peptides and Proteins

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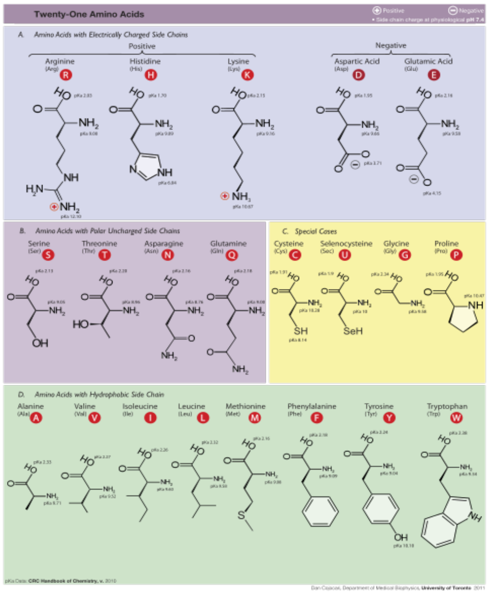
Amino acids



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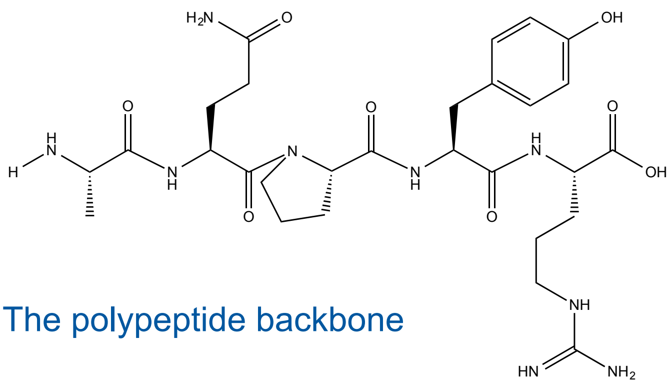
NMR of peptides and proteins



The polypeptide backbone

A Q P Y R

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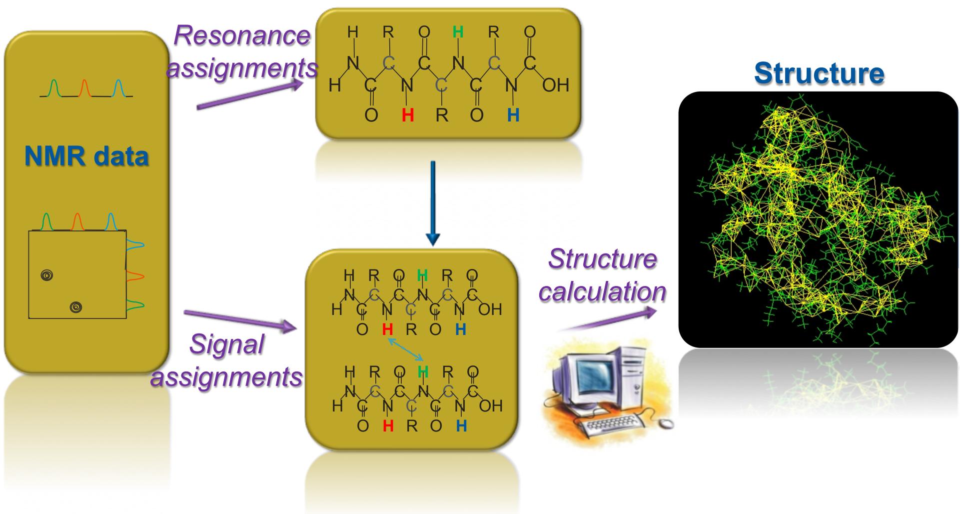
Protein Structure determination



Credit: Jakob T. Nielsen

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NMR of proteins: Homonuclear 2D NMR

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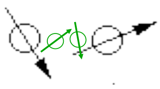
Energy transfer

1. direct dipolar coupling —through-space
   < 5 Å NOESY

d_{HH} ~ 100 kHz, responsible for relaxation



2. indirect dipolar coupling —through-bond
   ≤ 3 bonds COSY, TOCSY

J_{HH} ~ 10 Hz, responsible for spin-spin splitting

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Scalar coupling = *J*-coupling

without *J*-coupling:

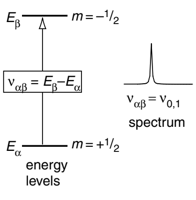

Fig. 2.4 The transition between the two energy levels of a spin-half is allowed, and results in a single line at the Larmor frequency of the spin.

with *J*-coupling:

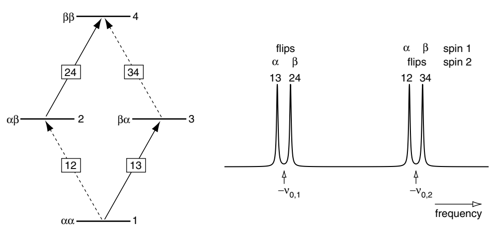

Fig. 2.7 On the left, the energy levels of a two-spin system; the arrows show the allowed transitions: solid lines for transitions in which spin 1 flips and dotted for those in which spin 2 flips. On the right, the corresponding spectrum; it is assumed that the Larmor frequency of spin 2 is greater in magnitude than that of spin 1 and that the coupling J_{12} is positive.

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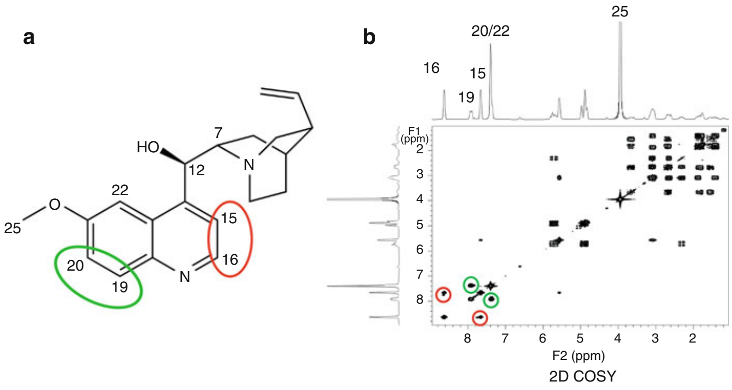
COSY = J_{HH}-correlation

Multidimensional NMR Spectroscopy



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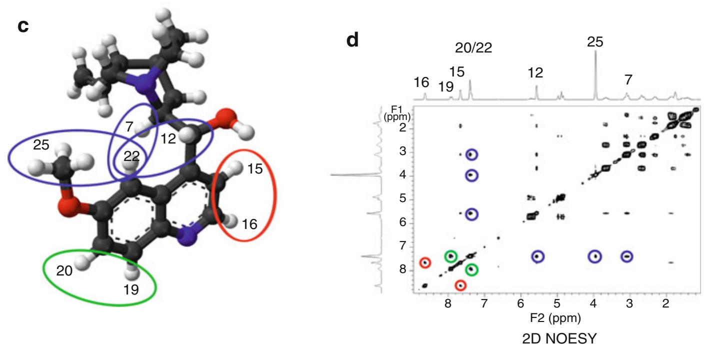
NOESY = d_{HH}-correlation



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NMR of proteins: Heteronuclear 2D NMR

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3D protein NMR and beyond

Resonance Assignments: Homonuclear Methods 253

Table 13.1. Molecular weight limitations for chemical shift assignments.

Mol. Weight	*Technique*	*Observed Spins*	*Dimensionality*
<10 kDa	Homonuclear	^{1}H	2D
10-15 kDa	^{15}N-homonuclear†	^{1}H, ^{15}N	3D, 4D
15-30 kDa	Triple Resonance‡	^{1}H, ^{15}N, ^{13}C	3D, 4D
30-60 kDa	Triple Resonance/deuterated$^{\parallel}$	^{1}H, ^{15}N, ^{13}C	3D, 4D
60-100 kDa	Triple Resonance/deuterated/TROSY$^{\parallel}$	^{1}H, ^{15}N, ^{13}C	3D, 4D

†Requires uniform labeling of protein with ^{15}N.
‡Requires uniform labeling with ^{15}N and ^{13}C.
$^{\parallel}$Requires uniform labeling with ^{15}N, ^{13}C and replacement of CH groups with CD.

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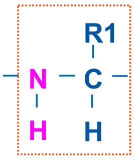
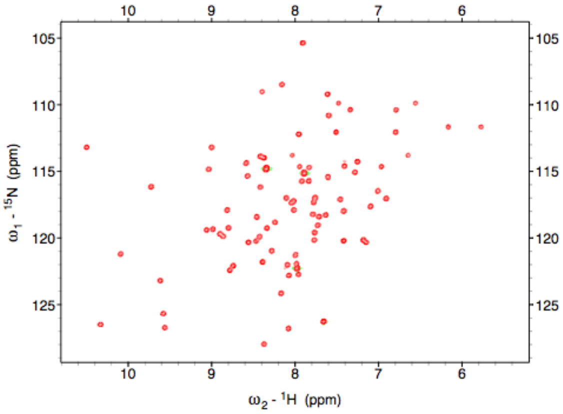
Heteronuclear NMR
→ Indirect ^{15}N NMR





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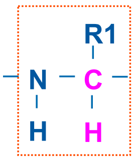
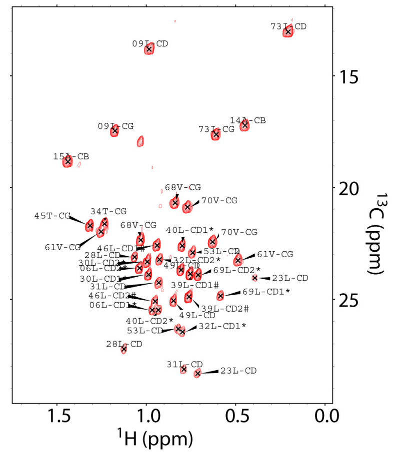
Heteronuclear NMR
→ Indirect ^{13}C NMR





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Effects of dynamic events on the NMR spectrum

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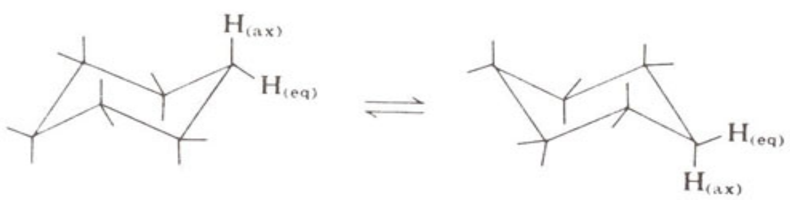
Slow motions (μs – s)



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Slow motions (μs – s)

› $k_{ex} = k_1 + k_{-1}$
› Symmetric two-site exchange: $\delta\omega = \omega_B - \omega_A$
› Slow exchange: $k_{ex} \ll \delta\omega$
› Intermediate exchange: $k_{ex} \approx \delta\omega$
› Fast exchange: $k_{ex} \gg \delta\omega$
› Line shape analysis software provides k_{ex} and $\delta\omega$

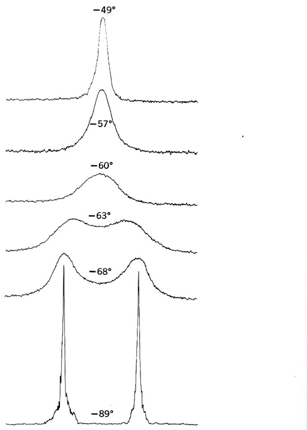

Fig. 5.13. The spectrum of the lone proton of d_{11}-cyclohexane (50% vol./vol. in CS_2) as a function of temperature (°C) observed at 60 MHz with deuterium irradiation at 9.4 MHz.

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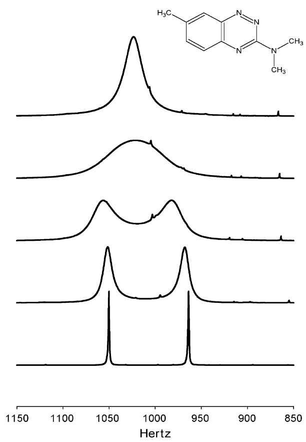
Slow motions (μs – s)

› Conformational exchange is widespread in chemistry and in biology

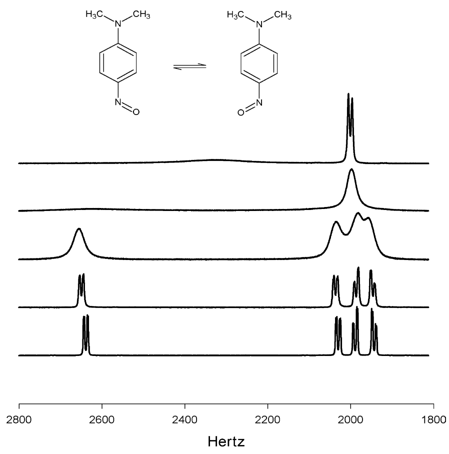
› N,N-dimethyl groups and other partial N–C double bonds



Examples taken from: Alex Bain, Prog NMR Spectr (2003)

Slow motions (μs – s)

› Conformational exchange is widespread: tautomers, *cis-trans* isomers, ring flips, sugar puckers, etc.



Examples taken from: Alex Bain, Prog NMR Spectr (2003)

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Slow motions (μs – s)

› Also chemical exchange = exchange of protons between *different* molecules

› Here: water and ethanol in acetone

68 *A.D. Bain / Progress in Nuclear Magnetic Resonance Spectroscopy 43 (2003) 63–103*

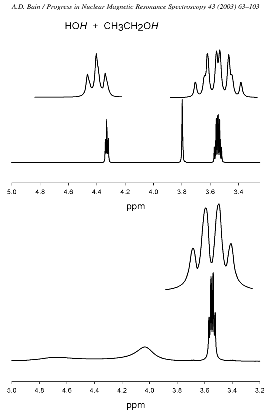

Fig. 5. Proton NMR spectra at 500 MHz of a mixture of ethanol and water, in acetone-d_6. The top spectrum is at 253 K, and the expansions show the coupling between the OH proton at 4.35 ppm and the CH_2 protons at 3.55 ppm. The signal at 3.8 ppm is due to the water. The bottom spectrum is at 333 K, and show the loss of coupling in the CH_2 group and the start of coalescence of the ethanol OH proton and the water peak.

Examples taken from: Alex Bain, Prog NMR Spectr (2003)

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