Introduction to solution NMR

Structural and biophysical methods for biological macromolecules in solution

06 – 14 December 2017 | Singapore, Singapore

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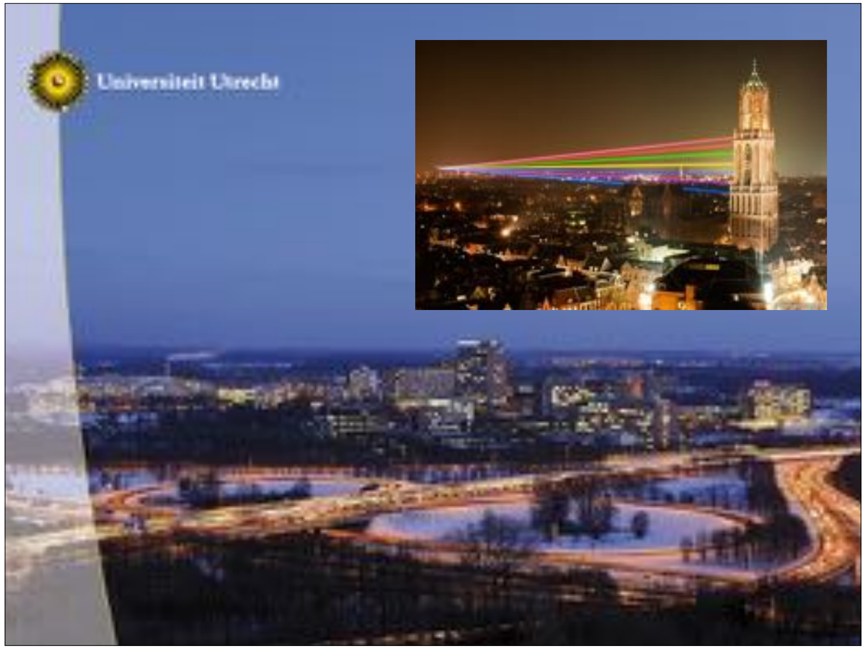


Solution NMR: 950, 900-cryo, 750, 600-cryo, 600US, 2x500 MHz **2017?: 1.2 GHz**
Solid-state NMR: 800WB-DNP, 400WB-DNP, 700US, 500WB MHz
e-infrastructure: >1900 CPU cores + EGI grid (>110'000 CPU cores)

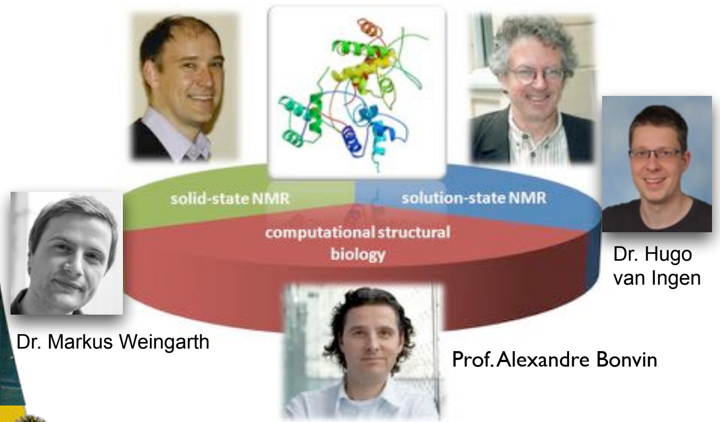
MoBrain
we-nmr
West-Life
bioexcel

National uNMR-NL **and European infrastructure** iNEXT

The NMR research group

Prof. Marc Baldus

Prof. Rolf Boelens



Dr. Hugo van Ingen

Dr. Markus Weingarth

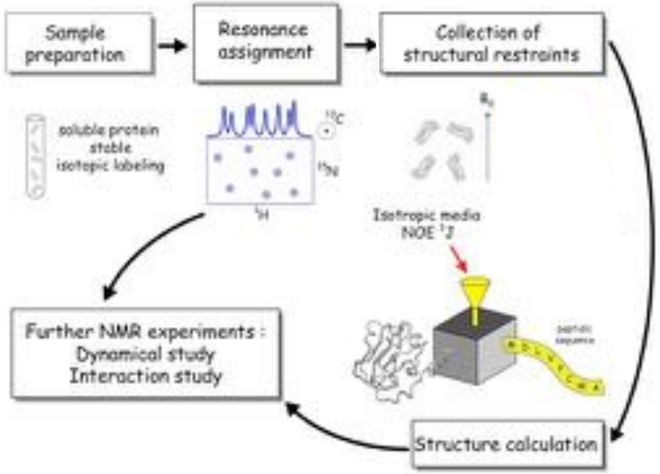
Prof. Alexandre Bonvin

Universiteit Utrecht

http://www.uu.nl/nmr

[Faculty of Science Chemistry]

NMR *'journey'*



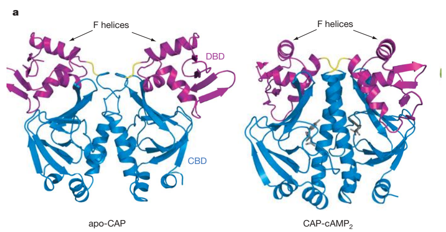
Topics

- **Why use NMR for structural biology...?**
- **The very basics**
- **Multidimensional NMR (intro)**
- **Resonance assignment (lecture Banci)**
- **Structure parameters & calculations (lecture Banci)**
- **NMR relaxation & dynamics**

Why use NMR.... ?

NMR & Structural biology

DYNAMICS

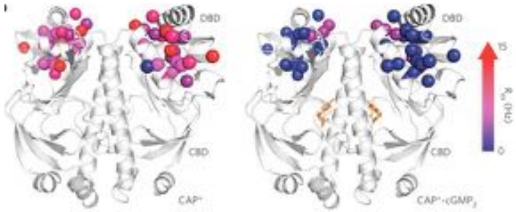


Dynamic activation of an allosteric regulatory protein *Tzeng S-R & Kalodimos CG Nature (2009)*

NMR & Structural biology

DYNAMICS

- **Allosteric regulation**
  - **Dynamic interaction between ligand-binding & DNA binding site**

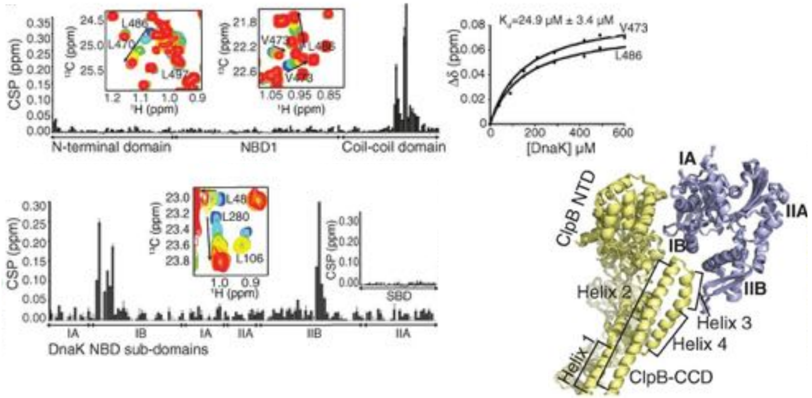


Dynamic activation of an allosteric regulatory protein *Tzeng S-R & Kalodimos CG Nature (2009)*

NMR & Structural biology

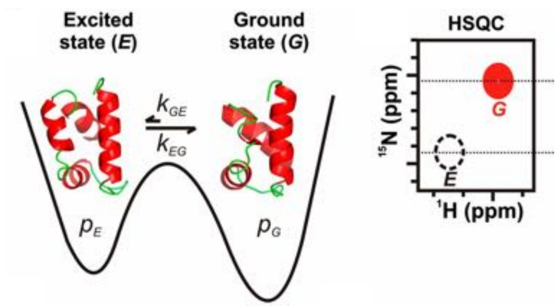
Biomolecular interactions

- **Even weak and transient complexes can be studied**



NMR & Structural biology

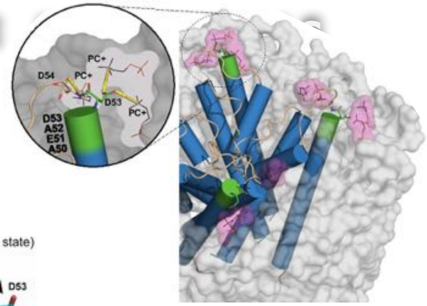
EXCITED STATES



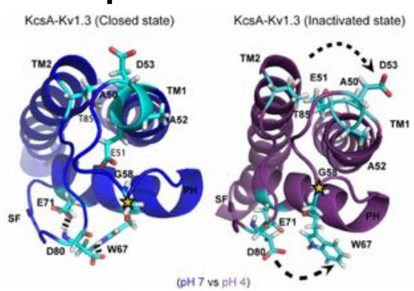
Shekhar & Kay PNAS 2013

NMR & Structural biology

MEMBRANE



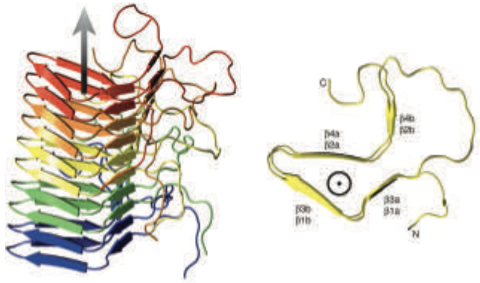
- **Native like environment**
  - **Structural changes due to lipid environment**



van der Cruijsen, ... & Baldus PNAS 2013

NMR & Structural biology

AMYLOID FIBRILS

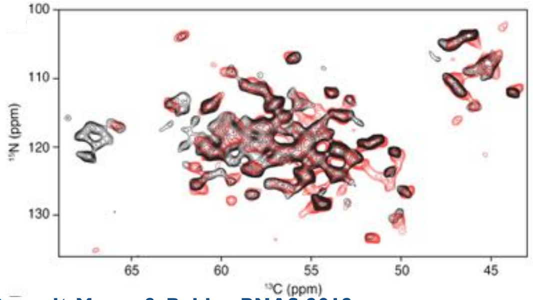


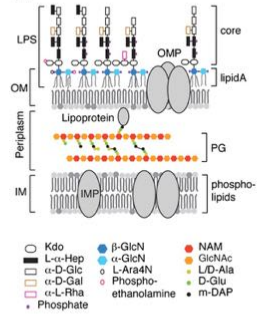
Amyloid Fibrils of the HET-s(218–289) Prion Form a β Solenoid with a Triangular Hydrophobic Core *Wasmer C. et al Science (2008)*

NMR & Structural biology

IN-CELL NMR

- **Study proteins in their native cellular environment**
  - **Outermembrane protein in bacterial cell envelop**





Renault M, ... & Baldus PNAS 2012

The very basics of NMR

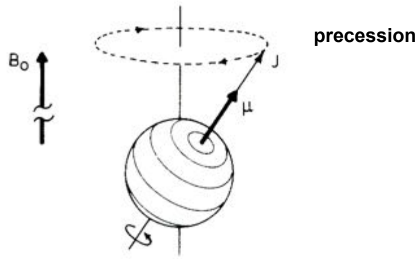
Universiteit Utrecht

[Faculty of Science Chemistry]

The NMR sample

- **isotope labeling**
  - ^{15}N, ^{13}C, ^{2}H
  - selective labeling (*e.g.* only methyl groups)
  - recombinant expression in *E.coli*
- **sample**
  - pure, stable and high concentration
    - 500 uL of 0.5 mM solution -> ~ 5 mg per sample
  - preferably low salt, low pH
  - no additives

Nuclear spin



$$E = -\vec{\mu} \bullet \vec{B} = -\mu_z B_z$$

$|\mu| = \gamma \hbar \sqrt{I(I+1)}$ — *I* = quantum number

$\mu_z = \gamma \hbar m$ — *m* = *I*, *I*-1, *I*-2… -*I* = allowed states

Nuclear spin

TABLE 1.1
Properties of Selected Nuclei[a]

Nucleus	I	γ (rad . T^{-1} . s^{-1})	Natural abundance (%)
^{1}H	$\frac{1}{2}$	2.6752×10^{8}	99.98
^{2}H	1	4.107×10^{7}	0.02
^{13}C	$\frac{1}{2}$	6.728×10^{7}	1.11
^{14}N	1	1.934×10^{7}	99.64
^{15}N	$\frac{1}{2}$	-2.712×10^{7}	0.36
^{17}O	$\frac{5}{2}$	-3.628×10^{7}	0.04
^{19}F	$\frac{1}{2}$	2.5181×10^{8}	100.00
^{23}Na	$\frac{3}{2}$	7.080×10^{7}	100.00
^{31}P	$\frac{1}{2}$	1.0841×10^{8}	100.00
^{113}Cd	$\frac{1}{2}$	5.934×10^{7}	12.26

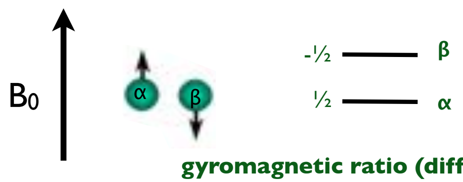
[a] The angular momentum quantum number, I, and the gyromagnetic ratio, γ, and natural isotopic abundance for nuclei of particular importance in biological NMR spectroscopy are shown.

Nuclear spin

- Nuclear magnetic resonance
  - Only nuclei with **non-zero spin quantum** number are "*magnets*"
  - Commonly used spins are spin ½ nuclei: **^{1}H, ^{13}C, ^{15}N, ^{31}P** *etc.*

quantum number I = ½

magnetic field strength



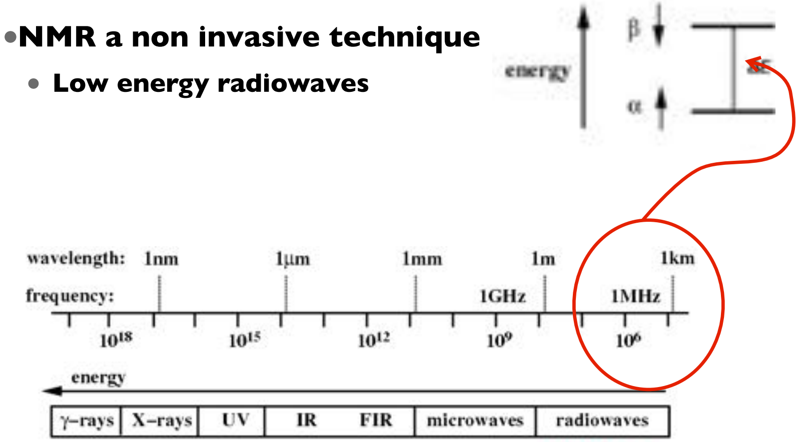
$$\Delta E = \gamma \hbar B_0$$

gyromagnetic ratio (different for each type of nucleus)

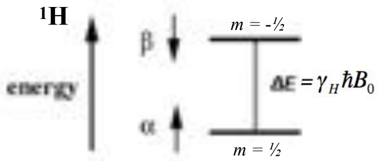
Larmor frequency $\nu = (\gamma B_0)/2\pi$

Nuclear spin & radiowaves

- **NMR a non invasive technique**
  - **Low energy radiowaves**



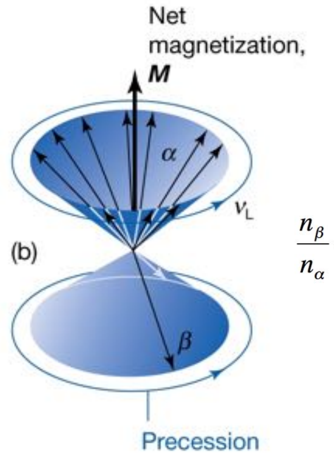
Boltzman distribution



$$\frac{n_\beta}{n_\alpha} = \exp\left(-\frac{\Delta E}{k_B T}\right) = \exp\left(-\frac{\gamma_H \hbar B_0}{k_B T}\right) = 0.9999$$

Example
- 20.001 spins
- Only 1 more spin in lower energy state

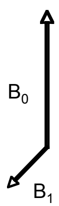
Net magnetization



$$\frac{n_\beta}{n_\alpha} = \exp\left(-\frac{\Delta E}{k_B T}\right) = \exp\left(-\frac{\gamma_H \hbar B_0}{k_B T}\right) = 0.9999$$

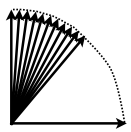
Pulse

- **Radio frequency pulses**
  - **Turn on an amplifier for a certain amount of time & certain amount of power (B_1 field)**



$$\nu_0 = \frac{\gamma B_0}{2\pi}$$

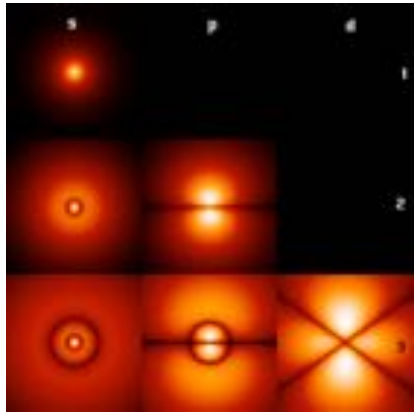
$$\nu_1 = \frac{\gamma B_1}{2\pi}$$



only rotation around B_1 is observed

<u>rotating frame</u>: observe with frequency ν_0

Chemical shielding

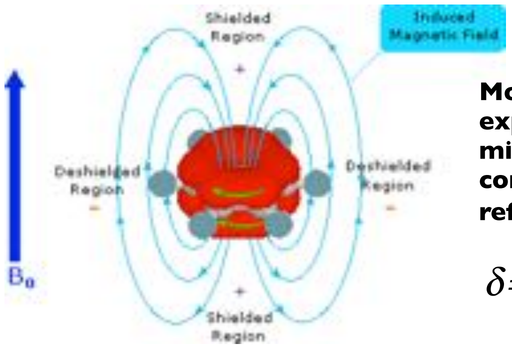


Local magnetic field is influenced by electronic environment
==> frequencies of nuclei will differ

Chemical shift

shielding constant

$$\nu = \frac{\gamma B_0}{2\pi}(1-\sigma)$$

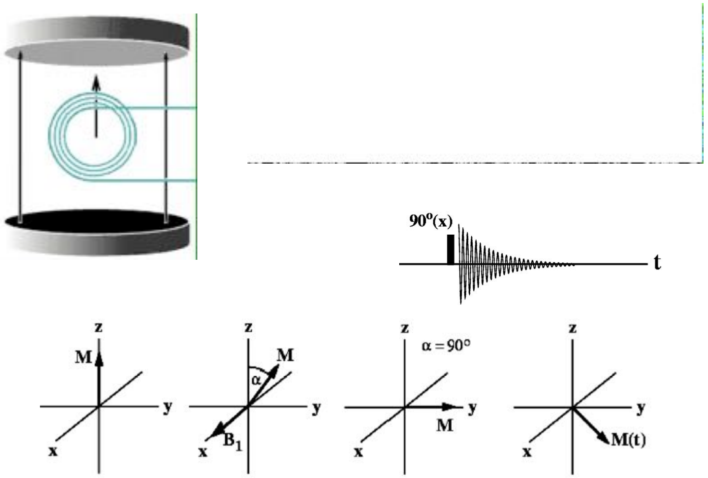


More conveniently expressed as part per million by comparison to a reference frequency:

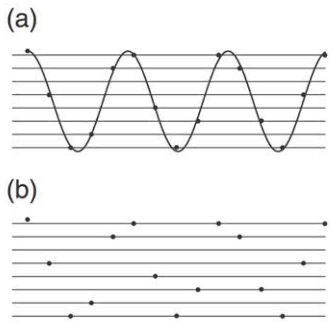
$$\delta = 10^6 \frac{\upsilon - \upsilon_{ref}}{\upsilon_{ref}}$$

The spectrometer

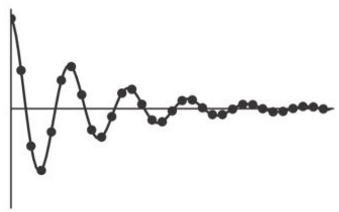
Free induction decay (FID)



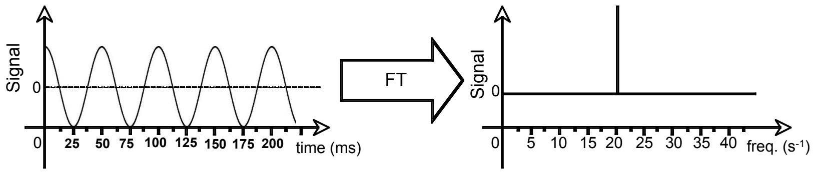
FID: analogue vs digital

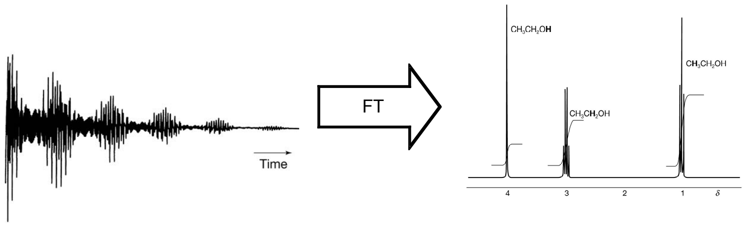


Free Induction Decay (**FID**)



Fourier Transform



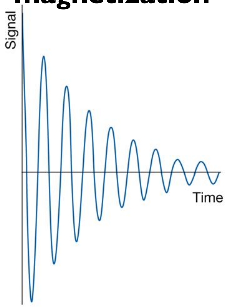


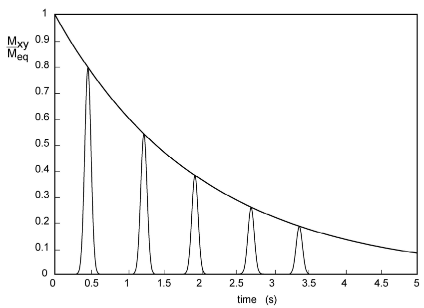
Relaxation

- **NMR Relaxation**
  - Restores Boltzmann equilibrium
- **T2-relaxation (transverse relaxation)spin-spin)**
  - disappearance of transverse (x,y) magnetization
  - contributions from spin-spin and T1 relaxation
  - 1/T2 ~ signal line-width
- **T1-relaxation (longitudinal relaxation / spin-lattice)**
  - build-up of longitudinal (z) magnetization
  - determines how long you should wait for the next experiment

Relaxation

- **Restoring Boltzmann equilibrium**
  - **T_2 relaxation: disappearance of transverse (x,y) magnetization**

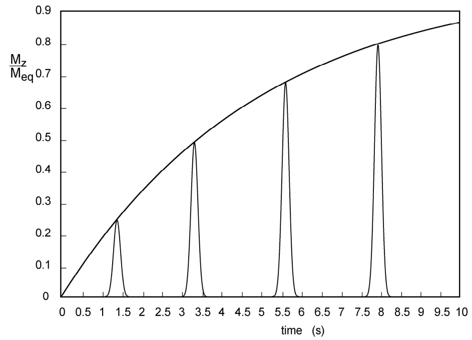




!! $1/T_2$ ~ signal line-width !!

Relaxation

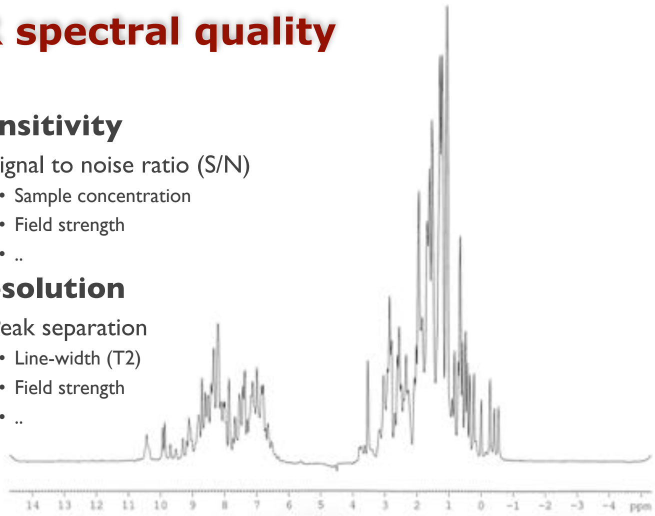
- **Restoring Boltzmann equilibrium**
  - **T_1 relaxation: build-up of longitudinal (z) magnetization**



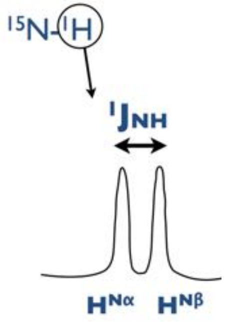
!! T_1 determines when to start the next experiment !!

NMR spectral quality

- **Sensitivity**
  - Signal to noise ratio (S/N)
    - Sample concentration
    - Field strength
    - ..
- **Resolution**
  - Peak separation
    - Line-width (T2)
    - Field strength
    - ..

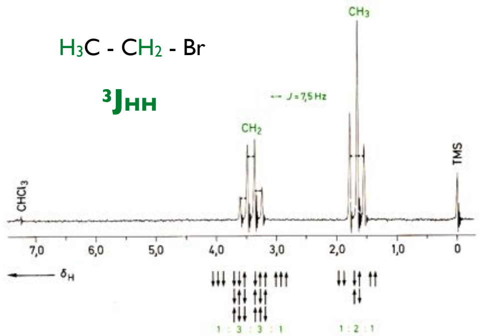


Scalar coupling / J-coupling



H_3C - CH_2 - Br

$^{3}J_{HH}$



Key concepts NMR

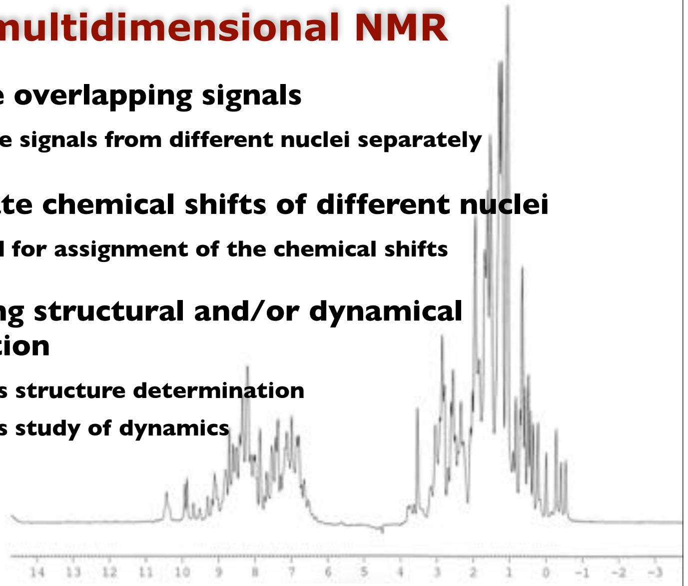
- **Nuclear magnetic resonance**
  - **In a <u>magnetic</u> field *magnetic* <u>nuclei</u> will <u>resonate</u> with a specific frequency**
- **FT-NMR**
  - **Pulse, rotating frame, FID**
- **Chemical shift**
  - **Electronic environment influences local magnetic field -> frequency**
- **NMR relaxation**
  - **T_1 & T_2**
- **J-coupling**

Multidimensional NMR

Universiteit Utrecht

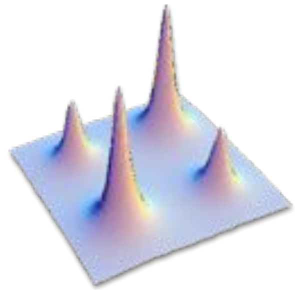
[Faculty of Science Chemistry]

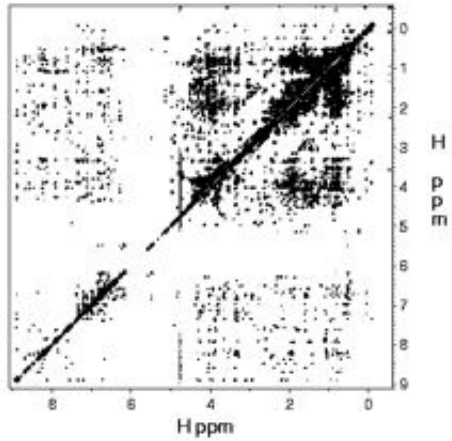
Why multidimensional NMR



- **Resolve overlapping signals**
  - **observe signals from different nuclei separately**
- **Correlate chemical shifts of different nuclei**
  - **needed for assignment of the chemical shifts**
- **Encoding structural and/or dynamical information**
  - **enables structure determination**
  - **enables study of dynamics**

2D NMR





3D NMR

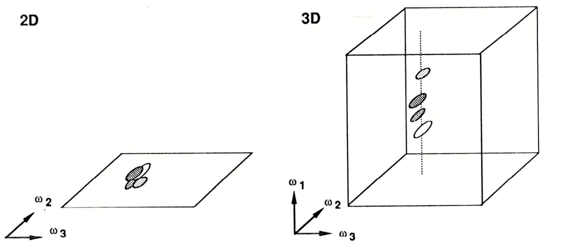
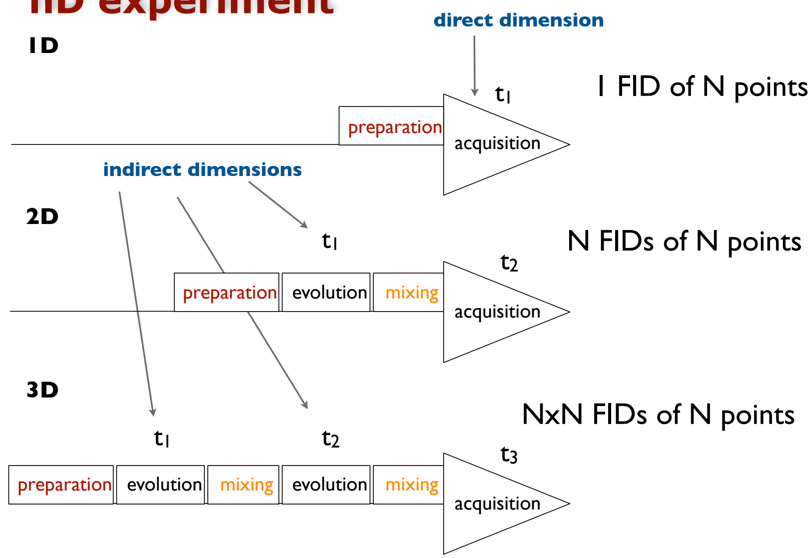


Figure 1. Illustration of the increase in resolution afforded by the increase in dimensionality. In the 2D spectrum, four cross-peaks overlap. By correlation with a third resonance frequency, each cross-peak obtains a different position along a line in the 3D spectrum, thus resolving the overlap problem.

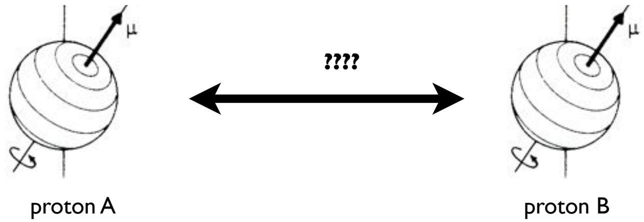
(Vuister, Ph. D. Thesis, 1991)

nD experiment



Encoding information

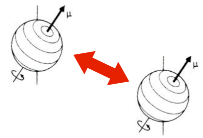
- mixing/magnetization transfer



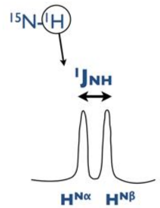
spin-spin interactions

Magnetization transfer

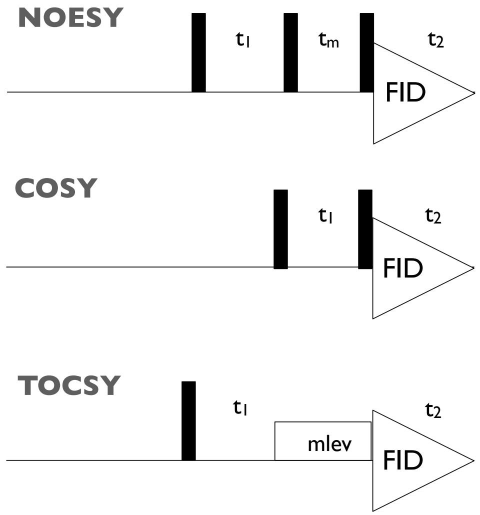
- **Magnetic dipole interaction (NOE)** dipole-dipole interaction
  - Nuclear Overhauser Effect
  - through space
  - distance dependent (1/r6)
  - NOESY -> distance restraints



- **J-coupling interaction**
  - through 3-4 bonds max.
  - chemical connectivities
  - assignment
  - also conformation dependent



homonuclear NMR



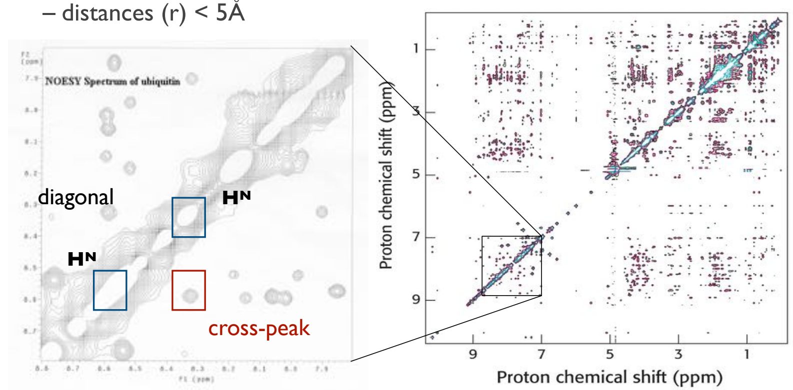
magnetic dipole interaction
crosspeak intensity ~1/r^6
up to 5 Å

J-coupling interaction
transfer over one J-coupling, i.e. max. 3-4 bonds

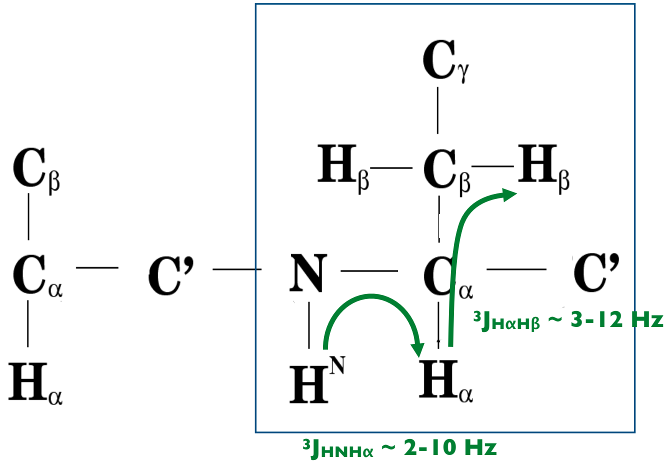
J-coupling interaction
transfer over several J-couplings, i.e. multiple steps over max. 3-4 bonds

2D NOESY

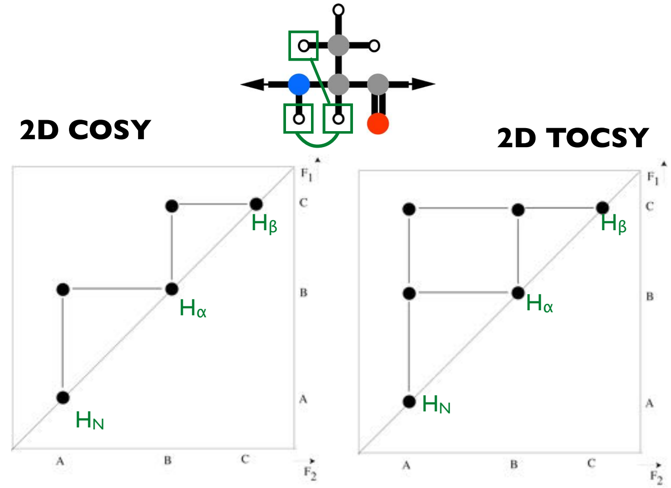
- **Uses dipolar interaction (NOE) to transfer magnetization between protons**
  - cross-peak intensity ~ 1/r^6
  - distances (r) < 5Å



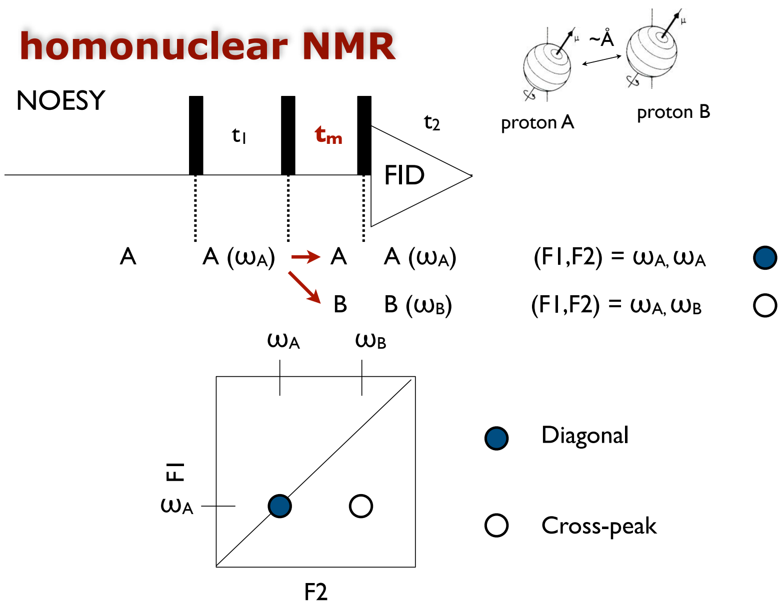
Homonuclear scalar coupling



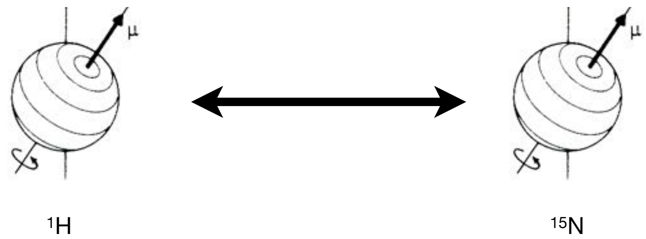
2D COSY & TOCSY



homonuclear NMR



heteronuclear NMR



- **measure frequencies of different nuclei; e.g. ^{1}H, ^{15}N, ^{13}C**
- **no diagonal peaks**
- **mixing not possible using NOE, only via J**

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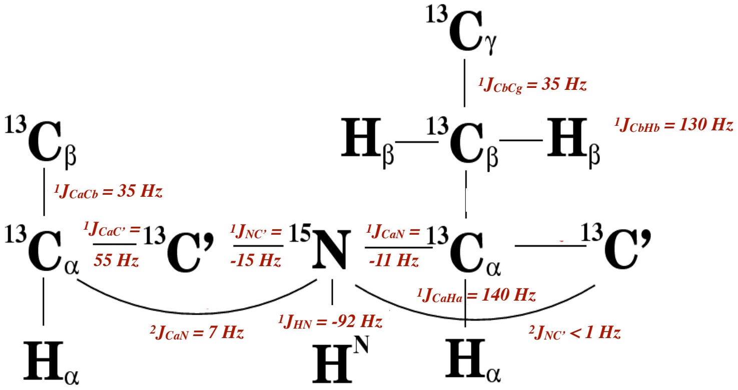
J coupling constants



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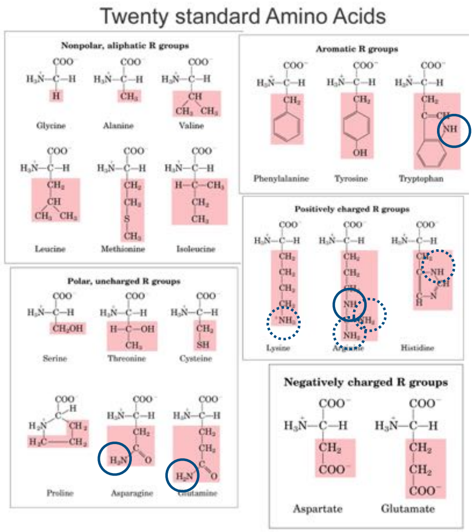
^{15}N HSQC

- Backbone HN
- Side-chain NH and NH_2



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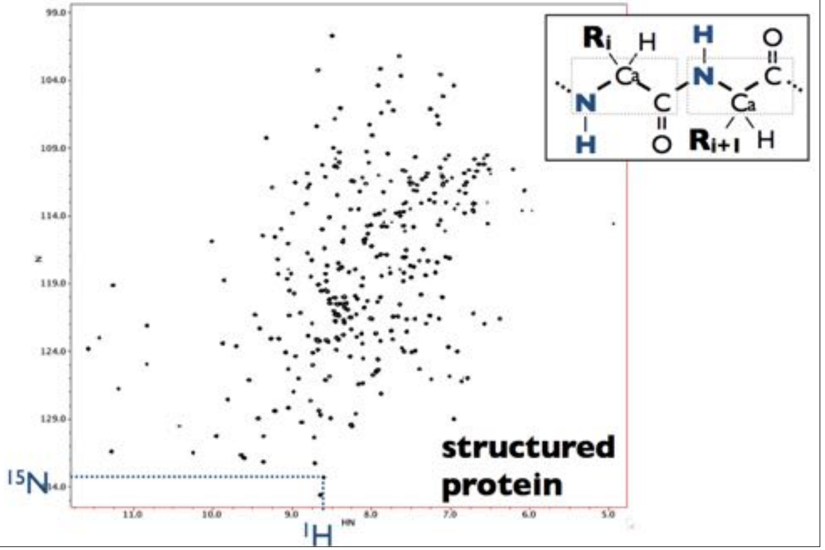
^{1}H-^{15}N HSQC: '*protein fingerprint*'



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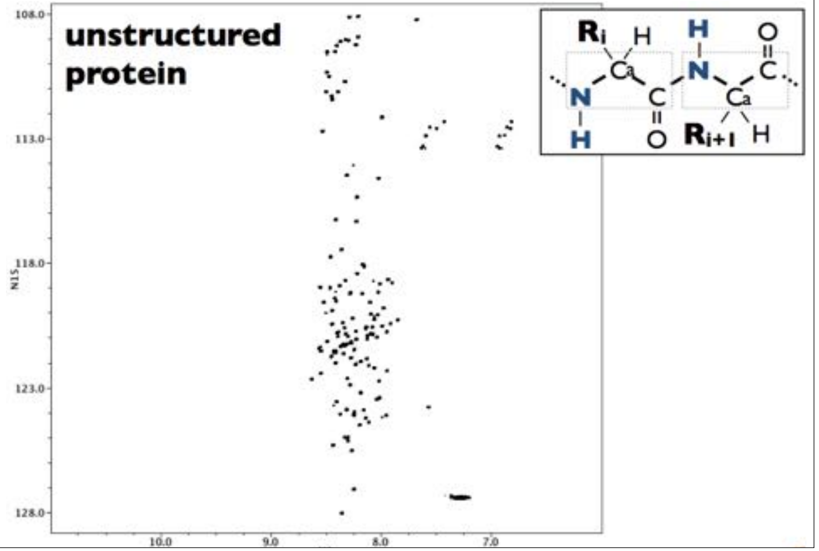
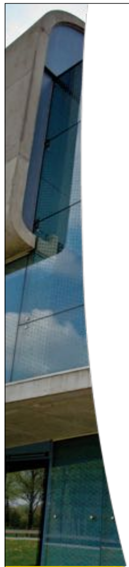
^{1}H-^{15}N HSQC: '*protein fingerprint*'



Key concepts multidimensional NMR

- Resolve overlapping signals
- Mixing/magnetization transfer
- NOESY, TOCSY, COSY
- HSQC
- 3D NOESY-HSQC, 3D TOCSY-HSQC
- Triple resonance



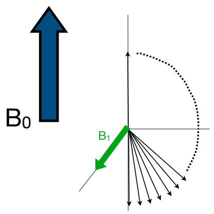
Relaxation & dynamics

Universiteit Utrecht

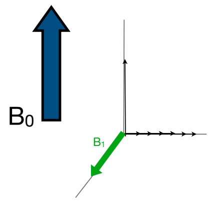
[Faculty of Science
Chemistry]

NMR relaxation

- **Return to equilibrium**
  - Spin-lattice relaxation
  - Longitudinal relaxation → T1 relaxation
    - Return to z-axis

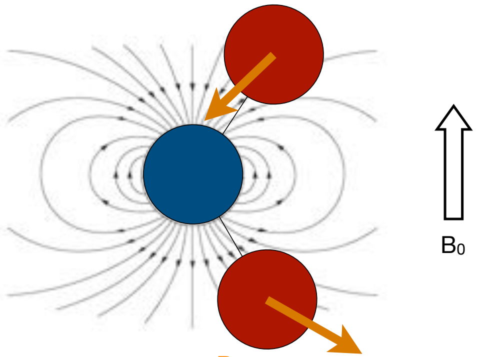


  - Spin-spin relaxation
  - Transversal relaxation → T2 relaxation
    - Dephasing of magnetization in the x/y plane + return to z-axis



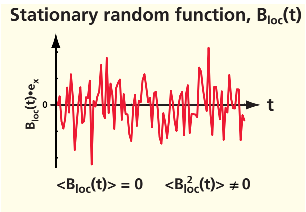
Relaxation is caused by dynamics

- **Fluctuating magnetic fields**
  - Overall tumbling and local motions cause the local magnetic fields to fluctuate in time



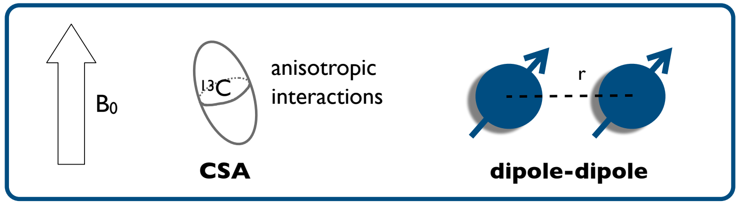
Relaxation is caused by dynamics

- **Fluctuating magnetic fields**
  - Overall tumbling and local motions cause the local magnetic fields to fluctuate in time
  - $B_{loc}(t)$ is thus time dependent
  - If $B_{loc}(t)$ is fluctuating with frequency components near ω_0 then transitions may be induced that bring the spins back to equilibrium
  - The efficiency of relaxation also depends on the amplitude of $B_{loc}(t)$



Local fluctuating magnetic fields

- **$B_{loc}(t) = B_{loc}[iso] + B_{loc}(t)[aniso]$**
  - Isotropic part is not time dependent
    - chemical shift
    - J-coupling
  - Only the anisotropic part is time dependent
    - chemical shift anisotropy (CSA)
    - dipolar interaction (DD)

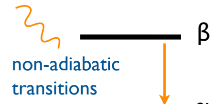


Local fluctuating magnetic fields

- **$B_{loc}(t) = B_{loc}[iso] + B_{loc}(t)[aniso]$**
  - Isotropic part is not time dependent
    - chemical shift
    - J-coupling
  - Only the anisotropic part is time dependent
    - chemical shift anisotropy (CSA)
    - dipolar interaction (DD)
- **Only $B_{loc}(t)[aniso]$ can cause relaxation**
  - Transverse fluctuating fields: $B_{loc}(t)\bullet e_x + B_{loc}(t)\bullet e_y$
  - Longitudinal fluctuating fields: $B_{loc}(t)\bullet e_z$

Components of the local field

- **$B_{loc}(t)\bullet e_{xy}$**
  - Transverse fluctuating fields
  - **Non-adiabatic**: exchange of energy between the spin-system and the lattice [environment]



T_1 relaxation

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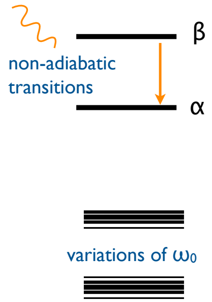
Components of the local field

- **$B_{loc}(t) \bullet e_{xy}$**
  - Transverse fluctuating fields
  - **Non-adiabatic**: exchange of energy between the spin-system and the lattice [environment]
  - Heisenberg's uncertainty relationship:
    - shorter lifetimes ⇔ broadening of energy levels



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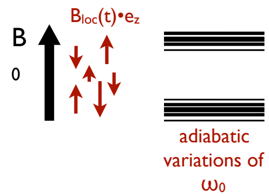
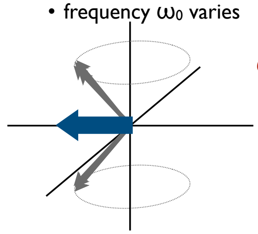
Components of the local field

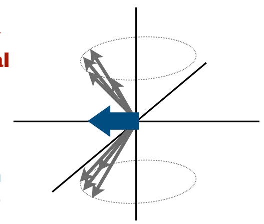
- **$B_{loc}(t) \bullet e_z$**
  - Longitudinal fluctuating fields
  - **Adiabatic**: NO exchange of energy between the spin-system and the lattice
  - Effective field along z-axis varies
    - frequency ω_0 varies





$B_{loc}(t) \bullet e_z$: frequency ω_0 varies due to local changes in B_0

$B_{loc}(t) \bullet e_{xy}$: transitions between states reduce phase coherence



T_2 relaxation

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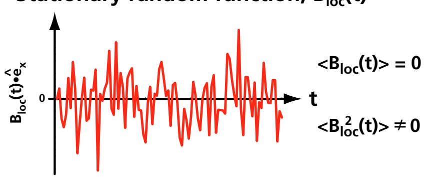
Correlation function

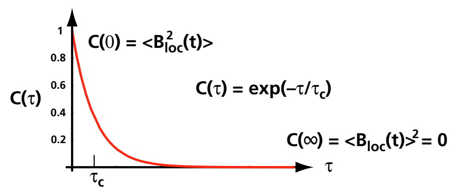
- **Describes the fluctuating magnetic fields**
  - correlation function $C(\tau)$ decays exponentially with a characteristic time τ_c

Stationary random function, $B_{loc}(t)$



Time correlation function, $C(\tau)$

$C(\tau) = <B_{loc}(t)B_{loc}(t+\tau)> = <B_{loc}(0)B_{loc}(\tau)>$



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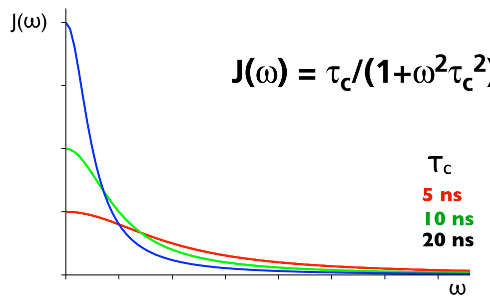
Spectral density function

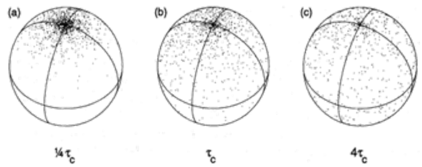
- **Frequencies of the random fluctuating fields**
  - Spectral density function $J(\omega)$ is the Fourier transform of the correlation function $C(\tau)$
  - $J(\omega)$ describes if a certain frequency can induce relaxation and whether it is efficient



Link to rotational motions in liquids

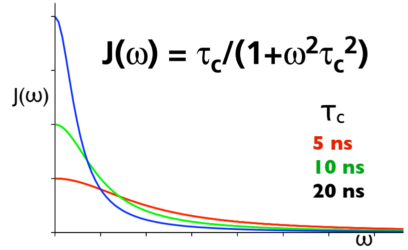
- **Molecules in solution "tumble"** (rotational diffusion combining rotations and collisions with other molecules)

- Can be characterized by a **rotational correlation time τc**
- τc is the time needed for the rms deflection of the molecules to be ~ 1 radian (60°)



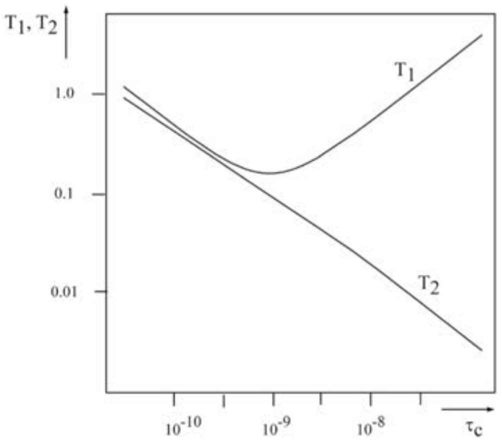
Link to rotational motions in liquids

- **Small molecules** (or high temperature):
  - smaller (shorter) correlation times (fast tumbling),
  - J(w) extends to higher frequencies - spectrum is flatter
- **Large molecules** (or low temperature):
  - larger (longer) correlation times (slow tumbling)
  - J(w) larger close to 0



Relaxation

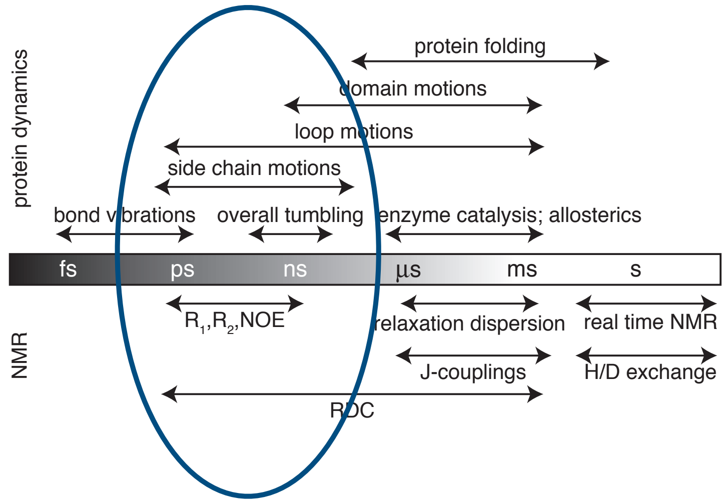
- **relaxation time is related to rate of motion**



$R_1 = 1/T_1$

$R_2 = 1/T_2$

NMR time scales



Protein backbone dynamics

- **^{15}N relaxation to describe ps-ns dynamics**
  - R_1: longitudinal relaxation rate
  - R_2: transversal relaxation rate
  - hetero-nuclear NOE: $\{^1H\}$-^{15}N

$$R_1 = d^2[J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N)] + c^2J(\omega_N)$$

$$R_2 = (d^2/2)[4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N) + 6J(\omega_H)] + (c^2/6)[4J(0) + J(\omega_N)]$$

$$NOE = 1 + [(\gamma_H/\gamma_N)d^2\{6J(\omega_H + \omega_N) - J(\omega_H - \omega_N)\}/R_1]$$

where $d^2 = (1/10)\,\gamma_H^2\gamma_N^2(h/2\pi)^2(\langle r_{NH}^{-3}\rangle)^2$ — dipole interaction

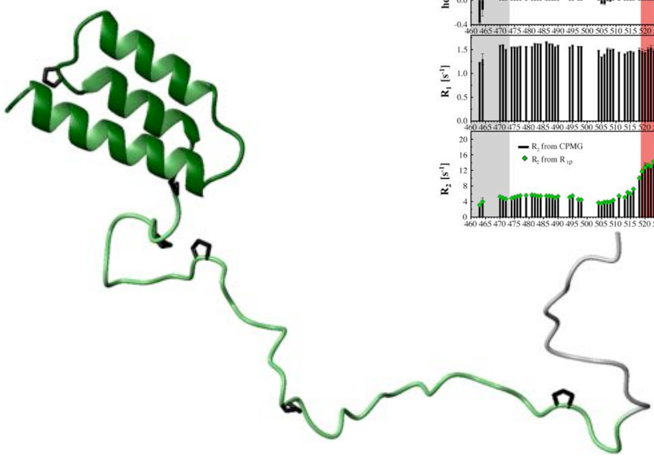
and $c^2 = (2/15)\,\omega_N^2(\sigma_\parallel - \sigma_\perp)^2$ — chemical shift anisotropy

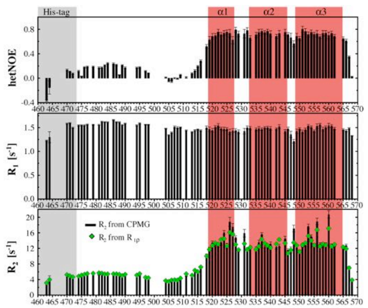
Protein backbone dynamics

- **^{15}N relaxation to describe ps-ns dynamics**
  - R_1: longitudinal relaxation rate
  - R_2: transversal relaxation rate
  - hetero-nuclear NOE: $\{^1H\}$-^{15}N

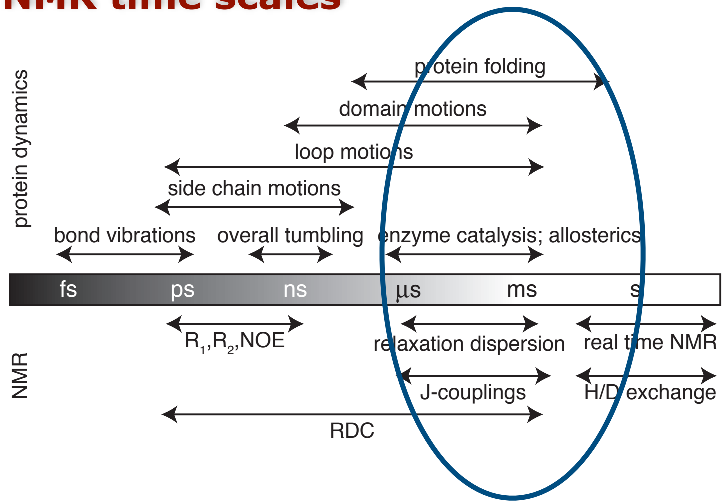
- **Measured as a 2D ^1H-^{15}N spectrum**
  - R_1,R_2: Repeat experiment several times with increasing relaxation-delay
  - Fit the signal intensity as a function of the relaxation delay
    - $I_0 \exp(-Rt)$
  - $\{^1H\}$-^{15}N NOE: Intensity ratio between saturated and non-saturated experiment

Relaxation rates

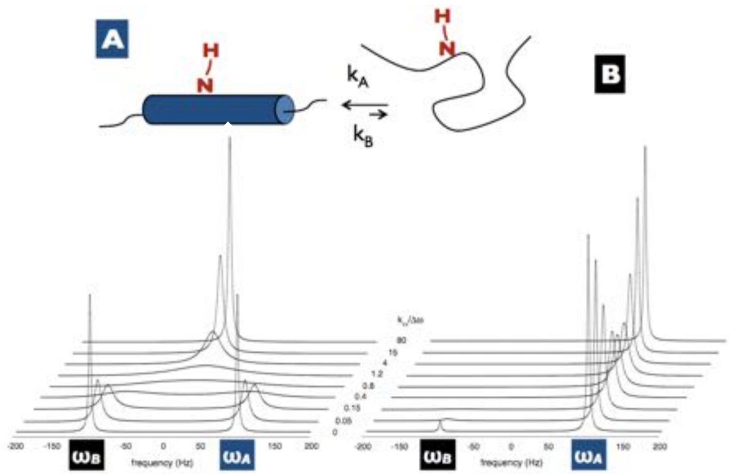




NMR time scales



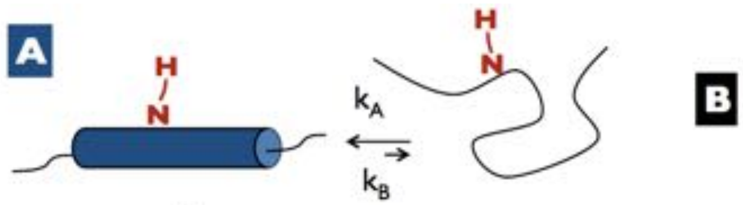
Conformational exchange



equal populations: $p_A = p_B$

skewed populations: $p_A >> p_B$

Conformational exchange



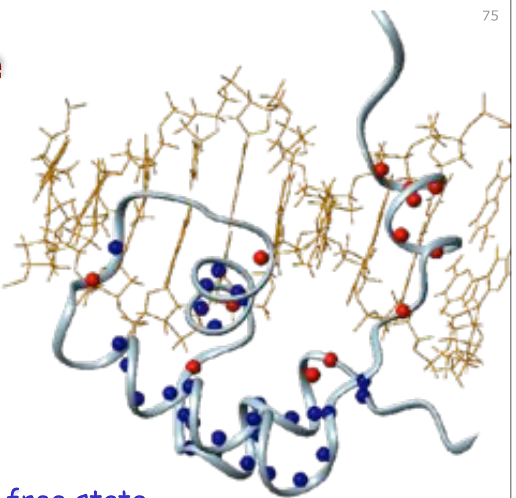
- **Causes line-broadening of the signals**

–$R_{2,eff} = R_2 + R_{ex}$

H/D exchange

Lac headpiece

Kalodimos et al. Science



- protected in the free state
- protected only in the DNA-bound state

Key concepts relaxation

- **time scales**

- **fluctuating magnetic fields**
- **correlation function, spectral density function**

- **molecular motions**
- **rotational correlation time (ns)**

- **fast time scale flexibility (ps-ns)**
- **slow time scale (µs-ms): conformational exchange**