

# Introduction to solution NMR

## Structural and biophysical methods for biological macromolecules in solution

19 – 26 June 2016 | Suwon, Korea

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*Bijvoet Center for Biomolecular Research*

with thanks to Dr. Klaartje Houben

Universiteit Utrecht

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  
 w@-nmr  
 West-Life  
 bioexcel  
 INEXT  
 uNMR\*NL  
 National and European infrastructure

## The NMR research group

Prof. Marc Baldus

Prof. Rolf Boelens

solid-state NMR

solution-state NMR

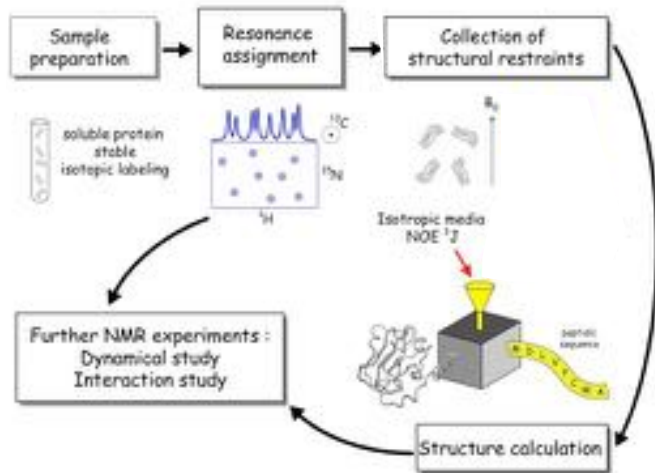
computational structural biology

Prof. Alexandre Bonvin

<http://www.uu.nl/nmr>

[Faculty of Science Chemistry]

## NMR 'journey'



## Topics

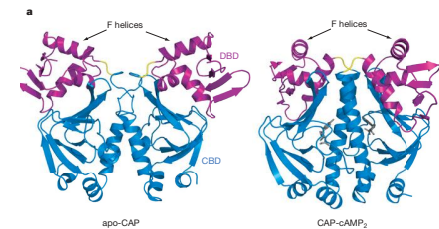
- Why use NMR for structural biology...?
- The very basics
- Multidimensional NMR (intro)
- Resonance assignment (lecture Banci)
- Structure parameters & calculations (lecture Banci)
- NMR relaxation & dynamics

## Why use NMR.... ?



## NMR & Structural biology

### DYNAMICS



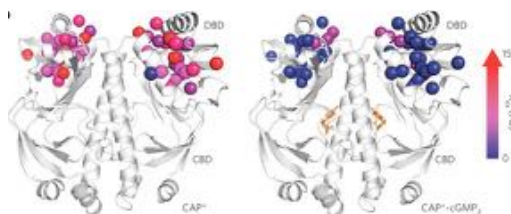
Dynamic activation of an allosteric regulatory protein Tzeng  
S-R & Kalodimos *CG Nature* (2009)

## NMR & Structural biology

### DYNAMICS

#### • Allosteric regulation

- Dynamic interaction between ligand-binding & DNA binding site

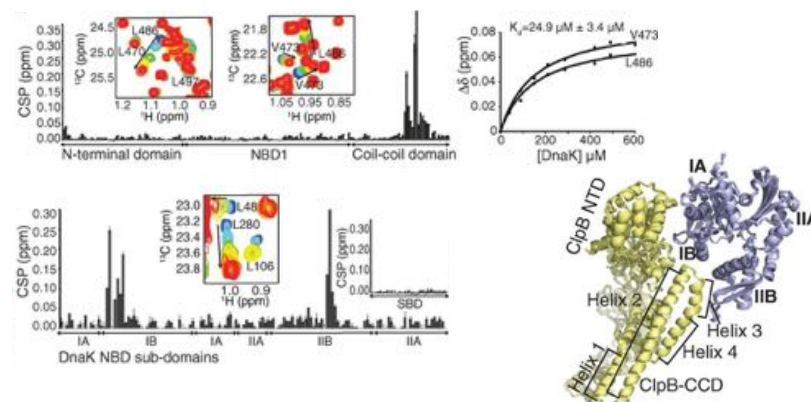


Dynamic activation of an allosteric regulatory protein Tzeng S-R & Kalodimos CG Nature (2009)

## NMR & Structural biology

### Biomolecular interactions

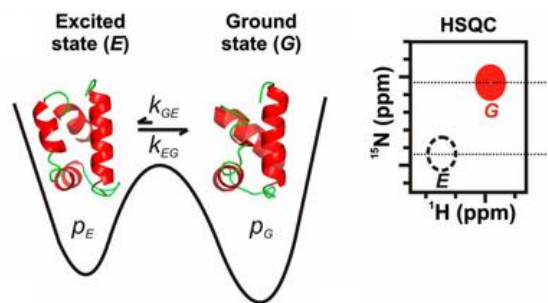
- Even weak and transient complexes can be studied



11

## NMR & Structural biology

### EXCITED STATES



Shekhar & Kay PNAS 2013

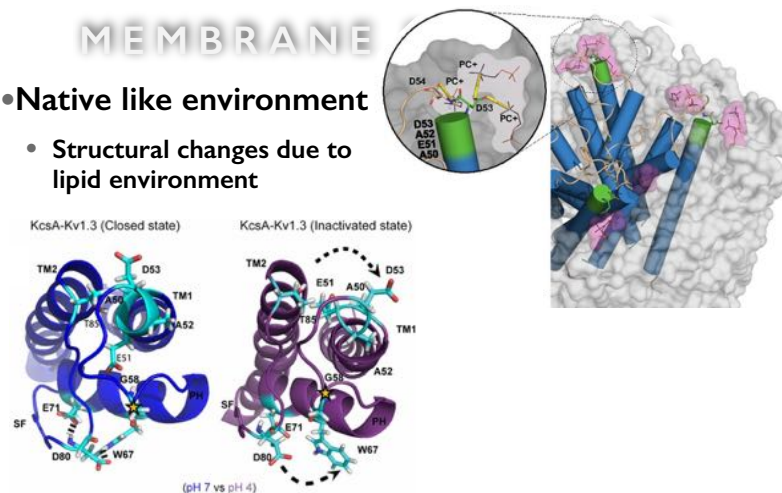
12

## NMR & Structural biology

### MEMBRANE

#### • Native like environment

- Structural changes due to lipid environment

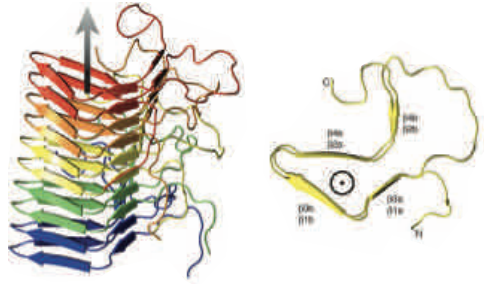


van der Cruisen, ..... & Baldus PNAS 2013



## NMR & Structural biology

### AMYLOID FIBRILS

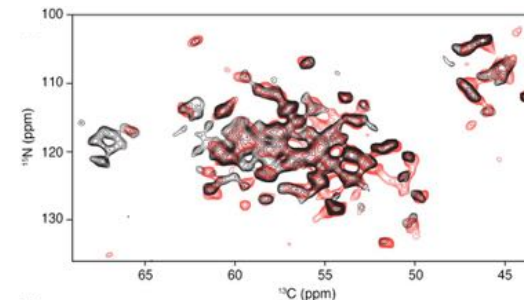


Amyloid Fibrils of the HET-s(218–289) Prion Form a  $\beta$  Solenoid with a Triangular Hydrophobic Core Wasmer C. *et al* Science (2008)

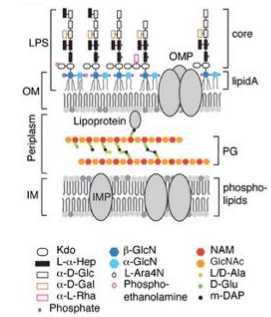
## NMR & Structural biology

### IN-CELL NMR

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



Renault M, ..... & Baldus PNAS 2012



## The NMR sample

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

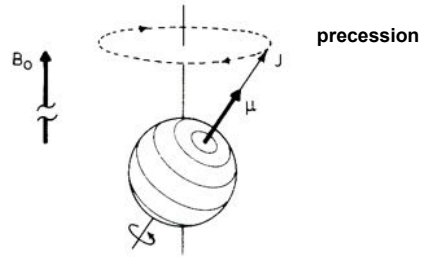


## The very basics of NMR

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# Nuclear spin



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

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

$$\mu_z = \gamma \hbar m \quad m = I, I-1, I-2 \dots -I = \text{allowed states}$$

# Nuclear spin

TABLE 1.1  
Properties of Selected Nuclei<sup>a</sup>

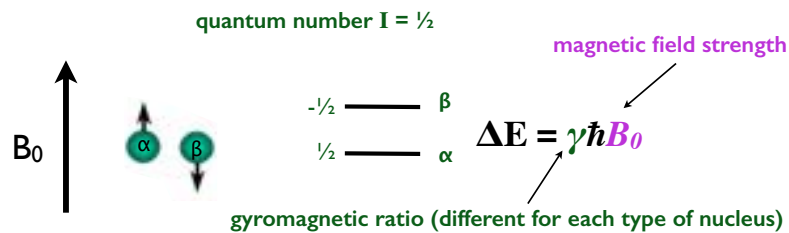
Nucleus	<i>I</i>	$\gamma$ (rad · T <sup>-1</sup> · s <sup>-1</sup> )	Natural abundance (%)
<sup>1</sup> H	1/2	2.6752 × 10 <sup>8</sup>	99.98
<sup>2</sup> H	1	4.107 × 10 <sup>7</sup>	0.02
<sup>13</sup> C	1/2	6.728 × 10 <sup>7</sup>	1.11
<sup>14</sup> N	1	1.934 × 10 <sup>7</sup>	99.64
<sup>15</sup> N	1/2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

<sup>a</sup> The angular momentum quantum number, *I*, and the gyromagnetic ratio,  $\gamma$ , 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 1/2 nuclei: <sup>1</sup>H, <sup>13</sup>C, <sup>15</sup>N, <sup>31</sup>P etc.

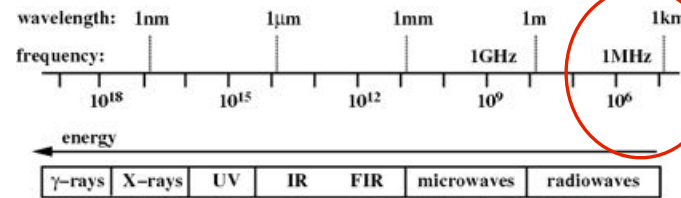
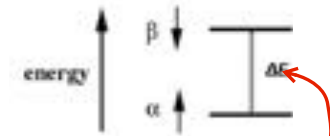


**Larmor frequency**      $\nu = (\gamma B_0)/2\pi$

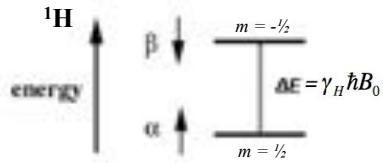
# Nuclear spin & radiowaves

## • NMR a non invasive technique

- Low energy radiowaves



## Boltzman distribution

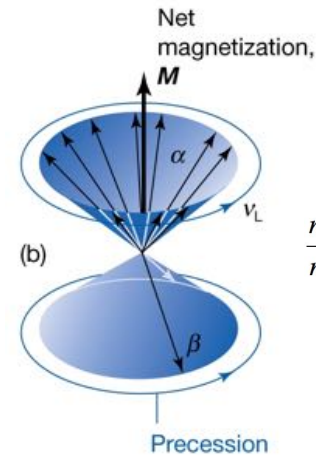


$$\frac{n_\beta}{n_\alpha} = \exp\left(-\frac{\Delta E}{k_B T}\right) = \exp\left(-\frac{\gamma_H \hbar B_0}{k_B T}\right) = 0.9999$$

### 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

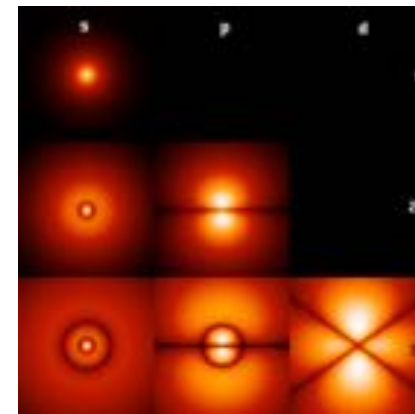
- Turn on an amplifier for a certain amount of time & certain amount of power ( $B_1$  field)



only rotation around  $B_1$  is observed

rotating frame: observe with frequency  $\nu_0$

## Chemical shielding

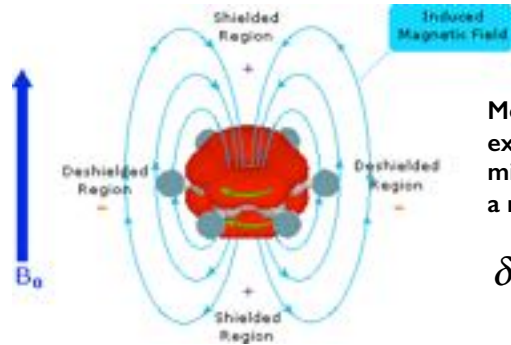


Local magnetic field is influenced by electronic environment  
 ==> frequencies of nuclei will differ

## Chemical shift

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

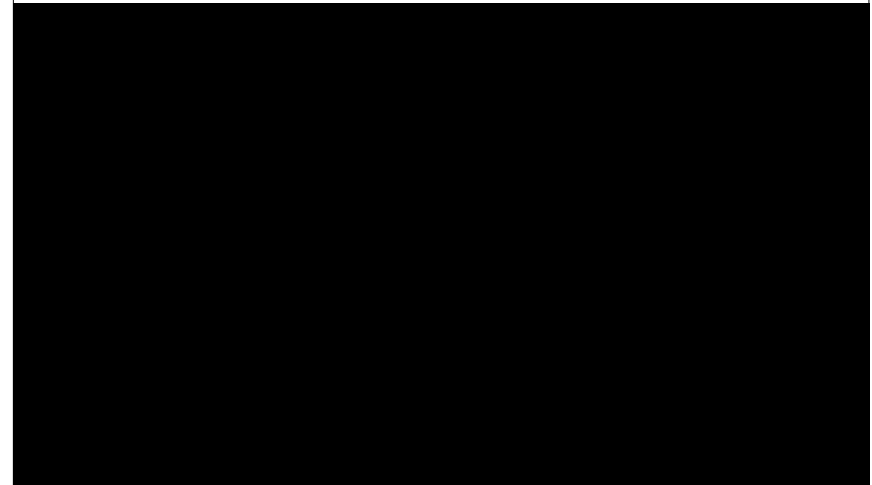
← shielding constant



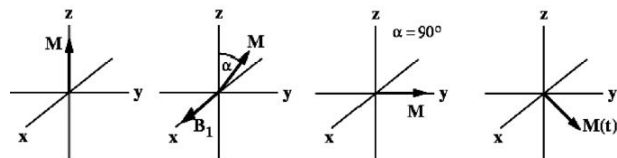
More conveniently expressed as part per million by comparison to a reference frequency:

$$\delta = 10^6 \frac{\nu - \nu_{ref}}{\nu_{ref}}$$

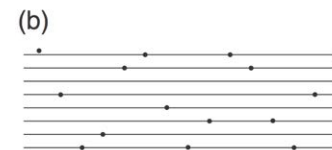
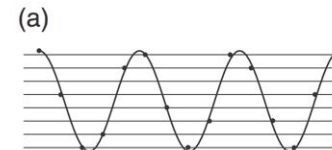
## The spectrometer



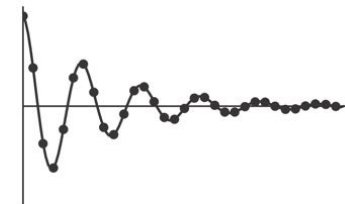
## Free induction decay (FID)



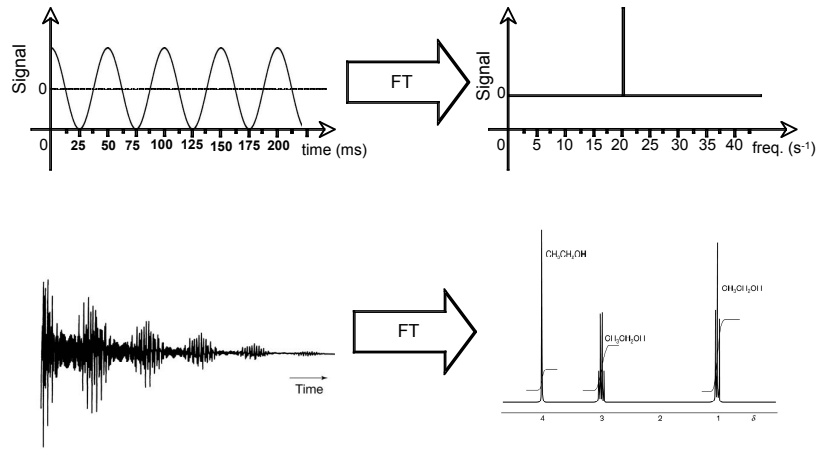
## FID: analogue vs digital



Free Induction Decay (FID)



## Fourier Transform

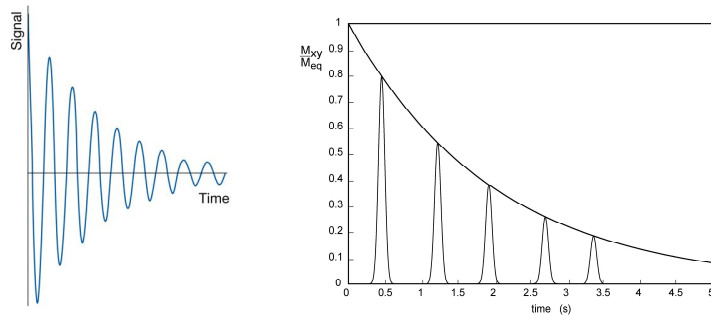


## Relaxation

- **NMR Relaxation**
  - Restores Boltzmann equilibrium
- **T<sub>2</sub>-relaxation (transverse relaxation) spin-spin)**
  - disappearance of transverse (x,y) magnetization
  - contributions from spin-spin and T<sub>1</sub> relaxation
  - $1/T_2 \sim$  signal line-width
- **T<sub>1</sub>-relaxation (longitudinal relaxation / spin-lattice)**
  - build-up of longitudinal (z) magnetization
  - determines how long you should wait for the next experiment

## Relaxation

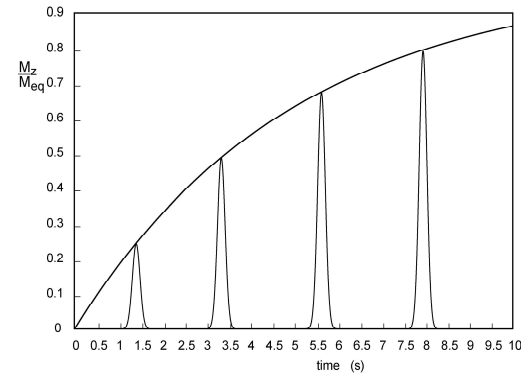
- **Restoring Boltzmann equilibrium**
  - T<sub>2</sub> relaxation: disappearance of transverse (x,y) magnetization



**!!  $1/T_2 \sim$  signal line-width !!**

## Relaxation

- **Restoring Boltzmann equilibrium**
  - T<sub>1</sub> relaxation: build-up of longitudinal (z) magnetization



**!! T<sub>1</sub> determines when to start the next experiment !!**



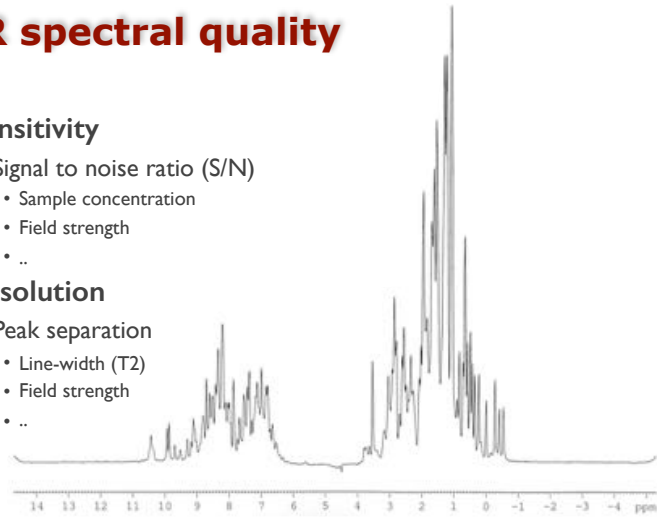
## NMR spectral quality

- **Sensitivity**

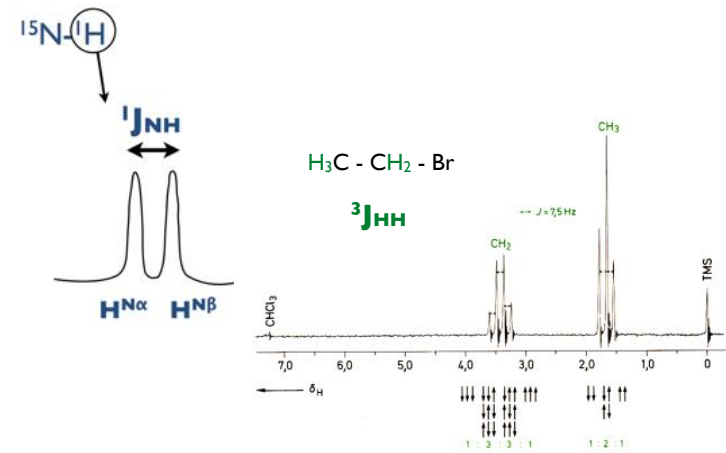
- Signal to noise ratio (S/N)
  - Sample concentration
  - Field strength
  - ..

- **Resolution**

- Peak separation
  - Line-width (T2)
  - Field strength
  - ..



## Scalar coupling / J-coupling



## Key concepts NMR

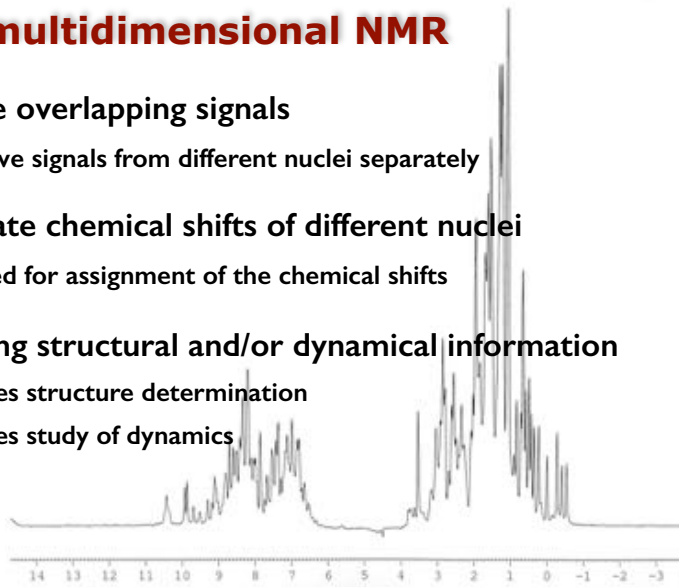
- **Nuclear magnetic resonance**
  - In a magnetic field magnetic nuclei will resonate with a specific frequency
- **FT-NMR**
  - Pulse, rotating frame, FID
- **Chemical shift**
  - Electronic environment influences local magnetic field -> frequency
- **NMR relaxation**
  - $T_1$  &  $T_2$
- **J-coupling**

## Multidimensional NMR

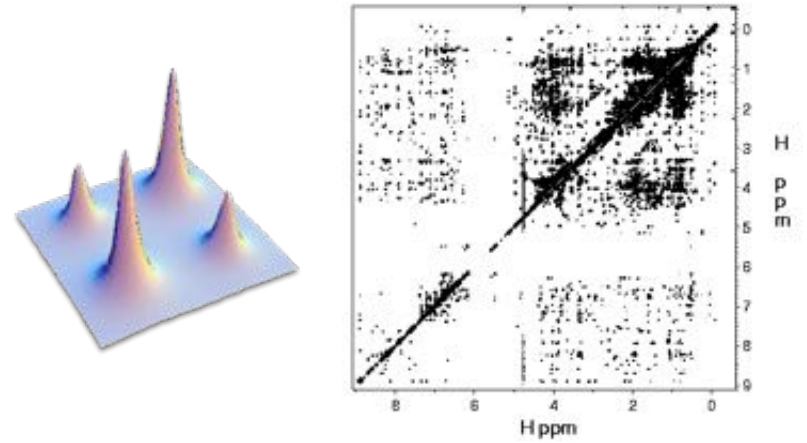


# Why multidimensional NMR

- 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

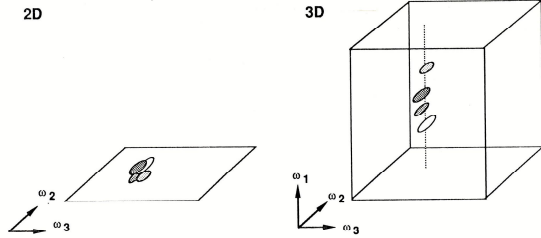
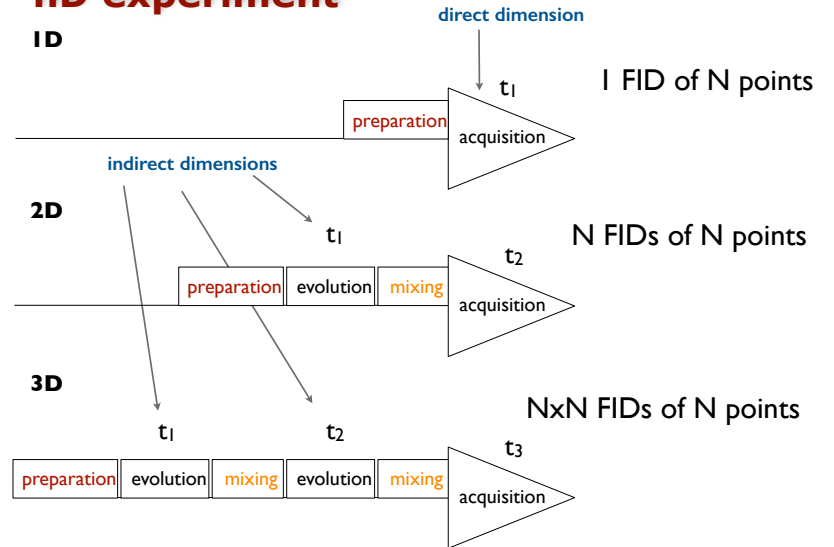


Figure 1. Illustration of the increase in resolution afforded by the increase in dimensionality. In the 2D spectrum, four cross-peaks overlap. By correlation with a third resonance frequency, each cross-peak obtains a different position along a line in the 3D spectrum, thus resolving the overlap problem.

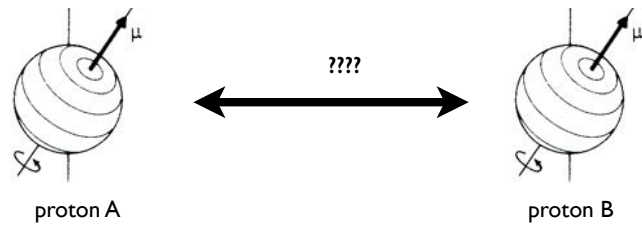
(Uuister, Ph.D. Thesis, 1991)

# nD experiment



## Encoding information

- mixing/magnetization transfer

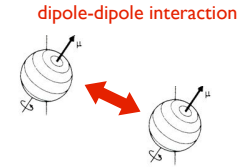


spin-spin interactions

## Magnetization transfer

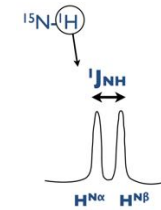
- **Magnetic dipole interaction (NOE)**

- Nuclear Overhauser Effect
- through space
- distance dependent ( $1/r^6$ )
- NOESY  $\rightarrow$  distance restraints



- **J-coupling interaction**

- through 3-4 bonds max.
- chemical connectivities
- assignment
- also conformation dependent



## homonuclear NMR

### NOESY



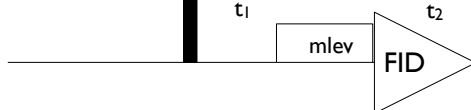
**magnetic dipole interaction**  
crosspeak intensity  $\sim 1/r^6$   
up to 5 Å

### COSY



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

### TOCSY

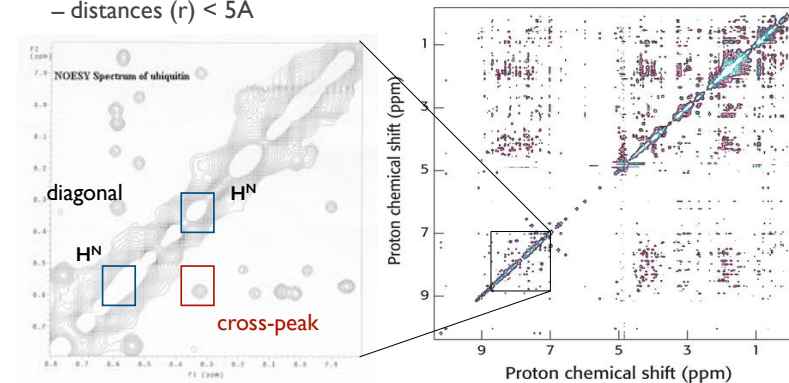


**J-coupling interaction**  
transfer over several J-couplings, i.e. multiple steps  
over max. 3-4 bonds

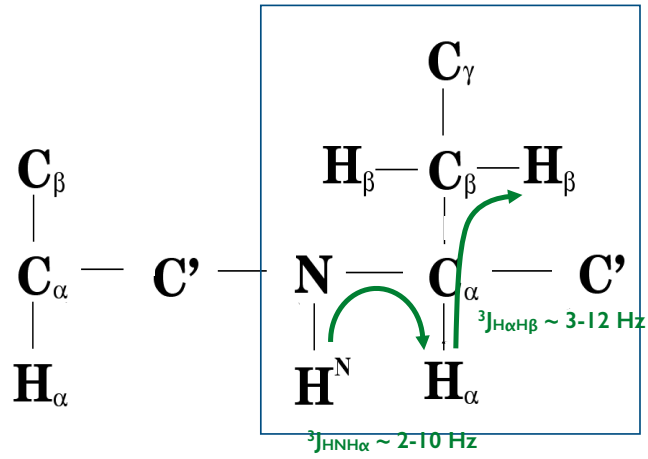
## 2D NOESY

- Uses dipolar interaction (NOE) to transfer magnetization between protons

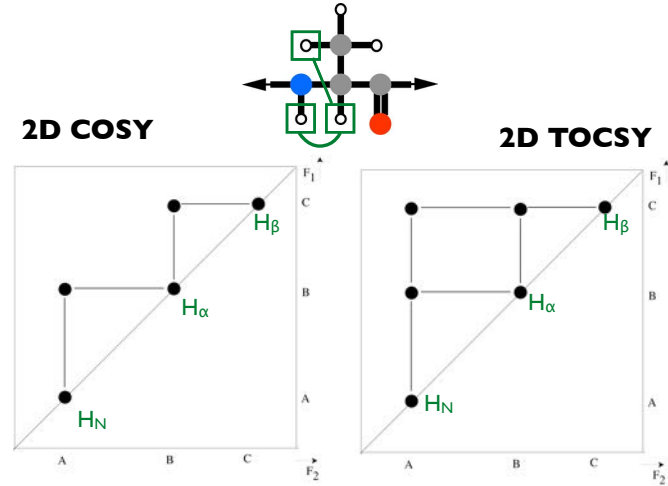
- cross-peak intensity  $\sim 1/r^6$
- distances ( $r$ ) < 5 Å



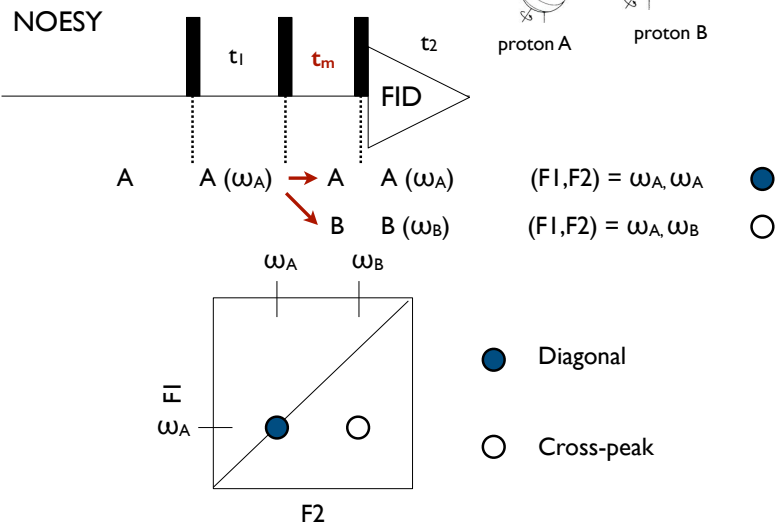
### Homonuclear scalar coupling



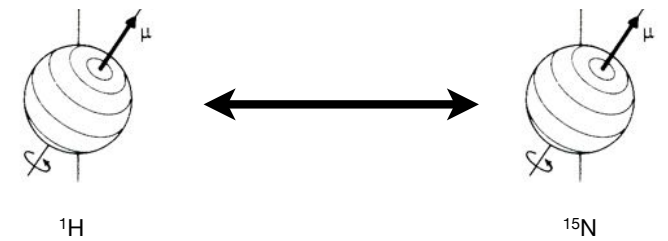
### 2D COSY & TOCSY



### homonuclear NMR

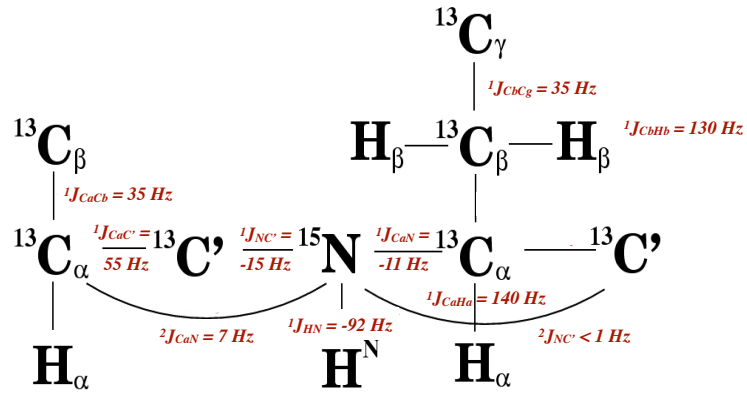


### heteronuclear NMR



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

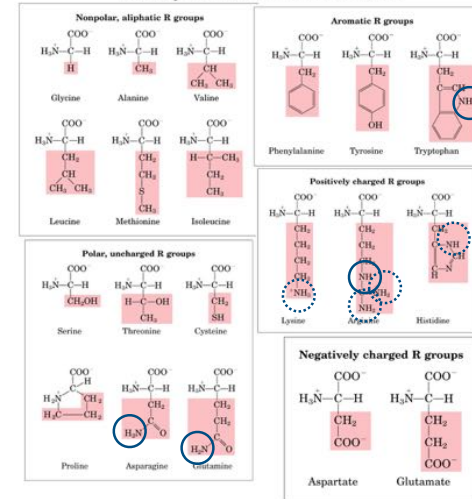
# J coupling constants



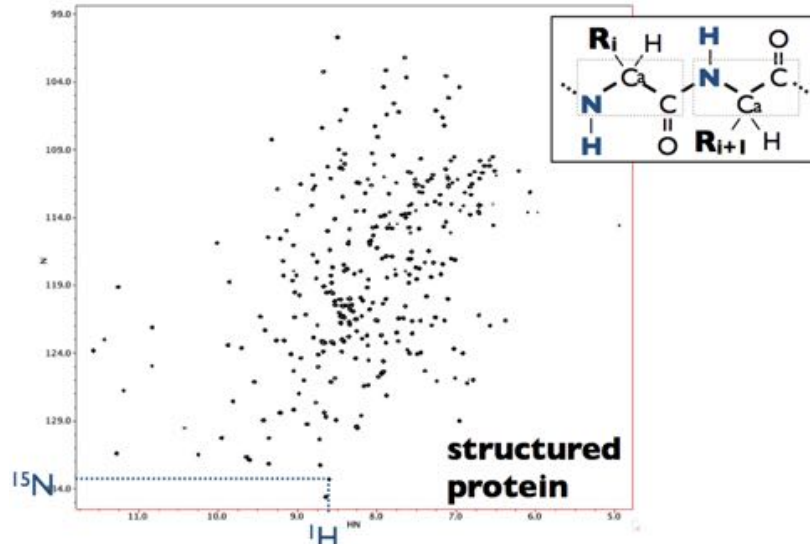
# 15N HSQC

- Backbone HN
- Side-chain NH and NH<sub>2</sub>

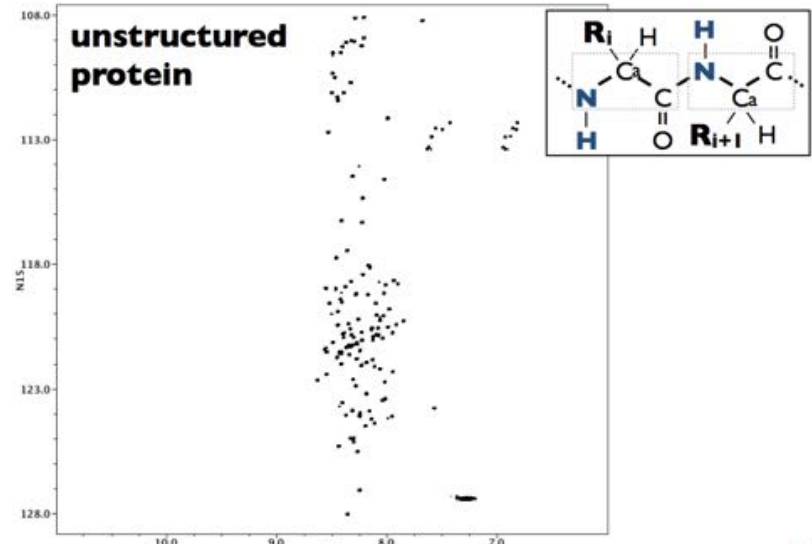
Twenty standard Amino Acids



# 1H-15N HSQC: 'protein fingerprint'




# 1H-15N 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



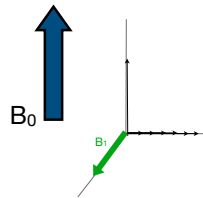
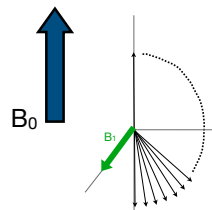
## Relaxation & dynamics

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[Faculty of Science  
Chemistry]

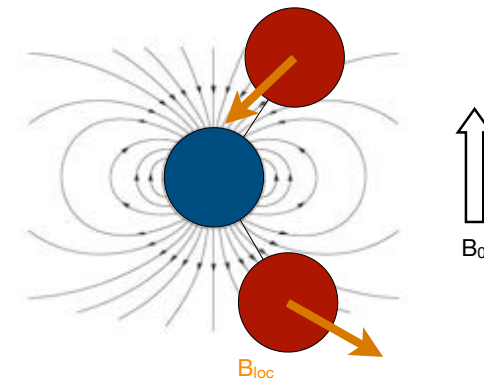
## NMR relaxation

- 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

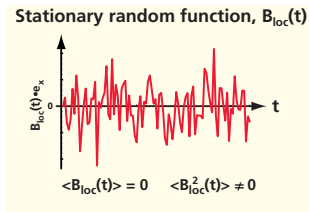
- Fluctuating magnetic fields
  - Overall tumbling and local motions cause the local magnetic fields to fluctuate in time



## 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  $\omega_0$  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)

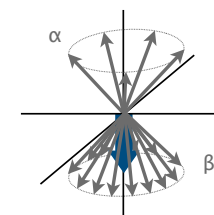
### • Only $B_{loc}(t)[aniso]$ can cause relaxation

- Transverse fluctuating fields:  $B_{loc}(t) \cdot e_x + B_{loc}(t) \cdot e_y$
- Longitudinal fluctuating fields:  $B_{loc}(t) \cdot e_z$

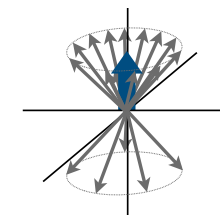
## Components of the local field

### • $B_{loc}(t) \cdot e_{xy}$

- Transverse fluctuating fields
- **Non-adiabatic**: exchange of energy between the spin-system and the lattice [environment]



transitions between states restore Boltzmann equilibrium



$T_1$  relaxation

## Components of the local field

- $B_{loc}(t) \cdot e_{xy}$

- Transverse fluctuating fields
- **Non-adiabatic:** exchange of energy between the spin-system and the lattice [environment]

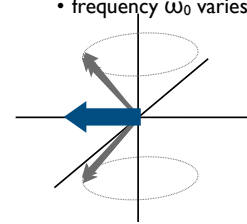
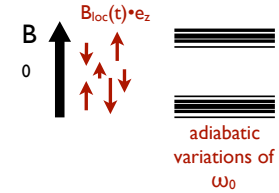


- Heisenberg's uncertainty relationship:
  - shorter lifetimes  $\leftrightarrow$  broadening of energy levels

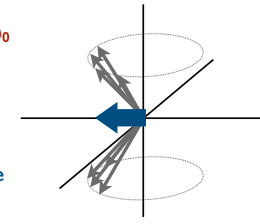
## Components of the local field

- $B_{loc}(t) \cdot e_z$

- Longitudinal fluctuating fields
- **Adiabatic:** NO exchange of energy between the spin-system and the lattice
- Effective field along z-axis varies
  - frequency  $\omega_0$  varies



$B_{loc}(t) \cdot e_z$ : frequency  $\omega_0$  varies due to local changes in  $B_0$

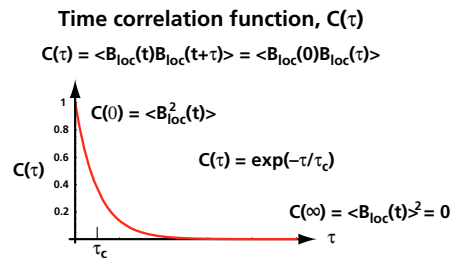
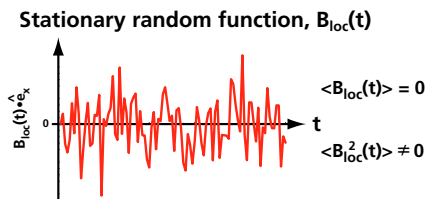


$T_2$  relaxation

## Correlation function

- Describes the fluctuating magnetic fields

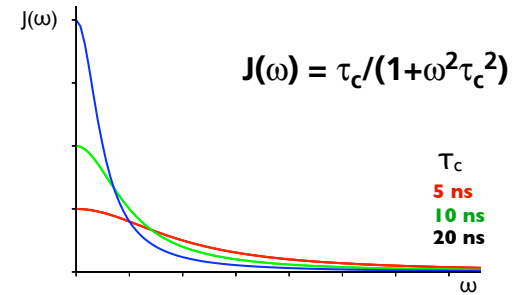
- correlation function  $C(\tau)$  decays exponentially with a characteristic time  $\tau_c$



## Spectral density function

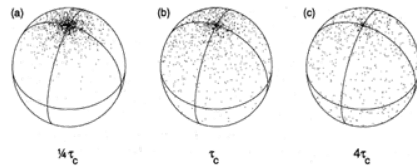
- Frequencies of the random fluctuating fields

- 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



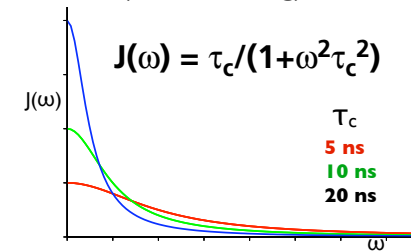
## Link to rotational motions in liquids

- **Molecules in solution**  
“tumble” (rotational diffusion combining rotations and collisions with other molecules)
- Can be characterized by a **rotational correlation time  $\tau_c$**
- $\tau_c$  is the time needed for the rms deflection of the molecules to be  $\sim 1$  radian ( $60^\circ$ )



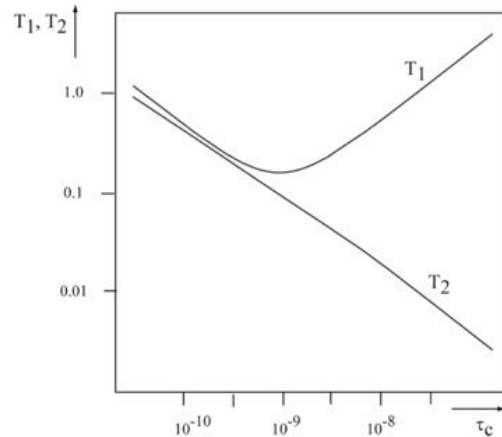
## Link to rotational motions in liquids

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



## Relaxation

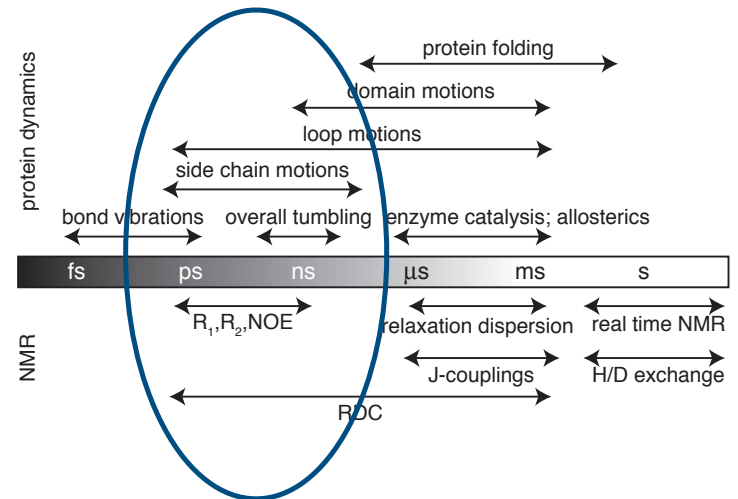
- relaxation time is related to rate of motion



$$R_1 = 1/T_1$$

$$R_2 = 1/T_2$$

## NMR time scales



## Protein backbone dynamics

- <sup>15</sup>N relaxation to describe ps-ns dynamics
  - R<sub>1</sub>: longitudinal relaxation rate
  - R<sub>2</sub>: transversal relaxation rate
  - hetero-nuclear NOE: {<sup>1</sup>H}-<sup>15</sup>N

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

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

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

where  $d^2 = (1/10) \gamma_H^2 \gamma_N^2 (\hbar/2\pi)^2 \langle \sigma_{NH} \rangle^2$  **dipole interaction**  
 and  $c^2 = (2/15) \omega_N^2 \langle \sigma_H, \sigma_N \rangle^2$  **chemical shift anisotropy**

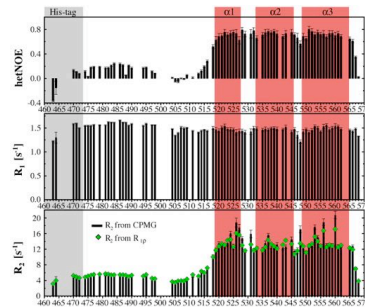
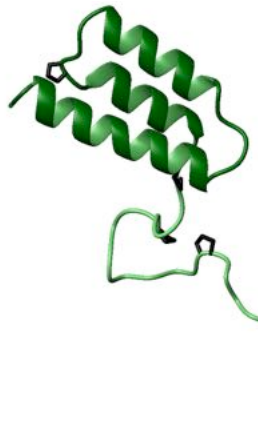
## Protein backbone dynamics

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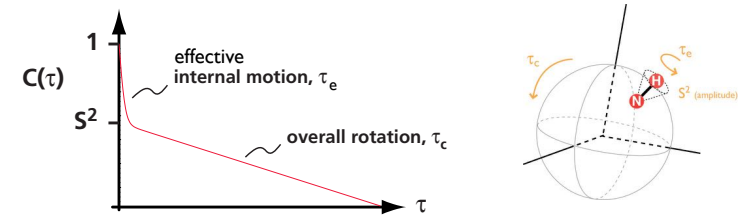
### Measured as a 2D <sup>1</sup>H-<sup>15</sup>N spectrum

- R<sub>1</sub>, R<sub>2</sub>: Repeat experiment several times with increasing relaxation-delay
  - I<sub>0</sub> exp(-Rt)
- Fit the signal intensity as a function of the relaxation delay
  - {<sup>1</sup>H}-<sup>15</sup>N NOE: Intensity ratio between saturated and non-saturated experiment

## Relaxation rates



## Lipari-Szabo MODELFREE



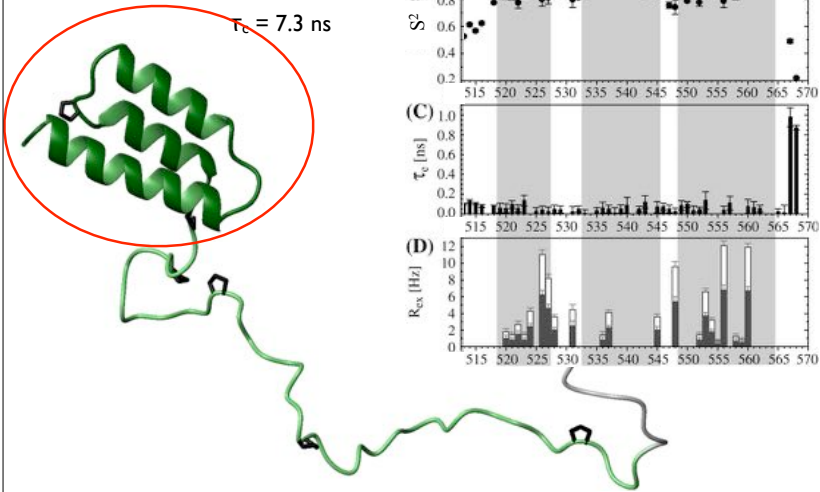
- Overall and local motion are considered to be uncorrelated
- S<sup>2</sup> = order-parameter

$$\left\{ \begin{array}{l} C_l(\tau=0) = 1 \\ C_l(\tau \rightarrow \infty) = S^2 \\ \int_0^\infty C_l(\tau) d\tau = \tau_e \end{array} \right\} \rightarrow C_l(\tau) = S^2 + (1 - S^2)e^{-\tau/\tau_e} \quad J(\omega) = \frac{2}{5} \left[ \frac{S^2 \tau_c}{1 + \omega^2 \tau_c^2} + \frac{(1 - S^2) \tau}{1 + \omega^2 \tau^2} \right]$$

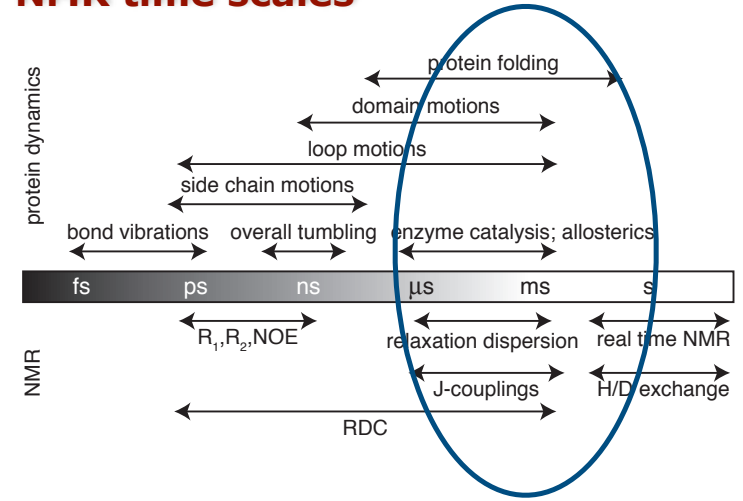
$$\tau^{-1} = \tau_c^{-1} + \tau_e^{-1}$$



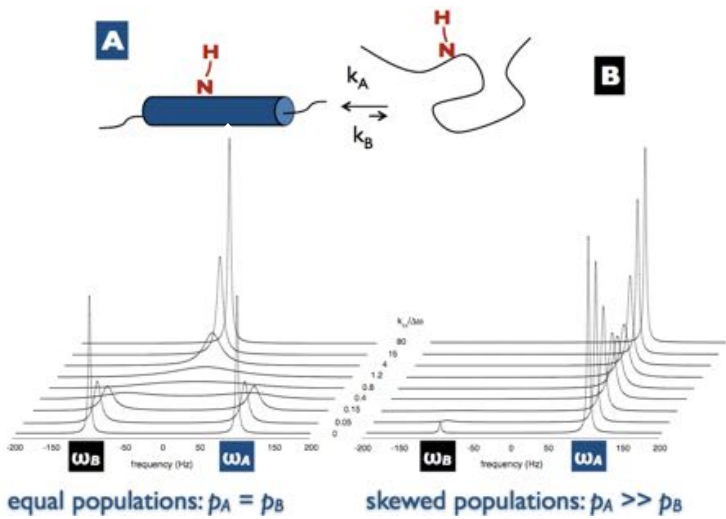
### Modelfree analysis



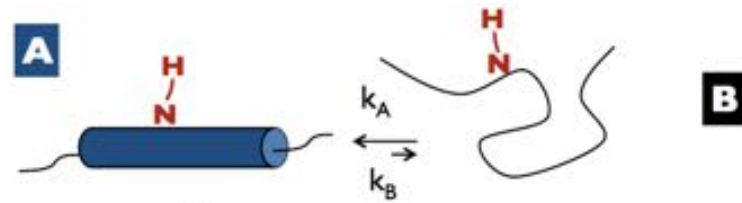
### NMR time scales



### Conformational exchange



### Conformational exchange



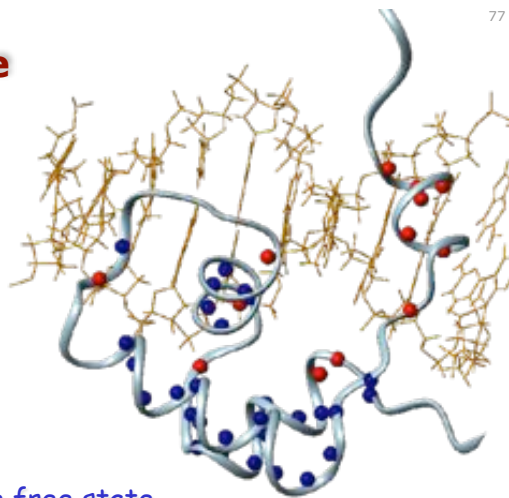
- Causes line-broadening of the signals

$$-R_{2,eff} = R_2 + R_{ex}$$

## H/D exchange

Lac headpiece

Kalodimos et al. Science



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

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## 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 ( $\mu$ s-ms): conformational exchange

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**The End**

**Thank you for your attention!**