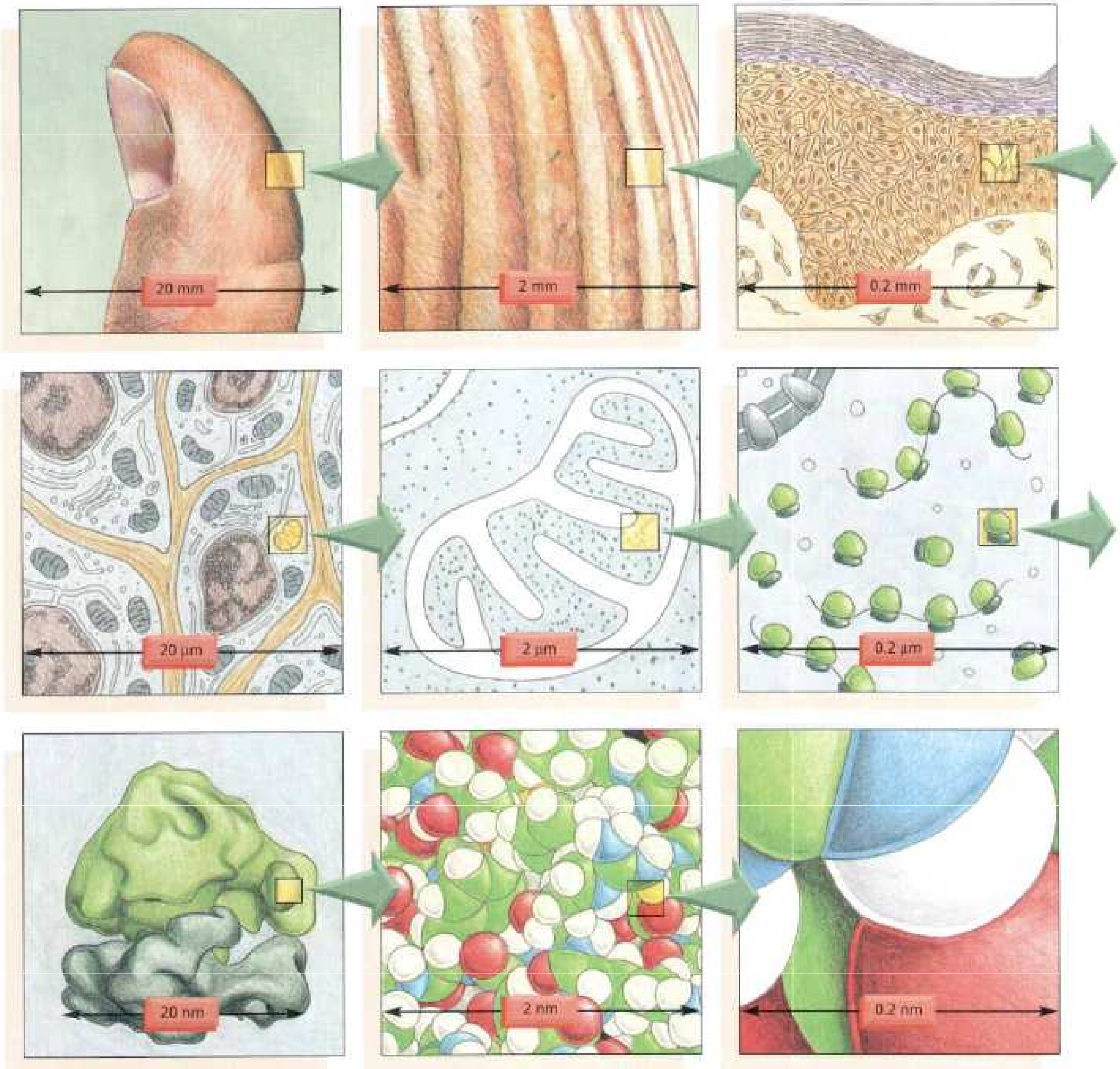
NMR-based Structural Biology for Studying Biomolecular Interactions

Karel Kubíček



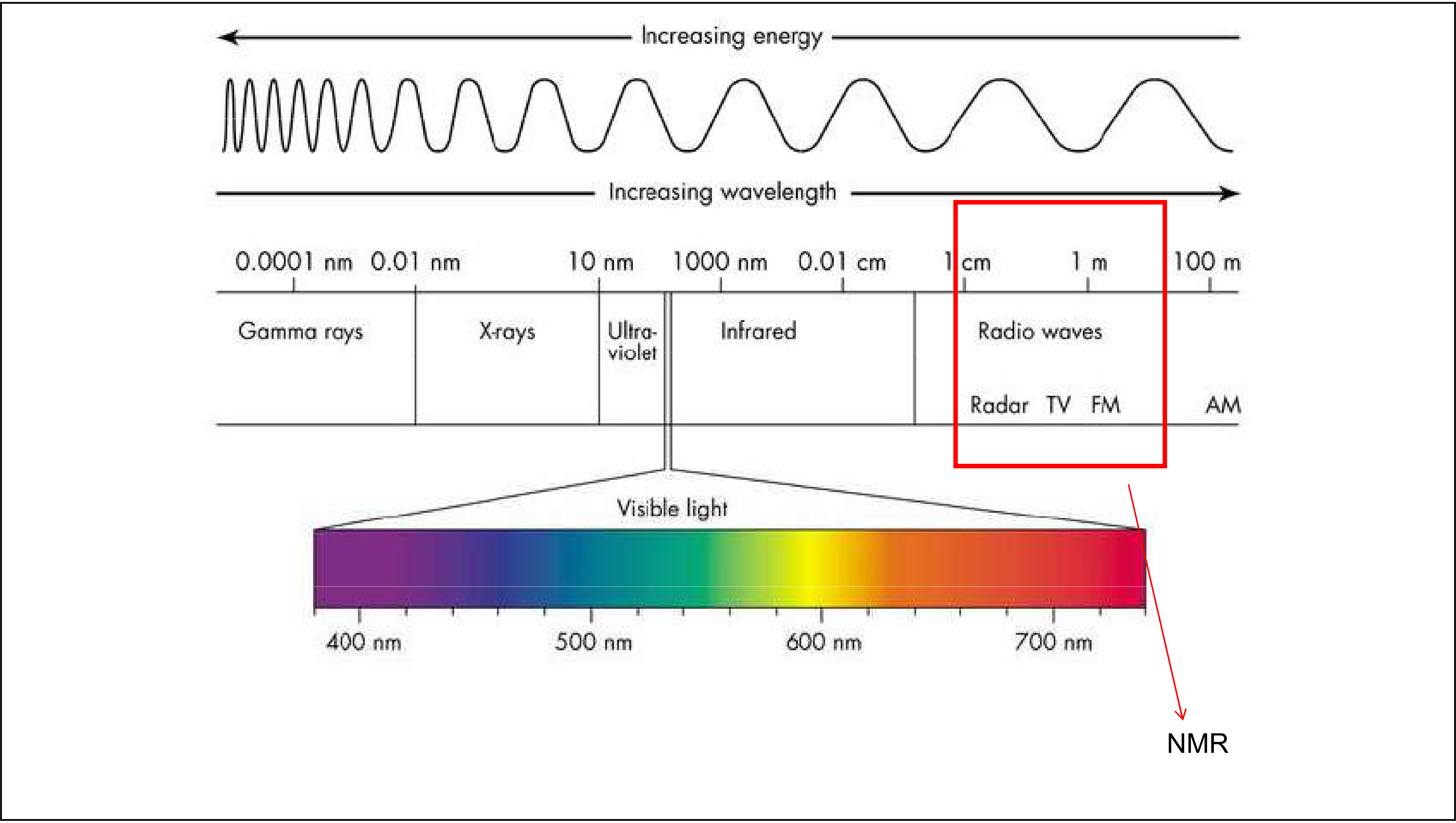
20 mm
2 mm
0.2 mm
20 µm
2 µm
0.2 µm
20 nm
2 nm
0.2 nm

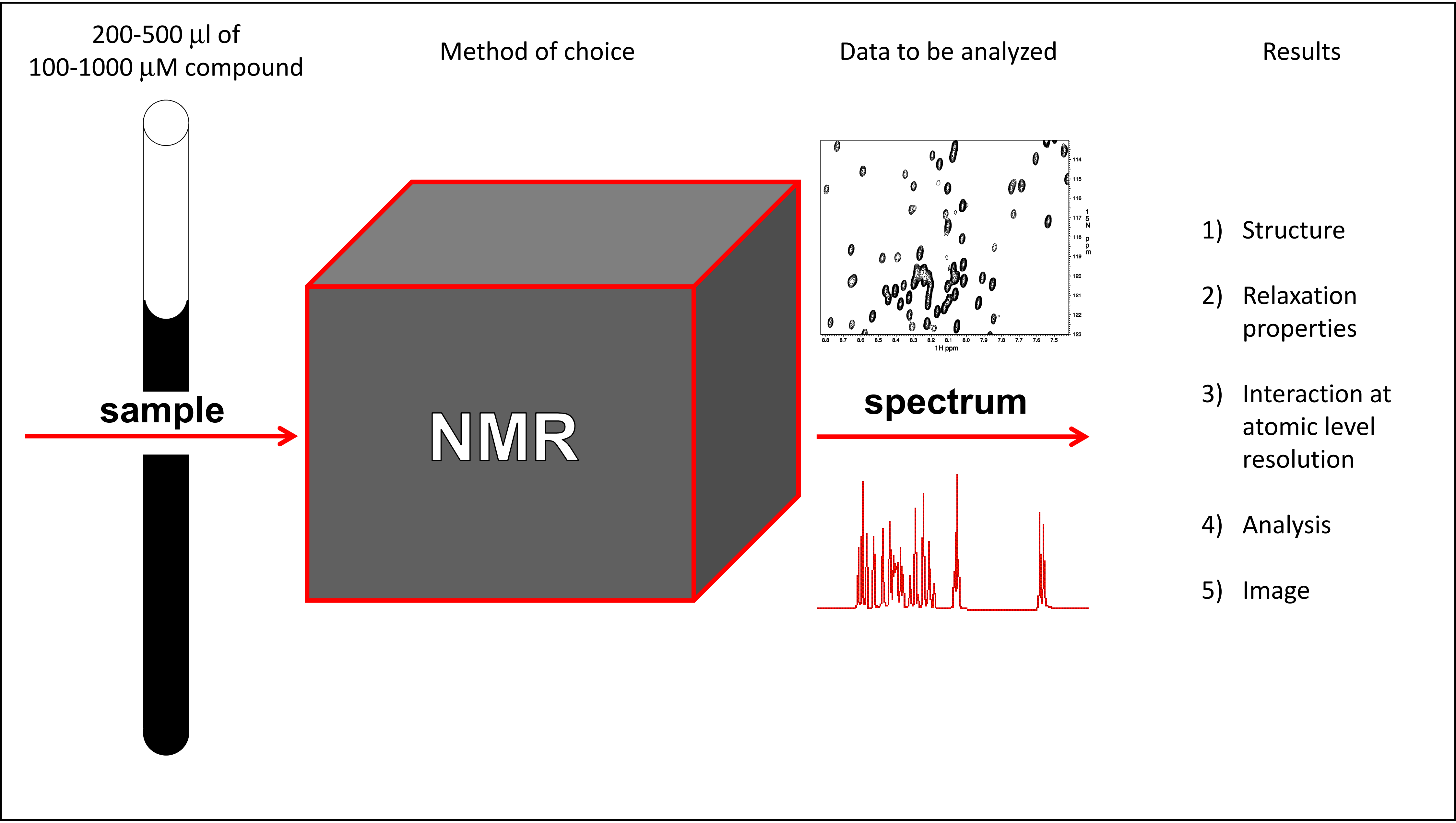
Composition of the Earth's Crust, Seawater, and the Human Body*

Earth's Crust		Seawater		Human Body†	
Element	%	Compound	m*M*	Element	%
O	47	Cl^-	548	H	63
Si	28	Na^+	470	O	25.5
Al	7.9	Mg^{2+}	54	C	9.5
Fe	4.5	SO_4^{2-}	28	N	1.4
Ca	3.5	Ca^{2+}	10	Ca	0.31
Na	2.5	K^+	10	P	0.22
K	2.5	HCO_3^-	2.3	Cl	0.08
Mg	2.2	NO_3^-	0.01	K	0.06
Ti	0.46	HPO_4^{2-}	<0.001	S	0.05
H	0.22			Na	0.03
C	0.19			Mg	0.01

*Figures for the earth's crust and the human body are presented as percentages of the total number of atoms; seawater data are millimoles per liter. Figures for the earth's crust do *not* include water, whereas figures for the human body do.

†Trace elements found in the human body serving essential biological functions include Mn, Fe, Co, Cu, Zn, Mo, I, Ni, and Se.


Increasing energy
Increasing wavelength
0.0001 nm
0.01 nm
10 nm
1000 nm
0.01 cm
1 cm
1 m
100 m
Gamma rays
X-rays
Ultra-
violet
Infrared
Radio waves
Radar
TV
FM
AM
Visible light
400 nm
500 nm
600 nm
700 nm
NMR



200-500 µl of
100-1000 µM compound
Method of choice
Data to be analyzed
Results
sample
NMR
spectrum
1) Structure
2) Relaxation properties
3) Interaction at atomic level resolution
4) Analysis
5) Image

NMR hardware

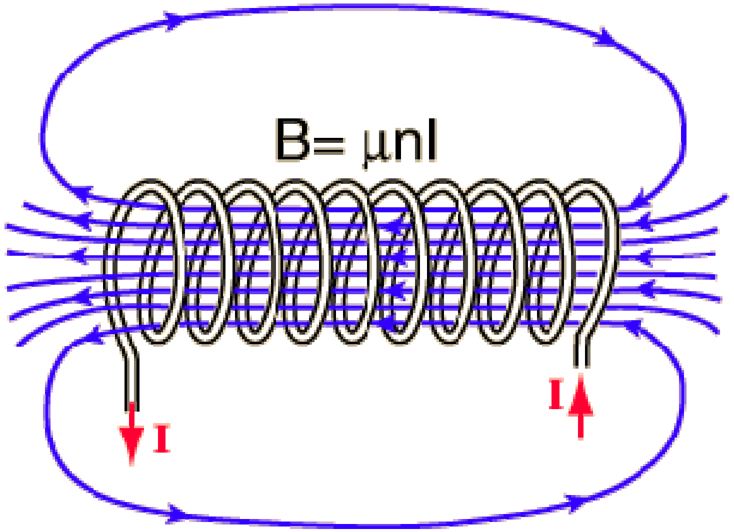
1)Magnet
2)Spectrometer
3)Control units

NMR spectrometer

Earth's Magnetic Field

~ 50μT

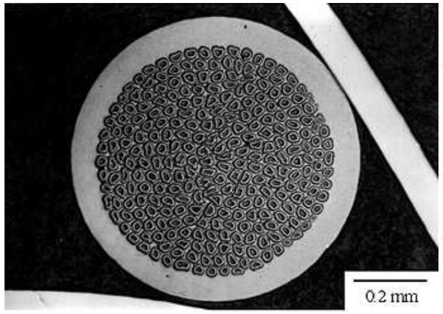
Ampere's law & solenoid

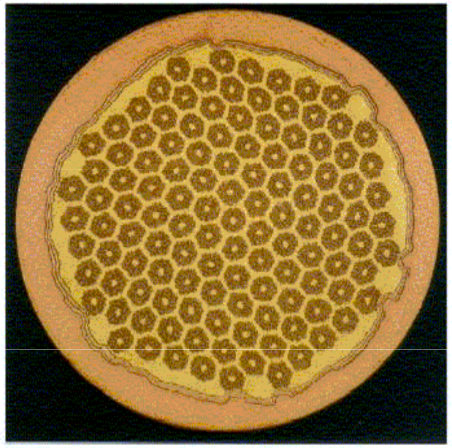


The magnetic field is concentrated into a nearly uniform field in the center of a long solenoid. The field outside is weak and divergent.

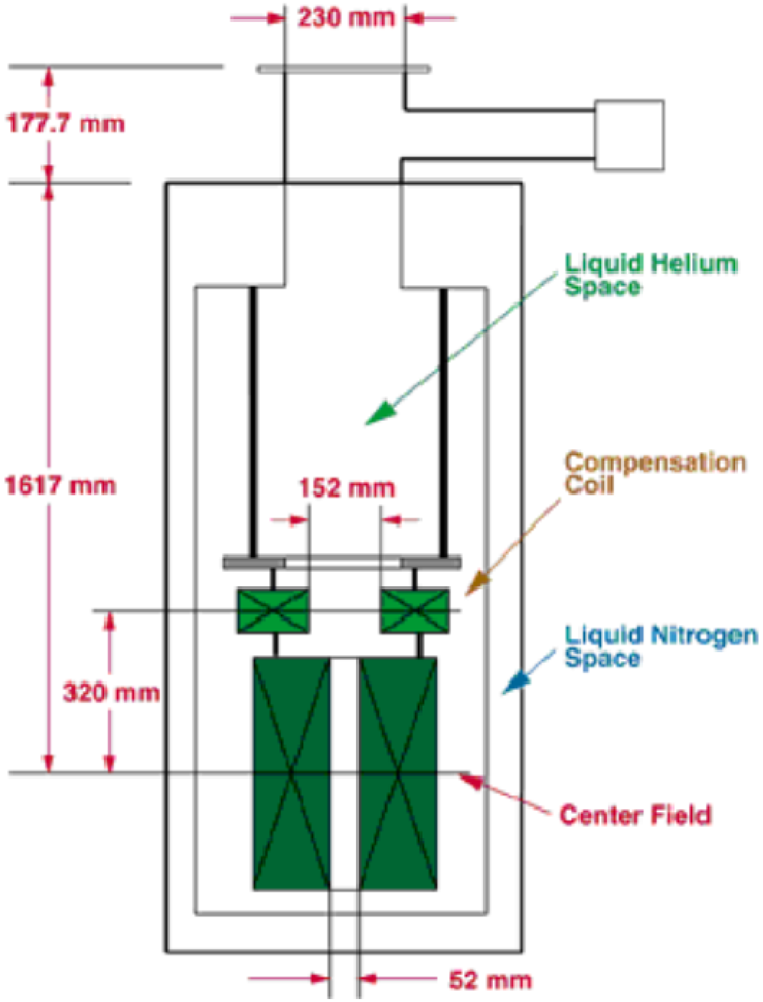
Magnet

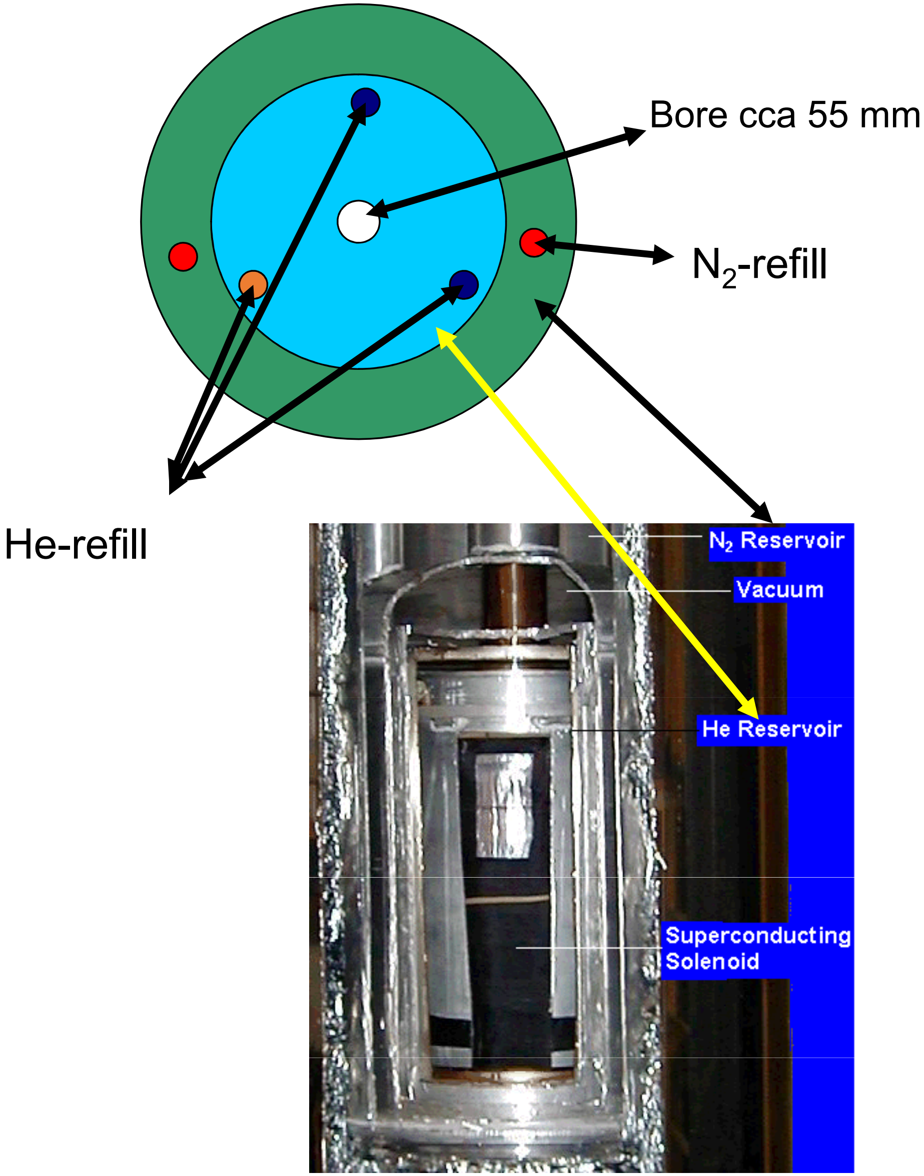
- superconducting solenoids immersed into He bath
- He-bath **~4 K** further improved to **~2.1 K** with J-T pump
- field strength 25-28 Tesla
- $(Nb, Ta)_3Sn$ superconductor of 0.81 mm with ~271 filaments buried in OFHC copper matrix





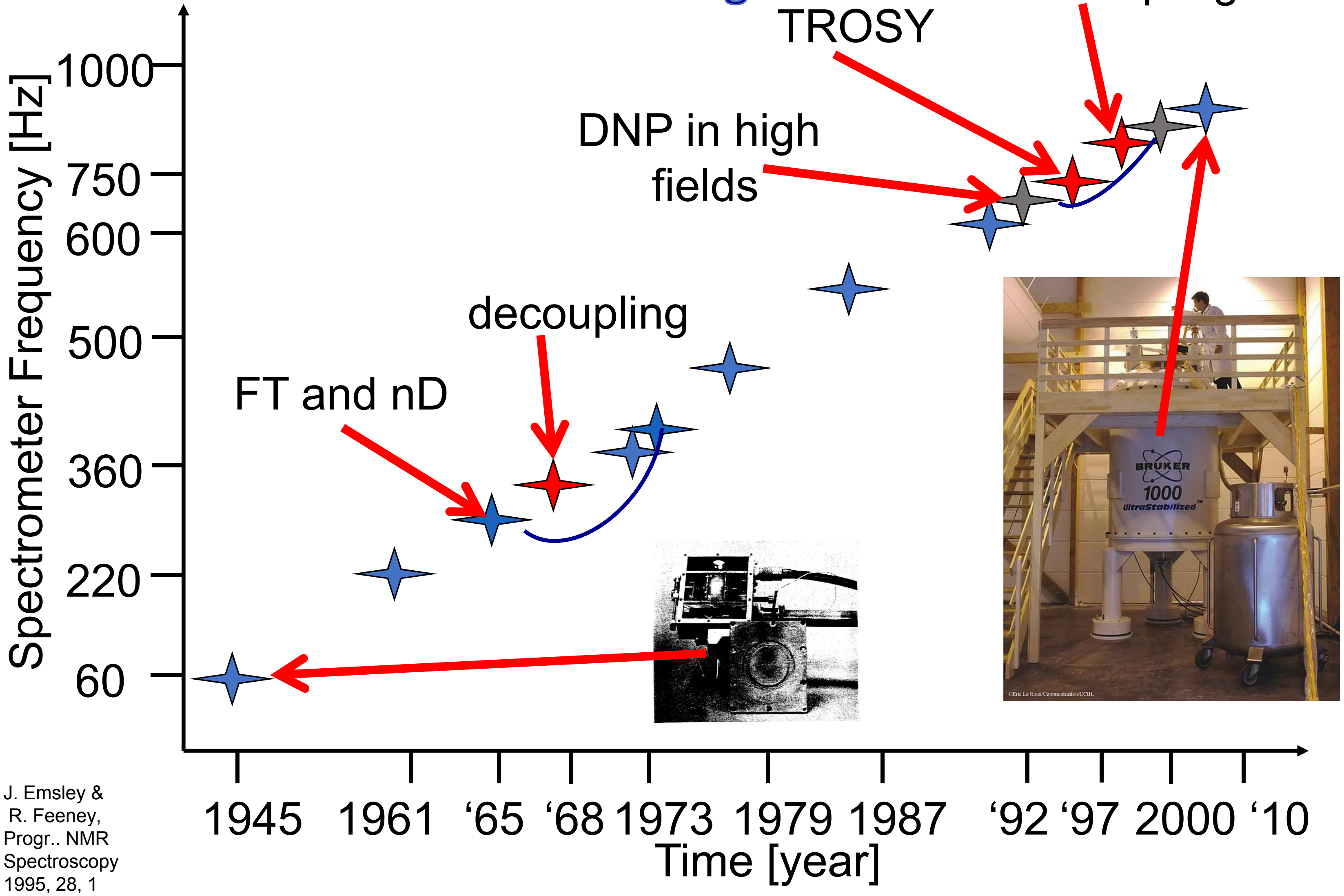
20T Superconducting Magnet





Bore cca 55 mm
N_2-refill
He-refill
N_2 Reservoir
Vacuum
He Reservoir
Superconducting Solenoid

Improvement of sensitivity, resolution and signal-to-noise ratio Of NMR magnets



J. Emsley & R. Feeney, Progr.. NMR Spectroscopy 1995, 28, 1

Quench

an **abnormal** termination of magnet operation

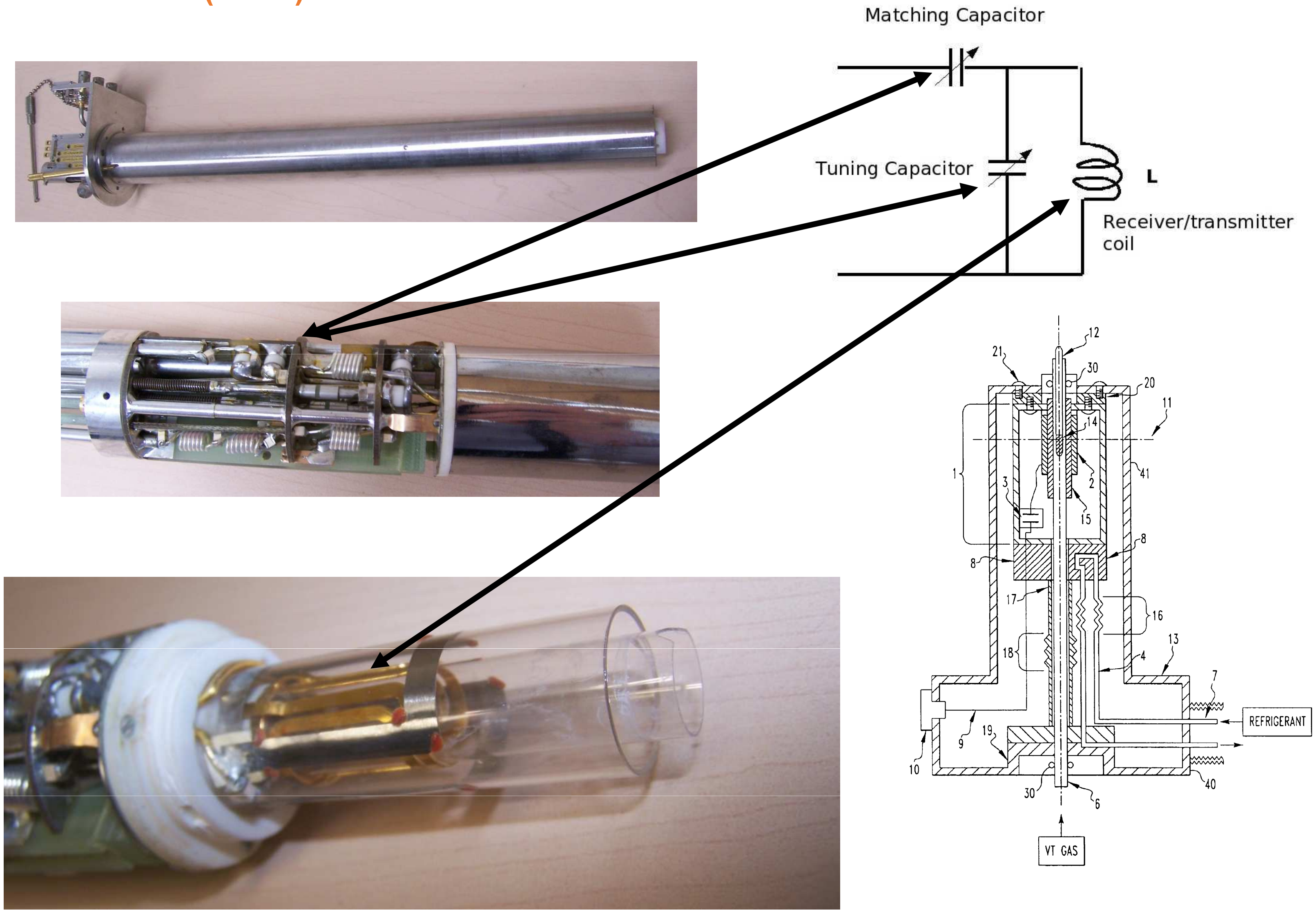
Occurs when part of the superconducting coil enters the normal (resistive) state.

This can **occur**

i) because the field inside the magnet is too large
ii) the rate of change of field is too large (causing eddy currents and resultant heating in the copper support matrix)
iii) or a combination of the two.
iv) a defect in the magnet can cause a quench.

MOVIE: https://www.youtube.com/watch?v=d-G3Kg-7n_M

NMR Probe(head)



Spectrometer

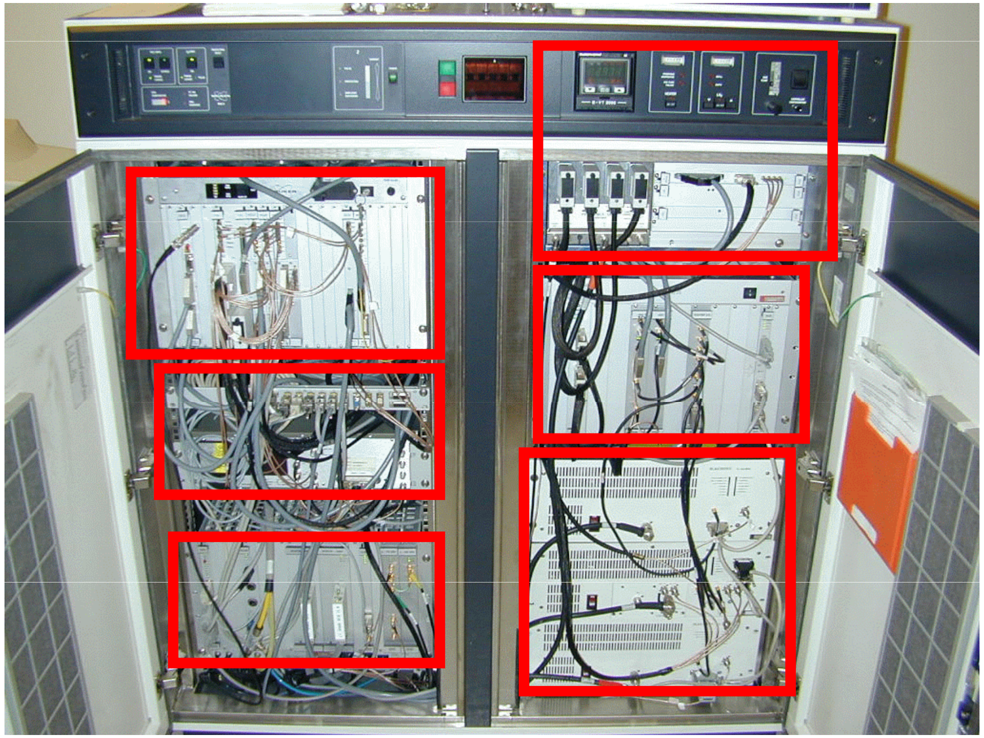
CBU

Control board unit

FGU

Frequency gen. u.

Shimms

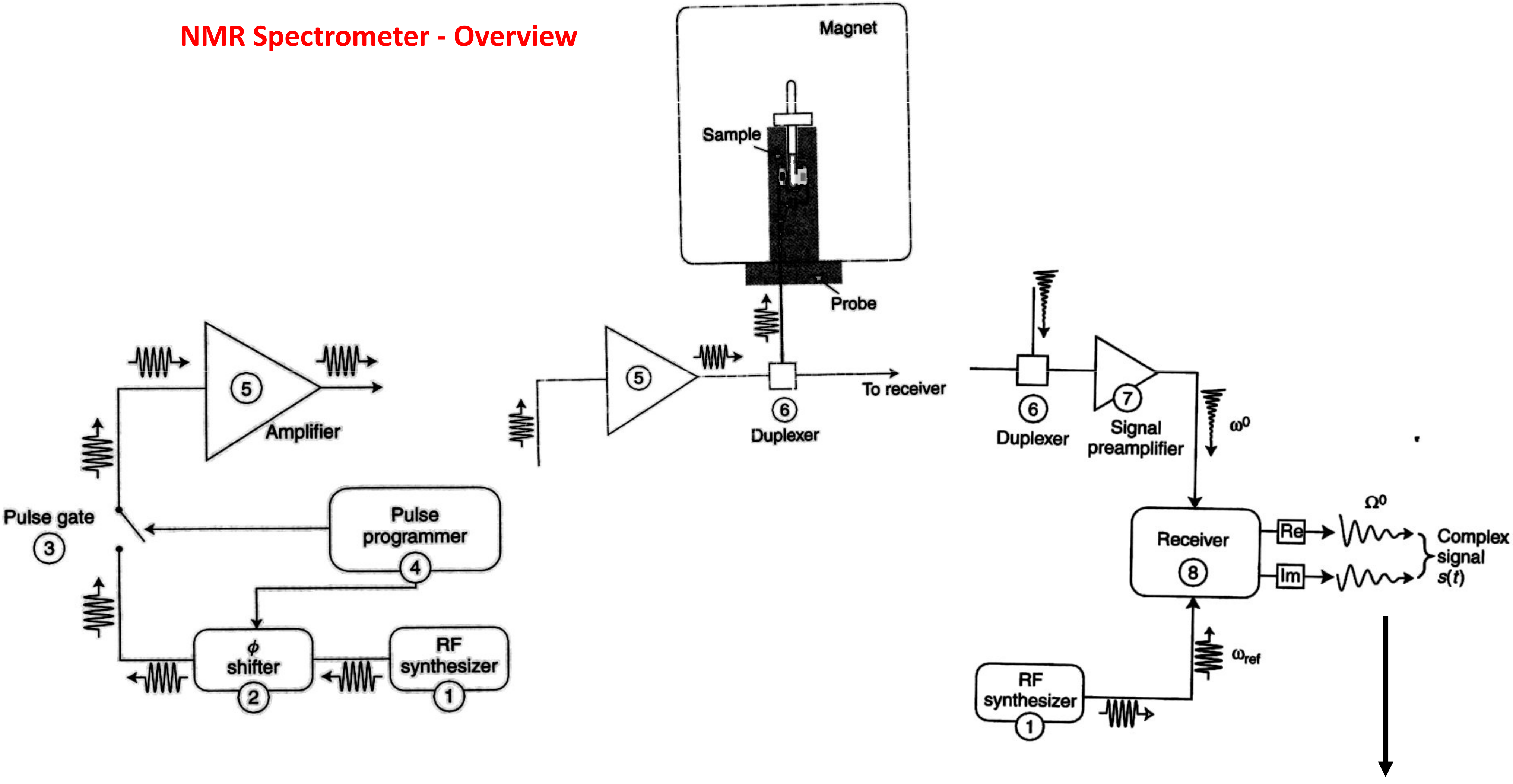


Temperature Unit

AcquisitionController

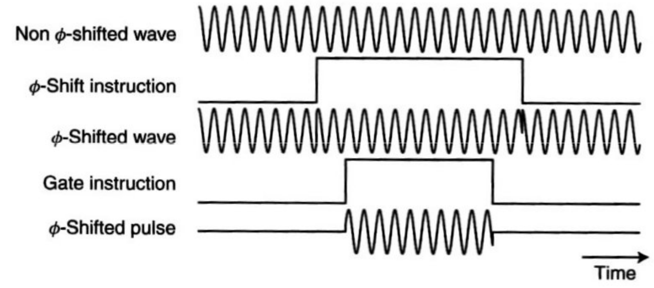
Transmitter

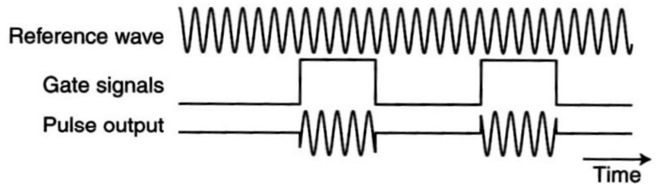
NMR Spectrometer - Overview



Signal - $s_l(t)=\Sigma\, s_l(t)$

NMR radiofrequency pulse





R.f. phase	Jargon
$\phi = 0$	'x-pulse'
$\phi = \pi/2$	'y-pulse'
$\phi = \pi$	'$\bar{x}$-pulse' or '$-x$-pulse'
$\phi = 3\pi/2$	'$\bar{y}$-pulse' or '$-y$-pulse'

Pulzy:

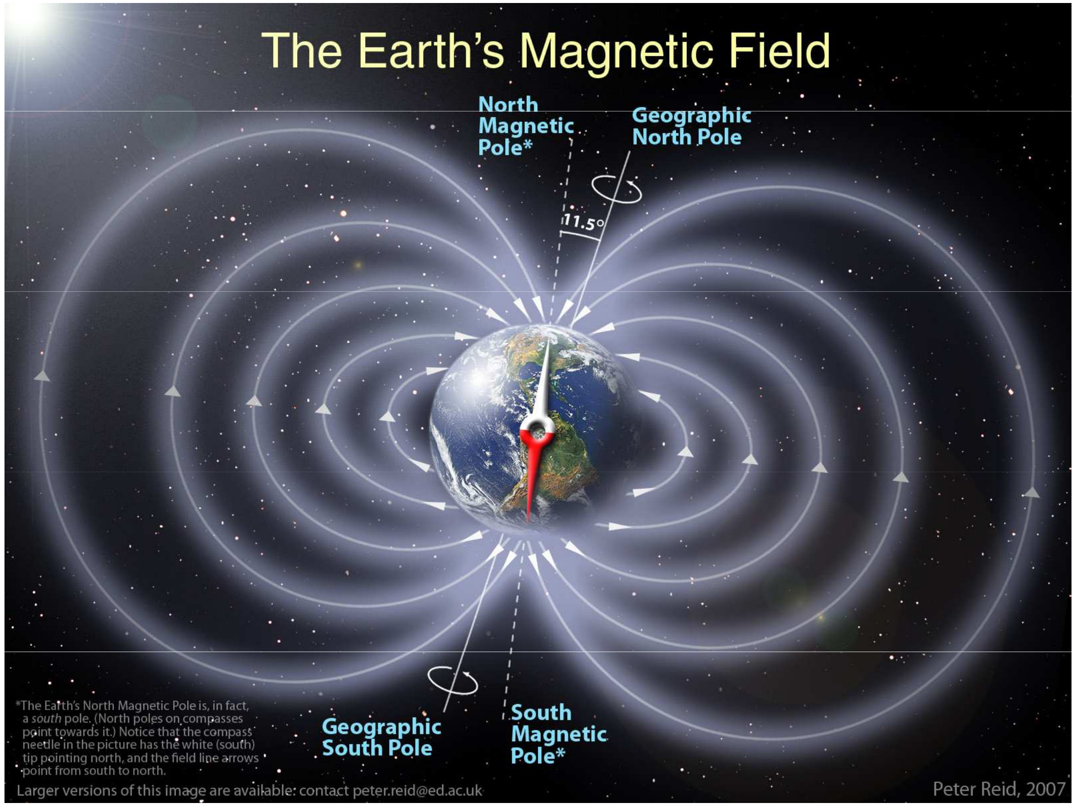
a) tvrdé – 7-30 μs@-3~+3dB

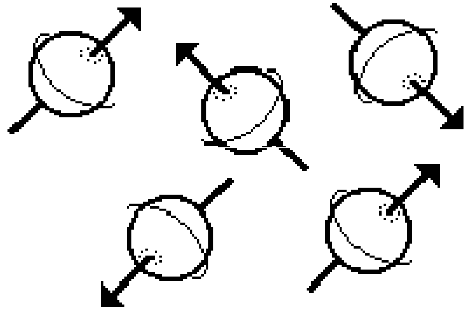
b) selektivní – ms~s@>30db

c) adiabatické

For NMR, nuclear spin is needed!!!

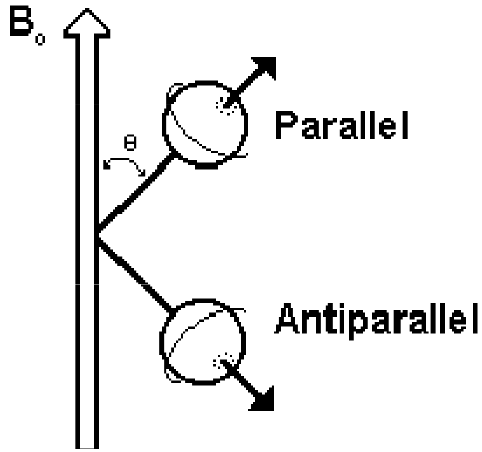
Spin analogy to a compass needle





Randomly oriented nuclear magnetic moments

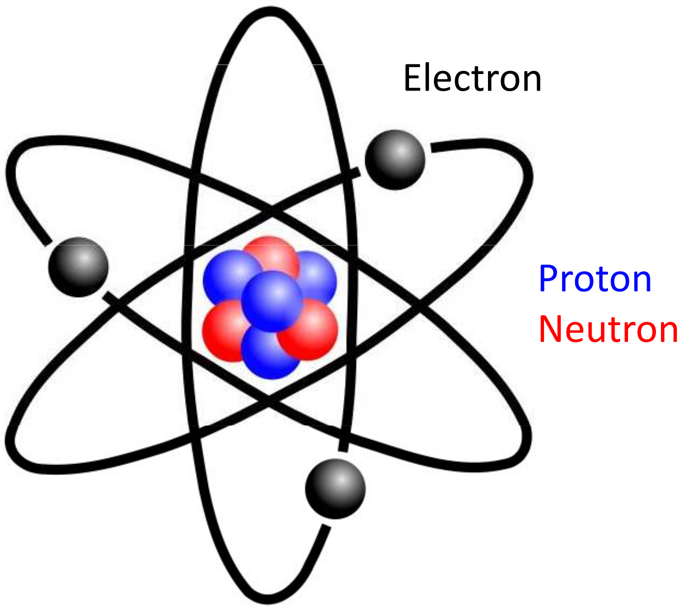
magnetic field = 0



Nuclear magnetic moments in the presence of an external field

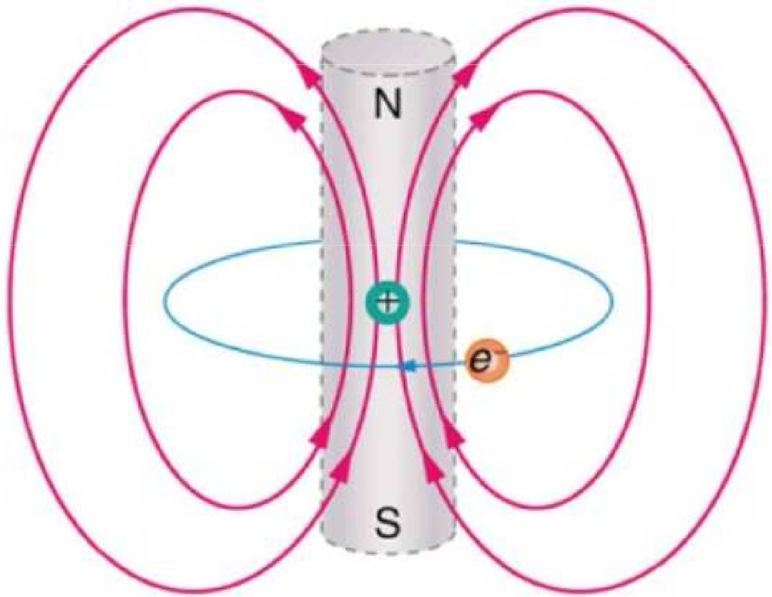
magnetic field > 0

Atom



=

In the planetary model of the atom, an **electron orbits a nucleus**, forming a closed-current loop and **producing a magnetic field** with a north pole and a south pole.



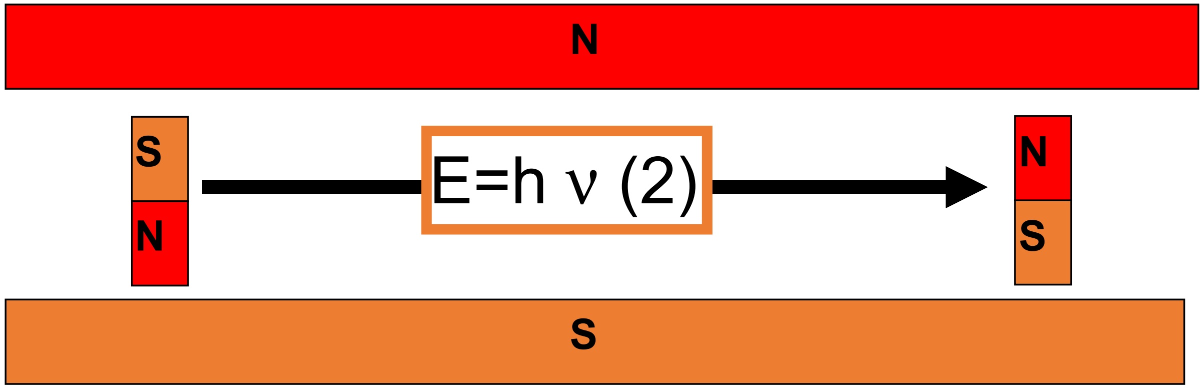
Molecule is hence a group of small magnetic fields and each atom within the molecule experiences different local magnetic field.

NMR - Refresh

1) nuclear spin ≠ 0 (^{1}H, ^{13}C, ^{15}N, ^{31}P)
   - number of neutrons **and** the number of protons **both even** ⇒ **NO** nuclear **spin**
   - number of neutrons **plus** the number of protons **odd** ⇒ **half-integer spin** (i.e. ½, 3/2, 5/2)
   - number of neutrons **and** the number of protons **both odd** ⇒ **integer spin** (i.e. 1, 2, 3)

2) $\nu=\gamma * B$ **(1)** - when placed in a magnetic field of strength **B**, a nuclei with a net spin can absorb a photon, of frequency ν. The frequency ν depends on the gyromagnetic ratio, γ of the nuclei

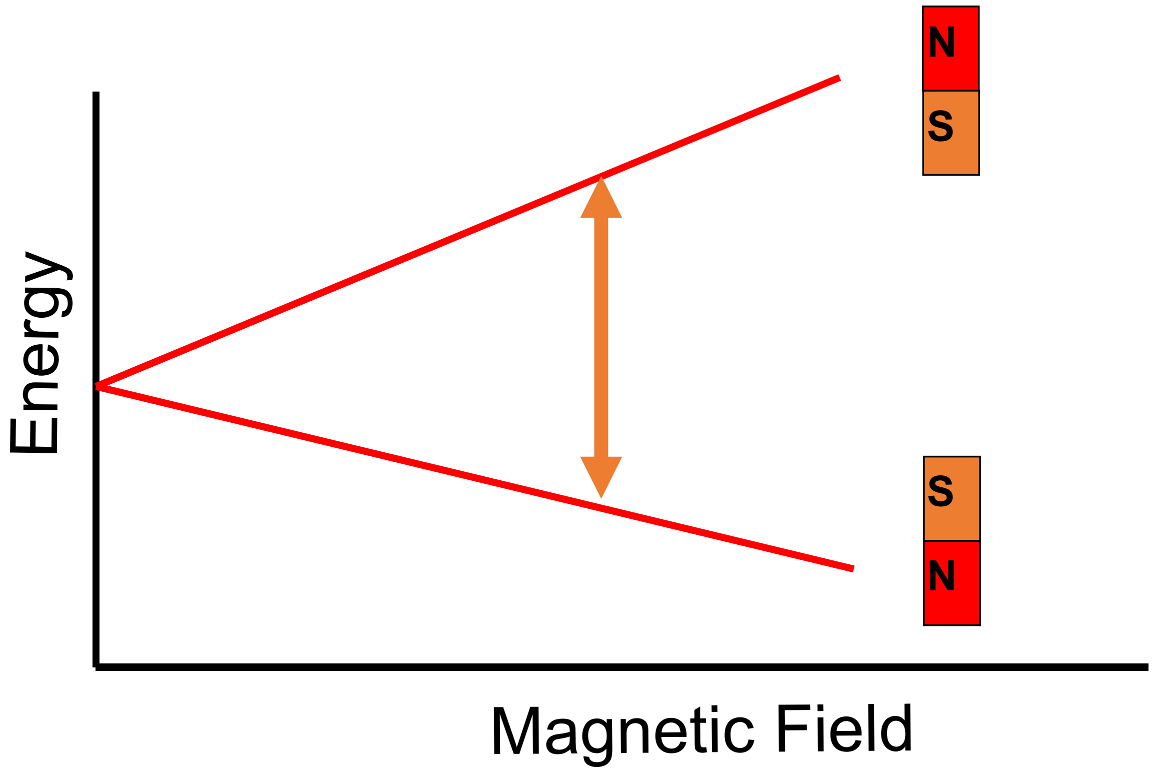
3) from quantum mechanics we know that nucleus with spin ***I*** can have ***2I +1*** orientations ⇒ **nuclei with a spin ½** can have **two orientations in** an external **magnetic field**– **low / high energy**

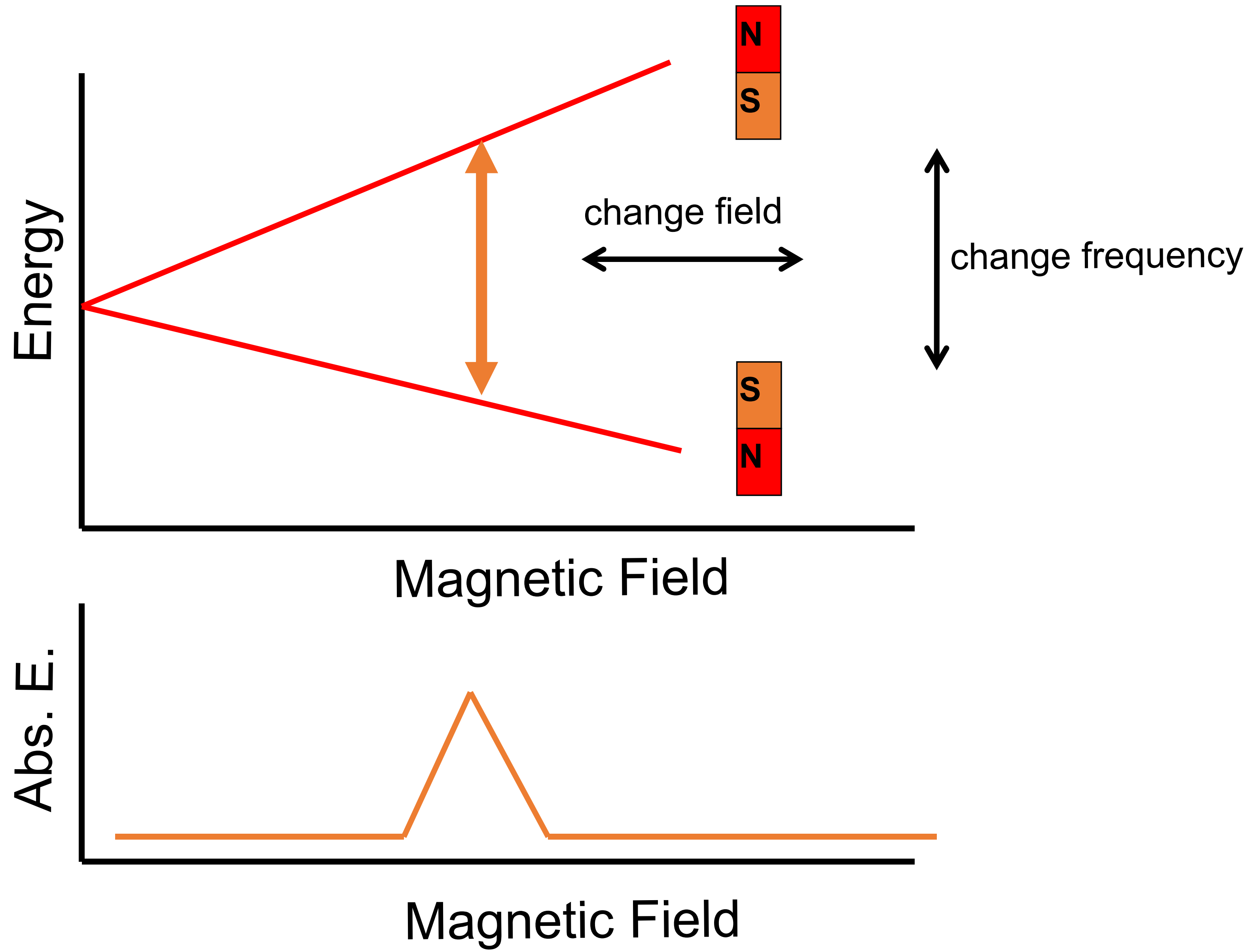


Nuclear Magnetic Resonance

Refresh

From (1) and (2): E=h γ B




N
S
change field
change frequency
S
N
Energy
Magnetic Field
Abs. E.
Magnetic Field

CW vs. Fourier transform NMR

Problem of NMR

the magnitude of the energy changes in NMR spectroscopy small ⇒ **sensitivity is a major limitation**

Solution I.

increase sensitivity by recording many spectra, and then add them together; because **noise is random**, it adds as the square root of the number of spectra recorded.

For example, if **100** spectra of a compound were recorded and summed, then the ***noise would increase*** by a factor of **10**,

but the ***signal would increase*** in magnitude by a factor of **100**
⇒ large increase in sensitivity.

However, if this is done using a **CW-NMR**, the time needed to collect the spectra is very large (one scan takes **2** - **8** minutes).

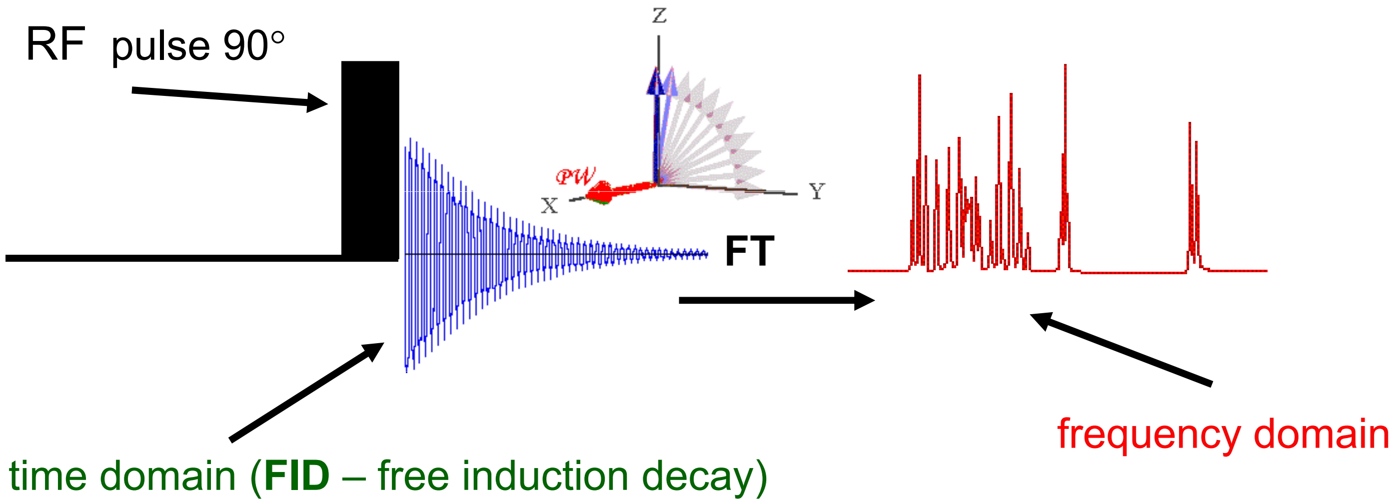
CW vs. Fourier transform NMR

Solution II.

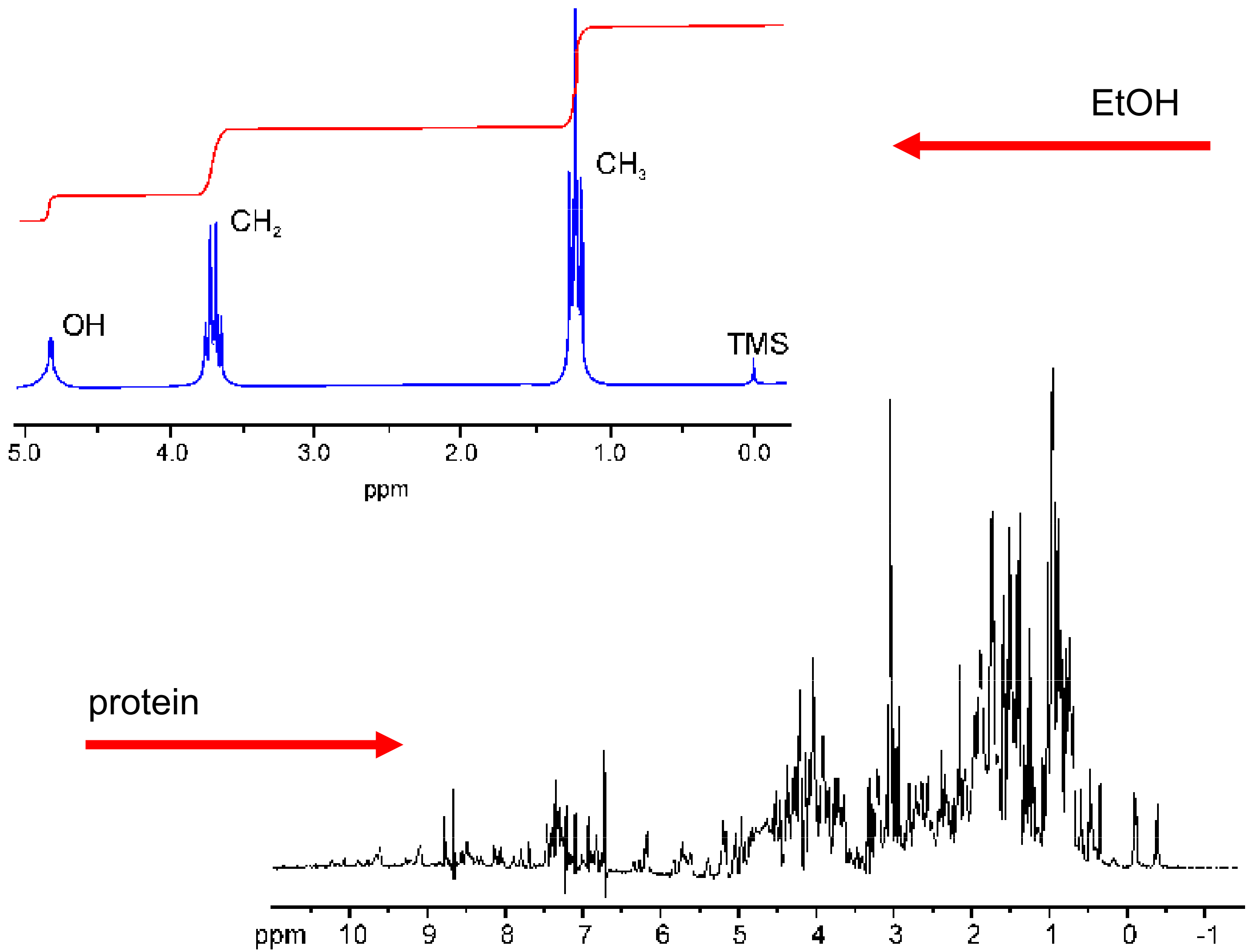
FT-NMR ⇒ ***all frequencies*** in a spectrum are ***irradiated simultaneously*** with a radio frequency pulse.

Following the pulse, the nuclei return to thermal equilibrium. A ***time domain*** emission signal is recorded by the instrument as the nuclei relax.

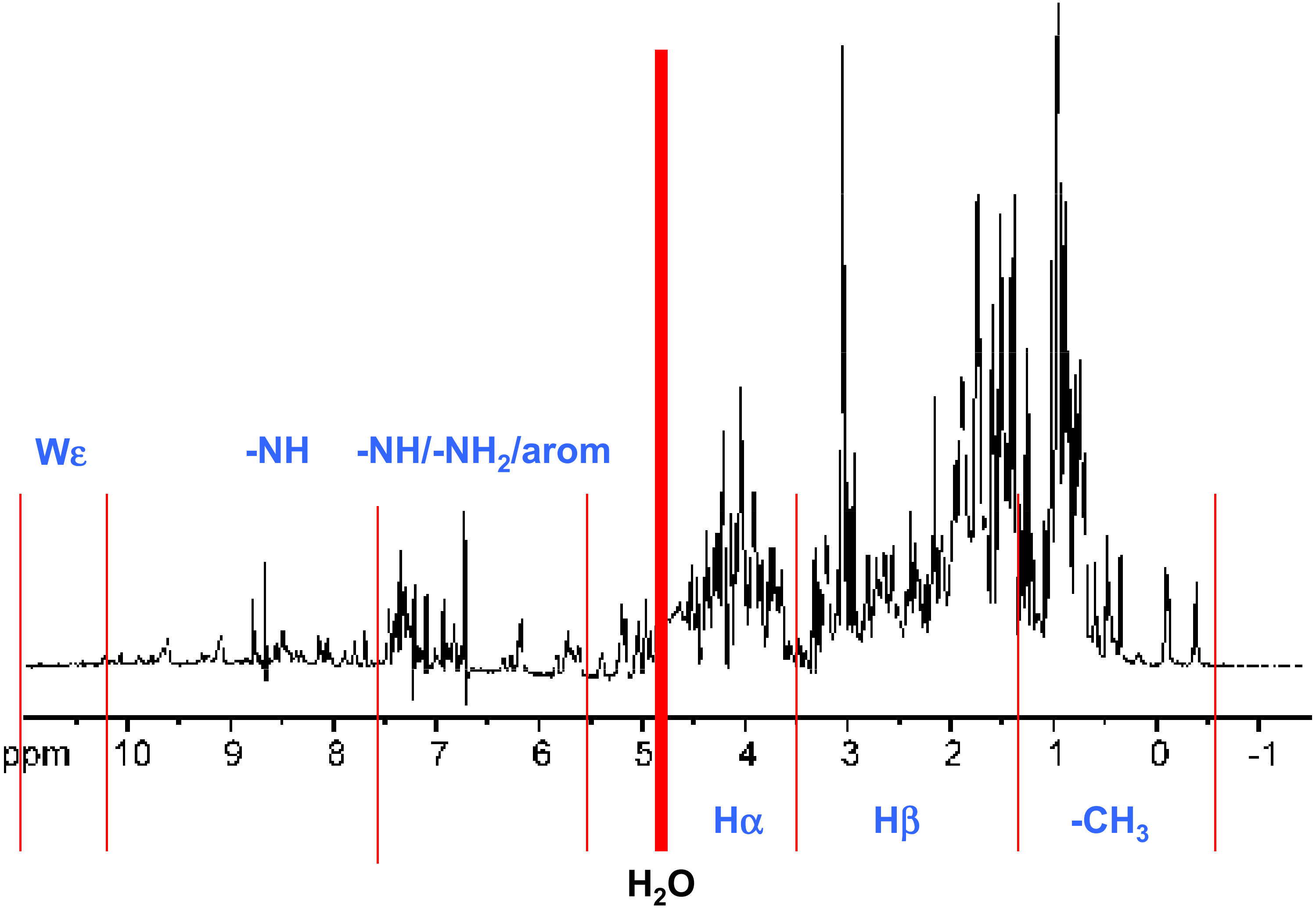
A ***frequency domain*** spectrum is ***obtained by Fourier transformation***.



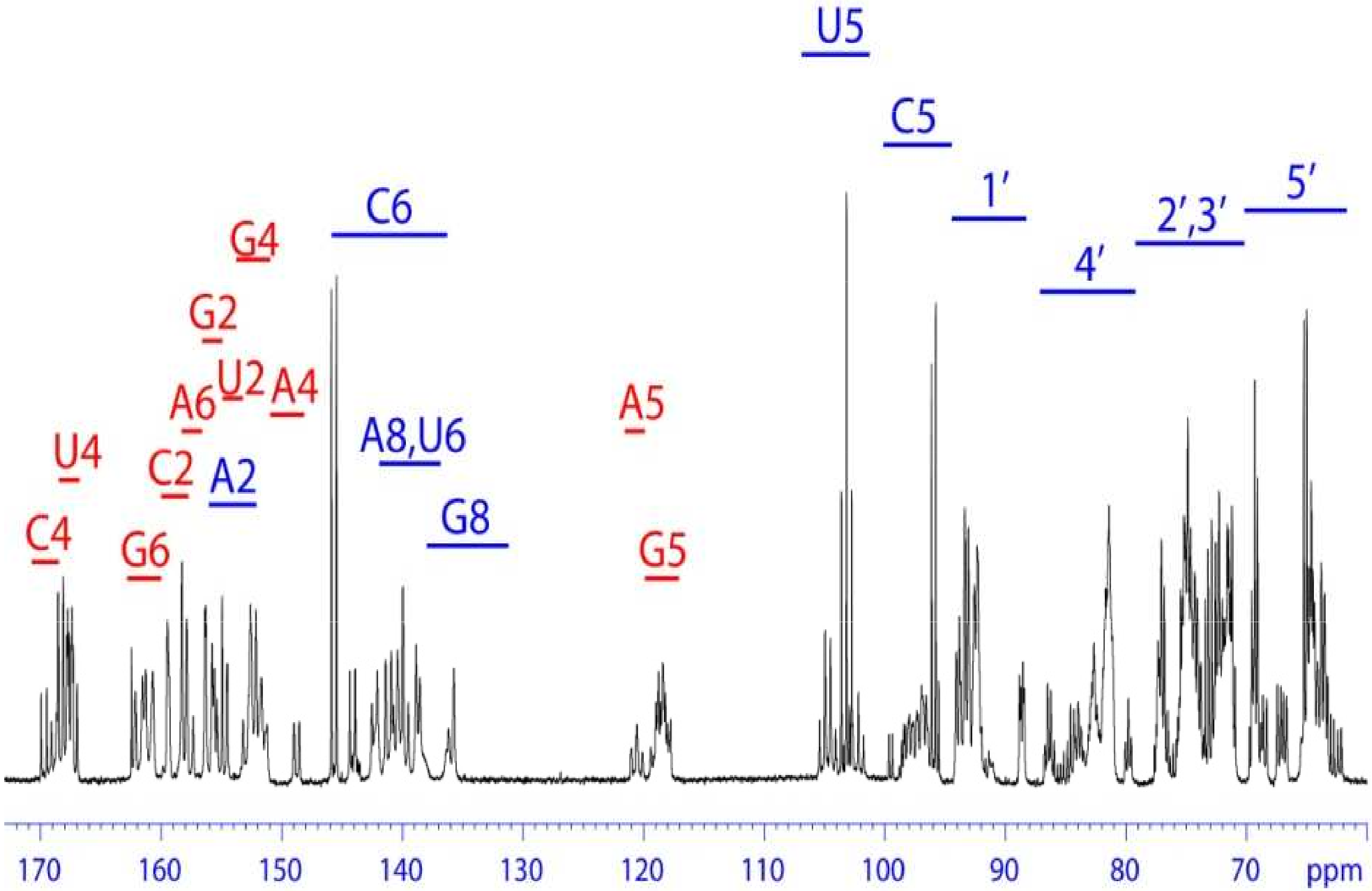
Each proton = 1 NMR signal

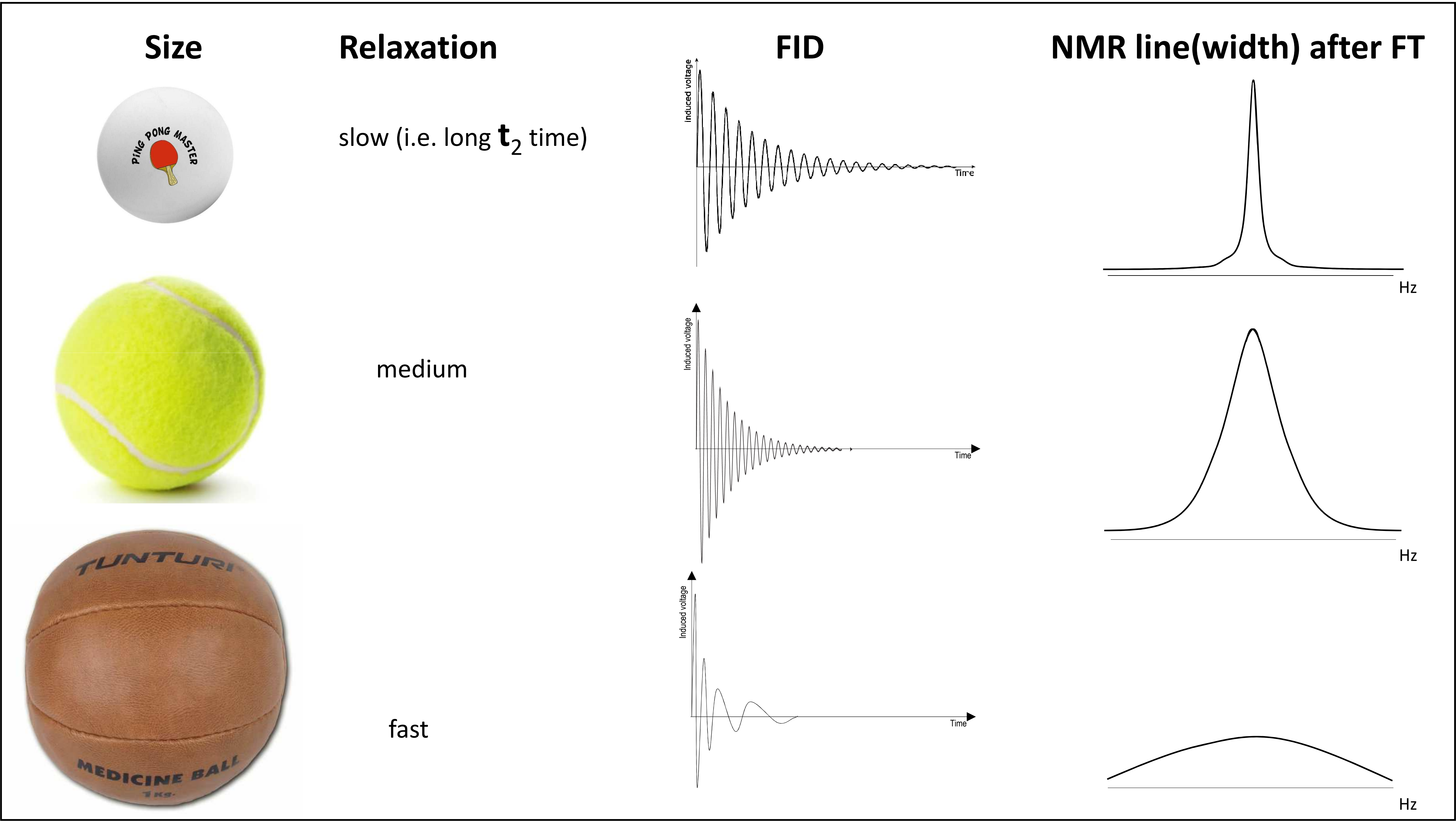


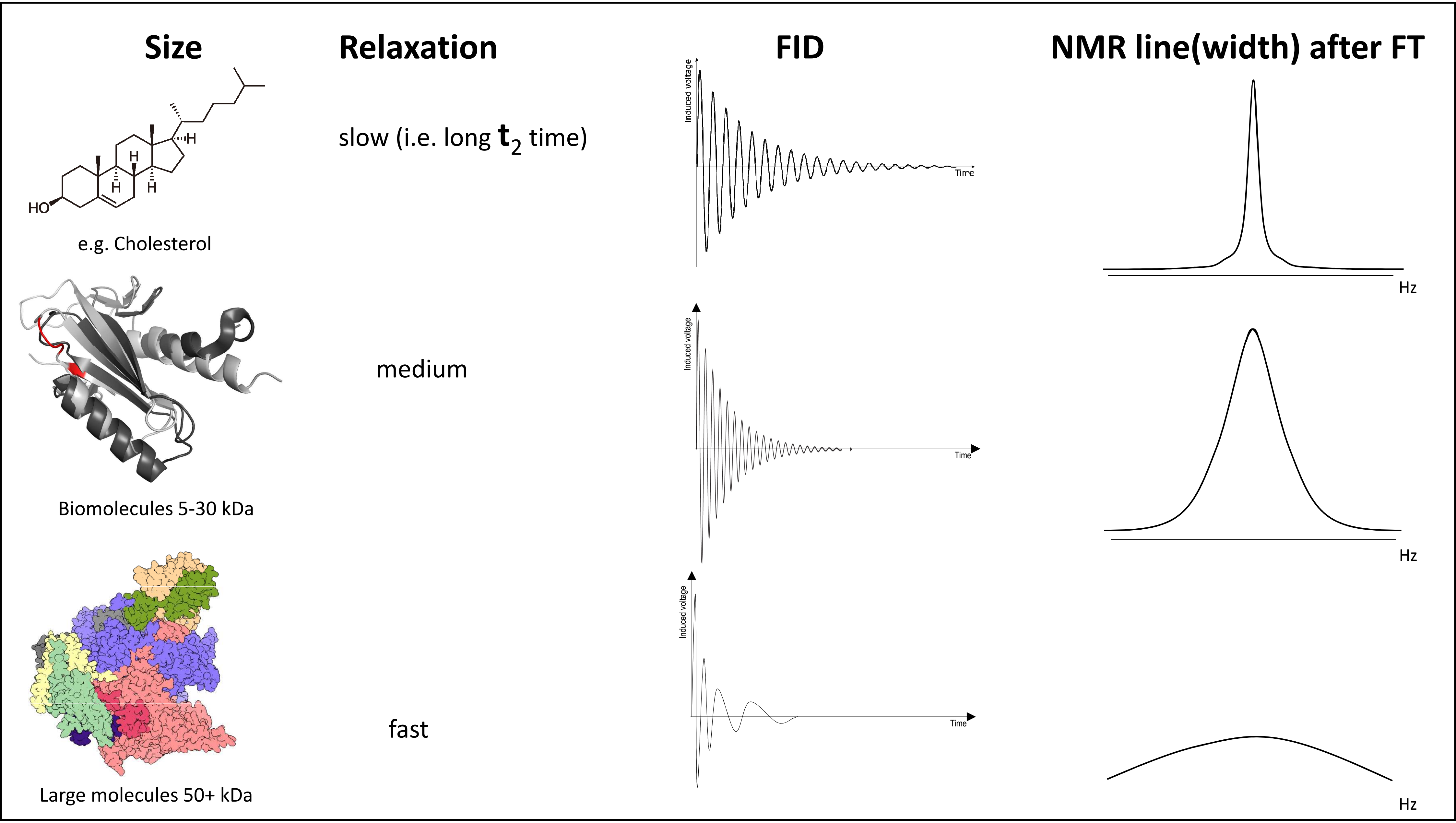
Each (non-exchangeable) proton = 1 NMR signal



Each (non-exchangeable) proton = 1 NMR signal

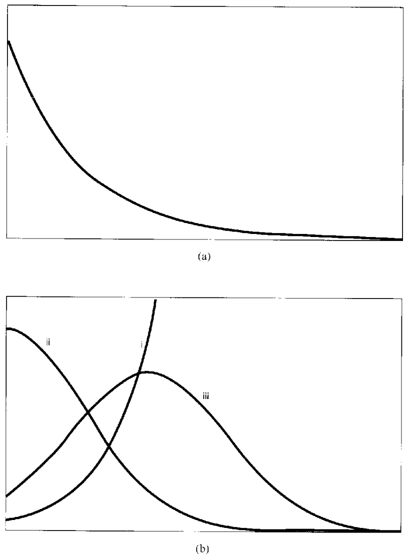



Size
Relaxation
FID
NMR line(width) after FT
PING PONG MASTER
slow (i.e. long t_2 time)
Induced voltage
Time
Hz
medium
Induced voltage
Time
Hz
TUNTURI
MEDICINE BALL
1 Kg.
fast
Induced voltage
Time
Hz


Size
Relaxation
FID
NMR line(width) after FT
HO
H
e.g. Cholesterol
slow (i.e. long t_2 time)
Induced voltage
Time
Hz
Biomolecules 5-30 kDa
medium
Induced voltage
Time
Hz
Large molecules 50+ kDa
fast
Induced voltage
Time
Hz

NMR data processing

NMR data processing

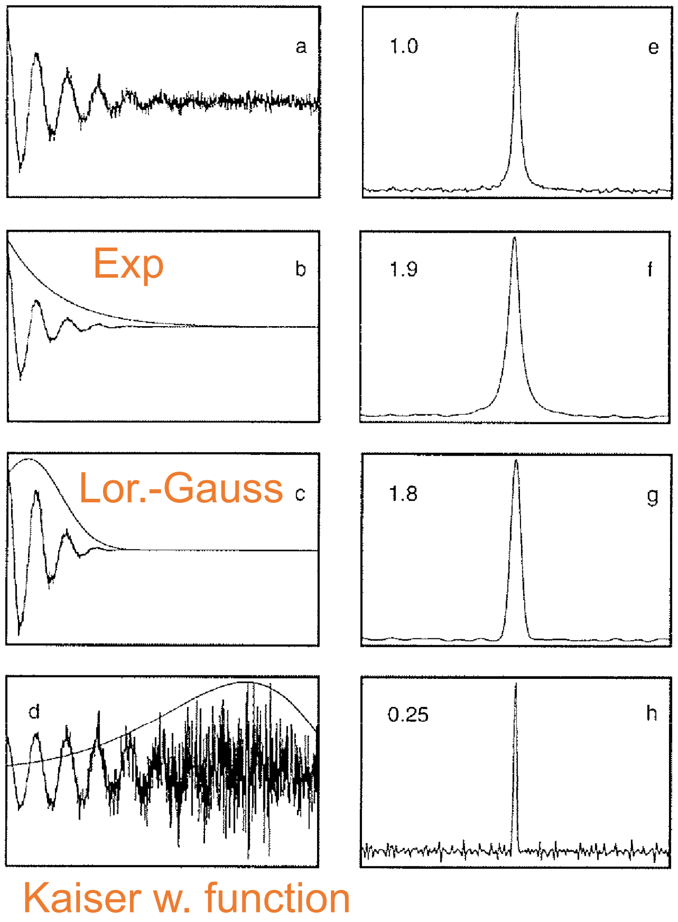


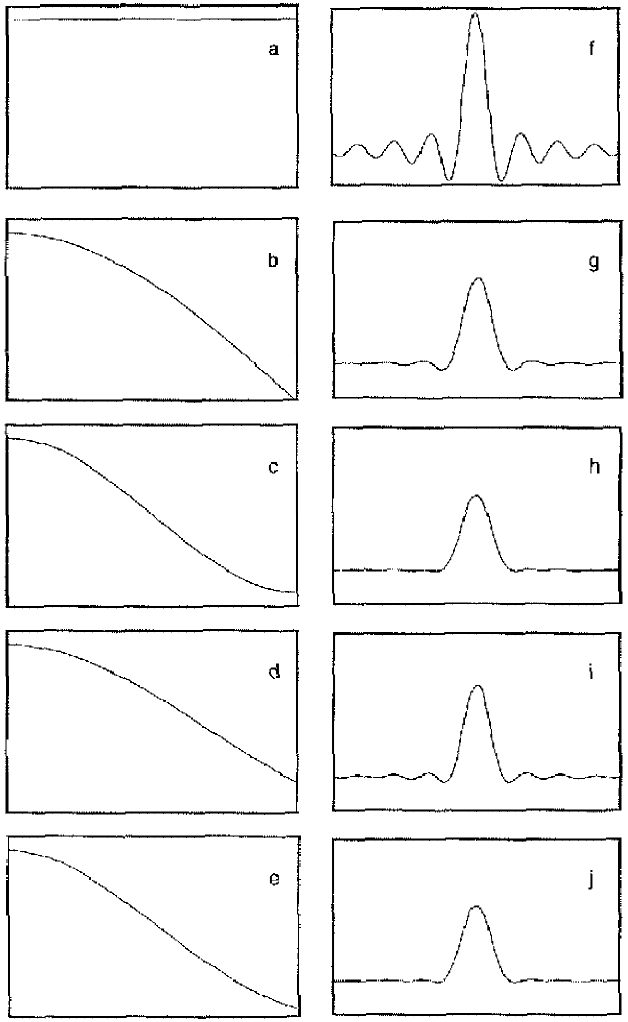
Window functions:

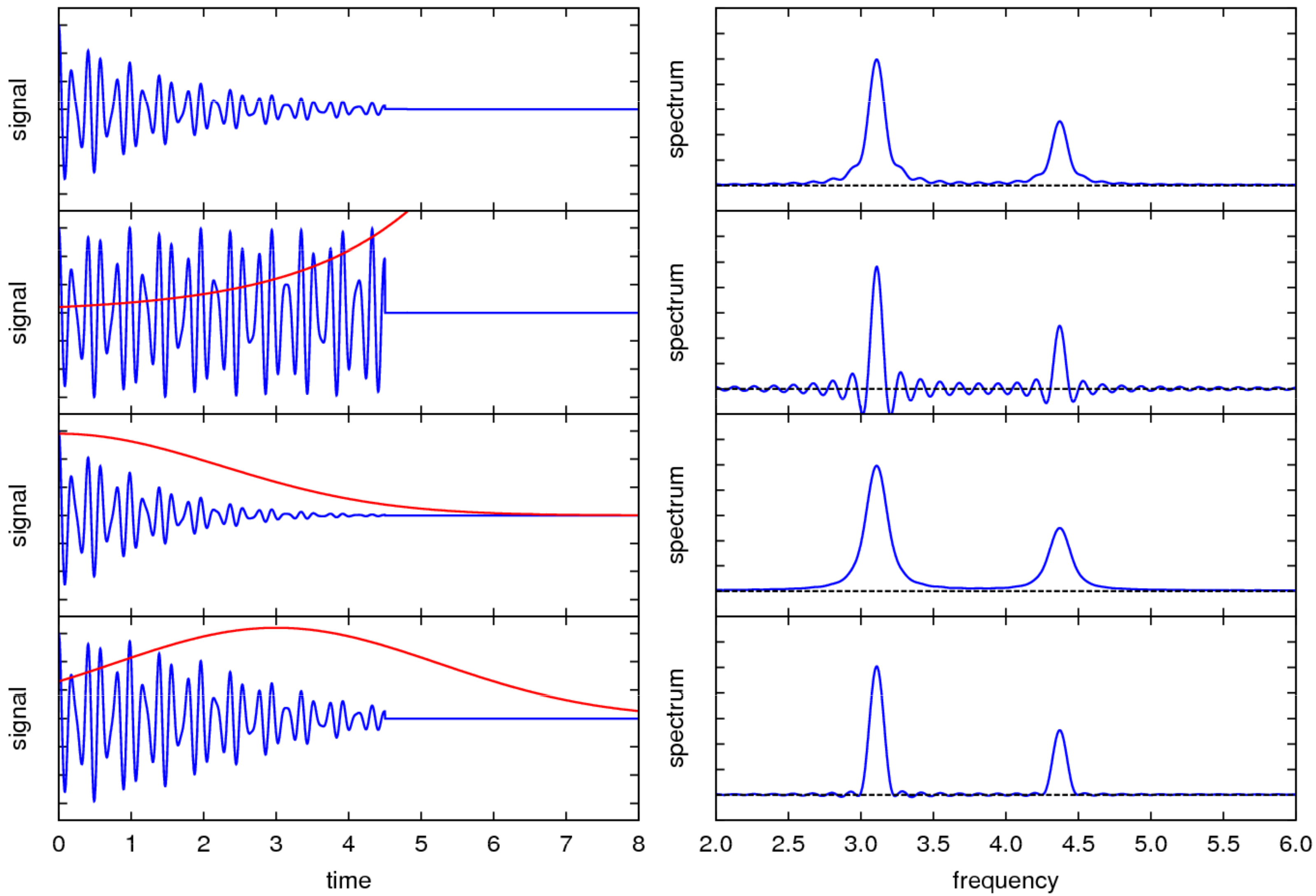
1) improvements od S/N ratio

2) increasing resolution

NMR data processing – window functions – apodization



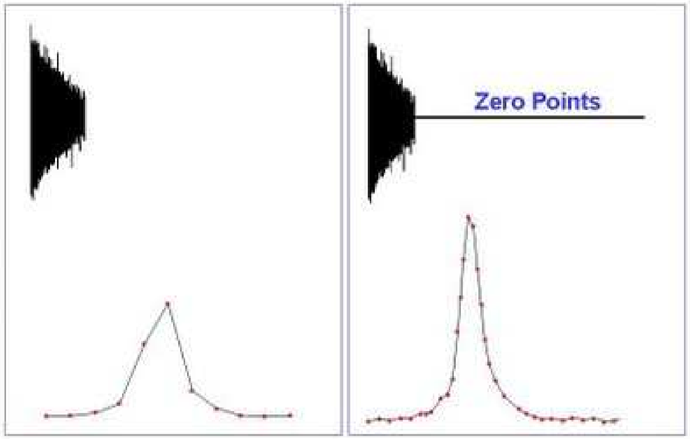




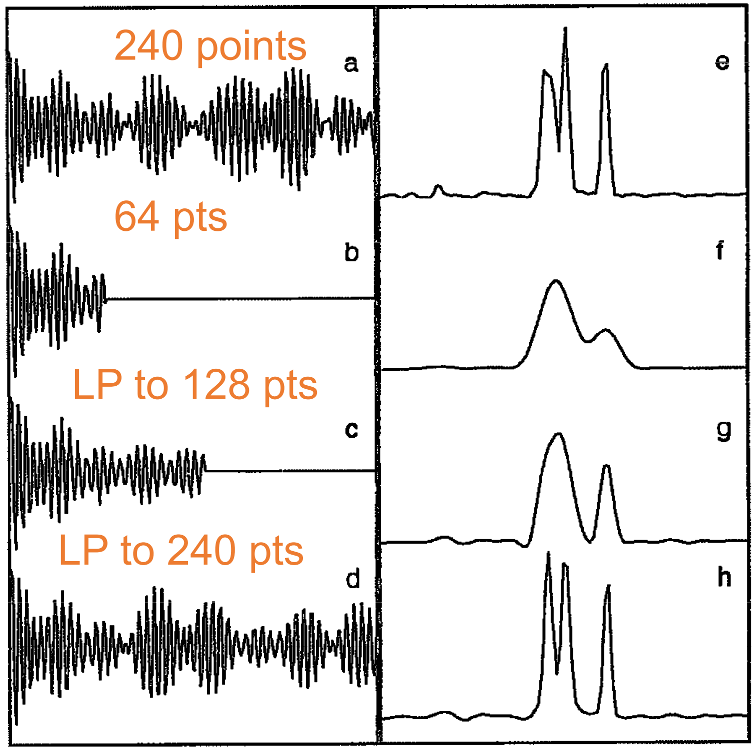
signal
signal
signal
signal
spectrum
spectrum
spectrum
spectrum
0
1
2
3
4
5
6
7
8
time
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
frequency

NMR data processing – Zero Filling, Linear prediction

Zero filling



Linear prediction



NMR data processing - summary

I) Solvent suppression

II) Window function

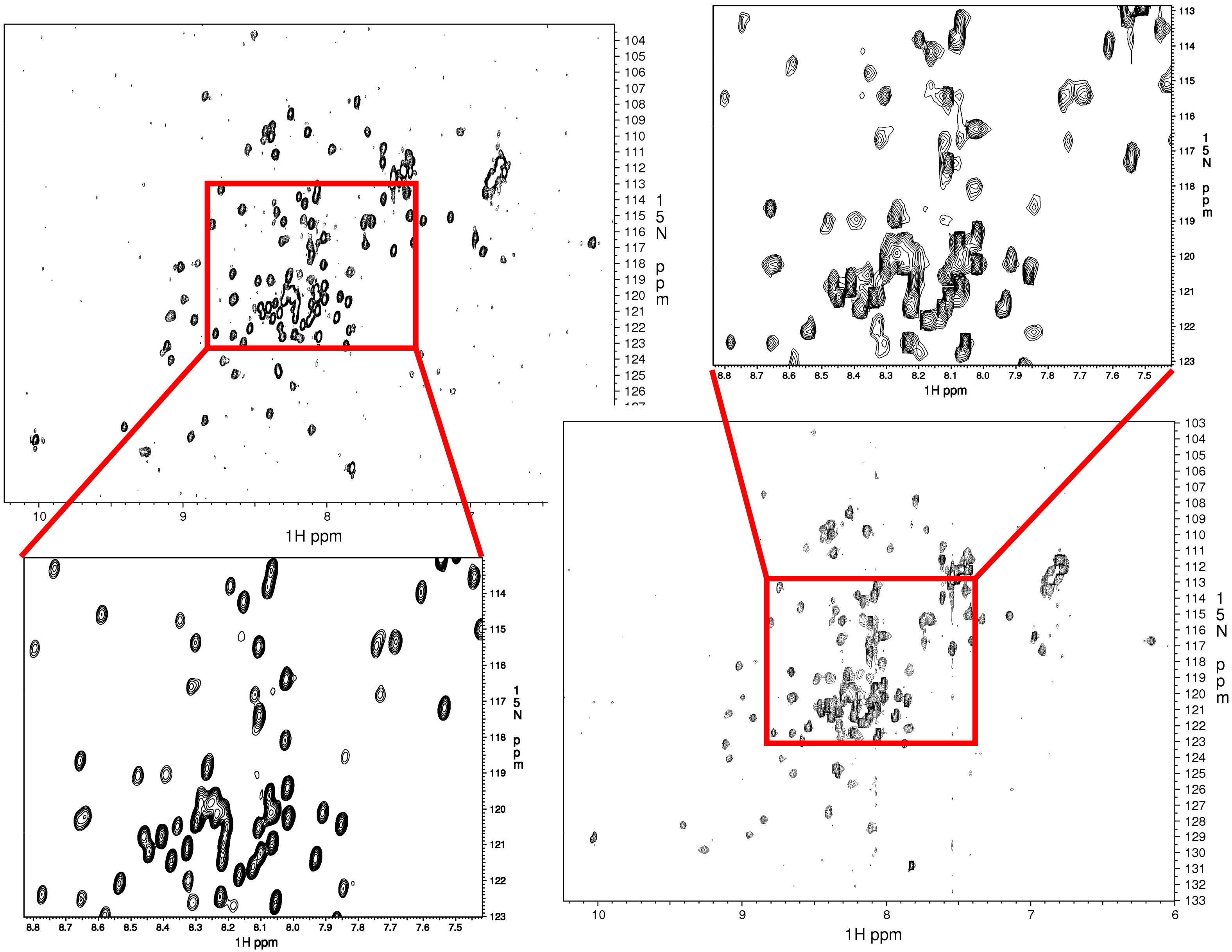
III) Zero-filling

IV) FT

V) Transpose (in case of multidimensional spectra)

```
|nmrPipe -fn POLY -time \
|nmrPipe -fn SP -off 0.33 -end 0.98 -pow 2 -c 1.0   \
|nmrPipe -fn ZF -size 2048 \
|nmrPipe -fn FT -auto \
|nmrPipe -fn PS -p0 -76.0 -p1 0.0 -di \
|nmrPipe -fn EXT -x1 11.0ppm -xn  6.0ppm -sw \
|nmrPipe -fn POLY -ord 3 -auto \
|nmrPipe -fn TP \
```

F2


1H ppm
15N ppm
1H ppm
15N ppm
1H ppm
15N ppm
1H ppm
15N ppm

NMR as a tool for study **structure**, **dynamics** and **interactions** of biomolecules

1) Structure determination of NAs and proteins
2) **Protein – metal interaction**
3) **Protein – ligand interaction**

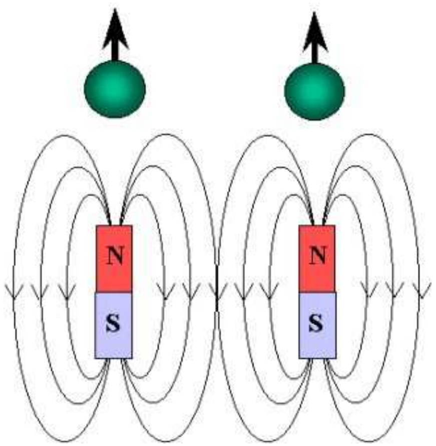
For most of the modern applications, enrichment by ^{13}C, ^{15}N and often ^{2}H needed!

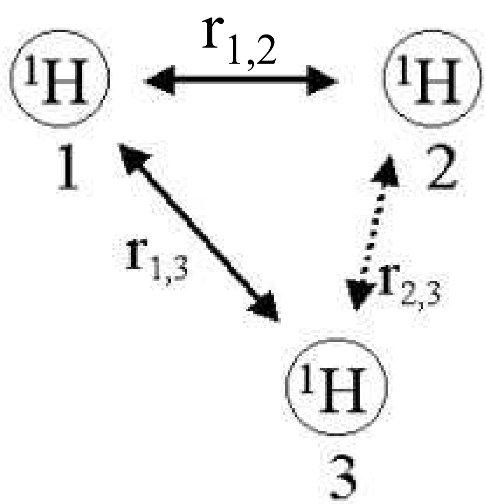
Isotope	Ground state spin	Natural abundance [%]	Rel. Sensitivity
^{1}H	½	~100	$1.00 \times 10^{+0}$
^{13}C	½	1.10	1.59×10^{-2}
^{15}N	½	0.37	1.04×10^{-3}
^{19}F	½	100	8.30×10^{-1}
^{31}P	½	~100	6.63×10^{-2}
^{12}C	0	98.90	-
^{16}O	0	~100	-

NMR as a tool for study **structure**, **dynamics** and **interactions** of biomolecules

0) AA/NA sequence, resonance assignment, standard chemical shifts
1) Structure determination of proteins/NAs
2) NMR can provide detailed information about the structure at the atomic level resolution relying on the spatial proximity of two interacting protons – nuclear Overhauser enhancement (NOE)
3) Additional structural information can be obtained (residual dipolar couplings – RDCs, *J*-couplings, backbone chemical shifts - CSI)

NOE:

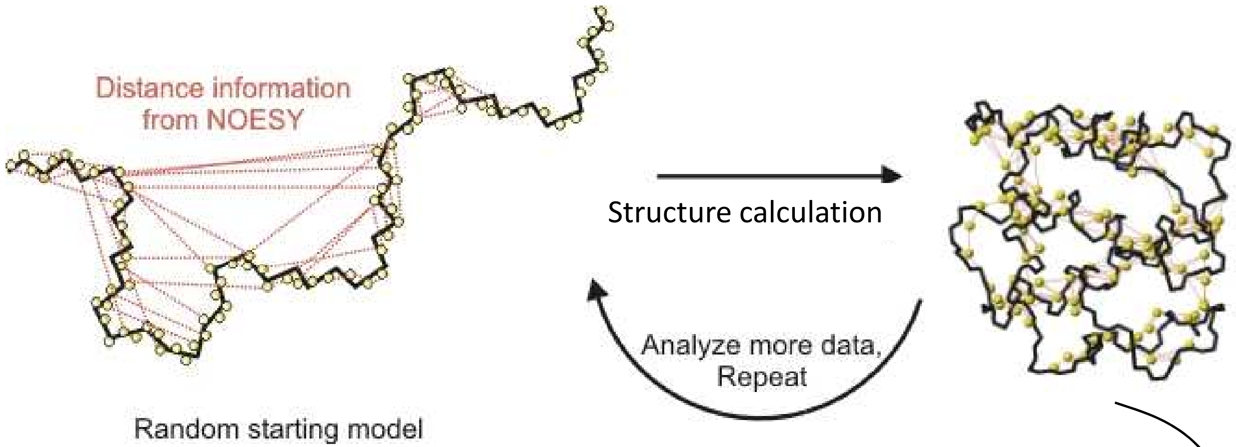




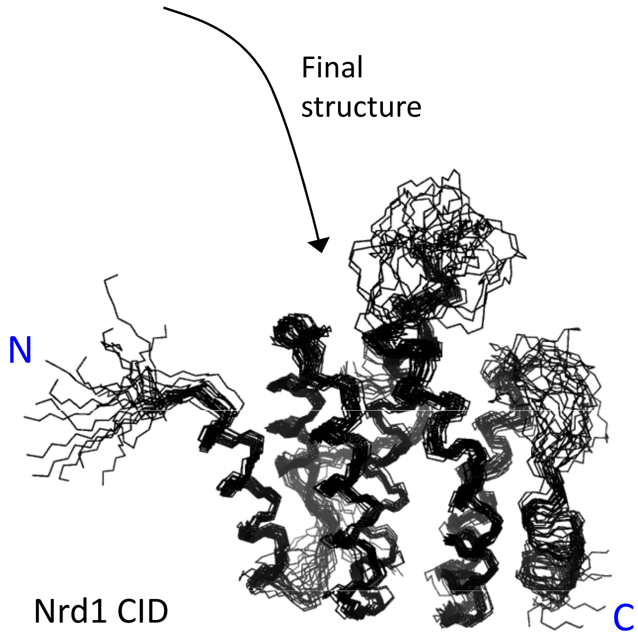
$$\mathbf{r_{1,2;}\ r_{1,3;}\ r_{2,3} \leq 6\ \text{Å}}$$

1Å=1.10^{-10}m

Iterative procedure of structure determination by NMR



Uncertainty of the final structure represented as a family of 10-20 structures with deviation among individual members indicated by RMSD (typically <1.5 Å^2)



Nrd1 CID
PDB ID: 2LO6

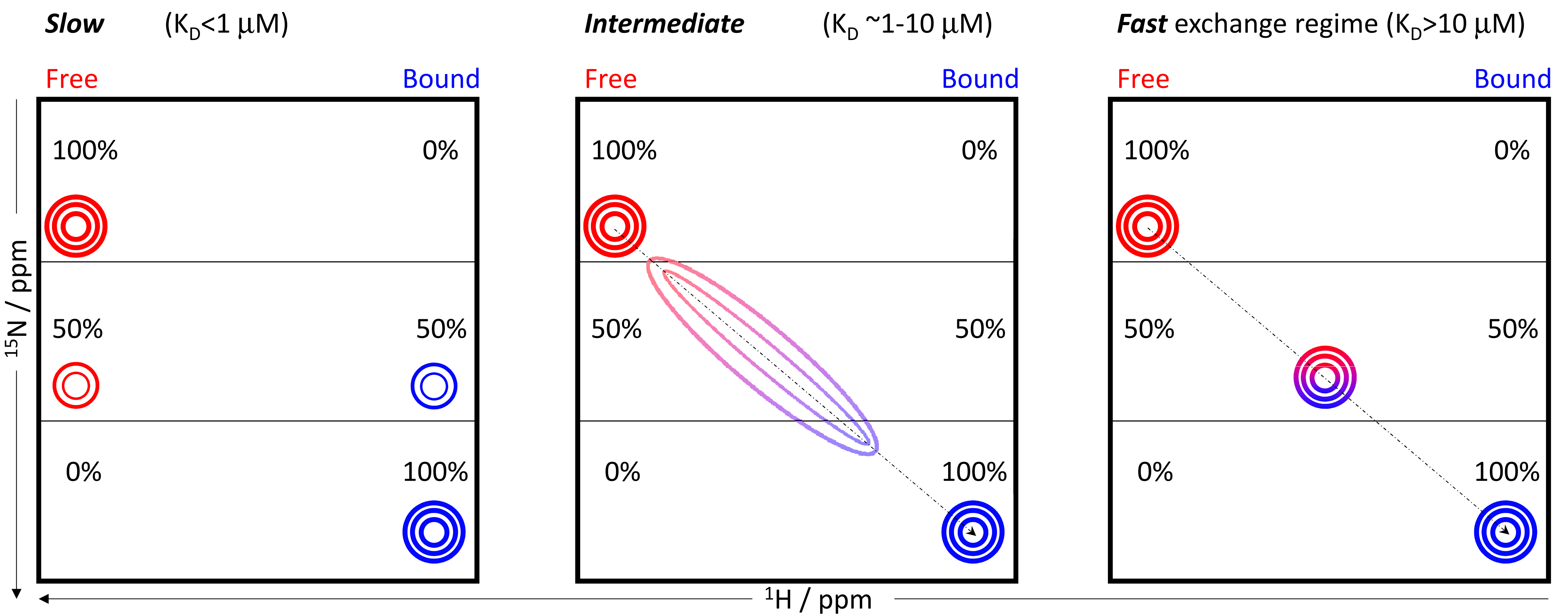
http://www.fbreagents.com/basics_nmr/9proteins.htm

Studying interactions by NMR titration

1) **Slow** exch. regime (on the NMR timescale) – individual peaks for each of the studied states (e.g. free / complexed forms of a protein), peak intensity representing population of a given state

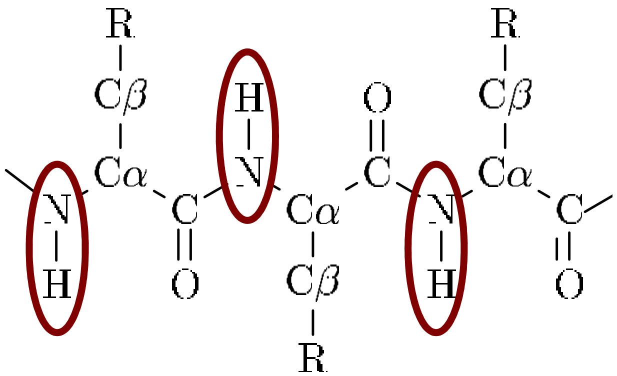
2) **Intermediate** exchange regime

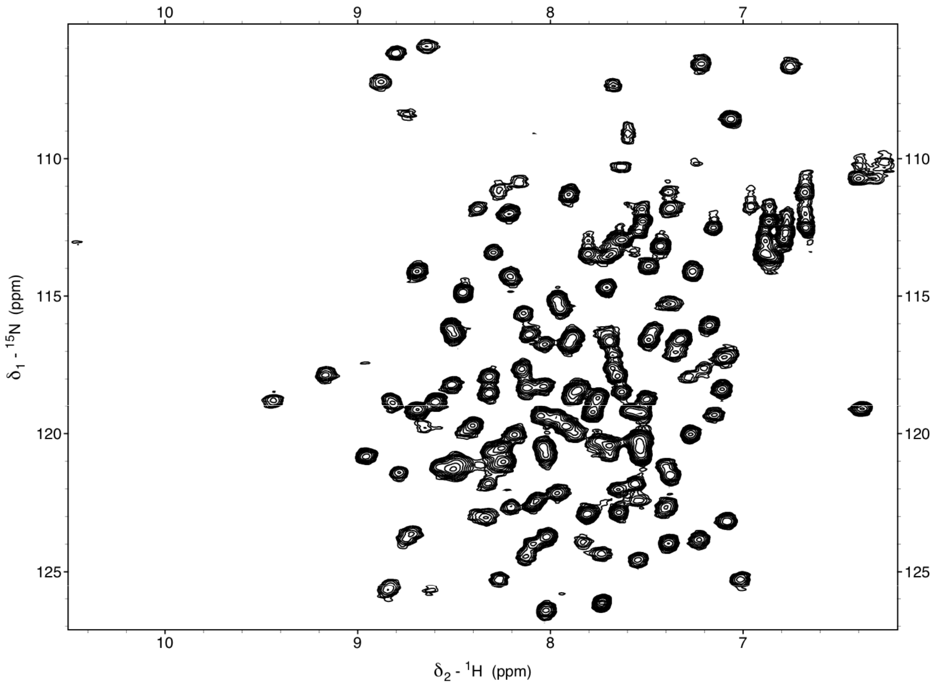
3) **Fast** exchange regime – single peak whose chemical shift position is given by the molar ratio of the states present in solution



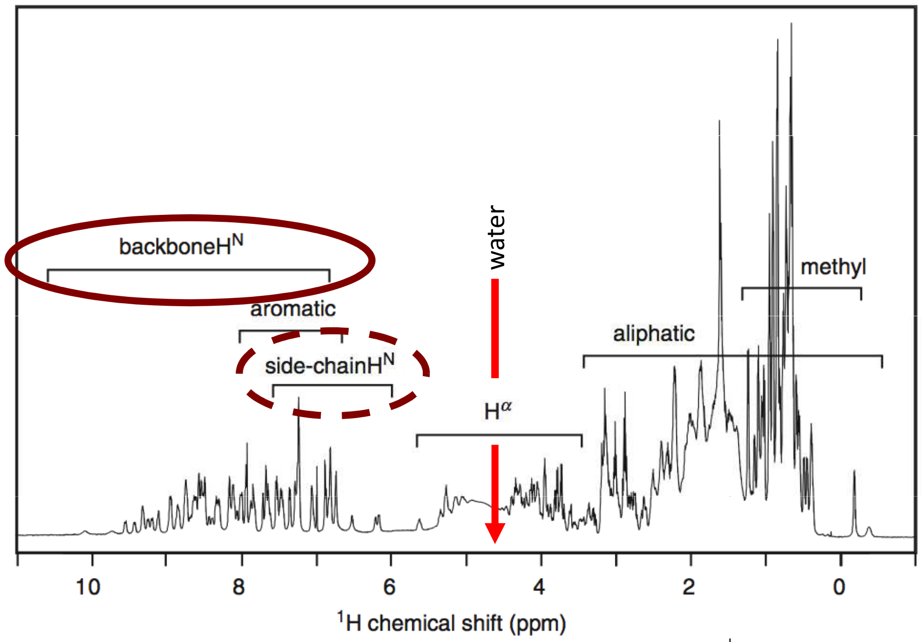
^{15}N-^{1}H HSQC – Heteronuclear Single Quantum Coherence

1) 1 peak ≅ 1 amino acid
2) good estimate of the protein folding status
3) no information about sequential assignment (which peak is which amino acid)
4) for sequential assignment third dimension needed (^{13}C)
5) once assignment of the peaks known – HSQC is optimal tool for monitoring interactions by NMR through titrations (i.e. stepwise addition of small amounts of ligand to the nearly constant volume solution with the isotopically enriched molecule)

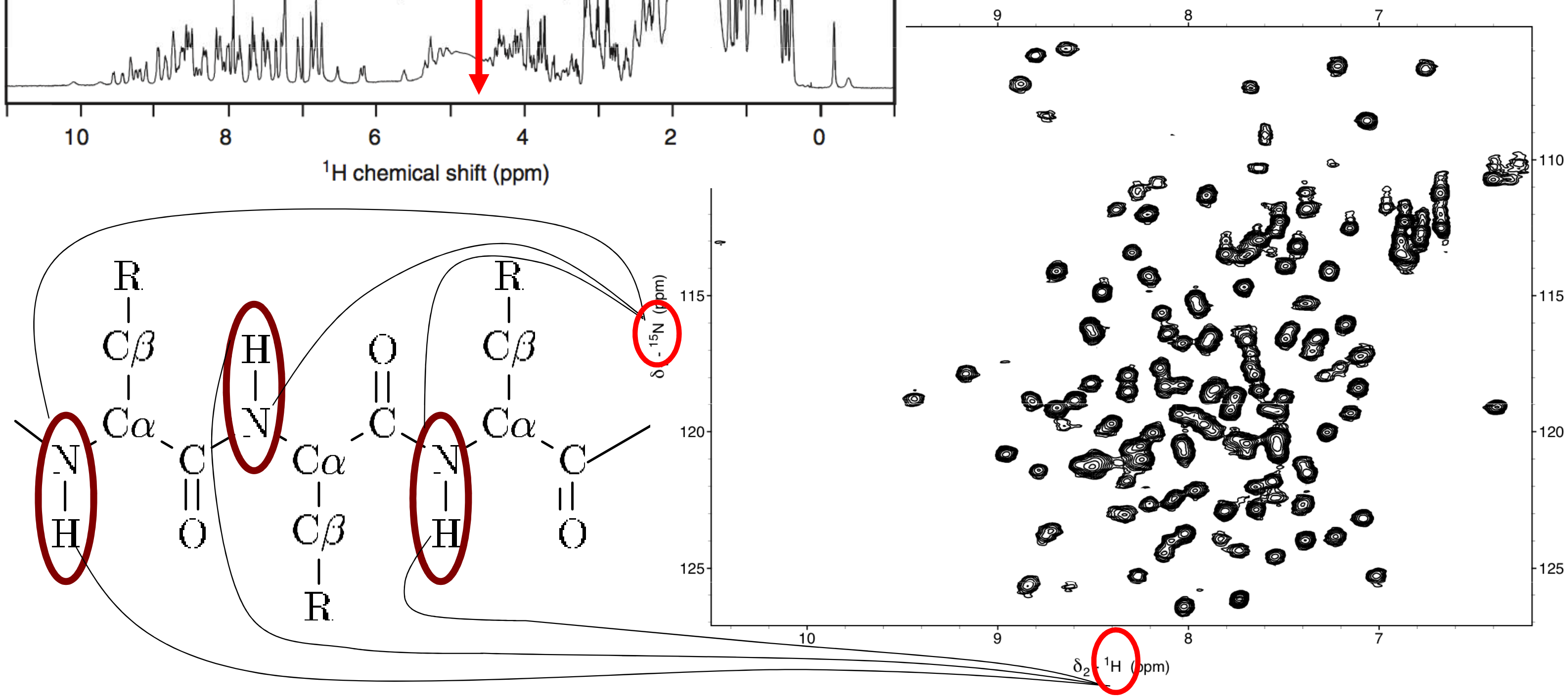




^{1}H-^{15}N HSQC, cca 155 aa, well folded, 600MHz, 293K



^{1}H 1D, Cavanagh et al., 2007



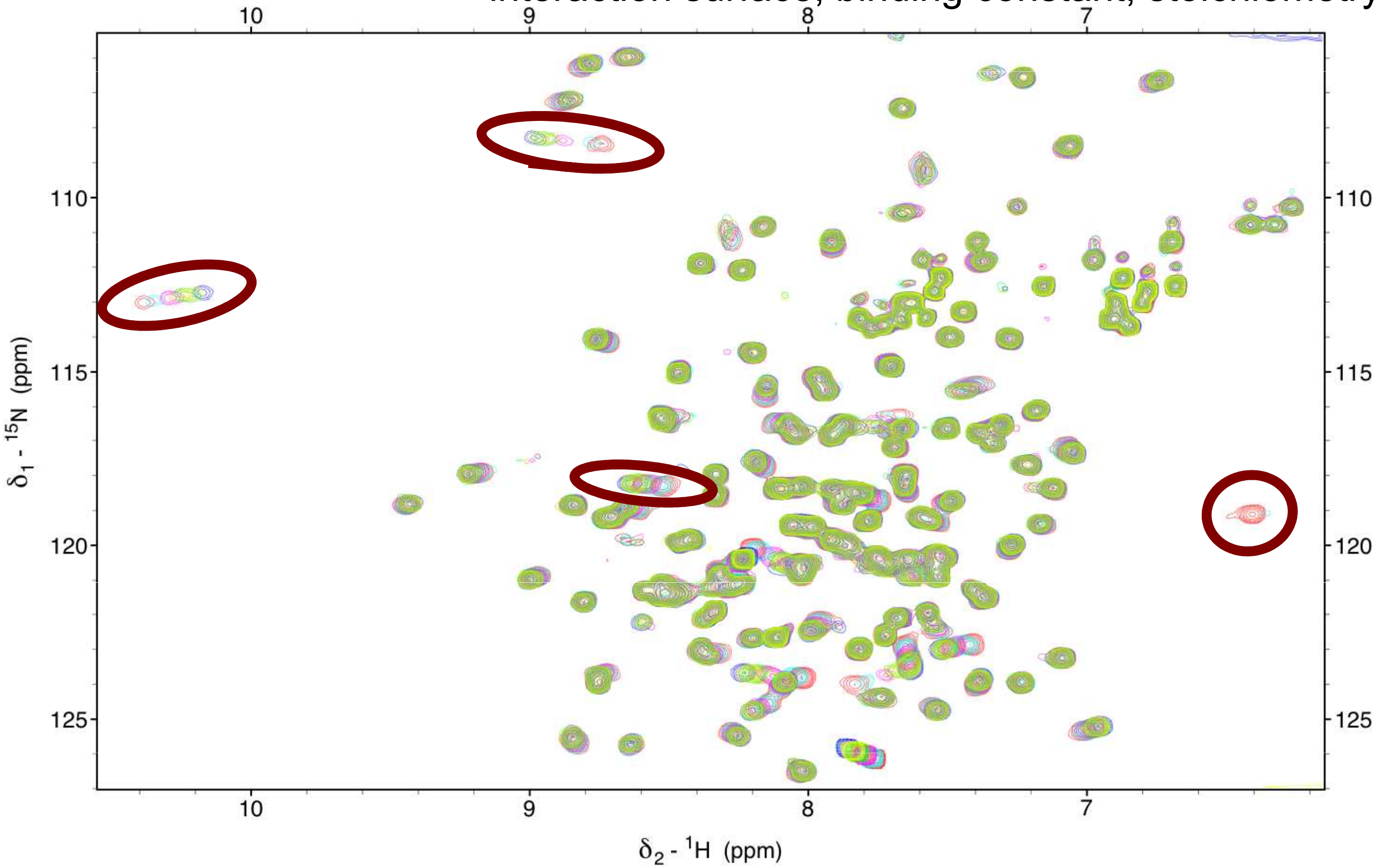
^{1}H-^{15}N HSQC, cca 155 aa, well folded, 600MHz, 293K

Interaction of Nrd1-CID with C-terminal domain (CTD)

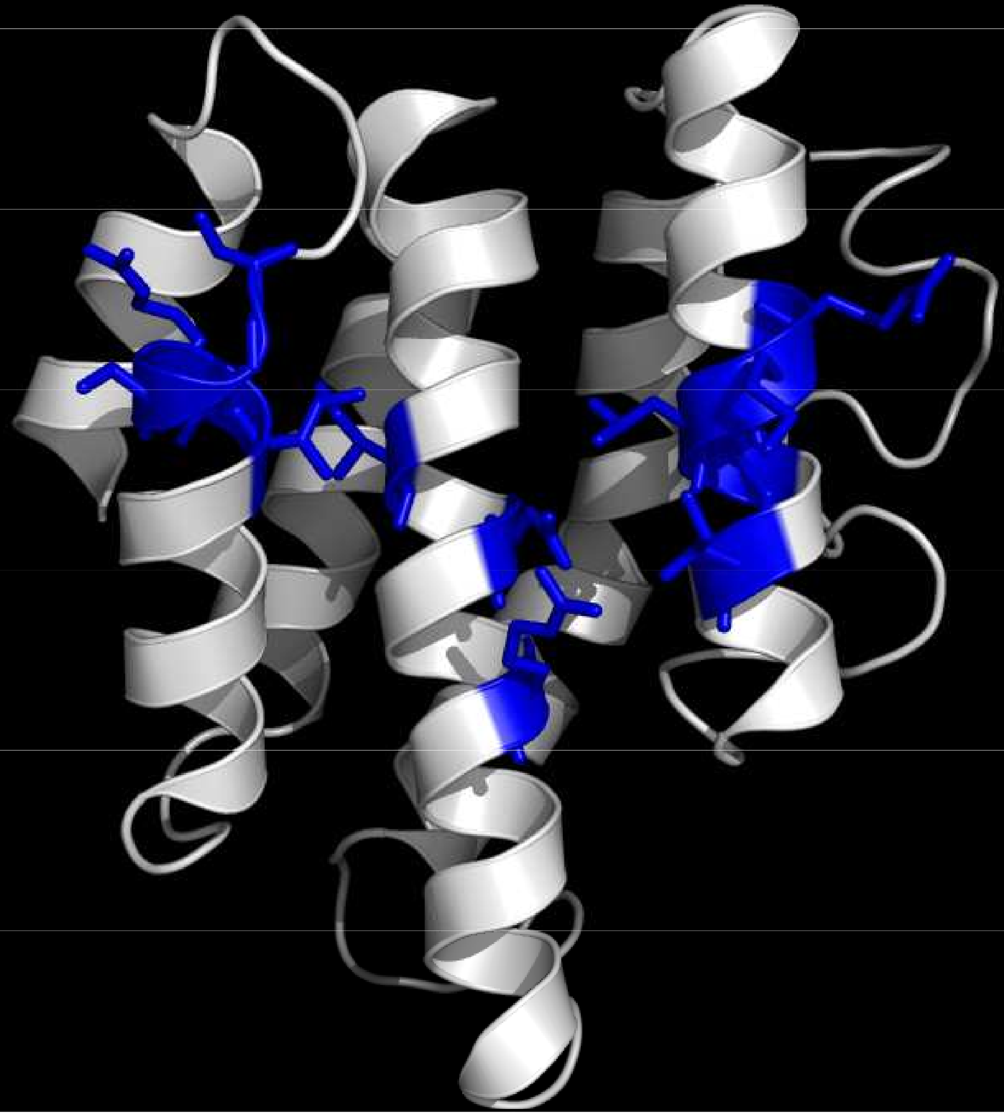
NMR Titration - ^{15}N enriched CID + unlabeled CTD-Ser5P in ***n***-steps, **n=6** in our case

- peaks corresponding to the interacting residues of CID change their chemical shift (position in the spectrum)

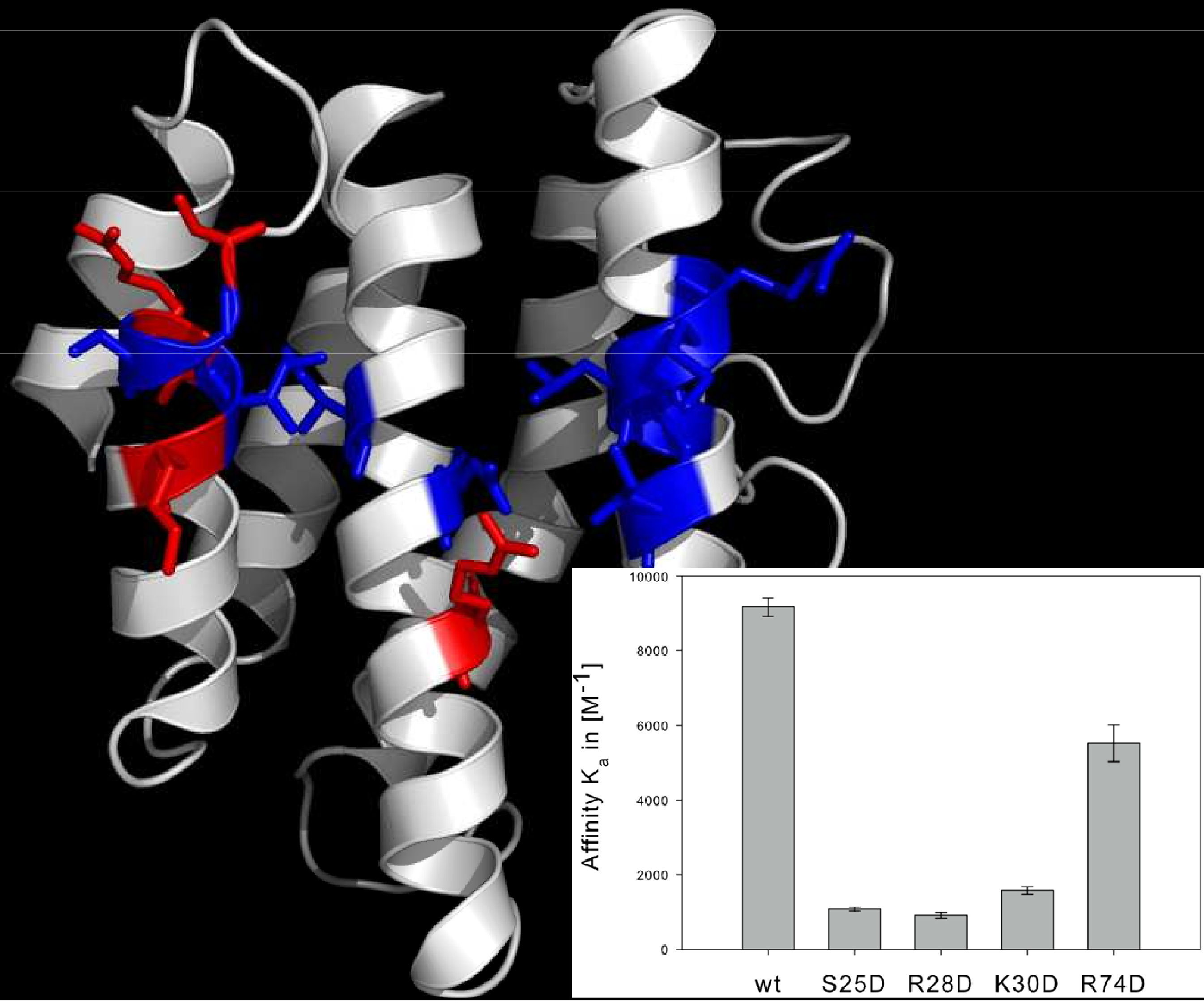
=> interaction surface, binding constant, stoichiometry



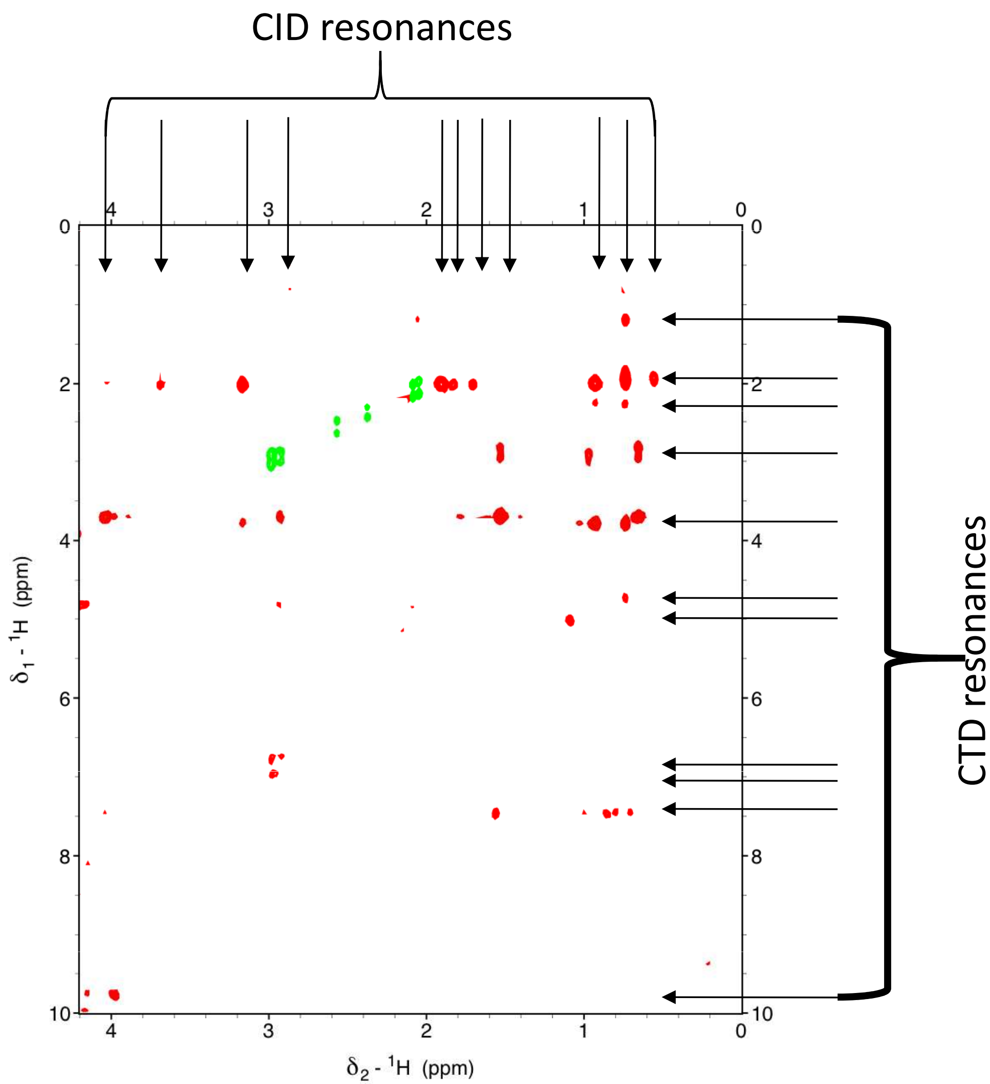
Nrd1 CID interaction surface – CID residues experiencing the largest chemical shift variations upon the interaction with 5-phospho-Ser CTD shown in blue with side-chains in stick representation



CTD-CID interaction with mutants studied by fluorescence anisotropy



Interligand NOEs between CID and CTD – 900MHz, 150ms, 293K

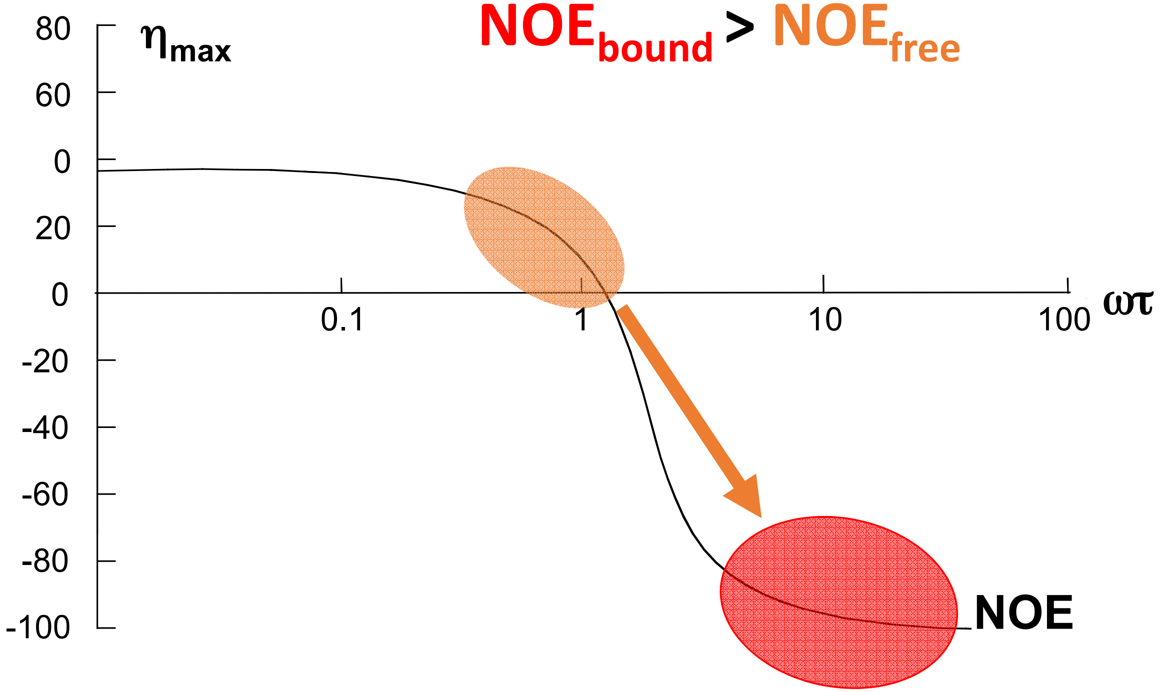


Transferred-NOE

$$NOE = p_{bound} \cdot NOE_{bound} + p_{free} \cdot NOE_{free}$$

$$\tau_{c,bound} >> \tau_{c,free} \quad (\text{and} \;\; p_{L,free} >> p_{L,bound})$$

$$NOE_{bound} > NOE_{free}$$

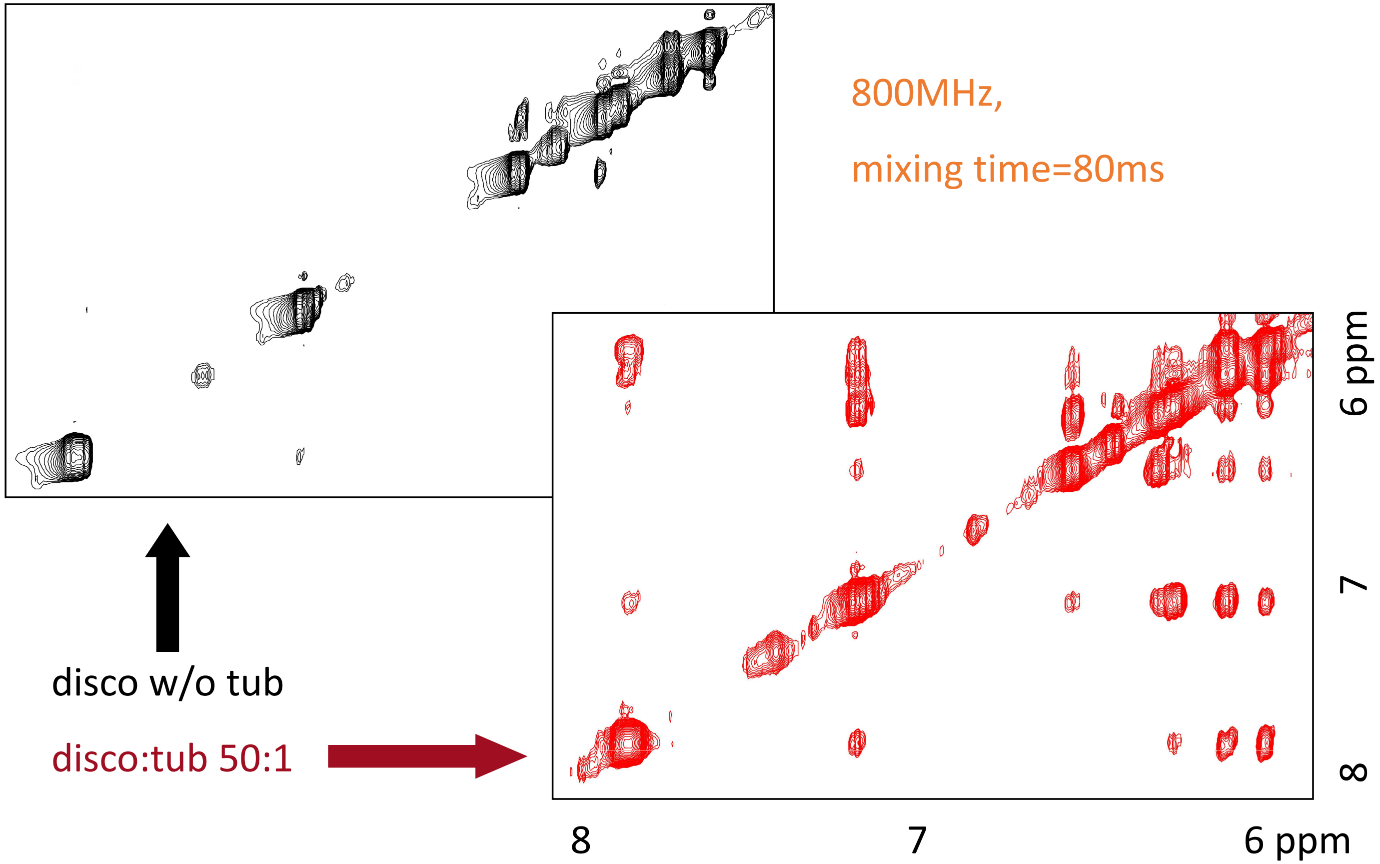


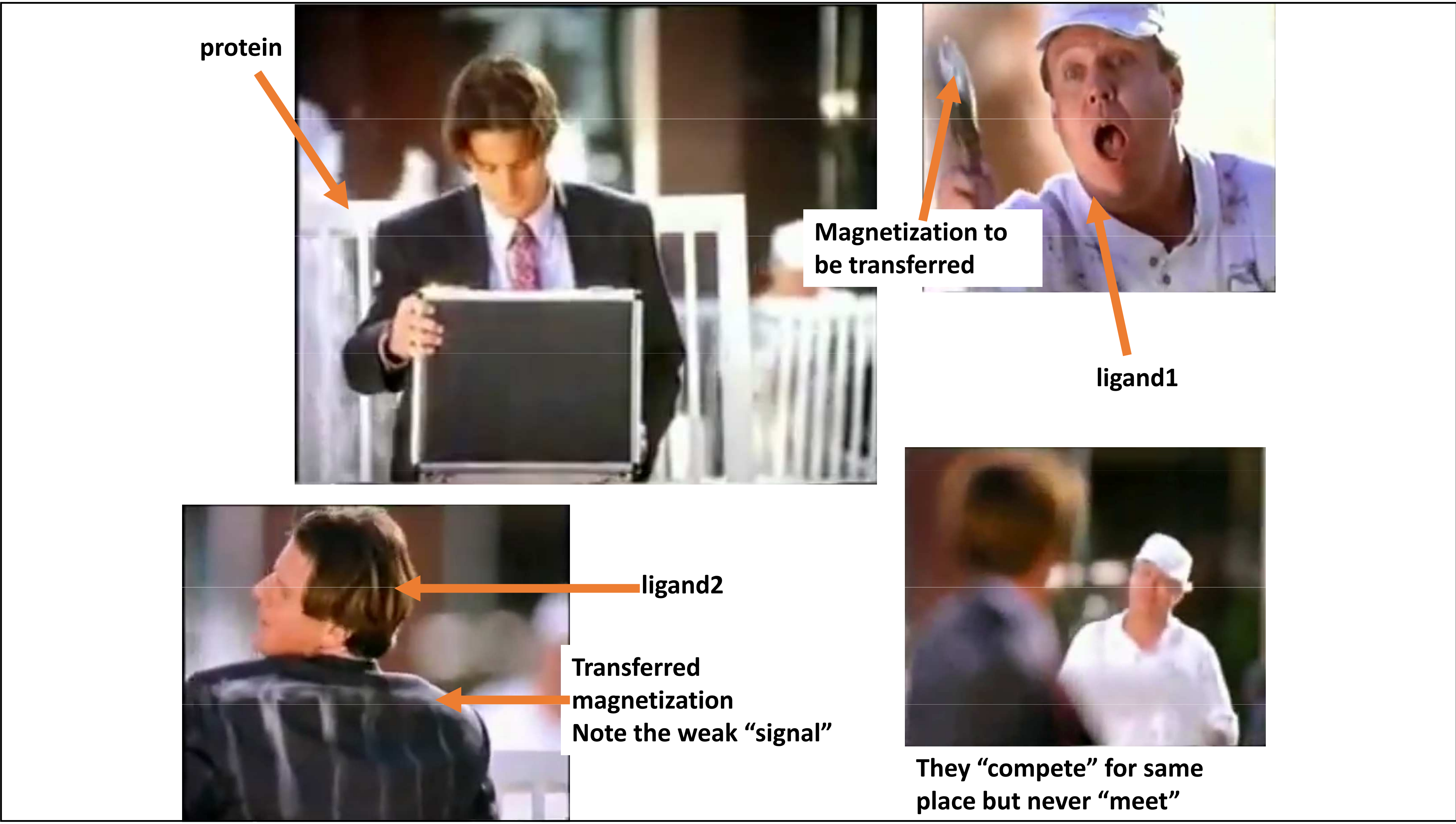
Transferred NOE Experiments

tr-NOESY~600µM Discodermolide **without** and **with ~12µM tubulin**

800MHz,

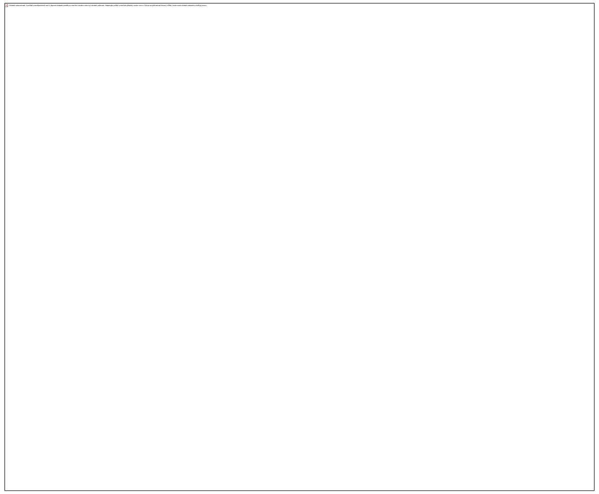
mixing time=80ms





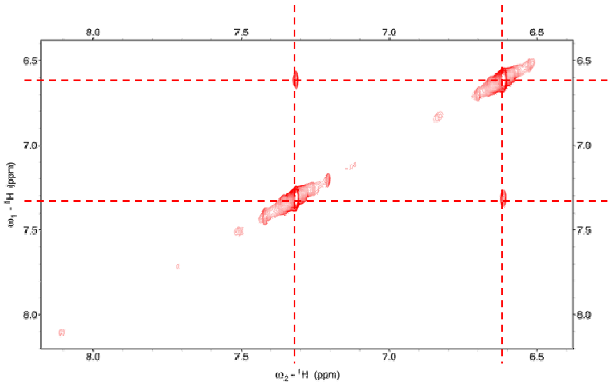
protein
Magnetization to be transferred
ligand1
ligand2
Transferred magnetization
Note the weak "signal"
They "compete" for same place but never "meet"

interligand NOE Experiments

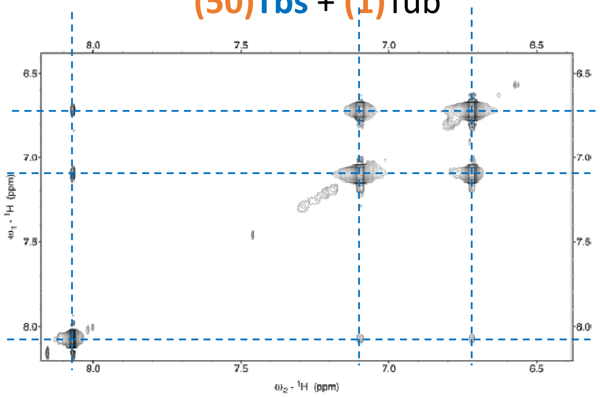


interligand NOE Experiments

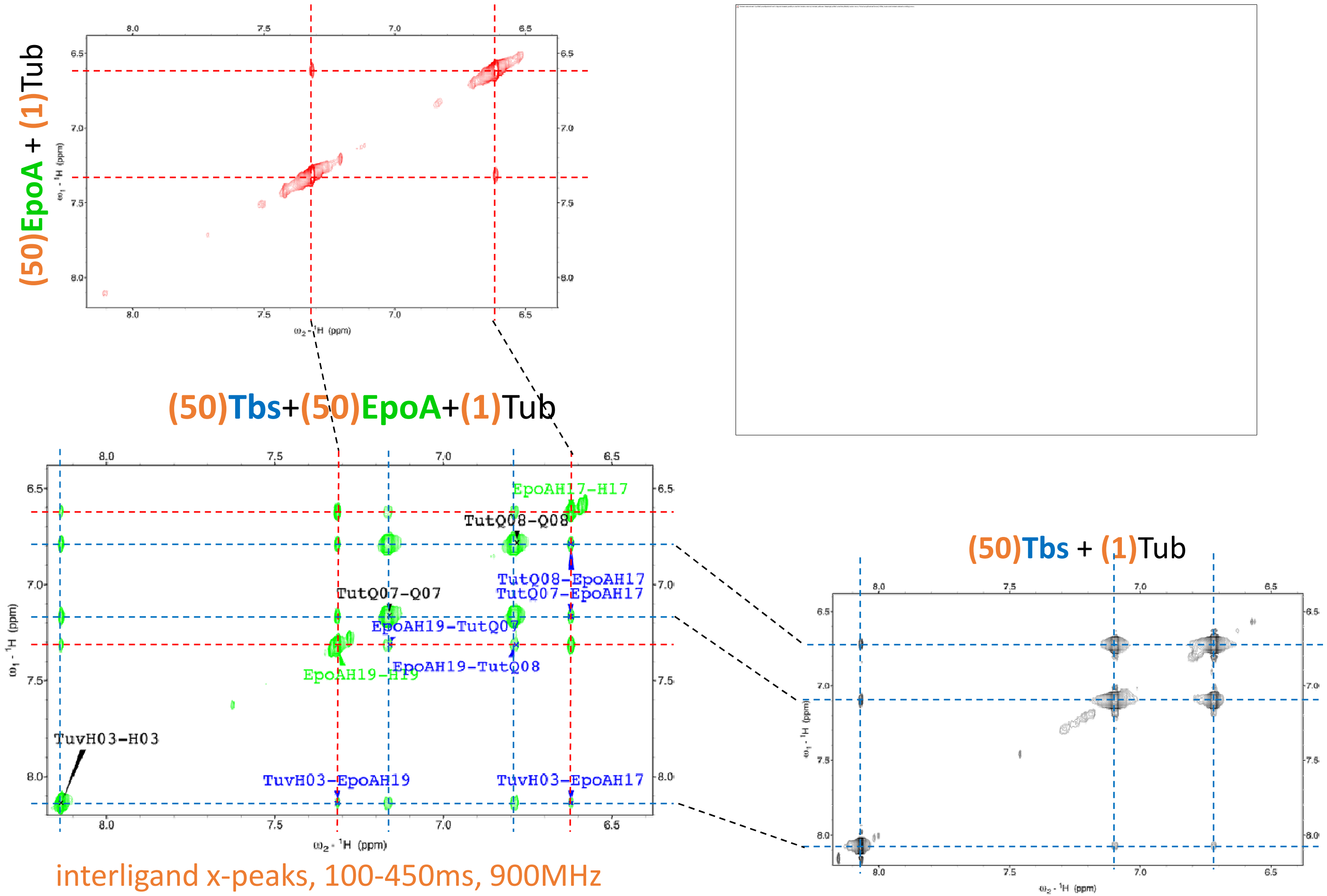
(50)EpoA + (1)Tub



(50)Tbs + (1)Tub

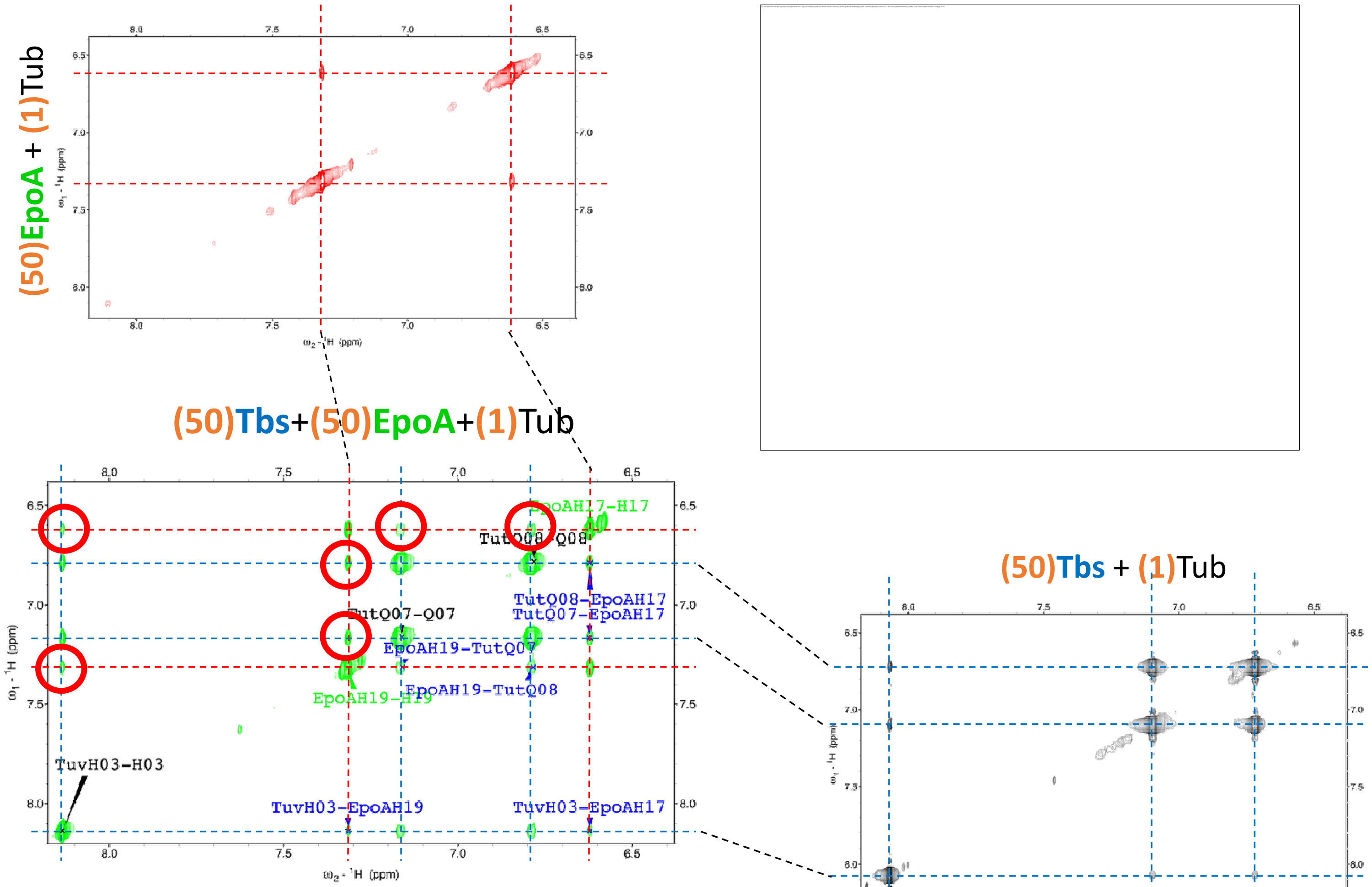


interligand NOE Experiments



interligand x-peaks, 100-450ms, 900MHz

interligand NOE Experiments



interligand x-peaks, 100-450ms, 900MHz