What is the medical importance of NMR?
(see lecture No. 13. in this semester!)

Physical bases, spins and nuclei

Properties of nuclear spin
Nucleus; proton and neutron

Two magnetic moments (spin) pointing in opposite
directions $\Rightarrow$ zero net magnetic moment!

nuclei with even $Z$ and even $N$ have nuclear spin $I=0$
(isotopes are important)

If there are unpaired protons or neutrons in a nucleus,
then the net nuclear spin (the intrinsic nuclear magnetic
moment) differs from zero.

Spins and nuclei
Protons and neutrons (and also the electrons) are:
particles having a spin angular momentum of $1/2$.

If the number of protons or neutrons is add in the
nucleus, then the total nuclear spin will not be zero!

NMR: Nuclear Magnetic Resonance
Miklós Nyitrai, April 6, 2016
Atoms for NMR

\[ ^1\text{H}, ^2\text{H}, ^3\text{H}, ^{12}\text{C}, ^{13}\text{C}, ^{14}\text{C}, ^{14}\text{N}, ^{15}\text{N}, ^{16}\text{O}, ^{17}\text{O}, ^{19}\text{F}, ^{23}\text{Na}, ^{31}\text{P} \text{, etc.} \]

The most frequently applied nuclei:

\[ ^1\text{H}, ^{13}\text{C}, ^{15}\text{N}, ^{17}\text{O}, ^{19}\text{F}, ^{31}\text{P} \]

Signal depends on:

- Magnitude of the magnetic moment
- Concentration of the isotope

The split of the energy states (Zeemann)

- α-spin state, favourable, lower energy
- β-spin state, unfavourable, higher energy

The energy difference between the spin states does depend on the strength of the external magnetic field.

Orientation and reorientation on the microscopic level

\[ M = \Sigma \mu \]

H=0; M=0

H>0; M>0

\[ m: \text{magnetic moment of the individual atom} \]

Graphical representation

- α-spin state
- β-spin state

\[ \Delta E_1 < \Delta E_2 \]

Remember, the energy is proportional to the frequency!

An example: CH\textsubscript{4}

We can probe the energy difference of the α - and β - state of the protons by irradiating them with EM radiation of just the right energy. In a magnet of 7.05 Tesla, it takes EM radiation of about 300 MHz (radio waves). So, if we bombard the molecule with 300 MHz radio waves, the protons will absorb that energy and we can measure that absorbance. In a magnet of 11.75 Tesla, it takes EM radiation of about 500 MHz (stronger magnet means greater energy difference between the α - and β - state of the protons).

What is the problem with this concept?

It is difficult to compare the data from two instruments with different strengths of magnetic fields.

Let’s use the chemical shift!

What is this? Much simpler than it may sound...
Chemical shift or $\delta$

We need a reference sample to standardize our instruments!

1. Measure the absorbance frequency of the standard $f_r$.
2. Measure the frequency for your own sample $f_s$.
3. Let's calculate the difference between the two: $\Delta f = f_r - f_s$.
4. Normalize the difference with the frequency of the reference: $\delta = \Delta f / f_r$.

The $\delta$, i.e., the chemical shift will be characteristic for your sample, but will not depend on the parameters of the instrument used for the experiments!

Why? Let's take an example!

Imagine that we have a magnet where our standard absorbs at 100,000,000 Hz (100 megahertz), and our sample absorbs at 100,000,300 Hz. The difference is 300 Hz, so we take $\frac{300}{100,000,000} = 1/1,000,000$ and call that 1 part per million (or 1 PPM).

Now let's examine the same sample in a stronger magnetic field where the reference comes at 450,000,000 Hz, or 450 megahertz. The frequency of our sample will increase proportionally, and will come at 450,000,450 Hz. The difference is now 450 Hz, but we divide by $\frac{450}{450,000,000} = 1/1,000,000 = 1$ PPM.

(We do not have to calculate all these, the NMR machine does it for us!)

NMR spectrum

The absorption is proportional to the concentration of the corresponding nuclei.

The NMR spectrum is the energy absorbed by the system as the function of the frequency $f$ of the excitation energy $\Delta E$ or the magnetic field $H, B$.

Due to local effects and fields the excitation energy (and thus frequency) is different for different nuclei.

Applications of NMR

- Structure of organic molecules and substances;
- Interactions of organic molecules;
- Structure of macromolecules (proteins, nucleic acids);
- Biological and artificial membranes;
- MRI: Magnetic Resonance Imaging.
SUMMARY

Jablonski diagram

The definition of absorption

How to measure absorption?
A scheme of a photometer.

The absorption of proteins
Kasha’s-rule

The emission of the fluorescence light is always starting from the lowest vibrational level of the first excited level ($S_1$).

Michael Kasha
December 1, 1920 - June 12, 2013

Definition of fluorescence and phosphorescence

$S \rightarrow S$ in the ns range

$T \rightarrow S$ in the > ms range

Basic fluorescence parameters

- Fluorescence spectrum, intensity;
- Quantum efficiency
- Fluorescence lifetime
- Polarisation

Stokes-shift

The difference (measured in nm) between the peak of the excitation and the emission spectrum (energy loss).

Stokes-shift diagram

Scheme of a spectrofluorometer

Non linear arrangement !!!

Photoselection
Scheme of a spectrofluorometer

Emission anisotropy

\[ E = \frac{(I_{VV} - G I_{VH})}{(I_{VV} + 2 G I_{VH})} \]

- dimensionless
- depends on rotational motion of the fluorophore
- additive

Remember!
\[ I_{sum} = I_{Z} + I_{X} + I_{Y} \]
\[ I_{sum} = I_{VV} + I_{VH} + I_{HH} \]
\[ I_{sum} = I_{VV} + 2 I_{VH} \]

Conditions of FRET

- A fluorescent donor molecule.
- The appropriate orientation of the donor and acceptor absorption spectra.
- Overlap between the donor emission and acceptor absorption spectra.
- Distance range of 2-10 nm!

FRET efficiency

\[ E = 1 - \left( \frac{F_{DA}}{F_{D}} \right) \]

where
- \( F_{DA} \): donor intensity with the acceptor
- \( F_{D} \): a donor intensity without the acceptor.

Can also be calculated with lifetimes!
\[ E = 1 - \left( \frac{\tau_{DA}}{\tau_{D}} \right) \]

Distance dependence of the FRET efficiency

\[ E = \frac{R_{0}^{6}}{R_{0}^{6} + R^{6}} \]

Applications of FRET

- The determination of FRET distances
- To study the establishment of interactions between molecules
- To study intra-molecular structural changes.
Vibration modes of water molecules

Infra and Raman spectroscopy

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Thank you!