

BIOMOLECULAR STRUCTURAL DYNAMICS

MIKLÓS KELLERMAYER

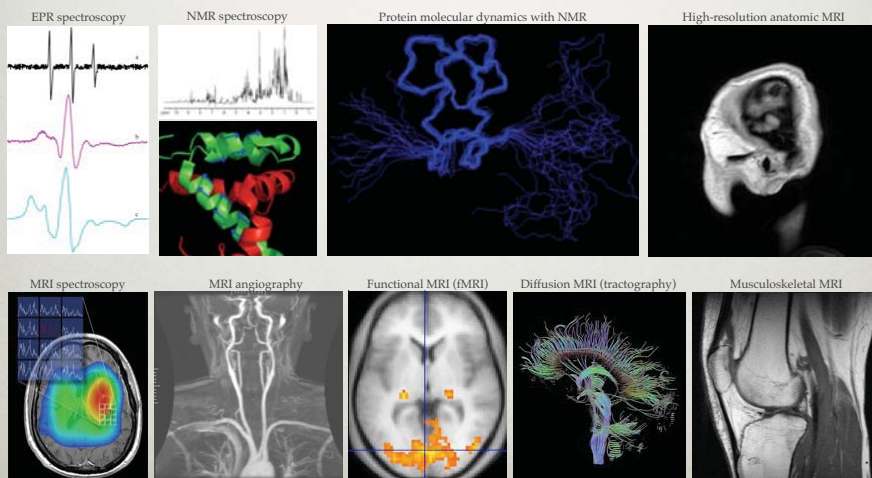
OBJECTIVES OF INVESTIGATION

- Understanding biomolecular **structure**
- and **function** (processes, states, transitions, interactions, etc.)

“RADIOSPECTROSCOPIES”:

REVOLUTIONIZED PHYSICS, CHEMISTRY, BIOLOGY AND MEDICINE

- Electrons spin resonance (*ESR*, electron paramagnetic resonance - *EPR*)
- Nuclear magnetic resonance (*NMR*, *MRI*)



ATOMIC, MOLECULAR SYSTEMS MAY BEHAVE AS ELEMENTARY MAGNETS

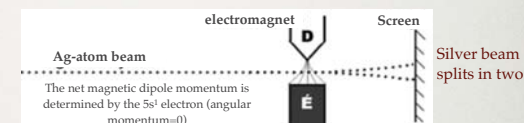
Stern-Gerlach experiment (1922)



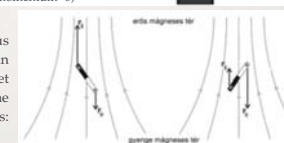
Otto Stern
(1888-1969)



Walther Gerlach
(1889-1979)



In an inhomogeneous magnetic field, in addition to torque, net force arises on the magnetic dipoles:



The spin magnetic moment may take on two values (+1/2, -1/2)

Nuclear magnetic resonance, NMR) Nobel-prize, 1952



Isidor Rabi
(1898-1988)



Felix Bloch
(1905-1983)

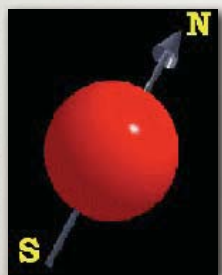


Edward Mills Purcell
(1912-1997)

Magnetic resonance: resonance-type absorption of electromagnetic energy by a sample placed in magnetic field.

SYSTEMS WITH NET SPIN: ELEMENTARY MAGNETS

- Elementary particles (p, n, e) have *spin* (due to angular momentum).
- Based on the number of elementary particles and certain organizational principles within the system (e.g., Pauli exclusion principle), *net spin* may arise.
- Nucleus: odd mass number - half nuclear spin (^1H , ^{13}C , ^{15}N , ^{19}F , ^{31}P); even mass number, odd atomic number - nuclear spin is whole number; even mass and atomic numbers - nuclear spin is zero.
- Electron: net electron spin only in molecules containing stable unpaired electrons (e.g., free radicals).
- Because of spin and charge, the system possesses *magnetic momentum*.



Top

Nuclear magnetic momentum:

$$M_N = \gamma_N L$$

γ_N = gyromagnetic ratio (ratio of magnetic momentum and angular momentum)

L = nuclear spin ($L = \sqrt{l(l+1)}\hbar$), l = net spin quantum number.

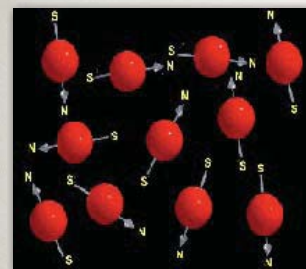
Electron magnetic momentum:

$$M_e = -g\mu_B \sqrt{S(S+1)}$$

g = electron's g-factor (dimensionless ratio that describes the relationship between magnetic momentum and gyromagnetic ratio)

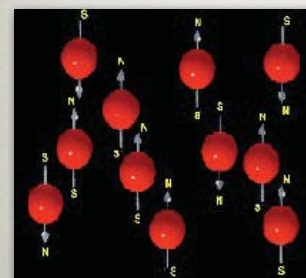
μ_B = Bohr magneton (unit of the electron's magnetic dipole momentum)

S = spin quantum number



In the absence of magnetic field:
orientation of elementary magnets is random

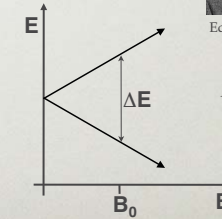
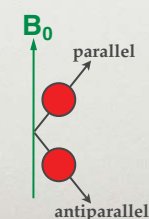
Paramagnetism: magnetism arises upon placing the sample in external magnetic field (magnetic dipoles become oriented).



In magnetic field:

elementary magnets
become oriented

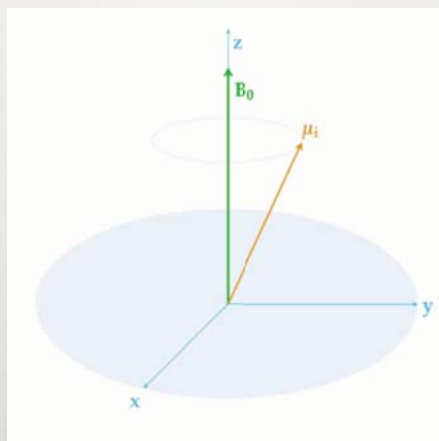
energy
levels split



Edward Purcell,
1946

Resonance
condition:
 $\Delta E = hf$

NUCLEAR MAGNETIC RESONANCE: SPIN PRECESSION



Precession or
Larmor frequency:

$$\omega_0 = \gamma B_0$$

$$f_{\text{Larmor}} = \frac{\gamma}{2\pi} B_0$$

Resonance condition:

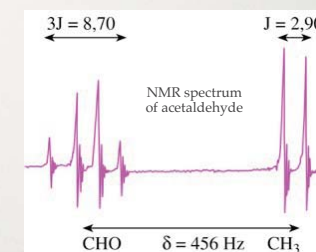
$$\Delta E = \frac{h\omega_0}{2\pi}$$



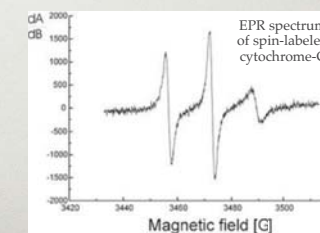
Felix Bloch, 1946

NMR AND EPR SPECTROSCOPY

- NMR spectrum: intensity of absorbed electromagnetic energy as a function of frequency.
- Area under the "NMR-line" is proportional to the number of nuclei.
- Presence (and structure) of local electron cloud affects the local magnetic field: frequency condition is shifted ("chemical shift"). It opens the possibility of chemical structure determination.

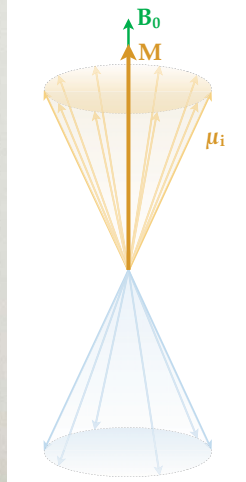


- EPR spectrum: intensity of absorbed electromagnetic energy as a function of magnetic field.
- Magnetic field is smaller, and frequency of EM radiation is greater (microwave) than in NMR.
- Spin-labeling: attachment of a molecule containing stable unpaired electron (radical).
- Rotational dynamics in the time range of 10^{-4} - 10^{-2} s may be measured.



NET MAGNETIZATION DUE TO SPIN ACCESS IN DIFFERENT ENERGY STATES

Low energy state
parallel in case of proton



B_0 = magnetic field
 M = net magnetization

High energy state
antiparallel in case of proton

Ratio of magnetic spins in high-
(antiparallel) and low-energy
(parallel) states:

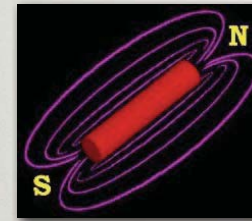
$$\frac{N_{\text{antiparallel}}}{N_{\text{parallel}}} = e^{-\frac{\Delta E}{k_B T}}$$

Boltzmann distribution

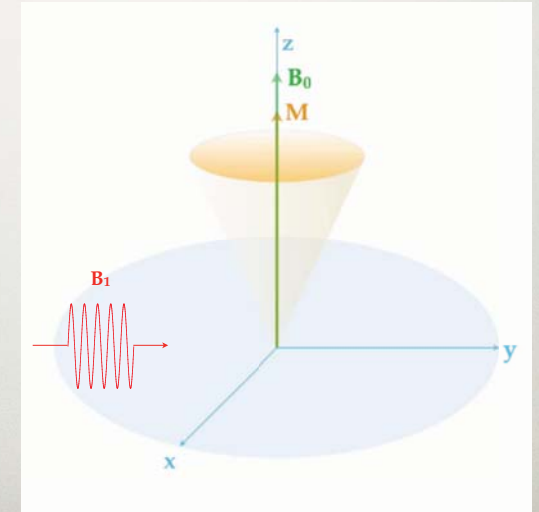
EXCITATION

USING RADIO FREQUENCY RADIATION

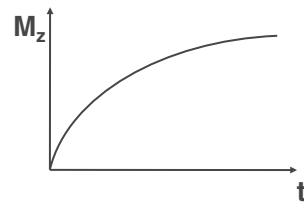
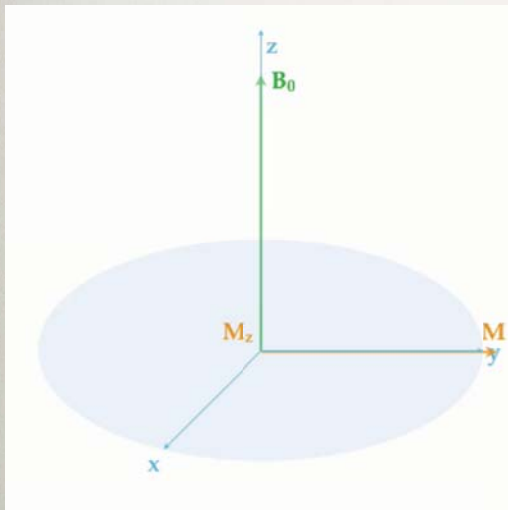
Resonance condition: Larmor frequency



B_0 = magnetic field
 M = net magnetization
 B_1 = irradiated radio frequency wave

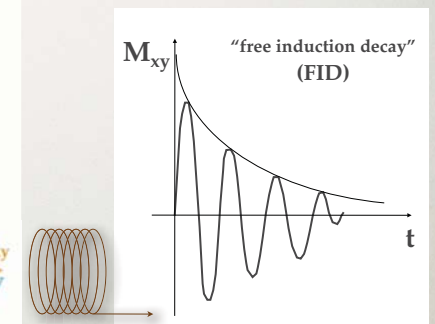
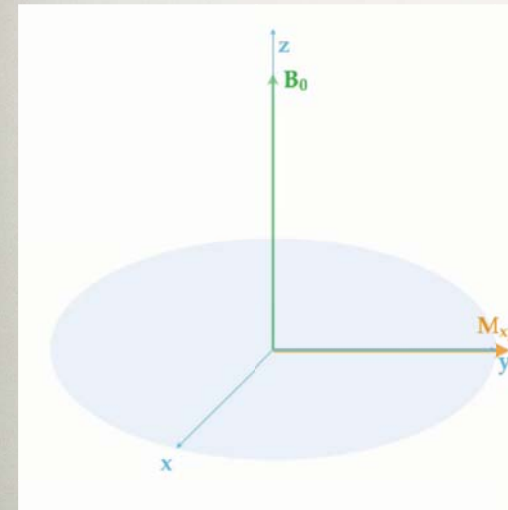


SPIN-LATTICE RELAXATION T1 OR LONGITUDINAL RELAXATION



T1 relaxation time:
depends on interaction
between elementary magnet (proton)
and its environment

SPIN-SPIN RELAXATION T2 OR TRANSVERSE RELAXATION



T2 relaxation time:
depends on interaction between
elementary magnets (protons)

MRI IS A NON-INVASIVE “TOMOGRAPHIC” METHOD



MRI:

NET MAGNETIZATION OF THE HUMAN BODY IS GENERATED



Raymond V. Damadian
(1936-)

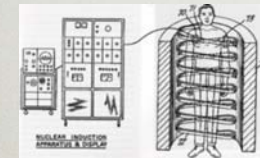
Nobel-prize (2003)



Paul C. Lauterbur
(1929-)



Peter Mansfield
(1933-)

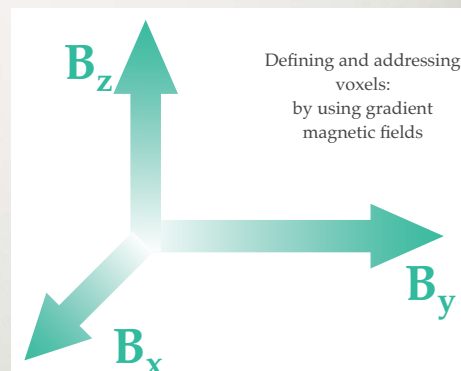
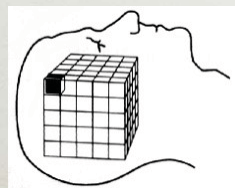
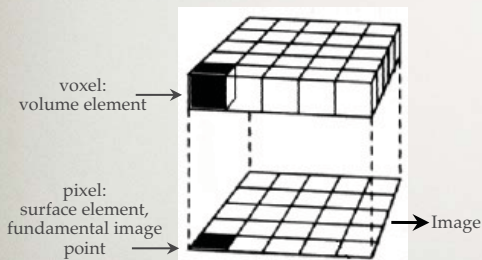


Damadian's patent figure

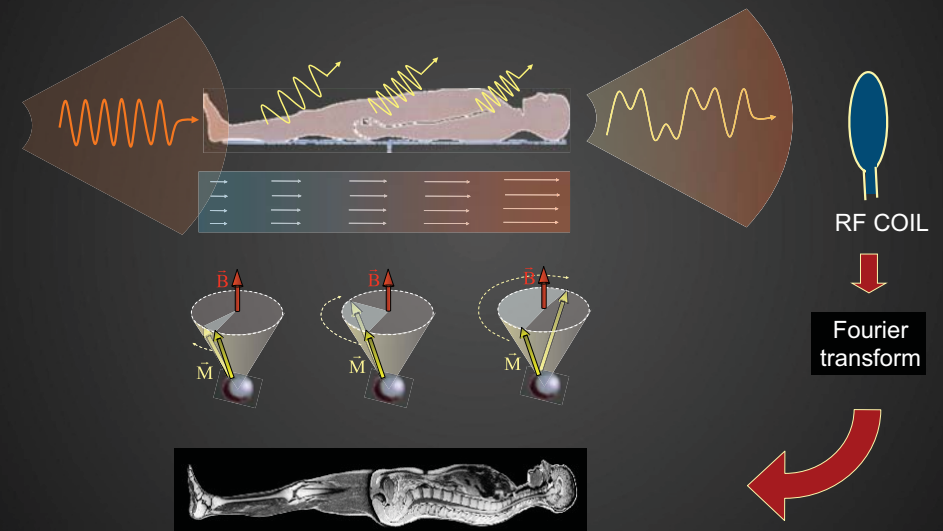


"Indomitable"

MRI IMAGING I: SPATIAL RESOLUTION

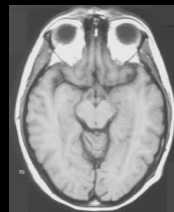
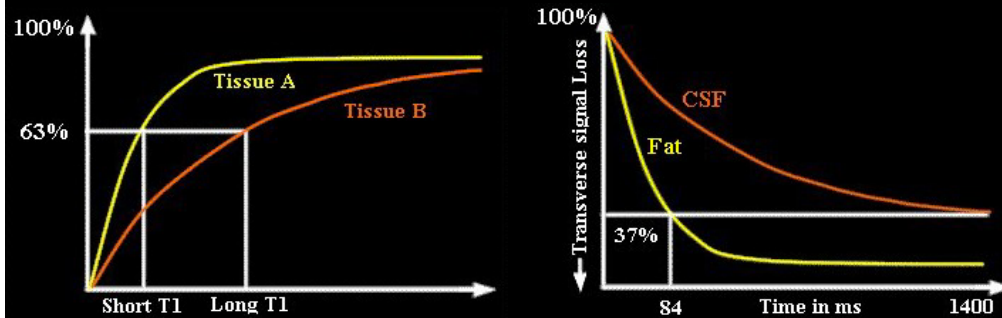


SPATIAL ENCODING OF THE NMR SIGNAL IS BASED ON FREQUENCY CHANGES IN THE PRECESSION

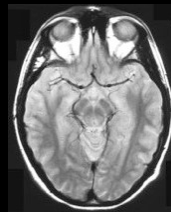


MRI IMAGING II:

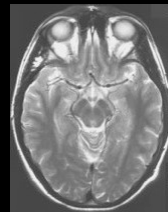
COLOR DEPTH BASED ON RELAXATION TIMES



T1-weighting

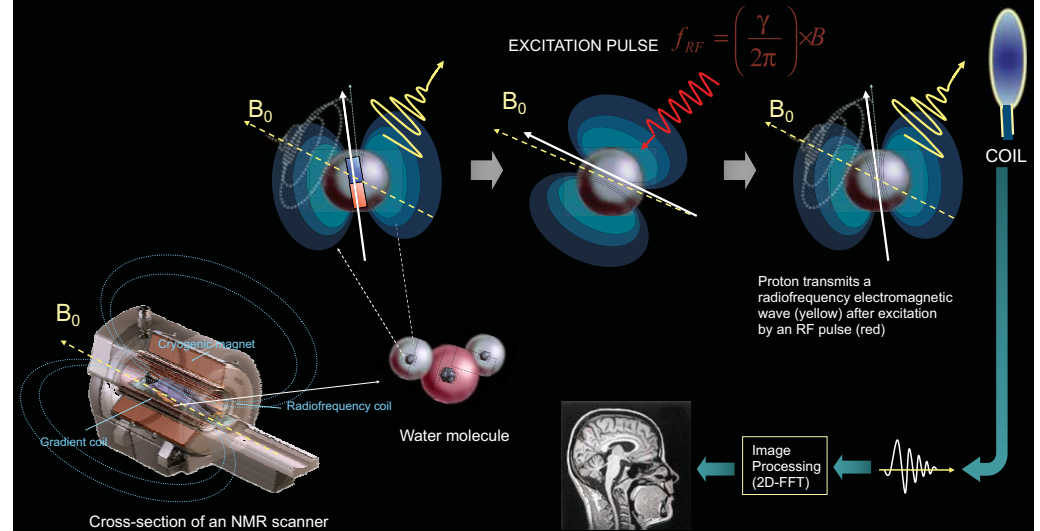


Proton density-weighting



T2-weighting

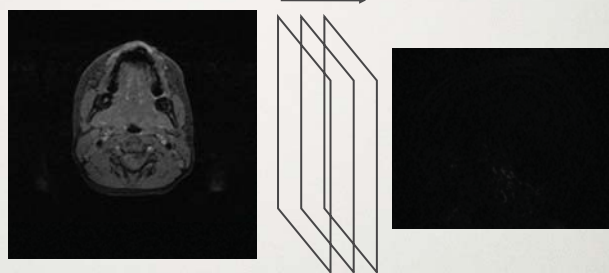
NUCLEAR MAGNETIC RESONANCE IMAGING: SUMMARY



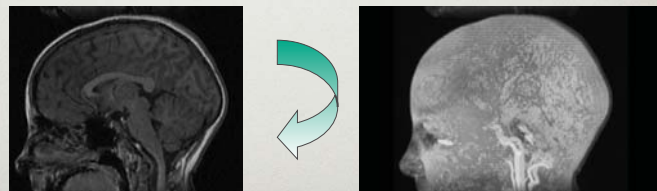
MRI:

IMAGE MANIPULATION

Reslicing in perpendicular plane

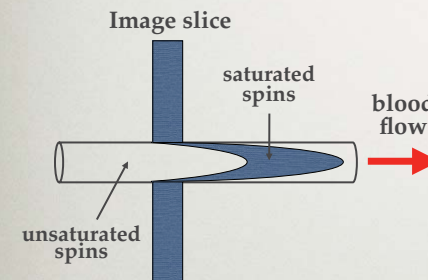


Spatial projection („volume rendering“)



MRI:

NON-INVASIVE ANGIOGRAPHY



MRI MOVIE

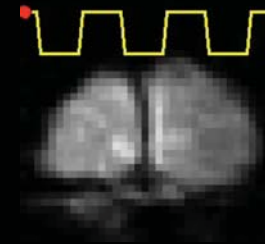
BASED ON HIGH TIME RESOLUTION IMAGES



Opening and closing of aorta valve

FUNCTIONAL MRI (FMRI)

HIGH TIME RESOLUTION IMAGES RECORDED
SYNCHRONOUSLY WITH PHYSIOLOGICAL PROCESSES



Effect of light pulses on visual cortex

SUPERPOSED MRI AND PET SEQUENCE



PET activity: during eye movement
Volume rendering

APPENDIX

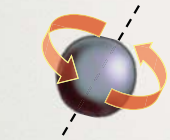
ATOMIC NUCLEI WITH NUCLEAR SPIN: ELEMENTARY MAGNETS



Otto Stern

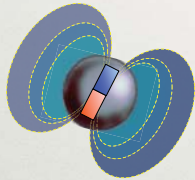


W. Gerlach



Atomic nuclei
have mass:

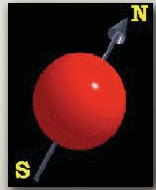
$$m_{\text{proton}} = 1,67 \cdot 10^{-24} \text{ g}$$



Atomic nuclei carry
angular momentum:

$$L = \sqrt{l(l+1)}\hbar$$

l = spin quantum number



Atomic nuclei
carry charge:

$$q_{\text{proton}} = 1,6 \cdot 10^{-19} \text{ C}$$

Atomic nuclei possess
magnetic moment:

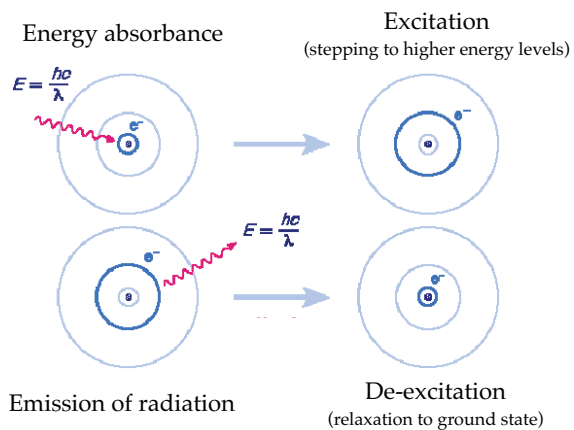
$$\mu_i = \gamma L$$

γ = gyromagnetic ratio
 L = angular momentum

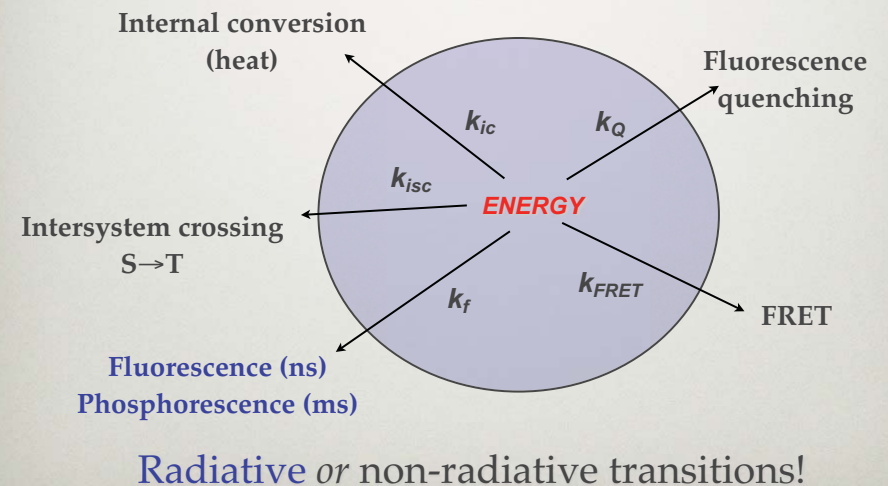
LUMINESCENCE

- Relaxation from excited state followed by light emission
- Radiation emitted by matter in excess of thermal emission
- "Cold light"
- Processes of fluorescence and phosphorescence

SIMPLIFIED STEPS OF LUMINESCENCE



FATE OF ABSORBED ENERGY



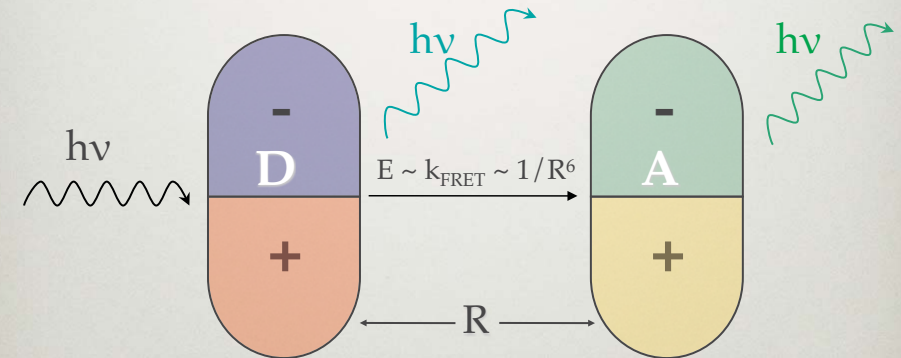
FLUORESCENCE RESONANCE ENERGY TRANSFER

In general:

- Occurs by non-radiative dipole-dipole interaction between an excited *donor* and an proper *acceptor* molecule under certain conditions (spectral overlap and close distance).
- Fluorescence Resonance Energy Transfer (FRET): if the participants of the transfer are fluorophores.

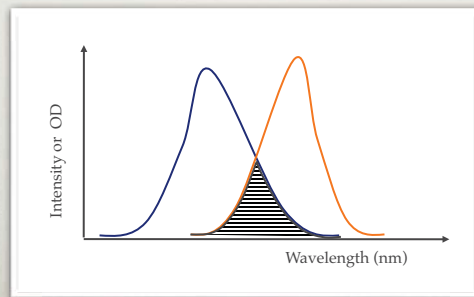
FRET

- Acceptor emission contributes to the relaxation of the excited donor molecule.



CONDITIONS OF FRET

- **Fluorescent** donor and acceptor molecules.
- The distance (**R**) between donor and acceptor molecules is 2-10 nm!
- **Overlap** between the emission spectrum of the donor and the absorption spectrum of the acceptor.

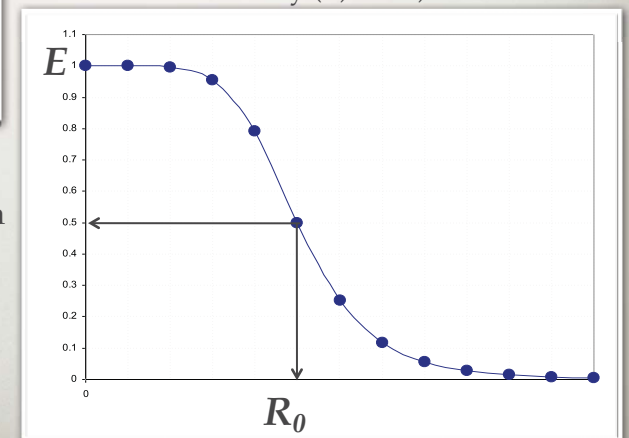


DISTANCE DEPENDENCE OF FRET

$$E = \frac{R_0^6}{R_0^6 + R^6}$$

Actual distance between fluorophores

Förster-distance
(Distance at which transfer efficiency (E) is 0.5)



APPLICATIONS OF FRET

- **Molecular ruler:** distance measurement on the nm (10^{-9}m) scale.
- High sensitivity!
- **Applications:**
 - Measurement of *interactions* between molecules.
 - Measurement of *structural* changes on molecules.