Fluorescence resonance energy transfer

Fluorescence methods in life sciences

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Energy transfer

Overview of the lecture

1. The origin of resonance energy transfer
2. Characteristic values of FRET
3. FRET applications for biomolecules

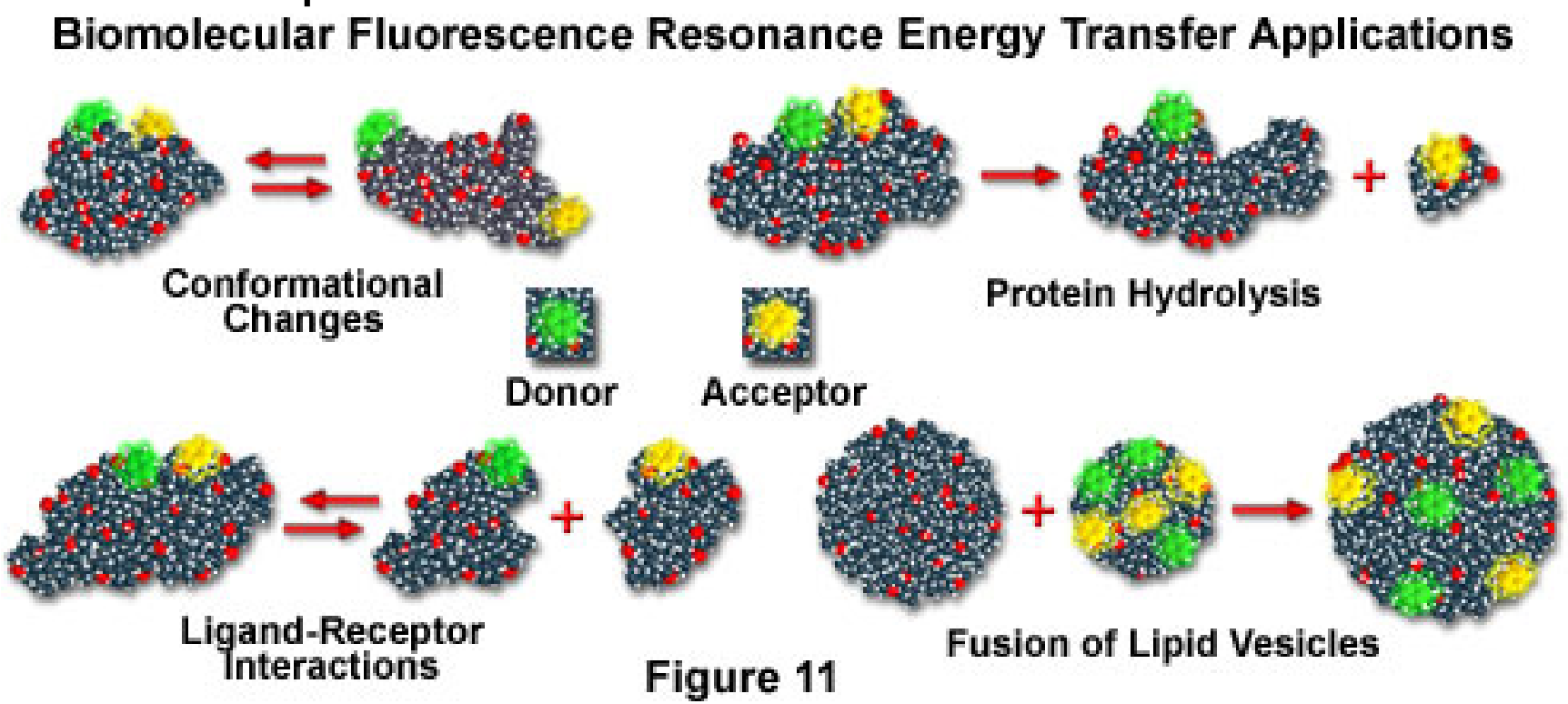
1. Excitation energy transfer

- Electron transfer of energy takes place by **radiative** or **non-radiative** mechanisms.
- The **radiative** (trivial) energy transfer occurs when the excited donor molecule emits radiation which is subsequently reabsorbed by the acceptor molecule.
  The photon is exchanged.

- Excitation by **non-radiative energy transfer (fluorescence resonance energy transfer, FRET)** occurs when only **donor** molecules absorb in the mixture of molecules. As a result, excited **acceptor** molecules that don't absorb the excitation radiation. During this transfer of energy the donor does not emit the light .
  There is no photon exchanged!

What is resonance energy transfer good for?

- Conformation changes
- Binding of a ligand and a receptor
- Cleavage of a protein/DNA
- Membrane fusion

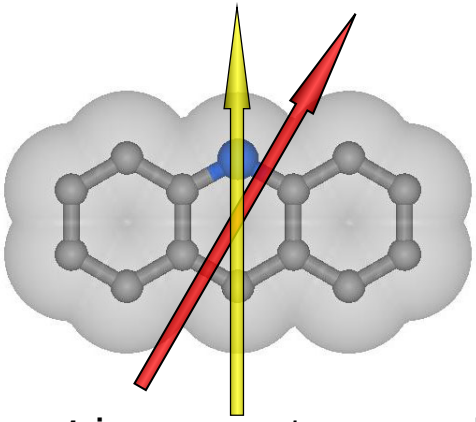


Resonance energy transfer

- The analogy with the energy transfer between tuners

- In the case of molecules, transfer of energy occurs due to dipole-dipole interactions

Transition dipole moment



Absorption

Emission

- **Transition dipole moment** is a quantum mechanical issue. It is not a real dipole moment. It is given by immediate state of electron shell of the molecule. The size of the dipole moment of transfer indicates the ability of the given molecule state to absorb or emit the light. The direction of the dipole moment of transfer indicates the direction in which the light is preferably absorbed or emitted by the molecule.
- The molecules preferentially absorb the light whose electric component oscillates in the same plane as **absorption transition dipole** of electron into a higher energetic level.
- Molecules preferably emit light in the same plane as the **emission transition dipole** of electron transition into a lower energetic level.

Interaction of transfer dipole moments of Donor and Acceptor

Donor

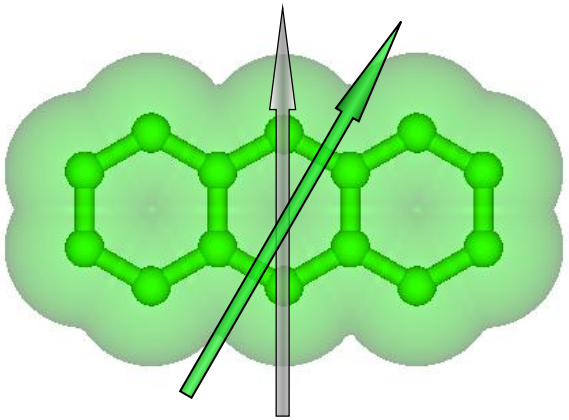
Absorption of donor

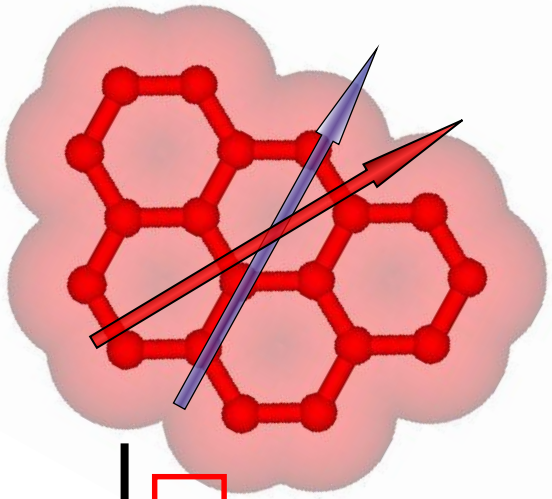
Emission of donor

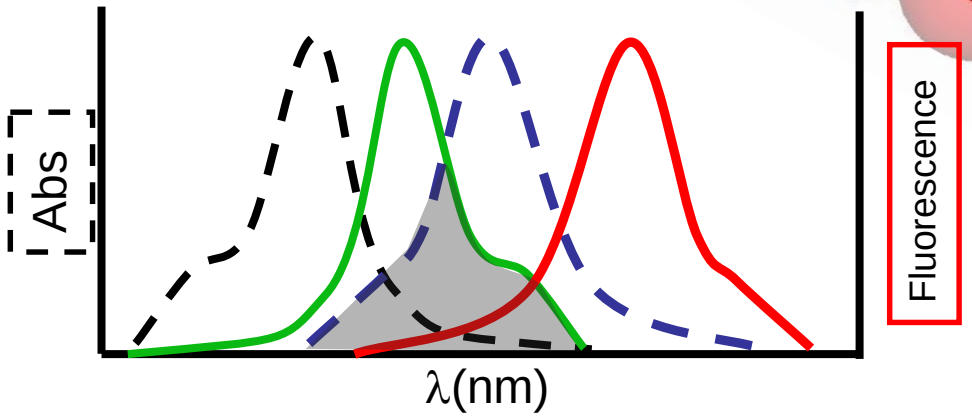
Acceptor

Absorption of acceptoru

Emission of acceptoru







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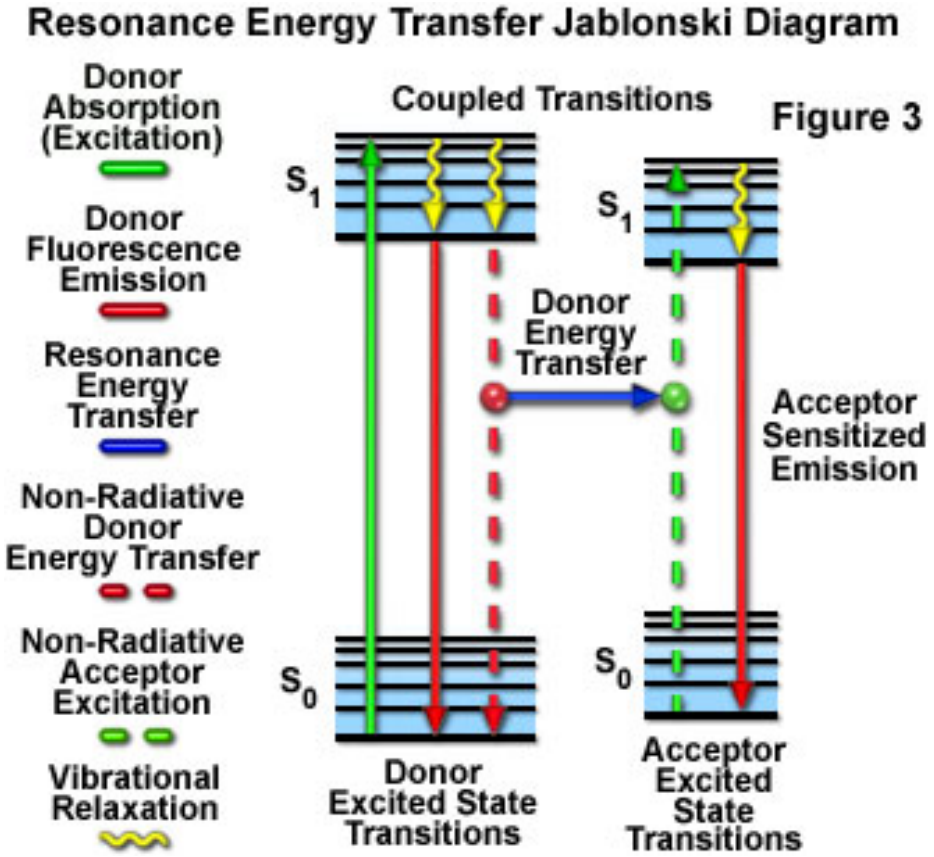
FRET – non-radiative energy transfer

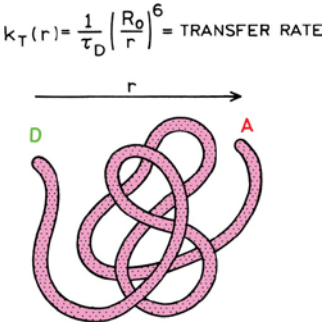


- In the presence of a suitable acceptor, a donor can transfer energy of the excited state directly to the acceptor without emission of a photon.
- The excited acceptor then emits the energy that was originally absorbed by the donor.

Fluorescence resonance energy transfer (FRET)

Resonance energy transfer can be characterized by the rate constant ($\mathbf{k_{DA}}$), which expresses **the probability of transfer**; the dipole-dipole energy transfer is the determining component for that Förster formula has been derived (in the case of the weak bond when mutual donor and acceptor interactions do not affect the optical spectra) in the form

$$k_{DA} = \frac{1}{\tau_D} \cdot \left(\frac{R_0}{r} \right)^6$$



τ_D – time decay of donor fluorescence , R_0 – the distance at which the probability of energy transfer is equal to the probability of internal deactivation of the excited state of a molecule, r – the distance between the donor and the acceptor.

Resonance energy transfer is highly dependent on the distance of the donor and acceptor.

Transfer efficiency E

Is determined by the relative amount of photons that are absorbed by the donor and subsequently emitted by the acceptor.

$$E = \frac{k_{DA}(r)}{\tau_D^{-1} + k_{DA}(r)} \qquad E = \frac{R_0^6}{R_0^6 + r^6} \qquad E = 1 - \frac{F_{DA}}{F_D}$$

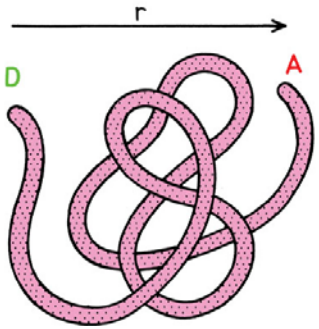
It is measured by determining of the relative intensity of donor fluorescence in the absence (F_D) and in the presence of acceptor (F_{DA}).

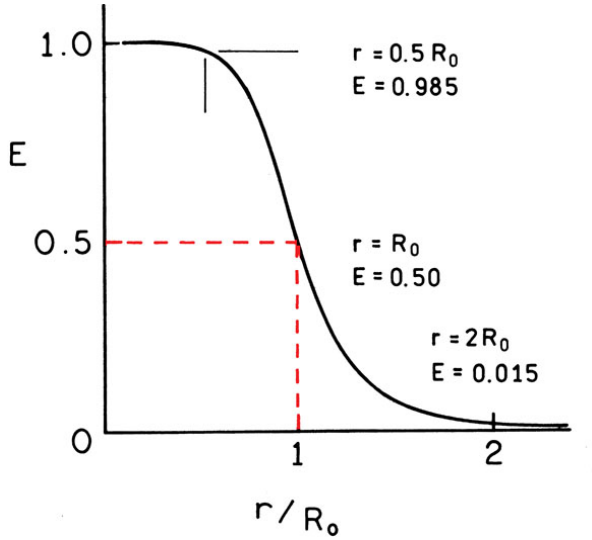
Rapidly decreases with the 6 power of the distance!

R_0 Förster distance

- R_0 is the distance of the donor and acceptor at which the transfer efficiency E is 50% = half
- Size of R_0 is usually 2 – 6 nm, which is comparable with the size of biomolecules

$$k_T(r) = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6 = \text{TRANSFER RATE}$$





The dependence of transfer efficiency E on distance r is the highest (the fastest change) in the case that the donor and acceptor distance is close to R_0.

R_0 indicates middle distance on which donor-acceptor pair can communicate – R_0 is an unique characteristic of the pair of fluorophores

The dependence of transfer efficiency on distance

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

$r = 0.1 \times R_0$	$E = 0.999999$
$r = 0.5 \times R_0$	$E = 0.984615$
$r = 2.0 \times R_0$	$E = 0.015384$
$r = 4.0 \times R_0$	$E = 0.000244$

Suitable distances for measurement: 0.5 - 2 R_0

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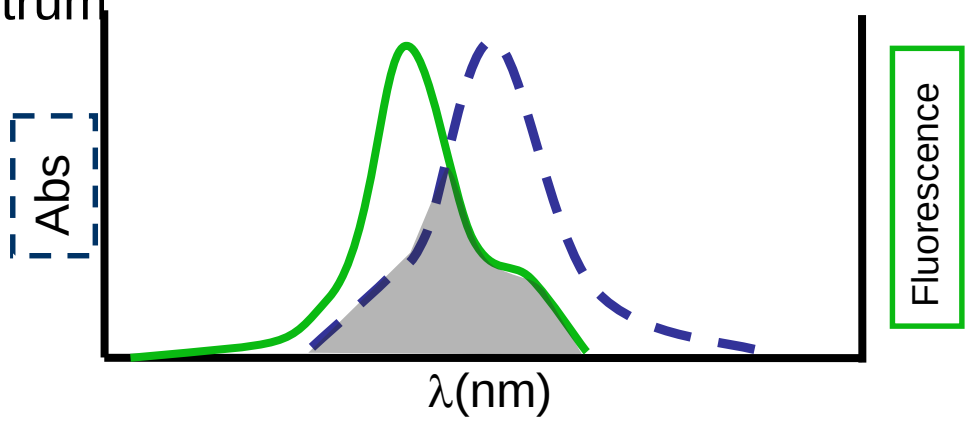
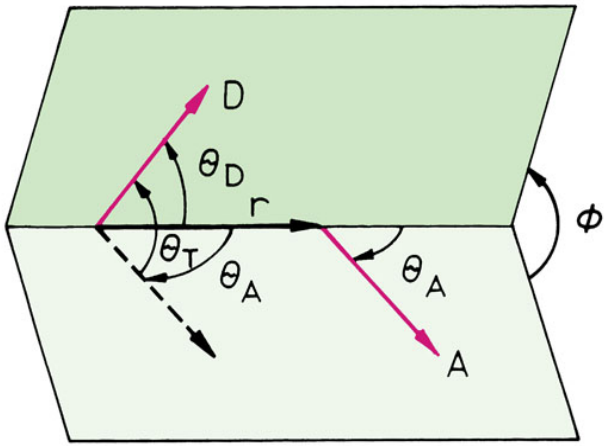
What does R_0 depend on?

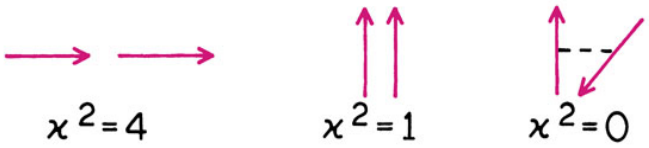
$$R_0 = 0.211\left(\kappa^2 n^{-4} Q_D J(\lambda)\right)^{1/6} \quad (\text{v } \overset{\text{o}}{\text{A}})$$

- On the orientation of the transfer dipole moments for the donor emission and acceptor absorption (the average value of 2/3 is used for factor κ^2)
- On the optical properties of environment (refractive index of water n_{H20}= 1.33)
- On the quantum yield of the donor Q_D
- On the degree of overlap J (λ) of the donor emission spectrum and the acceptor absorption spectrum



Indicative factor κ^2 dependence on the orientation of transfer dipole moments





$$\chi^2 = (\cos\theta_T - 3\cos\theta_D \cos\theta_A)^2$$

$$\chi^2 = (\sin\theta_D \sin\theta_A \cos\phi - 2\cos\theta_D \cos\theta_A)^2$$

For the random arrangement of transfer dipole moments

$$\kappa^2 = 2/3$$

This value is commonly used in common practice.

In the case that the transfer dipole moments are perpendicular $\kappa^2 = 0$

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Dependence of R_0 on spectral overlap

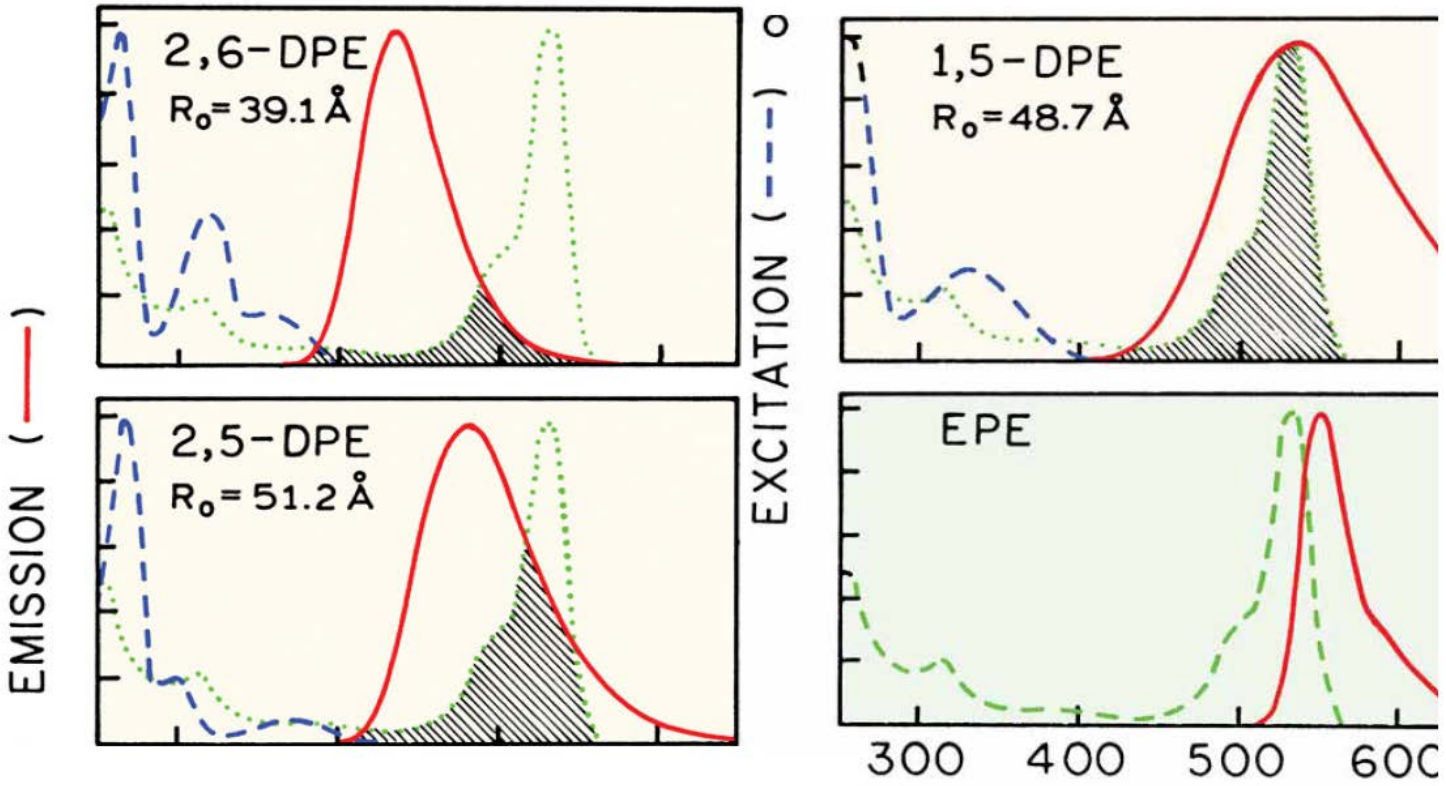


Table 13.1. Calculated R_0 Values for RET from Structural Isomers of Dansyl-Labeled Phosphatidylethanolamine (DPE) to Eosin-Labeled Phosphatidylethanolamine (EPE) and from 2,6-DPE to 2,5-DPE

Donor	Acceptor	Q_D	J (M^{-1} cm^3)	J (M^{-1} cm^3 $(nm)^4$)[d]	R_0 (Å)[a]
1,5-DPE[b]	EPE[c]	0.37	2.36×10^{-13}	2.36×10^{15}	48.7
2,5-DPE	EPE	0.76	1.54×10^{-13}	1.54×10^{15}	51.2
2,6-DPE	EPE	0.71	3.31×10^{-14}	3.31×10^{14}	39.1
2,6-DPE	2,5-DPE	0.71	1.3×10^{-15}	1.3×10^{13}	22.8

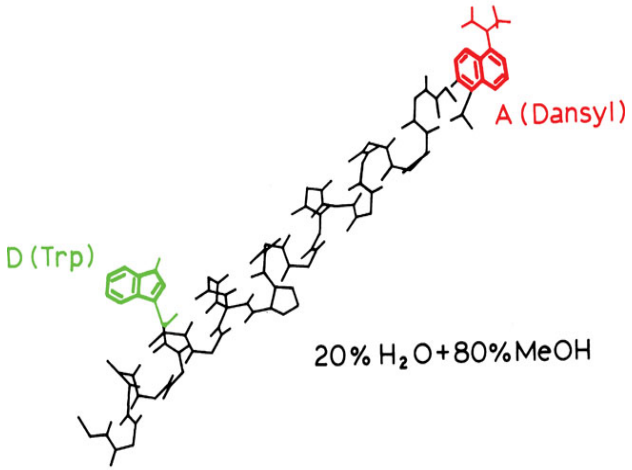
[a]From [5]. R_0 was calculated using $n = 1.4$ andy $\kappa^2 = 2/3$.

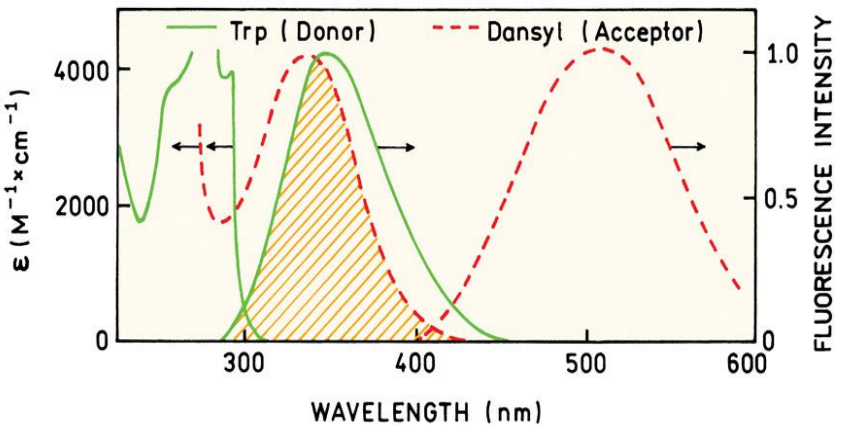
[b]Dansyl-labeled phosphatidylethanolamine.

[c]Eosin-labeled phosphatidylethanolamine.

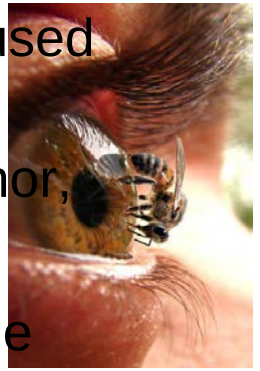
[d]The factor of 10^{28} between $J(\lambda)$ in M^{-1} cm^3 and M^{-1} cm^3 $(nm)^4$ arises from 1 nm = 10^{-7} cm, raised to the fourth power.

Distance measurement using FRET

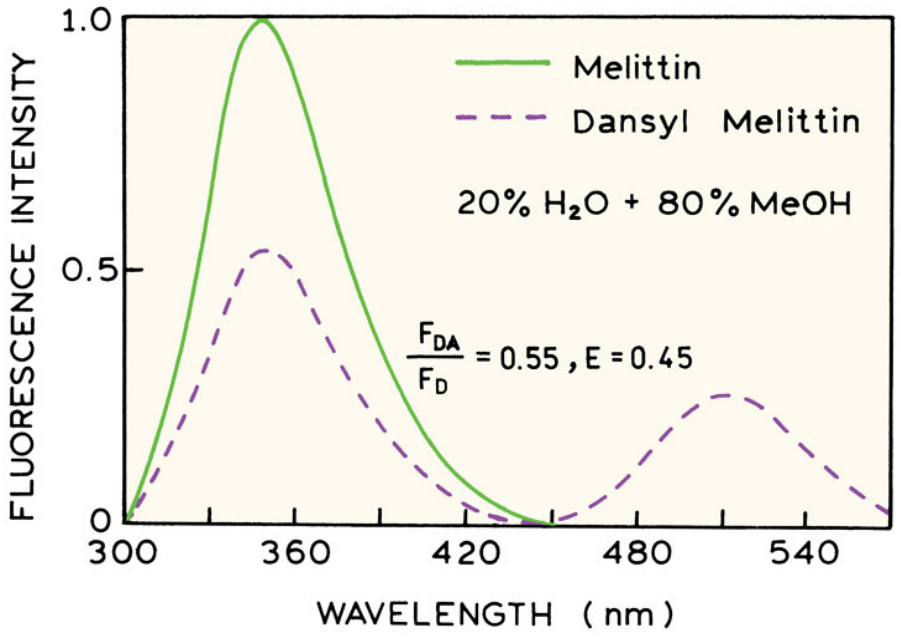




- Melittin is the most important component of honeybee venom (damaging the cell structure, decomposes the white and red blood cells, it causes death of cells). Its anti-inflammatory effects are used in the treatment.
- Melittin consists of 26 AK, tryptophan at position 19 was the donor,
- Dansyl at the N-terminus was the acceptor
- After separate spectral characterization of melittin with Trp as the donor and Dansyl as the acceptor the value R_0=2.36 nm was determined .



Determination of the Trp distance from the N-terminus of melittin using FRET



$$E = 1 - \frac{F_{DA}}{F_D}$$

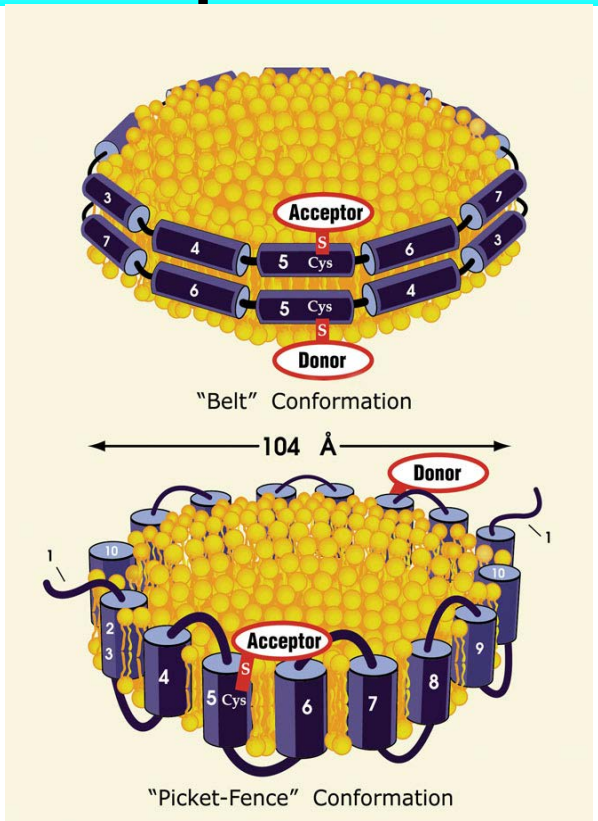
$$E = \frac{R_0^6}{R_0^6 + r^6}$$

- Based on the decrease in fluorescence intensity in the presence of the acceptor it is possible to determine the transfer efficiency and hence donor (Trp) distance from the acceptor (N-terminal).
- r = 2.44 nm

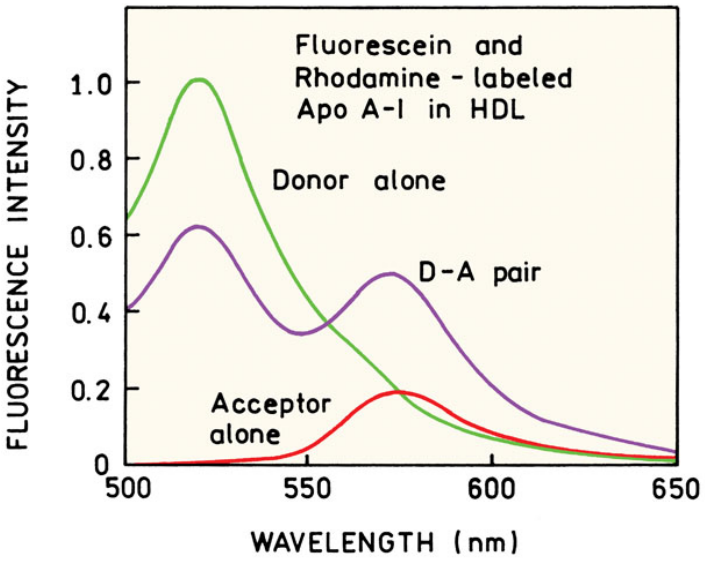
FRET advantages

- Efficiency of energy **transfer is not dependent** on environment between the donor and acceptor
- Measurement of intensity ratio enables the use of FRET analysis **independently** on the concentration
- For most applications, it is **not necessary to know R_0**

FRET allows to distinguish between multiple variants of protein folding

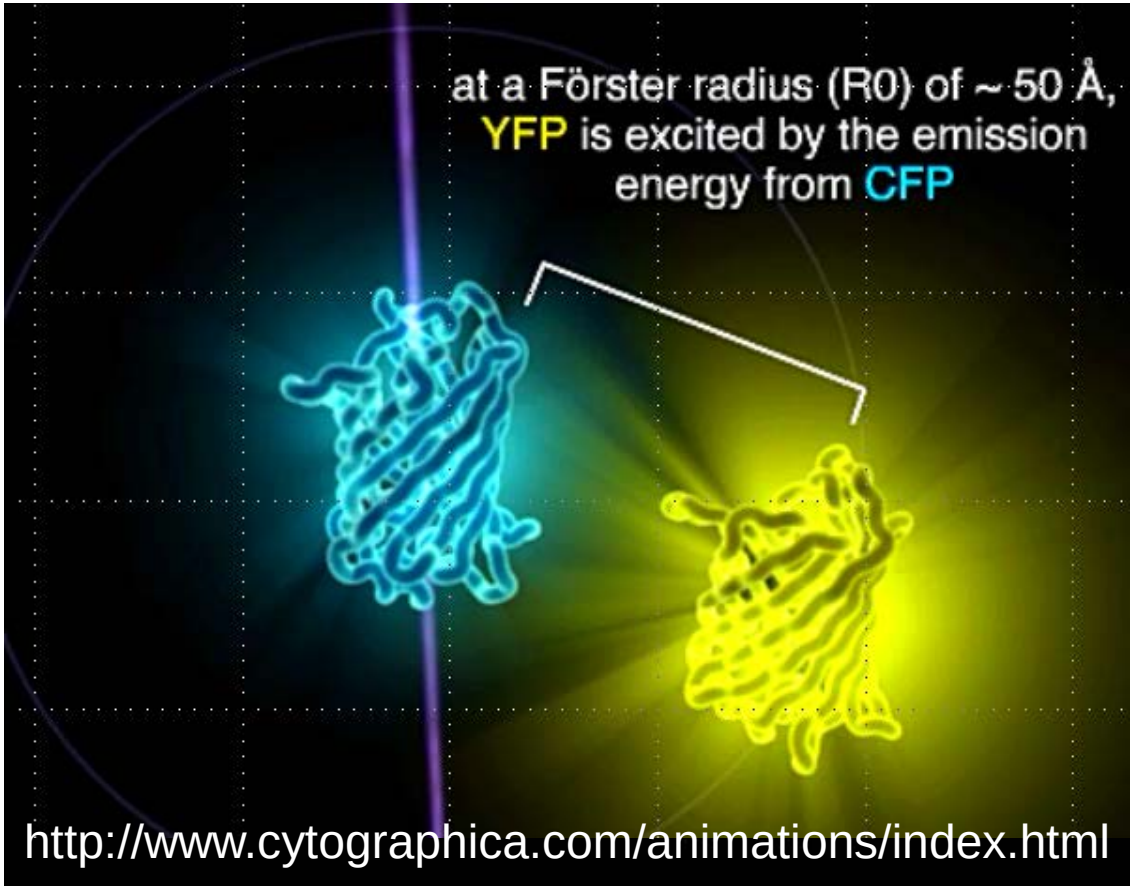


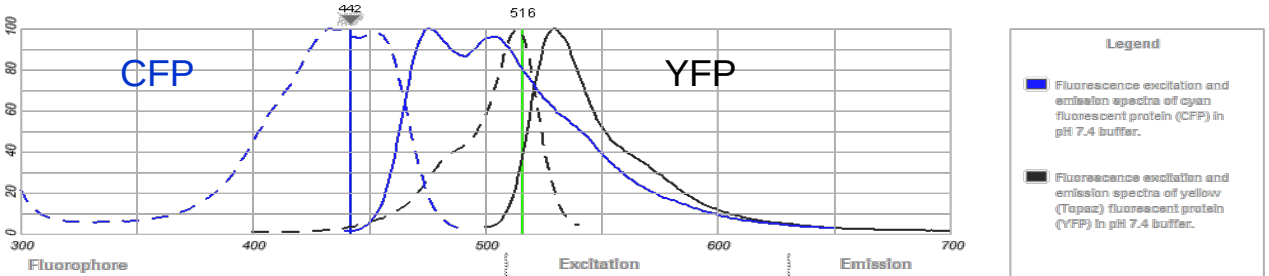
- apoA-I protein regulates cholesterol metabolism.
- apo A-I binds to a lipid membrane
- two possible arrangements of proteins in the membrane were designed
- structural methods can not be used because of the flexibility of lipids
- one of the chains of apoA-I proteins was labeled by fluorescein (Donor) and second one by tetramethylrhodamine (Acceptor)



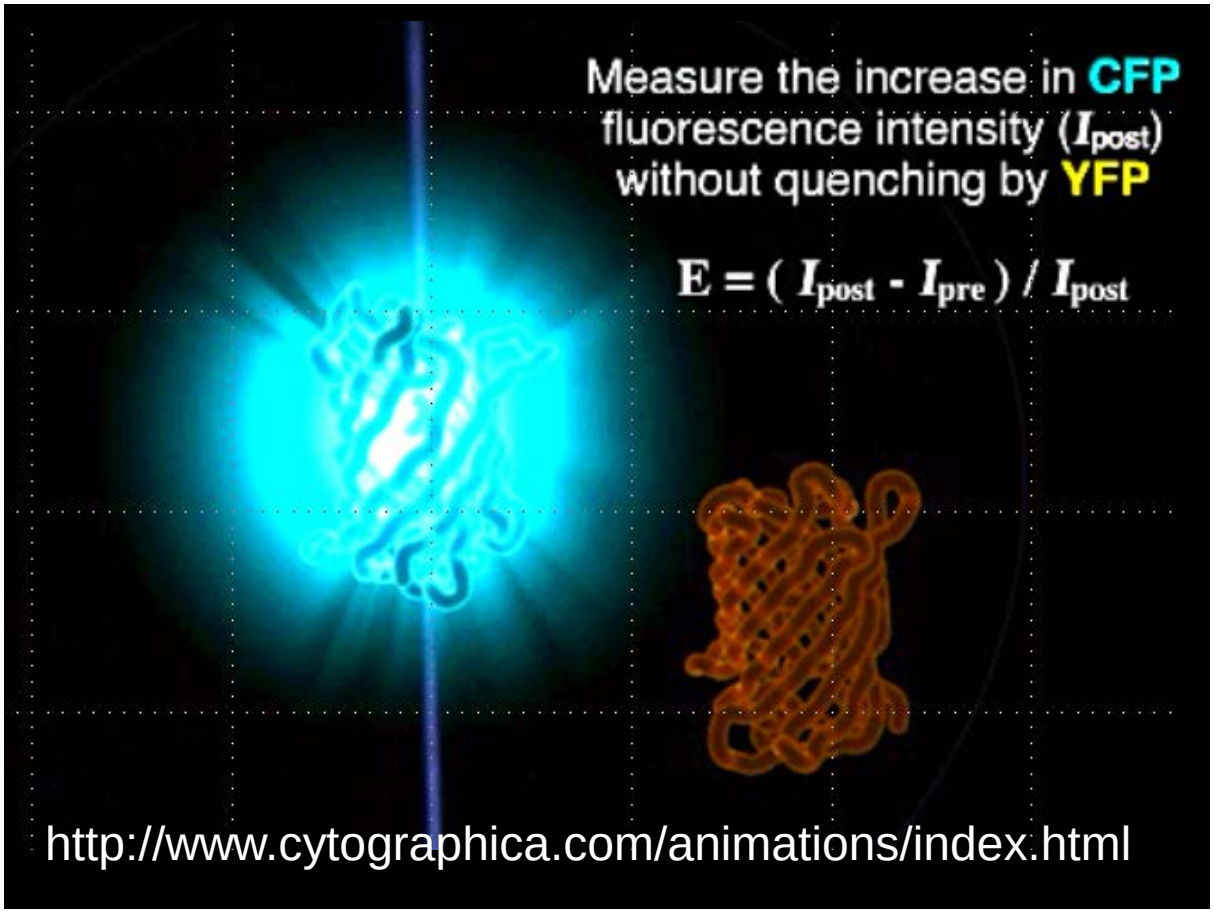
- Firstly, the emission spectrum of the donor itself in one chain of ApoA-I was measured
- Decrease of the intensity of donor fluorescence was observed after addition of the second chain with the acceptor
- There was a significant resonance energy transfer
- This proved a relatively small distance between the donor and the acceptor
- It was confirmed belt conformation of ApoA-I http://pubs.acs.org/doi/abs/10.1021/la402727a

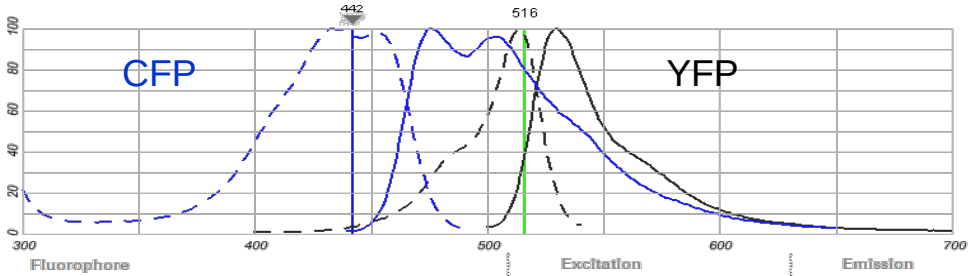
FRET between CFP and YFP

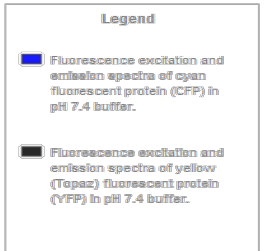




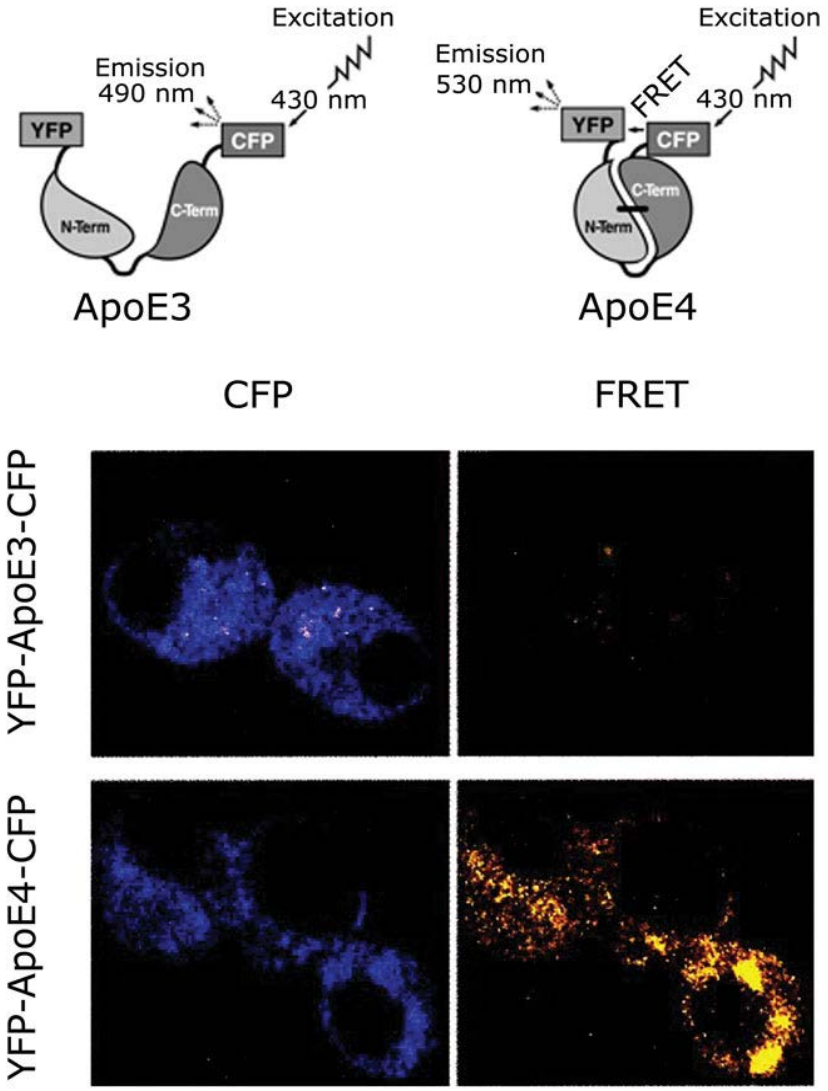
FRET quenching by photobleaching







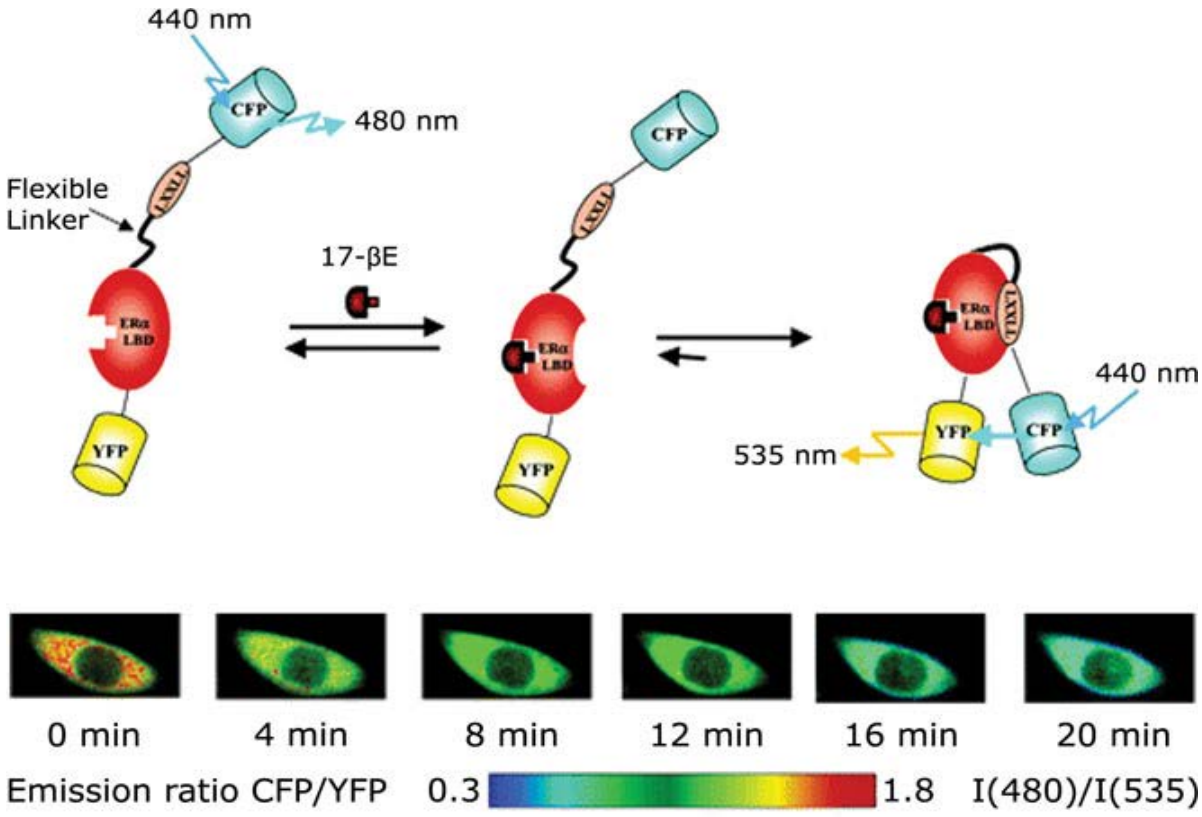
Protein folding *in vivo*



- ApoE4 is associated with Alzheimer's disease and binds to nerve cells
- ApoE3 has a similar sequence of AK, but does not bind to nerve cells
- It has been suggested that the binding ability to the nerve cells is related to the different arrangement of domains of the ApoE proteins
- For verifying nerve cells were transfected with constructs of ApoE
- FRET analysis showed that ApoE4 in conformation with associated domains binds to nerve cells, while ApoE3 does not have its domains in close proximity and does not bind to the nerve cells

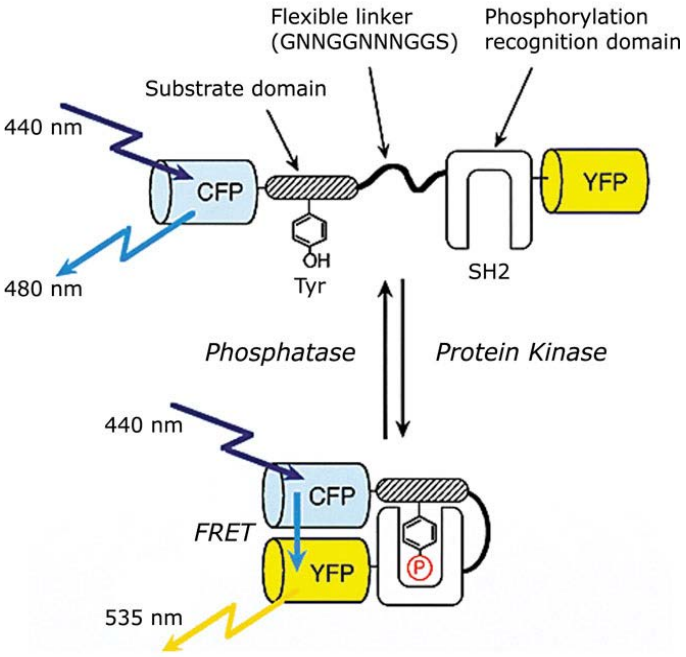
FRET sensors

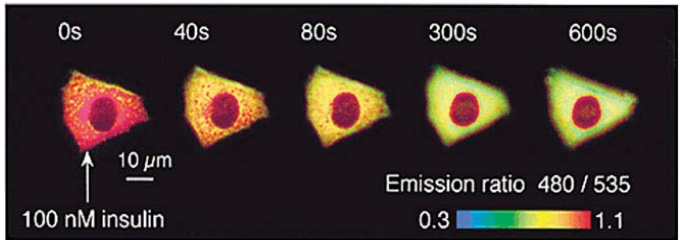
- Estrogen detection



Monitoring of protein phosphorylation

Monitoring of insulin processing



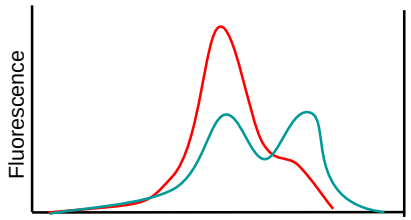


Insulin receptor is a tyrosine kinase which phosphorylates tyrosine in the recognized sequence, which is subsequently bound to the SH2 domain of another protein.

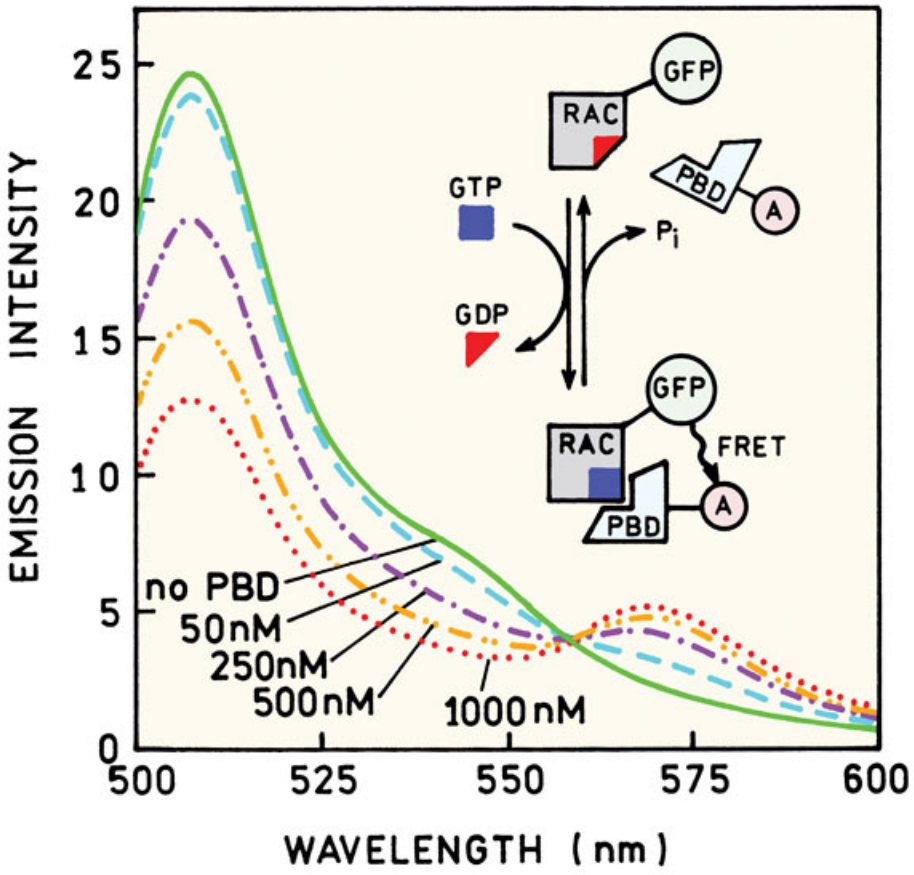
This binding causes the CFP and YFP approach and induces FRET.

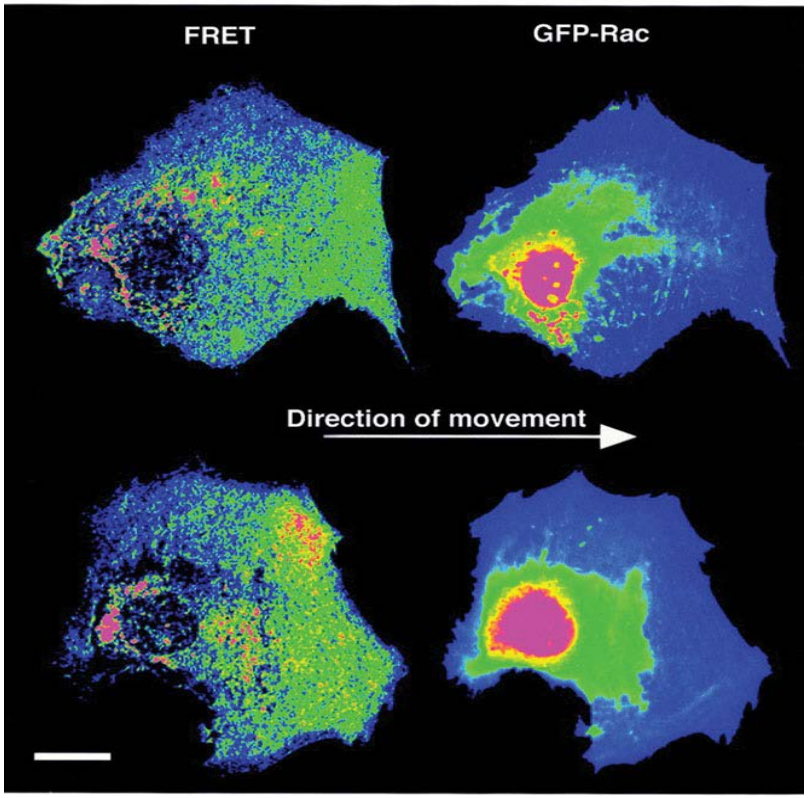
The protein construct was expressed in cells.

Transfer energy was increased gradually after exposure of cells to 100 nM insulin.



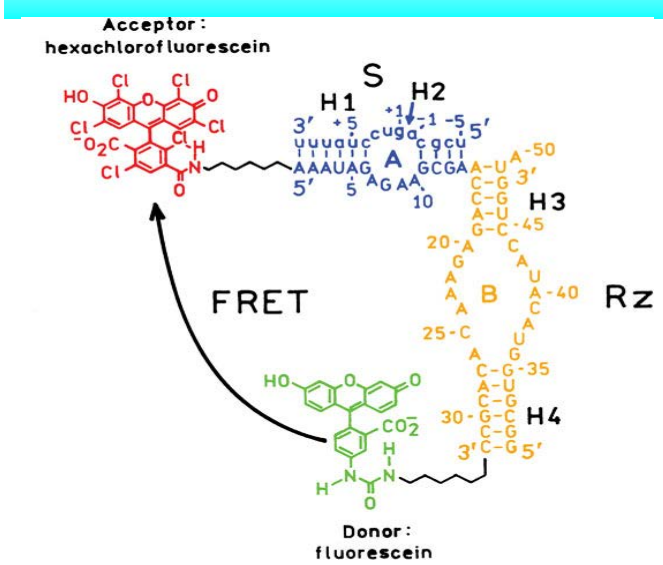
Monitoring of morphological changes of cells

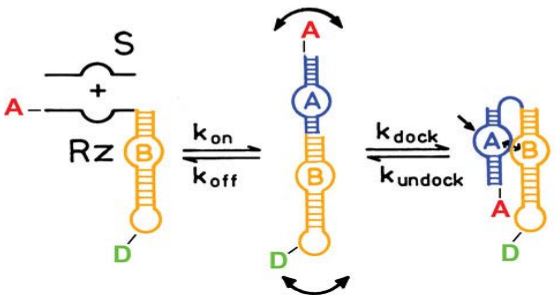


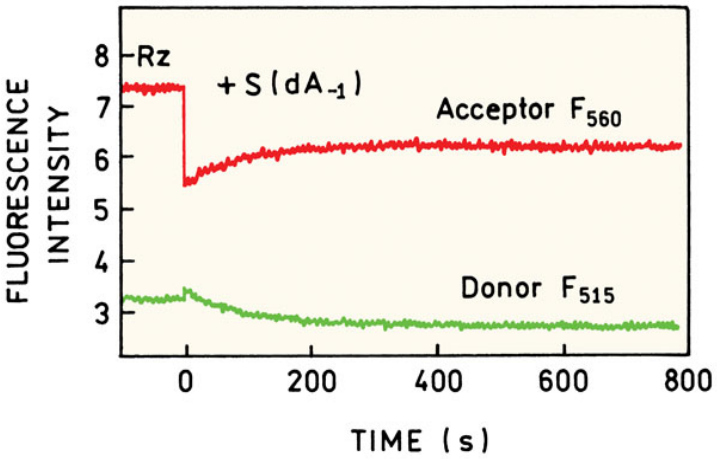


The most of GTP is processed in the green region of FRET and the cell also moves in this direction.

Ribozyme cleavage



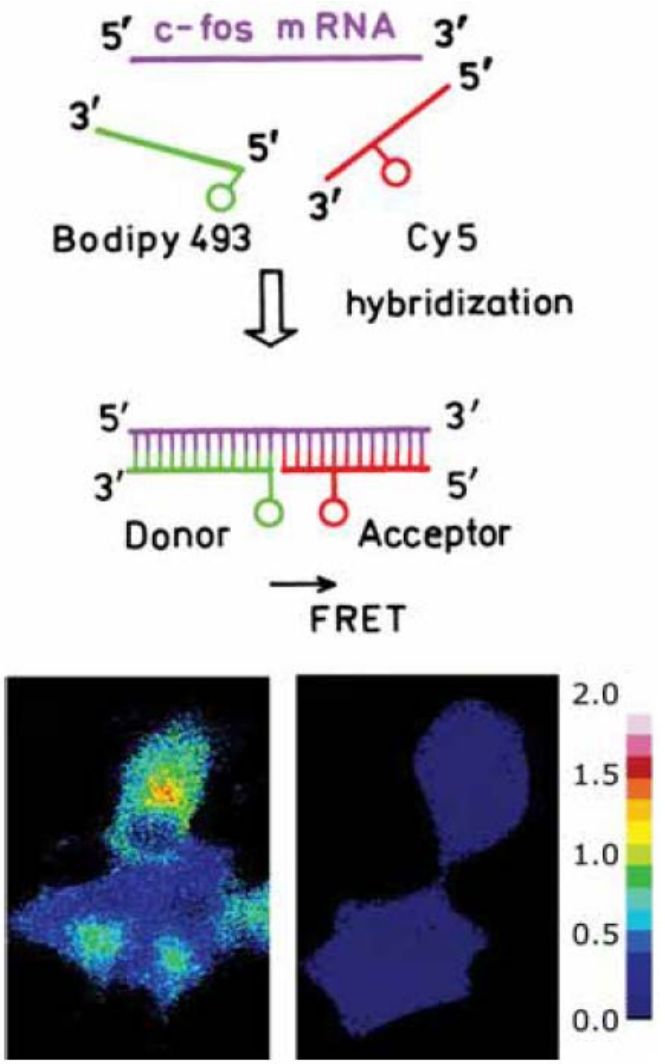




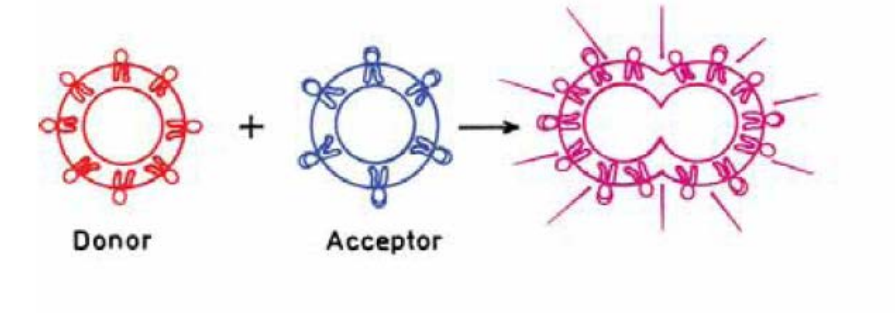
- Parts A and B get near upon binding of the ribozyme to a substrate resulting in increased FRET

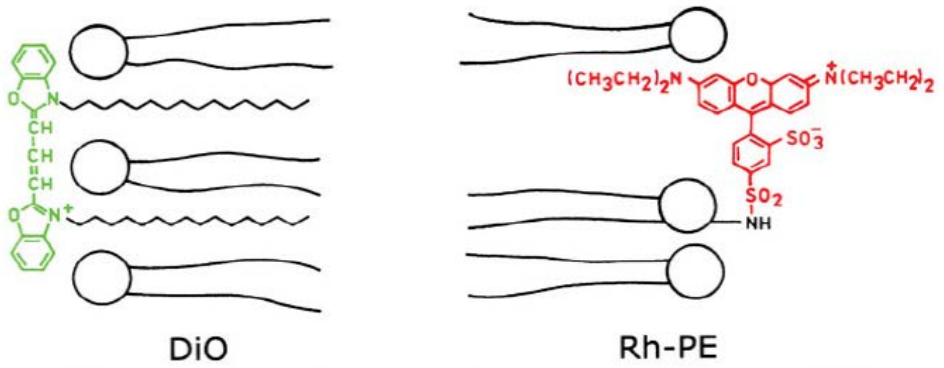
Detection of the presence of c-fos mRNA

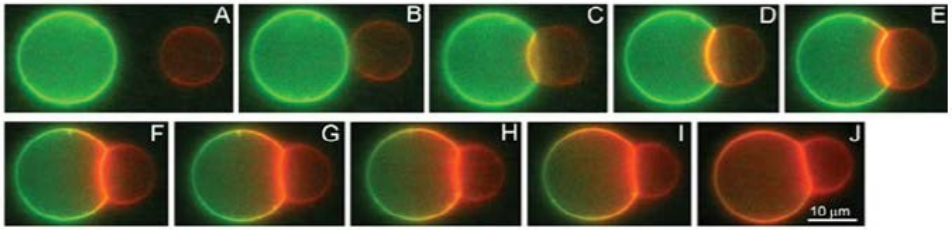
- c-fos mRNA is a transcription factor that affects cell cycle and differentiation
- FRET probes were used to detect the presence of c-fos mRNA in the cell culture
- The presence of c-fos mRNA in activated cells (left) was demonstrated



Membrane fusion

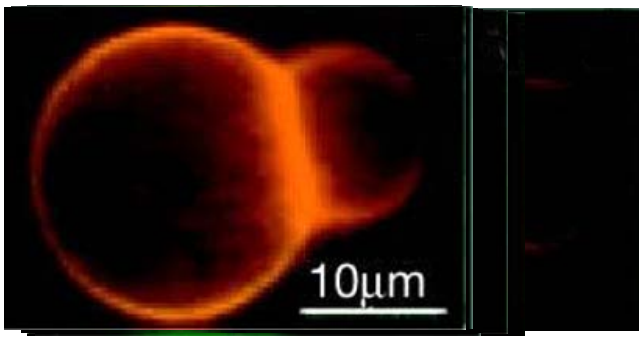


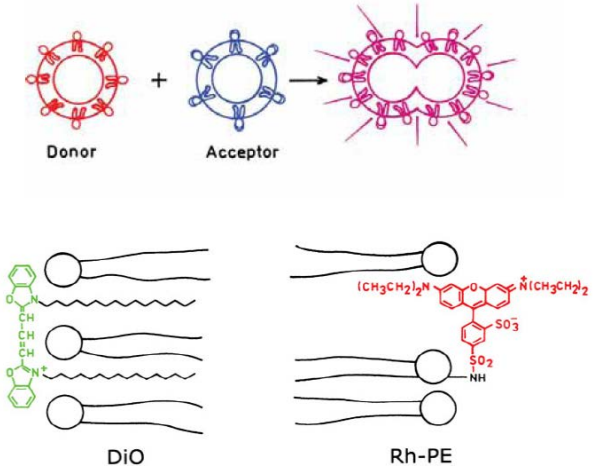




- Individual membranes contain a donor or an acceptor
- in the case of membrane fusion we observe FRET

Application of FRET microscopy





Lei, G and MacDonald, R.C., Biophys J. 2003

FRET was applied for visualization of lipid bilayer vesicle fusion:

Intermediates Captured by High-Speed Microfluorescence Spectroscopy

Literature

- Lakowicz J.R.: Principles of Fluorescence Spectroscopy. Third Edition, Springer + Business Media, New York, 2006.
- Fišar Z.: FLUORESCENČNÍ SPEKTROSKOPIE V NEUROVĚDÁCH http://www1.lf1.cuni.cz/~zfisar/fluorescence/Default.htm

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Intrinsic fluorescence of proteins