Fluorescence resonance energy transfer

Fluorescence resonance energy transfer (or **Förster resonance energy transfer**) describes an energy transfer mechanism between two fluorescent molecules. A fluorescent donor is excited at its specific fluorescence excitation wavelength. By a long-range dipole-dipole coupling mechanism, this excited state is then nonradiatively transferred to a second molecule, the acceptor. The donor returns to the electronic ground state. The described energy transfer mechanism is termed "Förster resonance energy transfer" (FRET), named after the German scientist Theodor Förster. When both molecules are fluorescent, the term "fluorescence resonance energy transfer" is often used, although the energy is not actually transferred by fluorescence.

Theoretical basis

The FRET efficiency is determined by three parameters:

. The distance between the donor and the acceptor.

0. The spectral overlap of the donor emission spectrum and the acceptor absorption spectrum.

. The relative orientation of the donor emission dipole moment and the acceptor absorption dipole moment.

The FRET efficiency E, which is defined as

$$E = 1 - \tau'_{\mathrm{D}}/\tau_{\mathrm{D}}$$

where τ'_{D} and τ_{D} are the donor fluorescence lifetimes in the presence and absence of an acceptor, respectively, or as

$$E = 1 - F'_{\mathrm{D}}/F_{\mathrm{D}}$$

where F'_{D} and F_{D} are the donor fluorescence intensities with and without an acceptor, respectively. E depends on the donor-to-acceptor separation distance r with an inverse 6th order law due to the dipole-dipole coupling mechanism:

$$E = \frac{1}{(1 + (r/R_0)^6)}$$

with R_0 being the Förster distance of this pair of donor and acceptor at which the FRET efficiency is 50%. The Förster distance depends on the overlap integral of the donor emission spectrum with the acceptor absorption spectrum and their mutual molecular orientation as expressed by the following equation:

$$R_0{}^6 = 8.8 \times 10^{23}\ \kappa^2\, n^{-4}\, Q_0\, J$$

where κ^2 is the dipole orientation factor, n is the refractive index of the medium, Q_0 is the fluorescence quantum yield of the donor in the absence of the acceptor, and J is the spectral overlap integral calculated as

$$J = \int f_{\mathrm{D}}(\lambda)\, \epsilon_{\mathrm{A}}(\lambda)\, \lambda^4\, d\lambda$$

where f_D is the normalized donor emission spectrum, and ε_A is the acceptor extinction coefficient. If either the donor or the acceptor is freely rotating (or both), $\kappa^2 = 2/3$ is assumed. On this condition, the R_0 value is determined only by the combination of the donor and acceptor molecules.

Applications

In fluorescence microscopy, fluorescence confocal laser scanning microscopy, as well as in molecular biology, FRET is a useful tool to quantify molecular dynamics in biophysics, such as protein-protein interactions, protein-DNA interactions, and protein conformational changes. For monitoring the complex formation between two molecules, one of them is labeled with a donor and the other with an acceptor, and these fluorophore-labeled molecules are mixed. When they are dissociated, the donor emission is detected upon the donor excitation. On the other hand, when the donor and acceptor are in close proximity (1-10 nm) due to the interaction of the two molecules, the acceptor emission is predominantly observed because of the intermolecular FRET from the donor to the acceptor. For monitoring protein conformational changes, the target protein is labeled with a donor and an

acceptor at two loci. When a twist or bend of the protein brings the change in the distance or relative orientation of the donor and acceptor, FRET change is observed. If a molecular interaction or a protein conformational change is dependent on ligand binding, this FRET technique is applicable to fluorescent indicators for the ligand detection.

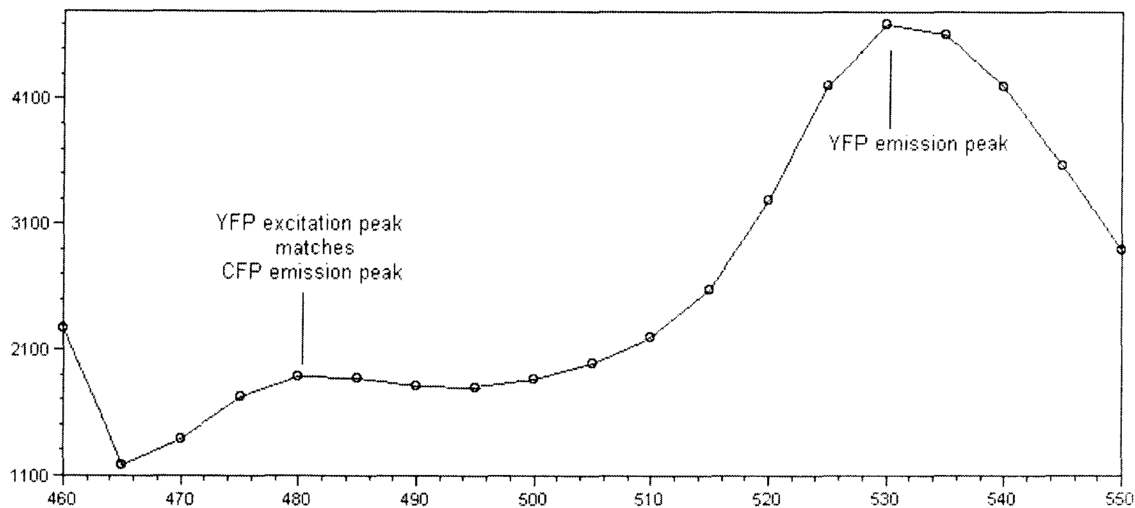
The most popular FRET pair for biological use is a cyan fluorescent protein (CFP)-yellow fluorescent protein (YFP) pair. Both are color variants of green fluorescent protein (GFP). While labeling with organic fluorescent dyes requires troublesome processes of purification, chemical modification, and intracellular injection of a host protein, GFP variants can be easily attached to a host protein by genetic engineering. By virtue of GFP variants, the use of FRET techniques for biological research is becoming more and more popular.

There is a newer type of FRET called BiFC where two halves of a YFP are fused to a protein(Hu, Kerppola et al. 2002). When these two halves meet they form a fluorophore after about 60s - 1 hr.

A limitation of FRET is the requirement for external illumination to initiate the fluorescence transfer, which can lead to background noise in the results from direct excitation of the acceptor, or photobleaching. To overcome this difficulty, Bioluminescence Resonance Energy Transfer (or BRET) has been developped. This technique uses a bioluminescent luciferase (typically purified from *Renilla Luciformis*) rather than CFP to produce an initial photon emission compatible with YFP.

FRET and BRET are also a common tools in the study of reaction kinetics and molecular motors.

A different, but related, mechanism is the energy transfer of Dexter type.



Example of FRET between CFP and YFP (Wavelength vs. Absorption): a fusion protein containing CFP and YFP excited at 440nm wavelength. The fluorescent emission peak of CFP overlaps the excitation peak of YFP. Because the two proteins are adjacent to each other, the energy transfer is significant–a large proportion of the energy from CFP is transferred to YFP and creates a much larger YFP emission peak. (Data: Used with permission from Isaac Li of IBBME, University of Toronto)

[edit]

References:

Joseph R. Lakowicz, "Principles of Fluorescence Spectroscopy", Plenum Publishing Corporation, 2nd edition (July 1, 1999)

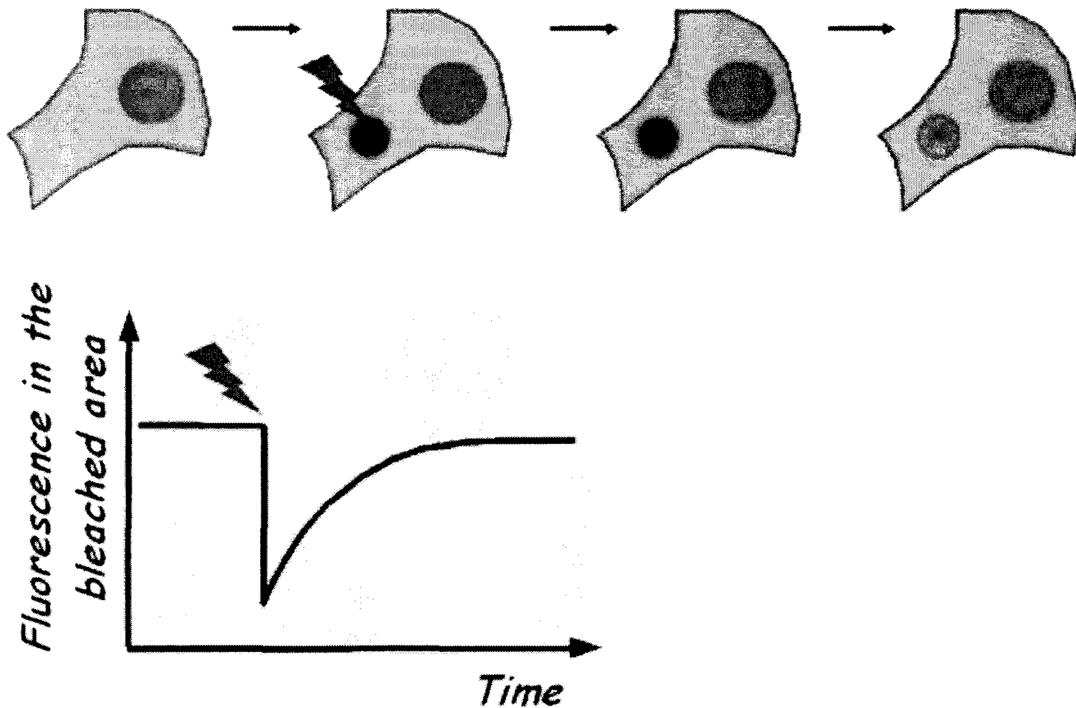
Fluorescence recovery after photobleaching

From Wikipedia, the free encyclopedia.

Principle of FRAP

Fluorescence recovery after photobleaching (FRAP) is a technique used in cellular imaging where a fluorochrome attached to a molecule is destroyed on purpose with an intense flash of light (by a laser). This is done in a well defined area to study the repopulation of this area with peripheral molecules still fluorescent. This methods allows measurement of the speed of diffusion of molecules in living cells. FRAP is usually done with confocal microscopes. This technique is commonly used in conjunction with green fluorescent protein

(GFP), fusion proteins, where the protein that is under study is fused to a GFP. GFP is a fluorescent protein that was isolated from jellyfish (*Aequorea victoria*). When excited by a specific wavelength of light, the protein will fluoresce. When the protein that is being studied is produced with the GFP, then the fluorescence can be tracked. Photodestroying the GFP, and then watching the repopulation into the bleached area can reveal information about protein interaction partners, organelle continuity and protein trafficking.



Fluorescence correlation spectroscopy

Fluorescence correlation spectroscopy (**FCS**) is a type of spectroscopy based on the measurement of fluorescence intensity and the analysis of it fluctuations, which can be due to the diffusion of the observed fluorophore in the excitation volume or to changes in the fluorescence quantum yield arising from chemical reactions. Measurement are usually made on only a few molecules at a time - of the order of 10 milliseconds - which is achieved by illuminating tiny volumes (around 1 femtoliter), available by employing two-photon excitation.

FCS is used to measure:

0. translational diffusion coefficients of macromolecules
0. the number of fluorescent molecules under observation
   the relative fluorescence yield of different molecules in a (inhomogeneous) solution, which gives the amount of each species