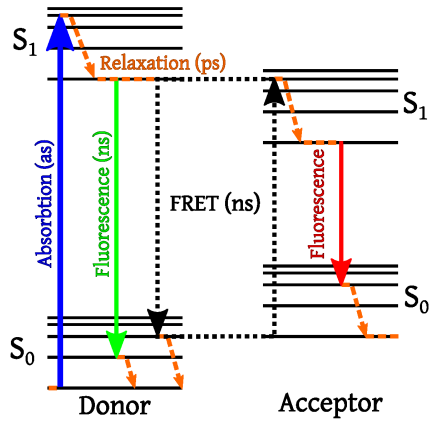
Förster resonance energy transfer

Förster (Fluorescence) resonance energy transfer (FRET), **resonance energy transfer** (**RET**) or **electronic energy transfer** (**EET**), is a mechanism describing energy transfer between two chromophores.[1] A donor chromophore, initially in its electronic excited state, may transfer energy to an acceptor chromophore through nonradiative dipole–dipole coupling.[2] The efficiency of this energy transfer is inversely proportional to the sixth power of the distance between donor and acceptor making FRET extremely sensitive to small distances.[3]

Measurements of FRET efficiency can be used to determine if two fluorophores are within a certain distance of each other.[4] Such measurements are used as a research tool in fields including biology and chemistry.



Jablonski diagram of FRET with typical timescales indicated.

FRET is analogous to near field communication, in that the radius of interaction is much smaller than the wavelength of light emitted. In the near field region, the excited chromophore emits a virtual photon that is instantly absorbed by a receiving chromophore. These virtual photons are undetectable, since their existence violates the conservation of energy and momentum, and hence FRET is known as a *radiationless* mechanism. Quantum electrodynamical calculations have been used to determine that radiationless (FRET) and radiative energy transfer are the short- and long-range asymptotes of a single unified mechanism.[5][6]

Terminology

Förster resonance energy transfer is named after the German scientist Theodor Förster.[7] When both chromophores are fluorescent, the term "fluorescence resonance energy transfer" is often used instead, although the energy is not actually transferred by fluorescence.[8][9] In order to avoid an erroneous interpretation of the phenomenon that is always a nonradiative transfer of energy (even when occurring between two fluorescent chromophores), the name "Förster resonance energy transfer" is preferred to "fluorescence resonance energy transfer;" however, the latter enjoys common usage in scientific literature.[10]

Theoretical basis

The FRET efficiency (E) is the quantum yield of the energy transfer transition, *i.e.* the fraction of energy transfer event occurring per donor excitation event[11]:

$$E = \frac{k_{ET}}{k_f + k_{ET} + \sum k_i}$$

where k_{ET} is the rate of energy transfer, k_f the radiative decay rate, and the k_i 's are the rate constants of any other de-excitation pathways.[12]

The FRET efficiency depends on many physical parameters that can be grouped as follows:

- The distance between the donor and the acceptor
- 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.

E depends on the donor-to-acceptor separation distance r with an inverse 6th power 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, i.e. the distance at which the energy transfer efficiency is 50%.[12] 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.[13][14]

$${R_0}^6 = \frac{9\,Q_0\,(\ln 10)\kappa^2\,J}{128\,\pi^5\,n^4\,N_A}$$

where Q_0 is the fluorescence quantum yield of the donor in the absence of the acceptor, κ^2 is the dipole orientation factor, n is the refractive index of the medium, N_A is Avogadro's number, 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 molar extinction coefficient.[15] κ^2 =2/3 is often assumed. This value is obtained when both dyes are freely rotating and can be considered to be isotropically oriented during the excited state lifetime. If either dye is fixed or not free to rotate, then κ^2 =2/3 will not be a valid assumption. In most cases, however, even modest reorientation of the dyes results in enough orientational averaging that κ^2 = 2/3 does not result in a large error in the estimated energy transfer distance due to the sixth power dependence of R_0 on κ^2. Even when κ^2 is quite different from 2/3 the error can be associated with a shift in R_0 and thus determinations of changes in relative distance for a particular system are still valid. Fluorescent proteins do not reorient on a timescale that is faster than their fluorescence lifetime. In this case $0 \le \kappa^2 \le 4$.[15]

The FRET efficiency relates to the quantum yield and the fluorescence lifetime of the donor molecule as follows:[16]

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

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

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

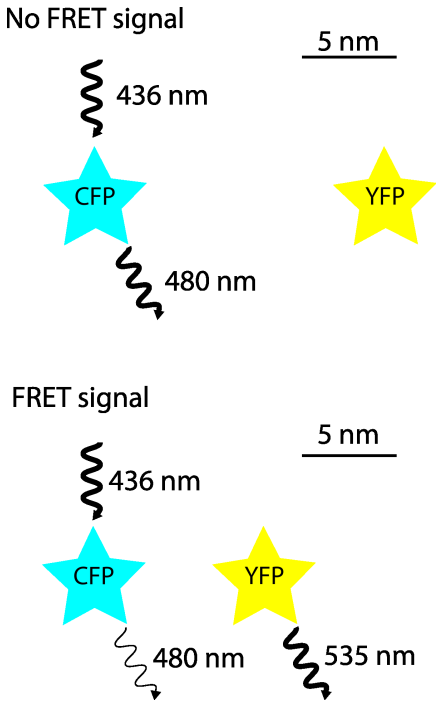
where F'_{D} and F_{D} are the donor fluorescence intensities with and without an acceptor, respectively.

Experimental Confirmation of the Förster resonance energy transfer theory

The inverse sixth power distance dependence of Förster resonance energy transfer was experimentally confirmed by Stryer and Haugland[17] using a donor and an acceptor separated on an oligoproline helix. Haugland, Yguerabide and Stryer[18] also experimentally demonstrated the theoretical dependence of Förster resonance energy transfer on the overlap integral by using a fused indolosteroid as a donor and a ketone as an acceptor.

Methods to measure FRET efficiency

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 and biochemistry, 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. The FRET efficiency is measured and used to identify interactions between the labeled complexes. There are several ways of measuring the FRET efficiency by monitoring changes in the fluorescence emitted by the donor or the acceptor.[19]



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.

Sensitized emission

One method of measuring FRET efficiency is to measure the variation in acceptor emission intensity.[14] When the donor and acceptor are in proximity (1–10 nm) due to the interaction of the two molecules, the acceptor emission will increase 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.

Photobleaching FRET

FRET efficiencies can also be inferred from the photobleaching rates of the donor in the presence and absence of an acceptor.[14] This method can be performed on most fluorescence microscopes; one simply shines the excitation light (of a frequency that will excite the donor but not the acceptor significantly) on specimens with and without the acceptor fluorophore and monitors the donor fluorescence (typically separated from acceptor fluorescence using a bandpass filter) over time. The timescale is that of photobleaching, which is seconds to minutes, with fluorescence in each curve being given by

$$(\text{background}) + (\text{constant}) * e^{-(\text{time})/\tau_{\text{pb}}}$$

where $\tau_{\text{pb}}$is the photobleaching decay time constant and depends on whether the acceptor is present or not. Since photobleaching consists in the permanent inactivation of excited fluorophores, resonance energy transfer from an excited donor to an acceptor fluorophore prevents the photobleaching of that donor fluorophore, and thus high FRET efficiency leads to a longer photobleaching decay time constant:

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

where $\tau'_{\mathrm{pb}}$and $\tau_{\mathrm{pb}}$are the photobleaching decay time constants of the donor in the presence and in the absence of the acceptor, respectively. (Notice that the fraction is the reciprocal of that used for lifetime measurements).
This technique was introduced by Jovin in 1989.[20] Its use of an entire curve of points to extract the time constants can give it accuracy advantages over the other methods. Also, the fact that time measurements are over seconds rather than nanoseconds makes it easier than fluorescence lifetime measurements, and because photobleaching decay rates do not generally depend on donor concentration (unless acceptor saturation is an issue), the careful control of concentrations needed for intensity measurements is not needed. It is, however, important to keep the illumination the same for the with- and without-acceptor measurements, as photobleaching increases markedly with more intense incident light.

Lifetime measurements

FRET efficiency can also be determined from the change in the fluorescence lifetime of the donor.[14] The lifetime of the donor will decrease in the presence of the acceptor. Lifetime measurements of FRET are used in Fluorescence-lifetime imaging microscopy.

Fluorophores used for FRET

CFP-YFP pairs

One common pair fluorophores for biological use is a cyan fluorescent protein (**CFP**) – yellow fluorescent protein (**YFP**) pair.[21] Both are color variants of green fluorescent protein (**GFP**). Labeling with organic fluorescent dyes requires purification, chemical modification, and intracellular injection of a host protein. GFP variants can be attached to a host protein by genetic engineering which can be more convenient.

BRET

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 to photobleaching. To avoid this drawback, Bioluminescence Resonance Energy Transfer (or **BRET**) has been developed.[22] This technique uses a bioluminescent luciferase (typically the luciferase from *Renilla reniformis*) rather than CFP to produce an initial photon emission compatible with YFP.

Homo-FRET

In general, "FRET" refers to situations where the donor and acceptor proteins (or "fluorophores") are of two different types. In many biological situations, however, researchers might need to examine the interactions between two, or more, proteins of the same type—or indeed the same protein with itself, for example if the protein folds or forms part of a polymer chain of proteins[23] or for other questions of quantification in biological cells.[24]

Obviously, spectral differences will not be the tool used to detect and measure FRET, as both the acceptor and donor protein emit light with the same wavelengths. Yet researchers can detect differences in the polarisation between the light which excites the fluorophores and the light which is emitted, in a technique called FRET anisotropy imaging; the level of quantified anisotropy (difference in polarisation between the excitation and emission beams) then becomes an indicative guide to how many FRET events have happened.[25]

Applications

FRET has been used to measure distance and detect molecular interactions in a number systems and has applications in biology and chemistry.[26] FRET can be used to measure distances between domains a single protein and therefore to provide information about protein conformation.[27] FRET can also detect interaction between proteins.[28] Applied in vivio in living cells, FRET has been used to detect the location and interactions of genes and cellular structures including intergrins and membrane proteins.[29] FRET can be used to obtain information about metabolic or signaling pathways.[30] FRET is also used to study lipid rafts in cell membranes.[31]

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

Other methods

A different, but related, mechanism is Dexter Electron Transfer.

An alternative method to detecting protein–protein proximity is the bimolecular fluorescence complementation (BiFC) where two halves of a YFP are fused to a protein. When these two halves meet they form a fluorophore after about 60 s – 1 hr.[32]

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External links

- Browser-based calculator to find the critical distance and FRET efficiency with known spectral overlap (http://www.calctool.org/CALC/chem/photochemistry/fret)
- FRET effect in a thin film (https://www.youtube.com/watch?v=3bmb_oDl6ws) on YouTube
- Free software program to calculate overlap integrals and Förster distances (http://www.setabiomedicals.com/software.php)

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