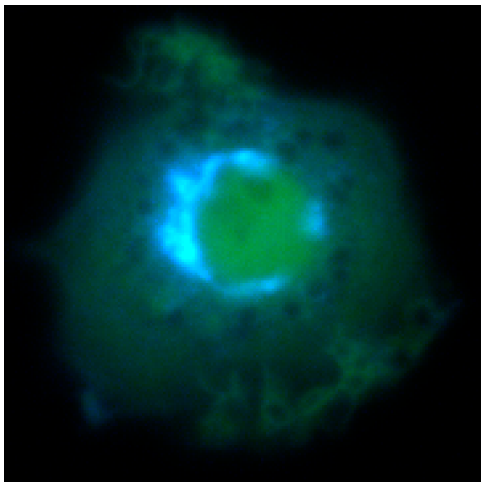
Förster resonance energy transfer



Fluorescently-labeled guanosine 5'-triphosphate hydrolase ARF reveals the protein's localization in the Golgi apparatus of a living macrophage. FRET studies revealed ARF activation in the Golgi and in the formation of phagosomes.[1]

Förster resonance energy transfer (abbreviated **FRET**), also known as **fluorescence resonance energy transfer**, **resonance energy transfer** (**RET**) or **electronic energy transfer** (**EET**), is a mechanism describing energy transfer between two chromophores.

A donor chromophore, intially in its electronic excited state, may transfer energy to an acceptor chromophore (in close proximity, typically <10nm) through nonradiative dipole-dipole coupling. This mechanism is termed "Förster resonance energy transfer" and is named after the German scientist Theodor Förster.[2] When both chromophores are fluorescent, the term "fluorescence resonance energy transfer" is often used instead, although the energy is not actually transferred by fluorescence.[3],[4] In order to avoid an erroneous interpretation of the phenomenon that (even when occurring between two fluorescent chromophores) is always a nonradiative transfer of energy, the name "Förster resonance energy transfer" is preferred to "fluorescence resonance energy transfer" - although the latter enjoys common usage in scientific literature. 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. From quantum electrodynamical calculations, it is determined that radiationless (FRET) and radiative energy transfer are the short- and long-range asymptotes of a single unified mechanism.[5],[6]

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:

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

where k_{ET} is the rate of energy transfer, k_f the radiative decay rate and the k_i are the rate constants of any other de-excitation pathway.
The FRET efficiency depends on many 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%. 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:

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

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. 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 $0 \le \kappa^2 \le 4$.
The FRET efficiency relates to the quantum yield and the fluorescence lifetime of the donor molecule as follows:

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

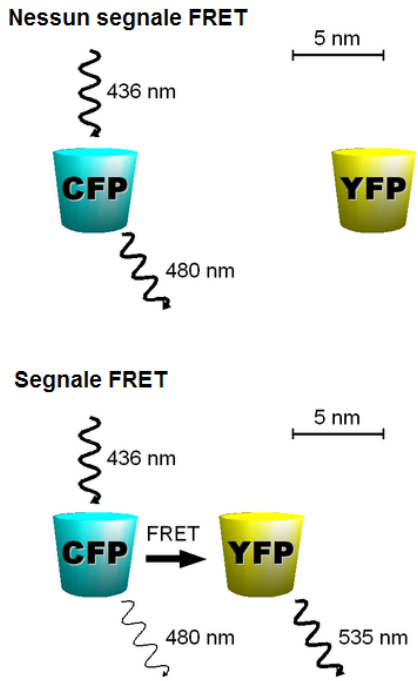
where τ'_D and τ_D are the donor fluorescence lifetimes in the presence and absence of an acceptor, respectively, or as

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

where F'_D and F_D are the donor fluorescence intensities with and without an acceptor, respectively.

Methods

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, 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 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.



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.

FRET studies are scalable: the extent of energy transfer is often quantified from the milliliter scale of cuvette-based experiments to the femtoliter scale of microscopy-based experiments. This quantification can be based directly (sensitized emission method) on detecting two emission channels under two different excitation conditions (primarily donor and primarily acceptor). However, for robustness reasons, FRET quantification is most often based on measuring changes in fluorescence intensity or fluorescence lifetime upon changing the experimental conditions (e.g. a microscope image of donor emission is taken with the acceptor being present. The acceptor is then bleached, such that it is incapable of accepting energy transfer and another donor emission image is acquired. A pixel-based quantification using the second equation in the theory section above is then possible.) An alternative way of temporarily deactivating the acceptor is based on its fluorescence saturation. Exploiting polarisation characteristics of light, a FRET quantification is also possible with only a single camera exposure.

CFP-YFP pairs

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.

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. This technique uses a bioluminescent luciferase (typically the luciferase from *Renilla reniformis*) rather than CFP to produce an initial photon emission compatible with YFP.

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

Photobleaching FRET

FRET efficiencies can also be inferred from the photobleaching rates of the donor in the presence and absence of an acceptor. 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 τ_{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:

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

where τ'_{pb} and τ_{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.[7] 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.

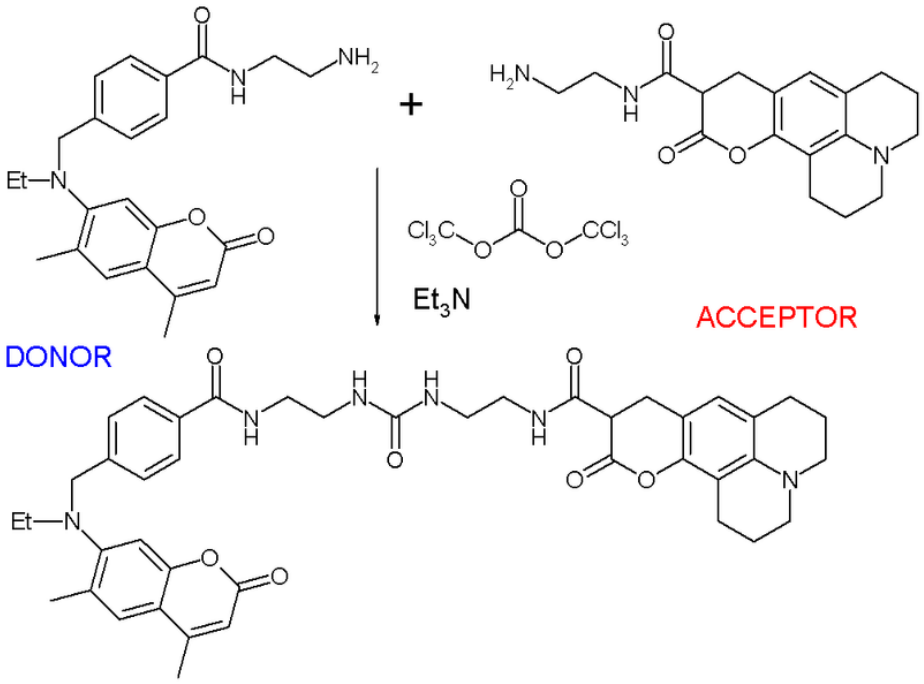
Other methods

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

An alternative method to detecting protein-protein proximity is 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 60 s - 1 hr.

Applications

FRET has been applied in an experimental method for the detection of phosgene. In it, phosgene or rather triphosgene as a safe substitute serves as a linker between an acceptor and a donor coumarine (forming urea groups).[8] The presence of phosgene is detected at $5x10^{-5}$M with a typical FRET emission at 464 nm.



MISTAKE: The chromophore on the right must be also coumarine (double bond is missing)

FRET is also used to study lipid rafts in cell membranes.[9]

External links

- Browser-based calculator to find the critical distance and FRET efficiency with known spectral overlap [10]
- FCS[11] [12] [13] [14] [15] .

References

[1] *Inconspicuous Consumption: Uncovering the Molecular Pathways behind Phagocytosis.* Inman M, PLoS Biology Vol. 4/6/**2006**, e190. doi: 10.1371/journal.pbio.0040190 (http://dx.doi.org/10.1371/journal.pbio.0040190)

[2] Förster T., Zwischenmolekulare Energiewanderung und Fluoreszenz, *Ann. Physik* **1948**, *437*, 55. doi: 10.1002/andp.19484370105 (http://dx.doi.org/10.1002/andp.19484370105)

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

[4] FRET microscopy tutorial from Olympus (http://www.olympusfluoview.com/applications/fretintro.html)

[5] D. L. Andrews, "A unified theory of radiative and radiationless molecular energy transfer", *Chem. Phys.* **1989**, 135, 195-201. doi: 10.1016/0301-0104(89)87019-3 (http://dx.doi.org/10.1016/0301-0104(89)87019-3)

[6] D. L. Andrews and D. S. Bradshaw, "Virtual photons, dipole fields and energy transfer: A quantum electrodynamical approach", *Eur. J. Phys.* **2004**, 25, 845-858. doi: 10.1088/0143-0807/25/6/017 (http://dx.doi.org/10.1088/0143-0807/25/6/017)

[7] Jovin, T.M. and Arndt-Jovin, D.J. FRET microscopy: Digital imaging of fluorescence resonance energy transfer. Application in cell biology. In Cell Structure and Function by Microspectrofluometry, E. Kohen, J. G. Hirschberg and J. S. Ploem. London: Academic Press, 1989. pp. 99-117.

[8] *A FRET approach to phosgene detection* Hexiang Zhang and Dmitry M. Rudkevich Chem. Commun., **2007**, 1238 - 1239, doi: 10.1039/b614725a (http://dx.doi.org/10.1039/b614725a)

[9] Silvius, J.R. and Nabi, I.R. Fluorescence-quenching and resonance energy transfer studies of lipid microdomains in model and biological membranes. (Review) *Molec. Membr. Bio.* **2006**, *23*, 5-16. doi: 10.1080/09687860500473002 (http://dx.doi.org/10.1080/09687860500473002)

[10] http://www.calctool.org/CALC/chem/photochemistry/fret

[11] Diaspro, A., and Robello, M. (1999). Multi-photon Excitation Microscopy to Study Biosystems. European Microscopy and Analysis., 5:5-7.

[12] Bagatolli, L.A., and Gratton, E. (2000). Two-photon fluorescence microscopy of coexisting lipid domains in giant unilamellar vesicles of binary phospholipid mixtures. Biophys J., 78:290-305.

[13] Schwille, P., Haupts, U., Maiti, S., and Webb. W.(1999). Molecular dynamics in living cells observed by fluorescence correlation spectroscopy with one- and two- photon excitation. *Biophysical Journal*, **77**(10):2251-2265.

[14] Near Infrared Microspectroscopy, Fluorescence Microspectroscopy,Infrared Chemical Imaging and High Resolution Nuclear Magnetic Resonance Analysis of Soybean Seeds, Somatic Embryos and Single Cells., Baianu, I.C. et al. 2004., In *Oil Extraction and Analysis.*, D. Luthria, Editor pp.241-273, AOCS Press., Champaign, IL.

[15] Single Cancer Cell Detection by Near Infrared Microspectroscopy, Infrared Chemical Imaging and Fluorescence Microspectroscopy.2004.I. C. Baianu, D. Costescu, N. E. Hofmann and S. S. Korban, q-bio/0407006 (July 2004) (http://arxiv.org/abs/q-bio/0407006)

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