Förster resonance energy transfer

Fluorescence resonance energy transfer (FRET = Fluorescence Resonance Energy Transfer or Förster resonance energy transfer) is a physical phenomenon describing the transfer of energy between two fluorophores. With appropriately chosen fluorophores (need to overlap the emission spectrum of the donor and the absorption spectrum of the acceptor), the donor, which is in an excited state, can transfer energy to the acceptor with the help of a non-radiative dipole - dipole interaction. The result is an excited acceptor molecule that then emits the energy originally absorbed by the donor. In FRET, there is no emission of a photon by the donor, so it is a non-radiative energy transfer. The efficiency of this transfer is inversely proportional to the sixth power of the distance between the donor and the acceptor, making FRET extremely sensitive to small distances.

Determining the FRET efficiency makes it possible to distinguish whether two fluorophores are within a certain distance of each other. This is what biological and chemical research uses.

Terminology

Förster resonance energy transfer is named after the German scientist Theodor Förster. Since both fluorophores are fluorescent substances, the term "Fluorescence Resonance Energy Transfer" is often used, even though energy is not actually transferred by fluorescence. In order to avoid misinterpretation of this phenomenon, the essence of which is non-radiative energy transfer, the term "Förster resonance energy transfer" is preferred over "Flourescence resonance energy transfer", however, in the scientific literature we can meet the latter more often.

Theory

Resonant energy transfer is characterized by a rate constant (kDA), which expresses the probability of transfer; the determining component is the dipole–dipole energy transfer, for which the Förster formula was derived (in the case of weak coupling, when the mutual interaction of the donor and acceptor does not affect the optical spectra) e.g. in this form:

$k_{DA} = (1/\tau_D)\ (R_0/r)^6$

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

Resonant energy transfer is therefore strongly dependent on the donor-acceptor distance.

Energy transfer efficiency

The efficiency of energy transfer is determined by the relative amount of photons that are absorbed by the donor and subsequently emitted by the acceptor.

$$E = \frac{K_{DA}(r)}{\tau D^{-1} + K_{DA}(r)}$$

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

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

It is measured by determining the relative fluorescence intensity of the donor in the absence (F_D) and in the presence of the acceptor (F_{DA}). It decreases rapidly with the 6th power of distance.

Transmission efficiency is determined by many parameters that can be summarized in these points:

- distance between donor and acceptor (typically in the range of 1–10 nm)
- spectral overlap of the emission spectrum of the donor and the absorption spectrum of the acceptor
- relative orientation of the emission dipole moment of the donor and the absorption dipole moment of the acceptor

Förster distance (R_0)

R_0 is the donor-acceptor distance at which the energy transfer efficiency is 50%. The size of R_0 is usually 2–6 nm, which is comparable to the size of biological molecules. The dependence of the transfer efficiency E on the distance r is most pronounced (fastest changing) when the donor-acceptor distance is close to R_0. R_0 indicates the average distance to which a given donor-acceptor pair can communicate - it is a characteristic of a pair of fluorophores.

Advantages of FRET

- The energy transfer efficiency does not depend on the environment between the donor and the acceptor
- Intensity ratio measurement allows FRET analysis to be used independent of concentration
- For most applications it is not necessary to know R_0

Use

FRET is used to measure distance and detect the interaction of molecules, and thus has many applications in biological and chemical research. It is used to measure the distance between the protein's own domains and thus provide information about its conformation (it enables, for example, the monitoring of protein denaturation changes, protein folding). It is also used to detect interactions between proteins. In vivo in cells, FRET is used to localize and detect the interaction of genes and factors regulating their expression. Furthermore, it is possible to use this phenomenon to investigate metabolic and signaling pathways. It is also widely used to study lipid rafts in cell membranes and to investigate the spatial distribution of membrane receptors. FRET is also a common tool in the study of enzyme reaction kinetics and molecular motors.

Reference

Resources

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