

Science Education Collection

Förster Resonance Energy Transfer (FRET)

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Abstract

Förster resonance energy transfer (FRET) is a phenomenon used to investigate close-range biochemical interactions. In FRET, a donor photoluminescent molecule can non-radiatively transfer energy to an acceptor molecule if their respective emission and absorbance spectra overlap. The amount of energy transferred—and consequently the overall emission of sample—depends on the proximity of an acceptor-donor pair of photoluminescent molecules. FRET analysis is combined with other biochemistry techniques to obtain detailed information of biomolecular structures and interactions from this “spectroscopic ruler.”

This video covers the principles and concepts of FRET analysis. The procedure focuses on preparing samples for FRET and ways to present and interpret data. Finally, the applications include monitoring conformational and cellular processes by labeling parts of a cell or protein, monitoring enzyme reactions that alter protein structures, and using FRET to monitor aggregation of monomers expressed by cells.

Förster Resonance Energy Transfer, or FRET, is a non-radiative transfer of energy between light-emitting molecules, and is often used to investigate close-range biochemical interactions. FRET only occurs when fluorescent molecules are spaced within 10 nm of each other. FRET analysis can be combined with other techniques to obtain detailed structural information. This video will introduce the underlying principles of FRET, summarize a protocol and data presentation, and discuss some biochemical applications.

A photoluminescent molecule such as a fluorophore is excited by absorbing electromagnetic radiation at a wavelength in its absorption spectrum. As it relaxes, it emits light at a wavelength within its emission spectrum. For more information about fluorescence, see JoVE's video on fluorescence microscopy. Different fluorophores absorb and emit light at different wavelengths, which frequently overlap. If the emission spectrum of a fluorophore overlaps significantly with the absorption spectrum of another fluorophore, the “donor” will release a virtual photon, which is absorbed by the “acceptor”. When an excited donor is within 10 nm of an acceptor, energy is transferred from donor to acceptor by dipole-dipole interactions. The release of energy by emission of light from the donor correspondingly decreases. Meanwhile, the excited acceptor emits light at its emission wavelength. The FRET response is evaluated in terms of efficiency, or the percentage of energy released from the donor by FRET rather than by fluorescence or other radiative processes. The efficiency depends strongly on the distance between the donor and acceptor, which allows FRET to act as a ‘molecular’ or ‘spectroscopic’ ruler.

In biochemistry, FRET is often used qualitatively to observe conformational changes in molecules by monitoring fluorophores as they move in and out of FRET range of each other. Similarly, cellular functions can be studied with molecules containing a FRET pair. If the labeled molecule is cleaved by enzyme activity, FRET stops and the observed fluorescence wavelength changes.

Now that you understand the principles behind FRET, let's look at an overview of a protocol and a few ways to present and interpret the data.

Prior to the experiment, the biomolecules of interest, typically DNA or proteins, are engineered with fluorescent tags, using molecular biology techniques. Common ways to introduce the modified genetic material into the cells include transfection and electroporation.

Then, the cells are prepared for FRET visualization on a fluorescence microscope. For instance, the molecules may be immobilized on a slide for single-molecule FRET, or samples are loaded into wells for high-throughput screening.

Then, the excitation lasers, microscope, and associated equipment are prepared. (A) FRET experiments often involve powerful lasers; (B) so appropriate PPE and safety procedures should be used. The sample is then placed in the instrument and illuminated with the excitation laser.

For experiments monitoring cell behavior, color images showing differences or changes in emission intensity are used. Donor and acceptor emission intensities are plotted together to track FRET response over time.

FRET data can also be fitted to various functions for more complex analyses. Depending on the experiment, data may be presented in multiple ways to best represent the results, making FRET a flexible experimental tool.

Now that you're familiar with the basics of running and analyzing a FRET experiment, let's look at some applications of FRET in biochemistry research.

FRET can be used to study conformational changes or cellular processes by labeling parts of the protein or cell predicted to move within 10 nm of each other with a FRET pair. For example, protein sensors are prepared by labeling receptors with a pair of fluorophores. The FRET response is monitored live by confocal microscopy. Variation of emission wavelength and intensity indicate conformational changes.

FRET can also be used by preparing molecules with an active FRET pair and observing changes in the response. When the substrate is cleaved, FRET is disrupted, causing an increase in donor emission and a decrease in acceptor emission. The emissions are analyzed to determine contributions by donor, acceptor, and FRET. Once the direct emission factors are calculated for the cyan and yellow fluorescent proteins, the concentration and kinetic parameters of the substrate can be determined.

Cells designed to express monomers containing either of a FRET pair function as ‘sensors’ for interactions between those monomers. If aggregation of those monomers is induced, a FRET response is observed. This can be used to investigate protein aggregation triggered by ‘seeding’ of misfolded proteins. Here, cells were transduced with aggregates of the protein of interest, incubated, and analyzed with flow cytometry.

You've just watched JoVE's video on Förster Resonance Energy Transfer, or FRET. This video contained the underlying principles of FRET, preparation and analysis of a FRET experiment, and a few biochemical applications.

Thanks for watching!