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Fluorescence Resonance Energy Transfer (FRET)

Fluorescence resonance energy transfer (FRET) is a distance-dependent interaction between the electronic excited states of two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule *without emission of a photon*. The efficiency of FRET is dependent on the inverse sixth power of the intermolecular separation,[REF] making it useful over distances comparable with the dimensions of biological macromolecules. Thus, FRET is an important technique for investigating a variety of biological phenomena that produce changes in molecular proximity.[REF] When FRET is used as a contrast mechanism, colocalization of proteins and other molecules can be imaged with spatial resolution beyond the limits of conventional optical microscopy.[REF]

Primary Conditions for FRET

- Donor and acceptor molecules must be in close proximity (typically 10–100 Å).
- The absorption spectrum of the acceptor must overlap the fluorescence emission spectrum of the donor (see **Figure**).
- Donor and acceptor transition dipole orientations must be approximately parallel.

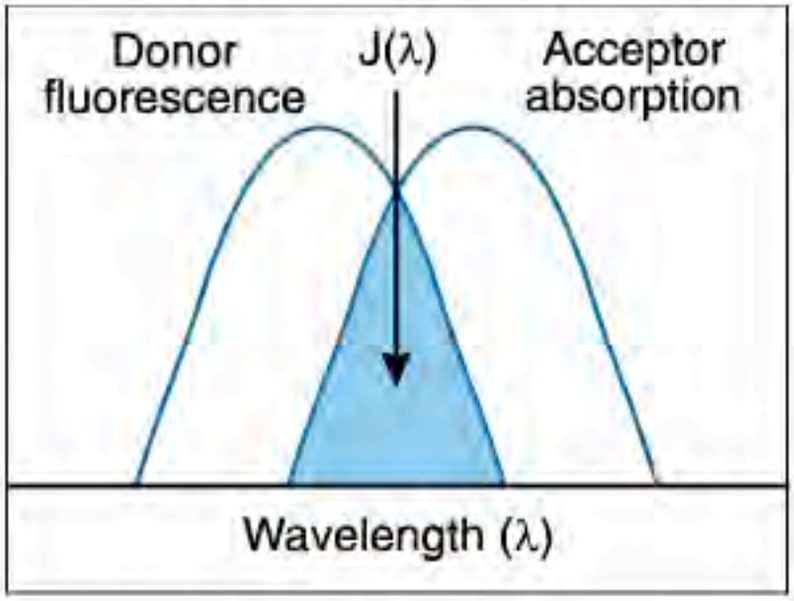

Figure. Schematic representation of the FRET spectral overlap integral.

Förster Radius

The distance at which energy transfer is 50% efficient (i.e., 50% of excited donors are deactivated by FRET) is defined by the Förster radius (R_0). The magnitude of R_0 is dependent on the spectral properties of the donor and acceptor dyes (see **Table**):

$$R_0 = [8.8 \times 10^{23} \cdot \kappa^2 \cdot n^{-4} \cdot QY_D \cdot J(\lambda)]^{1/6} \text{ Å}$$

where κ^2 = dipole orientation factor (range 0 to 4; $\kappa^2 = 2/3$ for randomly oriented donors and acceptors)
QY_D = fluorescence quantum yield of the donor in the absence of the acceptor
n = refractive index
$J(\lambda)$ = spectral overlap integral (see figure)
$= \int \epsilon_A(\lambda) \cdot F_D(\lambda) \cdot \lambda^4 d\lambda \text{ cm}^3\text{M}^{-1}$

where ϵ_A = extinction coefficient of acceptor
F_D = fluorescence emission intensity of donor as a fraction of the total integrated intensity

Table. Typical Values of R_0.

Donor	Acceptor	R_0 (Å)
Fluorescein	Tetramethylrhodamine	55
IAEDANS	Fluorescein	46
EDANS	Dabcyl	33
Fluorescein	Fluorescein	44
BODIPY FL	BODIPY FL	57
Fluorescein	QSY 7 and QSY 9 dyes	61

Donor/Acceptor Pairs

In most applications, the donor and acceptor dyes are different, in which case FRET can be detected by the appearance of sensitized fluorescence of the acceptor or by quenching of donor fluorescence. When the donor and acceptor are the same, FRET can be detected by the resulting fluorescence depolarization. REF Some typical values of R_0 are listed in the table above and a more extensive compilation is in Table 1.4 and Table 1.10. Note that because the component factors of R_0 (see above) are dependent on the environment, the actual value observed in a specific experimental situation is somewhat variable. Extensive compilations of R_0 values can be found in the literature. REF Nonfluorescent acceptors such as dabcyl and our QSY dyes (Table 1.9) have the particular advantage of eliminating the potential problem of background fluorescence resulting from direct (i.e., nonsensitized) acceptor excitation. FRET efficiencies from several donor dyes to the QSY 7 quencher in molecular beacon hybridization probes have been calculated. REF Probes incorporating fluorescent donor–nonfluorescent acceptor combinations have been developed primarily for detecting proteolysis REF (Figure 10.9) and nucleic acid hybridization REF (Figure 8.109, Figure 8.110).

Selected Applications of FRET

- Structure and conformation of proteins REF
- Spatial distribution and assembly of protein complexes REF
- Receptor/ligand interactions REF
- Immunoassays REF
- Probing interactions of single molecules REF
- Structure and conformation of nucleic acids REF
- Real-time PCR assays and SNP detection REF (Figure 8.111, Figure 8.112, Figure 8.113)
- Detection of nucleic acid hybridization REF (Figure 8.109)
- Primer-extension assays for detecting mutations REF (Figure 8.112)
- Automated DNA sequencing REF
- Distribution and transport of lipids REF
- Membrane fusion assays REF (Lipid-Mixing Assays of Membrane Fusion)
- Membrane potential sensing REF
- Fluorogenic protease substrates REF
- Indicators for cyclic AMP REF and zinc REF