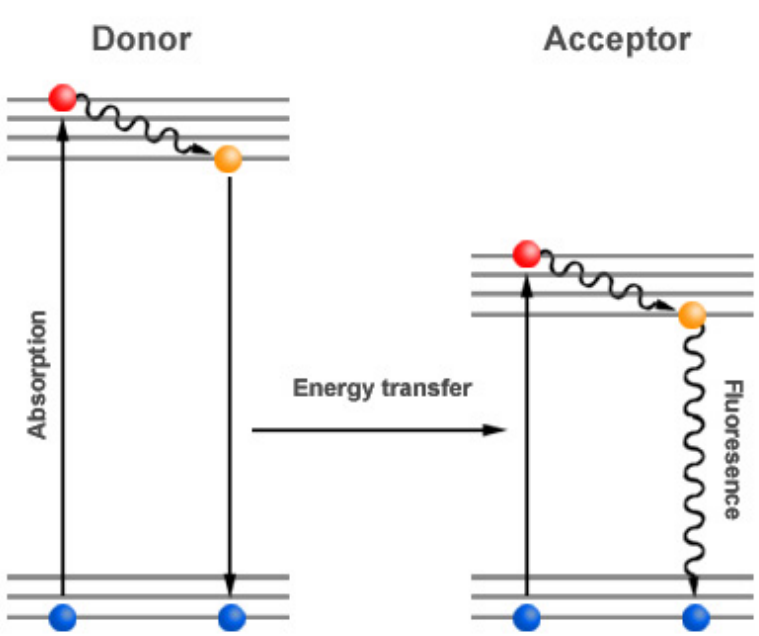
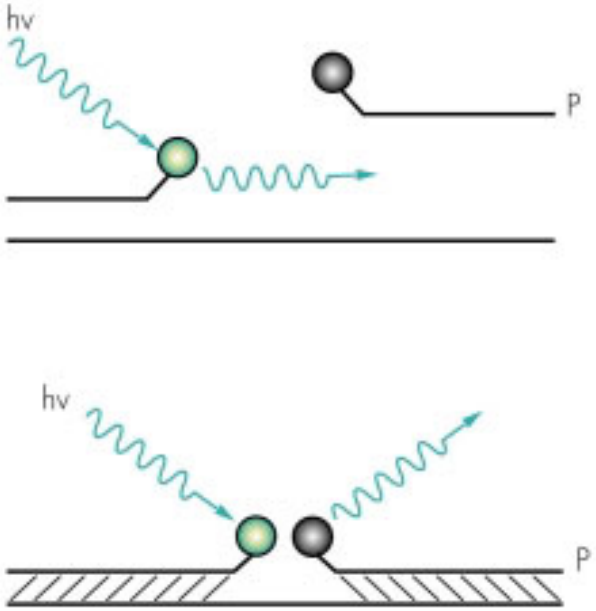
Fluorescence Resonance Energy Transfer (FRET)





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 (R0).

Applications of FRET in Biology

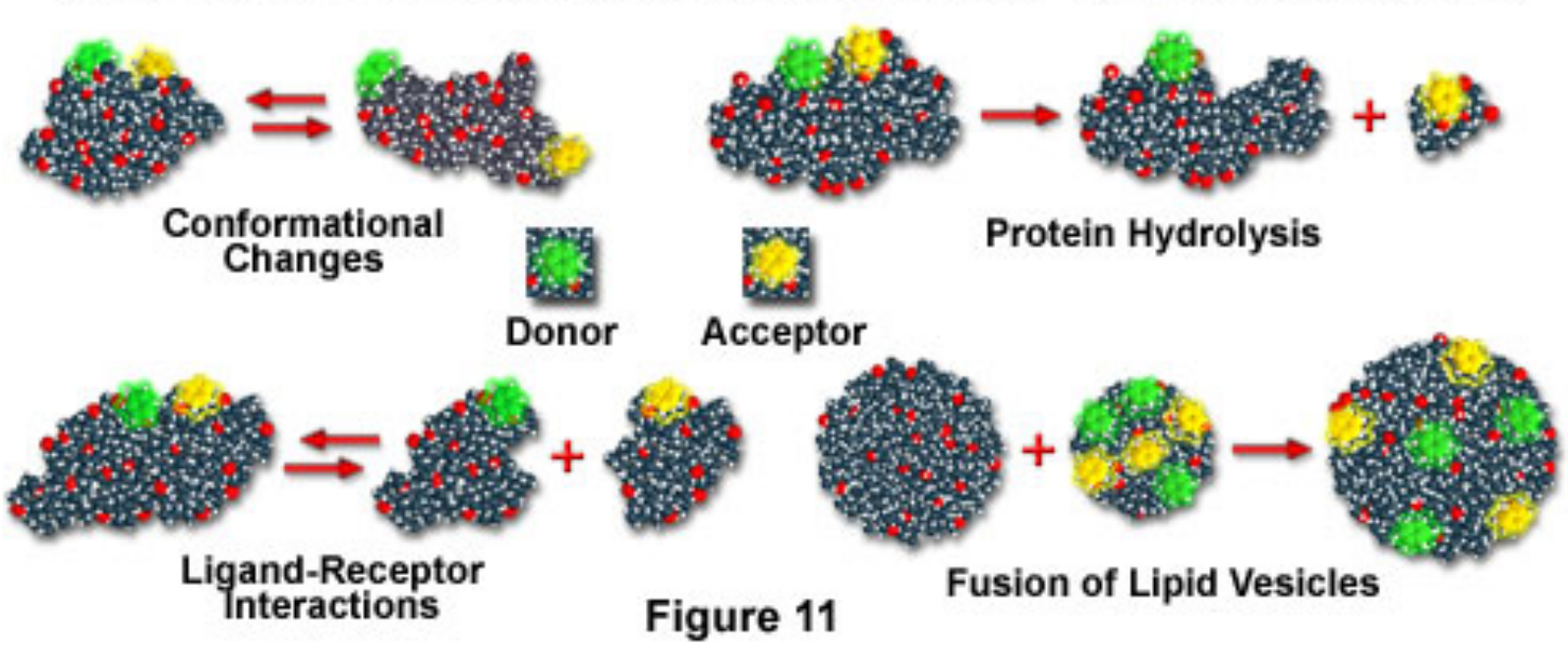
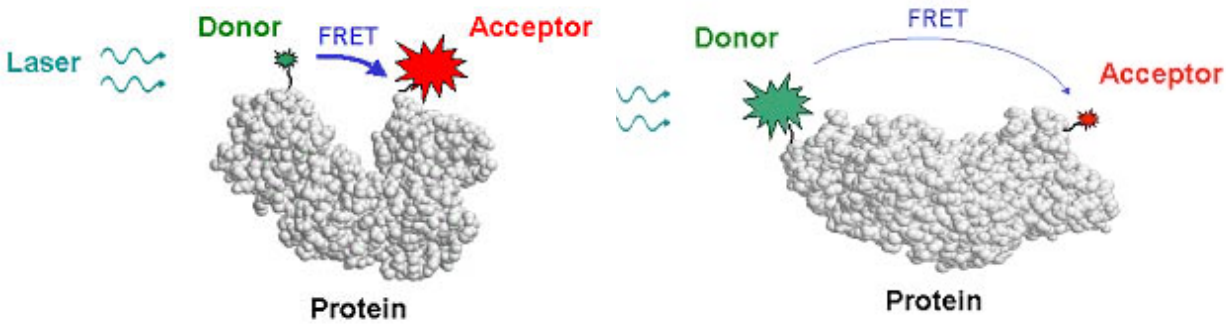


Figure 11

Survey of FRET-Based Assays

- Protease activity
- Calcium Ion measurements
- cAMP
- Protein tyrosine kinase activity
- Phospholipase C activity
- Protein kinase C activity
- Membrane potential

FRET probes conformational changes



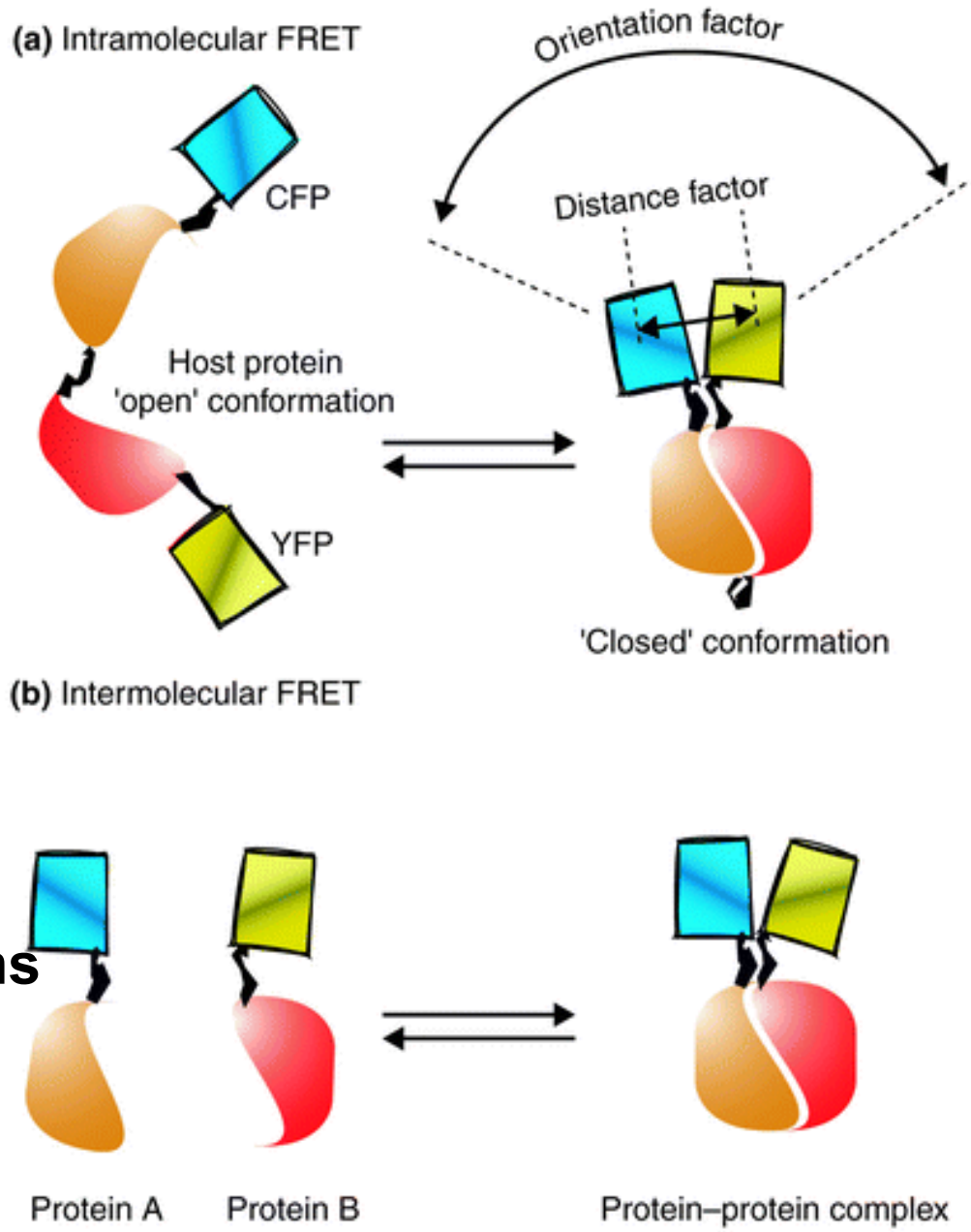
Different conformation gives
Different FRET signature

Inter and Intramolecular Forms of FRET with Proteins

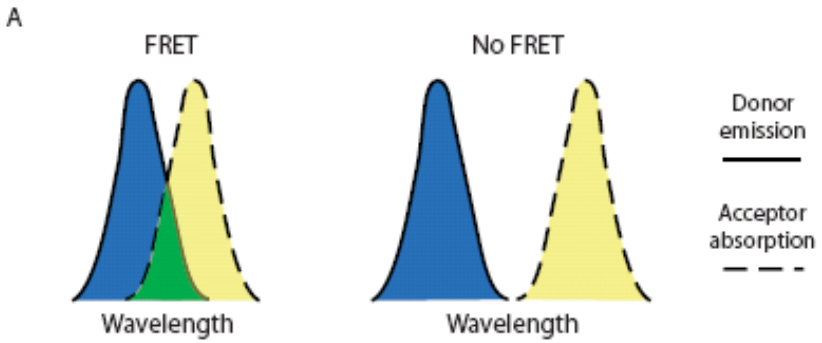
CFP-YFP good combo

FRET increases
In both cases

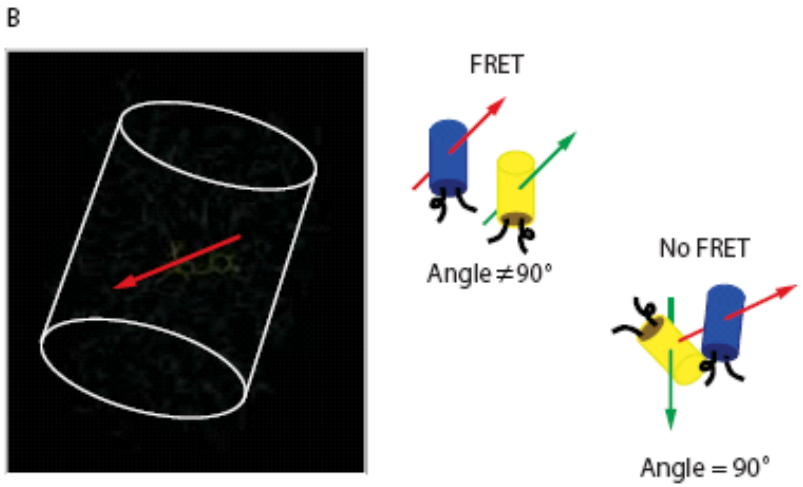
Protein-Protein Interactions
In cytoplasm and membranes



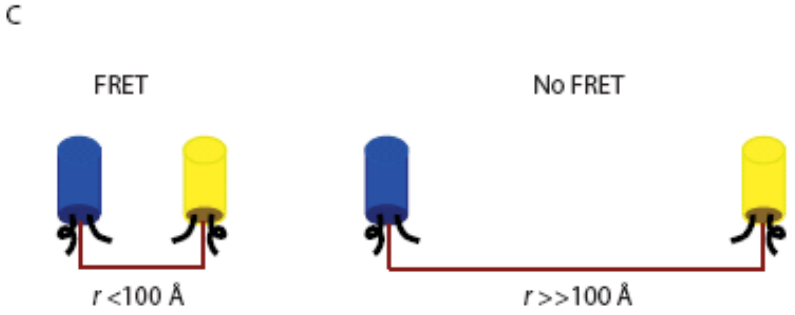
When FRET Occurs



No FRET for
No overlap of donor emission, acceptor absorption



No FRET for
Orthogonal dipole orientation



No FRET for molecules more than 10 nm apart

Number of FRET Publications since 1989

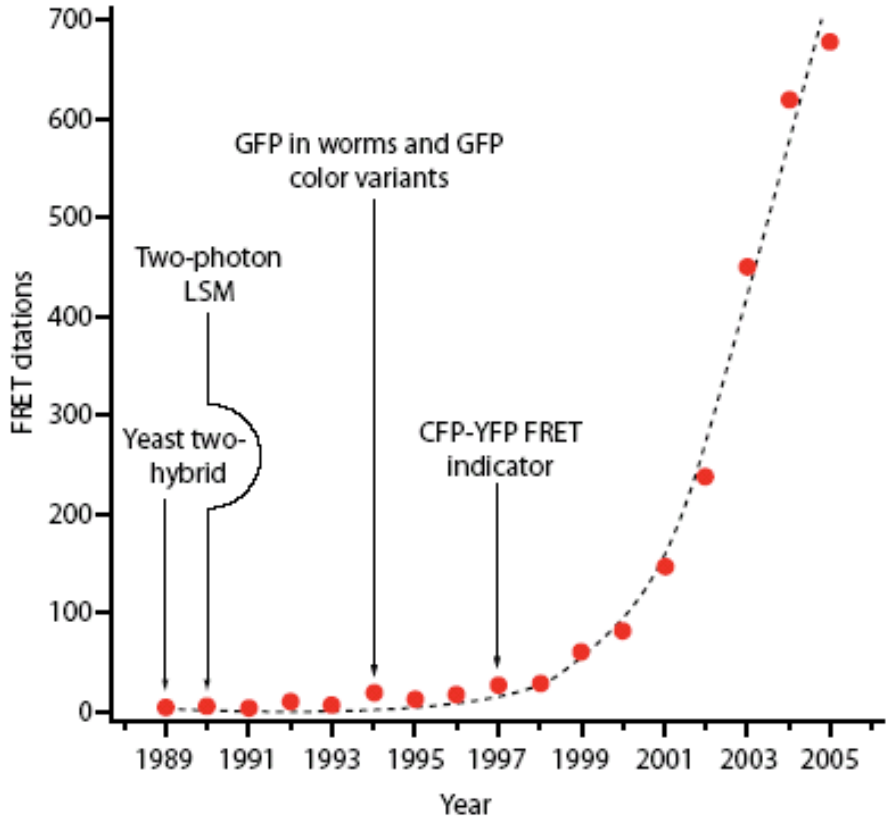
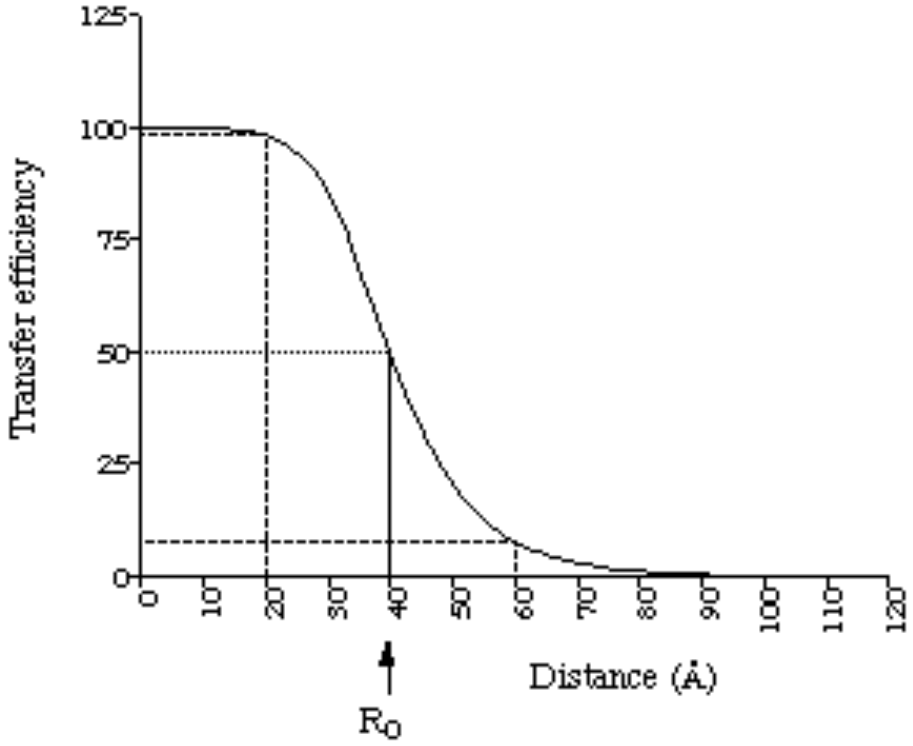


Fig. 1. The growth of PubMed (http://www.pubmedcentral.nih.gov/) citations containing the keyword "FRET" in the title or abstract over the past 16 years. Dates of important technological developments relevant to FRET are indicated with arrows.

Fluorescence Resonance Energy Transfer - Detection of Probe Proximity

$$FRET = \frac{F_D^0 - F_D}{F_D^0} = \frac{F_A - F_A^0}{F_A^{Max} - F_A^0} = \frac{\tau_D^0 - \tau_D}{\tau_D^0}$$



R_0 typically 40-50 Angstroms

50% transfer

$$FRET = \frac{R_0^6}{R^6 + R_0^6}$$

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

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

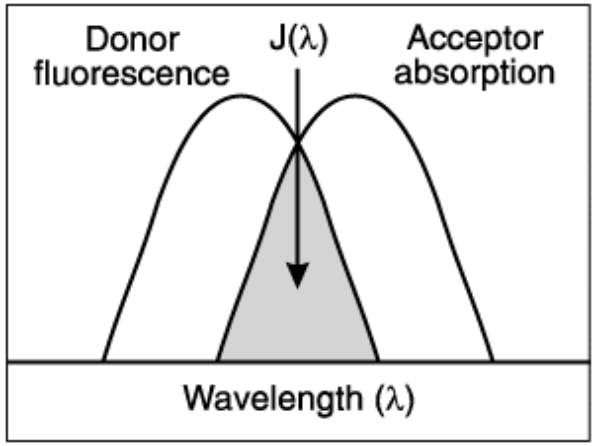
n = refractive index

$J(\lambda)$ = spectral overlap integral (see figure)

$$= \int \epsilon_A(\lambda) \bullet F_D(\lambda) \bullet \lambda^4 d\lambda \text{ cm}^3\text{M}^{-1}$$

where ϵ_A = extinction coefficient of acceptor

F_D = fluorescence emission intensity of donor as a fraction of the total integrated intensity



Typical Values of R_o

green

Donor	Acceptor	R_o (Å)
Fluorescein	Tetramethylrhodamine	55
IAEDANS	Fluorescein	46
EDANS	DABCYL	33
Fluorescein	Fluorescein	44
BODIPY FL	BODIPY FL	57
Fluorescein	QSY 7 dye	61
Cy3	Cy5	53
CFP	YFP	50

red

GFPs and other colored "FPs have transformed FRET microscopy

FRET Considerations:

1. Spectral overlap
2. Chromophore orientations
3. Distance dependence (Eff. ~ $1/R^6$)
4. How to quantify?

$$E = \frac{f_{acceptor}}{f_{donor}}$$

$$E = \frac{f_{acceptor} - f_{baseline} - f_{A-spillover}}{f_{donor} - f_{D-spillover}}$$

Practical Challenges to FRET Quantitation

- Emission from A contaminates D channel (filters)
- Emission from D contaminates A channel
- Unknown labeling levels for D and A
- Signal variation due to bleaching
  - Complicates kinetic studies
  - Bleaching rate of D can actually be slowed by FRET

Solutions:

- Separately labeled D and A controls to define bleedthrough
- Acceptor destruction by photobleaching to establish F_D^0
- Dual wavelength ratio imaging to normalize away variations in label levels and bleaching effects