

Fluorescence resonance/Förster energy transfer (FRET) is the radiationless transfer of energy between two molecules, which can occur if they are very close to each other ( $< 10$  nm), see Fig.1.

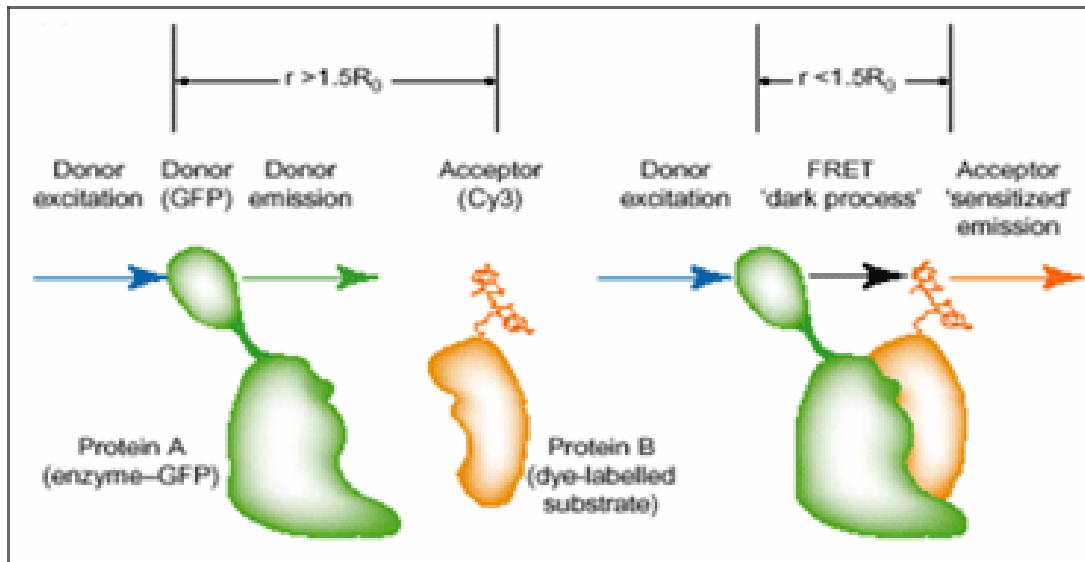


Fig.1 The acceptor and donor fluorophores must be closer than  $\sim 10$  nm for the energy transfer to occur (adopted from P. I.H. Bastiaens and R. Pepperkok, TIBS 25 (2000) 631).

Therefore FRET makes it possible to measure if two molecules, for example a ligand and a receptor, interact with each other. For FRET to happen the fluorescence emission spectrum of the donor has to overlap with the adsorption spectrum of the acceptor and the donor, see Fig. 2, and the transition dipole orientations must be approximately parallel.

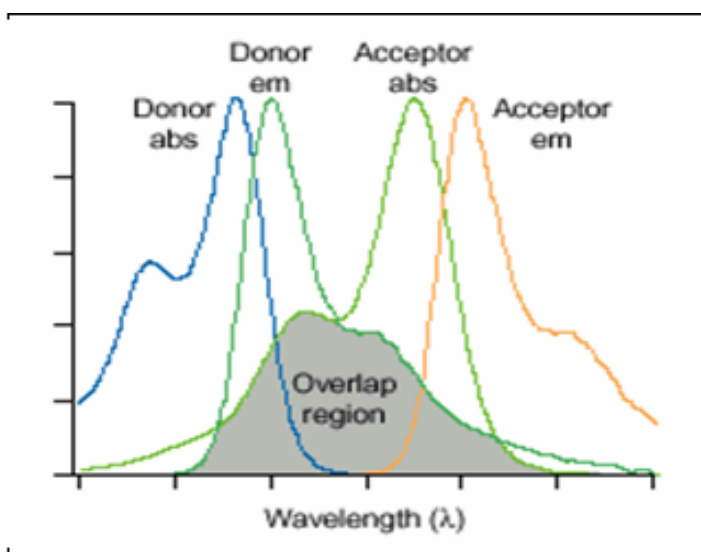
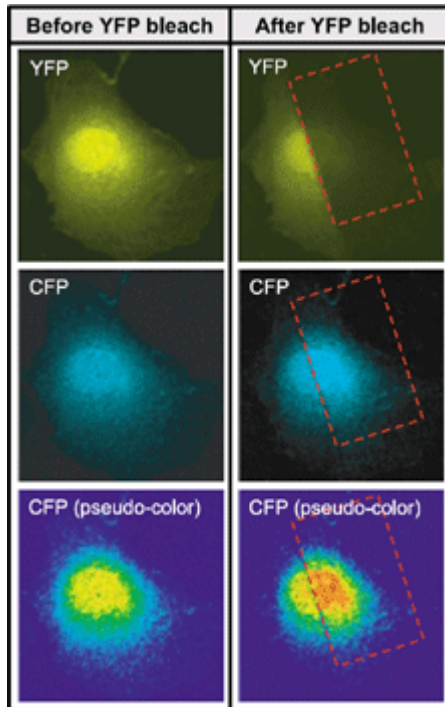


Fig. 2 The emission spectrum of the donor must overlap with the excitation spectrum of the acceptor (adopted from P. I.H. Bastiaens and R. Pepperkok, TIBS 25 (2000) 631).

The transfer can be measured by looking at the quenching of fluorescence of the donor in presence and absence of the acceptor, see Fig. 3 for an example of the acceptor bleaching method, or by excitation of the donor and then looking at the fluorescence of the acceptor.



*Fig. 3 An example of the acceptor bleaching FRET technique with CFP as the donor and YFP as the acceptor (R. Ankerhold, *Imaging & Microscopy* 2 (2001) 13-15).*