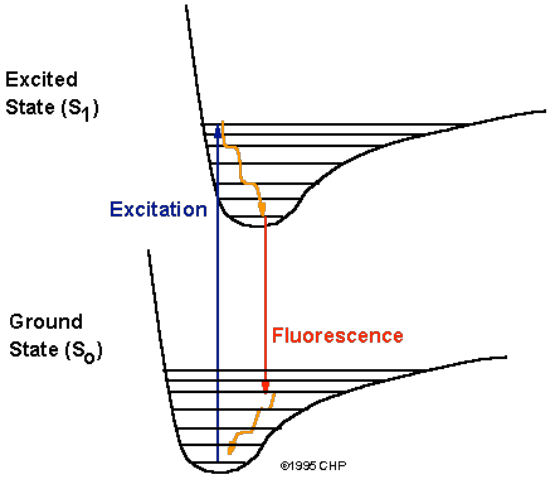
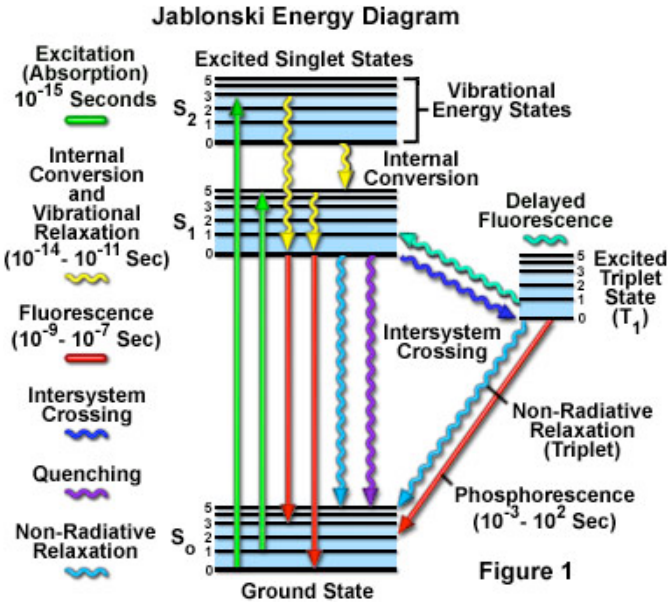
Fluorescence



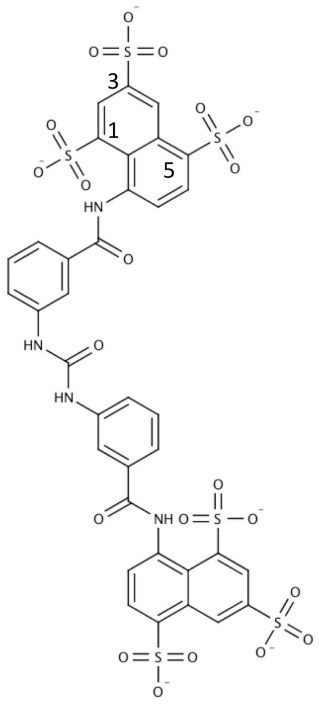


Trp fluorescence

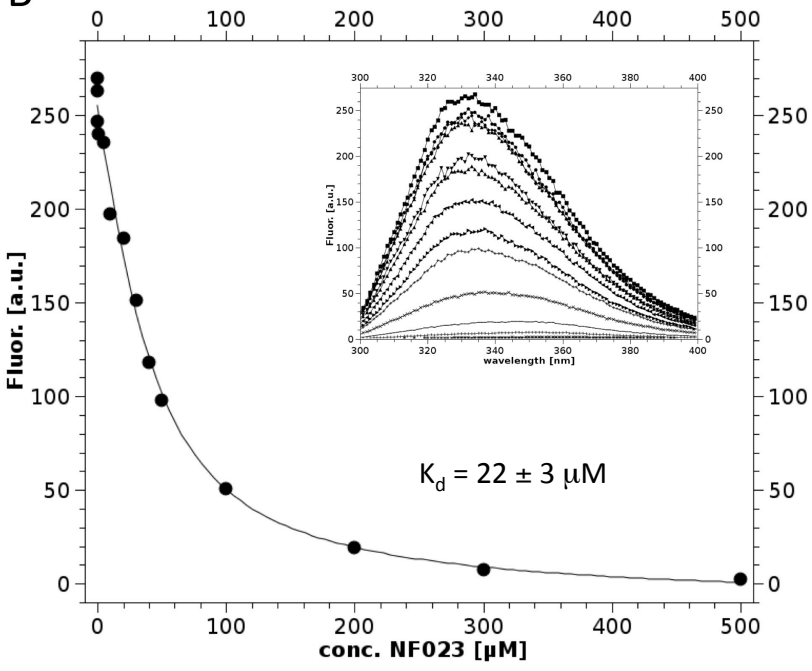
Ass=280 nm; Emiss=340 nm

$$F = M - \frac{(M-m)}{[P_T]}[PI]$$

A



B



K_d = cost. di dissociazione

$$F = M - \frac{(M-m)}{[P_T]}[PI] \qquad P + I \Leftrightarrow PI \qquad K_d = \frac{[I][P]}{[PI]}$$

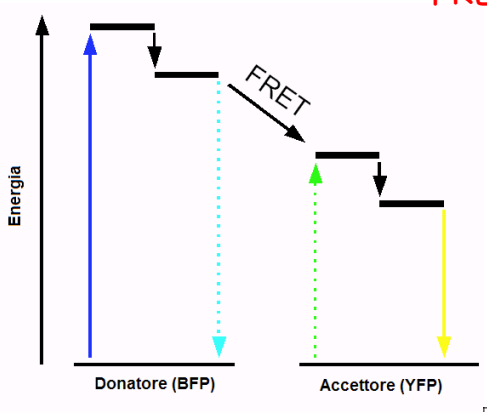
$$\begin{cases} [I_T] = [I] + [PI] \\ [P_T] = [P] + [PI] \end{cases} \Rightarrow \begin{cases} [I] = [I_T] - [PI] \\ [P] = [P_T] - [PI] \end{cases}$$

$$K_d = \frac{[I][P]}{[PI]} \qquad [PI]K_d = ([I_T] - [PI])([P_T] - [PI]) = [PI]^2 - [PI]([I_T] + [P_T]) + [I_T][P_T]$$

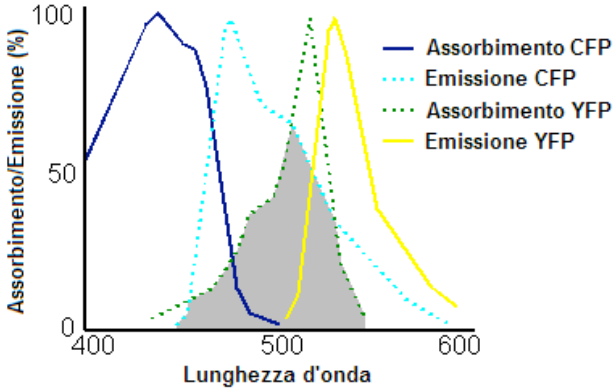
$$[PI]^2 - [PI]([I_T] + [P_T] + K_d) + [I_T][P_T] = 0$$

$$[PI] = \frac{([I_T] + [P_T] + K_d) - \sqrt{([I_T] + [P_T] + K_d)^2 - 4[I_T][P_T]}}{2}$$

FRET

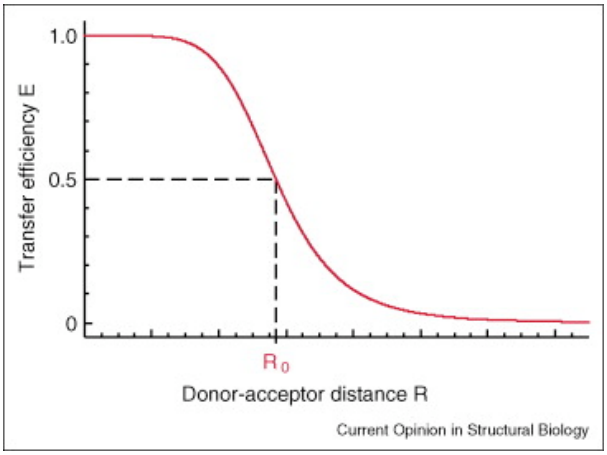


K_f decadimento radiativo



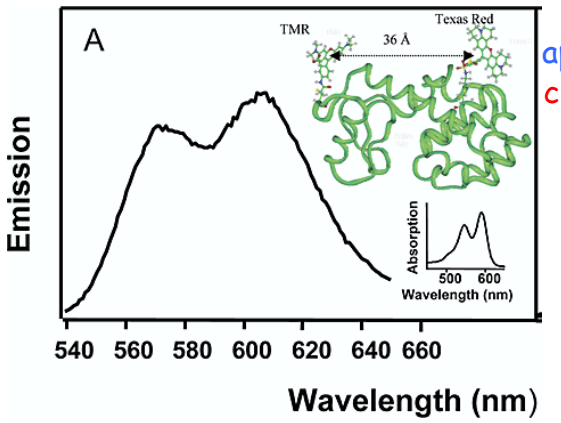
efficienza FRET

$$E = \frac{1}{\left(1 + (R/R_0)^6\right)}$$

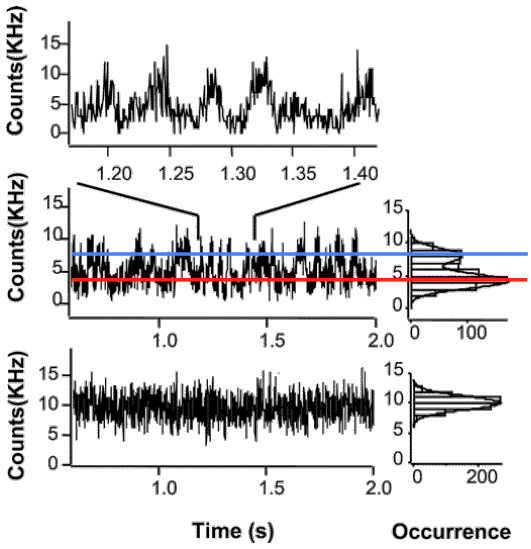


Probing Single-Molecule T4 Lysozyme Conformational Dynamics by Intramolecular Fluorescence Energy Transfer

Fluorescence spectra of tetramethylrhodamine- and Texas Red-labeled T4 lysozyme excited at 530 nm.



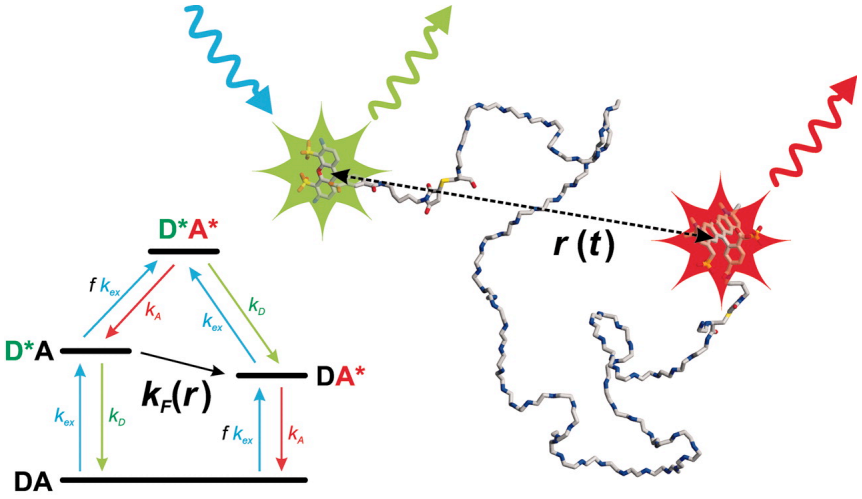
aperto
chiuso



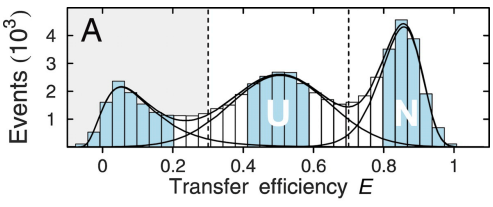
The upper panel shows donor fluorescence of a donor–acceptor-labeled single T4 lysozyme. The lower panel shows a portion of a trajectory recorded from a donor-only-labeled enzyme.

Yu Chen et al., *J. Phys. Chem. B*, 2003, **107**, 7947–7956

Ultrafast dynamics of protein collapse from single-molecule photon statistics



(*A*) Transfer efficiency histogram fit with three peaks corresponding to the native (**N**; high E) and unfolded (**U**; E ≈ 0.5) subpopulations, and molecules lacking an active acceptor (E ≈ 0).



Nettels et al., *Proc Natl Acad Sci U S A.* 2007, **104**, 2655-60.