Supporting Information

Diffusion-enhanced FRET and the Effect of External Quenchers and the Donor Quantum Yield

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1.1. The quantum-yield dependence of the effective distance

The quantum yield dependence of the effective distance was found to be:

$$R_{eff}\left(q\right)=\Phi_{\mathrm{D}}^{1/6}R_{F}\left(\left(1-\int p\left(r\right)\frac{r^{6}}{r^{6}+\Phi_{\mathrm{D}}R_{F}^{6}}dr\right)^{-1}-1\right)^{1/6}.$$

The derivation begins with the definition of the effective distance.

$$E_{0}=\frac{R_{0}^{6}}{R_{0}^{6}+R_{eff}^{6}}\qquad\text{or}\qquad R_{eff}=R_{0}\left(\frac{1}{E_{0}}-1\right)^{1/6}.$$

Given a distance probability density distribution, $p(r)$, the ETE in the absence of diffusion obeys

$$E_{0}=\left\langle E_{0}\right\rangle=\int p(r)E_{0}\left(r\right)dr$$

(Recall the definition of the average of an observable, A, $\left\langle A\right\rangle=\int A(x)p(x)dx$, the observed ETE has to be identical to the distance average of the local efficiency, $E_0(r)$.)

Using $E_{0}(r)=\frac{R_{0}^{6}}{R_{0}^{6}+r^{6}}$ and that the average of a constant is the constant itself, we can form

$$E_{0}=\int p(r)\frac{R_{0}^{6}}{R_{0}^{6}+r^{6}}dr=\int p\left(r\right)\left(1-\frac{r^{6}}{r^{6}+R_{0}^{6}}\right)dr=1-\int p\left(r\right)\frac{r^{6}}{r^{6}+R_{0}^{6}}dr\ .$$

Inserting this expression into the definition of the effective distance, we obtain

$$R_{eff}=R_{0}\left(\left(1-\int p\left(r\right)\frac{r^{6}}{r^{6}+R_{0}^{6}}dr\right)^{-1}-1\right)^{1/6}.$$

The quantum-yield dependence is obtained after the replacements $R_{0}^{6}=\Phi_{\mathrm{D}}R_{\mathrm{F}}^{6}$ and $R_{0}=\Phi_{\mathrm{D}}^{1/6}R_{\mathrm{F}}$.

For a single, static distance, R_{DA}, the distribution is close to a δ-function and the integral $\int p\left(r\right)\frac{r^{6}}{r^{6}+R_{0}^{6}}\mathrm{d}r$ becomes $\frac{R_{DA}^{6}}{R_{DA}^{6}+R_{0}^{6}}$. Insertion yields correctly that $R_{eff}=R_{DA}$. We see that the quantum-yield dependence of the effective distance is a consequence of the distance distribution:

It does not apply to the case of a single, static distance that describes, for instance, a short, inflexible polyproline peptide.

1.2. The quantum-yield dependence of the effective FRET rate

Although the elementary FRET rate constant, $k_T(r)$ does not depend on the quantum yield, the effective FRET rate, k_{FRET}, shows a weak dependence, because it is experimentally defined through the observed efficiency, E_{obs}. In the absence of diffusion, an analytical treatment is possible. The quantum yield dependence of the effective FRET rate was found to be:

$$k_{FRET0}\left(\Phi_D\right) = \frac{k_{rad}}{\Phi_D}\left(\left(\int p(r)\frac{r^6}{r^6+\Phi_D R_F^6}\mathrm{d}r\right)^{-1} - 1\right).$$

We recognize the consistency of the obtained expressions with $k_{FRET0} = k_D \frac{R_0^6}{R_{eff}^6}$.

To instill trust in and familiarity with this formula we offer two alternative derivations. 1) The effective FRET rate in the absence of diffusion ($E_{obs} = E_0$) is defined by

$$E_0 = \frac{k_{FRET0}}{k_{FRET0}+k_D} \text{ or } k_{FRET0} = k_D\left(\frac{1}{E_0} - 1\right)^{-1}.$$

Using $E_0 = \int p(r)\frac{R_0^6}{R_0^6+r^6}dr$, we obtain

$$k_{FRET0} = k_D\left(\left(\int p(r)\frac{R_0^6}{R_0^6+r^6}dr\right)^{-1} - 1\right)^{-1} \text{ or } k_{FRET0} = \frac{k_{rad}}{\Phi_D}\left(\left(1-\int p(r)\frac{r^6}{\Phi_D R_F^6+r^6}dr\right)^{-1} - 1\right)^{-1}.$$

2) In the second derivation we use again the definition $E_0 = k_{FRET0}/(k_{FRET0}+k_D)$ but also that E_0 is given by $E_0 = (A_D - A_{DA0})/A_D)$, the areas under the kinetic traces, where $(A_D - A_{DA0})$ is the fluorescence quenched by FRET. Both equations are consequences of the ETE being the fraction of donor deactivation events caused by FRET. Eliminating E_0, we obtain for k_{FRET}

$$k_{FRET} = k_D\left(\frac{A_D}{A_{DA0}} - 1\right) = \frac{1}{A_{DA0}} - k_D,$$

where we used that $A_D = \tau_D = k_D^{-1}$.

The time-course of the donor emission intensity of the donor-acceptor peptide in the absence of diffusion is given by

$$I_{DA0}(t) = \int p(r) \exp(-(k_D + k_T(r))t \cdot dr,$$

where $I_{DA0}(t=0)$ is 100%.

The area, which presents the measure of the total emitted fluorescence, becomes

$$A_{DA0} = \int_t \int_r p(r) \exp\left(-\left(k_D + k_T\right)t\right) dr dt$$

or, when we switch the order of integration

$$A_{DA0} = \int_r p(r) \int_t \exp\left(-\left(k_D + k_T(r)\right)t\right) dt dr .$$

The solution of the inner integral is $1/(k_D+k_T)$.

$$A_{DA0} = \int_r p(r) \frac{1}{k_D + k_T(r)} dr .$$

We use the Förster distance law $k_T(r)=k_D R_0^6/r^6$ and $k_D^{-1}= \tau_D$ to obtain

$$A_{DA0} = \int p(r) \frac{1}{k_D + k_D \frac{R_0^6}{r^6}} dr = \tau_D \int p(r) \frac{1}{1 + \frac{R_0^6}{r^6}} dr = \tau_D \int p(r) \frac{r^6}{r^6 + R_0^6} dr .$$

Insertion into $k_{FRET} = 1/A_{DA0} - k_D$ yields

$$k_{FRET} = \frac{1}{A_{DA0}} - k_D = \frac{1}{\tau_D \int p(r) \frac{r^6}{r^6 + R_0^6} dr} - k_D \text{ or}$$

$$k_{FRET} = \left(\tau_D \int p(r) \frac{r^6}{r^6 + R_0^6} dr\right)^{-1} - k_D = k_D \left(\left(\int p(r) \frac{r^6}{r^6 + R_0^6} dr\right)^{-1} - 1\right) \cdot$$

Both, k_D and R_0, depend on the quantum yield (eqs. 19 and 23 in main text), and the dependencies do not cancel out completely:

$$k_{FRET0}(\Phi_D) = \frac{k_{rad}}{\Phi_D} \left(\left(\int p(r) \frac{r^6}{r^6 + \Phi_D R_F^6} dr\right)^{-1} - 1\right)$$

When the distribution narrows and approaches a δ-function, the Förster distance law is recovered and the quantum-yield dependence vanishes:

$$k_{\mathrm{FRET0}} = k_{\mathrm{D}}\left(\left(\int p(r)\frac{r^6}{r^6+R_0^6}\mathrm{d}r\right)^{-1}-1\right) = k_{\mathrm{D}}\left(\left(\frac{r^6}{r^6+R_0^6}\right)^{-1}-1\right) = k_{\mathrm{D}}\left(\left(\frac{r^6+R_0^6}{r^6}\right)-1\right) = k_{\mathrm{D}}\frac{R_0^6}{r^6} = k_{\mathrm{F}}\frac{R_{\mathrm{F}}^6}{r^6}$$

The results of both derivations are identical (use $\int p(r)\frac{R_0^6}{R_0^6+r^6}dr = 1-\int p(r)\frac{r^6}{R_0^6+r^6}dr$):

$$k_{\mathrm{FRET0}} = k_{\mathrm{D}}\left(\left(\int p(r)\frac{R_0^6}{R_0^6+r^6}dr\right)^{-1}-1\right)^{-1} = k_{\mathrm{D}}\left(\left(\int p(r)\frac{r^6}{r^6+R_0^6}\mathrm{d}r\right)^{-1}-1\right).$$

2. Pseudo-monoexponential kinetics in time-resolved FRET measurements

We observed in the experiments that the donor decay kinetics of the donor-acceptor peptides NAla–$(GS)_6$–Dbo and FTrp–$(GS)_6$–Dbo could agreeably be fitted to monoexponential decay functions. Secondly, we noted in the simulations that the effective rate constant, k_{FRET}, is virtually independent of the quantum yield, in contrast to the more pronounced dependency in the absence of diffusion. Both observations are related.

When the donor emission decay in the donor-acceptor peptide proceeds monoexponentially, its time-dependent intensity has to follow $I_{\mathrm{DA}} = I_0 \exp(-(k_{\mathrm{D}} + k'_{\mathrm{FRET}})t)$. Neither k_{D} nor k'_{FRET} depend on the distance. The monoexponential time course is a consequence of a time-invariant shape of the distribution $N^*(r,t)$. Whereas $N^*(r,t)$ decreases with time, the normalized distribution $p(r,t)$ is time invariant. For this invariancy to be possible, the rate constant of the emission decay cannot change with distance. We show shortly that k'_{FRET} is identical to the effective FRET rate, k_{FRET}, as defined by $E_{\mathrm{obs}} = k_{\mathrm{FRET}}/(k_{\mathrm{FRET}}+k_{\mathrm{D}})$ and calculated by $k_{\mathrm{FRET}} = k_{\mathrm{D}}\,E_{\mathrm{obs}}/(1-E_{\mathrm{obs}})$. The area A_{DA} under the kinetic trace obeys $A_{\mathrm{DA}} = \int_t \exp(-(k_{\mathrm{D}}+k'_{\mathrm{FRET}})t)\,\mathrm{d}t = (k_{\mathrm{D}}+k'_{\mathrm{FRET}})^{-1}$. With that result used in the equation $k_{\mathrm{FRET}} = 1/A_{\mathrm{DA}} - k_{\mathrm{D}}$, we obtain $k_{\mathrm{FRET}} = (k_{\mathrm{D}}+k'_{\mathrm{FRET}}) - k_{\mathrm{D}} = k'_{\mathrm{FRET}}$.

The shape invariance of the distribution also means that the rate constant k'_{FRET} is identical to the average of the distance-dependent FRET rate, $\langle k_T \rangle$, [78] which excludes any quantum-yield dependency:

$$k'_{FRET} = \langle k_T \rangle = \int k_T(r)\, p(r) \mathrm{d}r = \int k_D (R_0^6 / r^6)\, p(r) \mathrm{d}r = k_{rad} R_F^6 \langle r^{-6} \rangle$$

It requires unrealistically large diffusion coefficients for the initial shape of the distance distribution to undergo not the slightest change. But an already sufficient condition for the experimental decays to appear monoexponentially is that the stationary probability distribution is attained very soon after excitation, and that the initial and stationary distribution differ only slightly (Fig. 8a in main text).

3. Symbols and Abbreviations

A	Index that denotes the FRET acceptor
$\boldsymbol{A_D}$	Area under the time course of donor emission in the donor-only peptide
$\boldsymbol{A_{DA}}$	Area under the time course of donor emission in the donor-acceptor peptide
$\boldsymbol{A_{DA0}}$	Area under the time course of donor emission in the donor-acceptor peptide in the absence of diffusion ($D = 0$)
$\boldsymbol{D}$	Diffusion coefficient
D	Index that denotes the FRET donor
ETE	Energy transfer efficiency
$\boldsymbol{E_{obs}}$	Observed energy transfer efficiency
$\boldsymbol{E(r)}$	Distance-dependent energy transfer efficiency
$\boldsymbol{E_0}$	Observed energy transfer efficiency in absence of diffusion
$\boldsymbol{\Delta E_{FDE}}$	Increase of E_{obs} caused by diffusion
$\boldsymbol{E_{EQ}}$	Fraction of donor-deactivation events attributable to equilibrium distance distribution
$\boldsymbol{E_M}$	Fraction of donor-deactivation events attributable to donor-acceptor diffusional motion
FDE	FRET diffusion enhancement

FRET	Förster resonance energy transfer
φ_{EQ}	Fraction of FRET events attributable to the equilibrium distance distribution
φ_{M}	Fraction of FRET events attributable to the equilibrium distance distribution
Φ_{D}	Donor-quantum yield
HSE	Haas-Steinberg equation
I_{D}	Intensity of the donor fluorescence in the donor-only peptide.
I_{DA}	Intensity of the donor fluorescence in the donor-acceptor peptide.
$k_{T}(r)$	Distance-dependent rate constant of Förster transfer
k_{FRET}	Effective FRET rate constant
k_{FRET0}	Effective FRET rate constant in absence of diffusion
k_{rad}	Rate constant of donor deactivation at a quantum yield of unity and in absence of FRET
$N^*(r,t)$	Distance distribution after donor excitation in the donor-acceptor peptide
$N_0^*(r)$	Normalized initial distance distribution at t = 0
$p(r)$	Normalized equilibrium distance distribution
$p_s(r)$	Normalized stationary distance distribution attained after donor excitation
r	Donor-acceptor distance in a distribution
R_{DA}	Single donor-acceptor distance in a rigid system
R_{eff}	Effective donor-acceptor distance
R_0	Förster radius at a specific donor quantum yield
R_F	Förster radius at a quantum yield of unity, radiative Förster radius
τ_0	Radiative donor lifetime at a quantum yield of unity
τ_D	Experimentally determined donor lifetime in the donor-only peptide
τ_{DA}	Experimentally determined donor lifetime in the donor-acceptor peptide