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# Site-to-site distance distribution in flexible molecules: theoretical evaluation of the donor and/or acceptor fluorescence decay function

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## Abstract

We present the theoretical expression describing dependence of the fluorescence intensity decays on the distance distribution $P(r)$between energy donors and acceptors in flexible bichromophoric molecules. The expression allows for multiexponential fluorescence decay of the donor- and acceptor-only molecules and takes into account the possibility of incomplete labeling of the molecules by acceptors. It is assumed that the donors and acceptors are static in space and do not move relative to each other during the excited-state lifetime. The potential application of the obtained expression is evaluation of the parameters of the function $P(r)$.

### Keywords

Intramolecular energy transfer; Distance distribution; Fluorescence decay

## 1. Introduction

The phenomenon of nonradiative resonance energy transfer (RET) has been widely used to recover the distance distribution $P(r)$ between donor (D) and acceptor (A) sites on macromolecules [1]. These applications of RET rely on time-resolved measurements of covalently linked D–A pairs and require theoretical modeling of the fluorescence decay function, $I(t)$, of the investigated system. Although, in principle, either the donor or the acceptor decay could be used to recover the distance distribution [2,3], in most of the cases just the donor fluorescence is analyzed. One of the reasons for omitting the acceptor emission in this kind of analyses is the lack of consistent and sufficiently general theoretical description of fluorescence properties of bichromophoric systems with donors and acceptors emitting simultaneously.

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The aim of this work is to obtain an expression describing the fluorescence decay function, $I(t)$, of the linked D–A system in the case when both donor and acceptor can emit fluorescence. Our considerations will be carried out with possible heterogeneity of the donor- and acceptor-only fluorescence decays taken into account. Additionally, it will be assumed that the considered macromolecules may be incompletely labeled by acceptors.

To avoid excessive complexity of our considerations, we will assume that in our system just simple, D→A, and no reverse, A→D, transfer of the excitation energy can occur. The complete theoretical calculations taking the reverse process into account seem to be possible only in the case of homogeneous, single-exponential fluorescence decays of the donor- and acceptor-only molecules. A similar type of kinetics has been described in the literature with reference to certain chemical reactions [4] or transient reaction kinetics of excimer formation [5]. In the case when the reverse A→D energy transfer has to be taken into account, the procedure of solution of the appropriate system of kinetic equations becomes very complicated. From our attempts based on the application of the Laplace transformation, we conclude that the discussed system of kinetic equations could eventually be solved in the Laplace domain, but the inversion of the so obtained expressions to the time domain would pose a rather difficult task.

In flexible molecules, RET can be influenced by molecular diffusion. In this work we will limit ourselves to the systems in which the relative diffusive displacement of chromophores during the donor fluorescence lifetime can be neglected.

## 2. Theory

Consider a solution of molecules, each containing the donor chromophoric group and suppose that the fraction $L$ of the molecules is labeled by another chromophoric group which can play the role of acceptor. Because of flexibility of the D–A linker, the solution is characterized by the distribution of D–A distances $P(r)$. The function $P(r)$ characterizes the system at the equilibrium and the value of $P(r)\,\mathrm{d}r$ is equal to the probability that for the given molecule the D–A distance $r$ is placed in the interval between $r$ and $r+\mathrm{d}r$. Both chromophoric groups, D and A, may become excited by the incident light, so after a pulse excitation at time $t=0$, one has in the solution up to three kinds of fluorescent centers: D*A, DA*, and D* (* marks the excited chromophore). These centers at later time can emit fluorescence. Assume that the fluorescence decays $I_{\mathrm{D}*}(t)$ of the donor-only molecules and $I_{\mathrm{A}*}(t)$ of the acceptor-only molecules are multiexponential:

$$I_{\mathrm{D}*}(t) \propto \sum_{i=1}^{n_{\mathrm{D}}} (f_{\mathrm{D}i}/\tau_{\mathrm{D}i}) \exp(-t/\tau_{\mathrm{D}i}), \tag{1}$$

$$I_{\mathrm{A}*}(t) \propto \sum_{j=1}^{n_{\mathrm{A}}} \left(f_{\mathrm{A}j}/\tau_{\mathrm{A}j}\right) \exp\left(-t/\tau_{\mathrm{A}j}\right). \tag{2}$$

where $f_{Di}$ and $f_{Aj}$ are the fractions of molecules emitting fluorescence with lifetimes $\tau_{Di}$ and $\tau_{Aj}$, respectively, and $n_D$ and $n_A$ denote respective numbers of these fractions. The important feature of the considered system is that inside the D*A centers there can appear the resonance energy transfer from donor to acceptor. We assume that there are no conditions suitable for reverse energy transfer from acceptor to donor in the system. Suppose, similarly as in Refs. [6,7], that the rates of resonance energy transfer from the $i$th donor fraction to an arbitrary acceptor fraction is given by

$$k_{DAi}(r) = \tau_{Di}^{-1}(R_0/r)^6, \tag{3}$$

where $R_0$ is the Förster radius. It should be noticed that expression (3) does not have strict theoretical or experimental grounds. Its form seems to be suitable, because similarly as for fluorophores with single-exponential decay, it makes the transfer rate being depended on the inverse of donor lifetime. According to the division of all fluorescent centers into three kinds, D*A, DA*, and D*, the fluorescence intensity decay of the system consists of three components:

$$I(t) = I_{D*A}(t) + I_{DA*}(t) + I_{D*}(t)\,. \tag{4}$$

At any time instant $t$ each of the total numbers of the D*A, DA*, and D* centers may be divided into $n_D$ or $n_A$ subpopulations containing the respective numbers of molecules $N_{D*Ai}(t)$, $N_{DA*j}(t)$, and $N_{D*i}(t)$. These subpopulations are characterized by fluorescence lifetimes $\tau_{Di}$ or $\tau_{Aj}$. Hence Eq. (4) may be rewritten in the form

$$I(t) = C_0\left[F_D Q_D \sum_{i=1}^{n_D} \frac{N_{D*Ai}(t)}{\tau_{Di}} + F_A Q_A \sum_{j=1}^{n_A} \frac{N_{DA*j}(t)}{\tau_{Aj}} + F_D Q_D \sum_{i=1}^{n_D} \frac{N_{D*i}(t)}{\tau_{Di}}\right], \tag{5}$$

where $C_0$ is a constant, $F_D$ and $F_A$ are values of the normalized emission spectra $\left(\int_0^\infty F_D(\lambda)\mathrm{d}\lambda = 1, \int_0^\infty F_A(\lambda)\mathrm{d}\lambda = 1\right)$ at the observation wavelength, and $Q_D$ and $Q_A$ are the quantum yields of the donors in the absence of acceptors and acceptors in the absence of donors, respectively.

In Eq. (5) the number $N_{D*Ai}(t)$ of the excited D*A centers belonging at time $t$ to the $i$th fraction may be understood as an integral

$$N_{D*Ai}(t) = \int_{r_{min}}^{r_{max}} n_{D*Ai}(r,t)\mathrm{d}r, \tag{6}$$

where $n_{D*Ai}(r,t)\mathrm{d}r$ represents the number of D*A centers characterized at time $t$ by the D–A distance present in the interval $r$ to $r + \mathrm{d}r$, and $r_{min}$ and $r_{max}$ are the minimum and maximum D–A distances. The density $n_{D*Ai}(r,t)$ decays due to the radiative and nonradiative relaxation

with the rate $\tau_{Di}^{-1}$ and due to nonradiative energy transfer with the rate $k_{DAi}(r)$. One can easily see that the time and distance dependence of $n_{D*Ai}(r,t)$ is given by

$$n_{D^*Ai}(r,t) = n_{D^*Ai}(r,0)\exp\left\{-\left[\tau_{Di}^{-1} + k_{DAi}(r)\right]t\right\}. \tag{7}$$

The initial distance distribution, $n_{D^*Ai}(r,0)$, of the $i$th fraction of the D*A centers may be calculated as

$$n_{D^*Ai}(r,0) = C_1 L f_D^{abs} f_{Di} P(r), \tag{8}$$

with $C_1$ being a constant and $f_D^{abs}$ being the donor-absorbed fraction of excitation photons. After combining Eqs. (6)–(8) one obtains

$$N_{D^*Ai}(t) = C_1 L f_D^{abs} f_{Di} \exp(-t/\tau_{Di}) \int_{r_{min}}^{r_{max}} P(r)\exp[-k_{DAi}(r)t]. \tag{9}$$

Thus, after taking into account Eq. (5) the first term of Eq. (4) may be rewritten in the form

$$I_{D^*A}(t) = C L f_D^{abs} F_D Q_D \Phi_{D^*A}(t), \tag{10}$$

with $C = C_0\, C_1$ and

$$\Phi_{D^*A}(t) = \int_{r_{min}}^{r_{max}} P(r) \sum_{i=1}^{N_D} f_{Di}\tau_{Di}^{-1} \exp\left\{-\left[\tau_{Di}^{-1} + k_{DAi}(r)\right]t\right\} dr. \tag{11}$$

In the second term of Eq. (5) the function $N_{DA^*j}(t)$ represents the number of DA* centers characterized at time $t$ by the decay time $\tau_{Aj}$. It decreases due to the radiative and nonradiative relaxation with the rate $\tau_{Aj}^{-1}$ and increases due to nonradiative energy transfer in the D*A centers ( D*A→DA* ) with the rate (3) according to the equation

$$\frac{dN_{DA^*j}(t)}{dt} = f_{Aj}\sum_{i=1}^{n_D} \int_{r_{min}}^{r_{max}} k_{DAi}(r) n_{D^*Ai}(r,t) dr - \tau_{Aj}^{-1} N_{DA^*j}(t), \tag{12}$$

where the density $n_{D^*Ai}(r,t)$ is given by Eqs. (7) and (8). The initial condition of Eq. (12) is

$$N_{DA^*i}(0) = C_1 f_A^{abs} f_{Aj}, \tag{13}$$

where $C_1$ is the same constant as in Eqs. (8) and (9) and $f_A^{abs}$ denotes the acceptor-absorbed fraction of excitation photons. Under these conditions the solution of Eq. (12) is

$$N_{\mathrm{DA}*j}(t) = C_1 f_{\mathrm{A}j}\Big[L f_{\mathrm{D}}^{\mathrm{abs}} \Psi_{\mathrm{DA}}(t) \otimes \varphi_{\mathrm{A}*j}(t) + f_{\mathrm{A}}^{\mathrm{abs}} \varphi_{\mathrm{A}*j}(t)\Big], \tag{14}$$

with

$$\Psi_{\mathrm{DA}}(t) = \int_{r_{\min}}^{r_{\max}} P(r) \sum_{i=1}^{n_{\mathrm{D}}} f_{\mathrm{D}i} k_{\mathrm{DA}i}(r) \exp\Big\{-\Big[\tau_{\mathrm{D}i}^{-1} + k_{\mathrm{DA}i}(r)\Big]t\Big\} \mathrm{d}r \tag{15}$$

and

$$\varphi_{\mathrm{A}*j}(t) = f_{\mathrm{A}j} \exp\left(-t/\tau_{\mathrm{A}j}\right). \tag{16}$$

In Eq. (14) $\otimes$ represents the convolution operator. Hence, after taking into account Eq. (5), the second term of Eq. (4) may be rewritten in the form

$$I_{\mathrm{DA}*}(t) = C F_{\mathrm{A}} \eta_{\mathrm{A}}\Big[L f_{\mathrm{D}}^{\mathrm{abs}} \Phi_{\mathrm{DA}*}(t) + f_{\mathrm{A}}^{\mathrm{abs}} \Phi_{\mathrm{A}*}(t)\Big], \tag{17}$$

where

$$\Phi_{\mathrm{DA}*}(t) = \Psi_{\mathrm{DA}}(t) \otimes \Phi_{\mathrm{A}*}(t) \tag{18}$$

and

$$\Phi_{\mathrm{A}*}(t) = \sum_{j=1}^{n_{\mathrm{A}}} f_{\mathrm{A}j} \tau_{\mathrm{A}j}^{-1} \exp\left(-t/\tau_{\mathrm{A}j}\right). \tag{19}$$

Taking into account Eqs. (18), (15) and (19) function $\Phi_{\mathrm{DA}*}(t)$ may be expressed as

$$\Phi_{\mathrm{DA}*}(t) = \sum_{j=1}^{n_{\mathrm{A}}} \frac{f_{\mathrm{A}j}}{\tau_{\mathrm{A}j}} \sum_{i=1}^{n_{\mathrm{D}}} f_{\mathrm{D}i} \times \int_{r_{\min}}^{r_{\max}} \frac{P(r) k_{\mathrm{DA}i}(r)}{[\tau_{\mathrm{DA}i}(r)]^{-1} - \tau_{\mathrm{A}j}^{-1}} \Big\{\exp\left(-t/\tau_{\mathrm{A}j}\right) - \exp\left[-t/\tau_{\mathrm{DA}i}(r)\right]\Big\} \mathrm{d}r, \tag{20}$$

where

$$\tau_{\mathrm{DA}i}(r) = \Big[\tau_{\mathrm{D}i}^{-1} + k_{\mathrm{DA}i}(r)\Big]^{-1}. \tag{21}$$

In the third term of Eq. (5) the function $N_{\mathrm{D}*i}(t)$ represents the number of $D^*$ centers characterized at time $t$ by the decay time $\tau_{\mathrm{D}i}$. This number just exponentially decreases with time:

$$N_{\mathrm{D}*i}(t) = C_1 (1 - L) f_{\mathrm{D}}^{\mathrm{abs}} f_{\mathrm{D}i} \exp\left(-t/\tau_{\mathrm{D}i}\right). \tag{22}$$

In consequence, the third term of Eq. (4) takes the form

$$I_{\mathrm{D}^*(t)} = C(1-L) f_{\mathrm{D}}^{\mathrm{abs}} F_{\mathrm{D}} Q_{\mathrm{D}} \Phi_{\mathrm{D}^*}(t), \tag{23}$$

where

$$\Phi_{\mathrm{D}^*}(t) = \sum_{i=1}^{n_{\mathrm{D}}} f_{\mathrm{D}i} \tau_{\mathrm{D}i}^{-1} \exp(-t/\tau_{\mathrm{D}i}). \tag{24}$$

The total decay function is given by the sum of Eqs. (10), (17) and (23). The final expression may be simplified by introducing the quantities $f_{\mathrm{D}}^{\mathrm{em}}$ and $f_{\mathrm{A}}^{\mathrm{em}}$ defined as

$$f_{\mathrm{D}}^{\mathrm{em}} = \frac{F_{\mathrm{D}} Q_{\mathrm{D}}}{F_{\mathrm{D}} Q_{\mathrm{D}} + F_{\mathrm{A}} Q_{\mathrm{A}}}, \tag{25}$$

$$f_{\mathrm{A}}^{\mathrm{em}} = \frac{F_{\mathrm{A}} Q_{\mathrm{A}}}{F_{\mathrm{D}} Q_{\mathrm{D}} + F_{\mathrm{A}} Q_{\mathrm{A}}}. \tag{26}$$

Assuming that $C(F_{\mathrm{D}} Q_{\mathrm{D}} + F_{\mathrm{A}} Q_{\mathrm{A}}) = 1$, we finally obtain

$$\begin{aligned} I(t) = {} & f_{\mathrm{D}}^{\mathrm{abs}} [L \Phi_{\mathrm{D}^*\mathrm{A}}(t) + (1-L) \Phi_{\mathrm{D}^*}(t)] f_{\mathrm{D}}^{\mathrm{em}} \\ & + \left[ L f_{\mathrm{D}}^{\mathrm{abs}} \Phi_{\mathrm{DA}^*}(t) + f_{\mathrm{A}}^{\mathrm{abs}} \Phi_{\mathrm{A}^*}(t) \right] f_{\mathrm{A}}^{\mathrm{em}} \end{aligned} \tag{27}$$

with functions $\Phi_{\mathrm{D}^*\mathrm{A}}(t)$, $\Phi_{\mathrm{D}^*}(t)$, $\Phi_{\mathrm{DA}^*}(t)$, and $\Phi_{\mathrm{A}^*}(t)$ given by Eqs. (11), (24), (20), and (19).

## 3. Discussion

The main result of this work is Eq. (27). It describes the fluorescence decay of the solution of bichromophoric D–A molecules fulfilling the assumptions discussed in the introduction. The first line of Eq. (27) describes the decay of the donor emission and the second line describes the decay of the acceptor emission. The distance distribution $P(r)$ needed for evaluation of the functions $\Phi_{\mathrm{D}^*\mathrm{A}}(t)$ and $\Phi_{\mathrm{DA}^*}(t)$ may take different forms. Most often $P(r)$ is assumed to be a Gaussian:

$$P(r) = \frac{1}{Z} \exp\left[ -\frac{(r - r_{\mathrm{av}})^2}{2\sigma^2} \right], \tag{28}$$

where $Z$ is the normalization factor fulfilling the condition $\int_{r_{\mathrm{min}}}^{r_{\mathrm{max}}} P(r)\mathrm{d}r = 1$. In such a case, the parameters which we are interested in evaluating by fitting Eq. (27) to the fluorescence decay obtained experimentally are the mean D–A distance, $r_{\mathrm{av}}$, and the standard deviation s or the half-width of the distribution given by $hw = \sigma\sqrt{8 \ln 2}$. Other parameters of Eq. (27) can be evaluated from independent experiments, with the exception of the fractions $f_{\mathrm{D}}^{\mathrm{em}}$ and $f_{\mathrm{A}}^{\mathrm{em}}$ which can be difficult for precise experimental evaluation. Fractions $f_{\mathrm{D}}^{\mathrm{em}}$ and $f_{\mathrm{A}}^{\mathrm{em}}$ are defined by Eqs. (25) and (26) and belong to the main parameters determining relative

contributions of the donor and acceptor intensities to the total fluorescence intensity $I(t)$ of the sample. Their values depend on the observation wavelength and on the quantum yields of the chromophores. Setting the observation wavelength sufficiently low or choosing the acceptor with a negligibly small value of the quantum yield, one can get the case where $f_D^{em} = 1$ and $f_A^{em} = 0$. Then Eq. (27) reduces to an equation commonly used in the donor fluorescence analysis (see Ref. [1] and the references therein). Because of long tails on the long wavelength side of the fluorescence spectra of chromophores, the opposite situation, where $f_D^{em} = 0$ and $f_A^{em} = 1$, is difficult to achieve. Assuming that the donor decay and the acceptor decay contain slightly different information about the distance distribution $P(r)$, one can expect that the global analysis of several total fluorescence decays registered at different observation wavelengths can lead to increased resolution of the distance distribution parameters. Our preliminary calculations seem to confirm this expectation, at least in certain specific circumstances. Because of limited space assigned to this article, we cannot discuss this topic here in more detail.

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