Influence of the energy transfer in a single donor–acceptor pair on the photon distribution functions in its fluorescence

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A B S T R A C T
Fluorescence of a single donor–acceptor pair is considered. A donor molecule is described by a three-level energy scheme. It has a ‘dark’ state, which makes donor fluorescence blinking. An acceptor molecule is described by two-level scheme. Its fluorescence is of continuous type. Strong dependence of the distributions \( w_D^n(T) \) and \( w_A^n(T) \) for photons emitted by the donor and the acceptor on the electronic excitation energy transfer rate \( F \) between the two molecules is demonstrated. For instance, at \( F > 1/T_D \), where \( 1/T_D \) is the donor fluorescence rate, the fluorescence of the two-level acceptor starts blinking. The photon number distributions at \( F \ll 1/T_D \) and \( F \gg 1/T_D \) are considered as well.

The process of electronic excitation energy transfer is of high importance in complex molecular systems, for instance, in photosynthesis [1]. We can state that the transfer is taking place whenever we observe that the acceptor molecules start to fluoresce without direct excitation. During the recent 50 years the energy transfer process was investigated in mixed ensembles of numerous donors and acceptors. However, it is evidently difficult to gain the necessary information on the mechanism of the transfer in individual pairs observing the fluorescence of many acceptor molecules [2,3].

With the development of the single molecule spectroscopy [4–7] there appeared fundamentally new opportunities for investigating the energy transfer process within single donor–acceptor pairs. New possibilities opening here were discussed by Weiss [6]. Such works with single pairs allow clarifying many energy transfer peculiarities. This transfer is normally detected by the fluorescence intensity of an acceptor single molecule [8–12].

However, single molecule fluorescence intensity always fluctuates. If a single molecule is excited by a CW-laser, the time instants of both laser photon absorption and fluorescence photon emission are random. Therefore, a different fluorescence photon number \( N \) will be detected at equal bin times \( T \), so the fluorescence intensity will indeed fluctuate.

There are several ways to characterize the fluorescence intensity fluctuations of a donor–acceptor system: for instance, either to use the fluorescence auto correlation function [13] or to measure various kinds of distribution functions [14]. Theories for the distribution function for the energy transfer efficiency [15] and for the photon distribution function [16,17] have been recently proposed. Photon distribution functions for donor and acceptor fluorescence will be considered in this Letter.

Single molecule irradiated by CW-laser light will emit train of fluorescence photons. Time instants of photon emission are random. Therefore we will count various numbers of photons \( N \) on equal time intervals of duration \( T \). Probability \( w_N(T) \) of finding time interval \( T \) with \( N \) photons is the photon distribution function.

According to the formula

\[
I = \frac{<N(T)>}{T} = \frac{1}{T} \sum_{N=0}^{\infty} NW_N(T),
\]

where \( <N(T)> \) is the average photon number over the time interval \( T \), the fluorescence intensity \( I \) is the first moment of the photon number distribution function \( w_N(T) \). Evidently the photon distribution function naturally gives far more information about the dynamics of the fluorescing system than its first moment does. Therefore, if the distribution functions for both the donor and the acceptor are measured or calculated, we will be able to find out more about the energy transfer process compared to the case when we consider only the fluorescence intensity.

The goal of the present Letter is to derive the expressions for the functions \( w_D^n(T) \) and \( w_A^n(T) \) – the distributions for photons emitted by the donor and the acceptor – and to find out how the value of the energy transfer rate \( F \) influences these distributions.

We will consider a three-level donor molecule with two singlet electronic levels and a non-fluorescent ‘dark’ level between them. The optical transitions occur with the electronic transitions between those two singlet states. The dark state can be, for example, a triplet non-fluorescent state. Such a donor–acceptor system is illuminated by a CW-laser in the spectral domain of the donor absorption.

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If the donor absorption rate $k$ is several tens times less than the rates $1/T_D$ and $1/T_A$ of donor and acceptor fluorescence, then the probability of simultaneous excitation of both molecules is low, so we can neglect the state where both the donor and the acceptor are excited. In this case the donor–acceptor pair is described by the energy scheme of a bimolecular system consisting of a three-level donor and a two-level acceptor shown in Figure 1:

Here 0, 1, 2 and 3 numerate electronic levels of a donor–acceptor pair, $\Gamma$ and $\gamma$ are the rates of getting into the dark state and leaving it, $D$, $D'$ and $D^2$ denote the ground, the first excited and the triplet donor states accordingly, $A$ and $A'$ denote the ground and the first excited state of the acceptor. The following system of rate equations corresponds to this scheme:

$$
\dot{\rho}_0 = -k\rho_0 + \rho_1/T_D + \gamma\rho_2 + \rho_3/T_A,
\dot{\rho}_1 = k\rho_0 - (1/T_D + \Gamma + F)\rho_1,
\dot{\rho}_2 = \Gamma\rho_1 - \gamma\rho_2, \dot{\rho}_3 = F\rho_1 - \rho_2 - \rho_3/T_A.
$$

(2)

These equations describe the dynamics of the donor and the acceptor electronic excitations taking into account the transfer of electronic excitations between $D$ and $D'$ with a rate $F$. The following system of rate equations corresponds to this scheme:

$$
\dot{\rho}_0 = -k\rho_0 + \rho_1/T_D + \gamma\rho_2 + \rho_3/T_A,
\dot{\rho}_1 = k\rho_0 - (1/T_D + \Gamma + F)\rho_1,
\dot{\rho}_2 = \Gamma\rho_1 - \gamma\rho_2, \dot{\rho}_3 = F\rho_1 - \rho_2 - \rho_3/T_A.
$$

(3)

The distribution is expressed solely via the function $s(t)$. It is called a start–stop correlator, as $dS(t) = s(t)dt$ is the probability to emit a fluorescence photon at the time interval $(t, t + dt)$ under the condition that the preceding photon was emitted at time $t$. The start–stop correlator is the probability density of finding two photons emitted one after another separated by the time interval $t$. The constant

$$
\tau_0 = \int_0^\infty \left(1 - \int_0^t s(x)dx\right)dt = \int_0^\infty ts(t)dt
$$

(5)
determines the average interval between two photons emitted one after another, $s(\lambda)$ is the Laplace transform of the start–stop correlator $s(t)$. The time-dependent function $s(\lambda)^N$, is the inverse Laplace transform of $s(\lambda)^N$.

The start–stop correlator is determined by the microscopic model of the fluoroc resonance system. There are two types of start–stop correlators, $s_0(t)$ and $s_3(t)$, for a donor–acceptor pair with donor and acceptor fluorescence. These correlators can be found with the help of the following rate equations:

$$
W_0^0 = -kW_0^0 + 0 + \gamma W_2^0 + W_3^2/T_A,
W_1^0 = kW_0^0 - (1/T_D + \Gamma + F)W_0^1,
W_2^0 = \Gamma W_0^0 - \gamma W_2^0,
W_3^0 = FW_0^0 - W_3^2/T_A,
$$

(6)

and

$$
W_0^1 = -kW_0^1 + W_1^0/T_D + \gamma W_2^1 + W_3^0 = 0,
W_1^1 = kW_0^1 - (1/T_D + \Gamma + F)W_0^1,
W_2^1 = \Gamma W_0^1 - \gamma W_2^1,
W_3^1 = FW_0^1 - W_3^2/T_A.
$$

(7)

The transition of photons from a closed system to an open system is described in detail in chapter 3 of Ref. [7].

![Figure 1. The energy scheme of a bimolecular system consisting of a three-level donor with a triplet level 2 and a two-level acceptor.](image)

'Zeros' in Eqs. (6) and (7) show which terms were omitted in Eq. (2) to obtain Eqs. (6) and (7). These equations describe evolution of the so-called open systems, where the total probability is not constant. On the contrary, Eq. (2) describe the evolution of a closed system, where the total probability equals unity. We derive Eqs. (6) and (7) from Eq. (2) omitting the terms $\rho_1/T_D$ and $\rho_2/T_A$, respectively in the first line of equations (2). The sums of the left hand side terms of Eqs. (6) and (7), i.e.

$$
\sum_{j=0}^3 W_j^0 = -W_0^0/T_D = -s_0(t), \sum_{j=0}^3 W_j^1 = -W_0^1/T_A = -s_3(t)
$$

(8)
determine the probability to leave the open systems, that is, determine the expressions for the start–stop correlators. The transition from a closed system to an open system is described in detail in chapter 3 of Ref. [7].

Carrying out the Laplace transformation in Eqs. (6) and (7) and solving the obtained algebraic equations with the initial conditions $W_0^0(0) = 1, W_1^0(0) = W_2^2(0) = W_3^2(0) = 0$ (these conditions are determined by the assumption that a donor/acceptor photon was emitted at zero time moment), we arrive at the following expressions for the Laplace transform of the start–stop correlators:

$$
s_0(\lambda) = \frac{\lambda_0\lambda_1\lambda_2\lambda_3}{2/TA} \left(\frac{\gamma - \lambda}{1/TA - \lambda}\right), (9)
$$

$$
s_3(\lambda) = \frac{\lambda_0\lambda_1(\gamma - \lambda)\lambda_2\lambda_3}{(\lambda_0 - \lambda)(\lambda_1 - \lambda)(\lambda_2 - \lambda)(1/TA - \lambda)}.
$$

(10)

Here $\lambda_1$ and $\lambda_2$ are the roots of the determinants of Eqs. (6) and (7).

Substituting these expressions into formula (3), we can calculate the photon number distributions $s_0(t)$ and $s_3(t)$ for the donor and the acceptor fluorescence, the way the calculations of $s(\lambda)$, are carrying out can be found in Refs. [16,17]. The calculations have been carried out with the help of the following set of parameters:

$$
1/T_D = 2 \times 10^8 \text{s}^{-1}, 1/T_A = 10^5 \text{s}^{-1}, k = 10^5 \text{s}^{-1},
\Gamma = 10^8 \text{s}^{-1}, \gamma = 10^8 \text{s}^{-1}
$$

(11)

and for different values of the electronic excitation energy transfer rate $F$. The results of these calculations are presented in Figure 2.

The distribution function on each panel satisfies the following equation $\sum_{j=0}^3 W_j^2(\lambda) = 1$.

Slow energy transfer is realized at $F \ll 1/T_0$. The first line of the graphs in Figure 2 corresponds to this case. The donor fluorescence intensity, i.e. $I = <N> / T$, is much larger as compared to the acceptor fluorescence intensity. Weak acceptor fluorescence is described by the Poisson distribution. The donor photon emission distribution is significantly different from a pure Poisson one. That is, the distribution is of super-Poissonian type. Such a photon distribution with a narrow peak and a wide plateau stretching to small photon numbers $N$ is telling feature of blinking fluorescence.
cases are responsible for the plateau of the distribution at small photon numbers \(N\).

Medium energy transfer rate is realized at \(F \approx 1/T_D\). The distribution functions for this case are shown in the third line of Figure 2. If the energy transfer can be attributed to Förster Resonance Energy Transfer (FRET) we can write \(F = F_0/R_{DA}^2\) where \(R_{DA}\) is a distance between the donor and acceptor molecules. Equation \((F_0/R_{DA}^2)T_D = 1\) enables one to find so-called Förster radius \(R_{DA} = R_F\). In this case the donor and the acceptor fluorescence intensities are comparable. As the energy transfer rate \(F\) increases from \(F \ll 1/T_D\) up to \(F \approx 1/T_D\), the donor fluorescence remains of super-Poissonian type, while the acceptor fluorescence gradually changes its character and becomes of super-Poisson type as well. The second and the third lines of Figure 2 show the appearance of the plateau in the acceptor distribution functions. Hence, the acceptor starts blinking, though it has no own dark state. The explanation for this fact is the following: while the donor gets to its dark state, it cannot transfer electron energy to the acceptor. So there is no way for the acceptor to be excited, that is why it blinks simultaneously with the donor.

At \(F \gg 1/T_D\) the donor fluorescence dramatically decreases, and the acceptor fluorescence intensity exceeds it considerably. This is represented at the fifth line of Figure 2. At the same time the shapes of both distributions change considerably. While the energy transfer rate \(F\) increases from \(F \ll 1/T_D\) to \(F \gg 1/T_D\), they both approach the Poissonian distributions (lines 4 and 5 of Figure 2). Therefore, at high energy transfer rates the donor dark state does not reveal itself. It happens because the energy transfer from the excited donor to the acceptor in the ground state becomes much more probable than the transition of the excited donor to the triplet state. It results into longer donor on-intervals, that is why its fluorescence looks as that of a two-level molecule. Thus, as the energy transfer becomes faster, the blinking of the donor is smoothed over, and both the donor and the acceptor distributions approach the Poissonian shape.

What advantage we acquire if photon distribution functions are measured instead of fluorescence intensity? By measuring the donor/acceptor fluorescence intensities \(I_D\) and \(I_A\) we are able to determine only the efficiency \(E = I_A/(I_A + I_D)\) of the energy transfer. However, it is unclear the way we can find value of the Förster rate constant \(F = F_0/R_{DA}^2\) from measured efficiency. As usual a relation between \(E\) and \(F\) is taken in the following form [14]

\[
E(F) = \frac{F_0 T_D}{F_0 T_D + R_{DA}^2} = \frac{FT_D}{FT_D + 1} \quad (12)
\]

The efficiency described by this function is shown in Figure 4 by solid line.

If we measure value \(E\) of FRET efficiency we can find with the help of solid line value of the Förster constant \(F\).

Another way for finding \(F\) we can use if we measured the distribution functions \(w_D(T)\) and \(w_A(T)\). By using the theory presented here we can calculate the distribution functions for various values of \(F\) and find at which value of \(F\) the calculated distribution coincides with the measured one. This method is more complex as compared with the method using solid curve in Figure 4. However, the method using distribution functions enables one to find true value of \(F\).

**Figure 2.** The distribution functions \(w_D(T)\) and \(w_A(T)\) at the time interval \(T = 1\) ms at various energy transfer rate values (solid lines); Poisson distributions – dotted lines. Numerals on each panel show values of \(w_D^0(1\) ms), \(w_A^0(1\) ms) and \(F T_D\).

**Figure 3.** The photon sequence of blinking fluorescence and the two types of time intervals at which photons are counted.
In order to compare our method of finding $F$ with the method based on relation (12) we have calculated fluorescence intensities $I_D$ and $I_A$ with the help of Eq. (1) and with the distribution functions $w_D^N (1 \text{ ms})$ and $w_A^N (1 \text{ ms})$ shown in Figure 2. The result for $E(F) = I_A/(I_A + I_D)$ is shown by dashed line in Figure 4. Solid and dashed lines differ considerably. This means that Eq. (12) cannot provide true value of Förster constant $F$.

The results of our calculations given above let us make the following conclusions:

1. There is a strong dependence of the fluorescence photon number distributions $w_D^N (T)$ and $w_A^N (T)$ on the energy transfer rate $F$.
2. If the energy transfer rate $F$ is less than the donor fluorescence rate $1/T_D$, i.e. $F \ll 1/T_D$, the distribution $w_D^N (T)$ has a complex shape typical for blinking fluorescence, and the distribution $w_A^N (T)$ is of Poissonian type.
3. At $F \approx 1/T_D$ the fluorescence of a two-level acceptor becomes of blinking type, i.e., the dark state of the donor reveals itself in the acceptor fluorescence.
4. At $F \gg 1/T_D$ both the donor and the acceptor photon distributions become of Poissonian type, as the fast process of the energy transfer does not give donor enough time to get into the dark state.
5. Comparison of the measured distribution functions $w_D^N (T)$ and $w_A^N (T)$ with those calculated enables one to find more reliable value of the transfer rate $F$.

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