Extending the range of FRET—the Monte Carlo study of the antenna effect

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Abstract The problem of extending the utilizable range of Förster resonance energy transfer (FRET) is of great current interest, due to the demand of conformation studies of larger biological structures at distances exceeding typical limiting distance of 100 Å. One of the ways to address this issue is the use of so-called antenna effect. In the present work, the influence of the antenna effect on the FRET efficiency is investigated by the Monte Carlo analysis. The previously published results Bojarski et al. (J Phys Chem B 115:10120–10125, 2011) indicate that using a simple model of donor linked with a protein labeled with multiple acceptors, significantly increases the transfer efficiency in comparison with donor–single acceptor system. The effect is stronger if the transition moments of acceptors are mutually parallel. In this work, to extend the scope of possible biological systems to be analyzed, different distributions of donor–acceptors distance are analyzed, as well as the size and shape of the attached molecule.

Keywords Enhanced transfer efficiency · FRET · Monte Carlo · Multiple acceptors

Introduction

Förster resonance energy transfer is the radiationless transfer of energy occurring between excited molecule (donor) and ground-state molecule (acceptor) via long range dipole–dipole interaction [1]. FRET plays an important role in all fields of science in which fluorescence phenomena are used, especially in understanding structure and functions of biological macromolecules. The features making FRET so widely used are a strong distance and orientation dependence of transition moments of interacting molecules, which creates a possibility to use it as a ‘spectroscopic ruler’ to measure intermolecular distances [2].

One of the most challenging problems in using FRET in this way, is that the usable range of the interaction does not exceed 100 Å, since the FRET efficiency strongly decreases with the increasing distance. Seeking ways to allow the use of FRET at longer distances is of great current interest [3–6].

One of the ways to achieve this is to study donor–acceptor system in the vicinity of incorporated metal nanoparticles or a metal nanolayer [7–10]. It occurs that the existence of metal substantially modifies the electromagnetic field in the neighborhood of excited molecules which leads, under specific conditions, to significant FRET enhancement. Studies in this area [7, 10] have shown that the proximity of the silver particles to the donor and acceptor bound to double helical DNA resulted in an increase of the Förster distance from 35 to 166 Å.

Another approach is the use of strong orientation dependence of FRET in existing biological systems, like photosynthetic systems as plants and some bacteria [11, 12], as well as by manipulating the organization of fluorophores in different kinds of matrices [13, 14].

In the context of some biological systems, so-called antenna effect can occur and be useful [1, 6]. In this approach the single acceptor molecule is replaced by many linked and closely located molecules to which resonance energy transfer is much more effective. The enhancement of transfer efficiency at a given distance means that the usable range of FRET can be extended in the experiments. In other
words, in the case of donor–multiple acceptors system, the same value of FRET efficiency as in the donor–single acceptor case can be obtained at longer distances.

We proposed the advanced Monte Carlo (MC) analysis of antenna effect. By means of MC simulations we mimic the biological antenna systems and, as a result, we can simply obtain not only the average value of FRET efficiency for a given system, but also the decay curve of donor and acceptor emission, which can be helpful in the interpretation and design of FRET experiments. The existing models [1, 6] allow to simulate systems characterized by a fixed distance between donor and the group of acceptors. Our aim is to extend the scope of biological systems possible to be analyzed, by introducing distribution function of distance between interacting fluorophores, as well as different shape of a protein carrying multiple acceptors. The simple antenna model, c.f., Fig. 1, is built by placing the donor molecule, D at the center of Cartesian coordinates system and the group of acceptors, A, randomly positioned on a surface of a globular protein, approximated by sphere with radius r. The donor and the protein are connected via link of a certain length, R.

The vectors of donors and acceptors transition moments, as well as the explicit distances between donor and each acceptor, are determined during each step of the MC simulation.

The most straightforward approximation, regarding the distance R is to use a rigid linker connected to the donor and the protein labeled with randomly positioned acceptors [1, 6]. In this manner, the distances between donor and each of the acceptors, \( R_i \), depend only on the acceptors distribution over the protein surface and the radius of the protein. \( R_i \) can be calculated as:

\[
R_i = \left( (R + r \sin \theta_i \cos \phi_i)^2 + (r \sin \theta_i \sin \phi_i)^2 + (r \cos \theta_i)^2 \right)^{1/2},
\]

where the angles \( \theta_i \) and \( \phi_i \) describe the \( i \)th acceptor location on the sphere with respect to the center of protein.

The absolute distance between fluorophores can vary from \( R \), for the closest located acceptor, to \( R + 2r \) in the case of molecule attached to the opposite part of the sphere.

Another extreme case follows from the assumption of an ideally flexible linker between the donor and the protein. The only restraint is the length of the linker, \( R_{\text{max}} \), which allows the distance to vary from 0 to \( R_{\text{max}} \). This, quite unrealistic in biological systems, model demands some conditions to be fulfilled. First of all, the existence of the linker does not affect the movement of the donor and acceptor. Second, all relative donor–acceptor positions are equally probable within the allowed range. The probability density function (PDF) that the protein will choose the position on the sphere with radius \( R \), among all equally probable positions within the ball with radius \( R_{\text{max}} \) is

\[
P(R) = \frac{3}{R_{\text{max}}^3} R^2.
\]

The donor molecule is placed in the center of this ball. The real distribution of end-to-end distances in flexible molecules can be obtained using several experimental methods like the time-resolved fluorescence spectroscopy [15–17], frequency domain data [18, 19] or steady-state measurements of the efficiency of fluorescence energy transfer [20]. In most of the cases it is necessary to introduce the end-to-end distance distribution (by proposing the mathematical function describing this distribution) to properly describe experimental data. Although several different functions can be used [16, 21], for most purposes the Gaussian distribution,

\[
P(R) = \frac{1}{\sigma \sqrt{2\pi}} \exp\left[ -\frac{1}{2} \left( \frac{R - \bar{R}}{\sigma} \right)^2 \right],
\]

is regarded to be adequate and best suited to describe end-to-end distribution in macromolecules [6]. The donor and acceptor molecules labeled on a native, rigid protein are expected to be relatively immobile during the excited-state lifetime, which leads to the possible \( R \)
values narrowly distributed around the average distance, $R$. For flexible or denatured peptides, either the donor and acceptor, or the peptide itself are more likely to rotate and diffuse. In this case the range of possible distances increases which leads to the wide $P(R)$ distribution.

Introductory Monte Carlo simulations of FRET efficiency and fluorescence decay of donor, published recently by Bojarski et al. [22], show, that the usable range of FRET can be extended even up to 200 Å if multiple acceptors with mutual parallel orientations are used. In this article we would like to show how the changes in attached proteins size and shape influence FRET. Moreover it is shown that the use of distribution function of distance results in the changes in the exponential character of donor fluorescence decay.

Monte Carlo simulations

To examine the influence of different parameters in antenna system on FRET efficiency, we use a simple Monte Carlo (MC) algorithm based on the following deactivation scheme

$$D + A_1 + \ldots + A_n + h\nu \rightarrow D^* + A_1 + \ldots + A_n,$$

$$D + A_1 + \ldots + A_n + h\nu \rightarrow D + A_1 + \ldots + A_n + h\nu_D,$$

where $k_D$ is the radiative decay rate of donor and $k_{DA}$ is the transfer rate for FRET process. The symbol $i$ distinguishes particular acceptor from the group of acceptors. $h\nu$ and $h\nu_D$ denote the energy of the exciting photon and photons emitted by donor. The kinetics of the system described by this scheme, can be written in terms of probability $p_D(t)$ that the donor is excited at time $t$ as follows:

$$p_D = -\left(\sum_i k_{DA_i} + k_D\right) p_D,$$

where dot above $p_D$ stands for derivative with respect to time. The radiative rate coefficients is calculated using the natural lifetime of the donor ($\tau_D$) as

$$k_D = \frac{1}{\tau_D}.$$

To calculate the FRET rate we use the well-known Förster formula, including the electromagnetic coupling between fluorophores in the dipole approximation

$$k_{DA} = \frac{1}{\tau_D} \left(\frac{R_0}{R^6}\right),$$

where $R_i$ is the distance between the middle of donors and acceptors transition dipoles and $R_0$ is Förster radius (the distance at which the probability of transfer is equal to the sum of all other deactivation probabilities) which depends on spectral characteristics of fluorophores and their mutual orientation. The literature values of Förster radius ($R_{F0}$) for different pairs of fluorophores are usually calculated for the case of fast isotropically rotating donor and acceptor molecules, which corresponds to the average $<\kappa^2> = \frac{3}{4}$. During the simulations, instead of assuming averaged values of the orientation factor, we calculated the real values for any pair of interacting fluorophores. Such a procedure allows to minimize the error resulting from arbitrary assuming any limiting value of $\kappa^2$ obtained as a result of averaging. To obtain the correct value for the real $\kappa^2$ we need to define $R_0$ as

$$R_0 = \frac{3}{2} R_{F0} \kappa^2,$$

where the orientation factor is expressed as:

$$\kappa^2 = (\cos\theta_T - 3\cos\theta_D\cos\theta_A)^2,$$

where $\theta_T$ is the angle formed by transition moments, $\theta_D$ is the angle between donors transition dipole and the vector joining the centers of donors and acceptors moment, and $\theta_A$ is the analogue angle for the acceptor.

The transfer rates are additive, therefore the total rate for both radiative and nonradiative energy transfer will be a simple sum of the rates for each acceptor. Based on the values of the transfer rates, the probability of a given event is calculated for each process: the transfer of energy ($p_{DA}$) and the emission of photon ($p_D$). For the donor,

$$p_{DA} = \frac{\sum_i k_{DA_i}}{k_D}, p_D = \frac{k_D}{k_D},$$

where $k_D = k_D + \sum_i k_{DA_i}$ means the sum of the rates for all processes possible in this case.

The choice of a process realized in each MC step is performed by generating a random number from a uniform distribution in the range $[0, 1]$. In the case of radiative deexcitation, simulation in this MC step is finished and fluorescence event is recorded. When FRET occurs, energy of excitation is transferred into the acceptors ensemble, which also finishes the simulations step and the transfer event is recorded. To simplify the calculations, we assume, that reverse energy transfer does not occur (i.e., there is no overlap between acceptor emission and donor absorption). Moreover, the possibility of clusters formation in the population of acceptors is neglected.

The efficiency of FRET is calculated as the relation of the number of energy transfer events ($n_{FRET}$) to the total number
of events (radiative-$n_r$ and non-radiative),

$$E_{\text{FRET}} = \frac{n_{\text{FRET}}}{n_{\text{FRET}} + n_\gamma}.$$  \hspace{1cm} (10)

The emission decay curve is obtained by relating all emission events with the particular moment of time at which investigated process occurs [22]. It is calculated by inverting the distribution function of the probability, $P(t, P_k)dt$, so that if at time $t$ the molecule is excited, then the process $P_k$ appears in the time interval $(t, t + dt)$,

$$p(t) = \sum_k p(t, P_k) = k_t \exp(-k_t t),$$  \hspace{1cm} (11)

where $k_t = k_{\text{rad}} + k_{\text{nr}}$ is the sum of transfer rates for each process which is possible in the case of donor or acceptor molecule.

In each MC step, a random number, $x$, is generated from the $(0-1)$ uniform distribution, and the time at which any process takes place is obtained by inverting the distribution function of the probability $p(t)$,

$$\int_0^t p(t) dt = x \rightarrow t = \frac{1}{C_0} \ln(1 - x).$$  \hspace{1cm} (12)

The set of lifetimes of photons, recorded during the simulation, is combined into a histogram.

The Monte Carlo simulations were performed over a suitably high number of simulation’s step ($10^5$ times), to obtain well averaged results.

**Results and discussion**

In the current work, we proposed the development of a simple antenna model, which has potential to become a valuable tool in design and interpretation of FRET experiments. Our model successfully predicts the enhancement of transfer in the presence of multiple acceptors. Moreover, by replacing the fixed distance between donor and attached protein by mathematical function of distance distribution and by assuming different shape and size of the protein, we can extend a range of systems possible to simulate using our MC model.

As shown in the work of Bojarski et al. [22], the parameters strongly influencing the FRET efficiency are both the orientation and number of fluorophores. In this study we focus only on two specific fluorophores orientations. First, the molecules are randomly oriented with the simulated average value of $< \kappa^2 >$ close to the theoretical value of $2/3$ (dynamic case) and second, where molecular transition moments are fixed in space with perfect parallel orientation ($< \kappa^2 > = 4$). The proposed method can be easily applied also to describe partly ordered systems [23], important from a biological and biophysical point of view.

To examine the effect of the number of acceptors on the FRET efficiency, suitable simulations were carried out. Figure 2 shows the dependence of the FRET efficiency in the presence of 1, 5 and 10 acceptors attached to the spherical protein with 40 Å diameter, as a function of the distance $R$. $R_0$ was assumed to be 80 Å, accordingly to recently available fluorescence probes [5, 24] and the distance is approximated by the rigid linker. Two orientation cases, random (black lines) and parallel (light blue lines), were considered. It can be seen, that increasing the number of acceptors to only five, for the distance $R=150$ Å results in a FRET efficiency of 6 %, and for ten acceptors it is 11 % (almost 40 % in the parallel case), whereas for a single acceptor the value of FRET efficiency is close to zero. This means that using multiple acceptors, it is possible to obtain the measurable values of FRET efficiency even at the distances of 150 Å, which can extend the useful range of energy transfer as a spectroscopic ruler. Moreover, the system with parallely oriented fluorophores, can act as a powerful antenna system and strongly increase the efficiency of collecting the excitation energy from the donor molecule.

Another aspect, we would like to consider is the influence of the size and shape of the protein. Due to the strong dependence of transfer efficiency on the absolute distance between donor and each acceptor, one can expect, that the FRET value changes as the relative distribution of acceptors over a protein varies. In Fig. 3, we present the simulations results for a different shapes of attached protein. We assumed the fixed donor–protein distance $R=120$ Å and the Förster radius of 80 Å. The volume of attached protein

![Fig. 2 Transfer efficiency as a function of the distance $R$ for different number of acceptors (1, 5, 10) for random (so called dynamic case) and parallel orientation of acceptor transition moments. The shape of attached protein is assumed to be spherical (radius of protein $r=20$ Å) with fixed donor–protein distance](image-url)
remains the same in all cases and equals to volume enclosed by a sphere with radius $r=20\,\text{Å}$. This volume is regarded to be adequate to the volume of typical miniantibody [5].

The simulations were performed for four different shapes of attached macromolecule: a sphere, a cylinder with height $h=60\,\text{Å}$ and radius $r=13.34\,\text{Å}$, a prolate elipsoid with semi-major and semi-minor axis equal 55.56 and 12 Å and an oblate elipsoid with axis $a=12\,\text{Å}$ and $b=25.82\,\text{Å}$. These protein shapes can correspond with, for example, a cylindrical chaperonin GroEL [25] and elipsoidal N-terminal domain of enzyme I (EIN) [26]. The effect of protein shape results in discrepancy in FRET efficiency values both in the case of parallel (light blue lines) and randomly distributed transition moments of acceptors (black lines). The highest values of transfer efficiencies are obtained for the oblate elipsoid and spherical shape of attached molecule, what results from relatively shorter distances between donor and each acceptor.

The simulations of expected FRET as a function of protein’s size are shown in Fig. 4. To simplify the calculations, the spherical shape of the protein and fixed donor–protein distance are assumed. In the case of a small protein ($r=10\,\text{Å}$) the presence of five acceptors results in FRET efficiency of 10 %. To obtain the same value of FRET for larger molecule, like fragment of an antibody (diameter of 55 Å), the number of fluorophores randomly attached to the sphere should increase to ten.

The effect of different functions of distance distributions can be clearly seen in Fig. 5, where an intensity decay of donor for four cases is shown. The decay curve in the absence of acceptors is, in the simplest case, described by a normal function approximating the distribution of the distances $R$. The shape of attached protein is assumed to be spherical with $r=20\,\text{Å}$. 

![Fig. 3](image1.png)

**Fig. 3** Transfer efficiency as a function of the distance $R$ for different shapes of attached protein, in the presence of five acceptors for a random and parallel orientation of molecules. The distance between the donor and the protein is fixed

![Fig. 4](image2.png)

**Fig. 4** Transfer efficiency as a function of globular protein diameter in the presence of multiple acceptors (1, 5, 10) for a random and parallel orientation of molecules. The distance between the donor and the protein is fixed

![Fig. 5](image3.png)

**Fig. 5** Time-resolved fluorescence intensity decay of donor in the donor-only and multiple acceptors case, for different distributions of the distance $R$. The shape of attached protein is spherical

![Fig. 6](image4.png)

**Fig. 6** Comparison of intensity decays of donor in the case of different values of standard deviation $\sigma$ for a normal function approximating the distribution of the distances $R$. The shape of attached protein is assumed to be spherical with $r=20\,\text{Å}$
a single exponential function, shown by black, dashed line. Adding the acceptors into the system results in shortening of the average lifetime of donor due to the energy transfer. While the distance between the donor and the protein is fixed, the intensity decay function remains single exponential (light blue, solid line). It is noteworthy that using the distribution function instead of the fixed distance results in changes of the nature of the decay. The range of distances results in a range of decay times. Due to this, the donor decay curve becomes more complex than a single exponential (light blue, dashed line and black, solid line). The shape of attached protein is assumed to be spherical with radius $r=20\,\text{Å}$. Ten acceptors were attached to the protein.

The effect of donor intensity decay on the half width ($hw$) parameter in the normal distribution of the distances is shown in the Fig. 6. Simulations were performed for the spherical shape of attached protein with radius $r=20\,\text{Å}$, labeled with five acceptors. As mentioned above, using normal distribution resulted in non-exponential decay curve. The effect is more visible as the half width parameter increases. The wider distribution function means, that acceptors are generally more closely located. As a result, the rate of energy transfer calculated for the donor and each acceptor is increased which leads to overall higher transfer efficiency and shorter decay curve (light blue, solid line and black, dashed line). For the narrow distribution, the nonexponential effect is less visible, and decay curve goes to the single exponential function as $hw$ goes to zero (black, solid line).

In order to test our model and show the influence of using different distance distributions on the final results, we used experimental results described by Maliwal et al. [5] As the experimental antenna system they used the 30-mer ds oligonucleotide with biotin on the 3′-end and Alexa-568 (donor, A568) on the 5′-end. Earlier studies have shown that the distance between opposite ends of the DNA fragment varies around 100 Å in the absence of conformational changes [27]. To build the antenna system, the avidin molecule was labeled with different numbers of fluorophore acceptors DyLight-649 (DL649) and DyLight-750 (DL750). Results was compared with the simple non-antenna system created by labeling the donor-oligonucleotide system with single acceptor. Table 1 presents the results of our simulations compared with the experimental data of Maliwal et al. [5]. Simulations were performed for the oligonucleotide labeled with donor and single acceptor (sample 1) and for the antenna model with spherical protein attached (samples 2–4). In the first case, the effective donor–acceptor separation $R$ used in simulations was increased by the length of fluorophores (5 Å added by both the donor and acceptor molecules) and equal 110 Å for the approximation of rigid chain. The avidin molecule attached to the system, increased the average distance between donor and acceptor by another 25 Å (the avidin radius). In different environments or at different temperatures the same macromolecule with the attached donor acceptor pair can appear in different conformations. Therefore we performed calculations for a couple of distribution parameters. We calculated the FRET efficiency for four distributions of donor to acceptor distance: a narrow normal distribution determined by standard deviation of $\sigma=5$, the wider normal distribution with $\sigma=18$, the ideally flexible and rigid chain. The average donor lifetimes were calculated for the simplest case of fixed distance between the donor and the protein.

Another aspect, we would like to mention is the influence of the size and shape of attached fluorophores. In our model the length of donor and acceptor is simply added to the distance. $R$ is increased by 10 Å for both the donor and the acceptor in the single acceptor case and by 5 Å in the donor-avidin system [5]. The influence of the shape of fluorophores is neglected in our model, nevertheless it could have a significant influence for systems in which sizes of a donor and an acceptor are comparable with attached protein.

**Conclusions**

The antenna effect significantly extends the range of using FRET in measurements of fluorophores distance. In standard systems the upper limit for distance measurements via energy transfer effect is 80–100 Å. However, by replacing single acceptor with the group of closely located acceptors, FRET simulations were performed for the donor–protein distance described by Gaussian distribution with two different $\sigma$ values and for the case of ideally flexible and rigid linker.
efficiency can be measurable even at the distances exceeding 150 Å, especially if acceptors form an organized structure (parallel transition moments). It occurs that the shape of the macromolecule strongly affects the energy transfer efficiency. The same separation energy transfer differs strongly for oblate ellipsoid, prolate ellipsoid, cyndindrical shape or sphere. The maximal expected difference in the energy transfer efficiency can even exceed 50%.

By means of the Monte Carlo simulations, we can easily adjust the parameters of the antenna system, to improve the designing and interpretation of FRET experiments. We demonstrated the theoretical potential of our variant of the MC approach. Instead of assuming the fixed distance between the donor and the group of acceptors [1, 6], we proposed using the different distance distributions and shapes of attached protein, depending on the characteristics of simulated systems. This approach allows to analyze the variety of larger biological systems and can be used in designing specific FRET experiments above 100 Å.

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