Fluorescence blinking of single major light-harvesting complexes

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Abstract. Recent time-resolved studies have revealed the switching behavior of single photosynthetic light-harvesting complexes. In this work, we suggest a conceptual diffusion-controlled model, which is able to describe essential protein dynamics underlying this switching phenomenon. The calculated blinking statistics is compared with the experimental results measured under various experimental conditions and not only reproduces the power-law behavior at intermediate times, but also follows the experimentally observed deviations from such behavior on a shorter timescale. We find that even under ordinary light-harvesting conditions, some antenna complexes are quenched and their fraction noticeably increases in a more acid environment. As a result, the lability of the protein scaffold allows the coexistence of light-harvesting and excitation-quenching states and therefore gives rise to regulatory switching known as non-photochemical quenching.

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1. Introduction

Already by the first experimental observations [1], single-molecule spectroscopy (SMS) has proven to be a valuable tool to inspect the subtle properties of optical transitions of individual molecules not obscured by the ensemble average. Indeed, traditional spectroscopic methods allow researchers to measure only some statistically averaged quantities, describing the system as a whole. In this case, the probability distribution of various quantities describing the system, their dynamic and/or static variations caused by the heterogeneity of the system characteristics, such as, for instance, fundamental interactions between distinct molecules and their proximate environment, remain undetermined. The ability to reveal such properties using SMS and therefore to obtain much more new information resulted in growing interest in the possible applications of SMS techniques not only to simple fluorophores like dye molecules [2, 3] or colloidal semiconductor quantum dots (QDs) [4] but also to complex biological systems such as green fluorescent proteins [5] or even pigment–protein light-harvesting complexes (LHCs) [6–11]. SMS methods have also been successfully applied in labeling experiments incorporating simple fluorophores being attached to complex macromolecules, thus providing valuable information about molecular interactions, reaction kinetics, conformational dynamics or molecular motion [12, 13].

SMS measurements have also revealed several unexpected properties of the individual molecule, such as spectral diffusion—the phenomenon when transition frequencies of a molecule change due to some variations of its local surroundings. Another intriguing effect discovered by SMS is the so-called fluorescence intermittency, or blinking. In virtually all fluorescing systems studied to date at the single-molecule level, the measured fluorescence intensity fluctuates rapidly and abruptly despite continuous illumination [14, 15]. The sudden and uncorrelated fluctuations occur mostly between two well-defined strongly and non- or weakly emitting levels (the corresponding states are commonly referred to as on- and off-states, respectively) and usually serve as a simple signature of single emitters. In the early studies of fluorescence blinking of single molecules in molecular crystals [16], it was found out that the probability of various time intervals spent by the system in the on- and off-states (on- and off-times) to a large extent could be described by a single-exponential distribution, as predicted by the quantum jump theory of transitions between singlet and metastable triplet states [17]. Later,
blinking behavior with much longer off-times that could not arise from intersystem crossing was also discovered [15]. In most cases off-times varied across almost all experimentally accessible timescales, typically spanning over four orders of magnitude or even from microseconds to several hours in the case of semiconducting QDs [18]. Moreover, in almost all these very diverse systems the dwell times $t$ of both on- and off-states were not exponentially distributed: their probability densities $P(t)$ followed an inverse power law of the form $P(t) \propto t^{-m}$, with the exponent $m$ typically lying between 1 and 2 [15]. Despite much research in this field, the explanation for probably one of the most intriguing riddles of SMS—why all these diverse systems of various complexities exhibit very similar blinking statistics leading to the absence of a typical time scale and even to weak ergodicity breaking [17]—still remains unanswered.

In order to resolve (at least partially) this problem, several models describing fluorescence blinking in semiconducting QDs have been proposed so far. In these models, the dark state of the QD is associated with the photoejection of an electron. According to the so-called trap models [14, 19, 20], the electron can tunnel through a barrier to a trap located nearby, and the dark period ends when the trapped electron hops back. Alternatively, power-law blinking statistics naturally arises if one considers a one- or two-dimensional random walk involving a first-passage time. In 2005, Tang and Marcus [21, 22] suggested a diffusion-controlled electron-transfer model, where a light-induced one-dimensional diffusion in energy space was considered. In addition, some more models of power-law statistics have been proposed, but none of the existing theories can explain all the experimental observations of the phenomenon of blinking. Moreover, no or very limited theoretical background regarding fluorescence blinking in other—biological—systems exists.

Recently, new data on fluorescence intermittency in the single major LHCs (LHCII) of green plants have been collected [10, 11]. These complexes comprise the major part of the photosynthetic light-harvesting antenna and are composed of aggregates of pigment molecules (chlorophylls (Chls) and carotenoids) bound to a protein scaffold. The arrangement of the pigments within the complex and of the complexes within the whole photosystem II (PSII) ensures the optimal collection of solar energy and its delivery to the reaction center (RC) of PSII, where water-splitting reactions take place and the excitation energy is then stabilized in the form of a trans-membrane electrochemical potential necessary for the subsequent stages of photosynthesis [23, 24]. From the point of view of the efficiency of the process of excitation energy transfer to the RC, the allowed spectral variability cannot be too large, and a contribution from quantum coherence to some extent may counteract the intrinsic protein disorder [25]. On the other hand, under bright sunlight this highly efficient light-harvesting process has a negative impact on photosynthetic organisms, since although being very fast, the turnover rate of the RCs is still finite, and the increased illumination levels just saturate the process of charge separation in the RCs. Long-living excitations, in their turn, can induce the formation of free radicals and/or singlet oxygen capable of damaging the whole PSII. Over long ages of evolution, plants ‘have learned’ how to deal with this excess excitation energy at the molecular level and, when needed, safely dissipate it as heat [26]. Firstly, the protein scaffold of the RCs is expected to adapt to the varying external electromagnetic field by efficiently regulating the process of charge separation [27, 28]. In addition, another regulatory process, termed non-photochemical quenching (NPQ), takes place in the LHCs with the involvement of their carotenoid molecules [29–32]. As a result, the LHCII complexes are able to switch quickly and reversibly between an almost perfect light-harvesting state and an almost perfect excitation-quenching state, as was demonstrated by means of a simple thermodynamic approach [33–35],
and therefore ensure high efficiency and robustness of plant photosynthesis under fluctuating light, even at very high intensities. 

Surprisingly, SMS fluorescence measurements [10, 11] of single LHCII complexes with a very complex internal biomolecular structure revealed very similar blinking behavior as in structurally much simpler fluorescent dyes or QDs. Furthermore, like in other systems, the dwell times of both on- and off-states were found to be distributed according to a power law and strongly dependent on various environmental conditions, such as acidity level or illumination intensity [10, 36]. Taking into account the important physiological functions of LHCII complexes responsible for very efficient light harvesting and excitation energy transfer as well as regulation via NPQ, it is very unlikely that this functional significance and fluorescence properties of the LHCIIIs can be unrelated. Since the models describing fluorescence intermittency in QDs cannot be directly applied to these biological complexes, the physical mechanisms underlying power-law blinking of single LHCII still remain unclear. A conceptual model based on the dynamic self-organization of the intrinsic structure of the LHCII was introduced recently [37]. It was assumed that due to some specific conformational change of the protein scaffold the energetic equilibrium in the LHCII trimer can be distorted and new paths for excitation energy transfer from the excited Chl molecules to the carotenoid lutein 1 (Lut 1) can be opened [31], resulting in very efficient fluorescence quenching. This NPQ scenario is supported by recent studies based on quantum-chemical calculations of the properties of corresponding molecules [38]. In this work, this conceptual model is further developed and a more detailed mathematical description is given, supplemented with numerical simulations and discussion on the fundamental molecular origin of NPQ.

2. Model for fluorescence blinking

In order to describe fluorescence intermittency in the LHCII complexes, we assume that as a consequence of the precise protein structural arrangement and dynamics, the LHCII trimer can be found in two equilibrium states: either in a light (on) state, in which the fluorescence signal from the irradiated LHCII trimer is clearly detected, or in a dark (off) state, in which the fluorescence is strongly quenched. Switching between these two states is probably related to a subtle conformational change of the protein, which disturbs the energy balance between different pigments. The occurrence of such conformational switches was recently demonstrated experimentally by measuring the effect of exciton annihilation in LHCII complexes imbedded in various environments [39]. Variability of the distance between the Lut 1 molecule and the cluster of three Chl a molecules, Chl a610–611–612, located nearby and constituting the lowest energy exciton states [40–42] can be considered as the dominant modulation factor of this energy balance. Another possible effect could be the lowering of the dark $S_1$ state of Lut 1 [43, 44]. In both cases, the self-regulating process of the formation of centers responsible for excitation energy quenching would shorten (or prolong) the mean excitation lifetime of the Chl molecules and consequently, the intensity of the measured fluorescence would vary in time.

To summarize, when the LHCII is in the on-state, the configuration of Lut 1 and Chl a610–611–612 molecules is unfavorable for excitation quenching to occur. Owing to the fast intramolecular dynamics of the Chls and their interactions with the protein vibrations (phonons) the energy of this state fluctuates rapidly in the vicinity of the minimum of the potential well of the on-state. Let us denote the generalized coordinate which describes the manifold of all these fast vibrations as $X$. As a result of a ‘slow’ deformation of the protein (let us introduce another
Figure 1. Potential surfaces of the on- and off-states in the phase space of the $X$ and $Y$ coordinates. $k_1$ and $k_2$ denote the relaxation rates of the downward $\text{on} \rightarrow \text{off}$ and $\text{off} \rightarrow \text{on}$ transitions, respectively.

generalized slowly varying coordinate $Y$ representing the slow structural change of the protein), the protein configuration in the vicinity of the cluster of Chl molecules changes, resulting in a decrease of the mean fluorescence intensity. Thus the system undergoes a transition into the off-state, corresponding to another potential well in the phase space of the coordinates $X$ and $Y$. Since the LHCII trimer can be in only one state at a time, the random walk in phase space will lead to a random and rapid switching between on- and off-states, which will qualitatively resemble the experimentally obtained effect of fluorescence intermittency (a somewhat similar concept was employed to describe fluorescence blinking in semiconducting QDs [22] and structural regulation in biological macromolecules [45, 46]).

In the simplest harmonic approximation the potentials of light and dark states can be written as

$$U_1(X, Y) = \frac{1}{2}\lambda_1 X^2 + \frac{1}{2}\gamma_1 Y^2,$$

$$U_2(X, Y) = \frac{1}{2}\lambda_2 (X - X_0)^2 + \frac{1}{2}\gamma_2 (Y - Y_0)^2 + U_0,$$

(1)

where indices ‘1’ and ‘2’ denote on- and off-states, respectively; $X$ and $Y$ are the generalized coordinates responsible for fast intramolecular vibrations of the Chls and slow structural conformations of the protein, respectively; $\lambda_i$ and $\gamma_i$ describe reorganization energies in the $i$th potential along the corresponding coordinate; $X_0$ and $Y_0$ determine the equilibrium position of the second potential surface; $U_0$ is the vertical shift between the potential minima. The schematic relative position of these potentials is represented in figure 1. If $Y \approx 0$, a common set of parameters will yield $U_1(0, 0) < U_2(X_0, 0)$, hence, the system will more probably be in the on-state. The vanishing probability of the excitation quenching in this scheme corresponds to the activated transition from the phase space point $(X, Y) \approx (0, 0)$ with the energy $U_1(0, 0)$ on the on-state potential to the same point with the energy $U_2(0, 0) \gg U_1(0, 0)$ on the potential.
of the off-state. After such a transition only the coordinate \( X \) changes rapidly (variations of \( Y \) occur on a much longer timescale), so the system, keeping the \( Y \) coordinate at fixed position \( Y = 0 \), quickly ‘diffuses’ to the potential minimum \( U_2(X_0, 0) \). From here the system will rapidly ‘fall’ back to the on-state potential with the energy \( U_1(X_0, 0) \ll U_2(X_0, 0) \) and finally ‘diffuse’ to the initial phase space point with the energy \( U_1(0, 0) \), which will lead to the recovery of fluorescence intensity.

Owing to some specific, environmentally induced conformational change of the protein, the \( Y \) value can increase, and in the vicinity of the \( Y \approx Y_0 \) configuration we will get \( U_1(0, Y_0) > U_2(X_0, Y_0) \). As a result, the system will probably switch to the off-state and therefore the fluorescence signal from the LHCII trimer will become quenched. Similar to the case discussed above, there exists a very small probability that for some reason the excitation energy quenching will terminate, which is represented by the activated transition \( U_2(X_0, Y_0) \rightarrow U_1(X_0, Y_0) \), after which the system rapidly ‘diffuses’ along the \( X \) coordinate to \( U_1(0, Y_0) \). However, at that point there is a very high probability for the system to relax to the much lower energy \( U_2(0, Y_0) \) and ‘diffuse’ back to the potential minimum \( U_2(X_0, Y_0) \).

For the sake of simplicity, we can also assume that the transitions between the on- and off-states occur strictly vertically, i.e. during the transition, the coordinates \( X \) and \( Y \) do not change. These transitions are shown in figure 1. In addition, let us also assume that the rate of relaxation (downward transition) from the phase space point \( A \) on the on-state potential to the phase space point \( B \) with the same coordinates on the off-state potential is equal to \( k_1 \) and does not depend on the position of the points \( A \) and \( B \). Similarly, the rate of downward transition \( C \rightarrow D \) from the off- to the on-state is denoted as \( k_2 \). The rates of the upward transitions are renormalized by taking into account the corresponding Boltzmann factor \( \exp(-|\Delta U|/(k_B T)) \), which introduces the dependence on the coordinates of the points \( A \) and \( B \) as well as \( C \) and \( D \). To summarize, the rates of the downward and upward transitions are assumed to be defined in the following way:

\[
\begin{align*}
A \rightarrow B & : \quad k_1, \\
C \rightarrow D & : \quad k_2, \\
B \rightarrow A & : \quad k_1 \exp\left(\frac{U_2(X,Y_0)-U_1(X_0,Y_A)}{k_B T}\right), \\
D \rightarrow C & : \quad k_2 \exp\left(\frac{U_2(X,Y_0)-U_1(X_0,Y_D)}{k_B T}\right),
\end{align*}
\]

where \( k_B \) is the Boltzmann constant, and \( T \) is the temperature.

From the energy gap law [47], which describes the non-radiative transitions between different energy levels by taking into account the interactions with the environmental phonons, it follows that in order to calculate the real (effective) transition rates, the ones defined in (2) should be multiplied by the factor

\[
\exp\left(-\alpha \frac{|U_1(X,Y) - U_2(X,Y)|}{\hbar \omega_0}\right),
\]

where \( \omega_0 \) is the dominating frequency of the phonons taking part in the transitions between the on- and off-states, and \( \alpha \) is some function, weakly (logarithmically) depending on the potential energy difference \( |\Delta U| \), so that we can treat it as some constant parameter (\( \alpha \approx 1–3 \)). This exponential factor ensures that both upward and downward transitions occur mainly in the vicinity of the line \((X^{(s)}, Y^{(s)})\), which corresponds to the intersection of the two potentials: \( U_1(X^{(s)}, Y^{(s)}) = U_2(X^{(s)}, Y^{(s)}) \). Upon moving away from this line, the potential energy difference \( |\Delta U| \) increases rapidly, so that the number of phonons taking part in the transition also rises and the transition probability decreases exponentially.
In order to reduce the number of unknown parameters and to simplify the set of equations, let us scale the potential energy in thermal units and thus introduce non-dimensional potentials

\[ V_i(X, Y) = \frac{U_i(X, Y)}{k_B T}, \quad i = 1, 2 \]  

as well as non-dimensional coordinates

\[ x = \sqrt{\frac{k_B T}{\lambda_1}} X, \quad x_0 = \sqrt{\frac{k_B T}{\lambda_1}} X_0, \quad y = \sqrt{\frac{k_B T}{\gamma_1}} Y, \quad y_0 = \sqrt{\frac{k_B T}{\gamma_1}} Y_0. \]  

Now, by denoting

\[ \frac{\lambda_2}{\lambda_1} = \lambda, \quad \frac{\gamma_2}{\gamma_1} = \gamma, \quad \frac{U_0}{k_B T} = V_0, \]  

we obtain the simplified expressions for the potential wells:

\[ V_1(x, y) = \frac{1}{2} \lambda (x - x_0)^2 + \frac{1}{2} \gamma (y - y_0)^2 + V_0. \]

\[ V_2(x, y) = \frac{1}{2} \lambda (x - x_0)^2 + \frac{1}{2} \gamma (y - y_0)^2 + V_0. \]

The time-dependent probability densities \( \rho_i(x, y, t) \) for finding values \( x \) and \( y \) at time \( t \), when the system is either in the on- or in the off-state, obey slightly modified Smoluchowski diffusion equations in the potential field [22, 48]:

\[
\frac{\partial}{\partial t} \rho_1(x, y, t) = (D_x \mathcal{L}_x + D_y \mathcal{L}_y) \rho_1(x, y, t) + \exp \left( -\alpha \frac{|\Delta U|}{\hbar \omega_0} \right) \\
\times \left[ k_2 \rho_2(x, y, t) - k_1 e^{V_1(x, y) - V_2(x, y)} \rho_1(x, y, t), \quad V_1(x, y) \leq V_2(x, y), \right. \]

\[
\left. k_2 e^{V_1(x, y) - V_2(x, y)} \rho_1(x, y, t) - k_1 \rho_2(x, y, t), \quad V_1(x, y) > V_2(x, y); \right. \]

\[
\frac{\partial}{\partial t} \rho_2(x, y, t) = (D_x \mathcal{L}_x + D_y \mathcal{L}_y) \rho_2(x, y, t) + \exp \left( -\alpha \frac{|\Delta U|}{\hbar \omega_0} \right) \\
\times \left[ k_1 e^{V_1(x, y) - V_2(x, y)} \rho_1(x, y, t) - k_2 \rho_2(x, y, t), \quad V_1(x, y) \leq V_2(x, y), \right. \]

\[
\left. k_1 \rho_1(x, y, t) - k_2 e^{V_1(x, y) - V_2(x, y)} \rho_2(x, y, t), \quad V_1(x, y) > V_2(x, y), \right. \]

where \( D_x \) and \( D_y \) are the diffusion coefficients along \( x \) and \( y \) directions, respectively, while \( \mathcal{L}_x \) and \( \mathcal{L}_y \) are the corresponding diffusion operators defined as follows:

\[ \mathcal{L}_z \rho_i(x, y, t) = \left[ \frac{\partial^2}{\partial z^2} + \frac{1}{k_B T} \frac{\partial}{\partial z} \frac{\partial U_i(x, y)}{\partial z} \right] \rho_i(x, y, t), \quad i = 1, 2, \quad z = x, y. \]

The coupled diffusion-controlled rate equations (7) and (8) describe the unconditional evolution of the probability density of the on- and off-states after some initial population. To compare the calculated blinking statistics for an on- (or off-) event with the experimental data, we need to calculate the conditional probability density \( P_i(t) \) (or \( P_2(t) \)) for the system to remain in the on- (or off-) state for the whole period \( t \). These conditional probability densities can be easily obtained if we decouple equations (7) and (8) by setting the population of the
off-state in (7) and the population of the on-state in (8) to zero during the whole observation time interval \( t \):

\[
\frac{\partial}{\partial t} \rho_1(x, y, t) = \left( D_x \mathcal{L}_x + D_y \mathcal{L}_y \right) \rho_1(x, y, t) - k_1 \exp\left( -\frac{|\Delta V|}{\Omega} \right) \rho_1(x, y, t)
\]

\[
\times \begin{cases} 
  e^{V_1(x,y) - V_2(x,y)}, & V_1(x, y) \leq V_2(x, y), \\
  1, & V_1(x, y) > V_2(x, y), 
\end{cases}
\]

\[
\equiv \left( D_x \mathcal{L}_x + D_y \mathcal{L}_y - k_1 H_1(x, y) \right) \rho_1(x, y, t),
\]  

\( (10) \)

\[
\frac{\partial}{\partial t} \rho_2(x, y, t) = \left( D_x \mathcal{L}_x + D_y \mathcal{L}_y \right) \rho_2(x, y, t) - k_2 \exp\left( -\frac{|\Delta V|}{\Omega} \right) \rho_2(x, y, t)
\]

\[
\times \begin{cases} 
  1, & V_1(x, y) \leq V_2(x, y), \\
  e^{V_2(x,y) - V_1(x,y)}, & V_1(x, y) > V_2(x, y), 
\end{cases}
\]

\[
\equiv \left( D_x \mathcal{L}_x + D_y \mathcal{L}_y - k_2 H_2(x, y) \right) \rho_2(x, y, t),
\]  

\( (11) \)

where we denote

\[
\Omega = \frac{\hbar \omega_0}{\alpha k_B T}
\]

and introduce new functions

\[
H_1(x, y) = e^{-|\Delta V|/\Omega} \cdot \min \left\{ 1, e^{V_1 - V_2} \right\},
\]

\[
H_2(x, y) = e^{-|\Delta V|/\Omega} \cdot \min \left\{ 1, e^{V_2 - V_1} \right\}.
\]  

\( (12) \)

As already mentioned, we assume that the diffusion along the \( x \) coordinate is much faster than that along the \( y \) coordinate, hence (10) and (11) can be reduced. Indeed, by assuming that \( D_x \gg D_y \) and denoting

\[
\eta = D_x / D_y \gg 1, \quad \tau = D_y t, \quad K_i = k_i / D_y,
\]

(10) can be rewritten as

\[
\frac{\partial}{\partial \tau} \rho_1(x, y, \tau) = \left( \eta \mathcal{L}_x + \mathcal{L}_y - K_1 H_1(x, y) \right) \rho_1(x, y, \tau).
\]  

\( (13) \)

Since \( \eta \gg 1 \), the dependence of \( \rho_1 \) on \( x \) can be evaluated by using the adiabatic approximation similar to the Born–Oppenheimer approximation in quantum mechanics. In the zero-order approximation the motion along the \( y \) coordinate can be considered completely frozen, hence, by taking into account the definition of \( \mathcal{L}_x \) (see (9)) and potential \( V_1(x, y) \) (see (6)), the last equation (13) is reduced in the following way:

\[
\frac{\partial}{\partial \tau} \rho_1(x, y, \tau) \simeq \eta \mathcal{L}_x \rho_1(x, y, \tau) = \eta \frac{\partial}{\partial x} \left( x + \frac{\partial}{\partial x} \right) \rho_1(x, y, \tau).
\]  

\( (14) \)

Owing to a large \( \eta \), the solution of this equation very quickly converges to a stationary (Gaussian) distribution along the \( x \) coordinate, so that we can approximate it as

\[
\rho_1(x, y, \tau) \simeq \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}x^2} \rho_1(y, \tau),
\]
Figure 2. Potential energies $V_i$ and initial probability density $\tilde{\rho}_i(y, \tau = 0)$ calculated according to (19) using the parameters obtained by fitting the experimental data collected at pH 8. The arrows indicate the corresponding vertical axis.

where $\tilde{\rho}_1(y, \tau)$ satisfies the equation

$$\frac{\partial \tilde{\rho}_1(y, \tau)}{\partial \tau} \equiv \frac{\partial}{\partial \tau} \int dx \, \rho_1(x, y, \tau) = (L_y - \kappa_1(y)) \tilde{\rho}_1(y, \tau).$$

Similarly, for the off-state we obtain

$$\frac{\partial \tilde{\rho}_2(y, \tau)}{\partial \tau} \equiv \frac{\partial}{\partial \tau} \int dx \, \rho_2(x, y, \tau) = (L_y - \kappa_2(y)) \tilde{\rho}_2(y, \tau).$$

Here, the new functions defined as

$$\kappa_1(y) = K_1 \frac{1}{\sqrt{2\pi}} \int dx \, e^{-\frac{1}{2}x^2} H_1(x, y),$$

$$\kappa_2(y) = K_2 \sqrt{\frac{\lambda}{2\pi}} \int dx \, e^{-\frac{1}{2}x^2(x-x_0)^2} H_2(x, y)$$

are introduced.

The initial conditions needed to solve equations (15) and (16) can be chosen as follows. Firstly, we define the stationary solution $\tilde{\rho}_1^{(st)}(y)$ of (15) when the transition to the off-state is inactive. Then, we multiply this steady-state solution (the Gaussian distribution) by the effective rate $\kappa_1(y)$ given by (17). The obtained function determines the initial distribution of the population of the off-state: $\tilde{\rho}_2(y, \tau = 0) \propto \tilde{\rho}_1^{(st)}(y)\kappa_1(y)$. Similarly, the initial probability density for the population of the on-state is given by the product $\tilde{\rho}_2^{(st)}(y)\kappa_2(y)$. It is noteworthy that after substituting the expressions for $H_i(x, y)$ (see (12)) and normalizing, both initial distributions coincide:

$$\tilde{\rho}_i(y, \tau = 0) \propto \int dx \, \exp\left(-\frac{|\Delta V|}{\Omega_2}\right) \min\{\exp(-V_1(x, y)), \exp(-V_2(x, y))\}. \tag{19}$$

A more detailed numerical analysis reveals that (19) defines a very sharp distribution with the maximum located at the intersection point $y^{(0)}$ of the one-dimensional functions $V_1(x = 0, y)$ and $V_2(x = x_0, y)$ (see figure 2).
After numerically solving equations (15) and (16), we need to calculate the survival probabilities of the on- and off-states, $S_i(\tau)$, which define the probabilities that after the transition the system is still in the same on- or off-state during the whole observation period $\tau$. These survival probabilities can be found by integrating $\bar{\rho}_i(y, \tau)$ over $y$:

$$S_i(\tau) = \int d y \bar{\rho}_i(y, \tau).$$

Finally, the density of this survival probability, which corresponds to the experimentally gathered blinking statistics and determines the probability $P_i(\tau)$ that a transition from one state to another occurs within the time interval $(\tau; \tau + d\tau)$, is defined as

$$P_i(\tau) = -\frac{dS_i(\tau)}{d\tau}, \quad i = 1, 2. \quad (21)$$

### 3. Modeling results

As follows from the model description, after introduction of the non-dimensional variables only eight undefined parameters have been left: five of them describe the potential surfaces ($\lambda$, $\gamma$, $x_0$, $y_0$ and $V_0$) while the other three determine the transitions between the on- and off-states ($k_1$, $k_2$ and $\Omega$). All of these parameters were varied by fitting the experimental data of the pH-dependent blinking statistics [10]. We note that the diffusion coefficient $D_y$ only determines the timescale of the protein conformation dynamics and therefore does not change the shape of the $P_i(t)$ distributions on a logarithmic scale but, since

$$\log P_i(\log t) = \log \left( -\frac{d}{d\tau} S_i(\log t) \right) = \log P_i(\log \tau - \log D_y) + \log D_y,$$

only shifts these distributions along the time and probability density axes. Thus, after calculating the blinking statistics with a particular set of the eight mentioned parameters, the obtained curves were shifted along the logarithmic $t$ and $P(t)$ axes to match the experimental distributions, and from the magnitude of this shift the diffusion coefficient was determined. The obtained fitting results for the data collected under different acidity conditions of the environment are shown in figures 3(a) and (b), and the corresponding fitting parameters are presented in figure 4. The value of pH 8 represents the natural physiological conditions corresponding to the state of strong fluorescence of the isolated trimers, while the lower pH values reflect the NPQ conditions. Finally, the survival probabilities of both states, $S_i(t)$, are depicted in figure 3(c). We see that at first they rapidly decrease (the survival probability in the off-state decreases much faster than that in the on-state), but after several seconds they approach an almost constant value.

### 4. Discussion

#### 4.1. Sensitivity of the model

As is clear from figures 3(a) and (b), the distributions of the dwell times, calculated according to the proposed model, not only reproduce the power-law behavior at intermediate times but also follow the experimentally observed deviations from such behavior at shorter timescale. However, we would like to point out that the calculated probability densities exhibit different sensitivities to some variations of different model parameters. This is demonstrated in figure 5,
Figure 3. (a), (b) Experimental (symbols) and simulated (lines) probability densities of the dwell times in bright (a) and dark (b) states for four different acidity levels of the environment. For visual clarity, each dataset was offset upwards by a factor of 100. (c) Survival probabilities of the on- and off-states, $S_{i}(t)$.

Figure 4. Fitted model parameters.

where the effect of an increase (dash-dotted lines) or reduction (dashed lines) of a particular parameter by 50%, compared with the value corresponding to the case of pH 8 and presented in figure 4, is shown. We notice that the most significant variations of the probability densities are produced by changing $y_0$ and $\gamma$, while the influence of all of the other parameters is less pronounced. The curves corresponding to $\lambda$, $x_0$ and $V_0$ are not shown since the mentioned 50% variations of these parameters did not produce any noticeable changes in the probability densities. Such insensitivity to these parameters most probably resulted from averaging over the fast coordinate $x$ performed in (17) and (18) as well as the rather small value of $V_0$ (see figure 4). In order to obtain some noticeable deviations from the fitted curves, at least two-fold changes of these parameters should be assumed.
Figure 5. Sensitivity of the model parameters. Thick black lines correspond to the simulated probability densities of the dwell times in bright (a) and dark (b) states for pH 8 (the same as in figures 3(a) and (b)); the color lines represent calculated probability densities when the corresponding parameter (see legend) was reduced (dashed lines) or increased (dash-dotted line) by 50% while all the other parameters remained unchanged.

4.2. Potential energy surfaces

By analyzing the parameters describing the potential surfaces of the bright and dark states, we see that the slopes of these surfaces along the $x$ coordinate differ several times, and this difference becomes less pronounced as the environmental acidity increases (see the black line in figure 4(a) representing the ratio of these slopes, $\lambda$). On the other hand, the ratio of the slopes of the potential surfaces along the slow $y$ coordinate, $\gamma$, in the near-neutral environment (around pH 6–8) is almost insensitive to the pH variations and approximately equals 1.3 (red line in figure 4(a)). A very weak pH dependence in that region is also obtained for $y_0$, which describes the position of the minimum of the potential energy of the dark state (see the red line in figure 4(b)). These results lead to the assumption that the generalized coordinate $y$, describing slow conformational changes of the protein, is also related to the distance between some particular pigments. Indeed, protonation of some specific protein residues does not directly influence the interaction between the pigments, which mostly depends on their relative arrangement. In such a case, neither the $\gamma$ parameter, which describes the reorganization energy relating to the change of the intermolecular distances, nor $y_0$, defining the inter-pigment arrangement that corresponds to the most effective excitation energy quenching, should exhibit any notable sensitivity to the acidity of the environment. On the other hand, when the environmental acidity increases further, approaching pH 5.5, both $\gamma$ and $y_0$ change noticeably, which can be a manifestation of some kind of phase transition. The almost two-fold increase of $\lambda$ at this pH just confirms this conclusion. The pH dependences of $x_0$ and $V_0$ are slightly non-monotonic (see the black and blue lines in figure 4(b)), which is probably due to some uncertainties in the obtained values. However, we see that generally in the more acid environment the energy difference between both minima decreases more than three times when compared with the physiological conditions. Obviously this would correspond to the increased probability for the system to switch to the non-fluorescing dark state.
4.3. **Diffusions and transitions between the states**

While analyzing the model parameters that determine transitions between the on- and off-states, from the pH dependence of $\Omega$, we first notice that the energy $\hbar \omega_0$ of the dominating phonon mode taking part in the transitions is of the order of $k_B T$ and is almost insensitive to the pH level. This relatively high value of $\Omega$ can also explain the discussed steadying of the survival probabilities of the on- and off-states. As was already mentioned, while modeling the diffusion in the potential wells, the initial probability distribution position $\tilde{\rho}_i(y, t = 0)$ was chosen near the intersection point $y^{(0)}$ of the on- and off-potentials. Owing to the presence of the exponential factor, $\exp(-|\Delta V|/\Omega) \approx 1$, the initial transition probability is rather high, which is reflected in the fast drop of the states’ survival probabilities (see figure 3(c)). During the following time periods the distribution is not only broadening along the $y$ coordinate, but also the position of its maximum is shifting toward the minimum of the corresponding potential. Since the distance $y_0 \approx 7$ is rather big, after some time $\tilde{\rho}_i(y, t)$ diffuses far away from $y^{(0)}$, and wherever $\tilde{\rho}_i(y, t)$ differs from zero, the factor $\exp(-|\Delta V|/\Omega) \rightarrow 0$, so that the transition probability drops almost to 0 and the corresponding survival probability approaches its steady-state regime. On the other hand, due to diffusion $\tilde{\rho}_i(y, t)$ is still expanding, and when the far wing of the probability density reaches $y^{(0)}$, the transition probability will rise again, leading to a further decrease of the population of both on- and off-states. With the current diffusion rates this effect would only become apparent after several tens of minutes, hence, due to photobleaching it was not possible to reveal it during SMS experiments.

As shown in figure 4(c), the rates of transitions from the on- and off-states differ significantly. The rate of the on$\rightarrow$off transition quickly increases upon lowering the pH level, while the rate of the backward transition remains virtually the same, so that the ratio varies between 40 and 50. Such a high ratio of the transition rates in opposite directions obtained for the whole studied pH range reveals why the population of the off-states, $S_2(t)$, decreases in time much faster than that of the on-states ($S_1(t)$, see figure 3(c)). After the transition to the dark state the system generally lives there only for a very short time, so that the measured fluorescence intermittency resembles very short blinking rather than short flashes. At first glance, the higher rate of the on$\rightarrow$off transitions in the more acidic environment would indicate an increased number of short-living on-states. However, the $\sim 1.4$-fold increase of the diffusion coefficient (in the pH 6–6.5 region) determines faster diffusion of the $\tilde{\rho}_i(y, t)$ toward the corresponding potential minimum, so that the overall effect is the experimentally observed stabilization of long-living bright states. This fact is also supported by the slightly higher value of the steady-state survival probability of the on-state (figure 3(c)).

On the other hand, in very acidic surroundings (pH 5.5) the rate of diffusion again decreases very notably, giving another confirmation of the mentioned phase transition occurring in this environment. Qualitatively, the trends of the pH dependence of the diffusion coefficient could be explained in the following way. In the near-neutral environment (around pH 7) the decrease of pH results in the protonation of those protein residues, which are most intimately related to the transitions between the on- and off-states and therefore electrostatic repulsion might increase the rate of those specific deformations responsible for such transitions. However, in a much more acidic environment the protein becomes almost homogeneously protonated, which mitigates the mentioned effect; moreover, the increased protein mass also slows down its deformations, so that finally the diffusion coefficient is reduced. The absolute value of the diffusion coefficient (several seconds) determines the timescale of the conformational dynamics of the protein.
scaffold. The time needed for the system to completely switch from one conformational state to another (the diffusion time) can be evaluated as $T = y_0^2/(2D_y) \approx 7$ s, which is of the same order of magnitude as the times required for conformational changes observed in other photoactive pigment–protein complexes [49]. Rapid forward and backward switching between these two states observable on a much shorter timescale is achieved due to fast molecular fluctuations.

4.4. Dynamic self-organization of light-harvesting complexes II

It is clear that the description of all of the possible conformational changes in the LHCII using only one generalized coordinate as well as simple harmonic potential wells cannot reveal all the subtle details of fluorescence intermittency. However, the rather good fitting results shown in figure 3 suggest that the major properties of the blinking phenomenon are preserved even in such a simplified model. The slight misfitting obtained for the on-state implies that several minima corresponding to the bright state might be expected to co-exist on the potential energy surface. This assumption is supported by the experimental observation [10, 11] revealing several distinct mean fluorescence intensities, which were attributed to the same bright state, with many transitions occurring just among them without switching to the off-state. Taking into account the trimeric structure of the LHCII complexes and the finite [41, 50] inter-complex excitation transfer rate, these multiple bright states can correspond to the varying number of fluorescence quenchers per LHCII trimer, i.e. some monomers may switch to the quenched state while others not. As a result, one would observe different fluorescence intensities and the dark state will correspond to the case when all the monomers become quenched.

Having obtained all of the parameters describing the potential surfaces as well as the switching between them, we can return to the system of coupled equations (7) and (8) and analyze its stationary solution, when $\frac{\partial}{\partial t} \bar{\rho}_i^{(st)}(x, y, t) = 0$, which yields

$$\kappa_1(y) \bar{\rho}_1^{(st)}(y) = \kappa_2(y) \bar{\rho}_2^{(st)}(y).$$

Then the integral $S_i^{(st)} = \int \bar{\rho}_i^{(st)} dy$ represents the probability for the system to be either in the bright ($i = 1$) or in the dark ($i = 2$) state (so that $S_1^{(st)} + S_2^{(st)} = 1$) after dynamic equilibrium between these states has been achieved. For the fitted parameters presented in figure 4, we observe that upon increasing the acidity level from pH 8 to 5.5, the ratio $S_1^{(st)}/S_2^{(st)}$ linearly drops from 150 down to 50. This means that even under ordinary light-harvesting conditions on average one per 150 LHCII trimers is quenched. Such a conclusion can partially explain the experimentally observable excitation energy quenching as well as the heterogeneity of the fluorescence lifetimes in the LHCII aggregates [34, 39, 51, 52]. Indeed, there is nonzero probability that in the aggregated supercomplex at any given time, one or several LHCII trimers will be in their quenching state, which due to the inter-trimer excitation transfer will lead to a shorter fluorescence lifetime compared to the case of the disconnected trimers. Under more adverse environmental conditions the average number of quenchers exhibits a three-fold increase.

Another interesting outcome of our model is the ability to precisely represent the power-law behavior of the probability density for the off-events with the exponents close to $-2$ [10]. Since ordinary one-dimensional random-walk models considering first-passage time distribution predict this exponent to be equal roughly $-3/2$ [53], one needs to assume anomalous diffusion to obtain different exponents [22, 54]. Over the last 20 years it was discovered that many disordered systems, especially biological ones, exhibit either a faster or slower increase in time
of the mean variance than classical Brownian motion predicts, corresponding to the so-called super- and subdiffusion, respectively [53, 55]. It was demonstrated, for example, that in living cells membrane proteins experience subdiffusive behavior [56], since due to the interaction with other particles their mobility decreases. However, the asymptotic distribution of the first-passage times in the case of one-dimensional subdiffusion is proportional to either $t^{-1-\mu}$ or $t^{-1-\mu/2}$, depending on whether the diffusion is bounded or unbounded [53]. Here, $\mu$ describes the rate of diffusion: $\langle (\Delta x)^2 \rangle \propto t^{\mu}$ with $0 < \mu < 1$ (classical diffusion corresponds to $\mu = 1$). We see that even anomalous diffusion could not explain the power-law blinking of single LHCII trimers with the exponent slightly greater (in absolute value) than 2 [10]. However, the model proposed in this work and based on dynamic self-organization processes in the pigment–protein complexes is able to overcome such difficulties and reproduce experimental observations reasonably well. It is also worth noting here that the drop of the absolute value of the mentioned exponent below 2 (as observed at pH 5.5 [10]), when anomalous diffusion can in principle be applied, indirectly confirms our previous assumption about the possible phase transition taking place in the very acidic environment.

5. Conclusions

To summarize, the proposed simple two-state diffusion-controlled model can quantitatively explain the major properties of fluorescence blinking in single LHCII trimers. The model is based on two-dimensional diffusion on the energy surface of the LHCII, with one coordinate reflecting fast inter-molecular vibrations, while the second coordinate is determined by some slow specific structural change of the protein and has a modulating effect on the potential energy surfaces. The reasonably good reproduction of the experimental data strongly suggests that the intrinsic ability of the LHCs to act as an environmentally controlled switch makes them suitable candidates to govern NPQ by fast reversible transitions between almost perfect energy-transfer and quenching states.

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