Biochimica et Biophysica Acta 1797 (2010) 106–112

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Effect of the P700 pre-oxidation and point mutations near A_0 on the reversibility of the primary charge separation in Photosystem I from *Chlamydomonas reinhardtii*

Wojciech Giera [a], V.M. Ramesh [b,c], Andrew N. Webber [b,c], Ivo van Stokkum [d], Rienk van Grondelle [d], Krzysztof Gibasiewicz [a,*]

[a] Department of Physics, Adam Mickiewicz University, ul. Umultowska 85, 61-614, Poznań, Poland
[b] School of Life Sciences, Arizona State University, Tempe, AZ, USA
[c] Center for Bioenergy and Photosynthesis, Arizona State University, Tempe, AZ 85287-4501, USA
[d] Department of Physics and Astronomy, Vrije Universiteit, De Boelelaan 1081,1081 HV Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Received 30 April 2009
Received in revised form 31 July 2009
Accepted 9 September 2009
Available online 15 September 2009

Keywords:
Time-resolved fluorescence
Streak camera
Photosystem I
Chlamydomonas reinhardtii
Electron transfer cofactors
Chlorophyll
Photosynthesis

ABSTRACT

Time-resolved fluorescence studies with a 3-ps temporal resolution were performed in order to: (1) test the recent model of the reversible primary charge separation in Photosystem I (Müller et al., 2003; Holwzwarth et al., 2005, 2006), and (2) to reconcile this model with a mechanism of excitation energy quenching by closed Photosystem I (with P700 pre-oxidized to $P700^+$). For these purposes, we performed experiments using Photosystem I core samples isolated from *Chlamydomonas reinhardtii* wild type, and two mutants in which the methionine axial ligand to primary electron acceptor, A_0, has been change to either histidine or serine. The temporal evolution of fluorescence spectra was recorded for each preparation under conditions where the "primary electron donor," P700, was either neutral or chemically pre-oxidized to $P700^+$. For all the preparations under study, and under neutral and oxidizing conditions, we observed multiexponential fluorescence decay with the major phases of ~7 ps and ~25 ps. The relative amplitudes and, to a minor extent the lifetimes, of these two phases were modulated by the redox state of P700 and by the mutations near A_0: both pre-oxidation of P700 and mutations caused slight deceleration of the excited state decay. These results are consistent with a model in which P700 is not the primary electron donor, but rather a secondary electron donor, with the primary charge separation event occurring between the accessory chlorophyll, A, and A_0. We assign the faster phase to the equilibration process between the excited state of the antenna/reaction center ensemble and the primary radical pair, and the slower phase to the secondary electron transfer reaction. The pre-oxidation of P700 shifts the equilibrium between the excited state and the primary radical pair towards the excited state. This shift is proposed to be induced by the presence of the positive charge on $P700^+$. The same charge is proposed to be responsible for the fast $A^+A_0^- \rightarrow AA_0$ charge recombination to the ground state and, in consequence, excitation quenching in closed reaction centers. Mutations of the A_0 axial ligand shift the equilibrium in the same direction as pre-oxidation of P700 due to the up-shift of the free energy level of the state $A^+A_0^-$.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Photosynthetic reaction centers (RC), in which the energy of electronically excited molecules is converted into the energy of charge separated states, are equipped with two branches (A and B) of electron transfer cofactors embedded in a protein matrix. In a classical view, the primary electron donor is a dimer of more or less strongly interacting molecules of (bacterio)chlorophylls, depending on the organism, positioned at one end of the two branches. Whereas in purple bacterial RCs this view is commonly accepted [1], in Photosystem I (PSI) it was recently proposed that the true primary donor is in fact the accessory chlorophyll (A) positioned in between the chlorophyll dimer (P700) and the chlorophyll serving as the primary electron acceptor (A_0) [2]. According to this model, P700 is a secondary electron donor and gives the electron to A^+ only in the secondary electron transfer step, forming the state $P700^+A_0^-$. A similar sequence of primary electron transfer events was also proposed for Photosystem II [3–5].

Resolving the primary electron transfer steps in Photosystem I is difficult because it binds as many as 90 antenna Chls, in addition to 6 electron transfer Chls [6] and excitation dynamics occurs on the same time scale as that of the primary electron transfer events [7,8]. Decay of the excited states coupled to electron transfer occurs on a 20- to 30-ps time scale [9–11], whereas intrinsic primary charge separation

Abbreviations: A_0, primary acceptor; A_1, secondary acceptor; Chl, chlorophyll; LHCI, light-harvesting complex I; P, primary donor; PSI, Photosystem I; RC, reaction center; WT, wild type

* Corresponding author. Tel.: +48 61 8295265.
E-mail address: krzyszgi@amu.edu.pl (K. Gibasiewicz).

0005-2728/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2009.09.006

from the excited primary donor is much faster and is estimated to occur on the subpicosecond to single picoseconds time scale [12–15]. Formation of the state $P700^+A_0^-$ is followed by 10- to 30-ps electron transfer from A_0^- to A_1, the secondary phylloquinone electron acceptor [12,14,16–18]. Only the next electron transfer step, from A_1^- to first iron-sulfur cluster, F_x, occurs on a slower, nanosecond time scale [19–24], and can be easily separated from the excitated state dynamics. Electron transfer in PSI occurs along both A and B branches of cofactors [22–30].

The different kinetic schemes for excited-state dynamics in PSI discussed in the literature usually assume an irreversible character of the primary charge separation and can be divided, in general, into two models called trap-limited and transfer-to-trap-limited, respectively [31]. In the trap-limited model, the energy equilibration between the antenna and the RC is assumed to be very fast and completed before the trapping event (caused by charge separation in the RC) occurs. In the transfer-to-trap-limited model, the excitation energy transfer to the RC is assumed to be slower than the charge separation step, and in consequence, energy equilibration between the antenna and the RC is not established before trapping occurs. In recent studies of PSI from *Chlamydomonas reinhardtii* and higher plants, the trap-limited model was proposed to describe the observed excited-states dynamics [11,32–35]. The overall kinetics in cyanobacterial PSI complexes was suggested by different groups to be either trap-limited [36,37] or transfer-to-trap-limited [9,10,14,15,38]. Recently, models assuming reversibility of the primary charge separation in PSI, and demonstrating the impact of this reversibility on the excitation dynamics, have been proposed [32,33,35,37].

In order to best resolve the kinetics of pure electron transfer reactions in PSI, not contaminated by the excitation energy dynamics, a few groups have the approach of subtracting the time-resolved transient absorption spectra recorded for the closed state of PSI (P700 pre-oxidized to $P700^+$) from those recorded for the open state (P700 neutral) [39–42]. This approach assumes that the excitation dynamics is identical for the two states, an assumption that is supported by experiments showing roughly similar monoexpoenential excited state decay in these two cases [39,43–47]. To rationalize this similarity, it is assumed that the quenching efficiency of P700 and $P700^+$ are identical, although the mechanism underlying the quenching mechanism by $P700^+$ is unknown. The reversible model for the primary charge separation in PSI, implying a more complex multiexponential decay of excited states [2,32,33], is at odds with the idea that P700 and $P700^+$ have identical quenching properties, and challenges the correctness of the subtraction procedure described above.

In order to critically test the recent models of energy transfer and reversible charge separation in *C. reinhardtii* PSI, and to gain a deeper insight into the mechanism of the excitation energy quenching in closed PSI, we have performed time-resolved fluorescence measurements on PSI-core preparations from wild type (WT) and two mutants (MHB and MSB, or PsaB: M664H and M664S) with open or closed RCs. In the mutants, the methionine axial ligand to the primary acceptor A_0 in the B-branch of the electron transfer cofactors was replaced by histidine (MHB) or serine (MSB). By using optical methods, these mutations have been previously shown to block the electron transfer from A_0^- to the secondary electron acceptor A_1 by modifying the properties of A_0 [28–30] (similar mutations were also characterized by using EPR [25–27]). Both pre-oxidation of P700, and mutations of the A_0 axial ligands, were expected to influence the primary electron transfer events.

The dynamics of the excited states strongly depends on the presence of so-called red chlorophylls in the investigated PSI particles that makes the interpretation of the experimental data more difficult. However, in contrast to what is observed in PSI from cyanobacteria and higher plants [9,10,35,37,48], no indications of red Chls absorbing above 700 nm were found in the PSI core particles isolated from CC 2696 strain of *C. reinhardtii* [11,49], which were investigated in this contribution.

2. Materials and methods

The experiments were performed on wild type (WT) and on MHB and MSB mutants in which the methionine axial ligand to the primary electron acceptor A_0 in branch B of electron transfer cofactors (Met B664) was replaced with histidine (MHB) or serine (MSB) using methods described previously [50,51]. The *C. reinhardtii* strain for transformation was CC2696 in WT and mutants, and was obtained from the *Chlamydomonas* Culture Collection at Duke University. The CC2696 strain carries a deletion in the chloroplast *psbA* gene that causes a complete loss of Photosystem II, and also contains the DS-521 nuclear mutation leading to a 90% reduction in LHC II content. The cells were grown in CC liquid medium [51] and then the thylakoid membranes were isolated according to the method presented in [52]. The PSI complexes were extracted from thylakoid membranes and purified using protocols described previously [28], and finally suspended in a buffer containing 50 mM HEPES (pH = 7.2), 5 mM $MgCl_2$, 12 mM $CaCl_2$, 20% glycerol (v/v), 1 mM benzamidine, 1 mM aminocapric acid, 1 mM EDTA, and 0.03% dodecyl maltoside. During experiments the primary donor was kept neutral (open RC) by addition of 20 mM sodium ascorbate and 20 μM phenazine methosulfate or oxidized (closed RC) by addition 3 mM $K_3(FeCN)_6$.

The time-resolved fluorescence measurements were carried out with a Streak camera setup. Excitation pulses of 400 nm with a time duration of ~100 fs and were generated in a system composed of a titanium:sapphire laser (Coherent, Vitesse), a regenerative amplifier (Coherent, RegA) and a double pass optical parametric amplifier (Coherent, OPA). The sample was excited with vertically polarized 1.2-nJ pulses (which corresponds to ~0.1 excitations per PS1) and a repetition rate of 125 kHz. The fluorescence was detected without any polarizer at a right angle with respect to the excitation beam using a spectrograph (Chromex 250IS) and streak camera (Hamamatsu C5680), and recorded by a CCD camera (Hamamatasu C4880). The temporal width of the detection system response function was ~3 ps (FWHM). The sample was placed in a rotating cuvette to ensure that each laser pulse illuminated a fully relaxed sample. The exposure times per image were 8–12 min and 4–6 min for the time windows of 200 ps and 500 ps, respectively. The detected streak images from both time windows were analyzed globally together from 635 to 780 nm with 5 nm resolution and decay-associated spectra (DAS) were obtained. The data were also modeled using the target analysis method [53,54].

3. Results

Fig. 1 shows decay associated spectra (DAS) of three preparations: WT and two A_0 mutants of PSI from *C. reinhardtii* in either the open or closed state. DAS are the wavelength-dependent pre-exponential factors, $A_i(\lambda)$, of the multiexponential fluorescence (Fl) decay components, $Fl = \Sigma\ A_i(\lambda)\exp(-t/\tau_i)$, associated with particular exponential lifetimes, τ_i. They were determined from the global fitting performed simultaneously for all wavelengths from the recorded spectral range of fluorescence emission (see [53,54] for further details on global fitting). The subpicosecond spectra are assigned to internal conversion of Chls from their Soret to Q_y state. The negative amplitudes of these spectra are due to the appearance of fluorescence in Q_y region. The 4.5- to 5-ns spectra are assigned to Chls uncoupled from the electron transfer reactions, whereas the ~100- to 200-ps spectra with very small amplitudes are assigned to a minor fraction of PSI particles showing either slow excitation energy transfer to RCs or nonphotochemical quenching. On the basis of the spectra presented in Fig. 1, the contribution of PSI particles showing these two types of slow decay is estimated to be below 15%. Both these phases were often observed in PSI core preparations from *C. reinhardtii* [11,29,32] and will not be discussed further here.

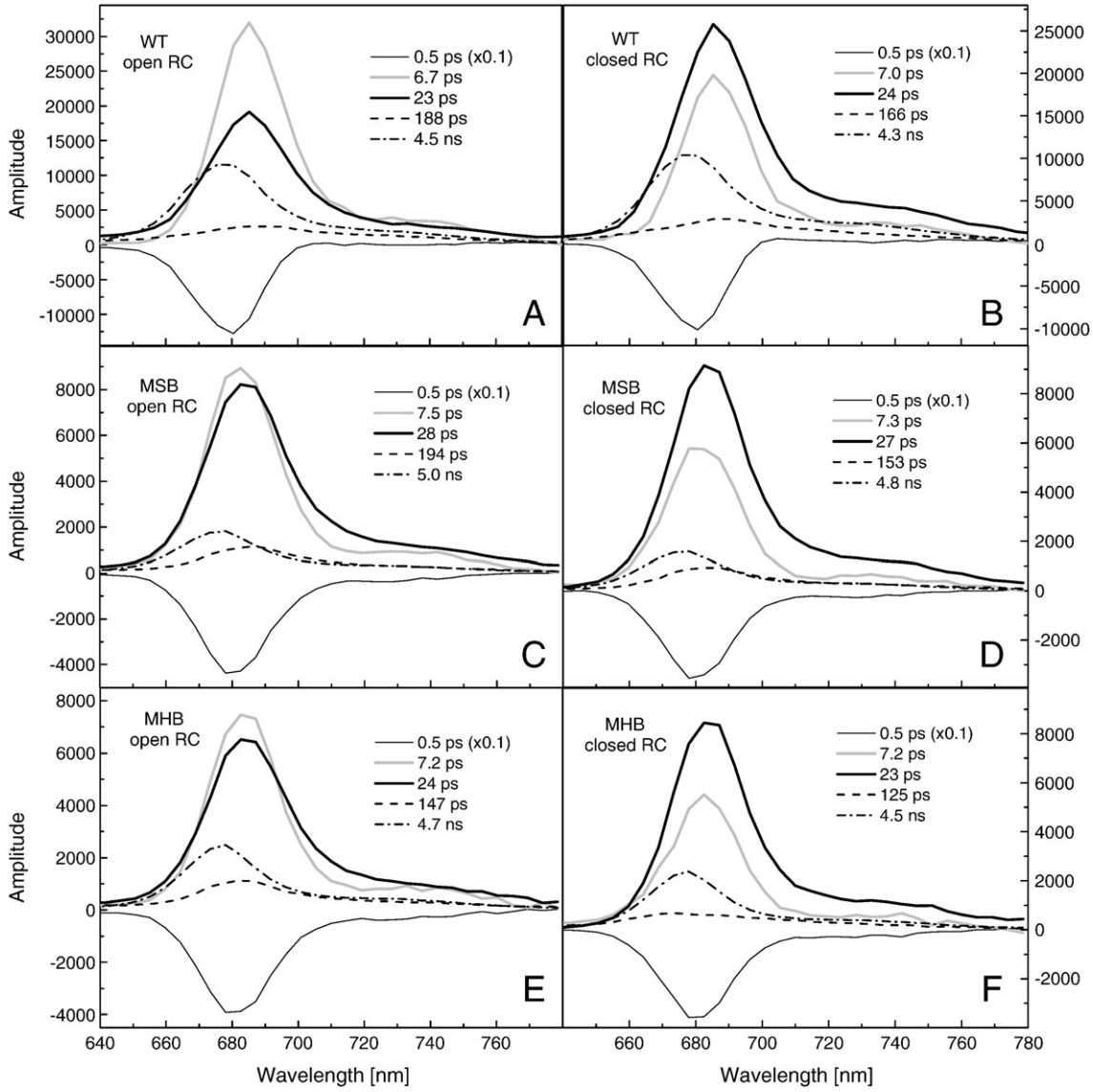

Fig. 1. Fluorescence decay associated spectra of WT and two mutant (MSB and MHB) Photosystem I core preparations from *C. reinhardtii*. (A, C, E) - reaction centers in open state (P700 neutral); (B, D, F) reaction centers in closed state (P700 chemically pre-oxidized to $P700^{+}$).

The most prominent feature of all three preparations shown in Fig. 1, both in the open and closed state, is a biexponential decay occurring with ~7-ps and ~25-ps lifetimes. Similar fluorescence lifetimes were previously observed for PSI from *C. reinhardtii* [33], cyanobacteria [9,10,37], and green plants [35]. However, unlike in PSI from cyanobacteria and green plants, in which the few-picosecond component is clearly related to energy transfer from bulk to red Chls, in the PSI core from *C. reinhardtii* which lacks far-red Chls [49], the ~7-ps DAS, positive at all wavelength, clearly reveals excited state decay.

In open WT PSI, the amplitude of the 6.7-ps DAS is significantly larger than that of the 23-ps spectrum (Fig. 1A). Both these spectra peak at the same wavelength, ~685 nm, and the slower DAS is slightly wider, which is related to the equilibration of excitation over a broader spectral distribution of Chls over a longer time scale. Upon chemical pre-oxidation of P700, the relation between the two DAS is reversed (Fig. 1B): the amplitude of the slower component (24 ps) is larger than that of the 7-ps DAS, whereas the maxima and the shapes of the DAS are not changed. This observed change in the relative amplitudes of the ~7- and 25-ps components in closed RC is qualitatively in agreement with results of fluorescence studies on PSI from *Synechococcus elongatus* [48]. In ref. [48], a 12% increase in fluorescence quantum yield, and a slight increase in fluorescence lifetime, from 34 to 37 ps, was reported upon P700 oxidation at room temperature. Overall, slower excitation decay in closed WT PSI from *C. reinhardtii* is also demonstrated in Fig. 2A.

The effects of both amino acid mutations are very similar to each other. The fast, ~7-ps excitation decay is no longer dominating the overall decay in the open state (Fig. 1C, E). Instead, the contributions of the ~7-ps and ~25-ps spectra are similar. Chemical pre-oxidation of the primary donor yields a further relative decrease of the ~7-ps DAS, qualitatively similar to what was observed in WT PSI. In effect, the overall decay of excited states is slightly slower in closed RCs (Fig. 2B).

4. Discussion

The observation of two phases of excitation decay (~7 and ~25 ps) may be explained either by a trivial heterogeneity model, or by a reversible model. In the latter model, the forward reaction leads to decay of the emitting excited state ((Ant/RC)*) and formation of a non-emitting charge-separated state (S1), which then may evolve irreversibly into a secondary non-emitting state (S2), or undergo a

back-reaction to the emitting state (the nature of the states S1 and S2 is discussed below):

$$(\mathrm{Ant}/\mathrm{RC})^* \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} \mathrm{S1} \overset{k_2}{\rightarrow} \mathrm{S2} \quad (1)$$

It is worth noting that in contrast to an earlier report [33], in our preparation we did not resolve two spectrally distinct emitting states, which were previously assigned to excited antenna (maximum at ~685 nm) and excited RC (maximum at ~714 nm) [33]. A possible origin of this difference may be more uniform excitation at 400 nm of all PSI Chls, including those in the RC, in this work compared to selective 675-nm excitation of antenna Chls in [33]. Thus, in our case, the energy transfer from the antenna to RC may be more difficult to resolve. However, the difference in the shapes of the ~7- and ~25-ps DAS may be due to a small contribution from the excitation energy transfer from antenna to RC to the ~7-ps spectrum. Since the effect is small in our experiment, we model fluorescence dynamics with only one emitting state, including contributions from both excited antenna and excited RC, (Ant/RC)*.

In the frame of the scheme (1), the fast ~7-ps excitation decay is an effect of energy equilibration between the initially excited state (Ant/RC)* and the non-emitting state S1. This equilibration leads to a decrease in the concentration of the emitting state and the appearance of the charge separated state S1. The ~25-ps decay is directly related to the decay of the both of the equilibrated states, (Ant/RC)* and S1, due to the S1 → S2 electron transfer reaction. The

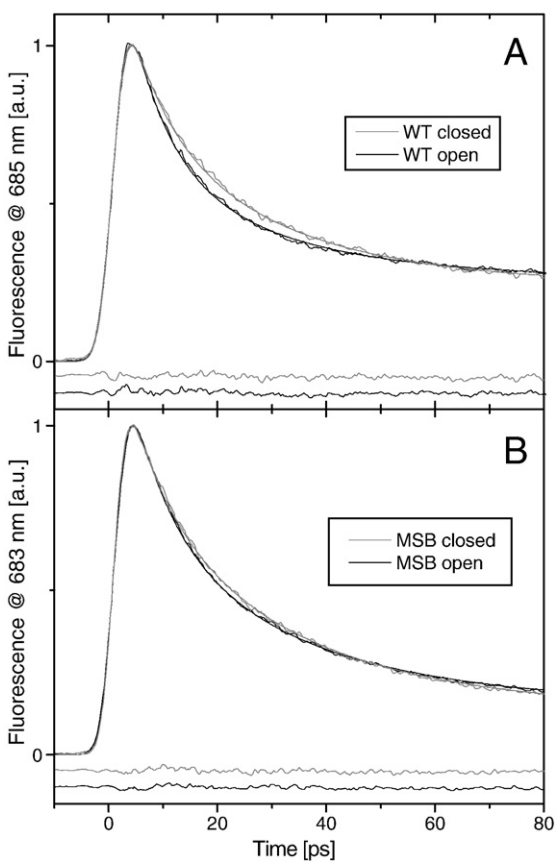

Fig. 2. Kinetic traces, fits, and residuals of fluorescence decay at maximum of fluorescence transient bands for open and closed WT PSI (A), and open and closed MSB PSI mutant. The parameters of the fits are the same as in respective panels in Fig. 1. At longer times (50–60 ps) the kinetic traces for open and closed RCs crosses due to overall faster decay of open RCs. Because of limited temporal resolution of the setup faster signals are "cut off" to a higher extent than the slower signals and therefore, after normalization of both traces to the same peak amplitude, an apparent higher contribution of the slow decay is observed in open RCs.

different relative contributions of the ~7- and ~25-ps components in open and closed PSI, in all three preparations under study, may be qualitatively easily explained by the shift in the (Ant/RC)* ↔ S1 equilibrium towards the emitting state as a result of pre-oxidation of the primary donor (Fig. 1). Such a shift results in a decrease in the equilibrium concentration of the S1 state and a decrease in the relative contribution of the ~7-ps component (see the results of target analysis and energetic calculations below). However, this explanation implies that the charge separated state S1 is formed independently of the redox state of P700, and consequently that P700 is not the primary electron donor. In the opposite case, i.e. if P700 is the primary donor, the identity of S1 in scheme 1 would be different for open and closed RCs, or scheme 1 would be fundamentally wrong at least for closed RCs. In the following, we argue that in the frame of scheme 1, with S1 being the same charge separated state for open and closed RCs,, we are able to explain all of our results and, moreover, that this scheme is consistent with a recent model of PSI in which the accessory Chl A plays the role of the primary electron donor [2].

According to the model put forward by Holzwarth and coworkers [2], the primary charge separation in PSI occurs between the accessory Chl A and Chl A_0, forming the primary charge separated state $A^+A_0^-$. In the second step, the electron is transferred from P700 to A^+, forming the secondary charge separated state $P700^+A_0^-$. Adapting this assignment, scheme (1) may be rewritten for open RCs:

$$(\mathrm{Ant}/\mathrm{RC})^* \leftrightarrow A^+A_0^- \rightarrow P700^+A_0^-. \quad (2)$$

In closed RCs, P700 is oxidized but we postulate that the primary charge separation between A and A_0 is still possible. The secondary electron transfer step characteristic for open RCs, from P700 to A^+, is naturally not possible. Instead, we propose that the charge on $P700^+$ induces fast charge recombination between A^+ and A_0^- to the ground state, which occurs with an apparent lifetime of ~25 ps—similar to the lifetime of the secondary electron transfer step in open RCs. It remains to be theoretically verified whether these postulates are physically plausible. Conceptually, however, they offer an attractive mechanism of excitation energy quenching in closed RCs. According to the postulated scenario, scheme (1) may be rewritten in the following way for closed RCs:

$$(\mathrm{Ant}/\mathrm{RC})^* \leftrightarrow A^+A_0^- \rightarrow AA_0. \quad (3)$$

The shift of the (Ant/RC)* ↔ $A^+A_0^-$ equilibrium towards the left upon closing the RCs postulated above, could be explained by an effect of the charge localized on the neighboring species $P700^+$. This charge is expected to slow down the primary charge separation between A and A_0, due to the electrostatic field being unfavorable for this reaction, and accelerate charge recombination between A^+ and A_0^- to the state (Ant/RC)*AA_0, due to the electrostatic interactions with the electron on A_0^-. On the other hand, the same charge on $P700^+$ induces fast charge recombination between A^+ and A_0^- to the ground state, as stated above.

Our results do not offer a direct proof for the proposed mechanism of excitation energy quenching in closed RCs and, in principle, direct quenching by $P700^+$ cannot be completely ruled out. However, in this case it would be difficult to consistently explain the biphasic excitation decay observed in closed RCs similar to that observed for open RCs. In fact, the idea of direct quenching by $P700^+$ arose from the observation of similar monophasic decays of antenna exited states in both open and closed RCs [39,43–47].

Similar to the effect observed by closing RCs, the different relative amplitudes of the ~7- and ~25-ps spectra found for open WT and open mutant PSI may also be explained by a simple shift in the (Ant/RC)* ↔ $A^+A_0^-$ equilibrium towards the emitting state in the mutated samples (compare Fig. 1A to Fig. 1C, E). It was previously reported that electron transfer leading to formation of $P700^+A_0^-$

occurs on a picosecond time scale in both branches of WT and A_0 mutant PSI [28–30]. In both branches of WT PSI, A_0^- is reoxidized within 20–30 ps by electron transfer to A_1 whereas in the mutated branch of the A_0 mutants, reoxidation of A_0^- is slowed down to 1–2 ns. This dramatic slowing down of A_0^- reoxidation was suggested to originate from the significant modifications of the A_0^-/A_0 redox potential caused by replacement of the methionine A_0 axial ligand by other amino acids [55].

The shift of the $(Ant/RC)^* \leftrightarrow A^+A_0^-$ equilibrium towards the emitting state in the mutants can be rationalized in two ways. First, the modified A_0^-/A_0 redox potential is expected to shift the free energy level of the state $A^+A_0^-$. Apparently this shift is related to slower primary charge separation (decrease in k_1 value, scheme (1)) and/or faster primary charge recombination (increase in k_{-1} value) to the emitting state. Second, energy transfer from the antenna to RCs is expected to be less efficient, and slower, in the mutants due to loss of excitonic coupling in the mutated branch of the electron transfer cofactors. Such a loss of excitonic coupling leading to a decrease of RC absorption was demonstrated previously [28,29,56]. It should be noted that the rate of the reaction $(Ant/RC)^* \rightarrow A^+A_0^-$ may depend on the time of excitation equilibration over the antenna system, on the time necessary for excitation energy transfer from equilibrated Ant* to RC, and on the intrinsic primary charge separation rate. In this study we do not determine the contributions of these three steps to the overall rate of the $(Ant/RC)^* \rightarrow A^+A_0^-$ reaction. However, observation of the effect of mutations and pre-oxidation of P700 on the observed rates (see below) implicates that the two latter steps may be rate limiting.

In order to treat the problem more quantitatively, we performed a target analysis of the data by using locally developed software. Detailed theory underlying the target analysis may be found in [53,54]. Briefly, target analysis is fitting the experimental decays of the whole investigated spectral region with intrinsic rate constants related to a real physical model (in our case, that presented in schemes 1–3) rather than with a simple sum of exponential decay components (presented in Figs. 1–2). The rate constants resulting from this target analysis are presented in Fig. 3. For WT PSI from *C. reinhardtii*, electron transfer reactions occur symmetrically in both branches of electron transfer cofactors on the subnanosecond time scale of our experiment [28–30]. Therefore, there is no need to double the number of compartments modeling each of the states in the A and B branches. For mutant PSI, in principle, separate sets of states related to each of the branches should be constructed and a separate set of rates should be calculated for each branch. However, this leads to an increase in the number of parameters that exceeds any possibility of their experimental verification. Therefore, only a single set of states is considered in the modeling of the results from the mutants, but the obtained values of the rates should be treated as average values for the mutated and non mutated branch (Fig. 3). Assuming that the rates in the branch which does not carry a mutation are the same as those in WT PSI, we can then conclude how the particular rate constants in the mutated branch are influenced by the mutations.

Rate constants for open WT PSI compare well with those published previously (Fig. 3A and B, [33]). The rates of the reversible reactions cannot be compared directly, since there is an extra RC* compartment in the cited paper. However, the free energy gap between the states Ant* (or (Ant/RC)* in our case) and S1, calculated from the forward and backward rates assuming the Boltzmann distribution, is not very different in both studies: 34 meV in our study vs. 41 meV in the previous study [33]. The secondary electron transfer step is also described by similar rates of 74 and 80 ns^{-1}, respectively.

In closed WT PSI, the value of k_1 (compare scheme (1) and Fig. 3B) decreases and the value of k_{-1} increases as compared to open PSI. Thus, changes of both rates contribute to a shifting of the equilibrium towards the excited states in agreement with the expectations expressed above on the basis of the qualitative analysis of the DAS presented in Fig. 1. A similar trend is also observed in both mutant PSI preparations. Apparently, the primary radical pair formation is a shallower excitation trap in closed than in open PSI RCs (Table 1). On the other hand, in closed RCs, the value of k_2 increases both in WT and in mutant preparations. The fact that the value of k_2 is different for closed and open RCs is not surprising since, as mentioned above, it describes two very different reactions in these two cases.

Comparison of the rate constants for open WT and open mutant PSI leads to the conclusion that the value of k_1 is smaller in mutants, whereas the mutations have little effect on the value of k_{-1}. The net effect of both mutations is a shift in the $(Ant/RC)^* \leftrightarrow A^+A_0^-$ equilibrium to the left (see also Table 1). Since, as noticed above, the value of k_1 in the open mutant PSI is expected to be an average value for the mutated and non mutated branch, the k_1 value in the mutated branch is expected to be even lower.

Finally, we would like to mention an alternative scheme in which the reversible step is excitation energy transfer between the antenna

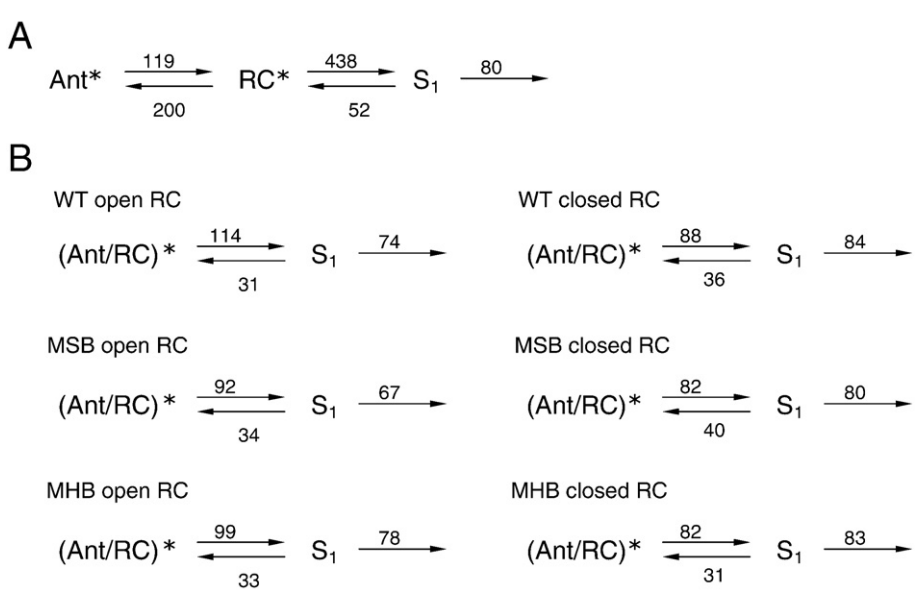

Fig. 3. Kinetic schemes of excitation energy and electron transfer reactions in Photosystem I core preparations from *C. reinhardtii* modeling the experimental results obtained: (A) by Holzwarth and coworkers (2005) [33]; (B) by us. The molecular rate constants were calculated from the target analysis and are given in ns^{-1}. S1—primary radical pair.

Table 1
Free energy gap between the excited state (Ant/RC)* (or Ant* in the case of [33]) and primary charge separated state RP1 in WT and two mutant Photosystem I core preparations from *C. reinhardtii* (in open and closed reaction centers). The gap was calculated from the Boltzmann distribution: $\Delta G = k_B T^* \ln(k_1/k_{-1})$; k_B—Boltzmann constant, T—absolute temperature, k_1 and k_{-1}—molecular rate constants explained in scheme (1).

	ΔG [meV]	
	Open RCs	Closed RCs
WT (Holzwarth et al. 2005)	41	
WT	34	23
MSB	26	19
MHB	29	25

and RC, and the primary charge separation occurs irreversibly between P700 and A_0 according to the classical model [57]:

$$\text{Ant}^* \leftrightarrow \text{RC}^* \rightarrow \text{P700}^+ \text{A}_0^- . \quad (4)$$

Formally, this scheme is identical to scheme 1. However, in order to yield biphasic fluorescence decay, the state RC* should be a much poorer fluorescence emitter than the state Ant*. Such a postulate is not supported by any experimental observation. In fact, it was shown that the RC* state is an efficient emitter [33]. For these reasons we discard model (4) as less likely.

5. Conclusion

In this contribution, a reversible primary charge separation model was successfully applied to both open and closed PSI from both wild type and samples containing a mutation of the A_0 axial ligand. It was demonstrated that moderate modulation of the free energy level of the primary charge separated state, $A^+A_0^-$, by the mutations near A_0^-, as well as by the charge on $P700^+$, leads to slight deceleration of the overall excitation decay. On the other hand, the positive charge on $P700^+$ exerts a pronounced effect on the $A^+A_0^-$ charge recombination rate to the ground state. This fast charge recombination process is proposed to be a mechanism for effective excitation quenching by closed PSI.

Acknowledgements

K.G. gratefully acknowledges financial support from the Polish government (scientific project no 1 P03B 003 29) and from Netherlands Organisation for Scientific Research (NWO; grant B 81-734). W.G. is a scholarship holder within the framework of Activity 8.2 of the Operational Programme *Human Capital* cofinanced by the European Social Fund of the European Union and the state budget. A.W. acknowledges support from DOE award number DE-FG02-99ER20349.

References

[1] N.W.T Woodbury, J.P. Allen, The pathway, kinetics and thermodynamics of electron transfer in wild type and mutant reaction centers of purple nonsulfur bacteria, in: R.E. Blankenship, M.T. Madigan, C.E. Bauer (Eds.), Anoxygenic Photosynthetic Bacteria, Kluwer Academic Publishers, Dordrecht/Boston/London, 1995, pp. 527–557.
[2] A.R. Holzwarth, M.G. Müller, J. Niklas, W. Lubitz, Ultrafast transient absorption studies on Photosystem I reaction centers from *Chlamydomonas reinhardtii*. 2: mutations near the P700 reaction center chlorophylls provide new insight into the nature of the primary electron Donor, Biophys. J. 90 (2006) 552–565.
[3] M.E. van Brederode, R. van Grondelle, New and unexpected routes for ultrafast electron transfer in photosynthetic reaction centers, FEBS Lett. 455 (1999) 1–7.
[4] V.I. Prokhorenko, A.R. Holzwarth, Primary processes and structure of the Photosystem II reaction center: a photon echo study, J. Phys. Chem. B. 104 (2000) 11563–11578.
[5] M.L. Groot, N.P. Pawlowicz, L.J.G.W. van Wilderen, J. Breton, I.H.M. van Stokkum, R. van Grondelle, Initial electron donor and acceptor in isolated Photosystem II reaction centers identified with femtosecond mid-IR spectroscopy, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 13087–13092.
[6] P. Jordan, P. Fromme, H.T. Witt, O. Klukas, W. Saenger, N. Krau, Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution, Nature 411 (2001) 909–917.
[7] K. Brettel, Electron transfer and arrangement of the redox cofactors in photosystem I, Biochim. Biophys. Acta 1318 (1997) 322–373.
[8] K. Brettel, W. Liebl, Electron transfer in photosystem I, Biochim. Biophys. Acta 1507 (2001) 100–114.
[9] B. Gobets, I.H.M. van Stokkum, M. Rögner, J. Kruip, E. Schlodder, N.V. Karapetyan, J.P. Dekker, R. van Grondelle, Time-resolved fluorescence emission measurements of Photosystem I particles of various cyanobacteria: a unified compartmental model, Biophys. J. 81 (2001) 407–424.
[10] B. Gobets, I.H.M. van Stokkum, F. van Mourik, J.P. Dekker, R. van Grondelle, Excitation wavelength dependence of the fluorescence kinetics in Photosystem I particles from synechocystis PCC 6803 and Synechococcus elongates, Biophys. J. 85 (2003) 3883–3898.
[11] K. Gibasiewicz, V.M. Ramesh, A.N. Melkozernov, S. Lin, N.W. Woodbury, R.E. Blankenship, A.N. Webber, Excitation dynamics in the core antenna of PS I from *Chlamydomonas reinhardtii* CC 2696 at room temperature, J. Phys. Chem. B 105 (2001) 11498–11506.
[12] G. Hastings, F.A.M. Kleinherenbrink, S. Lin, R.E. Blankenship, Observation of the reduction and reoxidation of the primary electron acceptor in Photosystem I, Biochemistry 33 (1994) 3193–3200.
[13] S. Kumazaki, I. Ikegami, H. Furusawa, S. Yasuda, K. Yoshihara, Observation of the excited state of the primary electron donor chlorophyll (P700) and the ultrafast charge separation in the Spinach Photosystem I Reaction Center, J. Phys. Chem. B 105 (2001) 1093–1099.
[14] S. Savikhin, W. Xu, P. Martinsson, P.R. Chitnis, W.S. Struve, Kinetics of charge separation and $A_0^- \rightarrow$ A1 electron transfer in Photosystem I reaction centers, Biochemistry 40 (2001) 9282–9290.
[15] B. Gobets, R. van Grondelle, Energy transfer and trapping in photosystem I, Biochim. Biophys. Acta 1507 (2001) 80–99.
[16] S. Kumazaki, M. Iwaki, I. Ikegami, H. Kandori, K. Yoshihara, S. Itoh, Rates of primary electron transfer reactions in the Photosystem I reaction center reconstituted with different quinones as the secondary acceptor, J. Phys. Chem. 98 (1994) 11220–11225.
[17] N.T.H. White, G.S. Beddard, R.G. Thorne, T.M. Feehan, T.E. Keyes, P. Heathcote, Primary charge separation and energy transfer in the Photosystem I reaction center of higher plants, J. Phys. Chem. 100 (1996) 12086–12099.
[18] K. Brettel, M.H. Vos, Spectroscopic resolution of the picosecond reduction kinetics of the secondary electron acceptor A1 in photosystem I, FEBS Lett. 447 (1999) 315–317.
[19] C.H. Bock, A.J. van der Est, K. Brettel, D. Stehlik, Nanosecond electron transfer kinetics in Photosystem I as obtained from transient EPR at room temperature, FEBS Lett. 247 (1989) 91–96.
[20] P. Setif, K. Brettel, Forward electron transfer from phylloquinone A1 to iron–sulfur centers in spinach photosystem I, Biochemistry 32 (1993) 7846–7854.
[21] W. Leibl, B. Toupance, J. Breton, Photoelectric characterization of forward electron transfer to iron–sulfur centers in Photosystem I, Biochemistry 34 (1995) 10237–10244.
[22] P. Joliot, A. Joliot, In vivo analysis of the electron transfer within Photosystem I: are the two phylloquinones involved? Biochemistry 38 (1999) 11130–11136.
[23] M. Guergova-Kuras, B. Boudreaux, A. Joliot, P. Joliot, K. Redding, Evidence for two active branches for electron transfer in photosystem I, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 4437–4442.
[24] Y. Li, A. van der Est, M.G. Lucas, V.M. Ramesh, F. Gu, A. Petrenko, S. Lin, A.N. Webber, F. Rappaport, K. Redding, Directing electron transfer within Photosystem I by breaking H-bonds in the cofactor branches, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 2144–2149.
[25] W.V. Fairclough, A. Forsyth, M.C.W. Evans, S.E.J. Rigby, S. Purton, P. Heathcote, Bidirectional electron transfer in photosystem I: electron transfer on the PsaA side is not essential for phototrophic growth in Chlamydomonas, Biochim. Biophys. Acta 1606 (2003) 43–55.
[26] S. Santabarbara, I. Kuprov, W.V. Fairclough, S. Purton, P.J. Hore, P. Heathcote, M.C.W. Evans, Bidirectional electron transfer in Photosystem I: determination of two distances between $P700^+$ and A_1^- in spin-correlated radical pairs, Biochemistry 44 (2005) 2119–2128.
[27] S. Santabarbara, I. Kuprov, P.J. Hore, A. Casal, P. Heathcote, M.C.W. Evans, Analysis of the spin-polarized electron spin echo of the $[P700^+A_1^-]$ radical pair of Photosystem I indicates that both reaction center subunits are competent in electron transfer in cyanobacteria, green algae, and higher plants, Biochemistry 45 (2006) 7389–7403.
[28] V.M. Ramesh, K. Gibasiewicz, S. Lin, S.E. Bingham, A.N. Webber, Bidirectional electron transfer in Photosystem I: accumulation of A_0^- in A-side or B-side mutants of the axial ligand to chlorophyll A_0, Biochemistry 43 (2004) 1369–1375.
[29] V.M. Ramesh, K. Gibasiewicz, S. Lin, S.E. Bingham, A.N. Webber, Replacement of the methionine axial ligand to the primary electron acceptor A_0 slows the A_0^- reoxidation dynamics in Photosystem I, Biochim. Biophys. Acta 1767 (2007) 151–160.
[30] W. Giera, K. Gibasiewicz, V.M. Ramesh, S. Lin, A. Webber, Electron transfer from A_0^- to A_1 in Photosystem I from *Chlamydomonas reinhardtii* occurs in both the A and B branch with 25-30-ps lifetime, Phys. Chem. Chem. Phys. 11 (2009) 5186–5191.
[31] R. van Grondelle, J.P. Dekker, T. Gillbro, V. Sundstrom, Energy transfer and trapping in photosynthesis, Biochim. Biophys. Acta 1187 (1994) 1–65.

[32] M.G. Müller, J. Niklas, W. Lubitz, A.R. Holzwarth, Ultrafast transient absorption studies on Photosystem I reaction centers from *Chlamydomonas reinhardtii*. 1. A new interpretation of the energy trapping and early electron transfer steps in Photosystem I, Biophys. J. 85 (2003) 3899–3922.
[33] A.R. Holzwarth, M.G. Müller, J. Niklas, W. Lubitz, Charge recombination fluorescence in Photosystem I reaction centers from *Chlamydomonas reinhardtii*, J. Phys. Chem. B 109 (2005) 5903–5911.
[34] A.N. Melkozernov, H. Su, S. Lin, S. Bingham, A.N. Webber, R.E. Blankenship, Specific mutation near the primary donor in Photosystem I from *Chlamydomonas reinhardtii* alters the trapping time and spectroscopic properties of P700, Biochemistry 36 (1997) 2898–2907.
[35] C. Slavov, M. Ballotari, T. Morosinotto, R. Bassi, A.R. Holzwarth, Trap-limited charge separation kinetics in higher plant Photosystem I complexes, Biophys. J. 94 (2008) 3601–3612.
[36] A.N. Melkozernov, Excitation energy transfer in Photosystem I from oxygenic organisms, Photosynth. Res. 70 (2001) 129–153.
[37] C. Slavov, E. El-Mohsnawy, M. Rogner, A.R. Holzwarth, Trapping kinetics in isolated cyanobacterial PS I complexes, Chem. Phys. 357 (2009) 163–170.
[38] S. Savikhin, W. Xu, P.R. Chitnis, W.S. Struve, Ultrafast primary processes in PS I from Synechocystis sp. PCC 6803: roles of P700 and A_0, Biophys. J. 79 (2000) 1573–1586.
[39] A.M. Nuijs, V.A. Shuvalov, H.J. van Gorkom, J.J. Plijter, L.N.M. Duysens, Picosecond absorbance difference spectroscopy on the primary reactions and the antenna-excited states in photosystem I particles, Biochim. Biophys. Acta 850 (1986) 310–318.
[40] G. Hastings, F.A.M. Kleinherenbrink, S. Lin, T.J. McHugh, R.E. Blankenship, Observation of the reduction and reoxidation of the primary electron acceptor in Photosystem 1, Biochemistry 33 (1994) 3193–3200.
[41] N.T.H. White, G.S. Beddard, J.R.G. Thorne, T.M. Feehan, T.E. Keyes, P. Heathcote, Primary charge separation and energy transfer in the photosystem I reaction center of higher plants, J. Phys. Chem. 100 (1996) 12086–12099.
[42] S. Savikhin, W. Xu, P. Martinsson, P.R. Chitnis, W.S. Struve, Kinetics of charge separation and A0 →A1 electron transfer in photosystem I reaction centers, Biochemistry 40 (2001) 9282–9290.
[43] V.A. Shuvalov, A.M. Nuijs, H.J. van Gorkom, H.W. Smit, L.N.M. van Duysens, Picosecond absorbance changes upon selective excitation of the primary electron donor P-700 in Photosystem I, Biochim. Biophys. Acta 850 (1986) 319–323.
[44] T.G. Owens, S.P. Webb, R.S. Alberte, L. Mets, G.R. Fleming, Antenna structure and excitation dynamics in photosystem I. I. Studies of detergent-isolated photosystem I preparations using time-resolved fluorescence analysis, Biophys. J. 53 (1988) 733–745.
[45] D.R. Klug, L.B. Giorgi, B. Crystal, J. Barber, G. Porter, Energy transfer to low energy chlorophyll species prior to trapping by P700 and subsequent electron transfer, Photosynth. Res. 22 (1989) 277–284.
[46] S. Turconi, G. Schweitzer, A.R. Holzwarth, Temperature dependence of picosecond fluorescence of a cyanobacterial Photosystem I particle, Photochem. Photobiol. 57 (1993) 113–119.
[47] S. Savikhin, W. Xu, P.R. Chitnis, W.S. Struve, Ultrafast primary processes in PS I from Synechocystis sp. PCC 6803: Roles of P700 and A_0, Biophys. J. 79 (2000) 1573–1586.
[48] M. Byrdin, I. Rimke, E. Schlodder, D. Stehlik, T.A. Roelofs, Decay kinetics and quantum yields of fluorescence in Photosystem I from Synechococcus elongatus with P700 in the reduced and oxidized state: are the kinetics of excited state decay trap-limited or transfer-limited? Biophys. J. 79 (2000) 992–1007.
[49] K. Gibasiewicz, A. Szrajner, J.A. Ihalainen, M. Germano, J.P. Dekker, R. van Grondelle, Characterization of low-energy chlorophylls in the PSI-LHCI super-complex from *Chlamydomonas reinhardtii*. A Site-Selective Fluorescence Study, J. Phys. Chem. B 109 (2005) 21180–21186.
[50] A.N. Webber, H. Su, S.E. Bingham, H. Käss, L. Krabben, M. Kuhn, E. Schlodder, W. Lubitz, Site-directed mutations affecting the spectroscopic characteristics and midpoint potential of the primary donor in Photosystem I, Biochemistry 35 (1996) 12857–12863.
[51] H. Lee, S.E. Bingham, A.N. Webber, Specific mutagenesis of reaction center proteins by chloroplast transformation of Chlamydomonas, in: Methods in Enzymology, Vol. 297, Academic Press, Boca Raton, FL, 1998, pp. 311–319.
[52] N.H. Chua, P. Bennoun, Thylakoid membrane polypeptides of *Chlamydomonas reinhardtii*: wild-type and mutant strains deficient in photosystem II reaction center, Proc. Natl. Acad. Sci. U. S. A. 72 (1975) 2175–2179.
[53] A.R. Holzwarth, Data analysis of time-resolved measurements, in: J. Amesz, A.J. Hoff (Eds.), Biophysical Techniques in Photosynthesis, Kluwer Academic Publishers, Dordrecht, The Netherlands, Advances in Photosynthesis Research, 1996, pp. 75–92.
[54] I.H.M. Van Stokkum, D.S. Larsen, R. van Grondelle, Global and target analysis of time-resolved spectra, Biochim. Biophys. Acta 1657 (2004) 82–104.
[55] P. Fromme, P. Jordan, N. Krauss, Structure of photosystem I, Biochim. Biophys. Acta 1507 (2001) 5–31.
[56] K. Gibasiewicz, V.M. Ramesh, S. Lin, K. Redding, N.W. Woodbury, A.N. Webber, Excitonic interactions in wild-type and mutant psi reaction centers, Biophys. J. 85 (2003) 2547–2559.
[57] B. Gobets, L. Valkunas, R. van Grondelle, Bridging the gap between structural and lattice models: a parameterization of energy transfer and trapping in Photosystem I, Biophys. J. 85 (2003) 3872–3882.