Redox potential of quinones in both electron transfer branches of Photosystem I

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Abbreviations:

* A. variabilis, *Anabaena variabilis*;
* Chl, chlorophyll a;
* bRC, bacterial reaction center;
* C. reinhardtii, *Chlamydomonas reinhardtii*;
* DMF, dimethylformamide;
* ENDOR, electron nuclear double resonance;
* EPR, electron paramagnetic spectroscopy;
* ESEEM, electron spin echo modulation;
* ESP, electrostatic potential;
* ET, electron transfer;
* FTIR, Fourier transform infrared;
* GG, monogalactosyl-diglyceride;
* NHE, normal hydrogen electrode;
* LPB equation, linearized Poisson-Boltzmann equation;
* PDB, protein data bank;
* PG, phosphatidyl-glycerole;
* phyllo-QA(B), phylloquinone in the A(B)-branch of the wild type PSI;
* PSI, photosystem I;
* PSII, photosystem II;
* P700A(B), Chl of P700 in A(B)-branch;
* A-1A(B), accessory Chl located between P700 and A0 in A(B)-branch;
* A0A(B), Chl A0 electron acceptor in the A(B)-branch;
* A1A(B), phylloquinone in the A(B)-branch of wild type PSI;
* QA(B), ubiquinone in A(B)-branch of the bRC from *Rhodobacter sphaeroides*;
* Rb. sphaeroides, *Rhodobacter sphaeroides*;
* SP, special pair in the bRC from *Rhodobacter sphaeroides*;
* S. elongatus, *Synechococcus elongatus*;
* ubi-Q, ubiquinone in bacterial reaction center;
SUMMARY

The redox potentials of the two electron transfer (ET) active quinones in the central part of photosystem I (PSI) were determined by evaluating the electrostatic energies from the solution of the Poisson-Boltzmann equation based on the crystal structure. The calculated redox potentials are –531 mV for $A_{1A}$ and –686 mV for $A_{1B}$. From these results we conclude: (i) Both branches are active with a much faster ET in the B- than in the A-branch. (ii) The measured lifetime of 200 – 290 ns of reduced quinones agrees with the estimate for the A-branch and corroborates with an uphill ET from this quinone to the iron-sulfur cluster as observed in recent kinetic measurements. (iii) The EPR data refer to the A-branch quinone where the corresponding ET is uphill in energy.

The negative redox potential of $A_1$ in PSI is primarily due to the influence from the negatively charged $F_X$, in contrast to the positive shift on the quinone redox potential in bRC and PSII that is attributed to the positively charged non-heme iron atom. The conserved residue Asp-B575 changes its protonation state after quinone reduction. The difference of 155 mV in the quinone redox potentials of the two branches were attributed to the conformation of the backbone with a large contribution from Ser-A692 and Ser-B672 and to the side chain of Asp-B575, whose protonation state couples differently with the formation of the quinone radicals.
INTRODUCTION

The crystal structure of Photosystem I (PSI) from *Synechococcus elongatus* (*S. elongatus*) that is now available at atomic resolution of 2.5 Å (1) solved the riddle of the microscopic structure of this membrane protein and has promoted the challenge to understand its function. PSI consists of 12 polypeptide chains. The central part of PSI is composed of the homologue chains PsaA and PsaB, followed by chain PsaC. The former two host as redox-active cofactors a dimer of chlorophylls (chlorophyll a, Chl) (P700), two accessory Chls, (A-1), two more distant Chls (A0), two phylloquinones (phyllo-Q, A1) and one iron-sulfur cluster (FX), while PsaC contains two iron-sulfur clusters (F_A and F_B) (Figure 1). Similar to the bacterial photosynthetic reaction centers (bRC) these cofactors are arranged in two branches (A and B) related by \( C_2 \) symmetry with an axis going through the P700 dimer and FX, the iron-sulfur cluster closest to P700. Both branches start with the dimer P700 and end in the iron-sulfur cluster FX, which they have in common. These cofactors are bridged by the four Chls A_{1A}, A_{1B}, A_{0A}, A_{0B}, two for each branch and the two quinones A_{1A}, A_{1B}. In spite of the apparent \( C_2 \) symmetry, the electron transfer (ET) in bRC occurs predominantly in the A-branch. A likewise asymmetric behavior was observed in PSI. For instance, EPR studies resulted in reduced A1 localized in the A-branch only (2) as also suggested by mutagenesis studies (3). However, kinetic investigations showed a biphasic forward ET from P700 to A1 (4) and also from A1 to FX (5,6), which can be interpreted as the action from two active branches. This view is also supported by point-mutagenesis studies replacing the tryptophans Trp-A697 and Trp-B677 in the vicinity of each of the quinones (Figure 2) by His, Leu and Phe residues. These two tryptophan residues in the A- and B-branch were shown to be related to the slow and the fast phase of the ET process from A1 to FX, respectively (7-10). Therefore, the idea that in PSI both branches are active ET pathways has been put forward, contrary to the conservative idea of a single ET active branch as found in bRC.

Knowing the redox potentials of the cofactors in the two branches provides a thermodynamic basis to understand these ET processes in more detail. Therefore, it would be quite helpful to measure directly the redox potentials of those cofactors individually in each ET active branch and to evaluate the corresponding free energy differences between the electron donor and acceptor groups. However, the complexity of the PSI membrane protein complex, the large number of possible variably charged groups (redox-active and titratable) and the unknown interactions among them make it difficult to attribute experimentally measured signals uniquely to a specific redox-active cofactor. Another problem is that the redox potentials of cofactors in PSI can often not be
controlled directly by the solvent redox potential. This is particular the case for Chl and phyllo-Q. For the accessory Chls (A\(_{-1}\)), located between P700 and the A\(_0\), it is even not clear whether they serve as electron acceptor or donor in the ET chain or whether they are involved directly in the charge separation process.

In this report, we show that by computing the redox properties for the phyllo-Q (A\(_1\)) participating in the ET pathways of PSI, we can clearly distinguish between the cofactors in the A- and B-branch. Our computed redox potential of A\(_{1A}\) is higher than of A\(_{1B}\) and assumes a significantly more positive value than the value of about –800 mV estimated from experimental data more than 10 years ago (11). This may be very important to finally solve the riddle whether one or both branches of redox-active cofactors are ET active and helps to interpret diverging conclusions that are based on data obtained with different experimental methods. EPR studies (2,3) are in favor of a single ET active branch, whereas kinetic studies (4-6) support two active branches. Our calculated redox potentials are capable to explain both the kinetic and the EPR studies and thus resolve the apparent conflict between these different experimental data.

We try to identify the factors that shift the quinone redox potentials in PSI. Recently, it was suggested that the charge state of the iron complexes is responsible for the difference in the quinone redox potentials between bRC, PSII and PSI (12). Comparing details of those factors in the structurally known proteins PSI and bRC and recognizing their differences may provide clues to understand why in PSI the quinone redox potentials are dramatically more negative than in bRC and PSII.

**COMPUTATIONAL PROCEDURES**

*Coordinates.* For our computations, we used the crystal structures of the trimeric PSI from *S. elongatus* at a resolution of 2.5 Å (PDB; 1JB0) (1) in the revised version, where the coordinates for the P700\(_A\) Chl were corrected \(^1\) (footnote). The calcium ion, which is at a distance of more than 35 Å from the closest quinone and more than 20 Å from the porphyrin ring system of the closest Chl was deleted as well as the three negatively charged phosphatidyl-glycerole (PG) and one neutral monogalactosyl-diglyceride (GG) that are present in the crystal structure. However, to study the influence of these four lipids on the values of the quinone redox potentials we also performed computations where the PSI structure included them. Furthermore, we neglected in our computations the subunit Psak, since it is very distant from the central part of the reaction center and coordinates are available for backbone atoms only. Since there are no hydrogen atoms in the crystal structures, they were generated and their positions were energetically
optimized with CHARMM (13). During this procedure the positions of all non-hydrogen atoms were fixed, all titratable groups were kept in their standard protonation state (i.e. the acidic groups ionized and the basic groups protonated) and the Chls of the P700 dimer, of A_2, and A_0 as well as the quinones were kept in the oxidized neutral charge state.

There is no explicit experimental information available on the position of the hydrogen atoms of water molecules found in the crystal structures. Generating these coordinates by modeling depends critically on the protonation pattern of titratable residues in the neighborhood and often more than one conformation is possible. To avoid this uncertainty in previous treatments, we normally did not consider crystal waters explicitly. In the absence of water, their influence was considered implicitly by the high dielectric constant used for the resulting cavities. There are a number of studies to evaluate electrostatic energies in protein-water systems based on the solution of the linearized Poisson Boltzmann (LPB) equation, which considered the influence of explicit water molecules, which generally yielded no improvement (14-21). In the present application we deviated slightly form this general procedure and considered also the action of crystal water and in particular of one specific water (W-37) in PSI. This water molecule forms a hydrogen bond with one of the acidic oxygens of Asp-B575 that was found to change its protonation state coupled with the redox states of the quinones as discussed later. If not otherwise stated, the presented results refer to computations that include this crystal water.

Atomic partial charges. Atomic partial charges of the amino acids were adopted from the all-atom CHARMM22 (22) parameter set. But, instead of attaching an explicit hydrogen atom on one of the oxygens of the protonated carboxyl group of glutamate or aspartate, the charges of these two oxygens were both increased symmetrically by +0.5 unit charges to account implicitly for the presence of the proton. A similar procedure was used for the basic groups of arginines and lysines, where instead of removing a proton explicitly in the deprotonated state the charges of all protons at the corresponding basic group were diminished symmetrically by a total unit charge. For cofactors and residues whose protonation states are not considered in the CHARMM22 parameter set, we used appropriate charges that were computed before (23). Since phyllo-Q has the same aromatic ring structure as menaquinone except for the non-polar phytol chain, the charges computed for the latter were used (23).

For the iron-sulfur clusters, we used the atomic charges (ESP charges) of the model system \([Fe_4S_4(SCH_3)_4]\) given in ref. (24). We considered F_X, F_A and F_B in the oxidized
charge state $[\text{Fe}_4\text{S}_4(\text{SCH}_3)_4]^{2-}$, while in the reduced state the iron-sulfur cluster corresponds to the charge state $[\text{Fe}_4\text{S}_4(\text{SCH}_3)_4]^{3-}$ (25-27). A detailed discussion for the redox states of the iron-sulfur clusters can be found in ref. (25). The atomic charges of the two different lipids PG and GG and of the Chl molecules (the latter in both redox states) (as listed in the SUPPLEMENTAL DATA) were determined from the electronic wave functions obtained in Hartree-Fock approximation with 6-31G* basis set by fitting the resulting electrostatic potential in the neighborhood of these molecules by the RESP model (28). The charges for the $\beta$-carotene molecules and the phytol chain of the Chls and quinones were assigned in agreement with CHARMM22 parameters with a standard charge of +0.09 for non-polar hydrogen atoms and vanishing total charge for each CH$_n$ group (22,29).

In the computation of the PSI charge pattern with an oxidized P700$^+$ the positive unit charge was located on the B-branch Chl monomer (P700$_B$) of the P700 dimer only. This model is in agreement with ENDOR and ESEEM studies from which a largely asymmetric spin density distribution was deduced (30-34). In a more recent combination of ENDOR and ESEEM experiments (35) it was found that the spin density distribution is completely localized on P700$_B$. Measurements on P700 where the axial ligands of both Chl monomers in the P700 dimer were mutated from histidine to Gln or Ser, for P700$_A$ and to Gln, Gly, Asn, or Cys for P700$_B$, demonstrated that the replacement of the axial ligand on the B-branch had a much stronger impact on the redox potential and the spin density distribution of P700 (36). Recent molecular orbital calculations of P700 corroborated the results for the spin density localization on P700$_B$ (37). Therefore, the redox potentials of the quinone cofactors were calculated with a positively charged Chl monomer P700$_B$ while the other Chls were kept in the neutral charge state.

**Computation of protonation pattern.** The computation of the energetics of the protonation pattern is based on the electrostatic continuum model by solving the LPB equation with the program MEAD from Bashford *et al.* (38). The ensemble of protonation patterns was sampled by a Monte Carlo (MC) method where we used our own program *Karlsberg* (39). The detailed procedure that we used is described in refs. (18,19). The dielectric constant was set to $\varepsilon_P = 4$ inside the protein and $\varepsilon_W = 80$ for water. Discussions about the appropriate choice of the dielectric constant can be found in refs. (23,40-43). All computations were performed at 300 K with pH 7.0 and an ionic strength of 100 mM. The LPB equation was solved using a three-step grid-focusing procedure with a starting grid resolution of 2.5 Å, an intermediate grid resolution of 1.0 Å, and a final grid resolution of
0.3 Å. The MC sampling yields the probabilities \([A_{\text{ox}}]\) and \([A_{\text{red}}]\) of the states of the redox-active group \(A\). With these probabilities, the redox potentials can be calculated from the Nernst equation

\[
E = E^\circ + \frac{RT}{F} \ln \frac{[A_{\text{ox}}]}{[A_{\text{red}}]},
\]

where \(F\) is the Faraday constant, \(E\) is the solution redox potential and \(E^\circ\) is the standard redox potential of the redox-active group \(A\). To minimize the statistical error in evaluating the redox potential from the Nernst equation (1) we applied a bias redox potential to the redox-active group \(A\) such that we obtained an equal amount of both redox states ([\(A_{\text{ox}}]\) = \([A_{\text{red}}]\)). The resulting bias potential is just the calculated value of the redox midpoint potential of that group.

Error estimate. The error in the computed redox potentials has statistical, numerical and systematic contributions. The standard deviation in the protonation probability of a single titratable group estimated from the MC sampling procedure accounting for correlation between subsequent MC steps as described in ref. (44) was much smaller than 0.01 protons. The sum of the standard deviations of protonation probabilities from all titratable groups was for each redox state of the cofactors that we considered about 0.02 protons similarly as in former applications (18,45). The error in the electrostatic energy values resulting from a grid resolution of 0.3 Å that we used was estimated to be considerably smaller than 10 meV (45). The resulting estimate of the combined numerical and statistical error of about 10 mV for the absolute values of the computed redox potentials is in the same range as typical uncertainties in experimental values for conventional molecular systems if measured under favourable conditions. But, this may not be fulfilled in the present case where redox-active cofactors are deeply buried in a large membrane-bound protein complex and require redox mediators to change their redox states.

The computational method involves also systematic errors, which are generally difficult to estimate and which can have a major influence on the final value of the computed cofactor redox potential. These errors can be due to uncertainties in atomic partial charges, the value of the dielectric constant used inside the protein and the atomic coordinates. Regarding the coordinates there are a number of error sources. In the present application we used a single coordinate set taken from the crystal structure. A statistical average of different conformations would be more appropriate but a suitable ensemble of conformations is not readily available. Conformations of crystal waters are generally
uncertain with regard to hydrogen atom positions and are likely to involve a number of energetically equivalent conformations. Therefore, they were removed in our past treatments. The distance dependence of electrostatic interactions is also for inhomogeneous dielectrics roughly proportional to the inverse of the distance between charges, which is a rather mild distance dependence. Therefore, the influence from different conformations is generally not so critical. But, this may no longer be the case in the presence of variably charged groups (titratable or redox-active) that are close to the considered redox-active group and are about to change their charge state. Under these circumstances, subtle changes in conformation may lead to shifts in the charge state of that group, which in turn changes the electrostatic interactions with the considered redox-active group and shifts its potential. In these cases shifts of the redox potential by 100 mV or more can be observed.

Nevertheless, we demonstrated in many different applications, where experimental redox potentials are known that our computational procedure is reliable and yielded redox potential values that often agree with experimental results within 20 mV (17,19-21). For convenience, the computed redox potentials are given with a 1 mV accuracy, albeit this does not suggest that the last digit is significant.

Computation of redox potentials of the cofactors. To obtain the in situ absolute values of the redox potentials of the cofactors, we need a reference model system of the cofactor (preferentially in solution) where the experimental value of the redox potential is known. By calculating the electrostatic energy difference of the two redox states of the considered cofactor in the reference model system and in the protein environment we obtain the shift in the redox potential between the two systems. This shift is added to the experimental value of the redox potential in the reference model system to yield the absolute value of the redox potential in the protein environment. As reference model system for phyllo-Q in PSI we considered the same quinone for the redox states (0/−1) solvated in dimethylformamide (DMF) where the measured redox potential is −463 mV versus normal hydrogen electrode (NHE). The latter value was obtained by converting the corresponding value measured versus standard calomel electrode (46). Note that the computational conversion of this redox potential value from DMF to water as solvent yields −453 mV, if we account for the dielectric constant of $\varepsilon_{\text{DMF}} = 37$ for DMF and $\varepsilon_{\text{W}} = 80$ for water. Henceforth, we will provide values of redox potentials relative to NHE, if not otherwise stated. The reliability for a series of those quinone redox potentials measured in DMF (46) has been demonstrated in our previous bRC study, where we successfully calculated the redox potential of ubiquinones (ubi-Q) (21) in agreement with
experimentally measured values, starting from the value in DMF. The phyllo-Q redox potential in DMF taken from the same reference (46) may help to clarify uncertainties arising from different conditions of the measurements. It also facilitates a direct comparison of our calculated A1 redox potentials in PSI with the calculated ubi-Q redox potentials obtained in bRC (21).

**Computation of the “direct” contribution of residues/cofactors on the A1 redox potentials.**

To characterize factors that influence A1 redox potentials, we discriminate between “direct” and “indirect” contributions on A1 redox potentials, which are due to a specific residue/cofactor as was done in similar investigations before (17). The former is due to the charges of the considered residue/cofactor that interact with A1 and thus lead to a shift in the redox potential. This "direct" contribution on the redox potentials of A1 can be turned off by setting the charges of the considered residue to zero, while fixing the charge states of all the other titratable and redox-active groups except of A1. Hence, the negative value of the shift obtained by setting the charges of a specific residue/cofactor to zero is the “direct” contribution from this residue. If in contrast we allow the charge states of all other groups to equilibrate, we obtain the sum of the “direct” and “indirect” contributions. The latter is due to changes in the protonation pattern or redox states that is induced by setting the charges of the specific residue/cofactor under consideration to zero. Note that by setting the charges of a specific residue to zero no cavity is generated, since the corresponding volume remains occupied by the atoms of that residue, such that the dielectric constant remains at \( \varepsilon = 4 \) as in the other parts of PSI.

**RESULTS AND DISCUSSION**

**Protonation pattern in PSI.** First, we like to discuss aspects of the protonation pattern, which may have an impact on the quinone redox potentials in PSI. A study based on FTIR difference spectroscopy indicated that one glutamate is perturbed upon formation of A1\(^{-}\). As possible candidates the glutamates A699, A702, B679 and B682 were proposed (47). The two pairs of glutamates Glu-A702/Glu-B682 and Glu-A699/Glu-B679 are located in equivalent positions in the A- and B-branch, respectively (Figure 2). We investigated the protonation pattern of titratable residues for each of the three different charge states P700\(^{+}\)A1\(^{-}\)A1\(^{0}\)A1\(^{-}\), P700\(^{+}\)A1\(^{0}\)A1\(^{-}\)A1\(^{0}\) and P700\(^{0}\)A1\(^{0}\)A1\(^{0}\)A1\(^{0}\). Among the four glutamates only Glu-B682 showed a notable albeit small increase of protonation from fully ionized for A1\(^{-}\) to 0.15 H\(^{+}\) with the formation of A1\(^{-}\). An interesting analogy is Glu-L212 in bRC from *Rb. sphaeroides*, where a significant change of protonation probability was found upon formation of Q\(_{B}^{-}\) (21,48,49), although Glu-L212 is fully protonated in the redox state Q\(_{B}^{-}\) (19,21,48,50-53).
The most striking effect in protonation pattern occurs at Asp-B575 from PsaB that is close to A1B (Figure 2) and has no symmetry counterpart in PsaA where we find Gln-A588 instead. One acidic oxygen of Asp-B575 is hydrogen bonded to the crystal water W-37 (Figure 2). Its protonation probability increased significantly from fully ionized to 0.85 H⁺ and 0.17 H⁺ upon formation of A1A⁻ and A1B⁻, respectively. In the absence of W-37 these protonation probabilities change to 1.00 H⁺ and 0.97 H⁺, respectively, while Asp-B575 is partially protonated in the state A1A0A1B0 where it carries 0.32 H⁺. As will be discussed later, these differences in the degree of protonation of Asp-B575 that are due to the explicit interaction with water W-37 invoke considerable shifts in the quinone redox potentials. Asp-B575 is conserved among a series of PSI from different species (e.g. *Anabaena (A.)* variabilis and *Chlamydomonas (C.)* reinhardtii). Regarding these drastic changes in protonation one might conclude that Asp-B575 is crucial for the asymmetry of the energetics of the ET process along the ET chain coupling more strongly to the A-branch than to the B-branch quinone.

Review of experimental results on redox potentials of phyllo-Q in PSI. Since a direct measurement of the redox potential of A1 was never successful, its value was estimated based on measurements of the triplet yield of P700 and additional assumptions yielding relatively low values around −800 mV (11). These extremely negative values were widely accepted, since the next acceptor FX in the ET chain of PSI, was known to have a very low redox potential too. Using knowledge on the redox potential of quinones in different solvents and the FX redox potential value of −700 mV suggested from experimental data in PSI the redox potential of A1 was evaluated to be −754 mV (54). Hence, according to the preceding estimates of the redox potentials of A1 and FX the ET from A1 to FX should be downhill in energy.

Estimate of the A1 redox potential based on kinetic and structural data. Considering an exergonic ET process from A1 to FX we can estimate the redox potential of A1 by evaluating the following empirical rate expression, which describes ET processes for edge-to-edge distances greater than 3.6 Å that are downhill in energy (55)

\[
\kappa_{\text{exergonic ET}}^{T=300K} = 10^{(13 - 0.6(R - 3.6) - 3.1(|\Delta G| + \lambda^2/\lambda))},
\]

where \(\Delta G\) is the redox potential difference between the electron donor and acceptor group. Note that in this expression energy parameters (\(\Delta G\) and \(\lambda\)) are given in units of eV and distances (\(R\)) in units of Å. The edge-to-edge distance of donor and acceptor group \(R\) taken from the latest crystal structure of PSI at 2.5 Å resolution (1) is \(R = 6.8\) Å, while in the previous crystal structure at 4 Å resolution this distance was with \(R = 11.3\) Å.
considerably larger (56). For the reorganization energy \( \lambda \), we took the value \( \lambda = 1.0 \) estimated from the kinetics of the ET process from A\(_1\) to F\(_X\) (57) that is not too far from the canonical value of 0.7 eV. The lifetime of the reduced state A\(_1^-\) was measured to be about \( 1/k_{ET} = 280 \) ns (6,58-61). The measured values of the redox potential of F\(_X\) vary between –705 mV and –650 mV (62-65). Using rate expression (2) that is valid for exergonic ET processes with these parameters yields a redox potential for A\(_1\) of –497 mV and –442 mV that is more positive than the value for F\(_X\). Hence, the ET from A\(_1\) to F\(_X\) is uphill in energy, such that we have to reevaluate the redox potential of A\(_1\) using the appropriate rate expression for endergodic ET processes (55).

\[
\frac{k_{\text{end}}}{k_{300K}} = 10^{(13 - 0.6(R - 3.6) - 3.1(|\Delta G| + \lambda)^2/\lambda - |\Delta G|/0.06)}.
\]  

(3)

According to equation (3) we estimated the redox potential of A\(_1\) to be between –577 mV and –522 mV considering the redox potential of F\(_X\) to be between –705 mV and –650 mV (62-65), respectively. It may be surprising that these estimates indicate that the forward ET from A\(_1\) to F\(_X\) is uphill in energy as compared to the estimate of –800 mV for the redox potential of A\(_1\) (11,66) that is considerably more negative.

**Computational results of A\(_1\) redox potentials.** The calculated redox potentials of the phyllo-Q (A\(_{1A}\) and A\(_{1B}\)) in PSI are –531 mV and –686 mV (–438 mV and –604 mV without water W-37) for the A- and B-branch, respectively (Figure 3, Table 1). Even more negative A\(_1\) redox potentials (–629 mV for A\(_{1A}\) and –776 mV for A\(_{1B}\)) were obtained in the presence of all crystal waters. But, these values may vary considerably due to the uncertain hydrogen positions of the waters. The A\(_{1A}\) redox potential obtained from electrostatic energy computations is in good agreement with the estimate between –577 mV and –522 mV computed from the rate expression (3). This lends the idea that the radical state A\(_1^-\) observed in EPR spectroscopy (2) refers to A\(_{1A}\), which is also supported by mutagenesis studies (3). Based on the calculated redox potential of A\(_{1A}\) and the available estimates for the redox potential of F\(_X\) (–705 mV to –650 mV (62-65)) the forward ET from A\(_{1A}\) to F\(_X\) is uphill in energy by about 119–174 mV, which can be correlated with results from EPR spectroscopy that probe the photo-accumulated A\(_1^-\) state from the A-branch quinone only (2). On the other hand, our computational results indicate that the ET reaction from A\(_{1B}\) to F\(_X\) is of ambient character varying from weakly exergonic (36 meV driving energy) to weakly endergonic (19 meV activation energy). This is in perfect agreement with the latest kinetic studies on temperature dependence of the ET from A\(_1\) to F\(_X\), revealing two components a slow and a fast corresponding to activation energies of 110 mV and 15 mV, respectively (10). Note that time resolved optical spectra for forward ET from A\(_1\) to F\(_X\) are biphasic, which may be due to the
co-existence of a fast ET on the B-branch and a slow ET on the A-branch (5-7,67). Using the rate expression (2) and (3), we calculated the lifetime of $A_1^-$ referring to the ET from $A_1$ to $F_X$ yielding 220 ns, 375 ns and 964 ns for the A-branch and 6 ns, 8 ns and 17 ns for the B-branch, depending on the value assumed for the redox potential of $F_X$ that is −650 mV (64,65), −670 mV (63) and −705 mV (62), respectively (Table 1). The lifetime ($t_{1/e} = 1/k_{ET}$) of $A_1^-$ was measured to be between 206 ns and 355 ns [206 ns and 231 ns (7), 216 ns (6), 220 ns (61), 231 ns (5), 289 ns (60), 355 ns (9)] for the A-branch, which is expected to correspond to the slower ET phase. From kinetic studies, the lifetime of $A_1^-$ in the B-branch, corresponding to the faster phase, was estimated to be 10 ns (68), 26 ns (5) and 36 ns (6). Some authors provide values of halftime ($t_{1/2}$) instead of lifetime ($t_{1/e}$), which were converted according to $t_{1/2} = \ln(2) \cdot t_{1/e}$. Our estimated rates for the ET process from $A_1$ to $F_X$ are for both, the fast and the slow phase, in good agreement with the lifetimes measured for $A_1^-$. 

Results from EPR studies seem to be in conflict with results obtained from kinetic studies. For instance, combined with a mutagenesis approach photo-accumulation of the redox state $A_1^-$ was observed for a single quinone only (3). Time-resolved EPR spectroscopy identified the appearance of the radical state $A_1^-$ with the charge-separated state $P700^+A_1^-$ (69). However, the measurements were sensitive only for times longer than a few $10^{-8}$ s such that a faster phase observed in UV/VIS spectroscopic studies could be not detected (70). In conclusion, both branches are probably ET active and the redox potential difference by 155 mV between $A_{1A}$ and $A_{1B}$ leads to a biphasic ET process, which is fast in the B-branch and slow in the A-branch.

It is of interest to calculate the contribution of the lipid molecules on the $A_1$ redox potentials that are at relatively short distance from both $A_1$ quinones. One of the three PG closest to the $A_{1A}$ side possesses a shortest distance to $A_{1A}$ of about 11 Å. The GG is close to the $A_{1B}$ side with a shortest distance to $A_{1B}$ that is also 11 Å. The negative charge on PG is expected to down-shift the $A_{1A}$ redox potential relative to the uncharged GG that is close to the $A_{1B}$ side. To check the influence of these lipids on the $A_1$ redox potentials, a PSI structure was prepared where all four lipid molecules of the crystal structure were considered and the $A_1$ redox potentials were calculated again. But, only a relatively small shift in the $A_1$ redox potential (8 mV down-shift for $A_{1A}$ and 5 mV up-shift for $A_{1B}$) was observed. A study of ET related photosynthetic activity on a mutant of *Synechocystis* sp. PCC 6803 with disrupted *pgxA* gene that reduces the PG content influenced the overall photosynthetic activity. But, this effect was attributed to a decrease of activity in PSII rather than in PSI (71). The small shifts of $A_1$ redox potentials due to these lipids obtained
in our computations are not in conflict with this mutagenesis study.

*Role of tryptophans near the A₁ binding sites.* Trp-A697 and Trp-B677 are in π-stacking conformation with the quinones of A₁A and A₁B, respectively (Figure 2). In the crystal structure, the inter-planar distances between the indole ring and the quinone ring vary between 3.0 Å and 3.5 Å (1,67) or 3.7 Å in ESEEM studies (72). These tryptohans were point-mutated to Phe, His or Leu and the changes of kinetics in the ET process were investigated by UV/VIS absorption spectroscopy (7) and EPR studies (8,9,73).

We calculated the “direct” contribution of the tryptophan side chain on the shift of the A₁ redox potentials (for a definition of “direct” contribution on the shift of the A₁ redox potentials see above in Computation of the “direct” contribution of residues/cofactors on the A₁ redox potentials). Accordingly, the tryptophans Trp-A697 and Trp-B677 were found to be responsible for a down-shift of the redox potential by only 27 mV in both cases for the corresponding quinone (Table 2). As expected the tryptophan that is close to one quinone had no influence on the redox potential of the other quinone in agreement with experiments (7-9,73) (Table 2).

These results contrast with recent quantum chemical computations from which it was concluded that the redox potential of quinone is down-shifted by 50–150 mV due to the π-stacking between Trp and quinone (74). However, this computation was performed just for quinone, Trp and a backbone NH group forming a hydrogen bond with one carbonyl oxygen of quinone with dielectric constant of ε = 1. Hence, the molecular system was practically considered under vacuum conditions, where π-stacking effects may be overestimated as compared to the situation in the protein environment.

Trp-B673 from subunit PsaB between A₁B and Fₓ has no symmetry counterpart in PsaA (Figure 2). It was speculated that this asymmetrically placed tryptophan may be responsible for the differences in the ET rates between the two branches (1). Therefore, we also calculated the “direct” contribution of the Trp-B673 side chain on the shift of the A₁ redox potentials. As a result (Table 2), the quinone redox potentials practically did not change yielding a vanishing shift for A₁A and a down-shift by only 4 mV for A₁B in our computations. Hence, these small “direct” contributions on the shift of the A₁ redox potentials from the Trp-B673 indole ring does not support this hypothesis. However, the Trp-B673 side chain may support the ET between the quinones and Fₓ, since it bridges the gap between them. The contribution from Trp-B673 to the A₁ redox potential by causing a distortion in the coordinates of PsaB as compared to the corresponding region of PsaA is discussed at the end of the next paragraph.
Asymmetry of the quinone redox potentials. Different factors contribute to the asymmetry of 155 mV that we computed for the quinone redox potentials in PSI. Although the iron-sulfur cluster FX is very close to the quinones and carries a negative charge of minus two, it does not contribute to the asymmetry of their redox potentials. We calculated the “direct” contribution of FX on the shift of the A1 redox potentials. The computed shifts in the A1 redox potentials that can be attributed to FX are 237 mV and 256 mV for A1A and A1B, respectively. The lack of asymmetry in the shift of the quinone redox potentials is also obvious from the symmetric placement of FX between the two quinones (identical edge-to-edge distance for FX – A1A and FX – A1B of 6.8 Å). Similarly, we estimated the contribution of the iron-sulfur cluster FA to the asymmetry of the quinone redox potentials. FA is placed asymmetrically with respect to the quinones but is also more distant from the quinones (edge-to-edge distance for FA – A1A and FA – A1B is 20.6 Å and 16.6 Å, respectively) than FX. Hence, it contributes only 16 mV to the asymmetry of the quinone redox potentials in PSI (Table 2). The contribution of the more distant FB (edge-to-edge distance for FB – A1A and FB – A1B is 24.6 Å and 28.9 Å, respectively) was negligible (Table 2).

However, we found that the largest portion of the asymmetry of the quinone redox potentials is generated by the following two factors; (i) the aspartate Asp-B575 that is closer to A1A than to A1B (minimum O – O distance with A1A and A1B of 8.9 Å and 13.7 Å, respectively) whose protonation state is more strongly coupled with the formation of the reduced state A1A•− than A1B•− (ii) the backbone conformation of PsaA and PsaB, which stabilizes the reduced state of the quinones A1A•− and A1B•− by different amounts.

In contrast to the glutamates Glu-B682 and Glu-B679 from subunit PsaB, which have Glu-A702 and Glu-A699 as symmetry counterpart in subunit PsaA, the symmetry counterpart to Asp-B575 in subunit PsaB is the non-titratable residue Gln-A588. Furthermore, we found that among all titratable residues in PSI Asp-B575 is the only residue that changes its protonation state considerably if one of the quinones is reduced. Asp-B575 is fully ionized, while both quinones are oxidized, but becomes protonated by 0.85 H+ and 0.17 H+ upon formation of the reduced states A1A•− and A1B•−, respectively. However, the protonation state of Asp-B575 relevant for the determination of the quinone redox potentials refers to the situation where the considered quinone is to 50 % reduced only. The protonation state of Asp-B575 corresponding to that situation is 0.44 H+ and 0.09 H+ for A1A and A1B, respectively (Figure 4). This is about half of the total change in protonation, which occurs between the fully oxidized and reduced state of the corresponding quinone.
We expect that the presence of the negative charge at Asp-B575 is down-shifting the A1 redox potentials considerably. This should be more pronounced for A1A than for A1B, since Asp-B575 is closer to A1A than to A1B. The “direct” contribution of the Asp-B575 charges on the shifts of the A1 redox potentials can be calculated by first considering the fully ionized Asp-B575 in PsaB and for symmetry reasons also of the equivalent neutral Gln-A588 in PsaA. These to two symmetry related residues yield a down-shift of 192 mV and 154 mV for the redox potentials of the quinones A1A and A1B, respectively, which, surprisingly, is rather symmetric although Asp-B575 is charged and Gln-A588 is neutral.

However, the situation is even more complex, since Asp-B575 changes its protonation state with the reduction of A1. The protonation is more significant with the formation of A1A– than of A1B–, which may induce an asymmetry in the quinone redox potentials leading to a larger down-shift of the A1B than of the A1A redox potential. This influence can be investigated by calculating the shift in quinone redox potentials if the protonation of Asp-B575 changes from fully ionized to fully protonated, while keeping the charge state of all other variably charged groups fixed. This procedure yielded redox potential up-shifts of the two quinones, which are 214 mV and 135 mV for A1A and A1B, respectively. The corresponding up-shifts for the partial protonation of Asp-B575 that we found if the considered quinone is to 50% reduced are accordingly 0.44 * 214 mV = 94 mV and 0.09 * 135 mV = 12 mV for A1A and A1B, respectively, yielding a net difference in the quinone redox potentials of 82 mV, which is due to the change in the protonation of Asp-B575 (Figure 4).

Surprisingly, we found that the backbone charges contribute differently to the stabilization of the two quinone anions A1A– and A1B–. The “direct” contribution from backbone charges on the A1 redox potentials was recorded by setting zero charges on all backbone atoms in PSI, while keeping all titratable groups in a fixed charge state. As a result, we found that the redox potentials of both quinones were up-shifted by the backbone charges by 173 mV and 129 mV for A1A and A1B, respectively yielding an asymmetry of 44 mV for the quinone redox potentials. Interestingly, the backbone charges of the symmetry equivalent pair of conserved residues Ser-A692 and Ser-B672 yield a marginal up-shift of the A1A (17 mV), and a down-shift of the A1B redox potential (48 mV). The resulting contribution of 65 mV from these serines to the asymmetry of the A1 redox potentials is diminished by the other parts of the backbone.

Analyzing the shifts from these serines in more detail we found that the distance from the backbone nitrogen of serine to the nearest carbonyl oxygen of the corresponding quinone are for both ET branches almost identical (O A1– NSer-backbone distance 5.7 Å and 5.4 Å for...
A- and B-branch, respectively). However, the corresponding distances of the backbone carbonyl oxygens of serine differ significantly by 2.2 Å (O_{A1} – O_{ser-backbone} distance 6.2 Å and 4.0 Å for A- and B-branch, respectively). The corresponding serine backbone CO groups exhibit quite a different orientation relative to its nearest quinone (Figure 2). The calculated “direct” contribution to the difference in the quinone redox potentials that is due to these two CO groups amounts to 65 mV. Mutating Ser-A692 to cysteine showed a 5 % decrease in the hyperfine coupling of the methyl group of the A1 ring, while mutating Ser-B682 to cysteine yielded the same spectra as for wild type PSI (75). However, such mutagenesis studies may not be able to assess the effect of the backbone orientation of the corresponding CO groups, unless the backbone conformation is changed in a controlled fashion. The different orientations of the CO groups of the equivalent serines Ser-A692 and Ser-B672 may be related to the location of a crystal water molecule (W-132) within hydrogen bond distance from Ser-A692 (O – O distance 2.9 Å), while no corresponding crystal water is close to Ser-B672. Another reason could be the difference in the neighbour residues. In PsaA a glycine is located at A693, while in PsaB the corresponding neighbour residue at B673 is a bulky tryptophan.

**Difference of quinone redox potentials in PSI relative to PSII and bRC.** It is still an open question which mechanisms shift the redox potentials of the quinones in PSI to those extremely negative values (between about –690 mV and –530 mV in the present study) relative to the quinones in bRC (between about –180 and –100 mV) (21,76-79) and in PSII (–80 mV) (80,81) (Figure 5). This difference cannot simply be explained by the difference in the solution redox potentials of naphthoquinone present in PSI (phyllo-Q; –463 mV in DMF (46)) and the two different benzoquinones used in PSII (plasto-Q; –369 mV in DMF (82)) and in bRC (ubi-Q; –360 mV in DMF (46)) (Figure 5). According to the crystal structure of PSI, only one of the carbonyl oxygen atoms for each of the two quinones accepts a hydrogen bond. These are formed with the backbone amid groups of Leu-A722 (N – O distance 2.7 Å) and Leu-B706 (N – O distance 2.8 Å) for A_{1A} and A_{1B}, respectively (1,67). Nevertheless, two serines are located close to the carbonyl oxygen atoms of A_{1A} and A_{1B} that are not engaged in hydrogen bonding. These are Ser-A692 (O – O distance 3.3 Å) and Ser-B672 (O – O distance 3.4 Å), respectively, which do not form hydrogen bonds with the corresponding carbonyl oxygen of the quinones, because of unfavorable hydrogen bond angles. This corroborates with data from EPR spectroscopy from which also only a single hydrogen bond per quinone was deduced (83). On the other hand, inspecting the crystal structure of bRC from *Rb. sphaeroides* one observes that both carbonyl oxygens of Q_A and Q_B are involved in hydrogen bonding (84). In EPR spectra of
bRC an asymmetric spin density distribution was found for QA\(^{-}\) (85). This is indicative for an asymmetry in the hydrogen bonding strength. Presumably, the H-bond with His-M219 is stronger, since the imidazole ring is polarized by the positive charge of the non-heme iron. Based on quinone replacement studies, hydrogen bonding of the quinones was suggested to be weaker in PSI than in bRC, since the orientation of the quinones varies in PSI, whereas in bRC different quinones that occupy the QA binding site adopt practically the same orientation (86,87).

The hydrogen bonding pattern of the quinones in PSI from *S. elongatus* contrasts with results form ENDOR studies on PSI from other species where two hydrogen bonds were found for A\(_{1A}\) in PSI from *A. variabilis* (88) and for A\(_{1A}\) and A\(_{1B}\) in PSI from *C. reinhardtii* (9,89). While the crystal structure of PSI likely refers to the charge neutral state, EPR and ENDOR that probe the quinone orientation by its radical form consider the charge-separated state. Hence, the additional hydrogen bonds involving the carbonyl oxygens of A\(_{1}\) as found in PSI from *A. variabilis* and *C. reinhardtii* may form in the photo-accumulated state of A\(_{1}\)^\(-\) only.

In quantum chemical computations of small quinone model systems for PSI O’Malley found a symmetric spin distribution if both carbonyl oxygens are involved in hydrogen bonding (90) and an asymmetric spin distribution if only one hydrogen bond prevails (91). An analog asymmetry in the spin density was observed in EPR spectra of A\(_{1}\)^\(-\) in PSI (92). Furthermore, O’Malley found by these computations that the redox potential of quinone can be up-shifted by about 250 mV by adding a hydrogen bond to one of the carbonyl oxygens (91,93). Based on these results and the difference in hydrogen bonding pattern of the quinones found in crystal structures of bRC (84) and PSI (1), it was speculated that the quinone redox potentials are much lower in PSI than in bRC due to the lack of a second hydrogen bond at the quinones in PSI (94,95). However, the quantum chemically calculated 250 mV up-shift of the quinone redox potential by adding a hydrogen bond may be overestimated, since the model on which these computations are based involves only a small fraction of the molecular groups of the whole protein environment and is considered at \(\varepsilon = 1\), i.e. under vacuum conditions. Similarly, it was found recently for cytochrome bo\(_{3}\) that an asymmetric spin density distribution of quinone does not need to go along with a strong down-shift in redox potential (96,97).

If the extreme negative values of the quinone redox potentials in PSI are due to incomplete saturation of the carbonyl oxygens with hydrogen bonds, the formation of an additional hydrogen bond should up-shift the corresponding quinone redox potential considerably. To investigate this influence theoretically, we modeled with a minor
conformational change in the crystal structure of PSI an additional strong hydrogen bond between the serine side chains Ser-A692 and Ser-B672 and the corresponding carbonyl oxygens of the quinones A1A and A1B, respectively. Note that in the crystal structure these two serine side chains provide the closest candidates of hydrogen bond donors to the quinones A1. However, the resulting up-shift of the quinone redox potentials was at most about 70 mV (data not shown). Therefore, according to our electrostatic energy computations the difference between PSI and bRC in hydrogen bonding pattern cannot account for the extreme negative shift of the redox potentials of the quinones in PSI relative to bRC.

According to our electrostatic energy computations, the quinone redox potentials undergo positive shifts by 190–270 mV being placed from DMF solution in one of the two binding sites in bRC from *Rb. sphaeroides* (21), whereas they experience negative shifts by 86–223 mV if placed in the A1 sites of PSI. Up-shift (down-shift) in the redox potential implies the presence of positive (negative) charges in the neighborhood of the considered redox-active group. We revealed that in the light-exposed structure of bRC from *Rb. sphaeroides*, the positive charge (+0.8) situated directly at the non-heme iron is responsible for a strong up-shift of the redox potential, which for QB was calculated to be 169 mV (21). Now we also calculated the corresponding value for QA and obtained 165 mV. One may consider the iron-sulfur cluster FX in PSI to play a similar crucial role for the redox potentials of the A1 quinones as does the non-heme iron for the quinones in bRC. One of the significant differences lies in the formal net charge of the two complexes, which is +1 for the non-heme iron complex and −2 for FX in the resting charge state, i.e. for [Fe4S4(SCH3)4]2− (25-27). While the quinone redox potentials in bRC are up-shifted by the presence of the positive charge from the non-heme iron complex (21) the quinone redox potentials in PSI are down-shifted by the iron-sulfur cluster carrying two elementary negative charges. This becomes evident by evaluating the “direct” contribution to the shift of the quinone redox potential which is due to the FX cluster, yielding a down-shift of 237 mV and 256 mV, for A1A and A1B, respectively (Table 2). Note that this is the largest shift of the redox potential of the A1 quinones that we observed in our electrostatic computations for PSI. Hence, the large difference in redox potentials of the quinones in bRC and PSII relative to PSI is mostly due to the oppositely charged non-heme iron complexes and only to a lesser extend due to the different hydrogen bonding patterns.

CONCLUSIONS

Our electrostatic computations provided values of the redox potentials of the quinone
cofactors in both ET branches of PSI that are based on the latest crystal structure at a resolution of 2.5 Å (1). Experimentally a discrimination of the redox potentials of the A₁ quinones in the A- and B-branch was not unambiguously possible. It is remarkable that the redox potentials of A₁ obtained in our computations are considerably less negative than the unusually negative value of –800 mV (11,54) estimated from experimental data. We could justify our computed values of the redox potentials by re-estimating the redox potential (55) from measured kinetic data (6,58-61) and the latest structural data (1), where we also observed a significant up-shift of the quinone redox potentials as compared to the former estimates (11,54).

Based on our computations we found that besides the contribution of the iron-sulfur cluster Fₓ to the down-shift of the quinone redox potentials the backbone of the serines Ser-A692 and Ser-B672 and the side chain of Asp-B575 play a significant role in discriminating the values of the quinone redox potentials between A₁A and A₁B. Asp-B575 changes its protonation state coupled with the formation of the A₁A and A₁B anion state, having a stronger effect on the A-branch than on the B-branch quinone. Further experiments will be useful to clarify the function of Asp-B575 and of the backbone part of Ser-A692 and Ser-B672 in more detail. The A₁A redox potential is by 155 mV more positive than that of A₁B, which is due to electrostatic interactions with the backbone and the conserved residue Asp-B575. The influence of the asymmetrically distributed lipids on the A₁ redox potentials turned out to be small in agreement with a recent mutant study (71), in spite of the negative net charge on PG.

Our computations corroborate: (i) Both branches are active for forward ET from A₁ to Fₓ as suggested by kinetic studies (5,7,8). The lifetime of A₁⁻ derived from the calculated redox potentials is in good agreement with the measured lifetime (5-7,9,60,61,68), demonstrating that the ET process in the B-branch (5,6,68) is much faster than in the A-branch (5-7,9,60,61). (ii) The biphasic kinetics of ET from A₁ to Fₓ can be explained by the redox potential difference between the two quinones in the A- and B-branch (10). The observed lifetimes of A₁⁻ for the slow (5-7,9,60,61) and fast (5,6,68) ET component are in agreement with values that were estimated for A₁A⁻ and A₁B⁻ based on the computed redox potentials of the A₁ quinones and appropriate model assumptions (55). (iii) The observed EPR data for A₁⁻ (2) can be attributed to the A-branch quinone.

The physiological relevance of the two ET branches in PSI and their different activity is still an open question. For a PSI mutant where Trp-B673 was replaced by phenylalanine, the ET rate from A₁ to Fₓ became slower for fast phase but remained still faster than slow phase. Nevertheless, the photosynthetic activity showed no observable changes relative
to the wild type PSI (7). Hence, in this case changing the ET process from $A_1$ to $F_X$ may physiologically not be critical for PSI function or this change might be compensated by other factors, for instance a corresponding change of the $F_X$ redox potential.

From ENDOR studies on PSI from *A. variabilis* and *C. reinhardtii* it was concluded that both carbonyl oxygens of quinone $A_1$ are engaged in hydrogen bonds with the protein environment (9,88,89). This finding disagrees with the crystal structure of PSI from *S. elongatus*, where only one hydrogen bond is present (1). However, this apparent discrepancy may be resolved by considering that in the reduced state $A_1^-$ a subtle conformational change may occur around $A_1$ that allows the formation of an additional hydrogen bond with the carbonyl oxygen of $A_1$ that would stabilize the radical state $A_1^-$ but is not present in the state $A_1^0$. Based on our computations, we found a relatively small up-shift in the quinone redox potential by modeling an additional hydrogen bond to $A_1$ in PSI, which cannot explain the large difference in the quinone redox potentials between bRC and PSII versus PSI. Instead, we found that the unusual negative redox potentials of the $A_1$ quinones in PSI as compared to bRC (21,76-79) and PSII (80,81) are due to the negative charge of the iron-sulfur cluster $F_X$ as compared to the positive charge of the non-heme iron complex present in bRC and PSII.

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REFERENCES


FOOTNOTE
1 N. Krauß, personal communications.
FIGURE LEGENDS

FIGURE 1: Arrangement of cofactors of the ET chains in PSI.

FIGURE 2: Quinone binding site, in PSI from *S. elongatus*. Trp-A697 and Trp-B677 are in a π-stacking conformation with the ring part of the quinones A1A and A1B, respectively. Oxygen and sulfur atoms are displayed in dark gray. The indole ring of the tryptophans with the carbon atom connecting to the backbone is depicted as stick model. Backbone atoms are shown only for the serines B672 and A692 with enlarged spheres for the carbonyl groups. The oxygen atom of the crystal water W-37 is shown as dark large sphere.

FIGURE 3: Energy scheme based on the calculated quinone redox potentials in PSI for the ET from A1 to Fx. The measured redox potentials of Fx range from –705 mV to –650 mV (62-65).

FIGURE 4: Shifts of A1 redox potentials upon protonation of Asp-B575. As Asp-B575 becomes protonated upon formation of A1-, the A1 redox potential shows a positive shift.

FIGURE 5: Comparison of the quinone redox potentials in solution (DMF) and in the protein complexes of PSI and bRC. The values for bRC from *Rb. sphaeroides* were taken from the computational results in ref. (19), which were obtained with the same methods as the present results on PSI.
Table 1: ET lifetime $1/k^{\text{ET}}$ from $A_1$ to $F_X$ in PSI estimated from the rate expressions of $k^{\text{ET}}$, eq. (2) and (3), based on the calculated redox potential value for $A_1$ and the measured value for $F_X$.

<table>
<thead>
<tr>
<th>redox potentials [mV]</th>
<th>1/$k^{\text{ET}}$ [ns]</th>
</tr>
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<tbody>
<tr>
<td>calc. $E_m(A_1)$</td>
<td>exp. $E_m(F_X)$</td>
</tr>
<tr>
<td>(-705^a)</td>
<td>(-670^b)</td>
</tr>
<tr>
<td>(-670^b)</td>
<td>375$^d$</td>
</tr>
<tr>
<td>(-650^c)</td>
<td></td>
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<td>(-705^a)</td>
<td>17$^d$</td>
</tr>
<tr>
<td>(-686)</td>
<td>(-670^b)</td>
</tr>
</tbody>
</table>

$^a$ See Ref. (62).
$^b$ See Ref. (63).
$^c$ See Ref. (64,65).
$^d$ Estimated by equation (3).
$^e$ Estimated by equation (2).
$^f$ See Ref. (5-7,9,60,61).
$^g$ See Ref. (5,6,68).

Table 2: Direct contributions to the shift of the $A_1$ redox potentials $\Delta E_m$ from different residues and cofactors. The shifts were calculated for a fixed protonation state of PSI by considering two situations where the atomic charges of the considered residue were kept (charged) or set to zero (zero).

<table>
<thead>
<tr>
<th>residue</th>
<th>$\Delta E_m$(charged - zero) [mV] $^a$</th>
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<tr>
<td>$A_{1B}$</td>
<td>$A_{1A}$</td>
</tr>
<tr>
<td>Asp-B575 &amp; Gln-A588</td>
<td>(-154)</td>
</tr>
<tr>
<td>Trp-A697 $^b$</td>
<td>(+1)</td>
</tr>
<tr>
<td>Trp-B677 $^b$</td>
<td>(-27)</td>
</tr>
<tr>
<td>Trp-B673</td>
<td>(-4)</td>
</tr>
<tr>
<td>$F_X$ $^c$</td>
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</tr>
<tr>
<td>$F_B$ $^c$</td>
<td>(-12)</td>
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$^a$ The value refers to the shift in the redox potential of $A_1$ in wild type PSI.
$^b$ These residues correspond to Trp-A693 and Trp-B673 in PSI from *C. reinhardti*
i and are in symmetry equivalent positions of subunit PsAa and PsAB, respectively.
$^c$ oxidized charge state $[\text{Fe}_4\text{S}_4(\text{SCH}_3)_4]^2$ of the iron-sulfur cluster.
FIGURE 1
FIGURE 2:

FIGURE 3
FIGURE 4

protonation  redox potential

$E_m(Q_B)$  12 mV  82 mV  94 mV  214 mV

$E_m(Q_A)$  0.00  0.44  1.00
FIGURE 5

The diagram illustrates the energy levels of PSI and bRC components, with specific electron transfer processes indicated by the arrows. The energy levels are labeled as follows:

- **PSI**
  - \(E_{m_{A1B}}\) at \(-700\) mV
  - \(E_{m_{A1A}}\) at \(-600\) mV
  - **phylllo-Q**
- **bRC**
  - **ubi-Q**
  - \(Q_A\)
  - \(Q_B\)
SUPPLEMENTAL DATA

Atomic partial charges for PG (1,2-dipalmitoyl-phosphatidylglycerole), GG (1,2-distearoyl-monogalactosyldiglyceride), and Chl a in the (–1/0/+1) charge states. The atomic charges of the aliphatic chain part not given here were assigned according to CHARMM22 parameters with a standard charge of +0.09 for non-polar hydrogen atoms and vanishing total charge for each CH₃ group (22,29).

Table S1: Atomic partial charges of PG

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Table S2: Atomic partial charges of GG

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Table S3: Atomic partial charges of Chl

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*a The atomic partial charges for Chl(–1) were not used in the present computation.