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The free energy difference between the excited primary donor $^1P^*$ and the radical pair state P^+H^- in reaction centers of *Rhodobacter sphaeroides*

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The Gibbs free energy difference between the excited primary donor and the relaxed radical pair state P^+H^- in quinone-depleted reaction centers of *Rhodobacter sphaeroides* has been determined from various measurements of the delayed fluorescence to be 0.25 eV. Comparing this value with other energy data an entropy change of $\Delta S(^1P^* - P^+H^-) \simeq 1.9 \cdot 10^{-4}$ eV/K is expected, giving only a small contribution at room temperature. Furthermore an upper limit, $\Delta G(^1P^* - P^+B^-) < 0.09$ eV, is determined.

Introduction

Primary charge separation in photosynthetic reaction centers (RCs) proceeds from the excited special pair state ($^1P^*$) to a bacteriopheophytin molecule (H) within a few picoseconds [1,2]. Both sequential [1,3–8] and unistep superexchange electron transfer mechanisms [2,9–14] have been invoked to explain this extremly fast rate over a center-to-center distance of 17 Å. Essential for any theoretical treatment of the kinetics is the free energy difference between the initial state, $^1P^*$, and the final radical pair (RP) state, P^+B^- or P^+H^-.

Delayed fluorescence due to recombination of P^+H^- back to $^1P^*$ gives direct access to the free energy change $\Delta G(^1P^* - P^+H^-)$, giving at least an upper limit for the free energy release on the very first electron-transfer step. The time resolved fluorescence measurements reported in Refs. 15 and 16 both agree that, in RCs of *Rb. sphaeroides* blocked by depletion of the quinone, at least two delayed fluorescence components are detectable in addition to the prompt fluorescence (unresolved in time). The slowest component has the same time constant (> 10 ns) as the decay of the radical pair P^+H^- detected in transient absorption experiments [17–20] and apparently originates from charge recombination of the radical pair state to the $^1P^*$. The slowest decay times detected both in absorption and in fluorescence are reduced on application of a magnetic field of several hundred gauss [15–20]. This fact certainly gives further confirmation that both signals refer to the same radical pair state. Two additional intermediate components are resolved in Refs. 16, while in Ref. 15 only one is detected due to the limited time resolution. This feature seems to be universal to RCs as so far observed, since it appears also in reduced RCs of *Rb. sphaeroides* [15,21,22] *Rb. rubrum* S1 [22] and *Rps. viridis* [23]. Furthermore, similar components have been seen in chromatophores [24], suggesting that these are not artifacts associated with the isolation procedure. In quinone-depleted RCs, the major of these components is 2 ns [15] or 3.1 ns [16], the other one slightly below 1 ns in lifetime. (Recently, very interesting time-resolved fluorescence measurements at very high magnetic fields have been published [50], also showing intermediate fluorescence components. The results have been interpreted in the frame-work of a relaxation model [51].)

Several reaction schemes have been proposed to explain these additional fluorescence components [21,25]. In the following I will discriminate between three principle models which can account for the observations.

(a) Relaxation model. Short-lived states are situated as earlier intermediates between $^1P^*$ and P^+H^- in a sequential reaction path. Since there are no absorption measurements which correlate in time with the relatively slow intermediate fluorescence components, the possible intermediate states must be spectroscopically indistinguishable from the P^+H^- state. Such states could be a form of the P^+H^- state being energetically nonrelaxed with respect to nuclear movements of the

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protein [21]. The slowest fluorescence component then reflects the relaxed state.

(b) Branching model. Starting from the initial state, $^1P^*$, the reaction path branches, one branch leading to P^+H^-, and the second one leading to a new different state [21,25]. X-ray structure data [26,27] of the RC revealing two branches of electron acceptors (indexed A and B, in the following) provide possible candidates for such a state. The bacteriopheophytin H_B on the B-branch was suggested to be a possible electron acceptor alternative to H_A on the A-branch [25]. Thus, one of the intermediate fluorescence components could reflect the lifetime of $P^+H_B^-$ decaying via a back reaction to the ground state and to $^1P^*$.

(c) Contamination model. Due to the very low fluorescence quantum yield to $4 \cdot 10^{-4}$ for intact RCs [28], slight traces of contaminating pigments can give comparatively strong signals in time resolved fluorescence measurements.

Referring to different models different values $\Delta G(^1P^* - P^+H^-) = 0.26$ eV [15] and 0.148 eV [16] have been published from different laboratories. The first value is derived from the slowest component of delayed fluorescence and is analysed in terms of the branching model (b). The second one also comprises the additional faster components and refers to a nonrelaxed RP state, $P^+H^-_{\text{nonrelaxed}}$, in terms of the relaxation model (a).

It is the purpose of this paper to obtain free energy values from both sets of measurements making them directly comparable. In Appendix I an approximate relation for $\Delta P(^1P^* - P^+H^-)$ is derived for the relaxed and nonrelaxed RP states. Alternatively it is shown in Appendix II, that $\Delta G(^1P^* - P^+H_A^-)$ derived in terms of the branching model is approximately equal to $\Delta G(^1P^* - P^+H^-)_{\text{relaxed}}$ derived in terms of the relaxation model. The results are compiled in the following. The obtained values are compared with the other energy data concerning $^1P^*$ or P^-H^- and consequences for the temperature dependence of $\Delta G(^1P^* - P^+H^-)_{\text{relaxed}}$ are discussed. Finally, an upper limit for the free energy change associated with primary charge separation is estimated.

Results and discussion

According to Ref. 21, we label the RP states in the relaxation sequence with index i, the first nonrelaxed state resolvable in fluorescence measurements having index $i = 1$ and the final, relaxed RP state labeled $i = N$. The free energy difference between the fluorescing state $^1P^*$ and the RP state P_i^F is given in good approximation by (see Appendix I):

$$\Delta G(^1P^* - P^+H^-)_i = -kT \ln\left[\frac{1}{\tau_i k_1}\frac{\Phi_i}{\Phi_0}\right] = -kT \ln\left[\frac{A_i}{\Phi_0 k_1}\right] \qquad (1)$$

$\Phi_i = A_i\tau_i$ are the quantum yield, the amplitude and the time constant of the corresponding fluorescence component. Φ_0 is the prompt fluorescence yield and k_1 is the primary charge separation rate. For $i = N$, i.e., referring to the final, relaxed RP state, this expression is the same as one would obtain for a single step charge separation and subsequent delayed recombination, without any side reaction. For the first nonrelaxed state ($i = 1$) an exact relation equivalent to Eqn. 1 of Ref. 21 can easily be derived. In this case A_1 in Eqn. 1 merely has to be replaced by ΣA_i. As shown in Appendix II, $\Delta G(^1P^* - P^+H^-)$ in terms of a branching model is approximately equal to $\Delta G(^1P^* - P^+H^-)_N$.

Aside from the primary charge separation rate, k_1, determined in fs time-resolved transient absorption measurements, the only experimental parameters involved in Eqn. 1 are the amplitudes of the delayed fluorescence relative to the prompt fluorescence yield, but not the absolute values. These are obtained from a single fluorescence decay trace in one measurement of one sample, since the procedure of decomposition into the various components scales the amplitudes so that $A_i\tau_i$ gives Φ_i relative to Φ_0 [21]. As will be discussed later, the most serious source of error will be the relative value of Φ_0, since at the given time resolutions it might not be truly all 'prompt' but might contain unresolved delayed fluorescence. Unfortunately, k_1 has not been measured on quinone-depleted RCs. Since the apparent prompt fluorescence of such RCs is only 10% higher than in untreated ones [16], we assume k_1 to be the same in both and use $k_1 = 1/(3.5\text{ ps})$ [1].

For comparison, in Ref. [16,21] $\Delta G(^1P^* - P^+H^-)_1$ is determined more indirectly, by relating the delayed fluorescence amplitude of blocked RCs to the total fluorescence yield of a different sample of untreated RCs. The values are determined in two independent measuring sessions and normalized to the absolute fluorescence yield of untreated RCs taken from the literature [28]. As in the treatment suggested above, one has to assume that the yield of untreated RCs is purely 'prompt'. The difficulty to reliably determine absolute quantum yields, however, supervenes.

Using Eqn. 1 we obtain the free energy differences shown in the following table. The values in the first row are based on experimental fluorescence data, i.e., amplitudes, yields and RP lifetimes as given in Table II of Ref. 16, the second row is derived from data in Table I of Ref. 15.

Fluorescence data from:	Ref 16	Ref. 15
$\Delta P(^1P^* - P^+H^-)_N$ (relaxed)	0.249 eV	0.258 eV
$\Delta G(^1P^* - P^+H^-)_2$ (nonrelaxed)	0.213 eV	0.202 eV
$\Delta G(^1P^* - P^+H^-)_1$ (nonrelaxed)	0.166 eV	–

The reliability of fluorescence data

The free-energy data derived for the nonrelaxed RP states are, of course, valid only under the supposition that these states really exist and that they are associated with the respective fluorescence components. The possibility of uncovering such 'hidden' states makes fluorescence experiments so attractive, while at the same time it is almost impossible to trace definitely most of the fluorescence components to their origin giving them the quality of a final proof. Indeed, we have no additional experimental evidence to decide on the origin of the intermediate fluorescence components referred to in this context and hence we have no data in support of any one of the models. On the other hand, the fluorescence data referring to the relaxed RP state appear to be quite reliable, as will be discussed in the following.

Various measurements indicate that emission at the blue side of the fluorescence spectrum has a different origin than the rest of the band. Microwave-induced low temperature fluorescence spectra of blocked RCs change sign at the blue side of the fluorescence band [29–31]. Time-resolved spectra of the early fluorescence (up to 2 ns) are blue-shifted with respect to fluorescence delayed by more than 10 ns in reduced RCs at room temperature [21]. Time-resolved fluorescence measurements of untreated RCs reveal a nanosecond component in addition to a 6 ps one, which becomes more intense at short wavelengths [32]. Though these results may have different causes, at least in the last case contamination appears to be the most likely one.

To avoid problems with possible contamination of the intermediate fluorescence components, I resort to discussing the validity of the slowest and of the prompt components only. The lifetime of the slowest component is markedly longer than the fluorescence lifetime of bacteriochlorophyll in various solutions typically ranging between 1.6 and 3.1 ns [33–35]. It is identical to that of the RP state measured with transient absorption methods in both reduced and quinone-depleted RCs respectively and exhibits the same magnetic field dependence, while its amplitude is unaffected. This quantitative correlation clearly identifies this fluorescence component as recombination emission and excludes significant contaminating contributions. The amplitude of the slowest fluorescence component of RCs blocked by reduction essentially goes to zero at lower temperatures [21]. This component therefore is not likely to contribute to the strange features in the microwave-induced fluorescence spectra. Its lifetime rises at lower temperatures, as the RP lifetime does. For the prompt fluorescence of any kind of contaminants, one would expect a constant amplitude and a quantum yield decreasing simultaneous with the lifetime due to any kind of quenching mechanism. According to Ref. 16, quinone-depleted RCs behave qualitatively the same way as reduced RCs [21]. Due to the quinone extraction treatment, quinone-depleted RCs could be even more susceptible to contamination [16]. Due to the arguments just given, this can affect only the faster components, however. Indeed, it is difficult to exclude the possibility that contamination contributes to the 1 ns component, since it is also detectable in untreated (open) RCs and persists even after chemical oxidation of the primary donor [32]. On the other hand, we are not in a position to value the purity of preparations from different laboratories.

Throughout this paper it is assumed that all the fluorescence which is faster than the instrumental response (< 100 ps [16,21]) is identical to the prompt fluorescence Φ_0. Since this emission component as well is apt to be contaminated with additional fluorescence, we will investigate how large such a contribution can be. Measurements at higher time resolution did not show a kinetic component between $\simeq 6$ ps and 1.2 ns [32]. Moreover, the absolute quantum yield of the 'prompt' fluorescence in Ref. 16 can be traced to $4.4 \cdot 10^{-4}$ and can be compared with the theoretical value of the prompt fluorescence $\Phi_0 = k_F/k_1$. With $k_F = 8.5 \cdot 10^7$ s^{-1} [16] and $k_1 = 1/3.5$ ps [1] (we assume that the charge separation rate is the same in untreated und in quinone-depleted RCs), we obtain $\Phi_0 = 3 \cdot 10^{-4}$. The consistency with the experimental quantum yield is within the error of measurement. A contamination of the 'prompt' fluorescence is therefore not expected to exceed 50%. Consequently, we expect the experimental data to produce $\Delta G(^1P^* - P^+H^-)$ values for the relaxed RP state being better in accuracy than 10% if analysed in the way shown in this paper, while statements concerning the hypothetical nonrelaxed RP state may suffer from the discussed experimental uncertainties. Due to the decreasing quantum yield of the delayed components, considerably larger errors have to be envisaged at low temperatures.

Comparison with other energy data

In the following I wish to compare $\Delta G(^1P^* - P^+H^-)$ with other energy data available for the involved states. The enthalpy difference $\Delta H(^1P^* - {}^3P^*)$ is essentially calculated from the difference in fluorescence and phosphorescence wavelengths [36]. However, appreciable shifts of the fluorescence band have been observed, depending on the preparation. RCs embedded in PVA films have fluorescence peaks located at 900 nm (280 K) and 913 nm (20 K) [36] while samples in solution (75% glycerol) have their emission maximum at about 911 nm (295 K) and at 925 nm (163 K) [15,21]. The absorption band experiences even a larger shift from 868 nm (PVA, 20 K) [36] to 890 (solution, 150 K). Unfortunately, the corresponding shifts of the phosphorescence band are not known. Thus, enthalpy differences between $^1P^*$ and $^3P^*$ in the range 0.42 eV to 0.435 eV have been employed [17,37–39].

The magnetic field dependences of the $^3P^*$ lifetime and of the RP lifetime completely resemble one another [40]. This fact and the temperature dependence of the $^3P^*$ lifetime have given evidence that this state partially decays via renewed charge separation to the state P^+H^-. From the theoretical evaluation of the magnetic field dependence of this activated process $\Delta G(P^+H^- - {}^3P^*) = 1.65$ eV [17,38] and 0.17 eV [37,39] (both at 290 K) have been determined from low- and high-field data, respectively. Together with $\Delta G(^1P^* - P^+H^-)$ we get $\Delta G(^1P^* - {}^3P^*) = 0.414$–$0.425$ eV being almost equal to $\Delta H(^1P^* - {}^3P^*)$. This is not trivial, since the electronic structure of $^1P^*$ and $^3P^*$ seems to be considerably different, as summarized in Ref. 36, thus making the interaction of the different charge distributions on the special pair molecules with the surrounding unpredictable.

The temperature dependence of $\Delta G(P^+H^- - {}^3P^*)$ in the range between 185–290 K is weak, due to a small contribution from entropy with $\Delta S(P^+H^- - {}^3P^*) = -1.32 \cdot 10^{-4}$ eV/K and an enthalpy value $\Delta H(P^+H^- - {}^3P^*) = 0.130$ eV [17]. Subtracting this value from $\Delta H(^1P^* - {}^3P^*)$ we obtain $\Delta H(^1P^* - P^+H^-) = 0.290$–$0.303$ eV. This apparently constitutes the major contribution to $\Delta G(P^* - P^+H^-)$ while $\Delta S(^1P^* - P^+H^-) = (1.41\text{–}1.86) \cdot 10^{-4}$ eV/K is comparatively small. In both charge separation reactions: $^1P^* \rightarrow P^+H^-$ and $^3P^* \rightarrow P^+H^-$ we find a small entropy decrease of the same amount. A slight entropy increase was determined in Ref. 39 for the second reaction from measurements in high magnetic fields. It would be interesting to compare these results with the temperature dependence of $\Delta G(^1P^* - P^+H^-)$ determined from the temperature dependence of the fluorescence measurements, since in reduced RCs an unusual temperature dependence has been observed [21]. A large entropy increase at temperatures below 200 K has been derived from such measurements. The temperature dependence of $\Delta G(^1P^* - P^+H^-)$ above 200 K is difficult to interpret, since it first increases and then decreases again.

Negligible entropy contributions $\Delta S(^1P^* - {}^3P^*)$ and $\Delta S(^1P^* - P^+H^-)$ have been anticipated in Ref. 39 when discussing the initial charge separation energetics, and proposing $\Delta G(^1P^* - P^+H^-) = 0.263$ eV. That is the reason why the value is somewhat larger than the one determined in this paper directly from the experimental fluorescence data.

In case electron transfer proceeds according to the sequence:

$$^1P^* \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} P^+B^- \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} P^+H^- \qquad (2)$$

we need independent information for the relevant free energy change $\Delta G(^1P^* - P^+B^-)$ responsible for the very first step. From the linewidth of the magnetic field dependent triplet yield an upper limit for the back hopping rate $k_{-2} > 10^9\ s^{-1}$ can be extracted [3,10,13,41]. Together with $k_2 = 1/0.9$ ps [1] we get $\Delta G(P^+B^- - P^+H^-) > 0.173$ eV. This contrasts with a value of $\Delta H(P^+B^- - P^+H^-) \simeq 0.025$ eV derived for reduced RCs from a temperature dependence of transient absorption measured at early times [42]. This temperature dependence was present in reduced RCs but not in quinone-depleted RCs. According to our value of $\Delta G(P^+B^- - P^+H^-)$, however, the maximum population of P^+B^- relative to P^+H^- is not expected to exceed 10^{-3} at room temperature. Such small concentrations should give considerably smaller signals than the temperature-dependent spectral features observed in Ref. 42. The observed temperature dependence must therefore be due to different reasons. From $\Delta G(^1P^* - P^+H^-) > 0.26$ eV and from $\Delta G(P^+B^- - P^+H^-) > 0.17$ eV we get $\Delta P(^1P^* - P^+B^-) > 0.09$ eV [13]. Note that the magnetic field data always refer to the relaxed RP state. In case relaxation occurs between between initial charge separation and the lifetime of the relaxed state P^+H^-, the upper limit for $\Delta G(^1P^* - P^+B^-)$ should change accordingly. Unfortunately, there is no independent evidence for such comformational relaxation of the protein.

Alternatively, if single step charge separation proves to be effective [2], the primary charge separation rate and the triplet recombination rate can be directly compared and one would expect the reorganisation energies to be roughly similar. Since both reactions are activationless, ΔG values should be similar as well. This expected similarity indeed would be better for the proposed nonrelaxed RP state than for the relaxed one, though considerable deviations from the condition for activationless electron transfer are tolerable [43].

Summarizing, it has been shown that the free energy difference between the excited state of the primary donor $^1P^*$ and the relaxed RP state P^+H^- derived from time-resolved fluorescence data is $\Delta G(^1P^* - P^+H^-) = 0.2493 - 0.26$ eV. Nearly identical values have been derived from different experimental data [15,16]. The results obtained do not depend on whether a relaxation [21] or branching [25] model is introduced to explain additional delayed fluorescence components. The results are consistent with energy data of the states $^1P^*$, P^+H^- and $^3P^*$ derived from other types of measurement [17,36–39]. Finally, an upper limit $\Delta G(^1P^* - P^+B^-) > 0.09$ eV was determined, which should be relevant for the first electron transfer step in a sequential mechanism.

Appendix I

$\Delta G(^1P^* - P^+H^-)$ in the relaxation model

The fluorescence yield due to delayed emission from

the RP state P_i^F is given by:

$$\Phi_i = k_F \left[\frac{[^1P^*]_{eq}}{[P_i^F]_{eq}} \right] \int_0^\infty [P_i^F](t)dt \quad (A1)$$

k_F being the inverse radiative lifetime, [](t) and $[]_{eq}$ indicating the time-dependent and equilibrium concentrations of the various states, respectively. Since any of the precursor states decays by more than a factor of 3 faster than any of the states P_i^F considered [16], it is assumed to be created almost instantaneously, while the precursor states have reached equilibrium within 5% deviation. Thus, the component with the lifetime τ_i in a multiexponential fluorescence decay trace can be assumed to directly reflect the decay of P_i^F. The integral in Eqn. A1 then evaluates to τ_i since in a strict sequential reaction scheme the quantum yield of P_i^F formation is $\simeq 100\%$ [44]. Due to microscopic reversibility the equilibrium $[^1P^*]_{eq}/[P_i^F]_{eq}$ between the initial state $^1P^*$ and the state P_i^F then is given by the product of the equilibrium constants k_{-j}/k_j $(j = 1 - i)$ between the successive states in the cascade, being connected by the forward and backward rates k_j and k_{-j} respectively.

$$\frac{[^1P^*]_{eq}}{[P_i^F]_{eq}} = \prod_{j=1}^{i} \left[\frac{k_{-j}}{k_j} \right] = \prod_{j=1}^{i} e^{-(\Delta G_j/kT)} = e^{((-\Sigma_{j-1}^{i} \Delta G_j)/kT)} \quad (A2)$$

The terms $[^1P^*]/[P_i^F]$, $k_F \simeq \Phi_0 k_1$ and the integral over $[P_i^F](t)$ can be eliminated from Eqn. A1. Then the total free energy gap between $^1P^*$ and P_i^F $\Delta G_i = [\Sigma_{j-1}^{i} \Delta G_j]$ can be expressed by Eqn. 1.

Appendix II

$\Delta G(^1P^* - P^+H^-)$ in the branching model

The main evidence for a branching mechanism being responsible for the fluorescence component with 2.5 ns lifetime was drawn from the fact that this component was also detected in untreated RCs [15]. Since ET proceeds forming $P^+Q_A^-$ in about 200 ps [45] in such RCs, according to a sequential model, no delayed fluorescence component slower than this should be detected. Only in a branching model (or in a contamination model), can energy be stored beyond the $P^+H_A^-$ lifetime. Upon reduction of Q_A the $^1P^*$ lifetime changes from 2.8 ps to 4.4 ps [46], i.e., by a factor of 1.6, and so does the prompt fluorescence yield (factor 1.5 [21]), since this rate is the yield determining quenching rate. If we assume that Q_A reduction does not change the energy of $P^+H_B^-$ i.e. if the recombination rate does not change significantly, the 2.5 ns delayed component should rise as well. According to [15] the 2.5 ns component rises by a factor of 2.8 on reduction. In a contamination model the 2.5 ns yield is not expected to change significantly. (Note that for quinone-depleted RCs the 2.5 ns yield is expected to be similar to the one from unreduced RCs.)

Other experimental work does not support such a branching model. In [22] a nanosecond component in untreated RCs has explicitly been excluded by the experimental data. Woodbury et al. have done this implicitly as well, by using the fluorescence trace of untreated RCs as the response function of the apparatus [16,21]. Visual inspection of this trace in Fig. 1 of Ref. 21 shows a slight asymmetry. Comparing this trace with simulated traces of a 2.5 ns decay function folded with a 1 ns response function shows that a 10% amplitude (corresponding to the one determined in Ref. 15) seems to be tolerable.

Careful inspection of the two alternative candidates, H_A and H_B, in transient absorption spectroscopy gave no evidence of H_B bleaching with the appropriate nanosecond lifetime in untreated, in reduced and in quinone-depleted RCs, restricting the maximum population of $P^+H_B^-$ to less than 5% at low temperatures [47]. Rates deduced in Ref. 15 would predict initial concentrations of $P^+H_B^-$ as large as 25% at room temperature. Such an increase of $P^+H_B^-$ formation is well compatible with thermaly activated charge separation to the B-branch. Charge separation in RCs trapped in the PH_A^- state is about 200-times slower to the B-branch than it is to the A-branch in untreated RCs at room temperature [48]. Such a comparison should be handled with caution, due to the unknown influence of the excess charges. After all, conformational changes following reduction of H_A have been observed [49]. Note that these measurements do not exclude recombination from $P^+B_B^+$, an alternative candidate for branching of primary charge separation. In the following we therefore show that $\Delta G(^1P^* - P^+H_A^-)$ as determined in terms of a branching model is identical with $\Delta G(^1P^* - P^+H^-)_{relaxed}$ as determined in terms of the relaxation model (Eqn. 1). Thus, we are free to postpone the final decision on this topic until additional data are available.

Within the branching model the time dependence of the excited state is given in Eqn. 5 of Ref. 25. Integrating over the first and the third term in that equation the quantum yield of the prompt fluorescence component:

$$\Phi_0 = \frac{k_F}{^Bk_1 + {}^Ak_1} \quad (A3)$$

and the quantum yield of the slowest component having a lifetime of τ_N are given:

$$\Phi_N = \tau_N \frac{k_F}{\frac{^Bk_1}{^Bk_{-1}} + \frac{^Ak_1}{^Ak_{-1}}} \quad (A4)$$

Indices A and B denote the charge separation rates ($^{A,B}k_1$) and the recombination rates ($^{A,B}k_{-1}$) on the A and B branch, respectively. As already noted, this branching model can only account for one of the inter-

mediate fluorescence components. Eliminating k_F from Eqns. A3 and A4 we get:

$$\frac{{}^{A}k_{-1}}{{}^{A}k_{1}} = \frac{\Phi_N}{{}^{A}k_1\Phi_0\tau_N + {}^{B}k_1\left[\Phi_0\tau_N - \frac{\Phi_N}{{}^{B}k_1}\right]} \quad \text{(A5)}$$

The second term in the denominator can be neglected, if $|{}^{B}k_1/{}^{A}k_1 - A_N/A_0| \ll 1$. This is the case, since

(a) there is no evidence of significant transient bleaching of Q_x band of H_B at any temperature for untreated [47,48], quinone-depleted and reduced RCs (Volk, M., Ogrodnik, A. and Michel-Beyerle, M.E., unpublished data), implying ${}^{B}k_1/{}^{A}k_1 \ll 1$.

(b) $A_N/A_0 \simeq 7 \cdot 10^{-5}$ [15,16].

Comparing with Eqn. A2 the free energy change can be expressed by Eqn. 1 and is independent of the model assumed.

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