VU Research Portal

Dynamic Stark Effect in Two-Dimensional Spectroscopy Revealing Modulation of Ultrafast Charge Separation in Bacterial Reaction Centers by an Inherent Electric Field

Ma, F.; Romero, E.; Jones, M.R.; Novoderezhkin, V.I.; Yu, L.-J.; Van Grondelle, R.

published in

Journal of Physical Chemistry Letters
2021

DOI (link to publisher)

[10.1021/acs.jpcllett.1c01059](https://doi.org/10.1021/acs.jpcllett.1c01059)

document version

Publisher's PDF, also known as Version of record

document license

Article 25fa Dutch Copyright Act

[Link to publication in VU Research Portal](#)

citation for published version (APA)

Ma, F., Romero, E., Jones, M. R., Novoderezhkin, V. I., Yu, L.-J., & Van Grondelle, R. (2021). Dynamic Stark Effect in Two-Dimensional Spectroscopy Revealing Modulation of Ultrafast Charge Separation in Bacterial Reaction Centers by an Inherent Electric Field. *Journal of Physical Chemistry Letters*, 12(23), 5526-5533. <https://doi.org/10.1021/acs.jpcllett.1c01059>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

vuresearchportal.ub@vu.nl

Dynamic Stark Effect in Two-Dimensional Spectroscopy Revealing Modulation of Ultrafast Charge Separation in Bacterial Reaction Centers by an Inherent Electric Field

Fei Ma,^{*} Elisabet Romero, Michael R. Jones, Vladimir I. Novoderezhkin, Long-Jiang Yu,^{*} and Rienk van Grondelle

Cite This: *J. Phys. Chem. Lett.* 2021, 12, 5526–5533

Read Online

ACCESS |

Metrics & More

Article Recommendations

Supporting Information

ABSTRACT: Despite extensive study, mysteries remain regarding the highly efficient ultrafast charge separation processes in photosynthetic reaction centers (RCs). In this work, transient Stark signals were found to be present in ultrafast two-dimensional electronic spectra recorded for purple bacterial RCs at 77 K. These arose from the electric field that is inherent to the intradimer charge-transfer intermediate of the bacteriochlorophyll pair (P), $P_A^+P_B^-$. By comparing three mutated RCs, a correlation was found between the efficient formation of $P_A^+P_B^-$ and a fast charge separation rate. Importantly, the energy level of P^* was changed due to the Stark shift, influencing the driving force for $P^* \rightarrow P^+B_A^-$ electron transfer and hence its rate. Furthermore, the orientation and amplitude of the inherent electric field varied in different ways upon different mutation, leading to contrasting changes in the rates. This mechanism of modulation provides a solution to a long-lasting inconsistency between experimental observations and activation energy theory.

Capture of the energy of photons through the separation of electric charge is the crucial step in the use of sunlight as a clean and safe energy source. In biology, photosynthetic reaction center (RC) pigment–protein complexes carry out this photoelectric conversion with a quantum yield that is close to an unity.¹ This is achieved within an electron transfer (ET) chain comprising multiple (bacterio)chlorin molecules, many basic features of which are conserved across diverse photosynthetic bacteria, algae and higher plants.^{1,2} In the RC of the purple bacterium *Rhodospirillum rubrum* (*Rb.*) *sphaeroides*, the ET cofactors are a pair of strongly excitonically coupled bacteriochlorophyll (BChl) *a* (P), two monomeric BChl *a* (B), two bacteriopheophytin *a* (H) and two ubiquinone molecules symmetrically arranged in two branches. At 77 K, electrons are transferred along the active A chain, sequentially from P to B_A, H_A and Q_A with time constants of 1–2, 0.5–1, and ~200 ps, respectively.^{3,4} The initially formed photoexcited electronic state of P (P^*) was found to have a substantial intradimer charge-transfer (CT) character ($P^*P_A^{\delta+}P_B^{\delta-}$) via Stark spectroscopy.⁵ In addition, a CT state intermediate, $P_A^+P_B^-$, was thought to be an ET precursor for the $P^* \rightarrow P^+B_A^-$ primary charge separation.⁶

Despite very extensive investigation of both wild-type and mutated purple bacterial RCs,^{7–11} many aspects of the fundamental mechanisms of primary charge separation are still unclear. One important problem is deviation of the experimentally determined rate of the primary $P^* \rightarrow P^+B_A^-$ step at cryogenic temperature from that predicted by activation

energy theory.^{9,10,12} Proposed explanations include vibrational mode coupling¹³ that helps to overcome the activation barrier or a reaction-diffusion model¹⁴ in which primary ET is determined by protein dynamics rather than by barrier crossing. However, neither of these explanations takes into account the real starting event of primary ET, namely the photoinduced charge separation within the P BChl dimer to create a CT state. In actuality it is this step that provides the initial driving force for electron movement, and the $P^*P_A^{\delta+}P_B^{\delta-}$ state is sensitive to the electronic charge distribution in its environment owing to its substantial electronic polarizability.¹⁵ A model that includes these factors is required to achieve a thorough understanding of the physical mechanism behind the high photoelectric conversion quantum yield in this RC.

The Stark effect refers to a change in the energy levels of an electronic state when a molecule is exposed to an electric field.¹⁶ The consequent change of transition frequency $\Delta\omega$ in response to the electric field $\vec{F}$ is given by $\Delta\omega = -\Delta\vec{\mu} \cdot \vec{F} - \frac{1}{2}\vec{F} \cdot \Delta\alpha \cdot \vec{F}$, where $\Delta\mu$ and $\Delta\alpha$ are the change in dipole moment and polarizability, respectively, between the ground and excited

Received: April 2, 2021

Accepted: May 20, 2021

Published: June 7, 2021

state of the transition.¹⁷ The $\vec{F}$ can be externally applied or inherent, and thus $\Delta\omega$ not only reflects the property of the electronic transitions but also the effective electric field strength and the dielectric nature of the local environment. The Stark effect has been exploited in different experimental scenarios to explore the charge separation process in RCs. Linear Stark spectroscopy recording the difference in absorption in the presence and absence of an external electric field, which manifests as a derivative shape,¹⁷ has revealed that CT–exciton mixing is significant for RCs^{5,15,18,19} and antenna complexes.^{20–22} Transient absorption experiments of RCs have shown that the CT states $P^+H_A^-$ and $P^+Q_A^-$ generate inherent electric fields and induce dynamic Stark shifts in the ground-state bleach (GSB) signal of B.²³ The application of a large ($\sim 10^6$ V/cm) external electric field can even influence the rate of ET in RCs.^{24,25} Two-dimensional electronic spectroscopy (2DES) of RCs has revealed derivative-shaped peaks that have been attributed to the Stark shift due to CT intermediates,^{26–28} and recently, a method for carrying out differential 2DES in both the presence and absence of an external electric field was developed and was applied to the Photosystem II RC.^{29,30}

Previous studies of the dynamics of Stark effects in RCs have focused only on the longer-lived CT intermediates $P^+H_A^-$ and $P^+Q_A^-$.^{23,31,32} Although the field intrinsic to the $P_A^+P_B^-$ state is expected to induce a distinct Stark signal, no evidence of this has been reported to date. This is likely due to the expected short lifetime (100–200 fs) of a signal located in a region of high spectral congestion, as well as ultrafast spectral artifacts that may blur a Stark signal. The application of 2DES with simultaneous high temporal and spectral resolution can potentially overcome these difficulties.³³ Accordingly, in the present work we looked for evidence of a Stark effect associated with the $P_A^+P_B^-$ state through the application of 2DES to three mutated *Rba. sphaeroides* RCs with different rates of primary charge separation. By comparing the shapes and dynamics of detected Stark signals arising solely from the inherent electric fields generated during light-induced charge separation it was shown that the efficient formation of a $P_A^+P_B^-$ CT state is essential for fast primary charge separation. Through these means valuable information can also be obtained about the relative orientation and strength of the internal electrostatic field interacting with the cofactors, revealing how it is able to influence the rate of primary charge separation.

All three RCs studied contained the AM260W mutation that disassociates the Q_A quinone acceptor (Figure 1 and the Supporting Information, Figure S1a), blocking long-lived ET and guaranteeing that the RCs remain in an active condition during 2DES measurements. In the single AM260W RC (dubbed M1), the rate of primary ET at 77 K is identical with that in the wild-type RC, $(1.4 \text{ ps})^{-1}$.^{28,34} RCs M2 and M3 had an additional YM210W or GM203L mutation, respectively. The YM210W mutation causes a small change in the relative orientations of the P and B_A BChls³⁵ (Figure S1b) and slows primary ET to $(260 \text{ ps})^{-1}$.^{28,36} The GM203L mutation removes a water molecule which directly links P_B and B_A through hydrogen-bonds³⁷ (Figure S1c) and slows primary ET to $(25 \text{ ps})^{-1}$.^{11,28} The mutations have small effects on the absorbance spectrum of the RC bacteriochlorins at 77 K (Figure 1), which comprises P, B and H bands.

The P band has a smaller extinction coefficient than the B band, so an excitation pulse covering both P and B would elicit

Figure 1. Normalized absorption spectra of M1 (solid line), M2 (dashed line) and M3 (light solid line) at 77 K overlaid with the laser spectrum (gray shaded area). Inset shows the structure of ET cofactors.

2DES data dominated by fast (200 fs) $B^* \rightarrow P$ energy transfer.²⁶ To focus on $P^* \rightarrow P^+B_A^-$ primary ET therefore, an excitation pulse was used that predominantly excited P with only minor excitation of B (Figure 1). Diverse derivative-shape signals appeared in response to this excitation at 77 K, not only on the B diagonal as reported previously,²⁶ but also at the (B, P) cross peak position (highlighted with rectangles in Figure 2a–c). In the 2DES spectra, positive bands normally correspond to GSB of the original electronic transition while negative bands to absorption of the newly appeared species, such as excited states, reaction intermediates or products. The decays of the bands with different signs are usually asynchronous. However, the positions of the positive and negative peaks of the observed derivative-shape signals were nearly symmetrical around the B diagonal GSB and the (B, P) cross peak positions, along the ω_t axis. And the both peaks evolved synchronously. These features indicated an appearance of Stark shift effect. Therefore, they were assigned to the GSB of the original transition and the absorption of the Stark-shifted transition. The appearance of Stark shifts showed that the cofactors were under the influence of the ET-induced electric field, which should be fixed relative to the different orientations of the transition dipoles of the cofactors. Equivalent signals were not observed at room temperature (RT) (Figure S2). Although line-width broadening upon increasing temperature might obscure the spectral shift, the more important reason for this temperature-dependent behavior is that the immobilized cofactor molecules in a frozen protein matrix can give sizable Stark effect signals that become much smaller in liquid solution due to protein conformational changes and solvation effects.³⁸

Derivative-shaped peaks around the B diagonal position arise from a Stark shift of B absorbance. Take the prominent signal in the 50 fs 2D spectrum of M1 (Figure 2a, top) for example, the positive $(12300, 12080) \text{ cm}^{-1}$ peak was attributable to the GSB of the original B transition and the negative $(12300, 12500) \text{ cm}^{-1}$ peak to the absorption of a new-formed level, the Stark-shifted B transition (B') (see Figure S3 for Liouville pathways for this feature). Note that the diagonal peak position of $(12300, 12300) \text{ cm}^{-1}$ deviated from the maximum of the B absorption at 12490 cm^{-1} because the excitation pulse did not cover the entire B absorption thus distorting its peak shape.³⁹ B' formed when B experienced an additional electric field produced by the CT intermediate.²⁶ Its appearance indicated a change of the electric field exerting on B which was induced by

Figure 2. 77 K absorptive 2D spectra of **M1** (a), **M2** (b), and **M3** (c) at short (top) and long (bottom) population times showing positive (red) and negative (blue) features. The rectangles highlight derivative-shape signals at the (B, P) cross peak position. (d) Double-sided Feynman diagrams of Liouville pathways contributing to the highlighted positive (red) and negative (blue) cross peak signals. Terms g and e (g' and e') denote the ground and excited state without (with) a Stark effect. Terms ω_P and ω_B (ω_P' and ω_B') are the energies of the original (Stark-shifted) transitions of P and B, respectively.

the excitation of P at 50 fs and by the formation of the CT intermediate $P^+H_A^-$ at 1 ns.

The derivative-shape peaks around the (B, P) cross peak position (Figure 2a–c, rectangle highlight) have a complicated origin because each peak involves two molecules, i.e., the excitation of B and the probe of P. They reflected the energy and/or electron transfer between B and P, and so can provide more useful information than the diagonal Stark signals. Furthermore, the opposite signs and different formation and decay dynamics (Figure 3) of the cross-peak Stark signals observed between the three mutant RCs provide a means to unveil mechanistic detail.

In **M1** (Figures 2a and 3a), the derivative-shape peaks around the (B, P) cross peak position, positive at (12300, 11020) cm^{-1} and negative at (12300, 11630) cm^{-1} , appeared shortly after excitation and remained until 500 fs (the time constant for this process is ~ 200 fs). Then they evolved into a single positive peak at (12300, 11290) cm^{-1} , which was typical for the (B, P) cross peak.^{26,27} This short-lived Stark effect was indicative of an ultrafast induced electric field, which blue-shifted the original P transition to the Stark-shifted P transition (P') and exhibited the positive P GSB and negative P' absorption peaks. Their two-molecule Liouville pathways are

Figure 3. Kinetic curves at (B, P) cross peak positions: (a) (12300, 11020) cm^{-1} (red) and (12300, 11630) cm^{-1} (blue) for **M1**, (b) (12300, 11000) cm^{-1} (red) and (12300, 11620) cm^{-1} (blue) for **M2**, and (c) (12300, 11400) cm^{-1} (red) and (12300, 10810) cm^{-1} (blue) for **M3**. (d) Kinetic curves at the B diagonal positions reflecting B $\rightarrow$ P energy transfer.

Figure 4. $\Omega_{\tau} = 12300 \text{ cm}^{-1}$ slice spectra (gray) and their fitting curves (black) for **M1** (a), **M2** (b), and **M3** (c) at 50 fs (top) and 1 ns (bottom). Each contribution of P (green) and B (pink) consisted of original and Stark-shifted transition spectra.

listed in Figure 2d. The positive band arose from excitation and de-excitation of the ground state of B and P, without a participation of their excited states. The negative band involved B^* and the ground state of P' . During the time the Stark effect remained, the excited state of B decayed mainly through the energy transfer to P. The time constant of the induced electric field, ~ 200 fs, was consistent with the lifetime of the $P^*P_A^{\delta+}P_B^{\delta-}$ CT state⁴⁰ and the $B \rightarrow P$ energy transfer process.²³ Although energy transfer from B to P could induce a change in electric field, it can be ruled out as the origin of the Stark effect because the associated signals were much slower in **M2** despite the rate of $B \rightarrow P$ energy transfer being similar in the three mutant RCs (Figure 3d). It was the specific exciton-CT mixing character of P^* interacting with B^* that induced the ultrafast dynamic electric field and caused the Stark effect.

The Stark signals disappeared in concert with ET from P to B, the subsequent time constants being $P_A^+P_B^- \xrightarrow{1.4 \text{ ps}} P^+B_A^- \xrightarrow{0.5 \text{ ps}} P^+H_A^-$. At 1 ns, another pair of derivative-shape signals, positive at $(11300, 12120) \text{ cm}^{-1}$ and negative at $(11300, 12560) \text{ cm}^{-1}$, appeared at the (P, B) cross peak position. Similar signals have been observed previously²⁶ and were attributed to a Stark effect on B in the $P^+H_A^-$ electric field. They, and the absorption peak at $(11300, 10760) \text{ cm}^{-1}$, therefore represented the formation of $P^+H_A^-$.²⁸

In **M2** (Figure 2b and 3b), at 50 fs only a single positive $(12250, 11270) \text{ cm}^{-1}$ peak appeared at the (B, P) position, but this evolved into positive $(12250, 11000) \text{ cm}^{-1}$ and negative $(12250, 11620) \text{ cm}^{-1}$ peaks at 1 ns (highlighted with rectangle). The rise and decay time constants were 0.5 and 260 ps, corresponding to the scheme $P^* \xrightarrow{260 \text{ ps}} P^+B_A^- \xrightarrow{0.5 \text{ ps}} P^+H_A^-$. Because the peaks had very similar

frequencies as those in **M1**, they can also be attributed to the GSB of P and the absorption of P' . The much slower dynamics of P' compared to **M1** pointed to a varied dynamic electric environment in which the CT state formed considerably more slowly. According to the dynamics of the Stark signals, we tentatively propose a model for primary ET in **M2** of $P^* \xrightarrow{260 \text{ ps}} P_A^+P_B^- \xrightarrow{k_2} P^+B_A^- \xrightarrow{0.5 \text{ ps}} P^+H_A^-$ where k_2 is much faster than $(260 \text{ ps})^{-1}$, and formation of $P_A^+P_B^-$ is the rate-limiting step. Owing to the slow primary ET rate, no signals attributable to $P^+H_A^-$ and its induced Stark effect were observed at the maximal population time of 1 ns.

In mutant **M3** (Figure 2c and 3c), the dynamics of the Stark peaks were similar to those in **M1**. The prompt appearance upon excitation and the ~ 200 fs time constant of the Stark signals indicated the same origin, namely P' . However, the peaks were opposite in sign compared to **M1** and **M2**; i.e., the negative $(12300, 10810) \text{ cm}^{-1}$ peak was below the positive $(12300, 11400) \text{ cm}^{-1}$ peak at 50 fs, indicating that P' is red-shifted with respect to P in **M3**. The overall time constant of $P^* \rightarrow P^+B_A^-$ primary ET was 25 ps. At 1 ns, the (B, P) position was dominated by a relatively strong single peak at $(12300, 11250) \text{ cm}^{-1}$, corresponding to considerable $P^+B_A^- \rightarrow P^*$ reverse ET.²⁸ Due to this effective reverse ET, no observable amount of $P^+H_A^-$ and its induced Stark effect were present at 1 ns.

The Stark shift appeared along the ω_i axis. To derive the transition frequency of P' , multi-Gaussian-peak fitting was applied to the $\omega_{\tau} = 12300 \text{ cm}^{-1}$ slice spectra using the formula:

Figure 5. Scheme for modulation of energy levels by the inherent electric field and the resulting influence on the primary ET rate in **M1** (a) and **M3** (b). The notations can be found in the text. (c) Kinetic curves for the stimulated emission of P at 77 K (top) and room temperature (bottom). Calculated temperature-dependent ET time constant k^{-1} for **M1** (d), **M3** (e), and **M2** (f) according to eq 2 with ΔE taken as -550 cm^{-1} and λ as 800 cm^{-1} .

$$\sum_{i=P,B} A_+(i) \exp\{-[\omega - \omega_+(i)]^2 / \sigma(i)^2\} - A_-(i) \exp\{-[\omega - \omega_-(i)]^2 / \sigma(i)^2\} \quad (1)$$

where A_+ and A_- were the amplitude of the positive and negative peak, ω_+ and ω_- were the frequency of the original and the Stark-shifted transition, $\Delta\omega = \omega_- - \omega_+$ was the Stark shift, and σ was the full width at half-maximum (fwhm) and was set to be equivalent for the original and Stark-shifted peaks. The results of this fitting are shown in Figure 4. Each slice spectrum contained a contribution from P and B, but it should be noted the decomposition of B may have been less accurate than P, owing to its spectral distortion. Focusing the analysis on P, at 50 fs P' was blue-shifted by 110 cm^{-1} in **M1** and red-shifted by 255 cm^{-1} in **M3**, while at 1 ns the existence and shift of P' were negligible in both **M1** and **M3**. In **M2**, P' did not exist at 50 fs and was blue-shifted by 66 cm^{-1} at 1 ns.

The Stark shift $\Delta\omega$ in the RC is dominated by $-\Delta\vec{\mu} \cdot \vec{F}$ while $\Delta\alpha$ is relatively small.^{21,23} Hence a red shift or blue shift occurs when $\vec{F}$ is aligned with or against $\Delta\vec{\mu}$, respectively. The value of $\Delta\omega$ is equal to $|\Delta\vec{\mu}||\vec{F}|\cos(\theta)$, where θ is the orientation angle between $\Delta\vec{\mu}$ and $\vec{F}$. For the P dimer in **M1** and **M3**, $\Delta\vec{\mu}$ was not expected to change much considering the nearly identical X-ray crystal structures. As a result, the differently orientated $\vec{F}$ exerted on P ($\vec{F}_P$) should be responsible for the opposite Stark shifts at 50 fs, i.e., θ was in a $[0, 90^\circ]$ range for **M1** but in a $[90^\circ, 180^\circ]$ range for **M3**. In **M2**, θ was in the $[0, 90^\circ]$ range, but the appearance of $\vec{F}_P$ was much later and slower.

The YM210W mutation present in **M2** brings about a $\sim 52 \text{ mV}$ ($\sim 420 \text{ cm}^{-1}$) increase in the midpoint redox potential of P/P⁺, although the structure of P and its environment does not undergo any major changes.³⁵ The two-order of magnitude

decrease in the rate of primary ET produced by this mutation has been attributed to a decrease in the activation energy gap between P* and P⁺B_A⁻.⁸ However, comparing the 2D spectra of **M2** with the other two mutant RCs, a new mechanism is apparent in which the P*P_A^{δ+}P_B^{δ-} or P_A^{δ+}P_B^{δ-} CT intermediate exhibiting a sizable Stark effect is the precursor of P⁺B_A⁻, and efficient formation of P_A^{δ+}P_B^{δ-} is necessary for the primary ET to be fast. The effect of the YM210W mutation is therefore to slow formation of P_A^{δ+}P_B^{δ-}. Our previous analysis has shown that coherent components favoring the formation of P_A^{δ+}P_B^{δ-} are missing in **M2**,²⁸ which may explain why charge separation inside P is less efficient.

In both **M1** and **M3**, as indicated above, an ultrafast inherent electric field was created nearly immediately upon excitation. It arose from the partial CT character of P* and its interaction with B and the protein environment. During the 300 fs it lasted, the CT state P_A^{δ+}P_B^{δ-} was created as a precursor to subsequent ET to B. The electric field induced a shift of the excited-state of P, to either higher or lower energy level, which had a significant influence on the rate of primary ET at 77 K. Recently, similar observation and mechanism in halide perovskites were reported that a dipole field created by a polaron pair can shift the exciton transition to higher energies.⁴¹

The descriptive schemes in parts a and b of Figure 5 show how the Stark shift modulated ET rates at 77 K. In **M1** (Figure 5a), $\vec{F}$ exerting on P ($\vec{F}_P$) was aligned with $\Delta\vec{\mu}$ and as a result the energy level of P' was about 110 cm^{-1} higher than P. Accordingly the Stark shift resulted in a larger free energy gap for the P* $\rightarrow$ P⁺B_A⁻ ET. In **M3** (Figure 5b), the situation was opposite, with $\vec{F}_P$ aligned against $\Delta\vec{\mu}$ leading to an approximately 255 cm^{-1} lower energy for P' and thus a smaller free energy gap.

To appraise the influence of the inherent electric field, the ET rate k was estimated using the Marcus equation and by taking into account the Stark shift energy, $h\Delta\omega$:

$$k \propto (4\pi\lambda RT)^{-1/2} \exp[-(\lambda + \Delta E - h\Delta\omega)^2/4\lambda RT] \quad (2)$$

The free energy difference between P^* and $P^+B_A^-$, ΔE , was taken as -550 cm^{-1} and the reorganization energy λ as 800 cm^{-1} .⁴² The temperature-dependence of the resulting values for k^{-1} is shown in Figure 5d–f. For **M1**, when $\Delta\omega$ was zero the time constant k^{-1} was 3.4 ps at RT and 2.2 ps at 77 K (Figure 5d). However, when the dynamic Stark effect produced a $\Delta\omega$ of 110 cm^{-1} at 77 K, k^{-1} decreased to 1.7 ps, which was close to the value of 1.4 ps obtained from experiment (Figure 5c). The influence of the Stark effect was more prominent for temperatures lower than 50 K, where the variance between values k^{-1} calculated either with or without $\Delta\omega$ increased drastically. As can be seen in Figure 5d, the conversion point of the Marcus curve moved in the direction of lower temperature as a result of the positive Stark shift. This behavior could explain the long-lasting question over why primary ET at 10 K is twice as fast as that at RT, a severe deviation from the Marcus theory.^{9,12} This scheme was consistent with the view of Parson¹³ that the $P_A^+P_B^-$ state should be above the lowest exciton transition in the wild-type RC (which **M1** represents).

For **M3**, when $\Delta\omega$ was zero the time constant k^{-1} was 10.0 ps at RT and 6.7 ps at 77 K (Figure 5e), and so exhibited the same temperature dependency as **M1** in the absence of a Stark effect. However, the negative $\Delta\omega$ pertaining to **M3** reversed this dependency. When $\Delta\omega$ was -255 cm^{-1} the value of k^{-1} increased to 22 ps at 77 K, close to the experimental value of 25 ps (Figure 5c). Furthermore, under this condition, the back reaction of the reversible $P^* \rightarrow P^+B_A^-$ ET with a rate k^{-1} became competitive (Figure 5b). Computational studies have shown that the absence of a bridging water molecule in the GM203L RC causes an increase in the driving force for charge recombination that is detrimental for stabilizing $P^+B_A^-$,^{43,44} consistent with our scheme. All evidence shows that the water molecule directly linking P_B and B_A is an important factor for determining the orientation and possibly also the amplitude of $\vec{F}_P$, and removing it leads to a rotation of $\vec{F}_P$, producing the contrast illustrated in parts a and b of Figure 5.

In **M2**, the temperature dependence of the experimental k^{-1} , 38 ps at RT and 260 ps at 77 K, was opposite in trend to that in **M1** (Figure 5c), despite the $\Delta\omega$ also being positive with a value of 66 cm^{-1} at 77 K. As the midpoint potential of P/P^+ is increased by $\sim 420 \text{ cm}^{-1}$ by the YM210W mutation in **M2**,^{8,36} the free energy difference between P^* and $P^+B_A^-$, ΔE , was taken to be $-550 + 420 = -130 \text{ cm}^{-1}$. The resulting temperature dependence of k^{-1} is shown in Figure 5f. When $\Delta\omega$ was zero, the value of k^{-1} was 38.0 ps at RT and 143 ps at 77 K, and a $\Delta\omega$ of 66 cm^{-1} led to a further increase of k^{-1} to 249 ps at 77 K, close to the experimental value of 260 ps.

The fact that all calculated temperature-dependent ET rates were consistent with the experimental values for the three diverse mutant RCs is a robust validation of the proposed mechanism. Summarizing, an ultrafast inherent electric field induced by the interaction of the CT character of P and the excited-state B modulates the energy level of the CT precursor through a Stark shift and changes the primary ET rate. Alterations in protein–cofactor structure that change the orientation and/or amplitude of the transient electric field

therefore modulate the ET rate. For **M1**, which can be viewed as equivalent to the wild-type RC, this mechanism guarantees that primary ET is conducted effectively at normal temperatures and explains why it is also conducted effectively at cryogenic temperatures.

Ultrafast Stark signals arising solely from the inherent electric environment were identified in 2DES spectra of *Rba. sphaeroides* RCs and revealed an influence of the CT state of the P bacteriochlorophyll dimer. By comparing the dynamics and Stark shifts of three mutated RCs with drastically different rates of primary ET, a mechanism was proposed that explains how an inherent electric field was able to modulate the rate of primary ET in these RCs. The findings not only provide a new insight into long-standing questions over the temperature-dependence of photochemical charge separation in this RC but also show the insights that follow from identifying transient Stark signatures in 2D spectra. Furthermore, the concept of an inherent electric field modulating the ET rate that is realized in nature through the specific spatial organization of the cofactors in the *Rba. sphaeroides* RC could provide guidance for the design of new artificial photoelectric conversion systems with defined ET rates and efficiencies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jpclett.1c01059>.

Experimental methods, structure of mutant RCs, room-temperature 2DES spectra, and double-sided Feynman diagrams for diagonal signals (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Fei Ma – Key Laboratory of Photobiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China; Department of Biophysics, Faculty of Sciences, VU University Amsterdam, 1081 HV Amsterdam, The Netherlands; orcid.org/0000-0002-7607-6429; Email: fma@ibcas.ac.cn

Long-Jiang Yu – Key Laboratory of Photobiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China; Email: longer@ibcas.ac.cn

Authors

Elisabet Romero – Department of Biophysics, Faculty of Sciences, VU University Amsterdam, 1081 HV Amsterdam, The Netherlands; Present Address: Institute of Chemical Research of Catalonia, Barcelona Institute of Science and Technology, Av. Països Catalans 16, 43007 Tarragona, Spain

Michael R. Jones – School of Biochemistry, University of Bristol, Bristol BS8 1TD, U.K.; orcid.org/0000-0002-8063-0744

Vladimir I. Novoderezhkin – A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, 119992 Moscow, Russia

Rienk van Grondelle – Department of Biophysics, Faculty of Sciences, VU University Amsterdam, 1081 HV Amsterdam, The Netherlands

Complete contact information is available at: <https://pubs.acs.org/doi/10.1021/acs.jpclett.1c01059>

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Natural Science Foundation of China (No. 21903086), the National Key R&D Program of China (No. 2019YFA0904600), the European Research Council through an Advanced Investigator grant (No. 267333, PHOTPROT), the Biotechnology and Biological Sciences Research Council of the U.K. (Project BB/I022570/1), and the Russian Foundation for Basic Research (No. 18-04-00105).

■ REFERENCES

- (1) Parson, W. W.; Warshel, A. Mechanism of charge separation in purple bacterial reaction centers. *Adv. Photosynth. Respir.* **2009**, *28*, 355–377.
- (2) Romero, E.; Augulis, R.; Novoderezhkin, V. I.; Ferretti, M.; Thieme, J.; Zigmantas, D.; van Grondelle, R. Quantum coherence in photosynthesis for efficient solar-energy conversion. *Nat. Phys.* **2014**, *10*, 676–682.
- (3) Zinth, W.; Wachtveitl, J. The first picoseconds in bacterial photosynthesis-ultrafast electron transfer for the efficient conversion of light energy. *ChemPhysChem* **2005**, *6*, 871–880.
- (4) van Brederode, M. E.; Jones, M. R. Reaction centers of purple bacteria. *Subcell. Biochem.* **2000**, *35*, 621–676.
- (5) Lockhart, D. J.; Boxer, S. G. Stark effect spectroscopy of *Rhodobacter sphaeroides* and *Rhodospseudomonas viridis* reaction centers. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 107–111.
- (6) Khatypov, R. A.; Khmelnskiy, A. Y.; Khristin, A. M.; Fufina, T. Y.; Vasilieva, L. G.; Shuvalov, V. A. Primary charge separation within P870* in wild type and heterodimer mutants in femtosecond time domain. *Biochim. Biophys. Acta, Bioenerg.* **2012**, *1817*, 1392–1398.
- (7) Fleming, G. R.; Martin, J. L.; Breton, J. Rates of primary electron transfer in photosynthetic reaction centers and their mechanistic implications. *Nature* **1988**, *333*, 190–192.
- (8) Nagarajan, V.; Parson, W. W.; Davis, D.; Schenck, C. C. Kinetics and free energy gaps of electron-transfer reactions in *Rhodobacter sphaeroides* Reaction Centers. *Biochemistry* **1993**, *32*, 12324–12336.
- (9) Haffa, A. L. M.; Lin, S.; Katilius, E.; Williams, J. C.; Taguchi, A. K. W.; Allen, J. P.; Woodbury, N. W. The dependence of the initial electron-transfer rate on driving force in *Rhodobacter sphaeroides* reaction centers. *J. Phys. Chem. B* **2002**, *106*, 7376–7384.
- (10) Huppman, P.; Arlt, T.; Penzkofer, H.; Schmidt, S.; Bibikova, M.; Dohse, B.; Oesterheld, D.; Wachtveit, J.; Zinth, W. Kinetics, energetics, and electronic coupling of the primary electron transfer reactions in mutated reaction centers of *Blastochloris viridis*. *Biophys. J.* **2002**, *82*, 3186–3197.
- (11) Potter, J. A.; Fyfe, P. K.; Frolov, D.; Wakeham, M. C.; van Grondelle, R.; Robert, B.; Jones, M. R. Strong effects of an individual water molecule on the rate of light-driven charge separation in the *Rhodobacter sphaeroides* reaction center. *J. Biol. Chem.* **2005**, *280*, 27155–27164.
- (12) Woodbury, N.; Allen, J. P. Thermodynamics of Electron Transfer in Wild Type and Mutant Reaction Centers of Purple Nonsulfur Bacteria. *Anoxygenic Photosynthetic Bacteria* **1995**, *2*, 527–557.
- (13) Parson, W. W. Dynamics of the excited state in photosynthetic bacterial reaction centers. *J. Phys. Chem. B* **2020**, *124*, 1733–1739.
- (14) Wang, H.; Lin, S.; Allen, J. P.; Williams, J. C.; Blankert, S.; Laser, C.; Woodbury, N. W. Protein dynamics control the kinetics of initial electron transfer in photosynthesis. *Science* **2007**, *316*, 747–750.
- (15) Middendorf, T. R.; Mazzola, L. T.; Lao, K.; Steffen, M. A.; Boxer, S. G. Stark effect (electroabsorption) spectroscopy of photosynthetic reaction centers at 1.5 K: evidence that the special pair has a large excited-state polarizability. *Biochim. Biophys. Acta, Bioenerg.* **1993**, *1143*, 223–234.
- (16) Liptay, W. Optical absorption of molecules in liquid solutions in an applied external electric field (electrochromism). *Ber. Bunsenges. Phys. Chem.* **1976**, *80*, 207–217.
- (17) Bublit, G. U.; Boxer, S. G. Stark spectroscopy: Applications in chemistry, biology, and materials science. *Annu. Rev. Phys. Chem.* **1997**, *48*, 213–242.
- (18) Steffen, M. A.; Lao, K. Q.; Boxer, S. G. Dielectric asymmetry in the photosynthetic reaction center. *Science* **1994**, *264*, 810–816.
- (19) Romero, E.; Diner, B. A.; Nixon, P. J.; Coleman, W. J.; Dekker, J. P.; van Grondelle, R. Mixed exciton-charge-transfer states in photosystem II: Stark spectroscopy on site-directed mutants. *Biophys. J.* **2012**, *103*, 185–194.
- (20) Ma, F.; Yu, L.-J.; Wang-Otomo, Z.-Y.; van Grondelle, R. The origin of the unusual Qy red shift in LH1-RC complexes from purple bacteria *Thermochromatium tepidum* as revealed by Stark absorption spectroscopy. *Biochim. Biophys. Acta, Bioenerg.* **2015**, *1847*, 1479–1486.
- (21) Boxer, S. G. Stark realities. *J. Phys. Chem. B* **2009**, *113*, 2972–2983.
- (22) Romero, E.; Mozzo, M.; van Stokkum, I. H. M.; Dekker, J. P.; van Grondelle, R.; Croce, R. The origin of the low-energy form of photosystem I light-harvesting complex Lhca4: mixing of the lowest exciton with a charge-transfer state. *Biophys. J.* **2009**, *96*, L35–L37.
- (23) Guo, Z.; Lin, S.; Woodbury, N. W. Utilizing the dynamic Stark shift as a probe for dielectric relaxation in photosynthetic reaction centers during charge separation. *J. Phys. Chem. B* **2013**, *117*, 11383–11390.
- (24) Franzen, S.; Goldstein, R. F.; Boxer, S. G. Electric field modulation of electron transfer reaction rates in isotropic systems: long-distance charge recombination in photosynthetic reaction centers. *J. Phys. Chem.* **1990**, *94*, 5135–5149.
- (25) Lockhart, D. J.; Kirmaier, C.; Holten, D.; Boxer, S. G. Electric field effects on the initial electron-transfer kinetics in bacterial photosynthetic reaction centers. *J. Phys. Chem.* **1990**, *94*, 6987–6995.
- (26) Niedringhaus, A.; Policht, V. R.; Sechrist, R.; Konar, A.; Laible, P. D.; Bocian, D. F.; Holten, D.; Kirmaier, C.; Ogilvie, J. P. Primary processes in the bacterial reaction center probed by two-dimensional electronic spectroscopy. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 3563–3568.
- (27) Ma, F.; Romero, E.; Jones, M. R.; Novoderezhkin, V. I.; van Grondelle, R. Vibronic coherence in the charge separation process of the *Rhodobacter sphaeroides* reaction center. *J. Phys. Chem. Lett.* **2018**, *9*, 1827–1832.
- (28) Ma, F.; Romero, E.; Jones, M. R.; Novoderezhkin, V. I.; van Grondelle, R. Both electronic and vibrational coherences are involved in primary electron transfer in bacterial reaction center. *Nat. Commun.* **2019**, *10*, 933.
- (29) Loukianov, A.; Niedringhaus, A.; Berg, B.; Pan, J.; Senlik, S. S.; Ogilvie, J. P. Two-dimensional electronic Stark spectroscopy. *J. Phys. Chem. Lett.* **2017**, *8*, 679–683.
- (30) Loukianov, A. D. Development of two-dimensional Stark spectroscopy for the investigation of photosynthetic charge separation. Doctoral dissertation; University of Michigan: 2017.
- (31) Vos, M. H.; Rischel, C.; Jones, M. R.; Martin, J. Electrochromic detection of a coherent component in the formation of the charge pair P+HL- in bacterial reaction centers. *Biochemistry* **2000**, *39*, 8353–8361.
- (32) Breton, J.; Martin, J. L.; Fleming, G. R.; Lambry, J. C. Low-temperature femtosecond spectroscopy of the initial step of electron transfer in reaction centers from photosynthetic purple bacteria. *Biochemistry* **1988**, *27*, 8276–8284.
- (33) Brixner, T.; Mancal, T.; Stiopkin, I. V.; Fleming, G. R. Phase-stabilized two-dimensional electronic spectroscopy. *J. Chem. Phys.* **2004**, *121*, 4221–4236.
- (34) Pawlowicz, N. P.; van Grondelle, R.; van Stokkum, I. H. M.; Breton, J.; Jones, M. R.; Groot, M. L. Identification of the first steps in charge separation in bacterial photosynthetic reaction centers of

Rhodobacter sphaeroides by ultrafast mid-infrared spectroscopy: electron transfer and protein dynamics. *Biophys. J.* **2008**, *95*, 1268–1284.

(35) McAuley, K. E.; Fyfe, P. K.; Cogdell, R. J.; Isaacs, N. W.; Jones, M. R. X-ray crystal structure of the YM210W mutant reaction centre from *Rhodobacter sphaeroides*. *FEBS Lett.* **2000**, *467*, 285–290.

(36) Vos, M. H.; Jones, M. R.; Breton, J.; Lambry, J.-C.; Martin, J.-L. Vibrational dephasing of long- and short-lived primary donor excited states in mutant reaction centers of *Rhodobacter sphaeroides*. *Biochemistry* **1996**, *35*, 2687–2692.

(37) Ermler, U.; Fritzsche, G.; Buchanan, S. K.; Michel, H. Structure of the photosynthetic reaction centre from *Rhodobacter sphaeroides* at 2.65 Å resolution: cofactors and protein-cofactor interactions. *Structure* **1994**, *2*, 925–936.

(38) Liptay, W. Electrochromism and solvatochromism. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 177–188.

(39) Gelzinis, A.; Augulis, R.; Butkus, V.; Robert, B.; Valkunas, L. Two-dimensional spectroscopy for non-specialists. *Biochim. Biophys. Acta, Bioenerg.* **2019**, *1860*, 271–285.

(40) Khatypov, R. A.; Khmelnskiy, A. Y.; Khristin, A. M.; Shuvalov, V. A. Femtosecond absorption band formation at 1080 and 1020 nm as an indication of charge-separated states $P_A^{\delta+}P_B^{\delta-}$ and $P^+B_A^-$ in photosynthetic reaction centers of the purple bacterium *Rhodobacter sphaeroides*. *Dokl. Biochem. Biophys.* **2010**, *430*, 24–28.

(41) Tran, N. L.; Elkins, M. H.; McMeekin, D. P.; Snaith, H. J.; Scholes, G. D. Observation of charge generation via photoinduced Stark effect in mixed-cation lead bromide perovskite thin films. *J. Phys. Chem. Lett.* **2020**, *11*, 10081–10087.

(42) Yakovlev, A. G.; Vasilieva, L. G.; Khmelnskaya, T. I.; Shkuropatova, V. A.; Shkuropatov, A. Y.; Shuvalov, V. A. Primary electron transfer in reaction centers of YM210L and YM210L/HL168L mutants of *Rhodobacter sphaeroides*. *Biochemistry (Moscow)* **2010**, *75*, 832–840.

(43) Ivashin, N. V.; Shchupak, E. E. Mechanism by which a single water molecule affects primary charge separation kinetics in a bacterial photosynthetic reaction center of *Rhodobacter sphaeroides*. *Opt. Spectrosc.* **2012**, *113*, 474–486.

(44) Eisenmayer, T. J.; Lasave, J. A.; Monti, A.; de Groot, H. J. M.; Buda, F. Proton displacements coupled to primary electron transfer in the *Rhodobacter sphaeroides* reaction center. *J. Phys. Chem. B* **2013**, *117*, 11162–11168.