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Delocalized electronic excitations and their role in directional charge transfer in the reaction center of *Rhodobacter sphaeroides* F

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Sabrina Volpert ; Zohreh Hashemi ; Johannes M. Foerster ; Mario R. G. Marques ; Ingo Schelter ; Stephan Kümmel ; Linn Leppert



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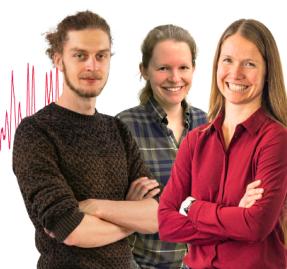
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Sabrina Volpert,¹ Zohreh Hashemi,¹ Johannes M. Foerster,¹ Mario R. G. Marques,¹ Ingo Schelter,¹ Stephan Kümmel,¹ and Linn Leppert^{1,2,a)}

AFFILIATIONS

¹ Institute of Physics, University of Bayreuth, 95440 Bayreuth, Germany

² MESA+ Institute for Nanotechnology, University of Twente, 7500 AE Enschede, The Netherlands

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^{a)}Author to whom correspondence should be addressed: l.leppert@utwente.nl

ABSTRACT

In purple bacteria, the fundamental charge-separation step that drives the conversion of radiation energy into chemical energy proceeds along one branch—the A branch—of a heterodimeric pigment–protein complex, the reaction center. Here, we use first principles time-dependent density functional theory (TDDFT) with an optimally-tuned range-separated hybrid functional to investigate the electronic and excited-state structure of the six primary pigments in the reaction center of *Rhodobacter sphaeroides*. By explicitly including amino-acid residues surrounding these six pigments in our TDDFT calculations, we systematically study the effect of the protein environment on energy and charge-transfer excitations. Our calculations show that a forward charge transfer into the A branch is significantly lower in energy than the first charge transfer into the B branch, in agreement with the unidirectional charge transfer observed experimentally. We further show that the inclusion of the protein environment redshifts this excitation significantly, allowing for energy transfer from the coupled Q_x excitations. Through analysis of transition and difference densities, we demonstrate that most of the Q-band excitations are strongly delocalized over several pigments and that both their spatial delocalization and charge-transfer character determine how strongly affected they are by thermally-activated molecular vibrations. Our results suggest a mechanism for charge-transfer in this bacterial reaction center and pave the way for further first-principles investigations of the interplay between delocalized excited states, vibronic coupling, and the role of the protein environment in this and other complex light-harvesting systems.

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I. INTRODUCTION

In natural photosynthesis, the energy of sunlight is converted into chemical energy in highly efficient excitation- and charge-transfer processes.¹ Absorption of light happens primarily in antenna complexes, which funnel the excitation energy toward the reaction center (RC), where a charge-separation step initiates a cascade of electron-transfer processes resulting in a proton gradient that drives the biochemical reactions of photosynthesis. In purple bacteria such as *Rhodobacter sphaeroides*, the fundamental design principles of these pigment–protein complexes are well

understood due to a wealth of experimental and computational techniques that give access to detailed structural and spectroscopic information.^{2–9} In this respect, the bacterial RC can also be understood as a model system for the RC of more complex photosynthetic organisms because its structure is highly conserved across bacteria, algae, and plants.¹⁰ Its main building blocks, shown in Fig. 1, are arranged along two pseudosymmetric branches A and B and consist of a strongly coupled dimer of two bacteriochlorophyll (BCL) molecules dubbed the special pair (P), two accessory BCLs (B_A, B_B), two bacteriopheophytins (H_A, H_B), and two quinones (Q_A, Q_B) embedded in a transmembrane protein matrix.

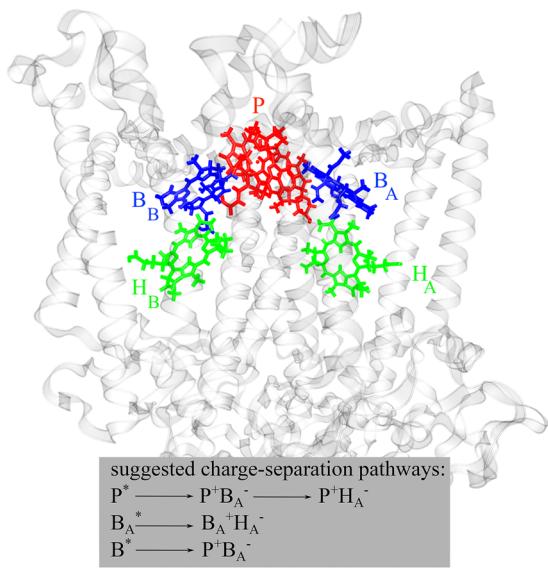


FIG. 1. Proposed charge-separation pathways^{7,15,19–22} and structural model of the RC of *Rhodobacter sphaeroides*, including the special pair (*P* in red), two accessory BCLs (B_A , B_B in blue), and two bacteriopheophytins (H_A , H_B in green). Protein chains are shown in transparent gray. Hydrogen atoms are omitted for clarity.

Despite the similar, but not exactly symmetric, structure of the *A* and *B* branches, it is well-established that the primary charge separation reaction only proceeds along the *A* branch with near-unitary quantum efficiency.^{11–13} The ultrafast timescales on which the primary energy- and charge-transfer processes occur in the RC, in combination with broad overlapping absorption peaks originating from the coupling of multiple pigments and their protein environments, have posed significant challenges to spectroscopic techniques. Two-dimensional electronic spectroscopy (2DES) has become one of the primary experimental techniques for studying the bacterial RC^{7,14–16} and other photosynthetic systems.^{17,18} For the RC of *Rhodobacter sphaeroides* and similar RCs, 2DES has been used to propose models for the kinetics of the primary charge separation. In these models, it is usually assumed that charge separation is initiated through the excitation of *P* (denoted as P^*), leading to a charge-transfer intermediate $P_A^-P_B^+$, which is followed by a charge-separated state $P^+H_A^-$ via an ultra-shortlived intermediate $P^+B_A^-$.^{7,15} However, alternative charge-separation pathways have been suggested as well, for example, starting from an excitation localized on one or both accessory BCLs, i.e., B_A^* or B^* ^{19–22} (Fig. 1).

Computational modeling has played an important role in helping to unravel the intricate factors affecting excitation and charge transfer in the antenna complexes and the RC. Calculations based on model Hamiltonians and advances in the semiempirical modeling of long- and short-range coupling between chromophores have allowed for the simulation of excitation processes in large pigment-protein complexes.^{23–25} However, also computationally more demanding first-principles calculations, primarily based on (time-dependent) density functional theory (TDDFT), have been employed to study systems of growing complexity and size. The focus of many studies has been the origin of the unidirectionality

of the charge-separation process in bacterial RCs and photosystem II of plants.

For the RC of photosystem II, a large model system was used by Frankcombe, including four (truncated) chlorophyll molecules, two pheophytin molecules, and two plastoquinone molecules using TDDFT and a polarizable continuum model (PCM) to account for effects of the protein environment.²⁶ Later, Sirohiwal *et al.* reported TDDFT calculations using a range-separated hybrid functional on chlorophyll monomers, dimers, and trimers of the photosystem II RC showing that the lowest-energy charge-transfer excitation corresponds to $B_A^+H_A^-$ and is strongly affected by protein electrostatics.²⁷ Low-energy charge-transfer excitations were also reported by Kavanagh *et al.* in TDDFT calculations using a hexameric model of the photosystem II RC, which included parts of the protein environment explicitly. Similarly, Förster *et al.* observed an excitation with a partial $B_A^+H_A^-$ charge-transfer character using the GW + Bethe-Salpeter Equation approach.²⁸

For the RC of *Rhodobacter sphaeroides*, (TD)DFT calculations including the special pair and some of its neighboring amino-acid residues were reported in 2011 by Wawrzyniak *et al.* and indicated that protein induced distortions of the special pair geometry lead to an asymmetric ground-state electron density.²⁹ A similar model system was employed by Eisenmayer *et al.*, who performed molecular dynamics simulations based on constrained DFT and showed that the electron-density asymmetry is dynamical and coupled to a low-frequency vibrational mode related to the rotation of a histidine residue close to B_A .³⁰ Later, Eisenmayer *et al.* included the B_A in their constrained DFT simulations showing the coupling of proton displacements to the primary electron-transfer step from *P* to B_A .³¹ Aksu *et al.* combined TDDFT with a tuned range-separated hybrid functional with PCM and showed that spectral asymmetries arise from locally different dielectric environments along the *A* and *B* branches.³² The initial charge-transfer excitations of *P* were also studied by Aksu *et al.*, employing the same methodology.³³ (TD)DFT calculations by Mitsuhashi *et al.*, in which the environment of *P* together with either B_A or B_B was represented using a QM/MM/PCM scheme, further indicated that the lowest unoccupied molecular orbital (LUMO) of B_A is lower in energy than the LUMO of B_B , suggesting that B_A is the primary electron acceptor.³⁴ Another study, in which some of the same authors used a diabatization scheme to evaluate electronic couplings between *P* and B_A and B_B , respectively, pointed to the particular importance of a tyrosine residue close to B_A as being responsible for the directionality of charge transfer.³⁵ Brüttling *et al.* investigated the primary charge separation step in the quasi-symmetric reaction center of *Helioibacterium modesticaldum*, with an emphasis on revealing the influence of nuclear motion on the relative energetic positions of different electronic excitations.³⁶

To the best of our knowledge, explicit TDDFT calculations on a reaction center model of *Rhodobacter sphaeroides*, including all six primary pigments and parts of the environment, have not been reported yet. Furthermore, while previous studies have provided detailed insight into the effects of the protein environment and molecular vibrations on excited states, little attention has been directed at the delocalized, correlated multi-particle nature of these excitations. One may wonder whether these characteristics can be

properly captured by TDDFT, as the wave functions obtained in TDDFT have no rigorous physical meaning. We here show, however, that one can analyze the excitations reliably based on transition densities and difference densities, i.e., quantities that have a solid foundation in TDDFT.

To this end, we use TDDFT with an optimally-tuned range-separated hybrid functional to study a hexameric model of the RC, including the primary pigments, i.e., the special pair P , the accessory BCLs B_A and B_B , and the bacteriopheophytins H_A and H_B . We also explicitly model the effect of close-lying amino-acid residues on the excited states by including them in our TDDFT calculations. We clarify which amino acids are responsible for significant changes in excited-state energies and compare our results with QM/MM calculations. Our calculations show that a distinction between localized excitations on the one hand and charge-transfer excitations on the other hand is of limited usefulness to understand the excited state structure of this system of strongly coupled pigments. Instead, we find excitations without charge-transfer character that are delocalized across several pigments and that cannot readily be classified as coupled excitations of individual monomeric units. Partial charge-transfer states between the special pair pigments ($P_A^-P_B^+$) are low in energy, mix with these delocalized states, and are a consequence of the strong coupling between the pigments. The lowest-energy charge-transfer state that transfers an electron into the A branch can clearly be classified as $B_A^-H_A^+$ and is significantly lower in energy than charge-transfer into the B branch. This is in agreement with previous first-principles calculations on the photosystem II reaction center but not in line with experimental reports suggesting charge-transfer through an intermediate $P^+B_A^-$ state. The $B_A^-H_A^+$ excitation is ~ 20 meV higher in energy than the highest-energy Q-band excitations. Although we cannot rule out that including further parts of the environment might lower its energy further, such a small energetic separation suggests that the vibrational modes of the pigments and/or the environment could couple this charge-transfer state to the delocalized Q-band excitations.

II. COMPUTATIONAL METHODS

A. Structure of model systems

All our calculations are based on the experimental crystal structure of the wild-type RC of *Rhodobacter sphaeroides* with Protein Data Bank file ID 1M3X.³ The pigment–protein complex has two main protein chains called L- and M-chains, which form the backbone of the A and B branches, respectively. We are interested in the primary charge-transfer process and, therefore, have included P , B_A , B_B , H_A , and H_B in all our computational models. For approximating the effect of the protein environment on energy- and charge-transfer excitations, we added amino-acid residues explicitly to our model structures, as described in more detail in Sec. III B. Hydrogen atoms are not resolved in the experimental crystal structure and are, therefore, added with the module HBUILD in CHARMM³⁷ and energetically optimized using the CHARMM force field³⁸ as described in Ref. 39. In all model systems, we cropped the phytol tails of the BCL molecules and saturated the carboxyl group with a hydrogen atom. Using a methyl group to saturate the phytol tail does not change the main conclusions of this paper, as shown in Fig. S1 of the supplementary material. Furthermore, we cut the bonds between the

amino-acid residues and the polypeptide chains between C_α and C_β and saturated them with hydrogen atoms.

B. TDDFT calculations

We performed linear-response TDDFT (LR-TDDFT) calculations using Q-CHEM, version 5.2.2.⁴⁰ Vibrational normal modes were calculated with TURBOMOLE version 7.5^{41,42} and QM/MM simulations with ORCA version 5.0.2.⁴³ We used the Pople basis set 6-31G(d,p) for which the Q_y and Q_x excitation energies of a single BCL a molecule are converged to within 50 meV.³⁹ We also tested the accuracy of the basis set for the special pair P , as discussed in the supplementary material (Table S1). The exchange-correlation energy is approximated using the optimally-tuned ω PBE functional,⁴⁴ which has been shown to properly capture the coupling between BCLs³⁹ and to be *on par* with Green's function-based many-body perturbation theory for a wide range of single chromophores.^{45,46} Range-separated hybrid functionals have also been demonstrated to accurately describe electrochromic shifts due to the protein environments of various biochromophores in an extensive benchmark of DFT approximations by Sirohiwal *et al.*⁴⁷ In the optimally-tuned ω PBE functional, the range-separation parameter determines the length scale at which short-range semilocal exchange goes over into exact long-range exchange. Such functionals significantly improve the description of charge-transfer excitations⁴⁸ and lead to excellent agreement with experimental photoemission spectroscopy for a broad range of systems, from molecules to solids.^{49–55} In the optimal-tuning procedure, the range-separation parameter ω is varied such that the difference between the HOMO eigenvalue ε_{HOMO} and the negative ionization potential of both the neutral and the anionic system is minimized.⁵⁶ Here, we use $\omega = 0.171a_0^{-1}$ based on tuning for one BCL a performed by Schelter *et al.*³⁹ We confirmed that the deviation of the ionization potentials from $-\varepsilon_{HOMO}$ of P and of a single BCL a with coordinating histidine is negligible, and we do not perform a separate tuning procedure for each of our model systems. This approach is also supported by more general arguments: Using the same ω for each model system allows us to compare the electronic and excited state structures of our model systems on the same footing. Furthermore, optimal tuning of conjugated systems of increasing size leads to artificially low values of ω and, thus, a dominance of semilocal exchange at long range, which deteriorates the description of charge-transfer excitations,^{53,57} as shown for model structures of increasing size in Fig. S2. For our LR-TDDFT calculations, we used the Casida approach and did not make the Tamm–Dancoff approximation (TDA) unless otherwise noted. We provide further information regarding the numerical convergence of our calculations in the supplementary material. Details of our QM/MM LR-TDDFT calculations with ORCA can also be found in the supplementary material.

C. Classification of charge-transfer excitations

Since the transition density vanishes for charge-transfer states, we calculated the difference density $\Delta n_i = n_i - n_0$ between the excited (n_i) and the ground-state density (n_0) for every excitation i . The excited-state density n_i is calculated as the diagonal part of the excited state density matrix $\gamma^{ii}(r, r') = N \int \Psi^i(r, r_2, r_3, \dots, r_N) \Psi^i(r', r_2, r_3, \dots, r_N) dr_2 \dots dr_N$, where N is

the number of electrons and Ψ^i is the approximate excited-state wavefunction that consists of a sum of Slater determinants of generalized Kohn-Sham orbitals with coefficients obtained from LR-TDDFT.⁵⁸ There is no formal guarantee that this wavefunction is equal to the exact excited-state wavefunction. However, it has been shown that eigenvalues and orbitals from accurate exchange-correlation potentials are an ideal basis for describing molecular excitations,⁵⁹ and the Kohn-Sham Slater determinant can be rigorously interpreted as the zeroth-order approximation to the true wavefunction in Görling-Levy perturbation theory.⁶⁰ For organic molecular systems, it has been found that approximating the true wavefunction by the Kohn-Sham Slater determinant is often justified. This is seen, e.g., in the successful interpretation of photoemission data using Kohn-Sham wavefunctions^{61,62} and the great success of the concept of natural transition orbitals.⁶³ To quantify the magnitude of charge transfer, we integrated over subsystem difference densities. For this purpose, we subdivided the volume containing the difference densities of our full model systems into subsystem volumes, each containing one pigment. Note that P is separated into P_A and P_B to enable the characterization of internal charge-transfer states of type $P_A^+P_B^-$. Our aim is to assign each grid point of the difference-density grid to its closest pigment molecule. To achieve this, we tested two methods for assigning grid points to subsystem volumes: In method 1, we used the distances between grid points and each molecule's atomic coordinates (including hydrogen atoms). In method 2, we used distances between grid points and each molecule's geometrical center of gravity. Both methods result in the same trends, although the absolute values of the integrated subsystem densities differ slightly.

III. RESULTS AND DISCUSSION

In the following discussion, our aim is to elucidate a mechanism for charge-transfer in the RC of *Rhodobacter sphaeroides* and to probe the effect of explicitly including amino-acid residues in the vicinity of the primary pigments. We start with a hexameric model system in Sec. III A consisting of P , B_A , B_B , H_A , and H_B . If amino acids are added to this system, the number of excited states that needs to be calculated to observe charge-transfer is too large to be computationally feasible. We, therefore, use two different types of model systems to study the addition of amino acids: in Sec. III B, we construct a tetrameric model system consisting of P , B_A , and B_B . We systematically add amino acids to establish the minimal model necessary to account for the static effects of the protein environment. However, this model does not include the bacteriopheophytins H_A and H_B and, therefore, does not allow us to observe all relevant low-energy charge-transfer excitations. In Sec. III C, we, therefore, use models of the A and B branches, including P , B_A , H_A , and P , B_B , H_B , respectively. We show that the A and B branch structures reproduce the main features of the hexameric model (Sec. III A) and probe the effect of adding amino acids to these models on the relevant charge-transfer states.

Since our goal is to isolate the direct electronic effects of the amino-acid environment on the excited states, we do not perform geometry optimizations for each model system. In other words, differences between the excitation spectra of our model systems

can be fully attributed to the electronic effects of the amino-acid environment and are not related to additional structural effects.

A. Absorption spectrum and excited state character of the bare hexameric RC model

We start our discussion by inspecting the absorption spectrum of a hexameric model of the RC based on the crystal structure as described in Sec. II A and without including any parts of the environment, as shown in Fig. 2(a). For this model, we were able to calculate 16 excitations, which correspond to the energy range depicted in Fig. 2(a). This energy range is dominated by Q-band excitations, i.e., excited states that originate from the coupling of the Q_y and Q_x excitations of the individual BCL and bacteriopheophytin molecules. However, because of the spatial proximity of these pigments in the RC, not all excitations can clearly be classified as coupled Q_y or Q_x , as

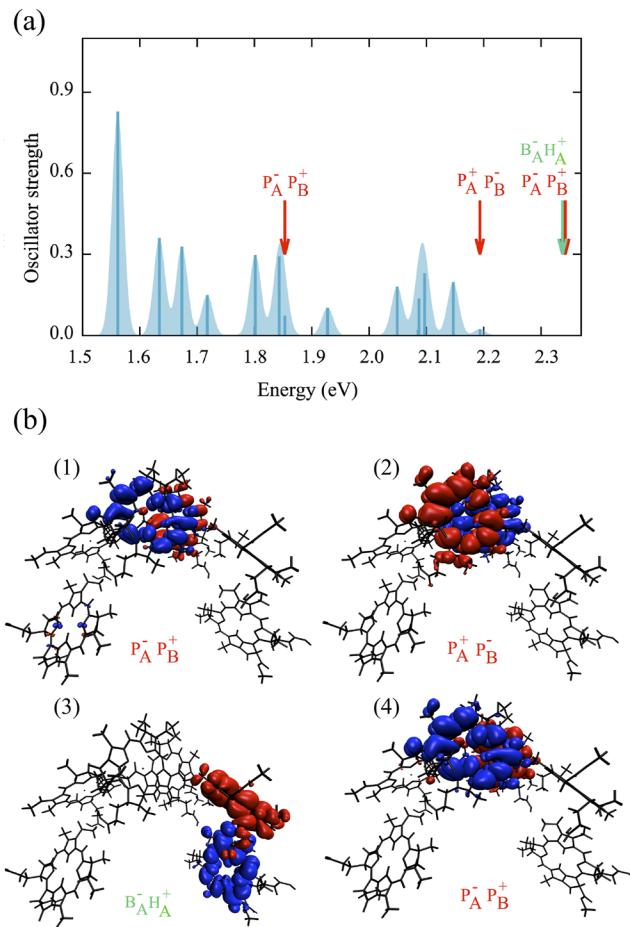


FIG. 2. (a) LR-TDDFT absorption spectrum of the bare hexameric RC model. Arrows mark excitations with low/vanishing oscillator strength and (partial) charge-transfer character. The shaded areas are calculated by folding the excitation energies into Gaussian functions with a width of 80 meV as a guide to the eye. (b) Difference densities of the four charge-transfer excitations in this energy range. Isosurface values correspond to $-0.0001\alpha_0^{-3}$ (red) and $0.0001\alpha_0^{-3}$ (blue), respectively.

apparent from their transition densities shown in Fig. S3. These transition densities also show that the majority of Q-band excitations are spatially strongly delocalized across several pigments, with some of them spreading over the entire RC model. This is the first main result of our study. A list of excitation energies, oscillator strengths, and spatial character as determined from the transition densities (and difference densities in the case of charge-transfer excitations) can be found in Table I. In this table and in the rest of the text, the notation $(PBH)^*$ corresponds to an excitation delocalized across P , B_A , B_B , H_A , and H_B , while $P_A^+P_B^-$ denotes a charge-transfer excitation from P_A to P_B .

We find four excitations with charge-transfer character in this energy range. The difference densities of these excitations are depicted in Fig. 2(b) (all other difference densities for this structural model can be found in Fig. S4). Here and in the following, positive difference density values indicate a region of space in which the electron density (i.e., negative charge density) increases as a consequence of the excitation (shown in blue), whereas negative values indicate regions of space in which the electron density decreases (shown in red). Numerical values based on the integration of difference densities as described in Sec. II C are listed in Table S3. The three charge-transfer excitations within the special pair, corresponding to $P_A^+P_B^-$ and $P_A^-P_B^+$ arise as a consequence of the strong coupling of P_A and P_B . In particular, the first $P_A^+P_B^+$ excitation mixes strongly with other excitations at ~ 1.85 eV and, therefore, exhibits partial charge-transfer character, in which 0.69 of an electron is transferred from P_A to P_B . Strikingly, we also find a charge-transfer excitation from B_A to H_A in this energy range. This is the lowest-energy pure charge-transfer state we find in our calculations (0.99 of an electron is transferred from B_A to H_A). The second main result of our study is that the appearance of this charge-transfer state at ~ 2.3 eV is a consequence of the spatial arrangement of the pigments in the bacterial RC alone. We will discuss how the energy of this state is affected by including environmental effects in Sec. III C.

TABLE I. Excitation energies (in eV), oscillator strengths, and spatial delocalization/charge-transfer character of the first 16 excitations of the bare hexameric RC model structure.

No.	Energy	Oscillator strength	Character
1	1.56	0.83	$(PBH)^*$
2	1.63	0.36	$(PBH_B)^*$
3	1.67	0.33	$(PBH)^*$
4	1.71	0.15	$(PBH)^*$
5	1.80	0.30	$(BH_B)^*$
6	1.84	0.29	$(PB_AH)^*$
7	1.85	0.07	$P_A^-P_B^+$
8	1.92	0.10	$(PBH)^*$
9	2.04	0.18	$(PBH)^*$
10	2.08	0.02	$(P_BBH_B)^*$
11	2.08	0.13	$(PBH)^*$
12	2.09	0.23	$(PBH_A)^*$
13	2.14	0.20	$(PBH_B)^*$
14	2.19	0.02	$P_A^+P_B^-$
15	2.33	0.00	$B_A^-H_A^+$
16	2.34	0.00	$P_A^-P_B^+$

B. Effect of the protein environment on a tetrameric RC model

The importance of the protein environment and its impact on charge transfer were recognized already in early studies of the bacterial RC.^{64–66} Proposals for how the surrounding proteins affect charge transfer in the RC have primarily included asymmetries in the dielectric environment and in the protein electrostatic fields that A and B branch cofactors experience.^{65,67–70} Our goal here is to explicitly include parts of the protein environment in our LR-TDDFT calculations to elucidate which amino-acid residues electronically couple to the primary RC pigments. For this purpose, we start by studying tetrameric models of the RC, including the special pair P and the accessory BCLs B_A and B_B , and systematically increase the number of amino-acid residues in our calculations.

We construct four model systems, as shown in Fig. 3(a): model system M_1 consists of the four BCL molecules P_A , P_B , B_A , and B_B . For a direct analysis of the influence of the closest lying amino acids, the histidine molecules that coordinate each of the BCLs (HIS M202, HIS M182, HIS L173, and HIS L153) were included in model system M_2 . Our largest model system, M_4 , contains all amino acids in a radius of 3 Å around the BCLs. These 32 amino acids were determined by constructing spheres with a radius of 3 Å around each atom of the four BCL molecules (excluding hydrogen atoms and the phytol tail). A complete list (Table S4) and all structure files can be found in the supplementary material. We calculated the electronic density of states (DOS) of these model systems and found two occupied states localized on amino acids TRP M157 and MET L248, respectively, energetically close to the highest occupied molecular orbital of M_4 (see Figs. S5 and S6). However, a model system M_3^* consisting of the four primary BCL molecules, the coordinating histidines, and these two amino acids features an electronic DOS distinctly different from that of M_4 (Fig. S7). We therefore additionally included the two main symmetry breaking amino acids PHE M197 and HIS L168 as suggested by Eisenmayer *et al.*³¹ to construct model system M_3 with a DOS in very good agreement with the DOS of M_4 in the relevant energy range.

The LR-TDDFT Q-band spectra of M_1 – M_4 comprising the ten lowest-energy excitations are shown in Fig. 3(b) and Tables S5 and S6. The first four excitations of these model systems can be seen as arising from a coupling of the Q_y excitations of P , B_A , and B_B . We provide a detailed analysis of the origin of these excitations in the supplementary material (Figs. S9–S11). Inspection of their transition densities (Fig. S9) shows that only the first excitation is localized on P , while excitations 2–4 are coupled Q_y excitations spread across all four BCLs. Among the following six excitations of M_1 , 5, 6, and 7 can clearly be assigned to P . Two of these excitations (6 and 7) have coupled Q_x character; excitation 5 has Q_y character, but integration over the difference density corresponding to this state also shows substantial charge-transfer character. States 8 and 9 of M_1 are Q_x excitations associated with B_A and B_B . Excitation 10 of M_1 is nearly dark and corresponds to a $P_A^+P_B^-$ charge-transfer state.

Inclusion of the histidines in M_2 hardly affects the first four excitations. Only when further amino-acid residues are added do we observe a noticeable redshift: the first excitation of M_4 is 40 meV lower in energy than that of M_1 . The character of these excitations is not changed by the environment. The average difference density

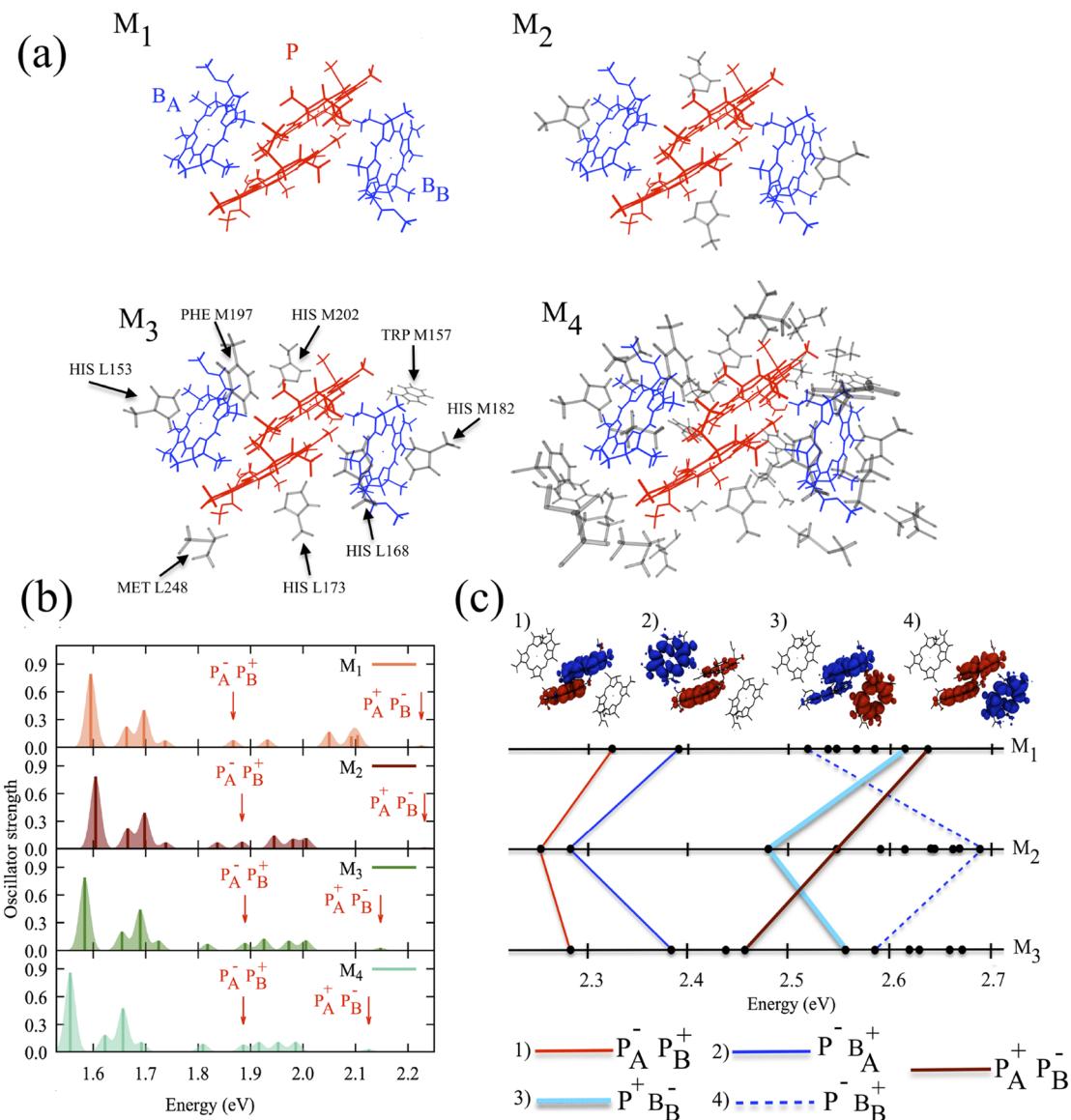


FIG. 3. (a) Representation of model systems M_1 , M_2 , M_3 , and M_4 as described in the main text. (b) Absorption spectra of M_1 – M_4 in the energy region where coupled Q_y and Q_x excitations are expected. Red arrows mark excitations with charge-transfer character. (c) Energy of dark excitations (zero oscillator strength) of M_1 – M_3 and difference densities of selected charge-transfer excitations of M_1 . The red surface of the difference density shows the isovalue $-0.0001\alpha_0^{-3}$ and the blue surface shows $0.0001\alpha_0^{-3}$.

of excitations 1–4 is barely affected by the addition of the environment, as shown in Fig. S12. We observe more significant energy differences for the next six excitations. Excitations with Q_x character are redshifted by ~ 100 meV through addition of the coordinating histidines, and the coupled Q_x excitations of P are redshifted by another 20 meV for model system M_3 , while the Q_x excitations of the accessory BCLs are stable. We note that the significance of the coordinating histidines for these excitations can also be seen in the

difference density (Fig. S12). In systems M_2 – M_4 , there is a clear transfer of positive charge from P to the coordinating histidines.

The energy of the (partial) charge-transfer excitations 5 and 10 of M_1 is also affected by adding the protein environment. Excitation 5 of M_1 is redshifted by ~ 60 meV and becomes excitation 6 in M_4 , and excitation 10 is redshifted by ~ 100 meV. The magnitude of charge transfer is only slightly affected by the addition of the environment. The amount of charge transferred from P_A to P_B in

excitation 5 of M_1 decreases slightly when adding the environment while in excitation 10 it remains the same. Overall, the character of excitations 5–10 is only slightly affected by the amino-acid environment, as can be seen in Fig. S13, which shows the average difference density of excitations 5–10 for systems M_1 – M_4 . Importantly, Fig. 3(b) demonstrates that the spectrum of M_4 is very similar to that of M_3 with the same order of states and only a small global redshift as compared to M_3 . We, therefore, conclude that the eight amino acids considered in M_3 reproduce the main (static) effects of the amino-acid environment. Therefore, they constitute a “reasonable minimal environment” that should be explicitly included in future calculations.

At energies above the Q-band and below the Soret band, which starts at ~ 3 eV,¹⁰ we observe a range of dark states with charge-transfer character. Here, we only discuss five states in this energy region that lead to a clear charge transfer between different BCL molecules. The energy and difference densities of these states are shown in Fig. 3(c). (Integrated) difference densities of all states are shown in Fig. S14 and Table S7. Due to the large size of M_4 , we only calculated 12 excitations with high numerical accuracy for this system. Since the effects of the environment are well-represented by M_3 , as shown before, we do not discuss the charge-transfer excitations of M_4 in detail. However, Tables S5 and S6 show that the energies of the charge-transfer excitations 11 and 12 of M_3 and M_4 are in very good agreement.

In similarity to the findings for our hexameric model of the RC, we find a $P_A^- P_B^+$ excitation in M_1 – M_3 (excitation 11). The addition of the histidines redshifts this state by 80 meV, while the additional amino acids in M_3 lead to a blueshift of 26 meV. The 12th excitation corresponds to $P^- B_A^+$. This state is redshifted by 120 meV through the addition of histidines, while further amino acids blueshift the excitation back by 110 meV. The energy gap between $P^- B_A^+$ and the next charge-transfer excitation is substantial: ~ 130 meV in M_1 , ~ 210 meV in M_2 , and 85 meV in M_3 . In the 13th excitation of M_1 , we observe a backward charge transfer from the B branch corresponding to $P^+ B_A^-$. The first forward charge transfer into the B branch ($P^+ B_B^-$) occurs at ~ 2.6 eV, i.e., at significantly higher energies than $P^- B_A^+$. The addition of amino acids affects this excitation in a similar way as $P^- B_A^+$. These calculations show that the inclusion of a (static) protein environment in the system studied here changes excitation energies substantially but hardly affects the character and spatial delocalization of states. For our tetrameric model systems, addition of the protein environment also does not lead to a mixing of Q-band excitations and experimentally relevant charge-transfer states.

To probe the effect of structural fluctuations on the energy of the excited states, we also calculated the normal mode spectrum of model system M_1 in TURBOMOLE using the B3LYP functional and def2-SVP basis sets. We then distorted the structure along each of the normal modes with a distortion amplitude corresponding to 300 K and calculated the LR-TDDFT excitation spectra in QCHEM with ω PBE as before. The high-frequency modes of M_1 correspond to intramolecular vibrations such as C–C and C–H stretch modes, which are not thermally activated and only have a small effect on the energy of the delocalized excitations that we are interested in here. We, therefore, only calculated the effect of normal mode distortions on excitation energies for wavenumbers below 85 cm⁻¹. Low-frequency modes correspond to intermolecular

vibrations that change the orbital overlap between neighboring BCL molecules and are thus expected to have a more substantial effect on the excitation energies of delocalized and charge-transfer excitations.⁷¹

Our results, shown in Fig. S15, confirm this intuitive picture: The first excitation, corresponding to a coupled Q_y excitation of P , exhibits mode-dependent energy changes of up to 20 meV, while excitations 2–4 are much less sensitive to these distortions with energy changes of $\lesssim 10$ meV in line with their spatial delocalization across B_A and B_B , which are far apart. A similar observation holds for the coupled Q_x excitations of P , B_A , and B_B . On the other hand, excitations with (partial) charge-transfer character between neighboring molecules are highly sensitive to low-frequency vibrations, exhibiting excitation-energy changes of up to 30 meV for charge transfer between P_A and P_B and up to 50 meV for charge transfer between P and B_A or B_B . These excitation-energy changes can result in both red- and blueshifts, as shown in Fig. S15. Nonetheless, our analysis indicates that the inclusion of inter- and intramolecular vibrations does not change our overall result that, in a tetrameric model of the bacterial RC, charge-transfer excitations into the A-branch are energetically well-separated from the Q-band excitations. A more complete picture of the effect of thermal fluctuations could be obtained through a statistical analysis of the excitation spectra of structure “snapshots” from molecular dynamics simulations. Such simulations were performed for the RC of *Helio bacterium modestum caldum* in Ref. 36. There, it was found that the excitation energies changed quantitatively, but the conclusions about the relative ordering of the excitations based on the ensemble-averaged excitation spectrum agreed with the conclusions that were drawn based on a single spectrum.

Finally, we tested whether the inclusion of further parts of the protein environment through a QM/MM scheme would change our main conclusions. Figures S16 and S17 show the full LR-TDDFT spectrum and the TDA spectrum of M_1 with and without the QM/MM environment. Inclusion of the QM/MM environment leads to changes in the absorption spectrum of comparable size as in our explicit model M_4 . In particular, we also observe a redshift of the first charge-transfer excitation of ~ 200 meV. However, the redshift of the coupled Q_y and Q_x excitations due to the protein environment is smaller in the QM/MM model, and some of the detailed changes in the partial and full charge-transfer excitations are also not captured by the MM environment.

C. Effect of environment on A and B branch excited states

Due to the large size of the hexameric model of the RC discussed in Sec. III A (494 atoms), an LR-TDDFT calculation including the relevant charge-transfer states for a structural model that also includes significant parts of the protein environment as performed for the tetrameric model in Sec. III B is computationally not feasible. The largest hexameric RC model that we could run full LR-TDDFT calculations for includes the coordinating histidines close to P_A , P_B , B_A , and B_B (as in M_2) plus two leucines close to H_A and H_B . Table S2 shows that including these amino-acid residues has similar effects as those observed in Sec. III B, but the calculation of more than the first 13 excitations was not feasible for this system.

However, given the large spatial separation between the A- and B-branch accessory BCLs and bacterioopheophytins, we can assume that parts of the spectrum of the full RC arise as combinations of the A-branch and B-branch excitations, respectively. To test this assumption, we constructed two further structural models, A_1 and B_1 , shown in Fig. 4(a), comprising P , B_A , and H_A for the A branch and P , B_B , and H_B for the B branch, respectively. We compare the excitation spectrum of the hexameric model with that of A_1 and B_1 , respectively, in Fig. 4(b). As expected, excitations associated with P appear in all three spectra, albeit at different energies (e.g., the first excitation and the charge-transfer states $P_A^-P_B^+$ and $P_A^+P_B^-$). On the other hand, excitations that are localized on the A- or B-branch can clearly be assigned to either A_1 or B_1 . In particular, in our calculation of the spectrum of A_1 , we find the charge-transfer state $B_A^-H_A^+$ at the same energy (~ 2.3 eV) as in our hexameric model. We can therefore study the effect of adding amino acids to the energy of this and other relevant charge transfer states using our structural models A_1 and B_1 as a starting point.

A comparison of the excitation energies of the relevant charge-transfer states in the A- and B-branch is shown in Fig. 4(c) and listed in Tables S9 and S10. The $B_A^-H_A^+$ state is significantly lower in energy than $B_B^-H_B^+$, in agreement with the experimentally observed directionality of charge-separation along the A-branch. By adding the coordinating histidines and leucines in model systems A_2 and B_2 , both states are redshifted by more than 100 meV. Charge-transfer states from P to B_B and P to B_A are significantly higher in energy, in particular after the addition of the coordinating histidines and leucines. Adding further amino-acid residues (listed in Table S11), in analogy with M_3 in Sec. III B, leads to a further redshift of $B_A^-H_A^+$, bringing this charge-transfer state within 25 meV of the coupled Q_x excitations (see Table S9). It is, therefore, likely that the addition of further parts of the protein environment in concert with thermally-activated molecular vibrations could lead to a mixing of this and other charge-transfer states and the delocalized coupled Q_x excitations. This is supported by earlier studies using polarizable continuum models, which suggest that differences in the dielectric

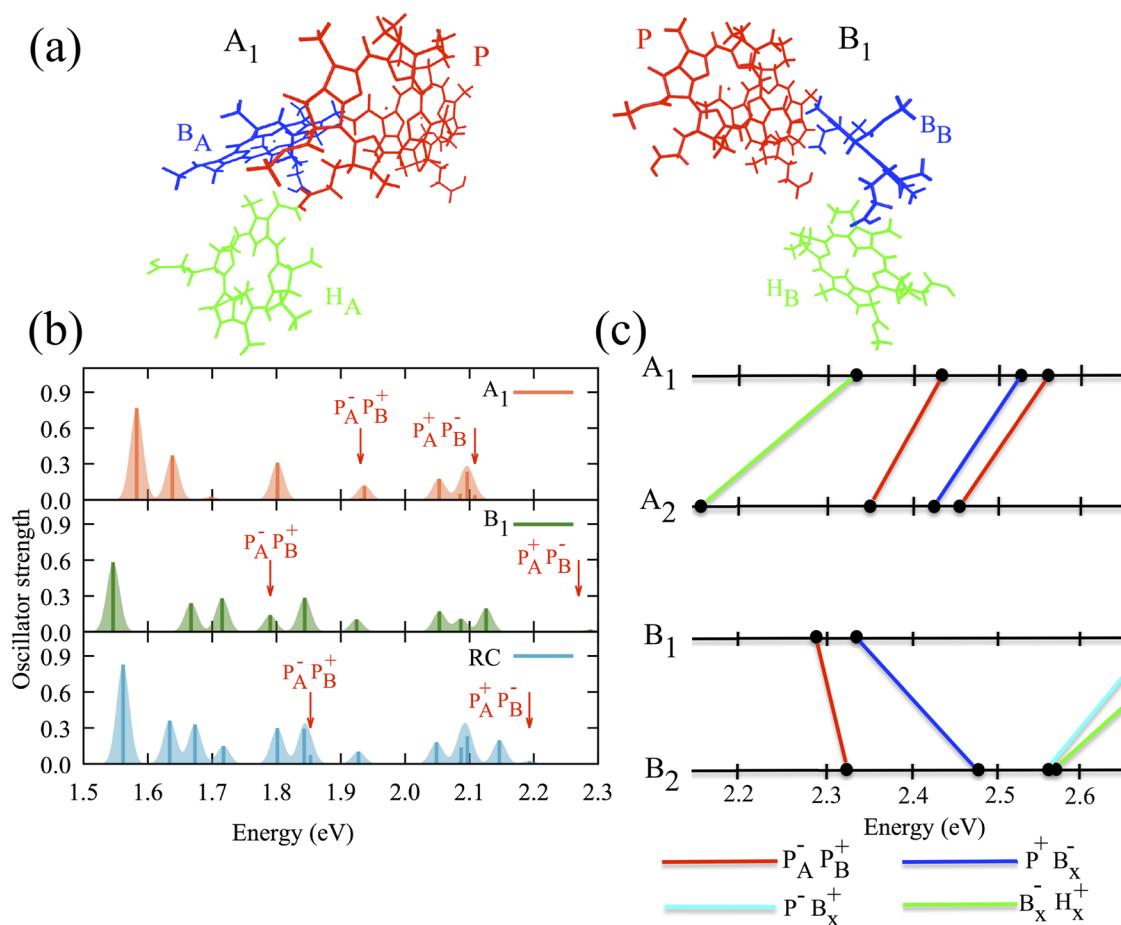


FIG. 4. (a) Structures of model systems A_1 ($P + B_A + H_A$) and B_1 ($P + B_B + H_B$). (b) Absorption spectra of A_1 , B_1 , and the full hexameric model system ($P + B_A + B_B + H_A + H_B$) in the energy region where coupled Q_y and Q_x excitations are expected. Red arrows mark excitations with charge-transfer character. (c) Energy of dark excitations (zero oscillator strength) in A_1 , B_1 and A_2 , B_2 . The latter correspond to A_1 and B_1 , respectively, but in addition include the coordinating histidines and leucines.

environment lead to a stabilization of charge-transfer excitations in the A branch in comparison with the B branch.³²

IV. SUMMARY AND CONCLUSIONS

Our first principles calculations show a pronounced effect of the protein environment on the electronic structure and excited states of the six primary pigments comprising the RC of *Rhodobacter sphaeroides*. By systematically adding relevant amino acids in the vicinity of these chromophores, we find a significant redshift of the coupled Q_y and Q_x excitations. Charge-transfer excitations are observed in the form of dark excitations starting at ~200 meV above the coupled Q_x excitations. These charge-transfer states are strongly affected by direct inclusion of the protein environment with energy changes of up to ~0.2 eV. However, contrary to the coupled Q_y and Q_x excitations, the protein environment affects charge-transfer states of different characters differently. In particular, the lowest-energy charge transfer state in our calculations corresponds to $B_A^- H_A^+$ and is significantly lower in energy than other excitations that move charge into the A branch. It is also almost 500 meV lower than an equivalent excitation in the B-branch. The $B_A^- H_A^+$ excitation is redshifted by the inclusion of close-lying amino-acid residues and can mix with the coupled Q_x excitations.

Our calculations suggest that charge-transfer along the RC A branch of *Rhodobacter sphaeroides* is energetically favored and demonstrate the complex excited state landscape of the RC's chromophores. Analyzing the transition and differences densities of the excited states allows for several conclusions. While most of the Q-band excitations can be understood as a consequence of the coupling of Q_y and Q_x excitations of the special pair BCLs P and the accessory BCLs B_A and B_B , the close spatial proximity of these molecules leads to strong coupling, mixing in (partial) charge-transfer states of the type $P_A^- P_B^+$ at relatively low energies. Furthermore, only the first high-oscillator strength excitation of the spectrum can be interpreted as a coupled Q_y excitation of the special pair. All other excitations are strongly delocalized, some of them with significant transition density on all six primary pigments. The presence of strongly delocalized excited states corresponding to both energy- and charge-transfer excitations is relevant because delocalized excitations are expected to be more strongly affected by thermally activated molecular vibrations than localized excitations, as shown previously by Alvertis *et al.* for the oligo-acene series.⁷¹ In our study, we have approximately shown this effect by calculating the exciton renormalization energies of tetrameric model structures distorted along vibrational normal modes. Based on our own calculations and previous literature,^{30,31,34,35} we expect yet more pronounced effects for larger structural models that also include the vibronic coupling to the protein environment.

Finally, our calculations allow us to comment on the interpretation of experimental spectroscopy of pigment-protein complexes like the bacterial RC. Our results suggest that because of the delocalized nature of energy- and charge-transfer excitations in these systems, the assignment of spectroscopic features to linear combinations of localized excitations on individual pigments is not always justified. Care should be taken when modeling the strongly coupled pigments of the bacterial RC in terms of their constituting elements.

SUPPLEMENTARY MATERIAL

See the supplementary material for additional convergence data, excitation energies, difference densities, and transition densities not shown in the main text, a discussion of the spectral origin of the coupled Q_y and Q_x excitations, details on the QM/MM calculations, results for vibrationally excited structures, and structure files.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Sabrina Volpert: Data curation (lead); Formal analysis (lead); Investigation (lead); Visualization (lead); Writing – review & editing (equal). **Zohreh Hashemi:** Data curation (equal); Formal analysis (equal); Investigation (equal); Visualization (equal); Writing – review & editing (equal). **Johannes M. Foerster:** Investigation (supporting); Validation (supporting); Writing – review & editing (equal). **Mario R. G. Marques:** Investigation (supporting); Writing – review & editing (equal). **Ingo Schelter:** Methodology (supporting); Software (supporting); Validation (supporting); Writing – review & editing (equal). **Stephan Kümmel:** Conceptualization (supporting); Formal analysis (supporting); Supervision (supporting); Writing – review & editing (equal). **Linn Leppert:** Conceptualization (lead); Funding acquisition (lead); Project administration (lead); Resources (lead); Supervision (lead); Writing – original draft (lead).

DATA AVAILABILITY

The data that support the findings of this study are available within the article and its supplementary material.

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