Probing structure–function relationships in early events in photosynthesis using a chimeric photocplex

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The native core light-harvesting complex (LH1) from the thermophilic purple photosynthetic bacterium Thermochromatium tepidum requires Ca2+ for its thermal stability and characteristic absorption maximum at 915 nm. To explore the role of specific amino acid residues of the LH1 polypeptides in Ca-binding behavior, we constructed a genetic system for heterologously expressing the Tch. tepidum LH1 complex in an engineered Rhodobacter sphaeroides mutant strain. This system contained a chimeric pufBALK gene cluster (pufBA from Tch. tepidum and pufLM from Rba. sphaeroides) and was subsequently deployed for introducing site-directed mutations on the LH1 polypeptides. All mutant strains were capable of phototrophic (anoxic/ light) growth. The heterologously expressed Tch. tepidum wild-type LH1 complex was isolated in a reaction center (RC)-associated form and displayed the characteristic absorption properties of this thermophilic phototroph. Spheroidene (the major carotenoid in Rba. sphaeroides) was incorporated into the Tch. tepidum LH1 complex in place of its native spirilloxanthins with one carotenoid molecule present per αβ-subunit. The hybrid LH1-RC complexes expressed in Rba. sphaeroides were characterized using absorption, fluorescence excitation, and resonance Raman spectroscopy. Site-specific mutagenesis combined with spectroscopic measurements revealed that α-D49, β-L46, and a deletion at position 43 of the α-polypeptide play critical roles in Ca binding in the Tch. tepidum LH1 complex; in contrast, α-N50 does not participate in Ca2+ coordination. These findings build on recent structural data obtained from a high-resolution crystallographic structure of the membrane integrated Tch. tepidum LH1-RC complex and have unambiguously identified the location of Ca2+ within this key antenna complex.


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Significance

Phototrophic bacteria have provided fundamental insight into the biological mechanism of solar energy conversion. Early events in photosynthesis are carried out by the antenna apparatus for light-harvesting (LH) and the reaction center (RC) for charge separation. Here we describe a system for expressing a chimeric LH1-RC complex from two phylogenetically distant photosynthetic purple bacteria: the LH1 from Thermochromatium tepidum and the RC from Rhodobacter sphaeroides. This system is exploited to definitively localize Ca2+ within the Tch. tepidum LH1 complex. The hybrid photocplexes also provide powerful new tools for probing photosynthetic energy transfer, identifying intrinsic LH1–RC interactions, monitoring altered behavior of carotenoids in a nonnative environment, and linking specific amino acid residues to the specific spectroscopic properties of different phototrophic organisms.

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To positively identify the $\text{Ca}^{2+}$ coordinating residues and to reveal their contributions to the LH1-$Q_y$ shift, we constructed a genetic system for heterologously expressing the Tch. tepidum LH1 complex in a deletion strain of Rhodobacter sphaeroides, a phylogenetically distant relative of Tch. tepidum that is readily amenable to genetic manipulation. By exploiting this system, we functionally expressed the Tch. tepidum LH1 complex in Rba. sphaeroides despite the two phototrophs sharing $<$40% sequence identity in their LH1 $\alpha$- and $\beta$-polypeptides. In addition, the carotenoid composition of Tch. tepidum LH1 was replaced with that of Rba. sphaeroides. Site-directed mutants obtained from these constructions were then used to rigorously characterize the Tch. tepidum LH1 complex and unequivocally identify how $\text{Ca}^{2+}$, a cation that influences both the thermal stability and unique spectral properties of Tch. tepidum LH1 (8, 11), is positioned within the precise structure of this important photocatalyst. Although this system is shown to verify the Ca-binding site within the precise structure of this important photocatalyst, the hybrid photocatalysts also provide powerful new research tools for probing photosynthetic energy transfer and quinone transport pathways, identifying intrinsic LH1–RC interactions, monitoring altered behavior of carotenoid molecules in a nonnative environment, linking specific amino acid residues to the specific spectroscopic properties of different phototrophic organisms, and advancing artificial photosynthesis.

Results

Construction of a Heterologous Expression System for Synthesizing the Tch. tepidum LH1 Complex and Production of Site-Directed Mutants.

A mutant of Rba. sphaeroides strain IL106 lacking the RC, LH1, and LH2 complexes (designated strain DPP1) was used to construct a genetic system for synthesizing the Tch. tepidum LH1 polypeptides (12) (SI Appendix, Materials and Methods). The constructed strain (designated strain TS2) contains a chimeric pufBALM gene cluster with pufBAL4 from Tch. tepidum and pufLM from Rba. sphaeroides. Using this strain, site-specific mutations in Tch. tepidum LH1 were designed with the following rationale. An alanine residue was inserted at position 43 of the Tch. tepidum $\alpha$-polypeptide (SI Appendix, Fig. S1), designated $\alpha$-ins43A, to mimic the sequence of the LH1 complex ($Q_y$ at 889 nm) of Allochromatium vinosum, a mesophilic relative of Tch. tepidum, and to examine the effect of this deletion on the LH1–$Q_y$ transition. The amino acids $\alpha$-Asp49, $\alpha$-Asn50, and $\beta$-Leu46 in Tch. tepidum LH1 were replaced by alanines (designated $\alpha$-D49A, $\alpha$-N50A, and $\beta$-L46A, respectively), because these residues had been tentatively assigned as Ca-coordinating ligands in the LH1-RC crystal structure (10). Finally, to specifically assess the role of the C-terminal residue of the $\beta$-polypeptide in $\text{Ca}^{2+}$ binding, a deletion mutant of the $\beta$-Leu46, designated $\beta$-L46del, was prepared.

Expression and Properties of the Chimeric LH1-RC Complexes. All Rba. sphaeroides strains harboring the chimeric pufBALM gene clusters grew phototrophically at virtually similar growth rates with significantly different LH1 polypeptides and spectroscopic properties from those of the native Rba. sphaeroides LH1. It also implies that the LH1-$Q_y$ position is primarily defined by the structures of the $\alpha$- and $\beta$-polypeptides.

The LH1 polypeptides in strains TS2 and $\alpha$-N50A were well assembled with BChl $a$, whereas mutant strains $\alpha$-43insA, $\alpha$-D49A, $\beta$-L46A, and $\beta$-L46del contained variable levels of BChl $a$ that were not properly incorporated within the LH1 polypeptides, as shown by their absorption characteristics between 750 and 800 nm (SI Appendix, Fig. S3). There was a tendency for strains expressing LH1 complexes with $Q_y$ bands more blue-shifted from that of the native Tch. tepidum LH1 to have higher proportions of misassembled complexes; this implies that the site-specific mutations that induce larger structural changes have more detrimental effects on pigment assembly. However, most of the misassembled components could be removed from the crude ICMS by mild detergent-treatment (Fig. 1). Unless stated otherwise, the detergent-treated ICMS of $\alpha$-43insA, $\alpha$-D49A, $\beta$-L46A, and $\beta$-L46del were used in subsequent measurements.

Introduction of different site-specific mutations into the Tch. tepidum LH1 polypeptides resulted in LH1 complexes with differing $Q_y$ transitions (Fig. 1). The LH1 complex of strain $\alpha$-N50A showed a $Q_y$ at 915 nm, the same as that of the native Tch. tepidum LH1. This indicates that the asparagine residue, the 50th residue from the N terminus in the $\alpha$-polypeptide (Asn50), does not participate in Ca binding in the Tch. tepidum LH1 complex, in agreement with preliminary results from a 1.9-Å structure of the Tch. tepidum LH1-RC (14). In contrast, the LH1 from strain $\alpha$-43insA, which contains an Alc. vinosum-like $\alpha$-polypeptide sequence (15), exhibited a blue-shifted $Q_y$ band at 899 nm. Comparison of this with the native Alc. vinosum LH1-$Q_y$ (889 nm) (16) suggests that the deletion at position 43 in the Tch. tepidum $\alpha$-polypeptide contributes significantly to the $Q_y$ red shift. Mutation of the $\alpha$-Asp49 to Ala (strain $\alpha$-D49A) resulted in a blue-shifted $Q_y$ band at 892 nm. This result agrees with the assignment of this residue as a Ca-binding ligand in the crystal structure (10), and indicates that this residue also plays an
important role in maintaining the red-shifted $Q_y$ of the *Tch. tepidum* LH1 complex. Both deletion and substitution of the $\beta$-Leu46 residue resulted in blue-shifted $Q_y$ transitions, highlighting the importance of this C-terminal residue. In fact, the $\beta$-L46A substitution yielded the most blue-shifted $Q_y$ transition of all mutations introduced in this study (Fig. 1).

Spectroscopic Characterization of the Strain TS2 Chimeric LH1-RC Complex. The LH1 complex from strain TS2 (Fig. 1) was sufficiently stable to be solubilized in an LH1-RC form and was obtained at high purity. Fig. 2 compares the spectra of purified LH1-RC complexes from strains TS2, DP2 (an LH2-deficient *Rba. sphaeroides* strain; SI Appendix, Materials and Methods), and native *Tch. tepidum*. Typically, the chimeric LH1-RC from strain TS2 displayed an LH1-$Q_y$ band in the range of 915–917 nm. A remarkable feature is the incorporation of spheroidene (the major carotenoid in *Rba. sphaeroides*) into the LH1 complex of strain TS2 in place of spirilloxanthin, which is present in the native *Tch. tepidum* LH1 complex (13). Notably, however, fewer spheroidenes per LH1 complex were incorporated than in wild-type *Rba. sphaeroides* LH1. Quantitative carotenoid analyses revealed the presence of $17.6 \pm 1.0$ carotenoid molecules per LH1-RC of strain TS2, indicating that each LH1 $\alpha\beta$ pair incorporated only one carotenoid molecule. This contrasts with the two carotenoid molecules per $\alpha\beta$ pair in the LH1 complex from strain DP2, the same as in wild-type *Rba. sphaeroides* (17).

Table 1 shows the carotenoid composition of purified LH1-RC complexes from the *Rba. sphaeroides* expression system. Spheroidene was the major component in all complexes, although there were variations in the composition of spheroidene derivatives among the complexes. The spheroidene-dominant carotenoids in the LH1 complex of strain TS2 exhibited absorption maxima at 443, 470, and 503 nm, slightly blue-shifted from those of the carotenoids in the LH1 complex of strain DP2 (447, 472, and 505 nm). This may reflect a difference in the protein environment between the two LH1 complexes (18).

The existence of Ca-binding sites in the LH1 from strain TS2 was probed using EDTA titration experiments. Fig. 2, Inset shows a typical example in which on addition of EDTA, the LH1-$Q_y$ band blue-shifted from 915 nm to 895 nm and displayed a broadened band shape. The absorption maximum then red-shifted back to 912 nm following the addition of excess CaCl$_2$. These data provide additional evidence that the Ca-binding sites in *Tch. tepidum* LH1 are also present in the heterologously expressed LH1 complex.

Fig. 3 compares the steady-state fluorescence excitation spectrum of the purified LH1-RC complex from strain TS2 with the spectra from strain DP2 and from *Tch. tepidum*. The overall efficiency of excitation energy transfer from the nonnative carotenoids to BChl $a$ in the LH1 complex from strain TS2 was calculated as 44%. This value is significantly lower than that of the native *Rba. sphaeroides* LH1 complex from strain DP2 (71%) despite a similar carotenoid composition, but is much higher than that of the spirilloxanthin-containing *Tch. tepidum* LH1 complex (23%). This result suggests that energy transfer efficiency may be affected not only by the conjugation length of the carotenoid, but also by the protein environment, as well as the gap between the absorption maxima of the carotenoid and BChl $a$ molecules and the number of carotenoids present in the LH1 complex. The efficiencies for the LH1 complexes from strain DP2 and native *Tch. tepidum* are compatible with those reported for other spheroidene- and spirilloxanthin-containing LH1 complexes, respectively (19–22).

The characteristics of chimeric and native LH1-RC complexes were further examined by resonance Raman spectroscopy. On excitation at 532 nm in the absorption region of carotenoids, the LH1 from strain TS2 yielded a spectrum essentially identical to that from strain DP2 (Fig. 4B), a spectrum characteristic of all-trans spheroidene. The intense bands at 1,518 cm$^{-1}$ and 1,154 cm$^{-1}$, assigned to the $\nu_{C=C}$ and $\nu_{C=C/6CH}$ modes of all-trans spheroidene, respectively (23), were shifted by 16 cm$^{-1}$ and 8 cm$^{-1}$ from those of all-trans spirilloxanthin in *Tch. tepidum* LH1 (24) due to the relatively shorter CC bond lengths. The intense peak at 1,518 cm$^{-1}$ in strain TS2 LH1 contained a shoulder around 1,505 cm$^{-1}$, which became more apparent or split into two peaks in the spectra of mutant LH1 complexes (Fig. 4).

Interactions between BChl $a$ and LH1 polypeptides were investigated by near-infrared resonance Raman spectroscopy (Fig. 5). The C3-acetyl C=O stretching band of the TS2 LH1 at 1,640 cm$^{-1}$ was up-shifted by 3 cm$^{-1}$ from that of native *Tch. tepidum* LH1 (24) but down-shifted 7 cm$^{-1}$ from that of strain DP2 LH1. This result indicates that the interaction between the C3-acetyl group of BChl $a$ and the LH1 polypeptides in strain TS2 is slightly weaker than that in the native *Tch. tepidum* LH1 complex but is stronger than that in the wild-type *Rba. sphaeroides* LH1 complex. As for the BChl $a$ C=O stretching band at 1,676 cm$^{-1}$ for strain TS2 LH1, this is close to that of wild-type *Tch. tepidum* LH1, but significantly blue-shifted by 10 cm$^{-1}$ from that of strain DP2 LH1. These data suggest a very weak (if any) hydrogen-bonding interaction between the C13-keto group and the LH1 polypeptides of strain TS2, whereas such hydrogen bonds likely form in the strain DP2 LH1 complex (25).

Characterization of Site-Specific Mutants of *Tch. tepidum* LH1 Complexes. Because spheroidene was incorporated into the chimeric LH1-RC complexes as the major carotenoid, the resonance
Raman spectra excited at 532 nm for the mutants were basically identical to those of wild-type Rba. sphaeroides LH1-RC. However, distinct differences were observed for the LH1 mutants in the C=C stretching region (Fig. 4), in which the intense bands around 1,518 cm\(^{-1}\) split into two bands, one around 1,505−1,508 cm\(^{-1}\) and the other around 1,516−1,519 cm\(^{-1}\). The relative intensity of the former to the latter increased in the order of α-N50A > α-D49A > α-N50A > α-ins43A > TS2. These results likely reflect a distorted conformation of the spheroidene molecules from a planar geometry along the C=C backbone in the mutant LH1 complexes (26). If this is so, then such distortion could influence the properties of BCHl \(\alpha\) in the LH1 complex and can be detected by resonance Raman spectroscopy with excitation at 1,064 nm. The results presented in Fig. 5 show that α-N50A with its LH1-\(Q_y\) band at 915 nm gave a C3-acetyl C=O stretching band at 1,640 cm\(^{-1}\), whereas this band shifted to 1,642 cm\(^{-1}\) for α-ins43A and β-L46del and to 1,647 cm\(^{-1}\) for α-D49A.

A linear relationship exists between a downshift in the C3-acetyl stretching mode, which is sensitive to hydrogen bonding, and a red shift in the LH \(Q_y\) maximum (25, 27) (SI Appendix, Fig. S4). This indicates that hydrogen-bonding interactions indeed occur in the LH1 complexes of the chimeric mutants. Moreover, the ratio of the splits in the carotenoid \(\nu\)C=C bands (∼1,505 cm\(^{-1}\)−1,519 cm\(^{-1}\)) also reveal a correlation with the LH1-\(Q_y\) red shift. This finding indicates that conformational changes of carotenoid molecules have little if any effect on LH1 absorption properties, but do influence the LH1-\(Q_y\) transition energy.

**Discussion**

The *Tch. tepidum* LH1 complex has been successfully expressed in *Rba. sphaeroides* mutant strains. In addition to their use in probing the coordination and function of Ca in *Tch. tepidum* LH1, these strains are valuable new tools for exploring several key primary processes in photosynthesis in greater detail. These include, but are not limited to (i) producing various hybrid LH1-RC complexes with vastly different biochemical and spectroscopic properties; (ii) investigating the intrinsic nature of the LH1–RC interactions; and (iii) verifying roles of specific amino acids in energy transfer-coupled electron/proton transport processes. Because the LH1 complex surrounds the RC, it serves as both the entrance route of excitation energy to the RC and the exit route of reduced ubiquinones from the RC to the quinone pool and cytochrome bc\(_1\) complex (2). Therefore, the chimeric LH1-RC complexes constructed herein also provide excellent subjects for probing the dynamic processes of energy and electron transfers in photosynthesis by time-resolved spectroscopy.

At present, no genetic manipulation or expression system is available for *Tch. tepidum*, while several heterologous expression systems exist in other species and have been used to study various functions of the bacterial photosynthetic apparatus (28–33). However, our construction differs from other chimeric LH1-RCs in at least three major ways: (i) the complex contains components from distantly related purple bacteria, the purple sulfur bacterium *Tch. tepidum* (γ-Proteobacteria) and the purple nonsulfur bacterium *Rba. sphaeroides* (α-Proteobacteria); (ii) the LH1 transferred to *Rba. sphaeroides* differs significantly in primary structure from its native LH1 complex (37% sequence identity for both α- and β-polypeptides); and (iii) the \(Q_y\) transition of the chimeric LH1 absorbs light of significantly longer wavelength (225–230 nm) as compared to its native counterpart (180–210 nm) (22). Therefore, *Tch. tepidum* LH1-RC complexes are great tools for exploring various aspects of photosynthesis.
BChl results show that the hydrogen-bonding interactions between the sequences (strain TS2) was virtually identical to that seen in the heterologously expressed LH1 complex containing wild-type in photosynthesis. The PufX polypeptide in *Rba. sphaeroides* is known to have important roles in supporting photosynthetic growth, structural organization of the LH1-RC core complex, and cyclic electron transfer (34). In this work, the *Rba. sphaeroides* pufX gene was present and unaltered in the mutant derivatives (SI Appendix, Fig. S5); however, its expression product could not be detected in the chimeric LH1-RC complexes. While we are still attempting to detect PufX in the chimeric complexes, our results thus far suggest that the PufX polypeptide, if expressed, is not required for (or is unable to promote) assembly of the *Tch. tepidum* LH1 in *Rba. sphaeroides*, and thus is not incorporated in the LH1-RC complex.

Site-specific mutagenesis of this study provided an ideal tool for clarifying uncertainties in the assignments of the crystallographic electron density map for the Ca-binding residues in the *Tch. tepidum* LH1 complex. Both α-Asp49 and α-Asn50 were tentatively assigned as the Ca-binding residues in the 3.0-Å structure (10). Mutation of the α-Asp49 to Ala resulted in a blue shift of the LH1-Q transition to 892 nm, whereas mutation of the α-Asn50 to Ala did not alter the Q position. These results are completely consistent with recent findings from both high-resolution Ca-bound (14) and Sr(Ba)-substituted *Tch. tepidum* LH1-RC structures (35) that point to α-Asp49 as a key metal-binding residue; in contrast, it is now clear that α-Asn50 does not participate in Ca coordination. In addition, our results for the two β-L46 mutants highlight the importance and sensitivity of this C-terminal residue. Substitution of the C-terminal β-Leu46 residue resulted in a blue-shifted LH1-Q transition (Fig. 1). This result would be difficult to interpret if the C-terminal carboxyl group serves as a ligand to Ca, as has been tentatively assigned in the 3.0-Å structure (10). Recently, it has become clear from a high-resolution structure of the *Tch. tepidum* LH1-RC (14) that β-Leu46 is not directly involved in Ca binding, but does participate in an extensive hydrogen-bonding network with its neighboring β-polypeptide and water molecules around the C-terminal end. Deletion of β-Leu46 also resulted in a blue-shifted LH1-Q band to 897 nm, which can be attributed to a disruption of the hydrogen-bonding network and a conformational change in the C-terminal region of the β-polypeptide.

All of the modified LH1 complexes produced in this study supported anaerobic, phototrophic growth of *Rba. sphaeroides*, and similar growth rates as seen in strain DP2 were observed for all mutant strains. These findings indicate that light energy absorbed by the modified LH1 complexes is indeed transferred to the RC for charge separation, even though these are energetically “uphill” processes (~490 cm⁻¹) occurring within a structurally nonnative LH1-RC complex. Phototrophic growth also demonstrates that the electron and proton transports necessary to support photophosphorylation are operational in each mutant derivative. Thus, in addition to the applications listed previously, the chimeric photocomplexes described here make available a powerful platform for experimentally dissecting the mechanism of uphill energy transfer and related early events in photosynthesis.

Fig. 5. Near-infrared resonance Raman spectra with excitation at 1.064 nm for the purified LH1-RC complexes from *Tch. tepidum* (A) and strain DP2 (B), and ICM from strains TS2 (C), α-43insA (D), α-N50A (E), α-D49A (F), and β-L46del (G).

The major difference between the native and heterologously expressed *Tch. tepidum* LH1 complexes was their carotenoid compositions. As spheroidene was biosynthetically incorporated into the expressed LH1 complexes in place of the native spheroidene and/or spirilloxanthin, our heterologous LH1 expression system could also serve as a powerful tool for probing the behavior of different carotenoids in a nonnative environment, as evident from the resonance Raman results (Fig. 4). Splitting of the vC=C bands in the 1,500–1,520 cm⁻¹ region were observed for carotenoids in the expressed LH1 complexes, indicating either that some of the spheroidenes in the expressed LH1 complexes differ slightly in their conformations from those in the native *Rba. sphaeroides* LH1 complex or that minor carotenoids such as demethyl-spheroidene and/or spirilloxanthin may contribute to the low-λ ω transition of LH1-RC complexes. While we are still attempting to detect PufX in the chimeric complexes, our results thus far suggest that the PufX polypeptide, if expressed, is not required for (or is unable to promote) assembly of the *Tch. tepidum* LH1 in *Rba. sphaeroides*, and thus is not incorporated in the LH1-RC complex.

The characteristic feature of the LH1-Q transition in native *Tch. tepidum* was reproduced in the heterologously expressed LH1 complex containing wild-type sequences, indicating that the spectroscopic properties of BCHl α molecules in LH1 complexes are ultimately determined by the primary structures of α- and β-polypeptides. As revealed by resonance Raman spectroscopy (Fig. 5), the interaction between BCHl α and LH1 polypeptides in the heterologously expressed LH1 complex containing wild-type sequences (strain TS2) was virtually identical to that seen in the native *Tch. tepidum* LH1 complex. The near-infrared Raman results show that the hydrogen-bonding interactions between the BCHl α C3-acetyl group and LH1 polypeptides in the native *Tch. tepidum* (24) are also major factors regulating the Q transition of the expressed LH1 complexes.

A final benefit of our use of site-directed mutagenesis in this study is that it allowed direct verification of a long-standing hypothesis on the effect of a deletion in the *Tch. tepidum* LH1 α-polypeptide (8, 9, 11). Despite the high degree of sequence identities between *Tch. tepidum* and *Alc. vinosum* LH1 polypeptides (SI Appendix, Fig. S1), a deletion is present at position 43 in the *Tch. tepidum* LH1 α-polypeptide where an Ala exists in the corresponding *Alc. vinosum* LH1 polypeptide (15). Insertion of an Ala residue into the *Tch. tepidum* LH1 α-polypeptide at this position (strain α-43insA) resulted in a blue shift of its LH1-Q band to 899 nm (Fig. 1). This indicates that the inserted Ala in the *Tch. tepidum* LH1 α-polypeptide prevents formation of a proper Ca-binding pocket and subsequent incorporation of Ca into the complex; this is not required in the mesophilic *Alc.*
**Materials and Methods**

Construction of a Rba. sphaeroides mutant lacking genes encoding the LH1-RC (puf/BALM) and LH2 (pufBA) polypeptides (designated DPP1) was performed using Rba. sphaeroides strain IL106 (12). A mutant lacking only LH2, designated DPP2, was generated as well. The chimeric puf operon consisting of Tch. tepidum pufBA and Rba. sphaeroides pufLM was cloned with a suicide vector pIP5603 and introduced into the Rba. sphaeroides mutant strain DPP1 by conjugal transfer from *Escherichia coli* SM17-tpi-1. The mutant recovering phototrophic growth by incorporation of the chimeric puf construct to the genomic DNA through a homologous recombination was named TS2. Site-specific mutagenesis and other genetic manipulations on the Tch. tepidum LH1 polypeptides were performed as described in **SI Appendix, Materials and Methods**. All expression strains were grown under phototrophic (anoxic/white light) conditions and, for certain experiments, with 850-nm LED illumination. The LH1-RC complexes from strains DPP2, TS2, and α-N50A were purified by solubilizing the IC with 0.23% lauryldimethylamine N-oxide in 20 mM Tris-HCl (pH 8.5), followed by anion-exchange chromatography (Toyopearl 6505; TOSOH) with 0.1% n-dodecyl β-maltopyranoside at 4 °C. Details of sample preparation and spectroscopic measurements are provided in **SI Appendix, Materials and Methods**.

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