Three-dimensional Reconstruction of a Membrane-bending Complex

THE RC-LH1-PufX CORE DIMER OF RHODOBACTER SPAEHOIDES

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A three-dimensional model of the dimeric reaction center-light harvesting I-PufX (RC-LH1-PufX) complex from Rhodobacter sphaeroides, calculated from electron microscope single particle analysis of negatively stained complexes, shows that the two halves of the dimer molecule incline toward each other on the periplasmic side, creating a remarkable V-shaped structure. The distribution of negative stain is consistent with loose packing of the LH1 ring near the 14th LH1 α/β pair, which could facilitate the migration of quinone and quinol molecules across the LH1 boundary. The three-dimensional model encloses a space near the reaction center QB site and the 14th LH1 α/β pair, which is ~20 Å in diameter, sufficient to sequester a quinone pool. Helical arrays of dimers were used to construct a three-dimensional membrane model, which matches the packing lattice deduced from electron microscope analysis of the tubular dimer-only membranes found in mutants of Rba. sphaeroides lacking the LH2 complex. The intrinsic curvature of the dimer explains the shape and ~70-nm diameter of these membrane tubules, and at least partially accounts for the spherical membrane invaginations found in wild-type Rba. sphaeroides. A model of dimer aggregation and membrane curvature in these spherical membrane invaginations is presented.

Photosynthetic bacteria provide an ideal system for investigating the conversion of light energy into chemical energy in nature. Only three different protein complexes are required for this conversion; the first is a so-called “core” complex composed of light-harvesting I (LH1) and reaction center (RC) complexes. Light energy absorbed by LH1 is transferred to the RC, where photochemical charge separation takes place (1, 2). This charge separation induces electron transfer coupled to production and migration of quinols to the cytochrome bc1 complex (3, 4). Pumping of protons across the membrane forms an electrochemical potential, which drives various cellular processes including synthesis of ATP (5). Most of the purple bacteria contain a second light-harvesting complex, LH2; each subunit of LH2 consists of two integral transmembrane polypeptides called α and β, which bind the bacteriochlorophyll (BChl) and carotenoid pigment molecules (6). The bacterial photosynthetic membrane is composed of closely packed arrays of LH2 and RC-LH1 complexes; atomic force microscopy (AFM) of membranes from several photosynthetic bacteria has revealed the organization of these arrays (7, 8). As a result of structural, functional, and AFM studies these are among the best characterized of any biological membrane. Recently, it has been possible to combine the data from structural and functional approaches to construct three-dimensional models of an entire membrane vesicle, comprising over a 100 complexes, at the atomic level (9).

Despite these advances, several important aspects of structure and function remain: it is not known how quinol molecules, the product of photochemistry, pass through the apparently closed LH1 rings that encircle RCs, nor how they migrate to the cytochrome bc1 complexes. Previous work has provided clues regarding the first of these issues, namely quinone/quinol exchange across the LH1 boundary. LH1, an oligomer of α/β transmembrane polypeptide pairs (10, 11), which can be reversibly dissociated into its αBChl, (B820) components (12), forms an encircling, ring-like structure with the RC in the center (13, 14). High resolution cryo-electron microscopy (cryo-EM) projection maps of the LH1-only complex reconstituted from B820 subunits from Rhodospirillum (Rsp.) rubrum, and of monomeric RC-LH1 core complexes both from wild and mutant type Rsp. rubrum, showed a closed LH1 ring consisting of 16 α/β subunits (11, 15, 16). Jamieson et al. (15) solved two different projection structures for RC-LH1 complexes of Rsp. rubrum; the circular and elliptical LH1 rings that enclose the RCs were suggested to reflect the flexibility of the LH1 complex, implying the possibility of quinol/quinone diffusion through the closed LH1 ring. Subsequent AFM studies of the RC-LH1 complex from Rsp. rubrum, as well as that from Blastochloris viridis, confirmed the flexibility of LH1 (17–19).

The core complex of Rhodobacter (Rba.) sphaeroides, the most intensively researched photosynthetic bacterium, is a dimer (8, 20–22). The presence of a small polypeptide, PufX, is required for photosynthetic growth (23), and it plays a key role in the formation of the (RC-LH1-PufX)2 dimeric core complex (24–26). A 8.5-Å resolution cryo-EM projection map clearly showed that 28 α/β LH1 subunits associate with two RCs to form a (RC-LH1-PufX)2 complex (22). A strong density within this map, indicating a transmembrane helix, was considered to be a good candidate for the location of PufX although an alter-
native location at the interface between the two halves of the dimer has also been proposed (7, 21, 27).

The observation that membranes composed nearly entirely of RC-LH1-PufX dimers form elongated tubular structures (28–30), with a degree of order sufficient to enable calculation of a low resolution projection map of the complex in its native membrane (20, 26, 30), allows this dimer structure to be used as a exemplar for induced membrane curvature by an intrinsic membrane protein. It is not known how intrinsic membrane proteins impose curvature on the membrane in which they sit, although several fascinating examples have emerged (31–33). To establish a link between membrane protein structure and membrane curvature, it is necessary to obtain a three-dimensional model of the dimer core complex, and to reconcile this information with the structural parameters of the native tubular membrane. In this work, we performed single particle analysis of negatively stained RC-LH1-PufX dimers and obtained a three-dimensional model at 25-Å resolution, the structure and shape of which reveal the basis for its capacity to impose membrane curvature, and enable the construction of a tubular membrane from its dimer building blocks. This membrane, which exhibits the same packing and geometry as the tubular membrane isolated from an LH2-minus mutant of Rba. sphaeroides, also explains in part the curvature of wild-type photosynthetic membrane. In this work, we performed single particle analysis of negatively stained RC-LH1-PufX dimers and obtained a three-dimensional model at 25-Å resolution, the structure and shape of which reveal the basis for its capacity to impose membrane curvature, and enable the construction of a tubular membrane from its dimer building blocks. This membrane, which exhibits the same packing and geometry as the tubular membrane isolated from an LH2-minus mutant of Rba. sphaeroides, also explains in part the curvature of wild-type photosynthetic membrane.

**Experimental Procedures**

**Cell Culture and Purification of the RC-LH1-PufX Dimer—**

An LH2\(^{-}\) mutant strain of Rba. sphaeroides (DD13/DG2 [pRKEH]) (34) was cultured in M22 medium anaerobically at 34 \(^{\circ}\)C for 5 days. Preparation, and solubilization of membrane, and purification of the dimer complex were followed according to the method of Qian et al. (22). The quality of the final purification of the dimer core complex was judged by the \(A_{280}^{\text{nm}}/A_{280}^{\text{nm}}\) absorbance ratio; fractions with an absorbance ratio greater than 1.9 were pooled. The purity of the sample was checked using SDS-PAGE and electron microscopy (EM). The purified dimer sample was stored at \(-80^{\circ}\)C before use.

**Isolation of Tubular Membranes—**

Isolation of the tubular membrane followed the method described previously (26) with modifications. Briefly, washed cells were passed through a French press twice under a pressure of 16,000 p.s.i. Unbroken cells were removed by centrifugation for 30 min at 15,000 \(x\) g. The supernatant was loaded on a three-step sucrose gradient (20/30/40% sucrose in buffer solution) and centrifuged for 4 h at 96,000 \(x\) g. A band rich in tubular membranes was collected at the interface between 30 and 40% sucrose. It was used for EM observation directly without further purification.

**Two-dimensional Crystallization—**

Protein concentration for two-dimensional crystallization was adjusted to 0.5 mg/ml. It was mixed with 4 mg/ml extract of Escherichia coli lipids (Avanti Polar Lipids) solution to a lipid/protein ratio from 0.3 to 0.6 (w/w). Each 100-\(\mu l\) aliquot of these mixtures was dialyzed against buffer solution (150 mm NaCl, 20 mm HEPES, 0.01% Na\(_2\)N, pH 7.5) at 20 \(^{\circ}\)C for 10 days using a homemade continuous flow device (35).

**Electron Microscopy—**

Isolated native tubular membranes, reconstituted two-dimensional crystals, and dimeric RC-LH1-PufX samples were used for electron microscopy data collection. Samples were applied to glow discharged, carbon-coated copper grids (Agar Scientific), blotted, stained with 0.75% (w/v) uranyl formate, blotted, and dried in air. Electron micrographs were recorded on a Philips CM100 electron microscope fitted with 1K \(\times\) 1K Gatan Multiscan 794 CCD camera at \(\times28,500\) magnification for native tubular membranes and \(\times61,000\) for purified RC-LH1-PufX. The magnification was calibrated from an image of negatively stained catalase crystals (Agar Scientific).

**Image Processing—**

Images of isolated native tubular membranes and two-dimensional crystals were processed using the MRC suite of programs (36) and DigitalMicrograph (Gatan) as described (15, 26).

For analysis of the single particles we used the IMAGIC-5 software package (Image Science Software GmbH) (37, 38). In total, 4852 single molecule projections were boxed from electron micrographs using a box size of 100 \(\times\) 100 pixels (1 pixel represents 3.92 Å) using the program XIMDISP (36). All single particle images were aligned directly to a total average after normalization and band pass filtering. Cycles of multivariate statistical analysis were then performed (39). A few easily interpretable classes were selected for multireference alignment calculation and further classification (40, 41). An initial three-dimensional reference model of the dimeric RC-LH1-PufX complex was produced by the use of three easily interpretable classes, viz. a projection parallel to the membrane plane (“side view”), a projection perpendicular to the periplasmic face (“top view”), and a projection perpendicular to the cytoplasmic face (“bottom view”). The Euler angles of these three views were defined as (0, 90, 0), (0, 0, 0), and (0, 180, 0), respectively. Re-projections from this reference three-dimensional model were used for Euler angle assignment of all averaged classes (42). The initial three-dimensional reconstruction incorporating all class averages was then calculated from the classes that together represented about 94% of the whole data set. The three-dimensional reconstruction was refined by further cycles of re-projection, multivariate statistical analysis, and multireference alignment until no further improvements could be seen.

**Orientation Fitting of the RCs in the Dimer—**

The atomic resolution model of the RC from Rba. sphaeroides (43) was used for the calculation of RC orientation in the dimeric RC-LH1-PufX complex. The approximate orientation in the X-Y plane of the dimer molecule was as determined by Qian et al. (22). On the Z-X plane, the RC orientation was fitted against the side view of the three-dimensional reconstruction model of the dimer by rotating the RC around its \(x\) axis. Projection maps were calculated for various angles of tilt of the RC out of the membrane plane and compared qualitatively with the experimentally determined projection map of Ref. 22 using the CCP4 program suite (44).

**Results**

Purification of the RC-LH1-PufX Dimer and Native Tubular Membranes—

Coomassie-stained SDS-PAGE of the purified dimer demonstrated only six bands; three from the RC L-, M-,
and H-subunits, two from LH1 $\alpha$ and $\beta$, and a poorly stained one from PufX (see supplementary data). The purity of the preparation was further confirmed by EM; electron micrographs of diluted, negatively stained complexes showed that the majority of the particles are dimers. The scale bar represents 50 nm. B, some of the selected single particles used for image processing. Each panel is 39.2 nm square.

FIGURE 1. Single particles of the dimeric RC-LH1-PufX complex. A, negatively stained electron microscopy of purified RC-LH1-PufX complexes shows that the majority of the particles are dimers. The scale bar represents 50 nm. B, some of the selected single particles used for image processing. Each panel is 39.2 nm square.

The tubular membrane fraction harvested from the sucrose step gradient is a mixture of tubular membranes and normal smaller, curved membrane fragments. The average width of the isolated tubular membrane when flattened on the carbon support film of the EM grid was 113 nm.

Three-dimensional Model of the RC-LH1-PufX Dimer—The 25 averaged classes used to construct a low resolution three-dimensional model of the dimer core complex are displayed in Fig. 2A. With reference to the cryo-EM projection map of the dimer (22), class 25, for example, can be identified as a side view, looking parallel to the membrane plane, class 1 as a view from the cytoplasmic side looking perpendicular to the membrane plane, and class 2 as a view from the periplasmic side. To con-

FIGURE 2. Classification of single particles of the dimeric RC-LH1-PufX complex. A, 25 averaged classes of the particles used for three-dimensional reconstitution. All classified images are aligned along the $y$ axis. B, reprojection images from the final three-dimensional model of the RC-LH1-PufX complex. Each reprojection image has a counterpart in the image classes and the Euler angles in $A$. The size of each box is $39.2 \times 39.2$ nm.
firm the consistency of the reconstruction with the original averaged data, re-projection images were produced from the final three-dimensional dimer model (Fig. 2B). All re-projections are consistent with the original averaged classes with correlation coefficients between 0.90 and 0.98.

The final three-dimensional model (Figs. 3, A–F) reveals some of the notable structural characteristics of this dimer molecule. The two central densities that protrude from the surrounding LH1 complexes can be assigned to RCs, based upon the previous assignment of the corresponding densities in the cryo-EM projection map (22). The threshold for surface rendering was chosen so as to form a tight fit round the RC, which has a molecular mass of 104 kDa; the dimer complex is predicted to be 487 kDa. This has several important consequences for the shape of the LH1 ring; although most of the wall of LH1 αβ subunits has a constant height of ~7.4 nm, corresponding to the second 12th αβ subunits assigned previously (22), there is enough contrast to distinguish a weaker region of density for the LH1 ring, in a region corresponding to the 14th αβ subunit. This region of density, indicated by a red arrow in Fig. 3, A–C, corresponds to the “gap” identified in earlier projection images of negatively stained membranes (20, 26).

A clear 2-fold symmetry axis relates the two halves of the dimer. The side view of this dimer clearly shows that the two halves of RC-LH1-PufX are inclined toward each other at an angle of ~146°, forming a distinct “V” shape. The height of the dimer measured from the cytoplasmic side to the periplasmic side is 9.5 nm. The length of 20.4 nm and width of 12.8 nm are consistent with the result from the projection structure determined by cryo-EM (22). Finally, the structures in Fig. 3, A and C, show that there is a “hole” in the density map, adjacent to the RC, and separated from the exterior by that part of the LH1 boundary denoted by the arrow.

Fig. 4A shows a superposition of the low resolution three-dimensional structure from the current single particle analysis.
Three-dimensional Reconstruction of the Core Dimer Complex

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FIGURE 5. Negative stain EM of reconstituted and native tubes composed of RC-LH1-PufX dimers. A, negatively stained two-dimensional crystals, following dialysis of purified dimers, flattened on a carbon film coated on an EM grid. The red lines denoted ag, gf, and fe indicate a single helical curve of dimeric molecules aligned through contacts made between long dimer axes. The red dashed line represents the curve at the back of the tube. 2πc is the vertical separation, i.e., the pitch of the helical curve; w is the width of the flattened tube; and α represents the gradient of the helical curve against horizontal line ab. The size of the green square is 80 × 80 nm. The inset is the gray level projection map of the flattened tube. Dimer molecules are arranged in p22,2, symmetry. One unit cell is outlined, a = 130.3 Å, b = 407.3 Å, γ = 90°. The rows of dimers have alternating topologies, i.e., periplasmic face uppermost, then cytoplasmic face uppermost. B, negative stain EM of a native tubular membrane flattened on a carbon support film. The longer red line indicates the direction of the helix, in which the dimer molecules are arranged, as in A, with contacts made between long axes. This helix has a longer pitch. The red dotted line represents the shorter pitch helix, in which molecules make contact between short dimer axes. The size of the green rectangle, as in A, is 80 × 80 nm. The inset is the projection map from the flattened native tubular membrane. The dimer molecules in the native tubular membrane all have the same topology, i.e., the cytoplasmic face of the complex is uppermost. The molecular arrangement obeys p2 symmetry with a = 125.0, b = 202.0, γ = 77.0°. The red arrows indicate the unit cell.

and the projection map calculated from frozen two-dimensional crystals (22), which gives a good qualitative match of the positions of the transmembrane helices with the three-dimensional model. Closer inspection of this superposition shows that the weaker region of LH1 density in the three-dimensional model, identified by the red arrows in Fig. 3, A–C, and also in Fig. 4A, corresponds to the αβ14 pair in the projection map. With the hindsight of this tilted dimer structure, it is likely that in the cryo-EM analysis of two-dimensional crystals (22), the relatively strong densities for the αβ subunits at the central interface region of the dimer reflect a perpendicular orientation to the support carbon film on the grid, whereas the weaker densities at the outer edges of the LH1 ring in the cryo-EM projection map are consistent with tilted LH1 helices. The side view of the V-shaped three-dimensional model (Fig. 4B) shows the extent of this tilt; here, two atomic resolution RC models (43) are shown fitted into the EM three-dimensional model; it is clear that an optimal fit must include a tilt of the RC. To provide a rough test of this tilt, a projection map of the tilted RC in Fig. 4B was calculated and then superimposed (in red) on the actual projection of the RC obtained in the cryo-EM projection map (22). The calculated projection map of a non-tilted RC gives a poor match with the observed density (results not shown). In contrast, the good qualitative agreement between the model and experimental projections shows that the projection in Ref. 22 is entirely consistent with the RC being tilted relative to the direction of view down the dimer axis. In particular, the strong density of the single transmembrane helix of the RC H-subunit in the cryo-EM map is now explained; the tilting of the whole complex orients this helix so it becomes more perpendicular to the support carbon film (see left-hand RC, blue helix, in Fig. 4B).

Calculation of Structural Parameters for Tubular Two-dimensional Crystals and Native Membranes, each Composed of Dimeric RC-LH1-PufX Complexes—When purified dimeric RC-LH1-PufX molecules are mixed with lipids in buffer solution and dialyzed against detergent-free buffer solution they can form an artificial tubular membrane. When flattened on a carbon film support, such tubes form two crystalline layers, each composed of a single molecular layer of dimer core complexes, which can be treated as two-dimensional crystals for EM structural analysis (22). It is straightforward to observe the arrangement of dimer molecules in the flattened tubule using negative stain. The projection map of the two-dimensional crystal showed that the dimeric molecules are assembled with a p22,2, symmetry (see Fig. 5A) (22). The dimer molecules make contact so as to stack in a direction parallel to their short axis, in a direction indicated by the shorter of the two red arrows in the inset to Fig. 5A, to form a linear array of dimers that follows the red line in the view of the flattened crystal in the main part of Fig. 5A. The dimer molecules in neighboring arrays take an up-down-up orientation (see Fig. 5A, inset), which results in alternating darker/lighter stripes of negative stain in the crystal. Given that the assembly of dimeric RC-LH1-PufX forms a perfect helical curve, prior to flattening on the EM grid, it can be described using the following parametric equation,

\[
\begin{align*}
& x = r \cos(t) \\
& y = r \sin(t) \\
& z = ct
\end{align*}
\]  

(Eq. 1)

where \( r \) is the radius of the helix, \( t \) is the angle the point \((x, y, z)\) makes with the \( x \) axis (projected to the \( xy \) plane), and \( 2\pi c \)
The Biological Significance of the Bent Dimer Structure and Its Contribution to Membrane Curvature—Membrane curvature is clearly an advantage in that it allows an increase in effective surface area for light gathering. The membranes of wild-type *Rba. sphaeroides* cells demonstrate this very well, with the cytoplasmic membrane invaginating to form numerous tightly curved spherical buds (45). Curved membranes are found in many types of cells and in the interaction of animal viruses with host cells; they facilitate motility, communication between intracellular compartments, cell division, vesicle trafficking, and viral membrane fusion (46). Membrane curvature can be affected in a variety of ways, such as through lipid composition, extramembraneous cytoskeletal or scaffold proteins interacting with the membrane, the shallow insertion of small amphipathic domains into the membrane, and lipid-protein interactions (47–51). In the case of membranes containing tightly packed integral proteins, the shape of these proteins can also exert a strong effect, for example, the nicotinic acetylcholine receptor (52) and the voltage-dependent K⁺-channel (53). However, given the small number of known structures for this class of proteins, there are very few cases where the role of shape has been definitively described for a protein deeply embedded in the membrane. The dimeric RC-LH1-PufX complex adds to this small list of integral membrane proteins capable of curving membranes. The AFM topographs of native membranes (8) showed that core and LH2 membrane protein complexes partition into domains, a process recently shown to be driven by depletion-induced attractions of the kind predicted by colloid theory, which involve size asymmetry of the core and LH2 complexes, as well as curvature mismatch between the interacting faces of adjacent complexes (54). The striking “bent” conformation of the core dimer is very likely a major driver of the formation of banded, near-spherical intracytoplasmic membranes in *Rba. sphaeroides* (Fig. 6, C and D), although LH2 complexes must also contribute to this, because membranes composed of only LH2 complexes are spherical (28). A linear dichroism study of the organization of complexes in intact wild-type membrane vesicles concluded that rows of just a few dimers

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**TABLE 1**

| Parameter                        | Reconstituted tube (n = 18) | Native tube (n = 22) |
|----------------------------------|----------------------------|---------------------|
| r (radius)                       | 361 nm                     | 36 nm               |
| 2π (helical pitch)               | 2π × 416 nm (2612 nm)      | 2π × 144 nm (902 nm) |
| s (arc length of one helical repeat) | 3459 nm                   | 929 nm             |
| n (molecules in one helical repeat) | ∼265                      | ∼73                |
| τ (angular separation of molecules) | 1.36°                      | 4.92°              |

is the pitch of the helix (Fig. 5A). The arc length is given by Equation 2.

\[ s = \sqrt{r^2 + c^2}t \]  

(Eq. 2)

Because 4 = w/π and c = r tan(α), where w is the width of the tubule flattened on the EM grid and α is the gradient of the helix, the arc length of one helical repeat is given.

\[ s = \frac{(w/\pi)^2}{(1 + \tan^2(\alpha))} \cdot 2\pi \]  

(Eq. 3)

With the unit cell parameters, \( a = 13.1 \text{ nm} \), \( b = 41.0 \text{ nm} \), \( \gamma = 90^\circ \) determined previously (22) the total number of molecules in one helical repeat can be calculated as \( n = s/a, \) and the angular separation of each molecule in the helix, therefore, is \( \tau = 360/n. \)

The same analysis can be applied to determining the helical structural parameters for the native tubular membrane (Fig. 5B). A previous analysis of purified native tubular membranes from *Rba. sphaeroides* showed that it contains only dimeric RC-LH1-PufX complexes (26); other proteins, if present, were below the limit of detection for SDS-PAGE. Here, to simplify the calculation, we assume that the native tubular membrane consists only of RC-LH1-PufX dimers. Analysis by EM of negatively stained samples showed that the arrangement of dimers that comprises the tubular membrane follows p2 symmetry with lattice parameters of \( a = 12.5 \text{ nm}, \) \( b = 20.2 \text{ nm,} \) \( \gamma = 77.0^\circ. \) All dimer molecules are assumed to have the same orientation with the periplasmic side facing the inside of the tube (see Fig. 5B, inset). The width of the flattened native tubule is 113.3 nm (\( n = 22 \)), and the gradient of the single helical curve is 82.0°. With these parameters, the coordinates of each dimer in the tube can be obtained. Therefore, the three-dimensional structure of the tubular membrane can be constructed.

Table 1 shows the results of these calculations. Under our experimental conditions, the diameter of isolated native (i.e. not flattened) membrane tubule is ∼72 nm, which is only one-tenth the diameter of the tubular two-dimensional crystal reconstituted from purified dimers. In terms of the cross-sectional area, the reconstituted tubular crystal is about 100 times larger than the native tube. Approximately 265 RC-LH1-PufX dimer molecules, aligned so their long axes make contact with one another (in the direction indicated by the short red arrows in insets to Fig. 5), are needed to complete one circle (360°) in the reconstituted tubular crystal but only ∼73 dimer molecules are needed in the native tubular membrane. These two arrangements reflect the fact that in these two different tubes each molecule is rotated by a different angle against the next one, and they are a consequence of a slight twist along the dimer array of 1.36° in the two-dimensional crystal, but 4.92° in the native tubule.

The final three-dimensional model of RC-LH1-PufX dimers in the native tubular membrane is depicted in Fig. 6. It clearly shows that dimeric core complexes in the native tubular membrane can conveniently be described as forming two different helices. The helix formed by dimers stacking in a direction perpendicular to their long axes, shown in green in Fig. 6A, has the longer pitch. Thirteen such helices form a complete membrane tube. The short pitch helix (Fig. 6B), consists of eight repeats of the helix along the z axis, which passes along the length of the tube, in the center. The curvature of this membrane, however, arises from only one feature of the dimer, namely the 146° angle made as the two halves of the dimer incline toward each other. Thus, in other contexts, such as membranes that also contain LH2 complexes, this feature of the dimer will still drive membrane curvature, but with a different overall architecture (Fig. 6, C and D).

**DISCUSSION**

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were capped by LH2-rich domains (55). The model in Fig. 6, A and D, shows that LH2 complexes separate rows of core dimers, as well as forming more extensive LH2-only domains. Both types of LH2 array were observed in AFM topographs of wild-type membranes (8).

Siebert et al. (26) showed that a mutant of Rba. sphaeroides lacking LH2 was capable of assembling long tubular membranes consisting entirely of RC-LH1-PufX dimers. Interestingly, in a mutant also lacking PufX, no such tubes were assembled; instead, large areas of membrane formed, composed exclusively of monomeric hexagonally packed RC-LH1 complexes. These membranes showed only a very gentle curvature compared with tubes, further suggesting that dimerization is essential for tight membrane bending. Structural analysis of the quasi-crystalline dimer-only membrane tubes revealed the packing of the dimers in the lattice (26).

The present work allows us to reconcile this known packing with the inherent curvature and size of the three-dimensional dimer structure. Modeling of a membrane tubule in Fig. 6 shows that we can explain the shape, diameter, and packing of tubular membranes of Rba. sphaeroides using only the three-dimensional dimer structure. Remarkably, the $\sim 146^\circ$ angle inherent to the complex leads directly to a tube of $\sim 70$ nm diameter, if we assume little or no relative tilt between LH1 helices in nearest-neighbor complexes within the lattice. AFM topographs of a native (core dimers plus LH2 complexes) photosynthetic membrane from Rba. sphaeroides (8) allowed visualization of individual molecules of the RC-LH1-PufX dimers. The membrane patches used for AFM were flattened onto mica for imaging, and the AFM topographs clearly show no evidence of a V shape for the dimer.

Thus, the molecule must be flexible at the interface between the two halves of the dimer. Circumstantial evidence for such flexibility, and even lability, in this region of the complex is also provided by the preparation of homogeneous populations of monomer core complexes, using detergents that differ from the $\beta$-dodecyl maltoside used in this study. In this view, monomers could arise from a symmetrical splitting of the dimer into two monomers as in Ref. 56.

Comparison of the Reconstruction with Previous Analyses—The current single particle analysis provides the first three-dimensional view of the RC-LH1-PufX dimer and in doing so it clarifies one issue, namely that of the gap in the LH1 ring. The projection map of the dimer calculated from negatively stained tubular membranes from Rba. sphaeroides revealed two RCs, each surrounded by an LH1 ring, forming an apparent "S"-shaped molecule leaving each ring slightly open. The existence of this gap, and its width, led to the conclusion that each LH1 ring consists of $\sim 12$ $\alpha\beta$ subunits (20). The same conclusions...
were drawn in a negative stain EM and AFM study of two-dimensional crystals of this complex (21). Subsequently, the 8.5-Å resolution cryo-EM projection map revealed that each ring was more likely to have at least 14 LH1 αβ units, albeit with a more diffuse density associated with the putative 14th LH1 αβ pair (22); pigment analysis of the purified dimeric RC-LH1-PufX complex supported this conclusion. It was clear from the 8.5-Å resolution map that the apparent gap does not form a well-defined break in the LH1 ring density, rather an area of poorly defined, possibly mobile, density more accessible to the negative stain. Indeed, the level of staining is dependent on the concentration of uranyl formate used. 0.75% Uranyl formate produced an apparent gap of ~43 nm as reported in Ref. 26, whereas 0.35% uranyl formate reduces this gap to ~35 nm (see supplementary data). With further reduction of the stain level to 0.04%, this gap approaches ~27 nm, a value also obtained from unstained (cryo-EM) analysis of two-dimensional crystals. Thus the apparent gap can appear to differ in size by the equivalent of at least a whole αβ dimer, depending on the staining level.

With the more uniform staining of the entire molecule in the present analysis, the LH1 appears as a complete ring. Nevertheless, more stain does penetrate near the αβ14 pair, as indicated by the arrows in Figs. 3 and 4A, reflecting increased mobility and flexibility of the transmembrane helices in this part of the ring, and more penetration of the negative stain on the periplasmic side of the membrane. Superposition of the cryo-EM projection map and the three-dimensional structure in Fig. 4A clearly shows the correlation between more diffuse density in the cryo-EM map, and the thinner (i.e. more heavily stained) part of the LH1 ring.

Another three-dimensional view of the LH1αβ polypeptide pairs in the dimer molecule is provided in Fig. 7; the αβ14 pair is differentiated from the others (red) by coloring in blue. Both the cryo-EM two-dimensional projection map used to position the helices and the three-dimensional reconstitution model, used to provide the mesh that defines the overall shape of the dimer, reflect the averaged results of thousands of flexible molecules. This explains why the density of the αβ14 pair is relatively weak, and also why the distance of β14 to its neighbor, β2, on the other half of the dimer, is relatively large (27.5 Å) in comparison with the average β-β distance of 22.0 Å. We suggest that this looser packing and flexibility of the transmembrane helices could aid the shuttling of the quinone molecules across the LH1 ring.

A Possible Vestibule for Accommodation of a Captive Quinone Pool within the Core Dimer—The RCs have provided three essential internal checks for our cryo-EM and single particle analyses. First, it was possible to establish the orientation of this complex in the horizontal plane, and therefore the location of the RC Q8 site (22). Second, in the present study (Fig. 4C), the tilt of the RC in the vertical plane is consistent with the RC projection density in Ref. 22. Third, the threshold value set for the three-dimensional RC-LH1-PufX model is based on the known size and shape of the enclosed RCs. This last constraint on the threshold reveals a space within the LH1 ring, colored yellow in Fig. 7A and near the LH1 αβ14 subunit colored in blue. The favored position of PufX assigned in Ref. 22, but still subject to some debate, is indicated by the single helix colored in magenta. A space-filled yellow RC Q8 molecule is also shown. The proximity of RC Q8, PufX, the LH1 αβ14 pair, and this apparent space is intriguing, especially in view of recent studies of quinones and lipids bound to this complex. The presence of a tightly bound quinone pool retained by purified dimers has been demonstrated by a number of workers (57−59). A stoichiometry of 10−15 ubiquinone molecules and 80−90 phosho-
lipid molecules per RC has been measured for purified, detergent-washed core dimer complexes (59). The three-dimensional model of the dimer shows that there is space between the inner face of the LH1 ring and outer surface of the RC, which could accommodate lipids. An area of ~250 Å² is required to accommodate ~10 quinone molecules within each half of the core complex, and lipid molecules are also likely to be present in this region, because they form a flexible environment for quinone movement (60). Each of the spaces identified in yellow in Fig. 7A is roughly equivalent to a circular area diameter of ~20 Å. This space appears to have been penetrated by stain, which may reflect the more open and mobile nature of the quinone/lipid environment compared with the dense hydrophobic stain-excluding interior of the protein. The ability of stain to penetrate lipidic areas has been noted previously (e.g. Ref. 61). If this really is a holding area or vestibule for quinones, it is a good strategic location, because it is adjacent to both the RC Q₉ site and the loose region of the LH1 ring, where the flexibility of the αβ transmembrane helices could facilitate the exit and ingress of quinol/quinone molecules. Clearly, higher resolution three-dimensional structural data are required to examine this point.

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2 The abbreviations used are: LH, light harvesting; RC, reaction center; AFM, atomic force microscopy; EM, electron microscopy.
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