P
hotosynthesis, the main source of energy for the biosphere (1, 2), is initiated when the thousands of pigments that cooperate to form an interconnected photosynthetic unit (PSU) harvest and transfer solar energy before its conversion to a charge separation. Peripheral pigment–protein complexes deliver energy to a reaction center (RC) (3–6), where it is used for ATP synthesis. The structures of the key protein complexes involved in this process have been solved both for oxygenic photosynthetic organisms, such as cyanobacteria, algae, and plants (9–14), and for anoxygenic photosynthetic bacteria (15–24). This recent progress makes it possible to study the biophysical processes involved in photosynthesis in atomic detail all the way down to the quantum mechanical level (25–31). However, it remains a challenge to understand how a biological membrane comprising hundreds of photosynthetic complexes functions with great efficiency. To address this challenge we embarked on the in silico construction of an entire photosynthetic membrane, namely the purple bacterial PSU (32), based on a combination of cryo-EM (24, 33–35), NMR (22), x-ray crystallography (18, 19, 23, 36), and atomic force microscopy (AFM) (37–41) data.

The purple bacterial PSU displays remarkable simplicity compared with its eukaryotic, oxygenic analogues, being evolutionarily more primitive (42). It contains six different kinds of proteins that work cooperatively: LH2 antenna complexes (18, 19, 23) capture photons and transfer the resulting electronic excitation to another antenna complex, LH1, and finally to a RC (22, 36, 40, 43, 44). Subsequently, the RC initiates transmembrane electron transfer to the small, membrane-diffusible electron carrier quinone, reducing it to hydroquinone.

In *Rhodobacter sphaeroides*, LH1 and RC, together with the small polypeptide PufX, form dimeric supercomplexes as seen in negative-stain EM of intact membranes and in cryo-EM projection maps of purified complexes (24, 33, 35, 39, 45). The hydroquinones released from the RC diffuse through the cell membrane to the ubiquinol–cytochrome *c*~2~ oxidoreductase (*bc*~1~ complex), which oxidizes hydroquinone by transferring the electrons to the cytochrome *c*~2~ complex and at the same time pumps protons across the membrane. The resulting proton gradient is finally used by ATP synthase (46–48) for the synthesis of ATP. Cytochrome *c*~2~ shuttles the electrons back to the RC, thus resetting the system. All aforementioned components are embedded in the cell membrane, with the exception of cytochrome *c*~2~.

EM studies revealed that the membrane-embedded PSUs form intracytoplasmic protrusions. The assembly of intracytoplasmic membranes is a complex process, initiated by the clustering of the LH2 and RC–LH1–PufX pigment–protein complexes, which has been studied with radiolabeling, biochemical, and spectroscopic techniques (49–51). In *R. sphaeroides* and some other bacteria a combination of aggregation of the protein complexes and lipid biosynthesis induces membrane curvature, thus causing the budding or invagination to form the intracytoplasmic membrane (49, 52, 53).

Spectroscopic data on membrane vesicles suggested the existence of quantified energy transfer domains comprising ∼3,000 bacteriochlorophyll (BChl) molecules surrounding and interconnecting ∼30 RCs (50). Modeling the collective behavior of the PSU requires knowledge of the relative stoichiometry and spatial distribution of photosynthetic complexes. Recently, the supramolecular organization of the PSU of several purple bacteria has been revealed through AFM (37, 38, 41) and linear dichroism (LD) (39) studies. The AFM data obtained for *R. sphaeroides* membranes (37) permits the construction of atomic models of the PSU needed for a detailed description of the successive processes of light absorption, excitation migration, electron transport, quinone diffusion, proton transport, and ATP synthesis.

**Results**

**Constituents and Overall Architecture of the PSU.** AFM images of *R. sphaeroides* membranes showed that the PSU consists of linear
arrays of RC–LH1–PufX dimers separated by domains of LH2 complexes (37). However, the AFM data alone do not permit an unambiguous three-dimensional reconstruction of intact chromatophore vesicles because the vesicles were flattened to form patches onto a mica surface. The reconstruction process therefore utilizes input from LD spectroscopy of native, intact membrane vesicles (39), revealing the orientation of the RC–LH1–PufX and LH2 complexes in the intact vesicle. This leads to a model in which linear assemblies of dimeric RC–LH1–PufX complexes lie along the long axis of the vesicle connecting its farthest extremity with the cytoplasmic membrane (39).

The bc1 complex and the ATP synthase have eluded imaging by AFM and still have to be assigned. A recent model of the *R. sphaeroides* vesicle, which considers the location and stoichiometries of electron transport components (54), concludes that as few as five bc1 complexes suffice to maintain adequate rates of ATP synthesis, but earlier studies suggest one bc1 complex per two RCs (55) and nine bc1 complexes per chromatophore (56).

About one ATP synthase is present per vesicle with some vesicles lacking this complex (57).

In light of the evidence described, we propose the following tentative picture for the architecture for an intracytoplasmic membrane (chromatophore) vesicle. The vesicle, connected at its “south pole” to the cell membrane, develops around linear arrays of RC–LH1–PufX dimers, oriented south–north consistent with ref. 39; LH2 complexes assemble around these arrays, consistent with the observed kinetics of LH2 incorporation (49, 52, 53), and mainly fill space in the equatorial and “northern” region. In mutants lacking LH2 continued formation of linear core dimer arrays results in the formation of elongated tubes (33, 35, 45). The proposed vesicle geometry is shown in Fig. 1.

Some bc1 complexes are proposed to populate the neck region of the vesicle, because it is known that a noninvaginated cytoplasmic membrane fraction can be purified from photosynthetically grown cells and that these membranes not only lack photosynthetic complexes, but are also enriched in respiratory components (58). However, other bc1 complexes may be located equatorially closer to the RC–LH1–PufX complexes. Because preparations of chromatophores do catalyze cyclic electron transport and ATP synthesis, bc1 complexes and ATP synthases must be present in at least some of the vesicles. Their schematic, peripheral placement in Fig. 1 reflects the lack of their direct detection by AFM. The in silico reconstruction of intracytoplasmic membrane vesicles presented herein is based on combining multiple small domains of AFM images consisting solely of RC–LH1–PufX dimers and LH2 complexes with the help of area-preserving maps between planar and spherical patches (see Fig. 1 d–f and Methods).

**Fig. 1.** Architecture and constituents of a spherical chromatophore vesicle from *R. sphaeroides* constructed from AFM/LD data (37, 39). (a) Light-harvesting complexes, LH2 (green) and LH1 (red), absorb light and transfer the resulting excitation to the RC (blue), which subsequently initiates electron transfers reducing quinone to hydroquinone (not shown); the bc1 complex (yellow) oxidizes hydroquinone to create a proton gradient across the membrane, which in turn is used by ATP synthase (orange) for ATP production. Electrons are shuttled back to the RC by cytochrome c2 (not shown). The current study focuses solely on the light-harvesting process within the vesicle, and accordingly, bc1 complexes and ATP synthase are not considered, being depicted schematically peripheral to the chromatophore, although other bc1 complexes may be located within the vesicle closer to the RC–LH1–PufX complexes. The ratio of surface area covered by RC–LH1 versus LH2 complexes is 1:1.31 for the first vesicle (shown) and 1:3.23 for the second vesicle (Fig. 2 d and f). (b) BCHls (represented by their porphyrin rings) of the atomic model for the RC–LH1 complex constructed for this study based on cryo-EM data (24). The PufX polypeptide is not included. (c) BCHls of the LH2 complex based on *R. acidophila* (23). AFM images (d) (37) are used to identify the arrangement of pigment–protein complexes within planar patches (e). An area-preserving map from the plane on to the sphere, the inverse-Mollweide projection (89) (Eq. 1), is then used to position pigment–protein complexes on the vesicle surface (f). To minimize distortions, multiple planar patches were used, whose sizes are small compared with the inner diameter of the reconstructed vesicle (60 nm). (a–c were made with the program VMD (Visual Molecular Dynamics) (90).)
olution data available, and from the monomeric RC–LH1 model derived from cryo-EM, NMR, and AFM data (22, 34, 40), but was not refined.

Several difficulties need to be faced before an atomic-level model for the vesicle can be constructed. For example, the preparation process distorts, stretches, and sometimes tears vesicle patches. Therefore, the original flattening process itself is not likely to be area preserving. If unaccounted for, such artificial stretching will reduce the pigment density and, as a result, the calculated overall quantum efficiency of the system.

Spherical vesicles were constructed by orienting planar strips containing the RC–LH1–PufX dimer arrays and their immediate LH2 neighbors along the north–south direction. The spaces between RC–LH1–PufX strips were filled with LH2-rich regions. An area-preserving map, the inverse-Mollweide transformation (see Methods, Eq. 1), was used to map planar regions onto spherical ones. Structural conflicts were subsequently removed and gaps that arose between the pigment–protein complexes as a result of the projection process were removed by manually shifting the center points of the proteins on the chromatophore sphere to achieve a tight packing. The “southern polar” region was left empty as a potential contact zone with the rest of the membrane. In this manner, two sample vesicles shown in Fig. 2 were constructed that have an inner diameter of 60 nm, chosen to be consistent with EM images. The first vesicle contains 18 dimeric RC–LH1 complexes and 101 LH2 complexes with a total of 3,879 BChls; the second vesicle contains nine dimeric RC–LH1 complexes and 144 LH2 complexes with a total of 4,464 BChls. The total number of RCs is chosen to be consistent with earlier estimates (50) and to correspond with moderate- and low-light growth conditions, respectively, for the two vesicles. The overall BChl:RC ratios for these vesicles of between 108 and 248 are also consistent with those obtained for wild-type chromatophores (59).

Fig. 2. Electronic interactions and excitation energy transfer across a chromatophore vesicle. BChls are represented by their porphyrin rings and colored as follows: blue, LH2 B800; green, B850; red, LH1 B875; purple, RC/accessory; orange, RC/special pair. (a) Electronic couplings (see text) between BChls of the reconstructed chromatophore vesicle. For the sake of clarity, only couplings > 3 cm⁻¹ are shown on a logarithmic scale. (b) The rate of excitation transfer (Eq. 2) between the BChl groups of the LH2 B850 ring (green) and the 5-shaped LH1 assembly (red), represented as bonds connecting the respective center of mass of each BChl group. For clarity, only strong connections are displayed on a logarithmic scale and the transfers involving other BChl groups, such as LH2 B800 BChls or the RC BChls, are not shown. (c) Excitation lifetime as a function of the initially excited BChl for the first vesicle (compare with a and b). (d) Excitation lifetime as a function of the initially excited BChl for the second, LH2-rich, vesicle. The cross-transfer probability between RCs, i.e., the probability that an excitation which has just been detrapped from a RC will be trapped at a given RC, is displayed in e and f for the first and second vesicles, respectively, for a detrapping event at the RC pair shown at the center. The probability is color-coded according to the color bar shown. Notably, excitation sharing between RCs arises mainly between adjacent RCs. The distribution of excitation lifetimes (compare with c and d) are shown in g and h for the two vesicles as a function of distance to the nearest RC (filled, B850 BChls; open, B800 BChls). The continuity of the distributions in g and h indicates that all BChl clusters are functionally connected. The distribution of lifetimes is reminiscent of random walks on graphs. [a and b were made with the program VMD (Visual Molecular Dynamics) (90).]
Excitation Migration over the Intracytoplasmic Membrane Vesicle.

Reconstruction of the intracytoplasmic membrane vesicles allows a detailed description of the excitation transfer within these massive pigment networks, which is achieved in an effective Hamiltonian formulation (28, 31, 60–68) (see Methods). The couplings between neighboring BCHls in the B850 ring of LH2 and the B875 “ring” of LH1 are strong enough for excitonic delocalization and subsequent spectroscopic shifts to play a prominent role in the excitation migration process. Indeed, a Markovian random walk of excitations localized on individual BCHls fails to give a satisfactory account of excitation migration in the pigment network and it is necessary to localize excitations on clusters of BCHls by using Eq. 2. Such a description reproduces observed excitation migration in small segments of the PSU (63–66) and in photosystem I in cyanobacteria and plants (29, 31, 68). An alternative formulation of excitation transfer processes, the modified Redfield theory is expected to yield a more accurate description, especially of strong coupling, short time scale events (69–71).

The BCHl clusters in LH2 consist of rings of 9 B800 BCHls and 18 B850 BCHls; RC–LH1 dimers consist of an S-shaped array of 56 B875 BCHls, and the RCs each have two special pair and two accessory BCHls. The effective Hamiltonian parameters describing the site energies and the coupling strengths of these BCHl clusters are based on earlier work conducted on smaller models of the PSU (27, 28, 63–66, 72). Specifically, the coupling between the special pair BCHls is assumed to be 500 cm$^{-1}$ according to refs. 72 and 73. The alternating nearest-neighbor couplings of the LH2 B850 ring are taken to be 363 cm$^{-1}$ and 320 cm$^{-1}$ (27, 28), whereas the alternating nearest-neighbor couplings of the LH1 B875 “S-band” are taken to be 300 cm$^{-1}$ and 233 cm$^{-1}$ (72, 74). All other couplings are computed in the dipolar approximation $v_{ij} = C(d_{ij}/r_{ij}^3) - 3(r_{ij}/d_{ij})(r_{ij}/d_{ij})/r_{ij}^2$ with a coupling constant $C$ of 348,000 Å$^2$ cm$^{-1}$ chosen to reproduce LH2 excitation spectra (28, 75). Inter- and intraprotein pigment couplings are computed according to identical formulæ. Couplings between the BCHls of the first vesicle are illustrated in Fig. 2a. Site energies of BCHls are based on earlier spectroscopic data (28, 66, 72–74, 76).

The excitation transfer times between neighboring BCHl clusters of B800, B850, and B875 BCHls in LH2 and LH1 are similar to those obtained in earlier studies (63–66, 72). Excitation flows quickly (<1 ps) from the B800 ring to the B850 ring in LH2 and is rapidly transferred to another LH2–B850 ring or to an LH1–B875 S-shaped assembly within ~10 ps, depending on the distance of the neighboring cluster. The cluster–cluster transfer rates computed based on Eq. 2 are shown in Fig. 2b.

Notable differences exist between the dimeric arrangement of the RC–LH1–PufX core complexes and the circular cores considered earlier (63–66, 72). First, for a circular LH1, most oscillator strength is accrued in the doubly degenerate second and third eigenstates as a result of symmetry (72), whereas for the S-shaped dimer, the degeneracies are broken, even though the third eigenstate still contributes the highest oscillator strength. The forward-transfer time (B875 $\rightarrow$ RC) is 20 ps, which is comparable to 15 ps computed in earlier studies (66, 72). These rates are largely consistent with the trapping lifetime of 37 ps of RC–LH1 complexes (77) that includes detrapping and retrapping events.

The back-transfer time (RC $\rightarrow$ B875) of the S-shaped complex computed with the aforementioned Hamiltonian parameters adopted from the circular RC–LH1 complex is too short (1.4 ps) compared with the empirically supported value of 8 ps (66, 72). This back-transfer time is sensitive to the energies and couplings of RC BCHls, which are impossible to determine accurately without a detailed atomic-level structure. Furthermore, the assumptions about Boltzmann equilibration and delocalization become increasingly more suspect as the size of the BCHl cluster increases and may not be the best suited tool for the description of LH1 excitons, which are likely to undergo a higher degree of thermal localization (65). Keeping these points in mind, we heuristically adopt a back-transfer time (RC $\rightarrow$ B875) of 8 ps, but consider also the effects of a 1.4-ps back-transfer time. The detrapping probability that corresponds to a back-transfer time of 8 ps is 27%, in agreement with the observed probability of ~20% (78–80). Direct transfer events between clusters that do not have any BCHls within 50 Å of each other are safely neglected. The transfer network thus constructed permits computation of excitation lifetime and quantum efficiency for each BCHl initially excited in either of the model vesicles (see Fig. 2).

The average excitation lifetime associated with the first vesicle is 50 ps corresponding to a high quantum yield of 95%. The second vesicle, which contains relatively more LH2 complexes, as seen in some AFM images, exhibits a longer lifetime, 162 ps, and a smaller quantum yield, 84%. The variant of the models with a 1.4-ps back-transfer rate results in lifetimes of 88 ps and 222 ps and quantum yields of 91% and 78%, respectively, for the two vesicle architectures. The increase in lifetime and the decrease in quantum yield for this variant arise from the increased prominence of detrapping events caused by faster back-transfer from the RC. The continuous distribution of lifetimes and BCHl–RC distances shown in Fig. 2 g and h indicates that none of the pigment clusters is functionally disconnected from the rest, indicating that each vesicle constitutes an efficient energy transfer network.

Multiple detrapping and subsequent retrapping events result in the excitation effectively being shared among neighboring RCs. Excitation sharing cannot stem from direct transfer between RCs, which is observed to be rather slow (250 ps) even for nearest neighbor RCs; instead, it would arise from back-transfer (detrapping) events followed by subsequent migration of excitation to a nearby RC. Excitation sharing in a network of RCs can be analyzed by the sojourn expansion (29, 31) that expresses the excitation transfer process in terms of an expansion of repeated detrapping events. In particular, the cross-transfer probability $Q_{jk}$ of an excitation that has just been detrapped from RC$k$ to be trapped at RC$j$ provides a measure of connectivity between RCs. These probabilities are shown in Fig. 2 e and f for the two constructed vesicles. The sum over all RCs, $\sum_j Q_{jk}$, is approximately equal to the quantum yield of the system. The probability of the excitation being detrapped from the RC shown at the center of the dimer array to be eventually trapped at that same RC is as low as 13% with the excitation being shared with the neighboring RCs within the same dimer cluster. As a result of the short transfer times between neighboring LH1 “rings” (as low as 4 ps), the neighboring linear clusters of dimers share only a very small portion of the detrapped excitation, as a comparison between the two vesicles shows.

Discussion

The in silico reconstruction of a chromatophore vesicle presented here elucidates efficient energy transfer and trapping at the scale of an entire PSU containing thousands of BCHls. Experimental data going back >50 years had shown the interconnected nature of energy transfer in bacterial photosynthetic membranes, with 20 or more reaction centers interconnected by many light-harvesting complexes, representing hundreds or even thousands of BCHl molecules (50, 81–84). The model presented here attempts to reconcile such data with the atomic-level structure of a whole membrane vesicle (chromatophore).

A remarkably short average excitation lifetime of 50 ps is computed across the entire vesicle corresponding to a quantum yield of 95%. This finding is in agreement with the observed excitation lifetime and trapping efficiency in purple bacterial antenna systems, typically of 60 ps and 95%, respectively (reviewed in ref. 25).
This high efficiency is achieved by more than simply a high pigment density, namely, by spectral tuning of the various pigment clusters in relation to one another. A significant degree of excitation sharing is observed between the RCs of a RC–LH1–PufX dimer array as a result of the close packing of the dimer clusters. This arrangement might have implications for maintaining a high quantum efficiency when one or more RCs in a linear array are in the closed state, the likelihood of which depends on the coupling of RC photochemistry to downstream processes such as quinol production and coupling to turnover at the bc1 complex. It is possible that the organization of LH2 and core complexes, which is now known to be highly variable (85), depends less on considerations of light harvesting than on coupling to the bc1 complex and coping with RCs in a closed state. This could dictate either the necessity for excitation sharing within rows of dimeric RC–LH1–PufX complexes, as seen in R. sphaeroides, or for the more diffuse arrangements of monomeric cores, each one surrounded by LH2, as visualized in AFM topographs of membranes from Rhodospirillum photometricum (86).

Reconstruction of the supramolecular organization of cellular domains is likely to become commonplace in connecting atomic-level structural information to cryo-EM cellular tomography data (87, 88). The PSU model constructed here ably explains light-harvesting kinetics and efficiency; it will also assist in describing subsequent photosynthetic processes, such as quinone diffusion coupling to the bc1 complex, generation of a proton gradient, and ATP synthesis. Eventually, it should be possible to integrate the present level of information into an integral model for PSU assembly.

Methods

An Area-Preserving Transformation Between Planar and Spherical Regions. We used the inverse-Mollweide projection (89) from geography to map planar regions to spherical patches. The Mollweide projection maps infinitesimal circles at the center of the transformed region onto circles without distortion. It is defined by the following mapping from a point (x, y) on the plane onto a point (φ, λ) on the sphere, where φ is the latitude and λ is the longitude:

\[
\phi = \sin^{-1}\left(\frac{2 \theta + \sin(2\theta)}{\pi}\right), \quad \lambda = \frac{\pi x / R}{2 \sqrt{2 \cos(\theta)}}.
\]

[1]

Here, we define \( \theta = \sin^{-1}(y/\sqrt{2R}) \); R is the radius of the target sphere.

The transformation (Eq. 1) was applied to determine the locations of the center points and the relative orientations of the proteins involved and not directly to the individual atomic coordinates. Thus, no artificial structural deformations of the constituent pigment–protein complexes are introduced. Many small planar patches were used to construct a spherical model, which was then manually cleaned of steric clashes and gaps (see Fig. 1 e and f).

Excitation Transfer Between Pigment Clusters. Excitation migration within the pigment network, because of resonant energy transfer (60, 61), was described through an effective Hamiltonian as applied earlier to smaller photosynthetic systems (28, 29, 31, 63–66, 68). For the sake of completeness, we provide a brief summary to this approach. The effective Hamiltonian is defined in terms of the site energies \( e_i \) and the electronic couplings \( v_{ij} \), which in the induced dipole-induced dipole approximation that applies typically for interpigment distances larger than 10 Å is given by

\[
\begin{align*}
\sum_{i \in D} |D^i \rangle \langle D^i| & = \sum_{i \in A} |A^i \rangle \langle A^i| + \sum_{i \in D, j \in A} |D^i \rangle \langle A^j| + |A^j \rangle \langle D^i| + \sum_{i \in D, j \in D} |D^i \rangle \langle D^j| + |D^j \rangle \langle D^i|,
\end{align*}
\]

[2]

\( D \) denotes the donor cluster and \( A \) the acceptor cluster; \( E^i_D \) and \( E^i_A \) are eigenvalues of the Hamiltonians \( H_D \) and \( H_A \) corresponding to \( D \) and \( A \), respectively. The integral represents the spectral overlap between donor \( S^i_D \) and acceptor \( S^i_A \) emission and absorption spectra, respectively. The coupling term \( U^i_{mn} \) in Eq. 2 between the \( m \)th excited state of \( D \) and the \( n \)th excited state of \( A \) is

\[
U^i_{mn} = \sum_{\alpha \in A} c^\alpha_i \sum_{\beta \in D} c^\beta_m H_{\alpha \beta},
\]

where \( H_{\alpha \beta} \) are matrix elements of the effective Hamiltonian that couple donor and acceptor pigments, \( c^\alpha_i \) and \( c^\beta_m \) being the respective eigenvector coefficients. The excitation lifetime \( \tau \) and quantum yield \( q \) of the system for the initial state \( |i \rangle \) can be expressed in terms of the transfer rates (Eq. 2) (29, 31, 66)

\[
\tau = \frac{1}{M} \sum_{i \in D} |K^i - |i \rangle, \quad q = \frac{1}{M} \sum_{i \in A} k_{CS}(RC|K^i - |i \rangle),
\]

[3]

where \( M \) is the number of pigment clusters \( (M = 256 \) and \( M = 315 \) for the first and second vesicle, respectively; \( B800 \) and \( B850 \) BChls are considered separate clusters). The constant \( k_{CS} = (3 \text{ ps}^{-1}) \) is the rate of electron transfer from the excited RC special pair of BChls. \( K \) denotes the rate matrix with elements \( K_{ij} = T_{ij} - \delta_{ij}(k_{CS} + k_{diss} + \Sigma_{i} \Delta t_{i}) \), where \( k_{diss} = (1 \text{ ns}^{-1}) \) is the dissipation rate in the system. The indices \( i,j \) run over pigment clusters and not individual pigments.

The sojourn expansion (29, 31) expresses excitation transfer processes in terms of repeated detrapping events from RCs followed by subsequent retrapping. In particular, the cross-transfer conditional probability between two RCs is given by

\[
Q^\lambda_{jk} = -k_{CS}(RC|K^i - |i \rangle)
\]

[4]

where \( T_{ik} \) is the state populated immediately after detrapping from \( RC_k \). The sum \( \Sigma_{i} Q^\lambda_{jk} \) equals the quantum yield corresponding to an initial state given by \( |T_{i} \rangle \).

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