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Self-Assembly of Photosynthetic Membranes

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Abstract

Bacterial photosynthetic membranes, also known as chromatophores, are tightly packed with integral membrane proteins that work together to carry out photosynthesis. Chromatophores display a wide range of cellular morphologies; spherical, tubular, and lamellar chromatophores have all been observed in different bacterial species, or with different protein constituents. Through recent computational modeling and simulation, it has been demonstrated that the light-harvesting complexes abundant in chromatophores induce local membrane curvatures via multiple mechanisms. These protein complexes assemble to generate a global curvature and sculpt the chromatophores into various cellular-scale architectures.

Introduction

Sunlight harvested via photosynthesis is the primary energy source of the biosphere. The multi-step process is carried out by a collection of proteins. In photosynthetic purple bacteria, the photosynthetic proteins aggregate and form distinct membrane indentations with a size scale of ~100 nm that can be observed under a light microscope. These membrane indentations, known as chromatophores, serve as the simplest prototype of a photosynthetic machinery, and have been studied intensely to characterize various aspects of photosynthesis. In this article we consider the overall architecture of chromatophores: how their distinct shapes arise and, in particular, how computational methods have helped to address this question. Studies on the formation of cellular-scale photosynthetic chromatophores coincide with a rising interest in the morphology of cellular membranes.[1, 2, 3, 4] The computational methods discussed here are not restricted to photosynthetic proteins and can be similarly applied to other membrane-bending molecular assemblies.[5, 6, 7, 8]

Chromatophores: the photosynthetic machineries

Using light and electron microscopy, microbiologists have long noticed the large protrusions populating photosynthetic bacterial membranes.[9, 10, 11, 12] The shape of these membrane protrusions were observed to vary significantly with the growth environment or upon the removal of certain proteins.[10, 11, 12, 13, 14] Later, through biochemical analyses and imaging techniques such as electron microscopy (EM) and atomic force microscopy (AFM), photosynthetic proteins were found to be the primary constituents of these membrane protrusions, termed chromatophores (for reviews see [15, 16]).

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In photosynthetic bacteria, light is first absorbed by peripheral light-harvesting (LH) complexes, which transfer the excitation energy to a reaction center (RC). The reaction center, together with another membrane protein, cytochrome bc_1, use the excitation energy to produce a transmembrane charge gradient, which is utilized by the protein complex, ATP synthase, to produce ATP. Each photosynthetic chromatophore is thought to be equipped with all these proteins at a certain stoichiometry. For example, in the case of *Rhodobacter* (*Rba.*) *sphaeroides*, on average, ten dimeric RC-light-harvesting complex I supercomplexes (RC-LH1),[17, 18] 100 light-harvesting complex II (LH2), five bc_1,[19, 20] and 1–4 ATP synthase [21, 22, 23] per chromatophore are expected, the ratio depending heavily on environmental factors, like light intensity.

Although all these membrane-bound photosynthetic protein complexes (RC-LH1, LH2, bc_1, and ATP synthase) are believed to be present in chromatophores, so far imaging studies have only identified RC-LH1 and LH2 (Figure 1a). In addition, studies have shown that the presence and concentration of RC-LH1 and LH2 are the dominant factors in determining the shape and size of the *Rba. sphaeroides* chromatophores. Indeed, the concentration of LH2 in *Rba. sphaeroides* increases with decreasing light intensity, leading to chromatophores with smaller radii.[13, 14] Also, deletion of LH2 in *Rba. sphaeroides* results in tubular chromatophores populated with orderly arranged dimeric RC-LH1 complexes, and the tubular chromatophores can extend and elongate the bacterial cell (Figure 1b).[10, 11, 24, 25, 26] These experiments prompt the consideration that both LH2 and RC-LH1 are curvature-inducing elements in chromatophores, with the former causing spherical curvature and the latter tubular curvature. The dynamic process of membrane remodeling is difficult to observe experimentally, but is possible with computational modeling even at atomistic resolution, given that structural information for many photosynthetic proteins has become available. Here we review computational studies elucidating the formation of photosynthetic chromatophores.

Curvature properties of LH2

LH2 is a ring-shaped membrane protein complex found in the chromatophores of photosynthetic bacteria. The function of LH2 is to absorb light via its protein-bound pigments, i.e., bacteriochlorophylls and carotenoids, and to pass the energy on to LH1 and the RC. As noted above, it is suspected that the aggregation of LH2 in the membrane plays a role in the development of the spherical chromatophore shape seen in *Rba. sphaeroides*, as this shape persists even in LH2-only mutants of *Rba. sphaeroides*.[13, 14]

To determine the membrane-curving properties of aggregates of LH2 complexes, arrays of seven hexagonally-arranged LH2s were modeled computationally at atomistic detail and were subjected to equilibrium molecular dynamics (MD) simulations.[28] The hexagonal arrangement of LH2 complexes was revealed in AFM images [29, 30, 23] and, therefore, is biologically realistic. While crystal structures of LH2 are available for two species, *Rps. acidophila* and *Rs. molischianum*,[31, 32] none are available for LH2 from *Rba. sphaeroides*. Because LH2s from different species are quite similar in sequence and structure, homology modeling is feasible and was applied to construct an all-atom model for *Rba. sphaeroides* LH2.[28] The resulting simulation system contains, in addition to the protein array, also the membrane environment and the solvent in order to mimic the physiological conditions as closely as possible, and consists of one million atoms in total, nearing current computational limits for all-atom MD.[28, 33] The membrane is decoupled from its periodic neighbors through additional water, eliminating resistance to curvature at the boundaries (Figure 2a). Equilibration of the system showed that the LH2 proteins pack together and tilt with respect to their neighbors to produce an overall curvature on a timescale of 20 ns (Figure 2b).[28] The observed tilt angle for *Rba. sphaeroides* LH2s of

~13° corresponds to a radius of curvature of approximately 32 nm, within the range of the size of native chromatophores (25–55 nm).[10, 11, 24, 25, 26]

MD simulations of arrays of LH2s from *Rps. acidophila* and *Rs. molischianum* were also performed, permitting comparison of the curvature effects from different species of LH2.[28, 34] Such a comparison is particularly interesting because *Rps. acidophila* and *Rs. molischianum* possess lamellar, folded chromatophores, unlike the spherical chromatophores in *Rba. sphaeroides*. Surprisingly, the packing of *Rps. acidophila* and *Rs. molischianum* LH2s also resulted in a net membrane curvature (Figure 3).[28, 34] These results indicate that the distinct chromatophore shapes observed are not due to differences in LH2-LH2 interactions between species, but rather due to other factors, e.g., possibly presence of other proteins, membrane composition or ion concentration.[35, 34]

The question of what molecular mechanisms drive the tilting of LH2s remains. Based on sequence alignments, highly-conserved charged residues at the cytoplasmic side of the LH2s were identified for all three species, the electrostatic interactions of which could induce neighboring LH2s to tilt away from each other on the cytoplasmic side (Figure 2b).[34] To determine their role in the curvature of LH2 patches, charged residues were mutated to either alanines or neutral analogs, and the same MD protocol was applied.[34] Indeed, for LH2s without the charged residues, the curvature of the patches was seen to be greatly reduced (Figure 3).[34] Further analysis of the simulations suggests that the presence of the charged residues causes the LH2s to pack less efficiently on the cytoplasmic side than on the periplasmic side, contributing to the overall curvature. In addition to the influence of charged residues, the physical shape of the LH2 also affects curvature, with the more wedge-shaped LH2s (*Rs. molischianum*) curving the membrane more than the more cylindrical LH2s (*Rps. acidophila*), and with the alanine-replacement mutants curving the membrane less than the neutralized versions due to the reduced bulk on the cytoplasmic side.[34] These studies suggest that LH2s in all species induce spherical curvature via a collective behavior, namely packing of the protein complexes mediated by electrostatic and steric interactions.

Curvature properties of *Rba. sphaeroides* RC-LH1-PufX

The RC-LH1 complex in *Rba. sphaeroides* is dimeric, and contains an additional polypeptide, PufX. The large RC-LH1-PufX molecular assembly is approximately 10 nm along the dimer's short axis and 20 nm along the long axis (Figure 4a). MD simulations of RC-LH1-PufX first faced the obstacle of incomplete structural information. Unlike LH2, there is no high-resolution structure available for a complete RC-LH1. For this reason computational studies on RC-LH1-PufX made use of several modeling techniques, incorporating structural data at different resolutions. An initial *Rba. sphaeroides* RC-LH1-PufX model was constructed combining homology modeling, solution structures, and crystal structures. [28] The protein components were assembled into a dimeric complex guided by an 8.5-Å-resolution cryo-EM projection map reported in Qian et al., 2005,[36] the highest resolution structural information available for the complex (Figure 4a). All-atom MD was employed to equilibrate the resulting RC-LH1-PufX model. The simulation revealed that the dimeric complex spontaneously bends slightly at the dimerizing interface ($\theta_{\text{dimer}} \sim 172°$) due to the relative orientation of the two RCs (Figure 4b).[28] The bent dimer was observed to pull its surrounding membrane along, thereby inducing a local curvature.

The bending reported in Chandler et al., 2008[28] rationalizes how the large RC-LH1-PufX dimer can fit into a curved chromatophore membrane, but the bending produced is actually insufficient to sustain the size of a *Rba. sphaeroides* chromatophore ($\theta_{\text{dimer}} \sim 172°$ corresponds to a radius of curvature of 72 nm, whereas a typical chromatophore has radius

25–55 nm [10, 11, 24, 25, 26]). A three-dimensional cryo-EM map of the *Rba. sphaeroides* RC-LH1-PufX dimer was published in a single-particle analysis study,[26] and revealed that the complex indeed is bent, but with a sharper bending angle ($\theta_{\text{dimer}} \sim 148°$) than was observed computationally (Figures 5a and b). It became necessary to improve the atomistic model of the *Rba. sphaeroides* RC-LH1-PufX complex to better reflect the quaternary structure unveiled by the EM density map, even though the low resolution of the map disallows direct extraction of atomistic data.

In this situation computational methods combining structural information at different resolutions is useful, the particular methodology employed was the molecular dynamics flexible fitting (MDFF) method.[38, 39, 40] MDFF was developed to transform, in a realistic manner, an all-atom structure, obtained either through crystallography or modeling, to an alternative configuration defined by a lower-resolution EM density. In an MDFF simulation two potentials, in addition to the force field describing the interactions between atoms, are incorporated into the MD simulation. One of the potentials steers atoms into high-density regions of the EM map, while the other maintains the secondary structure of the protein. With properly tuned parameters for these two potentials, a biomolecule can be fitted into an EM map within ns timescales, revealing the atomic structures and interactions within protein complexes in physiologically relevant conformations.[41, 42, 43, 44]

In addition to aiding the generation of new atomistic structures, MDFF permits the inclusion of additional molecules not coupled to the EM map in simulations. This feature of MDFF is directly applicable for the case of protein-induced membrane curvature, since lipid molecules need to be described in the simulated systems but should not in general be coupled to the EM map. Employing MDFF, an all-atom RC-LH1-PufX model conforming to the geometry of the EM density map was obtained. [45, 46] The membrane patch surrounding the protein complex was found to also bend, with a radius of curvature (~45 nm) in agreement with the size of chromatophore vesicles (Figure 5c). In addition, the local membrane curvature property of *Rba. sphaeroides* RC-LH1-PufX was observed to be asymmetric; namely, while bending the membrane around its short axis, the RC-LH1-PufX complex also twists the membrane slightly around its long axis. This membrane-bending behavior of RC-LH1-PufX offers a microscopic rationalization for the helical packing of the dimers seen in EM images of tubular chromatophores comprised solely of RC-LH1-PufX (Figure 1b);[24, 25, 26] detailed analysis of local curvature properties and their relationship to the global arrangement of RC-LH1-PufX is described in Hsin et al., 2009.[46]

Membrane curvature due to *Rba. sphaeroides* RC-LH1-PufX is a direct result of its highly bent geometry, which is unique to dimeric RC-LH1-PufX. In PufX-deficient *Rba. sphaeroides*, or in other species lacking PufX, the RC-LH1 complex is monomeric and ring-like, and does not possess a bent geometry.[47, 48, 49] The question remains what is the molecular basis for the dimerization and the resulting bent geometry of the *Rba. sphaeroides* RC-LH1-PufX complex. Recently, computational methods were applied in an attempt to address this issue. The important structural element considered was the PufX protein, which is a single transmembrane helix known to play the determining role in RC-LH1 dimerization,[50, 16, 51] but its location within the complex has yet to be precisely established.

One proposed placement of PufX is at the dimerizing junction of RC-LH1-PufX.[37] Since two PufX helices are expected per RC-LH1-PufX complex,[52] this placement requires the dimerization of PufX, yet only monomeric structures of PufX have been solved.[53, 54] To test if a dimeric PufX is feasible, computational modeling and MD simulations were performed.[55] A model for PufX dimer was constructed based on the suggestion that there is a dimerization motif in the PufX sequence.[56, 57] Subsequent all-atom MD simulations

of this PufX dimer model, placed in a membrane and water box, demonstrated that the dimer remained structurally stable over the 50 ns simulated, and that the PufX helices maintained a uniform crossing-angle of $\theta_{PufX} \sim 38°$ (Figure 6).[55] Assuming that such a PufX dimer indeed serves as the nucleation point of the RC-LH1-PufX assembly, and that the LH1 helices remain parallel to the PufX monomer on each side of the RC-LH1-PufX dimer, the overall bending of the RC-LH1-PufX complex arises naturally. This scheme explains the geometry of the *Rba. sphaeroides* RC-LH1-PufX dimer; the definitive answer to the question where PufX is located in the LH1-RC-PufX dimer, however, requires a full atomic-level structure of the complex.

Formation of chromatophores upon assembly of LH complexes

Computational studies have established that both *Rba. sphaeroides* LH2 and RC-LH1-PufX complexes are membrane-curving elements.[28, 34, 46] Assembly of these complexes should then produce chromatophores with a global curvature. Demonstration of the dynamic chromatophore formation process requires simulation of hundreds of proteins, and becomes computationally costly if performed at atomic resolution. The problem of computational cost can be avoided by using coarse-grained representations, assuming that the simplified model correctly replicates the membrane-curving properties of the protein complexes. An example of a successful application is the shape-based coarse-graining method used in demonstrating the membrane-sculpting effect of an array of BAR domain proteins.[5, 7, 8]

For the case of assembly of the photosynthetic chromatophores, a set of Monte Carlo simulations were performed.[58] In each simulation, a collection of membrane-curving “beads” mimicking the size and geometry of the LH2 and RC-LH1-PufX complexes were placed on a flexible surface. Each bead, as well as certain bead-to-bead interaction, was designed to produce a specific local curvature; bending of the membrane was ascribed an energetic cost calculated through the Helfrich free energy.[59, 2] An originally flat membrane consisting of a random mixture of LH2 and RC-LH1-PufX beads was seen to become vesicular as it approached equilibration, forming segregated domains.[58] A separate set of coarse-grained MD simulations similarly showed that in a membrane populated with curvature-inducing capsids, a vesicle spontaneously formed as attractive interactions between capsids were observed.[60, 61] Altogether, the formation of chromatophores is possibly a result of the spontaneous aggregation of the protein elements, which occurs to minimize the energy penalty of bending the membrane (Figure 7).

Why do photosynthetic chromatophores self-assemble?

Computational studies over the past few years on the curvature properties of photosynthetic proteins [58, 28, 34, 46, 55] are providing a plausible mechanism for the self-assembly of the chromatophores that can be a general principle for the formation of cellular organelles. The protein components of the chromatophore not only serve photosynthetic functions, but each also possesses unique membrane-shaping properties. Since bending the membrane comes with an energetic cost, the optimal arrangement for these particular membrane-bending elements is a tightly-packed aggregate, resulting in the formation of a well-defined functional unit.

Formation of the photosynthetic chromatophore is not only relevant in terms of membrane architecture, but also provides functional advantages. For example, the electronic excitation resulting from light absorption has to be transferred to an RC for charge separation and further processing. A closer packing between the light-harvesting complexes and the RC facilitates this process by lowering the excitation transfer times between complexes, and as a result increasing the overall quantum efficiency of the chromatophore.[27, 58, 45] Another example concerns the subsequent steps of photosynthesis, which involves diffusions of

quinones and cytochrome c2. These processes are also likely more efficient in a connected, compact compartment of the cell such as the chromatophore as opposed to having the constituent proteins loosely distributed over a large membrane area.[16] The advantages of compartmentalization become readily evident by a comparison with the plant and cyanobacterial systems.[64, 65] In evolutionarily more advanced oxygenic photosynthetic species,[66] not only do the individual light-harvesting complexes display a greater packing density of pigments (per amino acid), but also the constituent proteins are organized in a dense arrangement in the form of thylakoid membranes.[67] Formation of densely packed cellular compartments seem to arise as a result of evolutionary pressures. It appears that cellular functional units arise readily upon the assembly of their protein components, and the formation of functional units in turn facilitates the work of the proteins, interweaving structure and function into a complex relationship.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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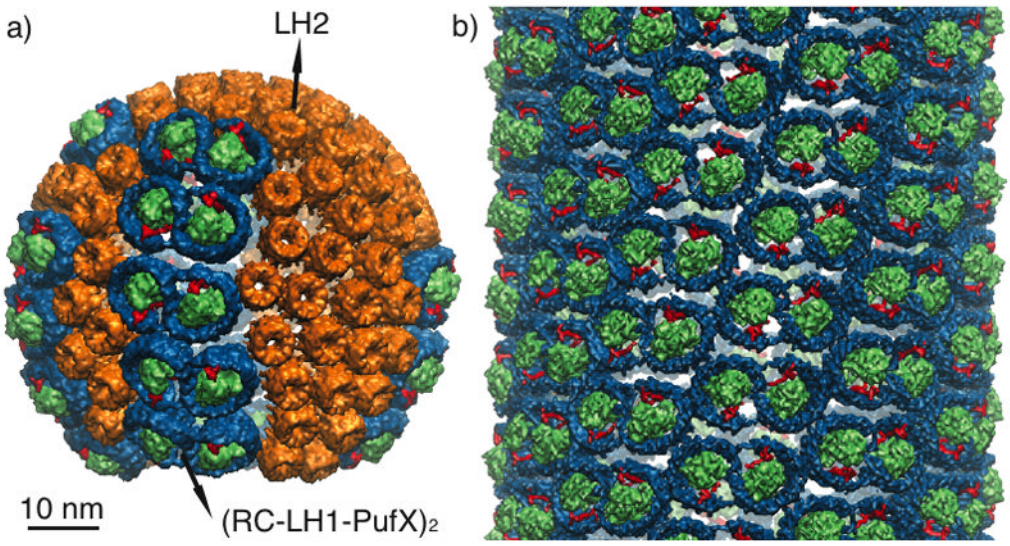

Figure 1.

Two examples of chromatophore shapes. a) Spherical chromatophore (radius 30 nm) found in wild-type *Rba. sphaeroides* containing LH2 (orange) and the RC-LH1-PufX supercomplex (RC: green, LH1: blue, PufX: red). Placement of the proteins was performed in [27] using a combination of structural and imaging data. b) Mutant *Rba. sphaeroides* lacking LH2 possesses tubular chromatophores, which are populated with helically ordered RC-LH1-PufX.[26] The tubular chromatophore shown here has a radius of 36 nm.

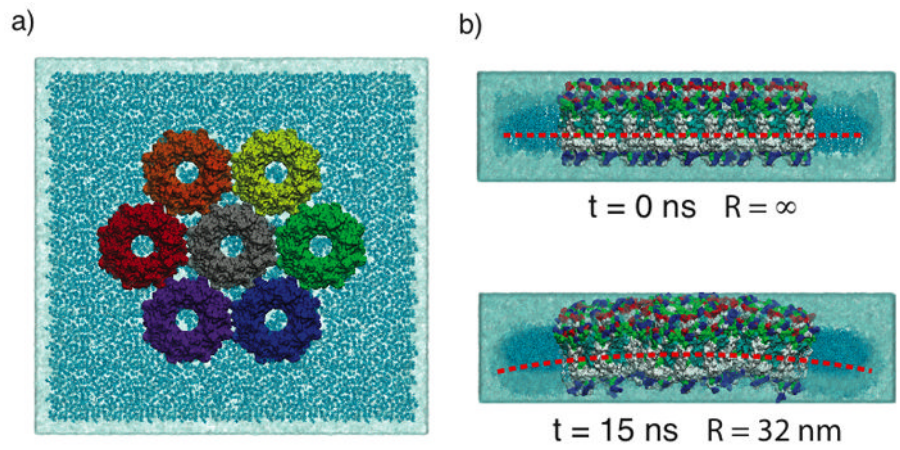

Figure 2.

a) Top view (perpendicular to membrane plane) of a setup of an LH2 simulation containing seven LH2s packed hexagonally in a membrane patch. Each LH2 is colored differently for distinction. Membrane is shown in blue, and water box in transparent blue. b) Side view (along the membrane plane) of the *Rba. sphaeroides* LH2 patch before and after equilibration.[28, 34] LH2s are colored according to residue type, with red being negatively-charged residues and blue positively-charged. Here, as well as in all the following protein-membrane systems shown in side views, the proteins are orientated with their cytoplasmic sides pointing upward. The dashed red lines indicate the membrane center to illustrate the curvature arising during the simulation.

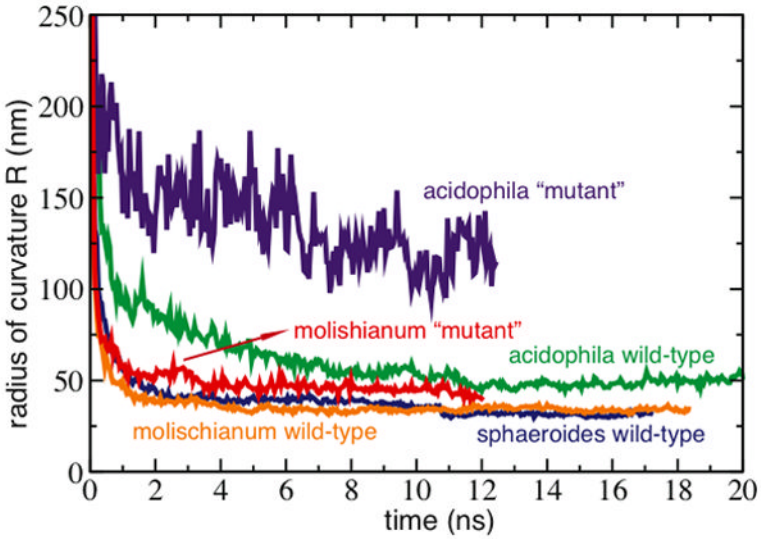

Figure 3.
Curvature induced by patches of LH2s from wild-type vs. alanine-replacement mutants. The mutated LH2s, in which the charged residues were changed to alanines, curved less than their wild-type counterparts.

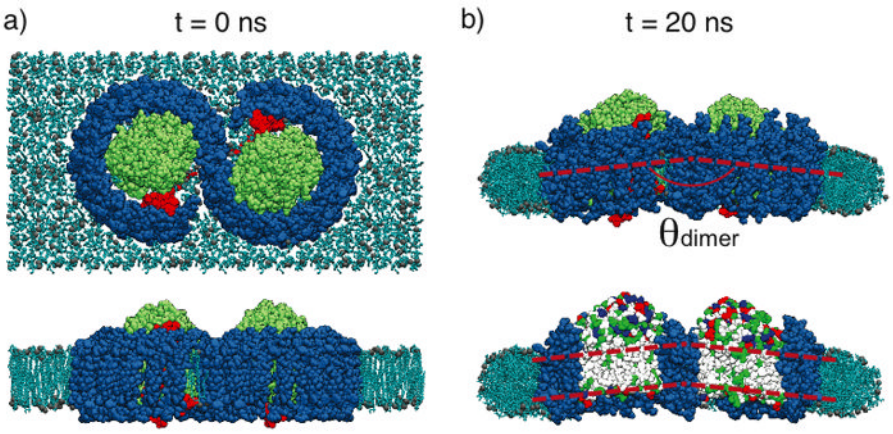

Figure 4.

Rba. sphaeroides RC-LH1-PufX model and equilibration.[28] a) Top and side view of the RC-LH1-PufX complex immersed in a membrane patch before MD equilibration. LH1 shown in blue, RC in green, PufX in red, and membrane in light blue. Water is not shown for clarity. For the side view, some lipid molecules are hidden to show better the protein complex. An alternative placement for PufX is discussed in Scheuring et al., 2004.[37] b) Simulation system after a 20-ns equilibration. Top panel shows the complex attaining a bending angle $\theta_{\text{dimer}} \sim 172°$. On the bottom panel, some LH1 helices are hidden to show the relative orientation of the two RCs, colored by residue types, which form a bent hydrophobic region.

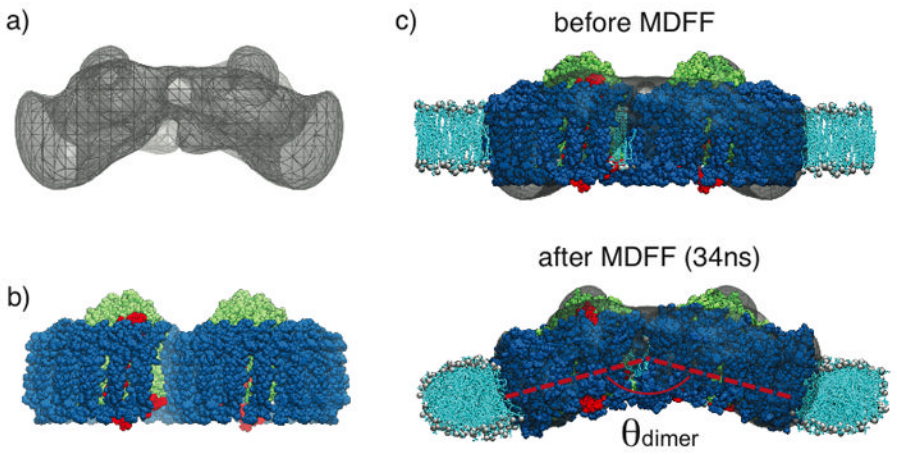

Figure 5.

Application of MDFF for the membrane-bending properties of *Rba. sphaeroides* RC-LH1-PufX complex. a) EM envelope obtained in a single-molecule analysis study, showing a large bending.[26] b) Modeled RC-LH1-PufX complex from Chandler et al., 2008,[28] which is initially flat. c) RC-LH1-PufX complex fitted into the EM map employing MDFF, resulting in a much more prominent θ_{dimer}. The membrane curves along with the complex. [46]

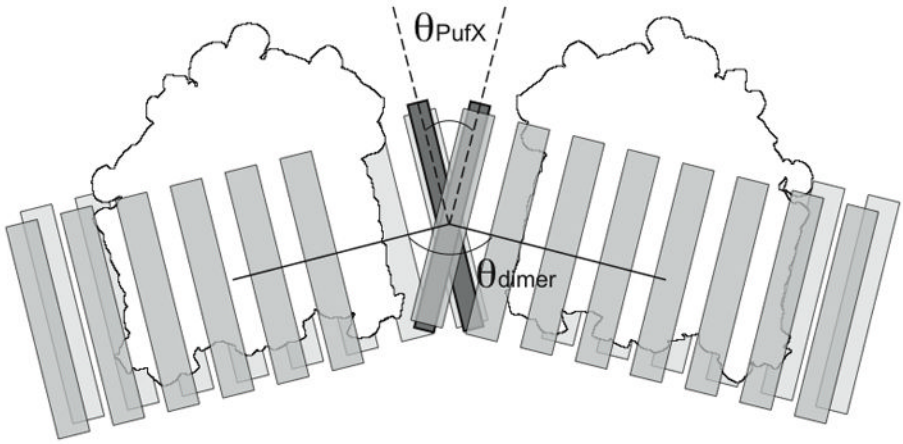

Figure 6.

PufX dimerization and the arrangement of *Rba. sphaeroides* RC-LH1-PufX complex. A pair of dimerized PufX helices are shown to be located at the center of the RC-LH1-PufX complex. In this scheme, the crossing angle of the PufX helices, θ_{PufX}, leads to the bending angle of the RC-LH1-PufX, θ_{dimer}.[55] PufX shown in dark gray, LH1 in light gray, and RC in white.

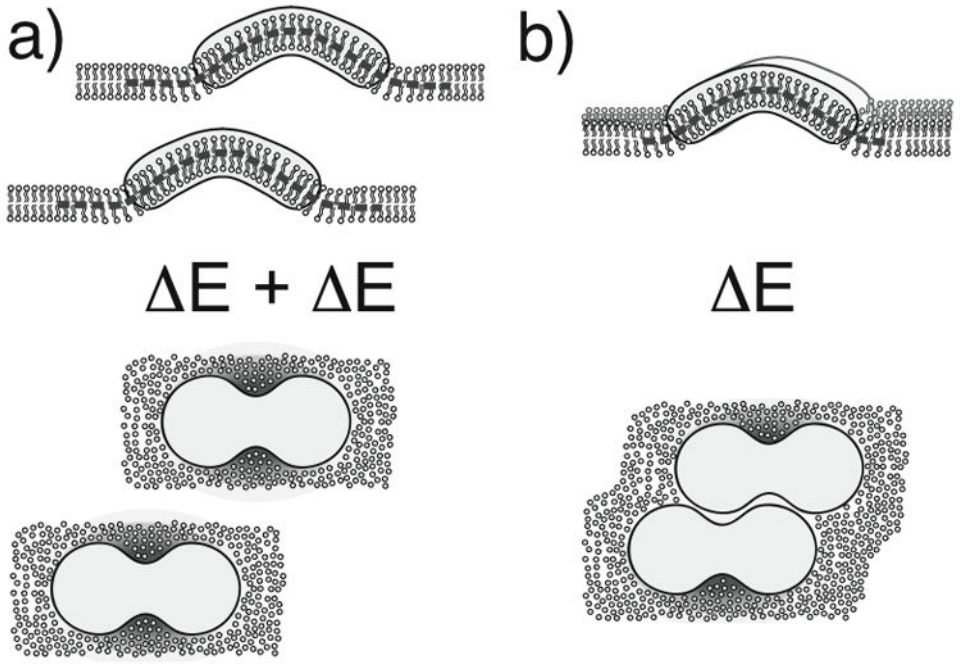

Figure 7.

Schematic diagram displaying curvature-mediated “attraction” between two membrane-bending proteins. In a), two proteins mimicking the curvature effect of the *Rba. sphaeroides* RC-LH1-PufX complex are placed far apart in the membrane, with each protein bending its surrounding membrane that requires energy ΔE. The shaded area in the membrane indicates the most prominent local curvature. In b), the two proteins are stacked together, and in this arrangement there is less membrane bending, indicated by fewer shaded areas. The energy required to bend the membrane is, therefore, less than the scenario depicted in a). Detailed theoretical calculations of the interactions between membrane-curving proteins can be found in several studies, e.g. Kim et al., 1998 and Chou et al., 2001.[62, 63]