Forces guiding assembly of light-harvesting complexes 2 in native membranes

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SUPPLEMENTAL INFORMATION

Supplementary Information 1

For the calculation of the equilibrium free energy difference ΔG following Jarzynski's equality, in a first step the non-equilibrium work W is integrated over each subunit unfolding process (**Figure S1A**).

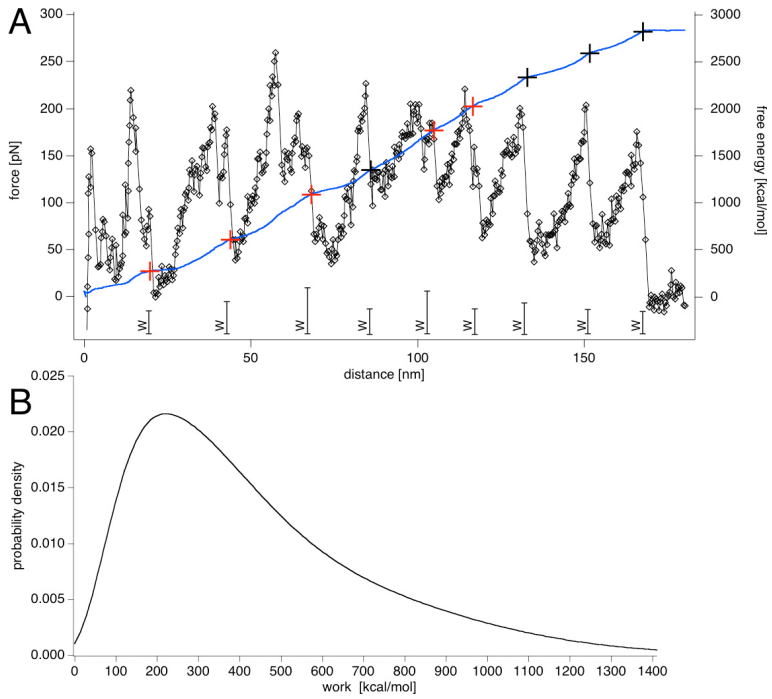

Figure S1. (**A**) Force distance curve (black line and markers) and non-equilibrium work (blue line) performed upon LH2 unfolding. Black and red crosses indicate the non-equilibrium work per subunit unfolding for the one-step and the two-step unfolding events, respectively. At the bottom of the graph is illustrated the non-equilibrium work W integrated at the end of each unfolding event. (**B**) Probability density of the dissipated work upon LH2 unfolding (n = 300).

Following Jarzynski (1), the work W invested in a non-equilibrium single molecule experiment is usually larger than the free energy difference ΔG of the equilibrium states before G_A and after G_B unfolding: $W \geq \Delta G = G_B - G_A$, because the system has to be pulled over an energy barrier between the two states.

However, if the experiment is performed many times (**Figure S1B**), in some rare cases the thermal bath 'aids' the unfolding effort, therefore occasionally experiments are performed in which the work W equals the

free energy difference ΔG, or is even lower. However, it is at first impossible to know what the free energy difference ΔG between the two equilibrium states G_A and G_B is.

If one could observe the process in both directions, pulling the system from either side G_A and G_B over the energy barrier to end up in G_B and G_A respectively, then Crooks' symmetry relation (2) applies, in which $\rho(W)_{(G_A \to G_B)}$ and $\rho(W)_{(G_B \to G_A)}$ are the work distributions measured for the process in both directions:

$$\frac{\rho(W)_{(G_A \to G_B)}}{\rho(W)_{(G_B \to G_A)}} = e^{1/k_B T \cdot (W - \Delta G)}$$

Thus, where $\rho(W)_{(G_A \to G_B)}$ and $\rho(W)_{(G_B \to G_A)}$ are equal, $\frac{\rho(W)_{(G_A \to G_B)}}{\rho(W)_{(G_B \to G_A)}}$ is 1, and the work W equals the free energy difference ΔG.

In other words, when the molecule is pulled out of the lower energy state (here G_A) up to the same free energy level like G_B, then the probability that thermal fluctuations pull the system across the energy barrier is equivalent from both sides, and the work W invested to get there must be the free energy difference ΔG.

Lacking the possibility to measure experimentally the refolding process, Jarzynski states that the non-equilibrium work relation:

$$\left\langle \; e^{-1/k_B T \cdot W} \; \right\rangle = e^{-1/k_B T \cdot \Delta G}$$

is valid, implying that the logarithm of the average exponentially weighted measured non-equilibrium work equals the free energy difference between the two states (3).

Supplementary Information 2

The LH2 complex from *Rsp. photometricum* presents a typical LH2 overall architecture, a ring with nine-fold symmetry (4), consistent with the X-ray structure of LH2 complexes from *Rps. acidophila* (5-7), but also with lower resolution data of several other species like *Rb. sphaeroides* (8, 9), *Rb. blasticus* (10), *Rps. palustris* (11), *Rvi. gelatinosus* (12). For sequence comparison of the *Rsp. photometricum* LH2 polypetides, we have sequenced the *pucA* and *pucB* genes. The LH2 sequences are similar especially in the transmembrane regions (**Figure S2**). It can be reasonably assumed that the structure of LH2, the length of the transmembrane α-helices, is in all species very similar as in the *Rps. acidophila* structure. The sequence identity and similarity of *Rsp. photometricum* LH2 α-polypeptide compared to LH2 α-polypeptides from other species are: vs *Rps. acidophila* (29%, 58%), vs *Ph. molischianum* (44%, 100%), vs *Rb. sphaeroides* (42%, 64%), vs *Rb. capsulatus* (33%, 78%), vs *Rps. palustris* (37%, 57%), vs *Rvi. gelatinosus* (50%, 75%). The sequence identity and similarity of *Rsp. photometricum* LH2 β-polypeptide compared to LH2 β-polypeptides from other species are: vs *Rps. acidophila* (26%, 50%), vs *Ph. molischianum* (45%, 63%), vs *Rb. sphaeroides* (36%, 47%), vs *Rb. capsulatus* (35%, 54%), vs *Rps. palustris* (36%, 51%), vs *Rvi. gelatinosus* (40%, 53%). (For comparison, aquaporin sequences revealed an identity of 25% (13) and have structures that are similar within a few Angstrom root mean square deviation of the protein chain backbone atoms (14)). According to the sequence similarity and the fact that LH2 complexes in *Rps. acidophila* appear as nonamers (5-7), in the present work, we used the structure of LH2 from *Rps. acidophila* to interpret the unfolding process and protein interactions of *Rsp. photometricum* LH2 studied by AFM single molecule force measurements.

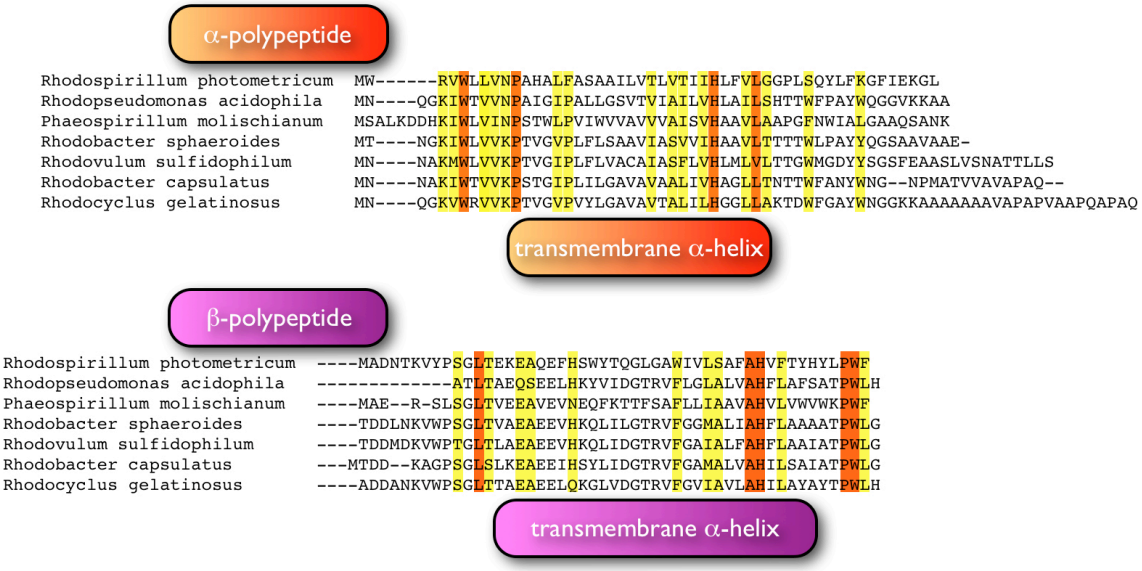

Figure S2. Sequence comparison of LH2 α- and β-apoproteins from *Rsp. photometricum, Rps. acidophila*, *Ph. molischianum*, *Rb. sphaeroides*, *Rb. capsulatus*, *Rps. palustris*, and *Rvi. gelatinosus*. Highly conserved residues are highlighted in red, and homologous residues in yellow, among all sequences. The transmembrane α-helix stretch in the *Rps. acidophila* structure is indicated below the sequences, using the same color code than in the main manuscript.

Supplementary Information 3

For indirect additional evidence for the structural stability between the α- and the β-polypeptides in the cytoplasmic region (15), we have recorded the near infrared spectrum of the native photosynthetic membrane from *Rsp. photometricum* upon addition of detergent (n-octyl-beta-D-glucoside, OG). The presence of OG influences the structure of membrane protein complexes, because of the integration of detergent molecules into the native membrane. First the membrane pressure is decreased due to detergent insertion into the membrane (8), and then the intercomplex contacts are loosened approaching solubilization. Complete solubilization might impact the ring integrity. We found, following addition of detergent, shifts of the absorption maxima of ~7 nm for the B850 bacteriochlorophylls (BChls) (**Figure S3A**), in contrast of only ~1.5 nm for the B800 BChls (**Figure S3B**). This is indirect evidence related to two topics in the main manuscript: first, the cytoplasmic side of the LH2 subunit is structurally very stable, as documented by the absorption conservation of the B800 BChls close to the cytoplasmic face. This structural stability might explain the subunit integrity during unfolding. Second, the fact that the B850 BChls absorption strongly shifts when the membrane pressure is alleviated (upon detergent addition), indicates that the two helices of the α- and the β-polypeptides can structurally 'open', when the membrane integrity, vicinity of other complexes is removed. This goes along with the finding that subunits that have membrane space unfold in the one-step process, while the β-apoprotein is stabilized as long as the molecular environment is crowded, resulting in a two-step unfolding process (see **Figures 2** and **3** in the main manuscript).

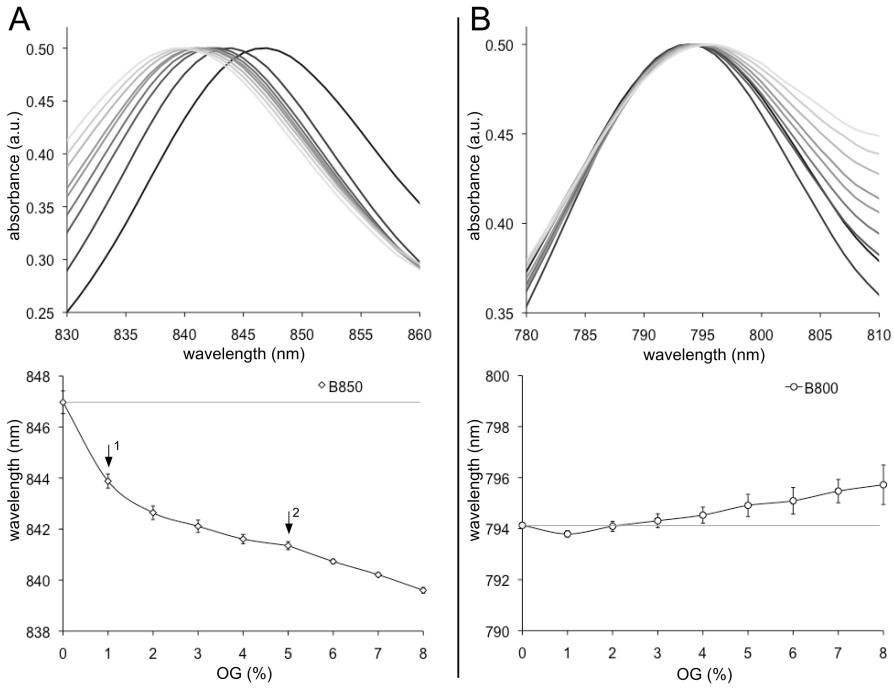

Figure S3. Absorption shift of the B850 and the B800 absorption bands upon addition of detergent (n-octyl-beta-D-glucoside, OG, 0-8% w/v) to native chromatophores of *Rsp. photometricum*. (**A**) B850 and (**B**) B800 absorption of the LH2 complexes in the native membrane (darkest grey line) following detergent addition (increasingly lighter grey lines). Bottom: The B850 absorption shifts readily strongly upon first detergent addition (arrow 1). In this regime, the membranes get enriched with detergent molecules. At higher detergent concentration an increased absorption shift is detected, probably reflecting membrane disruption (arrow 2). The B800 absorption shifts only slightly.

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