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Supplementary Information for

Dimers of mitochondrial ATP synthase induce membrane curvature and self-assemble into rows

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Figures S1 to S3
Captions for movies S1 and S2
Stills for movies S1 and S2

Other supplementary materials for this manuscript include the following:

Movies S1 and S2

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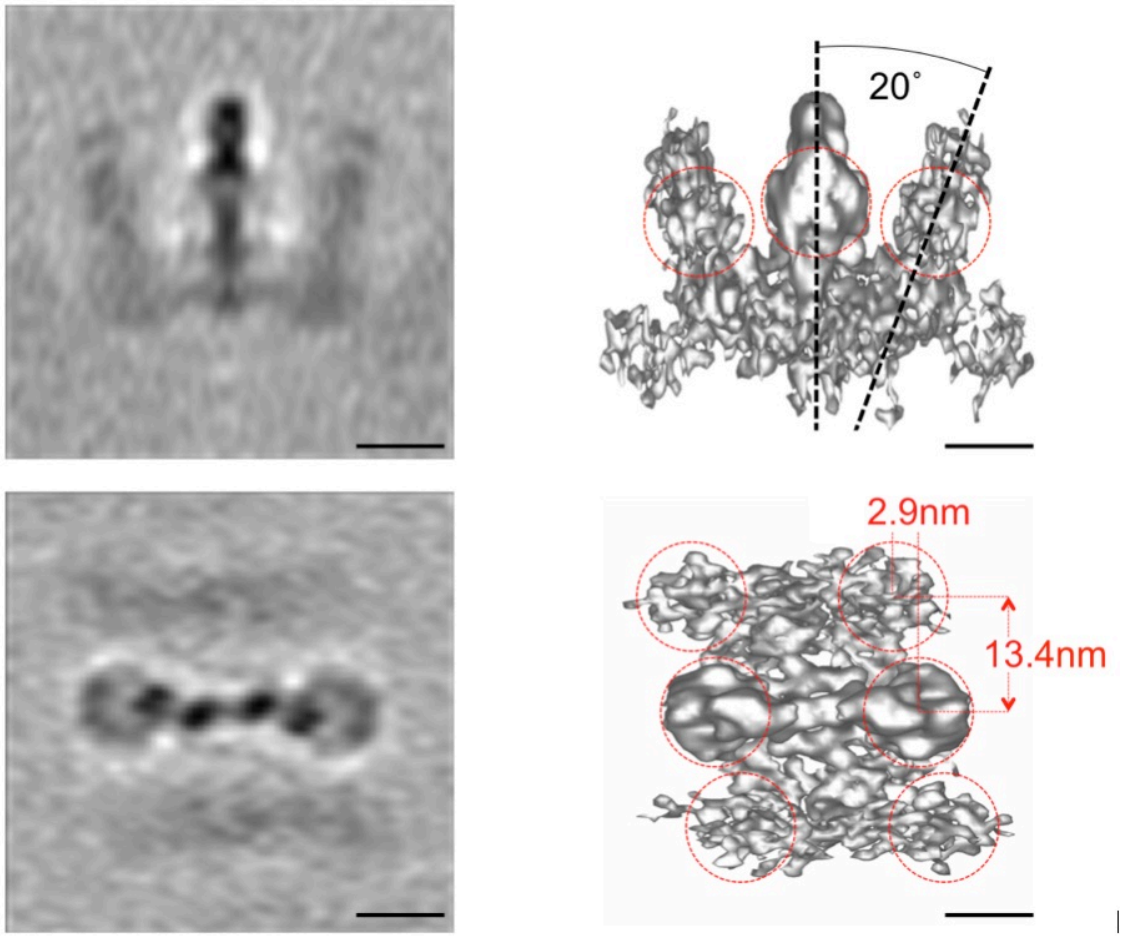


Figure S1
Subtomogram average volume of reconstituted *Polytomella* dimers. Two orthogonal views perpendicular to the dimer rows are shown. Tomographic volumes were aligned on the central dimer and averaged. The central dimer is well-defined, whereas neighboring dimers appear fuzzy, indicating variable inter-dimer distances and angles. Scale bars: 10 nm.

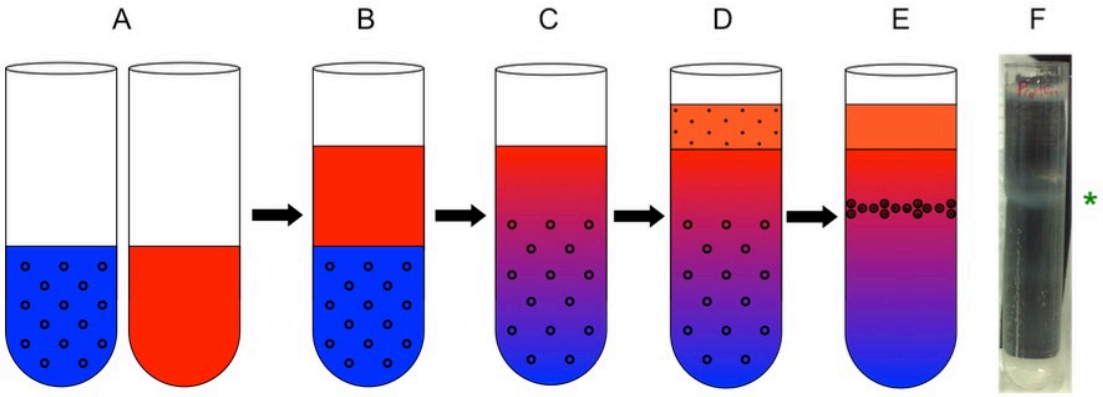


Figure S2
GRecon preparation of proteoliposomes. (A, B) Buffer with 0.3 M saccharose (red) is layered on the buffer with 1.3 M saccharose containing destabilized liposomes (black circles) and cyclodextrin (blue) in a centrifuge tube. (C) A linear gradient is prepared in a gradient maker. (D) Detergent-solubilized ATP synthase dimers (black dots) are layered onto the gradient. (E, F) During centrifugation, detergent is removed by dilution and adsorption to cyclodextrin. The protein incorporates into the liposomes (black circles with dots). Proteoliposomes migrate to a position in the gradient depending on their protein content (asterisk).

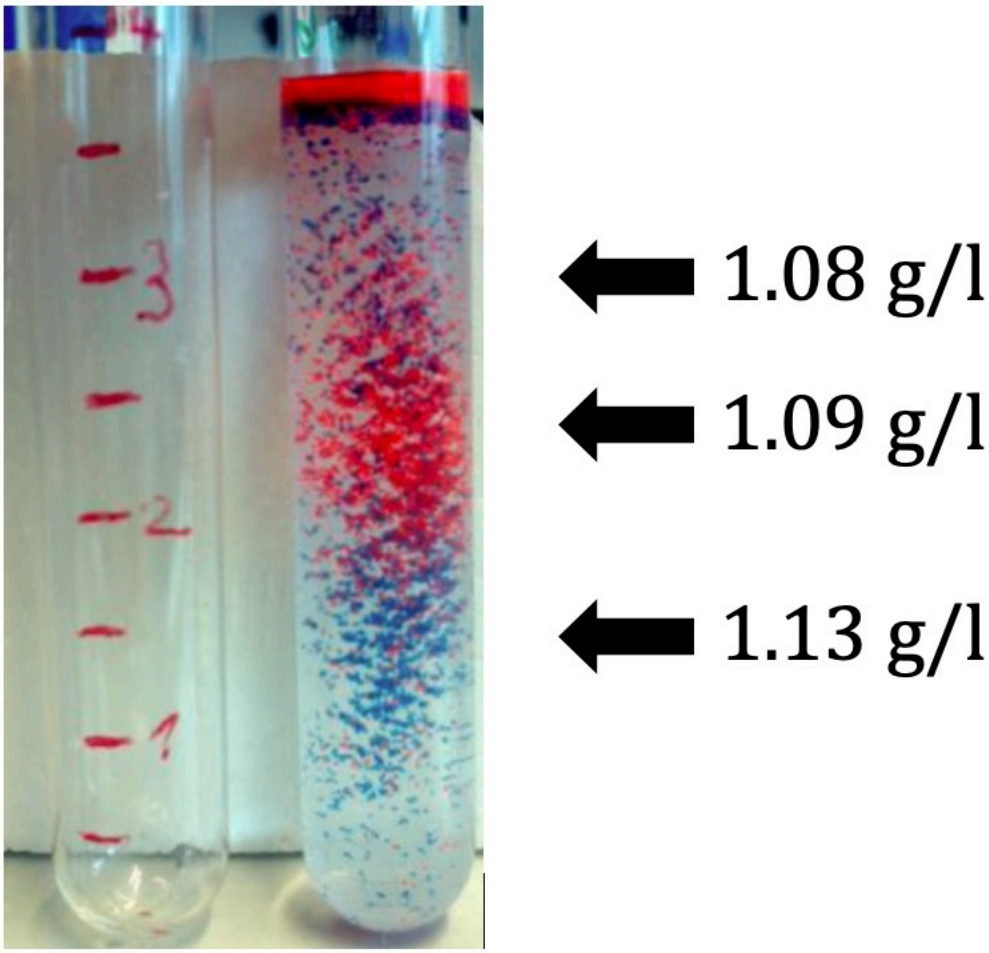


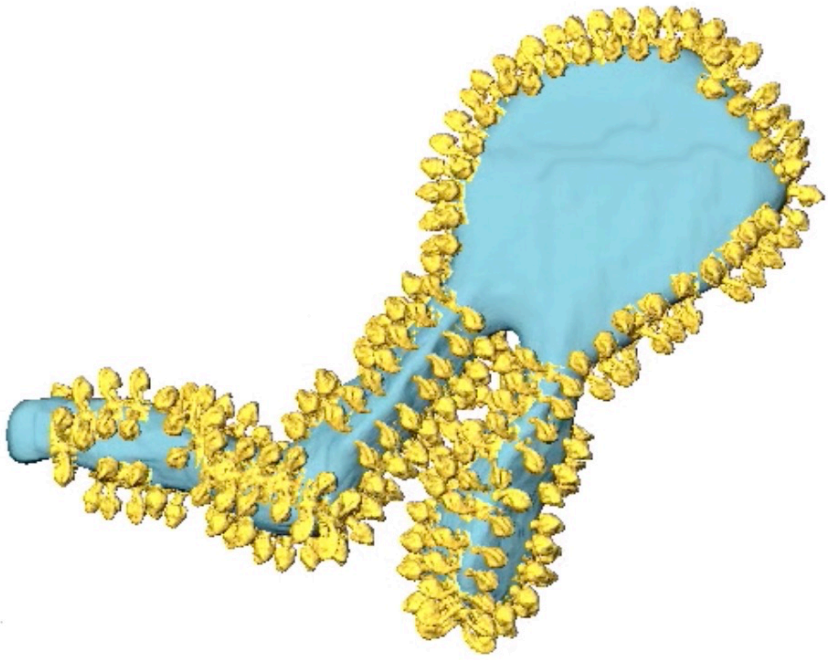
Figure S3
Gradient density. To determine the gradient density, microspheres (Cospheric) were added to a mock gradient. Upon centrifugation, the microspheres migrate to a gradient position that indicates their approximate density.

Movie S1 Electron cryo-tomography (cryo-ET) of ATP synthase dimers reconstituted into liposomes. V-shaped ATP synthase dimers (yellow) isolated from the yeast *Yarrowia lipolytica* insert themselves into preformed, destabilized *E. coli* polar lipid liposomes upon detergent removal on a sucrose density gradient. Reconstituted dimers spontaneously associate into rows that distort the lipid bilayer (light blue) into ridges. The irregular spacing of dimers along a ridge indicates the absence of specific protein-protein interactions, which are not required for row formation. The ridges run around the edge of the flattened vesicle that resembles lamellar cristae of mitochondria. If dimers insert into the membrane unidirectionally, parallel ridges can result in narrow tubular vesicles, as in this tomogram. If the dimers insert bi-directionally, they can shape the lipid bilayer into a corrugated sheet, as in Movie S2.

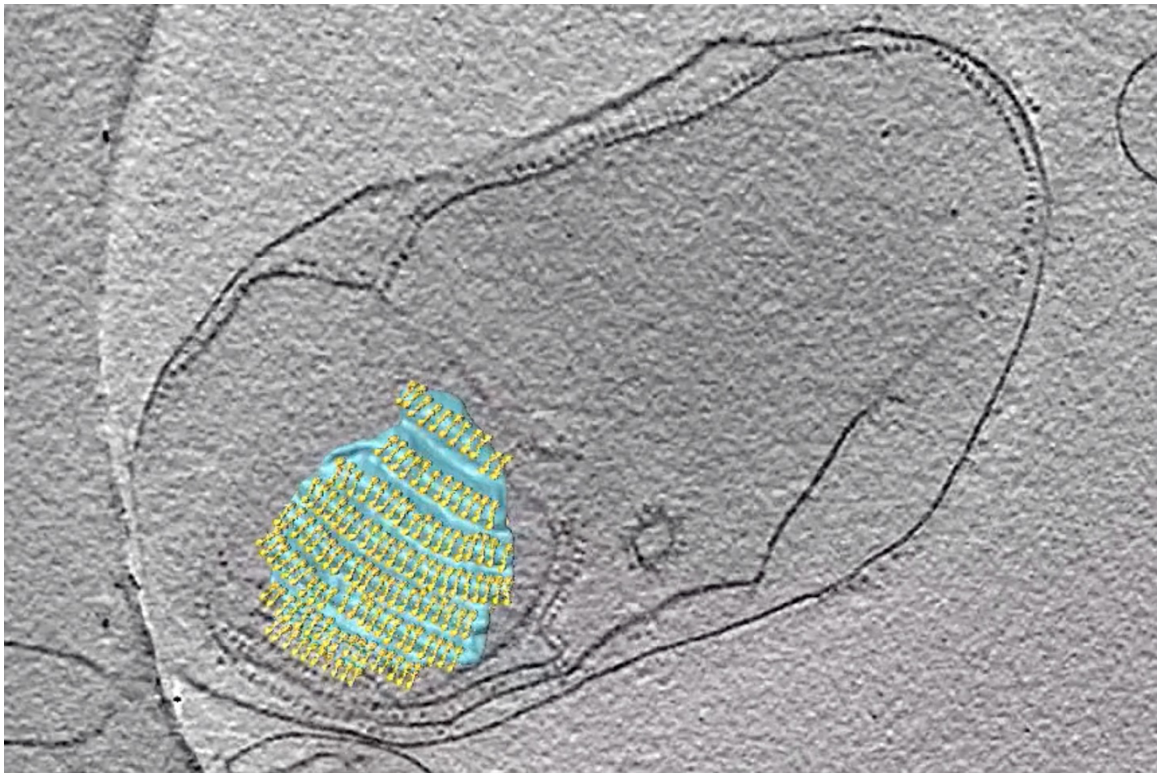
Note that the flat region of the lipid bilayer of the lamellar vesicle does not contain any inserted dimers. Note also that membrane insertion appears to be cooperative, as the two concentric vesicles seen at the lower right of the tomographic volume are completely devoid of inserted protein.

Movie S2 Cryo-ET of *Y. lipolytica* ATP synthase dimers (yellow) reconstituted into *E. coli* polar lipid liposomes, as for Movie S1. Movie S2 indicates bidirectional insertion of dimers into the lipid bilayer. ATP synthase dimers assemble spontaneously into rows, driven by the energy of elastic membrane deformation that results from the insertion of each dimer. Due to bidirectional insertion, rows form on both sides of the lipid bilayer (light blue). As each row bends the membrane locally by approximately 90°, rows on opposite surfaces shape the lipid bilayer into a corrugated sheet.

The tomogram shows several nested liposomes, the largest of which measures about 1 μm. Note that (a) most of the large vesicle surface is devoid of inserted dimers; (b) nearly all of the inserted dimers have assembled into rows; (c) cross sections through individual rows indicate a ~90° bend in the membrane; (d) the dimers tend to segregate laterally into one crowded membrane region, e.g. in the corrugated sheet.



Movie S1 still.



Movie S2 still.