Supporting Information

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Fig. S1. Cristae morphology in different species. Sections of segmented, surface-rendered tomograms of *Saccharomyces cerevisiae* (*A*), and bovine heart mitochondria (*B*), with different types of cristae morphology and cristae junctions. Mitochondria from *S. cerevisiae*, potato, and the other fungi had lamellar cristae, which merge with the inner boundary membrane in slit-like junctions (red arrows). Bovine heart mitochondria contained a complex network of cristae with each crista consisting of small lamellar disks (black arrowheads) connected by tubes. These cristae merged with the inner boundary membrane via a number of small circular junctions of 35 ± 5 nm diameter (black arrows).



Fig. S2. ATP synthase dimers in a whole plant mitochondrion. (*A*) Tomographic section through a whole mitochondrion isolated from potato tubers. ATP synthase dimers (yellow arrowheads) are visible where the crista membrane bends sharply by approximately 90°. (*B*) Three magnified views of the tomogram in *A* showing ATP synthase dimers at the apex of highly curved membranes. Scale bar, 100 nm.



Fig. S3. Rows of dimeric ATP synthases in a whole mitochondrion from bovine heart. (*A*) Slice through a tomogram of a whole bovine heart mitochondrion showing a row of ATP synthases (yellow arrowheads). (*B*) A rendered subvolume of the mitochondrion shown in *A* showing the location of ATP synthases (yellow spheres). As in mitochondria from *Podospora anserina* (Fig. 1), pairs of ATP synthases are found above regions of highly curved cristae membranes. Gray, outer membrane; blue, cristae membranes; blue-transparent, inner boundary membrane; yellow, ATP synthase. Scale bar, 200 nm



Fig. S4. F_1 head distances. Histogram of distances between F_1 heads in the F_1 - F_o ATP synthase dimers from bovine heart (orange), *P. anserina* (lilac), *S. cerevisiae* (blue), *Yarrowia lipolytica* (light blue), and potato (green). Separate Gaussian fits were calculated for the combined data from bovine and fungi (purple curve), and from potato (green curve). Data were normalized to compensate for differences in sample size. Average distances, standard deviations, and sample size (parentheses) for each species were as follows: bovine heart, 28.6 ± 3.0 nm (127); *Y. lipolytica*, 28.0 ± 2.4 nm (223). *P. anserina*, 28.1 ± 2.1 nm (76); *S. cerevisiae*, 28.4 ± 2.5 nm (175); potato, 33.6 ± 2.2 nm (76).



Movie S1. Tomographic volume of a small, intact *P. anserina* mitochondrion shown in Fig. 1. Rows of ATP synthase *F*₁ heads along cristae membranes are marked by yellow arrowheads or by small yellow spheres in the segmented, surface-rendered volume. All densities identified as ATP synthase heads are arranged in rows of dimers along the sharply curved cristae edges. Outer membrane, gray; cristae membranes, blue; inner boundary membrane, blue-transparent; ATP synthase, yellow.

Movie S1 (MP4)



Movie 52. Box-shaped crista vesicle isolated from *P. anserina* mitochondria shown in Fig. 4. Two rows of ATP synthase dimers (yellow and red) in the membrane (gray) run along the tightly curved box edges. Densities of nonregularly arranged complex I or other respiratory chain complexes (green) are confined to the flat membranes on the sides of the box. The ratio of complex I to ATP synthase is 1:3.6

Movie S2 (MP4)



Movie S3. Tubular crista vesicle isolated from potato mitochondria shown in Fig. 3C. Two rows of ATP synthase dimers (yellow) in the membrane (light blue) run along the long axis of the tube, and around its hook-like end. Green densities attributed to complex I or other respiratory chain complexes are found only in the membrane regions between the dimer rows. The largest of these densities are likely to correspond to respiratory chain supercomplexes.

Movie S3 (MP4)