Macromolecular organization of ATP synthase and complex I in whole mitochondria

Karen M. Davies1,1, Mike Strauss2,1, Bertram Daum1,1, Jan H. Kiefi,1, Heinz D. Osiewacz1, Adriana Rycovskia, Volker Zickermann* and Werner Kühlbrandt* 2

1Department of Structural Biology, Max Planck Institute of Biophysics, Max-von-Laue Strasse 3, 60438 Frankfurt am Main, Germany; 2Mitochondrial Biology, Medical School, Goethe University Frankfurt am Main, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany, and Deutsche Forschungsgemeinschaft Cluster of Excellence Frankfurt “Macromolecular Complexes”, 60438 Frankfurt, Germany; Department of Molecular Membrane Biology, Max Planck Institute of Biophysics, Max-von-Laue Strasse 3, 60438 Frankfurt am Main, Germany, and Medical Faculty, Molecular Bioenergetics, Goethe University, Theodor-Stern-Kai 7, 60590 Frankfurt am Main, Germany

Edited by Richard Henderson, Medical Research Council Laboratory of Molecular Biology, Cambridge, United Kingdom, and approved July 1, 2011 (received for review March 7, 2011)

We used electron cryotomography to study the molecular arrangement of large respiratory chain complexes in mitochondria from bovine heart, potato, and three types of fungi. Long rows of ATP synthase dimers were observed in intact mitochondria and cristae membrane fragments of all species that were examined. The dimer rows were found exclusively on tightly curved cristae edges. The distance between dimers along the rows varied, but within the dimer the distance between F1 heads was constant. The angle between monomers in the dimer was 70° or above. Complex I appeared as L-shaped densities in tomograms of reconstituted proteoliposomes. Similar densities were observed in flat membrane regions of mitochondrial membranes from all species except Saccharomyces cerevisiae and identified as complex I by quantum-dot labeling. The arrangement of respiratory chain proton pumps on flat cristae membranes and ATP synthase dimer rows along cristae edges was conserved in all species investigated. We propose that the supramolecular organization of respiratory chain complexes as proton sources and ATP synthase rows as proton sinks in the mitochondrial cristae ensures optimal conditions for efficient ATP synthesis.

The two large complexes occur at an approximate ratio of one molecule of complex I per 3.5 ATP synthase monomers (9). The ATP synthase is easily identified in mitochondrial membranes by its characteristic 10-nm F1 head connected to the membrane by a 5-nm-long stalk (10–12). Blue-native polyacrylamide gel electrophoresis (BN PAGE) of the mitochondrial F1,Fo ATP synthase has shown that the complex forms dimers or larger oligomeric assemblies when solubilized with mild detergents (13). Single-particle electron microscopy indicated angles of 40° (14, 15) or 70–90° (16, 17) between the long axes of monomers in the dimer. Freeze-fracture deep-etch replicas suggested that in Paramecium these dimers form rows along the edge of helical cristae tubes (18). More recently, cryoelectron tomography (cryo-ET) of cristae membranes from bovine heart or rat liver mitochondria has shown that the ATP synthase dimers form long rows along highly curved membrane ridges (12). The dimer rows appear to play a major role in cristae formation and morphology. Deletion of the dimer-specific subunits e, f, or the first helix of subunit b in the peripheral stalk results in the formation of mitochondria with onion-like cristae (19, 20).

BN PAGE has suggested that bovine heart complex I forms supercomplexes with cytochrome c reductase (complex III) and cytochrome c oxidase (complex IV) (21). 3D maps of this supercomplex have been obtained by single-particle electron microscopy of negatively stained samples (22), and, most recently, cryo-EM. The three respiratory chain complexes are oriented in the supercomplex in a way that appears to be optimal for electron transfer and substrate shuttling. Each works as a proton pump, and together they generate the proton motive force (pmf) that drives ATP synthesis. The mutual arrangement of electron transfer complexes as proton sources and ATP synthase complexes as proton sinks in the membrane is therefore of fundamental interest and importance for understanding mitochondrial energy conversion.

Author contributions: W.K. designed research; K.M.D., M.S., B.D., J.H.K., A.R., and V.Z. performed research; electron cryotomography was performed by K.M.D., B.D., and M.S.; subtomogram averaging was performed by M.S. and K.M.D.; complex I labeling was performed by K.M.D.; mitochondria were prepared by J.H.K. (Saccharomyces cerevisiae), A.R. or K.M.D. (Yarrowia lipolytica), B.D. and K.M.D. (potato), and M.S. or K.M.D. (bovine heart); H.D.O provided Podospora anserina mitochondria; Y. lipolytica complex I was isolated and reconstituted by V.Z.; tomograms were analyzed by K.M.D. and B.D.; figures and supplementary movies were prepared by B.D., K.M.D., and M.S.; W.K. initiated and directed the study; K.M.D. and B.D. analyzed data; and K.M.D., B.D., and W.K. wrote the paper.

The authors declare no conflict of interest. This article is a PNAS Direct Submission. Freely available online through the PNAS open access option.

K.M.D., M.S., and B.D. contributed equally to the work.

1To whom correspondence should be addressed. E-mail: werner.kuehbrandt@biophys.mpg.de.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1103621108/*DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1103621108

PNAS | August 23, 2011 | vol. 108 | no. 34 | 14121–14126
To study the macromolecular organization of ATP synthase and complex I in the inner mitochondrial membrane, we imaged mitochondrial membranes and whole mitochondria from mammals, fungi and plants by cryo-ET. Long rows of ATP synthase dimers in cristae membranes were a universal feature of all mitochondria we investigated. The rows were confined to the highly curved edges of lamellar cristae and were formed by dimers with a constant $F_1$ head distance. These dimers corresponded to those with a dimer angle of $>70^\circ$ observed by single-particle analysis (16, 17). Complex I in the membrane was identified by its shape, or by specific quantum-dot labeling. Unlike the ATP synthase, this complex was irregularly distributed on the mostly flat membrane regions on either side of the ATP synthase dimer rows. The organization of these two large complexes was the same in all species investigated and is thus conserved during evolution. We propose that the conserved organization of respiratory chain complexes into regions that are either rich in ATP synthase or in complex I, and the extended rows of ATP synthase dimers on the cristae edges has important implications for ATP synthesis in mitochondria.

**Results and Discussion**

**Cryo-ET of Whole Mitochondria.** Cryo-ET is the method of choice for visualizing the three-dimensional organization of protein complexes in the membrane. Using this technique, we previously discovered long rows of ATP synthases dimers in membrane fragments of bovine heart and rat liver mitochondria (12). To find out whether this striking arrangement occurs also in intact, functional mitochondria and perhaps extends to other kingdoms, we performed cryo-ET on whole mitochondria from bovine heart (*Bos taurus*), potato tubers (*Solanum tuberosum*), and three species of fungi (*Saccharomyces cerevisiae*, *Yarrowia lipolytica*, and *Podospora anserina*).

In tomographic reconstructions, mitochondria appeared as dense, oblate ellipsoids with a diameter around 600–1200 nm (average 900 ± 165 nm) for all species except *P. anserina*, which were smaller. The mitochondria contained a complete outer membrane with an inner to outer membrane distance of 12 ± 2.5 nm and an intercristae membrane distance of 20 ± 4 nm, consistent with previous studies (11, 23). For potato and the three species of fungi, the cristae consisted of distinct flattened sacs, which merged with the inner boundary membrane in long slit-like junctions (20–300 nm) (Fig. S1A). At their edges in the matrix, cristae often had square corners where the membrane bent abruptly by approximately 90° (Fig. S2). This feature was common in *P. anserina* and potato but also occurred in *S. cerevisiae*. In bovine heart, the cristae formed networks of small lamellar patches connected to each other by short tubular segments, as in neuronal mitochondria (24). Cristae were connected to the inner boundary membrane by small circular junctions of 35 ± 5 nm diameter. The tubular cristae segments always originated from the edges of lamellar patches rather than from flat membrane regions (Fig. S1B).

**Rows of ATP Synthase Dimers in Whole Mitochondria.** *P. anserina* mitochondria proved to be the most suitable for examining the arrangement of the ATP synthase in undisrupted organelles. Mitochondria prepared from synchronized, 6-d-old *P. anserina* cells measured only 300 to 600 nm (average 450 ± 80 nm) in diameter and hence were more transparent compared to those from other species, allowing large protein complexes to be visualized in the tomographic volume. Biochemical assays of similar preparations (25) showed tightly coupled respiration, indicating that the isolated mitochondria were undamaged and active.

In *P. anserina* mitochondria we found double rows of 10-nm particles approximately 5 nm above the membrane, along the highly curved cristae edges (Fig. 1 and Movie S1). The particles were connected to the membrane by a stalk of 3–4 nm, giving them the characteristic lollipop shape of the $F_1$-Fo ATP synthase (10–12). The center-to-center distance between particles across the dimer rows consistently measured approximately 28 nm, whereas the head-to-head distance along rows varied from 12 nm to greater than 20 nm. We conclude that the double rows are loose arrays of ATP synthase dimers, arranged with their long axis perpendicular to the direction of the row. No ATP synthase dimers or higher oligomers were detected in the flat cristae regions, which suggests that, at least in this species, ATP synthase dimers are confined exclusively to the cristae edges.

In the larger mitochondria from bovine heart, potato, and *S. cerevisiae*, detailed membrane features were usually obscured by the dense matrix. Occasionally, rows of ATP synthase dimers were visible and could be traced for short distances (Figs. S2 and S3).
The arrangement of ATP synthase in these mitochondria was similar to *P. anserina*, although the distance between the *F₁* heads in a dimer from potato was larger. The occurrence of ATP synthase dimer rows in whole mitochondria shows that the linear arrays previously observed in mitochondrial membrane fragments (12) were not due to membrane disruption but are a fundamental feature of intact, active mitochondria.

**Subtomogram Averages.** To compare the membrane arrangement of ATP synthase dimers in more detail, we collected tomographic volumes of isolated cristae membranes and calculated subtomogram averages of dimers from all five species (Fig. 2). The signal-to-noise ratio in such preparations was considerably higher than in intact mitochondria, because the membranes were surrounded by dilute aqueous buffer rather than the dense mitochondrial matrix. Cristae membrane fragments were either found amongst intact mitochondria, or created by successive freeze-thaw cycles. Although cristae were predominantly lamellar in whole mitochondria (Fig. 1 and Fig. S1), the isolated membrane fragments were mostly tubular (fungi and potato) or disk-like (bovine heart). As in whole mitochondria, the dimer rows were more or less straight, and followed the undulations of the cristae edges. The distance between *F₁* heads in a dimer matched those measured in whole mitochondria (Fig. S4). This suggests that the dimer rows are not easily disrupted by mechanical force, and either remain unperturbed or reassemble in isolated cristae membranes. Subtomogram averages of the two yeasts and *P. anserina* partially resolved the peripheral stalk, which extended from the dimer interface in the membrane toward the *F₁* heads (Fig. 2B). The angle between monomers in the dimer was approximately 80° for bovine heart and the three species of fungi, and approximately 115° for potato correlating with the difference observed in *F₁* head distance of the dimers.

Single-particle analyses of detergent-solublized ATP synthase dimers from *S. cerevisiae* have indicated two different conformations: one with an angle >70° between the long axis of the monomers, and another with a smaller angle of approximately 40° (14–17). Clearly, all dimers we found in whole mitochondria and cristae fragments were of the former class with the wider dimer angle. Dimers with the smaller angle have been proposed to reflect the interaction of monomers along the rows (17). However, a dimer angle of 40° in this direction would result in an approximately 20-nm radius of curvature along the row, which we did not observe. Alternatively, the 40° dimers might be due to an interaction with the *IF₁* factor that binds to the *F₁* heads and inhibits ATP hydrolysis. *S. cerevisiae* dimers with the 40° angle were isolated at pH 7.0. It is known that mammalian *IF₁* is mainly dimeric at pH 6.5 (26). It is thus conceivable that the 40° dimers formed by association with dimeric *IF₁* during isolation. Indeed, single-particle analysis of bovine heart ATP synthase revealed a similar 40° dimer with a bridging density between the *F₁* heads (15), which was attributed to the *IF₁* protein. Accordingly, the dimers in the linear arrays we found in whole mitochondria and isolated cristae membranes would be the uninhibited dimer, whereas the 40° dimers seem to be the inhibited *IF₁* complex.

**Dimer Rows are Ubiquitous in All Mitochondria.** The linear rows of ATP synthase dimers contrast with the helical rows observed in the two protist species *Paramecium* and *Polytomella* (18, 27), which have been proposed to induce the formation of helical tubular cristae from flat membranes (28). The dimer rows in these species appear to be more regular, such that each dimer interacts specifically with its neighbors, each offset by a small angle [9° in *Polytomella* (27)]. This gives rise to a helical arrangement on the outer edge of spiralling tubular cristae (18). In the six species we investigated [including rat liver, (12)], no offset was observed between dimers, and thus the dimer arrays were never helical. Therefore, although details of molecular interaction within the dimer or between dimers along rows may vary, the linear arrays of ATP synthase dimers are a ubiquitous, fundamental feature in mitochondria of all eukaryotes, including protists (29).

Linear dimer rows induce tight bends or ridges in the inner membrane, often extending for several hundred nanometers. Our observation that the tightly bent membrane regions persist after disruption of the mitochondria supports our earlier conclusion that the dimers shape the membrane, rather than the other way round. The dimer rows thus exert a bending force on the lipid bilayer, and any newly added dimers converge at the point of highest membrane curvature, where the bending energy exerted by each dimer would be minimal. This suggests that the linear rows of ATP synthase dimers are sufficient to create cristae ridges, whereas other factors are probably required to generate or maintain cristae junctions.

**Complex I in Cristae Membranes.** Rectangular particles rising approximately 15 nm above the inner membrane surface were often seen in tomographic volumes of isolated cristae membranes of all species investigated except *S. cerevisiae* (Fig. 3). Comparison with tomograms of complex I reconstituted into proteoliposomes suggested that these densities were most probably complex I.

![Fig. 2](image-url)
like cristae vesicle, giving a ratio of complex I to ATP synthase of 1:3.6, very similar to what has been found biochemically (9). In the cristae, complex I thus occupies the membrane regions on either side of the dimer rows, which are more or less flat. The other two mitochondrial proton pumps, complex III and IV, which are too small to be detected by present cryo-ET techniques, most likely also occur in these membrane regions, and may form supercomplexes with complex I. Indeed, some of the complex I densities in potato and bovine heart resemble the shape and size of the supercomplex (22) (Fig. 3 C and D and Movie S3). We can exclude that, in the mitochondria we investigated, these complexes form respiratory strings (30–32), which would be easily visible by cryo-ET.

**Functional Implications.** ATP production in mitochondria is powered by the pmf, manifest in the electrochemical transmembrane gradient. The resulting current of protons through the $F_0$ rotor of the ATP synthase drives ATP production by rotary catalysis in the $F_1$ head on the matrix side of the membrane. The pmf is generated by complexes I, III, and IV, the proton pumps of the respiratory chain, which translocate protons across the membrane from the matrix side into the cristae space. The pmf has two components, one resulting from the pH difference across the membrane ($\Delta pH$), and the other from the membrane potential ($\Delta \psi$). In mitochondria and chloroplasts, the pmf is roughly 180 mV. In chloroplasts, this is mostly accounted for by the $\Delta pH$, whereas in mitochondria the $\Delta pH$ contribution is comparatively small. Given that the matrix pH is 7.9 (33), the cytosolic pH is 7.35 (34), and the outer membrane is freely permeable to ions, the nominal $\Delta pH$ across the mitochondrial inner membrane is 0.55 units, equivalent to 32 mV. However, in vitro studies indicate that the mitochondrial, chloroplast and bacterial ATP synthases all need a $\Delta pH$ close to 2 units, equivalent to approximately 120 mV, and a p-side pH close to 6, to produce enough ATP to sustain life (35–38). We propose that the apparent paradox of a $\Delta pH$ that is sufficiently low and a p-side pH that is too high for efficient ATP production is resolved by the special geometry of the mitochondrial cristae.

In a previous study (12), we calculated that the high degree of membrane curvature at the cristae ridges could accommodate a higher charge density for a given membrane potential, resulting in a local $\Delta pH$ increase of up to 0.5 units. Furthermore, an earlier modelling study by Cherepanov et al (39) found that protons...
released on the membrane surface encounter a potential barrier of 0.1 to 0.15 mV. The potential barrier makes it more difficult for the pumped protons to escape to the bulk solvent, resulting in a higher proton concentration, and consequently a lower pH, at distances up to 1 nm from the membrane surface. The effective pH in this boundary layer has been estimated at approximately 6, independent of the pH of the bulk solvent (39). The boundary layer, in combination with the higher charge density at the cristae ridges, would establish a proton gradient along the mitochondrial membrane, from the matrix through the ATP synthase rotor, driving ATP synthesis.

Matrices and Methods

Culture Conditions. Yarrowia lipolytica strain E129 (41), and Saccharomyces cerevisiae strain BY4741 (EUROSCARF, Germany) were grown under standard conditions in YPG and YPEG medium respectively. Podospora anserina strain "s" (42) was grown as described (43). Young P. anserina mitochondria were isolated from liquid cultures inoculated with 6-d-old mycelium.

Purification of Mitochondria. P. anserina and S. cerevisiae mitochondria were isolated as described in refs. 25 or 44, respectively. Y. lipolytica mitochondria were obtained by either method. Bovine heart mitochondria were isolated as described in refs. 45 and 46. Potato mitochondria were obtained as described in ref. 47 followed by a Percoll step gradient (26/46%) Whole mitochondria were fragmented by successive freeze-thaw cycles at −20°C.

Complex I Reconstitution and Labeling. Complex I was purified from Yarrowia lipolytica strain PIPO and reconstituted into liposomes as described (48). Labeling was performed on submitochondrial vesicles obtained from Y. lipolytica by osmotic shock. Membrane extracts were incubated for 1 h at 4°C with a primary antibody (Y37F3) against the matrix arm of complex I from Y. lipolytica (49), followed by a secondary anti-mouse IgG antibody conjugated with a quantum-dot emitting at 650 nm (Invitrogen). Unbound antibodies were removed by an Optiprep step density gradient centrifugation (20/24%, 80,000 × g, 30 min).

Cryo-ET and Subtomogram Averaging. Tomography was carried out as described (12, 43). All mitochondria samples were resuspended in trehalose buffer (250 mM trehalose, 10 mM Tris-HCl pH 7.4) immediately before plunge-freezing. Subtomograms were averaged in IMOD (50) after preorientation using manually picked contours which described the positions of the F1-Fo parts relative to the membrane. The final volume was 2-fold averaged.

ACKNOWLEDGMENTS. We thank Angelika Horst and Thorsten Blum for assistance in collecting tomograms; Alexandre Werner for preparing mitochondria from P. anserina; Julian Langer, Stephen Marino, Ilka Wittig and Götz Hofhaus for assistance in preparation of bovine heart mitochondria; Deryck Mills for maintaining the electron microscope facility; and Ernst Bamberg for helpful discussions. This work was supported by the Max Planck Society (W.K., K.D., M.S., B.D., and A.R.), the Deutsche Forschungsgemeinschaft (A.W., H.O., and V.Z.), and the Deutsche Forschungsgemeinschaft-funded Cluster of Excellence Frankfurt “Macromolecular Complexes” (W.K., H.O., and J.K.).
tion state of the bovine F1-ATPase inhibitor protein, IF1, by pH. J Biol Chem
275:25460–25464.
organization of ATP synthase in intact mitochondria determined by cryo-electron
drial F1FO-ATP synthase and role of Aα7 protein in stability and oligomycin resistance of
oxidative phosphorylation system by structural analysis of respiratory supercomplexes
electrophoretic analysis of respiratory supercomplexes from Yarrowia lipolytica.
Proteomics 9:2408–2418.
of cytosolic, mitochondrial, and Golgi pH in single living cells with green fluorescent
Δψ catalyzed by the active, reduced H(+)-ATPase from chloroplasts.
Biochim Biophys Acta
275:275–278.
by the H(+)-ATPases from Escherichia coli or chloroplasts reconstituted into liposomes.
tivity of water at the membrane interface: Effect on the energy coupling mechanism in
along the membrane surface and retarded surface to bulk transfer. Nature
42. Rizet G (1953) Impossibility of obtaining uninterrupted and unlimited multiplication
of the ascomycete Podospora anserina. C R Hebd Seances Acad Sci 237:838–840
(in French).
in Podospora anserina. Aging Cell 9:761–775.
b2 and cytochrome c peroxidase are located in the intermembrane space of yeast
45. Hackenbrock CR (1972) Energy-linked ultrastructural transformations in isolated liver
mitochondria and mitoplasts. Preservation of configurations by freeze-cleaving
46. Crane FL, Glenn JL, Green DE (1956) Studies on the electron transfer system. IV. The
oxidoreductase) from Yarrowia lipolytica reconstituted into proteoliposomes. Biochim
49. Zickermann V, et al. (2003) Functional implications from an unexpected position of the
50. Kremer JR, Mastronarde DN, McIntosh JR (1996) Computer visualization of three-
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, $28.6 \pm 3.0$ nm (127); *Y. lipolytica*, $28.0 \pm 2.4$ nm (223); *P. anserina*, $28.1 \pm 2.1$ nm (76); *S. cerevisiae*, $28.4 \pm 2.5$ nm (175); potato, $33.6 \pm 2.2$ 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 S2. 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)