Preparation of Bovine Heart Mitochondria. Beef heart was obtained from the slaughterhouse immediately after killing and stored on ice. All further steps were done in the cold room at 7 °C as described in Löw and Vallin (1) with slight modifications. Connective tissue and fat were removed and the heart was cut into pieces and ground in a kitchen blender. The ground meat was resuspended in buffer, containing 0.25 M sucrose, while the pH was continuously adjusted to 7.5 with 1 M Tris. The suspension was filtered through the double layer of cheesecloth and the heart cells were aliquoted to 500 g fractions. Each aliquot was resuspended in 1 L of 0.25 M sucrose, 0.015 M EDTA. The pH was adjusted to 7.4 with 1 M Tris. The cell suspension was homogenized in a kitchen blender at maximal speed for several minutes. The homogenate was centrifuged for 20 min at 1,200 × g. The red supernatant was filtered through a double layer of cheesecloth to remove lipids and centrifuged for 15 min at 10,000 × g. The red-brown sediment was resuspended in buffer, containing 0.25 M sucrose, 0.01 M Tris-HCl, pH 7.4, and homogenized manually with a glass-teflon homogenizer. The homogenate was centrifuged for 10 min at 17,000 × g. The sedimented brown layer of mitochondria was resuspended in 0.25 M sucrose and shock-frozen in liquid nitrogen.


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**Fig. S1.** (A) Fourier shell correlation (FSC) functions for all the cones around the unit sphere. A number of separate lines are indicated with different colors for clarity. Red line indicates the 0.5 information threshold criterion. (B) Distribution of resolution values from A. The horizontal axes expresses the resolution values and the vertical axes describes the number of cones with corresponding resolution. (C) Distribution of resolution values in different directions on unit sphere with the corresponding heat map.
Fig. S2.  (A) Euler angles conventions. The fixed xyz system is shown in blue, the rotated XYZ system is shown in orange, and the lines of nodes are shown in blue-green. (B) Angular distribution of ice-embedded respirasomes adsorbed on carbon support film. Most of respirasomes show a preferred top-view orientation (central cloud). The angle $\varphi$ (horizontal axis) describes the rotation around internal axis of respirasome and $\theta$ (vertical axis) describes the orientation of the molecules relative to the grid. The $\varphi$-$\theta$ scatter plot represents a 2D surface on a 3D sphere, allowing larger sampling space in the areas of the equator.

Fig. S3. Fitting of the density map of complex I from Yarrowia lipolytica (yellow) before erasing density of neighboring complex I molecules, X-ray structures of bovine complex III$_2$ (green), and complex IV (purple) to 3D cryoelectron EM map of respirasome (semitransparent gray).
**Fig. S4.** A classification of 3D subvolumes into five classes (1–5, Left). From the best three classes, the central sections 9–16 (out of 32) are shown. (Bottom Right) Difference map between reconstructions of classes 5 and 4, and shows the highest structural difference at the site of the hydrophilic arm of complex I (red asterisk).

**Fig. S5.** Fitting of the X-ray structures of complex I from *Thermus thermophilus* (yellow), bovine complex III (green), and complex IV (purple) to 3D cryoelectron EM map of respirasome (semitransparent gray).