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The Structure of Eukaryotic and Prokaryotic Complex I

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Abstract

The structures of the NADH dehydrogenases from *Bos taurus* and *Aquifex aeolicus* have been determined by 3D electron microscopy, and have been analyzed in comparison with the previously determined structure of Complex I from *Yarrowia lipolytica*. The results show a clearly preserved domain structure in the peripheral arm of complex I, which is similar in the bacterial and eukaryotic complex. The membrane arms of both eukaryotic complexes show a similar shape but also significant differences in distinctive domains. One of the major protuberances observed in *Y. lipolytica* complex I appears missing in the bovine complex, while a protuberance not found in *Y. lipolytica* connects in bovine complex I a domain of the peripheral arm to the membrane arm. The structural similarities of the peripheral arm agree with the common functional principle of all complex Is. The differences seen in the membrane arm may indicate differences in the regulatory mechanism of the enzyme in different species.

Keywords

NADH Oxidoreductase; 3D reconstruction; Electron Microscopy; Single particle reconstruction; Electron transport complex I; Mitochondria; Electron transport chain; bovine complex I; bacterial complex I; *Yarrowia lipolytica*; *Aquifex aeolicus*

Introduction

Complex I is the largest enzyme in the respiratory chain and is located either in the inner mitochondrial membrane of eukaryotes or in the cytoplasmic membrane in prokaryotes. Complex I oxidizes NADH, transfers two electrons to ubiquinone and translocates four protons across the membrane. The reduced ubiquinol is transferred to the bc₁ complex (III), which pumps protons to the outer membrane space and reduces cytochrome c (Cyt c). In cytochrome c oxidase (complex IV), Cyt c is oxidized, in connection with proton pumping. As a result of this process a membrane potential is created, which drives the ATP synthase

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(complex V) that phosphorylates ADP to produce ATP (for review see e.g. (Friedrich and Bottcher, 2004; Yagi and Matsuno-Yagi, 2003; Brandt, 2006).

The functional principles of complex I are still controversial. They include a direct coupling mechanism for electron transfer and proton translocation (Degli Esposti and Ghelli, 1994; Brandt, 1997), as well as an active proton pumping mechanism, powered by long range conformational changes that are initiated by the oxidation of NADH (Brandt et al., 2003), or a combination of both (Friedrich, 2001). In addition a conformationally driven gated proton pumping mechanism has been proposed by (Ohnishi and Salerno, 2005).

Complex I is a membrane protein with a molecular mass of close to 1 MDa in eukaryotes and above 550 kDa in prokaryotes. The eukaryotic complex consists of more than 40 individual subunits, while the minimal bacterial complex I has only 14. The 14 bacterial subunits are conserved throughout species, thus the eukaryotic complex consists of 14 subunits homologous to the bacterial subunits and more than 26 accessory subunits. There are functional differences between bacterial and mitochondrial complex I. For example, vertebrate complex I shows a deactive to active state transition when substrates are added (Minakami et al., 1964b; Minakami et al., 1964a; Kotlyar and Vinogradov, 1990; Maklashina et al., 2003; Galkin et al., 2008), while bacterial and many non-vertebrate complex Is do not. Complex I from *Y. lipolytica* shows this transition; however, the transition is faster than for bovine complex I. While many of the subunits in bovine and *Y. lipolytica* complex I are equivalent, *Y. lipolytica* complex I has at least three fungus specific accessory subunits that are not present in *Bos taurus* (bovine) complex I and bovine complex I contains ten mammalian specific accessory subunits with no counterpart in *Y. lipolytica* complex I (Morgner et al., 2008).

A high-resolution structure of the complete enzyme is still lacking. Most, if not all, of the structural information on the holo-enzyme has been obtained by 3D electron microscopy (Guénebaut et al., 1997; Guénebaut et al., 1998; Grigorieff, 1998; Böttcher et al., 2002; Radermacher et al., 2006; Clason et al., 2007). Only the X-ray structure of the hydrophilic region of complex I from *Thermus thermophilus*, containing seven of the conserved subunits has been solved (Hinchliffe and Sazanov, 2005; Sazanov and Hinchliffe, 2006).

Complex I when isolated shows large structural variation in electron micrographs. Eukaryotic complex I mainly shows differences in conformation, most obvious in the variation of the angle between matrix and membrane arm. Preparations of bacterial complex I are less stable, and in addition contain many fractured particles. The better preservation of eukaryotic complex I may be attributable to the presence of the accessory subunits, which stabilize the complex. Some of the remaining variability may be caused by destabilization of the enzyme when solubilized in detergent; however, parts of the conformational variations should be related to its function. If complex I contains a conformationally driven active proton pumping mechanism, then oxidation of NADH in the matrix arm would initiate conformational changes that are transferred to the proton pumping subunits found in the membrane arm.

The high flexibility of the enzyme requires the use of 3D reconstruction techniques that are proven to correctly handle large-scale heterogeneity. If such methods are not employed, the combination of images from different conformations can lead to artifactual structures. All techniques in 3D electron microscopy that are proven to yield correct results and have been successfully employed for the structure determination of asymmetrical particles with a mixture of local and global heterogeneity as seen in samples of complex I, require tilting. These techniques are tomography (Hoppe et al., 1986; Oettl et al., 1983; Knauer et al., 1983), random conical reconstruction (Radermacher, 1988; Radermacher et al., 1986) and

orthogonal tilting techniques (Leschziner and Nogales, 2006). When combined with extensive image classification (van Heel and Frank, 1981; Frank and van Heel, 1982; Marabini et al., 1996; Samsó et al., 2002) these techniques allow the selection of homogeneous subsets of particles and permit the separation of different conformations.

Previously, we have determined the structure of *Y. lipolytica* complex I (Radermacher et al., 2006). This 3D reconstruction revealed for the first time a clearly defined domain structure (Fig. 1). There are six major domains visible in the matrix arm (labeled 1–6), two protuberances (labeled CMP and DMP) on the matrix facing surface of the membrane arm and two protuberances that face the inter-membrane space (IP1, IP2). In 2D average images of *Y. lipolytica* complex I (not shown) occasionally a weak thin connection between domain 5 and the membrane arm protuberances could be observed which led to one of our functional models, where conformational changes in the matrix arm are transferred via a tether to the protuberances on the membrane arm presumed to be close to the proton pumping subunits (Fig. 2). In an alternative model the conformational changes would be transferred internally.

From difference imaging of the intact complex with a subcomplex lacking the 24 kDa and 51 kDa subunits, it was possible to assign the locations of these subunits to domain 1 (Clason et al., 2007). Fitting of the X-ray structure of the hydrophilic arm of complex I from *T. thermophilus* into the *Y. lipolytica* complex I structure has been ambiguous thus far and left five possible placements for subunits other than the 24 kDa and 51 kDa subunits (Table 1). Fit 1, with the 49 kDa subunit residing in domain 2 and the 75 kDa subunit in domain 5, fit 2 with the 75 kDa subunit in domain 5 and the 49 kDa subunit in domain 4, pointing downwards in the connection between matrix and membrane arm, leaving domain 2 empty, fit 3 with the 75 kDa subunit in domain 2 and the 49 kDa subunit in domain 5, fit 4 with the 75 kDa subunit in domain 2 and the 49 kDa subunit in domain 4, leaving domain 5 empty, and fit 5 with the 75 kDa subunit in domain 4 and the 49 kDa subunit in domain 2, again leaving domain 5 empty. These fits implied that the domains left empty should contain accessory subunits not present in the bacterial complex I.

In this paper we report the 3D reconstruction of bovine complex I and the 3D structure of complex I from *A. aeolicus*. The new structures reduce the possible positioning of the hydrophilic subunits. From the comparison of the three structures, a coherent structure for complex I in all species is emerging. In addition, differences in the membrane arm structure of eukaryotic complex I are becoming apparent.

Material and methods

Bovine heart mitochondria were solubilized using dodecylmaltoside (2.5 g/g protein) and bovine complex I was isolated by BN-PAGE essentially as described in (Schägger, 2003).

A. aeolicus cells were obtained from the Archaeenzentrum, Regensburg University, Germany. Purification of complex I was carried out as described in (Peng et al., 2003) with a modification in a final step: The detergent was exchanged to 0.2 % (w/v) Decyl- β -D-maltoside.

Samples were deep stained using a variety of different stains (Uranyl acetate, Phosphotungstic acid (PTA) (pH 7.2), Ammonium Molybdate (pH 7.2), NanoVan, NanoW, (Nanoprobes, Yaphank, NY)) were tested for best preservation and embedding results. For bovine complex I the best stain embedding was obtained with PTA and for *A. aeolicus* with NanoW. For deep stain preparation (Radermacher et al., 2001; Stoops et al., 1992; Ruiz and Radermacher, 2006), the samples were diluted to approximately 15 μ g/ml, applied in 5 μ l drops to continuous carbon coated grids, previously glow-discharged in air to render them hydrophilic. The excess liquid was blotted off and the grids were dried fast under a stream of

dry nitrogen. Embedding thickness was controlled by the flow rate of dry nitrogen, which controls the drying time.

Images were recorded in an FEI Tecnai 12 transmission electron microscope, equipped with a LaB₆ filament at 100 kV and a nominal linear magnification of 52kX. Tilt pairs were recorded on Kodak SO163 film, with the first image taken at a tilt angle of 55° to 60° and the second at 0°. All images were recorded under low-dose conditions with a dose of approximately 10 e⁻/Å² (Fig. 3).

Images were digitized with an Intergraph SCAI flatbed scanner using a pixel size of 7 μm. The images were converted to SPIDER format, binned down in size by a factor of 3 and converted to optical densities. The final pixel-size was calibrated using the power spectrum of tobacco mosaic virus, which had been mixed in with the sample. The resulting pixel size was 4.02 ± 0.03 Å. The microscope contrast transfer function (CTF) including defocus, astigmatism and amplitude contrast was determined for each micrograph as described in more detail in (Radermacher et al., 2001): A single transfer function was determined for the 0° micrographs. The tilt images were divided into smaller areas and the CTFs for each area were determined. From these values a function of an inclined plane was derived that describes the CTF in each point of the tilt image.

Image processing was carried out using SPIDER version 5.0 (Frank et al., 1996) with modifications, following a similar method as in (Radermacher et al., 2006). The 0° images were processed first, by centration, reference free alignment (Marco et al., 1996) multiple rounds of correspondence analysis (Frank and van Heel, 1982; van Heel et al., 1982), classification (Diday's method with moving centers (Diday, 1971), followed by hierarchical ascendant classification) and multi-reference alignment. Classification was always the last step before 3D reconstruction. All alignments were carried out using the simultaneous translational/rotational alignment between 2D Radon transforms (Radermacher, 1997; Radermacher, 1994). After classification of the 0° images, the tilt images were centered and for each class 3D reconstructions were calculated. Each initial 3D reconstruction was followed by at least two rounds of refinement. First a translational refinement of the tilt images was carried out, followed by a combined small range angular and translational refinement. For some reconstructions, the refinement procedure was iterated. For all refinements, a common line method was used, implemented as cross-correlations between the 2D Radon transforms of the projections and the 3D Radon transform of the reference volume (Radermacher, 1994; Radermacher, 1997). This cross-correlation algorithm contains a provision that areas in the Radon transform of the volume that do not contain data (missing cone, or any other irregular missing data) are removed from the cross-correlation, whose normalization is adjusted accordingly.

Volumes that were similar and differed mainly in orientation were aligned and their projection sets were merged. For volume alignment, one volume was selected as reference and Radon transformed in three dimensions. From all other volumes 0° projections were calculated. The 2D Radon transforms of the 0° projections were aligned in two steps to the reference 3D Radon transform (Ruiz et al., 2001). The first step was a rough alignment over the entire rotational range in 10° increments combined with a 10 pixel shift range, the second step was a fine alignment around the previous orientation within ± 28°, in 4° increments and a shift range of 10 pixels. For those data sets that were merged, the projection angles were updated with the Euler rotations of the volumes and reconstructions were calculated from the merged set, followed by translational and rotational projection refinements. All resolution values were measured with the Fourier ring correlation in 2D or the Fourier shell correlation in 3D (Saxton and Baumeister, 1982; Harauz and van Hell, 1986). The cutoff criterion used was 0.3 according to (Rosenthal and Henderson, 2003).

Results

Bovine complex I

The best staining conditions for bovine complex I were found using PTA. 78 tilt pairs were evaluated which yielded a total of 7729 single particle pairs (Fig. 3). The data set of bovine complex I exhibited fewer variations than observed previously in the sample of *Y. lipolytica* complex I (Radermacher et al., 2006). Therefore, the data set required fewer iterations of classification and multireference alignment. Alignment and classification yielded five major classes of particles (Fig. 4). The 2D averages of the five classes show obvious differences. The 2D average of class 3 with only 41 images has the lowest resolution with 42 Å. The resolutions of the 2D averages of classes 1 (203 images), 2 (1947 images) and 4 (1585 images) are close to 25 Å and the class 5 average (3715 images), which shows the particle flipped relative to the other classes has a resolution of 17.5 Å. The 3D reconstructions of the five classes, calculated from the corresponding tilt images show the differences more clearly (Fig. 5). The resolutions of the 3D reconstructions after refinement are: 40 Å for class 1, 34 Å for class 2, 88 Å for class 3, 36 Å for class 4 and 32 Å for class 5. Fig 5a shows the volumes without any relative alignments and fig. 5b shows the top view of all reconstruction. From the comparison of both views one can see the major differences between the class reconstructions. The main difference between the volumes determined for classes 1 and 2 is a relative rotation. The reconstruction of class 3 particles shows a rather undefined volume. In addition to a rotation with respect to the other volumes (Fig. 5a), the top view of the reconstruction of class 4 (Fig. 5b) shows a substantially more curved and broadened membrane arm than observed in the reconstructions of the other classes. The reconstruction of class 5 shows complex I turned over relative to the reconstructions of classes 1, 2 and 4. The major difference between classes 1, 2 and 5 is the orientation of the particles while the overall shapes are very similar. Therefore the three volumes were aligned as described above, and the data sets were merged. A reconstruction from the merged data set was calculated, and refined resulting in a 3D reconstruction with 27 Å resolution (Fig. 6). Clearly visible are the same domain structures previously seen in the 3D reconstruction of *Y. lipolytica* complex I, most obvious in the matrix arm. The distal membrane protuberance of bovine complex I appears larger than seen in the *Y. lipolytica* structure. Striking is the connection between domain 6 and a proximal protrusion on the membrane arm (labeled PMP in fig. 6), which had not been observed in the *Y. lipolytica* complex. Viewed from the intermembrane side, three protuberances, labeled IP1, IP2 and IP3, are visible.

Aquifex aeolicus complex I

The best staining of *A. aeolicus* complex one was obtained using NanoW. 89 tilt pairs were evaluated, yielding a total of 4537 single particle image pairs. The sample for the reconstruction of *A. aeolicus* complex I was substantially more heterogeneous than the samples from either bovine complex I or *Y. lipolytica* complex I as can easily be seen observed in the micrograph (Fig. 7). Because of the high heterogeneity and the tendency for aggregation even at high dilutions, the yield of good image pairs was substantially lower than for bovine complex I. In addition, the heterogeneity resulted in a separation of the data into a larger number of classes and the much lower number of projections in each class has led to a final 3D reconstruction at substantially lower resolution than obtained for *Y. lipolytica* or bovine complex I.

Correspondence analysis of the complete data set gives an overview over the variations observed (Fig. 8a). The first classification resulted in three major classes with 2D class-averages with resolutions of 30 Å, 33 Å, and 30 Å respectively (Fig. 8b). Class 1 and 3 show complex I flipped relative to each other. Class 2 shows a less defined particle with a smooth bend and no clear substructure. The differences are more clearly seen in the 3D

reconstructions calculated from the corresponding tilt data of each class (Fig. 8c). The reconstructions confirm the opposite orientation of the class 1 and class 3 particles. The 3D reconstruction of the class 2 data shows a smoothly bent particle that, when compared to the other two classes, seems to have two major domains in the peripheral arm missing. The resolutions of the three volumes were 49 Å, 49 Å and 47 Å. Inspection of the class members within each major class still showed substantial variability, and the data sets for classes 1 and 3, showing the most complete particles were separately processed further.

Class 1 was separated into 7 classes (Fig. 9a,b) and class 3 into 11 classes (Fig. 9c,d, Table 2). Missing of domains in the peripheral arm can be observed in subclasses 1.1–1.4, and 3.2–3.7 and 3.9; differences in the angle between matrix and membrane arm in subclasses 1.2, 1.3, 3.5; and global changes to the particle in classes 1.3, 1.5. Also differences in the length of the membrane arm can be observed. The low number of particles belonging to each class significantly limits the resolution. Therefore we decided to combine classes 3.8, 3.10 and 3.11, which show substantial similarity. Subclass 3.1 was not included because it exhibits a substantially shorter membrane arm. 3D alignment and the combined reconstruction of the merged classes 3.8, 3.10 and 3.11 were carried out as before. The final structure of the combined subclasses contains 431 particles and has a resolution of 45 Å (Fig 10).

The structure of *A. aeolicus* complex I is an L-shaped molecule. The major domains 1,2 and 5 observed in bovine and *Y. lipolytica* complex I are clearly visible also in the bacterial enzyme. If the minor domains 3, 4 and 6 are present cannot be decided at this limited resolution. The membrane arm of *A. aeolicus* complex I shows a similar slight curvature as had been observed for the eukaryotic complex.

In comparison (Fig. 11), all three complexes are L-shaped molecules, with clearly identifiable domains in the matrix arm. The matrix arms exhibit small differences in rotation around their axis and small differences in the angle between the two arms. These relative rotations are not significant differences between complex I from the different species, but variations of these angles can be found within each sample. In *Y. lipolytica* complex I we have previously observed rotations around the matrix arm axis within a range of $\pm 10^\circ$ and variations in the angle between matrix and membrane arm of more than 50° . All three complexes show a slightly curved membrane arm. In eukaryotic complex I distinct protrusions on both sides of the membrane arm are visible.

Discussion

Our structure of bovine complex I is in excellent agreement with the structure of complex I from *Y. lipolytica*. All the domains (1–6) found in the Matrix arm of the *Y. lipolytica* enzyme are also present in our reconstruction of the bovine complex.

Except for the basic L-shape, the previously published structure of bovine complex I (Grigorieff, 1998) does not agree with our results. The lack of detail in the matrix arm of the earlier published bovine structure is most easily explained by a lack of classification methods used at the time for the 3D reconstruction of this structure. In addition, the 3D reference used in this earlier work had the assignment of membrane and matrix arm reversed.

On the membrane arm of eukaryotic complex I a distal protuberance (labeled DMP in figs. 1 and 6) and an additional, either central (labeled CMP in fig. 1, *Y. lipolytica*) or proximal (labeled PMP in fig. 6, *B. taurus*) protuberance can be observed. In the top view one can recognize that PMP is in a position closer to the center/opposite side of the membrane arm when compared to CMP in *Y. lipolytica*. PMP is connected to domain 6 in the matrix arm,

which also can be confirmed in a true density display. In the *Y. lipolytica* enzyme, this connection between domain 6 and the membrane arm cannot be observed even at very low threshold levels. The different location of the membrane protuberances could indicate that they are formed by subunits that are not in common to the two complexes. This difference would imply that they are not involved in essential parts of the proton pumping mechanism common to all versions of complex I. A transfer of conformational changes from the matrix to the membrane arm mediated by a tether, therefore, may not be part of the essential mechanism (Fig. 2). Alternatively, although unlikely, the subunits comprising CMP and PMP could be fragile and may have been lost in the different purification procedures. CMP, however, could still be present in bovine complex I, since it shows a more extended distal membrane protuberance, which is sufficiently large to accommodate both the distal and central membrane protuberances observed in the *Y. lipolytica* complex.

Both, bovine and *Y. lipolytica* complex I exhibit a slow transition from an inactive to an active form upon the addition of substrates. This transition is faster in *Y. lipolytica* and slower in bovine complex I. The connection of domain 6 could be involved in this mechanism and might slow down this transition in the bovine complex (Vinogradov, 1998).

More differences can be observed when viewed from the intermembrane space. *Y. lipolytica* complex I contains one larger protrusion towards the distal end (IP1, Fig. 1), and a ridge at the matrix arm end of the complex (IP2, Fig. 1). In bovine complex I the protrusion at the distal end appears divided into two smaller domains labeled IP1 and IP3 (Fig. 6c). The ridge visible near the matrix arm connection of the *Y. lipolytica* complex, IP2, becomes a strongly pronounced protrusion in bovine complex I. While most of the subunits in both, bovine and *Y. lipolytica* complex I are known, at this time there is insufficient information about their location to identify them with specific protrusions. Only 3D labeling studies could allow for a unique assignment of these domains.

The overall shape of our reconstruction of *A. aeolicus* complex I is in good agreement with the early reconstruction of *Escherichia coli* complex I (Guenebaut et al., 1998), the main difference being the clearer definition of the major domains in the matrix arm in the reconstruction of complex I from *A. aeolicus*. We have not found any subset of particles in any of our classes that would result in a U-shaped bacterial complex I, as was reported for the *E. coli* complex I in (Böttcher et al., 2002). For comparison we also imaged a sample of *E. coli* complex I (data not shown). Similarly to *A. aeolicus*, *E. coli* complex I presents a quite fragile sample, resulting in high heterogeneity when observed in the electron microscope. We have observed many complexes dissociated into membrane and matrix arm, as well as other fragments. Some intact particles showed the typical L-shape and densities indicating the presence of domains 1, 2 and 5.

All three complexes presented here are L-shaped particles, and all three clearly show the domain containing the 24 and 51 kDa subunits (domain 1), and domains 2 and 5. The fact that domain 2 is present in bacterial complex I removes one of the possible fits of the X-ray model of the hydrophilic portion of complex I from *T. thermophilus* to *Y. lipolytica* complex I, presented in our previous study (Clason et al., 2007). Fit number 2 left domain 2 empty and would have required this domain to consist of accessory subunits present only in eukaryotes. Since this domain is present in the bacterial complex, fit 2 can now be discarded. The current low resolution of our 3D reconstruction of *A. aeolicus* complex I, however, does not justify a more detailed study of possible fits. At this resolution the sizes of the domains are still unreliable, and the connection to the membrane arm is insufficiently resolved to decide if one or two connections are present. However, when the X-ray structure is manually placed in a position that fills all three visible domains and keeps iron sulfur cluster N2 closest to the membrane, corresponding to an intermediate position relative to our

previous fits 3 and 4 (Clason et al., 2007), the distance between N2 and the surface of the membrane arm is still ~ 30 Å. This is larger than required for a model of direct coupling between electron transfer and proton pumping and is consistent with our previous results (Clason et al., 2007).

We have determined the 3D structure of complex I from bovine heart mitochondria and from the bacterium *A. aeolicus*. The comparison of the two reconstructions with the earlier reconstruction of complex I from *Y. lipolytica* shows that the basic shape of the complex is preserved throughout species. The main domains in the peripheral arm of complex I can be observed in eukaryotes and bacteria. Differences in the domains visible in the membrane arm of bovine and yeast complex I may point to a different regulatory behavior of these two enzymes. In addition, the differences in the membrane arm protrusions may suggest that the central (*Y. lipolytica*) and proximal (*B. taurus*) membrane arm protuberances do not contain any of the 14 central subunits, common to eukaryotes and bacteria.

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Figure 1.

Structure of *Y. lipolytica* complex I (Radermacher et al., 2006). The matrix arm clearly shows six domains (numbered 1 – 6). The membrane arm shows a distal membrane arm protuberance (DMP) and a central membrane arm protuberance (CMP). The surface facing the inter-membrane space shows two major protuberances IP1 and IP2.

Figure 2.

Hypothetical model of a conformationally driven proton pumping mechanism. As observed occasionally in 2D averages of *Y. lipolytica* complex I domain 5 may be connected via a tether to the membrane protuberances, facilitating the transfer of conformational changes in the matrix arm to locations close to the proton pumping subunits in the membrane arm. Alternatively the conformational changes may be transferred through a more internal conformational coupling mechanism. (from <http://physiology.med.uvm.edu/radermacher/>, with permission)

Figure 3. Parts of a tilt pair of bovine complex I. a) tilt image, b) 0°-image. Dashed line tilt-axis. Arrows indicate typical particle pairs that were selected from the micrographs. Scale bar 100nm.

Figure 4.

a) Results of correspondence analysis of the 0°-images of bovine complex I. Shown is the visual representation of the map 1 vs. 2. Each image represents an average image of all single images whose coordinates fall within the same square. b) The five class averages (numbered 1–5) obtained after moving center classification followed by hierarchical ascendant classification. Scale bar 10 nm

Figure 5.
3D volumes of the five classes shown in figure 4b (same numbering). a) All five volumes without any relative alignment. b) Top views of the five reconstructions, aligned visually. The main difference between classes 1,2 and 5 is orientation. Scale bar 10 nm

Figure 6.

The 3D structure of bovine complex I. The same domains as found in the *Y. lipolytica* complex I appear in bovine complex I (numbered 1–6). The distal membrane protuberance is present (DMP). A second, proximal membrane protuberance (PMP) appears in a location different from CMP found in *Y. lipolytica* complex I, and connects to domain 6. Protrusion DMP is larger than in the *Y. lipolytica* enzyme and may include CMP. The intermembrane face of bovine complex I shows three protuberances IP1 – IP3. IP1 and IP3 in bovine complex I may correspond to the single IP1 in *Y. lipolytica* complex I. Scale bar 10 nm.

Figure 7. Parts of a tilt pair of complex I from *A. aeolicus*. a) tilt image, b) 0°-image. Dashed line tilt-axis. The 0°-image most clearly shows the high heterogeneity of the sample and the frequent overlap of particles. Arrows indicate typical particle pairs that were selected from the micrographs. Scale bar 100nm.

Figure 8.

a) Results of correspondence analysis of the 0°-images of complex I from *A. aeolicus*. Shown is the visual representation of the map 1 vs. 2. b) The three class averages obtained after moving center classification followed by hierarchical ascendant classification (numbered 1–3). c) Volumes reconstructed from the tilt images belonging to the same three classes. Volumes in their original relative orientations. No volume alignment has been applied. Scale bars 10 nm.

Figure 9. Sub-classification of classes 1 and 3 shown in Fig. 6 of the image data of *A. aeolicus*. a) b) Class 1 divided into seven subclasses. a) Class averages numbered 1.1 – 1.7, b) 3D reconstructions for classes 1.1 – 1.7. c, d, Class 3 was divided into 11 subclasses. c) Class averages numbered 3.1–3.11, d) 3D reconstructions for classes 1.1 – 1.11. Scale bars 10 nm.

Figure 10.
3D reconstruction of complex I from *A. aeolicus* calculated from the merged data set from classes 3.8, 3.10 and 3.11. Clearly recognizable are domains 1, 2 and 5. Scale bar 10 nm.

Figure 11.

Comparison of the three structures of complex I from the yeast *Y. lipolytica*, the mammalian bovine complex I and the bacterial complex I from *A. aeolicus*. Resolutions from left to right: 24Å, 27Å, 45Å. Scale bar 10 nm.

Table 1

Overview of the prior results of fitting the X-ray structure of the seven preserved hydrophilic subunits of complex I from *T. thermophilus* to the EM structure of complex I from *Y. lipolytica*. The numbers indicate which major subunit (bovine nomenclature) is located in which domain of *Y. lipolytica* complex I for each of the five fits.

Domain	Fit 1	Fit 2	Fit 3	Fit 4	Fit 5
1	51	51	51	51	51
2	49	---	75	75	49
4	---	49	---	49	75
5	75	75	49	---	---

“—” indicates that this domain is left empty, and therefore should contain accessory subunits in eukaryotic complex I.

Table 2

Results of the sub-classification of primary classes 1 and 3 of *A. aeolicus* complex I data. Class 1 was split into 7 subclasses; class 3 was divided into 11 subclasses. Indicated are the resolution of the 2D image average of each class, the resolution of the 3D reconstruction of each class and the number of images in each class. No 2D average could be obtained for the combined classes, because the particles are in different orientations.

Subclass	2D resolution in Å, FRC with cutoff 0.3	3D resolution in Å, FSC with cutoff 0.3	Number of images
1.1	40.0	63.2	223
1.2	36.1	55.8	146
1.3	33.6	61.7	118
1.4	51.4	65.8	139
1.5	50.5	out of limit	32
1.6	33.2	58.6	244
1.7	29.3	51.9	404
3.1	30.1	49.0	181
3.2	50.2	67.4	41
3.3	33.8	65.4	61
3.4	33.2	55.9	250
3.5	35.6	94.2	51
3.6	35.5	56.0	110
3.7	31.6	53.2	210
3.8	29.8	59.9	158
3.9	34.6	83.7	50
3.10	29.7	50.8	163
3.11	31.0	51.8	110
combined 3.8, 3.10, 3.11	n/a	45.2	431