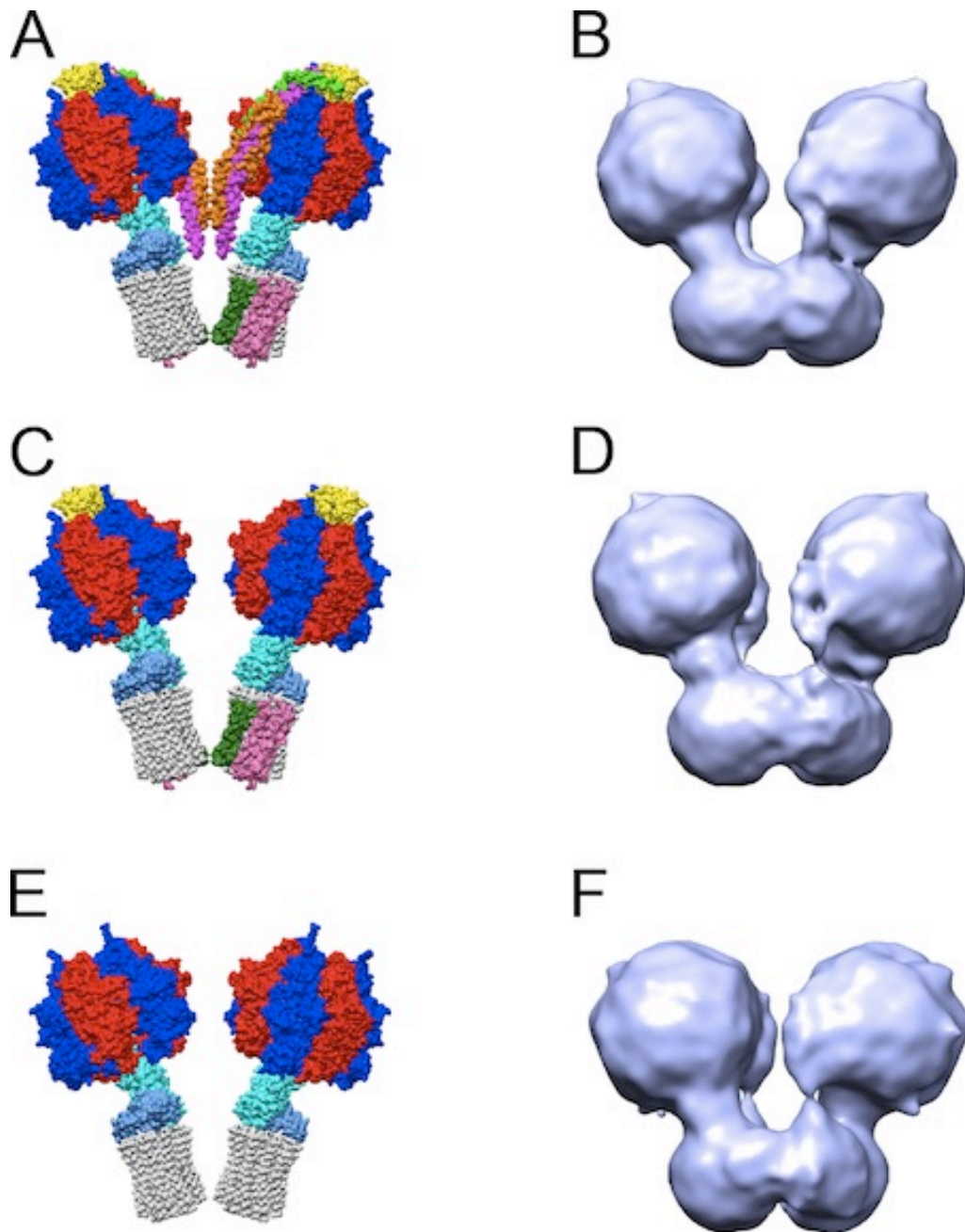
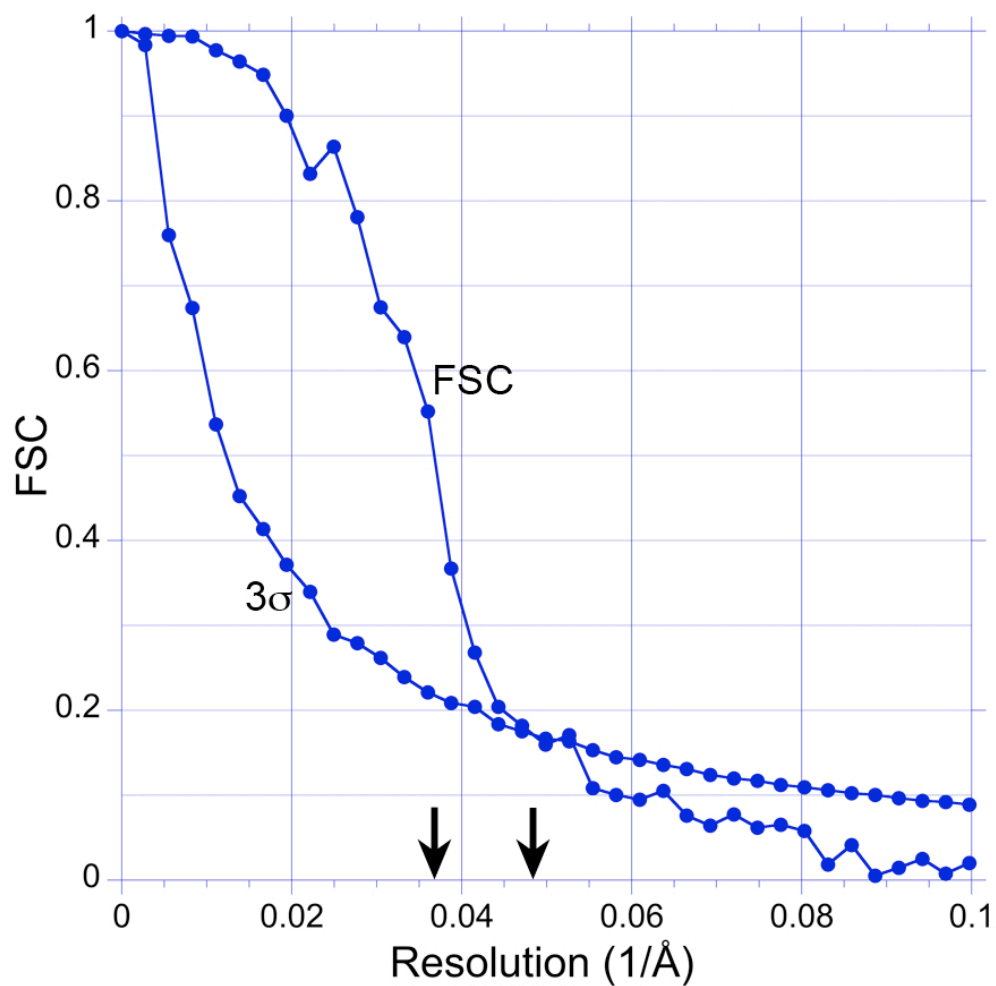


**Figure S1:** Three-dimensional reconstruction of the ATP synthase dimer using the atomic model shown in Fig. S2 to start up the alignment. As can be seen in (A,B), the atomic model fits well except for the peripheral stalks (see arrows and arrowheads in (B)) and a slightly different dimer angle as evident from the unfilled volume in (see arrowheads in (A)). The atomic model was subsequently ‘re-fitted’, (C,D) guided by the EM density (for the lower portion of the peripheral stator) and by the recent crystallographic structure of the  $F_1$ -stalk complex (2wss of ref. 53; for the upper portion of the stator); in this alignment cycle subunits *a* and *b* from *E. coli* were omitted from the atomic reference model. Note that deposited coordinates for the  $F_1$ -stalk structure (2wss of ref. 53) only contain coordinates for parts of the peripheral stalk subunits (*b*, *d* and  $F_6$ ), while the earlier crystal structure of the isolated peripheral stalk complex (2cly, ref. 48) is more complete. See main text for cited references.



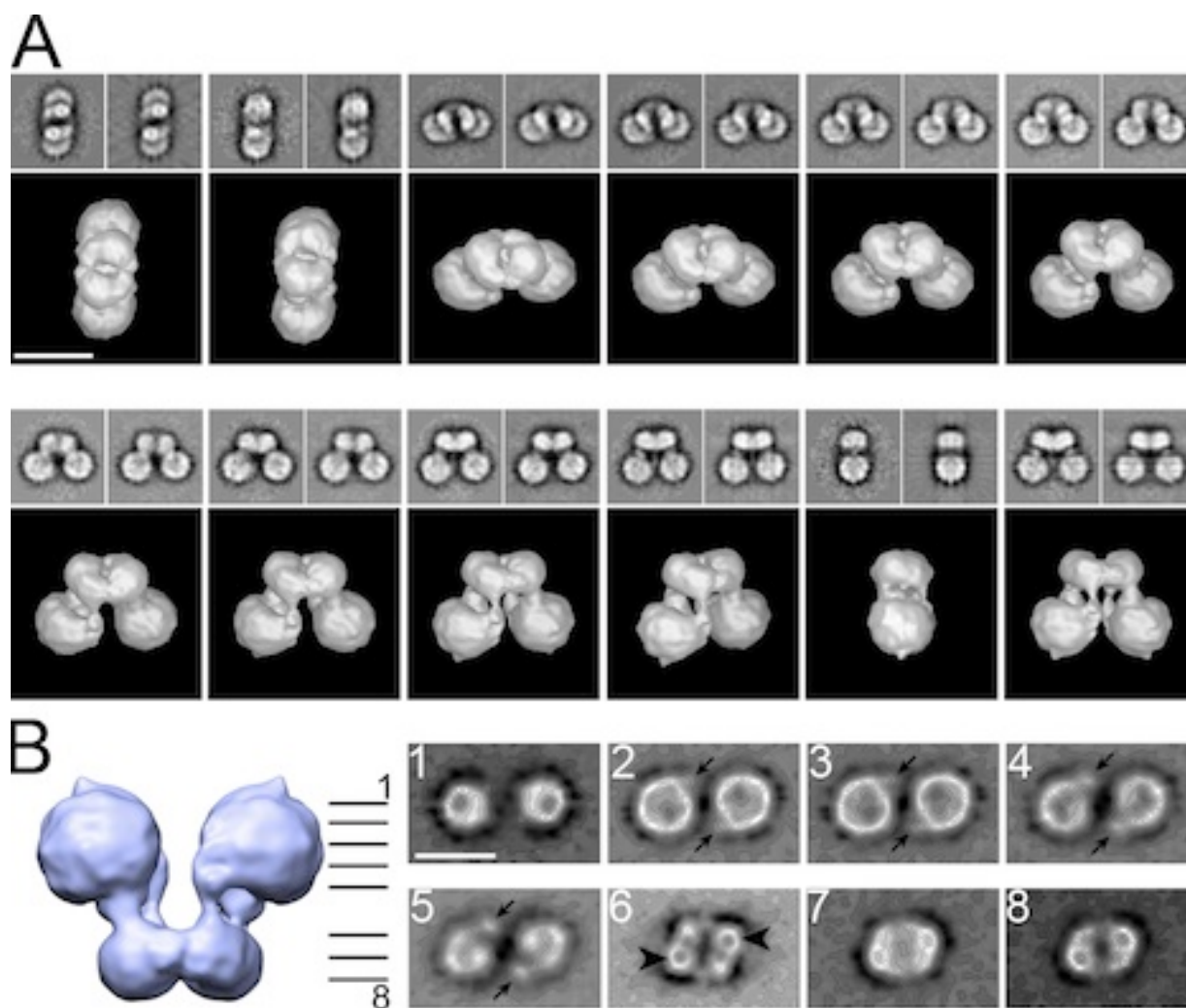
**Figure S2:** (A,C,E) atomic models of dimeric ATP synthase used for alignment of the electron microscopic images. The models in (A,C,E) contained  $\alpha_3\beta_3\gamma\delta\text{OSCP}_{nt}ab_{nt}b_{ct}df_6c_{10}$ ,  $\alpha_3\beta_3\gamma\delta\text{OSCP}_{nt}ab_{ct}c_{10}$  and  $\alpha_3\beta_3\gamma\delta c_{10}$ , respectively. (B,D,F), 3-D reconstructions obtained by using projections from the corresponding atomic models shown in (A,C,E). For PDB codes and references see main text.

Figure S3



**Figure S3:** Resolution of the final model estimated by Fourier shell correlation. At 0.5 correlation, the resolution is 27 Å and at  $3\sigma$ , the resolution is 21 Å.

Figure S4



**Figure S4:** (A) Surface representations with corresponding input (left) and re-projection (right) above the images. The top left view is from the inter membrane space side and the bottom right image is the side view. (B) 3-D surface representation with contoured cross sections as indicated by the lines 1-8. The arrows in images 2-5 indicate the position of the peripheral stalk while the arrowhead in image 6 points to the position of the *c* subunit ring.