

Supplementary Figure S1: F-type rotation axes are off-center from  $(\alpha\beta)_3$  axes

F-type rotation axes off-center from  $(\alpha\beta)_3$  axes (see Table 1 for values of inclination). Crystal structures of yeast and bovine  $F_1c_n$  complexes, with the central axis through the catalytic  $(\alpha\beta)_3$  head and the axis through the center of the c-ring labelled. (**a**)  $F_1c_{10}$   $(2WPD)^{28}$ , (**b**)  $F_1c_{10} (2XOK)^{27}$  and (**c**)  $F_1c_8 (2XND)^{29}$ .



Supplementary Figure S2: PS1, PS2 and PS3 fitted to the EM density of the proximal peripheral stalk

PS1 (a), PS2 (b) and PS3 (c) fitted into the EM density of the proximal peripheral stalk<sup>10</sup>. Correlation coefficients for the fit calculated by Chimera<sup>35</sup> are 0.86, 0.83 and 0.92 respectively. (d) EM density of intact  $Tt_A$ -ATPase, with composite model showing good fit of the PS1 structure into distal peripheral stalk EM density. (e) EM density of intact  $Tt_A$ -ATPase, with composite model showing the inferior fit of PS1 into proximal peripheral stalk EM density. (f) EM density of intact  $Tt_A$ -ATPase, with composite model showing the inferior fit of PS1 into proximal peripheral stalk EM density. (f) EM density of intact  $Tt_A$ -ATPase, with composite model showing improved fit of the PS3 model into proximal peripheral stalk EM density.



Normal Mode Analysis of PS2. Comparison of crystal and EM models with their simulated NMA counterparts using modes 7 ( $\omega = 15.9 \text{ cm}^{-1}$ ) and 8 ( $\omega = 31.2 \text{ cm}^{-1}$ ) applied to PS2. (**a**) PS1 crystal structure in green and PS2->PS1 NMA model (A = -124 and 82) in cyan. (**b**) PS3 EM model in green and PS2->PS3 NMA model (A = -282 and 86) in cyan.

Supplementary Figure S4: Right-handed coiled-coil domain architecture of the peripheral stalk complex



Right-handed coiled-coil domain architecture of the peripheral stalk complex (**a**) as encoded in hendecad (11-mer) and quindecad (15-mer) sequence repeats in subunits E (**b**) and G (**c**) – adapted from reference (26).

## Supplementary Figure S5: Flexibility of a right-handed coiled-coil



Example of right-handed coiled-coil consisting of pure hendecad repeats leading to parallel helices (pdb entry 1fe6<sup>61</sup>) with arrow indicating the direction of greatest flexibility according to NMA. Parallel coils are flexible in a plane perpendicular to the broad face of the coil (which has the least cross-sectional area), similar to the mechanical properties of a rectangular beam or that of a ruler.

## **Supplementary Figure S6: Example electron density**



Stereo view of the 2Fo-Fc electron density map of the peripheral stalk from  $Tt_ATP$  as to 2.25 Å resolution. Region shown corresponds to residues 37-61 of subunit E and 58-84 of subunit G, with  $\sigma$  cut-off set to 1.0.

## **Supplementary References:**

- 61. Stetefeld, J. et al. Crystal structure of a naturally occurring parallel righthanded coiled coil tetramer. *Nature structural biology* **7**, 772-6 (2000).
- 62. Drory, O., Frolow, F. & Nelson, N. Crystal structure of yeast V-ATPase subunit C reveals its stator function. *EMBO reports* **5**, 1148-52 (2004).