Structure of ATP synthase under strain during catalysis

Hui Guo \(^1,2\) & John L. Rubinstein \(^1,2,3\)

ATP synthases are macromolecular machines consisting of an ATP-hydrolysis-driven F\(_1\) motor and a proton-translocation-driven F\(_0\) motor. The F\(_1\) and F\(_0\) motors oppose each other’s action on a shared rotor subcomplex and are held stationary relative to each other by a peripheral stalk. Structures of resting mitochondrial ATP synthases revealed a left-handed curvature of the peripheral stalk even though rotation of the rotor, driven by either ATP hydrolysis in F\(_1\) or proton translocation through F\(_0\), would apply a right-handed bending force to the stalk. We used cryoEM to image yeast mitochondrial ATP synthase under strain during ATP-hydrolysis-driven rotary catalysis, revealing a large deformation of the peripheral stalk. The structures show how the peripheral stalk opposes the bending force and suggests that during ATP synthesis proton translocation causes accumulation of strain in the stalk, which relaxes by driving the relative rotation of the rotor through six sub-steps within F\(_1\), leading to catalysis.
ATP synthases use a transmembrane electrochemical proton motive force (pmf) to generate adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (Pi). The enzyme complex consists of two molecular motors positioned to oppose each other’s action on a shared rotor subcomplex (Fig. 1a, left). The membrane-embedded F_O motor is driven by proton translocation across the membrane through two offset half channels\(^1,2\) while the soluble F_1 motor is powered by ATP hydrolysis. In *Saccharomyces cerevisiae*, the FO region contains subunits a, e, f, g, i/j, k, 8, part of subunit b, and the c\(_{10}\)-ring of the rotor\(^3\), while the F_1 region includes a trimer of catalytic subunit \(\alpha\beta\) pairs and subunits \(\gamma\), \(\delta\), and \(\varepsilon\) from the rotor\(^4\). Coupling between F_1 and FO requires that the two motors are held stationary relative to each other by a peripheral stalk subcomplex (Fig. 1a, green structure), which in yeast is formed from subunits b, d, h, and OSCP (the oligomycin sensitivity conferral protein).

During ATP synthesis, proton translocation through FO at the interface of subunit a and the c-ring causes the \(\gamma\delta e c_{10}\) rotor (Fig. 1a, outlined in black) to turn. Rotation of subunit \(\gamma\) within F_1 causes the \(\gamma\) subunit to turn in the opposite direction, rotating the proton-carrying c-ring against subunit a in FO and pumping protons across the membrane. Even with the rotor turning at hundreds of revolutions per second\(^5,6\) there is little or no ‘slip’\(^7\) and the \(H^+:ATP\) ratio remains constant. In *S. cerevisiae* this ratio is 10:3 due to the ten proton-carrying c subunits in FO and three catalytic sites in F_1\(^8\). With this \(H^+:ATP\) ratio, when 10\(\times\) the free energy of proton translocation (\(\Delta \mu_{H^+}\)) is more negative than 3\(\times\) the free energy of ATP hydrolysis (\(\Delta G_{ATP}\)) the FO motor overpoweres the F_1 motor, forcing it to synthesize ATP.

![Fig. 1 Rotation in ATP synthase.](image-url)
3 × ΔG_{ATP} is more negative than 10 × Δμ_{H^+}, F_1 overpowers F_0 and the enzyme hydrolyzes ATP to pump protons.

Synthesis or hydrolysis of each ATP molecule is associated with a ~120° rotation of the γ subunit within F_1, leading to conformations of the enzyme known as rotational State 1, 2, and 3 Ref. 5. During ATP hydrolysis, which is better studied than ATP synthesis, this ~120° rotation is broken down into a ~40° sub-step as the enzyme transitions from a 'catalytic dwell' to a 'binding dwell', and an ~80° sub-step as the enzyme transitions to the next catalytic dwell13-15,12 (Fig. 1b, clockwise). ATP hydrolysis likely occurs during the ~40° sub-step while ATP binding likely occurs during the ~80° sub-step12,14. Consequently, the expected sequence of states for a 360° rotation of the rotor during ATP synthesis is State 1-binding → State 1catalytic → State 2-binding → State 2catalytic → State 2binding → State 3catalytic (Fig. 1b, counter-clockwise). The mismatch between these six sub-steps in F_1 and the ten proton-translocation steps in F_0 suggests that the enzyme cycles between strained and relaxed conformations during catalysis15,14.

Early cryoEM noted that the peripheral stalks of mitochondrial ATP synthases have a left-handed curvature17,18 (Fig. 1a, left). However, torque applied to the rotor following proton translocation through F_0 (Fig. 1a, right, blue arrows) would tend to rotate the αβ₃ hexamer in the same direction as the torque, inducing a right-handed curvature of the peripheral stalk as it resists the rotation. Similarly, the opposite torque applied to the opposite end of the rotor by ATP hydrolysis in F_1 (Fig. 1a, right, red arrows) would tend to rotate the membrane-embedded region of F_0 along with the c-ring, also inducing a right-handed curvature of the peripheral stalk as it resists the rotation17. Previously observed structures were obtained in the absence of a pmf or free ATP17-20, suggesting that the peripheral stalk may act as a spring that has a left-handed curvature when relaxed but a right-handed curvature under strain during catalysis17.

Results and discussion

The peripheral stalk shows pronounced bending under strain during ATP hydrolysis. We purified S. cerevisiae ATP synthase with the detergent n-Dodecyl-β-D-Maltopyranoside (DDM), which results in a monomeric preparation of the enzyme30,31, and determined its structure by cryoEM (Supplementary Figs. 1 and 2, Supplementary Tables 1 and 2). A high-resolution map of the intact complex was generated by combining multiple maps from focused refinements (Fig. 1c and Supplementary Fig. 1c). In this map, the peripheral stalk shows the left-handed curvature seen previously. Three-dimensional (3D) classification allowed particle images to be separated into six rotor positions, corresponding to the catalytic and binding dwell structures for each of the three main rotational states. These conformations resemble recent catalytic and binding dwell structures for an isolated bacterial F₁ subcomplex imaged during ATP hydrolysis, where the absence of the peripheral stalk resulted in all catalytic dwell structures being identical and all binding dwell structures being identical32. From ATP synthase imaged without ATP, the catalytic dwell structures show αβ₃high either in the open conformation lacking nucleotide or in a closed conformation with weak nucleotide density, and the binding dwell structures show αβ₃high only in an open conformation without nucleotide (Supplementary Fig. 3). The existence of αβ₃high in an open conformation without visible nucleotide density is likely an artifact from loss of ATP during the purification of the enzyme. Further classification of the State 1catalytic conformation resulted in classes distinguished by variability in the position of the peripheral stalk and a slight rotation of the rotor relative to subunit a. These classes were designated as State 1catalytic(a) (Fig. 1d, light blue) to State 1catalytic(d) (Fig. 1d, dark blue) in order of increasing straightening of the peripheral stalk (Supplementary Movie 1, ‘no ATP’ condition). As these structures were determined in the absence of free ATP, they likely represent energetically similar conformations that can be reached by thermal fluctuation of the enzyme structure20,28.

To test the hypothesis that the peripheral stalk of ATP synthase deforms under strain, we next added ATP to the preparation and froze cryoEM specimens. The presence of ATP in the cryoEM sample buffer introduces a concentration-dependant background noise and loss of contrast in images of DDM-solubilized ATP synthase (Supplementary Fig. 5A). Therefore, a delicate balance is required to provide sufficient ATP in solution to ensure that the enzyme is active as grids are frozen, while simultaneously maintaining sufficient contrast and image quality for image analysis. Initially, a small dataset of images was collected with a screening electron microscope where 10 mM ATP was added to the preparation at 4 °C and cryoEM specimens were frozen within 10 s. Analysis of this dataset revealed conformations of the enzyme not seen in the absence of free ATP (Supplementary Fig. 4). Therefore, a large dataset was collected for the specimen with a high-resolution microscope (Supplementary Fig. 5). Classification of the resulting dataset yielded maps showing six different F₁ states, corresponding to the catalytic and binding dwell structures from each of the three main rotational states. Subclassification of these populations separated each catalytic and each binding state into conformations with increasing rotation of the rotor relative to subunit a, and increasingly strained peripheral stalks, designated as ‘a’, ‘b’, ‘c’, etc. Overall, 27 unique conformations were identified: State 1binding(a to d), State 1catalytic(a to b), State 2binding(a to b), State 2catalytic(a to c), State 3binding(a to c), and State 3catalytic(a to e). Overlaid the eight State 1catalytic(a to h) structures reveals that during ATP hydrolysis the peripheral stalk exhibits a large bending motion, transitioning from a left-handed curvature (Fig. 1e, light purple) to the predicted right-handed curvature (Fig. 1e, dark purple; Supplementary Movie 1, ‘During ATP hydrolysis’ condition). Without ATP and during ATP hydrolysis, the left-handed curvature of the peripheral stalk remains the most highly populated conformation of the enzyme (Fig. 1f).

The flexible peripheral stalk accommodates rigid rotation of the rotor during ATP hydrolysis. To facilitate comparison of the ATP synthase conformations that occur during ATP hydrolysis, backbone models of the protein structure were fit flexibly into each of the 27 maps (Fig. 2a). Remarkably, the α₃β₃γδε₁₀ models from all 18 catalytic dwell conformations could be overlaid with high-fidelity (Fig. 2b, left), as could the nine α₃β₃γδε₁₀ models from binding dwell conformations (Fig. 2b, right), with some limited flexibility at the interface between F₁ and the c-ring. This observation shows that, other than being in a catalytic or binding dwell conformation, the differences between the structures are mostly due to deformation of the peripheral stalk subunits and the rotation of the c-ring relative to subunit a in F₀. Comparison of the eight State 1catalytic models shows that the α₃β₃γδε₁₀ rotor can turn ~80° against subunit a in F₀, or more than one-fifth of a complete revolution, before transition to the next binding dwell conformation (Fig. 2c). Bending of the peripheral stalk and not the central rotor of the complex supports suggestions that the peripheral stalk is the most compliant part of the enzyme and stores energy during rotary catalysis32,22,26,28,33,34.

The peripheral stalk bends by deformation of subunits d, f, and h. The peripheral stalk of yeast ATP synthase contains subunits b, d, h, and OSCP (Fig. 3a). Although atomic models for subunits b, d, and OSCP have been constructed from previous cryoEM of ATP synthase30, model quality for the 92-residue subunit h in
earlier structures was low due to flexibility in both the peripheral stalk overall and subunit h specifically. Focused refinement of the peripheral stalk in the current structure resulted in continuous density for most of subunit h, allowing for construction of an atomic model of residues 1 to 62 based on predictions from AlphaFold35 (Fig. 3a, blue; Supplementary Fig. 6). Interestingly, despite density immediately C-terminal of His62 in subunit h appearing disordered, an additional density that interacts with subunits a, d, f, and g indicates that the C terminus of the protein reaches the membrane surface, as suggested previously36 (Fig. 3a, dashed box). Therefore, subunit h spans the entire distance from F1 to FO, a role usually attributed only to subunit b, and different from subunit F6, the shorter mammalian homolog of subunit h29.

Aligning the eight structures corresponding to State 1catalytic by their FO regions reveals that the dramatic bending of the peripheral stalk is facilitated mainly by deformation of subunits b, d, and h (Fig. 3b; Supplementary Movie 1, 'During ATP hydrolysis' condition). In conformations that show only slight bending of the peripheral stalk, such as State 1catalytic(b to d), deviation from the relaxed State 1catalytic(a) conformation is mediated primarily by a pivot point in subunits b and d close to the membrane surface (Fig. 3c, red arrow). In the more strained conformations like State 1catalytic(h), a second pivot point in subunit b at the top of subunit d is apparent (Fig. 3c, yellow arrow). The two pivot points are located at either end of subunit d, indicating that subunit d controls where the peripheral stalk bends and likely acts to oppose the bending force, inducing the left-handed curvature of the peripheral stalk when it is not under strain. The structure of subunit d, with an α-helical hairpin that allows it to push against subunit b, is ideal for its role of applying a force that attempts to restore the relaxed conformation of the peripheral stalk during ATP hydrolysis or synthesis (Fig. 3d; Supplementary Movie 1, orange subunit). Subunit h contains two disordered regions close to the two pivot points defined by subunit d, which allows it to withstand the large conformational changes that occurs around the pivot points (Fig. 3d, blue asterisks). In contrast with the spring-like peripheral stalk seen here for the yeast ATP synthase, the unusually large peripheral
stall of algal ATP synthase from *Polytomella* sp., although imaged in the absence of substrate, appears mostly rigid, with the OSC subunit that connects the catalytic domain to the rest of the peripheral stalk showing the most flexibility.26

### Overall rotation cycle of yeast ATP synthase.**

Despite the presence of a high concentration of ATP in the buffer used for freezing specimens during ATP hydrolysis, State 1_catalytic(a), the least strained of the State 1_catalytic conformations, appears to have MgADP bound in its αβ_light site (Fig. 4a, left). In contrast, refinement of the F1 region with particle images combined from State 1_catalytic(e to h), the four most strained of the State 3_catalytic conformations, resulted in a structure similar to State 1_catalytic(d) but with what appears to be MgATP bound to αβ_light (Fig. 4a, right). In the presence of free ATP, ATP hydrolysis occurs at the αβ_light site and MgADP within the site is expected to inhibit this hydrolytic activity. Therefore, the presence of MgADP in αβ_light of the non-strained conformation suggests that many of the complexes that faith conformation are in the well-known MgADP inhibited state.43,37. Inactive complexes have been detected previously in the presence of free ATP.38,39 In contrast, the structures that show the more strained peripheral stalks appear to be calculated from images of active enzyme particles. Density for the binding dwell conformations suggests that they contain MgADP with Pi in the αβ_light site (Supplementary Fig. 7a), as was seen in the bacterial F1 region during ATP hydrolysis.32

To place the 27 conformations of ATP synthase observed during ATP hydrolysis into a rotational sequence, the positions of subunit γ relative to αβ3 in F1, (Fig. 4b) and of the c-ring relative to subunit a in F1O (Fig. 4c) were measured and plotted on circles that represent a 360° rotation. As described above, the αβ3γεδε3 subcomplex is found in three catalytic dwell conformations and three binding dwell conformations, resulting in only six unique positions of subunit γ relative to αβ3 in F1 (Fig. 2b). Consistent with the isolated bacterial F1 region,32,33 ATP hydrolysis in αβ_light of the yeast catalytic dwell conformation appears to induce a slightly more open conformation of the αβ pair and a ~36° rotation of the rotor (Supplementary Fig. 7b), leaving the enzyme in a binding dwell. MgADP and Pi are then released from the αβ_light site and ATP binding to the αβ_open site drives an ~84° rotation of the rotor to the next catalytic dwell conformation. Repetition of this process two more times completes the 360° rotation cycle for ATP hydrolysis (Fig. 4b, clockwise arrows), while for ATP synthesis the reverse reaction is driven by rotation of the rotor in the opposite direction (Fig. 4b, counter-clockwise arrows).

In contrast to the six unique positions of subunit γ relative to αβ3 in F1, there are 27 unique positions of the c-ring relative to subunit a in F1O. Plotting the angle of the c-ring relative to subunit a in F1O produces a series of arcs that show the range of rotation of the ring within each catalytic or binding dwell state (Fig. 4c, black, blue, yellow, green, red, and cyan arcs). These arcs reveal that as the c-ring rotates in the ATP hydrolysis direction, each state exhibits a decreasing strain on the peripheral stalk (Fig. 4c, clockwise arrow). For example, for State 1_catalytic (Fig. 4c, black arc), rotation of the c-ring in the ATP hydrolysis direction occurs during the transition from State 1_catalytic(h) → State 1_catalytic(a). As ATP hydrolysis in a catalytic αβ_light site causes the transition from a catalytic dwell to a binding dwell, the order of states indicates that the power stroke of ATP hydrolysis forces the peripheral stalk into a more strained conformation (e.g., State 1_catalytic(a) → State 1_binding(d)). This strain subsequently relaxes as the c-ring continues to turn in the ATP hydrolysis direction (e.g., State 1_binding(d) → State 1_binding(a)). Conversely, rotation of the c-ring in the direction driven by proton translocation during ATP synthesis (Fig. 4c,
counter-clockwise arrow) leads to increasing strain on the peripheral stalk (e.g., State 1_binding(a) → State 1_binding(d)), which relaxes as ATP is formed in the catalytic site and the enzyme transitions from a binding dwell conformation to a catalytic dwell conformation (e.g., State 1_binding(d) → State 1_catalytic(a)).

Notably, the most strained conformation of some of the states show less rotation of the c-ring in the ATP hydrolysis direction than less strained conformations of the preceding state (Fig. 4c, shaded areas). For example, the transition from State 1_catalytic(a) to State 1_binding(d) during ATP hydrolysis would involve the c-ring rotating 23° in the ATP synthesis direction. The same apparent ‘backstepping’ can be seen at the transition from State 3_catalytic → State 3_binding and State 2 Binding → State 1_catalytic. This backstepping of the c-ring would bend the peripheral stalk in the opposite direction of the applied force and is physically unlikely. Therefore, the unstrained conformations appear to show inactive complexes that are not part of the rotary sequence during substrate turnover. By extension, these data suggest that during rotary catalysis the peripheral stalk becomes strained and does not relax fully until catalysis stops. Construction of a movie showing rotation in the hydrolysis direction based on the most strained conformation of the enzyme illustrates the amount of deformation that can occur during ATP hydrolysis (Supplementary Movie 2, ‘ATP hydrolysis’ cycle). Similarly, a video can be constructed showing rotation in the ATP synthesis direction based on the most strained conformations (Supplementary Movie 2, ‘ATP synthesis’ cycle). Together, these data illustrate how in active ATP synthase the peripheral stalk can serve as a buffer for energy that deforms under strain. ATP synthase c-rings from different species can have between eight and 17 c subunits40 and the flexibility of the peripheral likely allows this variability. The storage of energy during rotation would also be expected to smooth the transmission of power between the F1 and F0 motors despite symmetry mismatch, which is thought to be essential for the high turnover rate of the enzyme.11,42. In the fully active enzyme, the peripheral stalk likely remains deformed as the enzyme runs, with the degree of bending dependent on the rate of turnover, and with the enzyme only becoming fully relaxed in the absence of ATP or a proton motive force. At present cryoEM appears uniquely capable of providing high-resolution insight into changes in enzyme conformation that occur during catalysis. Experiments to quantify strain within ATP synthase in solution conditions, such as single-molecule fluorescence resonance energy transfer,43 could complement the findings reported here and reveal how strain changes within individual molecules during each rotation cycle.

### Methods

**Yeast growth and ATP synthase purification.** Yeast strain USY006 containing a 6 × His tag at the N terminus of the β subunits was grown in YPGD media (1% [w/v] yeast extract, 2% [w/v] peptone, 3% [v/v] glycerol, 0.2% [w/v] glucose) with a 11 L fermenter (New Brunswick Scientific) for ~48 h at 30 °C until saturation. All purification steps were performed at 4 °C. Yeast cell walls were broken with bead beating, and cell debris was removed by centrifugation at 5000 × g for 30 min. Mitochondria were collected by centrifugation at 25,000 × g for 30 min, before being washed with phosphate buffer (50 mM sodium phosphate pH 9.0, 5 mM 6-aminocaproic acid, 5 mM benzamidine, 1 mM PMFS) for 30 min. Washed mitochondria were collected by centrifugation at 184,000 × g for 30 min, before being resuspended in buffer (50 mM Tris-HCl pH 7.4, 10% [v/v] glycerol, 1% [w/v] DDM [Anatrace], 5 mM 6-aminocaproic acid, 5 mM benzamidine, 1 mM PMFS) and solubilized with gentle shaking for one hour. Insoluble material was removed by centrifugation at 184,000 × g for 30 min, and supernatant containing solubilized protein was supplemented with 40 mM imidazole and 300 mM NaCl before being loaded onto a 5 mL HisTrap column (Cytiva) equilibrated with HisTrap buffer (50 mM Tris-HCl pH 7.4, 10% [v/v] glycerol, 0.05% [w/v] 40 mM imidazole, 300 mM NaCl, 5 mM 6-aminocaproic acid, 5 mM benzamidine, 1 mM PMFS) and washed with HisTrap buffer. ATP synthase was eluted with HisTrap buffer containing 300 mM imidazole and was loaded onto a Superose 6 Increase column (Cytiva) equilibrated with buffer (20 mM Tris-HCl pH 7.4, 10% [v/v] glycerol, 0.05% [w/v] DDM, 100 mM NaCl, 5 mM...
MgCl₂). Fractions containing ATP synthase were pooled, and the protein was concentrated to ~15 mg/ml prior to cryoEM grid freezing or storage at −80 °C.

CryoEM specimen preparation. Glycerol in the ATP synthase preparation was removed with a Zeba Spin desalting column (Thermo Fisher Scientific [TFS]) before freezing cryoEM specimens. Holey gold films with ~2 µm holes were nanofabricated on 300 mesh Maxtaform copper-rhodium grids (Electron Microscopy Sciences). Specimens with 10 mM ATP were prepared by first applying 0.4 µL of 50 mM ATP in buffer (70 mM Tris-HCl pH 7.4, 0.05% [w/w] DDM, 100 mM NaCl, 55 mM MgCl₂) onto a grid that had been glow-discharged in air for 2 min. Freshly prepared ATP synthase (1.6 µL) was mixed quickly with the ATP solution on the grid before blotting for 1 s in an EM GP2 grid freezing device.
consistent with MgADP in the increasingly relaxed as the c-ring rotates in the ATP hydrolysis direction. Transition between catalytic and binding dwell conformations would require back-binding or catalytic dwell conformation the peripheral stalk becomes increasingly strained as the c-ring rotates in the ATP synthesis direction and increasingly relaxed as the c-ring rotates in the ATP hydrolysis direction. Transition between catalytic and binding dwell conformations would require back-binding of the c-ring (shaded areas) if every conformation occurred during the rotary cycle. Colors in (b) and (c) are defined as in Fig. 1b.

Fig. 4 Sequence of conformations in the ATP hydrolysis and ATP synthesis cycles. a The unstrained conformation of State 1catalytic shows density consistent with MgADP at the αβ5iγδε catalytic pair (left, yellow), suggesting an ADP-inhibited state, while the strained conformations show density consistent with MgATP (right, purple) suggesting an active state. b Measurement of the rotation of the rotor subunit γ within the F1 region shows 36° and 84° sub-steps between catalytic and binding dwell conformations. c Measurement of the rotation of the c-ring relative to subunit a in F0. Within each binding or catalytic dwell conformation the peripheral stalk becomes increasingly strained as the c-ring rotates in the ATP synthesis direction and increasingly relaxed as the c-ring rotates in the ATP hydrolysis direction. Transition between catalytic and binding dwell conformations would require back-binding of the c-ring (shaded areas) if every conformation occurred during the rotary cycle. Colors in (b) and (c) are defined as in Fig. 1b.

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Sobti, M., Ueno, H., Li, C.-B. & Noji, H. Rotary catalysis of bovine mitochondrial F₁-ATPase studied by single-molecule experiments. Proc. Natl Acad. Sci. USA 117, 1447–1456 (2020).

Soga, N., Kanura, K., Kinosita, K., Yoshida, M. & Suzuki, T. Perfect chemomechanical coupling of F₆-F₅-ATP synthase. Proc. Natl Acad. Sci. USA 114, 4960–4965 (2017).

Stock, D., Leslie, A. G. W. & Walker, J. E. Molecular architecture of the rotary motor in ATP synthase. Science 286, 1700–1705 (1999).

Zhou, A. et al. Structure and conformational states of the bovine mitochondrial ATP synthase by cryo-EM. eLife 4, e10180 (2015).

Noji, H., Yasuda, R., Yoshida, M. & Kinosita, K. Jr. Resolution of distinct rotational substeps by submillisecond kinetic analysis of F₁-ATPase. Nature 410, 898–904 (2001).

Martin, J. L., Ishmukhametov, R., Hornung, T., Ahmad, Z. & Frasch, W. D. ATP synthase hexamer assemblies shape cristae of the mitochondrial F₁-ATPase studied by single-molecule experiments. J. Biol. Chem. 286, 36261–36266 (1999).

Pänke, O. & Rumberg, B. Kinetic modeling of rotary CF₀F₁-ATP synthase: Anatomy of F₁-ATPase powered rotation. Proc. Natl Acad. Sci. USA 111, 3715–3720 (2014).

Steel, B. C. et al. Comparison between single-molecule and X-ray crystallography data on yeast F₁-ATPase. Sci. Rep. 5, 8773 (2015).

Nishizaka, T. et al. Chemomechanical coupling in F₁-ATPase revealed by subrotation substeps through tetramerization. Nat. Commun. 11, 5342 (2020).

Gu, J. et al. Cryo-EM structure of the mammalian mitochondrial ATP synthase tetramer bound with inhibitory protein IF1. Science 364, 1068–1075 (2019).

Guo, H., Suzuki, T. & Rubinstein, J. L. Structure of a bacterial ATP synthase. eLife 8, e43128 (2019).

Hahn, A., Vonck, J., Mills, D. J., Meier, T. & Kühnlbrandt, W. Structure, mechanism, and regulation of the chloroplast ATP synthase. Science 360, eaat4318 (2018).

Hahn, A. et al. Structure of a complete ATP synthase dimer reveals the molecular basis of inner mitochondrial membrane morphology. Mol. Cell 63, 445–456 (2016).

Mühleip, A. et al. ATP synthase hexamer assemblies shape cristae of the mitochondrial ATP synthase. J. Biol. Chem. 321, 613–619 (2006).

Sobti, M., Ueno, H., Noji, H. & Stewart, A. G. The six steps of the complete F₁-ATPase rotary catalytic cycle. Nat. Commun. 12, 4690 (2021).

Rabinstein, J. L. & Walker, J. E. ATP synthase from Saccharomyces cerevisiae: localization of the OSCP subunit in the peripheral stalk region. J. Mol. Biol. 321, 613–619 (2001).

Sobti, M., Ueno, H., Noji, H. & Stewart, A. G. The six steps of the complete F₁-ATPase rotary catalytic cycle. Nat. Commun. 12, 4690 (2021).

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Sobti, M., Ueno, H., Noji, H. & Stewart, A. G. The six steps of the complete F₁-ATPase rotary catalytic cycle. Nat. Commun. 12, 4690 (2021).

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