Supplementary information

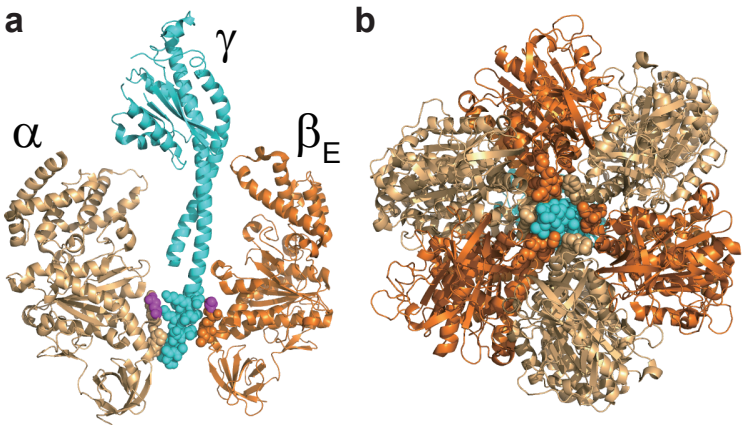
A change in the radius of rotation of F_1-ATPase indicates a tilting motion of the central shaft

Mitsuhiro Sugawa, Kaoru A. Okada, Tomoko Masaike & Takayuki Nishizaka

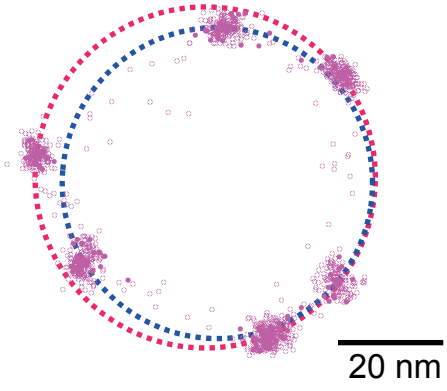
Supplementary table-1

Diameter of probe (nm)	Rotation rate (revolutions/sec ± S.D.)
40	0.75 ± 0.16 (41)
108	0.61 ± 0.14 (34)
220	0.50 ± 0.17 (17)
489	0.37 ± 0.13 (31)

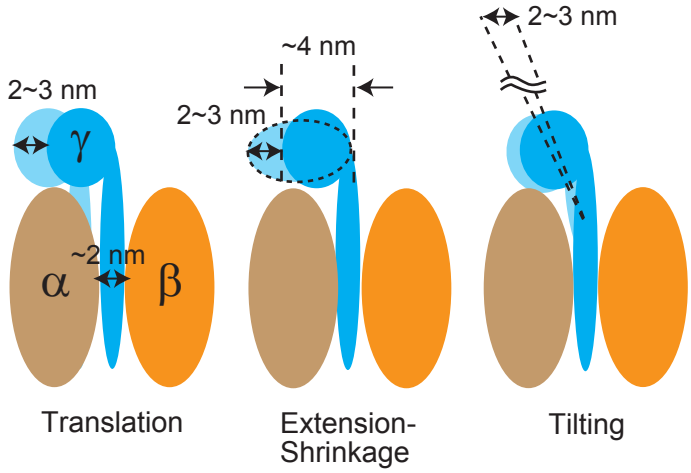
Values in parentheses represent the number of molecules.
Rotation assays using the F_1(βE190D) were performed at 1 μM ATP at 30 frames/sec.



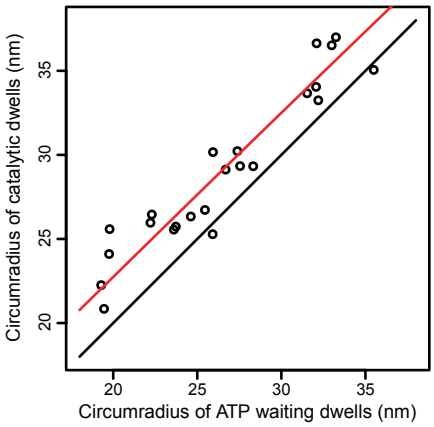
Supplementary figure-1. a) Structure of α (light orange), β (orange) and γ (cyan) by modifying bovine mitochondrial F_1-ATPase (PDB 1e79). Residues 288-292 of α, residues 274-278 of β and residues 258-273 of γ in 1e79 are indicated by space filling models. The distance between Cα of E292 of α (magenta) and Cα of A278 of β (magenta) is 1.7 nm. **b**) Viewed from the outside of membrane.



Supplementary figure-3. Observation of rotation of the F_1(βE190D) with a single 108-nm polystyrene bead at 1 μM ATP at 30 frames per second. Purple filled circle represents the trace of a single bead rotation. Blue and red dotted lines represent the circumscribed circles of the three ATP-waiting dwells and the three catalytic dwells, respectively. The two circles are not concentric, but the the circle of the catalytic dwells is outside that of the ATP-waiting dwells.



Supplementary figure-2. Three candidates of the conformational changes of F_1-ATPase explaining the change in the raidus of rotation. (Left) The translation is unlikely because of the narrow space of the hole of the $\alpha_3\beta_3$ crown. (Middle) The extension and shrinkage of the protrusion domain of the γ subunit is also unlikely because of the folding stability of the protrusion domain. (Right) The tilting is likely and reasonable because the change in the radius of rotation can be explained by relative smaller conformational changes.



Supplementary figure-4. Evaluation of the tilt angle of γ with a single 108-nm polystyrene bead in the presence of ATPγS. Plot shows relationship between the circumradius of the ATP waiting dwells and that of the catalytic dwells. Each point represents each molecule. Red line represents the fitting line by the formula (see text). The tilt angle obtained by fitting was 2.8°.