

**Supplementary Information for:**

***In situ* structure of the *Vibrio* polar flagellum reveals distinct outer membrane complex and its specific interaction with the stator**

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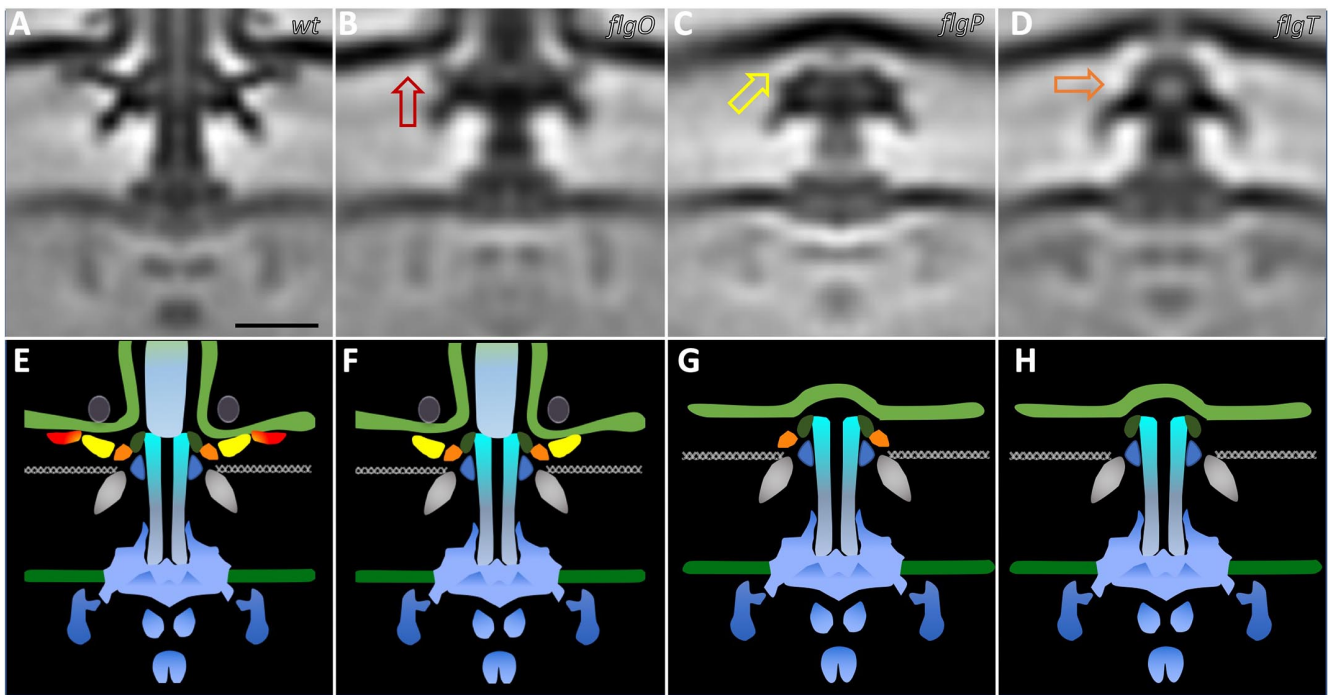
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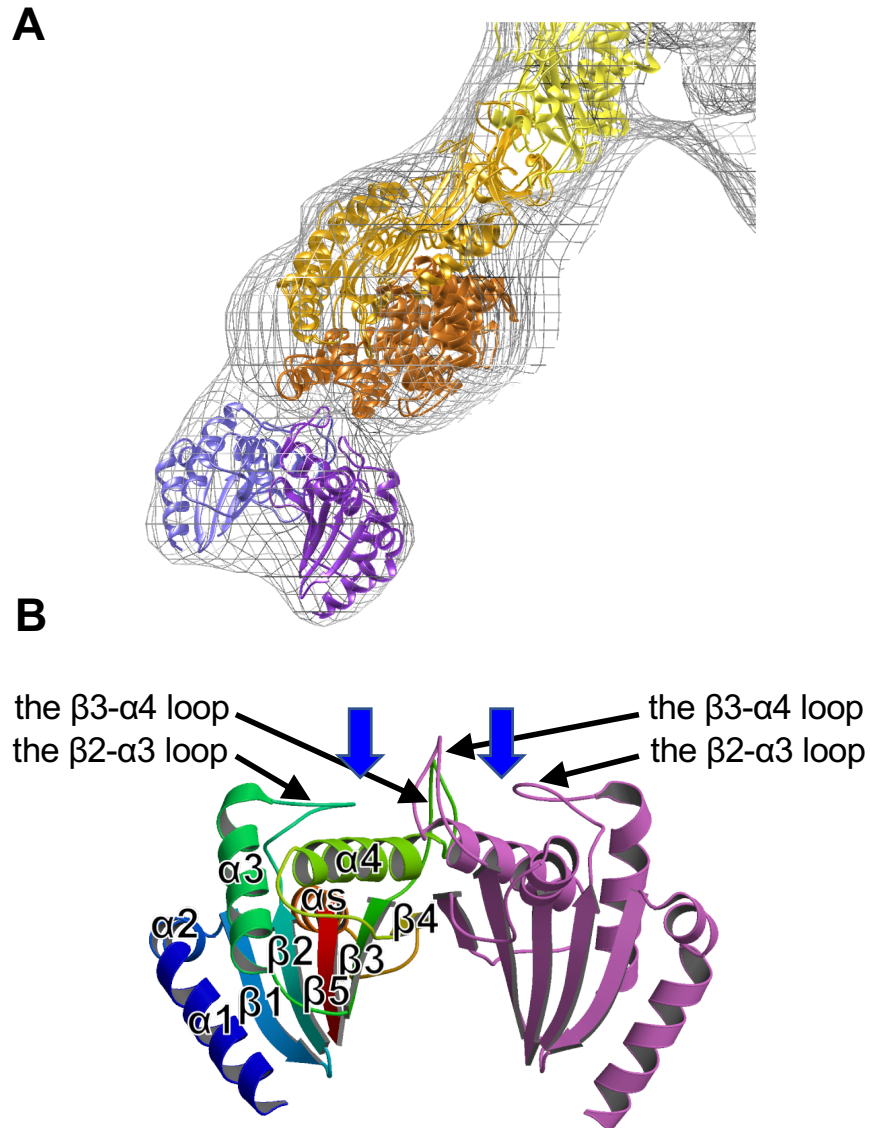
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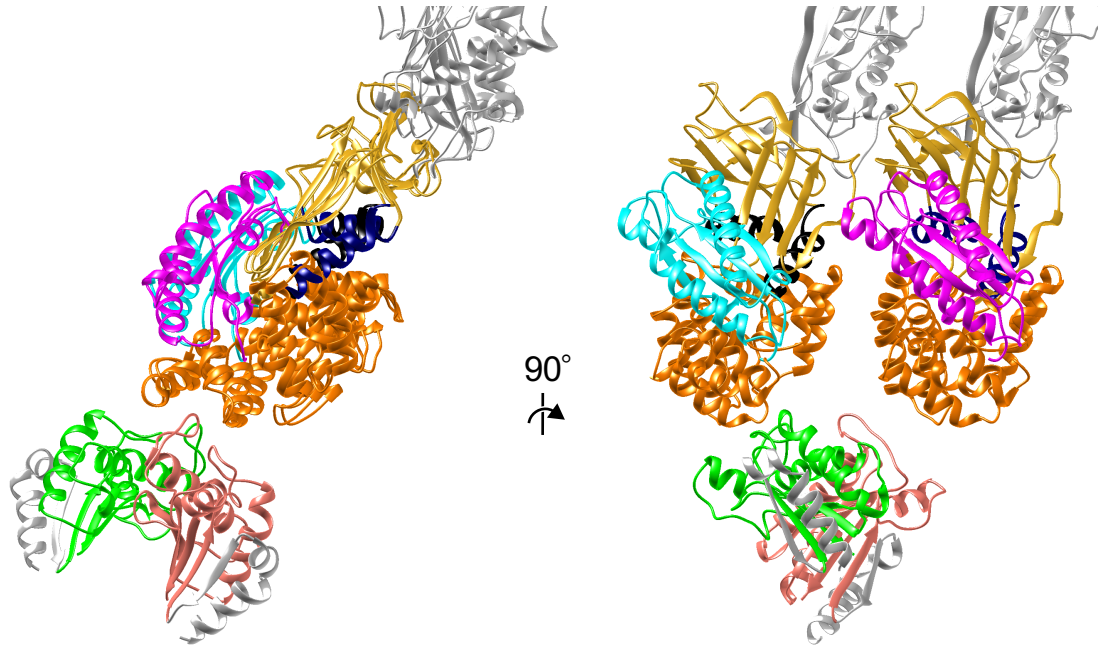
Email. g44416a@cc.nagoya-u.ac.jp or jliu@yale.edu



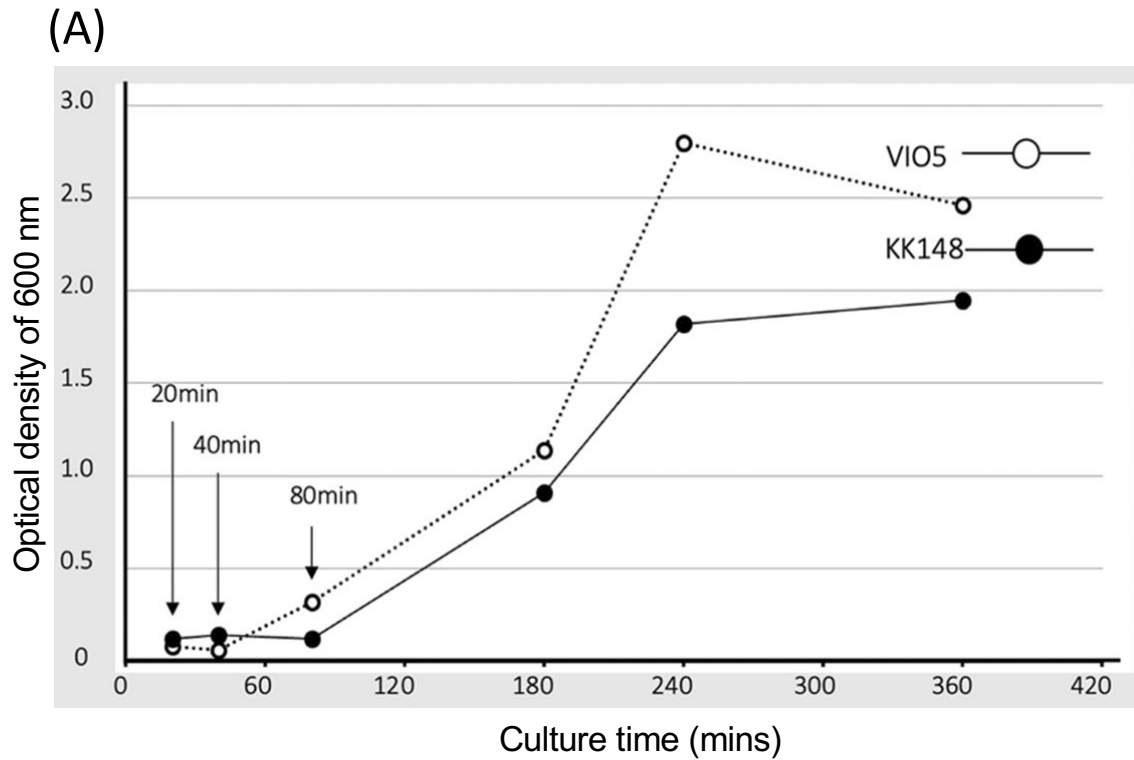
**Fig. S1.** Characterization of the H-ring components. (A~D) A 2D slice of sub-tomogram averaged structure of the top part of *Vibrio* motor in KK148-*wt*, KK148- $\Delta$ *flgO* (NMB337), *Vibrio fischeri*  $\Delta$ *flgP* (EMDB-3162) and KK148- $\Delta$ *flgT* (TH7). (E~H) The cartoon models of (A~D). Bar is 20 nm.



**Fig. S2.** (A) Enlarged figure of Fig. 3F. (B) The ribbon diagram of the atomic model of PomB<sub>C</sub> as a dimer (PDB ID:3WPW). The putative peptidoglycan binding sites previously suggested and the loop structures included in the binding sites are indicated by blue and black arrows, respectively.



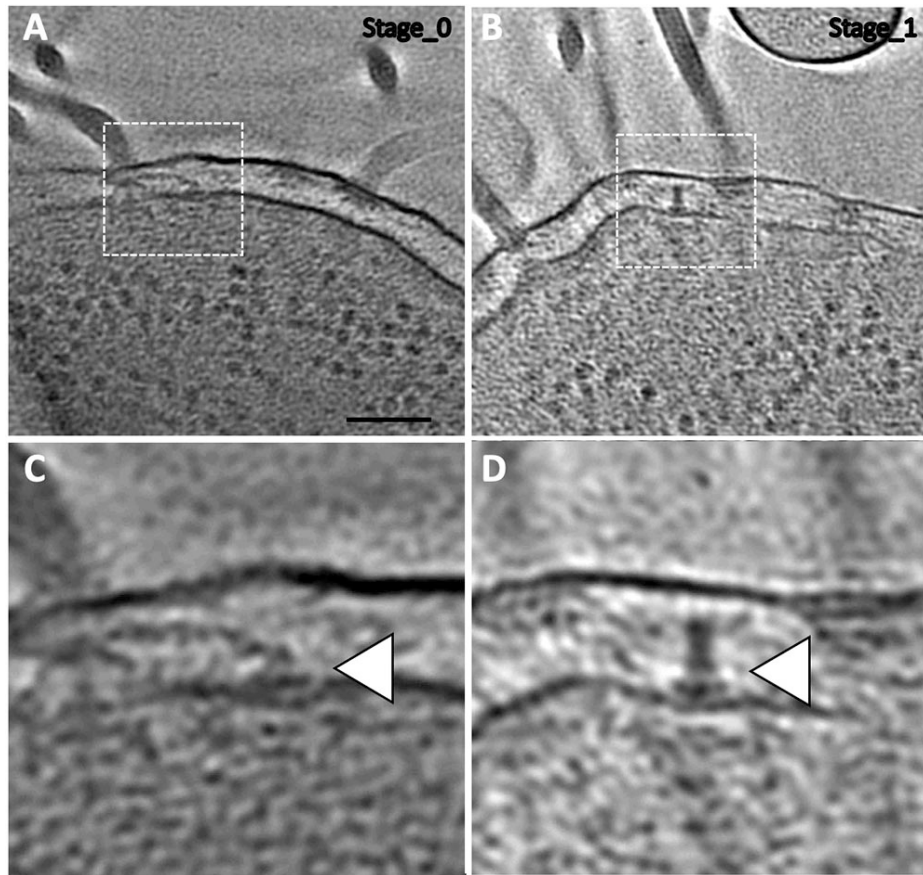
**Fig. S3.** The putative location of PG-binding motif of PomB and of MotY  $\alpha$ -helices which is close to MotX. The figure was enlarged from Fig. 3E and 3F and to give the colors which are magenta and cyan for PG-binding motifs of adjacent MotY, which are navy blue and black for  $\alpha$ -helices of MotY located near MotX, or which are green and coral for PG-binding motifs of PomB dimer.



(B)

Time resolved data	State1	State2	State3	State4
20 mins	4	1	0	0
40 mins	3	2	6	4

**Fig. S4.** (A) Growth curve comparison between single polar flagellated strain VIO5 and hyper-polar flagellated strain on time-coursed cryo-ET. (B) The number of the states observed time-coursed cryo-ET during flagellar assembly.



**Fig. S5.** Capturing initial state of flagellar assembly. (A) MS/C-rings together with export apparatus are visible in raw-tomograms. (B) Intermediated state assembled with MS/C-rings, export apparatus together with the flagellar rod is visible. (C, D) A zoom-in the view of squared area in (A) and (B).