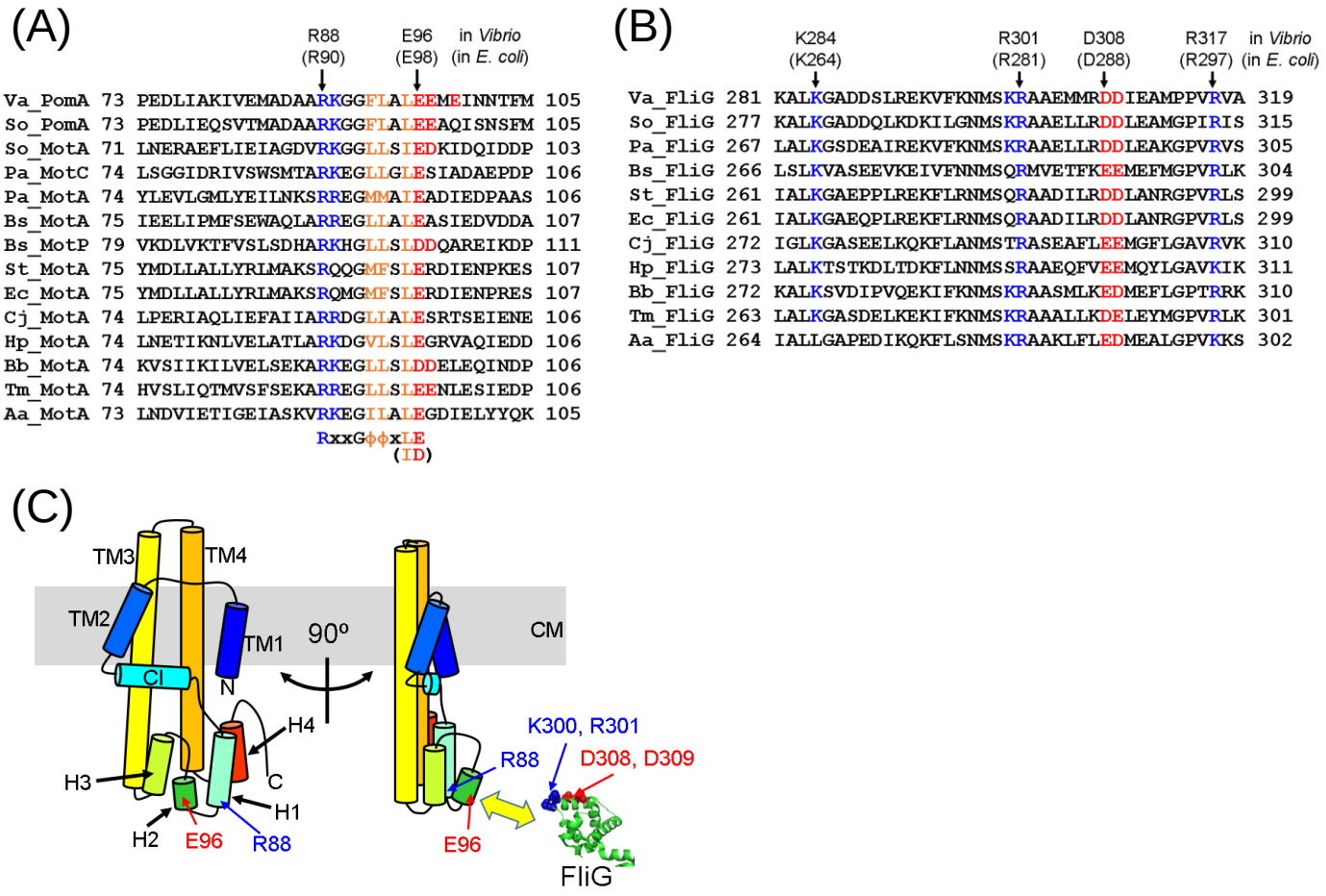


Fig. S1. (A, B) Alignments of the amino acid sequences of PomA and MotA (A) and FliG (B) from various species. The charged residues thought to be important for the motor function were shown in blue (positive) and red (negative). The conserved hydrophobic residues in the RxxGΦΦxLE motif were shown in orange. Abbreviations: Φ, hydrophobic residues; Va, *Vibrio alginolyticus* VIO5; So, *Shewanella oneidensis* MR-1; Pa, *Pseudomonas aeruginosa* PAO1; Bs, *Bacillus subtilis* subsp. *subtilis* 168; St, *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* LT2; Ec, *Escherichia coli* K-12 MG1655; Cj, *Campylobacter jejuni* subsp. *jejuni* NCTC 11168; Hp, *Helicobacter pylori* 26695; Bb, *Borreliella burgdorferi* B31; Tm, *Thermotoga maritima* MSB8; Aa, *Aquifex aeolicus* VF5. (C) The schematic drawing of the PomA monomer based on the cryo-EM structure of *C. jejuni* MotA/MotB complex (1). It was shown as a cartoon model in rainbow color. The mutations were introduced in H1, H1-H2 linker, H2 and H2-H3 linker. The C-terminal region of FliG from *A.aeolicus* was shown as a cartoon model (PDB ID: 3HJL). The important charged residues corresponding to *V. alginolyticus* FliG K300, R301, D308 and D309, were shown in blue (positive) and red (negative) spheres. TM1~TM4: transmembrane segments, CI: cytosolic interface helix, H1~H4: cytosolic helices.

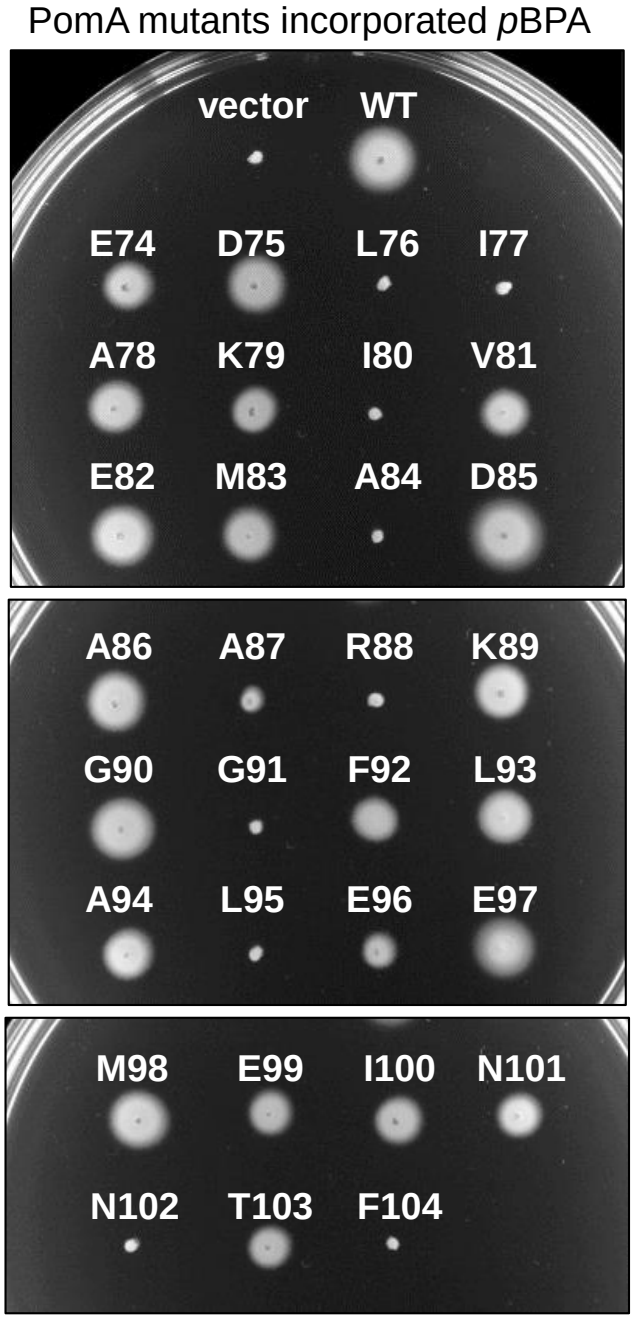
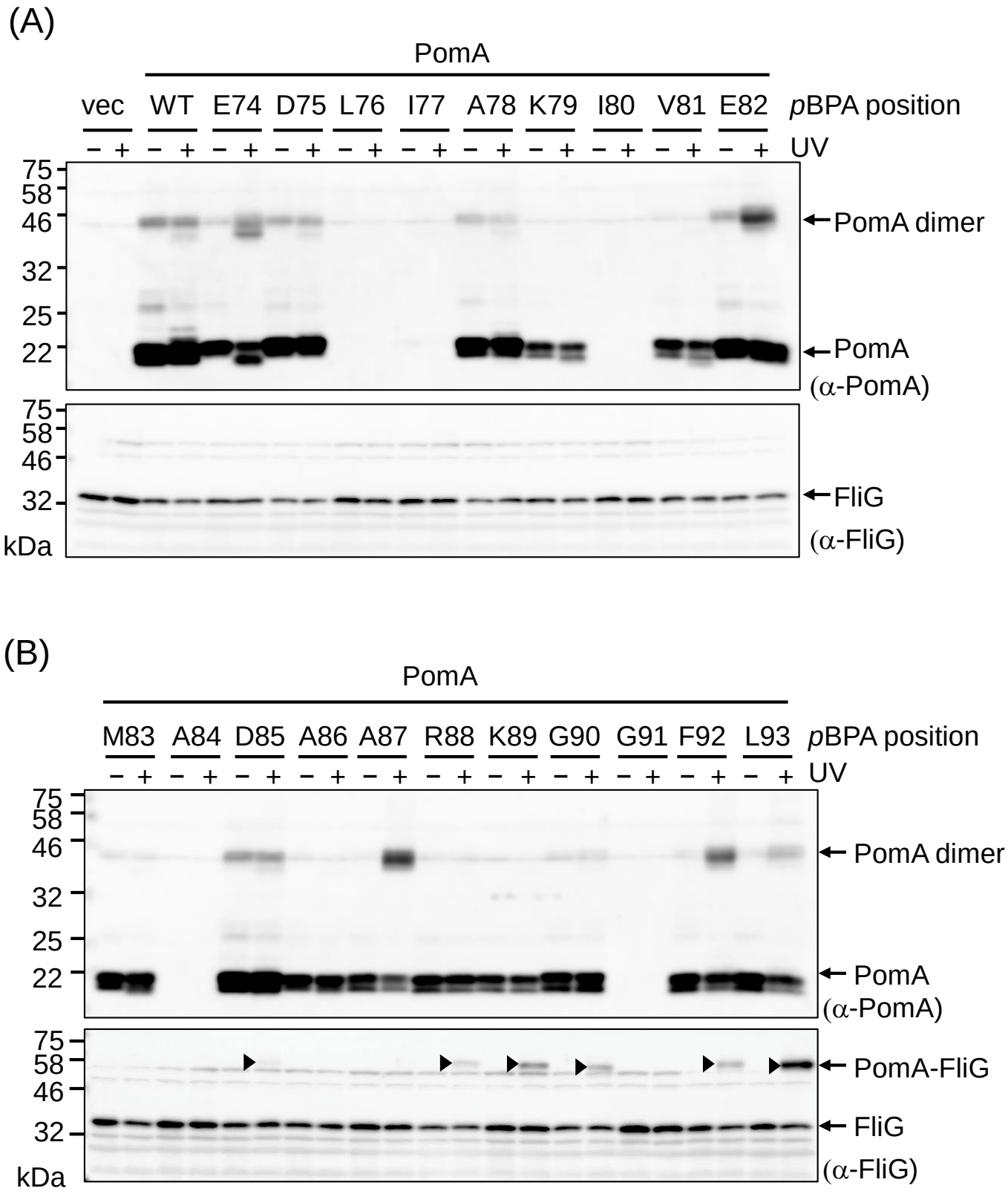


Fig. S2. Motility of *E. coli* Δ*motAB* cells expressing *p*BPA-incorporated *V. alginolyticus* PomA and chimeric PotB in a soft-agar plate. The cells were inoculated in TG 0.3% (w/v) bactoagar with 0.02% (w/v) arabinose plate at 30 ºC for 24 hrs. The *E. coli* Δ*motAB* strain is RP6894. The vector plasmid is pBAD24. PomA/PotB were expressed from pYS3 that harbors *pomA* and *potB* genes in pBAD24 backbone. *p*BPA-incorporation into an amber codon was carried out by the amber suppressor tRNA and the mutated tyrosyl-tRNA synthase expressed from pEVOL-pBpF.


(A)
PomA
vec
WT
E74
D75
L76
I77
A78
K79
I80
V81
E82
pBPA position
− + − + − + − + − + − + − + − + − + − +
UV
75
58
46
32
25
22
PomA dimer
PomA
(α-PomA)
75
58
46
32
FliG
(α-FliG)
kDa
(B)
PomA
M83
A84
D85
A86
A87
R88
K89
G90
G91
F92
L93
pBPA position
− + − + − + − + − + − + − + − + − + − +
UV
75
58
46
32
25
22
PomA dimer
PomA
(α-PomA)
75
58
46
32
PomA-FliG
FliG
(α-FliG)
kDa

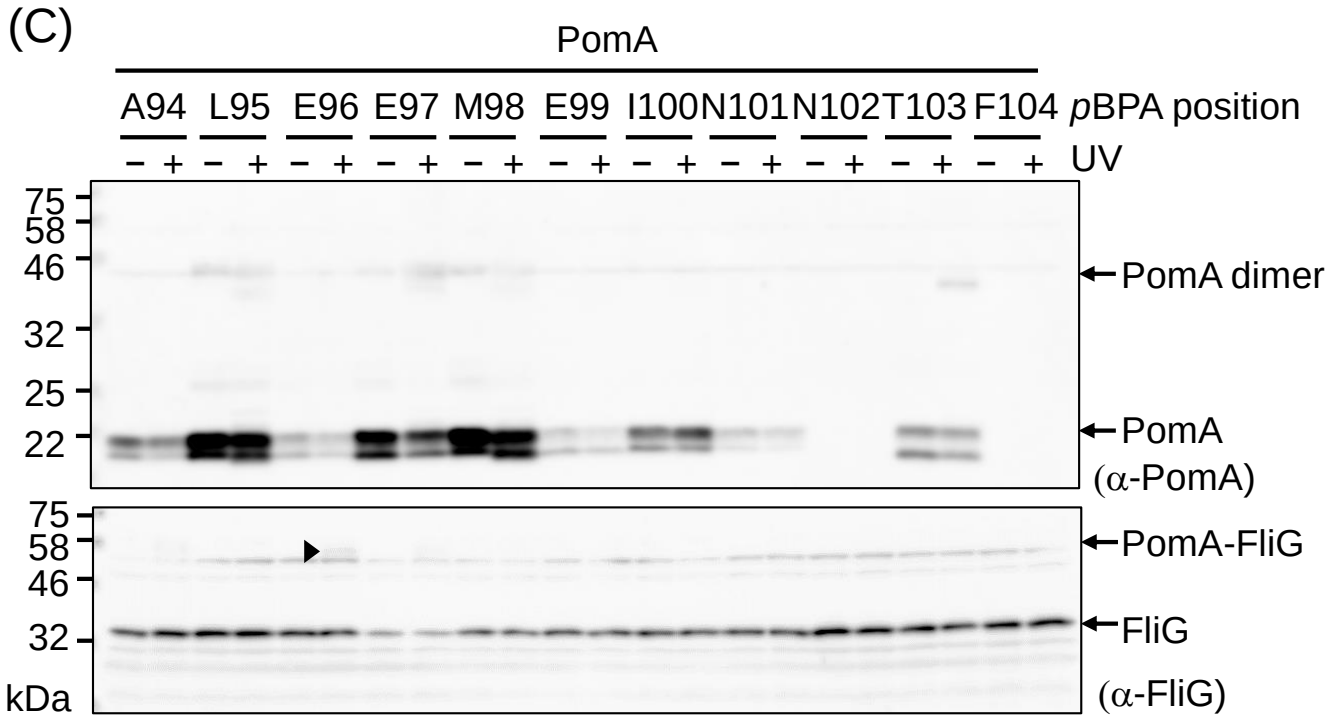


Fig. S3. Protein expression of *p*BPA-introduced, plasmid-borne *V. alginolyticus* PomA and photo-crosslinking between those PomA and endogenous *E. coli* FliG (A-C). SDS-PAGE samples were prepared from whole cell lysates. *V. alginolyticus* PomA and chimeric PotB were expressed from plasmid pYS3, and the amber suppressor tRNA and the mutated tyrosyl-tRNA synthase were expressed from plasmid pEVOL-pBpF, in the *E. coli* Δ*motAB* strain, RP6894. Upper and lower panels showed immunoblot images by using anti-PomA and anti-FliG antibodies, respectively. The crosslinked products were marked by black arrow head.

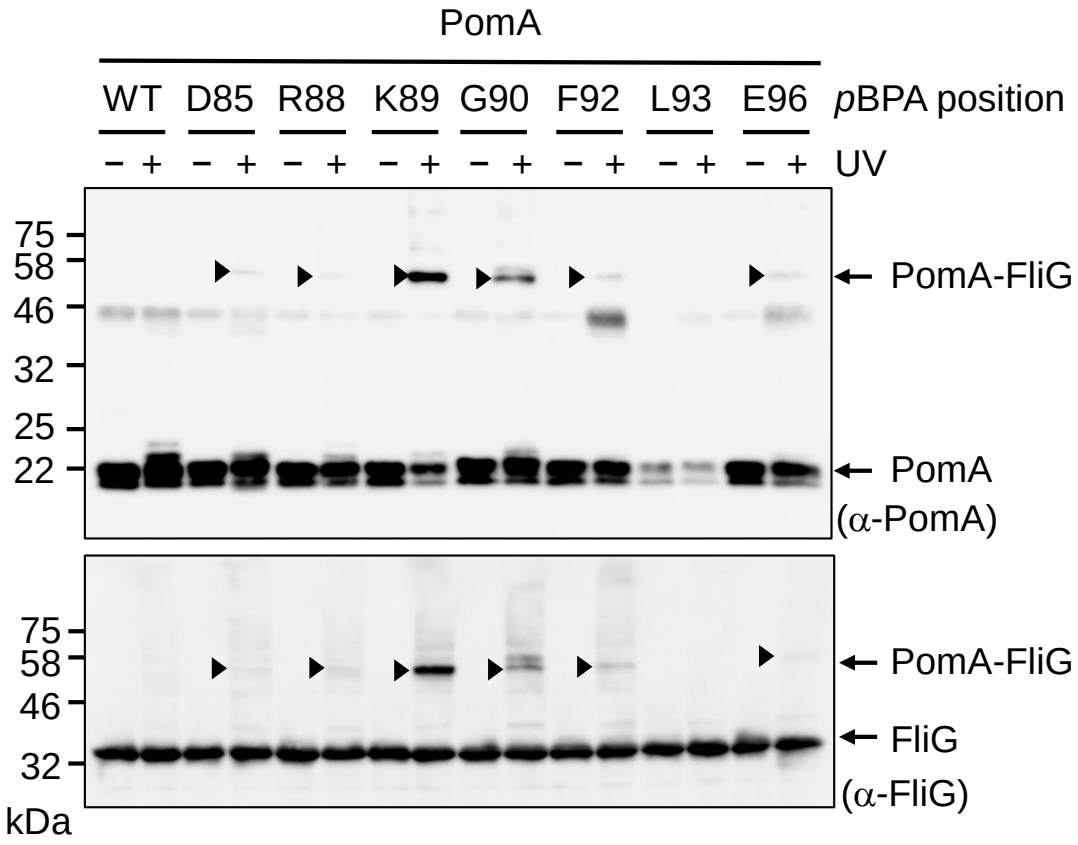


Fig. S4. Photo-crosslinking between plasmid-borne *V. alginolyticus* PomA and *E. coli* FliG in the *E. coli* Δ*flhDC* strain, RP3098. *V. alginolyticus* PomA, chimeric PotB and *E. coli* FliG were co-expressed from plasmid pTSK170, and the amber suppressor tRNA and the mutated tyrosyl-tRNA synthase were expressed from plasmid pEVOL-pBpF. Upper and lower panels showed immunoblot images by using anti-PomA and anti-FliG antibodies, respectively. The crosslinked products were marked by black arrow head.

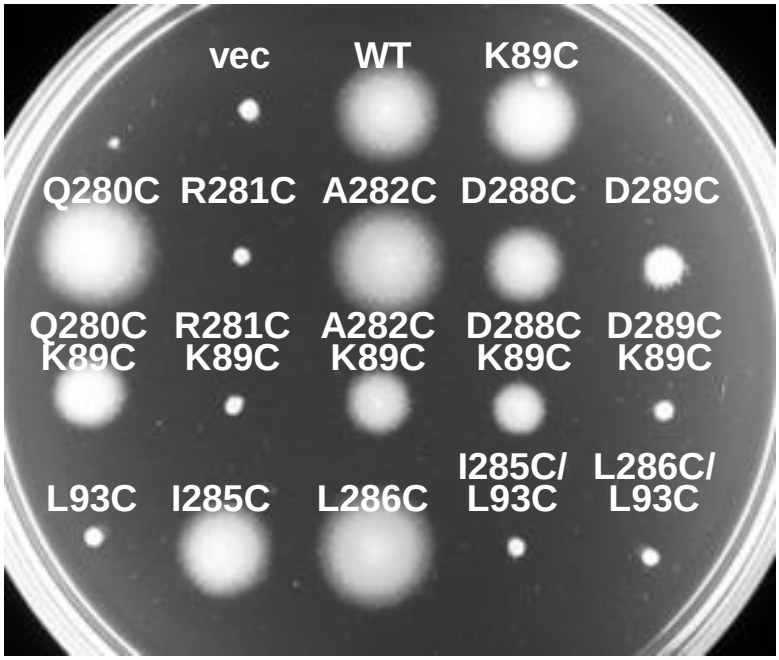


Fig. S5. Motility of *E. coli* Δ*motA*Δ*fliG* cells expressed *V. alginolyticus* PomA, and chimeric PotB and *E. coli* FliG in a soft-agar plate. The cells were inoculated in TG 0.3% (w/v) bactoagar with 0.02% (w/v) arabinose plate, and incubated at 30 ºC for 24 hrs. The *E. coli* Δ*motA*Δ*fliG* strain is DFB245. The vector plasmid is pBAD24. PomA, PotB and FliG were expressed from pTSK170, in which *pomA*, *potB* and *fliG* genes were cloned into pBAD24.

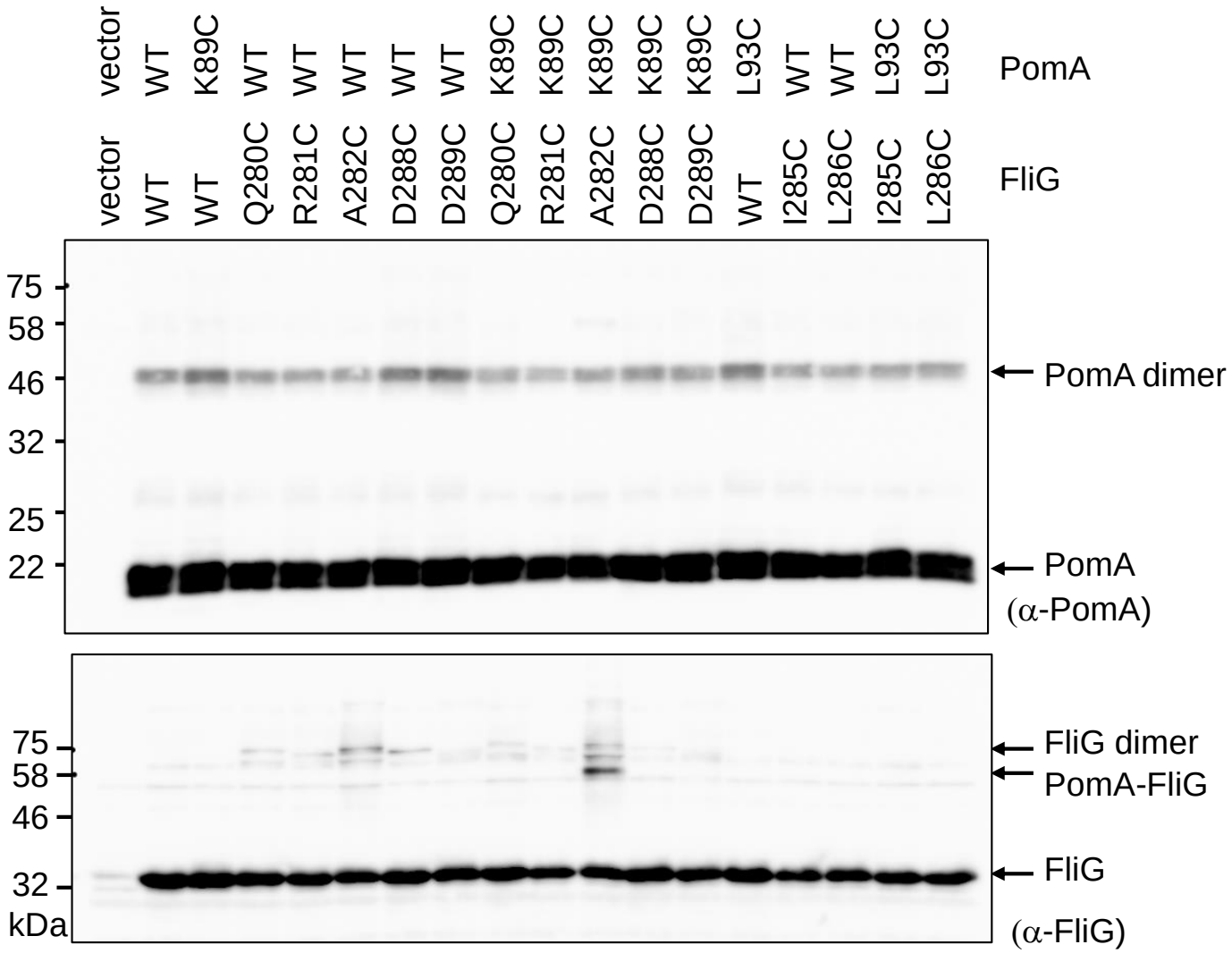


Fig. S6. Immunoblotting of the disulfide crosslinked samples with reduced treatment by β-mercaptoethanol. *V. alginolyticus* PomA, chimeric PotB and *E. coli* FliG were expressed from plasmid pTSK170, in the *E. coli* Δ*motA*Δ*fliG* strain, DFB245. Upper and lower panels showed immunoblot images by using anti-PomA and anti-FliG antibodies, respectively. A small amount of the crosslink product of FliG A282C/PomA K89C was detected even upon treatment with reducing agent.

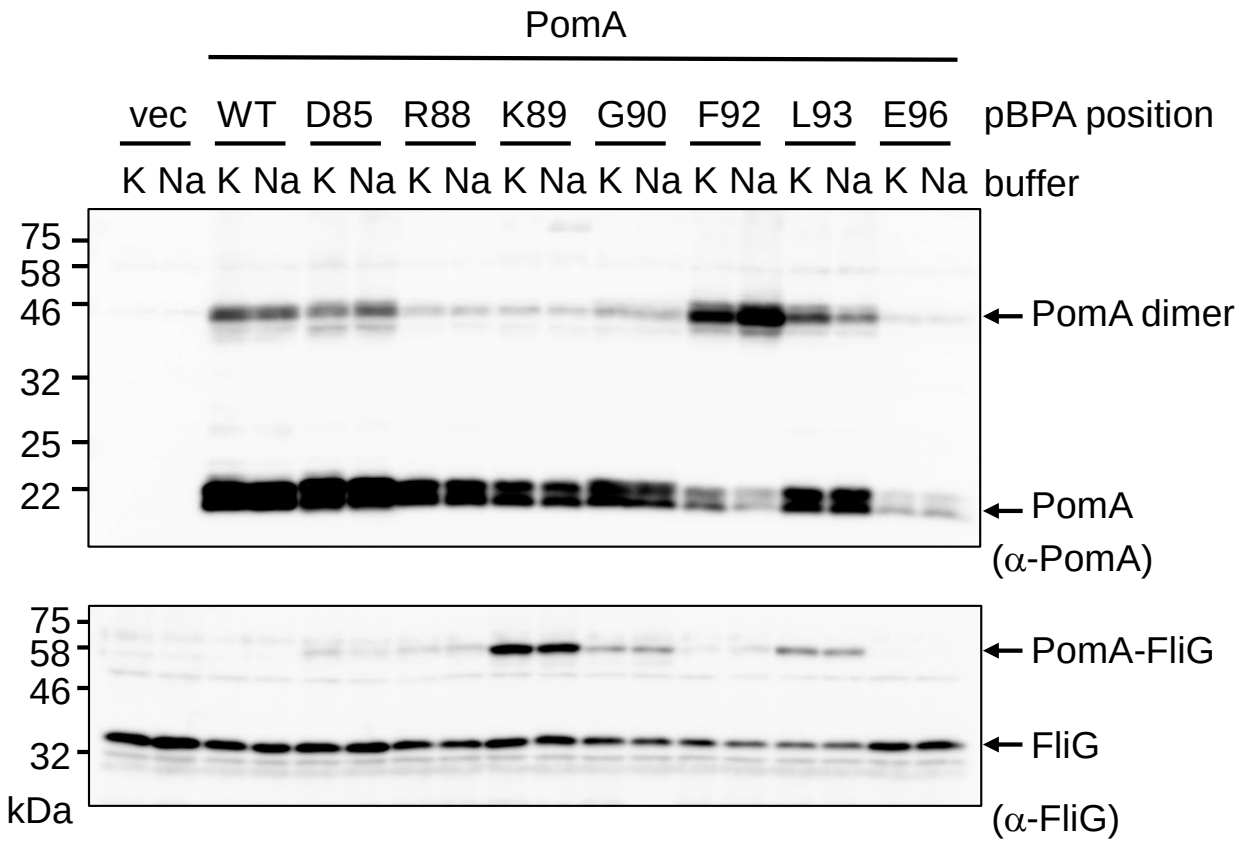


Fig. S7. Photo-crosslinking between plasmid-borne *V. alginolyticus* PomA and endogenous *E. coli* FliG in the presence of sodium buffer or potassium buffer. *V. alginolyticus* PomA and chimeric PotB were expressed from plasmid pYS3, and the amber suppressor tRNA and the mutated tyrosyl-tRNA synthase were expressed from plasmid pEVOL-pBpF, into the *E. coli* Δ*motAB* strain, RP6894. Upper and lower panels showed immunoblot images by using anti-PomA and anti-FliG antibodies, respectively.

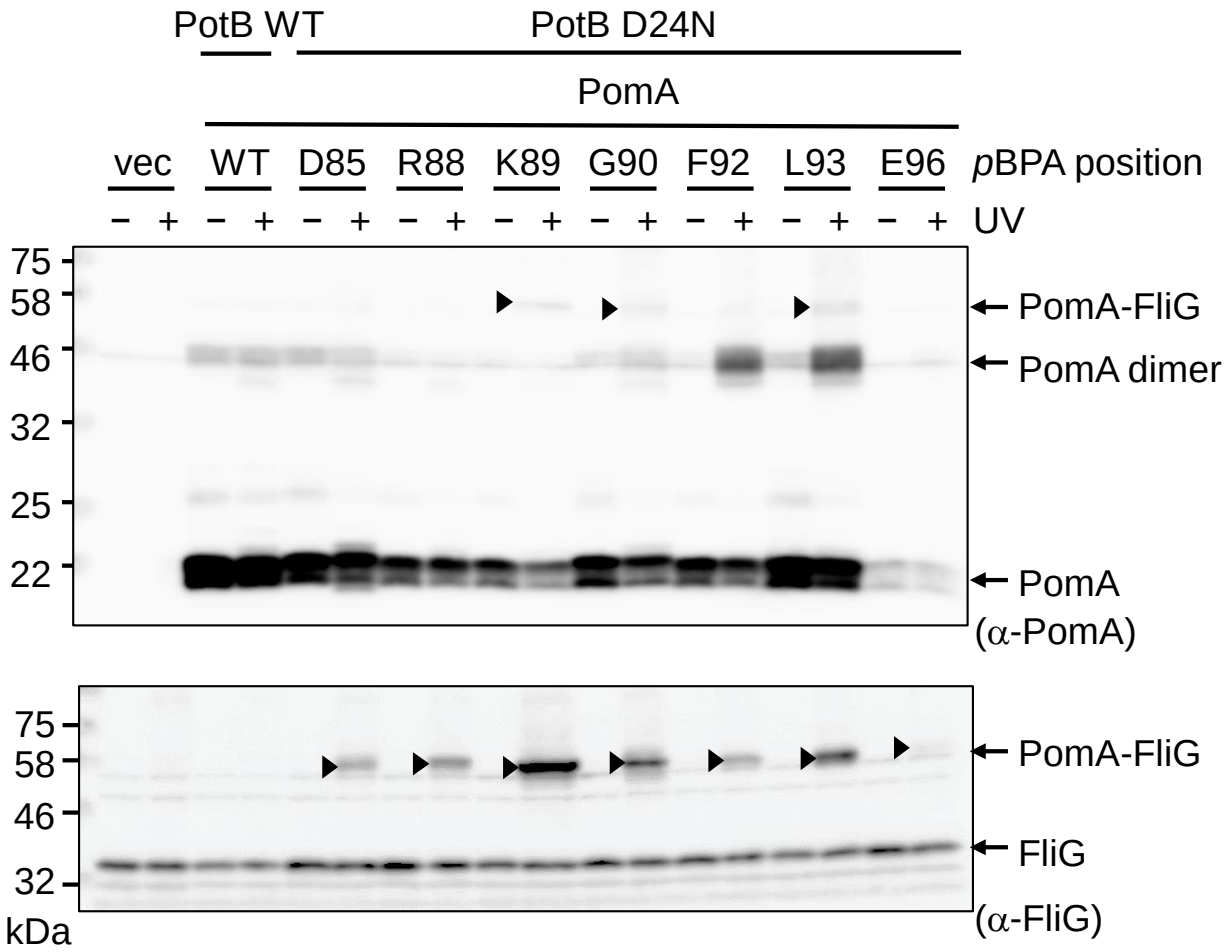


Fig. S8. Photo-crosslinking between plasmid-borne *V. alginolyticus* PomA and endogenous *E. coli* FliG in the background of PotB D24N. *V. alginolyticus* PomA and chimeric PotB were expressed from plasmid pYS3, and the amber suppressor tRNA and the mutated tyrosyl-tRNA synthase were expressed from plasmid pEVOL-pBpF, into the *E. coli* Δ*motAB* strain, RP6894. Upper and lower panels showed immunoblot images by using anti-PomA and anti-FliG antibodies, respectively. The crosslinked products were marked by black arrow head.

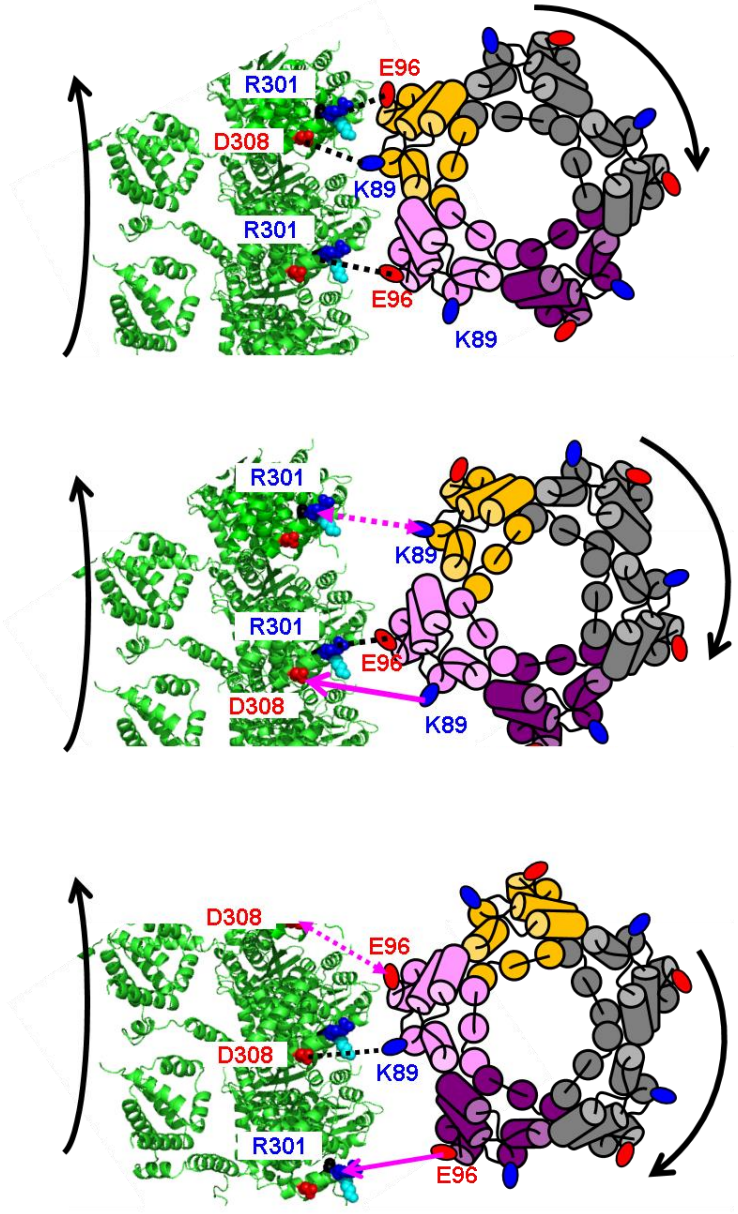


Fig. S9. The interaction model between C-ring and PomA. The schematic diagram of the PomA pentamer based on the cryo-EM structure (1) was shown from the top view. The C-ring model of *Vibrio alginolyticus* was based on the model previously reported (2) is shown from the top view. The positions of PomA K89 and E96 were shown in blue and red circles, respectively. FliG R301 and D308 in *V. alginolyticus* corresponding to R281 and D288 in *E. coli* were shown by space filling residues.

Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or description	Reference or source
E. coli		
DH5α	F^- Φ80d*lacZ* ΔM15 Δ(*lacZYA-argF*)U169 *deoR recA*1 *endA*1 *hsdR*17(r_K^-, m_K^+) *phoA supE*44 λ^- *thi*-1 *gyrA*96 *relA*1	(3)
RP437	*Wild-type strain*	(4)
RP6894	*motA* and *motB* null strain	(5)
RP3098	*flhD* and *flhC* null strain	(6)
DFB245	*motA* and *fliG* null strain	(7)
DFB225	*fliG* null strain	(8)
Plasmids		
pBAD24	pBR322-derived vector, *araBAD* promoter, Amp^r	(9)
pBAD33	pACYC-derived vector, *araBAD* promoter, Cm^r	(9)
pYS3	*V. alginolyticus pomA* and chimeric *potB* in pBAD24	(10)
pTSK170	*E. coli fliG, V. alginolyticus pomA* and chimeric *potB* in pBAD24	This study
pTY801	*E. coli fliG* in pBAD24	This study
pEVOL-pBpF	Plasmid for the incorporation of photo-reactive amino acid, *p*BPA, into the amber codon.	(11)
Amp^r, ampicillin-resistant; Cm^r, chloramphenicol-resistant.		

Reference

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