Supplementary Information

High pressure inhibits signaling protein binding to the flagellar motor and bacterial chemotaxis through enhanced hydration

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Table S1. The free energy difference between the active and inactive form of CheY monomers, $\Delta G_{\mathrm{M}} = \Delta G_{\mathrm{inactive}} - \Delta G_{\mathrm{active}}$.

Model	Pressure (MPa)	ΔG_{M} (kcal/mol)
aCheYp	0.1	4.5
	100	4.5
iCheYp	0.1	2.9
	100	1.2
aCheY	0.1	0.1
	100	1.1
iCheY	0.1	-2.4
	100	-0.1

Table S2. Effect of high pressure on the CheY and $FliM_N$ structures and various properties.

Model	State[a]	Pressure [MPa]	RMSD[b] [Å]	SASA[c] [10^3 Å^2]	V_{ex}[d] [10^4 Å^3]	V_{cav}[e] [Å^3]	κ^{ef}[f] [GPa^{-1}]	H_{PP}[g]	H_{PW}[h]	H_{PWP}[i]	r_{1SW}[j]	r_{1IW}[k]	r_{2SW}[k]	r_{2IW}[m]
aCheY	M	0.1	-	7.1 ± 0.1	2.41 ± 0.02	6 ± 5	0.2 ± 0.3	57 ± 6	208 ± 10	18 ± 3	-	-	-	-
		100	0.9	7.1 ± 0.1	2.40 ± 0.01	3 ± 3	0.2 ± 0.2	57 ± 6	215 ± 10	19 ± 4	1.07 ± 0.04 (31 ± 17)	1.03 ± 0.08 (3 ± 8)	1.04 ± 0.03 (32 ± 21)	1.03 ± 0.07 (6 ± 17)
iCheY	M	0.1	-	7.2 ± 0.2	2.43 ± 0.02	6 ± 5	0.3 ± 0.4	57 ± 6	210 ± 11	18 ± 4	-	-	-	-
		100	2.0	7.1 ± 0.1	2.41 ± 0.01	5 ± 5	0.2 ± 0.3	54 ± 6	221 ± 9	21 ± 4	1.06 ± 0.04 (26 ± 18)	1.07 ± 0.11 (7 ± 11)	1.02 ± 0.04 (18 ± 26)	1.00 ± 0.07 (1 ± 17)
aCheYp	M	0.1	-	7.0 ± 0.1	2.41 ± 0.01	8 ± 5	0.2 ± 0.3	58 ± 5	208 ± 9	20 ± 4	-	-	-	-
		100	2.9	7.2 ± 0.3	2.41 ± 0.03	6 ± 4	0.7 ± 0.7	60 ± 6	211 ± 11	19 ± 4	1.12 ± 0.05 (54 ± 24)	1.22 ± 0.12 (21 ± 10)	1.09 ± 0.05 (66 ± 35)	1.17 ± 0.11 (39 ± 24)
	D	0.1	-	7.0 ± 0.1	2.41 ± 0.01	5 ± 2	0.2 ± 0.3	58 ± 5	211 ± 10	21 ± 4	-	-	-	-
		50	1.2	7.0 ± 0.1	2.40 ± 0.01	4 ± 2	0.1 ± 0.2	58 ± 5	216 ± 8	19 ± 3	1.05 ± 0.04 (23 ± 17)	1.10 ± 0.09 (9 ± 8)	1.04 ± 003 (28 ± 22)	1.05 ± 0.06 (10 ± 13)
		100	1.2	7.1 ± 0.1	2.39 ± 0.01	3 ± 4	0.1 ± 0.2	58 ± 5	214 ± 8	18 ± 3	1.08 ± 0.04 (34 ± 17)	1.16 ± 0.09 (15 ± 8)	1.06 ± 0.03 (42 ± 22)	1.10 ± 0.06 (22 ± 14)
iCheYp	M	0.1	-	7.0 ± 0.1	2.40 ± 0.01	9 ± 6	0.2 ± 0.3	59 ± 5	207 ± 8	19 ± 4	-	-	-	-
		100	0.9	7.1 ± 0.2	2.40 ± 0.02	7 ± 6	0.3 ± 0.4	59 ± 5	215 ± 10	20 ± 4	1.08 ± 0.04 (34 ± 19)	1.09 ± 0.10 (9 ± 9)	1.06 ± 0.04 (43 ± 25)	1.04 ± 0.08 (8 ± 18)
$FliM_N$	M	0.1	-	1.7 ± 0.1	0.35 ± 0.01	0	0.5 ± 0.6	3 ± 2	39 ± 4	2 ± 1	-	-	-	-
		50	0.5	1.7 ± 0.1	0.35 ± 0.01	0	0.6 ± 0.8	4 ± 2	39 ± 4	3 ± 1	1.07 ± 0.13 (7 ± 14)	1.06 ± 0.15 (5 ± 12)	1.05 ± 0.11 (14 ± 28)	1.06 ± 0.12 (12 ± 25)
		100	0.3	1.7 ± 0.1	0.35 ± 0.01	0	0.3 ± 0.4	4 ± 2	39 ± 4	2 ± 1	1.09 ± 0.12 (11 ± 13)	1.08 ± 0.14 (7 ± 12)	1.07 ± 0.11 (17 ± 26)	1.04 ± 0.11 (8 ± 23)
	D	0.1	-	2.0 ± 0.1	0.37 ± 0.01	0	0.2 ± 0.3	2 ± 1	31 ± 4	1 ± 1	-	-	-	-
		50	1.1	1.9 ± 0.2	0.36 ± 0.01	0	0.9 ± 1.2	4 ± 2	28 ± 4	1 ± 1	1.11 ± 0.07 (12 ± 7)	1.11 ± 0.09 (9 ± 7)	1.21 ± 0.09 (89 ± 33)	1.20 ± 0.10 (65 ± 28)
		100	0.6	1.9 ± 0.1	0.36 ± 0.01	0	0.2 ± 0.2	3 ± 1	29 ± 5	1 ± 1	1.00 ± 0.06 (0 ± 7)	1.01 ± 0.08 (0 ± 6)	1.01 ± 0.07 (6 ± 28)	1.03 ± 0.08 (9 ± 25)
aCheYp–$FliM_N$	C	0.1	-	7.3 ± 0.1	2.64 ± 0.01	6 ± 6	0.2 ± 0.3	73 ± 6 (6 ± 2)	229 ± 10	22 ± 5 (2 ± 1)	-	-	-	-
		50	1.1	7.5 ± 0.2	2.66 ± 0.01	8 ± 4	0.2 ± 0.3	72 ± 7 (5 ± 2)	234 ± 11	23 ± 4 (2 ± 1)	1.06 ± 0.04 (27 ± 20)	1.06 ± 0.09 (7 ± 9)	1.04 ± 0.04 (30 ± 29)	1.05 ± 0.06 (12 ± 16)
		100	1.1	7.5 ± 0.1	2.64 ± 0.02	4 ± 2	0.2 ± 0.3	73 ± 6 (6 ± 2)	239 ± 11	22 ± 4 (2 ± 1)	1.08 ± 0.04 (36 ± 18)	1.08 ± 0.09 (8 ± 10)	1.06 ± 0.04 (46 ± 27)	1.04 ± 0.06 (11 ± 14)

[a]M: Monomer state started from monomeric structure, D: Dissociated state generated by PaCS-MD, C: Complex state. The average values and the standard deviation (values after '±') for the second half of the MD trajectories (M and C), and those for the PaCS-MD trajectories in Phase 3 (D) are shown. [b]RMSD of the representative structure at 100 MPa from that at 0.1 MPa calculated for all C_α atoms except for two terminal residues. [c]Solvent accessible surface area. [d]Excluded volume defined as the space inside of SASA. [e]Cavity volume inside the proteins. [f]Isothermal compressibility. [g-i]H_{PP}, H_{PW}, and H_{PWP} are the number of hydrogen bonds connecting protein–protein, protein–water, and protein–water–protein. For H_{PP}, the values in the parentheses indicate the number between aCheYp and $FliM_N$. For H_{PWP}, the values in the parentheses show the number for aCheYp–water–$FliM_N$. [j]Ratio of the number of water molecules in the first hydration sphere relative to 0.1 MPa. [k]Ratio of the number of water molecules around the complex interface in the first hydration sphere relative to 0.1 MPa. [l]Ratio of the number of water molecules in the second hydration sphere relative to 0.1 MPa. [m]Ratio of the number of water molecules around the complex interface in the second hydration sphere relative to 0.1 MPa. [j-m]The values in the parentheses indicates actual increase in the number of waters.

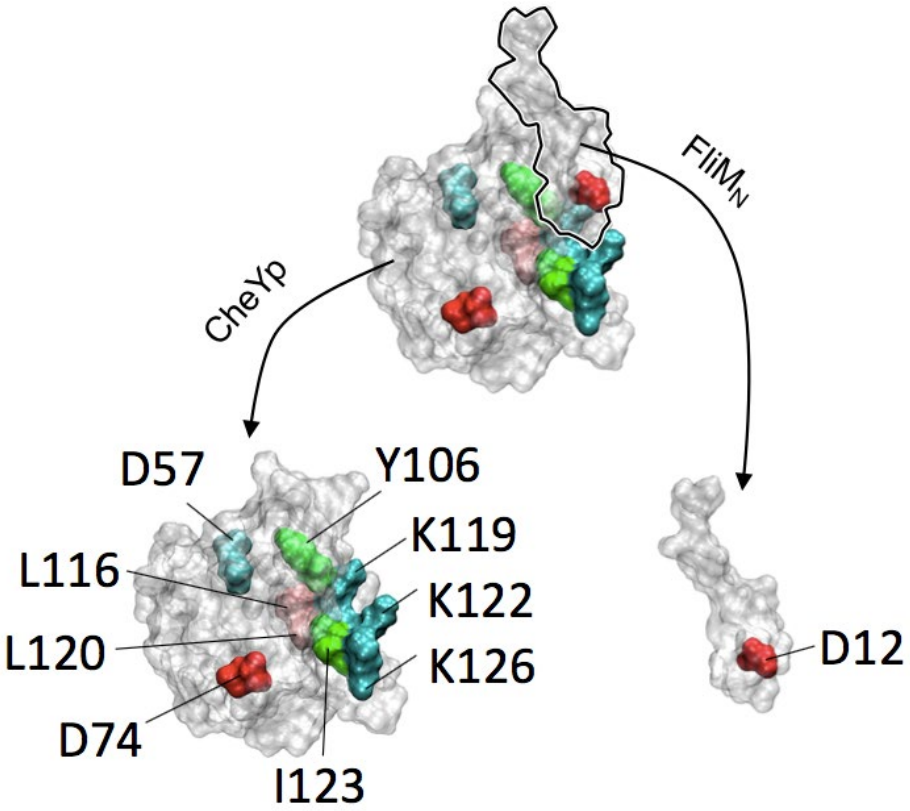

Figure S1. The residues mentioned in the main text. Phosphorylated D57, interface residues (Y106 –K126 of CheY and D12 of FliM$_N$), and D74 that underwent side-chain flip at 100 MPa in iCheY are shown. These residues are colored by residue types and the other residues are shown in transparent white so that residues in the back side are visible, e.g., D12.

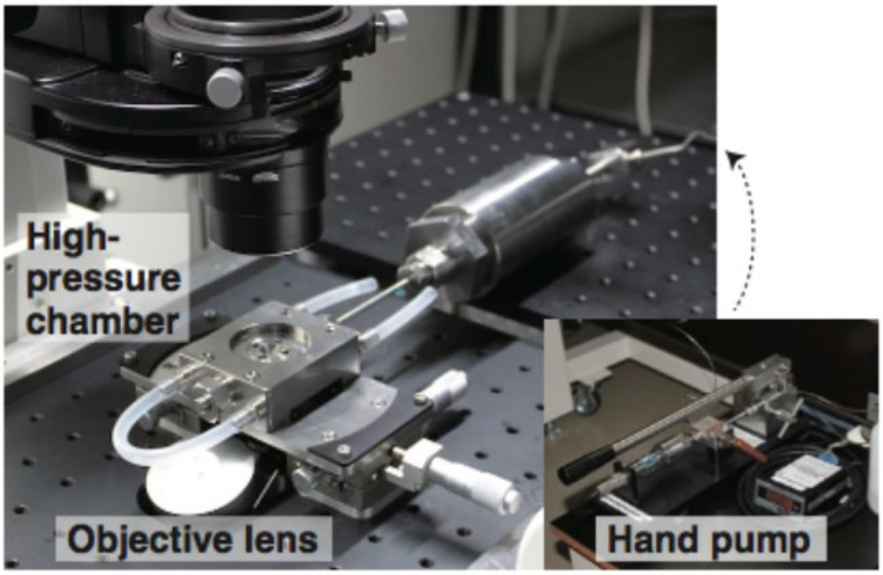

Figure S2. Photograph of the experimental setup.

Movie S1. Dissociation process of CheYp-FliM$_N$ complex at 0.1 MPa. Colorings are the same as in Fig. 3.

Movie S2. Dissociation process of CheYp-FliM$_N$ complex at 50 MPa. Colorings are the same as in Fig. 3.

Movie S3. Dissociation process of CheYp-FliM$_N$ complex at 100 MPa. Colorings are the same as in Fig. 3.