Supplemental Material For:

Title: Structural Basis of Directional Switching by the Bacterial Flagellum

Authors: Steven Johnson[1*+], Justin C. Deme[1+], Emily J. Furlong[2], Joseph J.E. Caesar[2,3], Fabienne F.V. Chevance[4], Kelly T. Hughes[4] & Susan M. Lea[1*]

Affiliations:

[1] Center for Structural Biology, CCR, NCI, Frederick, MD 21702-1201 USA

[2]Sir William Dunn School of Pathology, University of Oxford, Oxford, UK.

[3]Central Oxford Structural Molecular Imaging Centre, University of Oxford, Oxford, UK.

[4]Department of Biology, University of Utah, Salt Lake City, Utah, USA.

+ equal contributions

[*]Correspondence to: susan.lea@nih.gov or steven.johnson2@nih.gov

Table S1. Species equivalence of residues specified in text and figures.

	E. coli	*S*. typhimurium	*C. sporogenes*
MotA	R90	R90	R92
	Q91	Q91	R93
	M92	Q92	E94
	G93	G93	G95
	M94	M94	L96
	F95	F95	L97
	E98	E98	E100
	R131	R131	R116
	S135	S135	D120
	A253	A253	N236
	P255	P255	R238
FliG	R245	R245	V249
	Q248	Q248	Q252
	Q252	Q252	R256
	S256	S256	T260
	E257	E257	K261
	M278	M278	Q282
	S279	S279	S283
	Q280	Q280	K284
	R281	R281	R285
	I285	I285	S289
	D288	D288	E292
	D289	D289	D293
	N292	N292	F296

Table S2. Bacterial strains and plasmids used in this study

Strain	Genotype	Reference
E. coli MT56		Baumgarten *et al*., 2017
*S.*Typhimurium TH25631	Δ*flgE7659 flhD8070 flhC8092 fliA5225* Δ*fliB-T7771 fliG8835*(ΔPAA) Δ*rflM8403 fljB*^{enx} *vh2*	Johnson *et al*., 2022
*S.*Typhimurium TH25455	Δ*flgE7659 flhD8070 flhC8092 fliA5225* Δ*fliB-T7771* Δ*cheY8562* Δ*rflM8403 fljB*^{enx} *vh2*	This study

Plasmid	Description[a]	Reference
pT12_csMotAB	*Clostridium sporogenes motAB*$_{TEV2xStrepII}$	Deme *et al*., 2020
pT12_csMotA-FliG$_C$-MotB	*Clostridium sporogenes motAB*$_{TEV2xStrepII}$ with in-frame insertion of a $(GGGS)_3$ linker and *C. sporogenes fliG*$_{239-333}$ after S262 of MotA. Generated by Gibson assembly of the PCR products of the following two primer/template pairs: cs_short_FliGc_FWD + cs_short_FliGc_RVS from *C. sporogenes* 388 (DSM 795) genomic DNA cs_fliG_backbone_FWD + cs_fliG_backbone_RVS from pT12_csMotAB	This study

Table S3. Oligonucleotides used in this study

Primer	Sequence 5'-3'	Used in the construction of
cs_fliG_backbone_FWD	GGGGAAGGAGCAGCTCAAAATG	pT12_csMotA-FliG$_c$-MotB
cs_fliG_backbone_RVS	AGATACTTGTACTTTACTATAAGCTTGTCTTTCTG	pT12_csMotA-FliG$_c$-MotB
cs_short_FliGc_FWD	ATAGTAAAGTACAAGTATCTGGTGGAGGTTCTGGCGGTGGAAGCGGAGGGGGGTCGGTATTCGAAGATATA ATAACTCTAGACGATG	pT12_csMotA-FliG$_c$-MotB
cs_short_FliGc_RVS	TTTTGAGCTGCTCCTTCCCCCTAATCCTCGCCACCTCTTGATATTAC	pT12_csMotA-FliG$_c$-MotB