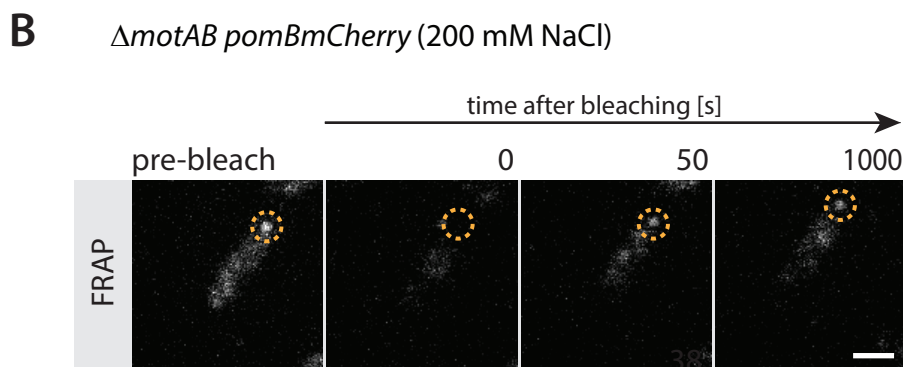
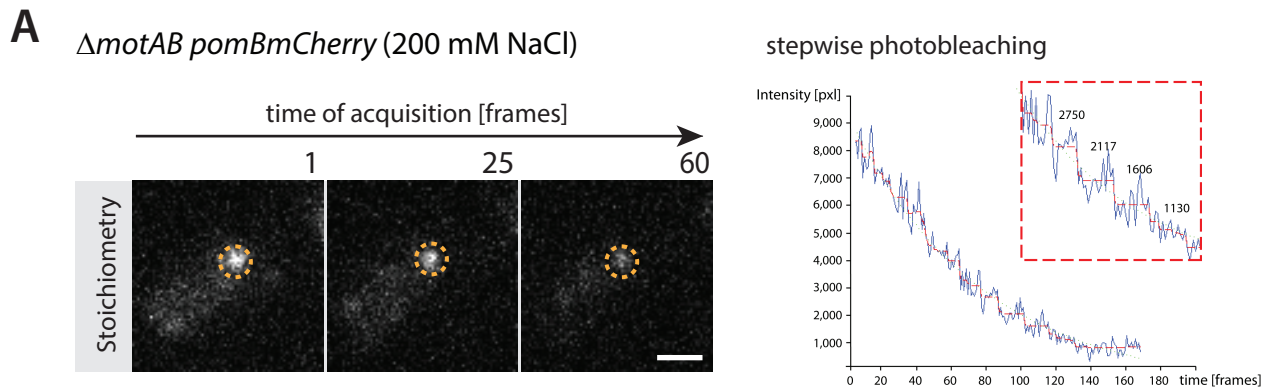
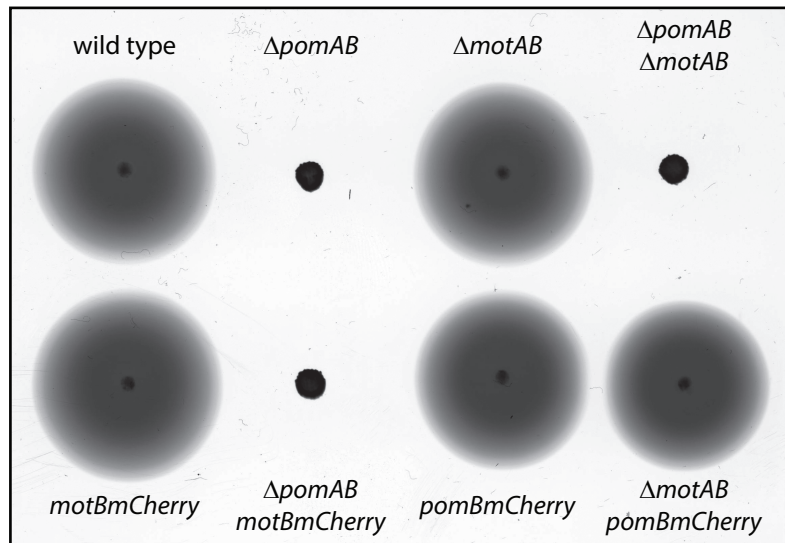


**Figure S1: Detection of MotB-mCherry and PomB-mCherry fusion proteins by immunoblot analysis.** Cell lysates of the indicated strains grown under appropriate conditions were subjected to SDS-PAGE followed by immunoblot analysis using an antibody raised against mCherry. Arrows with asterisks mark the positions corresponding to the estimated molecular masses of PomB-mCherry (61 kDa), MotB-mCherry (57 kDa) and mCherry (29 kDa), respectively. Wild-type cells not labeled with mCherry were used as negative control.



**Supplemental Figure 2: Analysis of stator stoichiometry and turnover by fluorescence microscopy**

**A) Determination of stator molecules.** Displayed are representative examples of fluorescence micrographs during photobleaching at the indicated timepoints (frames) of  $\Delta motAB pomBmCherry$  in 4M buffer supplemented with 200 mM NaCl (left) and an example for the stepwise photobleaching of fluorescence intensity using Matlab (right). **B) FRAP analysis:** Fluorescence micrographs for FRAP analysis of  $\Delta motAB pomBmCherry$  at 200 mM NaCl at the indicated timepoints (s); left panel pre-bleach, right panels micrographs after bleaching. Micrographs in (A) and (B) were modified in ImageJ, using the same contrast settings. Polar localization of stator complexes is highlighted by yellow circles. Scale bar, 2  $\mu$ m.



**Supplemental Figure 3: Spreading efficiency of cells with labeled stators in soft agar.**

3  $\mu$ l of exponentially growing cultures of the indicated strains were placed on 0.3 % soft agar and allowed to spread for 18 h.  $\Delta pomAB$  strains were non-motile under these conditions but exhibited robust movement when observed by light microscopy.

**Table S1:** Statistics for the determination of stator stoichiometry by fluorescence microscopya) Stator numbers in dependence of Na<sup>+</sup> concentrations

strain	mM	N	Mean	SD	Median	P25	P75	P-Value <sup>1</sup>	P-Value <sup>2</sup>
WT-PomBmCherry	200	107	7.79	2.16	7.22	6.39	9.27	<0.01	0.0002
	0	88	6.53	2.17	6.48	5.25	8.20	>0.15	
$\Delta$ <i>mot</i> -PomBmCherry	200	103	7.41	2.22	7.13	5.98	8.67	0.033	0.0043
	0	133	8.32	2.87	8.26	6.29	10.54	0.060	
WT-MotBmCherry	200	51	2.49	1.27	2.32	1.54	3.27	0.086	<0.0001
	0	54	4.77	2.00	4.67	3.11	5.71	>0.15	
$\Delta$ <i>pom</i> -MotBmCherry	200	69	8.39	3.07	7.98	6.11	9.64	0.032	<0.0001
	0	96	10.94	3.05	10.61	8.71	12.57	>0.15	

Abbreviations: SD=Standard deviation; P25= 25<sup>th</sup> percentile; P75=75<sup>th</sup> percentile

<sup>1</sup>P-value of Kolmogorov-Smirnov test of goodness of fit. If p-value below 0.05 mean variable is **not normally distributed**.

<sup>2</sup>P-Value of Mann Whitney test. If p-value below 0.05 means medians of variables are **significantly different**.

## b) Differences of stator numbers in the wild type compared to mutants with only a single stator

strain	mM	N	Mean	SD	Median	P25	P75	P-Value <sup>1</sup>	P-Value <sup>2</sup>
WT-PomBmCherry	200	107	7.79	2.16	7.22	6.39	9.27	<0.01	0.0660
$\Delta$ <i>mot</i> -PomBmCherry	200	103	7.41	2.22	7.13	5.98	8.67	0.033	
$\Delta$ <i>mot</i> -PomBmCherry	0	133	8.32	2.87	8.26	6.29	10.54	0.060	<0.0001
WT-PomBmCherry	0	88	6.53	2.17	6.48	5.25	8.20	>0.15	
WT- <i>mot</i> BmCherry	200	51	2.49	1.27	2.32	1.54	3.27	0.086	<0.0001
$\Delta$ <i>pom</i> MotBmCherry	200	69	8.39	3.07	7.98	6.11	9.64	0.032	
$\Delta$ <i>pom</i> -MotBmCherry	0	96	10.94	3.05	10.61	8.71	12.57	>0.15	<0.0001
WT- <i>mot</i> BmCherry	0	54	4.77	2.00	4.67	3.11	5.71	>0.15	

Abbreviations: SD=Standard deviation; P25= 25<sup>th</sup> percentile; P75=75<sup>th</sup> percentile

<sup>1</sup>P-value of Kolmogorov-Smirnov test of goodness of fit. If p-value below 0.05 mean variable is **not normally distributed**.

<sup>2</sup>P-Value of Mann Whitney test. If p-value below 0.05 means medians of variables are **significantly different**.

**Table S2: Statistical analysis of FRAP data for stator exchange**

strain	mM	N	Number of Clusters <sup>1</sup>	Exchange half-time <sup>2</sup>	SE	Exchange recovery rates <sup>2</sup>	SE	Exchange half-time <sup>3</sup>	R-square <sup>4</sup>
WT-PomBmCherry	200	15	5	24.52	2.81	0.041	0.0061	26.52	0.84
PomAB and MotAB	0	17	6	8.95	0.67	0.090	0.0055	8.18	0.82
<i>Δmot</i> -PomBmCherry	200	15	5	30.80	2.57	0.023	0.014	29.88	0.78
(PomAB only)	0	27	9	32.59	1.5	0.029	0.00312	34.73	0.94
<i>Δpom</i> -MotBmCherry	200	18	6	16.36	1.5	0.055	0.005	13.45	0.92
(MotAB only)	0	18	6	36.10	3.16	0.030	0.0059	36.01	0.81

Abbreviations: SE=Standard error

<sup>1</sup> cells were clustered according to their plateau of recovery of fluorescence intensity

<sup>2</sup> exchange half-time was derived by averaging the fit to clusters of three cells with a similar range of plateau for fluorescence recovery

<sup>3</sup>exchange half-time derived from the average fit of all analysed cells

<sup>4</sup> R-square was derived for the fit to the average of the normalised fluorescence intensity data for FRAP as shown in figure 1

**Table S 3:** Statistics of the swimming speed analysisa) Swimming speed [ $\mu\text{m/s}$ ] of different strains in dependence of  $\text{Na}^+$  concentrations

strain	NaCl [mM]	N	Mean	SD
<i>S. oneidensis</i> MR-1	200	120	53.2	16.0
	0	120	30.6	17.4
$\Delta\text{motAB}$	200	120	52.4	19.1
	0	120	16.6	5.1
$\Delta\text{pomAB}$	200	120	34.8	10.0
	0	120	40.5	15.6

Abbreviations: SD=Standard deviation

b) Comparison of the swimming speed [ $\mu\text{m/s}$ ] of different strains at high and low  $\text{Na}^+$  concentrations.

strain	NaCl [mM]	P-Value <sup>1</sup>	NaCl [mM]	P-Value <sup>1</sup>
<i>S. oneidensis</i> MR-1 $\Delta\text{motAB}$	200	0.981556	0	<0.0001
	0		0	
<i>S. oneidensis</i> MR-1 $\Delta\text{pomAB}$	200	<0.0001	0	<0.0001
	0		0	
$\Delta\text{motAB}$ $\Delta\text{pomAB}$	200	<0.0001	0	<0.0001
	0		0	
<i>S. oneidensis</i> MR-1	200	<0.0001	0	
	0		0	
$\Delta\text{motAB}$	200	<0.0001	0	
	0		0	
$\Delta\text{pomAB}$	200	0.372937	0	
	0		0	

<sup>1</sup>P-value of ANOVA test. If p-value below 0.05 mean variables are **significant different**.

**Table S4:** Strains, plasmids and oligonucleotides used in this study

strain	relevant genotype or phenotype	source or reference
<i>E. coli</i>		
DH5 $\alpha$ pir	<i>recA1 gyrA (lacIZYA-argF)</i> (80d <i>lac</i> [ <i>lacZ</i> ] M15) <i>pir</i> RK6	(1)
WM3064	<i>tbrB1004 pro tbi rpsL hsdS lacZ</i> $\Delta$ M15 RP4-1360 $\Delta$ ( <i>araB:AD</i> ) 567 $\Delta$ <i>dapA</i> 1341::[ <i>erm pir</i> (wt)]	W. Metcalf, University of Illinois, Urbana-Champaign
<b><i>In frame deletions</i></b>		
<b><i>S. oneidensis</i> MR-1</b>	<b>wild type</b>	<b>(2)</b>
$\Delta$ <i>pomAB</i>	$\Delta$ SO_1529-30	(3)
$\Delta$ <i>motAB</i>	$\Delta$ SO_4287-86	(3)
$\Delta$ <i>pomAB</i> / $\Delta$ <i>motAB</i>	$\Delta$ SO_1529-30; $\Delta$ SO_4287-86	(3)
<b><i>In frame insertion of fluorescence protein fusions</i></b>		
<i>motB-mCherry</i>	<i>motB::mCherry</i> ; Km <sup>r</sup> ; C-terminal fusion <i>mCherry</i> to <i>motB</i>	This work
<i>pomB-mCherry</i>	<i>pomB::mCherry</i> ; Km <sup>r</sup> ; C-terminal fusion <i>mCherry</i> to <i>pomB</i>	This work
$\Delta$ <i>pomAB</i> <i>motB-mCherry</i>	$\Delta$ SO_1529-30; <i>motB::mCherry</i> ; Km <sup>r</sup> ; C-terminal fusion <i>mCherry</i> to <i>motB</i>	This work
$\Delta$ <i>motAB</i> <i>pomB-mCherry</i>	$\Delta$ SO_4287-86; <i>pomB::mCherry</i> ; Km <sup>r</sup> ; C-terminal fusion <i>mCherry</i> to <i>pomB</i>	This work
<i>fliN-Gfp</i>	<i>fliN::gfp</i>	This work
<b>plasmid</b>		
pCR2.1-mCherry-SO	synthesized <i>mCherry</i> (monomeric), codon usage <i>S. oneidensis</i> MR-1 in pCR2.1 blunt end inserted	GenScript (USA)
pET21-sfGfp	fast maturing <i>gfp</i>	(4)
pNPTS138-R6KT	pUC origin pNPTS138 exchanged with $\gamma$ -origin from pUC18R6KT-mini-Tn7T	(3)
<b>Fluorescent protein fusion constructs</b>		
pNPTS-C- <i>pomB</i> (GGS)- <i>mCherry</i>	C-terminal fusion of <i>mCherry</i> to <i>pomB</i> ; linker (GGS) inserted upstream	This work
pNPTS-C- <i>motB</i> (GGS)- <i>mCherry</i>	C-terminal fusion of <i>mCherry</i> to <i>motB</i> ; linker (GGS) inserted upstream	This work
pNPTS-R6KT- <i>fliN-gfp</i>	C-terminal fusion of <i>gfp</i> to <i>fliN</i>	This work
<b><i>In frame deletion constructs</i></b>		
pGPSac28Km- $\Delta$ <i>pomAB</i>	<i>in frame pomAB</i> deletion fragment in pGPSac28Km; Km <sup>r</sup>	(3)
pGPSac28Km- $\Delta$ <i>motAB</i>	<i>in frame motAB</i> deletion fragment in pGPSac28Km; Km <sup>r</sup>	(3)

Km<sup>r</sup>, kanamycin resistance

oligonucleotide	Sequence 5'-3'	Restriction endonuclease
<b><i>Fluorescent protein fusions</i></b>		
PspOMI-pomB-C-mCherry-up-fw	CTC ATA <u>GGG CCC</u> TTG GCT ACA TTT GCC GAT TTG ATG	<i>PspOMI</i>
pomB-C-mCherry-up-rev	TGG AAA CGC TGC CGC CAT TTG GTT TAT CCA CTT GAA TCT CTT CC	-
pomB-C-mCherry-OL-fw	AAT GGC GGC AGC GTT TCC AAA GGG GAA GAG GAC AAT ATG	-
pomB-C-mCherry-OL-rev	TGA GGA CGT GTT ATT TGT ATA ACT CAT CCA TAC CAC CAG	-
pomB-C-mCherry-dwn-fw	T AAC ACG TCC TCA TAT TCA GCC GTG	-
NheI-pomB-C-mCherry-dwn-rev	<u>TGC TAG CAA</u> GCC ACC TAA ACC TTC GAT ACG	<i>NheI</i>
PspOMI-motB-C-mCherry-up-fw	CTC ATA <u>GGG CCC</u> ACC AGA AAA TCA TGA GCG TTG G	<i>PspOMI</i>
motB-C-mCherry-up-rev	CTT TGG AAA CGC TGC CGC CCT CAG GAA TGG GAA TAT GGC TTT C	-
motB-C-mCherry-OL-fw	TGA GGG CGG CAG CGT TTC CAA AGG GGA AGA GGA CAA TAT G	-
motB-C-mCherry-OL-rev	AGG AGT ATT CTT TAT TTG TAT AAC TCA TCC ATA CCA CCA G	-
motB-C-mCherry-dwn-fw	TAA AGA ATA CTC CTT CTT AGA TGT GTT TTA ATT TGA C	-
NheI-motB-C-mCherry-dwn-rev	<u>TGC TAG CTA</u> ACT GGC TTA TCT ATT ATG TTC TTA ATC	<i>NheI</i>
FliN-I-fw-SphI	CAA <u>TGC ATG</u> CGC CAC CAT TGT CAG CCC AAC CGA AG	<i>SphI</i>
FliN-I-rv-Eco	CAT <u>CGA ATT</u> CCA TCT CAC TTC ACC TTT ATA ATT CTG	<i>EcoRI</i>
FliN-II-fw-Bam	AAG <u>TGG ATC</u> CAG TAC AGA TGA CGA TTG GGC AGC	<i>BamHI</i>
FliN-II-rv-Pst	GTT <u>ACT GCA</u> GCC GTT GCC GCA CTA CCT TCA TTG	<i>PstI</i>
Gfp138-fw-Eco-NL	CTT <u>GAA TTC</u> CGT AAA GGA GAA GAA CTT TTC AC	<i>EcoRI</i>
Gfp138-rv-G-Bam	GAA <u>GGA TCC</u> TCC TCC GCC TCC TTT GTA TAG	<i>BamHI</i>
<b>“check” Primer</b>		
pomB-C-fluo-chk-fw	ATG GCT AAG TGC AAC TGT CCA CC	
pomB-C-fluo-chk-rev	ATA CGC CCG AGT CGA AAC CAC	
motB-C-fluo-chk-fw	TAA CTG GTA TCG CTG ACG GTG AG	
motB-C-fluo-chk-rev	AAC CTG ACA CAG AAT TAT GAA CAG CC	
chk-pomAB-SO-rv	GCA CGC CAA TCG CAT CGG TAA	-
chk-pomAB-SO-fw	TGC ATT GAC TAA CAC GCT GAT TCG	-
chk-motAB-SO-fw	ACG TTA ATG GAG CGT CAC TTT AGT TC	-
chk-motAB-SO-rv	CTG ACA CAG AAT TAT GAA CAG CCT CT	-

#### Additional References

- (1) **Miller VL, Mekalanos JJ.** 1988. A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*. *J Bacteriol* **170**:2575-2583.
- (2) **Venkateswaran K, Moser DP, Dollhopf ME, Lies DP, Saffarini DA, MacGregor BJ, Ringelberg DB, White DC, Nishijima M, Sano H, et al.** 1999. Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. *Int J Syst Bacteriol* **49**:705-724.
- (3) **Paulick A, Koerdt A, Lassak J, Huntley S, Wilms I, Narberhaus F, Thormann KM.** 2009. Two different stator systems drive a single polar flagellum in *Shewanella oneidensis* MR-1. *Mol Microbiol* **71**:836-850.
- (4) **Pedelacq JD, Cabantous S, Tran T, Terwilliger TC, Waldo GS.** 2006. Engineering and characterization of a superfolder green fluorescent protein. *Nat Biotechnol* **24**:79-88