

Mechanosensitive recruitment of stator units promotes binding of the response regulator CheY-P to the flagellar motor

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Supplementary Note 1: Predicting CW_{bias} versus [CheY-P] relationship from CW_{bias} distributions

The random variables B and C represent the variability in CW_{bias} and [CheY-P] in a cell population, respectively. The random variable C (CheY-P level) is assumed to be normally distributed with a mean μ and standard deviation σ . The bias B at each C is calculated from the Hill function:

$$B = \frac{C^h}{C^h + K_D^h} \quad \text{Equation 1}$$

where h and K_D represent the Hill coefficient and the dissociation constant, respectively. We derive the probability density for the bias from the following expression ¹:

$$P(B) = f(\phi) \frac{d\phi}{dB}$$

where,

$$\phi(B) = C = K_D \left(\frac{B}{1-B} \right)^{\frac{1}{h}} = K_D \left(\frac{1}{B} - 1 \right)^{-\frac{1}{h}}$$

and $f(\phi)$ is the Gaussian function that describes the [CheY-P] distribution.

The expression for the CW_{bias} distribution is:

$$P(B) = \frac{K_D}{hB^2\sigma\sqrt{2\pi}} \left(\frac{1}{B} - 1 \right)^{-\frac{1}{h}-1} \exp \left(-\frac{1}{2} \frac{\left(K_D \left(\frac{1}{B} - 1 \right)^{-\frac{1}{h}} - \mu \right)^2}{\sigma^2} \right) \quad \text{Equation 2}$$

We assumed $h = 10$ and $K_D = 3.1 \mu\text{M}$ for motors with a full complement of stator units (high torque group) based on an earlier work ². This is a reasonable assumption as the measurements of Cluzel and co-workers were performed at high loads where the stator carries a full complement of stator units. We used a nonlinear least-square approach to fit equation 2 to the CW_{bias} distribution in the high torque group. This yielded

$\mu = 5.3 \pm 0.5 \mu\text{M}$ (mean $\pm$ SEM) for the *$\Delta cheR cheB cheZ$* cells. For the medium and low torque groups, we assumed that the μ and σ remained fixed while K_D was a free parameter. The fitted values of K_D for the different groups are shown in **Supplementary Table 1**. The fits to the CW_{bias} distributions are indicated in the plots.

The nature of K_D 's dependence on torque did not change when we assumed different values for μ and σ .

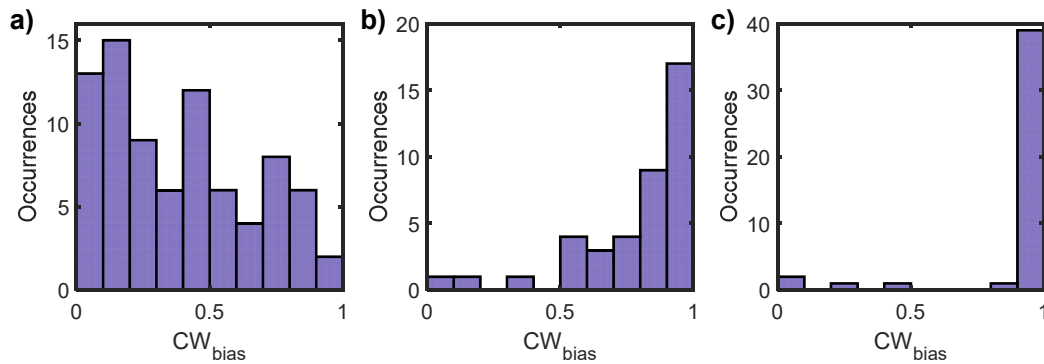
Supplementary Note 2: Estimation of CW_{bias} at varying viscous loads

The CW_{bias} was calculated from CCW-to-CW ($k_{CCW \rightarrow CW}$) and CW-to-CCW ($k_{CW \rightarrow CCW}$) switching rates from previous works^{3,4}:

$$CW_{\text{bias}} = \frac{k_{CCW \rightarrow CW}}{k_{CCW \rightarrow CW} + k_{CW \rightarrow CCW}}$$

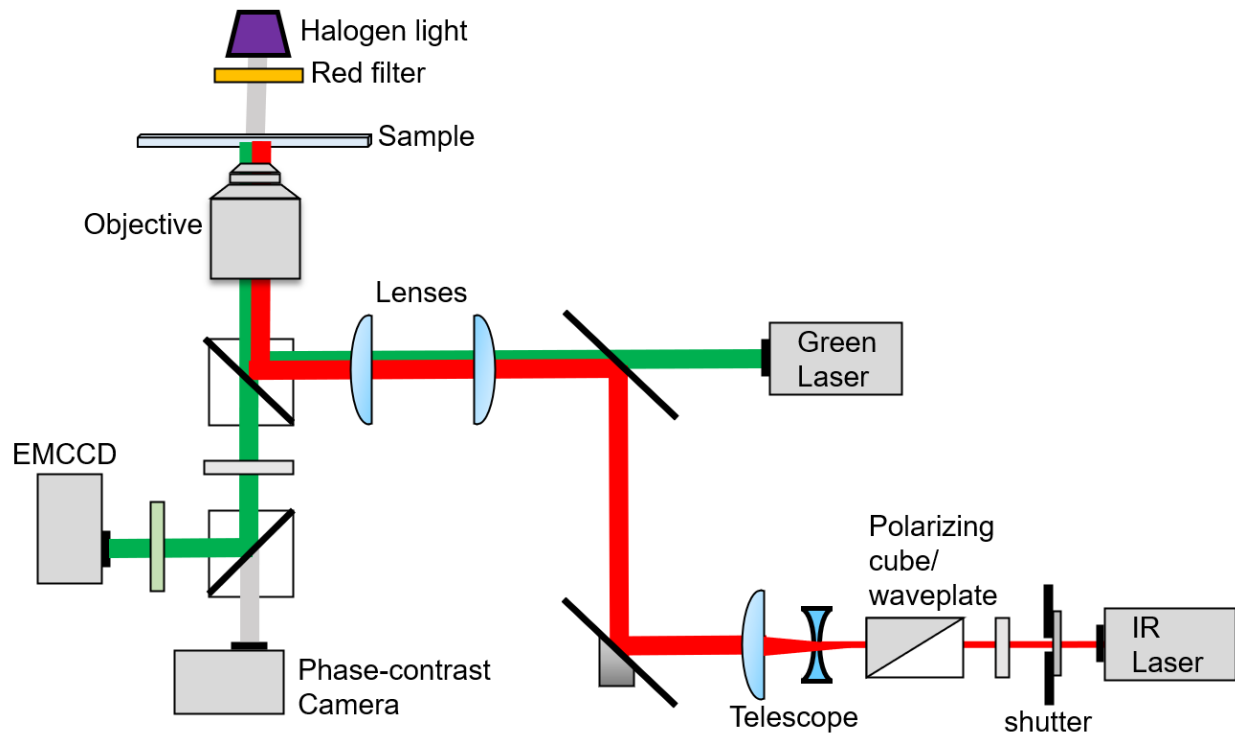
Supplementary Figures

Raw data for CW_{bias}



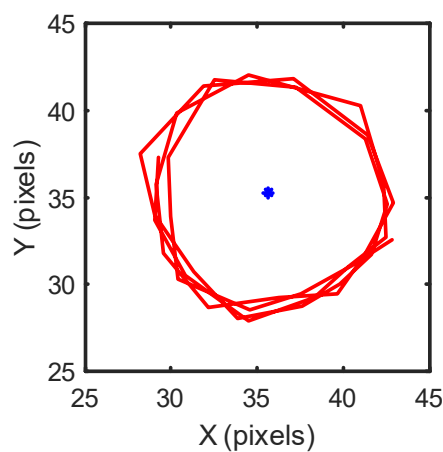
Supplementary Figure 1. Raw data for **Fig. 2B**: CW_{bias} histograms for the populations with **a)** low, **b)** medium, and **c)** high N_{st} . Source data are provided as a Source Data file.

Optical Trapping and TIRF Setup



Supplementary Figure 2. Setup for combined optical trapping (red laser) and TIRF illumination (green).

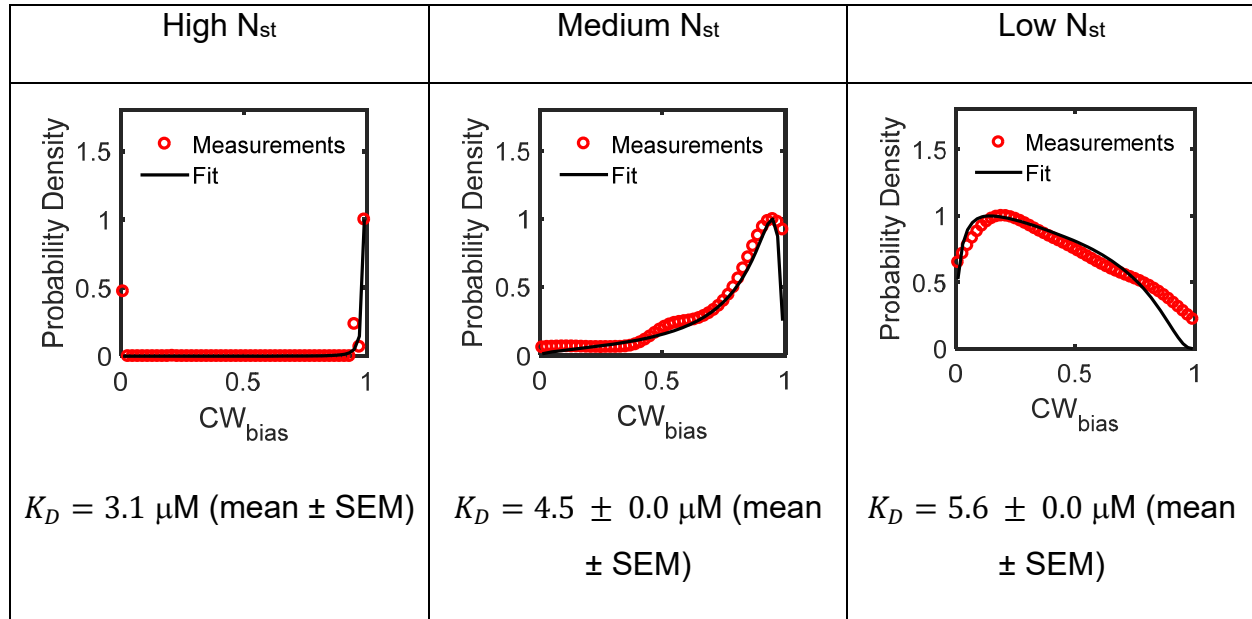
Point of tether in tethered cells



Supplementary Figure 3. The red trajectory shows the movements of the center-of-mass of a tethered cell. The center-of-mass was obtained from each image by fitting an ellipse to the cell. The tether point (motor location) coincides with the center of a circular fit to the trajectory. Source data are provided as a Source Data file.

Supplementary Tables

Supplementary Table 1: Nonlinear least-square fits



Supplementary Table 2: Plasmids

Plasmid	Plasmid #	Resistance	Cloning Sites
pBAD34- <i>motAmotB</i>	pPL39	Chloramphenicol	KpnI & Sall
pTrc99A- <i>eyfp-cheY</i>	pPL29	Ampicillin	KpnI & XbaI

Supplementary Table 3: Strains

Strain name	Lab Strain#	Genotype	Plasmid
MotAB+, Fig 1B	PL240	<i>fliCst ΔcheRcheBcheYcheZ</i>	pTrc99A- <i>eyfp-cheY</i>
Δ <i>motAB</i> , Fig 1B	PL239	<i>fliCst ΔcheRcheBcheYcheZ, ΔmotAmotB</i>	pTrc99A- <i>eyfp-cheY</i>
Δ <i>motAB</i> /p(empty), Fig 1C	PL349	<i>fliCst ΔcheRcheBcheYcheZ, ΔmotAmotB</i>	pTrc99A- <i>eyfp-cheY</i> , pBAD34
Δ <i>motAB</i> /p(MotAB), Fig 1C	PL350	<i>fliCst ΔcheRcheBcheYcheZ, ΔmotAmotB</i>	pTrc99A- <i>eyfp-cheY</i> , pBAD34- <i>motAmotB</i>
MotAB+, Fig 1D	PL233	<i>fliCst fliM-eYFP(A206K)-fliM ΔcheY</i>	
Δ <i>motAB</i> , Fig 1D	PL333	<i>fliCst fliM-eYFP(A206K)-fliM ΔmotAmotB ΔcheY</i>	
Δ <i>cheRcheBcheZ</i> , Fig 2A, B	PL254	<i>fliCst ΔcheRcheBcheZ ΔmotAmotB</i>	pBAD34- <i>motAmotB</i>

$\Delta cheRcheB$, Fig 2E	PL138	<i>fliCst ΔcheRcheB</i>	
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Supplementary Table 4: Primers

Primer	Sequence	Product/purpose
motAB_fwd_KpnI	CGTGGTACCGTCAACAGTGGAAAGGATG	pBAD34- <i>motAmotB</i>
motAB_rev_Sall	TTAGTCGACTCACCTCGGTTTCGGC	
EYFP_KpnI_fwd cheY_rev_XbaI	GGTACCCCATGGTGAGCAAGGCTCTAGAGCTCACATGCCAGTTT	pTrc99A- <i>eyfp-cheY</i>
CheRCheBCheZ_P1 NewP4_UsewithOIdP1	CTGATCGAAGAGTCAGTCAAT GCCAAACCAGATAACACTAAC	Amplification of $\Delta cheRcheBcheYcheZ$, $\Delta cheRcheB$, and $\Delta cheY$ DNA for construction of chemotactic mutants
MotAB_seqP1 motAB_seqP2New	AAGCAGAAGAAGGACCACTGC GCGCGAAAGGATAATTCGTCG	Amplification of $\Delta motAmotB$ DNA for construction of <i>mot</i> mutants

Supplementary References

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4. Yuan, J., Fahrner, K. A. & Berg, H. C. Switching of the bacterial flagellar motor near zero load. *J. Mol. Biol.* **390**, 394-400 (2009).