Supplementary Information for:

Signaling events that occur when cells of *E. coli* encounter a glass surface

Alina M. Vrabioiu and Howard C. Berg

Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138

Supplementary Figures

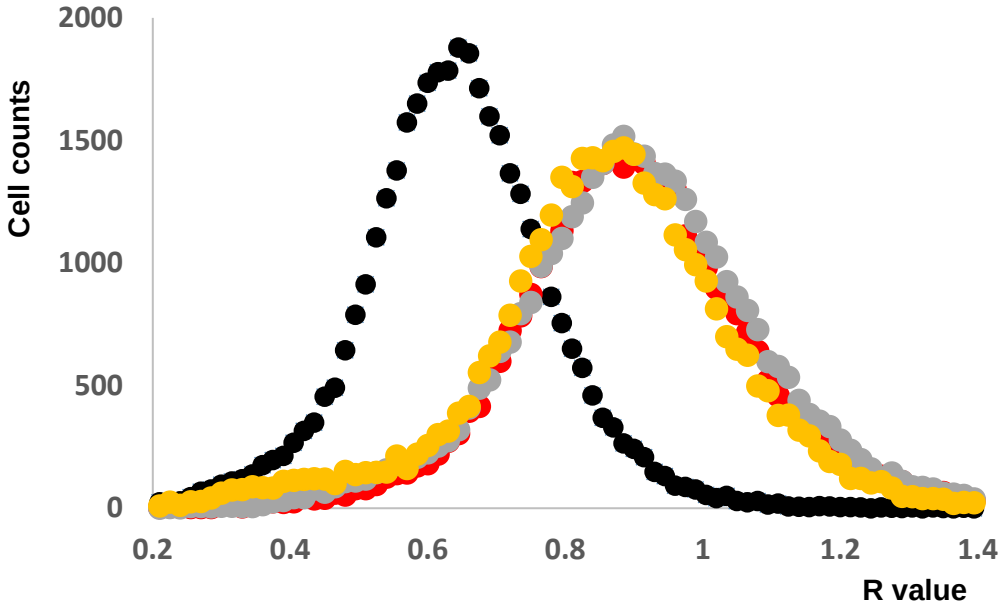


Fig. S1A

The sensor's dynamic range for high level expression of PdeH and WspR

Cell populations expressing the Sensor or the Sensor* platform and either the phosphodiesterase PdeH or the diguanylate cyclase WspR:D70E were induced for 4 hours with 100 μM IPTG and analyzed via flow cytometry. The data is presented in the histogram above; black is Sensor-expressing cells that overexpress WspR:D70E, red is Sensor-expressing cells that overexpress PdeH, and grey and orange are corresponding Sensor*-expressing cells.

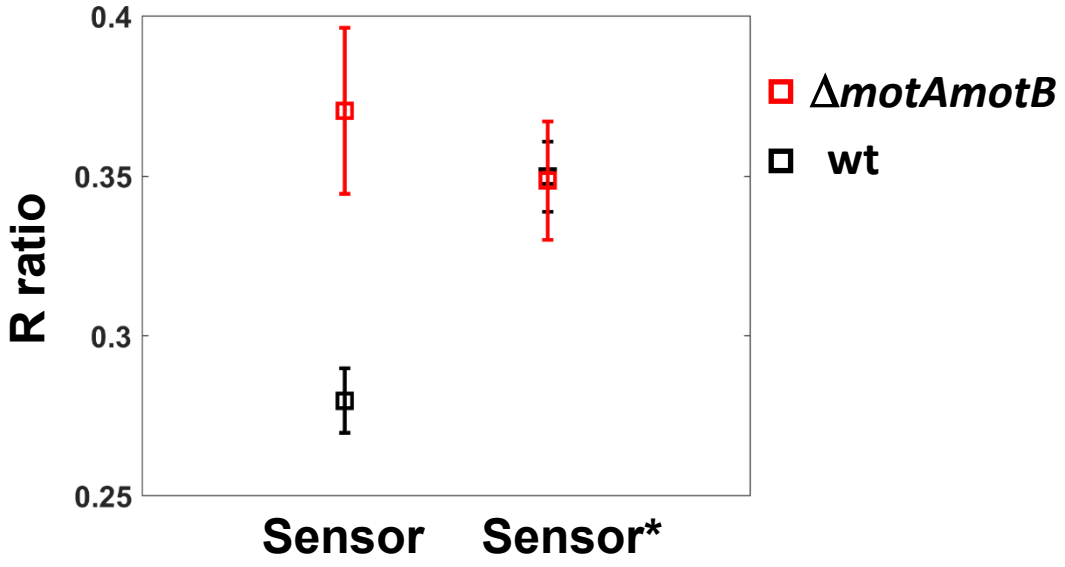


Fig. S1B

Cells that lack stators (ΔmotAmotB) have lower than wild type intracellular c-di-GMP

Wild type and Δ*motAmotB* cells in motility buffer with lactate (expressing either Sensor or Sensor*) were imaged 1 min after addition to channel slides. Average R values were computed from each category along with 95% confidence intervals. Number of cells varied between each category, as follows: 146 cells for wild type cells expressing Sensor, 120 cells for wild type cells expressing Sensor*, 68 for Δ*motAmotB* cells expressing Sensor, and 61 for Δ*motAmotB* cells expressing Sensor*. Black squares represent wild type cells, and red squares Δ*motAmotB* cells. Expression of either Sensor or Sensor* is indicated below each average measurement.

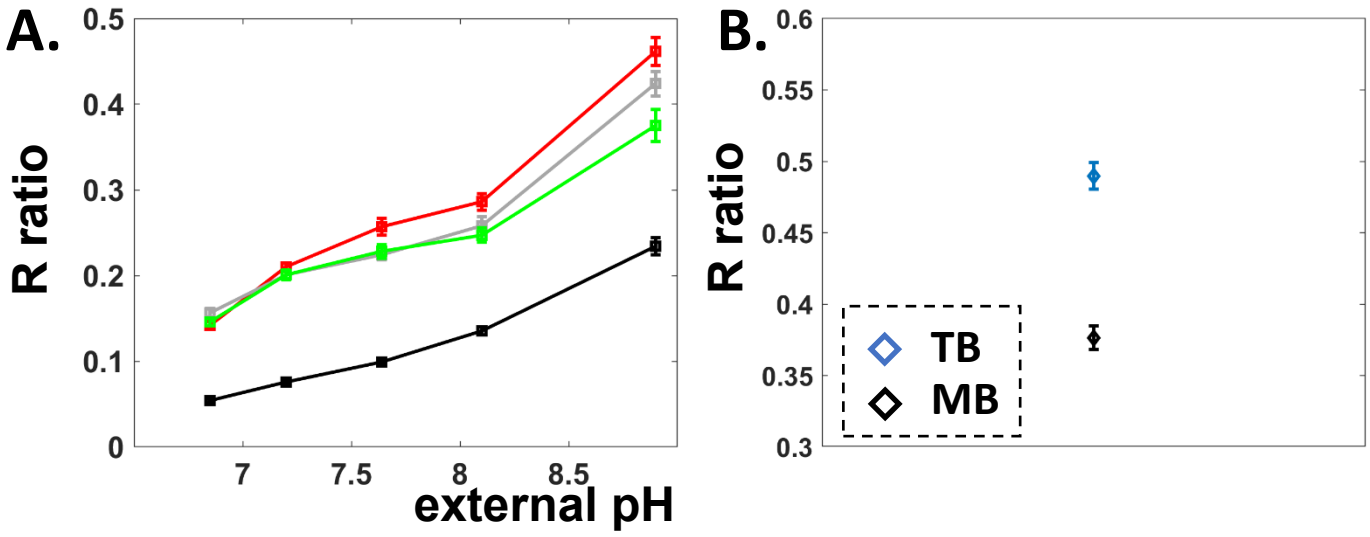


Fig. S2

The sensing platform is pH sensitive, and intracellular pH depends on the extracellular buffer

A. Both the Sensor and the Sensor* platforms are pH sensitive. Cells grown in tryptone broth were resuspended in 20 mM phosphate buffers of different pHs and attached to channel slide coverslips. 100 μM CCCP was added to reduce the transmembrane pH gradient. The average ratio R is plotted as a function of the external buffer pH for cells expressing the Sensor (green), and Sensor* (gray) platforms. In addition, measurements from Sensor cells overexpressing PdeH (red) or WspR:D70E (black) are also plotted. Brackets represent 95% confidence intervals.

B. Cells in tryptone broth have higher internal pH than cells in motility buffer, and the Sensor*'s R values vary accordingly. The blue diamond represents the average measurement in tryptone broth, and the black diamond the average measurement in motility buffer. 299 cells were averaged in each case. Brackets represent 95% confidence intervals.

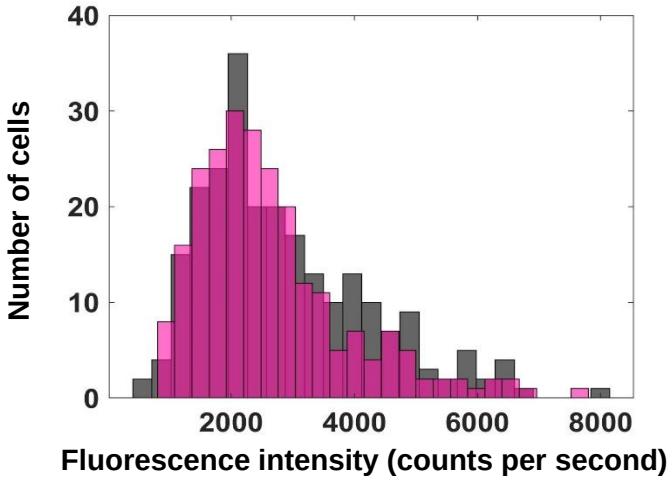


Fig. S3

Intracellular mScarletI intensity has a similar value distribution at different pHs

mScarletI intensity was measured for single cells attached to coverslips at external pH 7.2 and 8.1 (100 μM CCCP was added in both cases, as in Fig. S2A). The distribution of the measured values is shown in magenta (pH 7.2) and black (pH 8.1).

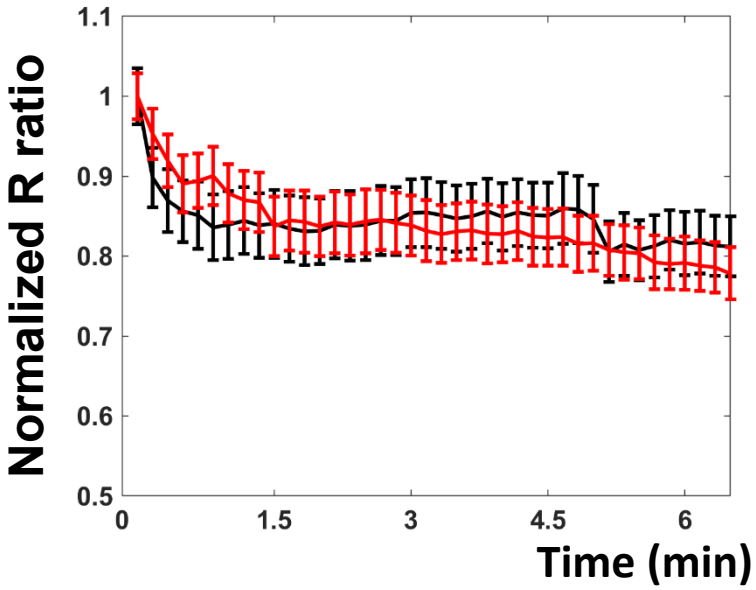


Fig. S4

c-di-GMP does not increase following surface attachment in the presence of 40 mM potassium benzoate and 40 mM methylamine

Images were acquired every 10 seconds following the attachment protocol from Fig. 2B, however the added motility buffer with lactate (pH= 7.5) contained 40 mM potassium benzoate and 40 mM methylamine to collapse the transmembrane proton gradient. Notice that the R values for both Sensor- and Sensor*-expressing cells decrease at a similar rate, suggesting that the c-di-GMP increase requires a transmembrane pH gradient.

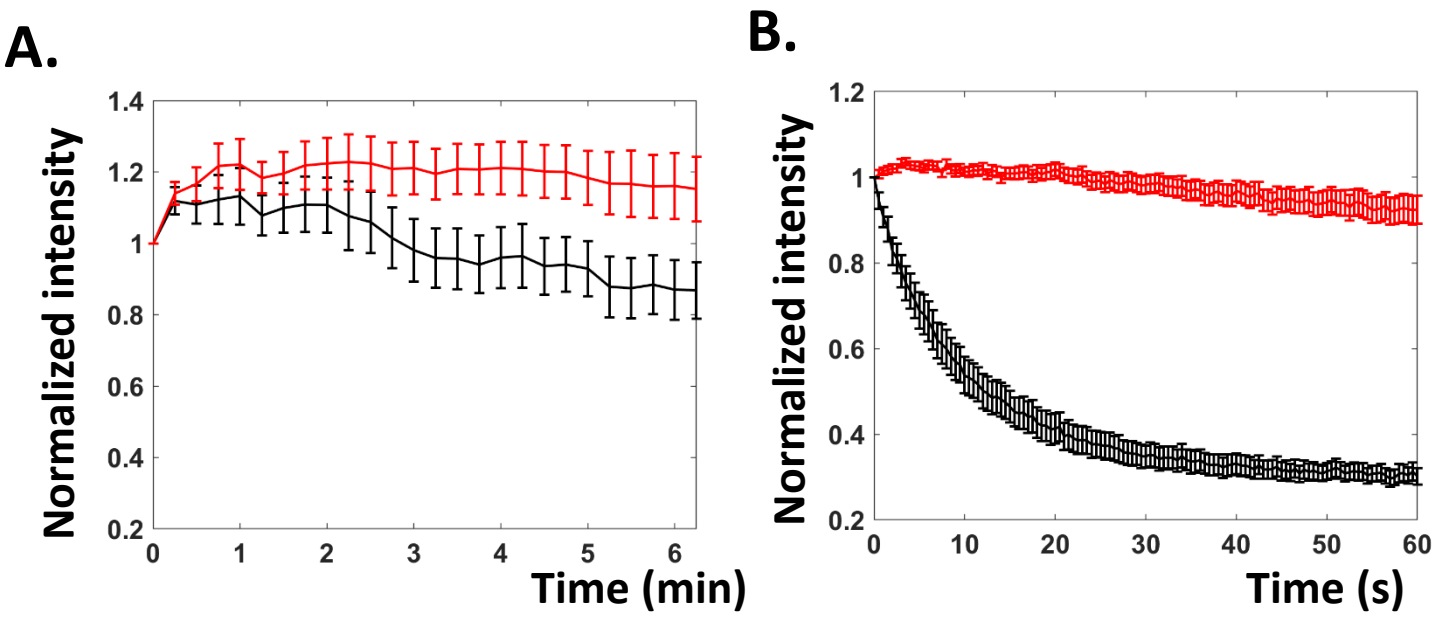


Fig. S5

mVenus^{NB} and mScarletI bleach at different rates under our experimental setup

A. Sensor*-expressing cells in motility buffer with lactate were imaged every 15 s, one minute after they attach to the coverslips. mVenus^{NB} fluorescence, averaged from 50 cell measurements and normalized to the initial value, is plotted as a function of time (black line). Similarly, mScarletI fluorescence measurement is depicted by the red line. The brackets at each time point represent 95% confidence intervals.

B. Sensor*-expressing cells in motility buffer with lactate were imaged every 0.5 s, 6 min after they were added to the channel slides. As above, the normalized average fluorescent measurement from 50 cells is depicted in red for mScarletI and in black for mVenus^{NB}. Brackets represent 95% confidence intervals.

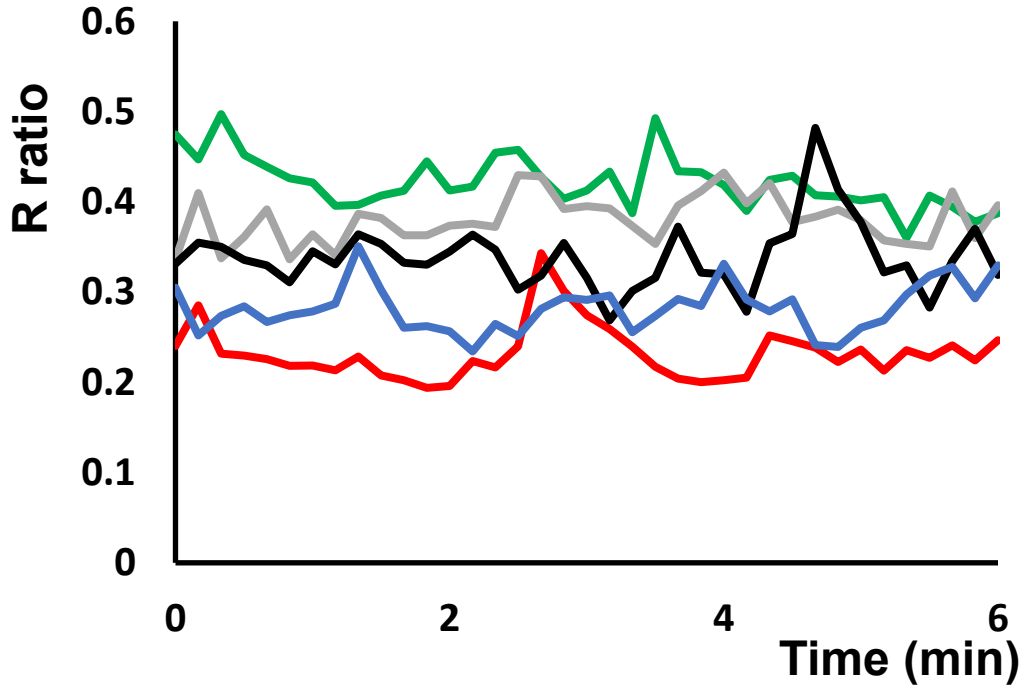


Fig. S6

Sensor* cells spike, but they lack the subsequent rapid downward decrease

Sensor*-expressing cells in motility buffer with lactate were imaged as they attached to coverslips every 10 s. The ratio R for individual cells was plotted as a function of time.

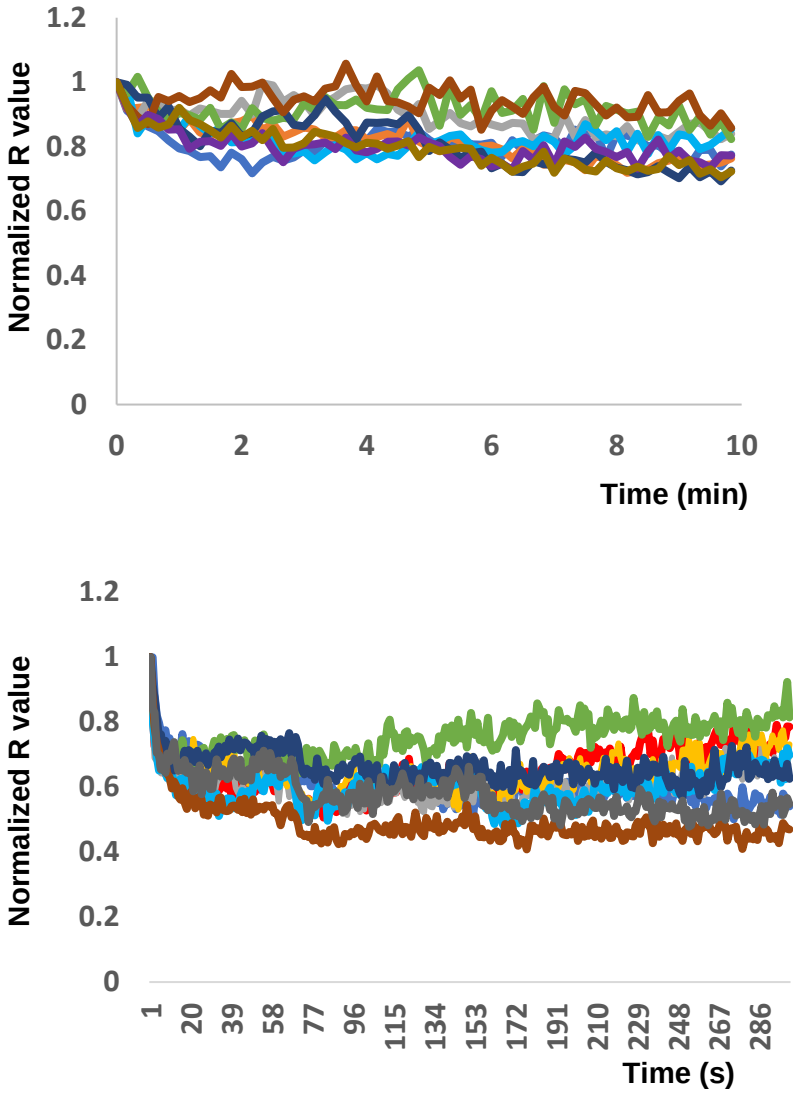


Fig. S7

No spikes were observed in lactate motility buffer when internal pH is clamped

Images were acquired every 10 s (top), and every 1 s (bottom) for cells in lactate motility buffer with 40 mM potassium benzoate and 40 mM methylamine. Individual R values (normalized to the initial value) are plotted as a function of time.

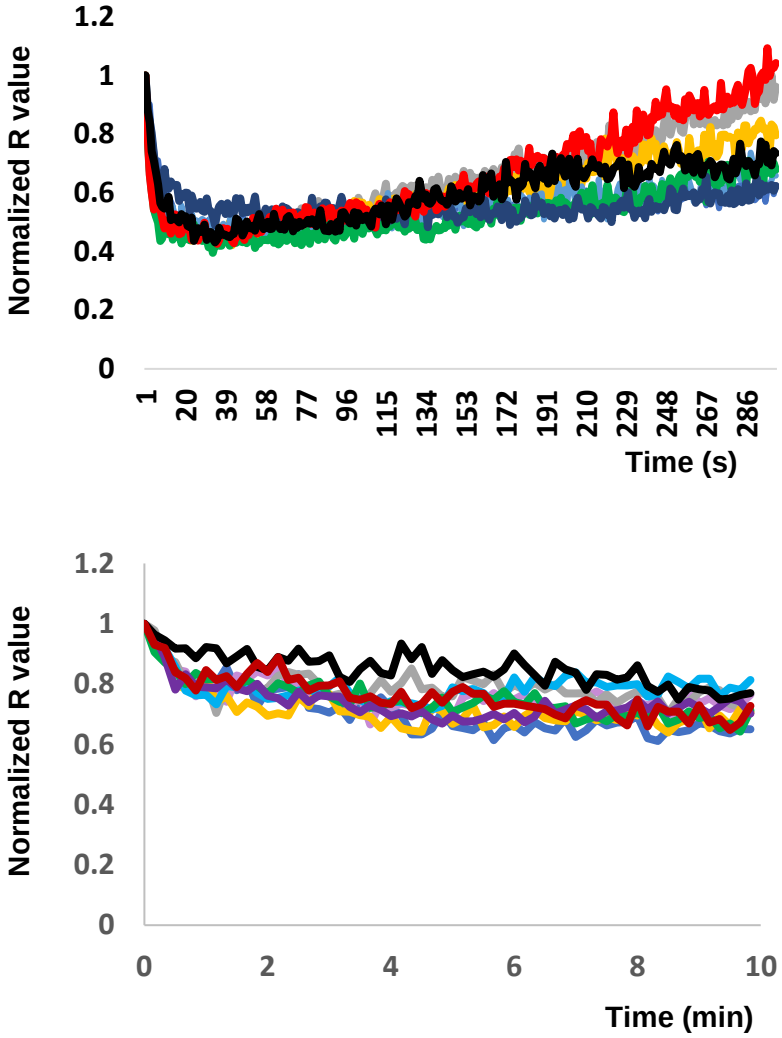


Fig. S8

No spikes were observed in glucose motility buffer when internal pH is clamped

Images were acquired every 1 s (top), and every 10 s (bottom) for cells in glucose motility buffer with 40 mM potassium benzoate and 40 mM methylamine. Individual R values (normalized to the initial value) are plotted as a function of time.

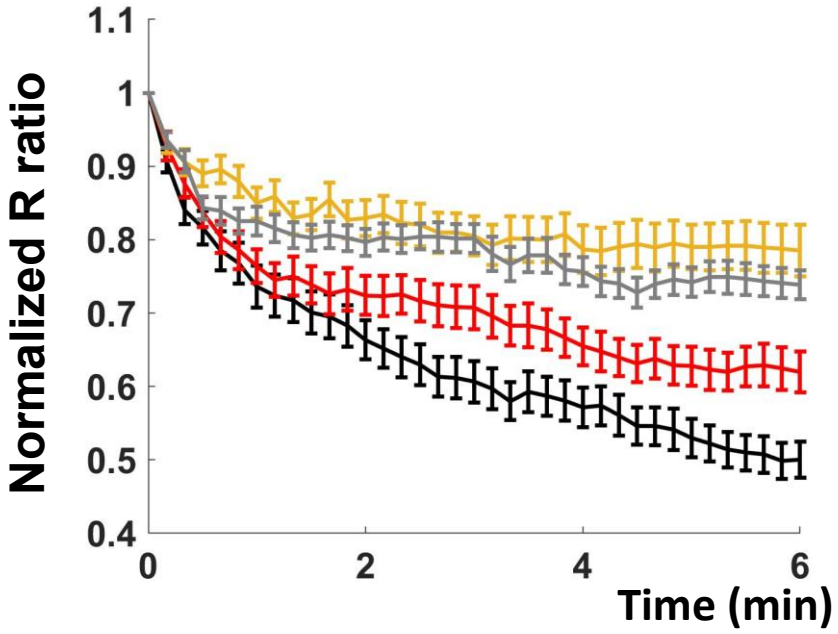
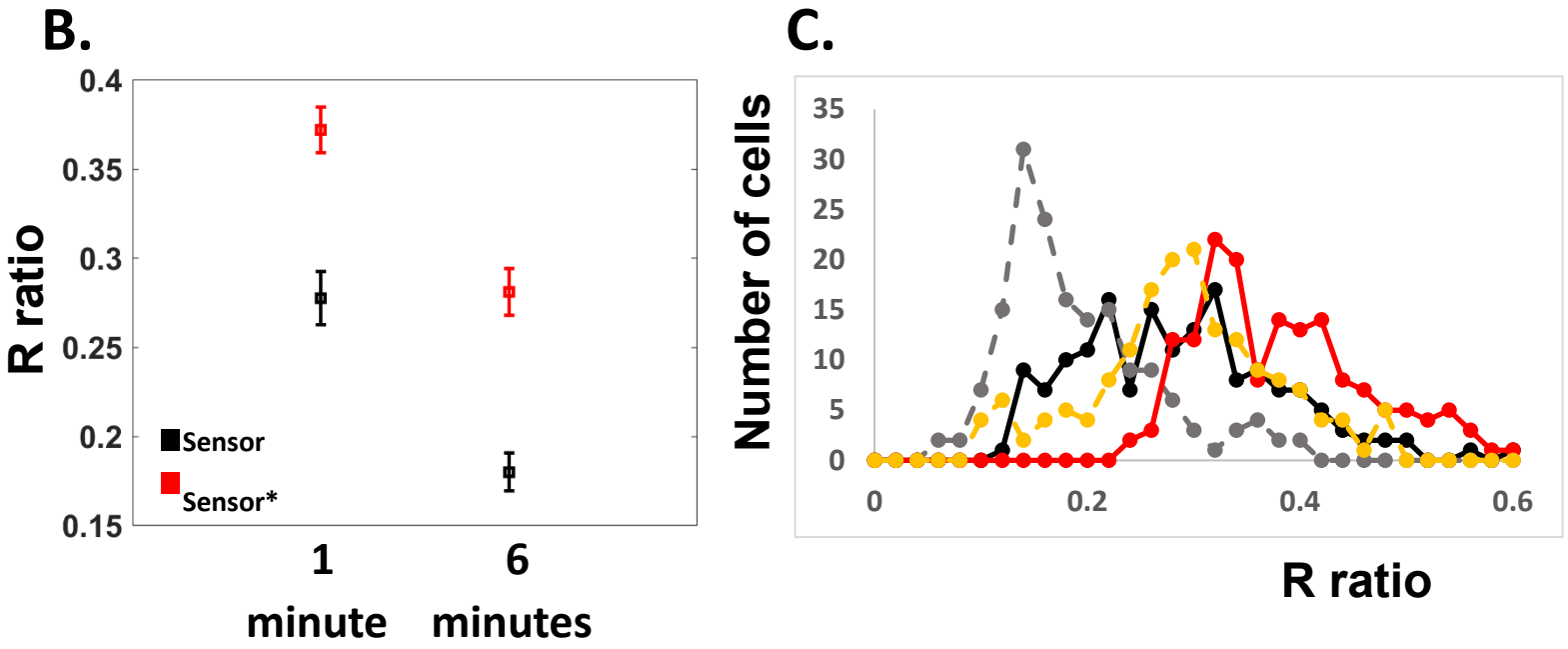


Fig. S9A

c-di-GMP increases modestly, and pH decreases when cells attach in motility buffer with glucose

E. coli cells in motility buffer with glucose were added to channel sides. Cells attached for ~45 seconds, and excess motility buffer with glucose was added to the channels. Image acquisition (every 15 s) was started ~1 min after cell addition. The normalized average ratio R is plotted as a function of time for Sensor- (black line), and Sensor*-expressing cells (red line). The grey (Sensor) and orange (Sensor*) lines correspond to experiments when the motility buffer with glucose contained 40 mM potassium benzoate and 40 mM methylamine to collapse the transmembrane proton gradient. 95% confidence intervals are shown on the plot for each time point.



Figs. S9B, C

B. Cells expressing Sensor or Sensor* were imaged just as they attached and 5 min later in motility buffer with glucose. The average R value for 160 cells is shown as black squares for Sensor-expressing cells and red squares for Sensor*-expressing cells along with corresponding 95% confidence intervals. Note that this is a population experiment, with no relation between the just attached (labeled 1 minute on the abscissa), and the 5 min later cells (labeled 6 minutes on the abscissa).

C. A histogram for the cells averaged in Fig. S9B is shown. Sensor-expressing cells at 1 minute -black, Sensor expressing cells at 6 minutes -gray, Sensor*-expressing cells at 1 minute -red, Sensor*-expressing cells at 6 minutes -orange.

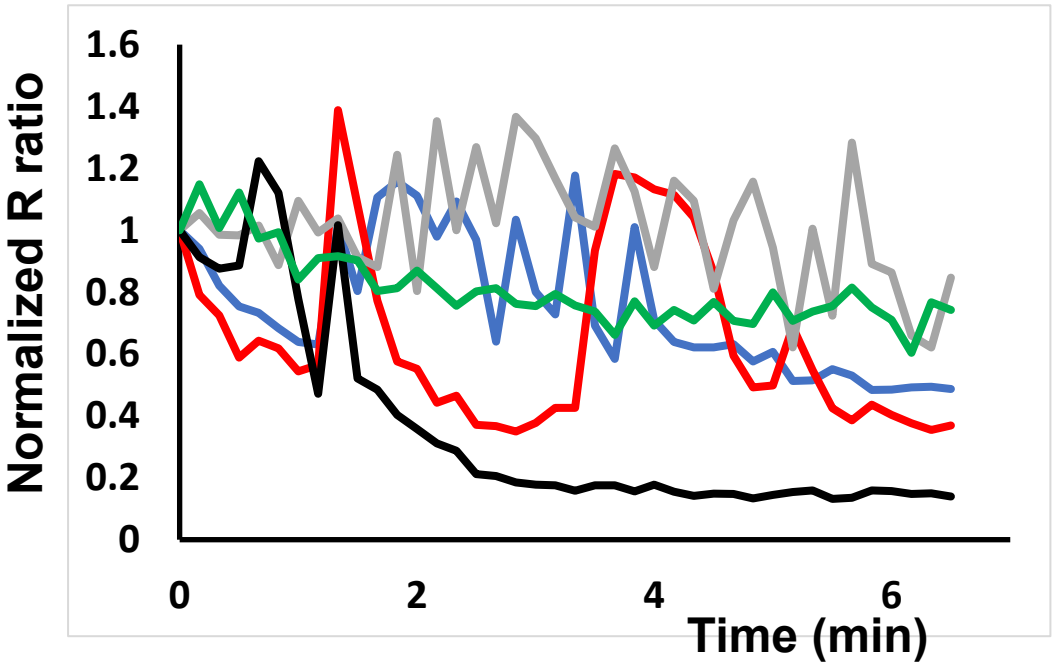


Fig. S9D

Individual R ratio traces from Sensor-expressing cells are shown following attachment in motility buffer with glucose (Fig. S9A data).

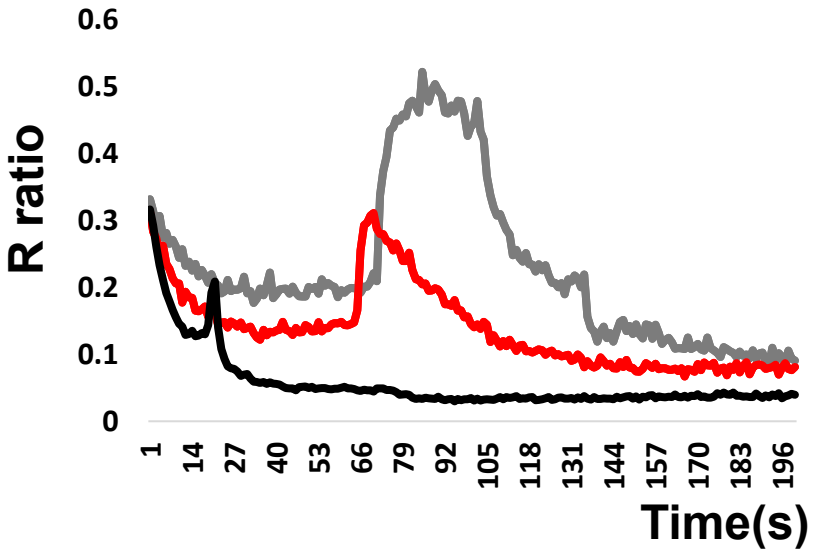


Fig. S10

E. coli does not require functional flagellar motors for the generation of alkaline spikes

Images were acquired every 1 second for Δ*motAmotB* cells that just attached to glass coverslips. The Individual R ratio traces display spikes for both Sensor*- (black and gray traces) and Sensor- (red trace) expressing Δ*motAmotB* cells, consistent with transient changes in pH upon surface attachment.

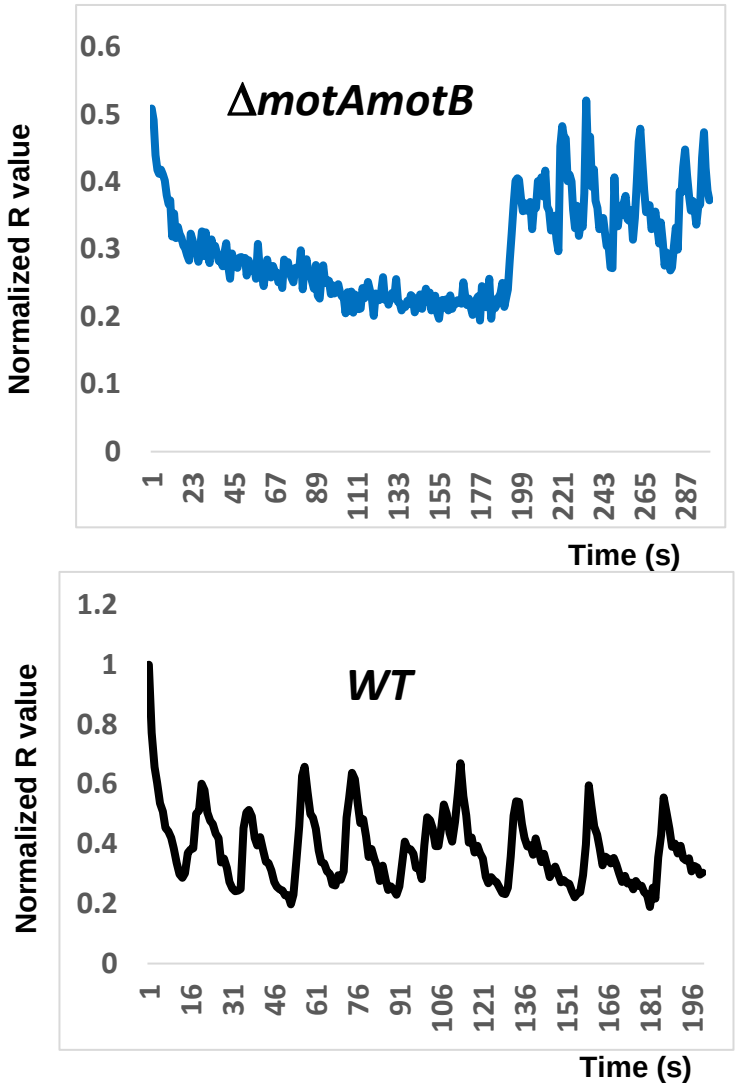


Fig. S11

ΔmotB and ΔmotAmotB cells do not display clear, periodic events in motility buffer with glucose

Out of 116 Δ*motB* and Δ*motAmotB* cells, 36 spike during the first 5 minutes following attachment in motility buffer with glucose. However, only two traces suggest a possible periodic burst (see blue trace). None of the cells display clearly separated spiking events as in the case of wild type cells (see black trace).

Buffer with Lactate				**Buffer with Glucose**			
Attached < 1 minute		**Attached 5 minutes**		**Attached < 1 minute**		**Attached 5 minutes**	
Total	Spiking	Total	Spiking	Total	Spiking	Total	Spiking
57	41	35	4	38	15	35	11
25	12	43	6	40	16	76	11
44	22	37	3	45	32	68	32
42	17	90	10	49	17	45	8
33	10						
201	102	205	23	172	80	224	62
	50.7%		**11%**		**46.5%**		**27.7%**

Supplementary Table

The percentage of cells with detectable alkaline spikes decreases over time

For individual channel slides, the total number of cells, and the number of cells with detectable alkaline spikes were logged. Each line shows the data from one channel slide, with experimental conditions indicated above (lactate buffer or glucose buffer). Imaging fields were assessed either when the cells were attached for less than 1 min, or when the cells were attached for 5-6 min. All the cells express the Sensor* system (Fig.1B) that responds only to pH changes.

Supplementary Note

Changes in proton flux through the flagellar motor could transiently impact intracellular pH

Using the buffering capacity equation $\mathbf{J_H = \beta * dpH/dt}$, where J_H is proton flux, β is buffering capacity of the cell, we can estimate that a change in proton flux of ~50,000 H^+/s (which corresponds to stalling 1 motor) would change the internal pH by 0.1 units in 10 s (using values from the literature for buffering capacity β ~50 nmol/pH/mg protein ~10^{-8} nmol/pH/cell ~$10^{-8} * 10^{-9} * 6 * 10^{23}$ =6,000,000 protons/pH (1 cell ~$0.2 * 10^{-9}$ mg)). Note that the buffering capacity β was determined using lysed cells (1), and that the buffering capacity of a living cell could be quite different.

1. E. Zychlinsky, A. Matin, Cytoplasmic pH homeostasis in an acidophilic bacterium, Thiobacillus acidophilus. *J Bacteriol* **156**, 1352-1355 (1983).