Supplemental Material for:

Assessment of a weak mode of bacterial adhesion by applying an electric field

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Supplemental Movie S1: A movie clip showing the motion of a number of bacteria on or near the capillary surface under 100V applied voltage. Note the slow and dragged motion of one bacterium in the midst of several cells quickly drifting to the right, as well as a number of cells anchored on the surface. Several frames of this clip were illustrated as Figure 2 in the main text, with more experimental details provided in the caption therein.

Supplemental Movie S2: A movie clip showing the motion of polystyrene beads on or near the capillary surface under 15V applied voltage. Note among a number of beads drifting to the right with roughly constant speed, one near the bottom transiently stopped about ⅔ into the play time. The traces of this bead and a freely drifting bead are indicated in comparison on the first image of the movie clip, shown in Figure 6 of the main text. See caption therein for more experimental details.

1. Suppression of fluid flow, electro-osmotic effect, and microbubbles

In our experimental design, illustrated as Figure 1 in the main text, the agarose seal on both ends of the capillary efficiently inhibits the fluid flow within the capillary. The surface of the type of capillaries we used (specified in Materials and Methods) effectively suppressed electro-osmotic flow. Thus, we noticed no rapid flow of dirts or tracer particles near the surface when the electric field was applied during the course of our experiment. Using channels of comparable dimensions with uncoated plastic (polyethylene, for instance) or glass surfaces, in contrast, we routinely noticed streaming motion of dirt or tracer particles when focusing on the surface vicinity. Therefore, our selection of the particular capillary coated with polyethylene and tissue culture treated with plasma avoids additional complications due to electro-osmotic effects.

We noticed that applying voltages over several minutes invariably caused gas bubbles and even cracks of the agarose gel near the electrodes, where chemical reactions occur. The bubbles produced in these regions progressively altered the electric current. Microbubbles also formed over time in the region of observation. When non-motile cells encountered them, their trajectories showed bumps over otherwise straight lines. Those aberrant trajectories were not considered in our analysis. To avoid these adverse effects, most data were acquired within five mins following an applied voltage.

2. Measurement of the current vs voltage behavior (I-V curve)

We measured the electric current in the capillary channel originated with application of the external direct current (DC) voltage. The capillary channel is expected to behave as an Ohmic conductor. The purpose of measuring an I-V curve is at least twofold: 1. By repeating such measurements over multiple samples, the extent of variation informs us how sensitively the actual field strength in the middle of the capillary depends on the exact positions of the electrodes as they are manually inserted into the capillary at both ends; in other words, how significantly the contact resistance and its variation affect the electric current through the sample. 2. By monitoring the current over time, we learn about how electrochemically stable the bacteria-containing liquid is, which sets the time limit for the experiments we perform. For 1, we realized that small changes in the electrodes inserted into the channel indeed lead to significant variation on the electric current. This amount of variation, by as much as 20-30%, sets the limit of accuracy for this experimental design. For 2, we noted a time dependence of the current in the sense that over ~30 seconds under a fixed applied voltage, the resulting current tended to increase over time. This increase was probably due to the ionic effects that occur near the electrodes. The current then stabilized for two or three minutes before it started decreasing over a longer time. The slow drop in current was attributable to the air bubbles that formed close to the electrodes, which we observed repeatedly.

As a consequence of this time dependence, we noted that when measuring current as a function of voltage in incremental steps, the results from previous measurements affected the subsequent measurements. In particular, the measured current increased over time, following an applied voltage. In order to eliminate the effect of drift on the shape of measured I-V curve, we did three series of measurements of current with stepwise increases of voltage (+10 V per step) and then repeated the measurements 3 times with stepwise deceases, i.e. starting from the highest voltage (120 V) down to 0 V in decreasing steps (-10 V per step). The plots for the two sequences of measurements indeed show opposite curvatures, but averaging them yielded a proportional I-V curve, as it should be. Figure S1 shows the plot of the I-V curve obtained by averaging all measurements ramping up and down in measurement steps over 3 cycles. The notably large error bars are due to different values between those acquired from the stepwise up as opposed to stepwise down measurements.
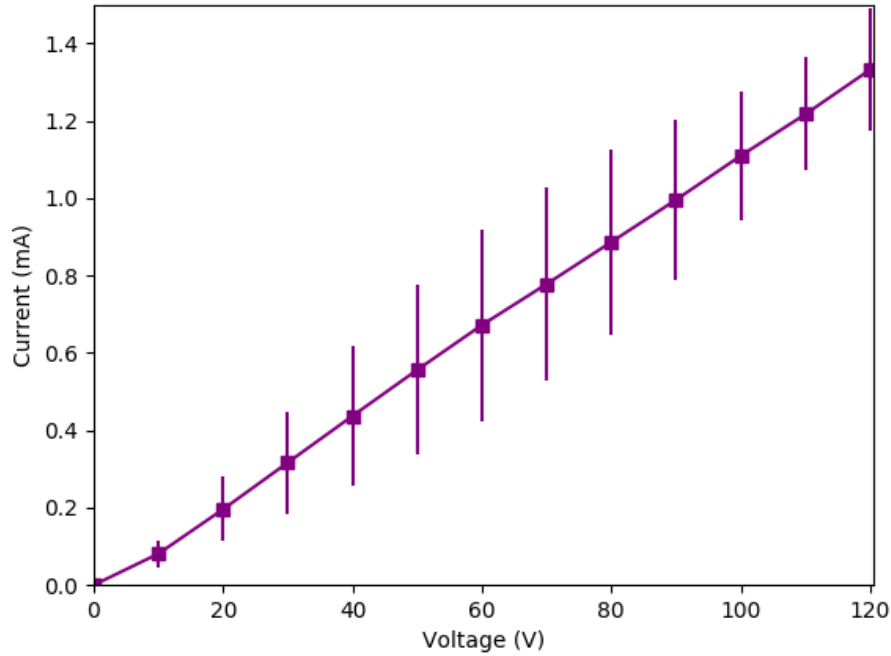
Figure S1: The measured I-V curve. Each data point was the average of six measurements: three with stepwise increases and three with stepwise decreases in voltage. Error bars represent the standard deviation. The solid line is a trend connecting the data points.

3. Estimating the electrophoretic mobility of *C. crescentus* based on measurements of drift velocity

When driven by an electric field (E), the ratio between the velocity of motion (v) of a charged particle and the field is defined as the particle's electrophoretic mobility (μ). In mathematical terms:

$$\mu = v/E \qquad\qquad \text{Eq. 1}$$

We measured the average drift velocity of six non-motile cells driven by the electric field of three applied voltages (80 V, 100V, 120 V), with the results shown as a bar graph (Figure S2). We estimated the electric field as the applied voltage divided by the length of the capillary channel (5 cm), ignoring the voltage drop in the proximity of the electrodes due to the effect commonly known as contact resistance. Thus, we were able to estimate the electrophoretic mobility of *C. crescentus* using Eq. 1. The results obtained are shown in Table S1, which average to -1.4 µm cm $V^{-1}s^{-1}$. This value is comparable to what is reported as -1.199 µm cm $V^{-1}s^{-1}$ for *E. coli* grown in L-medium [1].
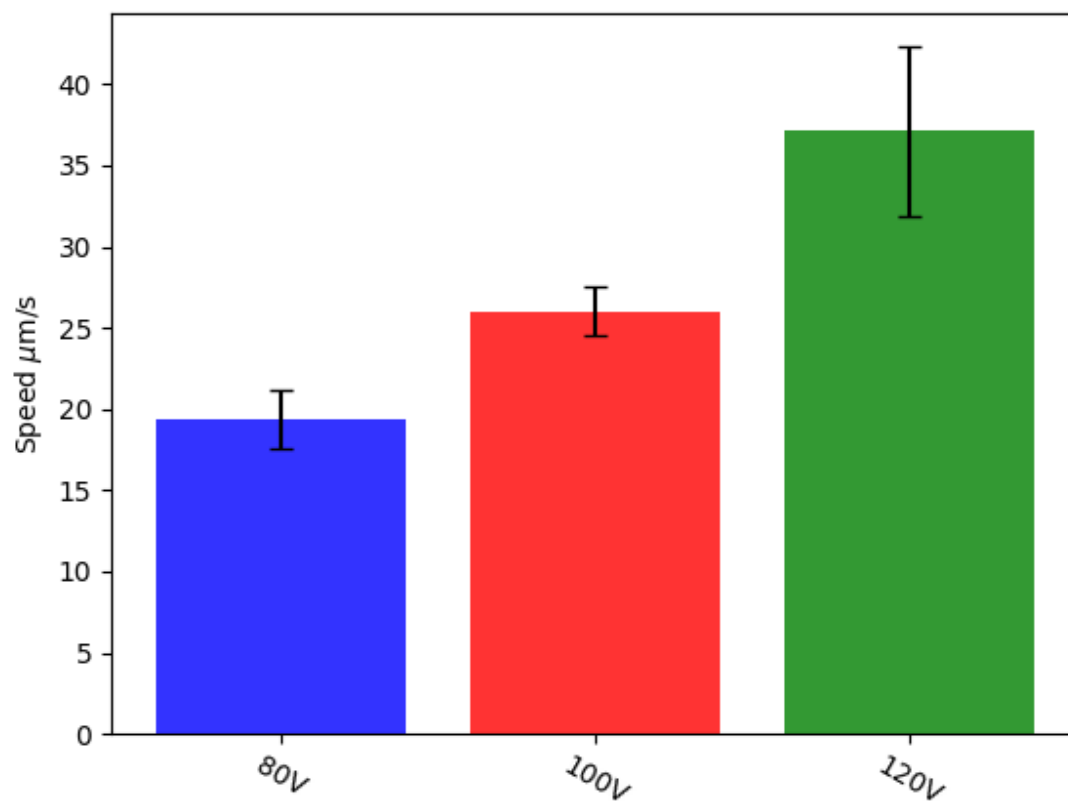
Figure S2: Average drift velocity for non-swimming cells driven by the applied electric fields. The error bars represent standard deviations. The averages were taken for 6 cells at each voltage.

Voltage (V)	Electrophoretic mobility (µm cm V^{-1}s^{-1})
80	-1.2
100	-1.4
120	-1.5

Table S1: Electrophoretic mobility measured at three applied voltages.

4. Estimating the electric force on bacteria

We estimate the electric force (F) exerted on the bacterial cells by applying the Stokes law for spherical particles moving in a viscous medium:

$$F = 6\pi\eta r v \qquad\qquad \text{Eq. 2}$$

Here, η is the fluid viscosity, r is the radius of a spherical particle and v is the velocity of the driven motion. Taking 1.0 µm as a typical radius for a bacterium (and approximating it as being spherical), the dynamic viscosity of water ($\eta = 0.89$ mPa.s) at room temperature and an average drift speed of 26 µm/s for non-swimming cells measured at 100 V (see Figure S2), we obtain a driving force of about 0.4 pN (picoNewtons) at this voltage. This force is rather small. It can be provided by a few polymers transiently and perhaps non-specifically tethered on the solid surface. This crude estimate also explains why we noted only a small number of cells occasionally dislodged by the field applied in our experiments. With stronger binding, the electric field applied might be insufficient to detach most attached cells.

5. Comparison between the electric force and shear force on bacteria

Intuitively, sub-pico newton force can also be applied by shear in a typical microfluidic device. In a recent such study on bacterial adhesion [2], we noted that their device generated a force much weaker, by about 2-3 orders of magnitude. Specifically, the particular flow device they used produced a shear stress on the order of 1 mN/m^2, which would exert a force of only 10^{-15}N on an estimated cross-sectional area of roughly (1µm)^2 for a bacterium. Such a comparison explains why many species of bacteria are able to attach and remain attached to surfaces in those flow experiments. It also suggests that we might be identifying those rare cases of the few bacteria that are attached stronger than the majority of them that sit on the surface non-adherently. Those non-adherent cells can be readily dislodged by moderate flow or much lower applied voltages.

Supporting References

[1] M. E. Bayer and J. L. Sloyer Jr. The electrophoretic mobility of Gram-negative and Gram-positive bacteria: an electrokinetic analysis. Journal of General Microbiology (1990), **136**, 867-874.

[2] S. Sharma and J. C. Conrad. Attachment from flow of *Escherichia coli* bacteria onto silanized glass substrates. Langmuir (2014). **30**, 11147−11155.