# The maximum number of torque-generating units in the flagellar motor of *Escherichia coli* is at least 11

Reid *et al*. 10.1073/pnas.0509932103.

## Supporting Information

**Files in this Data Supplement:**

Supporting Text
Supporting Figure 7
Supporting Figure 8
Supporting Figure 9

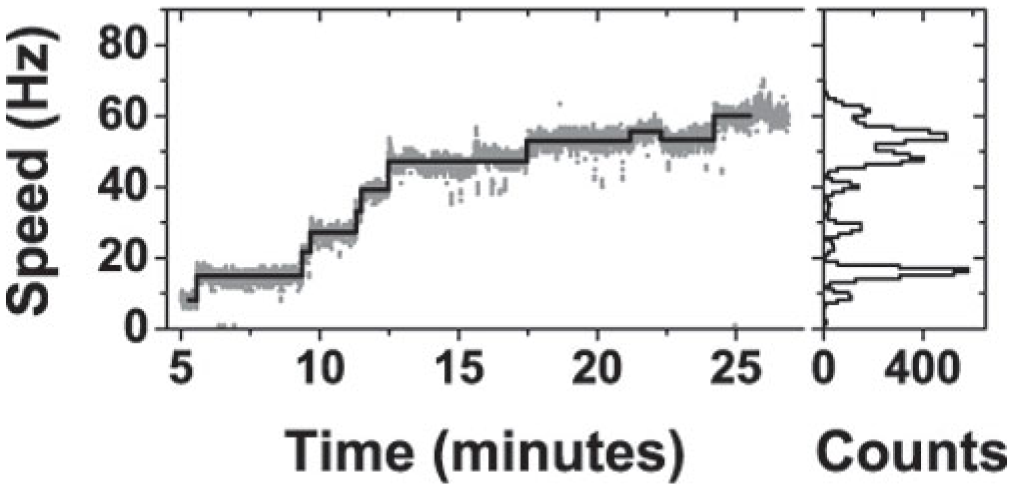


**Fig. 7.** A typical resurrection trace for the MotA strain in motility buffer containing lactate and methionine. Levels are superimposed and speed histograms are shown on the right. This cell resurrects from levels 1-9 (possibly 10).

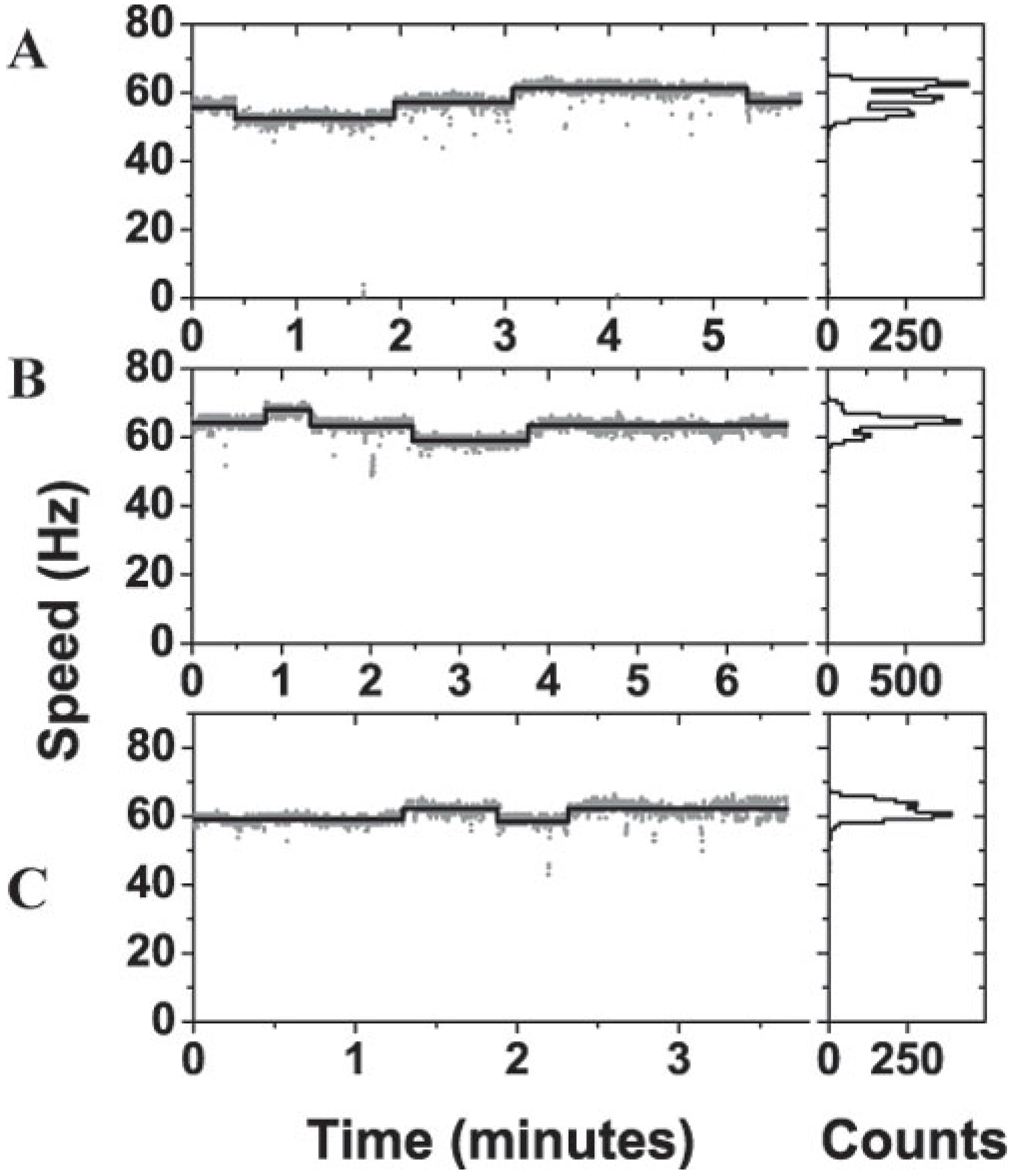


**Fig. 8.** Examples of extended speed measurements of fully induced cells for the MotA strain (*A*), the MotAB strain (*B*), and the WT strain (*C*), with levels superimposed and speed histograms shown on the right. The average step size in a set of 17 traces lasting a total of 294 min and containing 159 steps was 4.5 ± 0.4 Hz.

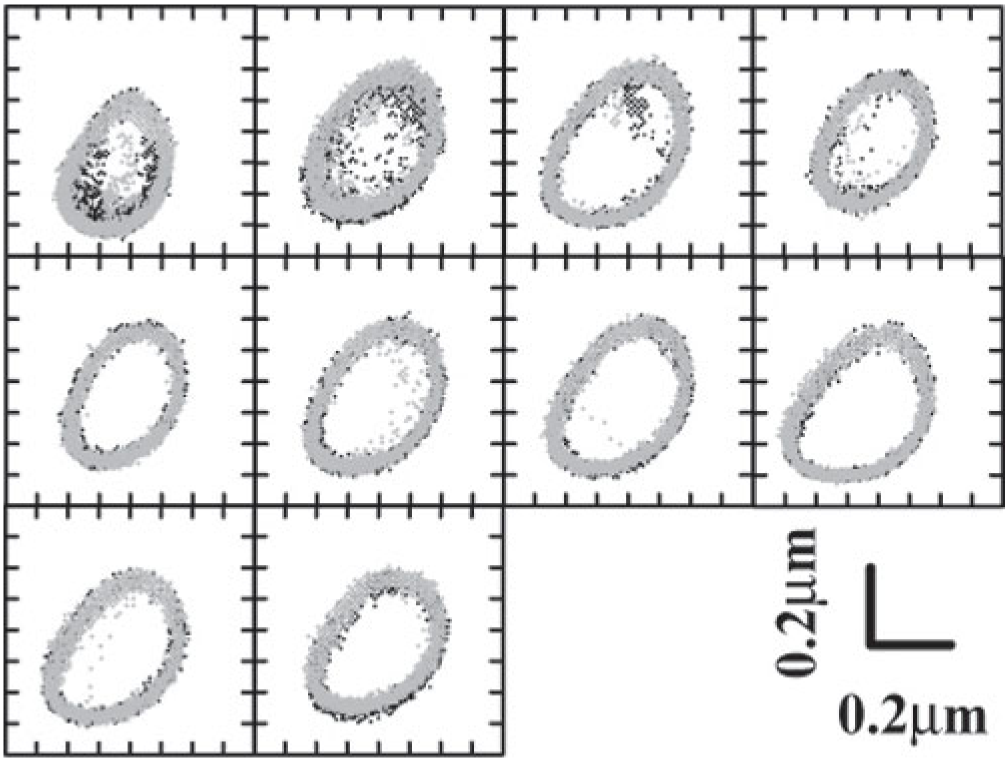


**Fig. 9.** Bead trajectory (*X* against *Y*) for the MotA resurrection trace of Fig. 3*A* is shown for 2 s on either side of each detected step. Beginning from top left and reading across steps are from levels 1 to 2, 2 to 3, 3 to 4, and so on. In most cases the light data points (after the step) obscure the dark data points (before the step), illustrating identical trajectories.

## Supporting Text

### Contributions to the Uncertainty in Estimates of Speed Levels from Steady-State Induction Experiments

The width of the peaks in Fig. 2 is caused by both variations from cell-to-cell and variations in the speed of each cell. Speed measurements lasted for 20.4 s, producing 194 separate data points. The SD of these 194 speed values was typically similar to the variation from cell-to-cell of the average speed. For example, the level-2 peak for the MotA strain is fitted as 15.1 ± 1.9 Hz (Fig. 2 and Table 2). Of 14 cells that showed a steady level 2 (i.e., no level changes or pauses) the average SD within each measurement was 1.3 Hz, attributed to speed fluctuations of each motor. The mean values for each measurement varied with a SD of 1.1 Hz attributed to variations in protonmotive force and viscous drag coefficient. Adding these two contributions in quadrature gives the observed peak width of 1.9 Hz. Thus we conclude that approximately half of the variation is within a single cell measurement, half between different cells.

## Full-Speed Estimates

The full-speed estimates used to calculate the number of torque-generating units were calculated by Gaussian fits to the largest high induction peaks of Fig. 2 *A* and *B*. These values were 61.7 ± 7.8 Hz (MotA) and 64.0 ± 6.9 Hz (MotAB) from Table 2, giving an average of 63 ± 7 Hz. This finding is consistent with the WT full-speed peak 62 ± 6 Hz and is also consistent with the maximum speeds reached during resurrection: the average of all 32 maximum speed levels found by the step-finding algorithm in the full data set was 63 ± 6 Hz; in the 23 trace "level 3" normalized subset it was 62 ± 7 Hz.

## Controls to Test Possible Alternative Explanations for the Reduction in Step Sizes at High Levels

We carried out the following experiments to demonstrate that the reduction of speed increments at high resurrection levels was a feature of the motor and not caused by de-energization of cells because of protein expression in a low-nutrient buffer or caused by speed-dependent changes in the bead orbit and thus in the viscous drag coefficient.

**Resurrection with Lactate and Methionine Present.** We performed resurrections of the MotA strain by using our current protocols but with an additional 70 mM sodium chloride, 1 mM sodium lactate, and 1 mM L-methionine added to the motility buffer, as used by Chen and Berg (1) to measure the torque-speed curve and differing only in L-methionine concentration from the buffer used in the previous resurrection work (2). There was no significant difference between resurrections in this medium and those in our normal motility buffer. In particular, resurrections up to at least level 10 were observed, and resurrection steps at higher levels were smaller than those at lower levels.

This data set was too small (six traces) to perform the detailed analyses of Figs. 5 and 6. Of the six traces, three reached level 10, five of the six traces contained a clear level 2, and all six contained a clear level 3. The average single torque-generating unit speed based on level 2 was 7.0 ± 0.5 Hz, the average based on level 3 was 6.6 ± 0.5 Hz (consistent with the level-3 average from the combined MotA and MotAB data set). The average step size calculated by fitting to the main peak of a step-size histogram of all of the found steps in this data set was 5.9 ± 0.7 Hz. These numbers illustrate the decreasing trend in step size, in particular that later steps are smaller than early steps, consistent with observations from the combined MotA and MotAB data set.

A typical trace with levels superimposed is shown in Fig. 7. Steps are evident from levels 1-9, possibly 10. The speed at level 1 is 8.2 Hz and at level 9 is 60.1 Hz, corresponding to 7.3 units under the assumption of equal torque per unit compared to the nine levels clearly observable.

**Step Size in Fully Induced and WT Cells.** To control for the possibility of de-energization, the speeds of WT cells (five traces) and fully induced cells grown with high inducer levels (five of MotA strain, seven of MotAB strain) were measured for extended periods totaling 294 min. Short sections from typical measurements are shown in Fig. 8. Speed steps were seen in all traces, with an average step size of 4.5 ± 1.4 Hz, which compares to an average step size of 5.2 ± 1.4 Hz for steps at speed levels above 5 in resurrection data. The average step size in these traces was 13 times smaller than the average speed in the same traces, confirming the conclusion that motors can contain more than eight torque-generating units.

**Bead Trajectory During Resurrection.** One possibility for the reduced step size at higher speed is a change in viscous load on the motor, possibly mediated by distortion of the hook or filament. This would result in an abrupt change in the bead trajectory during the resurrection step. No such abrupt changes were observed in any resurrection (Fig. 9). Gradual changes in bead trajectory were caused by drift in the apparatus, primarily in the microscope focus. Where this drift was observed it was corrected periodically by manual refocusing. Based on the typical observed radius of ~0.20 μm for a bead orbit, an accumulated change of ~0.024 μm in radius at each step would be necessary to account for the ~20% difference between step sizes at low and high levels (Fig. 6*B*). Over a whole resurrection of 10 steps this accumulates to a change of 0.24 μm compared with an uncertainty of ~0.03 μm in the radius of a typical bead orbit.

1. Chen, X. & Berg, H. C. (2000) *Biophys. J.* **78,** 1036-1041.

2. Blair, D. F. & Berg, H. C. (1988) *Science* **242,** 1678-1681.