Supplementary Material

Instability at alkaline pH_{ex}

Figure S1 *A, B* show the results of experiments in which motor speed and V_m were measured in the same cell and pH_{ex} was increased to 8. In these experiments we measured the speed of 1 μm diameter beads attached to motors by video analysis of movies taken at 2000 frames per second (10), and there were 10 s delays between successive measurements of speed and V_m. When we changed pH_{ex} from 7 to 8, some motors speeded up stably and V_m measured in the same cell increased to around -165 mV, as predicted by equations 10 and 11 (Fig. S1 *A*, $[Na^+]_{ex}$ = 85 mM, smf = -210 mV). However, as illustrated in Fig. S1 *B, C*, many motors were unstable at pH_{ex} = 8. The motor of Fig. S *B* had stopped rotating by the time of the first speed measurement, 5 minutes after the switch to pH_{ex} = 8, indicating collapse of the smf. The slow decay of V_m measurements reflects the slow response of the dye to a collapse of V_m which was probably complete within 5 minutes of the pH change. To investigate in more detail the behavior of cells at pH_{ex} = 8 we measured the speed of 0.5 μm diameter beads attached to the motor, using the bfp method (Fig. S1 C). V_m was not measured in these cells, due to technical limitations. The motor of Fig. S1 *C* speeded up as we changed pH_{ex} from 7 to 8, as in Fig. S1 *A*. Within 2 minutes, however, the speed had dropped to zero as in Fig. S1 *B*. After restoring pH_{ex} = 7 the motor recovered in a stepwise manner, similar to the resurrections observed by Sowa et al after transient de-energizations caused by removal of $[Na^+]_{ex}$ (10). Out of 51 cells observed after shifting from pH_{ex} = 7 to pH_{ex} = 8, 11 maintained rotation for at least 20 min (similar to Fig. S1 *A*), 40 stopped rotating within ~5 min (similar to Fig. S1 *B*). Thus it appears that many cells are unable to maintain rotation of chimeric flagellar motor at the high smf associated with pH_{ex} = 8, leading to a reversible collapse of both smf and V_m.

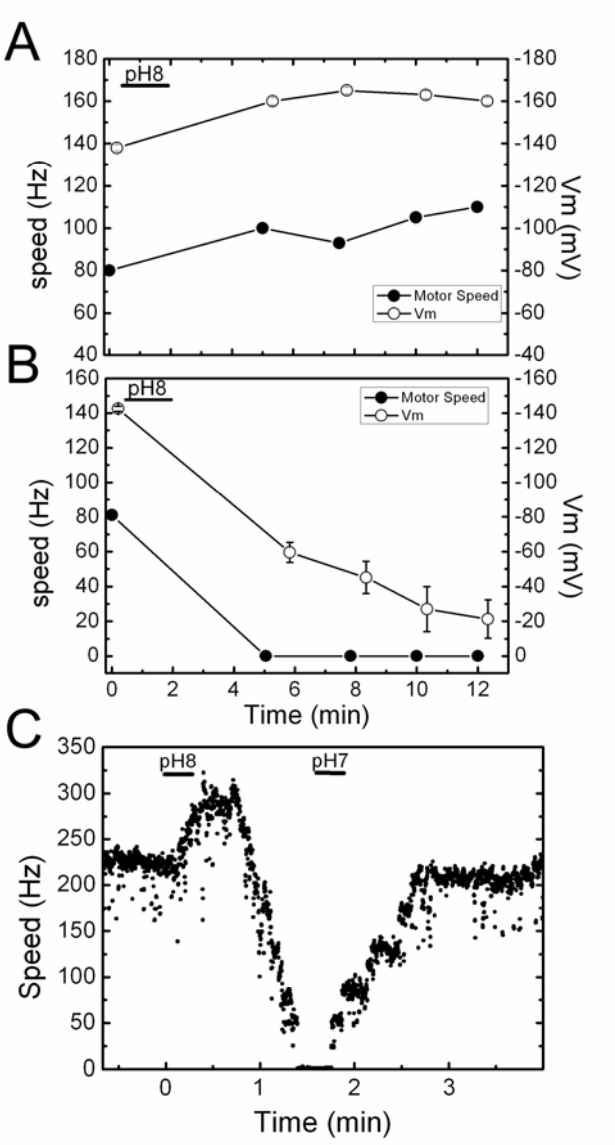

Figure S1

Transient responses to high pH_{ex}. *(A, B)*. V_m and speed of 1 μm diameter beads attached to the motor, versus time, measured in the same cell. The bar indicates the flow of buffer with pH_{ex} = 8; initially pH_{ex} = 7. After the change to pH_{ex} = 8, the motor and cell in *(A)* maintained increased speed and V_m, wheras the motor in *(B)* stopped, indicating zero smf and V_m. The apparent slow decay of V_m in *(B)* is due to slow equilibration of the dye. *(C)*. Speed of a 0.5 μm diameter bead attached to the motor. The change to pH_{ex} = 8 caused a transient increase in speed followed by a rapid collapse to zero. Return to pH_{ex} = 7 caused step-wise recovery to the original speed, typical of re-activation of stator units following transient de-energization.