Supplemental Information for:

Stochastic Growth Bursts Enable Burden-Free Flagellum Production in a Single Bacterium

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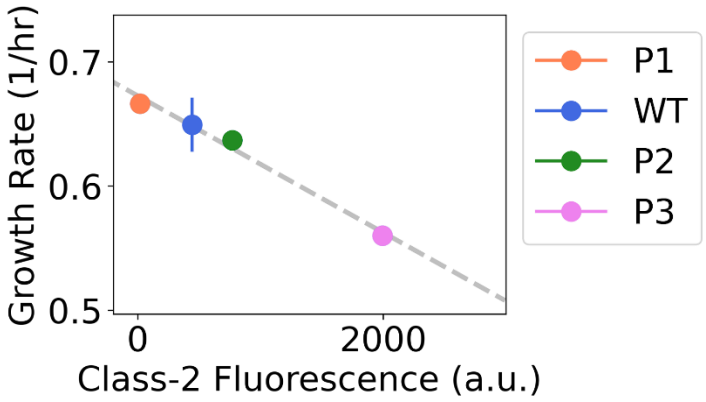

Figure S1. Bulk measurements show a decrease in Growth Rate when flagellar proteins are overexpressed in E. coli. We calculated the growth rate for a set of strains with varying levels of flagella expression (using different synthetic promoters with various strengths controlling Class-1 expression, P1-P3; see Table S2). The strains were grown on 96-well plates, and their OD600 was measured to calculate the slope of their growth curve during exponential growth. Each strain was also grown in bulk, and flow cytometry was used to measure fluorescence, which indicates the activity of the Class-2 promoter, *fliFp*. The figure shows the growth rate of each strain as a function of the corresponding mean fluorescence. The dashed line represents the best linear fit of the data. The data reflects the averages of experiments with two biological replicates, and the error bars represent the standard deviation.

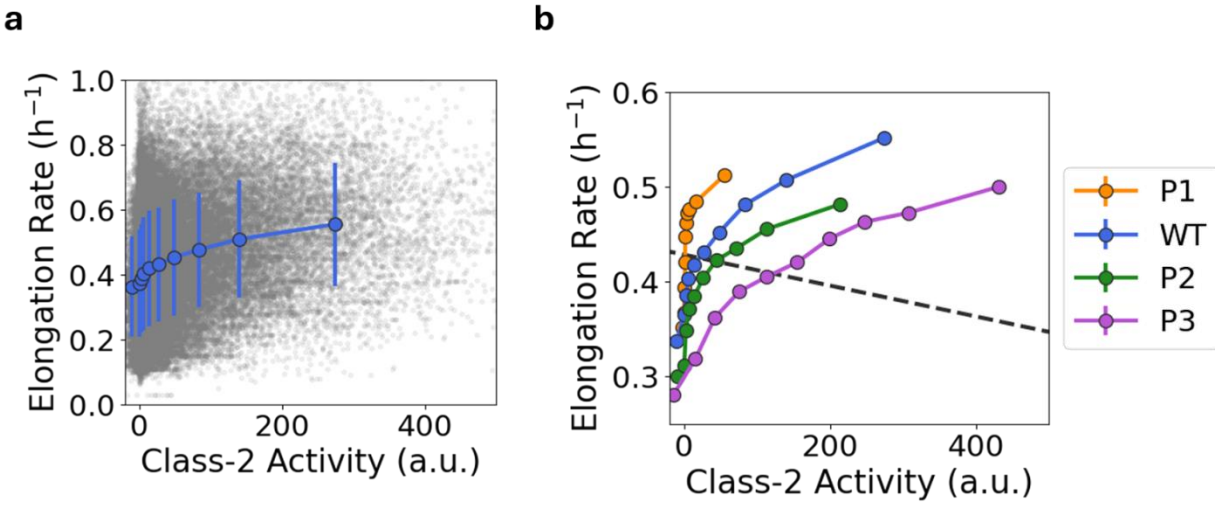

Figure S2. Elongation Rate vs Promoter Activity scatter plot and binning. (**a**) The scatter plot in gray illustrates the relationship between Elongation rate and Class-2 Activity for the strain with the native promoter controlling Class-1 (label WT, strain E98KFD), with each point representing a distinct time point. The entire dataset is divided into 10 bins according to the Class-2 Activity, each containing an equal number of points. The mean and standard deviation for each bin are calculated and represented in blue. The dataset consists of a total of n=57,592 data points, corresponding to n=107 mother cell lineages monitored over approximately 47 hours. (**b**) Mean for the binned Elongation rate as a function of Class-2 Activity, for the different strains that have different flagellar levels, i.e., different promoter controlling Class-1 Activity with a synthetic promoter, P1, P2 and P3, and the native promoter, WT. The number of time traces per strain are n=69, 96, 67 for P1, P2 and P3, respectively, all with a similar duration of 47 hours.

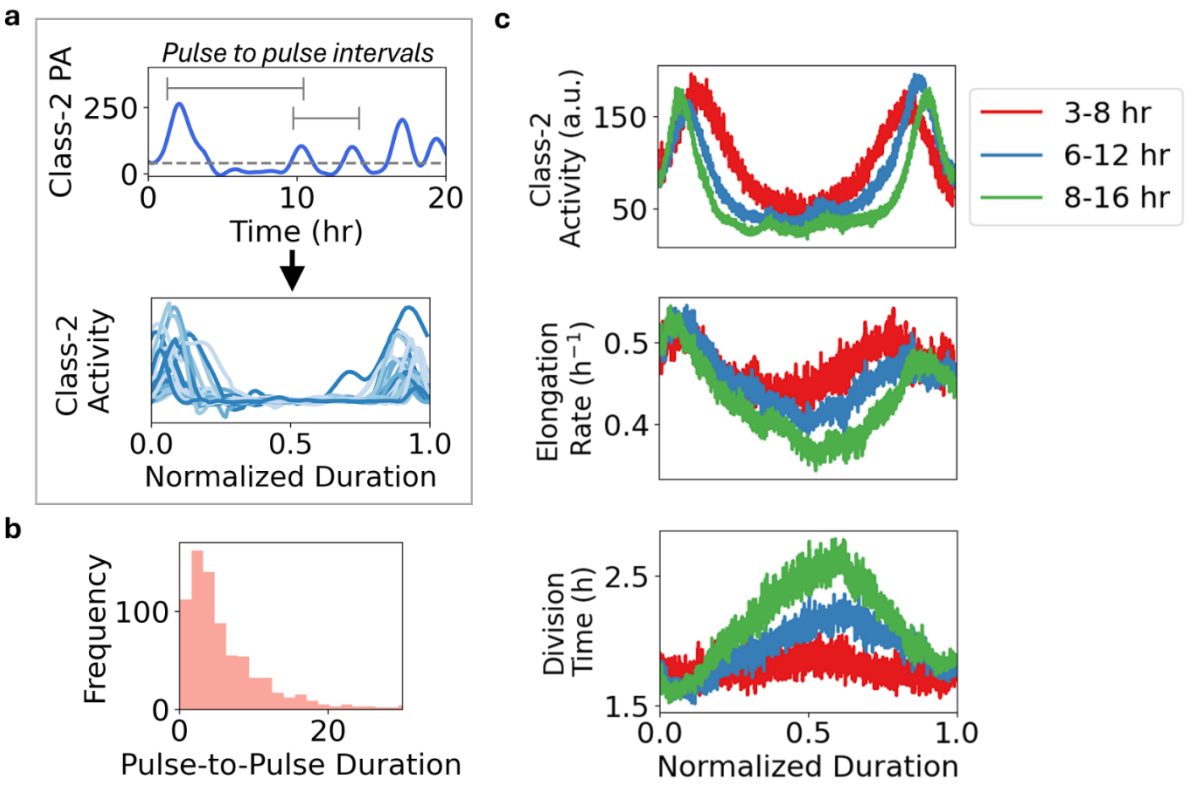

Figure S3. Class-2 pulse-to-pulse and Elongation rate analysis in *E. coli* MG1655. (**a**) The Class-2 Activity time series were divided in intervals that consisted of a pulse of activity followed by off state and another pulse. We select all the intervals with similar duration (within a range of no more than 6 hours). The pulses were defined by the period for which the Activity is above an empirical threshold (t=50). Then, the duration of the intervals of similar duration are normalized for them to have a duration from 0 to 1, in order to average them. (**b**) Histogram of the duration of the pulse-to-pulse durations. (**c**) Average of different variables for pulse-to-pulse intervals of different durations, red: from 3-8 h, blue: 6-12 h and green: 8-16 h. From Top to bottom: Class-2 Activity, Elongation rate and Division Time. (n=(262, 126, 103) different intervals for the three ranges of durations 3-18 h, 6-12 h, 8-16 h, respectively).

Table 1. List of Plasmids

Plasmid	Source	Description
pPro2-Venus	[40]	SC101ori-KanR-Pro2_mVenusNB
pPro4-Venus	[40]	SC101ori-KanR-Pro4_mVenusNB
pProB-Venus	[40]	SC101ori-KanR-ProB_mVenusNB
pProC-Venus	[40]	SC101ori-KanR-ProC_mVenusNB
pProD-Venus	[40]	SC101ori-KanR-ProD_mVenusNB

Table 2. List of Strains

Strain name	Promoter Label	Source	Description
E98KFD	WT	[21]	MG1655 IntS::ZeoR-PRNA1-mCherry E98K galK::AmpR-fliFp-CFP flhDC-YFP
E98KFD Pro2_T7	P1	[21]	MG1655 IntS::ZeoR-PRNA1-mCherry E98K galK::AmpR-fliFp-CFP flhDC-YFP flhDp::Pro4
E98KFD Pro4_T7	P2	[21]	MG1655 IntS::ZeoR-PRNA1-mCherry E98K galK::AmpR-fliFp-CFP flhDC-YFP flhDp::Pro5
E98KFD Pro5_T7	P3	[21]	MG1655 IntS::ZeoR-PRNA1-mCherry E98K galK::AmpR-fliFp-CFP flhDC-YFP flhDp::Pro2
MGPro2-Venus	P1	[40]	MG1655 E98K + pPro2-Venus
MGPro4-Venus	P2	[40]	MG1655 E98K + pPro4-Venus
MGProB-Venus	P4	[40]	MG1655 E98K + pProB-Venus

MGProC-Venus	P5	[40]	MG1655 E98K + pProC-Venus
MGProD-Venus	P6	[40]	MG1655 E98K + pProD-Venus
MC*	-	This work	MC4100 E98K ΔIS5_flhD flhD corrected galK::AmpR-fliFp-CFP flhDC-YFP