Supplementary Information

Blue light is a universal signal for *Escherichia coli* chemoreceptors

Tatyana Perlova, Martin Gruebele and Yann R. Chemla

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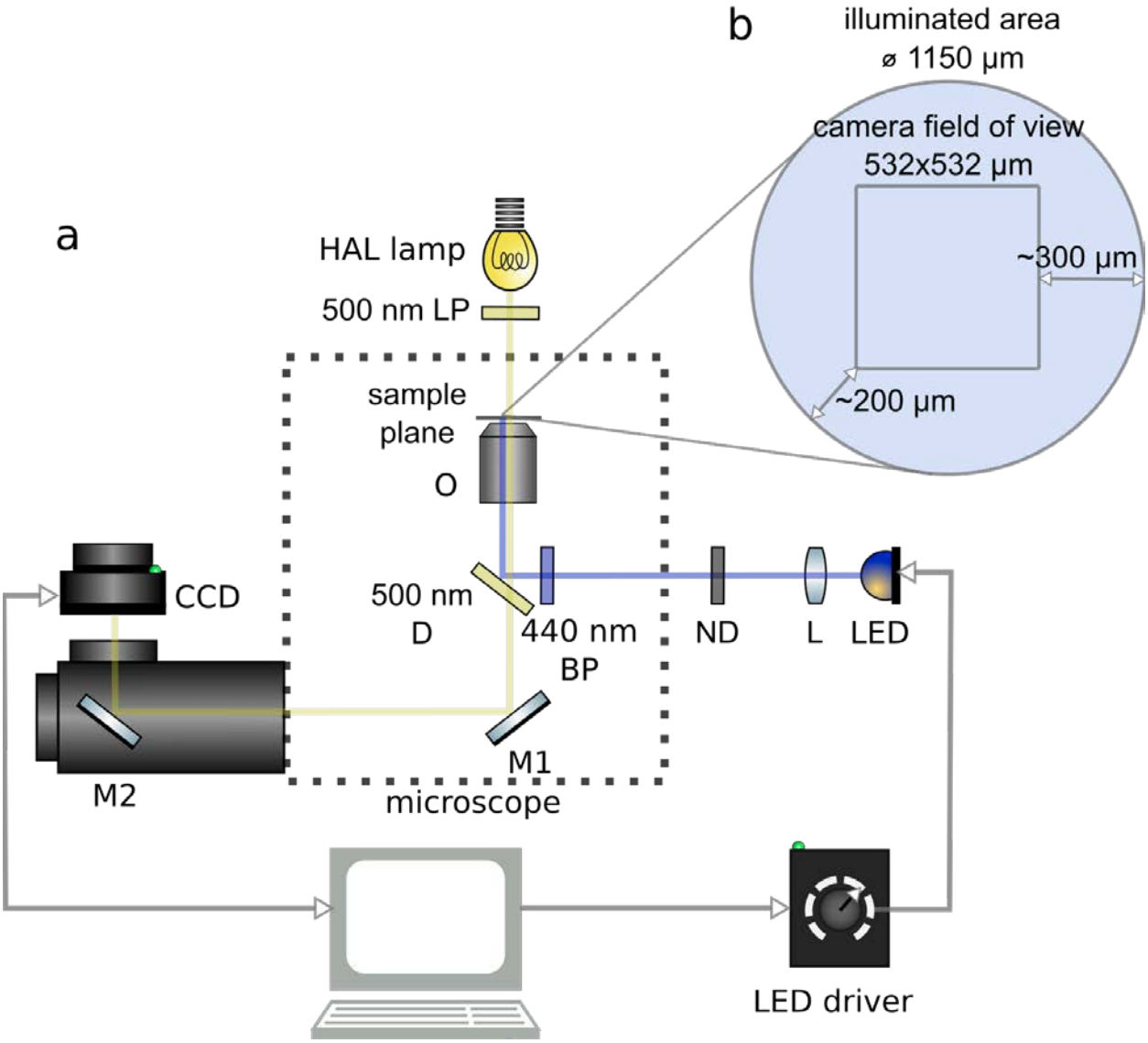
Effect of growth substrate on the blue light response in *E. coli*

Taylor *et al.* have shown that phototaxis requires a functioning electron transport chain and therefore can only be observed in anaerobically or aerobically respiring bacteria (1). Because of this potential coupling between the phototactic response and metabolism, we grew bacteria with a defined mode of metabolism, which can only be controlled by providing a specific substrate. We used succinate as a substrate in the motility buffer as it is non-fermentable and can support only aerobic respiration in the absence of alternative electron acceptor (2). For the same reason, we also used succinate as a substrate in the growth medium, obviating the need to switch carbon sources from growth to motility experiments. We chose not to use glycerol as a substrate because, unlike succinate, it is fermentable. In the presence of a fermentable substrate, cells could potentially switch metabolic state from respiration to fermentation, a concern as oxygen is likely depleted during motility measurements in the sealed sample chambers (see Materials and Methods).

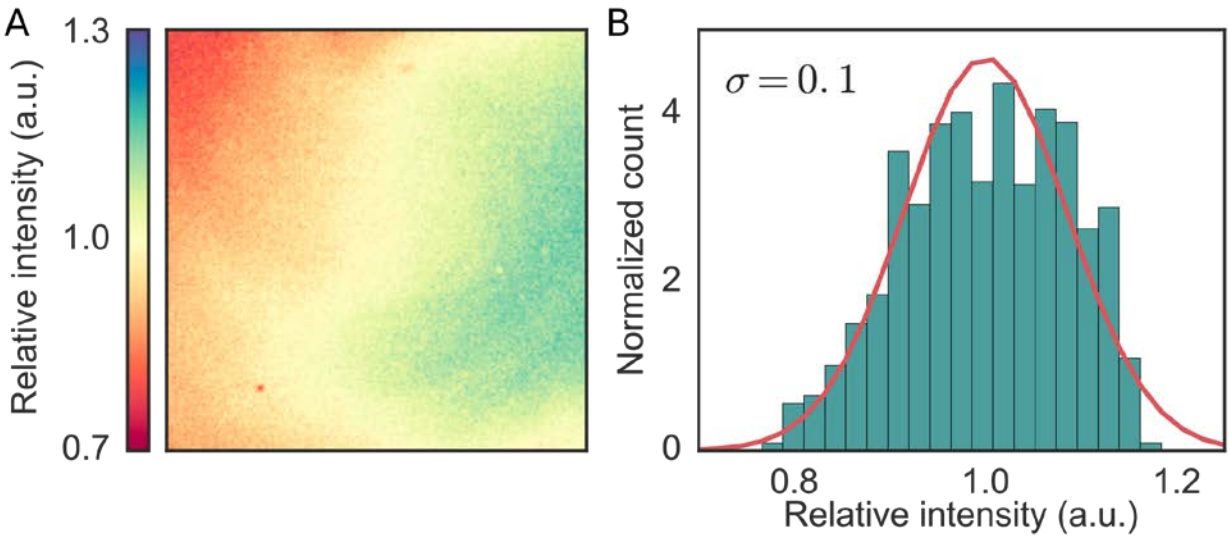
In previous work on phototaxis, Wright *et al.* used fermentable glycerol in the growth medium and non-fermentable lactate in the motility medium (3). We find that the growth substrate has an effect on the phototactic response amplitude (compare Fig. 1 with Supplementary Figure 5), but not motility medium (data not shown). There are several potential explanations for the effect of growth substrate on the light response:

1. It has been previously shown that the relative receptor abundances depend on the density of bacteria (4). The density of bacteria when they are harvested during exponential growth phase depends on the type of growth substrate. It is plausible that bacteria grown in different growth substrates have varying abundances of different receptor types and as a result exhibit light responses of different amplitude.
2. Carbon sources are sensed by Aer and Tsr receptors through changes in the electron transport chain (5). Bacterial metabolism in general and the electron transport chain in particular depend on whether bacteria are grown on a glycolytic (glycerol) vs non-glycolytic (succinate) carbon source. It is possible that Aer and Tsr respond differently to succinate versus glycerol and therefore have different steady-state methylation states in different growth substrates.
3. The proton motive force (PMF), which has been previously suggested as a signal sensed by receptors during phototaxis (3), is coupled to central metabolism and therefore may depend on the growth substrate.

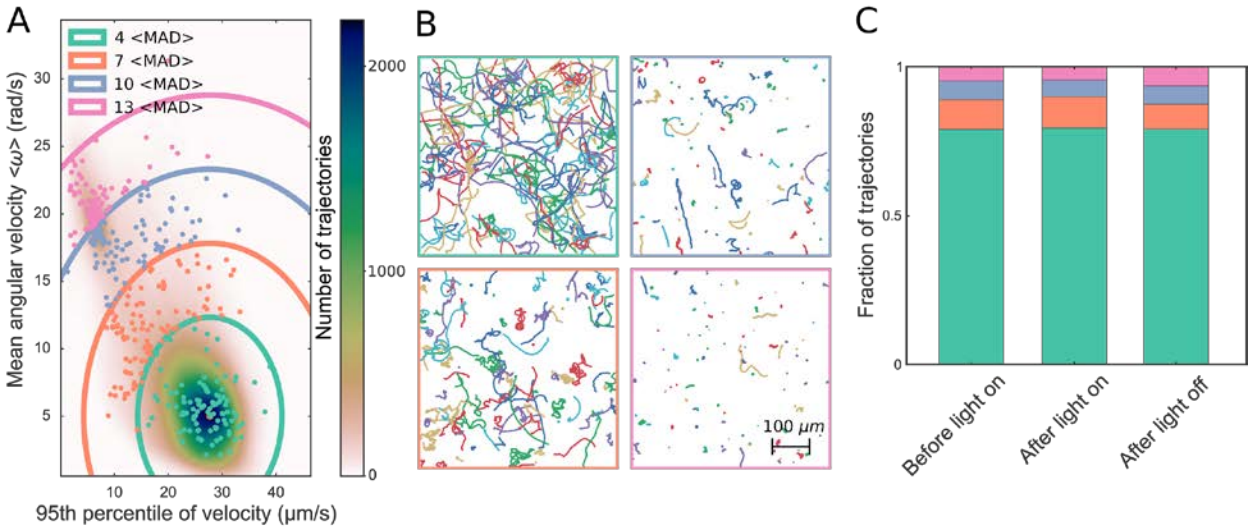
Supplementary figures



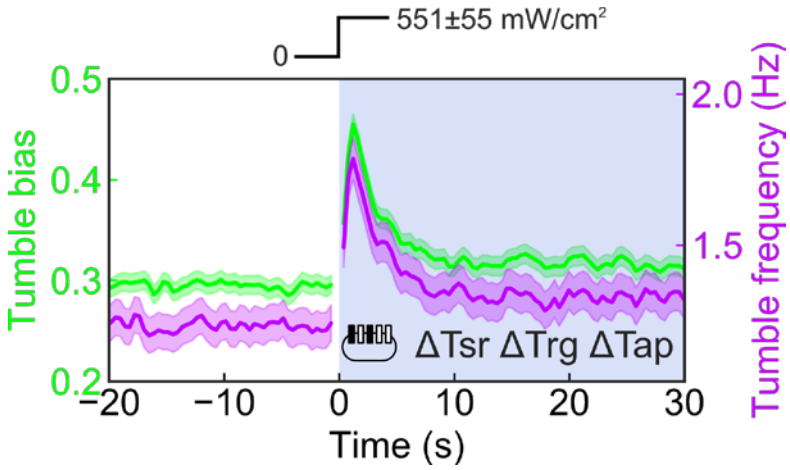
Supplementary Figure 1. Experimental setup. (A) Schematic of the setup with the light paths indicated by yellow (wide-range visible light from HAL lamp) and blue (blue LED) lines. Components are labeled as follows: LP - long-pass filter, O - 20x objective, D - dichroic, BP - bandpass filter, ND - neutral density filter, L - collimating lens, LED - light emitting diode, M1, M2 - fully reflective mirrors, CCD - Charge-Coupled Device Camera. LED power output is controlled by the current from the LED driver, which is controlled by modulating the voltage through a LabView interface. (B) Area captured by the camera compared to the total illumination area, which is equal to the objective's field of view. Minimal and maximal distances that unexposed bacteria need to swim to reach the observation area are shown.



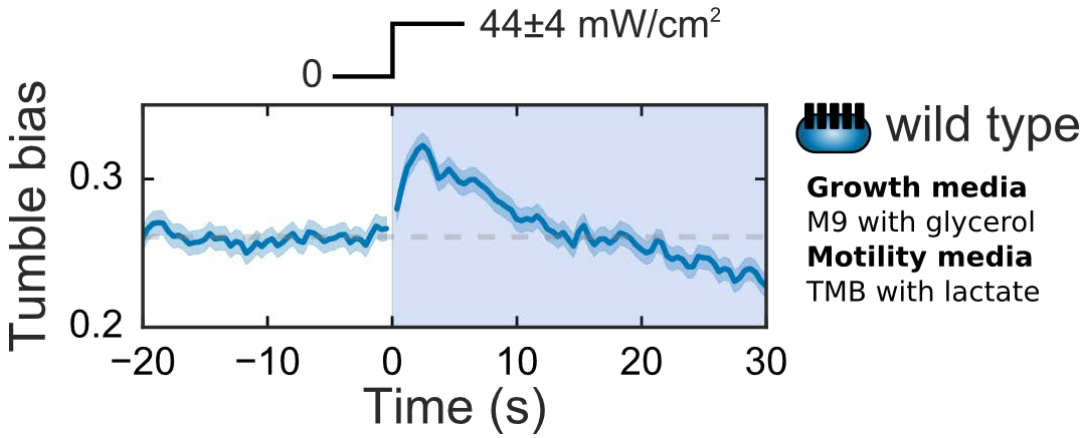
Supplementary Figure 2. Estimating evenness of the Illumination profile from the brightness of the image captured by the camera. (A) Blue light illumination profile captured by the CCD camera. Color indicates normalized brightness of each pixel (brightness divided by the average brightness). (B) Distribution of the normalized pixel brightness. The standard deviation of the distribution is ~ 0.1.



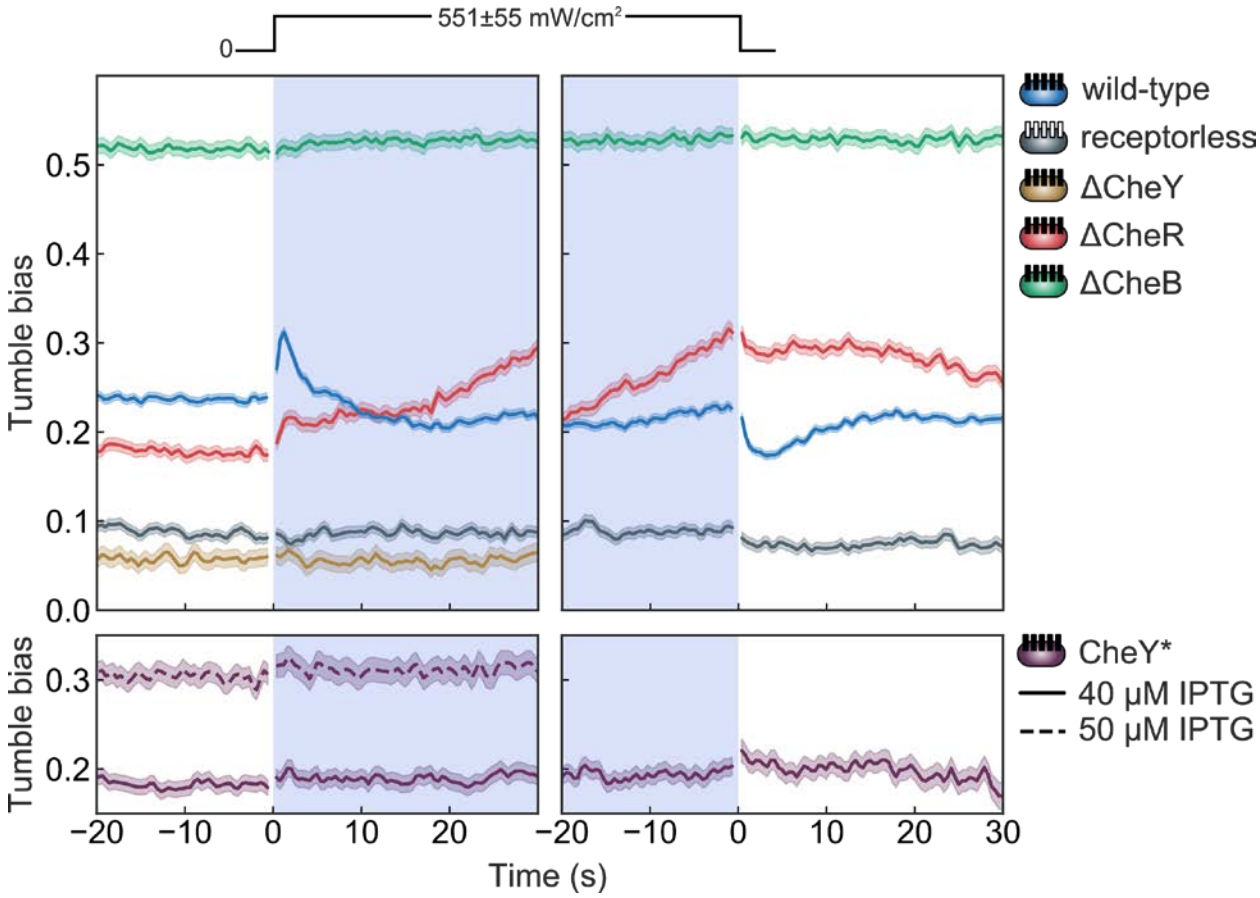
Supplementary Figure 3. Filtering spurious trajectories. (A) Representative two-dimensional distribution of ~200,000 trajectories from wild-type *E. coli* plotted as 95th percentile of velocity *vs* mean angular velocity. Trajectories outside the green contour with radius $R = 4 \langle MAD \rangle$ are removed from further analysis (Materials and Methods). Dots indicate randomly selected trajectories shown in panel (B). (B) Each panel contains 100 randomly selected trajectories from within each contour in panel (A). The color of the panel frame indicates which region the trajectories come from in panel (A). (C) Fraction of trajectories within each contour as indicated by the color, before, during, and after light exposure.



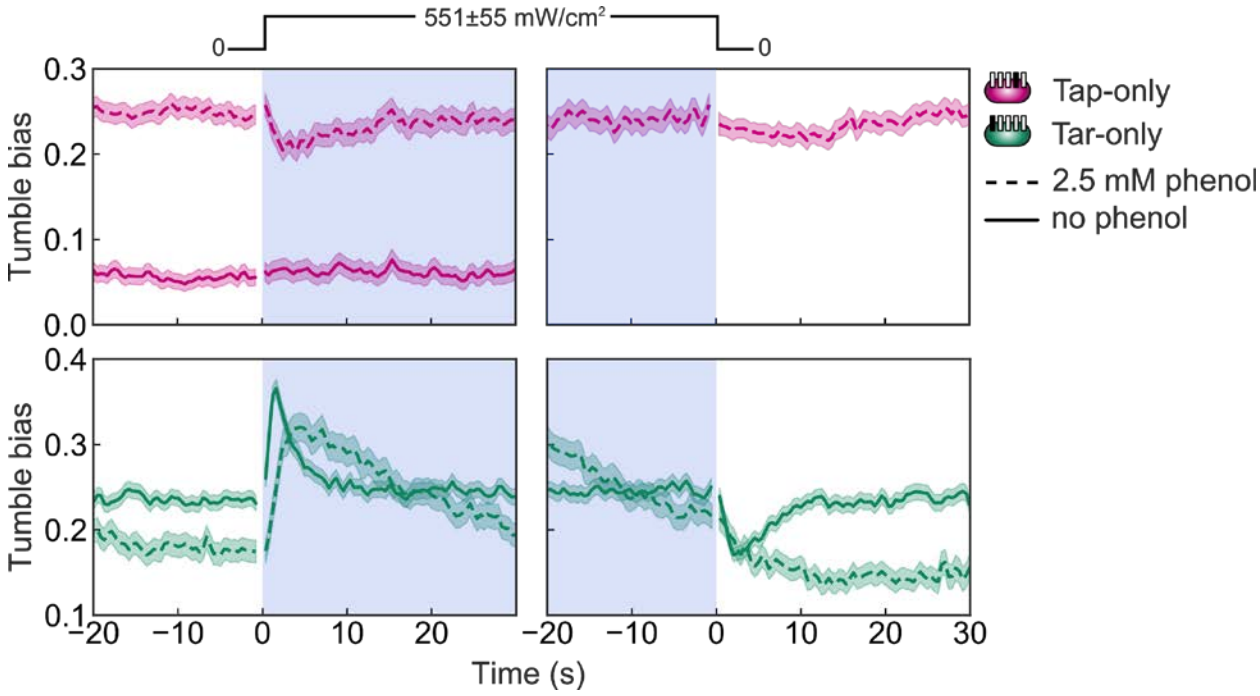
Supplementary Figure 4. Tumble bias *versus* tumble frequency. Blue light response of ΔTsrΔTrgΔTap strain shown as tumble bias (green) and tumble frequency (purple) time traces. Both tumble bias and tumble frequency show similar trends in response to light exposure. The prestimulus value of tumble frequency, ~1.2 s^{-1}, isin agreement with that reported in literature (6). The response was measured at a blue light intensity of 551 ± 55 mW/cm^2 as indicated by the light intensity profile.



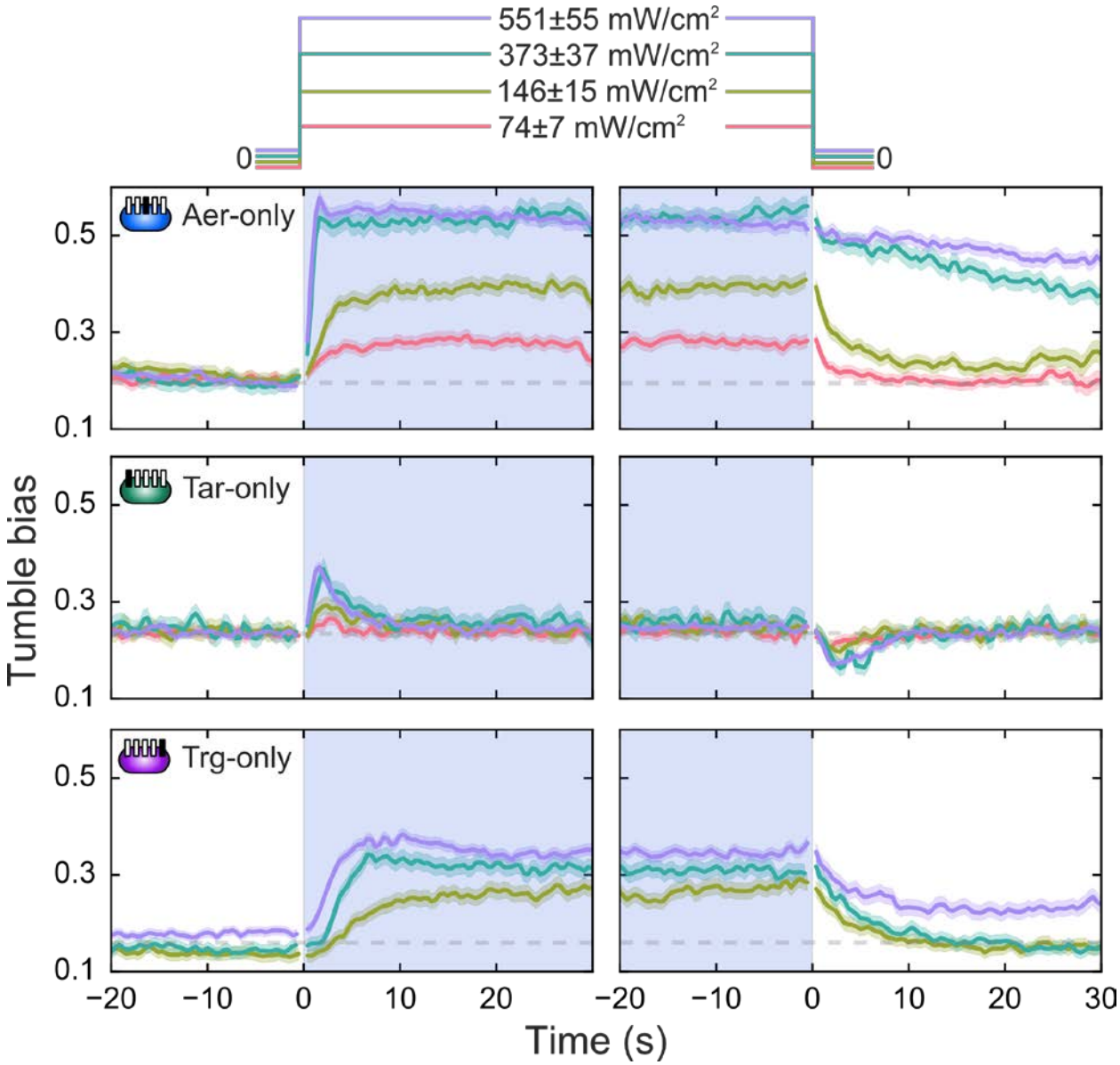
Supplementary Figure 5. Blue light response under different growth conditions. Tumble bias trace for the wild-type strain RP437 grown in M9 minimal medium with glycerol and resuspended in motility buffer with lactate (Materials and Methods). ~3000 trajectories were used to calculated average tumble bias at each time point. Response to a turn-on of blue light of intensity 44 ± 4 mW/cm^2 reproduces previous results on *E. coli* grown with the same carbon source (3).



Supplementary Figure 6. Response to light in *E. coli* is mediated by the chemotaxis network. Tumble bias traces for *E. coli* mutants lacking different components of the chemotaxis network (Table 1): receptorless strain with all receptor types deleted (grey) (7), ΔCheY strain lacking functional CheY (yellow) (8), ΔCheR strain lacking methylesterase CheR (red), ΔCheB strain lacking methyltransferase CheB (green), strain with constitutively active CheY at two induction levels, 40 and 50 μM ITPG (4). ~500 - 3000 trajectories were used to calculated average tumble bias at each time point. The wild-type response trace is shown for comparison (blue). All responses were measured at a blue light intensity of 551 ± 55 mW/cm^2 as indicated by the light intensity profile.



Supplementary Figure 7. Effect of phenol on the light response in Tap-only and Tar-only strain. Tumble bias traces for Tap-only (magenta) and Tar-only (green) strains without phenol (solid line), with 2.5 mM phenol (dashed line). ~200 - 2000 trajectories were used to calculated average tumble bias at each time point.



Supplementary Figure 8. Blue light responses at different intensity levels for Aer-only, Tar-only, and Trg-only strains. Light intensity is indicated by the trace color. Grey dashed lines show prestimulus tumble bias for turn-on and turn-off responses. Light exposure is indicated by the shaded area as well as by the light intensity profile above the plot. ~200 - 2000 trajectories were used to calculated average tumble bias at each time point.

References

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