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PROFILING BACTERIAL KINASE ACTIVITY USING A GENETIC CIRCUIT

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Phosphorylation is a post-translational modification that regulates the activity of several key proteins in bacteria and eukaryotes. Accordingly, a variety of tools has been developed to measure kinase activity. To couple phosphorylation to an *in vivo* fluorescent readout we used the *Bacillus subtilis* kinase PtkA, transmembrane activator TkmA and the repressor FatR to construct a genetic circuit in *E. coli*. By tuning the repressor and kinase expression level at the same time, we were able to show a 4.2-fold increase in signal upon kinase induction. We furthermore validated that the previously reported FatR Y45E mutation¹ attenuates operator repression. This genetic circuit provides a starting point for computational protein design and a metagenomic library-screening tool.

1. Design: sensing phosphorylation

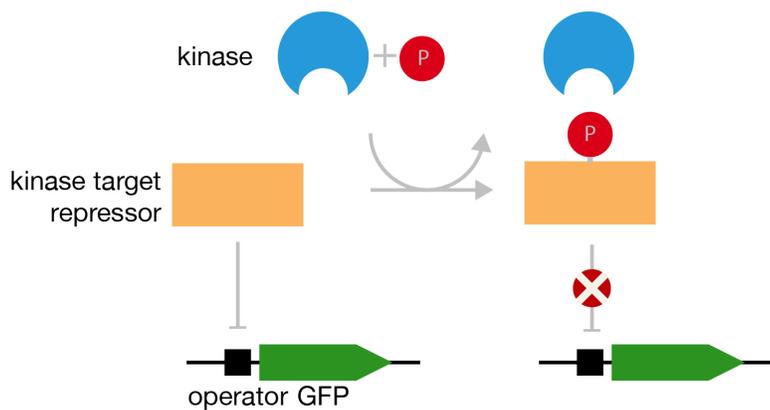


Figure 1a: General concept of kinase activity measurement through GFP fluorescence. The repressor binds the operator site upstream of *gfp*, thereby preventing transcription. The kinase phosphorylates the repressor, this attenuates binding of the repressor to the operator site, leading to transcription of *gfp* and thus fluorescence.



Figure 1b: An abstraction of the genetic circuit where the first inverter gate represents the phosphorylation of FatR by PtkA-TkmA on the post-translational level. The second inverter gate represents the transcriptional regulation by the binding of FatR to the operator upstream of *gfp*.

2. Build: circuit on two plasmids

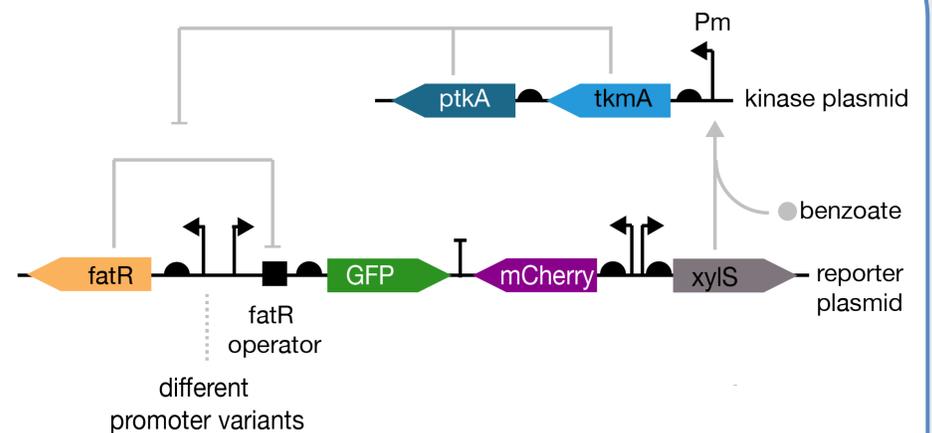


Figure 2: Outline of the implementation with FatR as repressor and PtkA as kinase. The kinase PtkA and kinase-activator TkmA expression is induced by the benzoate/XylS system. The PtkA-TkmA complex can phosphorylate the repressor FatR, which attenuates the DNA binding of FatR to the operator site in front of *gfp*. Derepression of the operator site leads to expression of GFP and thus an increase in GFP/mCherry ratio. Different promoter strengths are used to drive the transcription of the *fatR* repressor. mCherry fluorescence is used to normalize the fluorescent signal of GFP.

3. Test: using Fluorescence Activated Cell Sorting (FACS)

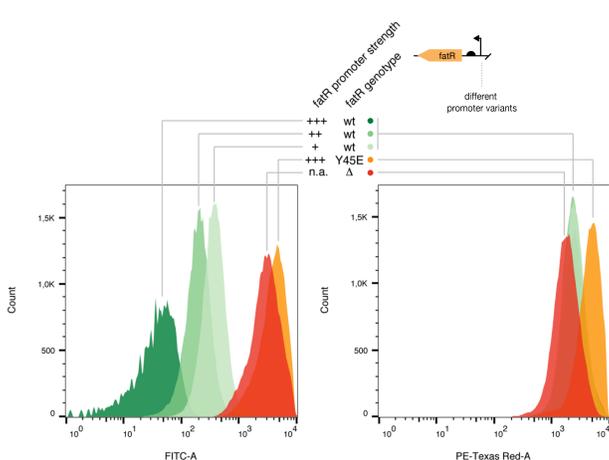


Figure 3a: Raw FACS counts of 5 different strains with various *fatR* (repressor) genotypes under conditions where the kinase operon is not expressed. These strains each display a different *fatR* activity, which is reflected in the signal in the GFP channel (FITC-A). A strong promoter driving *fatR* results in a low GFP signal (green), whereas the strain containing no *fatR* shows a high GFP signal (red). Only one biological replicate of each strain is shown for clarity. This is the data used to calculate the values in Figure 3b.

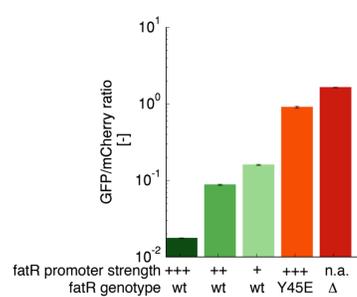


Figure 3b: GFP/mCherry ratio measured of 5 different strains with various *fatR* (repressor) genotypes (based on data from Figure 3a). The two negative control reporters *fatR*:Y45E and $\Delta fatR$ show an expected high ratio of GFP/mCherry. A stronger promoter driving *fatR* transcription results in more repression of GFP and thus a lower GFP/mCherry ratio as expected.

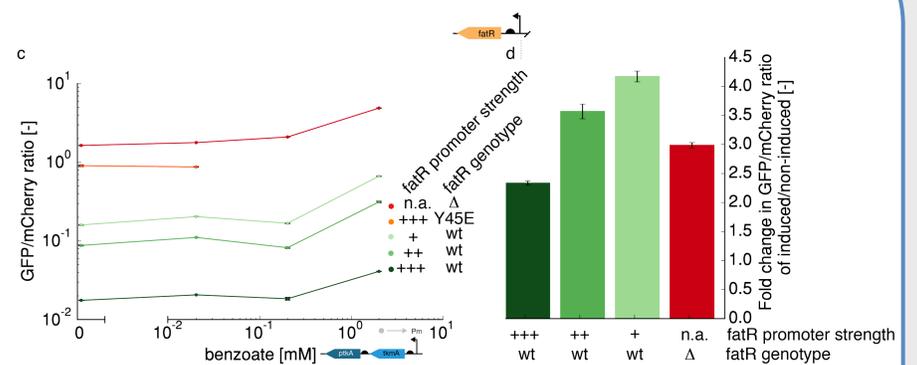


Fig 3c: Circuit response of *E. coli* containing both the platform plasmid and the kinase plasmid. Increasing amounts of benzoate induces the expression of the kinase PtkA and activation domain TkmA. Error bars represent SD of 3 biological replicates. **d:** The fold increase of GFP/mCherry between 0 and 2 mM benzoate. In the negative control strain ($\Delta fatR$, red) the fold increase in GFP/mCherry ratio is 2.99 ± 0.05 upon kinase induction. The strains containing a functional *fatR* gene (green shades), (++) and (+), show a 3.57 ± 0.13 and 4.17 ± 0.09 -fold increase in the GFP/mCherry ratio upon kinase induction, as expected. The strain containing (+++), with the strongest promoter driving *fatR* transcription shows only a 2.33 ± 0.04 -fold increase in GFP/mCherry ratio by inducing the kinase. Error bars denote SD of three biological replicates.

[1] Derouiche A, et al. *Nucleic Acids Res* 2013, 41:9371–81

