

# A light-inducible GadX transcription factor for gene network characterization and control

Caroline M. Blassick<sup>1,2</sup> and Mary J. Dunlop<sup>1,2</sup>

**Short Abstract — The *Escherichia coli* glutamate decarboxylase (*gad*) network has been implicated in cell survival under acid and antibiotic stress. Characterizing the dynamics of this network requires precise control over network components in order to make perturbations on key regulators and observe the effects on neighboring genes. However, it can be difficult to achieve the necessary control of these regulators through conventional means such as chemical induction. Here, we present a synthetic version of the Gad network regulator GadX, which is induced with blue light. This method will enable precise perturbations and control to be applied to the network.**

## I. PURPOSE

THE glutamate decarboxylase (Gad) network is a complex gene network found in *Escherichia coli* which helps cells survive acid stress [1]. Randomly occurring, dynamic activation of *gadX*, a key regulator of the network, has also been shown to help single cells tolerate transient antibiotic stress [2-3]. To better understand the mechanism of this effect and its implications on cell survival, it would be useful to dynamically control *gadX* in a way that mimics its spontaneous activation in wild type cells, and quantify the effects on the rest of the network. However, *gadX* can be difficult to control through conventional means. When the gene is placed under control of a chemically inducible *lac* promoter, even relatively low leaky expression far exceeds the levels normally found in wild-type cells. Furthermore, even if chemical induction could be optimized, it would still be difficult to use this method to recreate dynamic, single-cell pulses of *gadX* expression. Optogenetic, or light-inducible, gene expression systems could be used to this effect, but many suffer similar problems with leakiness. To solve this, we have developed a synthetic version of GadX that can be activated with light.

## II. RESULTS

A chimeric GadX transcription factor was constructed by fusing GadX with the Vivid (VVD) photodimer [4]. When exposed to blue light, the VVD domains undergo a conformational change that allow them to dimerize, bringing the GadX monomers together into their active form. In the absence of blue light, the VVD domains lose affinity for each other and dissociate. This results in a synthetic GadX transcription factor that can be switched on and off with light.

To test the efficacy of the synthetic GadX transcription

factor, we transformed it into cells which had the native *gadX* gene deleted from the chromosome, along with one of two different gene reporter plasmids [5]. These plasmids consist of the promoter regions of either *gadB* or *gadE* fused upstream of the gene encoding superfolder GFP (sfGFP). Both *gadB* and *gadE* are known to be activated by GadX, so activation of the synthetic GadX transcription factor with blue light should result in upregulation of both genes, as observed by increased sfGFP expression. This is indeed what was observed when cells were cultured under continuous exposure to blue light. Importantly, in the absence of light, cell fluorescence was comparable to that of cells not expressing the synthetic GadX transcription factor, indicating that the system has very low leakiness.

## III. CONCLUSION AND FUTURE DIRECTIONS

With the construction of a light-activable GadX transcription factor that allows for precise Gad network activation in bulk culture, the next step is to optimize a method for achieving the same level of control in single cells within a microfluidic device. Then, either RNA sequencing or additional gene reporter plasmids can be used to quantify network changes after various dynamic perturbations. Additionally, different expression patterns can be induced prior to exposure to acid or antibiotic stress to determine which factors are important for cell survival. This strategy for precise protein-level control can also generally be applied to other dimerizable transcription factors, as has already been demonstrated with AraC [6].

## REFERENCES

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