**Crosstalk between diverse synthetic protein degradation tags in *Escherichia coli***

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*Short Abstract* — Recently, a synthetic circuit in *E. coli* demonstrated that two proteins engineered with LAA tags targeted to the native protease ClpXP are susceptible to crosstalk due to competition for degradation between proteins. To understand proteolytic crosstalk beyond the single protease regime, we investigated a set of synthetic circuits designed to probe the dynamics of existing and novel degradation tags fused to fluorescent proteins. We quantified the degradation rates of each tag in isolation. We then tested for crosstalk between two distinguishable fluorescent proteins engineered with identical or different degradation tags. We demonstrated that proteolytic crosstalk was apparent between diverse tags.

*Keywords* — synthetic biology, queueing theory, protease, crosstalk, bottleneck.

Proper cell behavior is maintained using a finite pool of processing resources, such as the limited pool of enzymes required for gene transcription and protein translation\(^1\). Natural biological circuits are largely thought to have evolved to buffer against the effects of limited resources, but we are beginning to understand how processing machinery can form a bottleneck that is in fact leveraged as a control or signaling mechanism\(^2,3\). Proteolytic (protein degrading) pathways, in particular, have been found to form functional bottlenecks in a native *E. coli* network regulating the stationary phase sigma factor S (σ^S\(^5\)). The protein σ^S\(^5\) is degraded by the ClpXP proteolysis system (ClpXP protease and its chaperones) much faster during exponential growth phase\(^4,5\) than stationary phase\(^6\), and the corresponding buildup of σ^S\(^5\) in stationary phase acts as a signal triggering the stress response system for starvation\(^7\). An explanation for increased stability of σ^S\(^5\) in stationary phase is that there are an increased number of mistranslated proteins targeted for degradation by ClpXP. Mistranslated proteins are targeted to ClpXP because they have a C-terminal SsrA tag (LAA tag)\(^8\). These proteins compete for a limited number of proteases, especially ClpXP, which results in the formation of a “queue” of substrates for the protease that increases the apparent half-life of σ^S\(^5\).

The complexity of natural proteolytic pathways serves as a barrier to understanding this phenomenon, and so synthetic circuits offer a valuable alternative approach\(^9\). It was predicted based on the theoretical modeling of a synthetic oscillator that overexpression of LAA-tagged proteins could lead to saturation of proteolytic machinery\(^10,11\), i.e. that proteolytic machinery could be limiting.

Using synthetic circuits, we demonstrated that crosstalk could arise between several different proteolytic pathways. We first characterized a collection of amino acid sequences (tags) that target proteins towards active degradation. Proteins fused to these tags were expressed in a common strain using identical promoters and identical plasmid origins, which allowed us to compare fairly the effectiveness of these tags as degradation signals. This initial characterization of molecular parts is itself of value to synthetic biology. We then co-expressed YFP and CFP with different tags to determine crosstalk between pathways. The LAA tag was particularly prone to exhibiting crosstalk with itself and other tags. Other tag combinations demonstrated a range of crosstalk, though not as strong as we measured with LAA. Since many current synthetic systems rely on proteins engineered with LAA tags (targeted to ClpXP)\(^12\), our results strongly suggest that proteolytic crosstalk may be a major hurdle to scalability, indicating that new protease tags are required. Our select pairs of degradation tags with minimal to moderate crosstalk may have future applications in this direction, e.g. in the development of the first synthetic orthogonal to semi-orthogonal oscillators. Of course, tags with strong crosstalk may still be of value, as they could be used for more coordinated modules.

REFERENCES


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