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Improving Biomanufacturing Production with Novel ELP-Based Transcriptional Regulators

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ABSTRACT

Microbes can be used to produce valuable drugs, chemicals, and biofuels, but their potential has not been fully realized due to low production yields. To improve biomanufacturing processes and yield, we are developing novel, transcriptional regulators using biosynthesis technology in order to improve cellular health and overall production. Our regulator contains elastin-like polypeptides (ELPs), which make ideal sensors since they exhibit a sharp, inverse phase transition to indicators of cell health such as intracellular pH and ionic strength, and external stimuli such as temperature. We hypothesize that ELP can be fused to transcription factors to control expression of target genes. As proof of concept, Tet repressor protein (TetR) was fused to ELP to control expression of the red fluorescent protein mCherry, which was cloned under a Tet repressible promoter. The ability of TetR-ELP to control expression was determined by measuring fluorescence above and below the transition temperature (Tt) of ELP. Below the Tt, TetR is free to repress mCherry production, but above the Tt, ELP should aggregate, preventing TetR from repressing mCherry. However, our results showed that fluorescence was not affected as expected. We hypothesize that the observed behavior is due to either TetR having temperature sensitivity or that the binding affinity of TetR to DNA is much stronger than ELP aggregation. Further steps include proving this hypothesis and finding alternative transcription factors to test. These tested gene regulators will allow us to optimize production yield of microbe and bring development in manufacturing of drugs, chemicals, and biofuels.

KEYWORDS

Microbes, Elastin-like polypeptides, Biosynthesis, Gene Expression, Biomanufacturing