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Advanced biofuels offer a promising alternative fuel source to gasoline and diesel because they are renewable sources of energy that are compatible with existing fuel infrastructures. Engineered microbes can synthesize these biofuels, but can only tolerate limited concentrations in their environment. To increase biofuel yield, and to make biofuels an economically competitive fuel source, microbial biofuel tolerance must be increased. Previous research has shown that efflux pumps are an effective tolerance mechanism for cell growth in biofuels. These pumps are complexes of proteins that identify harmful compounds and remove them from the cell. However, overexpression of these pumps can also inhibit cell growth and, therefore, decrease biofuel production. Biofuel and pump expression toxicity must be balanced with each other in order to maximize cell growth.

Our research goal is to design a synthetic feedback loop that will be able to detect biofuel using a sensor protein, which will then trigger the expression of the efflux pump in the host *E. coli.* The protein MexR, native to *Pseudomonas aeruginosa*, was chosen as the biosensor because it detects oxidative stress, such as that caused by certain biofuels, in cells. The gene *mexR* has been codon optimized for *E. coli* in order to limit the strain on the cell. In the feedback loop, MexR represses the expression of the efflux pump by binding to synthetic promoter regions. Our initial studies, which use *rfp* for biosensor characterization, show that the sensor responds to biofuel. These results lay the groundwork for the synthetic feedback loop design where the biosensor will regulate efflux pump expression.