Living Material Production Architecture in Bacterial and Mammalian Cells

MIT iGEM Team 2010

Toggle and Switch Overview

Diagram of our toggle circuit, cI and lacI mutually repress one another such that IPTG can push the system into a high cI state and UV light can shift the system to a high lacI state. High levels of cI result in repression of all proteins excepting GFP. High levels of lacI result in repression of GFP and expression of both our protocol and an AHL synthase that functions to amplify material production.

R0065 leakiness and improvement

We discovered an error in R0065, the Plux/cI-OR promoter, and modified the promoter to remove a second transcription initiation site. Our new PLux/cI-OR promoter, K415032, shows significant reduction in leakiness.

Phage polymer output

To control M13 phage polymer formation, we took advantage of phage display technology. We envisioned a phage material composed of polyphage filaments, produced by cells that produce phage proteins on the polyphage coat. Specific pairs of coils dimerize. These linking interactions lead to material formation.

Cellular iPad

Mechanical stress processing osteogenesis

Creating self-constructing mammalian tissues requires mechanical feedback. As a proof of principle, we envisioned creating a cellular touchpad. Our goal is to engineer a mammalian cell line that can respond to pressure by differentiating into bone.

Results

To test our circuit, we used transient transfection to express our constructs in HEK endothelial cells. 41 hours after transfection, PonS was added to the culture medium to switch the circuit to the 'on' state. We observed a significant increase in EYFP fluorescence, shown on the left, indicating stable activation of our circuit.

Bone formation output

To test our system, cells were exposed to human recombinant BMP2 protein at either 100 ng/mL or 300 ng/mL, for five days to assay for differentiation. We observed a significant increase in EYFP fluorescence, shown on the left, indicating stable activation of our circuit.