Topologies of synthetic gene circuit for optimal fold change activation in mammalian cells

Doaa Ali-Naffaa, Ramez Daniel

Faculty of Biomedical Engineering, Technion, Haifa, Israel

Biological regulatory networks comprise feedforward and feedback loops, allowing cells to perform sophisticated tasks such as DNA repair, cell division, and apoptosis. During the past decades, feedforward and feedback loops have been introduced into living cells to scale -up the computational complexity of synthetic gene circuits. For example, mutual inhibition regulated by a double-negative feedback loop was the first network implemented in *Escherichia coli* to create a bistable switch (Gardner et al., 2000). Recently, an incoherent feedforward loop (Segall-shapiro et al.,2018) and an integral feedback controller (Aoki et al., 2019) have been applied to achieve a robust adaptation in *Escherichia coli* cells and mammalian cells (Frei et al., 2020). Here,we implemented a new genetic regulatory network, namely an indirect coherent feedforward loop (ICF), into *Escherichia coli* (Litovco et al., 2021) and mammalian cells (current study) to improve the fold change activation of induced promoters (ON/OFF ratio). We experimentally demonstrated that such a network could reduce the basal level while keeping the maximum activity high. In mammalian cells, we implemented the ICF loop using a small hairpin RNA (shRNA), which inhibits the expression level of the gene of interest (GOI), and decoy protein-binding RNA sites, named binding site sponge (BSS). The BSS includes multiple repeats of the same matching RNA sequence, creating a competition-derived repression of the shRNA repression. The GOI and BSS expression is induced by doxycycline (DOX), and the shRNA is expressed constitutively. On the one hand, when the DOX is low, the shRNA level is high, repressing the basal level for deficient levels. On the other hand, when the DOX level is high, shRNA binds to the BSS without inhibiting the GOI level. Our results demonstrated a five-fold increase in the ON/OFF ratio when incorporating the ICF loop. Similar systems are being developed with DAPG-(2,4-diacetylphloroglucinol) induced and cancer-sensitive promoters.