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Steady-state operation of a cell-free genetic band-detection circuit

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Over the past decade, synthetic gene networks have been used extensively to explore principles of biological pattern formation as they play a decisive role during biological growth and development processes. Pattern-forming circuits are also of great interest for the development of future biomaterials that respond to and differentiate autonomously with respect to their environment.
Here, we report on a bottom-up approach to design and analyze a cell-free genetic circuit based on an incoherent feed forward loop (IFFL-2), which is expected to produce a three-stripe pattern in response to an input gradient. In our work, we first simulated the behavior of the circuit and explored relevant parameters using a genetic algorithm approach. We then separately investigated the behavior of the three nodes comprising the IFFL-2 network in a bacterial cell-free gene expression system which was produced from a genome-engineered bacterial strain lacking LacI expression. We showed that the genetic circuit functioned as expected under non-equilibrium conditions in microfluidic ring reactors, whereas it fails to perform in bulk experiments in closed reactors. We showed that the non-equilibrium conditions are of necessity to establish the double-repression cascade which was the essential element of the genetic circuit. We used six neighboring ring reactors to establish a "virtual"morphogen gradient by supplying the reactors with decreasing amounts of the transcription factor σ28, corresponding to the different positions within an exponential morphogen gradient. We finally demonstrated that our IFFL-2 circuit, when operated in the microfluidic system, shows the correct gene expression response that is required for stripe-formation in a spatial context.

As the operation in microfluidic reactors would be at least laborious and time consuming to use for the realization of biomaterials that can differentiate autonomously in response to externally supplied chemical cues, the next step is to work on materials with simple and efficient supply lines (like vasculature). Those will be needed to implement cell-free metabolic processes and self-regeneration to enable operation of these systems over longer periods of time under non-equilibrium conditions.

Category

Other

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