Laboratory of Systems and Synthetic Biology
www.wur.nl\ssb
www.systemsbiology.nl

Sequence to Function: Modelling the relationship between sequences and synthetic circuit behaviour

Supervisors: Robert Smith

Contacts: robert1.smith@wur.nl

Type of thesis: Computational

Required competences: Knowledge of ODE models for synthetic biology (e.g. from SSB50806, SSB30806 or SSB31806) and ability to code in Python (e.g. from INF22306, BIF30806, or the SSB courses mentioned).

Acquired competences: Model fitting to data. Knowledge of synthetic biology construct design. If applicable, stochastic simulation methods.

Date: 04-10-22

Description

An important strand of synthetic biology is to design circuits to study system behaviour or create new applications. In the first instance, a classic example is building networks such as toggle switches that are prevalent in biological systems. In the second instance, networks can be created for specific purposes like biosafety mechanisms. In both instances, we aim to have control over how the system behaves and reduce uncertainty. This, generally, means that networks have two features: reduced leaky (unintentional) expression of system components, and inducibility such that the system only functions in tightly controlled, specific conditions.

In the case of toggle switches, CRISPRi-based networks have recently been developed that respond faster to inputs and provide less burden on a cells metabolism than classical protein-based systems (Santos-Moreno et al.). The system functions in response to chemical inputs to produce one of two outputs. Given one chemical inducer the accumulation of specific guide RNAs is promoted. These guide RNAs, when bound to Cas proteins, inhibit production of one system output and allows production of the second output. By adding a second chemical inducer, a different set of guide RNAs are produced

that "flip the switch" such that the production of second output is inhibited and the system produces the first output.

In the case of biosafety mechanisms, inducible riboswitches have been described in the literature (Isaacs et al., Gallagher et al.). Due to the tight folding of RNA strands, leaky transcription is prevented. By including inducible RNA unfolding and/or binding of complementary strands, a system's functionality can be tightly controlled. However, data obtained from the SSB lab suggests that experimental context matters – the same system shown to work in *E. coli* does not function when put into *P. putida*, both important species for synthetic biology applications.

Mathematical models are used to understand (and hypothesise) how differences in experimental conditions can lead to altered system functionality. Srinivas et al. have already shown how such DNA/RNA-based networks can be constructed mathematically, and we will use these ideas as a starting point to develop our equations for one of the systems above. Alongside our equations, we need to understand how fast transcription and translation processes occur to "parameterise" our model. Recent developments in synthetic biology have led to the ability to predict transcription/translation efficiency from a DNA or RNA sequence and structure (Fornace et al., Hossain et al., LaFleur et al.). We will combine these tools with data-fitting strategies to build a model that matches the data we have available – possibly including machine learning methods as proposed by (Rackauckas et al.). With this model to hand, we will then explore whether system performance can be optimised by changing the sequence of guide RNAs or riboswitches in the system.

It is rare to find such sequence-based mathematical models of synthetic circuits in the published literature, so we have a lot of scope to discuss and develop new methodology pipelines.

References

Fornace, M. E., et al. (2020) *ACS Synthetic Biology* **9:** 2665-2678

Hossain, A., et al. (2020) *Nature Biotechnology* **38:** 1466-1475

LaFleur, T. L., et al. (2022) *Nature Communications* **13:** 5159

Gallagher, R. R., et al. (2015) *Nucleic Acids Research* **43:** 1945-1954

Isaacs, F. J., et al. (2004) *Nature Biotechnology* **22:** 841-847

Srinivas, N., et al. (2017) *Science* **358:** eaal2052

Rackaukas, C., et al. (2020) *arXiv* **2001**.04385