Modelling RNA devices in a cellular context

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As part of the RiboNets project (www.ribonets.eu) a pipeline was created to de-novo design RNA molecules which can act as logic gates or devices within the cellular context of bacteria.
For a better assessment of the quality of our computational designs we evaluate the dynamic characteristics of the computationally designed RNA devices by embedding them into a network context, which models important cellular processes such as transcription, translation and degradation as system of ordinary differential equations (ODEs) with a more or less ”realistic” parameter set.

Minimal core system

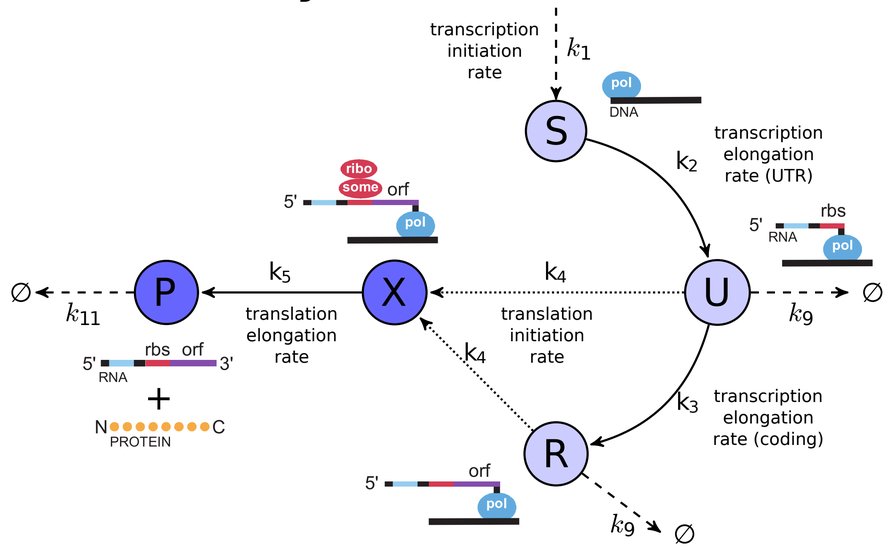

Figure 1: The core of the ODE system contains species and rates for transcription, translation and degradation processes. While unbroken edges depict direct transitions between the species, dashed lines represent a modulator on the target species. The translation initiation rate (k_4) requires to be a modulator to account for a polysomal translation. DNA, polymerase, ribosomes, nucleotides and amino acids are not explicitly included in the model as they mostly do not occur in rate limiting concentrations.

sRNA regulated translational OFF system

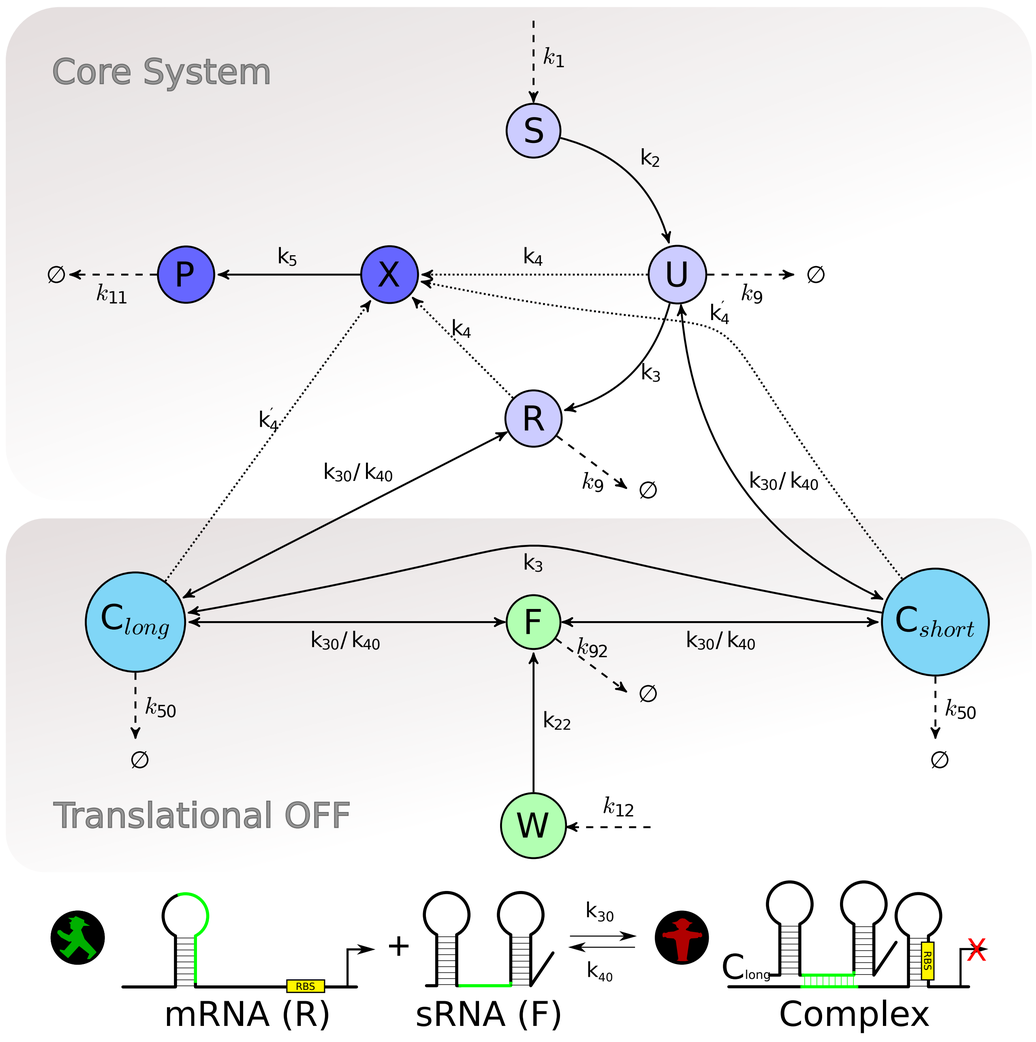

Figure 2: To model a sRNA regulated translational OFF system, four more species in addition to the core system are introduced: The sRNA initiation complex (W) which, with the initiation rate k_{22}, leads to the full sRNA (F). The sRNA and the 5'UTR of the mRNA (U/R) can then react to form RNA hybrids (C_{short}/C_{long}) with rates k_{30}/k_{40} for the complex formation and dissociation rate respectively. This process leads to the RBS being captured in a stable stem structure and therefore can only be translated with a very small rate.

Solving a translational OFF model with optimal complex formation and RBS accessibility values leads to a functional switching behaviour with a ~27-fold decrease of protein expression in the off state shown in Figure 3.

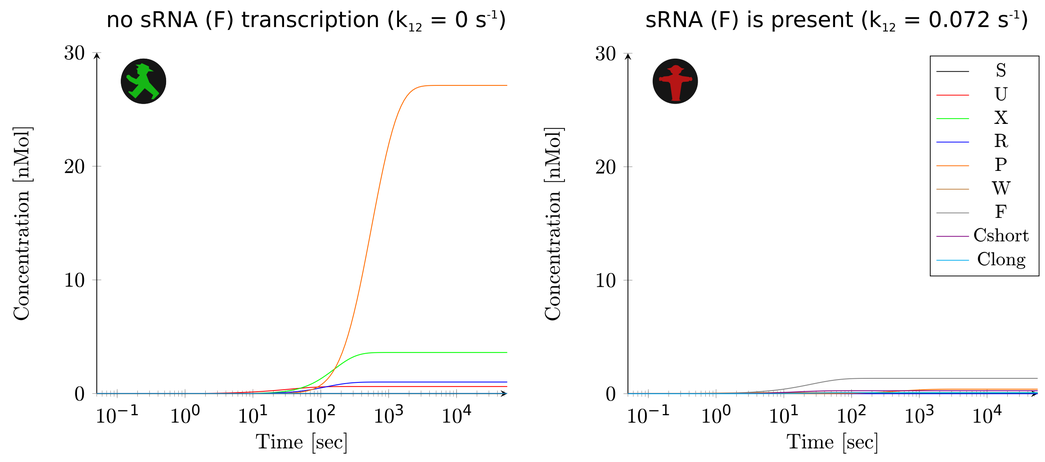

Figure 3: Solving of the previous introduced sRNA regulated translational OFF system leads, in absence of the sRNA initiation complex (W), to a stable protein production (ON state). As soon as we enable sRNA transcription, RNA-RNA complexes are formed between the sRNA and the 5'UTR of the mRNA, capturing the RBS site in a stable stem structure. This OFF state only shows a negligible protein expression.

Sequence dependent parameters can be estimated *in-silico*

In order to produce meaningful statements using such an ODE model, it is necessary to incorporate accurate rates. We use different approaches to obtain these values:
While some rates are derived from an exhaustive literature research, some device specific rates can be estimated from the RNA sequence and therefore energy landscape of the molecule. For the translational OFF system, the accessibility of the ribosome binding site and thus the efficiency of the translation initiation rate (k_4) is calculated using the RNAup approach, while the complex formation and dissociation rates (k_{30}/ k_{40}) are derived from the binding energy between the products of the reaction (1) and the equilibrium constant (2). Note that the k_{40} pre-factor α is an unknown variable and still needs to be identified.

$$R + F \rightleftharpoons C_{long} : k_{30}/k_{40} \quad (1)$$

$$K = \frac{[R \cdot F]}{[R][F]} = \frac{Z^{RF}}{Z^R \cdot Z^F}$$
$$Z^{RF} = Z^{cofold} - Z^R \cdot Z^F \quad (2)$$
$$Z = e^{-\frac{\Delta G}{RT}}$$
$$K = e^{-\frac{G_{cofold}-(G_R+G_F)}{RT}} - 1 = \frac{k_{30}}{k_{40}}$$

$$k_{40} = \alpha \cdot e^{-\frac{(-1)\cdot \Delta G_{Binding}}{RT}} \quad (3)$$
$$k_{30} = K \cdot k_{40}$$

How important are precise parameters?

While the individual modulation of many rates does not change the qualitative statement of the system, it is clear that a veridical balance between all rates is crucial for a quantitative prediction of the functionality of de-novo designed RNA devices. It is an ongoing challenge to not only measure, but also to fit unconfirmed rates using fluorescence data from already published designs[3].

References

[1] Carothers, J. M., Goler, J. A., Juminaga, D., and Keasling, J. D. (2011). Model-Driven Engineering of RNA Devices to Quantitatively Program Gene Expression. Science, 334(6063):1716–1719.
[2] Shimoni, Y., Friedlander, G., Hetzroni, G., Niv, G., Altuvia, S., Biham, O., and Margalit, H. (2007). Regulation of gene expression by small non-coding RNAs: a quantitative view. Molecular
[3] Green, A.A., Silver, P.A., Collins, J.J., and Yin, P. (2014). Toehold Switches: De-Novo-Designed Regulators of Gene Expression. Cell 159, 925–939.

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