APPENDIX

1. Equations for tx-tl ODE simulation

$$
\begin{aligned}
\text{mRNA balance:} \quad & \frac{dm_j}{dt} &&= \hat{r}_{X,j} - k^d_{X,j} m_j \\
\text{protein balance:} \quad & \frac{dp_k}{dt} &&= \hat{r}_{L,k} - k^d_{L,k} p_k \\
\text{Rate of mRNA transcription:} \quad & \hat{r}_{X,j} &&= k_{E,j} R_{X,T} \left(\frac{\mathscr{G}_j}{\tau_{X,j} K_{X,j} + (\tau_{X,j}+1)\mathscr{G}_j} \right) \cdot u_j \\
\text{Rate of protein translation:} \quad & \hat{r}_{L,j} &&= k^L_{E,j} R_{L,T} \left(\frac{m_j}{\tau_{L,j} K_{L,j} + (\tau_{L,j}+1) m_j} \right) \cdot w_j \\
\text{Transcription control function:} \quad & u_j &&= \left(\frac{W_{R_T,j} + \sum_{n\in\{+\}} W_{nj} f_{nj}}{1 + W_{R_T,j} + \sum_{d\in\{+,-\}} W_{dj} f_{dj}} \right) \\
\text{Fraction of transcription factor bound:} \quad & f_{ni} &&= \frac{I^m_{ni}}{K^m_{D,i} + I^m_{ni}}
\end{aligned}
$$

Here, m_j and p_k are the mRNA and protein concentrations, $\hat{r}_{X,j}$ and $\hat{r}_{L,k}$ are the transcription and translation rates (defined below), and $k^d_{X,j}$ and $k^d_{L,k}$ are the mRNA and protein degradation rates, respectively. In the equations for $\hat{r}_{X,j}$ and $\hat{r}_{L,k}$, $k_{E,j}, k^L_{E,j}, R_{X,T}$ and $R_{X,T}$ are the mRNA elongation rate constant, protein elongation rate constant, RNAP concentration and Ribosome concentration, respectively.

$\mathscr{G}_j$ is the gene concentration. τ and K denote the time and saturation constants (related to transcription and translation), respectively. In order to capture the regulatory network, the formulations developed by Moon et al.[1] were used. In the equations above, they are denoted by u_j and f_{ni}. Briefly, they describe the effect of the inducers (or repressors), I^m_{ni}, on the different promoters they interact with. The weight functions, $W_{nj,dj}$ and $W_{R_T,j}$ together with the binding function, f, describe the probabilities of the different microstates of the inducer-promoter-RNAP system. The repressors are included in the denominator of u_j. $K^m_{D,i}$ is the dissociation constant, and n is the cooperativity.

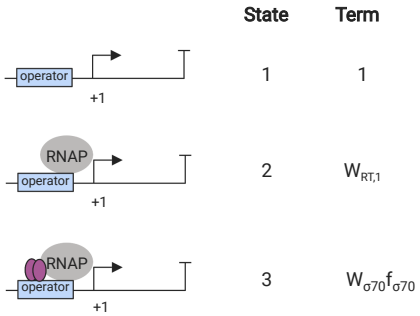

Figure 1: Schematic of the binding states of the inducible promoters. Adapted from Moon et al.[1]

2. Equations for Flux Balance Analysis

$$
\begin{aligned}
\text{Component mass balance:} \quad & \frac{d\mathbf{X}}{dt} &&= \mathbf{S}\cdot\mathbf{v} = \mathbf{0} \text{ or } \mathbf{b} \\
\text{Bounds on flux values:} \quad & \mathscr{L}_i &&\leq \mathbf{v} \leq \mathscr{U}_i \\
\text{Objective function:} \quad & \max_{\mathbf{v}} \quad (v_{\text{export}} &&= \mathbf{c}^T \cdot \mathbf{v})
\end{aligned}
$$

S is the stoichiometric matrix generated from the set of reactions occurring in the CFPS, **v** is the flux through each reaction and **b** is the flux of species into or out of the system. $\mathscr{L}$ and $\mathscr{U}$ are the lower and upper bounds, respectively. The objective function was set to maximizing the export of the three proteins: σ_{28}, CIssrA and deGFPssrA. $mathbfc$ is a vector of weights that indicates the contribution of each flux to the objective.

References

1. Moon TS, Lou C, Tamsir A, Stanton BC, Voigt CA. Genetic programs constructed from layered logic gates in single cells. Nature. 2012;491(7423):249.