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Supplementary Methods

Simplified model of the *Pseudomonas putida* TOL network

This model is based on the network described in de Las Heras et al, 2012. The aim is to simulate the behaviour of the TOL transcriptional network and modifications made to it. In the experimental studies, the induction of the TOL pathway is followed by the expression of the *lux* reporter gene under the control of the master transcriptional regulator of the TOL pathway, XylR. XylR is normally present on cells at stationary phase in an inactive form, which can be activated by the optimal inducer m-xylene and by sub-optimal inducers, such as 3MBA. Upon activation, XylR induces transcription of other proteins involved in the TOL pathway, and in this case, the *lux* reporter gene. In the wild type network activated XylR also represses its own transcription and its concentration is therefore regulated through a negative feedback loop. This regulation was experimentally modified by de Las Heras et al (2012) by changing the negative to a positive feedback loop in which XylR activates its own transcription. This was done by exchanging the endogenous XylR promoter, P_R, by one of two XylR-activated promoters, the strong promoter *Pu* and the weaker one *Ps*.

The model described below is a simple two-ODE model that follows only two variables, the concentration of XylR, and the concentration of the lux reporter, which is induced by the activated form of XylR. We also include the possibility of increasing the RNA polymerase factor σ^{54} as a factor controlling transcription from the *Pu* promoter.

Model description and assumptions

- XylR, denoted by R_T, is assumed to be synthesized at a constant background rate k'_{sR} and degraded also at a constant background rate, k_{dR}. In the wild type network and modified networks XylR synthesis is also a function *f* of XylR activity, denoted here as a_R. Therefore, we write a preliminary differential equation for XylR:

$$\frac{dR_T}{dt} = k'_{sR} + f(a_R) - k_{dR}R \qquad 1$$

- We assume that the activated form of XylR is bound to the inducer in a complex. Compared to protein synthesis and degradation, we expect the binding and unbinding of the inducer to XylR to be fast, so we can assume that the concentrations of the complex, R_c, and free XylR, R_f, are at equilibrium. Also, the sum of the complex and the free forms is equal to the total amount of XylR:

$$R_T = R_c + R_f \qquad 2$$

- Therefore, the concentration of the XylR-inducer complex, R_c can be described by the following expression:

$$R_c = R_T \frac{I}{K_D + I} \qquad 3$$

where *I* represents the concentration of inducer added to the cells, and $K_D$is the dissociation constant of the XylR-inducer complex.

- To model the effects of optimal and sub-optimal inducers, we consider that the complexes with different inducers have different levels of transcriptional activity. This is because it appears that the optimal and sub-optimal inducers have similar affinities for XylR (de Las Heras et al, 2012). We also allow the possibility that the free form of XylR can have a small, background level of activity. Therefore, we can define the activity of XylR, a_R as a weighted sum of the free and complex forms:

$$a_R = \lambda R_c + \alpha R_f \qquad 4$$

Where normally $\lambda > \alpha$, and λ is a parameter that indicates how strongly a particular inducer activated XylR, and α indicates the background activity of free XylR.

Thus, combining equations 3 and 4 we obtain an expression to describe the level of activity of XylR:

$$a_R = R_T \left(\alpha + (\lambda - \alpha)\frac{I}{K_D + I}\right) \qquad 5$$

- Using equation 5, we can then define the function $f(a_R)$ from equation 1. We assume that as most transcription factors, active XylR activates or inactivates transcription in a non-linear way, described here with Hill equations. The shape of the function also depends on whether XylR acts as a transcriptional inhibitor or as an activator.

Then, for the model of the wild type network, where XylR inhibits its own transcription in a negative feedback loop, the function f_n from equation 1 is defined as:

$$f_n(a_R) = k''_{sR}\frac{J_R^{nR}}{J_R^{nR} + a_R^{nR}} \qquad 6$$

Where k''_{sR} is the maximum rate of XylR synthesis (when $a_R = 0$), J_R is the a_R level resulting in half-maximal XylR-dependent transcriptional inhibition and n_R is the Hill coefficient which determines how steep is the transcriptional inhibition.

For the network with a positive feedback loop, the $f(a_R)$ function describing transcriptional activation of XylR by XylR is:

$$f_p(a_R) = k''_{sR}\sigma\frac{a_R^{nR}}{J_R^{nR} + a_R^{nR}} \qquad 7$$

Where $k''_{sR}\sigma$ is the maximum rate of XylR synthesis; k''_{sR} represents the contribution to the rate made by active XylR and σ takes into account the amount of the σ^{54} factor present in the cell. For simplicity we assume that increasing the levels of this factor leads to a linear increase in the transcription rate from the *Pu* promoter. J_R is the a_R level resulting in half-maximal XylR-dependent transcriptional activation and n_R is the Hill coefficient which determines the steepness of the activation.

Finally, we write a differential equation for the concentration of the Lux reporter, whose synthesis is activated by XylR.

$$\frac{dL}{dt} = k'_{sL} + k''_{sL}\sigma\frac{a_R^{nL}}{J_L^{nL} + a_R^{nL}} + k_{dL}L \qquad 8$$

Where k'_{sL} is a small background synthesis rate $k''_{sL}\sigma$ is the maximum rate of XylR and σ^{54}-dependent Lux synthesis. J_L is the a_R level resulting in half-maximal XylR-dependent transcriptional activation of *lux* and n_L is the Hill coefficient which determines the steepness of XylR transcriptional activation.

We used MATLAB to simulate these systems of ODEs:

- For the model with the original system architecture involving a negative feedback loop the system of ODEs is composed of ODEs 1 and 8 and algebraic equations 5 and 6 and
- For the system with XylR controlled by a positive feedback loop, the system is composed of ODEs 1 and 8 and algebraic equations 5 and 7.

Parameters values are shown in the Table below:

Parameter	Value
k'_{sR}	0 (Fig 2), 0.1 (Fig 6)
k_{dR}	1
K_D	1
λ	1
α	0.01
k''_{sR}	1 (Fig 2), 2 (Fig 6)
J_R	0.2 (Fig 2), 0.3 (Fig 6)
nR	3
k'_{sL}	0.1
k''_{sL}	1
k_{dL}	0.5
J_L	0.3
nL	3
σ	1 (Fig 2b, 6a), 2 (Fig 1c, 6b)
I	0 (before induction), 20 (after induction)