–SynBio3D–
Engineering gene circuits in both space and time

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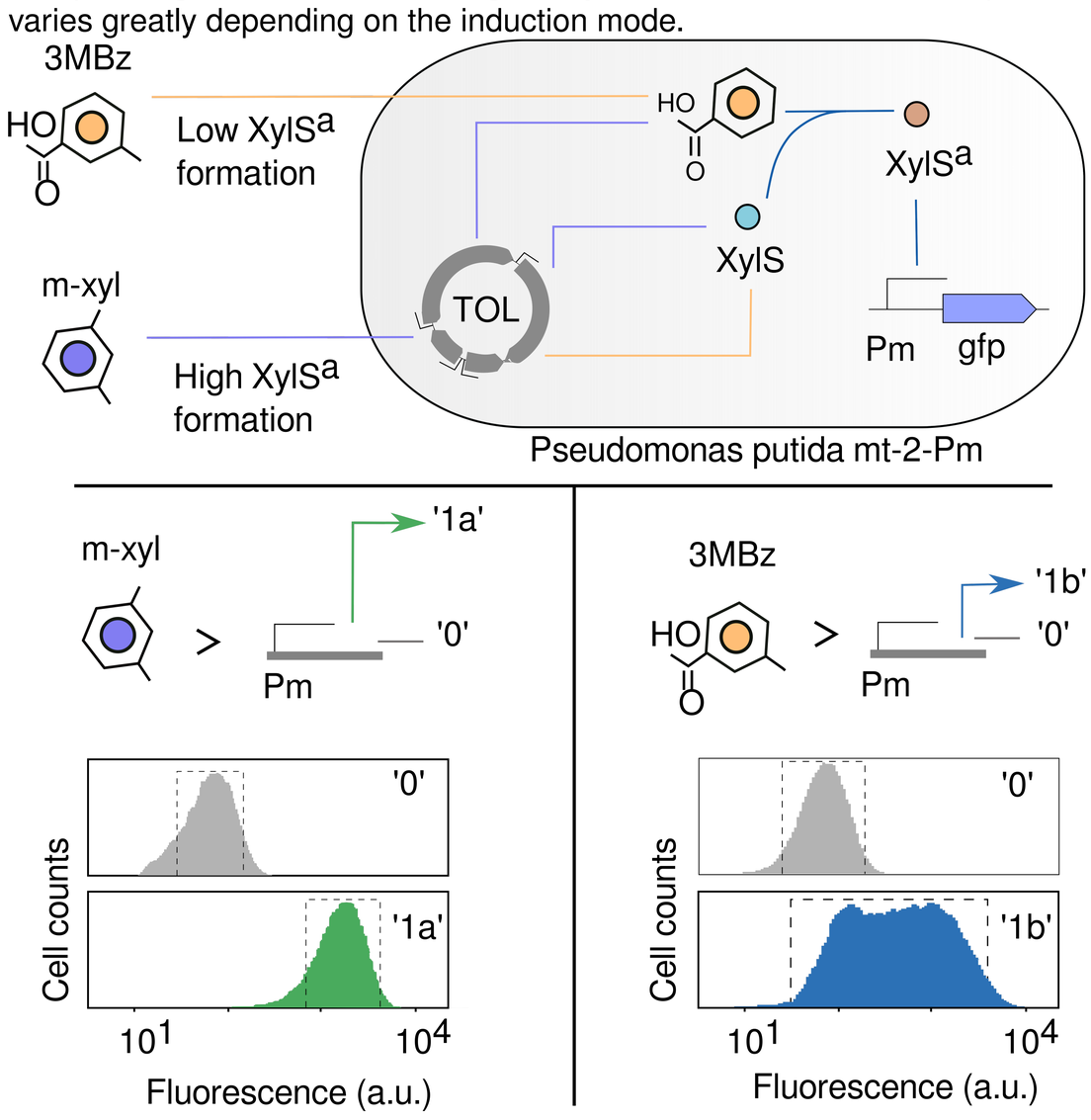
Abstract

We studied Pm promoter activity of the environ- mental bacterium Pseudomonas putida and its cognate regulator XylS by following expression of Pm-GFP fusions in single cells. Using mathematical modeling and computational simulations, we determined the kinetic properties of the system and used them as a baseline code to interpret promoter activity in terms of upstream regulator dynamics. Transcriptional noise was predicted to depend on the intracellular physical distance between regulator source (where XylS is produced) and the target promoter. Experiments with engineered bacteria in which this distance is minimized or enlarged confirmed the predicted effects of source/ target proximity on noise patterns. This approach allowed deconvolution of cytometry data into mechanistic information on gene expression flow. It also provided a basis for selecting programmable noise levels in synthetic regulatory circuits.

Differences in GFP expression

The function of the GFP-promotor (Pm) in Pseudomonas putida mt-2 is that of an OR logic gate where either input, 3MBz or m-xyl, can trigger its activity. The puzzling feature of the Pm regulatory node is that the Pm noise pattern varies greatly depending on the induction mode.

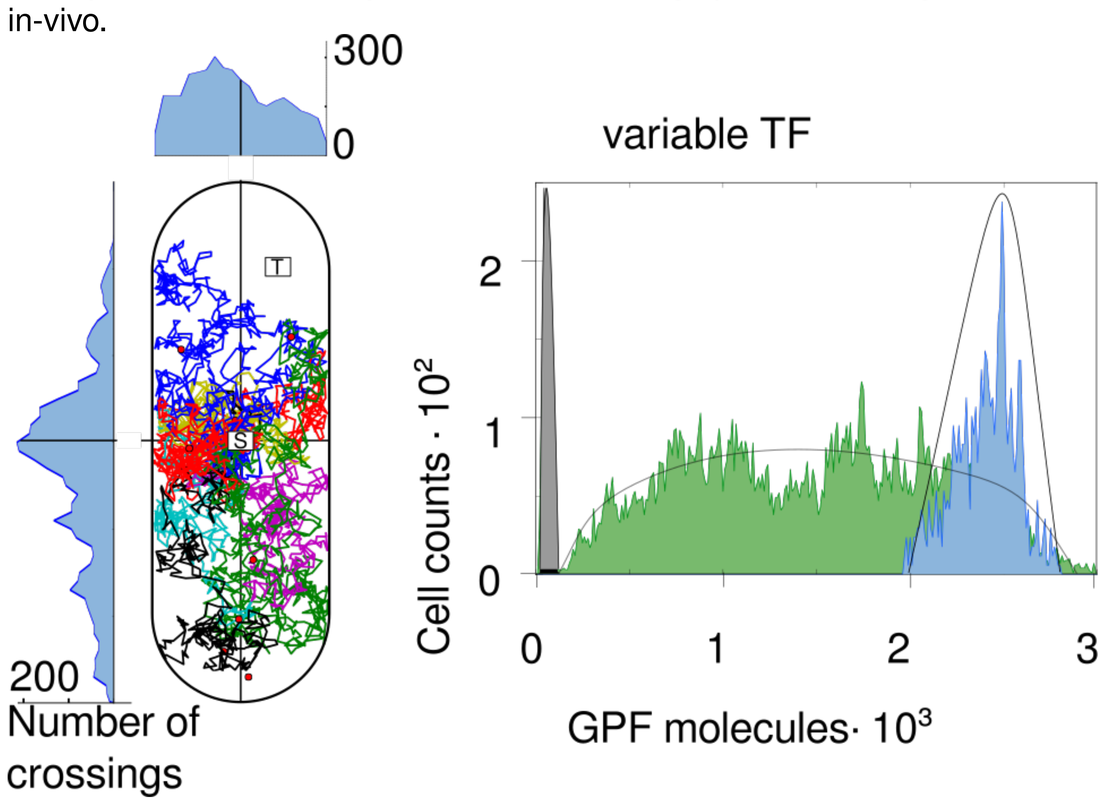


In these experiments, XylS molecules are produced by the TOL plasmid borne by the P. putida mt-2-Pm, whereas the target Pm-GFP reporter fusion is inserted in the chromosome ; that is, the source of the TF and its target promoter are nonadjacent and encoded in separate monocopy replicons.

Reference: Goñi-Moreno, A., Benedetti, I., Kim, J., and de Lorenzo, V. (2017). Deconvolution of Gene Expression Noise into Spatial Dynamics of Transcription Factor-Promoter Interplay. *ACS Synthetic Biology.*

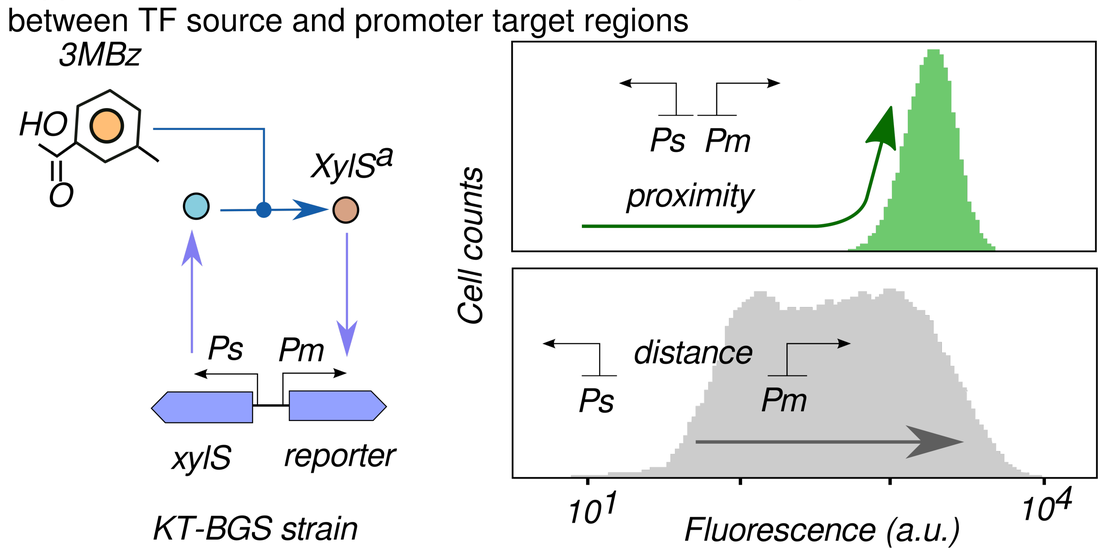
Simulating spatial reaction dynamics

To simulate diffusion of the TF through the cell, a simple 2d brownian motion algorithm was used. The crossing of the TF-trajectories through a subsection shows the availability in that area.
These number of crossings updated the TF availability variable within a single Gillespie simulation.This generates the wide-rage plateau- like signal as seen in-vivo.



Empirical spatial effects

We positioned the Ps-xylS and Pm-GFP sequences within the frame of a mini-Tn7 transposon vector, which was delivered to the single attTn7 site of P. putida KT2440. In these engineered bacteria, the two components of the regulatory device (Ps-xylS/Pm-GFP) were designed to be adjacent, in monocopy and at a fixed chromosomal site, with an artificially minimized distance between TF source and promoter target regions



Conclusion

Our model, validated by the experiments shown above, indicates that the physical distance between the regulator source and the target promoter is translated into specific noise patterns that change radically depending on promoter-TF proximity.