Supplementary Information

A transcription factor-based biosensor for detection of itaconic acid

Erik K. R. Hanko[1], Nigel P. Minton[1], Naglis Malys[1]*

[1]BBSRC/EPSCR Synthetic Biology Research Centre (SBRC), School of Life Sciences, Centre for Biomolecular Sciences, The University of Nottingham, Nottingham NG7 2RD, United Kingdom

*Author to whom correspondence should be addressed, E-Mail: naglis.malys@nottingham.ac.uk

Supplementary Tables and Figures

Table S1. List of primers that were used in this study. Restriction sites that were incorporated for cloning are underlined.

Primer name	Primer sequence (5'→ 3')
EH011_f	aatccaagcgtttaaacggaggcagacaaggtatagggc
EH012_r	tctgcctccgtttaaacgcttggattctcaccaataaaaaacgc
EH015_f	gcccagtctttcgactgagcctttcgttttatggcgcgccaggccggccgatatctggcgaaaatgagacgttg
EH075_r	aaggccatcctgacggatggccttttcctgcaggtcatcccaggtggcact
EH078_f	gttgtcataacctaggtataaacgcagaaaggcccacc
EH079_r	tgcgtttatacctaggttatgacaacttgacggctacatcat
EH083_f	aaaaggccatccgtcaggatggccttctatgtatatctccttcttaaaagatcttttgaattcca
EH190_r	aggctcagtcgaaagactgggcctttcgttttatgacgtctcaaggaaacacggtcaggaca
EH191_f	gctactcgccatatggttcctcctccaacttcgct
EH293_r	cggtttccctctagaaataatttttggaattcaaaagatcttttaagaaggagatataaccatgaccaagcaatctgcgga
EH294_f	tgcaacaggccccagtttctgcccatatccaatcaccctg
EH295_r	gtgattggatatgggcagaaactggggcctgttgca
EH296_f	gcgcacatttccccgaaaagtgccacctgggatgacctgcaggaaaaggccatccgtcaggatggccttctttataccagtggcgatttcacg
EH302_r	tcgttttatgacgtccttcatatccaaaagcaattaaacacac
EH311_r	tcgttttatgacgtcctacaggctgtctcccaccct
EH312_f	gctactcgccatatgtggcttcccatgacctaggg
EH313_r	tcgttttatgacgtcgggcactccatggcgttc

Figure S1. Nucleotide sequences of intergenic regions containing putative itaconate-inducible promoters. The (A) *Y. pseudotuberculosis* YPIII *itcR/ccl* and (B) *P. aeruginosa* PAO1 *itcR/ich* intergenic regions. Translational start sites are italicized. Transcriptional start sites of genes *ccl* and *ich* were predicted using program NNPP[1] and are underlined and in bold. The putative promoter -35 and -10 sequences were annotated manually on the basis of typical characteristics of bacterial promoters and are shaded in gray. The putative ItcR binding site in the *P. aeruginosa itcR/ich* intergenic region is underlined. Speculative annotation of the putative ItcR binding site is based on palindromic sequences upstream to the putative promoter -35 and -10 boxes. Palindromic sequences were identified using mfold web server.[2]

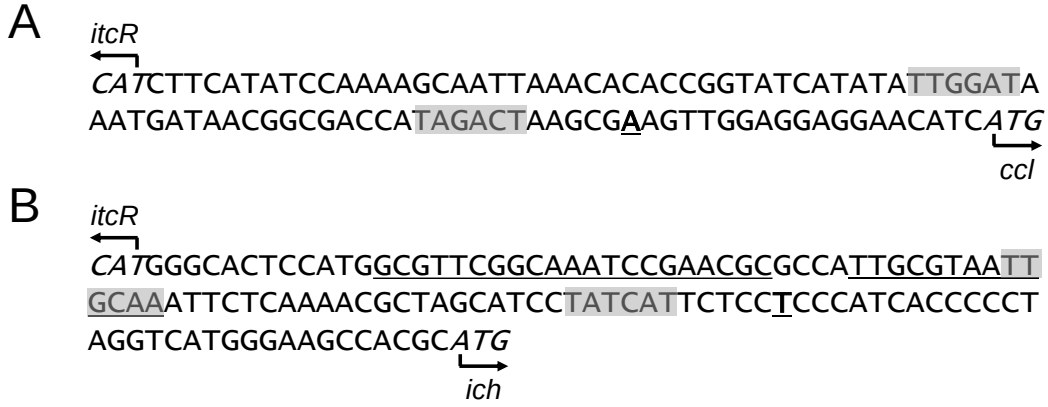


Figure S2. Gene cluster putatively involved in itaconate degradation in *Cupriavidus necator* H16. *C. necator* H16 gene cluster encoding the *P. aeruginosa* PAO1 Ict, Ich and Ccl homologs. An ItcR homolog is located in close proximity to the gene cluster involved in itaconate degradation and in the same orientation. Gene names and locus tags are shown. Protein sequence identity and coverage is indicated in brackets.

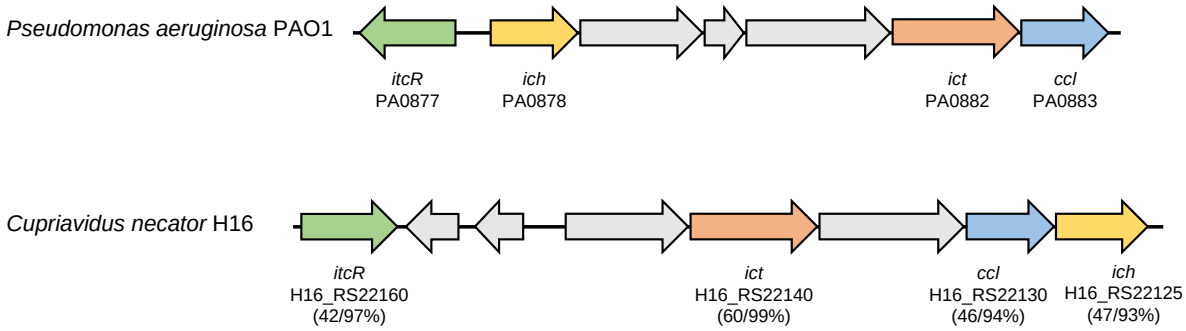


Figure S3. Dose response of the *Yp*ItcR/P$_{ccl}$ inducible system in rich medium. Dose response curve of the *Yp*ItcR/P$_{ccl}$ inducible system in *Escherichia coli* MG1655 grown in LB medium. The graph illustrates the correlation between itaconate concentration and fluorescence output four and eight hours post induction (hpi). Error bars represent standard deviations of three biological replicates.

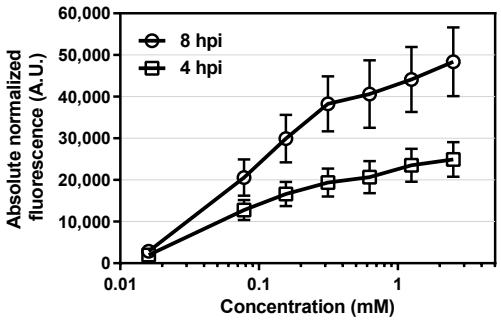


Figure S4. Specificity of the *Yp*ItcR/P$_{ccl}$ inducible system. Normalized fluorescence (in %) of *E. coli* MG1655 harboring the *Yp*ItcR/P$_{ccl}$ inducible system twelve hours after addition of different compounds at a final concentration of 10 mM, relative to the fluorescence output obtained by adding 5 mM itaconate (second bar). (-), uninduced sample. Error bars represent standard deviations of three biological replicates.

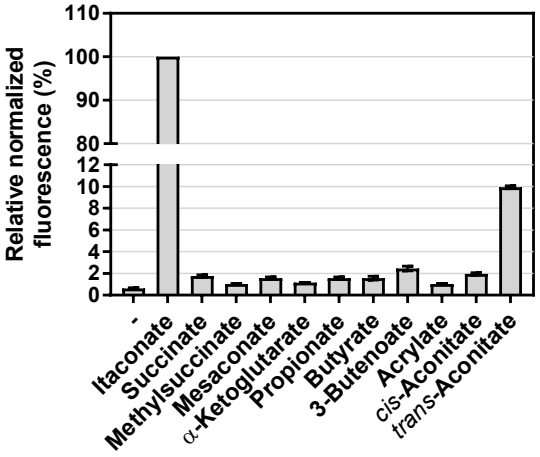


Figure S5. Dynamic range in response to different inducers. Dynamic range of the *Yp*ItcR/P$_{ccl}$ inducible system in *E. coli* MG1655 in response to different concentrations of itaconate, mesaconate, *cis*-, and *trans*-aconitate six hours after inducer addition. The dynamic range was fit to the corresponding inducer concentration using a Hill function. The maximum dynamic range (μ_{max}) is indicated for itaconate. For the other three inducers, the values for μ were extrapolated to reach μ_{max} using the available data points. The inducer concentration mediating half-maximal RFP expression (K_i) is indicated for each compound. Error bars represent standard deviations of three biological replicates.

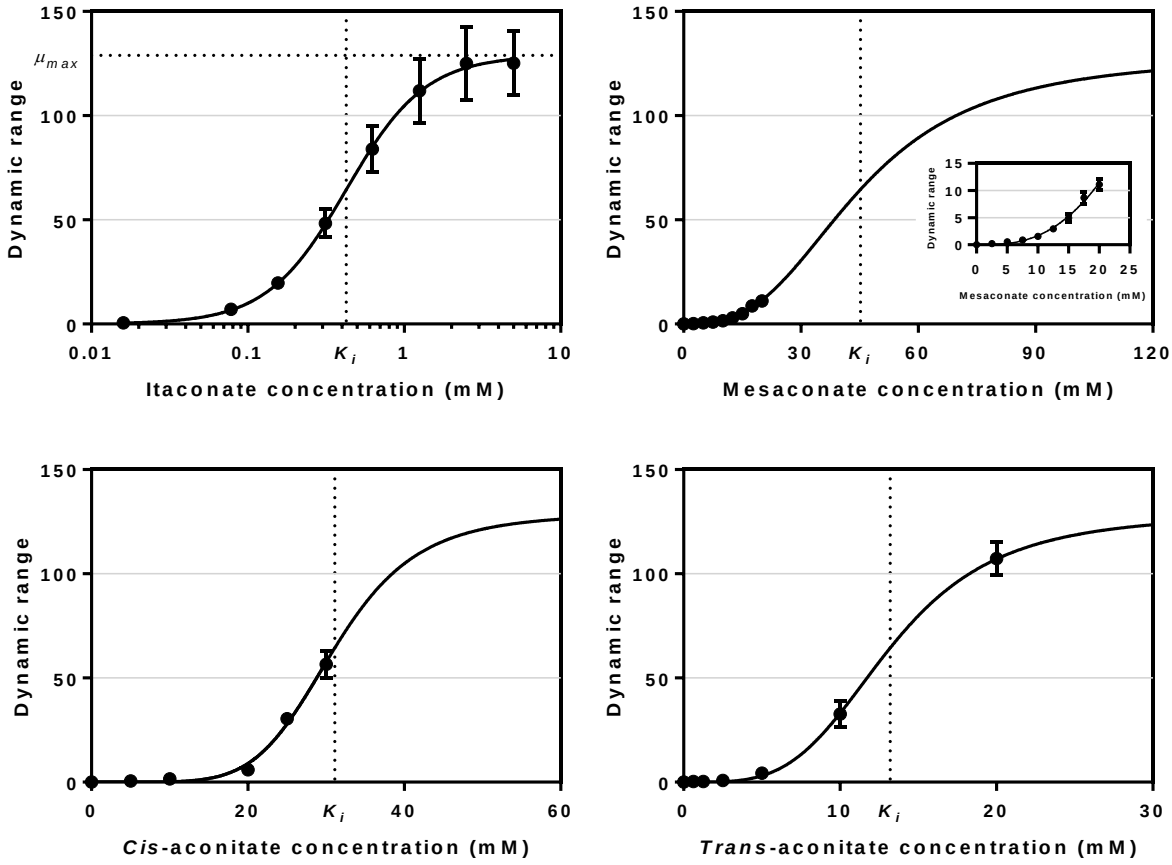
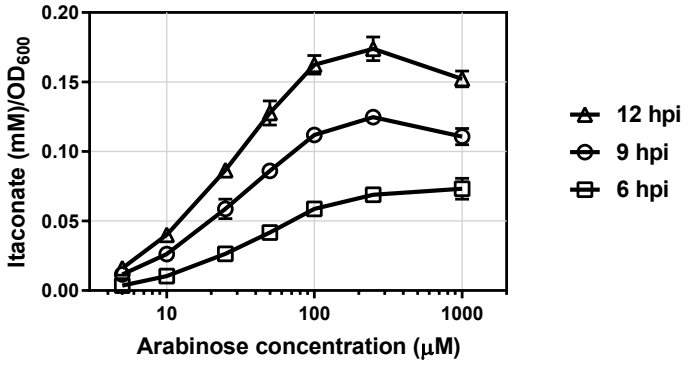


Figure S6. Biosensor-assisted improvement of itaconate production. OD-normalized itaconate titers of *E. coli* TOP10 harbouring pEH165, grown in small-volume cultures, six, nine, and twelve hours post induction (hpi) with 5, 10, 25, 50, 100, 250, and 1000 μM of L-arabinose. Error bars represent standard deviations of three biological replicates.



References

(1) Reese, M. G. (2001) Application of a time-delay neutral network to promoter annotation in the *Drosophila melanogaster* genome. *Comput. Chem. 26* (1), 51-56.

(2) Zuker, M. (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res. 31* (13), 3406-3415.