README

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This repository contains scripts of the models and analyses reported in published work:

Mannan, A. A & Bates, D. G. Designing an irreversible metabolic switch for scalable induction of microbial chemical production. *Nature Communications* (2021)

If any scripts of the models or analyses are used for other works, in whole or partially, **please cite the above paper**.

Here, we briefly describe what each script that performs analysis does. All analysis scripts reproduce the plots of the paper and are named "A[#1]_... .m", and can simply be run in MATLAB without any additional inputs.

We do not describe the scripts of the subfunctions used in the analysis scripts (what we call the method scripts, named "m_ ... .m"), but they are nevertheless well commented.

For **any further requests**, please **email d.bates"at"warwick.ac.uk**, the corresponding author.

Important notes:

- *Scripts were developed in MATLAB 2018a and use functions from the Ordinary Differential Equations solvers, Optimization Toolbox, Global Optimization Toolbox, Statistical Toolbox and perhaps others.*
- *Many scripts also use MATLAB parallel pools or optimization option UseParallel to speed up computation.*
- *Please take care if running in more recent versions of MATLAB as some functions may be discontinued or replaced.*

Folder: Model1_RevSwitch

Subfolder: 0_GlobalSensitivityAnalysis

Scripts for the global sensitivity analysis (GSA) of the reversible bistable-switch model, defined in Methods and Supplementary Notes S2. GSA is performed using the eFAST method[1] using the MATLAB scripts developed by the Kirschner lab[2] (enclosed in the folder).

- **A1_AllAnalysis.m**
  Runs eFAST GSA for the circuits with NAR and PAR, and computes and plots the first-order sensitivity indices for the induction threshold (IT), reversion threshold (RT) and bistable range (BR), using script **A1_efast.m** modified from [2] for our model, defined in scripts **Model1.m**, **Model1_Params.m**, **Model1_Jacobian.m** and **Model1_Obj_NullclinesAndSS.m**. ***Reproduces Supplementary Figure 2(a)***.

Subfolder: 1_SteadyStateAnalyses

Scripts performing local sensitivity analysis, i.e. how dose-response is affected for variations in circuit parameters of the circuits with NAR and PAR. Also contains script for calculating eigenvalues to determine reversion rates.

- **A1_RunLocalSensitivityAnalysis.m**
  Simulates and plots the dose-response curves for variations in parameters, for the circuits with NAR and PAR, using function **A1_DoseResp_VaryParams.m**. ***Reproduces Supplementary Figure 2(c)***.
- **A2_Plots_ParamsAffectBistRng.m**
  Simulates and plots dose-response characteristics for variations in parameters for circuits with NAR and PAR. ***Reproduces Fig. 1(c) and (d)***.
- **A3_SymbEigs.m**
  Calculates symbolic vector of eigenvalues of the model to determine system reversion rates. Input required is "arch" which defines circuit topology as 'NAR' or 'PAR'. ***Reproduces results in Supplementary Notes S4***.

Subfolder: 2_SimulatingDynamics

Scripts simulating and plotting the dynamics during induction, for retaining the induced production phenotype, and assessing the circuits performances, i.e. total OA used and switch time.

- **A1_Run.m**

  Simulates and plots the total OA used vs switch time for switches with NAR and PAR, using function **A1_VarOAFeedIn.m**, and plots dynamics for keeping systems at induced production phenotype. ***Reproduces all plots of Fig. 2***.

- **A2_Run.m**

  Simulated how Pareto front of total OA used and switch time is affected by variation in circuit parameters using function **A2_ParamsEffectOnObj1VsObj2Curve.m**. ***Reproduces all plots of Supplementary Figure 3***.

Folder: Model2_IrrevSwitch

Subfolder: 1_SteadyStateAnalyses

Scripts searching over the constrained parameter space of the modified circuit shown in Fig. 3(a), to identify regions of the space where it behaves as a reversible or irreversible switch. Also, plots the dose response for the parameter regime where all circuit topologies considered in Fig 3(a) behave as irreversible switches.

- **A1_RUN_IrrevDesignSpaceAndDoseResp.m**

  Assesses part of the defined parameter space where each of the circuit topologies illustrated in Fig. 3(a) can behave as reversible or irreversible switches. Also plots dose-response of circuits in parameter regime where they behave irreversibly. ***Reproduces plots in Fig 3 and Supplementary Figure 6***.

Subfolder: 2_SimulatingDynamics

Scripts simulating the dynamics of the irreversible switch, defined with parameters **Params_Eng_IrrevSw.mat** as reported in Supplementary Table 4, and performing the multi-objective optimization to determine optimal parameters that will minimize inherently opposing objectives total OA used and switch time.

- **A1_MultiObjOpt_FindOptIndRegime.m**

  Performed the multi-objective optimization for the circuit topology defined as COMP in Figure 3(a), to ***reproduce plots of Fig. 4(b)***.

- **A2_TimeCourseSim.m**

  Simulates time course of temporal OA induction for the optimal induction regime highlighted as a black dot in Fig. 4(b), to ***reproduce Fig. 4(a)***.

- **A3_HowParetoAffectedByCircParams.m**

  Simulates and plots how the Pareto front seen in Fig. 4(b) is affected by ±25% change to circuit parameter values, to ***reproduce Supplementary Figure 7(a)***.

- **A4_HowParetoAffectedByTuning_bD.m**

- Same as **A3_HowParetoAffectedByCircParams.m** except that it simulates and plots changes in Pareto front for changes to b_D from 1 to 10-fold its nominal value from Supplementary Table 4, to ***reproduce Supplementary Figure 7(b)***.

Subfolder: 3_ComparisonWithToggleSwitch

Scripts of the canonical bistable toggle switch (bTS), with raw data for its parameterization from Xu *et al* (2009)[3] given in the two Microsoft Excel files, and analyses of its engineering and performance (total IPTG used and switch time), for comparison with the proposed irreversible metabolic switch, as detailed in Supplementary Notes S10.

- **A1_RUN_IrrevParamSpaceForTS.m**

  Explores a defined space of the bTS circuit parameters to assess regimes where it behaves as an irreversible or reversible switch, to ***reproduce plots in Supplementary Figure 10***.

- **A2_MultiObjOpt_FindOptIndRegime.m**

  Performs multi-objective optimization to find Pareto front of optimal designs balancing total IPTG used and switch time, ***to reproduce plots in Supplementary Figure 11(a)***.

- **A3_TimeCourseSims.m**

  Simulated and plots time course dynamics of temporal induction of the bTS, to ***reproduce Supplementary Figure 11(b)***.

- **A4_HowParetoAffectedByCircParams.m**

  Simulating and plotting how Pareto front is changed for ±10% variation in bTS parameters, and for scaling of promoter strengths from 10% to 10-fold their nominal values in Supplementary Table 5, to ***reproduce plots in Supplementary Figure 12***.

References

1. Marino, S., Hogue, I. B., Ray, C. J. & Kirschner, D. E. A methodology for performing global uncertainty and sensitivity analysis in systems biology. *J. Theor. Biol.* **254**, 178–196 (2008).
2. Marino, S., Hogue, I. B., Ray, C. J. & Kirschner, D. E. Uncertainty and sensitivity functions and implementation. (2008). Available at: http://malthus.micro.med.umich.edu/lab/usadata/.
3. Xu, J. & Matthews, K. S. Flexibility in the Inducer Binding Region is Crucial for Allostery in the Escherichia coli Lactose Repressor. *Biochemistry* **48**, 4988–4998