# Multi-scale modelling of *E. coli* metabolism

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**Abstract**

In response to environmental and genetic perturbations, micro-organisms may regulate their metabolism both metabolically – via metabolite–enzyme interactions – and hierarchically – via modulating enzyme capacities. In this report, we develop a combined metabolic and genetic regulatory model of *E. coli* that may be used to test this multi-scale response.

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## Metabolic module

A number of kinetic models of *E. coli* metabolism are available in the literature [1, 2, 3, 4, 5], varying both in their level of detail and in their availability in standardised formats. As a scaffold, we use the metabolic model of Chassagnole *et al.* [1], available from the BioModels database [6] in SBML format [7] with accession number BIOMD0000000051. Some changes are made to this model, to enhance its use:

- co-metabolites AxP and NAD/P/H are allowed to vary
- dilution of intracellular pools due to growth, which have a negligible effect on system dynamics, are removed
- glucose uptake is set to an experimentally-determined initial rate of $1.23\,\mathrm{mM\,s^{-1}}$, of which 22% enters the pentose phosphate pathway [8]
- `rMax` values are rescaled to ensure the system starts at steady state, as was done in the original paper
- biomass-producing branches (that is, those other than `GPS`, `PDH` and `PPC`) are coupled via a single reaction, to simulate growth at an initial rate of $1.67 \times 10^{-4}\,\mathrm{s^{-1}}$. This ensures that all the metabolic building blocks are used in stoichiometric proportions dictated by the cell composition, and links metabolism to overall cell physiology.

## Integration of metabolism with regulation of gene expression

The model of Nishio *et al.* [5] contains a detailed description of the glucose phosphotransferase system (PTS) – the mechanism via which *E. coli* uptakes glucose – and its genetic regulation. We encoded their model in SBML format, now available from the BioModels database with accession number MODEL1501300000.

We found that the Nishio model shows a non-monontonic response of glucose flux to changes in glucose levels: as glucose concentration increases beyond approximately 0.01 mM, glucose uptake rate decreases. This non-physiological response appears to be due to an imbalance between the "accelerator" and "brake" modules. Since this balance is controlled by the "computer" module, we focused on this module to rebalance the two others. By increasing the affinity of CYA for IIAP – changing the parameter `kb` in reaction `binding_IIA_P_Cya` from 100 to $5000\,\mathrm{mM^{-2}}$ – this non-physiological behaviour is alleviated.

We rescale glucose flux, as above, to rate $1.23\,\mathrm{mM\,s^{-1}}$. This means that Nishio, and the metabolic module set out above, are compatible models: their overlapping elements – the PTS system – have equal fluxes, and the two models may be easily merged by removing the simple PTS reaction from the metabolic model, and appending the entire Nishio model.



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## Expansion of the regulatory module

The model is then expanded through inclusion of transcription and translation for all the metabolic enzymes. This is achieved by using the transcriptional and translational reactions of Nishio to guide us as to typical parameter values for these processes. The approach of building models using typical parameter values has been used previously to derive large-scale metabolic models [9, 10]; the justification is that model is largely driven by its structure, and hence approximate parameter values are often sufficient to produce correct overall behaviour. Typical parameter values are set out in Table 1.

We finally added transcriptional regulation, in addition to that already in present in Nishio. Included regulatory interactions are set out in Table 2.

## Evaluation of predictive capabilities

The model developed here simulates the steady-state metabolic operation of *E. coli* growing under abundant glucose. To test the predictive capabilities of this model, we simulate both the time-course response of *E. coli* to a sudden decrease of glucose level (from 2 mM to 10 $\mu$M, Figure 1), and the steady-state reached by the system under a large range of glucose levels (from 10 $\mu$M to 10 mM, Figure 2).

Firstly, the metabolic and regulatory modules show different dynamics in response to a sudden decrease of glucose concentration. While the metabolic module responds rapidly (in the second to minute range), the regulatory module shows a slower response (in the minute to hour range), as expected.

Secondly, both the steady-state glucose uptake and growth rates monotonically increase with the glucose level. This is qualitatively consistent with observations, although the predicted growth rate is higher than measured at low glucose levels. Expanding the model with additional metabolic pathways and/or regulatory interactions, as well as performing a new round of calibration, may improve these predictions. Regarding intracellular fluxes, the partition of carbon at the glycolysis-pentose phosphate pathway node is stable for all glucose levels, which is in excellent agreement with experimental data [11].

## Conclusion

We present a combined metabolic and genetic regulatory model of *E. coli*. Simulation results indicate that, while the model is developed and calibrated using experimental data collected under a unique metabolic steady-state, the predicted flux responses to perturbations are consistent with current knowledge. Its predictive capabilities will be improved by refining parameters and expanding it with additional metabolic and regulatory processes (e.g. post-transcriptional regulation).

This model may be useful in the field of systems biology, to investigate the specific roles of metabolic and hierarchical regulation in the long-term response of *E. coli* to environmental



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and genetic perturbations. It may also assist the design of more efficient and robust cell factories in biotechnology.

The model outlined here is available from the BioModels database with accession number MODEL1503050000.

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Table 1: Typical parameter values, derived from Nishio *et* al..

| parameter | value | units |
|---|---|---|
| [gene] | $2.43 \times 10^{-7}$ | mM |
| [mRNA] | $10^{-4}$ | mM |
| [protein] | $10^{-2}$ | mM |
| [regulator:metabolite] | $10^{-4}$ | mM |
| transcription | 1 | $s^{-1}$ |
| translation | 0.183 | $s^{-1}$ |
| mRNA degradation | $2.43 \times 10^{-3}$ | $s^{-1}$ |
| protein degradation | $1.83 \times 10^{-3}$ | $s^{-1}$ |
| effector:site binding | $10^{5}$ | $mM^{-1}$ |

Table 2: Transcriptional regulatory interactions included in the model. Those in the top half are taken from Nishio *et al.*.

| regulator | gene target | effect |
|---|---|---|
| Mlc | *ptsG* | – |
| | *ptsH* | – |
| | *ptsI* | – |
| Crp:cAMP | *crp* | + |
| | *cyaA* | – |
| | *mlc* | +/– |
| | *ptsG* | + |
| | *ptsH* | + |
| | *ptsI* | + |
| Crp:cAMP | *pdh* | – |
| Cra | *pfk* | – |
| | *ppc* | – |
| | *ptsH* | – |
| | *pyk* | – |
| | *tpi* | – |
| | *zwf* | – |
| PdhR:PYR | *pdh* | – |



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Figure 1: A sudden decrease of glucose levels (from 2 mM to 10 $\mu$M) is applied to the model at t = 100 s. The glucose uptake flux immediately decreases after the decrease of glucose concentration (A). This results in an increase of cAMP production (B) and of its concentration (C). In turn, the concentration of Crp:cAMP complex increases (D) and regulates the transcription of genes encoding metabolic enzymes (e.g. cyaA, E) and global regulators (e.g. crp, G). The dynamics of mRNA levels (e.g. cyaA, F, and crp, H) are slower.

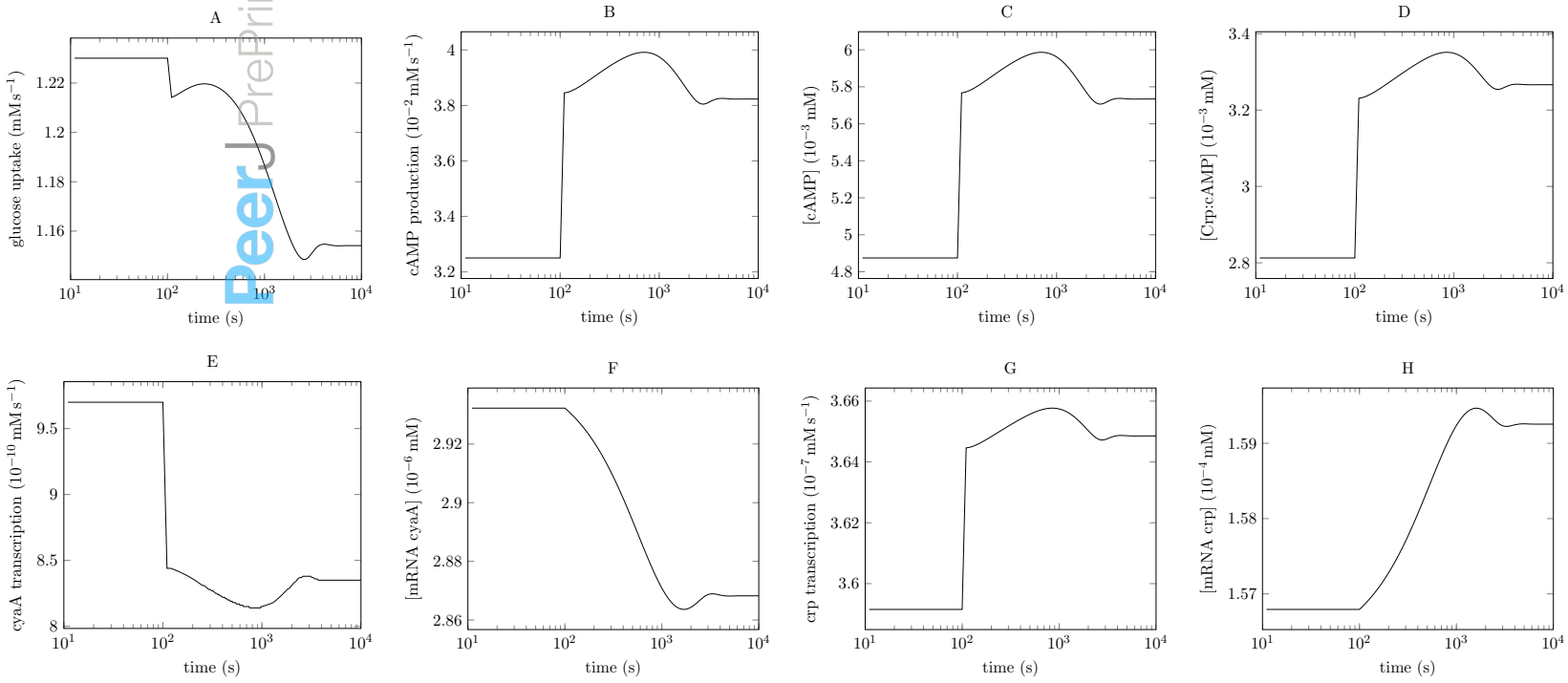






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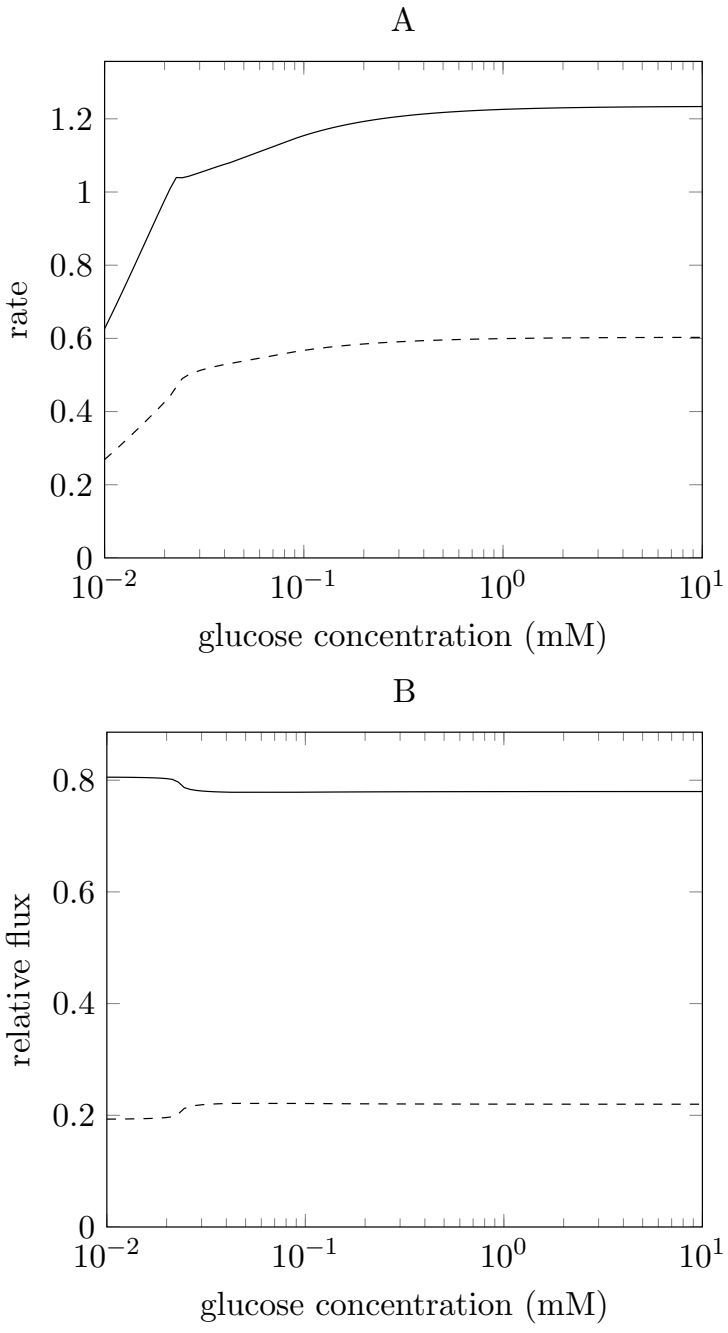
Figure 2: Steady-state response of the glucose uptake rate (solid, $\mathrm{mM\,s^{-1}}$) and growth rate (dashed, $\mathrm{s^{-1}}$) (A), and of the partition of carbon at the glycolytic (solid) – PPP (dashed) node (B) when glucose concentration is varied between 10 $\mu$M and 10mM.





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