Hierarchical control/sensitivity analysis and modularization

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Scalable Modeling and Analysis Techniques to Study Emergent Cell Behavior — understanding the *E. coli* stress response —
SCIENTIFIC BACKGROUND
The regulation of ammonium assimilation in *Escherichia coli* is tightly linked to the cellular carbon status\(^1\). In the past we have developed a detailed kinetic model of the metabolic subnetwork responsible for ammonium uptake\(^2\). We are currently integrating this model with carbon metabolism by linking it to kinetic models of the TCA cycle and glycolysis. The TCA cycle model we have developed in the past with a master student and is currently unpublished. The model of glycolysis that we use is the model developed by Chassagnole et al\(^3\) augmented with a detailed model of the PTS system for glucose uptake\(^4\). The resulting model is huge; it has 50 variable metabolites, 81 reactions, and 331 kinetic parameters (fixed metabolites, Vmax values, equilibrium constants, and affinity constants). This model is currently under in the phase of parameter adjustment given experimental data for Vmax values, fluxes, and some metabolite concentrations (mostly, metabolite concentration regimes). One approach to make such models manageable is to use a modular approach, either focusing on control and response theory\(^5-7\) or supply demand analysis (using the method described in Preez et al\(^8\)).

AIM
To develop a modular framework for the analysis of the regulation of ammonium assimilation in *Escherichia coli*.

RESULTS AND DISCUSSION
The carbon status affects the regulation of ammonium assimilation through its effect on the concentrations of glutamine and \(\alpha\)-ketoglutarate. Especially, the latter species is thought to be a carbon signal\(^1,2\) as it both a signal molecule in the ammonium assimilation network and an intermediate in the TCA cycle. Conversely, we can also consider this species as an ammonium status signal as it is known to be an important regulator of the TCA cycle and provided the ammonium assimilation network has considerable control on the concentration of \(\alpha\)-ketoglutarate. This is one of the questions we would address with the large model, which was introduced above. One way to do this would be to make a supply demand analysis around \(\alpha\)-ketoglutarate. This will not be reported in this deliverable as it is current work. What matters for this deliverable is our strategy to decompose metabolism into modules to allow for an
insightful analysis of the control properties of central carbon and nitrogen metabolism by making the complexity of the large model manageable.

In figure 1, a modular decomposition of the ammonium assimilation network is shown. In our current version of the ammonium assimilation network we can analyze the system in terms of these modules.

Figure 1. A modular decomposition of the ammonium assimilation network in *Escherichia coli* according to the modular decomposition criterion outlined in Bruggeman et al. Modules are defined as network segments that do not exchange mass flow but solely regulatory influences. Five modules are distinguished. Three modules form together a signaling supra-module. The two remaining modules are metabolism and gene expression.

In Figure 2, we show a calculation of the modular interactions in the metabolic ammonium assimilation model of Bruggeman et al. The regulation of the
adenylylation state of GS through glutamine is important at low concentrations of ammonium whereas the regulation of the PIKG₁ by glutamine is important throughout the entire range of ammonium concentrations.

Figure 2. Calculation of the modular interaction strengths of the ammonium assimilation model reported in Bruggeman et al² at two levels of α-ketoglutarate (0.2 mM (left) and 1.0 mM (right)). The importance of the interaction strengths depend on the external ammonium concentration.

The systemic response coefficients of the metabolic intermediates to a change in the ammonium concentration (N) is given by the following equation,

\[
\begin{bmatrix}
R^p_N \\
R^{PU}_N \\
R^{GS}_N \\
R^{A}_N \\
R^{NIP}_N \\
R^G_N \\
R^K_N
\end{bmatrix} =
\begin{bmatrix}
-1 & 0 & 0 & 0 & 0 & r^p_G & r^K_N \\
0 & -1 & 0 & 0 & 0 & r^{PU}_G & r^{PU}_K \\
r^G_P & r^{GS}_P & -1 & 0 & r^G_{NIP} & r^{GA}_G & 0 \\
r^A_P & r^{PU}_A & 0 & -1 & r^{GA}_P & r^{GA}_{NIP} & 0 \\
r^{NIP}_P & 0 & 0 & -1 & 0 & 0 & 0 \\
0 & 0 & r^G_{GS} & r^G_{GA} & 0 & -1 & 0 \\
0 & 0 & r^K_{GS} & r^K_{GA} & 0 & 0 & -1
\end{bmatrix}^{-1} \begin{bmatrix}
0 \\
0 \\
0 \\
0 \\
0 \\
0 \\
r^{G}_N \\
r^{K}_N
\end{bmatrix}
\]

The strengths of the modular interactions are denoted by the ‘r’’s in the previous equation and their dependence on the external ammonium concentration can be found in Figure 2.
Figure 3. The strength of signaling pathways in the ammonium regulation network at two concentrations for α-ketoglutarate. Strength of the three signaling pathways starting at glutamine and terminating at the adenylylation state of GS via PIIKG$_1$, via GLN, and via PIUMP$_3$KG$_3$ quantified as the products of local response coefficients along the pathways, i.e. for effect of GLN on n$_{AMP}$ via PIIKG1 the strength is calculated as $r_{n_{AMP}}^{PIIKG_1} r_{GLN}^{1}$. (left) at 0.2 mM α-ketoglutarate. (right) at 1.0 mM α-ketoglutarate.

The response of the ammonium uptake rate by GS can be expressed in terms of the systemic response coefficients and the modular control coefficients (the ‘c’$'$s, which are defined on the level of a module, all extramodular intermediate concentrations are held fixed),

$$ R^J_{G_S} = \frac{c_{S_{G_S}}^{J_{G_S}} c_{S_{G_S}}^{V_{G_S}}}{s_{S_{G_S}}^{J_{G_S}}} R^A_{G_S} + \frac{c_{S_{G_S}}^{J_{G_S}} c_{S_{G_S}}^{V_{G_S}}}{s_{S_{G_S}}^{J_{G_S}}} R^G_{G_S} + \frac{c_{S_{G_S}}^{J_{G_S}} c_{S_{G_S}}^{V_{G_S}}}{s_{S_{G_S}}^{J_{G_S}}} R^G_{G_S} $$

By analyzing the model in this fashion we can take a quantitative bird’s eye of the regulation of the network without having to focus on all kinetic detail. This modular analysis also has a possibility for variable scope.$^5$

As soon as the large model is finished, we will apply these modular methods to address the regulatory interplay between carbon and nitrogen assimilation.
REFERENCES


