Robustness by design? Structural analysis of dynamic metabolic models

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SUMMARY:

Dynamic metabolic models are describing the cellular responses of microorganisms under changing environmental conditions. Despite the enormous extracellular perturbations, robustness is observed, as important quantities, like the cell energy charge, do not react to the dynamics and remain practically constant. We propose a combined experimental and theoretic approach to rigorously investigate the structural sources of this robustness, as well as the trade-off between metabolic robustness and efficiency, and experimentally validate our mathematical findings. Identifying structures and mechanisms that guarantee robustness by design will streamline the synthesis of robust and efficient cell factories (GMO's) for the production of metabolites.

Introduction & scientific questions

Microbial life is constantly exposed to **dynamic environmental conditions** – temperature, nutrient supply and pH **vary in time**. Microbial cells appear to be **robust towards many perturbations**, like substrate pulses or changes in oxygen supply. How this robustness is generated from kinetic and stoichiometric properties, and which trade-offs between **efficiency and robustness** occur, remains to be elucidated. Mathematical tools are being developed to assess whether a dynamical system exhibits a **peculiar behaviour regardless of specific parameter values,** i.e.: robustness is guaranteed **by design**, in view of the **system inherent structure**.

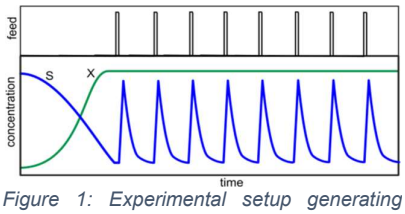

Figure 1: Experimental setup generating rapid feast/famine conditions. The blockwise feeding (20s) leads to gradients in substrate concentration (S). X is the biomass

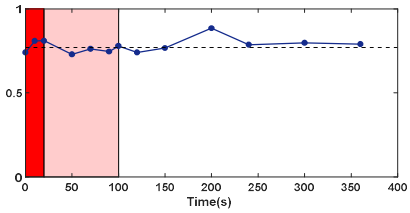

Figure 2: Energy charge (EC) during a feast/famine cycle, which is very stable although the extracellular environment (Fig. 1) changes more than 50-fold. The red region is the feeding phase (20s) and the pink is the feast phase.

Dynamic experiments with repetitive perturbations show that **biomass and product yields are reduced** compared to less dynamic conditions. Yet, even in a bioreactor subject to periodic feeding, resulting in feast/famine cycles (Figure 1), some fundamental values, such as the **energy charge** of the cells, remain surprisingly **constant** even though drastic changes occur in their environment (Figure 2). In our joint project the aim is to compare different organisms with different metabolic kinetics, using a model-based approach, that will help us address the following questions:

- Can **models** reproduce and quantify the **robustness towards perturbations** that is suggested by experimental observations?
- Which **parameters and structures** are essential for a strong robustness?
- Which mechanisms ensure a **constant energy charge** under highly varying flux conditions?

Approach – Duo student work-package

(1) Develop **reduced mathematical models** from published kinetic models (LST)
(2) **Analyse** the underlying interaction structures and **assess robustness** to parameter variations with mathematical methods tailored for the **structural analysis of dynamical systems** (DCSC)
(3) Identify putative mechanisms that can lead to **stable energy charge ratios** (current models are unable to reproduce this feature)
(4) Identify environmental conditions (e.g. cycle length/perturbation intensity) that increase **yield loss**
(5) **Validate** model predictions **experimentally**
(6) Identify mechanisms and **structures** that could increase/ensure **robustness**
(7) Exploiting the insight achieved through the structural analysis, design a **robust-by-design Genetically Modified Organism** to form a product (example: 1,4-butanediol)

Our innovative approach will provide new insight into dynamic phenomena in large-scale bioreactors by bringing together the strong experimental expertise of the Wahl group and the novel mathematical tools for structural analysis recently developed by the Giordano group.

Collaboration benefits

The Giordano group and the Wahl group have complementary skills that are essential to perform this project – unique theoretical approaches for the analysis of dynamic systems and experimental expertise for calibration and validation of models. Modelling techniques and experimental methods for model validation (LST) will be profitably combined with mathematical methods for the robustness analysis of dynamical systems, time-scale separation and structural properties (DCSC).

The project is ambitious from both a theoretical and an experimental point of view, therefore we propose to rely on the collaboration between two students from Life Science and Technology and Systems and Control. This generates a unique opportunity to collaborate with complementary skills, broaden their education portfolio and pursue an exciting multidisciplinary goal.

The BioDate funding will enable us to disseminate the results of this joint project at conferences and perform the respective validation experiments. We believe that this project will have a significant impact on the systems biology community, generating new knowledge on the dynamics and the structural features of cellular networks, and new approaches for data integration. It will also give us a chance to start a fruitful long-term collaboration between the two groups, focused on projects that tackle challenging biological problems with a combined theoretical and experimental approach.