IMAGINE BioSecurity: Metabolic Modeling-Enabled Biocontainment Redesign in Microbial Chasses

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Project Goals: Genetically modified organisms (GMOs) are widely used in agriculture and bioenergy industries, raising biosafety concerns about unintentional cellular proliferation or accidental environmental spread of synthetic genes. Supported by DOE BER Scientific Focus Area (SFA) program, we aim to design predictable and generalizable biocontainment strategies to prevent the potential hazards caused by GMO microbes.

Abstract Text: Our research goals in this SFA subtask are twofold. *First*, we focus on the development of new tools that can characterize metabolic responsiveness of microbial hosts to genetic safeguards as well as biocontainment constraints in controlled and simulated ecosystems. In this respect, we have successfully developed machine learning (ML)-^{13}C-fluxomics in which ML algorithm "learns" intrinsic relationship between ^{13}C-labeled metabolites and metabolic flux. Thus, the tool can read out the fluxomic phenotypes "directly and immediately" from isotopically metabolomic dataset [1]. One key merit of this tool is that the training dataset for ML can be generated by algorithm, such that it does not have to rely on experimental data, which is not always sufficiently available nor covering all realistic scenarios. The solvability of metabolic flux depends on the linear dependence of the adjoint labeling patterns of precursor metabolites, which is determined by the topology of the network and the labeling strategy of substrates. In our tool, screening for solvability is automatically performed. It eliminates the invalid variables and therefore increases the accuracy of prediction. The presented ML approach greatly reduced computation time for metabolic flux estimation down to <1 s in an exemplary *E. coli* model. Employing it for large scale flux analysis, high-throughput metabolic phenotyping and strain screening are enabled. Development of this efficient fluxomics approach for biocontainment hosts will promote iterative Design-Build-Test-Learn cycle and help biosystems redesign for safe production of next-generation biofuels.

Second, we are developing novel biosecurity strategies specifically in industrial clostridia. One strategy is based on metabolic robustness control, a generalizable design principle from inherent properties of metabolism [2]. This approach offers a unique capability to predict and govern the fitness of a dynamic biological system, especially when the system exhibits differential behavior in laboratory and natural conditions. In a modeled biocontainment circuit (Figure 1), for example, the GMO microbe will exhibit steady-state metabolism when a laboratory effector stabilizes the function of a key enzyme. In an uncontrolled environment, where the effector is absent, the targeted enzyme will be down regulated, thus depleting the pool of the vital metabolite(s) and resulting in instability of the GMO during escape. Metabolic robustness can thus be analyzed in the context of biocontainment modules by ensemble modeling, where a set of models with different kinetic data are parameterized and perturbed by varying maximum rate

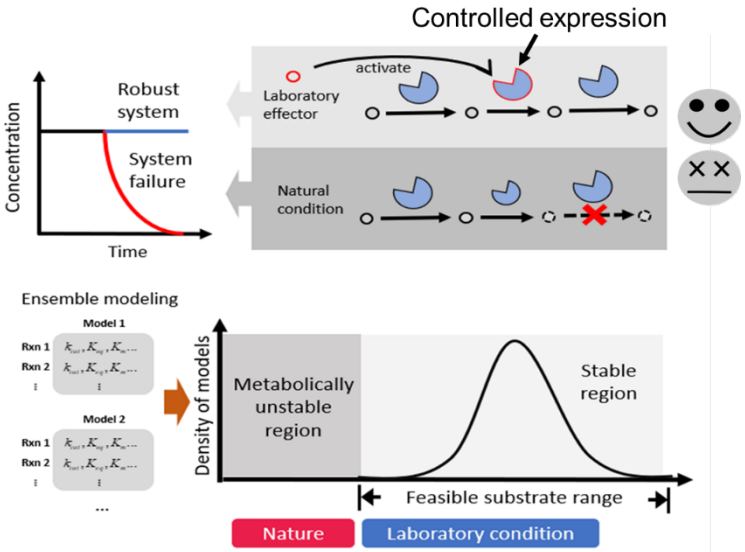

Figure 1 Predictive biosecure design based on ensemble modeling and robustness control [2].

(V_{max}), which is largely proportional to the control level of the enzyme. This approach counts the probability of system failure per perturbation. Such approaches can ultimately pinpoint new metabolic targets for optimal biosecure design, as well as assess viability of a modified laboratory organism in response to environmental changes. Currently, we have developed Ensemble Modeling for Robustness Analysis (EMRA) and are identifying in *Clostridium ljungdahlii* the most likely gene targets, modulation of which may predictably affect cell growth. Experimentally, we are developing a state-of-the-art multiplexed genome editing approach as well as inducible switch-offs for robustness control of clostridia. The aim is to enable simultaneous targeting of multi-sites for growth arrest, and to minimize escape efficacy.

References/Publications

1. Chao Wu, Jianping Yu, Michael Guarneri, Wei Xiong. A Computational Framework for Machine Learning-Enabled ^{13}C-Fluxomics. ***ACS Synthetic Biology.*** 2021 10.1021/acssynbio.1c00189 (Cover Art)
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