**Synthetic Biology Project: Building transcriptional controllers in *S. cerevisiae* (Master thesis, 6 months)**

**Background and Goal**

Our lab developed transcriptional control systems (Well Tempered Controllers) in yeast that allow us to control protein levels precisely within the yeast cell[1], [2]. To build WTC systems, we design new eukaryotic promoters that can be repressed by bacterial repressors (like TetR) and induce expression using small molecules (like anhydrotetracycline). These have wide ranging applications from investigating gene-gene interactions to building complex synthetic circuits within yeast.

Currently we are building one such complex circuit in yeast that will be able to evolve functional antibodies towards G-protein coupled receptors, which are important drug targets. Although we have already developed three orthogonal WTC systems that allow orthogonal control over three separate proteins within the cell, this complex circuit requires even more orthogonal systems to achieve control over different components. The aim of this project will be to design, test, and validate more orthogonal inducer-bacterial repressor pairs that can be used to build WTC systems.

**Tasks**

1. Understand the development process of the existing WTCs and review the literature to identify bacterial repressors that are likely to work in this context.
2. *Implementation in the lab*.
	1. Clone all necessary constructs and integrate them into yeast for testing.
	2. Design experiments based on fluorescence and flow cytometry to determine which repressor-inducer pairs are worth pursuing.
	3. Perform dose response experiments in high through-put format to better characterize the systems.
3. Depending on preliminary data, optimize the design of promoters and repressors to achieve high expression levels and tight repression.
4. Once new systems are built and characterized using fluorescence, you will be able to assess their performance further. You could place interesting endogenous yeast genes under their control, for example the uncharacterized protein Pbr1. While its function is unknown, it is thought to be a part of cell wall production pathway. Simultaneous control of Pbr1 and other cell wall production proteins (e.g. Kre5/9) can reveal more about its role through analysis of gene-gene interactions. Another option is to implement the new WTC systems directly within the context of the complex evolution system we are developing. The choice will be yours depending on what interests you more.
5. *Reporting.* You will be required to summarize your results in a written report and an oral presentation.

**Skills you will acquire**

You’ll learn how to review the literature and quickly extract the information you require for a given application, and how to draw on different resources to be able to build successful synthetic circuits. You’ll learn how to design efficient experiments, how to troubleshoot these and how to critically analyze your own data.

On the technical side, you’ll be proficient with yeast handling, yeast transformations, high-through put flow cytometry, various molecular cloning methods and Sanger sequencing by the end of the project. Depending on interest, you can also gain experience in western blotting. You will also learn how to analyze and present data using R.

**Requirements**

Motivation and a willingness to learn are more important than your background. If you don’t fit the formal requirements below, but you find the project interesting, you are still encouraged to apply.

1. Previous experience in a wet lab through lab courses or short projects. Ideally already familiar with basic methods such as PCR, gel electrophoresis, cell plating etc.
2. Basic knowledge in (synthetic) biology.
3. Any coding experience is a plus but not necessary.

**Supervision**

If interested, send an email to Dr. Asli Azizoglu (asliazizo@hotmail.com ) and Prof. Dr. Jörg Stelling (joerg.stelling@bsse.ethz.ch ).

**References**

[1] A. Azizoğlu, R. Brent, and F. Rudolf, “A precisely adjustable, variation-suppressed eukaryotic transcriptional controller to enable genetic discovery,” *Elife*, vol. 10, 2021, doi: 10.7554/ELIFE.69549.

[2] Brent, Roger., Azizoğlu, Asli., Loureiro, Christina., Venetz, Jonathan. “Autorepression‐Based Conditional Gene Expression System in Yeast for Variation‐Suppressed Control of Protein Dosage,” *Curr Protoc*, vol. 3, no. 1, 2023, doi: https://doi.org/10.1002/cpz1.647.