Our global community deserves access to healthcare and nutrition currently available to only the most fortunate among us. Thanks to synthetic biology applications exploiting the yeast chassis, valuable compounds such as anti-malarial drugs and specialty chemicals can now be produced inexpensively. Using Golden Gate assembly designed for BioBrick compatibility, we have developed two tools to improve yeast expression of non-native pathways. First, we engineered an ethanol control system that reduces yeast’s endogenous stress response and diverts more cellular resources towards product synthesis. Second, we constructed a light-induced system for instantaneous gain- and loss-of-function at the protein level. These tools will allow engineers to optimize heterologous pathways by monitoring toxic intermediates or regulating flux in a controllable, time-dependent manner. We hope our ideas will shape the future of industrial cell-based manufacturing.

Ethanol Control

- Yeast is a cost-effective chassis for producing high value compounds.
- Yields can be maximized by reducing ethanol stress during fermentation.
- We propose an ethanol control system in yeast (figure 1a,b).
- The design depends on the human cytochrome p450 CYP2E1 gene, whose encoded protein converts ethanol to acetaldehyde (figure 1b).
- CYP2E1 will break down ethanol produced during fermentation, thereby reducing ethanol stress.
- The system is controlled by ethanol induced promoter so CYP2E1 is only expressed when ethanol is in the system.

Effective yeast industrial applications depend on accurate control and understanding of the relevant biomolecular pathways.

Current standard of pathway control depends on use of chemical agents, which is slow and imprecise.

Optogenetic Control Protein

- Optogenetic control is fast, spatially precise, and additive neutral, allowing individual steps of pathways to be modulated instantaneously.
- We propose the use of TULIP system, which provides tunable control at the protein level.

- TULIPs consists of two domains: LOVpep, which looses and exposes a binding epitope upon exposure to 470nm (blue) light; and ePDZb, which binds to the exposed epitope (figure 2).

- We designed two strategies to implement TULIPs:
  - Strategy 1: Splitting a functional protein into two domains. Each is attached to either LOVpep or ePDZb. Upon binding, the split sections form a whole functional protein (figure 6 left).
  - Strategy 2: Tethering LOVpep to a subcellularly localized protein and ePDZb to a protein of interest. Upon excitation the ePDZb domain with protein attaches to the localized protein through LOVpep (figure 6 right).

Optogenetic Protein Control

Application to Cell Cycle Arrest

- We propose applying TULIPs to cell cycle arrest as a proof of concept of both strategies.
- We applied strategy 1 to proteins known to cause cell cycle arrest. Activation causes cell cycle arrest.
- Strategy 2 was applied to proteins essential to cell cycle progression.
- We made nine constructs for strategy 1 and seven constructs for strategy 2.
- Expression of both fusion proteins and correct sub-cellular localization of tethers were confirmed (figure 7).

Future Research

- We plan to test the optogenetic system with exposure to blue light, using a custom blue LED construct (figure 11).
- We plan to compare election reduction by CYP2E1 to that achieved by endogenous yeast pathways (e.g. ADH1 overexpression).