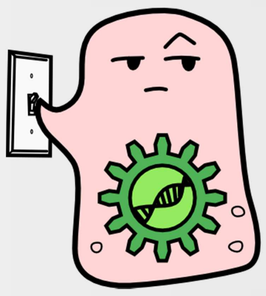
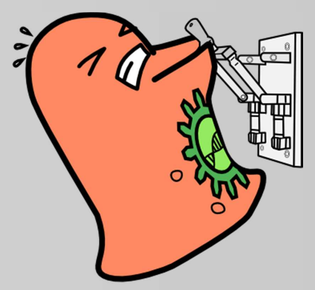


Switch: Building a Mammalian Light-Operated Genetic Controller



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Abstract: In the pharmaceutical industry, various therapeutic proteins are produced in mammalian cells because of their post-translational modification abilities. These proteins can sometimes be toxic to the producing cells, killing or damaging them during the synthesis. To obtain a reasonable efficiency, a conditional expression and close control of the product is necessary. Currently, this is done by using promoters responding to chemical products. This technique involves complex bioreactors designed to keep a uniform activator concentration across the tank. There has been some work on light-based conditional expression (using diverse photosensitive domains), but this has mostly been limited to bacteria. Fussenegger et al. have come up with a system using melanopsin and the calcium-NFAT pathway. We propose a system based on the LovTAP construct (from the 2009 EPFL iGEM team) adapted to mammalian hosts which should achieve higher efficiencies.

What is iGEM ?

International Genetically Engineered Machine (iGEM) is a synthetic biology competition.

Teams from many different universities worldwide develop projects in genetic engineering, using and developping new standardized biological parts.

These standardized parts are called Biobricks and are part of iGEM's registry, open for every team to use and add new constructs.

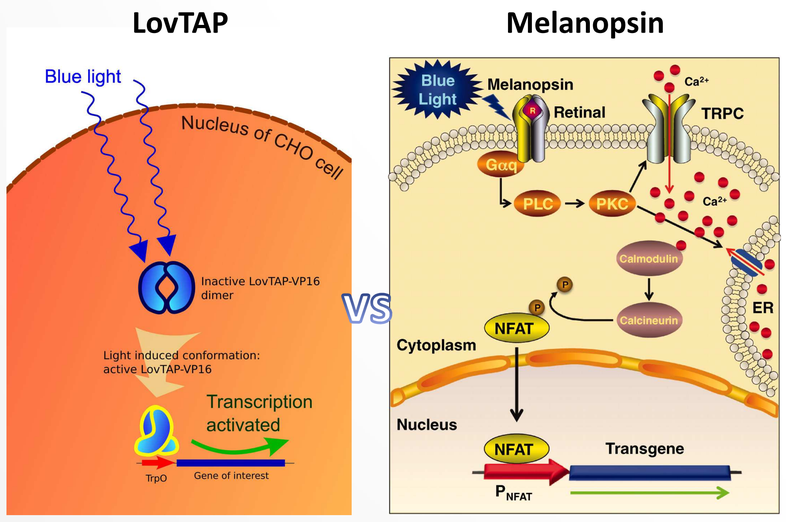
The teams' projects are judged by a jury at Regional (for Europe, Amsterdam) and Worldwide (Boston) finals.

Our Project: Switch

The aim of the "Switch" project is to express two different types of genetic switches able to respond to light. The main goal is to control protein expression in mammalian cells by simply turning light on and off.

LovTAP-VP16 is the main switch we are currently developing. We shall compare it to a more complex but already experimentally tested switch: the melanopsin cascade, described by Martin Fussenegger. We aim to quantify their respective expressions with appropriate readouts.

The Two Switches



The LovTAP protein is a combination of the photosensitive Lov domain, which was first discovered in a plant, and a TAP DNA-binding element. This protein has been engineered by Strickland et al. and used by the EPFL 2009 iGEM team. This year, we are working with LovTAP-VP16, a modified version, suitable for expression in mammalian cells.

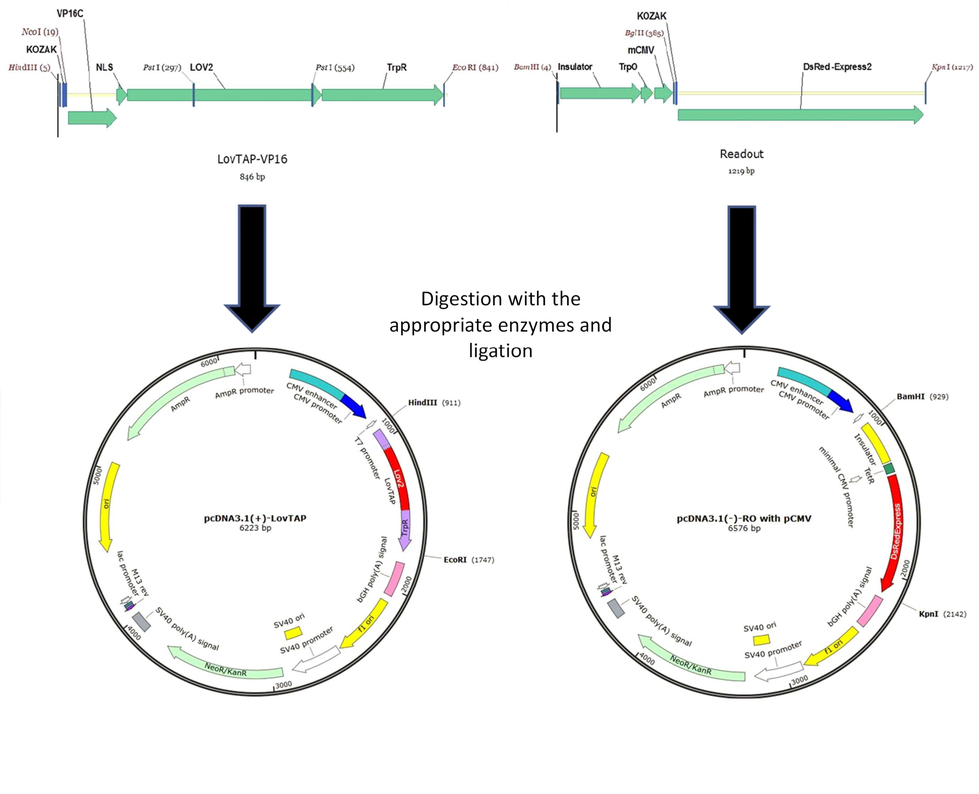
The light-induced expression using melanopsin described by Fussenegger et al. is a complex pathway involving calcium channels and several signaling molecules.

LovTAP-VP16 activates the transcription directly in response to blue light and does not rely on endogenous signaling pathways.

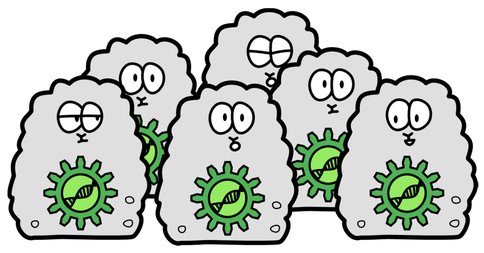
The Cloning

The VP16C-LovTAP construct, as well as its readout including TrpO and an insulator to block any CMV promoter, were made by direct synthesis.

These constructs should be cloned into a mammalian vector such as pcDNA3.1(+/-) or pMP, providing a mammalian promoter and a polyA signal for a successful expression of the protein.



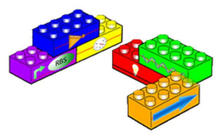
In parallel, the readout genes to re-enact Martin Fussenegger's melanopsin cascade experiment should be cloned downstream of an NFAT-inducible promoter, which can be found on the vector pGL4.30.



Biobricks are genetic constructs that contain a gene of interest surrounded by several specific restriction sites recommended by iGEM. They are listed in the Parts Registry (*partsregistry.org*).

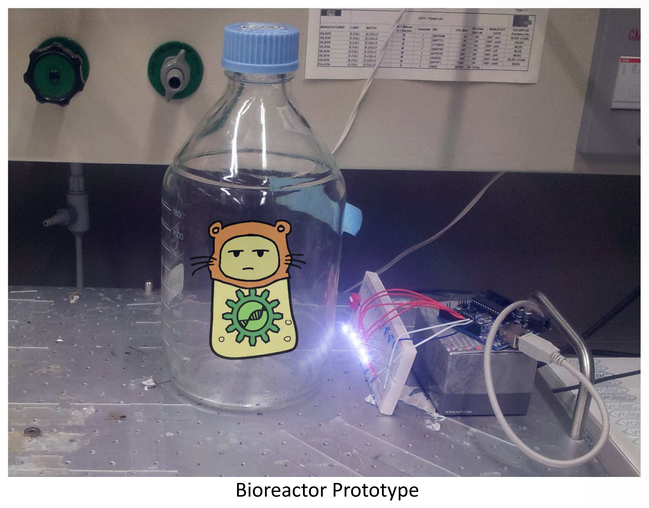
Biobricks we wish to submit:

1. Complete LovTAP-mammalian construct
2. Complete readout construct
3. Melanopsin gene
4. NFAT response element
5. Two readout genes: TNFR and SEAP



Bioreactor Design

We also look into the design of a bioreactor with the appropriate conditions for the culture of mammalian cells containing LovTAP or melanopsin. To do so, we need to develop a circuitry to illuminate the reactor (or the culture tubes) with blue LEDs of the appropriate wavelength (465 nm), and a microcontroller to be able to control the activity of the LEDs.



Bioreactor Prototype

The aim of such a setup is to regulate the readout levels by changing the LovTAP expression level with different illuminations, and to find the ideal setup for a maximized readout expression.

It can also be used to compare the efficiency of the two switches.

Future Applications

Producing complex therapeutic proteins requires biosynthesis in mammalian cells to obtain the desired product. Such products sometimes have some levels of toxicity for the cells, limiting the production possibilities. Therefore, our project could have a direct application in the pharmaceutical industry, providing an easy way to control the productivity of cells in a bioreactor, and allowing a synchronized synthesis of toxic compounds that maximizes the output without killing the cells.

If our construct is fully functional, it would only require a stable transfection in mammalian cells to be ready to use.

Mammalian Expression

After the cloning steps (bacterial), we are going to use Chinese Hamster Ovary (CHO) cells for mammalian expression. In the LovTAP-VP16 experiment, they will be co-transfected with LovTAP and its readout, dsRed. We could thus detect LovTAP activity by measuring red fluorescence. We could also perform a Western Blot or a qPCR.

For the melanopsin switch, three readout vectors are available. Their levels can be measured: fluorescence for GFP and ELISA for protein accumulation of SEAP and TNFR.

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