A LIGHT-RESPONSIVE GENETIC SWITCH

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Introduction

Recent discoveries of photoreceptors in many organisms got us excited about the possibility of using light-responsive genetic tools in synthetic biology[1]. Indeed, such tools could in principle induce phenotypic changes in a more localized, more precise and faster fashion than currently available chemical-methods. To evaluate the biotechnological potential of such tools, we specifically aimed to induce a change in gene expression, more specifically to directly turn a gene on or off, in a living organism, in response to a light stimulus.

For this purpose, we used a light-sensitive chimeric DNA binding protein "LovTAP"[2] to convert a light input into a chosen output, here fluorescence generated by the GFP reporter gene.

Background

Our experiments are based on the LovTAP system:

The light-sensitive input module is the LOV domain - the absorption of a photon through a flavin cofactor triggers a conformational change leading to the displacement and unfolding of a helix, mediating the activation of an attached output module, TrpR. The latter can in turn bind its operator DNA as a homodimer.

The LOV domain is shown in light blue, the TrpR domain in orange, and the operator DNA in grey. The shared helix is shown in dark blue when contacting the LOV domain and in red when contacting the TrpR domain.[1]

LovTAP can thus be found in two states: the dark-state and the light-state. We refer to the ground state as the dark state, and the photosensitive state as the light state.

BioBricks

First we created a LovTAP biobrick under control of an inducible promoter.

Second we created two Read-Out systems to assess the functionality of our protein with GFP as an output signal:

Read-Out 1: The construct behaves as expected. The expression of GFP compared to the negative control.

Read-Out 2: The system is light-responsive. (A) In the presence of light there is a clear increase in fluorescence as compared to the negative control. (B) The system is rapidly induced after 5 min of light exposure in 10 min analysis. Standard deviations were calculated by four internal repeats. Each experiment was repeated at least twice.

Human Practices

The goal was to inform a broad audience about iGEM and synthetic biology. This was divided into 3 parts: a large scale survey to gather opinions, talks to high schools and companies, and articles in newspapers.

What is your impression when you get a challenge? (Do you know what synthetic biology is?)

Wild type I427F L453G IN: 29,79% 57,2% 31,16% OUT: 70,21% 42,8% 68,84% 22,02%

Molecular Modeling

The aim of this part is to discover mutations that favor the formation of the light-activated state through molecular dynamic simulations. We validated a set of parameters for both dark and light state simulation.

- After light excitation, a covalent bond between the cytochrome 450’s sulfur atom and a flavin mononucleotide (FMN) carbon atom is formed.
- The cysteine 450 side chain shows two different conformations: in the IN conformation the side chain points towards the FMN with a mean distance of 3 Å and in the OUT state points away from the FMN with a distance of 5.1 Å. Our simulations reproduce data obtained from x-ray crystallography for the LOV domain.[2]

We then mutated two neighboring residues to promote the “IN” conformation by altering steric properties near active site.

Isoleucine 427

We mutated ILE 427 to a phenylalanine (I427F) to push the cysteine side chain toward the cofactor.

Results:

- Phenylalanine pushes C450 towards the IN conformation
- A higher “IN” % results in a higher chance of forming a covalent bond upon light absorbance of FMN.
- A general stabilization of the light activated state of the protein

Lysine 453

The Leu 453 was replaced by a glycine (L453G) to make space for the cytochrome.

Results:

- The I427F, L453G double mutation shows a further increase in the IN state percentage as compared to the I427F single mutation

Applications

Bioreactors: use the reversibility & non-invasiveness of the system to have a tight control over protein production. Local and spatial control: using optic fibers to control activation of proteins in vivo.

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Reference:

Summary

- Created LovTAP into a BioBrick
- Characterized two read-out systems
- Showed functionality of LovTAP in vivo

Molecular Modeling

- Generated Molecular Dynamic Simulations of the LovTAP protein
- Partially understand what limits the stability of the light state
- Found possible mutations to improve switching rates

References: