A light-responsive genetic switch
Swiss Federal Institute of Technology, Lausanne
Background
Why use light to control genetic networks?

• Photo-sensitive domains exist natively in many organisms

• Non-invasive control

• Easy to use & accessible

→ Sosnick et al. engineered a light-sensitive fusion protein: LovTAP

The LovTAP protein

LovTAP protein: fusion of TrpR and LOV domain

- Light-activated
- Reversible
- Chimeric protein

IGEM Team EPF-Lausanne 2009
LovTAP protein: illumination $\rightarrow$ conformational change
$\rightarrow$ Binding to DNA
Our goals

GOALS

• Make a BioBrick out of the LovTAP

• Show that it works in vivo

• Use it for induction of complex genetic circuit

• Optimize its effect via molecular modeling tools
Read-out system n°1

TrpR

LOV

Trp promoter

RFP

Fluorescence

Time

Strategies

Background	Results	Molecular Modeling	Future Directions	Human Practices

IGEM Team EPF-Lausanne 2009
Read-out system n°2

Strategies

Background Results Molecular Modeling Future Directions Human Practices

TrpR
LOV
Trp promoter
TetR
TetR promoter
RFP

Fluorescence
Time

IGEM Team EPF-Lausanne 2009
Results

- Height line
- Distance from left

Results
Experimental Setup

- Dark box
- Plate reader
- Incubator with illumination system
- LEDs
- qPCR machine
Read-Out 1:

→ Part works
Read-Out 2:

- **Trp**
- **TrpR**
- **ATC**

**Graph:**
- **Normalized fluorescence** vs. **Time [min]**
- 3 lines:
  - Red: + ATC
  - Blue: + Trp
  - Green: Negative control
- **Arrow:** Part works
Entire System :
Read-Out 2 + LovTAP
static measurement
Plate reader – 2h30
exposure
→ RO2 & LovTAP work

Fluorescence / O.D. 600
Entire system: plate reader, time-course measurement, delay for induction

→ Fast induction
Molecular Modeling
**Goal:** Favor the formation of light-activated state

**Dark state:** not bound to DNA

**Light-activated state:** bound to DNA

**Molecular Dynamics simulations:**
- *Long computer calculations* (~1 month/80 ns run)
- Simulation output:
  - *trajectory file:* defines atom’s movement and protein geometry over time

**Simulations were performed on LOV2 domain**

**LovTAP fusion protein:** LOV2 domain / TrpR / Chromophore / DNA
Active site major components:
- Cystein 450 (C450)
- Cofactor: flavin mononucleotide (FMN)

After light excitation:
- Covalent bond formation (cystein-FMN)

Conformation of cystein residue in the dark state:
- **IN**: C450 points toward the FMN
- **OUT**: C450 toward the opposite side of the FMN

Active site: LOV2 domain / Cystein 450 / FMN
Molecular Modeling: Cystein 450 analysis

Results

IN conformation = 29.8%  OUT conformation = 70.2%

These results confirm Halavaty et al. crystal occupancy for CYS450: IN = 30%, OUT = 70%.
REMINDER: Wild type results
- IN conformation = 29.8%
- OUT conformation = 70.2%

**I427F**
ILE 427 mutated into PHE.

<table>
<thead>
<tr>
<th>Results</th>
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<tbody>
<tr>
<td>IN conformation = 57.2%</td>
</tr>
<tr>
<td>OUT conformation = 42.8%</td>
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**L453G**
LEU 453 mutated into GLY.

<table>
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<tr>
<td>IN conformation = 31.2%</td>
</tr>
<tr>
<td>OUT conformation = 68.8%</td>
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</table>

**I427F & L453G**

<table>
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<tr>
<td>IN conformation = 78.0%</td>
</tr>
<tr>
<td>OUT conformation = 22.0%</td>
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Color code: ILE427 / CYS450 / LEU453 / FMN
Future Directions
Molecular Modeling

Study the molecular details of the LOV2 domain to understand the light induced conformational change of the J-alpha helix

Implement results of the modeling

We have generated LovTAP mutants: \textit{I427F, L453G, V416I}.

➔ To do

• See if predicted mutations from molecular modeling could improve the stability of the light state
• Can it be measured?
• Run time-course experiments
We have studied the LOV domain and understood its photochemical activity

→ Make new TrpR mutants to change specificity

→ Make new chimeric proteins:

   Fuse to other Transcription Factors

   Fuse to a broader range of protein domains with chosen activities
**Future Directions: Possible applications**

**Spatio-temporal control**: using focused light to control activation of proteins *in vivo* in a targeted cell

**Bioreactors**: use the reversibility & non-invasiveness of the system to have a tight control over protein production in bioreactors
Human practices

Human Practices
**Goals**: Share our experience with iGEM and the project with a broader community. Learn about people’s feelings towards genetic engineering

- **Talks**: High school students, Merck-Serono (EMD-Serono), Singapore General Hospital

- **Media**: Le TEMPS, La Tribune de Genève, 24 heures, Le Nouvelliste, Le Flash
We did a survey to evaluate people’s feeling toward genetic engineering and synthetic biology in relation with their background & level of education

- **469** responses
- 49% women, 51% men
- Age range: 14 – 30+

### Do you know what synthetic biology is?

- 69% No
- 31% Yes

### What is your impression when you hear “cloning”?

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<thead>
<tr>
<th>Neutrality</th>
<th>Scientific background</th>
<th>Non-scientific background</th>
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<tbody>
<tr>
<td>42%</td>
<td>24% Positive</td>
<td>12% Positive</td>
</tr>
<tr>
<td>34%</td>
<td>54% Negative</td>
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### Summary

<table>
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<th>Now</th>
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<tr>
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<td><strong>Molecular Modeling</strong></td>
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<td>✓ MD modeling of LovTAP protein</td>
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<td>✓ Found possible mutations to stabilize the light state</td>
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**April**

- ✓ 7 BioBricks submitted
- ✓ Characterization of two read-out systems
- ✓ Showed functionality of LovTAP *in vivo*

**Molecular Modeling**

- ✓ MD modeling of LovTAP protein
- ✓ Partly understood what governs the stability of the light state
- ✓ Found possible mutations to stabilize the light state

**Human Practices**

- ✓ Talked about our project to a broad range of people
- ✓ Survey with 469 responses
- ✓ Media relations
Prof. Bart Deplancke: Laboratory of Systems Biology and Genetics

Prof. Sebastian Maerkl: Laboratory of Biological Network Characterization

Prof. Matteo Dal Peraro: Laboratory for Biomolecular Modeling

Instructors: Nicolas Dénervaud, Carine Gubelmann, Enrico Spiga, Marco Stenta

Sponsors
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Wet lab: Mutagenesis

Exposure time [s]

Fluorescence / OD 600nm

WT

ILE427PHE

WT

ILE427PHE

WT