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- SYNBIOCOMM
- GeneArt
- Wissenschaftliche Gesellschaft, Freiburg
iGE-Machine

- Mechanical, technical aspect
- Given: protein-design lab working on split enzymes
  - Expression of complementing enzyme fragments
  - Enzyme becomes active if both parts are brought together
Split enzymes:

- Dihydrofolate reductase: essential for biosynthesis of thymine
- ß-lactamase: confers resistance against antibiotics
- Both available as used in cell-survival enzyme assays in our lab
The idea...

...attach split enzyme-halves to sensor-proteins, thus coupling receiving and execution in one molecule.
Signal input

Switch: on/off

Inactive state

Active state

Sensor-protein

Fragment 1

Fragment 2

??
...the sensor should:

- Be a single protein with a strong conformational change upon an external signal or
- A dimer - monomer system...
- ... featuring easy access and control
Possible „trigger“-proteins for the regulation of split enzymes:

- proteins that can change conformation upon light irradiation...
- proteins which can be regulated by chemical components:
  - Maltose binding proteins
  - **Calmodulin**: a calcium sensing protein performing strong conformation change upon binding calcium
...why calmodulin:

- Truong, Ikura et al. have already fused CFP and YFP to the ends of a modified calmodulin ... (CFP and YFP have almost the same size as our enzyme fragments)
- ...and were so friendly to send it to us!
Principle for a calcium mediated enzyme activation

Enzyme fragment 1

Calmodulin

Enzyme fragment 2

Active state

Conformational switch

Inactive state

\( \text{Ca}^{2+} \)
anyway, we couldn’t have our parts synthesized as

Enz 1  Sensor  Enz 2
3D-Model of „blac1-calmo-blac2“:
3D-Model of “DHFR1-calmo-DHFR2A”:
...we could induce the expression of this construct:

<table>
<thead>
<tr>
<th>IPTG-Induced Expression of DHFR1-Calmodulin-DHFR2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHFR1-4GlyCalmo4Gly-DHFR2A</td>
</tr>
<tr>
<td>DHFR1-6GlyCalmo6Gly-DHFR2A</td>
</tr>
</tbody>
</table>
In-vivo-testing:

- TMP
- DHFR construct

- Ca\(^{2+}\), TMP

- Ca\(^{2+}\), TMP
- DHFR construct
Dependence of growth on calcium:
Molecular light switch for executing devices
**Phytochrome A (PhyA):**

- Red light photoreceptor in plants → affects growth
- Changes between two stable conformations
  \[ \text{Pr} \xleftrightarrow{650 - 670 \text{ nm}} \xrightarrow{705 - 740 \text{ nm}} \text{Pfr} \]
- Activated PhyA acts as a transcription factor
- Uses a chromophore (Phytochromobilin) as photoconverter, we used PCB instead works as well

_Arabidopsis thaliana_
**PhyA - Chromophore**

Photoconversion
Far red elongated hypocotyl 1 (Fhy1):

- Binds P_{fr} form and is responsible for nuclear accumulation of PhyA
- Binds PhyA reversible (far red light causes dissociation)
- Only the use of the binding domain is necessary, thus, we used a truncated version reduced to the binding domain

Arabidopsis thaliana
Arabidopsis thaliana  =  ????

**PCB biosynthesis:**
- heme
  - ↓ HO1
  - biliverdin
  - ↓ PcyA
  - phycocyanobilin

**Codon usage:**
- Codons were optimized for *E. coli*

**Protein could be toxic in *E. coli***

PCB Plasmid obtained from J.C. Lagarias (PNAS, 2001, 98, 10566)
**Principle of PhyA – Fhy1 light switch**

- **PhyA**
  - Device 1: e.g. CFP, DHFR Fragment 1, ß-Lactamase Fragment 1
  - Device 1: e.g. YFP, DHFR Fragment 2, ß-Lactamase Fragment 2

- **Fhy1**

**Light Switch Mechanism:**

- **Pr** state: active state
- **Pfr** state: inactive state

**red light**

**far red light**

**active state**

**light switch**

**inactive state**
Current state:

- 3 Plasmids were cloned/transformed
- Fhy1-YFP fusion, AmpR
- PhyA-CFP fusion, CmR
- PCB enzymes, KanR
Suggestion to extend
Limitations of standard iGEM cloning

- Building fusion proteins made up of 2 or more different peptides is not possible with the iGEM biobrick system because of a stop codon after each protein:

- For building functional fusion proteins, stop codons between the parts must be avoided:

```plaintext
Part 1  STOP  Part 2  STOP  Part 3  STOP
Part 1  nnnnnn  Part 2  nnnnnn  Part 3  STOP
```
Combining 2 Biobrick-Parts via Spe I and Xba I results in a stop codon.
BioBrick ´Version 3.0´:

Combining 2 Biobrick 3.0 parts via the new inserted AgeI/NgoMIV cutting sites

<table>
<thead>
<tr>
<th>Prefix</th>
<th>Part</th>
<th>Suffix</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoRI</td>
<td>NotI</td>
<td>Xbal</td>
</tr>
<tr>
<td>NgoMIV</td>
<td>AgeI</td>
<td>SpeI</td>
</tr>
<tr>
<td>NotI</td>
<td>NgoMIV</td>
<td>Pstl</td>
</tr>
</tbody>
</table>

**AgeI**

ACCGGT
TGGCCCA

**NgoMIV**

GCCG GCC
CGGCC CGG

Thr

Gly
Improvements of BioBrick:

- Fusing BioBrick 3.0 parts does not result in a stop codon in the scar.
- Encoded amino acids (Threonine and Glycine) act as linker.
- Creating fusion proteins with different parts is possible by using BioBrick 3.0.
- It is completely compatible with common iGEM BioBricks.
Submitted iGEM Parts Freiburg: A Protein-Fusion-Kit

- Fluorescence markers: YFP (Venus), CFP (Cerulean)
- Split enzyme 1: DHFR 1, DHFR 2
- Split enzyme 2: β-Lactamase 1, β-Lactamase 2
- Purification: Strep-Tag, His-Tag
- Light sensor: Fhy1 (small), Fhy1 (big), PhyA
- Ca2+ Sensor: Calmodulin

→ all parts feature the BioBrick extension for fusion proteins
The End