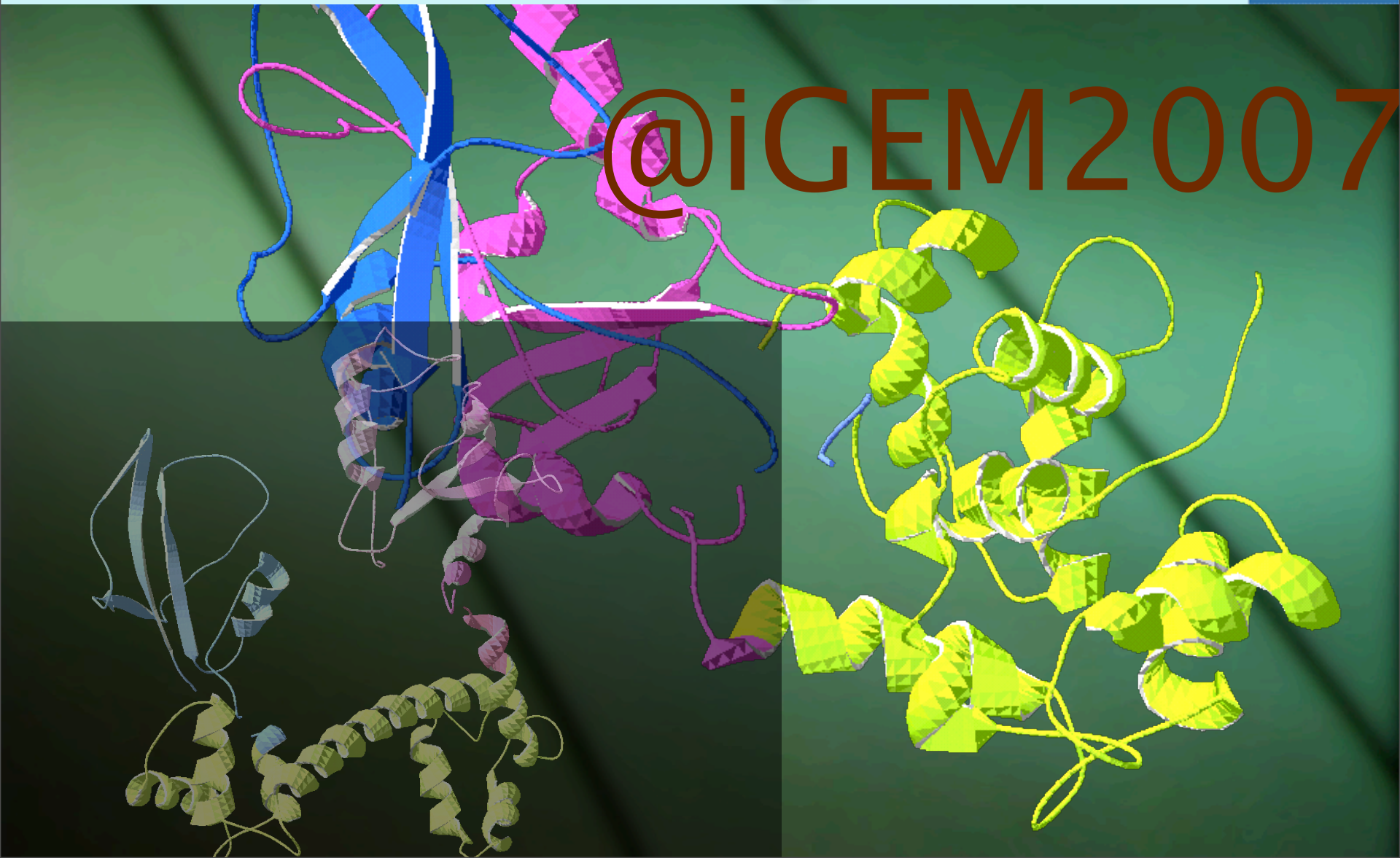
ALBERT-LUDWIGS-
UNIVERSITÄT FREIBURG

Albert-Ludwigs-Universität Freiburg

550 Jahre
Albert-Ludwigs-
Universität Freiburg
1457 – 2007



Members:

- Instructors:
  - Kristian Müller
  - Katja Arndt
- Students:
  - Maximillian Mauler
  - Moritz Busacker
  - Corinna Gruber
  - Natalia Maier
  - Dario Hermida Aponte
  - Philipp Mappes
- Advisors
  - Andreas Hiltbrunner, Eberhardt Schäfer
  - Michael Reth

Support:

- SYNBIOCOMM
- GeneArt
- Wissenschaftliche Gesellschaft, Freiburg

iGE-Machine

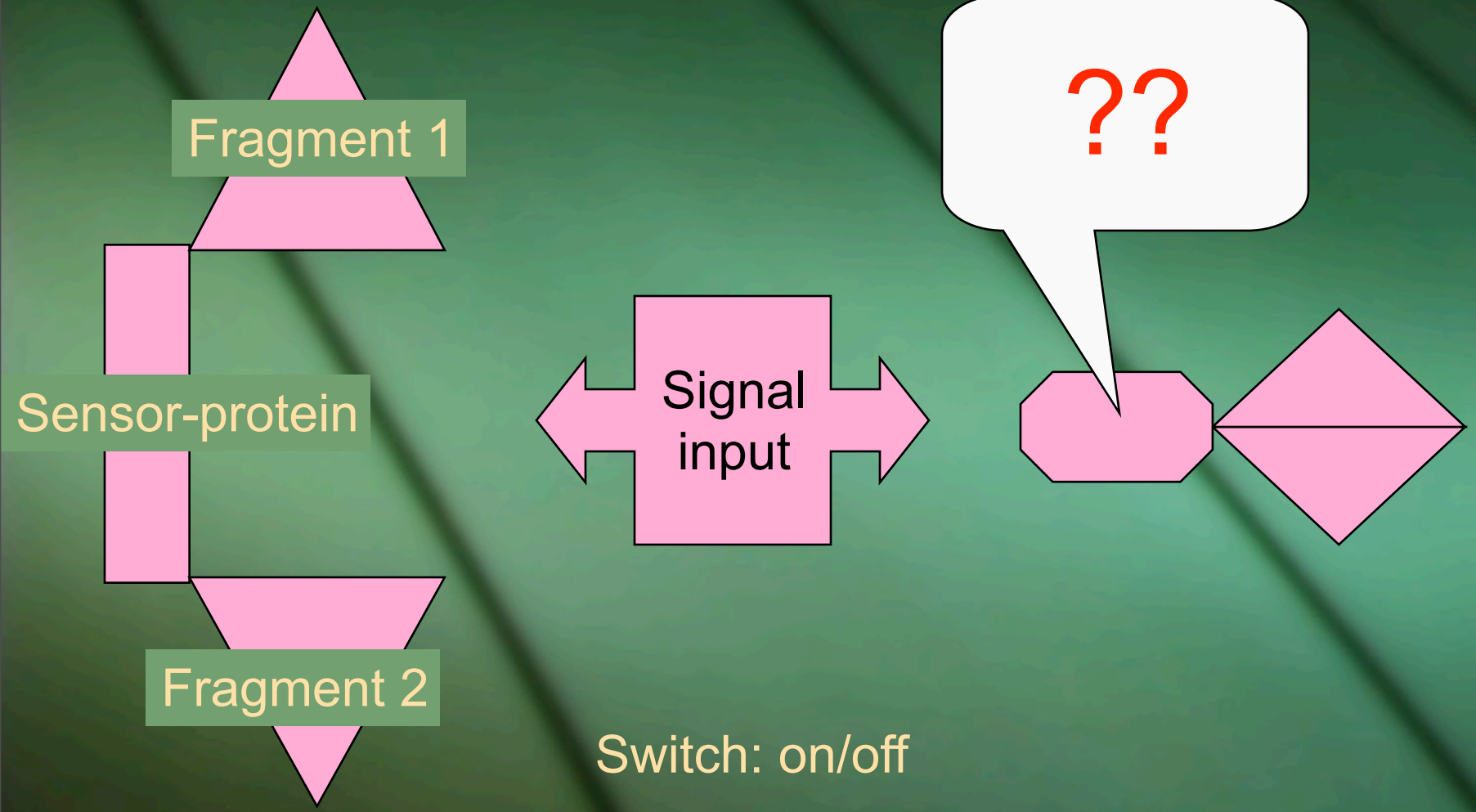
- Mechanical, technical aspect
- Given: protein-design lab working on split enzymes
  - Expression of complementing enzyme fragments
  - Enzyme becomes active if both parts are brought together

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-halfs to sensor-proteins, thus coupling receiving and execution in one molecule


Fragment 1
Sensor-protein
Fragment 2
Signal input
??
Switch: on/off
Inactive state
Active state

...the sensor should:

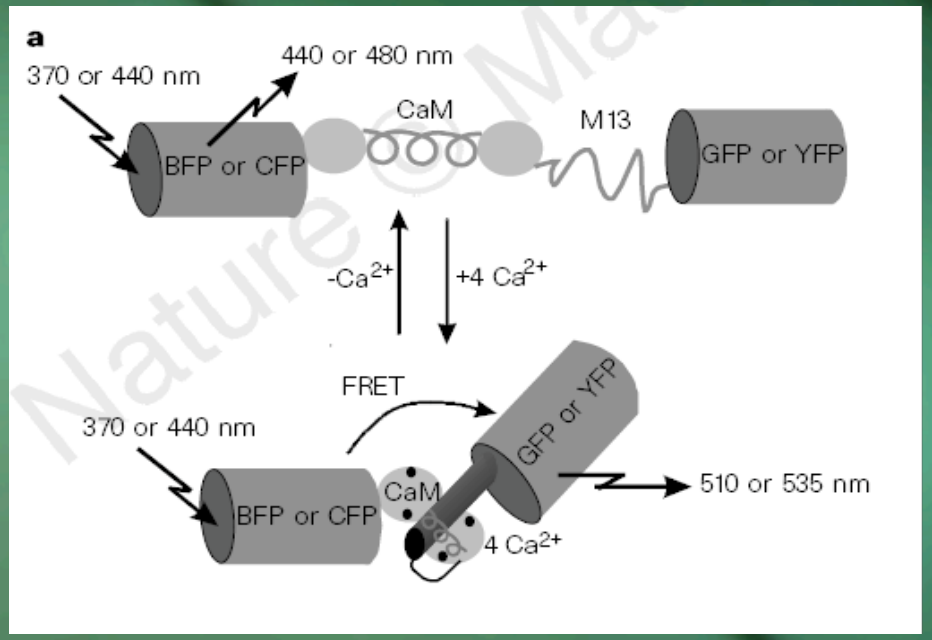
- Be a single protein with a strong conformational change upon an external signal or
- A dimer - monomer system...
- ... featuring easy access and control

Possible „trigger“-proteins for the regulation of split enzymes:

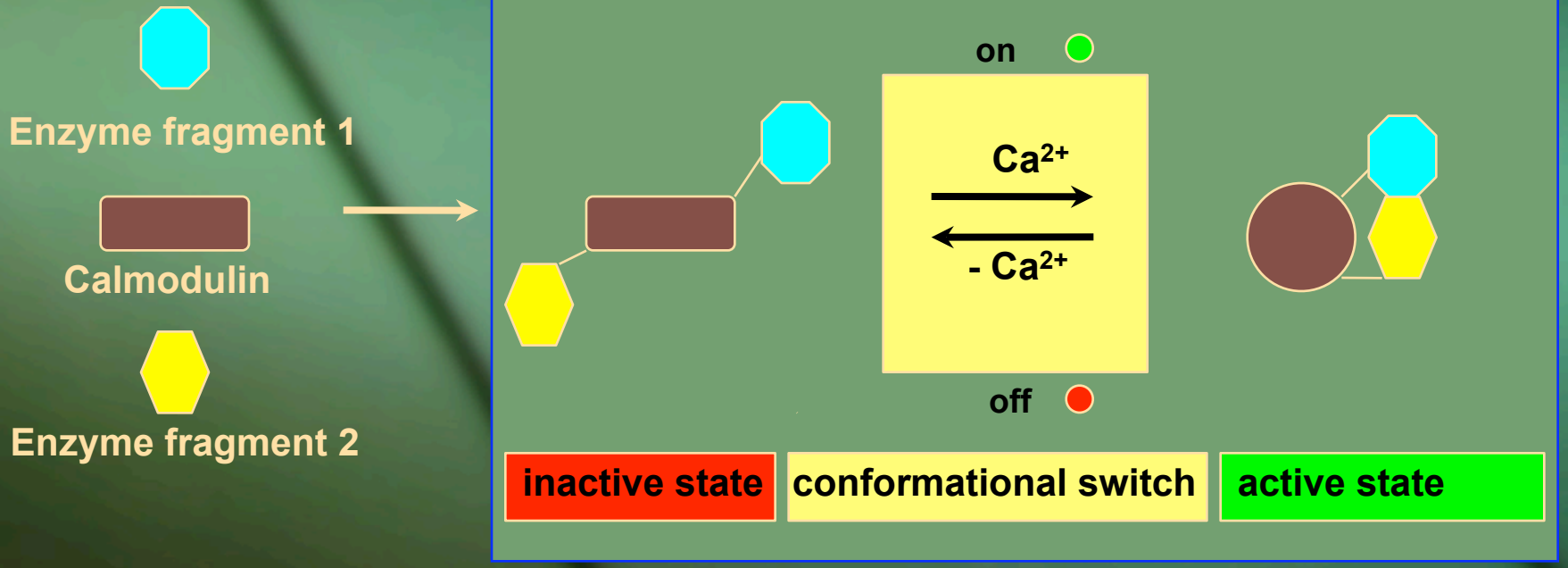
- proteins that can change conformation upon light irradiation...
- proteins which can be regulated by chemical components:
  - Maltose binding proteins
  - **Calmodulin:** a calcium sensing protein performing strong conformation change upon binding calcium

...why calmodulin:

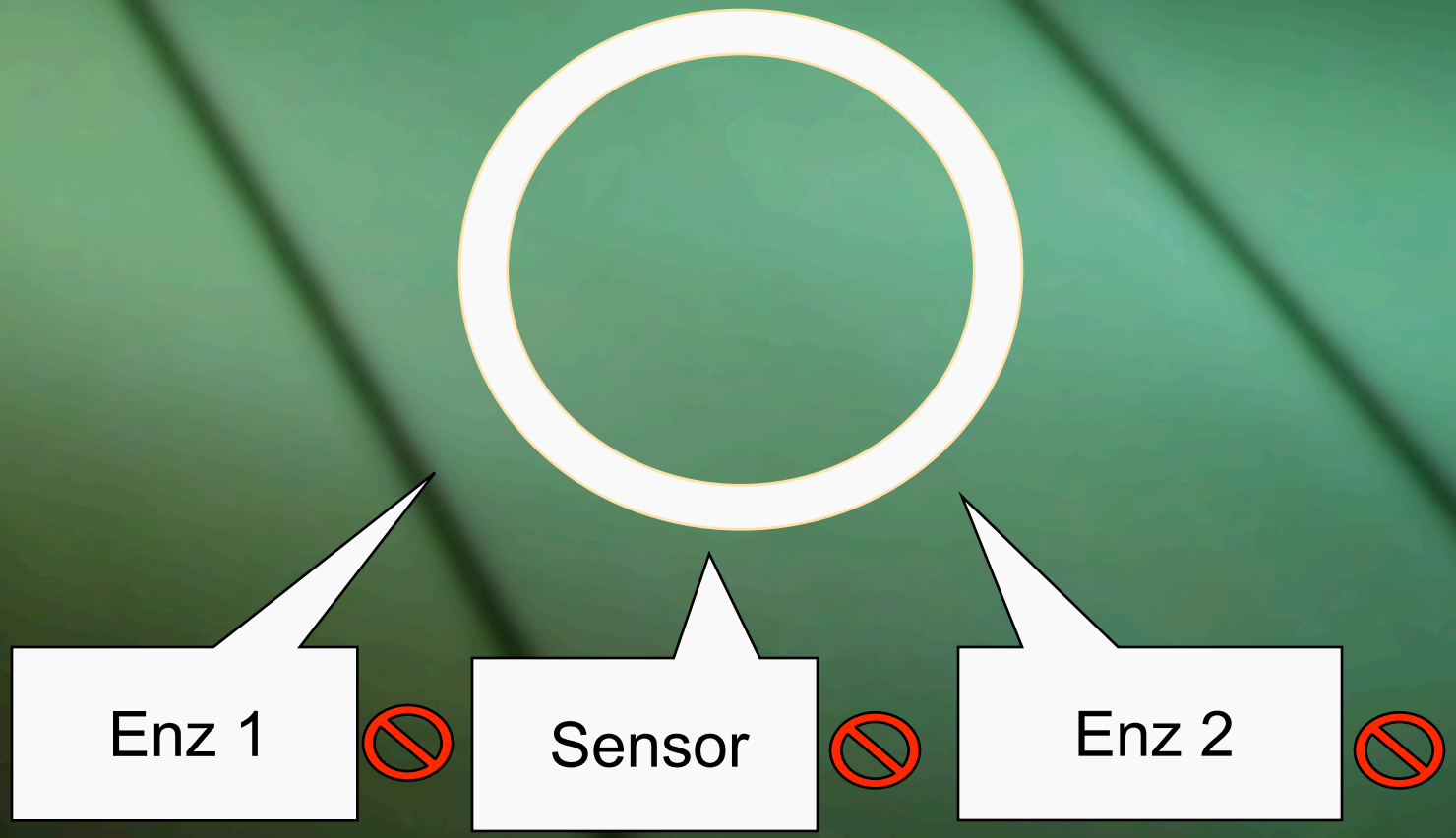
- Truong, Ikura et al. have already fused CFP and YFP to the ends of a modified calmodulin ...
  (CFP an YFP have almost the same size as our enzyme fragments)
- ...and were so friendly to send it to us!



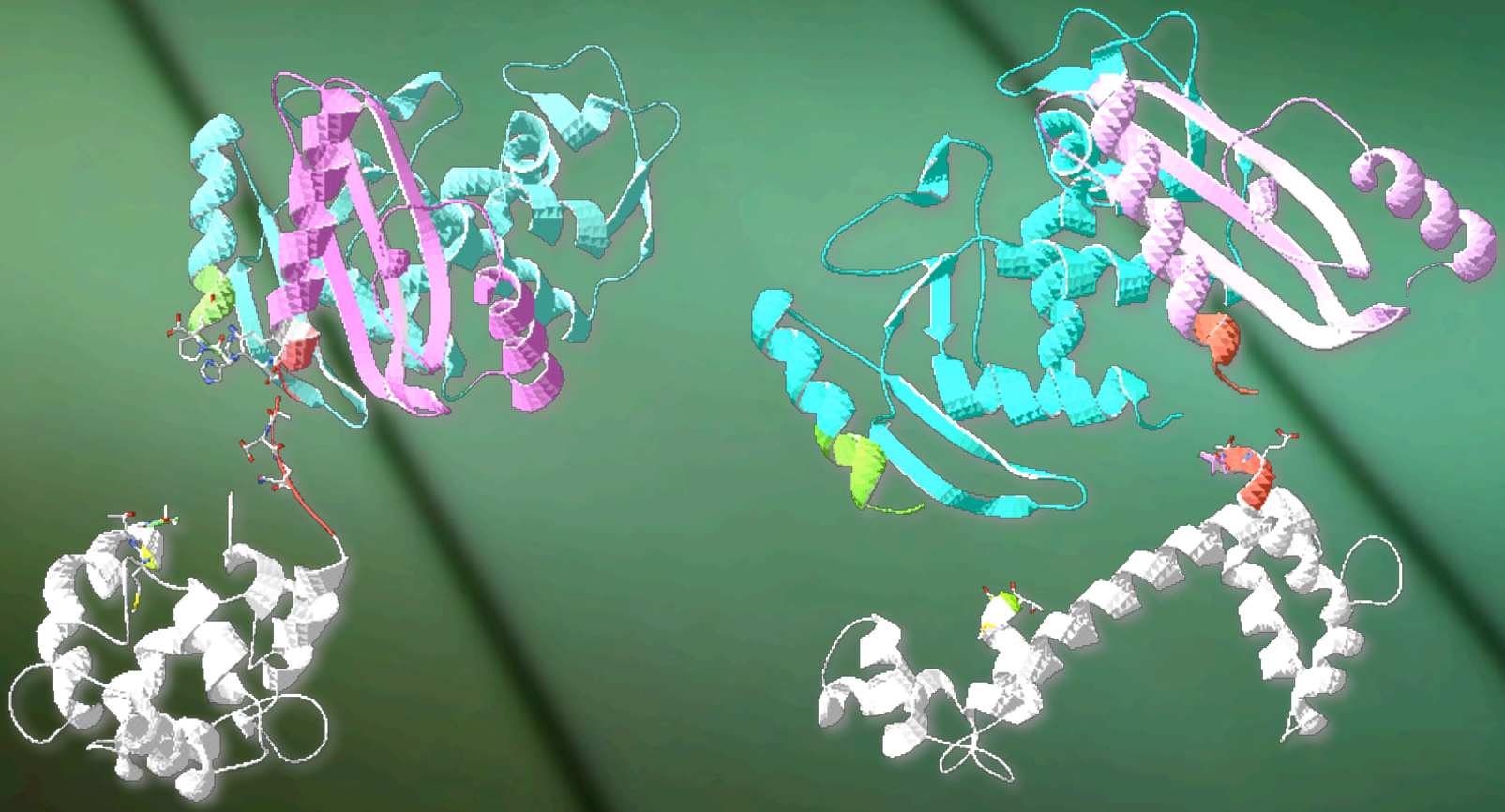
Principle for a calcium mediated enzyme activation



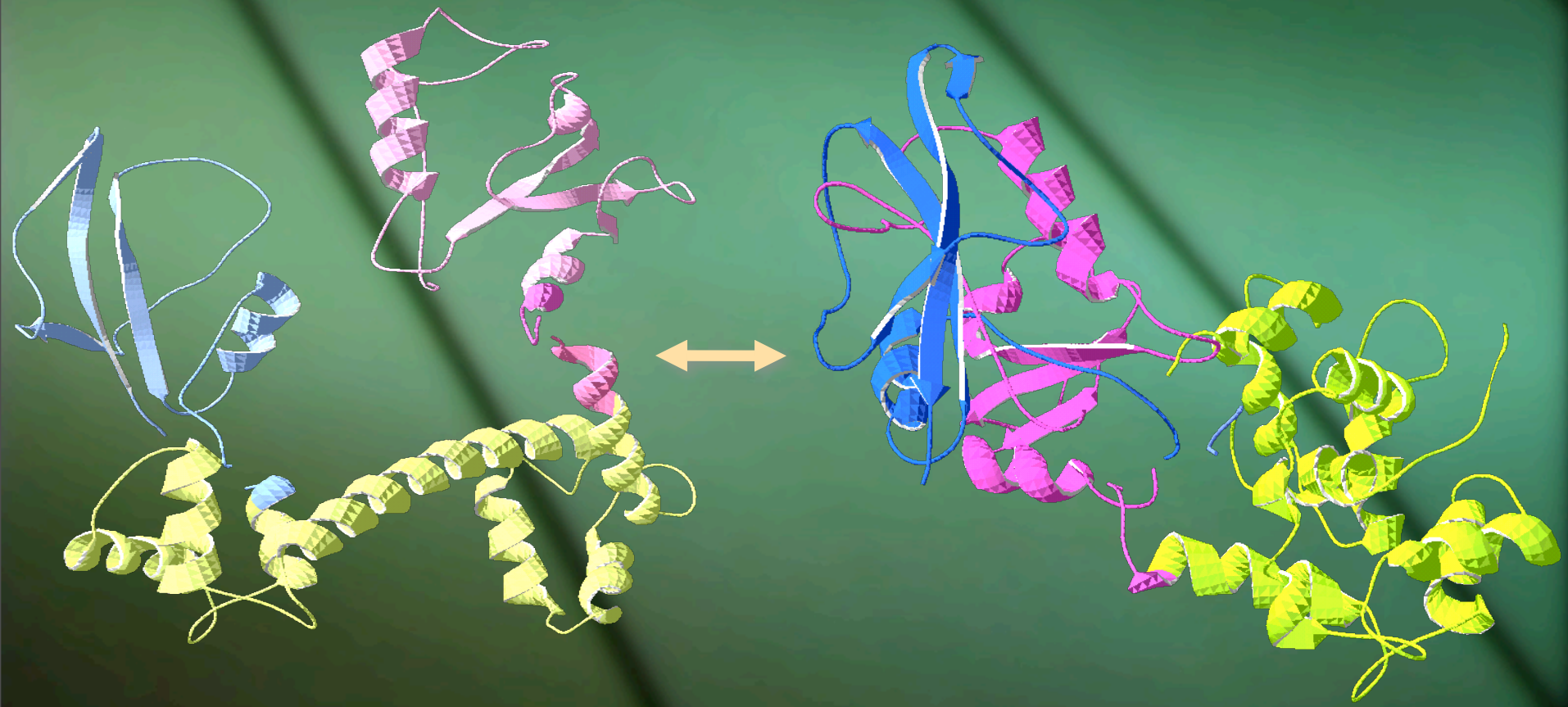
...anyway, we couldn´t have our parts synthesized as



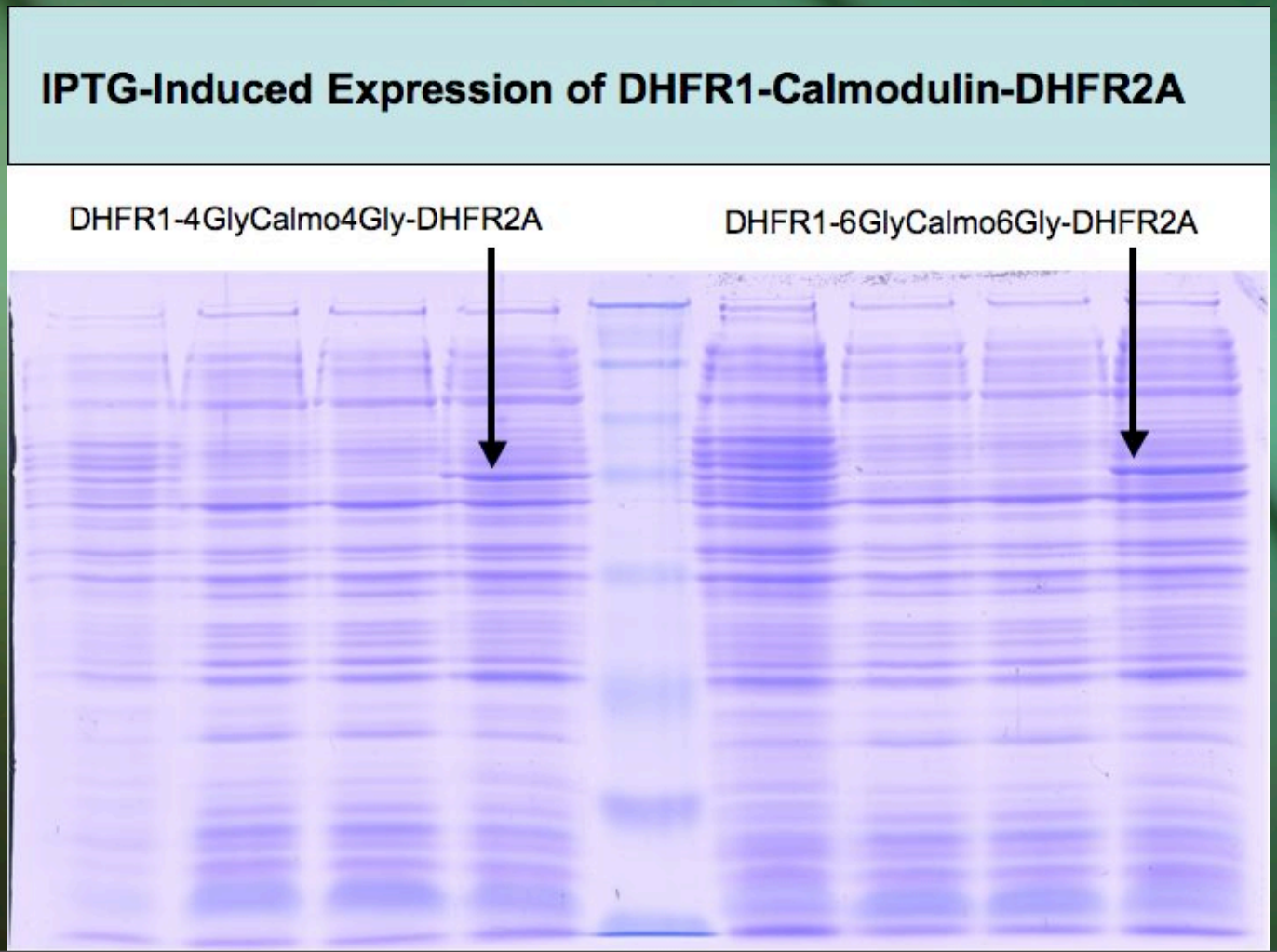
3D-Model of „blac1-calmo-blac2“:



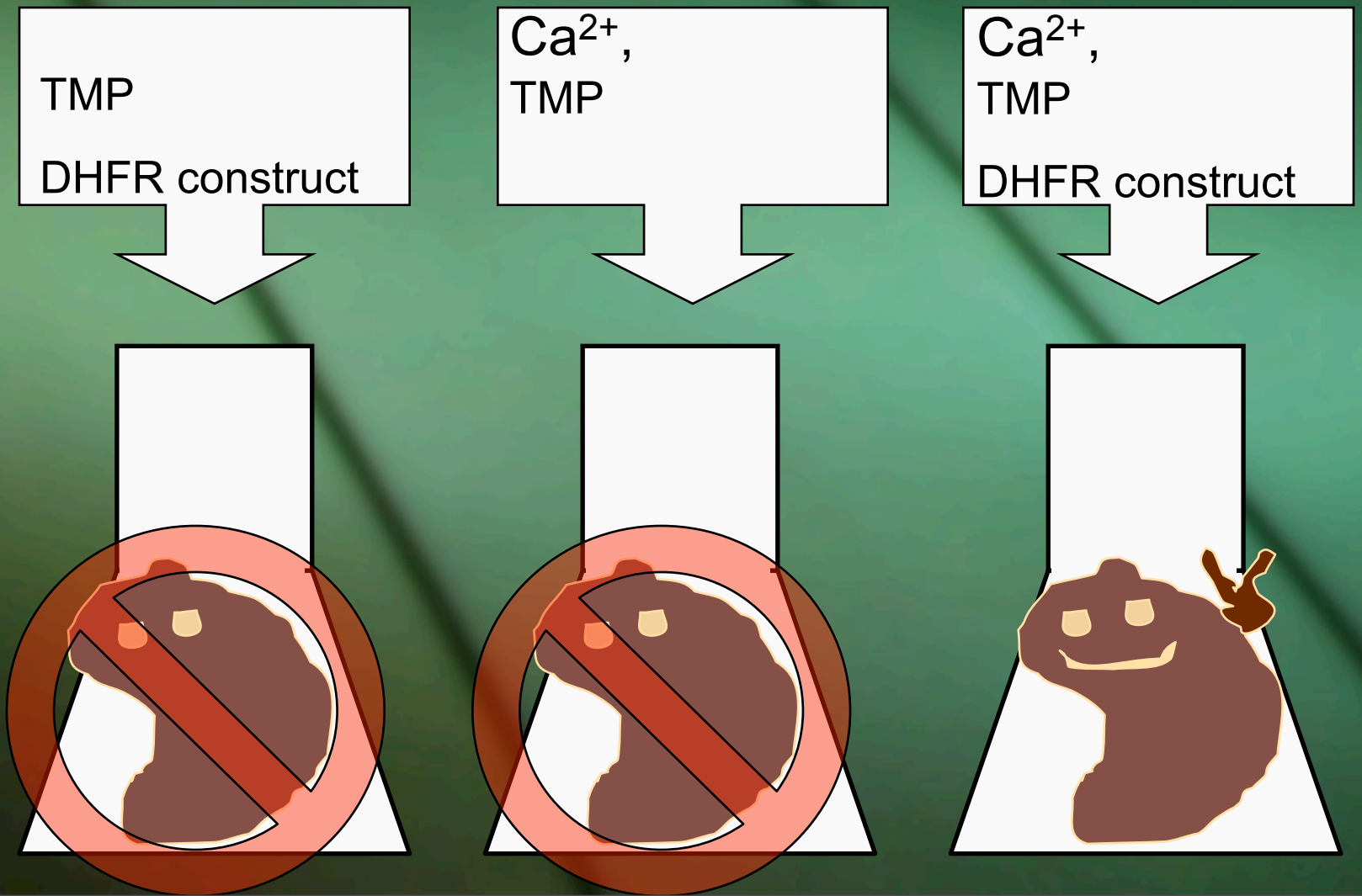
3D-Model of „DHFR1-calmo-DHFR2A“:

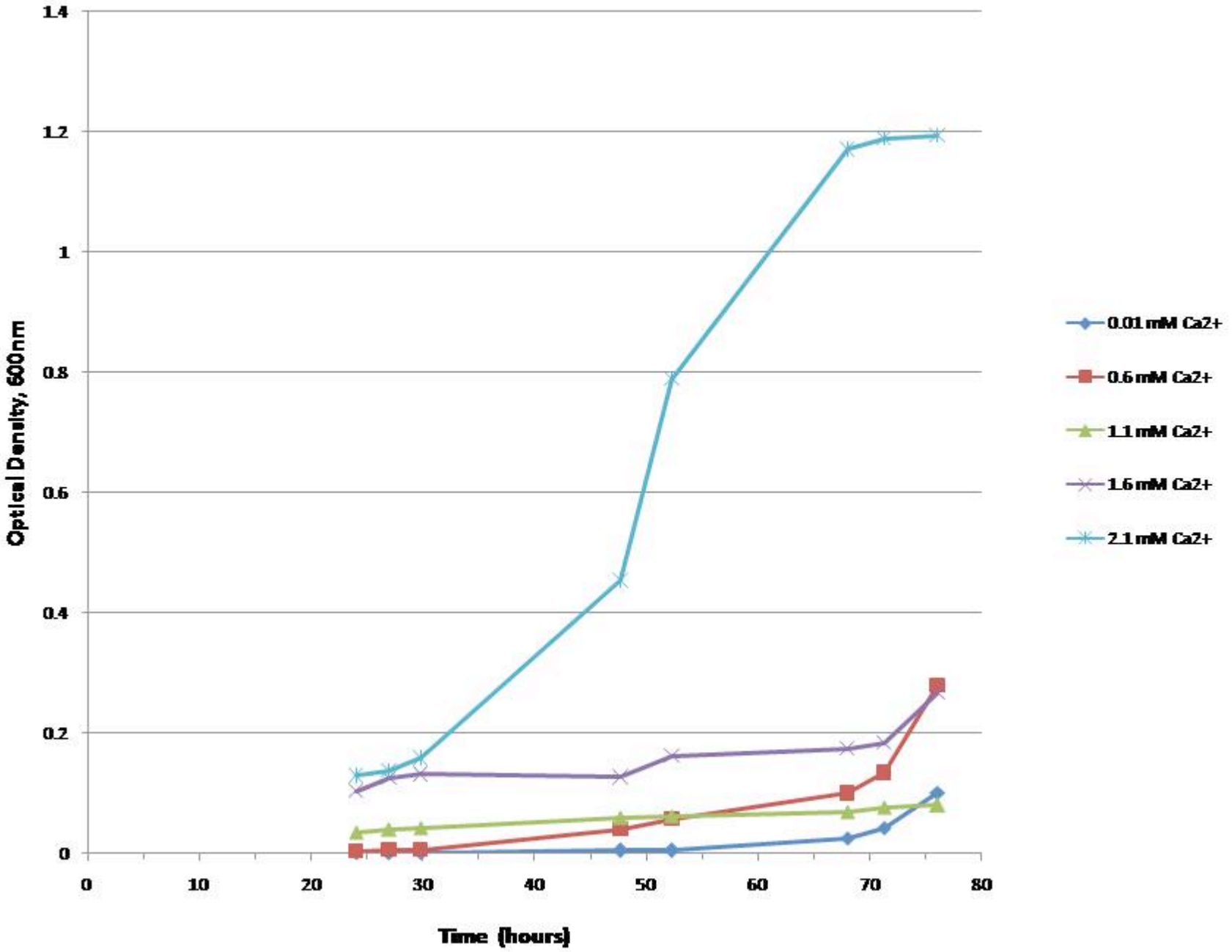


...we could induce the expression of this construct:



In-vivo-testing:




1.4
1.2
1
0.8
0.6
0.4
0.2
0
Optical Density, 600nm
0
10
20
30
40
50
60
70
80
Time (hours)
0.01 mM Ca2+
0.6 mM Ca2+
1.1 mM Ca2+
1.6 mM Ca2+
2.1 mM Ca2+

Molecular light switch for executing devices

Phytochrome A (PhyA):

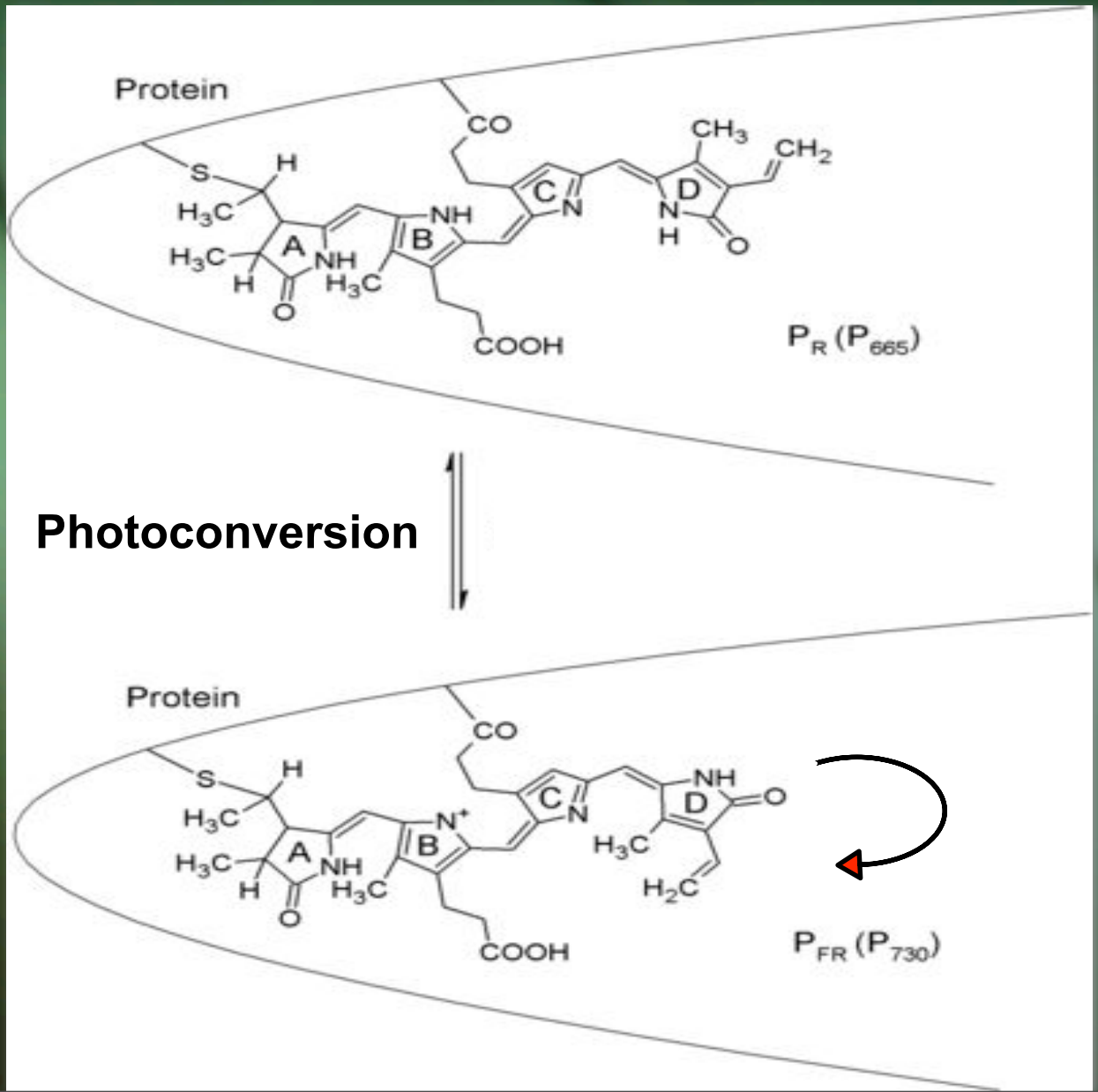
- Red light photoreceptor in plants → affects growth
- Changes between two stable conformations

$$P_r \underset{705 - 740\ nm}{\overset{650 - 670\ nm}{\rightleftharpoons}} P_{fr}$$

- Activated PhyA acts as a transcription factor
- Uses a chromophore (Phytochromobilin) as photoconverter , we used PCB instead
  → works as well

Arabidopsis thaliana

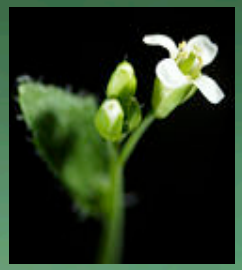
PhyA - Chromophore



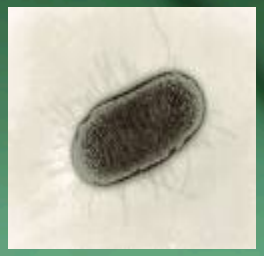
Far red elongated hypocotyl 1 (Fhy1):

- Binds P_{fr} form and is responsable 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



E. coli

= ???

PCB biosynthesis:

heme

↓ HO1

biliverdin

↓ PcyA

phycocyanobilin

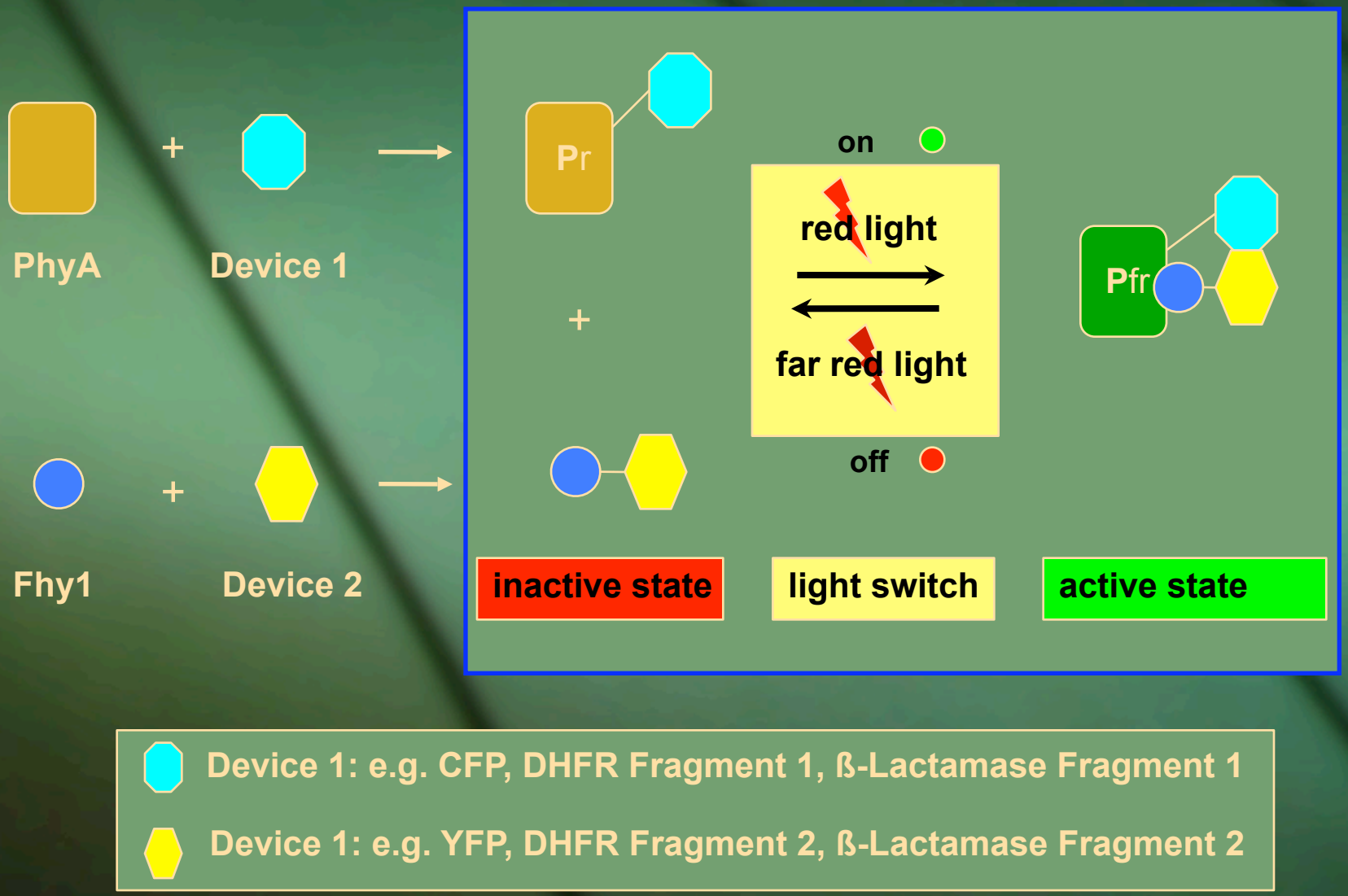
Codon usage:

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



Device 1: e.g. CFP, DHFR Fragment 1, ß-Lactamase Fragment 1

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

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

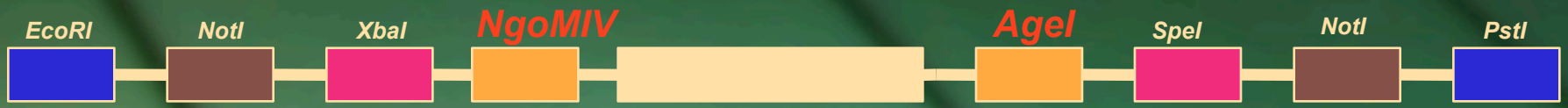
- Building fusion proteins made up of 2 or more different peptides is not possible with the iGEM biobrick system because of a stop codon after each protein:

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

Common BioBricks:

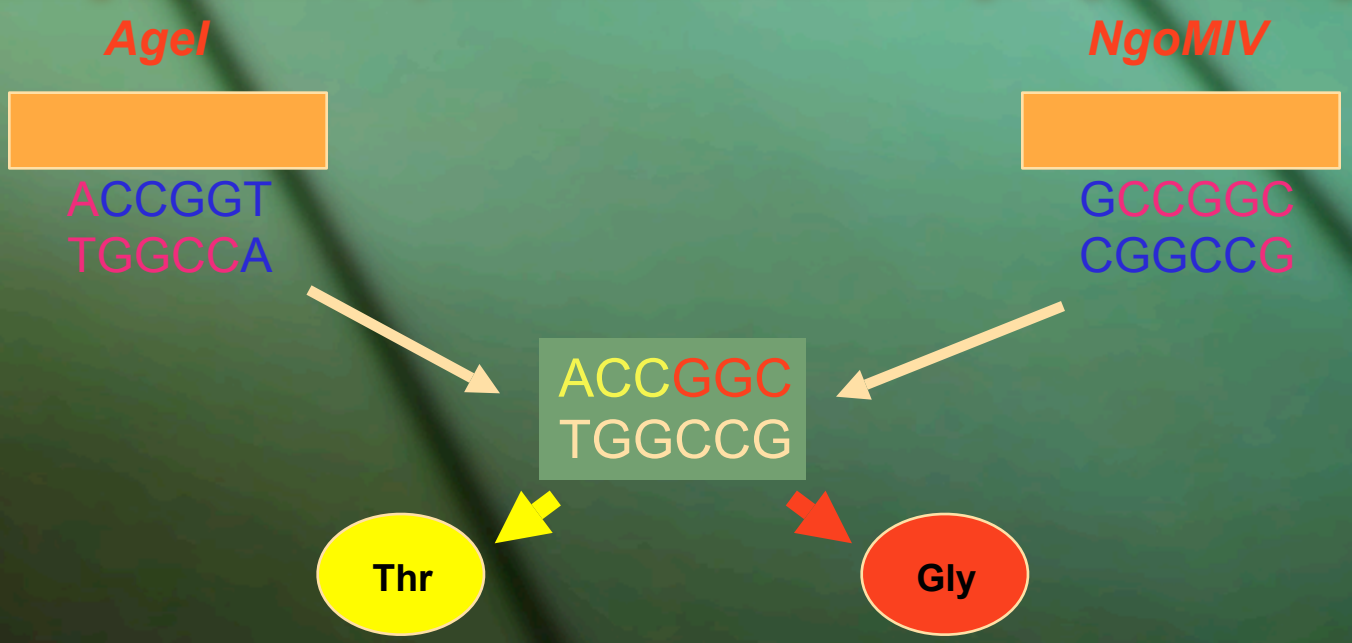
- Combining 2 Biobrick-Parts via Spe I and Xba I results in a stop codon

BioBrick ´Version 3.0´:



Prefix **Part** **Suffix**

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



Improvements of BioBrick :

- Fusing BioBrick 3.0 parts does not result in a stop codon in the scar
- Encoded aminoacids (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: ß-Lactamase 1, ß-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