Modular Synthetic Receptor System

ALBERT - LUDWIGS - UNIVERSITÄT FREIBURG, GERMANY
iGEM-Team Freiburg 2008, equal contribution: Kathrin Pieper, Sabine Jägle, Simone Weber, Normann Kilb, Michael Kneib, Robert Gawlik, Moritz Busacker, Philipp Mappes, Daniel Hautzinger
Instructors: Janina Speck, Mahima Swamy, Wolfgang Schamel, Katja M. Arndt, Kristian M. Müller
Correspondence should be addressed to: K. M. Müller, Department of Biology III, Schänzlestr. 1, 79104 Freiburg; kristian@biologie.uni-freiburg.de

Modular Synthetic Receptor System:

Signaling through membranes is a characteristic of life. Transmembrane proteins control proliferation, differentiation, and cellular response and are key for the formation of multicellular organisms. Controlling such proteins enables modifying cellular behavior and ultimately programming cells at will. The complex rules for transmembrane signaling often require engagement of several proteins in a fine-tuned spatial and temporal manner.

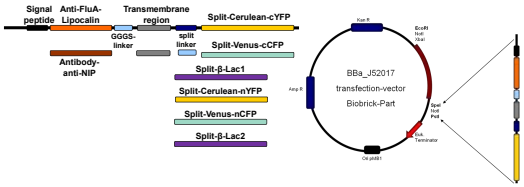
To tap possibilities of transmembrane programming, the Freiburg 2008 iGEM team provides an extensible system comprising an external framework with spatial resolution, a concept for modifying natural receptors, and a modular set of fusion-Biobricks for the construction of synthetic receptors. Spatial resolution in the nanometer scale is provided by DNA-Origami modified with distinct patterns and combinations of ligands. Receptors are decoupled from their natural ligands by fusion with artificial binding domains. The Biobrick collection for a working system contains signal sequences, binding domains, transmembrane domains, and effector domains featuring split enzymes and split fluorescent proteins for immediate readout.

Ultimately, we were able to stimulate cells with a green fluorescent molecule, and cells expressing our receptor constructs respond with a blue fluorescent output.

Cloning Strategy

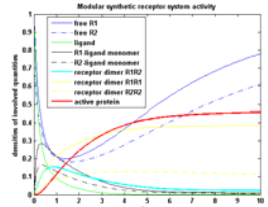
All our constructs feature the extended pre- and suffix for fusion proteins according to the BioBrick 3.0 proposal (Freiburg 2007) and were cloned in plasmid "pMA" (part Bba_K157000). The broad combinatorial range given by the modular concept was almost fully exploited, resulting in the submission of 13 basic and 28 composite parts.

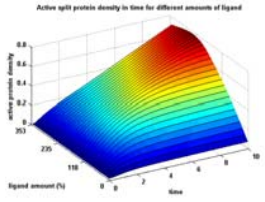
Once a construct was completed, it was cloned downstream of the CMV-promoter in our vector part Bba_K157040 and transfected in 293T-cells.



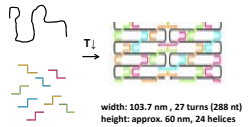
Modeling: Split Protein Activity of Two Kinetically Different Receptors

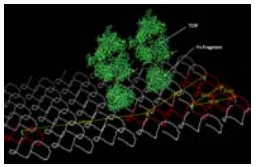
Assuming that receptor R1 is binding the ligand with a higher affinity, its binding rate is higher and its dissociation rate is lower than those of receptor R2. Furthermore, assuming not only a higher turnover rate S1, but also a higher dimerization and a lower dimer-dissociation rate for receptor R1 and a lower initial free receptor density for R2, the following theoretical result is received:





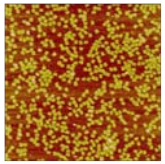
The Input Device: DNA-Origami as Nano Breadboard



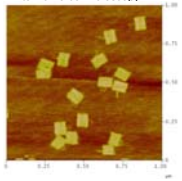


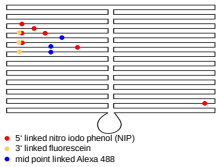
3D-Model of DNA-Origami

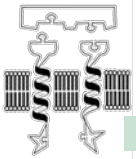
In 2006 Paul Rothemund demonstrated that the M13 phage single-stranded DNA can be formed in various flat shapes, by adding oligonucleotides as staples, which connect specific parts of the long single-stranded DNA. According to his work, we generated DNA-squares of about 100 x 60 nm size, in which the staple oligonucleotides form a grid with an approximate 6 nm spacing. Using modified staple oligonucleotides we were able to generate specific patterns of haptens or ligands, respectively.
As haptens we used nitro-jodo-phenol (NIP), fluorescein, and Alexa 488. The quality of the DNA origami was checked by atomic force microscopy. We also tested buffers for compatibility with origami stability and cell viability.



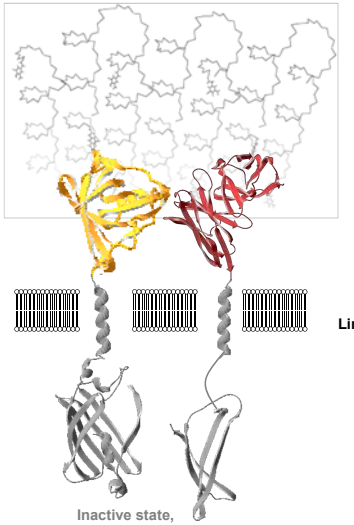
Atomic-Force-Microscopy







Transducer and Executor: Synthetic Receptors

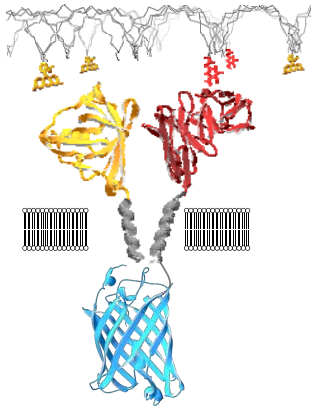


Haptens fused to origami:
-NIP (red)
-Fluorescein (yellow)

Extracellular modules:
-B1-8 sc-Fv-Fragment (red)
-FluA-anticalin (yellow)

Transmembrane region:
-EGFR
-BCR

Linkers (extra- or intracellular):
-GGGS-linker
-"Split-Fluo-linker

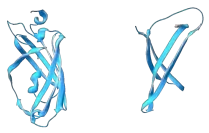


Inactive state, protein split

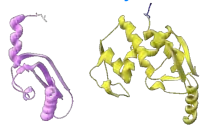
Active state, protein regains functionality

Intracellular modules:

Split CFP „Cerulean"



Split YFP „Venus"

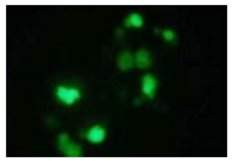


Split ß-lactamase

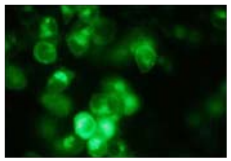
Cellular Results

Membrane localization
Membrane integration has been shown for our FluA-anticalin constructs using YFP as reporter:

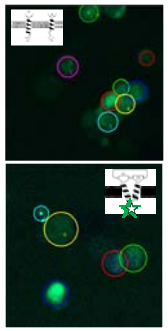
Left: cytosolic expression of YFP in 293T cells (CMV-promoter)



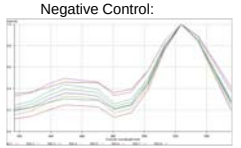
Right: membrane localization of part Bba_K157032-YFP in 293T cells



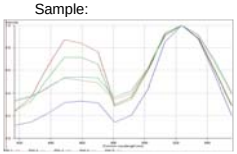
Synthetic receptor activation I
Split-ß-lactamase as intracellular domain allows detection of dimerization via enzyme activity assay using CCF4-AM, a substrate that is converted to a fluorescent product. The following pictures (inverse confocal fluorescence microscopy) show the activation of the synthetic receptor-pair built by part Bba_K157032 and Bba_k157033:



Negative Control:

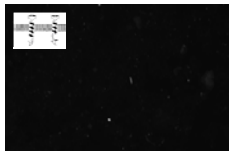


Sample:

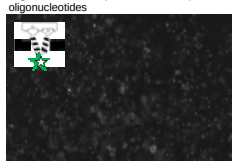


Synthetic receptor activation II
Part Bba_K157036 and Bba_K157037 feature halves of split-CFP "Cerulean" as intracellular domains and, thus, fluorescence as direct, measurable output. The pictures below show 293T cells transfected with these parts.

Left: without input



Right: stimulation by fluorescein-coupled oligonucleotides



Outlook:

Synthetic receptor-like molecules - or molecule-systems - imply a wide range of future applications including biosensors, biological nanobots or simply model systems for foundational research. DNA-Origami certainly holds potential as programmable input device for various further applications.

Support: