MARSS

Modulated Acetosyringone Receptor Sensor System

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Foto: Oliver Schwabe

The VirA Receptor

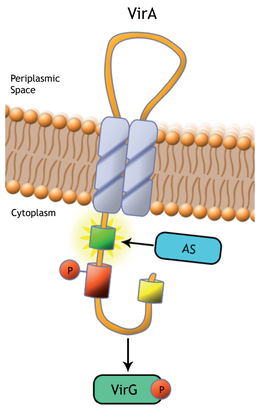

Figure 1:
The VirA receptor in *Agrobacterium tumefaciens*.

The original receptor was taken from the soil bacterium *Agrobacterium tumefaciens*, which is known as a phytopathogen causing crown gall disease in dicotyledonous species. This process is initiated by the VirA receptor, after it bound to a phenolic substance secreted by wounded plants called acetosyringone.
The binding domain of VirA, the so called linker region, is located in the cytoplasm. When binding is established, the kinase domain of VirA becomes active and catalyses the phosphorylation of the intracellular response regulator VirG. In its active state, the transcription factor VirG recognizes a specific short DNA-sequence called *vir*Box and enhances the expression of the virulence genes.

According to the literature the core structure of an inducing molecule must be a phenol with at least one methoxy group in *ortho* position. Moreover, the binding affinity to VirA seems to be empowered by a second methoxy group in *ortho* position and the presence of a chain with high potential capacity in *para* position.

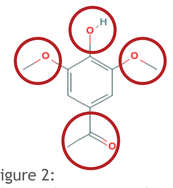

Figure 2:
Chemical structure of acetosyringone and its functional groups relevant for binding to the VirA receptor.

Final Construct

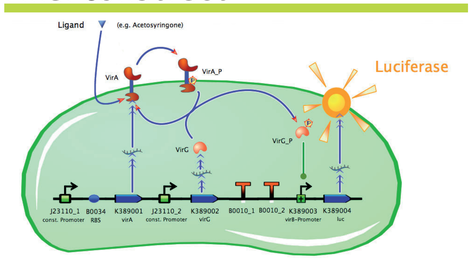

Figure 3: Main construct of acetosyringone inducible luciferase expression system containing constitutive expression of the two component receptor system (*virA+virG*).

The complete construct assembled during the project includes parts from different organisms brought into *E. coli*. The general sensor system is established by the constitutively expressed genes *virA* and *virG* from the soil bacterium *A. tumefaciens*. Once the VirA receptor has sensed a target molecule, it phosphorylates VirG which becomes an active transcription factor. This activated VirG initiates the expression of any gene set under control of the *virB*-promoter. Multiple versions of the complete system were constructed, within which different reporter genes (mRFP, luciferase and kanamycin resistance) were used in combination with the *virB*-promoter.

Abstract

In our MARSS (Modulated Acetosyringone Receptor Sensor System) project we introduced the VirA/G two-component receptor system originating from *Agrobacterium tumefaciens* C58 to *Escherichia coli*. The receptor from *A. tumefaciens* detects acetosyringone, a secondary metabolite of plants which attracts these bacteria. Binding to the receptor, acetosyringone induces an intracellular signal transduction. The receptor, the response regulator and an inducible promoter were successfully cloned into *E. coli*, and the signaling cascade was coupled to different reporter genes to measure the induction profile.
In a further setup we tried to alter the binding region of the VirA receptor via directed evolution in order to enable the detection of other compounds than the native inducer acetosyringone. As an exemplary substance we chose capsaicin, a molecule that is responsible for the spiciness in chili, pepper and hence in a lot of food. The idea is to make the spiciness of food visible via a light signal. The modulated system is supposed to emit light of different intensities, depending on the spiciness of a tested sample. Besides capsaicin there are other potentially detectable compounds of interest, like dopamine, adrenaline or near derivates.
In the course of our project we established a heterologous two-component system in *E. coli* capable of quantifying acetosyringone. Thus we proved its suitability as a biosensor. The created construct serves as a starting point for engineering novel quantification devices targeting a range of small molecules.
Even though a new receptor for capsaicin could not be achieved to date, we succeeded in developing methods for the creation and identification of modulated receptors.

Characterization

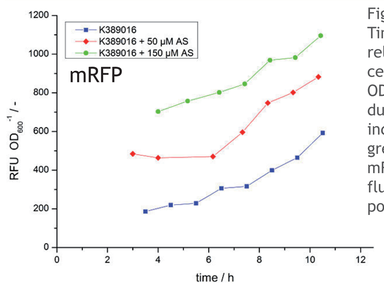

Figure 5:
Time course of relative fluorescence units per OD_{600} of uninduced (blue) and induced (red, green) cells. mRFP is used as fluorescence reporter.

To characterize our parts bacteria were grown in shake flasks and samples were drawn every hour. Depending on the reporter gene the fluorescence of mRFP or the relative light units (RLU) of luciferase were measured and normalized by the actual OD_{600} value. All figures shown in the following are based on multiple cultivations to gain high confidence levels of the derived values.

Bacteria with BBa_K389016 (main construct leading in mRFP expression) were cultivated with different concentrations of acetosyringone and the development of the mRFP to OD_{600} ratio was determined. The results (figure 5) indicate a significant basal transcription without inducer but clearly increased signal intensity with rising concentrations of acetosyringone.
In further analysis the transfer function of the main construct was tested, by using multiple concentrations of acetosyringone, for both readout systems (mRFP and luciferase). For statistical analysis of BBa_K389016 the specific production rate qp, which represents the slope of mRFP accumulation during exponential growth, was normalized by the qp of cultures without inducer. This normalized specific production rate is commensurate to relative promoter units (RPU) or PoPS (polymerase per seconds) and plotted against acetosyringone concentration in figure 6.

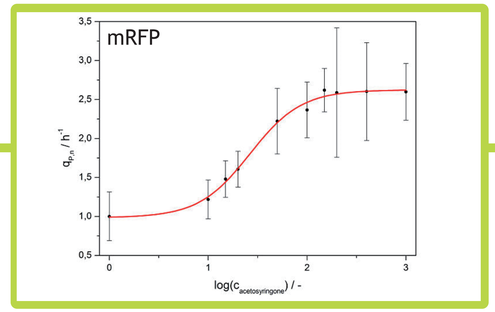

Figure 6:
measured normalized specific production rates $qP_{,n}$ plotted against the logarithm of the concentration of the inductor acetosyringone in µM.

The described transfer function was also determined for the main construct employing our luciferase readout (BBa_K389015). During analysis every RLU to OD_{600} ration was normalized by the analogue value of a non-induced culture during data analysis. The resulting figure 7 shows the response of the receptor system with the luciferase reporter to different levels of acetosyringone.

Sensitivity Tuners

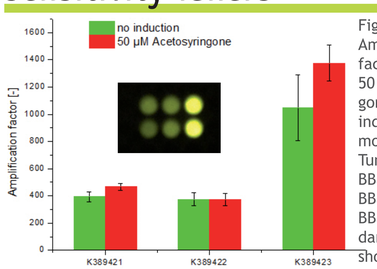

Figure 8:
Amplification factor of induced, 50 µM acetosyringone (red) and not induced (green) modified Sensitivity Tuner BBa_K389421, BBa_K389422 and BBa_K389423, Standard deviation shown.

Using inducible promoters often results in weak expression of reporter genes and thereby in a relative narrow spectrum of quantification. To achieve a broader range of quantification for our prototype test system, an amplification device was implemented, using so called sensitivity tuners. Within this system the *virB*-promoter regulated the gene for a phage activator, which in the following binds to a phage promoter upstream of a reporter gene. This additional molecular step generates a higher output signal which can be quantified as equivalent to the flow of RNA polymerase molecules along DNA (PoPs). To test the sensitivity tuners, three different devices with amplification factors from 10 to 35 were used in combination with the expression of the luciferase reporter gene.

Potential Candidates

When trying to detect other substances than acetosyringone, it must be considered, which chemical groups account for the activation of VirA (cmp. fig. 2). Figure 3 shows a small selection of molecules that fit the known requirements. Capsaicin is responsible for the spiciness in pepper. Dopamine is indicating misuse of doping agents and related to psychic disorders (parkinson disease, schizophrenia) as its degradation product homovanillic acid is. The latter is also important for the diagnosis of tumors (pheochromocytoma, neuroblastoma) in infants.

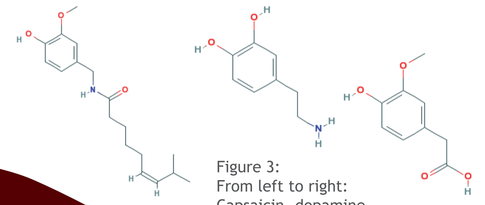

Figure 3:
From left to right:
Capsaicin, dopamine, homovanillic acid.

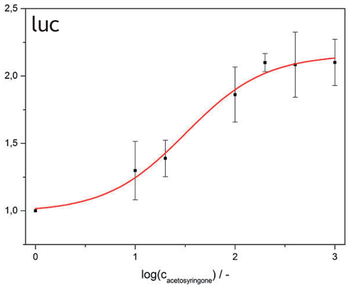

Figure 7:
Measured ratio Φ between induced and uninduced relative luminescence units per OD_{600} plotted against the logarithm of the concentration of the inductor acetosyringone in µM.

The observed results from both systems state that the main construct shows a sigmoid induction profile with rising concentrations of acetosyringone. The switch point of induction was found between 27 and 32 µM of inducing substance and the maximum of reporter gene expression was observed at concentrations above 200 µM of acetosyringone.

Receptor Modulation

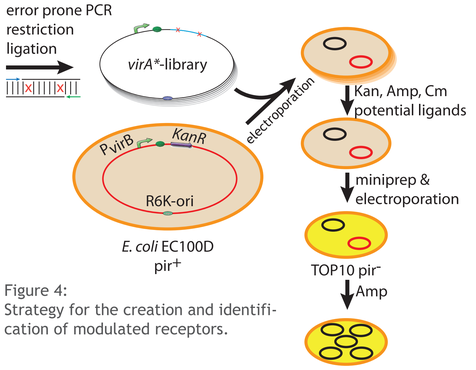

Figure 4:
Strategy for the creation and identification of modulated receptors.

Using error prone PCR a library of BioBrick-plasmids (pSB1AT3 backbone) containing randomly mutagenised *virA* genes can be created. These plasmids are brought into a pir^+ *E. coli* strain (EC100D) keeping a second plasmid with R6K-ori, encoding the response regulator VirG and a kanamycin resistance gene under the control of the *virB*-promoter. Selection on kanamycin, ampicillin, chloramphenicol and potential ligands then preserves cells with receptors inducible by the ligands of interest. Finally, plasmids with modulated *virA* can be seperated by electroporating both isolated plasmids into a pir^- strain (TOP10), in which the second plasmid with R6K-ori cannot be replicated. Only the plasmid including the desired variant of *virA* remains.

Science Communication

Synthetic biology and its tool genetics are often in a negative public focus. Therefore, altering the public opinion is hard to attain. Our team embraced the opportunity of the iGEM competition to bring synthetic biology to a public discussion. We do not appreciate science hiding behind closed doors, but bringing science to a broad range of society. Only a very open contact with the media can reduce prejudices against bacteria and genetic engineering.
The results of our strong emphasis on public relations are among others, two articles in local and national magazines, a radio series in three parts broadcasted by a big federal radio station and multiple appearance on German television. Moreover, we had a public discussion at “Science Café Bielefeld” with not less than 100 guests, in order to advance the people's opinion towards synthetic biology.

Acknowledgment

We would like to say thank you to Prof. Dr. Alfred Pühler, Prof. Dr. Karsten Niehaus, Dr. Jörn Kalinowski, Dr. Christian Rückert and all members of the Center for Biotechnology (CeBiTec Bielefeld) for the excellent and helpful working atmosphere we enjoyed.

Moreover, we want to express our thanks to our sponsors: