

Poster presentation

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ECOLITASTER: cellular biosensor

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from BioSysBio 2007: Systems Biology, Bioinformatics and Synthetic Biology Manchester, UK. 11–13 January 2007

Published: 8 May 2007

BMC Systems Biology 2007, 1(Suppl 1):P38 doi:10.1186/1752-0509-1-S1-P38

This abstract is available from: <http://www.biomedcentral.com/1752-0509/1?issue=S1>

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Background

Our project consists of designing a cellular biosensor [1]. We use as a chassis an *EnvZ* *E. coli* strain, and construct two different devices, a sensor and an actuator, assembled using *OmpR*-P as a standardized mediator. The sensor device contains a receptor protein and a synthetic two-component signal transducer, both implemented as new parts in the Registry of Standardized Biological Parts [2]. The device has been inspired on Hellinga's work [3], sensing TNT and other molecules by using a mutated periplasmic binding protein (PBP). Thus, our team thought in building a PBP that binds a vanillin molecule. The receptor protein has been computationally designed using a ribose binding protein as scaffold and finding the necessary mutations to change its ligand affinity towards vanillin, thus allowing the modified *E. coli* tasting flavors. Our computational methodology [4] can be used to test other molecules too. The synthetic two-component signal transducer was built by fusing the periplasmic and transmembrane parts of a *Trg* protein with an *EnvZ* kinase domain [5]. We expect this part to be very useful for future biosensors projects. When the PBP-vanillin complex binds *Trg*, an allosteric motion is propagated to the cytoplasmic *EnvZ* kinase domain, resulting in autophosphorylation and phosphate transfer to the *OmpR* transcription factor (*OmpR*-P), which then induces transcription of the *pOmpR* promoter. We use a synthetic genetic network as the actuator, which takes as input *OmpR*-P and as outputs GFP and RFP. At intermediate input levels, RFP will be expressed, whereas GFP is expressed proportionally to the

input to obtain a graded response if we superpose the outputs. This later device has been inspired on Weiss' work on pattern generation [6], but in this case we use a new two-regulator promoter to integrate the signals. This synthetic promoter is synergistically activated by two transcription factors *cl* and *CRP* (implementing an AND logic gate) [7], and it will be one of the first of its kind at the Registry [2]. We propose an extensive use of this type of

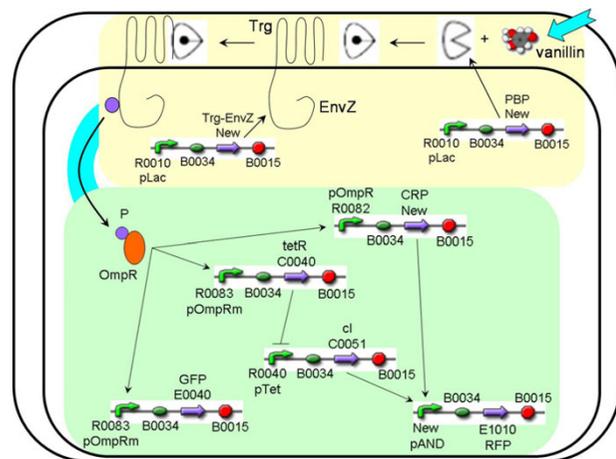


Figure 1

Biological scheme of the system using parts [2]. The yellow set shows the sensor device and the green one the actuator.

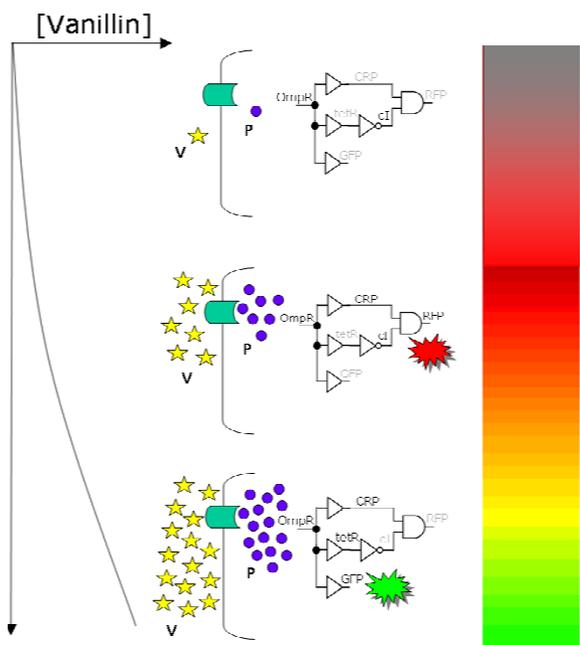


Figure 2
Mechanism of the system. Plot of the reporters according to the vanillin concentration.

promoters for future synthetic circuits, due to their small sequence size but sophisticated behavior.

Materials and methods

We have simulated our actuator device by using Matlab, and we have done a parametrical sensitivity analysis. On the other hand, we have obtained the sequence for the vanillin receptor by using a computational procedure [4]. We have used parts from the Registry [2] to implement our constructions by using standard assemblies [8]. Plasmids were derived from pUC containing antibiotic resistances such as ampicillin or kanamycin. Furthermore, we have used DNA synthesis to obtain the CDS of the vanillin receptor. For the synthesis of the AND promoter [7], we have used DNA synthesis from oligos [9]. The CDS for the CRP and Trg were obtained from genomic PCR. The growth medium was LB at 37°C with the corresponding antibiotic. The reporters fluorescence has been measured by using FACS.

Results

We show the whole system (Figure 1) and its functional mechanism (Figure 2). Nowadays, we continue working on this project. All parts are already available. Hence, tasks are concentrated in assembling them. Then, we will characterize the biological devices.

Acknowledgements

We thank A. Aparici, M. C. Aroca, J. Carrera, C. Edo, G. Fuertes, D. Giménez, C. Mata, J. V. Medrano, A. Montagud, C. Navarrete, E. Navarro, P. Tortosa, M. Bágüena, P. Fernández-de-Córdoba, A. Ferrando, J. Salgado, and J. Urchueguía as Valencia iGEM team members. In addition, we thank E. O'Connor and A. Moya for technical equipment access. This work was supported by the EU FP6 NEST SYNBIOCOMM project, by the Universitat Politècnica de Valencia, and by the Universitat de València.

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