Minutes of the fourth iGEM meeting

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We started the iGEM meeting with some organizational topics, followed by a discussion of the ideas from the last meeting and a presentation of new ideas.

1 Organization

1.1 Next meetings

The group decided to meet with the supervisors next wednesday, April 21 at 8 am. The next meeting for the students is scheduled for Friday April 23 at 4 pm.

1.2 Group outing

Inspired by Johnson's suggestion to have a group outing to get to know each other better, we decided to go out together next Friday, April 23. We planned to join the Biotec beer hour after our meeting and to have dinner in Neustadt at 8pm. Later we can go out to Neustadt.

1.3 Sponsoring

In order to collect enough money for the project and the trip to Boston we decided to appoint a "group finance minister" who is responsible for sponsorships. Mareike volunteered for this position. In order to find more sponsors, we had the idea to go to the job fair, which is organised by the student's initiative "bonding" and will take place from May 3-5 in Dresden.

2 Last week's ideas

2.1 Procalcitonin biosensor

We discussed the possibility of engineering E. coli biosensor to detect Calcitonin (NB: not Procalcitonin).

- It was intended for a protease and a human-derived Calcitonin receptor to be engineered on the surface of E. coli (or some other chasis).
- Procalcitonin, present during bacterial-induced sepsis, will be cleaved by the protease to produce Calcitonin, which will ultimately be detected by the human-derived Calcitonin on the outer membrane of E. coli.
- It is important at this point to note that Calcitonin is ubiquitous in healthy individuals and will be present at basal levels.
- However, during bacterial induced sepsis, it is expected that the increased concentrations of Procalcition will correlate to a higher concentration of Calcitonin which the engineered bacteria can detect.

We also discussed new problems with procalcitonin biosensor. In addition to the antibody based detection system we pointed out last week, we also found out that PCR has been actively used for detecting bacterial induced sepsis. This has been in use since 1997. Currently, multiplex and real-time PCR techniques are already in use to detect a broader range of bacterial pathogens and low quantities of bacterial genetic markers respectively. Given this, we ask ourself if this pro ject is still worth pursuing.

We contacted Dr. Bachmann from the Universittsklinikum Carl Gustav Carus Dresden who is an immunology specialist. He liked our idea a lot and is willing to meet with us to give us an insight of what we need to consider if we work on this project.

2.2 Bacterial based sequencing

There has already been much progress in the field of DNA sequencing and given the limited time for the project, we will not be pursuing this idea further. Nevertheless, a couple of students will be attending the upcoming Next Generation Sequencing seminar at MPI.

3 New ideas

The group came up with a lot of new ideas. That can all be found on the iGEM wiki discussion page. The two major new ideas with the most potential can found below.

3.1 Bacterial Metamorphosis

The fundamental idea is a cell that is phenotypically E. coli at the start transform into another species of bacteria after a given input. How would we accomplish this?

- E.coli contains two genomes, one corresponding to the genome of E.coli and another genome, say Salmonella spp.
- The E. coli genome will contain a Origin of Replication 1 (OriR1) and the Salmonella genome will contain a different one (OriR2)
- In order to accomplish the metamorphosis step we will need two events: 1. the deactivation of OriR1 and 2. the activation of OriR2. This will effectively prevent the E coli genome from replication while simulatiously allowing the Salmonella genome to be replicated
- We may have to use closely related bacteria so that the majority of the proteins already present will be compatible.
- Habib will ask Francis Stewart if this is feasible and will present this idea on Wednesdays meeting.

Major problems with this project: how are we going to get a bacterial with two genomes in the first place? Especially when it is already quite problematic transforming/introducing large plasmids.

3.2 Color adapting bacteria

Herein we attempt to build upon the idea of creating bacteria that can actively camouflage themselves. $http://en.wikipedia.org/wiki/Active\_camouflage$

3.2.1 Background

On given surfaces, we see only the color, or rather wavelengths of light that are not absorbed by the surface and are instead reflected. These are what our eyes can detect. For example, a leaf is green because it is able to absorb all the visible wavelengths of light expect those corresponding to green.

With respect to pigment-based colors, we have the three primary colors: yellow, red and blue. Secondary colors (orange, green and purple) can be derived by mixing two of the primary colors. Note that for light or optical color mixing, it's different.

3.2.2 Question: Can we create active camouflage based on bacteria?

We propose a bacterial-based system that produces the three primary colors in response to the input: The reflected color of interest. When our engineered cell is present on a colored surface, the reflected wavelengths (yellow

surface, therefore yellow light is reflected) will be the input telling the bacteria to produce yellow pigment. To this end, we will need photoreceptors that are sensitive to yellow, blue and red light and the corresponding colored pigments machinery in order to produce the required primary colors. Supposed the surface on which the bacteria is on is another color, say for example, green or purple. In order for our cell to create these colors, the principal of mixing two primary colors comes into effect. To this end, we will need some genetic trickery to accomplish this. For green color production, we will need both the yellow and blue color pigment production machinery to be on simultaneously. The biggest problem is, in our opinion, the ability of the bacteria to sense these intermediate (secondary color) wavelengths. Therefore, the broader the Gaussian curves of the absorbance spectrum of the photoreceptor, the better. This is because there is a greater chance that the photoreceptor may detect the overlapping wavelengths corresponding to two different colors.

The reasons we put forward the color mixing idea instead of encoding and engineering a photoreceptor and corresponding pigment production mechanism for every single color are apparent. 1.Simpler when we do not have to engineer so many different systems in the same cell, 2. Allows to fine tune secondary color production by regulating the simultaneous production of primary color. Major problem: Imagine that we want only blue pigment to be produced because the cells are on a blue surface. There is still visible white light present from everywhere. This is a problem because visible white light contains all the wavelengths (think prism) and will therefore trigger all three photoreceptors and the corresponding pigment production.

How can we prevent this and ensure that only blue pigment is produced (at least in excess)? Possible solution: We would suggest placing not only the production of the blue pigment under the control of the blue color photosensor but also two weak repressor systems. These two repressor systems will serve to limit/ repress the production of the other two primary color pigments. Note, the same system can be implemented for bacterial based color photography. All we need to do is to shine light of a certain color to trigger the cell to produce x color of interest.