Minutes of the eighth iGEM meeting

May 10th 2010

Participants: Rahul Akkinen, Habib Bukhari, Svea Grieb, Victor Gordeev, Sarah Mansour, Adithya Nagarakodige, Mareike Roth, Lucas Schirmer and Jonathan Tam

Supervisors: Annelie Oswald and Johnson Madrid

Organization:

1. The team attended a lecture on FRET on Monday the 10th of May by Mike Lorenz from the MPI. It was a very significant lecture and the team members had their questions answered concerning FRET microscopy.

2. Three of the team members will be attending a FRET practical course: Jonathan Tam, Adithya Nagarakodige and Habib Bukhari. The date is yet to be announced.

3. The team decided to choose three topics to focus on, the vote would take place during the next meeting on Friday when everybody had presented their ideas.

4. The team's first outing would take place on Wednesday the 12th of May where the team would go for bowling and dinner.

5. The team celebrated Habib's 22nd birthday!

6. Next meeting would take place on Friday the 14th of May where Victor and Charanya would present their own ideas to the groups followed by discussions.

Project ideas:

In this meeting, the team carried on with presenting their ideas. Four novel ideas for projects were presented by Svea, Jonathan, Rahul and Adithya. A presentation was given by Victor about PoPs. Finally Lucas added another idea to his previously presented project.

A powerpoint presentation was given by each of the members followed by a discussion with the team.

1. Phosphate extraction from waste water using bacteria/yeast:

This idea was presented by Svea, she introduced a method for retrieving phosphate due to its importance as well as its decreasing amounts in the near future.

Abstract for the presentation:

The amount of mineable phosphate that is needed in agriculture as a fertilizer decreases rapidly. Although municipal wastewater contains a lot of phosphate, it can not directly be applied on the fields because of decontamination with heavy metals. Thus, companies that buy the ash of the sludge use chemical compounds together with high temperature to remove the heavy metals.

The idea presented is to substitute this expensive treatment by using genetically engineered microorganisms that directly extract the phosphate out of the wastewater. Svea presented the specific phosphate transport system (PST) of E. coli and pointed out that it can easily be overexpressed in bacteria. Unfortunately, an unnaturally high accumulation of polyphosphates within the cell is cytotoxic and results in cell lysis.

Furthermore, the composition of wastewater differs widely (for example pH values from 2-12 are possible) which create an unfeasible environment for bacteria. This is why Svea concluded to engineer a yeast strain instead of utilizing bacteria. Benefits of this approach are that the phosphate could be stored in vesicles, that yeast is less sensitiv to changes in it's environment due to a thicker cell wall and that it is able to form spores in case the conditions get too bad. Additionally it was thought about introducing temperature stabilizing proteins so that the cells can efficiently take up phosphates even at low temperature.

2. PoPS (polymerase per second):

Victor presented a notion of PoPS (polymerase per second), its context and significance for synthetic biology, the standard assays used nowadays to derive the PoPS values for promoters using fluorescence proteins, mainly:

- via direct determination of the multiple parameters acting during transcription and translation
- via comparing the activity of a tested promoter to a standard reference promoter and expressing the PoPS in relative promoter units.

He also went through the abstracts of the last two years in iGEM projects to conclude that none of them dealt with direct determination of PoPS (some of the teams, however, like Heidelberg used a quantification method for mRNA during RT-PCR along with fluorescence protein determination).

3. In vitro PoPS measurement using FRET

Jonathan presented the second idea which was about PoPs measurement in vitro using FRET.

Abstract for the presentation:

Polymerase per second (PoPS) measurement is defined as the rate at which DNA-dependent RNA Polymerases (RNAP) travel past the last nucleotide of a given promter and is considered to be the ‚holy grail' of Synthetic Biology. Although many diverse methods to quantify mRNA after transcription have been described, they are indirect and accordingly invalid for PoPS measurement.

Herein, an FRET-based model for PoPS measurement *in vitro* is presented. PCR will be performed using forward and reverse primers that flank the promoter and ORF of interest. For signal detection, the use of a reverse primer with a FRET acceptor on the 3' end is necessary. Following PCR amplification, *in vitro* transcription with a RNAP-FRET donor construct will be carried out. Given that the spatial proximity between the FRET donor and acceptor is within the Förster radius (1nm – 10nm, depending on FRET pair), we can expect to see a ‚flash' or ‚blip' as the RNAP travels towards the 3' end of the DNA construct. Time-Correlated Single-Photon Counting FLIM presents itself as a viable detection system as it allows individual photons to be quantified with picosecond resolution.

In order to address concerns that fluoroscent labelling of RNAP could pose to be a problem, a paper describing the labeling of the Rpb4/7 subunit RNAP II at three different cysteine residues was presented. However, the use of T7 RNAP may prove to be a better candidate because it is a single subunit protein that does not require accessory proteins for transcription initiation.

4. Recovery of iron oxides from industrial wastes

This was an idea presented by Rahul where he proposed using genetically engineered organisms for iron oxides recovery from industrial wastes.

Abstract for the presentation:

Currently 100 million tons of iron ore ends up as waste from iron industries. There are some methods to recover iron from these wastes but they are chemically based. These methods are usually lengthy processes and produce by-products that are harmful for the environment. Using bacteria for this application would be cost effective and environment friendly.

Magneto tactic bacteria have the ability to uptake iron in large quantities (170 times higher than E.coli) for the formation of magnetosomes. This property can be used to trap iron from the wastes produced by iron industries. Once trapped, they can be separated and harvested by the application of magnetic field.

The genes that code for formation of magnetosomes can be cloned into E.coli to increase the iron uptake and to confer magnetic character. Also if we confer copper (or other metal) uptake ability to this E.coli and localize the trapped copper in another vesicle we can have integrated cell system that can recover different metals.

During the discussion, the team members suggested the following:
1. It would be better if the E.coli have photosynthetic ability as well.
2. Look for bacteria that can uptake iron more than magnetotactic bacteria.

5. GreenLife: Bacterial Scaffolding system for organizing functional materials for photo splitting of water.

Adithyia's motivation was creating a GreenLife, he presented his idea which is based on a bacterial scaffolding system.

The following points were triggered during his presentation:

-The bacterial system contains catalyst (IrO_2 hydrosol clusters)and Photosensitizes(ZnDPEG).
-IrO_2 chosen because of its know catalytic activity and stability under oxidation condition. ZnDPEG(Zinc deuteroporphyin IX bis- ethylene glycol) for its optical and electrochemical properties.
-IrO_2 binding bacterial system has to be designed.
Bacteria must secrete membrane proteins which can bind to IrO_2. The protein, with binding sequence AGETQQAM is found in M13 virus and can be cloned into the bacterial
system.
-To get the stability of the scaffold, Hyrdogels can be used.
For uniform distribution of the GreenLife in the hydrogel beads, micofludics systems can be implemented.
-Bacteria can also be designed for utilizing other carbon source for hydrogen production.(Parts available on the registry).

6. Lucas presented another approach to his idea presented in the previous meeting about "Sensor Brick"

The new approach was to use the proteolytic VDE sequence in yeast split and fused to two scFv with discrete binding site for a specific tumor antigen.
In case of binding to a tumor antigen, the two parts of the proteolytic sequences would come together and be autocatalytic cleaved in yeast.
After cleavage a transcription factor previously bound to one part of the VDE motive would be free an initiate the expression of a reporter gene or device.