Minutes of the fourteenth iGeM meeting

21/06/2010 and 22/06/2010

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

Supervisors: Johnson Madrid

Organization:

1. The next meeting would take place on 28 June, 2010 when the team members would vote for a final project which could be started working on, in the week after.

2. The voting of the final idea was decided to be done as soon as possible but with a lot of forethought and analysis of the problems and issues that were raised for each project idea.

Project ideas:

1. Initially, on the 21 June, 2010, Mareike presented the final review of her idea 'MouldEx' while Jonathan, Habib and Adithya presented the final version of the idea 'PoPs measurement'.

2. Next, as a continuation of the same meeting, the team met again on 22 June, 2010 wherein Lucas gave his final talk on Sensor Bricks summarizing the major idea and the concerns it involved.

3. The team actively participated in suggestion of ideas and attempts to solve some basic problems were made over delicious pizzas.

4. Following this, there was an intense brainstorming session on the various problems that the team could face experimentally in PoPs measurement during the course of the project.

5. The main points, major issues and problems that were discussed for each project idea are as follows:

MouldEx:

- PDMS was suggested to be used as the foil material. Also, we found that experts from MBZ are currently developing a membrane material. What process that we are going to adopt to make the film is also not exactly clear.

- The issue that PDMS is a hydrophobic substrate could prove to be problematic as it might lead to the denaturation of proteins. Change of this hydrophobic to hydrophilic substrate is not so feasible and clear (addition of hydrophilic ligands like in the case of biocompatibility could be a solution). Also, the concentration of PDMS influences the adhesion property to a great extent. Whether it is permeable to gases?

- We should take care that the microorganisms are not stable at the temperature in which PDMS is made. An issue is to analyze beforehand what would the fate of the bacteria at other temperatures!
- The thickness of the layer was proposed to be in the range of a few mm and surface modifiable.
- An alternative to PDMS could be polyurethane and to have an amphiphatic surface.
- It was suggested to make the surface rough by means of processes like salt leaching, addition of clay particles like bentonite, aragonite, etc. which would ultimately increase the surface area.
- Is the bacteria sequenced?
- Does the polymer surface form biofilms? This would decrease the enzyme secreting efficiency but has several other advantages!
- The availability of protocols for cloning/engineering the microorganism, its plasmid tolerance and copy number should all be taken care of.
- What kind of selection system to be adopted? (Incorporation of antibiotic resistant genes, resistant to aflatoxins).
- How tightly will essential oil bind to polymer surface?
- Lipases can be expressed by the bacteria -> kills fungi. Lipase would be released after the mold is gone. What if lipase gets released early??
- Why chitinase only from Serretia?
- Is it okay to use glucose or acetylated sugar moiety as promoter?
- Will the amount of bacterial replication affect the process in any way?
- The knowledge of the amount of enzyme per surface area, replication of the fluorescens, population control and the strength of the promoter/inducer would be helpful.
- Where our foil could be tested? – An option would be to test it on a piece of cloth coated with calcium carbonate and observe the degrading nature of the fungi under a microscope.

PoPs measurement:

We are focusing here on in-vitro single molecule PoPs measurement.

- The DNA strand with multiple probes could be ordered. Labelling of RNAP is a bit tedious and hence should be analysed and planned well before the start of the expt.

- Which part of the RNA pol will first encounter the donor? What effect would it create on the FRET signal? The alignment of the DNA molecule is important too!
- Which part of the RNA pol is the labeling done? (If the labeling is found at the DNA exit point as we want, then we could get it from Grill's lab).
- If we intend to increase the number of RNA pol molecules used, then it might increase the background noise. The concentration of RNA pol could control/change the elongation rate and hence it should be thought of.
- Attaching/immobilizing the DNA strand could be done by means of strep/biotin setup or by means of tweezers (is it actually required???)
- The G-C island would slow down the transcription rate but wrong incorporation might lead to no signal during FRET measurement. Also, when labeled NTPs are used, it would change the transcription rate again which might influence on the FRET.
- It is essential only to measure the relative strength of promoters and not the absolute value.
- In case of false positives for random dehybridization of dsDNA, normalization against RNA pol system could be carried out.
- The melting temperature is dependent on the G-C concentration; and hence the temperature we use will depend on the size of DNA we will use.
- Which promoters could we use and on what basis should we select them?
- One good point is that if all three sub projects work out, we could merge them all into a major idea!

<u>Talk with Seidel:</u>

- A tweezer is not really required as it is not important for the DNA to be linear to analyze FRET measurements. It should be enough to connect one side of DNA to a glass slide and use it for further measurements.
- A talk with Mike for a more practical knowledge on FRET is important. Reading the article on PoPs that Adithya sent would be useful for solving issues in background noise and photo bleaching issues (concentration of RNAp essential in this context).
- Other issues on fluorophore lifetime, stability of the fluorophore under specific conditions is very important.
- When we use several flurophores and quenchers, the appearance of many signals and the difficulty in distinguishing between them is the problem.

- An experimental control with just the polymerase and another with just the fluorophore could be maintained to analyze and separate background noise.
- Major things that need to checked out:

1. The donor – acceptor pair with high quantum yield (Cy3-Cy5 maybe?)
2. Standard fluorophores with high lifetimes (emission 500 – 600 nm?)
3. Promoters for FRET
4. Check for company that makes DNA with Donor-Acceptor of our interest.
5. Use of less Laser Power? (This also has drawback).

Sensor Bricks:

- Expression of the fusion protein on the yeast cell surface would be a concern.
- How do we cleave the fusion protein from the yeast surface eventually?
- It is important to make sure that only the bound Abs remain finally and hence the washing.
- It is essential to engineer the Ab fragment that it binds to LuxI.
- From where do we get the parts for each of the sub – projects discussed?
- Team Chiba already has the LuxR mechanism implemented in iGeM'09 which would be useful for us.
- The British Columbia team (2009) has the traffic light quantification system which our E.coli would use.
- What if the promoter is leaky? (normalization with background is a possible solution).
- In the washing step, how could we ensure that all of the fusion protein is washed away?