MINUTES OF THE FIFTH IGEM MEETING

04/23/2010

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from supervisors: Annelie Oswald

During the meeting, besides the organizational issues discussed, the team reviewed the situation on sponsorship, talked about potential funding opportunities, presented the progress on some old ideas and improved them. Some new ideas were presented as well.

ORGANIZATIONAL PART

MINUTES

Regarding the minutes we agreed to circulate this task among the members of the group so that one different person is responsible for each meeting. In addition we agreed to set up a template for the minutes to be posted on the wiki so that everybody can write down the things he/ she talked about during the meeting. In this way the minutes would be available earlier for the entire team and the task for the minutes-taker will be reduced to grammar checking, minor rearrangements of the material, addition of the organizational part and some other issues.

The team discussed the possible time frames when they are free to meet the PI's (check the email).

OTHER ISSUES

INFO ON PRIZES AND MEDALS

Victor talked about the possible categories of prizes and medals pointing to the requirements necessary to be fulfilled for the bronze, silver and in particular for the gold medal (where actions towards the integration of the IGEM projects should be visible like improvement, characterization of an already existing Biobrick, etc…). The information about the number of finalists, grand prizes and the entire spectra of Special prizes was included demonstrating the need to adopt the right strategy in order to be eligible for more of these categories.

SPONSORSHIP

Mareike presented the report on the sponsorship part. We talked about the opportunity to split in subgroups and go all to the Bonding event on the 3rd and 4th of May (Hörsaal Zentrum) – about 130 companies, most German will be present there, some of them specialized in Biology and Natural Sciences, plus companies that can sponsor aspects apart from scientific (Lufthansa). The team will

have a briefing how to present themselves on the exhibition in front of the companies next week. Additionally a Curriculum Vitae of the iGEM team will be created to hand over to the companies.

Some hopes were stated with respect to a foundation from TU-Dresden (Gesellschaft von Freunden und Förderern) to support young scientist.

We decided also to contact some local companies unrelated to science but interested in being advertised.

OLD IDEAS' PROGRESS

PROCALCITONIN DETECTION IDEA AFTER MEETING WITH DOCTOR BACHMANN

Lucas and Svea presented the results of the meeting with Dr. Bachmann.
The original idea was to engineer the human calcitonin receptor and protease onto the surface of a biomarker cell that could turn into a more cost effective and easier (than the antibody assay) way of detecting bacterial sepsis and thus distinguishing bacterial infections from viral. However, Dr. Bachmann stated that it could be difficult to express the functional human calcitonin receptor on the surface of the biomarker cells.

He objected that it can be really difficult to use the human calcitonin receptor in *E. coli* and to put the working receptor on the cell surface **to have the transmembrane domain in the bacterial membrane, the ligand binding domain on the outside and the signaling domain inside the cell.** He also noted that we have to bring the whole G-protein coupled signal transduction in the *E. coli*.

Asked how useful an assay for bacterial infections could be, he confessed that a doctor would normally see the difference in the symptoms of the disease. It would be more useful in a therapy to detect its state and the amount of remaining pathogens (for instance during an antibiotic treatment). There are a few diseases where you can't differentiate but normally plating cells would tell you more about the kind of the disease.

NEW IDEAS

1) SUGGESTIONS FROM PROF. BACHMANN wiki link

1.1) Toll-like receptors for bacterial/ viral sensing

The idea of Prof. Bachmann was to use Toll-like receptors (TLR) like TRL 9 that detects unmethylated, neighboring Cytosine and Guanine islands (CpGs) as bacterial markers or TLR 3 that detects double-stranded RNA as viral markers. So we could use one or more TLRs on the bacterial surface (of course not for bacterial detection) or in eukaryotic cells. He also said that his group works with eukaryotic cells which stably express TLRs on their plasma membrane and he offered us to use them. Useful TLRs for our purposes could be TLR 7, 8 and 9.

1.2) Building of an artificial receptor

One of his ideas was to build an artificial sensor from a known cell-surface receptor of *E. coli* having its ligand-binding domain replaced by the variable domain of an antibody coupled with a serine-glycine polypeptide (referred to as a single chain variable Fragment or scFv). We could get this scFvs from his workgroup.

1.3) Sensing tumor cells

Dr. Bachmann had another idea about engineering tumor sensing bacteria for instance to measure the amount of cancer cells in the blood in order to detect metastases or a variety of acute monocytic leukemia with CD33 as antigen. So a doctor could easily detect the stage of a tumor and the success of antitumor therapy. The advantages compared to the use of a fluorescently marked antibody coupled with fluorescence activated cell sorting (FACS) analysis could be the higher sensitivity and the costs. We even could use the color fading system to output the signal in an easy detectable way. Along with FACS this assay would be a really useful quantitative monitoring system for tumor cell antigens.

This idea was viewed by the team as having a bigger potential compared to the viral and bacterial detection systems. It became also obvious that such a system could be easier engineered using a yeast system vs a bacterial one because we can use the signaling pathways available instead of having to engineer them *de novo* plus it seems easier to achieve the proper orientation of the desired artificial receptors.

A potential problem could be represented by the difficulty of fusing the transmembrane and the intracellular parts of a receptor with a Single Chain Variable Fragment (scFv) specific for Cd33 (tumor marker) in order to create a FUNCTIONAL receptor able to transduce the signal upon and ONLY UPON binding of the specific Cd33 (or other tumor marker).

In this context Jon also pointed to the possibility of using fluorobodies instead of engineering a signaling pathway into the marker cells. These were an attempt with limited success (rejected article in Nature because not sufficiently convincing) to fuse antibodies to fluorescent proteins so that they emit the signal upon binding the antigen. The possibility of using this trick would absolve us from the need to deal with the signaling pathway to produce an output.

1.4) Build a multi antigen sensor in one receptor

Another exciting idea from the interview was to build a platform for many antigens for example by using an epitope coupled scFv (so this is scFv type a) to any other desired epitope in the media and on the other hand by building a receptor with a scFv-Domain (type b) able to recognize the scFv type a. This would represent a multi-purpose antigen sensor system. He offered us to use his scFv-s or complete cells and stabilized tumor cells that express these antigens all the time.

A significant problem would be to avoid false positives because normally either in the presence or in the absence of the antigen the scFv type a will bind the scFv type b triggering the response, unless the epitope that acts as a linker is not exposed by scFv type a only upon binding the target antigen.

Mareike and Lukas agreed to work further on the tumor sensing cells idea.

2) POPS MEASUREMENT *IN VITRO* BASED ON FRET wiki link

Below is the model generated by Jon based on a previous idea of measuring transcription in PoPS units (polymerase per second) by FRET (Fluorescence Resonance Energy Transfer):

Previous approaches to measure PoPS have been indirect, usually involving the measurement of protein synthesis and ultimately relating this back to transcription levels.

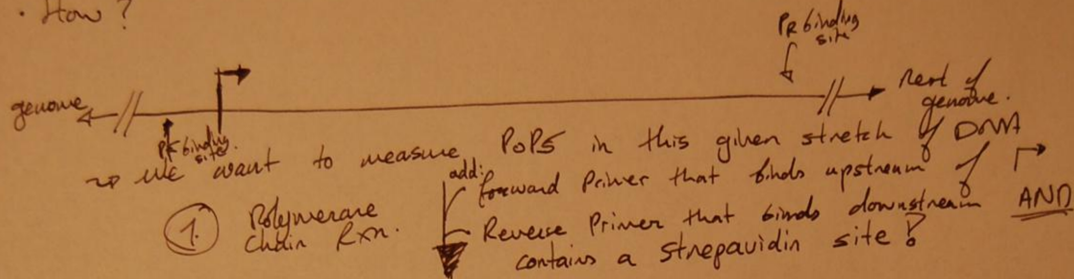
At this meeting we discussed the possibility of engineering an in vitro system for PoPS measurement from a single promoter using a system based on Fluorescence Resonance Energy Transfer.

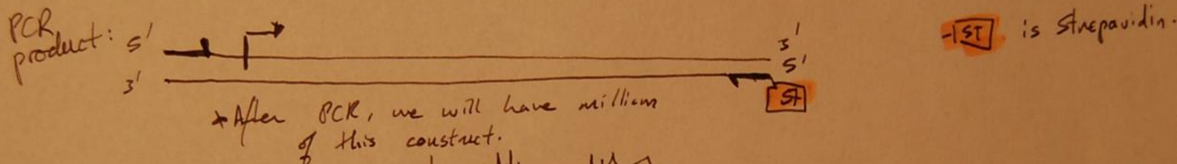
The PoPs measurement would include the following steps:

1. PCR amplification of a gene from a template using a forward primer and a reverse primer with a streptavidin tag attached (on the image P_F and respectively P_R).
2. Incubation with biotin-tagged FRET acceptor
3. In vitro incubation of the product from step 2 with a fully functional RNA polymerase containing the FRET donor attached
4. Detection by FRET and the derivation of PoPS

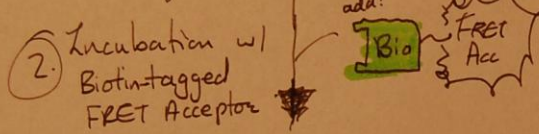
PoPS Measurement in vitro based on FRET.

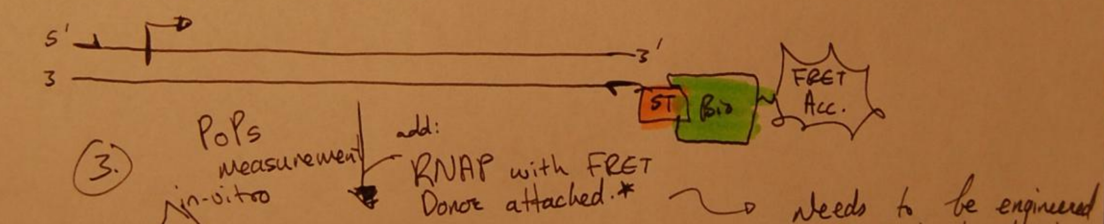
- fundamental question to be answered:
  - Q: How many RNA Polymerases (RNAP) passes along a given stretch of DNA under the control of promoter X (↱) over a period of time?
- Idea: PoPS Measurement in-vitro based on FRET.
- How?



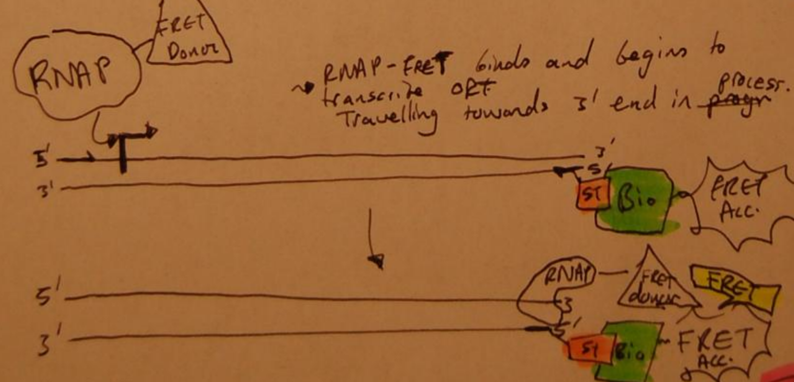


-[ST] is Strepavidin.





→ Needs to be engineered such that transcription is not jeopardized.



Emitted λ that we can detect as blips/flashes due to FRET.

Please note that the output were intended to be blips or flashes as the RNAP-FRET Acceptor approaches the 3' end.
If we can quantify the number of blips/ flashes over a period of time, we can easily derive the rate. This rate is PoPS.

Problem 1: We are only interested in the PoPS from a single promoter present on *a single copy of the PCR construct.* We have, however, millions of identical PCR constructs present in the same test tube. How can we determine PoPS from a single promoter?

Solution 1a: Should we choose to quantify PoPS exclusively *in vitro*, we can use serial dilutions and standard curves to determine the relative (or absolute if other parameters are known) rate of a single construct by extrapolation.

Solution 1b: There is always the possibility of cloning individual PCR constructs onto single copy plasmids and measuring PoPS after transformation.

Problem 2: Is there sufficient *spatial proximity* between the FRET donor and acceptor to initate FRET? Moreover, will the RNAP remain at the 3' end sufficiently long enough for the event to occur?

We will arrange appointments with PIs at the various institutes to find out more about this. As of now, we can only speculate.

Problem 3: The use of the streptavidin-biotin binding system to anchor the FRET acceptor to the PCR construct can pose a problem due to their relatively large structures.

Solution: We will need to look into alternative methods for anchorage. A possible alternative would be the Taqman-based probe used in the chemistry of probe-based real-time Polymerase Chain Reaction.

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Problem 4: Some students expressed concerns of false positive events that occur by the coincidental proximity of the FRET donor and FRET acceptor in solution. These events have a very low probability of occurrence but can pose a problem at higher concentrations.

Solution: To this end, a negative control, in which only the FRET donor-RNAP and Reverse Primer-FRET acceptor are present, is required. This is also opportune because it also allows for *normalization* of the signal to basal levels.

3) Rahul presented his ideas and the team discussed them:

3.1) Bacteria producing packaged plasmids for transformation

Bacteria producing plasmids and packaging them into vesicles in order to fuse these vesicles with the other bacteria to be transformed (a way to replace the transformation steps for example via electroporation that is not always successful and involves procedures for making the cells electrocompetent). It would be possible then to produce different transforming strains (one bacteria can transform with one kind of plasmid).

3.2) Magnetotactic bacteria used for protein separation

Magnetotactic bacteria can be engineered to produce certain receptors on their surface so that after addition into the medium they will bind the desired cargo protein and can be extracted out of the solution.

3.3) DNA sequencing by measuring the conformational change of a DNA polymerase during replication

The supposition is that the DNA polymerase changes its conformation by travelling along each of the nucleotides of the template sufficiently in order to modify the conformation of a fluorescent protein attached to it (fusion proteins) and produce fluorescent signals of varying intensity.

Supposing that the system works a potential problem could be the high speed of the replicating DNA polymerase that wouldn't allow the detection of separate signals.

4) GETTING PHOSPHORUS OUT OF SLUDGE

Svea came up with the following idea:

The world is running low on phosphorus. For instance only in the last few years the prices for it raised with 700%. There is a new system in Germany that uses clearing sludge (urine contains phosphorus) to produce ash with about 20% phosphorus than can be subsequently used as fertilizer. The technology of obtaining this ash includes two burning steps that are necessary to eliminate the toxic heavy metals out of the ash. Thus a lot of energy and expenses are linked to these steps. So is it possible to engineer bacteria able to import phosphorus in most or at least one predominant oxidation states or chemical compounds via an engineered or natural transport system? Then probably collecting the bacteria out of the reservoir won't be a problem since the buoyancy can be controlled by engineering the air vesicles producing genes or the cells can be modified to effectively aggregate when triggered. Possible improvements could also be related to engineering a phosphorus storage protein along with the import system. Sharing the team's enthusiasm about this idea Svea agreed to continue the investigations.