Minutes of the ninth iGeM meeting

14/05/2010

Participants: Rahul Akkineni, Habib Bukhari, Charanya Sampathkumar, Svea Grieb, Victor Gordeev, Adithya Nagarakodige, Mareike Roth, Lucas Schirmer.

Supervisors: Johnson Madrid

Organization:

1. The criteria on whose basis the ideas should be voted was discussed and the group gave top priorities to the following:
   - Which category of prize does the idea fall in?
   - Existence of previous biobricks relevant to the current idea
   - Applications of the idea
   - Timeline
2. Johnson gave a few valuable suggestions including:

   - It would be beneficial for the entire team if each team member shares the literature and background study of his/her idea with everyone and the remaining team members also participate actively by suggesting related articles/literature.
   - It would be useful and interesting to read and get to know about the concept of "Brainbows" which is basically the process in which the individual neurons of the brain are mapped to fluorescent proteins.

3. Organization of some PI talks was discussed and the group decided to contact Prof. Francis Stewart and Prof. Bernard Hoflack regarding the same. The members responsible for this are Habib and Mareike.

4. Johnson also brought to the team's notice that Prof. Petra Schwille and Eric Schaffer would give PI talks in the near future, the dates yet to be decided.

5. The team's first outing first took place on Wednesday the 12th of May when the team went out for bowling and dinner. The outing proved really productive for the team members wherein all of them interacted well with one another on an intellectual and informal basis, bringing them closer than ever!

6. The designing of the real Wiki webpage was talked about and Lucas offered that he already has some ideas about it and the remaining team would help him do it successfully.

7. It was generally decided to take snaps of the iGeM 2010 team which could also be used for uploading onto the Wiki page.

8. The group outing was a success and all the members felt that a team building activity along the same lines would be good for the team. Kletter Garten won the most number of votes and the date of the outing would be decided soon.

9. A brief introduction of the Filezilla application was given by Lucas for the purpose of storing all the presentations onto a common space; about which he would elaborate in the next meeting.

10. Mareike offered to email Susan about the requirements of application for the US Visa.

11. The Spring workshop in Paris organized by the iGeM 2010 would take place on the 12th and 13th of June and one instructor and a team member would attend it; who would be voted for in the next meeting.

12. The next meeting would take place on Monday, the 17th of May where further developments in each person's idea would be presented and discussed.

Project Ideas:

In this meeting, the final presentation of ideas took place. Charanya and Victor presented their ideas and Mareike added a few updates on her idea. A powerpoint presentation was given by the corresponding member and a discussion followed.

1. Recycling of carbon dioxide into hydrocarbons for fuel production.

Charanya presented her idea which was about the recycling of carbon dioxide into useful hydrocarbons and liquid fuels.

Motivation:

The past year 2009 was the warmest year in a record of 130 years of global instrumental temperature records. The upper safety limit for atmospheric CO2 is 350 parts per million (ppm) and atmospheric CO2 levels have stayed higher than 350 ppm since early 1988. This is indeed an endangering fact! Having this motivation in mind, several groups have been working towards the recycling of carbon dioxide.

Previous work:

- Photosynthetic bacteria *Rhodobacrer sphaeroides* and *Rhodobacrer capsulatus* were engineered to produce ethanol from CO_2.

- Biodiesel from algae has been proposed as one of the most efficient ways of generating biofuels; involve several intermediate stages for recycling CO_2 into usable fuels or chemicals, which increases production costs.
- Ethanol has also been produced from CO_2 and water using an engineered *S. elongatus* strain.

Abstract for the presentation:

The bacterium of interest could be engineered with the RuBiSCo enzyme to trap CO2; and provisions could be made to trap sunlight (in case of bacteria other than cyano/photosynthetic bacteria). The conversion of CO2 to isobutyraldehyde, ethanol and methanol could be mediated by engineering the bacteria with the necessary genes responsible for the production of the enzymes involved in the formation of intermediate products and ultimately, to isobutyraldehyde. This could then be converted in a biological catalyzed process, into isobutanol and other useful products which shall serve as fuels and substitutes for gasoline. In case of isobutyraldehyde, easy product recovery and from previous work done, production rate is high as well and comparable to the biodiesel production of algae.

Advantages:

- Isobutyraldehyde has a low boiling point (63 °C) and a high vapor pressure (66 mm Hg at 4.4 °C), and so it can be readily stripped from microbial cultures during production.
- Isobutyraldehyde can be readily converted to various hydrocarbons currently derived from petroleum.
- An attempt to produce different fuel sources such as isobutanol, ethanol, methanol in a single bacterium could be done.
- The bacterium could be compartmentalized so as to store each product separately for subsequent recovery.
- CO production can be induced as well by CODH enzyme (as is obvious from previous attempts).

Drawbacks:

- The cost of separating ethanol at low concentration remains a formidable problem.
- Already, photosynthetic bacteria exist for trapping CO2 alone.
- It is currently easier to use an existing and relatively inexpensive chemical catalysis process to convert isobutyraldehyde gas to isobutanol.
- Product recovery decides the cost effectiveness and hence is ambiguous!

2. An alternative genetic tool acting at posttranslational level using the prion concept.

Victor presented the second idea which was something really novel exploiting the concept of prions.

Background

To design an alternative genetic tool acting at posttranslational level using the prion concept and to apply this tool in yeast cells for building memory-enabled sensors or as an advanced goal “yeast stem cells” (other applications possible).

Prions (proteinaceous infectious agents) are proteins that can “replicate” their misfolded, usually disease-associated state using the normally folded protein of the same amino-acid sequence. In mammals it is associated with a range of deadly, incurable diseases like mad-cow disease, Creutzfeldt-Jacob, scrapie etc.

The misfolding can happen spontaneously creating nucleation sites and leading to exponential conversion of the pool of normal proteins into prion conglomerates but is also dependent on the amino acid sequence of the proteins, for instance a higher number of oligopeptide repeats triggers misfolding at much higher rates than the usual number (several orders of magnitude).

In yeast after a spontaneous nucleation event a specific translation termination factor called Sup35 that is also a prion is sequestered from the cell pool of normally folded Sup35 proteins and converted into aggregates of misfolded Sup35 proteins thus allowing higher probability of read-through past ochre stop codons in a wide range of transcripts (normally Sup35 prevents read-through allowing proper termination at stop codons).

Because a translation termination factor is affected the translation of a wide range of transcripts in the entire cell is modified thus leading to a significant change in phenotype while the same genome is preserved and this is viewed as an evolutionary advantage since each of these phenotypes is responsible for the organism survival on different carbon sources or different antibiotics (but probably also other traits).

The prion phenotype can be reversed by treatment with guanidyl hydrochloride or by knock-out or over expression of HSP104 (a chaperone) and this can serve as a memory “eraser”.

AIM

As an **easier goal** we can design a multisensor device where the sensing promoters can be coupled each to the sequence coding a prion that would misfold at a very high rate triggering the misfolding of the pool of wt proteins in the cell containing almost the same coding sequence (figure below). Thus the yeast cells should turn from red to white and pass this trait from now on to the next generations (individual white cells however can appear from time to time). But the cells can again become red if guanidine hydrochloride is added or HSP104 levels are manipulated thus erasing the memory regarding the activation event on one of the promoters.

As an **advanced** goal cells can be designed to stably switch on and off at translational level entire packages of engineered genes towards the accomplishment of a specific metabolic or synthetic task

in analogy to how yeast can render functional some of their silent transcripts by acquiring the prion state. (The pattern of the genes where read-through events happen in presence of the prion form of Sup 35 must be used for the switchable genes we want to engineer). A proof of concept on just two switchable genes should be sufficient.

Advantages/ Potential

- The system is native to yeast
- this system provides a heritable, erasable „long-term memory“
- stable over generations but reversible in the presence of specific factors (guanidine HCl, Hsp104)
- selection of the prion state is possible by growth medium
- New prions can be engineered *„de novo“for* specific aims or already existing prions can be used including the translation termination factor Sup35.

Challenges

- a single protein can adopt multiple wrong conformations as a result of its misfolding these conformation variants determine the efficiency with which amyloid fibers form, based on this weak and strong prion phenotypes are possible
- the number of nucleation sites determines the time needed for the transition of the entire pool of convertible proteins to prion variants, so triggering might be less efficient by expression of soluble misfolding proteins (as in our case) and not fragments of amyloid fibers (which act like real nucleation sites).
- The prion phenotype in yeast also happens in response to stress although with a low frequency.

3. Mareike added a few updates on her previously presented idea regarding Antimolds.

After the presentation on 07.05.2010 it was suggested that the modified microorganism is directly included in wall paints or wall paper. Wall paints are composed of 3 major ingredients: pigments, binder and solvent. Pigments determine the color and texture of the paint. The binder is the ingredient that confers adhesion to the wall and binds pigments together. The solvent is used to adjust the viscosity of the paint but is evaporated once the paint is dried. During curing the binder polymerizes which displays the second step of "drying". A thorough study of the ingredients led to the conclusion that this is not a suitable living environment for microorganisms. This would need to sustain a very long time which means to also provide nutrients over that time. Therefore the preventive treatment in this means does not show more advantages. Application of a foil in order to treat already existing mold seems more promising. An enzyme called, chitinase, can be used to target the mold. It has already been expressed in E.coli and seems to affect several species.