Protocol of the second iGEM meeting (04/01/2010)

The meeting started with a discussion about a **group wiki**. We will be provided with a wiki by the iGEM homepage but since this will be accessible for anyone we decided to also create an own group wiki. Lukas will set everything up as soon as we get some free space on the Biotec-server.

Then every group member presented his/her favorite project out of the ten iGEM projects from 2009 he/she studied:

Svea presented the project of team **Valencia**. They were making a **"bio-screen" of voltage-activated cells**, where every "cellular pixel" produces light. The team used the electricity sensibility of calcium channels to produce yeast luminescence as a response to electrical stimulous. Therefore they worked with a genetically modified yeast strain expressing aequorin. Aequorin is a bioluminiscent protein responsible of the emission of light in jellyfish. In order for this protein to produce light, it has to be coupled to a prostetic group: coelenterazine. Coelenterazine is oxidized when calcium ions bind to the aequorin-coelenterazine complex and, as a result, light is emitted. To sum it up, they induced a membrane depolarization by supplying transient electricity to their yeasts, thus opening voltage-dependent calcium channels to produce a calcium entry to cytosol and, as a result, get light emission from aequorin.

Sarah found interesting the project of the team from **Shanghai** because it was related to biological clocks. They created a prokaryotic bioclock based on a toxin-anti toxin system. The system based on E-coli that alternates between activity and dormancy compared to hibernation in some eukaryotes. RelE toxin basically inhibits translation and colony formation, and RelB antitoxin inhibits those effects. Possible applications of such a project were the preservation of scientifically important bacteria and since those in the dormancy state highly preserve their genetic information, it can be important.

She also presented her original idea: **creating a biological clock made of circular microtubules with Kinesin motors** revolving around it. The system should have an input to switch it off and on, and an output to calculate the exact position of the motors. The team discussed that a possible switch could be he addition of ATP and that we could vary the speed of the kinesin by modifications of the microtubules since the step size of kinesin depends on the alpha and beta units of the tubulin.

Lukas idea was to build a kind of **biological clock based on the genetic parts the Team Todia-Tokyo left**. It should be quite easy to build the whole thing on a genetic oscillator assembled of lacI and araC with a lacI/araC sensitive promotor. This would lead to an oscillation auf lacI and araC with an interval of approximately 10 min. It would be possible to build a round 10 well plate with the bacteria and a GFP underlying a araC promotor to build a kind of minute hand. The major problems are to start the bacteria in each well 10 min after the previous well or to synchronize the bacteria. There was also a switch to turn the system on by UV-light and off after a specific amount of time.
The group discussed this idea for a while and came to the point that it would be a great idea to use this system from Tokyo since they worked on it for 3 month and were close to finish it. Problems would be that these kinds of oscillators often are not consistent and that we would need a reliable system to switch the „clock" on and off.

Viktor presented briefly all the projects he read about:
MoWestern_Davidson: Making cells solve mathematics by joining certain AND and OR gates to make a system of living cells always pose the TRUE value (a fluorescence protein expressed) as an output (solving the satisfiability problem).
Team **NTU-Singapore: Alternative treatment for atherosclerosis**. A system was first modeled into E. coli with the further intention to introduce it into a macrophage chassis. The system can sense plaque build-ups and activate the expression of 3 elements : a cholesterol degrading enzyme, a reporter protein (for in-vivo imaging) and vasodilator.
Team Osaka: Programming cells to automatically form various pattern and gradation of colors by sensing cell identity and density by means of quorum sensing – result: cells can create artistic masterpieces.
Paris: Engineering a communication platform by controlling outer membrane vesicles (OMV) production by destabilizing membrane integrity through overexpression of specific periplasmic proteins (e.g., TolR). A targeting system was developed, based on the outer-membrane expression of Jun/Fos leucine zippers to control the vesicle flux between donor and recipient cells. Once received, the signal from incoming vesicles is transduced through a modified Fec pathway, whereby the receptor is provided by the OMV. Such reliable communications systems have wide biotechnological implications, ranging from targeted drugs delivery and detoxification to advanced division of labor or even cell-based computing.
Team **PKU_Beijing**: Developing a series of AND gates with a memory module that allow cells to convert the information about the concurrence of two signals (conditioned and unconditioned) into its memory. After the memory module is switched and given the "conditioned stimulus", the E. coli cells will pass the information to the reporter module and thus exhibit the "conditioned response" (just like in higher organism).
Team **NCTU_Formosa: A genetic circuit working as an OR gate** producing an output signal when any of the two or both input factors are present. The output is a red fluorescent protein (RFP) and the input signals are either the amount of lactose or the presence of certain bacterial proteins. The lactose amount serves as a timer triggering the transcription of RFP when the amount of pre-added lactose decreases. The bacterial proteins that is an indicator of bacterial contamination also triggers the transcription of RFP. The project can be applied to storage warning signs of fresh food, contact lens, and wound dressing.
NYMU-Taipei: Designing inoffensive Virocatcher cells to express antibodies and cell surface receptors (CD4 (for HIV), Integrin (for various viruses), Sialic Acid (for Influenza), and Antibodies (for Influenza)) that will bind viruses, sense this event and transduce the signal for a feed-back control, and remove the cell with the cargo by triggering digestion by macrophages.
Team Nevada: The objective of this project is to **engineer the cinnamaldehyde (an insecticide against mosquito) biosynthetic pathway into E. coli**. By introducing the genes encoding for certain enzymes necessary for transforming phenylalanine into cinnamaldehyde the system was proved to be working in E. coli and intended to be engineered into duckweed (an aqueous plant on which mosquito feed) - excellent vehicle to deliver cinnamaldehyde for mosquito control.
Purdue: CD133+ hunting machines were created by genetically engineering microglial cells (BV-2) – dynamic cells responsible that stand as the main immune cells in brain. The engineered BV-2s were equipped to locate the specific GBMs and label the targeted cells with a tat-GFP fusion protein. Once labeled, they can be destroyed. They were thinking for the future to introduce an apoptotic factor in all this game.

Rahul spoke about the **Team Missouri_miners** with their project „**A Synthetic Biology Apporach to Microbial Fuel Cell Development Utilizing E. Coli**". In this project E.coli was manipulated to release electrons into aerobic environment. Extracellular pilin, MacA, OmcB, OmcE, OmcS, and many other cytochromes were isolated from Geobacter sulfurreducens and introduced into E. coli to have microbial fuel cell apparatus. The pathway is as follows: electrons are produced by NADH Dehydrogenase in cytoplasm. these electrons are trapped by protein MacA which are transferred to complex of cytochromes in periplasm. From periplasm the electrons are transferred to proteins OmcB, OmcE, OmcS in outer membrane. Finally, transferred to pilin to external environment. Such a system can be used similar to flurorescent protein markers, to monitor cell activity. Such bacteria can be used as cheap and effective power source in micro & nano electronics.
Team **MIT: Photolocalizer**. This project deals with reversible control of gene expression (post translational control and to specifically targeting protein in yeast) using pulses of light. Switchable promoter system consist of a phytochrome PhyB, bound to chromophore PCB. In red light, PhyB changes conformation into it?s active form and can bind to a transcription factor called PIF3. And in far red light the phytochrome is inactivated. Yeast was engineered to produce PCB and PhyB-PIF3 endogenously. Either PhyB or PIF3 is anchored to target location then the protein is localized at that location when pulsed with red light. Such a system can be used to synchronize a culture of cells without adding any chemicals etc. Provides an easy way to switch on-off expression of essential genes and also increase or decrease their expression quickly using light.

Jon presented the idea of the team **Brown „A Synthetic Approach to Treating Nasal Allergies".** Nasal allergies are in particular due to the excessive release of histamine in the nasal cavity. The normal treatment involves antihistamines that block the histamine receptors and also the brain receptors , which leads to drowsiness and poor concentration. The Brown team engineered *Staphylococcus epidermidis* – native to nasal flora – to secrete a recombinant histamine binding protein (rEV131) as a response to elevelated histamine levels during allergic response. Therefore competing with human receptor for ligand. The Idea is to have this engineered *S. epidermidis* strain act as a self-regulating drug factory in the nose.
How it works: Mast cells produce histamine during allergic response. Free histamine binds engineered histamine receptor on *S. epidermidis*. Signal Transduction. This histamine receptor has the intracellular EnvZ kinase domain, which in turn phosphlorylates the TF OmpR. Which leads to an increased expression of rEV131 downstream of OmpC promoter. REV131 is an histamine-binding protein that binds histamine with higher affinity than our receptors. It contains secretiong signal on N' Terminus which allows recognition by Sec secretion system. The transcription/ translation elevated as long as ligand present.
Problem: First, *S. epidermidis* is generally non-pathogenic but biofilm formation could cause problems. Second, normal Quorum Sensing: high density of cells leads to higher levels of auto-inducing peptides (AIP). AIP induces agr operon: more AIP produced, biofilm genes expressed, and (in some cases) pathogenic genes. Sol'n: insert DNA gyrase inhibitor gene under the same promotion of agr operon promoter, so that cells die off at dangerous densities.

Mareike presented the idea of the group **Harvard**. This team has constructed a system that allows for interspecies, **bacteria-to-yeast optical communication**. In this system, bacteria to communicate to yeast the presence of IPTG, which results in transcription of lacZ in the yeast cells. To permit bacteria to send an optical signal, we expressed in E.

coli a red firefly luciferase under IPTG induction. To allow yeast to receive the signal, they used a two-hybrid-system based on the interaction between the red-light-sensitive Arabidopsis thaliana phytochrome PhyB and its interacting factor PIF3. Interaction between PhyB and PIF3 is induced by the red light from the bacteria, resulting in transcription of the lacZ gene.

Adithya presented the work of three teams. **Team Aberdeen_Scotland had a Synthetic Biology Approach to Pipe Repair: The Pico-Plumber**. An E. coli circuit for pipe repair was designed. Pipe breach detection and the restoration of pipe integrity were implemented through exploitation of chemotaxis, and cell lysis that releases a two-component protein-based glue (lysyl oxidase and tropoelastin). For controlling AND gate is used with quorum sensing for the timing of the release of glue proteins.
Team Alberta synthesised an artificial genome for an artificial cell by modular biofabrication method (BioBytes).
Team BCCS-Bristol's project is called VESECURE. Gram-negative bacteria's outer member vesicles diameter in range 20-200nm. Using this system for cargo transport. They protect their cargo from the extracellular environment and deliver it to a multitude of target cells via membrane fusion.

Habib talked about the team **UNICAMP Brazil**. Their idea was **to build bacteria "guards"**. In normal biological synthesis there is big chance for contamination. In order to overcome this problem killer cells were designed. Their function is to find and destroy contaminants by AL2 which is released by contaminants. In the presence of contaminants ,killer cells will induce surrounding worker cells to differentiate into more killer cells by a CRE recombinase under the control of a promoter responsive to AI2. After Differentiation two killing systems can be activated
1) Killing by colicin gene, which are incoded in plasmid, and will be delivered to contaminants by conjugation.
2) And the Kamikaze system. Which is mediated by strong expression of the T4 endolysin as a result it lysis the killer cells, and they release an enzymes which destroy Gram-negative contaminants.

Annelie gave a definition for **PoPS.** PoPS stands for polymerase per second and describes the flow of RNA polymerase molecules along DNA (i.e., 'current' for gene expression). The PoPS level is set by the amount of RNA polymerase molecules that trundle past a specific position on DNA each second.

The group decided to have the next meeting on april 9 without the group leaders.