Analysis of gaseous molecules for diagnosis of disease and bananas

iGEM Project Snifferomyces

The onset of spring brings with it the sweet fragrance of the tulips heralding a new year and new beginnings. This meant the formation of a new team to compete in the 2012 version of the prestigious International Genetically Engineered Machine (iGEM) synthetic biology competition to be held at the Massachusetts Institute of Technology in Boston, to build on the successes of the 2009 'Bacterial Relay Race' team, winner of the best information processing project and the 2010 'Alkanivore' team, one of the six finalists and winner of the best presentation award for providing a green solution for oil spills. This year nine students from TU Delft and two students from Leiden University, including our own Origin editor Mark Weijers, along with seven advisors came together to form team 'Snifferomyces', with a goal of providing sniffing capabilities to yeast to achieve rapid, non-invasive and a cost effective diagnosis system for tuberculosis.

The TU Delft 2012 iGEM team

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The iGEM competition, yearly organized since 2003, is a design based team competition in Synthetic biology. Synthetic biology is a new area of biological research that encompasses a variety of different approaches, methodologies, technologies and disciplines, combining the areas of science and engineering with a common goal to design and construct new biological functions and systems not found in nature. Student teams receive a kit with more than five hundred biological parts (DNA plasmids) at the beginning of the summer from the registry of standard biological parts. Using these parts, and newly designed parts, teams build biological systems and operate them in living cells, thus providing an exceptionally motivating and effective teaching method. At the end of the summer, each team present their findings at the Jamboree, first at the European level (2012.igem.org/Europe) then, if qualified, at the world championship Jamboree. Each team will submit their new parts with a detailed characterization to the growing library of biological parts.

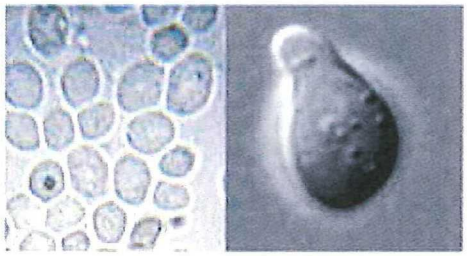
The 2012 project of this iGEM team draws inspiration from sniffer rats, which can be trained to sniff out for instance unexploded landmines or tuberculosis, based on a distinctive scent. Tuberculosis infects around eight million people a year and kills approximately two million. Drugs to treat tuberculosis can be quite effective, so a rapid diagnosis system is crucial to help curb the spread of the disease. The goal of this project is to develop a microbial-based system for detection. Several similar systems have been characterized, e.g. the I7 olfactory receptors in rats which can be coupled to microbial systems like the GPCR system in S. *cerevisiae*, or baker's yeast. Using bioinformatics and molecular tools, the team plans to develop a library of receptor proteins.

A tuberculosis infection is accompanied by a tar-like distinct odour, the abundant compound in which is methyl nicotinate. As part of this year's competition, the team is building a sensor capable of 'smelling' the presence of this compound. The sense of smell is one of the most complex and elaborate physiological systems in humans and other animals. Perception of odorants start with the stimulation of olfactory receptors(ORs) on the sensory neurons within the nasal olfactory epithelium, resulting in the activation of an adenylyl cyclase (AC), and subsequent opening of cyclic nucleotide-activated, non-selective cation channels. Electrical signals generated from OR activation is propagated in turn to the main olfactory bulb, the olfactory cortex,

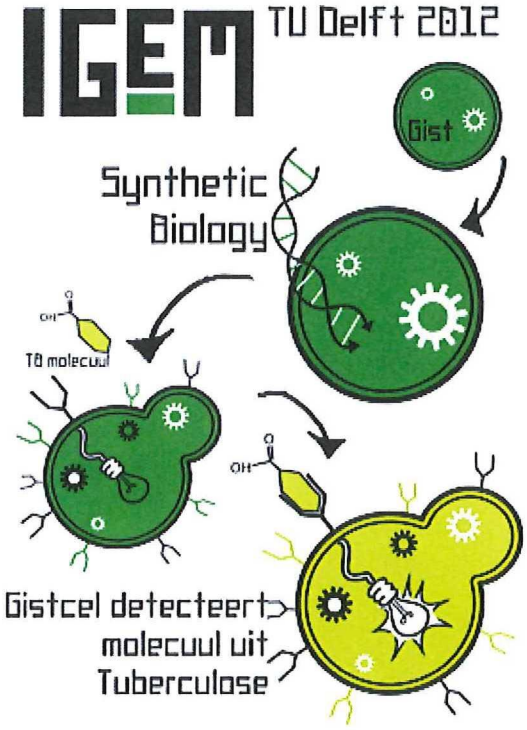
higher cortical areas and limbic structures of the brain, where eventually a perception of odour is formed.

The basic molecular mechanism of OR activation is conserved evolutionarily from yeast to humans. Haploid yeast cells exist in two cell types — '*a*' or 'α' cells, which can mate with each other to form diploids. Haploid yeast cells initiate mating with the opposite cell type upon 'smelling' the pheromone produced by the mating partners. The protein that 'smells' the pheromone released from the opposite cell type is a cell type specific GPCR, Ste2 in *a* cells or Ste3 in α cells. Stimulation of the yeast GPCR by pheromone leads to activation of a mitogen activated protein (MAP) kinase signal transduction pathway and the induction of several genes, including *FUS1*, a gene involved in cell fusion. The significant conservation of GPCR-activation-machinery between yeast and mammalian cells, the null background of mammalian GPCRs in the yeast system, the easy manipulation of the yeast genome genetically and molecularly, and the robust growth of yeast cells, all render yeast an excellent platform for the design of the proposed tuberculosis detection system.

In the first phase of the project, the yeast cells are tricked into activating the mating response for ligands other than pheromone (methyl nicotinate to be more specific) by introducing olfactory receptor gene fusions into *Saccharomyces cerevisiae*. In the second phase, the mating response signal is channelled into a light response by the use of GFP (Green Fluorescent Proteins). In this way, when the cells are exposed to the ligand they glow up. So whenever tuberculosis is in the air around the yeast culture, yeast cells will 'smell' its presence and begin to glow. A bio-chemical model of the pathway will be made use of to guide the experiments along with a bio-physics model of methyl nicotinate diffusion over a yeast culture to predict the amount of ligand concentrations available. To get a clear understanding of where the ligand binds in the receptor, and how to refine this cavity in order to let it bind more specifically to the desired compound, the team will also be making use of a structural model.



Normal yeast cells (left) and a yeast cell entering shmoo formation (right) (adapted from Wikimedia commons)



A working detection system not only has applications for sensing, but also for communication in non-aqueous environments between different species and kingdoms. The third and a more fundamental aspect of the project is to try and let yeast and *Escherichia Coli bacteria* communicate with each other. The first project of iGEM made a 'banana odour generator' plasmid, which creates a banana smell whenever it is transformed into *Escherichia Coli* cells. The yeast cells smell this odour and giving a communication signal back to the *Escherichia Coli* cells. The optimal communication system would be the one where *Escherichia Coli* and yeast cells regulate each other in their growth, by providing them with odour-inducible antibiotic resistance genes.

The olfactory system also showcases how an organism as ancient as yeast, with its rich tradition in industrial microbiology can be used, with the tools of synthetic biology, in designing systems which can contribute in an extraordinary way for the betterment of society.

See the progress of the team at 2012.igem.org/Team:TU-Delft