Introduction

An essential part of iGEM is to spread awareness about synthetic biology, which we have valued highly in this year's competition. Despite the implications of the Covid-19 pandemic, we have managed to arrange presentations and workshops about synthetic biology for high school students. In connection to those presentations, we have had several meetings with a project developer from Biotech Academy, who was also a part of the 2019 iGEM UCPH team, Iben Egebæk Nikolajsen, to promote the work that Biotech Academy does in relation to teaching high school students about synthetic biology through biosensor projects. While planning the high school visit to Borupgaard High School (Gymnasium), we got in contact with Biotech Academy to hear whether they were interested in providing us materials to present their biosensor cases that can be found at biosensor.dk (Danish) as well as taking the high school students into GMO class 1 laboratories that are required to perform the exercises that are detailed in the cases. The cases utilize a prokaryote in shape of E. coli as a model organism and teach the students about the central dogma and the basic components required for gene expression, including promoters, ribosomal binding sites, terminators, and get to know about plasmids, selection markers, reporter genes, transformations, and more. The students thus get to, in a safe and responsible manner, have their first experience with genetically modified organisms by performing their own transformations to make an effective biosensor, which they get to test themselves as well. Due to regulations caused by Covid-19, we could unfortunately not do the exercises that were included in the kits by Biotech Academy with the high school students. Instead, we took the interested students behind the scenes and in depth into the iGEM universe by introducing them to parts and system that constitute the biosensor used by us at CIDosis. As Biotech Academy were fascinated by how we went about making our biosensor, using a eukaryotic chassis instead of a prokaryotic one, we discussed and agreed to developing a biosensor case for them to be presented on their webpage, biosensor.dk, which utilizes Saccharomyces cerevisiae instead of E. coli for the biosensor purpose.

Why Saccharomyces cerevisiae as a chassis?

The budding yeast *Saccharomyces cerevisiae* was the first eukaryote to have its genome fully sequenced and has consequentially been utilized as a eukaryotic model organism ever since.[1] Following the first whole-genome sequencing of the human genome with a random shotgun method, with results first published in 2001[2], and the one of Saccharomyces cerevisiae in 1996, and since updated in 2010[3][4][5], it has been found that yeast possesses 23% homologous genes to humans, thereby making it a useful model for studies of gene function.[6] While humans and yeast

[1] Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., … Oliver, S. G. (1995). Life with 6000 genes. Science, 274(5287), 546–567. https://doi.org/10.1126/science.7542800
[2] The Sequence of the Human Genome, Craig Venter
[3] The Reference Genome Sequence of *Saccharomyces cerevisiae*: Then and Now
[4] Yeast as a model organism
[5] Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, et al. Life with 6000 genes. Science (New York, N.Y.) 1996; 274(5287):546–637.
[6] From *Saccharomyces cerevisiae* to human: The important gene co-expression modules

diverged from a common ancestor roughly 1 billion years ago, a plethora of evidence has been found for strong conservation of certain gene functions between the species. This includes several studies of protein function, both individually and in complex intracellular signaling pathways, including studies of metabolism, longevity, aging, neurodegenerative diseases, and genome stability[7].

While Saccharomyces cerevisiae is perhaps most known for its roles in baking and brewing through the process of fermentation, it is widely recognized in the scientific community as an efficient model organism for studying the physiology of specific human tissues and pathophysiology of humans[8]. S. Cerevisiae reproduces at rates comparable to bacterial cells in good to optimal conditions, and although they are unicellular eukaryotic cells, they share many of the same cellular systems as multicellular organisms, including cell walls, cell membranes, endoplasmic reticulum, mitochondria, vacuoles, vesicles, and nuclei. In addition, the protein post-translational modification- and folding system and gene expression system resembles mammalian cells markedly more than that in prokaryotes. Thus, utilizing a eukaryotic chassis for developing a biosensor case will give the students a closer insight to gene regulatory systems of human cells and provide them with a deeper understanding of the differences between prokaryotes and eukaryotes as model organisms for synthetic biology purposes. As a good student must know these key differences and how different model organisms may be more suited for some purposes than others, depending on the nature of the experiments, including the time and economy that can be spent on these, teaching students the differences between eukaryotes and prokaryotes in an experimental setting is essential for developing an understanding of their relevance to diagnosing and treating human diseases.

Practicality behind making a biosensor case (with yeast)

Firstly, biosensors are defined as devices that are used to detect the presence, or concentration, of one, or more, biological analyte, such as a biomolecule, which in the case of the biosensor used by the CIDosis team is IL-1-alpha, IL-1-beta, IL-6, and IL-10. The biosensors consist of three parts, with the first being a component that recognizes the biological analyte, which is typically a live cell, such as yeast, and produces a signal, a signal transducer/reporter system, and a technological device for reading the signal that is produced by recognition of the biological analyte [9][10]. As yeast can be made cheaply in an ”active dry” form and be stored for a long time and serve as a chassis for higher-eukaryotic sensing modalities, such as G-protein coupled receptors, and can tolerate harsh environments, compared to bacteria, this makes it an optimal component for recognizing a

[7] Longevity Regulation in *Saccharomyces cerevisiae*: Linking Metabolism, Genome Stability, and Heterochromatin

[8] Scope and limitations of yeast as a model organism for studying human tissue-specific pathways

[9] https://www.nature.com/subjects/biosensors

[10] Yeast-Based Biosensors: Current Applications and New Developments

biological analyte that we are interested in [11]. Through conversations with project developers at Biotech Academy and high school teachers from Borupgaard High School, it was evident that a major concern of the high school teachers was the time the experiments take to perform and analyze, as there is limited flexibility in the schedules of their students. Thus, it would be difficult to complete a case that requires laboratory work to be done over several days. Therefore, in agreement with Biotech Academy, we would attempt to prepare a case that takes a maximum of three to four days to complete, from start to end. Naturally, safety procedures must also be in place, as working with genetically modified organism require several installations in place, including the access to a GMO class 1 laboratory for white-listed, non-pathogenic organisms, such as S. cerevisiae, these are considered for, including potential carcinogenic and other dangerous chemicals that may be used. While such compounds may be used, to lessen the cost and potential hazards of the experiment, alternatives have attempted to be found for the proposed case.

As we at CIDosis have specialized in engineering a modular system in form of the yeast pheromone mating pathway that transduces an extracellular signal through a G-protein coupled receptor (GPCR), we found it fitting to make a biosensor case that also utilizes a modular system. Since the system is modular, this allows Biotech Academy to further develop the case in the future if they see it fitting, so that other biological may be analyzed, for example, or for modulating the sensitivity of the system. As GPCRs are involved in a variety of signaling pathways in mammalian cells and are found on the cell membrane, thereby not requiring the analyte to diffuse into the cytoplasm/nucleus to activate a receptor for the transducing activity, the pheromone pathway was agreed upon to be our pathway of interest for the case[12].

So, what is the plan, so far?

As of now, we have a clear plan of further developing our modified version of the yeast pheromone pathway. When fine-adjusted based on input from further tests of our biosensor from the wet lab, including the expression profile of our reporter gene of interest, we wanted to replace the interleukin receptors from our engineered pathway with other transmembrane receptors with cytosolic domains, suitable for the modularity of the pheromone pathway. As Biotech Academy is a non-profit organization, they try to reduce the cost as much as possible for the experiments while still having high standards for the kits they can offer. Therefore, a big issue was finding a transmembrane receptor that reacts to suitably cheap compounds while still also being appealing and relatable to the students that will be executing the experiments after preparation by their teacher. Figuring out which receptor this need to be is the last step needed for us to finish our case for Biotech Academy, as the case we present is, in essence, a direct continuation of our project. One example that we have discussed is making a carbohydrate-sensing chassis using different types of carbohydrate-sensing receptors. One such receptor could be gustary receptor for sugar taste 43a, also known as GR43A from Drosophila melanogaster, which is a transmembrane,

[11] Yeast-based biosensors: design and applications

[12] https://academic.oup.com/femsyr/article/15/1/1/543846

fructose-sensing receptor, that is not naturally present in yeast [13]. Another example could be the IR60b receptor, which seems to function as a transmembrane sucrose receptor [14].

By making different versions of Saccharomyces cerevisiae with a defined reporter gene, such as GFP, betanin, YFP, or any other colorful reporter gene that leads to a clear signal within hours of trancription being initiated, that is associated with each carbohydrate of interest, the students can go hunting for which carbohydrates are present in the solution they are given!

The receptors will be engineered to be linked with the yeast pheromone pathway, like our project. The binding of the ligand to the receptor of interest will thus lead to the intracellular association of a split TEV protease. By chance, the TEV protease will cleave our modified version of G-alpha/G-beta present in the cell. The students will have been handed out the same yeast models, but the key difference will be in the sensitivity of their chassis to react to the ligands of interest, based upon the interaction between G-alpha and G-beta/gamma. Depending on the modifications on G-alpha and G-beta, including which mutations are targeted at our mapped key interaction sites between the subunits, including where TEV insertion sites have been put to further disturb the interaction between the two subunits. In this way, the students will be able to detect whether the ligand(s) of interest are in the mixture that has been handed to them and need to be applied as "nutrients" for the yeast cells (in essence, a mix of nutrients and ligand(s) of interest with a defined concentration that the students must calculate beforehand). Following the pheromone pathway, the disruption of the interaction between G-alpha and G-beta will downstream lead to gene expression by binding of Ste12 to an inducible promoter, which in for us is the reporter gene of interest. Then, the students may see the color change, some seeing a more distinct color than others, and discuss whether this may be attributed to differences in concentration of our ligand of interest (which, as stated above, will be the same for all students), or which other reasons there may be for the difference in color intensity. In this way, the students get to reflect about more complex aspects of synthetic biology in relation to so-called "hijacked pathways", in a relatively easy manner.

As the case is more complicated than the ones that can be found on biosensor.dk, it would make sense for the students to attempt this case after having already established an understanding of what a biosensor is through one of the other cases. In this way, the students will get to work with both prokaryotes and eukaryotes, first in a relatively simple manner, to a more complicated case. As this is also representative for how eukaryotic organisms are generally more complicated than prokaryotic, this will allow the students to further reflect upon the different uses of eukaryotes compared to prokaryotes in synthetic biology.

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[13] A fructose receptor functions as a nutrient sensor in the *Drosophila* brain

[14] A receptor and neuron that activate a circuit limiting sucrose consumption