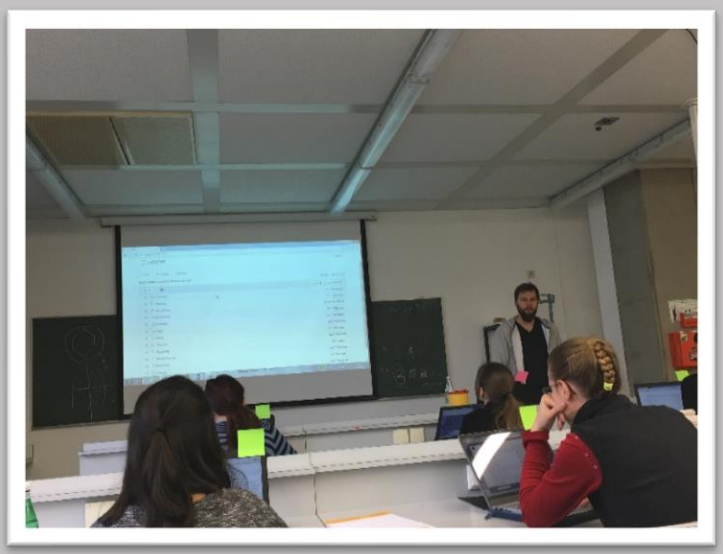
"The role of the infinitely small in nature is infinitely great"
- Louis Pasteur (1822-1895)

Laboratory start

Last month, our team finally started the lab work. Prof. Markus Pauly from Heinrich Heine University has kindly provided us with a laboratory in his institute and his continuous support.
Most of our members previously had little laboratory experience, so there was a brief period of familiarization in which day-to-day practices such as sterile work and handling laboratory equipment were being adopted.
After the division into Instructor and Member the formation of small subgroups took place for the three subprojects of our project idea.

For practical work in the various subprojects, so-called levels, the individual groups have chosen different cloning strategies. For that, the YTK Toolbox[1] and the *E. coli* MoClo Toolbox [2], as well as Gibson Assembly and Golden Gate in general are used.
With the YTK Toolbox and *E. coli* MoClo Toolbox we have now spent some time working intensively to prepare them with foresight in a joint effort of all team members for a quick and rational work. At the same time, our members were able to learn the laboratory routine in order to approach the upcoming cloning with great skill.



Further education

Not only in the laboratory, but also in the context of our scientific education, a lot has happened.
This month, our advisor Nicolas Schmelling has organized a V-module for many of our team members and other interested students. The topic of the module was the analysis as well as the graphical representation of large amounts of data using the programming language Python. These digital capabilities greatly simplify the later evaluation of our experimental data and have become a much used resource in the natural sciences.

Also in the field of biology, some team members at the Annual Conference of the Association for General and Applied Microbiology, VAAM short, have expanded their knowledge through many interesting lectures. In addition, some advisors and members were allowed to present their own projects during a poster session. Our PI (Principal Investigator), Prof. Ilka Axmann, presented her research on the circadian clock in bacteria during a lecture.

There was also a big theme evening of Synthetic Biology at Schloss Mickeln in Duesseldorf, where Philipp Rink, one of our advisors, and Marvin van Aalst, a member of last year's iGEM team, gave a talk and introduced the iGEM competition. Through the nice atmosphere experiences could be discussed

and talked about.
All in all, a massive exchange of knowledge took place that evening, as many professors from various disciplines were present and provided a good insight into new findings in synthetic biology, which can support and help us in our work through their lectures.

However, not only the people from the outside of iGEM lie in the reach of our networking specialists. iGEM team Makerere answered our call for getting in touch. During a very productive Skype meeting, all members could introduce themselves and get to know each other, as well as exchange ideas and suggestions. We intend to keep the exchange (constant) *consistent* and to organize further meetings.

Public relation

To strengthen our presence in the public, our team participated in a several big events this month.

One of the most important labware fair, Analytica in Munich, took place this month and three of our team members were there to learn about the latest methods of cultivating co-cultures and to get some companies' attention.

One the next day, on April 14, 2018, the March for Science took place, in which we saw an opportunity to show our passion for science and protest against the “fake-news” mentality. The iGEM teams from Aachen and Bielefeld were also there to march shoulder to shoulder with us. In addition, prominent speakers from science, politics and journalism gave their opinion on the topic of dealing with facts in general and the current situation in science. Afterwards, all the teams present got to know each other better during a meal together and exchanged their views.

The following week we organized a small information stand on the campus of HHU, where we also had waffles for sale. The goal was to introduce our team, our idea and the field of synthetic biology as a whole. In addition, a small quiz was used to capture the students' profound knowledge of biology topics. With a help of numerous discussions, we helped some people correct their misinformed opinions about genetic engineering.

Outlook

The next step is to make the virtually cloned plasmids in the lab, as the necessary primers for that have arrived at the end of this month.

Nutrients System:
This level deals with the organisms' dependence on essential nutrients such as nitrogen, phosphate and a carbon source, glucose, in our case.
The original state of the organisms is maintained with nutrient fixation as the only deviation. *E. coli* is no longer responsible for the fixation of phosphite but for the nitrogen fixation instead. The reverse applies for *S. cerevisiae* which is also going to be tasked with glucose delivery.

To date, gDNA has been isolated from *Synechococcus elongatus*, *Zymomonas mobilis*, *Corynebacterium glutamicum*, *E.coli* and *Pseudomonas putida*. In the next steps, the glucose transporter glf and the invertase invA are to be extracted from the *Z. mobilis*'s gDNA and introduced into the genome of *S. elongatus*. From the gDNA of *P. putida* the ptxD gene is to be extracted, which is responsible for use of phosphite and supply of phosphate for the other organisms. Subsequently, this is prepared by Gibson Assembly and introduced by a transformation in *Saccharomyces cerevisiae*.

Auxotrophie System
The aim of this system is the dependency of the same organisms as in the Nutrient System, but achieved through the exploitation of auxotrophies. *S. cerevisiae* is in our case auxotrophic for lysine produced by *E. coli*. At the same time, *E. coli* has an auxotrophy for arginine, which, in turn, is provided by *S. cerevisiae*. *S. elongatus* shows no auxotrophy and is only responsible for the production of sufficient glucose.

The further procedure for this system will now be the extraction of the Leu2 gene from the YTK Toolbox. This gene is going to be introduced into *S. cerevisiae* strain BY4742, as it is already lysine-auxotrophic.
For *E. coli*, a leucine auxotrophic strain is now being sought in which the genes ddh and lysC (isolated from *C. glutamicum*) are to be introduced.

Quorum sensing System
In this system we use the widespread quorum sensing method used by many bacteria for colony regulation to create an artificial dependence of our organisms. We focus on the sexual pheromone MAT, which can originally be found in *S. cerevisiae*.
If a cell recognizes this pheromone, it enters a cell cycle stop in the G1 phase. Normally, after an adaptation time, the cell would leave this G1 stop, but we use a cell stem that cannot perform this adaptation and remains in the G1 phase as long as the pheromone is present.
The aim of this system will be to determine the optimal regulation of the *S. cerevisiae* population by screening different promoters.
For this purpose, we employ the YTK Toolbox, as well as the *E. coli* MoClo Toolbox and the "GoldenGate" cloning process.

Since this system is extremely complex and some meetings have only been able to make small improvements, the next step is to revise the virtual cloning. Since the YTK Toolbox is used here, some special plasmids, so-called cassettes, have already been produced, which are necessary for further cloning.

We are happy to work on our project every day and share with you our strides. With your interest and support you are driving us towards delivering a great project with meaningful results in an even more motivated and ambitious way.

Many Thanks!

Your iGEM Team HHU 2018

1) CIDAR MoClo: Improved MoClo Assembly Standard and New E. coli Part Library Enable Rapid Combinatorial Design for Synthetic and Traditional Biology. Iverson SV, Haddock TL, Beal J, Densmore DM. ACS Synth Biol. 2015 Nov 4.

2) Dueber: A Highly-characterized Yeast Toolkit for Modular, Multi-part Assembly. Lee ME, DeLoache, WC A, Cervantes B, Dueber, JE. ACS Synthetic Biology 2015