# Participation of Bielefeld University Teams in the iGEM Competition of Synthetic Biology



UNIVERSITÄT BIELEFELD

CeBiTec Center for Biotechnology

# Welcome address of the CeBiTec Director

Over two decades, the international iGEM competition has gained worldwide recognition and relevance in the scientific community. It is therefore gratifying that since 2010 Bielefeld student groups are participating in this event with great success. It is very impressive to see how many individual prizes and gold medals went to Bielefeld University over the last twelve years. I therefore warmly welcome the idea of compiling the history of Bielefeld University's iGEM participation in this brochure. It provides an overview of the wide range of Bielefeld's iGEM topics and the course of their implementation.

As director of the Center for Biotechnology (CeBiTec), I am particularly delighted that the Bielefeld iGEM activities would not have been possible without the involvement of the CeBiTec. The CeBiTec has provided laboratory and meeting rooms to each of the iGEM teams, thus contributing to their great success. In addition, the iGEM teams were able to use the omics and bioinformatics platforms located at the CeBiTec and thus incorporate advanced techniques into their iGEM activities. It is also of particular note that the CeBiTec is involved in the Master program "Genome-Based Systems Biology" of the Faculty of Biology, from which a large number of students were recruited for the iGEM teams.

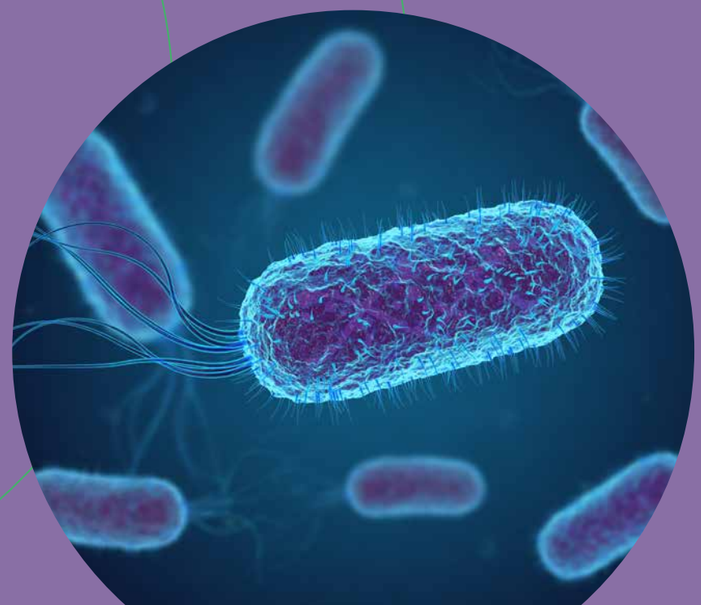
I wish the readers of this brochure much enjoyment in reading and I express my hope and trust that iGEM activities at the CeBiTec will continue in the coming years with great success

Prof. Dr. Olaf Kruse
Scientific Director CeBiTec

# Content


# 01

# Introduction to the iGEM competition



# Synthetic Biology – the core of the iGEM competition

The international Genetically Engineered Machine (iGEM) competition is dedicated to advancement in the field of Synthetic Biology. For this reason, the definition of this research field is briefly outlined here.

Synthetic Biology can be considered to build on the discipline Systems Biology which is defined as follows: ‘Systems Biology is an interdisciplinary approach that attempts to develop and test holistic models of living systems. A ‘top-down’ systems approach uses quantitative modelling to identify and describe the underlying biosynthetic and regulatory networks of a system, whereas a complementary ‘bottom up’ approach attempts to model the systems-wide phenotypes that emerge from component interactions.’

Synthetic Biology is seen as a multidisciplinary field of science that focuses on living systems and organisms, and applies engineering principles to develop new biological parts, devices, and systems or to redesign existing systems. A broader definition of Synthetic Biology is given by an expert group of the European Commission: ‘Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems which display functions that do not exist in nature. This engineering perspective may be applied at all levels of the hierarchy of biological structures from individual molecules to whole cells, tissues and organisms. In essence, synthetic biology will enable the design of biological systems in a rational and systematic way.’

Accordingly, Synthetic Biology aims to design or modify biological systems in the laboratory. To this end, it is being attempted to develop standardized components that can be easily combined to form new types of units. Synthetic Biology is a branch of the life sciences that uses engineering principles to specifically construct systems from biological parts (often named Bio-Bricks) and thus may provide solutions to global challenges such as disease, malnutrition, pollution and energy shortages.

*Further information on Synthetic Biology can be found here:*

**https://doi.org/10.1162/artl_a_00318**

**https://www.nature.com/articles/nrmicro3239**

**https://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_044.pdf**

# Facts about iGEM

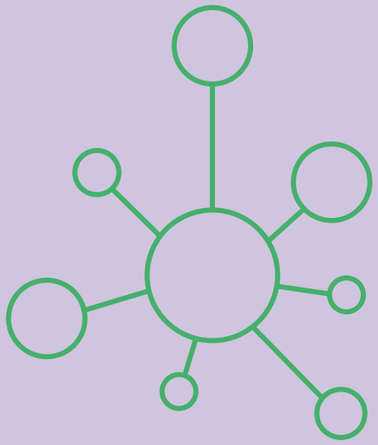
The international Genetically Engineered Machine (iGEM) competition is an international initiative for students in the field of Synthetic Biology. It has been organized by the iGEM Foundation since 2003, with the Massachusetts Institute of Technology (MIT) in Cambridge (USA) acting as the venue until 2014. In recent years, around 350 international teams from 45 countries have taken part every year.

## 3.600

So far, more than 3,600 projects were carried out by iGEM-teams in the field of Synthetic Biology. In the frame of these projects, Biological Parts, so-called BioBricks were constructed to build biological systems for operation in living cells. The resulting 'Genetically Engineered Machine' should feature new and unusual properties that contribute to solving societal challenges.

## 2003

**The iGEM competition was held for the first time in the year 2003.**



## continuously growing community for 20 years

**The iGEM competition grew out of student projects first conducted in 2003 at the Massachusetts Institute of Technology (Cambridge, Massachusetts, USA). Later, teams from other schools took part and in 2005, the competition was opened to participants from countries outside the United States. The iGEM competition and the associated community have been growing steadily since this year, so that 7,314 participants, organized in 350 teams, took part in the iGEM Jamboree in the year 2021.**

## 70.000

More than 70,000 iGEM team members from 45 countries have developed and carried out over 3,600 projects in synthetic biology to solve local challenges and advance this research field.




## 200 start-ups

Every year, numerous interesting project ideas were developed as part of the iGEM competition and then implemented as iGEM projects. Many of these projects have the potential for the development of innovative products, so that more than 200 start-ups have already emerged from iGEM. The iGEM Startup Program serves as a pre-accelerator for iGEM teams with promising project ideas.

**→ Further information can be found on the iGEM webpages: https://igem.org/**

## Responsibility

**Since iGEM projects involve the construction of 'Genetically Engineered Machines', responsible handling of the achievements from iGEM projects must also be taken into account. Synthetic biology works with very powerful technologies that, if used irresponsibly, may cause societal problems or even harm. For this reason, iGEM project ideas should also be considered beyond the laboratory work. In this context, biosafety, biosecurity risks and dual use issues play a role. Integration of a responsibility culture addresses these aspects. The iGEM Responsibility Program envisages a series of measures including risk management, education and social discussion.**

# Aspects of the international Genetically Engineered Machine (iGEM) competition

TEXT: NILS-CHRISTIAN LÜBKE

## General information on the iGEM competition

The international Genetically Engineered Machine competition – iGEM – is aiming for multidisciplinary student, high school or community lab teams realizing projects in the realm of synthetic biology. The independent, non-profit organization "iGEM Foundation" is running the competition annually. Teams need to conduct their own project to be presented to the community and to an international jury at the so called "Grand Jamboree". Starting out in 2003 as course at the Massachusetts Institute of Technology (MIT), Boston, USA, five teams in 2004 joined with nearly 50 participants as a "summer course" at the first ever iGEM competition at the MIT. In the year 2022, the competition gathered almost 3.500 participants at the Paris Expo and 3.000 participants online for the Grand Jamboree. Over the last 19 years, 46 countries and close to 70.000 participants have been part of the iGEM competition. The size and the growth of the competition can be estimated by the annually taken "iGEM from above" pictures of each year. Teams can apply for the competition in three divisions, namely High School, Undergraduate or Overgraduate teams. Although there is no age restriction to iGEM, the competition expects participants to be at least enrolled in High School – with the exception of community lab teams [1][2].

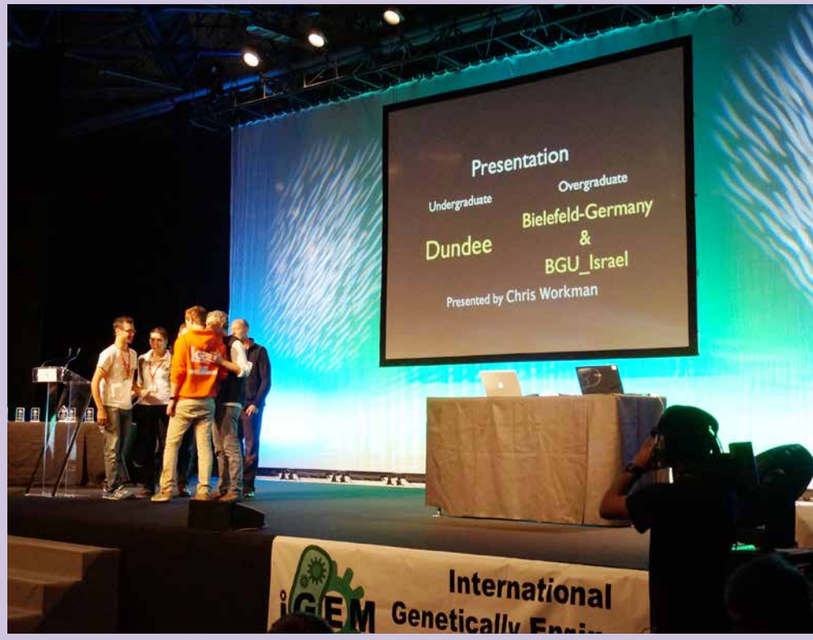
## Biological engineering with standardized biological parts – BioBricks

Synthetic biology incorporates the idea of engineering biological systems. The rationale underlying the iGEM competition is depicted through "BioBricks". BioBricks are standardized genetic parts, which can be combined to functional biological systems – called "composite parts" or "devices" – and easily shared among the community. These genetic elements introduce the engineering aspects into the idea of the iGEM projects. Every team needs to work with BioBricks and share their designed and constructed BioBricks with the other teams in iGEM's "Standard Registry of Biological Parts". Thus, future teams can build their ideas upon the BioBricks from all other teams which have ever participated in the competition. Based on this "get, give and share" and "information share" philosophy, over 20.000 documented "BioBricks" and devices thereof have been designed, created, characterized, and collected in the open "Registry of Standard Biological Parts" in 2022 [3].

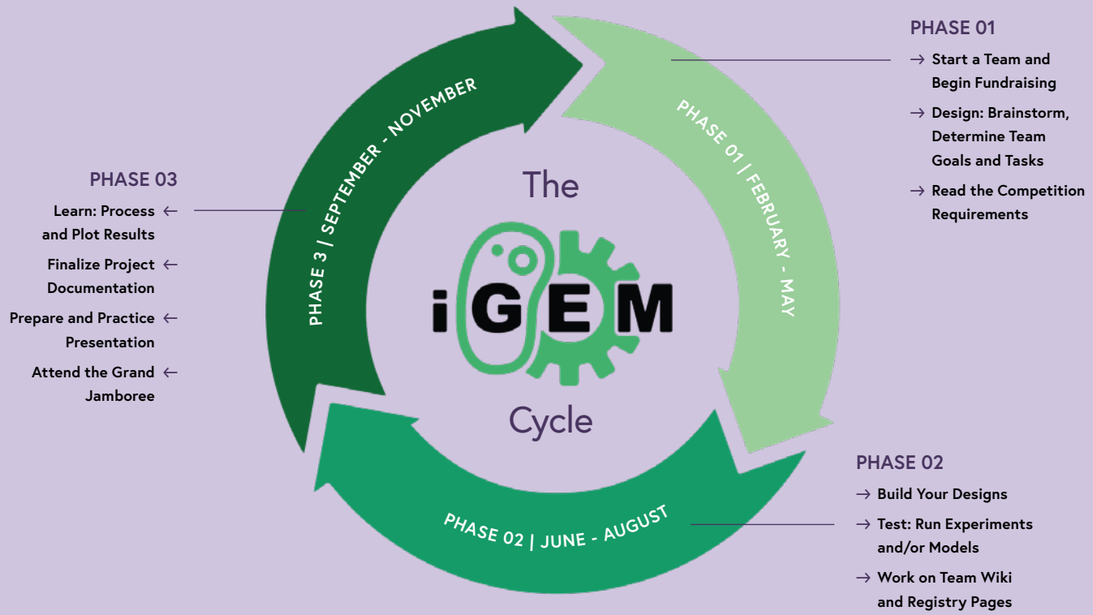
## A team's project in a nutshell

Registration for the competition usually opens in spring and the finale with the Grand Jamboree is held in autumn. In this time frame, the team members are recruited, the project idea is developed, the implementation and research is done, the application and societal aspects of the project are considered, and finally all results are compiled in the teams' wikis and presented at the Grand Jamboree. Further, teams need to cover funding, establish collaborations with other teams, seeking out advise from stakeholders, reflect their project for societal impact and structure the internal organization of their projects. Taking responsibility for one's work is an integral aspect of every iGEM team's project. Further, feasibility or applicability of the project's approach should be considered. These approaches are combined in the term "Human Practice" within the competition. In addition, teams should take time in modelling (parts of) their project and communicate their project and synthetic biology locally and beyond in outreach activities. →

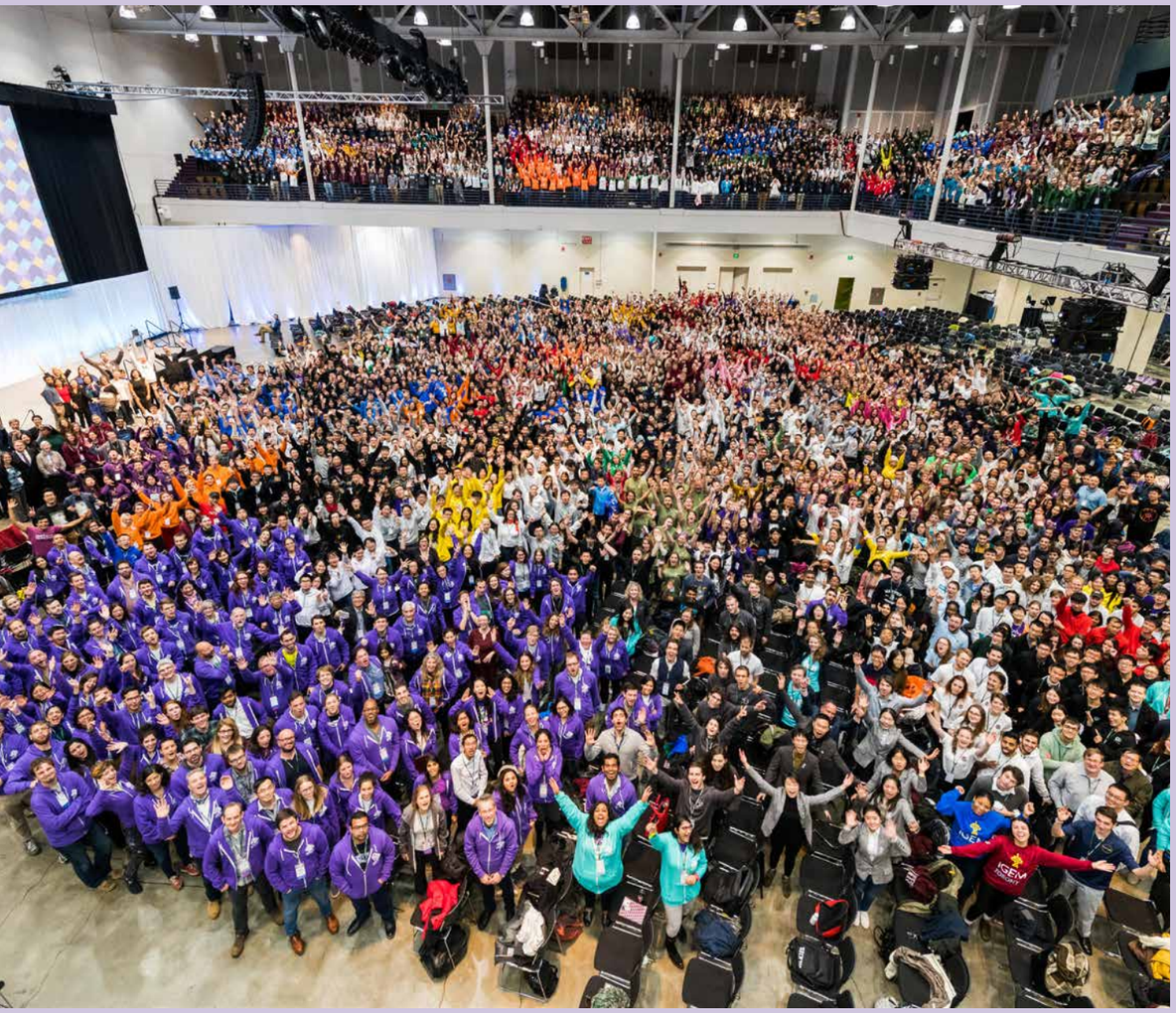

Members of the 2017 iGEM Bielefeld team holding the track prize for "Best Foundational Advance".




The iGEM cycle depicting the "iGEM year". Starting a team and securing funding at the beginning of each year, followed by an extensive project brainstorming session and the registration for the competition marks the first third of the iGEM year. During the summer, the teams test their designed ideas, finalizing them in autumn. Finally, the documentation of the project through the wiki and the preparation for the Jamboree takes place. The finale at the Grand Jamboree and coming back home for de-briefing marks the end of the "iGEM year".



*'Future teams can build their ideas upon the BioBricks from all other teams which have ever participated in the competition.'*



"iGEM from above" 2018, Hynes Convention Center, Boston, USA: The "IGEM from above" picture is a long lasting tradition of the competition. In 2018, the competition comprised 5790 participants in 340 teams. Depicted on "the iGEM from above" are only the attendees of the 2018 Giant Jamboree.




The iGEM Bielefeld team winning "1st Runner-up" at the Giant Jamboree 2013. The team's project "Ecolectricity – currently available" revolved around the construction of a microbial fuel cell with genetically modified *Escherichia coli*. The team and its supervisors are on stage of the Kresge Auditorium at the Massachusetts Institute of Technology, Boston, USA, with Judging committee member Karmella Haynes (on the right).

Teams document their ideas, project processes and progresses, protocols and results in their team wiki, which is a homepage designed by the teams themselves and hosted on the server of the iGEM foundation. Once the team wiki is "frozen", which happens close to the "Grand Jamboree", the team's wiki is no longer accessible anymore. Thus, teams need to complete their wiki before the actual "wiki freeze". Further, teams showcase their ideas in "Presentation videos" as well as in a presentation at the "Grand Jamboree".

## Judging – an integral part of the competition

Judging is an integral part of the competition to value the excellence and help celebrating the team's accomplishments. It is obvious that there is a large spectrum of working packages in place to be honored by "judging" in iGEM. A group of international Judges evaluates the teams' achievements after the "wiki freeze". Teams can win non-monetary prizes, which can be divided into Medals, Awards, Special Prizes, and the Grand Prize. It is noteworthy that all Medals – Bronze, Silver, Gold – are not awarded in comparison between teams but by challenging the teams themselves. Teams need to complete a set of deliverables for each Medal and convince the Judges about their work. Hence, more than one Medal is awarded during the competition and every team can be awarded with a Bronze, Silver or Gold Medal. Awards are given for the best executed project in a certain category, e.g., "Best Environmental Project". Special Prizes reward excellence in certain areas of the competition, e.g., "Best Education" or "Inclusivity Award". The judging process focuses solely on the given team's achievements. This holds also true for all of the divisions [4].

## The iGEM Bielefeld teams

In 2010, students from Bielefeld University decided to form an iGEM team. The support coming from the CeBiTec was right there in the beginning in person of Prof. Karsten Niehaus and Prof. Jörn Kalinowski. The latter should be in charge as the primary Principal Investigator (PI) for all 12 iGEM Bielefeld teams [5]. Starting as "Bielefeld – Germany" the geographically oriented name was exchanged to the "CeBiTec" attribution in 2014. From 2010 to 2021, 135 students took part in an iGEM Bielefeld team being mentored by more than 20 supervisors/instructors/advisors. The teams were awarded 12 Gold Medals, were two times among the best 16 teams of the competition (2011, 2012), Winner of the European Jamboree in 2013, First Runner-Up at the Giant Jamboree 2013, and have won several Track and Special Prizes. ●

[1] https://igem.org/
[2] https://competition.igem.org/
[3] https://parts.igem.org/Main_Page
[4] https://competition.igem.org/judging/introduction
[5] https://www.igem-bielefeld.de/de/igem

# Bielefeld University offers an excellent environment for iGEM activities

TEXT: ALFRED PÜHLER

In general, the participation of universities in the iGEM competition needs specific requirements. In particular, the field of synthetic biology should have a high priority in both teaching and research. This is the case for Bielefeld University, because various Master's programms, especially the Master's program "Genome Based Systems Biology" of the Faculty of Biology provides the basic knowledge, and the existing technology platforms at CeBiTec allow the molecular analysis of cellular building blocks.

## The Master's program "Genome Based Systems Biology", an important pillar for participation in the iGEM competition

At Bielefeld University, molecular-oriented students are educated in different Bachelor's programs of the Faculty of Biology and the Faculty of Technology. The Faculty of Biology teaches the molecular basis of living organisms, while the Faculty of Technology offers courses in the specialized fields of biotechnology and bioinformatics. The subsequent Master's programs then deepen the basic knowledge, in particular the Master's program "Genome Based Systems Biology (GBSB)" run by the Faculty of Biology provides an introduction to synthetic biology. The GBSB master program was founded in Bielefeld by Prof. Dr. Karsten Niehaus. In the meantime, Dr. Petra Lutter is in charge of this master program. In the past, both persons have worked hard for Bielefeld's participation in the iGEM competition and were therefore appointed as members of the recently founded iGEM steering committee.

The GBSB program emphasizes how omics technologies and bioinformatics as well as mathematical methods can be used to gain knowledge in molecular biology. It has to be mentioned that as a result of omics experiments, large amounts of data are obtained, the analysis of which can only be accomplished with bioinformatics programs. The establishment of whole genome sequences of individual organisms can serve as an example. After high-throughput sequencing of genomic segments, assembly programs help to generate the whole genome sequence. This whole genome sequence is then the starting point for the annotation process, that determines the ordered set of genes of the organism analyzed. After the knowledge of the whole genome sequence and the set of genes determined, the organism concerned can then be analyzed in terms of systems biology.

One obtains, for example, the network of characteristic metabolic processes. Synthetic biology now builds on these findings of systems biology. For example, synthetic biology is able to propose new metabolic pathways, e.g., for biotechnological application and thus expands the metabolic processes of the analyzed organisms. Importantly, the extension of metabolic processes is first done in a planning manner before a prototype can be constructed using genetic engineering techniques. Detailed experimental analysis of the prototype then allows conclusions on the original genetic planning step. This procedure is familiar from the engineering field. There, too, design drawings are first made before prototypes are built. For this reason, synthetic biology can also be considered as an engineering science. →

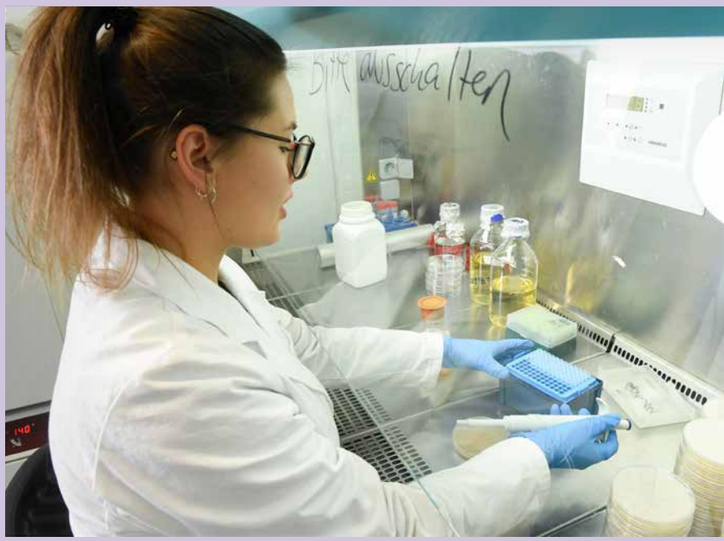
Mass spectrometer of the CeBiTec Technology Platform Genomics.





The Center for Biotechnology (CeBiTec) Laboratory building of Bielefeld University.

*'The CeBiTec, main home of Bielefeld University's iGEM activities.'*



Experimental work carried out during a GBSB master course.

*Selection of study programs at Bielefeld University from which iGEM teams are recruited.*






**Bioinformatics data analysis plays a major role in molecular biology oriented courses.**

## The omics technology platform of CeBiTec, a basis for experimental processing of iGEM topics

It is obvious that platforms for the generation of omics data and their bioinformatic analysis are a prerequisite for entering synthetic biology. Such platforms are available at CeBiTec of Bielefeld University and provide access to synthetic biology.

CeBiTec was founded in 1998 as a central scientific facility of Bielefeld University. A major DFG project enabled the establishment of two institutes at CeBiTec dealing with DNA sequencing and bioinformatic analysis. These two institutes determined the development of the omics platforms existing today. Thus, the latest sequencing equipment and mass spectrometers were procured to perform DNA sequencing, transcriptome, proteome or metabolome analyses. As the analysis of these large amounts of data requires sophisticated bioinformatics programs, CeBiTec is again well positioned in this respect thanks to an additional bioinformatics platform. In particular, due to a BMBF project a cloud structure has been installed that can be used to analyze any amount of life science data. The technology platforms located at CeBiTec thus form the basis for an experimental entry to work on projects in the field of synthetic biology. These technology platforms also make it understandable why all previous iGEM projects of Bielefeld University were located at CeBiTec. These favorable conditions have contributed to the fact that the 12 iGEM projects carried out so far have continuously been awarded gold medals. ●

## CeBiTec

The Center for Biotechnology – CeBiTec – is one of the largest faculty-spanning central academic institutions at Bielefeld University. Its purpose is to bundle the biotechnological activities and research projects at the university, to foster cross-linking of research approaches and technologies from different research fields and to develop innovative projects within its two main research areas: "Large Scale Genomics and Big Data Bioinformatics" and "Metabolic Engineering of Unicellular Systems and Bioproduction".

The availability of comprehensive technological infrastructure as being provided by CeBiTec's Technology Platforms is crucial for a successful scientific work. Academic training of graduates is fostered by the Graduate Center offering high-level PhD programs.

Furthermore, the CeBiTec considers itself as a central communication platform and a "think tank" of the university with respect to initiatives and activities with a dedicated biotechnological perspective.

# The organizational effort and public relation work of iGEM teams at Bielefeld University

TEXT: ALFRED PÜHLER

Bielefeld University.

The experimental parts of iGEM projects are carried out at CeBiTec laboratories.

The implementation of iGEM projects requires a high organizational effort to cope with the complexity of the task. For example, the experimental procedure, the documentation of the results and the acquisition of funding have to be managed. The iGEM group is also required to carry out extensive public relations work in order to make the content and purpose of its project known to the public.

## Bielefeld's iGEM projects require a great deal of organizational effort

Bielefeld University has participated in the iGEM competition a total of twelve times between 2010 and 2021. The iGEM teams were mostly composed of master students from the Faculty of Biology and the Faculty of Technology, with the GBSB master program acting as the nucleation seed. The iGEM groups developed in lengthy discussion sessions their own iGEM topic. Extremely interesting topics relevant to society were chosen, most of those had not been followed at the university before. The creativity of Bielefeld students was thus fully exploited in the selection of topics. Some topics and techniques dealt with during the Bielefeld iGEM projects are given in the figure next page. In this brochure, each individual iGEM project is described in detail on a double page. It is therefore worth paying a lot of attention to this part of the brochure in order to capture the creativity of the students involved in setting and solving the tasks.

Once the topic has been determined, the current iGEM group is challenged to organize the multi-faceted nature of the iGEM project. To do this, specific tasks must be distributed to individual group members. First, it is necessary to identify a core group that will take care of the experimental part of the project and the documentation of the results. In the experimental approach, it is also necessary to decide which biobricks of the iGEM organization can be used and which new biobricks should be created experimentally. Part of the iGEM group has to take care of raising funds needed for the overall project. Funding is, e.g., needed to cover the costs of the experimental approach and for participation in the "Grand Jamboree".

So far, the individual responsibility of the student members of the iGEM teams has been emphasized. However, the individual teams also received support and guidance from the professoriate and mid-level faculty. In particular, J. Kalinowski and his working group must be mentioned. He leads the sequencing platform at CeBiTec and is thus involved in both systems biology and synthetic biology projects. In the past, he has intensively supported the formation of iGEM teams and, above all, ensured that they were equipped with sufficient laboratory space to carry out iGEM projects. This location at CeBiTec also ensured that the existing technology platforms could be used to work on iGEM topics.

For several reasons, the continuation of a participation in the iGEM competition in 2022 was unfortunately interrupted. The main reasons were financial difficulties. The financing of a budget for material expenses, participation fees and travel expenses could no longer be secured. Efforts were therefore undertaken to solve this problem and a solution was found for the years 2023, 2024 and 2025. A larger amount of funding was made available by both CeBiTec and the Rectorate, which can be used to resume iGEM activities. A major change was also the formation of a steering group consisting of five members of the Faculty of Biology and the Faculty of Technology, who are very interested in the iGEM competition. The group has met several times in the meantime and is now in the process of launching the 2023 iGEM group. →


**The iGEM Steering Group at Bielefeld University (from left to right: Petra Lutter, Kristian Müller, Karsten Niehaus, Alfred Pühler [initiator of the iGEM restart], Jens Stoye, Jörn Kalinowski).**

**Selection of key words indicating topics and techniques dealt with in the Bielefeld iGEM projects.**






## The Bielefeld iGEM projects are characterized by extensive public relations work

When conducting iGEM projects, students are encouraged to document both the experimental procedure and the results obtained. For this purpose, the iGEM organization has set up an electronic procedure, a so-called "Wiki", in which all details of the iGEM project are collected. The iGEM organization then also uses this recorded information when evaluating the project. After the process is completed, all information about the implemented iGEM project is available to the public. The collected information on all implemented iGEM projects at Bielefeld University can be accessed on the iGEM website www.igem-bielefeld.de.

Independently of this basic procedure, the Bielefeld iGEM groups have used all possibilities to present their projects to the public. In addition to public events, radio and television broadcasts were primarily used to report on the successful participation in the iGEM competition. Over the years, radio broadcasts have been aired by Radio Bielefeld, Radio Herford, Radio Gütersloh and the university's own radio station. More supra-regional are the radio broadcasts on iGEM projects by broadcasters of Deutschlandradio or Westdeutscher Rundfunk. Television contributions were broadcast by Nano, Westdeutscher Rundfunk and Campus TV. The iGEM groups also played a privileged role in the organization of the annual CeBiTec Student Academy, dedicated to the topic of biotechnology/synthetic biology. Here, a discussion was held with students from pre-baccalaureate classes on genetic engineering and synthetic biology. With this public relations work, iGEM groups help to ensure that future-oriented topics such as the use of genetic engineering in the fields of biotechnology, medicine and agriculture are addressed and discussed extensively. The topics worked on by iGEM groups often yielded new scientific findings, which in some cases led to publications in peer-reviewed journals. The publications that have appeared are listed below.

# Publications of the Bielefeld iGEM-Groups

**High Quality *de Novo* Transcriptome Assembly of *Croton tiglium*.**
Haak M, Vinke S, Keller W, Droste J, Rückert C, Kalinowski J, Pucker B:
*Front Mol Biosci. 2018 Jul 5;5:62. doi: 10.3389/fmolb.2018.00062.*

**Synthetic Biology Ethics at iGEM: iGEMer Perspectives.**
Whitford CM, Lübke NC, Rückert C:
*Trends Biotechnol. 2018 Oct;36(10):985-987. doi: 10.1016/j.tibtech.2018.06.004.*

**A paper-based, cell-free biosensor system for the detection of heavy metals and date rape drugs.**
Gräwe A, Dreyer A, Vornholt T, Barteczko U, Buchholz L, Drews G, Ho UL, Jackowski ME, Kracht M, Lüders J, Bleckwehl T, Rositzka L, Ruwe M, Wittchen M, Lutter P, Müller K, Kalinowski J:
*PLoS One. 2019 Mar 6;14(3):e0210940. doi: 10.1371/journal.pone.0210940.*

**Auxotrophy to Xeno-DNA: an exploration of combinatorial mechanisms for a high-fidelity biosafety system for synthetic biology applications.**
Whitford CM, Dymek S, Kerkhoff D, März C, Schmidt O, Edich M, Droste J, Pucker B, Rückert C, Kalinowski J:
*J Biol Eng. 2018 Aug 14;12:13. doi: 10.1186/s13036-018-0105-8. eCollection 2018*


# 02

## The iGEM projects of Bielefeld University in the last 12 years

# 2010

## Modulated Acetosyringone Receptor Sensor System (MARSS) – Measurement of Spiciness in Food

TEXT: ANDREAS SCHLÜTER

**The project of the year 2010 was aimed at the detection of the substance capsaicin, which is responsible for the spiciness of food. For this purpose, the Vir (virulence) receptor system from the soil bacterium *Agrobacterium tumefaciens* was introduced into the enterobacterium *Escherichia coli* and trained for the target-substance capsaicin to be detected. For this purpose, a directed evolution approach was applied. The system can be linked to various receptors for possible use cases in medical diagnostics. To identify receptors for medically interesting ligands, a plasmid-based screening system was developed to facilitate the directed evolution approach.**

### Motivation

Synthetic biology is a new discipline in biological research and presents an active field, giving room for creative and innovative ideas. Every day, millions of people taste hot and spicy food, thereby making the market for hot sauces boom. Therefore, the development of a sensor for spiciness appeared to be a meaningful field of research and a worthwhile goal for an iGEM project. By now, the spiciness of food is scaled according to the Scoville test or assessed by taste: How many drops of water neutralize the spiciness of one drop of sauce? Obviously, that is quite subjective. This is why a biosensor was implemented to determine an objective value for the spiciness of food.

### Results

The basic idea of the project was to establish the complete VirA/VirG virulence signaling system from *Agrobacterium tumefaciens* in the bacterium *Escherichia coli*. For this purpose three new BioBricks were created, namely *virA*, *virG* and a *vir* promoter (see Figure). The VirA receptor recognizes the phenolic substance acetosyringone and transmits this information to the VirG response regulator representing a bacterial transcription factor which activates *vir* promoters. This natural VirA/VirG signaling system works in *E. coli* and was measured and characterized by using reporter genes.

Subsequently, modification of the VirA receptor by directed mutagenesis using the error-prone PCR (Polymerase Chain Reaction) method was addressed. This approach was aimed at the generation of VirA derivatives that detect substances other than acetosyringone, e.g. capsaicin. However, due to the shortness of time available, the set aim was not achieved. However, the basis for the development of a sensor-system for the detection of specific target substances was laid and my be further advanced in future projects.

### Towards application

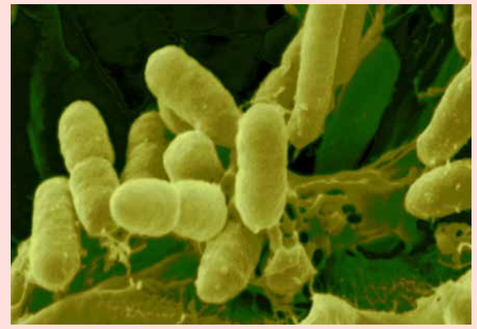
The analyzed VirA/VirG sensor system may be applied for different purposes. However, the main focus of this project was the detection of capsaicin, which is responsible for the spiciness of food. The construct for application may consist of immobilized bacteria on a test stick that can be used everywhere for easy and quick determination of the spiciness of food. Moreover, the modified bacterial sensor system could be implemented as part of food production processes.
The project was intended as proof of concept for directed mutagenesis of receptor molecules. Thought further, modified sensor systems could be used in the field of medical science. Moreover, there are possible applications regarding the detection of environmental pollutants, for example phenolic toxins. The presented system might be trained to detect these substances by using the developed directed evolution method.

### Awards

The iGEM Team of the year 2010 won a Gold Medal. ●

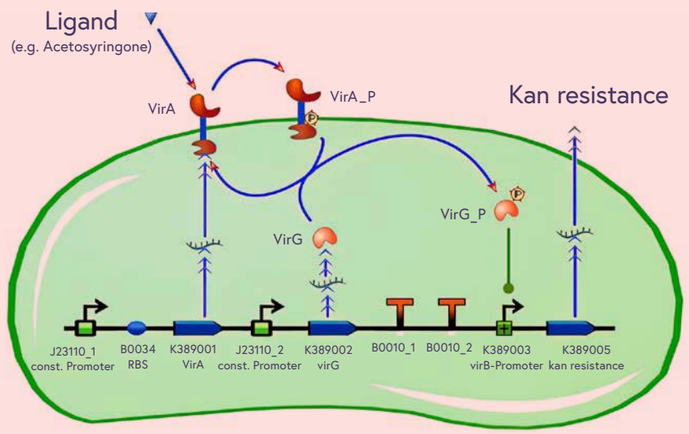
*Further information about the project can be found here:*

https://2010.igem.org/Team:Bielefeld-Germany

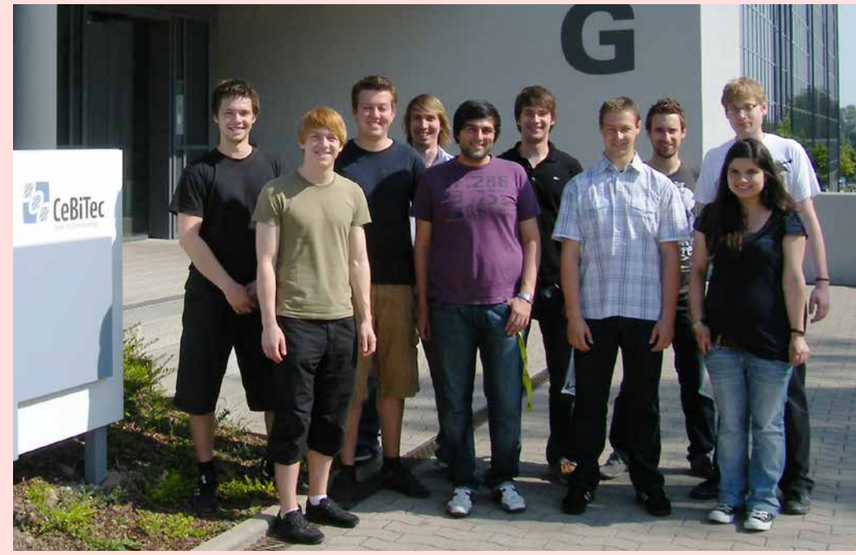


**The soil bacterium *Agrobacterium tumefaciens* is the natural host of the VirA/VirG virulence two-component regulatory system that was used for the construction of a BioBrick for the detection of target molecules.**

***A natural bacterial sensor system was used to measure the level of spiciness in food.***




**The VirA/VirG two-component sensory system as basis for the development of a module for the detection of capsaicin (ligand). Binding of the ligand (capsaicin) to the VirA receptor results in phosphorylation of VirA and subsequently, the phosphate moity (P) is transfered to VirG which in its phosphorylated form (VirG_P) acts as transcriptional activator at the promotor of the kanamycin resistance gene. Hence, binding of the ligand leads to kanamycin resistance which can be exploited for the detection of ligand binding.**




**iGEM-Team Bielefeld 2010: Back-row from left to right: Jonas Aretz, Timo Wolf, Nikolas Kessler, Frederik Walter, Jonas Marschall and Nils-Christian Lübke. Front-row from left to right: Simon Unthan, Armin Neshat, Frieder Hänisch, and Eva Brombacher.**

# 2011

## The Bisphenol A Team – Detection of the environmental toxin bisphenol A by a cell-free system

TEXT: ANDREAS SCHLÜTER

**The aim of the project was to detect the environmental toxin bisphenol A using a cell-free, biological system. For this purpose, three enzymes involved in the reduction of bisphenol A were linked to form a fusion enzyme. Bacterial surface proteins, so-called S-layer proteins, were used to immobilize the fused enzymes in a defined structure on the surface of small glass beads. The enzymatic reaction was detected *via* the by-product NAD⁺ and an enzymatic reaction dependent on it, as a result of which a light signal that can be perceived by the eye was generated.**

### Motivation

The development of sensitive and selective biosensors is an important research field in synthetic biology. Biosensors can be used in a wide range of applications, from the detection of environmental toxins up to clinical diagnostics. Since cells have to sense their surroundings, there are a lot of natural systems that function as biosensors. Natural cellular biosensors often show negative side effects that complicate any practical application. Common problems are the limited use outside of a gene laboratory due to the employment of genetically engineered cells, the low survivability of living cells in non-permissive environments and the appearance of undesired signals induced by endogenous metabolites. To solve these problems, the iGEM-Team Bielefeld of the year 2011 aimed to develop a cell-free bisphenol A (BPA) biosensor based on a coupled enzyme reaction fused to S-layer proteins for everyday use. Bisphenol A is a supposedly harmful substance which is used in the production of polycarbonate. To detect BPA, it was degraded by a fusion protein under formation of $NAD^+$ which can be detected by an $NAD^+$-dependent enzymatic reaction using a molecular beacon module. Both enzymes were fused to S-layer proteins which build up well-defined nanosurfaces and are attached to the surface of beads. By providing these nano-biotechnological building blocks, the system is expandable to other applications.

### Results

The bacterium *Escherichia coli* was enabled to degrade BPA *in vivo* by fusing the genes *bisdA* and *bisdB* encoding ferredoxin and cytochrome P450, respectively. In this way, a P450 monooxygenase system was created. As third enzyme in the redox cascade, ferredoxin-$NAD^+$ oxidoreductase (FNR) was implemented (see Figure). Moreover, it was demonstrated that the fusion protein featured a high specificity for BPA in comparison to other bisphenols and displayed a very high specific BPA degradation rate.

Bacterial S-layer proteins were intended to be used for immobilization of the fusion enzyme on the surface of glass beads. Four different S-layer BioBricks with different lattice structures were constructed. Two fluorescent S-layer fusion proteins from different organisms were purified and immobilized on beads, leading to a highly significant fluorescence enhancement of these beads. Accordingly, these S-layer proteins are available for the immobilization of the fusion enzyme designed to reduce BPA.

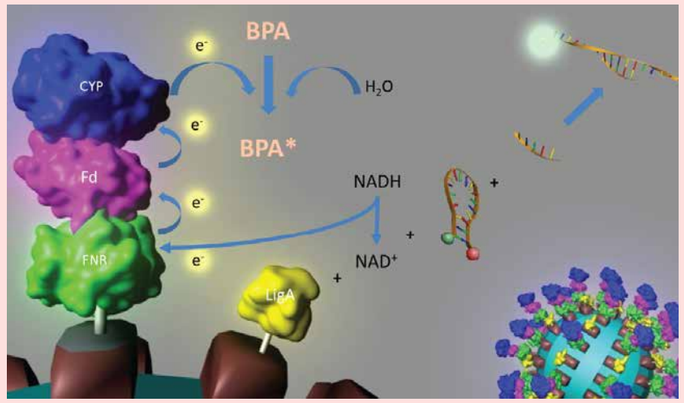
The enzymatic reaction at BPA was detected *via* the by-product $NAD^+$ and an enzymatic reaction using the latter molecule as co-factor. Finally, consumption of $NAD^+$ led to generation of a light signal that is visable by the eye.

### Towards application

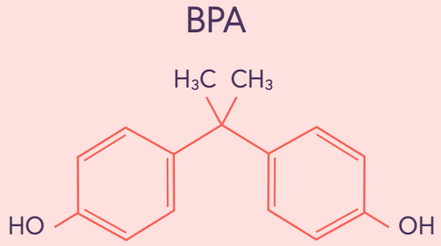
The ability of self-assembly and forming defined nano-structures enables the use of S-layer proteins as building blocks in a biomolecular construction kit for the generation of a universal nano-biotechnological matrix. By using the matrix for the attachment of further components or just the nano-structure itself, a great variety of applications in modular nano-biotechnology, biomimetics, bioanalytics and medical diagnostics are imaginable. A current topic in S-layer application is the design of more sensitive and improved optical and electrochemical sensors. In conclusion, S-layer proteins are one of the most innovative and promising discoveries for combining biology with engineering in the new field of nano-biotechnology.

**iGEM-Team Bielefeld 2011:**
**From left to right:**
**Simon Schäper, Panagiotis Papavasiliou, Michael Limberg, Katharina Thiedig, Timo Wolf, Anna Drong, Manuel Wittchen, Jonas Aretz, Robert Braun, Matthias Eder, Jan Schwarzhans.**

***Design of specific biosensors is an important research field in Synthetic Biology.***




**Enzymatic cascade used for the degradation (reduction) of bisphenol A (BPA) and its detection applying a molecular beacon based bioassay. BPA is reduced by an enzymatic redox cascade composed of the enzymes ferredoxin-$NAD^+$ oxidoreductase (FNR), a ferredoxin (Fd) and cytochrome P450 (CYP). Reduction equivalents for this reaction are delivered by NADH. The generated $NAD^+$ is used in an reaction catalyzed by *Escherichia coli* DNA ligase (LigA) resulting in ligation of two oligonucleotides that hybridize to a single-stranded DNA forming a stem-loop structure. The termini of the latter molecule are connected to a fluorophore (green bullet) and a corresponding quencher (red bullet). Opening of this molecular beacon results in generation of a fluorescent signal. All enzymes are attached to S-layer surface proteins of the bacterial cell envelope (brown structures).**




**Structural formular of the bisphenol A molecule.**

### Awards

The iGEM Team of the year 2011 won a gold medal at the European Regional Jamboree and was among the top 16 teams overall in the competition. ●

*Further information about the project can be found here:*

https://2011.igem.org/Team:Bielefeld-Germany

# 2012

## A Case for Laccase – Removing Estrogens from Wastewater

TEXT: ANDREAS SCHLÜTER

iGEM-Team Bielefeld 2012: Back-row from left to right: Robert Braun, Gabriele Kleiner, Kevin Jarosch, Moritz Müller, Hakan Geyik, Miriam Fougeras, Saskia Scheibler and Agatha Walla. Front-row from left to right: Sebastian Wiebe, Isabel Huber, Julia Schirmacher, Nadine Legros, Malak Fawaz, Derya Kirasi and Julia Voss.

**In 2012, the Bielefeld iGEM team aimed to develop an immobilized laccase-based system for the degradation of estrogens and other aromatic micro-contaminants from wastewater. In order to analyze the suitability of laccase enzymes occurring in many living organisms, the corresponding genes were isolated from various pro- and eukaryotic species and cloned by means of targeted integration into the production strains *Escherichia coli* and *Pichia pastoris*. Subsequently, the ability of the expressed laccases to degrade the target substances, namely estrogens and related aromatic molecules, was tested. Moreover, immobilization of the various laccases was analyzed and optimized to achieve the highest possible substrate binding and enzyme activity. The system was planned to be used in sewage treatment plants as a cell-free biological filter unit.**

### Motivation

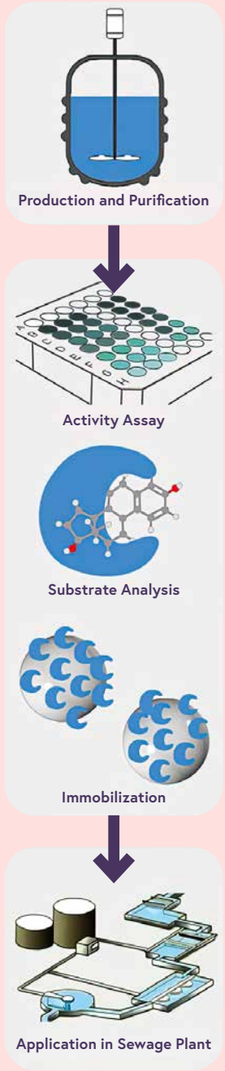
The pollution of wastewater by endocrine disruptors (endocrine active substances) is steadily increasing and is only inadequately filtered by sewage treatment plants. Municipal and industrial effluents contain a variety of pollutants, such as estrogens and xeno-estrogens, which can cause severe disorders in humans and animals. By using synthetic estrogens in the place of natural estrogen in hormonal contraceptives, the natural hormone degradation is slowed down and the substances remain longer in the waterbody. The effect of hormonally active substances on the environment and humans is very complex. They can cause reduced fertility in both humans and animals and result in feminization of males and hermaphrodism, especially in aquatic animals. Besides synthetic estrogen, other chemical substances can also have the same effects on the nature, e.g. bisphenol A. These so-called endocrine substances can also be detected in wastewater. They belong to the chemical class of polycyclic aromatic hydrocarbons (PAHs) emerging from burning of fossil fuels. They are bioaccumulating, toxic, and have a low biodegradability. Furthermore, numerous polycyclic aromatic hydrocarbons are cancerogen, which illustrates the danger they pose to the environment.
Synthetic estrogens and polycyclic aromatic hydrocarbons are among the substances, which cannot be removed sufficiently by conventional wastewater purification methods. These microcontaminants affect associated aquatic and/or downstream terrestrial ecosystems and may even contaminate groundwater. To reduce the concentrations of microcontaminants in the waterbody, it is necessary to find new methods and solutions. Synthetic biology and the use of laccases may offer solutions especially for such substances.

### Results

The iGEM Team successfully produced four active bacterial laccases and purified these enzymes. Their optimal activity conditions regarding pH, temperature, buffer solutions and organic solvent resistance were determined. Furthermore, it was demonstrated that the produced laccases can be immobilized maintaining their activity and degradation capacity for several micro-contaminants. All of the actively produced laccases are able to degrade estradiol and the two laccases from *Thermus thermophilus* and *Bacillus pumilus*, respectively, are able to degrade ethinyl-estradiol in combination with a mediator. First experiments showed successful production of the laccase from the fungus *Trametes versicolor*. A cheap alternative purification and immobilization method *via* a cellulose binding tag was also close at hand.

### Towards application

A plan for the application of the obtained project results had been developed. A next step is to test the system in a real sewage treatment plant under strict safety regulations. Furthermore, a lab-scale waste water system could be used to measure enzyme activities of the immobilized laccases in active sludge and in a fixed bed reactor under realistic conditions.




Model of the laccase of the fungus *Trametes versicolor* that was cloned and produced by the iGEM-team of Bielefeld University.

*Laccases degrade estrogens from wastewater.*

Workflow of the 'A Case for Laccase' project. The team produced and purified different bacterial and a fungal laccase. Activity assays were established to determine substrate profiles of the produced laccases. Laccases could be immobilized retaining their activity. It is planned to apply the developed laccase systems in sewage plants for the degradation of estrogens and other aromatic micro-contaminants.

### Awards

The iGEM Team of the year 2012 won a gold medal at the European Regional Jamboree and qualified for the world championship. Finally, they were among the top 16 teams. ●

*Further information about the project can be found here:*

https://2012.igem.org/Team:Bielefeld-Germany

# 2013

## Ecolectricity – Development of a Microbial Fuel Cell

TEXT: ANDREAS SCHLÜTER

**Building on the idea of providing an environmentally friendly alternative for energy recovery, the team developed a self-constructed microbial fuel cell (MFC) in 2013, the electrical energy of which is provided by an optimized strain of the bacterium *Escherichia coli*. In addition to the technical optimization of the fuel cell, different genetic approaches such as the integration of porins, the expression of cytochromes and the establishment of endogenous mediators *via* overexpression of the enzyme glycerol dehydrogenase and the riboflavin cluster were evaluated with regard to efficient electron transport within the bacterial cell and towards the anode of the fuel cell. For application of the microbial fuel cell outside the laboratory, the project was complemented by the development of a gene-based biosafety system and design of a 3D-printable MFC. The thematic relevance of the project is obvious since it addressed alternative energy recovery using regenerative raw materials.**

### Motivation

Today, there is a growing interest in the use of environmentally friendly alternative energy sources to combat the depletion of fossil fuels and an increasing pollution of the environment. Therefore, the iGEM-Team of the year 2013 developed a Microbial Fuel Cell (MFC) involving the modified bacterium *Escherichia coli* to recover electric energy. Applying strategies of synthetic biology, different BioBricks for bioelectricity generation were to be constructed. Specific electron transfer proteins were compiled from a variety of organisms, in order to design an *Escherichia coli* Fuel Cell platform. In other words, *E. coli* was to be transformed to an electro-active bacterium. For this purpose, different systems were implemented to achieve electron transfer from *E. coli* cells to the electrode of the fuel cell. Corresponding electron transport elements should increase electron transfer and bioelectricity generation (see Figure).

### Results

Different aspects and systems for technical and genetic optimization were implemented to use *E. coli* for direct electric energy recovery from energy-rich substrates. After extensive modifications, a well-functioning Microbial Fuel Cell was established and a protocol for measuring the power output of the electricity generating cultures was worked out. A stack of five Fuel Cells which was successfully used to power different LEDs and the motor of a small fan was built. Furthermore, a 3D model of a fuel cell was designed, which can be printed out using a 3D-printer.

### Towards application

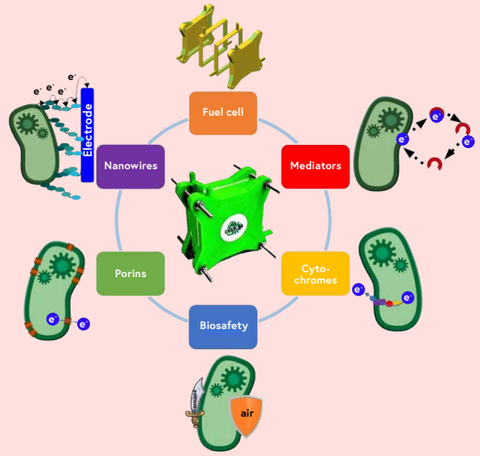
The most promising use case for microbial fuel cells is their deployment in sewage treatment plants. There, organic waste can be degraded while using the energy stored in different compounds of the sewage to recover electric power. With further work, the concept can also be inverted to allow MFCs to consume electricity to either produce biofuels or help with bioremediation of contaminants.

### Awards

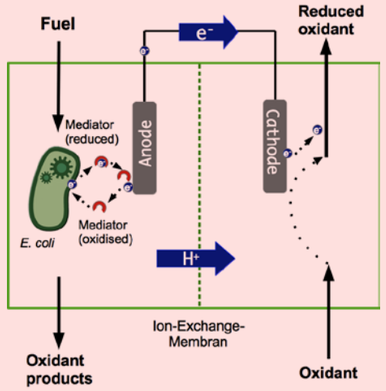
The 2013 iGEM Team won a gold medal, was European Champion and First Runner-up at the World Championship Jamboree. The team also won the award for the best presentation and took first place in the category Food & Energy Project (Overgraduate). ●

*Further information about the project can be found here:*

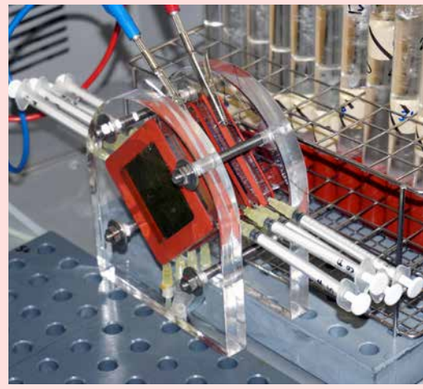
https://2013.igem.org/Team:Bielefeld-Germany




Tasks that were addressed in the frame of the 'Microbial Fuel Cell' project. Nanowires and porins were expressed in the bacterium *Escherichia coli* to facilitate electron ($e^-$) transport to the electrode of the fuel cell. Likewise, added mediators and implemented cytochromes mediate electron transport through the medium of the fuel cell and within the *E. coli* cell, respectively. Fuel cells were manufactured by 3D-printing. The project was complemented by the development of a gene-based biosafety system.




Schematic operating mode of a microbial fuel cell. The bacterium *Escherichia coli* utilizes energy-rich components of the medium (fuel) to supply its energy metabolism resulting in generation of reduction equivalents. Electrons (symbolized by $e^-$) may reduce mediators facilitating the transport of electrons to the anode of the fuel cell. At the cathode, electrons reduce an oxidant to yield the reduced form of the oxidant thus closing the flux of electrons. Charge equalization is ensured by hydrogen ions ($H^+$) flowing through an ion-exchange membrane.



Constructed microbial fuel cell device.

*'Living batteries' exploit bacteria to generate electricity.*

iGEM-Team Bielefeld 2013: Back-row from left to right: Lukas Rositzka, Timo Wolf, Robert Braun and Matthias Ruwe. Middle row: Sebastian Grenz, Tore Bleckwehl, Tom Tubbesing and Nils Lübke. Front-row from left to right: Fabian Thomas, Manuel Schüler, Thorben Meyer, Anna Korszanska and Nadiya Romanova.

# 2014

## The Transformers – From Carbon Dioxide to Biofuel (Production of isobutanol from carbon dioxide)

TEXT: NILS-CHRISTIAN LÜBKE

**In 2014, the iGEM Bielefeld-CeBiTec worked on a topic with relevance today more than ever. By introducing more and more renewable energy sources to cope with the increasing electricity demand, society tries to fight against the ongoing climate crisis. Today, like in 2014, certain amounts of this generated energy cannot be used due to the difficult task of transferring and saving generated energy. Beyond one major driver of the climate crisis is the increased amount of atmospheric carbon. The iGEM Bielefeld-CeBiTec 2014 team tackled these two challenges by introducing a genetically engineered *Escherichia coli* capable of using surplus energy and carbon dioxide to generate a storable energy supply. The aim of the project was to convey *E. coli* the ability to fixate carbon dioxide and electrical current to produce isobutanol. For this, the microorganism was equipped with an electron uptake system and a carbon dioxide fixation cycle. Finally, the isobutanol production in *E. coli* was established by using genes from *Lactobacillus lactis* and *Bacillus subtilis*. Isobutanol is an easy to store pre-cursor of fuel production. Thus, the team's project would make use of atmospheric carbon dioxide and unusable energy surplus to generate a storable fuel pre-cursor isobutanol. The team also thought about possible biosafety implications by introducing an antibiotic-free selection system. Beyond the microbiological work, the team modelled their approach, conducted knowledge exchange with stakeholders in the relevant fields and participated in a SYNENERGENE cooperation, in which the team depicted possible future social implications and applications of their project.**

### Motivation

The iGEM Bielefeld-CeBitec 2014 team's project core idea was to make use of atmospheric carbon dioxide and otherwise unutilized electric energy surplus. Renewable energy production comes with the pitfall to be dependent on environmental circumstances; e.g. sunshine for photovoltaic elements or a certain amount of wind for electricity production. Electricity is not always needed when produced, but storage of energy is still a problem today as the technology is lacking feasible approaches. Batteries that seize are not only economically unfeasible, but they are also hard to build at an industrial scale and lack the ability to store all of the generated surplus energy. The 2014 iGEM Bielefeld-CeBiTec team decided to engineer a microorganism capable of using the atmospheric carbon and the surplus energy to generate a storable energy source – isobutanol.

### Results

*Escherichia coli* was used as a chassis organism. The part of the project regarding the electric uptake system consisted of the creation of a reverse microbial fuel cell (rMFC). The fuel cell should be "loaded" with the team's engineered *E. coli* and provide the electric current via two electrodes to the organism. The *E. coli* should be capable of incorporating the provided electrons through the electrical current of the electrodes in its metabolism. In order to facilitate an electron uptake, the membrane protein OprF from *Pseudomonas fluorescencs*, constructed by the iGEM team Bielefeld 2013, was introduced to "open" the bacterial membrane for transport. Subsequently, different mediators for electron transport inside the cell were tested using different H-cell reactors – a specific form of a fuel cell – and different electrode material.

The second step within the project was the fixation of carbon dioxide. The team tried to implement the ribulose-1,5-bisohosphate carboxylase/oxygenase (RuBisCO) into *E. coli*. As the RuBisCO needs high levels of carbon dioxide, a microcompartiment called "carboxysome" from *Halothiobacillus neapolitanus* was also successfully integrated into *E. coli*. The team could not manage to combine both aspects for a successful carbon dioxide fixation inside the cell, although both components individually could be shown to work in *E. coli*.

The third and final part of the project consists of the establishment of an isobutanol production pathway like it has been described in literature. The team could show the increased production of isobutanol in their constructed *E. coli* strain after incorporating different BioBricks from former iGEM teams and genes from *Lactobacillus lactis* and *Bacillus subtilis*. To help ease the genomic work, an antibiotic-free selection system was implemented.

### Towards application

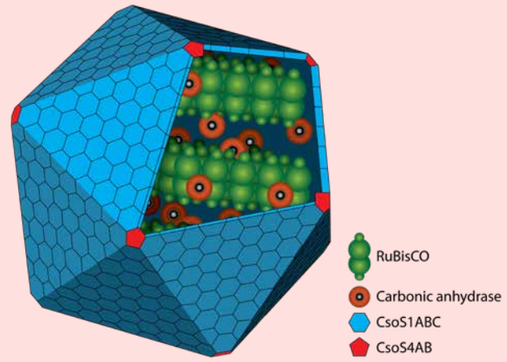
The team thought about possible applications and societal impacts of their project right from the start by searching the expertise of stakeholders in the field. During their cooperation with SYNENERGENE – a framework for responsible research funded by the European Union-, the team developed scenarios of their project being implemented in the near and far future.

### Awards

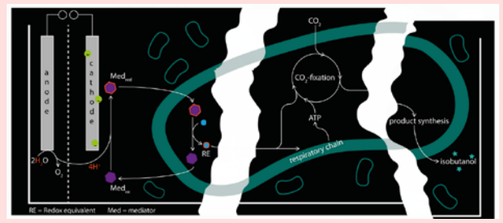
The team achieved a Gold Medal. ●

*Further information about the project can be found here:*

https://2014.igem.org/Team:Bielefeld-CeBiTec




**Scheme of the carboxysome harbouring the RuBisCO and carbonic anhydrases needed for carbon dioxide fixation. The fixation of the carbon dioxide should take place inside the carboxysome. The carboxysome can serve as a microcompartment in future microbiological works.**




**The project was divided into three sub-parts or modules. The first module depicted on the left exhibits the electron uptake and recycling within the organism. The second module is the carbon fixation system, whereas the third module consists of the isobutanol production.**


**iGEM-Team Bielefeld-CeBiTec 2014: Back-row from left to right: Janina Tiemann, Sandra Brosda, David Wollborn, Boas Pucker, Tore Blackwehl, Simon Riedl, Sebastian Blunk, Julian Droste. Front-row from left to right: Annika Fust, Birte Hollmann.**

# 2015

## Cell-free Sticks – it works on paper (Cell-free biosensor for detecting date rape drugs and heavy metals)

TEXT: NILS-CHRISTIAN LÜBKE

**The team's project in 2015 revolved on the detection of date rape drugs and heavy metals. Thinking about possible implications and applications of the to be built sensor, the iGEM Bielefeld team decided to work cell-free. Further, the sensor's handling needed to be robust and simple and the readout needed to be easily understandable. Thinking about possible real-world applications, the team decided to work a two-fold strategy – one for heavy metals used in water analysis as well as an application to fight the use of date rape drugs. Another reason for testing two such different categories of cell-free sensors was to illustrate the modularity of the sensor, but especially its expansion possibilities. For this purpose, the team investigated the cell-free protein synthetizes (CFPS) as well as Plasmid Repressor Interaction Assay (PRIA).**

### Motivation

Heavy metal contaminations are a worldwide problem bearing health risks and issues for those unaware or without no choice of using the contaminated water. Ideally, a test strip should combine different heavy metals in one test. So, the team worked with Arsenic, Chromium, Copper, Lead, Mercury, and Nickel. Those heavy metals are also regulated by the World Health Organization (WHO). Using already existing BioBricks and combining them with a robust read-out, the team accomplished the detection of Arsenic, Chromium, Copper, Lead, and Mercury.

Besides Heavy Metal contaminations, Date rape drugs are a concerning and frequently occurring issue. The term date rape drug is referring to sedatives, that may cause unconsciousness, loss of will, and ability to resist assault. The chemicals are easy to purchase and easy to apply. The victims of date rape drugs not only suffer from any assault on their body, but also sustain traumata after being attacked.

The team's goal, therefore, was to develop an easy to use, cell-free, paper based test strip, with a fast and easy to understand readout. The sensor should help to identify water contaminations like heavy metals or date rape drugs in beverages. Furthermore, the test strip should be modular, so it could be extended with possible other biosensors.

### Results

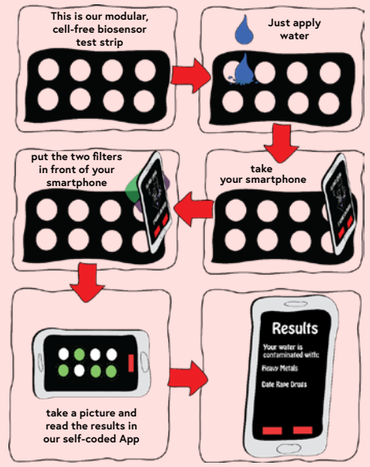
The cell-free protein synthesis (CFPS) is based on a one-pot-reaction combining *in vitro* the transcription and translation machinery with the DNA information of the given protein of interest – promoter and readout. Therefore, *Escherichia coli* KRX or ER2566 cell extract, water, nucleoside-triphosphates, amino acids, co-factors, Phosphoenolpyruvat (PEP), and the additive of interest are mixed. The team used a superfolder green fluorescent protein (sfGFP) readout, optimized the process and subsequently established a freeze-dried reaction on paper. By contact with water the CFPS machinery harboring a promoter sensitive to the substance to be detected was activated. This ensures the expression of the visible read out sfGFP as soon as the water is contaminated with the corresponding substance. Beyond the successful implementation of the CFPS, the team also tried to establish a plasmid repressor inactivation assay (PRIA). A repressor protein is bound to the promoter controlling the fluorescent protein gene. The repressor can be unbound by the detectable substance, thus de-controlling the promoter and the readout is established. The team managed a PRIA proof of concept.

Having established the foundation of the sensor with their CFPS protocols, the team worked on the incorporation of already existing heavy metal biosensors from the Registry of Standard Biological Parts into the CFPS system. Arsenic, Chromium, Copper, Lead, and Mercury detection were established with Nickel not gathering trustworthy results. All sensors were shown to work on a proof of concept level, but need to be further optimized for real-world applications.

Moreover, the team developed a date rape drug sensor by detecting γ-hydroxybutyric acid (GHB) and γ-butyrolactone (GBL). The blc operon from *Agrobacterium tumefaciens* was used and incorporated into the CFPS setup. Both chemicals could be detected in water samples.

Beyond the laboratory work, the team dealt with a common scientific dilemma called Dual Use Research of Concern (DURC). By working on a potential misuseable issue like date rape drugs, the researchers need to think about access to their work and their reach in public. The team accepted this challenges by working on their report on DURC shading light on the term and the dilemma itself, as well as the ethical and legal implications.


**iGEM Team Bielefeld 2015: Back-row left to right: Manuel Wittchen (advisor) Melissa Kracht, Gila Drews, Marta Jackowski, Luzia Buchholz, Anna Dreyer, Janina Lüders, Ursula Barteczko, Tobias Vornholt, Tore Blackwehl (advisor). Front row: Alexander Gräwe, Uyen Link Ho.**




**Workflow for the analysis of a specific compound in drinking water. The solution to be tested is applied onto the cell-free biosensor test strip. Two filters are amounted at a given smartphone. Using the app and the camera function of the smartphone, the results are easily processed.**

### Towards application

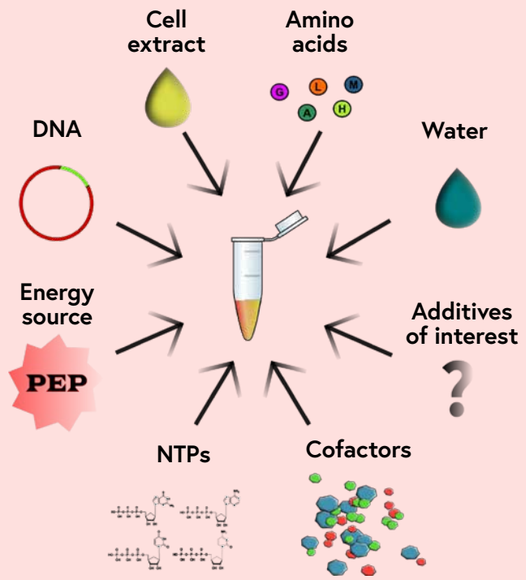
Independently on the substance to be monitored, the team's cell-free sensor, should be easy to use. The team was able to do this by creating a visual read out that is easy to evaluate with a smartphone using two light filters and an app they programmed themselves. This design makes the new biosensor a cost-effective alternative to laboratory testing or even cost-intensive on-site testing. In addition, it can also be used in environments such as a bar, clubs or discotheques, which is particularly relevant in the case of the detection of date rape drugs.

### Awards

The team won a Gold Medal and awards for the "Best Environmental Project", "Best New Composite Part", "Best Presentation", and "Best integrated Human Practice". Further, the team was awarded with the "Security Commendation" ●

*Further information about the project can be found here:*

https://2015.igem.org/Team:Bielefeld-CeBiTec




**The cell-free protein synthetizes (CFPS) is done a one-pot-reaction in a small scale. For the production, a DNA template with the given protein of interest, water, co-factos, NTPs, Cell extract – in this case from *Escherichia coli* – amino acids and PEP as an energy source is mixed.**

# 2016

## Evobodies – Molecular Speed Dating

## (Production of easy to modify binding proteins in *Escherichia coli*)

TEXT: NILS-CHRISTIAN LÜBKE


**iGEM-Team Bielefeld 2016: Back row from left to right: Carsten Hain, Pascal Schmidt, Fabian Roeloffs, Mikail Sahin, Niklas Hoffmann, Marten Linder, Sebastian Perez Knoche, Marius Schöller. Front row left to right: Cassandra Königs, Judith Kampa, Bianca Frommer.**

**Binding proteins are capable of attaching themselves to one or more specific targets. In 2016, the iGEM team Bielefeld set the scope of their activities towards implementing a novel and easy to use binding protein production system. The team used *Escherichia coli* and applied *in vivo* directed evolution onto the binding regions of synthetic antibody-like proteins. In this process, the binding region of the given protein is mutated, and subsequently, a selection process for increased binding takes place. The resulting system can quickly generate binding proteins – altered synthetic antibody like proteins - to specific targets. Those binding proteins are called "Evobodies".**

**The team created a system to generate improved binding proteins based on an initial library containing coding sequences of the proteins' specific binding regions. They modified the proteins constantly and selected the best candidates by antibiotic selection using a bacterial two-hybrid system. The selected proteins are directly injected into the mutation and selection process with higher antibiotic pressure. Thus, the proteins with the desired abilities are accumulated and improved by each process cycle. The results are verified through sequencing.**

**Next to the molecular biology work in the laboratory, the team thought about their project's impact. They conducted different interviews with various stakeholders discussing and shaping a possible industrial application of their project. The discussions resulted in process development and a business plan shared with the iGEM community. Further, the team created a synthetic biology dictionary and spread the word about their project as well as synthetic biology in different outreach activities.**

### Motivation

A specifically adapted binding protein can be used for a variety of applications. The key to the application is the specific interaction of the binding proteins with their target(s). Thus, the demand for those types of proteins is increasing, from basic laboratory to more advanced medical applications. Usually, the generation process and the specificity of the proteins are the bottlenecks of production. On top, after careful research, the iGEM team 2016 did not find any working systems for generating and adapting binding proteins in the iGEM registry of standard biological parts – at that time.

The team tried to solve these problems by creating an easy to apply and fast method to generate proteins with specific binding characteristics.

### Results

The project can be divided into different project parts – library, mutation, and selection. The library sets the base for further progress in the project. It is the starting point for the mutagenesis of the binding regions and, thus, the starting point of the altered shift towards an increased protein binding affinity. The library consisted of binding proteins with at least a low affinity binding to the target. Those binding proteins, or their corresponding binding region, to be more precise, were to be mutated and selected. In their endeavors, the 2016 team designed and constructed a library with over one hundred thousand Monobodies (a specific synthetic binding protein) and over one hundred sixty thousand Nanobodies – naturally occurring binding protein, respectively. They paved the way for future iGEM teams to build their own libraries on the iGEM Bielefeld's system.

The synthetic binding protein's ability to bind to the desired target should be enhanced by submitting the created library to multiple cycles of mutagenesis. The team used a falsified PCR reaction, called error-prone PCR, to introduce mutations in the specific binding region of the protein. Selection pressure was applied by antibiotic treatment, and subsequent selection was performed by a bacterial two-hybrid system. The bacterial two-hybrid system is based on the common yeast two-hybrid systems but optimized for bacteria like *E. coli*. For this purpose, a phagemid display using phagemids pAK100 and M13 derived from helper phages was used and coupled with antibiotic treatment. The team successfully created their "Evobody"-system and verified the gathered results by sequencing. All project parts worked individually. Hence, the team 2016 provided the iGEM community with a proof-of-concept for a protein modification system based on *in vivo* directed mutagenesis.

For future applications, the establishment of the fermentation process is essential. The team, therefore, conducted modeling approaches for best fermentation strategies. They gained first insights by shaking flask cultivations and during the usage of a self-made turbidostat.

### Towards application

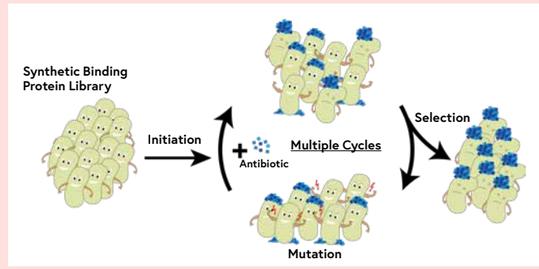
The team made efforts to create a process development and upscaling process as well as a business plan to fortify their project's industrial application. In this process, the team members investigated possible prerequisites, like legal aspects or the reduction of their process complexity, to manufacturing their Evobodies. In addition, they thought about upscaling and production on industrial scales using fermentation techniques. Finally, Evobodies should be used by researchers and diagnosticians worldwide.

### Awards

The team achieved a Gold Medal. ●

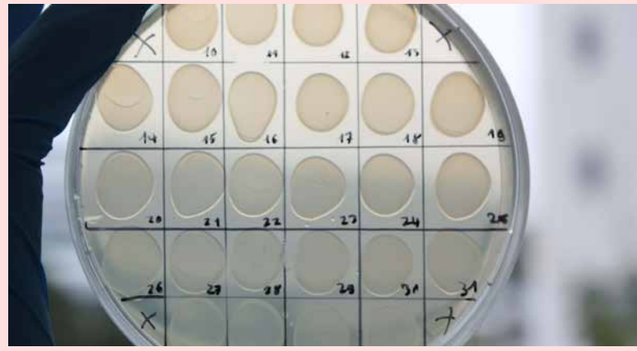
*Further information about the project can be found here:*

https://2016.igem.org/Team:Bielefeld-CeBiTec




**The generation of "Evobodies" in *Escherichia coli*. The binding proteins are passed through multiple *in vivo* directed mutagenesis cycles starting with a synthetic binding protein library. Then, the proteins are adapted and selected to their specific target using an antibiotic selection system.**

***The resulting system can quickly generate binding proteins to specific targets, called "Evobodies".***

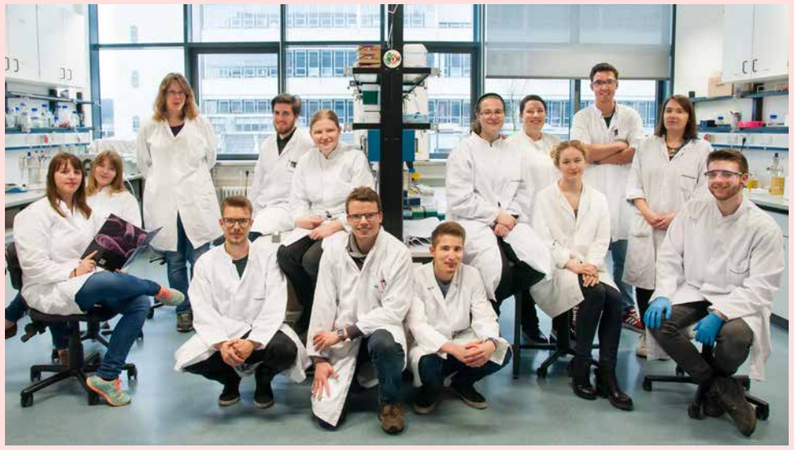


**A few of the many cloned colonies from the constructed library on a agar plate.**

# 2017

## Expand – Expanding the genetic code for introduction of non-canonical amino acids into proteins

TEXT: NILS-CHRISTIAN LÜBKE



iGEM-Team Bielefeld 2017: From left to right: Camilla Maerz, Saskia Dymek, Christina Drake, Daniel Bergen, Lennard Karsten, Michelle Liebers, Markus Haak, Maximilian Edich, Denise Kerkhoff, Olga Schmidt, Svenja Vinke, Christopher Whitford, Laura Schlüter, Yannic Kerkhoff.

**The iGEM 2017 team established various ways to incorporate noncanonical amino acids, amino acids beyond the standard used amino acid for protein synthesis, into the genetic code of the given organism. In this case the team worked with *Escherichia coli*. The genetic code is based on the four nucleo-bases adenine, thymine, cytosine, and guanosine. The bases are the foundation of the desoxyribonucleic acid (DNA) strand. The DNA is "read out" and transformed into the ribonucleic acid (RNA) during a process called transcription. The RNA contains a combination of the four bases with uracil as a replacement for thymine, arranged as base-triplets. Those triplets are used to build up proteins during the translation process. Every triplet "codes" for a specific amino acid to be inserted into the protein. Thus, the base triplets are called "codons". The incorporation of the amino acids into the protein chain is managed by so called transfer-RNAs (tRNAs). The mature proteins themselves fulfil different kinds of tasks in an organism. From their function as enzymes converting substrates into products to being outer membrane signaling proteins or binding proteins binding specific targets.**
**Re-coding the genetic code can inherit certain features and benefits. First off, being able to tailor a protein structure, which is determined by the amino acid composition, can help apply the proteins for broad variety of industrial and medical processes. Second, incorporating amino acids or newly nucleo-bases in the DNA may enhance the biosafety of a designed system as the system is easy recognizable and retrievable. Those approaches are called xenobiology ("alien-biology", from the Greek word "xenos") expanding the natural given system. Xenobiology is one of the major fields of synthetic biology.**
**The team worked on establishing the expanding of the genetic code in two approaches. One approach depicted the incorporation of a newly nucleobase in the DNA. The other approach exhibited the re-programming of an already in-frequently used codon to be used with newly introduced amino acid.**

### Motivation

Protein design is still today one of the major pillars of industrial used biotechnology and molecular medical diagnostics or therapeutics, respectively. If one can easily change the abilities of a protein towards a desired function, the applications of proteins increased drastically. Proteins can be used for their key enzymatic function of converting substrates into products or to bind a specific ligand or other proteins.
Beyond, incorporating noncanonical amino acids into the translational machinery – and with this doing xenobiology – could increase biosafety and biosecurity as those created systems are easily detectable and may be less interactive with natural occurring systems.

### Results

Using a rarely used codon in *Escherichia coli*, the team aimed to re-program the amber stop codon – UAG/ uracil, adenine, and guanosine – for their purposes. In doing so, they also need a modified tRNA-synthetase (aaRS). The transcriptional machinery needs to detect the re-programmed amber codon, subsequently makes use of the corresponding tRNA. The corresponding tRNA needs to be loaded prior to this process with the desired noncanonical amino acid. Hence, the team needed a modified tRNA-synthetase to load their noncanonical amino acid to the tRNA corresponding the amber codon UAG. Only then, the newly introduced noncanonical amino acid would be incorporated into the protein.
Based on wild-type aaRS from the bacterium *Methanococcus jannashii*, the team used a library approach applying mutation and selection process to optimize potential aaRS to bind the team's noncanonical amino acid. Results were tested by a selection system and Illumina sequencing. The library approach resulted in 27,672 possible usable aaRS. Further, they modeled their approach to prove feasibility of the experiments.
The second approach is based at the beginning of the protein production process by incorporating an unnatural base pair into the DNA. For this, the team had two possible choices: Either feeding *E. coli* with the unnatural base pairs through the cultivation medium or imparting *E. coli* the skill to synthesize the unnatural base pair itself. The overarching idea in both approaches is, that the unnatural base pair will be incorporated into the DNA and thus, will code for a new codon later on in the translational machinery for protein synthesis.
The uptake of the unnatural base pairs through the cultivation medium was achieved through the incorporation of transporter proteins from the algae *Phaeodactylum triconutum* into the outer membrane of *E. coli*. The approach to synthetize the unnatural base pair was facilitated by the isoguanosine – an unnatural base pair – synthetize pathway of the plant *Croton tiglium*. The pathway was successfully investigated in *Croton tiglium* and partly applied in *E. coli*.
In addition, the team modeled their approaches, established ways of selection and detection, developed a hardware tool and a corresponding android app for the manipulation of noncanonical amino acids, improved the analysis of nanopore sequencing to their needs, and wrote a comprehensive biosafety report regarding auxotrophy and xenobiology peer-reviewed published in Public Library of Science (PLoS).
Further, the team showed self-awareness of their research by conducting a comprehensive report including feedback from various stakeholders on "Chances and Implications of an Expanded Genetic Code" in their project.

### Towards application

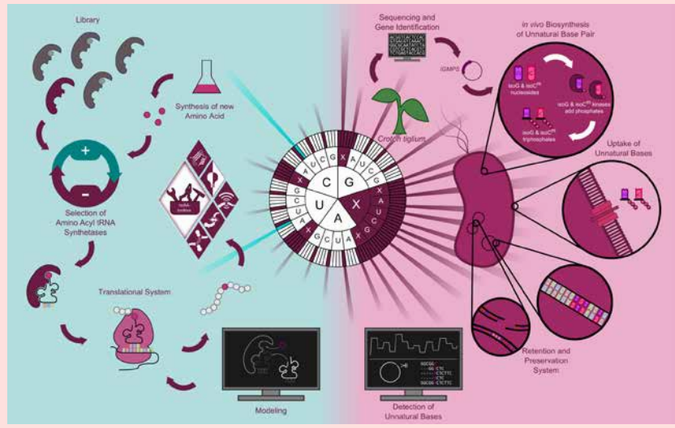
Xenobiology in protein production of a specific protein design can be applied in a broad variety of industrial and medical process. Every process involving a protein can, in theory, benefit from trailer-made proteins. The advantage of the xenobiological approach is the retrievability and detectability of the produced system as it exhibits noncanonical amino acids.
Beyond the human practice approach towards biosafety considerations should be incorporated in everyday research and may strengthen the ability of researchers to assess the impact of their research.

### Awards

The team achieved a Gold Medal and won the track prize "Best Foundational Advance" as well as the special prize for the "Best Basic Part". ●

*Further information about the project can be found here:*

https://2017.igem.org/Team:Bielefeld-CeBiTec




**The comprehensive workflow of the iGEM team Bielefeld 2017 project. Depicted on the left are the approaches to construct a tRNA-synthetase capable of loading a specific tRNA with their noncanocial amino acid and the corresponding modeling effort. On the right side, the efforts for an unnatural base pair as the base for noncanonical amino acids are shown. The approaches can be divided into the idea to supplement the organism with unnatural bases by the cultivation medium and to synthesize the unnatural base pair within the organism.**

# 2018

## nanoFACTORY – a combined system to scavenge metal ions in the environment

TEXT: IRENA MAUS

**Electronics consumption is increasing worldwide, in return, metal resources are becoming increasingly scarce. In today's era of electronics and overconsumption, it is almost impossible to imagine that the age of electronics will ever end. In this context, the Bielefeld iGEM team 2018 aimed to develop a system to scavenge heavy metal ions in the environment. The team optimized *Escherichia coli* cells to accumulate heavy metal ions inside the cytoplasm, such as copper and iron, by overexpressing specific import proteins and silencing the expression of heavy metal ions to export proteins simultaneously. Furthermore, the gene of the iron storage protein ferritin (bacterioferritin BfrB from *E. coli* and the human ferritin HuHF), the product of which can form nanoparticles, was cloned into the *E. coli* genome and successfully expressed, thus ensuring that the cell can form nanoparticles of a specific size which intercepted and exported accumulated heavy metal ions. The metals recycled inside nanoparticles can be recovered and used for various industrial applications.**

### Motivation

Raw material consumption worldwide amounts to 85 billion tons (Federal Ministry for Economic Affairs and Energy Germany, 2017). Without countermeasures, consumption will have doubled by 2050, and that is far more than is available to mankind. In this regard, the Bielefeld iGEM team was unanimous that they want to develop something that would secure resources – a system that would promote sustainability. The team came across the idea of purification of waste of any kind from metal ions. Followed by this motivation, the Bielefeld iGEM participants wanted to use synthetic biology to filter metal ions from the environment and use them as new resources – thus making the world a little better for us, but especially for future generations.

### Results

Recycling metals from electronic pollution and returning them to the community as valuable metal resources is the basic idea of the Bielefeld iGEM project. The participants used the tolerance of *E. coli* DH5α to heavy metals. They successfully cloned and expressed the human ferritin heavy chain (HuHF) BBa_K2638999 construct in these cells, which can bind different metal ions, e.g., iron and copper, and form nanoparticles. Thus, the new DH5α cell line was suitable for recycling different valuable metal ions. In the next step, the team increased iron nanoparticle formation through bacterioferritin BfBr overexpression and engineered HuHF to investigate stability changes in the presence of copper ions. Furthermore, they constructed HuHF mutants with the ability to form gold and silver nanoparticles.

### Towards application

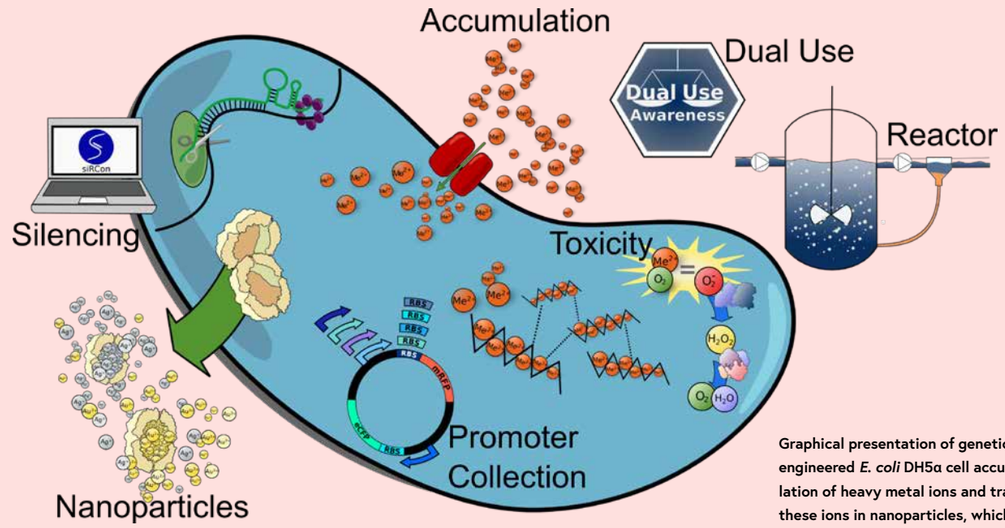
The nanoparticles produced with ferritin by the 2018 iGEM Team can be used inside the ferritin cage for molecular imaging. Furthermore, they can also be used as antibacterial agents and biosensors when extracted.

### Awards

The 2018 iGEM team won a gold medal at the European Regional Jamboree and Safety Commendation award and qualified for the world championship. ●

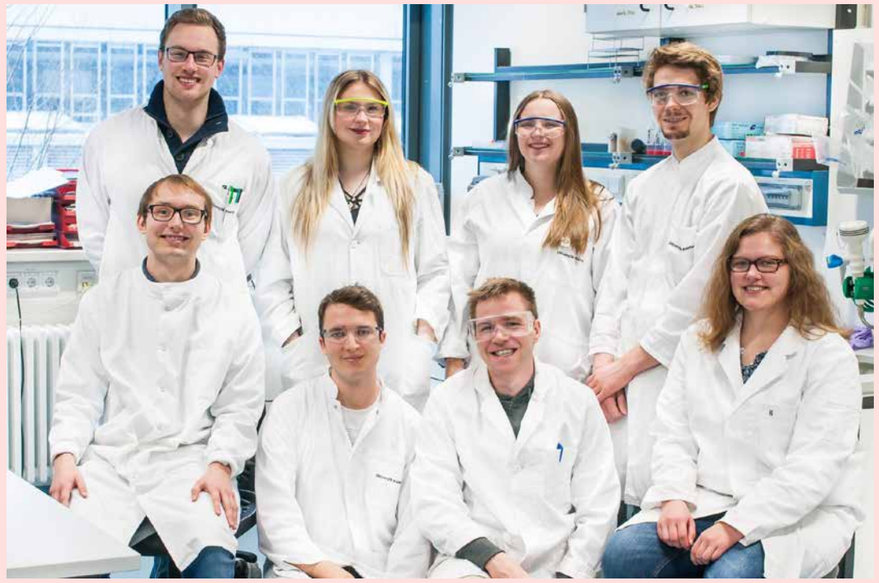
*Further information about the project can be found here:*

https://2018.igem.org/Team:Bielefeld-CeBiTec




**Graphical presentation of genetically engineered *E. coli* DH5α cell accumulation of heavy metal ions and trapping these ions in nanoparticles, which are then exported.**

***Effective pollution control has to minimize raw material use, thereby decreasing final waste and emissions.***



**The 2018 iGEM Team members (top row from left to right): Levin Joe Klages, Irina Rais, Vanessa Krämer, Antonin Lenzen (lower row from left to right): Matthias Otto, Johannes Ruhnau, Christoph Geske, Erika Schneider.**

# 2019

## Troygenics – a sophisticated bacteriophage-based platform system to transform eukaryotic microorganism

TEXT: IRENA MAUS

**Unicellular eukaryotic organisms are often difficult to handle and are only partially subject to genetic engineering. Transformation of those complex eukaryotes, e.g., fungi can unlock the treasures hidden in the genome of these enigmatic organisms and thus lead to new insights and industrial applications. In 2019, the Bielefeld iGEM team aimed to develop a platform system called Troygenics, which allowed to transform eukaryotic microorganisms. The so-called Troygenics represented the M13 bacteriophage with a modified major coat protein allowing selective endocytosis into the target cells. During this process, the gene of interest – packed up inside the Troygenic – is delivered into the cell of interest, thus transforming the cell transiently at first. Adding of homologous recombination sequences around the gene of interest enabled genomic integration and the generation of stable transformations. The system developed allows transforming even the most difficult eukaryotic targets, which will simplify not only many lab procedures but also has great potential in basic research.**

### Motivation

Many unicellular eukaryotic organisms are poorly transformed and therefore, troublesome to handle in the lab. Transformation of those complex eukaryotes can unlock the treasures hidden in the depths of these enigmatic organisms, for example, regarding the fascinating kingdom of fungi. We strived to develop a key to reach their secrets by designing Troygenics, a platform system that can be adapted to transform any eukaryotic cell.

### Results

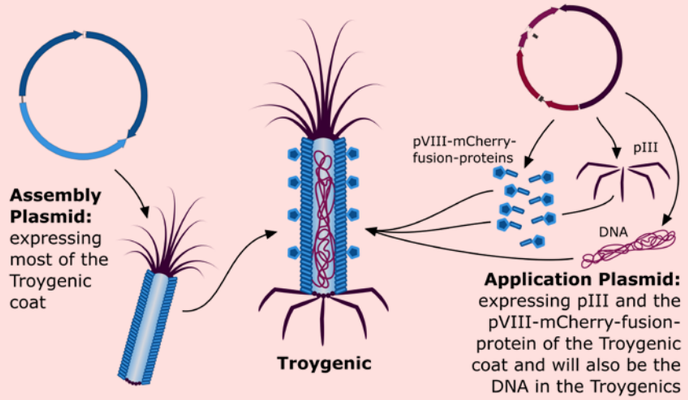
To transform eukaryotic microorganisms, the Bielefeld iGEM team 2019 developed a construct called Troygenics which basically represents two plasmids with genes mainly from the M13 bacteriophage allowing the generation of stable transformations leading to selective endocytosis into the target cells. The Troygenics were designed to aim at their target cell in a highly specific manner, leaving every other organism unaffected. To make the system as safe as possible, the team members split the Troygenic genome into two plasmids. The Assembly Plasmid coded for every protein necessary to replicate the Troygenic apart from the minor coat protein pIII, while the Application Plasmid coded for a shortened pIII, a modified pVIII and the gene of interest, which should be delivered into the target cell. By splitting up the essential genes into two plasmids, the team could ensure that Troygenics cannot be built with only one plasmid.

To get the system inside the target cell, the iGEM students used a fraction of the major coat proteins to target-specific ligands known to induce receptor-mediated endocytosis. After entering the cell via endocytosis, the DNA carried by the Troygenic integrated into the host cell's genome, so a gene of interest, in some cases Cas13a and some host-specific guide RNAs as the Cell Death Inducing System (CeDIS), are expressed. As soon as CeDIS detected any of its host-specific targets, it started collateral RNA cleavage, making the target unable to produce any protein, which ultimately results in cell death.

The system the Bielefeld iGEM team 2019 developed allowed them to transform even the most difficult eukaryotic targets, which will simplify many lab procedures and contribute to the primary research.

### Towards application

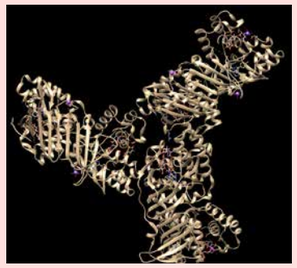
Troygenics can be adapted to fight several plant pathogenic fungi like *Phytophtera infestans, Puccinia graminis* and *Fusarium oxysporum* representing a huge threat to the world's food supply. Apart from fighting plant pathogenic fungi, Troygenics could emerge as a simplification of challenging task in the lab, e.g., the transformation of fungi and other eukaryotes. There are various of possible applications ranging from creating new production strains in the industry to fighting eukaryotic pathogens in the environment. The loss of these species would have a detrimental impact on entire ecosystems. Furthermore, *Trypanosoma*, which causes the African sleeping sickness and often results in the patients' death (WHO), is another challenge that Troygenics could tackle. In the best case, the Troygenics would specifically fight the *Trypanosoma* while having no effect on the human cells showing significant advantages to conventional treatments. A further application is needed to test this hypothesis.




**The two plasmids, namely the Assembly Plasmid and Application Plasmid, and their respective coded proteins, as well as the correctly built Troygenic as a product of these two plasmids. The Assembly Plasmid codes for all coat proteins except of pIII. This plasmid is only for the packing of the DNA in the protein coat. The Application Plasmid codes for a truncated version of pIII and a fusion protein of pVIII and mCherry (most times fused to an endocytotic ligand), as well as it is replicated as single stranded DNA which will be packed in the Troygenic as its DNA.**

### Awards

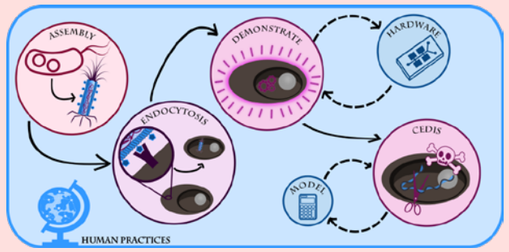
The 2019 iGEM team won a gold medal at the Jamboree and Best Measurement award. ●



**The 3D-structure of Aminoglycoside Phosphotransferase APH(3')-Ia as obtained from PDB. This protein conveys resistance to kanamycin.**

*Further information about the project can be found here:*

https://2019.igem.org/Team:Bielefeld-CeBiTec




**Experimental aspects of the Troygenics project, starting with the Troygenic assembly, endocytosis and Cell Death Inducing System (CeDIS).**

**The 2019 iGEM Team members (from left to right): Ina Schmitt, Astrid Többer, Johanna Opgenoorth, Niklas Fante, Alexander Schulze, Nefeli Chanoutsi, Isabel Conze, Katharina Wolff, Adila Apsara.**

# 2020

## Wavy Sense – the determination of the current human fertility status applying the surface acoustic waves technique

TEXT: IRENA MAUS

**Modern contraceptive strategies often include invasive methods, e.g., intrauterine devices or suppressing the natural menstrual cycle. This causes undesirable side effects in women such as migraine, weight change, depressive mood, mycosis or even thrombosis and strokes. The hormonal pill is the contraceptive most commonly used in Europe, but its functioning is not optimal. The Bielefeld iGEM team 2020 launched the WavySense project to develop a better non-invasive alternative to existing contraceptives that reliably indicates fertility in women under simple domestic conditions. The students created a device to determine the amount of hormones estradiol, progesterone and the luteinizing hormone (LH) in the morning urine to specifically determine the menstrual phases in women, thereby enabling the determination of the fertile phase in which a pregnancy is possible. By linking the hormone-based self-provided biosensor to the surface acoustic wave (SAW) technology within an app, the team quickly created a user-friendly application to display women's fertility status.**

### Motivation

Not all contraceptive methods are equally helpful for every woman; in some cases, they perform poorly, causing undesirable side effects or health impairment. There is a strong need for an alternative contraception strategy focus on natural contraception, with synthetic biology that could offer a key to this problem. The main idea of the iGEM project WavySense was to develop an alternative contraceptive method by using a simple and easily accessible way for women to display their ovulation in which women can get pregnant.

### Results

The duration of the menstrual cycle is between 25 and 30 days and can be divided into three phases: follicle phase (1), ovulation (2) and luteal phase (3). A new cycle begins with the menstruation within the follicle phase. In this phase, the follicle matures and the estrogen concentration rises, resulting in increasing LH release. During this time, the body begins to build up the endometrium. This leads to the second phase, the ovulation, in which a pregnancy is possible.
To detect hormone concentrations in the urine, the Bielefeld iGEM team 2020 used hormone-specific nanobodies, which were developed within this project, and single-chain variable fragments (scFv) combined with the surface acoustic wave (SAW) technology, that all in all delivered a sensitive and specific device for determining the fertility. Applying synthetic biology, the team first created specific nanobodies. The nanobodies showed a higher availability to tissue penetration and due to their smaller size, they trigger less immunological reaction, which leads to better pharmacokinetics. All these points were important properties for therapeutic options and diagnostic processes, e.g., molecular imaging.
The crucial role of the WavySense measurement was played by the innovative SAW method, enabling high sensitivity measurement. The (SAW) chip should carry immobilized nanobodies, which were developed in this project and are able to bind the sex hormones specifically. The SAW technique allows the device to measure slight weight differences which occur when the small sex hormones bind to the SAW-chip. Therefore, the piezoelectric effect is used, where an electric charge is created upon mechanical force. Due to the change in pressure caused by the weight of the bound antibodies, the electric charge is changed. WavySense should be able to detect these changes and correlate it with hormonal standards to identify the precise hormone concentration. The measuring chip is designed with separate measurement and reference channels for each hormone detection. The chip can also be connected to a mobile phone measuring device, which sends the measured data to the self-created WavySense-app, displaying the results to the user. The app can also record important additional information like the time and duration of the menstruation.

### Towards application

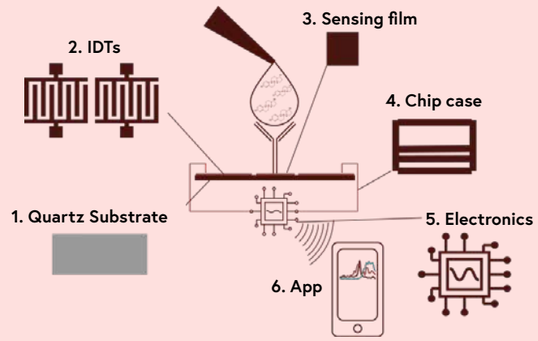
WavySense addresses women who want to prevent pregnancy. At the time of the project, only a prototype of the measuring device was designed but, due to the corona virus pandemic, could not be fully assembled. In the future, the reusability and durability of the SAW chip and the other components should be optimized to further safety and reliability of the product. In the end of this process, clinical testing and medical approval would be most important before a market launch in cooperation with medical experts, the German Ministry of Health and widely known official information platforms could be possible.

### Awards

The 2020 iGEM team won a gold medal at the Jamboree. ●

*Further information about the project can be found here:*

https://2020.igem.org/Team:Bielefeld-CeBiTec




**Graphical overview of the WavySense project starting from the application to the implementation and human practices. In the center is shown a surface acoustic wave (SAW) sensor. This sensor consists of quartz piezoelectric crystal (dark brown plate), IDTs (interdigital transducer) are built on the crystal (three light brown plates), the sensing film is located between the IDTs, the SAW Chip is inserted in a chip case which establish the connection to the electronics, the electronics are connected to the app to evaluate the data.**

***Tracking the hormonal cycle can be used as non-invasive and, at the same time, safe contraception method.***

**The 2020 iGEM Team members (from left to right): Laura Keller, Benjamin Julian Saalfeld, Ramon Luna Stollmeier, Tamara Folk, Philipp Kühnel, Leon Diel, Niko Klassen, Melina Müller, Marvin Geisler, Neele Kusch, Angelika Urban, Stephan Weber, Janine Dippel.**

# 2021

## P.L.A.N.T. – Plant-based Ligand Activated Noxious agent Tracker (A plant-based detection of specific chemical weapons in the soil)

TEXT: IRENA MAUS

The 2021 iGEM Team members (from left to right): Johnny Le, Marvin Hildebrandt, Lennart Lutz, Jonas Dauster, Jacob Sarx, Paul Goffing, Tim Prasun, Lucas Krause, Matthes Haese, Marie Schmiedekamp, Mareike Keßler, Julia Macholl, Louisa Lehmann.

**Chemical weapons are still stored in several unknown places all over the world and pose the threat of leakages. However, the storage sites are difficult to trace. Unfortunately, there is no significant effort from the German federal government to find, investigate and eliminate these toxic areas since this approach is cost- and time-intensive. The Bielefeld iGEM team 2021 intended to contribute to getting this problem under control by creating a detection system to identify contaminated sites that store chemical weapons. For this reason, the project "P.L.A.N.T – Plant-based Ligand Activated Noxious agent Tracker" was born in order to detect contaminations of specific chemicals in the soil. The system developed within this project allows the detection of chemicals by plants that can be seen visually through a change in the leaf's color. In their project, iGEM students worked with chemicals that are related to chemical weapons but do not contain the same risks.**

### Motivation

In Germany, contaminated sites are suspected in over 200 locations. They pose a toxic threat to humans and the environment if leakages occur. Tracing these sites is difficult because the exact location and the dangers can't be predicted in advance. Moreover, the German federal government makes no significant effort to investigate these sites and remove those chemicals. One of the critical factors here is the costs required to identify locations, and decontamination requires expensive equipment, time, and significant effort. Since no detection system is known for screening and surveillance of large suspect areas with chemical weapon degradation products, the Bielefeld iGEM team was motivated to create a cost-efficient and easy-to-use detection system with extensive area coverage to quickly identify these contaminated sites.

### Results

The team 2021 aimed to develop a two-component signaling system to detect several chemicals, namely methylphosphonic acid (MPA), diisopropyl methylphosphonate (DIMP), diethyl methylphosphonate (DEMP), thiodiglycol (TDG) and benzenetricarboxylic acid (BTCA) in tobacco plant *Nicotiana benthamiana*. These chemicals, related to chemical weapons but safe to work with, are stable and can potentially severely contaminate the environment and groundwater. Thus, *Nicotiana benthamiana* as hydroculture was cultivated in the lab to demonstrate chemicals uptake into the plant as well as its tolerance range against DIMP, DEMP, MPA and TDG.

The heart of the P.L.A.N.T. detection system was a signaling cascade split on two pMAP plasmids which was introduced in the plants. The first comprises the receptor ssTNT.R3, which specifically binds ligands, the membrane protein AtFLS-Trg-PhoB, and the second plasmid, consisting of the transcription factor PhoB-VP64 and the reporter RUBY to visualize the presence of DIMP, DEMP, TDG and BTCA in the soil. After detection of a chemical weapon degradation product and the activation of the signaling cascade in *N. benthamiana*, a visible output could be created. The plant exhibited a color change from green to red, caused by activating the RUBY reporter, which comprises genes for betalain biosynthesis, allowing the visible red coloration. Another fast-growing and easily manipulative model organism was required to perform initial tests with a signaling cascade designed in combination with computationally engineered receptors. The team decided to work with *Escherichia coli* for the first testing of a bacteria-adapted signaling cascade and proved the functionality and higher affinity of the engineered receptors to the chemical weapon-related molecule. To demonstrate its functionality, iGEM students tested the plant signaling cascade by transient expression in *N. benthamiana* and were able to record positive results.

### Towards application

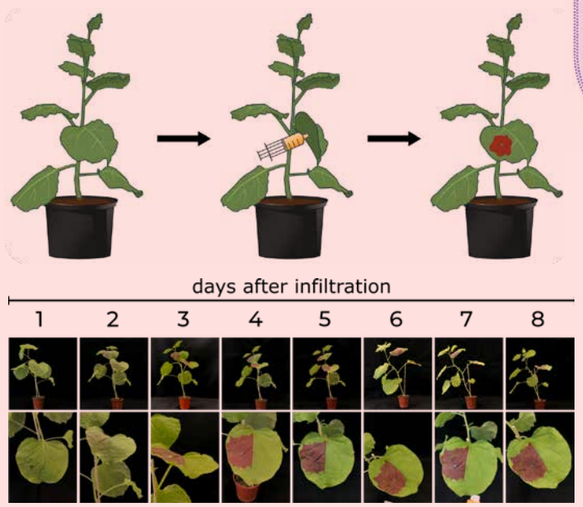
When appropriate safety and security features for gene-modified plants are established and their release into the environment is regulated in Europe, the P.L.A.N.T. system could be placed at suspected contaminated sites and then indicate the presence of these chemical weapon degradation products by shifting their color to red. The system is further adaptable for other chemical weapon-related chemicals by adapting the receptor.

### Awards

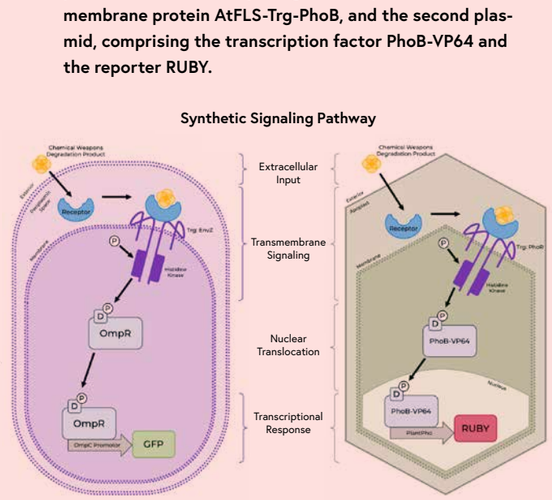
The 2021 iGEM team received a gold medal and was placed among the Top 10 teams at the Jamboree. The team was further nominated as Best New Application and Best New Basic Part. ●

*Further information about the project can be found here:*

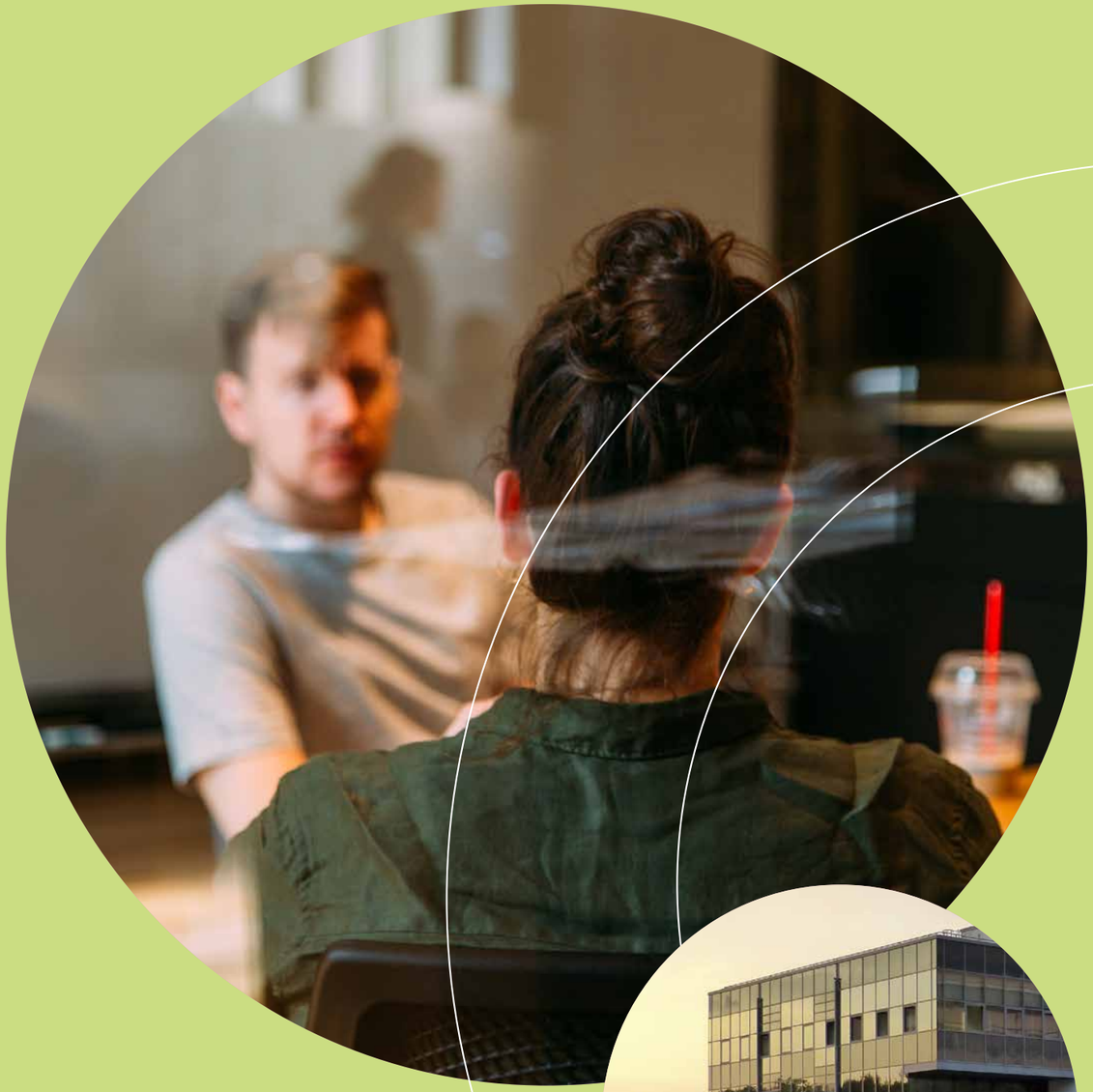
https://2021.igem.org/Team:Bielefeld-CeBiTec




The principle of P.L.A.N.T. application in *N. benthamiana*. A needleless syringe is used to infiltrate agrobacteria into the underside of the tobacco leaf. After two days, the RUBY reporter should be detectable by coloring contaminated leaf sides red. Infiltration with 35S:RUBY with agrobacteria for transient transformation resulted in a stable visible signal in *N. benthamiana*.




Schematic overview of the chemical detection signaling cascade in bacteria (left) and plants (right). The two-component system of the chemotaxis was engineered to allow recognition of a ligand of interest as a stimulus and resulted in a visible output. In bacteria, the stimulus were chemical weapon degradation products, and a green fluorescent protein (GFP) is used as response indicator. In plant, the signaling cascade was split on two pMAP plasmids. The first comprising the receptor ssTNT.R3, the membrane protein AtFLS-Trg-PhoB, and the second plasmid, comprising the transcription factor PhoB-VP64 and the reporter RUBY.



# 03

# Interviews of Bielefeld University members involved in iGEM projects

*'It just came naturally when a group of moleular biotechnology students approached me to ask whether I would support their iGEM participation.'*

PROF. DR. JÖRN KALINOWSKI

# History and highlights of iGEM at the CeBiTec

**Prof. Dr. Jörn Kalinowski is head of the Research Group 'Microbial Genomics and Biotechnology' and leads the Technology Platform Genomics of the CeBiTec. He also is a member of the CeBiTec's managing board. Prof. Kalinowski initiated iGEM activities at the CeBiTec in 2010 and since then was the driving force behind all iGEM projects that were carried out so far. He acted as supervisor and instructor of iGEM teams and helped to provide the financial resources needed to carry out iGEM projects. The following interview with him grants insights into different aspects of the iGEM initiative at the CeBiTec from his individual point of view.**





***What considerations led you to support the iGEM initiative at CeBiTec?***
It just came naturally when, in early 2010, a group of molecular biotechnology students approached me and asked if I would support an iGEM project. That was the starting signal, and the fact that the initiative came from the students themselves was, I think, the ideal precondition.

***How were the members of the annual iGEM teams interested and chosen?***
Several information events took place, to which bachelor and master students from the biology, chemistry and technical faculties were invited. Written applications were collected and applicants were asked to present their motivation for participating in the iGEM competition. If there were more applications than the size of the team allowed, individual interviews were held with the candidates and then a consensus was reached as to whether participation in the following year would be an option.

***What distribution of tasks had to be made in the iGEM team in order to achieve the different goals of the project?***
Roughly speaking, the tasks in the iGEM competition are divided into categories relevant for the evaluation and for organizing the team and resources. The latter also includes sponsorship, for which a small special team was always established very early in the competition period. Further division of tasks then takes place based on the knowledge and skills, but also the interests of the participants. Specializations are then possible in relation to the construction of biobricks, technical-analytical processes, mathematical modeling or even the area of 'human practices', i.e. interaction with society.

***How were the individual iGEM teams prepared for the evaluation process?***
This question can only be answered by considering what I would call the 'Bielefeld Model', because there are certainly many other possibilities. By being able to offer ex-iGEM participants Ph.D. projects, we established a system in which the new teams were supervised by experienced iGEM alumni and thus optimal preparation for the competition goals could be achieved. The supervisors also worked completely voluntarily, but were then allowed to accompany the team to Boston. Another important fact was availability of CeBiTec laboratory rooms that could be used around the clock. Equally critical was access to the genomics technology platform and post-doc expertise in my working group or elsewhere in the university. Ultimately, however, we also have to talk about finances. The teams were actually exceptionally successful in raising sponsorship funds. However, I was always able to help out if there was a need of further funding. In this respect, no team member or supervisor had to stay at home when the grand jamboree in Boston was coming up. I think it is extremely important to honor everyone's contributions.

***What role did participation in an iGEM project play for the students involved?***
I learned how important competitions in science are. Students with an interest in science in particular learn a lot of skills in a very short time that only play a minor role in the *curricula* of their particular course of studies: independence, time management, team building, electronic documentation using wikis and working under extreme pressures of time, competition and success. In my view, the relevant experiences and different interactions with internal and external team members and experts worldwide are very important for the team members. Furthermore, networks that are extremely helpful for the future career could be developed. Participation in iGEM was therefore a formative experience in the scientific career of every participant. All facets of scientific work were went through in fast forward mode.

***Why should teams from the CeBiTec and Bielefeld University take part in the iGEM competition in the future?***
The most important reason for the teams to participate in iGEM is that in this way, essential knowledge and experience is gained within a short period of time, which is crucial for an academic career and which is not taught within the framework of the studies or is taught outside of an immediate application context. iGEM is also helpful to develop international networks that may be of importance for further careers.
For the supervising doctoral students and post-docs, iGEM experiences are of importnace, because here the supervision of scientific teams can be learned and refined.
For me as a university teacher, the iGEM competition meets all the criteria of scientific training for students and employees. In addition, prospective doctoral students recommend themselves in this way. I have also been able to expand my scientific network and today have many scientific collaborations with iGEM supervisors at other locations. ●

*'My concern was to establish a concept for the continuation of the previously so successful iGEM activities at Bielefeld University.'*

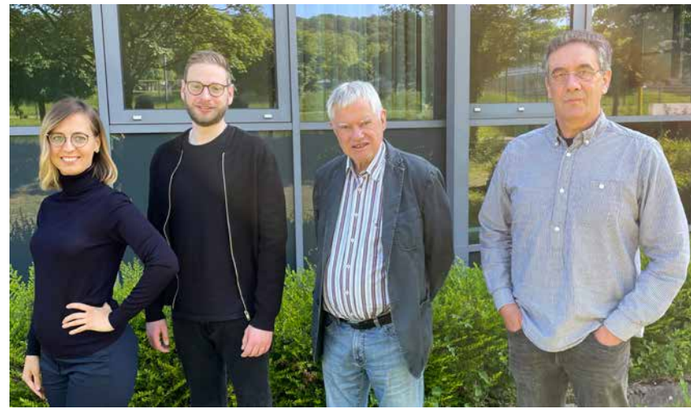
PROF. DR. ALFRED PÜHLER

# Restart of iGEM activities at the CeBiTec of Bielefeld University

**Until 2008, Prof. Dr. Alfred Pühler was employed as a full professor for genetics in the Faculty of Biology of Bielefeld University. His scientific work was highly recognized and he became member of three German academies. From 2008 on, he was further employed as Senior Research Professor for 'Genome Research of Industrial Microorganisms' at the CeBiTec of Bielefeld University. Currently, he is a honorary member of the senat of Bielefeld University and of the managing board of the CeBiTec. Prof. Pühler took the initiative to restart iGEM activities at the CeBiTec in the year 2022 and acquired the necessary financial resources for these efforts. The following interview sheds light on the background of his initiative to restart the participation of Bielefeld University for the next three years.**







**CeBiTec working group involved in the production of the iGEM brochure. From left to right: Irena Maus, Nils-Christian Lübke, Alfred Pühler, Andreas Schlüter.**

***The iGEM competition is focused on the newly developed research field 'Synthetic Biology'. What are your interests in this research field?***

My interests in Synthetic Biology are linked to my own research activities. Through my early engagement in genetic engineering, I got interested in omics technologies, i.e. in genome, transcriptome, proteome and metabolome research. These omics technologies provided all the requirements necessary to broaden my research activities towards the field of cellular-oriented systems biology. I therefore welcomed the CeBiTec's decision to install the master's course 'Genome-based Systems Biology', thus providing an advanced education in biology. Since genetic engineering, omics analyses and systems biology are the basis for synthetic biology, I got interested in this new research field. In 2009, I participated in the development of a statement on synthetic biology that was prepared by the German Research Foundation, the Academy of Science and Engineering and the Deutsche Akademie der Naturforscher Leopoldina. I therefore welcomed the fact that in 2010, a group of students of Bielefeld University decided to take part in the iGEM competition.

***What were the decisive reasons for restarting iGEM activities?***

The participation of iGEM student groups from Bielefeld University was extremely successful from 2010 to 2021. In 2022, there was a sudden interruption, which was due to changed conditions of participation, but also due to a lack of financing to ensure the implementation of the iGEM activities. I regretted very much this interruption and thought about how to get the financing problem solved. One possibility was to get the CeBiTec and the Rectorate of Bielefeld University interested to develop a joint concept to finance iGEM groups in the years 2023, 2024 and 2025.

***What structural changes were introduced to restart iGEM activities?***

In order to set up the iGEM activities at Bielefeld University on a broader basis, both a steering group and an advisory group have been established. The steering group, consisting of members of the Faculty of Biology and the Faculty of Technology with a CeBiTec involvement, should provide existing knowledge for the successful implementation of iGEM projects in the future. Furthermore, the advisory group, in which members of previous iGEM teams should have a seat, is intended to pass on knowledge and experiences from previous iGEM projects to current iGEM teams. Well-trained doctoral students and postdocs should be members of the advisory group for the guidance of experimental work in the laboratory. Moreover, participants from previous iGEM teams are particularly suitable as members of this advisory group.

***In the meantime, what steps have been taken to initiate the restart of iGEM activities?***

The newly implemented steering group is important as a cornerstone of future iGEM projects. It consists of the following scientists: Petra Lutter, Jörn Kalinowski, Kristian Müller, Karsten Niehaus and Jens Stoye. Petra Lutter has previously been involved in iGEM projects contributing to modelling issues. Jörn Kalinowski has been the driving force behind the implementation of all previous iGEM projects. Kristian Müller has followed the iGEM competition from the beginning and has accompanied the implementation of many iGEM projects. Karsten Niehaus was the head of the "Genome-based Systems Biology" master's program for a long time and is therefore familiar with the scientific principles of the iGEM competition. Finally, Jens Stoye is a representative of bioinformatics at Bielefeld University and can therefore offer assistance in the field of bioinformatics.

***How can the successes of the former iGEM projects be documented?***

During initial steps to resume iGEM activities at the CeBiTec, I tried to get an overview of the iGEM projects that have been carried out so far. I was surprised by the number of sophisticated topics and, above all, the continuous awards that had been achieved by the teams. Since these achievements should be documented, I decided to compile a brochure about the past twelve years of iGEM activities. I managed to put together a working group consisting of Nils-Christian Lübke, Irena Maus and Andreas Schlüter, who agreed to prepare an iGEM brochure. In addition, the members of the working group indicated their assistance in the future work of iGEM teams. Andreas Schlüter will be engaged in management and Irena Maus in public relation questions. Christian Lübke will be available with his specific iGEM knowledge. Concerning the iGEM brochure, the members of the working group invested a lot of time in finalizing the brochure. The overall organization was carried out by Andreas Schlüter. Together with the other members of the working group he participated in writing special text passages. The creation of the iGEM brochure was a lot of fun and was particularly beneficial due to the extensive documentation available. The iGEM brochure is intended to be used to facilitate the recruitment of new iGEM team members and to help with fundraising. ●

DR. CHRISTIAN RÜCKERT-REED

# Genome analysis as part of an iGEM project

**Dr. Christian Rückert-Reed is working in the research group "Microbial genomics and Biotechnology" at the Center for Biotechnology at Bielefeld University. He has a broad range of experiences with regards to molecular biology with a focus on research on Corynebactericeae, sequencing techniques and analyses of sequencing data, as well as genetic engineering. Christian has been involved in the iGEM Bielefeld projects right from the start in 2010. He supported the teams beyond being a supervisor for questions regarding molecular biology and project design. Christian is also active as a judge since 2015. In 2017, he received the "Best veteran Judge" award honoring his outstanding services as a judge in the competition. For Christian, judging is not just a mere means for a competition, but a way to help and support teams to grow from their judging feedback.**

***'Mining of genomes can deliver candidates for new and promising iGEM BioBricks.'***





***How did you get involved with iGEM and the supervision of the iGEM Bielefeld teams?***
That was back in the days when I was still working in the lab and had quite a bit of experience with cloning in *Escherichia coli*. After the first iGEM team had recruited Jörn Kalinowski and Karsten Niehaus as PIs, there came the time that the team ran into trouble in the lab. So Jörn approached me, telling me that there were some students who are working on a project on synthetic biology who need help in the lab.

***What are the key pillars of supervising an iGEM Team? Can you guide us through your task as an iGEM supervisor?***
With our teams in the last few years, there usually were several supervisors who manage different tasks. Some focus on giving practical advice in the lab or in modeling, others on teaching how to do Outreach, Human Practice, Sponsoring and all the other parts that make up an iGEM project these days. And at least one brave soul tries to keep overview over the whole project. They sound the alarm when the team is approaching a critical deadline and help the team leader(s) to make sure that all the different sub teams stay on track.

***What kind of experiences through an iGEM project or all of the iGEM project were helpful for you personally or in regards to your career, respectively?***
For me, iGEM has been extremely useful in many ways. There is the obvious: learning about new techniques, molecular tools, equipment, etc. and not only in theory but from people who tested all these things for half a year or more. That gave me lots of ideas for my own work. But even more useful is the more abstract knowledge I got, as iGEM taught me to keep the world outside the lab more in mind. Usually, if you study biology or bioinformatics, you do not really care about the implications what your work will do once you publish it. In iGEM, you learn to think about the potential impact your work might have. Some projects could be used with questionable intentions by others (dual use), but even well intentioned ideas might have unintended consequences. For example, if you use corn or grain as carbon source for your product, large scale production might raise the price of those materials and thus make them unaffordable for poorer people.
Finally, the most valuable thing I got from iGEM are the connections to lots and lots of interesting people. Building a network of persons with different capabilities is a prerequisite in science these days. Through iGEM I got to know a lot of great students, PhDs, and PIs, with whom I work outside iGEM these days.

***Genetic circuits and genetic engineering are the heart of the competition. Can you elaborate on the aspect of genome analysis as part of an iGEM project?***
While this has changed somewhat over time, the fundamental building block of an iGEM project is still the BioBrick, i.e., a piece of DNA that encodes o certain function, be it a promoter, an operator, a coding sequence or a non-coding RNA, to name but a few examples. And the largest repository of new building blocks is still nature and the millions of yet unknown (or unsequenced) organisms. So mining new genomes and transcriptomes can deliver good candidates for the "Best New Basic Part" award.

***You have been awarded the "Best Veteran Judge" in the competition 2017. Can you guide us through the judging process, how you became a judge and judging in iGEM?***
That are quite a lot of things to cover. Becoming a judge was basically due to my evolving role as a supervisor. In the first few years, I was quite involved in the day-to-day work of supervising the team. But as more and more of that was done by iGEMers from the previous teams and as my research work shifted from the lab to bioinformatics, my role changed into becoming the devil's advocate, i.e., picking apart the projects and offering constructive criticism on what and how to improve. Or, to use a nicer term, act as a mentor. As it turned out, that is exactly one of the things iGEM Headquarters is looking for in judges. Experience in synthetic Biology or metabolic engineering was another and knowledge of iGEM a third, so becoming a judge was an easy choice.
Concerning the judging itself, this usually starts with a lot of reading. Once the wikis are frozen, judges are assigned a slate of teams. The number of teams and tracks one gets varies each year, based on the number of available judges (as each team is reviewed by at least 6 judges) and potential conflicts of interest. Usually you have one week to read through the wikis of your assigned teams. Most wikis comprise dozens or hundreds of pages worth of text. Nowadays, you also have to watch the virtual presentations and often other videos made by the teams. Depending on the individual judge, you then also start to enter your votes on the various aspects you have to evaluate and take notes for the questions and answers section during the Jamboree.
During the jamboree, each team is interviewed by the assigned judges, after which they confer among themselves to resolve any remaining questions. While voting is not to be discussed during this "huddle", this discussion is very helpful to whether one has missed a critical detail while reading the wiki or watching the presentation. In former times, there was also a poster presentation which was a great format to discuss with the teams in a more relaxed setting over some food and drinks. Once you have gathered all information, you finalize your grades and write a "small" review for each team, highlighting areas of excellence as well as points to improve upon.

***Any advice for students interested to join an iGEM team in the future?***
I would start with a word of caution: Only join if you are willing to go the whole nine yards. iGEM is extremely demanding and tends to push you to your limits. While there are a lot of people around that will help you as good as they are able to, it is the responsibility of the team to get it to work.
That being said: If you are willing to spend long hours, cope with a lot of frustrations and get out of your comfort zone, doing an iGEM project can be among the best times of your life in science, as it is the rare opportunity to do what you (i.e., the team) want to do as a project. And you will learn things completely outside your fields of study and meet a lot of interesting people who might change your life. ●

*'Modeling nature is a way to understand nature.'*

DR. PETRA LUTTER

# Simulating biological systems as part of an iGEM competition

**Dr. Petra Lutter studied Technical Mathematics at Vienna University of Technology. After stops at the most renowned research institutions in Houston, Austin, Zurich, Frankfurt and Geneva, she has been teaching and researching at the Faculty of Biology of Bielefeld University since 2005. Currently, she is head of "Mathematical Modeling" within the department "Proteome and Metabolome Research", and is also responsible for the Master's program "Genome-Based Systems Biology" at Bielefeld University. Since 2011 she has supported the Bielefeld iGEM teams in terms of computer simulations and mathematical modeling. The following interview highlights the importance of mathematical modeling in the context of the iGEM competition and shows why it is becoming increasingly important in systems biology.**





***What is modeling?***
Mathematical modeling means expressing a real-world problem in the language of mathematics, for example, representing a biological process in abstract terms. This enables researchers to work out essential aspects of the problem and solve it by applying mathematical tools. Since the real problem is not yet available in a formalized form, a modeling process must be initiated. Starting from the hypothesis to be tested, a mathematical model is to be developed, e.g., by setting up a system of differential equations that can then be solved using mathematical methods. Hereafter, it is checked whether the mathematical solution makes sense in relation to the real problem and the model is validated, e.g., by comparison with experimental data. Ideally, there is a permanent interplay between "wet lab", e.g., a laboratory environment, and "dry lab", e.g., computer simulations.

***How did you get into mathematical modeling?***
For me, both mathematics and biology have always been exciting – in my eyes, the ideal combination to vent the secrets of living organisms. I really value interdisciplinary work, of course, you must go into detail within the respective discipline; interdisciplinarity does not work „superficially." Beyond that, it's about finding a common denominator, and this is where I think one of the great strengths of mathematics lies: it helps to figure out what you're talking about – it is definitional, so to say. On the one hand, I really fancy mathematics' abstract world, the structure in which everything interlocks so beautifully, on the other hand, I also love the large scope for creativity. For me, modeling means the foundation for a deeper understanding of a real problem. It offers the possibility to consider different scenarios through, e.g., computer simulations, which can ultimately lead to predictions that would be possible only with a considerably greater effort, if possible at all.

***How can one imagine mathematical modeling in the context of the iGEM competition?***
A great challenge – no doubt! Originally, the community believed that mathematical modeling was a "fancy add-on". So the teams were rather advanced when it came to their minds that mathematical modeling would be a „chic addition" to their project. Well, while on the one hand, you can sometimes explain how a device or an algorithm works in a reasonable time frame, on the other hand, modeling a whole biological system can be, of course, very time-consuming. Ideally, the modeling runs parallel to the lab work and is already envisaged from the start of the project.

***Do you remember your first contact with the Bielefeld iGEM team?***
To get advice from experts in the various life science disciplines, the Bielefeld iGEM students usually ask some faculty members for a consultation. Thus, sometimes, they knock on my office door and politely ask whether I have time to answer "a short question". By the way, this sentence makes me smile because this often happens to me in the context of mathematical modeling, independently from the iGEM competition. Usually, we can't even get by with an hour just for capturing and describing the task. Students often don't suspect it, but this "precise description" of the system already represents the first modeling step. So, as I said earlier in this interview, the time constraint for the first Bielefeld iGEM team asking for modeling support was significant.

***Did this first experience that modeling should be considered from the beginning of the project change the perspective of the following Bielefeld iGEM teams?***
Absolutely! The decision on whether to apply mathematical modeling in a project has always come earlier. And actually, the earlier the simulations started, the more successful the team models were. Fortunately, the Master's programs at Bielefeld University are already very broadly based, thus, some students bring a wide range of knowledge with them. If, in addition, a person who has applied a mathematical model as part of his/her Master's thesis and also has this in mind for the dissertation becomes part of the iGEM support team, that is, of course, ideal.

***If the framework conditions are right and everything runs ideally, how do the iGEM members realize this "wet lab" – "dry lab" interplay?***
I have observed that they often form subgroups of two to three students handling modeling tasks. It's a bit like a congress: the group of modelers is relatively small, but they know each other well because you regularly meet your "peers". Students, who represent the "modeling group" in the iGEM team, are additionally confronted with laboratory tasks – not least because of this, there is a connection between wet vs. dry lab. Of course, there is a regular information exchange between both subgroups. And, the team members who exclusively work in the laboratory also come to the meetings to get a clearer project view.

***What can the mathematical model achieve or what do the students take home?***
Well, of course, this depends on the scope. With a comprehensive model, you can run through various systems' scenarios on the computer and have results predicted under specified boundary conditions. But it is illusory to believe that at the end of the iGEM project, the team will have a model explaining all the phenomena of the biological system, allowing all desired simulations. But even if you don't use a mathematical model for predictions, the model creation itself offers great added value: abstraction and deep understanding of the biological system, and significantly improved laboratory performance, as I have often observed. The fact that mathematics is leaving its so-called ivory tower and turning towards practical applications is, of course, also noticed by the members of the iGEM teams. The systems biology approach, i.e., the interdisciplinary consideration of cells and organisms in their entirety to understand their functioning by capturing all dynamic processes at all levels, is now also part of everyday biological life. In this context, mathematical tools are becoming more and more self-evident. And if you "live" the interdisciplinarity in the project, i.e., you can work both theoretically and practically, then you also acquire essential "soft skills" such as project management, experimental design and the ability to communicate science well – mathematics then also finds itself in language. ●

SVENJA VINKE

# iGEM Biosafety and Dual Use Research of Concern

**Svenja Vinke is a PhD student in the research group "Microbial genomics and Biotechnology" at Bielefeld University's Center of Biotechnology (CeBiTec). Her research focus is set on amino acids, proteins, the genetic code, phages and viruses as well as sequencing techniques. Svenja joined the iGEM Bielefeld team in 2017 and supervised the teams 2018 and 2019. She has also judged at the competition in 2018, 2019, and 2020. Moreover, Svenja is actively advocating awareness of Dual Use Research of Concern (DURC) in the scientific landscape. In this regard, she is part of dual use research of concern program in the German Association for Synthetic Biology (GASB), is cooperating within iGEM, as well as taking DURC into account in her own research.**

*'The iGEM Responsibility Program was implemented to discuss Biosafety and Dual Use issues.'*

***How did you get involved with iGEM and the iGEM Bielefeld teams?***
I got in contact with iGEM even before I started to study. When I was 17 I joined a summer school at Bielefeld University about biotechnology. Different professors, PhD students and groups, including the iGEM team gave courses and the people from the iGEM team were the most fun! Long story short: After the summer school, I decided to study in Bielefeld with the aim to join an iGEM team one day. I did in 2017 and from 2018 on, I supervised the teams for the next three years.

***Can you elaborate on the motivation to participate at an iGEM-Bielefeld team?***
I actually struggled a lot in my biotechnology studies. I love science but just finding out why something happens does not challenge my creative site enough to keep me motivated. This could definitely be seen in my Bachelor degree's grades. This changed with iGEM and synthetic biology: We had our own project and we were designing something that has never been there in nature, the more creative the better. I fell in love with this kind of research and still work on it.

***Can you guide us through the "project idea" process and how you come up with the topic of "expanding the genetic code" in 2017?***
Finding a project in iGEM is hard! There have been thousands of teams before you with amazing ideas and in general all the ideas a team had have been there before in the research community. This does not exclude to work on it, but my team was crazy enough to set themselves the goal to find something completely new in regards to iGEM. We were researching for months, pitching ideas, reading literature, discussing pros and cons. In the end, one of our team members Lennard presented a related publication to our paper and a few team members, including me, fell in love with the project. After working out a project plan for this and two other projects, we voted for this project, which I still love to this day.

***How did you experience the time in the iGEM Bielefeld team personally? Can you elaborate on the good things and the difficulties to overcome?***
I loved my team. We had a lot of crazy people with high ambitions, willing to think outside the box and we grew very close. To this day some of the team members are my closest friends and will always be the first ones to hear about new research ideas, because I value their feedback more than any other in this scientific community. We spend the whole day together for months, which also introduces challenges, and I would lie saying there were none. During iGEM we worked more than we ever did in our lives, which was extremely rewarding for me, but it also includes that tensions arise between team members that want to work all day and others that need a better work life balance to function. This is normal and you should tolerate the pace and way your teammates work to have good working team and not expect them to work best the way you work best. This was hard for me to navigate while on the team, but taught me a lot of lessons looking on it in retrospect and supervising other teams later. It definitely shaped the way I work in teams now and I think my most valuable lesson.

***Biosafety and Biosecurity have been a key part of the iGEM Bielefeld teams you been affiliated to. In addition, you are now working in the field of biosafety and Dual Use research of concern questions beyond your daily work as a PhD student. How did you come up with the idea to investigate the field and how is the status of Biosafety and Dual Use research of concern in the iGEM competition?***
It honestly wasn't my idea. I got there by coincidence and from that time I just stumble from the current biosecurity project into the next biosecurity project. It all started with the team of 2018, which was the first team I supervised. They identified a potential of misuse in their research and started to work on raising awareness on how biosecurity issues are neglected within the scientific community. I had no idea about biosafety and biosecurity at that time, but getting in contact with this field through the team, I realized that this is a fundamental part of every research project and that we need to teach it that way. You as a researcher are responsible for the impact of your research on society. You are the key mechanism to make it hard to misuse and to make it safe. You are responsible for it being hard to misuse to hurt others or the environment. I think this is something that we get not taught enough at universities, so I decided that I would like to help to make this change. Part of that is that Irina Rais, one of the 2018 team members, and myself are still teaching Dual Use awareness workshops in iGEM. I hope these courses help to get more iGEMers involved in this discipline.

***You have also been Judge for several years now. Why did you apply in Judging and what are the benefits of being a Judge?***
Being a Judge means seeing the newest developments and crazy ideas in synthetic biology research first hand. I experienced it as extremely rewarding to talk to the next generation of iGEMers, encouraging them to believe in their ideas and to stay in science. Of course I did not know that in the beginning, I applied to give back to iGEM because of everything this competition has done for me. I am extremely grateful for my iGEM experience and I know that iGEM only works if people volunteer to help, so that is what I did.

***Any advice for students interested in Biosafety and Dual Use research of concern in and beyond the iGEM competition?***
Get involved and reach out to policy makers. It is totally normal to feel extremely insecure in the beginning (at least I did). They will throw biosecurity jargon words, names of treaties and research institutions at you, which you never heard of before and it is fine that you didn't! You will find out what's going on step by step. I experienced the biosecurity community as an extremely encouraging community who loves to get new people involved. Without the support of so many experts in the field, I would never be where I am now. If you are interested to start a project, reach out to the iGEM Safety and Security committee, the German Association of Synthetic Biology or your iGEM supervisor. They will make sure to get you involved. ●

LAURA SCHLÜTER

# The supervision of the iGEM teams

**Laura Schlüter is a PhD student in the research group "Microbial Genomics and Biotechnology" at the Center for Biotechnology with the focus on transcriptional regulation in *Actinoplanes* under the supervision of Prof. Dr. Jörn Kalinowski. In 2017, Laura came into contact with iGEM for the first time as part of the Bielefeld team with their project Expand – Expanding the Genetic Code", for which the team received the gold medal, two prices and four nominations. Laura stayed in contact with the Bielefeld iGEM teams in 2018 and 2019 as financial advisor. In 2020 and 2021, Laura joined the iGEM competition again as an expert and supervised the teams WavySense and P.L.A.N.T by advising and supporting the students on all aspects of the competition.**

*'As a student, I would never have thought that leading scientists would take their precious time to help students with their research projects.'*





***What motivated you to join an iGEM team in 2017 and how did you find out about the iGEM competition?***
In my course of study, the iGEM competition and the Bielefeld teams were known and it also was no secret that participation is associated with a lot of work. I really wanted to take on the challenge of developing independent research with major responsibilities and a high level of independent work you cannot have as a student. In addition, I wanted to strengthen critical soft skills such as project, time and team management, which would certainly help me in my further career. Of course, I also wanted to gain many good experiences during my iGEM year and having fun working in a team was also an excellent motivation for me.

***What advice can you give to future iGEM teams to make the process of an iGEM project successful?***
Each iGEM team is very individual, but there are a few general tips I can give. The motivation and ability to work in a team are skills that decide about the success of an iGEM project, whereas all biological and technical knowledge can be learned. I gained this in sight especially as supervisor of the iGEM teams. The choice of the project is also an important factor, saving much time when it allows to work on smaller work packages in parallel and to gain preliminary results. Of course, iGEMers should be willing to invest time in their research project, however, not every team member is able to invest the same amount of time. This can quickly lead to disagreements, especially with the deadline in sight, causing additional pressure. That is why clear communication is very important and the iGEM project is still fun.

***Which experiences from your participation in an iGEM project were important for your further scientific career?***
Participating in the iGEM competition has influenced me in many aspects, both in private life and career. Without the experience from participating in the contest and everything I had learned during this time, I probably would have decided against pursuing a PhD. My participation has completely changed my view of where I see myself professionally in my ongoing career. It revealed where I could improve and, most importantly demonstrated my skills and competencies. During my participation, I experienced how supportive life science can be to upcoming scientists and how positive the feedback to inquiries and scientific exchange is. As a student, I would never have thought that leading scientists would take their precious time to help students with their research projects. I was corrected that this assumption was wrong and scientists all over the world were willing to help us. Actively seeking contact with scientists also helped progress my research during the PhD.

***What arguments would you bring to convince students to take part in the iGEM competition?***
Participating in the iGEM competition entails an additional workload under time pressure on top of your own studies. I don't want to play that down. However, what I received from my participation outweighed this aspect. iGEM enables you to learn important soft skills, but also to acquire great expertise in your chosen field. The participation provides the framework to meet iGEMers from all over the world and is a unique opportunity to exchange ideas and connect with so many aspiring scientists. Finally, the most important argument for me is that participating is so much fun and I formed close friendships during that time. I also observed this in my two years as a supervisor. ●

*'Participation in iGEM is a unique opportunity to learn, grow, and make lasting connections in the Synthetic Biology community.'*

DR. TIMO WOLF

# The iGEM competition supports the development of career profiles

**After receiving his bachelor and master's degrees in molecular biotechnology from Bielefeld University, Timo finished his doctoral thesis in industrial biotechnology at Bielefeld University in 2017. After his PhD he transferred to Bayer working in the corporate function Engineering and Technology. Timo's scientific work reaches from genetic engineering and systems biology to biotech process development. Currently he is leading international projects focusing on prokaryotic and eukaryotic organisms, which are used by Bayer to produce enzymes, key-intermediates and final products. Recently he was appointed as a Bayer Science Fellow. In 2010, Timo was a founding member of the first iGEM Bielefeld team, member in 2011 and supervised the teams from 2012 to 2014. Further, he judged at the iGEM Jamborees 2013 and 2014 and returned to judging in 2022 at the Paris Expo.**





***How did you get involved with iGEM and the iGEM Bielefeld teams?***
In 2010, I was studying molecular biotechnology at Bielefeld University when I got asked to join the first iGEM Bielefeld team. As a student interested in synthetic biology and genetic engineering, I was immediately fascinated and enthusiastic about the iGEM opportunity. We worked hard over the summer to design and implement our project and were rewarded by traveling to the iGEM Jamboree in Boston to present our work and compete with teams from around the world. It was an amazing experience, and I will never forget the special vibe during these days which motivated me to stay actively involved in iGEM through one more year as a team member, three years as an advisor and two years of judging. After a break of several years due to focusing on other professional endeavors, I picked up my active iGEM participation again in 2022 as member of the judging team at the finals in Paris.

***What is the motivation and the benefit for a student to participate at iGEM?***
The motivation for participating in iGEM is the opportunity to learn about and work on cutting-edge technologies in synthetic biology, as well as the chance to collaborate with other students from different backgrounds and cultures. The benefits of participating in iGEM are numerous - it provides hands-on experience with molecular biology techniques, project management, and teamwork, as well as the chance to network with professionals in the field and present your work to a global audience. Additionally, the iGEM experience can help to build important skills such as critical thinking, communication, and problem-solving, which are valuable in any career. iGEM created many lasting memories which I will always remember, and which are quite unique.

***What kind of experiences you collected through iGEM were helpful for you personally or in regards to your career, respectively?***
Through iGEM, I gained a lot of valuable experiences that have been helpful both personally and professionally. On a personal level, I learned a lot about project management, leadership, and teamwork, and developed my communication and presentation skills through presenting at iGEM events. Professionally, my involvement in iGEM helped me to build a network of contacts in the synthetic biology community and opened up opportunities. In particular, my experience with iGEM was helpful in landing a job at Bayer, where I have been able to apply my knowledge of synthetic biology to real-world projects.

***Can you explain how you are still active in iGEM and how you benefit from your iGEM experience in your current job?***
Based on my long history with iGEM, I was just recently invited to be a judge at this year's Grand Jamboree in Paris. Through judging I evaluated, celebrated, and helped guide the next generation of synthetic biologists. The iGEM finals are not only the superb final of the competition but also the largest synthetic biology community of researchers, industry, investors, start-ups and policy makers. This is a great opportunity to stay up-to-date with the latest developments in synthetic biology, connect with experts from around the world and meet the brightest synthetic biology talents. Additionally, my experience with iGEM is very helpful in my current job at Bayer, where I work on projects related to biotechnology, molecular biology and synthetic biology. The combination of biology and data science, as it is applied in iGEM, is key for success and fundamental for my work as a project leader in international cross-divisional projects.

***Any advice for student interested to join an iGEM team in the future?***
iGEM is a unique opportunity to learn, grow, and make lasting connections in the synthetic biology community. Be proactive, take ownership of your project, network with other teams and take advantage of the resources and support available at the CeBiTec, Bielefeld University as well as through iGEM. Above all, have fun and enjoy the experience. ●

# 04

# Statements and Public Relations

# iGEM members' statements

Former iGEM team members commented on the iGEM competition and Synthetic Biology in general.

**Timo Wolf, Team 2010, Bayer AG:**

*'Synthetic Biology is an interesting and relatively new field of science, where young scientists have plenty of opportunities to pursue innovative ideas. With interdisciplinary approaches there is huge potential to develop new technologies and solve future problems.'*

**Robert Braun, Team 2012, scientist at the BIOTYPE GmbH, Dresden:**

*'Synthetic Biology - Do I need to say more? Working in this young, aspiring and rapid growing field of life science is challenging, but also fun. Having the chance to participate in iGEM is maybe the best chance to work in Synthetic Biology being a young scientist.'*

**Agatha Walla, Team 2012, PostDoc at Heinrich Heine University Düsseldorf, Plant Genetics (Düsseldorf, Germany):**

*'The reason why I participate in iGEM is that it gives young scientists the opportunity to deepen their knowledge in this very new field of synthetic biology. I hope that we get as much out of it as we can as a team.'*

**Eva Brombacher, Team 2010, PhD student at the Institute for Medical Biometry and Statistics (IMBI) at University Medical Center Freiburg:**

*'Concerning the medical sector, I am hoping for personalized medication and therapies through synthetic biology. Besides that, the potential of the latter could also serve the environment by designing bacteria eliminating polluting substances.'*

**Nadiya Romanova, Team 2013, Medical School OWL, Bielefeld (Germany):**

*'I strive to understand life using the tools of synthetic biology. iGEM offers a unique opportunity to develop organisms with novel properties.'*

**Tom Tubbesing, Team 2013, PhD-student in the Graduate School 'Digital Infrastructure for the Life Sciences (DILS)' at Bielefeld University:**

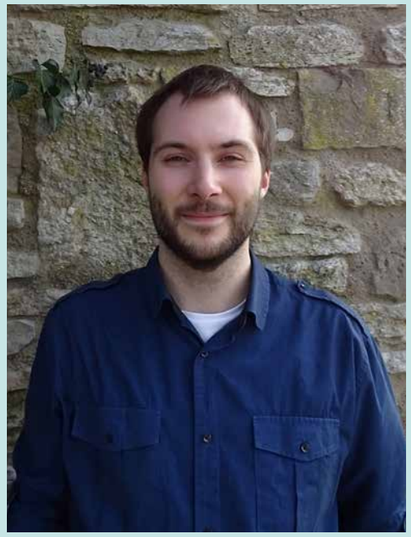
*'I am constantly fascinated by the rapid progress in the field of synthetic biology. I am convinced that the knowledge gained in this area, if used responsibly, can benefit everyone on earth. Now I'm taking part in the iGEM competition to implement our own ideas together with my team and thus show new applications for this young branch of science.'*

**Nefeli Chanoutsi, Team 2019, Graduate Research Scholar at University of California, San Francisco:**

*'I would describe myself as a person who is curious and open to new things, one who likes challenges and loves it when they can ultimately be overcome. With regard to the iGEM competition, I am fascinated by the variety of challenges it poses from start to finish. It offers the opportunity to plan your own project independently and to successfully achieve the common aim as a team. These are experiences that cannot be acquired in a regular study program. Such an opportunity should not be missed.'*

**Alexander Schulze, Team 2019, Master of Science, Bielefeld University:**

*'I participate in iGEM because I enjoy working on my own projects and realising my own ideas. The iGEM-Competition offers opportunities to do this that would be unimaginable during a normal course of studies. Also the possibility of getting to know the global science community and even taking part in the expansion of their scientific knowledge is a special treat for me.'*



**Laura Schlüter, Team 2017, PhD student at the CeBiTec of Bielefeld University:**

*'I am involved in the iGEM competition because I want to implement a project from the ground up. Not just research is at the forefront, but also important aspects such as public relations, human practice, and financing. I like this challenge.'*





**Birte Hollmann, Team 2014, Research Assistant at Dr. Wolff-Group:**

*'I participate at the iGEM-competition because the project is a unique chance for me to work on a research project related to synthetic biology within a group of students. It is exciting to collect ideas and to realize them afterwards. It is possible to gain a lot of experience and to establish contacts with other groups.'*


**Boas Pucker, Team 2014, Professor at TU Braunschweig, Institute of Plant Biology, Plant Biotechnology and Bioinformatics:**

*'Working with young and creative students on their own project from planning to presentation of the results is my main motivation. I would like to try out new methods of synthetic biology and to learn new skills.'*

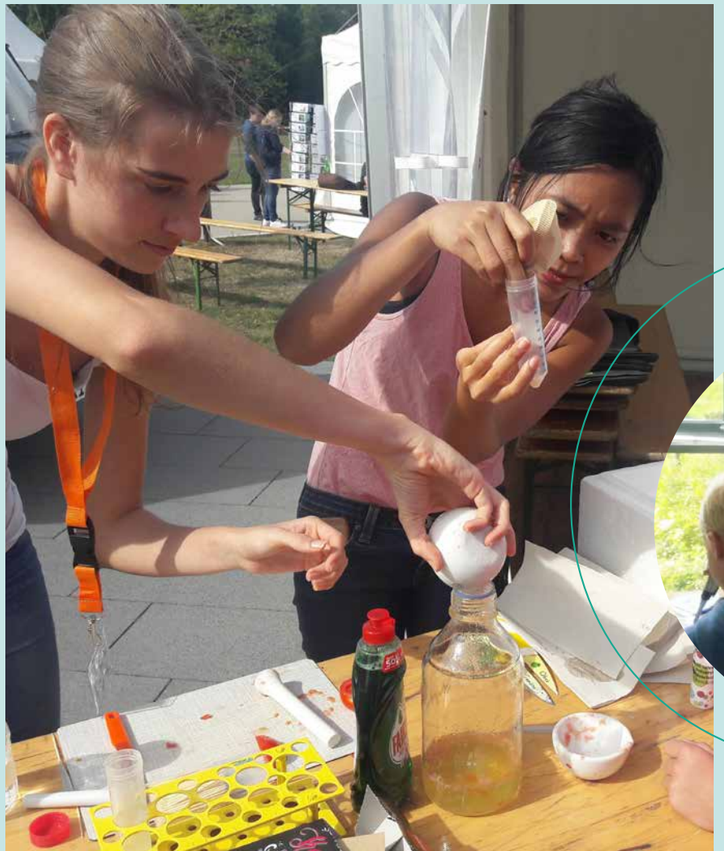
**Daniel Bergen, Team 2017, PhD Student at the Australian Institute for Bioengineering and Nanotechnology, University of Queensland:**

*'I consider my participation as an opportunity to work in a team on a self-determined project and contribute to research fighting current problems. Through international contact to other teams one can learn new perspectives, which can optimize the own work. The competition is an opportunity to make the world a better place and improve one's own abilities.'*

# Public relations: activities beyond the lab

Implementation of Synthetic Biology projects to address societal problems requires engagement and exchange with the public. In iGEM, these outreach activities are summarized under the term Human Practices. They involve thinking deeply and creatively about whether a Synthetic Biology project is responsible and advantageous for society. Issues such as Reflection, Responsibility and Responsiveness are of importance in this context.


**The iGEM-team of the year 2014 attended the NRW-day (North-Rhine-Westfalia day) and put up posters explaining the basics of synthetic biology and their project. They got in touch with a lot of visitors and led multiple discussions about synthetic biology, genetic engineering and the iGEM competition itself.**



**At a festival in the city of Bielefeld, the iGEM-Team of the year 2019 organized a stand to promote and inform people about Synthetic Biology, the iGEM competition and their individual project. Moreover, they offered different experiments for kids and distributed information materials. They let the kids isolate DNA and use pipettes to mix different kinds of food dye coloured water.**

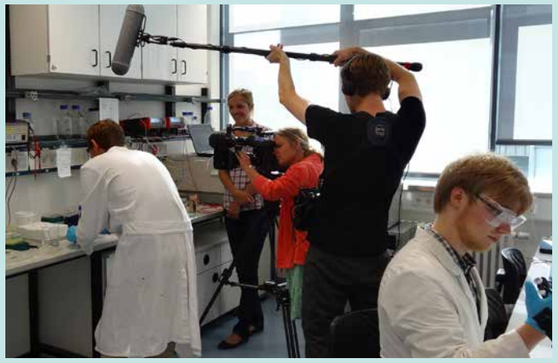
***Human Practice activities may include exchange of ideas with scientists and public representatives, communication, education, consultation of experts and stakeholders, engagement with the interested public and much more.***

**The iGEM-Team of the year 2013 particpated at the Synbio (Synthetic Biology) Day which is a street science event dedicated to educate children in a playful way but also to address their parents and adults in general. The team tried to explain Synthetic Biology to the interested public and aimed at getting as much feedback and opinion as possible.**

The iGEM-team 'P.L.A.N.T.' authored and recorded the 'Ask Niki' podcast to introduce listeners to Synthetic Biology in a few minutes without any additional effort. The podcast was distributed via social media channels and on the radio.


The 'Evobodies – Molecular Speed-Dating' iGEM project was presented at the German iGEM Meetup in Marburg. The event was organized so that the German teams could get to know each other and communicate their projects.

The iGEM-team of the year 2015 reported their project on TV in WDR (West Deutscher Rundfunk) which is one of the most important TV stations in western Germany. The team was also interviewed by the university online stream station, Campus TV Bielefeld. In this way, they reached a broad public to explain their project idea.



WDR

radio BIELEFELD 98.3 | 97.6

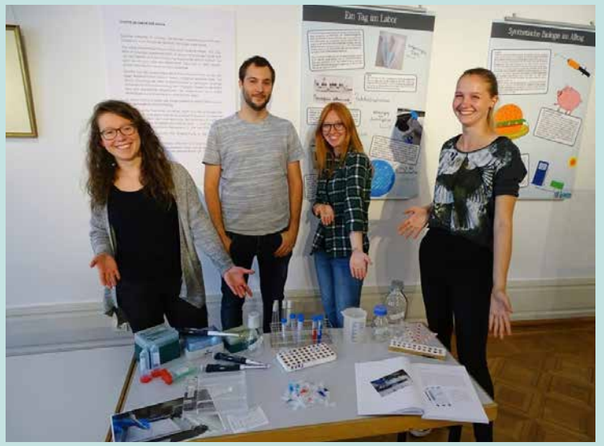

The iGEM team of the year 2014 visited the company Merck sponsoring their project. They presented project ideas and their scientific approaches to get feedback from experienced Merck researchers which was very helpful for further project planning.

The iGEM team of the year 2011 got an invitation to the BIO.NRW (PhD) Student Convention at the BayArena in Leverkusen (Germany). The congress gives lifescience students the opportunity to meet representatives from industry and academia. The iGEM team members of Bielefeld University, for the first time, presented their project to a broader audience.

Networking of the iGEM-team 2019 in The Hague (Netherlands). Fifteen European iGEM-teams met in The Hague to update each other on their projects and poster drafts. Participating teams had the opportunity to listen to inspiring talks and could attend workshops.



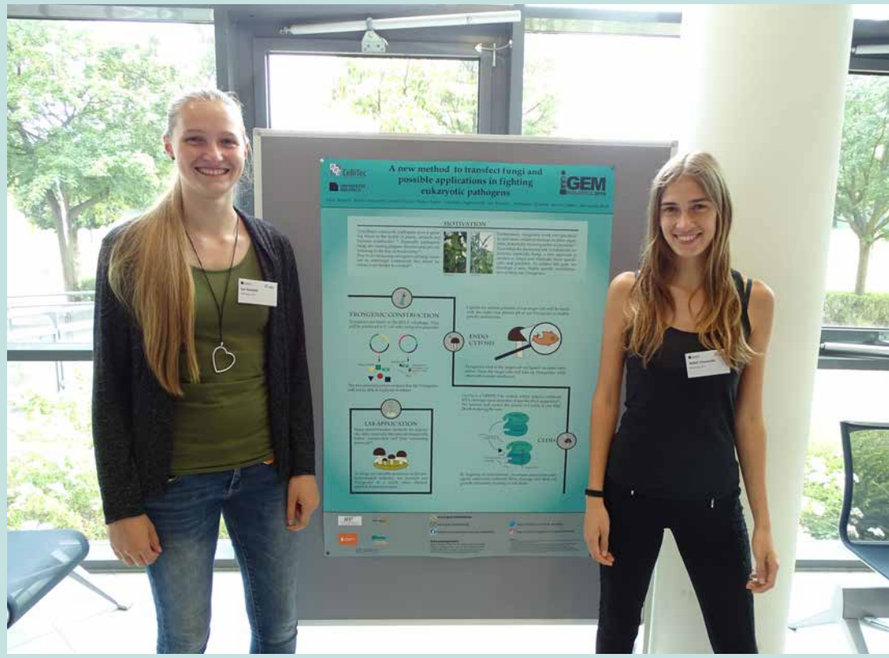
Selling biscuits and cake to finance their own iGEM project.




The iGEM-team Bielefeld worked together with the iGEM-team of the Heinrich-Heine University of Duesseldorf to organize an exhibition about Synthetic Biology in the Goethe Museum in Duesseldorf as part of their outreach program.

*The Bielefeld University iGEM teams used various events to present their projects to the interested public and in this way to promote Synthetic Biology.*

Poster session in the CeBiTec foyer. On the occasion of the scientific advisory board meeting, the iGEM team of the year 2019 participated in the poster session presenting their project idea to receive feedback from CeBiTec scientists and advisory board members.



# Imprint

**Prof. Dr. Alfred Pühler**
Bielefeld University
Center for Biotechnology (CeBiTec)
Universitätsstraße 27
33615 Bielefeld

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Prof. Dr. Alfred Pühler (CeBiTec)

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Nils-Christian Lübke, Dr. Irena Maus
and Dr. Andreas Schlüter (CeBiTec)

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