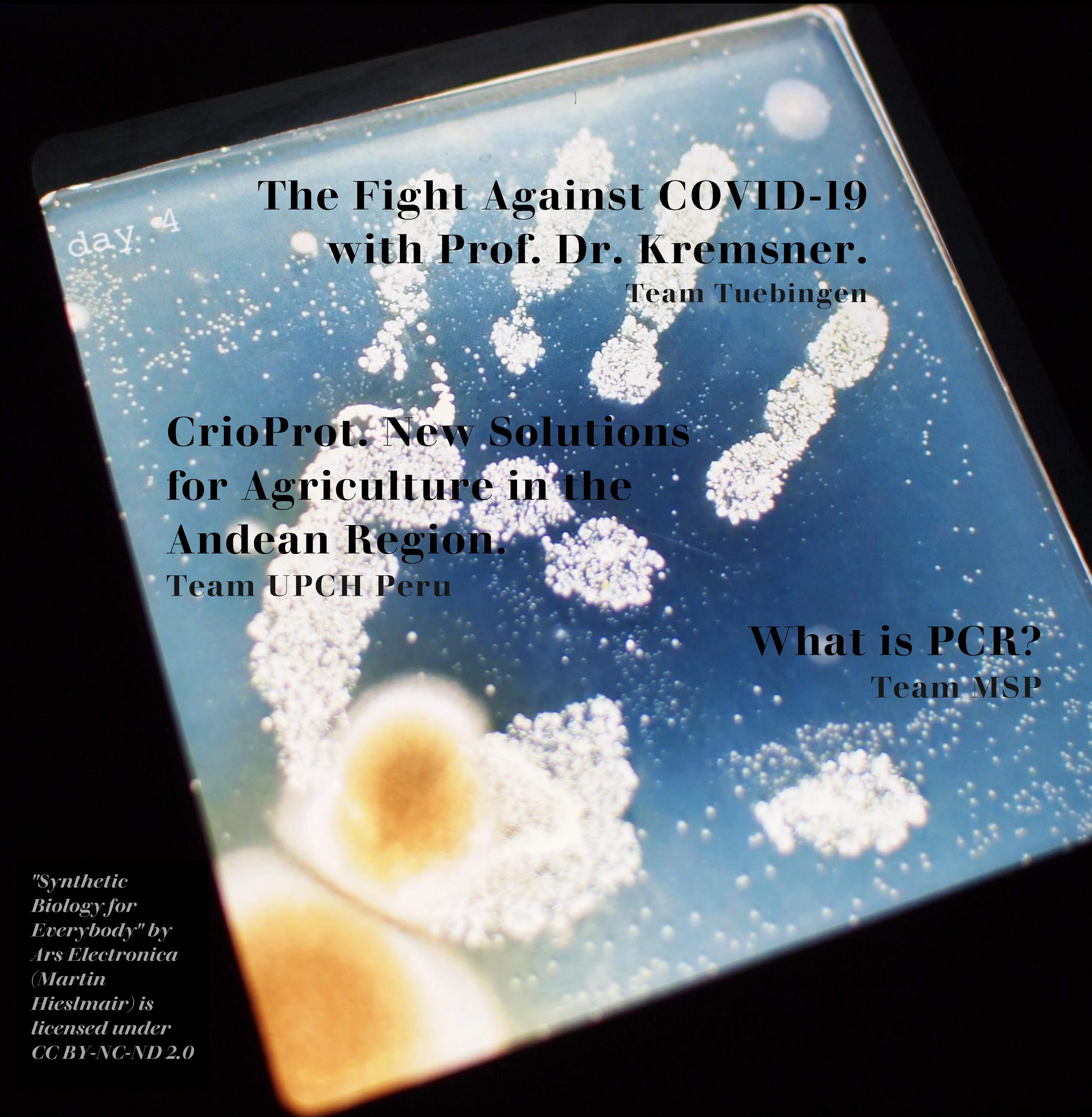
iGEM MUGGLE JOURNAL

The Unofficial iGEM Proceedings Journal for Everyone



The Fight Against COVID-19 with Prof. Dr. Kremsner.

Team Tuebingen

CrioProt. New Solutions for Agriculture in the Andean Region.

Team UPCH Peru

What is PCR?

Team MSP

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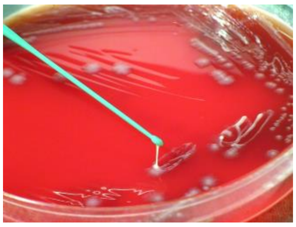

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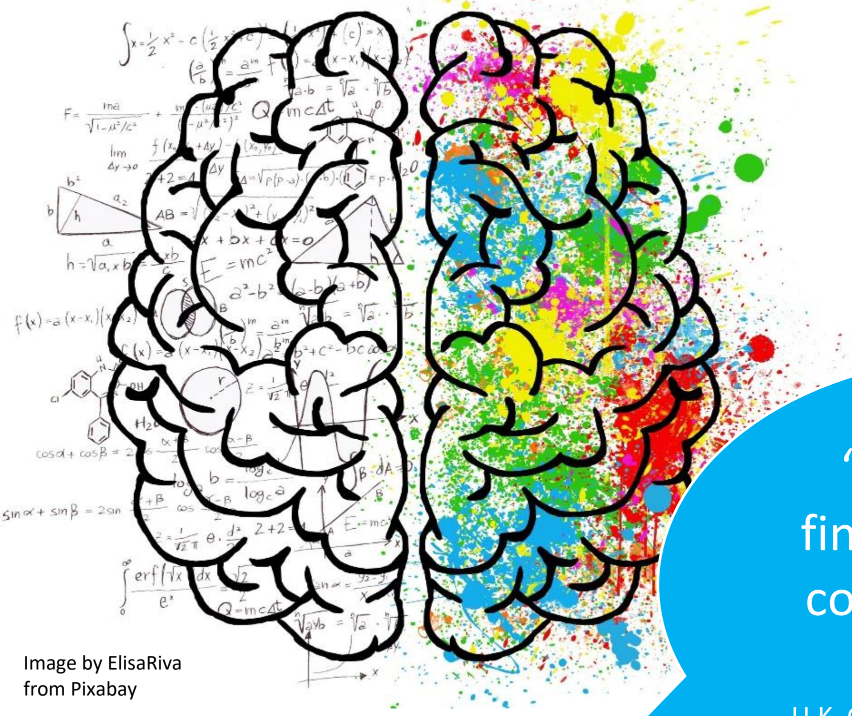


Image by ElisaRiva
from Pixabay

> "Science isn't finished until it is communicated."
>
> -Sir Mark Walport
> U.K. Government Chief Scientific

If you don't communicate the science, you may as well never have done it.

In today's world, it's not only pursuing science, but communicating about it effectively that's important. Science often has the problem that it stays in its own 'bubble' and the improvements , inventions and discoveries stay out of grasp for the general public. This is a large problem in this day and age as science is one of the most integral parts of society, at the same time scientific progress advances so fast that the general always lags behind.

Bridging the gap between the 'science world' and the general, non-scientist public that's crucial together with generating a dialogue and fostering a passion for synthetic biology in laymen. Informed and interested laymen are able to give feedback to the scientists, which leads science in new directions and aids scientists in making the best possible decisions in their research.

To make well-informed decisions, about our health, our environment and even politics, we all need accurate unbiased information. Scientists are the most direct and knowledgeable source of information, when it comes to science. They have the knowledge and credibility to counter misinformation and misconceptions (for e.g. "fake news"), which clutter public debate. That is why communication is an essential part of science.

This iGEM Muggle Journal is a scientifically simplified Journal, targeted to the general public (or Muggles borrowed from the Harry Potter word for non-magicians) with less experience reading scientific articles and research. Scientific articles can be challenging to read and understand for people who are not actively involved in the scientific community. With the Muggle Journal articles, more people are able to understand research! Have Fun exploring the Journal and getting inspired!

Larissa Markus, Editor in Chief

Team MSP-Maastricht

Dear scientifically interested people,

We are the MSP-Maastricht iGEM team consisting of 13 aspiring scientists from Maastricht University. Apart from one biomedical sciences bachelor's student and one student pursuing a master's in systems biology, we are all students of the Maastricht Science Programme and together we have a very broad background on all natural sciences. All together, we have nine different nationalities and speak twelve languages.

This year, team MSP-Maastricht's project is about fighting an invasive insect pest, the oak processionary caterpillar, which poses a local and continental threat. The oak processionary caterpillar not only defoliates trees and entire oak forests, feeding on their leaves. It also poses a serious health hazard especially in urban areas, as its toxic bristles can spread over hundreds of meters and cause skin rashes, eye complaints as well as respiratory issues for both humans and animals. Current methods and pesticides used to manage this pest are relatively inefficient, expensive and unspecific, so that a broad use of the pesticide is not possible due to its harmful effects on the ecosystem. This is why our team's project is to develop a safe biological pesticide that specifically targets the oak processionary and is therefore environmentally-friendly and presumably more efficient.

Team MSP-Maastricht decided that it is not only the general public that needs to learn more about synthetic biology and research. It is incredibly important to educate the general public about the new discoveries in synthetic biology and awake their interest for science. In these fast-changing times, it is hard to keep the overview of all great inventions that are made in synthetic biology and all of them should be acknowledged, as they can inspire new ideas in other people.

We hope you enjoy reading all these amazing texts from the 2020 iGEM teams and again a big thank you to all participating teams!

The MSP-Maastricht iGEM team 2020

What is iGEM?

The iGEM Foundation: Making the world a better place trough synthetic biology.

L. Markus[*], Raphaella Kosta[*]

[*] MSP-Maastricht, First Authors, Maastricht University

iGEM stands for International Genetically Engineered Machine and is a worldwide synthetic biology competition hosted by the iGEM Foundation. The foundation is an independent, non-profit organization with the goal to solve todays problems through synthetic biology and to foster a cooperative science community and friendly competition. The foundation has many different programmes from which the iGEM competition id the biggest and most known one.

Once a year, high school and university students (undergraduate and over graduate) from different fields and from all over the world, are organized in teams to work and collaborate on projects that can vary from local sustainable environmental solutions to medical-related ideas. The projects use synthetic biology to solve real-life problems, using the tools and technologies of genetic engineering.

"Team in front of the iGEM logo" *by the iGEM Foundation and Justin Knight taken on November 4, 2019 is licenced under Attribution 2.0 Generic (CC BY 2.0)*

There are several additional aspects such as public sensibilization and introduction to synthetic biology. The teams participating have the opportunity to push the boundaries of synthetic biology, think out of the box and choose their own problem they want to tackle. iGEM projects serve as proofs of concepts and prototypes, contributing to over 150 start-ups.

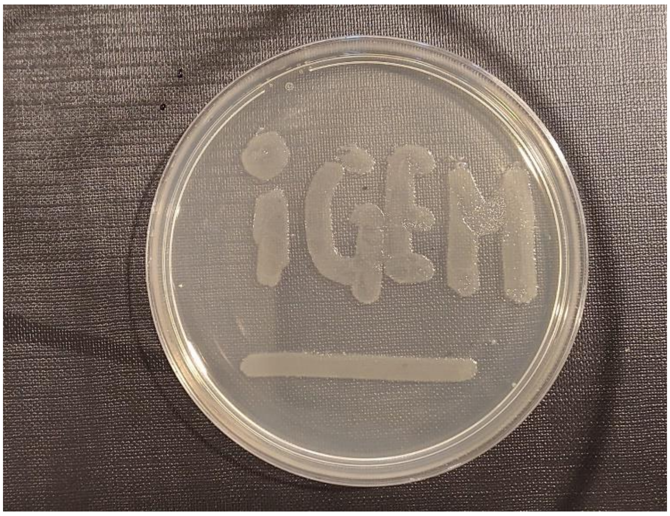
All the teams need to choose one of the tracks where their project fits best in. The tracks this year include diagnostics, energy, environment, food & nutrition, foundational advance, hardware, high school, information processing, manufacturing, new application, software, therapeutics and open; which is the track teams choose when they feel like their own idea does not fit in any of the other ones. Every year these tracks include many incredible projects, that have the potential to make this world a better place.

The iGEM Foundation runs the Registry of Standard Biological Parts and makes them available for every team to use during the competition.. The Registry has over 20,000 standard biological parts, and the teams can use standard interchangeable parts called BioBricks from the Registry to create their own synthetic organisms. The teams may also add new parts to the registry if the develop them and can win prizes like the BioBrick trophy for their design.

What is iGEM?

*“**Biobrick trophy**” by the iGEM Foundation and Justin Knight is licenced under Attribution 2.0 Generic (CC BY 2.0)*

The Foundation encourages responsible innovation trough efforts in biosafety and public outreach. In a cutting-edge field like synthetic biology ethical considerations and inclusion of the public in the development process are essential, therefore Human practices are an integral part of the competition and the participating teams not only do their research, but also contribute to society and integrates their opinions in their projects.



***iGEM logo grown on a plate.** Lab safety is incredibly important and all teams need to fill in safety forms about their lab, the project and their synthetic organism. Image by E.Thielechke and L.Markus*

All teams need to document their work on their team wiki page. The team wikis from 2020 iGEM competition can be found under: https://igem.org/Team_Wikis?year=2020

Every year, since 2003, the teams present their projects at the annual conference, the ‘Giant Jamboree’, in Boston, Massachusetts, where teams meet each other, celebrate their achievements and the winners of the competition are announced. The Teams present their work through posters and oral presentations, and compete for prizes and awards.

“*Team presenting at the Giant Jamboree” by the iGEM Foundation and Justin Knight taken on November 4, 2019 is licenced under Attribution 2.0 Generic (CC BY 2.0)*

There a trophys for many different disciplines including, best Educational project or the Inclusivity award, next to the trophies and medals for the best scientific projects.

“iGEM Trophies” by the iGEM Foundation and Justin Knight taken on October 28, 2018 is licenced under Attribution 2.0 Generic (CC BY 2.0)

What is iGEM?

Giant Jamboree" by the iGEM Foundation and Justin Knight taken on October 28, 2018 is licenced under Attribution 2.0 Generic (CC BY 2.0)

The last picture shows the participating teams of 2018. More and more teams from all around the world participate every year making the competition more diverse yearly. The different teams can be recognised by their unique hoodies.

Apart from the iGEM competition the iGEM Foundation had a few other projects which follow the same goal to make the world a better place through synthetic biology.

The Entrepreneurship Program Innovation Community (EPIC) supports the development of iGEMs entrepreneurial community through a range of global activities and the **After iGEM program** supports the competition's 40,000+ participants in their future as scientists, ambassadors and Researchers, who are setting the standards for synthetic biology on an international level. The Program includes Mentorship, Representative and ambassador programmes. Members of the iGEM community can also become part of an iGEM committee or write for the iGEM newsletter. More information about After iGEM can be found on the After iGEM website: https://after.igem.org/

For more information on the iGEM Foundation, the iGEM competition and the Foundations different programmes and goals check out the iGEM website: https://igem.org/Main_Page

You can also get involved and invest in the future by supporting iGEM programmes or becoming a part of the iGEM community yourself. Build a better world with iGEM!

You can contact iGEM via: hq AT igem DOT org

The Living Laser

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Lasers are devices that can amplify light by stimulated emission to generate coherent radiation. The light that has been emitted can be in various wavelengths, meaning that it corresponds to specific points in the electromagnetic spectrum. Different kinds of lasers can generate light at wavelengths ranging from millimeters to nanometers. However, we will be examining lasers which generate light in visible regions of the electromagnetic spectrum, wavelengths of roughly 400-700 nm.

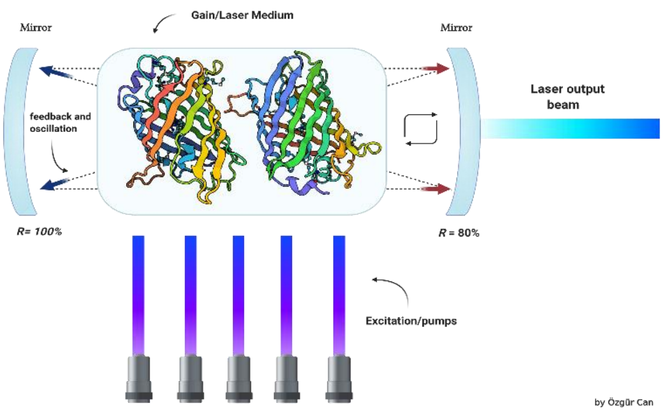

FIGURE 1: *Basics of a laser.*

A typical laser consists of three essential components as shown in *Figure 1*:

i) a gain medium consisting of active molecules, ions or atoms,

ii) an optical feedback structure which confines light inside by oscillations (it can be called as "resonator" or "cavity"),

iii) an energy source which excites molecules in the gain medium (it can be called as "excitation source" or "pump")

Although these main elements can be in a variety of different forms, we are focusing on biological counterparts of the gain medium and optical feedback structure to build a biological laser. It works like a regular laser, a gain medium, a resonator, and excitation energy are required.

Every working system needs the energy to run. For biolasers, the pump/excitation energy provides us that. It feeds the organic luminescent gain medium (such as a fluorescent protein), the protein along can emit light. However, mirror-like reflective surfaces/resonators must be used

to enhance the emitted light. The total internal reflection inside the resonator confines the emitted light and helps the amplification process of it to turn the biological system into a lasing system.

For a while, scientists used glass beads (containing fluorescent dyes) or inorganic materials as resonators. This would allow them to excite the gain medium from outside the cell that has engulfed them. Of course, this system didn't require a resonator because the bead itself is a resonator. With aid of total internal reflection, whispering gallery modes can be obtained from the resonator, and emitted light of the gain medium becomes laser like.

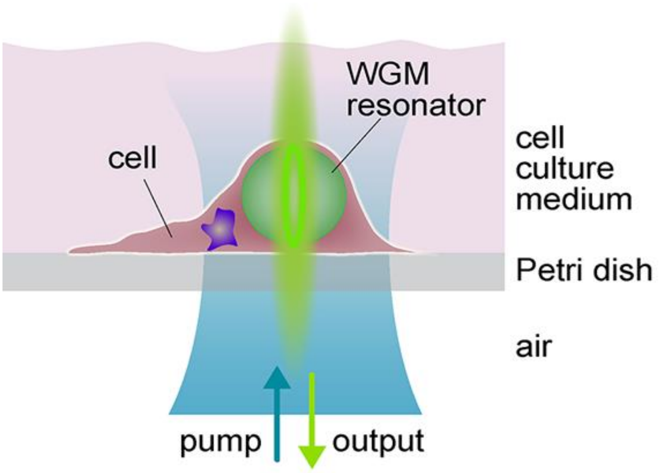

FIGURE 2: *Whispering gallery mode microresonator engulfed by a HeLa cell [Schubert et al. 2015].*

Aim and Future Experiments:

As the 2020 KU_ISTANBUL iGEM Team, we want to create better imaging methods for different samples of single cells, colonies of cells, and tissues. Minute differences inside a cell can be sensed with a cell laser; colonies of cells can be tracked by tagging them with micro/nano laser particles; differences in tissue composition can be determined by random lasers.

Creating a living cell laser without introducing artificial resonators into the cell is our main goal. We knew some former iGEM teams (TU_Delft 2016 and UiOslo_Norway 2017) and scientists around the world [Nizamoğlu et al. 2013] who did previous work on this topic too.

We want to cover the cell membrane with two different proteins, Silicatein and Reflectin, that can turn the entire cell into a resonator structure. When we overexpress the chosen fluorescent protein and excite it with a pump source; emitted light theoretically displays laser-like properties. The key part is converting the cell membrane or cell wall into a resonator. In this way, every component of the biolaser (except the pump) will be organic, biodegradable, and compatible with the cell's metabolism. We don't need any artificial beads or other inorganic materials.

For our experiments, we have decided to use *Escherichia coli* and *Saccharomyces cerevisiae* as our model organisms.

Both reflectin and silicatein, with their high refractive indices, can grant us mirror-like structure surfaces. For a start, yeast and E.coli are easier organisms to work with. However, in the future, we want to also work with oocytes and red blood cells to implement the same laser structure. The shape of the cell determines the biolaser output/emission. Change in the cell could also affect the cell's shape. These differences can be observed via biolaser cells. Basically in this way, improved cancer diagnostics and egg quality tests in IVF could be achieved.

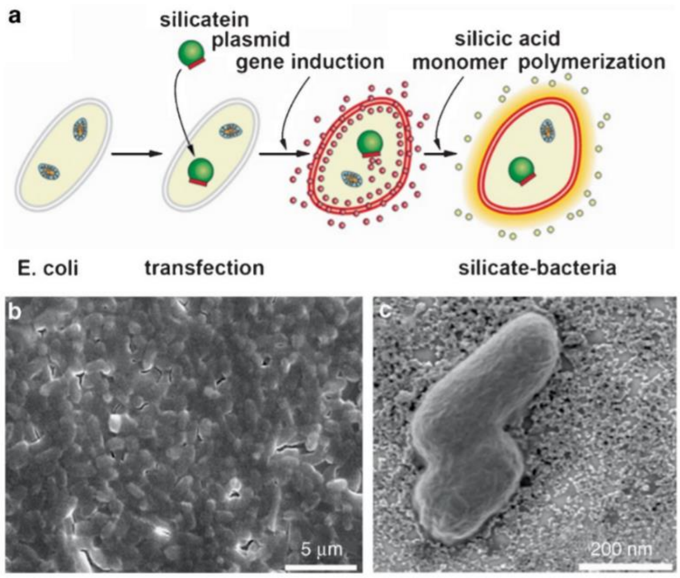

FIGURE 3: *SEM image of the polymerized silicatein molecules around E.coli Cell Membrane Surface [Shimizu et al. 1998]*

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Meet with Our Team:

Team members
Row 1: Cengizhan Büyükdağ, Zeliş Nergiz, Serena Özabrahamyan
Row 2: Fatih Uçar, Özgür Can, Oktay Göcenler
Row 3: Ali Doğa Yücel, Sueda Özcengiz, Ece Türksever
Row 4: İsmail Baturay Altay, Laşin Özbek, Ayşe Buket Pekşen

CRIO PROT
ANTIFREEZE PRODUCT

CrioProt

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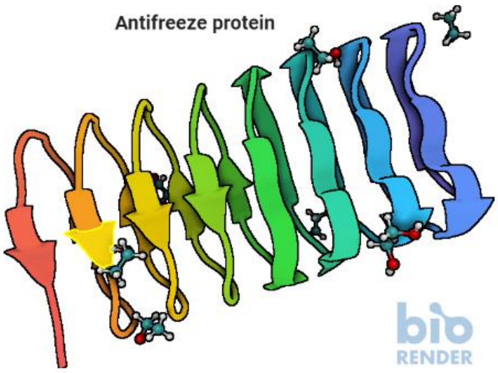
Every year, the agricultural sector in Peru faces many challenges related to frost season, which generally occurs in the Andean region and generates crop losses of up to 180 thousand hectares (*Instituto Crecer, 2018*). Frost is a meteorological phenomenon in which environmental temperature drops to 0ºC or less (*Senamhi, 2010*). This causes ice crystal formation in the intercellular space of plant cells, leading to dehydration (*Wei et al., 2017*), which limits plant distribution, growth, and productivity (*Yadollahpour, Bagheri, & Rahimina, 2016*).

Covering the plants with plastic blankets or setting up tall plantations on the perimeter of their properties, as a protective barrier against frost, are some of the current methods used by farmers to avoid frost damage. However, these options are expensive and increase environmental pollution. Hence, we decided to approach the problem using biotechnology. We want plants to be able to tolerate freezing temperatures, but given that transgenic plants aren't allowed in our country, we explored the possibility of developing an antifreeze product that will be sprayed on its leaves. This product will consist of a solution of recombinantly expressed and purified antifreeze proteins (AFPs). Their function is to prevent ice crystal growth by binding to its surface.

To produce the proteins, we designed several plasmids, which are portable DNA versions, that will be inserted into bacteria by a process called transformation - now we have a mutant! We choose to design each plasmid for two AFPs types from *Lolium perenne* and an AFP from *Tenebrio molitor*. Those former have a high capacity for crystal growth inhibition; the latter decreases the fluid freezing point (*Bredow et al., 2017; Middleton, 2011*).



The chassis, where all the action takes place, will function as an AFP fabric. For the first experimental stage, we are working with *E. coli*, but we plan to work with a new chassis that we have been characterizing: *Pseudoalteromonas nigrifaciens*. Given that this marine bacterium has an optimal growth at 4°C (*Baumann, Baumann, Bowditch, & Beaman, 1984*), it was selected for AFP production in low-tech environments, such as possible lack of sterile conditions and standard incubators for its proper growth. This chassis is a key component of the long-term implementation in which we aim to design an accessible low-tech growth system that allows AFPs production in the Andean region.

Currently, we are working on *E. coli* to test our system and characterize the effectiveness of the antifreeze protein. Also, we are working on *P. nigrifaciens* strain 217 characterization in order to optimize the AFP production in this organism.

ACKNOWLEDGEMENT

We thank the Laboratory of Individual Molecules for the reagents and space provided, and the Faculty of Sciences of Cayetano Heredia University for supporting our participation in the competition.

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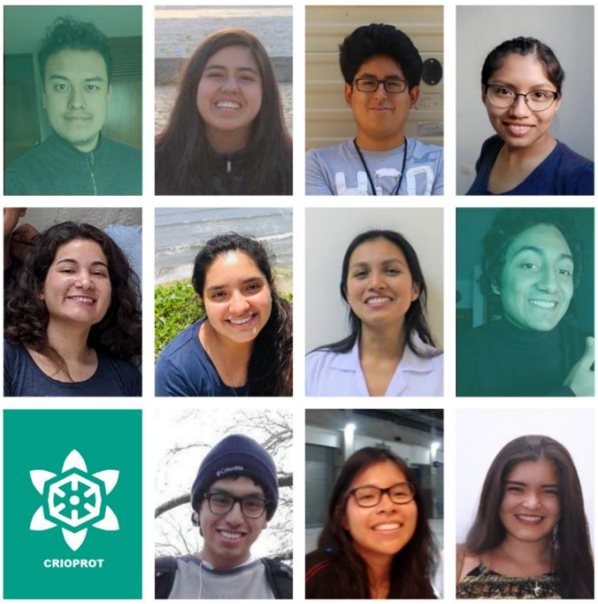

TEAM UPCH_Peru

Row 1: Diego Benites, Marité Castromonte, Oswlado Lescano, Ingrid Alarcón

Row 2: Marita Ortiz, Valeria Villar, Jesús Durand, Ruben Velasquez

Row 3: Our CrioProt Logo, Matías Rojas, Isabel Ruiz, Elizabeth Sánchez
Team iGEM UPCH Peru

Sudoku:

Time for a break. Train your brain with this fun sudoku riddle!

2		6		7				3
3	4	1		6	8	7	2	
9		7				8		4
6		3		8			5	7
	2		7			4	3	8
	7	4	3	5	2	1	9	6
7		9	5	4		6		2
5		2			7	3	4	
4	3	8	6					1

This is how it works: In the grid below, numbers must be filled in so that each row, column and 3x3 block contains the numbers from 1 to 9 exactly once.

Fun Fact:
Did you know that, after sudoku puzzles went viral in the Western World in 2004, pencil sales increased by 700%??

Solution

2	8	6	4	7	5	9	1	3
3	4	1	9	6	8	7	2	5
9	5	7	2	3	1	8	6	4
6	9	3	1	8	4	2	5	7
1	2	5	7	9	6	4	3	8
8	7	4	3	5	2	1	9	6
7	1	9	5	4	3	6	8	2
5	6	2	8	1	7	3	4	9
4	3	8	6	2	9	5	7	1

SPARKLE: Solar Potentiated Artificially Knitted Lipid Enclosures

Alissa Agerova, Avishan Aghayari, Amina Aliyeva, Nihat Aliyev, Norin Bhatti, Turan Badalli, Irina Borovko, Nadezhda Chulkova, Dmytro Fedorenko, Klāvs Jermakovs, Tatyana Kan, Valida Kazimova, Viacheslav Kiselev, Gleb Kovalev, Valeria Leoshko, Mykhailo Lytvynenko, Anna Makhotina, Dags Macs, Glib Manaiev, Frida Matiyevskaya, Mark Merzlikin, Juli Mukhadze, Alar Okas, Johanna Olesk, Aleksandra Panfilova, Aleksandrs Rebriks, Davit Rizhinashvili, Ekaterina Sedykh, Jhalak Sethi, Aleksandra Shabanova, Muazzamkhon Yusupova, Maksym Zarodniuk, Nastassia Shtaida, Mihkel Örd, Artemi Maljavin,
Ilona Faustova, Mart Loog
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Estonia TUIT, Institute of Technology, University of Tartu

Chemical production methods use non-renewable energy and raw materials and cause many ecological problems. To live happily tomorrow, we need to shift to a more sustainable way of producing things today.

Let's take lipids as an example. Lipids are widely used in different fields like cosmetics and pharmaceuticals (Athenstaedt & Daum, 2006). Their production in genetically modified microorganisms (or cell factories has a high potential to become an eco-friendly alternative to chemical one. Additionally, you can use waste as a source material, which enables recycling (Zhou et al., 2016).

That is why our team decided to create a yeast strain that can be used in lipids bioproduction (Ferreira et al., 2018). Let me show you how!

Here is a beautiful little yeast cell with a great potential (Fig.1 a). We genetically modify it to boost the lipid accumulation in the special cell structures called lipid droplets (Teixeira et al., 2018) (Fig1. b). Next, we increase the yield even more by fusing yeast with nanoparticles. This enables our yeast cell to use light as an energy source (Guo et al., 2018). Sounds cool!(Fig.1 c) One thing is to produce lipids, and the whole other thing is to extract them from rigid yeast cells. But don't worry, we got you covered. Our yeast produces special enzymes that make cells pop-up (Inokuma et al., 2020) and release the lipids (Fig1. d).

And this, dear friends, is SPARKLE: fast, easy, adjustable platform for lipids production. The developed yeast strain can be used as a base in research and cosmetics, pharmaceutical, fuel and other industries. In other words, our project aims to achieve sustainable bioproduction, thereby making the world a better place!

"From a small step to the global impact!"

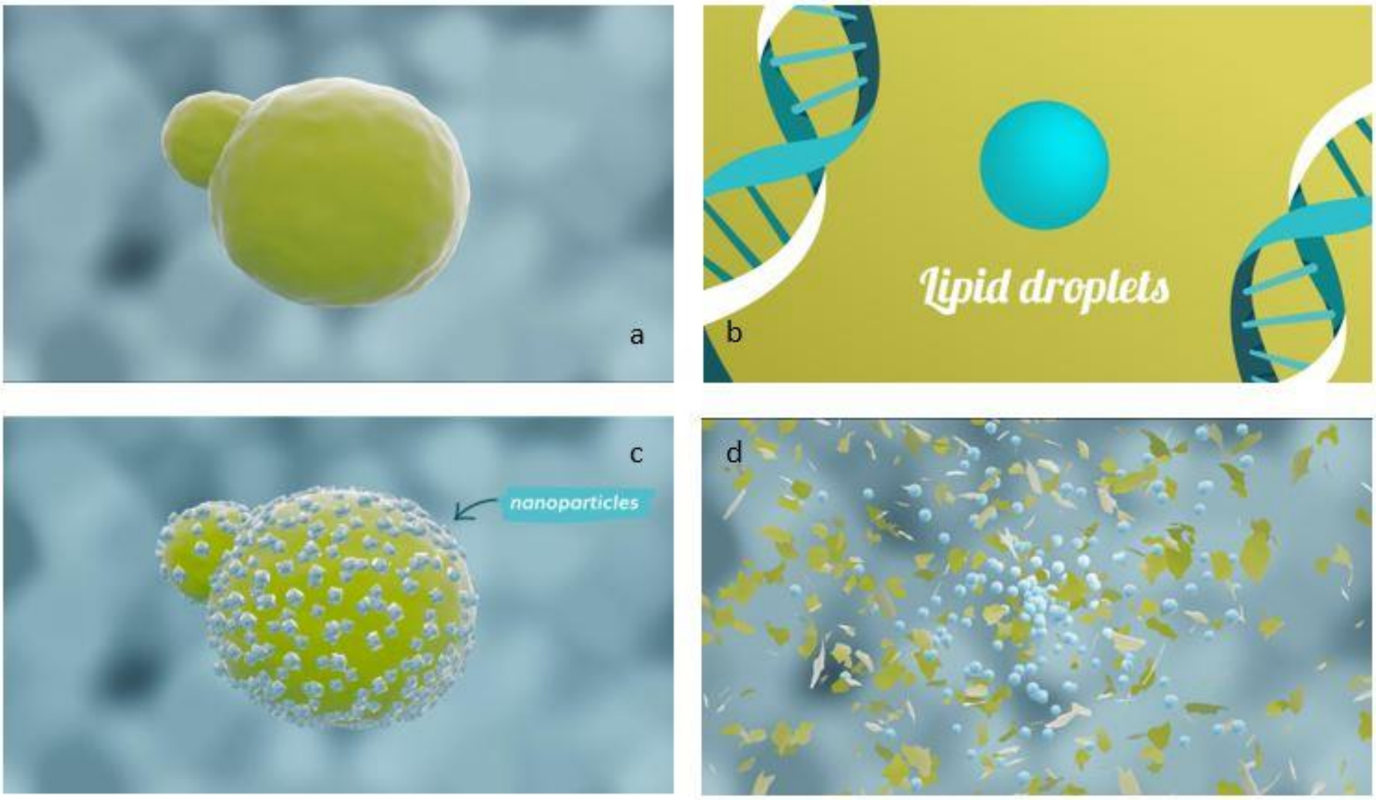

Figure 1: SPARKLE in Action

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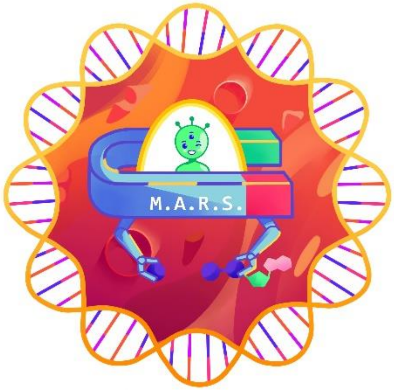

The Energy Of Life

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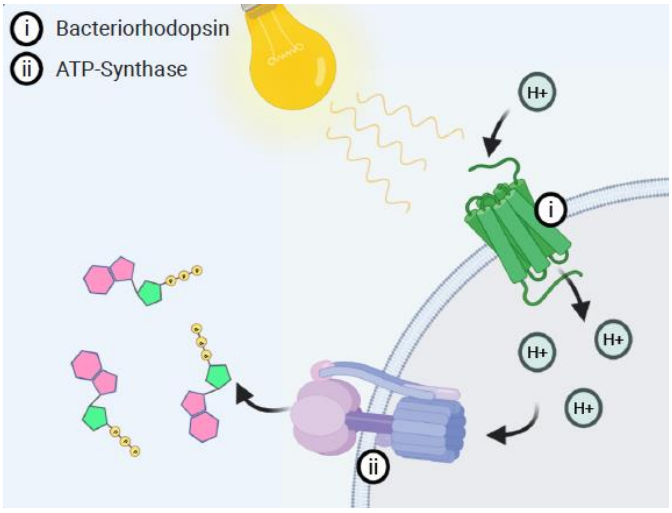
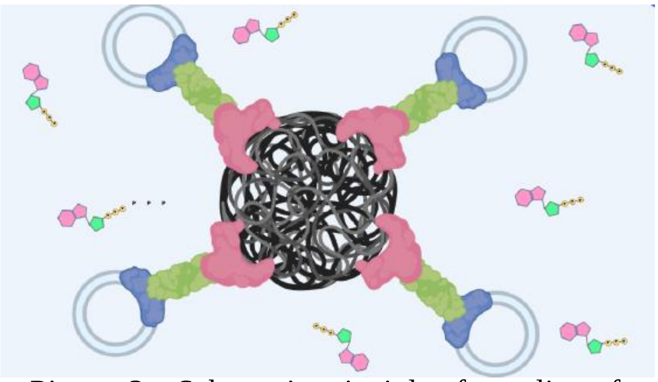
Every biological process in life needs energy. The same is true for the biological processes used in the biotechnological industry. Enzymes – the molecular machines that make chemical reactions happen – are the key players in every cell, organism and increasingly often in every industrial process, too. To work and to synthesize chemicals, pharmaceuticals and more, they need energy. More specifically, they need the energy of life called ATP.

The demand for safe, eco-friendly and cost-efficient ways of energy generation and supply is not only of great relevance in the macroscopic world of everyday life, but also in relation to the molecular level. As one of the most important energy sources for metabolic reactions and enzymatic pathways, Adenosine triphosphate (ATP) plays a vital role in various fields like production of medical drugs and fine chemicals. It currently takes several time-consuming and costly steps to meet the need for ATP in all these different areas of application. We, the iGEM Team of Aachen developed M.A.R.S – short for Magnetic ATP Recycling System, an innovative strategy to create light-powered, cell-like structures and a bioreactor that will recycle ATP in every industrial process.



Picture 2 – Schematic principle of ATP production by our cell-like chassis

Our system recycles used up ATP of enzyme reactions and feeds usable ATP back into the system instantly and continuously. Our cells are equipped with small magnetic particles so that we can direct their movement with common bar magnets to achieve the optimal results and recycling rates.



Picture 3 – Schematic principle of coupling of multiple chassis to a bigger magnetic particle

Biotechnology is all about harnessing the power of cells and their molecular machinery to our advantage. Just like we want the best molecules and mechanism to perfectly fit our demands, organisms evolve to achieve just that.

In a very harsh environment, we find the first of our main allies: Bacteriorhodopsin, kindly provided by *Halobacterium salinarum*. It lives under extremely salty conditions and can turn light energy into chemical energy via a highly intricate combination of Bacteriorhodopsin, creating a proton gradient, and ATP-Synthase using this gradient to generate ATP. The universal energy currency of each cell. With MARS, we emulate this natural mechanism in our bottom-up synthetic polymersomes. With a twist: We turn it all around, providing free ATP for the surrounding medium by using light rather than complicated and expansive reaction cascades.

ACKNOWLEDGEMENT

The team gratefully acknowledges the valuable advice of Professor U. Schwaneberg, Department of Biotechnology and DWI – Leibniz-Institute für Interaktive Materialien e.V., Professor L. Blank, Institute of Applied Microbiology, and Professor W. Wiechert, Forschungszentrum Jülich. The team also thanks Professor Schwaneberg for the possibility of using his laboratory space as well as for his generous gift of the different kinds of anchor peptides used in the experiments. Special thanks to Dr. Yu Ji for her patient and extensive guidance and support in the everyday laboratory work.

Team Aachen

Back Row: Marcel Wittmund,Sven Leisen, Slok Szatkowski, Lina Schmidt,Raphael Egging, Pia Erzinger, Florian Kroh, Kim Bütten, Vladyslav Los, Julia Gehrmann, Daniel Costard, Selma Busch

Front Row: Salim Atakhanov, Maximilan Dreimann

WORD PUZZLE

V	E	N	E	Z	U	E	L	A	B	N
S	A	A	C	A	C	E	U	R	E	P
O	E	S	N	I	C	C	A	T	L	T
A	R	U	A	V	Y	E	H	A	G	E
L	T	D	R	T	P	E	R	N	I	D
N	I	N	F	A	R	R	T	Z	U	N
E	R	A	P	L	U	G	P	A	M	A
D	E	L	A	I	S	L	Y	N	A	L
E	Y	N	A	M	R	E	G	I	L	E
W	D	I	C	H	I	L	E	A	I	R
S	L	F	A	R	L	E	A	R	S	I

BELGIUM
CHILE
CYPRUS
EGYPT

ERITREA
FINLAND
FRANCE
GERMANY

GREECE
IRELAND
ISRAEL
LAOS

LATVIA
MALI
NETHERLANDS
PERU

SWEDEN
TANZANIA
USA
VENEZUELA

REMAINING WORD, HINT 1:

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WORD PUZZLE

C	U	M	A	E	R	C	E	C	I	L
C	U	M	G	N	I	D	D	U	P	E
E	K	A	C	E	S	E	E	H	C	F
T	B	E	R	T	F	F	L	E	U	A
U	S	I	M	A	R	I	T	I	P	W
N	B	E	R	L	U	A	R	N	C	P
O	A	B	I	O	I	E	I	W	A	O
D	N	A	A	C	T	E	F	O	K	O
P	A	V	L	O	V	A	L	R	E	R
T	N	A	C	H	O	S	E	B	L	T
E	A	L	E	C	T	E	B	R	O	S

BANANA
BROWNIE
CHEESECAKE

CHOCOLATE
CUPCAKE
DONUT

ECLAIR
FRUIT
ICE CREAM

LAVA
NACHOS
PAVLOVA
PUDDING

SORBET
STROOPWAFEL
TIRAMISU
TRIFLE

REMAINING WORD, HINT 2:

_ _ _ _ _ _ _ _ _   _ _ _ _

_ _ _ _ _ _

WORD PUZZLE

Hint 2: Cucumber flea beetle

The striped cucumber beetle is a serious agricultural pest of plants in the family Cucurbitaceae in North America. The beetle feeds of cucumbers, zucchini and other plants that you might have in your garden. The beetles feed on flowers, leaves and fruit, which results in severe defoliation, which weakens the plants and reduces yield. In addition to feeding damage, the cucumber beetle is a vector for bacterial wilt diseases. The beetle belongs to the family of flea beetles which get their name from the fact that the beetles jump like fleas, if they are disturbed. (Evans, B.G. May 2018, EDIS UF/IFAS, "Striped cucumber beetle", entnemdept.ufl.edu/creatures/VEG/BEAN/striped_cucumber_beetle.html)

Striped Cucumber Beetle (Acalymma trivittatum)
Image by © 2016 Enriqueta Flores-Guevara & Lon Brehmer

Hint 1: Cinnabar moth caterpillar

In the picture below, you can see a cinnabar moth caterpillar. They can be found munching away on yellow-flowered ragwort, and their bold black-and-gold stripes make them easy to identify. Ragwort is toxic and well known for its potential to poison other animals. The toxins within the growing plant make it so bitter that it is usually avoided but the cinnabar caterpillars can easily digest it. They actually benefit from its toxicity by eating enough of it to become toxic themselves, and their colourful stripes are a warning to predators: I'm poisonous and taste terrible, don't try to eat me. Newly-hatched caterpillars are vulnerable at first so will cluster together, but as they grow bigger and develop their toxic defences they start to spread out. It takes about a month for them to develop fully, at which point they will go underground to pupate. They will remain there all winter, inside their cocoons, and complete their metamorphosis the following spring to emerge as beautiful black and red moths. (Owen, Charlotte. "Cinnabar Caterpillars." ,Sussex Wildlife Trust, 26 July 2018, sussexwildlifetrust.org.uk/news/cinnabar-caterpillars.)

Cinnabar moth caterpillar. Image by dendoktoor from Pixabay

Target Locusts from Within

Larissa Markus[*], Gabriela van Leersum[**]
Correspondence email – igem@tudelft.nl
[*] Interviewer, team MSP-Maastricht
[**] Team TUDelft

***Abstract*- This year, an outbreak of massive locust swarms has been categorized as the worst in recent decades. Locust swarms threaten croplands and food security in East Africa, Asia, and the Middle East. Records of locust plagues afflicting cities date as far back as ancient Egypt. However, this isn't just an issue of the past. This years' TU Delft iGEM team, Phocus, have chosen to concentrate their efforts on finding a solution to the locust crisis. We have invited them to answer some questions and bring awareness of this problem.**

We have invited them to answer some questions and bring awareness of this problem

Hi guys! Can you introduce yourself? Who are you (people that answer the questions) and what is your role in the team?

Thanks for having us, we are excited to have the opportunity to partake in your journal! My name is Gabriela van Leersum and I am one of the eleven enthusiastic students making up Phocus. Within the team we are all involved in developing the scientific aspects of the project, personally I fill the role of Outreach and PR manager.

Now let's get to the real questions:

How did you reach this topic and decided it would be the focus of your project?

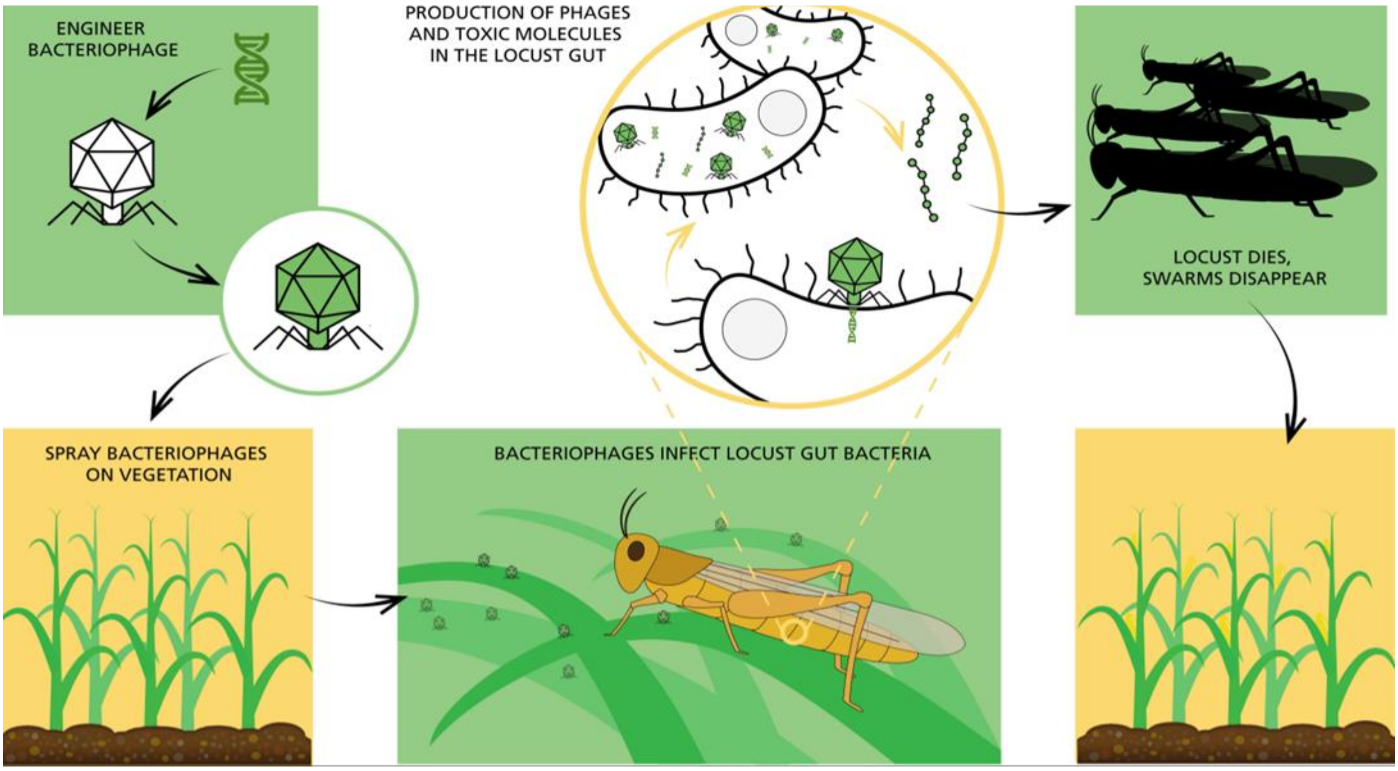
At the beginning of the competition we had many different ideas about potential projects. We realised that it would be important to take enough time to properly consider them all, before making a decision. After many interesting discussions, we found a topic that we were all motivated to work on; the locust crisis. The idea first came to us after reading a small news article on the damage locust swarms were causing in the Arabian Peninsula. From our discussions we learned that we wanted to make an impact and decided to contribute to finding an answer to the locust crisis.

Locusts (derived from the Vulgar Latin locusta, meaning grasshopper) are a collection of certain species of short-horned grasshoppers in the family Acrididae that have a swarming phase.

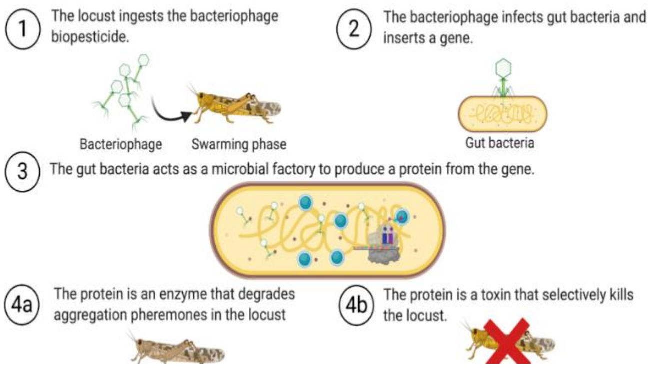
What can you tell us about the locust itself? and why is it so devastating for agriculture?
When you think about an individual locust, they may seem quite harmless, which is true. As is often said, there is strength in numbers. In the right conditions, as occurred in the past year, locusts gather in specific locations and form swarms. These swarms can grow to the size of Paris and eat the equivalent of half of France. In addition to swarming, they undergo a physiological change which allows them to develop wings, allowing them to travel large distances and grow in size. This year the United Nations' Food and Agriculture Organisation have estimated that the food security of 10% of the world's population is threatened by locusts.

What are some causes of these infestations?
This is a good question, however also difficult to answer. After speaking to multiple experts, including members of the FAO, it was clear that there is no consensus on one explanation. The process of swarm formation is known to be linked to weather patterns – namely due to drought after extended periods of rainfall. Yet the reason why the current upsurge is so large is still unclear. Some scientists have associated the changing weather cycles with the climate crisis, although there is no hard evidence to verify this.

What is the approach your team has worked on to provide a solution to the problem and how does it apply the concepts of genetics and synthetic biology?
Our aim is to design a specific, fast working and safe bio-pesticide. Specifically, we are using bacteriophage as a delivery method and engineering them to encode for toxic molecules. The phage is applied on vegetation in the affected areas and is ingested by the locusts. Once in the locusts' guts, the bacteriophages encounter bacteria into which they inject their DNA. This allows them to "hijack" their machinery and produce the encoded toxin. The bacterium eventually bursts, releasing the toxin into the gut and kills the locust from within.



Our mission is to tackle the locust crisis by developing a sustainable bio-pesticide through responsible innovation and collaboration.



What challenges have you encountered while working on this approach?

Similar to many of the other iGEM teams in the rest of the world, the restrictions associated to covid-19 have had a big impact on how we work. Especially, working with the restricted lab access has proven to require extensive planning and preparation. Nonetheless, we are confident that we will be able to present interesting results at the Jamboree (the final event of the competition).

This project has a direct effect on the global community, what human practices has your team worked on to engage with the public?

Our human practices involve engaging with a diverse range of stakeholders and we are proud to say that it has grown to be a substantial part of our project. We have talked to multiple experts and gained valuable insights into the reality of the current crisis. One of these discussions lead to our participation in the Mondial desert locust management Conference, hosted by TheWaterChannel. With a problem as widespread as the locust crisis, we have also been working hard to engage with the general public. Ranging from young to old, we have created material for *all*. An example would be the children's book about phages or the multiple lectures that we have presented at elderly homes.

Regarding iGEM itself, what is your team looking forward to the most?

Until now, we have already made many memories together that I know we will all look back on with pleasure. I think something that we are still looking forward to in the future would have to be the Jamboree. It's really exciting to think about the fact that we will come together with teams from all over the world to present our projects and learn from each other.

What has your team learned from participating at this competition and working on a research project like this?

iGEM is not a project that compares to a lab practical or a workshop at the university. The competition itself is incredibly broad and consists of merging skills from multiple disciplines into one project. This can be hard work however at the same time it is very fulfilling.

What other problems do you think could be tackled with synthetic biology and genetic engineering?

I speak for the whole team when I say that we believe in the potential for synthetic biology to come with solutions for many of the world's problems. To get a better idea of how broad this really is, I'd like to invite you to watch our synthetic biology video on YouTube!

https://www.youtube.com/watch?v=Yuj46jG4_Q4&t=4s

Last but not least. Is there anything that you as a team would like to share with the audience?

Partially due to the covid-19 epidemic, the locust crisis is not getting quite as much attention as it otherwise might. If you can, we would like to invite you to take 5 minutes to have a quick browse through google and see what you can learn about the areas that are currently dealing with locust swarms. Mention it to your friends and family, and together we can make sure that the people affected are heard!

Targeting the Oak Processionary Caterpillar Pest by Means of a New Bacterial Pesticide

R. Haikarainen [*], R. Kosta [*], D. Shumkova[*], L. Markus[*], S.Bonni[**], S. Björnör[**], L. Granston[**], J. Passariello-Jansen [**], L. Robeerst[**], M. Rubina[**], M. v.d. Schoot[**], C. Sébert[**], E. Thielecke[**]

[*] MSP-Maastricht, First Authors, Maastricht University
[**] MSP-Maastricht, Maastricht University

Severe itching, rashes, respiratory issues like asthma and eye complaints… All symptoms that affect over 100.000 people in the Netherlands alone as a result of the Oak Processionary Caterpillar (OPC) who returns with vengeance yearly. The MSP-Maastricht team therefore decided to tackle this local problem in the iGEM competition.

It is no secret that the OPC has become a major threat over the years, harming fauna around them as well as the flora they reside in. Apart from stripping trees bare to their bark, they form a serious health hazard for humans as well as animals. The allergenic protein thaumetopoein on the bristles triggers inflammatory responses of the skin and eyes as well as respiratory problems like asthma attacks (Rahlenbeck & Utikal, 2015). In serious cases, the bristles even have to be removed surgically from the eyes!

Figure 1: The Oak Processionary Caterpillar is the larvae of the Thaumetopoea processionea moth..

In the last 3 decades, climate change seems to be the OPC's best friend. It enabled the invasive species to cross its native borders in Central and Southern Europe, and spread towards all European countries and even parts of the Middle East (Koppert Biological Systems, 2020). It's quite the journey, isn't it?

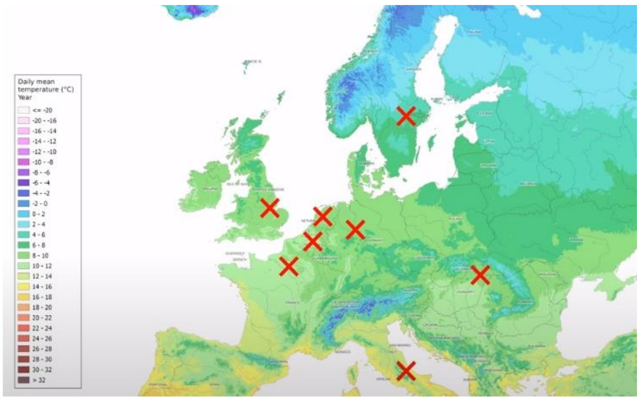


Figure 2: The spread of the OPC dues to climate change

Up until now, no insecticide has sealed the deal. Many of the current control methods are very costly, inefficient and non-specific. One of the control measures used at the moment is the biological control; bacterial insecticides (Bt) are sprayed on leaves releasing a toxin, which is a non-specific approach that also causes harm to other caterpillars and butterflies. Another method is the so-called nest removal; where the caterpillars are vacuumed up and burned. This is very dangerous for exterminators themselves and simultaneously also an expensive technique.

Figure 3: Removing the caterpillar is very dangerous for the exterminators.

With a team of 13 students from the University of Maastricht, we aim at tackling the problem by genetically engineering a bacterial pesticide that is specific for the OPC and on top of that, environmentally friendly. This biological pesticide would target specific and essential gene sequences in the caterpillar to reduce their growing population and avoid harming other species. For this, we plan to take advantage of the cellular mechanism of RNA interference. It is a form of gene silencing, where the selection and silencing of a critical target gene would lead to the OPC's death.

In order to develop a highly specific bacterial pesticide, we will identify essential sequences in the caterpillar not found in other organisms. With this information, we can design bacteria that produce silencing RNAs for these sequences (Niu et al., 2018). These modified bacteria will be sprayed on infected trees where the caterpillars will ingest them and prevent further reproduction of the caterpillars by blocking essential biological processes.

Figure 4: Experiment if the pesticide sticks to the trees. Testing it on oak branches in the laboratory.

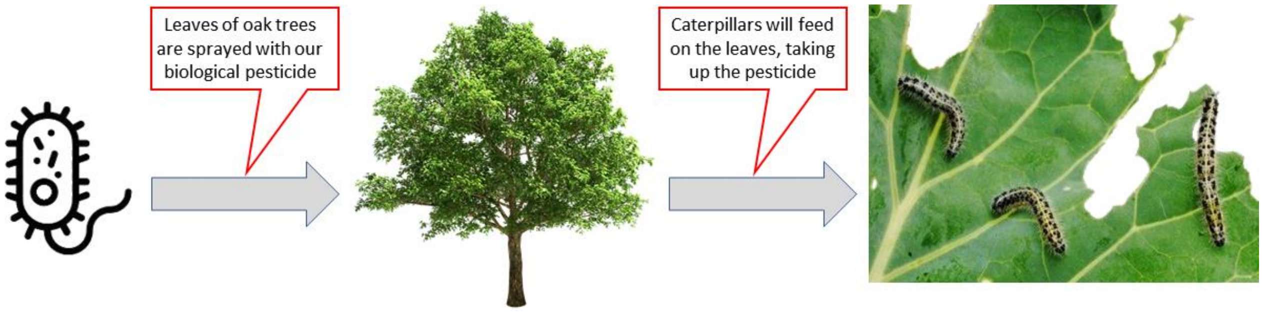


Figure 5: How our pesticide would be used on the invasive caterpillar.

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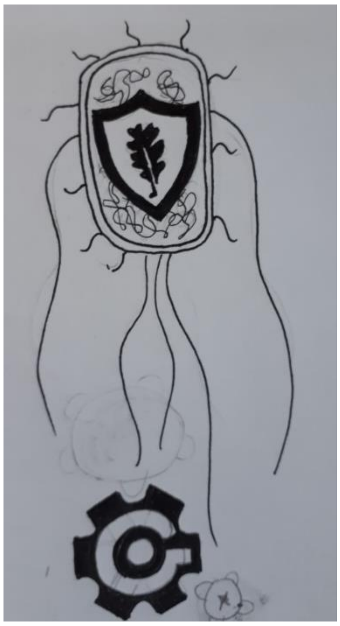
Rahlenbeck, S. & Utikal, J. (2015). The oak processionary moth: a new health hazard? Br J Pract, 65(637): 435-436. doi: 10.3399/bjgp15X686341

Why is a logo important?

Because it grabs attention, makes a strong first impression, is memorable, separates you from competition and is expected by your audience. That is why we put in a lot of work to create the perfect logos for our project. On this page you can see how our logo developed from a sketch to the nicely designed logo we now use as our main identifier.

DESIGN



<u>Step 1:</u> Brainstorm what the main purpose of the logo will be and what you want to signal with it

<u>Step 2:</u> Sketching a variety of logo concepts

<u>Step 3:</u> Create digital drafts in vector software

<u>Step 4:</u> Refine your logo design with feedback

POLYMERASE CHAIN REACTION (PCR)

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[*] Team MSP-Maastricht, Maastricht University

Polymerase Chain Reaction, (PCR), is a technique used to copy small segments of DNA. This procedure is used in research where the aim is to detect the presence of specific genes or to amplify the genetic material. Since substantial amounts of genetic material are needed to perform further experiments, PCR is an ideal technique that allows us to rapidly make millions of copies of even the smallest amounts of genetic material (1). This might become handy when a researcher is interested in a specific region of genetic material for which it might only have a small sample size.

HOW DOES IT WORK?

PCR uses the principle of DNA replication to amplify the target sequence; it makes use of a DNA polymerase enzyme – a protein that acts as a catalyst and accelerates chemical reactions – to replicate the sequence using the existing strands as templates for the copies (2).

The enzyme can only do its job if a primer is present; a primer is a sequence that indicates the starting point for the DNA polymerase to start the amplification, as it determines the region of DNA that will be copied (2).

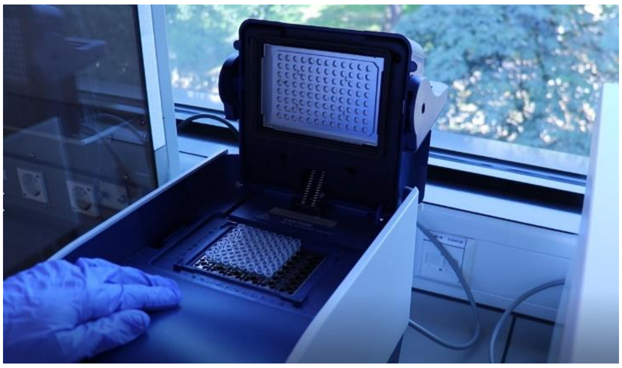

Figure 1. Thermocycler

STEPS (2)

1. Denaturation (96ºC): In this step, temperature is increased to separate the DNA strands and obtain the templates for the next step
2. Annealing (55-65ºC): Afterwards, the reaction is cooled, and the primers can now bind to their corresponding sequences on the templates
3. Extension (72ºCº): Finally, the temperature is increased once again so that the DNA polymerase extends the primers and amplifies the genetic material.

Figure 2. Thermocycler strip-tubes

This cycle is repeated approx. 25 to 40 times to obtain a sufficient amount of genetic material for further experiments.

The obtained amplified genetic material can then be further analysed via a process called *Gel Electrophoresis*. You can read more about this process on page (NUMBER).

(1)National Human Genome Research Institute (n.d.) *Polymerase Chain Reaction (PCR) Fact Sheet.* Retrieved from:https://www.genome.gov/about-genomics/fact-sheets/Polymerase-Chain-Reaction-Fact-Sheet

(2)Khan Academy (n.d.) *Polymerase Chain Reaction (PCR).* Retrieved from: https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/biotechnology/a/polymerase-chain-reaction-pcr

Why Are We Running Gels?

Ronja Haikarainen*

*Team MSP-Maastricht, Maastricht University

Gel electrophoresis, or as we call it "running some gels", is one of the basic techniques used in biology labs. Gel electrophoresis is a technique performed to get a separation of charged biomolecules, such as DNA, RNA, and some proteins (Reed, Holmes, Weyers, & Jones, 2016). In our experiments, the team MSP-Maastricht works with DNAs and RNAs.

The basic component of gel electrophoresis is the gel itself, as the name already suggests. These gels resemble flat bricks or sheets, and they are often made from agarose, which is a type of a polysaccharide (ThermoFisher Scientific, n.d.). At one end of the gel there are wells where the sample is inserted. Agarose gives a gel which has tiny holes that let molecules pass through.

Consequently, molecules get separated by their size. Smaller molecules move faster through the gel and larger ones move slower, as it is more difficult for them to pass through these holes.

What makes these molecules move? This is done by utilizing the chemical charge of the molecule. For example, the backbone of DNA and RNA are negatively charged, so when they are placed between two electrodes and an electrical current is applied, they will move towards the positive electrode (Reed et al., 2016). This also works the other way around, since opposites attract. Moving the molecules through the gel is is achieved by placing the gel into a tank with electrodes at both ends and some fluid to aid with the movement.

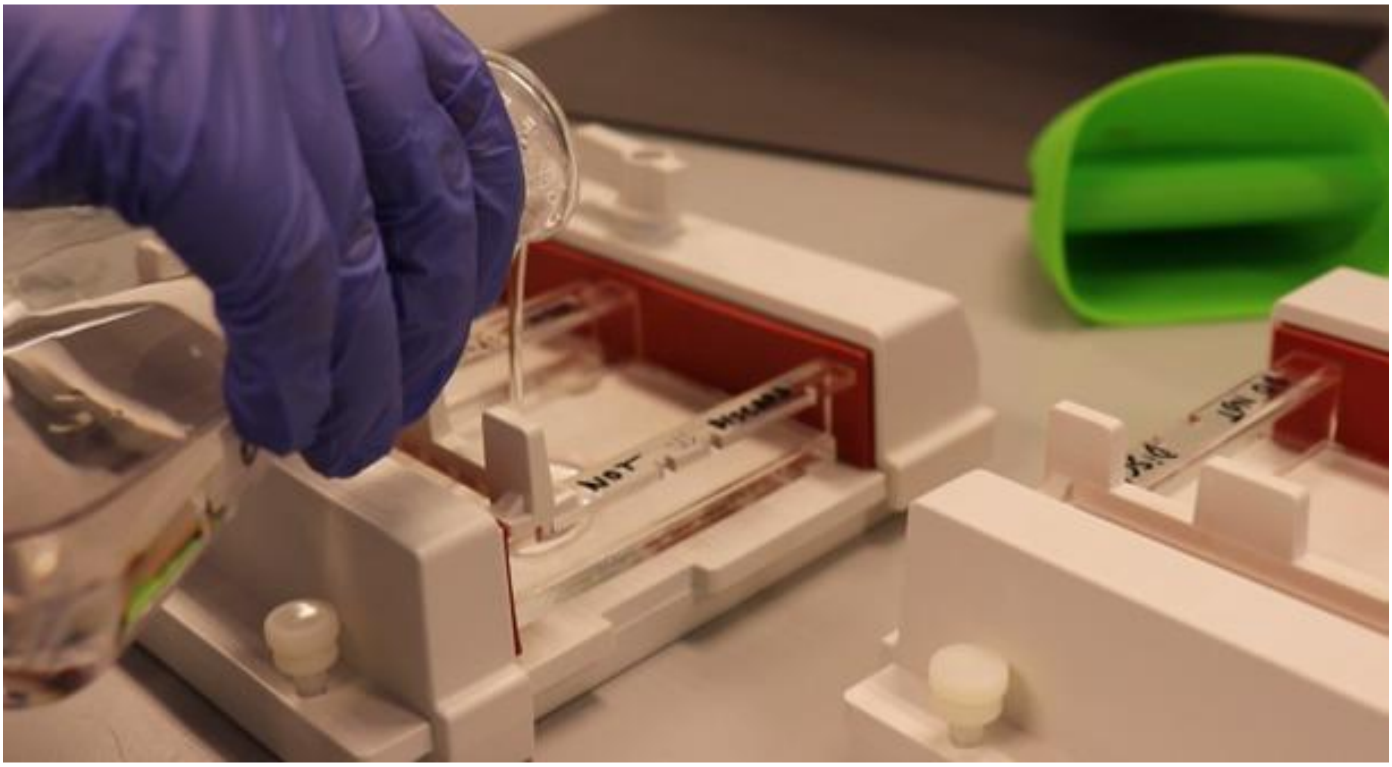

Figure 1*: Pouring hot agarose mixture into gel moulds. When the mixture cools down it turns into a gel.*

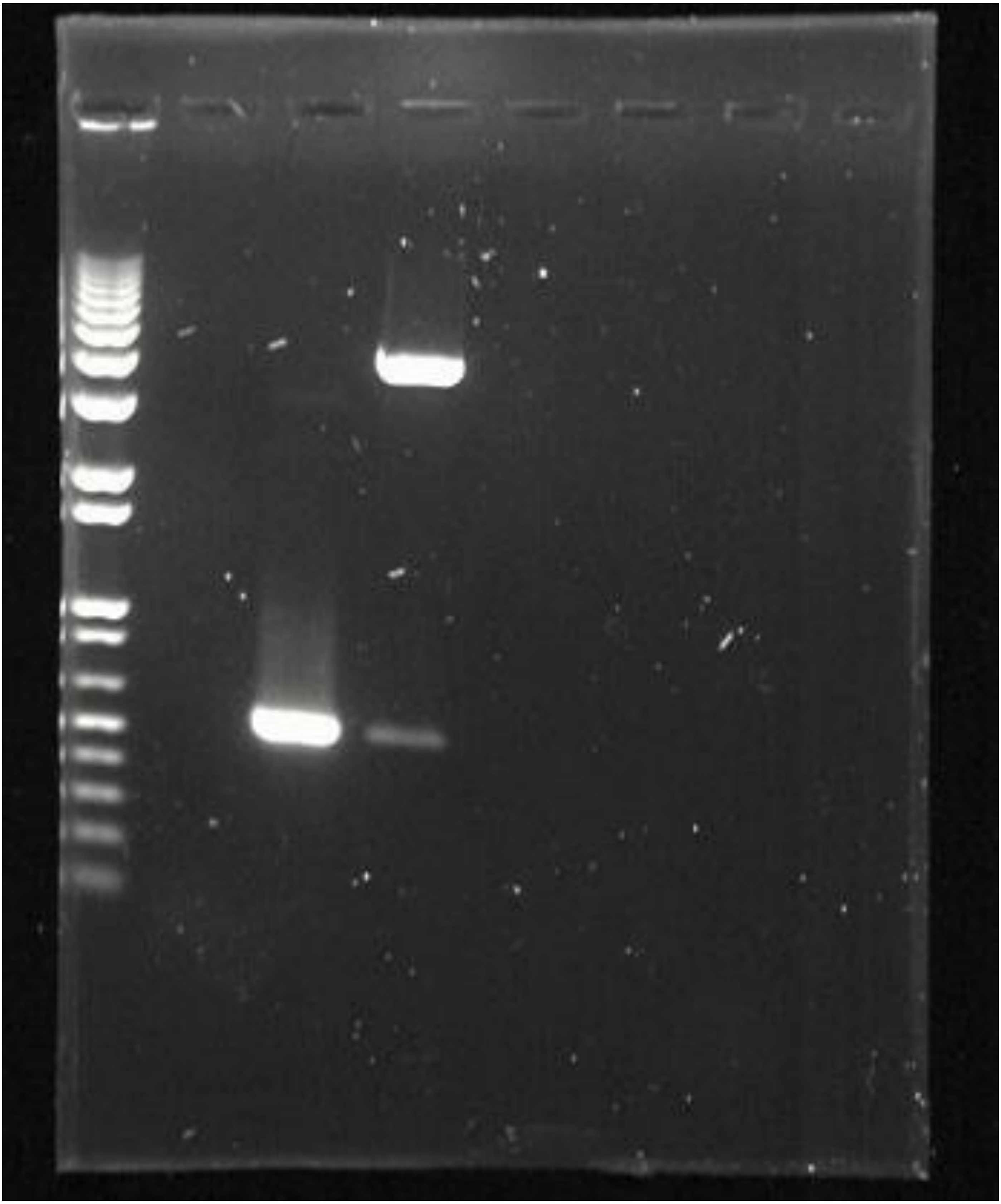

Figure 2: *Reference ladder (on left) and DNA fragment bands (right). © Photo by TransControl/WIKICOMMONS*

When the gel electrophoresis is complete, the gel can be imaged. The images reveal the position of the molecules in the gel. Because a reference, with multiple different fragment sizes, is placed next to the sample, we can easily determine the size of the molecule by comparing it to the known reference. In our experiments, we want to specifically determine the length of specific DNA or RNA fragments and use this information to identify if these fragments. Size of a molecule can tell you a lot about it. For example, we use gels to see if certain genes are present in the Oak Processionary caterpillar or if certain RNA molecules are being produced by our modified *E. coli* bacteria. Running a gel is one of the easiest and fastest ways to get this information! At this point we have probably run a full marathon with our gels!

Figure 3*: Staining of molecules is done for visualization*

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"Agarosegelphoto.jpg" by TransControl uploaded 2 may 2007 is licenced under Creative Commons Attribution-Share Alike 3.0 Unported license

iGEM Tübingen

The fight against COVID-19 with Prof. Dr. Kremsner

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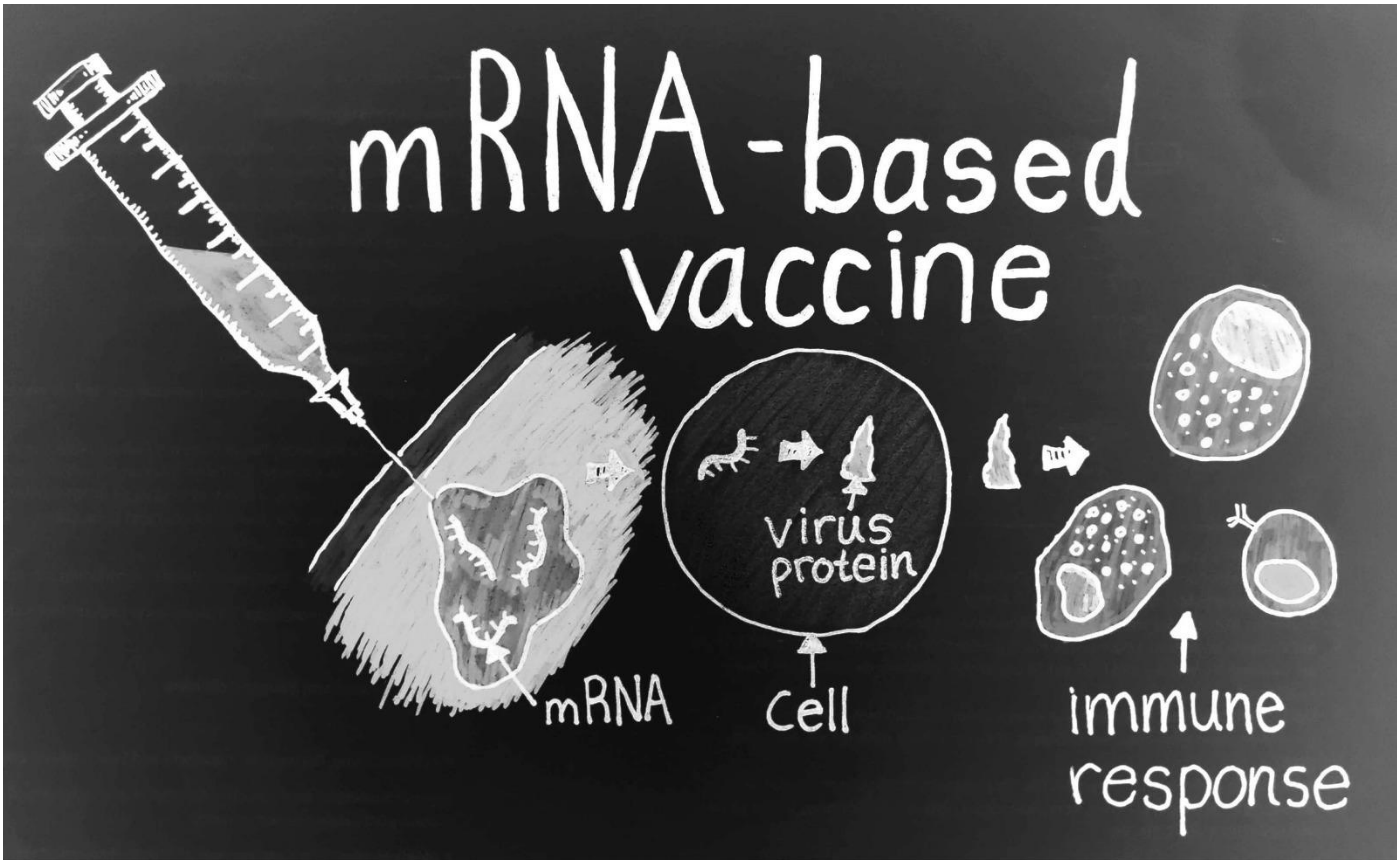
Correspondence Author – Aarón Alexander Refisch*, team@igem-tuebingen.de

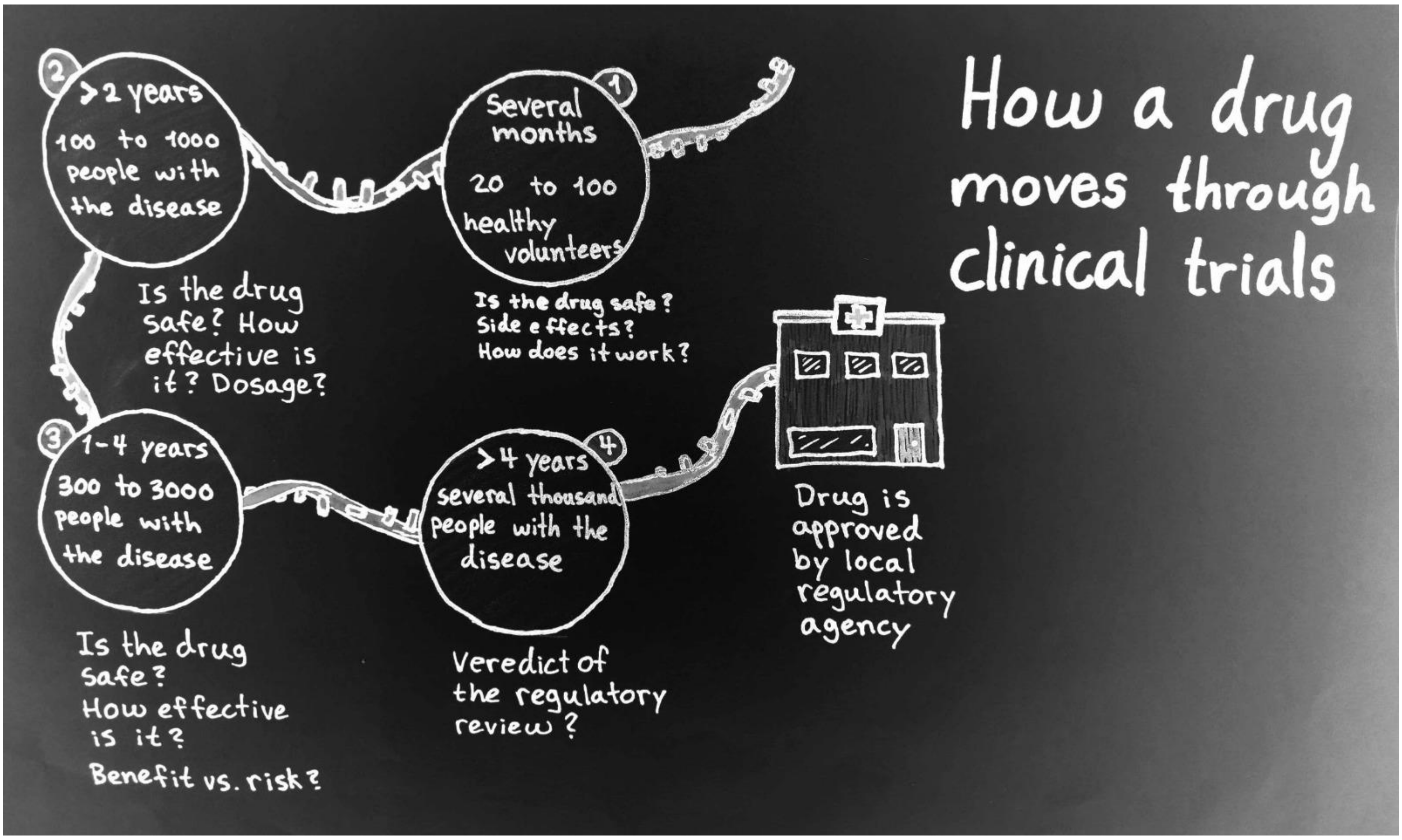
* iGEM Tübingen 2020

Over the last months, around 38.000.000 people worldwide have been infected with the virus SARS-CoV-2, which is the causative agent of the disease COVID-19, a.k.a. Coronavirus. Of those people, about 1.000.000 have lost their lives. In this interview, Prof. Dr. Kremsner evaluates the current situation of finding a cure, talks about the different approaches of designing a potential drug and gives personal insight into his academic career.

You are currently involved in three major vaccine studies. One that is an mRNA-based vaccine, another one that is based on the use of viral vectors, and a third one that uses virus-like particles. Could you explain to us the basic differences between the approaches a little more?

A so-called mRNA-based vaccine injects mRNA (the blueprints for a virus protein). The mRNA then penetrates the cells and is translated into a protein which sends out an alarm triggering an immune response.





Another vaccine study will be based on a previously researched malaria vaccine. At that time, we incorporated the malaria antigen, which is used for immunization, into virus-like particles. Now, this will be done analogously using the antigen of the coronavirus.

The third approach is based on Modified-Vaccinia-Ankara viral vectors in which the antigen of the coronavirus is incorporated.

Have you noticed a difference in working with other scientists since the pandemic has started? Did it bring all of you closer together or are you rather fighting in the search for an active compound?

At the moment, many groups are working together in the university area, but also keeping close contact with private initiatives. Especially in times like these, we as scientists have to move closer together. It usually takes 10 or even 20 years before a possible vaccine is designed and approved for selling. Decreasing this time to one to two years is only possible in a very close and well-coordinated cooperation. This is because despite the high-pressure work, no reduction in the very high standards of development, security testing, and especially in the work of the ethics committee can be made. I am very optimistic that we will find a vaccine soon.

When do you think the first COVID-19 vaccine will be available?

I believe that the CureVac vaccine will be approved by the end of next winter, mainly because of the joint efforts. Right now, we are in phase 1 of the drug trial. We aim to get the first results in the next two months, so that we can move on to phase 2 and, if possible, to phase 3 before the beginning of the next year.

Recently there were news reports that cured COVID-19 patients show a measurable decrease in neutralizing antibodies in the blood. This is seen as an indication that the permanently acquired immunity to SARS-CoV-2 may decrease. Do you see the vaccine effort at risk?

Of course, this is a phenomenon that still needs to be investigated, but the immune system and in particular the acquired immunity cannot simply be characterized by measuring the antibody concentration. To evaluate the chances for the effectiveness of an active ingredient, one has to examine the dynamics of the entire immune system. There are other viral diseases in which immunity can be acquired that is not necessarily detectable by an increased antibody concentration. Therefore, despite these reports, I do not consider efforts to get a vaccine at risk.

If you had yourself in front of you as a student, what advice would you want to give him for his career as a scientist?

The most important qualities in my experience are hard work, perseverance, and self-organization. I can remember how I always got up at six a.m. in my first semester to study as much as I could before the lectures started. This made it possible for me to spend my free time however I liked it. You will not be successful without discipline. It is also important to specialize in what interests you most as early as possible in your studies.

ACKNOWLEDGEMENT

Special thanks to Prof. Dr. Kremsner, for taking the time to answer our questions about the SARS-CoV-2 vaccine development and your insights into the scientific community in these testing times.

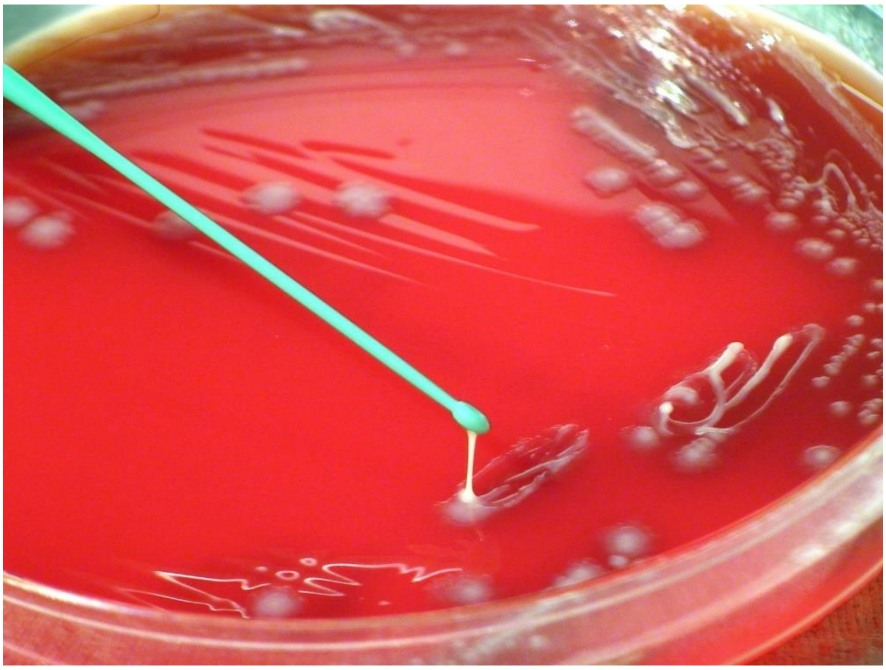
Thank you, David Keßler for getting in contact and conducting the interview and also to Katja Sievert for translating the original interview.

We also want to acknowledge all the support of the rest of the iGem Tübingen 2020 team, without you this would never be possible.

OUR SLIMEY FRENEMIES

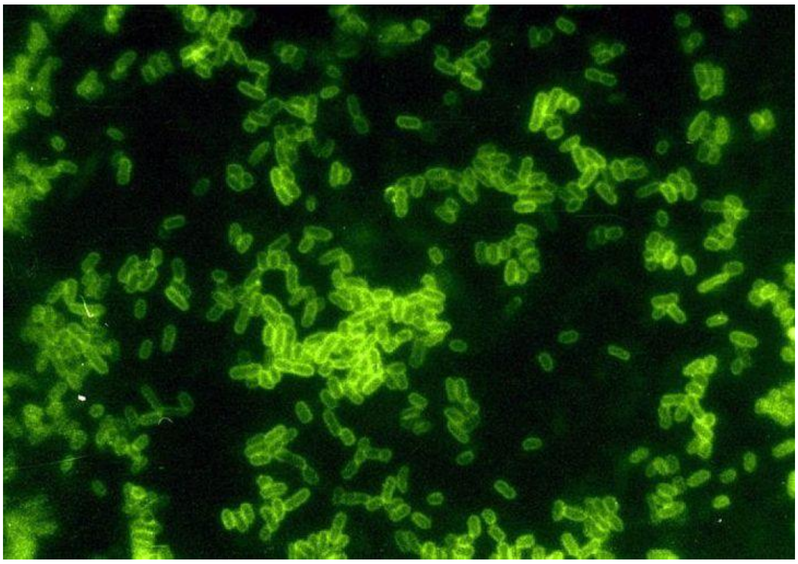
Have a look at this picture of gibberish slime pulled this blood agar sample plate. What is it?

It is a colony of *Yersinia pestis*, the pathogenic bacterium that causes plague, “the Black Death”, that has caused one third of the European population to die in the 14th century (Harvard Publishing, 2018). *Yersinia pestis* is an important example for pathogenic, disease-causing bacteria. Next to *Y. pestis*, there are countless other harmful bacteria that cause bacteria in humans.



***Yersinia pestis* grown on blood agar.** *By by Pete Seidel,Amanda Moore, MT, Todd Parker, PhD, Audra Marsh, USCDCP. Retrieved from https://pixnio.com/science/microscopy-images/*

Bacteria are ubiquitous microorganisms; they are almost everywhere. For this reason, it is important to maintain a good hygiene. Especially in times of a pandemic, the value of certain routines rises to a completely new level. Despite the fact that many bacteria are associated with pathogenicities, they are not all bad, on the contrary! For example, where you ever recommended to eat *probiotic* food by your doctor or advertisement? Probably. This is because your digestive system is highly dependent on the fermentation skills of the microbial friends (Jakob, 2014).



***Escherichia coli*, digitally colorized**. *By Dr. M.S. Mitchell, USCDCP. Retrieved from https://pixnio.com/science/microscopy-images/*

Now, let’s take a look at the picture to the left. Some people probably would say that these fellas still look a bit unpleasant to the eye. However, what you see here are *Escherichia coli* bacteria, who are an all-time superstar when it comes to synthetic biology.

E. coli live in our digestive tract and, although there are harmful strains as well, this bacterium is extremely convenient to use in biotechnological research and industry. It is super easy to grow and clone. Genetically engineered *E. coli* can for example be used as bacterial factories to produce human insulin, in vaccine development or as model organism for disease models (Science Learning Hub, 2014).

There are numerous other applications for genetically engineered microorganisms, as you can read about in other articles in this journal. So, now that we learned a bit about bacteria, their hazards and useful properties: Beware of and seek your bacterial frenemies!

Author: Eva Thielecke

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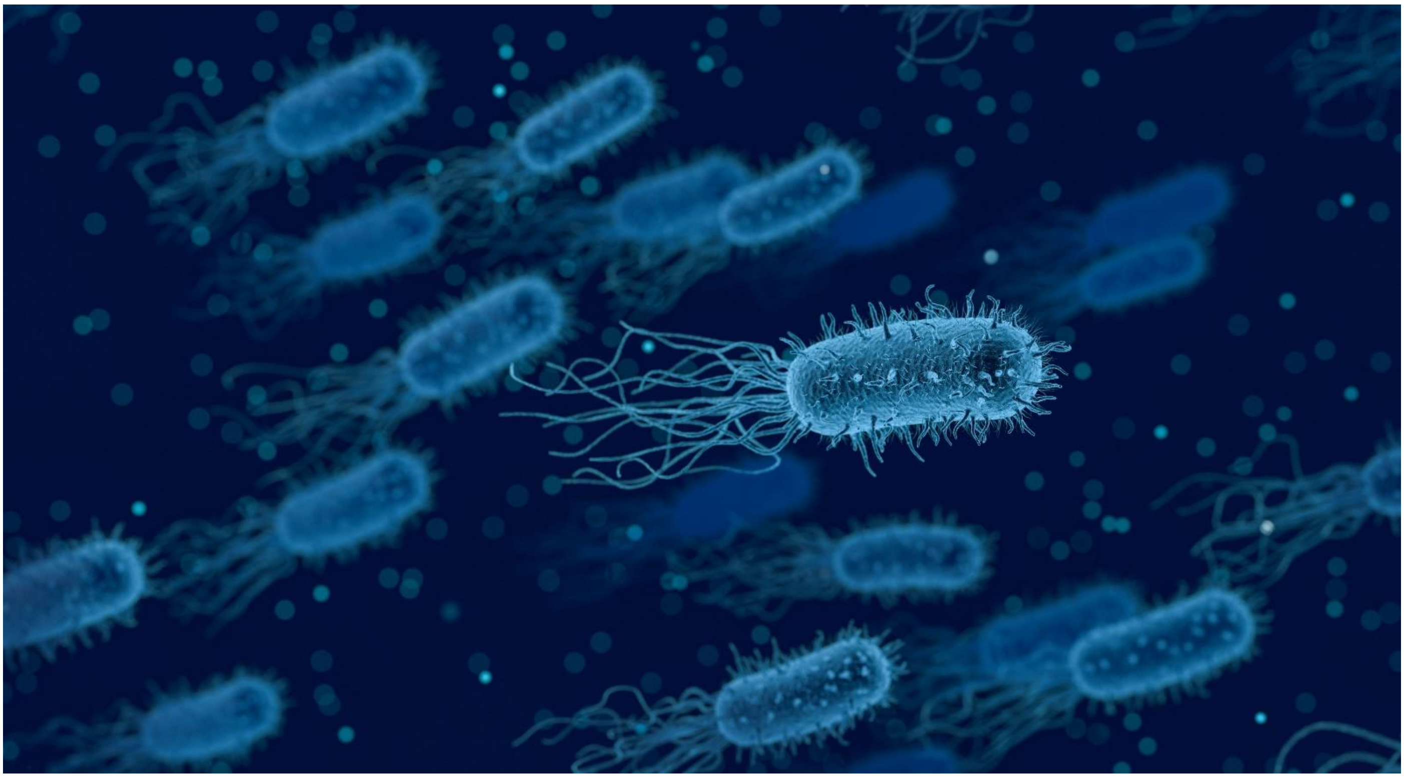

Artistic but realistic animation of bacterium*: Image by Arek Socha from Pixabay*

DIRTY HANDS

The picture take by Martin Hismaic shows a bacterial culture created from just a single hand print. The agar that the handprint was left on it is the perfect food for the bacteria living on our hands. When he placed his hand on the agar bacteria where transferred from his hand to the agar. Then the agar was incubated by 37°C for 4 days and the bacteria left to grow. The picture shows the growth of the bacteria on his hands after this incubation period. It is a beautiful picture, with important an important message. It can be difficult to comprehend just how clean or dirty our hands can be sometimes after all, it's not like we can see bacteria with the naked eye. Many of us don't quite realize what our hands could be harboring and how hand washing and hand sanitizers can actually help combat problems caused by unclean hands. Especially in times of a pandemic , personal Hygiene is incredibly important to prevent a disease from spreading. This picture is a good reminder of that, which is why we chose it for our cover.

Author: Larissa Markus

Fact: Germs can survive for up to three hours on your hands and there are between 2 to 10 million bacteria on your fingertips

Fact: The number of bacteria on your Hands doubles after you use the toilet and if you do not wash your hands you transfer germs to the food and drinks you eat.

Fact: Just 30 seconds of simple handwashing with soap and water reduces the bacterial count on your hands by 58%.

Fact: There are more germs on your phone than on your toilet seat. Cleaning it regularly with disinfectant will prevent bacteria from spreading.

Harvard Men's Health Watch. "Wash Your Hands." Harvard Health Publishing, July 2006. www.health.harvard.edu/newsletter_article/wash-your-hands.
Tri-County Health Care. "Gross! Hand Hygiene and Other Germy Facts.", 12 Dec. 2018, www.tchc.org/blog/2018/12/12/hand-hygiene-and-germ-facts/

"Synthetic Biology for Everybody" by Ars Electronica (Martin Hieslmair) is licensed under CC BY-NC-ND 2.0

Bioprinting: Printing Life

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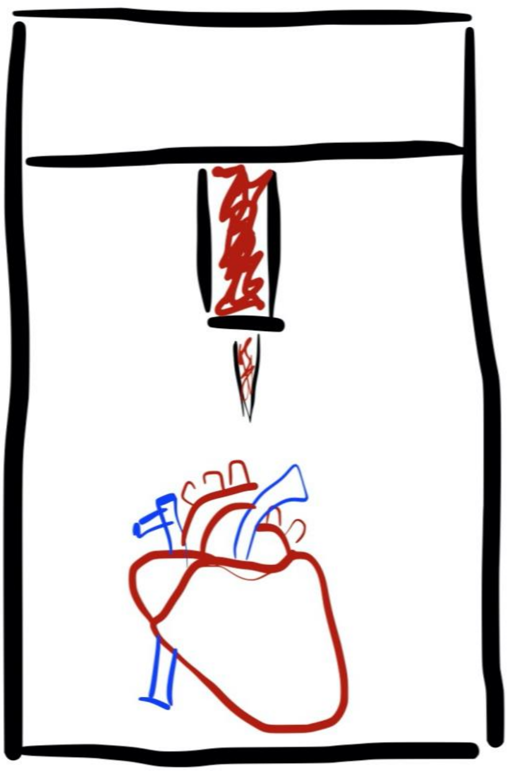
Bioprinting is a type of additive manufacturing process that uses biomaterials and living cells to create structures with various functions (Murphy & Atala, 2014). Currently, 3D bioprinting is mainly utilized for creating complex systems which can imitate natural tissues. Bioprinted artificial tissues can be used for medical and experimental purposes.

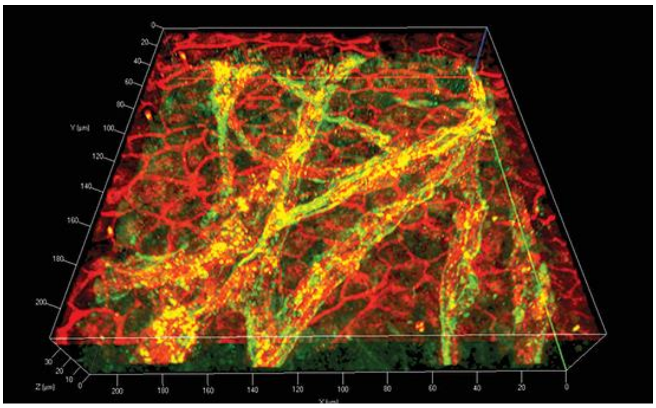


A 3D Bioprinted Heart by Leyla Yalçınkaya

In 3D bioprinting, materials used in the process need to be coherent with the process of printing and maintain certain mechanical and functional properties for aimed tissue constructs (Tappa & Jammalamadaka, 2018). Therefore, materials have to be based on naturally acquired or synthetic polymers.

3D-bioprinted tissues are important for drug discovery, toxicology, and organ/tissue transplantation because of their possibility of replacing animal testing (Murphy & Atala, 2014). This technology is far from perfect due to limitations of the materials used and administering technologies (Wan et al., 2020). Recently, there has been research on developing unique bioink materials for each application and 4D printing with dynamic shape changing! In the near future, bioprinting may be directly done on the patient's body by robotic surgical tools increasing the efficiency of the bioprinting process (Murphy & Atala, 2014). Bioinks made from special molecules delivering the cells to a gel framework is also an area of continuing research (Okamura et al, 2009; Yu et al., 2012). In 2019, light-activated bioinks were developed for minimal contact to the tissues (Skliutas et al., 2020). In 2020, intestinal and airway 3D printed tissues have been used in studying the effects of SARS-CoV-2 infection on real human tissues (Clevers, 2020).

Image of a 3-D bioprinted blood retina barrier tissue model by Min Jae



Song and Kapil Bharti.
Credit: NIH, NCATS, and National Eye Institute

ACKNOWLEDGEMENT

Thank you to our fellow team members Ömer Can Ergül & Tutku Muratoğlu who co-authored our original article for the Proceedings Journal. We would have been unable to transform our article into a format appropriate for the Muggle Journal without their help.

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Mother Nature gets a Detox: Laccase as a Solution to Micropollution

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* Team TU Kaiserslautern, Technical University of Kaiserslautern

Painkillers such as Ibuprofen or Diclofenac, are commonly used medication due to their antiinflammatory and analgesic properties. The advantages of local use in ointments for Diclofenac also make it exceedingly popular among doctors and patients. These micropollutants accumulate in wastewater due to overuse and poor disposal methods, contaminating the environment. The current treatment in sewage treatment plants, ozone, or carbon filtration methods, are expensive and complex.

Our vision is to make this process efficient and cost effective through the use of genetic engineering. Our project involves the modification of the green algae *Chlamydomonas reinhardtii*, enabling the chemical decomposition of Diclofenac and leading to its functional degradation. To achieve this, we will clone genes from two different laccases into the genome of our primary organism, *C. reinhardtii*, as well as into the bacterium *Escherichia coli* as a control.

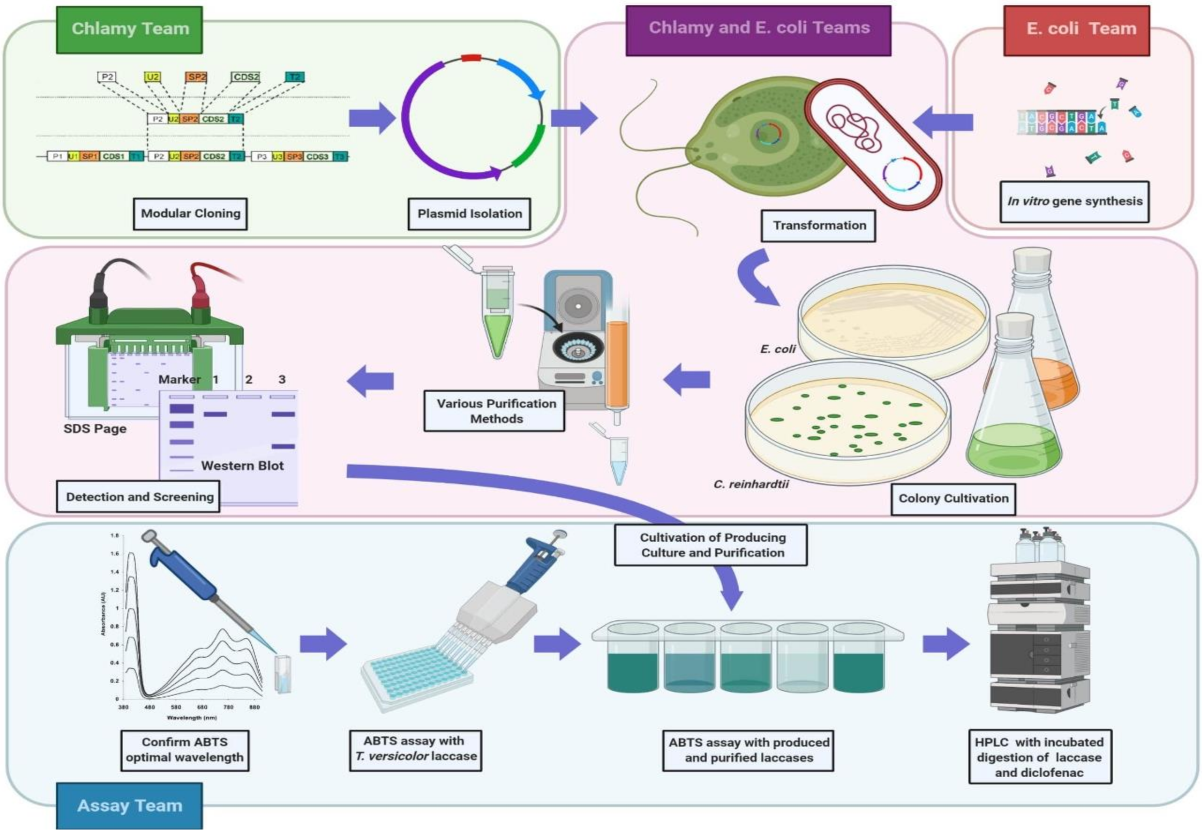

Fig 1: A summary of our team's collaborative effort throughout the project.

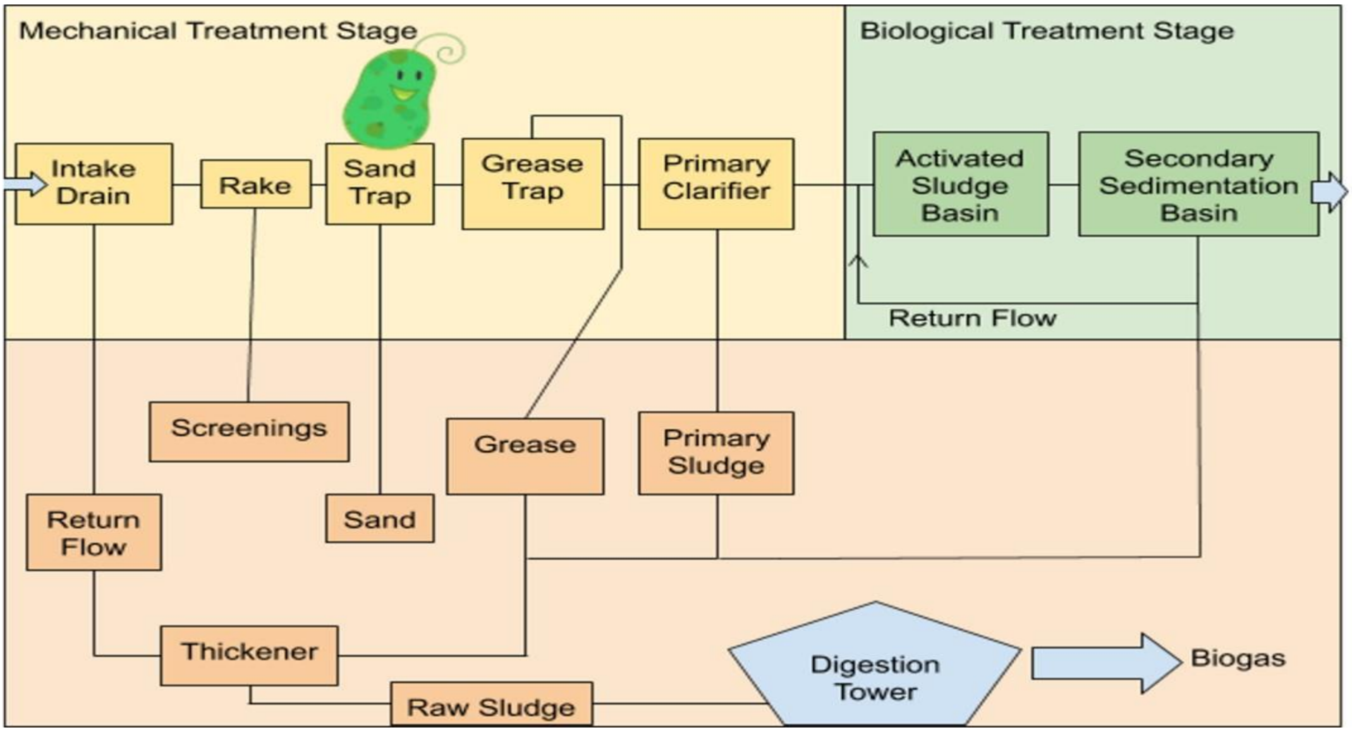

Fig 2: An overview of a German Wastewater Treatment plant and where we could implement our bioreactor.

Laccases are oxidases that have a multicopper center, allowing them to oxidize their substrates, As they take up an electron and transfer it to O2, it leads to the theoretical deactivation of the micropollutant and produces water as a byproduct. (Kittl *et al.*, 2012; Zerva *et al.*, 2019)

We split into three teams to best implement our experimental process: one team focused on producing proteins with our model organism (referred to as Chlamy Team), one focused on doing the same with our control organism (called *E. coli* Team), and our Assay Team who performed the confirmation activity assays with both previous teams' produced laccases.

Since our focus is to protect the environment with our innovations, we obviously want to prevent contamination of wastewater with our genetically modified organisms (GMOs). To ensure efficient enzyme production, *C. reinhartii* will be raised as a permanent culture in a bioreactor, outside the wastewater treatment plants. To separate the green algae from the wastewater, we want to create a filter between the bioreactor and the wastewater basin that only allows the enzymes to pass through, but not the GMOs.

The degradation products show no toxicity, therefore do not harm the environment. Since the laccases we have chosen do have activity at pH consistent with internal water treatment steps, we will be able to integrate our bioreactor directly into current wastewater treatment systems without the need for new additional steps as previously proposed by other teams working on laccase based solutions. This will make it more cost effective and efficient for smaller treatment plants all over the world to utilize, as compared to more expensive methods currently being used. We hope for a cleaner future for you and me!

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Academic Supervisors: Michael Schroda, Nicole Frankenberg-Dinkel, Felix Willmund
Figure 1 created with BioRender.com.

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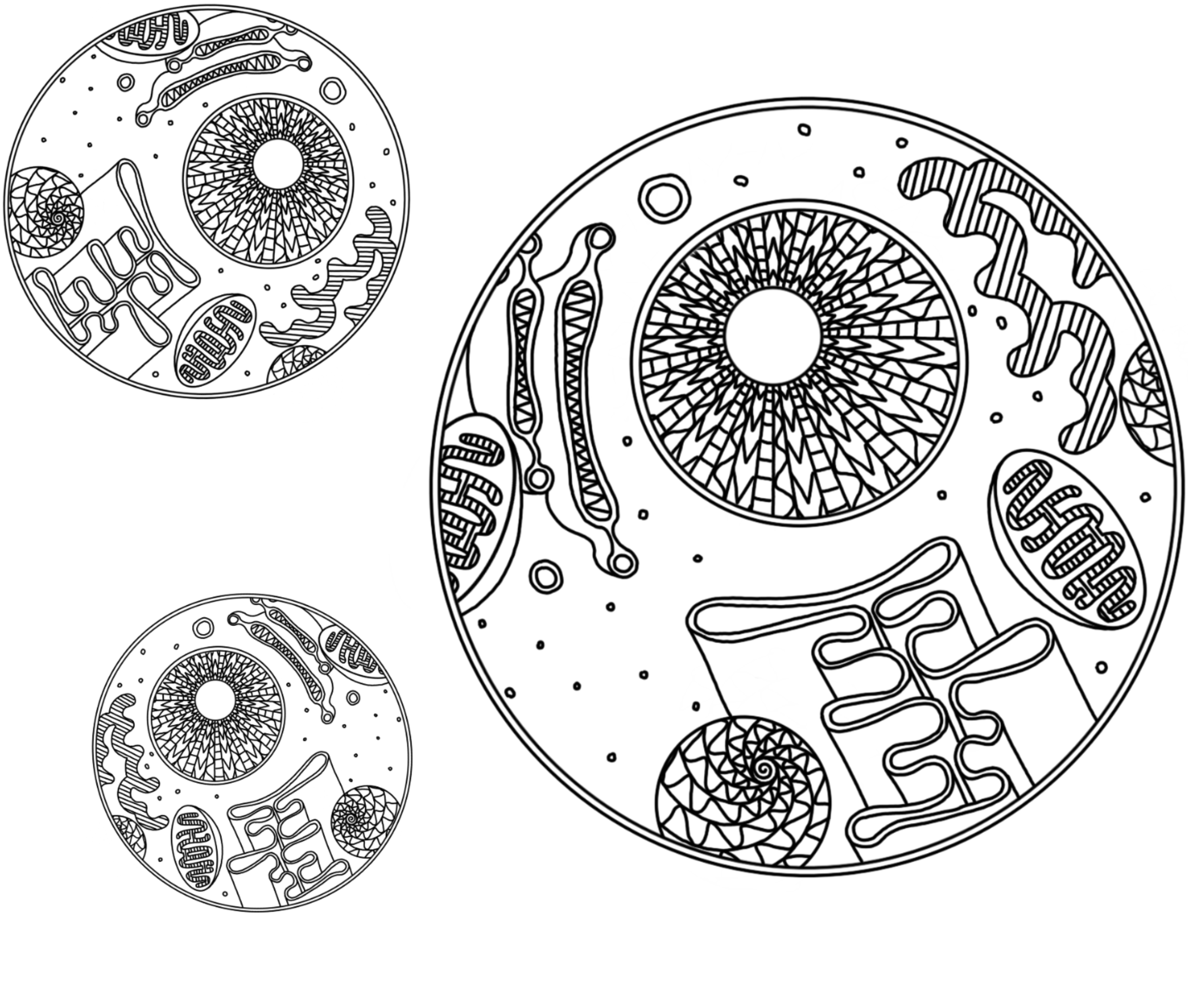
Back Row: Helena Schäfer, Yannik Schermer, Linda Müller, Nicolas Freche, Emily Becker
Front Row: Richelle Avers, Sarah Abdul-Mawla, Allyssa Hinkle, Stefanie Heinrich

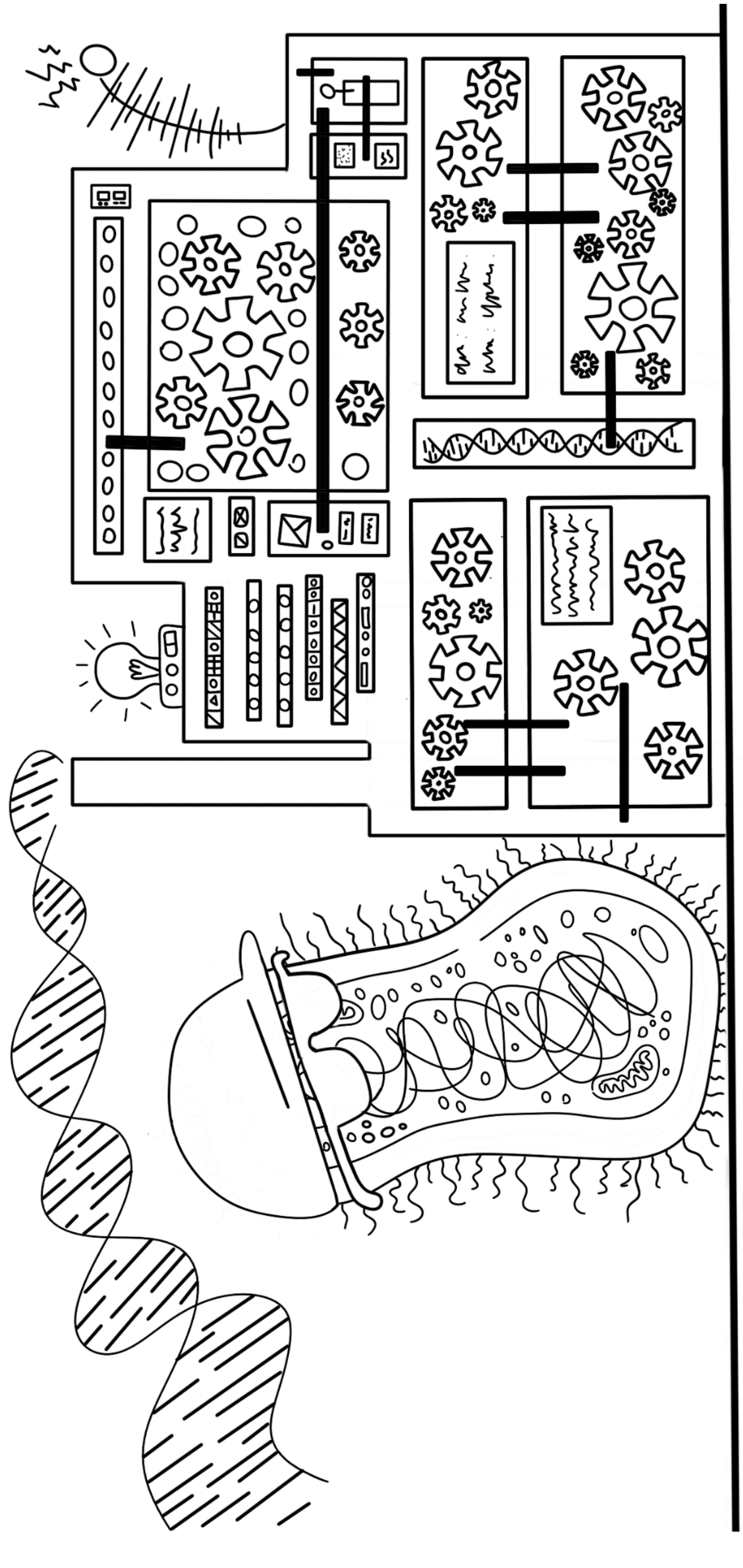
CREATIVE SCIENCE

The Colours of Synthetic Biology

These amazing drawings are part of the collaboration between the teams iBOWU-China and MSP-Maastricht for a children's coloring book. With the aim to spark curiosity on the many wonders of science, we invite you to take part in this collaboration too. It doesn't require fancy lab equipment, just some coloring tools. Be as creative as you witsh, try something new, and most importantly, have fun with discovering the Colours of Synthetic Biology!



The inner structure of a cell. By Angela Zhang / 章屹菲(Team iBOWU-China)



Engineering Bacterium. By Ronja Haikarainen (Team MSP-Maastricht)

In silico design and analysis of peptide inhibitors against *P. falciparum* malaria

Aleena Jose, Anantha Rao, Antony Kiran, Avadhoot Jadhav, Chinmay Patwardhan, Gunwant Patil

IISER-Pune-India, Indian Institute of Science Education and Research (Pune)

***Abstract*- Malaria, a mosquito-borne infectious disease caused by the parasite *Plasmodium sp.* is responsible for over 200 million cases and 400 thousand deaths per year. Current methods of treatment against the malarial parasite *Plasmodium falciparum* have started to fail because of the increasing drug resistance in the parasite population. One way to tackle this problem is to generate drugs based on proteins against the multiple *Plasmodium*-human protein interactions. Through our project we aim to develop a collection of peptide drugs which will inhibit these interactions. Once the parasites develop resistance against a drug chosen from this collection, a new one can be selected to combat the disease. We used computational techniques to design inhibitory peptides against two such interactions (PfEMP1-ICAM1 and CIDRa-CD36). These peptide inhibitors are small and hence are not guaranteed to be stable inside human body. So they will be grafted on another protein backbone called the cyclotides which are highly stable and resistant to degradation.**

***Index Terms*-** Cyclotide, Drug library, Drug resistance, Malaria, Peptide inhibitors, *Plasmodium falciparum.*

I. INTRODUCTION

Malaria, a mosquito-borne infectious disease caused by the *Plasmodium* parasite is responsible for a high mortality rate throughout the developing world with India alone contributing 77% of the total malaria cases in South-East Asia [1]. The most dangerous form of malaria is caused by *Plasmodium falciparum* which replicates quickly if not diagnosed and treated, results in a high rise of infection levels in a short period of time [2]. Over the past five decades, Plasmodium falciparum has gained resistance against drugs like chloroquine, sulfadoxine, quinine, and mefloquine, especially in Southeast Asia. Lately evidences of resistance against our first line of defence, artemsnin, have been reported from South-East Asia with the first case of artemisinin-resistant parasites reported in West Bengal, India in 2018 [3][4]. In recent times, protein-protein interactions (PPI) have emerged as potential and effective targets for medicinal and therapeutic studies [5]. Most functions inside our body are mediated by protein protein interactions. The malarial parasites interact with our proteins and use them in their advantage. PPI studies and modulation has helped to get a better understanding of host-pathogen interactions and in building novel peptide drugs that could inhibit these host-parasite protein interactions. To tackle the problem of malaria in an efficient and innovative manner, we are designing a library of inhibitory peptide molecules for various host-parasite interactions. Using two such interactions, we show that relatively small peptide inhibitors can be designed (~10 amino acids long) and characterised. These peptides can then be grafted on a cyclotide backbone, which are highly stable cyclised proteins, and thus our drugs can be made orally administrable, robust, cost-effective and resistant to degradation [6], [7].

II. MATERIALS AND METHODS

Due to pandemic situation we could not work in our labs hence we modelled the structure of our proposed drugs using computational techniques. These are the variuos methods we used in achieving it.

Selection of suitable interactions:

To model the structure of these peptide drugs we first had to find crystal structures of the protein interactions that we were trying to inhibit to fight malaria. The interaction complexes were obtained from the Protein Data Bank [8][9]. We selected two such interactions for our study, PfEMP1-ICAM1 and PfEMP1-CD36. In both these interactions one part is a *Plasmodium* protein and the other part is a humam protein. The role of these proteins in aggravating the infection is described in the table below

TABLE 1: The host-parasite protein interactions, chosen from amongst hundreds of interactions in malaria databases (PlasmoDB, PDB). Availability of the crystal structure of the host-parasite protein complex was an important parameter in choosing candidate interactions.

Parasite Protein	Human Protein	Function	PDB ID
PfEMP1 (Plasmodium falciparum Erythrocyte Membrane Protein 1)	ICAM-1 (Intercellular Adhesion Molecule 1)	PfEMP1s, predicted to bind to ICAM-1, is associated with increased risk of developing cerebral malaria [12].	5MZA
CIDRa domain of PfEMP1 variant 1 of strain MC	CD36 domain of Platelet glycoprotein 4	PfEMP1 proteins maintain the ability to tether to the endothelium and avoid splenic clearance by interacting with CD36 region.	5LGD

Identification of interacting regions:

To design peptide inhibitors that would prevent these interactions we used the human part of the protein. For this the structure of the interaction was visualised using a tool called Chimera [10]. All amino acids from the human protein that are in colse proximity to the parasite proteins are hypothesized to be the hotspot regions which mediate this interaction. Such continuous sequences of amino acids ie, peptides were identified.

Computational Saturation Mutagenesis and Scoring of Inhibitors:

After identifying hotspot regions, the basic unit of these peptide ie, amino acids from these model peptides were mutated. This was done by taking each amino acid residue in the peptide sequence, substituting them by the other 19 amino acids amd finding their structures [11]. For selecting the best peptide out of all the mutated peptide structuers obtained by single amino acid mutation, binding energy of each of these mutant peptides to the parasite proteins were found using another tool, **FoldX** [12]. Hybrid peptides were also made from the mutants by selecting and replacing each amino acid in the peptide sequence by the mutations having the least binding energy at that position.

MD simulation of PfEMP1 inhibitors:

Simulations can provide the ultimate detail concerning individual particles motions as a function of time. Thus, they can be used to address specific questions about the properties of a model system, often more efficiently than experiments on the actual system. Molecular Dynamic Simulations can be defined as a computer simulation technique that permits the prediction of the time evolution of a particular interacting system. After scoring the peptide inhibitors, the best scored mutants and hybrids were selected for further characterisation by Molecular Dynamic (MD) Simulations using Gromacs version 2019.1 [14]. MD simulations were run for each inhibitory peptide-protein complex from 5MZA and 5LGD for a duration of 80 and 100 ns respectively with a time step of 2 fs on the PARAM Brahma supercomputer (https://parambrahma.iiserpune.ac.in/). The MD simulation for each complex was repeated to ascertain the results obtained.

Analysis of MD simulation results:
Various analysis were done on the MD simulation results such as atomic root mean square calculations, radius of gyration of the complex. To visualise the simulations, snapshots of the simulations at a time interval of 0.5 ns for the entire simulations time were taken. Using these snapshots the distance between the centers of the protein and the peptide was calculated and plotted. We calculated the number of intermolecular Hydrogen bonds between the parasite protein and peptide over the entire duration to quantify its relative abundance.

Grafting of peptides on cyclotide backbone:
The best identified peptide sequence were thus grafted on the cyclotide backbone and the structure was studied for its stability. The grafting was made possible using MODELLER.

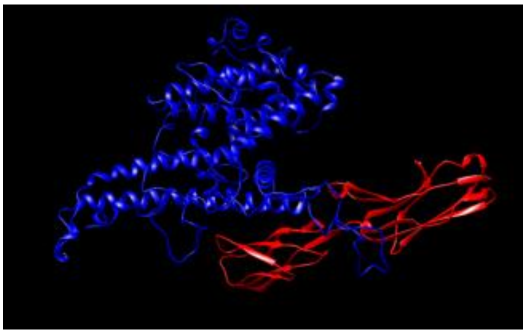
III. RESULTS AND FINDINGS

Identification of Interacting regions:
Peptide inhibitors for PfEMP1 were found using two complexes: PfEMP1-ICAM1 (5MZA) and PfEMP1-CIDRa (5LGD) (Fig. 1). We identified interacting peptide sequences for ICAM1 and CIDRa (Table 2).

Scoring of mutants:
For 5MZA [Appendix 1.2, 1.3] the most negative interaction energy was obtained when serine (S) 16 was mutated to isoleucine (I) (ILPRGGIVL, -8.64 kcal/mol) while for 5LGD [Appendix 2.2, 2.3] mutating serine (S) 160 to methionine (M) yielded the same (NQFVQMILNM, -18.82 kcal/mol). These were greater than the interaction energies of the initial sequences. The interaction energy is the free energy of binding, thus negative interaction energy means spontaneous protein-peptide binding. For both 5LGD and 5MZA the mutants with the least interaction energy scores were selected for MD simulations.

TABLE 2: Chosen Host-Parasite protein interactions.

Interaction	**Sequence (Initial sequence)**	**Residue Number**	**Interaction Energy (Kcal/mol)**
5MZA (wild type)	ILPRGGSVL	10-18 Chain B	-7.45405
5LGD (wild type)	NQFVQMILNS	151-160 Chain A	-16.5112

a. 

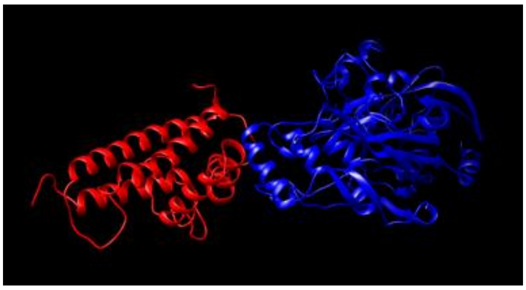
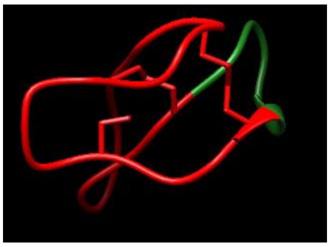
b. 

FIGURE 1. (a) PfEMP1-ICAM1 peptide interaction. Blue represents PfEMP1 and red represents ICAM1 [Appendix 1.1]. (b) PfEMP1-CD36 peptide interaction. Blue represents the

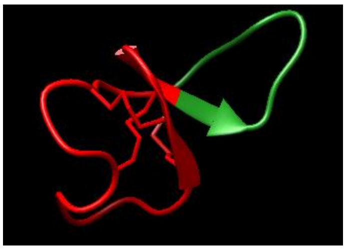
CIDRa domain and red represents the CD36 domain [Appendix 2.1].

MD Simulation:
While analysing the MD results we find that the distance between the protein and the peptide from 5MZA is around 30 A while that from 5LGD is larger than that indicating weak binding [Appendix 1.16, 2.14-2.15]. Hydrogen bonds between the peptide and the protein were also analysed[Appendix 1.10-1.13, 2.10-2.13]. The number of hydrogen bonds in 5MZA were more than that of 5LGD . The difference in the number and nature of the hydrogen bonds can explain the trends in their binding energy.

Grafting Results:
The structure of the inhibitor peptide sequences grafted on a cyclotide backbone are shown below. The region in green shows our peptide of interest.



a. Peptide from 5MZA



b. Peptide from 5LGD

IV. DISCUSSION

Towards designing peptide drugs against candidate host-parasite interactions in malaria, the relevant crystal structures were retrieved from the RCSB database and analysed for interacting host epitopes. 9 amino acid and 11 amino acid long peptides were respectively identified in this manner for 5MZA and 5LGD, which were then subjected to *in-silico* saturation mutagenesis. The mutant peptides obtained in this manner were screened for high affinity towards the parasite PfEMP1 protein. For the 5MZA (PfEMP1-ICAM1) interaction, the most efficient inhibitor (S16I) is found to form two additional hydrogen bonds *in silico* with the *P. falciparum* protein than the wild type. For 5LGD, a mutant CD36 peptide (S160M) yielded the greatest interaction energy. These results must be experimentally confirmed. The high affinity host-mimetic peptides were subjected to MD simulations. Further steps will involve mining databases and analysing interactions like PfRH5-Basigin, an important stage in blood stage of Malaria. To test the efficacy of these inhibitors, we plan to graft them into cyclotides and express them using plasmid vectors and standard biobricks. The circularisation of the cyclotide will be achieved with native chemical ligation (NCL) using the Split-Intein approach.

IV. CONCLUSION

We have described the *in silico* designing of peptide inhibitors against two candidate *Plasmodium falciparum*-human protein interactions. Also the various steps involved in the processing and analysis of peptide interactions- from retrieving structures from the PDB to obtaining the desired inhibitors were discussed. This approach may also be used to generate peptide inhibitors for other interactions, thus further contributing to the generation of the final peptide drug library. Furthermore, one can easily envision that these inhibitors can be developed into orally ingestible drugs using cyclotide scaffolds, for which animal and clinical trials would be necessary.

APPENDIX

Reference graphs and plots can be found here.
Direct link:
https://drive.google.com/drive/folders/11n_gymFqMGhczV2hbLOOqOMKrPAZZfNb

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AFTERWORD

"Alone we can do so little, together we can do so much." – Helen Keller

The first iGEM Muggle Journal exists because of the teamwork and effort provided by each and every one of the participating teams. This would not have been possible without the prolific engagement and enthusiasm each member brought to this initiative; excited to experience what is like to work on a research paper, the authors put forth their best effort, their most valuable content, and all those months of hard work, into a concrete article, to share with the world what they have contributed to the scientific community. The teams have also dedicated the time to help and support other participants by peer-reviewing each other's work, ensuring the quality of the content as well as providing advice to improve their final paper, a key process in the publishing experience that was only feasible because of their commitment to our initiative. We want to thank every team for helping us make our proposal a reality, and allowing us to reach the final product of a collaboration we hope establishes as a tradition in the iGEM competition every year, we truly appreciate your effort.

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AFTERWORD

If any specific article in this Journal has sparked your interest, you can check out the teams Page under the provided link. All their experimental data, detailed project elaborations, and more information on the team can be found on the following wiki pages.

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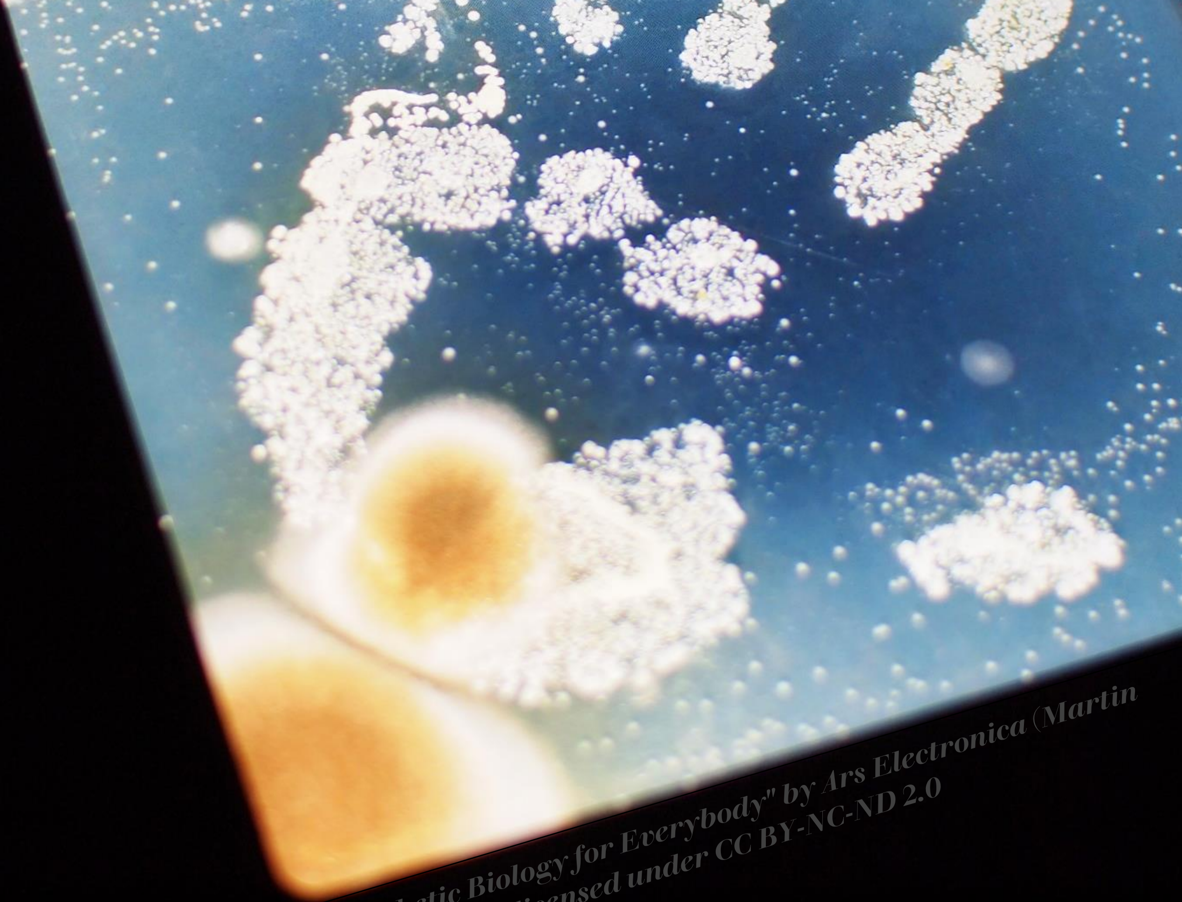

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