HUMAN PRACTICES REPORT

iGEM TU DARMSTADT
2019

INDEX

OUR MOTIVATION

At the beginning of the year we asked ourselves: How do we start our Human Practices approach? It was clear that people may think differently about our project than we do, and that we must consider their opinions. Now, as for us the time with iGEM 2019 is coming to an end, we thought about a way to show everything we learned and achieved and thank all the wonderful people who helped us improve our project. After reflecting the whole year in our team, we decided to write this report to show our journey in the Human Practices and why it is so important to communicate with people of other fields and ages. We hope that this report helps you to relive our experience and retrace our trains of thoughts regarding the improvement of our project and the steps we took to do so.

COMMUNICATION

For us it was important to consider the general acceptance of our project. Therefore, we have left the „science bubble“ and got in contact with the society. There we got to know the fears, impressions and ideas from the stakeholders and possible users. The output influenced the direction of our Human Practices work and the development of our project.

EXPERTS

After we did plenty of research for our project, we thought that it would be helpful to talk with experts of different faculties hoping they could help us improve our “Real Modular Virus-like Particles”. To widely cover the different aspects of our project we talked with many experts of fields like VLP applications, ethical problems behind our project and the safe and economical production of GMO products.

INTEGRATED AND BIOSAFETY

We included the output from our conversations with society and with experts into our project. Using new ideas, we created a modular platform where the modification degree can be controlled. Because of fears in context of the dual use aspect we designed a safety form which could help to prevent misuse.

WE WOULD LIKE TO THANK THE LOVELY PEOPLE THAT JOINED US ON OUR JOURNEY DEVELOPING “THE REAL MVP” AND HOPE YOU ENJOY OUR REPORT.

1. INTRODUCTION:

At the beginning of the year we started to figure out our project and to plan all the tasks and milestones in the wet lab. When our project gained more and more shape, and the topic of VLPs emerged, we asked ourselves: How do we start our Human Practice approach? It was clear that people may think differently about our project than we do, and that we have to consider their opinions. Therefore, we collected a lot of ideas from everyone in the team and started to plan events. We contacted a lot of people we wanted to talk with and were happy about the large number of experts and people from young to old who were responding to us and were interested in our work.

So, we set out and started our outreach, talking with fascinating people from all different fields of society and science. Although many of them could not always get all the details of our project, the discussions during the year were always interesting and intriguing. We really learned a lot through our Human Practice work, both inside and outside the lab.

Now, as for us the time with iGEM 2019 is coming to an end, we thought about a way to show everything we learned and achieved. Also, we were honored by how many people were happy to help us which is why we wanted to find a way to thank all of them. After reflecting the whole year in our team, **we decided to write this report** to show everyone how many new experiences we made and what big of an opportunity competing in iGEM is. With this report we want to show our journey in the Human Practices and why it is so important to communicate with people from other fields and ages. We hope that with this report, it gets easier for you to reconstruct our trains of thought and see the impact of the Human Practices work on our project. This report should help you to relive our experiences and follow our development throughout the year with iGEM and the wonderful people who helped us realize our ideas.

2. OUR PROJECT: A SHORT REMINDER

For our project we decided to use the exterior of the *Salmonella typhimurium* bacteriophage P22. The P22 Virus-like particle (VLP) is well characterized and has been used as a scaffold for modification before[1]. To assemble the P22 procapsid, so-called coat proteins (CP) and scaffolding proteins (SP) are needed. They ensure the self-assembly of the VLPs. After *in vivo* synthesis of the VLP, it can be modified on the outside through various methods, for example via an enzyme called sortase. This transpeptidase covalently binds the protein of interest to the VLP if both protein and CP have matching peptide tags.[2] Based on the publication about P22 modification with sortase we are going to mark our coat protein with a LPETG-tag via genetic engineering which can then be modified by the enzyme. As mentioned before, the nanoparticles cannot only be modified to present something on the outside but can also contain cargo on the inside. This could be achieved by designing a fusion protein consisting of the SP and the desired cargo. Our goal is to design a bacterial strain that expresses the VLP. After assembly the antigen or another cargo will be attached by the **Sortase A7M**. This bacterial strain will be easy to modify so that the antigen or cargo on the interior or exterior surfaces can be changed quickly depending on the desired utilization.

3. VLPS – WHERE DO WE STAND RIGHT NOW?

As a starting point, we wanted to get an overview of the current situation in the society we are trying to implement our project in. This involved research about the historical and the legal situation. We started with the application of Virus-like particles (VLPs) as vaccinations since it is the best investigated option. During our research we found out that vaccinations have been around for roughly 230 years[2], but still infectious diseases for which there is neither a way to prevent, nor to treat them, continue to harass humankind. Vaccinations are a controversial topic in society due to the growing community of anti-vaxxers. The WHO even declared anti-vaxxers to one of the ten biggest threats to global health. We wondered what we could do about this situation and decided to reach out and talk to as many different people as possible to better understand where those fears might come from.

Because of the aforementioned lack of disease treatment, we set out to develop a platform that can – for example – be applied as a tool in vaccination medicine for such diseases. However, instead of addressing vaccinations directly, which would require clinical testing for several years – something that lies beyond the scope of an iGEM project – we aimed to design an easy to use, highly modular platform based on modified Virus-like particles (VLPs). VLP provide a simple base for many possible applications. Besides functioning as vaccines, they can also be used for other purposes (e. g. targeted drug-delivery). Importantly, the range of possible modifications appears to be very broad, thus enabling everyone to modify our system to their liking. This also simplifies the legal situation. Usually a new medicine has to pass several steps of testing which require a lot of money and at least 9 years[3].

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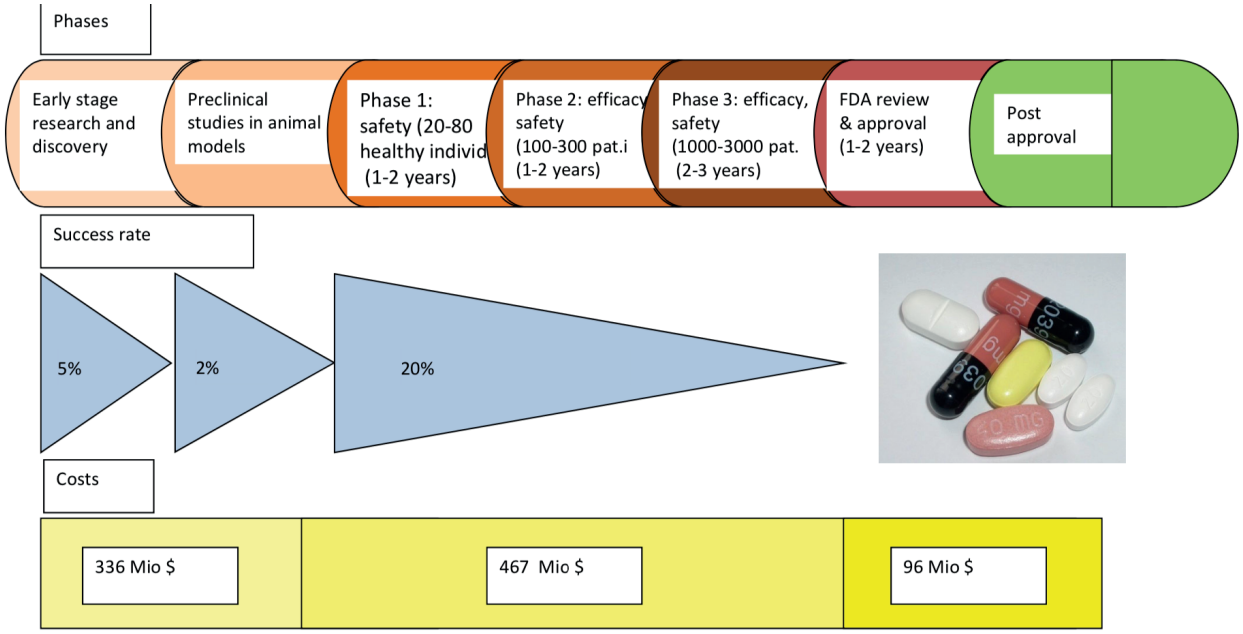
[1] Dustin Patterson et al., Sortase-Mediated Ligation as a Modular Approach for the Covalent Attachment of Proteins to the Exterior of the Bacteriophage P22 Virus-like Particle, Applied Bioconjugate Chemistry 2017 28 (8), 2114-2124 (DOI: 10.1021/acs.bioconjchem.7b00296)

[2] Alexandra Minna Stern and Howard Markel, The History Of Vaccines And Immunization: Familiar Patterns, New Challenges, Applied Health affairs mai/june 2005, https://doi.org/10.1377/hlthaff.24.3.611

[3] (https://www.who.int/emergencies/ten-threats-to-global-health-in-2019, 08/26, 4:38 pm)



Source: Cath O'Driscoll, Nature 2007

Overview of the costs and time needed for the development of new medicine.

This could be avoided by using our platform as a basis that has been approved beforehand. Although the details of these processes are hard to predict, such a platform could speed up the process of developing new vaccines as only the antigens on the particle have to be tested but not the particle itself. Likewise using our platform would make it easier to vaccinate children with mixed vaccines using only our particle which also supports the legal situation. The US does not have a vaccination law but all 50 states have laws requiring children to be vaccinated against diphtheria, tetanus, pertussis, polio, measles, rubella and varicella when entering school[1].

Another possible application for our VLPs is targeted drug delivery. The background of this application is that medication should be directed to diseased tissue without harming potentially healthy tissue in the rest of the body. At the moment the most used vehicles are liposomes or artificial DNA structures.[2][3]. Of course, this also needs to be approved by federal institutes and using our particles as a basis, this process might be accelerated.

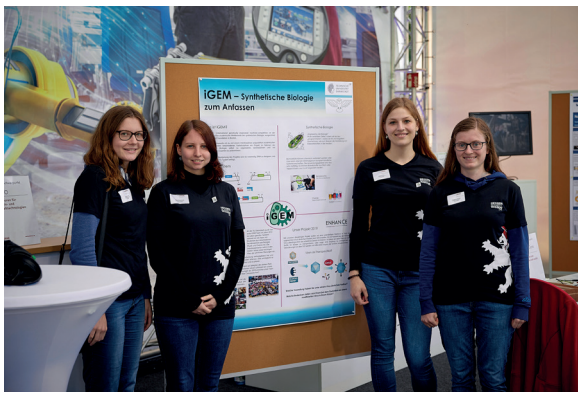
[1] (https://vaccines.procon.org/state-vaccination-exemptions-for-children-entering-public-schools/, 09/14, 11:05 am) Author: ProCon.org, State Vaccination Exemptions for Children Entering Public Schools, Last updated on: 7/26/2019

[2] Melody A Cobleigh et al. , A phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer, Seminars in Oncology, Volume 30, Supplement 16, October 2003, Pages 117-124, https://doi.org/10.1053/j.seminoncol.2003.08.013

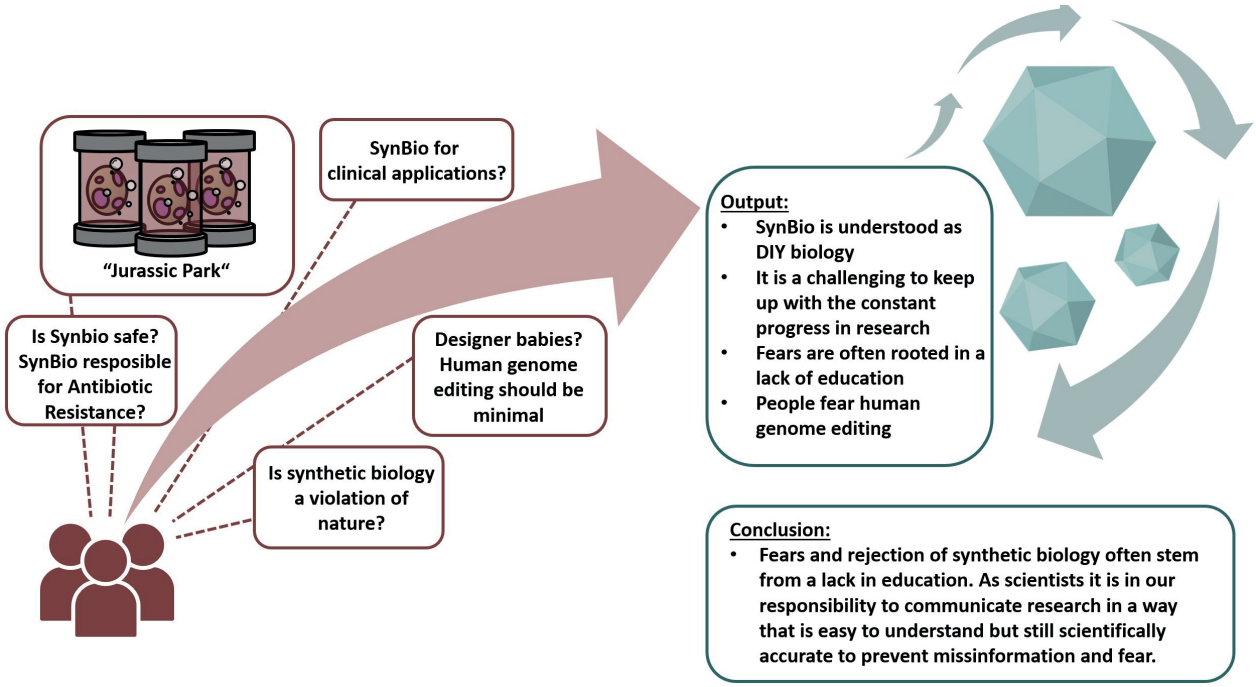
[3] Ebbe S. Andersen et al., Self-assembly of a nanoscale DNA box with a controllable lid, nature International journal of science, published 07 may 2009, Nature 459, 73-76 (2009), doi:10.1038/nature07971

4. FIRST STEPS: HESSENTAG

After collecting these background pieces of information, we were particularly interested in the impact our work with Virus-like particles (VLPs) has on society. As we wanted to get in contact with people of different ages to discuss synthetic biology and our project, we went to the annual "Hessentag" in Bad Hersfeld. It is a cultural festival happening in the state of Hesse (Germany) where traditions and modern lifestyles are linked. There are concerts, games and booths of non-governmental organizations and associations who are presenting themselves to a vast and diverse audience. Each year, thousands of people are coming to the "Hessentag" from many age groups with different levels of education regarding synthetic biology. Our main goal was, to explain what synthetic biology is about and which opinions people have about that topic. We wanted to gather some interesting ideas for making further progress on our project and to expand the knowledge of the people. We went there with some other members of the TU Darmstadt under the initiative "Hessen schafft Wissen" (Hesse creates knowledge), which wants to advertise Hesse, especially Darmstadt, as a city of science. To encourage the dialogue between us and society we were glad to accompany them on the "Hessentag". The research promotion program „ProLOEWE" which is part of the initiative „Hessen schafft Wissen", invited and sponsored us, so we could go to the "Hessentag". On our booth we showed what iGEM is about and explained the meaning of synthetic biology with a poster. We also talked about our project and some ethical problems in the context of science.
Here you can get a quick overview of our impressions and what we learned:



Team members in front of our poster



We had an interesting talk with a German politician, Sabine Bächle-Scholz, and her husband. During the conversation her husband stated that “synthetic biology” sounds paradox, because synthetic appears to him as something artificial and biology as something natural. Next, he asked us about the borders of science and if something like the reproduction of dinosaurs like in the movie “Jurassic Park” could become reality. The movie seemed to be a concrete connection between synthetic biology and the society but it also shifts the expectations of amateurs about genetic engineering. This example points out how people may gain fears they tend to justify based on knowledge extracted out of media. We hope that our explanations could clarify some of those prejudices, but this experience showed us that there is a lot of work left until synthetic biology becomes generally accepted in society as a chance of progress. Other conversations also lead to the impression that even people who studied scientific subjects handle the topic ‘synthetic biology’ with caution.

Explaining our modular platform

After all these talks on the first day we started to think about why people are so afraid of synthetic biology. One of the first answers that came up to our minds was, that they don´t know much about this topic. This guess was confirmed as someone argued that genetics is a fast-developing topic that has changed very much in the last few years. It seems that the constant progress in research has become overwhelming even to someone who studied something in the field of biology.

During our further Human Practice work, we will continue to deal with the issue of fast-changing research and because of that a growing lack of education. In our opinion, this is a point that needs to be considered more closely in order to bring society into harmony with science which is why we want to visit schools to educate more about those topics.

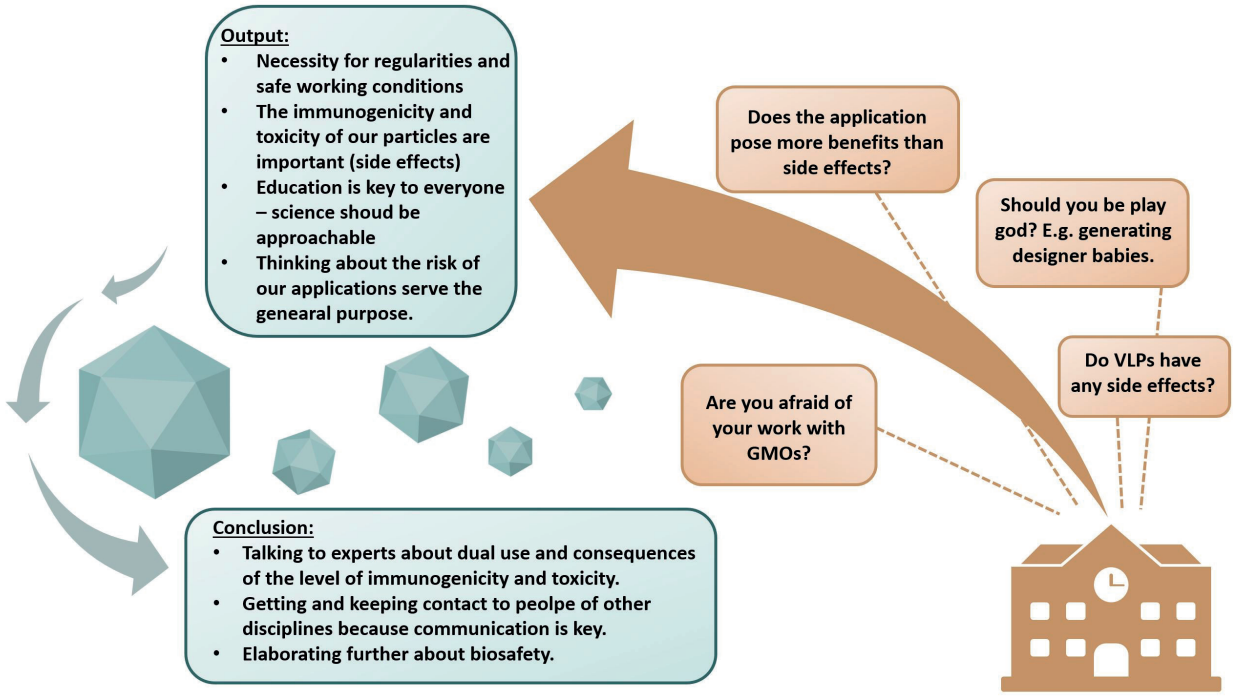
We were interested in what kind of application the people would think of for a VLP. One person immediately thought about clinical applications like vaccinations against HIV and influenza. There were many other ideas and we were not astonished since the flexibility of our modular platform is a huge aspect of our project. But one person also voiced particular fears about topics like designer babies which is why he asked us whether our intervention in the genomes was minimal. Another fear someone mentioned was that resistant organisms could get into the environment. We concluded that we should explain more about safety forms and how we deal with this. At this point we thought that for most people it may seem to be some unregulated “magic” that happens behind closed doors, with no thoughts about the consequences whatsoever. We need to open the conversation about those issues to the society and must not exclude them at scientific meetups and congresses.

Meeting Mrs. Bächle-Scholz

Something we also took from the “Hessentag” was to improve our explaining skills. Especially one term was very helpful. A young man studying in the field of information technology called synthetic biology: **“biology to build yourself”**. That is a very nice term to explain the topic with things that are closely related to society. Everybody knows building blocks. When kindergarten children came by later, we used the new metaphor to explain to them what a cell is. This worked very well and we will keep this metaphor in mind for future conversations. All in all, we have achieved our goal to get into conversations with people of different ages, from school age up to the retirement, and different experience levels (from only some biology lessons at school to a microbiology Ph.D.). During those conversations we expanded the knowledge of the people and got useful arguments for our future Human Practices work. We also got some helpful input, which we can use in our further research.

5. EDUCATION IS KEY: VISITING SCHOOLS

At the "Hessentag" we learned that **education is key to everyone.** Therefore, we wanted to go to schools and share our knowledge about synthetic biology and the perks of studying at a university. We visited multiple schools, one of them which was the Georg-Büchner-Schule in Darmstadt where we met a high-school class that has biology as a main subject in their A levels. Being there we held a presentation about iGEM and synthetic biology in general and later on discussed our project with the class. After giving some input and basic information in our presentation an interesting discussion arose as some pupils had the opinion that synthetic biology is dangerous and hard to control while others stated that this might be the future way to solve most of humanity's problems.



One of the concerns that came up was that this might be the tool for humans to "play god" considering the upcoming discussion about designer babies because of the recent incident in China . The class further agreed on boundaries regarding the possibilities of genetic engineering. They wanted even stricter laws for engineering human cells than for changing plant or bacterial cells because they thought that consequences are not really foreseeable. If more humans are healed by genetic engineering the problem of overpopulation could get even bigger. This could lead to engineering more plants to generate more food which ends up in a vicious circle. Because of this, the students argued that it would be more helpful to pay attention to the cause of the problems than to solve each one by itself.

[1] (Chinese Academy of Engineering calls for actions on the birth of gene-edited infants; Chen Wang, Xiaomei Zhai, Xinqing Zhang, Limin Li, Jianwei Wang, De-pei Liu; www.thelancet.comVol 393 January 5, 2019)

Referring directly to our project they had various ideas for medical applications that seemed to be touching for young people. Most students proposed therapeutic applications of which most of them were targeting cancer, HIV or diabetes mellitus. Besides all the good ideas the students had, they were also concerned that our modular platform could be misused or that it would not work as planned. Some commented that the labeling of the Virus-like particles (VLPs) could be incorrect if they are used for drug delivery and therefore the wrong cells in the body could be attacked by our system.

→ The discussion sensitized us for possible side effects and that we need to answer open questions about the immunogenicity and toxicity of our particles. As a direct result of the school talks, we began to reach out for experts like Dr. Stefan Schülke to talk about our VLPs.

Another topic that seemed strongly emphasized by the pupils was the issue of dual use of our modular platform. Although they had many concerns about the topic of synthetic biology and its applications, in the end they agreed that it would be better to take those risks if our VLPs were able to heal illnesses that are life-threatening or have worse the side effects than our VLPs themselves. Nevertheless, they still reminded us that it is better not to attack essential functions or organs of the body. All in all, most students said that they were not too afraid of the side effects of our project because they were used to normal vaccinations that are based on dead cell material. On the other hand some still preferred oral intake over injection into the body. Most students feared that after making our results public in the iGEM competition some people might misuse the modularity of our system.

→ After the input of this vivid discussion, we later decided to contact Prof. Dr. Sybille Gaisser who is an established scientist in biotechnology, recombinant pharmaceuticals and philosophy. We want to further discuss the aspects of dual use and the possible misuse of our platform with her.

In the end some of the students asked **whether we were afraid of the work we do**. We were taken by surprise by this question as we never thought about the option to fear our work but after reconsidering the discussion with the students, we saw that some aspects of our daily work should be thought about and treated carefully. Working with GMOs every day might have consequences that cannot be foreseen if the safety standards are not given enough attention. We think that some healthy respect for the dangerous sides of our work should be in our minds but fearing the work we are doing will stop the spirit of all life sciences.

Since the first presentation at a school gave us a lot of new input and we wanted to know more about the opinions of the younger generations, we decided to visit two other schools near Darmstadt. We contacted the former schools of some of our team members which also gave us the opportunity to bond with the students on another level and show them that **science is something reachable** for them. Science is a process and synthetic biology is now evolving rapidly. Future scientists are now in school. Therefore, it is necessary to wake the interest of them who are studying in schools at the moment. A few years from now, they have the opportunity to make their own research. Because of that, it is crucial to confront them with the necessity to think about ethical questions and dangers of big developments. The more these issues are discussed in an early stage, the better the young scientists are prepared for their work.

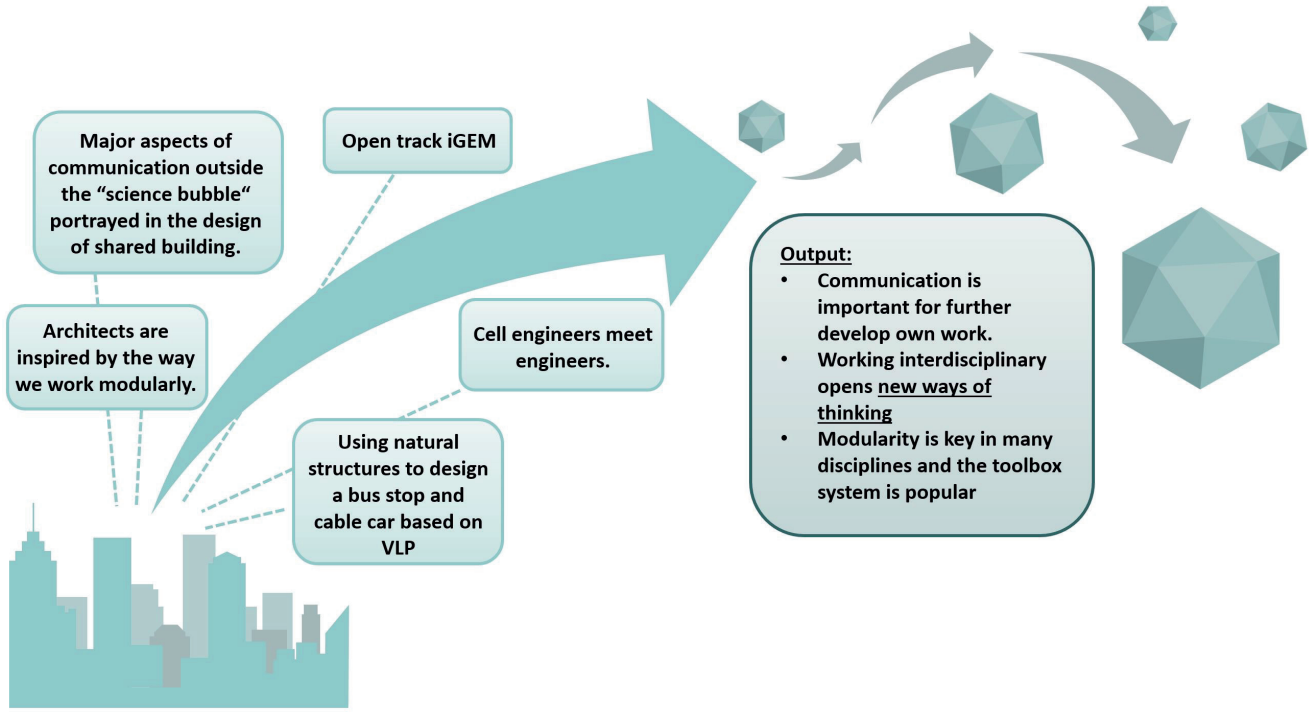
Therefore, we were very excited to welcome a class from a border school in Brazil that is mainly teaching in German. They were visiting the TU Darmstadt to be informed about the studies in Germany and wanted to be shown around our laboratory as they were particularly interested in the opportunities for practical work at our university. We told them about our project and the benefits of participating in iGEM. We were happy to see that so many young people are interested in our work allowing us to share our compassion with them.

6. MOLECULAR ENGINEER TEACH REAL ENGINEERS: INSPIRED

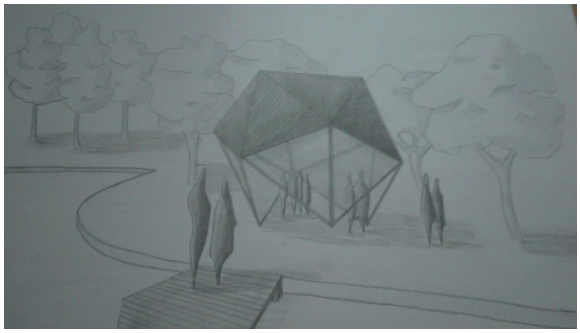
After talking with many people outside of our scientific world, we also wanted to communicate with other scientist as interdisciplinarity is a big issue in the iGEM community. Therefore, we wanted to broaden our minds by working together with scientists of different fields of our university. Under the motto: "cell engineers teach real engineers" we were supporting two groups of thirty people each consisting of mechanical engineers, material scientists, biologists and engineers in their lab work. We enjoyed showing how the golden braid system works and to see the positive outcome of their cloning. The comparison between the way of working as an engineer with machines and the way we work in our lab with "biological machines" was very helpful. Both modes of operation follow a certain protocol but differ in the way of thinking.

7. INTERDISCIPLINARY WORK: ARCHITECTURE

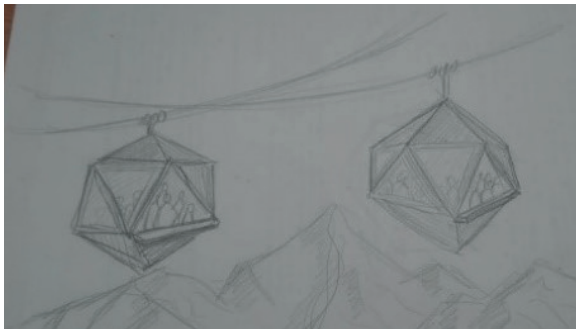
The work on our project had a huge impact on our daily life, so many conversations at home turned around iGEM. This leads to a lot of ideas coming from our families and friends. As we want to reach as many people as possible with our Human Practices and leave an impression, we were excited to hear that some of our loved ones were inspired by our work.



One of our team members who is responsible for our design was approached by a friend. She asked whether it would be okay to use our design for a project of hers. She is studying architecture at the TU Darmstadt and had the assignment to use a design of nature for her next building. After telling her about our project in more detail she sent us two drafts of the first ideas she had.



Design of a bus stop as a VLP



VLP design used for a cable car

We thought that the use of our capsid design as a cable car is very interesting since one of our possible applications is drug delivery. **We were delighted to see that our project inspires other people in their work.** Another aspect that made us think is the design as a bus station. It was not directly fitting to one of our possible applications but we thought it shows the inspirational core of our project that it is meant for everybody.

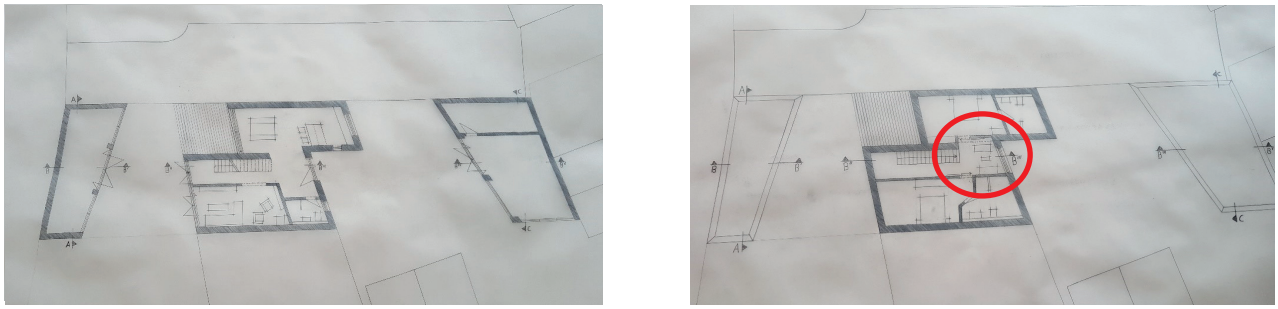
Nico Kühn showing his project

Another very surprising project for our Human Practice team was made by Nico Kühn, the brother of our team-member Angela. Nico is studying architecture at the "Hochschule Darmstadt - University of applied science". He told us, that after hearing about our work from his sister he felt inspired for his upcoming project. At first, we wondered, how our project could influence architecture, so we asked him and he explained:
In his current semester is a course which is called: "P2-country relish. Living and working in one place". The assessment was to design a place to live and work for a scientist and his/her family on a farm. Nico decided to work with the example of an ornithologist who would just be working in summer which is why he decided to implement another scientist into his house to work during the whole year. At this point Nico said he got inspired by our team and its work. Just like our team is inter-

disciplinary and therefore able to easily learn from each other, the scientists can cooperate and exchange ideas about their work. Our communication and discussion gave the impulse for these ideas through showing that conversations with others are important for the realization of one's own work.
This led to him thinking of an exchange between groups of different knowledge and the positive influence on the projects of the scientists.

From our working conditions Nico knows that there are special requirements for working and living spaces in the lives of scientists. One example of our work is the possibility to work sterile. Therefore, he decided that it would be best, when each scientist has his own working area which then left him with the question where the two scientists could meet each other during the day. The houses for working should be clearly separated which makes it the only option to interchange in the living areas.

For this problem Nico again found the answer by looking at our team. When we do our Human Practices work, we leave the lab and talk to other people. Our working place in the lab area is also divided into a defined lab space and a room for writing and chatting. Most conversations take place at the room next to the working area or when we meet other people outside the lab. The main exchange happens on collective property, such as corridors or staircases. Because of that, Nico got the idea for the concrete design of the living areas in one house.



Blueprints of the architectural project. The shared floor is marked.

In the middle of the two floor plans is the shared living house. The bedrooms are separated but the stairwell has to be shared and it is designed spaciously with an area including chairs and tables to provide space for exchange between the inhabitants in their daily routine which is shown in the red circle.

All in all, the finished design of the farm includes two working houses and one living house. **The project is called "inter living" which also stresses the aspect of communication between the scientist and their families.**

In general, we can say the architecture project is driven by the idea of exchange with society and the willingness to incorporate completely different ways of thinking into one's own work to improve the own project. This is highly inspired by the work we did over the year and we are proud of the level on which we were able to reach and inspire other people. **We**

are very excited, that we have such an impact on our environment that even a house was designed based on our work.

Project overview of the project „inter living“

DIGITAL DESIGN UNIT

The constructive exchange with Nico made us aware that architecture and biology may have more in common than we thought. Strikingly, we met another architect shortly after who confirmed this. As a big aspect of iGEM is the focus on interdisciplinary work, we were happy to collaborate with a working group of the faculty of architecture at our university called the "Digital Design Unit". One of their Ph.D. candidates (Bastian Wibranek) approached us after he overheard some team members talking about our project on the train on their way home. He wanted to know more about our work and thought we could seek some inspiration in each other's projects. Particularly fascinating, their working group also works with modular systems based on variable building blocks – in their case flexible changing bridges and buildings. Just as we do, they want to establish a toolbox useable for all architects. We agreed it would be a great idea to visit their offices and tell them about our modular system. We visited them and held a presentation about our work, focusing on modularity and the similarities between our work and theirs.

Afterwards, we had a fruitful discussion about the aspects of interdisciplinary work and the advantages of it. One of the members of the digital design unit stated that he thinks it will get more and more important to talk with experts of different fields to optimize their own work. **Through this talk we once again realized how important the interdisciplinary work and the communication with people outside of our field is.** Regarding synthetic biology, he thinks that it might be helpful to look at the natural designs and copy them with slight changes. Though, he stated that it is hard to exactly copy designs of nature since their structures are very complicated and it might not be possible to upscale the natural design. They are currently working with a machine that enables them to freely mold wet concrete while it is drying. This concept gives them the opportunity to produce bricks of all shapes which makes it easier to copy stable structures from nature and use them for sturdy buildings.

Team member Johny gets explained the concept of modular buildings

Hearing about our project also inspired them to think about copying mechanisms that work in a cell. They are developing a robot that can put defined bricks into every shape needed just like some enzymes put molecules in the right order to synthesize a bigger construct. This is very similar to Virus-like particle (VLP) assembly: VLPs are built out of small and simple proteins that all have the same shape and size but assemble to a rather big particle considering their size. Their view on the cells as machines that work precisely, with proteins being simple modules that can be put together, sounded so familiar (**iGEM: Genetically engineered machines**), that it made us think what other similarities or differences might lie in architecture and synthetic biology. Hearing them talk about copying designs and mechanisms out of nature, we wondered why those systems are not established yet. Mr. Wibranek then explained to us that in architecture they also have to consider the esthetic of the design and not only his functionality. This reminded us of the Open Track of the iGEM competition, where esthetic is a big aspect in the artistic designs. To a certain degree, this may take away some of the modularity that could be achieved by copying systems like ours. Moreover, they explained they have to design the buildings and bridges by hand which removes the factor of randomness in their work. On the other hand, in our synthetic biology lab it is sometimes key to include randomness in processes but we are also designing parts and even whole organisms based on what we want to achieve. Therefore, esthetic plays a minor role, however, iGEM has pioneered in this direction by establishing the Open Track, specifically the art and design track.

A strong similarity we saw was the way of working, on which we agreed during the discussion. Just like we do they work with computers to design as many stable bricks as possible and change as many parameters as possible to test their buildings. We do the same by choosing a specifically designed enzyme in advance and then changing parameters until we get the enzyme that does exactly what we want it to do. We call this process directed evolution. When they heard of this concept, they were inspired to transfer this way of working into their project. They discussed about “evolving” their buildings by modeling the possible conditions with their software and then choosing the best set of bricks to use in further tests. This also gives them the opportunity to imitate natural selection. One of the attendants commented on this process-oriented way of working. He argued that the creative aspect the work in architecture brings will be lost by optimizing buildings with computational modeling. We could understand this point of view and agreed that the process could only be used on buildings that have to fulfill certain parameters that do not include esthetics.

Mr. Wibranek explaining the benefits and downsides of copying nature

All in all, by visiting the working group of the „Digital Design Unit“ we learned a lot about process-oriented thinking and varying parameters to achieve your goal. We think that this might be useful to optimize the production and the properties of our VLPs to being more economic and stable. This collaboration also inspired us to think about how we can further establish a system that is based on “bricks” that can be combined in different ways so every scientist can base their work on toolboxes which will make it much easier to cooperate in the life sciences. The exchange of our projects once again encouraged us to work on our modular platform. Modularity seems to be a key aspect of future projects in all kinds of disciplines. It will get more and more important because modular platforms like our VLPs or the bricks the digital design unit uses will save money and time if established as a basis to work with. We were thankful for the fruitful discussion and once again were delighted to work with people of other disciplines. Thinking outside the box always is a major source for inspiration and problem solutions.

8. WHAT ABOUT THE SOCIETY? ETHICAL ASPECTS

Sibylle Gaisser:

- Professor for bioethics and biotechnology at the university of Ansbach since 2010
- Worked at the Fraunhofer Institute System and Innovation research for ten years
- Led a working group for assessing socioeconomic and ethical aspects of new technologies
- Was project manager of the TESSY project

- Create a safety form for risk evaluation
- Think about biosafety and realize safe working conditions.
- Open up dialogue and leave scince bubble
- Think about toxicity and immunology

→ Contact the PEI and talk to Dr. Schülke.

We had gained a lot of inspiring input from society and people of various age and education levels. But some professional insight concerning the depths of our project was still lacking. By the time we started with our first preliminary experiments in the lab, it became clear we had to talk with experts in our field.
First up we wanted to tackle the ethical problems of our project, so we decided to talk to Prof. Sibylle Gaisser. She led a number of projects on emerging technologies in life sciences where she was responsible for evaluating the societal, economic and ethical impact of innovations. In this time, she also worked closely with Prof. Engels who is teaching bioethics in Tübingen. Due to her career shown in the notes above we wanted to talk to her as her knowledge about biotechnology and philosophy is helpful for us to connect our project to the ethical questions the society might be troubled with.
Beforehand, we already noticed several times during the presentations at schools and at the "Hessentag" that our project needs to be discussed in society. Many people were troubled with questions we did not think about because working in synthetic biology is our daily life. In the years 2006-2008 Prof. Gaisser worked with a team on the TESSY (Towards a European Strategy for Synthetic Biology) project which had the goal to develop a road map for the future perspectives in synthetic biology for Europe. Therefore, she also told us about the current situation of synthetic biology in Europe in comparison to the plan they made in 2008. She said that due to the high rate of new developments it is hard to draft laws that are fitting for the current situation. Nevertheless, she thinks that the external conditions are

clearer than 10 years ago and that **we are on a good way to implement synthetic biology into our law system and our society.**
A major aspect she mentioned is that we as scientists of the life sciences have to be aware of what synthetic biology really is and that it is mostly seen as something between the already established sciences which is why it is that hard to draw up laws. Nevertheless, she stated that it is crucial the life science community opens up and intensifies a dialogue with the other disciplines and non-science society. This would help synthetic biology to become both, an everyday topic and something that should rather be controlled by law and regulated than just feared by people. She thinks that we have to leave our science bubble and talk to people who are not directly involved in science. As a cautionary example, she told us that scientists in the 1990s were trying to communicate about genetically modified crops but failed to integrate the society in the discussion, thus leading to the heavy dislike of the green biotechnology in the society we have today. At the same time, we should try to avoid provoking a too emotional discussion as for example Craig Venter did who was printed on the cover of Newsweek with the headline “Playing God”. The article was about the enormous progress that was made in the last years in synthetic biology but with the controversy headline he only tried to achieve maximum publicity without thinking about his integrity as a scientist. In this case, we agreed that the end does not justify the means, and that professionality should never be less important than publicity. She recommended us to inform ourselves about the ethical and cultural backgrounds of the interlocutors as well as their religious opinions. She thinks that the view on synthetic biology might differ from country to country based on their religion and cultural background which is why it is important to find a way of communication that does not leave room for misunderstandings.

At the beginning of the talk we first asked her to state her point of view on synthetic biology. She answered that she sees herself as the critical observer of the recent development of synthetic biology. She thinks that it is really **important to think about the advantages and disadvantages of every new technology** and that we as scientists have to assess whether it serves an important purpose that weighs out the disadvantages.

This directly led to our second question regarding the dual use aspect in science. We were interested in ther thoughts about whether scientists are responsible for creating a tool that could possibly harm the environment and its organisms. She told us that it is really important that one thinks about the possible applications of their invention since the one developing the tool is the one who knows best how it could cause harm. In context with our project this argument is really significant as we plan to design a modular platform for various applications which might be prone to misuse. Nevertheless, she also mentioned that there is no tool without side effects but **synthetic biologists should always have in mind that their science could be misused.** On the other hand, she argued that synthetic biologists are not the first humans to intervene in natural processes but that humans began with that thousands of years ago as they started domesticating animals and breeding them as they wished. She does not think that there is any difference to the work we do. She also told us to keep in mind that although synthetic biology is criticized now, other new technologies are not, pointing out that new development can be accepted by society. When Synthetic biology emerged, it was branded as bad and life scientists were branded as playing god. We now need to work hard to open up the dialogue and redirect the mainstream opinion about synthetic biology into a better one. She thinks we can only achieve that by arguing with the use

of every application for human society. This also means that it is necessary to do research based on the (medical) needs of society.

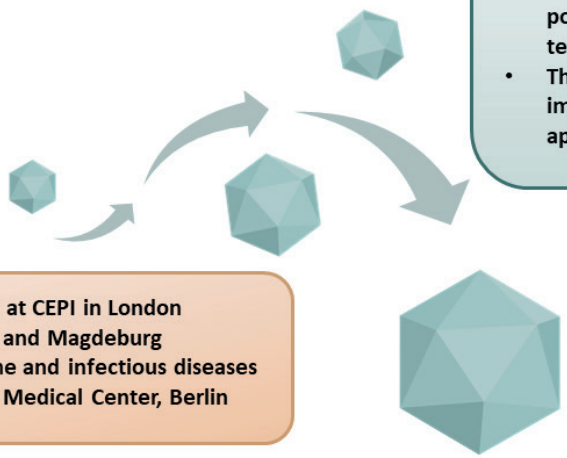
Thinking back to the "Hessentag" we also asked her whether synthetic biology is destroying or restoring the sensitive balance of the ecosystem. She said that most of our world is not in a balance anymore since humans influenced so many processes in the natural ecosystem that synthetic biology will not destroy the balance further. On the contrary, by using processes that are copied out of nature, we could help to get back closer to the former balance in the ecosystem. She told us that there is no difference in the arguments between bringing a plant from South America to Europe, or genetically engineering a plant to have the same characteristics and planting it in Europe. Nevertheless, we should be cautious with bringing GMOs out of contained systems like the laboratory since we cannot know exactly how they will interact with the ecosystem.
A big issue we were quarreling with was that we are developing a modular platform that could be used by researchers for any specific purpose they want. That leaves a great platform for misuse which we already started to discuss after the presentations of our project in schools. Keeping the pupils' questions in mind, we asked Prof. Gaisser how we could prevent some of the misuse. She mentioned that there are already safety forms that need to be sent when ordering DNA sequences of potentially dangerous organisms and that not everyone is able to simply order the genetic information for harmful parts of organisms e.g. endotoxins or certain enzymes that harm healthy tissue. She proposed that we could use a similar system that prevents people to order our parts for the VLPs without giving a reason what they are using them for in case our project develops into a company. With this method of bio-security assessments we could theoretically at least control which institutes are allowed to use our system and what for. Still an independent control layer would be necessary since we are not able to decide which application could be seen as misuse besides the clearly harmful ones.

All in all, she does not think that there are any problems with the "bringing GMOs out of the lab" aspect since our particles are not able to replicate by themselves so we do not have to worry about that. **In the end she also told us that it is crucial to test the immunogenicity and the toxicity of our particles in living systems.** To test these issues, she recommended to visit the Paul-Ehrlich-Institut in Langen. This is a research and control center for vaccines certified by the state. Furthermore, she suggested that we could talk with someone from the Fraunhofer Institute for Interfacial Engineering and Biotechnology in Stuttgart who also works with Virus-like particles (VLPs) and might have some expertise about it.

9. EXPANDING OUR KNOWLEDGE: EXPERT TALKS

9.1 DR. JACOB CRAMER

- Possible usage for our VLP could be a vaccination platform
- Next steps we'd need to take unitil we could establish our VLPs in a possible market are some toxicity tests
- The safety aspect is the most important aspect for an industrial application



- Head of clinical development at CEPI in London
- Studied medicine in Dresden and Magdeburg
- Specialist for tropical medicine and infectious diseases
- Worked in Charité University Medical Center, Berlin

Thinking about the possible applications of our Virus-like particles (VLPs) and wanting to know more about the current state of research PD Dr. med. Jakob Cramer. CEPI (Coalition for Epidemic Preparedness Innovations) calls itself "an innovative global partnership between public, private, philanthropic, and civil society organizations [...] to develop vaccines to stop future epidemics."[1]. Their goal is to "accelerate the development of vaccines against emerging infectious diseases and enable equitable access to these vaccines for people during outbreaks".
Having in mind that a possible usage for our VLPs could be a vaccination platform, we considered that an interview with a representative of an international company like CEPI could give us an idea of the characteristics our product would need in case of further development of our project. Furthermore, we wanted to ask for regulations and steps we would need to take until we could establish our VLPs in a possible market.

[1] https://cepi.net/about/whyweexist/ , 07/20, 14:12)

HOW DO YOU ADVERTISE WHEN YOU BRING OUT A NEW VACCINE?

PD Dr. med. Jakob Cramer (JC): The advertising is the work of the Commercial Department. The most important thing is to get a recommendation so that it is part of the program of medical insurances.

DO "ANTI-VAXXERS" AND PEOPLE WHO ARE AFRAID OF VACCINATIONS PLAY A MAJOR ROLE IN PROJECT DEVELOPMENT?

JC: No, not for my work. But there are people responsible for public relations.

HOW ECONOMICAL WOULD OUR PARTICLE HAVE TO BE TO THEORETICALLY SUCCEED?

JC: The most important things are the cost of goods sales. They contain the costs occurring during the production of the good, in our case during the production of the vaccination. This doesn´t just include the costs of the vaccine itself but the costs of all materials like stabilizations and so on… Everything that is in a dose.

> PROBABLY IT IS THE VACCINATION METHOD OF THE FUTURE.

HOW MUCH DOES THE DEVELOPMENT GENERALLY COST?

JC: This is hard to say. Modeling the development could be a good idea at this point.

WHAT ARE THE PRECONDITIONS FOR A VACCINE UNTIL IT IS READY TO USE? WHAT ARE THE DIFFERENT STEPS?

JC: First of all, you need to do in vitro experiments. The next step would be toxicity tests and experiments with animals. On this point you are testing if a sort of overdosing is possible and what the effects would be. Only if these tests were positive you start to do first compatibility and efficiency tests on humans. The safety aspect is the most important aspect in these research studies. At next you would test different doses and check the immune response. The last step before releasing a new vaccine would be a big field research with a placebo check.

DID YOU ALREADY WORK WITH VLPS AS PLATFORM FOR VACCINATIONS? AND IF SO, DO YOU THINK IT IS WORTH DOING RESEARCH ON IT?

JC: Yes, I did. And yes, VLPs are definitely worth doing more research about. Probably it is the vaccination method of the future. It can work as important adjuvant in some vaccinations.

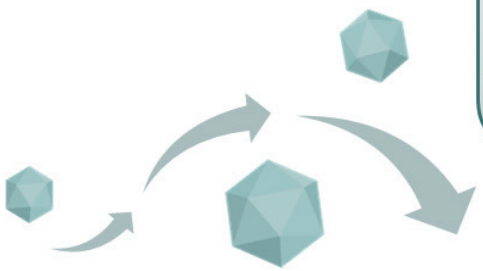
WHAT ADVANTAGES DO YOU EXPECT FROM A VACCINE BASED ON OUR MODULAR PLATFORM USING VLPS?

JC: The big advantage of using a modular platform for vaccinations is that you can do multiple vaccinations simultaneously. This could decrease the percentage of people that are too lazy to visit the doctor multiple times. Besides it could also decrease the money needed for the vaccines overall.

We want to thank Dr. Cramer for his advice. After all, we learnt that our VLPs could possibly be used as a platform for vaccines but they still need a lot of development such as tests for toxins. Furthermore, to actually produce them we would need to find a way to keep the costs low.

9.2 DR. JÖRG MAMPEL

- To analyze the structure of our VLPs we could use electron microscopy
- Need to do future investigations to reach safety standards suited for pharmacological applications
- It is important to communicate our knowledge and work to the public on an accessible and open level



- Studied microbiology at the university of Konstanz
- Project manager of metabolic engineering, metagenome screenings, biofilm formation and anti-microbials
- Heading the technology platform centered on the molecular biology / engineering of pro- & eukaryotic microorganisms

Looking for experts to talk with at the beginning of our project we were happy to encounter Dr. Jörg Mampel at an event in our university. After telling him about our project he informed us that he previously worked with biological nanoparticles. We then visited him at his office in the BRAIN company in Zwingenberg and talked to him about various aspects of our project. Being interested especially in the way our particles assemble and how to purify them after, we asked about his opinion. Based on his experience with bacterial microcompartments he assumed that phage derived particles also tend to assemble into stable compartments in- and outside of the cell. Also, they would most likely not enclose foreign matter without external stimuli meaning that we would have no major problems with accidentally enclosing cell compartments that might restrict the applications of our particles. By using the Sortase as this outside stimulus, we could show both, the activity of the Sortase and the integrity of our compartments.

To visually examine the structure of our Virus-like particles (VLPs), he suggested using an electron microscope, rather than an atomic force microscope (AFM). Electron microscopy would yield adequate results, while an electron microscope operation could be learned and applied by us.

We further acquired Dr. Mampel's knowledge of homeostatic balance of the scaffold protein, coat protein and the sortase. We were especially interested in the application of this balance in vivo. In his work he has found the induction of cells to be more exact and reliable when using arabinose, rather than isopropyl-ß-d-1-thiogalactopyranoside (IPTG). After establishing our modular platform, we have begun testing arabinose as an alternative inducer.

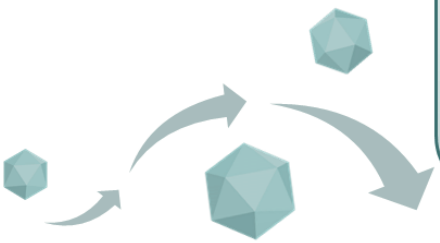
Through his experience in working for a biotechnological company, Dr. Mampel gave us insight into the industrial-level production of therapeutics using bacteria. To reach safety standards suited for pharmacological applications we would need to assess our VLPs for pyrogens. These pyrogens are produced by bacteria and causing a febrile response, like infectious symptoms, in patients. Keeping the importance of pyrogene control in mind we then encountered a lot of people asking us about the immunogenicity and toxicity of our VLPs. This led us to talking to Dr. Schülke from the Paul-Ehrlich-Institut in Langen to whom we were referred to a lot by other experts in the field of vaccines and VLPs.

Dr. Mampel stressed the importance of communicating our knowledge and work with the public on an accessible and open level. He found that scientists often tend to live in a science bubble. The public's concerns might not be seen in their importance, as they might be benign to a scientist. We also followed this thought again in our conversation with Professor Gaisser about ethics in sciences. Further, we responded to this problem by talking to students from various high schools, hearing and answering their questions.

[1] https://www.brain-biotech.com/de/blickwinkel/personen/, 06/11, 10:23)

9.3 PROF. DR. SUSANNE BAILER

- VLP as a basic structure, Modularity
- Chemical conjugation with a fusion protein after assembly of the particles
- Modification degree
- Assembly could only be verified via ultracentrifugation and following electron microscopy
- Large-scale production
- To see if our particles are safe to be used in pharmacy, she reffered us to the Paul-Ehrlich Institute.



- Adjunct professor at the University of Stuttgart
- Leader of the Department Biological-Medical Interfaces at the Institute of Interfacial and Biochemical Process Engineering IGVP
- Head of the Innovation field "Virus-based therapies and technologies" at the Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB in Stuttgart
- Research topics:
  - Infection diagnostics
  - Virus-based therapies (oncolytic viruses, Phage therapy, virus-like paricles)

In our previous Human Practices work we have seen that most of the people we talked to, even in our generation, are not averse to a future usage of Virus-like particles (VLPs) developed by us. This leaves the question how we prepare our project for industrial requirements, and how we convince people to approve VLPs.

To see how all these problems are handled in industry and how a project like this would be started, we reached out to talk to specialists from an institute with industrial experience.

During our research about VLPs for commercial application we found that the Fraunhofer IGB also worked on VLPs. Fraunhofer is a German research organization focused on applied research. We wanted to know about the motivation to work with VLPs and how they used and modified them. We contacted the institute and were happy to get the opportunity to talk to Prof. Dr. Susanne Bailer. She is head of the innovation field "Virus-based therapies and technologies" at Fraunhofer IGB in Stuttgart. Among other topics, the group of Prof. Bailer focuses on a project named: "Virus-like Particles - Biocontainer for drug delivery and vaccination".[1] This project is about the development of a VLP which can be equipped with antigens depending on the intended function. Together with her group, she aims to use the **VLP as a basic structure** that is functionalized with a protein of interest. This fits exactly to our project since we started with the same idea of a basic structure for multiple applications including vaccination. Because they already performed more research on the modification and usage of VLPs, we asked Prof. Bailer which problems we might encounter. She explained that their approach is to assemble the VLPs in vitro based on recombinantly expressed fusion proteins, but faced problems with proper VLP assembly. She stated that homogenous

assembly may be hindered by steric changes of the fusion proteins, as observed in centrifugation and electron microscopy experiments. Unfortunately, VLP heterogeneity caused a disruption of their function. Prof. Bailer liked the idea of our native basic structure and the chemical conjugation with a separately expressed protein and saw the potential that this approach may prevent the aforementioned problems. She thinks that the conjugates are less likely to disturb the integrity of the pre-assembled VLP core and allow for a modular and versatile system potentially used for many different applications. Comparing the methods, however, she thinks that pros and cons exist. Conclusively, we think that we have developed a good system for the desired modularity since we are able to functionalize VLPs with proteins more freely than they do by genetic encoding of the fusion proteins.

We also addressed the point of **modification degree**. After starting with our project, we figured that for some applications and for the integrity of the VLP core it might be necessary to control the degree of the VLP surface modification. This might also have an impact on the assembly and the stability of the particle afterwards. The team from the Fraunhofer IGB also thought about this but did not address this issue. They suspected that reducing the degree of modification may contribute to a better assembly in their case because the steric hindrance would be less dramatic, e.g. by mixing fusion proteins with non-fused proteins during assembly.

We also wanted to know what aspects we have to consider in the production of the VLPs. To verify how successful our VLP production is, we wanted to determine the yield of assembled VLPs. Prof. Bailer told us that ultracentrifugation and subsequent scanning electron microscopy can be applied, however, this way we would likely lose some of the product and have just a section of the actual yield. For the future application, for example as vaccines, large-scale production is necessary, therefore efficient synthesis is crucial. The synthesis of the VLPs could be like a classical synthesis: first the protein is expressed and after purification assembled to particles. The resulting particles need to be chromatographically separated and subsequently purified in various cleaning steps to make sure that no cellular or process contaminants, that potentially cause side effects, are present when the VLPs are injected into the human body.

Thinking of what we need to overcome following the purification of our VLPs for the respective application, we wondered how such a new medication is handled. First of all, a pharmaceutical company must be interested in our project, then lots of regularities for pharmaceutical products will follow. It is possible that each type of VLPs needs to get an own approval by state authorities which could cause a lot of work and problems. Prof. Bailer told us that in her perception, the vaccine industry is rather conservative in developing medication. Furthermore, not many VLPs are currently developed and applied as vaccines. Nevertheless, this is just an outlook for our project and the way how the authorities handle it is still in progress, so there is no need to consider it too strongly or be inhibited by this aspect.

Regarding the production of our VLPs we also asked ourselves whether it would be possible to continue to use our current *E. coli* expression system. Prof. Bailer told us, that with respect to the production of a pharmaceutical product, *E. coli* is well suited as an expression system but also mentioned that those are not the only organisms potentially used. The team from the Fraunhofer Institute worked with the yeast *Saccharomyces cerevisiae* as an expression

system in case of the VLPs, however the scientists also see the possibility of using mammalian cells. She especially stressed that working with yeasts like *Pichia pastoris* may allow for secretion of the proteins which would help to preserve the cells instead of destroying them and facilitate the harvest of VLPs from the extracellular milieu.

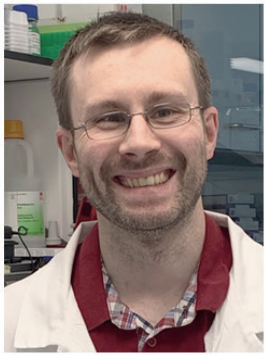
Thinking about other applications besides vaccinations such as drug delivery, we had the idea to add a tag of positively charged amino acids to the inside of the VLP in order to pack a negatively charged cargo. This way, a cargo could easily be enclosed for drug delivery purposes. With a selected target sequence on the particle surface, the VLPs could be transported specifically to the targeted cells and release the cargo at the point of need. Prof. Bailer thinks this should work in principle. She said the simpler the method the more effective and robust it likely is. However, packaging just based on charge interactions might compromise the specificity of the packaging.

One last important aspect we wanted to talk about regarding our previous human practices work was the biosafety and the impact on the health while using the VLPs as vaccines. Prof. Bailer does not think that bioterrorism is the primary problem in the usage of VLPs. But if we want to use the VLPs for pharmaceutical applications, we need to make sure that they will not be destroyed by macrophages when present in the blood after systemic injection. In addition, no side effects should occur during the use which is why we should test the immunogenicity of our product. When used as a drug delivery system it is important to establish a target-focused control. **To see if our particles are safe for use in drug delivery, she referred us to the Paul-Ehrlich-Institut**. There we might have the chance to do some tests to see if the usage of our system causes immunological rejections.

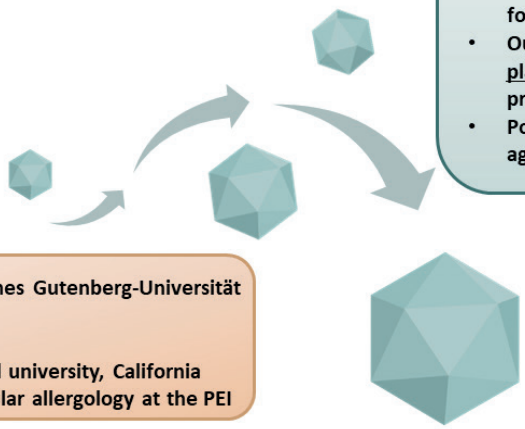
To sum up, it was a great opportunity to talk to Prof. Bailer because we got great input for our project. After the talk we got to know that our project has the benefit that the VLPs are modified after the assembly which should preserve their function. During the finding-phase of the project it was an important aspect for us that the usage of VLPs is currently limited because there is no standardized synthesis that produces the VLPs as a modular system to be used for various applications. [1] We read this on the homepage of the Fraunhofer IGB during our research for the project and it has inspired our project idea as well as it later impacted the implementation. **Furthermore, we can see that VLPs are a topic that will be relevant in the future and additional research will be done to further develop the platform. Many companies and institutes also see the potential of the particle and invest money in** exploring it. Thus, it is possible that our modular system will be of use for pharmaceutical application in the near future.

[1] (Quelle: Fraunhofer IGB:https://www.igb.fraunhofer.de/de/forschung/kompetenzen/molekulare-biotechnologie/infektionsbiologie-array-technologien/therapeutische-viren/virus-like-particles.html 27.09.19 um 10:58)

9.4 DR. STEFAN SCHÜLKE



- Studied biology at the Johannes Gutenberg-Universität in Mainz
- PhD in allegoly
- Visiting instructor in Stanford university, California
- Leader of a project in molecular allergology at the PEI



- Virus-like particles without modifications are not immunogenic because they do not contain viral DNA
- Consequently, they do not pose a healh risk for potential patients
- Our idea of using the P22 VLP as a modular platform would simplify the production process for vaccines
- Potential of VLPs for passive immunization against potentially fast-acting diseases

One possible application area for our Virus-like particles (VLPs) is vaccination. Vaccination is a highly controversial topic in our society and there are many people who are strictly anti-vaccinations because they fear dangerous side effects. As we already noticed at the Hessentag many people fear synthetic biology in general because of its high potential to be abused for biological weapons. To make sure that our particles are posing no danger to the human body or rather conversely to get a confirmation that VLPs have a high potential in the field of immunology we wanted to talk to an expert in the field. As other experts and discussions suggested the Paul-Ehrlich-Institut (PEI) as a good point of contact, we decided to talk to Dr. Stefan Schülke who works at the PEI in Langen, Germany. The PEI is a federal institute for vaccines and biomedical drugs. The regulatory tasks of the PEI include the marketing authorization of particular groups of medicinal products and the approval of clinical trials. Dr. Schülke is a great contact in terms of information about VLPs since he already worked with different VLPs in several cases[1][2][3]. Therefore, he has extended knowledge about both, immunology in general as well as the benefits and downsides of virus-like particles.

The first thing we wanted to know was whether our VLPs could cause immunogenic response without modification on the outside or inside. Dr. Schülke confirmed that Virus-like Particles without modifications are usually not very immunogenic because they only consist of the virus shell but lack the immune-activating viral genetic material packaged within the

[1] Anzaghe, M., Schülke, S. & Scheurer, S. Curr Allergy Asthma Rep (2018) 18: 71. https://doi.org/10.1007/s11882-018-0827-1, First Online25 October 2018, DOIhttps://doi.org/10.1007/s11882-018-0827-1

[2] Patricia Gogesch et al. , Modular MLV-VLPs co-displaying ovalbumin peptides and GM-CSF effectively induce expansion of CD11b+ APC and antigen-specific T cell responses in vitro, Molecular Immunology, Volume 101, September 2018, Pages 19-28, https://doi.org/10.1016/j.molimm.2018.05.017

[3] Katharina M. Uhlig et al., Lentiviral Protein Transfer Vectors Are an Efficient Vaccine Platform and Induce a Strong Antigen-Specific Cytotoxic T Cell Response, American Society for Microbiology Journals, Published online August 3, 2015, https://doi.org/10.1128/JVI.00844-15, PubMed 26085166

[4] doi:10.1038/s41541-018-0082-4 (einfach zur vorhandenen quelle dazu)

particles. Consequently, they should not pose a health risk for potential patients if purified expediently after production. Generally, VLPs already have a history of safe use in the industry which is demonstrated in the fact that the PEI has released a publication concerning Virus-like Particles in conjunction to vaccinations in the past years[4]. Moreover Dr. Schülke told us that there are already VLP-based vaccines authorized for human usage, for example against infection with the human papillomavirus (HPV) or Hepatitis B. Being interested in those already existing vaccines, this input led us to contact the Nobel laureate Prof. zur Hausen later on.

Next Dr. Schülke emphasized the **big potential of VLPs for active immunization against potentially fast-acting diseases.** Such development is in general a lengthy and expensive process. Here, our system could provide a fast and efficient platform to develop vaccination strategies against newly emerging or fast mutating pathogens. Indeed, the time required for vaccine production is one big argument for evolving a modular vaccine platform based on VLPs. At the moment, all particles used in the industry come from different organisms and have variable immunological and biochemical properties which leads to the fact that every VLP used in vaccinations has to pass through wide-ranging, complicated, and time-consuming tests to get approved. Our idea of using the P22 VLP as a modular vaccine platform would simplify this process because the capsid without any modifications would serve as a basic platform for the tests. Consequently, there could in theory be one approved basic construct that could be modified in all possible ways. Nevertheless, it is necessary to test all VLPs as soon as they are modified but this could be way faster than developing and testing new vaccines each time.

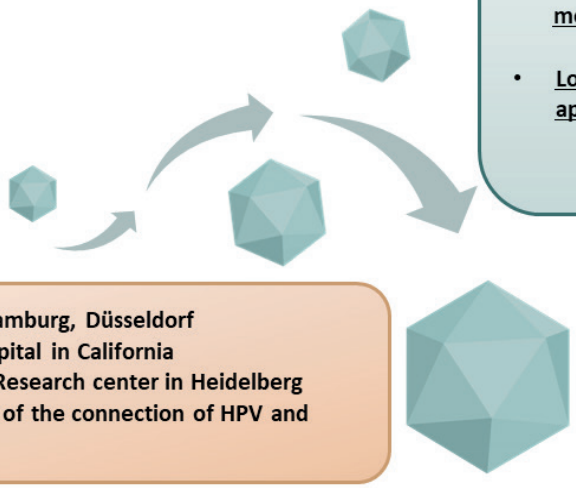
Another topic we talked about was mixed vaccinations and whether our platform is suitable for this purpose or not. According to Dr. Schülke, our modular particles would definitely be suitable for such approaches. Moreover, he pointed out that it would not be necessary to put multiple antigens on the surface of the particles, since mixing and co-administering differently modified VLPs would likely suffice to achieve the desired immune response. Here, the big advantage of a modular platform is that once the general production strategy is established, the creation of multiple differently modified VLPs as well as mixing them afterwards to create polyvalent vaccines is both easy and cost-efficient. This could simplify the process of vaccine production which would lead to a decrease in overall vaccination costs.

All in all, our conversation with Dr. Stefan Schülke really encouraged us in our project idea and confirmed that our concept has a huge potential in the field of immunology.

Tobi and Katha visiting Dr. Schülke in PEI

9.5 PROF. DR. HARALD ZUR HAUSEN

- Studied medicine in Bonn, Hamburg, Düsseldorf
- Worked in the Children's Hospital in California
- Head of the German Cancer Research center in Heidelberg
- Nobel prize for the discovery of the connection of HPV and cervical cancer



- Development of vaccines is a long process

→ could be shortened by the modularity of our System

- Lower costs for industrial applications

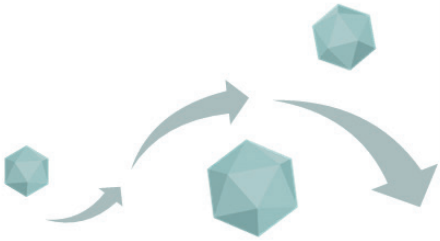
Based on professor zur Hausen's discovery of the role human papillomavirus (HPV) plays in the development of cervical cancer, the development of standardized prophylactic vaccines against the cancerous and most common HPV strains was possible[1]. These vaccines use a Virus-like particle (VLP) designed and produced through recombinant technology. As already pointed out, a possible application of our modular platform is the development of vaccines.
Through our conversation with Professor zur Hausen we hoped to gain insight in the process of the development of a vaccine, the communication of information about vaccines to the public, his thoughts on our project and his experiences in the scientific community.

Vaccines using VLPs may be safer, given the lack of viral DNA and therefore lack of infectiousness. The development of vaccines is a long process we hope could be shortened by the availability of our expression system and enable the addition of desired antibodies in as little as one cloning step.
Zur Hausen spoke to vaccine-costs being high due to the long development and was optimistic about our platform lowering the costs of vaccine-development through shortening the developmental phase.
Further we spoke about the government's role in educating the public on vaccines, the effect media can have on conveying science and securing funding for a research project.

[1] (Karin B Michels, Harald zur Hausen, HPV vaccine for all, The Lancet Volume 374, Issue 9686, 25–31 July 2009, Pages 268-270, https://doi.org/10.1016/S0140-6736(09)61247-2)

9.6 PROF. DR. LUCA SANTI

- Professor of plant virology at the university Tor Vergata Rome
- PhD in genetics at Max-Planck Institute, Cologne
- Assistant professor for biotechnology of plants at the Arizona State University



- Could be necessary to produce and purify VLPs on a large scale
- Purifying the particles via size exclusion increases the capacity
- To screen for the correctly assembled particles we could use ultracentrifugation followed by an ELISA
- It is important to control the modification level
- Control the modification rate by regulating the ratio of tagged and untagged coat potein
- Perform western blots for analyzing the exact particle modification level.

To advance our knowledge about working with Virus-like particles (VLPs), we contacted Prof. Luca Santi from the "Dipartimento di Scienze Agrarie e Forestali (DAFNE)" in Italy. His research focuses mainly on genetic engineering to produce biopharmaceuticals, in particular vaccines, for humans and animals. We were especially interested in his work and experience with VLPs[1]. We presumed he could give us professional insight concerning purification and potential impact on society. The desired application of his VLPs are novel vaccines. He imitates the already existing biphasic vaccines by expressing proteins for a native virus capsid with the fitting antigens displayed on the outside particle surface[2].

When we talked to him via skype, we were especially interested in the way he purified the particles and whether he was able to upscale the process of purification to a commercial level. For a pharmaceutical application it would be necessary to produce and purify VLPs in a larger scale. He answered that the sucrose gradient method was his method of choice at the beginning but there is no way of upscaling this process. Instead he suggested purifying the particles via size exclusion because that increases the capacity. Taking first steps to upscale our project we tried purifying our particles via size exclusion which was very successful, as you can see at our wiki. This question led us directly to the details about his Virus-like particles. As he is using leaves of different plants as expression systems[3], we thought it might be harder to purify them but he explained to us that he is separating the particles from the other cell fragments by ultracentrifugation followed by an ELISA to screen different frac-

[1] Luca Santi et al., Virus-like particles production in green plants, Methods, Volume 40, Issue 1, September 2006, Pages 66-76, https://doi.org/10.1016/j.ymeth.2006.05.020
[2] Luca Santi et al., Virus-like particles production in green plants, Methods, Volume 40, Issue 1, September 2006, Pages 6-76, https://doi.org/10.1016/j.ymeth.2006.05.020, page 7
[3] Luca Santi et al., Virus-like particles production in green plants, Methods, Volume 40, Issue 1, September 2006, Pages 66-76, https://doi.org/10.1016/j.ymeth.2006.05.020, page 5

tions thus selecting the correctly assembled particles.

Fascinated by the idea of synthesizing VLPs in plants, we wanted to know how exactly he worked in his research. Prof. Santi said that he prefers the expression in whole plants as tobacco. We wondered about how long the whole purification process takes from the first working step to the assembled particle and were told that including all purification steps it takes about ten to fourteen days, from constructs to assembled nanoparticles.

One crucial factor for our project is the **level of modification** of the particle-surface. We asked him: Would it be practical to completely modify the surface, or would modification to a lesser extent be more feasible? Using his particle as an example that requires only one protein for assembly in comparison to our which consists of two different, he told us about the benefits and downsides of various modification levels. Of his VLPs one particle consists of 180 coat proteins and he explained, that the fusion has length constraints and they might not assemble if he functionalized all of the 180 proteins with fusion proteins. He transferred this issue to our P22 platform and suggested **that it might not be a good idea to completely modify the whole particle surface.** With less modification, the capsid could probably assemble without any disturbance and the particle would keep its integrity. Furthermore, Prof. Santi told us that the density of modified coat proteins on the surface of the particle is highly dependent on the wished application. For vaccines as an example, he stated that not every single coat protein needs to be modified, in contrary it might harm the function of the particle. **This underlines how important it is to control the modification level.** Moreover, he discussed that a tight control over the modification level might be beneficial when other applications are intended: Particles used for drug delivery might need more proteins attached to the surface than those used as vaccines. He suggested to perform western blots for analyzing the exact particle modification level.

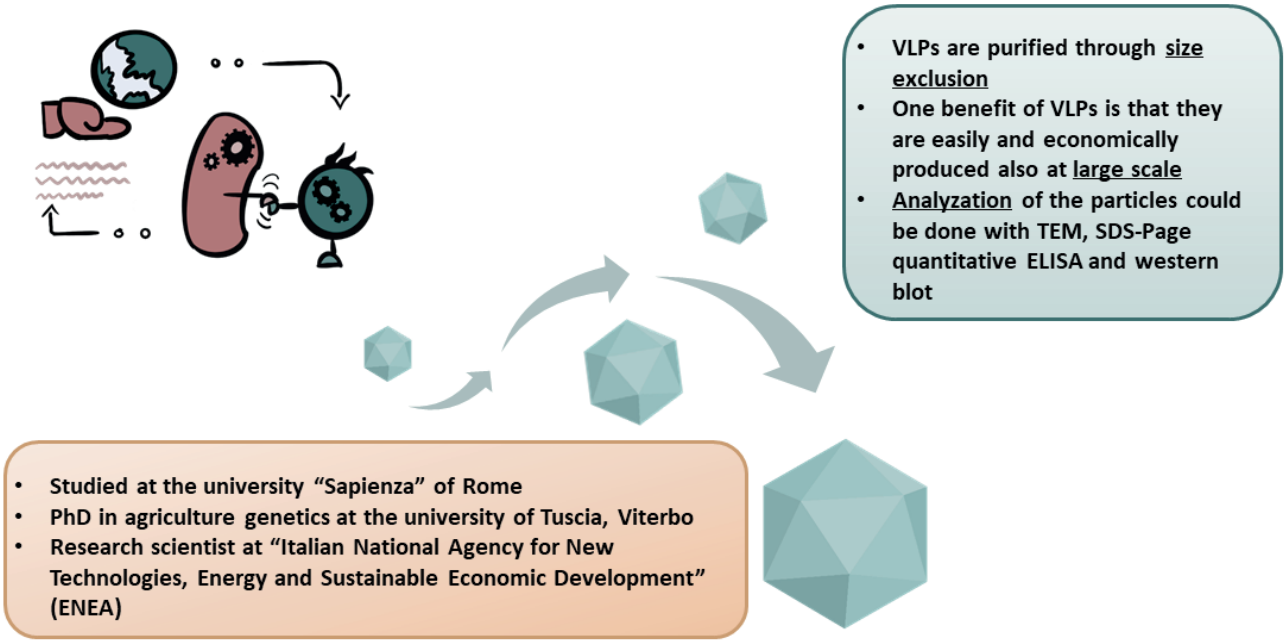
The moment of particle modification is different for our P22-platform and Prof. Santi's. In our case we use Sortase to attach the proteins of interest to the surface after particle assembly while he is expressing fusion proteins of coat protein and the protein of interest which means that at his setup, the modification happens before the assembly. With our setup, we can control the modification rate by regulating the ratio of tagged and untagged coat protein.

Another aspect of the modification we were interested in was the question whether it might cause a problem to modify the coat proteins before the assembly of the particles. Due to the flexibility of our system this would be possible if the VLPs are assembled in vitro. This question troubled us since Prof. Bailer told us, that they had some problems with the assembly due to the sterical constrains induced by the fusion proteins. Prof. Santi told us that assembly problems by the pre-assembly modification could appear but that it might differ from capsid to capsid. Furthermore, he told us that it also depends on the size of the protein that is fused to the coat protein. In the case of his virus it is not a problem to have a fusion protein with up to 60 amino acids on the coat protein before the assembly. Anyhow, any protein bigger than this could harm the homogeneity of the VLP if expressed as a fusion protein. Connecting this to our platform, it should be possible for us to control the modification ratio by employing LPETGG-tagged and untagged coat protein. This ensures that not all coat proteins get functionalized.

Regarding our aim of *in vivo* VLP assembly and modification we were wondering if endogenous proteins could accidentally get packed into the particles during assembly. Prof. Santi explained that the natural systems are optimized for specific packaging based on recognition motifs, therefore unspecific packaging should not occur. We became more optimistic because a very specific packaging would be important for nearly all downstream applications, especially drug-delivery intentions. Prof. Santi suggested electron microscopy to analyze the packaging state of the particles. In case of an empty VLP the constructs can diffuse into the particle causing it to appear black. However, if something large was packed inside this would not happen and we would see a brighter particle.

For a possible future application in the human body we were interested whether our VLPs are suitable for oral intake and how stable he assumes they are. Furthermore, we were worried that they might get destroyed in the body. Prof. Santi stated that we need to test different proteins and their stability in different pH values of the digestive system. In the past, he also analyzed this with his VLPs by making tests on humans and animals where he measured the immune response. Since some natural virus capsids are designed to be stable in the body, we are assuming ours would be stable in the same environment. Nevertheless, it became clear that investigations regarding the properties of our particles are essential.

9.7 DR. CHIARA LICO



Prof. Santi encouraged us to speak to Dr. Chiara Lico with whom he was working together for several projects[1][2]. She also is an expert for recombinant Virus-like particle (VLP) production of chimeric capsids in plants[3]. Following is the interview with her condensed to the main question part:

[1] Chiara Lico et al., Viral vectors for production of recombinant proteins in plants, Journal of Cellular Physiology, First published: 10 March 2008 https://doi.org/10.1002/jcp.21423
[2] Chiara Lico et al., The use of plants for the production of therapeutic human peptides, Plant Cell Reports, March 2012, Volume 31, Issue 3, pp 439–451, https://doi.org/10.1007/s00299-011-1215-7
[3] Chiara Lico et al., Peptide display on Potato virus X: molecular features of the coat protein-fused peptide affecting cell-to-cell and phloem movement of chimeric virus particles, JOURNAL OF GENERAL VIROLOGY Volume 87, Issue 10, First Published: 01 October 2006 https://doi.org/10.1099/vir.0.82097-0

HOW ARE VLPS CURRENTLY PRODUCED, WHAT ARE THE UPSIDES AND DOWNSIDES?

Chiara Lico (C.L.): VLPs are typically obtained producing the coat proteins as recombinant proteins in a heterologous expression system, based on cell cultures in bioreactors. The system is relatively cheap, nearly optimized, approved by regulatory organization, but there is always the risk of contaminations by pathogens dangerous for human health.

> VLPs ARE SELF-ASSEMBLING STRUCTURE, EASILY AND ECONOMICALLY PRODUCED ALSO AT LARGE SCALE

HOW ARE VLPS PURIFIED ON AN INDUSTRIAL SCALE?

C.L.: VLPs are purified through size exclusion chromatography, diafiltration and ultrafiltration techniques.

IS IT POSSIBLE TO PURIFY VLPS VIA DIALYSIS? IF SO, DOES IT HELP TO GET A HIGH PURITY?

C.L.: Dialysis is typically used to change the buffer with an increment of the final volume and the need of a concentration step. At small scale it is possible to use to this aim some concentrators provided with some membrane, of different compositions, with a specific cut-off. In this way, during the run in the centrifuge, the sample can be concentrated and retained by the membrane, removing at the same time the proteins of small molecular weight that will pass through the membrane, but the final product is only partially purified, and at industrial scale this is not feasible.

WHICH ARE THE BEST METHODS TO VERIFY THE SYMMETRY OR QUALITY OF VLPS?

C.L.: A Coomassie stained SDS-PAGE - even better a silver stained gel - can be useful to check the purity of the purified batch, searching for other proteins different from the ones forming the capsid of the VLPs. A DLS analysis can verify if the VLPs are monodispersed and homogenous in size (if VLPs are of spherical shape). A TEM image is the final confirmation of shape and dimension of the VLPs.

ARE THERE QUANTITATIVE METHODS TO ANALYZE HOW MANY CAPSOMERS HAVE BEEN MODIFIED E.G. COVALENTLY CONNECTED TO OTHER PROTEINS?

C.L.: Through a Coomassie stained SDS-PAGE it is possible to identify the modified capsomer at a higher molecular weight in comparison to the unmodified one, and to quantify the band in comparison to a quantified protein used as standard and loaded on the same gel at different concentrations. If the new protein fused to the capsomer is recognized by an antibody (or if it has a tag) it is possible to use a quantitative ELISA, producing a standard curve with the purified/quantified new protein not fused to the capsomer, or a western blotting using the densitometric analysis of the bands in comparison with the bands obtained by the purified protein loaded on the same gel at different concentrations.

WHAT ARE THE BEST METHODS TO ANALYZE THE CONCENTRATION OF VLPS?

C.L.: If the batch is well purified it is possible to evaluate the total protein concentrations, amenable univocally to VLPs capsomers, through a Bradford or a BCA assay or through a spectrophotometer. The most accurate way probably is always a quantitative ELISA if antibodies are available.

WHAT ARE THE BENEFITS AND DOWNSIDES OF VLPS COMPARED TO OTHER BIOLOGICAL NANOPARTICLES E.G. NANO-COMPARTMENTS?

C.L.: VLPs are self-assembling structure, easily and economically produced also at large scale. They can be modified chemically or genetically, on the surface as well as in the inner, and they are very different in shape and dimensions. For all these reasons they are very versatile for nanotechnology applications (biomedical in particular). A possible drawback is their protein nature, that renders VLPs biocompatible and biodegradable, but also recognizable by the immune system for example, or with some problem of stability.

IS IT POSSIBLE TO ENCAPSULATE SMALL MOLECULES E.G. DRUGS INTO VLPS AND DO THEY DIFFUSE OUT OF THE VLPS?

C.L.: Yes, it is. VLPs can be opened to diffuse in the inner the molecule of interest, and closed in a reversible manner to entrap the molecule; or, alternatively, coat proteins can be assembled around the molecule of interest. In particular pH and salinity conditions, VLPs can then be induced to relax their capsid structure (swelling) or to totally disassemble to release the molecule in the medium.

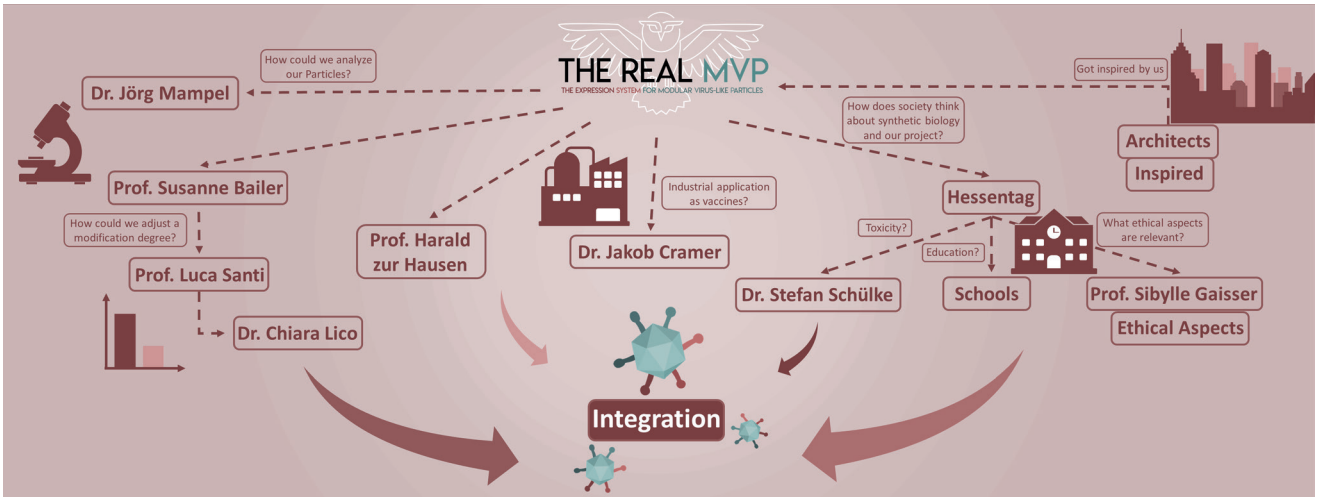
Talking to Dr. Lico confirmed most of our thoughts. We are delighted that the conversations with all of our experts led to a bigger picture of our particles. She once again confirmed that VLPs serve as a good platform and pointed out that due to the self-assembly of the particles they are easy to produce and the main costs lie in the purification.

10. THE ROAD SO FAR: SHORT SUMMARY

During the year, we spoke to a lot of stakeholders who are either possible future users of our modular Virus-like particle (VLP) platform or experts in possible application areas.

As you read in the previous chapters, we got valuable feedback from all the people we met. Many aspects of this feedback were later implemented in our lab work and project in general. Now, as our iGEM year comes to an end, we can conclude that our outreach inspired us as well as others.

Before we take a closer look at the integration, we would like to shortly overview and summarize our Human Practice work. For the overview, we have created this chart. Of course, there are even more cross-links between the individual points, but here we highlight what drove us to the particular expert or event. Afterwards, we have a closer look at how the main feedback points influenced the design of our MVP.



Overview about the individual workstations during our Human Practices and what question or impression leads us to them. Additionally, you can see that we used the feedback from these stations to design our project.

The different stations of our Human Practice outreach are highlighted by boxes on the left, while you can find a condensed overview of the respective key aspects on the right.

HESSENTAG

We went to the „Hessentag“ to explain what synthetic biology is about and to get an impression of what opinions people have about that topic. Also, we were particularly interested in the impact our work with VLPs has on society. During our conversations we realized that it is challenging to keep up with the constant progress in research. As a consequence, we thought that fears are often rooted in a lack of education, for example the fear about synthetic biology is connected to human genome editing.
The „Hessentag“ helped us to expand our explaining skills and we got the impression that synthetic biology is understood as DIY biology. In addition, we conclude from the output of the conversations that we need to do more education and go on communicating with others to leave the science bubble.
Some questions and fears lead us to think more about the safety of our work and the impact of our VLPs in case of a pharmacological application with regard to the toxicity.

SCHOOL

The observation that some fears appear due to the lack of education encouraged us to go to schools and communicate about synthetic biology and our project. We held a presentation and discussed afterwards with the students.
The discussion sensitized us for possible side effects and that we need to answer open questions about the immunogenicity and toxicity of our particles. This motivated us to talk to experts about the risks of our project, the immunogenicity and toxicity of our VLPs and whether the possible applications serve the general purpose.
Another topic that seemed strongly emphasized by the pupils was the issue of dual use of our modular platform. As that was also a topic in the conversations on the „Hessentag“, we decided that we should think more about regularities, biosafety and dual use aspects.
Once more we learned that education and communications is key to prevent fears and present science as something reachable. Because of that we wanted to keep and to get contact to other disciplines and people.

KEY ASPECTS AND IMPORTANT FEEDBACK

Need for more education and **COMMUNICATION**

Think about **SAFETY** aspect

Find out more about **TOXICITY** of the particles

IMMUNOGENICITY and **TOXICITY** of our particles is important

Need to think about **BIOSAFETY** and **DUAL USE**

Should encourage **COMUNICATION** with others

ARCHITECTS INSPIRED

During the first months, architects stumbled upon our synthetic biology project and became inspired by certain facets. One of them even created a whole project called "inter living" in which the aspect of communication between the scientists and their families is stressed. Another architect got inspired by the icosahedral shape of our VLP and designed a bus station as well as a cable car in this shape. We feel honored to be an inspiration and proud that our lab work, as well as our Human Practice work has such an impact on the people around us. Furthermore, we got an interdisciplinary exchange about modular "toolbox"-like systems. We discussed the challenges and chances of standardized building blocks with an architecture group. They are working with modular construction material to build flexible changing bridges and buildings. From all these great experiences we once again learned the importance of interdisciplinary communication to explore new ways of thinking. In addition, the architects showed us that modularity is key in many areas and that the toolbox and "LEGO" idea is very popular.

MODULARITY seems to be a key aspect of future projects in all kinds of disciplines

COMMUNICATIONS with other disciplines lead to new inspiration and opens new ways of thinking

ETHICAL ASPECTS

PROF. SIBYLLE GAISSER

Our first conversations (at „Hessentag" and in schools) made us more aware of biosafety, dual use and ethics in general. To get to know more about those topics we decided to talk to Prof. Gaisser, a biotechnology expert and professor who teaches about ethical aspects in biology. In case of our modular system we and society worried about possible dual use of the VLPs. Prof. Gaisser thinks that it is really important to consider advantages and disadvantages of every new technology and that synthetic biologists should always be aware of the possibility of intentional misuse by others. During our talk she mentioned a way to minimize the risk of the dual use of our modular system, in case our project develops into a company in the future. She proposed that we could establish a safety form that prevents people to order our parts for the VLPs without giving a reason what they are using them for. With this method of biosecurity assessment, we could theoretically at least control which institutes are allowed to use our system and what for.

Should take care of **DUAL USE** and create a **SAFETY FORM**

Regarding people's fears, she stated that it is crucial the life science community opens up a dialogue with the other disciplines and non-science society. This would help synthetic

COMMUNICATE with society to prevent fears

biology to become an everyday topic and reduce associated fears, because science would become something reachable.

DR. JACOB CRAMER

Since we started to work on our project we thought about many possible applications. One possibility that came to our mind is the use of VLPs as vaccines. Upon leaving our lab to broaden our mind we encountered a lot of people having the same thoughts. Because of that possibility we reached out to talk with experts in that field, hoping to learn more about vaccine application and its requirements.
Therefore, we were delighted to talk to Dr. Jacob Cramer, head of clinical development at CEPI in London. He has, like us, the opinion that our system could serve as a reliable vaccination tool. We were interested in the future steps that would approach if our VLPs should be established as vaccines. Once more we were told that the toxicity of the particles is relevant and that the safety aspect is the most important aspect in these research studies. In addition, more research should be performed regarding the immune response our particles cause.

DR. JÖRG MAMPEL

Since we started our lab work we wondered how we could analyze the particles after producing them. To get to know some analyzing methods and what requirements were needed to use the VLPs for pharmacological applications, we reached out to talk to Dr. Jörg Mampel from the BRAIN AG.
He told us that a good method to analyze our particles would be electron microscopy. Regarding *in vivo* production, he suggested to use arabinose as an inducer instead of IPTG. He stated the induction of cells is more exact and reliable when using arabinose. In addition, based on his experience in working for a biotechnological company, Dr. Mampel gave us an insight into bacterial industrial production. He mentioned that we need to do future investigations to reach safety standards suited for pharmacological applications.Dr. Mampel stressed the importance of communicating our knowledge and work to the public on an accessible and open level, especially because in his experience, scientists often tend to live in a science bubble.

One possible application for our system could be vaccines

THE TOXICITY and the Since aspects of our particles are important

ANALYZATION of the VLPs via electron microscopy

For a future use in the industry the **SAFETY** is important

INDUCTION WITH ARABINOSE is more exact and reliable

We should leave the science bubble and go one **COMMUNICATING** with others

ZUR HAUSEN

As one of the possible applications for our VLPs is vaccination, we decided to talk to someone who is familiar with this topic. Prof. zur Hausen is a leading expert in this regard since he was awarded the Nobel prize for development of HPV vaccine which is based on VLPs. We decided to reach out to him, as previously Dr. Stefan Schülke mentioned the HPV vaccination in context of the already approved vaccinations based on VLPs.
According to Prof. zur Hausen the development of vaccines is a long process and this leads to high vaccine costs. The required development time could be shortened by the modularity of our MVP system and this would lead to lower costs for industrial applications.

BAILER

One of the first challenges that occurred during the work on our project was the way of analyzing the assembly success. To get help from someone who also did some research about VLPs we decided to talk to Prof. Bailer from the Fraunhofer Institute. She is, like we are, interested in establishing VLPs as a basic structure that can be modified as required, although she uses other VLP scaffolds. She mentioned that pre-assembly modification of coat protein can result in heterologous particles. Therefore, she thinks that the chemical conjugation with a fusion protein after assembly of the particles could help avoiding incorrect assembly.Prof. Bailer also mentioned that it will be necessary to do the production at large scales for a future application in industry. To see if our particles are safe to be used in pharmacy, she referred us, as other experts did before, to the Paul-Ehrlich-Institut.

SCHÜLKE

In nearly every conversation the people mentioned some fears about possible side effects of the VLPs while using them for clinical applications. This aspect of the potential toxicity first appeared at the „Hessentag“ and from this point the toxicity question accompanied our work. The Paul-Ehrlich-Institut was suggested multiple times as a recommendable contact point. There we talked to Dr. Stefan Schülke. According to him the VLPs without modifications are not immunogenic because

There are alredy approved vaccinations based on VLPs

The **MODULARITY** of the VLPs shortened time and lower costs to the development of vaccines

MODIFICATION after assembly promotes the correct assembly

A **LARGE SCALE PRODUCTION** could be necessary

We need to take care about **TOXICITY**

Unmodified particles are not **IMMUNOGENIC**

There are alredy approved vaccinations based on VLPs

they do not contain viral DNA. He also explained to us that there is an already approved vaccination based on VLPs against HPV.
Dr. Schülke mentioned that our idea of using the P22 VLP as a modular platform would simplify the production process for vaccines because the necessary tests for many applications will be shortened. The potential of VLPs for passive immunization against potentially fast-acting diseases was also a topic we talked about. In conclusion we were informed that VLPs are well suited for the development of vaccines, however, many experiments are necessary to reach a state where they could be approved for real-life usage.

The **MODULARITY** simplify the production process of vaccines

SANTI

As Prof. Bailer mentioned, it could be useful to adjust the degree of modification. Therefore, we contacted another international expert who is familiar with VLPs. Prof. Santi from the DAFNE in Italy helped us with this topic. He has, like us, the opinion that it is important to control the modification level. In addition, he had an idea how to manage this problem. We could control the modification rate by regulating the ratio of tagged and untagged coat protein. To make an industrial application possible, he stressed purity of the particles has to be extraordinarily high. This could be achieved for example via size exclusion. Moreover, large scale production would be obligatory.

We should try to adjust the **DEGREE OF MODIFICATION**

This could be done with a tagged and untagged coat protein

We could use **SIZE EXCLUSION** for purification

LICO

Because of our great interest to talk to national and international experts who work with VLPs, Prof. Luca Santi referred us to Dr. Chiara Lico. She answered some of our questions about the analysis and purification of our particles. Similar to Prof. Santi, she advised us to purify our product via size exclusion. In addition, she suggested us some analysis methods like TEM (transmission electron microscopy) and coomassie stained SDS-PAGE. During the conversation about the benefits of VLPs she mentioned that they are easily and economically produced also at large scale. This supports our long term goal of an industrial production of our MVPs.

We could **PURIFY** the VLPs via size exclusion

For **ANALYZATION** we could use SDS-PAGE

VLPs can be produced in **LARGE SCALES**

We were glad to get the opportunity to talk to many potential MVP users and experts. Everyone we spoke to is a possible stakeholder because our system poses a great variety of possible applications, which means possibly everyone could be interested in it. Nevertheless, the main focus lies on pharmacological and clinical applications and potentially, upscaling for industrial purposes, since companies will most likely build the main part of the stakeholders. The information and impressions we got from the Human Practices work were very helpful and we enjoyed to talk to all these intriguing people. After all these interviews, conversations and discussions we think we got a really good impression what society thought regarding our project.
But to get society's approval it is not enough to only listen and understand their needs. It is very important to adapt our project so that it is designed to the welfare of the general public and future perspectives. Over the course of the year, we therefore attempted to incorporate all the valuable information and suggestions from experts and society into our MVP project. By this, we managed to improve our entire VLP platform by several magnitudes. To give you a clear overview of our Human Practice integration, we summarized the main points in the following section. We divided the section in six keywords as these kept turning up as recurrent themes throughout our conversations.

11. WHAT ABOUT US? INTEGRATION INTO OUR PROJECT

MODULARITY & MODIFICATION DEGREE

The first key word which was mentioned in nearly every conversation was "communication". Communication with other disciplines, with experts and society in general is really important to get new ideas and impressions which we included in the development of our project. In particular, experts like Dr. Jörg Mampel and Prof. Sibylle Gaisser stressed that it is important to leave the science bubble and educate, because this would help synthetic biology to become an everyday topic and reduce fears in society. For example, talking Dr. Schülke and Prof. Bailer made us think about the safety aspect and the toxicity of our Virus-like particles (VLPs). All the experts suggested methods for analyzation and purification to us, which we included in our lab work. Another often mentioned aspect was the potential use for a industrial application and the biggest topic was the modification degree and the modularity of our MVPs. All these main points appeared often during our Human Practices work and this led us to our final project. In the following you can read how we integrated the main points, which resulted from the communication with others.

MODULARITY & MODIFICATION DEGREE

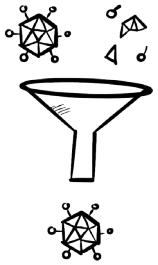
Modularity has always been the key of our project idea. It poses many advantages in all different fields of life as we learned during the conversation with the architects of the „Digital Design Unit". Our system was inspired by the concept of modularity. The question that was raised as we started our work with VLPs was: How do we actually implement this modularity? The concept of sortase modification was the first and important aspect of our project, but would it be possible to reach even better modularity? Experts, like Dr. Stefan Schülke and Prof. Susanne Bailer, suggested the modification degree.

Dr. Schülke from the Paul-Ehrlich-Institut stated that it is important to functionalize the particles so that they present as many fusion proteins as possible but not as much that the integrity of the particle is disturbed. Later Prof. Bailer mentioned that they were already thinking about adjusting the degree of modification, but have not tried it yet. We see this as a very important aspect in the development of our modular system. In the talk with Prof. Santi from the DAFNE we learned that there is a quite simple method to adjust the degree of modification. He explained to us that we could solve the problem by cloning the coat protein with a LPETGG-Tag and without one, so we have taken the first step to make sure, that not all coat proteins get functionalized. We used this in our wetlab and cloned the coat protein with and without the tag to generate a VLP where only some fusion proteins can be connected to the particle via sortase.
Because of the laborious purification process for *in vivo* produced VLPs we could not test modification ratios on VLPs themselves but rather tried to generate data about the expres-

sion levels of our promoters in dependence of the inducer concentration to develop strategies for adjusting the modification ratio of *in vivo* production.
Therefore, we started using a dual expression system as described on our wiki. All in all, the results we obtained from reporter protein expression suggest that it is possible to adjust the expression ratio of the included ORFs. The ideas of the experts contributed therefore significantly to our vision of a "Real MVP". We can imagine that a future system of dual expression plasmids containing tagged and untagged CP could serve as a suitable platform to produce different VLPs *in vivo*.

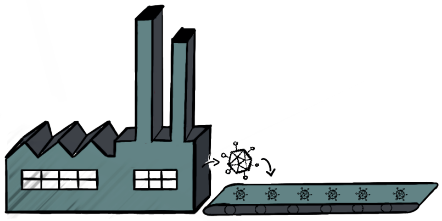
PURIFICATION & ANALYSIS

During our lab work we thought of how to analyze whether the product consists of correctly assembled VLPs. Nearly all of our experts told us that the ultracentrifugation and the following electron microscopy would be a good method for that. We employed their expertise and used their methods to purify and analyze the VLPs. Their input boosted our confidence, as we were not sure in the beginning if these methods were still state-of-the-art.
Regarding advanced VLP purification, Prof. Bailer told us that an industrial production would require various cleaning steps to make sure that no cell-material might cause cross-reactions when the VLPs are injected into the human body. In addition, we would have to get rid of the excess proteins. We were interested in methods to separate the assembled VLPs from unassembled proteins or our Sortase A7M and the proteins which were not connected to the particles. According to Prof. Santi and Dr. Chiara Lico a purification via size exclusion could be a good idea so we tried this method with our *in vivo* generated VLPs. The suggested purification steps led to intact VLPs. Dr. Chiara Lico and Dr. Stefan Schülke also recommended us to use DLS (dynamic light scattering) to analyze the size and purity of our particles. We got the opportunity to visit the PEI and be present while a DLS was performed with our particles. We were able to see that modified particles are larger than unmodified ones.
To conclude, the advice from the experts were very helpful and made us aware of the demanding downstream processes in the industry.

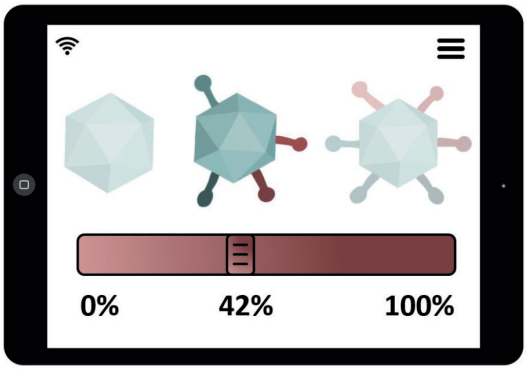


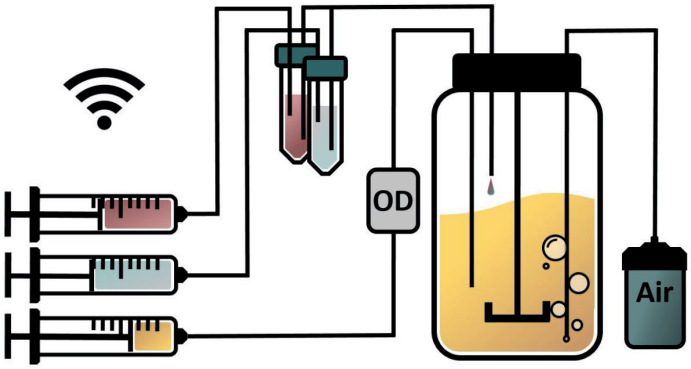
INDUSTRIAL APPLICATION

When thinking of possible applications, we thought about how we could achieve a production in an industrial scale. This is important so that the costs and the effort are low enough so that the production pays off for a company. Prof. Santi told us that it would be necessary to produce and purify the VLPs in a large scale. In addition, Dr. Chiara Lico mentioned that one of the benefits of VLPs is that they can be easily and economically produced also at large scale due to their self-assembly. So, we decided to design an automated self-inducing bioreactor to somehow simulate a large-scale production. With this system it is also possible to control the induction ratio what might be important according to the setting of the modification degree. For further information see the tech part at our wiki.



Unfortunately, assessing our MVP production quantitively was not possible to a satisfying degree. Prof. Bailer mentioned that VLP quantification generally is complicated, as there is no sophisticated method to date. However, we got a clear idea about the aspects and challenges in industrial production of new therapeutics.





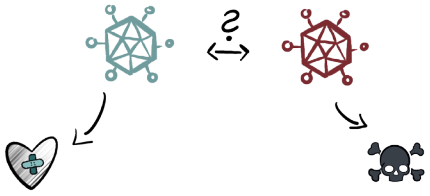
IMMUNOLOGY & TOXICITY

During our Human Practices work many people mentioned the aspect of the toxicity of the particles. We were often referred to the Paul-Ehrlich-Institut (PEI) to have a closer look at this topic. There we talked to Dr. Stefan Schülke from the PEI in Langen, Germany. During the conversation he confirmed that Virus-like Particles (VLPs) without modifications are usually not very immunogenic because they only consist of the virus shell but lack the immune-activating viral genetic material packaged within the particles.
When we visited the PEI, we also did an endotoxin test and the results showed us that we would need more purification steps until an application in the human body will be possible as a production in *E. coli* produced a high rate of endotoxins. However, with pure proteins and *in vitro* assembly, the endotoxin levels were lower, showing the potential of additional purification steps.

Dr. Schülke mentioned the potential of VLPs for passive immunization against potentially fast-acting diseases. We conclude that our focus on vaccines as a possible application was justified. Additionally, Prof. Bailer mentioned that VLPs as particles do not need adjuvants, which could cause problems like side effects. She explained that there is also the possibility to expose many antigens what makes them potentially highly effective. Dr. Schülke also mentioned that there are already existing VLP-based vaccines authorized for human usage, for example against infection with the human papillomavirus (HPV) or Hepatitis B. Being interested in those already existing vaccines, this input led us to contact the Nobel laureate Prof. zur Hausen later on.

DUAL USE & BIOSAFETY

Biosafety is an immensely important topic for us, as we used genetically modified organisms (GMOs) in our laboratory work. These organisms, containing antibiotic resistances, should not be released into the environment. People outside of our science bubble often do not know about our practices in the lab, about our use of GMOs, or about our safety practices. They may then develop fears towards synthetic biology. We experienced this at the "Hessentag", where we were able to talk to diverse groups of people who all agreed on the need for strict safety policies regarding our work.
This impression was further confirmed in conversation with the schools we visited and with Prof. Sibylle Gaisser, a professor of ethics and biosafety. Beyond our considerations on safety we learned about the aspects of safety in the context of an industrial application. This not only concerns the aspect of toxicity, but also the biosafety in our lab. We therefore decided to discuss this topic extensively, as you can see on our wiki under the safety point.



As our VLPs are designed as a modular platform, to be modified to one's liking, safety problems inherently appear. We were concerned about the possibility of dual use of our MVPs, and adamant about wanting to reduce such misuse. In our conversation Prof. Bailer, of the Fraunhofer Institute in Stuttgart, she reassured us that she does not see the extreme misuse of our VLPs like bioterrorism as a substantial problem of our project. Nonetheless we took up Prof. Gaisser's idea of establishing a safety form for our modular platform. After some research, we came up with the following sheet that addresses the main concerns like the proposed use, the protein sequence, and possible risks for humans or the environment. We exemplified the form with our collaboration project with the iGEM Freiburg team. The safety form for our VLPs requires the user to state their intentions, therefore helping to prevent misuse, as proposed projects may be assessed before starting.

Safety form for the use of VLPs

Why do I need to answer these questions?

Biosafety is an important topic in the scientific community, especially in synthetic biology. Every new invention requires a risk evaluation. To make sure our modular platform is not misused we ask you to fill out this safety form. This will help to retrace the use of VLPs for various applications, and will give us an overview whom is using our invention for which application. (See below for example texts.)

Questions:

FILL IN YOUR PERSONAL DATA:

Name, Academic Title, Date of Birth, Institute, City, Region, Country

WHAT IS THE EXPECTED APPLICATION?

We are seeking to employ the VLPs as vehicles for targeted drug delivery. With this, we want to target cancer cells by attaching a protein to the surface that binds to the sigma-1 receptor.

SEQUENCE OF SURFACE-PROTEINS?

ATGAAAGGGCCCTGTACTGGTTACGATACCGGGATAGCATCGTAAGGCAGTTGA

WHAT ARE THE PROPERTIES OF THE SURFACE-PROTEINS?

The surface protein will bind to the sigma-1 receptor through hydrophobic interactions. It does not seem to target any other receptors or cell types besides cancer cells. The protein is stable in temperature variations up to 60°C for 10 minutes, and pH stable between pH 4 and pH 9. It poses no threat to the healthy human body, as it does not show any signs of immunogenicity or toxicity.

WHAT ARE POSSIBLE SAFETY RISKS FOR YOUR APPLICATION?

The proteins meant to be attached to the particle show no sign of side effects that might be harmful to the body. They are neither toxic nor do they trigger any immunological response. They are meant to bind to the target receptor and so not have any other purpose or property that could interfere with the desired application. Nevertheless, the same test with modified particles need to be done extensively.

WHAT COULD BE A POSSIBLY HARMFUL APPLICATION OF YOUR DESIRED VLPS?

As the VLPs are used for targeted drug delivery it is possible that other cells could be harmed by the enclosed medicine. Since the medicine is meant to lead to apoptosis of the targeted cells, other important cells like the heart-muscle-cells could be attacked if the targeting protein on the surface of the VLPS is changed.

Detailed project description:

PLEASE FILL IN A DETAILED DESCRIPTION OF THE USE OF YOUR VLPS.

We are aiming to specifically target cancer cells by using surface proteins that bind to the sigma-1 receptor. With this method we hope to solely eliminate diseased tissue.

12. CONCLUSION

Our Human Practices work really helped and positively influenced the development of "The Real MVP". The conversations with society, non-scientific people and also with experts helped us to improve our project and get an impression of what is required for pharmaceutical production and application. On one hand we now have a good way of modifying the particles with the sortase after assembly, which leads to an easy assembly of the Virus-like particles (VLPs) on the other hand we established a way of adjusting the degree of modification. Additionally, we got some good ideas about analyzing and purifying the particles. Also, we started to create an automated way of a large-scale production which is relevant to lower the costs for the industry. The fears of the society led to that fact that we were more concerned with biosafety, so we designed a VLP safety form that could help to prevent dual use.

As you can see in our outlook, there is still a lot of work to do until „The Real MVP" is available in the trade for various applications. Nonetheless, we achieved to include stakeholders, possible users and experts in the development of our project and established a modular platform that is designed for the welfare of the general public. We thank all of the people who talked to us and shared their thoughts, impressions, fears and ideas with us. We enjoyed working together with such intriguing people and are excited to continue our journey in the synthetic biology and become responsibly acting scientists.