Targeted drug delivery: Nanocillus - ‘cause spore is more!

iGEM FREIBURG 2016

The treatment of diseases while avoiding systemic side effects is still a major obstacle in modern medicine. After administration, conventional drugs are distributed throughout the **whole body** thus affecting both, diseased and healthy cells. Current strategies on targeted drug delivery are mainly based on the applications of antibody-drug conjugates or nanoparticles. However, both approaches revealed considerable challenges in their application due to short half-life and expensive production. We developed a **novel platform** for targeted drug delivery by implementing highly specific nanobodies directed against surface markers of affected cells. The combination with an enzymatic functionality facilitates the local activation of prodrugs, thus preventing unnecessary side effects by systemic drug dispersal. By engineering the spores of probiotic ***Bacillus subtilis***, a member of the human microbiome, we establish a low-cost carrier for a well-tolerated treatment.

Attributions

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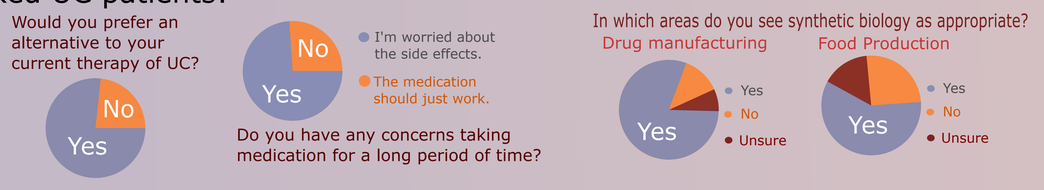
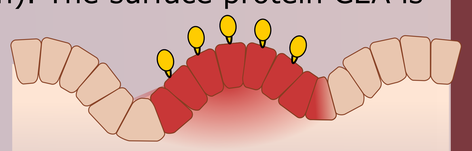
Ulcerative colitis

Ulcerative colitis (UC) is an autoimmune disease that leads to inflammations and ulcers in colon and rectum. The first symptoms include bloody diarrhea and highly frequent bowel movements. Current therapy is immunosuppressive or anti-inflammatory medication. In our survey we've asked UC patients:

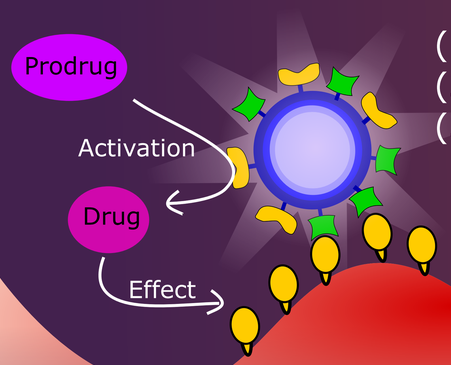


The results of this survey had a huge impact on the design of our project. The better acceptance of synthetic biology in drug manufacturing changed our idea of an Nanocillus-yoghurt to Nanocillus-capsules.

Many disease have their own and very characteristic markers. In the case of UC this marker is CEA (carcinoembryonic antigen). The surface protein CEA is expressed in higher quantities in cells affected by UC. Therefore, spores targeting CEA will ultimately bind to the inflammation caused by ulcerative colitis.



In short:



(1) Nanocillus binds to a desired structure.
(2) A prodrug gets activated locally.
(3) The drug has an effect on diseased cells.

No active drug without Nanocillus!
No side effects with Nanocillus!

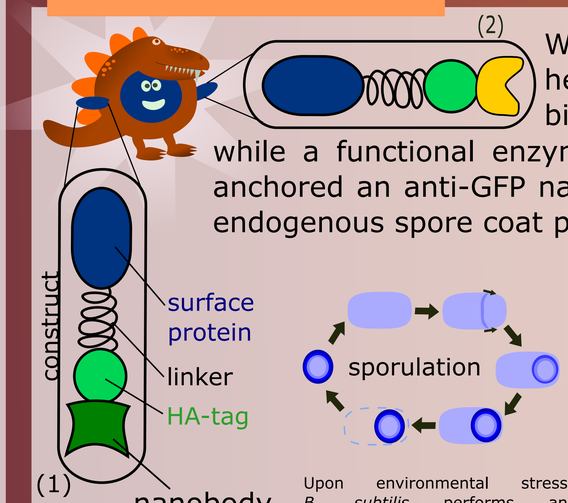
Public relations

Obviously, the community benefits of the results and progress of today’s science. Nevertheless, science depends on the public. Society defines the goals of scientific research and, therefore, makes science possible in the first place.
Motivated by all these points we’ve conducted 3 comprehensive surveys that asked people from all backgrounds and ulcerative colitis patients about their opinion on our approach and synthetic biology in general. With the help of the collected feedback, we contacted experts about their opinion on our project and its feasibility. The idea for Nanocillus was born.

Safety

When you want to use GMOs outside the laboratory, it is mandatory to look at the safety precautions. For additional safety we modeled two kill switches and analysed two germination deficient strains of Bacillus subtilis.

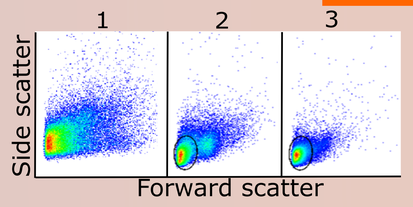
Nanocillus



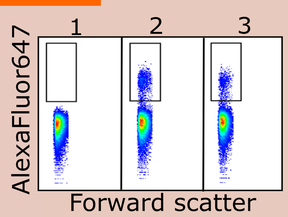
We engineered the spores of *B. subtilis* to display heterologous proteins for targeted drug delivery. A binding moiety ensures the targeting of a desired antigen while a functional enzymatic moiety enables the activation of a prodrug. We anchored an anti-GFP nanobody (1) and glutathione S-transferase (GST) (2) to endogenous spore coat proteins.

Upon environmental stress *B. subtilis* performs an asymmetric cell division. The resulting mother cell engulfs the forespore and synthesizes a spore coat.

RESULTS



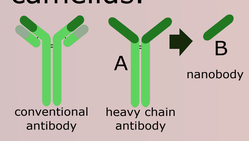
(1) vegetative cells (2) spores (3) purified spores Sporulation efficiency was evaluated by flow cytometry analysis. The vegetative cells exhibit a widespread population in the forward and sidescatter. After induction of sporulation the spores were treated with lysozyme to remove remaining vegetative cells.

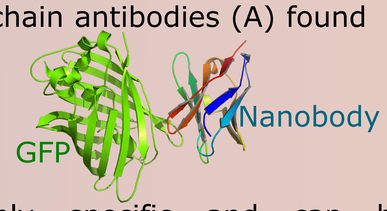


Surface display of designed proteins, anti-GFP nanobody (2) and GST (3) were validated compared to wild type spores (1) by FACS using an anti-HA antibody conjugated Alexa Fluor 647.

Binding

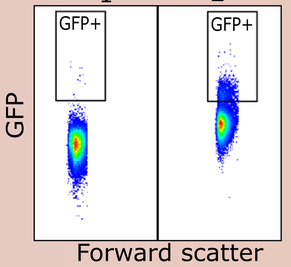
Nanobodies (B) are single domain antibodies engineered from heavy chain antibodies (A) found in camelids.



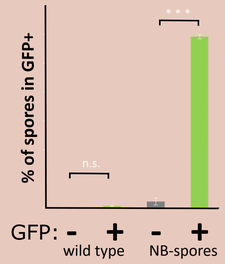


They are small, highly specific and can be synthesized in bacteria. Ultimately, our spores will bind CEA on epithelial layers of the colon. To validate the specific **binding of nanobody** displaying spores, we used GFP and anti-GFP nanobodies.

RESULTS



GFP staining of anti-GFP nanobody expressing spores (2) and wild type spores (1) in flow cytometry.
Flow cytometry has shown that our anti-GFP nanobody spores bind to GFP. Analysis of our data revealed that a signi-ficantly higher amount of GFP was bound by the modified spores compared to wild type spores.



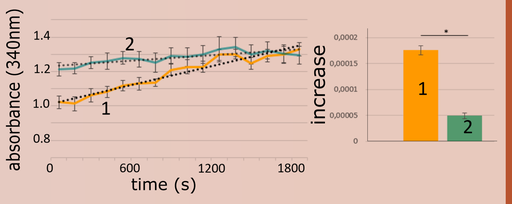
Bar chart: percentages of spores found in GFP+ gate from FACS analysis. GFP stained (+) and unstained (-) wild type and 2 modified spores were compared to see if the nanobody spore is functional.

Drug Delivery

The enzyme converting the prodrug azathioprine, which is commonly used in UC-therapy, is glutathione S-transferase (GST). A **GST-assay** with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate was conducted to validate the enzymatic activity of the spores. GST converts the glutathione (GSH) and CDNB to glutathione-SDNB which can be detected at 340 nm. The increase in absorbance is then translated into the conversion rate of the GST. The conversion rate of our **GST presenting spores** was significantly higher than for wild type spores.

RESULTS

GST Assay of GST spores (1) and wild type (2)



The GST assay was performed with 25 million GST displaying spores (1). The absorbance at 340 nm was monitored for a time course of 30 min. Unmodified wild type spores were used as reference (2).

Right: The increase of the absorbance in time is significantly higher for GST displaying spores compared to wild type spores.

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