Penn iGEM 2012
Targeted Bacterial Therapeutics

Engineering a Spatially Targeted Bacterial Cancer Therapeutic

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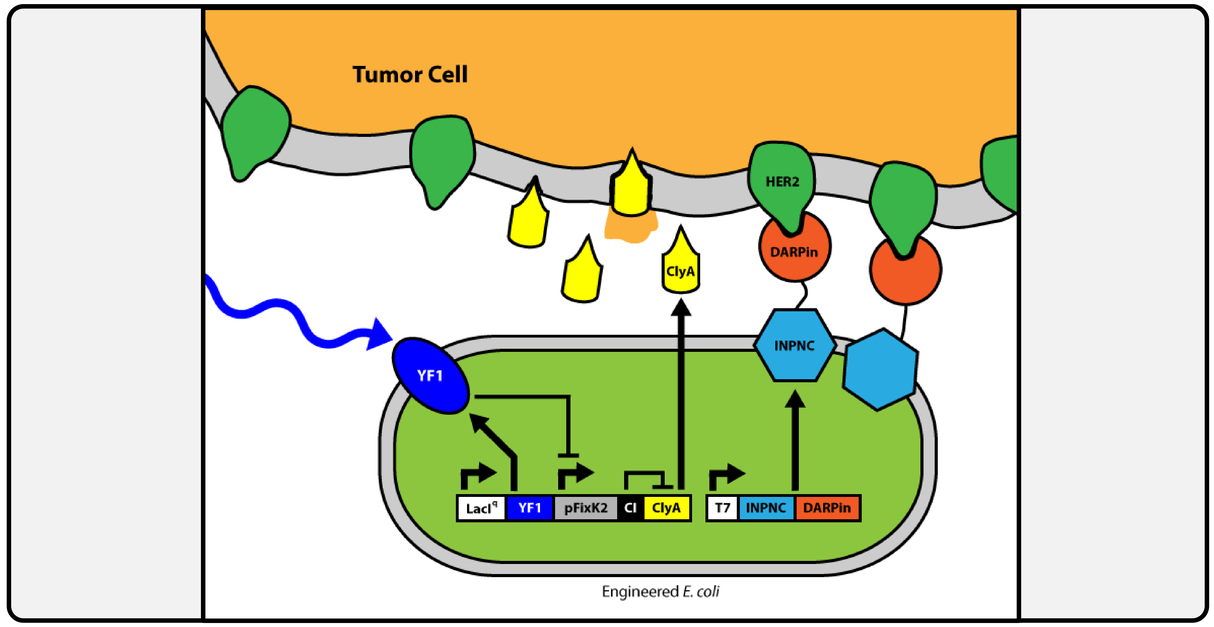
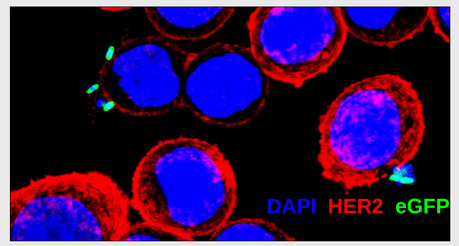
Introduction and Motivation

What if you could combine spatial targeting and cellular targeting into the same therapeutic? This idea is unprecedented but would allow for precise targeting of specific cells within a specific area, leaving healthy tissue intact and keeping side effects to a minimum. The 2012 Penn iGEM team has engineered a novel platform for targeted therapeutics which employs simultaneous spatial and cellular targeting. We have achieved spatial (and temporal) targeting with a blue light-switchable transgene expression system, and cellular targeting through display of an antibody-mimetic protein on the surface of E. coli for the first time. As a proof of concept, we applied our system to the treatment of cancer, a disease in which spatial and cellular targeting are of utmost importance. We displayed a high-affinity antibody-mimetic protein which targets Human Epidermal Growth Factor Receptor 2 (HER2), a protein commonly overexpressed in cancer cells. We combined this cellular targeting with a light-activated cytotoxic protein delivery system to successfully target and kill cancer cells.



Cancer Cell Targeting

To assay whether our DARPin-displaying E. coli bound to SKBR3 cells preferentially, we conducted experiments in which our bacteria were co-incubated with SKBR3 or HEK293T cells. The DARPin-displaying bacteria were co-transformed with eGFP for easy visualization. Our cells successfully bound to the HER2 on SKBR3 cells (below), and did not bind to low-HER2 HEK293T cells (not shown). In summary, our bacteria successfully target cancer cells!



Light-Activated Gene Expression

To engineer *E. coli* to deliver a drug in response to light, we first had to construct a light-activated gene expression system. This system would need to have very low background expression to reduce toxicity to untargeted tissue, and have very high expression levels when induced with light. The kinetics of the system should also be reasonable to be therapeutically relevant.; although protein expression generally occurs on the scale of hours or days, this system should not require more than a few hours of light to express enough toxin to kill surrounding cells. Several light sensors/effectors have already been engineered in *E. coli*, and we chose to work with the two-component YF1/FixJ system, which had the highest recorded on/off ratio and had already been assembled and optimized in a plasmid named pDawn.

To test the expression kinetics of pDawn, we cloned mCherry as the fluorescent output and illuminated *E. coli* BL21 cultures for various amounts of time and visualized red fluorescence (**Fig. 1**)

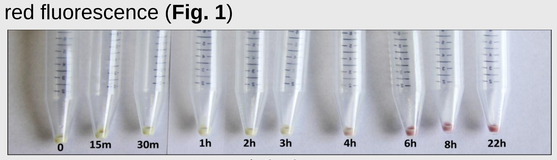


Figure 1: YF1/FixJ system with an mCherry fluorescent output allows light-activated transgene expression. Times are hours under blue light.

Light-Activated Drug Delivery

After verifying that the pDawn system was able to express mCherry in a light-dependent manner, we substituted our ClyA for the mCherry gene to prove that ClyA could be expressed in a light dependent manner. To assess this, we plated BL21 bacteria transformed with pDawn-ClyA or pDawn-mCherry on Columbia Agar plates supplemented with 5% Sheep Blood (BD). These plates are used to qualitatively detect hemolytic activity in bacteria by visually confirming lysis through a color change in the media as the blood cells are lysed. After plating the bacteria, cultures were grown in non-inducing conditions at 37C until visible colonies were present (~12 hours). Plates were then grown at 25C under either inducing or non-inducing conditions for 24 hours and imaged. These results are visible in **Fig. 2.**

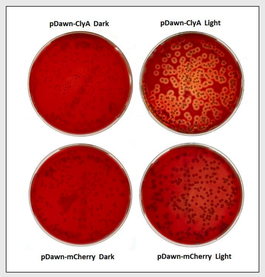


Figure 2: Light-activated lysis of blood cells and cancer cells.

Surface Display & Cancer Targeting

We sought to display the picomolar affinity, HER2 binding Designed Ankyrin Repeat Protein (DARPin) H10-2-G3 on the surface of our BL21 cells. This would allow for bacterial targeting to HER2 over-expressing cells, which could then be lysed in a spatially accurate manner using our light-activated ClyA expression system. Such surface display of a large antibody-mimetic protein is unprecedented.. We added a Human Influenza Aggregation (HA) tag to the N-terminal of DARPin, to allow us to use antibodies to assay whether it had been localized to the membrane. After expression and 48-hour induction of INPNC-DARPin-HA in BL21 cells using the pET26b expression vector, we immunostained cells using an anti-HA, antibody. Since antibodies are not permeable to the E. coli membrane, after washing the cells there should only be signal on those cells which have displayed the HA tag on their surface. DARPin was successfully displayed (**Fig. 3**). We also created a general Surface Display BioBrick that iGEM teams can use to display any protein (BBa_K811005).

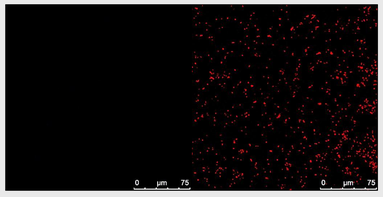


Figure 3: Surface Display of DARPin. Only the IPTG-induced group (right) was stained by the anti-HA antibody, indicating that these *E. coli* surface displayed DARPin-HA.

Human Practices: From Bench to Bedside

There have been over 75 iGEM Health and Medicine projects and many papers describing bacterial therapeutics engineered through synthetic biology. Why have none of them made it into the clinic? We conducted an analysis of the barriers facing engineered bacterial therapeutics, and gave specific guidelines to iGEM Health and Medicine teams in our presentation. We found that there are barriers in public perception, regulatory approval processes, and funding levels which have prevented these therapeutics from moving from the bench to the bedside. This analysis directly informed our experiments and outreach. We ported our light-activated drug delivery system into Nissle 1917, a “probiotic”, non pathogenic *E. coli* strain that is generally accepted by the public and is actually an over the counter medication in many countries. Our system worked in Nissle and we will be moving forward with this strain into trials in mouse models. We also conducted several outreach campaigns to improve public perception in the local community, including a fun seminar for kids where we showed them how to extract and visualize their DNA (below).