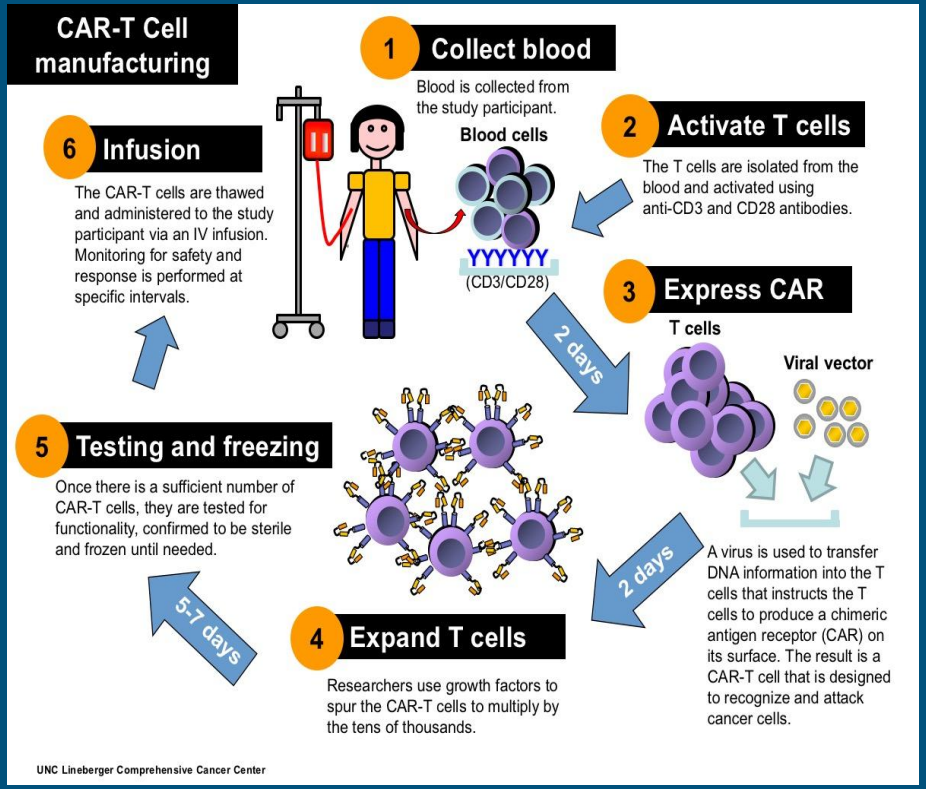
CU iGEM:
Brainstorming Ideas

T-cell Targeted Nociceptive Sodium Channels

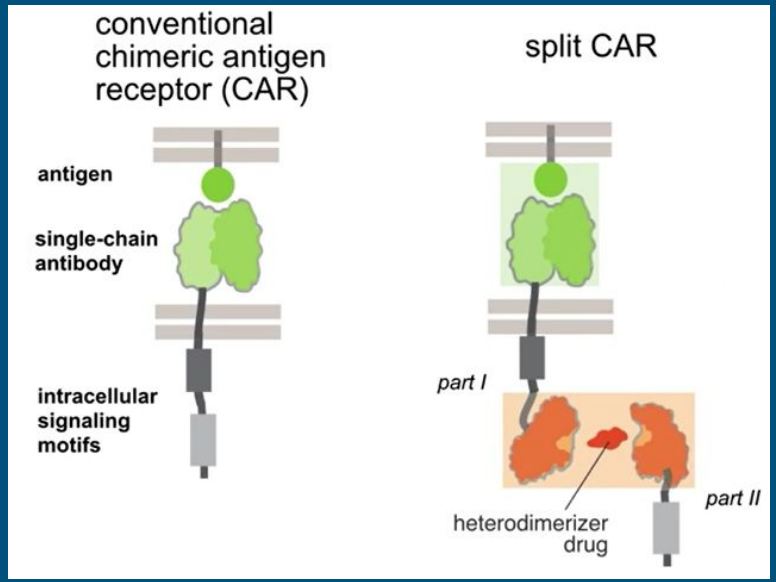
- Nav1.7 is a voltage-gated sodium channel expressed on nociceptive neurons, neurons responsible for transmitting pain signals from the PNS to the CNS.
- Nav1.7 is essential for normal pain sensation; if it is not expressed, the patient will not feel pain at all, and if it is overexpressed, the patient will experience chronic pain (20% of the population worldwide).
- Nav1.7 is therefore a prime therapeutic target whose blockage by an T-cell antibody would mitigate pain, with the T cell acting as a potential analgesic.
- A circuit would be designed so that once the T cell binds to the epitope, a chimeric antigen receptor (CAR) could be engineered to be drug-switchable so that the T cells are controllable, which is not currently the case.
    - This way, a physician could titrate cell activity and control timing with a drug, which is potentially safer.

Lee JH, L ee SY. “A monoclonal antibody that targets a NaV1.7 channel voltage sensor for pain and itch relief.” *Cell.* 2014 June

T-cell Targeted Nociceptive Sodium Channels



- Monoclonal Antibodies are already made

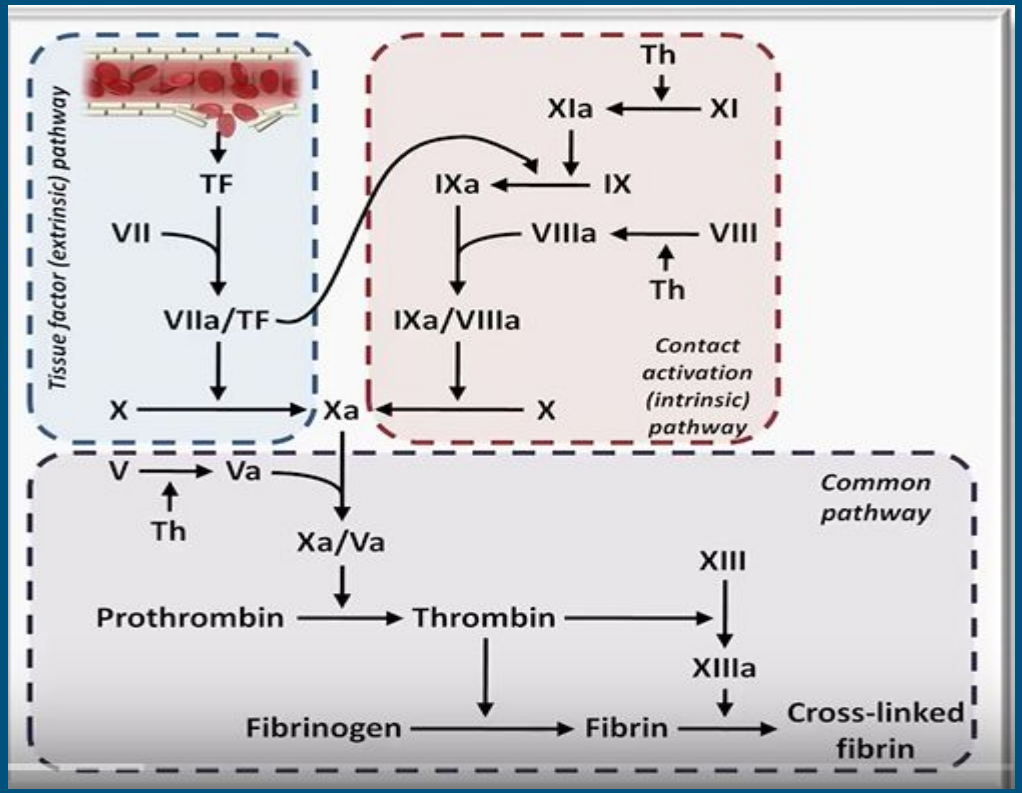


Mechanosensitive or Extrinsic-activated Coagulatory Cells

- There are products (such as polymer based gels) that *rapidly* stops blood loss
- However, they require physical application to the wound, meaning that they would not be able to stop internal bleeding and would not be practical to chronic or potentially-widespread bleeding such as hemophilia.
- Our cells would receive either a physical or chemical signal in response to a blood vessel being broken changing the extracellular pressure or releasing tissue factors through the extrinsic coagulation pathway, respectively.
- This would either induce a promoter, resulting in either a positive transcription loop or activation of an allosteric modulatory switch.
- The circuit would output cofactor 10a (FXa), the catalyst for the coagulation pathway.
- The system would be tested in cell culture and murine models.

Problem: Bacteria

Mechanosensitive or Extrinsic-activated Coagulatory Cells



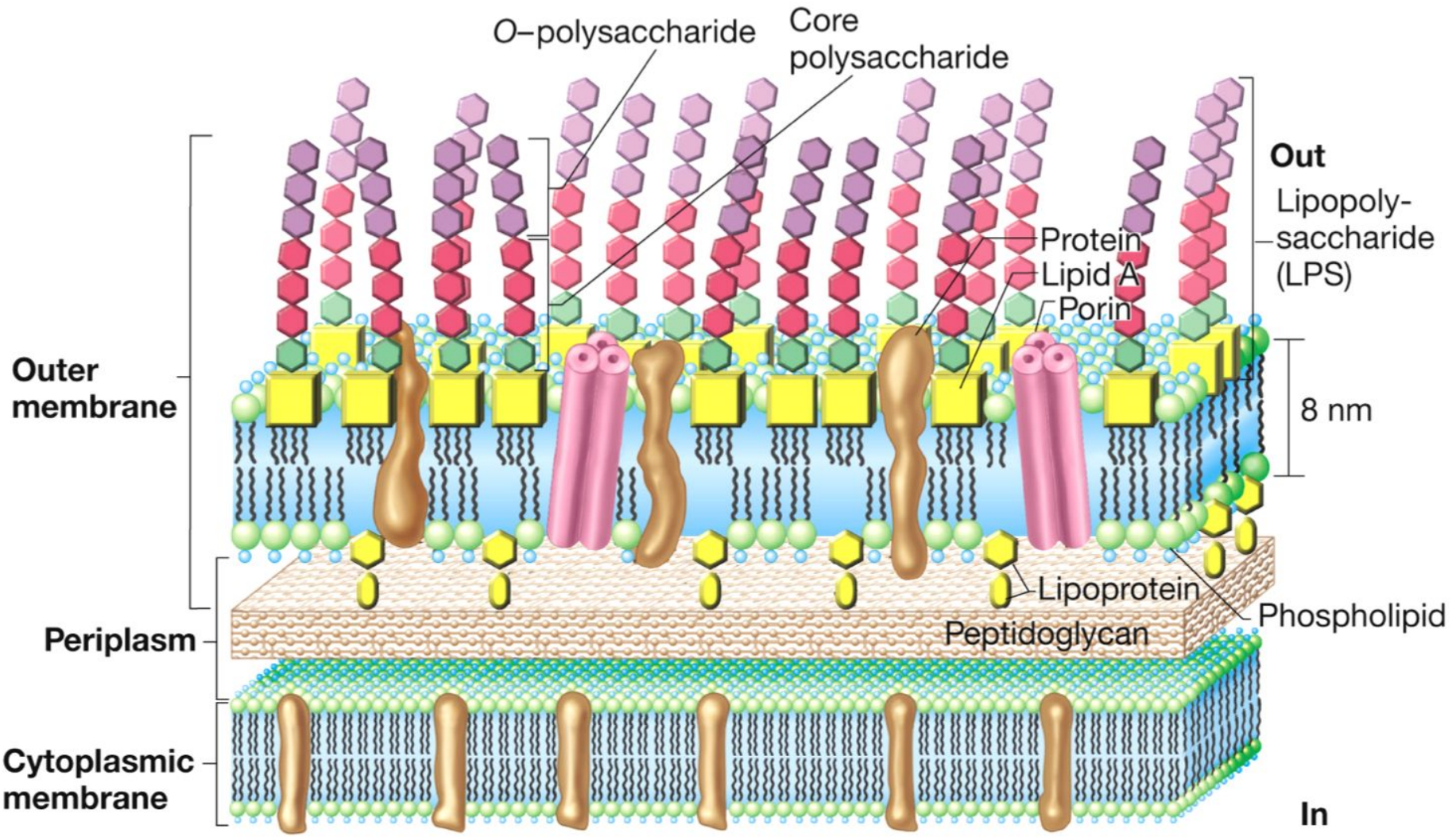
- Tissue Factor is released by the ruptured walls
- 1 Factor 10a (Xa) molecule can catalyze the formation of 1000 thrombin molecules

Fluorescent Eukaryotic Cells for Optical Measurement

- Many physiologists and neuroscientists use optical methods to measure cell behavior
    - 1)multiple portions of the sample could be monitored simultaneously, representing interactions between different cell species with different cellular functions
    - 2)measurements can be made real-time
    - 3)measurements can be relatively non-invasive.
        - Current methods involve extrinsic dyes or proteins that can cause photodynamic damage to the cells.
- Our pathway would receive a (neuro)chemical signal from a neighboring cell which would set off a signaling cascade that ultimately excites a fluorescent protein.
- CRISPR?
- The half life would have to be short and the circuit regulation cascading because both are fast and transient enough to allow for the next excitation by the next action potential.

https://www.wired.com/2014/07/neuron-zebrafish-movie/

Antibiotic Resistance


O–polysaccharide
Core polysaccharide
Out
Lipopoly-saccharide (LPS)
Protein
Lipid A
Porin
Outer membrane
8 nm
Lipoprotein
Phospholipid
Periplasm
Peptidoglycan
Cytoplasmic membrane
In

Ways bacteria can up their resistance:

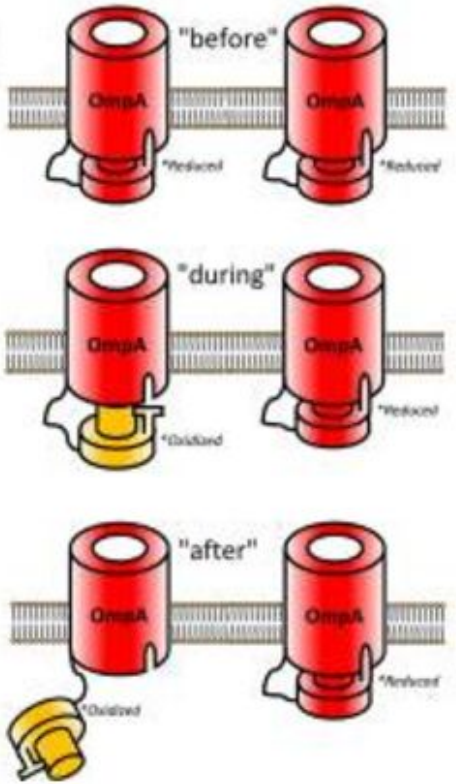
- Express more LPS’s
- Express less influx channel proteins
- Express more efflux channel proteins
- Enzymes in periplasm that digest antibiotics

Membrane permeabilizers for hydrophobic antibiotics

- Tris/EDTA*
- polymyxin B and polymyxin B nonapeptide (PMBN)*
- insect cecropins
- reptilian magainins
- dogfish shark squalamine
- …
- polymers of basic amino acids

Membrane permeabilizers for hydrophilic antibiotics

- Antimicrobial reactive oxygen species (ROS)
  - Hydrogen peroxide
  - Hydroxide ion
  - …



What are the advantages of taking a synthetic biology approach?

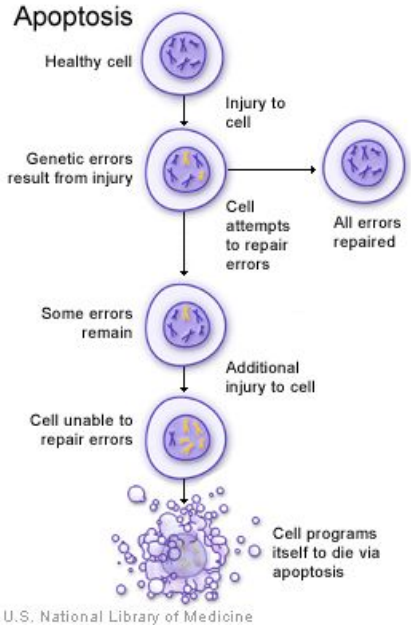
- Because bacterial resistance is so rapidly adapting, it is difficult to come up with new antibiotics every time bacterial populations grow resistant.
- We can engineer safe and insusceptible bacteria that can synthesize membrane-permeating substances in the medium in response to a pathogenic trigger
  - Reaction $\longrightarrow$ Action system
  - For example if we want to selectively localize antibiotic treatment (healthy vs. bad bacteria)

E. Coli Mediated Apoptosis of *Thiobacillus Ferrooxidans*

Nathan Lian and Tarun Srinivasan

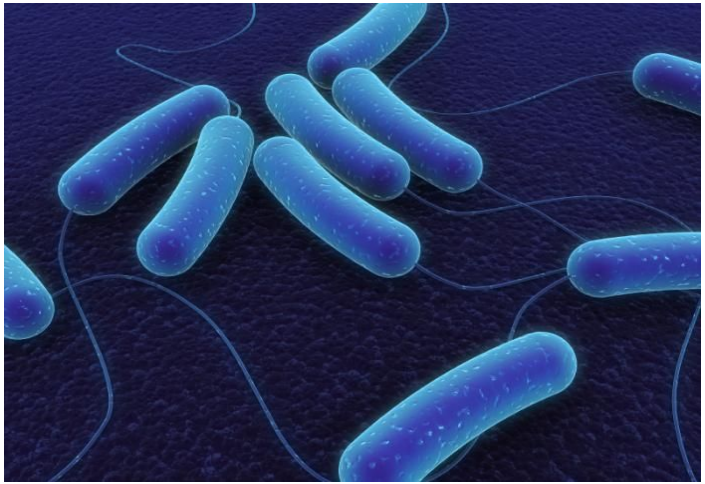
What is Programmed Cell Death?

- Programmed cell death (or PCD) is the death of a cell in any form, mediated by an intracellular program.

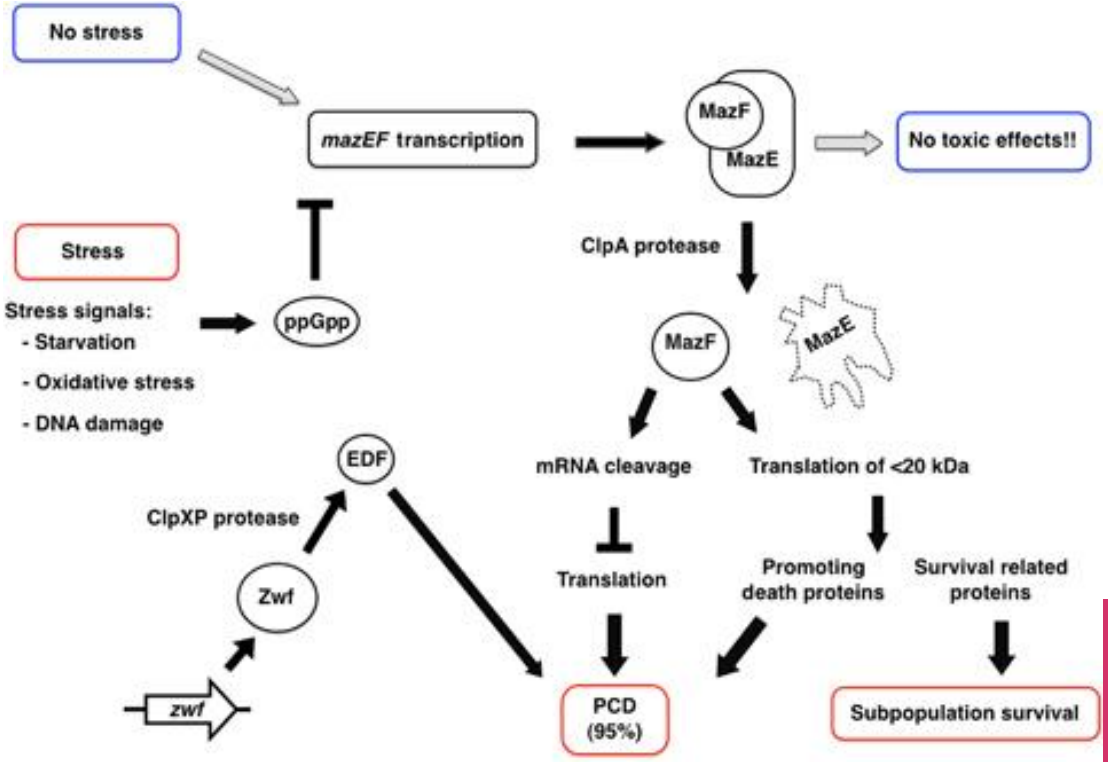


Mechanism of PCD in *E. Coli*

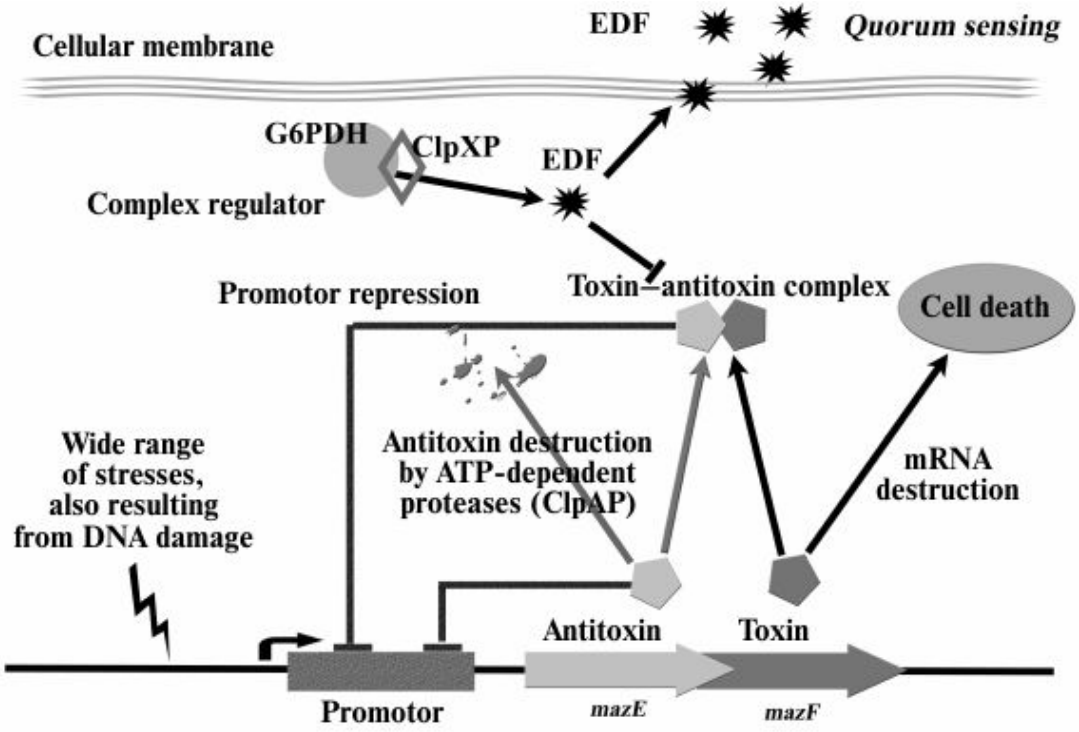
- It is mediated by the toxin–antitoxin system module *mazEF*.
- *mazF* encodes a stable toxin, MazF, and *mazE* encodes a labile antitoxin, MazE, which prevents the lethal effect of MazF.



Why E. Coli?

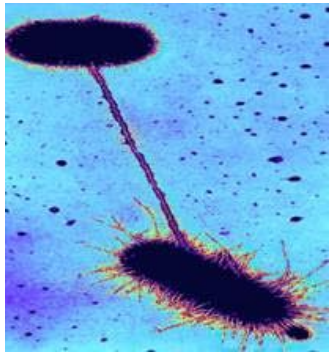

No stress
mazEF transcription
MazF
MazE
No toxic effects!!
Stress
Stress signals:
- Starvation
- Oxidative stress
- DNA damage
ppGpp
ClpA protease
MazF
MazE
EDF
ClpXP protease
Zwf
zwf
mRNA cleavage
Translation of <20 kDa
Translation
Promoting death proteins
Survival related proteins
PCD (95%)
Subpopulation survival

Why Synthetic Biology?

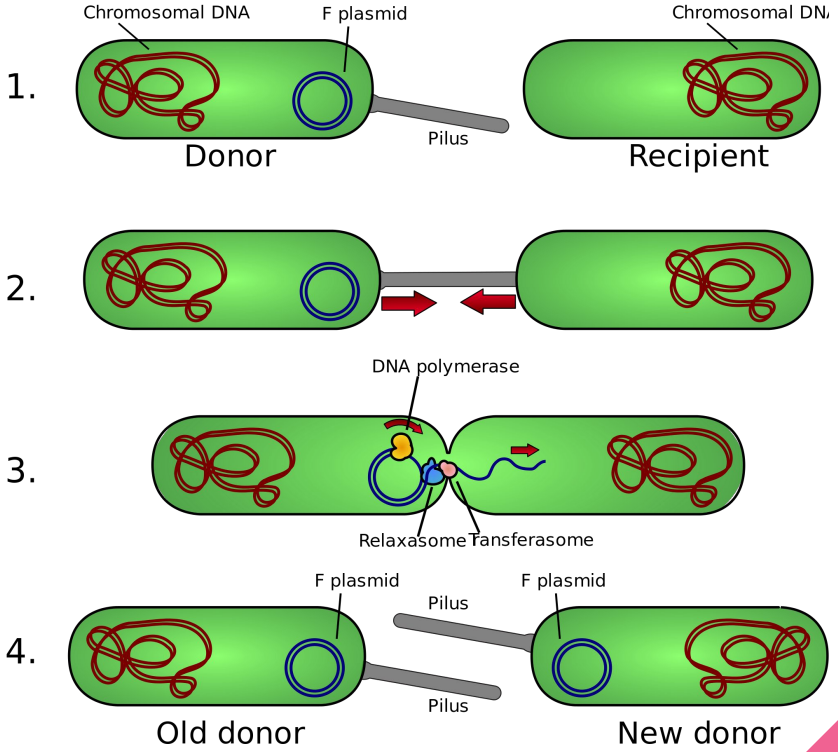


Project Description

- Testing whether bacterial conjugation can result in the Programmed Cell Death in other cells.
  - *Thiobacillus Ferrooxidans* offers a unique and easily quantifiable was to do this.
- Once the principle has been proven, there are multiple applications.



Therapeutic Implications



Bacteria as a drug delivery system

Applications: Cancer

Bacteria specifically recognize biomarkers on cancer cells

Drug delivery through simultaneous lysis of the entire population vs. continuous secretion in proliferating individuals

Simultaneous lysis minimizes adverse systemic inflammatory response from patient

Bacteria grows well in anaerobic environment

References: Anderson et al 2005, Zhou 2016, Din et al 2016

Applications: Cancer (challenges)

bacteria alone (whether engineered or not), are unlikely to eradicate tumours: treatment of mice with the engineered microbes in combination with chemotherapy did not destroy the tumour; instead tumours shrank for 18 days, after which regrowth occurred

A curative therapeutic approach: further improvements to engineered bacteria or using bacteria in combination with immunotherapy or other, more-powerful anticancer agents

References: Zhou 2016, Din et al 2016

Applications: other

diseases that require periodic dosing, such as diabetes and high blood pressure

Target natural niches for cyclical bacterial colonization

implantable, semipermeable cassette that can be traversed by proteins and small molecules but not by bacteria: host the engineered microbes

General challenge: bacterial-degradation by-products released in each lysis cycle might be absorbed into the blood and build up, causing toxic systemic effects (use less toxic or attenuated strains)

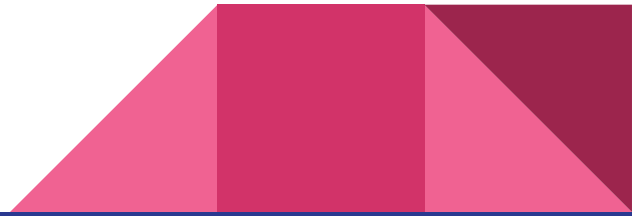
References: Zhou 2016

Release of elements in bacteria

Release cytotoxic agents

Release anti-cancer drugs

Release interference RNA



Synthetic biology: Bacteria synchronized for drug delivery

Shibin Zhou

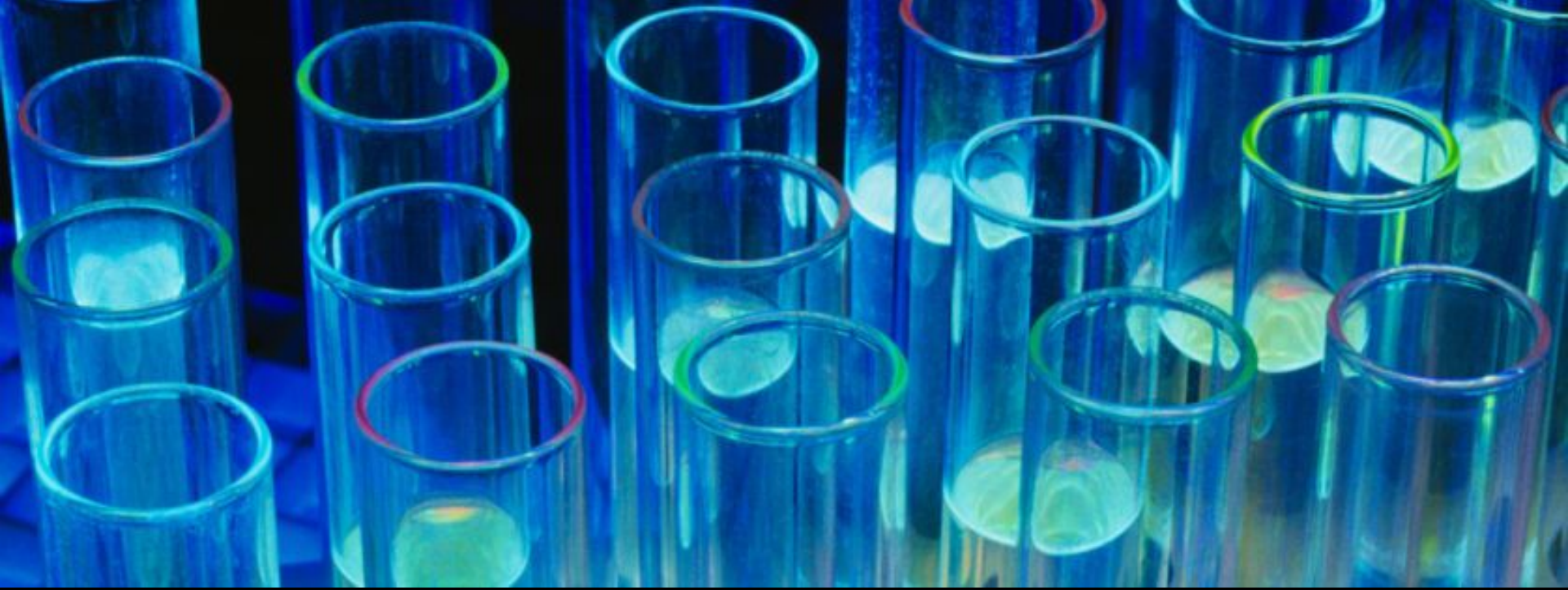
Nature **536**, 33–34 (04 August 2016) | doi:10.1038/nature18915
Published online 20 July 2016

A synthetic genetic circuit that mimics the quorum-sensing systems used by bacterial populations to coordinate gene expression enables bacteria to deliver drugs to mouse tumours in repeated and synchronized cycles. See Letter p.81

Subject terms: Synthetic biology · Cancer

Humans and bacteria have a long history of parasitic and symbiotic relationships. Now, Din *et al.*[1] exploit a relationship between bacteria and diseased human tissue for a therapeutic purpose. On page 81, the authors outline a system in which engineered bacteria acting as drug-delivery vehicles simultaneously break down, releasing an antitumour drug in synchronized cycles to maximize delivery efficiency and minimize toxicity.

In the body, some niches for bacteria — such as the anaerobic lumen of the intestines — have



CRISPR/Cas9 : A Potent Gene Editing Weapon against Cancer and Diseases

Brandon Cuevas, Panagiotis Oikonomou

Contents

- Main Ideas for specific Application
  1.) CRISPR & Breast Cancer
  2.) CRISPR & HIV
  3.) CRISPR & Mosquito Threat
- CRISPR Background Information
- Other ideas
- Discussion - Questions

Background Information – Gene editing methods

Reagents have been developed that are able to target specific nucleotide sequences
Multifarious clinical applications: genetic diseases, cancers, viral infections.
These reagents fall into three main categories:

i. Zinc-finger nucleases (ZFN)
   - DNA double-strand breaks (DSBs) → repaired by nonhomologous end joining (NHEJ)
   - significantly error-prone process.

ii. The transcription activator-like effector nuclease (TALEN) system,
   - fusion proteins, secreted by Xanthomonas bacteria

iii. The CRISPR-associated 9 (Cas9), the third and most powerful class, which provides unprecedented control over genome editing

Background Information – Why CRISPR?

Advantages

1. **Simplicity:**
   - The target sequence relies on RNA complex formation
   - gRNAs can be designed easily and inexpensively
   - They can target virtually any sequence in the genome.

2. **Efficiency:**
   - Modifications can be introduced by directly injecting the necessary components (Cas9 protein and gRNA)
   - No need of transfecting and selecting special cells that are required to create targeted mutant tissues

3. **Multiplexed Mutations:**
   - Multiple genes can be mutated simultaneously by co-injecting the endonuclease Cas9 with the corresponding gRNAs.

Function

- The CRISPR/Cas9 system consists of two distinct components: a gRNA and an endonuclease Cas9
- The gRNA is designed to contain a 20 base-pair guide sequence, which employs the gRNA/Cas9 complex to its target
- Protospacer Adjacent Motif (PAM), which is the trinucleotide sequence immediately following the target sequence.

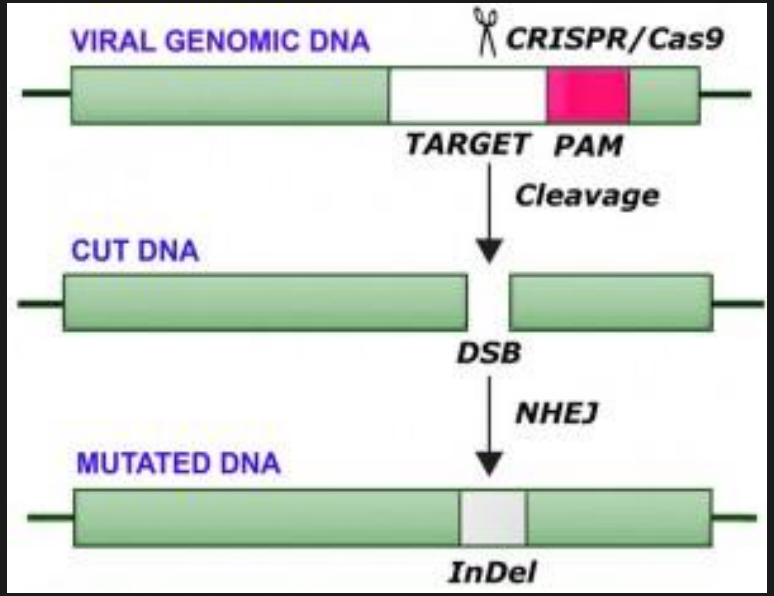
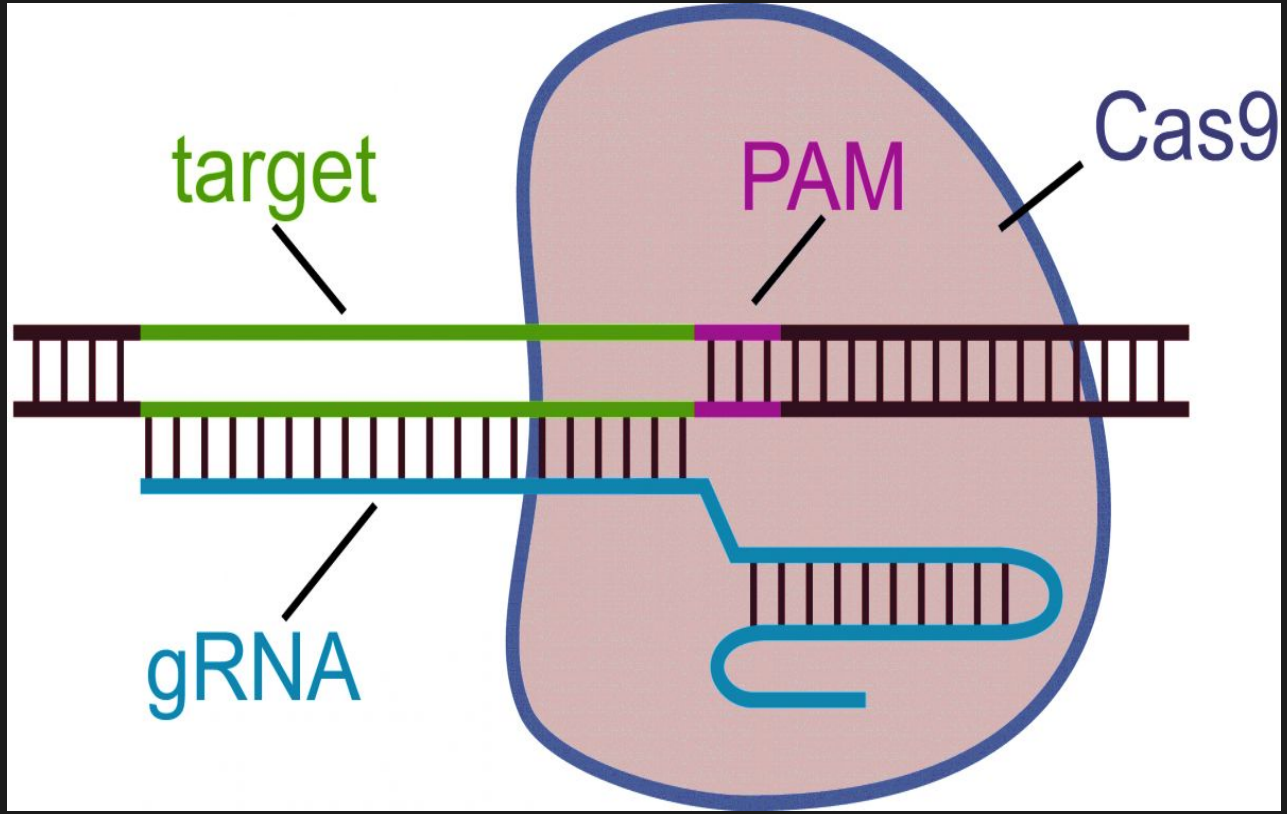


Figure 2.

Representation of mutagenesis of viral DNA by CRISPR/ Cas9.

Function



Implications of Function

- Almost exclusive on-target cleavage, given the large size of the human genome.
- Moreover, the Cas9 system continues to be modified and improved with respect to its genome editing efficiency and reduction of potential off-target effects.
- “paired Cas9 nickase strategy, which increases the site specificity DSB induction and can reduce off-target activity by 50- to 1,500-fold in cell lines.”
- In principle, eradication of viruses from cells by the CRISPR/Cas9 method should be possible to any DNA virus or RNA virus *

First Idea: Application of CRISPR to strengthen immune response against breast cancer

- Immune cells can be taken from patient and then modified by disabling a gene with CRISPR/Cas9 system
- Gene codes for PD-1 protein, which has the ability to halt any immune response; cancer cells take advantage of this.
- After modified cells are placed back into the body, immune cells should be able to respond to the tumor threat

Pros vs. Cons of CRISPR Immunotherapy

Pros	Cons
● Recent scientific advancements makes this process (CRISPR/Cas9 in general) somewhat reversible with anti CRISPR proteins	● Might be inferior to monoclonal antibody therapy
● No need to specifically target the tumor or any gene located in the tumor; immune cells will locate the tumor and attack	● Difficult to apply this modification to all immune cells (or at least enough) immune cells in human body
● No targeting of healthy, surrounding cells	● Difficult to test; cancer cell cultured and grown in GEMM for highly accurate results

Second Idea: (Viruses) Applications with both **therapeutic** and **prophylactic** potential

i. Human Papillomaviruses (HPV16 and HPV18)
ii. Hepatitis B virus (HBV)
iii. Epstein-Barr virus (EBV)
iv. HIV-1

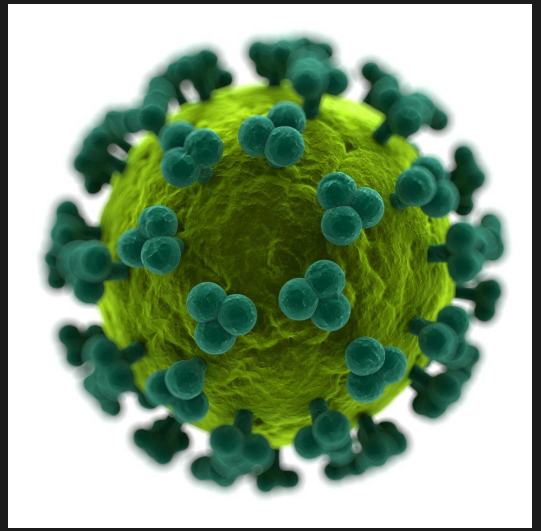
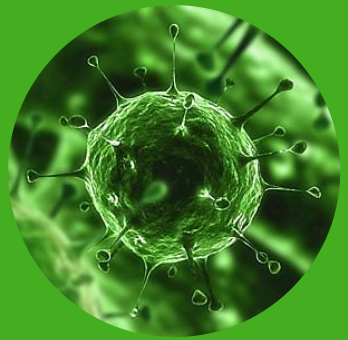


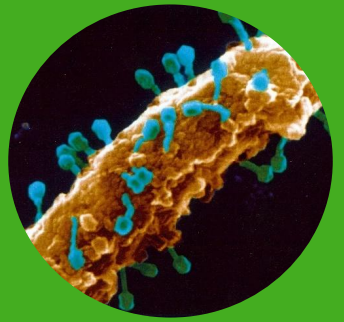
Figure 1. The HIV Virus

Intricacies of virus treatment



Viruses have intricate life cycles; they reproduce within the host cell and use “domestic” proteins. This makes it hard for our organism to identify and attack only the viral products.

The main reason why neutralizing viral pathogens has not been as effective as combating bacterial ones is their vastly different metabolism pattern.



Viruses can persevere by entering a latent state. During this latency period, they persist by remaining relatively inactive and producing very few proteins, rendering the targeting of merely their proteins futile.

Problem of Interest - Statistics (HIV)

- More than **1.2 million** people in the US are living with HIV, and about **1 in 8** are unaware of it.
- In 2014, an estimated **20,896** people were diagnosed with AIDS (an estimated **1,210,835** people have been diagnosed with AIDS since the beginning of the epidemic in the early 1980s).
- In 2013, there were an estimated **12,963 deaths** (due to any cause) of people with diagnosed HIV infection ever classified as **AIDS**, and **6,955 (~53%)** deaths were attributed directly to HIV.

(Statistics provided by the official U.S. government website, managed by the U.S. Department of Health & Human Services)

Discussion

- Potentially more effective than antiretroviral drugs
- Dealing with viral reservoirs
- Simplicity → integration in developing countries where sophisticated health care facilities are not always available

Third Idea: Application of CRISPR on Mosquito Threat

- Mosquitoes are well known to be carriers for a variety of diseases, some of which include Dengue fever, Zika virus, West Nile virus, Malaria, chikungunya, and yellow fever
- According to WHO, mosquitoes are responsible for taking more than 1 million lives annually.
- CRISPR can be used to engineer a gene drive that would force the new inserted gene--which makes the mosquito immune to the disease-- to become dominant over generations (almost always passed to offspring as opposed to 50% chance of being passed down)
- This technique can also be applied to other disease carriers other than mosquitoes, including fleas, ticks and flies

Pros vs. Cons

Pros	Cons
● Huge impact on the number of lives that can be saved	● Controversy over use of gene drive--bacteria might adapt and become an even bigger threat if change does not spread quickly enough.
● Usage of gene drive method nearly guarantees that mosquito offspring will have modified gene	● With this method, only a particular disease can be targeted, and mosquitoes carry many diseases.
● Modified genes can be created for many of the diseases that mosquitoes carry	● If entire mosquito species is targeted, debate over how the elimination of mosquitoes can affect the ecosystem would arise

Additional Ideas

- HPV - cancer related
- iGEM Past projects : eg. Coloring (team 2015 - A Light-inducible CRISPR/Cas9-mediated gene expression activation system in E. Coli and Yeast)
- Honeybee mysterious disappearance
- Genetic diseases such as Huntington’s Disease
- Aging
- Regeneration

References

White, Martyn K., Wenhui Hu, and Kamel Khalili. "The CRISPR/Cas9 Genome Editing Methodology as a Weapon against Human Viruses." Discovery Medicine. U.S. National Library of Medicine, Apr. 2015. Web. 01 Oct. 2016.

Park, Alice. Khalili, Kamel. "Cure for HIV Gets Closer as Researchers Snip Virus Out of Cells." Health Research. Time, 19 May 2016. Web. 05 Nov. 2016.

Yeadon, Jim, Ph.D. "Pros and Cons of ZNFs, TALENs, and CRISPR/Cas." The Jackson Laboratory. Jackson Laboratory, n.d. Web. 13 Nov. 2016.

"HIV IN THE UNITED STATES: AT A GLANCE." U.S. Statistics. U.S. Department of Health & Human Services, 02 Dec. 2014. Web. 13 Nov. 2016.
Boundless. “DNA Sequencing Techniques.” *Boundless Biology*. Boundless, 26 May. 2016. Retrieved 13 Nov. 2016

Lee and Fidock: CRISPR-mediated genome editing of Plasmodium falciparum malaria parasites. Genome Medicine 2014 6:63. doi:10.1186/s13073-014-003-9

http://www.nature.com/cgt/journal/v22/n11/full/cgt201554a.html

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4445958/

http://www.nature.com/news/2010/100721/full/466432a.html --mosquito ecosystem debate

http://www.nature.com/news/crispr-gene-editing-tested-in-a-person-for-the-first-time-1.20988 --CRISPR cancer application

http://www.livescience.com/57340-crispr-off-switch-discovered.html --CRISPR “offswitch”

https://www.youtube.com/watch?v=TnzcwTyr6cE video on CRISPR application to mosquito threat

https://www.hindawi.com/journals/bmri/2014/612823/-- HPV and CRISPR