F.B.I.

Featuring spiRNA & S.O.R.D. Secretor Of RNA Device

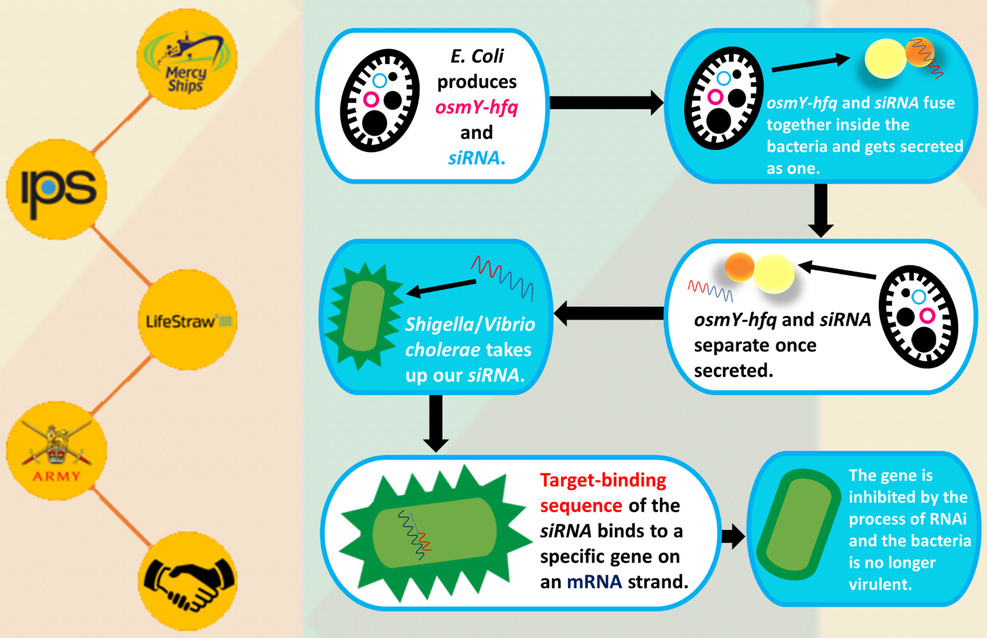
FIGHTING BACTERIAL INFECTIONS

Albert Brown Bartosz Witkowski Matthew Simmons Mia Wood Beth Rear Darryl Sime

MISSION BRIEF

To tackle the problem of waterborne bacterial infections in developing countries: namely, Cholera and Shigellosis.

These two targets have been selected due to the sheer scale of fatalities they cause via unaffordable or inaccessible cures.



BIOLOGY

HOW WILL WE ACHIEVE THIS?

We settled on combating these infections via RNA interference - but who could possibly help us in this enormously microscopic task?

This is where we decided to enlist the help of two brand new special agents:

S.O.R.D.
Secretor Of RNA Device

S.O.R.D. is our RNA secretion device, used to get our sRNA out of the *E. coli* that produced it and into its environment.

It is a fusion of the proteins OsmY (a protein naturally secreted by *E. coli*) and Hfq (RNA binding protein).

Therefore the role of the OsmY part of S.O.R.D. is to traffic the device out of the cell while the Hfq part binds to the spiRNA to allow the whole complex to escape from the producing cell

The function of the HA tag is so that it could be identified in experiments.

spiRNA

spiRNA is what we have nicknamed the sRNA secreted from our producing *E. coli* cell.

It can be modified to target any gene in a pathogenic bacterium; the spiRNA will bind to the mRNA transcribed by this gene, thus making it double stranded and unfit for translation.

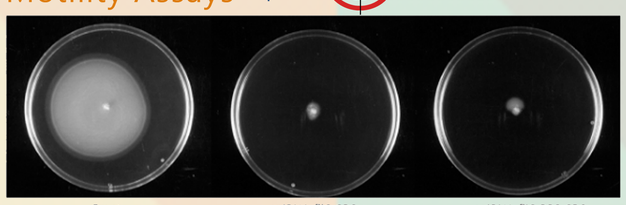
By targeting a gene vital to a bacterium's virulence or growth, the bacterium will die or be of no threat.

Each spiRNA consists of two main parts: a target-binding sequence (specific to the intended gene), as well as a section for binding to the Hfq in our fusion protein.

Target-binding Sequence

RESULTS

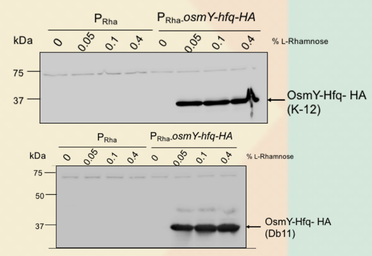
Motility Assays spiRNA



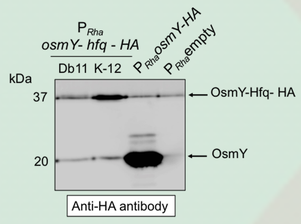
Empty spiRNA-fliC-CDS spiRNA-fliC-RBS-CDS

To test the targeting propertes of our spiRNA, we modified the target secquence so it was complimentary to *fliC*, a gene responsible for the proteins which make up the flagellum of *E. coli*.
The control plate shows that without our spiRNA, motility was maintained. Once added however, the motility was hugely decreased.
This proves that our spiRNA works and could be used to target any gene.

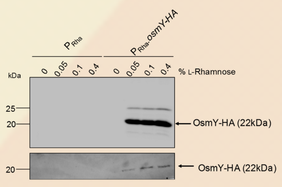
Western Blots S.O.R.D.
Secretor Of RNA Device



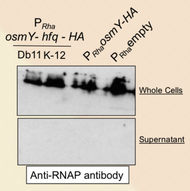
These western blots show that our fusion proteins (S.O.R.D.) OsmY-Hfq are being expressed inside the cell.



In this blot, we were checking if S.O.R.D. is being secreted from the cell

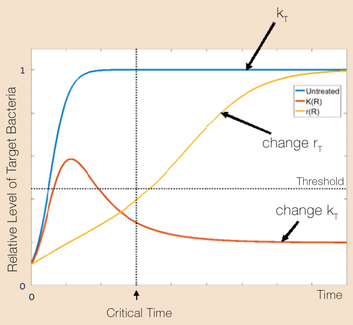


We performed a western blot on our characterised OsmY-HA tag to make sure it's being both expressed inside the cell and secreted out of it.



To ensure that the cells producing our OsmY-Hfq fusion were not undergoing lysis, we performed a western blot using an Anti-RNAP antibody.

MODELLING



Possible Equations

$\frac{dP}{dt} = r_P.P\left(1 - \frac{P}{k_P}\right)$ Rate of growth of produing bacteria and cell capacity

$\frac{dR}{dt} = k_R.P - k_D.R.T$ Rate of siRNA production, degradation minus the comsumtion by the target cell

$\frac{dT}{dt} = r_T(R).T(1 - \frac{T}{k_T(R)})$ Inhibition of target bacteria growth rate

By utilising mathematical modelling in our project, we have been able to use it to predict roughly how much spiRNA producing *E. coli* we would need in order to theoretically render any *Vibrio cholerae* or *Shigella* harmless.

It can also be used as a way of which to predict the outcome of an experiment without having to manually repeat it many times.

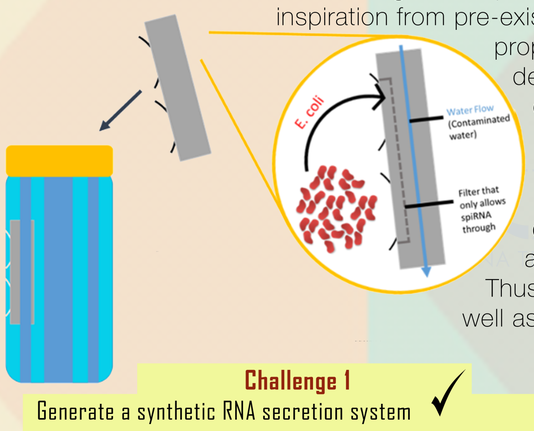
HUMAN PRACTICES

Our Final Product

After talking to multiple people and drawing inspiration from pre-existing products, our final proposal is a water bottle with a detachable filter that will hold our secretion system.

We talked to our local army medical squadron, who told us that they often handed out mosquito nets whilst on duty. They also told us water bottles are very much sought after.

Thus, we now have a useable product as well as a way by which to distribute it.



Challenge 1 ✓
Generate a synthetic RNA secretion system

Created our fusion protein S.O.R.D., which we have proved successfully secretes the RNA out of the cell.

Challenge 2 ✓
Design a modular RNA interference device

Created spiRNA, which we have proven can be modified to target and silence a specific gene.

Challenge 3 ✓
Find an efficient way to distribute our project

Found out from our local army squadron that they hand things out to villages when on duty; they could possibly take our bottle and filter.

FUTURE PLANS

In the lab there's still some unfinished work we would like to have completed. It would have been great to see if our spiRNA was actually being secreted out of the cell and if it could be taken up by the target bacteria. In the very far future it would also be good to test our idea on someone with Cholera or Shigellosis, to see if our product could function as a cure as well as a preventative.

We also would liked to have created a prototype of our filter, to get an idea of how much it would cost to manufacture.

Another plan we had was to see our product also being used in developed countries; for example, the town of Flint, Michigan is currently in crisis due to unclean water causing outbreaks of Cholera and Shigellosis. Our concept would be the same, but a payment would be involved and it would be just the filter on its own. This way, our product could potentially make a profit or at least sustain itself, ensuring our main goal of eradicating 3rd world countries of waterborne bacterial infections may actually be completed.