FOR iGEM HQ: DUE TO THE NATURE OF OUR ANIMATIONS, SOME SLIDES MAY BE UNCLEAR
E.R.A.S.E
Explosives Remediation by Applied Synthetic E.coli
**TNT AND NITROGLYCERIN POLLUTANTS**

**Direct Health**
- carcinogenic
- cause of cataracts
- cause of liver damage
- anemia

**Environmental**
- accumulation in water
- toxic to plants

**Economic**
- diminished crop yields
- cost of healthcare
- decreased land value
- cost of remediation
THE PROBLEM

TNT and NG waste sites
THE PROBLEM

In WWII 800,000 t TNT produced

For every ton: 40,000 L of water contaminated

Germany
THE PROBLEM

56 military sites

Hawaii: 1.4 g NG per Kg of soil

New Mexico: 2.5 g TNT per Kg of soil

Texas: 32 g TNT per Kg of soil
CURRENT DECONTAMINATION PROCESSES

Carbon adsorption columns

- Equipment is expensive
- HPLC required for confirmation
- Specialist training required
- Cannot be performed on site
CURRENT DECONTAMINATION PROCESSES

Contained incineration

Destroyed vegetation

Reduced soil fertility

Atmospherically polluting
Funding: £4 million.

Looking for short-term, proof-of-concept research proposals including:

“sensor technologies to detect chemicals, such as explosives...”

“... and decontamination approaches.”
IDEAL SYNTHETIC SOLUTION

Enzymes
Degrade explosive pollutants to harmless products

Kill Switch
Terminates lifecycle of organism upon completion of function

Promoter
Detects substrate and regulates specific response
IDEAL SYNTHETIC SOLUTION

**Enzymes**
Degrade explosive pollutants to harmless products

**Sensor**
Produces an observable output signal to confirm substrate presence

**Kill Switch**
Terminates lifecycle of organism upon completion of function

**Promoter**
Detects substrate and regulates specific response
PROJECT AIMS

Enzymes
Degrade explosive pollutants to harmless products

Promoter
Detects substrate and regulates specific response
MODELLING

To assist with the design, analysis and debugging our system.

Which properties should be characterised by experiment?

Provides an abstract representation of how our project works.
BIOCHEMICAL LEVEL

Degradation rate is most influenced by enzymes’ kinetic performance.

This model considers:  - Enzyme kinetics  - Substrate toxicity

Conclusion: Km, Vmax and toxicity must be experimentally determined to indicate how our system may perform.
MULTI-CELLULAR LEVEL  A spatial simulation of a population

Degradation rate \( \alpha \) [substrate] AND availability

Growth response to regions of varying [substrate]

Accounts for residual [substrate] remaining after cell death

Accounts for intracellular [substrate] in daughter cells after division

Probability cells dying or dividing (\( \alpha \) local [substrate])
The population may not degrade all TNT and NG in a sample where substrate distribution is non-uniform.
Our bacteria must be able to cope with samples where substrate distribution is non-uniform.

The maximum distance between islands of substrate can be altered by tuning different aspects of the bacteria:

Kill switch delay    Enzyme kinetics    and more
AIM 1: IDENTIFICATION OF AND CHARACTERISATION OF ENZYMES
IDENTIFICATION OF ENZYMES

PETN reductase enzyme is in the iGEM Registry
But was not completely characterised

Uncharacterised enzymes from scientific literature:

XenB (BBa_K1398001)
NemA (BBa_K1398003)

Higher affinities for both TNT and NG than PETN reductase
Similar to the comparatively well understood PETN reductase
PURIFIED NEMA DEGRADATION OF NITROGLYCERIN

Control experiments:

1. No protein

1,2,3. Protein minus cofactors

4. Cofactors + NemA protein:

90% of Nitroglycerin degraded after 15 minutes at room temperature

Error: standard deviation in Raman spectrum
IN VIVO DEGRADATION OF TNT

Degradation of the aromatic ring of TNT causes a distinctive set of colour changes to be observed in the sample:

Pablos et al. 2014: created a non biological sensor using the red coloured meisenheimer complex.

We observed red fading to yellow, indicating further degradation.
IN VIVO DEGRADATION OF TNT

Wild Type

Remains colourless

40 min  80 min  160 min
IN VIVO DEGRADATION OF TNT

XenB

40 min  80 min  160 min
IN VIVO DEGRADATION OF TNT

NemA

40 min  80 min  160 min
Vi as a function of [nitroglycerin]

Asymptote, $V_{\text{max}} \approx 21 \ \mu\text{mol mg}^{-1}\ \text{min}^{-1}$

$K_m \approx 6 \ \text{mM}$
NemA degrades nitroglycerin *in vitro*

NemA and XenB degrade TNT *in vivo*

Preliminary kinetic characterisation of NemA

Development of Raman and HPLC techniques

Quantification of TNT and NG was VERY challenging

This further highlights the need for a synthetic biological solution to quantification of these substrates
AIM 2: IDENTIFYING AND ENGINEERING A PROMOTER
TWO PROMOTERS BASED ON THE NEMR RESPONSE

NemR UIR
Responsive to the TNT-binding repressor protein NemR

Synthetic promoter
Minimal fluorescence in control cells

Constitutive expression for NemR UIR

Our inducible synthetic promoter responds positively to increasing TNT concentration
CONCLUSIONS OF PROMOTER ASSAYS

We have a promoter that responds to TNT.

This could facilitate use of:

• A biosensor
• A kill switch

In future: test linear response to increasing concentrations of TNT.
**IDEAL SYNTHETIC SOLUTION**

- **Enzymes**
  - Degrade explosive pollutants to harmless products

- **Kill Switch**
  - Terminates lifecycle of organism upon completion of function

- **Promoter**
  - Detects substrate and regulates specific response
IDEAL SYNTHETIC SOLUTION

Enzymes
Degrade explosive pollutants to harmless products

Kill Switch
Terminates lifecycle of organism upon completion of function

Sensor
Produces an observable output signal to confirm substrate presence

Promoter
Detects substrate and regulates specific response
Enzymes degrade explosive pollutants to harmless products.

Promoter detects substrate and regulates specific response.
OUTREACH: E.R.A.S.E THE GAME!

A mobile game on Google Play Education to show off the degradation side of our project!

Keep your bacteria alive by jumping the toxic molecules and gain health by collecting TNT! You will starve by 1 health every 5 seconds!

ACKNOWLEDGMENTS

Undergraduate Team
Ed Muir
Peter Reader
Bethany Hickton
Ben Miller
Katie Pearce
Max Smart
Martyn Bennett
Jessica Rollit
Elize Hernandez

Supervisors
Dr. John Love
Dr. Thomas Howard
Dr. Paul James
Dr. Lizzy Dridge
Dr. Christine Sambles