"Parasite detection with a rapid response"
PARASIGHT

Making the Invisible Visible
Synthetic biology: Solving problems in the developing world

Rapid detection for the real world

Schistosomiasis: 200 million infected

Consulted experts

Human Practices Panel Discussion

Specifications
Schistosomiasis is a major global health problem.
Schistosoma:
Complex lifecycle, problematic detection

Rapid detection for the real world

Schistosomiasis: 200 million infected

Consulted experts

Human Practices
Panel Discussion

Specifications

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Schistosoma:
Complex lifecycle, problematic detection

Rapid detection for the real world

Schistosomiasis: 200 million infected

Consulted experts

Human Practices Panel Discussion

Specifications
Multidisciplinary panel consulted to inform our design

Rapid detection for the real world

Schistosomiasis: 200 million infected

Consulted experts

Human Practices Panel Discussion

Specifications
Human practices defined our initial specifications

Specifications for a detection kit for water-borne parasites:

- Fast (minutes)
- Inexpensive
  - Production
  - Testing equipment
- Easy to use, store and transport
- Safe

- Rapid detection for the real world
- Schistosomiasis: 200 million infected
- Consulted experts
- Human Practices Panel Discussion
Our three stage synthetic biology device

Detection
- Input – Prarasitic protease
- Output – Autoinducing peptide

Signal transduction
- Input – Autoinducing peptide
- Output – TEV protease

Output
- Input – TEV protease
- Output – Coloured compound

Signal transduction
The Engineering Design Cycle

Human practices

Specification

Testing

Assembly

Modelling

Design

Optimization

S

T

A

M

D

1 2 3 4 5

S D M A T
Overview

- Detection
- Signal transduction
- Output

Detection → Signal transduction → Output

Signal transduction involves the conversion of an extracellular signal into an intracellular response. The process can be complex and involves a series of steps including receptor activation, G protein coupling, and downstream signaling pathways.
Bacillus subtilis can be used safely

Bacillus subtilis

Non-pathogenic
Sporulation

3 novel genomic integration vectors

AmyE  Starch catabolism
PyrD  Pyrimididine synthesis
Dif-sites  no spread of resistance
Module I

Detection

Signal transduction

Output
Reliable Detection

- Specific → single protease
- Robust → reduced noise
- Fast → efficient signalling
The surface protein contains an **Auto Inducing Peptide**

**Efficient**

**Linear peptide**

**No crosstalk**

*S. pneumoniae*
The cleavable linker confers specificity

Recognition motive

Specific
Cell Wall Binding Domain for attachment

Isolated from LytC-protein Cell Wall Binding Domain

Sequestration on cell wall
The detection module is highly efficient

Surface [AIP] = 1.3×10^{-3} M
Optimal [AIP] = 4.4×10^{-9} M

\[
\begin{align*}
E + S & \rightleftharpoons ES \rightarrow E + P \\
k_1 & \quad k_3 \\
k_2 & \\
\end{align*}
\]

\[
\begin{align*}
[\dot{E}] &= -k_1[E][S] + (k_2 + k_3)[ES] \\
[\dot{S}] &= -k_1[E][S] + k_2[ES] \\
[\dot{ES}] &= k_1[E][S] - (k_2 + k_3)[ES] \\
[\dot{P}] &= k_3[ES] \\
\end{align*}
\]
Threshold levels are easily reached

Using standard TEV-protease kinetics

Average amount of time needed for receptor activation after inducing the system with a known initial TEV concentration

Initial TEV concentration [nM]

Average time [min]

1.7 min
Assembly of the Detection Module

- Cell wall binding domain
- Surface protein

Surface protein testing construct with 6 alternative linkers

- Linker 1
- Linker 2
- Linker 3
The Modularity of our Fundamental Technology

- Schistosomiasis
- Chagas' disease
- C3 Convertase
- Leishmanolysin

Indicate acute infection

350 million people
Our Software Tool

Select Protease

Cruzipain

Generate!

Description
Currently, a lumbar puncture is necessary to diagnose the devastating Chagas' disease. However, a simple blood test could test for a specific protease called cruzipain which is produced by the Trypanosoma cruzi parasite.

Yellow - Biobrick Prefix/Suffix
Orange - Promoter
Testing allows determination of optimal linker-version

1. Salt elution and protein purification → Surface [AIP]

2. Protease exposure and protein purification → Cleavage efficiency
Summary: Detection module

Assembled and ready for testing
Module II

Signal Transduction

Detection  Signal transduction  Output
Specific \rightarrow single output

Robust \rightarrow reduced noise
S. pneumoniae Peptide Quorum Sensing in *B. subtilis*
ComE activation depends on [Auto Inducing Peptide] and [Receptor]

1. \[
\frac{d[AIP]}{dt} = -k_1[AIP][ComD] + k_{-1}[AIP - ComD]
\]

2. \[
\frac{d[ComD]}{dt} = -k_1[AIP][ComD] + k_{-1}[AIP - ComD]
\]

3. \[
\frac{d[AIP-ComD]}{dt} = k_2[AIP][ComD] - k_{-2}[AIP - ComD] + k_{-2}[AIP - ComD^*] - k_2[AIP - ComD][Phosphate] + (k_4 - k_{-4})[AIP - ComD^*][ComE^*]
\]

Rate(ComE_P) ≈ [Receptor]_0 + [AIP]_0

4. \[
\frac{d[ComE]}{dt} = -k_3[ComE][AIP - ComD^*] + k_{-3}[AIP - ComD^* - ComE]
\]

5. \[
\frac{d[AIP-ComD^*-ComE]}{dt} = k_3[ComE][AIP - ComD^*] - k_{-3}[AIP - ComD^* - ComE] + k_{-4}[AIP - ComD^*][ComE^*]
\]

Production rate of ComE-P

6. \[
\frac{d[ComE]^*}{dt} = k_4[AIP - ComD^* - ComE] - k_{-4}[AIP - ComD][ComE^*]
\]
Assembly of the Signal Transduction Module

K316013  K316014

ComE, ComD expression

ComE responsive promoter, optimized RBS, TEV-protease
Summary: Signal transduction

Specific

Robust

single output

reduced noise

Amenable to optimisation
Module III

Output
Specification

- Fast
- Simple
- Visual
Direct Transcription/Translation

DNA → Output Molecule (e.g. GFP)

Output vs. Time

Visibility Threshold
1 Step Enzymatic Amplification

DNA → Enzyme → Pre-synthesised Substrate

Output vs Time

Visibility Threshold
2 Step Enzymatic Amplification

DNA → Enzyme → Deactivated Enzyme → Pre-synthesised Substrate

Output vs Time

Visibility Threshold
Output Modelling

• Equations developed to describe system

• 1, 2 and 3 step amplifications modelled

1. \( T + sT_a + sT_b + sD \leftrightarrow T - sD \rightarrow T + D + sT_a + sT_b \)
2. \( T + sT_a + sT_b + D \leftrightarrow T - sT_a \rightarrow T + D + T_{sa} + sT_b \)
3. \( T + T_{sa} + sT_b + D \leftrightarrow T - sT_b \rightarrow T + D + T_{sa} + T_{sb} \)
4. \( T + T_{sa} + T_{sb} + D \leftrightarrow T_{sa} - T_{sb} \rightarrow T + D + T_s \)
5. \( T + sT_a + sT_b + sD \leftrightarrow T - sT_a \rightarrow T + sD + T_{sa} + T_{sb} \)
6. \( T + T_{sa} + sT_b + sD \leftrightarrow T - sD \rightarrow T + D + T_{sa} + sT_b \)
7. \( T + T_{sa} + sT_b + D \leftrightarrow T - sT_b \rightarrow T + D + T_{sa} + T_{sb} \)
8. \( T + T_{sa} + T_{sb} + D \leftrightarrow T_{sa} - T_{sb} \rightarrow T + D + T_s \)
9. \( T + sT_a + sT_b + sD \leftrightarrow T - sT_b \rightarrow T + sD + sT_a + T_{sb} \)
10. \( T + sT_a + T_{sb} + sD \leftrightarrow T - sD \rightarrow T + D + sT_a + T_{sb} \)
11. \( T + sT_a + sT_{sb} + D \leftrightarrow T - sT_a \rightarrow T + D + T_{sa} + T_{sb} \)
12. \( T + T_{sa} + T_{sb} + D \leftrightarrow T_{sa} - T_{sb} \rightarrow T + D + T_s \)
13. \( T + sT_a + sT_b + sD \leftrightarrow T - sT_a \rightarrow T + sD + T_{sa} + sT_b \)
14. \( T_s + T + sD \leftrightarrow T_s - sD \rightarrow T + D + T_s \)
Our Design

DNA → Deactivated XyE Enzyme → Catechol Substrate
Our Design
BBa_K316010

BBa_K316009
Extensive XylE characterisation

Existing part - BBa_J33204

Spectra of cultures after catechol addition

Michaelis-Menten Curve of Catalase(2,3)dioxygenase (in vitro - cell assay)
New Method for Characterising Promoters at Low Copy Numbers

Graph showing the relative promoter units over time for pVeg 3C/J23101 3C and pVeg 3K3/J23101 3K3 vectors.
Determination of visibility threshold
XylE-GFP fusion successfully limits enzyme activity

![Graph showing absorbance at 380nm over time. The graph compares natural XylE and GFP-XylE. Natural XylE shows a higher absorbance reaching a peak earlier and maintaining a steady state. GFP-XylE shows a slower absorbance increase, reaching the visibility threshold.]
TEV cleavage successfully activates GFP-XyIE
With characterisation data, our model shows 50% improvement.
Breakdown product attenuates cell growth.

**Xyle M9 Growth (600nm)**

- **Absorbance (600nm)**
- **Time (min)**

Lines represent different concentrations of Catechol:
- **1A 0 mM [Catechol]**
- **1D 0.5 mM [Catechol]**
- **1F 1 mM [Catechol]**
- **1G 1.5 mM [Catechol]**
- **1H 2 mM [Catechol]**
Output Summary

- New mechanism
- Faster than traditional systems
- Simple visual output
Specifications Achieved

- **Modular**: Customisable inputs and outputs
- **Fast response**: < 8 minutes
- **Easy to use**: Clear visual output
- **Easy to store & transport**: Spore-forming chassis
- **Inexpensive**: Low cost testing kit
- **Safe**: Non-pathogenic chassis, vectors & Dif excision
Building different prototypes allowed us to contextualise our detection kit.
Parasight
Achievements

- Real world application - Schistosomiasis
- Human practices defined our project
- Fast visual output
- Modular (surface protein – software tool)
- Characterisation of existing XyIE
- Characterisation of novel GFP-XyIE
- Submitted application to Gates Foundation

Extra data on the registry
Thank you
Any questions?

Our Team of 10 undergraduates