Polypeptide Digestion

Our solution has the potential to do this...
Applications

• Whole range of potential applications...

  Dietary Supplements

  Genetic disorders

  Chronic pain

• Projected sales in 2010 = $52 billion
Phenylketonuria (PKU)
- 1:10,000 sufferers worldwide
- Lack enzyme phenylalanine hydroxylase (PAH)

Phenylalanine
- Accumulation causes brain damage

PKU
- Severe mental retardation
- Behaviour problems

Currently no cure. Our project has the potential to treat this.
• Manufacture a polypeptide pill
• Deliver this past the stomach
• Deliver any biologically synthesisable compound
• Bypass the need for expensive storage, packaging and purification processes
Our Solution – The Engineering Approach

• Integrating key engineering concepts with the latest advances in Biology

• Creating a common platform using modular, reusable parts

• A multistage, integrated process
## Platform Specifications

The E.ncapsulator – Our versatile manufacture and delivery platform by which therapeutics can be reliably targeted to the intestine

1. **Polypeptide Production:** Must produce ANY polypeptide sequence
   - Use biosynthesis

2. **Polypeptide Protection:** Must protect polypeptide against pH 1-2 and dehydration
   - Use an acid resistant capsule
   - Use a preservative

3. **Safe & Acceptable:** Minimise risk
   - Non pathogenic chassis
   - Delete the genome

4. **Dosage & Usability:** Dosage must be tunable & pill must be usable
   - Use secondary encapsulation
We’ve solved this with a 5 step process...

By creating a manufacturing platform...
Our Engineering Control System

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>CHEMO-INDUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Polypeptide Production</td>
</tr>
<tr>
<td>0</td>
<td>Encapsulation</td>
</tr>
<tr>
<td>0</td>
<td>Genome Deletion</td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
Our Engineering Control System

- **Polypeptide Production**
  - 0: ON
  - 3: OFF

- **Encapsulation**
  - 0: ON
  - 3: OFF

- **Genome Deletion**
  - 0: ON
  - 3: OFF

- **Time (days)**
  - 0
  - 3

- **CHEMO-INDUCTION**
  - 0: ON
  - 3: OFF

- **AUTO-INDUCTION**
  - 0: ON
  - 3: OFF
Our Engineering Control System

Polypeptide Production

Encapsulation

Genome Deletion

Time (days)

0

CHEMO-INDUCTION

AUTO-INDUCTION

THERMO-INDUCTION
Chemoinduction

\[
\begin{align*}
\frac{d[mlacI]}{dt} &= k_{mlacI} - d_{mlacI} \times [mlacI] \\
\frac{d[lacI]}{dt} &= k_{placI}[mlacI] - d_{placI}[lacI]
\end{align*}
\]
Chemoinduction

Expression is controlled by LacI

\[ fh(lacl) = \frac{K^n}{K^n + [lacI]^n} \]

\[
\frac{d[lacI]}{dt} = k_{plac}[mlacl] - d_{plac}[lac] - k_1[lac][IPTG] + k_2[IPTG-lac]
\]

\[
\frac{d[lac-IPTG]}{dt} = k_1[lac][IPTG] - k_2[IPTG-lac]
\]
Data and Simulations

IPTG induction of Lac repressed promoter

Time (minutes)

OD Adjusted Fluorescence

Time (minutes)

Fluorescence

[ IPTG ]

- 1000μM
- 500μM
- 100μM
- 50μM
- 10μM
- 0 μM

Lac pI

RBS

GFP

T

T
System Overview

Module 1: Polypeptide Production
- Chemo-induction
- Auto-induction
- Thermo-induction

Module 2: Encapsulation
- Growth

Module 3: Genome Deletion
- Secondary Encapsulation
Cleavable Linker

Once liberated, the peptide is activated.
System Overview

Growth

Module 1
Polypeptide Production

Module 2
Encapsulation

Module 3
Genome Deletion

Secondary Encapsulation

Chemo-induction
Auto-induction
Thermo-induction
Building a Robust Timer

**Individual Timers**
- No idea!
- 1pm?
- 2pm?

**Population Timers**
- 3:53:32
Autoinduction

Glucose

Secondary Carbon Source

[g/l]

Time (hours)

[g/l]

Time (hours)
Glucose Tuneable Timer

![Graphic of glucose tuneable timer]

**Glucose to Xylose switch - 0.05% Glucose**

<table>
<thead>
<tr>
<th>Initial Glucose Concentration (%)</th>
<th>Time of switch (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>180</td>
</tr>
<tr>
<td>0.02</td>
<td>200</td>
</tr>
<tr>
<td>0.03</td>
<td>240</td>
</tr>
<tr>
<td>0.04</td>
<td>260</td>
</tr>
<tr>
<td>0.05</td>
<td>280</td>
</tr>
</tbody>
</table>

Plots part at 280 minutes
Which Carbon Sources?

GFP output from the PcstA promoter following overnight growth

- ON: 1% Casamino Acids
- OFF: 1% Glucose

Fluorescence / OD 600nm

- 7000
- 6000
- 5000
- 4000
- 3000
- 2000
- 1000
- 0
System Overview

Growth

Module 1
Polypeptide Production

Module 2
Encapsulation

Module 3
Genome Deletion

Secondary Encapsulation

Chemo-induction
Auto-induction
Thermo-induction
The Triple Hack

RcsB

Colanic acid synthesis

Rfal

YgiV

Biofilm formation

Tethered Export

Untethered Export

Untethered Export
RcsB Expression

RcsB predicted molecular weight = 23.67 kilodaltons
Colanic Acid Production

wt *E. coli*

RcsB +ve

---

Colanic Acid
Colanic Acid Protection

Cell survival after 4 hour pH 2 incubation

- **Control**
  - Before acid: $10^8$
  - After acid: $10^3$

- **Colanic Acid**
  - Before acid: $10^9$
  - After acid: $10^4$
Freeze Drying & Trehalose

Freeze Drying

Decomposition

Denatures Polypeptides & Damages membranes

Trehalose
System Overview

Growth

Module 1
Polypeptide Production

Module 2
Encapsulation

Module 3
Genome Deletion

Secondary Encapsulation

Chemo-induction
Auto-induction
Thermo-induction
**cl\textsubscript{857} Repression**

\[
\frac{d[E]}{dt} = k_E \times [mE] - d_E \times [E]
\]

\[
\frac{d[mE]}{dt} = k_1 \times \frac{K_{nm}^{nm}}{K_{nm}^{nm} + [Cl]^{ncl}} \times \frac{K_{nt}^{nt}}{T_{nt}^{nt} + K_{nt}^{nt}} - d_{mE} \times [mE]
\]
Part Characterisation

**J23114**

![Diagram showing the relationship between RBS, cl857, and GFP genes.]

- **RBS** (Regulatory Binding Site)
- **cl857**
- **Pλ** (Promoter Lambda)
- **GFP** (Green Fluorescent Protein)

**Gene Expression Model**

- cl587-GFP cells at 42°C
- cl587-GFP cells at 28°C
- Fitted gene expression model

**Graphical Representation**

- **Normalised Fluorescence** vs **Time after temperature shift (min)**

- Blue line: cl587-GFP cells at 42°C
- Red line: cl587-GFP cells at 28°C
- Green line: Fitted gene expression model

**Legend**

- cl587-GFP cells at 42°C
- cl587-GFP cells at 28°C
- Fitted gene expression model
Restriction Enzymes

DpnII

Taql

0ul  0.2ul  0.5ul  1.0ul

0ul  0.2ul  0.5ul  1.0ul
Restriction vs Methylation

[Image of a gel electrophoresis result showing bands for Marker, Dam +ve, and Dam -ve with different DpnII enzyme concentrations (0ul, 0.2ul, 0.5ul, 1.0ul).]
System Overview

Growth

Module 1 Polypeptide Production

Module 2 Encapsulation

Module 3 Genome Deletion

Secondary Encapsulation

Chemo-induction

Auto-induction

Thermo-induction
Secondary Encapsulation

1.5cm
Release In The Intestine

100,000,000,000,000 intestinal microorganisms

- Colanic acid – energy source
- Gut microflora (e.g. Commensal citrobacter) produce colanase
- Colanase breaks down colanic acid, releasing the polypeptide
Applications

Our E.ncapsulator opens up the possibility to treat a multitude of conditions...

Phenylketonuria

Chronic pain

Lactose Intolerance
BioBricks Submitted

- Created **30 new parts**
- Submitted **20** to the Registry
Human Practices

• Issues arose when designing the E.ncapsulator
• Therefore we added Genome Deletion Module

Workshops & interviews on 3 themes:

1) Make science understandable
2) Reflect on manipulating the materials of life
3) Address risk and safety in synthetic biology

Come visit our poster, read our reports and view our video!
Gold Medal Achievements

The Imperial College 2009 team have:

- Submitted **20 documented parts** to the Registry
- Characterised the **BBa_K098995 thermosensitive promoter & Part:BBa_K118011 glucose sensitive promoter**
- Submitted new coding regions for **PAH and Opiorphin**
- Designed new BioBricks using **novel genes**.

In addition we have:

- Explored **manufacturing considerations** for **The E.ncapsulator** as a viable product
- Conceptualised a **novel engineering technique** of integrating our 3 modules
- Explored **ethical issues**
- Helped **UCL** team and took part in the Valencia ethics survey.
The E.ncapsulator

- Attractive candidate for commercial pill development
- Demonstrates the manufacturing potential in Synthetic Biology
The Imperial Team

2 Biologists
2 Biochemists
4 Bioengineers
+ Advisors