A Synthetic Biology Approach to Bioremediation of Tailings Ponds

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Overview
Extraction of Resources: Worldwide Problem

Some of the human mining activity that creates slag and tailings ponds
The Tailings


Red mud (bauxite residue) dam, storing the waste stream of refining alumina http://www.flyertalk.com/forum/trip-reports

Industrial Mining Process

1. Raw Ore
2. Washing
3. Separation
   - Valuable Fraction
   - Waste
4. Further Processing
5. Centrifugation & Filtration
6. Tailings
In Our Backyard

- 141,000 square kilometers of land
- 170 billion barrels
- Over $12 trillion
Problem: Isolating the bitumen from the oil sands

Each grain of sand is surrounded by a layer of water and a film of bitumen.

Source: Canadian Centre for Energy Information
Targeting Catechol

- Phenol
- Benzoic Acid
- Phenoxyacetic Acid
- Naphthenic Acids

Catechol

Austen & Dunn, 1980
Catechol Degradation Pathway

Catechol

Catechol-2,3-dioxygenase (xylE)

2-Hydroxymuconate semialdehyde (2-HMS)
Catechol Degradation *in vivo*

1a – Control DH5α cells (hosting BBa_B0015) and 500µM catechol
1b – DH5α cells with BBa_K118021 and 500µM catechol
2 – Time course of catechol degradation *in vivo*
Catechol Degradation *in vitro*

**Graph:**

- **Y-axis:** 2-Hydroxymuconate Semialdehyde (μM)
- **X-axis:** Time (min)
- **Lines:**
  - Blue: Water
  - Red: 2μg S30 Extract
  - Green: 4μg S30 Extract

**Legend:**

- **BBa_K118021**

**Additional Elements:**

- Molecular structure of catechol
- Genes and regulatory elements (pcstA, RBS, xyE)
Segregating to Optimize

Co-localizing cellular processes in bacteria will improve the efficiency of the system.
Using Protein Compartments

- Lumazine Synthase
- *Aquifex aeolicus*
- Pentamers
- Microcompartments

Opposites Attract

- Opposite electrical charges attract
- Simple method to direct molecules
Charge Mutuation

- Lethbridge iGEM 2009 submitted mutant to parts registry
- Negative interior
- Use positive tag ($R_{10}$ Tail) to direct into cavity

Wild-type

Mutant
Localization of Fluorescent Proteins

- Proving localization is possible
- Which end to add positive charge onto?
- N-end rule

Excitation wavelength: 517 nm
YFP emission maxima: 524 nm
Fluorescence Resonance Energy Transfer

No FRET

439 nm

476 nm

~10 nm

FRET

439 nm

476 nm

~10 nm

527 nm
Localization of Fluorescent Proteins

- Construct to test assembly of microcompartment
- Plan targeting xylE
DNA Degradation

- BamHI from *Bacillus amyloliquefaciens*
- Submitted to the parts registry by UC Berkeley in 2007
Anticipating the Ethics of Synthetic Biology

• Four scientific discoveries were analyzed from ethical, environmental, economic, legal, and social perspectives.

• Ethical lessons can be applied to the relatively new field of synthetic biology.
Learning from the Past

• Important to act proactively and anticipate problems
• Information should be simplified to increase general public knowledge
• Public perception is vital

WWW  Antibiotics  Cloning  Nuclear Power
Interdisciplinary Interaction
Aesthetics

Visuals

Not Unlike ‘What you put into life”

Not Unlike ‘Love’

What Is The Sound of Three Hands Clapping?
Engaging the Public
Conclusions

• Performed initial kinetic analysis of xylE

• Created functional C-terminal tagged CFP and YFP

• Through ethical studies postulated methods to successfully introduce Synthetic Biology to the general public

• Created both visual and auditory art with our data
Submitted Parts

- BBa_K331023: RBS, CFP, R10 Tail
- BBa_K331027: pLacI, RBS, YFP, R10 Tail
- BBa_K331030: pTetR, RBS, R10 Tail, YFP
- BBa_K331031: pTetR, RBS, YFP, R10 Tail
- BBa_K331032: pTetR, RBS, R10 Tail, CFP
- BBa_K331033: RBS, CFP, R10 Tail
- BBa_K331034: RBS, R10 Tail, YFP, dT
- BBa_K331035: RBS, YFP, R10 Tail, dT
- BBa_K331039: RBS, YFP, R10 Tail
Characterized Parts

Chemical Degradation (Edinburgh 2008)
- BBa_K118021

Magnetic Nanoparticle Production (Lethbridge 2009)
- BBa_K249019

Compartmentalization Proof-Of-Principle (Lethbridge 2010)
- BBa_K331030
- BBa_K331031
- BBa_K331033
- BBa_K249004
- BBa_K249005
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