U of L
iGEM
The Team
Northern Alberta Tar Sands

* Images courtesy of Syncrude Inc. and Shell Scotford
Northern Alberta Tailings Ponds

*Image courtesy of Syncrude Inc.*
Analogy
Motility
The “Bacuum” Cleaner

- Locomotion
- Regulation
- Degradation
- Identification
Overview

• Locomotion
• Regulation
• Degradation
• Identification
Chemotaxis

Amino Acids

Carbohydrates

Running
Chemotaxis

Tumbling
Overriding chemotaxis

Amino Acids
Carbohydrates

CheZ
Flagella
CheY
P
CheR
CheW
CheA
CheB

Overriding chemotaxis
Controlling CheZ expression

Controlling CheZ expression

Motility assay

Motile
*E. coli* WT

Non-motile
*E. coli* Δ*cheZ*
Biobrick parts

- cheZ
  - BBa_K147000

Promoter + control + cheZ + T₁ + T₂
Overview

• Locomotion
• Regulation
• Degradation
• Identification
Riboswitch

• UTR of mRNA
  ○ Short (can be <100 nt)
• Binds target molecule, conformational change
• Regulates transcription termination, translation initiation or alternative splicing
• Fast response time
Types of riboswitches

- Widely diverse in nature
- Vary in ligands bound, methods of regulation
- Bacteria, archaea, eukarya
- Still a growing field of research

Riboswitch

- Theophylline aptamer
Biobrick parts

Low [ligand] →

High [ligand] →

Promoter + RS + gene + T₁ + T₂
Overview

- Locomotion
- Regulation
- Degradation
- Identification
2-chlorobenzoate

- Elimination of chloro substituents
  - Potential step in PCB degradation

\[ \text{2-chlorobenzoate (ortho-chlorobenzoate)} \rightarrow \text{catechol} \rightarrow \text{2-hydroxymuconic semialdehyde (coloured!)} \]

Biobrick parts

- **ohb operon** (*ohbA, ohbB, ohbC, ohbR*)
  - BBa_K147002 (*ohbA*)
- **xylE**
  - BBa_K147003
Overview

• Locomotion
• Regulation
• Degradation
• Identification
Identification

• Continually monitor modified bacterium
• Problem with reporter genes?
  o Expression limited under stressed conditions

• Solution?
  o Translation of essential genes
  o Initiated by unique sequence
rpsA TIR

- Initiates translation of S1 ribosomal protein
- Lacks Shine-Dalgarno and requirement of S1
- Unique watermark

Biobrick parts

- rspA TIR
Conclusions

Locomotion

Regulation

Degradation

Identification
Progress

- Modeling of CheZ function!
- Motility assay of WT vs. $\Delta$cheZ (RP1616)
- Submitted Biobricks cheZ, xylE, ohbA
  - Characterized cheZ part
- Isolated rpsA TIR
- Constructed theophylline riboswitch in Biobrick format (Nov. 6!)
Future work

• Continue with construct formation

• Modeling of reprogrammed bacuum motility

• Generate novel aptamer using SELEX
SELEX
Systematic Evolution of Ligands through Exponential Enrichment

Random nucleic acid synthesis

Tetracycline selection

Counter-selection with Ni^{2+}/Optimization

Nucleic acid with selected binding
Future work (cont’d)

- Characterize novel riboswitch function
- Assemble “Bacuum” Cleaner
- Test the system!
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