Aromatics Scouts

A Biosensor Toolkit for Profiling Aromatics in Environment

Peking iGEM 2013
Motivation
Motivation

Expensive Instrument

Specialized Skills

Confined in the lab
Motivation

Alternative Techniques

Biosensor Toolkit

- Inexpensive
- Accessible to the public
- In-field application
Biosensors

- XylS
- NahR
- DmpR
- HbpR
- XylR
- HcaR
Adaptor

Advanced Equipment

Biosensor

Undetectable Aromatic Compounds

Band-pass Filter
Band-pass Filter

Advanced Equipment

Adaptor

Band-pass Filter

Low Conc.  Medium Conc.  High Conc.
To Profile Aromatics in Practical Samples

A Collection of Biosensors for:

- Aromatic Hydrocarbons
- Aromatic Acids
- Phenols
- Biphenyls

A Bioinformatics Mining Method
**Biosensor Mining**

**Mining Criteria**

1. **TRs in Genetic-Context-Clear bacteria:**
   - *E. coli, B. subtilis, P. putida*

2. **Aromatics-related TRs:**
   - Keywords: aromatic, benzene, phenyl etc.

3. **Well studied TRs:**
   - Score the TFs by their citations
TRs in genetic context - clear bacteria

Aromatics-related TRs - feasible sensors

Well Studied TRs

Criterion 1

4, 362, 537

Criterion 2

21,096

Criterion 3

912

Effective

Efficient

Excluding false positive cases

Manual Adjustment

17

Promising Candidates

12

rate:9
name: tyrR
source: Escherichia coli (strain E12).

date:7
name: paaX
source: Pseudomonas sp. GM15.

rate:1
name: PM131_04889
source: Pseudomonas sp. GM5.

rate:1
name: PM131_00179
source: Pseudomonas sp. GM5.

rate:1
name: PM131_03336
source: Pseudomonas sp. GM5.

Protein analysis succeeded!
## Feasible sensing TRs

<table>
<thead>
<tr>
<th>Protein Names</th>
<th>Regulated Promoter</th>
<th>Sources</th>
<th>Reported Typical Inducers</th>
</tr>
</thead>
<tbody>
<tr>
<td>XylS</td>
<td>Pm</td>
<td><em>Pseudomonas putida</em> (Arthrobacter siderocapsulatus)</td>
<td>benzoic acid</td>
</tr>
<tr>
<td>XylR</td>
<td>Pu</td>
<td><em>Pseudomonas putida</em> (Arthrobacter siderocapsulatus)</td>
<td>m-Xylene</td>
</tr>
<tr>
<td>tyrR</td>
<td></td>
<td>Escherichia coli (strain K12)</td>
<td>tyrosine</td>
</tr>
<tr>
<td>nahR</td>
<td>Psal</td>
<td><em>Pseudomonas putida</em> (Arthrobacter siderocapsulatus)</td>
<td>salicylic acid</td>
</tr>
<tr>
<td>CapR</td>
<td></td>
<td><em>Pseudomonas putida</em> (Arthrobacter siderocapsulatus)</td>
<td>phenol</td>
</tr>
<tr>
<td>hcaR</td>
<td>Pc</td>
<td><em>Escherichia coli</em> (strain K12)</td>
<td>3-Phenyl-propionic acid</td>
</tr>
<tr>
<td>dmpR</td>
<td>Po</td>
<td><em>Pseudomonas sp.</em> (strain CF600).</td>
<td>phenol</td>
</tr>
<tr>
<td>pobR</td>
<td></td>
<td><em>Pseudomonas putida</em> (Arthrobacter siderocapsulatus)</td>
<td>p-hydroxybenzoic acid</td>
</tr>
<tr>
<td>CymR</td>
<td></td>
<td><em>Pseudomonas putida</em> (Arthrobacter siderocapsulatus)</td>
<td>4-Isopropyl benzoate</td>
</tr>
<tr>
<td>PaaX</td>
<td>Pz</td>
<td><em>Escherichia coli</em> (strain K12)</td>
<td>phenylacetyldetyl-CoA</td>
</tr>
<tr>
<td>hpaR</td>
<td>Pg</td>
<td><em>Pseudomonas putida</em> (Arthrobacter siderocapsulatus)</td>
<td>(3-hydroxy-phenyl)-acetic acid</td>
</tr>
<tr>
<td>mhpR</td>
<td></td>
<td><em>Escherichia coli</em> (strain K12)</td>
<td>(3-hydroxy-phenyl)-propionic acid</td>
</tr>
<tr>
<td>phhR</td>
<td></td>
<td><em>Pseudomonas putida</em> (Arthrobacter siderocapsulatus)</td>
<td>phenylalanine</td>
</tr>
<tr>
<td>bphS</td>
<td></td>
<td><em>Pseudomonas sp.</em> (strain CF600).</td>
<td>2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid</td>
</tr>
<tr>
<td>HbpR</td>
<td>Pc</td>
<td><em>Pseudomonas nitroreducens</em></td>
<td>2-hydroxybiphenyl</td>
</tr>
<tr>
<td>phcR</td>
<td></td>
<td><em>Pseudomonas putida</em> (Arthrobacter siderocapsulatus)</td>
<td>phenol</td>
</tr>
<tr>
<td>yodB</td>
<td></td>
<td><em>Bacillus subtilis</em> (strain 168)</td>
<td>2-methyl hydroquinone</td>
</tr>
</tbody>
</table>
Build Our Biosensor

Circuit Frame for Biosensors

TR

Constitutive promoter

sfGFP

Inducible promoter

pSB4K5

pUC57
Problem: High basal level

- **NahR**
  - Inducer Concentration Gradient
  - [Image of gradient]

- **XylS**
- **DmpR**
- **HbpR**
- **XylR**
- **HcaR**
- **HpaR**
- **PaaX**
  - [Image of gradient]
  - [Image of gradient]

**Inducer Concentration Gradient**
Fine-tuning of Biosensors

Round 1
Fine-tune TR Expression

J23113
J23109
J23117
J23114
J23105
J23106
Constitutive Promoters

Round 2
Fine-tune Reporter Amplitude

sfGFP

B0034
B0032
B0031
B0033
Ribosome Binding Sites
**Round 1: Fine-tuning HbpR expression level**

**Induction Ratio**

\[
\text{Induction Ratio} = \frac{\text{Induced Fluorescence}}{\text{Basal Fluorescence}}
\]

**Graph:**
- **2-HBP** and **2-ABP** bars for J23109, J23107, J23114, J23106.
- Induction ratio compared.
- Best, Optimized, Original categories.

*Constitutive promoter strength*
Round 2: Fine-tuning sfGFP expression amplitude

An Example: HbpR Biosensor
Fine-tuning Summary

- **NahR**
- **XylS**
- **DmpR**
- **HbpR**
- **XylR**
- **HpaR**
- **PaaX**
- **HcaR**

- **NahR**
- **HbpR**
- **XylS**
- **XylR**
- **DmpR**
- **HcaR**

- **XylS**
- **DmpR**
- **HbpR**
- **XylR**
- **HpaR**
- **PaaX**

- **HpaR**
- **PaaX**
Biosensor Characterization

XylS  NahR  DmpR

HbpR  XylR  HcaR

Fluorescence Intensity

Conc. of PPA

Conc. of inducers (μM)
Overall Biosensors
Biosensor Characterization

Practical Analysis

Synergistic
Orthogonal
Antagonistic
Orthogonality Assay

Data Plot

Orthogonality

Y-axis: A+ B+

Inducer

Irrelevant Aromatics

Biosensor

Slope=1

Synergistic

Regression Line

Orthogonal

Antagonistic

X-axis: A+ B-
Orthogonality

**Benzoates**

- DmpR: Phenols
  - Fluorescence Intensity (induced by Phi and 3-MeBzO)

**Salicylates**

- Fluorescence Intensity (induced by Phi and 4-MeSaA)

**Biphenyls**

- Fluorescence Intensity (induced by Phi and 2-HBP)

**XylS: Benzoates**

- Fluorescence Intensity (induced by 3-MeBzO & 4-MeSaA)

**Salicylates**

- Concentration of 4-MeSaA
  - Fitting Curve k=0.99

**Biphenyls**

- Concentration of 2-HBP
  - Fitting Curve k=1.15

**Phenols**

- Concentration of Phi
  - Fitting Curve k=0.98
## Multicomponent Analysis

<table>
<thead>
<tr>
<th></th>
<th>Benzoate</th>
<th>Salicylate</th>
<th>Biphenyl</th>
<th>Phenol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>XylS</strong>&lt;br&gt;Biosensor</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NahR</strong>&lt;br&gt;Biosensor</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HbpR</strong>&lt;br&gt;Biosensor</td>
<td></td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td><strong>DmpR</strong>&lt;br&gt;Biosensor</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>
Features of Biosensors

A Large set of aromatics biosensors

No Synergistic or Antagonistic effects

Practical Analysis
Using Biosensors to conduct Practical Analysis

**Water Sample**

- **Biosensor**
  - XylS
  - NahR
  - DmpR
  - HbpR
  - XylR
  - HcaR

**Calibration Curve**

- Fluorescence Intensity vs. Lg(Conc.)
- Fluorescence Output vs. Measured Conc.

**Evaluating Reliability**

Deviation Fold = \[ \frac{\text{Measured Concentration}}{\text{Real Concentration}} \]

- <<1: Bad
- ≈1: Good
- >>1: Bad
Multicomponent Analysis

XylS $\rightarrow$ 3-MeBzO $\rightarrow$ 4-MeSaA $\leftarrow$ NahR

HbpR $\rightarrow$ 2-HBP $\rightarrow$ Phl $\leftarrow$ Interference
## Multicomponent Analysis

<table>
<thead>
<tr>
<th></th>
<th>3-MeBzO 0 μM</th>
<th>3-MeBzO 10 μM</th>
<th>3-MeBzO 100 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-MeSaA 0 μM</td>
<td>4-MeSaA 1 μM</td>
<td>4-MeSaA 10 μM</td>
<td></td>
</tr>
<tr>
<td>4-MeSaA 1 μM</td>
<td>4-MeSaA 10 μM</td>
<td>4-MeSaA 10 μM</td>
<td></td>
</tr>
<tr>
<td>4-MeSaA 10 μM</td>
<td>4-MeSaA 10 μM</td>
<td>4-MeSaA 10 μM</td>
<td></td>
</tr>
<tr>
<td>4-MeSaA 50 μM</td>
<td>4-MeSaA 100 μM</td>
<td>4-MeSaA 100 μM</td>
<td></td>
</tr>
</tbody>
</table>

- **3-MeBzO**
  - XylS
  - NahR
- **2-HBP**
  - 0 μM
  - 10 μM
  - 100 μM
- **Phl**
  - Interferent

### Deviation Fold

- **93.4%**
- **Fold between 0.5 and 2.0**
Advanced Equipment

**Expanding The Detection Profile**

**Adaptor**

**Band-pass Filter**

**User-friendly Display**
Adaptor

Expand The Detection Profile?

Convert the Undetectable into the Detectable!
NahF: Adaptor for NahR

Adaptor

Constitutive promoter

Biosensor

NahF

Pr

Psal

sfGFP

NahR
Induction Ratio

\[
\text{Induction Ratio} = \frac{\text{Induced Fluorescence}}{\text{Basal Fluorescence}}
\]
XylC: Adaptor for XylS

Adaptor

Biosensor

\[
\begin{align*}
\text{CHO} & \quad \text{CH}_3 \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{C} & \quad \text{O} \\
\text{CHO} & \quad \text{CH}_3
\end{align*}
\]

\[
\begin{align*}
\text{CHO} & \quad \text{CH}_3 \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{C} & \quad \text{O} \\
\text{COOH} & \quad \text{CH}_3
\end{align*}
\]

Graph showing the induction ratio against the concentration of 3-MeBAD (µM). The graph compares XylS and XylS with Adaptor XylC.
Adaptors

Convert the Undetectable into the Detectable!
Expanding The Detection Profile

Adaptor

Band-pass Filter

User-friendly Display
How to Build a Band-pass Filter?

Incoherent Feed-forward Loop

Positive Loop

\[ \text{Output} \]

\[ K_1 \]

Input

Output

Negative Loop

\[ \text{Output} \]

\[ K_2 \]

Input

\[ K_1 \]

Input

\[ K_2 \]
Determining The Best Design Frame

Mathematical Analysis

Incoherent Feed-forward Loop

Most Robust
Parameter Sensitivity Analysis

High Hill Coefficient

Low Dissociation Constant

B → cl

C → φR73δ

B → A → C → D → B

Ligand

GFP
The Hybrid-promoter

- **Sensor**
- **IPTG**
- **SaA**
- **ϕR73δ**
- **cl**
- **sfGFP**
- **Hybrid Promoter**
- **Positive Loop (Fixed IPTG)**
- **Negative Loop (Fixed SaA)**

**Hybrid Promoter**

**Constitutive Promoter**

**Inducible Promoter**

**Positive Loop (Fixed IPTG)**

**Negative Loop (Fixed SaA)**

**Fluorescence Intensity vs. Conc. of SaA (μM)**

**Fluorescence Intensity vs. Conc. of IPTG (μM)**

- **A**
- **B**
- **C**
- **D**

**Ligand**

**GFP**
Construction of Band-pass Filter

- Determining The Design Frame
- Selecting Appropriate Proteins
- Engineering The Hybrid-promoter
- Characterizing The Hybrid-promoter
- Final Assembly
Expanding The Detection Profile

Adaptor

Band-pass Filter

User-friendly Display
Summary

Achievements

- ✔️ 6 High-quality Biosensors
- ✔️ 2 Adaptors
- ✔️ Band-pass Filter
- ✔️ 45 Biobricks Submitted

Future Plan

- ✔️ To accomplish Band-pass Filter Construction
- ✔️ More Adaptors
Perspective

Biosensors + Reporters = Pollution Monitoring

Biosensors + Degradation Pathways = Bioremediation
Aromatics-sensing Regulators for Metabolic Process Control

Aerobic Degradation of Aromatic Compounds

Dehalogenation

Perspective
Human Practice

Visiting a Printing Factory
Human Practice

Questionnaires
1. What are Aromatic Compounds?

- Benzene Series Compounds: 59%
- Fragrant Compounds: 33%
- Compounds with heavy metal ion: 1%
6. What do you think of biological prevention?

- 35% Totally for it
- 41% Acceptable
- 15% No idea
- 9% Totally Against it

Q1: 1% A, 7% B, 33% C, 59% D
Q2: 23% A, 48% B, 14% C, 15% D
Q3: 8% A, 12% B, 2% C, 78% D
Q4: 7% A, 24% B, 37% C, 32% D
Q5: 14% A, 37% B, 10% C, 39% D
Q6: 15% A, 35% B, 15% C, 35% D
Human Practice

Providing Guidance to a High School iGEM Team

Sow Synthetic Biologist for the future
Human Practice

Team Communication
Acknowledgements

Víctor de Lorenzo
Victoria Shingler
Jan Roelof van der Meer
Acknowledgements

• Dr. Chunbo Lou
• Prof. Qi Ouyang
• Prof. Luhua Lai
• Prof. Zhen Yang
• Prof. Junfeng Hu
• Prof. Chongren Xu
• Prof. Xinqiang He
• Prof. Chunxiong Luo
• Prof. Ge Gao
• Dr. Zailing Bai
Thank all the team members
Thanks for your attention