A Journey

Desert in Northwest China
Dead coral, Sanya, China
Incompact sand substrates!

This is the key issue for those problems...
Consolidate!

Traditional methods

Biological methods
Time for show!

Promotion of micro-consolidation tech
Euk. cement

iGEM-HUST-China-2015
Yarrowia Lypolitica

- Environmentally safe microorganism
- Live in marine environment
- Hydrophobic substrate
- Ability to grow fast
- High-density fermentation
- Express complex recombinant proteins
Genetic circuits

1. darkness induction system

2. silica binding system

3. flocculating system
1. Darkness induction system
-- Light control system based on Y2H
1. Dark induction system
2. Silica binding system
2. Silica binding system

cell

Si-tag

Silicon surface
3. Flocculating system

- cells
- sands
- silica-tag
- mcfps

consolidation

pAnb1

LIP2 prepro Mcfp-3

sand
Modeling & experiments
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Modeling & experiment

Ms. Experiment

Mr. Modeling
Light control systems modeling
Validation Test of Light-control Expression Part

β-galactosidase activity (Miller Units)

Test-light

Test-dark

Control-light

Control-dark

Light control systems

Modeling
Simulation of Surface Displayed Si-tag & Secreted Mcfp-3

- **Surface Displayed Si-tag**
- **Secreted Mcfp-3**

Protein level of Si-tag & Mcfp-3 vs. time (min)

- Time to Light ON
- Time to Light OFF
Fusion expression of 6xHis tag

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>Si-tag1L3-6xHis</th>
<th>regional</th>
<th>typical</th>
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</thead>
<tbody>
<tr>
<td>FITC anti 6xhis</td>
<td>true</td>
<td>true</td>
<td>true</td>
<td>true</td>
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<tr>
<td>Cells</td>
<td>false</td>
<td>true</td>
<td>false</td>
<td>false</td>
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<tr>
<td>Merge</td>
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<td>true</td>
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</tbody>
</table>

- Immunofluorescence
- Surface display
**Silica binding test**

- Glass surface binding
- Three different intensity

**Silica surface binding test**

<table>
<thead>
<tr>
<th>Loading before wash</th>
<th>Binding after wash</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>Si-tag 1</td>
</tr>
<tr>
<td>Si-tag 2</td>
<td>Si-tag 3</td>
</tr>
<tr>
<td>Si-tag 1+2</td>
<td>Si-tag 2+3</td>
</tr>
<tr>
<td>Si-tag 1+3</td>
<td>Si-tag 1+GSlinker+3</td>
</tr>
<tr>
<td>Si-tag 1+2+3</td>
<td></td>
</tr>
</tbody>
</table>

**Relative Silica Binding Ability**

- Control: low binding
- Si-tag 1: moderate binding
- Si-tag 2: high binding
- Si-tag 3: very high binding
- Si-tag 1+2: higher binding than control
- Si-tag 1+3: highest binding
- Si-tag 1+2+3: greatest binding among all
Experiment

Flocculating of secreted Mcfp-3

- Successful secretion
- Successful flocculating
Experiment

Consolidation test

• Mimetic seabed
• Successful cementation

Experiment Equipment

<table>
<thead>
<tr>
<th>macroscopical sample</th>
<th>microscopical sample</th>
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<tbody>
<tr>
<td>Si-tag123-JMY1212 + mcfp3-JMY1212 live cells treated sands</td>
<td></td>
</tr>
<tr>
<td>wildtype control JMY1212 live cells treated sands</td>
<td></td>
</tr>
</tbody>
</table>

(A) Experiment Equipment

(B) Macroscopical sample

(C) Microscopical sample

(D) Wildtype control JMY1212 live cells treated sands

(E) Macroscopical sample
Prof. J.J. Zheng
Huazhong University of Sci & Tech (HUST),
Wuhan, China
Expert in civil engineering

Offer harbors to marine organisms
Simulation of darkness induction system
Modeling

- Fast diffusion
- Semi-circle pattern

Diffusion models
Modeling

Design for diffusion

$L = k \ln V$
Safety

Implementation method revisited
Prof. Weiding Wang
Marine Fisheries Research Institute, Zhejiang, China
Expert in aquafarm and artificial reefs
Investigation in Zhoushan Islands, China
Human Practice

Exchanges and conferences in
- UC Berkeley @CA
- PKU @Beijing
- NCTU @Taiwan
- WHU @Wuhan

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### Light control parts

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Description</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_K1592005</td>
<td>GalBD-CRY2 Fusion for Yeast-Two-Hybrid</td>
<td>BD-CRY2</td>
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<tr>
<td>BBa_K1592006</td>
<td>GalAD-CIB1 Fusion for Yeast-Two-Hybrid</td>
<td>AD-CIB1</td>
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<td>BBa_K1592015</td>
<td>photoreceptor cryptochrome 2</td>
<td>CRY2</td>
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<tr>
<td>BBa_K1592016</td>
<td>a basic helix-loop-helix protein</td>
<td>CIB1</td>
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### Silica-tag protein

<table>
<thead>
<tr>
<th>Part Number</th>
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<tbody>
<tr>
<td>BBa_K1592007</td>
<td>LIP prepro + E. coli ribosomal protein L2 (1-60) + YLcwp3 Fusion</td>
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<tr>
<td>BBa_K1592008</td>
<td>LIP prepro + E. coli ribosomal protein L2 (61-202) + YLcwp3 Fusion</td>
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<tr>
<td>BBa_K1592009</td>
<td>LIP prepro + E. coli ribosomal protein L2 (203-273) + YLcwp3 Fusion</td>
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<td>BBa_K1592010</td>
<td>LIP prepro + E. coli ribosomal protein L2 (1-202) + YLcwp3 Fusion</td>
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<td>BBa_K1592011</td>
<td>LIP prepro + E. coli ribosomal protein L2 (1-202, 203-273) + YLcwp3 Fusion</td>
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<tr>
<td>BBa_K1592012</td>
<td>LIP prepro + E. coli ribosomal protein L2 (1-202, 203-273) + YLcwp3 Fusion</td>
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<td>BBa_K1592013</td>
<td>LIP prepro + E. coli ribosomal protein L2 (1-202, GS linker, 203-273) + YLcwp3</td>
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<tr>
<td>BBa_K1592014</td>
<td>LIP prepro + E. coli ribosomal protein L2 (1-273) + YLcwp3</td>
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### Flocculent protein

<table>
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<th>Description</th>
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<tr>
<td>BBa_K1592001</td>
<td>Mytilus californianus foot protein MCFP3 variant 3</td>
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<tr>
<td>BBa_K1592003</td>
<td>MCFP3 with prepro</td>
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<td>BBa_K1592017</td>
<td>MCFP3 with 203-273 pre</td>
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### Parts submitted

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<th>Abbreviation</th>
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<tr>
<td>BBa_K1592000</td>
<td>LIP2 prepro (signal peptide)</td>
<td>LIP2 prepro</td>
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<td>BBa_K1592002</td>
<td>Yarrowia lipolytica cell wall protein 3</td>
<td>YLcwp3</td>
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### Parts improved

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<tr>
<td>BBa_K1592020</td>
<td>Ptrp mutant 1</td>
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<td>BBa_K1592021</td>
<td>Ptrp mutant 2</td>
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<tr>
<td>BBa_K1592022</td>
<td>Ptrp mutant 3</td>
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### Composite parts

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<th>Description</th>
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<tbody>
<tr>
<td>BBa_K1592018</td>
<td>Pgαl1+rox1+cyc1_terminator</td>
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<td>BBa_K1592019</td>
<td>Panb1+XPR2 pre-Mcfp3</td>
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<tr>
<td>BBa_K1592023</td>
<td>Ptrp mutant1+RBS+GFP</td>
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<tr>
<td>BBa_K1592024</td>
<td>Ptrp mutant2+RBS+GFP</td>
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<tr>
<td>BBa_K1592025</td>
<td>Ptrp mutant3+RBS+GFP</td>
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### Others

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Description</th>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>BBa_K1592004</td>
<td>promoter hp4d</td>
<td>Php4d</td>
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</table>
Register, complete the judging form, wiki, poster and presentation, new BioBrick

Validated new BioBrick

Documented characterization of new parts and submit them

Abundant and effective human practice which is tightly related to our project

Cooperated with WHU & HZAU

Improved and further documented some previous parts
advisors: Professor Y.J. Ya, Professor K. Ning, Dr Y. Zhan, Dr Q. Ge
Wiki framework: Y. Xu
Art and design: Z.X. Yang
video: Y.X. Zheng J.Z. Yang
HP: J.T. Liu, S.Y. Tang, G.Z. Wu,
Professor X.Q. Zhao and Professor Jens Nielson providing Saccharomyces cerevisiae s288c.
Thanks Drs and masters for their direct guide in experiment.
Thanks Dr X.H. Gui, M Yulin Kuang Dr G.L. Wang, Dr W. Liu, Dr D.J. Pan
R.Y. Zhang
Team

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Euk.cement  iGEM2015:HUST-China
Thank you!

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