Communications

39 700 000 000 emails/day in 2009

Multi cellular:
• Inter organs communications
• Intra organ communications

Bacteria...
Bacterial communication

Quorum sensing

- Small molecules
- Specificity
- Continuum of information, based on concentration

A heart-shaped pattern on petri dish of E. coli as viewed by fluorescence microscope.

Need for specific long range communication?
Harness Vesicles

Need:
• Specific
• Robust
• Concentration independent message:
  **Quantum of information**
Need:
- Specific
- Robust
- Concentration independent

message:
**Quantum of information**

Our solution: Outer Membrane Vesicles
- Encapsulated
- Naturally used as a pathogen system
Harness Vesicles

Need:
- Specific
- Robust
- Concentration independent message: **Quantum of information**

Our solution: Outer Membrane Vesicles
- Encapsulated
- Naturally used as a pathogen system
Message in a bubble

Project: Divided in 3 parts
Message in a bubble

Loading message into vesicle
Message in a bubble

Producing the vesicle

Tol/Pal
Message in a bubble

Receiving the vesicle
Creating Vesicles: membrane destabilization

Tol/Pal system
Creating Vesicles: membrane destabilization

Tol/Pal system

Preserve membranes integrity

Destabilization of membrane integrity increases the production of vesicles.

1. Lloubès & Journet Research in Microbiology 2001
Creating Vesicles: membrane destabilization

Preserve membranes integrity

Destabilization of membrane integrity increases the production of vesicles.

Tol/Pal system

2 alternative approaches:
- TolR deleted strains.
- Overexpression of soluble parts of periplasmic domain of TolR controlled vesicle creation.
Creating Vesicles: membrane destabilization

Design

**fusion** between **OmpA signal** and the **soluble TolRII domain** to cross the inner membrane.

Target Construction

BBa_K257005  BBa_K257006

**Toolbox Improvement**

**OmpA signal peptide**

translocation of proteins to the periplasm.

*Silver 23 standard*
Biophysics model: vesicle formation

Unequal osmotic pressures create small blebbing

\[ \Delta P - 2\lambda + 2k_c(H + c_0)(2H^2 - c_0H - 2K) + k_c\nabla^2(2H) = 0 \]

Unequal osmotic pressure and Tol/Pal anchoring can explain blebs creation

Snapshot of our simulation

Blebing

Bleb Maturation

Vesiculation

Tol/Pal accumulates at the basis of nascent blebs

Diffusion can explain the increase of Tol-Pal complexes on the borders of blebs:

\[
\begin{align*}
\frac{dC_{Tol}}{dt} &= D_{Tol} \nabla^2 C_{Tol} - k_{Tol-Pal} C_{Tol} C_{Pal} + k_{Tol-Pal}^* C_{Tol-Pal} \\
\frac{dC_{Pal}}{dt} &= D_{Pal} \nabla^2 C_{Pal} - k_{Pal-Tol} C_{Pal} C_{Tol} + k_{Pal-Tol}^* C_{Pal-Tol} \\
\frac{dC_{Tol-Pal}}{dt} &= k_{TolPal} C_{Tol} C_{Pal} - k_{Tol-Pal}^* C_{Tol-Pal} + \Phi(P, C_{Tol-Pal})
\end{align*}
\]

1. Kumaran & Losick. PNAS USA 2009
Biophysics model: vesicle formation

Rings of accumulated Tol/Pal constricts blebs basis

Proteins fused to ClyA

Vesicles are naturally enriched in ClyA: good vehicle for the message

ClyA
Outer membrane protein
Pore-forming toxin (8/13 su)

Getting to the outer membrane: ClyA

Proteins fused to ClyA

Getting to the outer membrane: ClyA

Design

fusion between ClyA C_term membrane and Proteins.

Target Construction

Toolbox Improvement

ClyA BioBrick for protein fusion

Translocation of proteins to the outer membrane

Silver 23 standard
Getting to the outer membrane: ClyA

**Functional Test**

*E. coli* with pBad-ClyA-RFP in arabinose: fluorescence on the membrane.

**Fluorescence is localized on the membrane**

ΔTolR E. Coli with pBad-ClyA-RFP in arabinose.

**Vesicles**
The Delay Regulation Network
Modeling the delay system

Deterministic simulations were run to evaluate the delay system described by the following ordinary differential equations modeling non-linear production rate:

1. \[
\frac{d[P]}{dt} = -\gamma_{prot} [P] + \beta_{prot} \frac{([Ara]/K_{Ara})^\gamma_1}{1 + ([Ara]/K_{Ara})^\gamma_2}
\]

2. \[
\frac{d[LacP]}{dt} = -\gamma_{LacP} [LacP] + \beta_{LacP} \frac{([Ara]/K_{Ara})^\gamma_1}{1 + ([Ara]/K_{Ara})^\gamma_2}
\]

3. \[
\frac{d[TetR]}{dt} = -\gamma_{TetR} [TetR] + \beta_{TetR} \frac{1}{1 + ([LacP]/K_{LacP})^\gamma_2}
\]

4. \[
\frac{d[TolR]}{dt} = -\gamma_{TolR} [TolR] + \beta_{TolR} \frac{1}{1 + ([TetR]/K_{TetR})^\gamma_2}
\]

\(\Gamma\): dilution rates.
\(\beta\): maximum production rates
\(K\): Binding Constant

Delay of ~6 cell cycles
Specific fusion between vesicles and recipient bacteria?
Specificity & fusion enhancement

Fusion occurs **spontaneously**...

<table>
<thead>
<tr>
<th>Emission</th>
<th>Jun*</th>
<th>g3p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reception</td>
<td>Fos</td>
<td>pilus</td>
</tr>
</tbody>
</table>

**Signal peptide** | **Passenger** | **Linker** | **β-barrel** | **AIDA-based autotransporteur**

**Jun/Fos**
- Bba_K257001
- Bba_K257001

**g3p**
- Bba_K257001

The classical Fec pathway

Sensing and capturing Fe in the medium

\[(\text{Fe}^{3+}\text{citrate})_2\]

OM

Periplasm

CM

FecD \rightarrow FecC \rightarrow FecE

Fe\(^{2+}\)

ATP \rightarrow ADP + Pi

RNAP

pfur pacf \rightarrow feclR \rightarrow pfur pfecA \rightarrow fecABCDE

Amplification of the signal

Our strategy to **enhance** the robustness:

1. **FecA***
2. **FecR**
3. **FecI**
4. **pFec**
5. **FecR N ter**
6. **Fecl**
7. **RFP**

**Positive feedback loop**

Recipient bacteria: **FecA** -

Optimizing the reception

Stochastic simulations: 2 main features can explain non-robust response

- Probability of activation
- Activation delay

Solution
- Overexpression of FecR

Robust activation
pFec biobrick characterization

Measures realised in a 1mM [Fe] medium supplemented with different concentrations of Na Citrate

- Induction measured via microscopy and plate-reader assay:
  - Functional part
  - Optimal conditions to measure ferric citrate complex

LB medium

LB + Fe citrate (30mM)
Providing to the community numerous parts including very diverse functions necessary to implement our communication system.

- Export to the periplasm: OmpA signal
- Export to the OM: AIDA / ClyA N\textsubscript{ter} / OmpA Linker
- OM labeling: ClyA-RFP
- Fusion reporter: RFP C\textsubscript{ter} / RFP N\textsubscript{ter}
- Membrane destabilization: TolRII / TE3
- Specific Adhesion: Jun / Fos / g3p
- Transduction system: pFec / fecA* / fecR N\textsubscript{ter} / fecI
A student with social studies of sciences background included in the team
- How social studies of science can enlighten ethical challenges in synthetic biology?

Bibliography
- Following our day-to-day work
- Organizing daily debates to discuss synthetic biology concerns
- A report “Synthetics” available on the wiki
- Conclusion: ethics should be integrated to the daily scientific endeavour
Team & Sponsors
Collaboration

Come and join us @: http://wiser-u.net/