Engineered Mammalian Cell-Cell Communication Mediated by Synthetic Exosomal Cargoes

North American Regional
10/5/13
1. Introduction: Cell-cell communication with exosomes
2. Engineered miRNA-based cargo and results
3. Engineered protein-based cargo and results
4. Application to endogenous gene activation and results
5. Impact and human practices
Bottom-Up Composition in Synthetic Biology

Prokaryotic

Mammalian

Integrated Systems

Modules (Computation, Communication Oscillators)

Elements

Prokaryotic

Mammalian

Stem-Cell Differentiation

Tissue Homeostasis

Cellular Automata

Artificial Immune System

Communication?

Basu et al, Moon et al, Tamsir et al, iGEM
Cell-Cell Communication in the Literature

**Aim:** Engineer a scalable, tunable cell-cell communication system capable of short to long range signaling in mammalian systems.

- Diffusible molecule
- Ability to Engineer
  - Not orthogonal to cell
  - Not generalizable
- Receptor binding
- Ability to Engineer
  - Cross talk from delta/notch
  - Requires cell contact

*Sprinzak et al, Bacchus et al*
Engineering Exosomes for Communication

Mammalian Cell

Exosomes
Engineering Exosomes for Communication

Naturally formed and secreted
Choice of cargo
Shown to be uptaken by cells

- mRNA
- Small RNAs
- Protein
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Design and Implementation Considerations

Sender Cell

RNA Splicing Circuit

Packaging into Exosomes

Engineered Exosome

miRNA

Response to Signal

Receiver Cell

RNAi Sensor Circuit

Uptake of Exosomes

Choice of Signal

Generation of Signal Packaging into Exosomes

Response to Signal
Receiver Cell Circuit Testing

miRNA-451

<table>
<thead>
<tr>
<th>Input miRNA-451</th>
<th>Output eYFP</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>1</td>
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Exogenous Input Testing of Receiver Circuit

**siRNA-503**

### Graph

- **[eBFP] (a.u.)** vs. **[eYFP] (a.u.)**
- Data points for:
  - siRNA 503 (green)
  - siRNA 451 (orange)
  - Median (siRNA 503: blue, siRNA 451: red)

### Table

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**Diagram:**
- **Input** circuit: phEF1α eYFP
- **Output** circuit: phEF1α eBFP
- **siRNA-503** targeting sites: 4x451 target sites
- **BBa_K1179072**
- **BBa_K31800**
Exosomal Input Testing of Receiver Circuit

![Graph showing the comparison of [tagBFP] (a.u.) with and without exosomal miR451 treatment.](Image)

- **No Exosome Treatment**
- **With Exosomal miR451**

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Testing of Integrated Cell-Cell Communication System

![Graph showing the relationship between tagBFP and eYFP](chart.png)

- **[tagBFP] (a.u.)**
  - 0
  - 1
  - 2
  - 3
  - 4
  - 5
  - 10
  - 100
  - 1000
  - 10000
  - 100000

- **[eYFP] (a.u.)**
  - 0
  - 1
  - 2
  - 10
  - 100
  - 1000
  - 10000
  - 100000

- **Legend:**
  - Positive control
  - 0.5 million Jurkats
  - 1 million Jurkats
  - 2 million Jurkats

- **Table:**
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  - Output eYFP
  - 0
  - 1
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Engineering Exosomes for Communication

- Ability to Engineer:
  - Targeting mechanism not understood
  - miRNA crosstalk problems

- Ability to Engineer:
  - Orthogonal proteins available
  - Known targeting motif – Acyl-TyA
Acyl-TyA Targeting

Green: GFP     Blue: NucBlue
Red: Rh-PE     Blue: NucBlue
Acyl-TyA Targeting

Green: GFP  Blue: NucBlue  Red: Rh-PE
Acyl-TyA Targeting

Green: GFP  Blue: NucBlue  Red: Rh-PE
Acyl-TyA Export

Control Primary Antibody: anti-GAPDH (green)
Experimental Primary Antibody: anti-HIS (red)
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Circuit design for Cas9-VP16 mediated activation

- phEF1α
  - BBa_K1179002
- Cas9
- VP16
- pU6
  - gRNA(Cr9)
  - BBa_K1179015
- phEF1α
  - tagBFP
- Cas9
- VP16
- Cr9
  - Cr9
  - minCMV
  - BBa_K1179015
  - eYFP
Different concentration of gRNA cause various degree of EYFP activation.
Next Steps

1. Integrate the two parts of our project by creating Cas9-Acyl-Tya and exosomally communicate Cas9.

2. Choose target endogenous genes to target in non-engineered receiver cell.

3. Pursue end applications and their human practices implications.
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Impact

1) BioBricked mammalian cell-cell communication parts now available for novel mammalian systems.

2) Toolkit for engineering therapies based on exosomal remote control, including new cancer vaccines, antiviral resistance, and cell therapies.

3) The ability to build new systems will lead to advanced scientific understanding of how natural exosomal systems function.
Community engagement

High school outreach and lecture

Mentoring HS student team member

Student-led course simulcast to UAI Chile

Met with MDs and human practices experts early to guide research direction

Outreach to museum
Planning SB exhibit

Museum of Science
Integrated human practice approach

Application and impact

Clinical application:
e.g., In vivo immune engineering; Antibodies as cargos

Stem cell differentiation and tissue engineering:
Exosome mediated cell fate engineering with intact genome

Enquiry and Research
Risks and regulations

Expert in Human Practices
Doctors
Summary of Accomplishments

1. Addressable miRNA based cell-cell communication system

2. Localized protein-based cell-cell communication system

3. Endogenous gene activation application

4. Novel integrated human practice approach
Acknowledgments

Instructor:
Ron Weiss (faculty)

Advisors:
Jonathan Babb
Deepak Mishra
Brian Teague
Samira Kiani
Kristjan Eerik Kaseniit
Nathan Kipniss
Katie Bodner

Additional thanks:
Timothy Lu
Domiilla Del Vecchio
Alice M. Rushforth
Kristala L. Jones Prather
Roger Dale Kamm
Christopher Voigt
Feng Zhang
Jacquin Niles
Rahul Sarpeshkar
Natalie Kuldell

2013 MIT iGEM Team
Brandon Nadres, Chamille Lescott, Hao Xing, Hyodong Lee, Kyle Lathem, Lauren St. Hilaire, Molly Klimak, Mounica Paturu, Nathanael Ji, Nelson Hall

Thanks to our sponsors for their generous support!