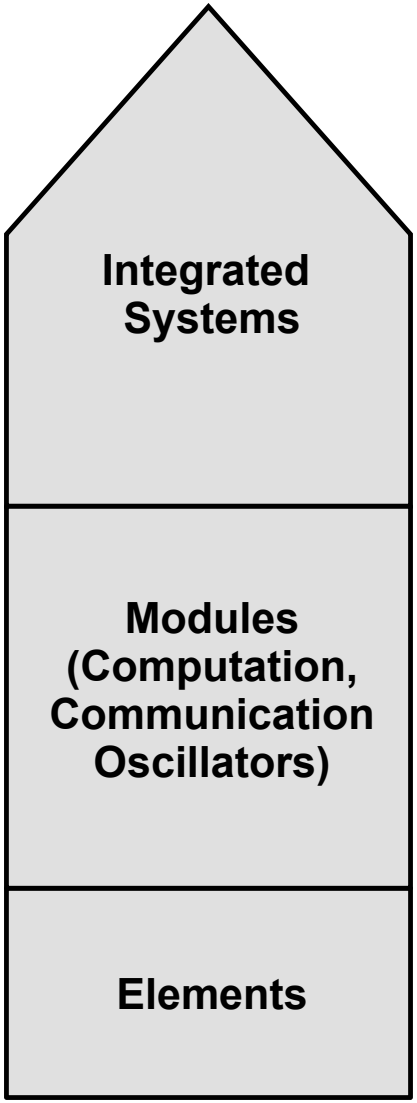
Engineered Mammalian Cell-Cell Communication Mediated by Synthetic Exosomal Cargoes

North American Regional
10/5/13

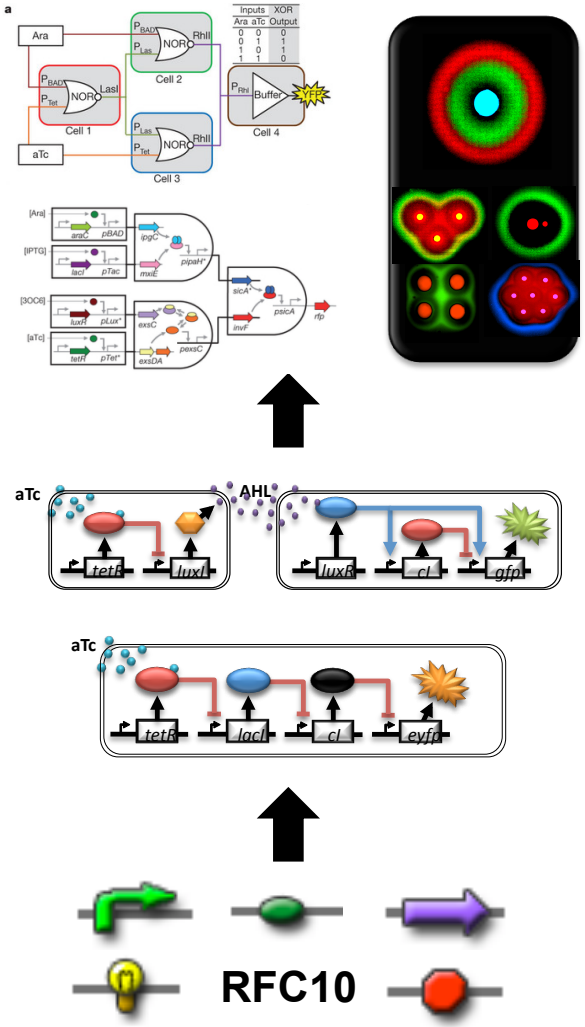
Outline

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

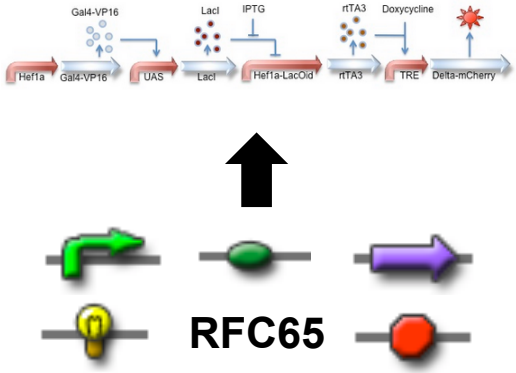
Stem-Cell Differentiation

Tissue Homeostasis

Cellular Automata

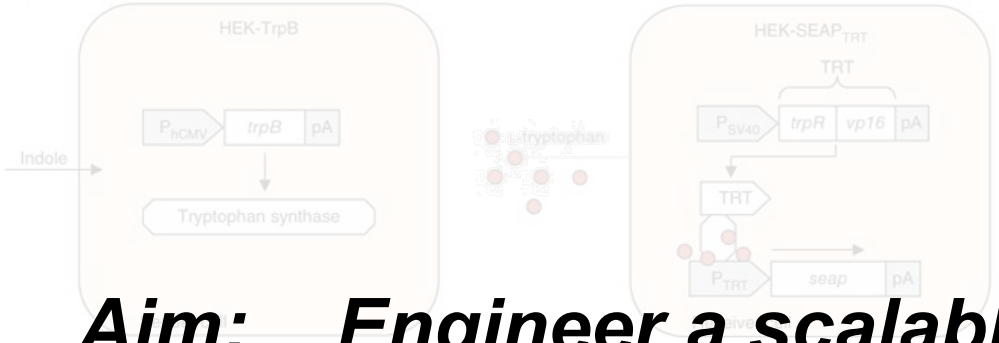
Artificial Immune System

Communication?



Basu et al, Moon et al, Tamsir et al, iGEM

Cell-Cell Communication in the Literature

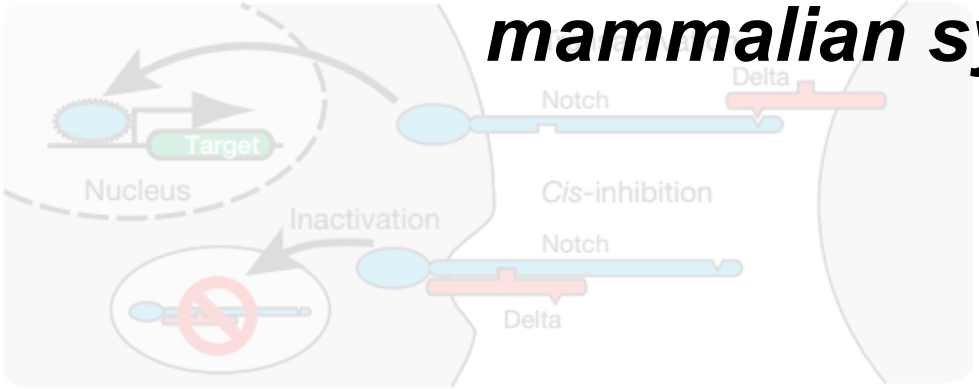


Diffusible molecule

Ability to Engineer

- Not orthogonal to cell
- Not generalizable

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



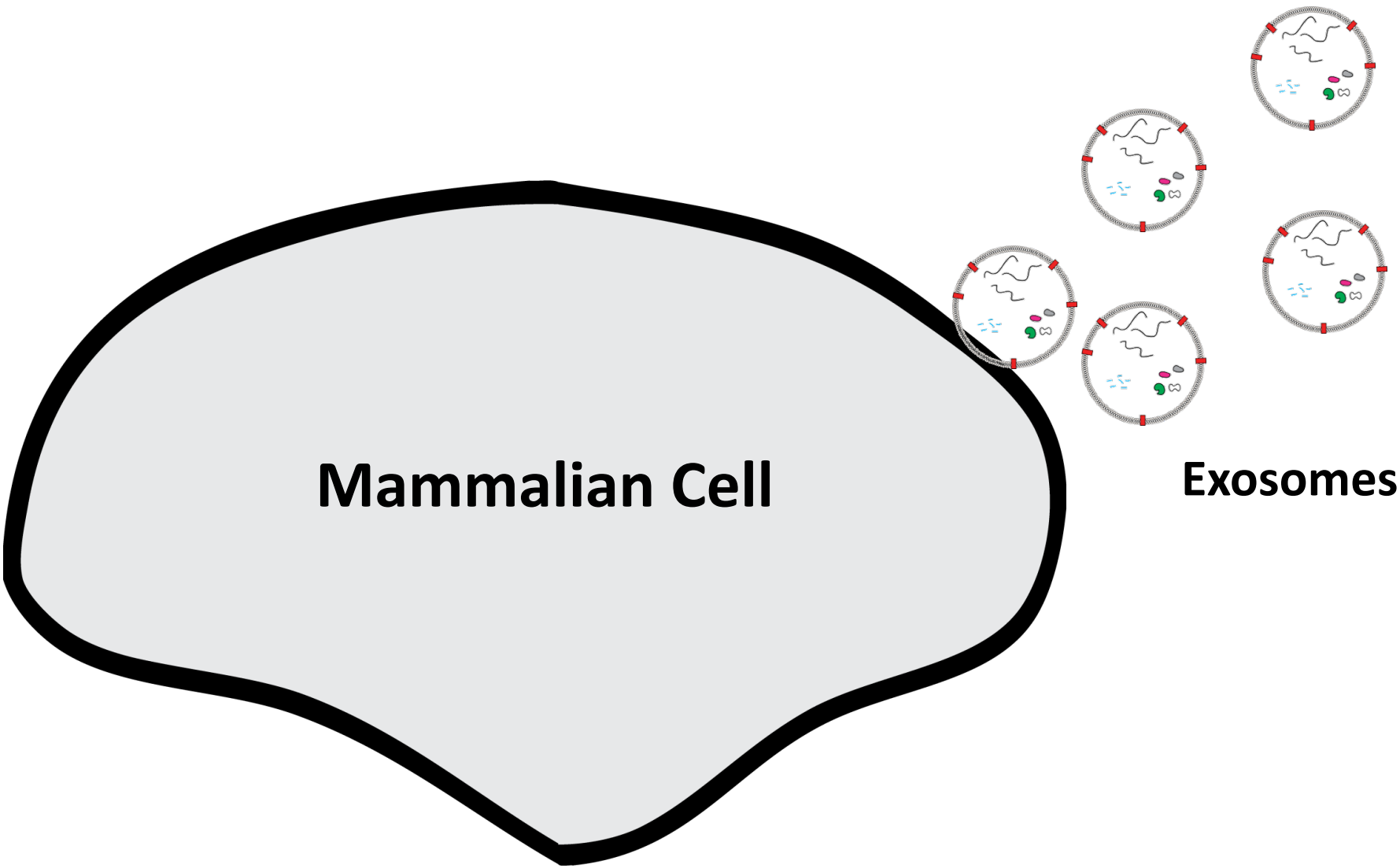
Receptor binding

Ability to Engineer

- Cross talk from delta/notch
- Requires cell contact

Sprinzak et al, Bacchus et al

Engineering Exosomes for Communication

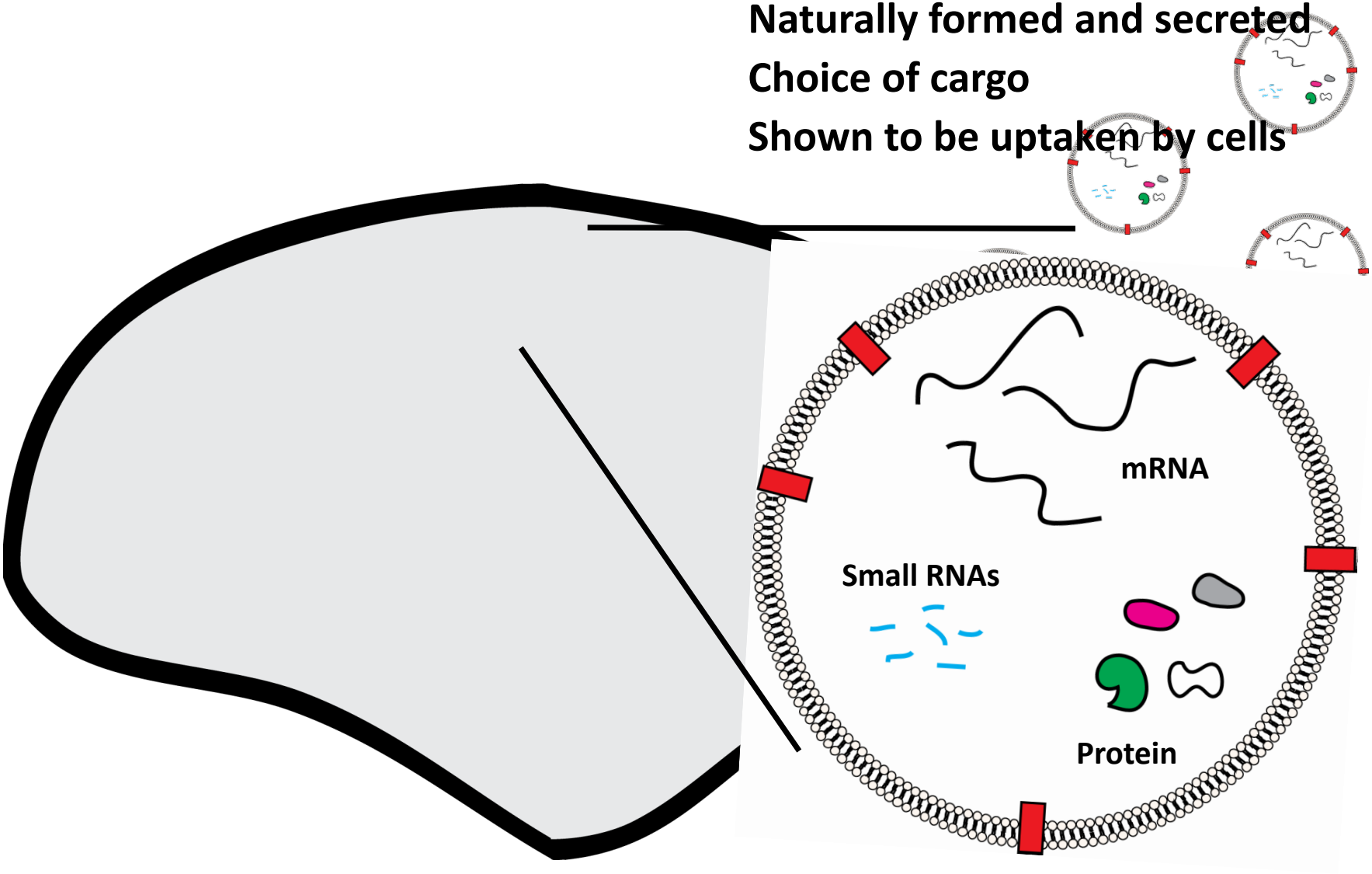


Engineering Exosomes for Communication

Naturally formed and secreted

Choice of cargo

Shown to be uptaken by cells



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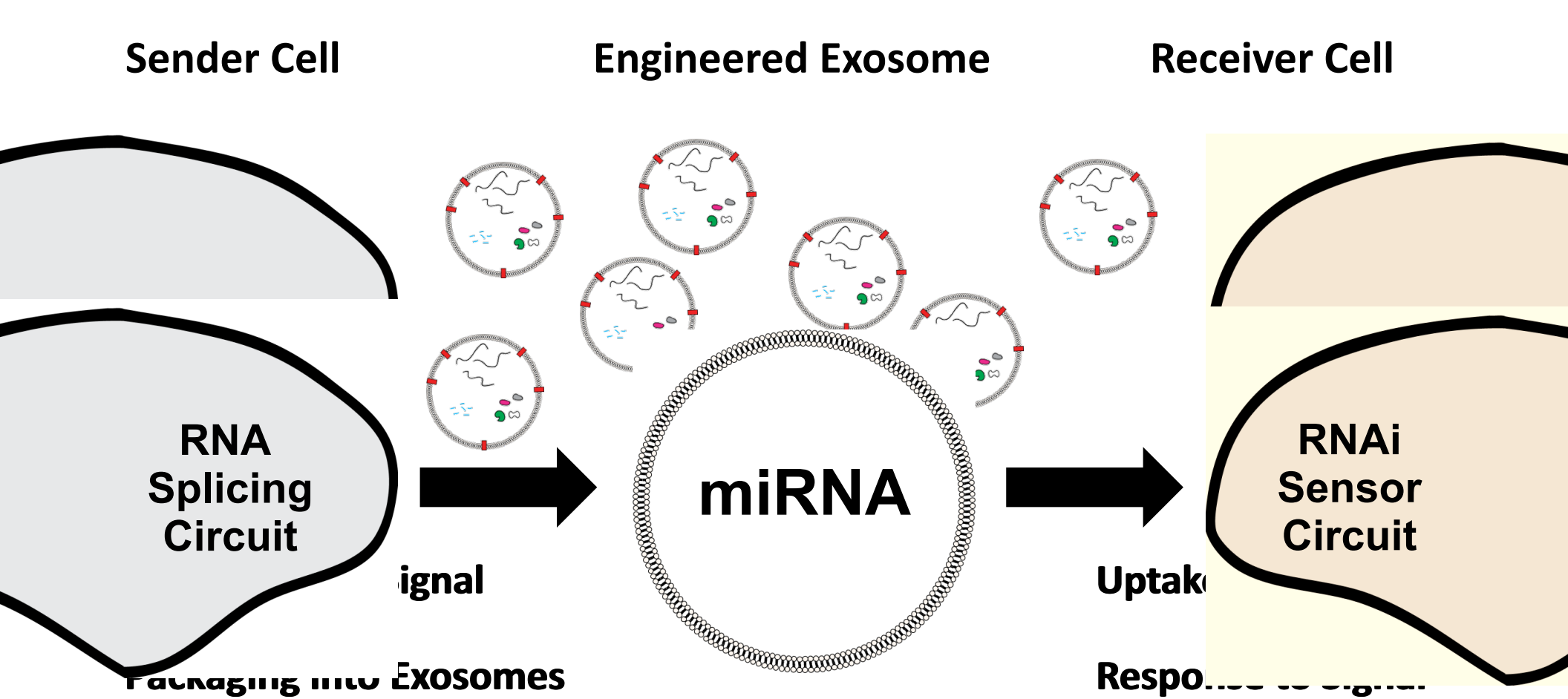
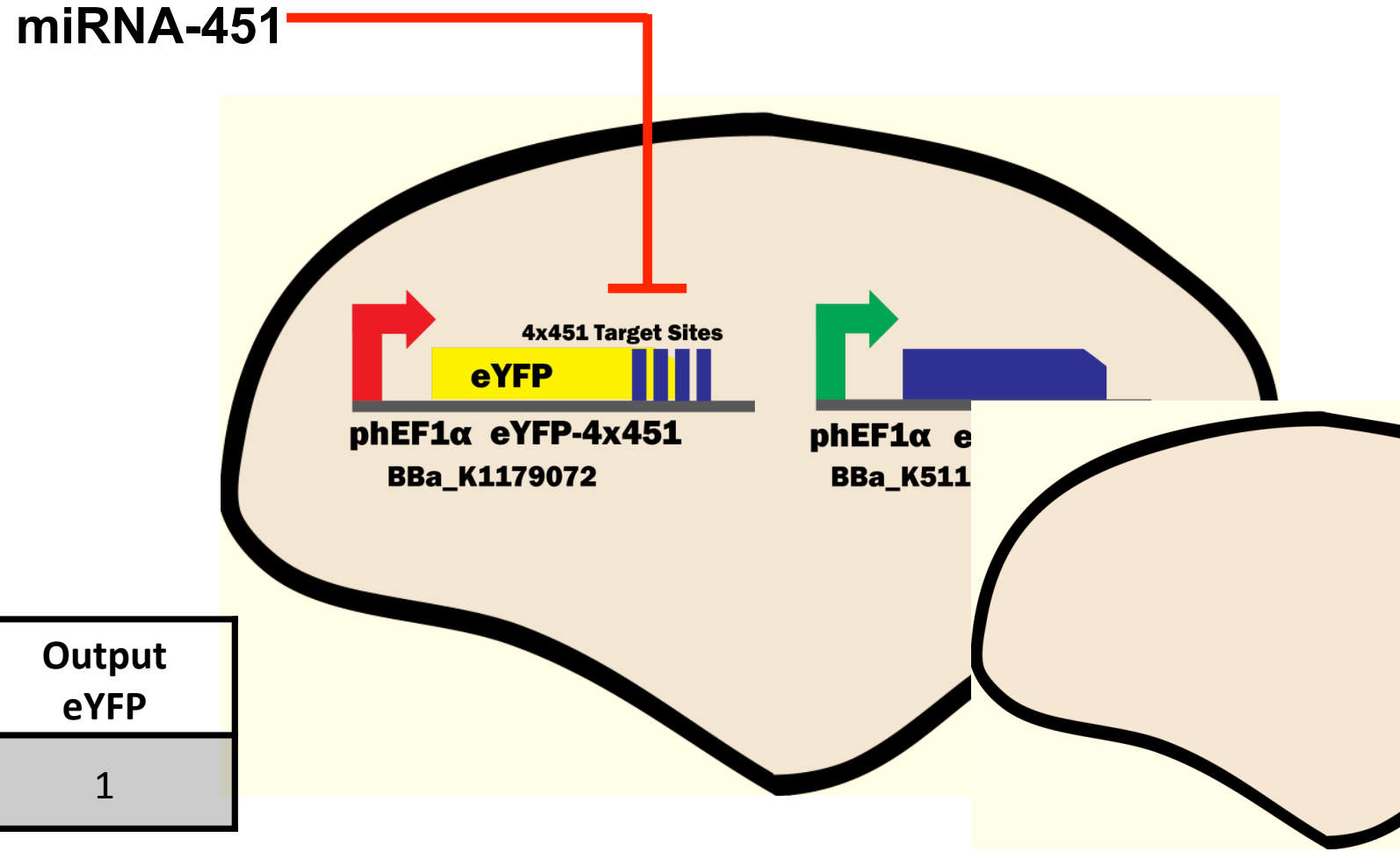
Design and Implementation Considerations

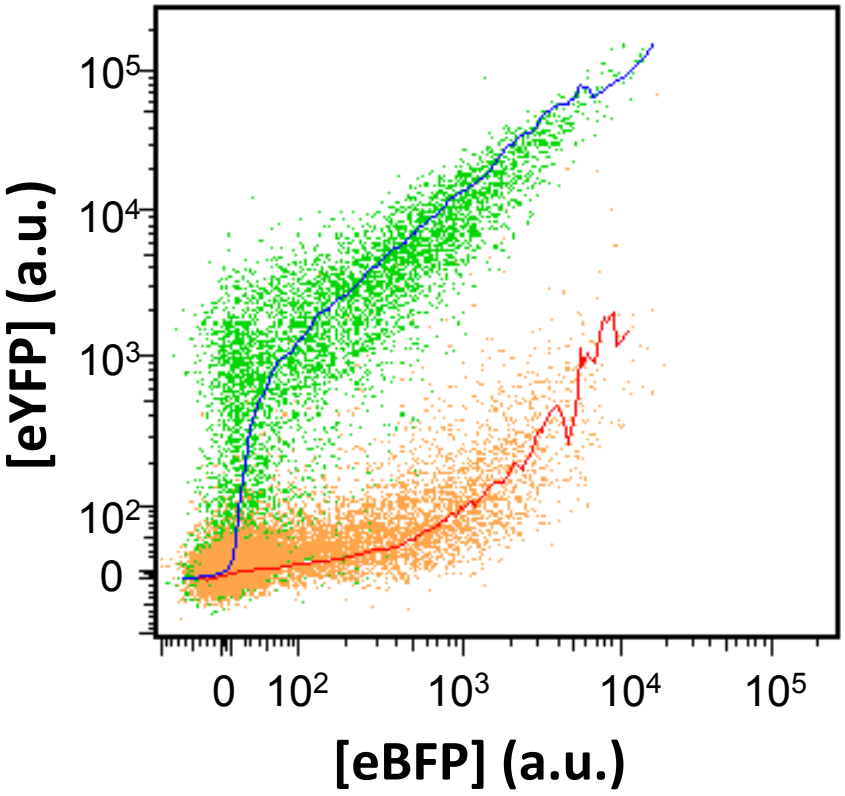


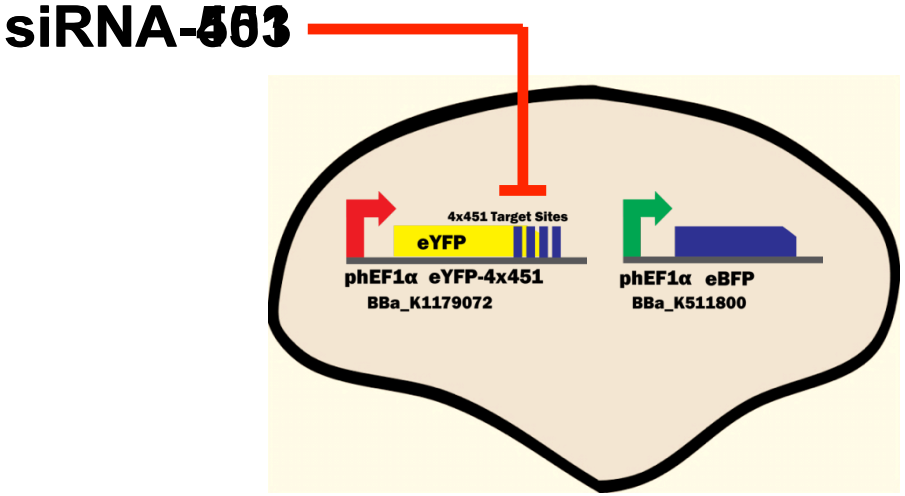
Receiver Cell Circuit Testing



Input miRNA-451	Output eYFP
0	1

Exogenous Input Testing of Receiver Circuit





Input miRNA-451	Output eYFP
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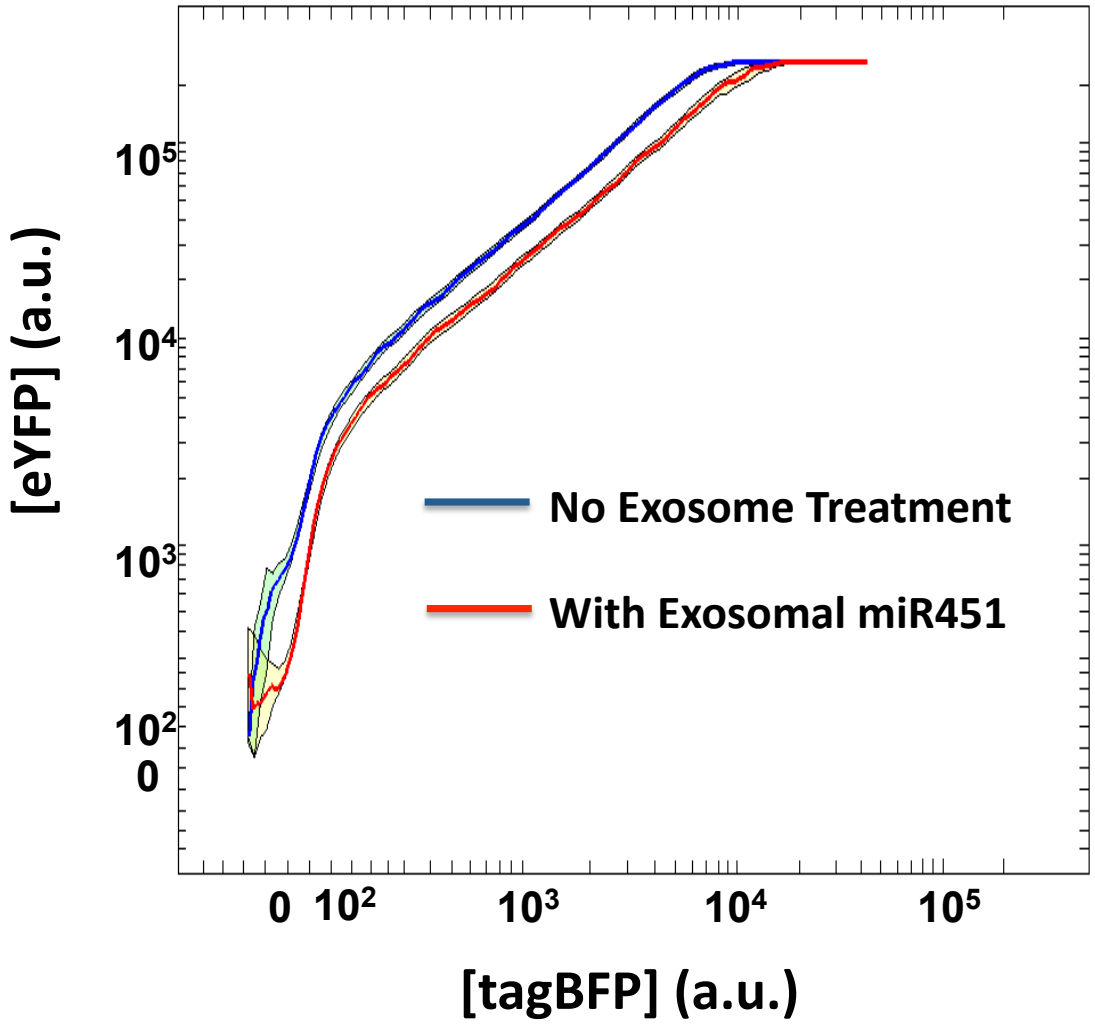
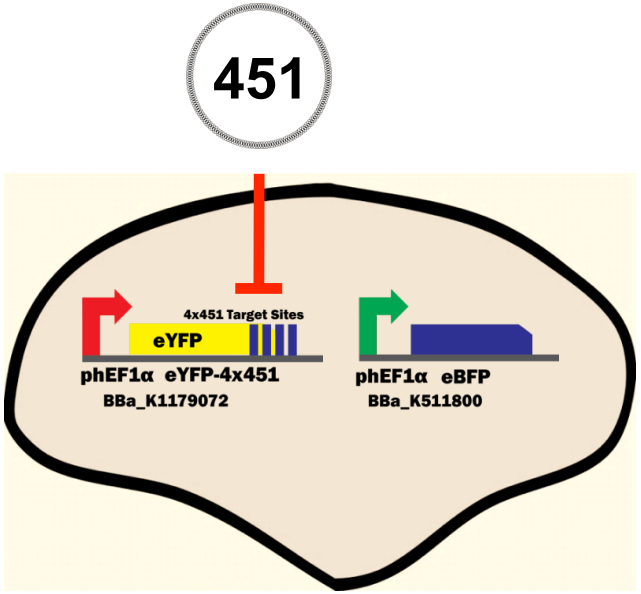
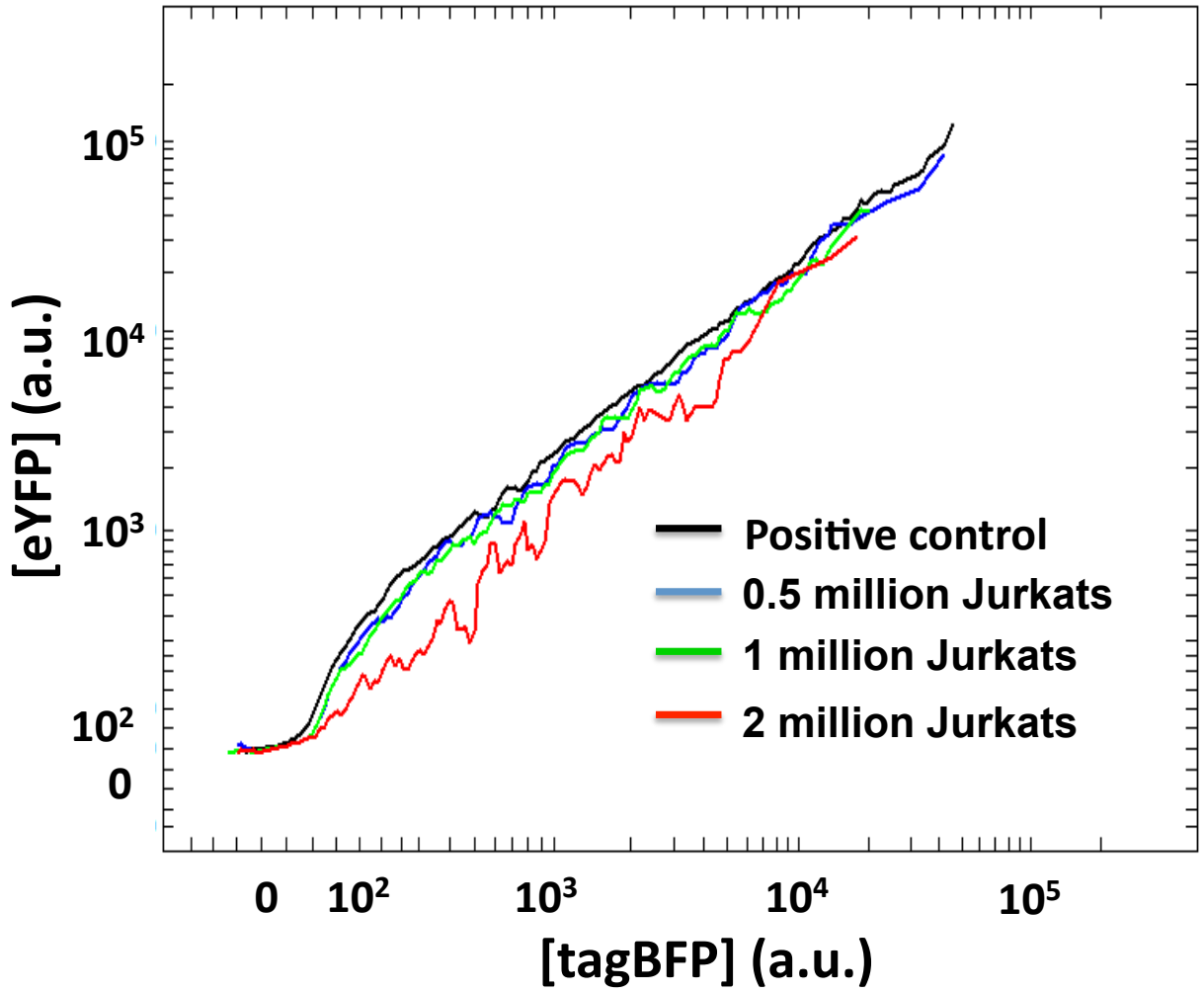
Exosomal Input Testing of Receiver Circuit

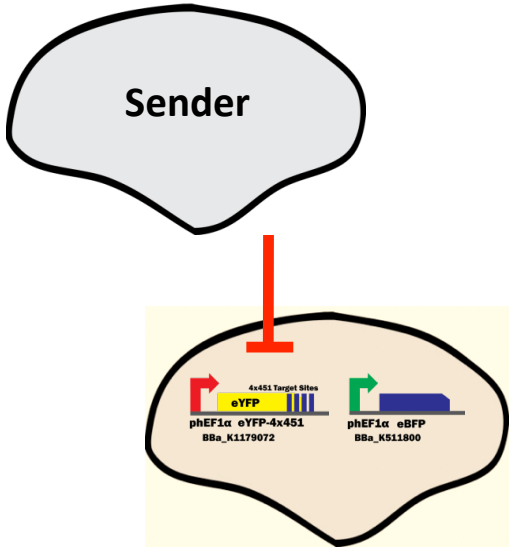




Input miRNA-451	Output eYFP
0	1

Testing of Integrated Cell-Cell Communication System





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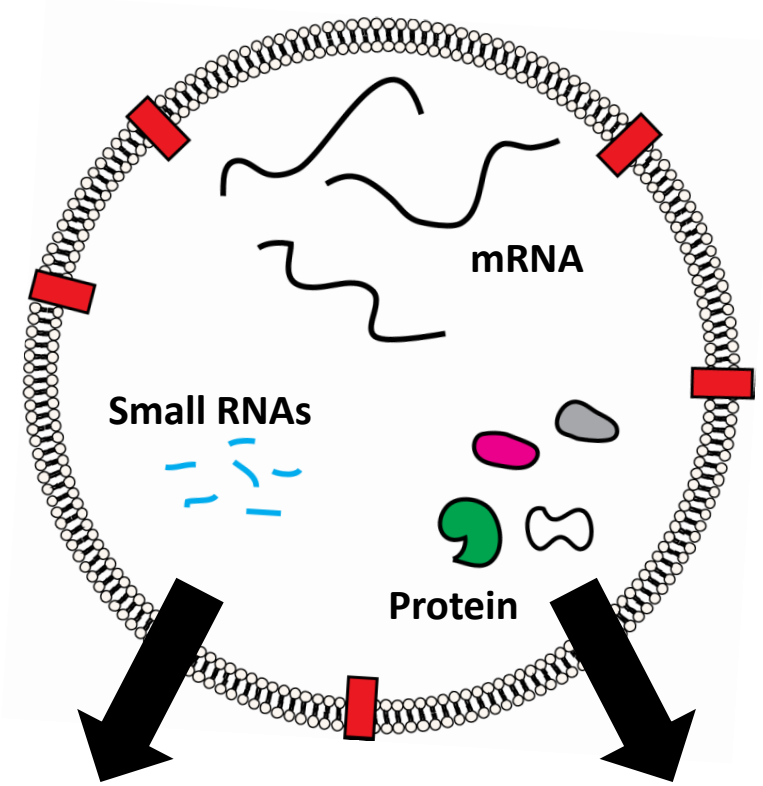
Engineering Exosomes for Communication



Ability to Engineer

Targeting mechanism not understood

miRNA crosstalk problems

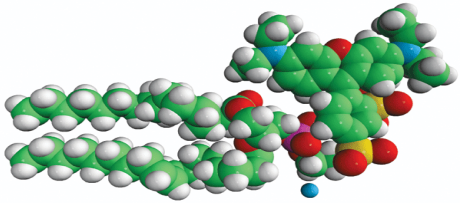
Ability to Engineer

Orthogonal proteins available

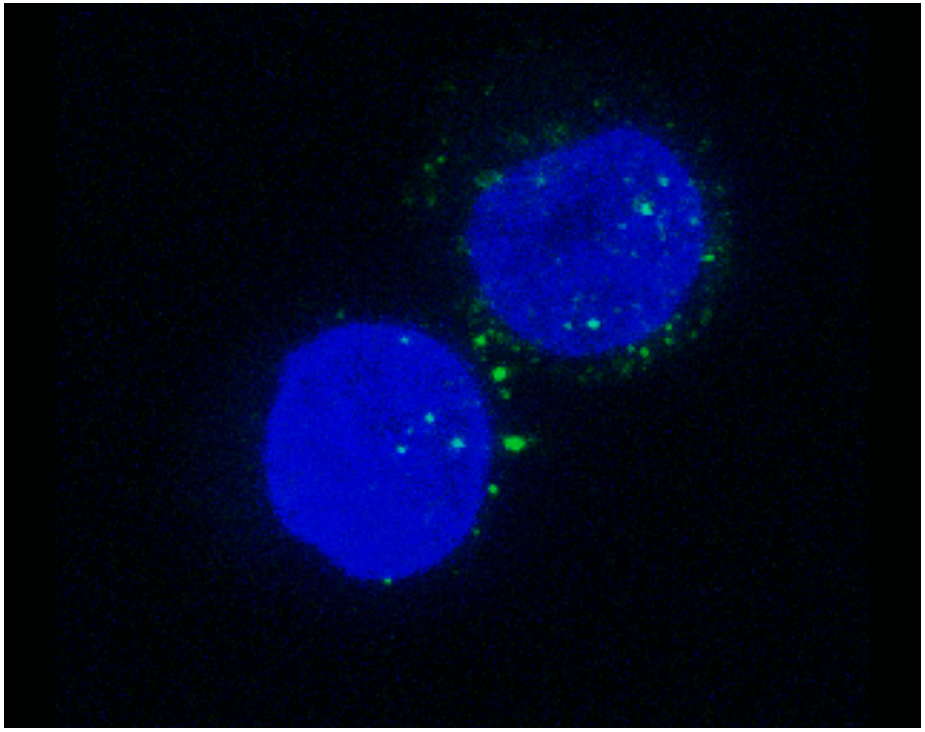
Known targeting motif – Acyl-TyA

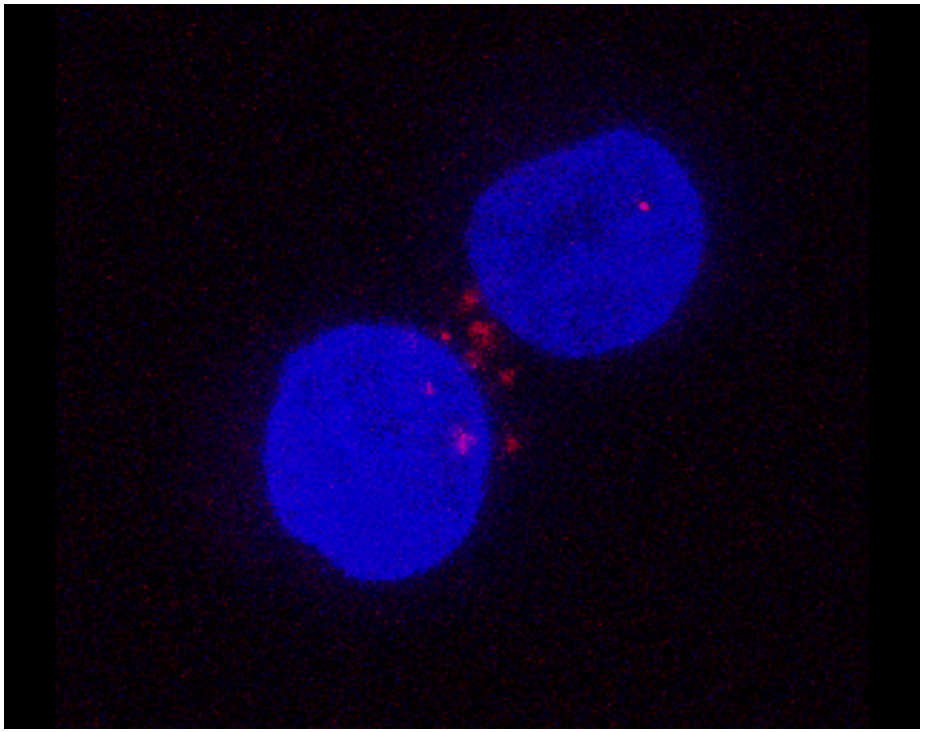
Acyl-TyA Targeting

BBa_K1179071



Rh-PE (Red)

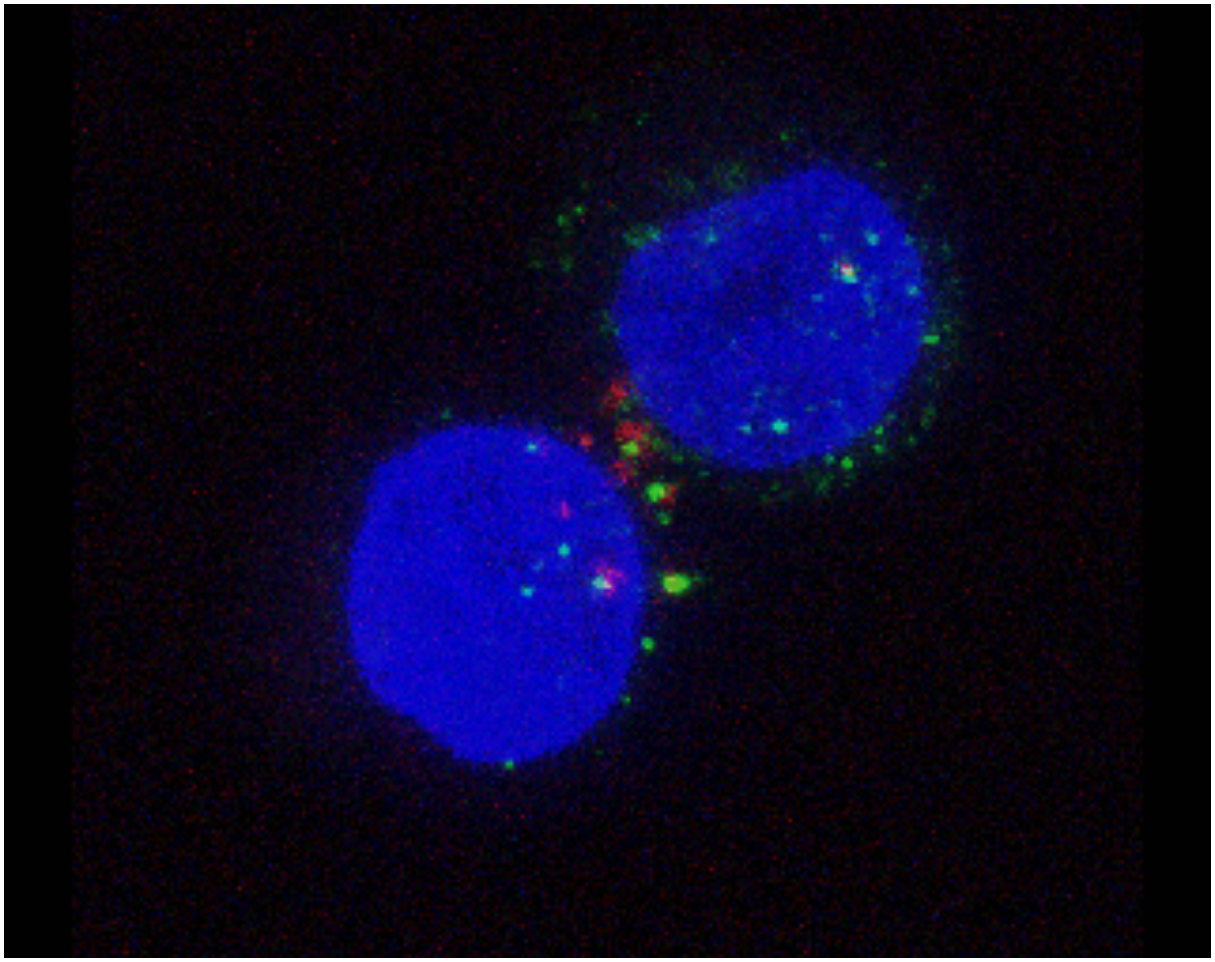




Green: GFP Blue: NucBlue

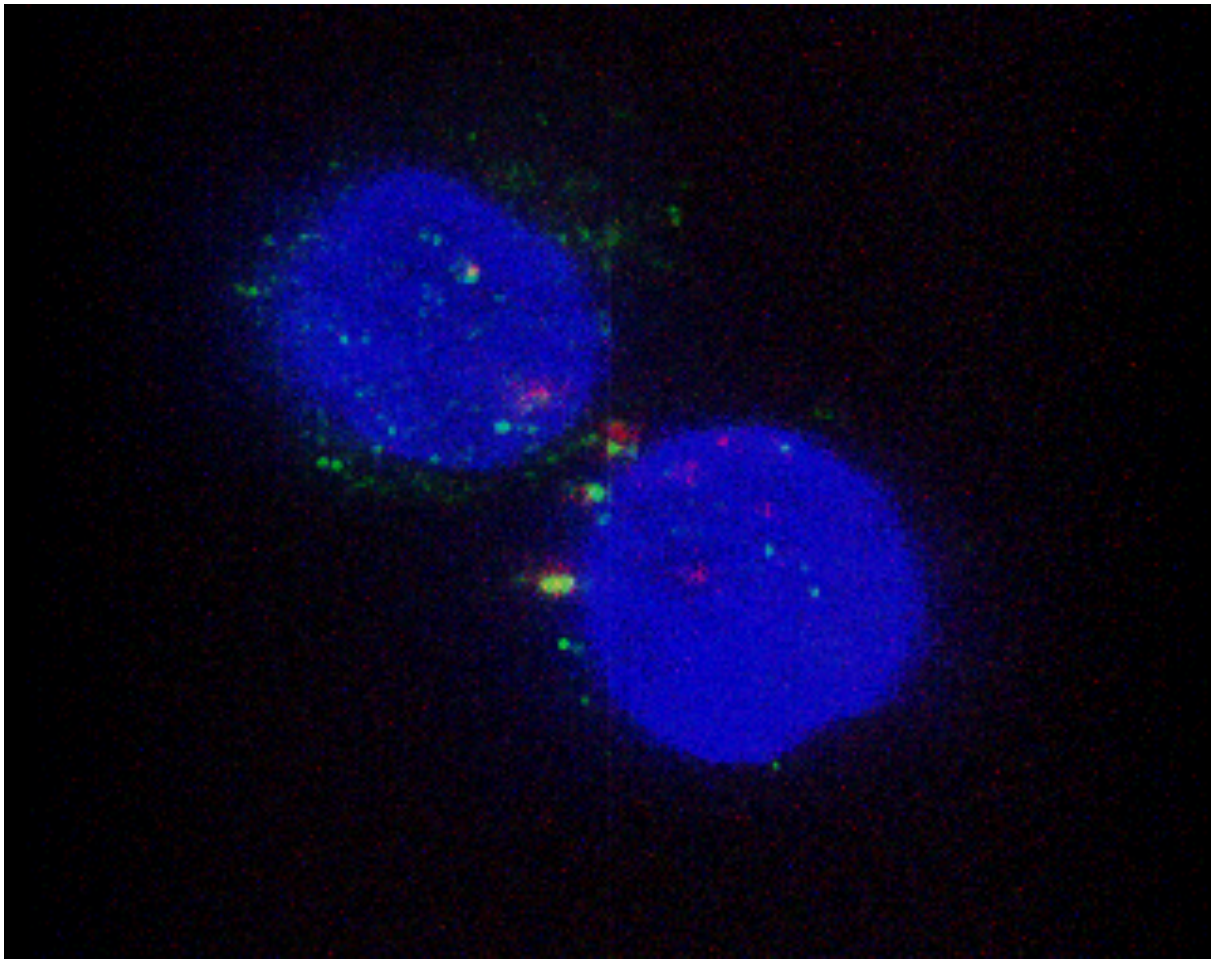
Red: Rh-PE Blue: NucBlue

Acyl-TyA Targeting



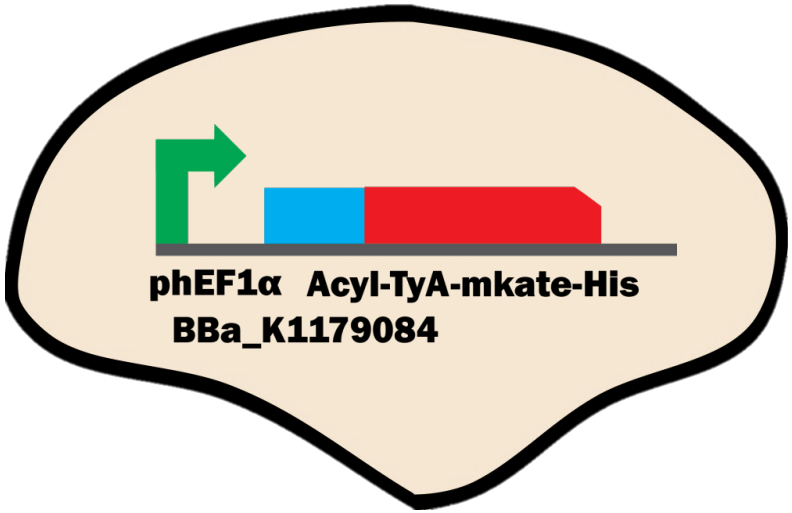
Green: GFP Blue: NucBlue Red: Rh-PE

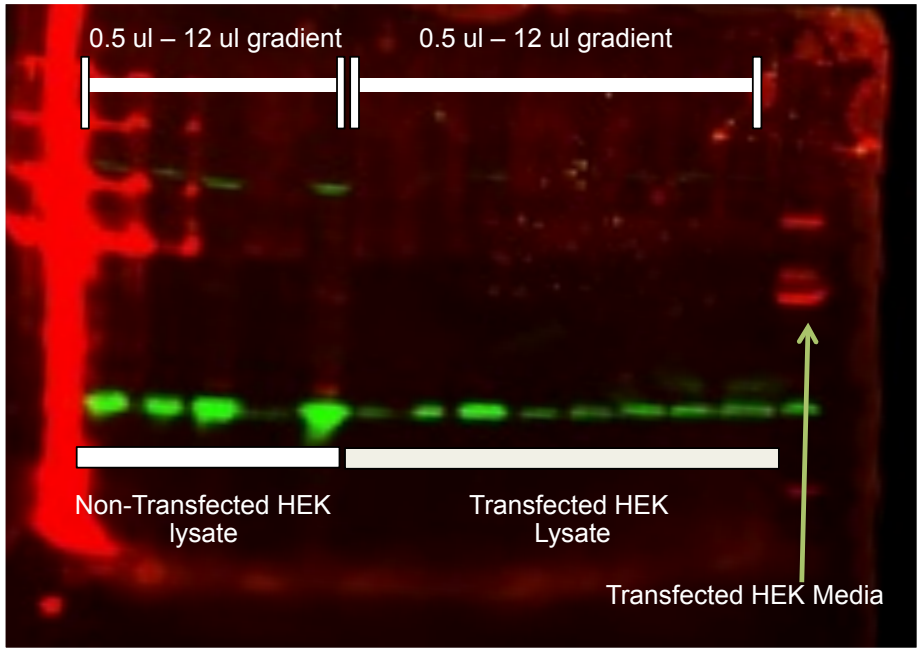
Acyl-TyA Targeting



Green: GFP Blue: NucBlue Red: Rh-PE

Acyl-TyA Export





Control Primary Antibody: anti-GAPDH (green)
Experimental Primary Antibody: anti-HIS (red)

HEK Cell Lysate

HEK Cell Media

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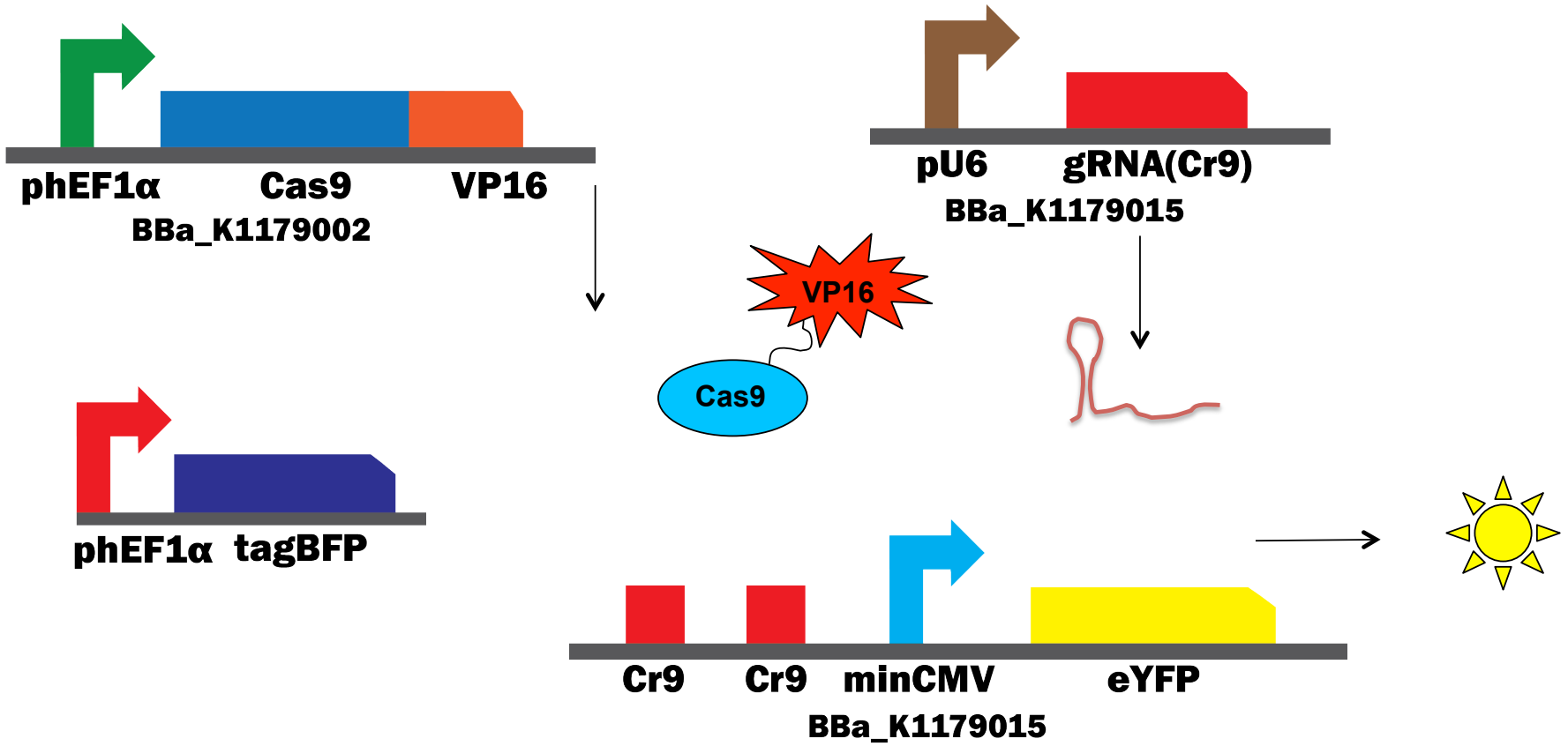
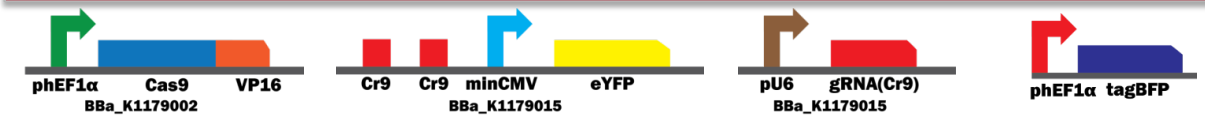
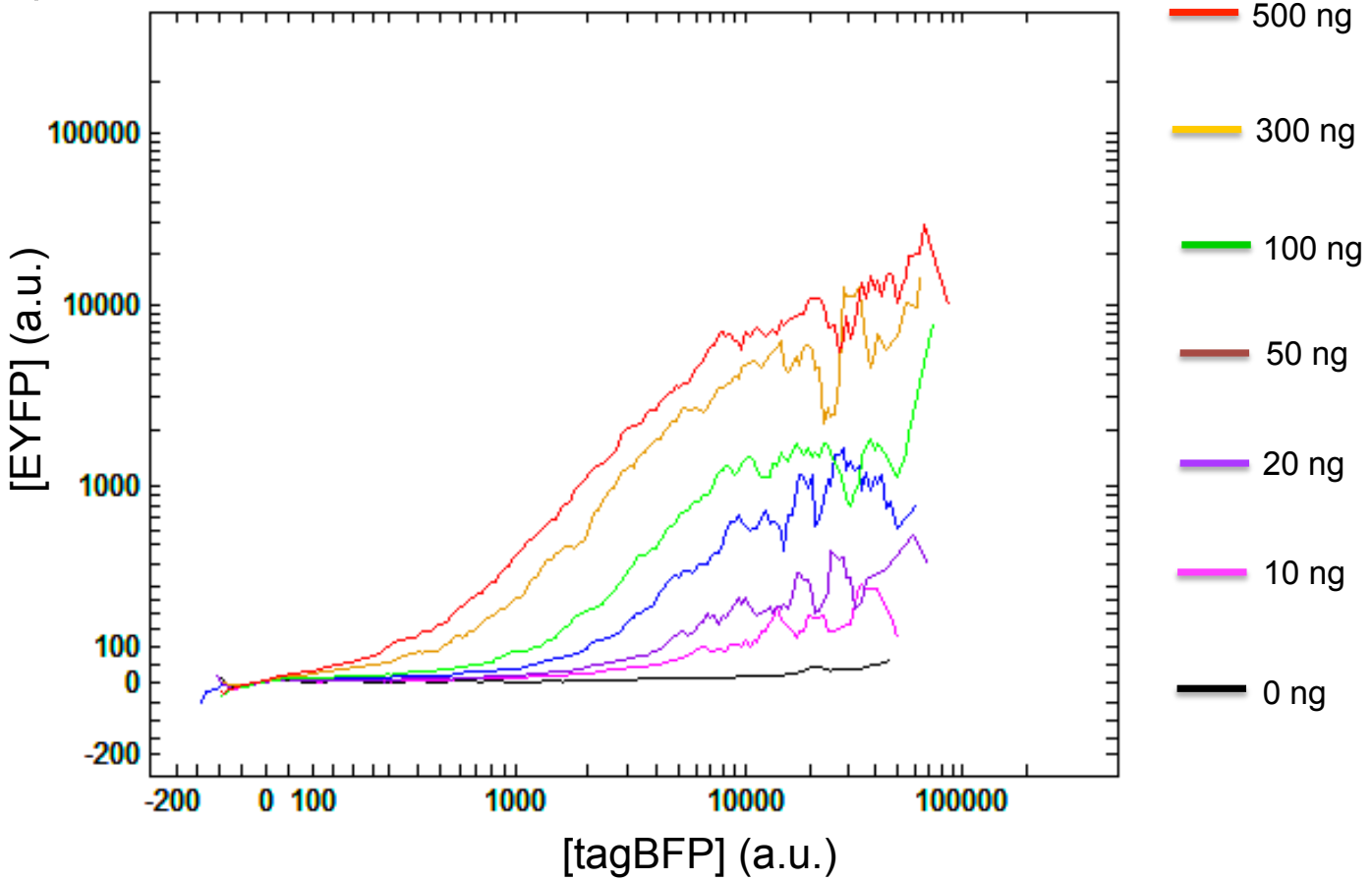
Circuit design for Cas9-VP16 mediated activation

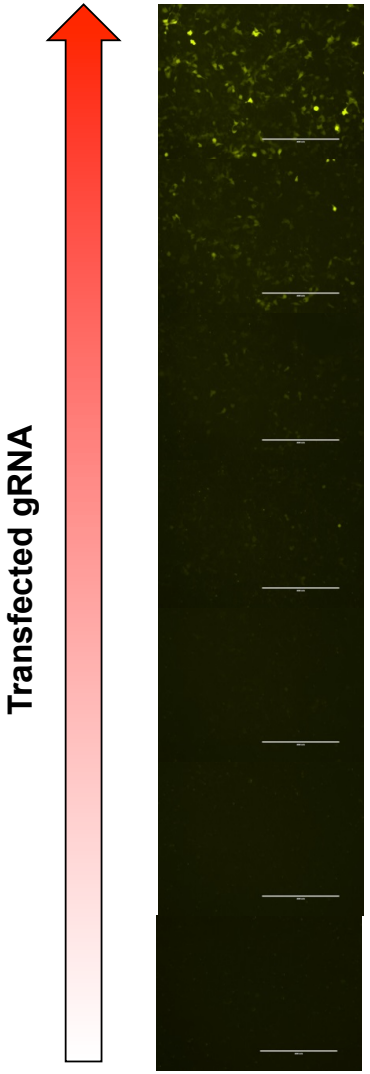


Cas9-VP16 mediated activation of EYFP



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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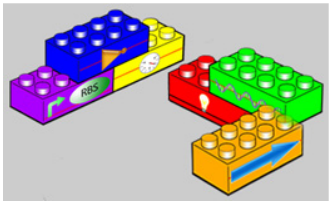
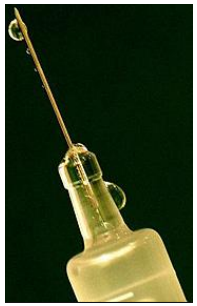
Impact

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

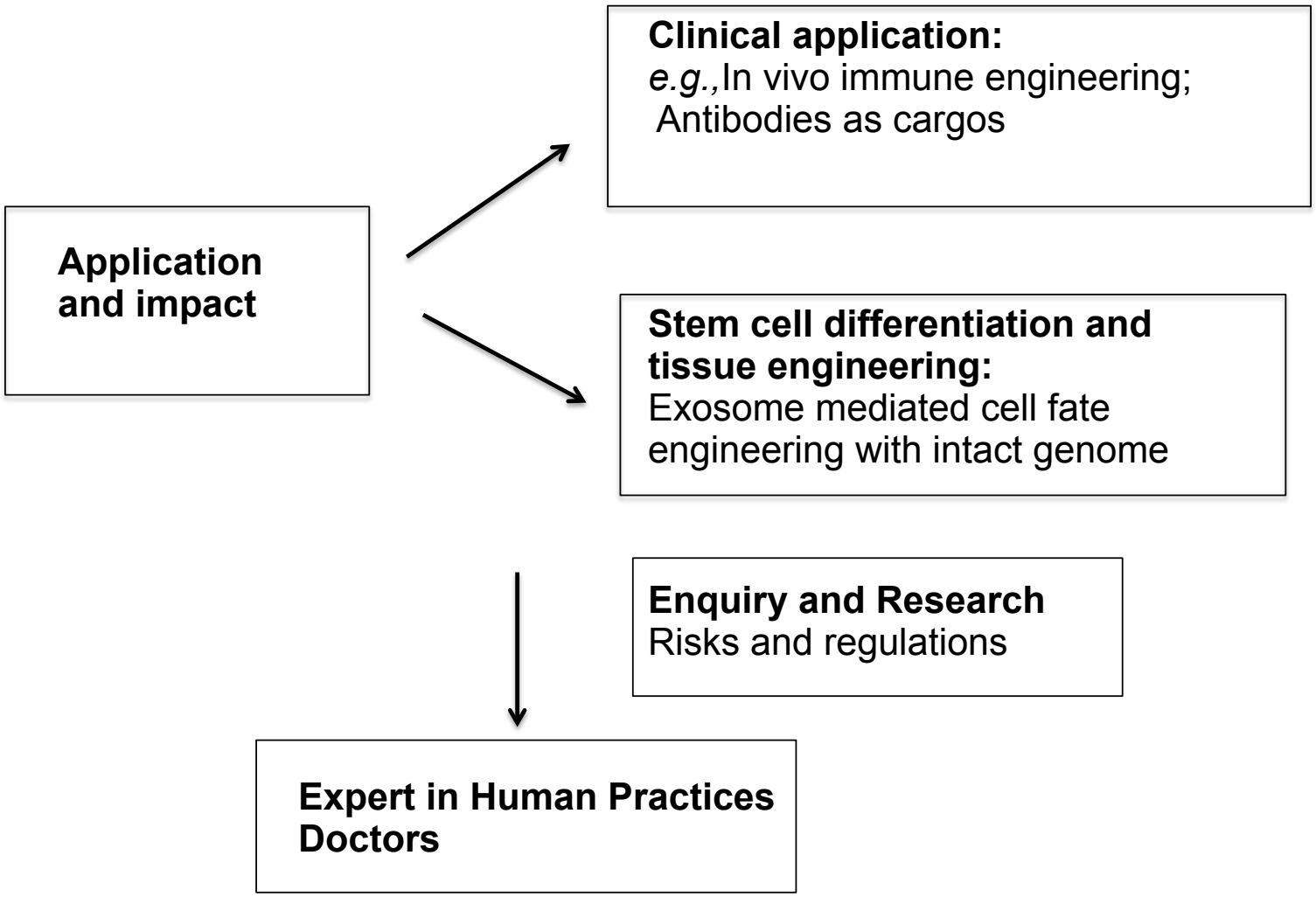
Student-led course simulcast to UAI Chile

Museum of Science®

Outreach to museum Planning SB exhibit

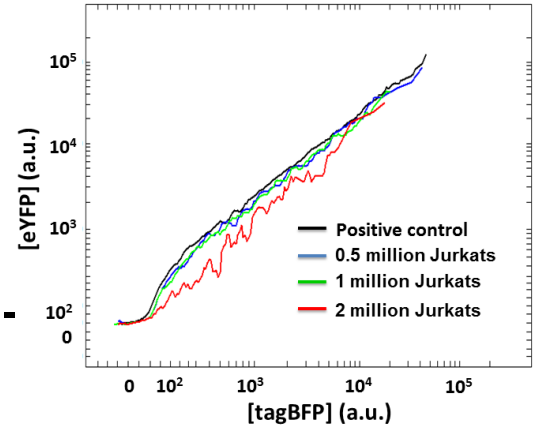
Met with MDs and human practices experts early to guide research direction

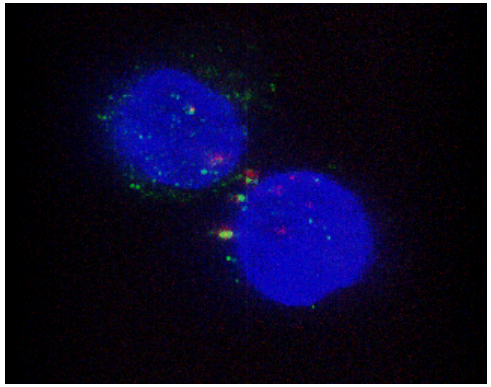
Integrated human practice approach

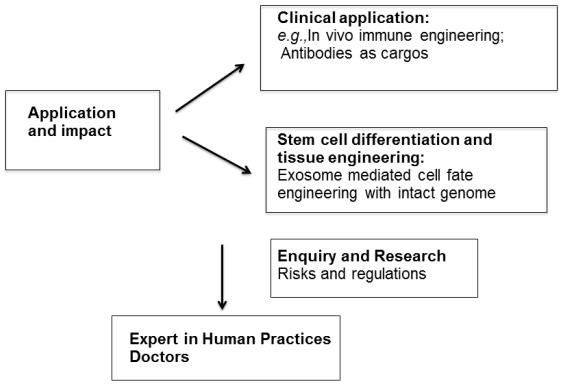


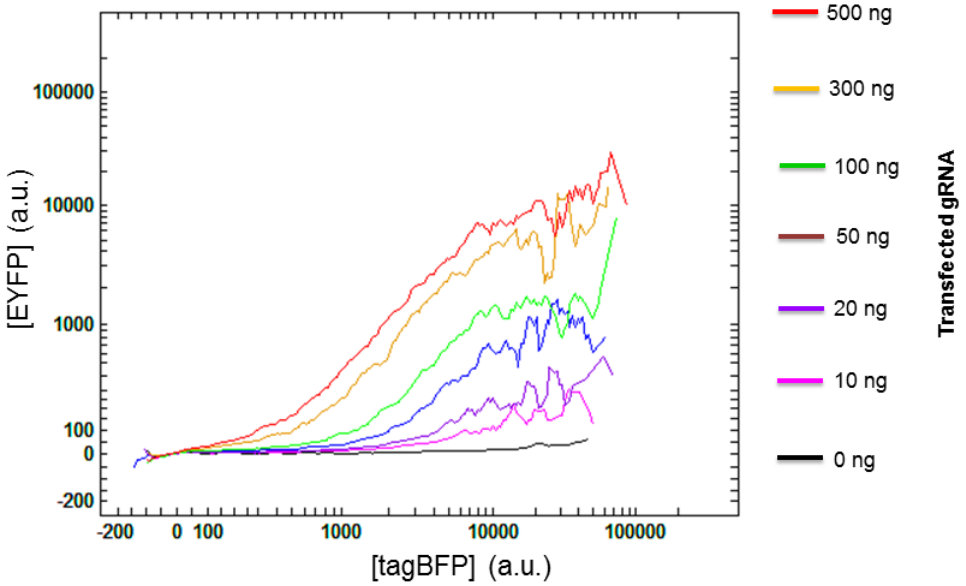
Summary of Accomplishments

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









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2013 MIT iGEM Team

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