Engineering genetic circuit interactions within and between synthetic minimal cells

CHEM-E8125 Synthetic Biology

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Engineering genetic circuit interactions within and between synthetic minimal cells

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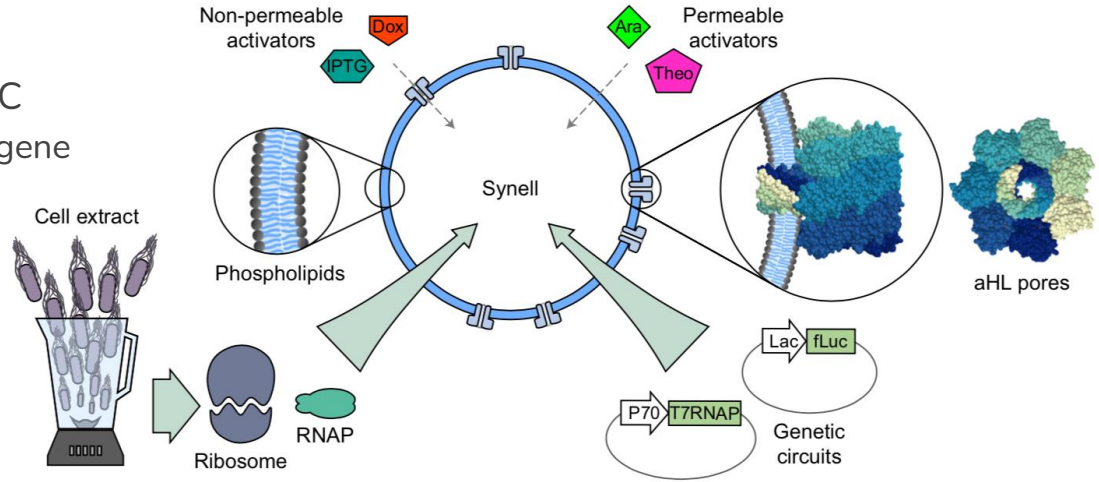
(Bio)chemical reaction cascades and genetic circuits in research and industry

- Characterize & model biological processes, biosensors, RNAs
- Engineer & produce small molecules and proteins
- Diagnostic tools

Introduction

Synells - Synthetic minimal cells

- Cell-free transcriptional/translational extracts encapsulated into liposomes
- Can be built to be SPECIFIC
  - Synthesizing a single-gene product



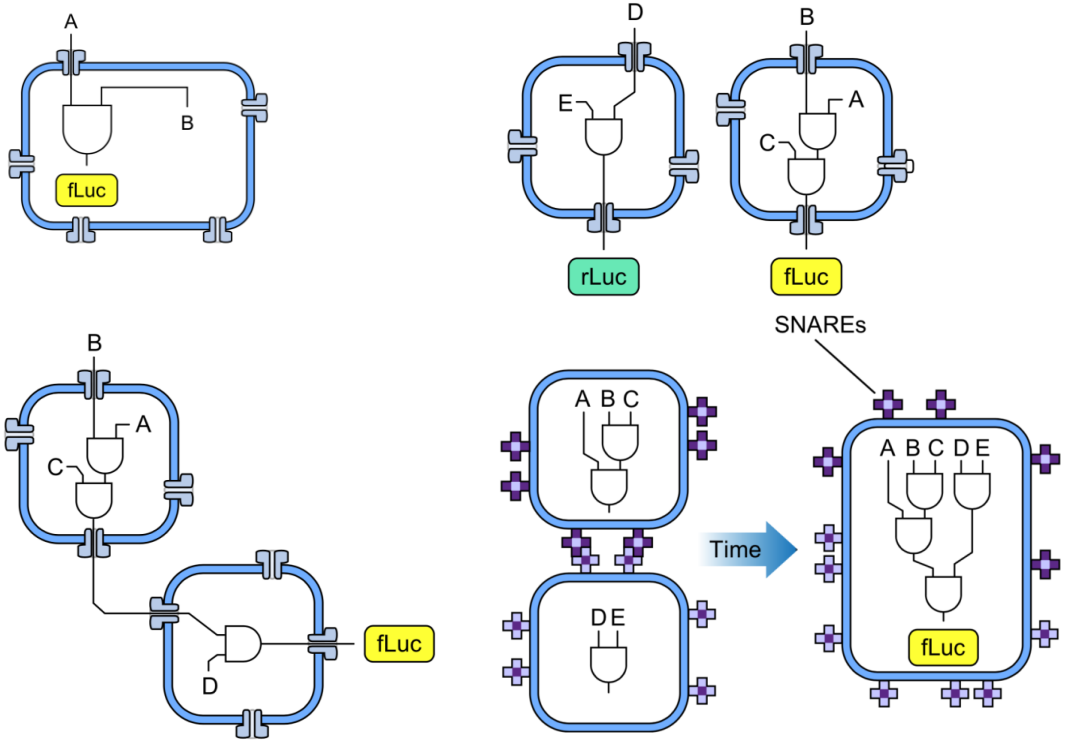
Main aim

- Integrating reactions and optimizing their scalability and flexibility
- To create modular, controlled compartmentalization of genetic circuits and cascades
- Creating synells that can operate in parallel, communicate or fuse

What was achieved

4 studies:

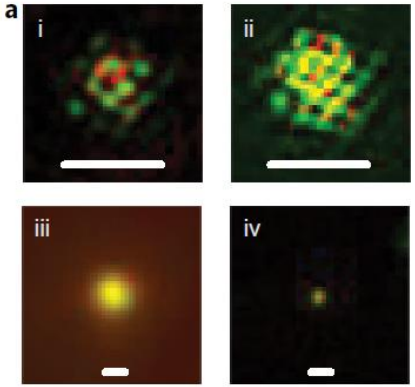
- Confinement
- Insulation
- Communication
- Fusion

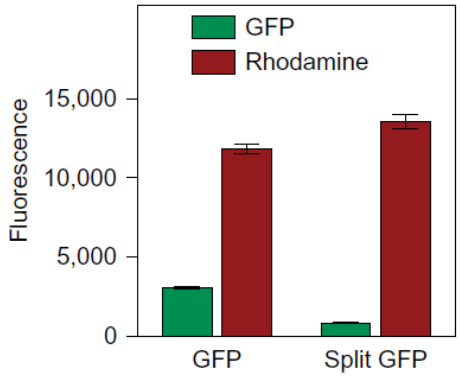


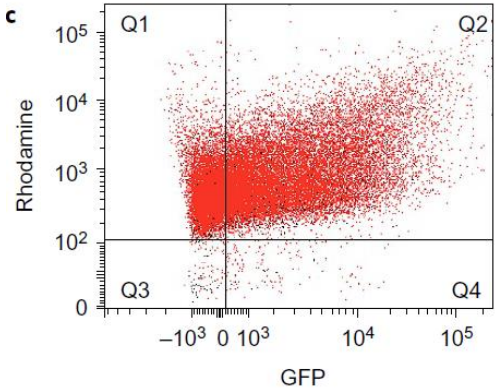
Confinement of genetic circuits in liposomes

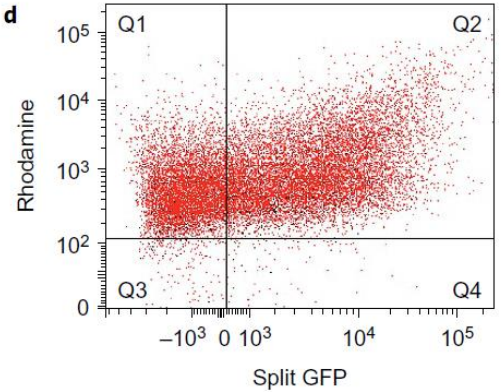
Characterization of basic functional and structural properties

- Labeled the liposomes with red dye
- Filled the liposomes with cell-free TX/TL extract (HeLa cells) & GFP or split GFP encoding DNA
  - Structured illumination microscopy → size of liposomes
  - Flow cytometry → quantify the function



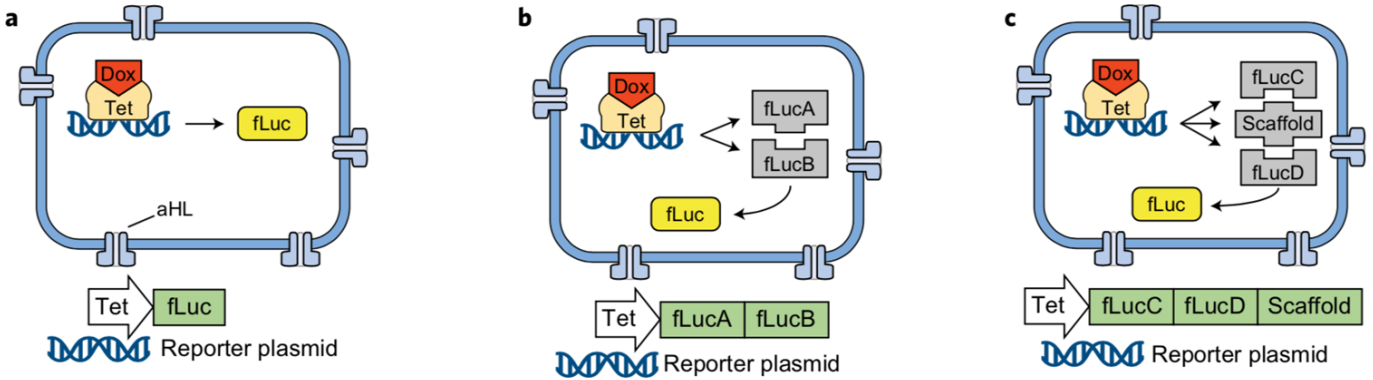






Facilitated reaction efficacy caused by molecular confinement

- Liposomal encapsulation can promote multicomponent genetic circuits and chemical reactions of higher order
    - The restricted movement of reagents increases the probability of the requisite multiway interactions

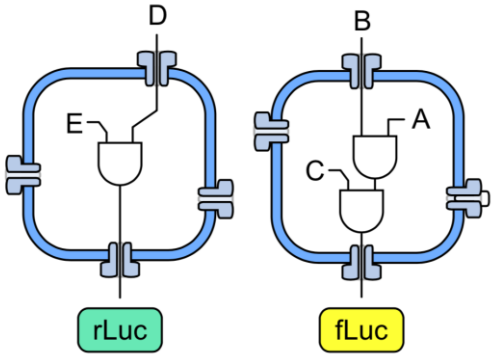


Insulation of genetic circuits operating in parallel liposome populations

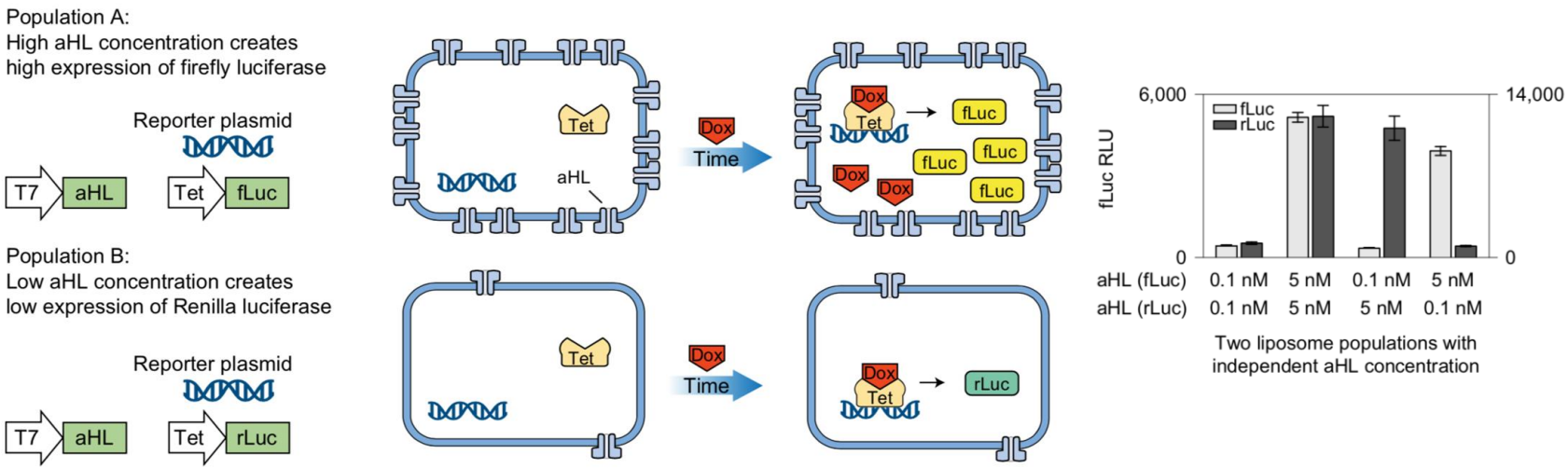
To determine if liposomes could be used to insulate multiple and potentially incompatible genetic circuits from each other, to operate in the same bulk environment.

Circuits could be optimized independently and contain chemical microenvironments that are not mutually compatible.

Populations of liposomes that would respond differently to the same external activators were created.



Insulation of genetic circuits operating in parallel liposome populations



Both populations operated independently and did not affect each other.

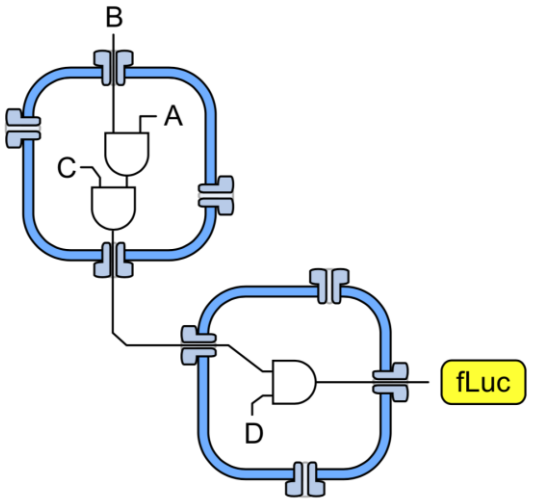
Communication between genetic circuits operating in multiple liposome populations

Objective:

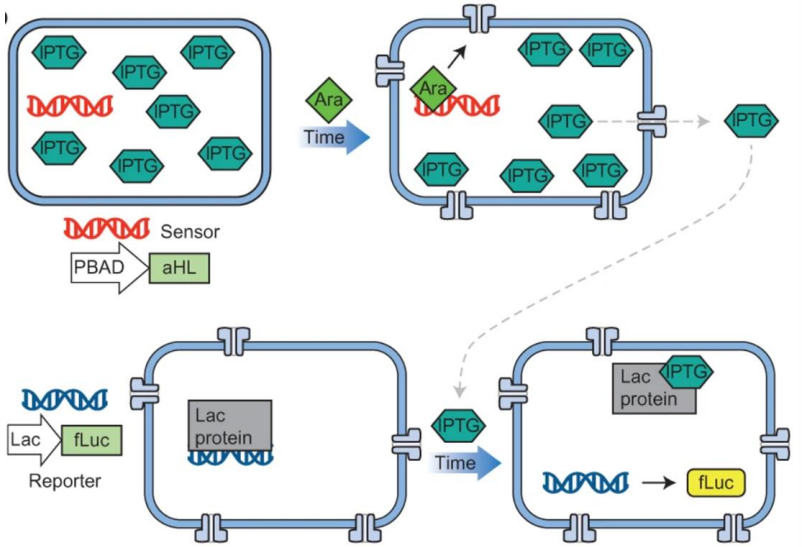
Can genetic circuits from different liposome populations communicate with each other? How is it if the populations are of different chemical microenvironments?

This experiment:

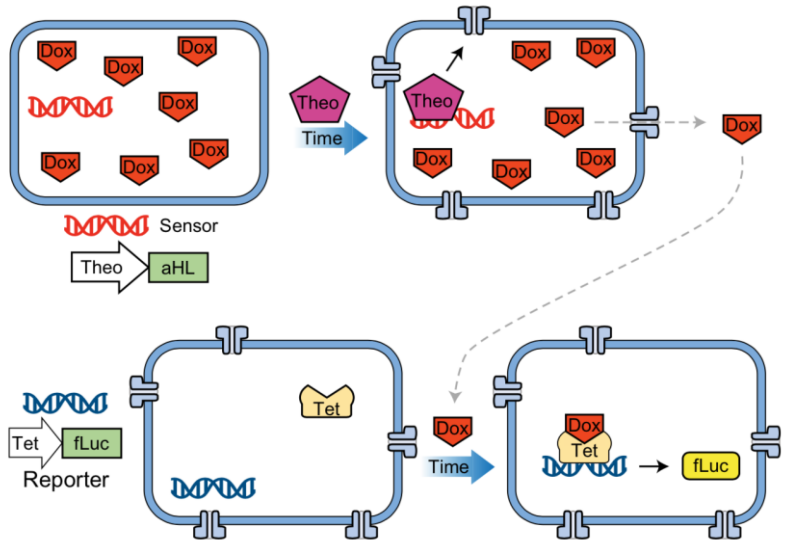
- 2 liposome populations
  - a **sensor** population that senses an external signal
  - a **reporter** population that receive a message from the sensor population and produce an output



1. Bacterial sensor and reporter liposomes

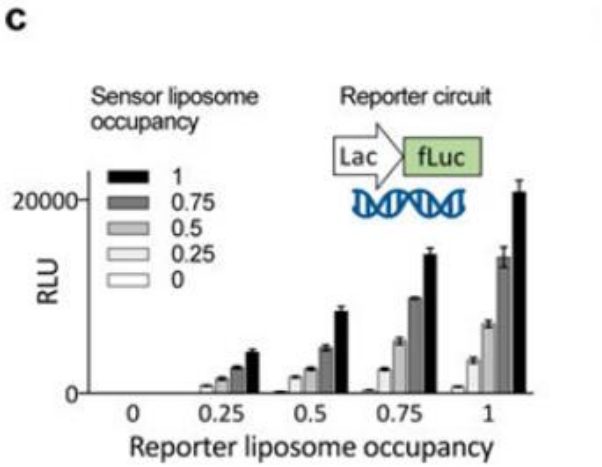


2. Bacterial sensor liposomes and mammalian reporter liposomes

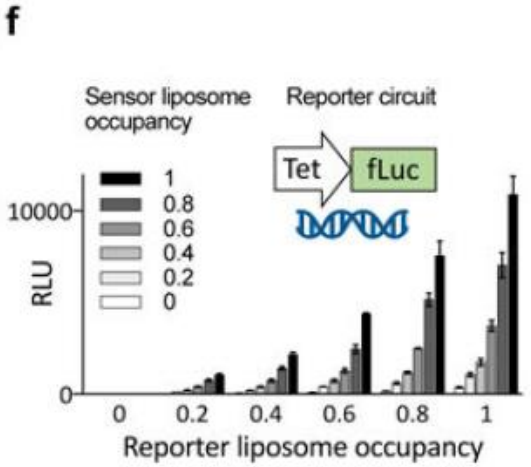


Result

- Both experiments worked, 1st one more efficiently
- Multicomponent genetic circuits with different chemical microenvironments can create coherent networks



1. Bacterial sensor and reporter liposomes



2. Bacterial sensor liposomes and mammalian reporter liposomes

Fusion of complementary genetic circuits

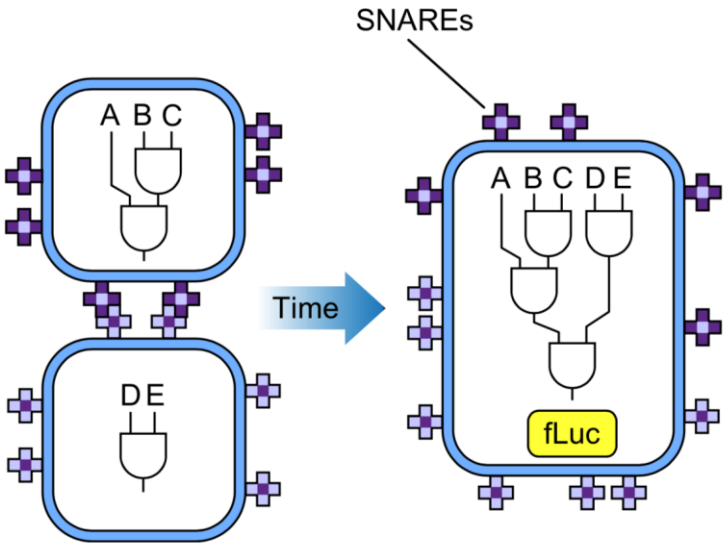
Objective:

To engineer synells capable of fusion to bring two genetic cascades in the same microenvironment in a programmable manner.

Motivation:

Two precursor molecules reacting together might have to be synthesized in different environments.

Example: Mammalian transcription and translation



Methods and approaches

Use of **SNARE-fusable liposomes.**

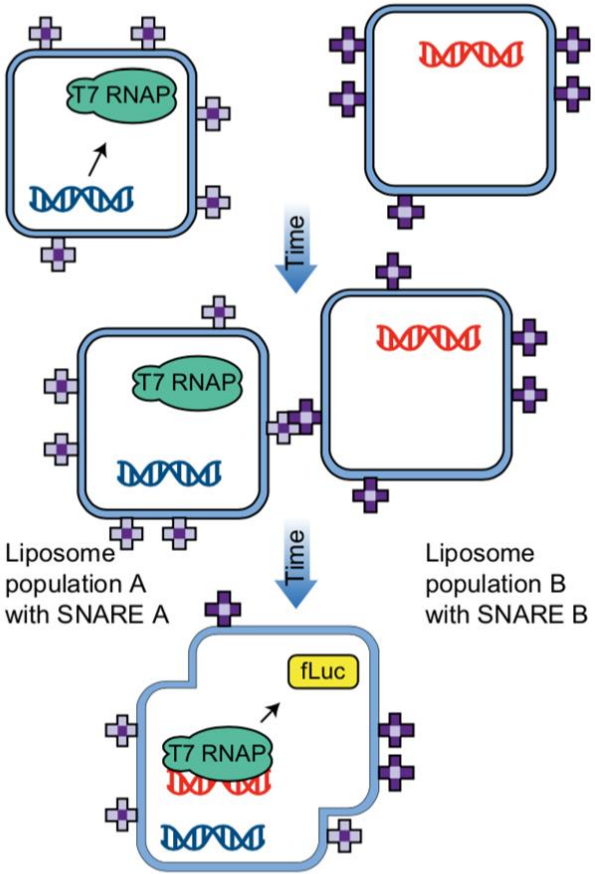
SNARE proteins mediate eukaryotic vesicle fusion.

SNARE protein mimics chemically synthesized by solid phase protein synthesis (Genscript) and fusion properties verified by SIM imaging and FRET.

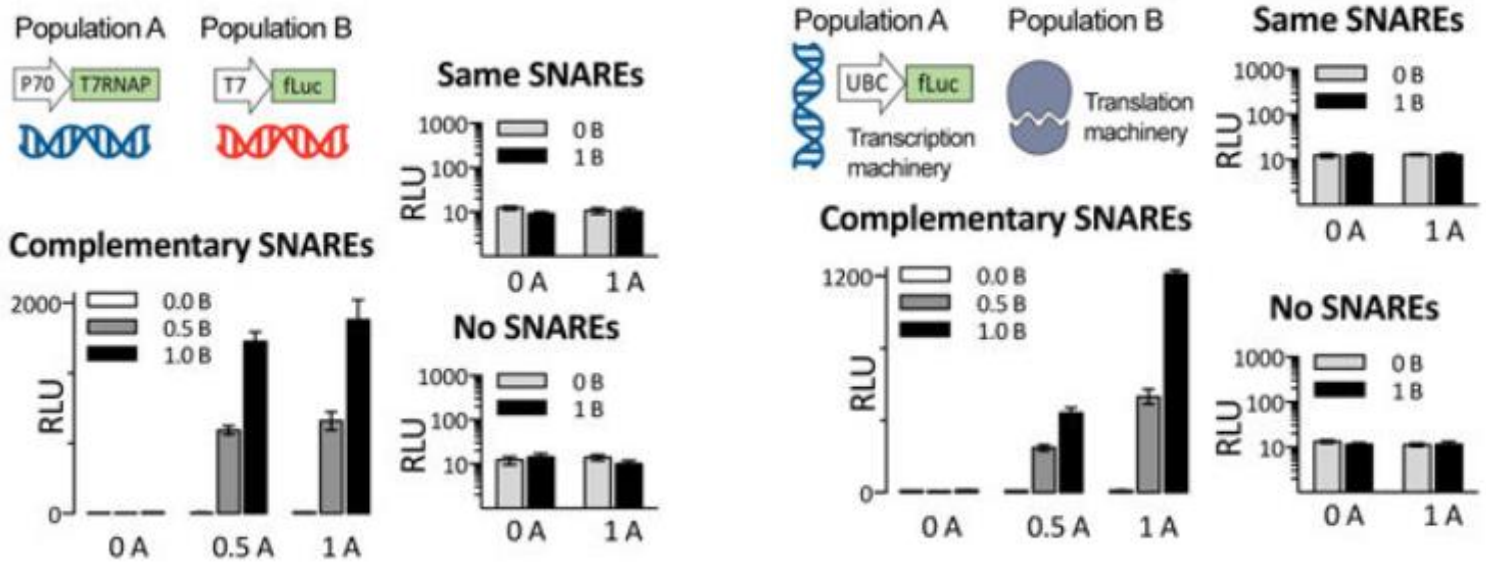
Only complementary SNAREs can mediate liposome fusion.

Liposome population A has SNAREs anchored in its membrane that are complementary to the SNAREs of liposome population B.

The reaction between A and B molecules can only occur after liposomes fusion.



Results for some of the experiments



Firefly luciferase (fLuc) activity was assayed using the Steady-Glo Luciferase Assay System (Promega).

In all experiments, the output (fLuc) was observed only when complementary SNAREs had mediated the two liposome populations fusion.

Importance and path forward

Synthetic minimal cells enable a new level of modularity for genetic circuit design and execution.

The method of compartmentalization is liposomal, so specialized hardware to mediate the communication and control of multiple interacting reaction systems is not needed.

Interacting encapsulated genetic circuits could enable the study of the more complex characteristics that have been proposed for the last universal common ancestor.

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References

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Thank you !

Questions ?