Engineering genetic circuit interactions within and between synthetic minimal cells

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Presentation Agenda

- Introduction to the article
- Aim of the study
- Methods and Approaches
- Discussion

Introduction

- Chemical systems that can perform biochemical reactions in the absence of live cells are utilized in research and industry
  - E.g, to model biological processes, produce small molecules, engineer proteins, etc.
- Cell-free production of biochemical products requires transcription/translation (TX/TL) extracts that can be obtained from many organisms
- Cell-free TX/TL extracts encapsulated within liposomes = bioreactors called synthetic minimal cells (synells)
  - Synells = liposomes containing genes as well as transcriptional and/or translational machinery
- So far, research/work on liposomal synells has focused on expression of single genes within a homologous population of liposomes
- However, one issue of synthetic biology is the low modularity of multi-component genetic circuits and cascades → liposomal compartmentalization enables modularity of genetic cascades
- These cascades can be created by first encapsulating genetic circuits and cascades within synells and then arranging them to either
  - Operate in parallel
  - Communicate with each other
  - Or fuse with each other in a controlled way
- Result: Genetic cascades can proceed in well-isolated environments while retaining the desired degree of control

Introduction

- This study presents design strategies for constructing these synell networks, which widens the scope of liposome technology and improves the modularity of synthetic biology
- Benefits: synell networks might support complex, higher order chemical reactions by providing high-fidelity isolation of multiple reactions from each other and controlled communication and regulatory signal exchange between said reactions
    - E.g., two typically incompatible reaction machineries can be brought together by the controlled fusion of two synell populations

Aim of The Study

- Challenge: How to maximize the modularity of genetic circuits/reaction cascades to enable the integration of different reaction networks and to optimize their scalability and flexibility
- Possible approach: encapsulate reactions within liposomes, which creates a well-isolated environment for chemical reactions to proceed
- The research group adapted liposome encapsulation to allow controlled compartmentalization of genetic circuits and cascades. The group demonstrated that:
  - Genetic circuit-containing synells can be engineered to contain multiple-part genetic cascades
  - These cascades can be controlled by external signals and inter-lipososomal communication without cross-talk
  - Liposomes with different cascades can be fused in a controlled way so that the products from incompatible reactions can be brough together
- Result: synells enable more modular creation of synthetic cascades which is an important step towards their programmability, which is the ultimate goal

Methods and Approaches

Confinement of genetic circuits in liposomes

- Before testing the control of the synells and the their communication, the basic structural and functional properties of individual synells were characterized
  - The liposome membranes were labeled with red dye and filled with cell-free TX/TL extract derived from HeLa cells, and DNA encoding either GFP or split GFP
  - **Sizes of the GFP liposomes** were measured using Structured illumination microscopy (SIM)
    - Diameters were between 100 nm and 1 µm. This result was confirmed with dynamic light scattering.
  - Functionality, meaning the **functional expression of genes by synells** was measured using flow cytometry
    - 68.4% of the GFP liposomes expressed fluorescence
    - 61.8% of the split GFP expressed fluorescence
  - Enzymatic activity of several reporters in the liposomes were characterized and Western blot was used for non-enzymatic characterization of luciferase expression

Confinement of genetic circuits in liposomes

- The performance of mammalian (HeLa) and bacterial (E.coli) TX/TL systems in the liposomes were compared

→Mammalian system was found to be slower and have a lower protein yield

→ The liposomes were proven to be of proper size and functionality

Confinement of genetic circuits in liposomes

Verification of well-known advantage of liposomal compartmentalization:

- Encapsulating reactants within a liposome facilitates the reaction efficacy
- Cell-free transcription/translation (TX/TL) reactions producing firefly luciferace (fLuc) from one, two, or three protein components were compared in bulk solution vs. synells:
  - →For all three orders of luciferase-producing reactions, the effect of dilution on fLuc expression was weaker for liposomes than for bulk solution
  - →The liposomes produced lower amounts of fLuc than the corresponding volume of TX/TL extract in bulk solution
  - →The liposome encapsulation resulted in a nearly equal efficacy to that of bulk solution for the third-order reaction
  - →The first-order and second-order reactions resulted in lower efficacy than in bulk solution

→As a result, it can be concluded that molecular confinement in liposomes may help facilitate higher-order reactions that require multiple chemical building blocks to be brought together

Synell Design Strategies

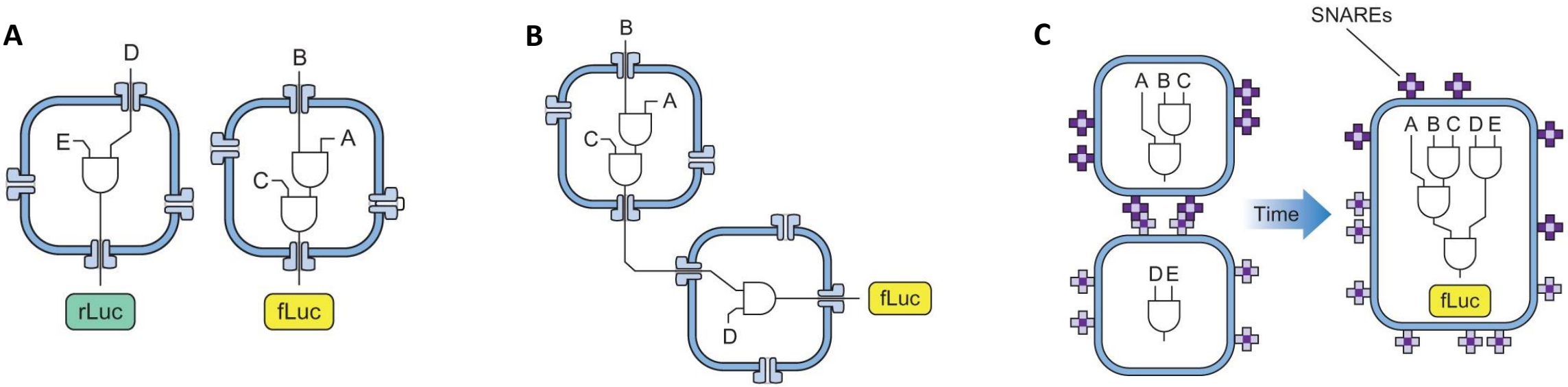
Synell design strategies

The article showed that synells can be designed so that…

A. The circuits can run in synell populations in the same container independently due to the insulation of the liposomal membrane

B. The circuits can communicate with one another through small molecule messengers

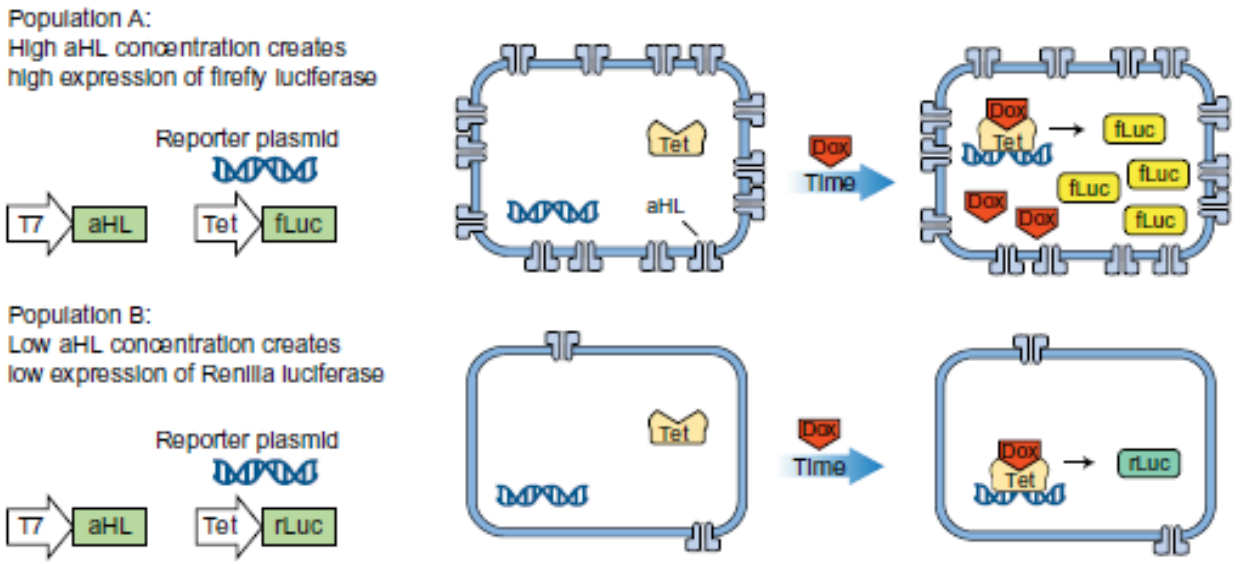
C. They can fuse together, enabling the direct union of separately synthesized reaction components



Insulation of genetic circuits operating in parallel liposome populations

The purpose of this experiment:

- Liposomal compartmentalization to create genetic cascades that take advantage of the modularity
    - Help support multicomponent genetic circuits
- Usage of liposomes to insulate multiple and potentially incompatible genetic circuits from each other so that they could operate in same bulk environment
- Modular design from this insulation, which also enables independent optimization of the circuits
- All circuits in the same living environment without interference
- Microenvironments in different liposome populations that are not otherwise genetically compatible



Insulation of genetic circuits that operate in parallel liposome populations (Adamala et. al., 2016)

Insulation of genetic circuits operating in parallel liposome populations

How was this done?

- Assessment of multiple liposomal circuits, whether they can operate in parallel without crosstalk
- Populations of liposomes that could respond differently to the same external activator
    - Two populations, one with mammalian TX/TL extract and the same amount of Dox-inducible luciferase DNA
    - Varied amount of HL DNA, which resulted to high-aHL and low-aHL synell populations
- The response to non-membrane-permeable Dox in the external solution was studied and no evidence of interference between the populations was observed
    - The populations reacted to external trigger in relation to their HL DNA concentrations
- Independent operation was confirmed with this experiment
- This enables pre-programming synells to have varying response levels to given triggers

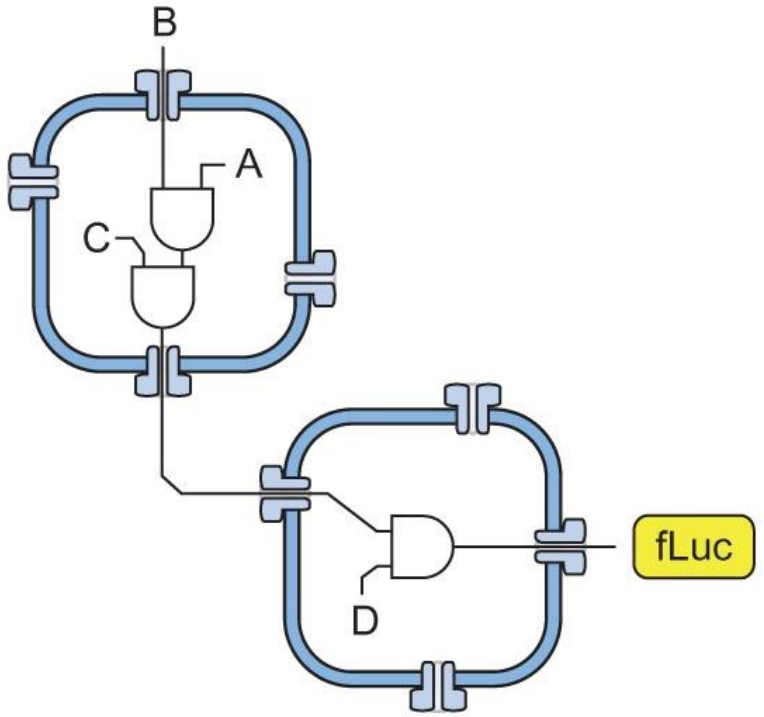
Communication between genetic circuits operating in multiple liposome populations

The aim

- Create controlled communication pathways between synell populations
- First create compartmentalized genetic circuits and then connect them to each other
- Physically separate the circuit elements into different liposomes

How was this done?

- Building two-component circuits: mixing together two liposome populations, ”a sensor”, and ”a reporter”
  - Sensor senses an externa small molecule signal
  - Reporter receives the signal from the sensor population and produces an output



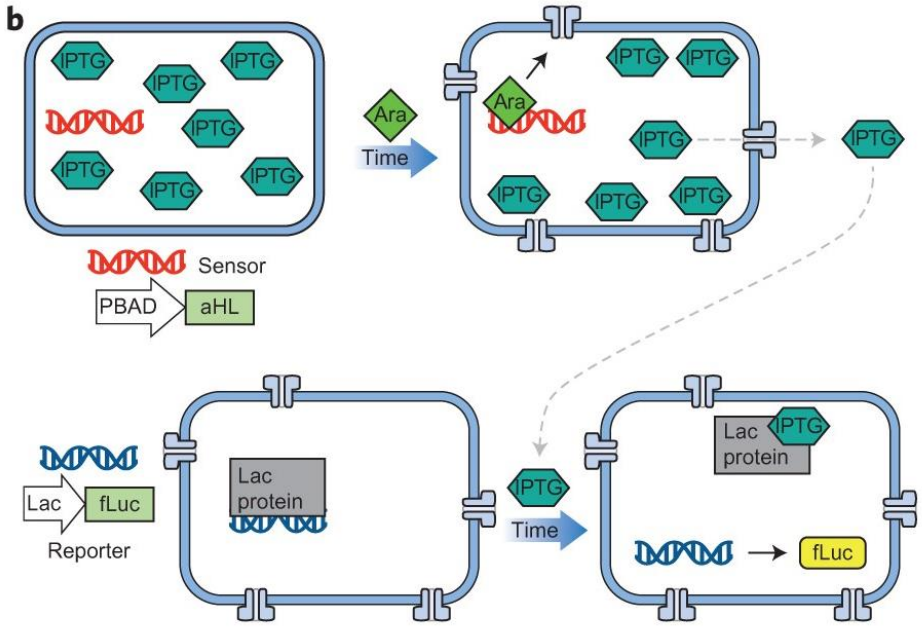
Communication between genetic circuits containing bacterial components

- Two of interacting populations: sensor and reporter
- Sensor liposomes contain the arabinose-inducible aHL gene and IPTG
  - IPTG is a small, non-permeable activator that induces the *lac* promoter
  - Arabinose is membrane-permeable
- Reporter liposomes contain machinery for fLuc expression and constitutively expressed aHL
  - fLuc expression controlled by the *lac* promoter
- During activation, arabinose (Ara) diffuses through the sensor liposome membrane

  → aHL expression induced

  → IPTG released through aHL channels

  → fLuc expression induced in the reporter
- **Result: multi-component compartmentalized genetic circuits operated as coherent wholes**



Communication between genetic circuits containing both bacterial and mammalian components

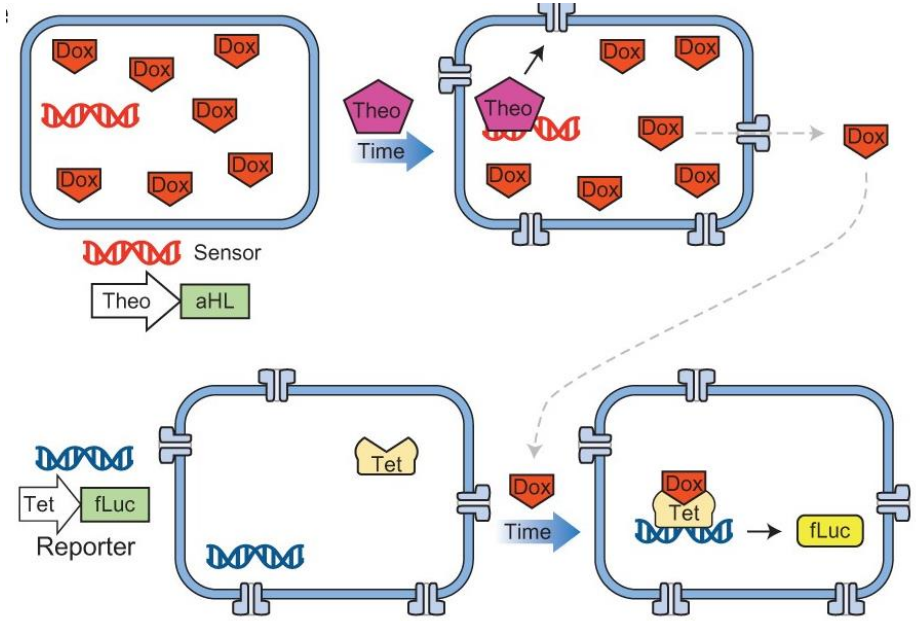
- Sensor liposomes contain the theophylline (Theo) triggered aHL gene and doxycycline (Dox)
  - Theo is membrane-permeable
  - Dox is non-membrane permeable
- Reporter liposomes contain constitutively expressed aHL and Tet, and Dox/Tet-driven fLuc
- During activation, Theo diffuses through the membrane of the activator liposomes

  → aHL expression induced

  → pores that release Dox from the activator created

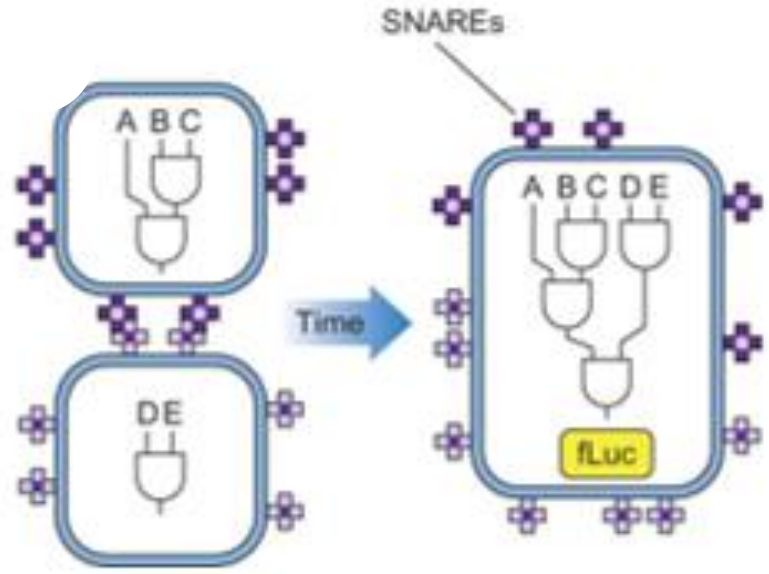
  → Dox released

  → fLuc expression induced in the reporter
- **Result: multi-component compartmentalized genetic circuits with different chemical microenvironments operated as coherent wholes**

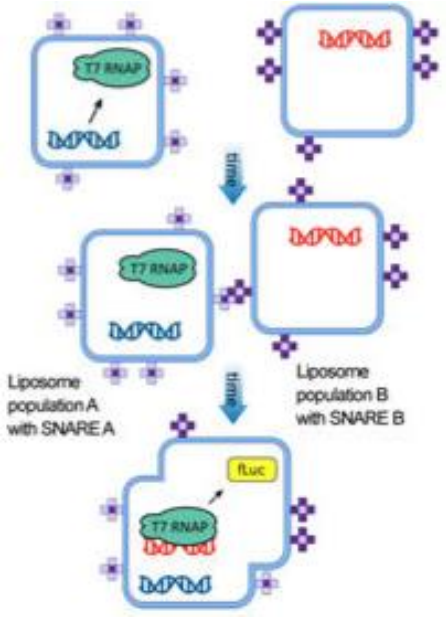


Fusion of complementary genetic circuits

- Encapsulate different genetic circuits or reaction cascades within synells and fuse together -> complex networks that can communicate and perform sophisticated functions.

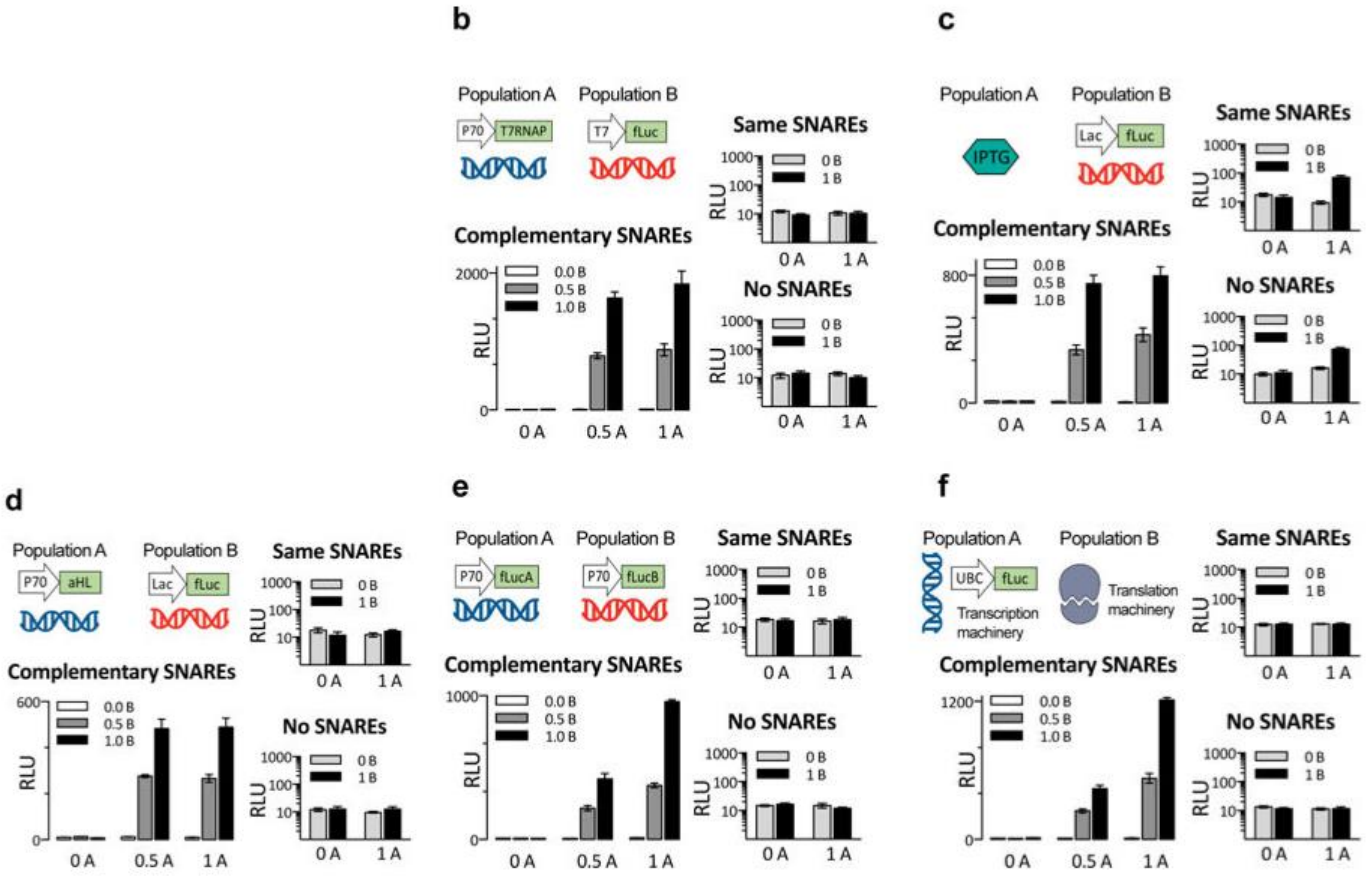


SNARE= Soluble NSF Attachmet Protein Receptor



- Two populations of liposomes, A and B, with SNARE protein mimics.
- The liposomes were membrane-labeled with rhodamine, bearing complementary SNARE pairs and fused for 4 hours.
- Maximum-intensity projections of structured illumination microscopy (SIM) z-stacks of liposomes

Fusion of complementary genetic circuits



- All liposomes in this figure contained bacterial TX/TL components (except f.)
- Illustrations show 5 different liposome fusion concept, exploring different ways to distribute genetic circuits across fusable liposomes
- 2 different populations of liposomes at 3 occupancy levels for each case.

The results:

- possible to maintain liposomes in high-integrity states despite being mixed
- synells fuse so that they could bring together two genetic cascades into the same environment in a programmable fashion.
- These findings demonstrate the potential for controlled fusion between synells containing different genetic circuits or reaction cascades.

Discussion

- **What was achieved**
  - The role for liposomal compartmentalization as a part of multicomponent genetic circuit function was experimented. In addition, transcriptional and translational machinery enables a great level of modularity for genetic circuit design.
  - The insulation of liposomal membrane was proven to be sufficient for non-interacting circuits
  - The fusion of synells together enabled new functions such as producing RNA encoding for fLuc and protein production with mammalian cells.
- **What did not work (if presented) / what is still unclear or needs further research**
- **Why and how is this important and path forward**
  - In the study, synells were proven to enable a great level of modularity for genetic circuit design and execution
  - Modularity is important in engineering: breaking complex biological systems into separate parts that can be controlled independently enables optimization of each individual part while retaining the cohesion of the whole system
  - The presented technology enables modularization of different synthetic biology problems and does not require specialized hardware
- **You can also list if there were major points you did not understand**
  - **Hard to interpret figures**