Engineering genetic circuit interactions within and between synthetic minimal cells

Adamala, K., Martin-Alarcon, D., Guthrie-Honea, K. et al. (2017)

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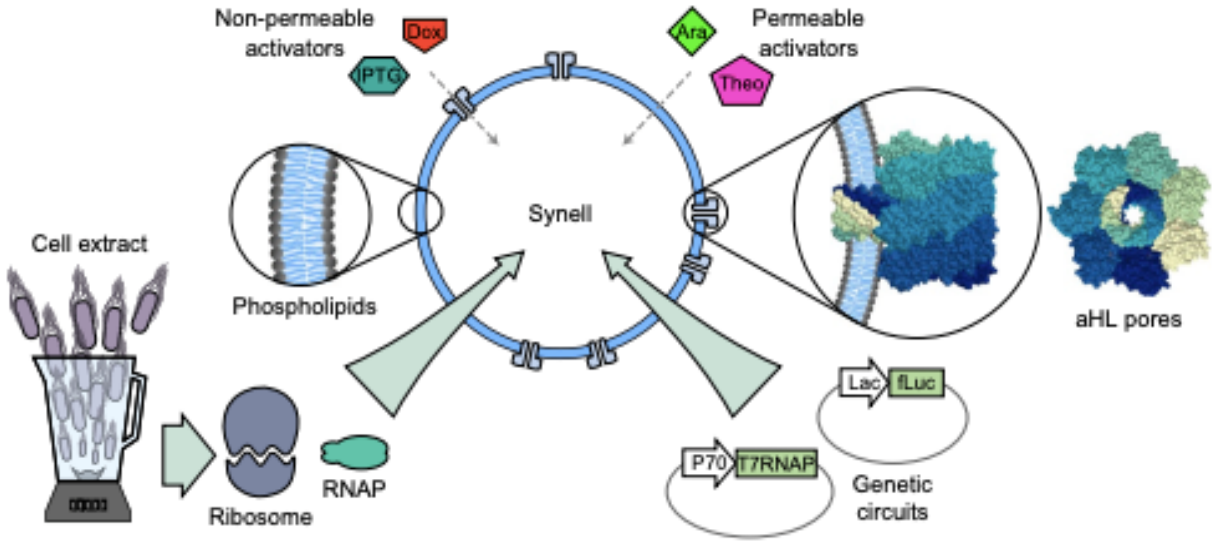
Contents

Introduction

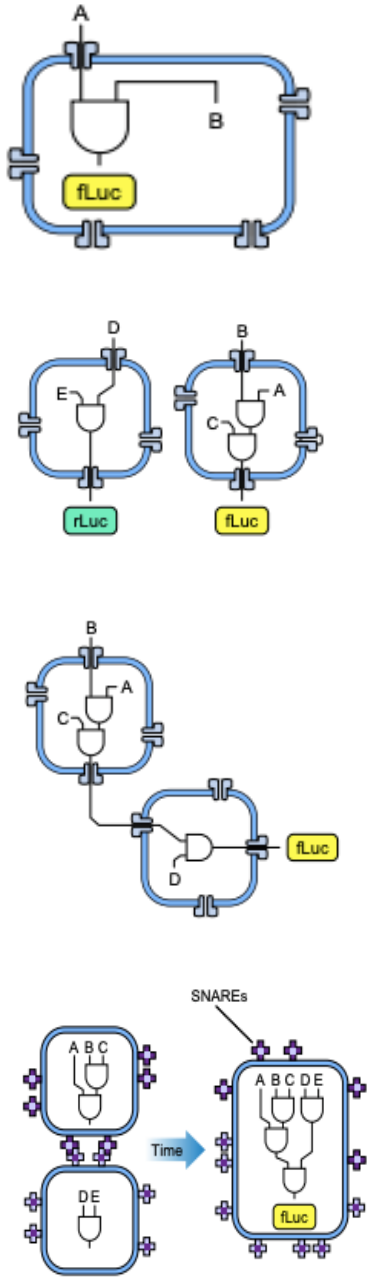
- Confronting a key issue in synthetic biology: the modularity of multi-component genetic circuits and cascades
- The utilization of synthetic minimal cells (synells) in synthetic biology
  - Artificial cells that are built from the same material as living cells and have a phospholipid bilayer membrane
  - Transcriptional and/or translational machinery from bacterial and mammalian cells
- Demonstrates how synells enable a great level of genetic circuit design and execution
  - Takes advantage of the modularity enabled by liposomal compartmentalization
  - The genetic cascades can proceed in well-isolated environments while permitting the desired degree of control and communication



(Figure 1a)

Aim

- Engineer liposomes containing different cascades that can be fused in a controlled way bringing together the products of incompatible reactions
- Demonstrate four novel competencies of synells:
  - Single synells in which all the components and operations take place within the same lysosome
  - Synells with parallel operations
  - Synells with serial operations
  - Synells enabling selective liposome fusion



(Figure 1b-c, rearranged)

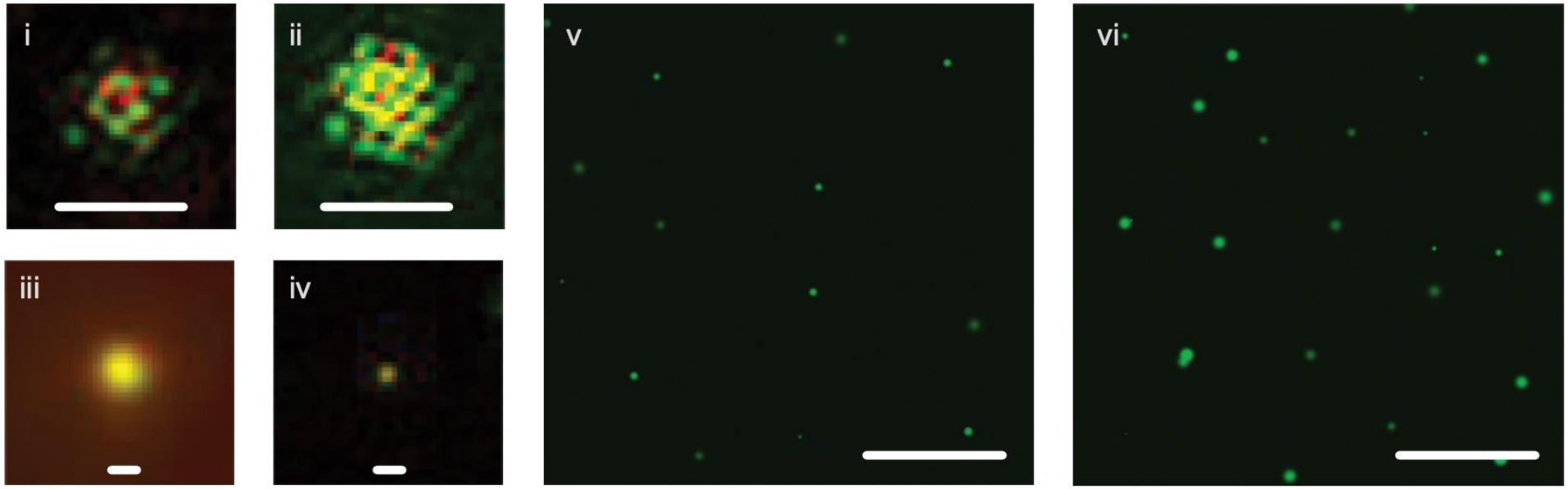
Common elements in the synells

- Transcriptional/translational machinery from bacterial or mammalian cells
- Added molecules:
  - Activators: permeable (Ara, Theo) and non-permeable (Dox, IPTG)
  - Helper proteins that mediate the activity of some activators (Tet, Lac)
  - Self-assembling membrane pores (aHL)
  - Reporter molecules firefly luciferase (fLuc) and Renilla luciferase (rLuc)
  - Membrane-anchored fusion peptides (SNAREs)

Methods & Results

Confinement of genetic circuits in liposomes

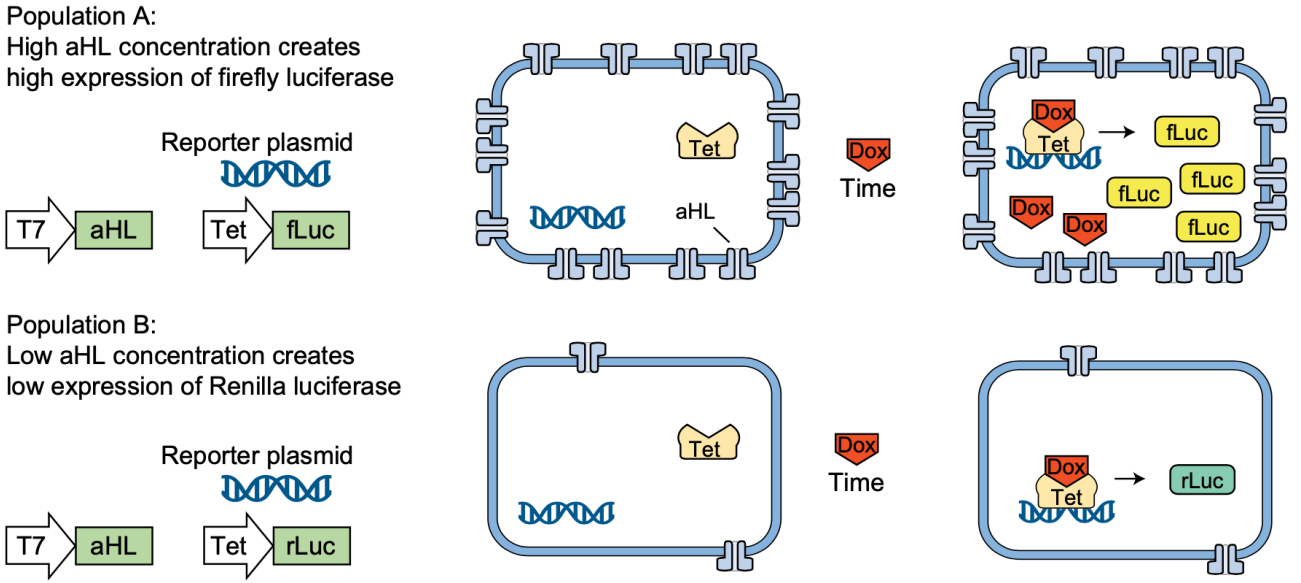
- Characterization of basic structural and functional properties of synells (size, functionality, reaction efficacy)
- Molecular confinement in liposomes may help support higher-order chemical reactions and multi-component genetic circuits



(Figure 2a)

Insulation of genetic circuits that operate in parallel liposome populations

- Testing whether liposomes could be used to insulate multiple, incompatible genetic circuits taking place in the same bulk environment
  - Created populations of liposomes responding differently to the same external activator
    - Two populations of liposomes carrying mammalian TX/TL extract and the same amount of Dox-inducible luciferase DNA
    - Different amount of aHL DNA to result in high-aHL and low-aHL synell populations

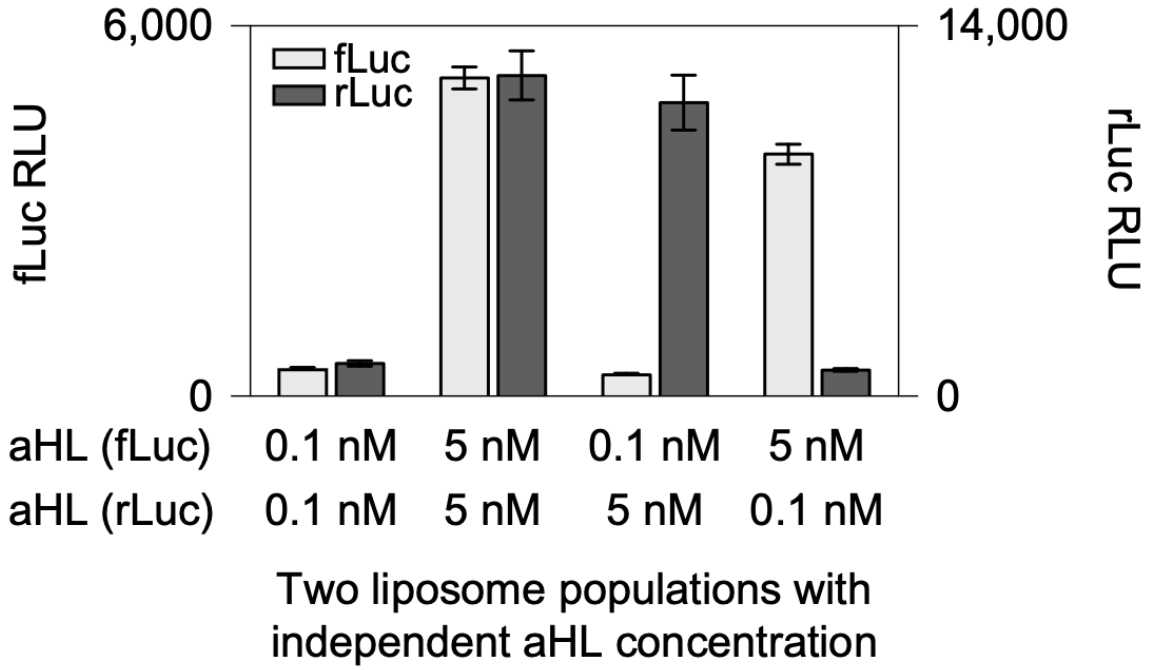


(Figure 4a)

Insulation of genetic circuits that operate in parallel liposome populations

- High-aHL and low-aHL synells responded to the external Dox concentration based on their own aHL concentration
- No evidence of cross-talk between different populations

→ Liposome populations can be programmed in advance to elicit varying responses and function simultaneously



(Figure 4b)

Communication between genetic circuits that operate in multiple liposome populations

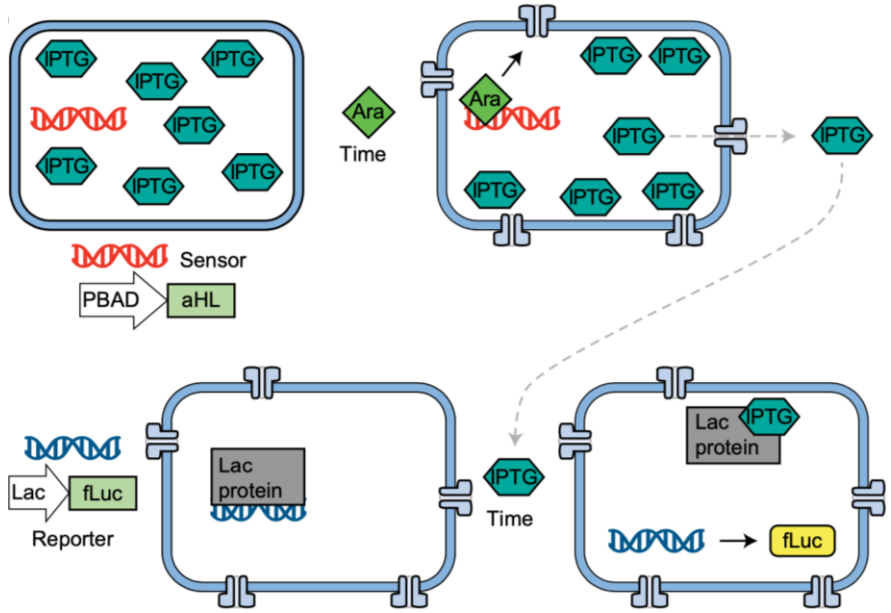
- Creating controlled communication pathways between populations of synells
  - Built two-component circuits by using two populations of liposomes
    - A ‘sensor population’ which senses a small external molecule
    - A ‘reporter population’ that receives a message from the sensor population and gives an output
  - First one built with bacterial TX/TL extract
  - Second one was constructed with both bacterial and mammalian components

Communication between genetic circuits that operate in multiple liposome populations

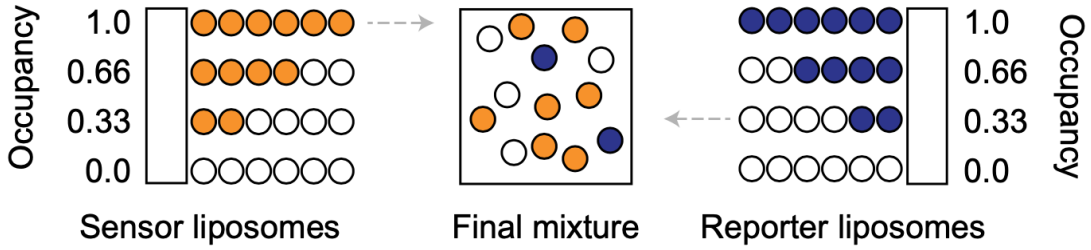
Bacterial TX/TL extract system:

- *Sensor liposomes:* contain IPTG (non-membrane-permeable activator, induces the lac promoter) and the arabinose inducible gene for aHL
  → Sense arabinose and release IPTG by expressing aHL channels
- *Reporter liposomes:* contain constitutively expressed aHL, in which fLuc is under the control of the lac promotereither directly or indirectly
  →Expression of fLuc

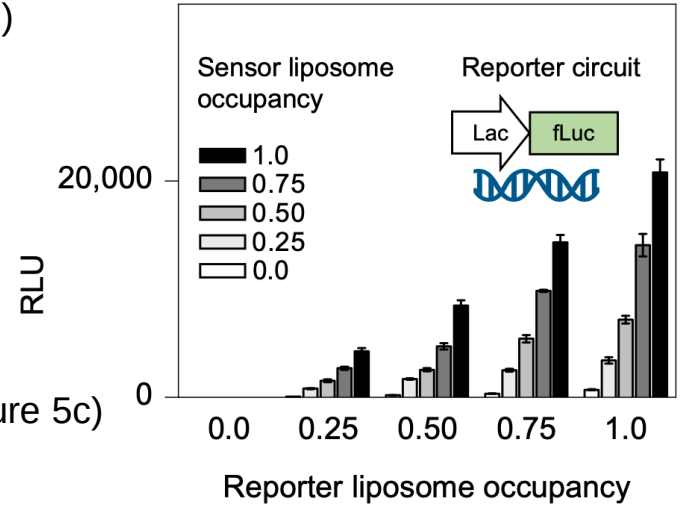
→ The constructed compartmentalized multicomponent genetic circuits operated as coherent entities



(Figure 5b)



(Figure 5a)

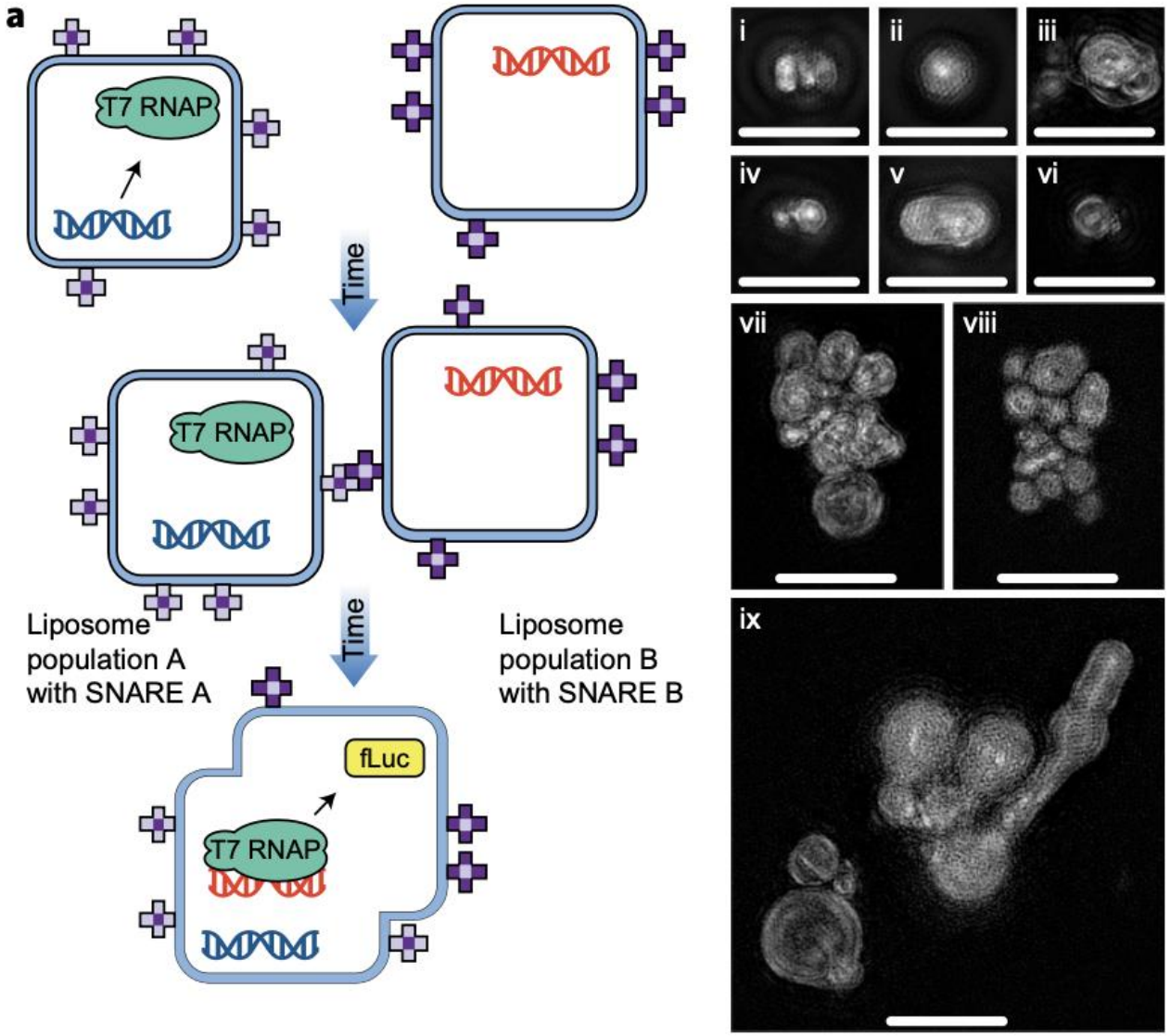


(Figure 5c)

Fusion of complementary genetic circuits

- Two liposome populations, A & B, were generated (on the right: a general representation of the fusion experiments)

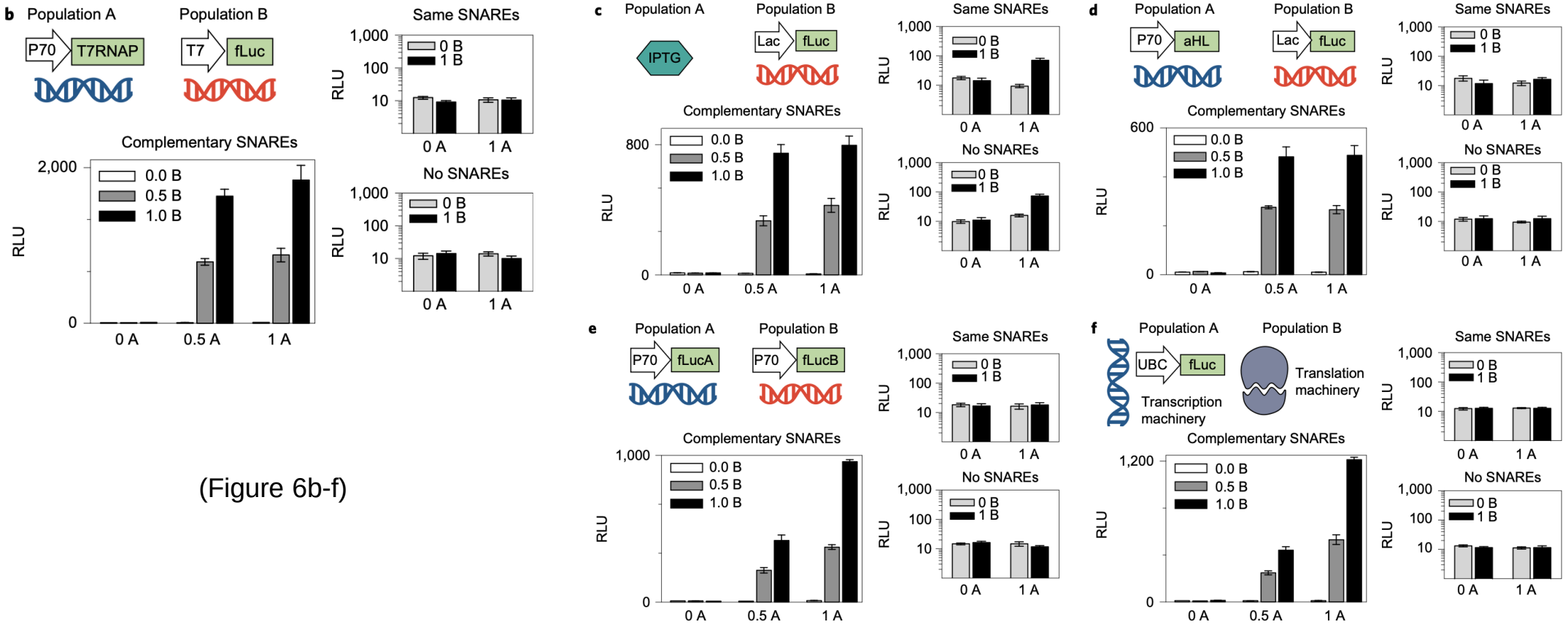
➔ The effect of SNAREs on liposome fusion resulting in successful production of the output of the genetic cascade was analyzed



(Figure 6a)

Fusion of complementary genetic circuits

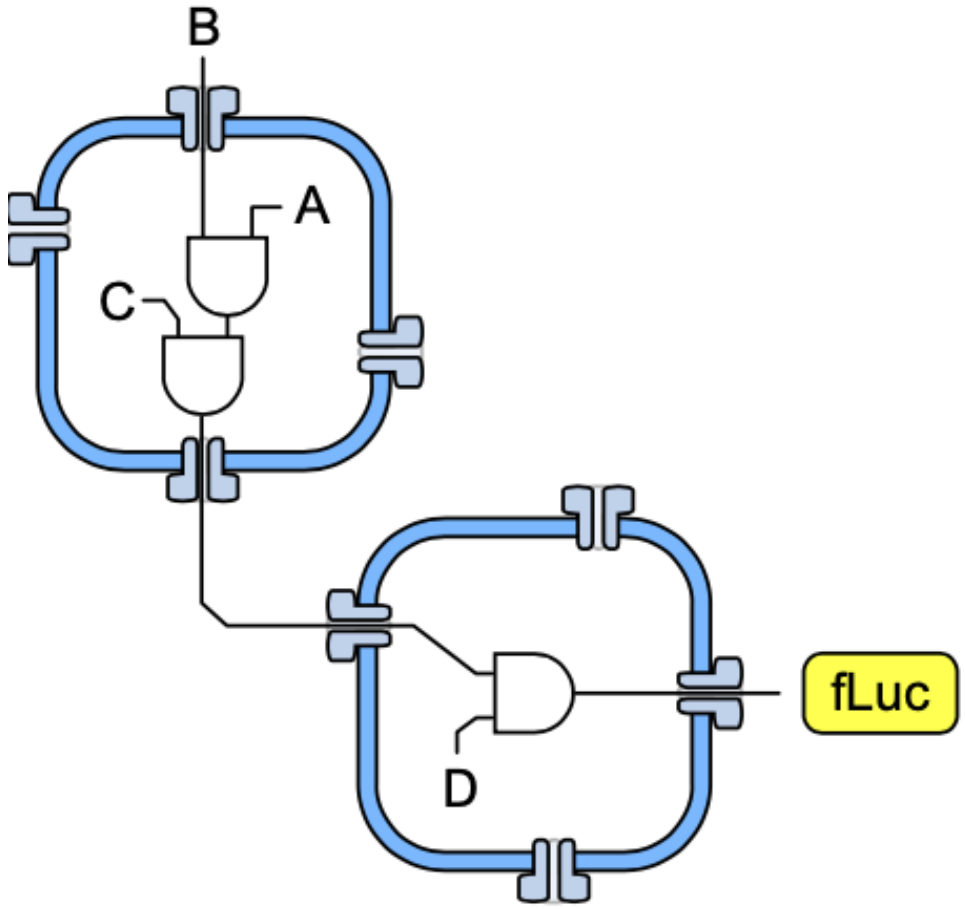
- Five different scenarios were tested, differing by the concept of liposome fusion and the SNAREs (complementary, same SNAREs, or no SNAREs at all)



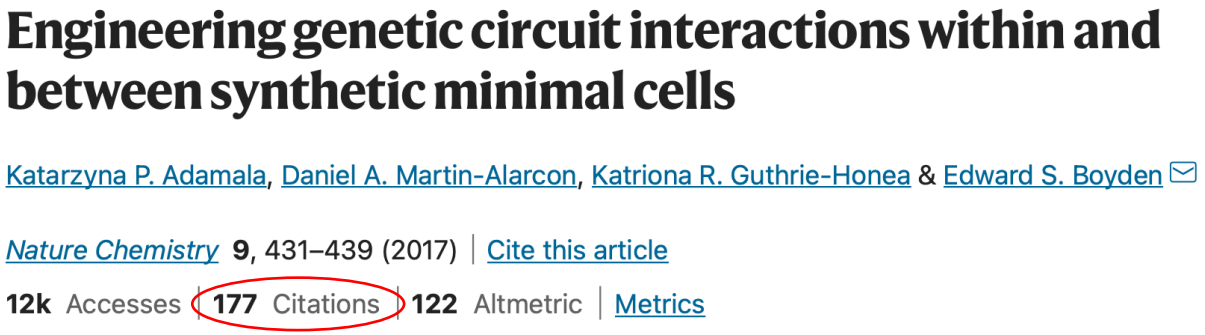
(Figure 6b-f)

Relevance & Further Research

- Synells enable a new level of modularity for synthetic biology
  - Modularity is crucial in bioengineering
- Synells can be used as a model for studying the origin and earliest evolution of early life



Relevance & Further Research



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Nature Chemistry **9**, 431–439 (2017) | Cite this article

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- Improving cell-free protein synthesis (Ayoubi-Joshaghani et al., 2020)
- Building larger pathways (Krishnan et al., 2020)
- Biomedical applications in drug delivery to suppress tumor growth (Krinsky et al., 2018)

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Thank you!
Questions?