On-Chip Synthetic Biology Platform for Diagnostics and Therapeutics

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Overview

Cell-free synthetic platforms that enable complex biological activities such as synthesis and self-assembly of biomolecules, can be employed for various applications, including drug discovery and diagnostics. Using synthetic genes, we provide a novel platform for cell-free synthetic biology on a chip which is a crucial step toward creating a self-replicating artificial cell and a general strategy for developing diverse multi-component macromolecular machines. This technology, done in a cell-free environment, has a strong potential in drug discovery and diagnostic applications.

Background and Unmet Need

The development of artificial cells capable of executing DNA programs has been an important goal for basic research and technology. The assembly of protein machines in cells needs to be precise, rapid, coupled to protein synthesis, and regulated in space and time. Natural and synthetic nanomachines assemble needs to be similarly controlled by genetic programming outside the cell. There is a requirement for such a technology in assembling 2D DNA compartments fabricated in silicon as ‘artificial cells’ capable of metabolism, programmable protein synthesis, and communication. Furthermore, the establishment of a self-replicating artificial cell requires ribosome biogenesis, an efficient and complex assembly process that has not been reconstructed outside a living cell so far.

The Solution

Prof. Roy Bar-Ziv and his team developed an on-chip artificial cell that mimics the cells’ biological function, providing a biosafe and rapid high-throughput platform for human disease therapeutics discovery and diagnostics.

Technology Essence

Prof. Roy Bar-Ziv and his team patterns biomolecules by UV lithography, thus enabling to immobilize different biomolecules anywhere on the chip to submicron resolution. Using a novel method developed by Bar-Ziv lab, the team developed ribosome biogenesis on a chip and confirm autonomous on-chip assembly1 of the E. coli small ribosomal subunit (SSU) revealing new insights into the assembly mechanism. The group develop quasi-two-dimensional (2D) silicon compartments that enable programming of protein assembly lines by local synthesis from surface-immobilized DNA brushes2. The local synthesis and surface capture of complexes provided high assembly yield and sensitive detection of spatially resolved assembly intermediates, with the 3D geometry of the compartment and the 2D pattern of brushes dictating the yield and mode of assembly steps.

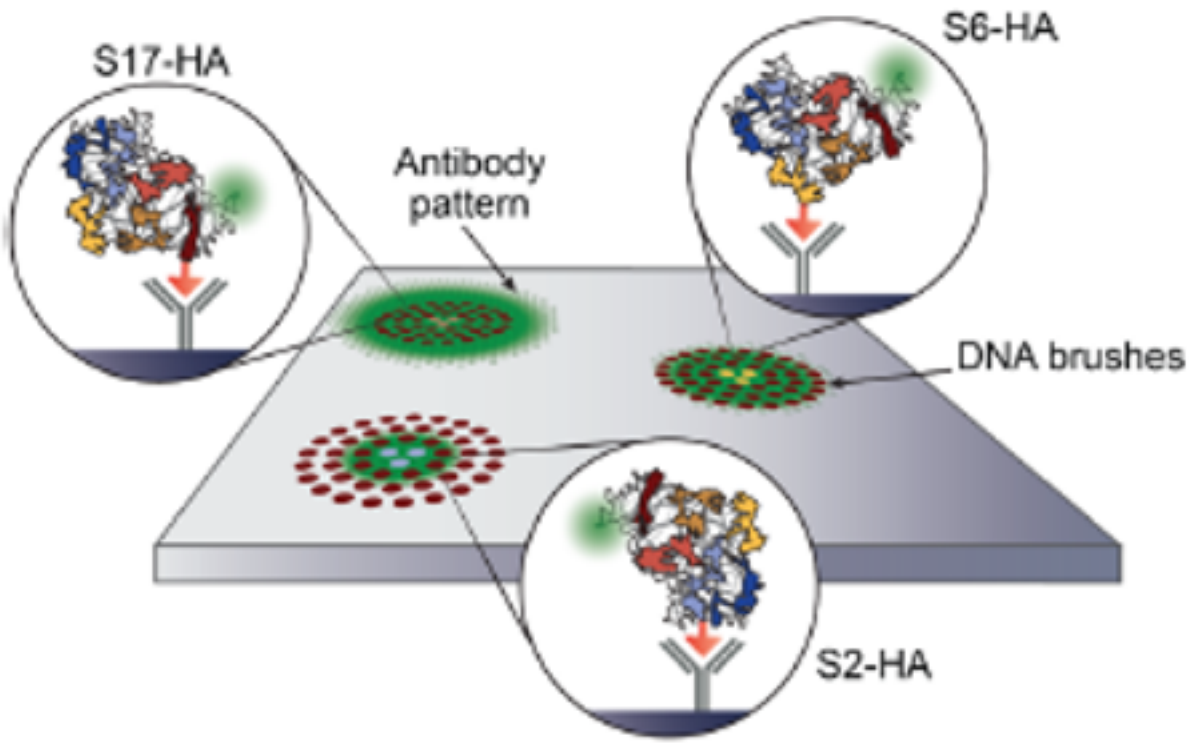

Figure 1. Ribosome (E. coli SSU) biogenesis on a chip - Twenty brush clusters coding for all SSU r-proteins, and the assembly factors Era, RsgA, RbfA, RimM, RimN, and RimP surround the central r-RNA and r-protein-HA brushes.

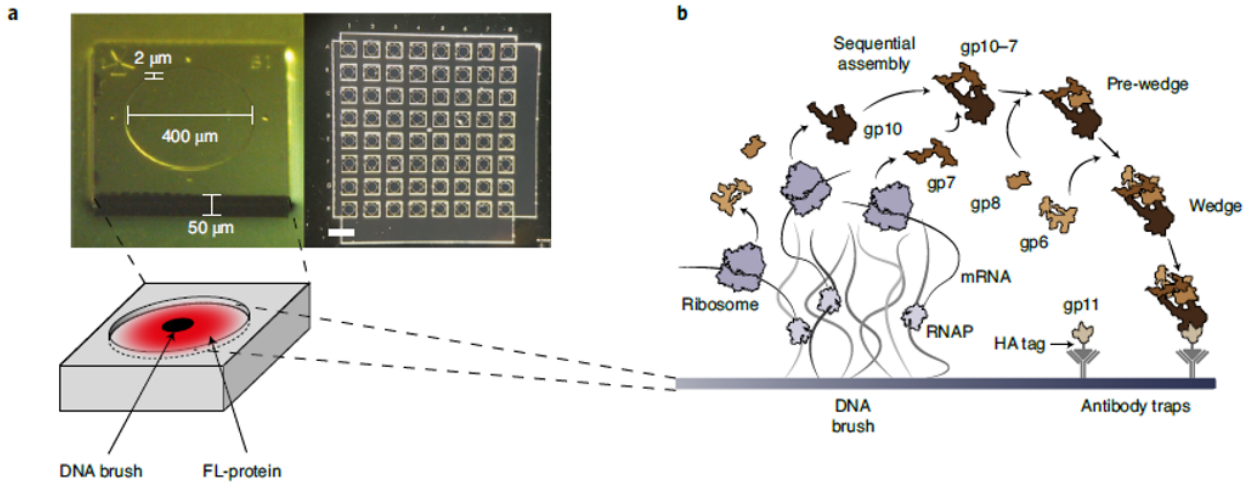

Figure 2. Synthesis and assembly in 2D compartments of Protein (T4) bacteriophage parts. a. Images and scheme of an array of silicon 2D compartments, with a central DNA brush (black circle) surrounded by captured wedges (stained by FL-protein, red). Scale bar in top right image, 1 mm. b, T7 RNAPs (light grey) and ribosomes (dark grey) localize to DNA brush and express wedge proteins (brown) that assemble sequentially and bind to gp11 (light brown) on surface antibodies.

Applications and Advantages

- Personalized pathogen diagnostics and serology
- Drug screening & discovery from COVID-19 POC to other diseases: pathogens, oncology, immuno-oncology
- Si-SiO2 micro-fabrication of miniaturized artificial cells
- Programmable, high-throughput chip generating personalized big data
- Scalable: High-throughput genetically encoded cell-free reactions
- Cell-free: Biosafe, non-hazard, cheap solution
- High capacity: thousands of cells, hundreds of genes per cell
- Genetically encode synthesis of biomolecules, ribosomes and other cellular machines

Development Status

Prof. Bar-Ziv and his team conducted autonomous synthesis and assembly of ribosomal subunit on a chip and programming of multi-protein assembly by 2D compartment geometry. They demonstrated a paradigm shift from bulk cell-free reactions to confined, quasi-2D, surface-localized reactions with a capacity for genome-scale synthesis, highly efficient and tunable assembly, capture of protein clusters, and resource partitioning, facilitating high yields of cascaded reactions. The group also demonstrated the technology capabilities on COVID-19 diagnosis, as a potential example of a real world application.

References:

Levy, M., Falkovich, R., Daube, S. S., & Bar-Ziv, R. H. (2020). Autonomous synthesis and assembly of a ribosomal subunit on a chip. Science Advances, 6(16), eaaz6020.? https://doi.org/10.1126/sciadv.aaz6020 [1]

Vonshak, O., Divon, Y., Förste, S. et al. (2020) Programming multi-protein assembly by gene-brush patterns and two-dimensional compartment geometry. Nat. Nanotechnol. 15, 783–791. https://doi.org/10.1038/s41565-020-0720-7 [2]

Patent Status

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