

A Supramolecular Chemical Approach to the Construction of Artificial Cells

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Exploring the boundary between living and non-living matter is one of the most challenging problems for contemporary scientists. To understand the cell, which is considered the smallest unit of life, a plausible strategy is to synthesize an artificial cell by using a supramolecular chemical approach, because simple molecular assemblies at one time evolved to create the simple cell on prebiotic earth. As shown in Figure 1, the key elements of a cell are the compartment, information, and a catalyst (*i.e.*, metabolism). We have attempted to construct a chemically based artificial cell endowed with these three elements.

In our laboratory, we attempted to construct two artificial cells by using giant vesicles (GVs) as the compartment. One, developed in collaboration with the Sugawara group (Kanagawa Univ.), is an artificial cell that can proliferate from generation to generation. Now, we have constructed a recursive vesicular artificial cell system with proliferation cycles. By using the vesicular transport system, the second generation GV, which contains no PCR reagents after self-reproduction, can be replenished by fusing them with conveyer GV bearing the PCR reagents by changing the pH of the dispersion. After the PCR reagents are replenished, the GV can self-reproduce again. This system could lead to an evolvable artificial cellular system. The other artificial cell is an artificial cell that contains

a catalyst-producing system. The GV system can generate catalysts and membrane molecules by transforming their respective precursors, thereby facilitating the proliferation of the GVs with the produced catalyst.

We are now tackling the creation of artificial cells that mimic cellular dynamics, such as cytoskeleton formation in the cell.



Artificial cell

- ✓ **Compartment** constructed by molecular assembly
- ✓ **Information** delivered to descendant
- ✓ **Catalyst** for chemical transformation

Figure 1. Artificial cell model. The replicating systems of the compartment and the information materials are combined. The reactions in the two replicating systems are accelerated by appropriate catalysts.

Selected Publications

- K. Kurihara, M. Tamura, K-I. Shohda, T. Toyota, K. Suzuki and T. Sugawara, "Self-Reproduction of Supramolecular Giant Vesicles Combined with the Amplification of Encapsulated DNA," *Nat. Chem.* **3**, 775–781 (2011).
- K. Kurihara, Y. Okura, M. Matsuo, T. Toyota, K. Suzuki and T. Sugawara, "A Recursive Vesicle-Based Model Protocell with a Primitive Cell Cycle," *Nat. Commun.* **6**, 8352 (2015).

1. An Artificial Cell Containing a Catalyst-Producing System

A cell is a self-organized system that can maintain its state via metabolism. Our previously developed artificial cellular system is robust, but it can self-reproduce only a specific state in the any environments.¹⁻³⁾

Here, our goal was to create a new artificial cellular system in which the GV self-organizes its composition spontaneously according to its environment. For a GV to self-reproduce (grow and divide spontaneously) and self-maintain, it is necessary to combine the metabolism and the compartment.⁴⁾ By introducing a cross-catalysis system (Figure 2), we constructed an artificial cell in which catalysts are produced. After addition of a membrane precursor aldehyde, the production of the catalyst and the membrane molecule was confirmed by nuclear magnetic resonance (NMR) and microscopic observation. In this system, the GV was reproduced by the catalyst, which catalyzed the production of the GV membrane lipid molecule. The GV membrane provides the field where the catalyst is synthesized.

In addition, by changing the composition of the vesicular membrane, the production of the catalyst and that of the membrane molecule fluctuated due to the components interacting each other; in effect, the artificial cell incorporated a negative feedback loop.

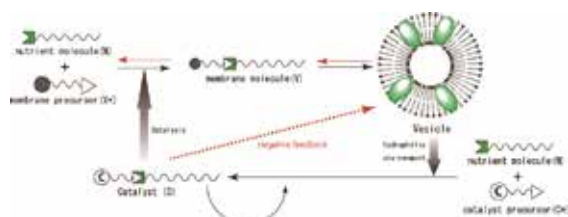


Figure 2. Scheme of our new artificial cellular system. The membrane molecules of the GV was synthesized by the catalyst produced in the GV.

We are also studying the oscillation in generation of membrane molecule and catalyst occurred by depletion of nutrient molecules.

2. An Artificial Cell Using a Self-Reproducing Oil Droplet as a Scaffold

Research on transforming oil droplets into vesicles by use of chemical reactions and self-assembly processes is expected to facilitate our understanding of the origin and definition of

life from a chemistry perspective.

The mixing of an aqueous solution of an aldehyde containing an imidazole hydrochloride group with octylaniline led to the spontaneous formation of autocatalytic oil droplets⁵⁾ (Figure 3). An aldehyde-bearing quaternary ammonium salt that does not react well with octylaniline was added to this autocatalytic droplet system. As a result, the catalytic molecules that formed within the oil droplets promoted the condensation reaction between the octylaniline and the non-catalytic aldehyde, which ultimately led to the synthesis of vesicular membrane molecules with imine functionality within the molecular aggregates; thus self-reproducible oil droplets were successfully transformed into vesicles upon the addition of the membrane precursor.

In this way, we created a protocell model that can construct boundaries by using this new process that relies on the formation of robust vesicles through the use of an existing autocatalytic, self-reproducing oil drop system as a scaffold.

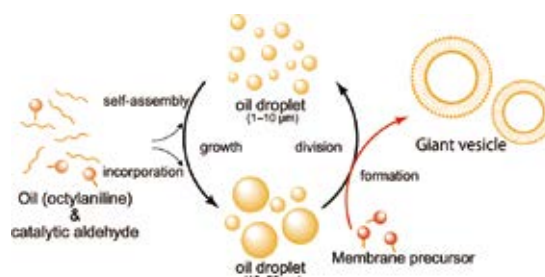


Figure 3. Scheme of the self-reproducing oil droplet (oil-in-water emulsion) and vesicular transformation system.

We will construct an oil droplet system that synthesizes peptides (simple proteins) and an oil droplet system that forms vesicles. The former is an oil droplet that incorporates amino acids and synthesizes peptides internally. In addition, peptide synthesis inside the vesicles is performed using a water-soluble condensing agent through our developed water-in-oil emulsion centrifugation method.⁵⁾

References

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