A Supramolecular Chemical Approach to the Construction of Artificial Cells

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The cell is the smallest unit of life, and the first simple cells evolved from simple molecular assemblies on prebiotic earth. To understand this transition from non-living to living structures, we use a supramolecular chemical approach. As shown in Figure 1, the key elements of a cell are a 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 constructed two types of artificial cells by using giant vesicles (GVs) as the compartment. The first, developed in collaboration with the Sugawara group (Kanagawa Univ.), is an artificial cell that can proliferate from generation to generation. We have improved this model by constructing a recursive vesicular artificial cell system with proliferation cycles. After self-reproduction, these second-generation GVs contain no PCR reagents by consuming and therefore cannot reproduce for a second time. However, the reagents can be replenished by using the vesicular transport system and changing the pH of the dispersion, resulting in the fusion of the GVs with conveyer GVs bearing the PCR reagents. After the PCR reagents are replenished, the GV can self-reproduce again. This system could lead to an evolvable artificial cellular system. The second type of artificial cell contains a catalyst-producing system. The GV system can generate catalysts and membrane molecules by transforming their respective precursors. The catalysts that are produced facilitate the proliferation of the GVs.

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

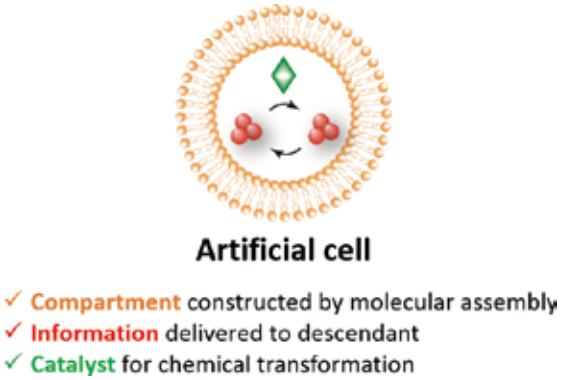

Figure 1. Artificial cell model. Materials containing heritable information are enclosed within a compartment. The reactions in the two replicating systems (compartment and information) are accelerated by appropriate catalysts The reactions in the two replicating systems are accelerated by appropriate catalysts.

Selected Publications

- Y. Natsume, E. Noguchi and K. Kurihara, "Spontaneous Localization of Particles in Giant Vesicles Owing to Depletion Force," *J. Phys. Soc. Jpn.* **88**, 033001 (2019).
- M. Matsuo, Y. Kan, K. Kurihara, T. Jimbo, M. Imai, T. Toyota, Y. Hirata, K. Suzuki and T. Sugawara, "DNA Length-Dependent Division of a Giant Vesicle-Based Model Protocell," *Sci. Rep.* **9**, 6916 (2019).

1. Spontaneous Formation of Liquid–Liquid Phase-Separated Droplets with Amino Acid Polymerization

In the prebiotic era, cooperative interaction between self-producing molecular aggregates and peptide polymers led to the emergence of primitive cells. Although the advanced membrane provides a field for catalytic reaction, it remains a mystery how cooperation between polymers and molecular aggregates occurred even in membraneless organisms like coacervate droplets. Therefore, we attempted to construct a liquid–liquid phase-separated droplet that self-reproduces by constructing a reaction system in which a peptide is produced by spontaneous polymerization of an amino acid derivative in water.

We synthesized an amino acid derivative (monomer) with two cysteine reactive sites at the N-terminus and a thioester at the C-terminus that spontaneously polymerizes in water to form a peptide. In order to prevent undesirable oxidation, a monomer precursor was obtained by crosslinking monomers with disulfide. After the addition of a reducing agent (dithiothreitol, DTT) to the monomer precursor solution, we observed the formation of droplets. We then added more precursor and DTT and examined the changes in particle size. The mean particle size increased and decreased rapidly immediately after the addition, confirming the self-reproducibility of the formed droplets. When the precursor and DTT were continuously added every 20 hours, the particle size of the droplets fluctuated recursively, indicating autocatalytic self-reproduction of the formed liquid–liquid phase-separated droplets. This autocatalytic droplet formation in this system is considered to be due to a physical mechanism: When a molecular assembly is created as the dehydration condensation proceeds and it forms a hydrophobic field, the assembly functions as a site for promoting dehydration condensation, thereby allowing the autocatalytic dehydration condensation to proceed.

The behavior of the interface formed by this chemical reaction replicates the autocatalytic self-reproduction that might have occurred in droplets formed by liquid–liquid phase separation on the primitive, prebiotic earth. In the future, we aim to construct the Droplet World Hypothesis by inducing the emergence of the primordial cell membrane via an internal chemical reaction or by functionally expressing biologically active molecular species, such as ribozymes, inside the droplet.

2. Self-Reproduction Model Using Particle-Localized Vesicles

Biopolymers inside cells change their structures around themselves, thereby increasing the free movement area. This entropic action is called the excluded volume effect. We prepared phospholipid vesicles containing densely packed colloidal particles as a model to show the excluded volume effect. When polystyrene beads of two different sizes were confined in the GV, a novel phenomenon was observed: Smaller beads were localized in the vicinity of the vesicle membrane. To explain this phenomenon theoretically, we assumed that an equilibrium osmotic pressure was realized between an outer phase containing a relatively large number of small particles and a separate inner phase.[1] We constructed a second model based on the depletion effect.[2]

Considering the relation between vesicular membrane area and the volume fraction of the particles, we hypothesize that increased membrane area would cause the vesicle to become unstable, and that stability could be restored by division into two spherical vesicles. We confirmed this by showing that when a fatty acid similar to a vesicular membrane molecule is added to particle-containing vesicles, the vesicles divide easily and frequently. To further assess this self-reproducing vesicle model, we are constructing a system can track morphological changes and analyze microscopy images.

The purpose of this vesicular system is to achieve cellular behavior such as internal structure or membrane deformation without sophisticated biomaterials. Because this vesicular system is simple, it is possible to extract and analyze the contribution of the crowding effect to cell deformation. For this reason, applying data to simulation and modeling will be relatively easy in this system compared with other systems. Because this model excludes biopolymers with specific properties, the biopolymers' unique functions do not appear. Therefore, this vesicular system is expected to act as a primitive cell in which simple molecules interact loosely to express their function.

References

1) Y. Natsume, Y. Komori, K. Itoh and K. Kurihara, *Trans. Mater. Res. Soc. Jpn.* **43**, 333–338 (2018).
2) Y. Natsume, E. Noguchi and K. Kurihara, *J. Phys. Soc. Jpn.* **88**, 033001 (2019).