# 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.

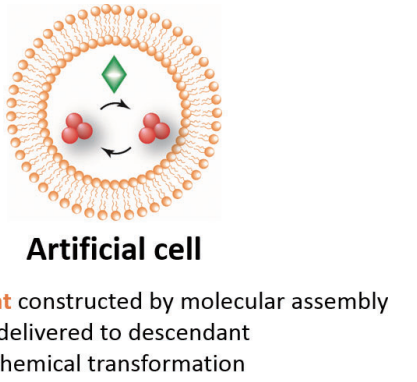


**Figure 1.** Artificial cell model. Materials containing heritable information are enclosed within a compartment. The reactions in the two replicating systems (compartment and information) are accelerated by appropriate catalysts The reactions in the two replicating systems are accelerated by appropriate 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 *et al.*, "Environment-Sensitive Intelligent Self-Reproducing Artificial Cell with a Modification-Active Lipo-Deoxyribozyme," *Micromachines* **11**, 606 (2020). doi:10.3390/mi11060606

## 1. Construction of a LLPS-Droplet Based Model Protocell

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. Since a coacervate droplet as a model of early life was created by Oparin about 100 years ago, interesting primitive cell models using coacervate droplets have been created. However, construction of the self-reproduction of coacervate droplets and the spontaneous formation of peptides, which are the constituents of the coacervate droplets has not been realized in the same environment. In the present study, we designed and synthesized a molecule that has both a peptide and a droplet formation site to enable the formation of the coacervate droplets. 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. A detailed analysis of the particle size distribution measurements based on dynamic light scattering revealed that the growth of the droplets can be classified into two stages: The initial autocatalytic droplet formation and the fusion of the 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.

Furthermore, we conceived that this liquid–liquid phase separation droplet would be useful as a place to integrate biomolecules representing other origin-of-life hypotheses (*e.g.*, RNA world, lipid world, *etc.*). Therefore, we attempted to investigate whether this droplet would incorporate those biomolecules. We added 20 mer RNA fragments, DNA fragments, and phospholipids to the droplets. By fluorescence microscopy observation and Raman microspectroscopy, it was found that the droplet was consisted of the hydrophobic center region and the hydrophilic peripheral region. The highly hydrophobic lipids were concentrated in the central region of the droplets and highly hydrophilic nucleic acids concentrated in the peripheral region. This hydrophilic and hydrophobic property was clear compared to the empty droplets. We suspect that the water contained in the hydrophilic region may have been replaced by nucleic acids.

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.