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Molecular robotics approach for constructing an artificial cell model

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Abstract

Prototype artificial cell models with designed functional molecules are presented here. Artificial molecular devices based on a giant liposome were prepared to obtain specific properties that cannot be obtained from natural cells. In this context, artificial cell research is seen an extension of "molecular robotics" research. Cooperative and integrated chemical systems will be constructed from the molecular devices. Here, we present the 3 aspects of the study model: (1) gene-expressing cell model encapsulated in the liposome to simulate membrane protein synthesis, (2) multirole molecular device with a designed DNA nanostructure on the cellular membrane, and (3) designed membrane peptide device for surface recognition. Although these devices are inspired by living cell functions, such goal-oriented systems are free from the constraints of natural history and evolution. These artificial devices may be integrated to develop novel tools for living systems.

Introduction

All living organisms are composed of cells, and cells are constructed from various molecules. Since the end of the last century, rapid progress in molecular science and bioengineering has enabled the analysis of complex living phenomena at a molecular level. Such a top-down approach has provided essential pictures of molecular systems, such as the entire human genome, proteome, or metabolome. However, constructive research has also been essential and is already used for evaluating biochemical reaction systems. Such a bottom-up approach also aims to build basic molecular systems from individual molecules. The goal of artificial cell research to create a cell-like structure in a spatiotemporal manner by using a designed molecular system[1]. Several research groups have reported to construct artificial cell models by using liposomes, that encapsulate biochemical reaction networks such as gene expression from a template DNA [2]. In the last decade, synthetic biology has been the main constructive approach, and living cell functions have been modified using standardized genetic components. The establishment of induced pluripotent cells [3] and total synthesis of the bacterial genome [4] are great milestones of this research area.

The goal of the bottom-up and top-down approaches is to construct the entire cell at the molecular level. Such realistic artificial cell studies seek to reproduce the history of the cell at the molecular level and may thus address the origins of life itself. However, we have also noted the "artificial" aspect of such cell models. It would be possible for the artificial structure in such models to perform a difficult task that is impossible for living cells. Such a capability might contribute to research in a different way from genetically modified cells.

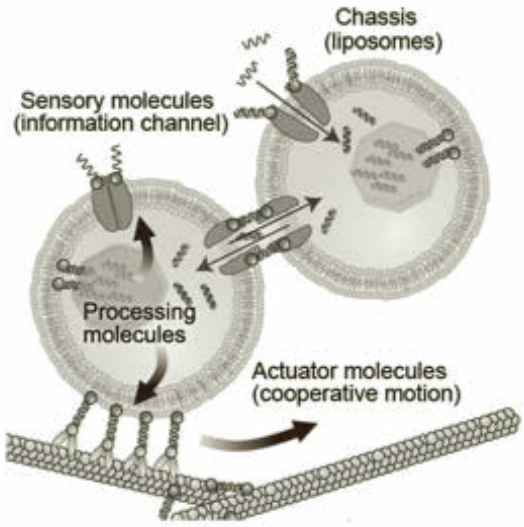

Fig. 1. Schematic illustration of a possible artificial cell model consisting of a designed molecular system. We call this system "molecular robotics."

We are also creating models with designed functional molecules. Such non-natural molecules confer specific properties (e.g., sensing, actuation, and computing) to the artificial cell compartment, which is a liposome. Such artificial molecular devices can be integrated into a complex molecular system to provide a "molecular robot" [5]. In such a context, artificial cell research is included as a subset of molecular robotics (Fig. 1.)

The study model has 3 aspects: (1) a gene-expressing cell model encapsulated in the liposome to enable membrane protein synthesis, (2) a multirole molecular device with a designed DNA nanostructure on the cellular membrane. (3) a designed membrane peptide device for surface recognition. Although these devices are inspired by living cell functions, such goal-oriented systems could become free from the constraints of natural history.

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Gene expression in an artificial cell

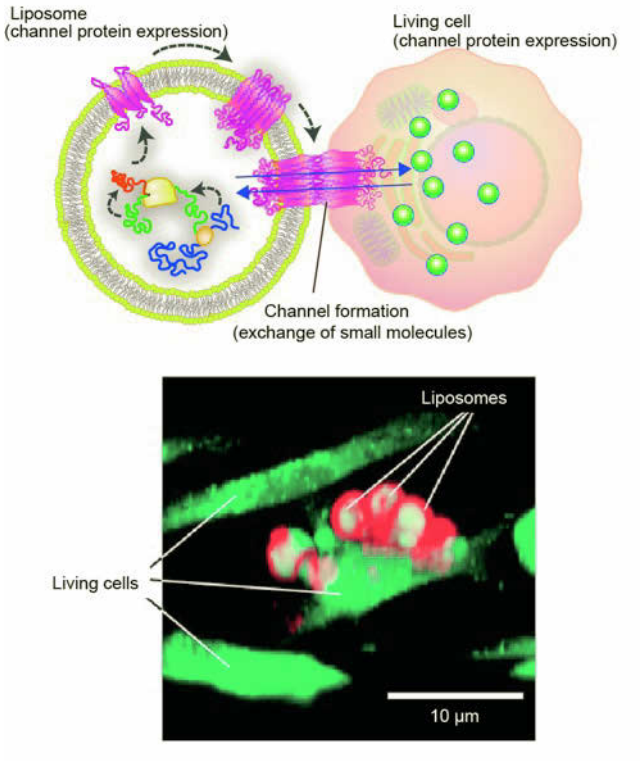

Fig. 2. Artificial cell model liposomes containing a gene expression system. Upper: Schematic illustration of the system. Lower: The membrane protein connexin was expressed and functioned on the liposome membrane. Liposomes were observed inside cultured cells.

We have constructed a series of artificial cell models based on giant liposomes. A giant liposome is a spherical structure that consists of a closed lipid bilayer membrane, with a diameter greater than several micrometers. The giant liposome membrane is known as the simplest model of the living cell membrane [6].
Several protein synthesis reactions with coupled transcription and translation have been reported by tntroducing various kinds of functional molecules into liposomes [2, 7, 8]. Expression of functional membrane proteins in the liposome has also been reported in successful [2f, 8, 9]. Connexin-containing liposomes were prepared by using a cell-free transcription/ translation system with a plasmid encoding connexin in the presence of liposomes. The nascently expressed membrane protein, connexin, was directly constituted to the liposome membrane on performing in vitro transcription and translation, thus generating pure membrane protein-containing liposomes. The hydrophilic dye calcein or peptides were efficiently transferred from connexin-expressing liposomes to cultured cells (Fig. 2).

One of our future goals in the expansion of this approach is total reconstitution of a living cell. Using extracted cell components from cultured cells, we are trying to completely reconstruct cellular components under conditions approximating those of living cells [10]. We adopt elemental molecular complexes without further processing if they are functional. These approaches may be termed as middle-out approaches. Such studies should indicate how functional components can be managed to obtain a complex life-like system.

Multirole molecular device based on designed DNA

Recently, DNA has been used as a programmable building material through self-assembly in DNA nanotechnology. Several methods have been proposed for the construction of nanostructures from DNA molecules such as DNA tiles [11], DNA origami [12], and DNA bricks [13]. We can design static nanostructures by using computer-assisted design software (e.g., caDNAno (http://cadnano.org). We designed an artificial molecule that can be used to attach exchangeable molecular devices to the cellular membrane. The X-type body consists of 4 individual single-stranded DNA (ssDNA) molecules with sticky ends, called "ARM sites." Molecular device attachments are designed to be complementary with ARM-site DNA sequences. The unit called the "ARM" can be attached to the corresponding ARM site of the DNA sticky end.

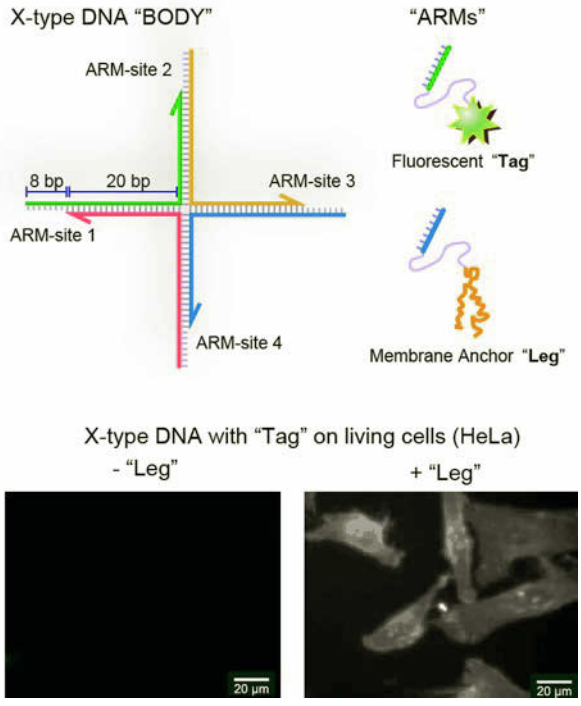

Fig. 3. X-type designed DNA molecules. Upper panel: schematic illustration of the molecular system. Lower panel: X-type DNA equipped with a "tag" was added to living human cultured cells.

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The ssDNA sequence was designed for attachment while mixing at room temperature. “Legs” formed from hydrophobic molecules can also be used to attach the DNA body to the cellular membrane (Fig. 3, lower). The DNA body was stained using a “tag” ARM and was thus found to move on the cell surface by two-dimensional diffusion. The diffusion constant was investigated by single-molecular tracking on the cell surface and found to be approximately 0.3 $\mu m^2/s$. The lipid bilayer is a universal molecular structure of the cell, and every receptor is located on a membrane. Similar to membrane proteins, membrane-localized molecular robots (molbots) may be used to control molecular information and compartmentalized conditions inside the cell. Simple molecular robots made of nucleic acids (DNA or RNA) such as those in the present study may be expressed in living cells by genetic engineering. When the molbot was appropriately designed with regard to Tm (melting temperature of double-stranded DNA or RNA), *in situ* production and function in the desired cell were achieved.

Designed membrane peptide device for surface recognition

As described in the previous section, nucleic acids such as DNA or RNA are attractive molecules for prototyping of molecular devices. If other types of biomolecules can also be designed easily, they will be useful for the construction of artificial functional cell models. Proteins are the main functional component of organisms and occupy over 16 wt% of the total mass of the human cell.

Compared to DNA nanostructure design, protein design is difficult because protein function depends not only on the linear sequence but also on folding states and post-translational modifications. However, small units of protein, i.e., peptides, can be easily designed and are easy to obtain as commercial molecules. Water-soluble peptides are commonly used as drugs or in the cosmetic field. Here, we aimed to design an artificial sensory molecule for attachment to the liposome. The transmembrane α-helix domain was designed based on a previous report [14]. A functional metal (Ti)-specific binding domain was prepared using a procedure reported in a previous study [15]. The designed amino acid sequence of the peptide can recognize the specific electrostatic potential of a metal surface and then bind to the surface. The designed amphiphilic peptide was also attached to a fluorescent molecule and mixed with a lipid solution (1 mM DOPC:DMPC:Chol = 6:1:2 with 50 nM peptide) to form a modified liposome with a diameter of 200 nm. The sample solution was placed onto glass with or without a titanium coating. Fluorescence microscopic observation clearly showed that the designed peptide embedded in the liposome membrane could attach to the titanium surface (Fig. 4). Functional designed peptides should also be synthesizable by gene expression in the giant liposome. Construction of a trigger system for expression control (e.g., riboswitches) is awaited.

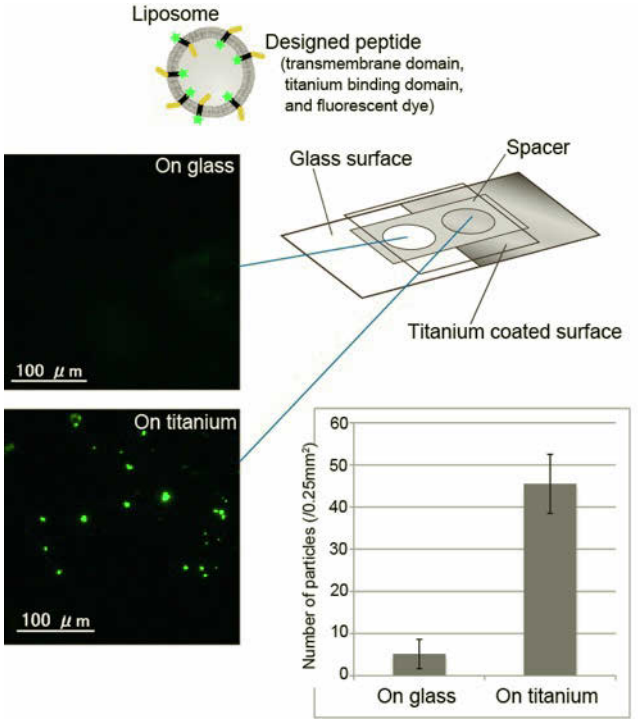

Fig. 4. Surface attachment of artificial liposomes equipped with the designed membrane peptides.

Conclusion

In this report, we have described our approach for constructing an artificial cell model, that is, encapsulation of biochemical reactants and artificially designed DNA and peptides. However, only combining the functional molecules can never give rise to functional structures; development of the molecular-processing system is a crucial step. If the model is compartmentalized, control of molecular input/output through the membrane is essential. To obtain reliable systems, the transduction mechanism needs to have both noise reduction and signal amplification. Implementation of multiple inputs and multiple outputs coupled with an internal chemical reaction network must also be considered. Given these critical issues, a self-reproducing system is a distant goal. Concerning about an artificial "cell", molecular robotics approach should also support an effort for a cell total reconstitution from natural materials [4, 10, 16]. Currently, undergraduate students have designed bacterial genetic circuits (http://igem.org/Main_Page) and DNA nanostructures (http://biomod.net). Thus, the current progress in this field indicates that it should be possible to obtain new artificial cell models in the near future.

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