Molecular Cybernetics: Challenges toward Cellular Chemical Artificial Intelligence

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Research on so-called “chemical artificial intelligence” (CAI) is an emerging field with the aim of constructing information-processing systems with learning capabilities based on chemical methodologies. This can be regarded as an attempt to reconstruct Cybernetics using molecular based systems. Many chemical reaction systems with computational abilities are proposed, but most are fixed functions that deliver molecular output for a given molecular input. On the other hand, chemical AI is a system with learning capability; namely, the output should be variable and gradually change upon repeated molecular inputs. In this paper, a compartmentalization approach for implementing cellular chemical AI using liposomes is discussed. The existing studies in terms of the methods used for assembling systems consisting of many liposomes with different functions, methods for achieving recursiveness and plasticity in chemical reaction systems, and methods for reconfiguring the network topology by liposome deformation are reviewed. Issues that must be addressed in order to realize chemical AI are also identified.

1. Introduction

In 1948, Norbert Wiener wrote a book entitled “Cybernetics”,[1] in which he described the communication and control mechanisms common to living things and artifacts; among them, the learning mechanism of the brain was one of the main issues. Molecular biology, which emerged around the same time, elucidated the mechanisms of living organisms and revealed a series of molecular mechanisms governing their formation and control. Advances in chemistry have made it possible to artificially synthesize DNA and proteins, which can be used to create a variety of functional molecules. Furthermore, in the field of DNA computing, it is possible to combine these molecules to perform complex logical operations. In other words, 70 years after Wiener’s cybernetics, it is now possible to design various molecules and assemble them into functional systems.

Research on chemical artificial intelligence (chemical AI) has begun with the aim of constructing information-processing systems with learning functions in chemical reaction systems. This is a truly challenging task; however, it is a research subject that has inevitably emerged against this background. In this review, the ongoing research on chemical AI is outlined, and research issues for realizing chemical AI are discussed.

Numerous computationally powerful chemical-reaction systems have been proposed.[2] These systems output the results of calculations, such as logical and arithmetic operations, for a certain molecular input. On the other hand, chemical AI is a chemical reaction system with “learning” capability, gradually changing its output by repeatedly accepting molecular inputs.

Let us consider the simplest learning process, Pavlov’s dog or conditioned reflexes.[3] This can be viewed as a task in which the dog’s brain associates two input signals, “bell ringing” and “meat.” By repeatedly presenting the system (the dog’s brain) with two inputs, bell ringing and meat, the system learns to associate them. In other words, initially, the system outputs the result of a YES operation (meat → saliva, but after learning, it outputs the result of an OR operation (meat OR bell → saliva).

Several properties are necessary to achieve this learning. First, the reaction system must accept repeatedly presented input. When an input is presented, the system produces an output as a result of a chemical reaction; however, after a while, the system must return to its original state and be able to accept the next input. This property is called “recursiveness”, or in other words, the function of forgetting. It is not easy to realize this property in chemical reactions because they proceed in the direction of decreasing free energy. It is necessary
to restore the state of the system in some way in order to accept input again. This can be achieved, for instance, by injecting physicochemical energy pulses as clocks from the outside or by using oscillatory chemical reactions. The second property is “plasticity.” If the system forgets everything after producing an output, without leaving any traces in the system state, the learning will not proceed. Therefore, the system must accumulate a certain number of molecules to represent the learning results.

There are many approaches to implement chemical AI. For example, fuzzy-logic-based learning systems have been proposed in,[4–6] utilizing various physicochemical phenomena such as photochemical and oscillatory reactions. In terms of the complexity of the information that can be handled, research is developing on DNA computers, i.e., computational systems based on hybridization reactions of DNA strands.[7] In the most typical system, a series of reactions called strand displacement reactions are performed in a single test tube (one-pot) for the DNA sequence inputs, and the calculation results are represented by the concentration of specific DNA sequence outputs.

Recently, approaches that make use of the spatial restrictions have been studied, such as systems that arrange computational molecules on DNA origami or distribute them on gels or beads.[8] In these systems, not only the concentration of the molecules but also their positioning and distribution convey information. However, in these approaches, environmental parameters are common to the entire system; therefore, combining reactions with different optimal conditions become more difficult as the number of reactions increases.

Compartmentalization is one of the most effective solutions to this problem. By dividing a complex chemical reaction system into a number of cells, it is easier to tune the reaction conditions in each cell. However, this division alone does not allow the system to function as a whole; therefore, a mechanism for integrating these cells is also necessary. This is achieved by the exchange of information between cells, that is, molecular communication. In this review, we focus on the implementation of cellular chemical AI with such “cellular architecture.”

In chemical AI research, the first thing to tackle is simple learning, such as Pavlov’s dog, but eventually, we will deal with more complex learning problems. A simple toy model for Pavlov’s dog should be able to solve complex learning problems with multidimensional inputs and outputs by extending the same architecture. This property is referred to as “scalability.”

A biological brain is an example of a scalable system. Every brain comprises a network of neurons. Each neuron responds to a stimulus that is transmitted to other neurons. The ability to learn is due to the variable nature of this transmission. The simplest brain is that of a nematode, which consists of 302 cells. The human brain, which consists of 100 billion cells, uses the same mechanism. Chemical AI is an attempt to artificially reconstruct such information processing using well-designed chemical reactions.

Learning in the biological brain is achieved by changing the topology of the network, that is, by neurons extending their axons to form synapses with other neurons. This property is called reconfigurability, which implies that the connectivity between cells is variable, and is essential for the realization of scalable chemical AI. The deformation of a compartment corresponds to nerve axon outgrowth, and synaptogenesis is the formation of new molecular communication channels between adjacent compartments.

This review is comprised of six sections. In Section 2, we discuss the learning of the DNA circuits. Computational reaction circuits with recursiveness and plasticity are central to chemical AI, and we discuss the progress in conventional DNA computing and how it needs to be extended. Section 3 describes the current status of research on liposome-based chemical reaction systems, which form the basis of cellular chemical AI. The scalability of liposome assembly technology and communication via molecular diffusion on liposomes are discussed. In Section 4, we describe the molecular communication technology between such liposomes. To guarantee scalability, it is necessary to adopt a communication method that does not mix the solutions between the connecting liposomes, and from the point of view of recursiveness, it is necessary to transmit signals multiple times. In Section 5, we discuss actuation techniques for liposome reconfiguration, in which compartments are transformed and combined with other compartments. Finally, in Section 6, we present our conclusions.

2. Principle of Learning in DNA Circuits

In this section, we describe reaction circuit design techniques for chemical AI. DNA computing technology is at the center of this technology. DNA computing is a research field that studies how to design a chemical reaction circuit with the desired dynamics, utilizing DNA strands as signaling molecules,[8–13] where the goal is to systematically construct various computational functions by combining reaction modules. In this section, we discuss recursiveness, plasticity, and other functions that are necessary for learning capability.

2.1. DNA Circuits

In a DNA reaction system, “inputs” and “outputs” are assigned to specific DNA strands, and their concentrations are defined as “signals.” By designing the reaction system to produce a specific input–output relationship, it can act as a functional “circuit” or module, hereafter referred to as a DNA circuit.[14,15] By “installing” DNA circuits that execute intelligent information processing in nano- and micro-machines, complicated tasks or circumstance dependent conditional decisions by these molecular machines or robots become possible.[16,17]

2.2. Design Methods of DNA Circuits

Various methodologies have been proposed for the rational design of DNA circuits. Typical design strategies are enzyme-driven circuits that use a set of enzymes to synthesize, cleave, and degrade DNA strands,[18–23] whereas enzyme-free circuits are composed of DNA-strand displacement reactions.[24–29] In either approach, there are three design requirements.[30] The first is modular systematic design. We usually construct
a circuit with the desired functionality by connecting small-scale circuits. This is possible because only signals containing meaningful information propagate through the connected circuits without attenuation. Second, energy efficiency is necessary in order to allow the execution of operations for extended amounts of time, which requires the management of waste strands (e.g., byproducts). Third, the system must be time-responsive in order to generate time-varying output signals in response to time-varying input signals. Time responsiveness can be rephrased as recursiveness in discrete learning tasks, such as Pavlov’s dog. It should be noted that a recursive circuit requires the ability to initialize the chemical reaction system after the input of a stimulus to prepare for subsequent stimuli. Design methods for DNA circuits that satisfy these requirements have been actively pursued and successfully developed in recent years owing to advances in DNA logic gates,[26,27,31,32] analog operations,[24,25,33–35] and feedback control laws.[36–40]

2.3. DNA Circuits for Learning

The development of DNA circuits that can dynamically adapt to unknown environments and make flexible decisions depending on a given situation is currently a major challenge in the field of DNA computing. The central issue in chemical AI is to realize learning by such dynamic DNA circuits (Figure 1).

In conventional DNA circuits, the circuit function is fixed when the input–output relationship is defined. To design a circuit with the ability to learn, the input–output relationship of the chemical reaction system must be dynamically and autonomously updated according to the input history. This implies that “plasticity” is an additional requirement of DNA circuits. How can a plastic DNA circuit be designed? Most DNA circuit designs that have been developed to date only assume a one-time response (one-shot operation); therefore, they cannot respond appropriately to secondary and further input stimuli. Therefore, it is essential to establish the design method for recursiveness as a fundamental property in realizing learning circuits. However, it is not straightforward to achieve the recursiveness in a chemical reaction system since the reactions proceed just in the direction of minimizing the free energy.

2.3.1. Recursiveness

Recently, the design of recursive DNA circuits has been investigated actively. A classic result is the “DNA tweezer” developed by Yurke et al.[15] The DNA tweezer transforms into an open state upon being provided with an input DNA strand, but it can be reset to form a closed state by inputting a fuel strand. A strategy in the design of such recursive devices is to apply a specific “resetting strand” after a steady state is reached, thereby updating the concentrations of the reaction system in preparation for the next input. For example, Eshara et al.,[41] Garg et al.,[42] and Genet et al.[43] proposed recursive designs for a “seesaw gate” (seg)[46,47] for details] and logic gates, where the circuits can be initialized by inputting resetting strands. On the other hand, designs of autonomously recursive DNA circuits without applying external resetting strands have also been proposed.[44,45] For example, an autonomous mechanism is achieved by separately defining the fuel strands used in the recursive process from those required to generate the output response and by carefully adjusting the rates of both reactions.[46] However, complete recursive mechanism is usually difficult; experiments have shown that the responses following resetting are weaker or stronger than the previous response.[40–43,45]

2.3.2. Functional Extension by Artificial Molecules

To realize chemical AI, in addition to the circuit design just with usual DNA molecules, new molecular materials that can extend and enhance the computational ability of chemical reaction system have been actively developed. In particular, the innovation of functional molecular materials is quite effective in designing recursive reaction mechanisms. For example, photo-responsive molecules such as azobenzene[46] and 3-cyanovinylcarbazole (cnvK)[47] switch their structural conformations when irradiated with UV light of specific wavelengths. The double helix of azobenzene-modified DNA can be destabilized by UV irradiation through photoisomerization.[46] Song et al.[48] proposed the design of a recursive seesaw gate with an azobenzene modification. The circuit is composed of a main response reaction, which generates the output, and a regeneration reaction, which returns the DNA circuit from the steady state to the initial state. Both reactions were controlled using UV irradiation via the functional molecular materials. To realize chemical AI, dependable design methods for recursive DNA circuits must be established, and a variety of new functional molecules must be developed. Cationic copolymers,[49] which dramatically increase the reaction rate of DNA-strand displacement, are one such type of functional molecule.

If an update law that can alter the reaction dynamics in a desirable direction can be added to the recursive mechanism, it will be possible to develop the chemical AI that performs learning tasks. Neural networks commonly used in the field of computer science employ the so-called back-propagation method as the update law (computational algorithm). However, this update law is too complicated to be applied to chemical AI as it is, because it requires the sophisticated modification of the coupling strength between nodes in the networks based on the gap between the current output and the target value. In the future, it is desirable to develop a simpler learning law that can be implemented in chemical AI, but the development of new molecular materials will be essential to achieve this goal.

3. Liposome Assembling Technologies for Cellular Chemical AI

The DNA computational circuits described in the previous section are all implemented as reaction circuits in a single test tube (“one-pot” reaction). There is a limit to superimposing many chemical reactions in a single reaction space and solution condition, and compartmentalization of the reaction space is necessary to improve the scalability of the system. In this section, we describe the micro reaction space using liposomes as artificial cells, including how to fabricate liposomes, how to
arrange a large number of liposomes, and how to express functions by liposome assembly.

3.1. Single-Functional Liposome

The construction of liposome-based artificial cells has undergone remarkable progress in the last two decades (Figure 2).

First of all, we briefly introduce the preparation methods of liposomes. When phospholipid molecules, with both hydrophilic and hydrophobic sections are dispersed in water, a closed bilayer membrane of phospholipid, known as a liposome, is formed. Not only nanometer-sized liposomes but also cell-sized liposomes (diameter > 1 µm) are formed in an aqueous dispersion. As a chemical model that mimics the structure of microorganisms and cells, liposomes have recently attracted interest in compartmentalization of reaction space of biological reaction systems. Nanometer-sized liposomes are relatively effortlessly homogenized in size, shape, and internal structure, hence, they are widely used in pure and applied sciences, such as in drug delivery systems. For over 50 years now, research on cell-sized liposomes has not been prevalent as they are considered difficult to handle quantitatively with good reproducibility. Cell-sized liposomes prepared using conventional methods have significant variations in size, shape, and internal structures. In recent years, the preparation methods of cell-sized liposomes have been greatly improved, and active research on the application of liposomes consisting of a single bilayer membrane (henceforth, this cell-sized liposome is referred to as liposomes) is being conducted.

Liposome preparation methods are usually associated with one of four methods: the thin film swelling method, water-in-oil (W/O) emulsion template method, jetting method, and water-in-oil-in-water (W/O/W) double emulsion templating method.

In 1969, the first preparation method of liposomes using a dry thin film of phospholipid was reported and this preparation was named the thin film swelling method. In this method, liposomes are formed by the swelling of the bilayer membranes on the thin film after water (or buffered solution) is added. A patterning technology that involves applying dry thin film to a glass substrate with a microfabricated stamp affords size-controlled liposomes.

The W/O emulsion template method involves wrapping W/O emulsion droplets (surrounded by a phospholipid monolayer) by a phospholipid monolayer formed at the interface between the emulsion and water phases. The use of centrifugation for the wrapping is common practice. Several research groups have established new hand-made devices and novel methods including the Continuous Droplet Interface Crossing Encapsulation (cDICE) method and Droplet-Shooting and Size-Filtration (DSSF) method, for controlling the size of emulsion droplets.

Along with the recent development of microfabrication techniques, the water-in-oil emulsion template method was improved, and new methods, including the jetting method and the W/O/W double emulsion template method, were established. The jetting method is based on a free-standing bilayer membrane (called as black lipid membrane) and a glass capillary. The liposomes are formed by a process resembling that of blowing soap bubbles. The W/O/W double emulsion template method involves producing uniform W/O/W double emulsion droplets in a microfabricated fluidic device and obtaining liposomes by utilizing the phase separation of oil from the droplets. Although there still exist some limitations to the versatility of these methods, such as use of reagents and obtaining the appropriate sizes, liposome preparation methods using microfabricated fluidic devices are expected to develop technologically and promote the application of liposomes.

Various types of functionalized liposomes that operate on their own have been proposed, such as sensors, processors, actuators (transformation, self-propulsion, or both), and combinations of these. Furthermore, proliferating liposomes that consume lipid precursors and exhibit growth and division through chemical conversion have been reported. Although the functionality of liposomes has been improved by sophisticated molecular synthesis and microfabrication technologies, there are still many constraints to be considered, such
as crossover interference between the internal solution and liposomal membrane conditions.

### 3.2. Multiple Liposome System

Living organisms have expanded their functions during biological evolution, either by developing intracellular organelles, such as eukaryotic cells, or by creating multicellular organisms. Mimicking this strategy has attracted attention, such as encapsulating liposomes inside liposomes\(^\text{[71]}\) or connecting liposomes for communication.\(^\text{[72]}\) The encapsulation of liposomes inside liposomes can be regarded as a model of eukaryotic cells. Within this strategy, various experimental systems, such as ATP and protein synthesis circuits in liposomes driven by light irradiation,\(^\text{[73]}\) actin-encapsulating liposomes exhibiting light-responsive transformation,\(^\text{[73]}\) and stochastic enzymatic reactions inside liposomes\(^\text{[73]}\) have been constructed. However, because the maximum size of liposomes reported so far is \(\approx2\) mm,\(^\text{[76]}\) without any structural reinforcement for the strength of liposomes using hydrogels or other macromolecular network,\(^\text{[77]}\) the larger liposomes are difficult to be realized. This size restriction could be a barrier for the implementation of a highly hierarchical structure inside a liposome. In the latter strategy of connecting liposomes, Bayley’s group developed hemi-fused multiple liposomes (hemi-fused liposomes share only a bilayer membrane between the connected liposomes) on the scale of several millimeters. They also showed information-transmitting ability among liposomes through a channel protein called \(\alpha\)-hemolysin inserted into the hemi-fused membrane.\(^\text{[78]}\) In principle, when the connected liposomes are reinforced with hydrogels to make them sufficiently rigid against hydrostatic pressure, there will be virtually no restrictions in scaling up the construction of liposomal tissues.

### 3.3. Liposomal Complex

Assuming that several types of functional liposomes are bound in suspension, how can scalable information processing be achieved? Adamala and Mann’s groups independently constructed modules for information processing by encapsulating DNA and proteins in liposomes or peptidosomes (capsule-like structures surrounded by amphiphilic peptides).\(^\text{[79,80]}\) Mann et al. placed micrometer-sized peptidosomes in a certain geometric pattern and obtained an interesting output that corresponded to the pattern. Van Hest et al. observed chemical traveling waves (adenosine monophosphate) on aligned liposomes.\(^\text{[81]}\) In these systems, the chemical output of each liposome was designed by numerical simulations, but the
signal transfer between liposomes was not controlled, only the diffusion of signal molecules.

To construct a scalable system capable of higher-order information processing, we need a system in which liposomes can be spatially aligned, connected, and communicate with each other, and the topology and mode of communication of the entire liposome must be tailor made depending on its own output. This is the strategy used by multicellular organisms to develop neuronal networks.

In fact, technologies for building liposomal complexes have been developed using external stimuli such as electromagnetic fields,[82,83] acoustic pressure,[84] radiation pressure,[85] and fluidic fields.[86–88] Orwar’s group also developed a technique for stretching membranes composed of liposomes into nanotubes. These tubes can be linked to another liposome membrane by using a capillary with a concentrated electric field at its tip.[89,90] This technique is powerful because it can build a network with a free geometry among the connected liposomes. However, each liposome must be manipulated individually, which requires a sophisticated technique of capillary manipulation. However, microfluidic devices have the advantage of being able to produce large numbers of liposomes of the same size and assemble them into an array ready for statistical analysis.

### 3.4. Molecular Communication in Liposomal Complex

Not only does passive lipid exchange occur between connected liposomes,[91] but chemical and electrical stimuli[92] can induce hemifusion and fusion between them. Without such structural changes of liposomes, Luisi’s group found that certain molecules can move among liposomes and termed this “vesicle colony”.[93] Ding et al. designed new amphiphilic molecules that could also move among liposomes.[94] Tomasi et al. encapsulated a solution of the Belousov–Zhabotinsky (BZ) reaction in liposomes and showed that chemical waves can be transmitted between adjacent liposomes.[95] This phenomenon probably occurs because less polar intermediates of the BZ reaction can pass through the membrane at a high permeability rate.

It is known that when the BZ reaction is induced in water-in-oil emulsions, the intermediates Br₂ (inhibitor) and BrO₂ (activator) produced during the reaction can diffuse into the oil phase, resulting in pattern formation with characteristics different than those of the BZ reaction in water.[96] A similar phenomenon was recently reported for geometric information processing using oil-in-water emulsions and DNA reaction systems within liposomes.[97–99] Ces et al. created hemifused liposome complexes by simultaneously passing multiple water-in-oil emulsions through the interface and used α-hemolysin to induce sequential chemical reactions across the liposomal compartments.[100] Bolognesi et al. collected multiple liposomes using optical tweezers and hemic fused them to induce sequential chemical reactions.[95]

In terms of the efficient development of chemical AI, a system that can simultaneously test the functionality of a large number of liposomal complexes is required, that is, a system that can apply various chemical signals to the liposomal complexes and observe the temporal changes in reactions occurring in each liposome in real time to obtain statistical analysis data. Such a system can use microfluidic devices made by microfabrication technologies such as 3D printers.[101]

### 3.5. Artificial Molecular Devices to Transmit Signals across the Membrane(s)

In the previous section, we described materials and techniques for compartmentalizing chemical reactions using liposomes and for fabricating assemblies consisting of many liposomes. Chemical AI aims to realize more advanced functions by connecting specific liposomes with each other in a desired order and pattern, rather than simply randomly arranging a large number of liposomes. To utilize the spatially ordered liposome system, molecular signals must be able to transfer across compartments to create an “awareness” of their neighbors. In this section, we describe membrane channels through which molecular communication can occur. In this section, we describe the designed membrane molecules for transmitting molecular information through the lipid bilayer of liposomes.

#### 3.6. Nanopore and Receptor

Both live and artificial cells based on giant unilamellar vesicles (GUVs) are membrane-compartmentalized systems. Chemical information in the compartment is only recognized by transmitting molecular signals across the membrane between the extra- and intra-cellular spaces. Various molecular devices have been developed to create multi-compartment artificial cell systems. The simplest device is a channel or functional pore (Figure 3, left) that directly connects the solution inside and outside the GUV. Early studies on artificial ion channels began with the design of synthetic peptides[102,103] or synthetic molecules[104] that can function on planar membranes. Reversible chain-like channels that mimic natural receptors and channels with artificial porous structures have been actively studied.[105] Recently, Kawano et al. reported a nanopore with de novo designed peptides capable of detecting a single molecule passing through a planar membrane.[106] GUVs with such nanopores and receptors can then be used to design and provide new smart materials that can operate by processing signals from the outside world or molecular signals that they use themselves. For example, by combining natural and artificial nanopore/receptors, Z. Chen et al. reported a so called artificial β-cell that could externally release insulin in response to elevated environmental glucose concentrations.[107]

#### 3.7. Nanopore Implementation on GUV

A number of studies have utilized α-hemolysin protein to create nanopores for the exchange of small hydrophilic molecules between the inside and outside of GUVs.[108] For de novo designed nanopores, artificial pores designed using DNA nanotechnology have attracted considerable attention[109] (Figure 4). Nanopores made of DNA origami with diameters as large as 30 nm[113] and DNA nanopores whose pore sizes can be changed...
by inputting DNA signals\cite{114} have been reported. Nanopores with external controllability will be useful for modeling biomolecular transmission mechanisms and achieving selective and efficient communication among artificial cells.

### 3.8. Receptor Implementation on GUV

Nanopores connect the internal space of GUVs to the external space. This means that the inside of the GUV is exposed to the external environment, even if partially or temporarily, causing leakage of molecular contents or chemical state homogenization. To avoid this, a device called a receptor (Figure 3, right) is required. The receptor receives molecular signals from the GUV and transmits information across the membrane through conformational changes. Various studies have reported receptors composed of synthesized organic molecules. A typical example is membrane-floating/sinking molecules that release signals from within vesicles in response to pH changes across the membrane (Figure 5a).\cite{115} Other responses include redox potentials, ions, small molecules such as inclusion compounds and chelating agents, and light stimulation.\cite{116}

DNA nanotechnology has also been applied to de novo designed receptors. Liu et al. designed a molecule that uses cholesterol-modified single-stranded DNA (ssDNA) as a hydrophilic transmembrane region and a hydrophilic ssDNA region at both ends for control (Figure 5b).\cite{117} When ssDNA is applied from the outside of the GUV, the pair of molecules undergoes hybridization and emits an output signal in the GUV. Chen et al. designed a pair of molecules with hydrophobized (hydrocarbon) DNA for the transmembrane region and DNA aptamers for the input site (Figure 5c). The distance between the molecules on the membrane was reduced by binding with ligands, such as ATP or lysozyme, inducing the formation of a G-quadruplex. This results in the output of a fluorescence signal from inside the GUVs.\cite{118}

### 3.9. Information Transmission Technology for Cellular Chemical AI

Artificial molecular devices inspired by biomolecular mechanisms are expected to be useful for obtaining spatiotemporal control of artificial and biological cell activities.\cite{119} However, none of these devices can be produced on site or used repeatedly. For chemical AI applications that require recursiveness of the system, it is necessary to develop tough and resettable devices for repeated use. In other words, the nanopores should be able to open and close, and the receptors should be reusable. To reset these devices, technologies such as reversible hybridization of DNA duplexes triggered by the trans–cis isomerization of azobenzene by light irradiation have been developed.\cite{120}

Some researchers have investigated signal transmission in artificial multicellular systems.\cite{121} It should be noted that two adjacent GUVs were separated by a four-layer lipid membrane. The aim was to develop a mechanism to transmit molecular signals across GUV membranes with no leakage. To realize molecular devices that can transmit signals across multilayered membranes, a cue can once again be taken from natural cells, such as multiple nuclear membranes, mitochondria, and gram-negative bacteria. In artificial cell engineering, self-assembly of de novo designed peptides onto membranes and the embedding of membrane proteins to interact with live cells as virus-like molecular systems have already been realized.\cite{122} Recently, a cell-sized hollow gel capsule composed of designed DNA segments was reported.\cite{123} This type of compartment was expected to allow permeation across the boundary. In the field of artificial molecular devices, the system design cannot limit the existing combinations of molecules in a live cell.

### 4. Molecular Actuation System for Reconfiguration of Liposomal Network

As mentioned in the introduction, cellular chemical AI requires development of a reconfiguration technology for liposome networks that mimics synapse formations in biological neurons to realize scalable learning functions. For this purpose, it is necessary to deform liposomes in a certain direction toward other liposomes, and to form channels when they come into contact. In this section, we describe how to make liposomes undergo large deformations upon receiving signals as outputs of molecular computational circuits (Figure 6). Although there are many possible macroscopic deformation mechanisms for artificial cells, we focus on the systems utilizing artificially structured and controlled molecular motors such as microtubules–kinesin pair.
Figure 4. Artificial nanopores designed de novo with DNA nanotechnology. a) DNA origami nanopore consisting of 54 helix bundles (HB) with 26 cholesterol anchors.[106] b) DNA origami T-shaped pore channel embedded on membrane via biotin/streptavidin bridges.[110] c) 6HB barrel nanopore with a membrane-spanning hydrophobic belt with phosphorothioate-ethyl anchors.[112] d) Ultrawide nanopores of DNA origami on GUV membrane. Reproduced with permission.[113] Copyright 2021, American Chemical Society. e) DNA origami nanopore with the ability to adjust its pore size. Reproduced with permission.[114] Copyright 2021, The Royal Society of Chemistry.
Figure 5. Artificial membrane receptors composed of small molecules and DNA nanotechnology. a) A synthetic signal transducer acted as a switchable catalyst, catalyzing the formation of surfactant molecules inside the vesicle in response to a change in external pH. Reproduced with permission.[115] Copyright 2017, American Chemical Society. b) DNA-based receptor analogues recognized external signals to drive two receptors into close spatial proximity to activate DNAzymes inside the vesicle. Reproduced with permission.[117] Copyright 2021, American Chemical Society. c) Transmembrane receptors made by cholesterol-modified DNA. An external molecular signal input induced the dimerization of two artificial receptors. Then, the pair formed a G-quadruplex, which served as a peroxidase-like enzyme in the vesicles. Reproduced with permission.[118] Copyright 2021, The Royal Society of Chemistry.
4.1. Liposome Deformation Mechanisms

It is known that encapsulation of concentrated cytoskeletons in liposomes often causes large deformations of the membrane.\cite{124,125} For example, microtubule-encapsulating liposomes form protrusions under ambient pressure (0.1 M Pa) owing to tubulin polymerization with them. When high pressure (60 MPa) is applied to the system, the protrusions rapidly shrink owing to microtubule depolymerization.\cite{124} Liposomes containing actin filaments exhibit other unique behaviors.\cite{125} When actin filaments are encapsulated in liposomes, the majority of the liposomes tend to be spindle-shaped, and occasionally, a string-like membrane tube extends from the spindle tips. Some liposomes deformed to triangle- or cruciform- (or quadrupole-) shapes. This deformation can be reversibly controlled by controlling the osmotic pressure of the outer solution. The hypertonic outer solution produces sharper spindles, and the hypotonic solution cancels the deformation. In addition, fluorescence-labeling of actin enables reversible control of the deformation triggered by light irradiation.

4.2. Artificial Molecular Muscles

One of the most feasible ways to realize precise control of deformation by molecular signals is to encapsulate “artificial molecular muscles,”\cite{126} which consist of molecular motors and cytoskeletons assembled via DNA hybridization, into liposomes. In contrast to natural muscles composed of filamentous actin and myosin, this system consists of DNA-modified microtubules, DNA origami comprising 6-helix bundles (6HB) with complementary strands, and kinesin tetramers assembled on streptavidin protein. Complexation between DNA-modified microtubules and 6HB results in the formation of an aster-like higher-order structure, and kinesin tetramers connect them to the millimeter-scale microtubule network. The addition of ATP, the chemical energy source for kinesin movement, to the system triggers a large-scale and rapid shrinking of the network to create muscle-like movements. Microtubules in the network are presumed to be radially oriented from the 6HB connection; therefore, the system corresponds to a smooth muscle model rather than a skeletal muscle model with a common sarcomere structure with highly ordered filaments in parallel orientation. A similar DNA-mediated assembly of microtubules was reported by Wollman et al.\cite{127} They modified kinesins with DNA-binding proteins and controlled the assembly of polar microtubule arrays using signals encoded in the DNA. Another system that harnesses the DNA-mediated assembly of microtubules is the “molecular swarm robot” system, developed by combining DNA-modified microtubes gliding on a kinesin-immobilized glass substrate.\cite{128–130}

4.3. DNA-Origami-Motor Protein Conjugates

The immobilization of motor proteins on DNA origami, on the other hand, has been accomplished mostly for the purpose of studying the dynamics of motor proteins.\cite{131–133} Derr et al. prepared 225 nm DNA-origami 6HB and introduced multiple dyneins or kinesins.\cite{131} Total internal reflection fluorescence microscopy (TIRFM) revealed that an increase in the number of motor proteins on the scaffold did not affect the velocity but significantly enhanced the total run length. These systems may also be applicable to the preparation of more efficient artificial molecular muscles. Iwaki et al. modified spring-like DNA origami\cite{132} or 10HB DNA-origami rods\cite{133} with myosins and successfully studied their motile properties in single-molecule or collective modes.
4.4. Liposome Deformation by Membrane-Binding DNA Origami

DNA origami is often modified with lipid-membrane binding moieties and is used to directly but statically deform liposomes.[134–137] As early as 2014, Simmel et al. reported rectangular DNA origami modified with cholesterol moieties.[134] They confirmed for the first time that such origami binds to the lipid membrane of GUVs. Zhang et al. later succeeded in shaping liposomes into tubular, toroidal, or twisted shapes using various stacks of DNA origami cages.[135] They designed DNA origami cages with cylindrical structures and tuned their diameter and height to control the shape of the captured liposomes. Franquelim et al. sculpted the shape of GUVs using DNA origami with curvatures.[136] Three types of cholesterol-modified DNA origami (i.e., linear, quarter-circle, and semi-circle), were prepared, and quarter-circle DNA origami was found to trigger outward membrane tubule formation. Zuraw-Weston et al. reported a similar vesicle deformation triggered by the binding of DNA origami rods.[137]

4.5. DNA Origami Actuators

Attempts to develop molecular actuation systems solely from nanomechanical DNA origami have been ongoing for decades. The direct attachment of such a molecular actuation system to the lipid membrane might realize the dynamic deformation of liposomes responsive to molecular signals. The first two DNA origami boxes, both reported in 2009, can be regarded as prototypes of DNA origami robots.[138,139] “DNA origami pliers” and “DNA origami forceps,” two DNA-origami pinching devices reported in 2012, were the first “nanomechanical DNA origami devices” that functioned only when their structural changes were induced.[140] The two lever-like portions were connected to each other at the fulcrum with free rotation. Such DNA-origami pinching devices can be used as indicator reagents to detect the presence of various bio-related molecules at the single-molecule level by utilizing their structural changes induced by specific ligand–receptor binding, such as biotin–streptavidin pairs.[140–143] Such bistable nanomechanical DNA origami devices with scissor-like designs have been used to prepare plasmonic nanodevices.[144–147] Kuzyk et al. modified such devices with gold nanorods and realized controlled switching of plasmonic coupling between the nanorods. Bistable DNA origami devices with a pivot by Dietz et al. utilized shape complementarity and nucleotide base-stacking to stabilize the closed state.[148–150] Both devices were observed under high-speed AFM, and structural changes were monitored in a single-molecule manner in real time.[145,149] Another unique bistable DNA origami developed by Suzuki et al. exhibits significant bending in response to DNA hybridization or quadruplex formation, which may be useful for dynamic liposome deformation.[151]

Bistable nanomechanical DNA origami, which can recognize and bind cells by logical operations, as reported by Douglas et al., was the first DNA origami system labeled as a “nanorobot.”[152] It had a barrel-shaped structure that could be selectively opened by specific aptamer–ligand binding events and loaded with antibodies. The nanorobot was later injected into living animals (cockroaches) and was confirmed to work correctly as programmed.[153] Similar bistable, tube-like DNA origami devices have been used for cancer therapy in vivo.[154,155]

The mechanical movement achievable with DNA origami nanomachines is not only bistable. Several systems with other modes of motion have been reported.[156–159] Ketterer et al.[156] and Kopperger et al.[157] independently developed sophisticated rotary systems for rotary DNA origami devices by assembling multiple origami units or simply from a single unit, respectively. In contrast, Benson et al.[158] developed a linear actuation system by applying ≈200 nm long DNA origami rotaxane structure. Stömmer et al.[159] also prepared a linear DNA origami actuation system by trapping a piston in a long DNA origami tube, which successfully realized linear transport up to 3 μm in length.

4.6. Toward Artificial Synapse Formation

As described above, neuronal plasticity is maintained by axonal outgrowth and synapse formation. To properly mimic this system, the artificial-molecular-muscle system has several issues that need to be resolved. For example, the achievement of repetitive reactions is still a big challenge; overcoming it will enable the realization of multiple and repetitive liposome deformations for the molecular learning process. The encapsulation of artificial molecular muscles into liposomes requires a continuous supply of ATP to the internal solution. In addition, the anchoring of molecular components to lipid membranes is desirable for the efficient conversion of artificial muscle actuation into liposome deformation. The precise prediction of liposome deformation is also necessary for an efficient connection between multiple liposomes.[160] The formation of an artificial gap junction (electrical synapse) requires connexin-like linker components.[161] Another choice may be to mimic chemical synapses that do not require direct physical connections; the recently reported long-range molecular-information transfer system would be a good candidate for this purpose.[162]

5. Conclusions

In this review, we discussed the current status of chemical AI inspired by the biological brain. Related research issues were raised, such as technology to produce and manipulate liposomes as a compartment to implement the chemical reactions, inter-liposomal (e.g., trans-membrane) molecular communication by artificial pores and receptors, DNA circuits with learning ability that require both plasticity and recursiveness, and actuation mechanisms to achieve reconfiguration among the compartments.

What lies ahead of the chemical AI? The closest application of chemical AI is as a diagnostic system. Considering that chemical AI was developed from DNA computing, it is obvious that RNA can be used as an input for chemical AI. Diagnosing the presence or absence of a disease using complex RNA combinations is within the scope of conventional DNA computing, but chemical AI can handle more complex relationships among
a wider variety of molecules. For example, it may be possible to search for an unknown set of disease-causing molecules from the molecular samples of a large number of patients. Another possibility is the exchange of molecular information between living organisms and intelligent systems designed by humans, such as artificial organs that are controlled by chemical AI. Chemical AI is an artificial cellular system; since it is mainly composed of biological materials, it can obtain its driving energy from the living body. In other words, it will be possible to implant chemical AI or to use artificial organs containing chemical AI for medical treatment or human augmentation. Experiments to exchange molecules by connecting cell-sized liposomes to living cells have already been initiated.\(^{[163,164]}\) The development of an intelligent device that is both biodegradable and capable of information processing, which is not possible with conventional electronic devices, may lead to a new industrial revolution in support of a sustainably developing society.

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**Conflict of Interest**

The authors declare no conflict of interest.

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[1] N. Winner, Cybernetics: Or Control and Communication in the Animal and the Machine, MIT Press, Cambridge, USA 1948.
[2] A. Adamatzky, B. De Lacy Costello, T. Asai, Reaction-Diffusion Computers, Elsevier, Amsterdam, Netherlands 2005.
[3] D. Coon, J. O. Mitterer, Cengage Learning 2008.
[4] P. L. Gentili, RSC Adv. 2013, 3, 2523.
[5] P. L. Gentili, M. S. Giubila, R. Germani, A. Romani, A. Nicoziani, A. Spalletti, B. M. Heron, Angew. Chem. 2017, 129, 7643.
[6] P. L. Gentili, Molecules 2021, 26, 5987.
[7] E. Katz, Ed., DNA- and RNA-Based Computing Systems, Wiley-VCH, Weinheim, Germany 2021.
[8] T. Corell, Nature 2011, 469, 45.
[9] K. Lund, A. J. Manzo, N. Dabby, N. Michelotti, A. Johnson-Buck, J. Nangreave, S. Taylor, R. Pei, M. N. Stojanovic, N. G. Walter, E. Winfree, H. Yan, Nature 2010, 465, 206.
[10] T. Omabegho, R. Sha, N. C. Seeman, Science 2009, 324, 67.
[11] P. W. K. Rothermund, Nature 2006, 440, 297.
[12] M. N. Stojanovic, D. Stefanovic, Nat. Biotech. 2003, 21, 1069.
[13] P. Yin, H. M. T. Choi, C. R. Calvert, N. A. Pierce, Nature 2008, 451, 318.
[14] Y. Benenson, T. Paz-Elizur, R. Adar, E. Keinan, Z. Livneh, E. Shapiro, Nature 2001, 414, 430.
[15] B. Yurke, A. J. Turberfeld, A. P. Mills, F. C. Simmel, J. L. Neumann, Nature 2000, 406, 605.
[16] S. Murata, A. Konagaya, S. Kobayashi, H. Saito, M. Hagiya, New Gener. Comput. 2013, 31, 27.
[17] Y. Sato, Y. Hiratsuka, I. Kawamata, S. Murata, S. M. Nomura, Sci. Robot. 2017, 2, eaal3735.
[18] K. Montagne, R. Plasson, Y. Sakai, T. Fujii, Y. Rondelez, Mol. Syst. Biol. 2011, 7, 466.
[19] T. Fujii, Y. Rondelez, ACS Nano 2012, 7, 27.
[20] K. Montagne, G. Gines, T. Fujii, Y. Rondelez, Nat. Commun. 2016, 7, 13474.
[21] M. Hagiya, N. Aubert-Kato, S. Wang, S. Kobayashi, Theor. Comput. Sci. 2016, 632, 4.
[22] A. Padirac, T. Fujii, Y. Rondelez, Proc. Natl. Acad. Sci. USA 2012, 109, E3212.
[23] N. Aubert, C. Mosca, T. Fujii, M. Hagiya, Y. Rondelez, J. R. Soc. Interface 2014, 11, 20131167.
[24] G. Seelig, D. Soloveichik, D. Y. Zhang, E. Winfree, Science 2006, 314, 1585.
[25] D. Zhang, A. J. Turberfield, B. Yurke, E. Winfree, Science 2007, 318, 1121.
[26] L. Qian, E. Winfree, Science 2011, 332, 1196.
[27] L. Qian, E. Winfree, J. R. Soc. Interface 2011, 8, 1281.
[28] D. Soloveichik, G. Seelig, E. Winfree, Proc. Natl. Acad. Sci. U.S.A. 2010, 107, 5393.
[29] D. Zhang, G. Seelig, Nat. Chem. 2011, 3, 103.
[30] E. Chinforooshan, D. Doty, L. Kari, S. Seki, LNCS 2011, 6518, 25.
[31] L. Qian, E. Winfree, J. Bruck, Nature 2011, 475, 368.
[32] K. Nishijima, T. Nakakuki, New Gener. Comput. 2020, 38, 285.
[33] T. Song, S. Garg, R. Mokhtar, H. Bui, J. Reif, ACS Synth. Biol. 2016, 5, 898.
[34] S. Kobayashi, K. Yanagibashi, K. Fujimoto, K. Komiya, M. Hagiya, 2014 IEEE 33rd International Symposium on Reliable Distributed Systems Workshops 2014, https://doi.org/10.1109/RSDSW.2014.7468414.
[35] K. Oishi, E. Klavins, IET Syst. Biol. 2011, 5, 252.
[36] B. Yordanov, J. Kim, R. L. Petersen, A. Shudy, V. V. Kulkarni, A. Phillips, ACS Synth. Biol. 2014, 3, 600.
[37] R. Sawlekar, F. Montefusco, V. V. Kulkarni, D. G. Bates, IEEE Trans. NanoBiol. 2016, 15, 443.
[38] N. M. G. Paulino, M. Foo, J. Kim, D. G. Bates, IEEE Control Systems Letters 2019, 3, 805.
[39] T. Nakakuki, J. Imura, SICE J. of Cont Mes and Syst Int 2016, 9, 60.
[40] T. Nakakuki, J. Imura, Automatica 2020, 114, 1.
[41] A. Eshra, S. Shah, T. Song, J. Reif, IEEE Trans. Nanotech. 2019, 18, 252.
[42] S. Garg, S. Shah, H. Bui, T. Song, R. Mokhtar, J. Reif, Small 2018, 14, 1801470.
[43] A. J. Genot, J. Bath, A. J. Turberfield, J. Am. Chem. Soc. 2011, 133, 20080.
[44] A. Goel, M. Ibrahim, Nat. Commun. 2011, 10, 467.
[45] N. V. DelRosso, S. Hewes, L. Spector, N. D. Dunn, Angew. Chem., Int. Ed. 2017, 56, 4443.
[46] H. Asanuma, X. G. Liang, H. Nishioka, D. Matsunaga, M. Z. Liu, M. Komiyama, Nat. Protoc. 2007, 2, 203.
[47] S. Nakamura, H. Kawabata, K. Fujimoto, ChemBioChem 2016, 17, 1499.
[48] X. Song, A. Eshra, C. Dwyer, J. Reif, RSC Adv. 2017, 7, 28130.
[49] N. Shimada, K. Saito, T. Miyata, H. Saito, S. Kobayashi, A. Maruyama, Adv. Funct. Mater. 2018, 28, 1707406.
[50] J. P. Reeves, R. M. Dowben, J. Cell Physiol. 1969, 73, 49.
[51] M. I. Miglena, D. S. Dimitrov, Faraday Discuss. Chem. Soc. 1986, 81, 303.
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