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Artificial Compartments Encapsulating Enzymatic Reactions: Towards the Construction of Artificial Organelles

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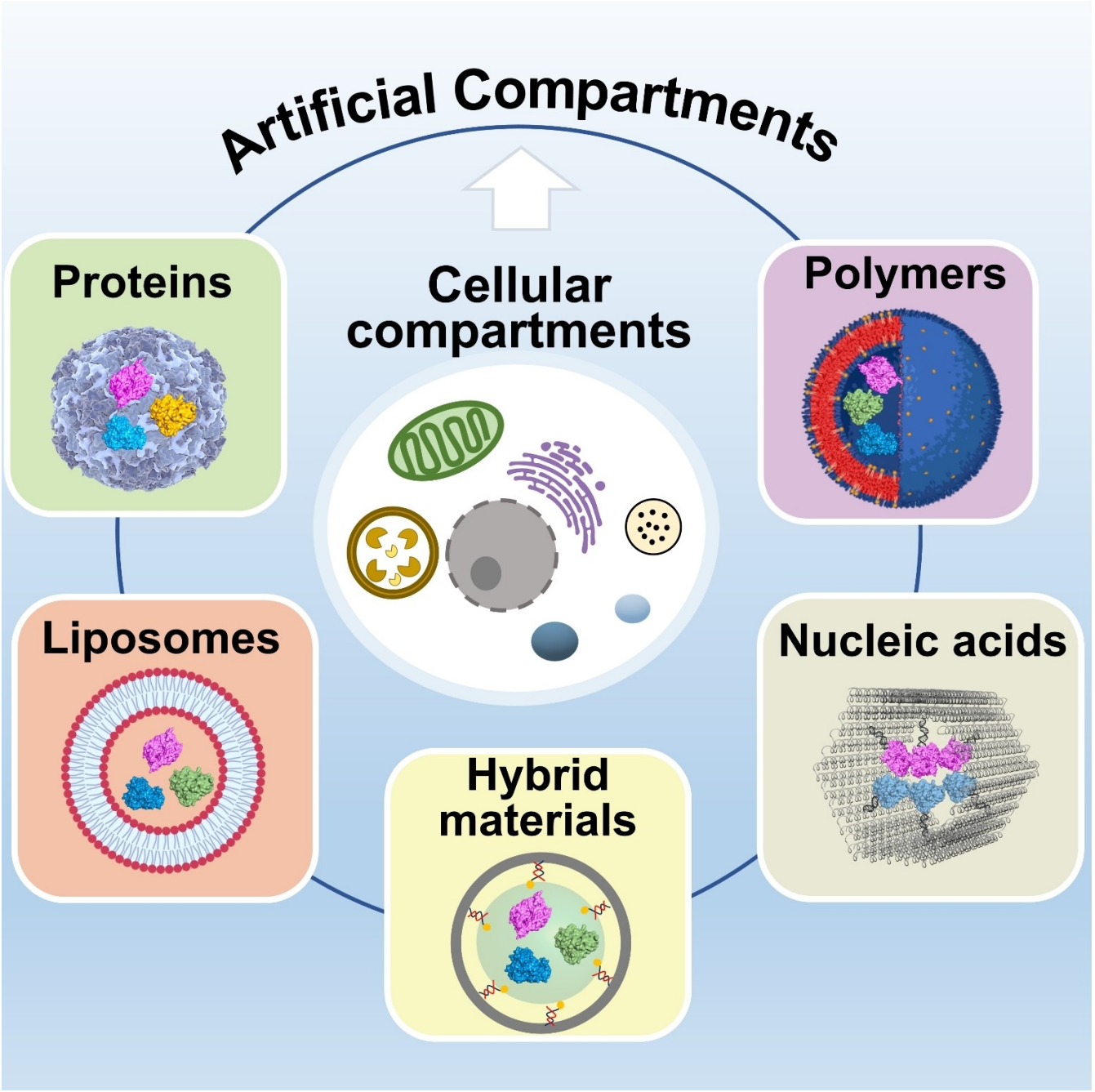

Cells have used compartmentalization to implement complex biological processes involving thousands of enzyme cascade reactions. Enzymes are spatially organized into the cellular compartments to carry out specific and efficient reactions in a spatiotemporally controlled manner. These compartments are divided into membrane-bound and membraneless organelles. Mimicking such cellular compartment systems has been a challenge for years. A variety of artificial scaffolds, including liposomes, polymersomes, proteins, nucleic acids, or hybrid materials have been used to construct artificial membrane-bound or membraneless compartments. These artificial compartments may have great potential for applications in biosynthesis, drug delivery, diagnosis and therapeutics, among others. This review first summarizes the typical examples of cellular compartments. In particular, the recent studies on cellular membraneless organelles (biomolecular condensates) are reviewed. We then summarize the recent advances in the construction of artificial compartments using engineered platforms. Finally, we provide our insights into the construction of biomimetic systems and the applications of these systems. This review article provides a timely summary of the relevant perspectives for the future development of artificial compartments, the building blocks for the construction of artificial organelles or cells.

1. Introduction

Living systems utilize well-organized enzymatic cascade reactions to implement most biochemical processes.[1] To carry out the complex metabolic pathways in the specific manner, compartmentalization has evolved as a fundamental and crucial strategy for efficient enzymatic reactions in cells.[2] The well-defined cellular compartments are formed with the following characteristics and advantages: (i) controlling the concentration of substrates and intermediates in a confined environment; (ii) protecting biological components from harsh external environment; (iii) reduction of side reactions and toxic by-products.[3] Compartmentalization of the metabolic pathways is an advantageous strategy to increase the efficiency of cascade reactions and the overall production.

Cellular compartments are categorized into membrane-bound organelles (e.g., endoplasmic reticulum, mitochondria, Golgi apparatus, and lysosomes), and membraneless organelles.[3] Cellular membranes are semi-permeable and highly functional, allowing the communications with the environment.[4] Many membraneless organelles are phase-separated biomolecular condensates of proteins and/or nucleic acids.[5] Nature's strategies for organizing a cell with membranes or condensates complement each other to provide maximum opportunities for organizing cell contents and maintaining cell functions.[6] Membrane-bound compartments can be used to provide long-term stability, while biomolecular condensates with distinct physicochemical properties could allow molecules to be concentrated and continuously exchanged with the environment without the difficulties of transport across membrane barriers.[6]

Bottom-up technology is a promising tool for assembling ordered biomimetic compartmentalized systems supported by a variety of artificial scaffolds (Figure 1).[7] Synthetic compartmentalization systems have been designed with the aim of enhancing a given biosynthetic pathway to increase the production of desired substances.[8] Liposomal self-assembled phospholipid vesicles have great potential in areas such as the development of artificial organelles with membrane functions and drug delivery.[9] Liposomes and polymersomes are two major types of soft materials used to mimic the membrane-bound compartments.[10] The artificial protein scaffold would be a promising material to mimic the membraneless compartments with colocalized enzymes and improved efficiency in various metabolic pathways.[11]

DNA origami is a technique that uses short DNA staples to fold a long single-stranded DNA into predesigned 2D (two-dimensional) or 3D DNA nanostructures with high folding yields.[12] With the superior properties of structural programmability and precise addressability, DNA scaffolds provide the unique platforms for spatial arrangement of biomolecules with control over intermolecular distance, molecular stoichiometry,

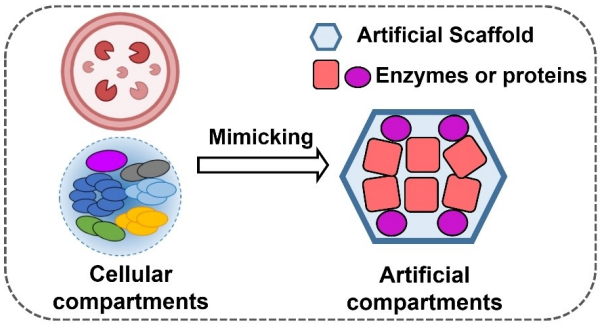

Figure 1. Use of artificial scaffolds to mimic cellular compartments. Cells have evolved compartmentalization strategies, both membrane-bound and membraneless, to implement the biological processes (left). Enzymes and/or proteins of interest are encapsulated in an artificial scaffold to physically separate them from the external environment to mimic the membrane-bound compartment (right). Assembling the enzymes and/or proteins of interest in a highly packed or dense state on an artificial compartment without the membrane mimics the membraneless compartment.

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and spatial location.[13] The DNA scaffold-based assemblies have been constructed with proteins, peptides or enzymes.[14] Various types of DNA scaffolds were constructed as the platforms or nanoreactors for the enzyme reactions.[15] The combination of DNA nanotechnology and soft materials technology enables the construction of artificial compartments with programmability and targeting functionalities.[16,17]

This review summarizes the current progress of artificial membrane-bound and membraneless compartments constructed by the artificial scaffolds of liposomes, polymersomes, proteins, nucleic acids, and scaffolded liposomes. The biomimetic systems with artificial metabolic pathways were also discussed. This review aims to provide an overview of the developments, applications and potentials of artificial compartments with enzymes as core components.

2. Compartmentalization in Cells

Cells have evolved compartmentalization strategies to implement the biological processes. Enzymes are spatially organized to perform specific sequential reactions within the compartments categorized into membrane-bound organelles and membraneless organelles. Enzyme complexes are often organized by the specific protein or membrane scaffolds to achieve the high efficiency of the reaction. Here, the typical examples of cellular membrane-bound organelles, membraneless organelles

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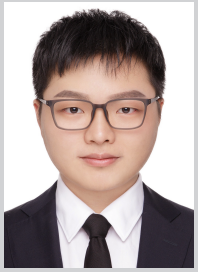

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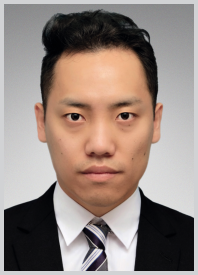

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(biomolecular condensates), and their biological processes or enzymatic systems were reviewed.

2.1. Membrane-Bound Organelles in Cells

Compartmentalization, a characteristic feature of eukaryotic cells, establishes microenvironments with distinct pH, ionic strength, or environmental polarity that promote optimal conditions for cellular biochemical reactions.[18] The membrane-bound organelles are formed with specific functions and biological processes.[19] The membrane-bound compartments such as nucleus, mitochondria, lysosome, vacuole, and peroxisome are formed with a boundary of phospholipid layers (Figure 2A).[18] These compartments share the common characteristics of sequestering components and providing a favorable microenvironment for many metabolic processes. Peroxisomes sequester reactions that involve toxic intermediates, such as hydrogen peroxide generated from oxidative reactions.[20] Lysosomes require a lower pH to facilitate degradation of internalized material. Membrane-bound proton pumps on the lysosome maintain this state.[21] Similarly, mitochondria require a large membrane surface area to efficiently generate ATP from electron gradients across their lipid bilayer.[22]

Cytochrome P450s (CYPs) are membrane-bound enzymes, mostly found in the smooth endoplasmic reticulum and some in mitochondria.[23a] In mitochondria, the inner membranes with large areas act as a scaffold for the proteins and enzymes involved in the cytochrome P450 electron transport chain (Figure 2B).[23b] Lipid membranes also have a compartmentalizing function in endocytosis, the uptake mechanism into cells. The membranes of endocytic compartments isolate the degradative enzymes inside and create the appropriate pH conditions for enzymatic activity through the membrane ion pump system.[24] Organelle membranes, composed of lipid bilayers, act as barriers between the lumens and the cytoplasm and harbor transmembrane proteins that interact with cytoskeletal elements and signaling pathways.[25] Understanding and identifying these components are critical to understanding organelle function.

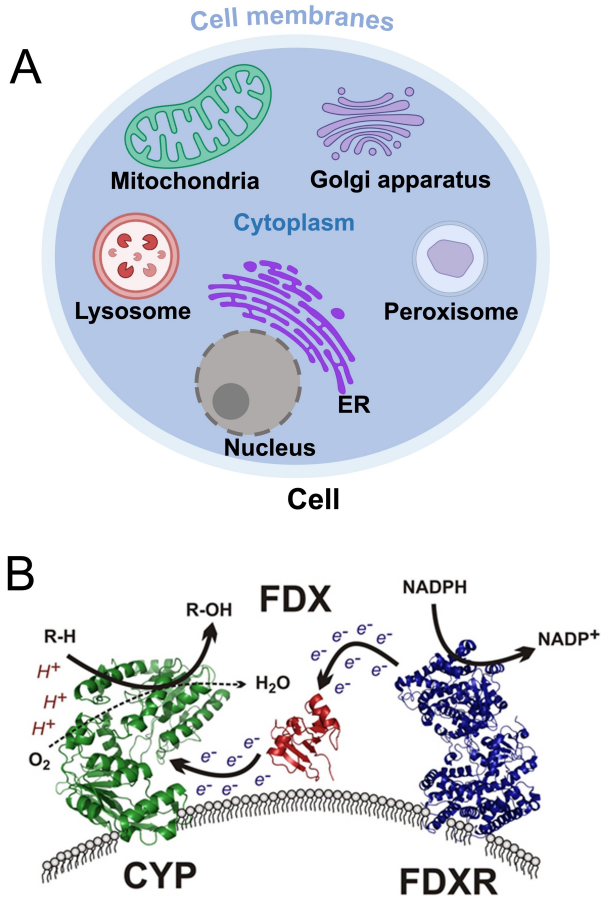

Figure 2. Membrane-bound compartments. (A) Common natural membrane-bound organelles in the cell. The typical membrane-bound organelles like mitochondria, Golgi apparatus, lysosome, nucleus, endoplasmic reticulum (ER), and peroxisomes are included. (B) The mitochondrial electron transport pathway of cytochrome P450. The membrane-associated flavin ferredoxin reductase (FDXR) accepts two electrons from NADPH to form $NADP^+$. These electrons are transferred to the iron-sulfur cluster of ferredoxins (FDX), then to the heme prosthetic group of the mitochondrial cytochrome P450 (CYP), which hydroxylates its target substrate (R–H) with protons and oxygen, resulting in the formation of hydroxylated product (R–OH) and water. Adapted from Ref. [23b], copy right (2023), with the permission from Frontiers Media.

2.2. Membraneless Organelles in Cells

Many membraneless organelles, such as stress granules, cajal body, and processing bodies (P-bodies), are phase-separated biomolecular condensates of proteins and/or nucleic acids (Figure 3A).[26] These membraneless compartments exhibit distinct physicochemical properties and would help to carry out the specific enzymatic reactions.[27]

P-bodies are cytoplasmic granules of ribonucleoprotein (RNP). They are mainly composed of translationally repressed mRNAs and proteins associated with mRNA decay (Figure 3B).[28] These granules are formed by liquid–liquid phase separation (LLPS) and are conserved in eukaryotes. However, the mechanisms of P-bodies in the repression of translation and/or in the mRNA decay are not clear.[28] The formation of biomolecular condensates is thought to modulate the local concentration of substrates or intermediates to influence metabolic pathways.[29] However, many questions about these unique natural systems remain unanswered. How are enzyme kinetics regulated in the condensate? How do protein-protein interactions contribute to the modes of regulation?[30]

Transcriptional condensates are membraneless compartments in nucleus. Transcription factors (TFs) play a central role in the gene transcription. TFs recruit coactivators, RNA polymerase II (pol II), chromatin remodelers, and the Mediator complex. Enhancers are regions of DNA that recruit TFs. TFs and coactivators commonly contain intrinsically disorder regions (IDRs) that can form the condensate. The transition of initial condensate to the elongation condensate is mediated by the phosphorylation of RNA Pol II (Figure 3C).[31]

Metabolons, which are intracellular metabolic enzyme complexes, efficiently promote and control metabolism through

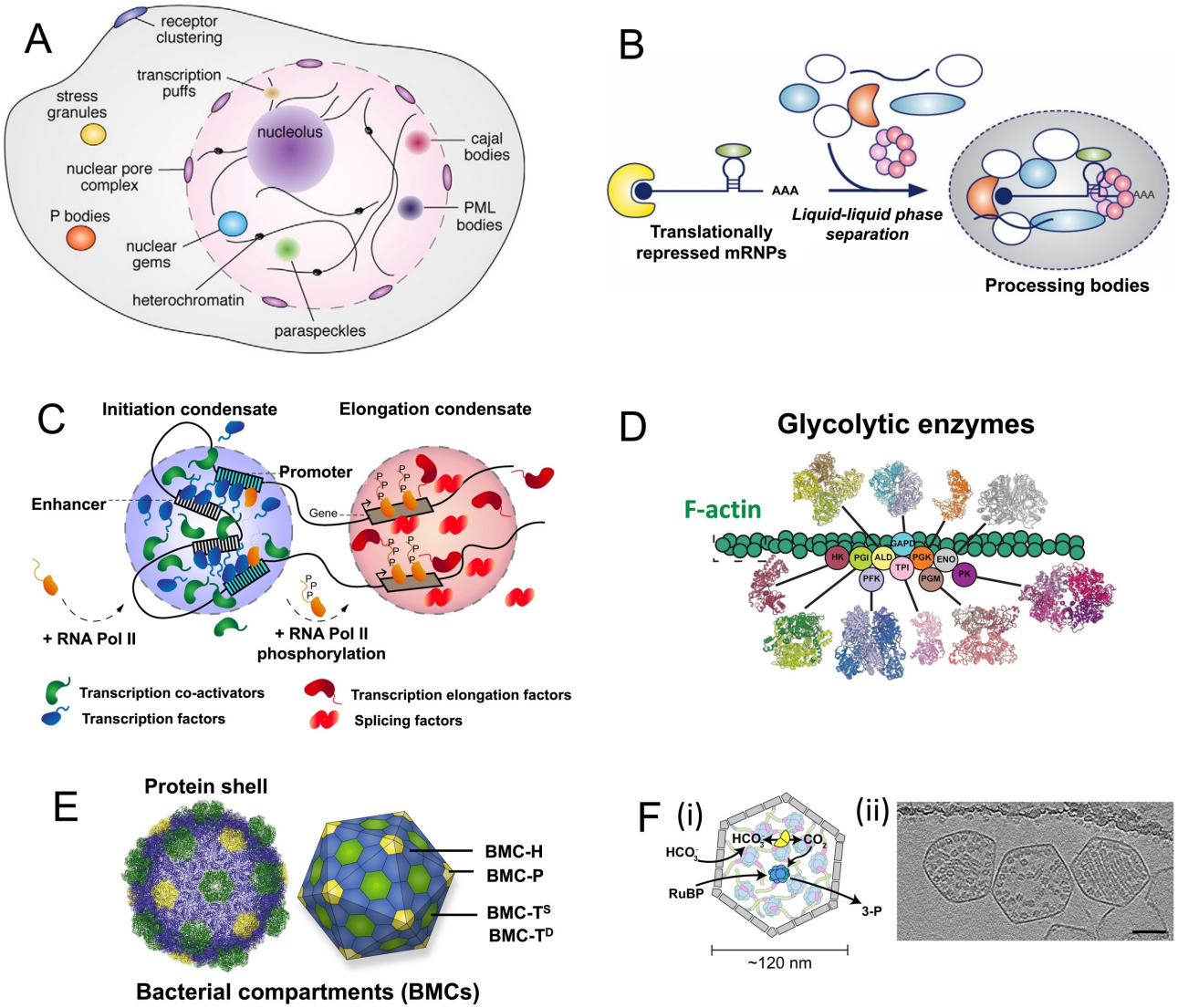

Figure 3. Natural membraneless compartments. (A) LLPS supports the biogenesis of a wide variety of membraneless organelles in cells; adapted from Ref. [26] copy right (2019), with the permission from Elsevier. (B) LLPS drives the formation of processing bodies; adapted from Ref. [28] copy right (2018), with the permission from American Chemical Society. C) The role of LLPS in gene transcription; adapted from Ref. [31] copy right (2020), with the permission from Springer Nature. (D) A hypothetical glycolytic metabolon; adapted from Ref. [34] copy right (2018), with the permission from Portland Press. (E) The structure of a BMC polyhedral protein shell from *Haliangium ochraceum* (left). BMC shells are formed by three groups of shell proteins: BMC-H (Pfam00936) to form hexagonal hexamers (blue); BMC-P (Pfam03319) to form pentagonal pentamers, and BMC-T (a tandem fusion of the Pfam00936) with two subtypes: trimers (BMC-T^S), and stacked dimers of trimers (BMC-T^D). A scheme of the icosahedral symmetry (right, BMC-H in blue; BMC-T^S and BMC-T^D in green; BMC-P in yellow); adapted from Ref. [38] copy right (2019), with the permission from the Springer Nature. (F) (i) The scheme of carbon fixation reaction in a carboxyso (ii) 4.4 nm thick orthosection showing three differently packed carboxysomes. From left to right: dense, sparse, ordered. Scale bar 50 nm; adapted from Ref. [43b] copy right (2022), with the permission from Springer Nature.

dynamic complex formation and dissociation of enzyme groups involved in metabolic pathways.[32] Metabolic reactions are thought to be controlled by spatiotemporal complex formation and dissociation of enzymes, but because interactions between proteins and enzymes involved in metabolism are transient, the composition and enzymatic reaction characteristics are still unclear.[33] An interesting example is the long-hypothesized glycolytic metabolon,[34] which is a series of enzymatic reactions catalyzed by ten enzymes that convert one molecule of glucose into two molecules of pyruvate. Association of glycolytic enzymes with F-actin increases the activity of each enzyme (Figure 3D), although the association patterns and protein-protein interactions of glycolytic enzymes are still unclear.[35] Interestingly, under hypoxic stress conditions, glycolytic enzymes form unique cytoplasmic granules called glycolytic (G) bodies driving by LLPS. RNA plays a role as scaffold in the formation of G-body.[36] Yeast G-body formation correlates with increased glucose consumption and cell survival,[36] but the mechanism of G-body formation and its effect on enzymatic reactions are unknown.

The metabolons are characterized by the dynamic assembly and disassembly of the enzyme complex. It has been proposed that the enzymes involved in *de novo* purine biosynthesis have been proposed to form a purinosome condensate in the LLPS

state.[37] This provides new insight into the mechanisms by which LLPS drives metabolon formation in human cells. It is thought that the transient nature of the protein assemblies allows intermediates to be passed from the active site of one enzyme to the next enzyme in the metabolic pathway, thereby improving the flux rate of enzyme cascade reactions.

In prokaryotic cells, there are organelles formed with permeable protein shells. There membranless organelles are named as bacterial microcompartments (BMCs).[38] BMC shells are formed by three groups of shell proteins: BMC-H (Pfam00936) to form hexagonal hexamers; BMC-P (Pfam03319) to form pentagonal pentamers, and BMC-T (a tandem fusion of the Pfam00936) with two subtypes: trimers (BMC-T^S), and stacked dimers of trimers (BMC-T^D) (Figure 3E).[38] BMCs encapsulated with enzymes that carry out the biological processes. The best studied bacterial microcompartment, the carboxysome, plays a key role in carbon fixation in autotrophic organisms such as cyanobacteria.[39] The carbon fixation reactions are carried out by two types of enzymes, carbonic anhydrase (CA) and ribulose 1, 5-bisphosphate carboxylase/oxygenase (RuBisCO), which are packed into the proteinaceous shell of the carboxysome and carry out the efficient carbon fixation reactions.[40] RuBisCO is one of the most abundant enzymes in the world. This enzyme catalyzes two directions of metabolic pathways with ribulose 1, 5-biphosphate (RuBP), carboxylation reaction using CO_2 as substrate, and oxygenation using O_2 as substrate.[41] RuBisCO show the disadvantage of low catalytic number for carbon fixation.[42] The reaction efficiency of RuBisCO would be improved by packing it with CA in carboxysome.[39] In this sequential reaction, CA converts bicarbonate HCO_3^- to CO_2, which accumulates inside the protein compartment, increasing the efficiency of the RuBisCO carbon fixation reaction and reducing side reactions with oxygen.[40] Recent studies have hypothesized that the RuBisCO molecules to form the condensate through the LLPS, suggesting the role of the biogenesis of the carboxysome.[43] It remains to be elucidated how the proteinaceous scaffold of the carboxysome functions in RuBisCO condensation. Metskas et al. used cryo-electron tomography to image α-carboxysomes. The arrangement of RuBisCO inside the carboxysome was determined. It was found that RuBisCO forms a lattice inside α-carboxysomes (Figure 3F).[43b]

2.3. Typical Structural and Functional Characteristics of Cellular Organelles

Membrane-bound organelles provide a membrane boundary to encapsulate, protect and sequester enzymes, proteins, nucleic acids, and small molecules for the implementation of efficient metabolic pathways and specific biological processes. The membrane, decorated with protein tranporters, acts as a selective channel for targeted molecules. Membraneless organelles are characterized by the biomolecular condensate of proteins and/or nucleic acids. While there are no physical boundaries as in lipid membranes, the condensate state of biomolecules provides a local microenvironment to enrich the local concentrations of substrates and intermediates for controlling metabolic pathways. We have summarized the typical structural and functional characteristics of membrane-bound and membraneless organelles as shown in Table 1 and Table 2. These characteristics are important factors for the construction of artificial compartments, organelles or cells.

Table 1. A summary on the typical membrane-bounded organelles.

Membrane-bound organelles	Structures	Locations	Functions	References
Cell membrane	Structure of bilayer phospholipids with associated proteins	In various types of organisms, including plants and animals	Involved in fixing the cell structures, providing a border to protect and organize the inner organelles, and acting as a selectively permeable membrane	Ref. [44]
Lysosomes	Single-layered membrane-bound organelles with the digestive enzymes inside the compartments.	In nearly all types of eukaryotic cells	Involved in digesting, removing wastes, and digesting damaged cells	Ref. [21]
Mitochondria	Oval-shaped, double-layered membrane-bound organelles	In the cytoplasm of eukaryotic cells	Involved in the cellular respiration and storage of energy by the formation of ATP	Ref. [22]
Endoplasmic reticulum	A network of membranous tubules	In the cytoplasm of eukaryotic cells	Involved in forming the skeletal framework of the cell and producing lipids and proteins.	Ref. [45]
Golgi apparatus	Membrane-bound, sac-like structures	In the cytoplasm of all eukaryotic cells, close to the nucleus	Involved in the secretion and intracellular transport.	Ref. [46]
Nucleus	A double-layered membrane-bound organelle found	In eukaryotic cells	Involved in controlling gene expression, storing DNA, and making ribosomes	Ref. [47]
Peroxisomes	Single membrane-bound organelles containing degestive enzymes and oxidative enzymes	In eukaryotic cells	Involved in various oxidative processes and lipid metabolism	Ref. [20]

Table 2. A summary on the typical membraneless organelles.

Membraneless organelles	Structures	Locations	Functions	References
P-body	Cytoplasmic ribonucleoprotein (RNP) granules comprised primarily of mRNAs in complex with proteins	In the cytoplasm of eukaryotic cells	Involved in the regulation of gene transcription and acting against stresses	Ref. [28]
Stress granule	Biomolecular condensates in the cytosol composed of proteins and RNAs	In the cytoplasm of eukaryotic cells	Involved in the regulation of translation and response to different stress stimuli	Ref. [48]
G-body	Membraneless granules (enzyme complexes) containing glycolytic enzymes	In yeast and human hepatocarcinoma cells.	Involved in the regulation of glycolysis	Ref. [35]
Cajal body	Nucleoplasmic structures containing coiled threads of the marker protein, and coilin.	In the nucleus of highly proliferative cells like cancer cells or neurons	Involved in the regulation of metabolic flux	Ref. [49]
Purinosomes	Dynamic metabolic complex es composed of enzymes	In higher eukaryotes cells	Involved in the de novo purine biosynthesis	Ref. [37]
Carboxysomes	Bacterial microcompartments (BMCs) consisting of polyhedral protein shells	In all cyanobacteria and parts of proteobacteria.	Involved in the concentration of carbon dioxdie	Ref. [40]

3. Artificial Compartments: Mimicking Cellular Compartments

To reconstruct the natural compartments, there are key requirements and factors for artificial compartments to resemble the functional and structural properties of natural organelles: (i) a boundary provided by membrane structure or condensate; (ii) the organization of biomolecules in the compartments to control the intermolecular distance and location of molecules; (iii) the loading capacity of compartments; (iv) the flexibility of soft materials to mimic the structure of natural compartments; (v) strategies for surface modification or functionalization of artificial scaffolds.

Compartments protect the catalysts from other competing molecules that may reduce the efficiency and/or selectivity of the reaction. At the same time, the fragile nature of enzymes requires their preservation for prolonged use, which is achieved by immobilization in/on synthetic mimics.[50] Various approaches and materials with application in materials science, chemistry, electronics, and medicine from the quest to mimic natural structures and functions for innovative materials design.[51] However, the variability among enzymes and their diverse requirements often pose challenges in adopting a general approach to enzyme compartmentalization. To mimic the action in the cell, many strategies have been developed for the assembly of single and multiple enzymes in and on vesicles[52] to create artificial compartments using a variety of materials. The artificial compartments have played a prominent role in encapsulating various cargoes, including therapeutics for drug delivery applications and enzymes to create nanoreactors.[53] Typical examples of artificial compartments constructed by using polymersomes, protein scaffolds, nucleic acid-based scaffolds, and hybrid materials, as well as recent advances, are described below.

3.1. Artificial Membrane-Bound Compartments Based on Liposomes or Polymersomes

The natural cell membrane consists of a phospholipid bilayer. A variety of proteins and small molecules, such as cholesterol and carbohydrates, are associated with the bilayer.[54] Liposomes and polymers are two major types of soft materials used to construct artificial compartments with membrane functions. Lipids and amphiphilic polymers have similar physical properties, and the surface of these materials can be modified.[55]

A liposome system can encapsulate several enzymatic reactions. A fatty acid synthesis and a cell-free gene expression system for the synthesis of acyltransferases are combined to provide a cell-free phospholipid synthesis system within phospholipid membrane vesicles.[56] Sequential reactions of the fatty acid synthesis and acyltransferase provide phosphatidic acids through the cell-free system, which would provide a platform for self-reproducing artificial cells by growing the membrane.

Enzyme reactions in the liposome can also be coupled with the target molecular recognition event of aptamers or antibodies on the surface. Liposomes encapsulating amyloglucosidease or invertase are modified on the surface with aptamers or antibodies against thrombin or C-reactive protein (CRP).[57] The translation of molecular recognition events on the surface results in the production of a large amount of glucose with the encapsulated enzyme. The resulting glucose is detected by using personal glucose meters (PGMs), leading to sensitive protein detection of disease biomarkers such as thrombin or C-reactive protein (CRP).

The inner aqueous compartment of liposomes also provides an efficient reaction environment for photoreactions. The conversion of nicotinamide adenine dinucleotide (NADH) to its oxidized form (NAD^+) by the photosensitized reaction within the compartment was accelerated by one order of magnitude compared to classical homogeneous reaction conditions[58] (Figure 4A). Such a system will be useful for constructing

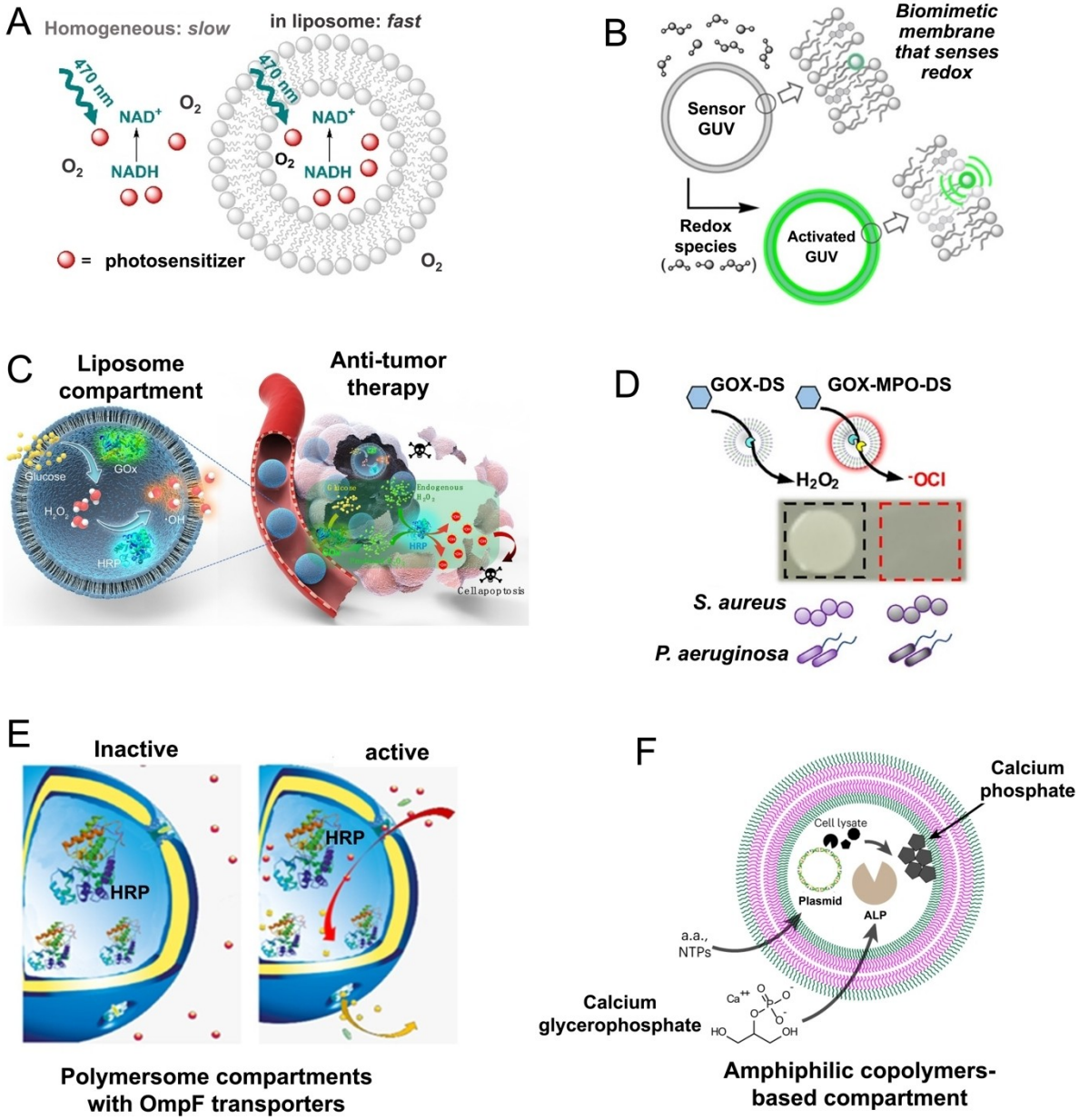

Figure 4. Artificial compartments based on liposomes or polymersomes. (A) Photosensitizer and substrate encapsulated in the inner aqueous interior of liposomes; adapted from Ref. [58], copy right (2022), with the permission from John Wiley & Sons. (B) Scheme of enzyme-encapsulated liposome-conjugated immunosorbent assay with a sandwich structure of bead/protein/liposome; adapted from Ref. [59], copy right (2024), with the permission from the American Chemical Society. (C) An illustration shows a liposome-encapsulated enzymatic nanoreactor (left) that mimics the tandem reactions of glucose oxidase and horseradish peroxidase to generate hydroxy radicals for antitumor therapy (right); adapted from Ref. [60], copy right (2023), with the permission from John Wiley & Sons. (D) Assembly of GOX-MPO-DS (glucose oxidase and myeloperoixase encapsulated in dendrimersomes) and the proposed bacteria-mediated switch-on mechanism; adapted from Ref. [61], copy right (2020), with the permission from the American Chemical Society. (E) Stimulus-responsive catalytic polymersome nanocompartmen. The redox stimuli-responsive bacterial transporter OmpF was embedded on the polymersome encapsulating horseradish peroxidase to regulate its activity with the external glutathione levels; adapted from Ref. [62], copy right (2018), with the permission from Nature Portfolio. (F) Diagram of GUV containing a plasmid and bacterial translation machinery for the production of ALP and subsequent ALP-catalyzed biomineralization of calcium phosphate; adapted from Ref. [63], copy right (2024), with the permission from Springer Nature.

artificial compartments coupled with different photochemical reactions. Various redox reactions have also been encapsulated in the artificial compartments to construct sensors and bio-reactors. Sensors for environmental redox cues have been constructed by using giant unilamellar vesicles (GUVs). with biomimetic lipid membranes[59] (Figure 4B). In this system, phospholipids modified with a fluorophore that responds to specific hydrogen sulfide (reductive state) or hydrogen peroxide (oxidative state) were incorporated into the membrane.

The redox enzymatic cascade reactions are encapsulated in the liposomes to construct the nanoreactors for possible use in inhibiting tumor growth in vivo.[60] Because of their ease of handling, glucose oxidase (GOx) and horseradish peroxidase (HRP) are often used to construct a simple model system of an

enzymatic cascade reactions. This system consumes glucose and oxygen, the tumor nutrients, produces toxic hydroxyl radicals (•OH). When these cascade enzymes are encapsulated in the liposomes, the local concentration of the intermediate product hydrogen peroxide (H_2O_2) for HRP would increase and the internal pH would decrease due to the generation of gluconic acid[60] (Figure 4C). These factors would accelerate the second step reaction of HRP to improve the efficiency of the cascade reaction of hydroxyl radical production that would result in tumor growth. The enzymes for the redox reactions were also encapsulated in the polymer-based compartment. A nanoreactor producing hypochlorite (ClO^-) upon glucose addition was constructed by encapsulating GOx and myeloperoxidase (MPO) in dendrimersome.[61] GOx and MPO were encapsulated in the central core of the dendrimersome due to its size-dependent permeability, allowing substrate/product passage. This nanoreactor was applied to the strategy of local production of cytotoxic ClO^- to exert potent broad-spectrum bactericidal activity against multidrug-resistant pathogens, suggesting the potential use of the nanoreactor for implant surface modification to prevent bacterial growth[61] (Figure 4D). Artificial compartments encapsulating redox enzymes are constructed by the polymersomes to detect and counteract superoxide radicals, a major contributor to various diseases (Figure 4E). By embedding a sequential enzymatic cascade in the vesicles and reconstituting channel proteins in their membranes, the system effectively converted radicals to harmless compounds, demonstrating its potential as an artificial organelle for enzyme therapy.[62]

In an effort to develop an artificial cell, self-assembling polymersomes are used as a compartment for protein expression (Figure 4F). Various types of micelles, including polymersomes and giant unilamellar vesicles, were formed with amphiphilic copolymers produced by myoglobin-induced polymerization. During the self-assembly of amphiphilic polymers, enzymes, nano- and microparticles, plasmids, and cell lysate were encapsulated into them, resulting in the formation of artificial compartments. The artificial compartments showed the characteristics of artificial cells, which carry out enzymatic reactions, biomineralization, and protein expression in the presence of amino acids.[63]

To increase the overall yield of a multistep reaction in the compartment, the incorporation of selective transporter or channel proteins is an effective approach. Enzymes associated with the three-step reaction to synthesize CMP-N-acetylneuraminic acid (CMP-Neu5Ac) were separately embedded in polymersomes. Because CTP, the substrate of the third reaction by CMP-sialic acid synthetase (CSS), strongly inhibits the first enzyme N-acyl-D-glucosamine 2-epimerase (AGE), N-acetylneuraminate lyase (NAL) and (CSS) were embedded in the polymersome membranes, and AGE with the allosteric activator ATP was encapsulated in the core of the polymersomes. A mutant of the channel protein OmpF was incorporated into the membrane to allow selective transport of substrates across the membrane and exclusion of CTP transport into the core of polymersomes.[64]

As shown above, positional control of enzymes also provides an effective approach to increase the efficiency of enzymatic cascade reactions. To construct a three-enzyme cascade with positional control of associating enzymes, porous polymersomes were functionalized with enzymes (GOx, CalB, HRP) at different positions (lumen, membrane, surface) by click chemistry and diazo transfer, which resulted in high efficiency of enzyme immobilization. While the model three-enzyme cascade utilized the common enzymes GOx and HRP, these enzyme-decorated polymersomes acted as nanoreactors and demonstrated a cascade reaction with HRP catalyzing the fastest step in the conversion of glucose acetate to $ABTS^{+\cdot}$.[65]

When artificial compartments are designed to serve as nanoreactors, the permeability of their membrane is a critical property.[66] This property is important to facilitate the passage of substrates and products for *in situ* reactions. Modification of the surface of liposomes by using synthetic polymers, such as PEG, PVP, and the components of biomembranes, such as cholesterol and sphingomyelin, is a common method to manipulate membrane properties and permeability.[67] However, the lipids used in vesicle formation have specific properties by nature and additional modifications require extensive procedures that can alter their phase transition properties.[68] There are several approaches to achieve membrane permeability. These include embedding membrane proteins and biopore formers,[69] and the use of stimuli-responsive building blocks to regulate and control permeability.[70]

The integration of transmembrane proteins into artificial compartments has attracted considerable interest in the development of selectively permeable structures.[71] The incorporation of membrane channel proteins while maintaining the vesicular structure is challenging due to variations in the length of the polymer chains that cause size of the hydrophobic domains of the polymer to mismatch with that of the protein.[72] The use of triblock polymers provides a suitable membrane for the embedding the membrane channel protein OmpF in polymersomes, a common strategy to provide permeability to the compartment. Membrane formation by the conformable hydrophobic block facilitated protein insertion with minimal energy loss.[73] This copolymer has been used to incorporate a variety of proteins, including ionophores, pore-forming proteins, receptors, and pump proteins. Control of protein orientation within these polymersomes remains to be established for directional transport, an area still under investigation.[74]

Current studies mostly use symmetric copolymers for protein insertion, but ongoing research, such as the incorporation of redox-responsive channels, shows promise for controlling molecular flow through these copolymer membranes.[75] A biomimetic strategy was introduced to create artificial organelles (AOs) by combining biomolecules with synthetic compartments, using protein gates inserted into polymersomes accommodating HRP. These AOs, which exhibited redox-responsive control of molecular flux across their membranes, maintained their structure and responded to intracellular glutathione levels in vitro. Functionality in zebrafish embryos demonstrated the potential of AOs as cellular implants in living organisms, which

could lead to the development of patient-focused protein therapy.[75] A method to construct 2D crystals and nanosheets of pore-forming membrane proteins within block copolymers would lead to the development of biomimetic membrane protein embedding- membranes by block copolymers.[76] These membranes exhibit exceptional molecular selectivity and water permeability, surpassing commercial membranes in the subnanometer to few nanometer range. This suggests their potential as high-performance alternatives for separations.

Cells respond dynamically to external stimuli, triggering a variety of biochemical reactions. To mimic such responses, the polymersomes or liposomes were prepared with stimuli-responsive blocks. The resulting compartments responded to changes in environmental factors such as temperature, light, solvent, and pH. These responses affected the permeability or phase separation dynamics of the polymersomes or liposomes to facilitate the control of enzymatic reactions within the compartments and in drug delivery. There are other excellent and typical examples of using lipid membrane to construct the artificial compartments.[77]

3.2. Artificial Compartments Based on Protein Shells

One promising strategy to increase the productivity of engineered eukaryotes is the compartmentalization of proteins into organelles.[78] Conjugation of enzymes of interest to the shell or capsid proteins of natural compartments, the protein cages and virus-like particles, provides a useful strategy for packaging them inside the compartments.

A self-assembling protein has been used to construct synthetic non-endogenous organelles in yeast.[79] A prokaryotic family of self-assembling proteins, encapsulins, has been shown to self-assemble into nanocompartments. By fusing a short targeting peptide of encapsulin, proteins of interest can be selectively packaged into the encapsulin compartments. In vivo proteolytic degradation can be prevented by encapsulating proteins within the encapsulin compartments, while co-localization within the compartment enhances the interaction between the split protein components[79] (Figure 5A). Substrate turnover was observed for an encapsulated yeast enzyme, suggesting that encapsulin compartments promote enzymatic catalysis. Thus, encapsulin compartments provide a modular platform that is orthogonal to existing organelles for the design

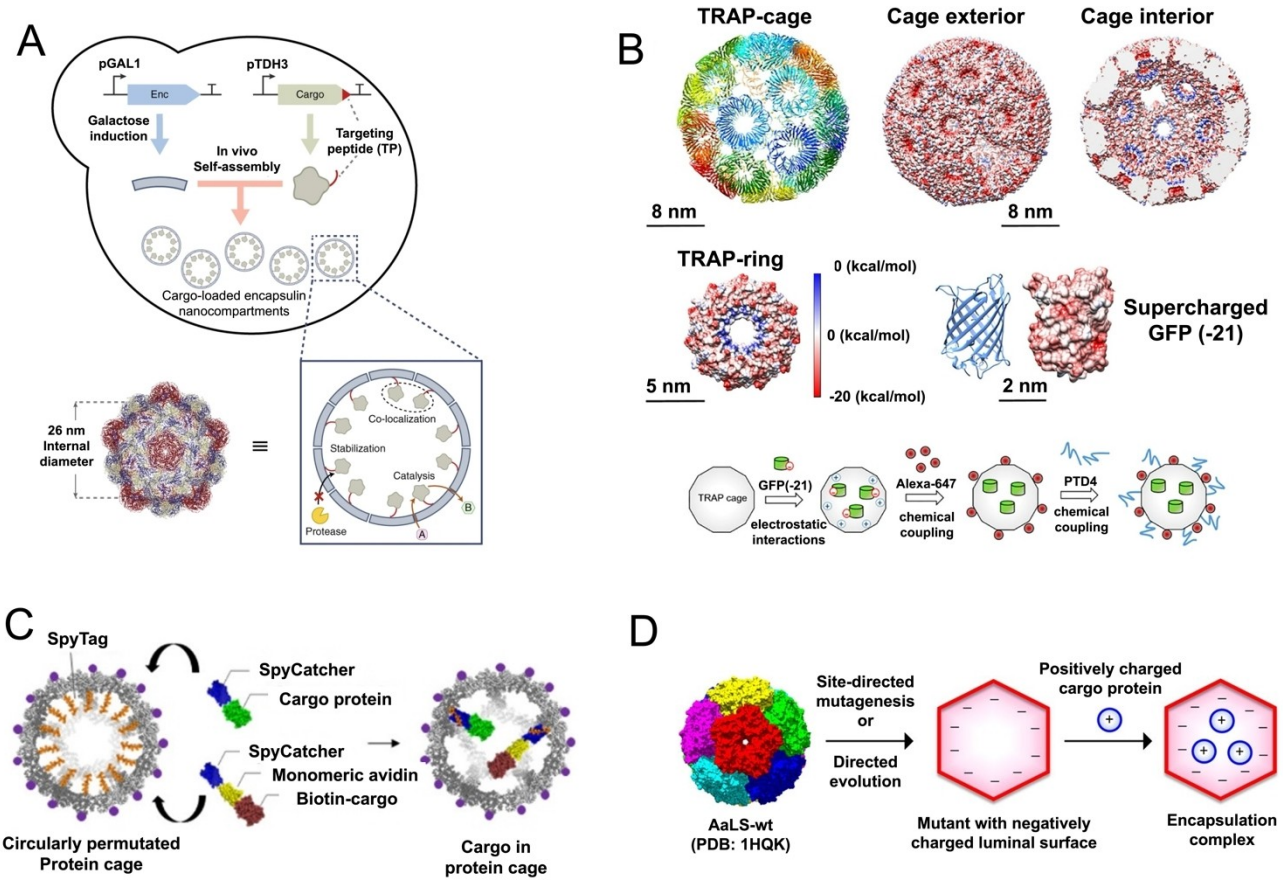

Figure 5. Artificial compartments based on protein cages. (A) Expression, assembly and cargo loading of encapsulins in yeast; adapted from Ref. [79], copy right (2024), with the permission from Springer Nature. (B) Schematic of the TRAP cage with each TRAP ring shown in a different color (top, left). Surface representation of the TRAP cage exterior (top, center) and interior (top, right), and the TRAP ring (middle, left). Encapsulation of negatively charged GFP(−21) (middle, left) in the TRAP cage (bottom) and external modifications with Alexa-647 dye and PTD4 peptide in the TRAP protein cage; adapted from Ref. [83], copy right (2021), with the permission from the American Chemical Society. (C) Schematic of the SpyTag-fused, circularly permutated mi3 cage for selective cargo encapsulation; adapted from Ref. [84], copy right (2022), with the permission from the American Chemical Society. (D) Design of the AaLS encapsulation system; adapted from Ref. [85], copy right (2017), with the permission from Springer Nature.

of synthetic compartments in eukaryotes. Similarly, the SpyTag-SpyCatcher system[80] was introduced to conjugate the coat proteins of the bacterial ethanolamine utilization microcompartment (EutM) to the enzymes of interest. By fusing the SpyCatcher protein to EutM and the SpyTag to alcohol dehydrogenase and amine dehydrogenase, the resulting EutM-enzyme conjugates formed protein fibrils. While the structure of the fibril protein scaffold and the spatial location of the co-immobilized enzymes remain to be elucidated, the dual enzymes modified protein fibrils catalyzed the enantioselective formation of chiral amines from the corresponding alcohols.[81]

The association of the enzymes of interest with the components of the protein cage can also be achieved by simple electrostatic interactions. RuBisCO and carbonic anhydrase (CA) were co-encapsulated in the protein cage to construct an artificial carboxysome.[82] RuBisCO and CA were converted to positively charged derivatives by fusing the hyper-positively charged (+36) variants of green and yellow fluorescent proteins. These positively charged constructs were encapsulated by the AaLS-13 capsids of lumazine synthase with a negatively charged luminal surface. Co-encapsulation of CA showed no significant kinetic effect on the reaction of RuBisCO under ambient or oxygen-saturated conditions. Nevertheless, this simple labeling system would be useful in the construction of artificial protein-enveloped compartments.

In addition to the above strategies of using the natural protein cages and virus-like particles, the use of artificial protein cages has been investigated to deliver proteins in the active form to cells.[83] The ring-shaped trp RNA-binding attenuation protein (TRAP) forms an artificial protein cage by bridging gold ions with an unusual geometry. The TRAP cage exhibits extremely high stability while disassembling upon addition of reducing agents, including the cellular reducing agent glutathione (Figure 5B). The TRAP cage was shown to be filled with a negatively hypercharged GFP protein. The TRAP cage was further modified on the surface with a TAT-based cell-penetrating peptide to promote its cell entry. The modified TRAP cage and the encapsulated GFP were successfully delivered intracellularly to the cell. Such artificial protein cages, whose constituent proteins do not naturally form a cage, have the potential to expand the application of protein-based compartments.

A porous protein cage was computationally designed based on the structure of the mi3 protein cage.[84] The RGD tripeptide was attached to the mi3 protein to project to the outer surface of the cage to target the cancer cell. The SpyTag was fused to the mi3 protein to protrude into the inner cavity of the cage to bind with SpyCatcher. Different proteins fused with SpyCatcher were encapsulated in the nanocage (Figure 5C). The structure of the protein cage and the encapsulated proteins was confirmed by cryo-electron microscopy (cryo-EM). The SpyTag protruding into the inner cavity was reacted with the SpyCatcher-modified monomeric avidin. This enabled the encapsulation of various biotin-modified molecules, such as the oligonucleotides and the anticancer drug. The engineered mi3 protein cage provides an effective cellular delivery strategy for various biomolecules.

Engineering the inner surface of the protein cage facilitated the encapsulation of cargo through the electrostatic interactions. The pentameric structure of lumazine synthase assembles into a 1 MDa dodecahedron of 12 subunits. By systematically increasing the negative charges on its luminal surface, the mutants were assembled into 3 and 6 MDa protein cages with 180 and 360 subunits, respectively, as characterized by cryo-electron microscopy structures[85] (Figure 5D). In contrast to the wild type, these enlarged cages adopted tetrahedral and icosahedral symmetric structures and formed pores in the shell, allowing encapsulation of positively charged cargoes. This example showed that the hypercharged modification not only allows the encapsulation of the complementary charged cargoes, but also changes the overall structures of the protein cages.

In addition to the above examples, self-assembling protein cages have been developed for use as nanoreactors to mimic the natural systems that would be useful in materials science and nanobiotechnology.[86]

3.3. Protein Assemblies on the DNA Nanostructures: Towards Membraneless Compartments

In 1982, Nadrian Seeman conceived the idea of using nucleic acids as the basic units for constructing functional structures and materials. This pioneering concept laid the foundation for DNA nanotechnology.[87] Since then, DNA nanotechnology has made remarkable progress, evolving from the assembly of small DNA structures to the construction of giant DNA superstructures. This evolution has moved from static configurations to intricate dynamic structures that respond to environmental cues. DNA origami is one of the nucleic acid self-assembly techniques that have been developed to create pre-designed DNA assemblies and hybrid nanomaterials in one, two, and three dimensions (1D, 2D, and 3D).[88]

DNA origami serves as a powerful tool for rapid prototyping and precise manipulation of molecular geometry, mechanics, and dynamics. Through targeted chemical modifications at specific sites, DNA origami structures provide a versatile engineering platform that allows programmable manipulation of nanoscale entities ranging from small molecular dyes to large protein complexes and from inorganic nanowires to 3D liposomes. This versatile platform has broad applicability and offers immense potential for engineering systems whose collective behavior depends on molecular organization.[89]

DNA origami provides a template for the construction of dense protein assemblies, which is difficult to realize in free solution especially in the case of intrinsically disordered proteins. The possible function of an intrinsically disordered protein was investigated by constructing a model the nuclear pore complex.[90] An intrinsically disordered protein (FG-Nups) rich in Phe-Gly (FG)-repeat domains of different types, such as FxFG and GLFG, forms a dense assembly at the nuclear pore, which is proposed to play a role in the permeability properties and the selective molecular transport to the nucleus. To mimic such dense assembly of FG-Nups, a 3D DNA origami ring was

used as a template to place different numbers and/or spatial positioning of NSP1, the yeast FG-Nups, at defined positions within the 34 nm wide ring (Figure 6A). NSP1 formed the dense aggregates within the ring template as revealed by cryo-EM imaging. Compared to the assembly of the hydrophilic mutant of NSP1, the parent NSP1 formed denser aggregates with

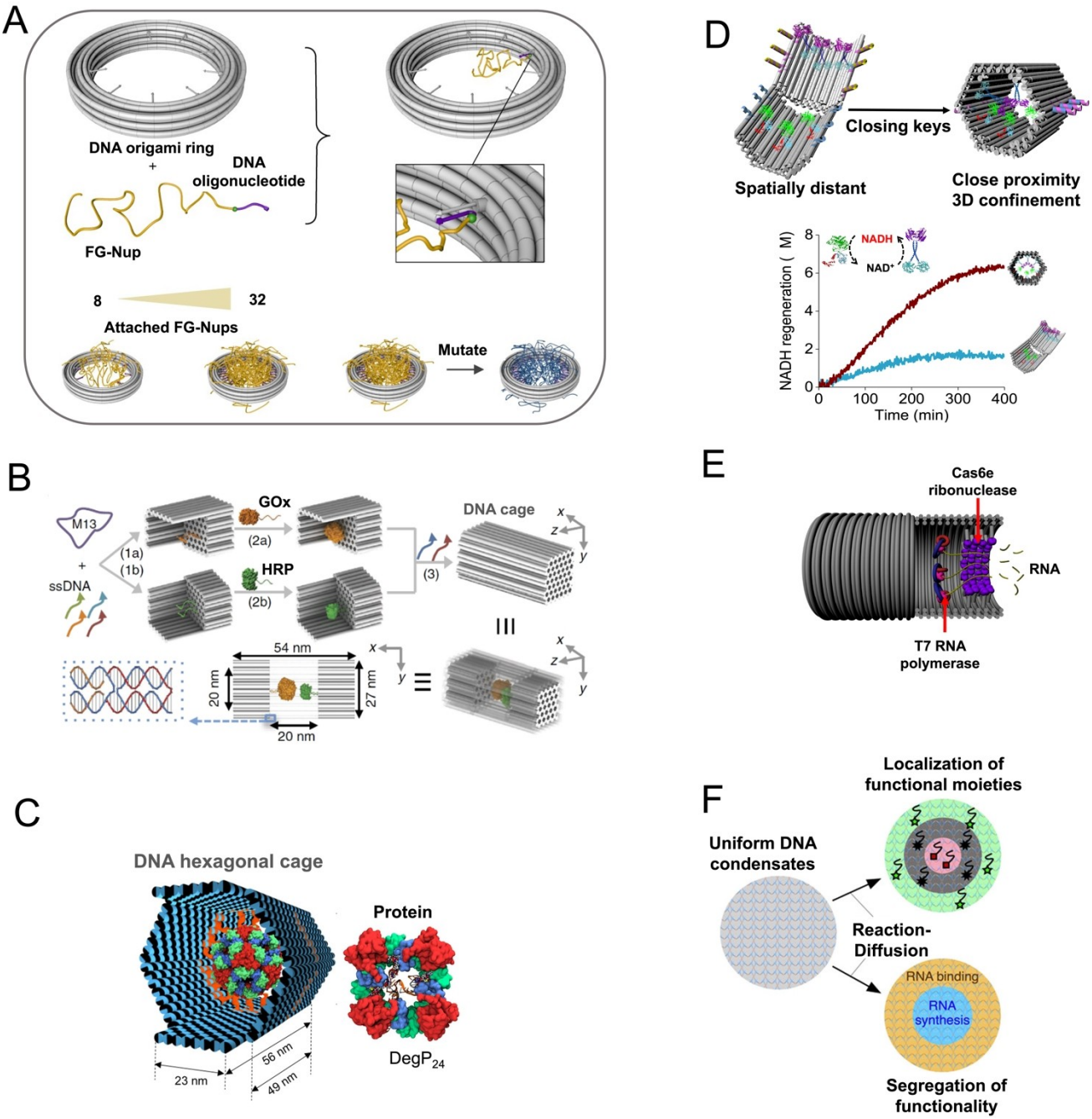

Figure 6. Artificial compartment based on DNA scaffold or DNA condensates. (A) DNA origami ring with attached nucleoporins (FG-Nups). DNA ring with one DNA modified NSP1 protein (a yeast FG-Nups) (top) and DNA ring with different number of FG-Nups and mutated FG-Nups; adapted from Ref. [90], copy right (2018), with the permission from Springer Nature. (B) DNA nanocage-encapsulated enzymes for 2-step reaction; adapted from Ref. [96], copy right (2016), with the permission from Springer Nature. (C) Encapsulation of the specific oligomer of the protein DegP in DNA origami scaffold using host-gest interaction (left) and an illustration of the specific 24-mer of protein guest DegP (right). the monomer of DegP consists of three domains, a protease (red), a PDZ1 (green) and a PDZ2 (blue) domain; adapted from Ref. [97], copy right (2017), with the permission from Springer Nature. (D) Schematic representation of a 2-step enzyme cascade reaction (XR-XDH) on a dynamically shape-transformable DNA scaffold; adapted from Ref. [106], copy right (2023), with the permission from John Wiley & Sons. (E) Schematic of RNA-extruding system constructed on DNA origami scaffold; adapted from Ref. [109], copy right (2020), with the permission from American Chemical Society. (F) Diffusion-controlled patterning of amphiphilic DNA condensates. The initially uniform DNA condensates were sequentially modified with hybridizing ODNs that differ in the diffusion rate within the condensates.to result in pattern formation to localize the functional moieties or to segregate the functionality; adapted from Ref. [110], copy right (2022), with the permission from the America Chemical Society.

higher ionic conductance. These results suggest that the hydrophobic interaction between NSP1 plays a role in the formation of dense aggregates, which is consistent with the molecular dynamics simulations.[90] The nuclear pore complex model described above was used to understand the mechanism of selective transport through the dense aggregates of FG-Nups, which are thought to act as a diffusion barrier for macromolecules between the nucleus and the cytoplasm. The DNA ring template allowed the variation of the number and position of the FG domain at the central pore to result in the formation of aggregates depending on the chemical property and number of the FG domain, suggesting that the aggregates of FG domain could correlate with its permeability properties.[91]

The configuration of the DNA nanostructure is transformed in response to external signals by appropriate design of the auxiliary domains. An autonomous DNA nanorobot delivers molecular cargo to cells, activated by sensing cell surface signals through aptamer-encoded logic gates. This prototype, loaded with specific antibody fragments, effectively stimulated cell signaling processes in tissue culture, suggesting the potential for diverse, selective designs with biologically active payloads in cell-targeting applications.[92] DNA origami-based nanorobots have been designed for targeted drug delivery to tumors.[93] These nanorobots, equipped with a nucleolin-binding aptamer on their surface and thrombin inside, successfully delivered thrombin to tumor-associated blood vessels in mouse models. Local thrombin induction led to intravascular coagulation, resulting in tumor necrosis and inhibited tumor growth. Importantly, these nanorobots were found to be safe and immunologically inert in mice and miniature pigs, demonstrating their potential as a precise and effective drug delivery system for cancer therapy.

A variety of 2D and 3D DNA nanoscaffolds have been reported as enzyme assembly platforms for cascade reactions. The catalytic compartments were constructed based on enzyme assembly on DNA scaffolds. The well-designed DNA-based enzyme cascade systems are used for further application and property studies.[94] 3D DNA nanostructure is also a powerful tool to study enzymatic cascade reactions within its confined 3D environment.

A 3D nanoreactor composed of spatially addressable DNA origami successfully demonstrated efficient enzyme cascade reactions between GOx and HRP within the reactor, producing hydrogen peroxide from glucose, facilitating TMB oxidation at the HRP enzyme site, and detecting TMB diamine (TMB*) by spectrophotometry.[95] Encapsulation of the enzymes in a DNA nanocage enhanced their catalytic activity and stability. By creating a highly controlled microenvironment with DNA nanocages, enzyme activity was increased (Figure 6B). DNA nanocages were potentially useful in biomaterials and biotechnology.[96] In the examples above and in the following enzyme assemblies, DNA hybridization and covalent bonds are used to locate the enzyme of interest at the defined position. A large protein complex could be held in a 3D DNA nanostructure by spatially defined supramolecular interactions consisting of a host-guest interaction. The inner surface of a 3D DNA hollow structure was modified with multiple guest ligands that symmetrically match the corresponding binding sites on the protein surface of a given protein oligomer (Figure 6C). DNA nanocontainers were tailored for specific oligomers based on the 1:1 host–guest recognition by customizing the size and the symmetric location of the host-guest interaction, providing a new method for protein caging.[97]

With the superior properties of structural diversity and precise addressability, DNA scaffolds provide unique platforms for the spatial organization of biomolecules. Enzymes or channel proteins can be precisely assembled on the DNA scaffold with control over inter-enzyme distance, number, and stoichiometry of enzyme, and their location.[98] By using zinc-finger protein (ZFP)-adaptors,[99] the Kir3 K^+ channel proteins were specifically assembled on the DNA scaffolds.[100] The modular adaptor method has been developed to assemble enzymes at the specific positions on the DNA scaffold with high enzyme loading efficiency.[101] The details of this method have been described in other reviews.[102]

Using the modular adapter method, RuBisCO and carbonic anhydrase (CA) were co-assembled on a DNA scaffold to construct an artificial carboxysome, but no obvious proximity effect of CA was observed for the RuBisCO reaction,[103] which is consistent with the previous result of protein cage encapsulation RuBisCO and CA.[82] Interestingly, when CA was assembled on a DNA scaffold with an inter-enzyme distance of less than 1 nm, i.e. in the packed state, an enhancement of the enzymatic reaction was observed compared to the reaction of the same enzyme assembled on the DNA scaffold in the dispersed state, in which the enzymes do not contact each other.[104] The reaction was more enhanced for substrates with higher hydrophobicity. The acceleration on the reaction rate of another enzyme, xylose reductase (XR), in the packed state further supported the initial finding of an accelerated reaction of CA in the packed state. Based on these results, the entropic force of water is likely to increase the local substrate concentration within the domain confined between the enzyme surfaces, thereby accelerating the reaction. This model provides a new insight to describe the reaction of the enzyme in the condensates in cells, although the detailed characteristics of the condensate remain to be elucidated.[104]

A 2D DNA scaffold carrying three cavities was constructed to study the enzyme cascade reaction derived from D-xylose metabolic pathways.[105] XR was fused to a modular adaptor (ZF-SNAP (ZS) to obtain ZS-XR, and xylitol dehydrogenase (XDH) was fused to GCN4 (G) to obtain G-XDH. ZS-XR and G-XDH were assembled on the scaffold with systematically varied inter-enzyme distance. The results showed quantitatively that the distance between the enzyme plays a critical role in the efficiency of the cascade reaction of XR and XDH.[105] A dynamic 3D DNA scaffold was further constructed to investigate the effect of the 3D confined environment on the metabolic reaction. ZS-XR and HG-XDH (modular adaptor, Halo-GCN4 (HG), fused XDH) were first specifically assembled on the DNA scaffold in the open state with an inter-enzyme distance of 60 nm. The enzyme loading yields were quantified by high-speed atomic force microscopy (AFM) images. The dynamic transformation of the DNA scaffold from the open to the closed

state was induced by short DNA strands which were designed by a toehold displacement mechanism. The closed state of the DNA scaffold resulted in an inter-enzyme distance of 18 nm. It was found that the cascaded enzymes encapsulated in the closed state of the DNA scaffold showed higher cascade efficiency compared to the enzymes assembled on the DNA scaffold in its open state (Figure 6D).[106] Two main factors have been proposed for the increased efficiency of the cascade in the closed state. The proximity of the enzymes in the closed state facilitated the efficient transport of intermediates from the first enzyme to the second enzyme. And the closed state provided a 3D confined environment for the enzyme reactions, which could reduce the diffusion of the intermediate. Compared with the same pair of enzymes assembled on a 2D DNA scaffold with a three-well cavity,[105] the cascade reaction of enzymes on the 3D DNA scaffold showed a higher turnover number. We proposed that the 3D DNA scaffold provided a more favorable microenvironment for the enzyme reactions. A high-density water layer formed near the negatively charged DNA scaffold surface may play an important role in regulating the enzyme reaction.[107]

Another pair of enzymes, XDH and xylulose kinase (XK), derived from the D-xylose metabolic pathway, was assembled on the 3D DNA scaffold capable of changing the structure from the open planner structure to the closed hexagonal prism structure. The XDH/XK cascade reaction was evaluated in the open and closed states and showed no significant change depending on the structural state. The lack of the inter-enzyme distance dependence for the XDH/XK cascade is presumably due to the much larger catalytic constant of the downstream enzyme XK. While the inter-enzyme distance is a critical spatial factor for the construction of the enzyme assembly when the kinetic parameters of the cascade enzymes are in balance, it is not the dominant factor for the cascade efficiency when the kinetic parameters of the downstream enzyme dominate the upstream enzyme.[108] The result provides important insights into the design and construction of artificial compartments with efficient enzymatic metabolic pathways. It was also shown that the spatial organization of enzymes regulates RNA production by the RNA extrusion machinery, in which RNA polymerases and RNA endonucleases are spatially organized on a 3D DNA origami-based barrel (Figure 6E).[109]

3.4. DNA- or Protein-Based Artificial Biomolecular Condensates

Chemically modified DNA nanostructures often form aggregates and hydrogels. Tuning the kinetics and/or stability of hybridization of the aggregate components provides an opportunity to define the composition or physical properties of DNA aggregates. DNA condensates with defined compositional patterns have been constructed using an amphiphilic DNA four-way junction conjugated with cholesterol moieties. One of the four arms acts as a hybridization site for short ODN, which was masked in the initial stage. The diffusion rate of the ODN complementary to the hybridization site, termed the patterning strand, depends on its length: the shorter patterning strand diffuses faster inside the aggregates than the longer one. The longer patterning strand is designed to replace the shorter one by the toeholding mechanism. The successive reactions of the initial uniform DNA condensates with the shorter and longer patterning strands resulted in the DNA condensates with spatial patterning of microenvironments, which would provide a platform for the construction of membraneless artificial cells. Using this platform, a system was developed to synthesize fluorescent RNA aptamers at the core part of the DNA aggregates, which then accumulate in the outer shell of the aggregates (Figure 6F).[110]

The physical properties of the DNA hydrogels were reversibly tuned by forming a dynamic network system of DNA nanostructures. Two bidentate units for chain elongation and two tetradentate units for both the chain elongation and crosslinking reactions were designed as toehold-modified components. Hybridization of these four units results in a hydrogel formation. The addition of an auxiliary effector increased the content of the tetradentate crosslinking unit, resulting in a higher stiffness of the hydrogel. Conversely, the addition of an effector to decrease the tetradentate crosslinking unit content results in the formation of a hydrogel with lower stiffness. Because the auxiliary effectors bind to each unit by hybridization, these re-equilibrations of hydrogel structures are reversible processes. Such hydrogels with the constitutional dynamic network could be useful for the design of self-healing and controlled drug release devices.[111] With the specific structural properties, DNA hydrogels were used for mimicking the biomolecular condensates.

By applying the mechanisms of phase-separation, the artificial biomolecular condensates have been constructed in vitro and in vivo. Molecular modules with the properties of intrinsically disordered proteins were genetically designed to form the condensate. These artificial condensates have been used to regulate the transcription processes transcription in bacteria and to modulate a protein circuit in mammalian cells.[112] Artificial biomolecular condensates were formed by intrinsically disordered cationic protein (GFP-K72) and single-stranded DNA, which was applied in the in vitro transcription-translation systems.[113] By using a modular combination of oligomeric proteins and peptides, the artificial phase-separated protein condensates were formed and applied in the recruitment of target proteins from the cytoplasm in cells.[114] There are updated reviews to further introduce the engineering synthetic biomolecular condensates.[115] With the developments of strategies to logically control the properties and functions of artificial biocondensates, these systems are promising for the applications in the fields of drug delivery, therapeutics, artificial organelles or cells.

3.5. Artificial Compartments Constructed by the Membrane Engineering

Membrane structures play an important role as functional interfaces that regulate structural organization and compart-

mentalization within cells or organelles.[116] Innovation in experimental strategies to mimic these membranes is critical. Artificial lipid membranes serve as powerful tools for studying membrane dynamics and exploring applications such as drug delivery.[117] Self-assembled DNA nanostructures provide precise templates for membrane engineering and offer a promising route to the construction of confined membrane structures.[118] Rationally designed DNA nanoscaffolds have been decorated with lipid molecules at specific positions, enabling the construction of artificial membranes.[119]

A new technique using a DNA "exoskeleton" was developed to limit the size of the liposomes. The inner surface of a DNA origami ring was modified with single-stranded DNA handles to which lipidated complementary DNA was hybridized. This allowed the growth of lipid "seeds" into precisely sized liposomes upon the addition of lipid and detergent.[120] This technology was used to organize liposomes at specific spacings, which allowed the study of lipid transfer through the synaptotagmin-like mitochondrial lipid-binding protein domain of extended synaptotagmin 1. The results supported the shuttle model of lipid trafficking and demonstrated the versatility of these DNA structures in the study of membrane-related processes.[121] The DNA "exoskeleton" was used to control the shape and dynamics of liposomes using modular DNA nanocages, allowing the precise construction and manipulation of various membrane structures such as tubes and toroids. Using DNA cage configurations, the research demonstrates the ability to mimic complex cell membrane curvatures in vitro and to systematically study membrane mechanics through controlled membrane fusion and bending.[122]

The membrane curvature plays a critical role in the cellular survival and life science.[123] The effects of membrane curvature on protein function have been also studied by a novel technique using cholesterol-modified DNA 'nanobricks' that sort diverse liposomes into uniform populations based on buoyancy density. The efficiency of this method, and its ability to produce leak-resistant, uniform liposomes, is of great benefit to membrane biology research and the development of targeted drug delivery.[124]

A double-stranded DNA platform was introduced across the DNA "exoskeleton" to assemble molecules of interest inside the nanoliposome.[125] A pH-sensitive fluorophore, 6-carboxyfluorescein (CF), was modified on the bridged platform to evaluate the environment inside the liposome. Upon formation of the nanoliposome, CF showed little or no fluorescent response to the pH change of the external buffer, indicating that the molecules assembled on the DNA platform across the DNA ring are effectively shielded from the external environment. Thus, the DNA "exoskeleton" equipped with the DNA platform therefore allows the assembly of functional molecules at the defined sites inside the nanoliposome while isolating them from the external environment (Figure 7A).[125]

In an approach inspired by virus assembly, a DNA octahedron was encapsulated in lipid bilayers (Figure 7B).[126] This DNA origami-guided liposome was used to construct an artificial compartment with size-selective molecular permeation function.[127] The liposome interior, including the internal DNA origami skeleton, was completely isolated from the external environment, and the internal DNA origami skeleton allowed the assembly of a defined number of molecules of interest at the specific sites inside and/or outside the compartment (Figure 7C).[127] Embedding a bacterial membrane protein OmpF into the liposome provided a permeation function to the artificial compartment. Only the molecule of interest with a molecular weight below 600 Da permeated between the external environment and the interior of the compartment.

The mechanism of DNA strand displacement was used to dynamically control the membrane conformation (Figure 7D).[128] With the extremely high specificity of Watson-Crick base pairing of DNA, DNA scaffolds are ideal building blocks for the hierarchical assembly of higher order structures. The DNA scaffold units are assembled into different patterns by DNA hybridization. Similarly, by applying the modification technology of oligonucleotides and liposomes, the liposome units can be rationally assembled.[129] In addition, heterogeneous assembly of DNA scaffold and liposome can also be realized.[130] By applying the methods of hierarchical assembly of DNA scaffolds and/or liposomes into network structures, the compartments with different cellular functions will be integrated into artificial organelles that can implement more complex biological processes.[131] Taking advantage of DNA nanotechnology and liposome technology, communication between compartments and transport of cargo will be realized.[132] Inspired by the assembly of the protein clathrin, DNA-coated liposomes were prepared by hydrophobic anchoring and subsequent linking of triskelion-like DNA nanostructures on the liposome surface (Figure 7E).[133] Successful DNA coating was verified by dynamic light scattering, zeta potential, confocal microscopy, and cryo-EM measurements. The disassembly of the DNA coats by a toehold-mediated displacement reaction indicated a possible application of coating and disassembly processes, such as the ordered arrangement of functional groups on the liposome surface. The DNA gel shell was also used as an artificial cytoskeleton for the liposomes (Figure 7F).[134] To reduce the repulsion between lipid membranes and DNA network, the cationic lipids were used. The DNA shell in the gel phase was shown to increase the stability of the liposome. Lipoproteins, including the high-density lipoprotein,[135] are composed of multiple proteins that transport lipids outside of cells. Inspired by the structural features of lipoproteins, porous protein cages have been used as templates for assembling lipids to form a hydrophobic surfactant-modified core to encapsulate poorly water-soluble small molecules. These lipoprotein-like complexes protect the encapsulated fluorescent probes and cytotoxic drugs from capture by serum proteins to enhance the cellular uptake (Figure 7G).[136] The further examples of proteinosome were shown in other references.[137]

Liposomes are considered as nano-sized containers for combinatorial screening. Stochastic fusion of a single-particle liposome was mediated by the lipidated DNA on the liposomes. Multiple fusion events were directly observed for surface-bound target liposomes with defined types of freely diffusing liposomes. Unique fusions and distinct fusion events were directly observed in real time using total internal reflection imaging.

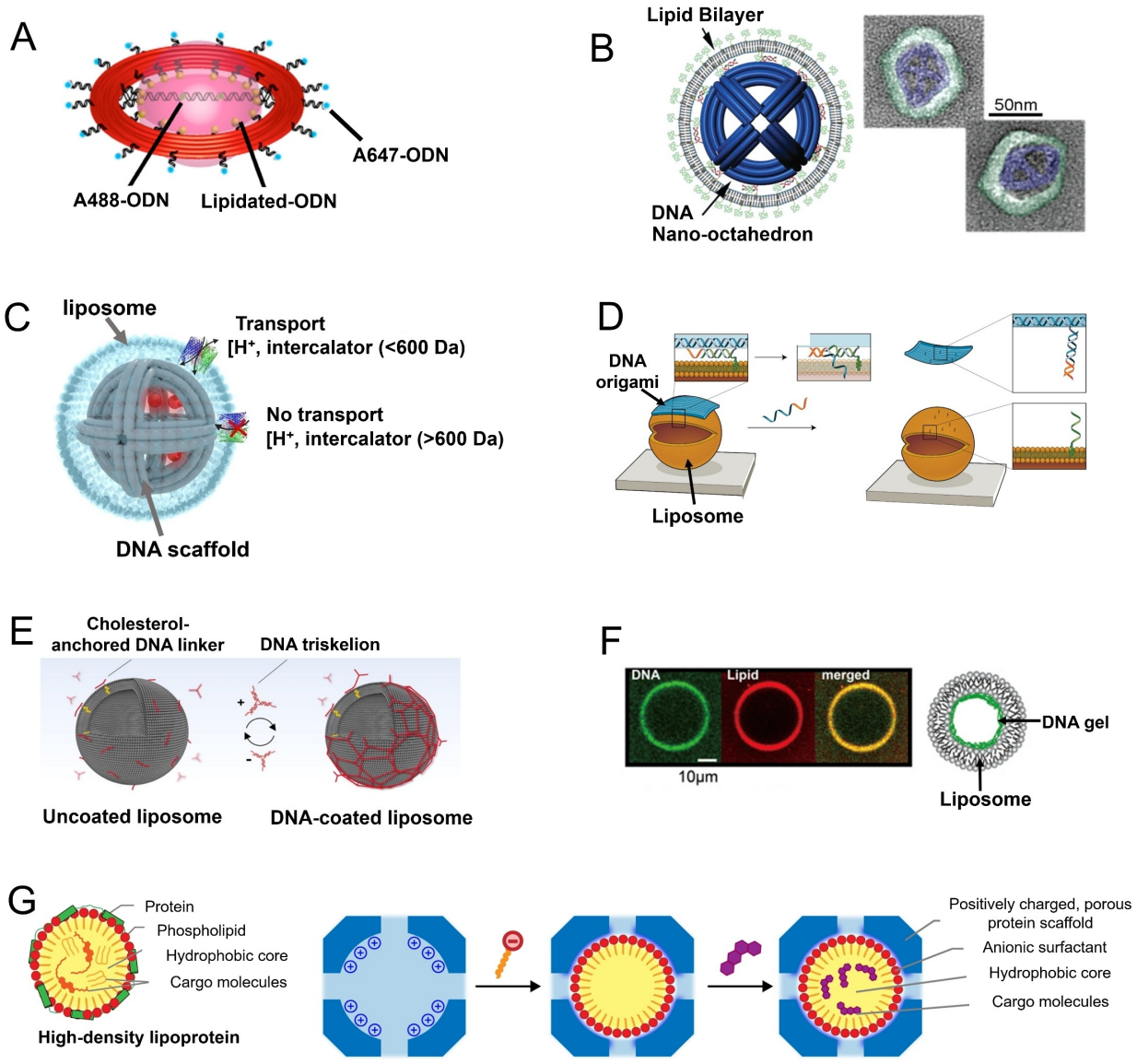

Figure 7. Artificial compartments based on hybrid materials. (A) Fluorophores encapsulated inside nanoliposome; adapted from Ref. [125], copy right (2023), with the permission from MDPI. (B) DNA nanostructures encapsulated lipid bilayer as nanoliposome; adapted from Ref. [126], copy right (2014), with the permission from the American Chemical Society. (C) DNA-scaffolded nanoliposome with transporter as size-exclusion molecular transportable artificial compartment; adapted from Ref. [127], copy right (2023), with the permission from John Wiley & Son. (D) Schematic of the binding of the DNA origami tile (blue) to a liposome (orange); adapted from Ref. [128], copy right (2021), with the permission from Oxford University Press. (E) DNA-coated liposome; adapted from Ref. [132], copy right (2020), with the permission from American Chemical Society. (F) Liposomes scaffolded by DNA gel shell; adapted from Ref. [134] copy right (2017), with the permission from the National Academy of Sciences. (G) Surfactant-encapsulated protein cage as a lipoprotein mimic; adapted from Ref. [136], copy right (2020), with the permission from Springer Nature.

The high-density arrays of surface-attached target nanocontainers provide highly efficient combinatorial screening using only small amounts of material.[138]

The use of natural and synthetic polymers, types of polymer-liposomes interactions on the surface, incorporation into liposomes and their effects on the pharmacological aspects have been reviewed in the references.[139] By combining the advantages of multiple materials, the hybrid nanosystems provide promising platforms for constructing artificial compartments with efficient enzyme reactions.

4. Summary and Outlook

The compartmentalization of the metabolic pathways is a favorable solution to increase the efficiency of the cascade reactions and to increase the overall output. This review discusses the construction and applications of biomimetic systems and provides the relevant perspectives for the future development of artificial compartments, the building blocks for the construction of artificial organelles or cells. Compartmentalization of enzymatic pathways is beneficial for enhancing the

production of value-added chemicals in the cell as well as localizing metabolic pathways to specific organelles.

The canonical examples of compartments in cells, including the recent studies on cellular membraneless organelles are reviewed. Membraneless organelles are often phase-separated biomolecular condensates of proteins and/or nucleic acids. These membraneless compartments would exhibit distinct physicochemical properties to modulate the local concentration of substrates or intermediates, which would affect the efficiency of metabolic pathways. However, much effort is needed to understand the properties of biomolecular condensates or membraneless compartments, such as the enzyme kinetics in the condensate. The development of appropriate model systems is necessary to understand the chemistry of the biomolecular condensates. Some prototypes of molecular models of biomolecular condensates or membraneless compartments are described in this review. Further understanding of the physical characteristics and chemistry associated with the membraneless compartments enable the usage of artificial membraneless compartments with defined chemical characteristics and size, which could be realized by using biomolecular scaffolds, such as the DNA nanostructures. These artificial membraneless compartments are necessary components to construct the construction of artificial cells.

Studies on the membrane-bound compartment, on the other hand, have recently made significant progress by combining traditional membrane technology and biomolecular templates, especially using the DNA nanostructures as platforms in the construction of artificial compartments, as summarized in this review. The artificial compartments constructed by using polymersomes, protein scaffolds, nucleic acid-based scaffolds, and hybrid materials have played a prominent role in encapsulating various cargoes, including therapeutics for drug delivery applications and enzymes to create nanoreactors.

Liposomes and polymersomes are two major types of soft materials that have been used to construct artificial compartments with a variety of membrane functions by taking advantage of the vast knowledge of these materials. Unfortunately, it is still difficult to control the number and location of the enzymes and other functional molecules inside the compartment and/or on the membrane surface of these compartments. However, the integration of transmembrane proteins into artificial compartments has attracted considerable interest in the development of selectively permeable compartments. Yet, the incorporation of membrane channel proteins while maintaining the vesicular structure in the given polarity is still a challenge. The artificial membrane-bound compartment constructed using the engineered platforms of DNA nanostructures hold promises to localize a given number of membrane proteins in a controlled polarity. In addition, the DNA platforms allow the localization of a given number of enzymes to be located on and/or within the compartment at defined spatial locations, allowing the quantitative study of enzymatic reactions and material transport in the compartment. The size limitation of the compartment is a major drawback for the use of the artificial membrane-bound compartment equipped with the DNA nanostructure platform. On the other hand, the combination of different types of artificial membrane-bound compartments with the DNA platform allows the programmable construction of higher-order compartment architectures, where each compartment is equipped with a defined number, location and type of enzymes and/or proteins. Such a fundamental understanding is necessary to establish the chemistry of the enzymatic reactions and molecular transport of the artificial compartment.

The membraneless compartments or the membrane-bound compartments by themselves would not exhibit complex functions justifying the artificial organelles or artificial cells. A possible method of constructing the higher-order compartment architectures is ecapsulation of the membraneless compartments with defined chemical and physical properties by the membrane-bound compartments. Understanding the chemistry of individual compartments leads to the design of a given function of the compartment and higher-order architectures of the artificial compartments that provide otherwise inaccessible functions of the artificial organelles and the artificial cells.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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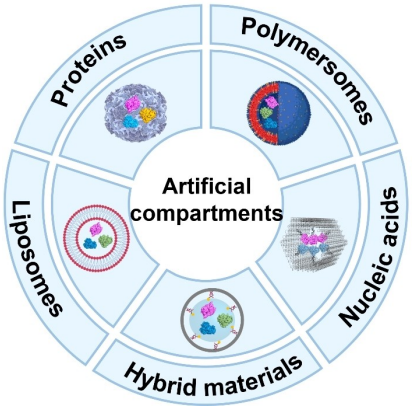
REVIEW

This review article summarizes the cellular compartments, and the current progress of artificial compartments constructed by using liposomes, polymersomes, proteins, nucleic acids, and hybrid materials. The perspectives for the future development of artificial compartments towards the construction of artificial organelles or cells were discussed.



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