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# Construction and application of artificial organelles *via* protein self-assembly

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## Abstract

Protein self-assembly represents a highly controllable and versatile strategy for the construction of artificial cells and their functional substructures. By harnessing or engineering the intrinsic assembly properties of natural proteins, diverse and structurally stable artificial organelles can be generated to enable spatial organization of enzymatic catalysis, metabolic pathways, and molecular transport. This review provides a comprehensive overview of representative protein self-assembly systems, including protein cages, scaffolds, and membraneless condensates with an emphasis on their assembly principles, structural characteristics, and emerging applications in enzyme catalysis, mass transfer, and metabolic engineering. Finally, we discuss the current challenges and future directions in the field, offering a conceptual and technical framework for the rational design of protein based artificial organelles.

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## Introduction

Through long-term natural evolution and selection, biological macromolecules have acquired the ability for specific recognition, dynamic interactions, and precise assembly, enabling the formation of intricate and biologically active functional systems[1, 2]. Among them, proteins, as essential biological macromolecules, can spontaneously form a variety of ordered supramolecular structures through self-interactions, such as viral capsids, bacterial microcompartments and actin filaments[3, 4]. With the rapid advancement of protein engineering, researchers have gradually elucidated the molecular mechanisms underlying protein self-assembly. Based on these principles, diverse artificial organelles have been designed and to mimic the functions of natural organelles, such as energy metabolism[5], material transport[6], and molecular synthesis[7, 8]. Progress in this field not only deepens our understanding of cellular structures and functions, but also provides new strategies and opportunities for synthetic biology, nanomedicine delivery, and innovative applications in biomaterials[2, 9].

Compared with traditional lipid-based self-assemblies, protein self-assembly systems exhibit increasingly prominent and unique advantages. Lipid molecules spontaneously form monolayer or bilayer structures driven by hydrophobic interactions, which can effectively mimic the cellular membrane environment; however, their structural diversity is relatively limited, regulatory strategies are constrained, and functional implementation largely relies on subsequent molecular modifications or membrane protein incorporation (Table 1). In contrast, protein assemblies not only possess inherent sequence programmability but can also construct multiscale architectures ranging from the nanometer to micrometer level through diverse interactions among amino acid residues, such as hydrogen bonding, hydrophobic interactions, electrostatic forces, and covalent modifications. More importantly, protein molecules often carry intrinsic catalytic, recognition, or signaling domains, enabling the realization of complex biological functions during the assembly process itself [10, 11].

In addition, protein self-assembly systems typically exhibit a high degree of dynamic tunability; for example, their assembly states can be reversibly switched in response to environmental conditions (pH, ionic strength, temperature) or external signals, thereby providing greater adaptability in the construction of artificial organelles[12, 13]. This review provides a systematic overview of recent advances in protein self-assembly, with a particular focus on its underlying molecular mechanisms（Table 2）. Based on differences in structural features and assembly modes, we classify artificial organelles derived from protein self-assembly into four categories: protein cages, protein scaffolds, membraneless organelles, and composite types (Fig. 1and Table 3), and highlight recent progress for each. In addition, we discuss the key challenges currently facing the field and propose potential strategies to address them, with the aiming of offering new insights and future directions for the design and application of protein-based artificial organelles.

Table 1 Core distinctions between lipid assemblies and protein self-assemblies

| Catalogs | Lipid assemblies | Protein self-assemblies | References |
|---|---|---|---|
| Designability & structure | Bilayers, vesicles, and rafts defined by lipid chemistry | Highly programmable, form nanocages, fibers, lattices, and droplets across scales | 10, 11 |
| Functionality | Provide basic compartmentalization; functionalization requires external modification | Spatial organization and compartmentalization, catalysis and reaction platforms, molecular recognition and signal regulation, mechanical support and structural scaffolding, transport and delivery, information storage and dynamic regulation | 12, 13 |

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| Catalogs | Lipid assemblies | Protein self-assemblies | References |
|---|---|---|---|
| Dynamics & responsiveness | High fluidity, reversible phase transitions, rapid morphology change under stimuli | Programmable, reversible assembly/disassembly, functional regulation under cues | 14, 15 |
| Applications & scalability | Clinically established (liposomes, LNPs) with robust stability | Engineered as artificial organelles or nanoreactors; customizable via synthetic biology | 16 |

## Non-covalent interactions in protein self-assembly

Non-covalent interactions among proteins constitute the fundamental basis of biological macromolecular organization, spanning from reversible and tunable microscopic pairings to large-scale supramolecular assemblies[17]. Their fundamental kinetic and thermodynamic properties are governed by a superimposable set of noncovalent force fields[18], including hydrophobic interactions, electrostatic interactions, hydrogen bonding, van der Waals forces, aromatic π–π stacking, and metal coordination[11].

### Hydrophobic interactions

Hydrophobic interactions in protein self-assembly are generally responsible for the "primary attraction" and solvent-exclusion–driven desolvation process[19] (Fig. 2a). Recent studies have highlighted that the ordering of water molecules, altered solvent dynamics, and interfacial dewetting play pivotal roles in this hydrophobic driving force[20]. At short-range, strongly hydrophobic interfaces, local water density depletion markedly lowers the free-energy barrier, allowing protein or peptide segments to rapidly approach and form a stable hydrophobic core[21, 22]. The reversibility, timescale, and interfacial architecture of this dewetting process critically regulate droplet viscoelasticity and maturation. For instance, in liquid-liquid phase separation (LLPS) systems, intrinsically disordered protein (IDP) sequences containing hydrophobic clusters can mediate initial condensation through weak, multivalent hydrophobic contacts[23-25]. Subsequent local structuring and accumulation of van der Waals contacts

increase droplet viscoelasticity, driving a transition toward a gel-like state[26, 27]. Engineering the length, distribution, and spacing of such hydrophobic motifs has been shown to precisely tune the phase diagram and critical concentration, thereby optimizing enzyme enrichment and substrate channeling within metabolic pathways[28, 29]. Both experimental and simulation studies on phase separation kinetics further reveal that hydrophobic assembly exhibits pronounced sensitivity to salt and temperature, enabling the design of thermo- and salt-responsive artificial organelles[23-25, 30]. Such tunable responsiveness offers direct utility in synthetic biology, where on-demand activation or repression of local metabolic activity is highly desirable.

**Electrostatic interactions**

Electrostatic interactions can function not only as long-range modulatory forces but also as determinants of selective pairing and reversibility within multivalent weak-binding networks[31] (Fig. 2b). The spatial distribution of charged amino acid side chains shapes the electrostatic potential across the protein surface, profoundly influencing the geometry of spherical cages, RNA-protein co-encapsulation, and the critical point of phase-separating systems[32]. For example, incorporating short, arginine- or lysine-rich cationic patches on protein surfaces markedly enhances attraction to anionic nucleic acids or polyacidic cofactors, thereby driving the formation of capsids or vesicles with defined size and cargo capacity[33]. Conversely, tuning the overall isoelectric point or introducing localized anionic groups can preserve reversible assembly and disassembly under high ionic strength conditions[33]. In synthetic metabolic pathways, electrostatic control has been exploited to create “charge channels,” which leverage electrostatic potentials to efficiently shuttle cofactors such as NAD(P)H between sequential enzymes, minimizing diffusive loss and enhancing catalytic coupling efficiency[34, 35]. When engineering such systems in vitro or in vivo, the use of electrostatics as a “guiding field” must be balanced with considerations of electrostatic screening, salt effects, and pH dependence to maintain the desired assembly state under varying environmental conditions[36].

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## Other non-covalent interactions

Hydrogen bonds and van der Waals interactions are indispensable for mediating specific pairing interfaces and establishing predictable subunit geometries. Hydrogen bonds confer directionality and selectivity, whereas van der Waals forces accumulate over large contact surfaces to provide stability[37-40] (Fig. 2c). In the design of isomorphic or quasi-symmetric protein cages, high-fidelity self-assembly is typically achieved by precisely engineering hydrogen-bond networks and complementary interfaces. For example, in *de novo* design protein nanocages, modular hydrogen-bonding motifs have been employed to constrain relative subunit orientations, thereby imposing predetermined symmetry and pore architecture[41]. Moreover, π-π stacking between aromatic residues exhibits strong directionality in the formation of one-dimensional fibers and layered supramolecular assemblies, and this interaction is frequently exploited to construct ordered superstructures with optoelectronic or photocatalytic functions[42]. Recent studies further indicate that certain "noncanonical" noncovalent interactions, such as CH-π and S-π contacts, can exert subtle yet crucial effects at tightly packed interfaces; collectively considering these weak forces is therefore essential for bridging molecular design and macroscopic function[43]. Metal coordination, as a semi-covalent and highly directional interaction, offers unique advantages in reversibility and functionalization. Metal-ligand interactions (e.g., $Zn^{2+}$–His, $Ni^{2+}$–His, $Fe^{2+}/Fe^{3+}$ with multidentate ligands) not only stabilize the geometric arrangement of multiple subunits but also introduce externally controllable switches[44], such as pH or ligand-induced reversible disassembly, enabling the construction of responsive nanocages, crystallized protein arrays, or ordered two- and three-dimensional superlattices[45]. Bioinspired examples, including shell and byssus matrices, demonstrate that metal-amino acid coordination can support repairable, self-healing supramolecular networks under aqueous conditions[46]. In artificial systems, metal-directed self-assembly has been leveraged to enhance the structural stability of protein cages and to impart catalytic or electronic transport functionality[47].

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## Covalent interactions in protein self-assembly

Covalent interactions play a dual role of reinforcement and functionalization in protein self-assembly. Compared with non-covalent interactions, covalent interactions offer high energy barriers, structural persistence, and controllability when engineering specific functions. Their drawbacks include potential irreversibility (unless reversible covalent bonds are employed) and the risk of folding or functional impairment[48]. Accordingly, modern designs of artificial organelles often adopt a "soft covalentization" strategy: leveraging the reversible, noncovalent-based assembly and dynamic behavior of protein systems while introducing reversible or triggerable covalent crosslinks to enhance long-term stability, mechanical robustness, or functional anchoring[49].

### Disulfide bonds

Disulfide bonds (Cys–Cys) are critical molecular scaffolds and regulators. Their function primarily relies on reversible redox chemistry[50] (Fig. 3a). During the folding of native proteins, disulfide bonds connect distinct cysteine residues within a polypeptide chain, stabilizing local or global tertiary structures, thereby promoting correct folding and preventing non-specific aggregation[51]. Virus-like particles (VLPs) exemplify this principle: inter-subunit disulfide bonds facilitate the formation of highly symmetric hollow architectures, enhancing both assembly efficiency and structural robustness[52] (Fig. 3a). Moreover, the reversibility of disulfide bonds allows protein assemblies to respond dynamically to environmental cues such as pH, temperature, or redox conditions[53]. For instance, in protein hydrogels (Fig. 3a), redox-driven disulfide bond "breakage and reformation" endows the material with reversible cross-linking and self-healing properties, enabling spatially selective control over the assembly state[54]. Despite their essential role, non-specific disulfide formation can lead to protein misfolding and aggregation, ultimately generating amyloid structures that impair function. In Alzheimer's disease, aberrant disulfide formation in β-amyloid peptides may promote aggregation into amyloid plaques, contributing to neurotoxicity[55, 56].

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Consequently, the design of protein self-assembly systems requires precise control over disulfide bond formation sites and conditions to avoid undesired aggregation.

**Thiol-ene click reactions**

Click chemistry has been widely employed for orthogonal modifications of materials and biomolecules due to its high selectivity, quantitative yields, and mild reaction conditions[57] (Fig. 3b). Among these, thiol-ene click reactions, which proceed efficiently under photoinitiated or thermal conditions, enabled the formation of stable covalent networks while preserving functional sites on proteins[58]. By exploiting this reaction, molecules bearing alkene groups can be conjugated to cysteine-modified proteins, facilitating the construction of protein assemblies with defined structures and functions[59] (Fig. 3b). Moreover, the reversible nature of the reaction allows dynamic regulation of assemblies under specific conditions. For instance, in cellular environments, redox-controlled modulation of the reaction enables the controlled disassembly of protein assemblies (Fig. 3b), providing a versatile tool for probing intracellular protein interactions[59, 60]. This strategy also allows the crosslinking of proteins with distinct functionalities into multifunctional complexes. Such as, proteins endowed with catalytic activity and receptor recognition capability can be linked via thiol-ene click reactions to generate dual-functional protein complexes[61]. Such assemblies hold potential for applications in biosensing, drug delivery, and beyond[61]. Additionally, studies have shown that protein nanostructures constructed via this reaction exhibit remarkable stability under elevated temperatures and extreme pH conditions[62]. The introduction of covalent crosslinks can further modulate protein functionality, such as enhancing catalytic activity or altering receptor recognition, offering new avenues for functional studies of proteins[57].

**Schiff base reactions**

The Schiff base reactions refer to the condensation of amines with aldehydes or ketones to form imine (Schiff base) structures[63] (Fig. 3c). In protein self-assembly, the amino groups of cysteine residues can react with aldehyde-containing molecules, such

as reducing sugars, to generate imine linkages, thereby connecting individual protein units[64]. By establishing stable connections between protein subunits, the Schiff base reaction enhances the structural stability of the resulting complexes[65]. For example, this strategy has been used to link distinct protein units, yielding protein nanostructures that exhibit remarkable stability under diverse environmental conditions[65]. Moreover, the reversibility of the Schiff base reaction allows protein complexes to dissociate or reorganize under specific conditions (Fig. 3c). A reversible protein assembly based on this chemistry has been engineered to disassemble under acidic conditions and reassemble under basic conditions[66]. Such reversibility provides a means for dynamic regulation, enabling protein complexes to function in different biological contexts (Fig. 3c). In addition, the reaction facilitates the incorporation of functional modules into protein nanostructures, thereby creating multifunctional protein materials[48] (Fig. 3c). For instance, catalytic enzymes and fluorescent probes have been conjugated onto protein nanostructures through Schiff base linkages, producing sensors capable of both catalysis and fluorescence output under defined conditions. The spatial selectivity of this reaction further enables targeted assembly of protein complexes at designated sites. A protein assembly designed with this principle can selectively organize at specific locations on the cell membrane, forming nanostructures with localized functions[67]. This spatially controlled assembly offers new opportunities for functionalizing protein complexes[64].

**Other covalent interactions**

In recent years, enzyme-catalyzed covalent modifications have emerged as a powerful strategy for constructing stable and controllable protein assemblies in protein engineering and materials science[68] (Fig. 3d). Such enzymatic cross-linking reactions often proceed under mild conditions with high selectivity and efficiency, endowing the resulting assemblies with superior mechanical stability and environmental resilience. Among these approaches, two of the most extensively studied and widely applied reactions are transglutaminase (TGase)-mediated amide bond formation and

tyrosinase/peroxidase-mediated dityrosine formation[69] (Fig. 3d). For instance, TGases catalyze the formation of covalent amide bonds between the γ-carboxamide group of glutamine residues and the ε-amino group of lysine residues, generating stable isopeptide linkages[69]. The high specificity and irreversibility of this reaction confer distinct advantages for constructing robust protein networks and assemblies. Microbial transglutaminase (mTGase), for example, has been used to cross-link gelatin, significantly enhancing the mechanical strength and thermal stability of the resulting hydrogels, which maintain their three-dimensional structures under elevated temperatures and varying pH conditions[70]. This strategy has been applied not only to the development of biomedical hydrogels, such as cell scaffolds and drug delivery matrices, but also shows potential in fabricating artificial collagen-based matrices and stable nanoparticles. Moreover, TGase can be exploited to design programmable protein assembly systems by introducing glutamine or lysine motifs at defined sites, enabling spatially controlled cross-linking that locks intersubunit interfaces. Compared with chemical cross-linkers, enzyme-mediated approaches avoid off-target reactions, preserving protein functionality while enhancing assembly stability[71].

Another widely used strategy involves tyrosinase- or peroxidase-mediated dityrosine formation[72, 73]. In this reaction, the phenolic hydroxyl groups of tyrosine side chains are oxidized to tyrosyl radicals, which subsequently couple to form dityrosine linkages. These enzymatic modifications can similarly achieve site-specific covalent connections under mild conditions, and the degree and pattern of cross-linking can be finely tuned by adjusting the oxidant concentration, reaction time, and enzyme type[74]. A prototypical example is horseradish peroxidase (HRP)-catalyzed dityrosine formation in the presence of hydrogen peroxide, which has been widely employed to produce protein-based hydrogels[72] (Fig. 3d). For instance, introducing multiple tyrosine residues into elastin-like polypeptides (ELPs) allows HRP-mediated rapid formation of physically robust and highly elastic hydrogels[75]. These hydrogels exhibit excellent performance in tissue engineering and drug delivery applications, and also hold promise

for injectable, self-healing materials. Unlike TGase-mediated cross-linking, HRP and related peroxidase-catalyzed reactions involve redox regulation, imparting dynamic and reversible properties to materials, thereby enabling responsiveness to diverse cellular microenvironments[72].

Table 2 Major mechanism in protein self-assembly

| Types | Representative molecules/methods | Functional consequences | References |
|---|---|---|---|
| Hydrophobic interactions | Hydrophobic clusters in IDPs, aromatic fragments | Trigger LLPS, increase local substrate concentration, modulate droplet viscoelasticity and maturation | 19, 21, 22, 76 |
| Electrostatic interactions | Lys/Arg-rich regions, RNA–protein charge complementarity | Control capsid size, RNA loading, cofactor guidance and channeling | 32, 34, 35, 77 |
| Hydrogen bonds / van der Waals forces | Designed interfacial H-bond networks, tight packing interfaces | Provide high-fidelity pairing, stabilize geometry, determine nanocage symmetry | 37-40, 47 |
| π–π / nonclassical weak interactions | Aromatic residues, CH–π, S–π | Drive fibrillation, influence optical/mechanical properties | 42, 43 |
| Metal-ligand interactions | $Zn^{2+}$–His, $Ni^{2+}$–His, $Fe^{2+}/Fe^{3+}$, etc. | Construct reversible lattices/repairable networks, enable catalysis or electron transport | 44, 45 |
| Disulfide bonds | Cys–Cys crosslinks | Enhance stability, enable environmentally selective depolymerization (redox control) | 50, 51, 78 |
| Thiol-ene click reactions | Thiol/alkene functionalization + photo/thermal initiation | Rapid and controllable crosslinking, in situ curing, drug delivery carriers | 57, 58, 79 |

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| Types | Representative molecules/methods | Functional consequences | References |
|---|---|---|---|
| Schiff base reactions | Amine–aldehyde forming imine | Reversible self-healing/stimuli-responsive hydrogels, controlled release | 59, 60, 63, 67 |
| Enzyme-mediated covalent bonds | SpyTag/SpyCatcher, transglutaminase, etc. | Highly selective site-specific linkage, modular assembly | 47, 68, 70 |

## Protein-based artificial organelles

Over the past few years, numerous protein-based artificial organelles have been developed by exploiting protein self-assembly mechanisms to mimic the functional roles of natural organelles. Based on differences in structural features and assembly modes, we classify artificial organelles derived from protein self-assembly into four categories (Fig. 1 and Table 1). These artificial organelles perform a range of vital functions, including compartmentalization of biomolecular processes to create distinct microenvironments; spatial confinement of metabolic reactions to increase local substrate concentrations and enzyme efficiency; physical segregation of incompatible or competing reactions to prevent cross-reactivity; and the modular organization of synthetic metabolic pathways to enhance flux, control, and overall productivity. Such advancements have propelled the development of artificial organelles and made them a research hotspot in the fields of biomedicine and bioengineering.

### Protein Cages

Protein cages are highly ordered, closed-shell nanostructures, typically exhibiting polyhedral geometry. Their unique architecture enables a range of biological and engineering applications, such as molecular encapsulation, protection, and targeted functional delivery[80]. These structures are characterized by uniform size, defined geometry, chemical and genetic modifiability, and excellent biocompatibility[10, 81-83](Fig.

4a). Carboxysomes, a class of protein-based organelles with icosahedral architecture, exemplify such assemblies[84]. They provide a stable anaerobic microenvironment and facilitate the precise encapsulation of cargo proteins through the action of a protein known as CsoS2, thereby protecting oxygen-sensitive enzymes from inactivation[85-88]. In one notable study, [FeFe]-hydrogenase was encapsulated within carboxysome to enhance its oxygen tolerance and catalytic efficiency[89]. Furthermore, TBAP-α was employed as a photosensitizer, non-covalently associating with the carboxysome–hydrogenase complex in solution[90]. This strategy enabled the construction of a novel photo-enzyme nanoreactor capable of visible-light-driven hydrogen evolution with high efficiency[90] (Fig. 4b). Motivated by principles of origami, the groups led by David Baker and Neil King innovatively introduced asymmetry into protein nanocage architectures, achieving the formation of exceptionally large structures up to 100 nm in diameter，corresponding to approximately 90 times the volume of an AAV capsid[91, 92]. Hydrophobic interaction-driven self-assembly yielded multi-enzyme protein compartments bearing β-galactosidase (β-gal), glucose oxidase (GOx), and horseradish peroxidase (HRP) on defined surfaces, mimicking organelle-like architectures and enhancing both mass transfer and catalytic efficiency[93, 94] (Fig. 5c). Collectively, protein cages, as naturally inspired nanoscale architectures, exhibit unique biological functions and significant potential for bioengineering applications. Their ability to self-assemble into closed, stable, and size-controlled three-dimensional shells makes them particularly advantageous for the encapsulation[89], protection[95], (Fig. 4b) and multienzyme proteinosomes[93, 94] (Fig. 4c).

**Protein Scaffolds**

Protein scaffolds are open, architecturally well-defined assemblies composed of protein subunits or domains (Fig. 5a), designed to spatially organize functional components such as enzymes, substrates, and signaling molecules[96-100]. They serve as structural frameworks that facilitate multivalent interactions, enhance catalytic efficiency, and enable precise spatial control within synthetic or natural biological

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systems[101, 102]. One example involves the use of an engineered tetratricopeptide repeat affinity protein (TRAP) as a scaffold for biocatalysis[103]. TRAP domains are genetically fused and programmed to selectively and orthogonally recognize peptide tags attached to enzymes, enabling the spatial organization of metabolic ensembles upon binding. Additionally, these scaffolds are encoded with binding sites that, through electrostatic interactions, selectively and reversibly sequester reaction intermediates such as cofactors, thereby increasing their local concentration and enhancing catalytic efficiency. This strategy has been applied to the biosynthesis of amino acids and amines involving up to three enzymes, resulting in a fivefold increase in specific productivity compared to non-scaffolded systems[104]. In another case, a protein scaffold system based on SH3-GBDPDZ (SGP)[12, 105] was used to assemble CYP82D26, P450 reductase, and an $NADP^+$-dependent aldehyde reductase into a multi-enzyme complex. The close proximity of catalytic centers, together with the electrostatic interaction between NADPH and the SGP scaffold, generated a "NADPH channeling effect," significantly improving the catalytic efficiency of the P450 enzyme. When this SGP scaffold system was expressed in *Escherichia coli*, it enabled the production of 240.5 mg/L daidzein, with a conversion rate of 86% for (2S)-naringenin, representing a 9-fold increase over the free-expressed P450 enzyme[106]. Moreover, based on the CipB scaffold protein from *Photorhabdus luminescens*[107], an electron conduction strategy was developed. The CipB protein was used to assemble multiple enzymes into a functional protein crystalline inclusion (PCI), thereby physically co-localizing enzymes involved in biosynthetic pathways[13]. This arrangement facilitated efficient electron transfer between P450s and reductases and enabled the engineered strain to produce high levels of lutein, (+)-nootkatone, apigenin, and L-DOPA[13]. In summary, utilizing protein scaffolds to co-localize multiple enzymes in close proximity can trigger substrate channelingand enhance electron transfer efficiency[34, 108] (Fig. 5b), thereby achieving efficient cofactor utilization[106], improving reaction rates[34, 109], and ultimately enabling spatial regulation and cascade catalysis[34, 110].

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## Protein droplets and Membraneless Organelles

Protein droplets, a condensed, dynamic, and reversible assembly of proteins, typically formed through liquid–liquid phase separation[111, 112], and membraneless organelles (MLOs), a cellular compartment that lacks a surrounding lipid membrane but still organizes specific biomolecules in space and time[113], have emerged as major research foci in cell biology and synthetic biology. Although conceptually related, they represent distinct organizational levels driven by multivalent weak interactions through LLPS[114]. Protein droplets emphasize the underlying physical process and its dynamic reversibility, whereas MLOs represent evolved, functional cellular structures built upon this principle. Functionally, protein droplets perform diverse roles in cells: under heat, oxidative, or toxic stress, they transiently sequester translation-suppressed mRNAs, thereby facilitating cellular adaptation. In these contexts, droplets provide spatial advantages such as molecular enrichment, accelerated reaction kinetics, and redistribution of metabolic fluxes[111, 112]. Conversely, aberrant droplet formation is closely linked to disease. In neurodegenerative disorders such as ALS and FTD, RNA-binding proteins including FUS, TDP-43, and hnRNPA1 fail to dissolve after stress, hardening into gel-like or fibrillar states that ultimately give rise to insoluble pathological inclusions[26, 56, 115-117]. In cancer biology, transcription factors such as Myc form droplet-like assemblies within the nucleus, locally concentrating transcriptional machinery to drive oncogene overexpression[118]. From an engineering perspective, researchers have begun to harness the dynamic controllability of protein droplets for designing artificial organelles. For instance, fusing intrinsically disordered regions (IDRs) to target enzymes can induce intracellular droplet formation, thereby enhancing metabolic flux. Artificial droplets constructed via FUS low-complexity domain (LCD) fusions have been shown to recruit specific enzyme cohorts in cells, markedly boosting the production of desired metabolites[119]. Moreover, controllable protein droplets have been explored as “molecular sponges” that capture and release drugs under defined conditions, highlighting their potential in therapeutic delivery[120].

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MLOs are a class of subcellular compartments recently discovered in eukaryotic cells[15]. They are primarily formed *via* LLPS of specific proteins or nucleic acid macromolecules[113] (Fig. 6a),. Lacking a surrounding lipid membrane, MLOs exhibit enhanced dynamic properties for material exchange and responsiveness to physiological stimuli, thereby playing vital roles in cellular processes[121, 122]. Researchers have exploited the phase separation capability of IDPs to reconstruct a membraneless organelle in *Saccharomyces cerevisiae* and developed two regulatory tools to control its size and mechanical properties. This system significantly improved methanol assimilation and 1-butanol production, while reducing the leakage of toxic intermediates and $CO_2$ emissions, thus offering a novel strategy for high-value chemical production in microbial cell factories[123]. Similarly, in the field of bioactive cargo delivery, scientists have engineered condensate vesicles formed via LLPS of cholesterol-modified DNA and histones. These vesicles lack a lipid bilayer and instead consist of a dense liquid shell enclosing an aqueous cavity. They can function as versatile carriers for diverse biotherapeutics, including viral particles, mRNA, cytokines, and peptides. Notably, such vesicles have been shown to enhance the delivery of oncolytic viruses and trigger potent antitumor immune responses in vivo[124]. In another example, a dipeptide copolymer was constructed to create a hydrophobic microenvironment suitable for intracellular biorthogonal catalysis in both natural cells and cell mimics. These copolymers not only effectively isolate and localize guest molecules but also significantly enhance enzymatic and photocatalytic efficiencies in aqueous environments, offering a new strategy for the stable dispersion and utilization of water-insoluble catalysts[125]. In summary, membraneless organelles assembled via protein-driven LLPS serve diverse roles in metabolic regulation by concentrating substrates and enzymes into locally enriched microenvironments, thereby accelerating reaction rates and minimizing side reactions[28] (Fig. 6b). In molecular delivery, their dynamic assembly and disassembly enable rapid responses to environmental cues and controllable transport of functional components[29] (Fig. 6c). Furthermore, by

modulating the spatial organization and reaction pathways of enzymes, MLOs facilitate the construction of multi-step catalytic platforms, highlighting their considerable potential in synthetic biology and artificial cell engineering[120, 126].

**Composite types**

In addition to classical artificial organelles based on protein cages, protein scaffolds, and membraneless organelles, composite organelles have emerged, such as protein cage–scaffold and membraneless organelle–scaffold systems[127-129](Fig. 7a). In cage–scaffold systems, protein subunits can be fused with interaction motifs to reassemble specific enzymes on the cage surface. For instance, a dodecahedral protein cage (Mi3)[130], derived from thermophilic aldolase, was engineered with SpyTag-based scaffolds to colocalize three lycopene biosynthetic enzymes, resulting in an 8.5-fold increase in lycopene production[131-133]. Similarly, "Mi3Chem" cages incorporating FKBP (FK506-binding protein)[134, 135]-Mi3 fusions recruited FRB (FKBP-rapamycin-binding domain) [134, 135]-tagged enzymes in response to rapamycin, creating a chemically inducible compartment that enhanced the specificity of deoxyviolacein biosynthesis by 2.6-fold[136]. In membraneless organelle–scaffold systems, phase-separating peptides are combined with protein interaction modules to recruit tagged enzymes into intracellular condensates[137]. For example, RGG-driven condensates[137, 138] integrated RIAD-RIDD (a short peptide interacting pair) interaction pairs[139] to assemble multiple enzymes from the 2'-fucosyllactose (2'-FL) pathway, increasing production by 2.4-fold and improving substrate conversion efficiency by 95%[140]. A similar strategy enhanced farnesene biosynthesis, with peptide-mediated enzyme clustering yielding higher titers than unassembled or condensate-free strains[139]. Together, these composite artificial organelles exemplify the advantages of protein self-assembly in metabolic engineering, enabling spatial confinement, minimizing intermediate loss or toxicity, and boosting pathway flux by locally enriching enzymes and substrates (Fig. 7b).

Table 3 Classification basis and characteristics of protein self-assembled artificial organelles

| Category | Classification Basis | Structural Features | Assembly Formations | Functional Characteristics | Application Highlights | References |
|---|---|---|---|---|---|---|
| Protein cages | Enclosed shell structure | Sphere<br>Polyhedral closed nanoshell | Icosahedral protein shell; multivalent scaffold protein CsoS2 | Physical isolation<br>Molecular encapsulation<br>Drug delivery | Visible-light-driven hydrogen evolution with enhanced stability | 141-143 |
| Protein scaffolds | Open framework structure | Fibrous<br>Rod-like<br>Mesh-like open architectures | Modular protein–protein interactions; electrostatic NADPH channeling | Enzymatic cascade reactions<br>Metabolic pathway optimization | 9-fold increase in daidzein production in *E. coli* | 12, 106, 144 |
| Membraneless organelles/ Protein Droplets | Driven by liquid–liquid phase separation (LLPS) | Lack fixed boundaries<br>Dynamic droplets<br>Condensates | LLPS of IDPs with tunable viscosity | Enzyme enrichment<br>Substrate isolation | Improved 1-butanol titers, reduced $CO_2$ emission | 14, 123, 145 |
| Composite types | Combining protein scaffolds and protein cages, or membraneless organelles | Combining protein scaffolds and protein cages, or membraneless organelles | Hybrid cage–scaffold fusion, inducible interactions | Combining protein scaffolds and protein cages, or membraneless organelles | 8.5-fold increase in lycopene biosynthesis; inducible pathway specificity | 131-133, 146, 147 |

Table 4 Latest Advances in Protein Self-Assemblies

| Assembly Type | Emerging Applications | Key Advances | Assembly Mechanism / Driving Force | References |
|---|---|---|---|---|
| Protein Cages | Drug delivery and targeted sensing | Metal ion-driven self-assembly constructs highly stable protein cages | Metal ion coordination, non-covalent interactions | 47, 148 |
| | Molecular capture and complex assembly | Programmable recruitment of different molecules for targeted delivery and biosensing | Electrostatic interactions, shape complementarity | 149, 150 |
| | Nanomaterial design | Study of multi-scale assembly behavior provides theoretical basis for nanomaterials and drug delivery | Hydrophobic and van der Waals interactions | 151, 152 |
| Protein Scaffolds | Multi-enzyme catalysis and synthetic biology | Programmable scaffold assembly generates multi-enzyme complexes | Genetic encoding, modular domain interactions | 7, 153 |
| | Hierarchical material construction | Protein scaffolds from *Methanococcus jannaschii* form extracellular hierarchical structures via disulfide bonds | Disulfide bonds, hydrogen bonding, shape complementarity | 154, 155 |
| | Biomaterial fabrication | Self-assembled protein scaffolds form robust macroscopic materials for biomaterial development | Covalent and non-covalent interactions, modular assembly | 156, 157 |

| Assembly Type | Emerging Applications | Key Advances | Assembly Mechanism / Driving Force | References |
|---|---|---|---|---|
| Membraneless Organelles/Protein Droplets | Intracellular organization and functional mimicry | Genetically encoded polypeptides form membraneless organelles in *E. coli*, demonstrating liquid-liquid phase separation | Multivalent weak interactions, liquid-liquid phase separation (LLPS) | 158, 159 |
| | Disease research and physiological mechanisms | Membraneless organelles play key roles in health and disease | Electrostatic, hydrophobic, and π–π interactions | 15, 160 |
| | RNA-mediated assembly | Novel architectural RNAs (arcRNAs) play critical roles in the assembly of membraneless organelles | RNA-protein interactions, multivalency | 161 |
| Composite types | Protein cage + scaffold for modular nanostructures | Combining protein cages with scaffolds enables controlled assembly of multi-component nanostructures for drug delivery and biosensing | Cage-scaffold docking, shape complementarity, metal coordination | 162, 163 |
| | Membraneless organelle + scaffold for functional compartmentalization | Scaffolds guide membraneless organelle assembly, improving spatial organization and enzymatic reaction efficiency | LLPS, scaffold-mediated multivalency, electrostatic/hydrophobic interactions | 131-133, 146, 147 |

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## Challenges and Future Directions

Artificial organelles constructed through protein self-assembly represent an emerging strategy for organelle engineering, demonstrating great potential in fields such as synthetic biology, metabolic engineering, and drug delivery. However, this technology is still in its early stages and faces numerous challenges that hinder its widespread application and in-depth investigation. Meanwhile, advances in synthetic biology and artificial intelligence are offering new opportunities to address these challenges.

### Challenges

Firstly, the structural design and control of protein self-assembly remain major bottlenecks[164]. The self-assembly process relies on highly specific interactions between protein molecules, such as hydrophobic interactions, electrostatic forces, hydrogen bonding, and van der Waals forces[165]. However, precise regulation of these non-covalent interactions is extremely challenging in practice[164, 166]. For example, a single amino acid mutation can destabilize or inactivate the entire assembled structure[167]. Additionally, self-assembled architectures are often complex, with spatial configurations and scales that are difficult to predict and fine-tune, which limits the consistency and reproducibility of structure-function relationships[168]. Therefore, a key challenge in current design strategies is how to precisely guide protein self-assembly while preserving biological activity. Secondly, the integration and regulation of functional modules add to the complexity. Natural organelles possess sophisticated compartmentalization and responsive mechanisms. Reproducing similar functional integration within protein-based artificial organelles remains a significant challenge. For instance, in multi-enzyme cascade reactions, it is necessary to precisely control the spatial localization, concentration, and sequential activity of each enzyme[169]. However, in membraneless protein self-assembly systems, these processes are highly susceptible to diffusion limitations and uneven spatial distribution. Responsive regulatory mechanisms (e.g., those triggered by pH, temperature, ions, or signaling molecules)

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currently lack universal design strategies[170]. Without the capacity for dynamic response and controllable activation, artificial organelles may struggle to adapt flexibly to the demands of complex biological systems. Furthermore, issues concerning stability and biocompatibility in physiological environments remain to be addressed. Although proteins are inherently biodegradable, their self-assembled structures are vulnerable to enzymatic degradation, oxidative stress, and pH fluctuations within cellular or in vivo environments, potentially leading to disassembly or functional loss[171, 172]. In addition, some engineered proteins may trigger immune responses or cytotoxicity, compromising their safety and longevity in biological applications[173, 174]. Thus, developing more stable and minimally invasive self-assembling units is a critical step toward advancing their translation into clinical and biomedical contexts.

**Future Directions**

Achieving a balance between structural assimilability and functional compatibility, as well as reconciling in vitro controllability with in vivo responsiveness, calls for the close integration of structural biology, computational design, and synthetic biolog[175, 176]. With continuous advances in cryo-electron microscopy, structural biologists are uncovering novel assemblies at the microscopic level. For example, through structural analysis and phylogenetic tree construction, a citrate synthase from *Synechococcus elongatus* was discovered to self-assemble into a Sierpinski triangle[177]. This is the first known protein in nature capable of forming a regular fractal pattern, significantly expanding our understanding of the structural diversity of protein molecules and providing a structural foundation for the construction of finely tunable protein assemblies. In addition, the development of computational biology and de novo protein design technologies has enabled researchers to create proteins with diverse functions and controllable assembly behaviors[178]. For instance, by mimicking silk protein domains and introducing histidine residues, a protein was rationally designed to undergo a sharp and reversible transition from assembly to disassembly within a narrow pH range of 0.3 units[179]. Upon acidification, the fibers disassembled in less than one

second. Cryo-EM structural analysis revealed that the optimized design closely matched the computational model in both subunit geometry and packing within the fiber, offering new avenues for developing controllable and highly compatible protein self-assembled organelles[180]. Taken together, although protein self-assembly serving as a bridge between biology and engineering still faces substantial challenges in advancing material science, medicine, and nanotechnology into new dimensions, the increasing convergence of multiple disciplines holds promise. In the future, this field is expected to move beyond simply "mimicking nature" to "surpassing nature," providing an innovative engine for sustainable development and precision medicine.

## Conclusions

In this review, the molecular mechanisms of protein self-assembly are comprehensively summarized, including hydrophobic interactions, electrostatic interactions, as well as other weak forces such as van der Waals interactions, hydrogen bonding, π–π stacking, and metal coordination. Based on differences in structural features and assembly modes, protein self-assembled organelles are classified into four major categories: protein cages, protein scaffolds, membraneless organelles, and composite types. The recent advances in applying these artificial organelles in areas such as enzymatic catalysis and metabolic engineering are systematically reviewed. Furthermore, the current challenges associated with protein self-assembly particularly in terms of controllable assembly, biocompatibility, and translational application are discussed. Finally, this review highlights the promising opportunities offered by integrating artificial intelligence and de novo protein design to address these challenges and advance the development of protein based artificial organelles.

## Author contributions

J. Sun: Conceptualization, Methodology, Investigation, Writing-original draft, Writing-review & editing. R. Gao: Conceptualization, Methodology, Writing-review & editing. Z. Yang: Conceptualization, Methodology. Z. Deng: Review & editing. L. Qin: Investigation, Review & editing. H. Jia: Investigation, Review & editing. C. Li:

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**Conflicts of interest**

The authors declare no other financial or commercial conflict of interest.

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## Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.