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

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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.<sup>1,2</sup> Among them, proteins, as essential biological macromolecules, can spontaneously form a variety of ordered supramolecular structures, such as viral capsids, bacterial microcompartments and actin filaments, through self-interactions.<sup>3,4</sup> 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 to mimic the functions of natural organelles, such as energy metabolism,<sup>5</sup> material transport,<sup>6</sup> and molecular synthesis.<sup>7,8</sup> 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.<sup>2,9</sup>

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 the micrometer level through diverse interactions, such as hydrogen bonding, hydrophobic interactions, electrostatic forces, and covalent modifications, among amino acid residues. More importantly, protein molecules often carry intrinsic catalytic, recognition, or signaling domains, enabling the realization of complex biological functions during the assembly process.<sup>10,11</sup> 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, and temperature) or external signals, thereby providing greater adaptability in the construction of artificial organelles.<sup>12,13</sup> 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 the 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. 1 and Table 3), and we highlight the recent progress for each. In addition, we discuss the key challenges currently faced by the

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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 and 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 and 13
Dynamics & responsiveness	High fluidity, reversible phase transitions, and rapid morphology change under stimuli	Programmable, reversible assembly/disassembly, functional regulation under cues	14 and 15
Applications & scalability	Clinically established (liposomes, LNPs) with robust stability	Engineered as artificial organelles or nanoreactors; customizable <i>via</i> synthetic biology	16

field and propose potential strategies to address them, with the aim of offering new insights and future directions for the design and application of protein-based artificial organelles.

## Non-covalent interactions in protein self-assembly

Non-covalent interactions among proteins constitute the basis of biological macromolecular organization, spanning reversible and tunable microscopic pairings to large-scale supramolecular

assemblies.<sup>17</sup> Their fundamental kinetic and thermodynamic properties are governed by a superimposable set of noncovalent force fields,<sup>18</sup> including hydrophobic interactions, electrostatic interactions, hydrogen bonding, van der Waals forces, aromatic  $\pi$ - $\pi$  stacking, and metal coordination.<sup>11</sup>

## Hydrophobic interactions

Hydrophobic interactions in protein self-assembly are generally responsible for the “primary attraction” and solvent-exclusion-driven desolvation process<sup>19</sup> (Fig. 2a). Recent studies have

Table 2 Major mechanisms 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 and 76
Electrostatic interactions	Lys/Arg-rich regions, RNA-protein charge complementarity	Control capsid size, RNA loading, cofactor guidance and channeling	32, 34, 35 and 77
Hydrogen bonds/van der Waals forces	Designed interfacial H-bond networks, tight packing interfaces	Provide high-fidelity pairing, stabilize geometry, and determine nanocage symmetry	37–40 and 47
$\pi$ - $\pi$ /non classical weak interactions	Aromatic residues, CH- $\pi$ , S- $\pi$	Drive fibrillation, influence optical/mechanical properties	42 and 43
Metal-ligand interactions	Zn <sup>2+</sup> -His, Ni <sup>2+</sup> -His, and Fe <sup>2+</sup> /Fe <sup>3+</sup>	Construct reversible lattices/repairable networks, enable catalysis or electron transport	44 and 45
Disulfide bonds	Cys-Cys crosslinks	Enhance stability, enable environmentally selective depolymerization (redox control)	50, 51 and 78
Thiol-ene click reactions	Thiol/alkene functionalization + photo/thermal initiation	Rapid and controllable crosslinking, <i>in situ</i> curing, and drug-delivery carriers	57, 58 and 79
Schiff base reactions	Amine-aldehyde forming imine	Reversible self-healing/stimuli-responsive hydrogels, controlled release	59, 60, 63 and 67
Enzyme-mediated covalent bonds	SpyTag/SpyCatcher and transglutaminase	Highly selective site-specific linkage, modular assembly	47, 68 and 70



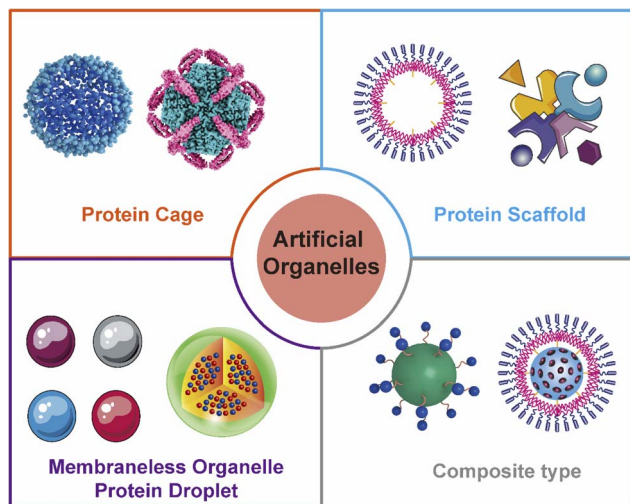


Fig. 1 Four classical categories of protein self-assembled artificial organelles.

highlighted that the ordering of water molecules, altered solvent dynamics, and interfacial dewetting play pivotal roles in this hydrophobic driving force.<sup>20</sup> 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.<sup>21,22</sup> 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.<sup>23–25</sup> Subsequent local structuring and accumulation of van der Waals contacts increase droplet viscoelasticity, driving a transition toward a gel-like state.<sup>26,27</sup> 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.<sup>28,29</sup> 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.<sup>23–25,30</sup> 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<sup>31</sup> (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.<sup>32</sup> 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 sizes and cargo capacities.<sup>33</sup> Conversely, tuning the overall isoelectric point or introducing localized anionic groups can preserve reversible assembly and disassembly under high ionic strength conditions.<sup>33</sup> 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.<sup>34,35</sup> 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

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, polyhedral closed nanoshell	Icosahedral protein shell; multivalent scaffold protein CsoS <sub>2</sub>	Physical isolation, molecular encapsulation, drug delivery	Visible-light-driven hydrogen evolution with enhanced stability	141–143
Protein scaffolds	Open-framework structure	Fibrous, rod-like, mesh-like open architectures	Modular protein–protein interactions; electrostatic NADPH channeling	Enzymatic cascade reactions, metabolic pathway optimization	9-Fold increase in daidzein production in <i>E. coli</i>	12, 106 and 144
Membraneless organelles/protein droplets	Driven by liquid–liquid phase separation (LLPS)	Lack fixed boundaries, dynamic droplets, condensates	LLPS of IDPs with tunable viscosity	Enzyme enrichment, substrate isolation	Improved 1-butanol titers, reduced CO <sub>2</sub> emission	14, 123 and 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 and 147



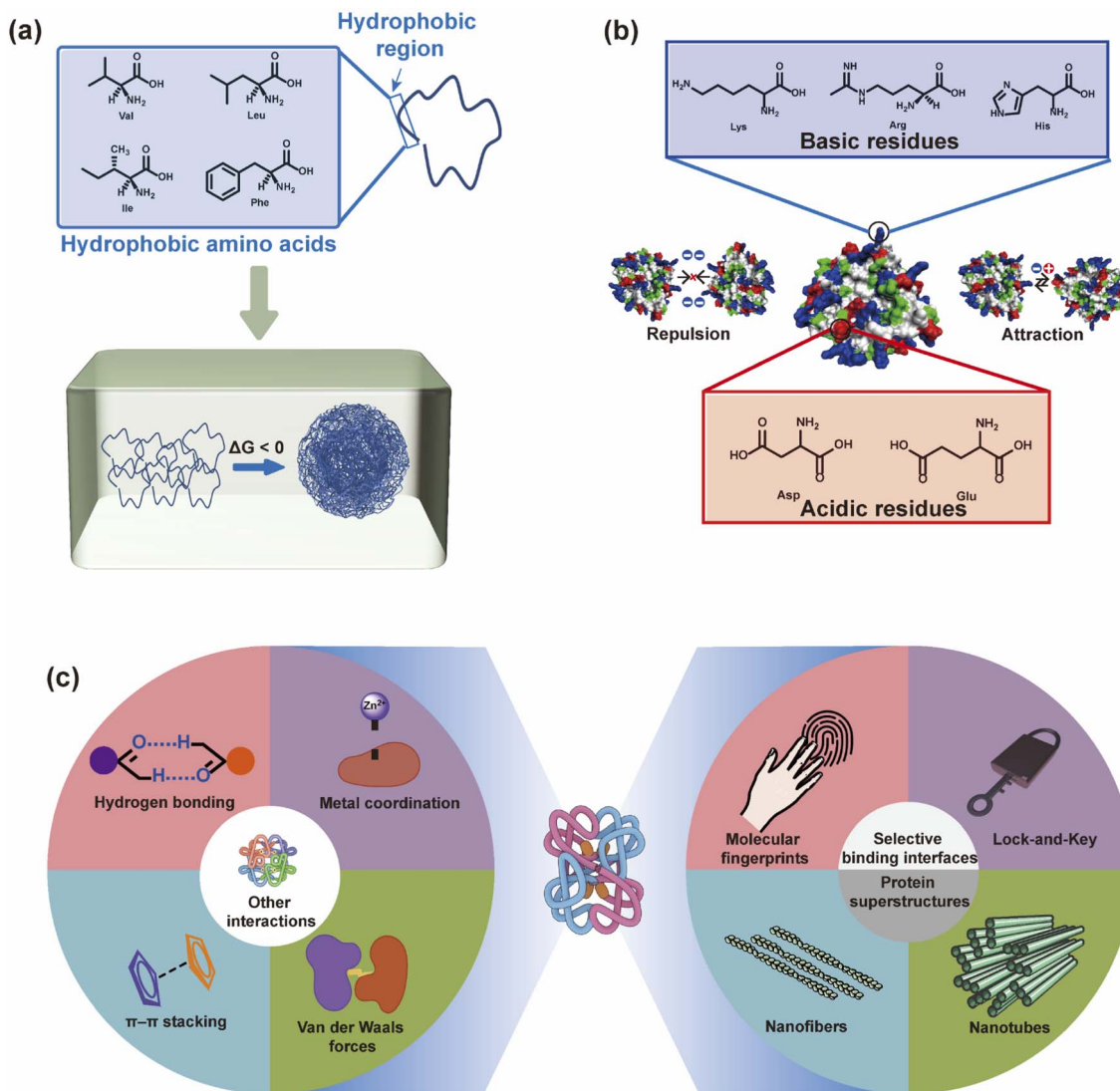


Fig. 2 Non-covalent interactions in protein self-assembly. (a) Proteins generating hydrophobic domains composed of hydrophobic residues, which drive their self-assembly through hydrophobic interactions; (b) protein self-assembly mediated by electrostatic interactions, which originate from the attractive and repulsive forces among acidic and basic amino acid residues; and (c) protein self-assembly governed by a range of non-covalent interactions, including hydrogen bonds, van der Waals forces,  $\pi$ - $\pi$  stacking, and metal coordination.

desired assembly state under varying environmental conditions.<sup>36</sup>

## 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<sup>37–40</sup> (Fig. 2c). In the design of isomeric 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*-designed protein nanocages, modular hydrogen-bonding motifs have been employed to constrain relative subunit orientations,

thereby imposing predetermined symmetry and pore architecture.<sup>41</sup> Moreover,  $\pi$ - $\pi$  stacking between aromatic residues confers 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.<sup>42</sup> Recent studies further indicate that certain “non-canonical” noncovalent interactions, such as CH- $\pi$  and S- $\pi$  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.<sup>43</sup> Metal coordination, as a semi-covalent and highly directional interaction, offers unique advantages in reversibility and functionalization. Metal-ligand interactions (*e.g.*, Zn<sup>2+</sup>-His, Ni<sup>2+</sup>-His, and Fe<sup>2+</sup>/Fe<sup>3+</sup> with multidentate ligands) not only stabilize the geometric arrangement of multiple subunits but





also introduce externally controllable switches,<sup>44</sup> such as pH or ligand-induced reversible disassembly, enabling the construction of responsive nanocages, crystallized protein arrays, or ordered two- and three-dimensional superlattices.<sup>45</sup> Bioinspired examples, including shell and byssus matrices, demonstrate that metal-amino acid coordination can support repairable, self-healing supramolecular networks under aqueous conditions.<sup>46</sup> In artificial systems, metal-directed self-assemblies have been leveraged to enhance the structural stability of protein cages and to impart catalytic or electronic transport functionality.<sup>47</sup>

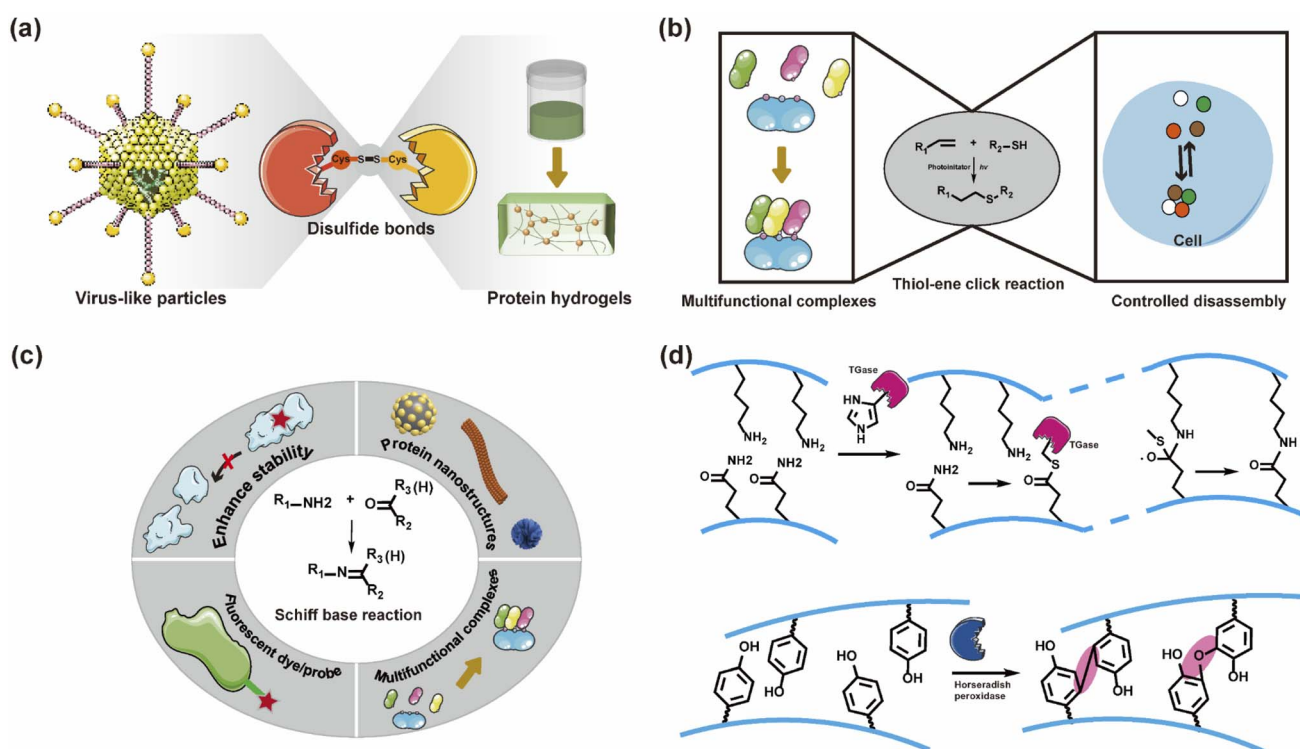
## Covalent interactions in protein self-assembly

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.<sup>48</sup> 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.<sup>49</sup>

## Disulfide bonds

Disulfide bonds (Cys–Cys) are critical molecular scaffolds and regulators. Their function primarily relies on reversible redox chemistry<sup>50</sup> (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.<sup>51</sup> 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<sup>52</sup> (Fig. 3a). Moreover, the reversibility of disulfide bonds allows protein assemblies to respond dynamically to environmental cues, such as pH, temperature, or redox conditions.<sup>53</sup> 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.<sup>54</sup> 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  $\beta$ -amyloid peptides may promote



**Fig. 3** Covalent interactions in protein self-assembly. (a) Disulfide bonds playing a crucial role in the assembly of protein complexes and protein hydrogels; (b) thiol-ene click reactions playing a crucial role in the assembly of protein complexes and controlled disassembly; (c) various protein nanostructures constructed *via* Schiff-base reactions, enabling the assembly of structurally stable protein complexes and the preparation of protein-based fluorescent probes; and (d) transglutaminase (TGase)-mediated amide-bond formation, tyrosinase/peroxidase-mediated dityrosine formation, and horseradish peroxidase (HRP)-catalyzed dityrosine formation in the presence of hydrogen peroxide.



aggregation into amyloid plaques, contributing to neurotoxicity.<sup>55,56</sup> 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<sup>57</sup> (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.<sup>58</sup> 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<sup>59</sup> (Fig. 3b). Moreover, the reversible nature of the reaction allows the 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.<sup>59,60</sup> This strategy also allows the crosslinking of proteins with distinct functionalities into multifunctional complexes. For instance, proteins endowed with catalytic activity and receptor-recognition capability can be linked *via* thiol-ene click reactions to generate dual-functional protein complexes.<sup>61</sup> Such assemblies hold potential for applications in biosensing, drug delivery, and beyond.<sup>61</sup> Additionally, studies have shown that protein nanostructures constructed *via* this reaction exhibit remarkable stability under elevated temperatures and extreme pH conditions.<sup>62</sup> The introduction of covalent crosslinks can further modulate protein functionality by enhancing catalytic activity or altering receptor recognition, offering new avenues for functional studies of proteins.<sup>57</sup>

## Schiff base reactions

Schiff base reactions refer to the condensation of amines with aldehydes or ketones to form imine (Schiff base) structures<sup>63</sup> (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.<sup>64</sup> By establishing stable connections between protein subunits, the Schiff base reaction enhances the structural stability of the resulting complexes.<sup>65</sup> For example, this strategy has been used to link distinct protein units, yielding protein nanostructures that exhibit remarkable stability under diverse environmental conditions.<sup>65</sup> 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.<sup>66</sup> 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<sup>48</sup> (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 the 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.<sup>67</sup> This spatially controlled assembly offers new opportunities for the functionalization of protein complexes.<sup>64</sup>

## 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<sup>68</sup> (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<sup>69</sup> (Fig. 3d). For instance, TGases catalyze the formation of covalent amide bonds between the  $\gamma$ -carboxamide group of glutamine residues and the  $\epsilon$ -amino group of lysine residues, generating stable isopeptide linkages.<sup>69</sup> The high specificity and irreversibility of this reaction provide distinct advantages in the construction of 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.<sup>70</sup> This strategy has been applied to the development of biomedical hydrogels, such as cell scaffolds and drug delivery matrices, and it shows potential for use 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.<sup>71</sup>

Another widely used strategy involves tyrosinase- or peroxidase-mediated dityrosine formation.<sup>72,73</sup> 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.<sup>74</sup> 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<sup>72</sup> (Fig. 3d). For instance, introducing multiple tyrosine residues into elastin-like polypeptides (ELPs) allows the HRP-mediated rapid formation of physically robust and highly elastic hydrogels.<sup>75</sup> These hydrogels exhibit excellent performance in tissue engineering and drug-delivery applications and also hold promise for use as 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.<sup>72</sup>

## 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.<sup>80</sup> These structures are characterized by uniform size, defined geometry, chemical and genetic modifiability, and excellent biocompatibility<sup>10,81–83</sup> (Fig. 4a). Carboxysomes, a class of protein-based organelles with

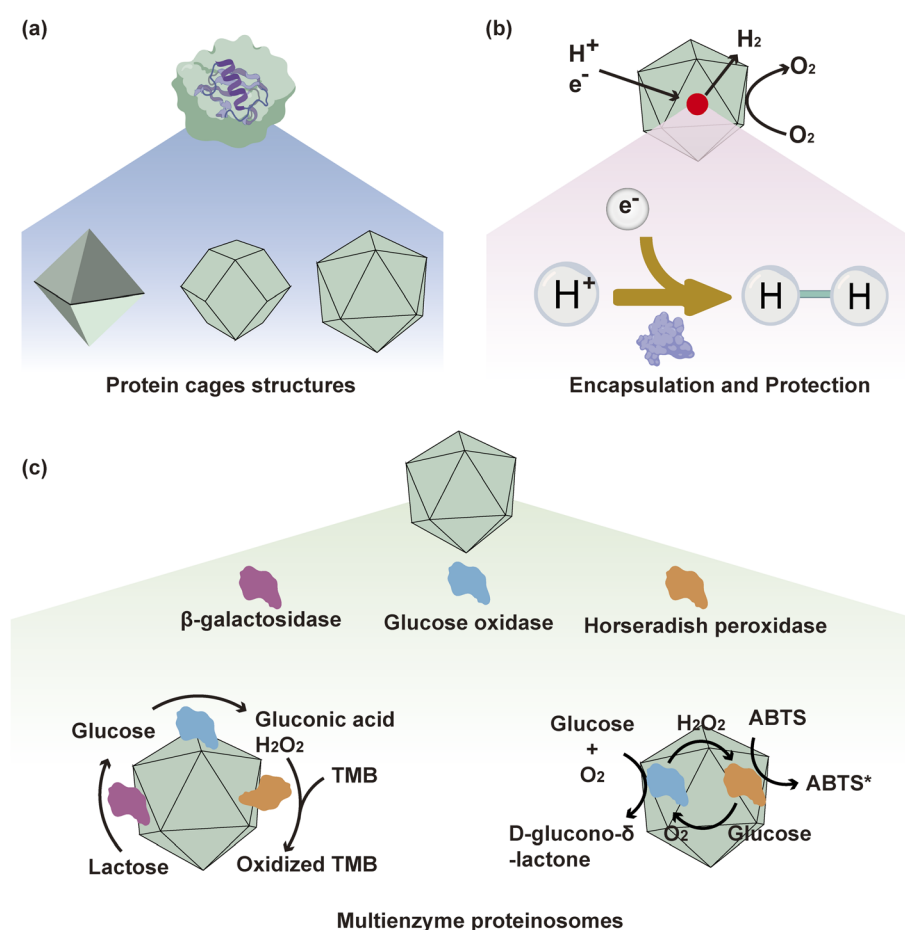


Fig. 4 Protein cage structures and applications. (a) Closed-shell nanostructures formed through the self-assembly of protein subunits; (b) encapsulation of the hydrogen-producing reaction within a protein cage to shield it from oxygen; and (c) construction of multi-enzyme cascade systems to enhance the catalytic efficiency.



icosahedral architecture, exemplify such assemblies.<sup>84</sup> They provide a stable anaerobic microenvironment and facilitate the precise encapsulation of cargo proteins through the action of a protein known as CsoS<sub>2</sub>, thereby protecting oxygen-sensitive enzymes from inactivation.<sup>85–88</sup> In one notable study, [FeFe]-hydrogenase was encapsulated within a carboxysome to enhance its oxygen tolerance and catalytic efficiency.<sup>89</sup> Furthermore, TBAP- $\alpha$  was employed as a photosensitizer, non-covalently associating with the carboxysome–hydrogenase complex in solution.<sup>90</sup> This strategy enabled the construction of a novel photo-enzyme nanoreactor capable of visible-light-driven hydrogen evolution with high efficiency<sup>90</sup> (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.<sup>91,92</sup> Hydrophobic interaction-driven self-assembly yielded multi-enzyme protein compartments bearing  $\beta$ -galactosidase ( $\beta$ -gal), glucose oxidase (GO<sub>x</sub>), and horseradish peroxidase (HRP) on defined surfaces, mimicking organelle-like architectures and enhancing both mass transfer and catalytic efficiency<sup>93,94</sup> (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,<sup>89</sup> protection,<sup>95</sup> (Fig. 4b), and formation of multienzyme proteinosomes<sup>93,94</sup> (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.<sup>96–100</sup> They serve as structural frameworks that facilitate multivalent interactions, enhance catalytic efficiency, and enable precise spatial control within synthetic or natural biological systems.<sup>101,102</sup> One example involves the use of an engineered tetratricopeptide repeat affinity protein (TRAP) as a scaffold for biocatalysis.<sup>103</sup> 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 the results for non-scaffolded systems.<sup>104</sup> In another case, a protein scaffold system based on SH3-GBDPDZ (SGP)<sup>12,105</sup> was used to assemble CYP82D26, P450 reductase, and an NADP<sup>+</sup>-dependent aldehyde reductase into a multi-enzyme complex. The proximity of the catalytic centers, together with the electrostatic interaction between NADPH and the SGP scaffold, generated an “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<sup>-1</sup> daidzein, with a conversion rate of 86% for (2*S*)-naringenin, representing a 9-fold increase over the free-expressed P450 enzyme.<sup>106</sup> Moreover, based on the CipB scaffold protein from *Photobacterium luminescens*,<sup>107</sup> 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

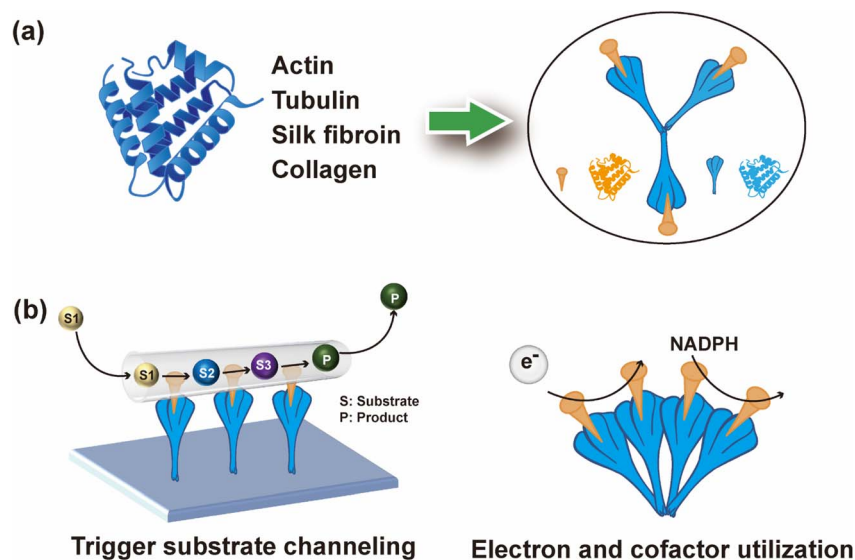


Fig. 5 Protein scaffold structures and applications. (a) Protein subunits or domains forming open structural frameworks through molecular recognition; (b) protein scaffolds improving the catalytic performance by promoting substrate channeling and enhancing the transfer of electrons and cofactors.





biosynthetic pathways.<sup>13</sup> 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.<sup>13</sup> In summary, utilizing protein scaffolds to co-localize multiple enzymes in proximity can trigger substrate channeling and enhance electron-transfer efficiency<sup>34,108</sup> (Fig. 5b), thereby achieving efficient cofactor utilization,<sup>106</sup> improving reaction rates,<sup>34,109</sup> and ultimately enabling spatial regulation and cascade catalysis.<sup>34,110</sup>

## Protein droplets and membraneless organelles

Protein droplets, a condensed, dynamic, and reversible assembly of proteins, typically formed through liquid–liquid phase separation,<sup>111,112</sup> and membraneless organelles (MLOs), a cellular compartment that lacks a surrounding lipid membrane but still organizes specific biomolecules in space and time,<sup>113</sup> 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.<sup>114</sup> 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.<sup>111,112</sup> Conversely, aberrant droplet formation is closely linked to diseases. 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.<sup>26,56,115–117</sup> In cancer biology, transcription factors, such as Myc, form droplet-like assemblies within the nucleus, locally concentrating transcriptional machinery to drive oncogene overexpression.<sup>118</sup> 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.<sup>119</sup> Moreover, controllable protein droplets have been explored as “molecular sponges” that capture and release drugs under defined conditions, highlighting their potential in therapeutic delivery.<sup>120</sup>

MLOs are a class of subcellular compartments recently discovered in eukaryotic cells.<sup>15</sup> They are primarily formed *via* LLPS of specific proteins or nucleic acid macromolecules<sup>113</sup> (Fig. 6a). Lacking a surrounding lipid membrane, MLOs exhibit enhanced dynamic properties for material exchange and

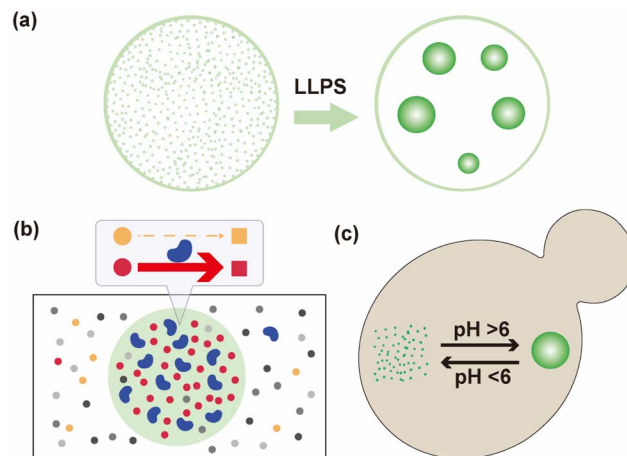


Fig. 6 Membraneless organelle structures and applications. (a) Protein subunits or domains forming membraneless organelles through LLPS; (b) membraneless organelles bring substrates and enzymes together, enhancing mass transfer and thereby increasing the reaction rate; and (c) membraneless organelles undergoing dynamic self-assembly in cellular factories in response to changes in pH.

responsiveness to physiological stimuli, thereby playing vital roles in cellular processes.<sup>121,122</sup> 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<sub>2</sub> emissions, thus offering a novel strategy for high-value chemical production in microbial cell factories.<sup>123</sup> 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*.<sup>124</sup> 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.<sup>125</sup> 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<sup>28</sup> (Fig. 6b). In molecular delivery, their dynamic assembly and disassembly enable rapid responses to environmental cues and the controllable transport of functional components<sup>29</sup> (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.<sup>120,126</sup>

## 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<sup>127–129</sup> (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),<sup>130</sup> 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.<sup>131–133</sup> Similarly, “Mi3Chem” cages incorporating FKBP (FK506-binding protein)<sup>134,135</sup>-Mi3 fusions recruited FRB (FKBP-rapamycin-binding domain)<sup>134,135</sup>-tagged enzymes in response to rapamycin, creating a chemically inducible compartment that enhanced the specificity of deoxyviolacein biosynthesis by 2.6-fold.<sup>136</sup> In membraneless organelle-scaffold systems, phase-separating peptides are combined with protein interaction modules to recruit tagged enzymes into intracellular

condensates.<sup>137</sup> For example, RGG-driven condensates<sup>137,138</sup> integrated RIAD-RIDD (a short peptide interacting pair) interaction pairs<sup>139</sup> to assemble multiple enzymes from the 2'-fucosyllactose (2'-FL) pathway, increasing production by 2.4-fold and improving substrate conversion efficiency by 95%.<sup>140</sup> A similar strategy enhanced farnesene biosynthesis, with peptide-mediated enzyme clustering yielding higher titers than unassembled or condensate-free strains.<sup>139</sup> 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 and Table 4).

## 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.

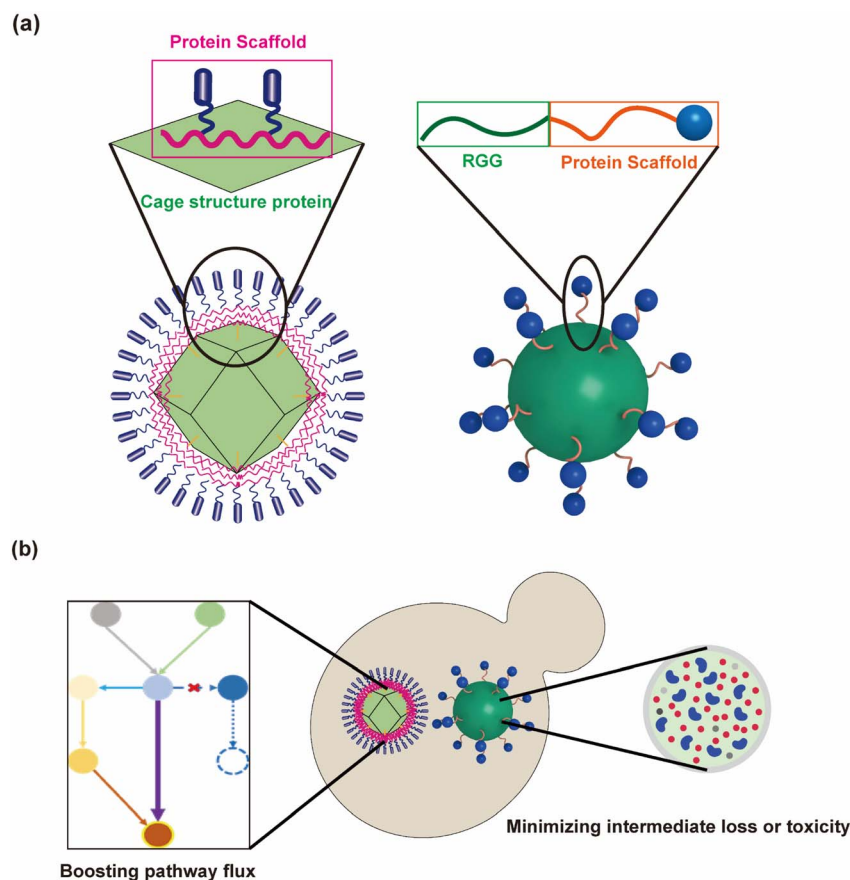


Fig. 7 Composite-type structures and applications. (a) Structures of composite organelles and (b) composite artificial organelles exemplifying the advantages of protein self-assembly in metabolic engineering.



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 and 148
	Molecular capture and complex assembly	Programmable recruitment of different molecules for targeted delivery and biosensing	Electrostatic interactions, shape complementarity	149 and 150
	Nanomaterial design	Study of multi-scale assembly behavior provides a theoretical basis for nanomaterials and drug delivery	Hydrophobic and van der Waals interactions	151 and 152
Protein scaffolds	Multi-enzyme catalysis and synthetic biology	Programmable scaffold assembly generates multi-enzyme complexes	Genetic encoding, modular domain interactions	7 and 153
	Hierarchical material construction	Protein scaffolds from <i>Methanococcus jannaschii</i> form extracellular hierarchical structures via disulfide bonds	Disulfide bonds, hydrogen bonding, shape complementarity	154 and 155
	Biomaterial fabrication	Self-assembled protein scaffolds form robust macroscopic materials for biomaterial development	Covalent and non-covalent interactions, modular assembly	156 and 157
Membraneless organelles/ protein droplets	Intracellular organization and functional mimicry	Genetically encoded polypeptides form membraneless organelles in <i>E. coli</i> , demonstrating liquid–liquid phase separation	Multivalent weak interactions, liquid–liquid phase separation (LLPS)	158 and 159
	Disease research and physiological mechanisms	Membraneless organelles play key roles in health and disease	Electrostatic, hydrophobic, and $\pi$ – $\pi$ interactions	15 and 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, and metal coordination	162 and 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 and 147

## Challenges

First, the structural design and control of protein self-assembly remain major bottlenecks.<sup>164</sup> 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.<sup>165</sup> However, the precise regulation of these non-covalent interactions is extremely challenging in practice.<sup>164,166</sup> For example, a single amino acid mutation can destabilize or inactivate the entire assembled

structure.<sup>167</sup> 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.<sup>168</sup> 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.<sup>169</sup> 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) currently lack universal design strategies.<sup>170</sup> 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.<sup>171,172</sup> In addition, some engineered proteins may trigger immune responses or cytotoxicity, compromising their safety and longevity in biological applications.<sup>173,174</sup> 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 biology.<sup>175,176</sup> 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.<sup>177</sup> 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.<sup>178</sup> 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.<sup>179</sup> 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.<sup>180</sup> 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,  $\pi$ - $\pi$  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: funding acquisition, project administration, supervision, writing-original draft, writing-review & editing.

## Conflicts of interest

The authors declare no financial or commercial conflicts of interest.

## 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.

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## References

- 1 L. Li and G. Chen, Precise Assembly of Proteins and Carbohydrates for Next-Generation Biomaterials, *J. Am. Chem. Soc.*, 2022, **144**, 16232–16251.
- 2 H. Guo, J. C. Ryan, X. Song, A. Mallet, M. Zhang, V. Pabst, A. L. Decrulle, P. Ejsmont, E. H. Wintermute and A. B. Lindner, Spatial engineering of *E. coli* with addressable phase-separated RNAs, *Cell*, 2022, **185**, 3823–3837.
- 3 M. Du, F. Zeng, Y. Wang, Y. Li, G. Chen, J. Jiang and Q. Wang, Assembly and Functionality of 2D Protein Arrays, *Adv. Sci.*, 2025, **12**, 2416485.
- 4 A. Solomonov, A. Kozell and U. Shimanovich, Designing Multifunctional Biomaterials via Protein Self-Assembly, *Angew. Chem., Int. Ed.*, 2024, **63**, e202318365.
- 5 A. Belluati, S. Jimaja, R. J. Chadwick, C. Glynn, M. Chami, D. Happel, C. Guo, H. Kolmar and N. Bruns, Artificial cell synthesis using biocatalytic polymerization-induced self-assembly, *Nat. Chem.*, 2024, **16**, 564–574.
- 6 F. Eweje, V. Ibrahim, A. Shajii, M. L. Walsh, K. Ahmad, A. Alrefai, D. Miyasato, J. R. Davis, H. Ham and K. Li, Self-assembling protein nanoparticles for cytosolic delivery of nucleic acids and proteins, *Nat. Biotechnol.*, 2025, 1–14.
- 7 X. Gao, G. Tang, J. Yan, S. Fang, K. Tian, F. Lu and H.-M. Qin, Programmed protein scaffold for multienzyme assembly empowering the biosynthesis of rare sugars, *Chin. J. Catal.*, 2025, **72**, 95–105.
- 8 G. Zhang, M. B. Quin and C. Schmidt-Dannert, Self-assembling protein scaffold system for easy in vitro coimmobilization of biocatalytic cascade enzymes, *ACS Catal.*, 2018, **8**, 5611–5620.
- 9 H. Wang, Y. T. Zheng, J. Zhang, Y. Gao, J. Chen, P. Cai, J. Wang, J. H. van Esch, X. Guo and H. Li, Synthesis of Abiotic Supramolecular Polymers Inside Living Cells via Organocatalysis-Mediated Self-Assembly, *Angew. Chem., Int. Ed.*, 2025, **64**, e202500998.
- 10 Y.-T. Lai, E. Reading, G. L. Hura, K.-L. Tsai, A. Laganowsky, F. J. Asturias, J. A. Tainer, C. V. Robinson and T. O. Yeates, Structure of a designed protein cage that self-assembles into a highly porous cube, *Nat. Chem.*, 2014, **6**, 1065–1071.
- 11 Q. Song, Z. Cheng, M. Kariuki, S. C. L. Hall, S. K. Hill, J. Y. Rho and S. Perrier, Molecular self-assembly and supramolecular chemistry of cyclic peptides, *Chem. Rev.*, 2021, **121**, 13936–13995.
- 12 J. E. Dueber, G. C. Wu, G. R. Malmirchegini, T. S. Moon, C. J. Petzold, A. V. Ullal, K. L. J. Prather and J. D. Keasling, Synthetic protein scaffolds provide modular control over metabolic flux, *Nat. Biotechnol.*, 2009, **27**, 753–759.
- 13 S. Y. Park, H. Eun, M. H. Lee and S. Y. Lee, Metabolic engineering of *Escherichia coli* with electron channelling for the production of natural products, *Nat. Catal.*, 2022, **5**, 726–737.
- 14 S. F. Banani, H. O. Lee, A. A. Hyman and M. K. Rosen, Biomolecular condensates: organizers of cellular biochemistry, *Nat. Rev. Mol. Cell Biol.*, 2017, **18**, 285–298.
- 15 Y. Li, Y. Liu, X.-Y. Yu, Y. Xu, X. Pan, Y. Sun, Y. Wang, Y.-H. Song and Z. Shen, Membraneless organelles in health and disease: exploring the molecular basis, physiological roles and pathological implications, *Signal Transduction Targeted Ther.*, 2024, **9**, 305.
- 16 X. Hou, T. Zaks, R. Langer and Y. Dong, Lipid nanoparticles for mRNA delivery, *Nat. Rev. Mater.*, 2021, **6**, 1078–1094.
- 17 S. Boeynaems, S. Alberti, N. L. Fawzi, T. Mittag, M. Polymenidou, F. Rousseau, J. Schymkowitz, J. Shorter, B. Wolozin and L. Van Den Bosch, Protein phase separation: a new phase in cell biology, *Trends Cell Biol.*, 2018, **28**, 420–435.
- 18 T. M. Zeigler, M. C. Chung, O. P. Narayan and J. Guan, Protein phase separation: physical models and phase-separation-mediated cancer signaling, *Adv. Phys.:X*, 2021, **6**, 1936638.
- 19 B. J. Berne, J. D. Weeks and R. Zhou, Dewetting and hydrophobic interaction in physical and biological systems, *Annu. Rev. Phys. Chem.*, 2009, **60**, 85–103.
- 20 P. Liu, X. Huang, R. Zhou and B. J. Berne, Observation of a dewetting transition in the collapse of the melittin tetramer, *Nature*, 2005, **437**, 159–162.
- 21 G. A. Papoian, J. Ulander and P. G. Wolynes, Role of water mediated interactions in protein–protein recognition landscapes, *J. Am. Chem. Soc.*, 2003, **125**, 9170–9178.
- 22 M. Ahmad, W. Gu, T. Geyer and V. Helms, Adhesive water networks facilitate binding of protein interfaces, *Nat. Commun.*, 2011, **2**, 261.
- 23 Y. Lin, Y. Fichou, A. P. Longhini, L. C. Llanes, P. Yin, G. C. Bazan, K. S. Kosik and S. Han, Liquid-liquid phase separation of tau driven by hydrophobic interaction facilitates fibrillization of tau, *J. Mol. Biol.*, 2021, **433**, 166731.
- 24 S. Das, Y.-H. Lin, R. M. Vernon, J. D. Forman-Kay and H. S. Chan, Comparative roles of charge,  $\pi$ , and hydrophobic interactions in sequence-dependent phase separation of intrinsically disordered proteins, *Proc. Natl. Acad. Sci. U. S. A.*, 2020, **117**, 28795–28805.
- 25 G. Krainer, T. J. Welsh, J. A. Joseph, P. St George-Hyslop, A. A. Hyman, R. Collepardo-Guevara, S. Alberti and T. P. J. Knowles, Reentrant liquid condensate phase of proteins is stabilized by hydrophobic and non-ionic interactions, *Biophys. J.*, 2021, **120**, 28a.
- 26 S. Ray, N. Singh, R. Kumar, K. Patel, S. Pandey, D. Datta, J. Mahato, R. Panigrahi, A. Navalkar, S. Mehra, L. Gadhe, D. Chatterjee, A. S. Sawner, S. Maiti, S. Bhatia, J. A. Gerez, A. Chowdhury, A. Kumar, R. Padinhateeri, R. Riek, G. Krishnamoorthy and S. K. Maji,  $\alpha$ -Synuclein aggregation nucleates through liquid–liquid phase separation, *Nat. Chem.*, 2020, **12**, 705–716.
- 27 A. Agarwal, A. Chandran, F. Raza, I.-M. Ungureanu, C. Hilcenko, K. Stott, N. A. Bright, N. Morone, A. J. Warren and J. Lautenschläger, VAMP2 regulates phase separation of  $\alpha$ -synuclein, *Nat. Cell Biol.*, 2024, **26**(8), 1296–1308.



- 28 L. Wan, Y. Zhu, W. Zhang and W. Mu, Recent advances in design and application of synthetic membraneless organelles, *Biotechnol. Adv.*, 2024, 108355.
- 29 M. Yoshikawa and S. Tsukiji, Modularly Built Synthetic Membraneless Organelles Enabling Targeted Protein Sequestration and Release: Published as part of the Biochemistry virtual special issue "Protein Condensates", *Biochemistry*, 2021, **60**, 3273–3276.
- 30 N. B. Rego, E. Xi and A. J. Patel, Identifying hydrophobic protein patches to inform protein interaction interfaces, *Proc. Natl. Acad. Sci. U. S. A.*, 2021, **118**, e2018234118.
- 31 H.-X. Zhou and X. Pang, Electrostatic interactions in protein structure, folding, binding, and condensation, *Chem. Rev.*, 2018, **118**, 1691–1741.
- 32 R. F. Garmann, M. Comas-Garcia, M. S. T. Koay, J. J. L. M. Cornelissen, C. M. Knobler and W. M. Gelbart, Role of electrostatics in the assembly pathway of a single-stranded RNA virus, *J. Virol.*, 2014, **88**, 10472–10479.
- 33 J. D. Perlmutter, C. Qiao and M. F. Hagan, Viral genome structures are optimal for capsid assembly, *eLife*, 2013, **2**, e00632.
- 34 Y. Zhang and H. Hess, Toward rational design of high-efficiency enzyme cascades, *ACS Catal.*, 2017, **7**, 6018–6027.
- 35 M. J. Kummer, Y. S. Lee, M. Yuan, B. Alkotaini, J. Zhao, E. Blumenthal and S. D. Minter, Substrate channeling by a rationally designed fusion protein in a biocatalytic cascade, *JACS Au*, 2021, **1**, 1187–1197.
- 36 M. D. Crabtree, J. Holland, P. Kompella, L. Babl, N. Turner, A. J. Baldwin and T. J. Nott, Repulsive electrostatic interactions modulate dense and dilute phase properties of biomolecular condensates, *Biorxiv*, 2020, 2010–2020.
- 37 M. Rossi, W. Fang and A. Michaelides, Stability of complex biomolecular structures: van der Waals, hydrogen bond cooperativity, and nuclear quantum effects, *J. Phys. Chem. Lett.*, 2015, **6**, 4233–4238.
- 38 T. Wu, J. Huang and Y. Yan, Self-assembly of aggregation-induced-emission molecules, *Chem.-Asian J.*, 2019, **14**, 730–750.
- 39 A. Siber and R. Podgornik, Influence of salt and viral protein charge distribution on encapsidation of single-stranded viral RNA molecules, *arXiv*, 2008, preprint, DOI: [10.48550/arXiv.0806.0138](https://doi.org/10.48550/arXiv.0806.0138).
- 40 S. Mohammad, N. Bisht, A. Kannan, A. Brandmeier, C. Neiss, A. Görling, M. Stöhr and S. Maier, Pyridyl-functionalized tripod molecules on Au (111): Interplay between H-bonding and metal coordination, *Surf. Sci.*, 2025, 122701.
- 41 S. E. Boyken, Z. Chen, B. Groves, R. A. Langan, G. Oberdorfer, A. Ford, J. M. Gilmore, C. Xu, F. DiMaio and J. H. Pereira, De novo design of protein homooligomers with modular hydrogen-bond network-mediated specificity, *Science*, 2016, **352**, 680–687.
- 42 R. M. Vernon, P. A. Chong, B. Tsang, T. H. Kim, A. Bah, P. Farber, H. Lin and J. D. Forman-Kay, Pi-Pi contacts are an overlooked protein feature relevant to phase separation, *eLife*, 2018, **7**, e31486.
- 43 V. A. Adhav and K. Saikrishnan, The realm of unconventional noncovalent interactions in proteins: their significance in structure and function, *ACS Omega*, 2023, **8**, 22268–22284.
- 44 E. N. Salgado, R. J. Radford and F. A. Tezcan, Metal-directed protein self-assembly, *Acc. Chem. Res.*, 2010, **43**, 661–672.
- 45 M. J. Harrington, F. Jehle and T. Priemel, Mussel byssus structure-function and fabrication as inspiration for biotechnological production of advanced materials, *Biotechnol. J.*, 2018, **13**, 1800133.
- 46 T. Priemel, G. Palia, F. Förste, F. Jehle, S. Sviben, I. Mantouvalou, P. Zaslansky, L. Bertinetti and M. J. Harrington, Microfluidic-like fabrication of metal ion-cured bioadhesives by mussels, *Science*, 2021, **374**, 206–211.
- 47 N. Osiński, K. Majsterkiewicz, Z. Pakosz-Stepień, Y. Azuma, A. P. Biela, S. Gawel and J. G. Hedde, Designed, Programmable Protein Cages Utilizing Diverse Metal Coordination Geometries Show Reversible, pH-Dependent Assembly, *Macromol. Rapid Commun.*, 2025, **46**, 2400712.
- 48 A. Bandyopadhyay and J. Gao, Targeting biomolecules with reversible covalent chemistry, *Curr. Opin. Chem. Biol.*, 2016, **34**, 110–116.
- 49 O. J. Lange and K. M. Polizzi, Click it or stick it: covalent and non-covalent methods for protein-self assembly, *Curr. Opin. Syst. Biol.*, 2021, **28**, 100374.
- 50 I. Condò, S. M. Giannitelli, D. Lo Presti, B. Cortese and O. Ursini, Overview of Dynamic Bond Based Hydrogels for Reversible Adhesion Processes, *Gels*, 2024, **10**, 442.
- 51 M. F. Mossuto, Disulfide bonding in neurodegenerative misfolding diseases, *Int. J. Cell Biol.*, 2013, **2013**, 318319.
- 52 A. Zeltins, Construction and characterization of virus-like particles: a review, *Mol. Biotechnol.*, 2013, **53**, 92–107.
- 53 I. Altinbasak, S. Kocak, R. Sanyal and A. Sanyal, Fast-forming dissolvable redox-responsive hydrogels: exploiting the orthogonality of thiol–maleimide and thiol–disulfide exchange chemistry, *Biomacromolecules*, 2022, **23**, 3525–3534.
- 54 X. Bian, Z. Luo, B. Peng, J. Chen, P. K. Lo, L. Zhou and Y. Wen, Engineered Bionanomaterials for Precision Delivery of Nucleic Acid Drugs, *Small*, 2025, e02667.
- 55 B. J. Krzesinski, T. J. Holub, Z. Y. Gabani and M. Margittai, Cellular Uptake of Tau Aggregates Triggers Disulfide Bond Formation in Four-Repeat Tau Monomers, *ACS Chem. Neurosci.*, 2024, **16**, 171–180.
- 56 X. Yan, D. Kuster, P. Mohanty, J. Nijssen, K. Pombo-García, J. Garcia Morato, A. Rizuan, T. M. Franzmann, A. Sergeeva, A. M. Ly, F. Liu, P. M. Passos, L. George, S.-H. Wang, J. Shenoy, H. L. Danielson, B. Ozguney, A. Honigmann, Y. M. Ayala, N. L. Fawzi, D. W. Dickson, W. Rossoll, J. Mittal, S. Alberti and A. A. Hyman, Intra-condensate demixing of TDP-43 inside stress granules generates pathological aggregates, *Cell*, 2025, **188**(15), 4123.
- 57 P. M. Kharkar, M. S. Rehmann, K. M. Skeens, E. Maverakis and A. M. Kloxin, Thiol–ene click hydrogels for therapeutic delivery, *ACS Biomater. Sci. Eng.*, 2016, **2**, 165–179.



- 58 M. D. Nolan and E. M. Scanlan, Applications of thiol-ene chemistry for peptide science, *Front. Chem.*, 2020, **8**, 583272.
- 59 L. A. Sawicki and A. M. Kloxin, Design of thiol-ene photoclick hydrogels using facile techniques for cell culture applications, *Biomater. Sci.*, 2014, **2**, 1612–1626.
- 60 B. Colak, J. C. S. Da Silva, T. A. Soares and J. E. Gautrot, Impact of the molecular environment on thiol-ene coupling for biofunctionalization and conjugation, *Bioconjugate Chem.*, 2016, **27**, 2111–2123.
- 61 J. Anthony Dicks and C. Woolard, Thiol-X Chemistry: A Skeleton Key Unlocking Advanced Polymers in Additive Manufacturing, *Macromol. Mater. Eng.*, 2025, 2400445.
- 62 A. Campaniço, M. Baran, A. G. Bowie, D. B. Longley, T. Harrison and J. F. McGouran, Chemical and photo-activation of protein-protein thiol-ene coupling for protein profiling, *Commun. Chem.*, 2025, **8**, 25.
- 63 J. Xu, Y. Liu and S.-h. Hsu, Hydrogels based on Schiff base linkages for biomedical applications, *Molecules*, 2019, **24**, 3005.
- 64 H. Wang and S. C. Heilshorn, Adaptable hydrogel networks with reversible linkages for tissue engineering, *Adv. Mater.*, 2015, **27**, 3717–3736.
- 65 Y. Liu, R. Xing, J. Li and X. Yan, Covalently triggered self-assembly of peptide-based nanodrugs for cancer theranostics, *iScience*, 2023, **26**, 105789.
- 66 K. Karakyriazis, V. Lühns, S. Stößlein, I. Grunwald and A. Hartwig, Synthesis and characterization of a Schiff base crosslinked hydrogel based on hyperbranched polyglycerol, *Mater. Adv.*, 2023, **4**, 1648–1655.
- 67 M. Sajjadi, R. Jamali, T. Kiyani, Z. Mohamadnia and A.-R. Moradi, Characterization of Schiff base self-healing hydrogels by dynamic speckle pattern analysis, *Sci. Rep.*, 2024, **14**, 27950.
- 68 R. Naranjo-Alcazar, S. Bendix, T. Groth and G. Gallego Ferrer, Research progress in enzymatically cross-linked hydrogels as injectable systems for bioprinting and tissue engineering, *Gels*, 2023, **9**, 230.
- 69 E. Lai, B. Bao, Y. Zhu and H. Lin, Transglutaminase-catalyzed bottom-up synthesis of polymer hydrogel, *Front. Bioeng. Biotechnol.*, 2022, **10**, 824747.
- 70 D. Gupta, J. W. Santoso and M. L. McCain, Characterization of gelatin hydrogels cross-linked with microbial transglutaminase as engineered skeletal muscle substrates, *Bioengineering*, 2021, **8**, 6.
- 71 M. Zhou, B. H. Lee, Y. J. Tan and L. P. Tan, Microbial transglutaminase induced controlled crosslinking of gelatin methacryloyl to tailor rheological properties for 3D printing, *Biofabrication*, 2019, **11**, 025011.
- 72 M. Khanmohammadi, M. B. Dastjerdi, A. Ai, A. Ahmadi, A. Godarzi, A. Rahimi and J. Ai, Horseradish peroxidase-catalyzed hydrogelation for biomedical applications, *Biomater. Sci.*, 2018, **6**, 1286–1298.
- 73 M. E. Carnes, C. R. Gonyea, R. G. Mooney, J. W. Njihia, J. M. Coburn and G. D. Pins, Horseradish peroxidase-catalyzed crosslinking of fibrin microthread scaffolds, *Tissue Eng., Part C*, 2020, **26**, 317–331.
- 74 J. W. Bae, J. H. Choi, Y. Lee and K. D. Park, Horseradish peroxidase-catalyzed in situ-forming hydrogels for tissue-engineering applications, *J. Tissue Eng. Regen. Med.*, 2015, **9**, 1225–1232.
- 75 I. K. Amma, R. S. J. Ingrole, G. K. Venkatesa Prabhu, R. Dominquez, D. Kong, S. C. H. Mangalala, G. B. McKenna and H. S. Gill, Di-Tyrosine Cross-Linking of Elastin-Like Polypeptides through Ruthenium Photoreaction To Form Scaffolds: Fine Tuning Mechanical Properties and Improving Cytocompatibility, *Biomacromolecules*, 2025, **26**, 1580–1594.
- 76 S. Rai, S. Pramanik and S. Mukherjee, Deciphering the liquid-liquid phase separation induced modulation in the structure, dynamics, and enzymatic activity of an ordered protein  $\beta$ -lactoglobulin, *Chem. Sci.*, 2024, **15**, 3936–3948.
- 77 Y. Li, R. Tian, Y. Zou, T. Wang and J. Liu, Strategies and Applications for Supramolecular Protein Self-Assembly, *Chem.-Eur. J.*, 2024, **30**, e202402624.
- 78 D. Fass and C. Thorpe, Chemistry and enzymology of disulfide cross-linking in proteins, *Chem. Rev.*, 2018, **118**, 1169–1198.
- 79 J. T. Sharick, A. J. Atieh, K. J. Gooch and J. L. Leight, Click chemistry functionalization of self-assembling peptide hydrogels, *J. Biomed. Mater. Res., Part A*, 2023, **111**, 389–403.
- 80 R. Sun and S. Lim, Protein cages as building blocks for superstructures, *Eng. Biol.*, 2021, **5**, 35–42.
- 81 I. W. Hamley, Protein assemblies: nature-inspired and designed nanostructures, *Biomacromolecules*, 2019, **20**, 1829–1848.
- 82 Y.-T. Lai, G. L. Hura, K. N. Dyer, H. Y. H. Tang, J. A. Tainer and T. O. Yeates, Designing and defining dynamic protein cage nanoassemblies in solution, *Sci. Adv.*, 2016, **2**, e1501855.
- 83 A. D. Malay, N. Miyazaki, A. Biela, S. Chakraborti, K. Majsterkiewicz, I. Stupka, C. S. Kaplan, A. Kowalczyk, B. M. A. G. Piette and G. K. A. Hochberg, An ultra-stable gold-coordinated protein cage displaying reversible assembly, *Nature*, 2019, **569**, 438–442.
- 84 P. Wang, J. Li, T. Li, K. Li, P. C. Ng, S. Wang, V. Chriscoli, A. Basle, J. Marles-Wright and Y.-Z. Zhang, Molecular principles of the assembly and construction of a carboxysome shell, *Sci. Adv.*, 2024, **10**, eadr4227.
- 85 L. M. Oltrogge, T. Chaijarasphong, A. W. Chen, E. R. Bolin, S. Marqusee and D. F. Savage, Multivalent interactions between CsoS2 and Rubisco mediate  $\alpha$ -carboxysome formation, *Nat. Struct. Mol. Biol.*, 2020, **27**, 281–287.
- 86 L. M. Oltrogge, A. W. Chen, T. Chaijarasphong, J. B. Turnšek and D. F. Savage,  $\alpha$ -Carboxysome size is controlled by the disordered scaffold protein CsoS2, *Biochemistry*, 2023, **63**, 219–229.
- 87 T. Li, T. Chen, P. Chang, X. Ge, V. Chriscoli, G. F. Dykes, Q. Wang and L.-N. Liu, Uncovering the roles of the scaffolding protein CsoS2 in mediating the assembly and shape of the  $\alpha$ -carboxysome shell, *Mbio*, 2024, **15**, e01358.
- 88 Y. Q. Tan, S. Ali, B. Xue, W. Z. Teo, L. H. Ling, M. K. Go, H. Lv, R. C. Robinson, A. Narita and W. S. Yew, Structure of a minimal  $\alpha$ -carboxysome-derived shell and its utility



- in enzyme stabilization, *Biomacromolecules*, 2021, **22**, 4095–4109.
- 89 T. Li, Q. Jiang, J. Huang, C. M. Aitchison, F. Huang, M. Yang, G. F. Dykes, H.-L. He, Q. Wang and R. S. Sprick, Reprogramming bacterial protein organelles as a nanoreactor for hydrogen production, *Nat. Commun.*, 2020, **11**, 5448.
- 90 J. Yang, Q. Jiang, Y. Chen, Q. Wen, X. Ge, Q. Zhu, W. Zhao, O. Adegbite, H. Yang and L. Luo, Light-Driven Hybrid Nanoreactor Harnessing the Synergy of Carboxysomes and Organic Frameworks for Efficient Hydrogen Production, *ACS Catal.*, 2024, **14**, 18603–18614.
- 91 S. Lee, R. D. Kibler, G. Ahn, Y. Hsia, A. J. Borst, A. Philomin, M. A. Kennedy, B. Huang, B. Stoddard and D. Baker, Four-component protein nanocages designed by programmed symmetry breaking, *Nature*, 2025, **638**, 546–552.
- 92 Q. M. Dowling, Y.-J. Park, C. N. Fries, N. C. Gerstenmaier, S. Ols, E. C. Yang, A. J. Wargacki, A. Dosey, Y. Hsia and R. Ravichandran, Hierarchical design of pseudosymmetric protein nanocages, *Nature*, 2025, **638**, 553–561.
- 93 Y. Li, L. Liu and H. Zhao, Enzyme-catalyzed cascade reactions on multienzyme proteinosomes, *J. Colloid Interface Sci.*, 2022, **608**, 2593–2601.
- 94 K.-L. Yang, Combined cross-linked enzyme aggregates of horseradish peroxidase and glucose oxidase for catalyzing cascade chemical reactions, *Enzyme Microb. Technol.*, 2017, **100**, 52–59.
- 95 S. Bhaskar and S. Lim, Engineering protein nanocages as carriers for biomedical applications, *NPG Asia Mater.*, 2017, **9**, e371.
- 96 N. Kobayashi and R. Arai, Design and construction of self-assembling supramolecular protein complexes using artificial and fusion proteins as nanoscale building blocks, *Curr. Opin. Biotechnol.*, 2017, **46**, 57–65.
- 97 J. Fan, K. Abedi-Dorcheh, A. Sadat Vaziri, F. Kazemi-Aghdam, S. Rafieyan, M. Sohrabinejad, M. Ghorbani, F. Rastegar Adib, Z. Ghasemi and K. Klavins, A review of recent advances in natural polymer-based scaffolds for musculoskeletal tissue engineering, *Polymers*, 2022, **14**, 2097.
- 98 L. Zhang, W. Zhang, Y. Hu, Y. Fei, H. Liu, Z. Huang, C. Wang, D. Ruan, B. C. Heng and W. Chen, Systematic review of silk scaffolds in musculoskeletal tissue engineering applications in the recent decade, *ACS Biomater. Sci. Eng.*, 2021, **7**, 817–840.
- 99 L. H. Beun, I. M. Storm, M. W. T. Werten, F. A. de Wolf, M. A. Cohen Stuart and R. de Vries, From micelles to fibers: balancing self-assembling and random coiling domains in pH-responsive silk-collagen-like protein-based polymers, *Biomacromolecules*, 2014, **15**, 3349–3357.
- 100 S. Gomes, I. B. Leonor, J. F. Mano, R. L. Reis and D. L. Kaplan, Natural and genetically engineered proteins for tissue engineering, *Prog. Polym. Sci.*, 2012, **37**, 1–17.
- 101 M. C. Huber, A. Schreiber, P. Von Olshausen, B. R. Varga, O. Kretz, B. Joch, S. Barnert, R. Schubert, S. Eimer and P. Kele, Designer amphiphilic proteins as building blocks for the intracellular formation of organelle-like compartments, *Nat. Mater.*, 2015, **14**, 125–132.
- 102 A. Solomonov, A. Kozell and U. Shimanovich, Designing multifunctional biomaterials via protein self-assembly, *Angew. Chem.*, 2024, **136**, e202318365.
- 103 E. B. Speltz, A. Nathan and L. Regan, Design of protein-peptide interaction modules for assembling supramolecular structures in vivo and in vitro, *ACS Chem. Biol.*, 2015, **10**, 2108–2115.
- 104 A. Ledesma-Fernandez, S. Velasco-Lozano, J. Santiago-Arcos, F. López-Gallego and A. L. Cortajarena, Engineered repeat proteins as scaffolds to assemble multi-enzyme systems for efficient cell-free biosynthesis, *Nat. Commun.*, 2023, **14**, 2587.
- 105 X. Wang, Y. Jiang, H. Liu, H. Yuan, D. Huang and T. Wang, Research progress of multi-enzyme complexes based on the design of scaffold protein, *Bioresour. Bioprocess.*, 2023, **10**, 72.
- 106 Z. Wang, Y. Dai, F. Azi, Z. Wang, W. Xu, D. Wang, M. Dong and X. Xia, Constructing Protein-Scaffolded Multienzyme Assembly Enhances the Coupling Efficiency of the P450 System for Efficient Daidzein Biosynthesis from (2 S)-Naringenin, *J. Agric. Food Chem.*, 2024, **72**, 5849–5859.
- 107 Y. Wang, R. Heermann and K. Jung, CipA and CipB as scaffolds to organize proteins into crystalline inclusions, *ACS Synth. Biol.*, 2017, **6**, 826–836.
- 108 L. F. Bugada, M. R. Smith and F. Wen, Engineering spatially organized multienzyme assemblies for complex chemical transformation, *ACS Catal.*, 2018, **8**(9), 7898–7906.
- 109 G. A. Ellis, W. P. Klein, G. Lasarte-Aragón, M. Thakur, S. A. Walper and I. L. Medintz, Artificial multienzyme scaffolds: pursuing in vitro substrate channeling with an overview of current progress, *ACS Catal.*, 2019, **9**, 10812–10869.
- 110 J. Wang, X. Ouyang, S. Meng, B. Zhao, L. Liu, C. Li, H. Li, H. Zheng, Y. Liu and T. Shi, Rational multienzyme architecture design with iMARS, *Cell*, 2025, **188**(5), 1349.
- 111 Z. Wang, Q. Yang, D. Zhang, Y. Lu, Y. Wang, Y. Pan, Y. Qiu, Y. Men, W. Yan, Z. Xiao, R. Sun, W. Li, H. Huang and H. Guo, A cytoplasmic osmosensing mechanism mediated by molecular crowding-sensitive DCP5, *Science*, **386**, eadk9067.
- 112 Y. Wang, S. Li, M. Mokbel, A. I. May, Z. Liang, Y. Zeng, W. Wang, H. Zhang, F. Yu, K. Sporbeck, L. Jiang, S. Aland, J. Agudo-Canalejo, R. L. Knorr and X. Fang, Biomolecular condensates mediate bending and scission of endosome membranes, *Nature*, 2024, **634**, 1204–1210.
- 113 B. Wang, L. Zhang, T. Dai, Z. Qin, H. Lu, L. Zhang and F. Zhou, Liquid-liquid phase separation in human health and diseases, *Signal Transduction Targeted Ther.*, 2021, **6**, 290.
- 114 Y. Shin and C. P. Brangwynne, Liquid phase condensation in cell physiology and disease, *Science*, 2017, **357**, eaaf4382.
- 115 A. Patel, H. O. Lee, L. Jawerth, S. Maharana, M. Jahnel, M. Y. Hein, S. Stoykov, J. Mahamid, S. Saha and T. M. Franzmann, A liquid-to-solid phase transition of the





- ALS protein FUS accelerated by disease mutation, *Cell*, 2015, **162**, 1066–1077.
- 116 B. Jia, Z. Shen, S. Zhu, J. Huang, Z. Liao, S. Zhao, H. Li, S. Chen, Y. Xu and Y. Wang, Shank3 oligomerization governs material properties of the postsynaptic density condensate and synaptic plasticity, *Cell*, 2025, DOI: [10.1016/j.cell.2025.07.047](https://doi.org/10.1016/j.cell.2025.07.047).
- 117 J. Zhang, J. Hu, R. Liu, T. Zhou, X. Luo, P. Liang, Z. Xie, Q. Zhao, Y. Chen, D. Du, C. Liu, Y. Zheng, D. Li and B. Wang, YAP maintains the dynamics of TDP-43 condensates and antagonizes TDP-43 pathological aggregates, *Nat. Cell Biol.*, 2025, **27**, 1148–1160.
- 118 B. R. Sabari, A. Dall'Agnesse, A. Boija, I. A. Klein, E. L. Coffey, K. Shrinivas, B. J. Abraham, N. M. Hannett, A. V. Zamudio and J. C. Manteiga, Coactivator condensation at super-enhancers links phase separation and gene control, *Science*, 2018, **361**, eaar3958.
- 119 W. Yu, K. Jin, D. Wang, N. Wang, Y. Li, Y. Liu, J. Li, G. Du, X. Lv and J. Chen, De novo engineering of programmable and multi-functional biomolecular condensates for controlled biosynthesis, *Nat. Commun.*, 2024, **15**, 7989.
- 120 S. Lim and D. S. Clark, Phase-separated biomolecular condensates for biocatalysis, *Trends Biotechnol.*, 2024, **42**, 496–509.
- 121 E. Gomes and J. Shorter, The molecular language of membraneless organelles, *J. Biol. Chem.*, 2019, **294**, 7115–7127.
- 122 W. Li, C. Jiang and E. Zhang, Advances in the phase separation-organized membraneless organelles in cells: a narrative review, *Transl. Cancer Res.*, 2021, **10**, 4929.
- 123 P. Zhou, H. Liu, X. Meng, H. Zuo, M. Qi, L. Guo, C. Gao, W. Song, J. Wu and X. Chen, Engineered artificial membraneless organelles in *Saccharomyces cerevisiae* to enhance chemical production, *Angew. Chem., Int. Ed.*, 2023, **62**, e202215778.
- 124 P. Wen, H. Huang, R. Zhang, H. Zheng, T. Liang, C. Zhuang, Q. Wu, J. Wang, F. Liu and K. Zhang, Coacervate vesicles assembled by liquid–liquid phase separation improve delivery of biopharmaceuticals, *Nat. Chem.*, 2025, 1–10.
- 125 S. Cao, T. Ivanov, J. Heuer, C. T. J. Ferguson, K. Landfester and L. Caire da Silva, Dipeptide coacervates as artificial membraneless organelles for bioorthogonal catalysis, *Nat. Commun.*, 2024, **15**, 39.
- 126 S. Shuyu, S. Wen, O. Xiaoyi and W. Ping, Phase separation in synthetic biology, *Quant. Biol.*, 2021, **9**, 378–399.
- 127 K. A. Ganar, L. Leijten and S. Deshpande, Actinosomes: condensate-templated containers for engineering synthetic cells, *ACS Synth. Biol.*, 2022, **11**, 2869–2879.
- 128 W. Mu, Z. Ji, M. Zhou, J. Wu, Y. Lin and Y. Qiao, Membrane-confined liquid-liquid phase separation toward artificial organelles, *Sci. Adv.*, 2021, **7**, eabf9000.
- 129 J. Liu, F. Zhorabek, X. Dai, J. Huang and Y. Chau, Minimalist design of an intrinsically disordered protein-mimicking scaffold for an artificial membraneless organelle, *ACS Cent. Sci.*, 2022, **8**, 493–500.
- 130 Y. Hsia, J. B. Bale, S. Gonen, D. Shi, W. Sheffler, K. K. Fong, U. Nattermann, C. Xu, P.-S. Huang and R. Ravichandran, Design of a hyperstable 60-subunit protein icosahedron, *Nature*, 2016, **535**, 136–139.
- 131 A. R. Swartz and W. Chen, SpyTag/SpyCatcher functionalization of E2 nanocages with stimuli-responsive Z-ELP affinity domains for tunable monoclonal antibody binding and precipitation properties, *Bioconjugate Chem.*, 2018, **29**, 3113–3120.
- 132 B. Zakeri, J. O. Fierer, E. Celik, E. C. Chittock, U. Schwarz-Linek, V. T. Moy and M. Howarth, Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, E690–E697.
- 133 W. Kang, X. Ma, D. Kakarla, H. Zhang, Y. Fang, B. Chen, K. Zhu, D. Zheng, Z. Wu and B. Li, Organizing enzymes on self-assembled protein cages for cascade reactions, *Angew. Chem., Int. Ed.*, 2022, **61**, e202214001.
- 134 J. Choi, J. Chen, S. L. Schreiber and J. Clardy, Structure of the FKBP12-rapamycin complex interacting with binding domain of human FRAP, *Science*, 1996, **273**, 239–242.
- 135 Y. Wang, S. F. H. Barnett, S. Le, Z. Guo, X. Zhong, P. Kanchanawong and J. Yan, Label-free single-molecule quantification of rapamycin-induced FKBP-FRB dimerization for direct control of cellular mechanotransduction, *Nano Lett.*, 2019, **19**, 7514–7525.
- 136 W. Kang, X. Ma, H. Zhang, J. Ma, C. Liu, J. Li, H. Guo, D. Wang, R. Wang and B. Li, Dynamic metabolons using stimuli-responsive protein cages, *J. Am. Chem. Soc.*, 2024, **146**, 6686–6696.
- 137 M. V. Garabedian, W. Wang, J. B. Dabdoub, M. Tong, R. M. Caldwell, W. Benman, B. S. Schuster, A. Deiters and M. C. Good, Designer membraneless organelles sequester native factors for control of cell behavior, *Nat. Chem. Biol.*, 2021, **17**, 998–1007.
- 138 S. Elbaum-Garfinkle, Y. Kim, K. Szczepaniak, C. C.-H. Chen, C. R. Eckmann, S. Myong and C. P. Brangwynne, The disordered P granule protein LAF-1 drives phase separation into droplets with tunable viscosity and dynamics, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 7189–7194.
- 139 Y. Wang, M. Liu, Q. Wei, W. Wu, Y. He, J. Gao, R. Zhou, L. Jiang, J. Qu and J. Xia, Phase-separated multienzyme compartmentalization for terpene biosynthesis in a prokaryote, *Angew. Chem.*, 2022, **134**, e202203909.
- 140 L. Wan, Y. Zhu, W. Zhang and W. Mu, Phase-separated synthetic organelles based on intrinsically disordered protein domain for metabolic pathway assembly in *Escherichia coli*, *ACS Nano*, 2023, **17**, 10806–10816.
- 141 T. W. Giessen, Encapsulins: microbial nanocompartments with applications in biomedicine, nanobiotechnology and materials science, *Curr. Opin. Chem. Biol.*, 2016, **34**, 1–10.
- 142 X. Yu, Z. Weng, Z. Zhao, J. Xu, Z. Qi and J. Liu, Assembly of protein cages for drug delivery, *Pharmaceutics*, 2022, **14**, 2609.
- 143 S. S. Nurttila, R. Becker, J. Hessels, S. Woutersen and J. N. H. Reek, Photocatalytic hydrogen evolution by a synthetic [FeFe] hydrogenase mimic encapsulated in a porphyrin cage, *Chem.–Eur. J.*, 2018, **24**, 16395–16406.



- 144 A. H. Chen and P. A. Silver, Designing biological compartmentalization, *Trends Cell Biol.*, 2012, **22**, 662–670.
- 145 H. Zhang, S. Elbaum-Garfinkle, E. M. Langdon, N. Taylor, P. Occhipinti, A. A. Bridges, C. P. Brangwynne and A. S. Gladfelter, RNA controls PolyQ protein phase transitions, *Mol. Cell*, 2015, **60**, 220–230.
- 146 E. M. Zhao, N. Suek, M. Z. Wilson, E. Dine, N. L. Pannucci, Z. Gitai, J. L. Avalos and J. E. Toettcher, Light-based control of metabolic flux through assembly of synthetic organelles, *Nat. Chem. Biol.*, 2019, **15**, 589–597.
- 147 H. Park, W. Wang, S. H. Min, Y. Ren, K. Shin and X. Han, Artificial organelles for sustainable chemical energy conversion and production in artificial cells: artificial mitochondrion and chloroplasts, *Biophys. Rev.*, 2023, **4**, 011311.
- 148 B. Piette, Bi-Symmetric Polyhedral Cages with Three, Four, Five or Six Connected Faces and Small Holes, *Symmetry*, 2025, **17**, 1537.
- 149 X. Ma, L. Yi, J. Li, J. Ma, W. Li, L. Wang, C. Liu, B. Li, N. Deng and W. Kang, Engineering a Self-Assembled Protein Cage for Targeted Dual Functionalization, *Nano Lett.*, 2024, **24**, 9237–9244.
- 150 W. Zhou, S. Ma, R. Gao, Y. Tang, H. Zhang, A. Liang, M. Yang, C. Ma, Q. Fan and X.-E. Zhang, Assembly of Matryoshka-Type Protein Nanocages for Compartmentalized Oxygen Sensing, *Nano Lett.*, 2025, **25**, 4433–4440.
- 151 J. Zhu, N. Avakyan, A. Kakkis, A. M. Hoffnagle, K. Han, Y. Li, Z. Zhang, T. S. Choi, Y. Na and C.-J. Yu, Protein assembly by design, *Chem. Rev.*, 2021, **121**, 13701–13796.
- 152 T. G. W. Edwardson, M. D. Lévassieur, S. Tetter, A. Steinauer, M. Hori and D. Hilvert, Protein cages: from fundamentals to advanced applications, *Chem. Rev.*, 2022, **122**, 9145–9197.
- 153 Z. Fan, Z. Gao, Y. Fu, Y. Liao, W. Xiao, Y. Ding, C. Yang, X. Li, G. Li and D. Su, Unlocking the Potential of Fruit and Vegetable Waste: Sustainable Biomanufacturing Strategies for Synergistic Nutrient Recovery and High-Value Bioproduct Synthesis, *Trends Food Sci. Technol.*, 2025, 105288.
- 154 Y. Meng, L. Peplowski, T. Wu, H. Gong, R. Gu, L. Han, Y. Xia, Z. Liu, Z. Zhou and Z. Cheng, A Versatile Protein Scaffold Engineered for the Hierarchical Assembly of Robust and Highly Active Enzymes, *Adv. Sci.*, 2025, **12**, 2500405.
- 155 E. Moreno-Reyes and J. M. Goddard, Hierarchical materials: an overview of synthesis methods and revolutionary impact on enzyme biocatalysis, *Colloids Surf., B*, 2025, 115086.
- 156 R. Zhang, S.-Y. Kang, F. Gaascht, E. L. Pena and C. Schmidt-Dannert, Design of a genetically programmable and customizable protein scaffolding system for the hierarchical assembly of robust, functional macroscale materials, *ACS Synth. Biol.*, 2024, **13**, 3724–3745.
- 157 J. B. Miller, C. H. Abrahamson, M. S. Lee, B. J. Palmero, N. W. Kennedy, C. E. Mills and D. Tullman-Ercek, Self-assembling protein materials with genetically programmable morphology and size, *bioRxiv*, 2025, 2007–2025.
- 158 A. T. Hilditch, A. Romanyuk, S. J. Cross, R. Obexer, J. J. McManus and D. N. Woolfson, Assembling membraneless organelles from de novo designed proteins, *Nat. Chem.*, 2024, **16**, 89–97.
- 159 T. Kortemme, De novo protein design—from new structures to programmable functions, *Cell*, 2024, **187**, 526–544.
- 160 M. Cao, X. Zhang, X. Wang, D. Zhao, M. Shi, J. Zou, L. Li and H. Jiang, An Overview of Liquid-Liquid Phase Separation and Its Mechanisms in Sepsis, *J. Inflammation Res.*, 2025, 3969–3980.
- 161 T. Hirose, N. Fujiwara, K. Ninomiya, T. Yamamoto, S. Nakagawa and T. Yamazaki, Architectural RNAs: blueprints for functional membraneless organelle assembly, *Trends Genet.*, 2025, **41**(10), 919–933.
- 162 Z. Wang, Y. Zhao, S. Zhang, X. Chen, G. Sun, B. Zhang, B. Jiang, Y. Yang, X. Yan and K. Fan, Re-engineering the inner surface of ferritin nanocage enables dual drug payloads for synergistic tumor therapy, *Theranostics*, 2022, **12**, 1800.
- 163 R. Bhujel, V. Enkmann, H. Burgstaller and R. Maharjan, Artificial Intelligence-Driven Strategies for Targeted Delivery and Enhanced Stability of RNA-Based Lipid Nanoparticle Cancer Vaccines, *Pharmaceutics*, 2025, **17**, 992.
- 164 G. Thakur and T. Thundat, Designing protein self-assembly for smart materials, *J. Nanomed. Res.*, 2017, **6**, 00154.
- 165 Y. Li, H. Lu, L. Lu and H. Wang, Engineering Peptide Self-Assembly: Modulating Noncovalent Interactions for Biomedical Applications, *Acc. Mater. Res.*, 2025, **6**, 447–461.
- 166 X. Zottig, M. Côté-Cyr, D. Arpin, D. Archambault and S. Bourgault, Protein supramolecular structures: from self-assembly to nanovaccine design, *Nanomaterials*, 2020, **10**, 1008.
- 167 H. Garcia Seisdedos, T. Levin, G. Shapira, S. Freud and E. D. Levy, Mutant libraries reveal negative design shielding proteins from supramolecular self-assembly and relocalization in cells, *Proc. Natl. Acad. Sci. U. S. A.*, 2022, **119**, e2101117119.
- 168 G. Chiesa, S. Kiriakov and A. S. Khalil, Protein assembly systems in natural and synthetic biology, *BMC Biol.*, 2020, **18**, 1–18.
- 169 Y. Wang and T. Douglas, Tuning properties of biocatalysis using protein cage architectures, *J. Mater. Chem. B*, 2023, **11**, 3567–3578.
- 170 B. C. Buddingh, A. Llopis-Lorente, L. K. E. A. Abdelmohsen and J. C. M. van Hest, Dynamic spatial and structural organization in artificial cells regulates signal processing by protein scaffolding, *Chem. Sci.*, 2020, **11**, 12829–12834.
- 171 S. Sarvepalli, S. R. Pasika, V. Verma, A. Thumma, S. Bolla, P. K. Nukala, A. Butreddy and P. K. Bolla, A Review on the Stability Challenges of Advanced Biologic Therapeutics, *Pharmaceutics*, 2025, **17**, 550.
- 172 N. Livnat-Levanon, E. Kevei, O. Kleifeld, D. Krutauz, A. Segref, T. Rinaldi, Z. Erpapazoglou, M. Cohen, N. Reis



- and T. Hoppe, Reversible 26S proteasome disassembly upon mitochondrial stress, *Cell Rep.*, 2014, **7**, 1371–1380.
- 173 M. Rahban, F. Ahmad, M. A. Piatyszek, T. Haertlé, L. Saso and A. A. Saboury, Stabilization challenges and aggregation in protein-based therapeutics in the pharmaceutical industry, *RSC Adv.*, 2023, **13**, 35947–35963.
- 174 J. Schuster, A. Koulov, H.-C. Mahler, P. Detampel, J. Huwyler, S. Singh and R. Mathaes, In vivo stability of therapeutic proteins, *Pharm. Res.*, 2020, **37**, 1–17.
- 175 R. Arai, Hierarchical design of artificial proteins and complexes toward synthetic structural biology, *Biophys. Rev.*, 2018, **10**, 391–410.
- 176 C. Guindani, L. C. da Silva, S. Cao, T. Ivanov and K. Landfester, Synthetic cells: from simple bio-inspired modules to sophisticated integrated systems, *Angew. Chem.*, 2022, **134**, e202110855.
- 177 F. L. Sendker, Y. K. Lo, T. Heimerl, S. Bohn, L. J. Persson, C.-N. Mais, W. Sadowska, N. Paczia, E. Nußbaum and M. del Carmen Sánchez Olmos, Emergence of fractal geometries in the evolution of a metabolic enzyme, *Nature*, 2024, **628**, 894–900.
- 178 H. Shen, J. A. Fallas, E. Lynch, W. Sheffler, B. Parry, N. Jannetty, J. Decarreau, M. Wagenbach, J. J. Vicente and J. Chen, De novo design of self-assembling helical protein filaments, *Science*, 2018, **362**, 705–709.
- 179 S. E. Boyken, M. A. Benhaim, F. Busch, M. Jia, M. J. Bick, H. Choi, J. C. Klima, Z. Chen, C. Walkey and A. Mileant, De novo design of tunable, pH-driven conformational changes, *Science*, 2019, **364**, 658–664.
- 180 S. Rammensee, U. Slotta, T. Scheibel and A. R. Bausch, Assembly mechanism of recombinant spider silk proteins, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 6590–6595.

