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Wider impact statement

The exploration of globular proteins as building blocks for functional-mechanical materials represents a paradigm shift in biomaterials science. Unlike traditional structural proteins, globular proteins offer unique opportunities for integrating molecular-level biofunctionality with engineered mechanical responsiveness. This reimagining opens up new design principles for creating adaptive, biodegradable, and biocompatible materials that respond to biological and environmental cues. By leveraging advances in protein customization, supramolecular assembly, and green processing, these materials hold promise for next-generation applications that demand both structural integrity and dynamic function—ranging from implantable devices to sustainable soft robotics. Importantly, the development of scalable strategies to harness the inherent programmability of globular proteins contributes to a broader movement toward more sustainable and life-integrated material systems, aligning with global needs for environmentally responsible innovation in biomedicine, electronics, and beyond.

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Data availability statement

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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Globular Proteins as Functional-Mechanical Materials: A Multiscale Perspective on Design, Processing, and Application

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Abstract

Globular proteins, traditionally regarded as non-structural biomolecules due to the limited load-bearing capacity in their monomeric states, are increasingly recognized as valuable building blocks for functional-mechanical materials. Their inherent bioactivity, chemical versatility, and structural tunability enable the design of materials that combine biological functionality with tailored mechanical performance. This review highlights recent advances in engineering globular proteins—spanning natural systems (serum albumins, enzymes, milk globulins, silk sericin, soy protein isolates) to recombinant architectures including tandem-repeat proteins—into functional-mechanical platforms. We discuss strategies such as sequence engineering, crosslinking chemistry, hybrid modulation, and hierarchical assembly to enhance mechanical properties. Diverse material formats including fibers, films, hydrogels, and porous scaffolds are examined, along with processing techniques like wet/electro-spinning, 3D printing, and self-assembly suited to the proteins' thermal and solubility constraints. Emerging applications span tissue engineering, soft electronics, and environmentally adaptive systems. Key challenges such as maintaining functional activity during reinforcement, achieving interfacial stability, and developing scalable, standardized processing methods are critically evaluated. By repositioning globular proteins as dynamic, tunable material platforms, this work aims to inspire new directions in the development of intelligent, biocompatible, and sustainable materials.

Keywords

Globular proteins; protein-based materials; structural–functional integration; molecular engineering; bioinspired design; sustainable biomaterials; hierarchical assembly.

Wider impact

The exploration of globular proteins as building blocks for functional-mechanical materials represents a paradigm shift in biomaterials science. Unlike traditional structural proteins, globular proteins offer unique opportunities for integrating molecular-level biofunctionality with engineered mechanical responsiveness. This reimagining opens up new design principles for creating adaptive, biodegradable, and biocompatible materials that respond to biological and environmental cues. By leveraging advances

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in protein customization, supramolecular assembly, and green processing, these materials hold promise for next-generation applications that demand both structural integrity and dynamic function—ranging from implantable devices to sustainable soft robotics. Importantly, the development of scalable strategies to harness the inherent programmability of globular proteins contributes to a broader movement toward more sustainable and life-integrated material systems, aligning with global needs for environmentally responsible innovation in biomedicine, electronics, and beyond.

1. Introduction

Protein-based materials are emerging as a versatile platform driving advances in biomedical engineering, sustainable manufacturing, and intelligent material technologies, owing to their inherent structural diversity, biodegradability, and environmental compatibility.[1-5] Among these, fibrous structural proteins—such as silk fibroin and collagen—have been extensively studied for the development of high-strength, biodegradable biomaterials, primarily due to their naturally aligned chain architectures and hierarchical supramolecular organization. These materials have given rise to well-established design paradigms that integrate structure, mechanical performance, and biological function.[6, 7] Notably, certain multi-domain globular systems—exemplified by tandem-repeat proteins like titin's immunoglobulin domains and fibronectin type III modules—demonstrate inherent load-bearing capacity through organized arrays of globular units. These natural molecular springs dissipate mechanical stresses under physiological conditions.[8-12] In contrast, the vast majority of globular proteins exist as single-domain monomers, which remain underexplored as structural materials despite their biochemical versatility. Representative natural monomeric globular proteins include serum albumins, such as human serum albumin (HSA), bovine serum albumin (BSA),[13-15] β-lactoglobulin (BLG),[16] ovalbumin (OVA),[17, 18] lysozyme,[19-21] sericin,[22, 23] and soy protein isolate (SPI)[24-26]. These proteins generally adopt compact and thermodynamically stable tertiary structures, enriched with reactive and polar surface residues and exhibiting favorable biochemical attributes,[16, 21-23, 26, 27] thereby underpinning their increasing utility in biomedical applications such as drug delivery, tissue repair, and bioactive scaffolding.[13, 23, 26, 28]

Nevertheless, when deployed as structural building blocks, monomeric globular systems face inherent architectural constraints. Unlike fibrous proteins that feature repetitive, long-range secondary structures (e.g., β-sheet nanocrystals in silk or collagen's triple helix),[1, 6, 29] monomeric globular proteins lack extended supramolecular order. Their discrete spherical architecture inherently limits intermolecular packing efficiency and effective stress distribution, resulting in materials with relatively low stiffness and fracture strength.[30, 31] Consequently, hydrogels composed of native serum albumins or β-lactoglobulin, for example, typically display inferior mechanical performance compared to their fibrous counterparts, limiting their applicability in load-bearing or mechanically demanding environments.[13, 32, 33] Overcoming these limitations necessitates the integration of rational design strategies to introduce hierarchical architectures and controlled crosslinking that compensate for the proteins' intrinsic structural disorder. Recent advances in protein engineering, covalent/non-covalent crosslinking, and multiscale material fabrication have enabled a shift toward structure–function co-optimization in globular protein-based materials. Through molecular-level sequence modification and folding control, mesoscale crosslinking strategies and domain organization, and macroscale structuring into three-dimensional constructs, researchers are endowing globular proteins with tunable mechanical and functional properties. As a result, these proteins are being transformed from passive biomolecular carriers into multifunctional material units with both mechanical integrity and bio-responsiveness—expanding their potential in areas such as dynamic tissue engineering, soft electronics, and sustainable bio-based materials.

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This review encompasses the engineering and application advances of both single-domain and multi-domain globular proteins as functional-mechanical materials. (**Fig. 1**). We systematically discuss the transition from functional proteins to structure–function integrated materials, focusing on four key design axes: molecular sequence engineering, crosslinking strategies, hybrid control, and hierarchical structural assembly. Additionally, we highlight their emerging roles in tissue regeneration, flexible functional devices, and environmentally compatible material systems. By redefining the position of globular proteins within the materials design landscape, this work aims to facilitate their transition from passive biological agents to active platforms for next-generation intelligent and bioinspired material systems.

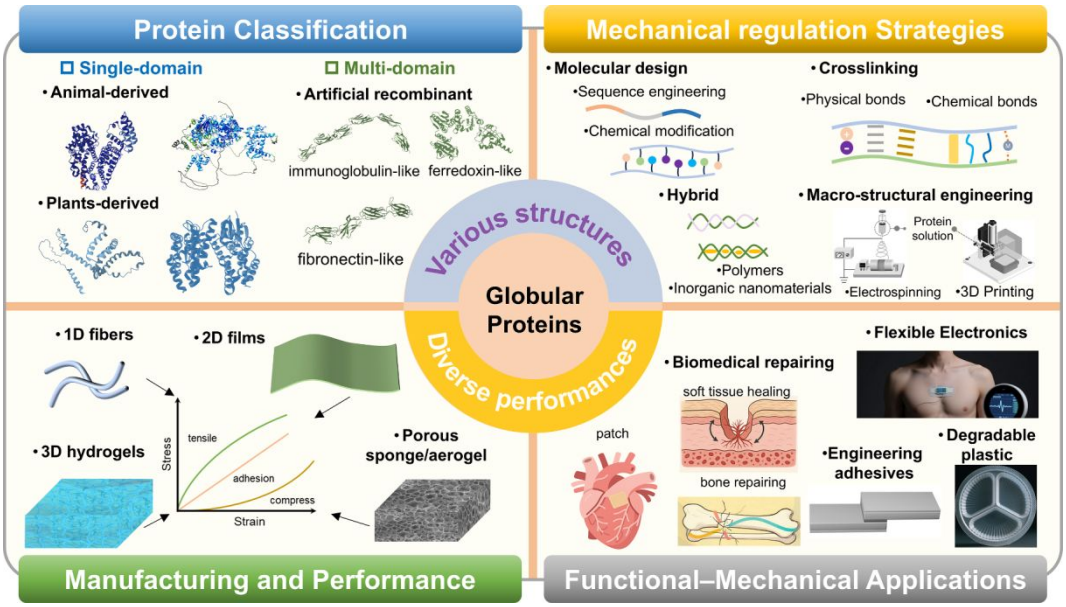

Fig. 1 Comprehensive overview of globular protein classification, mechanical regulation strategies, material manufacturing and performance, and functional–mechanical applications.

2. Structure–Function Basis of Globular Proteins

From a materials-design perspective, globular proteins can be considered in terms of three parameters: **(i)** domain organization (single-domain vs. multi-domain) defines the accessible conformational space and potential routes to mechanical coupling; **(ii)** the distribution of solvent-exposed residues, which governs crosslinking, hybrid, and interfacial interactions; and **(iii)** representative biological exemplars, which relate native roles to practical design requirements. Under this framework, architecture and surface chemistry are treated as controllable variables for directing assembly, stability, and function, providing a concise basis for the analysis that follows.

2.1 Molecular Conformation and Stability Mechanisms

As individually folded macromolecules, globular proteins adopt compact tertiary conformations formed through hierarchical folding, driven primarily by hydrophobic interactions. Hydrophobic residues—such as valine, leucine, and phenylalanine—aggregate to form an internal core, stabilizing secondary elements like α-helices and β-sheets. Additional structural integrity arises from intramolecular disulfide bridges (e.g., between cysteine residues), hydrogen bonding networks, and van der Waals interactions. While their cores are generally rigid, many globular proteins include flexible loops or terminal regions that allow conformational adaptability, critical for ligand binding or catalytic action.[27, 34-38] In commonly studied single-domain systems used for materials design, core hydrophobic packing and strategically placed disulfide bonds confer baseline conformational stability, whereas surface loops provide the limited flexibility needed for binding and processing. A representative example is human serum albumin,

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which consists of three homologous domains stabilized by 17 intrachain disulfide bonds and maintains structural resilience across a broad range of physiological conditions. This intrinsic structural stability, combined with selective flexibility, makes globular proteins well-suited for controlled material design at the molecular level.[31] Extending this molecular picture from single domains to concatenated arrays, multi-domain (tandem-repeat) proteins organize compact modules via defined linkers, introducing interdomain coupling and additional stabilization routes (e.g., domain–domain contacts and sacrificial interactions). Natural exemplars include titin (serial Ig domains with PEVK segments) and fibronectin (type-III modules), whose repetitive architectures permit cooperative conformational responses and, when assembled, can approach long-range order characteristic of fibrous systems. Thus, there exists a continuum from individually folded domains to engineered tandems, in which stability mechanisms operate at both intra- and interdomain levels and can be harnessed in subsequent materials design.[8-12]

2.2 Chemical Properties and Functional Groups

The chemical reactivity and tunability of globular proteins originate from the diversity of amino acids exposed on their surfaces. Single-domain proteins often present polar and charged side chains—such as lysine, glutamic acid, and arginine—thereby promoting solubility and facilitating interactions with ions, ligands, or surfaces. Surface-exposed functional groups including primary amines ($–NH_2$), carboxyls (–COOH), thiols (–SH), phenolic tyrosines, and hydroxyls (–OH) serve as key sites for chemical modification, crosslinking, or conjugation. Disulfide formation from thiol groups is particularly significant for redox-responsive behavior and structural reinforcement, while amine-reactive chemistries support functionalization with polymers, bioactive ligands, or nanoparticles. Additionally, aromatic and hydrophobic residues contribute to π-π stacking, hydrophobic assembly, or metal coordination, all of which are valuable for engineering responsive materials. These properties collectively enable fine control over protein–protein and protein–interface interactions, laying the groundwork for material assembly, stability, and dynamic functionality.[27, 34, 38-40] In multi-domain or tandem-repeat proteins, the same chemistries are present but distributed across repeated modules and linkers. Reactive sites are therefore more patterned at the domain level; many repeats have few accessible free cysteines, so practical crosslinking often relies on lysine/tyrosine pathways, with additional contributions from linker residues.[8, 41-45] This modular distribution can aid site selectivity, whereas single-domain proteins usually provide a higher density of uniformly accessible groups.

Table 1 Structural and functional signatures of representative globular proteins engineered as functional-mechanical building blocks.

	Protein Type	Main sources	Key Features	Native Biological Function
Single-domain	Serum Albumin (HAS, BSA, etc.)[13-15]	Plasma	Three-domain structure, 17 disulfide bonds, pH-responsive, hydrophobic binding pockets	Transport of small molecules, osmotic regulation
	BLG[16]	Bovine milk	Rich in aromatic residues, heat-induced aggregation, β-sheet dominant structure	Antibacterial defense, nutrient stabilization
	Sericin[23, 28, 46, 47]	Silkworm glands	Hydrophilic, disordered segments, flexible conformation	Silk shell adhesion, protection
	OVA[17, 18]	Egg white	Mixed α-helix/β-sheet structure; monomeric; heat-sensitive gelation upon heating	Reservoir of amino acids in eggs; antimicrobial and protease-inhibitory precursor
	Lysozyme[19-21]	Egg white/tears	Small size, high surface charge, catalytic site	Bacterial cell wall degradation, immune defense
	SPI[24, 25]	Soybean seeds	Acidic residues, hydrophilic, abundant and accessible	Nitrogen storage, seed germination control
Multi-domain	Ferredoxin-like polyprotein $(FL)_n$[41-43]	Recombinant	Small α/β fold, modular domains	Electron transfer mediator, redox reaction facilitator, iron-sulfur cluster carrier
	Immunoglobulin-like polyprotein $(IG)_n$[8, 44, 45]	Recombinant	β-sandwich fold, modular domains	Antigen recognition, neutralization, immune regulation or load-bearing functions

2.3 Representative Globular Proteins and Native Functions

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Based on domain count, globular proteins can be grouped into two categories: single-domain and multi-domain proteins (**Table 1**). Single-domain globular proteins are abundant in biological fluids and tissues, including blood plasma, milk, egg whites, and plant seeds. Each type performs distinct physiological roles based on its structure and biochemical profile. Serum albumin functions as a molecular transporter and regulator of osmotic pressure.[13-15, 31] β-Lactoglobulin, found in whey, binds to hydrophobic molecules and contributes to immune modulation.[16] Lysozyme, present in egg white and secretions, acts as an antimicrobial enzyme by degrading bacterial cell walls.[19-21] Plant-derived globulins, such as those in soybeans, serve as nutrient reserves and possess natural emulsifying and gelling capabilities.[24, 25] Beyond native single-domain proteins, recombinant multi-domain globular constructs modeled on tandem-repeat architectures have become attractive for materials design. Engineered proteins such as ferredoxin-like (FL) domains and immunoglobulin-like (IG) repeats represent modular scaffolds that combine biological activity with tunable mechanical or assembly behavior. These synthetic domains provide engineered coupling sites (e.g., SpyTag/SpyCatcher, cysteine residues, or tyrosine residues) and programmable mechanical responses, enabling their integration into materials with tailored functional properties (**Table 1**).[8, 41-45] Related ECM exemplars such as fibronectin (FN) also exhibit a modular repeat architecture; in materials studies, however, recombinant FN constructs are used mainly as bioactive ligands rather than as load-bearing scaffolds.[9, 48-51]

Collectively, these globular systems—whether native or engineered—exhibit high biochemical precision and versatile functionality. Yet the discrete spherical architecture of monomeric globular proteins inherently constrains intermolecular packing efficiency and stress distribution, thereby necessitating strategic interventions to achieve mechanically robust architectures.

3. Mechanical Regulation Strategies

By leveraging techniques from primary sequence modification to hierarchical structural assembly, researchers have enabled the creation of globular protein-based materials with tunable mechanical behavior tailored to diverse functional needs. This chapter reviews the key strategies for enhancing the mechanical properties of globular proteins, with a focus on molecular-level engineering and chemical modification, crosslinking approaches, hybrid system design, and macrostructural organization.

3.1 Molecular-Level Sequence Optimization and Chemical Modification

With the advent of advanced recombinant and protein-engineering techniques, “bottom-up” design of high-performance globular proteins has become increasingly feasible.[8, 41-45, 52-57] While industrial scale production of recombinant human serum albumins (r-HSA) is now well established, primarily for drug delivery and biomedical applications,[58] researchers are extending these methods to other globular scaffolds to tailor their mechanical properties through precise gene-level control. Recently, a series of recombinant globular proteins tailored specifically for mechanical regulation have been reported. For instance, integrating elastin-like peptide motifs into the red fluorescent protein mCherry enables precise control over its phase-separation behavior, thereby tuning the stiffness and Young’s modulus of the resulting vesicles (**Fig. 2a**).[59-61] Leveraging AI-driven structure prediction, researchers have redesigned a ferredoxin-like fold into a multirepeat protein scaffold that undergoes reversible, force-induced unfolding from its compact globular state and refolds upon unloading, endowing hydrogels with exceptional elasticity (**Fig. 2b**).[42, 43]

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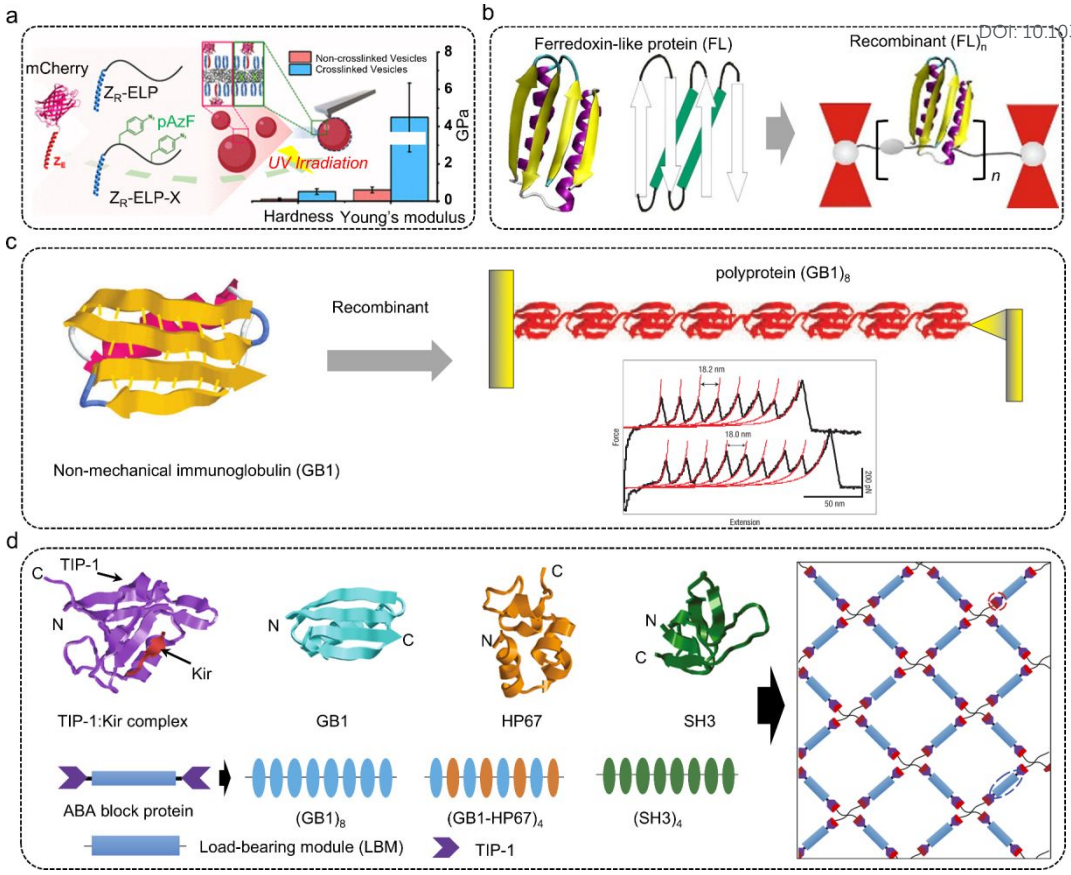

Fig. 2 Recombinant sequence engineering strategies for regulating the mechanical properties of globular proteins. (**a**) Integration of elastin-like peptide (ELP) motifs into the globular protein mCherry enables phase-separation behavior to be tuned. Adapted with permission from **ref. 60**, Copyright 2020, American Chemical Society (**b**) AI-guided design of ferredoxin-like fold proteins results in multirepeat globular scaffolds that unfold under force and refold upon unloading, serving as highly elastic building blocks for hydrogels. Adapted with permission from **ref. 42**, Copyright 2018, Springer Nature. (**c**) Tandem GB1 polyproteins exhibit stepwise unfolding behavior under tensile loading. Their fast folding, mechanical resilience, and low fatigue properties form the basis for further modular fusion. Adapted with permission from **ref. 44**, Copyright 2007, Springer Nature. (**d**) Fusing GB1 with folded domains (e.g., HP67, SH3), enables tailored control over the mechanical strength, elasticity, and modulus of the protein-based hydrogels. Adapted with permission from **ref. 45**, Copyright 2018, Springer Nature.

Immunoglobulin (Ig) domains also serve as versatile templates for mechanical tuning.[62] Li and co-wokers[41, 44] engineered tandem repeats of the GB1 domain (**Fig. 2c**), producing GB1-polymers that exhibit fast, high-fidelity folding kinetics, low mechanical fatigue, and resilience against residual stresses under tensile loading. By further integrating arthropod-derived elastin-like sequences,[53] the mechanical properties of GB1-based materials—such as strength, modulus, and elasticity—can be precisely tuned within a consistent molecular framework. Moreover, the incorporation of engineered functional domains like HP67 and SH3 (**Fig. 2d**),[45] or specific affinity modules such as SpyCatcher–SpyTag,[52] enables additional regulation of the hydrogel's mechanical behavior. Inspired by the long-range order of titin's Ig domains, another study employed directed polymerization of titin-derived Ig modules to form extended, ordered arrays, yielding non-fibrous protein fibers with both high strength and toughness.[8] In contrast to FL/Ig repeat–based scaffolds, which primarily target mechanical resilience, recombinant fibronectin (FN) modules are typically incorporated into repair-oriented hydrogels to coordinate integrin-mediated adhesion with heparin/growth-factor sequestration; in these systems FN acts chiefly as a

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biochemical presentation motif rather than as the load-bearing backbone.[48, 51] In summary, the modular workflow of "motif insertion–polymeric assembly–multifunctional fusion" via recombinant sequence engineering lays a fundamental molecular foundation for mechanical control of globular proteins, offering rich design opportunities for subsequent chemical modification and higher-order assembly

Beyond genetic sequence engineering, chemical modification serves as an indispensable strategy for fine-tuning both the mechanical performance and functional characteristics of globular protein materials at the molecular level. Globular proteins typically present a variety of reactive surface groups, with lysine ε-amines being particularly abundant and accessible.[13] For example, introducing additional carboxylate groups onto BSA increases its negative surface charge, thereby modulating interactions with four-arm PEG and significantly altering the compressive properties of the resultant hydrogel.[63] Similarly, phosphorylation of soy protein isolates (SPI) using diethyl chlorophosphate (DECP) integrates phosphate ester moieties without disrupting the native fold, markedly improving film tensile strength and toughness.[64] Furthermore, incorporation of thiol groups followed by oxidative disulfide formation or Ag^{+}-mediated coordination crosslinking enhances both elasticity and fracture resistance.[65, 66] To date, the most prevalent modification exploits lysine residues to graft vinyl groups (e.g., methacrylation),[13, 67-72] enabling free-radical polymerization—whether homopolymerized or copolymerized with other vinyl polymers—to build tunable crosslinked networks. In addition, polyphenolic moieties, such as the catechol groups found in DOPA, can also be conjugated to the surface of globular proteins, providing functional sites for subsequent metal-ion coordination crosslinking.[73] Collectively, these chemical tools allow precise control over crosslink density and network architecture, profoundly influencing stiffness, toughness, and dynamic responsiveness, and thus offer a powerful molecular toolbox for expanding the capabilities of globular protein hydrogels and related soft materials.

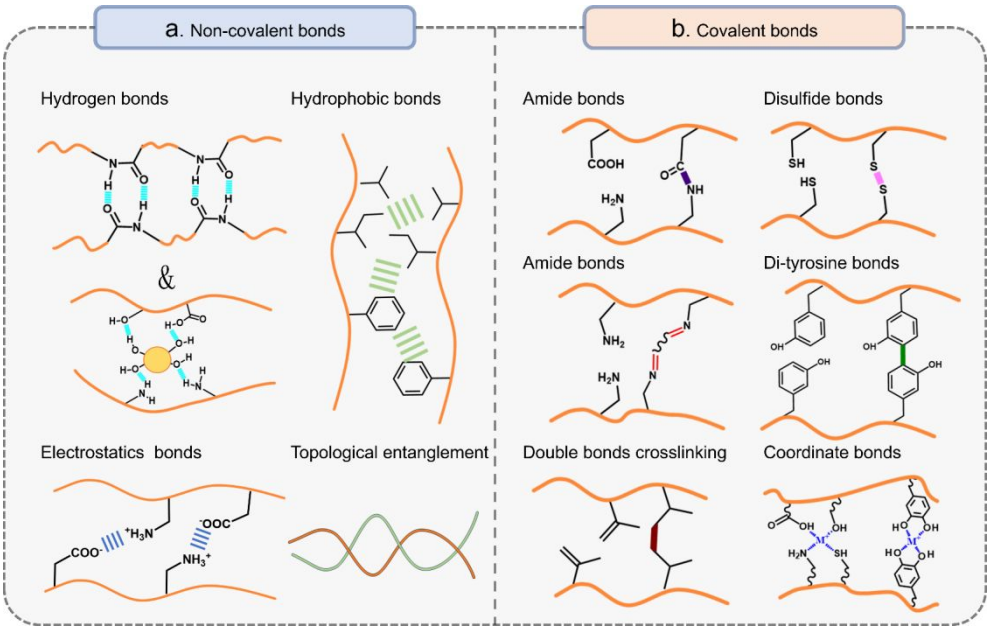

Fig. 3 Schematic illustration of common non-covalent and covalent crosslinking strategies for globular protein-based materials. (**a**) Non-covalent interactions, including hydrogen bonding, hydrophobic interactions, electrostatic interactions, and topological entanglement, provide reversible and dynamic crosslinking ideal for self-healing and responsive systems. (**b**) Covalent interactions, such as amide bond formation, disulfide bridges, dityrosine coupling, vinyl group polymerization, and coordinate bonding, introduce covalent crosslinks that enhance mechanical strength, stability, and multifunctionality in protein-based hydrogels and materials.

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3.2 Diversification of Crosslinking Strategies

Crosslinking plays a pivotal role in the mechanical reinforcement of globular protein materials by facilitating the formation of stable three-dimensional networks. a broad spectrum of crosslinking strategies—ranging from non-covalent interactions to covalent bonds—creates the three-dimensional networks that underpin their mechanical integrity (**Fig. 3**). Non-covalent include hydrogen bonds, hydrophobic associations, electrostatic attractions and topological entanglements, all of which can be reversibly tuned by environmental cues (**Fig. 3a**). In contrast, covalent crosslinks offer more permanent stabilization through the formation of amide bonds, Schiff-base (imine) linkages (e.g., glutaraldehyde), dityrosine bridges, vinyl double-bond polymerization, disulfide bonds, and metal–ligand coordination (**Fig. 3b**), each contributing to robust and tunable mechanical reinforcement.

3.2.1 Non-covalent Crosslinking Strategies

For non-covalent crosslinking，electrostatic interactions stand out as a prominent class of non-covalent forces, play a pivotal role in stabilizing protein structures and driving their self-assembly processes. These interactions not only govern protein folding and stability but also modulate aggregation behaviors and material properties. For instance, Tatsuya Nojima and colleagues developed an electrostatic-based strategy to achieve ordered assembly of globular proteins by leveraging charged surfactants. By designing surfactants with tailored charge properties and selecting counterionic surfactants based on the isoelectric point (pI) of target proteins, they successfully induced the formation of well-organized protein aggregates.[74] Subsequent studies demonstrated that thermal treatment of these protein-surfactant assemblies could further enhance their structural integrity, resulting in protein-based gel materials with exceptional compressive resistance (**Fig. 4a**).[75, 76] This approach highlights the potential of electrostatic engineering in creating functional biomaterials with tunable mechanical properties.

Beyond direct modulation of electrostatic interactions, an essential strategy for facilitating non-covalent crosslinking involves triggering conformational changes in globular proteins. These conformational changes are typically achieved by perturbing the native protein structure—thereby exposing hidden interactive domains such as hydrophobic patches, charged residues, or β-sheet-forming sequences—that can engage in intermolecular associations. This "conformational exposure–interaction assembly" mechanism is particularly effective for forming extended non-covalent networks and is central to the fabrication of protein-based hydrogels and scaffolds. For example， pH-induced aggregation and denaturation of bovine serum albumin (BSA) is a well-established method for preparing non-covalently cross-linked hydrogels. As the pH drops from neutral to near the isoelectric point (~4.7), surface charge neutralization reduces electrostatic repulsion, allowing protein molecules to approach and initiate weak aggregation. Further acidification to around pH 3.5 leads to partial unfolding of the protein from a compact globular state to an extended, cigar-like conformation. This structural remodeling is accompanied by a decrease in α-helix content and an increase in β-sheet formation, exposing hydrophobic domains and charged residues that promote intermolecular interactions—namely hydrophobic, ionic, and electrostatic associations—resulting in the assembly of non-covalently crosslinked hydrogel networks. Under alkaline conditions (pH > 10), BSA exhibits a distinct conformational shift toward a so-called "aged" form, with an increase in α-helical content and a reduction in β-structures, favoring gelation primarily via ionic interactions. (**Fig. 4b**).[77, 78] Overall, pH-induced non-covalent crosslinking exemplifies a “conformational exposure–interaction assembly” pathway, in which external pH cues reshape protein architecture to unlock hidden interactive motifs, allowing the construction of responsive and mild-condition-compatible protein materials. In contrast, thermal induction is another widely employed strategy for initiating non-covalent crosslinking in globular protein

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systems by promoting protein unfolding and structural reorganization. Heating BSA solutions above their denaturation temperature (~62 °C) facilitates the exposure of hydrophobic and charged residues, allowing proteins to form extensive non-covalent networks through hydrophobic associations, hydrogen bonds, and electrostatic interactions. The resulting aggregates stabilize into macroscopic hydrogel structures, with the extent of thermal denaturation and network formation being highly tunable based on protein concentration, temperature profile, and heating duration (**Fig. 4c**).[79]

Notably, combining heat treatment with pH adjustment enhances BSA gelation, highlighting the cooperative effect of dual stimuli in modulating protein conformation and assembly. In many studies, pH and thermal stimuli are employed synergistically to induce amyloid-like fibrillation of globular proteins, enabling more efficient unfolding and reorganization of protein structures. This dual-trigger strategy leverages the combined effects of electrostatic repulsion modulation and conformational mobility to facilitate the exposure of aggregation-prone regions. The resulting partially unfolded intermediates can assemble into highly ordered fibrillar architectures, offering a robust route for constructing mechanically enhanced protein materials.[80] For instance, lysozyme can undergo extensive conformational unfolding under acidic conditions (pH ~2) and elevated temperatures (~65 °C) over prolonged incubation. Upon subsequent dehydration, the denatured protein chains align and self-assemble into a highly ordered nanofibrillar membrane exhibiting remarkable stiffness and mechanical integrity.[81] This strategy has also been successfully applied to plant-derived proteins. In a recent study, researchers fabricated high-performance bioplastic films using soy protein isolate by heating it in aqueous acetic acid, which promoted its transition into amyloid-like nanofibrils. The resulting films demonstrated high tensile strength and optical transparency, highlighting the potential of this method for sustainable material development.[82] Similar protocols have also been applied to other globular proteins such as β-lactoglobulin and bovine serum albumin, where combined pH and thermal induction significantly enhance fibrillization efficiency and enable the formation of structurally robust protein-based materials.[83-91] Overall, the cooperative use of pH and heat serves as an effective strategy to overcome the intrinsic structural stability of globular proteins, facilitating their controlled transformation into high-performance fibrillar networks.

Beyond physical stimuli, chemical reagents can also be employed to induce the misfolding of globular proteins, thereby exposing hidden interaction domains and facilitating self-assembly. Disulfide bonds are critical in maintaining the tertiary structure of many globular proteins. These covalent linkages are reversible and can be selectively cleaved by various reducing agents, leading to partial or complete unfolding of the protein chains. Common thiol-based reductants—such as dithiothreitol (DTT), 2-mercaptoethanol, cysteine, and glutathione—break disulfide bridges through nucleophilic attack. While effective, these agents also generate free thiol groups, which may reoxidize and form uncontrolled intermolecular crosslinks under ambient conditions, potentially favoring random network formation over orderly self-assembly.[92] In contrast, tris(2-carboxyethyl)phosphine (TCEP), a non-thiol reductant, reduces disulfide bonds to the corresponding protein thiols via phosphine oxidation while introducing no additional low-molecular-weight thiols, offering broad pH efficacy and air stability that collectively mitigate thiol–disulfide exchange and undesired reoxidation.[93, 94] Leveraging this advantage, Yang's group systematically unfolded a series of disulfide-rich globular proteins using TCEP.[4, 81, 95-102] This strategy enabled the proteins to adopt extended conformations while avoiding premature aggregation or gelation. Remarkably, the unfolded chains underwent spontaneous self-assembly into continuous nanofibrous membranes, exhibiting high structural order and mechanical strength (**Fig. 4d**).[102] The method demonstrated broad applicability across various globular proteins, highlighting the general utility

of TCEP-induced unfolding for directing controlled supramolecular assembly rather than random crosslinking.

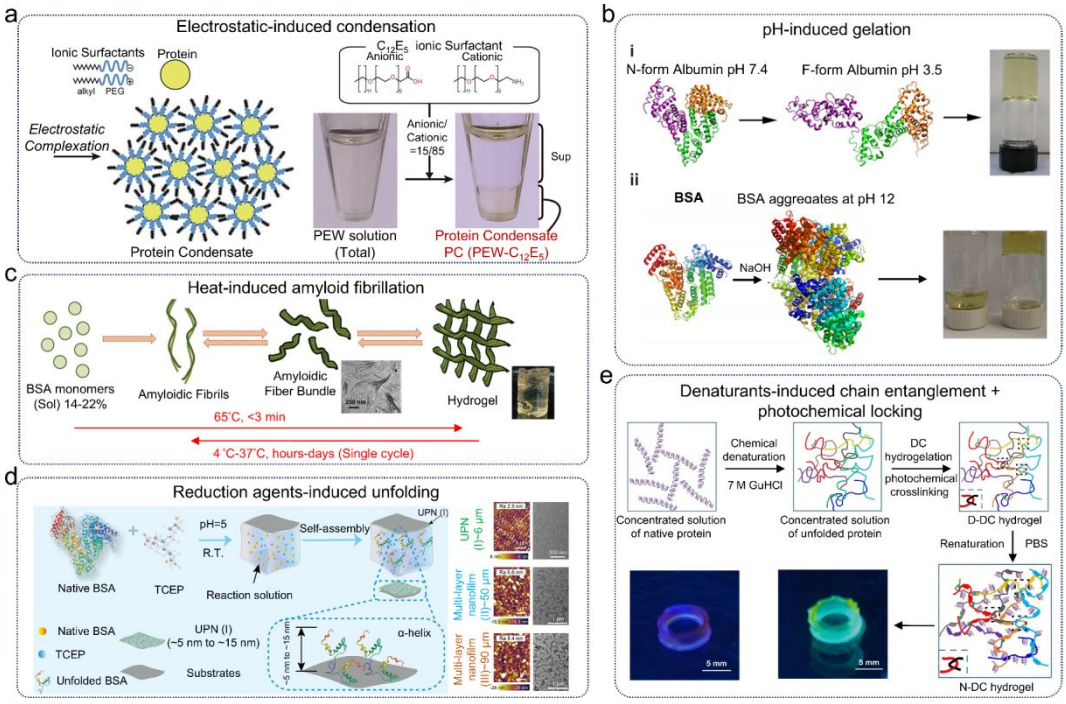

Fig. 4 Non-covalent crosslinking of globular proteins via supramolecular bonds. (**a**) Electrostatic protein–surfactant complexation in water induces liquid–liquid phase separation, forming dynamic condensates for gentle, tunable processing. Adapted with permission from **ref. 76**, Copyright 2022, Springer Nature. (**b**) pH-triggered partial unfolding of albumin enables fabrication of tough, flexible protein structures. Adapted with permission from **ref. 77** and **78**, Copyright 2014, American Chemical Society and Copyright 2018, John Wiley & Sons, Inc. (**c**) Mildly acidic heating converts BSA into amyloid fibrils and hydrogels in a single thermal cycle. Adapted with permission from **ref. 79**, Copyright 2021, Elsevier. (**d**) TCEP-mediated reductive unfolding of BSA yields unfolded protein nanoparticles that assemble into ordered nanofibrous membranes. Adapted with permission from **ref. 102**, Copyright 2024, Wiley-VCH GmbH. (**e**) Guanidine hydrochloride -induced denaturation of recombinant $(FL)_8$ extends and entangles chains; subsequent hydrogelation includes photochemical covalent crosslinking in addition to physical entanglement, and partial refolding yields tough, elastic hydrogels. Adapted with permission from **ref. 43**, Copyright 2022, Springer Nature.

Organic solvents and chaotropic agents have also been widely employed to disrupt the native fold of globular proteins. Organic fluorinated alcohols—such as trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP)—destabilize tertiary structures by solvating backbone amide groups and promoting α-helical content or aggregation-prone intermediates.[103-105] Similarly, chaotropic agents like guanidine hydrochloride (GuHCl) and urea disrupt intramolecular hydrogen bonds and hydrophobic interactions, especially at high concentrations, leading to extensive chain extension and structural unfolding.[106, 107] For example, Li et al. used concentrated guanidine hydrochloride to fully denature recombinant $(FL)_8$ proteins—an engineered globular protein construct—driving the chains into highly extended, highly-entangled conformations. Upon photochemical locking, a hydrogel network that integrates topological entanglement with partially refolded domains formed, yielding materials with exceptional mechanical strength and toughness (**Fig. 4e**).[43] This approach illustrates strong chemical denaturants can not only facilitate complete unfolding but also guide the post-denaturation refolding and assembly pathways to produce functional protein-based materials.

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3.2.2 Covalent Crosslinking Strategies

In contrast to non-covalent crosslinking strategies that typically rely on partial or complete unfolding of globular proteins to expose hidden interaction domains, covalent crosslinking often preserves the native globular conformation by directly targeting reactive surface groups for covalent coupling. This difference in approach confers several distinct advantages. By avoiding extensive structural denaturation, covalent crosslinking strategies can retain the intrinsic biochemical functions, binding specificities, and mechanical resilience encoded within the original protein folds. Among the various reactive groups present on protein surfaces, lysine residues are especially attractive due to their abundance and high nucleophilicity. For example, lysine accounts for approximately 10–12% of the amino acid composition in serum albumin and is similarly prevalent in other globular proteins such as soy protein, β-lactoglobulin, and lysozyme.[16, 17, 26, 108, 109] The primary amine side chains of lysine readily react with a broad spectrum of bifunctional crosslinkers to form stable covalent networks. A classic example is glutaraldehyde, a small bifunctional aldehyde that crosslinks proteins through Schiff base (imine) formation with lysine residues (**Fig. 5a**).[110] This dynamic covalent bonding strategy has been widely used to modulate the rheological properties of BSA hydrogels and underpins FDA-approved protein-based adhesives such as BioGlue®.[111] He et al. further expanded this system by integrating glutaraldehyde crosslinking with a wet-spinning process to fabricate BSA-based protein fibers exhibiting high mechanical strength, thereby extending its utility beyond traditional hydrogel platforms.[112] Alternatively, oxidized aldehyde-containing macromolecules have been utilized as multifunctional crosslinkers, forming imine-linked protein networks with enhanced scalability and design flexibility.[113-116] In addition to amine–aldehyde coupling, carboxyl groups—primarily from glutamic acid and aspartic acid residues—offer another avenue for crosslinking via carbodiimide-mediated condensation reactions. These groups can be activated using EDC/NHS chemistry to form amide bonds with lysine residues.[117] For instance, Chen et al. demonstrated that EDC/NHS coupling of BSA led to the formation of highly robust covalent hydrogels.[118, 119] In addition, the use of EDC alone to activate the carboxyl groups on the BSA surface is also sufficient to induce amide bond formation with amino groups, thereby establishing a covalent crosslinked network (**Fig. 5b**).[119] Similarly, NHS-activated polyethylene glycol (NHS-PEG-NHS) has been employed as a bifunctional crosslinker to bridge protein molecules via amide linkages, enabling the construction of HSA-based and BLG-based hydrogels with tunable properties.[33, 120] Furthermore, other crosslinking agents have been also explored to expand the available chemical toolbox. Epichlorohydrin, a heterobifunctional molecule containing both epoxide and chloromethyl groups, reacts efficiently with amines and has been used to fine-tune the mechanical properties of BSA hydrogels.[121] A more unconventional yet effective strategy involves the use of tetra(hydroxymethyl) phosphonium sulfate (THPS).[122, 123] In aqueous solution, THPS hydrolyzes to yield tris(hydroxymethyl)phosphine and formaldehyde. These intermediates undergo Mannich-type reactions with protein amines, forming Mannich bases or ammonium intermediates that further react to establish dense, amine-linked networks.

Recently, Alshakim Nelson and colleagues reported the fabrication of mechanically robust BSA-based hydrogels via a photoinduced aza-Michael addition reaction. In their system, long-chain poly (ethylene glycol) diacrylate (PEGDA) served as a multifunctional crosslinker, where the acrylate termini underwent a nucleophilic addition with the ε-amino groups of lysine residues on the BSA surface. This reaction—activated under UV light in the presence of a photoinitiator—enabled rapid and spatially controllable network formation, resulting in 3D-printed protein hydrogels with significantly enhanced mechanical strength.[124, 125] Moreover, many studies have explored the pre-functionalization of protein surfaces by grafting vinyl groups—such as acrylates or methacrylates—onto reactive residues like lysine,

allowing the introduction of pendant double bonds that serve as latent crosslinking sites (**Fig. 5c**).[71] Upon light activation in the presence of photoinitiators, these vinyl moieties undergo rapid radical polymerization, enabling the formation of dense, covalently bonded networks. This strategy significantly enhances the processability of globular protein materials, particularly in the context of digital light processing (DLP) and stereolithographic (SLA) 3D printing.[67, 69, 70, 126, 127] By endowing proteins with photocurable functionality, it becomes feasible to fabricate architected hydrogels with high spatial resolution, tunable mechanical properties, and application-specific geometries.

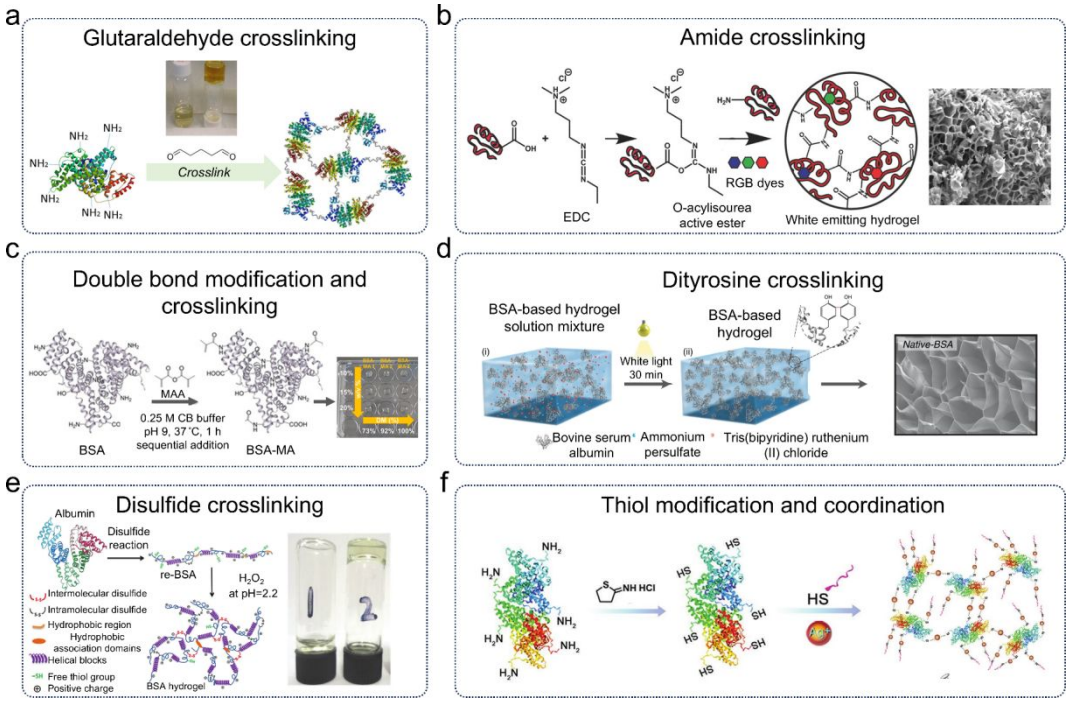

Fig. 5 Covalent crosslinking of globular proteins via chemical coupling at reactive functional sites. (**a**) Glutaraldehyde crosslinking of BSA forms imine (Schiff base) networks, yielding robust hydrogels. Adapted with permission from **ref. 110**, Copyright 2015, Springer Nature. (**b**) Carbodiimide (EDC) activation of surface carboxyls enables coupling to amines, forming amide crosslinks. Adapted with permission from **ref. 119**, Copyright 2017, Wiley-VCH GmbH. (**c**) Visible-light $Ru(bpy)_3^{2+}$-mediated oxidation of tyrosine forms dityrosine bonds, producing covalently crosslinked hydrogels. Adapted with permission from **ref. 71**, Copyright 2020, American Chemical Society. (**d**) Vinylation of BSA followed by radical polymerization generates tunable hydrogel networks. Adapted with permission from **ref. 128**, Copyright 2019, Springer Nature. (**e**) Reduction–reoxidation of native disulfides regenerates interprotein disulfide crosslinks to form self-supporting gels. Adapted with permission from **ref. 129**, Copyright 2016, Royal Society of Chemistry. (**f**) Surface thiolation introduces free thiols that coordinate with Ag^+ to form metal–thiolate crosslinks, yielding self-healing albumin hydrogels. Adapted with permission from **ref. 65**, Copyright 2020, Wiley-VCH GmbH.

In addition to lysine-targeted strategies, tyrosine residues offer another valuable reactive site due to their phenolic side chains.62 Under visible light irradiation, in the presence of tris(bipyridine) ruthenium (II) chloride ($[Ru(bpy)_3]^{\wedge 2+}$) and oxidants such as ammonium persulfate (APS), tyrosine residues undergo radical coupling to form dityrosine crosslinks (**Fig. 5c**).[128] This photoinitiated mechanism enables the formation of mechanically stable hydrogels in a spatially and temporally controllable manner.[41, 130-132] Slawinski et al. further showed that the inclusion of acetic acid in the reaction system generates carboxymethyl radicals that inhibit dityrosine formation, thereby offering a means to modulate the crosslinking density and mechanical properties of the resulting hydrogels.[133]

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Cysteine residues—featuring thiol (-SH) side chains—also offer a unique set of chemical reactivity that enables selective and versatile crosslinking strategies. Although cysteine is less abundant in most globular proteins, its thiol group is highly nucleophilic and prone to oxidation, making it an excellent target for redox-controlled or metal-coordination-based crosslinking. One widely employed method leverages the reversible nature of disulfide bond formation. Under reducing conditions, native disulfide bonds within proteins can be cleaved to expose free thiol groups. Upon subsequent re-oxidation, these thiols can recombine to form new inter- or intramolecular disulfide bridges, resulting in covalently crosslinked protein networks.[104, 105, 134] For instance, BSA hydrogels have been fabricated by first reducing the native disulfide bonds with agents such as dithiothreitol (DTT), followed by oxidative reformation of disulfide bonds (**Fig. 5e**).[129] Such redox-responsive systems are particularly attractive for biomedical applications where environmental cues such as redox gradients or reactive oxygen species (ROS) are present. In addition to redox-mediated crosslinking, thiol groups on cysteine residues also serve as effective ligands for coordination with soft metal ions. Silver ions (Ag^+) exhibit strong affinity toward thiol groups, enabling the formation of metal–thiolate bonds.[13] This metal–ligand interaction can be exploited to construct non-covalently and covalently robust hydrogels with self-healing properties. A typical strategy involves chemically introducing additional thiol groups onto the protein surface via thiolation reagents, such as 2-iminothiolane (Traut's reagent),[135] which converts surface-exposed amines into thiol groups (**Fig.5f**).[65] These introduced thiols subsequently coordinate with silver ions to form metal–thiol crosslinks, as demonstrated in the development of BSA-based self-healing hydrogels. The resulting materials exhibit shear-thinning behavior and dynamic mechanical recovery, making them ideal for injectable biomaterial applications or stimuli-responsive systems.[66, 136]

To optimize the mechanical performance of globular protein-based materials, synergistic integration of non-covalent and covalent crosslinking strategies has proven highly effective. For instance, subjecting proteins to thermal denaturation exposes internal functional groups and interaction motifs, which can then be further stabilized through subsequent covalent crosslinking—such as amide bond formation, dityrosine coupling, or radical polymerization of vinyl groups.[69, 118, 130] These dual-step strategies promote the formation of dense, hierarchical networks that combine the reversible adaptability of supramolecular interactions with the permanence and robustness of covalent linkages. Additionally, incorporation of polyphenolic or polyhydroxy compounds offers another layer of mechanical enhancement by introducing abundant hydrogen-bond donors and acceptors, thereby reinforcing the protein matrix through dynamic, multivalent hydrogen bonding.[69, 125, 137-142] Notably, phenolic hydroxyl groups can function as coordination donors, forming stable metal–phenol complexes with multivalent ions. These coordination crosslinks contribute additional crosslinking density and introduce energy-dissipative, reversible interactions that significantly improve material toughness and fatigue resistance. When combined with other non-covalent interactions—such as hydrophobic association and π–π stacking—these multiphase, cooperative mechanisms collectively enhance the mechanical strength, elasticity, and structural resilience of globular protein materials, thereby expanding their potential for use in mechanically demanding and dynamically responsive applications.[73, 131]

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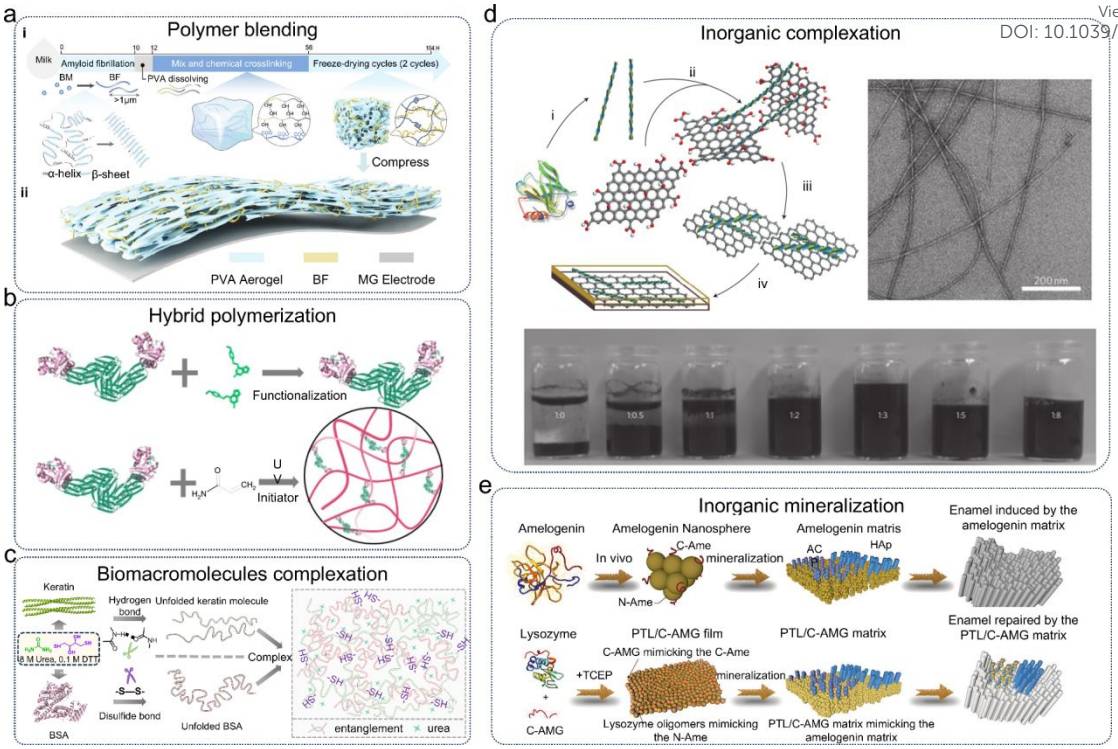

Fig. 6 Protein-based organic–inorganic hybrid strategies for tuning the mechanical properties of globular protein materials. (**a**) β-Lactoglobulin aerogel composite integrated with polyvinyl alcohol to enhance mechanical performance. Adapted with permission from **ref. 86**, Copyright 2024, Wiley-VCH GmbH. (**b**) Functionalization followed by ultraviolet-initiated crosslinking with acrylamide-type monomers forms protein networks, yielding hybrid hydrogels with tunable mechanics. Adapted with permission from **ref. 54**, Copyright 2020, Springer Nature. (**c**) Amyloid fibrils hybridized with graphene oxide nanosheets via π–π interactions and hydrogen bonding form aligned nanocomposites with increased strength and conductivity. Adapted with permission from **ref. 106**, Copyright 2025, Springer Nature. (**d**) Soy protein isolate and nanocellulose combined via amidation and epoxy crosslinking produce reinforced nanocomposite networks. Adapted with permission from **ref. 90**, Copyright 2012, Springer Nature. (**e**) Amelogenin-inspired lysozyme hybrid mimics enamel biomineralization; assembly into nanospheres and films guides hierarchical mineralization and strengthens mechanical integrity. Adapted with permission from **ref. 99**, Copyright 2020, Wiley-VCH GmbH.

3.3 Hybrid Strategies

To overcome the intrinsic mechanical limitations of globular proteins, hybrid offers a powerful strategy by integrating organic and inorganic phases at multiple scales. As shown in **Figure 6**, various protein-based hybrid systems have been developed that synergize the functional versatility of proteins with the robustness of synthetic or inorganic components.

3.3.1 Protein–organic hybrid systems

A variety of functional polymers have been employed to enhance the mechanical properties of globular protein-based materials by leveraging specific intermolecular interactions. Among them, cationic polyelectrolytes such as polyethyleneimine (PEI) and poly(L-lysine) (PLL) are widely utilized due to their high density of amine groups. These positively charged moieties can form electrostatic interactions with negatively charged residues on protein surfaces, while also participating in hydrogen bonding networks that reinforce the polymer–protein interface.[128, 143, 144] For instance, Popa et al. demonstrated that both PEI and PLL significantly increased the stiffness of bovine serum albumin (BSA) hydrogels, with PEI achieving up to a sixfold enhancement due to its branched architecture and higher charge

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density. This stiffening effect was accompanied by a reduction in pore size and water content, as well as an increase in pore wall thickness, indicating improved network compaction and crosslinking. Importantly, these modifications did not alter the native folding of the BSA domains, preserving their biochemical function.[128] Poly (acrylic acid) (PAA) represents another class of polyelectrolyte that can interact with globular proteins through covalent bonding. Its carboxyl groups can be activated using EDC/NHS chemistry to form amide bonds with protein lysine residues, resulting in covalently crosslinked hybrid gels with enhanced cohesion. This approach has been successfully applied to develop albumin-based adhesive patches with high wet adhesion and mechanical tolerance, where the covalent integration of PAA contributes to network toughness and interfacial stability.[145] In addition to charged polymers, poly (vinyl alcohol) (PVA) offers a hydrogen-bond-rich matrix that can synergistically interact with protein backbones and side chains. PVA's ability to form dense hydrogen-bonding networks, coupled with its semi-crystalline domains, lends significant mechanical reinforcement when hybridized with protein assemblies.[86, 91, 146-148] For example, Zheng et al. reported the fabrication of aerogel networks composed of fibrillated β-lactoglobulin and PVA, where careful modulation of hybrid parameters enabled the production of flexible films spanning a wide range of mechanical stiffnesses (**Fig. 6a**). The presence of PVA not only contributed to structural integrity via entanglement and crystallization but also facilitated solvent responsiveness and film processability.[86]

Beyond direct blending with preformed polymers, globular proteins can also serve as active components in in-situ free-radical copolymerization, where monomers are mixed with proteins and polymerized to yield hybrid hydrogels with tunable mechanics and functionalities. Common vinyl monomers include acrylamide (AAm), N, N-dimethylacrylamide (DMAA), N-(2-hydroxyethyl)-acrylamide (HEAA), and various glycidyl or methacrylate derivatives—each offering different hydrophilicity, crosslinking density, and network dynamics. When these monomers are co-polymerized in the presence of globular proteins, the resulting materials often form interpenetrating polymer networks (IPNs) or double networks (DNs), in which the protein domains provide reversible crosslinks (via thermal unfolding, hydrogen bonds or metal coordination) while the synthetic polymer network supplies a percolating scaffold for load transfer.[123, 139, 149-156] For example, Chen et al. mixed AAm with bovine serum albumin (BSA) and initiated polymerization to form a DN hydrogel: one network arising from thermally-unfolded BSA crosslinks, and the second from PAAm chains. This architecture led to dramatic improvements in adhesive strength, toughness, and strain tolerance of the composite hydrogel.[155] In another approach, tandem-repeat proteins (G8) were used as molecular crosslinkers within a conventional polyacrylamide matrix (**Fig. 6b**). Upon stretching, these polyprotein crosslinks unfold only near crack tips, dissipating energy locally and preventing catastrophic fracture. The resulting hydrogels simultaneously achieve ultrahigh stretchability, low hysteresis, and high fracture toughness, as well as exceptional anti-fatigue resistance over thousands of cycles—attributes difficult to reconcile in purely synthetic or purely proteinaceous gels.[54] Furthermore, globular proteins can be engineered to serve as initiator-rich macromolecular crosslinking centers for polymer growth. For example, Xu et al. chemically modified BSA with methacrylate groups (BSA-MA), introducing polymerizable double bonds on its surface. Subsequent oxidative polymerization of pyrrole (PPy) from these modified sites led to the formation of a BSA-MA-PPy conductive hydrogel, where BSA acted as a nucleating and crosslinking core. This structure not only endowed the hydrogel with high mechanical strength, but also imparted it with electrical conductivity and self-healing ability, showing great promise for applications in wearable sensors and soft bioelectronics.[68]

The hybrid of globular proteins with natural biopolymers has also emerged as a green, mild, and

functionally enriched strategy for mechanical enhancement. These composite materials retain the structural and functional advantages of proteins while incorporating the flexibility, biocompatibility, and network-forming capabilities of natural polysaccharides and proteins. This synergy significantly improves mechanical strength, bioactivity, and degradability.[1, 3, 13] Common natural components used in such hybrids include polysaccharides like cellulose,[157-159], Lignin,[160, 161] chitosan,[162] alginate,[100, 116, 163-166] dextran,[115] heparin,[136] pectin,[88] and gellan gum.[87, 114] These polysaccharides are rich in hydroxyl, amino, and carboxyl groups, enabling stable network formation with globular proteins through hydrogen bonding, electrostatic interactions, or covalent crosslinking. For instance, gellan gum, due to its anionic nature, can interact electrostatically with positively charged surfaces of lysozyme nanofibers formed under acidic conditions. This charge-driven complexation, followed by wet spinning, allows the fabrication of high-strength composite fibers.[57] Chitosan, a cationic polysaccharide, readily complexes with negatively charged regions of proteins such as BSA and casein. Its semi-crystalline backbone further enhances rigidity and mechanical stability, making it suitable for constructing bioadhesive and antibacterial hydrogels.[162] In addition, natural proteins such as gelatin,[167, 168] silk fibroin,[169-172] casein,[173] and keratin[106, 174-176] also form co-networks with globular proteins via hydrophobic interactions, hydrogen bonding, or enzymatic crosslinking. For example, Gao et al. developed a tough hydrogel by combining BSA with gelatin and crosslinking them with genipin, achieving high tensile and compressive strength.[168] More recently, He et al. proposed a novel strategy using wool keratin to regulate the mechanical behavior of globular proteins. Through denaturation, the protein chains were unfolded to form highly entangled states, and dynamic disulfide crosslinking was achieved via free thiol groups. This led to the formation of composite fibers with excellent strength and toughness. The same approach was successfully extended to other globular proteins, such as soy protein isolate, β-lactoglobulin (BLG), and ovalbumin (OVA), demonstrating broad versatility and scalability (**Fig. 6c**).[106]

3.3.2 Protein–inorganic hybrid systems

Building on the versatile co-assembly strategies involving polymers, the integration of globular proteins with inorganic components has significantly broadened the functional scope of protein-based materials. In contrast to their organic counterparts, inorganic nanomaterials offer intrinsic advantages such as high stiffness, chemical robustness, and unique electronic or bioactive properties. By engaging in specific interactions with amino, carboxyl, thiol, or phenolic groups on protein residues, inorganic domains can establish intimate associations with protein networks, giving rise to hierarchical soft–hard hybrid systems. These heterogeneous architectures leverage the complementary characteristics of flexible protein matrices and rigid inorganic frameworks, resulting in substantial enhancements in mechanical strength, environmental resilience, and multifunctionality. Generally, protein–inorganic integration follows two principal strategies: (1) the direct combination of pre-formed inorganic nanoparticles with protein assemblies, and (2) in-situ mineralization, where protein scaffolds direct the nucleation and growth of inorganic phases.

In the direct hybrid route, the Mezzenga's group has conducted extensive studies using globular proteins such as β-lactoglobulin and lysozyme, which are first denatured and assembled into amyloid-like nanofibrils. These nanofibrils are then combined with various inorganic nanomaterials—such as porous carbon frameworks,[177] $CaCO_3$,[178] hydroxyapatite (HAP),[179] and graphene oxide (GO)[90]—to fabricate functional hybrid aerogels and films. A representative example involves the integration of GO with β-lactoglobulin-derived amyloid fibrils to construct protein–graphene composites. In this system, thermally denatured β-lactoglobulin forms fibrillar templates that interact electrostatically with GO nanosheets at pH 2, exploiting the charge contrast between the two components (**Fig. 6c**). Subsequent chemical

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reduction with hydrazine restores the sp²-hybridized carbon network of graphene while retaining the supporting protein scaffold. Vacuum filtration of the hybrid dispersion yields free-standing composite membranes with outstanding mechanical performance—including high tensile strength and modulus—alongside excellent electrical conductivity.[90] Beyond graphene hybrids, other inorganic materials have also been employed to reinforce protein networks. Carbon quantum dots have been shown to modulate the nanostructure and enhance the mechanical properties of OVA-based hydrogels by strengthening their internal architecture.[180] Functionalized silica nanoparticles have acted as non-covalent cross-linkers in soy protein isolate matrices, dramatically improving adhesion strength and wet stability through hydrogen bonding and interfacial reinforcement.[181] Similarly, Laponite clay nanosheets have been blended with chemically unfolded BSA, forming nanocomposite films with remarkable strength and water resistance through strong electrostatic interactions.[182]

In parallel, in situ mineralization approaches exploit the templating ability of protein scaffolds to guide the formation of inorganic nanophases. Mezzenga and co-workers demonstrated that β-lactoglobulin and lysozyme amyloid fibrils can serve as structural templates for the in-situ generation of nanoparticles such as gold,[183, 184] silver,[185] and TiO_2,[85] leading to functional films and aerogels.[185] More recently, Yang's group has developed a suite of protein-templated hybrid systems based on amyloid-like globular proteins.[96, 98, 99, 186] In one example, BSA nanofibrils were used to guide hydroxyapatite mineralization via ion infiltration, producing hierarchically aligned HAP nanofibers with excellent mechanical strength and bioactivity.[96] In another biomimetic study, lysozyme fibrils were employed to emulate enamel biomineralization, directing the nucleation and growth of dense, homogeneous HAP coatings that exhibited exceptional hardness and scratch resistance (**Fig. 6e**).[99]

Collectively, these examples highlight the great potential of globular protein–inorganic hybrid systems. By harnessing the structural tunability and chemical functionality of protein scaffolds in tandem with the superior mechanical and multifunctional properties of inorganic components, such strategies enable the design of next-generation materials with precisely tailored strength, biofunctionality, and environmental robustness.

3.4 Hierarchical Structural Engineering

Hierarchical structural engineering offers an additional dimension of control over the mechanical and functional performance of globular protein-based materials. By organizing protein assemblies across mesoscopic and macroscopic scales, it becomes possible to modulate stress distribution, enhance anisotropic properties, and integrate directional responsiveness into the final materials.

At the mesostructural level, inducing alignment of protein building blocks represents a key strategy to impart long-range anisotropy and enhance mechanical robustness. Various external stimuli have been employed to control the spatial organization of proteins, thereby tuning the resultant material properties. One such method is the application of electric fields, which can guide the directional migration and orientation of charged protein molecules, leading to the formation of ordered hydrogel structures with anisotropic mechanical behavior and improved structural regularity. For instance, electrostatic induction can direct the orientation of SPI aggregates in hydrogel systems, producing anisotropic protein networks with enhanced mechanical integrity and structural regularity.[187, 188] Similarly, electrospinning techniques utilize high-voltage electrostatic fields in combination with a rotating collector to generate unidirectionally aligned nanofibrous mats. The resulting membranes exhibit improved tensile strength due to the high degree of molecular orientation along the fiber axis.[14, 189-193] Another powerful technique is directional freezing (freeze-casting), where ice crystals serve as a dynamic template to guide the assembly of proteins into oriented porous architectures. During the freezing process, protein molecules

are expelled from the advancing ice front and accumulate between growing ice lamellae. Upon subsequent sublimation of the ice, an anisotropic, often layered or radially aligned scaffold remains. This strategy has been successfully applied to fabricate globular protein-based materials with improved mechanical strength.[32, 159, 194] In addition to these physical guiding strategies, substrate-assisted alignment has also been explored. Hierarchical alignment of protein nanofibrils can be achieved by casting or depositing protein materials on structured substrates with grooved micro- or nanoscale patterns. These predefined topographies act as physical guides, enabling macroscale orientation of nanofibers and resulting in materials with highly ordered architectures and enhanced anisotropic properties.[195]

At the macroscopic level, three-dimensional structural design enables precise control over bulk mechanical behavior, including tensile, compressive, and shear properties. Emerging fabrication techniques such as 3D printing and direct ink writing (DIW) allow for programmable patterning of protein-based inks into complex geometries with tunable density and anisotropy. These methods not only preserve the biochemical functionality of the constituent proteins but also empower the construction of architected materials with tailored stiffness, elasticity, and deformation pathways.[67, 69, 97, 124-127]

Collectively, these hierarchical engineering strategies complement molecular and nanoscale design approaches by enabling structure–property integration across multiple length scales. Through the deliberate organization of nanofibrils, microarchitectures, and 3D frameworks, it becomes possible to unlock the full mechanical potential of globular protein materials for load-bearing, responsive, and biofunctional applications.

4. Manufacturing and Performances of Globular Protein-Based Functional–mechanical materials

4.1 Fabrication Strategies

The development of globular protein-based functional–mechanical materials with superior mechanical and structural functionalities heavily depends on precise and efficient processing strategies. Due to the thermal sensitivity, hydrophilicity, and complex folding behavior of globular proteins, conventional fabrication techniques must be adapted or innovated to preserve or utilize their structural integrity during material processing. Researchers have established a diverse set of methods to guide the transformation of globular proteins from molecular states to bulk forms, offering structural control across nano to macro scales. The following categorization summarizes current strategies based on their fabrication pathways:

4.1.1 Interfacial Self-Assembly into Nanofilms

Native globular proteins can unfold under mild reductive conditions (e.g., tris(2-carboxyethyl) phosphine, TCEP), exposing their hydrophobic domains. These unfolded proteins spontaneously assemble at the air–water interface into continuous, dense nanofilms due to interfacial tension and directional hydrophobic interactions. This process can be conducted under biocompatible conditions (neutral pH, ambient temperature) without the need for harsh solvents or high-energy input.[196, 197] Research by Yang et al. has demonstrated the generation of highly anisotropic protein nanofilms through additional application of shear force at the liquid surface, aligning protein domains directionally to enhance mechanical anisotropy (**Fig. 7a**).[96] Their studies further extended to self-assembly at various solid-liquid interfaces—including metals, glass, plant leaves, and fabrics—enabling the formation of protein coatings with diverse interfacial functionalities.[99, 101, 102, 186, 198, 199] These interfacial strategies yield nanomembranes with uniform morphology, tunable thickness, and excellent scalability, making them ideal for applications in flexible electronics, biosensors, and protective barriers.

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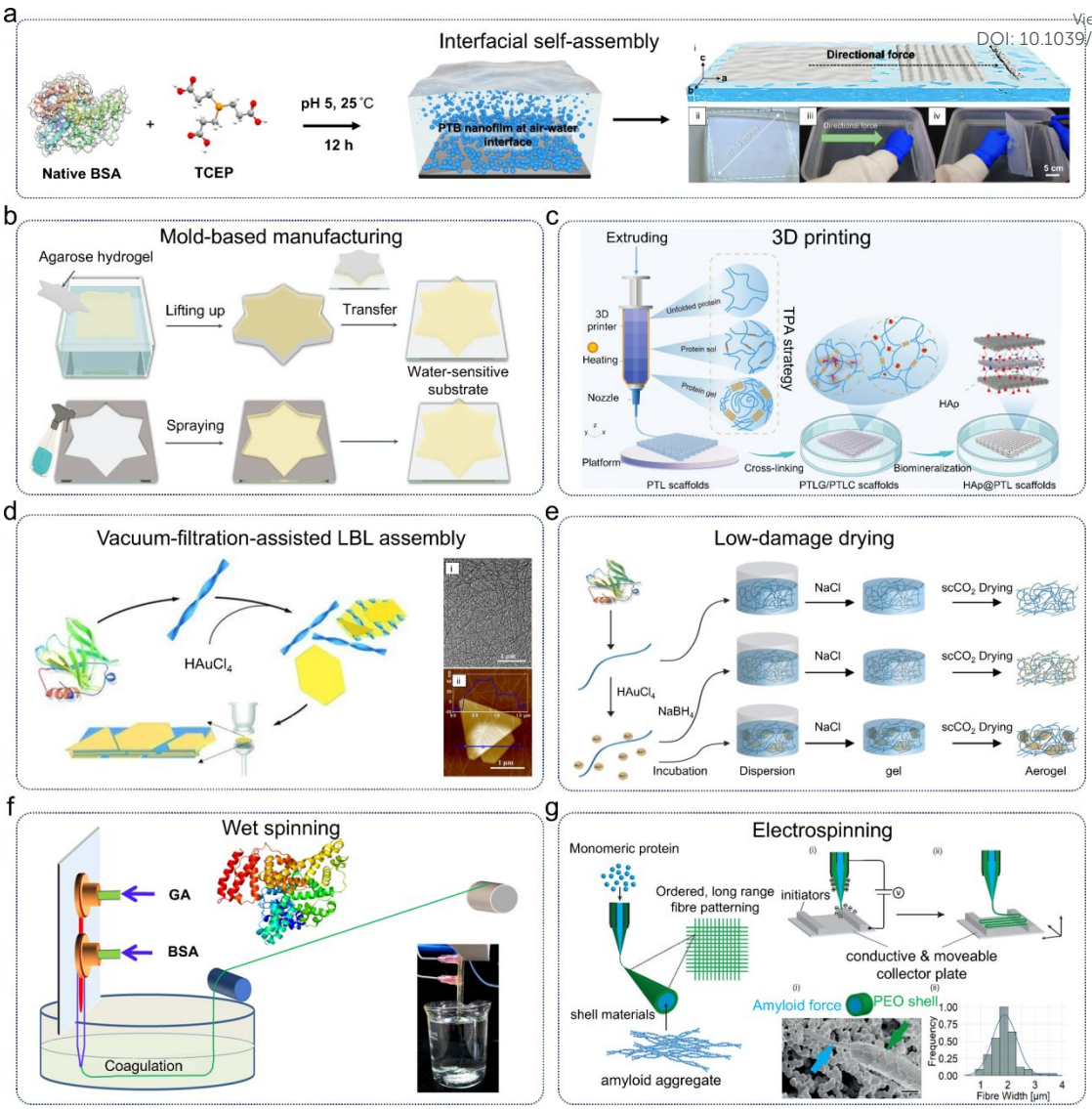

Fig. 7 Overview of fabrication strategies for globular protein-based functional–mechanical materials. (a) Interfacial Self-Assembly: BSA unfolds under mild reduction (e.g., TCEP) and assembles at the air–water interface into nanofilms; directional shear further aligns domains to strengthen the membranes. Adapted with permission from **ref. 96**, Copyright 2025, Wiley-VCH GmbH. (**b**) Mold-Based Shaping: protein solutions/gels are cast in water-sensitive molds and transferred/sprayed to shape films with precise geometry for scaffolds and coatings. Adapted with permission from **ref. 81**, Copyright 2024, Springer Nature. (**c**) 3D Printing: extruded protein inks solidified by thermal protein aggregation, followed by biomineralization, build hierarchical scaffolds with tunable mechanics. Adapted with permission from **ref. 97**, Copyright 2024, Wiley-VCH GmbH. (**d**) Vacuum-filtration–assisted layer-by-layer (LBL) assembly: filtered protein nanofibrils form dense or layered films with controlled thickness and alignment, suitable for barrier materials or flexible electronics. Adapted with permission from **ref. 183**, Copyright 2013, Wiley-VCH GmbH. (**e**) Low-Damage Drying: supercritical CO_2 drying preserves network structure during solvent removal, yielding highly porous aerogels with tunable mechanics. Adapted with permission from **ref. 184**, Copyright 2016, Wiley-VCH GmbH. (**f**) Wet Spinning: coagulation and drawing produce aligned protein fibers. Adapted with permission from **ref. 112**, Copyright 2019, Wiley-VCH GmbH. (**g**) Electrospinning: electrostatic jetting yields amyloid core–shell and aligned deposits, enhancing functionality. Adapted with permission from **ref. 89**, Copyright 2021, American Chemical Society.

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4.1.2 Mold-Based Shaping

Mold casting remains one of the most accessible and widely adopted strategies for fabricating protein-based materials. This technique involves pouring protein hydrogels or solutions into predesigned molds, followed by drying, curing, or gelation to form stable geometries (**Fig. 7b**).[81] By manipulating protein concentration, ionic environment, pH, and drying rate, self-assembly and crosslinking behavior can be tuned to obtain specific shapes and textures. Molded materials can range from cylindrical hydrogels and flat membranes to intricate 3D structures such as stars or tissue-mimicking scaffolds. This method is especially valuable for applications requiring precise shape conformity, such as tissue scaffolds, wound dressings, or soft implants. It is compatible with various material states, including wet gels, dried films, and aerogels, and can be further enhanced through pattern transfer techniques (e.g., microstamping, embossing) or composite layer integration.[41-43, 45, 82, 118, 128, 131, 143]

4.1.3 3D Printing

Three-dimensional (3D) printing introduces programmable spatial control in the fabrication of protein-based constructs, enabling the integration of structural complexity and mechanical gradients within the material. Two common printing modalities are employed for globular proteins: (**i**) Photopolymerization: Using photoinitiators such as ruthenium complexes and ammonium persulfate, crosslinking can be rapidly induced via dityrosine bond formation under light exposure.[172] Alternatively, vinyl-functionalized proteins or protein-monomer mixtures can undergo free-radical polymerization during stereolithographic printing, resulting in structurally robust constructs.[67, 69, 124-127] (**ii**) Thermal Protein Aggregation (TPA): Heat is applied to induce protein unfolding and intermolecular aggregation, forming a dynamic gel network. Upon cooling, the partially refolded proteins reassemble into nanostructured aggregates, which solidify into a stable matrix. This strategy allows fabrication of mechanically reinforced scaffolds with tailored porosity and stiffness (**Fig. 7c**). By fine-tuning the printing path, nozzle parameters, thermal profile, and post-crosslinking treatments (e.g., mineralization with hydroxyapatite), multiscale control over material density, pore architecture, and fiber orientation can be achieved.[97] This approach has significant promise in constructing load-bearing scaffolds for bone and connective tissue engineering.

4.1.4 Vacuum Filtration

Vacuum-assisted filtration is particularly effective after protein nanofibrillization. Proteins such as β-lactoglobulin or whey protein can be thermally or pH-induced to form β-sheet-rich nanofibers. These dispersions are filtered through membranes under vacuum, enabling the layer-by-layer accumulation of aligned or entangled fibril networks. and biofunctional membranes exhibiting excellent tensile strength, moisture sensitivity, and barrier properties. Advantages include straightforward processing, tunable film thickness and porosity, and compatibility with other assembly strategies like co-filtration or sequential deposition (**Fig. 7d**).[90, 177, 183, 200] It is highly suitable for fabricating large-area membranes and biomimetic nanostructures.

4.1.5 Low-Damage Drying Techniques

Drying proteins without denaturation or shrinkage is crucial for retaining the hierarchical structure and mechanical integrity of the final material. Two major techniques are employed: (**i**) Lyophilization (Freeze-Drying): Proteins in aqueous gels are frozen, and water is sublimated under vacuum. This retains the original network structure and introduces porosity.[86] By using directional freezing or freezing under thermal gradients, oriented ice templates can guide the formation of anisotropic, multilayered porous structures during sublimation.[32, 159] (**ii**) Supercritical CO_2 Drying: Water is gradually replaced with

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ethanol, then with liquid CO_2, followed by heating beyond the supercritical point. This process prevents capillary collapse and results in highly porous, low-density protein aerogels. Hybrid aerogels composed of protein nanofibrils and inorganic components have been developed by Mezzenga et al.,[91, 178, 185] demonstrating superior strength-to-weight ratios and enhanced thermal insulation or adsorption capabilities (**Fig. 7e**).[184] Both techniques offer environmentally friendly, scalable routes to fabricate ultralightweight, high-surface-area protein materials for biomedical, filtration, or sensor applications.

4.1.6 Wet Spinning

In wet spinning, concentrated protein solutions are extruded into a coagulation bath, where solvent exchange induces phase separation and solidification. The shear forces during extrusion align protein molecules, and additional post-stretching enhances chain orientation and crystallinity (**Fig. 7f**). For globular proteins, unfolding and partial refolding dynamics can be modulated by bath composition (e.g., salts, pH) and temperature. Crosslinking agents (e.g., glutaraldehyde) or hybrid with nanofillers can reinforce the resulting fibers. This strategy enables the continuous production of tough, elastic protein fibers with applications in wearable biomaterials, tissue sutures, and artificial ligaments.[8, 87, 88, 106, 112, 201, 202]

4.1.7 Electrospinning

Electrospinning leverages high-voltage fields to stretch protein solutions into ultrafine fibers, which are collected onto rotating or translating collectors. These fibrous mats exhibit nanoscale porosity, high surface area, and tunable anisotropy, making them ideal for wound healing, filtration, and bioactive coatings. Globular protein electrospinning often requires blending with carrier polymers or modifying solution parameters (e.g., viscosity, conductivity) to ensure stable jet formation.[14, 189-192, 203] Oriented fiber deposition and direct electro-writing enable precise patterning of fibers at micro to millimeter scales.[89] Advanced strategies include in-situ crosslinking, multiaxial collectors, and electrostatic guidance for hierarchical or gradient architectures (**Fig. 7g**).

Through a diverse suite of fabrication strategies—from interfacial self-assembly and 3D printing to advanced spinning and drying techniques—globular proteins can be effectively transformed into functional–mechanical materials with tunable mechanical properties, anisotropic architectures, and functional responsiveness. These methods address the inherent challenges of protein processing while unlocking their potential in biomedical devices, regenerative scaffolds, smart materials, and environmentally friendly composites.

4.2 Morphologies and Mechanical Performances of Globular Protein-Based Materials

Globular proteins can be processed into various material morphologies through precise non-covalent and covalent manipulation. These material forms include fibers, membranes, hydrogels, and porous structural materials, each exhibiting distinct structural features and mechanical responses. This section systematically discusses these material forms and their associated properties, with an emphasis on tensile, compressive, and adhesive behaviors such as strength and modulus (**Table 2-4**).

4.2.1 Fibrous Materials

Globular protein-based fibers, primarily produced via wet spinning and electrospinning, exhibit excellent mechanical performance due to the alignment of protein chains and the formation of dense hydrogen-bonding networks during processing. Wet-spun fibers are typically generated from concentrated protein solutions that are extruded and coagulated into continuous filaments. A representative example involves BSA-based fibers fabricated by glutaraldehyde crosslinking during wet spinning, which achieved tensile

strengths up to 279 MPa—the highest value reported for serum albumin-derived fibers.[112] This performance is comparable to spider silk and exceeds that of conventional collagen-based fibers. Recently, He et al. successfully developed a high-strength albumin-based composite fiber without the need for glutaraldehyde crosslinking agent by introducing a chain entanglement strategy (**Fig. 8a**). This fiber not only exhibits excellent mechanical properties (with a strength of approximately 250 MPa and a toughness as high as 70 MJ/m³), but also maintains good immunocompatibility.[106] In addition, globular protein composite fibers have been successfully developed by incorporating lysozyme nanofibers with gellan gum, yielding fibers with tensile strengths reaching 265 MPa.[87] More impressively, a recent study using recombinantly engineered multimeric globular proteins has achieved tensile strengths as high as 378 MPa, significantly surpassing the mechanical performance of previously reported protein-based fibers and demonstrating the potential of molecular design strategies.[8] For electrospinning, many globular proteins such as soy protein isolate require blending with other polymers to achieve appropriate viscosity and chain entanglement. However, BSA can be directly electrospun using mixed aqueous/organic solvents to form uniform nanofibers with tensile strengths of 30–60 MPa and Young's moduli of 1.5–2 GPa, representing excellent performance for pure protein-based fibers(**Table 2**).[189]

Table 2 Overview of representative globular protein-derived fibers, summarizing their compositions, processing protocols, mechanical properties, and reported applications.

Composition	Processing conditions and method	Mechanical properties	Applications	Reference
BSA	TFE/H_2O, β-ME; Electrospinning	TS: 30-60 MPa; TM:1.5-2 GPa	—	**189**
BSA	TFE/H_2O, β-ME; Electrospinning	TM:1.22 MPa	Cardiac patch	**190**
BSA	Glutaraldehyde crosslinking; Microfluidic spinning	TS: 279 MPa; TM:4.4 GPa	—	**112**
BSA	TCEP, pH, mineralization; Self-assembly, stretching	TS: ~143 MPa; TM: ~4.7 GPa	Cranial bone regeneration	**96**
BSA/keratin	Urea, DTT; Wet spinning	TS: ~250 MPa; TM: ~70 MPa	Surgical sutures	**106**
OVA	Glutaraldehyde crosslinking; Wet spinning	TS: ~60 MPa; TM: ~2.6 GPa	Biological suture.	**202**
BLG/PEO	Ph; Electrospinning	TS: 10-15 MPa; TM:1.1 GPa	—	**89**
BLG/pectin	pH, heating, $CaCl_2$ crosslinking; Microfluidic spinning	TS: 201 MPa; TM:7.0 GPa	—	**88**
SPI/PEO	Water; Electrospinning	TS: 1.0 MPa; TM: 41 MPa	—	**192**
Sericin/Cyclo-FF NWs	Ethyl alcohol dehydration; Wet spinning	TS: 180 MPa; TM:10 GPa	—	**201**
Lysozyme/Gellan gum	pH, heating; Wet spinning	TS: 265 MPa; TM:2.5-8 GPa	—	**87**
Poly (IG) protein	HFIP; Wet spinning	TS: 378 MPa; TM:4.2 GPa	—	**8**

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Abbreviations: TS, tensile strength; TM, tensile modulus.

Table 3 Overview of representative globular protein-derived films, summarizing their compositions, processing protocols, mechanical properties, and reported applications.

Composition	Processing conditions and method	Mechanical properties	Applications	Reference
BSA	TFE/H_2O, β-ME; Casting	TS: ~6.5 MPa, TM: 12 MPa	Large-scale sensing	**104**
BSA/Laponite	TCEP; Casting	TS: 21 MPa, TM: 2 GPa	—	**182**
Lysozyme	TCEP; Self- assembly	TS: ~150 MPa, TM: ~8.3 GPa	—	**204**
Lysozyme	pH, heating; Self-assembly, casting	TM: 5.2-6.2 GPa	—	**200**
Lysozyme	TCEP, pH, mineralization; Self-assembly	Hardness: 3.8 GPa	Dental repair coating	**186**
Lysozyme	TCEP, pH, mineralization; Self-assembly	Hardness: 4.5 GPa	Dental repair coating	**99**
Lysozyme/alginate	TCEP; Self-assembly	TS: ~70 MPa, TM: 4 GPa	OLED device	**198**
BLG/PVA	pH, heating; Casting	TS: ~24 MPa, TM: ~0.3 MPa	Flexible bio-sensors	**86**
BLG/PVA	pH, heating; Casting	TS: 17-22 MPa	Food package	**84**
BLG/GO	pH, heating; Self-assembly, vacuum filtration	TS: 6 MPa; TM: ~8 GPa	Enzyme-sensing	**90**
BLG/HAP	pH, heating; Self-assembly, vacuum filtration	TS: ~11 MPa; TM: ~1.4 GPa	—	**179**
SPI	Dehydration; Casting	TS: ~25 MPa		**64**
SPI	pH, heating; Casting	TS: 16 MPa, TM: 209 MPa	Food package	**82**
SPI/PEI	Heating; Casting	TS: 11 MPa	—	**144**
SPI/PEI	Heating, coordination; Casting	TS: 8.3 MPa	—	**143**
SPI/HPSA	pH, heating; Casting	TS: 15 MPa, TM: 174 MPa	—	**205**
SPI/FK	pH, heating; Casting	TS: 8.2 MPa	—	**175**
SPI/HBPE	pH, heating; Casting	TS: 13.7 MPa, TM: 376 MPa	—	**137**
Sericin	Ethanol, dehydration; Casting	TS: ~60 MPa	—	**206**
Sericin	Ethanol, dehydration; Casting	TS: 40.5 MPa, TM:2.8 MPa	—	**207**
Sericin/keratin	Dehydration; Casting	TS: 32 MPa, TM:656 MPa	—	**174**

Abbreviations: TS, tensile strength; TM, tensile modulus.

4.2.2 Film (Membrane) Materials

Globular protein-based membranes are typically produced by casting protein solutions into molds and allowing them to dry into flexible or rigid films. During this process, the network transitions from a hydrated gel to a denser, less hydrated solid, with thicknesses ranging from micrometers to millimeters. Mechanical assessments of these membranes consider not only tensile strength and modulus but also

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bending stiffness and fracture toughness.[90, 200] The mechanical performance of these membranes can vary significantly depending on residual water content and drying methods. For instance, fully dried β-lactoglobulin nanofibril films, especially when reinforced with graphene oxide, show high stiffness values (~8 GPa)[90] but exhibit brittle behavior at low strains due to tight molecular packing and low water content. In contrast, membranes prepared under ambient conditions retain more water and exhibit greater extensibility and toughness, though with reduced stiffness. Furthermore, the incorporation of plasticizers—such as glycerol—has been demonstrated to effectively modulate the mechanical properties of protein-based membranes by enhancing polymer chain mobility and facilitating hydrogen bonding interactions. For instance, the addition of glycerol to soy protein isolate (SPI)-based nanostructured membranes markedly reduces brittleness and significantly enhances ductility, enabling better mechanical adaptability (**Fig. 8b**).[82] In parallel, the incorporation of polymeric additives such as polyvinyl alcohol (PVA) can further enhance the mechanical properties of hybrid β-lactoglobulin nanofibril-based membranes. These membranes exhibit notable improvements in flexibility and stretchability, even under relatively low hydration conditions, owing to the synergistic effects of molecular interactions and phase compatibility (**Table S2**).[84, 86]

4.2.3 Hydrogel Materials

Hydrogels derived from globular proteins are among the most extensively studied forms due to their high-water content, tunable shape, and biofunctional potential. These 3D hydrated networks can be molded into diverse geometries such as cubes, spheres, cylinders, and rings, and exhibit varied mechanical properties depending on formulation and crosslinking strategies.[208] Typically, conventional globular protein hydrogels show relatively low tensile strength—often in the range of several pascals to a few megapascals—due to their high-water content and weak molecular interactions.[13, 29] However, recent advances have significantly improved their mechanical robustness. For instance, the incorporation of glycerol into BSA hydrogels has led to tensile strengths reaching 12 MPa, along with enhanced elasticity and fracture resistance.[105] In specific applications such as tissue engineering, compressive strength becomes the dominant performance metric, especially for bulk geometries. A notable example is the development of $(FL)_8$-based hydrogels for cartilage replacement, which demonstrated compressive strength values of approximately 68 MPa—a level adequate for load-bearing biological applications (**Fig. 8c**).[43] Moreover, 3D printing enables multiscale structural control, including specific pore architectures and mechanical gradients that enhance compressive and tensile properties (**Table 4**).[124]

Moreover, soft gels formed by the aggregation of unfolded proteins at high concentrations can develop strong amyloid-like β-sheet structures through hydrophobic interactions and hydrogen bonding. These structures enable robust covalent and non-covalent interactions at interfaces, resulting in excellent adhesive performance. Using this unfolding–aggregation strategy, Yang et al. developed globular protein-based gels with outstanding adhesion, achieving underwater shear strengths up to ~4 MPa—significantly outperforming many conventional adhesives (**Fig. 8d**).[103] In addition, several studies have developed polymer-hybridized organohydrogel systems that exhibit strong interfacial adhesion. The presence of abundant polar and non-polar groups within the gel enables multiple interactions with surfaces, leading to excellent shear and peel adhesion performance.[145, 150, 151, 155] In addition to conventional mechanical performance, many protein hydrogels and organohydrogels display self-healing capabilities, enabled by reversible covalent bonds or dynamic non-covalent interactions—such as disulfide bond, imine bond, metal coordination, hydrogen bond, and hydrophobic aggregation—which allow the network to restore its structure after damage.[78, 114, 121, 134, 135, 144, 209, 210] These self-healing protein-based hydrogels exhibit excellent adaptability to dynamic environments and outstanding

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resilience under mechanical stress, showing great potential in fields such as tissue engineering, wearable electronics, and soft robotics, where materials are frequently subjected to deformation and damage.[121, 144]

Shape-memory behavior represents another distinctive feature of protein-based hydrogels. Engineered with defined geometries and architectures, these materials can undergo reversible conformational transitions of globular protein domains in response to external stimuli such as humidity, solvent composition, ionic strength, and mechanical stress. For example, bovine serum albumin (BSA) embedded in a hydrogel matrix can reversibly unfold in 6 M guanidine hydrochloride (GuHCl) and refold in Tris buffer, enabling controlled, repeatable shape switching.[128] Similarly, such hydrogels can exhibit tunable mechanical properties—stiffening in high-salt conditions and softening in standard buffer solutions—thereby completing a full shape memory cycle based on environmental cues.[131] Moreover, in 3D-printed structured hydrogels, the globular protein domains can undergo stress-induced unfolding during deformation and subsequently recover their original conformation upon appropriate stimuli, such as thermal treatment or water-induced swelling.[124] These reversible morphological transformations offer exciting opportunities for designing reconfigurable soft materials with programmable shape memory behavior. [124, 128, 131]

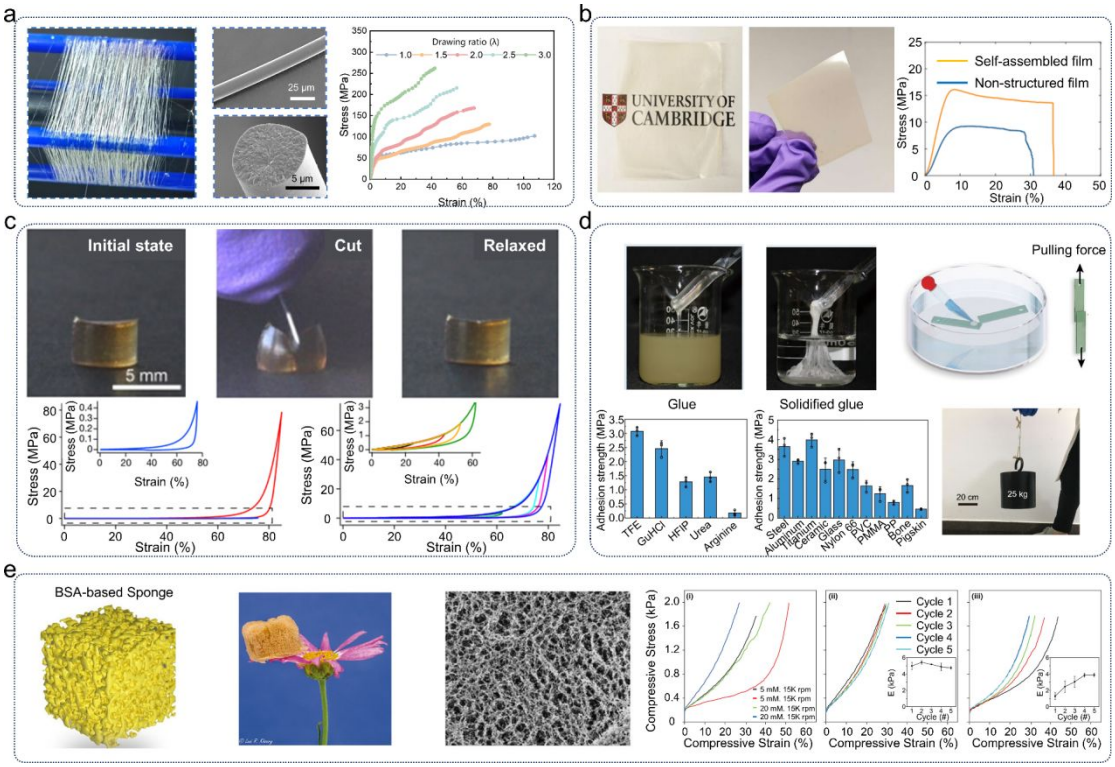

Fig. 8 Representative morphologies and mechanical behaviors of globular protein–based materials. (**a**) Wet-spun BSA fibers with aligned, hierarchical structure; tensile stress–strain curves showing high strength and toughness. Adapted with permission from **ref. 106**, Copyright 2025, Springer Nature. (**b**) Self-assembled PSI film with higher modulus and extensibility than non-structured films (stress–strain comparison). Adapted with permission from **ref. 82**, Copyright 2021, Springer Nature. (**c**) Entanglement-enhanced $(FL)_8$ hydrogel exhibits rapid mechanical recovery. Adapted with permission from **ref. 43**, Copyright 2023, Springer Nature. (**d**) Adhesive hydrogels from globular proteins via unfolding–aggregation show strong underwater adhesion. Adapted with permission from **ref. 103**, Copyright 2023, Springer Nature. (**e**) BSA-based sponge displays an interconnected porous network, enabling tunable resilience and energy dissipation. Adapted with permission from **ref. 132**,

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Table 4 Overview of representative globular protein-derived hydrogels, summarizing their compositions, processing protocols, mechanical properties, and reported applications.

Composition	Processing conditions and method	Mechanical properties	Applications	Reference
HSA	PEG-$(SS)_2$ crosslinking; Casting	TS: ~40 KPa; CS: ~273 KPa	Wound healing	**120**
MA-HSA	Double bond photocrosslinking; Casting	CS: ~158 KPa, CM: ~88 KPa	Xeno-Free Microneedle	**72**
BSA	Dityrosine photocrosslinking; Casting	TS: 33 KPa, TM: 191 KPa	—	**131**
BSA	Heating, dityrosine photocrosslinking; Casting	CS: 38 MPa, CM: 1.5 MPa; TS: 0.62 MPa, TM: 2.9 MPa	—	**130**
BSA	pH; Casting	CS: ~8 MPa, CM: 630 KPa;	Hemostatic	**211**
BSA	Heating, amide crosslinking; Casting	CS: 115 MPa, CM: 971 KPa; TS: 0.43 MPa, TM: 1.0 MPa	—	**118**
BSA	TFE/H_2O, β-ME; Casting	TS: ~12 MPa, TM: 50 MPa	Artificial Skin	**105**
BSA	TFE/H_2O or urea, GuHCl/TCEP; Collaborative assembly	AS: 0.46-3.6 MPa	Adhesives	**103**
BSA	BAC, Urea, l-cys; Collaborative assembly	AS: 0.1-0.4 MPa	Adhesives	**107**
BSA/PVA	GSH reduction, genipin crosslinking; Casting	CS: ~1.62 MPa	—	**148**
BSA/PVA	Tannic acid crosslinking; Casting, freeze−thaw	TS: ~9.5 MPa	—	**146**
BSA/PMAAm	Heating, coordination, polymerization crosslinking; Casting	TS: ~110 KPa, TM: ~550 MPa; CS: 2 MPa, CM: ~550 KPa	—	**153**
BSA/GEL	Genipin crosslinking; Casting	TS: 8.72 MPa, TM: 4.62 MPa; CS: 0.5 MPa	Wound healing	**168**
BSA/PEGDA	HPG crosslinking, photocrosslinking; 3D printing	YS: 1.8-50 MPa, TM: 29-1000 MPa	—	**125**
BSA/PEGDA	Photocrosslinking, heating; 3D printing	TS: 46 MPa	Scaffold, implants, stents	**124**
MA-BSA/PEGDA	TA crosslinking, heating; 3D printing	TS: 7.1 MPa, TM: ~115 MPa	Bioplastic screw	**69**
MA-BSA/PEGDA	Double bond photocrosslinking; 3D printing	CS: 6.3 MPa, CM: 2.4 MPa;	—	**67**
BSA/vinyl monomers	Polymerization crosslinking, Heating; Casting	CS: 50 MPa, CM: 1.0 MPa; TS: 0.48 MPa, TM: 0.8 MPa	Adhesives	**150**
BSA/PMAAm	Heating, polymerization crosslinking; Casting	CS: 1.5-3.5 MPa, CM: 100-150 KPa;	—	**152**
BSA/PAAm	Heating, polymerization crosslinking; Casting	TS: 410 KPa, TM: 650 KPa	Adhesives	**155**
MA-BSA/PAM/PPy	Double bond, polymerization crosslinking; Casting	TS: 5.36 MPa, TM: 3.13 MPa	Electrocardiogram sensing	**68**
BSA/PAA	alginate-dopa crosslinking; Casting	TS: ~8 MPa, TM: 0.3 MPa	Adhesive bandage	**145**
OVA	Heating, surfactant; Casting	TS: ~0.38 MPa, TM: ~250 KPa; CS: 34.5 MPa, CM: 117.8 KPa	—	**75**
Lysozyme	TCEP, heating; 3D printing	CS: ~57 KPa, CM: ~3 KPa	Skull bone regeneration.	**97**
Lysozyme/CNC	pH, heating; Casting, ice-templated freeze-drying	CM: ~400 KPa	—	**159**
SPI	TGA/WPF crosslinking; Collaborative assembly	AS (dry):1.56 MPa; AS (wet):0.77 MPa	Adhesives	**212**
SPI	SiO_2/PEI/DBA crosslinking; Collaborative assembly	AS (dry):2.6 MPa; AS (wet):1.46 MPa	Adhesives	**181**
SPI/CS	Multiple bonds crosslinking; Collaborative assembly	AS (dry): 2.73 MPa; AS (wet): 1.86 MPa	Adhesives	**162**
SPI/PAE	Tanic crosslinking; Collaborative assembly	AS (dry): ~2.5 MPa; AS (wet): ~1.0 MPa	Adhesives	**138**
SPI/AM	TA-Fe^{2+} crosslinking; Collaborative assembly	AS (dry): 1.4 MPa; AS (wet): 1.3 MPa	Adhesives	**139**
BLG	pH, heating, BTCA crosslinking; Casting, freeze-drying	CS: ~40 KPa, CM: 200 KPa	Cell scaffolds	**32**
Sericin	TA crosslinking; Collaborative assembly	AS: ~1.0 MPa	Wound healing	**141**
Sericin/PAAm	Polymerization crosslinking; Casting	TS: ~56.6 KPa, TM: ~9 KPa	Flexible sensing	**156**
MA-sericin/GO	PEGDA polymerization crosslinking; Casting	TS: ~142 KPa	Implantable bioelectronics	**213**
Sericin/GO/Alginate	HRP enzymatic crosslinking; Casting	CS: ~68 KPa	Bone regeneration	**163**
G8/PAA	Double bond crosslinking; Casting	TS:110 KPa, YM: ~80 KPa	—	**54**
$(FL)_8$	Denaturant-induced entanglement; Casting	CS: 68 MPa, CM: 1.7 MPa; TS: ~0.7 MPa, TM: ~0.7 MPa	Cartilage repairing	**43**
$(FL)_n$ series	Dityrosine photocrosslinking; Casting	TS: 33-48 KPa; TM:14-18 KPa	—	**42**
$(GB_1)_8$	Four-armed PEG crosslinking; Casting	TS: ~100 KPa, TM: 84 KPa	—	**45**

Abbreviations: TS, tensile strength; TM, tensile modulus; CS, compressive strength; CM, compressive modulus; YS, yield strength; AS, adhesion strength; SM, storage modulus.

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4.2.4 Porous Materials

Porous materials derived from globular proteins—such as sponges and aerogels—are typically fabricated by drying protein-based hydrogel precursors through methods like freeze-drying, foaming, or supercritical drying. These processes yield materials with micro- to nano-scale porosity, offering a combination of low density and high surface area, which is particularly advantageous for applications in filtration, thermal insulation, environmental remediation, and biomedical scaffolding. From a mechanical standpoint, these materials are primarily characterized by their compressive resilience, elastic recovery, and energy dissipation capacity. For example, BSA-based sponges produced via photochemical crosslinking followed by foaming and drying demonstrate moderate compressive strength and excellent shape recovery, making them ideal for applications that require deformation tolerance and structural durability such as absorbent pads (**Fig. 8e**).[132] Mechanical properties can be significantly enhanced by introducing amyloid-like protein nanofibers into the network. When processed using mold-guided ice-templated freeze-drying, these nanofiber-reinforced aerogels exhibit improved compressive strengths of up to ~40 kPa, along with high elastic modulus and excellent structural recoverability. These attributes make them particularly promising for high-performance scaffolds and adaptive biointerfaces.[32] Further improvements can be achieved through composite strategies. For instance, blending globular proteins such as β-lactoglobulin with synthetic polymers like polyvinyl alcohol (PVA) yields aerogels with compressive strengths reaching ~60 kPa, while also improving toughness and durability.[91] Moreover, recent advancements have demonstrated that templating protein nanofiber networks with inorganic nanoparticles, followed by supercritical drying, enables the fabrication of hierarchical porous aerogels. These materials exhibit multifunctional performance, combining enhanced mechanical strength with additional properties such as electrical conductivity, catalytic activity, or bioactivity, depending on the choice of incorporated nanomaterials.[178, 184, 185]

Overall, the morphological transformation of globular proteins into a diverse array of material forms enables finely tuned mechanical performance—ranging from ultrasoft, self-healing hydrogels to ultrastiff, high-strength fibers and membranes. By precisely adjusting formulation parameters, processing techniques, and crosslinking strategies, these protein-based materials can be engineered to meet a broad spectrum of functional and structural needs in areas such as tissue engineering, flexible electronics, soft robotics, and filtration. The versatility in achievable morphologies highlights the exceptional potential of globular proteins as next-generation building blocks for multifunctional, bioinspired materials.

5. Mechanical Applications of Globular Protein-Based Materials

Owing to their inherent structural diversity and tunable mechanical properties, globular protein–based materials have emerged as highly promising candidates for a broad spectrum of mechanically functional applications. Their unique combination of adaptability, resilience, and responsiveness supports their integration across both biomedical and non-biomedical fields. This section outlines the ways in which their mechanical attributes are utilized in various application contexts, laying the groundwork for a more detailed exploration of their roles in specific functional scenarios.

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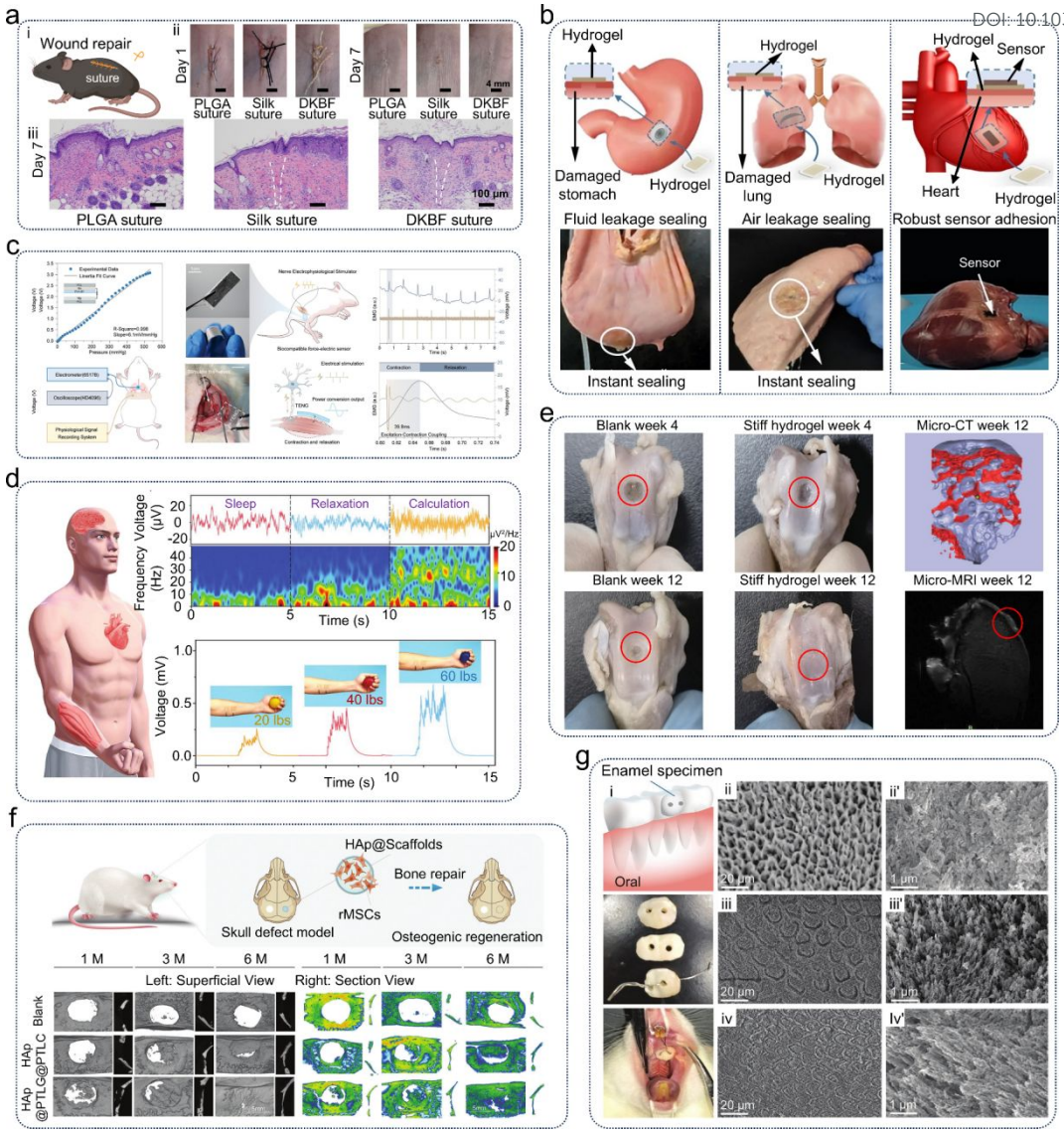

Fig. 9 Representative applications of globular protein-based materials in biomedical fields. (**a**) Chain entanglement–enhanced, disulfide-crosslinked keratin/BSA fibers show high tensile strength and toughness, supporting use as absorbable surgical sutures. Adapted with permission from **ref. 106**, Copyright 2025, Springer Nature. (**b**) Albumin hydrogel patches form robust adhesion to wet tissues within seconds, enabling rapid sealing of gastric, pulmonary, and cardiac defects. Adapted with permission from **ref. 145**, Copyright 2021, Springer Nature. (**c**) β-Lactoglobulin fibril porous membranes combine flexibility and durability, enabling implantable triboelectric nanogenerators for biomechanical energy harvesting and physiological sensing. Adapted with permission from **ref. 86**, Copyright 2024, Wiley-VCH GmbH. (**d**) Sebum-membrane-inspired BSA bioprotonic hydrogels function as artificial skin and human–machine interfaces. Adapted with permission from **ref. 105**, Copyright 2023, Wiley-VCH GmbH. (**e**) Engineered $(FL)_8$ hydrogels with chain entanglement display increased stiffness and fracture toughness, supporting cartilage regeneration in load-bearing settings. Adapted with permission from **ref. 43**, Copyright 2023, Springer Nature. (**f**) 3D-printed, biomineralized lysozyme–hydroxyapatite hybrids show strong osteoconductivity and mechanical resilience, promoting skull bone regeneration. Adapted with permission from **ref. 97**, Copyright 2024, Wiley-VCH GmbH. (**g**) Lysozyme-templated hydroxyapatite composites recapitulate the hierarchical nanostructure of enamel and dentin, supporting biomimetic dental regeneration. Adapted with permission from **ref. 99**, Copyright 2020, Wiley-VCH GmbH.

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5.1 Biomedical Applications

Globular protein-based materials are increasingly being explored for a wide range of biomedical applications, particularly where mechanical integrity is critical. These applications span from soft tissues—such as skin, blood vessels, and internal organs—requiring flexibility and wet adhesion, to hard tissues like bone and teeth that demand high stiffness and load-bearing capacity. By engineering globular proteins into diverse formats including fibers, films, hydrogels, and hybrid architectures, these materials can be finely tailored to meet the distinct physiological and mechanical requirements of targeted tissues, highlighting their potential in soft tissue repair, flexible bio-electronics, and load-bearing tissue regeneration.

5.1.1 Soft Tissue Sealing and Adhesive Interfaces

Globular protein-based materials offer valuable advantages in soft tissue sealing and wound closure due to their excellent biocompatibility, tunable mechanical properties, and strong adhesion under wet physiological environments. One prominent strategy involves the development of fibrous protein composites that serve as bioresorbable sutures. As illustrated in **Fig. 9a**, disulfide-crosslinked keratin/BSA fibers (DKBFs) exhibit high tensile strength, sufficient flexibility, and reliable in vivo degradation, making them effective for skin and soft tissue suturing.[106] Beyond suturing, soft hydrogel patches have emerged as promising adhesives capable of rapidly sealing wounds on internal organs. BSA-based hydrogel patches demonstrate high interfacial adhesion and burst pressure resistance. Their strong bonding to wet tissue surfaces enables effective sealing of perforated stomach, lung, and cardiac tissues. These materials benefit from cohesive hydrogel strength combined with adhesive functionalities such as hydrogen bonding, hydrophobic interaction, and covalent coupling (**Fig. 9b**).[145] Such strategies can be extended to other globular protein systems. For example, electrospun albumin nanofiber patches have been developed as cardiac patches for myocardial infarction therapy, showing enhanced cardiac function and tissue remodeling in vivo.[203] In addition, sericin–tannic acid (Ser-TA) hydrogels function as highly effective wet adhesives, forming conformal seals on dynamic organs via self-assembling nanostructures.[141] Another example includes a BSA–gelatin double-network hydrogel, which provides robust tissue adhesion while maintaining tissue compatibility and strong mechanical durability, making it suitable for gastric wall defect sealing.[168] These studies collectively highlight the potential of globular protein-derived fibers and adhesives in diverse soft tissue sealing and repair scenarios, offering rapid, strong, and safe adhesion for clinical use.

5.1.2 Bioelectronic Interfaces and Soft Sensing Systems

The integration of globular proteins into flexible bioelectronic systems enables new opportunities for implantable sensors and real-time physiological monitoring. Such systems require materials with excellent mechanical softness, biointegration capability, and functional responsiveness—attributes that globular protein-based materials can effectively fulfill. As demonstrated in **Fig. 9c**, β-lactoglobulin nanofibril membranes were employed in a triboelectric nanogenerator (TENG) capable of converting organ motion into electrical signals. These membranes provide soft, flexible interfaces that are compatible with dynamic biological environments. The device not only harvests biomechanical energy from visceral movement (e.g., leg muscle) but also acts as a self-powered physiological sensor. This work exemplifies that protein nanomaterials can bridge mechanical actuation and electrical response for in vivo applications.[86] Additionally, **Fig. 9d** presents a class of flexible, BSA-based electronic skin (e-skin) patches for health monitoring. These devices can conform to the contours of human skin and joints, continuously tracking physiological signals like pulse, respiration, and motion. One notable system

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integrates conductive elements into a soft protein matrix to ensure stable output and real-time response, even under repetitive deformation. This wearable device platform has shown promise in smart health diagnostics and rehabilitation monitoring.[105] Together, these developments establish the feasibility of protein-based soft bioelectronics for both implantable and epidermal applications, pushing the boundaries of personalized health monitoring and intelligent diagnostics.

5.1.3 Load-Bearing Tissue Repair and Regeneration

Regeneration of load-bearing tissues such as cartilage, bone, and dental enamel presents significant challenges, demanding biomaterials that can simultaneously provide mechanical strength, biological activity, and integration with host tissue. Globular protein-based materials—either as hydrogels, composites, or biomineralized scaffolds—offer a promising toolkit for addressing these challenges. As illustrated in **Fig. 9e**, mechanically reinforced protein hydrogels composed of tandem-repeat $(FL)_8$ polyproteins exhibit entangled-chain structures that afford high fracture toughness and elasticity.[43] These hydrogels mimic the load-bearing capacity of articular cartilage and can be directly injected into cartilage defects, promoting chondrogenesis and joint surface regeneration. In the context of bone regeneration, **Fig. 9f** shows the use of PTLG-mineralized hydroxyapatite (HAp@PTLG) scaffolds in a critical-sized skull defect model.[97] The hybrid scaffold supports both osteogenic differentiation of mesenchymal stem cells and new bone formation, facilitated by the nanoscale mineral alignment and protein-mediated cell signaling. These constructs successfully regenerate large bone defects over a 6-month period, highlighting the long-term regenerative potential of protein–inorganic hybrids. Lastly, dental tissue repair is addressed through biomimetic reconstruction of enamel. As presented in **Fig. 9g**, lysozyme-guided self-assembly leads to highly oriented hydroxyapatite nanocrystals that closely mimic natural enamel in both microstructure and mechanical properties. This approach holds great promise for enamel-like surface restoration and long-term dental protection.[99] Collectively, these examples underscore the capacity of globular protein-based materials to function as dynamic scaffolds for hard tissue regeneration, combining load-bearing resilience with regenerative bioactivity.

5.2 Non-Biomedical Applications of Globular Protein-Based Materials

Globular protein-based materials are increasingly being explored in non-biomedical fields due to their intrinsic biodegradability, structural versatility, and abundance of functional groups. For instance, bovine serum albumin (BSA)-based porous sponges have been developed as eco-friendly adsorbents for environmental remediation. Fabricated via optimized foaming protocols, these sponges exhibit high porosity, mechanical robustness, and effective pollutant removal—demonstrated by their ~80% adsorption efficiency for perfluoro octane sulfonate (PFOS) in near-neutral water (**Fig. 10a**). The adsorption process is characterized by strong surface interactions and reusability, underscoring their potential for broader applications in catalysis, drug delivery, and environmental systems.[132]

In engineering, albumin-based adhesives have shown strong bonding capacity on wood or metal surfaces while being fully biodegradable, offering a green alternative to conventional petroleum-derived glues (**Fig. 10b**).[107, 214] Similarly, soy protein-based adhesives are gaining traction in wood and composite bonding. Through chemical enhancements such as lignin crosslinking, melamine resin incorporation, and glycidyl ether modification, their water resistance and mechanical integrity have been significantly improved, expanding their potential in industrial and structural uses.[158, 161, 176, 215] In the field of sustainable packaging, high-performance protein films are emerging as biodegradable substitutes for conventional plastics. Self-assembled soy protein films demonstrate excellent tensile strength and environmental compatibility, making them ideal for eco-friendly food packaging.[82] Additionally,

composite membranes combining whey protein with polyvinyl alcohol (PVA) exhibit enhanced strength and biodegradability, further supporting their applicability in sustainable packaging solutions (**Fig. 10c**).[84] Beyond films and adhesives, globular proteins are also being applied in 3D printing. For example, soy protein blended with polylactic acid (PLA) has been used to create printable biocomposites that can be molded into biodegradable products such as flower pots, highlighting the material's potential in sustainable manufacturing and consumer goods.[216]

Collectively, these examples demonstrate the versatility of globular protein-based materials in advancing green material technologies, with broad implications for environmental protection, industrial adhesives, sustainable packaging, and bio-based product design.

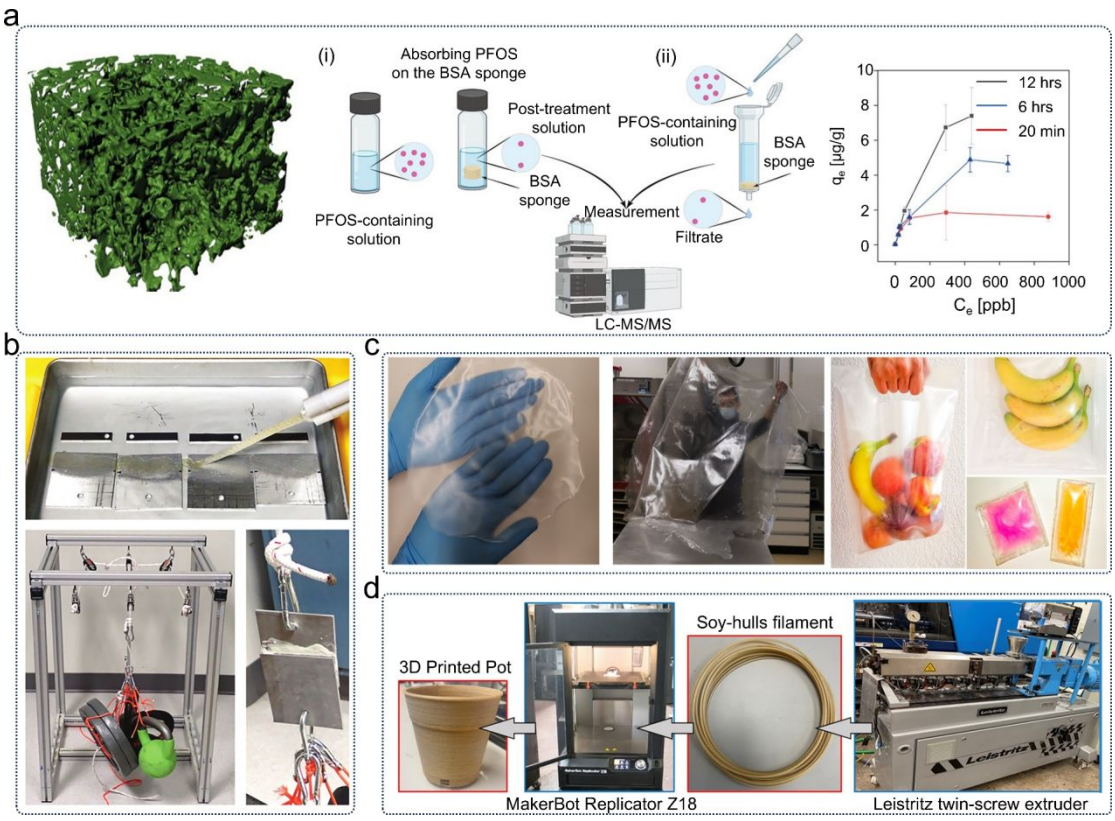

Fig. 10 Representative non-biomedical applications of globular protein-based materials: (**a**) BSA sponges remove perfluoro octane sulfonate (PFOS) via their porous structure and functional groups. Adapted with permission from **ref. 132**, Copyright 2025, Wiley-VCH GmbH. (**b**) BSA-derived adhesive bonds metal plates, showing strong adhesion in seawater and potential biodegradability for engineering use. Adapted with permission from **ref. 107**, Copyright 2024, Wiley-VCH GmbH. (**c**) β-Lactoglobulin/PVA composite films for biodegradable food packaging with improved mechanical strength and sustainability. Adapted with permission from **ref. 84**, Copyright 2021, American Chemical Society. (**d**) Soy protein/PLA composites 3D-printed into biodegradable flower pots, illustrating sustainable manufacturing. Adapted with permission from **ref. 216**, Copyright 2023, MDPI.

6. Conclusion and Outlook

In this review, we have systematically explored the transformation of globular proteins—traditionally classified as non-structural biomolecules—into functional-mechanical materials. These proteins, which include serum albumins, enzymes, milk globulins, silk sericin, soy proteins, and recombinant analogs, are characterized by compact tertiary structures and diverse biochemical functions. While not inherently mechanical in nature, their well-defined structures and modifiable surfaces offer a unique foundation for

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engineering multifunctional materials. We have highlighted key advances in molecular design, crosslinking strategies, hybrid control, and hierarchical assembly that enable the conversion of functional globular proteins into structurally robust systems. Emerging applications in tissue regeneration, soft robotics, and environmentally responsive materials further underscore their potential to bridge the gap between biological function and mechanical performance. However, despite this progress, the field remains at an early and exploratory stage. The successful translation of globular proteins into high-performance material systems requires addressing a set of fundamental scientific and technical challenges, which also define the directions for future research.

6.1 Bridging Challenges and Opportunities: From Fundamental Science to Application Viability

One of the most pressing challenges lies in the inherent trade-off between mechanical enhancement and functional preservation, which critically impacts material utility. Mechanical strengthening often induces irreversible conformational changes that compromise biological activity. Developing strategies that maintain protein function while imparting structural integrity is therefore a central issue for creating materials that deliver both mechanical performance and desired biofunctionality in applications like bioactive implants or responsive devices.

In addition, compared to naturally fibrous proteins, materials constructed from globular proteins often lag in strength, stiffness, and toughness. Although techniques such as chemical crosslinking, composite formation, and chain entanglement have shown promise, the design rules remain poorly established, with limited predictability and controllability of mechanical outcomes. This performance gap currently limits their competitiveness against established structural materials (e.g., synthetic polymers, silks) in demanding engineering applications, posing a significant barrier to broader market adoption.

The processing and fabrication of globular protein materials also present considerable hurdles with profound implications for scalability and cost-effectiveness, key determinants of commercial viability. Their thermal sensitivity and solubility in aqueous media render them incompatible with standard polymer processing techniques (e.g., melt extrusion, injection molding), necessitating specialized, often energy-intensive, and potentially costly alternative methods. While solution-based assembly methods (e.g., hydrogel formation, electrospinning) offer routes to material formation, they frequently face challenges in scalability, reproducibility, and achieving the high production rates required for economically feasible manufacturing. Furthermore, variations in protein source, extraction methods, and purification protocols can lead to significant inconsistencies in final material properties, compounding quality control difficulties and hindering standardization essential for commercial products.

Environmental and interfacial stability adds yet another layer of complexity that directly affects material lifespan, reliability, and suitability for intended applications. Most globular protein-based materials rely on weak physical interactions or labile chemical bonds, making them vulnerable to degradation, swelling, and instability in physiological or engineering environments—especially under long-term mechanical loading or fluctuating conditions. This susceptibility raises concerns about long-term performance and durability in real-world settings, potentially limiting their use in critical applications and increasing lifecycle costs. A deeper understanding of interfacial mechanics and strategies to enhance long-term durability is urgently needed to enable reliable deployment.

Collectively, these fundamental challenges—the function-performance trade-off, mechanical performance deficits, processing limitations, and stability concerns—translate into substantial barriers for the application and commercialization of globular protein materials. Overcoming these requires not

only scientific innovation but also a concerted focus on scalable manufacturing, cost reduction, stringent quality control, and demonstrating robust performance under real-world conditions to meet market demands and regulatory standards.

6.2 Future Directions: Toward Predictable, Scalable, and Functional Protein Materials

Progress will benefit from constraint-aware, multiscale design that simultaneously preserves biochemical function and upgrades mechanical response while improving predictability and development efficiency. Computational tools—such as AlphaFold, molecular dynamics, and multiscale modeling—can guide the rational design, simulation, and screening of candidate proteins to improve both folding stability and mechanical performance. At the sequence level, strategies like inserting flexible linkers, designing force-responsive motifs, or constructing multi-domain recombinant proteins can unlock novel mechanisms of stress dissipation and adaptive mechanical behavior. For instance, the polymerization of small globular protein domains has been shown to generate emergent mechanical properties absent in their native monomeric forms.[8, 41, 43-45, 53, 54]. Furthermore, incorporating mechanically robust domains from fibrous proteins—such as spider silk, silkworm fibroin, resilin, or squid ring teeth—into globular protein scaffolds may yield hybrid architectures with enhanced mechanical integrity and functional versatility.[1, 217-219] Importantly, unifying mechanical adaptability with biochemical activity opens new directions for creating intelligent, bioresponsive protein-based materials.

Beyond sequence- and domain-level engineering, recent supramolecular strategies show that globular proteins can assemble into highly ordered superlattices (protein nanocrystals, mesocrystals) with exceptional packing densities and symmetry-dependent functionalities, enabling applications in photonics, catalysis, and molecular sensing.[220, 221] Although such systems are predominantly explored for non-mechanical purposes, their dense packing and long-range order suggest mechanics-oriented opportunities. Two routes appear particularly promising: **(i)** post-assembly locking—via covalent/coordination locking, densification, or controlled dehydration—to convert fragile lattices into stiffer, more stable solids; and **(ii)** superlattice-based composites, wherein protein superlattices act as reinforcing phases or templates that translate nanoscale order into macroscale strength and toughness. We therefore refer readers to specialized reviews on protein superlattice engineering for fundamental assembly mechanisms, while this article maintains its focus on established mechanical design paradigms for globular-protein materials.[220-224]

At the processing level, building standardized and modular platforms for protein extraction, modification, and fabrication is essential for reproducibility and scalability. Emerging techniques like 3D printing, cryo-forming, and microfluidic assembly can support the development of reproducible, customizable, and multifunctional protein-based materials. Crucially, process development must increasingly prioritize scalability, energy efficiency, and cost-effectiveness from the outset to bridge the gap between laboratory innovation and industrial production.

In application domains, attention should also be given to the valorization of by-product proteins. For example, during the purification of serum albumin for high-end biomedical use, a substantial fraction of structurally intact yet pharmacologically non-compliant proteins is produced as a by-product. Although unsuitable for pharmaceutical-grade applications, these proteins often retain favorable mechanical processability and biocompatibility. They can thus be repurposed for low- to medium-risk biomedical uses, such as fiber suture, hemostatic sponges, and hydrogel-based wound dressings—offering both resource efficiency and a potential pathway to lower-cost products that address specific market needs. In parallel, non-medical applications—in food packaging, green composites, and environmental

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remediation—can leverage the low-cost, renewable nature of plant-based globular proteins to create biodegradable and carbon-conscious alternatives to synthetic materials. Ultimately, the successful translation of globular protein materials hinges on a holistic approach that concurrently advances fundamental understanding, material performance, scalable manufacturing processes, and clear market alignment. Addressing the cost, scalability, and stability hurdles identified in **Section 6.1** is as critical as achieving functional and mechanical excellence.

In summary, globular protein-based materials are undergoing a paradigm shift—from passive biochemical components to actively engineered platforms capable of mechanical function, biological responsiveness, and environmental sustainability. This transition marks a critical step in expanding the material utility of proteins beyond traditional boundaries. As interdisciplinary tools and technologies continue to evolve, globular proteins are poised to play a central role in the next generation of biomimetic, intelligent, and sustainable material systems—impacting fields from advanced healthcare to green manufacturing and beyond.

Author contributions

Conceptualization, Haonan He, Peng Zhang, and Jian Ji; Review methodology (scope definition, search strategy, inclusion/exclusion criteria), Haonan He and Peng Zhang; Literature curation and analysis, Haonan He and Peng Zhang; Visualization (figures/schemes/TOC graphic), Haonan He; Writing – original draft, Haonan He; Writing – review & editing, Haonan He, Peng Zhang, and Jian Ji; Supervision & project administration, Peng Zhang and Jian Ji; Funding acquisition, Peng Zhang, Haonan He, and Jian Ji.

Conflicts of interest

There are no conflicts to declare.

Data availability

This review did not generate any new experimental data. All data supporting the analysis and conclusions are derived from published literature, which is comprehensively cited in the manuscript.

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