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Protein-based bioactive coatings: from nanoarchitectonics to applications

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Protein-based bioactive coatings have emerged as a versatile and promising strategy for enhancing the performance and biocompatibility of diverse biomedical materials and devices. Through surface modification, these coatings confer novel biofunctional attributes, rendering the material highly bioactive. Their widespread adoption across various domains in recent years underscores their importance. This review systematically elucidates the behavior of protein-based bioactive coatings in organisms and expounds on their underlying mechanisms. Furthermore, it highlights notable advancements in artificial synthesis methodologies and their functional applications *in vitro*. A focal point is the delineation of assembly strategies employed in crafting protein-based bioactive coatings, which provides a guide for their expansion and sustained implementation. Finally, the current trends, challenges, and future directions of protein-based bioactive coatings are discussed.

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1. Introduction

Surface modification provides an effective means to confer new functionalities upon a bulk material without altering its

intrinsic properties.¹ To fulfill the demands in high-tech fields, such as electronics, biotechnology, and environmental science, a series of physical and chemical methods has been developed, ranging from physical adsorption and plasma treatment² to chemical vapor deposition³ and self-assembled monolayers.⁴ The creation of bioactive coatings is of paramount importance for biomedical materials, particularly because these coatings make direct contact with the organs of the body. These bioactive coatings are designed to promote specific biological responses, such as enhancing cell adhesion, reducing bacterial adhesion, and modulating organ behavior. Given these requirements, biomacromolecules, including proteins,⁵ peptides,⁶ and polysaccharides,⁷ have emerged as ideal building blocks for bioactive coatings. Their excellent biocompatibility and unique

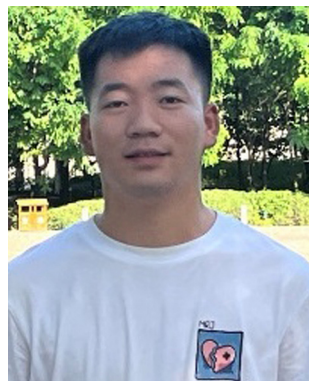
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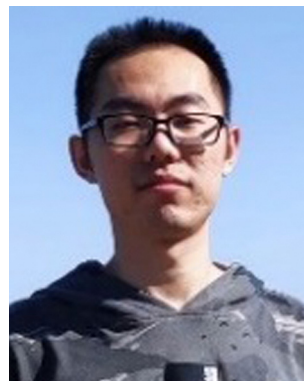
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biological functionalities make them particularly suited to this role.

The intricate interplay between proteins and their environment is fundamental to the functionality and survival of living organisms. Proteins, particularly those that interact at interfaces, are central to myriad biological processes and have a remarkable ability to regulate bodily functions, often even at extremely low concentrations. Despite comprising a small fraction of the total biomolecular constituents in organisms, interfacial proteins exert influence far beyond their relative abundance. For example, signaling proteins, such as hormones, can bind to specific receptors on the surface of a cell, triggering a cascade of events within the cell that can influence growth, metabolism, and other critical functions.⁸ Biofilm formation is a process in which bacteria adhere to surfaces in an aqueous environment and to each other to form a polymeric matrix. Even though the initial attachment is facilitated by proteins present at low concentrations, it is crucial for the development of biofilms, which have major implications in medical and industrial contexts.⁹ Moreover, in human joints, the glycoprotein lubricin present in the synovial fluid can be adsorbed on the cartilage surface and acts as an efficient lubricant to reduce the wear and tear of moving

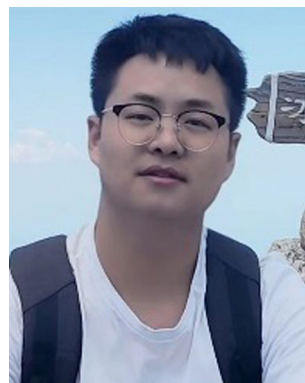
biological components.¹⁰ Hence, interfacial proteins, though often present in minute quantities, have significant roles in various biological systems, influencing vital life behaviors and functioning as key players in biological regulation. Apart from their multifarious functions, proteins exhibit high biocompatibility and elicit a minimal immune response. This is especially important in applications such as medical implants, where the coating is in direct contact with body tissues. In addition, proteins can be molecularly engineered to fine tune their properties and can self-assemble into well-ordered structures.¹¹ The latter is beneficial for creating coatings with precise nano- or micro-scale architectures. Recent advances in bioengineering and material science have sparked increased interest in understanding these interactions and harnessing them for practical applications, particularly in the realm of surface modifications for biomedical devices, environmental sensors, and many other technologies.

This review provides a comprehensive understanding of protein-based bioactive coatings, highlighting their preparation methods, interaction with surfaces, and potential applications. In Section 2, we delve into the fascinating realm of protein-based bioactive coatings, elucidating the mechanisms behind the interfacial behaviors of functional proteins in



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organisms. We explore various aspects of interfacial proteins, including their roles in lubrication, adhesion, and the regulation of biomineralization, which underscore their potential for bioactive-coating applications. Understanding the interactions between proteins and surfaces is crucial for the effective design of coatings. Various methods for the preparation of protein-based bioactive coatings are introduced in Section 3. We provide an overview of these methods and explore their potential and limitations in creating effective protein-based bioactive coatings. In Section 4, we show that the manipulation of surface properties through protein-based bioactive coatings opens a plethora of possibilities in various applications, including biomedicine and environmental monitoring. By harnessing the natural capabilities of proteins, scientists design surfaces with “tailored” properties to meet specific requirements, from antibacterial surfaces for medical implants to bioactive coatings that promote cell growth for tissue engineering. Finally, we conclude by highlighting the challenges and future perspectives of protein-based bioactive coatings and discuss the

emerging fields in which protein-based bioactive coatings are ideal candidates for contributing to their development. It is expected that such examples could inspire and forge new directions for the design, synthesis, and wider applications of materials for protein-based coatings (Fig. 1).

2. Interfacial behaviors of functional proteins in organisms

Proteins are vital fundamental building blocks within organisms. The mechanisms through which myriad functional proteins operate require exploration to demystify life processes. Intriguingly, even at extremely low concentrations, proteins can assemble at interfaces/surfaces and manipulate biological activities, exhibiting distinctive behaviors. The formation of a thin protein coating can enable cells or organs to undertake various physiological roles, ranging from acting as a lubricating layer in human joints and teeth,¹² to serving as adhesive proteins for cell attachment,¹³ or as

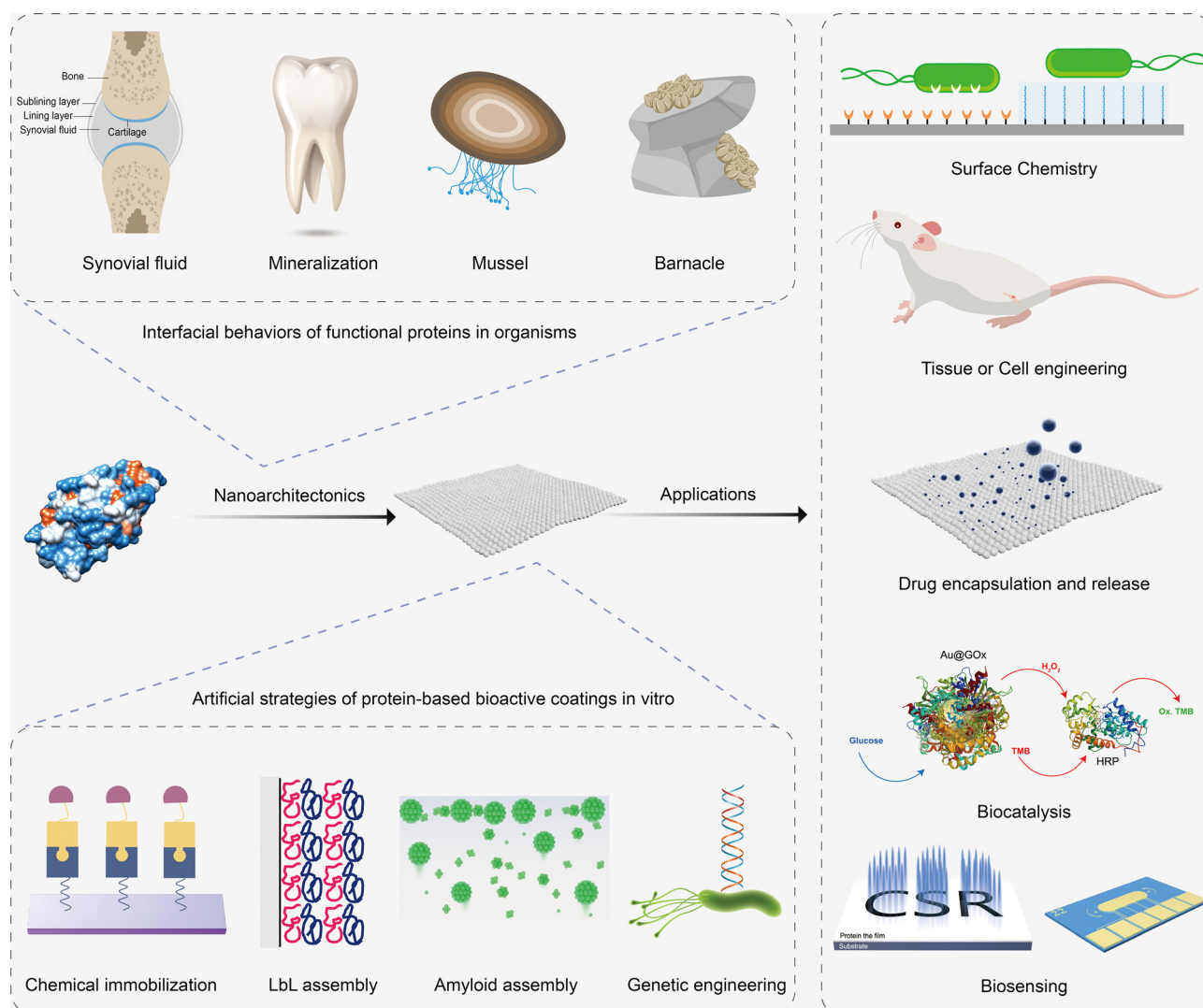


Fig. 1 A schematic representation of nanoarchitectonics and applications of protein-based bioactive coatings in organisms and *in vitro*.

chorion proteins shielding eggs from pathogens.¹⁴ On the other hand, protein adsorption in the bloodstream is a key factor for thrombosis.¹⁵ It can also lead to the adhesion of bacteria and further biofilm formation, which can promote inflammation cascades.¹⁶ Consequently, research on antifouling properties against protein adsorption continues to be at the forefront of surface functionalization. Hence, understanding the mechanisms and functions of interfacial proteins can shed light on biological behaviors but it can also inspire scientists to develop functionally enhanced surface materials. In this section, we discuss typical functional proteins that have pivotal roles at interfaces in biological systems, including boundary lubrication, surface adhesion, biomineralization regulation and antifouling properties. We believe that these examples among countless interfacial proteins underscore the importance of protein-based coatings and will further stimulate the development of coating materials.

2.1 Interfacial proteins for lubrication

The effectiveness of lubrication is paramount in safeguarding many organ surfaces, such as joints between hard tissues, eyelids, and the gastrointestinal tract.¹⁷ Moreover, the slipperiness of fish skin makes them difficult to catch by hand and reduces swimming drag.¹⁸ Lubrication layers in biological systems remain the most efficient when compared with artificial materials. These systems exhibit an extremely low coefficient of friction, reaching between 0.0005 and 0.04,¹⁹ but also endure millions of loading cycles throughout their lifespan. Therefore, extensive research efforts have been devoted to understanding the mechanisms behind natural lubrication.

2.1.1 Lubricin for boundary lubrication. Lubrication behaviors in synovial joints, such as the knee and shoulder, have been studied widely due to the high incidence of osteoarthritis in older and obese populations. Since the inception of hydrodynamic theory in the 1930s, several mechanisms have been proposed.²⁰ This theory suggests that as joint surfaces move toward each other, the pressure within the synovial fluid increases, compelling it to form a thin, pressurized layer between articulating surfaces.²¹ The efficiency of hydrodynamic lubrication is dependent upon the velocity of joint movement, viscosity of synovial fluid (dependent largely on the hyaluronic acid (HA) concentration),²² and the shape and relative roughness of articulating surfaces. If these conditions are optimal, hydrodynamic lubrication can be very effective in reducing wear and friction, thereby helping to protect joint surfaces and promote ease of movement. The hydrodynamic theory has led to the use of HA injections (also known as “viscosupplementation”) as a treatment for osteoarthritis, particularly for the knee.^{23,24} Nevertheless, experimental results revealed minimal changes in lubricating ability after HA removal, thereby refuting the high viscosity of synovial fluid *via* the application of testicular hyaluronidase. In contrast, lubricant action completely vanished upon treatment with trypsin and chymotrypsin, which removed 65% of proteins from the hyaluronate–protein complex in synovial fluid.²⁵ Similarly, Wilkins and colleagues observed the effects of enzymatic digestion on the ability of synovial lubrication.²⁶ Those findings highlight the crucial role proteins have in lubrication and have spurred the development of the

“boundary lubrication” mechanism of synovial joints. Boundary lubrication (also termed “boundary film lubrication”) is a leading theory formulated to elucidate joint lubrication. According to this theory, lubricating molecules are adsorbed onto the cartilage surfaces of articulating joints, creating a thin protective layer that prevents direct contact between opposing cartilage surfaces, thereby reducing friction and wear (Fig. 2a).²⁷ In the context of synovial joints, key boundary-lubrication molecules include lubricin (also known as “proteoglycan 4” or “PRG4” because it is encoded by the PRG4 gene in humans) and HA.²⁸ These molecules can adhere to cartilage surfaces and provide a slippery interface. Lubricin, in particular, plays a crucial part in boundary lubrication within a joint, which was first proposed in 1970.²⁹ It is a mucinous glycoprotein secreted by synovial fibroblasts and chondrocytes in the superficial zone of synovial joints. Lubricin can bind to the surface of articular cartilage to create a lubricating boundary layer.³⁰ Although the concentration of lubricin in synovial fluid (approximately 52–350 $\mu\text{g mL}^{-1}$) is lower than other components such as albumin (around 4–10 mg mL^{-1}), HA (approximately 1–4 mg mL^{-1}), and surface-active phospholipids (about 0.1–0.2 mg mL^{-1}), the unique structure of lubricin plays a critical part in boundary lubrication.³² Lubricin appears as an elongated glycoprotein with a length of 200 ± 50 nm and a diameter of a few nanometres (Fig. 2b).³³ The structure of lubricin is characterized by a central mucin-like domain flanked by two globular domains. The central mucin-like domain is rich in threonine and proline residues and contains many O-linked $\beta(1-3)\text{Gal-GalNAc}$ oligosaccharides (O-linked glycosylation), giving lubricin its highly hydrated, lubricating properties.³⁴ This domain is also characterized by several tandem repeats, which contribute to its viscoelastic properties.³⁵ The N-terminal is linked to somatomedin B-like domains, which are paralogs of vitronectin.³⁶ Therefore, the N-terminal can interact with the cluster of differentiation (CD)44 receptors on the surface of chondrocytes. CD44 is a cell-surface glycoprotein responsible for cell–cell interactions and cell adhesion, and is most notably involved in HA binding.³⁷ Conversely, the C-terminal end is connected to hemopexin-like (PEX) domains, which can mediate attachment to extracellular matrix (ECM) proteins such as collagen and fibronectin, given that PEX is part of the matrix metalloproteinase family.³⁸ Consequently, the globular domains at the N-terminal and C-terminal ends are instrumental in binding to various surfaces, including cartilage and other proteins.

The principal structure of lubricin is distinguished by a sizable central mucin-like domain, which is highly glycosylated, containing an array of carbohydrate chains affixed to the protein backbone.³⁹ More than two-thirds of the sugar groups are topped with a charged sialic acid, so lubricin exhibits a negative surface charge.⁴⁰ Consequently, lubricin displays a distinctive “bottle brush” structure, which contributes significantly to its lubricating properties. Primarily, this bottle-brush structure imbues lubricin with robust hydration properties because the glycosylation sites bind water molecules effectively, fostering a hydrophilic environment around the lubricin molecule.⁴¹ In the context of synovial fluid, this hydration layer enveloping the lubricin molecule enlarges the molecule efficiently by increasing

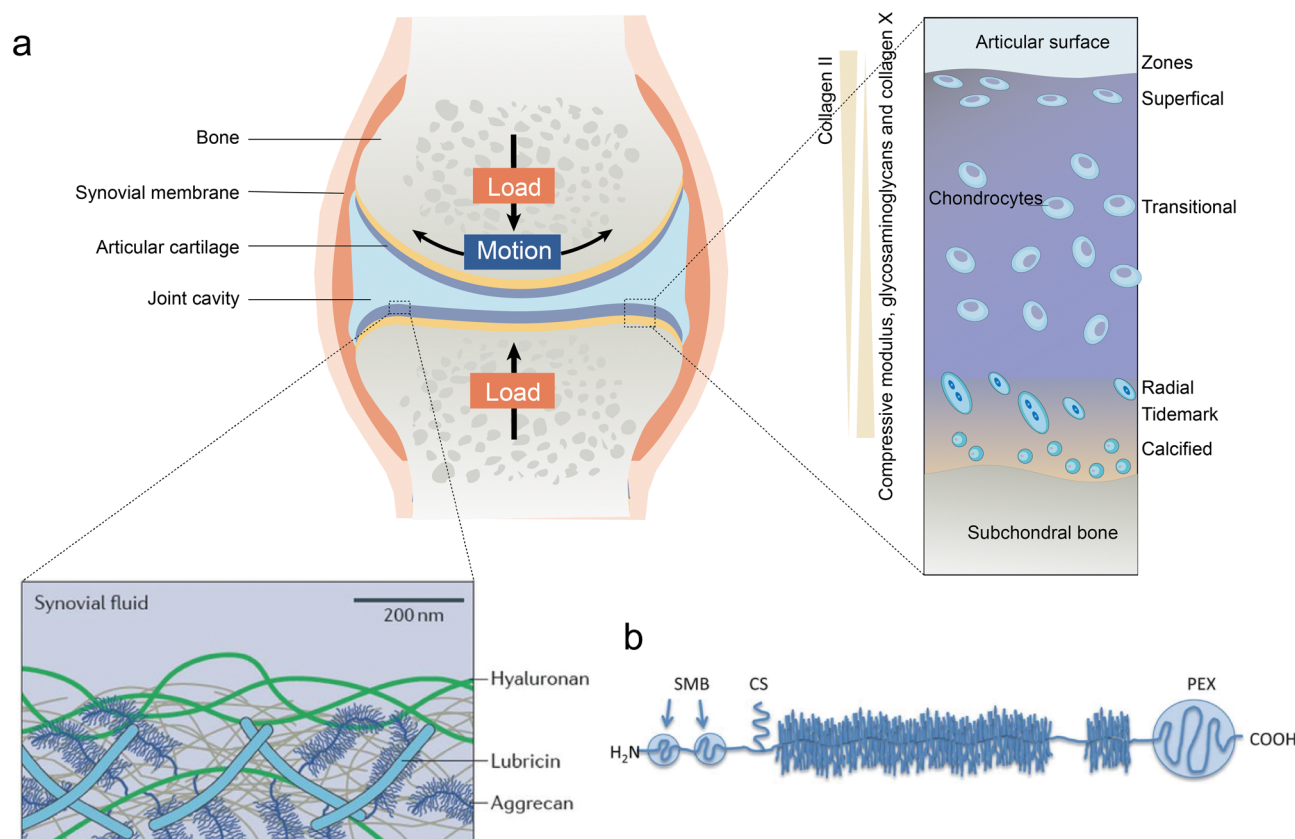


Fig. 2 (a) A schematic representation of the structure and constituents of articular cartilage. Reprinted with permission from ref. 31 Copyright 2021, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (b) A schematic representation of the brush-like structure of lubricin. Reprinted with permission from ref. 28 Copyright 2011, Royal Society of Chemistry.

its volume and size. This enlargement maintains the separation between opposing cartilage surfaces in the joint, averting direct contact and consequently reducing friction and wear. Furthermore, the hydration layer surrounding lubricin traps and immobilizes water molecules, thus creating a low-friction, slippery interface that further enables joint movement. This represents a form of boundary lubrication, whereby a molecular layer physically segregates two opposing surfaces, thereby significantly mitigating friction and wear.⁴² Apart from its role in boundary lubrication, the lubricin-associated hydration layer also functions as an antifouling coating to prevent unwanted protein deposition. This barrier precludes the deposition of other proteins, including those implicated in inflammation or calcification, thereby preserving joint health.^{43,44} Experimental evidence from lubricin-null mice (PRG4^{-/-} mice) revealed that the absence of lubricin led to protein deposition and cell overgrowth on cartilage surfaces, thus underscoring its critical role in preventing unnecessary protein and cell adhesion to the cartilage surface.⁴⁵

On the one hand, due to the densely packed glycosaminoglycan chains extending from the core protein, which carry negative charges, an electrostatic repulsion ensues if lubricin molecules are in proximity on the articular surface. This repulsion prevents surfaces from making direct contact, thereby minimizing friction and wear.⁴⁶ Furthermore, during separation, adhesion transpires between the lubricin-coated surfaces, likely a

result of chain disentanglement and molecular bridging.⁴⁷ These behaviors could be attributed to physical entanglement in elongated chains or covalent linkage *via* disulfide bonds between cysteine residues.⁴⁸ The bottle-brush structure of lubricin also enhances surface coverage, allowing it to effectively adhere to and coat cartilage surfaces within a joint.⁴⁹ In conclusion, the structural basis of lubricin endows it with superior properties for boundary lubrication, including end-group adhesion, hydration, enhanced surface coverage, surface repulsion, and protection from protein deposition. Lubricin has critical roles in boundary lubrication, but the synergistic interactions between lubricin and other components in synovial fluid are responsible for the low friction coefficient of articular cartilage under harsh conditions.³¹ For instance, the N-terminal domain of lubricin can interact with the CD44 receptor to bind HA and form a meshwork or gel-like structure, further enhancing its lubricating and load-bearing properties.^{32,50}

Lipids are vital components of synovial fluid, contributing to joint lubrication through their unique properties and interactions with other lubricating molecules. Phosphatidylcholine can assemble into micelles or bilayers in synovial fluid, effectively reducing friction between cartilage surfaces in the joint.^{51,52} The interaction between lipids and HA enables lipid multilayers to accumulate on the cartilage surface, ensuring an adequate supply of these bio-lubricants at sliding interfaces.^{53,54} Given our understanding of

boundary lubrication on the cartilage surface, a series of bio-mimetic polymers have been developed for the treatment of osteoarthritis. These include polymer brushes,^{55,56} zwitterionic ABA bottle-brush polymer,^{57,58} and hyaluronan backbones grafted with lubricin-like sulfonate-rich polymers.⁵⁹ Due to the synergistic effects of various lubricating agents in cartilage lubrication for high-efficiency boundary lubrication, further research is necessary to fully understand their contribution to the complex process of joint lubrication.

2.1.2 Mucin-related lubrication. Apart from joints, boundary lubrication is also important for soft tissues. Mucins are a family of high-molecular-weight, heavily glycosylated proteins (glycoproteins) produced by epithelial tissues in most animals.⁶⁰ They are a major component of the mucus that coats the surfaces of cells in the body, such as those in the ocular, digestive, and oral systems.⁶¹ The molecular structure of mucins also exhibits a bottle-brush structure, which is cell membrane-tethered and assembles to form mucus gels on epithelial surfaces. Such a gel layer acts as a size-exclusion barrier to molecules, particles, and pathogens, but it also acts as a lubricating agent to protect organs.⁶²

In the ocular system, mucins are secreted by the conjunctiva and are a vital part of the tear film that covers the cornea.⁶³ The primary mucin in the ocular system is known as mucin (MUC)5AC, a gel-forming mucin secreted by conjunctival goblet cells. Other mucins, such as MUC1, MUC4, and MUC16, are also found on the ocular surface. These are membrane-associated mucins produced by epithelial cells of the cornea and conjunctiva.⁶⁴ They help spread the watery layer across the surface of the eye, maintaining hydration and ensuring smooth and comfortable eye movements. This phenomenon also helps clear the surface of the eye of small foreign objects or particles.

Two primary types of mucins are found in saliva: MUC5B and MUC7.⁶⁵ Mucins and other saliva proteins are adsorbed on teeth. Then, the thin, proteinaceous layer that forms on the tooth surface is termed an “acquired salivary pellicle.”¹² The pellicle layer diminishes friction among opposing teeth as well as between teeth and mucosal surfaces. Remarkably, the introduction of salivary pellicles between solid surfaces leads to a reduction in the friction coefficient of up to 20. This marked friction reduction aligns with the inherent long-range purely repulsive characteristics of the normal forces at play between salivary films. Consequently, the lubrication mechanism can be attributed to the complete separation of sliding surfaces due to the presence of salivary films.⁶⁶ By lubricating the oral cavity, mucins help to facilitate the movement of food during chewing and swallowing. This lubrication also makes it easier to speak. When mucins are secreted on fish skin, they also contribute to the formation of the slippery, gel-like substance. The latter helps to provide a smooth, low-friction surface on the skin of fish, which reduces drag when they are swimming, making their movements more efficient. It can also help them escape from predators by making them harder to grasp.⁶⁷

Apart from the examples stated above, mucin-related lubrication is involved in reproductive,⁶⁸ digestive,⁶⁹ and respiratory systems,⁷⁰ which is crucial for the maintenance of life behaviors.

Lubricin exhibits a structural and lubricating mechanism similar to that of mucins, but the unique gel-forming property of mucins remains fundamental to their lubrication function.⁷¹ The gel formed by mucins can absorb and retain water, with water accounting for up to 95% of the total mass in the mucus gel.⁷² This property is attributable to mucin-associated glycans, which contribute up to 80% of the molecular weight of mucins and contain highly hydrated hydroxyl groups.⁷³ Comparative analyses between native mucins and deglycosylated mucins underscore the importance of these glycans. The removal of glycans from mucin results in a 3.5-fold decrease in hydration and causes an increase in friction by two orders of magnitude.⁷⁴ Thus, glycans significantly enhance the hydration and viscosity of mucus to create a smooth, slippery surface conducive to lubrication. In addition, the gel-forming ability of mucins facilitates their retention on tissue surfaces for extended periods. This prolonged presence establishes a continuous protective and lubricating layer, which effectively prevents dryness and irritation.

2.2 Adhesive proteins

Proteins readily adhere to surfaces through non-specific adsorption, a prevalent event implicated in numerous biological processes, such as the adsorption of fibrinogen that may contribute to thrombosis.⁷⁵ This spontaneous protein adsorption can form a layer, thereby altering surface behaviors substantially. However, in the realm of surface science and materials engineering, terms such as “coating” or “film” typically suggest a well-controlled, continuous layer designed to provide certain properties or withstand certain conditions. The non-specific adsorption of proteins may result in patchy, discontinuous coverage, particularly if the surface properties are heterogeneous. This contrasts with a controlled protein coating that aims for continuous, homogenous coverage. In addition, non-specific adsorption does not allow for the control of coating protocols, such as thickness, speed, and composition. Therefore, adhesive proteins could be used as coating materials based on several properties, including strong adhesion to various surfaces, stability under harsh conditions, and processability for different coating methods. Building on these principles, in this section we will discuss adhesive proteins found in organisms that hold potential for utilization in bioactive coatings.

2.2.1 Mussel-foot proteins (Mfps). Mfps are a family of proteins produced by marine mussels that enable them to attach to different surfaces under wet conditions. The remarkable adhesive properties of mussels were noted in the 1960s⁷⁷ but it was not until the late-20th century that Mfps were identified and their unique bioadhesive properties recognized. Since then, they have been the focus of numerous studies aimed at understanding their adhesive mechanism and harnessing it for practical applications.⁷⁸ In the biological process of mussel adhesion, proteins are secreted by a gland and polymerize rapidly to form a thread structure. The end of the thread that touches the substrate expands to form a flattened adhesive plaque (Fig. 3a). The thread is composed of multiple proteins. These provide mechanical strength as well as Mfps, which contribute to adhesion and protection.^{79,80} The average force needed to dislodge the California mussel (*Mytilus californianus*)

is estimated to be 250–300 N per mussel, with an average detachment force of 5–6 N per thread.⁸¹ The primary component responsible for the adhesive properties of Mfps is the 3,4-dihydroxyphenylalanine (DOPA) residue, a modified form of the amino acid tyrosine.⁷⁶ The adhesion mechanism of DOPA residues in Mfps is fascinating due to the balance of covalent and non-covalent interactions.

In the case of DOPA, non-covalent interactions include hydrogen bonding, hydrophobic (water-repelling) interactions, and coordination bonds with metal ions. Hydroxyl groups in DOPA can form hydrogen bonds with other polar molecules on the substrate. Hydrogen bonds are weaker than covalent bonds, but their cumulative effect across multiple DOPA residues can contribute significantly to the overall adhesive strength, which can promote its absorption to mucosal tissues⁸² and hydroxyapatite (HAP) surfaces.⁸³ The aromatic ring of DOPA also plays an important part in adhesion, particularly through interactions that rely upon π - π bonding (Fig. 3b). Aromatic rings contain delocalized π electrons above and below the plane of the molecule. These π electrons can interact with the π electrons from another aromatic ring or polarizable electrons on a surface, creating an attractive force, which improves the cohesion to the surface of aromatic compounds⁸⁴ (e.g., polystyrene) and gold substrates.⁸⁵ The catechol groups in DOPA can form coordination bonds with metal ions, especially those abundant in aquatic environments, such as calcium and iron. This coordination is strong and reversible, which can help crosslink proteins and reinforce the adhesive plaque.^{86,87}

These hydrophobic interactions also involve surface adhesion due to the hydrophobic regions that can interact favorably with hydrophobic surfaces, contributing to the adhesion.⁷⁶ Apart from the non-covalent interactions, covalent interactions are typically facilitated through reactions between DOPA and functional groups on the substrate. For instance, DOPA can

undergo oxidation to form reactive quinone intermediates,⁸⁸ which can then react with nucleophiles on the substrate surface to form covalent bonds (e.g., $-\text{NH}_2$, $-\text{SH}$, imidazole).⁸⁹ This process contributes to the long-term stability of the adhesion. By exploiting the balance of these covalent and non-covalent interactions, DOPA-containing proteins can adhere strongly and durably to a wide variety of surfaces. This dual mechanism also contributes to the versatility of DOPA-based adhesion, allowing mussels to attach to a diverse range of substrates in their aquatic environment.

2.2.2 Cement proteins from barnacles. Barnacles are infamous as marine-fouling organisms due to their exceptional adhesive capabilities that allow them to bind to a wide array of surfaces under challenging environmental conditions, such as in turbulent saline seawater. Their robust adhesion makes them exceedingly difficult to remove once attached to surfaces.⁹⁰ Barnacles and mussels can adhere to diverse materials (indicating that their adhesion mechanisms do not rely on specific interactions with particular substrates) but significant differences exist in the specifics of their adhesive systems and the individual proteins involved. These differences reflect their adaptations to distinct niches within marine environments and their unique biological characteristics. Barnacles and mussels use complex protein mixtures to adhere to surfaces: in mussels, these are known as Mfps, and in barnacles they are referred to as “barnacle cement proteins” (Fig. 4a). The lifecycle of barnacles is in four stages: nauplius, cyprid, juvenile, and adult. With regard to surface adhesion, the cyprid first produces a reversible adhesive footprint for exploring the substrate to decide whether to colonize on it.⁹¹ After this surface exploration, the cyprid produces cement for the attachment on a suitable substrate.⁹²

During the cyprid stage of barnacle development, temporary and reversible adhesion occurs, enabling these organisms to explore various surfaces. The exact mechanisms behind this

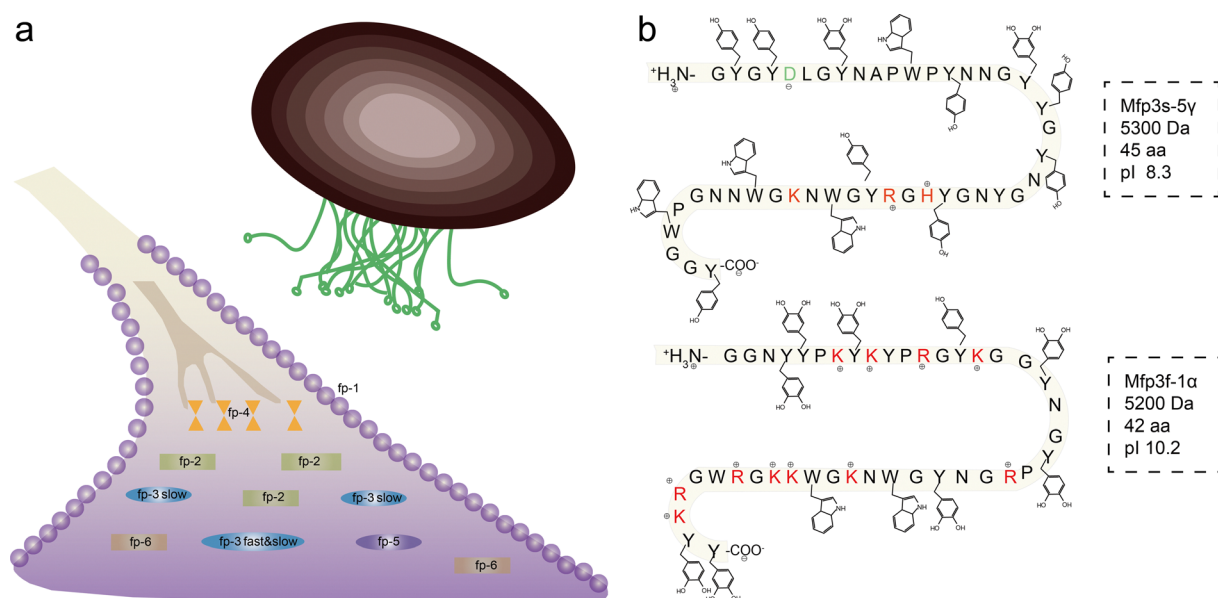


Fig. 3 Byssal plaque proteins of *Mytilus*. Reprinted with permission from ref. 76 Copyright 2012, American Chemical Society.

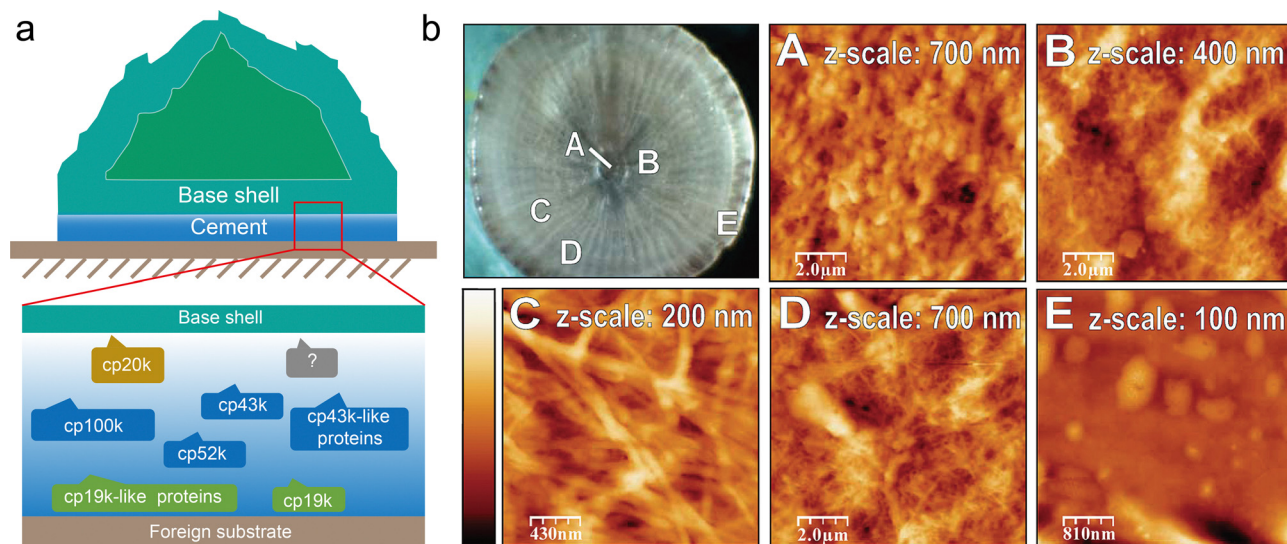


Fig. 4 (a) The protein composition and microscale structure of barnacle cement. Reprinted with permission from ref. 110 Copyright 2022, Frontiers in Bioengineering and Biotechnology. (b) The optical micrograph of a barnacle base plate; the upper left image shows the labelled area from where the AFM images was obtained. Reprinted with permission from ref. 104 Copyright 2010, American Chemical Society.

temporary adhesion involve complex synergistic effects ranging from biochemical to physical and behavioral factors, and they are the subject of ongoing research.⁹³ Upon settling on a suitable substrate, the cyprid matures into an adult and secretes cement proteins for permanent attachment. Initially, barnacle cement is a fluid secreted from specialized glands. However, it solidifies rapidly, anchoring the barnacle securely to the surface. The analysis of cured cement has revealed a series of proteins, including cp100k, cp52k, cp68k, cp19, and cp20k (where “cp” represents cement protein and “100k” is the molecular weight as estimated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis).⁹⁴ These diverse cement proteins seem to have distinct roles in surface adhesion. For instance, cp20k coordinates with calcium ions (Ca^{2+}), which suggests selective attachment to calcite. This behavior can be attributed to the sequences EED, EEDDGD, and DHHDDD in cp20k, which cluster and facilitate coordination between cp20k and metal ions *via* side-chain carboxyl groups.⁹⁵

Apart from the specific adherence of cp20k to calcite, the adhesion of cp19k and cp68k is dependent mainly on physical interactions, which enables non-specific adhesion to various substrates. Electrostatic interactions, hydrogen bonding, hydrophobic interactions, and van der Waals forces each have a role in the surface binding due to the amino-acid composition of cp19k.⁹⁶ Notably, six amino acids (Ser, Thr, Gly, Ala, Val, Lys) account for ~70% of the total residues in cp19k.⁹⁷ The arrangement of these amino acids resembles that of block copolymers: one part is rich in hydrophobic and charged amino acids (*e.g.*, Val, Lys), whereas the other contains a high abundance of Ser, Thr, Gly, and Ala.⁹⁸ This structure further enhances the surface adhesion of cp19k. Given that cp68k shares similar amino-acid compositions and sequence properties to cp19k, it has been hypothesized that cp68k has a similar role to cp19k in barnacle adhesion.

The permanent adhesion of adult barnacles relies heavily on a crucial step known as the “curing process,” which is as

integral as the presence of adhesive cement proteins. During this transition from a liquid state to a solid state, cement proteins enhance their mechanical strength significantly. This action allows the barnacle to remain adhered even under harsh environmental conditions, such as potent wave action, currents, or predatory assaults.⁹⁹ Furthermore, the cured adhesive demonstrates exceptional chemical resistance, thereby aiding the barnacle in enduring a broad spectrum of water chemistries, which includes changes in salinity, pH shifts, and potential pollutants.¹⁰⁰ The curing process is dependent primarily on the internal cohesion of bulk cement proteins. The formation of amyloids, crosslinking of disulfide bonds, and non-covalent interactions among cement proteins have pivotal roles in the curing process. Amyloids are proteins that are typically associated with neurodegenerative diseases in humans, such as Alzheimer’s disease and Parkinson’s disease. They are characterized by a specific form of protein folding into beta sheets, which aggregate into insoluble fibrils.¹⁰¹ The correlation between barnacle cement and amyloid fibrils was identified initially by Kamino and colleagues due to their similar insolubility and high β -sheet content.¹⁰² Subsequently, it was shown that cement proteins, including cp52k, cp100k, cp68k, and cp19k, could aggregate into insoluble amyloid fibrils under certain conditions (Fig. 4b).^{103,104} The amyloid state imparts cement proteins with insolubility, strength, elasticity, and resistance to extreme pH or ion strength,¹⁰⁵ which enables the adhesive to be robust and durable in the harsh marine environment. The high-aspect-ratio structure of amyloid fibrils further promotes the non-covalent interactions of cement proteins,¹⁰⁶ reinforcing the cohesion of barnacle adhesive and its adhesion to surfaces. Besides non-covalent interactions, covalent crosslinking is dependent primarily on disulfide bonds. According to analyses of the primary structure, all cement proteins contain cysteine, and a high concentration of reductant is expended in the dissolution of the cured cement.¹⁰⁷ Therefore, intermolecular disulfide bonds contribute to the

polymerization of barnacle cement. Raman spectroscopy has revealed intermolecular disulfide bonds to be absent in *Balanus crenatus* cement,¹⁰⁸ further affirming the role of disulfide bonds in the internal cohesion of bulk cement proteins. In this section, we have only briefly summarized the understanding of barnacle adhesion. The comprehensive theory of barnacle adhesion lacks experimental evidence from biological or chemical perspectives. Nonetheless, it continues to inspire scientists to develop protein-based coating materials or underwater adhesives, such as amyloid adhesive.¹⁰⁹ Moreover, comprehending the adhesion mechanisms of barnacles paves the way for the development of novel antifouling technologies.

2.2.3 Proteins for biofilm adhesion. Biofilm formation is a complex, multi-step process that allows microbial communities to adhere to surfaces and each other, providing numerous advantages like enhanced resistance to antibiotics and protection against environmental challenges. Bacterial adhesion can be categorized into two phases: initial attachment and irreversible adhesion.¹¹¹ In the initial phase, planktonic microbial cells are regarded as colloidal microparticles, with their attachment to surfaces influenced by factors such as chemotaxis, fluid currents, and gravity.¹¹² This behavior results from transient, reversible interactions with the surfaces. Once attached, microbial cells begin forming stronger, more specific adhesive interactions with the surface. This transition is often driven by changes in the expression of complex extracellular polymeric substance (EPS) matrix. In both phases, proteins play crucial roles in the bacterial adhesion.

Adhesins are surface-exposed molecules or molecular complexes on bacteria that mediate attachment to both biotic and abiotic surfaces.¹¹³ They can be proteins or glycoproteins and are commonly situated on the bacterial filamentous cell appendages such as pili (fimbriae), flagella, or nanofibres.¹¹⁴ The structure and functionality of adhesins can vary depending on the bacterial species and the target surface. In the case of abiotic surfaces, the long, slender, and hair-like structure of pili provides an increased surface area for interactions and maintains contact with surfaces even under dynamic conditions, like in the presence of fluid flow. The adhesins on the pili can mediate weak, non-specific interactions with the surface such as the van der Waals, acid-base interactions, electrostatic and hydrophobic forces.¹¹⁵ Adhesins can also recognize and bind to specific receptors on the target surface. This is particularly true for biotic surfaces where adhesins might recognize specific carbohydrate, protein, or lipid structures on host tissues. The interaction can be likened to a “lock and key” mechanism, where the adhesin is the “key” and the receptor on the target surface is the “lock.” For example, FimH is a well-known protein that serves as an adhesin. It's located at the tip of type 1 pili (or fimbriae) on certain strains of *Escherichia coli* (*E. coli*) and some other bacteria. The N-terminal domain of FimH (also known as the lectin domain) is responsible for binding to receptors on the host cell, while the C-terminal domain anchors FimH to the tip of the type 1 pilus. Due to the lectin domain, FimH can recognize and bind to terminal mannose residues on glycoproteins and glycolipids on the surfaces of host cells. FimH mediates the attachment of bacteria to host

tissues, particularly in the urinary tract, which can lead to urinary tract infections (UTIs).

Biofilm formation is central to bacterial persistence and virulence. Beyond adhesins, amyloids significantly influence bacterial adhesion. Notably, a range of enteric commensals, pathogens like *E. coli* and *Salmonella* spp., and various environmental Gram-negative bacteria express adhesive amyloids.¹¹⁶ Curli amyloids, consisting predominantly of CsgA and CsgB subunits at approximate ratios of 20:1 *in vivo*, exemplify this role. The innate amyloid core grants curli its distinctive fibrillar structure. This elongated, hair-like configuration presents an expansive interactive surface, enabling the fibrils to intertwine and adhere effectively to surfaces, augmenting non-specific bacterial-surface interactions. Furthermore, curli fibrils can specifically recognize and bind to host tissue components, such as the extracellular matrix proteins fibronectin, laminin, and plasminogen. This specificity is paramount for pathogenic bacteria, aiding their colonization and persistence in hosts. After the initial adhesion, curli fibrils bolster biofilm structural integrity and advocate bacterial aggregation, a pivotal phase in biofilm genesis. Hence, elucidating biofilm adhesion mechanisms is twofold: it paves the way for designing antifouling coatings and inspires the crafting of novel underwater adhesives. For instance, the universal adhesive properties of CsgA amyloid proteins present promising prospects for next-generation coating materials.¹¹⁷

2.3 Regulation of biomineralization

Biomineralization is a sophisticated biological process in which organisms synthesize minerals to harden or reinforce existing tissues. Interfacial proteins contribute significantly to this process, particularly during the early stages of mineral formation. The proteins involved in biomineralization are varied and govern the nucleation, growth, and assembly of minerals within biological systems. They accomplish this through specific interactions with the mineral phase to steer the mineralization process. These proteins commonly possess unique characteristics, such as high concentrations of acidic amino acids, repeated sequences, and the capacity to bind organic and inorganic substances. Notably, fewer than 5% of organic components inserted into aragonite platelets can direct the nucleation and growth of the inorganic phases of nacre, thereby facilitating the unique organization of the organic-inorganic complex.¹⁰⁹ This hierarchical configuration of organic and inorganic components enhances the fracture resistance of nacre by 2–3 orders of magnitude as compared with that of pure CaCO₃.¹¹⁸ Matrix proteins from *Pinctada* species, such as nacrein, MSI60, N16, and Pif, have a critical role in nacre formation.¹¹⁹ Moreover, type-I collagen in the ECM significantly influences the mineralization of bone, dentin, and cementum. Collagen fibrils provide a scaffold for mineral deposition, facilitating the organization and alignment of HAp crystals (the principal mineral component in these hard tissues).¹²⁰ In addition, phosphorylated proteins adhere to the existing matrix of collagen and mineral components, subsequently influencing the rate, size, shape, and orientation of mineral crystal growth.¹²¹ These examples have been explored extensively in various works,

but our focus herein is on the acquired salivary pellicle and its anti-mineralization properties because it offers a straightforward method for controlling mineral growth.

The acquired salivary pellicle (ASP) or acquired enamel pellicle (AEP) is a thin proteinaceous layer that forms spontaneously on tooth surfaces immediately following cleaning. This layer originates predominantly from salivary proteins but also incorporates proteins from gingival crevicular fluid and oral microbes.¹² The formation of a salivary pellicle is a highly selective process involving specific interactions between tooth HAp and certain salivary proteins. This process, in general, proceeds in two stages: the initial swift protein adsorption (within minutes of saliva exposure) followed by a slower phase of protein accumulation (over several hours) (Fig. 5a–c).¹²² The adhesion of ASP is facilitated primarily by pellicle precursor proteins such as statherin, histatins, and proline-rich proteins (PRPs). For instance, the N-terminus of statherin consists of five consecutive acidic residues: one aspartic acid (D), two phosphoserine (Sp), and two glutamic acid (E) residues. They exhibit specific HAp-binding properties, acting as anchors for surface immobilization (Fig. 5d).¹²³ Moreover, the N-terminus preferentially binds calcium ions over phosphate ions, thereby disrupting the correct stoichiometry needed for CaP nucleation. Full-length statherin further inhibits the spontaneous precipitation of calcium phosphate by keeping saliva supersaturated with calcium phosphate salts. To understand more deeply the mineralization properties of statherin, a peptide

(DR9) composed of nine amino acids (DSpSpEEKFLR) derived from the N-terminus was synthesized. The duplicate construct, DR9–DR9, demonstrated a stronger inhibitory property toward HAp growth as compared with that of native statherin, histatin, or other peptides derived from ASP proteins.¹²⁴ By altering the phosphoserine groups at positions 2 and 3 to serine (DSEEKFLR, DR9/2), the affinity and inhibitory effect toward HAp decreased rapidly, indicating the critical role of phosphorylation of serine residues 2 and 3 in the growth mechanism of HAp crystals.¹²⁵ In terms of PRPs, the N-terminal exhibits an affinity and inhibitory effect similar to that of statherin. The N-terminal 30-residue tryptic peptide can prevent the transformation of dicalcium phosphate dihydrate into basic calcium phosphate salts, thereby inhibiting HAp growth.¹²⁶ Those findings suggest that the inhibitory effect on HAp growth and the affinity for HAp can be attributed to the dense arrangement of negatively charged residues such as phosphate and carboxyl in the amino-acid sequence of pellicle proteins. This sequence first maintains the supersaturation of calcium-phosphate ions in saliva. Upon adsorption of the pellicle proteins on the tooth surface, they bind tightly to calcium ions and repel the phosphate needed for CaP nucleation and subsequent HAp deposition.

In addition to the role pellicle proteins have in inhibiting HAp growth, the ASP functions as a semi-permeable barrier, regulating the diffusion of acids as well as the transport of calcium and phosphate ions to and from the enamel surface. The ASP also crucially mediates the demineralization process of

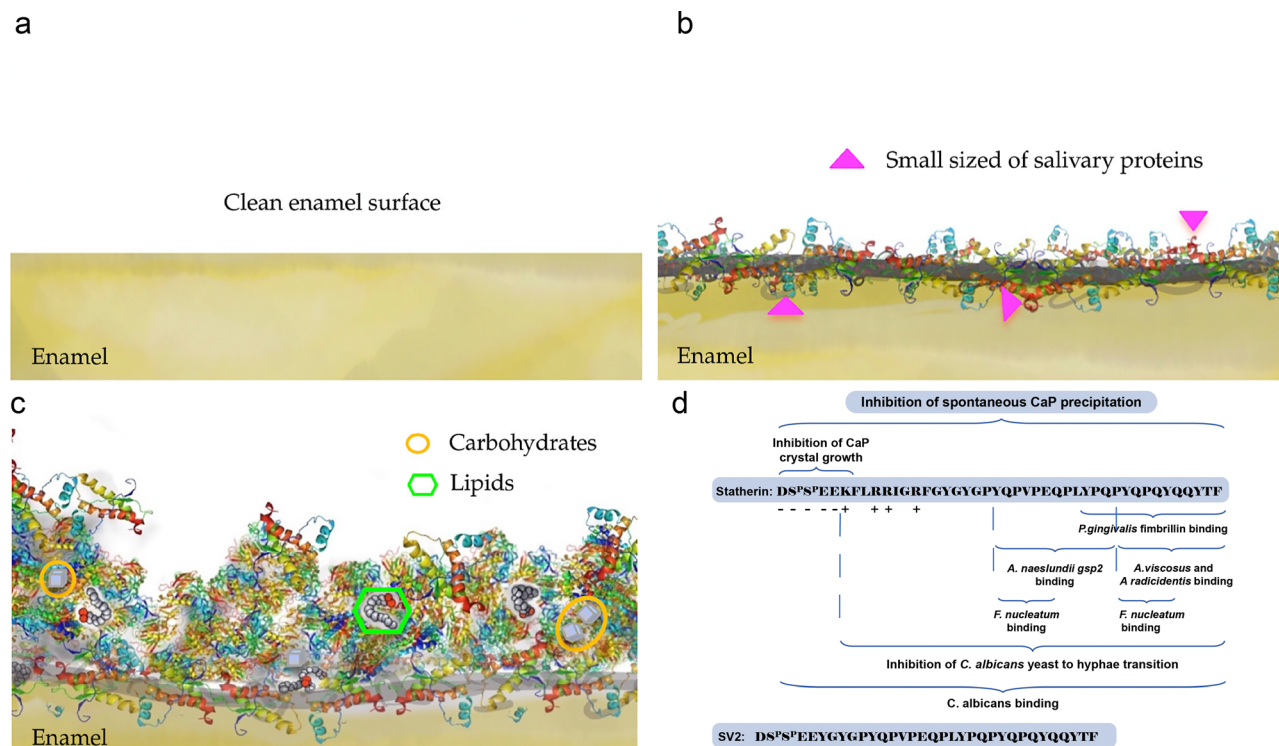


Fig. 5 The formation of the acquired salivary pellicle. (a)–(c) The process of protein adsorption and dissociation on the enamel surface. Reprinted with permission from ref. 122 Copyright 2021, Elsevier. (d) Structural and functional characteristics of statherin. Reprinted with permission from ref. 123 Copyright 2010, American Chemical Society.

enamel.¹²⁷ The inhibitory impact of ASP on HAP growth is a dynamic process influenced by various factors. Thus, the overall effect of ASP on tooth mineralization is complex and can differ under varying conditions. Drawing inspiration from ASP formation on the tooth, the development of protein-based coatings could be an effective strategy for modulating mineralization, which could reduce calcification. Furthermore, when considering restorative materials for tooth repair, ASP formation must be accounted for, given that its inhibitory effect may impact the mineralization of the restorative layer.

2.4 Resisting the fouling agents

To resist undesirable colonization by other organisms or fouling agents, several organisms produce interfacial proteins with antifouling properties. These proteins play critical roles in ensuring that these organisms remain free from unwanted microbial or macroscopic attachments, which could compromise their health or function. For instance, the bottle-brush structure of lubricin not only mediates boundary lubrication but also inhibits undesired protein deposition (discussed in Section 2.1.1). The S-layer (surface layer) is a paracrystalline protein or glycoprotein layer found on the surface of many bacteria and nearly all archaea.¹²⁸ This layer consists of a single protein or glycoprotein species and forms a two-dimensional lattice that completely covers the cell surface. S-layers can serve as a protective shield against environmental threats, such as pH changes, desiccation, osmotic stress, and even predatory bacteria.¹²⁹ From a structural perspective, various protein domains within S-layer proteins contribute to their unique structure and functionality. The S-layer homology (SLH) domain, prevalent in numerous bacterial S-layer proteins, is instrumental in tethering the S-layer protein to the cell exterior, frequently through interactions with secondary cell wall polymers in Gram-positive bacteria. It is reported that the glycosylation of S-layer proteins can affect their surface hydrophilicity and charge. A highly hydrophilic and periodically patterned surface, stemming from glycosylation, could thwart the non-specific adsorption of proteins and other molecules – a foundational step in fouling. Specific sugar residues might also offer steric hindrance or produce repulsive forces against encroaching particles or cells. Furthermore, native S-layer proteins from several *Bacillus* strains contain carboxyl groups, which are counterbalanced by equivalent amino groups, resulting in an outer surface with a neutral charge. Such polyelectrolytic properties imbue S-layer proteins with a tightly adhered water layer, establishing a robust physical and energetic impediment against protein adsorption. In this regard, the S-layer could prevent the nonspecific adsorption of macromolecules and maintain the permeability properties through the S-layer pores.

Elastin-like polypeptides (ELPs) originate from the recurring pentapeptide sequence in human tropoelastin, denoted as Val-Pro-Gly-X-Gly, where 'X' can be any amino acid excluding proline.¹³⁰ These polypeptides can be biosynthetically produced using recombinant DNA techniques, allowing for consistent and scalable production. ELPs can be easily modified to exhibit different properties by changing the sequence of amino acids. By altering the amino acid sequence, the properties of

ELPs can be tailored; for instance, substituting 'X' with serine transforms ELPs into hydrophilic polypeptide brushes.¹³¹ These specially engineered ELPs can establish a highly hydrated surface layer, which, in turn, serves as a physical barrier, providing steric hindrance to the adherence of fouling organisms or proteins.¹³² Given their natural origin, ELPs are often biocompatible, making them suitable for applications where bio-interfacial interactions are crucial, such as medical devices or implants.¹³³ Notably, ELPs demonstrate a reversible phase transition based on temperature, transitioning from water solubility to aggregation at designated temperatures.¹³⁴ We believe this unique behavior presents an opportunity to design surfaces with properties that can shift, offering fouling resistance under predetermined conditions.

3. Artificial strategies of protein-based bioactive coatings *in vitro*

In vitro strategies for the development of protein-based bioactive coatings revolve around the design and synthesis of protein coatings endowed with specific bioactive properties tailored for a range of applications.¹³⁵ Such coatings are intended to refine the interfaces of materials, including implants and medical devices, thereby enhancing their interactions within biological environments. This strategy encompasses various methods: chemical immobilization of proteins on surfaces *via* covalent bonds or ionic linkages; genetic engineering to anchor proteins onto cell surfaces; orchestrating proteins into well-ordered nano- or microstructures through self-assembly.¹³⁶ The overarching aim is to ensure meticulous and persistent orientations of proteins on surfaces to facilitate superior engagements with cells and tissues. This strategy, in turn, paves the way for heightened biocompatibility, seamless amalgamation of tissue, and optimized functionality in medical and biotechnological domains.¹³⁷ This chapter elucidates the *in vitro* methodologies for the assembly of protein-based bioactive coatings and is segmented into five areas: chemical immobilization; layer-by-layer (LbL) self-assembly; amyloid assembly; genetic engineering; other pioneering assembly methods.

3.1 Adhesion mechanisms of proteins to interfaces

Understanding the protein adhesion mechanism at interfaces presents a formidable challenge due to several intrinsic complexities. Proteins exhibit a diverse range of structures, spanning multiple scales from the atomic to macroscopic levels. This structural diversity is further compounded by the dynamic nature of proteins, with continuous changes in their conformations over time. The multitude of protein types existing in biological systems introduces an additional layer of complexity as each protein may manifest unique characteristics in the adhesion process at interfaces. The interplay of these factors makes it difficult to discern and generalize the mechanisms governing protein adhesion. To overcome the challenges posed by experimental limitations and gain a more comprehensive insight into the protein adhesion mechanism at interfaces, molecular dynamics (MD) simulation emerges as a powerful

and indispensable tool.^{138,139} By simulating the behavior of protein molecules under varying interface conditions, molecular dynamics simulations offer a detailed and dynamic view of protein interactions. This computational approach allows for the consideration of various forces, including electrostatic interactions, hydrophobic effects, and hydrogen bonds.

Consequently, molecular dynamics simulations provide a sophisticated means to unravel the intricate and multifaceted interactions between proteins and interfaces, offering valuable insights that might be challenging to attain through traditional experimental methods alone.¹⁴³ Moreover, chemoinformatics plays a pivotal role in drug discovery by allowing the prediction of binding affinities between protein-protein and protein-interface interactions through techniques like quantitative structure-activity relationship (QSAR)¹⁴⁴ models and molecular docking.^{145,146} In the realm of materials science, it contributes to the rational design of surfaces and materials, optimizing protein interactions for various applications, including medical implants. Additionally, chemoinformatics facilitates virtual high-throughput screening, accelerating the identification of potential drug compounds¹⁴³ or materials with desired protein-interface interactions, thereby reducing time and costs.¹⁴⁷ The integration and analysis of large datasets related to protein-interface adhesion are made possible by chemoinformatics, utilizing machine learning and data mining to uncover hidden patterns and discover novel strategies for enhancing or inhibiting protein adhesion. As our knowledge and computational tools advance, chemoinformatics continues to play a crucial role in

understanding and manipulating protein-interface adhesion for innovative solutions in scientific and technological domains.^{147,148}

First, adsorption is the initial step where proteins physically approach the interface, driven by factors such as concentration, temperature, and surface properties. This step is vital for the initial protein-interface interaction. Molecular recognition follows, involving a variety of intermolecular forces, including van der Waals forces, electrostatic interactions, hydrogen bonding, and hydrophobic interactions (Fig. 6a). The amino acid residues and functional groups of proteins play pivotal roles in recognizing and binding to specific regions on the interface.¹⁴⁰

Protein adsorption at surfaces is a multifaceted process influenced by dynamic interactions and conditions.¹⁴⁹ As illustrated in Fig. 6b, the dynamics of this system depend on a balance between the kinetics of initial protein adsorption and the kinetics of protein unfolding and spreading on the surface. Water is a vital component in the process,¹⁴⁸ as it complexes with the protein and surface, affecting protein adsorption stages.^{150,151} Importantly, water isn't inert but actively participates in the process. The presence of an irreversible step in adsorption implies that surfaces will become saturated with adsorbed protein over time. The organization of proteins on the surface, influenced by interactions with the surface and other proteins in the surrounding aqueous solution, determines the final state of the adsorbed protein layer. Subsequently adsorbed proteins are increasingly hindered from reaching the irreversible step by previously adsorbed proteins, explaining why a

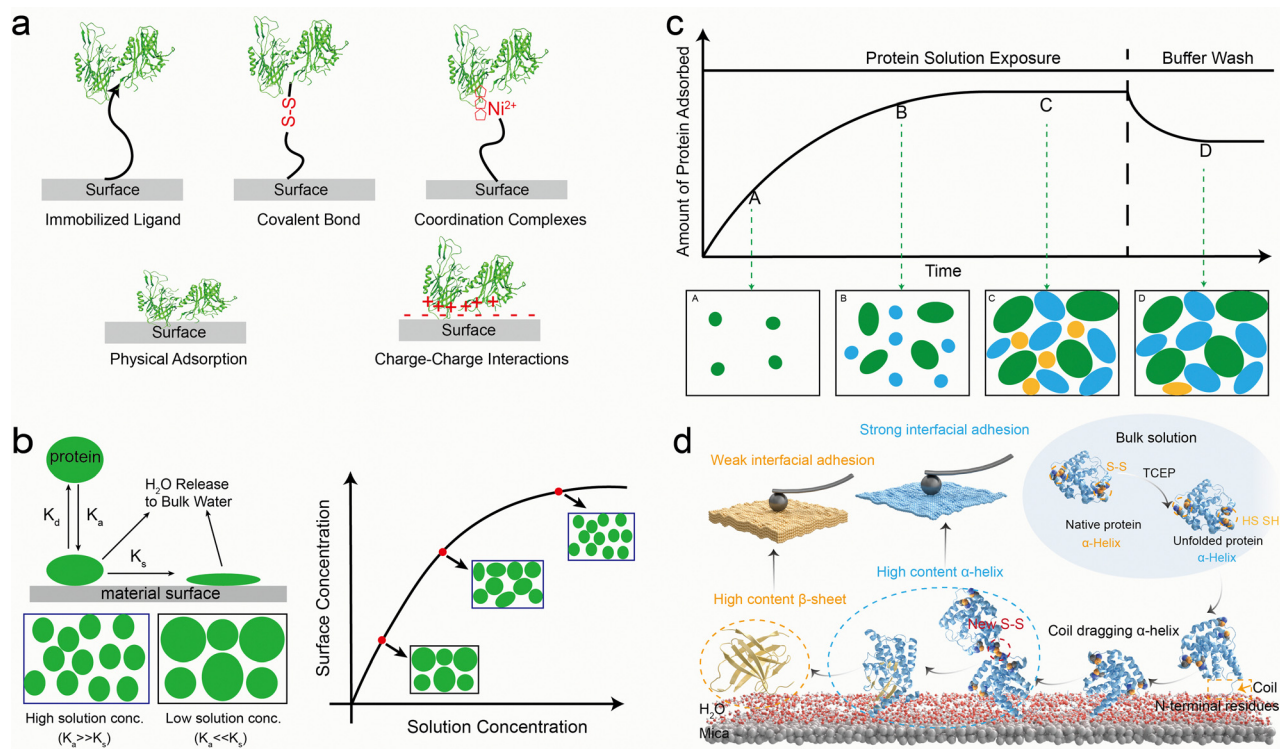


Fig. 6 (a) The way proteins attach to surfaces. Reprinted with permission from ref. 140, Copyright 2017, Biointerphases. (b) and (c) Illustration of the protein adsorption process and adsorption kinetics of single-component proteins. Reprinted with permission from ref. 141, Copyright 2005, Elsevier. (d) The α -helix-mediated interface adhesion model. Reprinted with permission from ref. 142 Copyright 2023, American Chemical Society.

fraction of the adsorbed protein layer can desorb upon exposure to pure buffer solution (Fig. 6c).¹⁵² Moreover, nonhomogeneous surfaces with different phases can contribute to the reversibility of protein adsorption by presenting areas with irreversible and reversible adsorbing properties. The history-dependent outcomes of protein adsorption can be attributed to factors like surface properties, protein concentration, and mass transport, all of which play a role in the complexities of protein-surface interactions. Understanding these intricacies is pivotal for accurate protein-surface interaction studies.¹⁵³

Conformational changes can occur during protein adhesion, where proteins undergo structural alterations to optimize their binding to the surface, providing a dynamic perspective on protein-interface interactions. Among the array of protein-adsorption models occurring at interfaces,¹⁵⁴ the phenomenon of β -sheet stacking, and consequent amyloid aggregation emerges as a pivotal determinant influencing potent interfacial adhesion. A prime illustration is offered by the curli fibril,^{155,156} an archetype of amyloid fibril protein synthesized by bacteria. Characterized by its distinctive β -sheet architecture, this protein engenders robust adhesion across an array of solid surfaces. Likewise, in another instance, barnacles employ their cement to achieve firm adhesion to reef or boat surfaces, employing a composition primarily dominated by amyloid-like components rich in β -sheet motifs.⁹⁸ In contrast to earlier reports outlining the surface adhesion of proteins mediated by β -sheet stacking, Zhang and Yang *et al.*¹⁴² proposed an α -helix-mediated interfacial adhesion model of proteins (Fig. 6d). They revealed that contrary to previous assumptions, β -sheet stacking does not initially form in the solution but instead takes shape at the interface subsequent to α -helix-mediated interfacial adsorption. This transformation involves a structural shift from α -helix to β -sheet stacking. By strategically disrupting the disulfide bonds within proteins to unlock high-energy α -helices, the adhesion of unfolded protein chains at interfaces was found to be governed by interactions between exposed functional groups derived from α -helices and the interface. α -helical structures exhibited robust interfacial adhesion, which gradually diminished as a transition from α -helices to β -sheets took place at interfaces. In addition, intermolecular disulfide crosslinking within the adsorption layer further contributed to this adhesion phenomenon.¹⁴² In summary, protein adhesion to interfaces involves a nuanced interplay of adsorption, molecular recognition, conformational changes, thermodynamics, and desorption. Biochemists play a central role in unraveling these complex mechanisms and applying their knowledge to diverse applications while addressing the challenges posed by the multifaceted nature of protein-interface interactions.

3.2 Chemical immobilization

Chemical immobilization of proteins is employed to covalently bond proteins to solid substrates. This approach entails modifying the substrate surface with designated chemical moieties that engage in reactions with functional groups present on the protein, thereby ensuring a robust and permanent linkage.¹³⁶ Standard chemical methods for protein attachment use reactive

entities such as amino, carboxyl, thiol, or aldehyde groups.¹⁵⁷ These entities can establish covalent interactions with their counterparts on the protein structure. The ubiquity of this method is evident in its deployment across different applications, from biosensors and biochips to medical implants,¹⁵⁸ where it serves to dictate protein orientation and bolster their stability and surface activity. Chemical immobilization presents the dual benefits of durable connections and meticulous control over the density and alignment of proteins on the substrate. This positions it as an indispensable instrument within biochemistry and biotechnology domains.^{137,159} Covalent immobilization of proteins refers to the irreversible binding of proteins to a solid substrate *via* the formation of covalent bonds.¹⁶⁰ This method ensures a durable attachment, preventing the leaching or desorption of proteins during subsequent use. Notably, covalently immobilized proteins maintain their native configuration and functionality,¹⁶¹ thereby facilitating consistent interactions with molecules^{162,163} such as antibodies,^{164,165} ligands, and receptors,¹⁶⁶ in various assays. Such immobilization is used in bioanalytical assays,¹⁶⁷ biosensors,¹⁶⁸ protein microarrays,^{169,170} and other biotechnological endeavors.^{171–179} Established methods encompass the coupling of N-hydroxysuccinimide esters,^{180,181} epoxy or aldehyde coupling,¹⁸² photoactivation diimide chemistry,^{183,184} click chemistry,^{185–189} hydrazine groups,¹⁹⁰ protein A/G/G' coupling,¹⁹¹ and aldehyde-assisted ligation¹⁹² (Fig. 7a). Beyond these traditional methods, a novel approach involves the site-specific immobilization of histidine-tagged proteins onto vinyl sulfone-bearing surfaces by covalent bonding, which ensures the optimal display of the bioactive domains of proteins to enhance their biological effects.¹⁹³ Karen *et al.*¹⁹⁴ employed electron-beam lithography to produce intricately arranged multicomponent protein nanopatterns in 2D single-layer or 3D multilayer configurations. Meanwhile, Raphel *et al.*¹⁸³ detailed the design of a modified elastin-like protein to form enduring coatings on titanium-based dental and orthopedic implants using innovative photo-crosslinking and solution-processing methodologies.

Cell therapy is a rapidly advancing field that exploits the therapeutic potential of cells for treating diseases, but optimizing cell therapeutics while reducing potential toxicity is essential. This involves cell engineering, where advances in gene technology, chemistry, and materials science provide tools to enhance cell functions.¹⁹⁵ Genetic engineering, such as the use of chimeric antigen receptors (CAR) in T cells, allows the precise targeting of tumor cells. Coating cell membranes onto nanoparticles creates biomimetic systems, and biocompatible scaffolds support cell viability after transplantation. Conjugating immunostimulants to immune cells enhances their activity. Among various engineering techniques, chemical ligation is a versatile approach for decorating cells with drugs, ligands, or other entities. Bio-orthogonal chemistry, particularly “click reactions,” has emerged as a means to operate rapidly under mild biological conditions without disrupting the functions of engineered cells or the biosystem.¹⁹⁶ The primary bio-orthogonal reactions suitable for cell engineering (Fig. 7b) include copper(i)-catalyzed azide-alkyne cycloaddition (CuAAC), strain-promoted alkyne-azide cycloaddition (SPAAC), and inverse-electron-demand Diels-Alder (IEDDA) reactions.¹⁹⁷

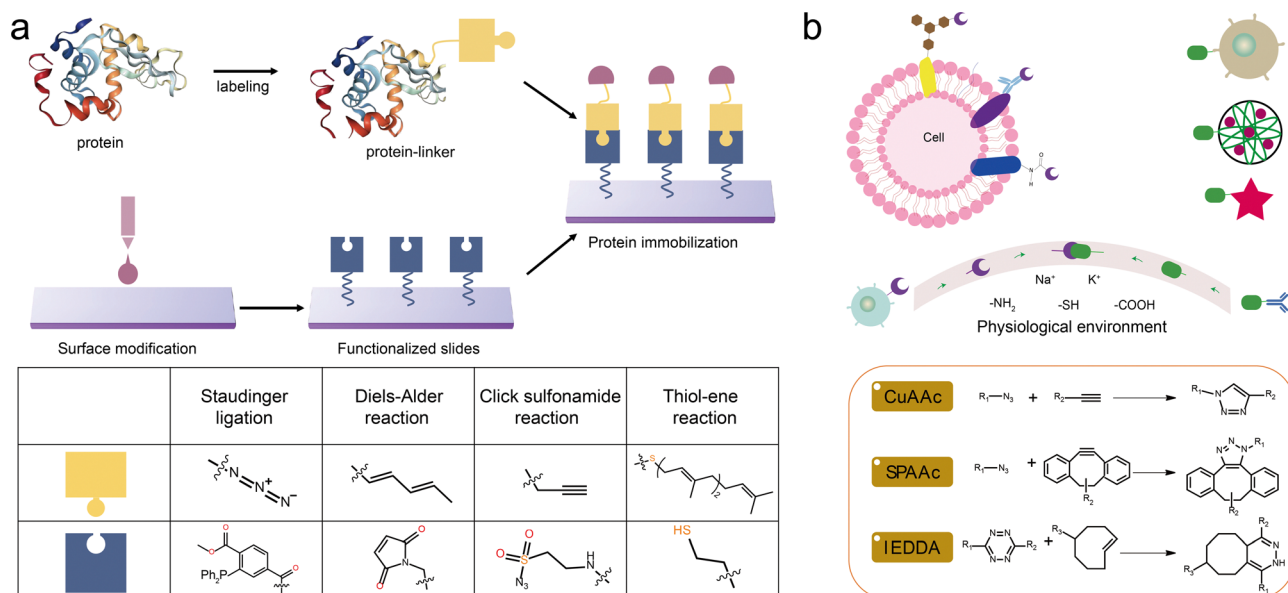


Fig. 7 (a) Bioorthogonal chemistry for site-specific labelling and surface immobilization of proteins. Reprinted with permission from ref. 160 Copyright 2011, American Chemical Society. (b) The principles of bioorthogonal chemistry for engineering cells. Reprinted with permission from ref. 197 Copyright 2022, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

These reactions allow the intentional introduction of reactive moieties onto cell surfaces, forming bio-orthogonal bonds selectively in aqueous media under physiological conditions. Bio-orthogonal chemistry has become an indispensable tool for manipulating cell functions and fate, both *in vitro* and *in vivo*, by engineering the surfaces of living cells and extending its utility to living organisms.

Protein biochips, often called “protein microarrays” or “protein chips,” are powerful tools in biochemistry and molecular biology. They enable the simultaneous analysis of numerous proteins, supporting the high-throughput examination of protein interactions, including protein-protein and protein-ligand binding.¹⁹⁸ Various innovative methods have been developed for efficient protein immobilization on these biochips. For example, Corgier *et al.*¹⁹⁹ introduced a method to directly graft noncatalytic proteins onto the surface of screen-printed graphite electrode biochips, allowing precise spatial immobilization. Wasserberg *et al.*²⁰⁰ developed a patterned protein array with a fluorogenic surface for immobilization detection, enabling the covalent anchoring of fluorescent proteins and other thiol-functionalized biomolecules. Kindermann *et al.*²⁰¹ introduced a universal strategy for the chemical immobilization of fusion proteins, while Liebich *et al.*²⁰² innovated a procedure for covalently anchoring proteins onto custom paper sheets, opening doors to controlled and efficient protein immobilization with a wide range of potential applications in the field. These advancements highlight the significance of protein biochips in the study of protein functions and interactions.

The immobilization of proteins on micro- and nano-surfaces, hydrogels, and cellular surfaces is of great significance, extending beyond traditional protein chips on planar surfaces.¹⁹⁸ These methods offer diverse opportunities in the fields of biochemistry and biotechnology. For instance, the development of multifunctional

silica nanoparticles (SiNPs) with amino, phosphonate, and thiol functional groups provides a zwitterion-stabilized surface that prevents non-specific adsorption and allows the targeted covalent immobilization of proteins.²⁰³ SiNPs have demonstrated their effectiveness in preserving the activity of tethered proteins, making them valuable for applications like glucose-binding protein biosensors and bi-domain cytochrome P450 enzymes due to their stability and monodispersity. Additionally, covalently anchoring cell-adhesive proteins to poly(vinyl alcohol) hydrogel surfaces using various chemical mediators has been explored, offering a robust method for protein immobilization with minimal dissociation.²⁰⁴ Leveraging genetic engineering for protein immobilization on cellular surfaces holds promise by securing and rejuvenating proteins, particularly surface proteins that play crucial roles in cellular adhesion, receptor actions, and enzymatic processes. These approaches shed light on cellular surface functionalities and their potential applications in various fields.²⁰⁵

3.3 LbL assembly

LbL assembly^{206,207} allows for the stepwise deposition of substrates into aqueous solutions of charged biomacromolecules (proteins) or polymers. This method facilitates the construction of controlled, nanometre-thick, multilayered ultrathin films. The primary mechanism underpinning multilayer growth is the overcompensation of the surface charge during the deposition of each layer. This action leads to a reversal of the surface charge, thereby enabling the adsorption of the subsequent layer bearing an opposite charge. Polyelectrolytes are charged soluble polymers and are employed predominantly for this purpose.²⁰⁸ However, when creating protein-based active coatings *via* LbL assembly, the interaction forces between multiple layers must be considered. Factors such as the surface charge of the protein can impact the scalability of such active coatings significantly.

The complexation of lysozyme with poly(styrenesulfonate) (PSS) results in protein–polyelectrolyte complexes (PPCs) with standardized charges.²⁰⁹ Detailed examinations have been conducted on the size and electrical properties of these PPCs, with a focus on understanding the underlying forces facilitating their complexation. Crucially, studies have highlighted that LbL assembly using PPCs hinges primarily on standard polyelectrolyte interactions. This phenomenon makes the charge state of the protein irrelevant, thereby streamlining the integration of proteins into multilayers. Interactions (*e.g.*, hydrophobic, covalent bonding, hydrogen bonding) can also be harnessed for nano/microfilm assembly (Fig. 8a).^{210,211} LbL assembly boasts nanoscale precision, simplicity, and adaptability. These features facilitate the coating of planar and particulate substrates across various domains, from optics and energy to catalysis, separations,^{212,213} and biomedicine.^{214,215} LbL assembly offers a myriad of benefits: controlled coating, cost-effective production, defined thickness, conditions amicable to biomolecules, and the ability to incorporate and regulate the release of therapeutic agents. Furthermore, LbL assemblies can serve as storage media for charged agents and growth factors.^{216,217} The self-assembly procedure provides meticulous functional oversight by modulating building blocks, pH, and salt concentration.²¹⁸ In essence, LbL assembly is an efficient method for crafting thin films with exactitude in thickness and functionality, marking its importance across various scientific and technological arenas.

Proteins can be co-assembled with biologically active substances to create drug-loaded nanoplateforms,²¹⁹ such as multilayered nanofilms using the layer-by-layer (LbL) assembly of silk fibroin (SF) and heparin (HEP). The construction of these nanofilms relies on molecular interactions between SF and

HEP functional groups. By controlling the β -sheet content within these SF/HEP LbL-assembled nanofilms, their hydrophobic nature is harnessed for interactions with hydrophobic drugs, directly affecting drug loading and release.²²⁰ This approach has been extended to integrate multilayers on nanofibrous scaffolds, allowing the precise modulation of bioactive molecule release. Multilayered polydopamine/graphene oxide/type-I collagen nanofilms have significantly augmented the osteogenic differentiation of stem cells and regulated the release of bioactive agents, providing a method for creating multifunctional bioactive coatings on implant surfaces.²²¹ Furthermore, interactions between proteins and polyphenols,²²² like tannic acid, have been explored for various applications, including catalysis, fluorescence imaging, and cell targeting. Multilayered bioactive coatings comprising tannic acid and lysozyme have been tailored to enhance antioxidative, antibacterial,²²³ and osteogenesis-promoting attributes,²²⁴ with further investigations into cell adhesion and proliferation.²²⁵

Layer-by-layer (LbL) films and capsules derived from proteins have been known for some time.^{226,227} However, employing the LbL assembly to create protein shells²²⁸ around cells has proven challenging, with only a few successful instances.^{229–231} Within the human body, the ECM and growth factors continually regulate cell functions.²³² LbL assembly is a versatile method in the realm of functional biomaterials, allowing precise bio-coatings at micro- and nanoscales, effectively emulating ECM microenvironments.²³³ In a seminal study, Matsusaki and colleagues²³⁴ expertly crafted cellular multilayers using nanoscale ECM films, approximately 6 nm thick, composed of fibronectin and gelatin. This innovative approach led to the development of xenogenic human-bilayer structures resembling blood vessels

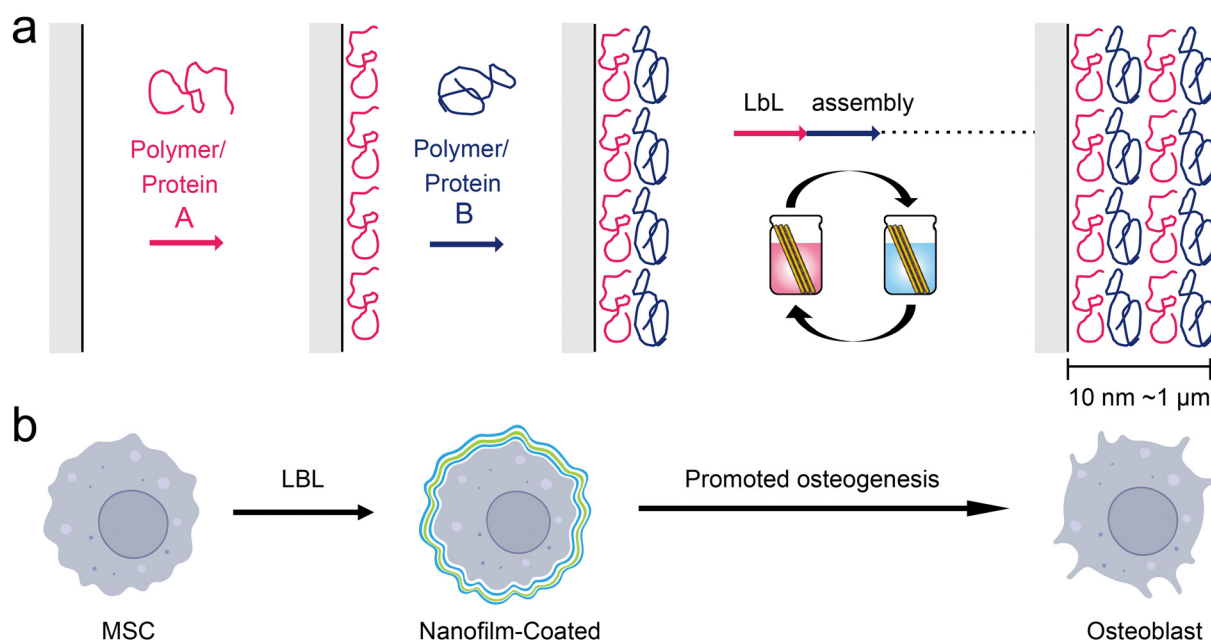


Fig. 8 (a) Schematic representations of the layer-by-layer (LbL) assembly. Reprinted with permission from ref. 231 Copyright 2014, Nature Publishing Group. (b) A schematic representation of an artificial cellular nano-environment formed by molecular adsorption on a single-cell surface. Reprinted with permission from ref. 235 Copyright 2019, Elsevier.

through the integration of nanofilms on cellular surfaces (Fig. 8b).²³⁵ These nanofilms created a synthetic cellular environment on cell membranes, facilitating the regulation of cell viability, morphology, and proliferation, especially in osteogenesis or bone formation.²³⁶

Moving beyond protein-based coatings on cellular surfaces, Li and colleagues²³⁷ developed an effective encapsulation strategy for probiotics using the LbL assembly method, leveraging electrostatic interactions and hydrogen bonds among fibrils from various sources. Encapsulated probiotics exhibited significantly improved survival rates during simulated gastrointestinal digestion and storage. This method holds promise for enhancing the delivery and adhesion of probiotics to the intestinal mucosa. In another innovative approach, Husteden *et al.*²³⁸ introduced a gene-activated surface coating, a pioneering strategy for smart biomaterials tailored for bone-tissue engineering. The foundation of this coating consists of polyelectrolyte multilayers of type-I collagen and chondroitin sulfate, constructed using LbL assembly. These multilayer structures were adorned with liposomes containing DNA encoding bone morphogenetic proteins, which are crucial for osteogenic differentiation of mesenchymal stem cells and tissue regeneration.

3.4 Amyloid assembly

Protein self-assembly is pivotal in biological functions but also serves as a flexible approach for crafting intricate architectures spanning sizes from nanometres to micrometers.^{239–241} Proteins can adopt a wide array of tertiary structures based on their amino-acid sequences. Proteins can form an extensive range of symmetric structures. By manipulating the intensity, count, or alignment of protein–protein interactions,²⁴² the self-assembly behavior of a protein can be modulated.²⁴³ This capacity for precise control enables researchers to engineer many supramolecular structures.²⁴⁴ Protein assembly is intrinsic to living organisms,²⁴⁵ as well as being integral to numerous cellular functions and biological processes.²¹⁰ The mechanisms underlying protein assembly are governed by kinetic and thermodynamic forces: kinetics controls the pace of assembly, and thermodynamics determines the stability and energy profile of the resultant structures.²⁴⁶ As proteins undergo conformational shifts during assembly, these changes are orchestrated by thermodynamic principles to reach an optimally stable configuration. A comprehensive grasp of the relationship between kinetics and thermodynamics is essential for unraveling the intricate dynamics of protein assembly, and offers insights beneficial for biotechnology, pharmaceutical design, and cellular-function research.²⁴⁷ This review delves into the mechanisms governing amyloid-protein assembly and provides a detailed comparison of the assembly processes between amyloid and amyloid-like proteins.

3.4.1 Amyloid-like nanofilm. Contrary to traditional amyloid-aggregation processes, which are slow and necessitate harsh *in vitro* conditions, Li and Yang *et al.*^{248–250} identified three fundamental factors for a milder amyloid-like aggregation process. These are a segment in globular proteins with a high predisposition to fibrillation, an abundance of α -helix structures, and intramolecular S–S bonds that stabilize the

α -helix. These factors are pivotal for the swift assembly of amyloid-like proteins. If the S–S bonds are reduced, the α -helix transitions swiftly, leading to the rapid formation of β -sheet-rich amyloid oligomers and protofibrils in minutes.¹⁰⁹ Subsequently, such assemblies generate a macroscopic nanofilm at the air/water interface and microparticles in a bulk solution. Those findings offer valuable insights for the advancement of amyloid-based materials and their potential applications. Recently developed amyloid-like proteins represent a novel class of surface-modified technical materials. Building on this, Wang and Yang *et al.*^{109,251} investigated the bioadhesive properties of amyloid-like nanofilms, thereby highlighting their exceptional interfacial adaptability regardless of the substrate type, shape, or size. Proficiently crafted macroscopic giant amyloid-like nanofilms of specific sizes and patterns using techniques such as immersion, film transfer, contact printing with a hydrogel stamp, and spraying were obtained.²⁵² Moreover, amyloid-like nanofilms could seamlessly coat objects ranging from the nanoscale to microscale-like Au NPs, silica NPs, polystyrene NPs, yeast, and CaCO₃ particles merely by incorporating them into the reactive solution.²⁵³ Analyses of these amyloid-like nanofilms disclosed their surface composition and identified functional groups. These groups facilitated a spectrum of binding interactions, such as metal–S, hydrogen bonding, hydrophobic, and electrostatic interactions, with the underlying substrate. This comprehensive binding capacity ensured sturdy interfacial adhesion.^{254,255}

3.4.2 Activity retention (one-step assembly). Proteins, as versatile biomolecules, are integral to a multitude of physiological and biochemical processes in living organisms.²⁵⁶ Their roles range from acting as enzymes, receptors, and structural components to serving as transporters and signaling molecules.²⁵⁷ However, when subjected to external conditions *in vitro*, proteins can rapidly denature, resulting in a loss of their biological functions. Variations in factors such as pH, salt ions, temperature, and metal ions can disrupt the secondary structure of proteins, presenting a significant challenge in preserving their activity.^{258,259} This is crucial for various applications in biochemical and biotechnological research. A central challenge in working with proteins is finding a method that not only maintains their structural integrity but also retains their biological functionality during assembly.^{256,260} The conventional approach of amyloid aggregation often leads to protein inactivation,^{261,262} creating a paradox. To address this, a novel concept known as chemoselective reaction-induced protein aggregation (CRIPA) has been introduced.¹⁰⁹ CRIPA focuses on the role of disulfide bonds in stabilizing protein structures.^{263,264} By selectively targeting and cleaving disulfide bonds away from the protein's active site using a reducing agent, this approach enables the formation of amyloid-like nanofilms, which function as bioactive coatings.²⁶⁵ Notably, this strategy has been successfully applied to specific proteins, such as lysozyme, without affecting other standard proteins. These amyloid-like nanofilms maintain the activity of encapsulated proteins and exhibit robust interfacial adhesion across various substrates (Fig. 9a). Offering a non-toxic means to create proteinaceous nanofilms or coatings on diverse material surfaces, this methodology holds significance for applications

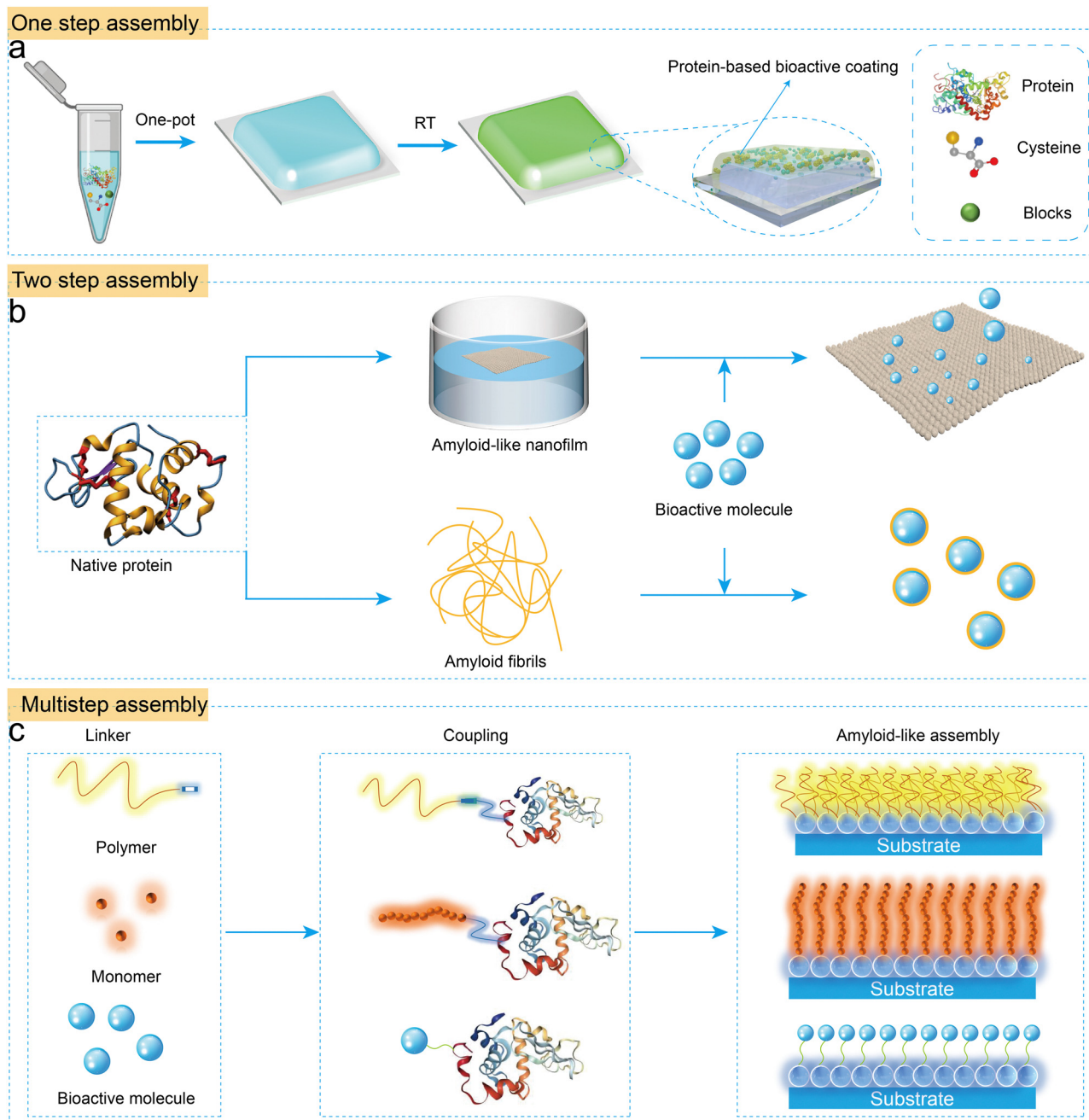


Fig. 9 (a) One-step assembly: protein and active drug molecules are co-assembled in one step to form a protein-based bioactive film. Reprinted with permission from ref. 265 Copyright 2020, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (b) Two-step assembly: the first step is to assemble the protein to form a nanofilm or nanofiber, and the second step is to combine the active drug with a molecular complex with protein assemblies to form protein-based bioactive coatings. (c) Multi-step assembly: the linker is introduced through bioactive molecules (such as polymers, monomers and drug molecules) and then chemically coupled to the protein surface and is finally formed on the surface of the substrate through amyloid-like assembly, in which the amyloid-like protein's main function is to act as an adhesive layer, and bioactive molecules can be released by breaking chemical bonds.

related to cellular control, cell culturing, and various biological research areas. Additionally, it introduces a strategy to immobilize and release proteins on surfaces without substantial loss of activity, paving the way for advancements in these critical domains of research.

3.4.3 Storage and release of active substances (two step assembly). In the development of bioactive molecule coatings,

two fundamental considerations come to the forefront. The first pertains to preserving the intrinsic activity of the bioactive molecule.^{133,266} This is of utmost importance, especially when dealing with therapeutic proteins or specialized enzymes. The coating process must be meticulously tailored to ensure that the structural and functional attributes of the molecule remain intact. Such preservation is crucial for the effective functioning

of the molecule, whether within a biological system or other applications (Fig. 9b). The second critical consideration revolves around protection and controlled release. Beyond preserving activity, protein coatings must act as protective barriers, particularly in *in vivo* applications. Unprotected bioactive molecules can be susceptible to degradation by native enzymes in the body, potentially compromising their efficacy. A well-designed protein coating serves to shield these molecules from enzymatic breakdown, enhancing their resilience during circulation or storage. Furthermore, these coatings can be engineered to regulate the timed release of the bioactive molecule, ensuring systematic delivery to targeted sites, a pivotal feature in drug delivery scenarios. In essence, proficient protein coatings strike a harmonious balance between preserving the activity of the bioactive molecule, safeguarding it against degradation, and orchestrating its controlled release.²⁶⁷ This equilibrium is indispensable for the utilization of bioactive molecules across a diverse range of biomedical and biotechnological domains. Additionally, the incorporation of polysaccharides in the protein assembly process demonstrates the potential for synergies between proteins and polysaccharides, opening new avenues in biomedicine and green chemistry.^{268–270}

3.4.4 Activity expansion (multistep assembly). Self-assembled protein nanofilms have been valuable for surface modification;²⁵¹ however, their limited functionality presents challenges in meeting specific interface property requirements. This has led to the development of protein-based active coatings but challenges remain regarding the controlled maintenance and consistent release of active factors. Surface functionalization, a well-established concept, provides a foundation for surface modification and confers distinct functional properties to interfaces. Protein coupling and interface modification methods, such as grafting functional molecules onto proteins, have emerged, anchoring them to interfaces *via* amyloid-like transformations to ensure controlled functionality and interface stability (Fig. 9c).^{271–277} For instance, grafting poly(ethylene glycol) (PEG) onto lysozyme rapidly forms an effective antifouling nanofilm, deterring oral bacteria adhesion.²⁷¹ Additionally, grafting polyzwitterions^{272,273} onto lysozyme and enhancing bactericidal properties by grafting chitosan onto lysozyme demonstrate the potential of these approaches in surface modification and biomedical applications.²⁷⁸ These developments are advancing the field of surface interface research, offering new tools and strategies for enhancing material properties and biological performance.

3.5 Genetic engineering

Protein-based materials have gained considerable attention due to their unique programmable and biocompatible features. Notably, mussel-inspired and amyloid proteins have been developed, highlighting their innate multifunctional characteristics. These proteins have been leveraged to formulate coatings with a range of applications. Advancements in synthetic biology have further enriched this trajectory by unveiling and designing functional modules inspired by natural phenomena.²⁷⁹ Specifically, an engineered fusion-protein coating is generated by anchoring a fusion protein onto a chosen surface. This fusion

protein is conceived through genetic engineering, amalgamating different functional domains or segments that possess distinct attributes. The coating emerges by binding the tailored fusion protein to a desired surface, whether it is a biomaterial, cell or solid substrate. Such fusion-protein coatings can fulfill diverse roles, from enhancing biocompatibility and facilitating cell adhesion to delivering therapeutic compounds or fostering specific molecular or cellular interactions. This methodology paves the way for “bespoke” coatings with designed functionalities, positioning it as an invaluable asset for clinical applications.

Marine mussels possess remarkable adhesive capabilities attributed to the catechol ligand DOPA,²⁸⁰ enabling robust substrate interactions through covalent and noncovalent bonds, such as hydrogen bonds and π -interactions. A mussel-inspired, bio-orthogonal approach was utilized by Zhang and colleagues²⁸¹ to design a 3,4-hydroxyphenylalanine-containing recombinant insulin-like growth-factor-1, which exhibited a strong binding affinity for titanium surfaces and promoted cell growth significantly. Innovative chimeric proteins like MP-KE²⁸² (Fig. 10a), combining mussel adhesive proteins with zwitterionic peptides, have been engineered for multifunctional coatings, rendering surfaces antifouling, antifogging, and self-cleaning.^{283,284} Multifunctional films created by depositing substrates with bioinspired protein MP-KE offer potential for innovative coatings with antifogging, self-cleaning, antimicrobial properties, and excellent hemocompatibility. Genetically engineered chimeric proteins have demonstrated effectiveness in serving as antifouling materials by directly anchoring onto various substrates, offering a one-step process for multifunctional coatings.²⁸⁵ Another chimeric protein, Mfp-AFP,²⁸³ combines mussel-inspired adhesive domains with antifreeze properties from a beetle, exhibiting biocompatibility and holding significant potential for applications in anti-icing coatings and biomedical materials.

Biofilm proteins, exemplified by the functional amyloid fibrils like curli, play a pivotal role in enabling bacteria to adhere firmly to surfaces and are essential in biofilm structure. The CsgA amyloid proteins found in *Escherichia coli* biofilms not only contribute to adhesion but also self-assemble into amyloid-fibril structures that enhance the structural stability of the biofilm matrix (Fig. 10b).²⁸⁶ These proteins possess a unique ability to form elongated, well-organized amyloid fibrils, controlled by specific amino-acid sequences and environmental factors.¹⁵⁵ The amyloid nanofibrils created by CsgA provide surfaces with increased mechanical strength and structural coherence.^{117,287} Recent studies revealed that genetically engineered CsgA fusion proteins have potential applications in underwater adhesives,²⁸⁷ nanoparticle-assembly scaffolds, patternable materials, biomimetic mineralization,^{288,289} and medical hydrogels.^{290,291} CsgA's adaptability allows for the incorporation of various functional peptides and protein domains, making it a promising platform for coatings with diverse functionalities. However, the full potential of genetically engineered CsgA-based coatings in various applications is yet to be explored fully.²⁹²

3.6 Other assembly methods

Protein-based materials provide a biocompatible and sustainable platform for the development of functional materials,

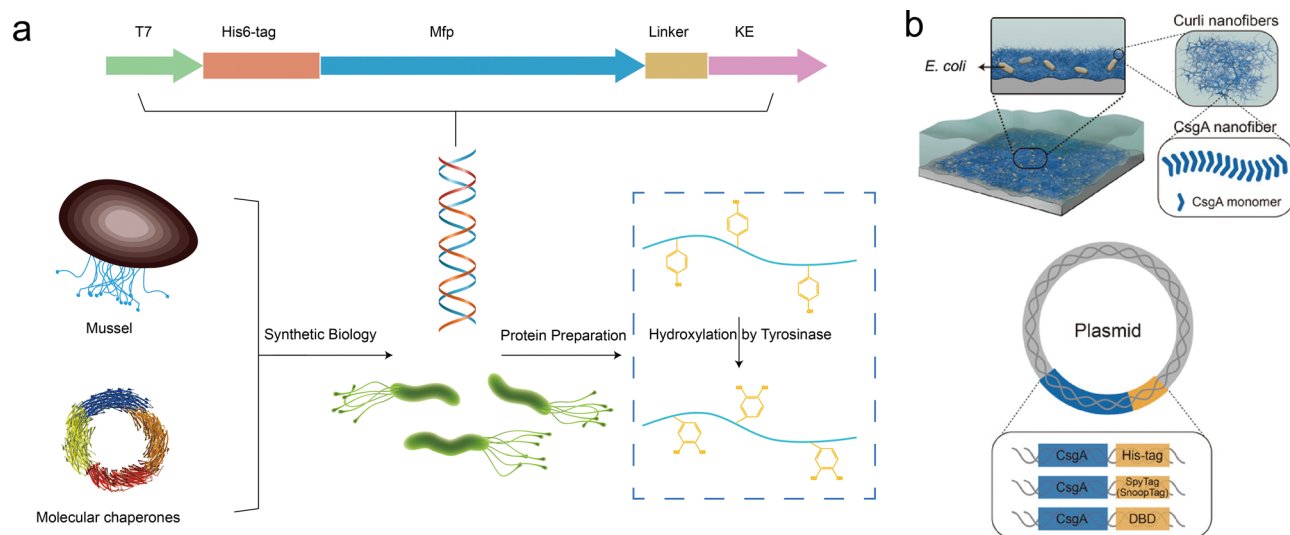


Fig. 10 (a) The construction of engineered multifunctional chimeric protein coatings. Reprinted with permission from ref. 285 Copyright 2018, Royal Society of Chemistry. (b) Inspired by *E. coli* biofilms, protein nanofiber coatings were prepared using genetic engineering. Reprinted with permission from ref. 117 Copyright 2020, American Association for the Advancement of Science.

thereby leveraging the innate structural and functional heterogeneity of proteins. Research has highlighted the effectiveness of protein films in bioelectronics, tissue engineering, and drug delivery. These films capitalize on their aqueous processability and minimal ecological footprint, underscoring their potential as environmentally friendly materials. Nevertheless, the creation of protein films that can resist aqueous degradation remains crucial, especially for applications such as tissue engineering and the controlled delivery of drugs. Soft micro/nanopatterned materials have garnered attention for their diverse applications in optical, mechanical, electronic, microfluidic, and optofluidic devices. Incorporating pure protein-based materials into this paradigm presents innovative prospects. SF, renowned for its exceptional optical clarity and biocompatibility, emerges as an optimal material. This paves the way for a groundbreaking technology platform harmonizing nanophotonic with biopolymeric and biocompatible materials, ushering in a new realm of device applications.²⁹³ Jeoung *et al.*²⁹⁴ introduced a scalable, additive-free nanoimprint-lithography method for crafting stable, patterned protein films. This method boasts versatility, accommodating a range of protein constructs while maintaining their inherent structure and properties. Film-surface charges can be customized by selecting specific proteins, a claim substantiated by findings from Kelvin probe force microscopy. Moreover, by modulating the processing temperature and pressure, the stability and degradation rates of these materials can be calibrated. Their utility is exemplified by the development of effective antifouling surfaces and regulated cellular adhesion through the judicious choice of protein precursors.

Conventional nanofabrication methodologies often resort to hazardous chemicals and intricate lithographic processes, thereby restricting their scalability. Using silk as a resist alleviates these constraints, given that the entire procedure is water-centric, spanning from the silk solution to the maturation of the exposed

silk film. The polymorphic crystalline composition of silk enables it to act as a positive and negative resist in electron-beam applications. The adaptability of silk as a resist is further accentuated when fabricating nanoscale photonic matrices using pure silk or silk combined with quantum dots, green fluorescent proteins, or horseradish peroxidase. Kim and colleagues²⁹⁵ advocated an innovative, environmentally conscious approach to nanofabrication, leveraging silk as a natural resist for electron-beam lithography. This sustainable methodology represents opportunities for expansive nanofabrication incorporating biologically active functional resists.

Electrospinning is a versatile method for creating protein-polymer coatings with various morphologies, allowing precise control over the coating thickness and structure by adjusting parameters like solution concentration, field strength, deposition distance, and deposition time.²⁹⁶ Curcumin-loaded coatings have been introduced through electrospinning, demonstrating their potential as food-contact layers suitable for active and bioactive food packaging.²⁹⁷ In contrast, electrophoretic deposition has gained popularity in biomedical applications due to its advantages, such as operating in a gentle aqueous environment, enabling the creation of uniform coatings on intricate or porous medical devices. The quality of the coating can be tailored through process parameters, and the method is time-efficient and scalable. A unique double-layered silk fibroin (SF) coating system has been developed through sequential electrophoretic deposition, with the base layer consisting of *Bombyx mori* SF (bmSF) molecules and nanospheres and the upper layer made of *Antheraea pernyi* SF (apSF) with Arg-Gly-Asp (RGD) peptide to enhance cellular interactions.²⁹⁸ This system also incorporates a model drug (doxycycline), offering the ability to control drug release rates and volumes by adjusting nanosphere concentration and deposition time. Biomimetic strategies leverage insights from natural processes and proteins to engineer coatings with

specific biological functionalities. Microfluidic devices play a crucial role in manipulating protein self-assembly, resulting in highly organized structures like amyloid fibrils, known for their remarkable stability, mechanical robustness, and biocompatibility. These properties make them promising candidates for applications in drug delivery and tissue engineering. Microfluidics also allows for the precise incorporation of functional biomolecules or nanoparticles within protein coatings, expanding their utility to multifunctional purposes. Dautel *et al.*²⁹⁹ have explored the modifiability of protein vesicles for stimuli responsiveness, highlighting their potential in domains ranging from synthetic biology to drug delivery and microreactors. Temperature-responsive amphiphilic protein-polymer hybrids known as “proteinosomes” autonomously assemble, resembling colloidosomes and offering a foundation for stimulus-responsive, biomimetic protocells.³⁰⁰ These proteinosomes exhibit features such as guest-molecule encapsulation, selective permeability, gene-directed protein synthesis, and membrane-regulated enzyme catalysis, holding promise for diverse bioengineering endeavors. Nanostructured mineral coatings have also been found to significantly enhance the stability of proteins for therapeutic applications, extending the biological potency of proteins released from such coatings as compared to conventional methods.³⁰¹

4. Applications of protein-based bioactive coatings

Protein-based bioactive coatings can enhance the functionality and performance of diverse surfaces across various applications.^{302,303} Protein bioactive coatings offer distinct advantages as compared to organic, inorganic, and other biological material coatings. Firstly, proteins are inherently biocompatible and often elicit minimal immune responses, making them ideal for various biomedical applications.³⁰⁴ Their ability to interact specifically with biological molecules, such as cell receptors, antibodies, or enzymes, enables precise targeting and controlled interactions, a feature not easily replicated by organic coatings. Moreover, proteins can facilitate cell adhesion, proliferation, and differentiation, making them valuable for tissue engineering and regenerative medicine.²⁹² The programmability of proteins, through genetic engineering or chemical modification, allows for the tailored binding and release of specific biomolecules, drugs, or growth factors.^{305,306} However, protein coatings can face challenges, including potential instability and denaturation, which may limit their long-term durability. Their complex structure and behavior may also require sophisticated engineering and precise control, which can be time-consuming and costly. In contrast, organic coatings, while versatile and cost-effective, might lack the same level of biocompatibility and specificity found in protein coatings. Inorganic coatings offer durability, heat resistance, and protection but often lack bioactive properties and the capacity for biological interactions. Coatings made of biological materials such as polysaccharides or DNA can be biocompatible and biodegradable, with potential applications in drug delivery, but they may not offer the same level of

specificity, complexity, or diversity as protein coatings, particularly concerning specific receptor or molecule targeting.^{307,308} The choice of coating material should be determined by the specific requirements of the application and the balance between these advantages and disadvantages. Future research should continue to explore ways to enhance the stability and functionality of protein coatings while expanding our understanding of their applications and limitations within the broader context of coatings science. This review offers a thorough assessment of recent advances and novel applications of protein-based coatings, highlighting their considerable potential in arenas from biomedicine to industrial biosensing. Concentrating on their design, fabrication, and practical applications, this review delves into the influence of these coatings on surface chemistry, cell or tissue engineering, drug delivery and delivery, biocatalysis, and biosensing (Table 1). By analysing recent research and technological innovations, we emphasize the pivotal role of protein-based bioactive coatings in advancing biochemistry and biotechnology. As the quest for advanced surface modifications intensifies, protein-based bioactive coatings present a solution to intricate challenges, paving the way for the evolution of next-generation biomaterials and biofunctionalized interfaces.¹³⁶

4.1 Surface chemistry

4.1.1 Antifouling. Biofouling, or biocontamination, poses a significant challenge across a wide range of applications, including surgical equipment, medical implants, biosensors,³⁰⁹ textiles, food packaging,³¹⁰ water purification systems, and industrial equipment.³¹¹ The issue arises from the nonspecific adsorption of proteins and microbes, leading to reduced sensitivity in applications like immunological assays and compromising the effectiveness of biological implants. In the medical field, protein adsorption on implants can result in complications such as thrombosis and biofilm formation, threatening patient safety. In other industries, including food and maritime, the attachment of microorganisms to surfaces can lead to biofilm formation, infection risks, and increased operational and maintenance costs. Therefore, addressing biofouling with effective antifouling measures is crucial to ensure the functionality, safety, and efficiency of various applications and devices while reducing healthcare and operational expenses.^{132,276}

Motivated by the impressive adhesive properties of mussels,³²⁸ attributed in part to the presence of DOPA in their adhesive proteins,²⁷⁹ substantial research efforts have been dedicated to the development of antifouling polymers coupled with mussel-inspired polymeric anchors.³²⁹ These polymers are affixed to target surfaces, resulting in extended and partially oriented polymer chains that minimize excluded volume effects, forming a layer akin to polymer brushes. The considerable antifouling capabilities of surfaces bearing such polymer brushes are primarily due to the robust hydration and steric repulsion of the polymer chains.^{283–285} Simultaneously, the development of antifouling materials has encountered challenges in creating robust coatings for diverse surfaces. Although synthetic materials like hydrophilic polymers have demonstrated antifouling properties, their inability to provide universal interfacial adhesion complicates coating

Table 1 Protein-based bioactive coatings and their applications

Assembly method	Materials	Applications	Ref.
Amyloid assembly	Lysozyme/antimicrobial peptides	Preventing dental caries	312
	Lyso/HLF/BSA	Cell-proliferative, antibacterial, Osseointegration	313–315
	HFL/HA/CsA	Controlled drug release	266, 268, 316 and 317
	BSA/SA		
	BSA/PL		
	β -Lactoglobulin/HA		
	Lysozyme and silver nanoparticles	Antibacterial and moisture management	278 and 318
	Lyso-chitosan		
	Lyso/C-AMG	Enamel remineralization	319
	Lyso-PEG		271–273
LbL	Lyso-polyzwitterionic	Antifouling and antibacterial fabrics	
	BSA or BSA-pSBMA		252 and 276
	CsgA proteins	Cell attachment, proliferation, and stabilization of the cytoskeleton	292
	Protein/polyphenol (TA)	Antioxidation, antibacterial and osteogenesis-promoting	222–225
	BSA/cationic antibiotics	Medical implant coatings	320
	BSA/baicalin/TA	Enhance osteogenesis and resist oxidative stress	321
	Collagen, heparin	Cell adhesion	322
	Gentamicin-silk protein	Antibacterial implants	323
	Silk fibroin/AgNP/peptides	Antibacterial and osseointegration	324
	Collagen/polydopamine/Go	Controlled release carrier of bioactive substances	221
Genetic engineering	Peptide-eGFP/EndLys/polymer Brush	“Kill and release” antibacterial	325
	Ag-MAP-KE	“Kill and release” antibacterial	326
	MZAgP, adhesive peptide and silver-binding peptide	Antibacterial coating of implants	279
	Elastin-like polypeptide fusion proteins	Drug delivery	327
	Chimeric protein Mfp-AFP	Anti-icing coating	283
Chemical immobilization	His-tagged proteins	Enzyme immobilization, biosensors and arrays, drug delivery	193
	S-Transferase fusion proteins	Protein microarrays	188
	Fibroblast growth factor, bone morphogenetic protein	Programmable cell-differentiation pathways	166
	Proteins-TC-tags/CrAsH	Microarray analytics	169

diverse surfaces. Introducing a novel approach, a class of anti-fouling materials known as amyloid-like protein aggregates has emerged. These materials combine interfacial anchoring and antifouling capabilities, providing strong adhesion to various surfaces without extensive pretreatment. This innovative strategy leverages the phase transition of bovine serum albumin (BSA), achieved by reducing its disulfide bond, resulting in an amyloid-like proteinaceous nanofilm (Fig. 11a).²⁵² This antifouling material exhibits excellent performance against a wide spectrum of contaminants, ease of preparation, and durable interfacial adhesion to a range of substrates, presenting a promising solution for various engineering applications.

In addition, through the conjugation of amyloid-like proteins with polymers, including the chemical coupling of hydrophilic polymers like PEG²⁷¹ and polyzwitterions,^{272,273} the antifouling properties of amyloid proteins can be substantially enhanced, resulting in improved protein utilization. The film created using this method is nearly a monolayer nanofilm, characterized by its simplicity, transparency, and bio-friendly nature. The co-assembly of proteins and polysaccharides has also garnered significant attention in recent research. For instance, sodium alginate and hyaluronic acid²⁶⁶ co-assemble with amyloid proteins,²⁶⁸ leading to the enhancement of various properties in protein nanofilms, such as strength, flexibility, and hydrophobicity. In recent years, there has been a growing focus on the development of smart surface coatings

with bactericidal release capabilities, particularly for clinical applications. While preventing bacterial adhesion is essential, complete sterilization and adhesion prevention are the ultimate goals. Consequently, a smart antibacterial approach known as “Kill-Release” functional conversion has been developed,³²⁵ resulting in the creation of a series of intelligent antibacterial surfaces. These surfaces typically integrate bactericidal components (e.g., bactericides) on the material surface along with responsive elements like stimulus-responsive polymers. This dual-action strategy effectively eliminates adhering bacteria while releasing deceased bacteria and their fragments upon the appropriate stimulus, thereby restoring the surface to cleanliness and ensuring a long-term antibacterial effect.

4.1.2 Antimicrobial. Protein-based antibacterial coatings represent an innovative approach in the field of biomaterials and medical devices to combat bacterial infections.³³⁰ These coatings are designed to inhibit the growth and colonization of bacteria on various surfaces, ranging from medical implants to everyday objects, thereby reducing the risk of infections and associated complications.³³¹ The concept revolves around using proteins with inherent or engineered antibacterial properties to create a protective layer that prevents bacterial adhesion, biofilm formation, and subsequent infection development.³³² Sarmiento *et al.*³²⁵ developed hybrid synthetic–natural water-soluble macromolecules. These macromolecules are assembled autonomously on dressing surfaces, generating an antifouling brush imbued

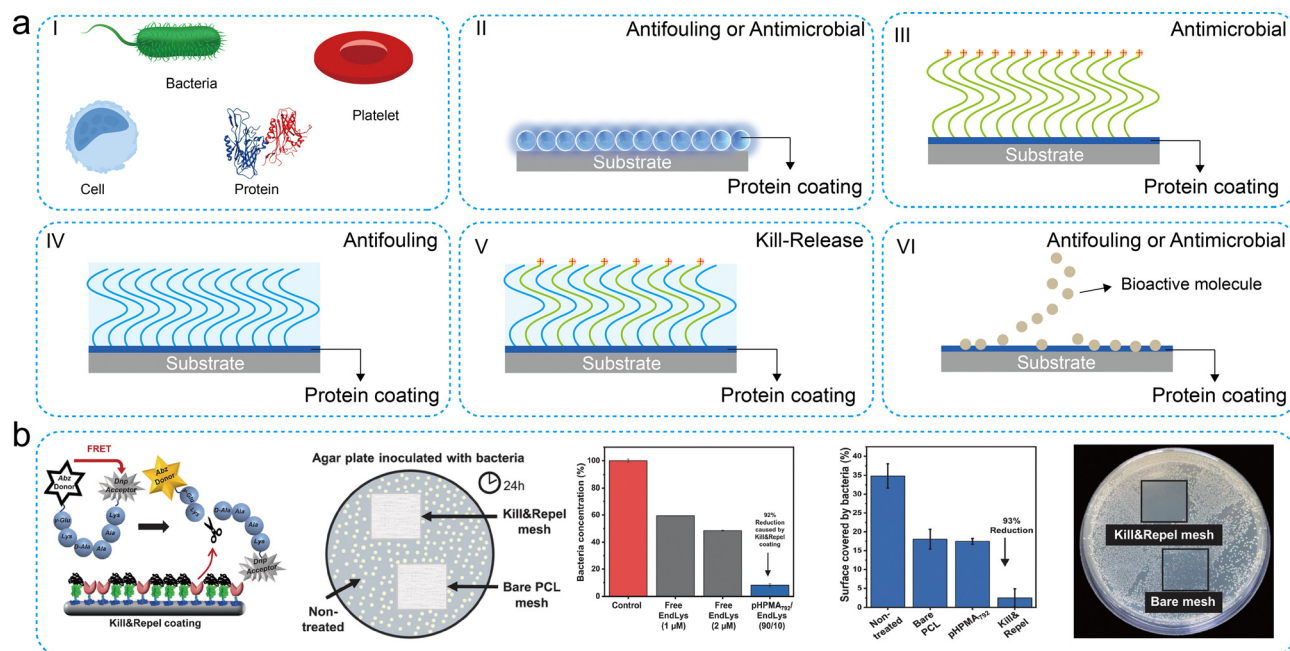


Fig. 11 Construction-types of protein-based bioactive coatings. (a) (I) Common biological contaminants (bacteria, platelets, cells, proteins). (II) Protein-based antimicrobial/antifouling coatings. (III) Antimicrobial coating prepared by coupling protein with cationic polymer. (IV) Antifouling coating formed by the coupling of protein and a hydrophilic polymer. (V) Intelligent "kill-release" coating formed by coupling proteins with block polymers. (VI) Functional coating formed by the protein coating of bioactive molecules. (b) Construction diagram and properties of the "kill and repel" multifunctional coating. Reprinted with permission from ref. 325 Copyright 2021, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

with endolysin (benign bactericidal enzyme). This "kill and repel" coating realized a 93% reduction in planktonic bacterial concentration, outperforming the standalone enzyme. This novel methodology holds great promise for enhanced safety and efficacy in wound infection management (Fig. 11b).³³³

Predominantly, antimicrobial coatings are designed to encapsulate and subsequently release the antimicrobial agent. Gu and Yang *et al.*³³⁴ introduced a series of environmentally friendly antimicrobial coatings derived from phase-transition lysozyme (PTL). These coatings consistently displayed broad-spectrum antimicrobial efficacy *in vitro* and *in vivo* against Gram-positive/negative bacteria and fungi. This pronounced antimicrobial performance was ascribed to the synergistic interplay between positively charged and hydrophobic amino-acid residues. On this basis, a PTL coating can be combined with various functional substances, such as metal ions,³³⁵ polyphosphates,³³⁶ polysaccharides, antimicrobial peptides^{317,337} and others.³³⁸ Lactoferrin is also a natural antimicrobial protein, demonstrating broad-spectrum activity against Gram-positive and Gram-negative bacteria.^{325,339} Wang *et al.*³²⁰ illustrated the employment of fluorinated protein-nanofilm coatings for antibiotic encapsulation aimed at exterminating bacteria on implant surfaces. In fluorinated settings, a thermally triggered protein-denaturation process yielded water-resilient thin films and preserved intrinsic protein-surface attributes such as charge and hydrophilicity.^{294,340,341} These films, post-formation, can be infused with antibiotics. The release rate is modulated through electrostatic interactions between the therapeutic substance and the protein film, culminating in a bactericidal surface.

Unexpected outcomes can also emerge from protein-based antimicrobial coatings.³⁴² For instance, Yang *et al.*³¹⁵ fabricated NaCa₂HSi₃O₉ (NCS) nanorods on titanium *via* micro-arc oxidation and hydrothermal processes. Subsequently, they coated these NCS nanorods with protein layers composed of varying proportions of BSA and lysozyme. This protein layer preserved the structure and phase composition of NCS nanorods, serving as a protective barrier against medium exposure, thereby decelerating their degradation. Consequently, enhanced biocompatibility was observed with MC3T3-E1 cells and human umbilical vein endothelial cells. In addition, the BSA-lysozyme combination imparted potent activities against *S. aureus* and *E. coli*.

4.2 Cell or tissue engineering

4.2.1 Cell adhesion. Protein coatings play a crucial part in facilitating cell adhesion, which is a fundamental process in biology with profound implications for various biological and medical applications.³⁴³ "Cell adhesion" refers to the attachment of cells to surfaces, whether it is other cells, ECM components, or synthetic materials.³⁴⁴ Protein coatings provide a bioactive interface that interacts with cell-membrane receptors, enabling cells to adhere, spread, and interact with their environment. In particular, protein coatings can mimic the natural microenvironment of a cell, regulate cell signaling,³⁴⁵ promote cell-cell and cell-matrix interactions,³⁴⁶ reduce non-specific binding, reduce immune responses, and have designable surface properties.^{347,348} In essence, protein coatings provide a biologically relevant interface for cells to adhere and interact with their surroundings, thereby facilitating studies on cell behavior, tissue engineering, regenerative medicine,

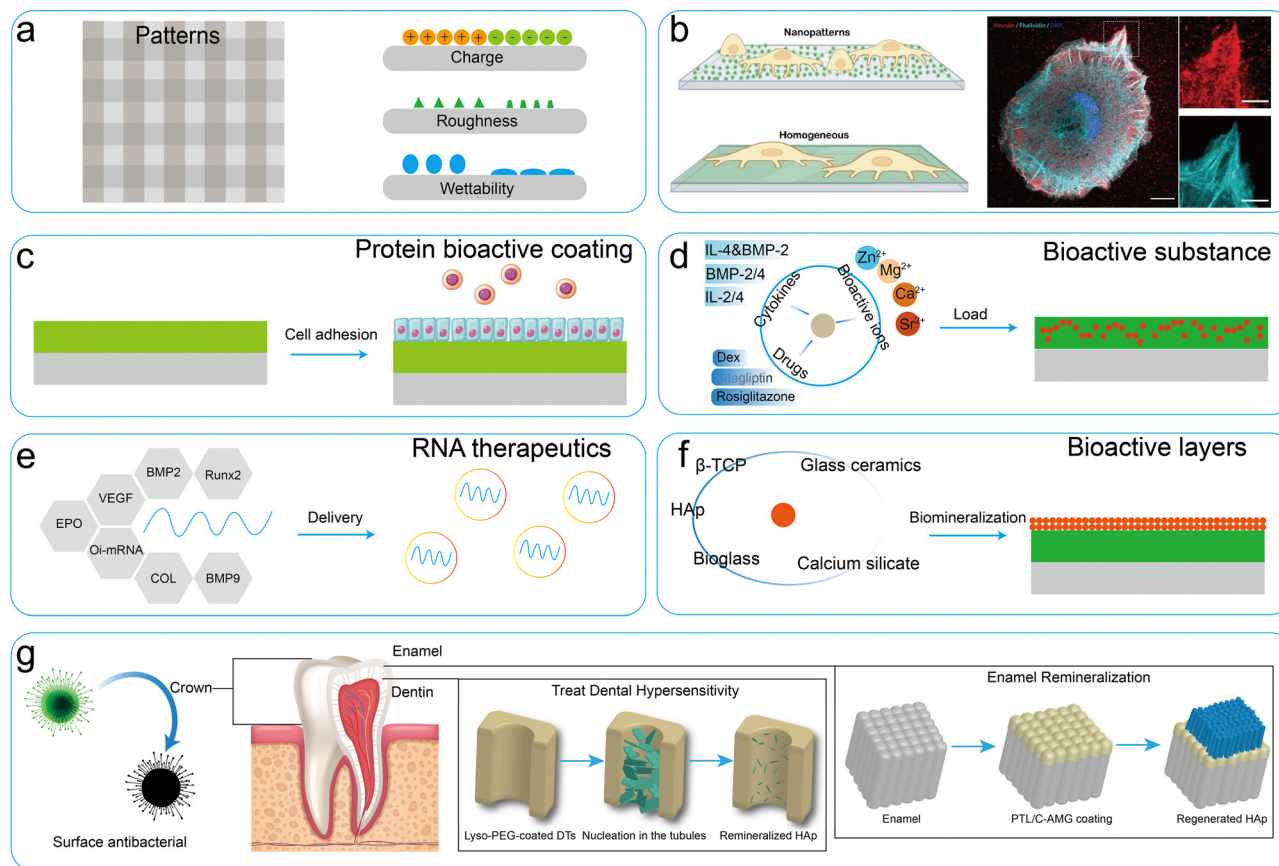


Fig. 12 (a) and (b) Schematic representation of cell spreading and adhesion processes on nanopatterns. Reprinted with permission from ref. 350 Copyright 2022, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (c)–(f) Application of protein-based bioactive coatings in bone tissue engineering. BMP: bone morphogenetic protein; COL: collagen; Runx2: runt-related transcription factor 2; VEGF: vascular endothelial growth factor; EPO: erythropoietin; OimRNA: osteoinductive mRNAs. (g) Application of protein-based bioactive coating in dental medicine.

and the development of medical devices.³²² Their ability to influence cellular responses makes them valuable tools in the field of biology and beyond.²⁹²

The effect of the surface pattern or morphology on cell adhesion influences cell behavior directly, including attachment, spreading, migration, and overall cellular responses (Fig. 12a). The topographical features of a substrate, ranging from the micro- to nanoscale, have a critical role in regulating cell adhesion and subsequent cellular processes.³⁴⁹ Ghorbani *et al.*³⁵⁰ developed nanopatterns using laminin 332 (Ln332) to guide and analyse the formation of hemidesmosomes (HDs) in adherent HaCaT cells. In contrast with cells on homogeneous Ln332 surfaces, which adhere *via* focal contacts/focal adhesions, those on Ln332 nanopatterns adhere through HDs. Notably, $\alpha 6$ integrin clustering is evident on nanopatterned Ln332 patches of size ≥ 300 nm. Cells on patterns with a diameter of 500 nm exhibit pronounced co-localization of $\alpha 6$ integrin with collagen type XVII (ColXVII) or pan-cytokeratin compared with those with a pattern of 300 nm or 1000 nm. This observation suggests a threshold for HD initiation that exceeds 100 nm, with a pattern-size preference for HD maturation. Hence, Ln332 patterning can dictate the choice of adhesion mechanism of a cell, with size influencing the initiation and maturation of HDs. This protein-nanopatterning

approach offers a novel *in vitro* method for investigating the role of HDs in cellular signaling and functionality (Fig. 12b).

In the context of engineering cardiac tissue for myocardial injuries, Wang *et al.*³⁵¹ developed a composite matrix that incorporates silk fibroin (SF) and graphene oxide (GO) to enhance substrate adhesion and guide the differentiation of mesenchymal stem cells (MSCs) into cardiac muscle cells. This matrix, referred to as P-GSF, was created through a process involving patterned SF films and plasma treatment to allow GO nanosheets to adhere to the patterned SF, resulting in a nanostructured surface with linear grooves and GO ridges. The P-GSF film demonstrated excellent protein absorption and desirable mechanical properties. It not only promoted early cell adhesion but also influenced MSC differentiation into cardiac cells, as confirmed by gene expression analysis and immunofluorescence imaging. This innovative approach holds promise for advancing cardiac tissue repair and engineering.^{352,353} Additionally, cell adhesion plays a crucial role in wound healing, and silk protein derived from the wild silkworm *A. pernyi*,^{354,355} enriched with the RGD sequence, can be modified to create wound dressings that enhance cell adhesion and expedite skin recovery (Fig. 12c). When combined with a polydopamine (PDA) layer, the *A. pernyi* silk protein film (PAF) showed significant improvements in cell adhesion, hydrophilicity,

and wound healing. PAF films supported the attachment and migration of mesenchymal stem cells, leading to faster wound healing *in vivo*, characterized by well-structured collagen distribution and minimal inflammation. This research suggests that PDA-coated AF films hold promise as effective wound dressings for skin tissue regeneration, highlighting their potential to promote wound healing.³⁵⁶

4.2.2 Response to foreign bodies. Protein coatings have emerged as a valuable approach to mitigate immune rejection in various biomedical applications, particularly in the context of transplantation and implantation. The immune response triggered by foreign materials can lead to graft rejection and inflammation, which can compromise the functionality of medical devices.³⁵⁷ Protein coatings offer a potential solution by modulating the immune response, mimicking the host's tissues, providing a physical barrier, and inducing tolerance.³⁵⁸ This can significantly enhance the biocompatibility and longevity of medical implants and transplants. As research in protein engineering, biomaterial science, and immunology advances, the application of protein coatings to combat immune rejection holds great promise for the field of transplantation and implantation medicine. A deeper understanding of bone physiology and immunology has shed light on the essential role of the immune response in bone regeneration.³⁵⁹ Immune cells, particularly macrophages, play a central role in regulating inflammation and bone dynamics following the implantation of biomaterials. Protein coatings that modulate the immune response have the potential to influence osteogenesis and osteoclast processes, contributing to improved bone regeneration and implant performance.^{360–362} For example, the titanium surface of a plant body was modified with a protein coating as the intermediate layer for coordination metal ions (*e.g.*, Mg^{2+}) to achieve bone immunomodulation (Fig. 12d).

Tao *et al.*³⁶³ focused on an innovative approach to mitigate immune and inflammatory responses in tissue engineering by integrating an albumin coating, achieved through dopamine modification, onto xenografts, particularly hyaline cartilage grafts. Using porcine chondrocyte-derived living hyaline cartilage grafts (LhCGs) and their decellularized counterparts, the researchers observed a significant reduction in immune and inflammatory reactions at cellular, protein, and gene levels following the application of the albumin coating. This strategy holds promise for improving immune tolerance within the realm of tissue engineering since it led to a decrease in inflammatory cells and a marked reduction in cytokine expression. Building on the theme of reducing pro-inflammatory effects, Dai *et al.*³⁶⁴ demonstrated that protein coronas formed on engineered particles from cell-conditioned media could distinctly alter particle attributes and protein adsorption as compared to those derived from unconditioned settings. They found that these coronas could affect the immune response by influencing the secretion of pro-inflammatory cytokines and the apoptosis of immune cells. This highlights the potential of protein coronas to fine-tune the immune response *in vivo* and reduce biotoxicity. In another study, Park *et al.*³⁶⁵ presented an innovative approach to coating silicon dioxide nanoparticles (SiNPs) with protein layers, which reduced complement activation and macrophage uptake.

Unlike traditional steric-blocking protein coatings, this strategy used functional proteins tailored to specific aspects of complement activation, providing a comprehensive understanding of the mechanisms behind SiNP-induced complement activation and its mitigation *via* protein-based coatings. This approach enhances the immunological performance of SiNPs and can be applied to a broader range of nanoparticles and related fields, reducing immune responses and enhancing biocompatibility.^{366–368}

Recent advancements in protein engineering technology^{221,369} have led to the development of protein-based coatings for implantable biomedical devices,³⁷⁰ addressing foreign-body reactions that can result from biomolecule, microorganism, and cell accumulation on device surfaces. One innovative approach draws inspiration from intrinsically disordered proteins (IDPs) that undergo liquid-liquid phase separation. By crafting a tetra-cysteine tagged FUS IDP sequence, Chang *et al.*³⁷¹ developed a low-fouling biomaterial that forms a densely entangled protein layer, enhancing surface hydration. These IDPFUS-coated surfaces effectively repel protein adsorption, deter cell adhesion, minimize platelet interaction, and impede bacterial attachment. Compared to PEG-coated counterparts, implants with this coating showed a significant reduction in foreign-body reactions, especially in thwarting collagen encapsulation. This method holds promise for improving coatings on biomedical implants, highlighting the potential of using other IDP proteins for similar applications. In a broader context, The utilization of protein-mediated RNA delivery for bone tissue treatment is a promising therapeutic approach.³⁷² Various methods are employed for the delivery of RNA using proteins as carriers.³⁷³ One common method involves the use of carrier proteins or nanoparticles designed to encapsulate and protect RNA molecules, ensuring their targeted delivery to bone tissues (Fig. 12e).^{374–376} These carriers, whether natural bone matrix proteins or engineered counterparts, often incorporate bone-targeting ligands and cell-penetrating peptides to enhance specificity for bone cells. Through self-assembly into nanoparticles, these carriers efficiently transport and release therapeutic RNA, offering a multifaceted strategy for precise and effective RNA therapy in bone-related disorders. Ongoing research focuses on optimizing these delivery methods, aiming to enhance therapeutic outcomes and contribute to advancements in regenerative medicine and bone health.³⁷⁷

4.2.3 Engineering of bone tissue. Protein-based bioactive coatings have gained significant attention in the field of bone tissue engineering due to their versatile applications and potential to revolutionize regenerative medicine.²⁵⁶ Bone tissue engineering aims to regenerate damaged or lost bone tissue using biomaterials, cells, or growth factors (Fig. 12f).³⁷⁸ Protein coatings play a crucial role in this process, offering a range of applications, including enhancing cell adhesion and proliferation, guiding osteogenic differentiation, mimicking the extracellular matrix (ECM), controlling the release of growth factors, preventing immune responses, improving implant integration, functionalizing scaffolds, and delivering therapeutic agents.^{379–381} The application of protein coatings holds great promise for advancing regenerative medicine and addressing bone-related disorders, injuries, and defects. A pioneering approach by Yang *et al.*²⁸⁹ involves the use of engineered curli nanofibrils derived from biofilms to

create multifunctional mineralization coatings on various substrates.³⁷⁸ These coatings utilize a peptide sequence (DDDEEK) to initiate biomineralization, and the engineered *E. coli* excretes this bio-inspired coating. Remarkably, this coating outperforms traditional Hap coatings in terms of mineralization and stability, leading to enhanced cell proliferation, osteogenicity, and osseointegration in animal studies. This innovative method shows significant potential for various biomedical applications and has the potential to revolutionize bone tissue engineering.³⁸²

Organisms have evolved sophisticated cell-mediated biomineralization processes, producing composite materials with structural nuances and “living” characteristics akin to their natural models. Wang *et al.*²⁸⁸ have crafted patterned and gradient composites inspired by natural gradient materials. They achieved this by using light-activated bacterial biofilm in combination with biomimetic Hap mineralization. This approach allows precise control over the placement and intensity of mineralization, maintaining cell viability, environmental adaptability, and dynamic responsiveness. After mineralization, there is a notable increase in Young's modulus, facilitating directed spatial restoration. This strategy provides insights into the formation of natural gradient composites and introduces responsive, malleable living composites. Another critical consideration in developing bioactive composites is enhancing and regulating adhesion at material interfaces. Many synthesized biomaterials lack interface bioactivity due to their inert nature. Ha and Yang^{314,383} pioneered a flexible method for biomimetic mineralization adaptable to virtually any scaffold material, initiating Hap crystal nucleation and growth by employing a polydopamine coating, addressing the challenge of functionalizing diverse materials for optimal biomineralization.

Functional protein coatings on implant surfaces have advanced significantly, with a focus on achieving a balance between antibacterial properties and osteogenic activity while preventing bacterial resistance. Zhou *et al.*³²⁴ developed Ag@AMP/SF coatings for titanium implants, combining antimicrobial peptides (AMPs) and osteogenic fragments. The “crown” structure of the protein on silver nanoparticles (AgNPs) provided a synergistic combination of antibacterial and osteogenic functionalities. These coatings exhibited robust antibacterial effects against *S. aureus* and demonstrated *in vitro* osteogenic potential and *in vivo* osseointegration capabilities when tested with bone marrow stem cells (BMSCs) and implanted in rat femurs. In a related approach, Jo *et al.*³⁸⁴ introduced a protein-infused SiNP coating to enhance osteogenesis on titanium implants by combining a recombinant adhesive protein from mussels with a silica-precipitating peptide (R5-MAP). This resulted in controlled microroughness through SiNP assembly, leading to improvement *in vitro* osteogenic responses and *in vivo* bone tissue development, promising enhanced implant stability and longevity. These innovations represent significant progress in developing implant coatings with dual functionality.

4.2.4 Tooth remineralization. Protein-based bioactive coatings have emerged as a promising avenue for promoting tooth remineralization, a critical process in maintaining dental health and combating tooth decay.³⁸⁵ Remineralization involves the restoration of minerals such as calcium and phosphate to tooth enamel, which helps reverse the early stages of demineralization caused by acids

produced by bacteria. Protein coatings play a significant part in this process because they can influence the interactions between minerals and the tooth surface.³⁸⁶ Protein coatings can provide several benefits that support remineralization: scaffolds for mineral deposition; regulation of mineralization; enhanced adhesion; protection against acid attack; reduced sensitivity; potential for drug delivery.^{387,388} As research in biomaterials and dental science continues to evolve, protein-based approaches to tooth remineralization are poised to have a significant impact on the future of dental care.³⁸⁹

For instance, Wang and Yang *et al.*³¹⁹ devised a functional coating integrating a protein peptide reminiscent of tooth enamel using phase-transition lysozyme as a foundation. This unique coat facilitated the *in vivo* and *in vitro* generation of HAP crystals, leading to the proliferation of HAP crystals, closely mirroring natural enamel (Fig. 12g). This innovative approach presents a viable avenue for intervention against dental caries. Furthermore, a dual-purpose membrane, amalgamating lysozyme with polyphemusin I (PI), aimed to suppress *S. mutans* and bolster tooth remineralization.³¹² Enamel treated with lysozyme demonstrated enhanced PI absorption, resulting in more effective *S. mutans* regulation and biofilm reduction. Such PI-coated enamel safeguarded against calcium depletion but also exhibited superior crystal reformation and a substantial decrease in lesion depth ($72.45 \pm 4.07 \mu\text{m}$) in comparison with the uncoated variant ($93.30 \pm 7.64 \mu\text{m}$) post *S. mutans*-induced demineralization. This multifaceted, biocompatible membrane has potential in thwarting dental caries.

Dental hypersensitivity (DH), marked by brief, acute pain stemming from exposed dentin, affects around 42% of individuals aged 18 to 35.³⁹⁰ Most current desensitizing treatments prove ineffective in addressing DH. These treatments primarily focus on sealing orifices near the DTs within a 10 μm range, rather than achieving deep occlusion. To address the shortcomings of the above-mentioned desensitizers, the rapid amyloid-like transformation of PEG grafted by lysozyme facilitates the formation of an ultrathin nanofilm on the intricate walls of DTs.²⁷¹ This nanofilm acts as an efficient barrier against oral bacteria, notably *S. mutans*, and fosters the *in situ* crystallization of HAP minerals. This action promotes remineralization within DTs, sealing their openings and mitigating dentin hypersensitivity. Biomimetic remineralization mediated by amyloid-like proteins introduces a novel approach for the sealing of pits and fissures. Unlike the traditional approach (which seals from the outside in), this approach delivers amyloid-like protein nanomembranes deep into fissure recesses, thereby driving intrinsic enamel remineralization. Aggregation of amyloid-like proteins could offer a pioneering approach to enamel remineralization by refining clinical-sealing methods and providing significant advancements in caries prevention.³⁸⁶

4.3 Encapsulation and release of drugs

Protein-based bioactive coatings have emerged as a game-changer in the encapsulation and controlled release of drugs. Integrating proteins with drug-delivery mechanisms creates a harmonious blend of biocompatibility, tailored release kinetics,

and therapeutic adaptability. This innovative approach bolsters the stability and protection of encapsulated drugs but also provides granular control over their release trajectories, thereby facilitating targeted and prolonged therapeutic actions.³⁹¹ The inherent adaptability of protein coatings enables them to be tailored to specific clinical requirements, thereby cementing their role in advancing safer and more efficacious drug-delivery modalities.³⁹² As investigations deepen into protein–drug synergies and coating optimization, bioactive coatings are poised to reshape pharmaceutical interventions, heralding a new wave of “personalized” and enhanced treatments.

The strategic application of coatings to medical-device surfaces, such as drug-eluting stents,³⁹³ external fixators,³⁹⁴ and implants,³⁸⁴ is a powerful method for the localized delivery of therapeutic drugs. For instance, urethral strictures can develop post-urethral injury due to abnormal extracellular matrix (ECM) buildup in submucosal and periurethral tissues. To tackle this issue, a “sandwich” nanofilm composed of amyloid-like protein and polysaccharide was created, providing resistance to bacterial biofilms and serving as a drug storage system for extended release over tens of days.³¹⁶ This innovation endows catheters with anti-fibrotic and anti-biofilm capabilities, with the potential for scalability to other implants. Impressively, the anti-fibrotic catheter significantly reduced the required rapamycin dose by 79.2%.³⁹⁵ Additionally, the layered multi-scale assembly of proteins and polysaccharides allows for the creation of versatile functional capsules, films, and fibers,²⁶⁸ with broad applications for drug encapsulation and delivery.³⁹⁷ In summary, the integration of protein coatings onto therapeutic contact lenses, as demonstrated by Qin and Yang,²⁶⁶ enables the active loading and controlled release of drugs, leading to a substantial increase in bioavailability. This marks a significant advancement in the use of amyloid-like coatings in the medical field. In an innovative approach, Wang *et al.*³⁰⁵ employed LbL assembly methods to create silk sutures with antibacterial and anti-inflammatory properties. These sutures, designed for continuous dual-drug delivery, effectively prevent surgical-site

infections. Coated with berberine and artemisinin, they not only deter initial biofilm formation but also maintain their antibacterial and anti-inflammatory functions over a 42 day period. Experiments in rats demonstrated the coating's ability to suppress pro-inflammatory cytokines, shorten the inflammatory phase, and promote angiogenesis. With their strong structure, optimal biocompatibility, and persistent antibacterial and anti-inflammatory properties, these innovative sutures hold significant potential for a wide range of surgical applications. NPs offer promise in targeted drug delivery, potentially reducing side effects and enhancing drug efficacy. However, the clinical application of NP-based therapeutics faces challenges in regulating interactions between NPs and biological systems. Oh *et al.*³⁹⁶ developed NPs that bind non-specifically to recombinant fusion proteins, reducing interactions with serum proteins. These modified NPs showed targeted delivery both *in vitro* and *in vivo* while evading macrophage clearance (Fig. 13). Another approach involved using chitosan-gold NPs enveloped in a silk fibroin (SF) coating to encapsulate and gradually release doxorubicin, achieving sustained drug release.³⁹⁸ In summary, NPs enhanced with protein modifications offer a potent avenue for precise and sustained drug delivery.

4.4 Biocatalysis

Protein-based bioactive coatings have risen to prominence in biocatalysis, ushering in a novel methodology that amplifies enzymatic activities across different applications.³⁹⁹ Comprising specialized proteins, these coatings imbue surfaces with distinct catalytic attributes, thereby markedly modulating reaction kinetics, selectivity, and stability. Within biocatalysis,⁴⁰⁰ they act as custom microenvironments that enhance enzyme–substrate interactions.⁴⁰¹ Leveraging the innate specificity of proteins, these coatings refine substrate binding, leading to efficient enzymatic conversions. Beyond traditional biocatalysis, the relevance of protein-based bioactive coatings permeates diverse fields such as pharmaceuticals, green chemistry, and sustainable energy. They offer a versatile foundation for enzyme immobilization,⁴⁰²

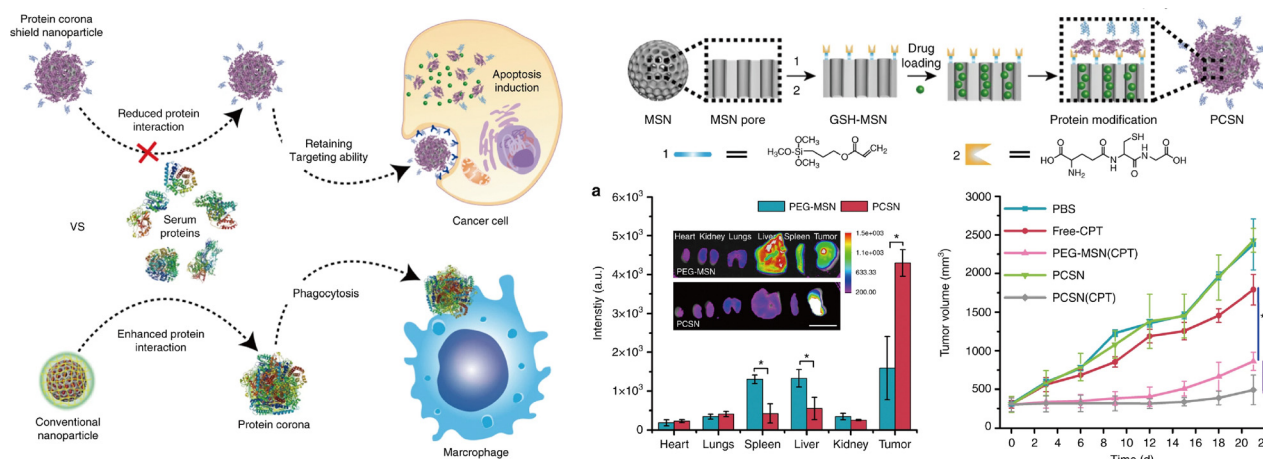


Fig. 13 Cloaking nanoparticles with a protein corona shield for targeted drug delivery. Reprinted with permission from ref. 396 Copyright 2018, Nature Publishing Group.

prolonging their functional lifespan and streamlining recovery processes. In addition, these coatings facilitate the formation of complex enzyme cascades and versatile biocatalytic systems, thereby broadening the horizons of synthetic transformations.^{403–405}

Männel *et al.*⁴⁰⁶ immobilized enzymes onto gold and iron oxide NPs *via* physical adsorption to produce catalytically active and stable NP systems. The robustness and activity of enzyme coatings depend on pH and specific enzyme properties. A direct relationship exists between colloidal stability and enzymatic performance, which facilitates enzymatic reactions at the colloidal level for near-homogeneous catalysis. Enzyme-coated NPs support inter-colloidal enzymatic cascade reactions, offering selective catalyst separation and opening avenues for novel design approaches (Fig. 14a). Beyond this inter-colloidal enzymatic cascade, amyloid fibrils can be optimal nanoscaffolds for enzyme immobilization in sustainable industrial applications. Boasting a wealth of exposed reactive groups, amyloid fibrils enhance enzyme stability against heat and acidic environments and bolster the reusability of biocatalysts as compared with their unbound counterparts.⁴⁰⁷ Beyond adsorbing enzymes on the surface of metal NPs, amyloid fibrils (particularly those composed of non-toxic, food-grade proteins with a wealth of exposed reactive groups) serve as exemplary nanoscaffolds for enzyme immobilization in sustainable industrial applications. Due to their proteinaceous composition, amyloid fibril coatings exhibit remarkable stability, positioning them as a prime choice as biocompatible nanoscaffolds. Such an immobilized

biocatalyst system might also facilitate direct phase separation in aqueous or aqueous/organic solvent reaction mixtures, thereby streamlining the recycling and recovery of enzymes and product purification.

Amyloid fibrils, if grafted covalently with laccase, can degrade rhodamine dye. A consistent rate of dye degradation exceeding 90% was observed across nine 24 h incubation cycles, with the resultant solution transitioning from a deep blue to a pale pink hue (Fig. 14b). During a catalytic cycle, acetosyringone is oxidized by laccase into free radicals, which then diffuse from the surface. Subsequently, these free radicals are transferred to rhodamine dye, whereupon the oxidized mediator reverts to its original form, catalyzing the degradation of rhodamine dye. Amyloid fibril coatings, serving as nanoscaffolds, can be functionalized through the covalent binding of enzymes. Such functionalization allows for significant dye removal and repeated use with minimal degradation. These findings support a general approach to multifunctional surface modification using amyloid proteins.⁴⁰⁸

4.5 Biosensing

4.5.1 Patterned printing. Patterning technology is experiencing significant advancements with the incorporation of protein-based coatings.⁴⁰⁹ Derived from the intricate nuances of biology, these coatings are set to redefine the precision and sophistication of printing methods. Capitalizing on the unique specificity of protein interactions, these coatings facilitate the

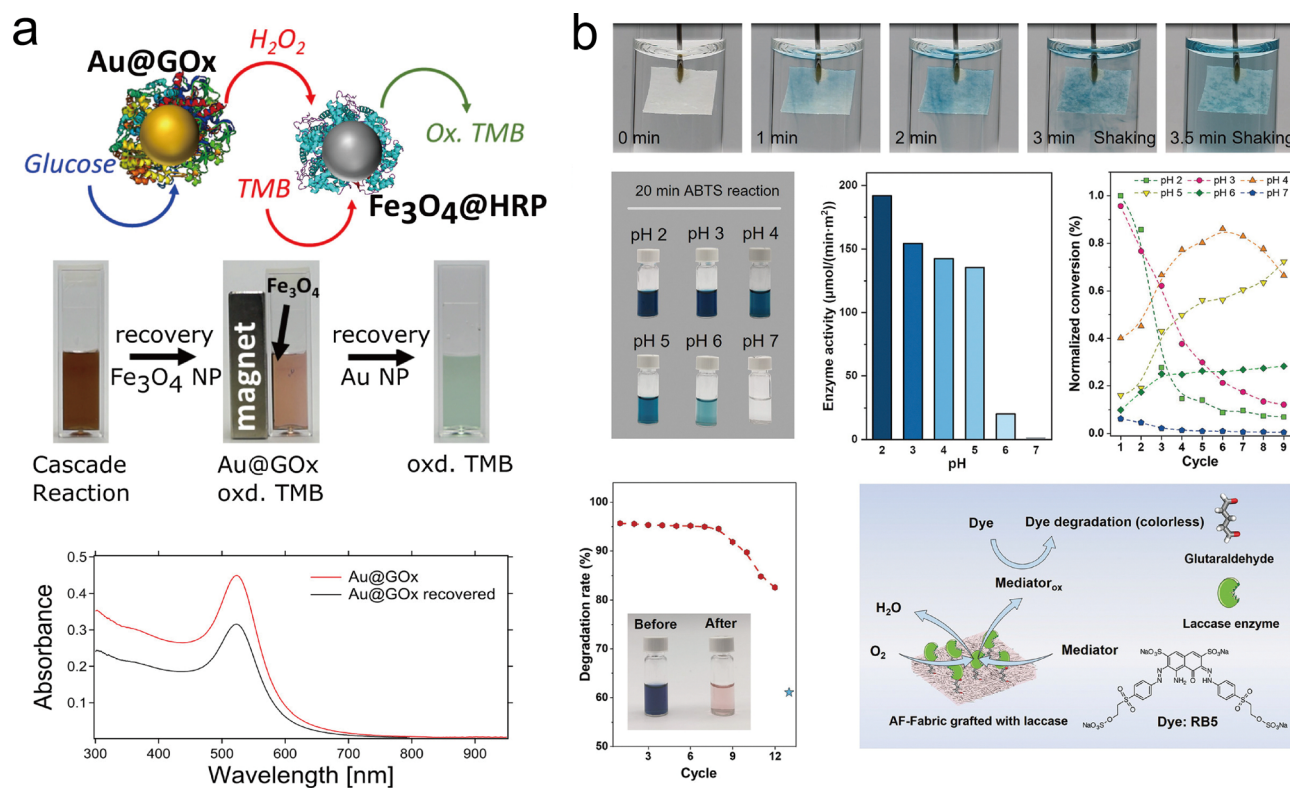


Fig. 14 (a) Enzymatic cascade reaction of Au@GOx NPs and Fe₃O₄@HRP NPs. Reprinted with permission from ref. 406 Copyright 2017, American Chemical Society. (b) Biocompatible performance of a laccase-grafted AF-fabric for enzyme catalysis. Reprinted with permission from ref. 408 Copyright 2022, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

generation of sharply defined patterns with meticulous spatial arrangement. Such innovations herald a range of potential applications, spanning tissue engineering to microfabrication, and provide a novel framework for conceptualizing functional surfaces. At this pivotal juncture, the amalgamation of protein-infused bioactive coatings with cutting-edge patterning methodologies could redefine the contours of additive manufacturing. Anticipation for the future encompasses unmatched intricacy, adaptability, and tailor-made capabilities in printed architectures, anchored by the harmonious convergence of biomolecular precision and print technology. Molecular printing, characterized by the exact deposition of molecules onto micron-scaled surfaces, has recently witnessed advancements. Micropatterned substrates are now a staple in biological research.⁴¹⁰ Strale *et al.*⁴¹¹ elucidated the application of light-induced molecular adsorption (LIMAP) for protein micropatterning. LIMAP deploys a water-soluble photoinitiator capable of altering the antifouling properties of polymer brushes *via* a photocleavage mechanism activated by exposure to near-UV light (Fig. 15a). This method furnishes a flexible methodology for the swift, high-resolution patterning of an array of biomolecules, marking a significant stride toward facilitating comprehensive, high-throughput studies of biomolecular interplays.

Most surfaces in specific applications remain static or possess “on” and “off” states. The development of reconfigurable surfaces that can adapt dynamically to swiftly evolving environments or needs remains a complex endeavor. Various photolysis and photocoupling reactions have been explored to modulate surface functions.^{412–414} Xie *et al.*⁴¹⁵ employed a

rubidium complex ($\text{Ru-H}_2\text{O}$) to modify substrates. To impart a specific functionality to the $\text{Ru-H}_2\text{O}$ -modified substrate, a functional thioether ligand was affixed to the substrate *via* Ru -thioether coordination. Modifying the surface function of the substrate involved detaching the affixed thioether ligand through visible light-induced ligand dissociation. This action was followed by the attachment of an alternative thioether ligand possessing a unique function. Introducing different thioethers resulted in different functional attributes on the surface. The use of this method allows for the reshaping of surface patterns, regulation of protein adsorption, and alteration of surface wettability. This pioneering approach paves the way for a generation of versatile surfaces with functionalities tailored to distinct demands. Green-light lithography provides a non-invasive approach to the meticulous micropatterning of functional proteins. Xu *et al.*⁴¹⁶ introduced a method to photo-pattern in an oriented fashion using green light within LbL multiprotein films. Leveraging the method of LbL multiprotein films, proteins of varying functions can be integrated systematically into distinct layers and tailored to specific applications. Green-light lithography can be employed to craft intricate protein patterns of diverse functionalities, offering promise for sophisticated endeavors in biotechnological research. The protein configurations crafted *via* this method are compatible with cell culture, especially photopatterned fibronectins, which facilitate the spatial modulation of cell adhesion. This method is a flexible and accurate means to micropattern histidine-tagged proteins in a directed fashion. This approach affords outstanding spatiotemporal control with non-invasive green light, thereby ensuring the retention of protein function.

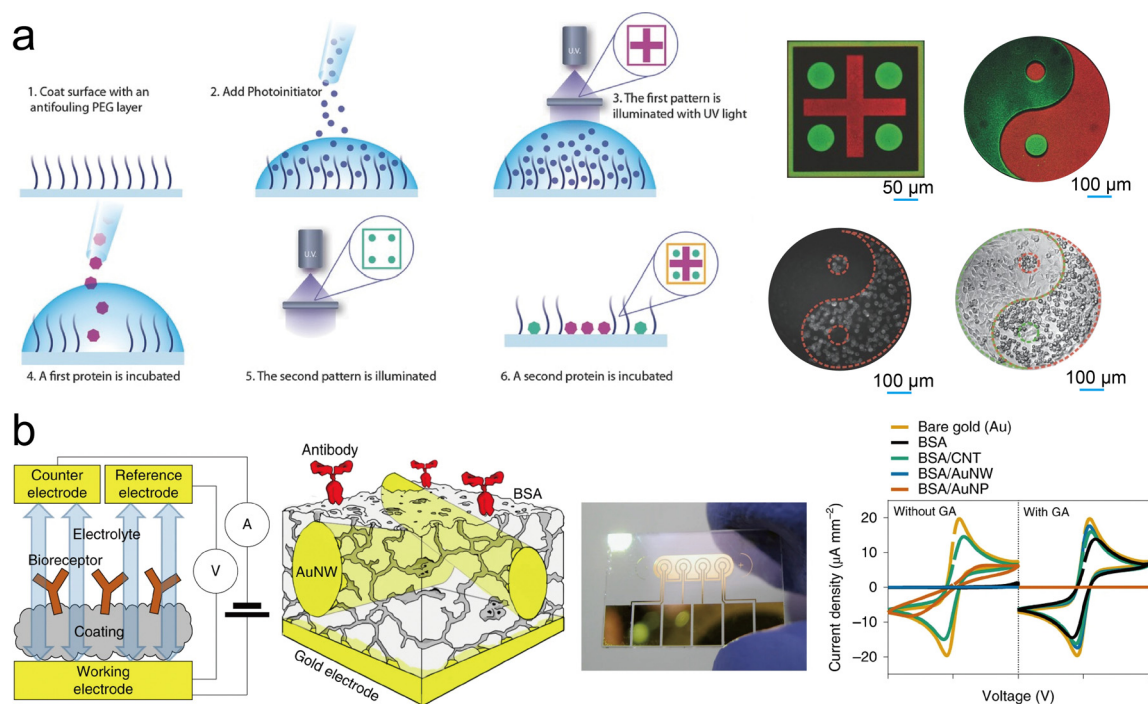


Fig. 15 (a) Schematics of the LIMAP patterning principle. Reprinted with permission from ref. 411 Copyright 2016, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (b) Schematic illustration of a protein-based antifouling coating that enables affinity-based electrochemical biosensing in complex biological liquids. Reprinted with permission from ref. 419 Copyright 2019, Nature Publishing Group.

4.5.2 Detection and sensing of biological molecules. The integration of protein-based bioactive coatings into the detection and sensing of biological molecules offers substantial potential for enhancing diagnostic and analytical capabilities. These coatings, celebrated for their unparalleled biorecognition attributes, provide a dynamic platform for devising sensitive and selective biosensors. Capitalizing on the natural affinity of proteins for distinct targets, this approach facilitates the development of rapid and precise detection instruments, essential in domains spanning from medical diagnostics to environmental surveillance.⁴¹⁷ The convergence of protein coatings with state-of-the-art sensing technologies lays the foundation for real-time, label-free, and multiplexed assays, thereby transforming the understanding of intricate biomolecular interactions.⁴¹⁸ The prospect of using affinity-based electrochemical detection within complex biological fluids can advance multiplexed point-of-care diagnostics, amplifying the viability of domestic healthcare solutions. Sabaté del Río *et al.*⁴¹⁹ integrated BSA with conductive nanomaterials such as gold nanowires, gold nanoparticles, and carbon nanotubes to engineer a 3D antifouling nanoelectrode coating. This innovative coating curtailed nonspecific interactions, thereby enhancing electron transfer to the electrode interface. Remarkably, it retained 1% of its initial signal after an extended (88-month) exposure to untreated human plasma. When augmented with specific antibodies, the coating facilitated the precise quantification of anti-IL6 in plasma (Fig. 15b).

Timilsina *et al.*⁴²⁰ combined crosslinked BSA coatings on electrochemical sensors with conductive pentaamine-modified graphene nanoflakes. This advanced integration led to unprecedented sensitivity and selectivity, thereby positioning these sensors for diagnostic endeavors.⁴²¹ Through a simple dip-coating method, the antifouling coating was affixed seamlessly to the chip, markedly enhancing electrode conductivity within untreated biological specimens. Impressively, this method demands only 15 μL of blood to identify clinically pertinent biomarkers. In minutes, sensitivities up to single-digit pg mL^{-1} can be attained in raw human plasma and whole blood, outperforming conventional enzyme-linked immunosorbent assays by a minimum factor of 50 in terms of rapidity and sensitivity. Furthermore, the elicited signal retains its stability and can be quantified even after 1 week in storage. Unlike before, electrodes modified with lubricin-reduced GO serve as size-discriminant molecular sieves to filter small analytes from larger contaminant molecules at the electrode–fluid boundary.⁴²² By mitigating protein contamination, these electrode coatings enable the precise and immediate measurement of clonazepam concentrations in untreated saliva. The employment of this electrode coating for the acquisition of swab samples presents an affordable and practical biosensing methodology to gauge electroactive drug levels in saliva within point-of-care contexts.

5. Conclusion and perspective

In this review, we have summarized recent advancements in protein-based active coatings. As a subset of protein materials,

the preparation and application of these coatings rely on (or are inspired by) the properties of natural functional proteins. Our exploration began with a brief study of functional protein layers occurring naturally within organisms. This section of the review serves a dual purpose. First, it underscores the pivotal roles that surface proteins have in various biological systems, illuminating their importance and versatility. Second, it provides a foundation for understanding how these natural phenomena can inform and enhance synthetic efforts in creating protein-based coatings. To that end, we delved into a detailed exposition of the strategies used to prepare such coatings. From physical adsorption and chemical binding to protein assembly, each method offers unique benefits and potential applications. This part of our review sought to dissect these methods, providing insights into their mechanisms, strengths, and areas of potential improvement. Subsequently, we shifted our focus toward the application of protein-based active coatings in a range of sectors. By examining fields as diverse as biomedical engineering, tissue engineering, catalysis, and biosensors, we highlighted the versatility of these coatings and their potential to promote multiple sectors.

Proteins have these unique advantages, but there can also be challenges in using proteins for bioactive coatings. Given their nature as soft matter, their stability (especially under varying conditions) is an area of concern. The vulnerability of protein-based coatings to factors like degradation, denaturation, or desorption cannot be overlooked, particularly if they are exposed to aggressive environments or subjected to frequent use. This becomes even more critical when considering coatings for medical implants, where longevity and reliability are paramount. Addressing this concern, research is pointing toward manipulating protein assembly. For instance, amyloid aggregates have demonstrated resilience due to their inherent low energy. This characteristic equips them to resist environmental stressors, be it pH changes, enzymatic interactions, or mechanical forces, making them promising candidates for durable coatings. Thus, deepening the comprehension of inherent protein properties is crucial for advancing the development of protein-based active coatings. Another challenge in protein-based coatings is the intricacies of large-scale production. Ensuring consistent bioactivity across different batches requires rigorous optimization, standardization, and quality assurance. Such production dynamics demand state-of-the-art methods, but also exacting standards with respect to the production, purification, and subsequent characterization of proteins. These stages, if handled with precision, ensure the effectiveness and safety of the final product. Moreover, the ever-evolving landscape of protein engineering and bioconjugation offers promise. With advancements in these fields, we are witnessing an expansion in the applications of proteins in designing bioactive coatings, broadening their scope and potential impact. However, as we chart this promising terrain, we must also be vigilant about potential biocompatibility issues. Protein coatings, given their biological origins, must be tested meticulously to ensure they do not trigger adverse immune or allergic reactions in users. In conclusion, while the journey of harnessing proteins

for bioactive coatings is lined with challenges, it is also rife with opportunities. As research progresses and technology evolves, we move closer to realizing the full potential of these coatings, heralding a new era in biocompatible and effective applications.

The future of synthesis and preparation methods for protein-based bioactive coatings in chemical biology holds great prospects for advancing diverse applications. Research in this domain is anticipated to focus on refining existing methods and innovating new techniques to enhance the efficiency, reproducibility, and scalability of these coatings. Integration with emerging materials like nanomaterials, biopolymers, and smart materials is likely to be a key avenue of exploration, aiming to create coatings with superior properties such as enhanced mechanical strength, durability, and responsiveness to environmental stimuli. The synergy of proteins with existing composite materials and the development of novel synthesis approaches will contribute to expanding the versatility of these coatings. Protein-based bioactive coatings, positioned at the convergence of biochemistry and materials science, form a dynamic field with extensive applications and transformative implications. This exploration provides a comprehensive overview while delving into emerging trends and directions.

(1) Biomedical applications and smart coatings: in advanced biomedical applications, these coatings exhibit substantial promise, evolving into “smart coatings.” These coatings not only enhance biocompatibility and tissue integration but also feature controlled release properties. This innovation holds the potential to revolutionize regenerative medicine and drug delivery.

(2) Personalized medicine and precision diagnostics: as personalized medicine gains traction, protein coatings can play a pivotal role in crafting patient-specific medical devices, minimizing rejection risks. In precision diagnostics, future iterations are anticipated to excel in biomarker capture and detection, offering multiplexing capabilities for simultaneous assessment of multiple disease indicators, particularly in oncology, infectious diseases, and chronic conditions.

(3) Environmental applications: beyond healthcare, protein-based coatings show promise in environmental protection. Specifically, designed coatings can reduce the environmental impact of shipping and offshore industries by preventing fouling. Moreover, these coatings can be adapted for efficient pollutant removal in water treatment systems, potentially transforming wastewater management.

(4) Renewable energy innovations: protein coatings contribute to innovations in renewable energy technologies, enhancing the efficiency of solar cells and biofuel cells. This application holds significant potential for sustainable energy solutions.

(5) Scalability and collaborative efforts: with research progress, scalability becomes crucial. Future directions should prioritize cost-effective, large-scale manufacturing methods for widespread adoption across industries. Collaboration with industry partners is essential to translate research findings into practical applications, preparing for the commercialization of these coatings.

(6) Challenges and considerations: despite immense potential, challenges persist. Specificity, stability, ethical considerations, and

scalability issues demand meticulous attention. Integrating protein-based coatings with emerging materials, including nanomaterials and smart materials, holds the potential to address these challenges.

(7) Interdisciplinary collaboration: interdisciplinary collaboration is imperative as protein-based coatings venture into uncharted territory. Biochemists, materials scientists, engineers, and medical professionals must synergize efforts to unlock the full potential of these coatings. Protein-based bioactive coatings are poised to introduce innovative solutions with far-reaching implications in healthcare, diagnostics, environmental sustainability, and materials science. Their versatility and potential for personalization indicate a future where medical implants are seamlessly integrated, diagnostic tests provide rapid results, and environmental challenges are effectively addressed. However, overcoming challenges related to specificity, stability, ethics, and scalability is crucial to realizing the full potential of protein-based coatings, ensuring an exciting and impactful future.

Conflicts of interest

The authors declare no competing interests.

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