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Perspective

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Engineering complexity into protein-based biomaterials for biomedical applications

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Abstract

Protein-based biomaterials are growing in popularity for biomedical applications, in part owing to their innate ability to interface with biological systems. These materials, in the form of fibres, nanoparticles and hydrogels, have shown promise as drug delivery vehicles, tissue scaffolds and vaccines. Moreover, the explosion of protein engineering tools and the inception of de novo protein design have transformed our ability to explore new protein structures, enabling the creation of novel materials with diverse properties and furthering their customization for various applications. In this Perspective, we explore the coming of age of protein engineering technologies and their impact on biomaterials. Starting with naturally sourced materials, we highlight common protein building blocks and fabrication methods, as well as recent applications of each. We subsequently explore rationally designed materials and conclude by discussing the potential impacts that de novo design will have on the biomaterials field.

Sections

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Introduction

Biomaterials have played a pivotal role in transforming medicine and biomedical research. Originally, the use of materials in medicine focused on wound healing (for example, sutures or staples) and implants (including heart valves, artificial joints or dental implants)[1]. However, progress in biomedical research has enabled the development of new approaches to disease prevention and treatment, facilitated by novel biomaterials such as nanoparticles and hydrogels[2,3].

In particular, protein-based biomaterials have grown in number and popularity in the past three decades, making their way into biomedical research as an alternative to traditional synthetic polymeric systems. The increasing use of protein-based materials in health applications can be partially attributed to their chemical and structural similarity to naturally occurring biological structures[4,5], properties that are difficult to emulate with purely synthetic species[3]. In native tissue, cells are surrounded by a complex milieu of proteins (among other biomolecules) and as such are innately equipped to interact with them. Proteins are often naturally biocompatible, inherently bioresorbable and the by-products of their degradation are generally harmless[5]. These properties are highly desirable when considering materials for use in medicine.

The first protein-based biomaterials were taken directly from nature and included silk, collagen and elastin. These biomaterials have proven over the years to be key components in many biomedical applications[6,7]. However, our relatively newfound ability to manipulate proteins using simple molecular biology techniques has led to the explosion of rationally designed protein biomaterials. Through a combination of primary amino acid sequence modifications and fusion proteins, we now have entirely protein-based biomaterials that respond to stimuli including light[8–10], temperature[11], pH[12], redox conditions[13], presence of metallic species[14–16] and more[17]. These materials have, in turn, been used in a wide range of applications, including as tools to enable a better understanding of cell biology through model systems, as vehicles for vaccination[18–20] and as improved delivery platforms for small molecule-based[21–23], protein-based[24,25] and cell-based[26,27] therapies.

We now stand on the cusp of another major milestone in protein engineering with the rise of computational de novo protein design[28]. By combining tools such as AlphaFold[29,30] for structure prediction, ProteinMPNN[31] for sequence design and RoseTTAFold diffusion[32] for backbone generation (Box 1), we now have the power to design new-to-nature proteins and access protein structures that previously existed only in the imagination of protein engineers. Although de novo protein design is in many ways still a nascent field, its successes so far have allowed us to envision how it will impact the future of many fields, including protein-based biomaterials.

In this Perspective, we outline how the development of protein-based biomaterials, from naturally occurring systems to rationally engineered constructs, and most recently, to de novo designed proteins, has expanded the strategies available for designing biomaterials and enabled new biomedical applications. We explore how each approach addresses limitations of the last, and how emerging tools are creating new possibilities for protein-based biomaterial design. To support the breadth of potential biomedical applications, we consider various material structures, including 1D and 2D structures (such as fibres and layers), finite 3D systems (such as nanoparticles) and macroscopic 3D materials (such as hydrogels) (Box 2). We begin with a discussion on biomaterials that have been developed using proteins from nature

Box 1 | Key computational protein engineering tools

Structure prediction

The initial release of AlphaFold2 (ref. 29) in 2021 has revolutionized protein structure prediction. AlphaFold2 is a machine-learning-based software trained on experimentally determined structures from the Protein Data Bank (PDB). A central principle of the model is the use of multiple sequence alignments, in which many sequences are aligned and compared with one another, to detect statistical dependencies between amino acid positions and convert these to distance constraints[29]. This information is combined with protein templates, or fragments of protein backbone that are similar across a set of proteins[29], and fed into a neural network that then predicts the 3D structure of a given protein directly from its sequence. The subsequently released AlphaFold3 (ref. 30) uses a similar architecture but with improved prediction accuracy and the ability to include predictions with non-protein components such as DNA or glycans. Other software, namely, ESMFold[216] and RoseTTAFold[220], also use machine-learning-based systems to predict protein structure, each emphasizing different balances of speed, flexibility and modelling depth.

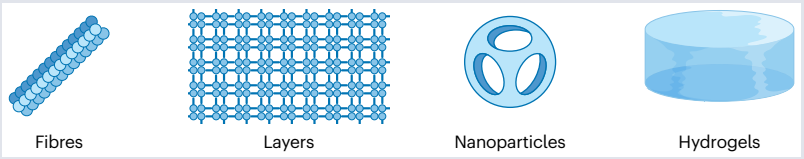
Sequence design

Engineering the optimal sequences for a given protein interaction or a protein backbone has always been a challenging task. With new tools, it has become much faster and easier to determine a robust set of residues that will best fit a given backbone. ProteinMPNN[31] is one machine-learning-based software that was trained on structures found in the PDB. In contrast to previous methods that use energy optimization to optimize a sequence, ProteinMPNN is based on a graph-based neural network[31]. Given a 3D protein structure, ProteinMPNN determines spatial relationships between residues and predicts optimal sequences to fit the backbone. Other deep-learning-based models, such as ThermoMPNN[221], ESM-IF1 (ref. 222) and ProGen2 (ref. 223), have also been developed for sequence prediction, each leveraging different combinations of stability metrics, structural context and sequence diversity.

De novo backbone generation

Many of the tools described previously require an existing protein backbone, scaffold or interaction for proper use. Designing new backbones not seen in nature is challenging owing to the vast number of possible structures. Historically, the most successful attempts have been those that use the Crick equations[197] to design relatively simple coiled-coil structures. With the development of RFdiffusion[32] and Chroma[224], tools now exist for freely generating new protein backbones. Such software typically uses diffusion models for protein structure that can denoise a noised protein backbone. They are trained on proteins found in the PDB and can accurately generate new proteins of various shapes and oligomeric states freely or with programmed constraints[32,224].

Box 2 | Material types



Fibres and tubes
Fibres and tubes are solid or hollow cylindrical structures where the length is much longer than the width. Widths can vary from several tens to hundreds of nanometres, and lengths from tens of nanometres to micrometres. In some cases, macroscopic materials are formed from such fibres to be used as highly porous tissue engineering scaffolds, although many applications primarily focus on drug delivery.

Layers
Layers are large arrays of lattice-like structures composed of regularly repeated geometric patterns. It is often difficult to define specific boundaries for these structures and their morphology can be quite heterogeneous even within a single sample. Most layers and sheets can be several thousand square nanometres in area, and many assemble onto specific surfaces, such as cellular membranes or other solid metals and polymers. These structures have been used as platforms for diagnostics and have potential for use as therapeutics.

Nanoparticles
Nanoparticles are fully bound protein assemblies that may or may not contain pores on the surface and are most often hollow. They typically form regular shapes of well-defined size and architecture, ranging from several nanometres in diameter to very large assemblies, more than 100 nm large. Display of antigens and other binding proteins on nanoparticle surfaces is typically required for many applications from drug delivery to vaccines.

Hydrogels
Hydrogels are macroscopic hydrophilic networks that absorb a large amount of water, resulting in material properties reminiscent of biological tissues. Material properties, including stiffness, viscoelasticity and degradation rate, can be selected through protein design and formulation and are often tailored to a given application. Hydrogels are most often utilized as tissue engineering scaffolds and as vehicles for controlled therapeutic delivery.

without modification to their amino acid sequences, termed 'natural protein-based biomaterials' (Fig. 1a). We then move on to discuss 'rationally designed protein-based biomaterials' (Fig. 1b), in which the source proteins are modified through point mutation, directed evolution, interface redesign or other methods to achieve specific properties or function. Finally, we cover 'de novo biomaterials' (Fig. 1c), composed of proteins with structures and sequences not previously observed in nature.

Natural protein-based biomaterials

Early approaches to forming protein-based biomaterials involved the use of natural proteins. Here, we define 'natural' protein-based biomaterials as ones whose primary sequence has not been modified by point mutation, directed evolution or computational methods. Despite their unmodified sequences, these materials can still be altered to provide application-specific functionality, by removing segments of their native sequence, through simple fusion with other native proteins or through chemical modifications[7,33]. Although more advanced approaches have broadened the scope and translational potential of protein-based biomaterials, this simpler approach can still be effective for treating and preventing diseases without directly relying on protein engineering tools.

Fibres and layers

A small number of naturally occurring proteins, including α-lactalbumin, COMP coiled-coil domain, lysozyme, SP1, PduA/B, pilin, flagellin and some viral coat proteins[34–41], can undergo self-assembly to form nanotubes or fibres, either on their own or through the addition of enzymes or small molecules. However, most natural proteins require additional engineering to obtain desirable fibre structures[5]. One widely used method involves spinning, in which protein solutions are converted into fibres through various processes, including applying a voltage to draw the solution into fine fibres (electrospinning)[42], extruding the solution and allowing it to dry out into fibres (dry spinning)[43] or inducing fibre formation in a liquid coagulation bath (wet spinning)[44] (Fig. 2a). Unfortunately, many of these approaches use solvents that may pose risks for biological applications, and the resulting fibres may lack the mechanical strength and other necessary characteristics for translation[45,46]. Electrospun collagen fibres, for instance, provide great similarity to the extracellular matrix (ECM) but are inherently soft, which can limit their usability outside compliant tissue applications or can require further processing — such as chemical crosslinking — to stiffen the material[47]. Although variations such as microfluidic spinning, where fibres are formed in liquid microchannels, could provide finer control over fibre structure, limitations in achieving desired biomaterial characteristics remain[48].

Nanotube structures can also be fabricated from natural proteins that would not otherwise self-assemble, such as human serum albumin, glucose oxidase, haemoglobin or collagen, through a process known as layer-by-layer assembly[49–51] (Fig. 2b). Here, alternating layers of oppositely charged proteins and poly amino acids are deposited within a template structure that is subsequently degraded. Nanotubes are of particular interest for drug delivery applications, as therapeutics can be loaded into their hollow cores using capillary forces, electrostatic interactions or specific molecular interactions such as affinity tags[49].

There are also some examples of native 2D protein layers, most prominently the surface (S)-layer protein lattices found in many prokaryotes. These proteins have the intrinsic ability to self-assemble into large, higher-order assemblies, often with regular square or hexagonal symmetries[52]. S-layer proteins can also be obtained through recombinant expression, whereby the corresponding genes are introduced into host organisms to produce the proteins artificially.

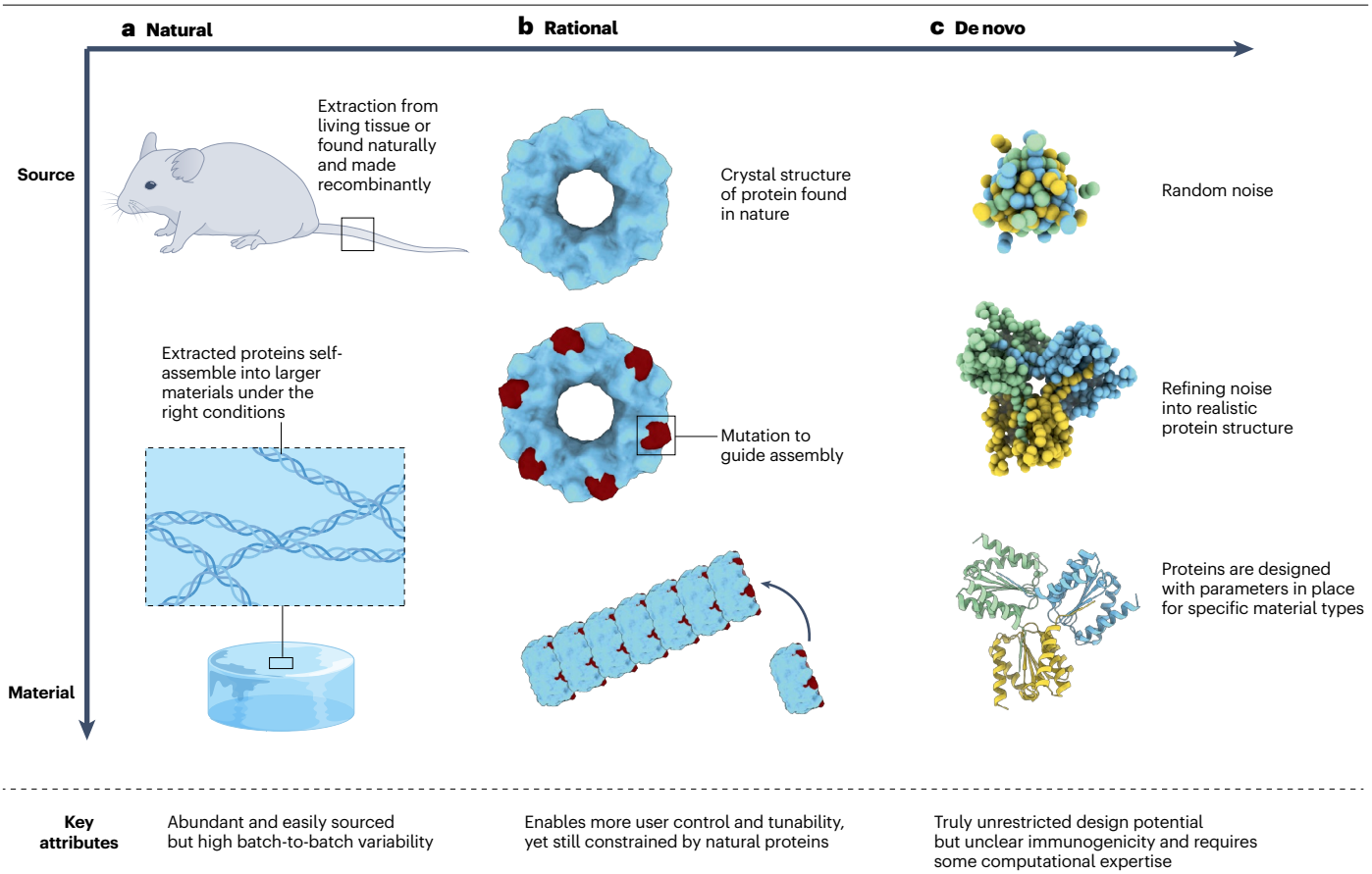


Fig. 1 | Overview of the three approaches to designing protein-based materials. a, Natural protein-based biomaterials were the first to be well studied and used in biomedical applications. Many of these materials consist of proteins that are sourced from living tissues, such as collagen, or made recombinantly. These proteins then self-assemble into microscopic and macroscopic materials, such as hydrogels. **b**, As the field progressed, biomaterials could later be made with rational design approaches. This often involves starting with a native structure from the Protein Data Bank and then designing mutations such that the native protein favours assembly into larger biomaterials, such as nanotubes. **c**, The newest stage of protein-based biomaterial design focuses on de novo approaches, which now use machine learning and often start with random Gaussian noise, which can, over a series of denoising steps, result in larger protein oligomers.

These recombinantly produced proteins can self-assemble into lattices in solution or large monolayers on solid supports[53] (Fig. 2c).

Applications. Although many protein-based nanotubes and layers have been reported, few have been utilized in a biological context. This limited progress towards functional implementation is likely due to difficulty in controlling the size of these unbounded assemblies, which often propagate to various degrees in one or multiple directions, yielding heterogeneous samples[54]. Regardless, electrospun materials in particular have been studied across a wide range of biomedical applications, primarily through early studies in research laboratories. For example, silk and zein nanofibres have been used for small-molecule drug delivery[55–57], and both silk and soy scaffolds have been used as cell-laden matrices for tissue regeneration[58,59]. Electrospun materials from collagen and other natural proteins have also been used as dressings to accelerate wound healing[60,61].

Beyond electrospun materials, other native protein-based fibres and layers have been explored for biomedical applications. For example, as a proof of concept, α-lactalbumin nanotubes and COMP coiled-coil domain nanofilaments have been used to deliver hydrophobic small-molecule drugs, which typically have poor absorption[21,41,62]. Additionally, layer-by-layer assembly is useful for creating nanotubes with decorated inner surfaces, providing more control over nanotube dimensions[63]. Biomolecule loading is often achieved by electrostatic incorporation as part of the layer-by-layer fabrication process[64,65] or through affinity-mediated capture after nanotube fabrication[66,67]. Such nanotubes have been decorated with small molecules[67], enzymes[66], nanoparticles[67], antibodies[64] and other virus-binding proteins[65], offering promise for future applications. For 2D layers, functional utilization is more sparse, although S-layers have been used to display proteins for delivery and diagnostics[68–70].

Nanoparticles

Some of the earliest recorded protein-based nanoparticles were virus-like particles (VLPs), formed by viral coat proteins[71]. Most VLPs consist of self-assembling proteins that form highly regular structures based on interactions between symmetrically arranged subunits. To synthesize VLPs, proteins are often recombinantly expressed and subsequently self-assemble independently or with other protein components[71]. Proteins from a diverse set of viruses, including cowpea

chlorotic mottle virus[72], tobacco mosaic virus[73] and hepatitis B virus[74], as well as bacteriophages such as MS2 (ref. 75) and Qβ[76], have been used to create VLPs. Beyond proteins sourced directly from viruses, many organisms form protein nanoparticles that perform important biological functions. Examples include ferritin[77], lumazine synthase[78], vaults[79], encapsulins[80] and some gas vesicles[81], spanning various symmetries and number of homomeric oligomers.

Most of the nanoparticles mentioned earlier take advantage of specific protein–protein interactions, including hydrophobic interactions, hydrogen bonding and electrostatic interactions, to promote

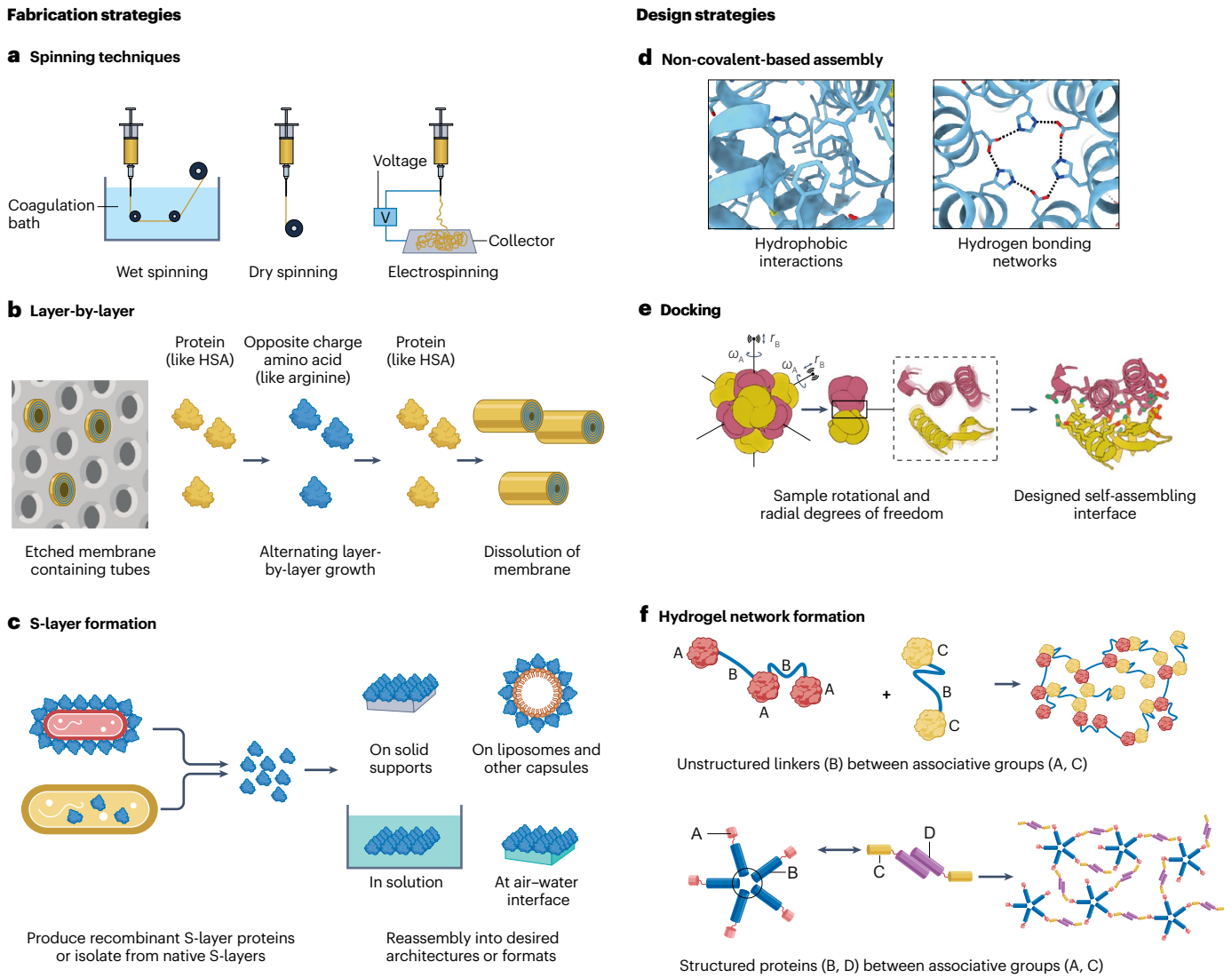


Fig. 2 | Examples of methods used to fabricate and design protein-based biomaterials. a, Overview of the process for spinning protein fibres: (left) wet spinning, in which fibres form in a liquid coagulation bath; (middle) dry spinning, in which extruded proteins in solvent dry out into fibres and (right) electrospinning, in which a voltage is applied to an extruded protein solution that forms fibres on a collection plate. **b**, The layer-by-layer method for creating nanotubes is a stepwise method that alternates between proteins and oppositely charged amino acid solutions to form multilayer tubes within a porous support membrane that is subsequently dissolved. **c**, S-layer arrays from recombinantly produced proteins or isolated from native S-layers can be assembled in various manners, for example, in solution, on solid supports, surrounding liposomes or at air–water interfaces. **d**, Representation of two key aspects of protein and biomaterial self-assembly. Hydrophobic residues can be interdigitated for optimal packing, and polar residues can form complex hydrogen bonding networks and other electrostatic interactions that promote assembly of components. **e**, An overview of a widely used computational nanoparticle design pipeline that uses docking algorithms to bring together components in space and sample their rotational and translational degrees of freedom. The resulting interfaces between components are then designed using computational tools such as Rosetta and ProteinMPNN. **f**, Two examples of how proteins that associate with one another can be used to create larger macroscale biomaterials such as hydrogels using both structured and unstructured linkers. For the unstructured linkers, a two-component system design consists of ABABA and CBC, in which A and C are proteins that associate with one another and B is a flexible linker. When the two systems are mixed, proteins A and C form a crosslink resulting in a macroscopic hydrogel. For the structured linkers, two different protein oligomers are each genetically fused to different associative groups that can then covalently or non-covalently crosslink to form larger macroscopic hydrogels. HSA, human serum albumin. Part **e** adapted with permission from ref. 218, PNAS. Part **f** adapted with permission from ref. 189, PNAS.

self-assembly (Fig. 2d). However, nanoparticles derived from natural proteins can also be produced by physically or chemically modifying the proteins or their environment[7]. Several different proteins can be used in this way, such as gelatin[82], silk fibroin[83], human serum albumin[84] and casein[85]. Although potentially promising, formation of such nanoparticles typically requires complex manufacturing steps and can result in polydisperse samples[54], neither of which are optimal for biomedical applications.

Applications. Generally, nanoparticles are suited for clinical translation in two major categories: vaccination and therapeutic delivery. Nanoparticle surfaces can be functionalized to promote interactions with cells and other components in vivo through covalent chemical conjugation[86] such as *N*-hydroxysuccinimide ester reactions and disulfide chemistries or through direct genetic fusion. These methods have been used to conjugate antigens, peptides and cellular receptors to many nanoparticles[87–90], although termini availability and geometric compatibility can limit options. Complementing exterior modification, many nanoparticles can also be directly loaded with therapeutics[7,23,91].

Nanoparticle vaccines allow for multivalent display of antigens and their larger size often leads to superior trafficking to germinal centres when compared with soluble antigens[92]. Several of the platforms described so far have been used to generate vaccine candidates, with some achieving regulatory approval, including licensed vaccines for human papillomavirus[93] and malaria[94]. In addition to VLPs, other protein-based nanoparticles such as ferritin and lumazine synthase have been engineered as vaccine scaffolds for antigens such as influenza haemagglutinin[89] and the germline-targeting engineered outer domain of HIV envelope (eOD-GT8)[95], respectively. Naturally occurring one-component protein nanoparticles have also been adapted for use with mRNA technologies to create mRNA-launched vaccines. This approach has been tested in a human clinical trial for HIV[96] and in a rodent study for rotavirus[97]. Although ferritin and lumazine synthase are historically the most studied and widely used protein nanoparticle platforms, owing to their properties such as structural stability, efficient expression and accessible termini for antigen display, alternatives scaffolds such as encapsulins have also been explored[98].

Hydrogels

Among the most common naturally occurring proteins used for hydrogel formation are fibrous proteins found in the ECM, including collagen or gelatin, elastin, laminin and fibrin[99]. These proteins are particularly attractive for biomedical applications as they natively surround cells in vivo. Other fibre-forming proteins, namely, silk fibroin, resilin and keratin, are also widely used[99]. Notably, because these proteins all natively undergo several levels of self-organization, they can readily form hydrogels when the proteins are subjected to the right conditions[6]. Globular proteins, such as albumin, glycinin, zein and lysozyme, can also be used to form hydrogels but generally require some level of post-extraction modification that enables their crosslinking into a network[33,100].

In most cases, natural proteins used for hydrogel formation are isolated from abundant biological samples that are rich in them[101]. This isolation typically requires chemical treatment to extract and solubilize the protein from the tissue[102,103], often yielding high batch-to-batch variation that can be an insurmountable barrier to clinical usage. Thus, recombinant protein production, using affinity purification tags and targeted overexpression, provides an attractive alternative for producing a purer and more homogeneous product[104,105].

After extraction, both fibrous and globular natural proteins may be physically, chemically or enzymatically modified to enable or reinforce network formation[33]. For example, silk and collagen hydrogels can be physically modified by differing pH, ionic strength and temperature conditions during formation[100]. A broad range of chemical treatments that modify various amino acid side chains are also commonly used, including primary amine-reactive glutaraldehyde and the less-toxic genipin, or disulfide bond-modifying dithiothreitol[33]. However, as many chemical treatments are toxic, enzymatic treatments such as tyrosinase and transglutaminase are often preferable when pursuing biomedical applications[33].

Other protein-based macroscopic 3D biomaterials have also been used in a biomedical context. These materials include protein-based foams and sponges with highly porous and permeable networks, which are well suited for facilitating fluid transport, cell infiltration and tissue integration[106,107]. Bioprinting with protein-based bioinks has also emerged as another effective strategy for fabricating macroscopic 3D structures[108].

Dynamically responsive materials — those whose structural, mechanical and/or chemical properties can be modified by user-controlled stimuli — are largely unobtainable with naturally occurring proteins. Although some have unique stimuli-responsive properties, they generally cannot form crosslinked networks on their own; chemical or enzymatic treatment would likely interfere with their functionality[109]. One exception is gelatin, which displays thermo-responsive behaviour[33].

Applications. Naturally derived protein hydrogels have shown substantial promise in the past couple of decades for supporting drug delivery, regenerative medicine and tissue engineering applications. Collagen is by far the most commonly utilized of the proteins covered in this section, attributed to its abundance in the ECM, native cell-attachment sites, biocompatibility and biodegradability[102]. However, other proteins are also promising, each with their (dis)advantages in preparation, mechanical strength, biocompatibility or resorption rates.[101] Composite materials, in which two different natural proteins are combined, can endow blended properties of materials, although reproducibility and batch-to-batch variability continue to hamper potential clinical translation[99].

In the past few years, there has been a distinct focus on directing neurogenesis for spinal cord injuries. Collagen, fibrin and silk/laminin hydrogels have been used for neuronal regeneration, serving as small-molecule delivery vehicles[22], therapeutic cell transplantation platforms[27,110] and on their own[111]. As vascularization has an important role in promoting the formation of innervated tissues, some have used collagen gels with silicone conduits[112] or polydimethylsiloxane spacers[113] to regenerate innervated and vascularized tissues in rodent defect models.

Hydrogels have also been used in wound-healing applications, as they are capable of effectively stopping bleeding, improving healing outcomes and minimizing infections, particularly in burn, diabetic and other full-thickness wounds[114]. They have also been used to load antimicrobial peptides and antibiotics[115–117] or have been formed from antimicrobial proteins (such as lysozyme) to afford broad-spectrum antibacterial properties[118,119]. Other developments have focused on anti-inflammation using silk sericin and gelatin/elastin[120–122], hemostasis using zein[123] or neovascularization through small-molecule-containing or cell-containing gels[124,125].

Beyond these applications, there is growing interest in utilizing natural protein materials to model or regenerate diverse tissues,

including cornea[104,126], intestinal epithelium[127] and myocardium[128]. Protein-based foams, sponges and bioprinted constructs are especially promising within this context as they have been used to regenerate muscle[129], bone[130] and soft tissues[131]. Natural protein biomaterials have also been used to support disease treatments, including as vehicles for insulin delivery in diabetes treatment[25] and as intraarticular supports for osteoarthritis[132].

Limitations of natural protein-based biomaterials

Natural protein-based biomaterials are often limited by a reliance on nature to provide building blocks that are well suited to solve nuanced biomedical challenges. For example, formation of nanotubes and layers relies heavily on clever manufacturing methods to create usable materials[51]. Biomedical applications of nanoparticles are inherently constrained by the available termini and morphology of natively formed particles. Hydrogels constructed from natural proteins can suffer from poor mechanical properties and batch-to-batch variation, making it challenging to mimic various tissues and hindering clinical translation[6,133]. Importantly, natural protein-based biomaterials do not take advantage of our ability to readily modify protein primary sequences to achieve a more diverse library of biomaterials intentionally tuned to specific biomedical applications.

Rationally designed protein-based biomaterials

Following the advent of more accessible genetic and protein engineering tools, rationally designed protein-based materials have become popular in biomaterials research. The routine use of techniques such as polymerase chain reaction, alongside the growing availability of custom gene synthesis, has revolutionized our ability to redesign proteins in the span of weeks. Furthermore, most of these designed proteins are produced recombinantly, minimizing the reliance on complex extraction, processing and fabrication techniques and instead allowing efforts to be directed towards the deliberate design of protein biomaterials through templated assembly for easier fabrication. Here, we define rationally designed protein-based materials as those taken from native protein sequences and subsequently modified through point mutagenesis (mutating a single native residue), directed evolution (iterative cycles of mutation and selection for desired attributes), computational interface redesign (mutating protein–protein interfaces for self-assembly) and other methods. These tools allow users to iterate on existing protein sequences to create new material-forming constructs with useful properties beyond those attainable with naturally occurring proteins alone.

Fibres and layers

Rational protein design has enhanced the diversity of nanofibre and nanotube structures by allowing for the bottom-up design of self-assembling units, inspired by those found in nature[5]. The simplest approach is to make single amino acid substitutions that guide structural assembly, for example, by mutating residues at the assembly interfaces of ring-like proteins such as HCP1 and trp RNA-binding attenuation protein to cysteines to guide inter-ring stacking via disulfide bond formation[13,134]. The resulting nanotubes have a fixed width and redox-controlled assembly and disassembly. Alternatively, the introduction of binding motifs can facilitate formation of protein nanowires as small as a single protein in width[135].

More advanced protein interface redesign techniques can be used to control assembly without the need to carefully select the location of a point mutation or affinity tag. For example, relocation of the self-assembling interface in naturally occurring ferritin protein cages has been used to create nanofilaments, nanorods and nanoribbons in the presence of calcium[136]. Computationally assisted redesign of protein cytochrome cb_{562} has also enabled the formation of several unique nanostructures[137]. Further modification of cb_{562} through the addition of cysteine and histidine residues has promoted zinc-mediated assembly of protein units into nanotubes of varying sizes[16].

Similar methods have been implemented to make even larger 2D arrays and sheets, although closer attention to the geometry and local flexibility of components is necessary. Earlier examples of these layers were designed by genetically linking components to form the desired asymmetric units[138]. Subsequently, it was shown that introducing key mutations in native proteins can drive the formation of large, dynamic 2D arrays based primarily on disulfide bonds or metal coordination instead of genetic fusion[17]. Computational tools such as Rosetta have also been used to sample the 3D space between subunits to identify optimal orientations and promote self-assembly into sheets[139,140].

Applications. Although rationally designed nanofibres, wires, tubes and layers can, in principle, be tailored to enable various biomedical applications, practical demonstrations of such applications remain largely unexplored, likely because of difficulties in producing homogeneous samples. Regardless, incorporating rational design into the creation of these novel biomaterials is promising as it allows for more user-defined tuning of material geometries and properties without an over-reliance on pre-existing structures that natively form lattices and tubes. For example, this flexibility could lead to intentional selection of building blocks that would result in optimally positioned termini for fusion to therapeutic cargos. One example recently showed a computationally designed two-component 2D layer that could be functionalized with cellular receptors to facilitate cell-surface attachment, endocytic blocking and receptor clustering[140]. This system proved to be highly ordered and enabled control over the timing of assembly, both unique attributes would have been extremely difficult to achieve if restricted to using protein layers and sheets found in nature[140]. Although these biologically relevant effects are interesting, the introduction of rational design has not eradicated problems with construct heterogeneity. Subtle unintended conformational changes can still lead to multiple assembly states, and a continued reliance on existing native structures limits the ability to control propagation in defined ways. These attributes make clinical translation difficult.

Nanoparticles

Designing and creating novel protein nanoparticles remains a challenging task, especially because the protein components themselves are constrained to adopt specific geometries. Although most nanoparticles described here have been designed using computational methods, directed evolution has also proven to be a successful strategy. A notable example is the evolution of the 60-subunit lumazine synthase cage into a 240-subunit nanoparticle capable of packaging its own RNA[141]. However, difficulties in setting up selection systems for nanoparticle structures and functions have likely prevented widespread usage of directed evolution to design nanoparticles to date.

A pioneering study in 2001 established the use of symmetric protein building blocks as a common theme in protein nanoparticle design and introduced a method of combining these blocks in specific geometries to generate target assemblies[54]. By selecting naturally occurring oligomers and genetically fusing them at optimal angles, single protomers were created that could assemble into larger

nanoparticles[142–144]. A similar approach has been strategically used to combine various building blocks with coiled-coil domains[145–148]. These principles have even been extended to include disulfide bonds between coiled coils to create at least one large self-assembling nanoparticle[149]. Although clearly powerful, this genetic fusion approach is limited by the exacting geometric requirements for the building blocks and the tendency to generate unintended assemblies between two domains owing to linker flexibility[150]. Alternatives to genetic fusion also exist; for example, self-assembling protein nanoparticles have been built out of elastin-like polypeptides primarily based on hydrophobic and hydrophilic domain interactions[151].

Building on the concept of using naturally occurring protein oligomers as building blocks, a general computational method for designing protein nanoparticles was first introduced in 2012 (ref. 152). In this method, naturally occurring protein building blocks that match a desired oligomeric state are first docked into a target nanoparticle geometry, and then new protein–protein interfaces are computationally designed between the protein building blocks to drive macromolecular self-assembly[152–155] (Fig. 2e). This method has been broadly implemented and adapted since, recently having been scaled to design very large pseudosymmetric particles – some up to 96 nm in diameter[156,157].

Protein nanoparticle interfaces can also be elegantly designed with metal coordination as the driving factor for assembly. One of the earliest demonstrations showed that ferritin nanoparticles could be engineered to assemble only in the presence of copper[158]. Since then, many examples of metal-mediated nanoparticle assembly have been demonstrated[159–162], underscoring this approach as a powerful rational design strategy for generating novel protein-based nanoparticles.

Applications. Rationally designed protein nanoparticles are highly promising candidates for vaccine scaffolds and drug delivery platforms. With respect to vaccines, most examples to date have used computationally designed two-component nanoparticles that can scaffold various antigens, such as F glycoproteins from respiratory syncytial virus[163], native-like HIV envelope trimers[164] and influenza haemagglutinin[165]. Notably, one of these designed two-component nanoparticles has recently been licensed as a SARS-CoV-2 vaccine under the name SKYCovione[166]. In parallel, single-component nanoparticles such as I3-01 have been adapted for use as mRNA-launched nanoparticle immunogens displaying the SARS-CoV-2 receptor-binding domain[167]. This type of multivalent display, in which multiple copies of an antigen are presented on a single nanoparticle, typically results in a potent neutralizing antibody response that is orders of magnitude larger than an equivalent dose of the soluble antigen[168]. However, even with such progress, there is a need for more intentionally designed nanoparticles that can be used to investigate the impact of nanoparticle size, geometry and antigen spacing.

Outside vaccines, computationally designed protein nanoparticles have enormous potential as drug delivery vehicles. This area of research has not been extensively explored, primarily because of classical obstacles in drug delivery, such as targeting a specific cell type and enabling nanoparticle and endosomal escape of the loaded drug. Some strategies have been implemented to begin addressing these challenges. For example, instead of loading protein therapeutics into a nanoparticle, it may be possible to specifically design nanoparticles that contain those therapeutics within the scaffold itself, eliminating the need for release from within the nanoparticle. This principle would be similar to what has already been done to create antibody nanoparticles, where antibodies are incorporated as structural components within the designed assembly[169]. Even so, these antibody nanoparticles have been redesigned to be pH-responsive, enabling delivery of packaged cargo intracellularly[12]. Computationally designed nanoparticles have also been used for tissue-specific delivery[170], small-molecule packaging[171] and small interfering RNA delivery[172].

Hydrogels

Although many diverse targets have been used to guide the rational design of protein hydrogels, the proteins used typically fall into three categories: unstructured proteins used as flexible linkers, associative species for crosslinking and components that impart stimuli-responsiveness. Among them, the most common unstructured protein linkers include elastin-like polypeptides, silk-like polypeptides and combinations such as silk–elastin-like polypeptides[173]. These proteins comprise short, (pseudo-)repeating peptide sequences from their parent proteins. Other unstructured linkers not based on parent proteins but rationally designed include XTEN and C10 (refs. 26,174–176).

Coiled coils, such as those in leucine zippers and cartilage oligomeric matrix protein (COMP), were perhaps the first associative proteins to be utilized as crosslinkers for hydrogels[174–178]. Other crosslinking options include tryptophan-rich WW domains and their proline-rich peptide partners[179] or metal-coordinating polyhistidine tags[15,24]. Although most associative proteins result in physical crosslinking, the SpyTag/SpyCatcher protein pair has enabled covalent crosslinking, improving hydrogel stability[180,181].

Various stimuli-responsive proteins have been utilized to create protein-based hydrogels with responsive properties[182]. To afford photoresponsiveness, proteins such as CarH, PhoCl, LOVTRAP and Dronpa have been incorporated[8–10,183,184]. Small-molecule-responsive proteins and peptide motifs such as calmodulin and histidine tags can also impart responsivity[14,185–187]. Elastin-like polypeptides and silk–elastin-like polypeptides are inherently thermoresponsive, with modifiable transition temperatures dictated by their primary sequences[173].

Rationally designed protein hydrogels are often created with an intended method of crosslinking, commonly achieved by arranging various associating domains[188]. One such design is an ABA ordering, in which an associative group (A) is present on both termini, separated by a flexible linker (B)[26,174–176]. For this single-component design to yield bulk materials, A must homomultimerize or self-assemble with multiple identical copies of the same protein, as is seen in many coiled-coil systems. A common two-component system design is ABABA and CBC, in which A and C are two proteins that interact to form a crosslink and B is a flexible linker[179–181] (Fig. 2f). Such arrangement allows proteins that associate with a single partner, such as SpyTag/SpyCatcher, to yield a structurally unbounded 3D gel. Multiple crosslinking domains can even be combined to create networks with unique properties[8,10,24,183,184]. Notably, the protein selected for each of these roles strongly influences the final material properties; length and type of linkers, valency of protein–protein interactions and strength of interactions all contribute to the physical properties of the hydrogel[189]. The incorporation of stimuli-responsive domains[190] can further enable modulation of gel formation, stiffness or dissolution[182].

Applications. For years, rationally designed protein hydrogels have been utilized for simple in vitro studies of cell survival or model protein release. Some have shown promise for tissue engineering applications

through their ability to maintain high viability of diverse cells types including human umbilical vein endothelial cells[179], stem cells[179,181] and fibroblasts[180,181]. Several photodegradable hydrogels, using both CarH and PhoCl proteins, support both 3D cell culture and light-mediated cell release[9,10,184]. Even metal ion-responsive systems, such as those based on mussel foot protein or calmodulin, can support cells without deleterious effects[15,186,187].

Investigations of the in vivo efficacy of rationally designed protein hydrogels have gained traction in recent years. Because they are relatively straightforward to translate to the clinic, simple materials using elastin-like polypeptides and silk–elastin-like polypeptides have been the most heavily used for in vivo tissue engineering and drug delivery applications. Such hydrogels have been used as hemostatic materials[191], cell delivery vehicles for cartilage regeneration[192,193] and carriers for sustained release of difficult-to-deliver drugs[11]. Silk–elastin-like polypeptides have even been used as a cancer treatment through arterial embolization[194]. Elastin-like polypeptides are also increasingly being incorporated into in vivo applications as components of responsive protein fusions, for example, as light-responsive materials that promote neural axon regrowth[24].

Coiled coils are similarly enabling for in vivo applications, owing to their self-healing nature that supports injection-based bioprinting and delivery. For example, coiled coils and adhesive mussel foot proteins have been combined to create printed bi-layer cardiac patches for treatment of myocardial infarctions[195]. Coiled coils have also been used as injectable hydrogels for delivery of sensitive cell types including hepatocytes[26] and exosome delivery for wound healing[196].

Limitations of rationally designed protein-based biomaterials

Although rational design, directed evolution and computational tools provide many opportunities to create new protein-based biomaterials with unique properties, these techniques rely on modification of sequences found in nature. Although continued iteration of proteins found in living systems provides an already large sample size, many opportunities to build new biomaterials remain unexplored through these approaches alone. For example, in nanotube and 2D layer design, identifying suitable capping proteins to minimize polydispersity and enhance drug loading or display remains challenging (Fig. 3a). Nanoparticle design at this stage is still heavily reliant on structures found in the Protein Data Bank, which considerably limits possibilities for clinical translation if the termini are not accessible or if the resulting size or geometry is unsuitable for the intended application. Even in hydrogel design, only a small number of proteins are being repeatedly used in varied arrangements, with opportunities for unique designs becoming more and more limited. Expanding beyond rational design tools may enable the discovery of novel, never-before-seen protein structures that could contribute a wealth of new properties to future materials.

De novo protein-based biomaterials

We define de novo proteins as entirely new species, designed computationally from the ground up using engineering principles rather than through modification of existing proteins or amino acid sequences found in nature. De novo proteins were originally created relying on Francis Crick's equations to define relatively simple coiled-coil helical bundles[197], leading to the emergence of several new species during the 1990s and into the 2000s[198,199]. The status quo has considerably changed with the exponential growth of machine learning and artificial intelligence. Up to this point, even the most advanced software, such as ProteinMPNN[31] for sequence design and AlphaFold3 (ref. 30) for structure prediction, had not realistically allowed for high-throughput, intentional protein backbone design. However, with the advent of RoseTTAFold diffusion (RFdiffusion)[32], it is now possible for de novo protein design to generate biomacromolecular backbones with specific functions in mind at a scale previously unimaginable. Since its introduction in 2023, RFdiffusion has already enabled the design of protein binders against snake toxins[200], as antagonists for cytokine storm inducers[201] and more. Much of the potential for new and transformative protein-based materials has yet to be realized; although important limitations still exist, de novo protein design offers the opportunity to unlock truly novel materials.

Fibres and layers

De novo design has enabled the spontaneous formation of protein filaments from folded polypeptides without the need for pre-existing internal symmetry[202]. Additionally, specifically designed capping proteins has enabled the controlled disassembly of these filaments[202]. More recently, de novo design has yielded protein nanofibres with buried histidine residues that exhibit pH-mediated (dis)assembly[203], a property that is difficult to achieve through rational design but common in natural systems. Collectively, these examples represent a jump forward in our ability to mimic the parameters that control native protein filament formation. These materials may also prove useful in future drug delivery applications, as new designs unlock more monodisperse assemblies. Although few published examples of de novo protein layers exist[204], we expect new approaches enabled by de novo design to address long-standing challenges in these systems, including the limited availability of protein building blocks and the inability to regulate layer size.

Nanoparticles

The primary focus of nanoparticle design has historically been on interface redesign, using pre-existing or slightly modified versions of native proteins. However, with de novo proteins, new nanoparticles can be designed without directly relying on pre-existing protein structures. Earlier approaches to nanoparticle design focused on connecting different protein subunits together with de novo components. Several examples exist in which helical bundles have been connected in such a way to create higher order tetrahedral, octahedral and icosahedral assemblies[155,205,206]. RFdiffusion has also been successful in connecting protein subunits[207] or even extending certain assemblies to make dihedral nanoparticles with two independently addressable faces at programmable distances[208]. In addition to using these tools to design nanoparticles themselves, de novo protein design also shows promise for more robust fusion of bioactive groups to nanoparticles[209] (Fig. 3b,c). Relying on simple fusion with glycine–serine linkers can be challenging and lead to uncertainty regarding surface orientation or distance from the nanoparticle, potentially impacting efficacy. With rigid linkers that can be designed with de novo techniques, many of these questions may be eliminated.

Although the examples mentioned earlier use de novo protein design to build components of nanoparticle assemblies, often in a hierarchal manner, the ability to design entire de novo protein nanoparticles in one step remains elusive. However, as protein design tools continue to develop, such rapid nanoparticle design may become common. Notably, this approach has already been applied to the design of a one-component nanoparticle[32], serving as an early demonstration of its potential for future applications.

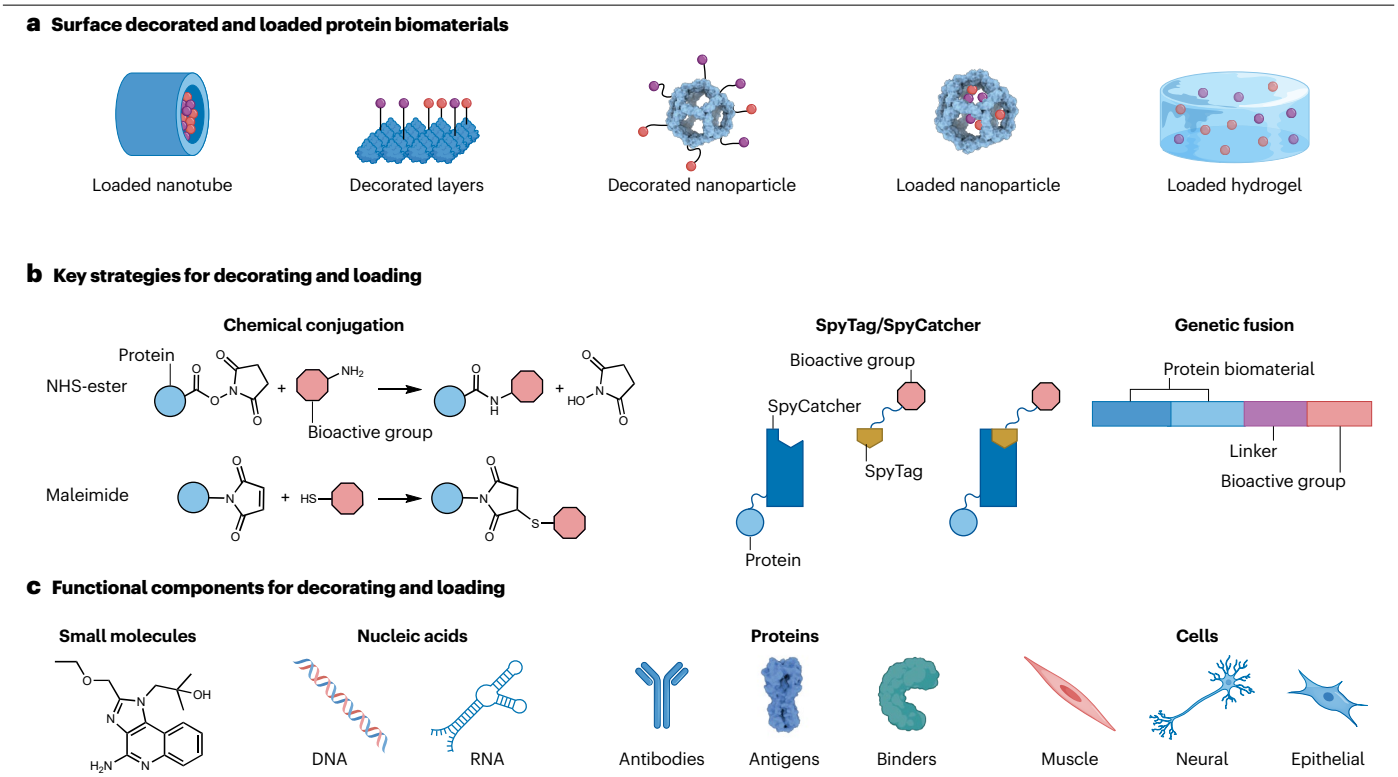


Fig. 3 | Functionalizing protein-based biomaterials for therapeutic effects. a, Some examples of the ways in which protein-based materials can be functionalized, including coating a nanotube, functionalizing a 2D array, decorating a nanoparticle, loading a nanoparticle and loading a hydrogel. Yellow and orange spheres represent any bioactive or therapeutic group. **b**, Key strategies for covalent decoration and loading of various protein-based biomaterials. Examples include *N*-hydroxysuccinimide (NHS)-ester and maleimide chemistries to attach an active group to a protein, SpyTag/SpyCatcher covalent bond-forming protein pairs to covalently link a bioactive group to a protein of interest and genetic fusion for expression of a protein and therapeutic as a single polypeptide chain. **c**, Examples of the many bioactive components that could be used, including small molecules, nucleotides, proteins and cells when loading or decorating protein-based materials. These components impart additional therapeutic effects that the materials may not have on their own. Part **b** adapted with permission from ref. 219, Taylor & Francis.

Hydrogels

De novo proteins can enable the design of bulk hydrogel materials that are unlike those that have been rationally designed. To date, only two examples of a bulk hydrogel created from de novo proteins have been reported. These hydrogels were built from self-assembling de novo multimers with varying assembly valencies, which were subsequently fused with either covalent or non-covalent crosslinking domains that react spontaneously[189] or with small-molecule addition[210]. Changes in the molecular structure of these components, such as valency, flexible linker length or multimer arm rigidity, were shown to contribute to changes in material viscoelasticity. This approach is promising for biological applications, including for cell encapsulation and intracellular formation of hydrogel-like structures in cells engineered to express both the multimeric scaffold and the crosslinking domain[189].

As de novo protein design continues to grow, we believe that additional examples of de novo protein hydrogels will be published in the coming years. Many de novo protein assemblies and binding pairs originally intended for other purposes may prove useful as structural or crosslinking domains for hydrogel materials[211,212]. Furthermore, continued study of the relationship between protein structural characteristics and bulk material properties may provide an unprecedented level of pre-programmed control over hydrogel function and responsiveness.

Outlook and future perspectives

Natural protein materials have been heavily explored in the past several decades, with recent work demonstrating the translation of well-characterized materials into applications. These materials continue to prove useful, especially in hydrogel development and tissue engineering applications. One advantage of naturally derived proteins, particularly in comparison with rationally and computationally designed materials, is that they require little expertise to implement. Many of the most utilized naturally occurring proteins for materials can even be purchased in ready-to-use states. These traits make natural protein materials attractive to researchers looking for materials to apply to translational applications. However, the manufacturing of these materials faces significant limitations, such as batch-to-batch variability, difficulty scaling extraction from animal tissues and thoroughly removing contaminants such as endotoxins, which can complicate translation[213,214]. Additionally, newly discovered native structures continue to surprise the scientific community, illustrating that there are still ample opportunities for new biomaterial discovery. For example, S-layer proteins are complex and found everywhere in nature, yet many have not been fully characterized. Working with newer experimental tools, along with the predicted structures in databases such as AlphaFoldDB[215] and ESMAtlas[216], could provide insights into the

specific requirements for certain protein assemblies and help guide future protein-based biomaterial design.

Rational design remains a popular area of focus for protein-engineering-minded materials development. We believe that this approach will continue to result in materials with interesting structures and useful responsive behaviours that go beyond what can be achieved with natural proteins. However, relying only on rational design is limiting and results in various materials that utilize a small set of protein building blocks with similar modifications or fusion architectures to achieve apparent variety in material properties. In this sense, utilizing computational tools to iterate on these protein building blocks, such as through interface redesign, may be the most fruitful way to add diversity to the rational design toolbox. Continuing to improve manufacturability is also another likely fruitful avenue of research, for example, by finding safer and more continuous methods to scale production of engineered materials[217]. In the coming years, we hope to see further pursuit of in vitro and in vivo application of these materials for biomedical uses. In particular, application of fibres and layers in biomedical contexts have been widely proposed, although functional utilization remains sparse.

De novo design stands to bring important changes to protein biomaterials in the coming decades. The newest computational approaches for de novo protein design allow for rapid generation of a near-limitless number of new biomaterials with different geometries and functions. De novo design holds the potential to bring true programmability to protein-based materials and to help bridge the gap in understanding between protein structure and material properties. However, whether these advances can be realized remains to be seen, as only a handful of examples have been published to date. As such, further empirical data are still needed to validate many prospective future directions[28].

The future of protein engineering will rely heavily on de novo protein design tools, and although this leaves room for innovation, there will also be significant challenges. Perhaps, one of the earliest challenges will be balancing the fundamental spirit of de novo design, in which backbones are freely generated, with specific constraints requested by a protein engineer. Having an easily accessible platform that allows biochemists and engineers, who may not be experts in machine learning or programming, to easily define their protein's desired characteristics (such as specifying secondary structure or constraining the protein in 3D space) will be critical to the future success of de novo protein design.

Another potential obstacle in de novo protein design will likely arise as these technologies are more regularly applied to larger protein assemblies. One-step computational methods for designing materials such as fibres, layers, nanoparticles and even hydrogels may be possible in the future, but large assemblies often come with additional design complexities. For example, protein-based hydrogels rely on unstructured linkers to provide flexibility between crosslinking points. However, current de novo design tools are poorly tuned towards the creation of unstructured linkers, as they are often trained on large data sets of folded protein structures that exist in nature[32]. Additionally, many of the larger assemblies will have much higher computational demands, which could quickly become a limiting factor.

Perhaps, the largest outstanding question for de novo proteins in a biomedical context is how they will behave as therapeutics, vaccines, tissue engineering scaffolds or drug delivery vehicles. To our knowledge, there has been no late-stage clinical trial that has tested fully de novo proteins in humans. Many outstanding questions remain about the immunological and pharmacokinetic impacts of using de novo proteins. Studies that analyse how de novo scaffolds may change innate and adaptive immune responses will be pivotal. We must also understand differences in both thermal and proteolytic stability as well as determine any changes to the clearance of these proteins in vivo. The broad testing of de novo materials in humans is essential, and the approval of such medicines will justifiably face serious scrutiny.

Although important challenges currently exist in de novo protein design, the future will undoubtedly allow for the creation of complex biomaterials. We ultimately envision technology progressing to one-step biomaterial generation. If fibres, sheets, nanoparticles and entire hydrogels can be reliably created with atom-level control spanning many orders of scale using one-step computational protocols, protein-based materials could be enlisted to overcome complex challenges. Although the technology is perhaps not yet ready to meet this vision owing to hurdles such as computational costs, difficulty predicting large symmetric or pseudosymmetric materials, and gaps in available tools to intentionally build materials with a pre-defined function, new work is already demonstrating that one-step biomaterial generation is possible[32]. As designing proteins becomes more accessible, it will be critical to define protein design goals clearly and realistically, ensuring that they align with the challenges we encounter. This necessity for a clear vision is especially true within the context of scaling and manufacturing these materials as these factors ultimately determine their utility and impact. Therefore, considering downstream factors earlier in the design pipeline (such as optimizing for expression or homogeneity) will be essential. Ultimately, machine-learning tools for designing de novo protein biomaterials, together with established approaches leveraging natural and rationally designed proteins, have collectively formed a powerful toolkit that establishes a new frontier set to revolutionize how we treat and prevent disease.

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