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Synthetic Biology for Multiscale Designed Biomimetic Assemblies: From Designed Self-Assembling Biopolymers to Bacterial Bioprinting

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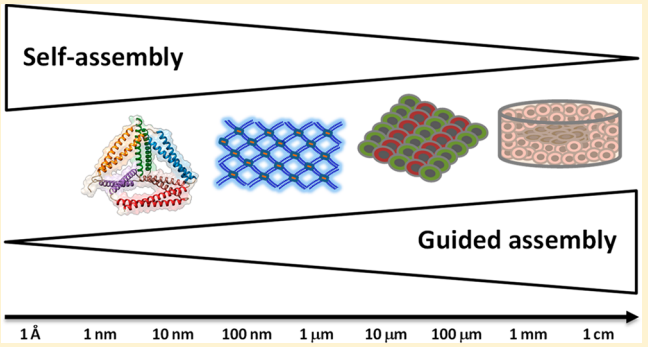
ABSTRACT: Nature is based on complex self-assembling systems that span from the nanoscale to the macroscale. We have already begun to design biomimetic systems with properties that have not evolved in nature, based on designed molecular interactions and regulation of biological systems. Synthetic biology is based on the principle of modularity, repurposing diverse building modules to design new types of molecular and cellular assemblies. While we are currently able to use techniques from synthetic biology to design self-assembling molecules and re-engineer functional cells, we still need to use guided assembly to construct biological assemblies at the macroscale. We review the recent strategies for designing biological systems ranging from molecular assemblies based on self-assembly of (poly)peptides to the guided assembly of patterned bacteria, spanning 7 orders of magnitude.



Biological systems are exceptionally complex and highly hierarchical in structure and span a broad scale over 10 orders of magnitude. Biological components range in size from nanometers to tens of meters, from single molecules to the largest organisms, respectively. Biological structures such as lipid membranes, higher-order structured proteins, or nucleic acids are formed exclusively through self-assembly, a bottom-up process in which atoms or molecules associate in well-defined and functional assemblies under physiological conditions.[1] Naturally occurring self-assembly represents an attractive comprehensive tool and is a technologically feasible and cost-effective strategy for the design of new biomimetic systems or materials, functional biomaterials, or devices. In fact, the design of novel protein nanostructures can be achieved on the basis of the fundamental biophysical principles of protein self-assembly, whereas the idea of modular design based on preformed building blocks has been central to the design of higher-order nanostructures and biomaterials.[2]

In recent decades, multiple methods and technologies have been applied to elucidate the principles of natural self-assembly processes and to design new approaches aimed at assembling biomolecules, cells, and tissues.[3−6] On the molecular scale, we have already designed or guided interactions between individual molecules, although self-assembly approaches typically produce results that are far superior. Self-assembly extends to the macroscopic scale in natural biological systems, although we currently lack a full understanding of the principles that define the structure and function of individual cells. The deficits in our knowledge are even greater for cellular differentiation and formation of multicellular tissues, organs, and whole organisms. Formation of multicellular systems is typically slow and may take days to years for the complete development of the organism. The structure of multicellular organisms is to a large degree hardwired within the genetic program, although external forces and epigenetic elements can have important effects on the shape and properties of the mature organism. The principles of self-assembly of complex multicellular organisms remain to a large degree unknown and will probably take several decades to fully understand before they can be applied to fundamentally redesigning the self-assembly of multicellular organisms.

While self-assembly is highly desirable for engineered biological systems, guided assembly aimed at imposing a desired arrangement of molecules and cells can also be used to direct the formation of biological systems. While nanostructures are too small to be produced efficiently by any other method apart from self-assembly, guided assembly based on coupling of selected physiochemical signals or the use of external fields or conditions can be applied to generate patterns that guide the ordering of cells. Although a rich diversity of complex multicellular organisms exists in nature, self-assembly

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can likely be used to guide only a limited number of structures. Additionally, the process of formation of a multicellular organism is very complex and difficult to engineer. Patterning by external inputs, such as light or acoustic or magnetic fields, can help compensate for our current inability to guide the self-assembly of multicellular organisms, enabling the formation of shapes that may be difficult if not impossible to reach by self-assembly and accelerating the process of macroscale shape formation. Therefore, multiscale synthetic biology approaches are being developed to direct the assembly of building blocks into hierarchically ordered structures using a combination of self-assembly and guided assembly strategies. This approach offers the potential to build designed, nanostructured biomaterials with greater complexity of structure and/or function on multiple length scales.

To create biomimetic systems, synthetic materials that can recapitulate the structural and functional complexity of biological materials must be developed. Such biomimetic assemblies could be formed by self-assembly of polypeptides or cells, without or in combination with different organic and inorganic compounds, nanoparticles, or scaffolds, or via assembly guided by external inputs or conditions. The design of biomimetic materials is ready to face more complex challenges such as the introduction of functions or the development of medical and nonmedical applications. Here we review recent examples and discuss self-assembly and guided assembly strategies in synthetic biology and their advantages and disadvantages for the design of novel biomimetic assemblies across different scales, from *de novo* designed biopolymers to guided assembly of patterned bacteria, and discuss future perspectives and potential applications.

DESIGNED SELF-ASSEMBLY OF BIOPOLYMERS

Self-assembling biomacromolecules, including nucleic acids, peptides, proteins, lipids, and carbohydrates, are the fundamental building blocks of life. Large macromolecular assemblies, composed of tens to thousands of polypeptide chains, are widespread in all cell types and come in many shapes and forms. Single-chain biomolecules range in size from hundreds of daltons to megadaltons, with the largest polypeptide chain, titin, composed of ~30000 residues that form 244 folded protein domains, exceeding 1 μm in length.[7] This example demonstrates that biological systems can produce quite large building molecules if their assembly is modular and folding is not a limiting factor.

One major approach for designing biologically active high-order structures or materials is the self-assembly of polypeptides. Polypeptide sequences often include different types of domains within the same chain and can rapidly self-assemble under normal physiological conditions into complex and well-defined structures with a precise spatial arrangement of functional groups.[8] Given their enormous variability in sequence and structure, proteins appear to have nearly unlimited functional potential under conditions conducive to life. While DNA nanotechnology has demonstrated the repurposing of natural building materials for the construction of simply and completely designed shapes, polypeptide nanotechnology has clear advantages due to the versatility of chemistries and geometries allowed in polypeptide building. Additionally, polypeptides can be efficiently, sustainably, and cost-effectively produced by cell factories via environmentally friendly methods.[9]

Proteins self-assemble on the basis of the formation of a large number of long-range weak cooperative interactions between atoms of a linear polypeptide chain, which may be difficult to accurately predict. Therefore, the modularity of the design can enable the construction of large designed proteins. Novel peptide and protein nanostructures can be developed by combining and re-engineering already-existing protein domains through protein fusion or protein interface design or by designing peptides and proteins *de novo*. Despite the complex interplay of interactions that determine the three-dimensional (3D) structure of proteins, researchers recently succeeded in making a breakthrough by designing bioinspired assembled protein structures that do not exist in nature.[4,10]

De novo design of multiple-chain peptide assemblies has yielded an array of increasingly complex nanostructures.[11] α-Helical bundles were one of the first *de novo* designed multiple-chain assemblies,[12,13] which were later used for the design of multiple multichain nanostructures, including cages[14] and nanotubes.[15] *De novo* design of multichain protein assemblies was developed to design protein assemblies employing natural oligomerizing domains, e.g., monomers, which assemble with a specific symmetry.[16−18] Yeates et al. used natural dimerizing and trimerizing domains fused into a single polypeptide chain, which self-assembled into protein cages with tetrahedral, octahedral, or icosahedral symmetry, layers, crystals, and filaments.[16] More recently, Baker et al. demonstrated self-assembly of oligomerizing domains, where cagelike proteins were designed with tetrahedral or octahedral symmetry (Figure 1A)[17] as well as a 600 kDa protein homododecamer that self-assembled into a symmetric tetrahedral cage.[18] Fallas and Hartgerink have described a multistate computational design protocol for the design of three peptides that fold into a highly stable ABC heterotrimer.[19] A related approach to design self-assembling nanostructures is based on the engineering of protein−protein interfaces. In this technique, computational methods are used to design the structural complementarity to direct the assembly, providing the driving force for the assembly and for the definition of the relative orientations of the building blocks. On the basis of this strategy, different 24-

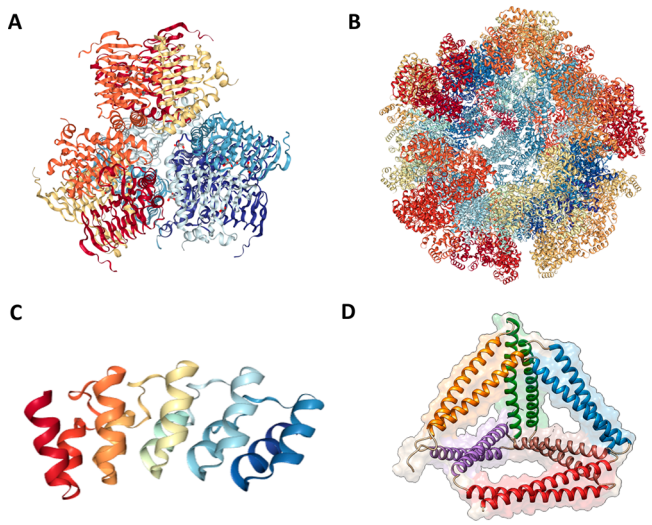

Figure 1. Different types of designed self-assembling proteins. (A) Fused oligomerizing domain tetrahedral cage.[17] (B) Icosahedral assembly based on a designed protein interface.[21] (C) Repetitive protein.[23] (D) Coiled-coil protein origami tetrahedral cage.[33]

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subunit cagelike protein nanostructures that combined trimeric subunits that co-assembled into a symmetric tetrahedral architecture were designed.[20] Two-component building blocks (pentamers and trimers) were combined to form 120-subunit icosahedral protein nanostructures (Figure 1B),[21] and self-assembled unilamellar spheres were constructed from building modules comprised of two noncovalent heterodimeric and homotrimeric coiled-coil bundles.[14] These achievements were made possible by the advances in computational molecular modeling tools, notably the Rosetta software pioneered by the Baker group.[22]

Modular designed single-chain protein assemblies were initially assembled from repeat proteins, where the regularity of their inner structure as assessed by repetitive short- and medium-range interactions defines the curvature and pitch of the assemblies (Figure 1C).[23−25] The first examples of nonrepetitive single-chain protein nanostructures made by this design strategy were α-helical bundles that relied on short-range interactions.[26] As an upgrade to introduce long-range intramolecular interactions to design complex modular structures, Jerala and colleagues translated the concept of DNA nanotechnology[27] into polypeptides.[28] In this approach, α-helical coiled-coil dimers, one of the best-understood protein structure motifs,[29,30] provided orthogonal pairwise-interacting modules reminiscent of the complementarity of nucleic acids. Designed Coiled-coil Protein Origami (CCPO) structures were based on coiled-coil segments as distinct structural elements that were arranged in a defined order to define the path of the polypeptide chain to form edges of a stable polyhedral protein cage. Dimers composed of coiled-coil peptides were formed by a polypeptide double Eulerian path, which promoted folding into a desired protein fold.[31] This strategy was first applied to design a single-chain polypeptide tetrahedral fold[32] and further utilized to design different single-chain coiled-coil protein origami structures of increasing complexity and size, i.e., polyhedron-shaped protein cages (Figure 1D). The largest cage (a triangular prism) consisted of 700 amino acid residues and represents one of the largest single-chain protein designs.[33] Additionally, supercharged orthogonal coiled-coil elements with negatively charged residues at non-interacting positions[34] were employed to chaperone the correct *in vivo* self-assembly under physiological conditions.

GUIDED ASSEMBLY OF BIOPOLYMERS

While self-assembly dominates the formation of complex biomolecules, molecular assembly can be also guided by the chemical activity of small or large molecules or by different physical signals. One frequently used strategy for guided molecular assembly is coordination through metal ions. α-Helical bundles, nanotubes, and two-dimensional arrays have been assembled through natural metal binding sites.[35] Additionally, *de novo* metal binding protein interfaces can be engineered into proteins.[36] Metal site design can also be used to control the assembly of coiled-coil peptides.[37] Environmental oxidation conditions have been used to guide redox-sensitive protein assemblies (e.g., self-assembly interfaces sensitive to the redox state in the cell, through disulfide bond formation).[38] The phosphorylation state can also influence the self-assembly of proteins,[39] which has allowed the design of reversible molecular switches.[40] In protease-responsive modules,[41] an inhibitory domain is cleaved off to endow responses to selective proteases that are active under certain cellular conditions or upon interaction with pathogens. This approach has been implemented in an example of designed cross-linked supramolecular filaments that dissociate into less stable micellar assemblies and monomers upon proteolytic activity of matrix metalloproteases-2[41] or by designed coiled-coil-mediated assemblies.[42] In chemically induced dimerization, a protein complex can assemble by the addition of a chemical signal triggering an interaction between the receptor domains (e.g., rapamycin-triggered FKBP-FBP or abscisic acid-triggered heterodimerization of Pyl-ABI), which can mediate the assembly of other protein domains.[43] The temperature-responsive behavior of designed proteins is based on the (de)stabilization of protein domains or assemblies.[44] The amino acid residues histidine, aspartic acid, and glutamic acid are frequently responsive to conformational changes over a range of pH values between 3 and 7.[45,46] Protein assembly can also depend on ionic strength,[47] solvent polarity,[48] or mechanical stimulation.[49] The light-triggered response of protein-based materials can be established by photochemical cross-linking reactions with polymeric materials,[50] based on the introduction of light-responsive LOV domains[51] or other light-inducible oligomerization domains.[52] The assembly of protein nanostructures can also be guided via noncovalently[53] or covalently[54] linked polymer−protein conjugates or the formation of hybrid protein−nanoparticle complexes.[55] Guided assembly can be combined with patterning or immobilization of protein molecules onto functionalized surfaces and used for the generation of complex bioactive scaffolds for applications such as drug discovery.[56]

DESIGNED PATTERNING OF BACTERIA

While designed intermolecular interactions can be used to guide the self-assembly of protein complexes, the large-range order required to create multicellular assemblies makes this approach still too challenging. Therefore, patterns may be imposed on populations of cells by external signals in combination with synthetic biology approaches to engineer specific cellular responses. Initially homogeneous groups of bacteria can be induced to express genes differentially under the control of chemical inducers, either externally applied or self-produced. This approach opens the possibility of employing a large range of natural abilities in new ways, resulting in emergent patterning that is comparable to the development of animal tissues. In one early example, *Escherichia coli* were designed to produce fluorescent proteins in response to a quorum-sensing signaling molecule diffusing away from a point source. Expression of the fluorophore within a lawn of bacteria was de-repressed only at intermediate concentrations of the chemical inducer, such that a bulls-eye pattern of fluorescence could be established.[57] Similar band-pass filters have also been implemented in lawns of *Lactobacillus lactis* in combination with applied masks of diffusing inducer chemicals, creating fluorescent line patterns of arbitrary two-dimensional geometries and tunable thicknesses (Figure 2A).[58] A cell density-sensing system was later employed to control the expression of a chemotaxis regulatory protein, such that the *E. coli* bacteria themselves became arrayed in rings of alternating high and low density at a tunable wavelength.[59] A higher degree of self-regulation of bacterial patterning was achieved when more complex genetic circuits acting as an AND gate were constructed in *E. coli* to create three-color patterns in response to self-produced inducers.[60] Recently, *E. coli* were engineered to express a synthetic genetic circuit that spontaneously creates

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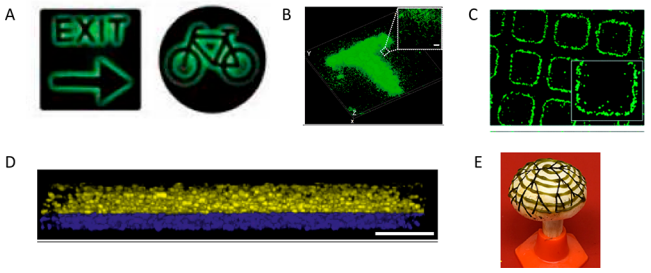

Figure 2. Bacterial patterning. (A) Genetically engineered band-pass filters that respond to chemical inducers.[58] (B) The letter "T" bioprinted in *P. aeruginosa* biofilms via optogenetics.[67] (C) Patterning of individual *Staphylococcus aureus* bacteria through selective surface modification.[69] (D) Layered bacteria achieved with 3D printing.[78] (E) 3D-printed bionic mushroom incorporating cyanobacteria and graphene for generation of photocurrent.[80]

stable disordered Turing patterns, exhibiting high spatial resolution and independence of pattern formation with weak control over the final shape and position of the pattern.[61]

While chemical inducers have proven to be useful tools in pattern formation, they are subject to the laws of diffusion, resulting in a slow spread over longer distances, loss of directionality, and poor control over the induction strength in different regions of the culture. A promising alternative to chemical inducers is the control of bacterial pattern formation with light, which can allow for high-resolution patterning with stringent control. In early proofs of principle of light-induced patterning in bacteria, light-sensing systems from cyanobacteria were transferred to engineered *E. coli* to create strains that acted as edge detectors[62] or that could upregulate the transcription of desired genes orthogonally in response to two different wavelengths of light.[63] Recently, light induction has been applied to regulate bacterial attachment via several different techniques. Proteins that interact via reversible photoswitching were exploited by expressing one of the proteins on the surface of *E. coli* and conjugating its partner to a glass substrate, after which the application of a photomask allowed for the bacteria to adhere to the substrate in controlled, reversible patterns.[64] Photoinducible expression of an adhesion gene in *E. coli* upon stimulation with patterned light was next used to create spatially patterned biofilms via a more streamlined but nonreversible approach.[65] Light-induced biofilm attachment has also been achieved in engineered *Pseudomonas aeruginosa* by introducing light-sensing systems to control the expression and/or degradation of biofilm signaling molecules (Figure 2B).[66,67]

Genetic engineering and control with chemicals or light to induce bacterial patterning will likely continue to be used extensively due to their proven efficacy in combination with the ever-increasing number of sequenced and examined genetic systems from which the parts and chassis may be chosen. However, the patterned bacteria need to be genetically engineered, noisy logic gates might be needed, the number of variants of, e.g., quorum-sensing proteins and sensors is limited, and control is realized by diffusing inducers. Together, these factors make fine-tuning and up-scaling of these patterning systems slow and laborious. In addition, the absolute dimensions of the produced bacterial patterns are typically small, usually in the millimeter- to centimeter-scale range in the $x-y$ plane but only in the tens of micrometer range in the z direction. Consequently, these methods are not currently suitable for patterning of bacteria in larger volumes, an important prerequisite for producing materials for practical applications.

GUIDED ASSEMBLY OF PATTERNED BACTERIA

Current methods for the designed patterning of bacteria excel at patterning cells with a high resolution, but they require genetic engineering of the bacteria and have thus far not been used to pattern more than one species at a time. To achieve the patterning of unmodified bacteria, several guided assembly techniques have been developed. To generate selective surface modification, substrates were etched with an ion beam to expose only specific areas for the attachment of a cross-linker. This cross-linker, in turn, bound antibodies specific to the fimbriae of *Salmonella enterica* serovar Typhimurium, allowing for the specific and patterned binding of *Salmonella* bacteria to the modified surface while other bacterium species in a mixed culture did not adhere.[68] If high selectivity is not required, then simpler surface modifications can be employed. For example, polystyrene surfaces were modified by applying a copper-grid mask while ultraviolet (UV) cross-linking an amphiphilic block copolymer onto the surfaces, resulting in defined areas of increased surface hydrophilicity. These surface structures allowed cultures of *Staphylococcus aureus* to bind selectively to these areas even down to a resolution of a single-bacterium width, despite the tendency of *S. aureus* to form clusters (Figure 2C).[69] A straightforward variation of this approach was used to pattern multiple species of bacteria on agar plates by exploiting their differential susceptibilities to antibiotics. Agar was supplemented with a photoactivated antibiotic that was selectively activated with a mask and a UV lamp, resulting in different antibiotic concentrations depending on the length of UV exposure. A mixture of *E. coli* and *Micrococcus luteus* plated onto these plates resulted in areas where *M. luteus* grew exclusively, due to its high resistance to the antibiotic, and mixed areas where both bacteria survived.[70]

Wild-type bacteria can also be patterned via encapsulation with photochemistry.[71] Using a laser-based lithography technique, bacteria were suspended within a solution containing gelatin and a photosensitizer. Multiphoton lithography was then applied to induce cross-linking at the focal points of the laser, allowing for the fabrication of arbitrarily shaped, 3D microenvironments with micrometer resolution. The structural components were permissive for small molecules such as nutrients and antibiotics while reliably containing bacteria within the compartments, allowing for investigation of bacterial interspecies interactions by surrounding a colony of one species by a colony of a second species.[71] Surface modification for selective attachment of bacteria and encapsulation via photochemistry both allow for very high resolutions without requiring genetic engineering of bacteria. Photochemistry-based encapsulation allows for a wide variety of geometries to be produced, and the selectivity of surface modification can be flexibly tuned depending on the application, from a simple increase in surface hydrophilicity to the use of highly selective antibodies, of which many are commercially available. Both approaches require highly specialized technologies, in the form of multiphoton laser writing or microfabrication techniques for substrate modifications or the production of masks, depending on the complexity and dimensions of the pattern applied.

The high precision of machines used in printing and microfabrication makes the small size of bacteria less of a

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confounding factor in patterning, allowing for approaches in which bacteria can be placed directly by a pumping system or tiny, microfabricated structures. Microfabrication has been used to create PDMS stamps with pillars as small as 1 μm in diameter to transfer cells from a lawn of *E. coli* onto an agarose substrate, producing arrays of groups of bacteria. Via reduction of the number of bacteria in the initial bacteria lawn, arrays of bacteria could be produced with single-cell resolution.[72] Fabricated PDMS stencils have also been applied to produce arrays of biofilms of several Gram-positive and -negative bacteria species on different substrates. This technique is suitable for batch cultures as well as a culture in microfluidic devices and has produced biofilms with dimensions in the tens of micrometers, with further down-scaling expected to be possible.[73] Microfabricated tools allow for very high resolutions, but scale-up and the use of different cell types can be a challenge. Furthermore, specialized microfabrication facilities and trained staff are required to produce the stamps and stencils.

To deposit bacteria via a more accessible approach, commercial inkjet printers have been employed to deposit suspensions of *E. coli* onto agar-coated glass slides, reaching resolutions of 100 colonies/cm^2 and achieving single cells per droplet by adjusting the concentration of cells in the printed suspension. Despite the high temperatures used in the printheads to generate droplets, the printed bacteria were viable.[74] A similar approach was later used to print bacterial dots with a diameter of 100 μm and a density of 400 dots/cm^2 onto agar-coated glass slides. Via the printing of gradients of antibiotic solutions, inkjet printing was used as a high-throughput replacement for the traditional disc diffusion method of determining minimum inhibitory concentrations.[75] Inkjet printing is a compelling technique, due to the low cost of commercial printers, their high precision, the ability of control with standard software, and the ease of modification for biological purposes. Challenges arise from cross-contamination when more than one species or strain is printed, the difficulty of sterilization of, e.g., the print head, and clogging through dried growth medium components or carrier substances.

For applications requiring macroscopic 3D structures and flexibility in the choice of bacterial species, additive manufacturing has recently emerged as a promising tool. The Meyer lab refitted a commercial 3D printing kit into a 3D printer for bacterial cells that extrudes bioink, a mixture of bacteria, nutrients, and an alginate solution that solidifies into a gel upon contact with a calcium ion-rich substrate. The printed *E. coli* cells could be arranged in stable, layered structures while still being accessible to nutrients and chemical inducers for the controlled activation of engineered genes.[76] Thereafter, applications of 3D-printed bacteria were demonstrated for bioremediation when *Pseudomonas putida* was printed and fixed in a UV-cross-linkable bioink, resulting in a mesh-shaped living material that successfully degraded phenol present in the surrounding medium. The potential for production of materials for biomedical applications was also demonstrated when *Acetobacter xylinum* was printed in a complex 3D shape to cover the face of a doll, which had been scanned to provide the printing coordinates. The bacteria subsequently produced cellulose *in situ* that remained in the printed shape after removal of the biological residues, showing a path toward using 3D-printed bacteria for the production of personalized skin grafts.[77]

Recently, K'NEX toys were used to create the lowest-cost 3D bioprinter to date. This 3D printer was used to extrude *E. coli* engineered to express curli fibers, the major proteinaceous component of *E. coli* biofilms, in the presence of a chemical inducer. After a postprinting induction period, the printed structures became resistant to the strong alginate-dissolving agent citrate, showing that exogenous control over biofilm formation can be up-scaled to the macroscale to generate stable, living materials (Figure 2D).[78] 3D printing techniques have also been applied to encapsulate engineered *Bacillus subtilis* biofilms inside of hydrogels through sequential printing of hydrogel and biofilm-producing layers. When 3D-printed *B. subtilis* biofilms were transferred onto a fresh agar plate, the printed samples were able to self-regenerate biofilm growth on the plate in the same geometrical pattern as the initial print, showing the potential of this approach to create long-term, transferrable bacterial patterning.[79] 3D printing has also been applied to create "bionic mushrooms" by 3D printing layers of cyanobacteria and graphene nanoribbons onto mushroom caps (Figure 2E). The mushrooms were able to nourish and sustain the cyanobacteria over an extended period of time, during which the bacteria produced electricity that was harvested via the conductive graphene ribbons. This creative work shows the potential benefits that can arise from creating an artificial, spatially patterned symbiosis between different kingdoms of life.[80]

CHALLENGES AND PERSPECTIVES

One of the most active areas of innovation for the development of novel polymeric nanomaterials is in self-assembly processes that can be tuned via external inputs into the system. In this Perspective, we review developments and strategies at two extremes of synthetic biology approaches, ranging from the design of new nanoscale molecular assemblies such as self-assembling polypeptides to guided macroscale patterning of bacterial cells to illustrate recent progress and current challenges (Figure 3). While we are already able to engineer self-organizing patterns of bacteria, macroscale patterning can still primarily be addressed by guided assembly approaches.

Building synthetic biological structures by assembling biomolecules in an organized way is a rapidly growing field of research involving intense interdisciplinary collaboration. Structure-based computational protein design strategies have been demonstrated to be a powerful tool for engineering new functional capabilities that extend beyond biomimicry. As we have illustrated, diverse approaches to the design of novel protein nanostructures with tunable dimensions, morphology, and functionality have recently emerged and are continuously being improved, where structural elements as small as short peptides can already form nanoscale-ordered assemblies.[10,4]

Among those approaches, one of the main strategies continues to be the *de novo* design of polypeptide assemblies, where reduction of the design complexity has been made possible by symmetry-aware algorithms, where (poly)peptide building blocks are docked together symmetrically, or modular design, which prioritizes the stability of the building elements. A wide range of self-assembling polypeptide nanomaterials has already been developed[81,82] and implemented in many biomedical applications for therapy, prevention, and diagnostics such as drug delivery and targeting,[83] epitope scaffolding for vaccination,[84] an alternative strategy for microvascular anastomosis using a peptide-based hydrogel,[85] alternative antimicrobial strategies using virus-inspired artificial

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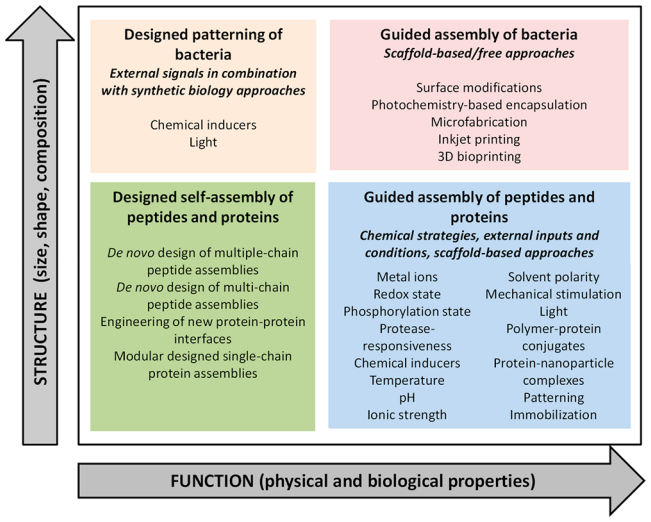

Figure 3. Strategies and methods for assembly of bionanostructures and biomaterials. The hierarchy of complexity of assembled biological structures increases from designed (poly)peptide nanostructures at the molecular scale and nanoscale to designed bacterial biomaterials at the micro- and macroscale. The complexity of physical and biological functions of designed assemblies of (poly)peptides and bacteria increases from self-assembly to guided assembly approaches.

capsids,[86] a strategy for the delivery of nucleic acids with artificial viruses,[87] and many others. Self-assembling polypeptide-based materials have also been used in applications in nonmedical areas such as the rational design of protein molecular machines (e.g., biocatalytic nanomaterials,[88] sensors for electrochemical applications,[89] or optical-biosensor applications[90]) and functional materials (e.g., protein nanowires[91] or free-standing protein films[92]). Protein self-assembly includes certain limitations (e.g., most proteins are vulnerable to extreme chemical conditions such as pH, ionic strength, and temperature), so the construction of ordered protein assemblies needs an adaptable design and accurate control under strict conditions. On the other hand, environmental responsiveness also represents an advantage for the construction of dynamic assemblies with advanced functionalities, as one of the ultimate goals in this field. The capability of engineered biomaterials for programmed responses to external stimulations can lead to a panoply of different designed functions, such as specific targeting, controlled release, or improved efficacy. These "smart" behaviors can be combined for the construction of highly selective delivery systems, for complex conformational changes in highly ordered structures, or for reversible phase transitions and mechanical properties[93,94] that are important for the design of dynamic features in advanced bionanostructures and biomaterials.

Synthetic biologists are increasingly turning their attention to the creation of large engineered biological structures, for example, eukaryotic cells assembled into artificial 3D tissues structurally organized on multiple levels. For building such large biological structures, biological building blocks, which currently fall into the nano- or microscale range, must be extended into the macroscopic range. The design of self-assembling macroscale assemblies poses a formidable challenge in synthetic biology. Nature has solved these problems both for multicellular organisms and at the scale of individual cells, where oscillations and concentration gradients can be used to define organization at the macroscale.[95,96] Biomolecular self-assembly from a diverse array of multiscale building blocks, from polypeptides to cells, is ultimately driven by noncovalent interactions.[1] It is currently easier to organize micro- or macrobiostructures such as artificial tissues by using external inputs, including magnetic fields,[97,98] acoustic waves,[99] geometric docking,[100] liquid-based templates,[101] or bioprinting,[102] that can trigger and/or drive self-assembly of building blocks. Field-directed self-assembly (i.e., controlled by light, magnetic fields, etc.) has an added advantage in that fields can be switched on or off and tuned dynamically, which enables improved long-range order and controlled orientation. Currently, other synthetic biology approaches are being developed for building larger structures composed of interacting cells, based, e.g., on the deployment of synthetic cell-surface receptors that sense

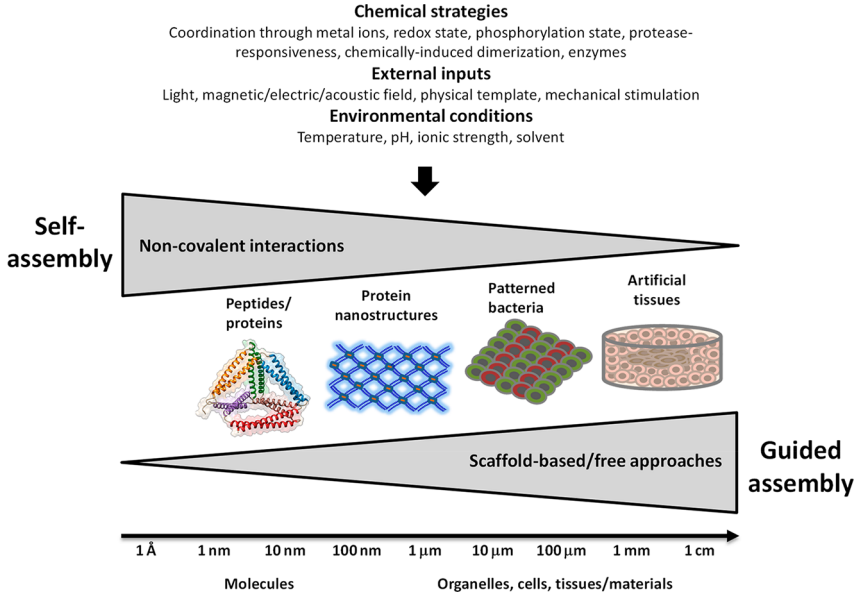

Figure 4. Self-assembly and guided assembly strategies for biomolecular construction can create designed biological systems spanning multiple length scales.

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the types of neighboring cells and trigger their own differentiation, as was recently demonstrated by a combination of synthetic Notch receptors and cadherins.[103]

Today, most tissue engineering approaches require the use of a patterned substrate (e.g., hydrogels) that mimics an extracellular matrix to assist cells in the assembly process. In contrast, a different type of approach uses spheroid formation to provide a scaffold-free environment for 3D cell culturing.[104] Between those extremes, there are multiple additional scales, such as the internal structuring of cells, where molecular assembly is being extended toward the microscale by diverse methods of pattern formation that can also exist under cell-free conditions.[95,96] The synergistic combination of both technologies, where self-assembly dominates ordering at the molecular level and guided assembly directs order at larger length scales with scaffold-based/scaffold-free approaches and the use of different chemical strategies, external inputs, or environmental conditions, promises to advance solutions for precision, repeatability, and high-throughput processing to develop complex assemblies at scales from the nano- to macroscale, from protein nanostructures to the patterning of bacteria or the formation of artificial tissues (Figure 4). In such an active and diverse field of research, where biotechnology meets nanotechnology and materials science, we have without question missed some interesting reports about novel protein nanostructures or biomaterials and their use in applications.

LONG-TERM VISION FOR MULTISCALE ASSEMBLY

Protein design and engineering in synthetic biology are moving toward the functional design of smart biological parts, devices, and systems that will be based on self-assembly and will be responsive to diverse signals. The future is likely to bring the development of structures and systems that will actively interface with a complex biological environment, for example, advanced bionanostuctures or biomaterials for therapeutic delivery that will shield the sensitive molecular cargo, target specific cells or tissues, and release cargo at the appropriate site of action.

An additional level of complexity will be the ability to coordinate multicellular events to assist in the organization of multiscale tissues, for example, in tissue integration and regeneration. One promising example in this area is nanocages, protein assemblies that show a great potential to be developed into artificial stimulus-responsive or programmable bionanomachines functioning as drug/gene carriers, biosensors, imaging agents, vaccine/immunomodulators, or nanoreactors for biocatalysis.[105] Currently, clinical applications of self-assembled protein nanocages are still limited,[106] but further engineering and introduction of responsiveness to molecular or environmental signals will bring solutions for the enhancement of their targeting capacity or penetration efficiency. We can also expect new abilities for nanocages to modulate the immune response and to feature enhanced biocompatibility and biodegradability via engineering of external cage surfaces.

For multiscale assembly, we can envision exciting potential in the introduction of dynamic features (e.g., incorporating degradation or signal responsiveness into biomaterials) or the introduction of nonbiological moieties to biological systems, such as bioinert micro- and nanocarriers. We also anticipate the realization of engineering paradigms that have been so far used only in nonbiological engineered systems, including standardization, reliability, and predictability. We expect numerous benefits based on the productive merging of features of the two worlds for the benefit of human health, industrial production, and the environment.

It seems at first glance that bioprinting might eventually not be required at all if self-assembly could be programmed into designed biological systems like the differentiation programs of plants and animals. Bioprinting could nevertheless provide a much faster means of assembling macroscopic tissues and fabricating shapes and functional assemblies that could be very difficult if not impossible to encode into the genetic program. To promote the accessibility of this promising new technology, attention should be paid to developing a new generation of 3D bioprinters that are dramatically less costly but can still accommodate multichannel printing and bioinks of a variety of viscosities with minimal sample heating.

For future construction of advanced complex biological and biomimetic structures, up to artificial tissues, we will need to combine both a deeper understanding of naturally evolved systems, from proteins to tissue architecture and differentiation, and advances in analytical and manufacturing technology and multiscale modeling. The fast-growing field of computation-aided structural protein design has already significantly enhanced efforts in protein-based nanomaterial design by automating structure and function prediction. In the future, massively parallel approaches for *de novo* protein design will transform computational protein design into a data-driven big science that will utilize deep learning algorithms and methods.[107] In addition to the Internet of Things (IoT), we may also move toward the Internet of Biological Things (IoBT), where synthetic biology and nanotechnology tools will allow the engineering of biologically embedded computing devices for a vast array of applications pertaining to health, energy, and the environment.

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Notes

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