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Multiscale Biofabrication: Integrating Additive Manufacturing with DNA-Programmable Self-Assembly

*Anna C. Jäkel, Michael Heymann, and Friedrich C. Simmel**

Structure and hierarchical organization are crucial elements of biological systems and are likely required when engineering synthetic biomaterials with life-like behavior. In this context, additive manufacturing techniques like bioprinting have become increasingly popular. However, 3D bioprinting, as well as other additive manufacturing techniques, show limited resolution, making it difficult to yield structures on the sub-cellular level. To be able to form macroscopic synthetic biological objects with structuring on this level, manufacturing techniques have to be used in conjunction with biomolecular nanotechnology. Here, a short overview of both topics and a survey of recent advances to combine additive manufacturing with microfabrication techniques and bottom-up self-assembly involving DNA, are given.

1. Motivation and Introduction

Spontaneous hierarchical assembly is a hallmark of life that hinges on intricate biomaterial structure-function relationships. Correct spatial organization of biological components requires cooperative self-organization across multiple scales. For instance, enzyme efficiency and specificity at the molecular level is codified by amino-acid side chain organization in the catalytic center through protein folding. Multiple proteins can assemble into filaments to scaffold molecular motors for muscle contraction. Lipids can organize into membrane bilayer systems to compartmentalize cells into diverse organelles. This sequence of self-assembly and hierarchical organization continues all the way to different organs and body parts in larger organisms. In stark contrast, our capacity to design and facilitate synthetic self-organization over multiple length scales remains limited.

Top-down microfabrication techniques and additive manufacturing, if suitably fused with bottom-up self-assembly approaches may allow integration of ever more useful biomaterial properties into promising new life-inspired technologies. Resulting applications may achieve sophisticated spatial and hierarchical organization that matches their biological counter-parts. For instance, 3D printing in tissue engineering allows to fabricate biocompatible scaffolds and matrices that after colonization with cells may further self-organize into multicellular assemblies and tissues. A low-cost bioprinting platform, however, may lack the precision to sufficiently resolve target tissue structure. Controlling biological organization at the highest possible cellular or even sub-cellular level in turn greatly increases process cost while also limiting top-down volume fabrication rates.

Biomolecular nanotechnology is ideally poised to complement top-down biofabrication techniques. Nucleic acid nanotechnology and especially the DNA origami technique can realize almost arbitrarily shaped molecular nanostructures of 10–100 nm in size. DNA and RNA self-assembly are sequence-programmable and compatible with chemical functionalization at nanoscale resolution. Also, micrometer scale super-assemblies from DNA tectons have been achieved to create extended, space-filling 3D materials. Unfavorable assembly defect rates suggest DNA gels to be a suitable alternative avenue to integrate nanoscale ordering with lower-resolution biofabrication.

This review surveys recent advances in additive manufacturing and microfabrication techniques for their capacity to synergize with bottom-up self-assembly to spatially organize biomolecular functions over multiple length scales. Especially 3D bioprinting and other biofabrication techniques can integrate nicely with structural DNA nanotechnology and other DNA-based approaches for controlled material and pattern formation. Fundamental limitations of the different techniques are analyzed to derive optimal process windows for complementary patterning strategies. Ideal biofabrication will define minimal boundary conditions to orchestrate suitable bottom-up self-assembly cascades. For this, top-down techniques should operate at the most affordable micro- to millimeter resolution as required by the final application. Resulting soft material templates are subsequently matured down to the nanoscale via molecular self-organization and self-assembly. Finally, successful recent examples of such "hybrid" fabrication approaches will be reviewed.

A. C. Jäkel, F. C. Simmel
School of Natural Sciences
Department of Bioscience
Technical University Munich
Am Coulombwall 4a, 85748 Garching b. München, Germany
E-mail: simmel@tum.de
M. Heymann
Institute of Biomaterials and Biomolecular Systems
University of Stuttgart
Pfaffenwaldring 57, 70569 Stuttgart, Germany
The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adbi.202200195.

DOI: 10.1002/adbi.202200195

2. Bioprinting

Additive manufacturing or 3D printing of objects with complex 3D topography proceeds by sequentially structuring voxel-volume elements from a print resin material source. Initially pioneered for inert plastic, metal, and ceramic materials, 3D printing has become common place for both rapid prototyping and also final product fabrication in many sectors, such as automotive and aerospace industries, civil engineering, architecture, and the arts. The rapidly expanding range of biological and biocompatible materials coined bio-inks that can be additively structured are highly sought after for biomedical applications, for example, to create bone grafts.[1] or to engineer tissue or blood vessels.[2]

2.1. 3D Printing Methods and Their Limitations

Bioprinting is the art of patterning aqueous bio-inks into 3D hydrogel scaffolds or adhesive structures for active biological components, such as cells. These include mechanical and optical structuring methods, such as extrusion-based (**Figure 1**a), inkjet droplet-based (Figure 1b), laser induced forward transfer (LIFT, Figure 1c), and stereolithographic 3D printing (Figure 1d). Specific hardware configurations may amplify application related advantages or disadvantages of the respective methods. For example, piston, screw thread, or pneumatically actuated extruder print heads and a plethora of needle tip configurations require their own specific bio-ink properties, such as viscosity or surface tension.

Print resolution and volume fabrication rates are inversely related and their tradeoff is a common concern to every additive manufacturing project. As a rule of thumb, doubling the resolution in all three principal directions requires $2^3 = 8$ fold longer print times to complete the same volume. Print resolution is commonly defined as a measure of voxels per distance, that is, droplets per inch (DPI), that are still distinguishable. The finest feature size is the smallest voxel that can be produced and is shaped by extrusion physics or optical limitations. Lateral (XY-) and vertical (Z-direction) resolution can hence diverge in additive manufacturing methods. For example, a laser is usually easier to focus in width than in depth. Resolution of how thin or thick a line may be deposited in mechanical filament extrusion is defined by the print head needle diameter together with its travel speed. Similar to the end grain and long grain orientation in wood, the print resin extrusion direction critically affects load-bearing strength of the resulting prints.

High-resolution 3D printing methods that approach and surpass sub-micrometer lateral voxel sizes include projection micro-stereolithography[3] with a few μm, two-photon stereolithography (also known as femtosecond direct laser writing) with several 100 nm, and electrophoretic deposition[4] with less than 100 nm lateral resolution.

To recapitulate soft tissue biomechanics, print scaffolds require suitable support during fabrication to prevent them from collapsing under their own weight.[5] Especially support baths are commonly used such that the hydrogel scaffold is printed directly into a supporting liquid medium.[6] Rather than printing the desired 3D objects themselves, 3D printing can also be used to print molds (i.e., "negatives" of the structures) that are then used for casting the finobjects from a different (potentially non-printable) material.

2.2. Printing Biological Matter

There are two main approaches for the 3D printing of biological matter: Bioprints may be functionalized before or after 3D printing, either by admixing cells, bacteria, or biomolecules to the bio-ink upfront and/or by later decorating scaffolds after printing(cf. Figure 1e,f). Prior functionalization of inks affords their seamless integration into the final structure with a high homogeneity throughout the print. Using several bio-inks in parallel (e.g., cell-laden and cell-free bioink), biofunctionalized voxels can also be placed at specific points within the structure. However, the printing process itself limits which components can be mixed into the ink beforehand. Due to high shear forces, which occur during extrusion of the bio-ink, fragile components such as cells can be easily torn apart. Printing conditions such as high or low temperatures and also the printing time limit the chances of cell survival or compromise the activity of biomolecules. In particular for applications involving cells, it is therefore often advantageous to infuse a printed object with a cell suspension only after the printing process.

A dedicated overview of bio-inks and their advantages and drawbacks, with a particular emphasis on their use in the context of cell-printing is already given in ref. [7]. The mechanical, rheological, and biological properties of a material are decisive for its applicability as a bioink. Bioinks consist of biogenic and synthetic biomaterials, or a combination of both. To obtain stable structures, most inks must be solidified during or after the printing process. Most bioinks are hydrogel-based, and they can be categorized in protein-based bioinks, polysaccharide-based bioinks, decellularized extracellular matrix (dECM)-based bioink, and synthetic polymer-based bioinks. In addition, also cell aggregate-based bioinks, composite bioinks or bioinks with bioactive molecules like for example, growth factors are under development.

The most commonly used hydrogel-forming compounds for 3D bioprinting are alginate, gelatin, chitosan, collagen, methylcellulose, agarose, and carrageen. In order to decide which material is best suited for a particular application, several factors have to be considered such as long-term stability and degradability of the material, cell viability within the print, and shape fidelity. Chemical considerations such as bonding between the individually printed voxels or layers play a role, and finally of course the rheological properties such as shear thinning behavior and thixotropy (cf. the review of ref. [8] on this topic).

Depending on the nature of the biological components hosted in the printed structures, different criteria have to be met. For instance, embedding DNA molecules or sturdy bacteria into 3D printed structures is less demanding than the incorporation of living mammalian cells, but still requires moderate printing temperatures, acceptable pH values, and salt concentrations.

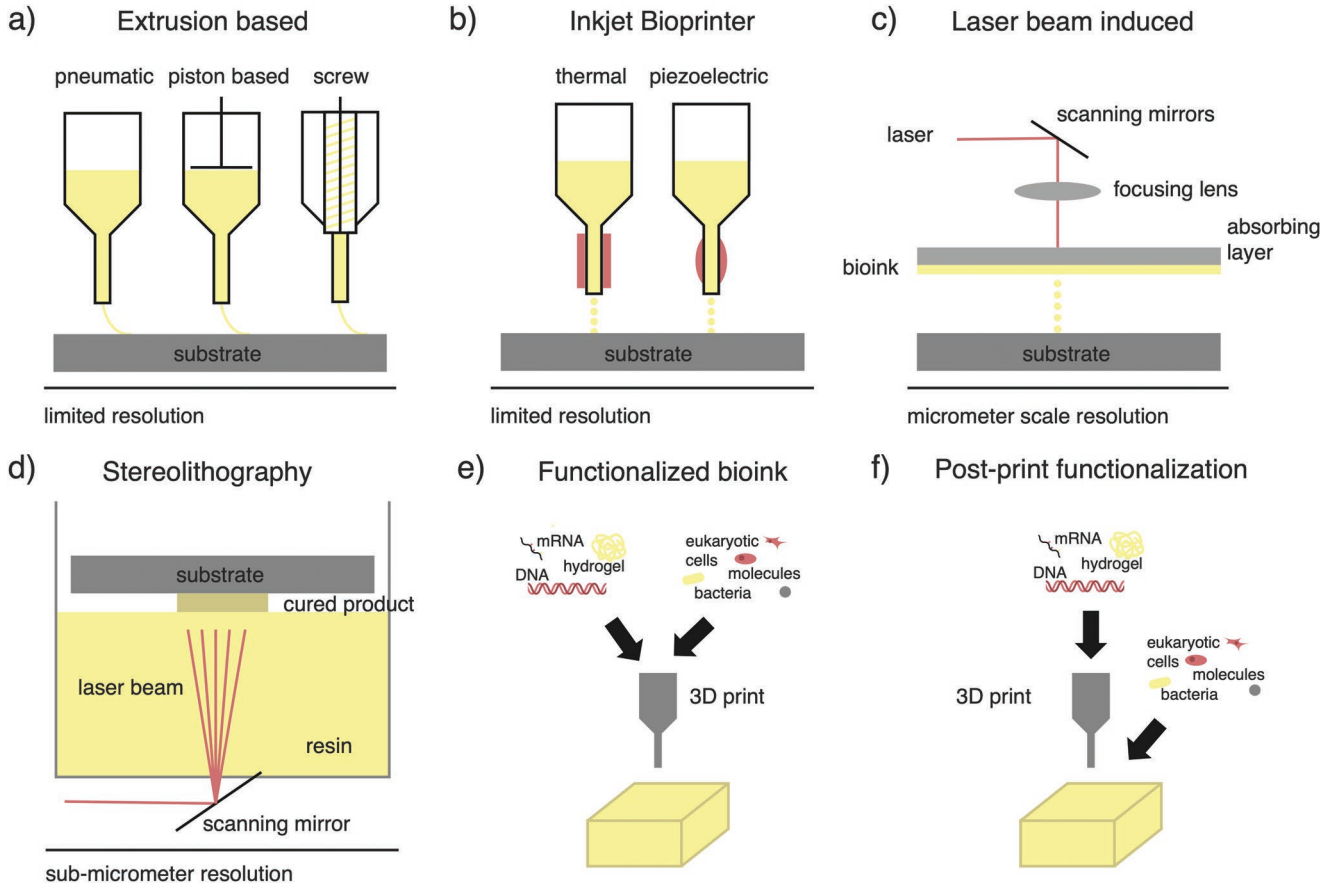

Figure 1. Schematic representation of different 3D printing techniques. a) Three major extrusion-based techniques exist, in which printed material is extruded either via application of pneumatic pressure, with a piston or with a screw. These techniques are particularly suited for printing of lines. b) In order to create structures out of small droplets, inkjet techniques can be used, which either use thermal or piezoelectric actuation. c) An alternative 3D printing method is laser-induced 3D printing, for which small droplets are released from a bioink layer with a laser. Here, the resolution is much higher compared to extrusion-based 3D and inkjet printing. d) Even higher resolutions can be achieved with stereolithography where a laser scanner is used to define structures by curing layer by layer from a resin bath. Two different approaches are used for 3D bioprinting: e) functional components—DNA/RNA/proteins for structure formation or control, bacterial or eukaryotic cells, function-enhancing molecules such as growth factors or inducers—and the bioink itself are mixed before printing of the object; or f) some of the functional components are added after printing and allowed to diffuse or grow into the object.

2.3. Other Methods to Structure Biomaterials

We note in passing that for many applications, 2D structuring of biomaterials is sufficient. For such applications, already a wide range of soft lithographic methods[9] (e.g., stamping proteins onto surfaces), inkjet-printing, spotting, etc. have been developed, which in many cases provide a better resolution than what is achievable in 3D at the moment. Planar structures can also be readily combined with microfluidics,[10] which can be used to supply the structures of interest with chemicals and remove waste products. Also methods for the direct lithographic structuring of molecules on a chip have been developed. For instance, utilization of photocleavable compounds can be employed to lithographically structure gene brushes (polymer brushes made from long gene-encoding dsDNA molecules) and thus enable spatial organization of cell-free gene expression on a chip.[11] This was applied to operate cell-free gene circuits on a chip,[12,13] and more recently to study cell-free expression and assembly of the ribosome.[14] Using electron-beam lithography, the method could even be extended to structure gene brushes in the sub-micrometer range.[15,16]

3. Bottom-Up Engineering with DNA

3.1. Structural DNA Nanotechnology

Structural DNA nanotechnology aims to realize molecular nanostructures by self-assembly of DNA molecules (**Figure 2**). The basic concept behind DNA nanotechnology is to utilize DNA base pairing to define which DNA segment interacts with another. Double-stranded DNA—with a persistence length of $L_p = 50$ nm—is a relatively rigid molecule, while single-stranded DNA is much more flexible. This allows to define molecular nanostructures composed of rigid and flexible elements—in contrast to the typical situation found in biology, multiple DNA molecules can be connected together via branched junctions, resulting in fabric-like molecular sheets or cage-like wireframe

structures (Figure 2a). Already in 1982 Nadrian Seeman, the father of DNA nanotechnology, conceived crystals composed of DNA junctions, which would aid in the arrangement of other molecules of interest into a crystalline pattern.[17] After the successful realization of 2D crystals in 1998,[18] he finally succeeded to create DNA crystals with sizes of several 100 μm in 2009.[19]

Today probably the best known variant of DNA nanotechnology is the DNA origami method,[24] which facilitates the creation of discrete molecular objects of almost arbitrary shape (Figure 2b,c). DNA origami is based on the folding of a long single-stranded DNA "scaffold" molecule into a desired shape by crosslinking the different parts of the scaffold with a large number of shorter "staple" strands. In its standard version, a scaffold of length 7000–8000 nt is used, which is derived from the single-stranded genome of phage M13. Folding requires about 200 staples of length ≈30–40 nt, resulting in molecular objects with linear dimensions in the range 10–100 nm. More recently, also other scaffolds with designed sequences have come into use, resulting in accordingly larger or smaller objects.[25] The DNA origami approach had been initially used to create flat single-layer structures,[24] but was quickly generalized to 3D multi-layer objects.[26] Next to DNA origami, also highly efficient scaffold-free techniques such as single-stranded tile assembly were developed.[27,28] Importantly, chemical modification of specific staple strands enables the precise geometric arrangement of other molecules on top of the DNA origami structures with nanometer precision.

In order to create larger structures, various approaches were explored to self-assemble multi-component assemblies from DNA origami subunits. Molecular objects with sizes up to several Gigadalton were created from origami building blocks with shape-complementary docking sites,[29] a technique, which also was used to create large molecular cages (with cavity diameters of up to 280 nm), which were inspired by the assembly of viral capsids.[30] An approach to compose DNA objects from multiple subunits in a hierarchical assembly scheme successfully resulted in the realization of micrometer-scale non-periodic structures.[31] More recently, also crystalline arrangements of DNA origami structures were demonstrated,[23] and a novel technique controlling the nucleation barrier for the realization ("crisscross polymerization") of grid-like molecular assemblies from DNA origami slats was shown to yield very large DNA assemblies with essentially zero background nucleation of undesired structures.[32]

More complex assemblies than simple periodic arrangements or cages can be created by implementing molecular algorithms to control their growth. To this end, the unique sequence-dependent interactions of DNA molecules have been utilized to implement cellular automata-like self-assembly of DNA tiles,[33] binary counters,[34] or iterated Boolean circuits.[35] Importantly, algorithmic assembly enables the efficient generation of complex patterns, or non-periodic and finite molecular structures based on a comparatively small number of building blocks. For instance, it is easy to realize periodic arrangements of building blocks by simple polymerization—but this will result in polymers (or crystals), which have a distribution of sizes and potentially many defects. Algorithmic DNA self-assembly has explored methods to prevent the generation of defects (using "proof-reading" tiles[36,37]), or to create finite patterns, for example, by counting.[38]

An interesting recent development is the integration of mechanical and information processing capabilities for the realization of DNA nanorobotic systems. Such systems were shown to be capable of programmably assembling gold nanoparticles,[39] or sort, move, and place molecules.[40,41] Recently, the first example of a "molecular printer" constructed from DNA was demonstrated.[42] For a more thorough overview of structural DNA nanotechnology and the DNA origami technique, the reader is referred to dedicated reviews such as refs. [43–46].

3.2. Programming Self-Organization with DNA

DNA has not only been used to create well-defined molecular nanostructures, but also to direct other self-assembly and self-organization processes, which are of relevance for biofabrication.

3.2.1. Gels and Liquids

In one range of applications, DNA tags have been used to only control the interactions between nanoscale building blocks and thus assemble them into amorphous superstructures. For instance, DNA three-way and four-way junctions (sometimes referred to as Y-DNA and X-DNA) have been extensively used to realize DNA gels (**Figure 3**a), in which the DNA tectons were connected without any long range order.[47,48] The pore sizes and rheological properties[49] of such gels can be programmed via the sequence, and chemical modifications can be used to trap and release other molecules using these gels or perform chemical reactions.[50] A related approach had been used earlier to generate gels from polyacrylamide modified with DNA-based crosslinkers (using acrydite modified gels), and also in this case the pore size and rheology could be controlled by DNA length and cross-linking density.[51] Also hybrid DNA-protein nanogels were developed using biotin-streptavidin interactions for cross-linking, where the gel properties could be controlled via the protein crosslinks rather than via DNA hybridization.[52] A large variety of methods have been employed to make DNA-based gels switchable and shape-changing, including DNA strand displacement,[53,54] pH,[55] ion responsive DNA motifs,[56] aptamers,[57] enzyme action,[58,59] or temperature.[60] For reviews of responsive DNA gels see also.[61,62]

In the past decade, it has become increasingly clear that phase separation and coacervation processes play an important role in cell biology (**Figure 4**).[65] Molecular condensates were found to form liquid droplets inside of living cells, which provide a means to concentrate and compartmentalize certain molecules without the need for a dedicated membrane enclosure. Many of these condensates—Cajal bodies, P-bodies, the spliceosome, etc.—involve RNA molecules interacting with multiple RNA binding proteins.[65,66] Liquid-like coacervates have also been created with simpler composition,[67] for example, using ATP and polyallyl amine,[68] or RNA and peptides.[69,70] By tuning the interactions between DNA tectons, one can also create liquid-like structures from Y-DNA and

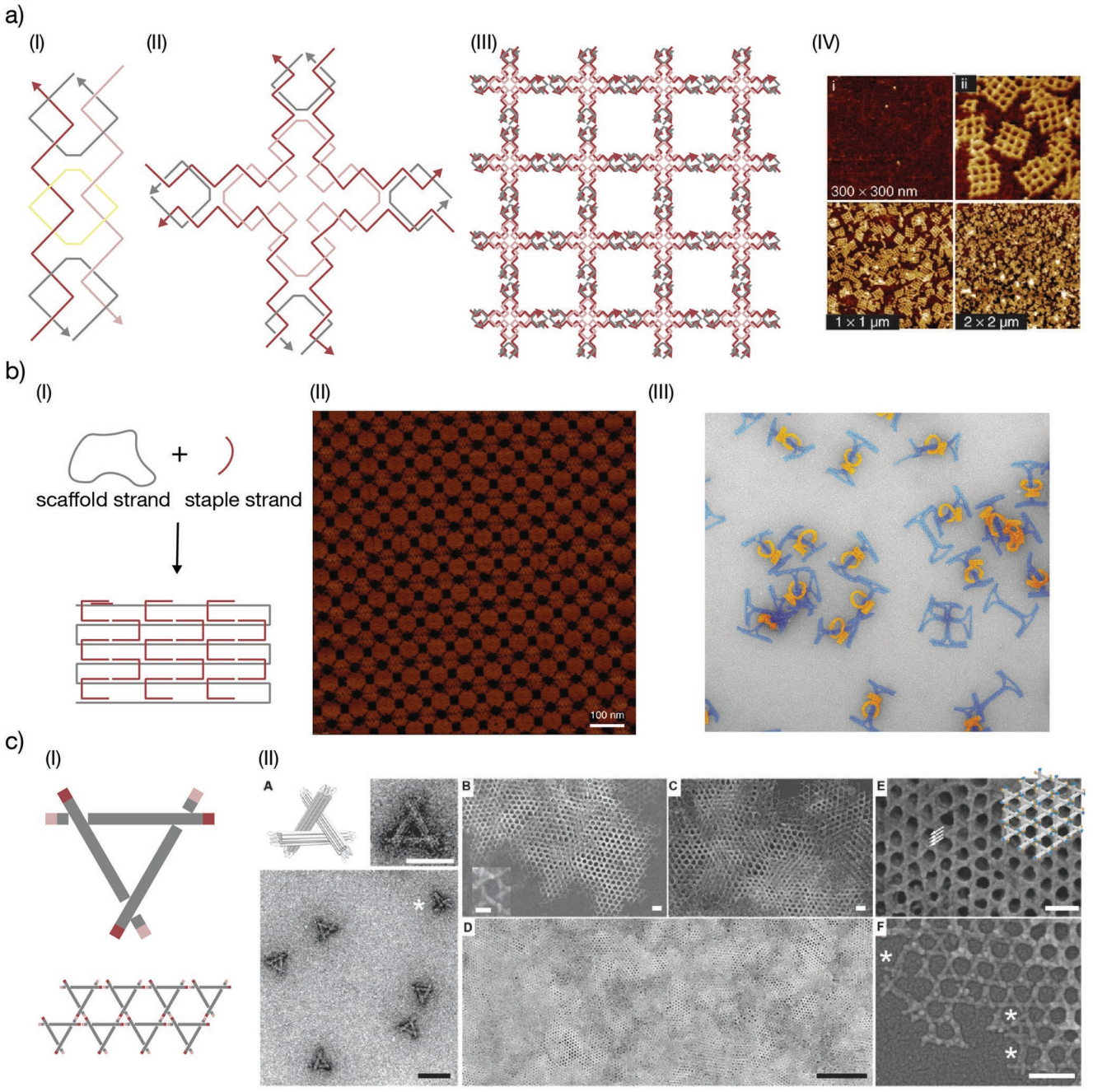

Figure 2. DNA as building material for 2D and 3D objects. Schematics and examples of a) DNA tile assembly, b) DNA origami, and c) crystals from DNA origami structures. In (a) a double-crossover DNA tile (I) and a DNA-cross tile (II) are shown. Through the concatenation of the cross tiles a planar grid (III) is formed. An AFM image of such a grid is shown in (IV). Reproduced with permission.[20] Copyright 2006, Wiley Online Library. b) DNA origami structures (I) are formed by site-specifically hybridizing a large number of DNA staple strands with a long single-stranded DNA scaffold. Origami structures can be used, for example, as tectons to generate planar 2D lattices (II). Reproduced with permission.[21] Copyright 2020, Nature Publishing Group. Also multicomponent 3D structures can be generated, the example shows a transmission electron microscopy (TEM) image DNA rotaxane constructs, which are composed of ring structures (yellow) threaded onto dumbbell structures (blue) (III).[22] c) DNA origami structures can also be connected periodically to form crystal-like structures. (I) shows a schematic illustration of triangular DNA origami building blocks and their assembly. Electron microscopy images of such origami crystals are shown in (II). Reproduced with permission.[23] Copyright 2018, Advanced Materials.

X-DNA rather than gels.[71] Finally, Y-DNA has been used to generate a "cortex" for synthetic cells,[72] and there have been attempts to generate DNA nanotube-based cytoskeletons inside droplets and artificial cells.[73,74]

3.2.2. Chemical Reaction Networks and Reaction-Diffusion Systems

DNA has also turned out to be extremely useful for the realization of synthetic biochemical reaction networks that can be

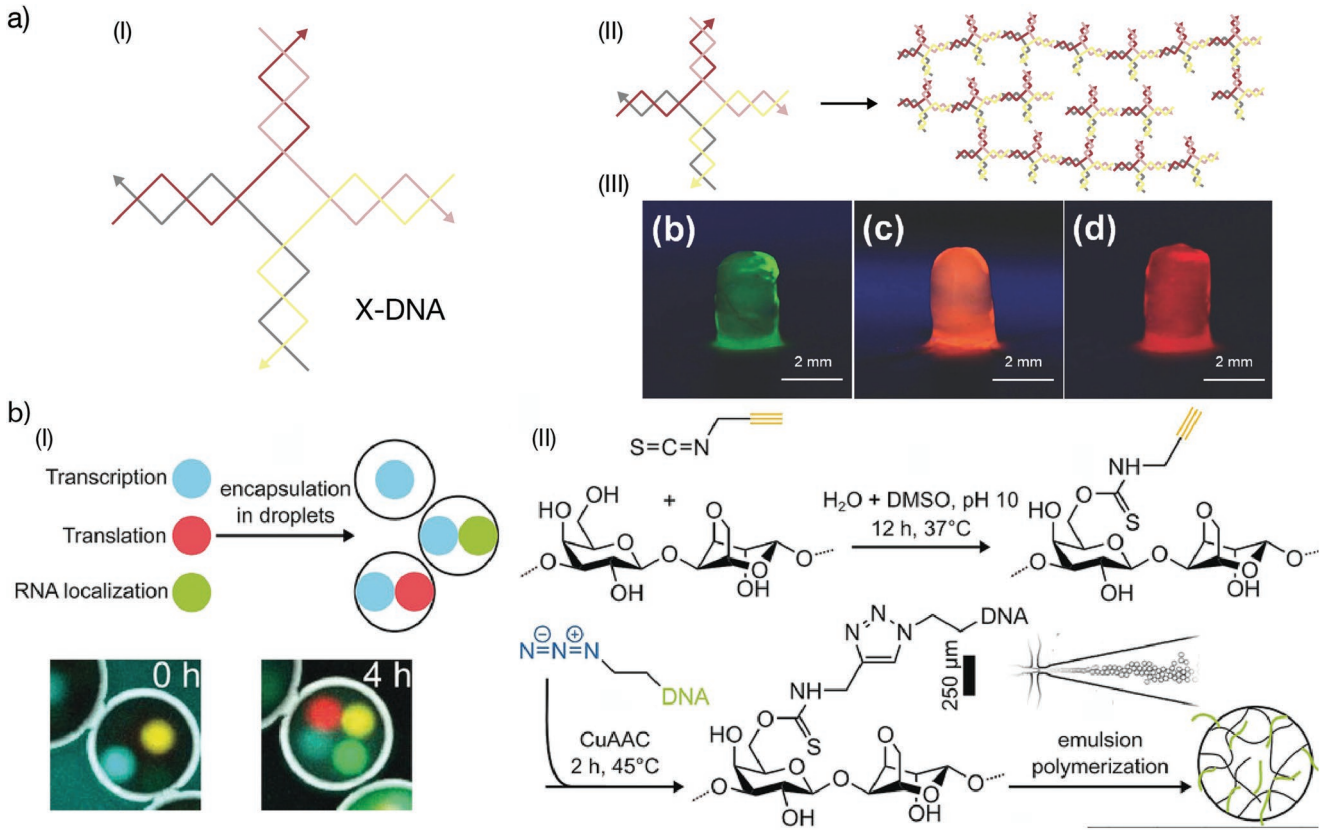

Figure 3. DNA functionalization of hydrogels. a) Gels composed entirely of DNA. DNA four-way junctions with sticky ends ("X-DNA" (I)) can be used to form DNA gels. The branched DNA monomers are linked via DNA hybridization to form extended gel networks (II). For visualization, X-DNA gels can be fluorescently labeled using fluorophore-conjugated ssDNAs that are complementary to the sticky ends of the X-DNA linkers (III). Reproduced with permission.[63] Copyright 2015, Wiley Online Library. b) Hybrid hydrogels functionalized with DNA. In this example, agarose microgel spheres are functionalized with DNA molecules to enable spatial organization of cell-free transcription and translation reaction (I). To this end, agarose is modified with PITC and coupled to azide-modified DNA, monodisperse microspheres are produced using droplet microfluidics (II). Reproduced with permission.[64] Copyright 2018, Wiley Online Library.

used to mimic biological networks, or used as sensors, controllers, and computers. One of the core processes used for the realization of such dynamical networks is the so-called DNA strand displacement process, in which an input strand binds to a DNA complex composed of a DNA duplex and a short, single-stranded extension called "toehold," and then displace the "incumbent" strand in a three-way branch migration process (for a review see ref. [76]). Such processes can be used to switch nucleic acid complexes between several mechanically distinct states, or activate/deactivate certain sequence domains by making them more or less accessible. In the so-called "genelet" approach, for example, a transcription template for T7 RNA polymerase is controlled by adding or removing a DNA strand that contains an essential part of the promoter sequence, which can be used to mimic gene regulatory processes found in naturally occurring gene circuits.[77,78] In a related approach called "DNA toolbox," similar control is exerted over the production of DNA (rather than RNA) molecules in a strand displacement amplification (SDA) process.[79,80] Genelets, DNA toolbox, and even strand displacement systems operated in the absence of enzymes have been successfully used to realize a variety of dynamical behaviors such as bistability,[77] chemical oscillations,[78,79,81,82] or transient dynamics.[83]

Of relevance for the realization of biomaterials, DNA-based reaction networks have also been utilized to generate spatial structure. In a spatial context, DNA-based feedback networks have been shown to generate spiral[84] and shock waves,[85] they have been used to realize propagating chemical pulses in networks of emulsion droplets,[86] or to implement the famous "French flag" pattern known from developmental biology.[87,88] It is also possible to couple DNA-based reaction networks to DNA-controlled assembly/disassembly processes, for example, by using DNA/RNA species occurring in the networks to displace connections between nanoparticles or DNA assemblies.[88] In this way, a French flag pattern could be transferred into a pattern of microparticles, whose density was spatially modulated in accordance with the pattern.[88] Inspired by the active, non-equilibrium processes involved in the growth of cytoskeletal filaments, a genelet-based oscillator was used to grow and destroy DNA-based nanotubes.[73] Similarly, genelets and the DNA toolbox were shown to dynamically direct the assembly and disassembly of colloids into large aggregates,[89,90] and even the movement of microparticles along colloidal scaffolds.[91]

While the well-defined structures developed in structural DNA nanotechnology provide a means to control the position of molecules with nanoscale precision, the complementary approaches

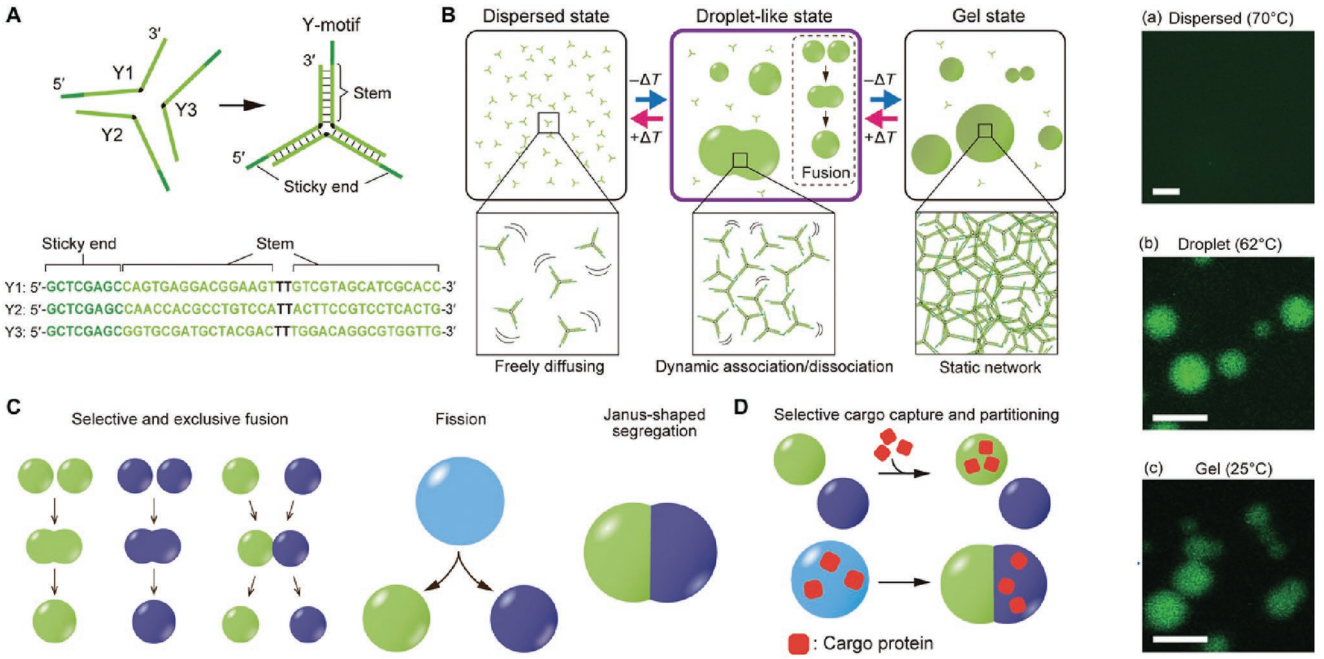

Figure 4. Branched DNA structures can be used to generate liquid phase separated structures. In the example shown here "DNA droplets" are formed from designed DNA nanostructures, whose properties and behavior can be controlled by their sequence. a) The droplets are formed from three-arm junctions with sticky ends ("Y-DNA"). b) The Y-motif complexes are dispersed in a solution at high temperatures and phase separation is induced via a temperature decrease, resulting in the formation of a liquid-like droplet state, which can further change to a gel state. Corresponding fluorescence images are shown on the right. c) DNA droplets can fuse and split depending on the Y-DNA sequences, and also d) cargo proteins can be captured inside the droplets. Reproduced with permission.[75] Copyright 2020, American Association for the Advancement of Science.

highlighted in this section are also of considerable interest for biofabrication. Gels and droplets can be formed faster, at larger scales and more easily than, for example, DNA origami structures. Further, they can create dynamic microenvironments, which allow the exchange of components with their surroundings via diffusion. Chemical reaction networks add another layer of control to biomolecular assembly processes. In principle, they could be used to direct growth processes, realize dynamically re-configuring materials, and materials responding to their environment in a context-dependent manner—thus opening up an approach toward the realization of "4D biomaterials."[89]

4. Limitations and Opportunities

Ultimately, we seek to control biological matter down to the finest possible scale, but what is the best way to achieve this? Both, "top-down" lithography and additive manufacturing methods or "bottom-up" mediated DNA self-assembly and other self-organization phenomena have their respective advantages and disadvantages. These pertain to physical limits, material incompatibilities, but also economic aspects such as speed or cost. Notably, a top-down approach can at most set the initial and boundary conditions, but by itself is insufficient to allow for growth, reconfiguration or dynamical responsiveness.

4.1. Length Scales

Every biofabrication method is inherently limited in the length scales that can be achieved or controlled, as well as the times necessary to create a given structure or to achieve such control. As noted above, additive manufacturing can realize 3D objects with a spatial resolution from 1 mm down to 100 nm, depending on the method. Extrusions are limited by the nozzle size and bio-ink properties, while two-photon polymerization is physically limited by the wavelength of the light used. All these voxel dimensions correspond to rather large molecular populations.

Scanning probe methods, in principle, enable manipulation and placement of molecules with nanometer precision, but are probably not useful for creating extended 3D structures. Lithographic methods—in 2D—such as photolithography or electron-beam lithography enable control of structures down to several 100 nm or a few nm, respectively. These structures are initially defined in an appropriate resist and subsequently have to be replaced by or filled with a material suitable for the desired application.[92] They may perhaps set initial conditions for growth processes starting from a surface. However, none of these techniques offers the spatial resolution to position a group of molecules with respect to each other with nanometer precision. Conversely, DNA nanostructures enable such control (the combination of lithography and DNA origami has, in fact, been utilized in nanotechnological applications,[93] for a review see ref. [94]).

In principle monolithic origami structures can be generated, in which the position of a specific DNA base or a molecule attached to one of the DNA staples is nanometer precise. Functionalization can be achieved at a specific base pair, which corresponds to a resolution of 0.34 nm in the context of a double helix. Structural irregularities and fluctuations in DNA origami scaffolds reduce this resolution accordingly. However, it is more difficult to translate this precision to larger structures.

Assume that a single molecule should attach with nanometer precision in a cube with a given side length of ≈100 μm. It is possible, for example, to create molecular crystals composed of DNA subunits,[19] but the arrangement of other molecules on these 3D scaffolds has so far been challenging due to the fragility of the DNA crystals.[95] Also arrangement of origami subunits and single-stranded DNA tiles into crystal-like assemblies was demonstrated,[23] potentially leading to more robust structures. These approaches, however, naturally lead to a periodic arrangement of many copies of the molecule, and not the precise positioning of an individual molecule at a specific point. While non-periodic structures may be programmed with DNA by using several types of DNA subunits, only some of these would be modified with the molecule in question. Non-periodic, but controlled arrangement can then be achieved by brute force (adding unique connectors to each component), by executing suitable molecular programs (e.g., using a molecular counter as in ref. [34]), or by using a smart hierarchical assembly approach.[31] However, defining all the interactions between the DNA subunits with distinct sequences is exceedingly more difficult or even impossible for large structures. While perfect crystalline assembly is important in several applications like X-ray diffraction studies or nanophotonics, it may not be required for the fabrication of "life-like" materials. After all, biological organisms are rarely crystalline.

4.2. Material Complexity: Mechanics and Supply

Nature pursues extreme spatial control only when it is functionally required, as for example, with microtubules or the flagellar motor. Other structures follow alternative ordering mechanisms that are not necessarily precise down to the nanoscale and also accept more irregular organization. In some cases only the spatial proximity of molecules is important. In other cases spatial organization involves compartmentalization and possibly rough positioning of the subcompartments in the cellular context. In other words, Darwinian evolution has intricately optimized such structure–function relationships with part counts often exceeding current biofabrication methods. For example, *Escherichia coli* flagella are assembled from 24 core proteins,[96] while multi-material biofabrication methods hardly exceed two or three different materials.[97]

When inkjet printing a picture, any color can be readily mixed from a few base colors. Respective dot patterns on the paper overlay to macroscopically represent the desired color. Such simplicity is elusive for most biomaterial properties. For instance, biomechanics result from detailed molecular interactions and cannot be simply formulated at will, for example, when seeking a particular shear-thinning or shear-thickening profile. Accordingly, often extensive material optimization such as bioink rheology is required to render it printable for a given technique. Free-standing structures then often have to be cross-linked to achieve enough stability. Admixture of particles that improve the rigidity of the final prints is limited due to jamming effects. This detailed optimization of extensive material chemistry and engineering in turn requires suitable quality control, all of which increase cost for often perishable bioink resins.

A combination of top-down and bottom-up approaches is highly promising. At the cellular scale, biological structures obtain their mechanical properties from the cytoskeleton, cell cortex, or (for bacteria and plant cells) the cell wall. Multicellular structures are held together and stabilized by an extracellular matrix, and larger organisms require bones, wood, or other support structures following a complex hierarchical assembly instruction. In analogy, a rigid biopolymer matrix may be grown during or after bioink deposition to provide additional support and flexibility for the final structure. Conversely, conventional fabrication methods may be used to generate large-scale scaffolds or "skeletons" for the soft biological components. Such in situ material differentiation will hinge on robust self-organization at the respective scales.

4.3. Reaction-Diffusion as Massively Parallel Computation

DNA molecules can "program" reaction-diffusion systems such as chemical waves or other types of self-organized spatial patterns (**Figure 5**). The spatial resolution of such processes is expected to be roughly on the order $\lambda = \sqrt{D/k}$, where D is a diffusion constant, and k the typical rate of a chemical process involved. For typical values such as $D = 10\ \mu m^2\ s^{-1}$ and $k = 0.1\ s^{-1}$, we expect spatial patterns on the order of 10 μm. In general, reaction-diffusion processes will only generate comparably large scale structures and hence are not suited for nanoscale positioning of objects.

The importance of such processes lies in the fact that they are *autonomous*, which suggests a different role for them in the context of biofabricated materials. Intriguingly, they may orchestrate further material sub-structuring *after* printing/fabrication. They could thus accommodate to specific environments and potentially change in response to external influences. Apart from pattern formation processes, also growth processes or dynamical reconfiguration of components could be of interest in this regard.

So far however, de novo design of suitable reaction-diffusion mechanisms remains challenging, as reaction rates and cross-catalytic sensitivities need to be carefully tuned. For instance, a canonical genetic switch operating with two mutual repressors[100] or the repressilator[101] exhibit phase portraits with complex bifurcation dynamics (Figure 5a). The perhaps most well understood biomolecular reaction-diffusion system is the MinDE oscillator, which is involved in defining the bacterial division plane and has been extensively studied mechanistically in vitro.[102] Intriguingly, also oscillatory and stationary patterns with defined wavelength have been accomplished.[103] In operating such molecular programs on 3D printed scaffolds (Figure 5b top),[98] they may define suitable downstream reaction cascades for selective material maturation. For instance, an elegant example of combined chemical and subsequent physical Turing type differentiation was achieved for chemical cells[99] (Figure 5b bottom). Here initially identical droplets encapsulating the oscillatory Belousov–Zhabotinsky reaction organized within minutes into reduced and oxidized states, which convert malonic acid substrate into carbondioxide at distinct rates. Over time this results in an increasing osmotic imbalance between both chemically differentiated droplet

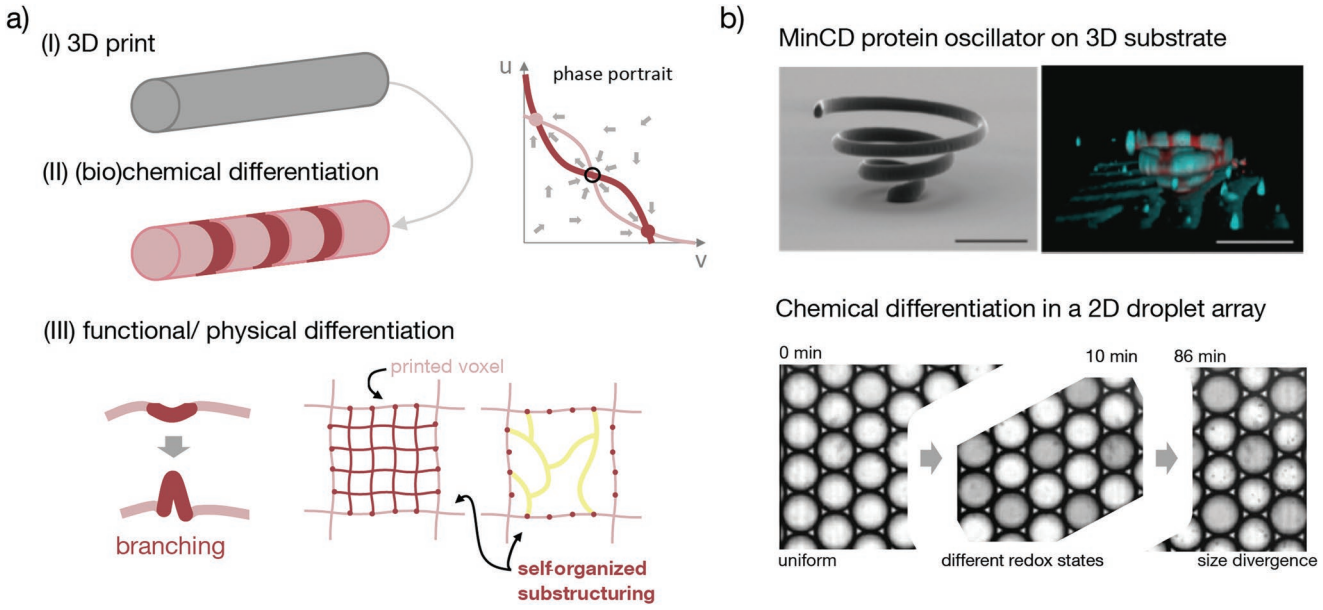

Figure 5. Morphogenetic differentiation of printed scaffolds. a) Morphogenetic molecular programs may further sub-pattern low resolution printed scaffolds (gray). Two morphogens u (light red) and v (dark red) act as molecular rulers to mark sectors via a tailored reaction-diffusion diffusion mechanism. This requires rationally designed reaction networks (phase portraits). Downstream processing then rewrites critical biomaterial properties in the scaffold accordingly. These may involve zonal biomineralization, scaffold rigidity adaptation, or higher resolution tiling through suitable self-organization processes. This may be accomplished, for example, via growth of DNA-based molecular grids acting as breadboards or coordinate systems with nanoscale resolution. b) The MinDE protein oscillator forms traveling waves on supported lipid bilayers. Different surface-to-volume ratios on the 3D spiral scaffold result in different patterns compared to a homogeneous 2D surface. Reproduced with permission.[98] Copyright 2021, Royal Society of Chemistry. Alternatively, a 2D voxel array assembled from about 60 µm diameter emulsion droplets encapsulating the Belousov–Zhabotinsky reaction of identical composition was shown to initially undergo a fast chemical differentiation via a Turing mechanism, followed by a slower physical differentiation through osmotic droplet volume adjustment. Adapted from ref. [99].

populations. Osmotic re-equilibration through water vapor diffusion between neighboring drops then results in noticeably distinct final droplet volumes for both states. A critical limitation of the Belousov–Zhabotinsky reaction is that only few biomolecules can tolerate its low pH. Promising efforts to rationally engineer desired reaction profiles are hence focused on nucleic acid programs[104] or computational protein design.[105]

4.4. Biofabrication Rates and Information Density

Another important physical consideration refers to the time it takes to build a specific structure. When printing voxels sequentially, this is simply the average print time needed per voxel times the number of voxels. By contrast, chemical self-organization processes like aggregation, growth, and reaction-diffusion patterns are limited by reactant diffusivity to their site of attachment and/or their speed of production and the assembly reaction involved. Diffusion coefficients are in the range of $D = 100 - 1000\ \mu m^2\ s^{-1}$ for proteins and small molecules under typical conditions, which translates to diffusion times $\Delta t \sim \Delta x^2/D$ on the order of 10–100 h and more in objects of cm scale. In dense gels and under conditions of molecular crowding, these time scales will be considerably longer.

Nature has exploited various strategies to circumvent diffusion limits in large organisms—including the use of active transport by molecular motors, but also by utilizing supply lines (vasculature) for tissues and by distributed production of the building blocks required for growth. For convenience of the argument, large organisms can be considered similar to an unicellular organism that has multiple cell nuclei, or a structure of many cooperating cells acting in concert. Top-down biofabrication may aid in speeding up growth processes by emulating these strategies—for instance, bioprinting could be used to define multiple active growth centers or seeds, from which growth processes can start in parallel. Also, biofabricated structures can be equipped with supply channels that ensure optimal transport of nutrients and dispose of waste products.

Typical growth or generation times of biological organisms can inform on achievable bottom up biofabrication rates. Bacterial generation times range from below 10 min under optimal conditions for certain bacteria such as *Pseudomonas natriegens* and can range up to several days.[106] The laboratory workhorse *E.coli* takes ≈20 min to double its size under optimal conditions (its genome takes ≈40 min to replicate).[107] With a size of ≈ 1.5 μm^3, *E.coli* contains ≈3×10^6 proteins, which have to be produced by about 20 000 ribosomes in the cell for each doubling. Cell cycle times of eukaryotes are on the order of hours (e.g., budding yeast) and days. Mammals take weeks, months, or even many years to develop into a full-grown organism. Plants vary in growth rates over six orders of magnitude.[108] These different growth rates reflect fundamental biosynthetic limitations on the one hand, but also the time it takes for complex developmental processes and adaptations to different environmental niches and resource limitations. In this sense,

bottom up "fabrication speeds" cannot be directly compared to top-down biofabrication methods.

4.5. Inscribing Biological Self-Organization and Feedback Locally

Top-down bioprinting techniques may generate large structures with low resolution and low complexity faster, but biological organisms in contrast achieve much more complex, finer detailed, dynamic, and responsive structures. A bioprinter can generate cell-sized voxels of a single (or a few types) of materials per printing step, but cells generate structures with millions of proteins of thousands of different types per each generation.

What is, thus, the best strategy to create a block of living material that is precisely defined from the meter to the nanometer scale? First, and foremost we need refined computer aided design tools to not only specify desired shapes, but also all the required self-organization and self-assembly processes. "Top-down" biofabrication tool-path planning languages, such as G-code, must hence be complemented with suitable "gene"-coded molecular program information. In fact, the ability of DNA to store information about the fabrication process itself was already recognized. Under the DNA of things paradigm, digital design shape information were compiled into DNA, which was then embedded into the 3D printing filament.[109] In this way, all key design and process information can be obtained by sequencing the DNA snippets embedded into the object itself. By further increasing the information stored in such DNA of things genomes, more and more regulatory elements and self-synthesis capacity may be achieved.

5. Biofabrication Meets DNA-Based Self-Organization

5.1. Going Macroscopic with DNA-Functionalized Gels

As argued in the previous sections, a combination of top-down methods with self-assembly and self-organization processes could be used to generate highly structured, resilient life-like materials with the ability to grow and respond to their environment in multiple, context-dependent ways. As also pointed out, DNA has already been used for the realization of 3D molecular objects in the nanometer to micrometer range, for tuning the properties of hydrogel structures and colloidal assemblies, for storing and processing of (non-biological) molecular information as well as for the realization of molecular control programs and reaction networks. DNA nanotechnology can thus potentially provide the molecular structures and control circuits required for the "bottom-up" self-organization aspect of the biofabrication process, while top-down methods can be used to set the boundary or initial conditions. Recent work has shown that indeed the two approaches can be fruitfully integrated with each other (for examples see **Figure 6**).

The ability of complementary strands of DNA to bind together has been exploited to provide a sequence-programmable adhesive in hydrogels for bioprinting. DNA can be used either as a crosslinker or to interconnect small building blocks to form larger objects, but also to change the mechanical parameters of the printed material itself. For instance, a DNA hydrogel was developed,[58] which formed by hybridization of Y-DNA as a scaffold and linker DNA, exhibited thermal and enzymatic responsiveness that could be controlled by properly tailoring the "sticky ends" of the DNA building blocks.

DNA modifications were not just used to change the parameters of a hydrogel suitable for printing, but were also utilized to direct the growth of larger structures through self-assembly. In an approach to use DNA-directed assembly to build larger structures from hydrogel blocks,[113] DNA was used as a sequence-specific glue to define the association of shape-controlled gel cubes into prescribed 3D superstructures, resulting in aggregates with sizes in the μm to mm range. A similar approach was used in another study[112] that used DNA-coated colloids to realize a gel, which was held together by DNA hybridization interactions (Figure 6d). The resulting colloidal gel was shown to be form-stable after deposition with a 3D printer and could be used to create objects with sizes of several millimeters.

As mentioned earlier, DNA hydrogels can be modified to become responsive to external stimuli, which can be used to influence their properties also after the printing process. For instance, a polypeptide-DNA hydrogel was developed,[114] which allows printing with two composites, which create a solid structure only after mixing. The resulting gel is degradable with proteases or nucleases and its mechanical properties can be adjusted via the conformation of the polypeptide backbone, as well as through an external stimulus such as a pH-change.

In recent years, DNA-based hydrogels have been increasingly applied in a biomedical context. For instance, multifunctionalized DNA gels were utilized for tissue regeneration,[115] whereas in another approach DNA hydrogels containing upconversion nanoparticles (UCNPs) were applied for cell engineering,[116] where the UCNPS provided protection of cells against irradiation damage.

5.2. Dynamics and Embedded Information

Most of the examples described so far only made use of DNA as a structural component, as a cross-linker or for the incorporation of chemical functions or nanomaterials. Research in dynamic DNA nanotechnology and DNA computing has indicated the immense potential of DNA beyond structural applications, in particular as a molecular material for information processing, realization of programmable dynamic reaction networks, and, of course, for information storage. In recent years, researchers have thus started to explore the integration of more advanced DNA-programmed functions into materials.

An important step in this direction was taken with the realization of shape-changing polyacrylamide hydrogels with sequence-programmable DNA modifications[110] (Figure 6a). The gels were structured using photolithography, which enabled the realization of various shapes and precise functionalization of different areas of the gel. Actuation itself was achieved using a DNA hairpin chain reaction (HCR),[117] which could be used to selectively expand specific gel regions, leading to movements and shape changes. This work thus already points toward applications in soft robotics, in which materials functions can be

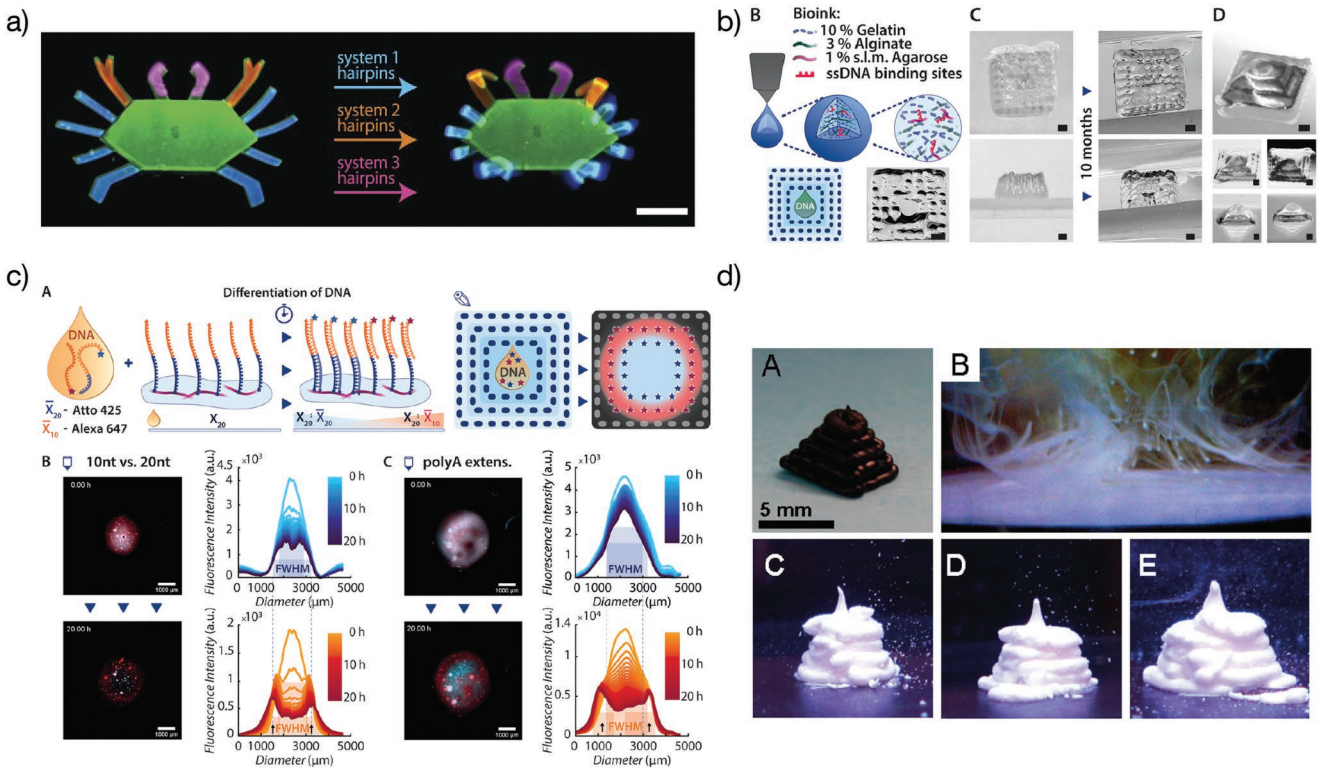

Figure 6. Combination of top-down manufacturing with DNA self-assembly and self-organization. Manufacturing techniques like photolithography or bioprinting are used to define macroscopic 3D objects and DNA is used to further functionalize these structures on a smaller lengthscale. a) Multilayer planar soft machines were built from polyacrylamide slabs that were structured using photolithography. The polyacrylamde was coupled to single-stranded DNA and cross-linked via DNA hybridization. Shape changes can be induced by changing the length of the linkers. Specifically, the degree of swelling of the gels can be increased by expanding the cross-links successively using DNA hybridization cascades. Reproduced with permission.[110] Copyright 2017, American Association for the Advancement of Science. b) Hydrogel structures were 3D-printed with an extrusion-based 3D bioprinter using DNA-functionalized bio-ink, which allows localization of DNA-tagged molecules at ssDNA binding sites inside the printed structures. Reproduced with permission[111] Copyright 2020, Wiley Online Library. c) These DNA-functionalized hydrogel structures can be used to implement simple reaction-diffusion patterning involving DNA hybridization and strand displacement reactions. In the image, fluorescently labeled DNA is added as a diffusable signal to the center of a gel print (A). DNA molecules of different length/degree of complementarity to DNA anchor strands linked to the gel then localize at different positions (B,C). Reproduced with permission.[111] Copyright 2020, Wiley Online Library. d) DNA can also be used to form gels from DNA-coated colloids using DNA:DNA interactions as a sequence-programmable "glue" to hold the shape after 3D printing of the structures. Reproduced with permission.[112] Copyright 2015, ACS Publications.

programmed via dynamic DNA processes. Notably, sequence-addressable molecular processes are mechanically amplified in such materials to generate macroscopic movements.

DNA-based chemical reaction networks have also been utilized to dynamically control the spatiotemporal properties of soft materials in other contexts. For instance, a light-responsive DNA circuit embedded into a gel, containing DNA components with photocleavable compounds, was shown to be capable of autonomous edge detection,[118] dynamically generating a sharp boundary between dark and illuminated regions of the gel. More recently, simple sender–receiver and reaction-diffusion processes were implemented in 3D-printed gel structures based on a DNA functionalized bioink[111] (Figure 6b,c). DNA signals diffusing through the gel from a source were selectivel immobilized in different DNA tagged regions of the gel, where they could also release secondary DNA signals via a strand displacement process. Even the genetic coding properties of DNA can be used in the context of hydrogels to spatially organize cell-free protein expression reactions. For instance, in ref. [64], double-stranded DNA gene templates were incorporated into small agarose gel beads, which enable the spatial organization of transcription and translation reactions (Figure 3b).

In a quite different approach also the information-storage capacity of DNA—rather than structural or dynamical properties—has been recently utilized in a materials context. The "DNA of things" concept mentioned above envisions the use of DNA to embed the information required to generate an object within the object itself.[109] Conceptually, this points toward materials of much higher complexity and information content than materials traditionally used in mechanical engineering.

5.3. Toward Life-Like Materials with a Metabolism

Utilizing DNA as a molecular tag to increase the information content of soft materials, or DNA-based equilibrium self-assembly to improve the spatial resolution of top-down fabrication methods benefit from the relative robustness of

DNA molecules compared to other types of biomolecules, and it can be expected that DNA-boosted materials will find increasingly widespread applications.

More complex functions involving growth processes, computation, actuation, reconfiguration, and attentiveness, however, will inevitably require the operation under non-equilibrium conditions, which demand for a continuous supply with nutrients and removal of waste products. Taking ATP consuming enzymatic reactions as an example, continuous operation would require a supply of the material with ATP and replenishment of the enzymes themselves (which likely will lose activity over time). One could also strive for generating these components in situ, which would require a metabolism to generate ATP, and cell-free expression of the enzymes. This in turn poses major additional hurdles, as the cell-free expression system itself would need to be replenished, which will be extremely costly for extended objects. Researchers worldwide are working on self-regenerating systems,[14,119–121] but these are, as of yet, out of reach. In fact, the problems described here are shared, to a large degree, with the challenges faced in projects aiming at the creation of synthetic cell structures.

In the absence of such systems, hybrid approaches may prove useful, in which biological cells or organelles are embedded into the materials, which would allow to utilize their biosynthetic and metabolic capabilities and would only require supply of "simple" nutrients contained in typical growth media. In the synthetic cell context, photosynthetic vesicles,[122] chloroplasts,[123] or mitchondria[124] were used to provide ATP. There also have been various attempts to embed live bacteria or even eukaryotic cells in 3D-printed gels, which might prove useful in this context.[125–127]

Even if there was a functioning metabolism in place, spatially extended life-like materials will require efficient ways to supply nutrients and purge waste products, which demands for a synthetic "vasculature"—the creation of such vasculature might in fact be achieved using a 3D printing approach.

6. Conclusion and Outlook

The combination of 3D bioprinting and DNA nanotechnology appears to be a suitable approach to overcome the limitations of ultra fine structuring with additive manufacturing techniques on the one hand and unfavorable high assembly defect rates on the other hand. With pre-structuring, through additive manufacturing to set boundary conditions for self-organization of the smaller elements to achieve resolutions of sub cellular level, these limits can be handled. This hybrid approach leads us to entirely new synthetic systems and opens up a wide range of possible applications.

DNA can be used to define and control the rheological parameters of bioinks, but also to create a grid on a level below the printing resolution. Spatial positions could be addressed to change the hydrogel's parameters locally or to position functionalization elements inside the printed 3D structure. Another possibility would be to incorporate live material like bacteria or eukaryotic cells into hydrogels in order to realize living materials and use the cells as small reactors that already contain metabolic machinery. Here, the supply with nutrients would become a limiting factor. Therefore, vascular structures would have to be incorporated in the 3D printed structures.

Acknowledgements

This work was funded by the Federal Ministry of Education and Research (BMBF) and the Free State of Bavaria under the Excellence Strategy of the Federal Government and the Länder through the ONE MUNICH Project Munich Multiscale Biofabrication and the TUM Innovation Network RISE (Robotic Intelligence in the Synthesis of Life). M.H. acknowledges the Ministry of Science, Research and Arts of Baden-Württemberg and the University of Stuttgart within the "Leistungszentrum Mass Personalization" and "3R-US."

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

additive manufacturing, biofabrication, DNA-nanotechnology, self-assembly, top-down fabrication

Received: July 13, 2022
Revised: September 23, 2022
Published online: November 3, 2022

[1] W. J. James, M. A. Slabbekoorn, W. A. Edgin, C. K. Hardin, *J. Oral Maxillofacial Surg.* **1998**, *56*, 512.
[2] E. Hoch, G. E. Tovar, K. Borchers, *Eur. J. Cardio-Thoracic Surg.* **2014**, *46*, 767.
[3] E. Behroodi, H. Latifi, F. Najafi, *Sci. Rep.* **2019**, *9*, 19692.
[4] I. Liashenko, J. Rosell-Llompart, A. Cabot, *Nat. Commun.* **2020**, *11*, 753.
[5] J. Jiang, X. Xu, J. Stringer, *J. Manuf. Mater. Process.* **2018**, *2*, 64.
[6] K. Zhou, Y. Sun, J. Yang, H. Mao, Z. Gu, *J . Mater. Chem. B* **2022**, *10*, 1897.
[7] P. S. Gungor-Ozkerim, I. Inci, Y. S. Zhang, A. Khademhosseini, M. R. Dokmeci, *Biomater. Science* **2018**, *6*, 915.
[8] H. Li, C. Tan, L. Li, *Mater. Des.* **2018**, *159*, 20.
[9] M. Mayer, J. Yang, I. Gitlin, D. H. Gracias, G. M. Whitesides, *Proteomics* **2004**, *4*, 2366.
[10] L. Y. Yeo, H.-C. Chang, P. P. Chan, J. R. Friend, *Small* **2011**, *7*, 12.
[11] A. Buxboim, M. Bar-Dagan, V. Frydman, D. Zbaida, M. Morpurgo, R. Bar-Ziv, *Small* **2007**, *3*, 500.
[12] E. Karzbrun, A. M. Tayar, V. Noireaux, R. H. Bar-Ziv, *Science* **2014**, *345*, 829.
[13] A. M. Tayar, E. Karzbrun, V. Noireaux, R. H. Bar-Ziv, *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 11609.
[14] M. Levy, R. Falkovich, S. S. Daube, R. H. Bar-Ziv, *Sci. Adv.* **2020**, *6*, 16.
[15] G. Pardatscher, D. Bracha, S. S. Daube, O. Vonshak, F. C. Simmel, R. H. Bar-Ziv, *Nat. Nanotechnol.* **2016**, *11*, 1076.
[16] G. Pardatscher, M. Schwarz-Schilling, S. S. Daube, R. H. Bar-Ziv, F. C. Simmel, *Angew. Chem., Int. Ed. Engl.* **2018**, *57*, 4783.
[17] N. Seeman, *J. Theor. Biol.* **1982**, *99*, 237.
[18] E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, *Nature* **1998**, *394*, 539.

[19] J. Zheng, J. J. Birktoft, Y. Chen, T. Wang, R. Sha, P. E. Constantinou, S. L. Ginell, C. Mao, N. C. Seeman, *Nature* **2009**, *461*, 74.
[20] S. H. Park, C. Pistol, S. J. Ahn, J. H. Reif, A. R. Lebeck, C. Dwyer, T. H. LaBean, *Angew. Chem.* **2006**, *118*, 749.
[21] A. Aghebat Rafat, S. Sagredo, M. Thalhammer, F. C. Simmel, *Nat. Chem.* **2020**, *12*, 852.
[22] J. List, E. Falgenhauer, E. Kopperger, G. Pardatscher, F. C. Simmel, *Nat. Commun.* **2016**, *7*, 12414.
[23] T. Zhang, C. Hartl, K. Frank, A. Heuer-Jungemann, S. Fischer, P. C. Nickels, B. Nickel, T. Liedl, *Adv. Mater.* **2018**, *30*, 1800273.
[24] P. W. K. Rothemund, *Nature* **2006**, *440*, 297.
[25] F. A. S. Engelhardt, F. Praetorius, C. H. Wachauf, G. Brüggenthies, F. Kohler, B. Kick, K. L. Kadletz, P. N. Pham, K. L. Behler, T. Gerling, H. Dietz, *ACS Nano* **2019**, *13*, 5015.
[26] S. M. Douglas, H. Dietz, T. Liedl, B. Högberg, F. Graf, W. M. Shih, *Nature* **2009**, *459*, 414.
[27] Y. Ke, L. L. Ong, W. M. Shih, P. Yin, *Science* **2012**, *338*, 1177.
[28] B. Wei, M. Dai, P. Yin, *Nature* **2012**, *485*, 623.
[29] K. F. Wagenbauer, C. Sigl, H. Dietz, *Nature* **2017**, *552*, 78.
[30] C. Sigl, E. M. Willner, W. Engelen, J. A. Kretzmann, K. Sachenbacher, A. Liedl, F. Kolbe, F. Wilsch, S. A. Aghvami, U. Protzer, M. F. Hagan, S. Fraden, H. Dietz, *Nat. Mater.* **2021**, *20*, 1281.
[31] G. Tikhomirov, P. Petersen, L. Qian, *Nature* **2017**, *552*, 67.
[32] D. Minev, C. M. Wintersinger, A. Ershova, W. M. Shih, *Nat. Commun.* **2021**, *12*, 1741.
[33] P. Rothemund, N. Papadakis, E. Winfree, *PLoS Biol.* **2004**, *2*, e424.
[34] R. D. Barish, R. Schulman, P. W. K. Rothemund, E. Winfree, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 6054.
[35] D. Woods, D. Doty, C. Myhrvold, J. Hui, F. Zhou, P. Yin, E. Winfree, *Nature* **2019**, *567*, 366.
[36] E. Winfree, R. Bekbolatov, *DNA Comput.* **2004**, *2943*, 126.
[37] H.-L. Chen, R. Schulman, A. Goel, E. Winfree, *Nano Lett.* **2007**, *7*, 2913.
[38] M. Cook, P. Rothemund, E. Winfree, *DNA Comput.* **2004**, *2943*, 91.
[39] H. Gu, J. Chao, S.-J. Xiao, N. C. Seeman, *Nature* **2010**, *465*, 202.
[40] A. J. Thubagere, W. Li, R. F. Johnson, Z. Chen, S. Doroudi, Y. L. Lee, G. Izatt, S. Wittman, N. Srinivas, D. Woods, E. Winfree, L. Qian, *Science* **2017**, *357*, eaan6558.
[41] E. Kopperger, J. List, S. Madhira, F. Rothfischer, D. C. Lamb, F. C. Simmel, *Science* **2018**, *359*, 296.
[42] E. Benson, R. C. Marzo, J. Bath, A. J. Turberfield, *Sci. Rob.* **2022**, *7*, 65.
[43] N. C. Seeman, *Annu. Rev. Biochem.* **2010**, *79*, 65.
[44] N. C. Seeman, H. F. Sleiman, *Nat. Rev. Mater.* **2017**, *3*, 17068.
[45] K. F. Wagenbauer, F. A. S. Engelhardt, E. Stahl, V. K. Hechtl, P. Stömmer, F. Seebacher, L. Meregalli, P. Ketterer, T. Gerling, H. Dietz, *ChemBioChem* **2017**, *18*, 1873.
[46] S. Dey, C. Fan, K. V. Gothelf, J. Li, C. Lin, L. Liu, N. Liu, M. A. D. Nijenhuis, B. Saccà, F. C. Simmel, H. Yan, P. Zhan, *Nat. Rev. Methods Primers* **2021**, *1*, 13.
[47] Y. Li, Y. D. Tseng, S. Y. Kwon, L. d'Espaux, J. S. Bunch, P. L. McEuen, D. Luo, *Nat. Mater.* **2004**, *3*, 38.
[48] S. H. Um, J. B. Lee, N. Park, S. Y. Kwon, C. C. Umbach, D. Luo, *Nat. Mater.* **2006**, *5*, 797.
[49] Z. Xing, A. Caciagli, T. Cao, I. Stoev, M. Zupkauskas, T. O'Neill, T. Wenzel, R. Lamboll, D. Liu, E. Eiser, *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 8137.
[50] N. Park, S. H. Um, H. Funabashi, J. Xu, D. Luo, *Nat. Mater.* **2009**, *8*, 432.
[51] D. C. Lin, B. Yurke, N. A. Langrana, *J. Biomech. Eng.* **2004**, *126*, 104.
[52] M. Mariconti, M. Morel, D. Baigl, S. Rudiuk, *Biomacromolecules* **2021**, *22*, 3431.
[53] T. Liedl, H. Dietz, B. Yurke, F. Simmel, *Small* **2007**, *3*, 1688.
[54] A. Cangialosi, C. Yoon, J. Liu, Q. Huang, J. Guo, T. D. Nguyen, D. H. Gracias, R. Schulman, *Science* **2017**, *357*, 1126.
[55] W.-C. Liao, S. Lilienthal, J. S. Kahn, M. Riutin, Y. S. Sohn, R. Nechushtai, I. Willner, *Chem. Sci.* **2017**, *8*, 3362.
[56] X. Yu, Y. Hu, J. S. Kahn, A. Cecconello, I. Willner, *Chem. - Eur. J.* **2016**, *22*, 14504.
[57] Z. Zhu, C. Wu, H. Liu, Y. Zou, X. Zhang, H. Kang, C. J. Yang, W. Tan, *Angew. Chem., Int. Ed. Engl.* **2010**, *49*, 1052.
[58] Y. Xing, E. Cheng, Y. Yang, P. Chen, T. Zhang, Y. Sun, Z. Yang, D. Liu, *Adv. Mater.* **2011**, *23*, 1117.
[59] J. Jin, Y. Xing, Y. Xi, X. Liu, T. Zhou, X. Ma, Z. Yang, S. Wang, D. Liu, *Adv. Mater.* **2013**, *25*, 4714.
[60] C. Wang, X. Liu, V. Wulf, M. Vázquez-González, M. Fadeev, I. Willner, *ACS Nano* **2019**, *13*, 3424.
[61] J. Liu, *Soft Matter* **2011**, *7*, 6757.
[62] J. S. Kahn, Y. Hu, I. Willner, *Acc. Chem. Res.* **2017**, *50*, 680.
[63] C. Li, P. Chen, Y. Shao, X. Zhou, Y. Wu, Z. Yang, Z. Li, T. Weil, D. Liu, *Small* **2015**, *11*, 1138.
[64] L. Aufinger, F. C. Simmel, *Angew. Chem., Int. Ed.* **2018**, *57*, 17245.
[65] A. A. Hyman, C. A. Weber, F. Jülicher, *Annu. Rev. Cell Dev. Biol.* **2014**, *30*, 39.
[66] S. C. Weber, C. P. Brangwynne, *Cell* **2012**, *149*, 1188.
[67] W. M. Aumiller, C. D. Keating, *Adv. Colloid Interface Sci.* **2017**, *239*, 75.
[68] E. A. Frankel, P. C. Bevilacqua, C. D. Keating, *Langmuir* **2016**, *32*, 2041.
[69] T. Y. D. Tang, C. R. C. Hak, A. J. Thompson, M. K. Kuimova, D. S. Williams, A. W. Perriman, S. Mann, *Nat. Chem.* **2014**, *6*, 527.
[70] W. M. Aumiller, C. D. Keating, *Nat. Chem.* **2016**, *8*, 129.
[71] Y. Sato, T. Sakamoto, M. Takinoue, *Sci. Adv.* **2020**, *6*, eaba3471.
[72] C. Kurokawa, K. Fujiwara, M. Morita, I. Kawamata, Y. Kawagishi, A. Sakai, Y. Murayama, S.-i. M. Nomura, S. Murata, M. Takinoue, M. Yanagisawa, *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 7228.
[73] S. Agarwal, M. A. Klocke, P. E. Pungchai, E. Franco, *Nat. Commun.* **2021**, *12*, 3557.
[74] P. Zhan, K. Jahnke, N. Liu, K. Göpfrich, *Nat. Chem.* **2022**, *14*, 958.
[75] Y. Sato, T. Sakamoto, M. Takinoue, *Sci. Adv.* **2020**, *6*, eaba3471.
[76] F. C. Simmel, B. Yurke, H. R. Singh, *Chem. Rev.* **2019**, *119*, 6326.
[77] J. Kim, K. S. White, E. Winfree, *Mol. Syst. Biol.* **2006**, *2*, 68.
[78] J. Kim, E. Winfree, *Mol. Syst. Biol.* **2011**, *7*, 465.
[79] K. Montagne, R. Plasson, Y. Sakai, T. Fujii, Y. Rondelez, *Mol. Syst. Biol.* **2011**, *7*, 466.
[80] A. Baccouche, K. Montagne, A. Padirac, T. Fujii, Y. Rondelez, *Methods* **2014**, *67*, 234.
[81] T. Fujii, Y. Rondelez, *ACS Nano* **2012**, *7*, 27.
[82] N. Srinivas, J. Parkin, G. Seelig, E. Winfree, D. Soloveichik, *Science* **2017**, *358*, eaal2052.
[83] J. Deng, A. Walther, *J. Am. Chem. Soc.* **2020**, *142*, 21102.
[84] A. Padirac, T. Fujii, A. Estévez-Torres, Y. Rondelez, *J. Am. Chem. Soc.* **2013**, *135*, 14586.
[85] A. S. Zadorin, Y. Rondelez, J.-C. Galas, A. Estevez-Torres, *Phys. Rev. Lett.* **2015**, *114*, 068301.
[86] A. Dupin, F. C. Simmel, *Nat. Chem.* **2019**, *11*, 32.
[87] L. Wolpert, *J. Theor. Biol.* **1969**, *25*, 1.
[88] A. S. Zadorin, Y. Rondelez, G. Gines, V. Dilhas, G. Urtel, A. Zambrano, J.-C. Galas, A. Estevez-Torres, *Nat. Chem.* **2017**, *9*, 990.
[89] J. Deng, A. Walther, *Nat. Commun.* **2020**, *11*, 3658.
[90] H. Dehne, A. Reitenbach, A. R. Bausch, *Nat. Commun.* **2021**, *12*, 6811.
[91] E. W. Gehrels, W. B. Rogers, Z. Zeravcic, V. N. Manoharan, *ACS Nano* **2022**.
[92] Y. Xia, G. M. Whitesides, *Annu. Rev. Mater. Sci.* **1998**, *28*, 153.
[93] A. Gopinath, C. Thachuk, A. Mitskovets, H. A. Atwater, D. Kirkpatrick, P. W. K. Rothemund, *Science* **2021**, *371*, eabd6179.

[94] I. V. Martynenko, V. Ruider, M. Dass, T. Liedl, P. C. Nickels, *ACS Nano* **2021**, *15*, 10769.
[95] Z. Li, L. Liu, M. Zheng, J. Zhao, N. C. Seeman, C. Mao, *J. Am. Chem. Soc.* **2019**, *141*, 15850.
[96] R. Liu, H. Ochman, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 7116.
[97] H. Ravanbakhsh, V. Karamzadeh, G. Bao, L. Mongeau, D. Juncker, Y. S. Zhang, *Adv. Mater.* **2021**, *33*, 2104730.
[98] H. Eto, H. G. Franquelim, M. Heymann, P. Schwille, *Soft Matter* **2021**, *17*, 5456.
[99] N. Tompkins, N. Li, C. Girabawe, M. Heymann, G. B. Ermentrout, I. R. Epstein, S. Fraden, *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 4397.
[100] T. S. Gardner, C. R. Cantor, J. J. Collins, *Nature* **2000**, *403*, 339.
[101] M. B. Elowitz, S. Leibler, *Nature* **2000**, *403*, 335.
[102] J. Denk, S. Kretschmer, J. Halatek, C. Hartl, P. Schwille, E. Frey, *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 4553.
[103] P. Glock, F. Brauns, J. Halatek, E. Frey, P. Schwille, *eLife* **2019**, *8*, e48646.
[104] N. Lobato-Dauzier, A. Baccouche, G. Gines, T. Lévi, Y. Rondelez, T. Fujii, S. H. Kim, N. Aubert-Kato, A. Genot, bioRxiv **2022**, https://doi.org/10.1101/2022.07.06.498929.
[105] S. Kretschmer, T. Kortemme, *Proc. IEEE* **2022**, *110*, 659.
[106] J. L. Weissman, S. Hou, J. A. Fuhrman, *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2016810118.
[107] S. Cooper, C. E. Helmstetter, *J. Mol. Biol.* **1968**, *31*, 519.
[108] A. C. White, A. Rogers, M. Rees, C. P. Osborne, *J. Exp. Bot.* **2016**, *67*, 31.
[109] J. Koch, S. Gantenbein, K. Masania, W. J. Stark, Y. Erlich, R. N. Grass, *Nat. Biotechnol.* **2020**, *38*, 39.
[110] A. Cangialosi, C. Yoon, J. Liu, Q. Huang, J. Guo, T. D. Nguyen, D. H. Gracias, R. Schulman, *Science* **2017**, *357*, 1126.
[111] J. Müller, A. C. Jäkel, D. Schwarz, L. Aufinger, F. C. Simmel, *Small* **2020**, *16*, 2001815.
[112] P. B. Allen, Z. Khaing, C. E. Schmidt, A. D. Ellington, *ACS Biomater. Sci. Eng.* **2015**, *1*, 19.
[113] H. Qi, M. Ghodousi, Y. Du, C. Grun, H. Bae, P. Yin, A. Khademhosseini, *Nat. Commun.* **2013**, *4*, 2275.
[114] C. Li, X. Zhou, Y. Shao, P. Chen, Y. Xing, Z. Yang, Z. Li, D. Liu, *Mater. Chem. Front.* **2017**, *1*, 654.
[115] L. Zhou, W. Pi, S. Cheng, Z. Gu, K. Zhang, T. Min, W. Zhang, H. Du, P. Zhang, Y. Wen, *Adv. Funct. Mater.* **2021**, *31*, 2106167.
[116] J. Tang, J. Ou, C. Zhu, C. Yao, D. Yang, *Adv. Funct. Mater.* **2022**, *32*, 2107267.
[117] R. M. Dirks, N. A. Pierce, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15275.
[118] S. M. Chirieleison, P. B. Allen, Z. B. Simpson, A. D. Ellington, X. Chen, *Nat. Chem.* **2013**, *5*, 1000.
[119] J. Li, W. Haas, K. Jackson, E. Kuru, M. C. Jewett, Z. H. Fan, S. Gygi, G. M. Church, *ACS Synth. Biol.* **2017**, *6*, 1327.
[120] B. Lavickova, N. Laohakunakorn, S. J. Maerkl, *Nat. Commun.* **2020**, *11*, 6340.
[121] K. Libicher, R. Hornberger, M. Heymann, H. Mutschler, *Nat. Commun.* **2020**, *11*, 904.
[122] K. Y. Lee, S.-J. Park, K. A. Lee, S.-H. Kim, H. Kim, Y. Meroz, L. Mahadevan, K.-H. Jung, T. K. Ahn, K. K. Parker, K. Shin, *Nat. Biotechnol.* **2018**, *36*, 530.
[123] B. V. V. S. P. Kumar, J. Fothergill, J. Bretherton, L. Tian, A. J. Patil, S. A. Davis, S. Mann, *Chem. Commun. (Cambridge, U. K.)* **2018**, *54*, 3594.
[124] K. Göpfrich, B. Haller, O. Staufer, Y. Dreher, U. Mersdorf, I. Platzman, J. P. Spatz, *ACS Synth. Biol.* **2019**, *8*, 937.
[125] M. Schaffner, P. A. Rühs, F. Coulter, S. Kilcher, A. R. Studart, *Sci. Adv.* **2017**, *3*, eaao6804.
[126] R. L. Martineau, A. V. Bayles, C.-S. Hung, K. G. Reyes, M. E. Helgeson, M. K. Gupta, *Adv. Biol.* **2022**, *6*, 2101070.
[127] J. Müller, A. C. Jäkel, J. Richter, M. Eder, E. Falgenhauer, F. C. Simmel, *ACS Appl. Mater. Interfaces* **2022**, *14*, 15871.

Anna C. Jäkel is a junior researcher at the Technical University of Munich (TUM). She obtained her M.Sc. in physics from TUM in 2020, under the supervision of Prof. Friedrich C. Simmel, where she continued her research as a Ph.D. candidate. She is specialized in 3D Bioprinting and cell-free gene expression circuits. Her research focuses on biofabrication and combining 3D bioprinting with synthetic biology.

Michael Heymann is an assistant professor at the University of Stuttgart. He graduated in Biology at Humboldt University Berlin and received a Ph.D. degree in Biophysics and Structural Biology from Brandeis University in 2014. After postdoctoral work at DESY, Hamburg, and MPI of Biochemistry, Martinsried, he has been heading the Intelligent Biointegrative Systems lab at the Institute of Biomaterials and Biomolecular Systems in Stuttgart since 2019. His interests focus on developing generative biofabrication processes that combine biological self-assembly with lithographic precision.

Friedrich C. Simmel is a full professor at the TUM School of Natural Sciences. He received his diploma and Ph.D. degree in Physics at Ludwig-Maximilians-Universität München in 1999. After postdoctoral employment at Bell Laboratories, Murray Hill, NJ, he returned to Germany in 2002 as a DFG Emmy Noether fellow. Since 2007, he has held the Chair of Physics of Synthetic Biological Systems at TUM.