Review

Strategies to Build Hybrid Protein-DNA Nanostructures

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Abstract: Proteins and DNA exhibit key physical chemical properties that make them advantageous for building nanostructures with outstanding features. Both DNA and protein nanotechnology have grown notably and proved to be fertile disciplines. The combination of both types of nanotechnologies is helpful to overcome the individual weaknesses and limitations of each one, paving the way for the continuing diversification of the structural nanotechnologies. Recent studies have implemented a synergistic combination of both biomolecules to assemble unique and sophisticated protein-DNA nanostructures. These hybrid nanostructures are highly programmable and display remarkable features that create new opportunities to build in the nanoscale. This review focuses on the strategies deployed to create hybrid protein-DNA nanostructures. Here, we will discuss strategies such as polymerization, spatial directing and organizing, coating, rigidizing or folding DNA into particular shapes or moving parts. The enrichment of structural DNA nanotechnology by incorporating protein nanotechnology has been clearly demonstrated and still shows a large potential to create useful and advanced materials with cell-like properties or dynamic systems. It can be expected that structural protein-DNA nanotechnology will open new avenues in the fabrication of nano-assemblies with unique functional applications and enrich the toolbox of bionanotechnology.

Keywords: DNA nanotechnology; Protein Nanotechnology; Self-assembly; Bionanomaterials

1. Introduction

The versatility and programmability of DNA in the nanoscale has been demonstrated by the notable growth and diversification that structural DNA nanotechnology has displayed in the last decades[1–4]. The continues growth and diversification of the structural DNA nanotechnology has been paved by the establishment of novel strategies to build DNA structures[3,5]. For example, the building of DNA-junctions and DNA-crossovers[1], pioneered by Seeman in the 80s, and the DNA Origami[6], developed by Rothemund in the early 2000s, have been important milestones. These building blocks have served as the foundation to build novel, complex and hierarchical nanostructures that inaugurated new sub-fields in the genealogy of structural DNA nanotechnology[3].

The scope of structural DNA nanotechnology has been further expanded by the incorporation of other type of building blocks[3]. For example, the incorporation of inorganic nanoparticles by Mirkin and his collaborators lead to the development of programmable DNA-based colloidal crystals with applications in photonics, electronics and self-assembly[5]. These hybrid nanomaterials combine ssDNA molecules with rigid templates made up of inorganic nanoparticles. The latter acts as the brick that organizes and dictates the shape, while the former works as “glue” by establishing directional “bonds”. DNA-based colloidal crystals are an early example of how the combination of DNA with other types of building blocks could generate a whole new area of programmable hybrid nanomaterials.

Similarly, the incorporation of proteins into structural DNA nanotechnology as a co-building block has further expanded the scope of DNA nanotechnology and sprouted new research avenues[7,8]. The use of proteins in DNA nanotechnology is indeed not new. Proteins have been implemented since the dawn of the structural DNA
Nevertheless, the accumulated advances in the understanding about protein self-assembly, design and engineering has increased the interest to integrate them into DNA nanotechnology. However, up to date the incorporation of proteins has mostly limited to equip DNA nanostructures with specific functionalities that DNA lacks, for instance molecular recognition[11] or catalytic activity[12]. This limited use of proteins contrast with the myriad of functions and capabilities that nucleoprotein complexes perform in nature (e.g. genetic switches, ribosomes, nucleosomes and viruses). Looking at the large structural and functional diversity of nucleoproteins, we can realize all the potential that proteins have to offer when working synergically with DNA building blocks.

More recently, the incorporation of proteins to control or enhance structural features or properties of DNA nanostructures has been implemented, however, it still remains largely unexplored. This review will focus on the structural roles that proteins offer for building hybrid nucleoprotein nanostructures through their combined self-assembly with diverse DNA building blocks (e.g. DNA origami, DNA junctions, plasmid, linear DNA). We will review seminal and recent work to show the strategies deployed to build hybrid protein-DNA nanostructures. We will demonstrate how the full integration of proteins into DNA nanotechnology, mainly through structural and mechanical roles, makes possible to build remarkable and unique nanomaterials and exploit all the potential benefits that these hybrid materials can offer. For reviewing the application of non-structural roles of proteins on DNA nanostructures (e.g. arrangement of proteins or enzymes on pre-assembled DNA nanostructures) as well as structural roles that DNA offers to build nanostructures, we suggest approaching to other reviews recently published[4,7,8,13].

2. Structural Protein-DNA nanotechnology

In comparison to DNA, proteins display complex and diverse functions such as catalytic activity, molecular recognition or allosteric regulation. Since the early developments of the structural DNA nanotechnology these functionalities have been harnessed to increase the functionality of DNA nanomaterials. They have provided advanced functionalities such as enhanced recognition for cellular ligands or enzymatic cascades. To achieve this, proteins are precisely positioning on a previously assembled DNA nanostructure (e.g. DNA Origami or DNA junctions) (Figure 1a) [9–11,14,15]. Besides only adding functional capabilities to, otherwise, inert DNA nanostructures, proteins can cooperate synergistically and bring important features to the final assembled hybrid nanostructure (Figure 1a-b).

Two distinctive approaches of how proteins are combined with DNA can be clearly distinguished: (1) proteins for function and (2) proteins for structure (Figure 1a). The first represents “Proteins-in-DNA nanotechnology”, whereas the second “Protein-DNA nanotechnology”. This review focuses in the second approach which we refer along the review as “structural protein-DNA nanotechnology”. In this hybrid protein-DNA nanotechnology, proteins and DNA act synergistically during the self-assembly process and serve as the foundation of the final nanostructure. Meaning that protein and DNA nanotechnologies show a high degree of structural integration.

By advantageously harnessing the biophysical and chemical properties from both biomolecules[16], structural protein-DNA nanotechnology has reduced the limitations that each molecule present when is used alone. Proteins have larger chemical and structural diversity than DNA and although proteins alone can build sophisticated nanostructures, their versatility and programmability are severely limited due to intricate sequence-structure relationships. On the other hand, DNA lacks the ample structural and chemical diversity seen in proteins but has more predictable folding than proteins due to readily programmable Watson-Crick interactions. Since protein-DNA nanotechnology aims to harness the different but highly complementary physical-chemical and structural properties of both biomolecules, their synergistic combination offers strategical benefits for the fabrication of nanomaterials.
Structural protein-DNA nanotechnology sets apart from the most common use of proteins in DNA nanostructures because proteins play important structural, mechanical and/or assembling roles. Although both DNA and proteins provide these roles, their degree of participation depends on the structural complexity of the starting and final structure. However, as we will show below, most of the literature shows that proteins use to be more operative than DNA. We consider that proteins and DNA have structural roles in a particular protein-DNA nanostructure when is not possible to achieve such final nanostructure without the co-participation of both building blocks (Figure 1a). Hence, the non-existence of one building block does not lead to acquisition of a particular shape, size, order, organization or certain mechanical or dynamic properties. This means that the removal or the absence of one of them (protein or DNA) disassembles the structure or largely compromises its stability or properties.

Due to their large structural and chemical diversity, proteins can bring multiple advantages when used for structural purposes (Figure 1b). They can orient spatially DNA in specific geometries and preserve DNA topology by coating and stiffening. Furthermore, proteins can establish strong and specific interactions with ssDNA but specially with DNA duplexes. This opens the opportunity to incorporate dsDNA into current DNA nanotechnology, which in turn relies in ssDNA (M13 virus plasmid and staple oligonucleotides)[17,18]. As proteins offer the advantage of working isothermally and at environmental temperatures, they can reduce the dependence on DNA molecules and multi-temperature assembly processes of DNA nanotechnology. Therefore, proteins have a large potential to significantly reduce the production costs and simplify assembly processes, which currently limits the large-scale use of DNA nanotechnology in many applications.

Figure 1. Overview of the structural protein-DNA nanotechnology. (a) “Proteins for function” versus “proteins for structure” in DNA nanotechnology. In the latter case there is much more synergy between the protein and the DNA building blocks. “R” means reactive and “P” product. (b) Structural roles of proteins in hybrid protein-DNA nanotechnology.

3. Proteins in hybrid nanotechnology

In order to form nanostructures with DNA, proteins need to establish strong and effective interactions with DNA building blocks. Several types of proteins have been used in structural hybrid nanotechnology. It encompasses enzymes, multimeric proteins, metal-binding proteins, coiled-coil peptides, cationic peptides, cationic polymer proteins, ribosomal proteins, transcription factors, viral proteins, nucleosomes, polymerases and others (Table 1). These proteins interact with DNA through two different approaches: (1) covalent conjugation or (2) non-covalent co-assembly (Figure 2). Covalent conjugation is usually done by chemically linking proteins and DNA through reactive groups (Figure 2a). The DNA can be an assembled nanostructure or oligos with complementary sequences. Covalent conjugation is frequently used because is straightforward, easy to control and render (bio)chemically stable conjugates[13]. This strategy also offers the possibility that practically any protein carrying the proper reactive group can be conjugated. On the other hand, non-covalent co-assembly requires to use proteins with DNA-binding
capabilities (Figure 2b). Since non-covalent interactions are tunable and reversible, they offer the possibility to create flexible, modular and highly dynamic hybrid nanostructures with advanced and complex functionalities that could mimic natural nucleoprotein complexes. However, the resultant complexes can have low stability and be more susceptible by the environmental conditions than chemically linked complexes; thus, the control of these type of interaction represents a great challenge.

Figure 2. Approaches to link proteins and DNA. (a) Covalent conjugation ("S-S" represents a disulfide bridge) and (b) Non-covalent interactions (dotted lines represent hydrogen bonds).

Reported proteins (lacking DNA-binding capabilities) conjugated covalently to DNA include (multimeric) enzymes used as structural templates[19–21], metal-binding proteins[22], coiled-coil peptides[23] or elastine-like peptides[24]. By contrast, proteins that exhibit DNA binding affinity in a sequence-dependent or independent mode include cationic peptides[25–28], cationic polymer proteins[29–31], ribosomal proteins[32], transcription activator-like (TAL) effectors[17], transcription factors[33], viral proteins[34–36], histones and polymerases[37]. Simpler options such as streptavidin or bioinspired cationic protein polymers made up of highly simple and repetitive amino acids that retain DNA-binding functionality or even virus-like properties have also been used[7,25,38]. These proteins have been used in combination with junctions, tiles, motifs or origamis and also single ssDNA or dsDNA molecules.

Table 1. Proteins used in structural protein-DNA nanotechnology.

| Building block          | Type of Protein                        | Building Strategy                      | Interaction¹ | Ref |
|-------------------------|----------------------------------------|----------------------------------------|--------------|-----|
| βGalactoside 1D-DNA conjugate | Enzyme | (1) Structural scaffold to attach DNA (2) Polymerization | C | [19] |
| GroEL-DNA conjugate     | Chaperonin                             | (1) Structural scaffold to attach DNA (2) Polymerization | C | [20] |
| RIDC3-DNA conjugate     | Engineered tetrameric metal-interacting cytochrome cb56 | (1) Structural scaffold to attach DNA (2) Polymerization | C | [22] |
| Drosophila Engrailed homeodomain (ENH) | Engineered transcription factor | (1) Polymerization | NC | [33] |
| Coiled coil-DNA conjugate | D_{*} e_{	ext{novo}} dimerizing peptide | (1) Polymerization | C | [23] |
| K3C6SPD                 | Engineered self-assembly β-sheet cationic peptide | (1) Polymerization | NC | [25] |
| CP++ and sCP            | Designed self-assembly cationic collagen mimetic peptides | (1) Polymerization | NC | [27] |
| Aldolase-DNA conjugate  | Trimeric enzyme                        | (1) Structural scaffold to attach DNA (2) Spatial organization | C | [21] |
| Protein/Peptide                     | Description                                                                                   | Functions                                                                 | Conjugation Type | References |
|------------------------------------|-----------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|------------------|------------|
| H2A, H2B, H3 and H4               | Histones proteins forming nucleosomes (Chromatin)                                             | (1) Spatial organisation                                                  | NC               | [39]       |
| Streptavidin                       | Tetrameric biotin binding protein                                                             | (1) Spatial organisation                                                  | NC               | [40, 41]   |
| Traptavidin                        | Engineered tetrameric biotin binding protein                                                 | (1) Spatial organisation                                                  | NC               | [42]       |
| B3V3A3G3K3                        | Engineered self-assembly β-sheet cationic peptide                                            | (1) No programmable folding of DNA                                        | NC               | [28]       |
| L7Ae                               | RNA-binding ribosomal protein                                                                | (1) Spatial organisation                                                  | NC               | [32, 43]   |
| Transcription activator-like (TAL) effector | Engineered bivalent proteins for recognition of specific DNA sequences                        | (1) Programmable folding of DNA                                           | NC               | [17]       |
| RecA                               | DNA-binding protein involved in the repair and maintenance of DNA                             | (1) Self-assembly                                                          | NC               | [44]       |
| Tobacco Mosaic Virus coat protein  | Viral RNA binding protein                                                                     | (2) Coating                                                               | NC               | [34, 35]   |
| Redβ                               | Single-strand annealing protein for homologous recombination in phages                       | (3) Rigidifying                                                           | NC               | [36]       |
| Cs-B^so/74                         | Engineered diblock protein polymer carrying a non-sequence specific dsDNA binding domain from archael origin | (1) Coating                                                               | NC               | [18, 31, 45] |
| Cs-Si0-BK12                        | Engineered triblock cationic protein polymer                                                  | (2) Rigidifying                                                           | NC               | [30]       |
| Ce-B^c12                           | Engineered diblock cationic protein polymer                                                   | (1) Coating                                                               | NC               | [29, 46]   |
| T7RNAP-ZIF                         | Engineered T7 RNA polymerase fused to a DNA-binding zinc finger motif                        | (2) Rigidifying                                                           | NC               | [37]       |
| (GVGVP)_40                         | Engineered elastin-like polypeptide                                                          | (1) Dynamic and responsive systems                                        | C                | [24]       |

1. Tables may have a footer. C: Covalent conjugation, NC: Non-covalent interaction.
4. Strategies to build protein-DNA nanostructures

In structural protein-DNA nanotechnology, proteins and DNA building blocks have been combined following a variety of strategies. The strategies reported up to date include polymerization, directing and spatial organization, bending, folding, self-assembly, coating, rigidizing and moving DNA parts (Figure 3). In them, DNA and proteins act synergically to build more complex structures. However, proteins use to carry a more active role during the assembly than DNA. Yet DNA plays an important role in the self-assembly of the final structure by operating as a structural template or scaffold for protein binding or anchoring. Below, we will discuss the mentioned strategies.

4.1. Polymerizing DNA

Perhaps the most basic strategy is to bring together DNA and protein components to polymerize them (Figure 4). Polymerization occurs through complementary DNA oligos attached to proteins or through protein-protein interactions. Proteins that polymerize with themselves and with DNA either by chemical conjugation to self-complementary oligos or through specific interactions have been used to create one-dimensional protein-DNA nanomaterials. A pair of dimerizing coiled coils were chemically conjugated to ssDNA oligonucleotides complementary to oligos located on the surface of a DNA origami (Figure 4a)[23]. When the DNA-coiled coil conjugates self-assemble into antiparallel dimers, DNA origamis were brought together and elongated megadalton-size nanostructures were obtained. Other reports have followed similar approaches. For example, protein dimers that co-assemble through metal-directed protein-protein self-assembly were further polymerized through complementary ssDNA strands (Figure 4b)[22]. Analogously, proteins covalently conjugated to ssDNA strands positioned on opposing faces have been harnessed to create large one-dimensional nanotubes or fibrous nanomaterials (Figure 4c)[19,20,47].
A more sophisticated strategy to build one-dimensional nanomaterials was reported by Mayo and his team[20]. They designed computationally a protein able to establish dual protein-protein homodimerization and protein-DNA interactions (Figure 4d). They combined a DNA-binding domain with the engrail *Drosophila* homeodomain to create a protein capable of establishing both interactions in opposing sides. In presence of dsDNA, the designed protein self-assembled in hybrid nanowires, consisting of two interactive proteins bridging dsDNA molecules on both sides.

![Figure 4](image_url)

**Figure 4.** Polymerizing proteins and DNA into 1D nanostructures. (a) Coiled-coils conjugated to oligonucleotides (top left) dimerize DNA origamis (bottom left). TEM image is shown (right). Scale bar: 50 nm. Adapted with permission from [23] Jin et al. Copyright 2019 American Chemical Society. (b) Metal-mediated proteins conjugated to DNA oligonucleotides self-assemble into 1D-DNA assemblies. Reprinted with permission from [22] Subramanian et al. Copyright 2018 American Chemical Society. (c) Enzyme β-galactosidase conjugated to complementary oligonucleotides in opposed faces polymerize into elongated nanostructures (left). TEM image (right). Scale bar: 200 nm. Reprinted with permission from [19] McMillan and Mirkin, Copyright 2018 American Chemical Society. (d) Rationally designed protein with protein-protein and protein-DNA interactions forms nanorods (left). TEM image (right). Adapted by permission from [33] Mou et al. Copyright 2015 Springer Nature.

### 4.2. Directing and organizing DNA on space

Using proteins to orient or arrange a number of DNA components spatially is another common strategy reported to build hybrid nanostructures (Figure 5). A very early attempt to create directional hybrid protein-DNA complexes was achieved by Chengde Mao and his group[40]. They attached four biotinylated dsDNA fragments to a central streptavidin tetramer, resulting in a cross-shaped complex, which nevertheless was too flexible to possibly build something (Figure 5a). Later on, this concept was further developed by conjugating four different short ssDNAs, instead of long dsDNA fragments, into traptavidin, a form of chemical and mechanical-resistant streptavidin (Figure 5b)[42]. The resultant protein-DNA complex was associated with magnetic beads and semiconductor nanoparticles through complementary ssDNA strands for applications in plasmonics.

In the search of increasing the dimensionality of hybrid nanostructures, the combination of proteins and DNA have been successful to produce 3D nanostructures. More sophisticated nanostructures than linear ones have been created using protein handlers. Several streptavidin tetramer proteins were grafted into each face of DNA polyhedras and biotinylated at each wire of the frame[41]. This rendered a more complex and richer 3D protein-DNA nanostructure than the original DNA polyhedra. Another example of constructing spatially defined and tunable 3D tetrahedral cages involved the self-assembly of a homotrimeric protein, covalently joined to three identical ssDNA handles (one in each protein) to a triangular DNA base carrying at each corner complementary ssDNA strands (Figure 5c). In other example, precise and regular geometrical 3D nanostructures were
built by harnessing the natural propensity of histones to form quaternary structures and bind specifically ssDNA[39].

**Figure 5.** Proteins direct and organize DNA on space. (a) Streptavidin tetramers binds to 4 copies of a biotinylated dsDNA (left). AFM image (right). Image adapted with permission from [40] Tian et al. 2006 Royal Society of Chemistry. (b) Traptavidin tetramer self-assembles four ssDNA oligonucleotides. Adapted with permission from [42] Kim et al. Copyright 2019 American Chemical Society. (c) 3D nanocages are formed between the assembly of a triangular DNA nanostructure carrying complementary oligos in its vertices with a protein trimer conjugated to complementary oligonucleotides. Adapted with permission from [21] Xu et al. Copyright 2019 American Chemical Society.

4.3. Shaping DNA through bending and folding

In this strategy proteins bring together different intramolecular parts of a nucleic acid building block to obtain a nucleoprotein complex with a regular and well-defined shape (Figure 6)[17,28,32,48]. These principles were elegantly harnessed by Dietz and his group to self-assemble remarkable DNA-protein hybrids into 2D and 3D nanoshapes (Figure 6a) [39]. They engineered dozens of TAL effector proteins, each one able to bind on two distant intramolecular positions of a flexible linear dsDNA molecule. Upon binding on the sites, the bivalent TAL effector proteins orchestrate the folding of the DNA molecule into a shape previously designed. The assembly of DNA-protein hybrid nanostructures was demonstrated in a cell-free system from genetic components codifying for the proteins, suggesting that such hybrid protein-DNA nanostructures could be biologically produced. On another interesting example, Hirohisa Ohno et al. used the ribosomal RNA-binding protein L7Ae to bend dsRNA molecules into geometrical shapes (Figure 6b). L7Ae bends a dsRNA by tightly interacting in particular sequences called K-turns and inducing a conformational change of approximately 60° on them[32]. Using this strategy they created a synthetic RNA–protein nanostructure shaped like an equilateral triangle. The group later extended this idea to engineer RNA-protein complexes with different nanoarchitectures and applied them for imaging and therapeutic applications[43,49].
Figure 6. DNA is shaped by proteins into nanostructures. (a) Dimeric TAL effectors were programmed to attach to a dsDNA molecule into specific positions and fold it into pre-designed hybrid nanostructures (left). TEM image (right). Scale bar: 20 nm. Reprinted with permission from [17] Praetorius and Dietz. Copyright 2017 AAAS. (b) An equilateral triangle is formed by bending RNA by RNA-binding protein L7Ae (left). AFM image (right). Adapted by permission from [32] Ohno et al. Copyright 2011 Springer Nature.

4.4. Protein self-assembly on DNA

Sometimes a DNA building block is used as a foundation for protein self-assembly on its surface (Figure 7). This strategy has been exploited to render hybrid nanostructures with new shapes and properties than the original DNA building block. Linear dsDNA molecules have templated the self-assembly of RecA protein filaments[44], virus-like β-sheet forming peptides[28] or Tobacco Mosaic Virus (TMV)-inspired proteins[30]. RecA protein allowed to pattern double-stranded DNA scaffolds in a programmable and site-specific fashion (Figure 7a). A TMV-inspired protein called C-S_{10}B_{12} self-assembled on dsDNA and condensed it into regular protein-DNA nanorods (Figure 7b). Other groups have used DNA nanostructures instead of monomolecular DNA templates. Sophisticated nanoarchitectures were created through the \textit{in situ} assembly of TMV coat proteins onto genome-mimicking RNA strands anchored to the sides of DNA nanotubes (Figure 7c) or the vertexes of DNA nanotriangles[34]. This was exploited to render hybrid nanostructures with new and different properties than the original DNA-nanostructure.

Figure 7. Protein self-assembly on DNA templates. (a) RecA protein self-assembles on a DNA oligonucleotide before forming complexes with a dsDNA (top). AFM images (bottom). Adapted with permission from [44] Sharma et al. Copyright 2014 American Chemical Society. (b) A tri-block C-S_{10}B_{12} protein polymer self-assembles onto dsDNA to form hybrid nanorods (top). AFM images
4.5. Coating and rigidizing DNA

When a single DNA block is coated by various copies of a protein, this results in a stiff complex (Figure 8). This simple and direct strategy can be exploited to assemble self-sustained topological protein-DNA nanostructures[18,31,36,45,50]. This happens because a DNA-binding protein increases the rigidity of very flexible DNA parts (usually double strand) in a DNA nanostructure. The protein coating reveals and sustain the previous floppy topology of the initial DNA nanostructure. The coating-and-rigidizing strategy has also the extra advantages of increasing the enzymatic and thermal stability of the protein-coated DNA building block.

A clear example of this strategy is the protein C8–BSso7d (Figure 8a). It consists of a colloidal stability block that has been attached to a non-sequence specific DNA binding affinity domain (Sso7d, 7 kDa). The considerable stiffening effect that this DNA-coating protein has over linear dsDNA molecules has been harnessed to build star-like DNA nanostructures from linear DNA molecules[18]. C8–BSso7d has also use to coat 2D and 3D DNA origamis without any observable structural perturbation. C8–BSso7d-coated DNA origamis presented higher biochemical stability, needed lower amounts of Mg2+ to be assembled and improve aqueous dispersion and decoration with gold nanoparticles than naked DNA origamis[31,45]. This strategy could be used to reduce the leakage of small size drugs encapsulated in the interior of 3D DNA nanostructures.
Similarly, other proteins have been used to coat DNA and create hybrid nanomaterials. For example, C4-BK12, a DNA-coating protein related to C8–BSq17d, has been used to resolve fluorescent markers with high spatial resolution in single DNA molecules inside nanochannels upon their binding and stiffening[46]. RecA protein filaments have been exploited to provide rigidity to DNA wires[44,50] and to build tetrahedral nanostructures with defined dimensions (Figure 8b)[50]. Similarly, the single-strand annealing protein Redβ was used to form rigid blunt-ended four-arm junctions[36].

4.6. Dynamic Nanostructures: Moving DNA Parts

Dynamic protein-DNA systems have a large potential because are able to carry very complex processes like the ones observed in complex viruses or in cellular components such as motors, compartments or ribosomes. Development of dynamic hybrid biomaterials are still in their infancy, however, the few examples reported here display the potential of this strategy. In this strategy proteins or DNA parts actuate on the other building blocks of the system, meaning that they act as switches or molecular motors (Figure 9). Without doubt, this is one of the most advanced function that proteins can bring to the field of hybrid protein-DNA nanotechnology.

Pirzer and his collaborators functionalized a rectangle DNA origami with elastin-like polypeptides which could reversibly fold the nanostructure upon hydrophilic-hydrophobic transition of the ELP base on salt concentration or temperature changes (Figure 9a) [24]. Famulok and collaborators achieved to build a hybrid nanoengine by coupling an engineered zinc finger - T7 RNA polymerase to a circular dsDNA (stator) catenated into a smaller and rigid circular dsDNA (rotor) (Figure 9b)[37]. The protein fusion, acting as an engine, anchored onto T7 promotor located in the rotor and the polymerase could move it. This particular system can bring novel nanomaterials with dynamic capabilities.

Figure 9. Dynamic hybrid nanostructures. (a) ELP open and closes a hinge-type DNA origami upon a stimulus. TEM images (left). Scale bars: 100 nm. Adapted from [24] Goetzfried et al. 2019. (b) Two intertwinned dsDNA rings harbored a dimerize Zinc finger and an RNA polymerase, causing the latter to rotate the ring with a T7 promotor. AFM images (right). Reprinted by permission from [37] Valero et al. Copyright 2018 Springer Nature.

In another outstanding study, the dynamic and step-wise assembly of TMV proteins was control by DNA nanostructures[35]. The genome-mimicking RNA anchored on triangular or barrel origami nanostructures was locked by a series of DNA strands on the template, preventing the protein to self-assemble on the RNA. Using toehold-mediated strand displacement, the viral genome was stepwise released, leading to in situ dynamic TMV assembly and the production of a DNA–protein hybrid nanostructure. This work points that by clever DNA strand displacement technologies, DNA could actively participate in creating dynamic hybrid nanostructures. Furthermore, combining proteins and DNA nanostructures can generate systems reassembling the packaging mechanisms...
observed in viral systems, meaning that the information flow leading to protein self-assembly can be regulated by using DNA nanotechnology. Although the combination of proteins and DNA to create successfully dynamic structures has been observed, the tip of the iceberg, in terms of capabilities, starts to be explored.

5. Perspectives

The structural protein-DNA nanotechnology will continue growing its capabilities and increasing the repertoire of hybrid nanostructures. This will bring new conceptual and technical developments into the field of nanomaterials, as well and new and useful applications. To materialize all the promises that novel hybrid nanotechnology offers and to further develop them, there are challenges that need to be addressed. One main limitation is the inherently limited knowledge about the proteins that are integrated into DNA. They are complex entities, and their mechanism are not well understood or available for fine-tuning. Another practical limitation is that the DNA nanostructure or DNA strands in the vicinity of the protein could affect undesirably the properties of the protein, making to lose their binding capabilities; or vice versa, the protein could affect the DNA structure. Highly charged proteins can change the structure of the DNA and the highly negative charge proteins or hydrophobic patches can alter protein folding or conformation, losing the desired functionality. When working with DNA origamis as building blocks, bulky proteins could interfere with the origami structure or with its assembly. This is true for proteins that kink or bend DNA upon binding. The physical properties of the DNA scaffold may also directly influence the activity of attached enzymes. Furthermore, the inherent asymmetry of protein surfaces can limit their application to form regular nanostructures when attaching oligos.

Design and engineering of optimized DNA-binding proteins will contribute to advance the emerging structural protein-DNA nanotechnology. For example, by increasing the toolbox of DNA-binding proteins able to work in wider conditions or being more stable or robust. Demonstrating practical applications of hybrid DNA-proteins nanostructures will also help to consolidate this emerging field[7,13]. The use of RNA-building blocks and RNA-binding proteins have been scarcely explored. They could follow a similar path as the one with DNA but also open new ways and offer unique advantages. Hybrid DNA nanotechnology can be explored beyond proteins or complemented by integrating other biomolecules such as RNA, carbohydrates or lipids. This could lead towards structures with cell-like functionalities.

6. Conclusions

Protein-DNA nanotechnology has moved beyond solely arrange proteins on the surface of DNA building blocks or nanostructures. Structural protein-DNA nanotechnology has combined synergistically both molecules, usually at room temperature, to build nanosystems with unique properties not seen when using the proteins or DNA independently. This hybrid nanotechnology is an amalgamation of molecular recognition and self-assembly capabilities of proteins with the Watson-Crick base pairing programming of DNA. A diverse selection of DNA-binding proteins from natural or artificial origins have been exploited or engineered to work together with DNA building blocks that vary in nature and features such as monomolecular templates as ssDNA, dsDNA and also self-assembled structures as DNA tiles or DNA origamis. In protein-DNA nanotechnology various strategies have been exploited to assemble unique nanomaterials and nano-entities, for example: DNA polymerization, spatial organization and orientation of DNA, shaping and bending DNA, coating and rigidizing DNA, protein assembly on DNA and moving DNA parts and creating dynamic structures or systems. The synergistic combination of DNA and proteins has been demonstrated to build highly ordered nanostructures with advanced functionalities and have the potential to accomplish functions similar to
the natural nucleoprotein counterparts or even surpass them. Taking fully advantage of structural protein-DNA nanotechnology can lead us to new horizons in nanotechnology.

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