Mechanical DNA Origami to Investigate Biological Systems

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Abstract

The ability to self-assemble DNA nanodevices with programmed structural dynamics that can sense and respond to the local environment could enable transformative applications in fields including mechanobiology, and nanomedicine. The responsive function of biomolecules is often driven by alterations in conformational distributions mediated by highly sensitive interactions with the local environment. In this review, the current state-of-the-art of constructing complex DNA geometries with dynamic and mechanical properties to enable a molecular scale force measurement are first summarized. Next, an overview on engineering modular DNA devices that interact with cell surfaces is highlighted detailing examples of mechanosensitive proteins and the force-induced dynamic molecular interaction on the downstream biochemical signaling. Finally, the challenges and an outlook on this promising class of DNA devices acting as nanomachines to operate at a low piconewton range suitable for a majority of biological effects or as hybrid materials to achieve higher tension exertion required for other biological investigations, are discussed.

Introduction

DNA is historically viewed as the inheritable material of living systems, encoding the genome of living organisms to pass to their progeny, and to direct gene expression. DNA is sufficiently flexible to be packaged into nuclei around chromosomes and be subjected to various processes, such as replication and transcription, while also behaving as an entropic spring (1, 2). Today, DNA is increasingly being used as a building material to construct nano-objects with defined shape and size. The principle of Watson-Crick complementarity permits the design and production of self-assembling macromolecular objects with custom 3D shape. Of particular importance, these nanodevices can serve as molecular machines

inspired by nature that can perform complex tasks dynamically and reliably using different design approaches. Creating these DNA nano-objects with specified shapes was first conceptualized more than 40 years ago by Ned Seeman [3] and experimentally achieved ten years later [4]. A significant breakthrough in the construction of nanometer-sized DNA objects occurred in 2006 with the introduction of the 2D DNA origami method by Paul Rothemund (5), and 3D DNA origami method introduced by William Shih in 2009 (6). Since these developments, DNA origami method has been thoroughly tested and applied to become, progressively, the gold standard method to build DNA nanostructures (7).

Self-assembled DNA origami are made from a scaffold strand of single-stranded DNA (ssDNA), typically M13 phage genomic DNA, which is folded by a complementary set of short oligonucleotide staple strands (5, 6, 8). Initial DNA origami structures were static objects, albeit able to incorporate functionalized chemistries and biomolecules in a site-specific manner with well-defined spatial arrangement (5, 9, 10). The well-characterized DNA-base pairing provides an easy means to control DNA interactions. This has allowed sequence programmability to rationalize the design into precisely defined geometries with increasingly complexity, including 2D (11), 3D lattice (12), and wireframe structures (13, 14) with defined functions and operations, with micrometer (15), and gigadalton scale multi-component assemblies (16, 17). DNA origami objects have now graduated to dynamic movements with mechanical articulation or actuation programmed to respond to specific stimuli, including devices that open (18, 19, 20, 21) or close (22) in response to target molecules, (Fig. 1). There are DNA origami devices that undergo multistage movement by strand-displacement reactions (23, 24). It is now possible for DNA origami devices to respond to electric fields (25, 26), and light (27, 28).

The ability to both program stimuli responsiveness and to create a multicomponent object able to communicate between the different components, to propagate information, and eventually to translate the triggering input into a predefined motion allows the creation of devices able to solve complex tasks. Gerling, T. *et al* used the principle of shape complementarity stabilized via stacking bonds to produce homo- and heteromultimeric objects as well as reconfigurable devices, including an actuator, a switchable gear, a nanobook, and a nanorobot. The conformation assemblies have been finely tuned by cation concentration, temperature, and strand-displacement reactions (29). Later Ke and co-workers introduced a rhombus-shaped DNA nanoactuator. The device is composed of four stiff rod-like arms connected into a rhombus shape using flexible ssDNA scaffolds in every corner. This design enables precise distance changes induced in one half of the rhombus (driver) to be propagated quantitatively to the other half (mirror) within one dimensional plane (30). Precise distances were achieved on the driver side using an adjustable strut composed of dsDNA with length under control of the investigator. Annealing of different oligonucleotide staples to this strut governed the distance extended between target molecules on the opposite side of the rhombus. The device was used as a sensor that responds to specific stimuli, including changes in buffer composition and the presence of restriction enzymes or specific nucleic acids.

In 2017, Song *et al*. employed base-stacking interactions as a driving force to perform long-range information relay on dynamic DNA molecular arrays. The array transformation is equivalent to a molecular “domino array” (23). Reconfiguration of DNA origami arrays allowed their use for information storage (31), even a reconfigurable system

that encodes information for secure cryptographic communication (32). More recently, a new strategy based on the reconfigurable DNA origami nanoarray from two dimensional (2D) to three dimensional (3D) was evidenced by Yan Liu *et al*. (33). These devices allowed the controlled configuration of structures by simple DNA "trigger" strand addition. Design malleability allowed a combination of several properties such as site-specific addressability and stimuli-responsiveness within one rationally designed architecture. This has allowed the study of complex dynamic behaviors and allosteric mechanisms observed in biological systems (34, 35, 36) and attracted increasing attention particularly in medicine as drug-delivery (18, 20 , 37) and imaging for diagnostics (38, 39, 40).

Within the last few years DNA origami-based mechanical nanodevices (Table 1) are becoming a technique for measuring and exerting forces unto themselves that can expand the palette of existing techniques. Uses of such nanodevices are permitting measurements of protein-DNA binding forces (35, 36) or mechanical sensing in cellular and non-cellular systems (41, 42). Here, we focus on DNA origami mechanical nanodevices for sensing, transmitting, and measuring precise forces that can regulate different biological processes. First, we introduce the design principles and properties of existing DNA nanoactuators. We then highlight prospects of this rapidly developing research field including modeling and implementations to enhance other microscopic and force spectroscopic methods. We discuss some recent applications of DNA origami nanostructures for studying a variety of ligand-receptor interactions. Finally, we discuss recent advances in the field to create DNA nanorobots able to actuate on cells or even within living organisms to activate targeted mechanosensitive proteins and their impact on the molecular functions.

1. DNA origami nanoactuators

The mechanical properties of DNA duplexes or hairpin nanostructures have been widely investigated by tuning the length, sequence, and geometry (43). This opened possibilities to study cell membrane interface and interactions. DNA molecules labeled with fluorophores have been used as reporters to measure force-sensitive structural changes (44, 45). DNA nanostructures were also integrated with existing techniques, such as force spectroscopy to improve their performance (46) by detecting transient molecular events (47), and measuring force-sensitive structural changes of mechanosensitive proteins (48). DNA helices assembled into compact bundles in DNA origami structures are orders of magnitude stiffer than an individual DNA helix (49) with a persistence length of 1.4µm for DNA nanotubes composed of six helices (persistence length of double-stranded DNA is 50nm (50) and only between 1 to 2nm for single-stranded DNA (51, 52). Ultimately the stiffness of DNA origami objects is the result of the number, locations, and stability of crossover points, as well as the overall geometry of the structure (8, 53, 54). The mechanical properties of static DNA origami objects were explored by just considering the bending persistence length (55, 56). Few studies have explored the stiffness of DNA origami to improve resolution of force spectroscopic methods or to apply controlled piconewton (pN) scale force on biological systems. Nickels *et al*. have created a force-clamp, a specific protein could be propped between the stiff DNA origami arms by ssDNA springs. The force applied to the target molecule could be controlled incrementally by changing the length of the ssDNA spring strands (35, 36). Other designs were created to apply a controlled force on mechanosensitive proteins such as the DNA origami nanospring, programmed with a

defined spring constant (41) or the multivalent DNA origami tension probe able to interface with specific cell receptors and generate forces to elicit biological responses in massive parallelization (42).

Newer generations of DNA origami with mechanical actuation or articulation offer a plethora of devices able to undergo programmed motion. DNA devices able to respond to defined signals as single-stranded DNA sequences have existed for over two decades as of this writing, such as a molecular clamp, able to open and close reversibly in response to single-strand exchange (57). Tremendous progress has been made in constructing molecular motors, including walking motors that autonomously move along a track and sort molecular cargoes (58, 59), molecular circuits that can compute tasks encoded in complex mixture of molecules (60), or catalytic amplifiers that can sense and amplify signals (61). The DNA origami technique is now rapidly moving into the area of nanorobotics with nano-mechanical components (62). The group of Castro *et al*. have developed a number of devices capable of articulation and motion in different directions (63). This group has developed prismatic joints that slide a piston linearly through a ringed cuff. Folding of this mechanism was achieved by optimizing the number of connector strands between the piston and cuff as well as staple strand length and melting temperature. The piston was able to slide freely through the cuff or could be locked into position by the addition of complementary hairpin staple strands to the connector strands (64). This design was united with a DNA origami hinge to generate a crank-slider mechanism able to produce both linear and bending motion simultaneously (63). A similar device was designed to measure compressive depletion forces to a resolution of approximately 0.1 pN (65).

DNA origami devices mediated by molecular interactions allowed detection and quantification of interactions between biomolecules. One of the main advantages of using three-dimensional DNA origami is that they can be designed to serve as a programmable scaffold with a precise control of number, spacing, and orientation of moieties attachment such as ligands, proteins, metal particles etc,. DNA origami nanostructures were used to investigate the dynamics between competing motor proteins such as kinesin versus dynein, wherein cargo transport velocity was unaffected by motor numbers (66), and myosin V versus myosin VI, which demonstrated motors regulation by adjusting engagement sites (67, 68). Control of molecular interactions is now possible using actuating DNA origami devices. Funke et al. (69) developed a hinged design able to position molecules with extreme precision using a torsional spring mechanism at the vertex. This positioning system also serves as a type of force spectroscope able to measure the energy landscape between pairs of interacting nucleosomes to better understand the action of chromatin compaction of the genome (70, 71). A direct measurement was assessed with this force spectroscope hinge origami *via* single-particle electron microscopy imaging and FRET measurement by integrating FRET pairs within the hinge origami (70, 72). Nanocalipers were useful in increasing transcription factor occupancy on nucleosomes (71) and tracking the unwinding forces of nucleosomes (73) (Fig. 2A). Several force spectroscopy techniques often investigate individual molecules, however with limited experimental throughput. These studies illustrate one significant advantage of DNA origami: experiments can be conducted in a massively parallel fashion.

DNA origami can now be used as force spectrometers replacing single-molecule force spectroscopy techniques (SMFS) (74) by applying tension forces to an internal

component using principles of stressed tensegrity structures (55). For example Nickels et al. designed a force clamp that exerts tension via the entropic elasticity of single strand DNA (ssDNA), (35). This pre-stressed arrangement was used to investigate the amount of force exerted by the TATA binding protein upon binding to the target sequence by folding differing clamps with varying amounts of exerted force. A similar design was used to interrogate the polymerase III pre-initiation complex, finding that the complex relies on stability provided by Bdp1 (36) (Fig. 2B). Another promising application of DNA origami is its integration with single molecule techniques to improve performance and sensitivity. Single molecule techniques are limited by the physical tethers connecting to the molecules of interest. Noise is introduced into the measurements due to thermal fluctuations perturbing these tethers and limiting the measurable force regime to above 10pN. To reduce noise DNA helix-bundles (HB) were mounted as tethers on the beads with the molecule of interest between them (46). The significantly higher persistence length of origami HB (49) establishes a tether unaffected by thermal fluctuations in the surrounding medium. This allowed finer resolution of force modeling results when tested on DNA hairpins. DNA origami nanostructures have also been employed to track rotational movements in biological processes. DNA origami are able to undergo rotation (75, 76, 77). Recently, a new origami rotor was designed with fluorescently labeled arms and tethered through the central shaft by a dsDNA helix to a given DNA motor protein. Rotation tracking of the arm movement during operation of the motor protein, such as RecBCD or RNA polymerase, revealed individual base pair unwinding (78) (Fig. 2C). The authors speculate that a similar design, united to the ability to control origami with electrical fields (26), could be a new technique for single-molecule torque force spectroscopy. Recently reported nanostructures utilize an electro-chemical gradient to power unidirectional rotation (79, 80). And a recent motor design developed by Pumm *et al* consists of a rotor arm component constrained sterically to uniaxial rotations around the pivot point within the plane of a triangular platform. The motor is powered by a simple external energy modulation that does not need any feedback or information supplied by the user to direct the motors. Backwards rotation of the rotor is prevented using DNA hinges that act as ratchets, ensuring rotation in only one direction. The authors compare the mechanism to the ATP synthase (81).

Modeling of newly designed DNA nanostructures constitutes an important step to cross-validate experimental observations. This is particularly important in the design/characterization of dynamic structures, where the observation of multiple conformations is often an indirect process (fluorescence reporter), or requires repeated sampling (electronic microscopy). Furthermore, force measurements are based on the evaluation of geometrical deformations (length or angle variations), which require detailed description at the nanometer scale. Many of the initial designs of DNA nanostructures are done with tools such as cadnano (82) or scadnano (83), where only idealized structural information is provided. Further information can only be reached with more realistic models, either at the atomic level or as coarse-grained models. While the former provide very detailed information, they are not suited for conformational exploration of large DNA nanostructures, due to severe limitations in time steps. To circumvent this problem, coarse-grained models have been developed for DNA (and RNA) nanostructures. In particular, oxDNA model (84) has been parameterized to realistically reflect mechanical properties of oligonucleotides. A large number of processes involved in DNA nanotechnology have been modeled in this way: walkers (85), strand displacement (86), hairpin opening (87), and sliding structures (88). It is interesting to note that detailed comparison between coarse-grained

models and detailed structures from cryo-TEM observations can show partial disagreement, which can be traced to experimental limitations or possible misfoldings (88).

Still, these achievements are far from describing the complexity of interactions between DNA nanostructures and even simple biological systems. From the perspective of this review, two major aspects still need further development. First, DNA-protein interactions are only partially taken into account in coarse-grained models (89) whereas the intra-protein interaction is very often an effective one (spring model) (90). A similar situation is found for the DNA-lipid interactions, only taken into account in a limited number of force fields (89). Second, the description of basic dynamic mechanisms such as the insertion of DNA nanostructures into lipid bilayers (91) or hairpin unzipping inside larger nanostructures will require dedicated methods for conformational sampling.

Table 1. Summary of the reviewed DNA origami nanodevices with mechanical actuation and articulation that exerts forces under specific interaction/stimulus.

DNA origami nanodevice	**Actuation mechanism**	**Threshold Forces**	**Probe**	**References**
Nanolever	Electric field actuation	N/A	Fluorescence	25
Plasmonic Light device	UV/VIS Light	N/A	Gold Plasmon	28
DNA origami pliers forceps	Target molecule binding	N/A	AFM, fluorescence	34
DNA origami-based force clamp	protein interaction: TATA and RNAP proteins	piconewton (0 to 4 pN) (2.6 to 6.6 pN)	FRET	35, 36
Plasmonic Molecule Detector	Small molecule ligand or temperature	N/A	Gold Plasmon	39
NanoDyn	Thermal actuation; crowding poly-ethylene glycol (PEG) molecules	sub-piconewton	FRET, TEM	65
DNA force spectrometer	Interaction between nucleosomes	N/A	FRET	70
Nanoclock	Strand exchange and DNAzyme	N/A	FRET	76
Nano DNA force spectrometer	Base pairing	(>10 pN)	FRET	92, 93
Sliding DNA origami filaments	Strand exchange	N/A	FRET	94
DNA Nanosprings	pH	(>11 pN)	AFM, Fluorescence	95

			microscopy	

2. DNA devices interacting with cellular receptors

For a DNA origami device to interact on a cell surface the critical first step is to adapt targeting strategies. Several strategies have been developed for coupling biomolecules to DNA origami in order to address the DNA origami to cell surfaces. Lipid-DNA linkers have been used as hydrophobic anchors such as cholesterol, synthetic fatty acids, cyclodextrin or tocopherol [96]. One of the main drawbacks of this approach is the hydrophobic aggregation that can affect structural control. To prevent that, efficient techniques include DNA sequences with minimal secondary structure, terminal poly-T section preceding the cholesterol tag instead of guanine nucleotides, and ssDNA overhangs adjacent to the cholesterol group (97).

However, in the case of targeting a specific receptor on a cell type of interest, it is important to couple the DNA origami with a ligand to specifically recognize and bind to the receptor. The strategy will affect how the DNA device interacts with the targeted receptor. Several considerations for the targeting strategies are molecular architecture of the receptor, binding affinity, efficient chemical conjugation, cell-buffer compatibility, ligand-based selective receptor binding, etc,. Today, there are two common strategies for DNA origami functionalization. The first is to design a DNA origami with staple strands bearing chemical moieties that can be directly incorporated during the DNA origami self-assembly such as biotin, receptor ligands, click chemistry functional groups, etc. The second is to design a DNA origami with ssDNA anchors that hybridize complementary sequences bearing a chemical-moiety or biomolecule-of-interest (98). Several ligands (99), aptamers (20), antibodies and nanobodies (100) can be coupled to DNA origami, for example *via* click-chemistry (101). Angelin et al. developed multiscale origami structures as cellular interfaces functionalized with epidermal growth factor (EGF) ligands to interact with EGF receptors. These interfaces allow tailoring of nano to microscale geometries and the implementation of multiplexed high-throughput applications (102).

Finally, DNA conjugation chemistry can be used to target bacteria cell surfaces, through covalent approaches (sodium peroxidate oxidation, photo-crosslinking) and non-covalent approaches (lipid hydrophobic insertions e.g. by attaching some cholesterol molecules to the terminus of a DNA strand, aptamer binding and coordination-based DNA-bacteria conjugation using genetically engineered bacteria with His-tag proteins expressed on the surface). While covalent approaches are more stable but also more harmful, non-covalent approaches are more biocompatible but less stable (103).

Thus, numerous strategies have been developed to address DNA origami on cell surfaces. It allows the nanostructures to achieve their purpose, for example biosensing and measuring forces. Indeed, researchers have been working to unite the realm of DNA-based nanotechnology to investigate mechanical forces at the nanoscale which are essential for numerous biological systems. Researchers are developing synthesized DNA automata able to interface with living cells to both measure and exert piconewton scale forces. DNA origami nanostructures have been created to detect certain disease states in conjunction with DNA-manipulating enzymes *in vitro* (104, 105, 106). Synthetic DNA-based transcription factors have also been developed that can ultimately be used for portable diagnostics (107). DNA nanotechnology is now at the level of being able to interact with living cells. DNA nanorobots

loaded with antibody fragments (18) or thrombin (20) were assembled with aptamers responsive to specific cell-surface receptors, such as nucleolin or platelet-derived growth factor (PDGF). With two different aptamers holding it closed, the device is required to bind both target surface receptors on the cell before it will open to release the payload, effectively using multi-aptameric locks to categorize and affect cells. In another study, DNA origami nanostructures tuned the nanoscale spacing of ephrin-A5 to direct EphA2 cell-receptors at defined positions to investigate receptor phosphorylation levels in human breast cancer MDA-MB-231 cells, resulting in reduced cell invasion (108). The same design was employed to show in a recent study that nanoscale spatial distribution of ligands modulate activation and downstream transcriptional responses upon ligand binding (109).

DNA aptamers are one promising avenue to target specific cell types and cell surface receptors with DNA nanodevices. Unfortunately, the number of aptamers against distinct cell surface receptors is fairly limited. In spite of this, a number of devices against certain cell types have been developed. The aforementioned tumor-targeting, cargoed nanorobots employed aptamers against nucleolin and PGDF (18, 20). Mela *et al*. developed a DNA nanostructure using aptamers against Gram-positive *B. subtilis* and Gram-negative *E. coli*. Loading the nanostructure with lysozyme retarded the growth of bacteria more significantly than lysozyme alone (110). Unfortunately, the component(s) of the membrane bound by the aptamer were not fully characterized (111), limiting the specificity of nanostructure binding.

3. DNA Origami to probe force effects on cells

The next logical step in the investigation of cell mechanoreceptors is the development of techniques to measure and exert forces on mechanoreceptors. The group of Dr. Khalid Salaita has developed a number of strategies to investigate forces exerted by cell integrins. DNA hairpins of a known length and GC content can serve as benchmarks for molecular forces able to unzip them (112). These have been employed successfully to investigate forces exerted by cells on a rigid surface. Previously, labeled double-stranded DNA tethers mounted on surfaces were able to discern the single molecule force thresholds for integrin and Notch engagement (113). A DNA hairpin with a fluorescent dye and quencher held in close proximity as digital tension probes were unzipped by cellular traction forces freeing the fluorescent dye from the quencher and allowing visualization of the precise position and force exerted by cells undergoing spreading or migration (114). To improve investigation of cellular traction forces a tunable, multivalent DNA origami tension probe was designed that is able to multiplex ligand-cell receptor interactions and report traction force via force-sensitive hairpins in a parallel fashion (42) (Fig. 3A). This platform could be tailored with multiple, tunable force tension gauges and an adjustable number of ligands. The tension exerted by platelets was observed to increase with the increasing number of ligands per origami as measured by hairpin unfolding.

To robotize the DNA-ligand, an approach using a DNA “nanospring” was designed wherein a single-stranded DNA underwent rolling circle amplification and was functionalized with RGD-labelsed oligonucleotides. On the surface of a cell the nanospring could allow proteins to aggregate or, upon addition of a complementary oligo, cause extension of the spring and increased tension between integrin-bound ligands. Downstream biochemical

signals were monitored through PI3K/Rac1 signalling and changes in cell morphology (115) (Fig. 3B).

Cells have a variety of mechanisms to sense and exert mechanical force in their local environment. This includes membrane receptors such as integrins and notch receptors (113) and proteins such as YAP that relocate from the cytoplasm to the nucleus when the cells are under force (116). Tools to interrogate forces exerted by cells and proteins have been mentioned above, but tools to exert forces have been lacking. Atomic force microscopy and optical or magnetic tweezers have been used to exert forces on cells and proteins (117), DNA nanostructures could be designed to exert precisely controlled forces to probe nanomechanical systems of living cells (118). Force exerting devices, including nucleic acid walking devices that traverse oligonucleotide tracks (58), generate forces in the tens of piconewtons. Multiple DNA hybridization events in multivalent devices coated in oligonucleotides complementary to “lawns” of RNA can roll with hundreds of piconewtons of force by RNAse H digesting the annealed RNA strands allowing the device to roll to fresh strands leaving a depleted path in the wake of the device preventing backwards movement (119, 120).

To address the absence of a force-exerting device on live cells we have recently contributed a new DNA origami design, the Nano-winch (121). The Nano-winch was inspired by the linear joint design of the Castro group (63, 64). It consists of a central piston domain encircled by a cuff or cylinder with six single-stranded scaffold connectors between the top of the piston and the top of the cylinder and six additional single-stranded connectors between the bottom of the cylinder and the tip of the piston. The piston tip can be functionalized with ligands for any target mechanical receptor. Two landing leg origamis are assembled on the sides of the cylinder and each have two projections emerging at 90° from the bottom of the assemblage allowing it to land on a cellular membrane surface without toppling. The single stranded connectors act as entropic springs to retract or extend the piston in an “autonomous” mode of operation, exerting low pN forces on any target membrane protein coupled to the tip of the piston. Complementary DNA hybridization on the single-stranded connectors between the backstop and the cylinder allowed the connectors on the bottom of the device to fully retract the piston away from the membrane surface with significantly greater force, termed “remote-activation” mode (Fig. 3C).

We used the “autonomous” mode of the Nano-winch to stimulate the activation of integrin, a force sensor on the surface of cells able to detect and respond to forces around ~10pN (114, 122, 123). Addition of a fully assembled Nano-winch robot functionalized with cyclic arginine-glycine-aspartic acid (cRGD) ligand to the integrin heterodimers caused the phosphorylation of the focal adhesion kinase (FAK), a protein that becomes phosphorylated upon force exertion (117, 124, 125). Control experiments using a non-interacting ligand, cRGE, cRGD ligand alone, a cRGD-functionalized piston without legs, and an undecorated Nano-winch all failed to elicit phosphorylation of FAK.

“Remotely-activated” Nano-winches were used to stimulate the mechanically-gated membrane protein, BtuB, from *Escherichia coli*. BtuB is a β-barrel channel occluded by a plug domain that requires a retraction force of approximately ~50pN to a distance of 20 nm to open sufficiently (126). BtuB reconstituted into planar lipid bilayers had detectable opening events after addition of the extension oligonucleotides to attached Nano-winches. The plug

refolded back into the channel after chemically detaching the Nano-winch from the membrane protein.

Without the need for the bulky, costly instrumentation required by optical and magnetic tweezers or atomic force microscopes nor highly experienced operators, the Nano-winch, and devices like it, can serve as an inexpensive, instrument-free method to investigate mechano-receptors. Directing it to target membrane proteins (127) will create a new method to stimulate mechano-receptors on living cells to investigate mechanotransduction systems.

4. Conclusions and Future directions

New DNA origami devices are being developed to address problems in biophysics and biomechanics. The capacity to manipulate and probe biological and synthetic materials systems at the nano- and microscale remains limited due to the difficulty of engineering probes with commensurate dimensions and force scales. With their nanometer dimensions, biocompatibility, autonomous activity, and robotic articulation, DNA nanotechnology is uniquely suited as a tool to investigate problems in mechanobiology *in cellulo*. We envision DNA nanorobots able to actuate either autonomously or upon user control on living cells to activate targeted mechanosensitive proteins. This has significant advantages over current techniques as it does not require the expensive or cumbersome equipment of existing techniques. The dimensions of this technology allow it to fit within areas other techniques cannot access, and its composition renders it largely biocompatible.

Further solutions need to be improved to increase the DNA nanodevices stability *in cellulo*. The presence of nuclease enzymes can rapidly degrade DNA nanostructures, although crossover placement and density can increase stability (128). DNA origami structures exposed to the conditions within cell culture are degraded (129). A number of strategies to circumvent this issue have been devised. Heat denaturation or treatment of serum with nuclease inhibitors can prevent degradation of DNA origami structures (130). Encapsulation of origami with viral capsid proteins improved the uptake of DNA origami into cells significantly over Lipofectamine-based transformation approaches (131). Viruses also served as the inspiration for Perrault and Shih in which a sphere-shaped DNA origami was functionalized with lipid-conjugated oligonucleotides and then reconstituted within a lipid bilayer. The protective coating offered by the lipid bilayer greatly improved the stability of the DNA nanostructure by preventing degradation from nucleases and improved the pharmacokinetics and bioavailability *in vivo* in mice (132). Direct chemical occlusion of the nanostructure can act to conceal the structure from nucleases and seal to prevent denaturation. Oligolysine coated nanostructures proved resistant to nuclease degradation as well as low magnesium salt concentrations but high concentrations of oligolysine caused aggregation. Changing to oligolysine-PEG improved nuclease resistance 1,000-fold and avoiding aggregation in a structure-independent method (133, 134). Chemical crosslinking at strand nicks to prevent nuclease attack has been employed (135) as has exposure to ultraviolet light to crosslink nearby thymidines (136). Additional methods for enzyme stabilization including stable design, crossover density, and tuning the location of restriction sites have been reviewed elsewhere (137,138, 139). A novel approach to extend the lifespan

of DNA nanostructures is to create nanostructures able to reassemble, self-heal, and regenerate when damaged. DNA nanotubes incubated in serum were able to dynamically heal when supplemented with DNA tiles. As nuclease degrades the nanotube the damaged individual tiles are replaced with intact tiles, counteracting the disassembly (129). DNA origami nanotechnology is moving into the realm of practical application. With time these devices will serve to further investigations of the mechanobiology as well as to act in diagnostic and therapeutic applications.

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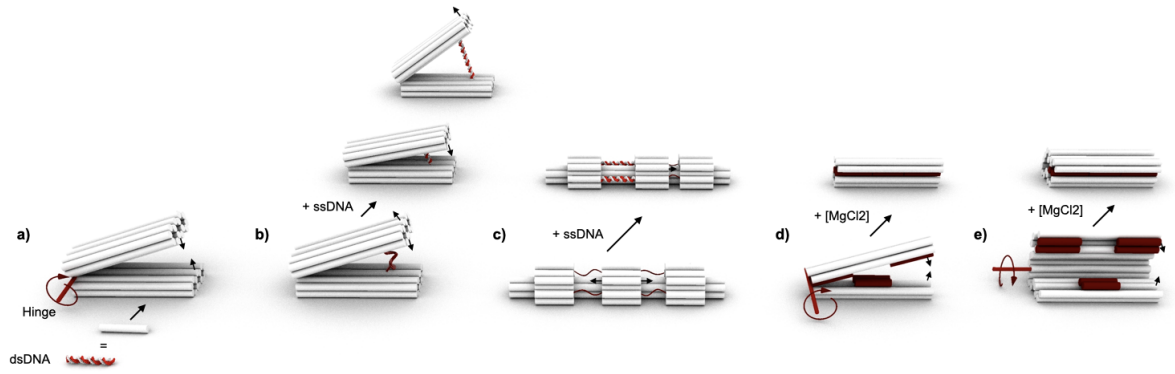


Figure 1. Schematic overview of the DNA origami articulation. a) DNA origami featuring a spring-loaded hinge. **b**) Transition of single-stranded DNA (ssDNA) to double-stranded DNA (dsDNA) allows the articulation of two structural domains of an origami. The number of base pairs located in the adjuster strand controls the angle between the two structural domains of the origami. **c)** DNA origami slider consists of a ring-shaped domain folded concentrically around a piston to facilitate linear motion. These domains are connected by ssDNA scaffold tethers. Addition of oligonucleotides complementary to these tethers induces linear motion by transitioning the tethers to rigid dsDNA. **d and e)** Dynamic origami that cycle from open to closed states with changes in divalent salt (MgCl2) concentration or temperature though nucleotide base-stacking interactions. These reconfigurable structures are designed with shape-complementary DNA double-helical domain protrusions and recessions, highlighted in red.

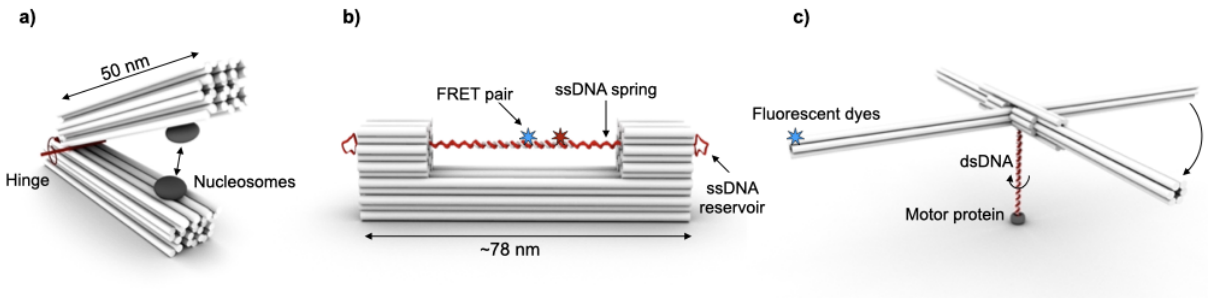
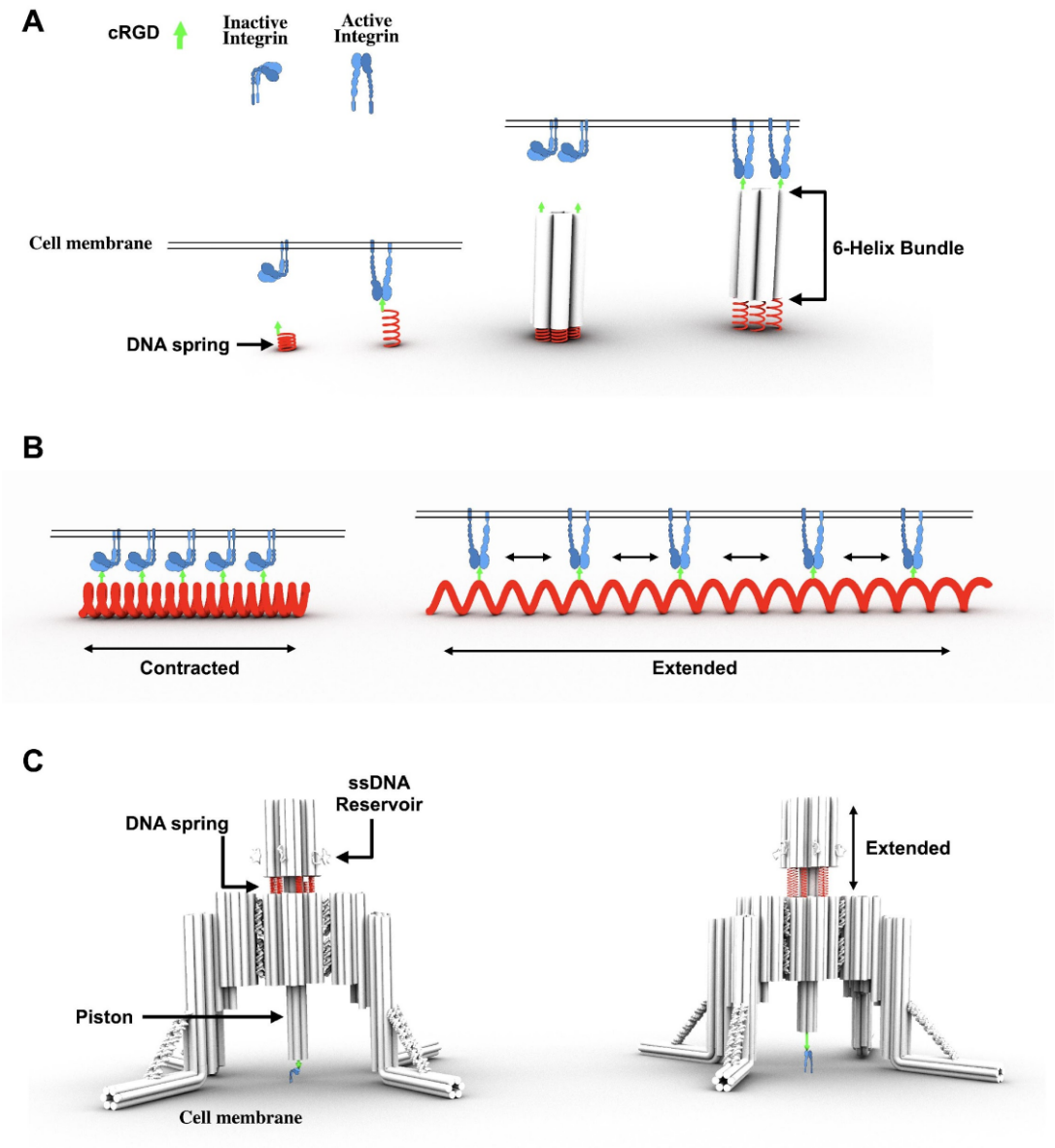


Figure 2. Tension translator and reporter. a) DNA force spectrometer with torque generated near the hinge, from Funke *et al.* 2016. **b**) DNA origami force clamp holding a single-stranded DNA spring under varying amounts of force, from Nickels *et al.* 2016. **c**) Rotational tracking origami with a strategically positioned dye on the arm tips allows movement detection, from Kosuri *et al.* 2019.

Figure 3. DNA Origami for Mechanotransduction Investigation. A) Classical DNA-based tension probes (red coils) with integrin ligands (green arrow) are less efficient than multivalent DNA origami tension probes, which allow a tunable number of tension probes and ligand peptides. **B**) The nano-spring is a strand of DNA studded repetitively with integrin ligands that can be extended by addition of complementary oligonucleotides. This extends the distance between bound integrin heterodimers to elicit cellular responses. **C**) The Nano-winch lands on the cell surface and binds integrin at the tip of a piston. The piston can then



extend to exert force on the bound integrin.