Peer-reviewed version available at *Int. J. Mol. Sci.* **2018**, *19*, 2114; doi:10.3390/ijms19072114

Review

Dynamic DNA Origami Devices

Heini Ijäs [1,2], Sami Nummelin [1], Boxuan Shen [1], Mauri A. Kostiainen [1,3] and Veikko Linko [1,*]

[1] Biohybrid Materials, Department of Bioproducts and Biosystems, Aalto University, P.O. Box 16100, 00076 Aalto, Finland; heini.ijas@aalto.fi (H.I.); sami.nummelin@aalto.fi (S.N.); boxuan.shen@aalto.fi (B.S.); mauri.kostiainen@aalto.fi (M.A.K.); veikko.linko@aalto.fi (V.L.)

[2] University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, 40014 University of Jyväskylä, Finland

[3] HYBER Center of Excellence, Department of Applied Physics, Aalto University, 00076 Aalto, Finland; mauri.kostiainen@aalto.fi

* Correspondence: veikko.linko@aalto.fi; Tel.: +358-45-673-9997

Abstract: Structural DNA nanotechnology provides an excellent foundation for diverse nanoscale shapes that can be used in various bioapplications and materials research. From all existing DNA assembly techniques, DNA origami has proven to be the most robust one for creating custom nanoshapes. Since its invention in 2006, building from the bottom up using DNA has drastically advanced, and therefore, more and more complex DNA-based systems have become accessible. So far, vast majority of the demonstrated DNA origami frameworks are static by nature, but interestingly, there also exist dynamic DNA origami devices that are increasingly coming into view. In this review, we discuss DNA origami nanostructures that perform controlled translational or rotational movement triggered by predefined DNA strands, various molecular interactions and/or other external stimuli such as light, pH, temperature and electromagnetic fields. The rapid evolution of such dynamic DNA origami tools will undoubtedly have a significant impact on molecular scale precision measurements, targeted drug delivery and diagnostics, but they can also play a role in development of optical/plasmonic sensors, nanophotonic devices and nanorobotics for numerous different tasks.

Keywords: DNA nanotechnology; DNA origami; self-assembly; molecular devices; mechanical movement; robotics

1. Introduction

In his idiosyncratic talk "There's plenty of room at the bottom" in 1959, Richard Feynman envisioned that it should be possible to build nanoscale machines that could carry out chemical synthesis by mechanical movement [1]. He also presented Albert R. Hibbs's idea of miniature surgical robots that could perform predefined tasks in the human body [1]. Now almost 60 years later, thanks to modern biology, we know that human body is actually a large-scale biofactory that is comprised of a great number of tiny and accurate nanomachines such as motor proteins and enzymes, as a result of billions of years of evolutionary processes on the Earth. However, we are not merely products of those natural nanomachines or simply hosts to them, but we are also able to look at them with our state-of-the-art microscopes, and even more interestingly, to create artificial and completely new nanodevices. In other words, we are putting Feynman's postulate into practice.

At the time of Feynman's talk, the structure of double-stranded DNA (dsDNA) had been resolved just 6 years ago [2]. However, it was known that DNA carries the genetic information and

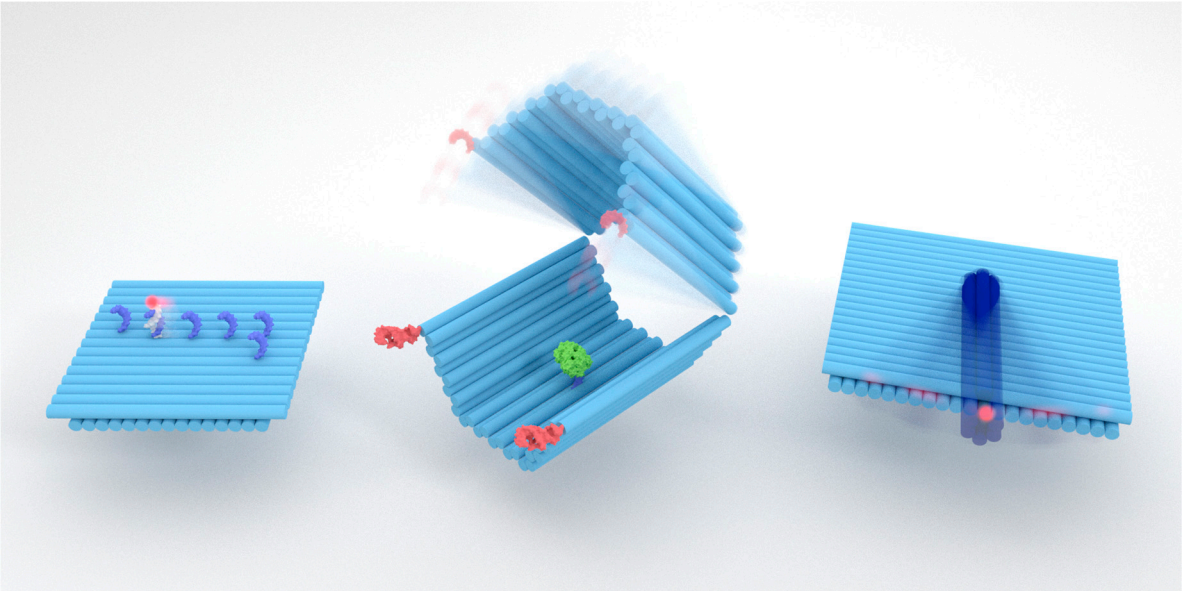
how DNA strands hybridize to each other following Watson-Crick base pairing rules [2]. Nevertheless, it took almost 30 years before the potential of DNA molecule as a programmable construction material [3] – and not merely as the genetic information storage – was proposed by Nadrian "Ned" Seeman [4]. Since then, the field of structural DNA nanotechnology has been constantly growing and it has started to truly flourish during the last decade [5,6]. Today, researchers routinely use DNA to build not only static two- and three-dimensional (2D and 3D) nanostructures [4–11] but also dynamic and precise nanodevices and robots [12,13] Feynman was only dreaming about. As a matter of fact, Feynman's statement "Biology is not simply writing information; it is *doing something* about it" [1] has been literally realized in the case of DNA molecule and the DNA nanotechnology.

Although the structural DNA nanotechnology has been constantly evolving during the last 35 years starting from Ned Seeman's vision of using DNA junctions and lattices to build DNA crystals [6,7], the recently witnessed big boom in the field started from the invention of a 2D DNA origami in 2006, a technique developed by Paul Rothemund [14]. DNA origami is based on a long single-stranded DNA (ssDNA) scaffold that is folded into a desired nanoscale shape with the help of dozens of short oligonucleotides [14]. Since 2006, the method has been extended to 3D shapes [15,16], designs with curvatures and twists [17,18], wireframe based and automatically designed structures [19–21] and assemblies that can reach micrometer or gigadalton scales [22,23]. Inspired by DNA origami, scaffoldless methods that are based on brick-like assembly have also been developed [24,25].

The benefits of using DNA origami technique are not only the virtues of the custom nanoscale shapes but the method also enables extremely accurate molecular scale positioning and patterning. These features can be used in controlling chemical reactions [26–28], creating tunable plasmonic systems [29,30] and building carriers for drug delivery [12,31–35]. Precise and addressable DNA origami can also be used in metrology and optical super-resolution imaging [36], forming crystals and nanoparticle superlattices [37,38] and creating metallic nanostructures [39]. Recently, it has also been observed that DNA origami structures are more resilient than previously understood [40] and that the mass production of DNA origami is affordable [41]. Therefore, highly versatile and modular DNA origami has become a standard molecular scale tool in many laboratories.

In this review, we discuss DNA origami nanostructures that can be used as dynamic and controllable nanodevices such as walking robots [42], logic-gated nanopills [12] and rotors [43] (see Scheme 1). The development of such molecular machines is based on tailoring the DNA sequences in such a way that the structures first self-assemble into desired shapes and are thereby able to perform predefined tasks *via* translational or rotational movement. In this respect, the dynamic DNA origami devices can be considered analogous to protein shapes and functions that are encoded in the sequences of the polypeptide and nucleic acids molecules [8]. Importantly, DNA origami provides a straightforward route from sequence design to actual shapes, unlike protein synthesis. However, *de novo* protein design allows synthesis of completely new proteins with tailored functions [44]. Combination of these two techniques would have potential to revolutionize biomedicine and molecular nanotechnology.

In many dynamic systems, the ability to simulate molecular motion and fluctuations come increasingly important. There are ways to predict DNA origami dynamics based on the rigid-beam models (CanDo) [45,46], atomistic molecular dynamics simulation [47], and coarse-grained models (oxDNA) [48,49]. Additionally, mass-weighted chemical elastic network models (MWCENM) and symmetry-constrained elastic network models (SCENM) [50] can be used to estimate the structural fluctuations.



Scheme 1. Artistic rendering of selected examples of dynamic DNA origami devices. **Left**: A cargo-sorting robot walking on a DNA origami-templated track [42]. **Middle**: A logic-gated DNA origami "nanopill" that selectively displays the loaded cargo [12]. **Right**: A DNA origami robotic arm that performs rotational movement under electric field [43].

Although the focus of this review is on the DNA origami-based devices, it is noteworthy to mention that diverse DNA-based molecular machines have been introduced already before DNA origami. Famous examples include a machine that performs movement based on a DNA conformation change (between B- and Z-forms) [51] and DNA tweezers that can be fueled by additional DNA strands to switch the configuration between open and closed states [52]. By taking advantage of simple DNA nanostructures, it is possible to form nanomechanical devices with different rotational or translational states [53,54] and also control their movement using *e.g.* RNA strands instead of DNA [55]. Later on, DNA nanostructure-based tweezers [56,57], whose arms can be further equipped with enzymes to facilitate control over chemical reactions have been proposed. There are also numerous DNA-based walkers that utilize strand displacement reactions and employ so-called toehold exchanges (toeholds are short ssDNA regions that first bind to reactant DNA molecules). Such devices have been extensively reviewed in Refs. [58,59].

Here, in Section 2 we first discuss DNA origami assemblies with DNA-DNA interaction, *i.e.* the mechanical design of DNA origami and the systems that take advantage of strand displacement reactions, transient DNA binding or stacking interactions. Section 3 is devoted to dynamic DNA origami devices that move due to some other molecular interaction. In other words, here the molecular interaction produces a desired movement or alternatively, interaction can be characterized using the device as a measurement tool. Section 4 reviews the DNA origami movement by external stimuli such as light, temperature, pH and electromagnetic field and the devices that can be utilized to probe multiple interactions. Section 5 concludes the discussion and gives future perspectives in this immensely growing field.

2. DNA-DNA Interaction -Based Movement and Imaging

Here, dynamic DNA origami systems based on DNA-DNA interactions are introduced. This section covers devices whose movements rely on base-pairing (strand displacement), systems that exhibit dynamic behavior based on DNA base stacking interactions and tight-fitting DNA components.

As early as 2009, Andersen et al. [15] fabricated a hollow DNA origami "cuboid" from six 2D origami sheets. The 3D box contained a controllable lid functionalized with "lock-key system"

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 July 2018 doi:10.20944/preprints201807.0033.v1

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 2114; doi:10.3390/ijms19072114

comprised of dsDNAs with sticky-end extensions. Fluorescent dyes Cy3 and Cy5 embedded to the opposite faces of the box, facilitated the detection of irreversible lid opening by strand displacement reaction using Förster resonance energy transfer (FRET). Three years later Zadegan et al. [60] demonstrated reversible opening and closing of a lid in a hollow 3D DNA origami box as a response to supplied opening and closing ssDNA keys. In 2017, Grossi et al. [61] constructed a DNA nanovault with a similar, reversible opening/closing mechanism (Figure 1a). They were able to encapsulate a single enzyme inside the vault and demonstrate that closing the nanovault resulted in notable reduction in enzyme activity.

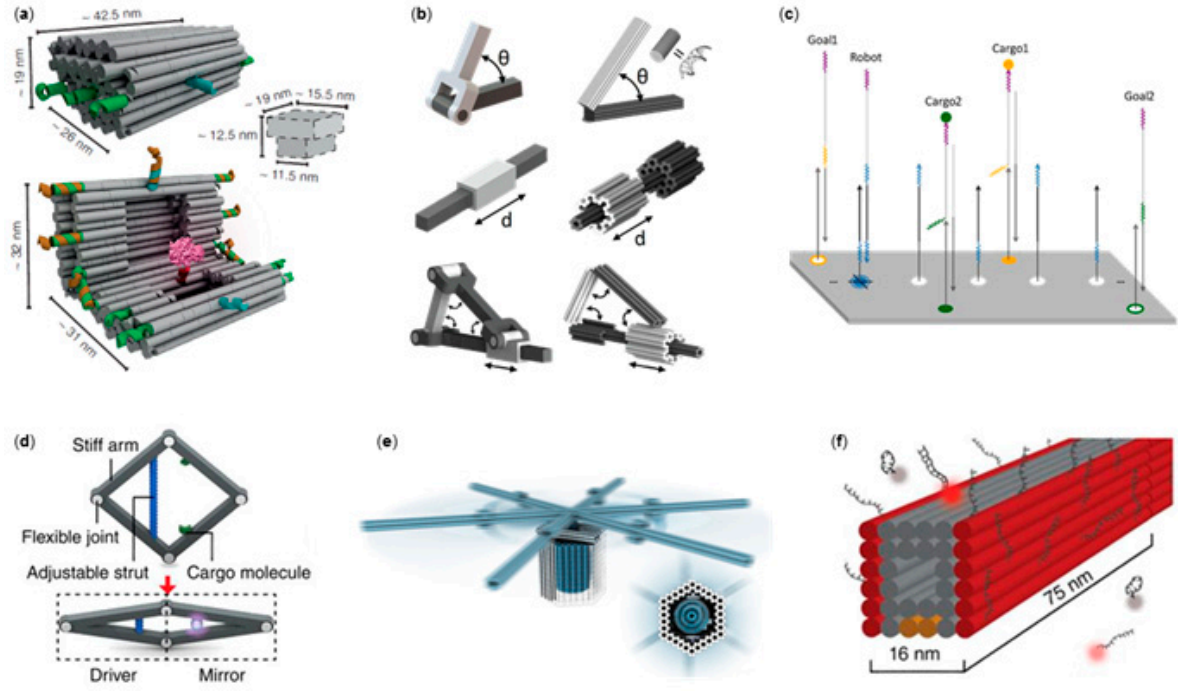


Figure 1. DNA origami mechanics by DNA-DNA interaction. (**a**) A DNA nanovault that displays a cargo when opened *via* strand displacement [61]. (**b**) DNA origami nanomechanics [66]. (**c**) A robot that picks up a cargo and delivers it to a goal on top of a DNA origami [42]. (**d**) DNA origami actuator; movement on the left (driver) side is mirrored to the right side [69]. (**e**) DNA origami rotary apparatus constructed from the tight-fitting components [74]. (**f**) Super-resolution imaging with DNA origami by taking advantage of transient DNA binding [77]. (**a**) is reproduced with permission from [61]. Published by Nature Publishing Group, 2017; (**b**) is reproduced with permission from [66]. Copyright National Academy of Sciences 2015; (**c**) is reproduced with permission from [42]. Copyright The American Association for the Advancement of Science, 2017; (**d**) is reproduced with permission from [69]. Published by Nature Publishing Group, 2016; (**e**) is reproduced with permission from [74]. Published by The American Association for the Advancement of Science, 2016; (**f**) is reproduced with permission from [77]. Copyright Nature Publishing Group, 2014.

Recently, Selnihhin et al. [62] applied a toehold-mediated strand displacement-based lock-key system in a dynamic DNA origami beacon designed for high-sensitivity biosensing. By functionalizing the device with high numbers of fluorophores interacting *via* FRET in a closed state device, they could demonstrate that opening the devices with target DNA keys caused a detectable decrease of FRET efficiency even at DNA concentrations as low as 100 pM.

To build dynamic devices, it is essential to understand the mechanical behavior of DNA origami and their responses under external physical forces. Numerous studies have exploited these aspects of DNA nanostructures [63], and for example, Zhou et al. [64] designed and characterized a tunable DNA origami structure with a compliant part able to bend into different angles under the tension caused by ssDNAs with various lengths. Later, they also studied a four-bar bistable

mechanical system with designed energy landscape, and showed that the conformational dynamics of the device can be controlled *via* strand displacement [65]. In 2015, Marras et al. [66] demonstrated mechanical designs of DNA origami inspired by macroscopic devices, including a hinge (rotational motion), a slider joint (translational motion) and a complex crank-slider mechanism integrated from the former two (see Figure 1b). In addition, a Bennett linkage which can be actuated *via* strand displacement was also characterized.

As another class of DNA-DNA interaction, a number of non-autonomous, autonomous and directed DNA walkers have been introduced and analyzed both experimentally and theoretically [58,59,67]. By taking advantage of the DNA origami addressability and programmability, the environment where the walker or robot is moving can be defined and precisely tuned. One example of such system was presented by Lund et al. [68], where "molecular spiders" of streptavidin body equipped with three catalytic deoxyribozyme legs were set to autonomously move along the predefined path on top of DNA origami template. A very recent and sophisticated example of DNA-assembled robotics on a DNA origami platform was created by Thubagere et al. [42]. They developed an algorithm for sorting two types of cargoes and their destinations on a DNA origami platform (Figure 1c). The DNA robot constructed from three functional domains was able to pick up the cargo and release it at the desired location. The movement of the robot is solely based on random walk and thus it does not require any additional energy to operate. The robot performed on average 300 steps during the cargo sorting, which is a huge improvement (one to two magnitudes) on previously reported DNA walkers that performed tasks while walking.

In 2016, Ke et al. [69] presented a nanoactuator design consisting of four DNA origami beams linked into a rhombus shape by flexible ssDNA joints (Figure 1d). Opening angle of the device was controlled by ssDNA lock strands of different lengths. Attaching two halves of eGFP to the device and closing the device with short locking strands was shown to bring the halves together and restore fluorescence of the protein. A similar working principle was amplified into a large-scale reconfiguration of a 2D origami lattice by Choi et al. [70]. They built a DNA accordion rack from long DNA beams connected by multiple flexible joints. Aspect ratio of the whole lattice could be controlled by adding DNA lock strands at selected positions in the structure. By applying toehold-mediated strand displacement processes, multiple rounds of conformational switching could be demonstrated.

Base stacking of blunt-ended dsDNA segments can form strong attractive interactions between different DNA nanostructures, or within a single device [71,72]. Gerling et al. [72] showed that transitions between different conformational states in various DNA origami designs could be controlled by adjusting the strength of base-stacking interactions between shape-complementary parts with cation concentration or temperature. Base-stacking interactions have also been used as a driving force in conveying information in large 2D DNA origami arrays [73]. The system constructed by Song et al. [73] consisted of multiple interconnected trapezoidal "antijunction units". A conformational change was initiated at selected units by addition of ssDNA strands complementary to ssDNA regions at the edges of the units. The change was seen to propagate through the whole assembly, as neighboring units switched into energetically more favorable conformations by maximizing the number of base-stacking interactions.

DNA-DNA interactions, including base stacking, can also be used to assemble complex nanomachines from multiple DNA origami elements, as demonstrated by Ketterer et al. [74]. They manufactured a miniature rotary apparatus analogous to F1F0-adenosine triphosphate (ATP) synthase (Figure 1e). The device was constructed from multiple tight-fitting DNA origami components by guiding the self-assembly with specific base-stacking interactions or DNA hybridization events. Based on single-particle fluorescence microscopy recordings, the devices were shown to exhibit random Brownian rotary motion. Controlling the movement of such devices with external triggers could lead to realization of intricate DNA-based nanomachines.

Recently, dynamic DNA devices have been combined with plasmonic systems, whose optical responses are extremely sensitive to the relative positions and orientations of the components. Kuzyk et al. [75] assembled a metamolecule from two gold nanorods (AuNRs) and two

interconnected DNA origami beams. The origami beams were connected by a single holliday junction in the middle, and ssDNA at the ends of the beams were used in strand displacement to switch the structure between a closed state and an open state. By switching the origami, the relative angle between the two AuNRs were altered, which resulted in a change in the circular dichroism (CD) signal (see Section 4 for similar systems with other stimuli). Besides dynamic DNA origami device, oligonucleotide-functionalized AuNR could also walk on a static origami *via* a strand displacement [76].

Although not being an actual device, it is worth to mention that the dynamic interaction between short oligonucleotides has promising applications in super-resolution imaging. The so-called transient binding describes a temporary binding of complementary DNA strands at a temperature close to the melting point of oligonucleotides with specific sequences and lengths. DNA origami with docking strands which allow transient binding of dye-labeled oligonucleotides have been used in DNA-point accumulation for imaging in nanoscale topography (DNA-PAINT) in super-resolution microscopy [36,77,78] (see Figure 1f). Furthermore, detection of multiple protein targets in fixed cells with super-resolution Exchange-PAINT technique has been recently realized [79].

3. DNA Origami Devices With Molecular Interaction

In this section, DNA devices with dynamic properties mediated by molecular interactions between the device and other molecules in the solution are discussed. The scale of the dynamic devices ranges from tools designed for measuring or detecting a specific molecular interaction to aptamer-functionalized objects for nanorobotics, computing, and drug delivery.

An early example of dynamic DNA origami devices used in molecular detection was the single molecule beacons presented by Kuzuya et al. in 2011 [80] (Figure 2a). These "DNA origami pliers" or "DNA origami forceps" consist of two rigid beams connected by a flexible DNA crossover region, which allows the arms to rotate relative to each other. When a target such as protein, metal ion, or human microRNA (miRNA) binds to both arms of the device, the arms are locked into a parallel orientation. Thus, single-molecule binding events are amplified into a major conformational change that can be detected by using transmission electron or atomic force microscopy (TEM or AFM).

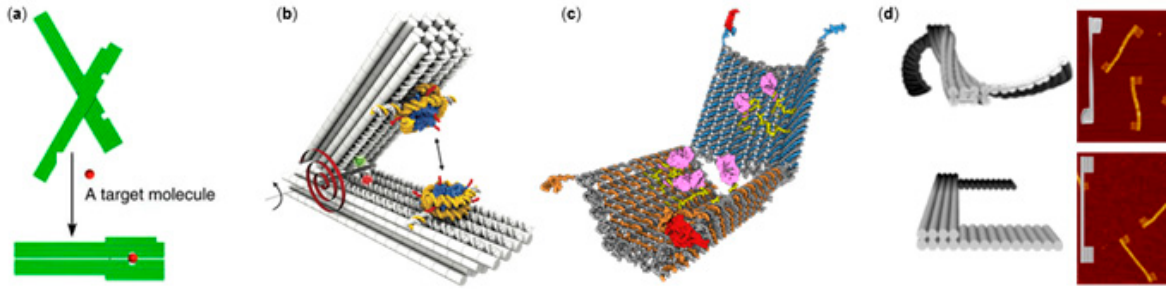


Figure 2. DNA origami devices with molecular interaction. (**a**) DNA origami pliers or forceps that exhibit conformational change upon a target molecule binding [80]. (**b**) DNA origami measurement device equipped by nucleosomes to probe nucleosome-nucleosome interaction [81]. (**c**) A logic-gated nanorobot that displays the cargo when specific antigens bind to aptamer-encoded DNA locks [12]. (**d**) DNA origami twisting and rotation by applying DNA intercalating molecules [87]. (**a**) is reproduced with permission from [80]. Published by Nature Publishing Group, 2011; (**b**) is reproduced with permission from [81]. Published by The American Association for the Advancement of Science, 2017; (**c**) is reproduced with permission from [12]. Copyright The American Association for the Advancement of Science, 2012; (**d**) is reproduced with permission from [87]. Copyright American Chemical Society, 2016.

Other neat examples showing the potential of DNA origami-based measurement tools are the various studies carried out with DNA origami devices and nucleosomes [81–83]. Funke et al. [81]

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 2114; doi:10.3390/ijms19072114

and Le et al. [82] both introduced measurement devices with similar hinge-like designs, where two DNA origami arms are joined together at one end by a flexible ssDNA hinge (Figure 2b). The system presented by Funke et al. was initially introduced as a static tool for placing molecules at set distances with extreme precision [84]. In the nucleosome studies, a molecular interaction under interest changes the opening angle of the device, which can then be used as a measure of the interaction strength. The device was used to study both nucleosome unwrapping at different ionic strengths [82] and the strength of attractive interactions between two nucleosomes [81]. Le et al. [83] used their device for probing various properties of nucleosome-DNA interaction, such as nucleosomal end-to-end distance, nucleosome conformation, and nucleosome stability.

The previous examples of molecular measurement devices all share a relatively similar working principle: molecular binding or interaction under interest converts the device into a discrete, relatively immobile orientation, which is then characterized. In contrast to this, Hudoba et al. [85] measured compressive depletion forces in a solution with a dynamic device that constantly fluctuates between an open (uncompressed) and a closed (compressed) state. Increased depletion forces caused by molecular crowding agents, particularly by poly(ethylene-glycol) (PEG), were observed to shift the dynamics of the device more towards the closed state.

DNA origami devices with specific interactions to other biomolecules of interest can be constructed with the help of aptamers. Aptamers are oligonucleotides, which bind a specific target molecule with a high affinity. One type of dynamic aptamer-functionalized systems is a container that is held closed by the aptamer regions hybridized to complementary DNA strands [12,86]. The container is released into an open state when the aptamers come in contact and bind to their target molecule, which creates intriguing potential to use these types of DNA devices as specifically targeted drug-delivery vehicles.

A famous example of an aptamer-functionalized DNA origami container is a logic-gated nanorobot introduced by Douglas et al. [12] (Figure 2c). The robots were equipped with different combinations of aptamers. The robot held in a closed state would open and release its cargo only when two different triggers were simultaneously encountered in the vicinity of specific cell lines, thus creating a logical AND-gate. In a later study, Amir et al. [88] developed the idea of recreating logical functions and performing molecular computing by DNA nanorobots even further. When aptamer-functionalized robots were mixed in defined molar ratios with robots that could either activate or deactivate the original robots by DNA-DNA interactions, the robot mixtures could emulate a variety of logical functions and perform rudimentary computing inside living cockroaches. Later it was demonstrated that a mixture of three interacting robots could behave according to Isaac Asimov's three laws of robotics (see Appendix) [89].

Recently, *in vivo* therapeutic potential of aptamer-functionalized DNA origami nanorobots was shown by Li et al. [86], who designed a tubular DNA origami device made out of a rolled up 2D origami sheet held together by nucleolin-binding aptamers. The devices were shown to unroll and expose active thrombin in the vicinity of targeted endothelial tumor cells, leading to inhibition of tumor growth.

Dynamic DNA origami devices are often constructed by linking rigid dsDNA elements with flexible hinges or pivot points formed of ssDNA. Chen et al. [87] studied the possibility to induce controllable dynamic behavior in structures consisting solely of dsDNA. They utilized DNA-binding adducts, such as ethidium bromide (EtBr), which intercalate between DNA base pairs and cause torsional deformation of dsDNA by unwinding the duplex. By increasing the intercalator concentration in the solution, they demonstrated a significant twist along the length of the whole DNA origami (Figure 2d). The change was shown to be fine-tunable by intercalator concentration and partially reversible when intercalators were removed by addition of competing DNA strands.

In addition to moving DNA origami devices, DNA origami itself can act as a template in a highly dynamic assembly. As an intriguing example of such system, a programmable DNA origami rod can be used as a cargo mimic for motor proteins [90]. By controlling the number and type of proteins linked to the cargo mimic, a molecular scale tug-of-war can be assembled for probing the collective motility of the selected motor proteins. Similarly, dynamic DNA origami diffusion can be

assisted by lipid bilayers [91–93]. This type of lipid-assisted diffusion is not fully controllable and is not exactly falling in the category of DNA origami devices, but interestingly, by employing lipid bilayers, DNA origami can be dynamically arranged into well-defined lattices and other higher-order assemblies. The DNA origami diffusion on top of a lipid layer can be tuned using cholesterol-modifications in DNA origami, taking advantage of lipid membrane phases (liquid-disordered or solid-ordered) or by adjusting cation concentration. Therefore, it is likely, that combination of DNA origami with proteins and lipids may find uses in developing dynamic nanomachines.

4. DNA Origami Devices Triggered by External Stimuli or Multiple Interactions

This section deals with DNA origami nanosystems, in which plasmonic effects, dynamic and controllable movement *e.g.* switching or rotation is induced using wide spectra of external stimuli ranging from photoregulation to pH and thermally directed assembly to electromagnetic fields.

Yang et al. [94] were among the first to show UV-controllable DNA origami structures. They demonstrated the assembly and disassembly of predesigned multi-orientational patterns constructed from rigid DNA hexagons with photoresponsive azobenzene-modified oligonucleotides inserted either in one, two or three edges. Later on, Kohman and Han [95] demonstrated light-triggered reconfiguration of a hollow spherical DNA nanostructure. The sphere was obtained from two hemispheres linked together through DNA scaffold, and the sphere was sealed from the equator using nine crossover strands modified with photolabile *o*-nitrobenzyl moieties. Irradiation at the specific wavelength of 302 nm resulted in almost quantitative and irreversible cleavage of nitrobenzyl groups showing tethered hemispheres in TEM images (Figure 3a).

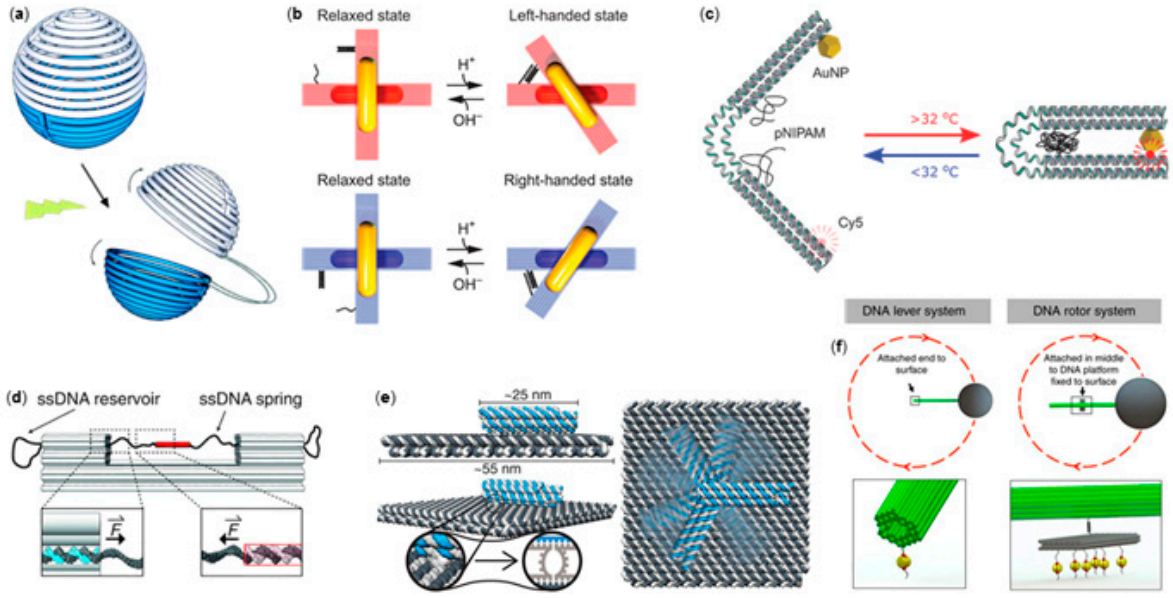


Figure 3. DNA origami movement using stimuli: (**a**) A spherical DNA origami container that can be opened by light [95]. (**b**) Reconfigurable chiral plasmonic metamolecules [97]. (**c**) Thermoresponsive actuator [99]. (**d**) Autonomous nanoscopic force clamp [100]. (**e**) Electric field directed robotic arm [43]. (**f**) Magnetic actuators [104]. (**a**) is reproduced with permission from [95]. Copyright The Royal Society of Chemistry, 2015; (**b**) is reproduced with permission from [97]. Published by The American Association for the Advancement of Science, 2017; (**c**) is reproduced with permission from [99]. Copyright John Wiley and Sons, 2018; (**d**) is reproduced with permission from [100]. Copyright The American Association for the Advancement of Science, 2016; (**e**) is reproduced with permission from [43]. Copyright The American Association for the Advancement of Science, 2018; (**f**) is reproduced with permission from [104]. Published by Nature Publishing Group, 2018.

Kuzyk et al. [96] designed an elegant, light-driven 3D plasmonic DNA origami nanostructure, which is based on reversible *cis-trans* photoisomerization of azobenzene units. They used similar design as described in Section 2 but now the design contained azobenzene units that were assembled to form a chiral template with an adjustable angle. Optical control between locked (*ca*. 50° angle) and relaxed (*ca*. 90° angle) conformational states was obtained by ultraviolet (UV) and visible (Vis) light illumination. Insertion of two AuNRs to the bundles resulted in a tunable plasmonic chiroptical response with large amplitude modulation upon light stimuli. Elaborated from the previous design [96], Kuzyk et al. [97] demonstrated that the plasmonic metamolecules can also be reconfigured *via* pH change which trigger the selective control over chiral locked or relaxed state (Figure 3b). The DNA "lock" is based on DNA triplex formation through pH-sensitive and sequence-specific parallel Hoogsteen interactions occurring between a ssDNA and a duplex DNA. Tuning of the relative contents of TAT/CGC triplets enabled programmability and discrimination of chiral quasi-enantiomers over a wide pH range.

Jiang et al. [98] introduced chiral plasmonic nanostructures by assembling AuNRs into predesigned L-type configuration with the aid of rhombus-type DNA origami templates. The template was obtained by joining two different triangular-shaped origami along the defined seams that respond to different stimuli. A clear dynamic plasmonic effect was detected from circular dichroism spectra when modular controller strands were probed by reversible *cis-trans* induced dissociation of azobenzene moiety that resulted in a stretching of G-quadruplex or pH-induced folding/unfolding of cytosine-rich i-motifs. Irreversible cleavage of disulfide-bonds by reduction of glutathione tripeptide or cleavage of restriction enzyme-sensitive DNA sequences was also demonstrated.

Turek and co-workers [99] created a thermoresponsive DNA origami tweezers in which the actuation is based on reversible temperature-induced coil-to-globule transition of the thermo-responsive polymer poly(N-isopropylacrylamide) (PNIPAM). Above lower critical solution temperature (LCST) of 32 °C PNIPAM becomes hydrophobic causing the folding of both rigid arms (Figure 3c) which is detected as an increased fluorescence of gold nanoparticle (AuNP) and Cy5 located at the tip of the tweezer in equivalent positions.

Nickels et al. [100] demonstrated that a folded bracket-shape DNA origami functions as nanoscopic force clamp for probing multiple different interactions. In this nanodevice, the system of interest (red rectangle, Figure 3d) is hooked between two immobile attachment points *via* ssDNA domains that function as entropic springs and exert constant force in piconewton (pN) scale over time. ssDNA reservoirs are residing on both sides of the clamp. Conformational transitions can be monitored *via* single-molecule FRET. The sensitive device equipped with (Cy3)-donor/(Cy5)-acceptor FRET-pair is able to characterize a movement of four-way Holliday junction between different states and also protein-induced DNA bending. Very recently, related to above-mentioned force spectroscopy, Dutta and co-workers [101] reported a DNA origami tension probe (DOTP) capable of depicting the traction forces generated by living cells. Various DOTP combinations were employed to map the forces applied by human blood platelets during initial adhesion and activation. Traction forces with piconewton (pN) resolution were measured utilizing tension-to-fluorescence transduction upon unfolding the DNA hairpin that was incorporated into the system.

In addition to the examples above, external electromagnetic fields can be used to manipulate DNA origami movement. Recently, Kopperger and co-workers [43] displayed a truly dynamic DNA origami platform for nanoscale (up to 400 nm) robotic arm controlled by electric fields. The system was composed of a rigid DNA origami plate equipped with 25 nm long 6HB arm attached *via* ssDNA scaffold crossovers (see the close up in Figure 3e). Flexible joint allowed stochasting switching of the arm due to transient binding, which was detected from FRET-signals generated by donor fluorophore on the tip of the arm and two acceptor dyes mounted on the rectangular base plate. Electrically controlled movement of the robot arm (angular movement up to 25 Hz) was measured when the system was mounted in a 4-way electrophoretic chamber. Further, ability to move inorganic nanoparticles, *e.g*. AuNRs, by the robotic arm was demonstrated. DNA origami

Peer-reviewed version available at Int. J. Mol. Sci. 2018, 19, 2114; doi:10.3390/ijms19072114

polarizability can also be used to direct and trap DNA origami by dielectrophoresis in a nonuniform electric field as shown by Kuzyk et al. [102] and Shen et al. [103].

Lauback et al. [104] introduced yet another way to control DNA origami movement by employing external magnetic fields. Three quasi-analogous nanostructures, *i.e*. lever, rotor and hinge systems (Figure 3f), having diverse angular movement paths were demonstrated. All constructs were assembled from three components: a base platform, a stiff 56-helix bundle rotor arm equipped with a micromagnetic bead on the free rotating end, and a ductile pivot anchoring the rotor to the base platform *via* biotin-streptavidin affinity. The concept allowed sustained rotational motion (up to 2 Hz), capability of operating up to 80 pN-nm of torque, and a definite control (±8° resolution) over the angular conformation.

5. Conclusions and Perspectives

"What are the possibilities of small but movable machines? They may or may not be useful, but they surely would be fun to make," pondered Feynman in his talk [1]. Here we have reviewed DNA origami-based nanomachines that exhibit translational and rotational motion when triggered by various types of stimuli. We have also addressed Feynman's question by discussing the usefulness of these nanomachines. The summary of stimuli/interactions, implementations and possible applications reviewed in this article are listed in Table 1.

Table 1. Summary of the reviewed types of DNA origami motion.

Interaction / stimulus	Implementation	Application
DNA oligonucleotides	Lock-key systems based on toehold-mediated strand displacement Transient binding	Containers [15,60,61] Biosensing [62] Reconfigurable plasmonics [75] Reconfigurable actuators and lattices [69,70] DNA-PAINT [77,78] Robotic walkers [42]
Entropic elasticity and steric effect	ssDNA as an entropic spring	Nanoscale mechanical devices [64,65,66] Force spectrometers [100]
DNA base stacking	Shape-complementary, blunt-ended dsDNA regions	Large-scale assembly [22,72] Reconfigurable devices [72] Information relay [73] Rotary devices [74]
Site specific binding of target molecules	Incorporation of residues with specific chemical reactivity Modified oligonucleotides Aptamers	Measurement devices [69,80–84,100] Drug delivery and nanorobotics [12,86,88,89]
Non-site specific interactions with other molecules	Mixing DNA origami with crowding agents (*e.g*. PEG) intercalators, lipid bilayers	Measurement of molecular crowding [85] Fine-tunable twisting motion [87] Lipid-assisted diffusion [91–93]
Light (UV/Vis)	Incorporation of photoresponsive molecules, *e.g*. azobenzenes	Photo-controllable assembly and disassembly of nanostructures [94] Photo-cleavable containers [95] Reconfigurable plasmonics [96]
pH change, Hoogsteen interactions	pH-sensitive DNA regions, *e.g*. i-motifs, G-quadruplexes, triplex-forming sequences	Reconfigurable plasmonics [97,98]
Temperature change	Thermoresponsive polymers	Thermoresponsive actuation [99]
Electric or magnetic field	Polarizability of DNA in electric field Magnetic beads linked to origami	Rotary devices, hinges and levers [43,104]

Although the future of the artificial DNA-based nanodevices seems bright, there are several challenges and problems that should be resolved. One obvious issue in Feynman's surgical nanorobot vision is that the DNA structures are prone to degradation in many biologically relevant conditions [40]. Therefore, researchers have introduced plenty of strategies for coating and protection of DNA origami [31,105-109], but the challenge is to ensure the functionality of the dynamic devices with such protection systems. Even in the static systems, for example in the confined enzyme reactors [110], the functionality – in this case enzymatic reaction – is suppressed due to the protective coating. Nevertheless, as the DNA nanotechnology field has already reached the enabled state [8], it is likely that DNA origami-based dynamic drug-delivery systems are increasingly coming into view [61,86]. Feynman's another postulate regarding mechanical machines has already become true, since DNA origami allows molecular scale precision measurements that are either challenging or not even achievable using other techniques [13]. These devices facilitate characterization of DNA stacking forces [111], nucleosome-nucleosome and nucleosome-DNA interactions [81–83] and for example, probing of protein-DNA interaction [100]. Moreover, employing external stimuli such as electric [43] or magnetic field [104], it is possible to bridge microscale manipulation to nanoscale devices and thereby control the movement of these nanomachines with short response times. Further engineering of these programmable and dynamic DNA origami nanomachines will lead this research field from proof-of-principle examples to actual utility.

Author Contributions: The article was written through contributions of all authors. H.I., S.N. and B.S. contributed equally to this work.

Funding: This research was funded by the Academy of Finland (grant numbers 286845 and 308578), Jane and Aatos Erkko Foundation, Sigrid Jusélius Foundation, Magnus Ehrnrooth Foundation and Emil Aaltonen Foundation.

Acknowledgments: This work was supported through the funding agencies above and it was carried out under the Academy of Finland Centers of Excellence Programme (2014-2019).

Conflicts of Interest: The authors declare no conflict of interest.

Appendix

"The Three Laws of Robotics" by Isaac Asimov [112]:

L1. A robot may not injure a human being or, through inaction, allow a human being to come to harm.

L2. A robot must obey the orders given it by human beings except where such orders would conflict with the First Law.

L3. A robot must protect its own existence as long as such protection does not conflict with the First or Second Laws.

In the paper "Molecular robots obeying Asimov's three laws of robotics" [89], the robots were similar two-state DNA origami devices as explained in the Ref. [88] (see also Figure 2c). A microRNA molecule (a human miR-16 analogue) was used as a damage signal. With the logic-gated DNA nanorobots and the selected damage signal, the authors were able to recreate Isaac Asimov's "Runaround" scenario using approximately 100,000,000,000 robots [89]: "It begins with L2 dominating, followed by a conflict between L2 and L3, causing an equilibrium to be reached. The equilibrium is terminated by the introduction of another conflict, between L1 and L2, in which L1 overrides L2."

References

1. Feynman, R.P. There's plenty of room at the bottom. *Engineering and Science* **1960**, *23*, 22–36 (Caltech, February, 1960).
2. Watson, J.D.; Crick, F.H.C. Molecular structure of nucleic acids. *Nature* **1953**, *171*, 737–738, DOI: 10.1038/171737a0.
3. Jones, M.R.; Seeman, N.C.; Mirkin, C.A. Programmable materials and the nature of the DNA bond. *Science* **2015**, *347*, 1260901, DOI: 10.1126/science.1260901.
4. Seeman, N.C. Nucleic acid junctions and lattices. *J. Theor. Struct. Biol*. **1982**, *99*, 237–247, DOI: 10.1016/0022-5193(82)90002-9.
5. Wang, P.; Meyer, T.A.; Pan, V.; Dutta, P.K.; Ke, Y. The beauty and utility of DNA origami. *Chem* **2017**, *2*, 359–382, DOI: 10.1016/j.chempr.2017.02.009.
6. Nummelin, S.; Kommeri, J.; Kostiainen, M.A.; Linko, V. Evolution of structural DNA nanotechnology. *Adv. Mater.* **2018**, *30*, 1703721, DOI: 10.1002/adma.201703721.
7. Seeman, N.C. DNA in a material world. *Nature* **2003**, *421*, 427–431, DOI: 10.1038/nature01406.
8. Linko, V.; Dietz, H. The enabled state of DNA nanotechnology. *Curr. Opin. Biotechnol*. **2013**, *24*, 555–561, DOI: 10.1016/j.copbio.2013.02.001.
9. Hong, F.; Zhang, F.; Liu, Y.; Yan, H. DNA origami: scaffolds for creating higher order structures. *Chem. Rev*. **2017**, *117*, 12584–12640, DOI: 10.1021/acs.chemrev.6b00825.
10. Bathe, M.; Rothemund, P.W.K. DNA nanotechnology: A foundation for programmable nanoscale materials. *MRS Bull*. **2017**, *42*, 882–888, DOI: 10.1557/mrs.2017.279.
11. Seeman, N.C.; Sleiman, H.F. DNA nanotechnology. *Nat. Rev. Mater*. **2017**, *3*, 17068, DOI: 10.1038/natrevmats.2017.68.
12. Douglas, S.M.; Bachelet, I.; Church, G.M. A logic-gated nanorobot for targeted transport of molecular payloads. *Science* **2012**, *335*, 831–834, DOI: 10.1126/science.1214081.
13. Castro, C.E.; Dietz, H.; Högberg, B. DNA origami devices for molecular-scale precision measurements. *MRS Bull*. **2017**, *42*, 925–929, DOI: 10.1557/mrs.2017.273.
14. Rothemund, P.W.K. Folding DNA to create nanoscale shapes and patterns. *Nature* **2006**, *440*, 297–302, DOI: 10.1038/nature04586.
15. Andersen, E.S.; Dong, M.; Nielsen, M.M.; Jahn, K.; Subramani, R.; Mamdouh, W.; Golas, M.M.; Sander, B.; Stark, H.; Oliveira, C.L.P., et al. Self-assembly of a nanoscale DNA box with a controllable lid. *Nature* **2009**, *459*, 73–76, DOI: 10.1038/nature07971.
16. Douglas, S.M.; Dietz, H.; Liedl, T.; Högberg, B.; Graf, F.; Shih, W.M. Self-assembly of DNA into nanoscale three-dimensional shapes. *Nature* **2009**, *459*, 414–418, DOI: 10.1038/nature08016.
17. Dietz, H.; Douglas, S.M.; Shih, W.M. Folding DNA into twisted and curved nanoscale shapes. *Science* **2009**, *325*, 725–730, DOI: 10.1126/science.1174251.
18. Han, D.; Pal, S.; Nangreave, J.; Deng, Z.; Liu, Y.; Yan, H. DNA origami with complex curvatures in three-dimensional space. *Science* **2011**, *332*, 342–346, DOI: 10.1126/science.1202998.
19. Benson, E.; Mohammed, A.; Gardell, J.; Masich, S.; Czeizler, E.; Orponen, P.; Högberg, B. DNA rendering of polyhedral meshes at the nanoscale. *Nature* **2015**, *523*, 441–444, DOI: 10.1038/nature14586.
20. Veneziano, R.; Ratanalert, S.; Zhang, K.; Zhang, F.; Yan, H.; Chiu, W.; Bathe, M. Designer nanoscale DNA assemblies programmed from the top down. *Science* **2016**, *352*, 1534, DOI: 10.1126/science.aaf4388.
21. Linko, V.; Kostiainen, M.A. Automated design of DNA origami. *Nat. Biotechnol*. **2016**, *34*, 826–827, DOI: 10.1038/nbt.3647.
22. Wagenbauer, K.F.; Sigl, C.; Dietz, H. Gigadalton-scale shape-programmable DNA assemblies. *Nature* **2017**, *552*, 78–83, DOI: 10.1038/nature24651.
23. Tikhomirov, G.; Petersen, P.; Qian, L. Fractal assembly of micrometer-scale DNA origami arrays with arbitrary patterns. *Nature* **2017**, *552*, 67–71, DOI: 10.1038/nature24655.
24. Ke, Y.; Ong, L.L.; Shih, W.M.; Yin, P. Three-dimensional structures self-assembled from DNA bricks. *Science* **2012**, *338*, 1177–1183, DOI: 10.1126/science.1227268.
25. Ong, L.L.; Hanikel, N.; Yaghi, O.K.; Grun, C.; Strauss, M.T.; Bron, P.; Lai-Kee-Him, J.; Schueder, F.; Wang, B.; Wang, P., et al. Programmable self-assembly of three-dimensional nanostructures from 10,000 unique components. *Nature* **2017**, *552*, 72–77, DOI: 10.1038/nature24648.
26. Linko, V.; Nummelin, S.; Aarnos, L.; Tapio, K.; Toppari, J.J.; Kostiainen, M.A. DNA-based enzyme reactors and systems. *Nanomaterials* **2016**, *6*, 139, DOI: 10.3390/nano6080139.
27. Gothelf, K.V. Chemical modifications and reactions in DNA nanostructures. *MRS Bull.* **2017**, *42*, 897–903, DOI: 10.1557/mrs.2017.276.
28. Grossi, G.; Jaekel, A.; Andersen, E.S.; Saccà, B. Enzyme-functionalized DNA nanostructures as tools for organizing and controlling enzymatic reactions. *MRS Bull*. **2017**, *42*, 920–924, DOI: 10.1557/mrs.2017.269.

29. Kuzyk, A.; Schreiber, R.; Fan, Z.; Pardatscher, G.; Roller, E.-M.; Högele, A.; Simmel, F.C.; Govorov, A.O.; Liedl, T. DNA-based self-assembly of chiral plasmonic nanostructures with tailored optical response. *Nature* **2012**, *483*, 311–314, DOI: 10.1038/nature10889.
30. Shen, B.; Linko, V.; Tapio, K.; Pikker, S.; Lemma, T.; Gopinath, A.; Gothelf, K.V.; Kostiainen, M.A.; Toppari, J.J. Plasmonic nanostructures through DNA-assisted lithography. *Sci. Adv.* **2018**, *4*, eaap8978, DOI: 10.1126/sciadv.aap8978.
31. Perrault, S.D.; Shih, W.M. Virus-inspired membrane encapsulation of DNA nanostructures to achieve in vivo stability. *ACS Nano* **2014**, *8*, 5132–5140, DOI: 10.1021/nn5011914.
32. Linko, V.; Ora, A.; Kostiainen, M.A. DNA nanostructures as smart drug-delivery vehicles and molecular devices. *Trends Biotechnol*. **2015**, *33*, 586–594, DOI: 10.1016/j.tibtech.2015.08.001.
33. Surana, S.; Shenoy, A.R.; Krishnan, Y. Designing DNA nanodevices for compatibility with the immune system of higher organisms. *Nat. Nanotechnol*. **2015**, *10*, 741−747, DOI: 10.1038/nnano.2015.180.
34. Ora, A.; Järvihaavisto, E.; Zhang, H.; Auvinen, H.; Santos, H.A.; Kostiainen, M.A.; Linko, V. Cellular delivery of enzyme-loaded DNA origami. *Chem. Commun*. **2016**, *52*, 14161–14164, DOI: 10.1039/c6cc08197e.
35. Zhang, Y.; Tu, J.; Wang, D.; Zhu, H.; Maity, S.K.; Qu, X.; Bogaert, B.; Pei, H.; Zhang, H. Programmable and multifunctional DNA-based materials for biomedical applications. *Adv. Mater*. **2018**, *30*, 1703658, DOI: 10.1002/adma.201703658.
36. Graugnard, E.; Hughes, W.L.; Jungmann, R.; Kostiainen, M.A.; Linko, V. Nanometrology and super-resolution imaging with DNA. *MRS Bull*. **2017**, *42*, 951–959, DOI: 10.1557/mrs.2017.274.
37. Julin, S.; Nummelin, S.; Kostiainen, M.A.; Linko, V. DNA nanostructure-directed assembly of metal nanoparticle superlattices. *J. Nanopart. Res*. **2018**, *20*, 119, DOI: 10.1007/s11051-018-4225-3.
38. Zhang, T.; Hartl, C.; Fischer, S.; Frank, K.; Nickels, P.; Heuer-Jungemann, A.; Nickel, B.; Liedl, T. 3D DNA origami crystals. *Adv. Mater*. **2018**, *30*, 1800273, DOI: 10.1002/adma.201800273.
39. Shen, B.; Tapio, K.; Linko, V.; Kostiainen, M.A.; Toppari, J.J. Metallic nanostructures based on DNA nanoshapes. *Nanomaterials* **2016**, *6*, 146, DOI: 10.3390/nano6080146.
40. Kielar, C.; Xin, Y.; Shen, B.; Kostiainen, M.A.; Grundmeier, G.; Linko, V.; Keller, A. On the stability of DNA origami nanostructures in low-magnesium buffers. *Angew. Chem. Int. Ed.* **2018**, *57*, DOI: 10.1002/acie.201802890.
41. Praetorius, F.; Kick, B.; Behler, K.L.; Honemann, M.N.; Weuster-Botz, D.; Dietz, H. Biotechnological mass production of DNA origami. *Nature* **2017**, *552*, 84–87, DOI: 10.1038/nature24650.
42. Thubagere, A.J.; Li, W.; Johnson, R.F.; Chen, Z.; Doroudi, S.; Lee, Y.L.; Izatt, G.; Srinivas, N.; Woods, D.; Winfree, E.; Qian, L. A cargo-sorting DNA robot. *Science* **2017**, *357*, eaan6558, DOI: 10.1126/science.aan6558.
43. Kopperger, E.; List, J.; Madhira, S.; Rothfischer, F.; Lamb, D.C.; Simmel, F.C. A self-assembled nanoscale robotic arm controlled by electric fields. *Science* **2018**, *359*, 296–301, DOI: 10.1126/science.aao4284.
44. Huang, P.-S.; Boyken, S.E.; Baker, D. The coming of age of de novo protein design. *Nature* **2016**, *537*, 320–327, DOI: 10.1038/nature19946.
45. Castro, C.E.; Kilchherr, F.; Kim, D.-N.; Shiao, E.L.; Wauer, T.; Wortmann, P.; Bathe, M.; Dietz, H. A primer to scaffolded DNA origami. *Nat. Methods* **2011**, *8*, 221–229, DOI: 10.1038/nmeth.1570.
46. Kim, D.-N.; Kilchherr, F.; Dietz, H.; Bathe, M. Quantitative prediction of 3D solution shape and flexibility of nucleic acid nanostructures. *Nucleic Acids Res*. **2012**, *40*, 2862–2868, DOI: 10.1093/nar/gkr1173.
47. Maffeo, C.; Yoo, J.; Aksimentiev, A. De novo reconstruction of DNA origami structures through atomistic molecular dynamics simulation. *Nucleic Acids Res*. **2016**, *44*, 3013–3019, DOI: 10.1093/nar/gkw155.
48. Sharma, R.; Schreck, J.S.; Romano, F.; Louis, A.A.; Doye, J.P.K. Characterizing the motion of jointed DNA nanostructures using a coarse-grained model. *ACS Nano* **2017**, *11*, 12426–12435, DOI: 10.1021/acsnano.7b06470.
49. Shi, Z.; Castro, C.E.; Arya, G. Conformational dynamics of mechanically compliant DNA nanostructures from coarse-grained molecular dynamics simulations. *ACS Nano* **2017**, *11*, 4617–4630, 10.1021/acsnano.7b00242.
50. Jo, S.; Kim, S.; Lee, B.H.; Tandon, A.; Kim, B.; Park, S.H.; Kim, M.K.Fabrication and characterization of finite-size DNA 2D ring and 3D buckyball structures. *Int. J. Mol. Sci.* **2018**, *19*, 1895, DOI: 10.3390/ijms19071895.
51. Mao C, Sun, W., Shen, Z.; Seeman, N.C. A nanomechanical device based on the B-Z transition of DNA. *Nature* **1999**, *397*, 144–146, DOI: 10.1038/16437.
52. Yurke, B.; Turberfield, A.J.; Mills, A.P. Jr.; Simmel, F.C.; Neumann, J.L. A DNA-fuelled molecular machine made of DNA. *Nature* **2000**, *406*, 605–608, DOI: 10.1038/35020524.

53. Yan, H.; Zhang, X.; Shen, Z.; Seeman, N.C. A robust DNA mechanical device controlled by hybridization topology. *Nature* **2002**, *415*, 62–65, DOI: 10.1038/415062a.
54. Simmel, F.C.; Yurke, B. A DNA-based molecular device switchable between three distinct mechanical states. *Appl. Phys. Lett*. **2002**, *80*, 883–885, DOI: 10.1063/1.1447008.
55. Zhong, H.; Seeman, N.C. RNA used to control a DNA rotary nanomachine. *Nano Lett*. **2006**, *6*, 2899–2903, DOI: 10.1021/nl062183e.
56. Liu, M.; Fu, J.; Hejesen, C.; Yang, Y.; Woodbury, N.W.; Gothelf, K.V.; Liu, Y.; Yan, H. A DNA tweezer-actuated enzyme nanoreactor. *Nat. Commun*. **2013**, *4*, 2127, DOI: 10.1038/ncomms3127.
57. Xin, L.; Zhou, C.; Yang, Z.; Liu, D. Regulation of an enzyme cascade reaction by a DNA machine. *Small* **2013**, *9*, 3088–3091, DOI: 10.1002/smll.201300019.
58. Bath, J.; Turberfield, A.J. DNA nanomachines. *Nat. Nanotechnol*. **2007**, *2*, 275–284, DOI: 10.1038/nnano.2007.104.
59. Pan, J.; Li, F.; Cha, T.G.; Chen, H.; Choi, J.H. Recent progress on DNA based walkers. *Curr. Opin. Biotechnol*. **2015**, *34*, 56–64, DOI: 10.1016/j.copbio.2014.11.017.
60. Zadegan, R.M.; Jepsen, M.D.E.; Thomsen, K.E.; Okholm, A.H.; Schaffert, D.H.; Andersen, E.S.; Birkedal, V.; Kjems, J. Construction of a 4 zeptoliters switchable 3D DNA origami box. *ACS Nano* **2012**, *6*, 10050–10053, DOI: 10.1021/nn303767b.
61. Grossi, G.; Jepsen, M.D.E.; Kjems, J.; Andersen, E.S. Control of enzyme reactions by a reconfigurable DNA nanovault. *Nat. Commun*. **2017**, *8*, 992, DOI: 10.1038/s41467-017-01072-8.
62. Selnihhin, D.; Sparvath, S.M.; Preus, S.; Birkedal, V.; Andersen, E.S. Multifluorophore DNA origami beacon as a biosensing platform. *ACS Nano* **2018**, *12*, 5699–5708, DOI: 10.1021/acsnano.8b01510.
63. Castro, C.E.; Su, H.J.; Marras, A.E.; Zhou, L.; Johnson, J. Mechanical design of DNA nanostructures. *Nanoscale* **2015**, *7*, 5913–5921, DOI: 10.1039/c4nr07153k.
64. Zhou, L.; Marras, A.E.; Su, H.-J.; Castro, C.E. DNA origami compliant nanostructures with tunable mechanical properties. *ACS Nano* **2014**, 8, 27–34, DOI: 10.1021/nn405408g.
65. Zhou, L.; Marras, A.E.; Su, H.-J.; Castro, C.E. Direct design of an energy landscape with bistable DNA origami mechanisms. *Nano Lett*. **2015**, *15*, 1815–1821, DOI: 10.1021/nl5045633.
66. Marras, A.E.; Zhou, L.; Su, H.-J.; Castro, C.E. Programmable motion of DNA origami mechanics. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 713–718, DOI: 10.1073/pnas.1408869112.
67. Khara, D.C.; Schreck, J.S.; Tomov, T.E.; Berger, Y.; Ouldridge, T.E.; Doye, J.P.K.; Nir, E. DNA bipedal motor walking dynamics: an experimental and theoretical study of the dependency on step size. *Nucleic Acids Res*. **2017**, *46*, 1553–1561, DOI: 10.1093/nar/gkx1282.
68. Lund K.; Manzo, A.J.; Dabby, N.; Michelotti, N.; Johnson-Buck, A.; Nangreave, J.; Taylor, S.; Pei, R.; Stojanovic, M.N.; Walter, N.G. ; Winfree, E.; Yan, H. Molecular robots guided by prescriptive landscapes. *Nature* **2010**, *465*, 206–210, DOI: 10.1038/nature09012.
69. Ke, Y.; Meyer, T.; Shih, W.M.; Bellot, G. Regulation at a distance of biomolecular interactions using a DNA origami nanoactuator. *Nat. Commun.* **2016**, 7, 10935, DOI: 10.1038/ncomms10935.
70. Choi, Y.; Choi, H.; Lee, A. C.; Lee, H.; Kwon, S. A Reconfigurable DNA Accordion Rack. *Angew. Chemie - Int. Ed.* **2018**, 57, 2811–2815, DOI: 10.1002/anie.201709362.
71. Woo, S.; Rothemund, P.W.K. Programmable molecular recognition based on the geometry of DNA nanostructures. *Nat. Chem.* **2011**, *3*, 620–627, DOI:10.1038/nchem.1070.
72. Gerling, T.; Wagenbauer, K.F.; Neuner, A.M.; Dietz, H. Dynamic DNA devices and assemblies formed by shape-complementary, non-base pairing 3D components. *Science* **2015**, *347*, 1446–1452, DOI:10.1126/science.aaa5372.
73. Song, J.; Li, Z.; Wang, P.; Meyer, T.; Mao, C.; Ke, Y. Reconfiguration of DNA molecular arrays driven by information relay. *Science* **2017**, *357*, eaan3377, DOI: 10.1126/science.aan3377.
74. Ketterer, P.; Willner, E.M.; Dietz, H. Nanoscale rotary apparatus formed from tight-fitting 3D DNA components. *Sci. Adv.* **2016**, *2*, e1501209, DOI: 10.1126/sciadv.1501209.
75. Kuzyk, A.; Schreiber, R.; Zhang, H.; Govorov, A.O.; Liedl, T.; Liu, N. Reconfigurable 3D plasmonic metamolecules. *Nat. Mater.* **2014**, *13*, 862–866, DOI: 10.1038/nmat4031.
76. Zhou, C.; Duan, X.; Liu N. A plasmonic nanorod that walks on DNA origami. *Nat. Commun.* **2015**, *6*, 8102, DOI: 10.1038/ncomms9102.
77. Jungmann, R.; Avendano, M.S.; Woehrstein, J.B. ; Dai, M.; Shih, W.M.; Yin, P. Multiplexed 3D cellular super-resolution imaging with DNA-PAINT and Exchange-PAINT. *Nat. Methods* **2014**, *11*, 313–318, DOI: 10.1038/nmeth.2835.
78. Schnitzbauer, J.; Strauss, M.T.; Schlichthaerle, T.; Schueder, F.; Jungmann, R. Super-resolution microscopy with DNA-PAINT. *Nat. Protoc.* **2017**, *12*, 1198–1228, DOI: 10.1038/nprot.2017.024.

79. Schueder, F.; Lara-Gutíerrez, J.; Beliveau, B.J.; Saka, S.K.; Sasaki, H.M.; Woehrstein, J.B.; Strauss, M.T.; Grabmayr, H.; Yin, P.; Jungmann, R. Multiplexed 3D super-resolution imaging of whole cells using spinning disk confocal microscopy and DNA-PAINT. *Nat. Commun.* **2017**, *8*, 2090, DOI: 10.1038/s41467-017-02028-8.
80. Kuzuya, A.; Sakai, Y.; Yamazaki, T.; Xu, Y.; Komiyama, M. Nanomechanical DNA origami "single-molecule beacons" directly imaged by atomic force microscopy. *Nat. Commun.* **2011**, *2*, 449, DOI: 10.1038/ncomms1452.
81. Funke, J.J.; Ketterer, P.; Lieleg, C.; Schunter, S.; Korber, P.; Dietz, H. Uncovering the forces between nucleosomes using DNA origami. *Sci. Adv*. **2016**, *2*, e1600974, DOI: 10.1126/sciadv.1600974.
82. Funke, J.J.; Ketterer, P.; Lieleg, C.; Korber, P.; Dietz, H. Exploring nucleosome unwrapping using DNA DNA origami. *Nano Lett*. **2016**, *16*, 7891–7898, DOI: 10.1021/acs.nanolett.6b04169.
83. Le, J.V.; Luo, Y.; Darcy, M.A.; Lucas, C.R.; Goodwin, M.F.; Poirier, M.G.; Castro, C.E. Probing nucleosome stability with a DNA origami nanocaliper. *ACS Nano* **2016**, *10*, 7073–7084, DOI: 10.1021/acsnano.6b03218.
84. Funke, J.J.; Dietz, H. Placing molecules with Bohr radius resolution using DNA origami. *Nat. Nanotechnol.* **2016**, *11*, 47–52, DOI: 10.1038/nnano.2015.240.
85. Hudoba, M.W.; Luo, Y.; Zacharias, A.; Poirier, M.G.; Castro, C.E. Dynamic DNA origami device for measuring compressive depletion forces. *ACS Nano* **2017**, *11*, 6566–6573, doi:10.1021/acsnano.6b07097.
86. Li, S.; Jiang, Q.; Liu, S.; Zhang, Y.; Tian, Y.; Song, C.; Wang, J.; Zou, Y.; Anderson, G.J.; Han, J.Y., et al. A DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger in vivo. *Nat. Biotechnol.* **2018**, *36*, 258–264, DOI: 10.1038/nbt.4071.
87. Chen, H.; Zhang, H.; Pan, J.; Cha, T. G.; Li, S.; Andréasson, J.; Choi, J.H. Dynamic and Progressive Control of DNA origami conformation by modulating DNA helicity with chemical adducts. *ACS Nano* **2016**, *10*, 4989–4996, doi:10.1021/acsnano.6b01339.
88. Amir, Y.; Ben-Ishay, E.; Levner, D.; Ittah, S.; Abu-Horowitz, A.; Bachelet, I. Universal computing by DNA origami robots in a living animal. *Nat. Nanotechnol*. **2014**, *9*, 353–357, DOI: 10.1038/nnano.2014.5.
89. Kaminka, G.A.; Spokoini-Stern, R.; Amir, Y.; Agmon, N.; Bachelet, I. Molecular robots obeying Asimov's three laws of robotics. *Artif. Life* **2017**, *23*, 343–350, DOI: 10.1162/ARTL_a_00235.
90. Derr, N.D.; Goodman, B.S.; Jungmann, R.; Leschziner, A.E.; Shih, W.M., Reck-Peterson, S.L. Tug-of-war in motor protein ensembles revealed with a programmable DNA origami scaffold. *Science* **2012**, *338*, 662–665, DOI: 10.1126/science.1226734.
91. Sato, Y.; Endo, M.; Morita, M.; Takinoue, M.; Sugiyama, H.; Murata, S.; Nomura, S.M.; Suzuki, Y. Environment-dependent self-assembly of DNA origami lattices on phase-separated lipid membranes. *Adv. Mater. Interfaces* **2018**, *5*, 1800437, DOI: 10.1002/admi.201800437.
92. Suzuki, Y.; Endo, M.; Sugiyama, H. Lipid-bilayer-assisted two-dimensional self-assembly of DNA origami nanostructures. *Nat. Commun*. **2015**, *6*, 8052, DOI: 10.1038/ncomms9052.
93. Kocabey, S.; Kempter, S.; List, J.; Xing, Y.; Bae, W.; Schiffels, D.; Shih, W.M.; Simmel, F.C.; Liedl, T. Membrane-assisted growth of DNA origami nanostructure arrays. *ACS Nano* **2015**, *9*, 3530–3539, DOI: 10.1021/acsnano.5b00161.
94. Yang, Y.; Endo, M.; Hidaka, K.; Sugiyama, H. Photo-controllable DNA origami nanostructures assembling into predesigned multiorientational patterns. *J. Am. Chem. Soc*. **2012**, *134*, 20645–20653, DOI: 10.1021/ja307785r.
95. Kohman, R.E.; Han, X. Light sensitization of DNA nanostructures via incorporation of photo-cleavable spacers. *Chem. Commun.* **2015**, *51*, 5747–5750, DOI:10.1039/c5cc00082c.
96. Kuzyk, A.; Yang, Y.; Duan, X.; Stoll, S.; Govorov, A.O.; Sugiyama, H.; Endo, M.; Liu, N. A light-driven three-dimensional plasmonic nanosystem that translates molecular motion into reversible chiroptical function. *Nat. Commun.* **2016**, *7*, 10591, DOI: 10.1038/ncomms10591.
97. Kuzyk, A.; Urban, M.J.; Idili, A.; Ricci, F.; Liu, N. Selective control of reconfigurable chiral plasmonic metamolecules. *Sci. Adv*. **2017**, *3*, e1602803, DOI: 10.1126/sciadv.1602803.
98. Jiang, Q.; Liu, Q.; Shi, Y.; Wang, Z-G.; Zhan, P.; Liu, J.; Liu, C.; Wang, H.; Shi, X.; Zhang, L.; Sun, J.; Ding, B.; Liu, M. Stimulus-responsive plasmonic chiral signals of gold nanorods organized on DNA origami, *Nano Lett.* **2017**, *17*, 7125-7130, DOI: 10.1021/acs.nanolett.7b03946.
99. Turek, V.A.; Chikkaraddy, R.; Cormier, S.; Stockham, B.; Ding, T.; Keyser, U.F.; Baumberg, J.J. Thermo-responsive actuation of a DNA origami flexor. *Adv. Funct. Mater*. **2018**, *28*, 1706410, DOI: 10.1002/adfm.201706410.
100. Nickels, P.C.; Wünsch, B.; Holzmeister, P.; Bae, W.; Kneer, L.M.; Grohmann, D.; Tinnefeld, P.; Liedl, T. Molecular force spectroscopy with a DNA origami-based nanoscopic force clamp. *Science* **2016**, *354*, 305–307, DOI: 10.1126/science.aah5974.

101. Dutta, P.; Zhang, Y.; Blanchard, A.; Ge, C.; Rushdi, M.; Weiss, K.; Zhu, C.; Ke, Y.; Salaita, K. Programmable multivalent DNA-origami tension probes for reporting cellular traction forces. *Nano Lett*. **2018**, *18*, DOI: 10.1021/acs.nanolett.8b01374.
102. Kuzyk, A.; Yurke, B.; Toppari, J.J.; Linko, V.; Törmä, P. Dielectrophoretic trapping of DNA origami. *Small* **2008**, *4*, 447–450, DOI: 10.1002/smll.200701320.
103. Shen, B.; Linko, V.; Dietz, H.; Toppari, J.J. Dielectrophoretic trapping of multilayer DNA origami nanostructures and DNA origami-induced local destruction of silicon dioxide. *Electrophoresis* **2015**, *36*, 255–262, DOI: 10.1002/elps.201400323.
104. Lauback, S.; Mattioli, K.R.; Marras, A.E.; Armstrong, M.; Rudibaugh, D.P.; Sooryakumar, R.; Castro, C.E. Real-time magnetic actuation of DNA nanodevices via modular integration with stiff micro-levers. *Nat. Commun*. **2018**, *9*, 1446, DOI: 10.1038/s41467-018-03601-5.
105. Mikkilä, J.; Eskelinen, A.-P.; Niemelä, E.H.; Linko, V.; Frilander, M.J.; Törmä, P.; Kostiainen, M.A. Virus-encapsulated DNA origami nanostructures for cellular delivery. *Nano Lett*. **2014**, *14*, 2196–2200, DOI: 10.1021/nl500677j.
106. Kiviaho, J.K.; Linko, V.; Ora, A.; Tiainen, T.; Järvihaavisto, E.; Mikkilä, J.; Tenhu, H.; Nonappa, Kostiainen, M.A. Cationic polymers for DNA origami coating – examining their binding efficiency and tuning the enzymatic reaction rates. *Nanoscale* **2016**, *8*, 11674–11680, DOI: 10.1039/c5nr08355a.
107. Argawal, N.P.; Matthies, M.; Gür, F.N.; Osada, K.; Schmidt, T.L. Block copolymer micellization as a protection strategy for DNA origami. *Angew. Chem. Int. Ed*. **2017**, *56*, 5460–5464, DOI: 10.1002/acie.201608873.
108. Auvinen, H.; Zhang, H.; Nonappa; Kopilow, A.; Niemelä, E.H.; Nummelin, S.; Correia, A.; Santos, H.A.; Linko, V.; Kostiainen, M.A. Protein coating of DNA nanostructures for enhanced stability and immunocompatibility. *Adv. Healthcare Mater*. **2017**, *6*, 1700692, DOI: 10.1002/adhm.201700692.
109. Ponnuswamy, N.; Bastings, M.M.C.; Nathwani, B.; Ryu, J.H.; Chou, L.Y.T.; Vinther, M.; Li, W.A.; Anastassacos, F.M.; Mooney, D.J.; Shih, W.M. Oligolysine-based coatings protects DNA nanostructures from low-salt denaturation and nuclease degradation. *Nat. Commun.* **2017**, *8*, 15654, DOI: 10.1038/ncomms15654.
110. Linko, V.; Eerikäinen, M.; Kostiainen, M.A. A modular DNA origami-based enzyme cascade nanoreactor. *Chem. Commun*. **2015**, *51*, 5351–5354, DOI: 10.1039/c4cc08472a.
111. Kilchherr, F.; Wachauf, C.; Pelz, B.; Rief, M.; Zacharias, M.; Dietz, H. Single-molecule dissection of stacking forces in DNA. *Science* **2016**, *353*, aaf5508, DOI: 10.1126/science.aaf5508.
112. Asimov, I. I, robot. Bantam Books, U.S.A., 2014; ISBN 9780553803709.