

Video Article

Design and Synthesis of a Reconfigurable DNA Accordion Rack

Yeongjae Choi*¹, Hansol Choi*¹, Amos C. Lee*², Sunghoon Kwon^{1,2,3,4}¹Department of Electrical and Computer Engineering, Seoul National University²Interdisciplinary Program for Bioengineering, Seoul National University³Institute of Entrepreneurial Bio Convergence, Seoul National University⁴Seoul National University Hospital Biomedical Research Institute, Seoul National University Hospital

*These authors contributed equally

Correspondence to: Sunghoon Kwon at skwon@snu.ac.krURL: <https://www.jove.com/video/58364>DOI: [doi:10.3791/58364](https://doi.org/10.3791/58364)

Keywords: Bioengineering, Issue 138, DNA nanomachines, DNA nanotechnology, DNA nanostructures, DNA Origami, Self-assembly, caDNAno, oxDNA

Date Published: 8/15/2018

Citation: Choi, Y., Choi, H., Lee, A.C., Kwon, S. Design and Synthesis of a Reconfigurable DNA Accordion Rack. *J. Vis. Exp.* (138), e58364, doi:10.3791/58364 (2018).

Abstract

DNA nanostructure-based mechanical systems or DNA nanomachines, which produce complex nanoscale motion in 2D and 3D in the nanometer to ångström resolution, show great potential in various fields of nanotechnology such as the molecular reactors, drug delivery, and nanoplasmonic systems. The reconfigurable DNA accordion rack, which can collectively manipulate a 2D or 3D nanoscale network of elements, in multiple stages in response to the DNA inputs, is described. The platform has potential to increase the number of elements that DNA nanomachines can control from a few elements to a network scale with multiple stages of reconfiguration.

In this protocol, we describe the entire experimental process of the reconfigurable DNA accordion rack of 6 by 6 meshes. The protocol includes a design rule and simulation procedure of the structures and a wet-lab experiment for synthesis and reconfiguration. In addition, analysis of the structure using TEM (transmission electron microscopy) and FRET (fluorescence resonance energy transfer) is included in the protocol. The novel design and simulation methods covered in this protocol will assist researchers to use the DNA accordion rack for further applications.

Video Link

The video component of this article can be found at <https://www.jove.com/video/58364/>

Introduction

Mechanical systems based on DNA nanostructures or DNA nanomachines^{1,2,3,4,5} are unique because they produce complex nanoscale motion in 2D and 3D in the nanometer to ångström resolution, according to various biomolecular stimuli^{2,3,6}. By attaching functional materials on these structures and controlling their positions, these structures can be applied to various areas. For example, DNA nanomachines have been proposed for a molecular reactor⁷, drug delivery⁸, and nanoplasmonic systems^{9,10}.

Previously, we introduced the reconfigurable DNA accordion rack, which can manipulate a 2D or 3D nanoscale network of elements¹¹ (**Figure 1A**). Unlike other DNA nanomachines that only control a few elements, the platform can collectively manipulate periodically arrayed 2D or 3D elements into various stages. We anticipate that a programmable chemical and biological reaction network or a molecular computing system can be built from our system, by increasing the number of controllable elements. The DNA accordion rack is a structure, in which the network of multiple DNA beams is connected to joints composed of single-stranded DNA (**Figure 1B**). The accordion rack generated by the DNA beams is reconfigured by the DNA locks, which hybridize to the sticky part of beams and change the angle between the beams according to the length of the bridging part of the locks (locked state). In addition, multi-step reconfiguration is demonstrated by adding new locks after formation of the free state by detaching DNA locks through toehold-based strand displacement^{12,13}.

In this protocol, we describe the entire design and synthesis process of the reconfigurable DNA accordion rack. The protocol includes design, simulation, wet-lab experiments, and analysis for the synthesis of the DNA accordion rack of 6 by 6 meshes and a reconfiguration of these. The structure covered in the protocol is the basic model of the previous research¹¹ and is 65 nm by 65 nm in size, consisting of 14 beams. In terms of the design and simulation, the structural design of the accordion rack is different from conventional DNA origami^{14,15} (*i.e.*, tightly packed). Therefore, the design rule and molecular simulation have been modified from traditional methods. To demonstrate, we show the design technique using the modified approach of caDNAno¹⁴ and the simulation of the accordion rack using oxDNA^{16,17} with additional scripts. Finally, both protocols of TEM and FRET for analysis of configured accordion rack structures are described.

Protocol

1. Design of the 6 by 6 DNA Accordion Rack with caDNA¹⁴

- Download and install caDNA¹⁴ software¹⁴ to design a DNA accordion rack (caDNA¹⁴ 2.5 is also available on <https://github.com/cadnano/cadnano2.5>). Open caDNA¹⁴ and click the **Square Tool** to add a new part with a square lattice.
- Number each beam of the accordion rack and draw on the left lattice panel of the caDNA¹⁴ (**Figure 2**).
- Click the pencil tool and draw each beam on the right edit panel on the caDNA¹⁴. Break beams every 32 bp, which is for joints between adjacent beams. Place staple crossovers in the same position as the joints. Use the **Insert Tool** and the **Pencil Tool** in caDNA¹⁴ to let the joints have additional single-stranded crossovers.
- Click the **Pencil Tool** and connect the joints. Each beam has seven joints.
- Generate scaffold crossovers to merge the scaffolds into the single loop by using the previously reported scaffold routing algorithm¹¹. Do not let the minimum binding domain between scaffold and staple strands be less than 8 bp (**Figure 3**).
- Place the scaffolds that are not used in the assembly at the vertices located on opposite sides of the accordion rack, as shown in **Figure 3**.
- Click the **Break Tool**. Break the strands where staple strands are circular or longer than 60 bp.
- Design the DNA lock strands.
 - Click the **Break Tool**. Break 8 bp of a staple DNA region to make a sticky part and delete 8 bp of a staple DNA region. There are 18 sticky parts (**Figure 1**) in the 6 by 6 accordion rack.
 - Place sequences that are reverse complementary to the sticky parts at both ends of lock strands and connect them by a bridging region, which consists of poly T strands of the desired length (**Figure 1B**).
 - For the reconfiguration, add 8 bp of toehold sequences at the end of DNA locks for strand displacement. The toehold sequence used is in **Table 2**.
 - Prepare poly A strands which are reverse complementary to the bridging region.
 - Design strands that are reverse complementary to the DNA locks for the reconfiguration experiment.
- Click the **Sequence Tool** and click scaffold DNA. Choose the scaffold as standard M13mp18. Click the **Export Tool** and save sequence in csv format (**Table 1**).

2. Simulate the Structure with the oxDNA

- Download and install the oxDNA^{16,17}. The latest source code is available on <https://sourceforge.net/projects/oxdna/files/>.
- Make starting configuration files from the caDNA¹⁴ file using python script 'cadnano_interface.py', which is provided in the oxDNA^{16,17} package. The usage is as follows: 'python cadnano_interface.py cadnano_file.json sq'. The topology file and configuration file are now generated.
Note: The topology file includes how many strands and nucleotides are in the structure and information regarding backbone-backbone bonds between nucleotides. The configuration file includes general information such as timestep, energy, and box size. Orientation information such as position vector, backbone-base vector, normal vector, velocity, and angular velocity of nucleotides is also included (**Figure 4**).
- Change the information in the topology and configuration file from caDNA¹⁴ to make them reflect the real structural information of the accordion rack. All beams are arranged in parallel when the topology and configuration files from caDNA¹⁴ are visualized. However, the accordion rack is a lattice structure so the distance between bonded nucleotides are far for simulation (**Figure 5**).
 - Rotate and move each beam to the desired lattice structure. The nine columns on the left in the configuration file are the position vector, backbone-base vector, and normal vector (**Figure 4**). To rotate a beam, rotate all of position, backbone-base, and normal vectors using rotational transformation. Then move a beam by changing the position vector to locate it as shown in **Figure 5**.
 - Relax the structure using the script provided in the oxDNA package (see example in \$oxDNA/EXAMPLES/RELAX_INITIAL_CONFIGURATION for further information).
- Run molecular dynamics simulation for ten million steps using the relaxed configuration file. The usage is as follows: './oxDNA <input>' Save data every 5000 or 10000 steps.
- Visualization
Note: The structures were visualized using cogli.
 - Download and install the latest version of the cogli (<https://sourceforge.net/projects/cogli1/>).
 - Run the cogli with the topology and configuration files from the oxDNA simulation. The usage is as follows: './cogli1 -t <topology file> <configuration file>' .
 - Hide the box by pressing **b**.

3. Synthesis of the Structure

Note: The synthesis method is adapted from the previous protocol^{15,18}.

- Purchase the designed DNA staples from an oligonucleotide provider.
- Adjust the concentration of these DNA staples to 100 μ M using nuclease-free water.
 - Pool each DNA strand that constitutes a 'free state' structure into one tube and adjust the concentration to 2 μ M for each strand.
 - Pool DNA lock strands by length and number of lock sites into tubes and adjust the concentration to 2 μ M for each strand. 18, 9, and 4 lock sites are used. Add poly A strands which are complementary to the bridging region at the same concentration.

3. Pool strands that are reverse complementary to the DNA lock strands by length into tubes and adjust the concentration to 2 μM for each strand.
3. Prepare MgCl_2 solution of 300 nM by mixing 70 μL of nuclease-free water and 30 μL of the 1 μM MgCl_2 solution. Prepare a 5x Tris EDTA solution by mixing 95 μL of nuclease-free water and 5 μL of the 100x Tris EDTA solution.
4. Add 2 μL of staple DNA, 1.1 μL of MgCl_2 solution, 2 μL of Tris EDTA solution, 7.6 μL of nuclease-free water and 7.3 μL of scaffold DNA of which the concentration is 110 nM to make 20 μL of mixed stock. Set the final concentration of the scaffold DNA to 40 nM, staple DNA to 200 nM, MgCl_2 to 16 mM and Tris EDTA solution to 0.5x.
5. Rapidly heat the mixed stock solution in a thermal cycler to 80 $^\circ\text{C}$ and cool to 60 $^\circ\text{C}$ at a rate of 4 min per $^\circ\text{C}$ and cool from 60 $^\circ\text{C}$ to 4 $^\circ\text{C}$ at a rate of 40 min per $^\circ\text{C}$.

4. Purification of the Structure

Note: Samples of all structures were purified before analysis. In this section, we describe the protocol of PEG purification, which is adapted from previous literature¹⁹. The sample can also be purified by gel electrophoresis as described in previous literature^{15,18}.

1. Prepare 5 M of NaCl and 100x Tris-EDTA.
2. Prepare precipitation-buffer by mixing 150 μL of PEG 8000, 500 μL of 100x Tris EDTA and 101 μL of 5 M NaCl and 249 μL of nuclease-free water.
3. Prepare target-buffer by mixing 5.5 μL of 300 nM MgCl_2 solution from Section 3.3, 10 μL of 5x Tris EDTA solution from Section 3.3 and 84.5 μL of nuclease-free water.
4. Mix 20 μL of the synthesized structure from Section 3 and 20 μL of precipitation-buffer from Section 4.2. Then spin the mixed stock at 16000 x g at 4 $^\circ\text{C}$. Remove the supernatant and dissolve pellet in the target-buffer from Section 4.3.

5. Reconfiguration of the Accordion Rack from a 'Free State' to a 'Locked State'

1. Synthesize the structure without DNA locks for the configuration experiment.
2. Prepare DNA lock strands from Section 3.
3. Add 2 μL of DNA lock strands of the desired length into 20 μL of the synthesized structure. The DNA lock strands' concentration is five times higher than the structure.
4. Incubate the sample for 0, 10, 25, 50, or 100 minutes to see how fast reconfiguration occurs.
 1. For the 100 minute incubation, incubate the sample at 50 $^\circ\text{C}$ for 30 minutes and slowly cool down to 25 $^\circ\text{C}$ at a rate of 0.33 $^\circ\text{C}/\text{minute}$.
 2. For the 50 minute incubation, incubate the sample at 50 $^\circ\text{C}$ for 15 minutes and slowly cool down to 25 $^\circ\text{C}$ at a rate of 0.66 $^\circ\text{C}/\text{minute}$.
 3. For the 25 minute incubation, incubate the sample at 50 $^\circ\text{C}$ for 7.5 minutes and slowly cool down to 25 $^\circ\text{C}$ at a rate of 1.32 $^\circ\text{C}/\text{minute}$.
 4. For the 10 minute incubation, incubate the sample at 50 $^\circ\text{C}$ for 3 minutes and slowly cool down to 25 $^\circ\text{C}$ at a rate of 3.3 $^\circ\text{C}/\text{minute}$.
 5. For the 0-minute incubation, store sample at 4 $^\circ\text{C}$ right after DNA locks strands are added.
5. Right after the attaching step, rapidly cool down the sample to 4 $^\circ\text{C}$ to prevent unwanted denaturation.

6. Reconfiguration of the Accordion Rack from a 'Locked State' to a 'Free State'

1. Synthesize the structure with DNA locks of the desired length for the configuration experiment.
2. Prepare reverse complementary strands from Section 3.
3. Add 2 μL of strands that are reverse complementary to the lock strands of the desired length into 20 μL of the synthesized structure. The DNA lock strands' concentration is five times higher than the structure.
4. Incubate the sample for 0, 12, 60, 120, 240 minutes to see how fast reconfiguration occurs.
 1. For the 12, 60, 120, 240 minute incubation, rapidly heat the sample to 40 $^\circ\text{C}$ and slowly cool down to 20 $^\circ\text{C}$ for the time corresponding to each. Right after the detaching step, rapidly cool down the sample to 4 $^\circ\text{C}$ to prevent unwanted denaturation.
 2. For the 0-minute incubation, store sample at 4 $^\circ\text{C}$ right after reverse complementary strands are added.

7. TEM Imaging

Note: TEM imaging protocol was adapted from previous literature^{18,20}.

1. Prepare 1.25 M NaOH solution by mixing 87.5 μL of nuclease-free water and 12.5 μL of 10 M NaOH solution.
2. Add 1 μL of 1.25 M NaOH solution to 50 μL of the 2% uranyl formate solution.
3. Vortex the solution for 3 minutes and centrifuge at max speed for 3 minutes. Deposit 3 μL of the purified sample on the glow-discharged TEM grid for 3 minutes and rapidly wash out with filter paper.
4. Deposit 7 μL of prepared uranyl formate solution for 30 seconds and rapidly wash out with filter paper.
5. Measure the length and angle of the accordion structure imaged by TEM.

8. FRET Analysis

1. Use Atto 550 and Atto 647N dye, for which the Förster distance is 6.5 nm. Replace Staple 58 and Staple 117 in **Table 1** with fluorescently labelled strands. Then synthesize the structure with fluorescently labelled strands by the method described in Section 3.
2. Measure the concentration of the purified sample.

3. Normalize the sample to 10 nM and load 50 μL to the 384 microplates well.
4. Excite the sample with donor and acceptor dyes at 550 nm and measure the fluorescence spectrum from 570 nm to 800 nm with a fluorometer.
5. Measure the fluorescence spectrum of the donor-only sample in the same way.
6. Excite the dyes of the sample at 650 nm and the fluorescence spectrum and measure from 670 nm to 800 nm. This is to measure the concentration of the acceptor.
7. Obtain the standard deviations by repeating the same experiment with three samples, which are synthesized and purified separately.
8. Calculate the FRET efficiency with the ratio A method as described by the equation below²¹.

$$(ratio)_A = \frac{[DA_{550}] - [D_{550}]}{[DA_{650}]}$$

DA_{550} : Acceptor peak fluorescence intensity of the sample with donor and acceptor at 550 nm excitation.

D_{550} : Fluorescence intensity at the acceptor emission range of the donor-only sample at 550 nm excitation.

DA_{650} : Acceptor peak fluorescence intensity of the sample with donor and acceptor at 650 nm excitation.

Representative Results

The designed 6 by 6 DNA accordion rack is simulated from the oxDNA^{16,17} and the results are shown in **Figure 6**. From the simulation result, it was confirmed that the intended structure is formed without distortion of the structure.

The TEM images in **Figure 7** are images of configured structures with a lock length of 2, 8, 13 and 20 bp. On the image, the angle of the structures (**Figure 8A**) is decreased as the length of the lock becomes longer. To statistically analyze the tendency, the average and the standard deviation of the angle (**Figure 8A**) of multiple configured structures were obtained (**Figure 8B**). Also, the FRET measurement results show structural change and its speed, in addition to the structural tendency shown in the TEM image (**Figure 8C**).

From these analysis procedures, characterization of the DNA accordion rack such as how many locks are needed for structural configuration and reconfiguration speed was determined.

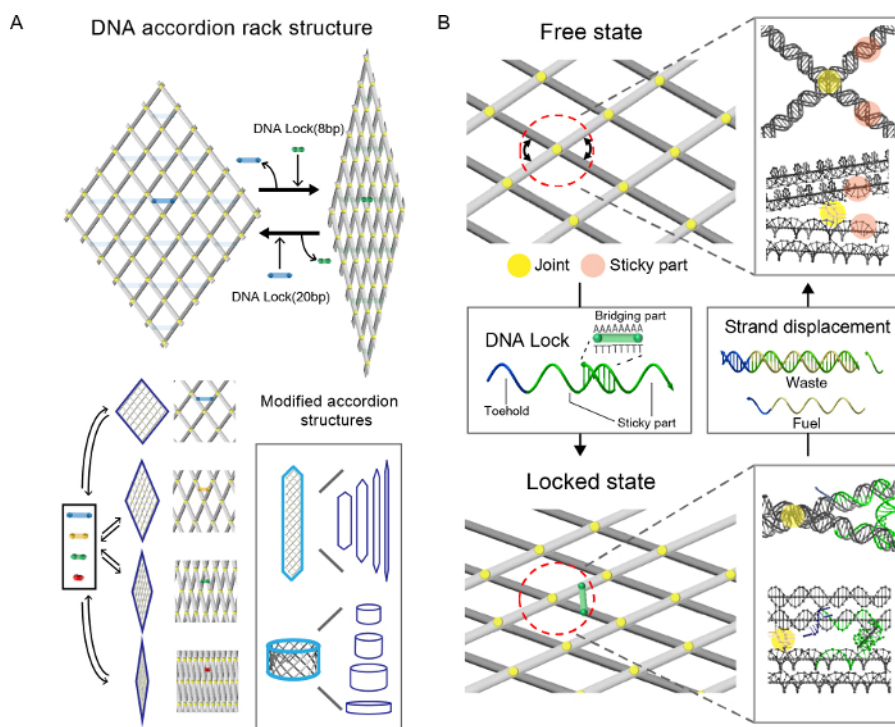


Figure 1. A reconfigurable DNA accordion rack. **A.** The DNA accordion rack is composed of rigid DNA beams and the multiple flexible joints that connect each beam. The accordion rack is configured differently according to the length of the DNA locks. Various 2D and 3D platforms can be designed by modifying the platform. **B.** The reconfiguration of the DNA accordion rack is achieved with hybridization of DNA locks with different lengths. In the 'free state' structure, when extra nucleotides are added to the crossovers (or joints, yellow), due to the loose connection between the DNA beams (comprising two helices), the angle can be changed (upper part). Hybridization of the DNA locks with various lengths of the bridge part to the sticky part of both adjacent beams generates the 'locked state' (Lower part). Strand displacement using the toehold parts (blue) of the DNA locks with the fuel strand detach the DNA locks from the locked state of the structure and change the state to the free. By repeating the process with various libraries of the DNA locks, the structure is reconfigured with different angles. This figure has been modified from the previous research¹¹. [Please click here to view a larger version of this figure.](#)

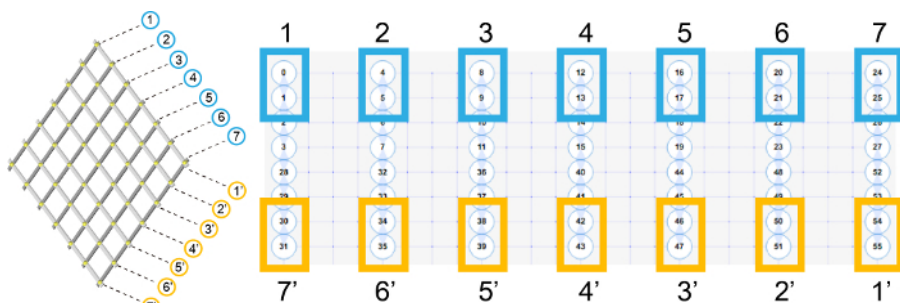


Figure 2. 6 by 6 accordion rack is designed using the caDNAo. The 14 beams of the DNA accordion rack are numbered and drawn on the lattice panel of the caDNAo. This figure has been modified from the previous research¹¹. [Please click here to view a larger version of this figure.](#)

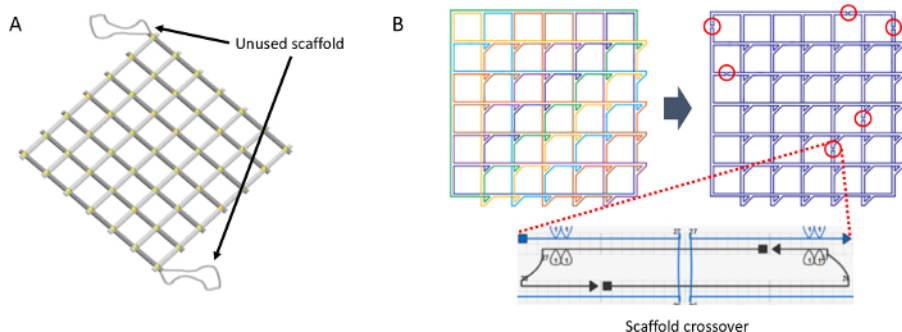


Figure 3. Scaffold routing algorithm. **A.** Scaffold that are not used in the assembly at the vertices located on opposite sides of the accordion rack. **B.** Seven closed loops are merged into a single loop by generating scaffold crossovers. At least six scaffold crossovers points are needed and the position of the scaffold crossovers is adapted from previous literature. This figure has been modified from the previous research¹¹. [Please click here to view a larger version of this figure.](#)

```

t = 0
b = 89.760000 89.760000 89.760000
E = 0.000000 0.000000 0.000000
21.3795554958 0.155291427862 1.11022302463e-16 -0.965925826289 -0.258819045103 0.0 0.0 0.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0
21.1952222484 0.451441447669 0.38976285513 -0.658703734041 -0.752402412782 0.0 0.0 0.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0
20.8772999334 0.594999764949 0.779525710261 -0.128833222413 -0.591666274914 0.0 0.0 0.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0
20.5332494795 0.537442238563 1.16928856539 0.444584200901 -0.895737064271 0.0 0.0 0.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0
20.2793633569 0.298223885404 1.55905142052 0.867272738558 -0.497039809007 0.0 0.0 0.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0
20.2014576025 -0.0417971097846 1.94881427565 0.997570662512 0.0696618496409 0.0 0.0 0.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0
20.3258651369 -0.367698265784 2.33857713078 0.790224771906 0.612817109639 0.0 0.0 0.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0
20.6105349749 -0.569300451659 2.72033998591 0.315775041854 0.948834086099 0.0 0.0 0.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0
20.9592458775 -0.578481417593 3.11810284104 -0.265409795827 0.964135695988 0.0 0.0 0.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0
    
```

Position vector
Backbone-base vector
Normal Vector

Figure 4. Example of a configuration file. The configuration file includes general information such as the timestep, energy and box size and orientation information. The nine columns on the left in the configuration file are the position vector, backbone-base vector, and normal vector respectively. The six columns on the right are velocities and angular velocities. [Please click here to view a larger version of this figure.](#)

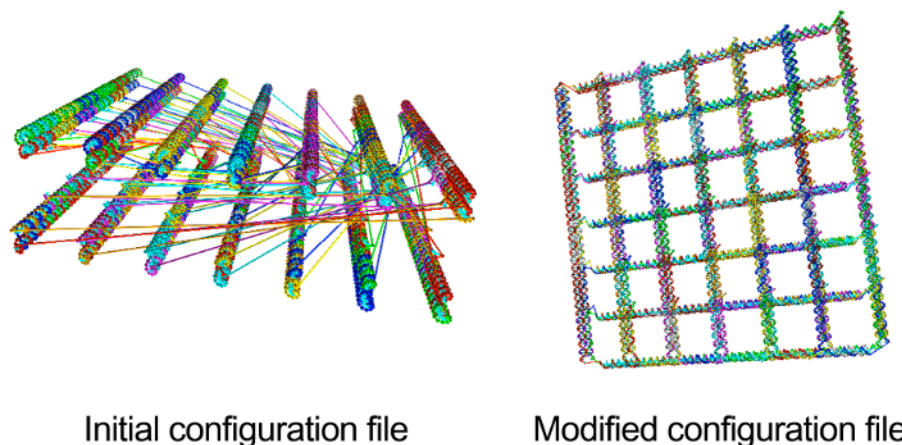


Figure 5. Visualization of the configuration file before and after the modification. Position, backbone-base, and normal vector was modified using rotational transformation to make the information in the configuration file reflect the structural information of the accordion rack. [Please click here to view a larger version of this figure.](#)

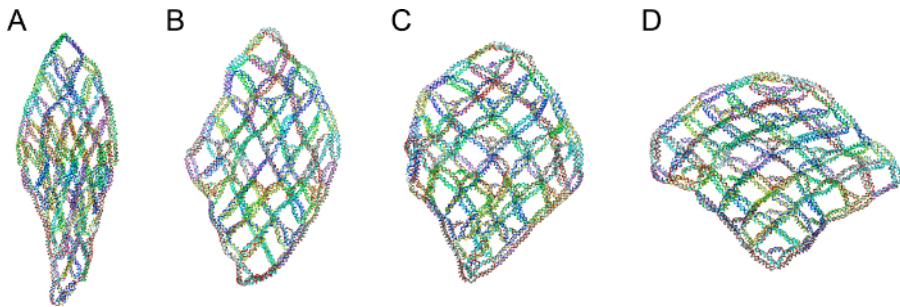


Figure 6. Simulation result from oxDNA. The accordion rack with DNA locks, of which the lengths are 2, 8, 13 and 20 bp, were simulated. 18 locking sites were used. A 6 by 6 accordion rack with a DNA lock of which the length is **A** 2 bp, **B** 8 bp, **C** 13 bp and **D** 20 bp are simulated and visualized by cogil. [Please click here to view a larger version of this figure.](#)

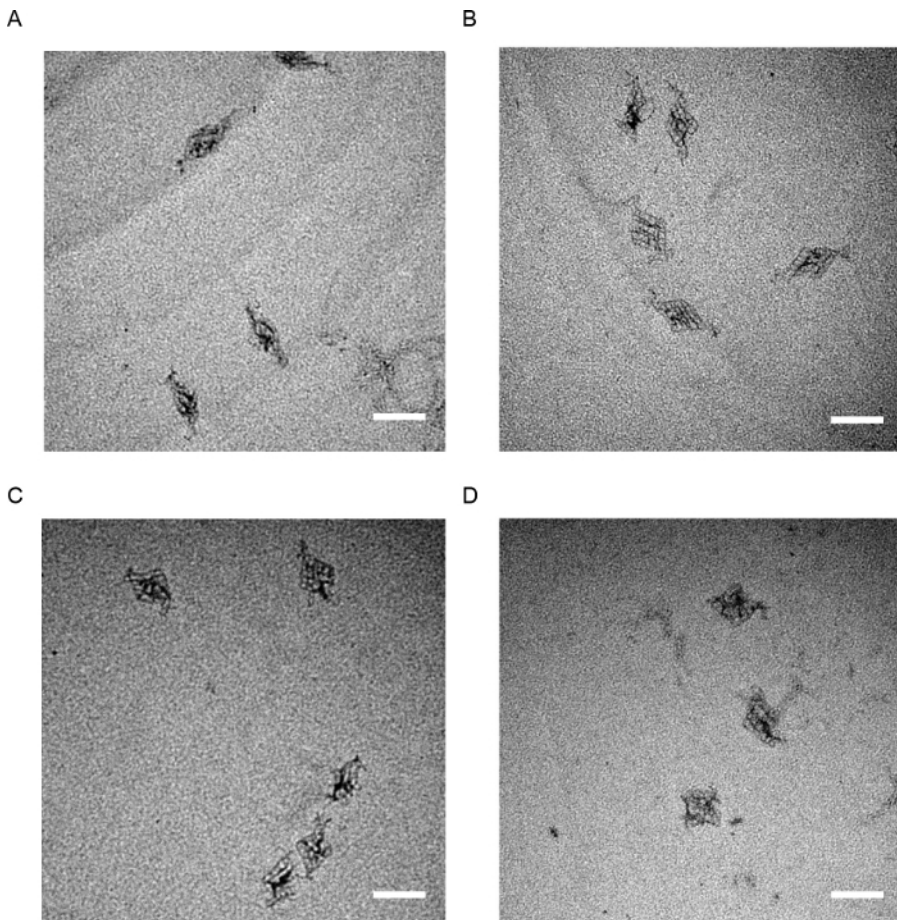


Figure 7. TEM images of configured 6 by 6 DNA accordion racks. Structures with lock lengths of **A** 2 bp, **B** 8 bp, **C** 13 bp and **D** 20 bp were imaged. 18 locking sites were used for this experiment. (scale bar: 100 nm). This figure has been modified from the previous research¹¹. [Please click here to view a larger version of this figure.](#)

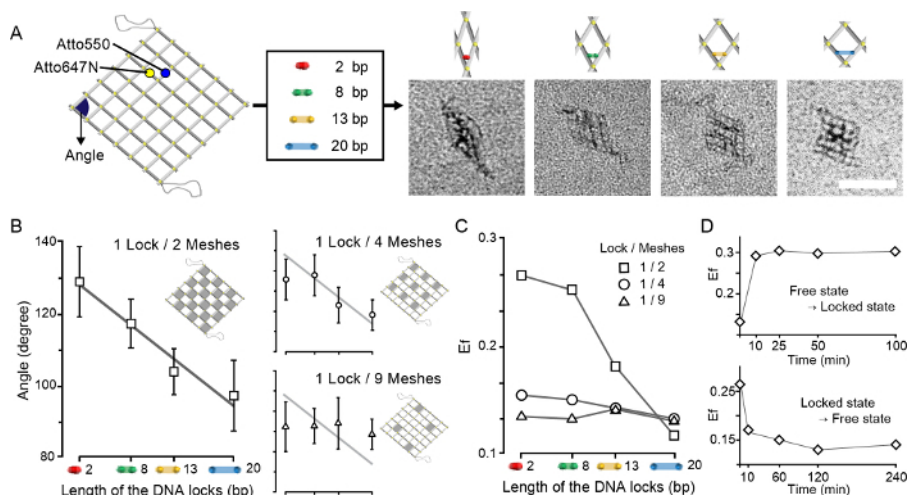


Figure 8. The angle control of the 2D accordion rack. The angle of the accordion rack comprised of 6-by-6 meshes was controlled by the length of the DNA locks. **A.** By adding DNA locks with lengths of 2, 8, 13 and 20 bp to the 18 locking sites of the accordion rack, the angle (blue) is configured as seen in the representative TEM images. The dye pair, Atto647N, and Atto550 is attached to the structure for FRET efficiency analysis. (scale bar: 100 nm) **B.** The distribution of the configured angles. The overlaid trend line (grey) is from the graph of the 1 lock per 2 meshes. Additionally, the grid of the x-axis corresponds to the same grid on the graph of the 1 lock per 2 meshes. Bars represent one standard deviation from the mean angle. **C.** The distribution of FRET efficiencies. Bars represent one standard deviation from the mean efficiency. **D.** FRET efficiency change while the state transitioned from free to locked or vice versa was measured according to different incubation times. The DNA lock with the length of 2 bp was used. Bars represent one standard deviation from the mean efficiency. This figure has been modified from the previous research¹¹. [Please click here to view a larger version of this figure.](#)

Name	Sequence	Length
Staple 1	ATAAGAGGTCATTTTTGCGGATGGATGTTACT	32
Staple 2	CAAAGTTACCAGAAGGAAACCGAGACATCGGG	32
Staple 3	ACAATGAAATAGCAATAGCTATCTCAAATAAA	32
Staple 4	CGCTAATATGAACGGTGTACAGACCAGGCGCATAGGCTGGGAACAAAGTCAGAGGG	56
Staple 5	CAGAAAACCCGGAATAGGTGTATCACCGTACTCAGGAGGTCCCCCTCAAATGCTTT	56
Staple 6	AGTGAGAATAGAAAGGTATGATATCAACCGTTCTAGCTGATAAATTATCAACAGT	56
Staple 7	ATTTGGGGCGCGAGCTATATGGTT	24
Staple 8	GCCTTTATTTCAACGCAAGGATAATTAATGGA	32
Staple 9	TCAATTACCCACTACGAAGGCACCGGTAATAAT	32
Staple 10	ATTCAGTGAATAAGGCTTGCCCTGAAAACAGA	32
Staple 11	AGAAACAATAACGGATTCGCCTGATAGCCGAA	32
Staple 12	ATCAATATCTGGTCAGTTGGCAAAAGTAACAA	32
Staple 13	CGGAGACAGTCAAATCACCATCAAGGGTTAGA	32
Staple 14	GAGAATTAAGTGAACACGAAACAAAGTACAACGGAGATTTGTATCATCGAAGCGCA	56
Staple 15	CGAGAGGGTTGATATAAGTATAGCATAGGAAC	32
Staple 16	CCCTTATTAGCGTTTGCCATCTTTTGGTGCCG	32
Staple 17	GTTTTCATCGGCATTTATGCCGGA	24
Staple 18	GTAGCAACTTCCAGTAAGCGTCATGTCTCTGA	32
Staple 19	AGCTAATGCAGAACGCAATAAACA	24
Staple 20	CCGTAATGGGATAGGTCACGTTGGGCCTATTT	32
Staple 21	ACCAGAGCCACCACCGCGAGAGGC	24
Staple 22	AGGCTCCAAAAGGAGCCGTTTACCAGACGACGATAAAAACCAAATAGAAAATCTC	56
Staple 23	GCCGGAAGGAAACGCAATAATAACGGAATACCCAAAAGAAGTCAACAATCCACACA	56
Staple 24	TTTTTCACCGGTGTCTGGAAGTTTCATTCCATATAACAGTACTAAAGGAATTGCGA	56
Staple 25	ATCAGGTCTTTACCCTCGCATTAAATTTTTGTTAAATCAGCTCATTTTTGACCATA	56
Staple 26	TGCTGCAAGGCGATTAAGTTGGGTGACATTCA	32
Staple 27	AATAAAGAAATTGCGTAGATTTTCAGCTGCTC	32
Staple 28	CCCAATCCTCGCACTCCAGCCAGCTTTCCGGCACCGCTTCAAATAAACAGCCATA	56
Staple 29	AAAGACTTCAAATATCATCAAAGAATAGCCCCGAGATAGGGTTGAGTGATTAAGAG	56
Staple 30	GTAGCGACAGAATCAAGTTTGCCCTGATTTAGA	32
Staple 31	GCCACCCTCAGAACCGCCACCCTCAACAAGAG	32
Staple 32	AAGCAAGCCGTTTTTATTTTCATCTCCTGATT	32
Staple 33	GAGGGTAGCTATTTTTGAGAGATCCGTCAGAC	32
Staple 34	TATGACCCTGTAATACTCCCATCCTAATTTACGAGCATGTAGAAACCAGTTGTACC	56
Staple 35	GATTAGAGAGTACCTTAACAGTGCCCGTATAAACAGTTAATGCCCCCTAACTCCAA	56
Staple 36	CGTCTTTCCAGACGTTGTAGGAATCATTACCGCGCCAATAGCAAGCAGCATCTAA	56
Staple 37	TTTAACGTCAAAAATGAGTGAGACGGGCAACAGCTGATTGCCCTTCAACAAGAAACG	56
Staple 38	TTTAACGGGGTCAAGTGCCTTGAGTATAAGGGA	32
Staple 39	AATTGTGTGCAAAATCCCTTATAAGGTGGTTC	32
Staple 40	ACCAGTAGCACCATTACCATTAGCTTTCAGGG	32
Staple 41	AAACATCAAGAAAACAAAATTAATTCACATTA	32
Staple 42	TTGGCCTTGATATTCATCATCTTT	24
Staple 43	TGCATCAATCAACAGTTGAAAGGAATTGAGGAAGGTTATCATTATAGTCAGAAGCA	56
Staple 44	GCCCCAGCAGGCGAAAATCCTGTTAGGGGACG	32

Staple 45	TCCTCATTAAAGCCAGAATGGAAAAGCAAGAA	32
Staple 46	GAGCCTAATTTGCCAGGGGGTAATAGTAAAATGTTTACTGATAGAACGAGCG	56
Staple 47	TAAGAGCAACACTATCATAACCCTTTAAACGTC	32
Staple 48	ATAGCAAGCCCAATAGGAACCCATGCAAAATC	32
Staple 49	CATAAAAACCTTAGAGCTTAATTGCTGAATATAATGCTGTATAGCAGCCTTTACAGA	56
Staple 50	ATTTACCGGGCTACAGAGGCTTTGGAACGAGG	32
Staple 51	GTGCCTAATGAGTGAGAATGCAGATACATAACGCCAAAAGGAATTACGAAGTGTA	56
Staple 52	ATACCGATAGTTGCGCATCGGCCAACGCGGGGAGAGGCGGTTTGCCTTTCTTAA	56
Staple 53	ATAACCGATACCGAAGCCCTTTTAAAGAAAAGTAAGCAGAAATGACAACAACCATC	56
Staple 54	TCAGAGCCGCCACCCTCAGAACCGGCTCAACA	32
Staple 55	TTTTGCAAAAAGAAGTTTTGCCAGAAATCAAAA	32
Staple 56	GGCCTCTTCGCTATTACGCCAGCTAAGAAACC	32
Staple 57	ACATGTTCAAAGACACCACGGAATCATATAAA	32
Staple 58	CTGTGTGAAATTGTTAGAGTAATGTGTAGGTAAAGATTCAAAGGGTGTGGTCATA	56
Staple 59	TTTCGAGGCCGGATATTCATTACCCAAATCAACGTAACAAAATTGTATCGGTTTAT	56
Staple 60	CTTTAGGAGCACTAACAATAATAAGCCCAA	32
Staple 61	AGATTAGTTGCTATTTTACAAAAGGCTATCAGGTCATTGCCTGAGAGTCTTGAAGCC	56
Staple 62	GAGCCGCCGCCAGCATTGACAGGACTGACCTT	32
Staple 63	GAAACCAGGCAAAGCGCCATTGCTCATAGCC	32
Staple 64	CATCAAGAGTAATCTTGACAAGAACCACCA	32
Staple 65	AACCTTGCTTCTGTAATCGTCGCTTATCAAC	32
Staple 66	TAGTTAGCGACAACCTCGTATAAATCCTTTGCCCGAACGTGTAGCATTCCACAGAC	56
Staple 67	ACCGATTGAGGGAGGGAAGGTAAAGGGGGATG	32
Staple 68	TACCAGCGCCAAAGACAAAAGGGCGTTTAGCT	32
Staple 69	TCATATATTTTAAATGCAATGCCTAAAACGAA	32
Staple 70	ACATTATCATTTTTCGCTATTGACG	24
Staple 71	CGGAACCTATTATTCTGAAACATGCGGATTGA	32
Staple 72	CCTGAGAGATATTTTGTAAAATTTAAATTGT	32
Staple 73	GAAACCATCGATAGCAAATCAGAT	24
Staple 74	CTCAGAGCCGCCACCATATTGGGC	24
Staple 75	CACCGACTTGAGCCATTTGGGAATACACTGAG	32
Staple 76	TCATGAGGAAGTTTCCATTAAACGTTTTCGGG	32
Staple 77	AAACAGGAAGATTGTATAAGCAAATAAAATAT	32
Staple 78	ATCGTCACCCTCAGCAGCGAAAGAAGACTTTT	32
Staple 79	ATCAACATTAATGTGTTGTCCA	24
Staple 80	TGCAGGGAGTTAAAGGAACGAAAGAGGCAAAAAGAAATACACTAAAACACTTCGGTGC	56
Staple 81	ACGTAATGCTGAGCAAAAGAAGATTATTCATT	32
Staple 82	AATCGATGAACGGTAATCGTAAAATTAGTACC	32
Staple 83	GACCCCGAGCGATTATACCAAGCGGAGGCAGG	32
Staple 84	CGAAATCCGCGACCTGGCCTGATA	24
Staple 85	GCCATCAAAAATAATTTCGCGTCTGAGTGCCGT	32
Staple 86	TCGGCTGTCTTTCCTTATCATTCCATTACCTT	32
Staple 87	CAGACCGGGGGCGCATCGTAACCGTGCATCTGCCAGTTTGTTTAATTTCGAGCTTCA	56
Staple 88	GTTTGGAAACAAGAGTCCACTATTACCTGTAGC	32
Staple 89	AACAGTACATAAATCAATATATGTCGGGAGAA	32
Staple 90	GACGTTGGGAAGAAAATCTACGCTGGCATG	32

Staple 91	CAAAATCGCGCAGAGGGCGGAAAC	24
Staple 92	ATTGCGTTGCGCTCACTGCCCGCTGATGAAAC	32
Staple 93	CCCCGGTTGATAATCACGTCCAAT	24
Staple 94	ATGCCTGCAGGTCGACAAGAACGGGTATTAAACCAAGTACCGCACTCACCAGTGCC	56
Staple 95	GTTTGGATTATACTTCTGAATAATTGATTCCC	32
Staple 96	ACCGAACTGACCAACTTTGAAAGATAATAAGT	32
Staple 97	GCCAGGGTGGTTTTTCTTTTCACCCCTCAGAG	32
Staple 98	CAGTTGAGATTTAGGAATACCACATACATTTA	32
Staple 99	TAGCCGGAACGAGGCGCAGACGGTTCCTTTTTG	32
Staple 100	AGTATTAGACTTTACAAACAATTCCGTAATCA	32
Staple 101	CTTGCGGGAGAACCGCCACCCTCAGAGCCACCACCCTCATGAGGCGTTTTAGCGAA	56
Staple 102	TTAAGAACTGGCTCATATCAATAA	24
Staple 103	CATACAGGCAAGGCAAAGAATTAGAAGTTTAT	32
Staple 104	ATTGAGTTAAGCCCAAACATGGCTTTTGATGATACAGGAGTGTACTGGGAGATAAC	56
Staple 105	ATAGAAGGCTTATCCGGTATTCTACGGAAACG	32
Staple 106	CTGTCGTGCCAGCTGCATTAATGATTTGAATA	32
Staple 107	CTTACCAAGATTAGAGCCGTCAATAGATAATACATTTGAGCCCAGCTACAATTTTA	56
Staple 108	ACCTTGCTGAACCTCAAAAAGTATTAAGAGGCTGAGACTCCTCAAGAGAAAAAATCT	56
Staple 109	TTTGTACAATCAATAGAAAATCCAATAAAT	32
Staple 110	CAATTCTATTCAACTTTAATCATTCTTGAGAT	32
Staple 111	ACGACAGTATCGGCCTCAGGAAGAGCTGGTTT	32
Staple 112	ACTGCGGAATCGTCATAAATATTCATGTCAAT	32
Staple 113	TTTCGTACCAGTACAACTACAAATCACCGT	32
Staple 114	AATTCTGCGAACGAGTAGATTTAGTCCTGATT	32
Staple 115	GAAATTATTCATTAAAGGTGAATTTTTAAAG	32
Staple 116	ACCAGAAGGAGCGGAATTATCATCTCGGTGCG	32
Staple 117	AATTCGTAACGAGAAACACCAGAACGAGTAGTAAATTGGGGAGGATCCCCGGGTAC	56
Staple 118	ATTAAGACTCCTTATTACGCAGTACCAGTCAG	32
Staple 119	GGTTTAATCTAATAGTAGTAGCATGGTGGCAT	32
Staple 120	AAACCGTCTTAGCGGGTTTTGCTCAGTACCAGGCGGATATGGACTCCAACGTCAA	56
Staple 121	TATCAAAATATTTGCAGAAAGGC	24
Staple 122	ACAACATTATTACAGGTAGAACCC	24
Staple 123	CCCGTCGGATTCTCCGTGGGAACAAACCCTCA	32
Staple 124	ACAATTCATTTGAATTACCTTTTAGATTCAT	32
Staple 125	GCTAAACACATTCAGGCTGCGCAACTGTTGGGAAGGGCGAAATGAATTTTCTGTAT	56
Staple 126	AATAGATAAGTCCTGAACAAGAAAGAGTGAAT	32
Staple 127	AAAGCTAATGTTAGCAAACGTAGAAAATACATACATAAAGTTAAGCAATAAAGCCT	56
Staple 128	TGTTTTAAATATGCAACTAAAGTACGCCTCCC	32
Staple 129	ATACAGTAACAGTACCAGGCATAG	24
Staple 130	GTA AACGTTTGACCATTAGATACATTTGCAAATGGTCACAGGGTTTTCCCAGTC	56
Staple 131	AGTTGCAGCAAGCGGTGCGCTGGC	24
Staple 132	ATGGCAATTCATCAATTCGAGAAC	24

Table 1. Sequence of the staple strands

Name	Sequence
Toehold_1	AGAAGTCG
Toehold_2	AGGTATGG
Toehold_3	CTCCACTC
Toehold_4	GTGTCCGA
Toehold_5	AAGTAGAC
Toehold_6	GAGTCGCT
Toehold_7	AGTGTCTA
Toehold_8	GTATCGTG
Toehold_9	CATCACAG
Toehold_10	CGAGACTT
Toehold_11	ACGGACGA
Toehold_12	GCCTACTC
Toehold_13	GATGTGGA
Toehold_14	GCGTGCGT
Toehold_15	GTGGACAA
Toehold_16	TACGACTG
Toehold_17	CAGCCGTG
Toehold_18	TGCCGCAT

Table 2. Toehold sequence for the strand displacement experiment

Discussion

This protocol introduces the entire process from design, simulation, synthesis, and analysis of the basic 2D DNA accordion rack. The modified design and simulation rules have been described because the design rule differs from that of standard DNA origami, in that the DNA accordion rack has additional nucleotides at the crossovers for flexibility^{14,15}. From this, we expect that the protocol can accelerate various researches using DNA accordion racks. In addition, the described protocol can also be applied to other research using DNA nanostructure rather than standard DNA origami.

For structures other than the basic 2D structure demonstrated in the original research paper, the design can be done with modifications of the described protocol. In brief, a boxing-glove-spring structure is designed by changing the beam number and length from the basic model. 3D nanotubular structures can also be created by connecting both ends of a 2D accordion rack structure. However, for simulating the 3D structures, the initial position of the beams should be considered in 3D space and the initial state of the beams is supposed to be bent. Therefore, more computational transformation is needed. Design and simulation of various 2D and 3D DNA accordion rack structures will be conducted by developing the computer-aided program in future research.

Speed and repeatability of actuation are also important factors in the field of dynamic DNA nanotechnology. Recently dynamic DNA structures that respond to external electrical or magnetic field are proposed and operated with high speed^{22,23}. While the DNA accordion rack has enabled collective actuation of multiple DNA beams, the reconfiguration speed was not significantly improved since actuation is based on DNA strand displacement. For further applications, response to external stimuli should be improved by optimizing DNA lock design or using another external stimuli.

As an application study for the reconfigurable accordion structure, various functional materials such as drug molecules, nanoparticles, or proteins can be attached to the structure. For this, the molecules can be connected to the staple that exists at the intended position. Overall, a variety of application studies can be conducted through this protocol and its modifications.

Disclosures

The authors have nothing to disclose

Acknowledgements

This research was partially supported by the Global Research Development Center Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Science and ICT (MSIT) (2015K1A4A3047345) and Nano-Material Technology Development Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (MSIT) (2012M3A7A9671610). The

Institute of Engineering Research at Seoul National University provided research facilities for this work. Authors acknowledge gratitude towards Tae-Young Yoon (Biological Sciences, Seoul National University) regarding the fluorescence spectroscopy for the FRET analysis.

References

1. Andersen, E.S. *et al.* Self-assembly of a nanoscale DNA box with a controllable lid. *Nature*. **459** (7243), 73-6 (2009).
2. Cha, T.-G. *et al.* Design principles of DNA enzyme based walkers: Translocation kinetics and photo-regulation. *Journal of the American Chemical Society*. **137** (29), 9429-37 (2015).
3. 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*. **347** (6229), 1446-1452 (2015).
4. Pinheiro, A. V., Han, D., Shih, W.M., Yan, H. Challenges and opportunities for structural DNA nanotechnology. *Nature nanotechnology*. **6** (12), 763-72 (2011).
5. Li, J. *et al.* Exploring the speed limit of toehold exchange with a cartwheeling DNA acrobat. *Nature Nanotechnology*. **1** (2018).
6. Krishnan, Y., Simmel, F.C. Nucleic acid based molecular devices. *Angewandte Chemie International Edition*. **50** (14), 3124-56 (2011).
7. Liu, M. *et al.* A DNA tweezer-actuated enzyme nanoreactor. *Nature communications*. **4**, 2127 (2013).
8. Douglas, S.M., Bachelet, I., Church, G.M. A logic-gated nanorobot for targeted transport of molecular payloads. *Science (New York, N.Y.)*. **335** (6070), 831-4 (2012).
9. Kuzyk, A. *et al.* Reconfigurable 3D plasmonic metamolecules. *Nature Materials*. **13** (9), 862-866 (2014).
10. Zhou, C., Duan, X., Liu, N. A plasmonic nanorod that walks on DNA origami. *Nature communications*. **6**, 8102 (2015).
11. Choi, Y., Choi, H., Lee, A.C., Lee, H., Kwon, S. A Reconfigurable DNA Accordion Rack. *Angewandte Chemie International Edition*. **57** (11), 2811-2815 (2018).
12. Chen, H. *et al.* Understanding the Mechanical Properties of DNA Origami Tiles and Controlling the Kinetics of their Folding and Unfolding Reconfiguration. *Journal of the American Chemical Society*. **136** (19), 6995-7005 (2014).
13. Han, D., Pal, S., Liu, Y., Yan, H. Folding and cutting DNA into reconfigurable topological nanostructures. *Nature Nanotechnology*. **5** (10), 712-717 (2010).
14. Douglas, S.M. *et al.* Rapid prototyping of 3D DNA-origami shapes with caDNA. *Nucleic Acids Research*. **37** (15), 5001-5006 (2009).
15. Castro, C.E. *et al.* A primer to scaffolded DNA origami. *Nature methods*. **8** (3), 221-9 (2011).
16. Ouldridge, T.E., Louis, A.A., Doye, J.P.K. DNA Nanotweezers Studied with a Coarse-Grained Model of DNA. *Physical Review Letters*. **104** (17), 178101 (2010).
17. Snodin, B.E.K. *et al.* Direct Simulation of the Self-Assembly of a Small DNA Origami. *ACS Nano*. **10** (2), 1724-1737 (2016).
18. Amir, Y., Abu-Horowitz, A., Bachelet, I. Folding and Characterization of a Bio-responsive Robot from DNA Origami. *Journal of Visualized Experiments*. (106), e51272 (2015).
19. Stahl, E., Martin, T.G., Praetorius, F., Dietz, H. Facile and Scalable Preparation of Pure and Dense DNA Origami Solutions. *Angewandte Chemie International Edition*. **53** (47), 12735-12740 (2014).
20. Wei, B., Vhudzijena, M.K., Robaszewski, J., Yin, P. Self-assembly of Complex Two-dimensional Shapes from Single-stranded DNA Tiles. *Journal of Visualized Experiments*. (99), e52486 (2015).
21. Clegg, R.M. Fluorescence resonance energy transfer and nucleic acids. *Methods in enzymology*. **211**, 353-88 (1992).
22. Kopperger, E. *et al.* A self-assembled nanoscale robotic arm controlled by electric fields. *Science (New York, N.Y.)*. **359** (6373), 296-301 (2018).
23. Lauback, S. *et al.* Real-time magnetic actuation of DNA nanodevices via modular integration with stiff micro-levers. *Nature Communications*. **9** (1), 1446 (2018).