Chapter 2

Computer-Aided Design of DNA Origami Structures

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Abstract

The DNA origami method enables the creation of complex nanoscale objects that can be used to organize molecular components and to function as reconfigurable mechanical devices. Of relevance to synthetic biology, DNA origami structures can be delivered to cells where they can perform complicated sense-and-act tasks, and can be used as scaffolds to organize enzymes for enhanced synthesis. The design of DNA origami structures is a complicated matter and is most efficiently done using dedicated software packages. This chapter describes a procedure for designing DNA origami structures using a combination of state-of-the-art software tools. First, we introduce the basic method for calculating crossover positions between DNA helices and the standard crossover patterns for flat, square, and honeycomb DNA origami lattices. Second, we provide a step-by-step tutorial for the design of a simple DNA origami biosensor device, from schematic idea to blueprint creation and to 3D modeling and animation, and explain how careful modeling can facilitate later experimentation in the laboratory.

Key words DNA, Nanotechnology, Origami, Biosensor, CAD, Software

1 Introduction

The methods for designing DNA nanostructures have been developing over the last three decades with the objective of creating well-ordered DNA lattices to organize and control matter at the nanoscale [1, 2]. When the DNA origami method was introduced in 2006 it provided many new features that were not previously achievable such as programmable overall shape, sequence-specific addressability on a large structure, and high self-assembly yield [3]. The trick to obtain these features was to fold a long DNA "scaffold" strand into a desired shape using several smaller DNA "staple" strands. The scaffold strand was obtained from a natural source, the M13mp18 bacteriophage single-stranded DNA genome with a length of 7,249 nucleotides, and the staple strands were obtained by chemical synthesis—usually about 230 DNA strands of 32 nucleotides each. The self-assembly was done by mixing all DNA strands in a standard buffer with high magnesium concentration followed by heat-annealing of the strands whereby

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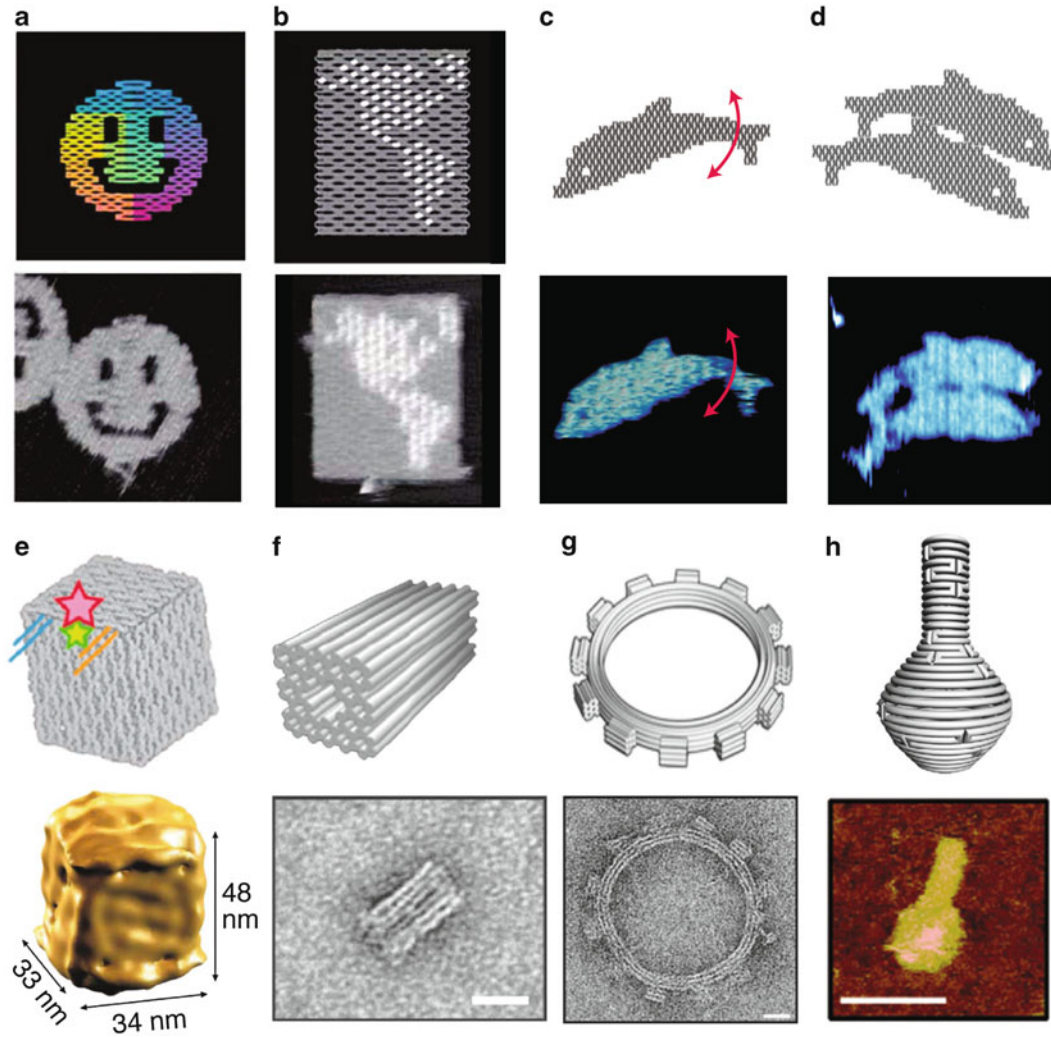

Fig. 1 DNA origami structures. (**a**–**d**) 2D DNA origami structures: (**a**) DNA smiley face strand path and corresponding AFM (atomic force microscopy) image [3]. (**b**) DNA rectangle with map and corresponding AFM image [3]. (**c**, **d**) DNA dolphin with specific shape recognition and corresponding AFM image [5]. (**e**–**h**) 3D origami structures: (**e**) DNA box with controllable lid model and cryo-EM reconstruction [8]. (**f**) Square nut model and TEM image with scale bar of 20 nm [9]. (**g**) Twelve-tooth gear model and TEM image [10]. (**h**) DNA origami flask model and AFM image with scale bar of 75 nm [11]

the desired structures form. Shapes with complicated folding paths of the scaffold strand could be obtained (Fig. 1a) and the addressability was demonstrated by the addition of "pixels" (projecting DNA structures) on the surface of the DNA origami sheets (Fig. 1b). The method was rapidly adopted by other research groups to produce new shapes [4, 5] and to label the structures with other materials [6, 7]. An early hint that DNA origamis could act as mechanical devices in solution was suggested by the design and demonstration of DNA origamis with flexible regions

(Fig. 1c) and post-assembly sequence/shape-recognition between structures in solution (Fig. 1d). A second development was the design of three-dimensional (3D) DNA origami structures. The first approach demonstrated that DNA origami sheets can be arranged in 3D by geometry-inducing crossovers to form a DNA origami box with a controllable lid (Fig. 1e) [8]. The second approach was to stack DNA origami sheets to form solid 3D shapes (Fig. 1f) [9], which could be further curved and twisted into more complicated shapes (Fig. 1g) [10]. The curvature and bending of single-layer DNA origamis could also be controlled by choosing crossover positions to enforce an overall shape (Fig. 1h) [11]. Several other design developments have been demonstrated later, e.g., the DNA origami gridiron [12], DNA origami with parallel crossovers [13], and single-stranded tiles with single crossovers that assembles efficiently in the absence of a long scaffold strand [14, 15].

Several computational tools have been developed to facilitate the design of DNA nanostructures [16]. The most relevant tools for designing DNA origami structures are listed in Table 1 and are often used in conjunction during the design process as illustrated in Fig. 2. The design process usually starts with an idea for a shape or device sketched out on paper, where the strand path and size of the object is roughly calculated (Fig. 2a). The second step is the creation of a digital blueprint of the strand path of the scaffold strand and the positions of the staple strands (Fig. 2b). Two software packages are available for this task: SARSE [5] and Cadnano [17].

Table 1
Software for DNA origami design and analysis

Name	Description	Link	References
caDNAno	Blueprint editor and 3D editor for DNA origami design	http://cadnano.org	[17]
CanDo	Coarse-grained modeling of strain and flexibility in DNA origami structures	http://cando-dna-origami.org	[21, 27]
NUPACK	Thermodynamic analysis of the annealing of multiple DNA strands	http://nupack.org	[33]
OxDNA	Coarse-grained modeling thermodynamic DNA hybridization	http://dna.physics.ox.ac.uk/	[24]
SARSE	2D editor for DNA origami design with output of atomic models	http://cdna.au.dk/software/	[5]
TIAMAT	3D editor with sequence symmetry minimization	http://yanlab.asu.edu/Resources.html	[18]
Uniquimer	3D editor with symmetry and energy minimization	http://ihome.ust.hk/~keymix/uniquimer3D/	[19]

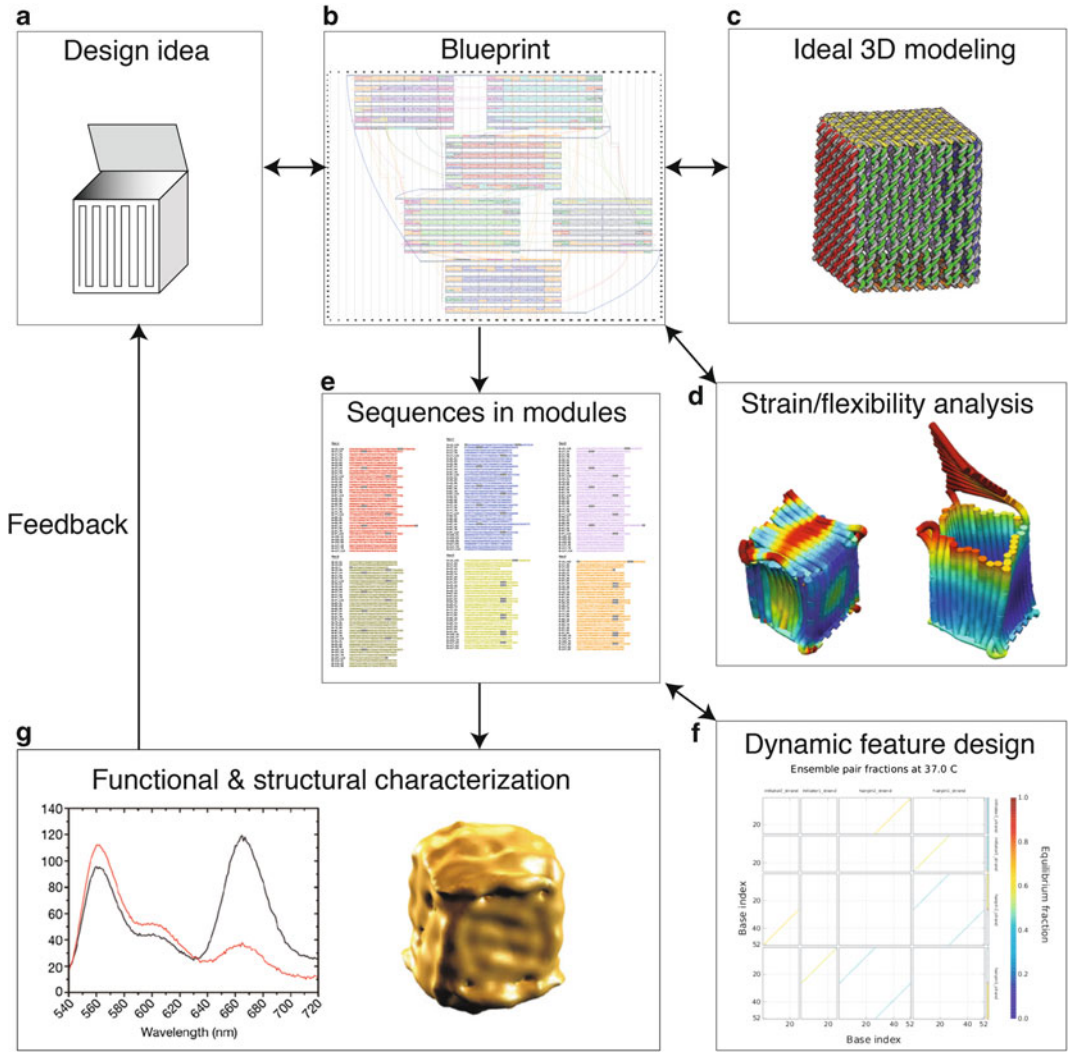

Fig. 2 DNA origami design procedure. Flow chart of the different steps in the design of a DNA origami box structure. (**a**) An initial idea is materialized by calculating the possible dimensions of the object given the length of the scaffold strand to be used. (**b**) A blueprint is constructed using the SARSE or Cadnano software packages [5, 17]. (**c**) 3D modeling is used to evaluate how modules are placed in relation to each other and used for the design of 3D staple strand connections. (**d**) Coarse-grained modeling using the CanDo program [21, 27] can be used to evaluate the strain and flexibility of the structure. (**e**) Sequences are exported from the blueprint program and sorted into functional modules that can be used to make different versions of the designed structure. (**f**) DNA strands used for strand displacement can be designed in dedicated software. (**g**) DNA is ordered, self-assembled, and tested in structural and functional experiments

The SARSE software allows automated creation of blueprints based on shapes specified in bitmap files, detailed editing of the blueprint, and export of sequences and atomic models. The software only works for flat DNA origami structures and has not been actively developed to accommodate new design principles. Cadnano allows

the manual construction of DNA origami blueprints and supports both square and honeycomb lattices. The software is actively developed and the json files have become the standard blueprint file format. 3D modeling software is another important tool for designing complicated 3D DNA origami structures (Fig. 2c). Cadnano has been integrated as a plug-in in the 3D modeling environment Autodesk Maya, which provides a live 3D CAD experience by linking 2D blueprint to 3D model. Other examples of dedicated 3D modeling software for construction of DNA nanostructures are TIAMAT [18] and Uniquimer3D [19], but general molecular visualization software like UCSF Chimera [20] can also be used for model building and rendering of models. When a blueprint is finished the 3D shape can be analyzed using the CanDo web server (Fig. 2d) that uses the crossover positions in the DNA origami blueprint to determine the overall shape using a finite element analysis method [21]. CanDo can both be used to detect unwanted distortions and to design bend and twisted shapes on purpose. When a satisfactory design is achieved the DNA staple strands are exported based on the blueprint and a choice of scaffold sequence (Fig. 2e). At this stage the DNA strands are normally sorted into modules that allow functional or structural elements to be substituted at the pipetting stage. Functional modules like strand displacement systems [22] can be designed in other software like NUPACK [23] and mechanistic details can be modeled in coarse-grained modeling software OxDNA [24]. After ordering of the final DNA sequences, the DNA origami structure can be self-assembled in the laboratory, characterized by biophysical techniques, and applied for the desired purpose (Fig. 2f). As always in design processes the testing stage can be used as feedback to improve the design if it does not meet the requirements.

Tutorials for designing DNA origami structures are found both on the websites of the software and published in the literature. The original DNA origami papers describe the design principles in detail [3, 9–15, 17, 25]. We have earlier provided a tutorial for the design of 3D DNA origami objects using the SARSE software [26]. Dietz and colleagues have provided a detailed step-by-step tutorial to DNA origami design using caDNAno and CanDo as well as experimental protocols [27]. In this chapter we start by describing how crossover positions in DNA origami structures are calculated and discuss the standard flat, square and honeycomb lattices. Next, we provide a simple design example and the step-by-step tutorial for designing the structure using caDNAno and for 3D modeling and animation using Maya. We have chosen this example to show how easy it is to make a functional mechanical device and hope it will provide a stepping stone for creating more complex and advanced devices.

2 Materials

1. Download and install cadnano 2.2.0 from http://cadnano.org.
2. Download and install Autodesk Maya 2012 from http://www.autodesk.com/education/free-software/maya.
3. Three button mouse.

3 Methods

3.1 Calculating Crossover Positions Between DNA Helices

To construct a crossover between two DNA helices we can work with a simplified 3D DNA model showing only the position of phosphate (P) atoms along the backbone (Fig. 3). The simplified model has a minor groove angle of 133.0° and a twist angle of 34.48°/bp, which corresponds to 10.44 bp/turn along the helical axis for a standard B-form helix [28]. A crossover is made by aligning two P atoms of the two DNA helices, breaking the bond on the same side of the P atoms, and rejoining the strands between the helices (Fig. 3a). The crossover operation results in a four-way junction that has two possible stacking conformations and some flexibility at the junction [29]. To fix the two helices rigidly in relation to each other a second crossover has to be made. The double crossover can be made in five different ways named DAE, DAO, DPE, DPON, and DPOW, where D stands for double crossover, A/P for antiparallel or parallel strand direction at the crossover, E/O for an even or odd number of half-turns between the crossovers, and N/W for major/wide or minor/narrow groove bridging in the parallel odd case [29]. Here we only describe crossovers between antiparallel strands because these are the ones used in the standard DNA origami structures discussed below. To calculate the position of the second crossover on the same strand (DAE) we use the twist angle of 34.48°/bp (*see* **Note 1**) and find that it has an optimal spacing of 21 bp (Fig. 3b). Where phosphates meet, a second crossover can be made using the same operation as described in Fig. 3a. To calculate the position of the second crossover on the opposite strand (DAO) we use the minor groove angle of 133.0° and the twist angle of 34.48°/bp (*see* **Note 1**) and find that it has an optimal spacing of 16 bp (Fig. 3c). As seen on the side views to the right of the models a DAE has the minor grooves on the same side, while a DAO molecule has the major and minor grooves on opposite sides at the crossovers, which leads to different pseudo symmetry axes [29]. Crossover positions do not always fit perfectly when calculated along the strand, but can work anyway since the DNA helix has a significant degree of flexibility and can accommodate suboptimal conformations.

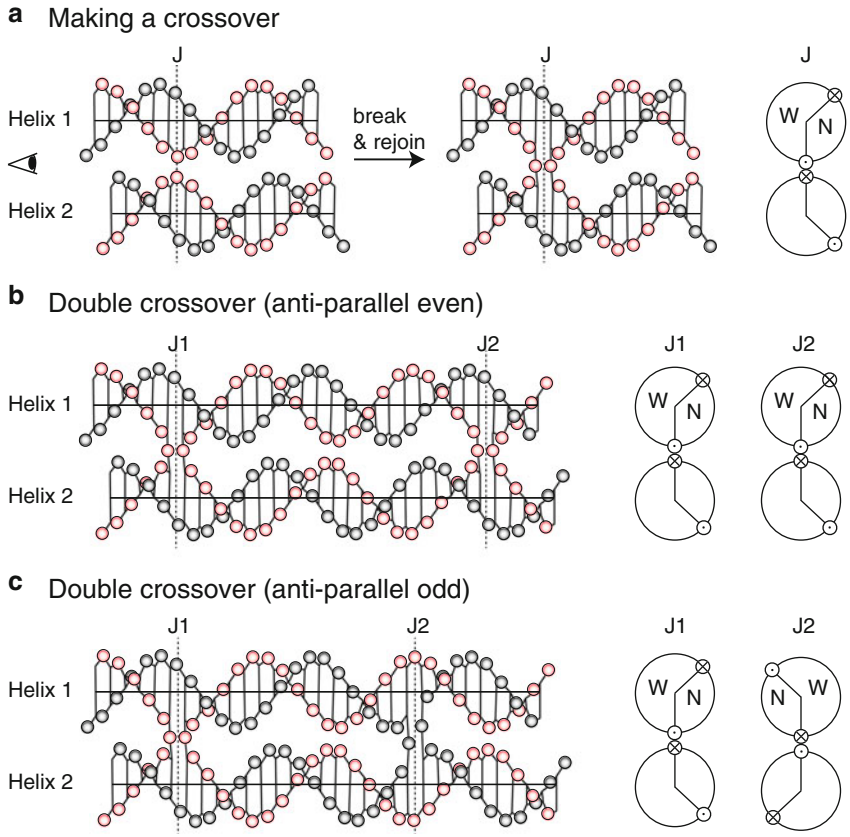

Fig. 3 Formation of crossovers between two DNA helices. (**a**) *Left*: Two phosphates are aligned in the plane between two helices. *Right*: Strands are broken and rejoined to form the crossover. (**b**) A double-crossover molecule with crossovers on the same strand with a spacing of 21 base pairs. (**c**) A double-crossover molecule with crossovers on the two complementary strands with a spacing of 16 base pairs. DNA helices are based on atomic models, but are shown here in a simplified representation, where the phosphates are shown as spheres, lines are drawn between P and C3 atoms along the backbone, and lines between two C3 atoms represent base pairs. The two complementary strands are colored with black and red. The 5′ end of the strands is seen as ending with a P sphere. Helical axes are shown and positions of crossovers are indicated by *dashed lines* (J = junction). Junctions are also shown in side view on the *right* as seen along the helical axis from the *left* of the models (*see* eye icon). The major/wide groove is marked with a W. The minor/narrow groove is marked with a N. The strand direction at the junction is shown as a *circle with a dot* (3′ end) and a *circle with a cross* (5′ end)

3.2 Scaffolded DNA Origami Crossover Patterns

In DNA origami structures the anti-parallel type of crossovers is used to connect multiple helices in several configurations (Fig. 4). The majority of crossovers in a DNA origami is made by the staple strands, which are all on the same strand in relation to each other and thus calculated as in Fig. 3b. The scaffold strand also makes crossovers as it threads through the structure and these crossovers are on the opposite strand and calculated as in Fig. 3c. The standard flat DNA origami structures [3] seeks to make all crossovers in the plane (Fig. 4a). It has a spacing of 32 bp (3 turns) between

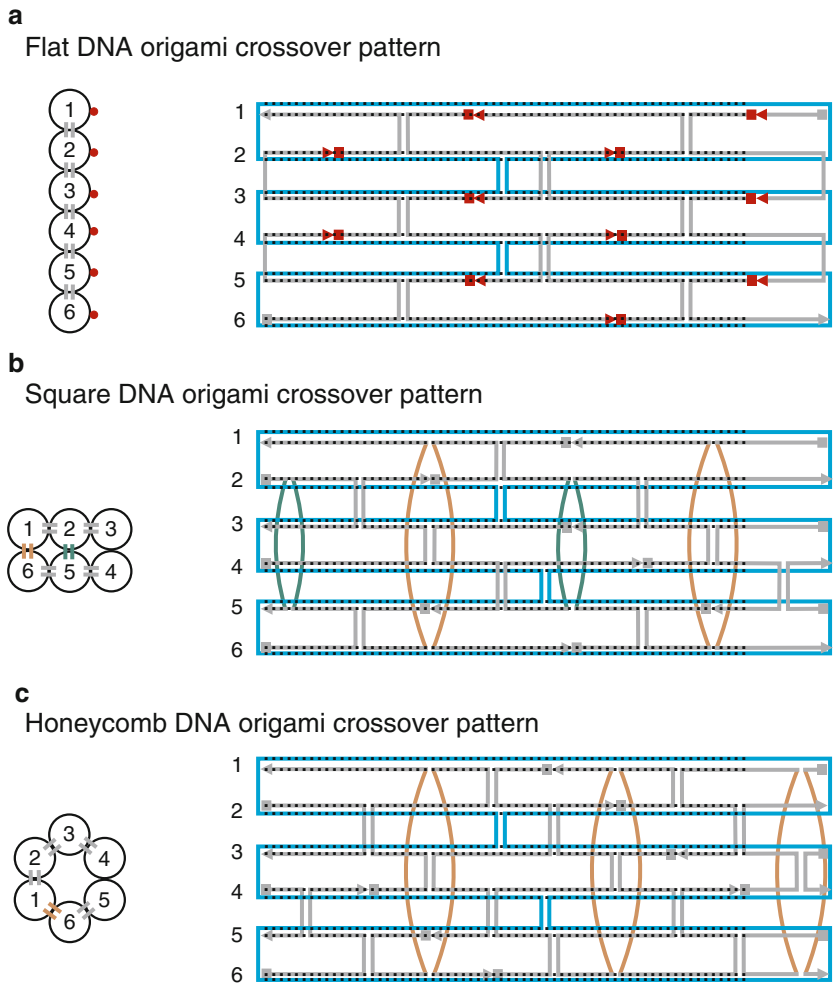

Fig. 4 DNA origami crossover patterns. *Left*: Numbered helixes shown in side view. *Right*: Blueprints with scaffold strand in cyan and staple strands in *grey*. Crossovers between distant helices are colored with *orange* and *green*. DNA bases are marked as *black dots on the strands*. The scaffold strand is circular and runs clockwise through the structure (Color figure online)

crossovers of the same two helices, and a 16 bp (1.5 turn) spacing when crossing over to a third helix in the plane. Thus, this architecture assumes 32 bp/3 turns = 10.67 bp/turn and a twist of 360°/10.67 bp = 33.74°/bp. Since the real value is 10.44 bp/turn [28] the resulting DNA structure will be undertwisted resulting in an overall distortion of the structure which can be investigated using the CanDo software (Table 1). The staple strands are often broken in the middle of the 16 bp region on every other helix which results in a 8-16-8 bp annealing region pattern. The pattern is repeated through the whole structure and only interrupted on the edges. The way the strands are broken in Fig. 4a makes all staple strands form an "S" shape and the 5′ and 3′ ends are all positioned on the

upper surface of the DNA origami sheet (marked in red in Fig. 4a). The staple strands can also be broken on alternate helices resulting in a "Z"-shaped staple strand. In this case the 5′ and 3′ end points to the other side of the DNA origami sheet. At last one can make combinations of S and Z staples to position the ends on both sides of the sheets. 5′ and 3′ ends are good for placing chemical modifications. Square DNA origami is a 3D version of flat DNA origami where the same helicity and spacing between crossovers are used. Scaffold strand segments that make up helix 1 and 6 are placed far apart on the scaffold strand and have to be connected together via staple strands for the structure to be stable. The first staple strand (from the left) of helix 1 has a crossover that connects it with helix 6 (orange crossover in Fig. 4b) and with helix 5 (Fig. 4b). This staple strand has to make 3/4 of a turn (8 base pairs) on helix 6 to be able to crossover to helix 5 after crossing over from helix 1 (side view in Fig. 4b). The same is valid for the crossovers connecting helices 2 and 5 (green crossovers in Fig. 4b). Honeycomb DNA origami uses 10.5 bp/turn, which is very close to the reported value of 10.44 bp/turn [28] (*see* **Note 2**), and allows it to have a spacing of 21 bp between the crossovers of the same strand (Fig. 3b). As seen in the side view in Fig. 4c the honeycomb arrangement of helices are linked by local crossovers (grey) and long-range crossovers between helix 1 and 6 (orange). Annealing regions in honeycomb lattices are 7 bp.

3.3 Design of a DNA Origami Device

Here we demonstrate the design process for making a DNA origami biosensor (Fig. 5). The structure consist of two six-helix bundles that are connected through a hinge at one end and held together via complementary oligonucleotides protruding the other end. Complementary oligonucleotides hold the structure together in a closed conformation. Donor and acceptor fluorophores are

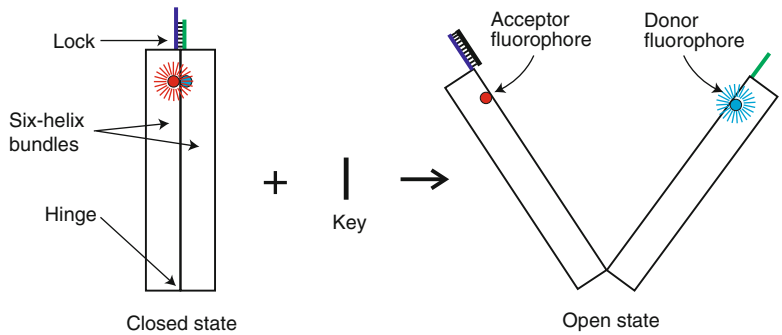

Fig. 5 The sketch of DNA origami six-helix bundle sensor. The sensor consists of two six-helix bundles that are connected in one end by a scaffold strand and held together by an oligo lock in other end. In the closed conformation there is FRET between donor and acceptor fluorophores that are positioned at the end of the each bundle. When the structure is in its open conformation the distance between the fluorophores is too large for FRET to occur

placed at the end of each bundle to detect the opening of the device by Förster resonance energy transfer (FRET). In the closed conformation FRET occurs from donor to acceptor fluorophore resulting in acceptor fluorescent signal when the donor fluorophore is excited. One of the locking strands is designed with a toehold for a "key" strand that can displace the shorter locking strand from lock helix by the strand displacement mechanism [22]. Upon the addition of a key the lock helix is opened and the biosensor opens due to the electrostatic repulsion between the DNA helices. Increased distance between fluorophores decrease FRET resulting in only donor fluorescence when it is excited. The key strand acts as a signal that can be measured by acceptor and donor fluorescence and the structure can be used as a biosensing platform for the key strand.

3.4 caDNAno Design

The DNA origami device described above can be implemented in different sizes. Here we choose a small size using 1,344 nucleotides to make an easy design process. The standard DNA origami structures consist of approximately 14,000 nucleotides.

1. Open the caDNAno program and get familiar with its interface (Fig. 6).
2. To begin, choose a honeycomb lattice from the main menu and draw a six helix bundle in the lattice window (Fig. 6a).
3. Click on the arrows just above the helices in the top right corner (Fig. 6g) to add 84 bp to the helices' length to 42 bp that are set by default.
4. Draw the scaffold strand as shown (Fig. 7d) using the "Pencil" tool from the tool box. Each bundle should be 61 bp long. A 5′ end can be connected with 3′ end using the "Pencil" tool. The scaffold strand can be connected only with another part of the scaffold strand and staple strands only with other staple strands. There is a more convenient way of drawing the scaffold path through the helices. Click with the left button of the mouse on helix "0" in the lattice window, hold the mouse button down and select all the other helices by moving the mouse clockwise. Release the button when the last helix is selected. The scaffold path centered on the "ruler" should appear connected via crossovers. Note that the cell array may not contain any drawn scaffold strands for this function to work properly. Correct automatic scaffold drawing according to the blueprint in Fig. 7d. This can be achieved by dragging the crossover elements using the "Select" tool. Remove any undesired crossovers by first selecting the crossover and then pressing the "Delete" key on the keyboard. Place 3′ and 5′ ends at desirable positions and use the "Pencil" tool to draw a crossover. The select tool only selects the elements that are defined in the "Selectable" menu above the blueprint window (*see* **Notes 3** and **4**).

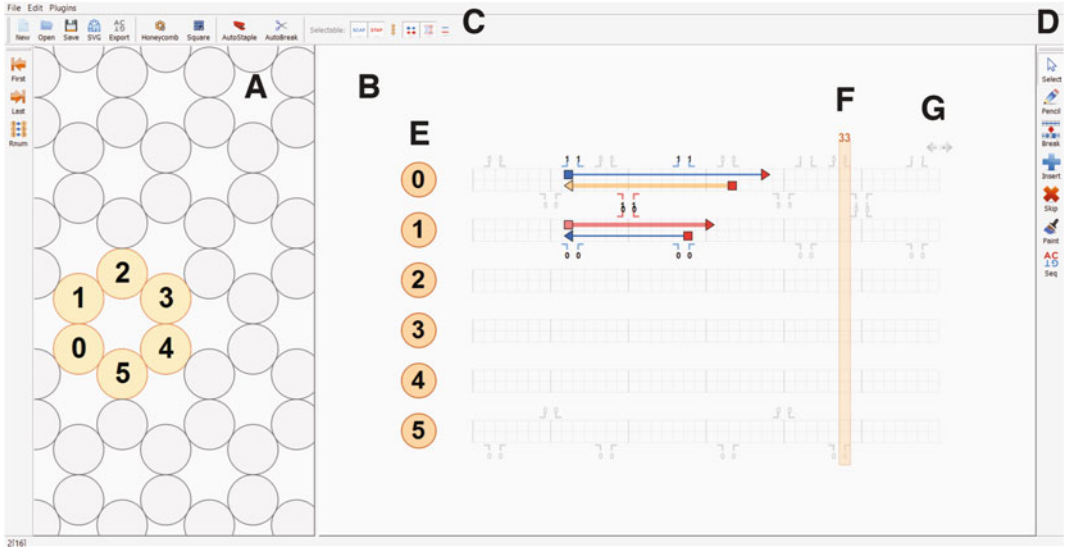

Fig. 6 caDNAno interface. (**a**) Lattice window shows how the helices are arranged in the designed structure. Honeycomb lattice is shown as an example. Each helix is numbered: the *odd* and *even numbers* represent the directionality of the scaffold/staple strand helices. Scaffold strand's directionality of the odd helices is opposite to directionality of the even ones. Alternatively a square lattice can be chosen. (**b**) Blueprint window shows how the crossovers between the helices hold the structure together. (**c**) Selectable menu allows to make the selection specific for defined elements. (**d**) Tool box: Select—use it to select elements in blueprint window, Pencil—allows to draw scaffold and staple strands in the cell arrays, Break—can be used to introduce breaks in the strands, Insert/Skip—allows to insert or skip a base on a desired strand, Paint—allows to change the color of the strands, Seq—sets the sequence of the scaffold strand and the staple strand sequence is generated automatically (**e**) Each helix is represented by 2xN cell array. The *top row* represents a scaffold strand and *bottom row* a staple strand (vice versa for odd numbered helices). Each cell represents a base of a DNA strand. (**f**) Movable ruler, (**g**) Addition/deletion of bases to the cell array

5. Click the "Autostaple" button—this will add staple strands automatically to the designed structure. Added staples are long strands that bind the scaffold strand in a regular pattern (*see* **Note 5**).
6. Use the "Break" tool from the tool box to cut the strands into shorter segments of 28–42 bp per strand. In the honeycomb lattice the staple strand pattern is usually 7-7-14-7-7 bp (or 7-7-7-14-7 bp etc.) where the number refers to the binding regions on the helix (*see* **Notes 6** and **7**).
7. Shorten the staples at the ends of each helix by two base pairs to remove the possible strain of the scaffold strand (Fig. 7e). More than one endpoint can be selected simultaneously by selecting "Staples" and "Endpoints" from the "Selectable" menu and hovering the arrow icon over the desired endpoints.
8. Now we will design oligonucleotides that will serve as the lock mechanism. Choose the two staple strands that will be functionalized with donor and acceptor fluorophores. Helices 0 and 5

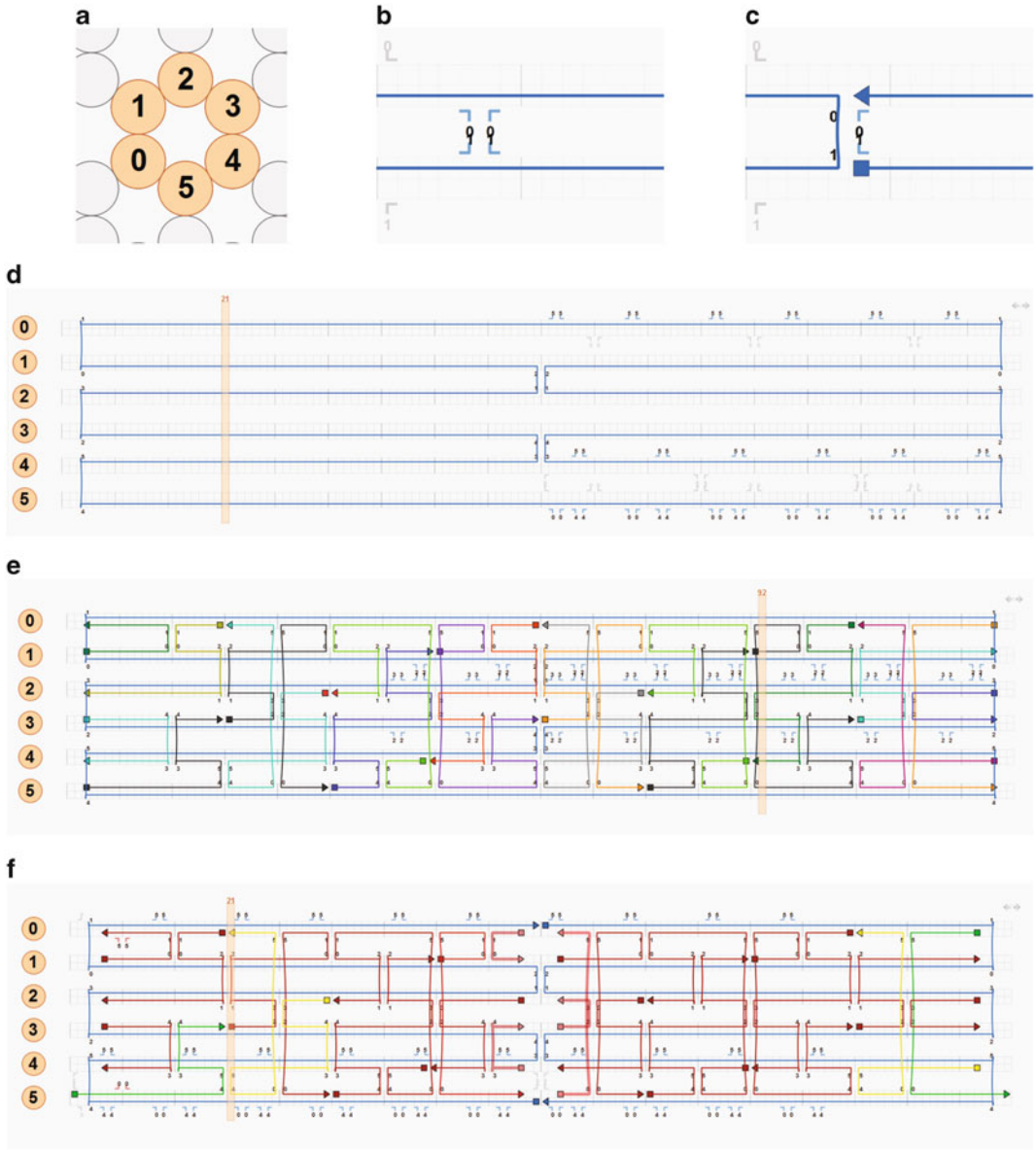

Fig. 7 Blueprint design in caDNAno. (**a**) Six-helix bundle drawn in lattice window. (**b**) Crossover markers. (**c**) One part of the (**d**) blueprint of scaffold strand. (**e**) Addition and cleavage of the staple. (**f**) Preparation of the design for Maya animation

connect the two bundles, determine the hinge axis, and are chosen for positioning the lock and FRET pair. Color all the staples with red by selecting the red "STAP" button in "Selectable" menu shown in Fig. 6c. Use both "Select" and "Paint" tool from the toolbox to color them all with red. Now color the lock staples with green and the fluorophore staples with yellow. Now make small changes on the lock and fluorophore staples as

shown (Fig. 7f). Last thing to do is to break the scaffold strand at the bending points of helices 0 and 5 (Fig. 7f) (*see* **Note 8**).

9. Open Maya and click on the caDNAno plug-in button in the right upper corner beneath the "Minimize" and "Close" icons. The screen now consists of three windows: two of them are the familiar caDNAno windows and the third is the Maya window.
10. Open your caDNAno design file through the caDNAno window. Essentially, one has the same possibilities in the plug-in as in caDNAno itself. You can use "Alt + the left mouse" key to rotate the view around your object in the Maya window.
11. Our lock strands were initially designed too short. Now we can make them a little bit longer in two ways:
    (a) Use the select tool of the caDNAno window and edit the lock strands in caDNAno.
    (b) Select one of the green helices in Maya window by clicking with the left button of the mouse and drag the blue rhomb (Fig. 8f, g).
12. Do the same with the green staple strand from the other end of the object. Extend the possible helix length if needed by extending cell arrays in the caDNAno blueprint window.
13. The rest of the modeling is done in Maya only (*see* **Notes 9** and **10**) and caDNAno can now be closed to get more workspace. Click the caDNAno icon in the top right corner to close the plug-in.

3.5 Maya Modeling

The structure that is made in caDNAno depicts only the DNA part and fluorophores have to be added separately in Maya. The fluorophores are going to be represented as spheres of different color: donor is colored with green and acceptor with red.

1. The object has lost its color and only a wireframe is visible. Click the "Smooth shade all" button from the panel tool bar to bring the colors back (Fig. 8b).
2. Select Create > NURBS Primitive and deselect "interactive creation" at the bottom of the drop down menu (press "ctrl + M" on the keyboard if the menu bar is invisible). Now select Create > NURBS Primitive > Sphere and click on the small square next to it. In the Sphere option menu change the radius from 1 to 0.5. Click "create." A sphere is now positioned at the center of the grid (the grid is invisible by default, but can be switched on by selecting Display > Grid). Press "Z" to undo an operation instead of the more common "Ctrl + Z" combination. "Shift + Z" will redo the operation.
3. The fluorophore has to be placed on the end of our yellow staple. This can be done with precision in 3D by viewing our object from x, y, and z directions in orthogonal view.

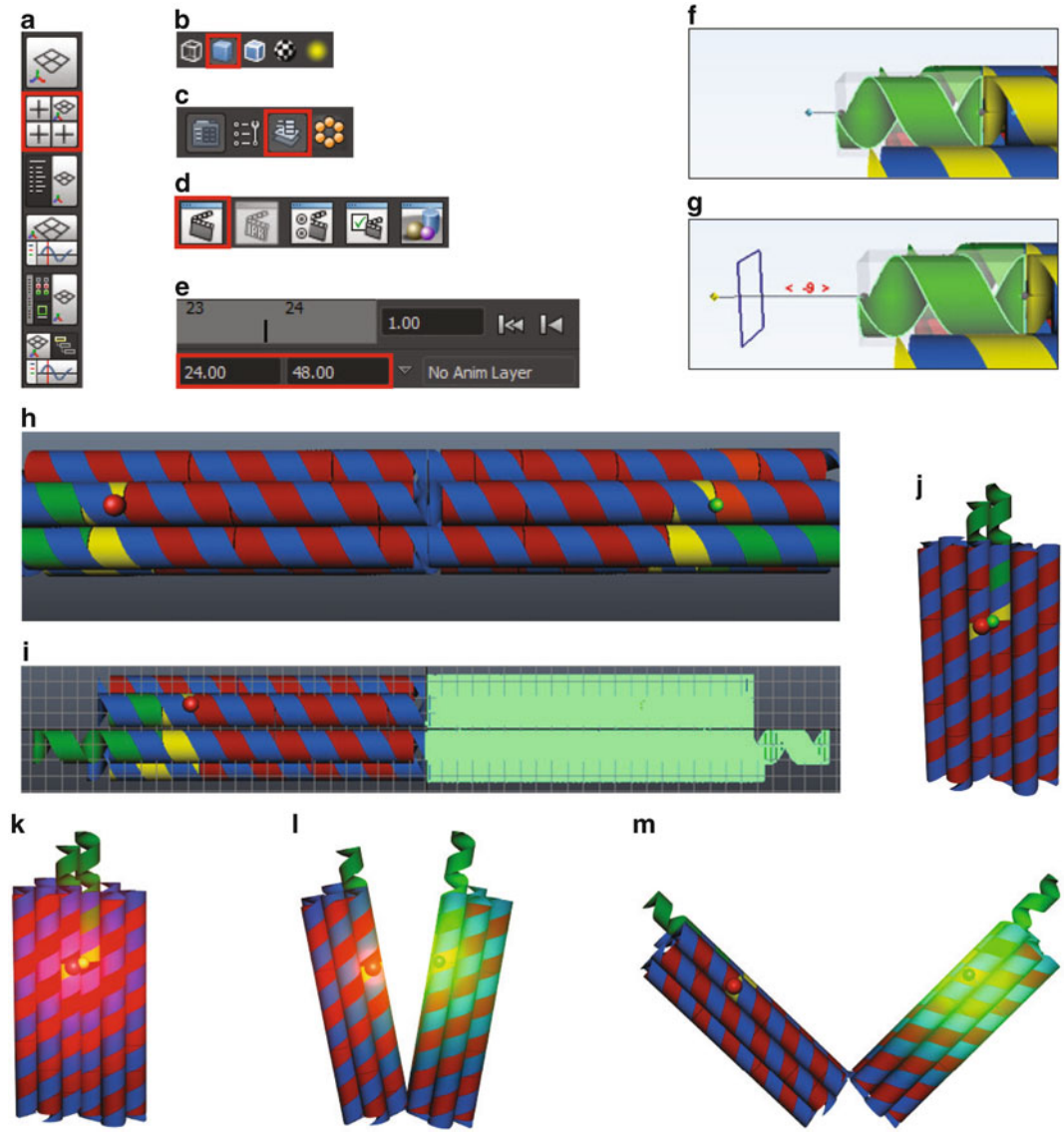

Fig. 8 3D modeling and animation in Maya. (**a**) Quick layouts buttons are placed beneath the tool bar menu. (**b**) Part of the panel tool bar. (**c**) Editor icons. (**d**) Rendering shelf. (**e**) Range slider. (**f**) Selection of a strand in Maya is shown as a *grey box*. (**g**) The length of the strand can be changed by moving the blue rhomb along the strand axis. (**h**) Adding fluorophores in Maya. (**i**) Grouping the selected elements in orthographic view. (**j**) Device in a closed state. (**k**–**m**) Animation of the opening of the device

The "quick layout" buttons allow you to change between different viewing modes. Choose the "Four view" option using the "quick layout" buttons just beneath the tool bar menu (Fig. 8a). The window is now split into four windows where the object is seen in three orthogonal views plus the perspective view.

4. Select the sphere with the select tool (it can be selected in any of the windows) and choose the move tool from the tool box on the left panel. Three arrows appear (red, green, and blue) each representing the direction that the sphere can be moved along the *x*, *y*, and *z* axes.
5. Drag the blue arrow with the left button of the mouse to the left. You can follow the movement of the sphere in all windows simultaneously. If the sphere disappeared in one of the windows use "alt + middle mouse button" to move the view in that window (scrolling the mouse wheel will zoom in/out). If a bigger view is needed in one of the windows press "space" while pointing on it with the mouse arrow. Press space again to return back to the four window view. Place the sphere on the 3′ end of the yellow staple on one six-helix bundle (use caDNAno to find out which end is 3′ and which is 5′).
6. Create the second sphere with a radius of 0.75 and place it at the 3′ end of the yellow staple on the other six-helix bundle. Both spheres are now placed at the right positions and will represent donor and acceptor fluorophores (Fig. 8h).
7. Click with the right button of the mouse on the sphere and while still holding the right mouse button choose "Assign a new Material" and release the button. Choose "Blinn" as a material and the red color for the big sphere and the green color for the small one.

   The initial state of the object is the closed one. The two bundles have to be brought together. Each bundle has a scaffold strand, multiple staple strands, and one fluorophore, which are grouped to treat them as one element in the animation.
8. Go back to the four view display and choose the right bottom window that shows the structure in side view. Select one of the halves (the selected area should turn green) and press "ctrl + G" to group all the selected elements into one group (Fig. 8i). Select and group the other half.
9. Select the Panels menu and choose Saved Layouts > Persp/Hypergraph. The view is changed to perspective and the Hypergraph window is now visible. The Hypergraph window depicts all the objects found in the workspace in a block form. When you group your objects they become associated under the "group" blocks. It is easier to navigate by selecting or deselecting objects using Hypergraph. Click on the group in the Hypergraph window which results in selection of the corresponding group in the workspace window. One can rename, hide or delete objects using Hypergraph.
10. Choose one of the groups and select the rotate tool from the tool box. You will see three circles of different color that, as with the moving tool, refer to the rotation around three axes.

Press the "Insert" key on the keyboard. The circles turn into a point—the pivot point. Use the arrows to move the pivot point from the middle of the bundle to the hinge axis that is found between helices 0 and 5. Using the four view window, place the rotating point on the outer edge of the helices 0 and 5.

11. Open the menu "Channel Box"—one of the Editor Icons found in the upper corner next to the caDNAno plug-in key (Fig. 8c). This menu provides you total control over the Move, Translate and Rotate operations. Depending on which part you have chosen first, it has to be rotated by either 90° or −90° around the *y*-axis. Repeat the operation for the other part.

    Our structure is now in the closed state (Fig. 8j). The next stage is to animate the object to the open state.

12. At the bottom of the window there are two sliders: the time slider and the range slider. The time slider shows which frame of the animation is currently shown, while the range slider tells how many frames there are in the animation and which part of it we are currently working on.

13. Change both numbers on the left of the range slider to 1 and on the right to 240 (Fig. 8e). Set the time slider to 1. Select one of the groups and press "S" on the keyboard—the red mark now appears on the first slide of the time slider. Select slide 240 on the time slider. Change the rotation around the *y* axis back to zero in the Channel Box and press "S" on the keyboard. Go back to the first frame on the time slider and press the play button in the lower right corner of Maya. You will see that one of the six-helix bundles is moving from the closed conformation to the open. Press stop and do the same for the other bundle.

14. Addition of fluorophore glow to the animation will make the function of the biosensor more visual. Go to slide 1 and click with the right button of the mouse on the red fluorophore. Choose "Material attributes ..." from the drop-down menu. From the Material attributes menu scroll down to the Special effects function. Set glowing intensity to 1 and click with the right button of the mouse on it. Choose "Set driven key" from the drop-down menu. Choose one of the groups in Hypergraph and click on "Load Driver" in the set driven key menu. Choose rotate Y for the Driver and click "Key" in "Set driven Key." Click on the "Blinn" in the "Driven" and move the time slider to frame number 100. In the "Attribute editor" on the right set the glow to 0 and click again "Key" in "Set driven Key." The degree of glowing is now dependent on the bundle rotation around its Y axis. The glow effect is invisible until the frames are rendered. Do the same for the green fluorophore where glowing is set to 0 in the first frame and to 1 in the 100th frame.

15. The whole animation has to be rendered into a short movie sequence. Go to frame 1 and press the "Render Current Frame" button (Fig. 8d). A new window opens with a rendered frame of the device in the closed state: only the red fluorophore is glowing (Fig. 8k). Do the same for the frame 50 and 100 and you should get the frames shown in Fig. 8l, m. In the 100th frame the distance between fluorophores is too large and only the green fluorophore is glowing.
16. Choose the angle from which the animation is going to be viewed—by default the perspective view is rendered. Now we can render the first 100 frames and put them together in a small animation. Click the "Render Current Frame" button on a random frame. Choose Options > Render Settings ...
    (a) Set "Render Using" to "Mental ray" software.
    (b) Name file sequence in the "File name prefix."
    (c) Set "Frame/Animation ext" to "name.#.ext."
    (d) Set "Image format" to "JPEG(jpg)".
    (e) "Frame padding" refers to the maximum size of the image sequence. Set it to 3 (it will run from 000 to 999, in total 1,000 images).
    (f) Set the "Start frame" to "1," "End frame" to "100" and "By frame" to "1". These options render each frame from 1 to 100.
    (g) Remember to note down the path to the directory where the rendered images will be saved.
17. Close the Render settings window and the Render window. Click "Render" on the menu bar and then "Batch render." One can follow the rendering process on the command line. It should take approximately 3–5 min to render 100 frames on a standard laptop computer. The sequence of 100 frames can be compiled into an animation. Close Maya. A PC user can use the already installed Windows Live Movie Maker while QuickTimePro for this purpose.
18. Open Windows Live Movie Maker and simply drag and drop your image sequence in the program. Go to the edit menu and change the duration of each frame to 0.05 s. Now save the movie and play it.

We have now created a 3D model and animation of a DNA origami device.

Here we have shown how to make a DNA origami blueprint in caDNAno and how to make a simple animation of a dynamical structure. The animation cannot be regarded as a simulation of the device but it makes it possible to get an idea about how the structure looks in 3D and how the dynamic parts might move in

relation to each other. Furthermore, the animation of the idea can be successfully used when complicated scientific designs should be presented for the broader public but also for communication between scientists.

3.6 Suggestions for Further Work

Next you can start designing more complex shapes based on models from the literature (*see* **Note 11**), start considering how to functionalize the device by attaching RNA or protein elements (*see* **Note 12**) or order the designed structure, self-assemble, and characterize it (*see* **Note 13**).

4 Notes

1. To get comfortable with the various crossover patterns it is advised to use a spread sheet to calculate the positions of phosphates for the DNA helices involved in a crossover complex. Make two columns for each strand of the helix and specify the strand direction. Set the angle of the first crossover to zero and use the twist angle to calculate the phosphate position along the helix. Find the position where the DNA strands meet again on the same strand or the opposite strand and mark these positions with a color. Now try extending this to the multihelix DNA origami crossover patterns and verify that it fits the crossover spacings shown in Fig. 3.
2. A sketch is always a good start for designing a structure or drawing the initial idea but it should be also tried out in caDNAno to see if the structure design is possible. One of the first things to decide when designing a new structure using the DNA origami method is what lattice one would like to use. There are two main lattices that are widely used: square that was introduced in original Rothemund paper [3] and the honeycomb lattices [9]. The square and honeycomb lattices refer to the arrangement of helices in the structure (Fig. 4). The advantage of the square lattice is its compactness compared to the honeycomb lattice and can be used in construction of very dense structures. The angles between the helices in the honeycomb lattice result in close to integer numbers of bases between the crossovers that leads to less strained self-assembled structures. The square lattice spacing does not come so close to the integer numbers and is therefore more strained (Fig. 4). The square lattice can be manipulated to be more relaxed by insertion and deletion of bases in the helices to come closer to the natural DNA helicity of 10.44 bp/turn [10]. Beside these two lattices one can also use the less usual gridiron lattices [12] or curved scaffold path [11].
3. caDNAno takes that into account and shows the favorable crossovers for a selected helix with other helices in the lattice

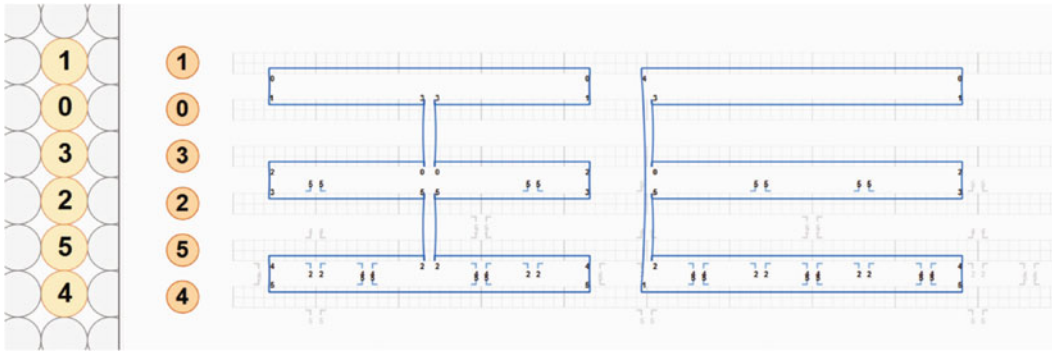

Fig. 9 Mid-seam and raster design strategies. Two blueprints proposed for rectangular DNA origami design. Blueprint on the *right* has fewer crossovers that only connect the ends of each helix with another. Blueprint on the *left* has extra crossovers that connect helices along the helix length—these crossovers are called "the seam"

as corners with numbers that show which helices are placed optimally for the crossover at that position (Fig. 7b). Crossovers are easily placed by clicking on these corners (Fig. 7c).

4. The scaffold that is used for DNA origami is usually a circular scaffold. That means that scaffold path should end where it starts. Imagine that you are designing a simple rectangular origami sheet. There are two ways that a scaffold can be wound through the structure using either raster or mid-seam design strategies (Fig. 9). The raster design proposed on the right has fewer crossovers that are only connecting the ends of each helix and includes a long crossover from helix 1 to 4 that should contain single stranded part long enough to span the length between these helices. The mid-seam design has crossovers not only placed on the ends of helices but also in between the helices. The crossovers connecting the scaffold helices inside the structure are called "the seam." The seam is another feature to consider before starting designing a DNA origami structure. The seam offers a possibility to introduce openings in the structure like the eyes and mouth of the original DNA origami smiley face [3]. The disadvantage of the seam is that it introduces breaks in the scaffold path that has to be linked with staple strands. A staple strand connecting such a break loses its long scaffold annealing region due to the break in the scaffold. The seam can be avoided by cutting the scaffold to make it linear [3]. The structure can also be designed so that it does not use or minimizes the use of the seam [30].
5. The average sequence distance between scaffold segments that are brought together by staple strands interaction is called contact order and was shown to have a big influence on self-assembly yield [31]. The maximum contact order that can be achieved depends on the design: 3D structures require smaller

contact order compared to 2D structures [3, 31]. This is especially important to have in mind when designing a structure, as introduction of the seam can reduce the contact order up to a factor of two. Consider our designs of rectangular origami sheet (Fig. 9). Both designs have the same dimensions of the structure but different contact order. Most helices are split into two parts in the mid-seam design that reduces contact order of these helices by a factor of two.

6. Another important step is the staple strand design. Staple strand design has been shown to have a great effect on self-assembly yield and stability of the structure [31, 32]. Designs where staple strands have only short 7 bp uninterrupted annealing regions on the scaffold strand do not self-assemble. Designs where staples have 11 or 12 bp stretches that anneal directly to the scaffold either do not assemble or assemble in a low yield. Designs that have the highest yields include the staple strand design where each staple has an at least 14 bp long uninterrupted region of complementarity to the scaffold and where most of the crossovers are preserved.
7. Addition of each base to a synthetic DNA oligonucleotide during chemical synthesis has a yield of 99 %. Longer oligos will result in lower quality. Therefore, staple strand lengths should be kept between 28 and 42 bp.
8. It is easier to model dynamical parts in Maya when hinge parts are not continuous—this can be done by introducing a break in the caDNAno design.
9. caDNAno2 also offers to show the position of the phosphates on the structure in the Maya window. Choose the menu: Edit > Modify mode. The black dots on the structure represent phosphate positions. These can be removed by pressing the "Modify mode" button once more.
10. Maya is commercial 3D animation software that offers a comprehensive creative feature set for 3D computer modeling, animation and simulation. As a student or educator one can get a free 3-year license.
11. Examples of DNA origami designs are found in the literature or on websites. There is currently no repository for such blueprint files, so one has either to reconstruct them in the program or ask the authors for their design files.
12. You can add molecular models of any structure from the Protein Data Bank (PDB) by using the Molecular Maya plug-in: http://www.molecularmovies.com/toolkit/. You can use the show phosphates option to find a spot where the protein can be attached on your structure.

13. In order to get the staple strand sequences for the designed structure one will need a scaffold strand sequence. Scaffold strand sequence is then imported into caDNAno using "Seq" function from the toolbox and clicking onto the scaffold strand. The scaffold strand may not have more than one break. The 5′ end of the scaffold will be a starting point of the sequence. One could use blueprint from Fig. 7e to introduce a break. Staple strand sequences will be generated automatically when the scaffold sequence is assigned. The staple strand sequences can now be exported to Excel readable csv format using "Export" function in the top menu. Every strand has a start and end coordinates for 5′ and 3′ ends respectively. The coordinates are given in the format xx[nn], where xx is a helix number and nn is a base number on the helix. One can easily trace the desired staple strand back to the blueprint using the coordinates. One can use movable ruler (Fig. 6f) for marking the right base number along the helix while helix number can be directly read (Fig. 6e). The sequence output provides the staple strand length and a specific color code. Color code can be used for sorting the sequences into separate modules for easier handling, e.g., lock strands and fluorophore strands. Staple strand sequence list can than be used for ordering oligonucleotides from a supplier of custom nucleic acids.

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