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Building DNA Brick Structures with LegoGen

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1 References

- Ke Y, Ong LL, Shih WM, Yin P (2012) Three-dimensional structures self-assembled from DNA bricks. Science 338(6111):1177–1183.

- Yoo, J. & Aksimentiev, A., 2012, Improved Parametrization of Li+, Na+, K+, and Mg2+ Ions for All-Atom Molecular Dynamics Simulations of Nucleic Acid Systems, The journal of physical chemistry letters, 3(1), pp. 45-50. http://dx.doi.org/10.1021/jz201501a

- Yoo, J., & Aksimentiev, A, 2013, In situ structure and dynamics of DNA origami determined through molecular dynamics simulations. Proceedings of the National Academy of Sciences of the United States of America, 110(50), 20099-104. doi:10.1073/pnas.1316521110

- Li, C., Hemmig, E. A., Yoo J., Hernández-Ainsa, S., Keyser, U. F. & Aksimentiev A., Ionic conductivity and structural deformation of DNA origami plates in electric field., *ACS Nano*, 2015, 9(2), pp. 1420-1433. http://dx.doi.org/10.1021/nn505825z

2 Introduction

In this tutorial, we will go through the steps for designing DNA structures using the DNA brick method. We will produce files for all-atom simulations of DNA bricks as well strand sequences for use in experimentation. DNA structures will be built out of individual cubes known as Voxels, which have a side length of ~2.5 nm, or 8 base pairs.

2.1 Definition of Commands

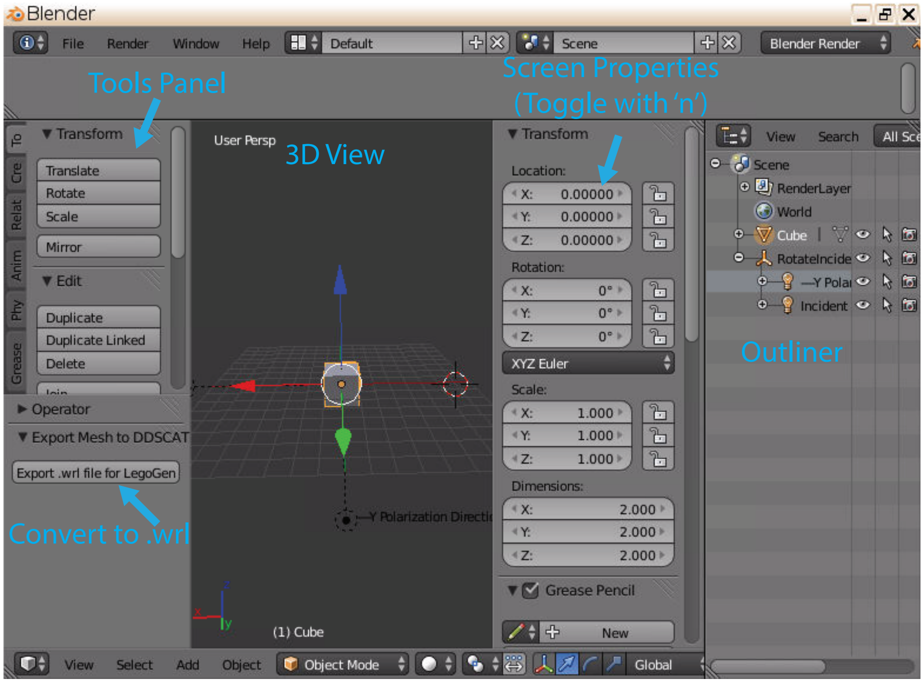
If at any time you see an icon such as Shift , that means you need to press the appropriate key on your keyboard, in this case "Shift." If you see LMB , MMB , or RMB , then you need to click the left, middle, or right mouse button respectively.

3 Building the 3D model

3.1 Preferred CAD software

We use Blender (www.blender.org) to design the initial 3D model of our DNA brick object, exported in VRML (.wrl) format. Tinkercad (www.tinkercad.com) can also be used to build CAD models exported in the VRML format, as well as any other CAD software that exports in the VRML format. Blender is integrated into LegoGen's interface, meaning no installations are required to use it with Legogen. We will discuss the use of Blender for building initial models.

3.2 Using Blender to build models



Transform

Location:
X: 0.00000
Y: 0.00000
Z: 0.00000

Rotation:
X: 0°
Y: 0°
Z: 0°

XYZ Euler

Scale:
X: 1.000
Y: 1.000
Z: 1.000

Dimensions:
X: 2.000
Y: 2.000
Z: 2.000

Figure 1: Blender Interface and Important Panels

Blender can either be downloaded through their website, the Steam distribution platform, or used inside the LegoGen tool. Keep in mind different distributions of Blender will have slightly different appearances and interfaces, Fig. 1. This manual assumes you are using the version built into the LegoGen tool.

To create structures for LegoGen, it is important to know certain rules and limits to structure design, similar to 3D printing. If regions are made too thin then DNA helices might not be properly connected to the main structure, and would not form in experiment. The main rules to avoid this are to never build structures too thin and to avoid sharp edges. Keep the depth of a structure at least 5 nm (two helices or voxels) thick, and try to use gradual shifts rather than sharp edges.

We have several tutorials on how to build structures with LegoGen, depending on your amount of skill with CAD software. If you are not very familiar with CAD software and would prefer to rapidly build using a template, several templates are provided and their use is explained in Section 3.2.1. If you would like to know how to build basic shapes such as spheres and cubes, and to convert these simple structures with LegoGen, go to Section 3.2.2. If you want to know how to build advanced structures made of numerous simple shapes, consult Section 3.2.3. If you would like a full understanding of the abilities of LegoGen, please consult all three sections. If you feel you are familiar with building models in Blender, please go ahead to Section 4 after you have made a structure.

If you have further questions about building with Blender there are numerous manuals and tutorials for it, giving many ways to build structures as you wish.

3.2.1 Template Design: Carving a Structure

Included with this tutorial are several template files in "Templates and NAMD files/blender_templates.zip", which are collections of cubes that are sufficient for use in LegoGen. These files can be uploaded to the LegoGen interface by clicking on "Download Files" on the main LegoGen screen. Upload "10voxels_shrunk.blend" and load it into Blender by clicking "3D Shape Generation", Fig. 2. You will already be in Edit mode, which can be toggled with Tab . You can drag and rotate the structure with the MMB until its in the orientation you want. To build structures using this template you'll need to carve away unneeded cubes until the intended structure is visible. Each cube represents a single voxel of the DNA brick structure, which is approximately 2.5 nm×2.5 nm×2.7 nm, with the longer helical axis being parallel to the Z-axis.

To delete individual cubes you will need to change your Select Type, which specifies what parts of the geometry you can select and modify. Choose all three Select Types (Vertex, Edge, and Face) by clicking all three while holding Shift

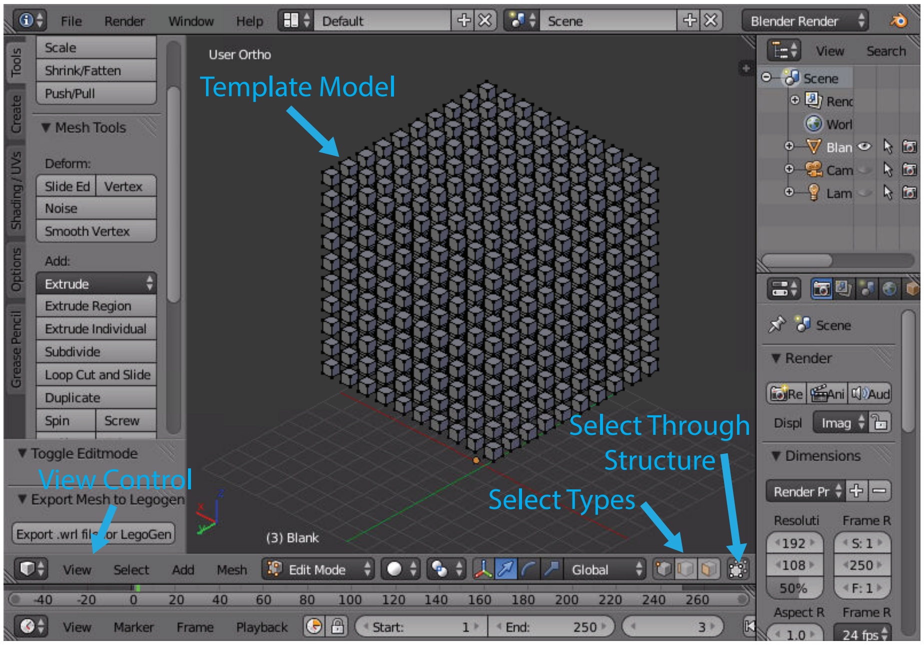


Figure 2: 10×10×10 template and important buttons for Template Design

. Now you can select all parts of the structure. To delete individual cubes you will have to enter Select mode with C . Select a cube with the LMB and it will highlight all parts of the structure inside the cursor. It is recommended to highlight at least two nonadjacent vertices or faces, but is not always necessary to remove the cube completely. If you accidentally highlight a portion you wish to preserve, you can deselect it with the MMB or by holding Shift while selecting. Once you've finished your selection delete it with Del and select "Faces" or go to "Delete" in the "Tools" tab. Your selection should be completely removed.

To carve all cubes in a specific row or column, we will need to change our selection method again. Click "Select Through Structure" which is right of the Select types. Normally you can only select from the visible portions of the structure, but now you will select all portions of the structure that are inside the circle, going through the structure. This can be very dangerous, letting you select large portions of the template without realizing it. To prevent this, change the View to the direction (Top, Front, Right, etc.) parallel to the intended row so that accidental selections won't be possible. Once you have made the intended selection, delete as before.

As you carve the shape remember the physical distances you are working with. Each cube is 2.5 nm wide and is 2.7 nm tall, so initially the template is

27×25×25 nm^3. If you intend to make a structure that has a ∼20 nm edge, you'd make it 8 cubes wide. Remember the longest dimension of your structure in terms of cubes, as you will need it in conversion. Once you have finished carving the template into the intended shape select "Export .wrl file for LegoGen." You can then exit or minimize Blender and proceed to the next step: conversion in Section 4. If you didn't dramatically change the template's edges, its longest dimension should still be 10 cubes.

3.2.2 Basic Design: Converting a Sphere

If you want to make a simple shape out of DNA, it is very easy to do. Open Blender like in 3.2.1, and you will be presented with the initial Blender window, where only a default cube exists. Delete this cube, and create a sphere by going to the Create Tab in the Tools Panel and selecting "UV Sphere." You can also select a different geometric shape if you would prefer, but we will assume you selected the sphere. You may move the sphere around but this will not change how the structure converts. Since the structure is so simple, it doesn't need any modifications, and is ready to convert to .WRL format. Select "Export .WRL file for LegoGen" and proceed to Section 4.

3.2.3 Advanced Design: Converting a Pillar

More advanced structures cannot be made automatically, and can be tedious to carve using the template method. We will make a pillar shape 40 nm tall, 28 nm thick at its base and top and 14 nm at the middle. In Blender's units, we will consider 1 unit equal to 14 nm, although any conversion ratio can be used as long as you are consistent.. The helices in the structure will be parallel to the pillar's orientation. To convert this into a DNA structure we must first determine how many voxels long the structure will be in each direction. In the helical direction, voxels are 2.7 nm thick, so the pillar will be 14.8 voxels tall, which we will round to 15. Any other direction the voxels are 2.5 nm wide, meaning the pillar's thicknesses will be 11 and 5 voxels respectively. Remember the longest of these values, 15, its orientation to the helical axis, parallel, for later.

Delete the default cube as in previous sections, and change the viewing orientation to "Front." First, we will build the base of the pillar. Create a cone from the Create tab of the Tools Panel. There will be a panel in the lower left called "Add Cone" that will let you change properties of the cone. Change its depth to .5, Radius2 to .5 and its location to (0,0,.25). Duplicate the cone with Shift-D and place it at (0,0,1.75) and rotate it 180 degrees along the Y axis. To rotate or move already generated shapes you need to open the Screen Properties panel with N . The bases of the cylinder are nearly done.

Next we will make the cylindrical portions of these bases. This is so the bases do not have sharp edges that might convert incorrectly. Create a cylinder and change its depth to .25 and location to (0,0,-.125). Either duplicate or create an identical cylinder and place it at (0,0,2.125). The bases of the pillar are complete. Create a new cylinder with different dimensions (Radius .5 and Depth 1) at (0,0,1). The pillar is now complete.

We will now join the portions of the pillar together and remove redundant faces. Select all portions of the structure by holding Shift while selecting them in the Outliner, Fig. 1. Join them together by selecting "Join" in the Tools tab of the Tools Panel. The overall shape of the structure pillar is now complete, Fig. 3A, but it is not yet ready to convert. If you attempt to voxelize the structure, like in Section 3.2.2, the structure might have large holes or potentially portions of it missing.

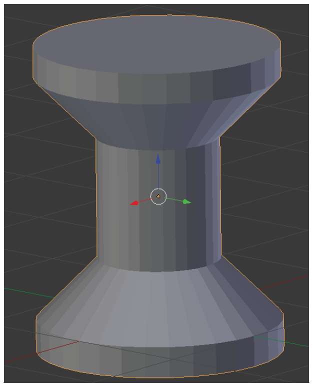
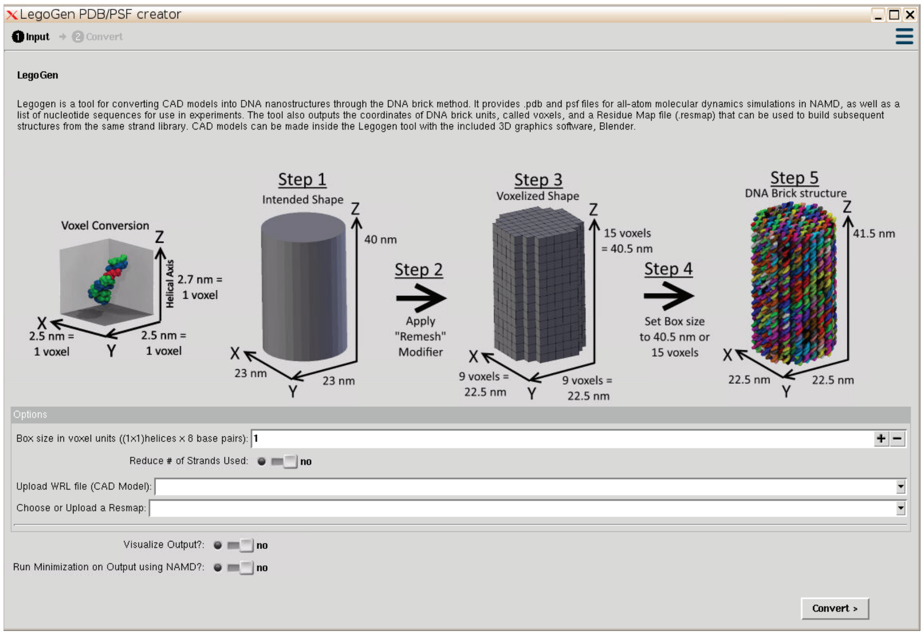


Figure 3: Completed Pillar

Enter Edit Mode and select all vertices with A . Select "Remove Doubles" in the Tools tab, and redundant vertices that were shared between adjacent shapes will be removed. However, the faces shared between shapes still exist as internal faces. These can easily be removed by going to Select in the lower right and selecting "Interior Faces." Delete what Blender selects and all interior faces have been removed. The structure can now be sent to LegoGen. Remember the distance in the longest direction, 15 voxels, and its orientation, parallel to the the helical axis, and proceed to Section 4.

4 Converting Models with LegoGen

Once your models are in WRL format they can be converted to a DNA brick model with LegoGen, Fig. 4. The only requirements are your converted WRL



LegoGen PDB/PSF creator

❶ Input → ❷ Convert

LegoGen

Legogen is a tool for converting CAD models into DNA nanostructures through the DNA brick method. It provides .pdb and psf files for all-atom molecular dynamics simulations in NAMD, as well as a list of nucleotide sequences for use in experiments. The tool also outputs the coordinates of DNA brick units, called voxels, and a Residue Map file (.resmap) that can be used to build subsequent structures from the same strand library. CAD models can be made inside the Legogen tool with the included 3D graphics software, Blender.

Options

Box size in voxel units ((1x1)helices x 8 base pairs): 1

Reduce # of Strands Used: ● no

Upload WRL file (CAD Model):

Choose or Upload a Resmap:

Visualize Output?: ● no

Run Minimization on Output using NAMD?: ● no

Figure 4: LegoGen conversion interface.

file and parameters you will enter now, with an optional residue map (resmap) file if you wish to use strands from a strand library. The important factor in conversion is the box size, which will affect both the size of our structure and how well its resolved in DNA bricks. A voxel unit is equal to 2.5 nm×2.5 nm×2.7 nm, with the longer end oriented with the DNA helix inside the voxel. Changing the box size changes the number of voxels in each direction. For example, with our initial sphere, a box size of 6 will make a sphere with approximate dimensions of 15 nm×15 nm×16.2 nm, so the sphere will be slightly longer in the helical direction. Doubling the box size will in turn double the size of the sphere in each direction. However, it is difficult to think in voxels rather than physical units of distance. To use physical units of direction we also must take the direction of the box size into account. Take the pillar from Section 3.2.3 for example. The pillar is intended to be 40 nm tall and 28 nm at its widest. The longest direction would be 40 nm, which is parallel to the helical axis. The orientation matters because the voxel is longer in the parallel orientation, meaning the pillar is 14.8 or 15 voxels tall. If it were longest in a non-helical direction, and 40 nm, then it would be exactly 16 voxels tall.

Remember from some of our earlier models that the box sizes that were used. If you used the recommended template from Section 3.2.1 and didn't change its maximum dimension, then you will have a box size of 10. For our pillar from Section 3.2.3, its 15. For the sphere from Section 3.2.2, we didn't specify a size initially, so you can scale the size as you wish. Keep in mind the maximum size for structures is 32 voxels, or 80 nm. Enter in the appropriate box size for your structure.

Next we can select whether to produce a blunt structure or a strand minimized structure. In a blunt structure, all DNA will be double-stranded, and 8 nt strands will be generated. This will produce a lot of strands, but there will be no exposed nucleotides. In a strand minimized structure, no 8 nt strands will be produced and additional connections will be made to make the average strand length at the edges longer. The structure will also have a buffer of 8 nt fragments of thymine on end strands, making the structure dimensions potentially larger than expected. Select the version of the structure you would prefer.

Next we will select our CAD model file. If your model was converted to WRL through the built-in version of Blender, select "Blender File" as the model to use. If bringing in a file externally, select "Upload." If you would like an example structure built, there is one available of Link from *The Legend of Zelda*. This model looks best at a box size of 16–32, although it is likely too complex to be able to be created practically.

Lastly, we can select a residue file, or resmap. Residue maps record the positions of nucleotides in the structure, allowing for different structures to share sequences if the same resmap is used. If you have one you may upload it now. If you don't there are two example residue maps, or if you select "Don't use a residue map" one will be generated for you.

You can switch "Visualize Output" to yes to get a glimpse of your produced structure in VMD before it saves. This will let you see if the structure was what you intended. If it isn't, you can easily go back to Blender and modify it as you please. If your intent is to use LegoGen for simulations, the structures can be minimized to streamline that step of simulation. Flip the "Run Minimization" switch to Yes to add this to conversion.

Once all files and inputs are selected, press "Convert" to start. The file will be automatically converted into DNA bricks, and can be downloaded afterwards in a compressed format. A list of sequences and the residue map used are also provided. Your structure is now ready to be created in a DNA self-assembly reaction or simulated in NAMD. The remaining portions of this section deal with specific advanced topics of conversion. If you are going to need to read the sequence list, go to 4.3. If you have built the structure you intended and wish to simulate your structure in NAMD, go to 5.

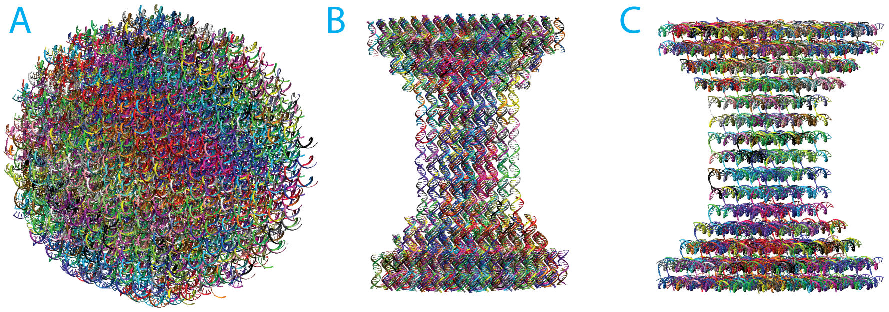


Figure 5: Converted Structures using Blunt Method. A. Converted Sphere. B. Converted pillar. C. Converted pillar with an extra rotation.

4.1 Importance of Model Orientation

It is important to keep track of the effects of orientation of the model on conversion. The sphere was largely independent of orientation, and converted to a generally spherical shape made of DNA strands, Fig. 5A. For the more complex pillar, there are notable effects from orientation. Depending on the orientation, the pillar's overall length might change. For the pillar exactly as it is in the tutorial, the helices are oriented to the direction of the pillar, with the heads of the DNA bricks oriented towards the base of the pillar, Fig. 5B. When the model was rotated 90 degrees in the Y direction, the structure looks visibly different, Fig. 5C. The structure is slightly shorter and stretched in the direction of the helices, caused by the fact that the voxels are slightly longer in the helical axis. Keep in mind these differences in orientation might cause your structure to be drastically different both in appearance and mechanics. The two pillars, while having the same overall shape, would behave differently when in tensile or compressive stress. Also keep in mind that in some CAD programs, the definition of orientations can be different, so if you have a preferred CAD software keep this in mind when converting.

4.2 Use of a Residue Mapping File and Preserving Sequences

A residue map preserves the sequences of strands associated with specific voxels By making minor changes that do not change the overall dimensions or number of voxels used, it is possible to reuse the same sequences for different structures, preserving the core structure. This can be of use if, for example, several cubical structures were designed with the same core shape, Fig. 6A, but each had different extensions or internal changes without changing the overall shape, then

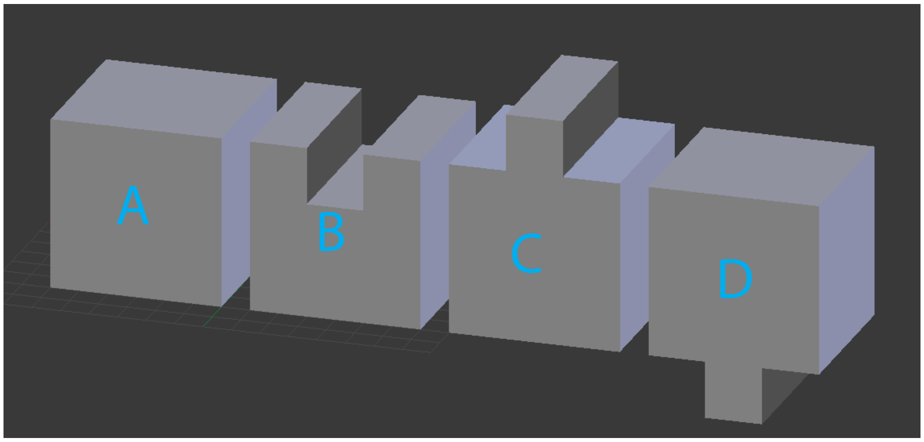


Figure 6: Representative cubes with different bounding voxel amounts. A. 3 voxels. B. 3 voxels. C. 4 voxels, due to the addition, but no shift in core sequences. D. 4 voxels, but a shift in core sequences

the unchanged strands would be identical between structures. Keep in mind this is dependent on several factors. If a structure is changed by the removal of sections, Fig. 6B, then there is no change to the overall mapping of strands and the strands in remaining sections will be identical If a structure has a large extension is added to it, the voxel box size will need to be changed and the unchanged regions may not be identical. It depends largely on the direction the addition is made. If the direction is made in the negative X direction or the positive Y or Z direction in Blender, then the overall structure will not be dramatically shifted and strand sequences will be identical, Fig. 6C. If it is in the opposite directions then there will be some differences in strands, Fig. 6D.

Residue map files can be changed directly if specific nucleotides are intended for selected regions of a structure. Residue map files are a list of coordinates and the nucleotide associated with those coordinates. The first 3 coordinates correspond to the voxel location, the fifth is the base pair in the nucleotide chain of the helix in that voxel, with 0 being the base pair closest to the negative-X side of the voxel and 7 being the base pair closest to the positive-X side. The fourth location is which of the nucleotides in that base pair is selected, with 0 being the nucleotide with its 3' end pointed to the negative-X side of the voxel and 1 being the nucleotide with its 3' end to the positive-X side of the voxel.

4.3 Understanding Sequence Output

The three outputs of the conversion will be the PDB and PSF files for use in simulations, the residue map file for future conversions, and a list of sequences used. The list of sequences per strand are paired with a sequence coordinate (0001, 0002, 0003, etc.), and a similar looking serial number (00019, 0002F, 000311, etc.). The sequence coordinate is that strands specific position in the structure. The first three are its coordinates in voxels and the last it is strand number. These can be specifically selected for viewing by opening the PDB and PSF files in VMD and selecting them with "segname '<coordinates>'" in Graphical Representation. The single quotation marks around the coordinates are important, as a coordinate of "0001" might be read as the number "1." The single quotation marks preserve your selection as a string. In structures with minimal differences, strands with identical coordinates should have identical or similar sequences, but to verify this we look at the serial number. If a structure's dimensions have been shifted, as warned about in the previous section, then there is a chance they will not be comparable. Be careful when building structures to avoid this if the goal is to preserve certain sequences.

The serial number, the second column, shares the first 3 digits with the sequence coordinate, but the rest will be unique to that strand. If two strands in a structure have the same serial number, they will have the same sequence. This lets you maintain a library of strands by their serial numbers, rather than having to look by sequence. This can also help you verify that your structure is as intended, and you didn't accidentally shift the structure as warned, as most of your serial numbers should still be the same. Once you've converted your structure, if you already have a library of strands defined by serial number, you can simply select them from the list and proceed with experiments! If you are more interested in simulation, please proceed to Section 5 below.

5 Preparation for simulations in NAMD

Now that our DNA structure has been generated, it needs to be prepared for simulation through several steps. Elastic constraints, minimization, and the addition of water and ions to the system are just some of the required steps. We will first apply constraints to the DNA bases for use in minimization and initial equilibration. We will assume you are using the structure generated from the Basic Design tutorial, and will refer to our PSF and PDB files as `sphere.pdb` and `sphere.psf`, respectively.

5.0.1 Elastic network model (ENM) constraints

Because we want to optimize only the DNA backbone conformations, we need to apply restraints to protect the DNA base conformations. In this tutorial, we add inter-nucleotide harmonic distance constraints between base heavy atoms within 8 Å using extrabonds functions in the NAMD package. See NAMD manual for details of extrabonds (e.g., `http://www.ks.uiuc.edu/Research/namd/2.7/ug/node26.html`). Because this approach resembles the elastic network model (ENM), we term this the ENM constraint. We provided a perl script (`pdb2enm.pl`) for generating extrabonds between base heavy atom pairs. The following command will take `sphere.pdb` as an input to generate `sphere.enm.extra` file that contains extrabonds entries. Note that we used a cutoff of 8 Å and a force constant of 0.5 kcal/mol/Å^2, which can be changed depending on the input system.

```
perl pdb2enm.pl --namd --k=0.5 --cut=8 sphere.pdb > sphere.enm.extra
```

Or using a tcl version (this may take a few minutes):

```
vmd -dispdev text -e pdb2enm.tcl -args 0.5 8 sphere.pdb sphere.enm.extra
```

5.0.2 Energy minimization using NAMD

Now we are ready to perform energy minimization using the prepared files. If you already minimized inside the LegoGen tool you may skip this step. We provided a sample NAMD input configuration file (`mini.namd`) that can be used to optimize the DNA backbone conformation. The extrabonds function are invoked by the following lines in the `mini.namd`.

```
extraBonds on
extraBondsFile sphere.enm.extra
```

Run NAMD by typing the following command on the terminal (this may take a few minutes):

```
namd2 mini.namd > mini.log &
```

This simply performs an energy minimization for 1,000 steps and writes the final conformation to a file named `sphere.mini.coor`. One can confirm that crossover conformations are optimized by loading the resulting coordinate file to VMD. To save the final conformation as a new PDB, `sphere_mini.pdb`, run the following line:

```
vmd -dispdev text -e mini_saveLast.tcl
```

5.1 Insertion of Mg-hexahydrate

Next, we would neutralize the DNA brick system with Mg-hexahydrate (MGHH) molecules. Mg parameters of the standard CHARMM force field result in artificially condensed phase of DNA as demonstrated in our reference (Yoo and Aksimentiev, JPCL, 2012). Thus, an improved model of Mg in the form of Mg-hexahydrate is essential for DNA structures as demonstrated in our reference (Yoo and Aksimentiev, PNAS, 2013).

Because Mg-hexahydrate molecules diffuse slowly, if we place them far from the DNA bricks, we would spend a lot of time waiting for Mg-hexahydrate molecules to diffuse into the DNA brick structure. So, we would use a customized script to place Mg-hexahydrate molecules close to the DNA brick strands. Run the following command in the terminal:

```
vmd -dispdev text -e add_mgh_ver2_3.tcl -args sphere_mini
sphere_MGHH 516
```

The first argument “sphere_mini” is the prefix for the input psf and pdb files, which you can change to “sphere” if you minimized inside LegoGen. The second argument “sphere_MGHH” is the prefix for the output psf and pdb files. The last argument “516” is the number of Mg-hexahydrate molecules to be added, which should be adjusted depending on the size of the final simulation box and the desired concentration. If you intend to neutralize keep in mind each strand will have a charge equal to the number of nucleotides plus 1.

5.2 Solvation

In reality, DNA brick structures are always prepared in solution. So, to simulate the sphere in a experimental condition, we need to put the sphere in solution. The `solIon.tcl` is used for solvating the system in water and adding Cl^- ions. Running the following command

```
vmd -dispdev text -e solIon.tcl
```

would produce the final solvated sphere system (`sphere_MGHH_WI.psf` and `sphere_MGHH_WI.pdb`). Note that the number of monovalent ions can be adjusted by modifying `solIon.tcl`.

5.3 Extrabonds for MGHH stability

Usually Mg-hexahydrate structure is so strong that the structure is stable with no constraints in a normal condition. However, the MGHH structure can be easily broken at the beginning of the DNA brick simulation because of large Coulombic forces between DNA and Mg due to charge imbalance. Thus, we add `extrabonds` constraints between Mg and water oxygen in MGHH to prevent undesired dissociation of MGHH. `mghh_extrabonds.pl` script will generate extrabonds definitions for NAMD.

```
perl mghh_extrabonds.pl sphere_MGHH_WI.pdb > mghh_extrabonds
```

Or, you can use the tcl version:

```
tclsh mghh_extrabonds.tcl sphere_MGHH_WI.pdb
> mghh_extrabonds
```

In NAMD script, the following lines are needed.

```
extraBonds on
extraBondsFile ./mghh_extrabonds
```

5.4 Equilibration using molecular dynamics

Equilibrating the DNA brick system is not a trivial task. There are two main factors which could break local DNA brick structure. One of them is the electrostatic repelling force between the backbones of the DNA strands. The other is the torsion at the heads of the DNA bricks. To avoid breaking the base-pairs while relaxing the global structure, we simulate the sphere with ENM constraints and gradually reduce the force constant. The following protocol will simulate the sphere with ENM constraints with k = 0.5, 0.1, 0.01 and 0 (kcal/mol/Å^2) continuously. Each simulation is 4.8 ns. Although this protocol applies to most small- to medium-sized DNA structures, the users may need to modify it for their own brick structures, especially larger structures at the limits of LegoGen's box size. First, run the following command to generate ENM extrabonds files with 3 different force constants:

```
./mk_extra.sh
```

For k = 0, we simply turn off the ENM constraints.

Then, run the simulations one after another:

```
namd2 equil_k0.5.namd > equil_k0.5.log &
```

```
namd2 equil_k0.1.namd > equil_k0.1.log &
```

```
namd2 equil_k0.01.namd > equil_k0.01.log &
```

```
namd2 equil_k0.namd > equil_k0.log &
```

Once your structures have equilibrated through all steps you are ready to proceed with any other simulations you intend to do. Look at the trajectory to make sure the structure has relaxed as intended, and that the MGHH molecules have penetrated the DNA brick structure.

6 Troubleshooting

6.1 When equilibration simulations crash

At the beginning, the system can be unstable. You need to check total energy. If total energy looks fine and your system is still unstable, you might try:

- Decreasing the timestep
- Turn off pressure coupling; do NVT.

Somehow both genbox and solvate put significantly less number of water in a box, resulting in bubbles in the box after equilibration in NVT.

Solutions:

1. Resolvation after equilibration.
2. NPT with DNA fixed.

When a DNA brick object is large, the number of inserted water molecules can be underestimated, causing a bubble after a short equilibration. Bubbles of moderate size can be removed by simply equilibrating under NPT ensemble. Sometimes large bubbles can cause abrupt change in box size, resulting in an instability of MD simulations. In this case, increasing langevinPistonPeriod and langevinPistonDecay by a factor of 10 can be helpful. By changing these options, we make changes in box size less responsive to the internal pressure to make the MD simulation more stable. After the box size becomes stabilized, those options can be reverted to the default values.