

Molecular Dynamics Simulations of Self-Assembled DNA Nanostructures

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1. Introduction

DNA self-assembly permits fabrication of nanoscale objects using tools of molecular biology. Incepted in 1980s, the field of structured DNA nanotechnology has grown dramatically in recent years fueled by demonstration of the DNA origami method and the development of the DNA origami design tools. While DNA origami remains the most popular framework for designing self-assembled DNA nanostructures, several alternative design strategies have been recently proposed and realized. Complementing development of these new self-assembly methods, several computer programs have been written to aid the design of new type of structures. In parallel to development of the structure design tool, a number of physics-based simulation approaches has been applied to study self-assembled DNA objects. One such approach is the all-atom molecular dynamics (MD) method that can characterize the structural and physical properties of DNA objects with atomic precision.

Building on our previous work that introduced the application of the all-atom molecular dynamics method to DNA origami objects, here we describe how all-atom MD simulation can be performed on structures built using the most popular design and simulation tools, including CanDo, Nucleic acid builder (NAB), oxDNA, Nanoengineer, vHelix, DAEDALUS and Tiamat. Specifically, we describe a collection of protocols for converting the structures designed or modeled using the above tools to all-atom representation suitable for performing all-atom MD runs. To generate all input parameters and topology files required to perform MD runs, we will be using extensively the *enrgmd* toolkit, which is a part of the *mrDNA* package recently developed in our lab and described in detail in the [Multi-Resolution DNA simulations tutorial](#).

This tutorial is organized as following. Section 2 describes the files and codes required to through this tutorial. Table 1 lists the software packages supported by this tutorial and points the reader to respective sections of the tutorial describing the use of the specific software package. Each section from 3.1A-3.1G can be done independently from one another and does not rely on

the output from the previous section. However, we strongly recommend going through our [DNA origami tutorial](#) first before attempting to complete this tutorial.

2 Materials

2.1 Software

This tutorial is an extension of our previous [DNA origami tutorial](#) that describes all steps necessary to perform an all-atom MD simulation of a hexagonal DNA bundle starting from its caDNAno design. Here, we expect that the users have completed our previous [DNA origami tutorial](#) before proceeding to this tutorial. We also assume that the readers already have access to software packages caDNAno, NAMD, VMD and Perl (described in the origami tutorial), which are necessary for performing MD simulation of DNA structures. Specific to this tutorial, the readers are asked to install the following two additional packages:

- 1. Python3 package.** User will be required to install the python3 programming environment. Anaconda distribution will be probably the easiest way to set python3.
- 2. mrDNA** Multi-resolution DNA (mrDNA) package is a utility toolkit for the modelling and simulation of DNA nanostructures. In order to run mrDNA, you will need a working python3 installed. Users can download/clone the toolkit from our [mrDNA git hub](#).

Shell > **git clone** <https://gitlab.engr.illinois.edu/tbgl/tools/mrdna.git>

Apart from that, this tutorial assumed that the reader has access to the software listed in the first column of Table 1.

Table 1: Simulation and design tools supported by this tutorial

Package Name	Website	Software Required (Optional)	Section
caDNAno	cadnano.org	Python3, mrDNA, (caDNAno)	3.1A
CanDO	cando-dna-origami.org	Python3, mrDNA	3.1B
NAB	casegroup.rutgers.edu/casegr-sh-2.2.html	Python3, mrDNA, (AMBERTOOLS)	3.1C
oxDNA	dna.physics.ox.ac.uk	Python3, mrDNA (oxDNA)	3.1D
Nanoengineer	github.com/kanzure/nanoengineer	Python3, mrDNA, (Nanoengineer)	3.1E
v-Helix	vhelix.net/	Python3, mrDNA, (Autodesk Maya)	3.1F
DAEDALUS	daedalus-dna-origami.org	Python3, mrDNA	3.1G
Tiamat*	yanlab.asu.edu/Resources.html	Python3, mrDNA (Tiamat)	3.1H

*Follow similar set of instruction as for given for CanDO i.e. section §3.1B

2.2 Required Files.

1. origamiTutorial package. It is assumed that the readers have access to the files from the [DNA origami tutorial](#). Please download and extract the folder in the file system of your computer. The folder name is origamiTutorial.

2. universal-all-atomTutorial Files. The output of all-atom pdb files obtained from various software packages are given in the folder named universal-all-atomTutorial. These files will be used in this tutorial and users can download the folder from this link <https://drive.google.com/drive/folders/193nAIw9ZSJ9at15wVCa1KCJuOotipejz>. Save the folder in the same directory where you have kept the origamiTutorial files.

3. Method

Our previous [DNA origami tutorial](#) explains the method of performing all-atom MD simulations with hexagonal DNA bundle from caDNAno in explicit water and ions. Here we describe protocols for preparing atomistic models of DNA nanostructure from the output of various other softwares. If you wish to design a DNA origami system from [caDNAno](#), go to section §3.1A. If you are using output structures from [CanDo](#), please go to section §3.1B. For proceeding with the output pdb of [nucleic acid builder \(NAB\)](#) please go to section §3.1C. For the output of [oxDNA](#), please go to section §3.1D. If you are using the structure from [Nanoengineer](#), please go to section §3.1E. For the output of [vHelix](#) please go to section §3.1F. If you are using the output pdb from [DAEDALUS](#), please go to section §3.1G. In these sections, we describe methods for converting an abstract design of a DNA origami system obtained from the given softwares to simulation files required for their atomistic MD simulations. Using these simulation files, the users can follow from the sections §3.2 onwards in our previous [DNA origami tutorial](#) to perform standard MD simulation of custom shaped DNA nanostructures.

3.1A All-atom model from caDNAno design using enrgmd

In our previous DNA origami tutorial, we have converted a caDNAno design to an all-atom structure using our online webserver. Here we introduce an alternative method, *enrgmd* tool-kit which will convert the caDNAno output json file to an all-atom pdb structure without using the webserver. The acronym *enrgmd* stands for *elastic network guided molecular dynamics* which has been described in detail in our previous [NAR article](#). We assume that the user has generated the caDNAno design from following our previous [DNA origami tutorial](#). Figure 1 shows a screenshot of the caDNAno interface. Here, we have chosen a design of hexagonal DNA origami bundle (**hextube.json**) which is given in the **universal-all-atomTutorial** folder.

1. Go to the **cadnano** subdirectory of the folder **universal-all-atomTutorial**
Shell > **cd universal-all-atomTutorial/cadnano**
2. You will find the hextube.json file in this directory which we have created from the caDNAno interface. User can also create their own design using caDNAno.
3. Launch the enrgmd tool-kit with the following commands to get the all-atom structure and topology
Shell > **enrgmd hextube.json -o hextube**

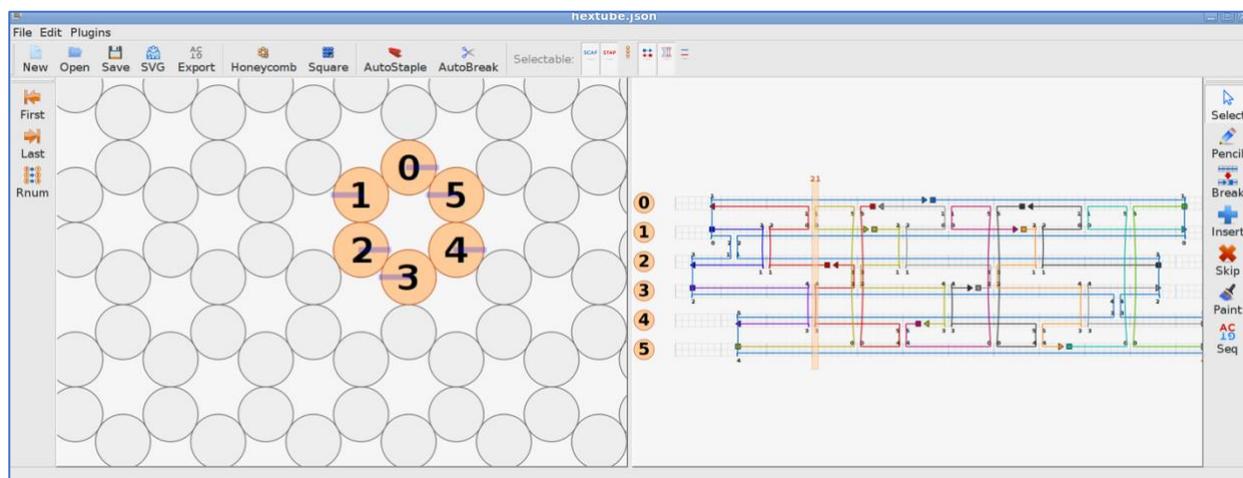


Figure 1: A snapshots of caDNAno interface showing the design of hexagonal DNA bundle.

This will create the following files:

- hextube.psf: The CHARMM format PSF file.
- hextube.pdb: The PDB format file of the all-atom structure.
- hextube.namd: The NAMD input file for the structure optimization simulations.
- hextube.exb: Extrabonds restraints for structure optimization.
- charmm36.nbfix: CHARMM36 force field files.

To perform the simulation and analysis of the obtained structure, proceed to section §3.2 of our previous [DNA origami tutorial](#).

3.1B All-atom simulation of DNA Nanostructures starting from the output of CanDo

Computer added engineering for DNA origami ([CanDo](#)) is a webserver which predict 3D structure of DNA origami structures using a finite-element based modeling approach. It can read the input design from *caDNAno*, *Tiamat* and *CanDo* file format and the output structure can be exported to multi-model files formats including a pdb file. Here we describe a procedure to create the necessary topology and parameter files so that the output pdb can be used for all atom MD simulations.

1. For this tutorial, we have already copied an atomistic pdb file from CanDo server, (**nextube_multimodel.pdb**) along with the caDNAno design and sequence file

(**hextube.json**, **hextube.csv**) inside sub-folder **cando** of the main folder **universal-all-atomTutorial**. In order to obtain the atomistic pdb from CanDo server for their own designs, users have to register to an online account using the webportal of CanDo at cando-dna-origami.org/registration. Next you load the caDNAno design (**hextube.json**) and sequence file (**hextube.csv**) of hexagonal DNA bundle to the online submission server of *CanDo*. A screenshot of the webportal is given in figure 2A. In the online server choose, Model resolution “fine”, Lattice type “Honeycomb” and also check the box “Yes” against “Would you like the atomistic model included” section. After submitting the files to this server, you will receive a link to download all-atom pdb structure (**hextube_multimodel.pdb**) and the images of the structure (figure 2B) to your registered email address. Download and extract the hextube_atomic.zip file and save it to your favorite location.

2. Go to the **cando** sub-directory inside main **universal-all-atomTutorial** directory. Copy the downloaded pdb file in to this directory.

Shell > **cd universal-all-atomTutorial/cando**

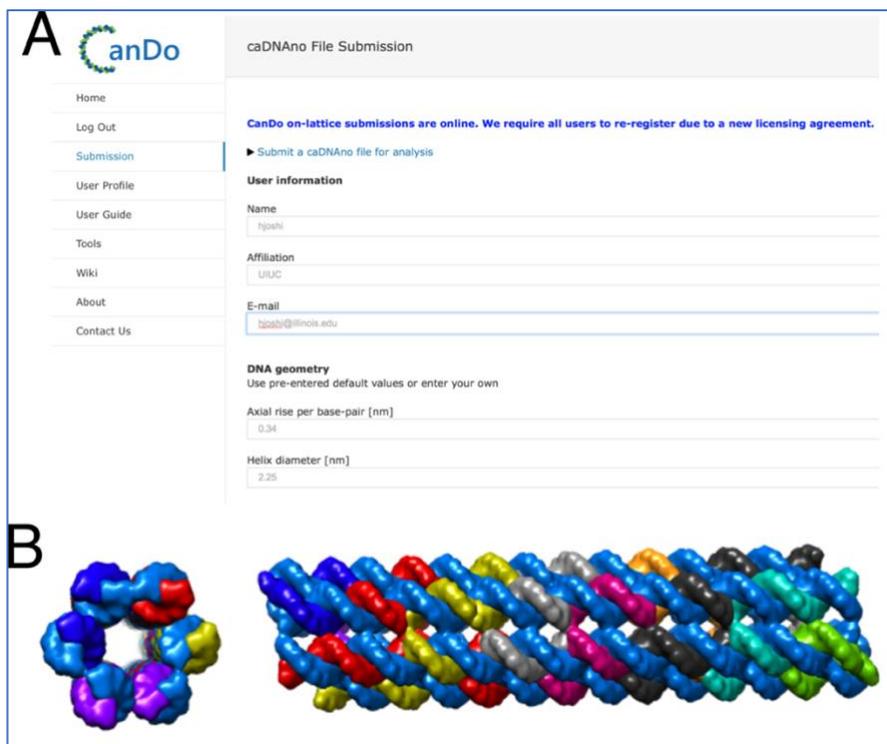


Figure 2. (A) A screenshot of the CanDo online file submission server. (B) The top view (left) and side view (right) of the structure obtained from the CanDo interface.

3. Use your favorite text editor to remove the redundant lines from the obtained pdb file. Basically, you need to delete all the line which **do not** start with “ATOM”. Here, we use *sed* command in a unix machine to do so as follows,

```
Shell > sed -i '/END/d' hextube_multimodel.pdb
```

```
Shell > sed -i '/MODEL/d' hextube_multimodel.pdb
```

4. Next, we use the *enrgmd* toolkit from the *mrdna* package to generate the necessary files to run the simulation. Type the following commands in the terminal

```
Shell> enrgmd hextube_multimodel.pdb -o hextube
```

The toolkit will create the following files

- hextube.psf: The CHARMM format PSF file.
- hextube.pdb: The PDB format file of the all-atom structure.
- hextube.namd: The NAMD input file for the structure optimization simulations.
- hextube.exb: Extrabonds restraints for structure optimization.
- charmm36.nbfix: CHARMM36 force field files.

Proceed to section § 3.2 of our previous [DNA origami tutorial](#) to continue with the MD simulation of these structure using NAMD.

3.1C CHARMM simulation files for DNA nanostructures generated using NAB

[Nucleic Acid builder \(NAB\)](#), a part of AMBERTools package, is a higher-level programming environment to create atomistic models of unusual nucleic acid structures. It is particularly useful to create atomistic model of custom shaped DNA nanostructures. To give an example, we have created a hexagonal DNA bundle using a sample NAB code *hextube.nab* given in **nab** sub-folder of the main directory **universal-all-atomTutorial**. If you want to create your own code to generate a custom shape DNA nanostructure, please go through the NAB manual in [AMBERTOOLS](#). In case you do not have AMBERTOOLS installed you can skip step 1 and 2 and proceed to step 3 with the pdb **hextube_nab.pdb** obtained from NAB and already available in **nab** sub-folder of the main directory **universal-all-atomTutorial**.

1. Go to **nab** subdirectory of the **universal-all-atomTutorial** directory.

```
Shell > cd universal-all-atomTutorial/nab
```

2. To obtain a pdb structure from the nab code, install AMBERTOOLS and type the following commands in terminal.

```
Shell > nab hextube.nab -o hextube.o
```

```
Shell> ./hextube.o
```

3. Next, using the **enrgmd** toolkit we generate the CHARMM simulation files for this atomistic pdb structure **hextube_nab.pdb** provided in the folder step1. The steps are given below and similar to what we followed for the CanDo pdb design in section § 3.1B.

```
Shell > enrgmd hextube_nab.pdb -o hextube
```

```
m_temp=copymolecule (m1);
mat_x = newtransform (0.,20.,0.,0.,0.,0.);
transformmol(mat_x,m_temp,NULL);
putpdb ("dna1.pdb",m_temp);
mergestr(m_final,"S","last",m_temp,"A","first");

m_temp=copymolecule (m1);
mat_x = newtransform (17.32,-10.,0.,0.,0.,0.);
transformmol(mat_x,m_temp,NULL);
putpdb ("dna3.pdb",m_temp);
mergestr(m_final,"B","last",m_temp,"A","first");

m_temp=copymolecule (m1);
mat_x = newtransform (-17.32,-10.,0.,0.,0.,0.);
transformmol(mat_x,m_temp,NULL);
mergestr(m_final,"C","last",m_temp,"A","first");
putpdb ("dna5.pdb",m_temp);

freemolecule (m_temp);
freemolecule (m1);
m1=newmolecule();
addstrand (m1,"A");
```

Figure 3. A snapshot of a small portion of the NAB code to generate DNA nanostructures.

The toolkit will create the following files

- hextube.psf: The CHARMM format PSF file.
- hextube.pdb: The PDB format file of the all-atom structure.
- hextube.namd: The NAMD input file for the structure optimization simulations.
- hextube.exb: Extrabonds restraints for structure optimization.
- charmm36.nbf: CHARMM36 force field files.

To proceed with the all-atom MD simulation of the structure using NAMD from these files, follow section § 3.2 of our previous [DNA origami tutorial](#).

3.1D Atomistic simulation files for coarse-grained oxDNA structures.

[oxDNA](#) is a recently developed coarse-grained DNA model which is relevant for simulating self-assembly and studying the thermodynamic properties of DNA based nanostructures. oxDNA treats DNA as a string of rigid nucleotides, which interact via some basic potentials such as backbone bonding, excluded volume, hydrogen bonding etc. Here we show how to perform all-atom MD simulation starting from the coarse-grained structure obtained from oxDNA. This utility can be particularly useful if one wants to run the atomistic simulation after performing coarse-grained oxDNA simulation.

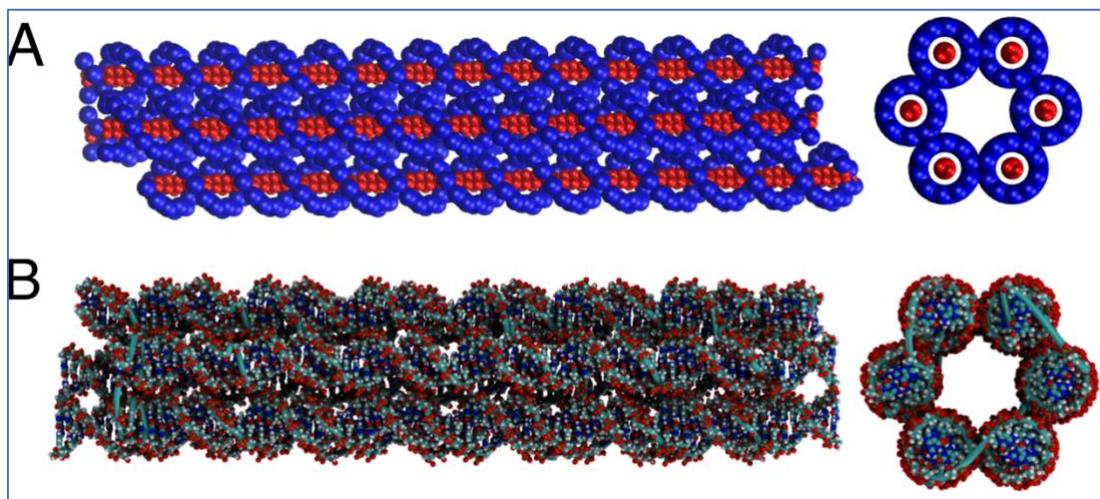


Figure 4. oxDNA to all-atom conversion of the hexagonal DNA bundle obtained from caDNAno design. (A) The coarse-grain oxDNA model of hexagonal DNA bundle (B) The corresponding all-atom structure obtained from **enrgmd** tool-kit. The right hand side panel shows the top view of the respective model.

We use the python utility codes provided in oxDNA package to obtain an atomistic pdb structure from the coarse-grained oxDNA topology and coordinate file. For the purpose of this tutorial, the input caDNAno file (*hextube.json*) and the obtained atomistic structure (*hextube_oxdna.pdb*) are provided inside the sub-folder **oxdna** in main folder **universal-all-atomTutorial/**. We have also

copied a folder UTILS inside the **oxDNA** subdirectory. This folder contains the python codes from oxDNA to perform the various inter file conversions as described below. If you have coarse-grained oxDNA simulation files from oxDNA skip step1 &2 and follow the step 3 with your custom topology and configuration files.

1. Go to the oxDNA sub-directory in universal-all-atomTutorial directory

```
Shell > cd universal-all-atomTutorial/oxdna
```

2. Run the python code **cadnano_interface.py** to obtain the oxDNA files from caDNAnano design as follows,

```
Shell> python2 UTILS/cadnano_interface.py hextube.json he 200
```

3. Step1 will give you the oxDNA topology (prova.top) and configuration (prova.conf) files. These coarse-grained model files can be converted into xyz using the python code as follows

```
Shell > python2 UTILS/traj2vis.py xyz prova.conf prova.top
```

It will create a xyz file “prova.conf.xyz” which can be loaded and visualized in vmd as shown in figure 4A.

```
Shell > vmd prova.conf.xyz
```

4. Run another python code named as **convert_to_atomic.py** to obtain a pdb structure from the oxDNA coarse grained simulation files as follows,

```
Shell> python2 UTILS/convert_to_atomic.py UTILS/dd12.pdb prova.conf prova.top> \  
hextube_oxdna.pdb
```

5. Next, run our *enrgmd* toolkit to create the input topology and parameter files as follows

```
Shell > enrgmd hextube_oxdna.pdb -o hextube
```

The toolkit will create the following files

- hextube.psf: The CHARMM format PSF file.
- hextube.pdb: The PDB format file of the all-atom structure.
- hextube.namd: The NAMD input file for the structure optimization simulations.
- hextube.exb: Exrabonds restraints for structure optimization.
- charmm36.nbfix: CHARMM36 force field files.

Figure 4B shows the all-atom structure of DNA bundle obtained from oxDNA models. Proceed to section § 3.2 of our previous [DNA origami tutorial](#) to continue with the MD simulation of the structure using NAMD.

3.1E Creating atomistic DNA nanostructure from Nanoengineer.

Nanoengineer is multi-scale modeling and simulation program with special support for structural DNA nanotechnology. The program creates the coarse-grained model of DNA nanostructure (mmp file). Figure 5A shows a snapshot of nanoengineer window. Here is the link to a [video tutorial](#) from our lab explaining the building of DNA motifs in nanoengineer.

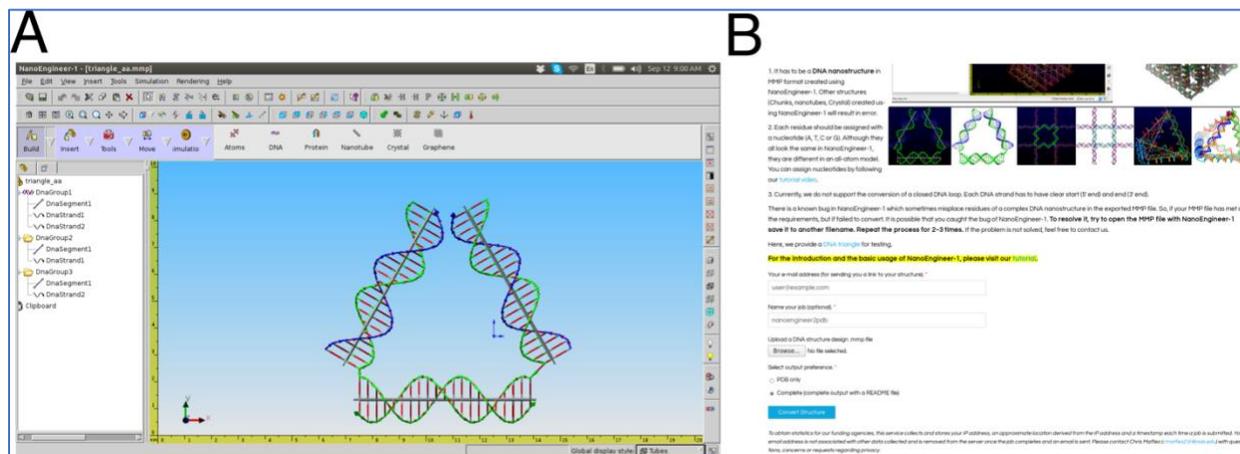


Figure 5. (A) The snapshot of a session of Nanoengineer1 interface. (B) A snapshot of our online server to convert the mmp files into atomistic structures.

Here, we have created a box of DNA in nanoengineer, the output file *box.mmp* is kept under the nanoengineer sub-directory of the universal-all-atomTutorial directory. Previously, we have provided a [webserver](#) for converting the output of Nanoengineer to an all atom structure. To generate the CHARMM atomistic simulation files to run NAMD, follow the steps given below

1. Open a web browser. Any web browser will work.
2. Open the website for converter file. Using you favorite browser, navigate to our [webserver](http://bionano.physics.illinois.edu/nanoengineer2pdb) (<http://bionano.physics.illinois.edu/nanoengineer2pdb>). An example of the web server session is shown in figure. 5B.
3. Provide your email address to receive the link to download the structure (optional).
4. Name the job. Type in the job title box.
5. Upload the mmp file. Click “Choose file” and select **box.mmp**.
6. Select the output preference to “Complete”.

7. Create simulation files. Clicking “Convert Structure” will initiate the conversion process, which will generate a download link upon completion.

Download the box.tar.gz file and extract it. The folder will contain several files including the following ones.

- a. box.psf: The CHARMM format PSF file.
- b. box.pdb: The PDB format file of the all-atom structure.

Copy the box.pdb file to the **nanoengineer** sub-folder of the main directory **universal-all-atomTutorial**. For the purpose of this tutorial, we have already copied the **box.pdb** in this folder. We can then use **enrgmd** toolkit to generate the simulation files in a similar way as we have generated in the previous sections § 3.1A-C.

1. Go to the sub-directory “nanoengineer” in the main directory universal-all-atomTutorial.

```
Shell > cd universal-all-atomTutorial/nanoengineer
```

2. Use the enrgmd tool-kit to generate the necessary files as follows,

```
Shell > enrgmd box.pdb -o box
```

This toolkit will create the following files

- box.psf: The CHARMM format PSF file.
- box.pdb: The PDB format file of the all-atom structure.
- box.namd: The NAMD input file for the structure optimization simulations.
- box.exb: Extrabonds restraints for structure optimization.
- charmm36.nbf: CHARMM36 force field files.

User can perform all-atom MD simulation in NAMD using these files in a similar way as described in section § 3.2 of our previous [DNA origami tutorial](#).

3.1F Creating atomistic structure from Vhelix design.

[vHelix](#) is a graphical tool to design complex DNA nanostructures. It is available as a plugin to Autodesk Maya software. The Högberg group at Karolinska Institutet Sweden has designed and experimentally synthesized many interesting structures using the vHelix software. The output of vHelix, a maya file can be read by our enrgmd toolkit to create the atomistic coordinate and topology file for MD simulations. For an example, we have provided the famous bunny structure

in the sub-directory **vhelix** in the main directory **universal-all-atomTutorial**. If you have “Autodesk Maya” software installed with vHelix plugin, you can load the bunny.ma structure in the and visualize/edit the design as shown in figure 6 A. Use the following step to generate the all-atom simulation files

1. Go to sub-directory **vhelix** of the main directory **universal-all-atomTutorial**.

Shell > **cd universal-all-atomTutorial/vhelix**

2. Run the energy md toolkit with the given maya file (bunny.ma) as follows

Shell> **enrgmd bunny.ma -o bunny**

The toolkit will create the following files

- bunny.psf: The CHARMM format PSF file.
- bunny.pdb: The PDB format file of the all-atom structure.
- bunny.namd: The NAMD input file for the structure optimization simulations.
- bunny.exb: Extrabonds restraints for structure optimization.
- charmm36.nbfix: CHARMM36 force field files.

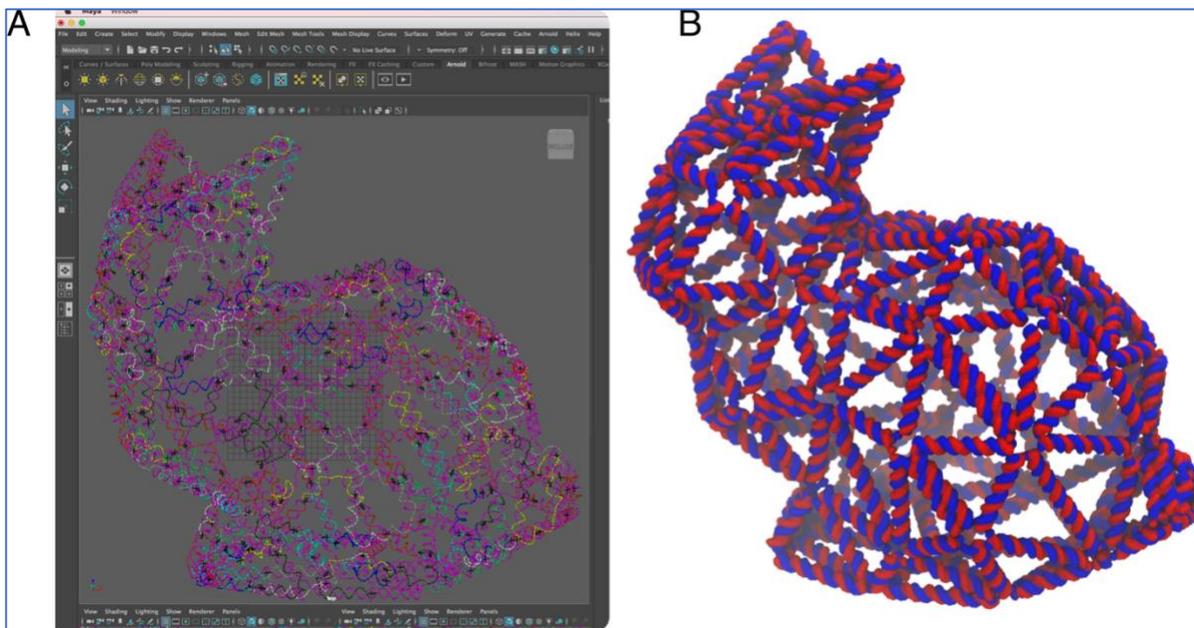


Figure 6. (A) A screenshot of vHelix session loaded with the design of bunny shaped DNA nanostructure. (B) The corresponding all atom structure of the vHelix design in vmd generated using enrgmd toolkit.

You can load the all-atom psf and pdb in VMD to visualize the structure as shown in figure 6B.

```
Shell > vmd bunny.psf bunny.pdb
```

Follow similar solvation, charge neutralization and equilibrium MD simulation protocol as described in section § 3.2 of our previous [DNA origami tutorial](#) to continue with the MD simulation of the structure using NAMD.

3.1G Atomistic simulation files for DAEDALUS structures

[DAEDALUS](#) (DNA Origami Sequence Design Algorithm for User-defined Structures) is online tool which converts any given 3d shape into a list of staple strand sequence and atomistic pdb file of the wireframe DNA nanostructure in that shape. You have to provide the scaffold sequence, the default is M13mp18 sequence. DAEDALUS is particularly helpful to design the sequence for any given 3D shape. It takes a simple polygon file format (.ply) for the shape structure as input and outputs the sequence suitable to design the particular DNA nanostructure. To this end, we submitted a simple tetrahedron ply file (*tetrahedron.ply*) and obtained the atomistic *tetrahedron_daedalus.pdb* file from DAEDALUS online server. These files are kept inside the **daedalus** subdirectory of the main **universal-all-atomTutorial** directory. In order to obtain the CHARMM files to run NAMD simulation of DAEDALUS pdb file, proceed with following steps.

1. Go to the Daedalus subdirectory of the main folder universal-all-atomTutorial.

```
Shell > cd universal-all-atomTutorial/daedalus
```

2. Remove the redundant lines from the pdb structure using your favorite text editor. Here we do it using *sed* command as follows

```
Shell > sed -i '/END/d' tetrahedron_daedalus.pdb
```

```
Shell > sed -i '/MODEL/d' tetrahedron_daedalus.pdb
```

```
Shell> sed -i '/REMARK/d' tetrahedron_daedalus.pdb
```

3. Finally, use the enrgmd toolkit from the **mrdna** package to generate the necessary files to run the simulation. Type the following commands in the terminal

```
Shell> enrgmd tetrahedron_daedalus.pdb -o tetrahedron
```

The toolkit will create the following files

- tetrahedron.psf: The CHARMM format PSF file.
- tetrahedron.pdb: The PDB format file of the all-atom structure.
- tetrahedron.namd: The NAMD input file for the structure optimization simulations.
- tetrahedron.exb: Extrabonds restraints for structure optimization.

- charmm36.nbfix: CHARMM36 force field files.

4. Load the obtained structure in vmd to visualize it as shown in figure 7B as follows,

Shell > **vmd tetrahedron.psf tetrahedron.pdb**

Users can follow similar solvation, charge neutralization and equilibrium MD simulation protocol as described in section § 3.2 our previous [DNA origami tutorial](#) to continue with the MD simulation of the structure using NAMMD.

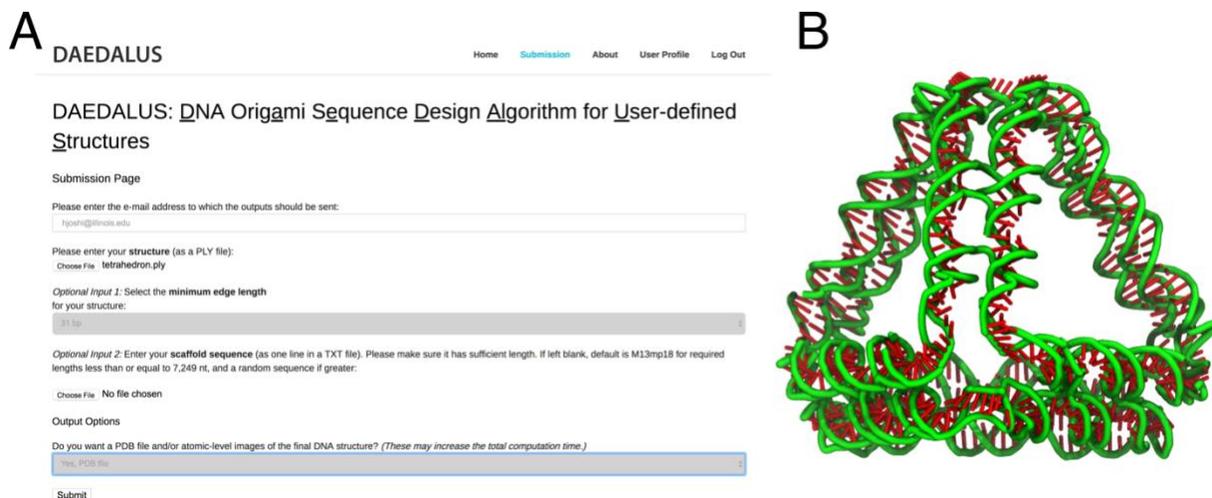


Figure 7. (A) A screenshot of the webserver of DAEDALUS online file submission session. (B) The vmd snapshot of DNA tetrahedron output all-atom structure of from **enrgmd** server has been loaded.

In case you want to create pdb structure of your own 3D design using DAEDALUS, follow the steps below,

1. Register to the online portal of DAEDALUS. <http://daedalus-dna-origami.org/registration/>
2. Log in to your account and load your 3D structure into the submission page as shown in the screenshot in figure 7 A.
3. After submission you will receive an email to download the sequence design and atomistic pdb structure of the structure. Download and extract the results folder to your favorite location in your workstation. You will find the pdb structure in the“input..” directory of the downloaded “job_submission..” folder.
4. Copy this file to daedalus subdirectory and follow the instruction given above to use **enrgmd** tool-kit to obtain the CHARMM simulation files of your structure.

3.1H. Atomistic simulation files from Tiamat

Tiamat is graphical user interface to create and edit model DNA nanostructure. As we described in section 3.1B, CanDo can take the output file of “Tiamat” design as an input to create the atomistic structure. Users can obtain the atomistic simulation files for Tiamat design using the similar steps as described in section 3.1B.

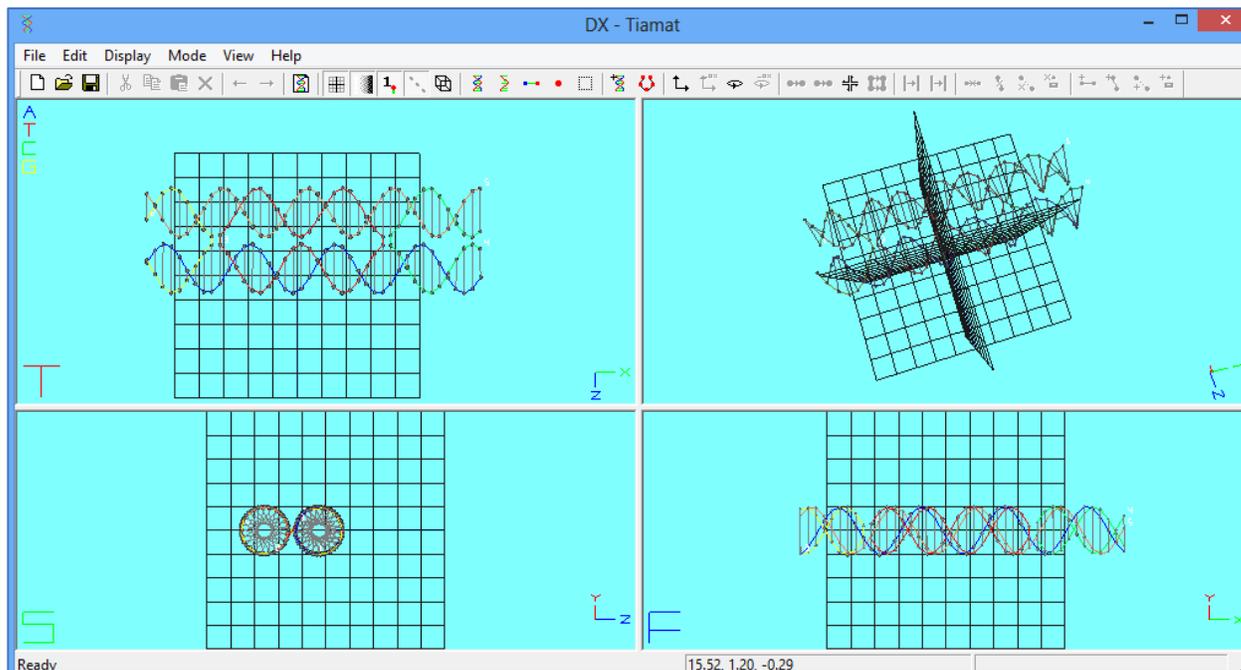


Figure 8. A screenshot of the Tiamat graphical interface with the design of double crossover molecule.