User Manual

3DNA 2.0
User Manual

Credits & Team

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Acknowledgements

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1. Basics of DNA Lego™ Modelling

1.1   Introduction

Sculpting has a long history and is often considered as one of the oldest art forms. In this era of DNA Nanotechnology it is now possible to create nano-sized sculptures out of blocks of DNA.

DNA Lego modeling is a robust technique for constructing 3D DNA nano structures by the combination of distinct single stranded DNA strands called DNA bricks in a fashion similar to that of Lego™ bricks.

This manual assumes that the user is familiar with the basics of base pairing of DNA bricks. The reader should refer to Three-Dimensional Structures Self-Assembled from DNA Bricks for further technical details on the language semantics.

1.2   Technique and details of the model

Each brick is 32nt long and composed of 4 domains of equal length. Each of these 8nt domains interacts with a local neighboring domain containing the complementary strand. Such an interaction for a single domain measures 2.5 by 2.5 by 2.7 nm and can be seen as a voxel in the molecular canvas. As seen in fig 1, each two-brick assembly forms a 90° dihedral angle via hybridization of two complementary 8-nt domains. By deselecting voxels from the molecular canvas of user defined dimensions, various shapes can be sculpted.
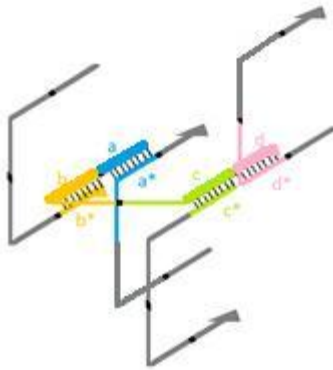
Figure 1 Self-assembly of DNA brick sequences in Lego™ like fashion. Each domain is represented by an and its complementary domain by alphabet*.

Four different types of DNA sequences are used to fully model a structure, these include:
   A. half bricks or 16 nt sequences
   B. full bricks or 32 nt sequences
   C. Protector bricks: full and half bricks which are partially poly 1T strands at the front and back faces of the 3D structure. They prevent unwanted hybridization of the DNA strands
   D. boundary bricks: 48 nt sequences formed by the concatenation of a full and a half brick found at the boundary surfaces of the structure

2. Why to use 3DNA

1.1 How 3DNA helps you
The general setup for designing and modeling 3D shapes using DNA Bricks mostly includes:

A. Use of heavy, licensed software for designing and visualizing 3D structures
B. Complex scripts for determining DNA sequences for each new structure
C. Integration of the scripts and DNA strand lists with experimental setup in laboratories

The aforementioned ad hoc approach could be error prone and cumbersome to work with. Scripts that are used to generate DNA sequences vary with their developers, thus creating documentation conflicts and poor communication across different labs. Finally, using proprietary licensed software to visualize 3D structures could be a challenge in itself. Although most of the 3D rendering engines are capable of rendering shapes with more than hundreds of sequences, it becomes confusing for the user to analyze and draw observations when the visualization is too cluttered.

3DNA as a software suite helps in providing solutions to such challenges in the following way:
A. Lightweight open source application
B. Intuitive and user friendly
C. Easy to work with even with minimal in-depth knowledge
D. Provides different visualization modules, customized specifically for viewing/analyzing DNA sequences

1.2 Organization
Efforts have been made to make the usage of the software intuitive and user friendly. The diagram below depicts the basic workflow of 3DNA.
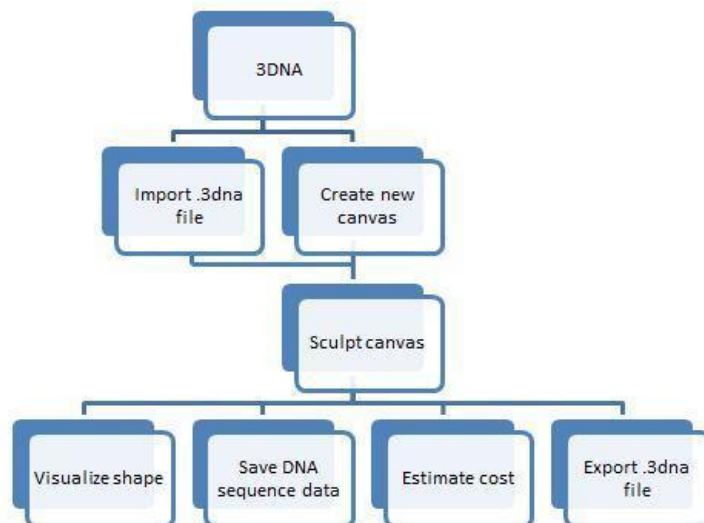

Figure 2 Software workflow

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3. Installation and download

3.1   Where to find it
3DNA can be downloaded from the **downloads** page of the software's official website at http://guptalab.org/3dna/ . You can also download supplementary data to get you started with the input and output formats of the software.

3.2   System requirements
3DNA is a cross platform software, which means it can be used on the majorly popular desktop environments i.e. Windows, Mac OS X and on various flavors of Linux. Usage of a mouse pointing device is recommended for modeling and analyzing 3D shapes.

3.3   Setup
Installation instructions for running 3DNA on a machine will vary from one operating system to another. Bundled binary (installer) obtained from the website contains the relevant instructions required to build 3DNA on the system. A compiled description on building 3DNA can be found below:
   A. On Windows:
       a. On executing the setup, a dialog box should appear with a short description of the software. Click on "Next" to proceed
       b. By clicking on Next button, the License agreement will appear. Please accept the license and click on Next to proceed
       c. A dialog box will appear asking the user to select the location for installing the software
       d. In the next dialog box, you will get an option to create desktop icon and start menu entry for the installation entry
       e. Finally, click on "Install" to install 3DNA software on your system.
   B. On Mac OS X:
       a. Open the 3DNA.dmg file and mount it on your disk
       b. Open the 3DNA.pkg file and follow the instructions to install the software
       c. Once the installation is complete you can run the software from your "Applications" folder
   C. On Linux:
       a. Extract the file "3DNA.zip" on your disk
       b. Open your system terminal
       c. Type "java -jar 3DNA.jar" and press **return** to run 3DNA on your linux desktop

3.4   Uninstalling:
   A. On Windows: Open the control panel and go to "Add/Remove programs". Locate 3DNA in the list of applications and click on uninstall to remove the 3DNA from your system
   B. On Mac OS X:
   C. On Linux: Simply delete the directory where 3DNA has been installed

4. Using 3DNA

4.1 3D Molecular Canvas:

Molecular brick canvas is one of the most integral parts of the software. The canvas is a cuboidal figure composed of individual molecular voxels. The dimensions of the canvas can be specified as an input by the user.

Figure 3: Input box for dimensions of molecular canvas

Once the dimensions of the canvas are entered you will be redirected to the actual canvas interface:
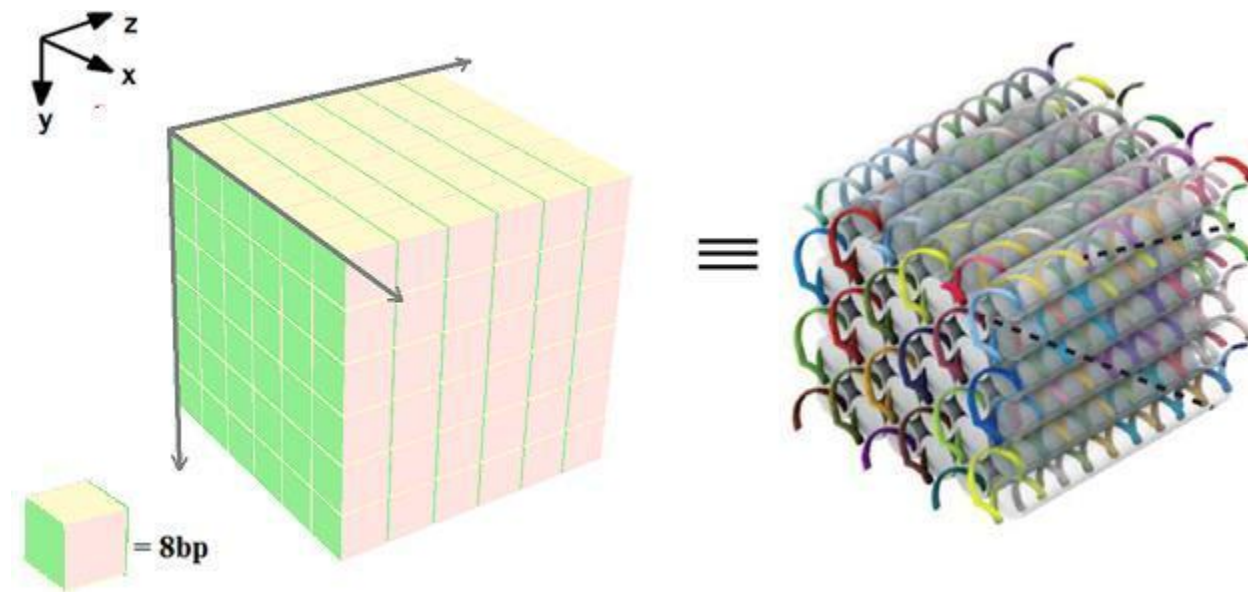

Figure 4 3D molecular canvas of dimensions 6H by 6H by 48bp and its corresponding DNA helix model

Each individual molecular voxel in the molecular canvas represents an interaction of a domain with its complementary strand. A voxel measures 2.5 by 2.5 by 2.7 nanometers. A collection of all such voxels forms the molecular canvas. The six faces of the molecular canvas are characterized with different colors to distinguish one from another (the XY plane is green in color, the YZ plane is grey and the XZ plane is yellow).Molecular axis originates from the top left corner of the first face,

denoting that point as origin and its associated voxel as the first brick with coordinates (0,0,1).

One key information to note here is that the first Z Axis plane has the plane number 1 as compared to X and Y Axis plane number which start with 0. Z Axis plane 0 is reserved for protector bricks (poly-1T) which can be enabled / disabled separately (explained in detail in **5.**).

4.2 Modeling / Sculpting Shapes

The molecular canvas has been designed to make the modeling and navigation as intuitive as possible. Individual molecular voxels can be deselected by simply clicking on them. User can interact with the molecular canvas either by mouse movements or by clicking the various buttons in the Left toolbar.

Mouse Navigation:

A. Rotation: Molecular canvas can be rotated canvas across its origin by long pressing the **left mouse button** and moving the pointer in the desired direction of rotation.
B. Translation: User can translate (move around) the molecular canvas by long pressing the **right mouse button** and moving the pointer in the desire direction of translation.
C. Zoom: User can zoom in and zoom out of the **canvas scene** by long pressing the **mouse scroll wheel** and zoom in by moving the pointer upward or zoom out by moving the pointer downward. Alternatively for pointer devices without the scroll wheel, a similar feature can be accessed by long pressing the **Alt key** on the keyboard along with the left mouse button.

Toolbar Navigation:

These features can also be used by clicking on the buttons present in the navigation bar on the left side of the main software interface. These buttons can be used to navigate the **canvas scene** to zoom in/out, move left/right/up/down, for resetting the molecular canvas to its original position and to **undo** any unwanted selection of a voxel.

Figure 5: Navigation toolbar

4.3 DNA Sequence Formats

Using the molecular canvas, various shapes can be created. The assembly process of the structure can be achieved using a set of random, yet unique sequences or a fixed set of sequences, which may be uploaded by clicking on the " **Change List"** button under the Choose DNA sequences list option on the advanced panel.

A. Random sequences: DNA bricks offers a simple and robust method for the self-assembly of shapes with respect to sequence composition. 3DNA chooses DNA sequences from a set of random, unique sequences with GC content: 40 - 60% and Hamming distance less than or equal to 6 per domain. This is the default option.

B. Fixed sequences: A set of fixed sequences with coordinates can be imported as .csv files. This feature is explained in more detail in 4. B (Import DNA Sequences).

4.4   Advanced Panel

Advanced panel can be invoked by clicking the ⬛button from the taskbar. Advanced panel comprises of various different functionalities which can either be used to further model the canvas or can be used to implement constraints and features to the 3D shape/DNA sequences.
These include:

A. Delete multiple voxels:

      a. Delete canvas plane
      b. Delete canvas row/column along a particular axis

B. Import DNA Sequences

User can import their own set of DNA sequences, to create shapes based on the sequences that they already have. Import sequence module supported by 3DNA only takes **.csv** as an input with a specific format. The 3 .csv file (one each for half brick, full brick and boundary bricks) has a separate format for each.

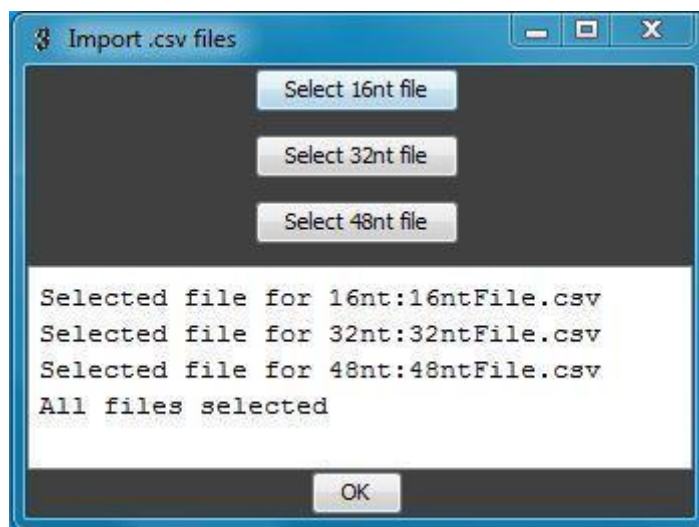The software takes 3 different files in its import framework:

Figure 6: Import sequence framework

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a. **16nt file:** A .csv file containing all the half brick sequences required for completing the molecular canvas. The general format of this file is:

[strand number, plate number, position on plate, domain1/3 DNA sequence, domain2/4 DNA sequence, helix 1 / helix 3, z1 / z3 coordinate, helix 2 / helix 4, z2 / z4 coordinate]

b. **32nt file:** A .csv file containing all the full brick sequences required for completing the molecular canvas. The general format of this file is:

[strand number, plate number, position on plate, domain1 DNA sequence, domain2 DNA sequence, domain3 DNA sequence, domain4 DNA sequence, helix 1, z1 coordinate, helix 2, z2 coordinate, helix 3, z3 coordinate, helix 4, z4 coordinate, check1, check2, check3, check4]

c. **48nt file:** A .csv file containing all the boundary brick sequences required for completing the molecular canvas. The general format of this file is:

[strand number, plate number, position on plate, domain1 DNA sequence, domain2 DNA sequence, domain3 DNA sequence, domain4 DNA sequence, domain5 DNA sequence, domain6 DNA sequence, helix 1, z1 coordinate, helix 2, z2 coordinate, helix 3, z3 coordinate, helix 4, z4 coordinate, helix 5, z5 coordinate, helix 6, z6 coordinate, check1*, check2*, check3*, check4*, check5*, check6*]

*Check (x) is a binary value (1 if domain(x) DNA sequence is a poly 1T ("TTTTTTTT") sequence and 0 if domain(x) DNA sequence is not a poly 1T sequence).

The imported files should also contain poly 1T domain protector strands if the user wants to have a structure with protector bricks. The software will choose a subset of correct DNA strands from the given set of files to form the prescribed shape on the molecular canvas.

C. Implementing advanced features on 3D structure

a. 48 nt Boundary bricks: This function can be enabled on different canvas planes which will result in the concatenation of 16nt boundary bricks and 32nt bricks along the left (Z-coordinate) to form 48nt boundary bricks.
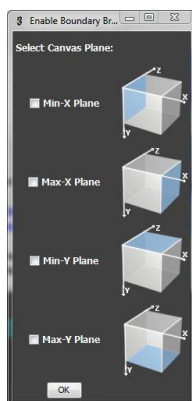

Figure 7: Enable Boundary bricks option menu

b. Protector bricks: The 32 nt bricks with poly-1T domains can be enabled / disabled at the extreme left and right (along Z-axis) by selecting the appropriate option.
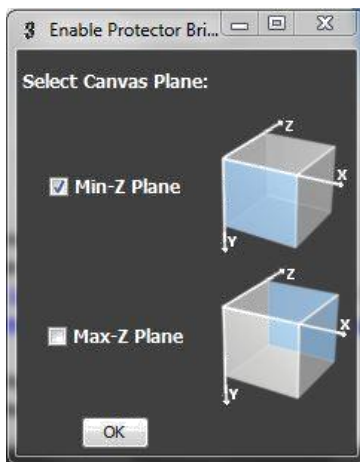

Figure 8: Enable Protector bricks option menu

c. Enable crystal(s): 3DNA also boasts the functionality to create DNA brick crystals elongating in X, Y and Z directions. These can be enabled by selecting the appropriate buttons.
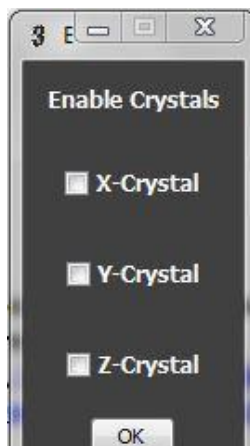

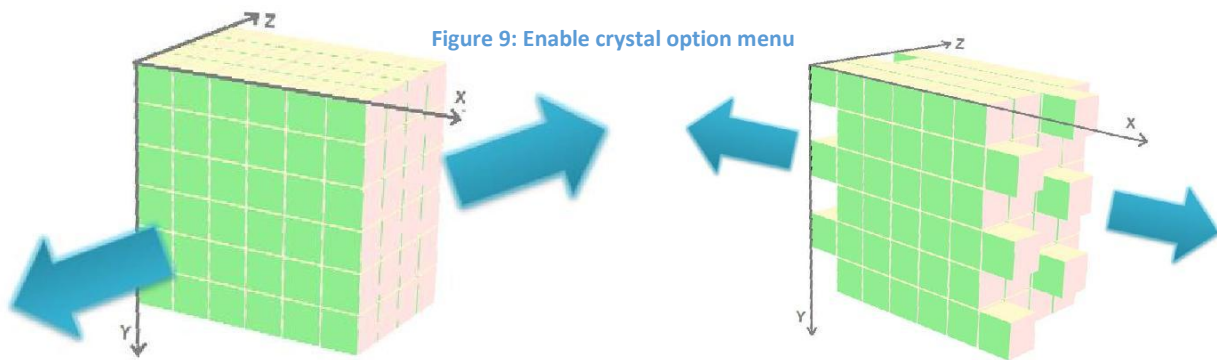Figure 9: Enable crystal option menu

Figure 10: z-crystal indicating growth along the z-axis   Figure 11: x-crystal indicating growth along the x-axis
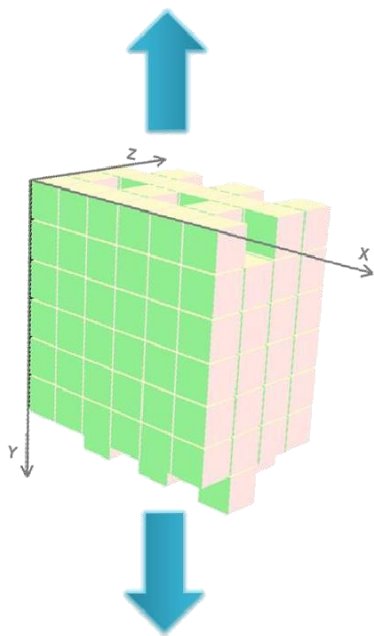
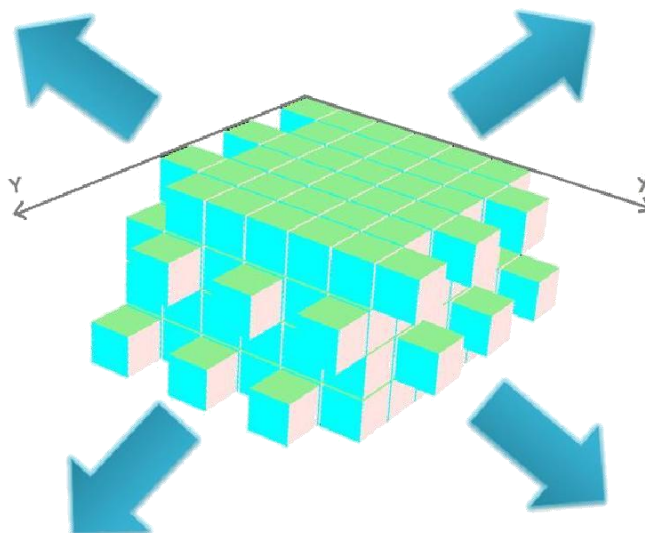**Figure 12: y-crystal indicating growth along the y-axis**   **Figure 13: xy-crystal indicating growth along the x and y-axes**

D. Implement advanced constraints on DNA Sequences

    a. Enable cavity boundary bricks: This function when enabled identifies and creates boundary bricks surrounding the internal cavity. This is an option to explore how the stability and formation is affected with the presence or absence of longer strands in different parts of the 3D structure.

    b. Enable poly-1T domains: In the case when coordinates / sequences cannot be mapped from an imported .csv file (section 4. B), they are considered as "removed / deleted" voxels. In this situation, the corresponding domains of single stranded DNA, which were to be complementary to these removed / deleted voxels can be replaced by poly 1T domains if this function is enabled. If not enabled, the complementary strands remain in their original state.

4.5 Saving options:

Output DNA sequences obtained from the software can be saved on the local storage in 2 different formats viz. **.pdf** and **.csv**. By default the name of these files are named as a derivative of the current project name. The output files contain all the data for the prescribed structure including the DNa sequence, coordinates in two formats: [x, y, z] coordinates and [helix,z-coordinate] for each domain. Thus, for example, if a full bricks is taken into consideration, it would consist of 4 domains. The coordinates would be in the form: [x1, y1, z1] [x2, y2, z2] [x3, y3, z3] [x4, y4, z4] and [helix1, z1, helix2, z2, helix3, z3, elix4, z4].

4.6 Cost Estimator

3DNA comes with an inbuilt estimator function, which can be used to calculate the cost of conducting a specific experiment. The module takes "cost per DNA base in USD" as the input variable and gives the total cost of DNA sequences in USD.

4.7 Visualization

3DNA comes integrated with visualization modules to verify and analyze the shape drawn using the molecular canvas. Considering the limitations of the graphic rendering hardware as well as the user's ability to clearly validate shapes, 3DNA provides 3 different types of visualizations to the user, these include:
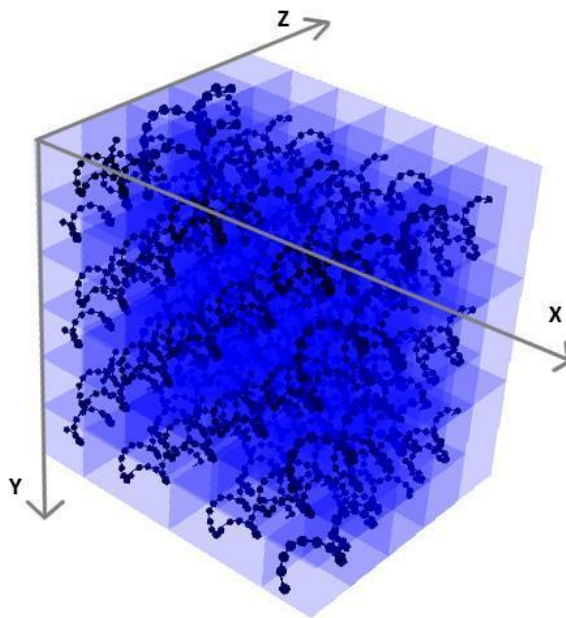

Figure 14: DNA helix model for structure of dimensions 6H x 6H x 40bp

A. **Full canvas visualization**: Helical representation of the interaction between individual single stranded DNA sequences and how they join in the Lego™ like fashion to make a full scaled DNA molecular canvas.

   Note: Full canvas visualization module renders individual DNA strands which might use large amount of CPU processing powers. To visualize canvases of dimensions higher than 6Hx6Hx48BP one should choose Individual plane visualization for analysis.

B. **Individual plane visualization**: Built as a lightweight version of full canvas visualization, individual plane visualization allows the user to focus on all the DNA bricks present on a single plane (or z-coordinate). The module loads comparatively faster than the full canvas visualization and is ideal for use on canvases with dimensions higher than 6H x 6H x 48BP.
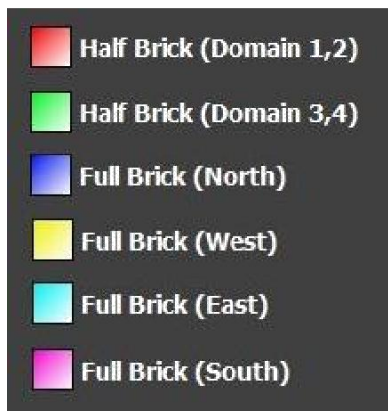
Figure 15: color encoding for visualization of Planes
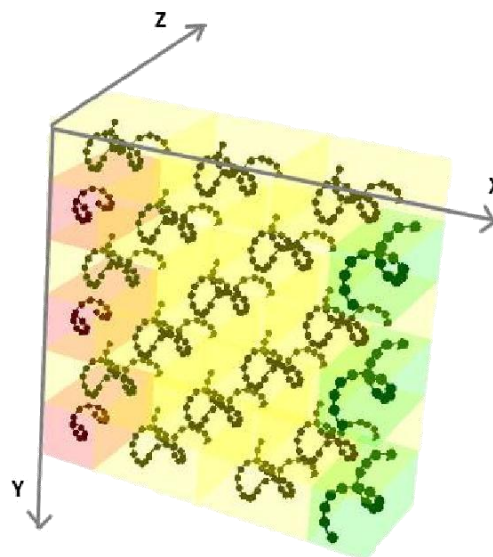
Figure 16: visualization of DNA Bricks for west plane from a structure of dimensions 6H x 6H

C. **Elementary visualization:** Elementary visualization uses an arrow like notation to denote DNA sequences rather than using a template strand. This module is easier for preliminary analysis of the whole canvas and can be used to check for cavities or crystal shapes.
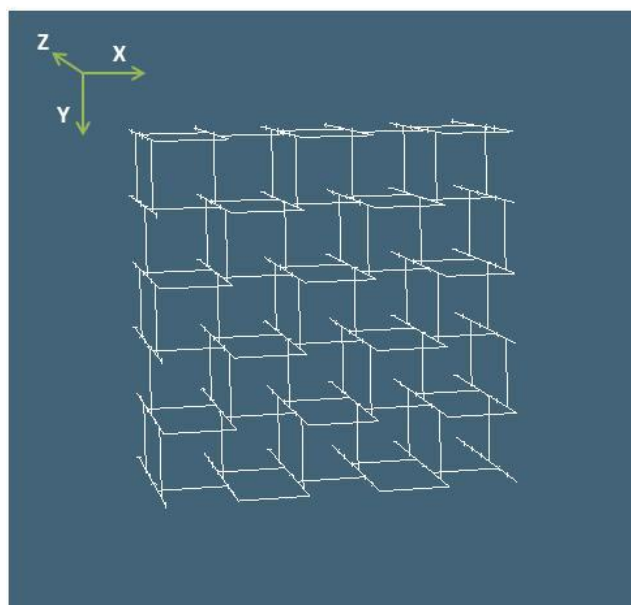

Figure 17: elementary visualization of a 5H x 10H x 80bp structure

4.8 Import and Export

To provide flexibility and ability to open and edit an existing project, the user may export a current project. By availing the Export button, the current project is saved as a .3dna file. An existing project which has been exported previously can be opened and modified/viewed through the Import functionality by clicking on the Import button. An existing project with .3dna extension can be imported.

5. Legal Stuff

5.1 Copyright/Distribution

3DNA

License for use and distribution

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http://www.guptalab.org/3dna

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Team

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Principle Investigator (Germany): Dr. David M. Smith
Graduate Mentor (India): Dixita Limbachiya
Graduate Mentor (Germany): Martin Sajfutdinow
Key Developers: Shikhar Kumar Gupta, Foram Meghal Joshi
Software Logo: Foram Meghal Joshi

Note: You can use 3DNA Pen on any computer, including a computer in a commercial organization. You don't need to register or pay for 3DNA.

5.2   Warranty

Licensor provides the Work (and each Contributor provides its Contributions) on an "AS IS" BASIS, WITHOUT WARRANTIES OR CONDITIONS OF ANY KIND, either express or implied, including, without limitation, any warranties or conditions of TITLE, NON-INFRINGEMENT, MERCHANTABILITY, or FITNESS FOR A PARTICULAR PURPOSE.

6. References and Feedback

Users are requested to contact team at the feedback page on the website www.guptalab.org/3dna for any issue with the software or they can also mail at 3dna@guptalab.org. Two platform specific installers (Windows and Mac) are available on the project home page along with source code.

7. Glossary

1. Boundary bricks

   These are short 16nt bricks which can be found on the boundary surfaces (along the X and Y-direction). Except for the boundary bricks at the front end of the structure, all of the short 16nt bricks can be connected with the 32nt bricks on their left (Z- direction) to form 48nt boundary bricks. See figure

2. Crystal

   It is continuous DNA bricks structure composed by the concatenation o f similar building blocks of smaller size. The **X-crystal** is formed by growth of the structure along the X-axis. Similarly, the **Y-crystal** grows along the Y-axis and **Z-crystal** along the Z-axis. It is also possible to design two-dimensional crystals which simultaneously grow along two axes. See figure

3. DNA brick

   A single stranded DNA sequence which is synthetic in nature. It is the main building block of the molecular canvas and is of 2 types: full brick (32nt), half brick (16nt).

4. Domain

   Each DNA brick is composed of segments of equal length (8nt) called domains. Full bricks have 4 domains and half bricks have 2.

5. Full Bricks

   These are 32 nucleotide single stranded DNA sequences. These full brick strands serve as building blocks of the 3D molecular canvas.

6. Half Bricks

   These are 16 nucleotide single stranded DNA sequences, found usually at the boundary surfaces of the 3D structure.
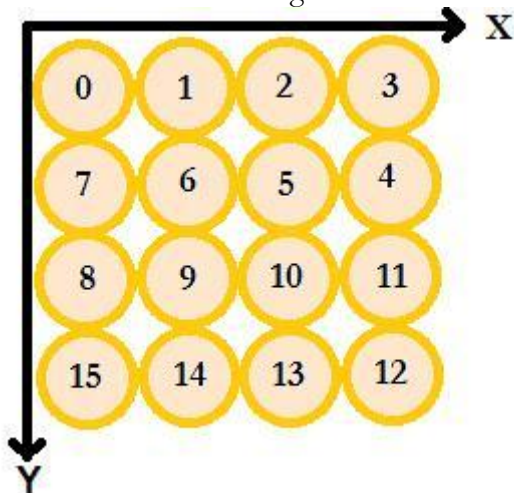
7. Head Domains

Domains present at the minimum z-coordinate for a given structure.

8. Helix numbers

Similar to the caDNAno design, 3DNA assigns the coordinates in the form of a combination of helix numbers and Z-coordinate numbers. See figure

9. Molecular Canvas

A master brick collection of voxels is a molecular canvas. Its dimensions can be defined by the user.

10. Plate number

The number associated with the plate for pipetting in the experimental setup.

11. Poly 1T domains

A domain which is entirely made up of Thymine i.e. the sequence "TTTTTTTT". These domains can be found in protector bricks or as domains which were complementary to the removed voxels in order to prevent unwanted hybridization.

12. Position on plate

The number associated with the position on a plate for pipetting in the experimental setup.

13. Protector bricks

These are 32 nt bricks with a special purpose. They contain poly-1T domains in order to prevent unwanted hybridization of DNA strands at the front and back faces (along Z-coordinate) of the 3D structure.

14. Strand number

A unique number associated with each strand.

15. Tail Domains

Domains present at the minimum z-coordinate for a given structure.

16. Voxels

Each 8–base pair domain interaction (between bricks) defines a voxel with dimensions of 2.5 by 2.5 by 2.7 nanometers.