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# Supplemental Materials

## for

# Visualizing the Invisible: A Guide to Designing, Printing, and Incorporating Dynamic 3D Molecular Models to Teach Structure–Function Relationships

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## Table of Contents

**(Total pages 9)**



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**Appendix 1: Video guide to design flexible DNA**

Video can be found at https://doi.org/10.1128/jmbe.v19i3.1663 and at https://digitalcommons.unl.edu/structuralmodels/22.

**Appendix 2: Supplemental Video Transcript and Directions**

**Introduction for Supplemental Video Transcript**

(00:00) In this video, I will walk you through designing and 3-D printing a long flexible DNA model, displayed here, that mimics the structure and function of DNA. We will do this by first building a single base pair with its backbone, then applying 2 kinds of modifiers that will repeat this base pair and add a twist to the DNA. However, by changing the initial building block that you create, you can make other polymers, as well.

(00:28) I am using Blender 2.79 to create this model. Blender is a free and open source 3-D computer graphics software that I already downloaded from the web at https://www.blender.org/download).

1. (00:44) Begin by downloading and opening Blender.
2. (00:47) First, I suggest adjusting the user preferences for more intuitive navigation. Under "File" click "User preferences". Then under the "input" tab: change the options to "Select With Left" and "Emulate Numpad". Save settings. And close the window.
3. (01:11) Blender has two viewing options: **perspective** (default)—which has depth—and **orthographic**—which is a fixed depth, where each line of 1 unit will appear to have the same length everywhere on the drawing. Hit 5 at any time to switch between these two views. However, especially as the drawing gets larger, I prefer to be in the User Orthographic view.
4. (01:57) Hit tab to enter edit mode. Then "X" → "Vertices" to delete the default cube.
    a. You must be in edit mode when you delete this object, or mesh; because if you delete the mesh in "object mode" you will lose the ability to enter "edit mode" when you add new meshes.

**(Orienting Blender's User Interface)**

(02:17) Each region of Blender's user interface responds to different key strokes. By default, Blender displays 5 editors: 1) the top bar is the info editor; 2) the main region of the screen is the 3D view editor {which is broken into the tool shelf (top left), operator panel (bottom left), properties panel on the right (which can be opened by hitting "N"), and the header (bottom strip), and the main region. This is where we will build the model. 3) The 3$^{rd}$ editor is the outliner editor on the top right. This region allows you to quickly select the mesh of interest. 4) The properties editor in the bottom right allows you to change lots of different qualities of your mesh. 5) The 5$^{th}$ editor is the timeline editor (at the bottom) that is not used in 3D printing.

(03:48) Because each of these different editors responds differently to keystrokes, ensure that your cursor is where you want it when you hit a hotkey. For the keystrokes we use, ensure that your cursor is on the main screen in order for Blender to respond.

**(Building the DNA)**

(04:04) Now, let's build our DNA.

5. (04:06) To build the initial base pair, add 2 cylinders, representing the bases, and 1 sphere, to give the model the structural stability that it will need for printing.
6. (04:15) In object mode (hit "Tab" to enter object mode), hit "Shift+A" to open the "add mesh" pull-down menu. First add 1 cylinder.
7. (04:30) You can set your dimensions and location in the bottom left corner immediately after adding the mesh. However, once you move the object you added, this tool is gone. Instead, you can adjust these criteria in the properties panel, in object mode. If this panel is not showing on your screen, hit "N" to open it.
8. (05:22) The cylinder will be 2 by 2 by 3 mms.
    - Blender operates in "Blender Units" an arbitrary unit that is equal to mm. 1 BU = 1 mm. (However, if you want to change the units in which you work, you can set your units in the "Scene" option on the rightmost properties editor). I prefer to keep the units in Blender units so that Blender doesn't adjust these units as I move and zoom in on my model.
    - Our first cylinder needs to be 2mm x 2 mm x 3 mm.
    - And the location needs to be x, 0.5; y, 1.5, z, 0.
    - The rotation needs to be set as x, 90; y, 0; and z, -18.
9. (07:04) To add the second cylinder, you can either repeat the previous steps: "Shift+A", add cylinder. Or, you can simply duplicate the first cylinder by hitting "Shift+D", and dragging the object, or clicking on the screen.
    - This cylinder will be the same size 2x2x3.
    - The location of the second cylinder will be x, 0.5; y, -1.5, z, 0.
    - The rotation will be set as x, 90; y, 0, and z, +18.
10. (07:58) Now add a sphere to join these bases.
    - "Shift+A" → "Mesh" - opens the "add mesh" pull-down
    - The size of the sphere will be 2 x 2 x 2; or radius = 1

- The location of the sphere will be x, 0.01112; y, 0, z, 0.
- The rotation will be x, 90; y, 0; z, 0
  - Hit “Z” to enter the wireframe mode to observe the change made in rotation
  - Then hit “Z” again to return to the solid render or navigating to the “viewpoint shading” and clicking “solid”.

11. (09:49) Now add the DNA backbone to the model. Hit “Shift+A” to add 2 oval cylinders for the backbone.
    - The first cylinder will be as follows: Dimension: [2.4, 1.6, 2 mm]; Location [1.07, 3.3, 0]; Rotation: [0, 0, -18 degrees] (Supplemental Figure 1).
    - The second cylinder will be as follows: Dimension: [2.4, 1.6, 2 mm]; Location [1.07, -3.3, 0]; Rotation: [0, 0, 18 degrees] (Supplemental Figure 1).

12. (11:26) To join all the objects into one, click the sphere so that it is the active object. Then press “A” twice or navigate “Select>select all by type>mesh” to select all, with the sphere remaining as the active object. The active object remains highlighted in a lighter orange than the rest of the objects. Then press “Ctrl+J” to join all parts into one object.
13. (12:12) Ensure that the mesh is manifold. Manifold means that the mesh is water-tight, with no holes, and no duplicate faces or edges, but that all vertices are connected. This is crucial so that the printer knows exactly where to deposit the material in 3-dimension.
    - Hit “Tab” to enter edit mode.
    - Ensure that you are on the vertex option (on the bottom ribbon when in edit mode).
    - Hit “A” once or twice until no vertices are selected. When nothing is selected, hit “Z” to enter the wireframe mode (or enter it by clicking the “viewpoint shading” menu), then “Ctrl+Alt+shift+M” will select any non-manifold vertices. Unlike the solid render, the wireframe mode allows you to see through your object.
    - If any non-manifold vertices exist, these will become selected or highlighted. To fix these you need to identify where the hole is and how to repair it. Numerous online tutorials and forums are available to assist with this, or you can contact us for tips.
      - One useful command is the “Make Face/Edge” option that you can find in the tool editor when in edit mode. Or hit “F” to make a face or edge between non-manifold vertex. However, in the case displayed here our mesh is manifold.

14. (14:16) In object mode (hit "Tab" to return to object mode): Press "Ctrl+A" and select "Rotation & Scale" to apply rotation and dimensions of the object so that future steps will refer to this presentation of the model.

15. (14:42) In order to match the model's scale to real B-form DNA measurements, temporarily add a few modifiers (Supplemental Figure 2). Recall that modifiers will alter your object to achieve different purposes. In our case, we want to extend the length of our DNA and add a twist to our helix. Modifiers can be found in the properties editor. Click the button that looks like a wrench. Then, "Add modifier".
16. (15:11) Add **Array modifier:** This modifier allows you to repeat your individual unit.
    - Count: # of bp desired. For now, use 21, which equals 2 complete turns of a DNA helix.
    - Select relative offset (checked): [0, 0, 1] so that the base pair is repeated along the z-axis (Supplemental Figure 2).
17. (16:17) Add **Simple deform modifier:** This modifier allows you to twist or bend your model.
    - Deform angle: 720 degrees, which equals 2 complete turns of the DNA helix: 360*2 (Supplemental Figure 2).

18. (17:25) Use simple math to adjust the scale so that the model measurements correlate to B-DNA (Supplemental Table 1).
    - Thus, adjust the x and y dimensions to be 12.35 mm.
    - Press "Ctrl+A" to apply the scale.
19. (18:50) Because this will make the DNA too thick and not as flexible as desired, scale dimensions down:
    - Set scale: [0.5, 0.5, 0.5], then press "Ctrl+A" to apply scale.
    - This would be the equivalent of starting with a base pair with a height of 1 mm (z-axis), or each cylinder [2.05, 2.512, 1 mm after rotation]. The new scale is displayed (Supplemental Table 2).
20. (20:10) Now adjust the **array** and **simple deform** modifiers to give the desired length of DNA. Because we intend to print this model in Elasto Plastic material from Shapeways we went to their website to determine the maximum bounding box for 3D printing with this material. The max bounding box for 3D-printing Elasto Plastic from Shapeways is [300, 300, 150 mm], with a hypotenuse of 449 mm from diagonal corners. Allow 10 mm extra if adding handles to each end. (You can also design different end attachments, such as a small box that holds magnets. Small magnets can be purchased from online vendors such as K&J Magnetics: www.kjmagnetics.com). To ensure an even number of complete turns, so as not to introduce an extra partial twist when joining ends after printing, the target length is 420 bps for **Array count** (40 turn * 10.5 bp) and twist is 14,400 degrees for **Simple deform angle** (40 turns * 360 degrees). Then apply the

modifiers. The order in which you apply modifiers is important: start from the top and apply down to achieve the intended results.

- Tip: to navigate around the screen to change your view on the model, hit "Shift + clicking middle mouse" as you move the mouse around the viewer. You can also hit "Shift + scroll middle mouse up and down" to scroll your screen up and down; or hit "Ctrl + scroll middle mouse up and down" to scroll your screen left and right.

21. (23:19) Add handles extending from the backbone, which will allow students to hold the ends of the DNA as they interact with the model: Press "Shift+A" to add 1 cylinder with dimensions: x, 2.7; y, 2.7; z, 5 mm. Move it to extend from one backbone hitting "G" (for grab) and "X", "Y", or "Z" to transform the cylinder along the respective axis.
    - Hit "Z" to enter wireframe view and "1" or "3" to view the cylinder and DNA along the y-axis or x-axis, respectively. Ensure that the cylinder is overlapping with the DNA so that it will be connected to the DNA as a single object during print.
    - Once satisfied with the location of this cylinder, hit "Shift+D" to duplicate the cylinder and orient it to extend from the adjacent backbone.
    - Select both cylinder handles, then hit "Shift+D" to duplicate both handles and "Z" to lock and drag them along the z-axis. Set the z-location for each of these handles as 420 in the properties panel. The make fine adjustments by selecting both cylinders and hit "G" then "Z". Because the helix makes complete turns, the top and the bottom ends will align.
22. (28:37) Once satisfied with the locations of the handles, select all the objects with the DNA as the active object, and press "Ctrl+J" to join all parts into one object.
    - Then rotate to extend the DNA from diagonal corner to diagonal corner: x, -15; y, 45; z, 0 degrees].
    - Press "Ctrl+A" to apply rotation and scale, ensuring that the mesh is within the bounding box in Shapeways.
23. (30:11) Export object as .stl file and upload to Shapeways.com. (Other export options are also available). You can then order the model to be 3-D printed and shipped to you. There are a variety of materials in which you can print this model, however, we find that the Elasto Plastic is convenient for simulating the flexibility of DNA.

(32:16) For the complete 3D file, or for links to print supporting proteins such as nucleosomes, histones, or transcription factors as referenced in the main text of the paper. Or for additional questions, contact the authors.

**Accompanying Video can be found in Appendix 1 and at https://digitalcommons.unl.edu/structuralmodels/22)**

**(Helpful Additional Resources)**

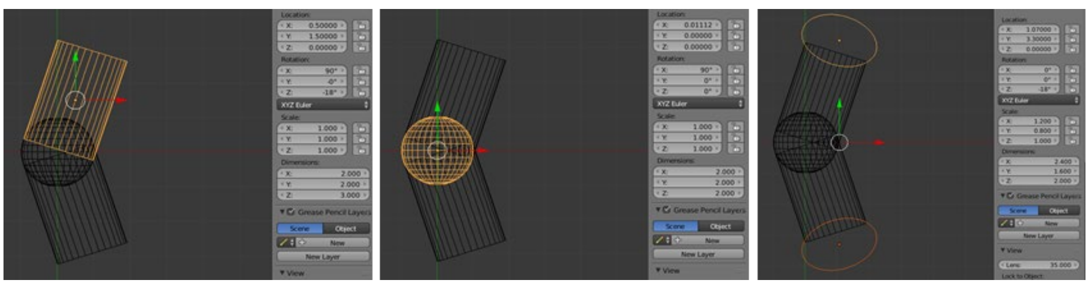
Below you will find links to a few useful tutorials to assist in your use of the Blender software. Additionally, you can find numerous video tutorials on [www.YouTube.com](www.YouTube.com) or with a simple Google search.

https://wiki.blender.org/

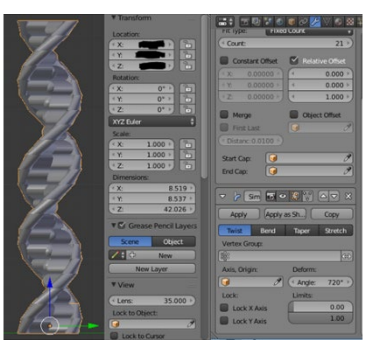
https://3dprinting.com/blender-tutorials/

https://www.shapeways.com/tutorials/prepping_blender_files_for_3d_printing

https://www.sculpteo.com/en/tutorial/prepare-your-model-3d-printing-blender/



**Supplemental Figure 1.** Properties panels of the cylinders and sphere that make up the initial DNA base pair and backbone in object mode.



**Supplemental Figure 2.** Array and Simple deform modifiers are added to create short helix.

**Supplemental Table 1.** Summary of calculations comparing B-form DNA to the model of the short helix in order to set the model to scale.

| Trait | B-form DNA molecule | 3D Model B-form DNA |
|---|---|---|
| 1 complete turn | 34 Å | 21 mm |
| 2 complete turns | 68 Å | 42 mm |
| Diameter | 20 Å | ***Should be 12.35 mm (not 8.5 mm)*** |
| Major Groove | 22 Å | 13.59 mm |
| Minor Groove | 12 Å | 7.41 mm |

**Supplemental Table 2.** Summary of measurements of real B-form DNA and the model scaled by 0.5 from the initial design.

| Trait | B-form DNA molecule | ***Smaller*** **3D Model B-form DNA** |
|---|---|---|
| 1 complete turn | 34 Å | 10.5 mm |
| 2 complete turns | 68 Å | 21.0 mm |
| Diameter | 20 Å | X = 6.163, Y = 6.176 |
| Major Groove | 22 Å | 6.8 mm |
| Minor Groove | 12 Å | 3.7 mm |