

## SUPPLEMENTARY MATERIAL

### 1. Preparing 3D model files for printing

#### 1.1) Generate a custom molecular model with UCSF Chimera

The first step in preparing a 3D printable model of a biomolecule is to locate or generate a 3D molecular data file. Several online databases host 3D molecular data files along with associated metadata.

- PubChem (<https://pubchem.ncbi.nlm.nih.gov/>): a repository of small molecules, 65 million of which include calculated 3D structures.
- Protein Databank (PDB) (<http://www.rcsb.org>): a repository of more than 100,000 experimentally derived 3D structures of biomolecules.
- Protein Model Portal (<http://www.proteinmodelportal.org>): a database of theoretical models of biomolecules, generated with molecular modelling software.
- Electron Microscopy Databank (EMDB) (<https://www.ebi.ac.uk/pdbe/emdb/>): hosts 3D models of macromolecular assemblies derived from EM density maps.

#### 1.2) Preparing a 3D printable representation of a biomolecule using Chimera

Chimera (<https://www.cgl.ucsf.edu/chimera/>) has a straightforward graphical user interface, complemented with a powerful command line. To access the latter, go to *Favorites > Command Line*. It will appear at the bottom of the screen. Below we explain how to prepare models for 3D printing using both the available pulldown menus as well as commands (the latter shown in text boxes). Use of the command line can save time, but requires the user to familiarize him/herself with the code.

The rotational orientation of the molecule can be adjusted by clicking and dragging the left mouse button. After making changes to your molecule, you can save the Chimera session with **File > Save Session** which is essential for back-tracking. Fundamental to all operations that customize display is selection, which is done with the options under the **select** menu. Selections can also be made graphically by using **ctrl + click** or **ctrl + drag** to select a rectangular region. Using **ctrl + shift** adds to a previous selection. The type of selection can be set to append, intersect, replace, or subtract with **Select > Selection Mode**. Selections can be saved by using **Select > Name Selection**. The saved selection can then be selected again with **Select > Named Selection [selection name]** or with the command **sel name**, where **name** is the name of your selection. All actions that change how the model is displayed are restricted to the atoms in the current selection and are essential to combining multiple representation types into a single model.

#### Name selection

**namesel NAME** - save a selection called NAME

**sel NAME** - re-select the saved selection NAME

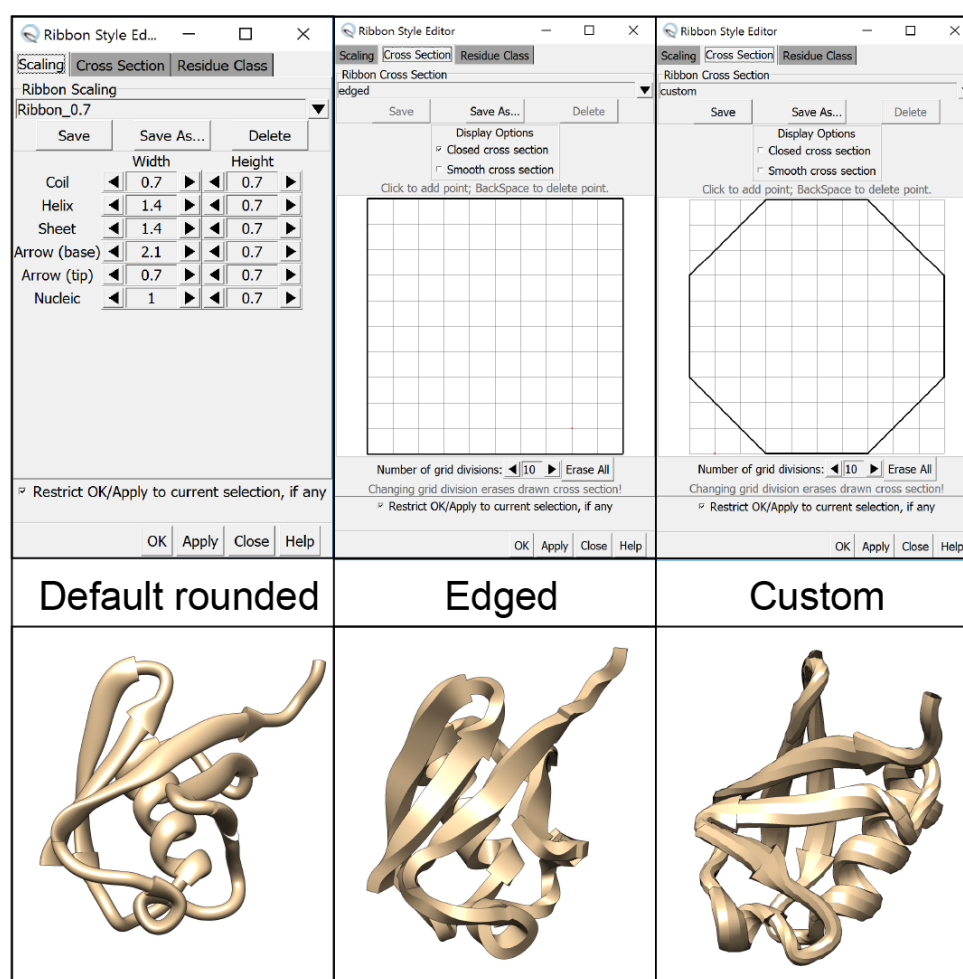
**Export scene**

## 1.2.1) Generating a 3D printable representation in 'ribbons'

### 1.2.1.1) Protein

Ribbon models display the structure of the protein backbone, distinguishing loops, alpha helices, and beta sheet as different conformations of the ribbon. However, ribbon models present a challenge to 3D printing. The shape of alpha helices and the often complex geometry of the backbone creates a large number of overhangs that must be supported. By generating ribbons with physical properties that facilitate printing, these challenges can be overcome.

Begin by using **Select > All**, then **Actions > Atoms/bonds > Hide**, and **Actions > Surface > Hide**. This will clear the scene so that no other representation types are displayed. Select the portion of the biomolecule that you would like to be a ribbon and use **Actions > Ribbons > Show**. To change the ribbon's appearance go to **Tools > Depiction > Ribbon Style Editor**. This menu allows several parameters of the ribbon's appearance to be specified (Figure S1). The thickness of the ribbon is the most important factor for printing success. Ribbons should be made *as thick as possible without obscuring the shapes of secondary structures*. The ribbon style editor can also be used to alter the ribbon cross section. This parameter determines the geometry of the ribbon exterior and also of the mesh that is exported from Chimera. The default cross section will produce a rounded exterior. Using other cross section types, such as squares or octagons, will reduce the number of polygons in the output file, which can be desirable when working with large files.



**Figure S1.** Ribbon style editor. **Top:** Ribbon style editor menu with suggested parameter, and examples of customizable cross section. **Bottom:** Resulting ribbons from shown parameters. For additional information on the Ribbon Style Editor, see: <https://www.cgl.ucsf.edu/chimera/current/docs/ContributedSoftware/ribbonstyle/ribbonstyle.html>.

### 1.2.1.2) Nucleic acid

Nucleic acids can also be represented as ribbons. However, the process of preparing nucleic acids is slightly different from that of protein, as nucleic acid is composed of two different representations for its backbone and base. The sugar phosphate backbone of nucleic acid is displayed as regular ribbons, which are edited in the ribbon style editor. While the nucleobases are *nucleotide objects* which have their own properties. To begin, select nucleic acids and use **Actions > Ribbons > Show**. Then use **Actions > Atoms/bonds > Nucleotide Objects > On**, and **Actions > Atoms/bonds > Nucleotide Objects > Settings**. Within this menu ensure that “show backbone as” is set to **ribbon** and change “show side (sugar/base)” from “**fill/slab**” to either “**ladder**” or “**atoms and bonds**”. When bases are displayed as ladders, the rung radius can be altered to improve printability; values of 0.8 or greater work well.

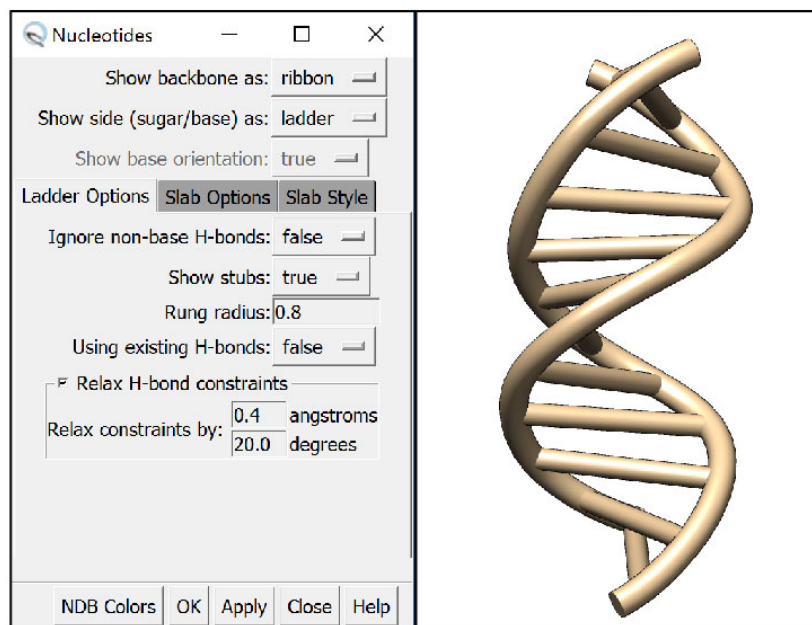


Figure S2. Nucleic acids helix rendering editor.

## 1.2.2) Generate a 3D printable ‘surface’ representation of the molecule

### 1.2.2.1) Adding hydrogens

If the depiction of every atom is important for the model of interest, such as when using spheres to represent a DNA model, hydrogens can be added using the menu: **Tools > Structure Editing > AddH**.

Adding Hydrogen atoms

addh

The surface representation displays the exterior of a molecule. Surfaces are simple to prepare and also straightforward to print. Chimera calculates the surface of a molecule by considering that each atom has a radius and then rolling a sphere of arbitrary radius over the model to see the depth accessible by the probe. If the probe radius is made small, the result is equivalent to the spheres representation - as shown in Figure S1.

Prior to generating a surface, all other components of the representation should be hidden. To do so, select everything and then use **Actions > Atoms/bonds > Hide**, and **Actions > Ribbon > Hide**. To render the surface representation use **Actions > Surface > Show**, or go to **Presets**

> **Interactive 3 (hydrophobicity surface)**, then **Presets > Publication**. Both options yield the same printed results, as silhouettes and shadows don't alter the information in the 3D model file.

#### Surface generation commands

The Van-der-Waals (VDW) radius must be set first, which determines the distance of the generated surface from the underlying atoms. To preserve differences between the radii of each element, it is optimal to increase the VDW radius for all atoms by the same amount.

**vdwdef - 0.5** decreases the surface distance by 0.5 Å

**~ vdwdef** - resets the VDW radius to default

**vdwdef + 2.0** increases the surface distance by 2.0 Å

If the model is composed of multiple components, they can be separated into chains using the **split** command. This allows the generation of surfaces for specific components of the molecule.

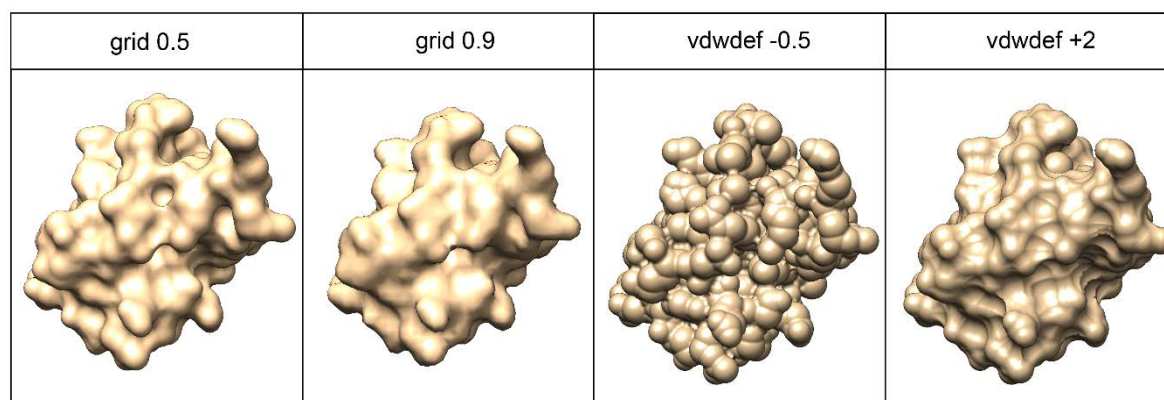
**Split** - separates each chain into a separate model

**split atoms selected** - splits the current selection into a separate model

#### Generate surface of atoms

To generate a surface, use the **surf** command. Include the **model #** to generate a surface for only that model. The **grid** argument is required, as it specifies the resolution of the exported STL. A grid value of 0.5 gives a result that is not too large, but can still be scaled without showing the individual faces in the print.

**surf #0 grid 0.5**



**Figure S3:** Surface renderings. **Left:** Two surfaces with different resolutions. **Right:** Two surfaces rendered using probe radius of 0.5 and 2.0 angstroms.

### 1.2.3) Displaying atoms

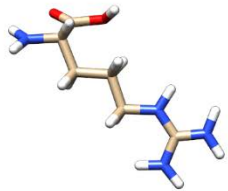
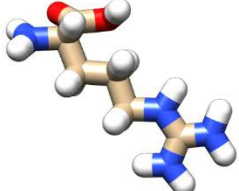
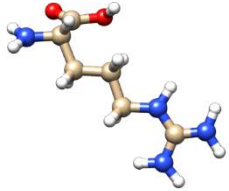
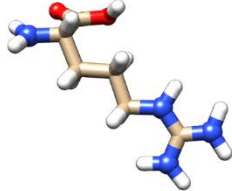

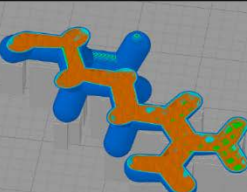
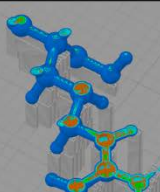
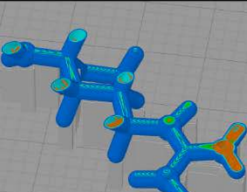




#### 1.2.3.1) Stick format

To highlight specific interactions or structural features, selected atoms in a molecule can be displayed. The relevant residues can be selected using selection tools, with the mouse, or in the sequence panel under **Favorites > Sequence**. With the correct atoms selected, use **Actions > Atoms/bonds > Show**, then **Actions > Atoms/bonds > Stick** to render the atoms as sticks.

To change the stick radius, select the entire model or the subset of atoms and open the menu **Actions > Inspect**. In the **Inspect** menu the **radius** can be changed. The default **radius** is 0.2. Increase to around 0.5 for easier printing depending on the scale of the model.

#### Changing atom stick radius

**setattr m stickScale 2** - increases bond radius for all atoms in the model

	Default sticks	Thickened sticks	Ball and stick	Custom sticks
Chimera				
Slicer				
Printed model				

**Figure S4.** Examples of printed results using different stick styles. Thickened sticks print more reliably and the final model is stronger.

#### 1.2.3.2) Sphere format

When using the spheres representation, the size of individual atoms or bonds can be altered. To change the radius of a selection of atoms, go to **Actions > Inspect**. Select **Inspect: Atom** and increase the radius. To change the radius of all atoms of a given type, first select them by using **Select > Chemistry > Element** and choosing the atom type.

### Changing sphere atom radius

The command **vdwdef** can be used to change atoms sphere radius by either setting it to a new value or increasing/decreasing them. It can be applied to the whole model, to specific atom type, or to the current selection **sel**

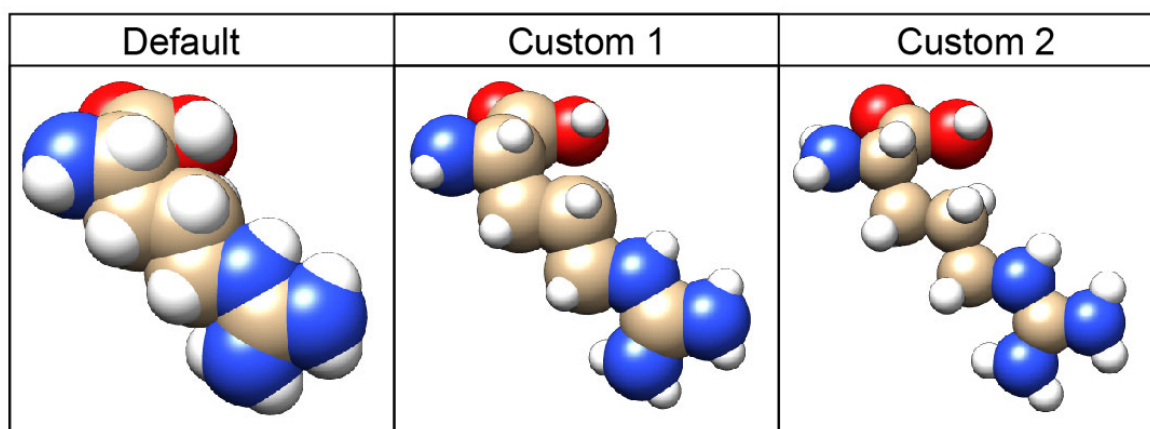
**vdwdef +0.5** - increases bond radius for all atoms in the model by 0.5 Å

**vdwdef -0.5 C** - decreases bond radius for all carbon atoms by 0.5 Å

**vdwdef 1.5 O** - changes bond radius of all oxygen atoms to 1.5 Å

**vdwdef 1.5 sel** - changes bond radius of selected atoms to 1 Å

**~vdwdef** - resets VDW radius to default



**Figure S5.** Chimera atom radius. Arginine molecule rendered with default atomic radius and with all radii reduced by 0.5 (Custom 1) and with carbon atoms further reduced by 0.3 and nitrogen atoms by 0.2 (Custom 2).

### 1.2.4) Displaying hydrogen bonds

The display of hydrogen bonds within a molecule adds important information about secondary structure and may potentially increasing the stability of the print. To show hydrogen bonds, use **Tools > Structure Analysis > Find H bond** and hit **apply**. By default, this will display the bonds as wires, which have no volume and cannot be included in an STL file. The wires must be displayed as sticks. Use **Select > Select All** and then **Actions > Inspect**. Use the Inspect menu to inspect **pseudobonds** and change the **bond style** to **stick**.

The default **radius** is 0.2 but should be increased to approximately 0.4. It is good practice to keep the hydrogen bond radius smaller than other chemical bonds so they can be easily distinguished. The same results can be achieved using the following commands:

### Creating hydrogen bonds

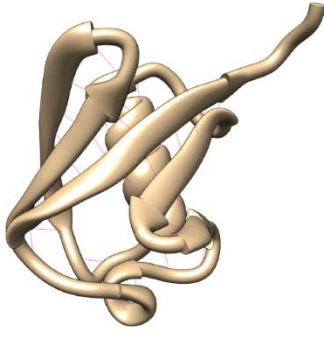
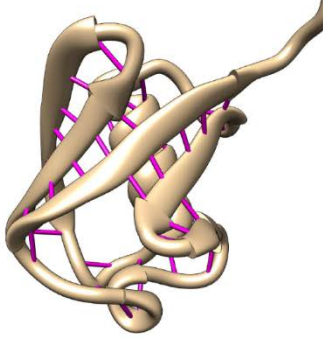
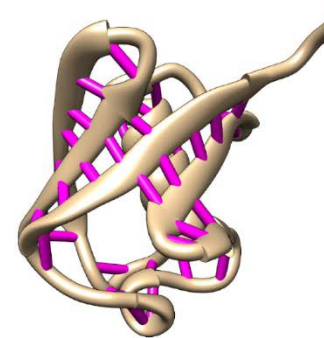
**hbond** - renders hydrogen bonds for all displayed atoms

**hbond selrestrict any** - renders hydrogen bonds for atoms in the current selection

**setattr p drawMode 1** - changes bond style from wire to stick

**setattr g stickScale 2.5** - thickens stick radius



<b>hbond</b> <i>Show hydrogen bonds</i>	<b>setattr p drawMode 1</b> <i>Draw bonds as sticks</i>	<b>setattr g stickScale 2.5</b> <i>Change stick radius</i>
		

**Figure S6.** Chimera H-bonds. Steps to produce hydrogen bonds using Chimera. Hydrogen bonds are displayed as wires by default (left), and must be converted into sticks (center) and then thickened (right).

### 1.2.5) Displaying struts

Even with the addition of hydrogen bonds, many ribbon models are still too delicate to be printed successfully. Struts are physical connections within the model that do not reflect any molecular property but add to the mechanical strength, thus facilitating printing and handling. Chimera offers a quick way to automatically add struts to a model with the strut command.

#### Adding automatic struts

To create blue struts of radius 1.0 Å in the carbon alpha of every 70 residues no further than 8 Å apart, use the command:

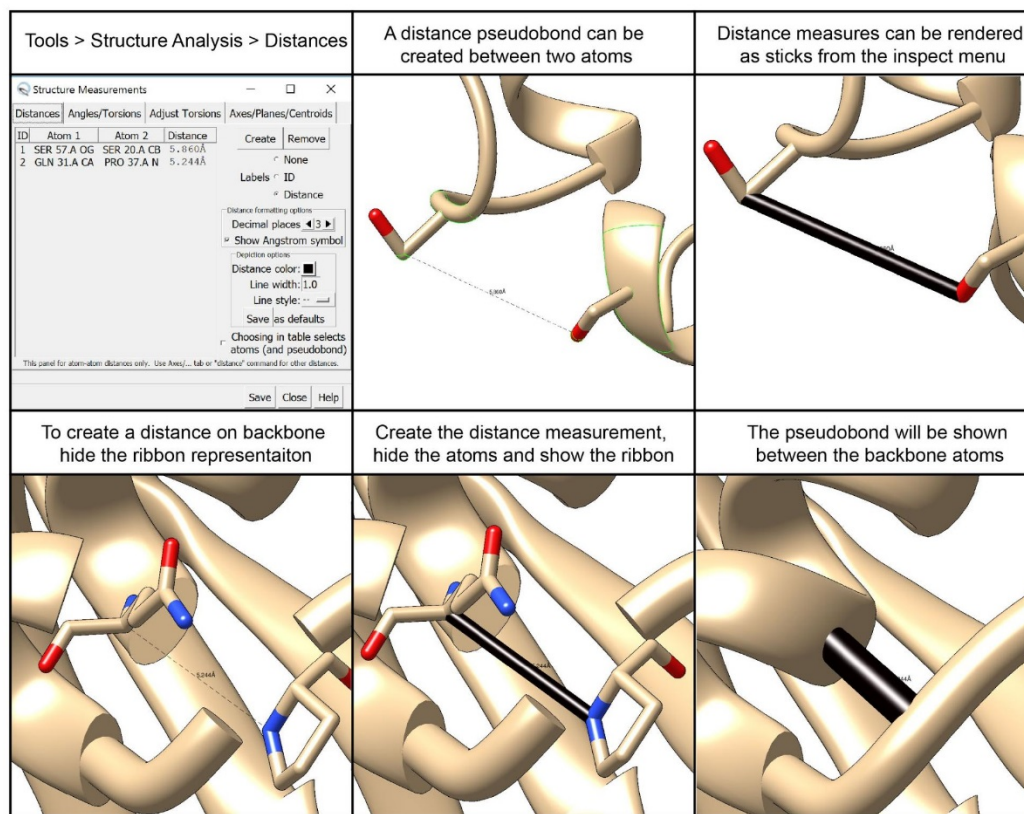
**struts @ca length 8 loop 70 color blue rad 1.0 fattenRibbon false**

Note: The struts command may contain a bug in the version of Chimera you are using. After the command is used, the direction of arrows in beta sheets is reversed. If so, open the ribbon style editor for a second time and click “apply” to correct the change.

Struts can be created manually in Chimera with the distance measure tool. The distances menu can be opened by going to **Tools > Structure Analysis > Distances**. From there, a distance measure can be created between any two atoms that are selected. To add a distance measure strut to the backbone atoms of a protein, the ribbon representation must first be turned off, and the atoms displayed and selected. After adding the distance pseudobond, the ribbon can be turned on again, and the atoms hidden, resulting in a strut between ribbons. Distance measures are a type of pseudobond and can be displayed as sticks and thickened with the inspect menu or the command line in the same manner as hydrogen bonds.

Note that in the workflow you must use the ribbon style editor to set ribbon scaling after using the struts command due to a bug in the struts command that reverses the direction of arrowheads.

Instead of distance, one can also create actual bonds by selecting 2 atoms, and using the command **bond sel**. The bond can be removed by selecting it and going to **Actions > Atoms > Delete**.



**Figure S7.** Chimera struts using distance tool. **Top:** Steps to create a distance measure pseudobond between two atoms. **Bottom:** to create a pseudobond between two residues, atoms from the backbone must be selected by hiding the ribbon representation of the desired residues, showing the atoms, creating the distance measure, and then showing the ribbon again.

## 1.2.6) Scaling and thickening for printing

Depending on the nature of your biomolecular representation, scaling of the model from the default magnification before printing may be essential to success. Most FFF 3D printers do not reliably print features smaller than 0.2 mm, which will occur in unscaled ribbon and stick models. Ribbon models generally require at least 200% scaling from the default to increase the stability of delicate ribbon structures and overhangs. Complex ribbon models may require multiple attempts to print correctly. Surface models can usually be printed as small as 50%; 100% scaling is more reliable.

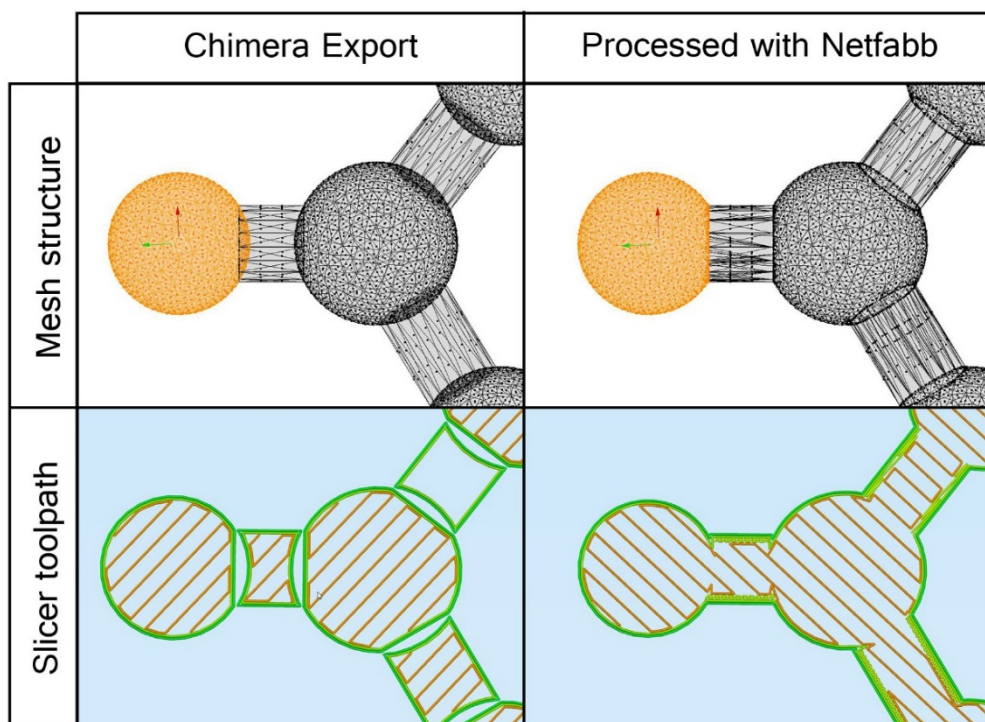
## 2. Processing STL files for printing

### 2.1) Repair STL files with Autodesk Netfabb

A 3D model file generated is often not continuous, but rather a number of pieces with intersecting geometries. For example, a hydrogen bond is a cylinder that overlaps with the closed surface of a ribbon. This can cause errors when the file is read by some slicing software, as regions where two geometries overlap can be interpreted as the exterior of the model. To correct this, utilize *cleaning software* to obtain a continuous mesh from the separate geometries within the file. Autodesk Netfabb is a mesh cleaning tool that will correct a number of common mesh errors. A limited version of Netfabb, called Netfabb Basic, is freely available



(<https://www.netfabb.com/products/netfabb-basic>). It lacks the mesh merging capability of the full version, Netfabb Standard. Autodesk offers free educational licenses for Netfabb Standard ([www.autodesk.com/education/free-software/netfabb](http://www.autodesk.com/education/free-software/netfabb)).



**Figure S8.** Repairing meshes for printing. Depiction problem that occurs with the STL model hydrogen bonds generated by Chimera before being run through Netfabb, and how it looks after.

## 2.2) Orient models for printing with Autodesk Meshmixer

Autodesk MeshMixer is a freely available tool with several functions for manipulating 3D meshes, used here to optimally orient models and minimize overhangs for printing. A correctly oriented model will print faster, require less support material, and be less likely to fail. To orient your model before printing, open Meshmixer and import your model, then use **analysis > orientation**. Four sliders are presented. The overhang angle determines which orientation of a triangle relative to the surface is required for it to be considered an overhang. It is acceptable to keep this value at the default of 45 degrees. The other three sliders are the part strength, support volume, and support area. Changing these sliders adjusts the priority (or weight) of optimizing that component. For example, the default setting with Support Volume at 100, Strength at 0 and Support Area at 0, will orient the part so that the total volume of supports is minimized. For molecular models it is most useful to minimize the number of overhangs. To do this set Support Area to 100, Strength to 0 and Support Volume to 0. Click accept and then use File>Export and select STL. It may be useful for the user to experiment with this feature to find optimal settings for their models.

## 3. Slicing and printing

### 3.1) Selecting a filament material

A wide variety of thermoplastic polymers are available for FFF printers. Select a material based on the properties required in the printed object and the capabilities of the available printers. The most commonly used filaments are polylactic acid (PLA) and acrylonitrile butadiene

styrene (ABS). Additionally, thermoplastic elastomers (TPE) are available and can be used to create flexible models.

We find that PLA is the most effective material for printing biomolecular models. With a low melting temperature and active cooling, PLA prints small parts and bridges gaps better than other filaments. Compared to ABS, it produces fewer and less harmful particulates and is therefore more suited for unventilated areas. Due to its low melting temperature PLA can be actively cooled during the printing process. The application of airflow to the point of extrusion causes the filament to solidify rapidly, preventing it from drooping or changing shape. This allows small features, overhangs, and bridges to be fabricated accurately. PLA can adhere to a build plate that is at room temperature and coated in kapton tape. However, it is advised to use a glass bed heated to ~60°C for best results. One drawback of PLA is that it causes more friction than ABS and can occasionally jam in printers that use a bowden tube to connect the extruder to the hot end. PLA is also less resistant to high temperatures and more brittle than ABS.

TPE has similar material properties to PLA but is more difficult to extrude. The use of flexible 3D printing filament enables the fabrication of models that can mimic some of the dynamic properties of biomolecules. Flexible models can assist in studies of protein folding, protein-protein binding, and ligand-protein binding. Using flexible filament adds utility to both ribbon and surface models. The material properties of flexible filament create some specific printing challenges. The softness of the filament makes it more likely to buckle when undergoing compression, which occurs between the driving gear and the hot end. For printers that use a long tube between the drive and hot end (a bowden tube) the filament can jam in the tube and prevent extrusion. Some bowden tube printers can print with flexible filament, but only at very slow speeds. The softness of the filament also causes issues with retraction. With other materials, the reversal of the drive gear is rapidly translated to the hot end and quickly pulls the filament away from the surface of the print. Flexible filament will stretch during retraction, causing a time lag between the action of the drive gear and the filament retraction at the hot end, thus retraction settings should be changed or eliminated for flexible materials.

	Nozzle Temperature	Bed temperature	Active cooling	Fumes	Flexibility
PLA	190- 210	60	Yes	Minor	Brittle
ABS	235-245	120	No	Hazardous, unpleasant smell	Minor
TPE	190-210	60	Yes	Minor	Significant

### 3.2) Generate G-code

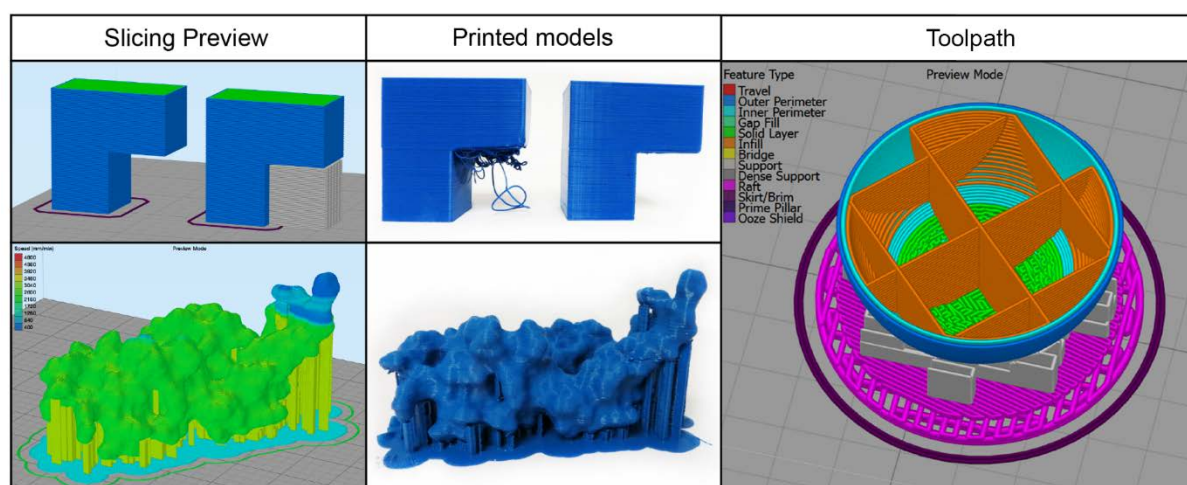
*Slicing* is the process of converting a 3D model file into instructions for a 3D printer to create a physical replica of the structure. To do this, a 3D shape is sliced into a stack of horizontal layers with sequential printer commands. The slicer software is used to define parameters important to the printing process, such as object location and orientation, printing temperature, layer height, printing speed, interior fill, and supports. These instructions are often referred to as G-code and .gcode is a common file type used by many desktop 3D printers. The slicer can stream instructions directly to the printer or save a .gcode file onto an SD card.

There are many types of slicing software available, including Cura (open source), Slic3r (open source), Craftware (freeware), and Simplify3D (proprietary). The instructions in the protocol

are for Simplify3D, which has a number of features that greatly assist the printing of biomolecular models. Simplify3D is the only slicer that allows customization of supports, which can be individually added or removed. This is often necessary when preparing ribbon models. Additionally, the software is compatible with virtually every desktop FFF printer and has optimized profiles for many of them. Although Simplify3D is the best solution for biomolecular models our instructions can be adapted to other slicing software, as many settings are equivalent or similar.

### 3.2.1) Simplify3D

Supports are temporary structures included in the G-code to enable printing of overhangs. An overhang is a part of a print that is at more than 45° away from vertical or a part where the lowest section has nothing underneath it. Because material extruded to form overhangs would not have anything to connect to, temporary support structures hold overhangs in place until they are connected to the rest of the print. Supports are generated by slicing software as the print is being prepared. The placement of supports is an important factor to the success of a print. They must be sufficient to hold all overhangs in place and must be easy to remove after printing.



**Figure S9.** Supports in Simplify3D. **Top Left:** slicing preview and printed result of a test model with and without supports, showing drooping that occurs. **Bottom Left:** Slicing preview and printed results of histone monomer surface prior to support removal. **Right:** Cross section of the toolpath generated by Simplify3D for a sphere, allowing the user to inspect for possible problems.

Simplify3D has very effective support structure generation and editing tools. Support structures should first be generated automatically, using an overhang threshold between 45° to 60°, depending on the properties of the printer. The resolution of the supports can be defined; finer resolution will provide better support, but will be more difficult to remove.

After automatic supports have been generated, the supports should be inspected and edited as necessary. The support generator will miss some sharp bottom angles, so extra supports should be added to the lowest points of ribbons. Supports should be removed from crevices where they could not be removed from the physical model, such as inside binding pockets on surface models, and inside alpha helices in ribbons.

The cross section tool can be used to visualize where supports are being added. It also makes it easier to remove supports within crevices when there is no direct line of sight from outside the model. Suggested support settings are shown on the table below.

Once supports have been finalized, the model must be converted into G-code using a specific process and saved. Click 'prepare to print' and select the specific process for the printer.

Simplify3D will use the profile settings in the process to construct a toolpath, which will be displayed in the primary window. The visible layers of the print can be selected with a slider, and the paths are colored by part type or toolhead speed.

It is important to preview and inspect the generated toolpath before exporting it as a .gcode file. This allows problems with the model to be detected before starting a print that would fail because of a bad toolpath. Errors that can be found in the toolpath include overlapping parts for models that have not been repaired and features that are too small to print. Features should be large enough so that both the external shell and one internal perimeter shell can be printed.

Support settings	
Support infill percentage	20%
Extra inflation	0
Dense support layers	1
Dense infill	70
Print support every	1
Horizontal offset	0.2
Upper vertical separation	1
Lower vertical separation layers	0

### 3.2.2) Simplify3D settings

**Extruder:** Retraction is the pulling of the filament towards the drive gear, to minimize oozing. Bowden tube printers should use retraction distances between 4-8 mm. When printing with flexible materials, retraction should be minimized.

**Layer:** Layer height is a tradeoff between print speed and resolution, a value of 0.2 mm is reasonable.

Solid top and bottom layers will prevent gaps between the interior and exterior of the model in the Z axis. Seven layers is ample.

The number of Perimeter shells determine the thickness of the exterior of the model in the XY axes. Values greater than two may be useful for very thin ribbon and stick models.

It is essential for the first layer to adhere to the build plate. Running the first layer at 50% of the default speed on the 'First layer settings' will reduce print failures.

**Rafts and brims:** Rafts and brims should always be included in the printer profile when printing molecular models. A brim is a thin outline of the shape of the model printed on the build plate, it ensures that the material in the hot end starts exiting the nozzle before critical parts of the print begin.

A raft is a flat structure at the base of a print, connecting the build plate to the supports and the printed part. By increasing the surface area between the part and the build plate, the raft increases adhesion and prevents the part from becoming dislodged during the printing process.

A raft is essential to printing any biomolecule. The efficacy of the raft can be increased by using 1 raft layer, 6 mm offset from part, 0.0 mm separation distance, 100% raft infill, and disabling raft base layers.

**Infill:** Infill is the material printed inside of the part. Surface models should be printed with 10-20% infill, and ribbon models with at least 20-30% infill.

**Cooling:** If printing with PLA or flexible filament, cooling should be turned on at the second layer.

#### **4. Post-production processing**

An alternative to manual support removal is use of a dissolvable support, but it requires a dual extrusion printer. In this process two filaments are used: (1) the model is printed with a primary material; (2) the supports with a secondary material. The secondary material is dissolved when the printed part is submerged in a solvent. Dual material printing is only possible with printers that use two nozzles or a hot end that can change materials. We find that printing with multiple materials greatly increases the number of possible points of failure in the printing process. The adhesion between different materials may not be strong enough to hold them together and the secondary nozzle may dislodge the print. The application of solvent is dangerous and requires a fume hood or outdoor space. The best of the bad combinations is an ABS model with PLA support, later dissolved using an aqueous base such as sodium hydroxide. We feel that the advantages of dissolvable support do not outweigh the costs.

### **5. Printing instructions for models shown in figure 4 of the protocol**

#### **5.1) HIV surface with glycoproteins and antibodies**

To simulate the HIV membrane (envelope), create a sphere with a radius of 100 nm and wall thickness of 10 nm in Autodesk Netfabb. To simplify the model and make it smaller, cut the sphere to give a spherical segment. Make the viral spikes by generating a surface model of the gp120 trimer ectodomain of the (PDB: 5FUU) and manually add a transmembrane and internal domain that mimics the 3 helices unresolved in the structure. The spikes were randomly distributed over the envelope at a frequency similar the native population measured by EM studies. Print the membrane (here in yellow) and the glycoprotein (here green) using a dual extrusion PLA printer (Flashforge Creator Pro in our case). Print the IgG antibodies (PDB: 1IGT) separately and attach using a spot of modeling clay or blue tack.

#### **5.2) HIV protein surface with antibody ribbons**

Download the coordinates of the crystal structure of the glycosylated HIV envelope trimer in complex with 3 broadly neutralizing antibodies (PDB: 5FYJ). Cut the model using planes in Netfabb to only the contact region between gp120 and the variable region of the antibody VRC01. Generate a surface model for the glycoprotein. Print the HIV protein fragment with 2 filaments - in our case blue for glycosylations and green for protein. Create a ribbon representation of the antibody fragment, add a disc for support, and reveal the side chains that contact the HIV protein. Add extensive support or the ribbons will not print correctly. Print the antibody in 2 colors corresponding to the heavy and light chain. Assemble the puzzle. There's only 1 position at which the antibody fits well over the HIV protein. Test your colleagues.

#### **5.3) Ribosomal complexes**

Download the structure of the thermus thermophilus 70S ribosome in complex with mRNA and 3 tRNAs occupying A-, P- and E-sites (PDB: 4V5D). Generate a molecular surface for all chains. **Left** - Print the model in 2 colors to represent ribosomal RNA ( here red) and protein components (blue) followed by printing tRNAs in flexible material (white). This will enable you to fit them into the rigid ribosome cavities. Alternatively (**right**), print the ribosome in one color



(white) and the transfer RNAs in another (green) to highlight their position. Add the mRNA (red) and the exiting polypeptide (blue) by gluing in 1.7 mm filaments at the right positions. The exit channel is located at the back of the large ribosomal subunit. If not blocked by support the green of the tRNA can be seen.

## **6. Failures, common problems, and solutions**

To alert the user to some of the challenges encountered when using desktop 3D printers, we have compiled common issues that occur and possible solutions. Comprehensive support resources are available online to help with troubleshooting, including Simplify3D support guides (<https://www.simplify3d.com/support/>).

### **6.1) Uneveled build plate**

**Problem:** If the build plate is horizontally levelled not leveled it will cause the extruder to be too close to it in some regions, preventing filament from being extruded, and too far in other regions, preventing filament from sticking to the build plate.

**Solution:** Level the build plate, usually done manually using knobs on the bottom, and ensure that when Z axis is on the home position there's a small distance between the nozzle and the build plate, sufficient for a piece of paper to be slid through with a little friction.

### **6.2) Print detaches from build plate**

**Problem:** Sometimes the print may detach from the build plate by being knocked off if it's base touching the build plate is too small, or if the adhesion to the bed is insufficient, as can happen in a heated build plate that's not hot enough.

**Solution:** Adding rafts during slicing greatly reduces chances of detachment. Some materials adhere to the bed better than others. PLA adheres especially well, while for other materials such as ABS, PET and Nylon it is much harder to obtain good layer adhesion.

### **6.3) Supports knocked off**

**Problem:** Since supports are often tall with small bases, they can easily be knocked off if the first layer adhesion is not good.

**Solution:** To avoid this, rafts can be used, as they provide a base to which supports adhere very well when extruded. Because molecular models have many supports, rafts are highly recommended.

### **6.4) Nozzle clog**

**Problem:** Clogs can have several causes, such as accumulation of degraded material on the walls of the extruder and reduces flow rate, or inconsistencies in the diameter of the filament, making it too thick to fit the hot end. Sometimes the filament may be ground by the driving gear, resulting in a piece of filament stuck in the hot end.

**Solution:** The best way to prevent clogs is to periodically drill the printer nozzle, so as to remove material that is adhering to the nozzle wall. Additionally, lubricating filament with mineral oil by having it pass through a sponge with a few drops of oil on top of the extruder also reduces clogging, because it reduces friction with the extruder walls and accumulation of material. When a clog occurs, if pushing the material down is not sufficient, the hot end must be disassembled and cleaned.

### **6.5) Filament tangle**

**Problem:** if there is a knot in the filament spool or if it tangles around the spool holder, material flow will stop, and if this happens even for one layer, the print will be compromised.

**Solution:** It is nearly impossible to prevent tangles when the filament spool has a knot and it happens in the middle of the print, unless someone sees the table and reacts in time. Ensuring that the spool holder is good and can rotate freely is the only other thing that can be done.

### **6.6) Material accumulation on the hot end**

**Problem:** If part of the printer is knocked off or it detaches, and extruded material starts adhering to the hot end, a large blob of material can form on the extruder if the print is left going for a long time (such as overnight prints). This is the worst kind of failure, and in rare occasions so much material may accumulate on the extruder that it becomes impossible to remove without damaging the wires, and the whole hot end needs to be replaced.

**Solution:** Other than checking the print periodically and using rafts to ensure it won't detach, there is not much else that can be done. This kind of clog can only break the extruder if left unattended for several hours as in overnight prints, so it is very rare.

### 6.7) Warping

**Problem:** If the model being printed has a large continuous surface, the contraction of the extruded material as it cools down can cause the entire model to warp.

**Solution:** Increasing room temperature diminishes warping. Industrial printers (which usually print in ABS) keep the chamber temperature at 80°C or above, preventing warping altogether. Warping also changes considerably depending on material. PLA is very good in this regard, whereas ABS, PET and Nylon are very prone to warp during cool-down.

### 6.8) Filament kink

**Problem:** For some extruder assemblies where there is significant space between the driving gear and the hot end tube, flexible material may kink and curl around the driving gear, instead of being pushed down. The same thing may happen with hard plastics if they heat too much and become soft prior to being pushed down the extruder due to insufficient cooling.

**Solution:** Some extruder assemblies, such as those using bowden tubes, generally can't print flexible materials. If PLA or other plastics are curling around the driving gear, something might be wrong with the insulation between the hot end and the driving gear assembly, or the cooling fan might not be working.

## 7. Uploading files to the NIH 3D Print Exchange

The NIH 3D Print Exchange is an online repository of biomedical models. It can be used to process 3D model files with the full Netfabb capabilities when models are uploaded to the repository.

- To use or upload a model to the NIH 3D Print Exchange, navigate to <http://3dprint.nih.gov>. Log in or create an account.
- Go to the 'Share' page on the 3D Print Exchange. Select the category that most clearly defines your model.
- Upload the 3D STL file exported from Chimera. Add relevant additional information, e.g. title, description, references, pictures, printing guidelines, authorship and licensing.
- On the 'Upload your files', mark the checkbox 'Ensure printability with Netfabb'. The 3D Print Exchange will automatically process the file and will host the file on a model page created for the upload. User must publish the model before the files are made available for download.