REVISION HISTORY

| DATE	DESCRIPTION OF CHANGE	OWNER	REVISION
12/07/2013	FIRST DRAFT	K. STEVENSON	1

SEQUENCE OF EVENTS
(S.O.E)

3D PRINTING PROTEIN STRUCTURES

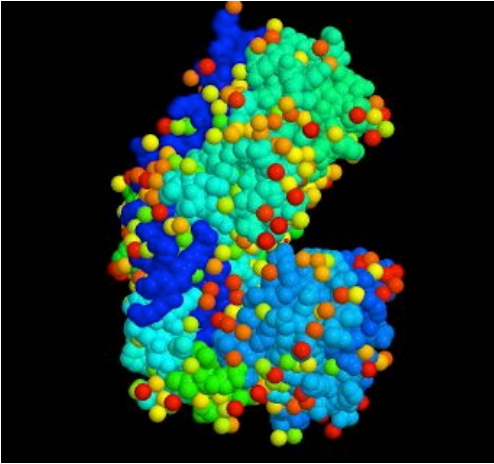**Prepared for: S. Bailey**, Professor of Biological Sciences, Mira Costa College
Prepared for: P. Clarke, Professor of Drafting & Design, Mira Costa College

Prepared by: K. Stevenson, Mira Costa College DESN 204 Student, Fall 2013

The proteins that started it all!
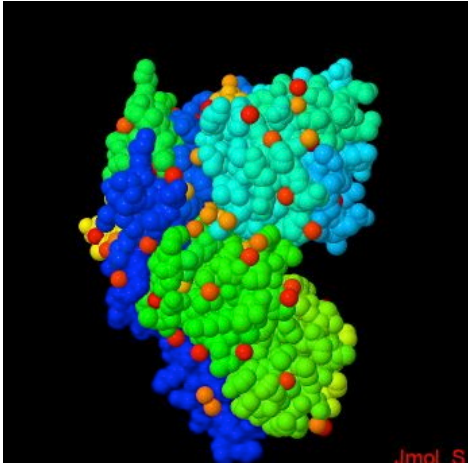
Hexokinase - Closed   Hexokinase - Open

Summary

Purpose

List the sequence of events necessary to print protein structures on a 3D printer and process them for enhancing the educational experience with protein structures that you can see and feel the difference.

Process Overview

Select and download the protein structure file (.pdb) from the Protein Database, PDB. Use PyMol software to convert the .pdb file into a .wrl file format. Use MeshLab software to convert the .wrl file format into a .stl file format for 3D printing. Print the protein structure. Remove any printing support material. Smooth the protein surface with an Acetone treatment. Paint protein structure as required.

Resources

A computer with an internet connection is needed along with certain software to perform the file conversions to the .stl file format for 3D printer software.

www.pdb.org
Internet site that hosts a database of protein structures for public use. Registration is not required but it is free and helpful.

- Requires JAVA plugin for web browser in order to be able to view the protein structure on the website and manipulate the different structural views and rotate the molecule on the webpage. Follow the instructions on the website if unable to view the protein structures. Need to burn PopUp Blocker off.
- The above is not required if you know the protein you want. You can just download the .pdb file.
- www.pdb.org

PyMOL
Is an open source software application for viewing molecular structures graphically.

- Runs on both Mac and Windows computers
- A free version for educators and students is available for download and use. Registration required along with meeting specific criteria.
- www.pymol.org/pymol

MeshLab
Is an open source software application for processing and editing unstructured 3D triangular meshes for 3D scanning and printing .

- Runs on both Mac and Windows computers
- Information and download at www.meshlab.sourceforge.net/

3D Printer
Printing for this project was performed on a Dimension 3D printer

1. Computer Software Setup

Software

Download and install PyMOL and MeshLab software. You may need to have administrative privileges in order to install PyMOL. Administrative privileges may be necessary for JAVA plugin and MeshLab installation as well. NOTE: S.O.E procedures written using a Mac computer for software installation, setup, and file conversion. Admin privileges were not reruired. Screenshots and events may vary with a Windows computer.

PDB website
Click on the link below to view the page for Hexokinase - Closed protein molecule. Download the .pdb protein file.

- http://www.pdb.org/pdb/explore/explore.do?structureId=1BDG

- Above the **"Biological Assembly"** picture of the protein, press the **"Download Files"** arrow and select **"PDB File (Text)"** or **"PDB File (gz)"**. File will automatically download to the **"Downloads"** folder on your computer.
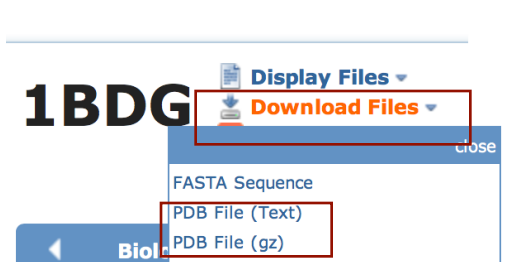
- If you want to view the protein structure in more detail, click on the **"3D View"** link under the picture of the

  protein, right side, lower left of the picture. There are many options for different views of the protein under **"Select Display Mode"**. You can press the Export 3D image button to export a .jpg of the current view if you like.

- If the protein is unable to be viewed, JAVA plugin is not installed or is the wrong version. Follow the website instructions on how to download, install and active the JAVA plugin in your web browser. NOTE: It took a couple of tries to active the JAVA plugin on a Mac.

S.O.E: 3D PRINTING PROTEIN STRUCTURES

PyMOL

Click on the link below to view the information page about PyMOL. There is the paid "incentive version" and an "Educational version"

- www.pymol.org/pymol . Web page for info. You can click on the links for the "incentive version" (purchase) or the "Educational subscriptions" version.
- COPY/PASTE FROM THE PYMOL WEBSITE: "Incentive PyMOL is a packaged software product that makes it easy to become a PyMOL user. By purchasing Incentive PyMOL, you support development and receive the following benefits. Educational subscriptions are available at no cost to full-time students and educators teaching full-time students."

- I followed the instructions/links for registering and downloading the Educational version: http://www.pymol.org/educational
- Once you register, you will be emailed a link for download access. Follow the instructions for downloading and installing the software.

MeshLab

Click on the link below to select the right version of the software to download. Right side of the page in the **PURPLE** section.

- http://meshlab.sourceforge.net
- Follow the instructions for the computer type you have.
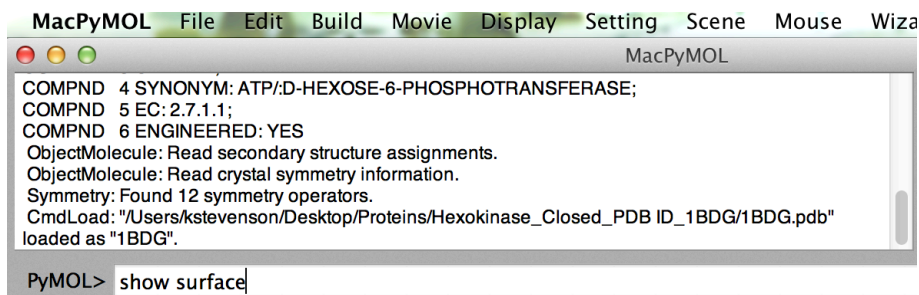
2. File Conversion

PyMOL & MeshLab
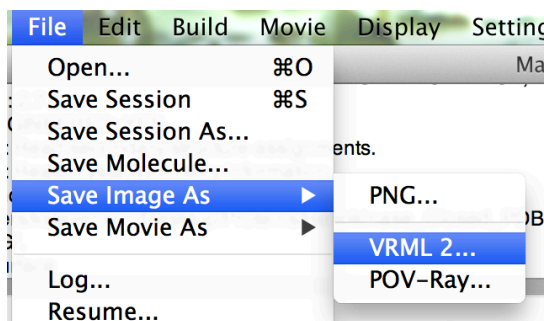
Open both applications, PyMOL and MeshLab.

PyMOL

Open the .pdb protein file in PyMOL. Use PyMOL to SAVE AS (Export) the .pdb file as a VRLM (.wrl) file.

- Go to **File>Open**
- Select the protein file ("proteinID".pdb) and press **Open**.
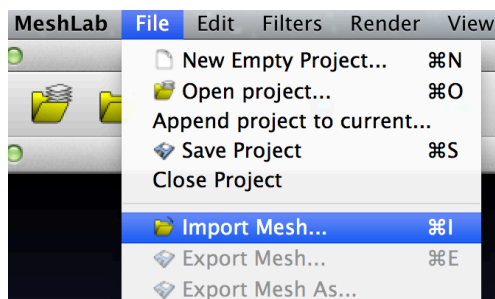- At the command line type "**show surface**" and press **Enter**.

- Go to **File>Save Image As>VRLM2** (.wrl). Name and save the file.
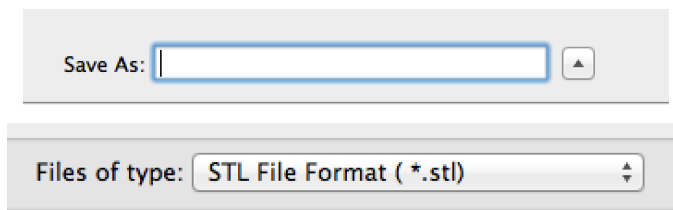

MeshLab

Open the .wrl protein file in MeshLab. Use MeshLab to SAVE AS (Export) the .wrl file as a .stl file.

- Go to **File>Import Mesh**. Select the protein .wrl file and press **Open**.

- Go to **File>Export Mesh As**. Select "**STL File Format (*.stl)**" file type in "**Files of type**" drop down menu. Name the file and press **Save**.

3. 3D Printing

3D Printer

Decide on a type of printer and material to use for printing the protein.

NOTE: Best results so far have been to use the Design Departments Stratasys Dimension printer using white ABS material.

**CatalystEX Dimension Software**

Find the computer in the Design Lab that has the Dimension CatalystEX software on it.

- Double Click the **CatalystEX** software shortcut on the Desktop.
- Go to **File>Open STL FILE>** select the protein to be printed and press **Open**.
- On the **General** tab, verify printer model is: **sst768PO7114 (Dimension SST/SST768)**
- On the **General** tab, verify printer status is Ready.
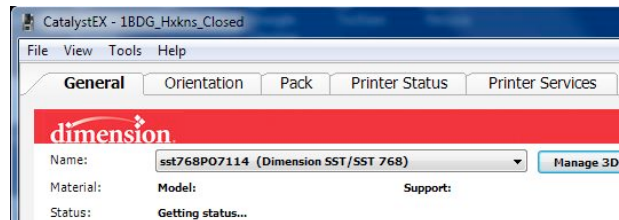
- On the **General** tab, verify the Model **Properties** settings: see screen shot below. **NOTE: Scale** - (Varies, currently using a scale of 2 for Hexokinase), also, **Number of Copies** may vary depending on how many need to be printed.
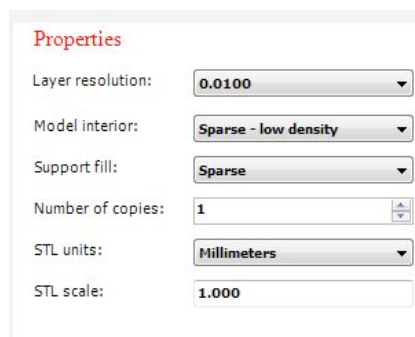
- On the **Orientation** tab, adjust/rotate the protein so that it is as flat as possible on its side to minimize the height. This will keep the amount of support material needed to reduce cost as well as post processing time to remove the support material.
- Adjusting is done by entering + or - degrees in the **Degree** and then pressing the X, Y, or Z button in the **Rotate** area. NOTE: I mostly adjusted in the Y direction, sometimes in the X direction. In 30, 15, 5 degree

increments, + & -. Also, pressing the scroll wheel on the mouse and moving it will rotate the image so you can see how well the protein is 'flat' on the print bed. Rolling the scroll wheel will zoom in and out as well.
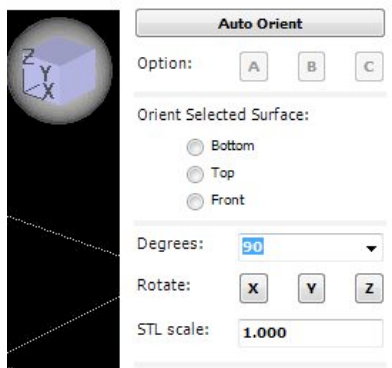
- On the **Orientation** tab, press the **Process STL** button to have the software calculate how to build the protein. May take some time, 5 to 10 minutes. Depends on complexity and scale size.

- Calculations are complete when the image in the looks something like the screen shot below, as well as the **BLUE** progress bar stops appearing/moving.
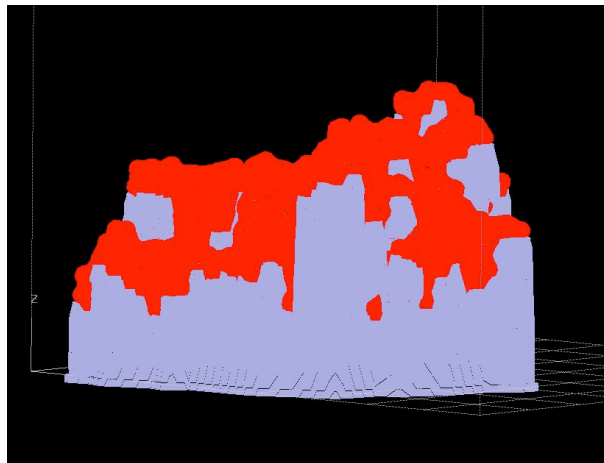
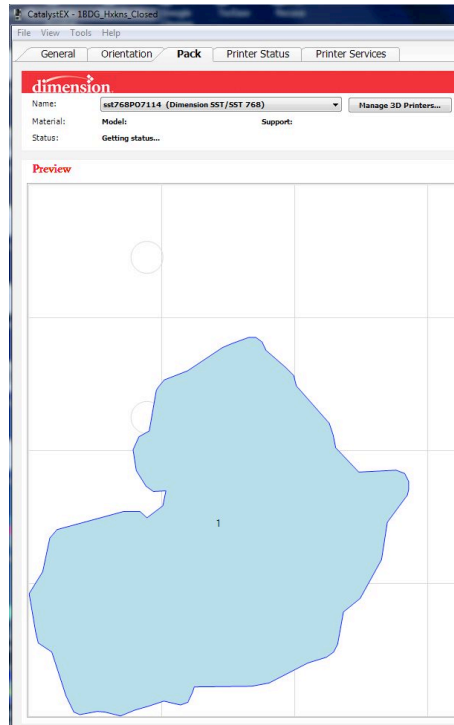- Press the Add to Pack button. Then go to the Pack tab and check the placement of the protein on the print bed. Move as desired.

- Go to the **General** tab and verify that there is enough **Material** and **Support** to complete the print job. Compare what is in the cartridges to the amount CatalystEX calculated will be used, **cubic inches**, for **Material** and **Support**.

  NEED DIFFERENT SCREEN SHOT TO SHOW QUANTITY OF MATERIAL AND SUPPORT IN THE PRINTER AND QUANTITIES CALCULATED TO PRINT THE PROTEIN
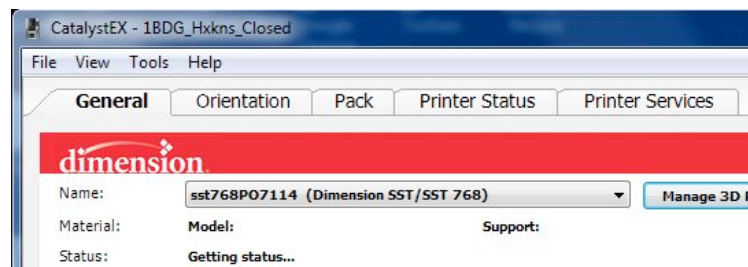
- Press the **Print** button (lower left corner) and come back after the amount of hours the software says it will take to print in the upper right corner of the **General** tab.
- When the print job is finished, the support material needs to be removed. Turn on the heated bath to 70 degrees Celsius and let it soak until all of the support material is dissolved. **ASK FOR HELP IF UNSURE. ALWAYS USE THE PROTECTIVE GLOVES WHEN WORKING IN THE BATH. IT IS AN ALKALINE SOLUTION.**
- Length of time for soaking in the heated bath varies with the amount of Support material used.
- Rinse and dry the protein once it is finished in the heated bath.

4. Surface Finishing

Acetone Treatment

There are 2 types, heated and room temperature treatments. The Acetone treatment is not really necessary, however, the protein looks a lot better and will be easier to paint.

NOTE: PERFORM IN A WELL VENTILATED AREA AND USE GLOVES WHEN HANDLING ACETONE. The "over the counter" kind from a DYI store is all that is needed.

Heated Acetone Treatment

Not a lot of Acetone is needed. Will need to find a container that is big enough to hold the printed protein and can also be heated on a hot plate.

- Find a way to fit the protein into a container, preferrably glass so you can see how the process is progressing. **DO NOT LET THE PROTEIN TOUCH THE ACETONE DIRECTLY...THIS WILL MELT/RUIN YOUR PRINTED PROTEIN.**
- Find a way to support the protein above a thin layer of Acetone in the bottom of the container. I used thin wire and bent it so that it would suspend the protein above the Acetone by hanging the wire on the edge of the glass jar. I also put a wire mesh with bolts in the bottom of the glass jar to prevent the protein from dropping accidentally into the Acetone.
- Ideally, a hot plate from the Chemistry Department that you can set the temperature on is best. Set the hot plate to 110 degrees Celsius.
- 1/4" to 1/2" layer of Acetone in the bottom of the container is sufficient. Put a cover **LOOSELY** over the opening of the container to allow the Acetone to heat up faster. When you see the condensation of the Acetone rising up the sides of the glass, put the printed protein in. May take 10 to 15 minutes for the Acetone to show signs of bubbling every so often to verify it is at the right temperature.
- **BE CAREFUL. THE CONTAINER WILL BE HOT. USE INSULATED GLOVES TO KEEP FROM GETTING BURNED.**
- 5 to 10 minutes is usually sufficient to smooth out the surface. May need to do the treatment in 2 phases, especially if you suspend the protein in the container. The part that is closer to the Acetone will smooth more/faster. After the 5 to 10 minutes, pull the protein out of the container and let it stand for about 10 minutes or more so that the surface is cool and a little tacky to the touch. Re suspend the end of the protein that was near the top of the container down closest to the Acetone. About 5 minutes or a little more is all that should be needed.
- Turn off the hot plate and let the protein cool.
- NOTE: THIS IS THE PROCESS I USED TO TREAT THE PROTEIN SAMPLES.

HEATED ACETONE TREATMENT

Room Temperature Acetone Treatment

Not a lot of Acetone is needed. Will need to find a container that is big enough to hold the printed protein and you will need to find a way to suspend the protein over the Acetone and allow it to be covered with some paper towels.

- Find a way to fit the protein into a container, preferably glass so you can see how the process is progressing. **DO NOT LET THE PROTEIN TOUCH THE ACETONE DIRECTLY…THIS WILL MELT/RUIN YOUR PRINTED PROTEIN.**

- 1/4" to 1/2" layer of Acetone in the bottom of the container is sufficient. Allow a paper towel to touch the Acetone. Suspend the protein over the Acetone and cover it up with the paper towels **LOOSELY** . Put a cover over the opening of the container to keep the Acetone vapors inside the container.

- It takes anywhere from 30 to 45 minutes to process the protein.

- Gently remove the protein from paper towel and the container. **BE CAREFUL BECAUSE THE ABS MATERIAL WILL BE SOFT AND PLIABLE**. Let the protein stand for several hours to re solidify.

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5. Painting (Optional)

What Kinds?

NOTE: I have not tried painting any of the samples yet so what you read next is what I have read works best.

Acrylics work best. Also, using a primer can help a great deal with the actual paint color adhering. Nail polish is also a favorite for small, delicate jobs.

Recommendation

Since the proteins are relatively small, acrylic spray primer and paint won't like work well to highlight the active and other sites on the proteins. Best to use nail polish. May need to cut the brush a little in order to have more control over where and how much paint to apply.

- Paint away, let it dry.
- Your done!

6. Close the Loop, R.A.R

Read, Apply, Revise

This S.O.E is a work in progress. Please revise as needed in order to preserve and keep the information current and correct.

Thank you.