# Segger – Loop Modeling (SegLoop)

Last updated: Nov. 24, 2015 (Segger v1.9.2, Chimera Version 1.11.0)

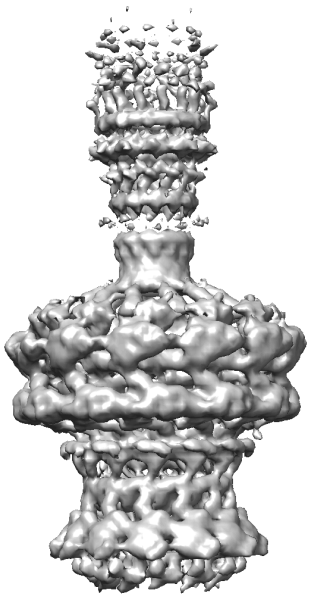
## Overview

In this tutorial, we will use the SegLoop tool to add a long missing loop to an atomic model, using segmented regions as guides.

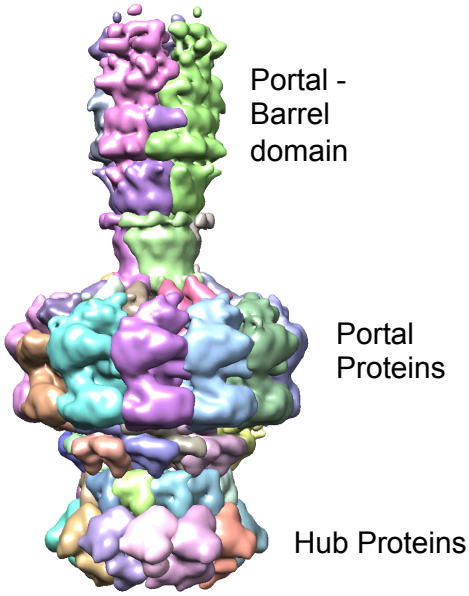
For this tutorial, download the following file from the following link:
https://www.dropbox.com/s/7rsnouhsjivfj2o/segger_tutorial_segloop.zip

## 1. Opening and segmenting the portal

- Open the file P22_c12_portal_hub.mrc.
    - Note that this is extracted from the P22_virion map, but after it was C12 averaged (with `e2proc3d –sym=C12` command). The symmetry application helps to make the protein densities smoother and easier to segment.
- Open Segment Map dialog, select the map in the Segment Map field, and press Segment. From a side view, we can see parts of the 12 proteins in the portal and 12 proteins in the hub segmented.



Portal and hub extracted from P22_virion.mrc.

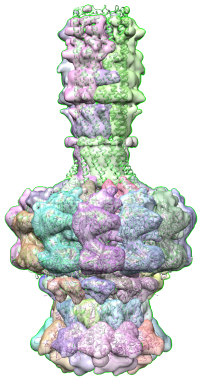



Segmented portal

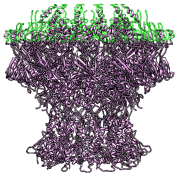
**2. Fitting Crystal Models into the Density**

- Each of the 12 proteins in the portal, as seens above, have complicated structure. We will use the crystal model to help segment them further.
- Open the files 3LJ5.pdb and 3LJ4.pdb.
- In the Segment Dialog:
    - Press the Select regions: **All** button to select all the regions – we will use these regions to quickly fit the crystal models to.
    - Open the Fit Dialog from the Shortcuts panel, press Other tools: **Fit**
- In the Fit Dialog:
    - Select 3LJ5.pdb in 'Structure of Map to fit'
    - Press the **Fit** button at the bottom of the Dialog.
    - Select 3LJ4.pdb in the Structure or Map to fit
    - Press the **Fit** button at the bottom again.
- In the main window, you should see the fitted crystal models, as shown below, in the first image on the left.
- Note that 3LJ5 contains only portal proteins (gp1), while 3LJ5 contains both hub (gp4) and part of the portal (gp1) proteins.
- We will remove the gp1 partial proteins from 3LJ5:
    - In Model Panel, hide all models except 3LJ4.
    - Ctrl+click+drag the top part of the model, as shown below, to select parts of the gp1 protein in 3LJ4. Press the up arrow twice, to select the entire gp1 proteins.
    - In the Command: at the bottom of the main window (open from Tools -> General Controls -> Command Line), type 'del sel' and press enter.

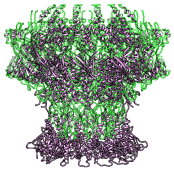
- Now in Model Panel, make 3LJ5 visible again. You should see the image on the right below, with both gp1 and gp4 proteins.



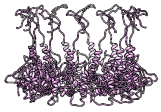
3LJ5 and 3LJ4 fitted to all portal segmented regions



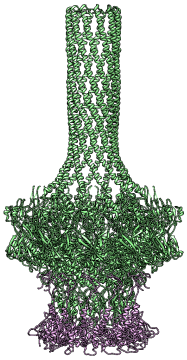
3LJ4, with top of gp1 proteins selected (use ctrl+click+drag)



After pressing up twice – all of gp1 proteins selected



After running 'del sel' to delete selected atoms



After showing 3LJ5 (gp1 – green) and what's left of 3LJ4 (gp4 – purple)

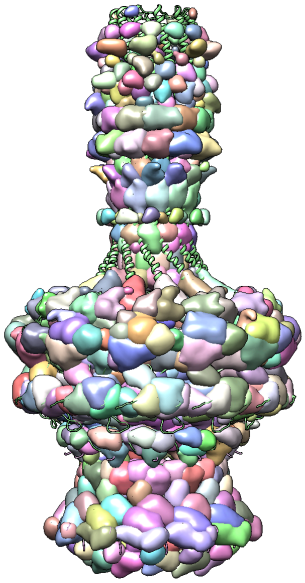
**3. Segmenting using fitted crystal models**

- In Segment Map dialog, open the Options panel, enter 0 for 'Smoothing steps', and press **Segment** at the bottom of the dialog. (Go to Model Panel to show the seg model if it is hidden).
- In the Fit To Segments dialog, from the Fit menu at the top left corner, select 'Group regions by chains in visible (Molecule) models.
    - Before doing this, make sure the 3LJ5 and 3LJ5 models are visible in the Model Panel, as in the images above, though with the segmentation on top of it as well (as in image below on left).
    - This operation will group the regions based on which chains each one overlaps the most. When done, you should see one region for each of the 12 portal proteins (gp1) and one region for each of the 12 hub proteins (gp4), as shown in the image below in the middle.
    - This operation will take about a minute – first a map is made for each chain, which is then used for overlap testing with each regions.
    - Please note that the only models considered by this operation are the ones that are visible in the main window, in other words have the boxes under S (Show) checked in the Model Panel dialog.

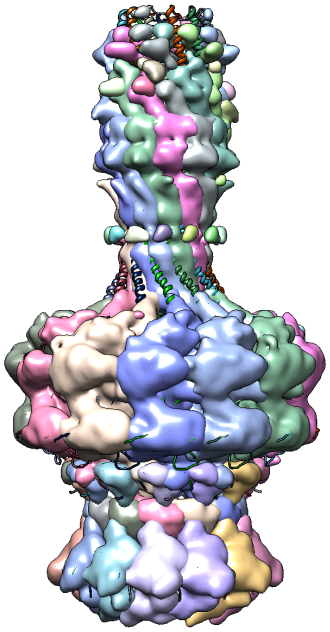
**4. Isolating a single gp1 chain**

- Ctrl+click on one of the portal (gp1) regions (the one with the long helical part in the barrel domain), and in Segment Map dialog, Shortcuts panel, click Show regions: **Only Selected**. You should then only see that region, as shown below on the right in orange.

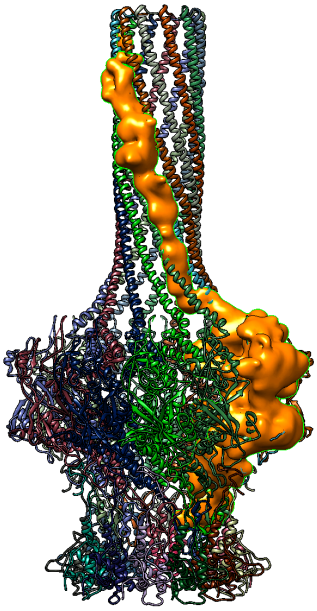
- Let's focus on only one of the gp1 proteins rather than 12, so:
  - ctrl+click on the ribbon for the chain inside the shown region (you can hide the seg model to do this, or you can follow visually where the chain is and try to find an atom that is sticking out of the region, there are a couple of places where the chain is not fully inside the region).
  - Press the up arrow twice on the keyboard to select the entire chain, then press the right arrow on the keyboard to select every other chain in the model.
  - In the Command at the bottom of the main window, type del sel and press enter.
  - Hide the 3LJ4 model in the model panel (or you can also close it, for the purposes of this tutorial we will leave it alone).



All regions in the portal+hub model after Segmenting with 0 smoothing steps.

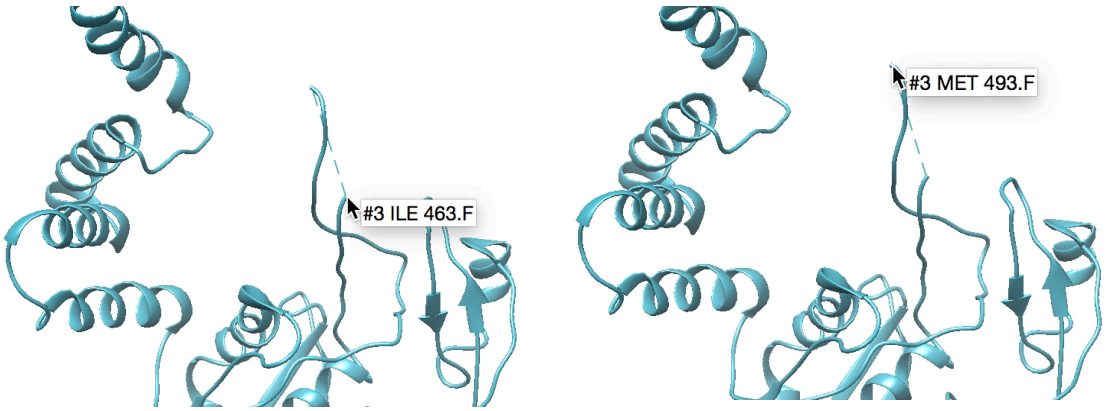


All regions after grouping based on chains in 3LJ5 and 3LJ4



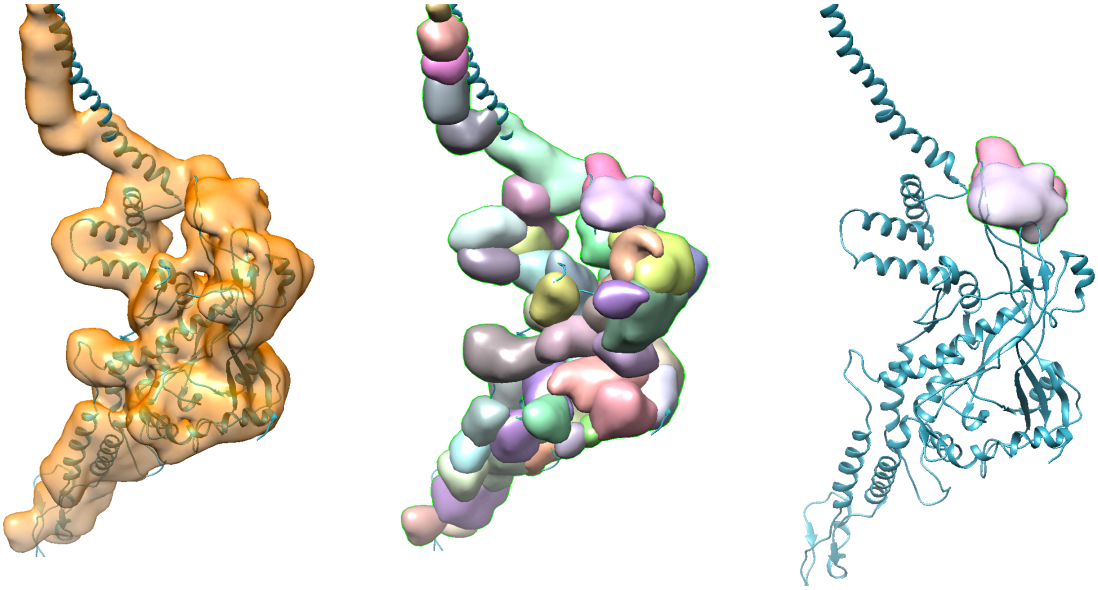
Only one region selected for a chain in 3LJ5, gp1.

- Now there should be only one portal gp1 chain and visble segmented region, as shown below.
- You'll notice there is a loop missing from the chain, as shown below. This is drawn as a dotted line along the ribbon for the chain.
  - Hide the seg model first, showing just the ribbon for the gp1 chain.
  - Move the mouse to the two ends of the dotted line to find out the residue numbers… you should see a white box with:
    - model (#3),
    - chain (.F here but you may have a different chain, which is ok),
    - residue (ILE 463, and ALA 493) as shown in the images below.

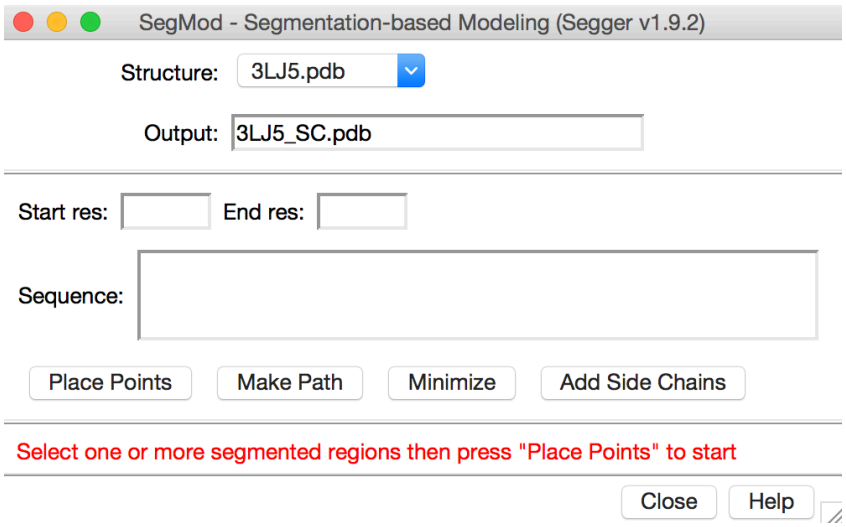



**5. Modeling the missing chain with SegLoop**

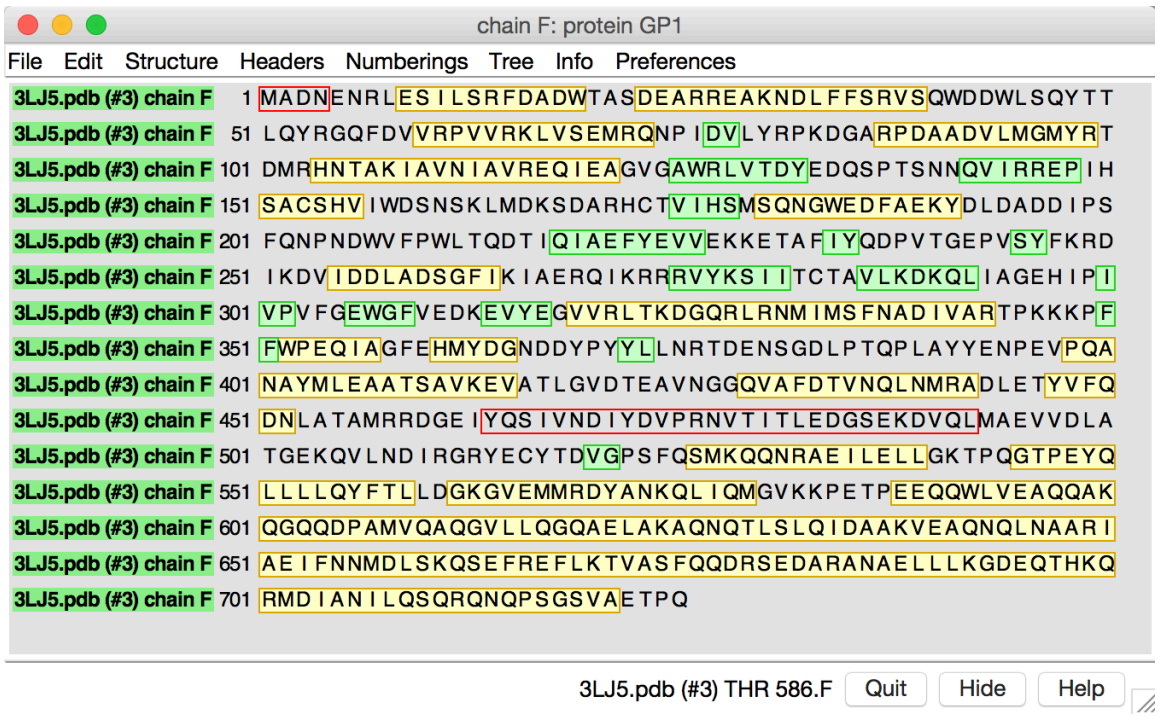
- Show the segmentation model again from the Model Panel (P22_c12_portal_hub.seg), which should have just the one region shown (as below).
- Select the region shown, and in Segment Map dialog, press **Ungroup** at the bottom.
- You should now see all the small regions that make it up.
- Select just the two regions close to where the missing loop is. To help see the missing loop part again, you can press **Transparent** in Segment Map dialog, Shortcuts panel, Selected regions:.
- In the Segment Map dialog, Shortcuts panel, press Show regions: **Only Selected**. You should then see just the two regions, as shown below on the right.



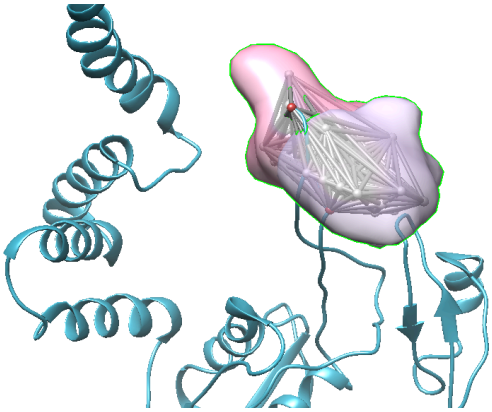
- In the Segment Map dialog, Shortcuts Panel, press **SegLoop** to the right of Other tools. This dialog is shown below:

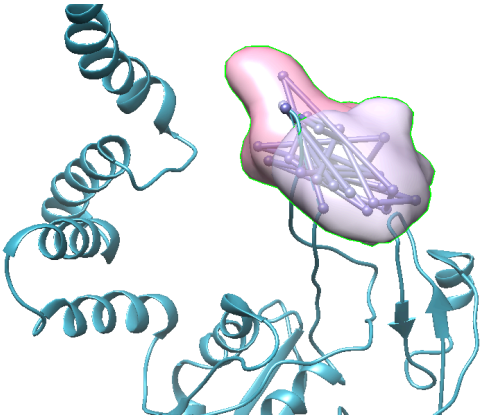



- In the SegLoop dialog, 3LJ5 is already selected as the 'Structure:', if not select it.
- In the 'Output:' field is the name of the structure after modeling.
- As we saw above, the last and first residues before the missing loop which are present in the structure are 463 and 493. So we want to build the missing residues, 464 to 492. In the SegLoop Dialog, enter:
    - 'Start res:' 464
    - 'End res:' 492
- A pdb file typically lists only the sequence of the residues that have atom positions, but they can also list the missing residues, and PDB:3LJ5 does this – you can see the missing residues by opening the PDB file, and look in the REMARKS section. You can also see the missing sequence by opening Tools -> Sequence -> Sequence, then select 3LJ5.pdb chain F (or whatever chain is left), then press the Show button in the dialog that pops up. You'll then see the sequence dialog:

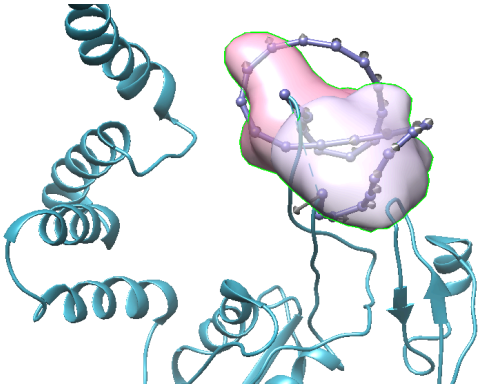



- Note the missing residues have a red box around them. You can click+drag in this dialog to select that part of the sequence, then copy that to the clipboard (Command+C on Mac, Ctrl+C on Windows/Linux).
- Paste (Command/Ctrl+V) the sequence into the 'Sequence:' text area on the SegLoop dialog:
    - YQSIVNDIYDVPRNVTITLEDGSEKDVQL
- In SegLoop dialog, press:
    - **Place Points**
    - **Make Path**
    - **Minimize**
    - **Add Side Chains**
- After each of the above operations, you should see something like in the following images, ending with a model that includes the missing residue and side chains.

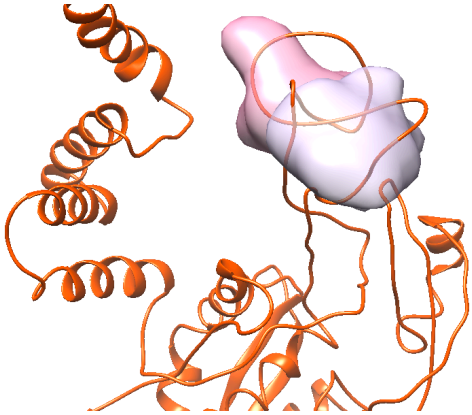




After pressing 'Place Points'. This adds a point for each missing residue in the selected regions.



After pressing Make Path. This finds a path through the points.



After pressing Minimize. This refines the path so it's smooth, and points are close enough to place a residue between them.

After pressing Add Side Chains. This adds the missing residues along with side chains to the model.

Note that while SegLoop tries to add residues so that they don't have any serious clashes, the final model should then be minimized using a Molecular Dynamics tool (such as NAMD/MDFF, or Tools -> MD -> Molecular Dynamics Simulation in Chimera, although the latter takes a long time for large structures such as this one).