Segger – Radial Segmentation (rSeg)

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

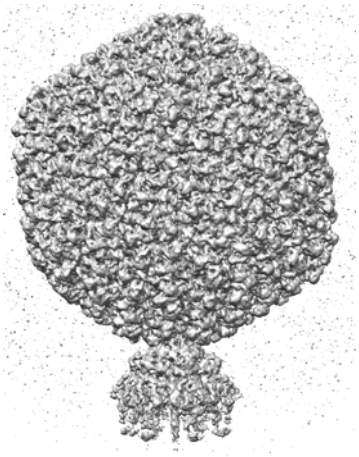
Overview

In this tutorial, we will use the rSeg tool to segment the coat, DNA layers, and portal-hub-adhesin complex in an ichosahedral virus.

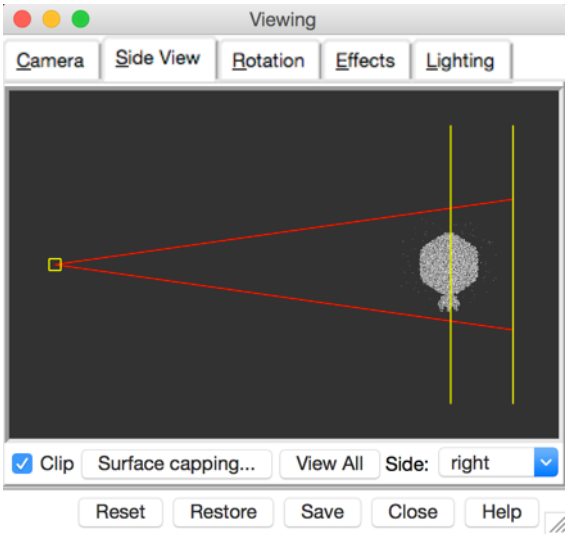
For this tutorial, download the density map for P22 virion from:
https://www.dropbox.com/s/c32huj2qjuhjkwq/segger_tutorial_rSeg.zip

1. (Spherical) Radial Segmentation

Open the map in Chimera, and set the threshold in the Volume Viewer dialog to 0.2. Turning the virus ~90° around x-axis should look like the image below.



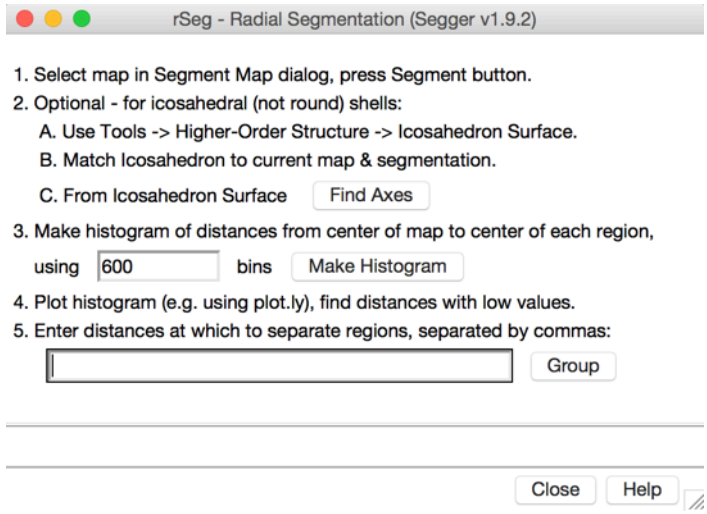
To see the inside of the virus, open Tools -> Viewing Controls -> Side View, and move the yellow line on the left to the middle of the virion, as such:

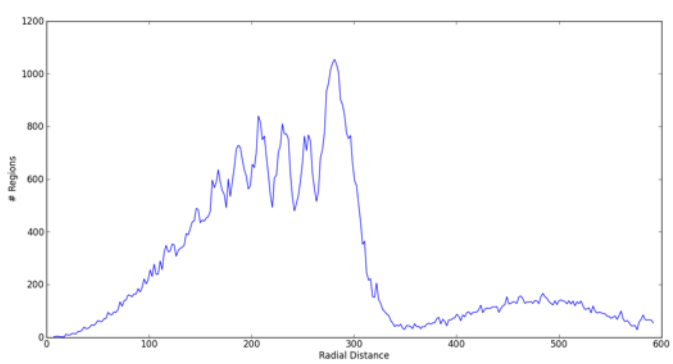


The icosahedral capsid can clearly be seen in the slice, and moreover the DNA layers inside themselves follow icosahedral geometry especially close to the capsid.

To segment the capsid and the DNA layers, we can first try radial segmentation, and see how well it does.

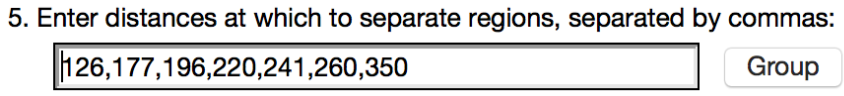
- Open the Segment Map dialog from Tools -> Volume Data, and select the map in the 'Segment map' field at the top.
- Then press the **Options** button at the bottom of the dialog.
- Enter 0 for 'Smoothing steps'.
- Because clipping can confuse Segger as it keeps track of regions surfaces, also press **View all** at the bottom of the Viewing dialog (above picture on the left).
- Press **Segment** at the bottom of the Segment Map dialog.
    - Note that this will produce ~80,000 regions, but Segger will not display them all, as that would slow down the display immensely.
- Press the **Shortcuts** button on the Segment Map dialog, and then press **rSeg** to the right of Other tools. This will bring up the rSeg Dialog:



- We've already done step 1, and for now we will not account of icosahedral geometry, so let's go on to step 3.
- Set the number of bins. A rule that seems to work well is to set it to $\sqrt{N}$, where N is the number of samples. In this case it's ~80,000 regions, and $\sqrt{80{,}000} \approx 300$, so entering 300 for number of bins works quite well.
- Press the **Make Histogram** button.
  - This will go through the ~80,000 regions and calculate the distance from the center of the map to the center of the region (the radial distance).
  - After the calculation is done, you will see a new dialog asking you to confirm where the histogram data file will be saved. Name it dist_hist_sphere.txt, and place it in the same path as where you uncompressed the downloaded file for this tutorial (path should have name segger_tutorial_rSeg).
  - In terminal cd to that path, and then run the python plot script:
    - `python plot_sphere.py`
  - You should see a plot like this:
  - 

  - Notice how there are obvious peaks and valleys. Moving the mouse over the plot, you'll notice the x and y values reported below the plot.

Let's read out where valleys are – this is where there are fewer regions with that radial distance, hence lower density shells:
    - 126,177,196,220,241,260,350
- Enter those numbers as above, with commas:



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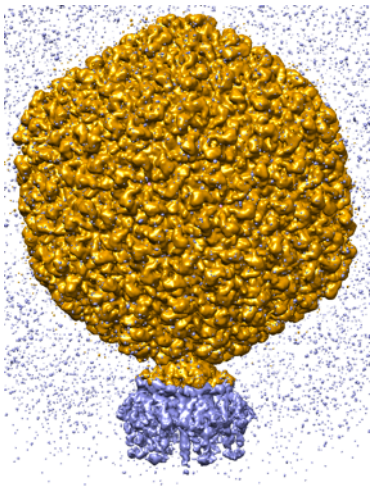
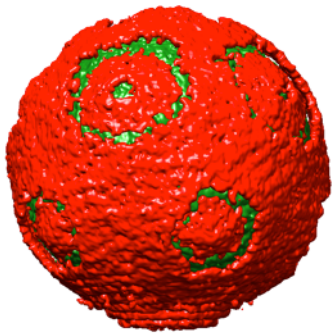
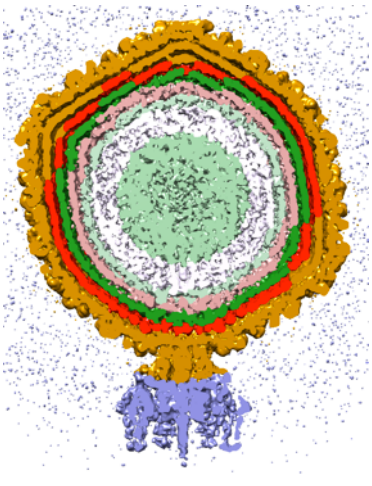
- Press the **Group** button.
- Going back to the Segment Dialog, you'll see now there are only 8 regions.
- The outermost layer is some noise outside the capsid and the tail (light blue in below images). Then the next layer is the 'capsid' (brown in the images below).
    - Hide these two layers by ctr+click on them, then Selected Regions: **Hide** in the Shortcuts panel. You'll see the first 'DNA' layer (red in images below).
- Using the clipping plane as above in the Viewing dialog, we can look at the segmented layers, as shown on the right below.



The outermost regions after grouping based on radial distance



The 3rd layer after hiding the outermost 2.

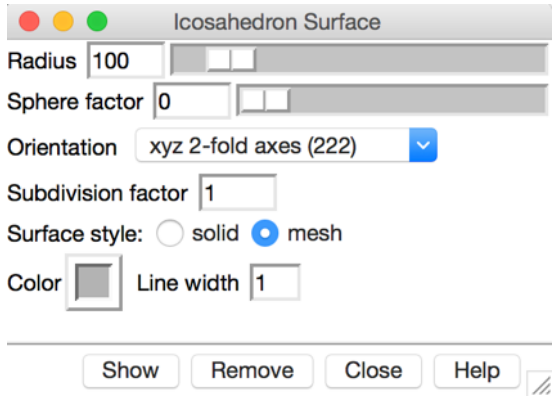


All layers shown by clipping through middle.

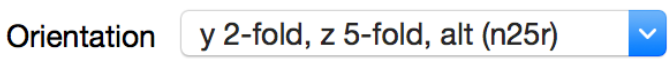
2. Icosahedrally-adjusted Radial Segmentation

We can see the layers above are not quite icosahedral – they are spherical. Let's correct the radial distances based on icosahedral geometry.

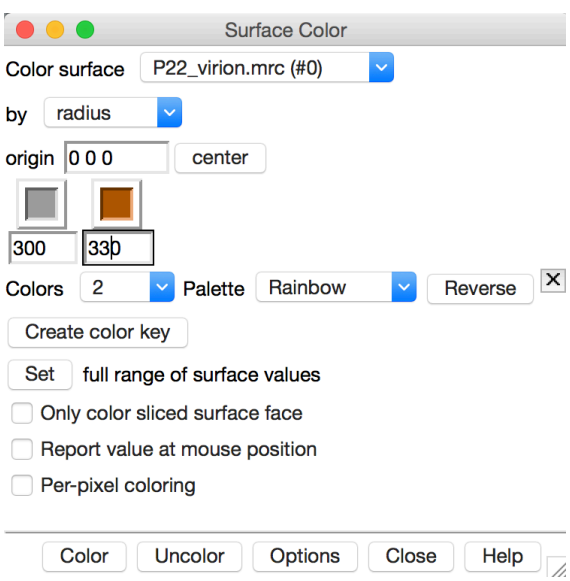
- In Model Panel, show the P22_virion.mrc model.
- Tools -> Higher-Order Structure -> Icosahedron Surface.

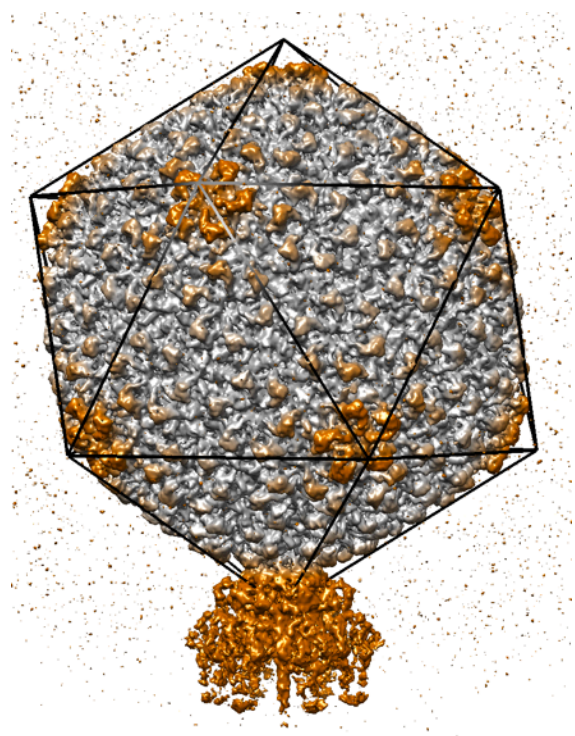


- In the Icosahedron Surface dialog, press the Show button, and then drag the Radius slider until you see the icosahedron wireframe appear, for a Radius of ~400.
- If you look carefully you'll notice the default icosahedron Orientation does not match up well with this map. The correct Orientation is the last entry in the list as below:

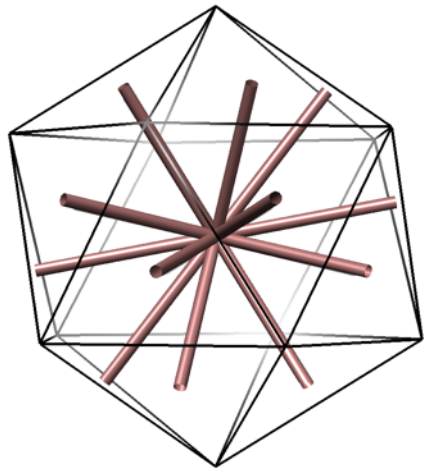


- In that orientation, you'll see the icosahedron aligns well with the 5-fold vertices in the capsid.
    - To better see the 5-fold vertices in the capsid, use Tools -> Volume Data -> Surface Color, and set as shown below on the left.

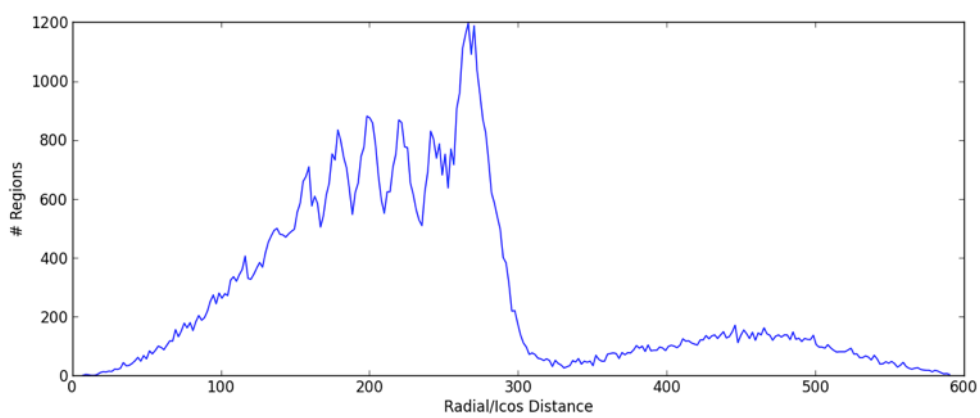




- Now let's use the icosahedral geometry to adjust the radial distances.
- In the rSeg Dialog, press the **Find Axes** button.
    - If you hide the map in Model Panel, you will see the axes as such:



    - These axes go from the center of the icosahedron to the midpoint of each of the 20 triangles that make up the icosahedron.
    - Then press Make Histogram again.
        - Now the radial distances are projected onto the closest axis, which adjusts for the icosahedral geometry.
        - Note the radius of the icosahedron doesn't matter, it's only the direction of the axes that is used.
        - Save the result as 'dist_hist_icos.txt'
        - In the terminal, run
            - `python plot_icos.py`
        - You should see a plot appear:

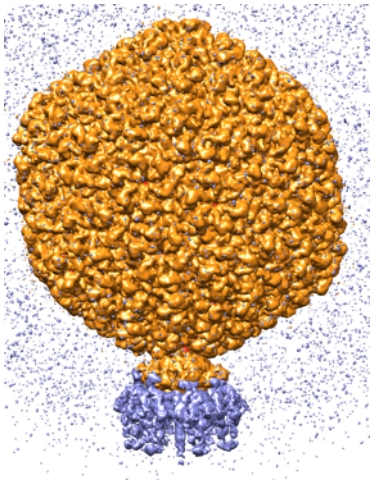


- Find and enter the x-values for the valleys in the rSeg dialog
    - 120,144,168,188,210,235,252,320

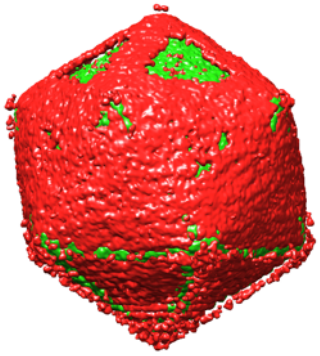
5. Enter distances at which to separate regions, separated by commas:

120,144,168,188,210,235,320 | Group

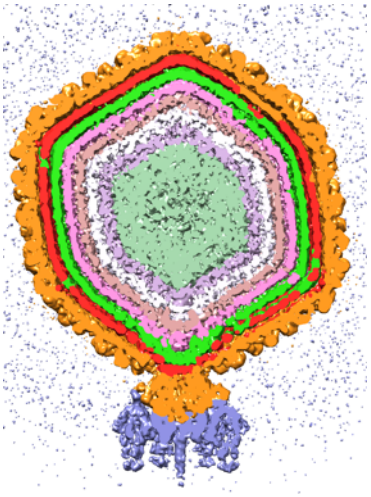
- Then press **Group**.



The outermost regions after grouping based on radial distance, with adjustment for icosahedral geometry.

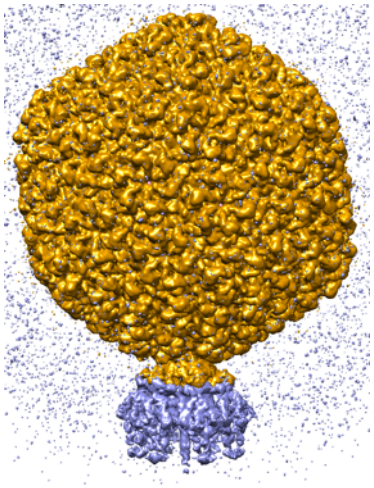


The 3rd layer after hiding the outermost 2.

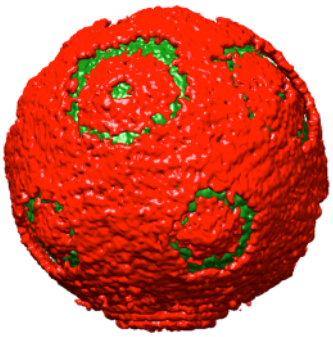


All layers shown by clipping through middle.

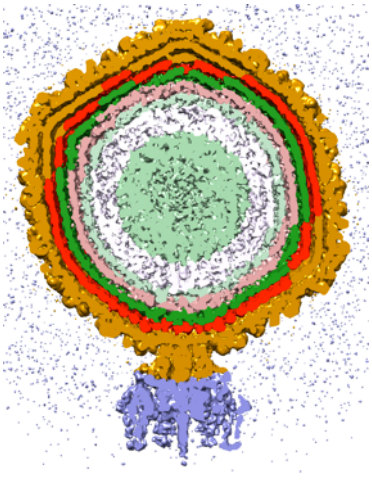
You'll notice that the segmented layers, as shown above, follow the icosahedral geometry much more closely than before. The segmetation based on spherical distance is shown below for comparison.



The outermost regions after grouping based on radial distance



The 3rd layer after hiding the outermost 2.



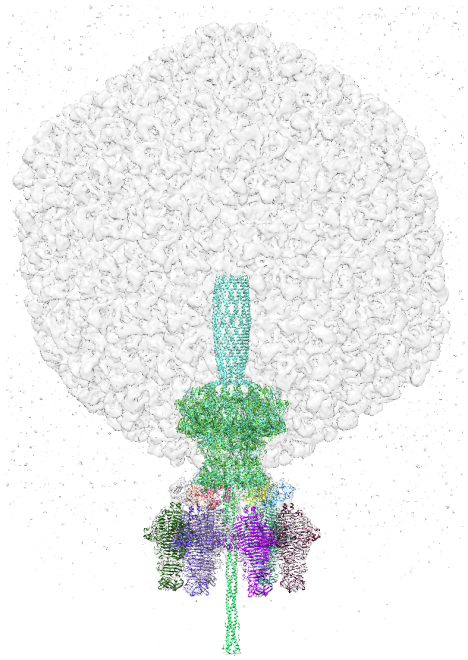
All layers shown by clipping through middle.

Even with the icosahedral adjustment, the segmentation is not perfect, as the virion itself in nature is not perfectly icosahedral, but the segmentation can be further adjusted interactively using group/ungroup operations.

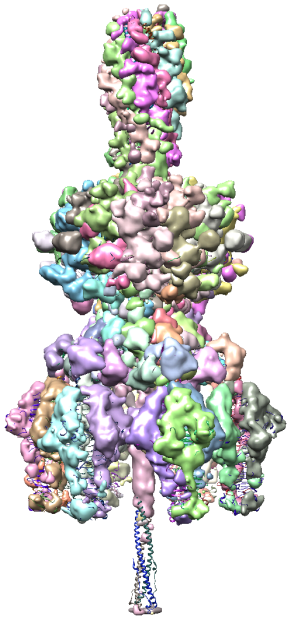
3. Segmenting the Portal, Hub, and Ahesin (Tail)

You'll notice above that we didn't talk about the portal and adhesin sticking out at one of the icosahedral vertices. Segmenting these out can be quite tough, as they are complex. We can use crystal models to help though.

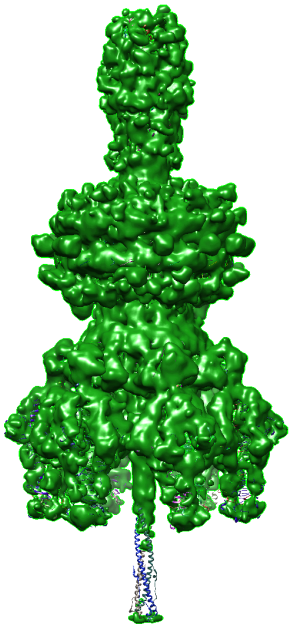
- In the downloaded file, there is a folder called docked_models. Open all the pdb files in that folder. You'll see all the crystal models docked inside the density:



- Undo the radial grouping done in previous steps
  - Unselect any regions by ctrl-click in the main window on an empty spot.
  - In the Segment Map dialog, press the **Ungroup** button at the bottom of the window.
- In the Segment Map dialog, open the Shortcuts panel and press the **Fit** button to the right of Other tools. The Fit Dialog will appear.
- In the Fit Dialog, select 'Group regions by chains in visible (Molecule) models' from the Fit menu at the top left corner of the dialog.
  - As the name suggests, this will group regions that overlap each chain in the Molecule models that are open and visible in the main view.
  - This will take a couple of minutes, so read your favourite news feed meanwhile… (Engadget, Redit, Macrumors, etc.).
  - You can check the status message in red at the bottom of the Fit Dialog to see when it's done.
- To see the resulting regions, and none of the others, press the **Grouped** button next to Show regions: in the Shortcuts panel of the Segment Map dialog.
- Select all grouped regions by ctrl+drag box around all the regions, then press **Group** in Segment Map dialog.

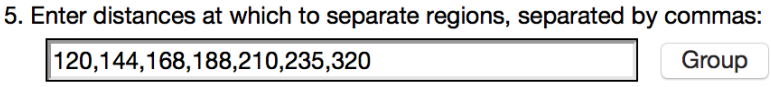


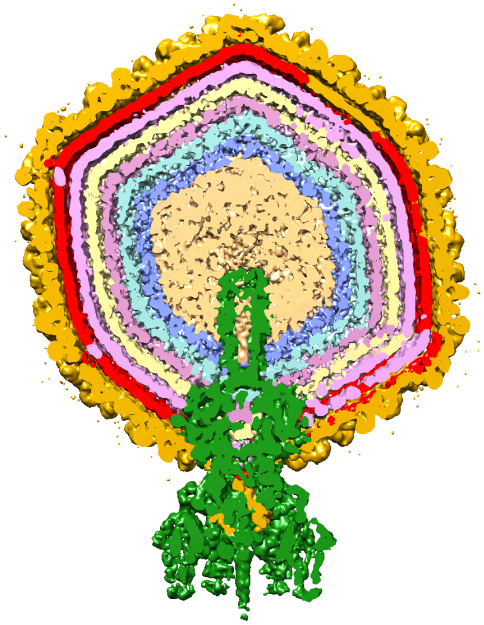
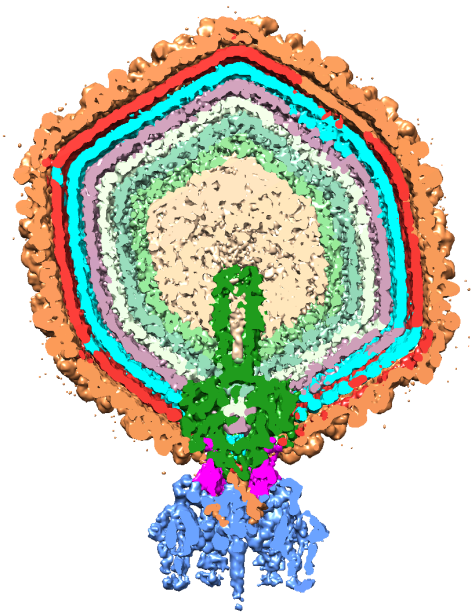
Segmented regions after grouping by chain



All chain-grouped regions further grouped into 1 region

- Now, going back to rSeg Dialog, do all steps as above again:
  - If you've left the dialog open, you can just start at **Make Histogram**
  - Otherwise go back, make the icosahedron surface, Find Axes, then Make Histogram.
  - Save the histogram file as dist_hist_icos.txt.
  - You can replot the histogram and pick out the valley x-values again, but they are mostly the same – nothing has changed other than the portal-hub-adhesin regions which are now grouped as one
  - With the values as before and below, press **Group**
    - 120,144,168,188,210,235,252,320



- So now we've combined the portal-hub-adhesin segmentation with the radial/icosahedral segmentation. The tail-hub-adhesin region will be part of one of the icosahedral layer. You can separate it out as follows:
  - Select the region wit the portal-hub-adhesin.
  - Ungroup the regions by pressing **Ungroup** in Segment Map dialog.
    - First, increase the number in 'Display at most ____ regions' in the Options panel in the Segment Dialog to 10000. We need each of the ungrouped regions to have a surface, so that we can group them back together after unselecting the portal-hub-adhesin region. This is inconvenient right now but is a limitation of only being able to select regions that have surfaces shown.

    - Because so many surfaces have to be created and shown, this step may also take a bit of time on the order of 10 seconds or so, not much more.
- Click-ctrl on the portal-hub-adhesin region to remove it from the selection.
- Press Group in Segment Map dialog to group all other regions.
- Now the segmentation should look more like below, left. With a bit more work, the portal-hub-adhesin regions can be ungrouped and regrouped into actual portal, hub, and adhesion components, as shown on the right.