Gorgon

Test data set: Rotavirus VP6, 3.80 Å resolution, 1.23 Å/pixel, Monomer segmented with Chimera, EMDB ID: 1461, X-ray structure: 1QHD.

1. Open volume file. File>Open>Volume "…Data-Sets/Gorgon/vp6-96o.mrc".
2. Build a density skeleton. Actions> Volume> Skeletonization> Binary skeleton; select a threshold where the separation of stands and loops can be first seen (~0.40) .
3. Calculate SSE score. Actions> Secondary Structure Elements> Identify SSEs. Set the threshold to ~0.40, resolution 8.00. (resolution does not directly correspond to volume resolution). Click on Find Scored Pseudoatoms (saved output file: "skeleton-vp6-b0.40.mrc").
4. Build SSEs. To add a helix, Ctrl+click on red Ca-atoms at beginning and end of helix and then click "Add Helix". Select Helix in visualization window and press Ctrl+f to refine fit to density. To add a sheet, Ctrl+click on blue Ca-atoms in a plane then Click "Add Sheet". Iterate until all visible SSEs are annotated.
5. Generate an SSE correspondence. Actions > Secondary Structure Elements > Find SSE correspondence. Enter the following files: Cryo-EM skeleton: "skeleton-vp6-b0.40.mrc", Sequence: "vp6.pdb", 3D Helix locations: "helices-vp6.vrml", 3D Sheet locations: "sheets-vp6.vrml". Click "OK".
6. Select correspondence. Evaluate correspondence by comparing lengths, percentiles and overall topology. Click on helices in visualization window to view correspondence. Constrain "good" matches by clicking on "Constrained" box. Click "OK" to re-run correspondence routine with desired selection.
7. Place atoms. Actions > C-alpha atoms > Semi-automated atom placement. Top panel is sequence viewer with SSE predictions. Current location in sequence is shown in grey bar and below in the zoom view. Bottom panel (atom panel) has 4 tabs for atom placement.
8. Add helices. Select "helix editor" in the atom panel. Ctrl+Click on a helix in the visualization window. Click on the corresponding helix segment in sequence viewer; sequence will be highlighted in black. Adjust length/position in the helix editor if desired. Click "Accept" to build a helix; atoms will appear in visualization window. Click on an assigned atom an locate it in the sequence view. If helix is reversed, flip helix by clicking on "flip" (at least 1 atom in the helix must be selected. Repeat until all helices are assigned
9. Build loops. Select "atomic editor" in the atom panel. Set C-alpha distance to 3.5Å. Click on a starting/ending atom in the vis window. Select direction in Atomic editor panel (next atom increments, previous atom decrements). Selected residue is highlighted, residue to be placed is in green. Cycle through the "Use choice" positions to find best placement, current position for the atom to be placed is in cyan. Click on "Accept". Repeat until next assigned atom or terminus is reached
10. Alternate loop placement. Select "loop editor" in the atom panel. Select residues between two assigned residues in the sequence window. Click "start loop placement". Select start point by Ctrl+ click on the desired start point. Move loop through density with alt+move. Click "End loop placement" when finished .
11. Adjust atom positions. Select residue (click). Adjust position by Ctrl+click+drag or use Position editor in Atom panel. Blue bonds are too short(<3.5Å). Red bonds are too long (>4.2Å). Relative sidechain size can be shown by selecting "mock sidechains". Repeat until all atoms are adjusted.

Pathwalking

Test data: Beta-Galactosidase, 3.2 Å resolution, 0.6375 Å/pixel, Monomer segmented with Chimera, EMDB ID: 5995, X-ray structure: 3j7h.

1. Preprocess the map. e2proc3d.py sub-A.mrc map.mrc --process normalize.edgemean --process threshold.belowtozero
2. Generate pseudoatoms. e2segment3d.py map.mrc --pdbout=pseudoatoms.pdb --process=segment.kmeans:ampweight=1:nseg=1022:verbose=1:minsegsep=1:pseudoatom=1:thr=10
3. Calculate an initial path. e2pathwalker.py pseudoatoms.pdb --mapfile=map.mrc --output=path0.pdb --solver=lkh --overwrite --dmin=1 --dmax=10 --mapthresh=12 --mapweight=200
4. Allow gaps. e2pathwalker.py pseudoatoms.pdb --mapfile=map.mrc --output=path1.pdb --solver=lkh --overwrite --dmin=1 --dmax=10 --mapthresh=12 --mapweight=200 --subunit=3
5. Fix the gaps. printf "870 1017\n827 829\n" > edge.txt
6. Rerun pathwalker with fixed edges. e2pathwalker.py pseudoatoms.pdb --mapfile=map.mrc --output=path2.pdb --solver=lkh --overwrite --dmin=1 --dmax=10 --mapthresh=12 --mapweight=200 --edgefile=edge.txt
7. Set the termini. e2pathwalker.py pseudoatoms.pdb --mapfile=map.mrc --output=path3.pdb --solver=lkh --overwrite --dmin=1 --dmax=10 --mapthresh=12 --mapweight=200 --edgefile=edge.txt --start=91 --end=956
8. Find and regularize helices. e2pwhelixfit.py --mapin map.mrc --pdbin path3.pdb --output hlx.pdb --denthr 13 --mapwohelix map_nohlx.mrc --minlen 4 --lenthr 10
9. Find and regularize sheets. e2pwsheetfit.py --pdbin hlx.pdb --output sheet_0.pdb --nsht 30 --minlen 3