Chimera Tutorial:
Fitting Molecular Models in Single-Particle EM Maps

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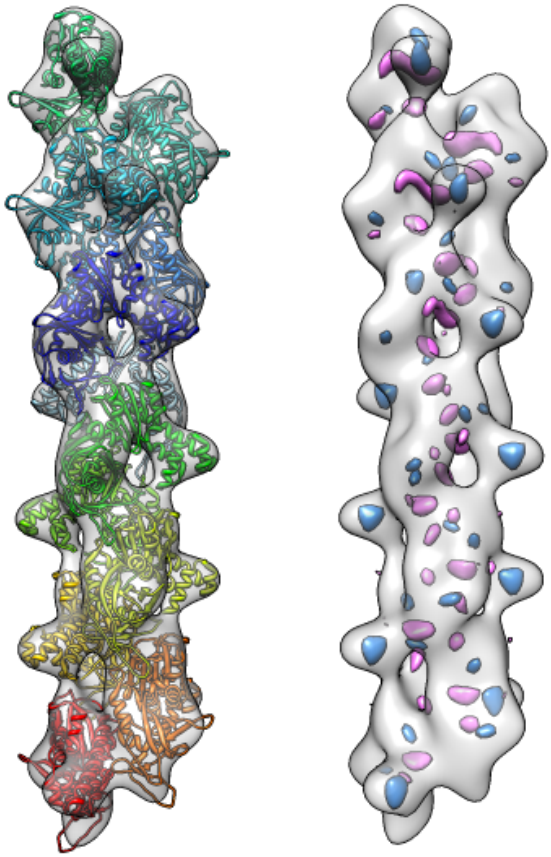
October 3, 2012

Topics

- Fitting molecules in maps with global search.
- Calculating map symmetry and creating symmetric molecule copies.
- Symmetric fitting to avoid molecular clashes.
- Calculating and displaying difference maps.



What does ParM do?

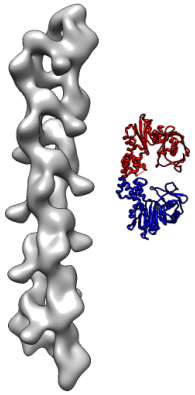
- The ParM protein forms filaments that segregates DNA plasmids prior to cell division.
- To partition low copy number DNA plasmids in E coli evenly during cell division between the two daughter cells, a plasmid is attached to each end of a growing ParM filament that pushes them to opposite sides of the mother cell.
- ParM filaments look similar to actin filaments.
- Filament growth is driven by ATP and filaments have dynamic instability like microtubles.

Modeling a ParM Filament

- ParM monomers bind ATP and can have the binding cleft open or closed.
- We'll build a model of the open state filament with the ATP binding site empty using x-ray structure 1mwk and cryoEM map EMDB 5129 (19 Angstroms).
- Closed state data is also available: x-ray model 1mwm and map EMDB 5128 (17 Angstroms). Won't have time to look at those.

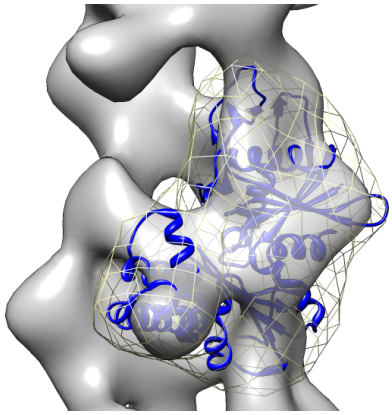
Analysis Steps

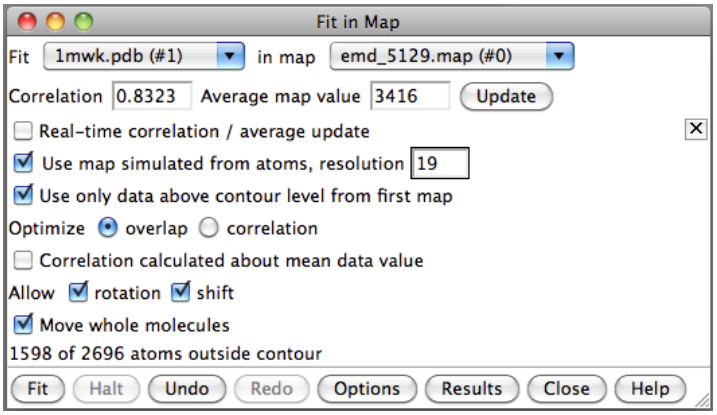
Show Molecule and Map



1. Open PDB 1mwk, EMDB 5129.
2. Show Command Line (Favorites menu).
3. Deactivate map (model 0) below command-line to move 1mwk away from map.
4. Command "rainbow chain" to color two ParM monomers.
5. Delete chain B. Use menu Select / Chains / B, then menu Actions / Atoms / Delete.

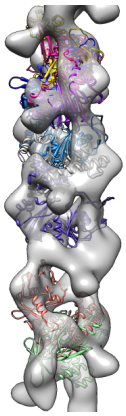
Fit Molecule in Map





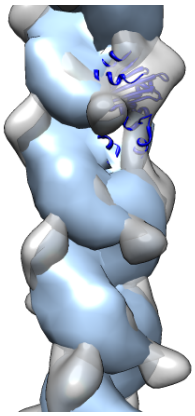
6. Fit 1mwk in map.
7. Move 1mwk into map.
8. Press Fit button in Fit in Map dialog (volume dialog Tools menu).
9. Fit using correlation: Fit dialog Options button, enable "Use map simulated from atoms..." resolution 19. Press Fit.
10. Show simulated map (Volume dialog eye icon) as mesh.
11. Correlation depends on domain of calculation. Change simulated map threshold and press Update in Fit dialog.
12. Spend a few minutes trying alternative fit positions.

Global Fit Search



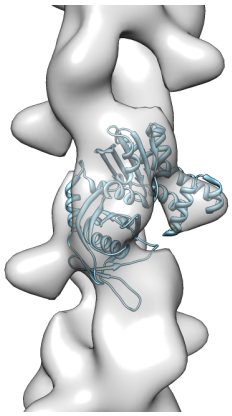
13. Search for best fit using 30 tries with command "fit #1 #0 search 30"
14. Fits appear all along filament, many are equivalent due to symmetry of the filament.

Calculate Map Symmetry



15. Determine map symmetry to eliminate equivalent fits.
16. Command "measure symmetry #0 helix 20,180,opt minimumCorrelation 0.95".
17. The "helix" option gives Chimera a hint about helical parameters.
18. The "minimumCorrelation" option accounts for this unusual map where the helix does not extend to the edges of the volume box.
19. View symmetry copies of molecule with command "sym #1 group #0 surf true".
20. Remove symmetry copies with "~sym #1" (note leading tilde character which means "undo" in Chimera commands).

Fit Asymmetric Unit

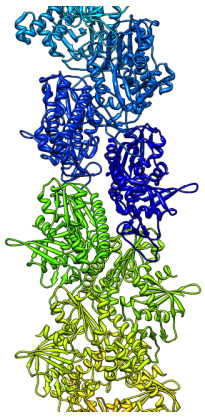


21. Clear fit list.
22. Rerun previous fitting command "fit #1 #0 search 50".
23. Clear fit list.
24. Use correlation optimization "fit #1 #0 search 50 res 19".

Make Filament Model

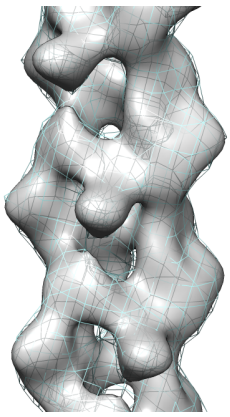
25. Show symmetric molecule copies for best fit. Command "sym #1 group #0 update true".
26. Color molecules distinctly. Command "rainbow model".

Reduce Molecular Clashes



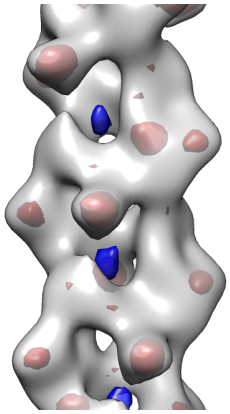
27. Inspect clashes between adjacent ParM molecules.
28. Fit asymmetric unit including all overlapped symmetric molecules. Command "fit #1 #0 sym true res 19"
29. Note increased space between molecules.

Calculate Predicted Map



30. Compute difference map between experimental map and predicted map for molecular model.
31. First delete extra ParM molecules outside experimental map. Ctrl-drag mouse to select outside molecules. Press up-arrow key to extend selection to full molecules. Menu Actions / Atoms / Delete to delete.
32. Calculate predicted map. Command "molmap #1,2, 19"

Difference Map



33. Subtract two maps, scaling the second to minimize difference. Command "vop subtract #0 #3 minRMS true"
34. Adjust difference map contour level. Add negative contour with ctrl-click on histogram. Adjust contour colors.
35. Hide molecular models with Model Panel (menu Favorites).

Compare "Open" and "Closed" Filaments.

36. Load "closed" filament (GDP bound), EMDB 5128, menu File / Open...
37. Flip closed map 180 degrees and fit to open map.
38. Compute closed map on same grid as open map. Command "vop resample #5 onGrid #0"
39. Use Morph Map (Tools menu of volume dialog) to morph between maps. Movie.