

Visualizing Molecular Assemblies and Cells by Electron Microscopy

Tom Goddard
November 19, 2007

Topics

Progress this year

- Software for interactive exploration of EM tomography.
- New tools for single particle reconstruction -- our main base of EM users and collaborators.
- Advertising Chimera EM capabilities: meetings and journal articles.

Focus areas for next year

- Density map masks: file formats, creating and applying masks
- Tomography filtering operations: bilateral, median, anisotropic diffusion.
- Seamless user interface for atomic models of molecular assemblies.

Chimera Use in the Electron Microscopy Community

Who are our current EM users?

Most Chimera EM users study molecular assemblies with single particle reconstruction (back-projecting thousands of 2-d particle images).

Chimera map visualization tools have been developed for this audience for 6 years with primary collaborator Wah Chiu, director of the National Center for Macromolecular Imaging (NCMI).

Chimera map capabilities are described in [Guide to Volume Data Display](#). Display, fitting, carving, and measuring maps and handling large multimeric atomic models (viruses, ribosomes, filaments, ...).

Estimate 500-1000 EM Chimera users based on email support requests from ~100 distinct users per year.

Estimate 50-100 EM tomography users based on email correspondence.

Why add EM tomography capabilities to Chimera?

Chimera has been used for exploring EM tomograms (calculated from tilt series of single specimens) although we did not develop tools specifically for this use until this year.

Strong encouragement from collaborators

- Manfred Auer (LBNL, stereociliar bundles)
- Wah Chiu (National Center for Macromolecular Imaging),
- Wolfgang Baumeister (MPI, cellular organization)
- Yifan Cheng (UCSF, clathrin vesicles)
- John Sedat (UCSF, drosophila chromosome structure)
- Gary Ren (UCSF, low density lipoprotein cholesterol packaging)

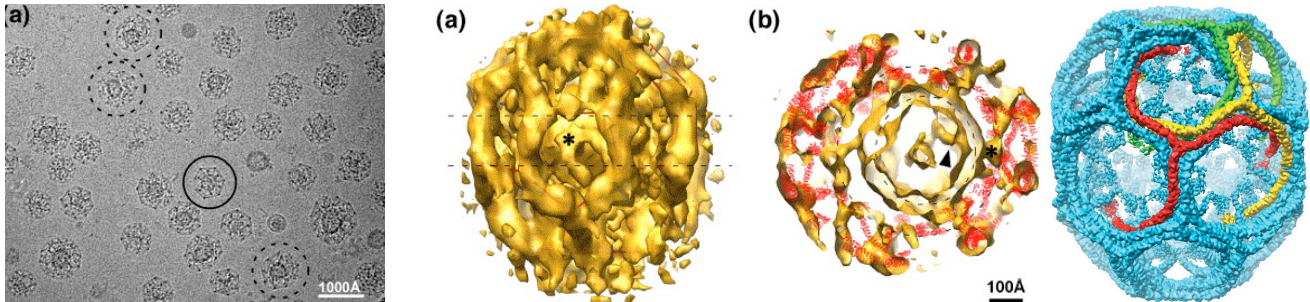
Growing literature of tomography applications to molecule-scale architecture, where Chimera map and atomic model capabilities have been used.

Molecular-scale EM Tomography Examples

Some published examples using Chimera for EM tomography (all completed before we added specific tomography support).

Clathrin vesicles, Yifan Cheng.

Cheng Y, Boll W, Kirchhausen T, Harrison SC, Walz T.
Cryo-electron tomography of clathrin-coated vesicles: structural implications for coat assembly.
[J Mol Biol. 2007 Jan 19;365\(3\):892-9.](#)

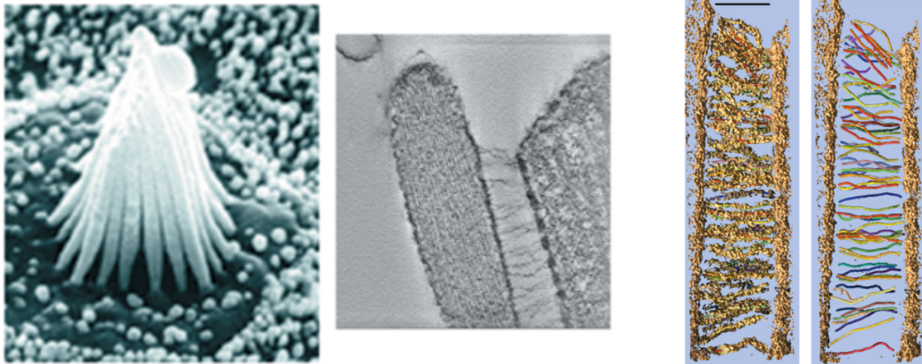


In vivo clathrin cages have many topologies.

Modeling a cage from tomogram.

In vitro prepared clathrin allows averaging.

Stereocilia molecular linkers (unpublished), Manfred Auer lab.



SEM of hair bundle

Tomogram section of tip links.

Traced links composed of a few molecules. Scale bar 100 nm.

HIV glycoprotein spikes, Roux lab.

Zhu P, Liu J, Bess J Jr, Chertova E, Lifson JD, Grise H, Ofek GA, Taylor KA, Roux KH.
 Distribution and three-dimensional structure of AIDS virus envelope spikes.
[Nature. 2006 Jun 15;441\(7095\):847-52.](#)

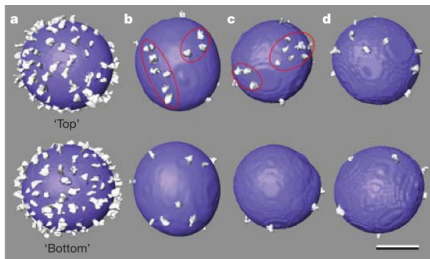
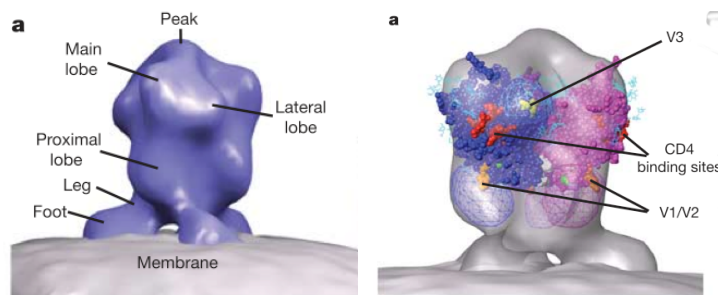
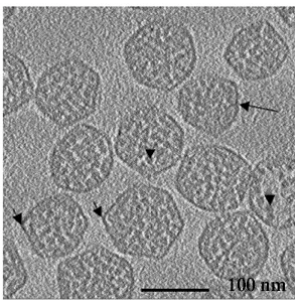


Figure 2 | Surface-rendered models of a representative SIV (a) and three HIV-1 (b-d) virions with highlighted (white) presumptive Env spikes.

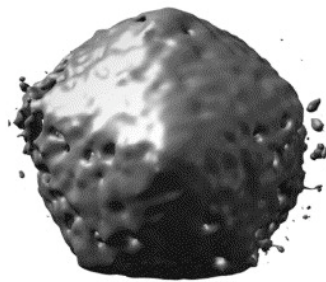


Carboxysomes, Michael Schmid (co-director of NCMI).

Schmid MF, Paredes AM, Khant HA, Soyer F, Aldrich HC, Chiu W, Shively JM.
 Structure of *Halothiobacillus neapolitanus* carboxysomes by cryo-electron tomography.
[J Mol Biol. 2006 Dec 1;364\(3\):526-35.](#)



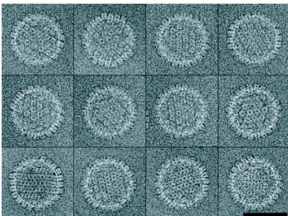
Carboxysomes encapsulate RuBisCO in cyanobacteria.



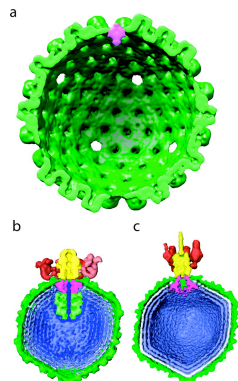
Averaging 21 tomogram particles with diameter ~100 nm suggests arrangement of capsid molecules.

Herpes virus portal, Wah Chiu lab.

Chang JT, Schmid MF, Rixon FJ, Chiu W.
 Electron cryotomography reveals the portal in the herpesvirus capsid.
[J Virol. 2007 Feb;81\(4\):2065-8.](#)



Herpes simplex virus.
 Tomographic sections of several particles.



One of 12 vertices occupied by a portal of nearly equal mass to 11 pentons.
 Tomographic reconstruction used to identify portal vertex and 13 particles averaged.
 Large Epsilon 15 and P22 virus portals shown for comparison below.

Demonstration of Tomography Capabilities

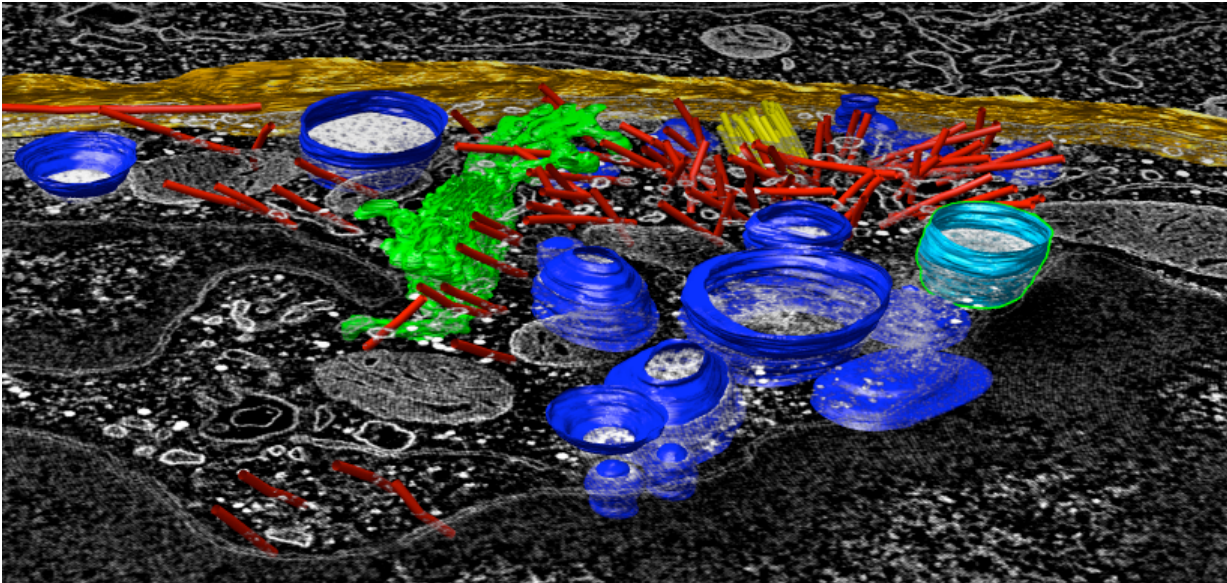
Example: Human cytotoxic T-cell killing neighboring cell.

Stinchcombe JC, Majorovits E, Bossi G, Fuller S, Griffiths GM.

Centrosome polarization delivers secretory granules to the immunological synapse.

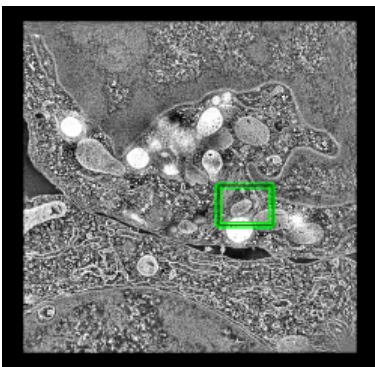
[Nature. 2006 Sep 28;443\(7110\):462-5.](#)

Displaying segmentations. Can read IMOD segmentation files, hide, show, split, color, measure enclosed volumes (with holes) and surface areas of objects.

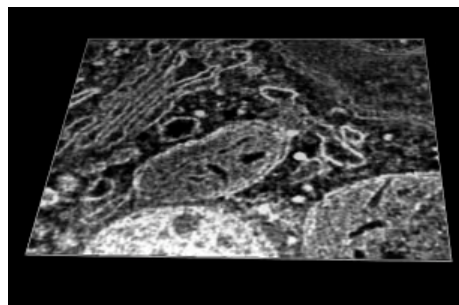


IMOD segmentation of tomogram: lysosomes (blue), microtubules (red), centrosome (yellow), golgi (green), cell boundary (orange).

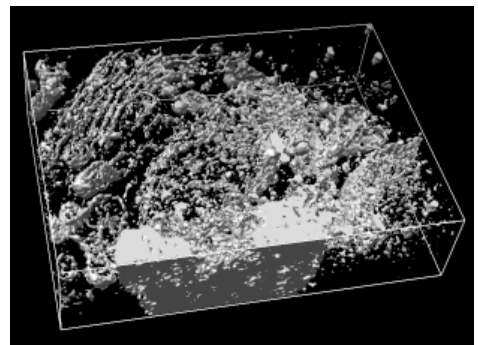
Single plane display is most effective view due to high noise level. Contour surfaces, the common display style for single particle reconstructions, are much less useful.



Full plane with subregion in green.

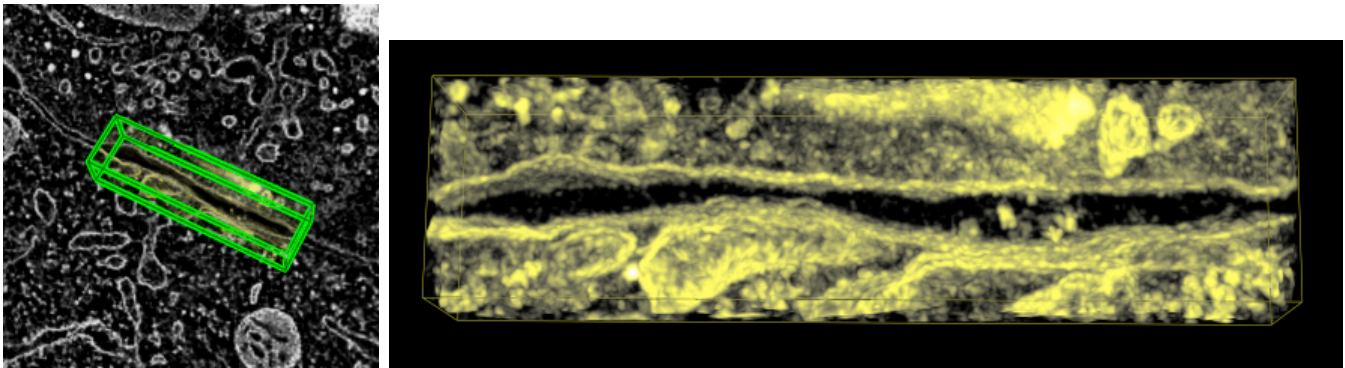


Single plane.

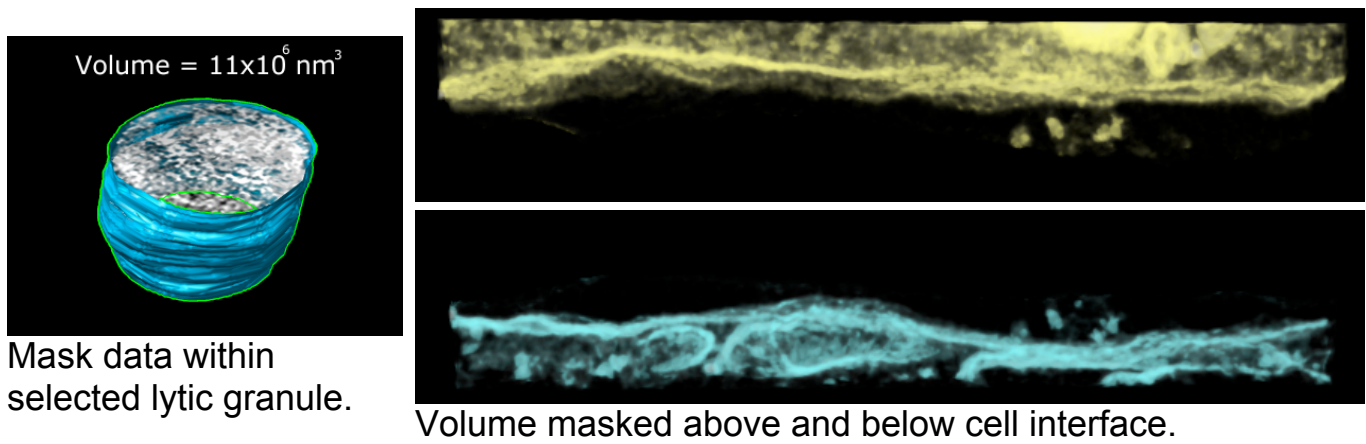


Contour surface.

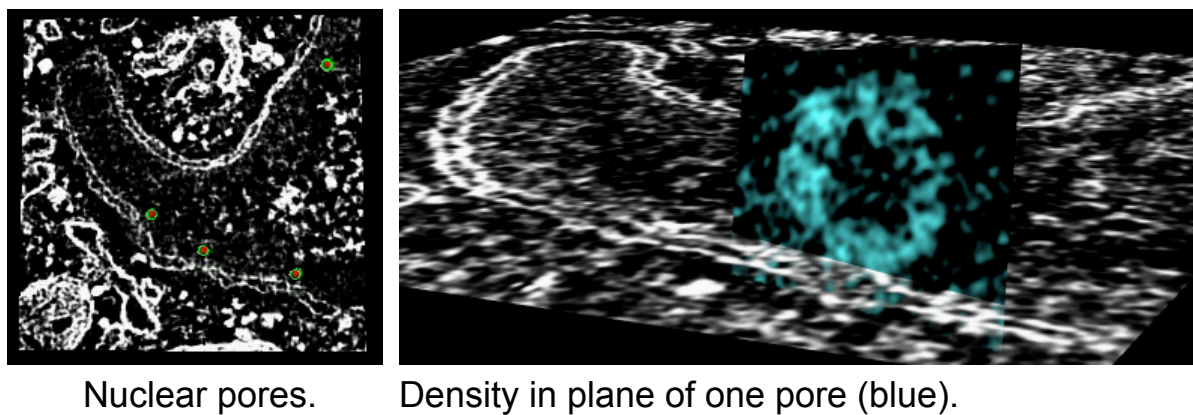
Resampling. Extract subregion with axes rotated relative to full data. Interesting structures are generally not aligned with tomogram axes.



Masking. Extract density bounded by IMOD segmentation surfaces.



Slice plane at any orientation.

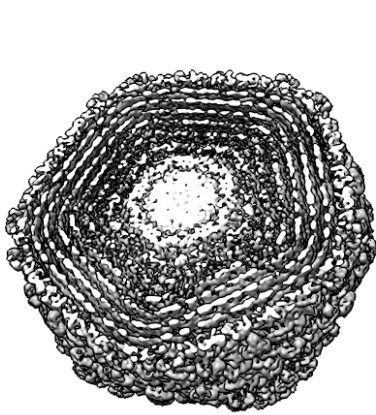


Nuclear pores.

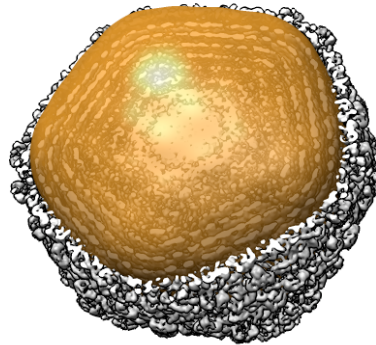
Density in plane of one pore (blue).

New Developments for Single Particle Reconstructions

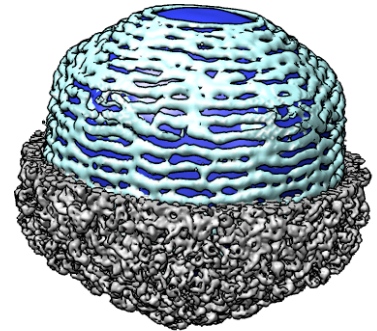
Masking capability for tomography is also useful for single particle reconstructions.



Phage K1E.



Contour surface of Gaussian smoothed data separates protein capsid from DNA genome.



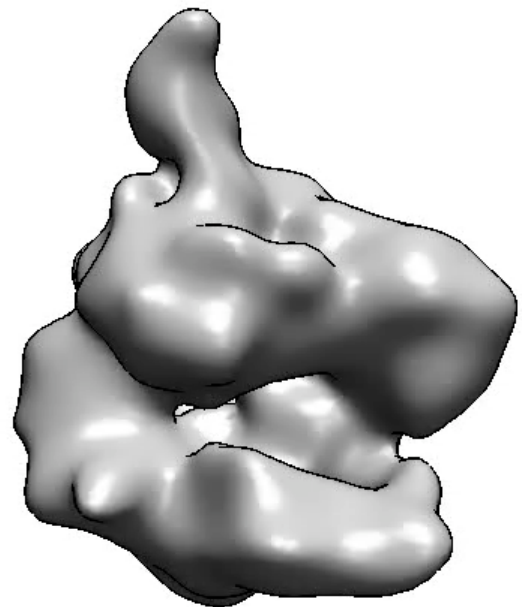
Masked DNA shows spiral packaging.

Morph Map

New morph map tool linearly interpolates between maps. Effective method for viewing differences between related maps. Most EM Databank maps belong to sets of related maps.

Original Chimera code contributed by Wei Zhang from Pawel Penzcek's lab.

Two conformations of human RNA polymerase II.

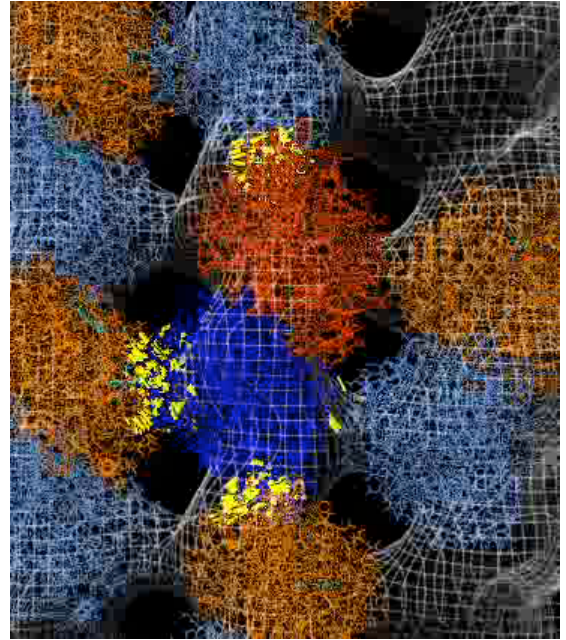


Clash Detection While Fitting

New detect clashes tools shows steric clashes as yellow lines while model is interactively hand-fit or locally optimized.

Symmetry copy command makes copies of contacting monomers.

Fitting alpha and beta tubulin into a microtubule map.



Promoting Chimera in the EM Community: Meetings and Journal Articles

We promote Chimera through demonstrations and hands-on tutorials at meetings:

- Workshop on Visualizing Cellular Complexity, Max Planck Institute for Biochemistry, Munich, December 13-15, 2006, Chimera presentation.
- Biophysical Society annual meeting, Baltimore, March 5-6, 2007, vendor booth presentation.
- EMAN workshop, Baylor College of Medicine, March 14-17, 2007, Chimera tutorial, ~50 participants.
- Bay Area cryoEM Meeting, UC Davis, May 4, 2007, Chimera tutorial and demo
- Workshop on Advanced Topics in EM Structure Determination, UCSD, Nov 14, 2007, Chimera demonstration

and through publications.

- Goddard TD, Ferrin TE.
Visualization software for molecular assemblies.
Curr Opin Struct Biol. 2007 Aug 27;
- Goddard TD, Huang CC, Ferrin TE.
Visualizing density maps with UCSF Chimera.
J Struct Biol. 2007 Jan;157(1):281-7.

Focus areas for next year

1) Masks for density maps: file formats, creating and applying masks.

- Can use map files with 0/1 values, 0 to N values, or bit masks for overlapping segments.
- Allow user naming of segments, and user interface to show/hide/color any set of segments.
- Create masks with watershed, volume painting (extending volume eraser).
- Tools that work on full map (fitting, filtering, calculating sdev, segmenting) should be usable on individual segments.

2) Tomography filters: bilateral, median, anisotropic diffusion.

- These are popular algorithms for denoising tomography data. Chimera already has Gaussian filtering.
- Allow filtering on small subregions for user to compare filters and determine best parameters interactively.
- Desirable for filters to run in separate thread with progress report and ability to cancel.

3) Seamless user interface for atomic models of molecular assemblies.

- Reduce hurdles to using PDB biological unit coordinates.
- Many PDB entries (32%) do not contain coordinates for the biological unit.
- Chimera currently has separate user interface for creating, show/hide, selecting, coloring, styles, ... of quaternary structure.
- Incorporate multimer handling into standard Chimera Actions / Selection / command user interface.
- Support multiple assembly specifications in mmCIF.

Beyond next year: Animations and EM Databank

Four software development areas of our molecular assemblies NCRR core project:

- Single particle reconstructions.
 - Data standards for EM maps, masks, and meta-data are being advanced as part of merging EM Databank into wwPDB.
 - We are advising and testing proposed standards (HDF maps).
 - Will have large impact on interoperability of software and reuse of archived data.
- Animations of assemblies.
 - Current animation support is piecemeal, e.g. morph map has its own movie recording option.
 - Future plan is for graphical animation editor and suite of commands for common animation transitions (movement, fade in/out, recolor, morph atomic models and maps, map slicing, ...).
- EM tomography.
- Atomic models of large molecular assemblies.