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Übung 4: Electron Density Maps[1]

In the lectures, a map of electron density was treated as a goal, but one is really interested in the atoms which give rise to this density. Normally, a crystallographer spends much time trying to fit atoms into the calculated density. Here, we will look at the simpler problem of fitting a ready-made molecule into density.
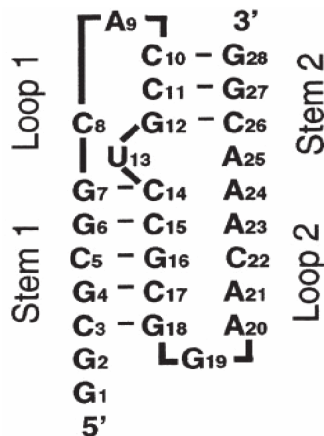
You will get to know the 1.25Å resolution X-ray structure of the frameshifting RNA pseudoknot from beet western yellow *leteovirus* (BWYV). It offers the most detailed view yet of the conformational preference and flexibility of an RNA pseudoknot. The structure was determined on the BL1-5 beamline at Stanford Synchrotron Research Laboratory.[2]

First, you should get used to the electron density map and some basic functions of UCSF Chimera for displaying volume data. You can think of a density map as a contour plot with isosurfaces. To understand this you may think of the weather forecast. There is one that displays isolines or isobars, that is lines are drawn at levels of equal air pressure. In these pictures the pressures are measured near the ground. If one would also collect air pressure data at higher altitudes (e.g. 1000m above sealevel) then the same isobars would appear at a different position on the map or even disappear. Now, if corresponding isobars (i.e. of same pressure) from all

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[1] Originally written by Gundolf Schenk

[2] M. Egli et al. (2002), *PNAS*, 99:4302-7.

altitudes were connected in 3D then the isosurfaces are constructed. Of course, for display it is reasonable to show only one isosurface at once.

Second, you will look at the model (pdb entry) that was derived from the electron density. You are supposed to superimpose this model onto the density map at a certain density level (i.e. isosurface). After you managed to do this you will learn how the structural properties of the model relate to the density map.

Documentation for *UCSF Chimera* can be found at
http://www.cgl.ucsf.edu/chimera/docindex.html

Please answer the following questions in a brief written report, and send it to
hansen@zbh.uni-hamburg.de
not later than **9 January 2012** with your full name written on it. Do not send TeX, openoffice or staroffice files. Plain ASCII or pdf files are preferred.

Tasks:

1. Load the file (*~hansen/teaching/density_maps/RNA_pseudoknot.omap*) into UCSF Chimera. It contains the unit cell (i.e. smallest entry in a crystal) of the electron density map of the RNA pseudoknot with some small ligands bound.
   Play with the Volume Viewer dialog. (Be careful: The rendering can become slow, when using too much detail.) Please answer briefly some questions about rendering.
   * What is the difference between surface and mesh?
   * What do you think is displayed when using solid?
   * What happens when you change Step?
   * What happens when you change the position of the bar on the histogram?
   * What does the histogram display?
   * What else did you find useful in the Volume Viewer dialog?

2. Colour the map according to density (In the Volume Viewer: Tools->Surface Color).
   Where can you observe high density in the unit cell? Why?

3. Now colour the map using radius.
   Can you identify the backbone or some nucleosides?
   Which ones and because of what did you identify them?
   What values did you use in the Volume Viewer dialog?

4. Write down the sequence of bases.... no, just kidding.

5. Until now, you should have familiarised yourself with the electron density map. Load another file (~*hansen/teaching/density_maps/RNA_pseudoknot.pdb*). It contains the PDB model structure that was derived from the density map.
It is now your task to fit this model into the map. To do so move the model onto the fixed density map by hand. Therefore, use left (rotation), middle (XY-translation) and Ctrl+middle (Z-translation) mouse buttons. To locally optimise the position, use the Fit Models entry in the Maps tool menu. You might want to select some atoms for the automatic fit in advance (for example backbone). If you achieved an average map value under 1.00 then probably your manual placement was not close enough. If you really don't succeed in properly positioning the RNA structure within the electron density map, then close this model (but not the electron density map) and directly load the RNA structure in the correct 3D positioning instead:
~*hansen/teaching/density_maps/RNA_pseudoknot_orig.pdb*
However, you should not give up too fast. You might want to use a different representation and colouring for the structure and map than default.
* How / with what did you orient yourself during the manual placement?
* Which atoms did you select for the local fit and what average map value did you achieve in the end? Please describe in a few words why your placement was successful or why you think it was not.

6. After the model is in its right place, highlight/colour atoms with high B-factor (@/bfactor>30).
* Which atoms did you highlight, where do they belong to ?
* What is different compared to others (not highlighted)? Why ?
* Can you confirm your findings with the corresponding electron density ?

7. Now have a closer look at the ligands. Where are they in the structures ?
* Which other molecules/atoms that are not part of the RNA can you find ?
* How can you distinguish between them in the electron density ?
* To which residues do they form non-covalent contacts?
* Describe their coordination geometry, if possible.
* What are their B-factors? Why?

8. Finally, make a picture (and include that in your short report) that highlights your findings (for example with coloured surface and/or displayed H-bonds). Be artistic. The best picture will be rewarded with a beer.