

Fully automated $C\alpha$ tracing for high- and low-resolution electron density maps

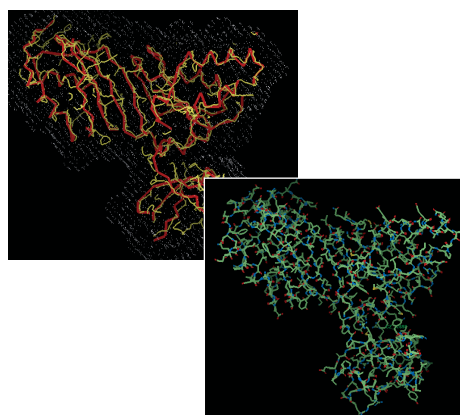
$C\alpha$ trace to fitted all-atom model in 3 to 4 clicks

Powerful 'knot analysis' tool for checking $C\alpha$ trace

X-AUTOFIT

X-AUTOFIT is a powerful X-ray crystallography application that enables you to trace and build protein model coordinates into electron density maps phased by single isomorphous replacement (SIR), multiple isomorphous replacement (MIR), or multi-wavelength anomalous dispersion (MAD). With X-AUTOFIT, what previously took weeks or months can now be accomplished in a single day. The new X-AUTOFIT module combines functionality previously available in X-AUTOFIT and X-POWERFIT. Functionality includes three fully automated protocols for tracing, and for sequence assignment, and model building.

X-AUTOFIT is a unique module that automatically determines the $C\alpha$ trace of a protein from MAD, MIR, or SIR electron density maps. New methods in this tool can speed up electron density fitting up to 500-fold over conventional methods. Fully automated protocols in X-AUTOFIT then allow you to proceed to sequence assignment and fitting of an all-atom model all with a few clicks of the mouse.



*$C\alpha$ trace and final structure of *truB*, a tRNA pseudo-uridine synthase. Data courtesy of Robert Fletterick at UCSF.*


Tracing an initial map is a time consuming and laborious process that can take many months of work, especially for large maps. The tracing process is also significantly slower for maps generated from data with low resolution or poor phases. X-AUTOFIT has been fully automated and optimized so that it traces high resolution maps extremely fast (in less than a second). Furthermore, for data in the 2-4 Å resolution

range, the automated protocols not only tease out features in the maps using a unique approach, but they do so in less than a minute. Once a $C\alpha$ trace is obtained, sequence assignment, generation of an all-atom model, and real-space refinement of the final model can all be done in a few clicks with fully automated protocols. X-AUTOFIT represents a complete solution for tracing of *de novo* electron density maps, both in the low- and high-resolution ranges.

About the Software

When using the X-AUTOFIT module, your electron density map is first skeletonized. X-AUTOFIT allows you to edit the skeleton (bones) so that it only covers one molecule in the map. You can then calculate a map mask from the bones, and use this as a boundary for subsequent calculations.

You then have two options for automatically determining the $C\alpha$ trace from the skeletonized density. The first option is geared towards high-resolution maps, generally between 2.2 and 1.5 Å. This is a pathway analysis method in which the program tries to identify a unique path through the skeleton in a single pass. The second method¹ is tailored for lower resolution maps (between 4.0 and 2.2 Å) and is based on the calculation and placement of vectors that represent the principle components of the secondary structural elements². These vectors are automatically converted into a $C\alpha$ trace. X-AUTOFIT then takes these $C\alpha$ traces and automatically extends them through the rest of the map. The end result is a



close-to-complete trace of the structure. Semi-automated tools within X-AUTOFIT can then be used to extend the C α trace into a complete structure. X-AUTOFIT also permits real space refinement of the C α atoms with respect to the map.

An additional option is available in X-AUTOFIT that automatically performs the low-resolution tracing protocol nine times with different starting parameters (bones sigma values). A 'consensus trace' is generated at the end of this process. This option saves time by automating a process that would otherwise have to be repeated manually, and it also ensures that the final trace is more likely to identify features in your low-resolution map that would otherwise have gone undetected in a single trace.

Once a C α trace is obtained, the sequence can be assigned to the trace with a single click (for high resolution maps). For low resolution maps, you are often able to identify characteristics of the amino acid side chain (e.g. large, medium, small, aromatic, or aliphatic). Using unique fuzzy descriptors, and any specific assignments, X-AUTOFIT quickly realigns against the protein sequence.

Fully automated tools also exist for building an all-atom model of your structure, and to fit this model into the electron density using real-space refinement techniques. These steps used to take significant time and effort, and involved many interactive steps. But now, the entire process has been completely automated.

The X-AUTOFIT application only requires an extended map. Although space group knowledge of the molecule under construction is highly recommended, it is not absolutely necessary.

Benefits

The availability of fully-automated tools means that you can quickly complete the *de novo* tracing of your structure. For high-resolution maps, the single pass tracing option is extremely reliable and allows you to trace a majority of the C α atoms in the structure with one click. For low-resolution maps, the automated tracing protocols enable you to arrive at a C α trace within one minute. Furthermore, X-AUTOFIT can initially provide qualitative structural information when fitting C α atoms into electron density. This can help you speed up the structure determination process in a number of ways, such as:

- by providing you a good starting point in a map which has been difficult to trace
- by providing a simple, fully-automated map fitting approach to accelerate the map fitting process
- by helping you to diagnose whether a map is worth the effort of model building, or whether you just need better data

Key Features

- Automatically generates a C α trace from skeletonized electron density map utilizing built-in intelligence of protein structure features³
- Provides three tracing options uniquely suited for either low or high-resolution maps
- Provides real space refinement of the C α trace
- Unique 'knot analysis' tool identifies incorrect tracing by checking for internal knots in the C α trace

- Automatically assigns the sequence to the C α trace
- For poor quality maps, quickly identifies unique fragments in the chain by using fuzzy descriptors for amino acids with forward and reverse automatic sequence alignment
- Builds and refines a full coordinate representation of each C α segment in seconds using one of four available methods, including a unique torsion angle real space refinement technique and a robust C α correlation method

Complementary Software

- X-BUILD contains functionality previously found in X-LIGAND that automatically fits ligand molecules to electron density of protein-ligand complexes. This module also features an extensive suite of tools that modify and construct the atomic model during rounds of crystallographic refinement
- X-SOLVATE for rapid searching and placement of water molecules into an electron density map
- CNX for x-ray and NMR structure determination
- CHARMM for simulation of biological macromolecules
- MODELER for automatic homology model generation

References

1. Oldfield, T.J., "Automated Tracing of Electron-Density Maps of Proteins," *Acta Cryst.*, **2003**, D59, 483-491
2. Oldfield, T.J., "Pattern-recognition Methods to Identify Secondary Structure within X-ray Crystallographic Electron Density Maps," *Acta Cryst.* **2002**, D58, 487-493
3. Oldfield, T.J. and Hubbard, R. E., "Analysis of C α Geometry in Protein Structures," *Structure, Function, and Genetics*, **1994**, 18, 324-337.

System Requirements:

QUANTA runs on Linux and SGI work stations.

Dial Box is supported for QUANTA on both the Linux and SGI IRIX platforms.

© 2006 Accelrys Software Inc. All brands or product names may be trademarks of their respective holders.