

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 Ca 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.

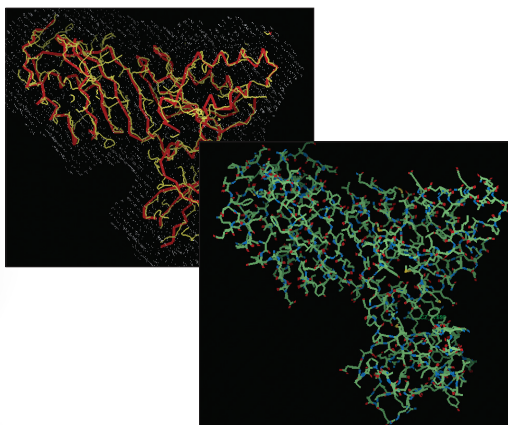
Tracing an initial map is a time consuming and laborious process that can take many months of work, especially for large maps. The tracing process is also significantly slower for maps generated from data with low resolution or poor phases. X-AUTOFIT has been fully automated and optimized so that

it traces high resolution maps extremely fast (in less than a second). Furthermore, for data in the 2-4 Å resolution X-AUTOFIT 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 Ca 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 Ca 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



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

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 Ca trace. X-AUTOFIT then takes these Ca traces and automatically extends them through the rest of the map. The end result is a close-to-complete trace of the structure. Semiautomated tools within X-AUTOFIT can then be used to extend the Ca trace into a complete structure. X-AUTOFIT also permits real space refinement of the Ca 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 Ca 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 Ca atoms in the structure with one click. For low-resolution maps, the automated tracing protocols enable you to arrive at a Ca trace within one minute. Furthermore, X-AUTOFIT can initially provide qualitative structural information when fitting Ca 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 Ca 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 Ca trace
- Unique 'knot analysis' tool identifies incorrect tracing by checking for internal knots in the Ca trace
- Automatically assigns the sequence to the Ca 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 Ca segment in seconds using one of four available methods, including a unique torsion angle real space refinement technique and a robust Ca 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

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REFERENCES:

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