OpenEye Scientific Software

Methods For Automated Structure Determination for Ligands Within a Protein-Ligand Complex

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Abstract

We present Afitt (OpenEye Scientific Software, Inc), a software package for automated ligand conformation generation and placement within algorithmically identified unfilled electron density. Following real space refinement, the ligand solution is sent for subsequent refinement by Refmac or CNX, via coordinate and dictionary files. We have validated Afitt on forty publicly available data sets, chosen because it contains examples of highly strained ligand conformations (J. Med. Chem. 2004, 47, 2499-2510). We show that Afitt can automatically generate low-strain ligand conformation and placement solutions for every example. Furthermore, in each case refinement of the new solution gives a fit equivalent to the original model.

Introduction

We have undertaken a validation of AFITT using a data set published by Perola and Charifson where they analyzed the local and global strain energies of 150 bound ligands for ligand conformations taken from crystal structures. Seven percent of the structures were calculated to have greater than 10 kcal/mol strain energy using the MMFF94s force field. We will show that for some structures in the data set there exist alternate ligand models that fit the data equally well or better than the original, and have a lower strain energy.

AFITT is used to generate new ligand models from crystallographic reflection or electron density map data. AFITT uses a novel protocol to solve ligand structures. All reasonable ligand conformers are generated in vacuo. Each ligand conformer is then matched to the ligand density to identify the closest likely solutions. Tens of possible ligand conformers are then allowed to adapt to the ligand density using an adiabatic fit procedure where the electron density is introduced incrementally to the force field during geometry optimization (see Figure 1).

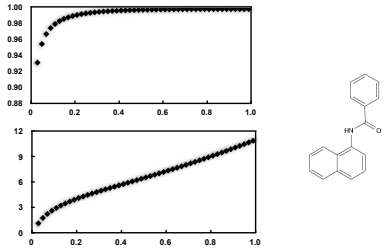

Figure 1: Strain energy and goodness of fit as a function of lambda (shape component) during an adiabatic fit. As lambda is increased shape fit increases exponentially while strain increases linearly, allowing for a maximization of fit with a minimal increase in strain.

Thus the maximum flexible fit is obtained before significant strain is introduced into the ligand conformation. The entire procedure is automatic, and typically takes less than one minute per ligand.

Methods

Of the 100 non-proprietary, 40 structures have publicly available structure factors (www.rcsb.org). The ligands were extracted from their corresponding PDB files, and automatically manipulated into their proper protonation and charge states using QuacPac. The resulting protein only files were refined using Refmac (v5.2.0019) to remove ligand bias. The reflection files generated were used for subsequent refinement using Refmac on the deposited ligand coordinates and AFITT generated solutions with refinement dictionaries generated by AFITT. Ligand strain was determined by summing the geometric and van der Waals components from a no optimization (single point) energy calculation using the MMFF94s force field.

Results

The majority of the solutions found by AFITT closely match the re-refined deposited coordinates; 29 out of 40 ligands differed by less than 0.4 Å RMS (see Figure 2). In two cases, AFITT generated solutions that differed trivially in the atomic positions but had a lower strain energy. In two cases, the AFITT solutions were significantly different and had much lower strain energies. Examples of AFITT solutions for high quality density, incorrect conformation, missing density, and a highly symmetric molecule are presented in Figures 3 to 6.

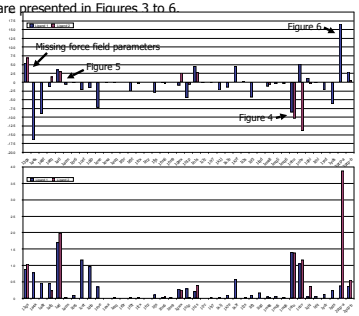

Figure 2: RMS distances and energy differences between re-refined crystal structures and the refined AFITT solutions. Ligand 2 designates those cases where more than one ligand is present within the asymmetric unit cell.

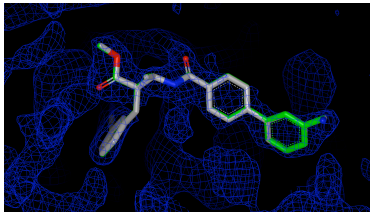

Figure 3: A typical AFITT result. The refined PDB structure of 1EZQ is shown in green. The AFITT solution in atom-colors. RMSD = 0.02 Å and Δ strain = -0.2 kcal/mol

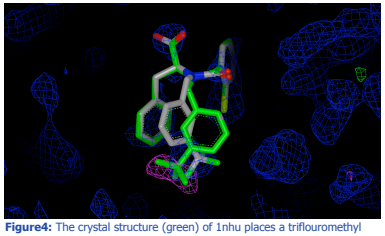

Figure4: The crystal structure (green) of 1nhu places a triflouromethyl group and part of an aromatic ring into negative density. The structure is strained due to a VdW clash. The AFITT solution (atom-color) both relieves the strain and avoids the regions of negative density. RMSD = 1.4 Å and the Δ strain = -8.5 kcal/mol.

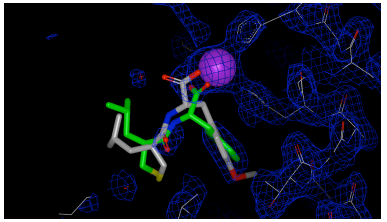

Figure 5: Superposition of the refined PDB (green) and AFITT (atom-color) solutions (1atl). AFITT was unable to regenerate the deposited solution. RMSD = 1.7 Å and the Δ strain = 1.9 kcal/mol. It is reasonable to assume that the missing density is partly to blame in this case.

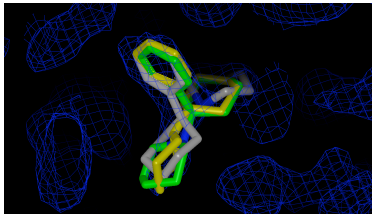

Figure 6: The top ranked AFITT solution (atom color) for one of the two monomers in the asymmetric unit cell was placed incorrectly. RMSD = 3.9 Å and Δ strain = 4.0 kcal/mol. The PDB structure (green) of 2PCP and the AFITT solution (yellow) from the other monomer of shown for comparison. RMSD = 0.17 and Δ strain = 4.0 kcal/mol.

Conclusion

AFITT appears to consistently generate ligand placements and/or geometries that are equivalent to, or in three cases better than, deposited solutions, as measured by fit-to-data and geometric strain. For the three exceptions (13gs, 1atl, 2pcp) poor electron density force field errors seem to hamper the procedure. AFITT provides a unique, rapid, solution to the challenge of ligand structure determination via automatically identifying unfilled density, generating conformations and initial placements, real-space refinement of those top ranked placements and finally generating refinement dictionaries for subsequent RefMac or CNX refinement.

References

Perola, E. and Charifson, P.S., "Conformational analysis of drug-like molecules bound to proteins: an extensive study of ligand reorganization upon binding" J. Med. Chem., 2004, 47, 2499-2510

Wlodek, S.; Skillman A.G.; Nicholls, A., "Automated Ligand Placement and refinement with a combined force field and shape potential" Acta Cryst., 2006, D62, 741-749.

Electron Density Server - http://eds.bmc.uu.se/eds/

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