Modern, rigorous macromolecular crystallographic refinement using mixed-QM/MM functional methods as implemented in DivCon

Oleg Y. Borbulevych[a] and Lance M. Westerhoff[a]
[a]QuantumBio Inc., State College, PA 16801, USA

Conventional macromolecular crystallographic refinement relies on stereochemistry restraints and rudimentary energy functionals to ensure the correct geometry of the model of the macromolecule, along with any bound ligand(s), within the experimental, X-ray density. Traditionally, these highly approximate functionals lack explicit, chemically rigorous terms for electrostatics, polarization, dispersion, hydrogen bonds, and other interactions, and they often rely on pre-determined parameters to capture the *a priori* understanding of the structure. Previously, we addressed this problem, especially for active sites and ligands, through the integration of our DivCon, linear scaling, semiempricial quantum mechanics software with the PHENIX package. However, this implementation was limited in that it still treated the remainder of the system using conventional, stereochemical restraints without taking into account, long-range interactions between this part and the QM region.

In order to address this deficiency and capture a more complete understanding of the structure, we have developed a fully automated approach for macromolecular refinement based on a two layer, QM/MM (ONIOM) scheme implemented within our DivCon plugin. This method consists of one or more user-defined, PM6 quantum mechanics regions (referred to as the "region layer") coupled with a "system layer" encompassing the rest of a protein described with our implementation of MM functional. This chemically complete approach is fully automated for amino acids, nucleotides, truncated residues, ligands, and cofactors, and it does not require user-provided stereochemical restraint files (CIF's) or other description files in order to correctly type the atoms within the structure.

We have validated this plugin on a population of 80 protein-ligand structures from the Astex Diverse Set. Across the entire population, our QM/MM plugin results in significantly lower strain energies and lower residual difference density around each ligand. Further, the tool leads to dramatic improvements of the overall structural quality as measured by MolProbity. For example, the MolProbity ClashScore averaged over 80 structures is 1.11 after DivCon:ONIOM refinement compared to 4.83 after conventional PHENIX refinement and to 5.52 after use of our previous, pure DivCon:QM region refinement.