MS02-P04

Quantum refinement of X-ray and neutron protein crystal structures

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Combining quantum mechanics and molecular mechanics (QM/MM) is one of the most important methods of studying the structure and function of proteins. This approach can also be applied in crystallographic refinement, as the geometry restraints used in refinement are in the form of an MM force-field. Thus, the MM potential for a small part of the molecule (for example, the active site of an enzyme) can be replaced by a QM potential, which can result in local improvement of protein crystal structures. We have developed such a method, *quantum refinement*, implemented in the ComQum-X program, which integrates a quantum chemical software with a crystallographic refinement software.

We present several recent applications of quantum refinement on both X-ray and neutron protein crystal structures. Firstly, we show how quantum refinement can help in determining the composition and geometry of the active site in metalloenzymes. For example, quantum refinement supports a model of particulate methane monooxygenase (pMMO) with only one copper atom in the active site instead of the two that the structure deposited in the PDB contains. (Cao *et al.*, 2018) Secondly, we show a protonation study of the active site of nitrogenase. Quantum refinement predicts the correct protonation state of the homocitrate residue (Cao *et al.*, 2017) and suggests that the FeMo-cluster is fully unprotonated in the resting-state crystal structure. Furthermore, we show some applications of the more recently developed ComQum-U (Manzoni *et al.*, 2018), which combines quantum chemistry with joint X-ray and neutron refinement. This is especially useful in refining structures of enzymes that catalyze reactions that occur with proton transfer, in which case the neutron data may be unclear in the active site. To this end, we apply quantum refinement on the crystal structure of substrate-free lytic polysaccharide monooxygenase (LPMO) and on the crystal structure of triose phosphate isomerase (TIM) in complex with an inhibitor.

References:

Cao, L., Caldararu, O., Rosenzweig, A. C. & Ryde, U. (2018). Angew. Chemie - Int. Ed. 57,.

Cao, L., Caldararu, O. & Ryde, U. (2017). J. Phys. Chem. B. 121,.

Manzoni, F., Caldararu, O., Oksanen, E., Logan, D. T. & Ryde, U. (2018). J. Appl. Crystallogr. Submitted

Keywords: quantum mechanics, refinement

MS02-P05

Present status of SPring-8 macromolecular crystallography beamlines

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At SPring-8, JASRI and Riken are collaboratively developing five beamlines dedicated to macromolecular crystallography. Each beamline shares to serve broad requests from beamline users and liaises to develop new applications for enhancing each characteristic property.

Undulator beamlines, BL41XU [1] and BL32XU [2], focus on cutting edge analyses exploiting high flux microbeam produced by high-magnification focusing optics. In BL41XU, the two step focusing achieved beam size of 2 μm × 2 μm - 35 μm (H) × 50 μm (V). The wide range of beam size allows both micro-crystallography and high-resolution data collection that makes efficient use of crystal diffraction volume. In addition to high-resolution analysis, ultra-high resolution (~0.4 Å) data could be collected by using higher energy X-rays in energy range of 20 keV to 35 keV focused by using compound refractive lenses. Meanwhile, BL32XU can provide the fine beam with typical horizontal size of 1 μm. This micro-beam is very suited to data collection from small crystals, especially membrane protein crystals grown in LCP that are important targets on the beamline. For such micro-crystals, we developed an automated data collection system, ZOO. It is performed in advance of multi-crystal data collection for microcrystals; the crystal alignment tool, SHIKA, provides 2D spot population map of raster scan, and KUMA program is a tool suggesting data collection strategy with mitigating radiation damage.

On the other hand, the bending magnet beamlines BL26B1/B2 and BL38B1 are focused for automation and routine data collection exploiting stable and easily tunable beam. The humidifiers have been installed for the HAG (Humid Air and Glue-coating) mounting method [3], which involves a combination of controlled humid air and water-soluble polymer glue for crystal coating. By this technique, most protein crystals can be kept at room temperature and are able to be cryo-cooled under optimized humidity. In Situ X-ray diffraction instruments using crystallization plate have also been developing. This system consists of plate goniometer, robot arm for plate mounting and plate hotel.

References:

[1] Hasegawa, K. et al. (2013). J. Synchrotron Rad. 20, 910-913.

[2] Hirata, H. et al. (2013). J. Phys.: Conf. Ser. 425, 1-4.

[3] Baba, S. et al. (2013). Acta Cryst. D69, 1839-1849.

Keywords: synchrotron x-ray crystallgoraphy, macromolecular crystallography