Poster

Enhancing structural refinement of macromolecules obtained from neutron crystallography

Lucrezia Catapano

MRC Laboratory of Molecular Biology

lucrezia@mrc-lmb.cam.ac.uk

Hydrogen atoms represent a large fraction of the total atomic content of macromolecules. They often play critical roles in enzyme catalysis, ligand recognition processes, and protein-protein interactions. However, their direct visualisation by diffraction techniques is challenging. Macromolecular X-ray crystallography affords the localisation of only the most ordered hydrogen atoms at (sub-)atomic resolution (around 1.2 Å or higher). However, many hydrogen atoms of biochemical significance remain undetectable by this method. Differently, neutron diffraction methods enable the visualisation of most hydrogen atoms, typically in the form of deuterium (2H) atoms at much more common resolution values (better than 2.5 Å). Thus, neutron crystallography, although technically demanding, is often the method of choice when direct information on protonation states is sought.

Novel refinement protocols have been implemented in the macromolecular refinement software REFMAC5 [1], one of the flagship packages of the Collaborative Computational Project Number 4 (CCP4) suite of programs [2]. One new feature for neutron data analysis in REFMAC5 is refinement of the protium/deuterium (1H/2H) fraction [3]. This parameter describes the relative 1H/2H contribution to neutron scattering for hydrogen isotopes. Additionally, stereochemical restraints, including accurate covalent bond distances between the hydrogen atom and parent atom nuclei suitable for neutron refinement, are now included in the CCP4 Monomer Library, the source of prior chemical information used by REFMAC5 [3-4]. The newly developed REFMAC5 algorithms were tested by performing the (re-) refinement of several entries available in the PDB and of one novel structure (FutA) [5] using either (i) neutron data only or (ii) neutron data supplemented by external restraints to a reference X-ray crystallographic structure. Furthermore, the refinement process of urate oxidase (UOX) in complex to a C5(S)-peroxo derivative of 9-methyl uric acid (MUA), a co-factor free enzyme that catalyses the degradation of uric acid (UA) to 5-hydroxyisourate is discussed. The varying protonation states of the catalytic residues provide insights into the enzyme mechanism.

[1] Murshudov, G. N., Skubak, P., Lebedev, A. A., Pannu, N. S., Steiner, R. A., Nicholls, R. A., Winn, M. D., Long, F. & Vagin, A. A. (2011). Acta Cryst. D 67, 355-367.

[2] Agirre, J., Atanasova, M., Bagdonas, H., Ballard, C. B., Basle, A., Beilsten-Edmands, J., Borges, R. J., Brown, D. G., Burgos-Marmol, J. J., Berrisford, J. M., Bond, P. S., Caballero, I., Catapano, L., Chojnowski, G., Cook, A. G., Cowtan, K. D., Croll, T. I., Debreczeni, J. E., Devenish, N. E., Dodson, E. J., Drevon, T. R., Emsley, P., Evans, G., Evans, P. R., Fando, M., Foadi, J., Fuentes-Montero, L., Garman, E. F., Gerstel, M., Gildea, R. J., Hatti, K., Hekkelman, M. L., Heuser, P., Hoh, S. W., Hough, M. A., Jenkins, H. T., Jimenez, E., Joosten, R. P., Keegan, R. M., Keep, N., Krissinel, E. B., Kolenko, P., Kovalevskiy, O., Lamzin, V. S., Lawson, D. M., Lebedev, A. A., Leslie, A. G. W., Lohkamp, B., Long, F., Maly, M., McCoy, A. J., McNicholas, S. J., Medina, A., Millan, C., Murray, J. W., Murshudov, G. N., Nicholls, R. A., Noble, M. E. M., Oeffner, R., Pannu, N. S., Parkhurst, J. M., Pearce, N., Pereira, J., Perrakis, A., Powell, H. R., Read, R. J., Rigden, D. J., Rochira, W., Sammito, M., Sanchez Rodriguez, F., Sheldrick, G. M., Shelley, K. L., Simkovic, F., Simpkin, A. J., Skubak, P., Sobolev, E., Steiner, R. A., Stevenson, K., Tews, I., Thomas, J. M. H., Thorn, A., Valls, J. T., Uski, V., Uson, I., Vagin, A., Velankar, S., Vollmar, M., Walden, H., Waterman, D., Wilson, K. S., Winn, M. D., Winter, G., Wojdyr, M. & Yamashita, K. (2023). Acta Crystallographica Section D 79, 449-461.

[3] Catapano, L., Long, F., Yamashita, K., Nicholls, R. A., Steiner, R. A. & Murshudov, G. N. (2023). Acta Crystallographica Section D 79, 1056-1070.

[4] Vagin, A. A., Steiner, R. A., Lebedev, A. A., Potterton, L., McNicholas, S., Long, F. & Murshudov, G. N. (2004). Acta Cryst. D 60, 2184-2195.

[5] Bolton, R., Machelett, M. M., Stubbs, J., Axford, D., Caramello, N., Catapano, L., Malý, M., Rodrigues, M. J., Cordery, C., Tizzard, G. J., MacMillan, F., Engilberge, S., von Stetten, D., Tosha, T., Sugimoto, H., Worrall, J. A. R., Webb, J. S., Zubkov, M., Coles, S., Mathieu, E., Steiner, R. A., Murshudov, G., Schrader, T. E., Orville, A. M., Royant, A., Evans, G., Hough, M. A., Owen, R. L. & Tews, I. (2024). Proceedings of the National Academy of Sciences 121, e2308478121.