Challenge to an extremity of X-rays crystal structure analysis

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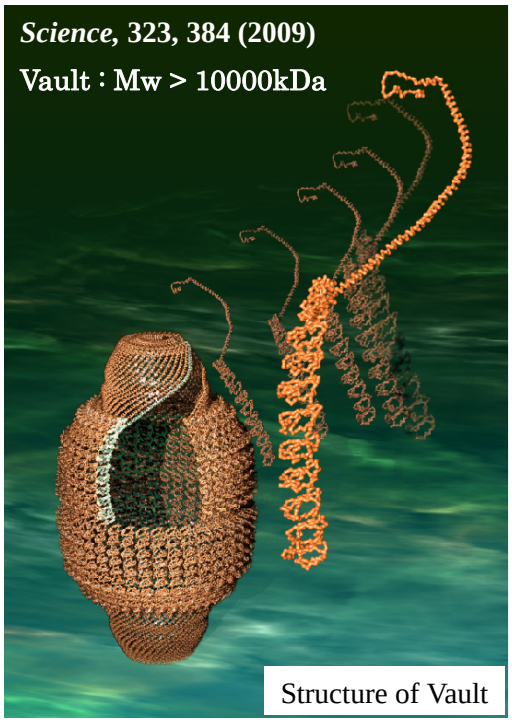
The protein structures are the very important keys to understand the puzzles of life process. The whole world was surprised when the crystal structure of the ribosome, a huge molecule with 3,000 kDa molecular weight (MW) was determined about 10 years age. Since then, the field of macromolecular crystallography has been rapidly developed in both sides of hardware and software. Recently, we cooperated with professor Tsukihara group of Institute for Protein Research, Osaka University and Department of Life Science, University Hyogo, to determine a crystal structure of Vault which is a huge protein complex with 10,000 kDa MW. This study was published in Science as it is the biggest structure ever solved by crystallography (figure)[(1)].



Structure of Vault

Vault is a large barrel-shaped ribonucleo-protein particle that is highly conserved in wide variety of eukaryotes. Although the cellular function of Vault remains unclear, the functions including roles in multidrug resistance, cell singling, and innate immunity have been reported. The group of Professor Tsukihara has got successful to grow the crystals of Vault and collect reflection data at 3.5 Å resolution. The phases were calculated by molecular replacement using the cryo-EM map as a search model followed by non-crystallographic symmetry averaging, and the initial model was partly built. In general, the refinement of protein is a time consuming step since the manual fitting and building are necessary. For example, it will take several weeks or months for the protein with 10-100 kDa MW. Therefore, it is nearly unrealistic to perform refinement of such huge macromolecule as Vault by a normal method. Here our program LAFIRE which is an automatic refinement software package[(2, 3)] made a major contribution.

We have developed the program LAFIRE as one important part for high-throughput crystal structural analysis, and it is designed to perform the whole process beginning from an initial model that can be approximate, fragmentary or even only main chain. In current study, we improved the LAFIRE for huge molecule, and the refinement at 3.5 Å resolution of Vault was performed in few weeks.

Reference

1. H. Tanaka, K. Kato, E. Yamashita, T.i Sumizawa, Y. Zhou, M. Yao, K. Iwasaki, M. Yoshimura, T. Tsukihara (2009) *Science*, **323**, 384-388
2. M. Yao, Y. Zhou, I. Tanaka (2006) *Acta. Cryst.*, **D62**, 189-196
3. Y. Zhou, M. Yao, I. Tanaka (2006) *J. Appl. Cryst*,. **39**, 57-63