Oral Presentation 4

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『構造生命科学』
Structural Bioscience

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O1-05

Protocol for Assessing the Optimal Pixel Size to Improve the Accuracy of Single-Particle Cryo-Electron Microscopy Maps

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Keywords: Cryo-EM, Protein structure modeling

With the advances of hardware and software technologies, detailed structural models of biomolecules can now be obtained from cryo-EM experiments. We have been developing algorithms and software to assist such modeling [1]. The three-dimensional volume obtained from the cryo-EM is given as data on a three-dimensional lattice. Here, the parameter that specifies the actual size of each lattice determines the size of the whole molecule and thus affects the structure modeling results. However, it is known that there can be an error of a few % in the estimation of this parameter. Therefore, in order to improve the accuracy of structural modeling, we have developed an algorithm that can estimate an accurate value for this lattice size parameter [2].

In this algorithm, many EM maps with different grid sizes are prepared and a large number of models are created by performing structure refinement against these maps. Then, the correct lattice size is estimated by evaluating the "quality" of each of these structures. Here, evaluation of the "quality" of the obtained structures needs to be performed independent of the fitting procedure. The algorithm was tested using the maps created by simulation for several model systems, and various "quality" evaluation approaches were examined. First, we showed that the general correlation coefficient, which measures the degree of agreement between the structure and the map as corresponding electron densities, is not sufficient to detect correct grid size. It was also found that the Molprobity index, which is commonly used to evaluate X-ray crystal structure models, is insufficient because there is no physicochemical aspect in the evaluation of interatomic interactions. Finally, we found that GOAP, which is an index used for protein structure prediction, is effective in evaluating the local structure and interatomic distance in proteins, which is the "quality" of the structure necessary to identify correct grid size. We applied the developed algorithm to experimental data and demonstrated the usability of the algorithm. The details of computational protocols and tools will be discussed.

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[2] Tiwari, S.P., Chhabra, S., Tama, F., Miyashita, O., *Computational Protocol for Assessing the Optimal Pixel Size to Improve the Accuracy of Single-Particle Cryo-Electron Microscopy Maps*, J Chem Inf Model. 2020;60:2570-2580

High-resolution neutron crystallography visualizes an OH-bound resting state of a copper-containing nitrite reductase

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Keywords: Neutron crystallography, Structural Biology, Quantum chemistry

Copper-containing nitrite reductases (CuNIRs) transform nitrite to gaseous nitric oxide, which is a key process in the global nitrogen cycle. The catalytic mechanism has been intensively studied to ultimately achieve rational control of this important geobiochemical reaction. However, accumulated structural biology data show discrepancies with spectroscopic and computational studies, and hence the reaction mechanism is still controversial. In particular, the details of the proton transfer involved in it are largely unknown. This situation has arisen from that even atomic resolution X-ray crystallography fails to determine positions of hydrogen atoms and protons, which play essential roles at the catalytic site of CuNIRs. We determined the 1.50 Å resolution neutron structure of a CuNIR from *Geobacillus thermodenitrificans* (trimer molecular mass of ~ 106 kDa) in its resting state at low pH [1]. Our neutron structure reveals protonation states of catalytic residues (deprotonated aspartate and protonated histidine), providing insights into the catalytic mechanism. We found that a hydroxide ion, not a water molecule, can exist as a ligand to the catalytic Cu atom in the resting state even at low pH. This OH-bound Cu site is unexpected given previous X-ray structures, but consistent with a reaction intermediate suggested by computational chemistry. Furthermore, the hydrogen-deuterium exchange ratio in our neutron structure suggests that the intramolecular electron transfer pathway has a hydrogen-bond jump, which is proposed by quantum chemistry. Our study can seamlessly link the structural biology to the computational chemistry of CuNIRs, boosting our understanding of the enzymes at the atomic and electronic levels.

[1] Fukuda, Y., Hirano, Y., Kusaka, K., Inoue, T., and Tamada, T., High-resolution neutron crystallography visualizes an OH-bound resting state of a copper-containing nitrite reductase, *Proc. Natl. Acad. Sci. USA*, 117:4071-4077, 2020.

O1-07

Innovative Small-Angle Scattering for Structural Analysis of Biomacromolecule in Solution

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Keywords: Small-Angle X-ray Scattering (SAXS), Laboratory-based Size exclusion chromatography SAXS System (La-SSS), Analytical UltraCentrifugation Small-Angle Scattering (AUC-SAS)

Small-Angle Scattering (SAS) offers overwhelming opportunities for structural analysis on various biomacromolecules in solution close to physiological condition. To reveal structure of a single biomacromolecule in multi-component solution, it is indispensable to selectively acquire scattering profile of the concerned biomacromolecule. In particular, contamination of undesirable aggregates could hinder the scattering profile and then lead to failure of the structural analysis. Therefore, we developed two methods for realizing the selective observation of multi-component systems. In addition, the developed methods are utilized with laboratory-based instruments, meaning that the problem on the limitation of the beam time of the synchrotron-based Small-Angle X-ray Scattering (SAXS) could be also solved.

The first development is Laboratory-based Size exclusion chromatography SAXS System (La-SSS) by utilizing a state-of-the-art laboratory-based SAXS instrument and optimization of various parameters [1]. The components with the different sizes in solution are separated with size exclusion chromatography just prior to SAXS measurements and then are directly injected into SAXS instrument. La-SSS makes the selective observation of the concerned component not only in solutions including aggregates but also in general multi-component solutions. The second development is a combination analysis method with Analytical UltraCentrifugation and SAS (AUC-SAS) [2]. The scattering profile of the concerned component is extracted from that of multi-component solution by utilizing the weight fraction of each component obtained with AUC.

In the aggregation-removal, La-SSS works even with the sample including large amount of aggregates, while AUC-SAS is applicable only to that with relatively small amount of aggregates (ca. < 15 % of weight fraction). On the other hand, AUC-SAS requires the smaller amount of sample (~ 0.2 mg of biomacromolecule for AUC-SAS, and > 2 mg for La-SSS in a typical case) and enables to observe a weakly-bound complex which dissociates in a SEC-column. Consequently, complementary use of La-SSS and AUC-SAS makes it possible to analyze various multi-component biomacromolecular solutions. In the presentation, we will demonstrate some applications of La-SSS and AUC-SAS to aggregation-prone systems and general multi-component systems such as solutions under the association-dissociation equilibrium.

[1] R. Inoue, T. Nakagawa, K. Morishima, N. Sato, A. Okuda, R. Urade, R. Yogo, S. Yanaka, M. Yagi-Utsumi, K. Kato, K. Omoto, K. Ito, M. Sugiyama, *Scientific Reports*, 9:12610, 2019.
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