平成 26 年 3 月 19 日

（独）理化学研究所
研究担当理事　川合 眞紀

平成 25 年度実施 主任研究員の中間レビューの結果について

准主任研究員制度設置規程（平成 25 年規程第 14 号）に基づき准主任研究員の中間レビューを踏まえ、レビューアーから送られた評価結果は以下のとおりです。

１．評価対象： 米倉生体機構研究室 米倉 功治 准主任研究員

１）評価体制

実施日：平成 25 年 12 月 16 日（火曜日）
4 名の所外有識者を評価委員とするヒアリングレビューを実施。
評価者：
Jianwei John MIAO, Professor
Department of Physics and Astronomy
University of California, Los Angeles, U.S.A.

Osamu NUREKI, Professor
Graduate School of Science,
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Graduate School of Life Science,
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２）評価結果の概要等

General comments:

【Reviewer 1】

The Biostructural Mechanism Laboratory, headed by Dr. Koji Yonekura, has developed advanced technologies of 3D electron crystallography and coherent X-ray diffraction imaging (CXDI), which are strong tools for elucidating biomolecular structures impossible to crystalize or extremely weak to X-ray and electron irradiation. Using these advanced techniques with some other conventional techniques such as EM single particle analysis and X-ray crystallography, Dr. Yonekura and his laboratory members have achieved a variety of outcomes such as 3D Coulomb potential maps of Ca^{2+} ATPase, high resolution structures of bacterial flagellar filaments, single particle analysis of a PomAB, complex crystal structure of Hfq and HPII, structural analysis of telomere complexes, and so on. These achievements can be evaluated to contribute to the advancement of our knowledge in the field of structural biology. They published six regular papers in international journals including high level one for

recent five years. Their techniques have great potential to elucidate the structure-function relationship of biomolecules including proteins of which X-ray crystallographic analysis are difficult. However, they have not yet bring out the full potential of their techniques. Dr. Yonekura said his future plan is to reveal working mechanisms of biological macromolecules. I think their targets are somewhat too dispersed. I expect they will focus their target and achieve a real breakthrough with their superior technology.

【Reviewer 2】

The Biostructural Mechanism Laboratory lead by Dr. Koji Yonekura aims to elucidate the working mechanisms of biological macromolecular machines by X-ray crystallography, coherent X-ray diffraction imaging (CXDI), and cryo–electron microscopy (cryo-EM). They have been developing several methods to overcome difficulties associated with structural determination of various biological objects.

Developing an electron crystallographic method for 3D nanocrystals, Dr. Yonekura in collaboration with Professor C. Toyoshima (The University of Tokyo) successfully obtained 3D Coulomb potential maps of Ca^{2+}-ATPase and catalase at resolutions of 3.4 Å and 2.8 Å, respectively. This achievement (manuscript in preparation) is a breakthrough in electron crystallography.

Additionally, Dr. Yonekura and colleagues have succeeded in the helical reconstruction of a bacterial flagella filament by cryo-EM. They have also realized EM single particle analyses of a membrane protein complex and an RNA-targeting complex. Moreover, they have applied cryo-EM tomography of biological objects to reveal the heterogenic structures. Due to their elite abilities in EM structural biology, many biologists are collaborating with them to determine the structures of biological macromolecular assemblies. Recently, they employed X-ray crystallography to determine the structure of an RNA chaperon Hfq and catalase HPII complex.

Dr. Yonekura has also contributed to coherent X-ray diffraction imaging (CXDI) at SACLA. In a collaborative project, he and coworkers developed a new cryo-specimen holder for CXDI, cryo-EM, and X-ray crystallography.

Dr. Yonekura's laboratory excels in EM structural biology, and utilizing XFEL they have advanced efforts toward elucidating the working mechanism of biological macromolecular machines. The reviewer believes that they will continue to realize significant scientific advances toward the development of a new methodology of XFEL.

【Reviewer 3】

Dr. Koji Yonekura's Biostructural Mechanism Laboratory aims to develop and implement a combination of correlative tools to tackle important biological problems. Over the past five years, his lab has made significant progress in the following four research directions. First, Dr. Yonekura and his collaborators obtained an atomic model of the L-type straight bacterial flagellar filament at ~ 4 Å resolutions and observed some new structure features. Second, he developed electron crystallography of 3D nanocrystals. Because the scattering power of electrons is more than five orders of magnitude higher than that of X-rays, this

new research area could potentially have significant impact to structural biology. Third, he and his collaborators have developed instrumentation and methodologies for coherent X-ray diffraction imaging (CXDI). This is especially important for the RIKEN SPring-8 Center as it hosts the world's second X-ray free electron laser (SACLA). Finally, Dr. Yonekura has applied electron cryo-microscopy (cryoEM) to biological macromolecular complexes such as telomere complexes, CRISPR-Cas complexes, the FliPR complex, and a TRP channel. Looking forward, Dr. Yonekura will combine cryoEM and CXDI to solve biological problems, and also take advantage of the unique facility at SPring-8 (i.e. SACLA). Overall, I was impressed by Dr. Yonekura's research broadness and depth and his willingness to develop new methodologies for structural biology. If Dr. Yonekura's laboratory can have more manpower, his research productivity would be further enhanced.

【Reviewer 4】

Dr. Yonekura is promoting electron crystallography, which means electron scattering for three dimensional crystals. The advantages of this new technique is to clearly elucidate charged state and protonation state in the Coulomb potential map, which is very difficult to elucidate in the normal X-ray crystallography. Dr. Yonekura combined the two complementary techniques, electron crystallography and X-ray crystallography, to uncover ionic state of phenylalanine residue coordinating to heme in catalase and protonation state of glutamate residue in Ca2+ pump. Electron crystallography has of course limit of the thickness of crystals, but he is developing a new device for the electron microscopy to overcome this limit. Dr. Yonekura is further promoting the research using combination of cryo-optical microscopy, EM and CXDI to elucidate macromolecular complex and bacterial cells. Therefore, Dr. Yonekura is developing the state-of-the-art technique to uncover the functional structure of various targets, which is really impressive. While his group is rather small, he well manages the three staffs and research assistants as well as good collaborators to promote the new research field.

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