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Structural Physiology Research Group Final Review

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評価結果の概要 General comments：

[REVIEWER 1]

The Structural Physiology Research Group with aim to understand the atomic to cell scale level physiologically, from the structural point of view, a regime is created by 4 research teams with different methods to deal with the research project in a complementary style. And the regime addresses the basic technology development to help structural understanding and the application of the research. Research method using high intensity X-ray source from SACLA and SPring-8, and the R&D which is complementally usable for the method are academically valuable to fill in the resolution gap between the best (ideal) and the actual situation of structural biology, and also the technologies produced by the research activities and applied to biological living samples are physiologically important for proteins associated with synapses and sensory perceptions, as well as cytoskeleton.
Bio-multisome Research Team, by using electron microscopes, addressed the research of filling the resolution gap between light microscopes and x-ray microscopes (used in x-ray crystal structural analysis). Especially the development of genetically encoded tags enabling protein detection by electron microscopes and the application of the encoded tags succeeded to observe the protein localization to synapse. Hereafter much further improvement is required for the general use of the microscopes, however the approach of the field of microscope R&D which is

important for the electron microscopes is highly evaluated. Also currently this team is working on a correlated light and electron microscope, and also it succeeds to identify the localization of acetylcholine receptors so that we can expect further future development. Such R&D will be gradually more significant to make the connection between atomic models created by the x-ray crystal structural analysis and the functions of the actual cells.

Three dimensional microscopy research team made efforts on two dimensional crystallization of membrane proteins and the crystallization was applied to the crystal structure analysis by electron beam, and the team was also collaborated with Molecular Signaling Research Team to address the three dimensional crystallization. About the electron beam analysis of crystal structure, the three dimensional conformational transporter of the AE1 (anion exchanger 1) was determined and also the three dimensional crystals of oxalate transporters are obtained so that the improvement of the crystallization are expected.

Molecular Signaling Research Team with an aim to understand the mechanism of the sensor molecular functions, the mechanism related to the protein structural determination and the functional analysis of the sensor molecules are addressed.

About acetylcholine-binding proteins which are homologous to the substrate-binding sites of acetylcholine receptors, complex molecule structures binding to some pesticides were determined. Such detailed structural analysis seems significant in order to develop the specific medical substances. Also heterodimers of T1T2 and T1T3 as gustatory receptors were structurally analyzed and also TRP channel as mechanosensitive channel were analyzed using NMR. For the protein crystallization screening, new techniques such as native electrophoresis and other methods were developed. Therefore future use of these methods is promising.

X-ray Structural Analysis Research Team structurally analyzed for F-actins and the F-actin capping protein complexes in diverse ways. First, high-resolution structure was determined by using fiber diffraction and mutant actins were analyzed. And to understand the motility, inelastic scattering measurements and molecular dynamics calculations were introduced. Also collaborative research in actin-related protein complex was conducted. Through these wide ranges of analysis, highly complementary results (figures) were obtained so that other fibril proteins such as amyloid fibrils are promisingly applied.

Some other collaboration researches within some groups are also in progress and all teams are engaged with some kinds of collaborative research so that it seems that the management system interactively activates entire research. Also each team has a clear and challenging research targets and these research are conducted in order to understand them. Although the research methods are widely varied, since two teams are mainly engaged with SPring-8 (synchrotron radiation) and other two teams are engaged with complementary methods (as using electron microscopes) and both ways are appropriately balanced. Moreover each group sets a challenging goal so that the future development will be positively expected.

[REVIEWER 2]

1. Research Subject

From the self-evaluation report, it is difficult to determine the main subject of this project. Summarizing the research activity of this project including the published peer reviewed papers, the focus seems to be a study of the mechanisms for cellular motility and communication at the molecular level. The research teams worked as

users employing various existing imaging techniques developed in the field of diffraction physics rather than as innovators seeking to develop original techniques for SPring-8, SACLA and electron microscopy. Thus, the research carried out in the seven-year project seems to center on sample preparation to allow visualization of cellular and molecular structures.

2. Scientific Results

This seven-year project, which spent a grant of approximately 750 million yen, is large enough to raise high expectations for important contributions to the fields of biological and biophysical science. Below, I tried to briefly summarize the original achievements of the four research teams. During the past 7 years, the scientific results from this project probably have little social impact.

The bio-multisome research team seemed to focus on sample preparation techniques using commercially available electron microscopes. The approach is important to develop methods using electron microscopy in biological science as well as done in some groups of national universities. As a result, this group published four papers closely related to the main theme. The research that was carried out mostly within the group is highly evaluated. Collaboration with research groups outside RIKEN is an important prerequisite for this team to publish papers in various fields.

Two of the research teams studied the structures of membrane proteins. The Three-dimensional Microscopy Research Team focused on the human erythrocyte anion exchanger 1 at low resolution, and published several papers in collaboration with some experts of electron microscopy. The Molecular Signaling Research Team received 161.4 million yen during the period from 2007 to 2012, and published probably two papers reporting the original sample preparation techniques concerning the project. The other publication are better to be categorized as not main but side projects of this team. The small number of original papers from these two research teams indicates the difficulties and risks in structural biology studies of membrane proteins. The researchers would reveal many three-dimensional structures of membrane proteins at high resolution in the near future based on the currently unpublished research results.

The X-ray Structural Analysis Research Team focused their research on the structure-function of actin. The scientific significance of the research subject is well known in biophysics and biology. This team worked well under the direction of Professor Maeda of Nagoya University and proposed a structural model of actin filament through very simple calculation of the fiber diffraction pattern obtained at SPring-8. In addition, by providing samples to the neutron scattering group at Tokai Institute of the Japan Atomic Energy Agency, this team contributed to measure the 'ensemble-averaged' structural fluctuation of actin filament. Judging from the number and quality of the peer-reviewed publications, this team was the most active among the four teams, despite receiving the smallest amount of grant money during the seven years.

Operations of the SACLA XFEL facility for public users started in 2012. The project leader emphasizes that their novel techniques in electron microscopy are suitable for XFEL experiments using biological samples. However, it is very surprising and unfortunate for RIKEN that no one in four research teams used SACLA during this project.

3. Management

This research group seems to be a simple assembly of four research teams of biophysicists, who studied different targets using different experimental techniques, although the group leader emphasized his efforts to organize scientifically the four teams, that were directed relatively young scientists without experiences in scientific researches and laboratory management. People in the biophysics field would sympathize with the group leader for his struggles to coordinate this disorganized group.

4. Summary

In summary, the research project contributed to structural biology of actin based on the previous research project conducted by Professor Maeda. The other three teams also carried out nice researches for structural studies of cellular structures and membrane proteins. The multi-some research must be quite difficult, judging from the small number of peer reviewed publications and invited lectures, which are closely related to the 7-years research project. Thus, it is very difficult to say simply this project, which spent the large grant of approximately 750 million yens, ends in failure except for a model to tentatively discuss the structure-function relation of actin. And, it is now a total mystery why managers of Harima RIKEN selected this project 8 years ago.

[REVIEWER 3]

The Structural Physiology Research Group, based on the seven-year-plan from April 2006, has been promoting the research development related to the structures of the membrane proteins and cytoskeletal proteins related to cellular information transmitting as well as the physiological functions of the proteins. This group consists of four teams and comprehensively addresses to combine three analysis methods of X-ray spectroscopy (structural analysis), electron microscopy, and light microscopy. Centering the analysis information transmission in the synapse, the four teams aimed to create the synergy effect by analyzing the various membrane proteins related to the information transmission and the three dimensional dynamic physiological function of the complexes.

Under the management of respecting the each distinctive research team, the research activity of this research group launched by collecting bright futures young researchers produced excellent outputs individually. On the other hand, since the direction of the whole group was not positive going, the enhancement of the collaborative research among the teams produced the synergy effect in a limited way. Therefore there was no remarkable creation of technology and academic finding as representing outcomes of RSC. The research project of RIKEN is expected to create innovative technologies and academic outcomes by utilizing the cutting edge facilities. From this point of view, the top management should be required. Also this group set the important and difficult themes to challenge aggressively, however the human power resource was not enough and some researchers transferred out in the middle of the project. As the result, the quantity of the research papers from the group was not sufficient so that the “manuscript in preparation” of papers should be in hurry. Considering how to build the framework to address the challenging project, the researchers should find a room for the improvement. Good things to note is that many of the team leaders and researchers who were remarkably active, found independent positions in universities and started their own research activities therefore the purpose of educating the next generation leaders

for the structural biology was accomplished.

[REVIEWER 4]

1. Research Objectives: novelty, specific significance

The Structural Physiology Research Group aims to study a dynamic physiology from three dimensional structure analyses of proteins and complexes, which are important for signaling, by an X-ray crystallographic analysis and an electron microscopy. Although the targets of this research project are diverse, this group shares common sense of research analysis, and their research objectives are appropriate.

2. Research Results: Originality, specific significance social impact

All of four teams obtained superior results. For instance, 1) Bio-multisome Research Team developed multiple novel techniques for observation by electron microscopy, such as STEM (scanning transmission electron microscope) tomography. 2) Three-dimensional Microscopy Research Team succeeded to obtain low-resolution structure of AE1, the most abundant membrane protein in human erythrocyte. 3) Molecular Signaling Research Team determined the crystal structure of LeuT, the highly-stable NSS (neurotransmitter sodium symporters) homolog, in two different functional state. 4) X-ray Structural Analysis Research Team clarified the nature of the globular- to fibrous-actin transition by creating a model of F-actin using X-ray fibre diffraction intensities. Each results has high quality of science, and their social ripple effect is very strong.

3. Management of the Laboratory: Laboratory member composition, researchers interaction etc.

Many talented scientists from different fields gather and form this unique group. Advantage of this group seems to be a respect of “bottom-up science”, and most of research subjects appeared to be derived from the researcher side. This may be due to a direction of Dr. Miyazawa, the leader of this group, who respects the individuality of each team leaders. One of the criticisms at the Midterm Review of this group in 2010 was a lack of cooperative works between four research teams. However, some high-level studies were performed cooperatively by multiple teams, and this criticism has been now improved.

4. Overall assessment

This group succeeded to obtain unique and superior results, each of which has high quality of science. This is mainly due to excellent talented scientists are gathered from various fields, and also due to the “bottom-up direction” by the leader of this group.

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