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Synchrotron Radiation System Biology Research Group Final Review

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評価結果の概要 General comments：

[REVIEWER 1]

Goal setting: Dr. Kuramitsu is a leading pioneer in science launched the project of "Whole-cell system of Thermus thermophiles" to understand all the 3D structures of proteins and reveal the functions and life phenomena of the proteins by biophysical methods using an extremely thermophilic bacterium, Thermus thermophilus HB8 as a model organism. This research is a one of significant pillars as utilizing SPring-8 for research. Also the project would not achieved the results without SPring-8 and the significance of SPring-8 became widely known by carrying it out, therefore his accurate goal setting is highly appreciated.

Research achievements: During the targeted period of this project outcome evaluation, this group, consisting of three teams such as Functomics Integration Research Team, Functomics Biology I Research Team, and

Functomics Biology II Research Team (Kumamitsu, Shinkai, and Bessho, team leaders respectively) carried through the research. As the group as a whole, despite the smaller numbers of research staff mainly affiliated with RIKEN, more than 20 papers have been published annually. Especially as recent research outcomes, the DNA Modification Enzymes for structural biological research press release on March 2010 and November 2011 really attracted/grabbed attention.

Mainly, on the project of "Whole-cell system of Thermus thermophiles", as the purpose of analyzing all the functions of proteins by genes, Team Kuramitsu constructed and developed new platforms which reach the purification, crystallization, and analysis of the proteins. Especially development of protein expression plasmids and their analysis data are compiled as database in order to disclose to the public, so that it seems helping a wide range of researchers.

Teams of Shinkai and Bessho are proceeding to target molecules related to transcription, translation, and modification which are important for organisms. Therefore the strong originality of the research group is highly esteemed.

Ripple effect: At the beginning of SPring-8 operation, there were only limited number of the facilities and human resources related to X-ray crystallographic analysis of the protein. Today, however, succeeding protein purification steps most likely leads to the understanding of the protein crystallization since the quality of domestic researchers are greatly enhanced by MEXT Protein 3000 Project as well as the important contribution of this project group.

Overall evaluation: By pursuing "whole-cell project of T. thermophiles HB8", this research group significantly contributed to the development of Japan's structural biological research and its devotion is highly evaluated.

[REVIEWER 2]

1. Research Object

This group took (1) traditional bottom-up (structural and functional analyses of molecules approaches of each molecule by the chemical and biophysical methods and (2) top-down approaches by the omics and whole-cell imaging methods at the same time to complement the weaknesses of the individual two approaches. This has the originality and the uniqueness to analyze the whole cell phenomena by using Thermus thermophilus HB8 as the sample.

2. Research Results

This group has published 154 original papers involving the collaboration with other research groups. After the interim review, the number of publication increased expectedly. Besides the publication, total 491 protein structures were analyzed and they still maintained the high success rate (over 22%). It showed the evidences that their protein preparation and other technologies must be established. Regarding unknown functional proteins, the group could predict functions of 60% of total unknown functional proteins. It makes a sense for the whole cell project using Thermus thermophilus HB8. Because these proteins exist commonly in various mammalian cells not limited in Thermus thermophilus HB8. In the interim review, especially two

technologies are very impressive. One is overproduction by the Mistic (membrane-integrating sequence for translation of integral membrane protein constructs) tag of Bacillus subtilis to their N-terminals. Twelve transmembrane proteins were tested for overproduction by the Mistic system, and eight of them were successfully expressed. I agree that he success rate of about 65% will be sufficient for the future membrane-protein project for structural and functional analysis. Another is the technology development for using of XFEL. Regadring XFEL method, this group has the obligation for the development of some technologies. Although I guess that there isn't enough time to establish new technology, it still didn't get the proof yet.

3. Management of the Research Group

After interim review, this group spread the collaborations with many researchers at RIKEN, universities, institutes, and companies. I think their efforts for some collaboration absolutely reflected to the results.

4. Comprehensive Review

i. Construction of resources for whole-cell analysis of a model organism

This group constructed the resources for structural and functional genomics using by using Thermus thermophilus HB8 as the sample. It achieved to establish some important technologies, especially for the protein expression and purification.

ii. Research and development of functional discovery of new proteins (genes)

This group constructed the platforms for top-down approaches, which includes transcriptome and proteome analyses. By using these platforms, they found new functions of important proteins (genes). Newly identified proteins must be important for interpreting the whole-cell imaging. In the aspect of developing for functional genomics, it is still under developing. The prediction rate of unknown-functional proteins would be more increasing by using the informatics involving the experimental data of interactions between various chemicals and unknown proteins.

iii. Research and development of bio-molecular imaging methods for XFEL

I think this technology is under development and didn't reach sufficient level yet.

I hope that these technologies will be developing to the common technologies by this clue in future...

[REVIEWER 3]

This project so called, "Whole-cell Project" launched to understand the ongoing biological life phenomenon of a cell at atomic level. In order to pursue the project, the integration of two way approaches involves a bottom-up approach to the protein analysis from the molecular-level to the upper macro level and a top-down approach from the cellular-level to the lower micro level. The model organism selected is an extremely thermophilic bacterium, Thermus thermophilus HB8.

1) Research goal setting:

On the way to develop for structural biological research, a deeper understanding of the life phenomenon have

been received attention from not only researching at the protein level but also targeting the upper level of the proteins' 3D structural and functional research. As part of the attention, this project promotes a way to approach the research from the molecule level to the cellular level uniformly by bring the whole-cell into a view at once. The target setting is positively aggressive and disciplinary implication is remarkably strong.

2) Research achievements:

T. thermophilus HB8 has about 2200 genes (proteins) and or 90% (2000 genes) of them have been successfully overproduced by using their protein-production system. And establishment of the foundation for the structural and functional research is highly evaluated. Furthermore, the approximately 500 protein structures were finalized by purifying and crystalizing the expressed proteins. Also there were 500 proteins out of 2,200 which have unknown functions, and the functions of the 140 out of the 500 were specified.

As the bottom-up approach to the protein analysis from the level of protein, the structures of 500 proteins were concluded as the result of devoting large efforts to the determination of the large amount of protein structures. About the proteins with unknown functions, since approximately 70% of the proteins are predictable to find the way how protein's main-chain is folded, 80% of the main-chain structures were identified to analyze in the whole project. This structural analysis will introduce a new platform which is usable for the upper level of system biology.

The proteins with identified structures can be categorized into specific functional groups. The transcription, translation, and DNA modification of these functional groups were also analyzed by the teams of Functomics Biology I Research and Functomics Biology II Research so that these three functional groups lead to the structural and functional research outcomes.

3) Research group management:

By holding symposium, the group actively made effort to research outcome presentations and interexchange among researchers.

4) Overall (comprehensive) evaluation:

Increasing research quantity at the level of protein compound is succeeded so that it seems that there is an achievement of positive results. Especially Protein 3000 Project showed the successful results by increasing the success rate for this structural prediction. However, if I have to point out the issue of this project with expectation, the suggestions are as follows.

It is hard to say that the development of analyzing each protein structures can obtains a better understanding of life phenomena in the upper level of the protein structures. Because it seems that this project only focused on increasing the large quantity of each specific protein structure. Despite attempting to understand the life phenomena widely and deeply by the extension of this project, it has been unsuccessfully performed. After Protein 3000, the project should have only focused on a strategy for the particular functions to understand more. The structural research at the early stage also should have shifted the direction of the mission into complex proteins and membrane proteins which are essential to understand the life phenomena. By doing so, we could have found further progress of the structural research related to the complex proteins which are necessary to

comprehend the functions.

In the research of the bottom up approach (the lower micro level from the cellular level), system biology research with the advantage of the protein structures would be possibly conducted. Moreover the possibility should obviously come true, however, within this size of the project group absolutely cannot help for pursuing the understanding.

Making continuous effort of analyzing T. thermophiles HB8 has been valuable assets. Therefore as the research using SACLA, the effective use of the analysis should be made. In this case, the SACLA should be used not only for technical evaluation of bio-molecular imaging but also operated by indicating the clarified purpose of biological significance.

[REVIEWER 4]

Synchrotron Radiation System Biology Research Group is based on the structurome project as structural genome project by using extremely thermophilic organisms. On the basis of the group results, for seven years from April 2006, conducting the comprehensive functional genome research and the functional analysis of individual proteins the group aims to enable the simulation of whole biological process. As the framework, "Functomics Integration Research Team" led by group leader Kuramitsu entirely coordinates the whole project to provide a pipeline as protein productions to protein structural analysis, at the same time, on the basis of platform technical development for the functional genome research mRNA, protein expression, and metabolic dynamics, furthermore, electron microscope tomography and x-ray fluorescent imaging were tentatively introduced. It is interesting that the plasmids or databases as the resources are provided to the community. Other two teams conduct structural functional analysis by paying attention to the different sub-systems among extremely thermophilic organisms. Group of Shinkai mainly focuses on the transcription, and group of Bessho focuses on transcription, translation, replication, and oxidative stress response. Enabling the simulation of all biological process in cells is one of the ultimate goals of the life science and the research of this group will make a significant contribution to scientific advance. As of October, 2012, despite there were members of eight including the team leaders, it is significantly notable that 157 of original papers were published for seven years and 125 of new structures were registered to protein databank. Although it is disappointing not to see the goal of understanding whole cells to confirm the promising future success at this time, there are many outcomes by analyzing individual protein structure and its function. This result is obvious by reading the newspaper article per attached so that it made a great impact on the public. As the summary, this project could not accomplish the initial goal perfectly however could create the significant results academically so that it succeeded in high level.

[REVIEWER 5]

1. Goal setting : By revealing the three-dimensional all protein structures contained in an extremely thermophilic model organism, Thermus thermophilus HB, the goal setting of this group had high-nobility with originality by understanding the life phenomenon in a cell, from system biological point of view and also provided academically significant information. Nowadays, the goal of this research is just a standard concept however when the research group launched, the goal seemed not reachable at all. However, over the short time period, the group gradually enabled to give a new insight into the life science of the next generation to lead the world

research so that the contribution of the group is quite large.

2. Research achievements：The group aimed to understand the functions of a cell by going through the three dimensional structures and functions of all the proteins in the cell, the outcomes acquired by the high originality research goal has a quite high originality as well. By the research results, 154 of the original paper publications were released on the internationally recognized magazines for seven years so that academic significance was also great. The information related to the genes and well-constructed overexpress protein plasmids which distributed to external researchers were enormously valuable as a way to promote protein science as well as life science research. Also the research outcomes that have been achieved were went into headlines and widely spread out through symposiums, so that the achievements had a profound effect on society.
3. Research group management：This group is consisted of three teams, and under a team leader each team is consisted of researchers, PhD researchers, and technical staff, so that it appears that the management was smoothly practiced. Also a large amount of visiting researchers and graduate students were affiliated, so that it shows the active research exchanges were established at annual research conference in where the big number of researchers assembled at once.
4. Overall evaluation：The research contribution can be highly evaluated that a series of system was created genomic analysis, protein expression related construction, protein overexpression, purification, crystallization, diffraction data collection, structural calculation, and functional analysis, also 490 structural proteomics analysis was completed for seven years. Furthermore, it is also profoundly appreciated that a great mark was left for human development and social contribution by accepting visiting researchers and holding research meeting. Therefore this project group surely played a remarkable role in the field of structural proteomics.

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