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RIKEN Harima Institute
Research Promotion Division

Reports on Structural Biophysics Laboratory Periodical Review

Laboratory Leader: Chief Scientist, Masashi Miyano (Dr. Sci.)
Date: December 3, 2007
Venue: RIKEN Harima Institute (SPring-8)
Reviewers:
Prof. Bi-Cheng Wang
(Biochemistry & Molecular Biology, University of Georgia, USA)
Prof. Haruki Nakamura
(Institute for Protein Research, Osaka University, Japan)
Prof. Shinya Yoshikawa
(Graduate School of Life Science, University of Hyogo, Japan)
Prof. Yoshinori Satow
(Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan)

Reports:

Reviewer1:

This research group has published articles in Science and Nature since 2000, suggesting extremely strong research activity as a whole. However, the reviewer does not understand why the chief scientist did not present any concrete future research plan (or dream) in the review meeting. Unfortunately, this seems to be consistent to the fact that it is unclear what the research objective by driving the four research subjects in the last 7 years is. Therefore, the reviewer strongly encourages for the chief scientist to show his concrete future plan to the member of his research group. All the members of Structural Biophysics Laboratory are quite talented and enthusiastic in their research subjects and thus, their future progress as the world-leading scientist is definitely promising. Setting the clear objective to these young research group members from the Chief Scientist would surely stimulate their research activities.

Reviewer2:

The most serious point of this research group is that the group leader does not make their research objectives clear, although there are several research topics performed by the group. To the reviewer's question at the periodical review meeting about the future goals, the group leader only answered that they are going to determine the crystal structures of membrane proteins as many as possible. In addition, it does not seem that this group has made much effort to develop or invent any novel approaches for the structure analyses of membrane proteins, which are widely known to be very difficult by the current conventional approaches. Probably, it is due to a lack of the scientific motivation, by which the group wants to reveal any particular biological phenomenon with the membrane proteins.

This field is now very hot in the world, and so international collaborations are critical. However, this group has very rare international collaborations, and has no foreign staffs. The abilities of English speaking of the members are not quite high except the group leader, as the Japanese researchers'

standard.

It is not clear that how many original papers have been actually published from this group since 2000, because the corresponding authors are not identified from the publication list correctly. However, when I assume that the corresponding authors are the first or the last authors of the papers in the list, the publication number becomes very few. In 2000, 1 (#10); in 2001, 1 (#17); in 2002, 0; in 2003, 1 (#31); in 2004, 3 (#36-38); in 2005, 3 (#43, #56, #58); and in 2006, 3 (#64, #66, #74). Total only 12 papers are considered to have been published from this group, although there are many collaborative papers with other researchers inside or out of RIKEN. My estimate of the corresponding authors may not be very correct, but it is true that the name of the group leader does not appear frequently as the first or the last author. Although the group published one Science and one Nature paper during the period, the resulted publications are not very satisfactory, considering the total research budget, 325 M yen, spent from years 2000 to 2006.

Thus, the reviewer does not consider that the current group leader is very suitable for continuing to have the leadership of the "Structural Biophysics Laboratory", which should have a very important position in RIKEN. This conclusion has also been confirmed by the fact that the group leader has not succeeded in obtaining research grants from outside of RIKEN in 2004-2006.

Reviewer3:

Research objectives of this Laboratory are mainly in the three-dimensional structural and functional studies of lipid-related proteins. The proteins intensively studied are bovine rhodopsin, human leukotriene C_4 synthase (LTC_4S), and *Bacillus celeus* sphingomyelinase (*Bc*-SMase). These are of biological and medical interest, hard to be structurally elucidated, and of high scientific significance. The laboratory members are also focusing their efforts on development and improvement of methods for protein crystallography using synchrotron radiation.

Rhodopsin is a G-protein coupled (photo)receptor (GPCR) embedded in the membrane. The crystals of rhodopsin were obtained by their collaborators, and its structure was promptly determined by this Laboratory in the early beginning of the laboratory startup. This structural study was in the forefront of the elucidation of GPCRs, breaking new ground in understanding its function, and is highly evaluated.

Leukotriene synthase is a membrane-associated protein involved in metabolism of eicosanoid and glutathione. Human LTC_4S is a membrane integral protein and a target in the structure-based drug design. They expressed LTC_4S in *Schizosaccharomyces* yeast and successfully determined its three-dimensional structures.

Sphingomyelinases cleave sphingomyelin in cell membranes into ceramide and phosphocholine. Its product ceramide is an important signaling molecule in cellular processes and also in diseases including cancer and diabetes. They determined the structure of *Bc*-SMase that contains two divalent metal ions of Co^{2+}, Mg^{2+}, or Ca^{2+}. They also determined several other proteins including long-chain fatty acyl CoA synthetase, which have high social impact because of biological and medical interest.

They contributed to the startup and the success of the Protein 3000 structural genomics project in RIKEN. In this project, they developed fully-automated crystallization and crystal-observation system TERA as well as protein expression procedures for structural and functional analyses. They also have made efforts to determine accurate structures at ultra-high resolutions. These achievements are highly remarked.

Management of the Laboratory appeared very good. Laboratory members are composed of talented and skilled professionals in excellently-managed scientific atmosphere, and collaborating with a wide variety of researchers. The members cover most of the technical fields required in modern protein crystallography: protein expression, purification, crystallization, functional analysis,

and structure determination. Laboratory facilities are well equipped and maintained. They are fully taking great advantage in using synchrotron X-rays of SPring-8 located in the same site.

Although future research plans were less spoken by the Chief Scientist of the laboratory in the Periodical Review meeting, their directions seem highly promising since their past scientific achievements are measured in excellence. Their cooperative involvement in synchrotron X-ray crystallography as well as in structural biology itself is fully anticipated. They are expected to play important roles in protein research activities which will be carried out by using powerful XFEL radiation in the near future.

Overall, the activities of the scientists of this Laboratory are assessed in a high and valuable rank.

Reviewer4:

The Structural Biophysics Laboratory, headed by Dr. Masashi Miyano, has continued to gain a strong reputation among national and international structural biology communities during the last seven years. The lab clearly possesses a number of strong and unique attributes, including but not limited to:

- Focus on lipid-related proteins and biologically important proteins.
- Providing enabling technology within and outside RIKEN institutes.
- A highly motivated and integrated team of experts.

During the past 7 years, the lab members have implemented the needed infrastructure related to their mission and level of support. Notably, their accomplishments include:

- Successfully implemented a hetero host expression system, *Schizosaccharomyces prombe* for membrane protein expression.
- Successfully determined several important lipid-related protein structures and a number of other biomedical relevant protein structures.
- Developed an automated high throughput robotic crystallization and observation system named TERA.
- Established successful collaborations with colleagues within RIKEN, and at other research institutions in Japan and the USA.
- 74 original published papers, 36 reviews and 5 book chapters; some of which were published in high impact journals.

The Miyano group has focused on several areas of biomedical research, and has produced excellent results in each of these areas. In close collaboration with colleagues at the University of Washington, for example, Miyano's group solved the first detailed structure of bovin rhodopsin in 2000. This work, which was published in *Science*, has now been cited over 2000 times. All other determined structures from Miyano's group are also from proteins that are of high biomedical relevance, and are targeted for potential drug discovery. This group of structures includes a recent paper on human leukotriene C4 synthase, published in *Nature*. Dr Miyano's group has also engaged in the development of high throughput structural genomics pipelines. In these areas, the TERA crystallization and observation system was developed in an impressively short period of time, and has partly served the critical need for high-throughput crystallography in Japan's structural genomics efforts.

As to the future plans, Dr. Miyano intends to keep the options open in searching for biologically significant molecules that may not be currently identifiable, and for methodology development to advance to the "Integral Life Science Research at SPring-8." While he will continue to work on obtaining the highest resolution possible for the lipid-related membrane structures that he is studying, he will also work to develop integrated methods, including EM and XFEL, for structural studies. The linking of XFEL with crystallography should be an important extension of the structural biology

capabilities at SPring-8, and is highly significant in view of the unique opportunities for using advanced XFEL at SPring-8 in the near future. Based on Dr. Miyano's past track record, enthusiasm and collaborative nature in scientific work, there is little doubt that he will be unsuccessful in his future research efforts.

With the completion of seven years' service as Chief Scientist, and the ending of the Tanpaku3000 project before advancing into new areas of research, now seems to be an excellent point in time for Dr. Miyano to consider a sabbatical experience for a period of 3 to 6 months. During this refreshing period, Dr. Miyano may want to devote 50% of his efforts towards exploring new collaborations and possibilities, while also keeping the other 50% available for supervising his current research projects remotely. This will likely add new insight and strength to the operations of the laboratory, which is already highly innovative and productive.