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RIKEN Harima Institute
Research Promotion Division

# Reports on Biometal Science Laboratory Periodical Review

**Laboratory Leader:** Chief Scientist, Yoshitsugu Shiro (D. Eng)
**Date:** January 25, 2008
**Venue:** RIKEN Harima Institute (SPring-8)
**Reviewers:**
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**Reports:**

**Reviewer1:**

This has been a very productive and forward thinking laboratory. The leader, Dr. Yoshi Shiro, is a well known investigator with a particularly strong and broad skill set. Studies focus on a number of different biometal proteins, most of which contain heme. The laboratory has been very successful in their studies of structure/function of these enzymes, particularly *in vitro*. There can be no doubt that this work will continue and be very highly regarded internationally. I believe that one particularly important piece for these studies are cell biology. In most biomedical research programs around the world biological features of structural studies are a very important piece of this research. Dr. Shiro is well aware of this change in research directions over the past 20 years. Today structural biology is considered a tool leading to understanding the biology of systems. The structural biology and *in vitro* studies in the Biometal Science Laboratory are as good as anywhere in the world. Dr. Shiro has included scientists with a strong biology background in his group, but there is no consistent source of this support for the studies. Certainly stronger collaboration with the Cell Biology Program at RIKEN might be desirable as would be recruiting investigators into the Biometal Science Laboratory at a tenured level in hopes of maintaining consistent biological background for the work by the protein chemists.

The international recognition of this laboratory is outstanding both in terms of novelty and scientific significance as clearly demonstrated by the number of important meetings around the world in which members are invited to participate. The high quality publications from the laboratory demonstrate both the originality and scientific significance of their work. I do not know how to assess the social impact of the work. Dr. Shiro is an excellent manager and allows his senior scientists to participate in this process as part of their training. The future research plans are very sound although as I indicate above, a more formal plan for assuring biological support for these projects is desirable. My overall assessment is that this is a truly outstanding research program which is most appropriate

for the overall research program at RIKEN. I believe that RIKEN's management is very supportive of the Biometal Science Laboratory and the review system is strong.

**Reviewer2:**

The Shiro group has been engaged in structural studies of heme containing metalloenzymes since its start. $P450_{BS\beta}$, $P450_{nor}$, indoleamine 2,3-dioxygenase, and human cytoglobin are some examples of those hemoproteins. Especially, the reduction of NO to afford $N_2O$ was a very novel system and the Shiro group has been intensively studied its detailed reaction mechanism by employing various spectroscopic methods and a proposed mechanism is very interesting. The X-ray facilities of the SPring-8 have been serving very much for these research projects. In addition, metalloproteins related to signal transduction are currently getting very important issue, while studies in this area are still pre-mature. Thus, the group is focusing their efforts on this subject. In conclusion, the research objectives as well as the results are very novel and significant. $P450_{BS\beta}$ is a highly potent heme enzyme as an oxidation catalyst applicable to industrial use, while it still needs more work.

The management by Dr. Shiro is really outstanding, i.e., many of the Lab members have been promoted at the associate professor level and junior researchers in the Lab are very active. Collaboration of the Shiro group with groups outside of RIKEN is also actively taking place and the collaboration is very important for research groups outside of RIKEN and SPring-8. In fact, without collaboration with several groups of RIKEN and SPring-8, this reviewer may not have proceeded protein structure projects. More importantly, many projects of the group have been supported by MEXT Grants, indicating that the originality and novelty of projects conducted by this group are also highly appreciated by peer reviewers. Research facilities of this group are well equipped.

**Reviewer3:**

Dr. Shiro has been working on the elucidation (1) of the structure and function relationships of metalloproteins involved in biological redox reactions and in cellular signal transduction, (2) of design and syntheses of de novo proteins, and (3) of the development and application of new technologies for protein structural determination. The strategy of his research is clear and well designed. He has achieved very good results in his research and contributed to the progress of the research field of bioinorganic chemistry. His research activity is very high. The number of the original papers he has published from 2000 to 2007 is 121. In each of these cases the work has been published in the leading journals such as PNAS, JACS, JBC and Biochemistry, which clearly shows that both of the productivity and quality of his research are top level in world standard.

In his research achievement, there are several topics that have a significant impact on the research field of bioinorganic chemistry. The structural determination of indoleamine 2, 3-dioxygenase (IDO) is one example among them. IDO is a historical enzyme Prof. Osamu Hayaishi found to establish the concept of "oxygenase" about 50 years ago. Although we have a long history of the research on heme-containing dioxygenases including IDO, the reaction mechanism of dioxygenation of their substrates is not fully understood because of the lack of the structural information of them. Dr. Shiro's work on IDO is the first structural determination of heme-containing dioxygenases in the world, which will be the beginning of the fully understanding of the structure-function relationships of heme-containing dioxygenases.

His works on cytochrome P450-type nitric oxide reductase (P450nor) and P450 peroxygenase are also very interesting because these P450-type enzymes are very unique compared with other P450s. Though P450nor has the same active site as does normal P450, the reaction catalyzed by P450nor, the

reduction of NO to $N_2O$, is totally different from that catalyzed by normal P450, which is monooxygenation by molecular oxygen. In the case of P450 peroxygenase, it uses $H_2O_2$ as a physiological oxygen donor instead of molecular oxygen that normal P450 uses. He has determined the crystal structures of these unique P450-type enzymes and elucidated the reaction mechanisms of them. These results have opened a door for the research of P450s to a new stage.

Future research plans he told in the interview are well designed and will be able to contribute to the development of bioinorganic chemistry. Given his achievement so far, there is no doubt of the success of his future plans. In overall, the originality and achievement of his research makes him a leading scientist in the research field of bioinorganic chemistry.

His management of the laboratory is going well. Young scientists in his group are motivated to do good science. The background of his group members is chemistry or biochemistry. If there are some scientists with the background of biology and medical science, it will be very beneficial for his group to expand his research.

**Reviewer4:**

Dr. Shiro has assembled an army of excellent junior investigators in his research group to elucidate the structure and function of metalloproteins physiological significance including cytochrome P450 enzymes, a dioxygenase heme enzyme, oxygen sensing FixL, and newly found globin molecules. These target proteins are carefully chosen based on their biochemical significance, a very smart approach. With his advantage to be proximity to SPring-8, majority of his work has been focused on the crystal structural determination of these proteins, as envisioned by the list of the proteins structures of which have been determined by him. However, quite different from many ordinary crystallographers who tend to describe biochemical mechanisms based solely on crystal structures, Dr. Shiro, due probably to his early training in physical chemistry and biophysics, appears to fully utilize all the power of various methodologies available to his work so as to obtain solid and significant conclusions highly useful to understand roles of metal centers in biochemical function displayed by his target proteins.

In order for me to report his all major accomplishment, the space allocated is too small, because he has accomplished many important contributions to the field of biological inorganic chemistry. Instead, let me provide a succinct assessment of his achievement. His work on a P450 type nitric oxide reductase unveiled for the first time how nitric oxide is enzymatically reduced and made a large impact in the research areas of nitric oxide and P450 biochemistry. Following this exciting success, he has solved crystal structures of novel P450 enzymes and this has put him one of the front runners in the field of P450 studies, despite the fact that he started P450 structural biology much later than many other investigators. More important accomplishment is the crystal structural determination of a dioxygenase, indole amine dioxygenase, which is not only one of the central enzymes in tryptophan metabolism but also recognized as one of the important players in clinical cancer biology. Dr. Shiro has triumphed to win the race to determine the first dioxygenase crystal structure by beating many investigators around the world. This outstanding accomplishment by Dr. Shiro will rewrite a field of tryptophan metabolism biochemistry. This is by no means one of the top outcomes from SPring-8. Dr. Shiro's research will be benefited by further extending his research endeavor into X ray spectroscopy, the method which will provide structural details of the active sites of the enzymes studied in his laboratory.

Dr. Shiro has been managing a sizable group of scientists and students. He has successfully sent his former group members to reputable faculty and research positions, an indication for his ability to deliver good training. I did not find any flaws in his management during the individual interview of

his current members whose independent ideas seem to be well respected. His laboratory is very well equipped and nicely funded as well.

The future plans are well thought out based on what has been accomplished. Some might be tricky. But based on his accomplishment, likelihood of success appears to be high. Given the proximity to SPring-8 and being in RIKEN, structure biology occupies a fairly large portion in this planned research. I think, however, that his research activity would be benefited by incorporating more “cell and molecular biology”. This will strengthen the structural biology component by clarifying physiological and biological significance of his future projects.

In general, Dr. Shiro has made important contributions to the field of metallobiochemistry and appears to continue to generate new and solid findings useful for our understanding the chemistry and biochemistry of enzymes of biochemical significance.

I found the RIKEN review system is reasonable by the Japanese standard. Also, this was an easy review due to the principal investigator' wonderful research record and excellent future plans.