

Vol.5 No.2

KEK News

Target Oriented Structural Genomics
Using Synchrotron X-ray Protein Crystallography

High Energy Accelerator Research Organization

A Message from DG

– from the speech given at New Year's Party on January 7th –

Happy New Year to everybody! I am very glad to be able to present my penultimate new year's message to you. We have a lot to expect in the year 2002.

First of all, we can expect new physics results from our B-factory after the 100 fb^{-1} of new data, preferably towards the end of this summer. We hope to achieve this goal together with our competing and collaborating laboratory, SLAC.

Secondly, K2K experimental group is certainly facing a crucial year. How fast it can understand the cause of the last year's disaster and how fast it can restart the experiment demonstrates the ability of the group and will determine its future.

Thirdly, JHF will enter into the second fiscal year of its construction. Our collaboration with JAERI must become more mature. We should also be prepared to help JAERI as a good friend in its reform process. We all know that the success of the JHF project will be judged by how many excellent scientific results it will produce and, perhaps, how fast. It is, therefore, very important that we set clear priorities at this stage and design the organizational and the technical systems to support these priorities.

Fourthly, our AR radiation facility is now ready to resume its operation. Together with the 2.5 GeV photon factory we are certainly one of the world centers in this field. The last frontier of material science, i.e. the study on living forms, has just begun. We must strengthen our activities in this field and reform our organizational and technical systems towards this goal.

In addition to these positive challenges the year 2002 will also be a difficult year. There are at least three very difficult decisions to be made. KEK must choose a new leadership team.

KEK, together with other inter-university institutes of Japan, must choose what sort of an independent agency it will become. KEK, together with the world community of high energy physicists, may have to decide how to proceed with the linear collider project.

First, let me make just one recommendation about the new leadership of KEK. Choose a young person who can serve for approximately 10 years, just like the Fermilab director or the SLAC director. If she or he has breath and depth of vision, she or he will need that much time to realize it and it is definitely wrong to choose a person without future vision. Personally, an old soldier like myself, should start preparing to disappear. An old soldier has been wounded, becomes tired, and feels he can no longer perform his duty joyfully; then he makes up his mind to fade away.

Next, let me touch on the issue of the governmental reform of national institutes into an independent agency. It is a political issue, and as is always the case with political issues, we have passionate people and apathetics, agitators and followers, reformists and oppositions, genuines and phonies, officials and officials' pets, opportunists and enthusiasts, etc. etc. We must constantly ask ourselves what we are and what we want to become.

Finally, let me mention my thoughts on the linear collider project, briefly. I believe that the selection of the technology is still premature, although some people, especially those in DESY, may not agree with me. But, quite independently from that, we may be able to agree on the organizational structure of the project. We all seem to agree that the project must be the global effort. The organizational structure, therefore, must also be global. We have many models to learn from: the CERN model, the ESRF model, the United Nations University model, etc. etc. It will be wonderful if I were able to report to you on the agreed-upon structure in my (last) new year's message next year. Thank you.

January 7, 2002

Hiroataka Sugawara, Director General of KEK

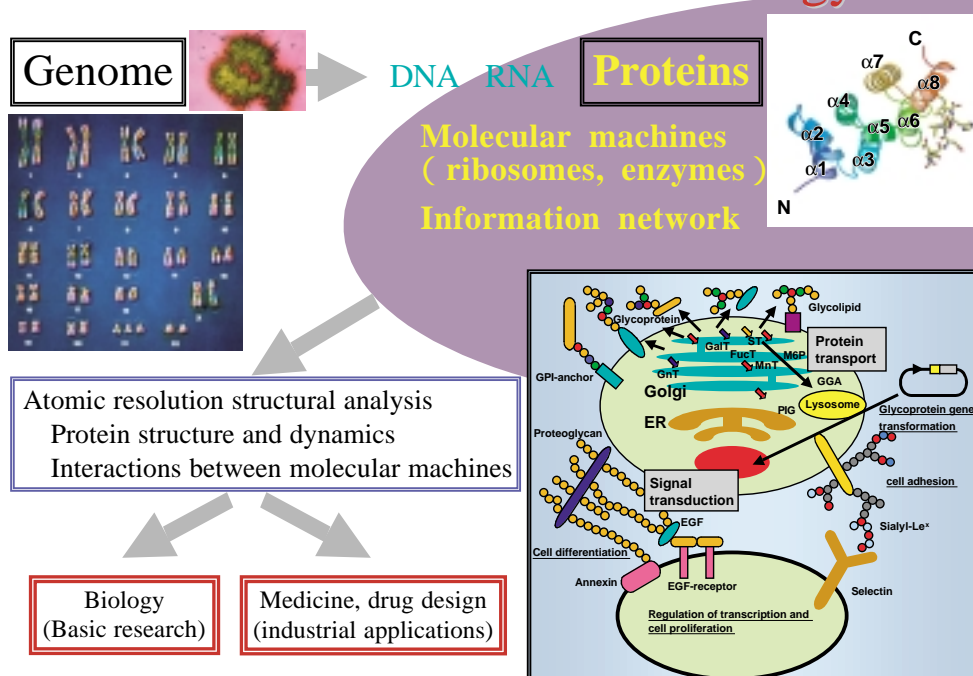
Target Oriented Structural Genomics

Using Synchrotron X-ray Protein Crystallography

The recent progress of the human and other genome sequencing projects has produced a tremendous amount of genetic information on the entire genomes. Subsequently, new scientific branches called post-genomic sciences started to take best advantage of the genome information and to use it to improve human life.

Proteins are the products of genes and they perform numerous functions in cells through intricate interplay between their amino acid residues and other molecules. Therefore, one important aspect of the post-genomic sciences is to study the network of proteins and other biological molecules. Here, elucidation of three-dimensional structures of proteins and their complexes is the key to fully understand their functions. Traditionally, structural biologists have been studying functions of specific proteins and nucleic acids of their interest using X-ray crystallography and/or nuclear magnetic resonance (NMR) techniques. In contrast, structural genomics (SG) is an endeavor to determine protein structures *en masse* in a systematic way, thus expected to play a major role in post-genomic sciences. The advancement in the past ten years at modern synchrotron radiation facilities has revolutionized the protein crystallography field. Highly collimated and energy-tunable X-ray photons from synchrotron beam lines have reduced the amount of time required for accurate structure determination by one to two orders of magnitude and made it possible to determine very large protein structures from extremely small crystals.

Role of Structural Biology



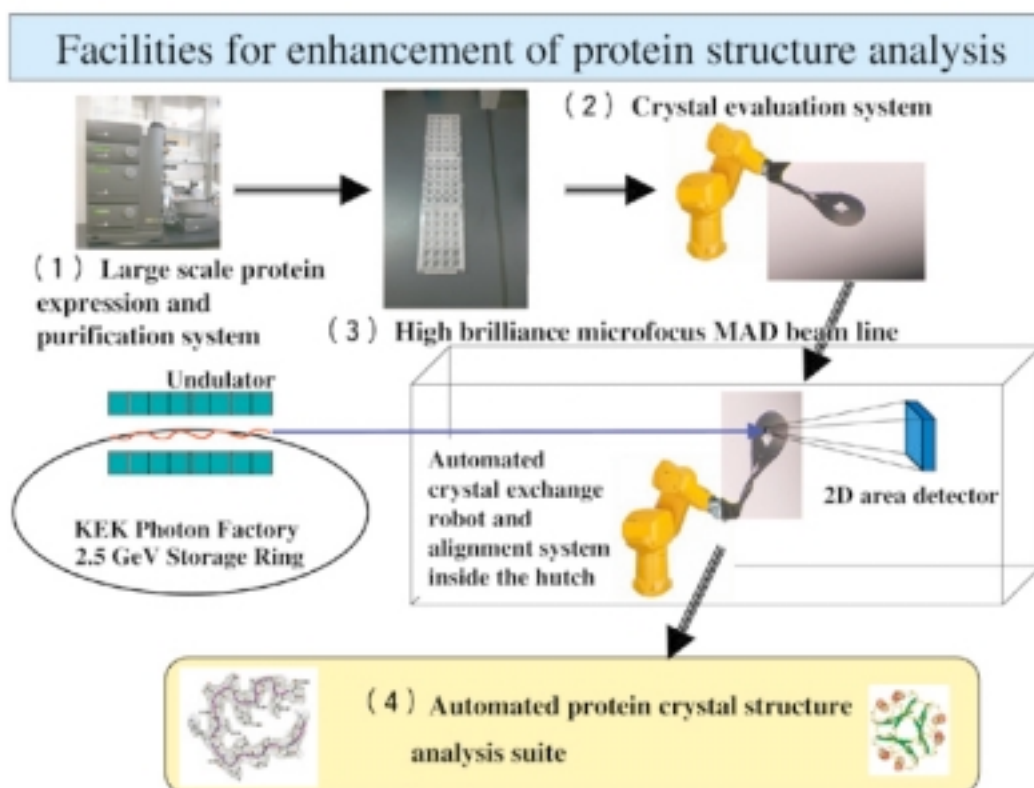
Combined with the technological advancements of genome analyses and proteomics, a science to study interaction of network of proteins, it is now foreseeable to solve thousands of protein structures and analyze their functions in a coordinated way worldwide. Clearly, the synchrotron radiation is indispensable for the SG projects. In this article, I will describe an example of SG project with specific biological targets and development of new X-ray crystallography techniques for highly efficient structure determination.

Target oriented structural genomics: research consortia concept

-- National project of determining 3000 unique folds in 5 years from FY2002

There are many national and international genomics projects started in the last few years. For instance, the National Institute of Health in the USA is funding 9 consortia, which pursue pilot SG projects with different targets. In Europe, there is a new initiative of coordinating European laboratories to focus on SG relevant to human health. In Japan, RIKEN has a major SG program based on the NMR and synchrotron X-ray techniques while the Biological Information Research Center of the METI concentrates on membrane proteins and National Institute of Agrobiological Sciences of the Ministry of Agriculture, Forestry and Fisheries is developing a SG project on rice.

The Life Sciences Division of the Ministry of Education, Culture, Sports, Science and Technology is starting a five-year project of determining 3000 structures or unique folds from April 2002. The SG project at RIKEN will carry out the major part of the Protein 3000 project. Their primary goal is to contribute to the worldwide effort of determining all the representative structures in the structure space by determining 2500 structures in the next five years.



On the other hand, structural biologists of the Japanese universities and the Photon Factory (PF) have been proposing a network of SG consortia to pursue target-oriented SG projects aimed at specific biological or medical targets.

Each consortium will consist of X-ray protein crystallography and NMR groups tightly coupled with researchers specialized in medical, pharmaceutical and biological sciences who share the same biological interests in their pursuit of structure-function relationships.

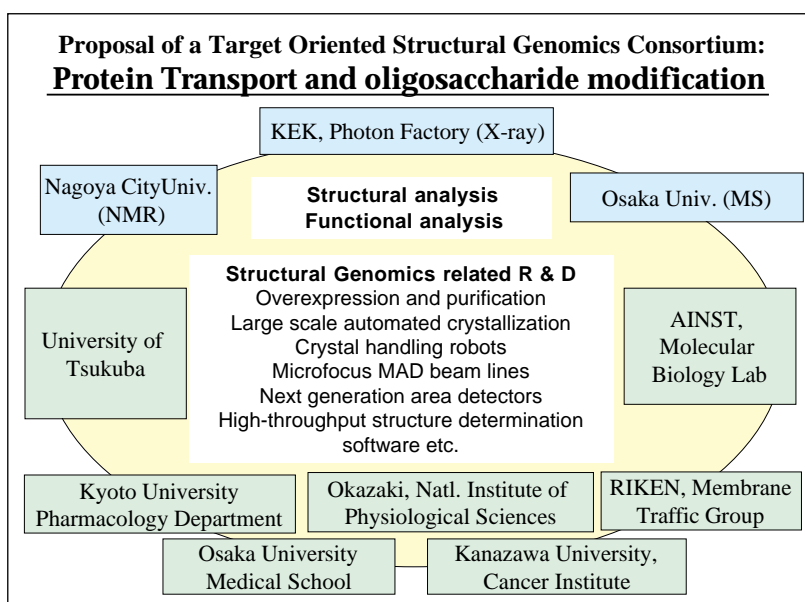
We emphasize following important factors in the preparation stage of our proposal:

- Initiative of the researchers of universities and research institutes
- Close collaboration with biochemistry, molecular and cell biology, medicine, pharmacology departments of the universities and research institutes
- Research and development of key technologies
- Improvement of the overall efficiency of structure determination by a factor of 10
- Determination of 500 biologically-important protein structures
- Education of the next generation of structural biologists

It is important to note that R&D of high throughput technologies is essential to achieve the goal. Moreover, the essential technologies are still not matured for truly high throughput operations, and therefore it is necessary for different SG consortia in the world to pursue R&D projects in parallel at this stage. Here, it is more efficient to concentrate on the R&D projects to solve the bottlenecks of the structure determination as shown in the diagram of our proposal of a target-oriented SG consortium.

Structural biology of protein modification and transport processes

Protein modification and sorting are essential for all living cells. In higher organisms such as human, newly synthesized proteins need to be modified and directed to their correct targets for proper functioning. Error in protein modification and mis-targeting of proteins into wrong compartments cause a number of human diseases. Furthermore, infection and proliferation of viruses such as HIV and influenza require host cell's transport systems. Hence, elucidation of detailed mechanisms of protein modification and transport is expected to lead to development of new drugs against diseases caused by viruses and mis-targeted proteins. In particular, two

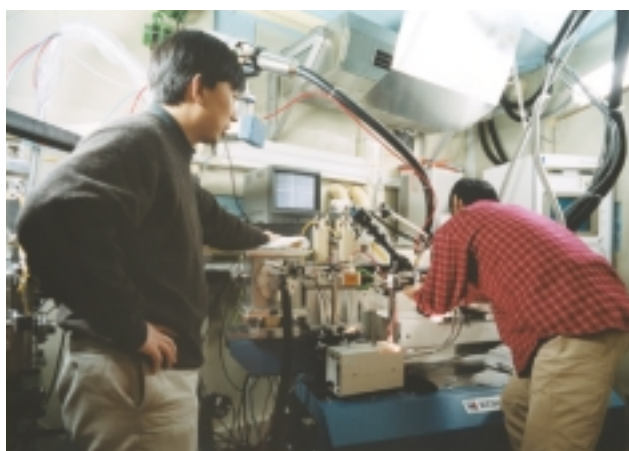


compartments called the endoplasmic reticulum and the Golgi apparatus are the key to protein modification and correct sorting. In analogy to chemical plants, these compartments serve both as a distillation tower and as a chemical reactor of the cell by sorting and modifying proteins. In the long run, full understanding of these mechanisms will be extremely useful in developing key technologies to produce medically/ biologically active human glycoproteins (proteins modified with short sugar chains) using lower organisms such as yeast.

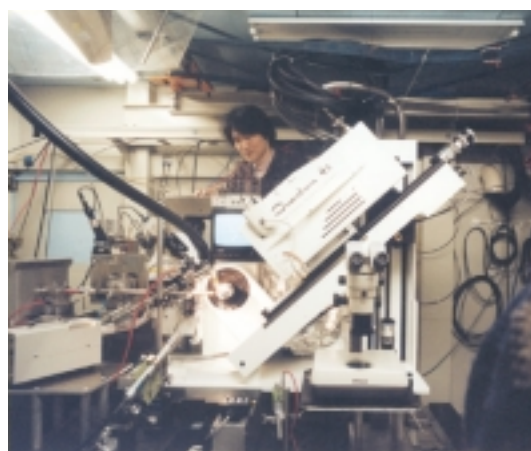
To pursue these lines of research, the PF structural biology group, together with five universities and three institutes, is proposing a SG project on protein transport and post-translational modification (which means modification after proteins are produced from the genes) of proteins as a consortium. The core of this consortium has already began in the form of the collaboration between the PF group and the K. Nakayama's group in University of Tsukuba and Y. Jigami's group in AINST, Tsukuba. The project includes systematic structural analyses of proteins and complexes involved in protein transport and post-translational modification processes, using synchrotron X-ray protein crystallography, NMR spectroscopy, and mass spectroscopy. The proposal will give high priority to development of various high-throughput protein crystallography techniques using synchrotron radiation.

Protein structure analysis using synchrotron X-ray beam lines

Three dimensional arrangements of proteins and large protein complexes can be studied in atomic detail by synchrotron X-ray crystallographic techniques. In the era of third-generation synchrotron facilities specializing in insertion device beam lines, the second generation facilities such as the PF face severe competitions. The protein crystallography beam lines in operation at the PF, BL6A (below left), BL6B, and BL18B (below right), remain very productive; 40 to 70 original structural papers are published each year. In fact, 80 to 90% of protein crystallography experiments can be performed on beam lines at any well set-up and maintained second generation synchrotron facilities. It is thus critical for the PF to keep developing new technologies for protein crystallography experiments and implement them in the facility. We are vigorously working on this by (1) constructing new insertion-device beam lines on the 6.5 GeV Advanced Ring (AR) and the 2.5 GeV PF ring, and (2) developing key technologies for high-throughput protein crystallography experi-



New experimental setup in BL6A



MAD Beam line BL18B

ments. They include a unified database for entire experiments (developed by Y. Gaponov), automatic capturing of protein crystals and cryogenic freezing, and a next generation X-ray area detector. For instance, the new experimental table and the single-bounce monochromator allows for anomalous experiments on BL6A (page 5 bottom left). The energy resolution of the monochromator is not ideal for multiple anomalous dispersion (MAD, a technique to determine protein structures rapidly and accurately using anomalous scattering effects of heavy atoms embedded in protein crystals) experiments, but the energy tuneability has improved the success rate of structure determination on the beam line. Indeed, we have already solved a structure of a domain of a protein involved in the vesicle transport using the MAD technique on BL6A (page 5, bottom right).



The first structure determined of a transport protein from a Se-MAD experiment using the new set-up of BL6A (T. Nogi et al., submitted to *Nature Structural Biology*).

Furthermore, a new section with an experimental floor, 1279 m², is soon to be completed along the northwest quadrant of the 6.5 GeV AR where we will construct a new MAD beam line. In collaboration with Profs. H. Kawata, M. Nomura, and Assoc. Prof. S. Yamamoto of the PF, the Structural Biology group has designed the beam line optics, hutches and experimental facilities as well as the adjacent preparation laboratory. The beam line will be constructed on one of the straight sections, NW12. A 40-mm period undulator will produce X-ray photons ranging from 7 to 16.8 KeV. The flux at the sample position is expected to be 2×10^{12} photons/sec through an aperture of 200 μm by 200 μm , which will surpass the intensity of bending magnet beam lines of the third generation synchrotron sources. As a next phase, we have a plan to build new insertion device MAD beam lines on the PF-Ring and are making vigorous efforts for realization of the new beam lines along with the reorganization of the PF-Ring to create more straight sections.

High throughput protein crystallography project

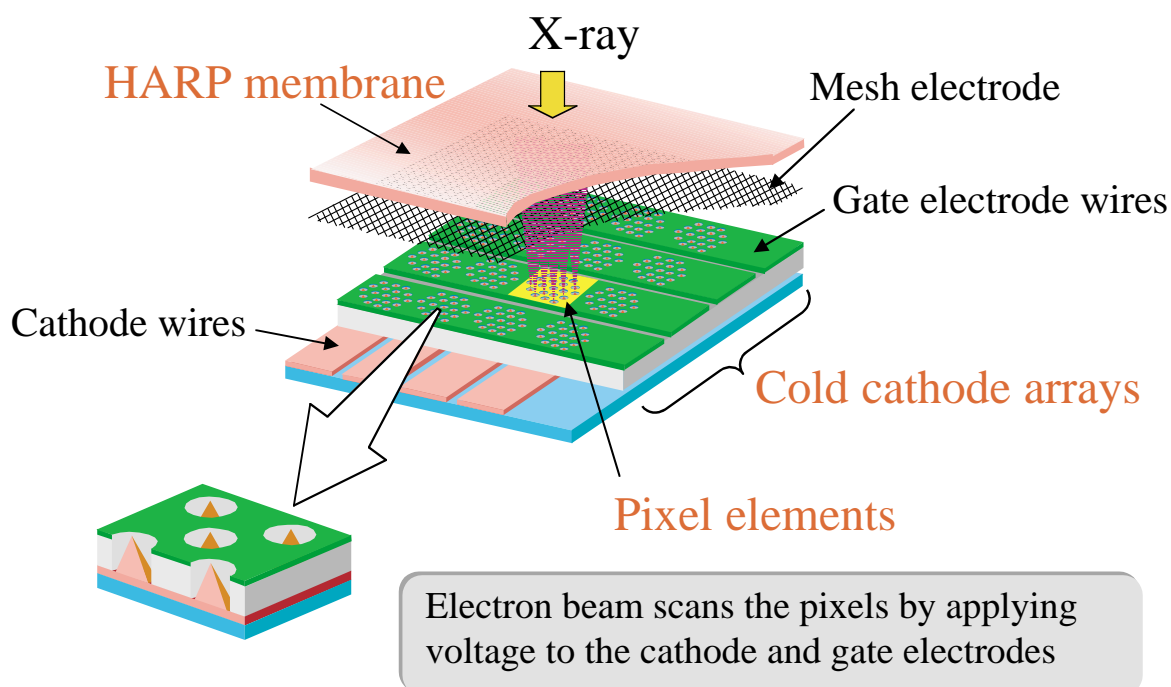
A key factor for high performance X-ray protein crystallography beam line is the overall duty cycle of the beam line including efficient optics alignment, automation of sample handling, crystal visualization and alignment, data collection and data analysis. Many synchrotron radiation facilities are now concentrating on these high-throughput projects, which will have an enormous influence on the overall throughput of the field. At the PF, we are pursuing various R&D projects in order to provide the best possible data collection facilities at the PF. We consider sources, optics, end station instrumentation including the choice of a detector, sample environment, data acquisition and data analysis, and a new beam time scheduling scheme for flexible and rapid access as integral parts of the beam line and try to unify them among the protein crystallography beam lines at our institute.

In August 2001, a competitive research grant was awarded from the Japanese government to

the group for a project to increase the efficiency of protein structure determination using the synchrotron facilities. The proposal includes 4 main R&D projects: (1) a large scale protein expression and purification system (2) a visualization system for micro crystals and a crystal freezing robot, (3) a high brilliance micro-focus MAD beam line, and (4) a software package for automated structure determination.

The first project is in collaboration with Profs. M. Tanokura and K. Saigo of the University of Tokyo, while the PF staff will use the outcome for producing proteins for structure determination. Prof. K. Miki of Kyoto University will join the effort to evaluate the crystal visualization system and the freezing robot. The PF staff will design and construct a MAD beam line for high throughput structure determination as part of the third project. Here we aim to develop an extremely precise sample rotation and automated sample alignment using a CCD microscope, an UV/visible illumination aided by an on-chip pattern recognition program. One of the most challenging projects of our proposal is the development of a HARP based X-ray area detector, which will be pursued by NHK Engineering Services, a subsidiary of the national broadcasting corporation. Assoc. Prof. S. Kishimoto of the PF and our group will evaluate the new detector and implement it into the experimental environment. The groups of Prof. I. Tanaka of Hokkaido University and Assoc. Prof. A. Nakagawa of Osaka University will play key roles in the fourth project joined by the PF staff on the development of a software package for automated structure determination. These technological developments will be coordinated with other synchrotron facilities of the world so as to provide the best experimental environment to users.

Coldcathode HARP area detector (by K. Tanioka, NHK)



The new Structural Biology Group

The new PF Structural Biology Group started in May 2000 with the emphasis on combining synchrotron beam line activities and competitive structural biology research. The group has currently 15 members (1 professor, 1 associate professor, 5 research associates, 1 computer scientist, 2 post doctoral fellows, 1 software engineer, 1 technician, 1 mechanical engineer from the KEK Mechanical Engineering Center, and 2 secretaries) covering a wide spectrum of the research fields ranging from the beam line development and operation, software development, biochemistry, and protein crystallography. At least four post-docs and two Ph.D. students will join the group from April 2002. We expect to open additional positions shortly and invite applications from abroad.

A new Structural Biology Building was completed in March 2001. The one-story 438 m² building houses two main biology laboratories for biochemistry and genetic engineering with ancillary laboratories for protein expression, purification and crystallization. It is also possible to carry out simple characterization of proteins and their interaction, for instance surface plasmon resonance experiment. The computer graphics room has several workstations for solving and analyzing protein structures.



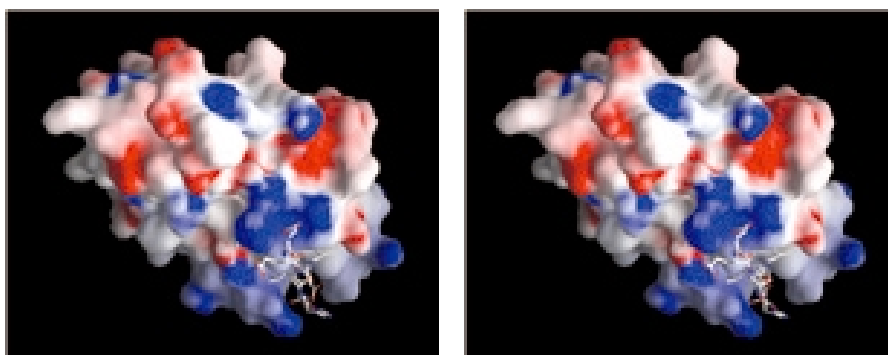
Since the completion of the building, the group has initiated a number of structural biology research projects focused on the intracellular protein transport and post-translational protein glycosylation. One of them is a structural study of a small domain called VHS of a human GGA1 protein involved in protein transport in cells. The human GGA protein has been the focus of intense research of many cell biologists in the US, Europe and Japan during the last 12 months. Several



groups including Prof. Kazuhisa Nakayama of Tsukuba University with whom our group has a close collaboration, simultaneously found the protein. There have been a number of important papers reported in the literature on the biological role of the protein during the past 12 months, notably one in *Cell* and two in *Science*. In short, the protein is involved in the interaction with sorting receptors and clathrin molecules, and therefore is believed to play a key role in the formation of transport vesicles.

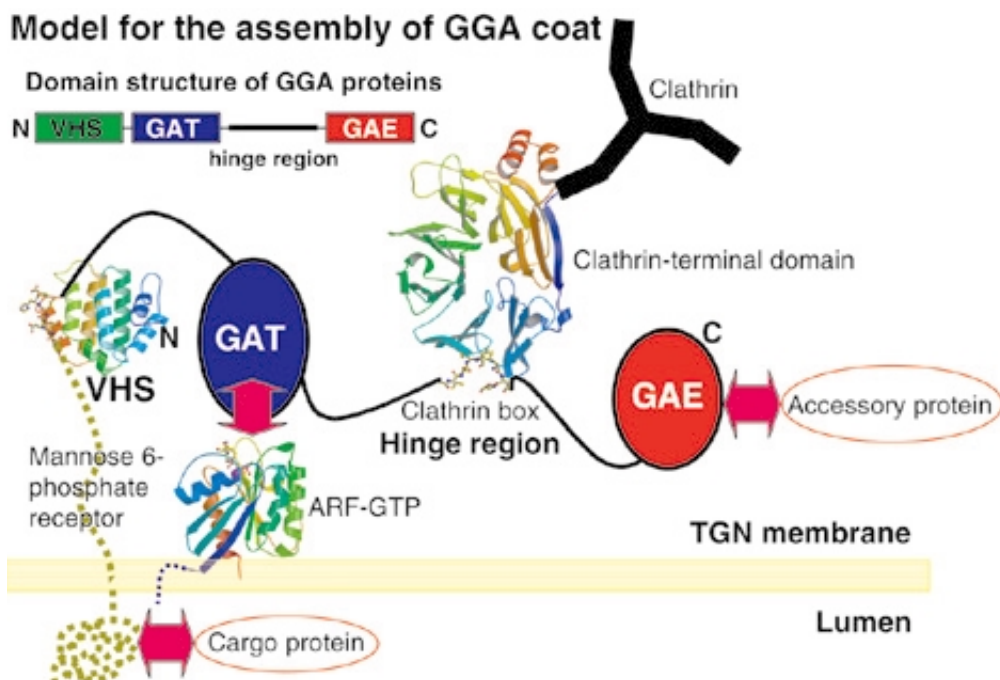
Protein preparation was started on 23 April 2001, the same month as the completion of the building, and the structure was solved five weeks later on 28 May 2001. In order to understand the mechanism of the signal peptide recognition, we subsequently tried to solve the structure of the domain in complex with the receptor fragment. By the time we finally obtained crystals of the complex, it was already August and many synchrotron facilities were shut down for summer recess. However, Dr. Thomas Earnest of the ALS, Berkeley, U.S.A., has offered to collaborate. So we sent frozen crystals to Berkeley at 5PM 13th August last year by Federal Express. As soon as he received the crystals, he collected diffraction data sets and 44 hours after the shipment from Tsukuba, I received the data by E-mail. We

were all extremely pleased when a quick calculation confirmed the binding of the signal peptide! The result was published in *Nature* vol. 415, 937-941, 21 February 2002. A group in the National Institute of Health in the USA also solved the structure of the complex from GGA3, 73 percent



Electrostatic surface potential (red: negative, blue: positive) of the GGA1-VHS domain in complex with the sorting receptor fragment shown as a stick model.

Above pictures are for a stereo-vision. Use your left eye for the left picture, right eye for the right picture and try to merge them together. This will give you a 3-dimensional view of the protein.



GGA proteins are composed of a VHS domain, a GAT domain, and a GAE domain. The GAT domain is linked to the GAE domain by a flexible hinge region. The VHS domain directly interacts with the acidic cluster dileucine motif in the cytoplasmic domain of sorting receptors. The GAT domain binds to the GTP-bound form of ARFs. The hinge region binds clathrin. The GAE domain binds to various accessory proteins.

identical to GGA1. Their paper appeared in the same issue of *Nature*. In the future, the structures of the other domains will help elucidate the molecular mechanism of the protein-protein interaction of this protein which is critical for correct transport of proteins from the terminal Golgi network to lysosomes (a compartment which degrades unwanted macromolecules) using sorting receptors. The group is extending this study to other related proteins and their complexes with the hope to gain atomic details of protein transport and modification processes.

Further information is available from Prof. Soichi Wakatsuki, IMSS, KEK, 1-1 Oho, Tsukuba, Ibaraki 305-0801, Japan, Soichi.Wakatsuki@kek.jp, Tel: +81-298-64-5648, Fax: +81-298-64-2801. Web site is under construction: <http://pfweis.kek.jp>



The author of this article, **Soichi Wakatsuki**, obtained a Ph.D. in Chemistry from Stanford University, worked as a PDF at the University of Oxford, UK. He then moved to the European Synchrotron Radiation Facility, France and lead the Structural Biology Group. After 16 years working abroad, he has made a full circle, at least for the time being, and assumed a position as the head of the new Structural Biology Group at the Photon Factory, IMSS, KEK in May 2000.

Salt of the earth, a story of a noble constructor



We stay in science because we believe in future and feel responsible for the people in the future. However, we often feel being unappreciated because fundamental science does not produce quick money. Similar situation can be found in architecture in Japan. Modern, good-for-mass-production houses have been shadowing on traditional Japanese houses. Modern houses are cheaper, more comfortable, although it is not necessary true in a long run. In fact, traditional Japanese houses can last a long time and more economical in a long run. But people seem to prefer lower down payment.

It is interesting to note that most traditional Japanese houses do not incorporate diagonal braces. This is intentionally done to keep the building structure resilient and many inventive lumber joints absorb earthquake energy. The traditional construction technology is the result of accumulated experiences through centuries. Nails are not usually used since nails do not last very long. The fact that buildings with such resilient (and not solid) structure have survived through many earthquakes (some over 1000 years) justifies the correctness of such structure in Japan. Unfortunately, there is no computer simulations (which people tend to trust more than facts) to

demonstrate the superiority of the traditional structure.

To save traditional houses from total extinction in Tsukuba, the city relocated and restored an old house donated by Yokota family. Now, the house not only serves to show how traditional houses were built, but have been used as a gathering place. It is not a museum to preserve the past, but a place alive to let people experience how nice a traditional construction is. (cover photos)

The leader of the restoration project, Mr. Fumio Tanaka, is one of the most respected carpenters in Japan. He has restored many ancient buildings. These buildings were built hundreds of years ago when no one was required to prove the structural strength for a building permit. Materials and essential structures for modern buildings were well tested and approved, but not so for these of ancient buildings. He has to actually build similar structures and go through a thorough test to get a permission for restoration, although the simple fact that many very old buildings and houses have survived through earthquakes and daily abuse for over centuries should be the living proof. Modern buildings could withstand earthquakes when they are relatively new, but often fail in a long run. But law does not care how it will be in a long run as long as it is safe for a while.

Mr. Tanaka was born in 1932. At his age of 69, he is as active as ever. He teaches and trains his young followers so that they could become familiar with design and technology of traditional Japanese buildings and inherit the disappearing art. He is also supporting a recently started university that teaches traditional technologies of Japan. He is found everywhere; persuading public office people, teaching students, giving talks to general public, directing at construction sites, designing buildings, investigating old building technologies, conducting tests to verify the safety of ancient structures. He was even in Norway in 1989, as he was invited to give a talk at Oslo School of Architecture. It was found that the extinct ancient architectural technology of Norway is still alive in Japan.

He tells his students that there are only 6 possible answers: "I understand", "I do not understand", "I can", "I can not", "I like it", and "I do not like it". No excuses, no wordiness. Just do it and prove it.



At work, he can be a ticking bomb, at coffee break, he is a loving father. I actually found him a very kind-hearted man. He is also very practical, rational and knows the limitation of the traditional technology very well.

None of his projects were easy and most of them do not have sufficient funds. There aren't so many people who care to preserve old buildings. But, he is willing to do it because he thinks someone has to do it and he is unhappy to see good and beautiful things disappear. Mr. Tanaka is one of the "salt of the earth". Researchers in fundamental science, you are not the only people fighting a lone battle. (TKO)

2001**September 15**

KEK Annual Open House was visited by over 2000 people.

October 1

Public Relations Office headed by Mr. Yuichi TAKAYANAGI started with 9 members.

Mr. TAKAYANAGI was a well-known TV commentator on science at Japan Broadcasting Corporation (NHK).

**October 6, 7**

KEK participated annual Tsukuba Science Fair for which over 5000 children and parents came.

**November 20-22**

The 5th Topical conference titled as "Frontiers in Flavor Physics" was held at KEK. Participants were 56 from overseas and 82 from Japan, totaling 138. (<http://kektc5.kek.jp/>)

November 22

The 15th annual Fuigo (forge) Festival was hosted by KEK Machine shop.

Traditional forging of an iron tool was demonstrated by a machine shop member.

**December 10-12**

Int'l Workshop on Nuclear and particle Physics at 50-GeV PS (NP01) was held at KEK. More than 100 participants discussed about the realistic experimental programs and facility layout of the 50-GeV PS.

December 13-16

The 1st Int'l Workshop on Neutrino-Nucleus Interactions in the few GeV Region (NuInt01) was held at KEK. (65 participants) There were lively discussions on neutrino experiments and on the possible future experiments for precise measurement of ν -N interactions.

December 16

Commemoration lectures titled as "40 years of Accelerator Science" were hosted by KEK at University of Tokyo.

2002

January 4

A fully optical computer network, Super-SINET, constructed by MEXT (ex-Monbusho) and NII (National Institute of Informatics) started service for High Energy Physics, Space/Astronomy, Genetics, Nano-technology and GRIDs boosting the network capability by ~100times.

January 7

KEK Director general, Hirotaka Sugawara, gave a new years resolution in English emphasizing that KEK is an international organization. (full text on page 1)

Notable visitors:

Nov.13 Minister of Education, Nov.15 Minister of Policy on Science and Technology.

Announcement:

On November 12, 2001, Super-Kamiokande had a sad accident losing about 60 % of both 20" PMTs (inner detector) and 8" PMTs (outer detector) so that the K2K run, originally scheduled to resume in January 2002, had to be deferred. As the spokesperson of the Super-Kamiokande Collaboration, Professor Totsuka, announced immediately after the accident, Super-Kamiokande detector will be rebuilt as quickly as possible. For now, the K2K experiment will restart within a year or so with half the original PMT density. (<http://www-sk.icrr.u-tokyo.ac.jp>) The half PMT density will not cause any serious deterioration in detector performance for the GeV neutrinos from KEK. The K2K Collaboration fully supports the recovery work of Super-Kamiokande. (<http://neutrino.kek.jp>)

(K. Nakamura)

Front and back covers

Cover photos shows the 250 years old farm house in the Tsukuba Central Park. (see page 11,12)



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