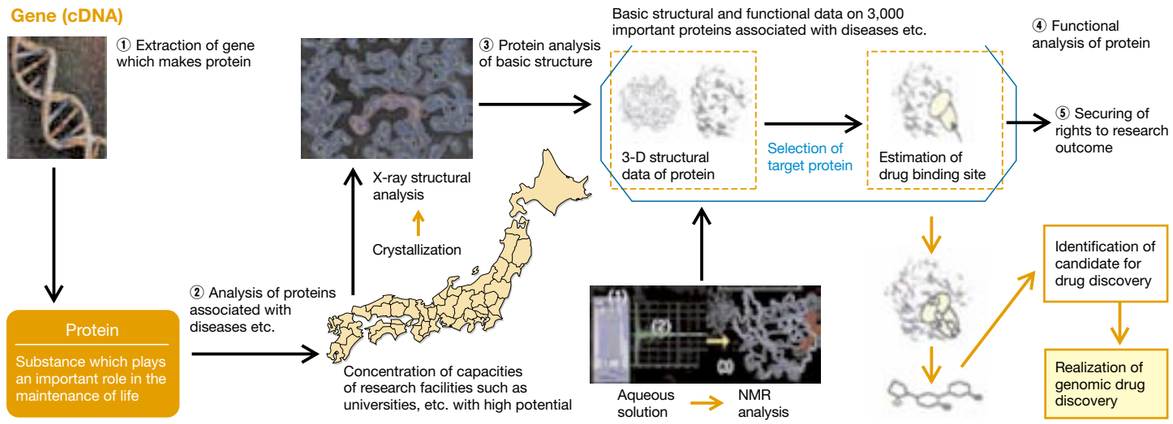
Project Overview: Results Achieved with a Japan-Wide Organization

Project Objectives & Overview

The analysis of protein structure is vital in order to understand life phenomena and create a basis for new drug development. If Japan is to achieve parity with Europe and the US in the field of structural biology (which involves the study of protein structure), public funds must be invested and research promoted systematically. Recognizing this, the National Project on Protein Structural and Functional Analyses (NPPSFA) was launched by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) in FY 2002, as a five-year project.

The project's aim is to determine the basic structure of over 3,000 proteins, from the standpoint of contributing to the international community. State-of-the-art Nuclear Magnetic Resonance (NMR) systems, large-scale synchrotron radiation facilities were improved (see pp. 3 - 4) and the full research capabilities of the nation were used to study the structure and functions of proteins, and technologies were developed and personnel trained in order to enable future protein research.



Researches ranging from the production of proteins based on genetic information to the structural and functional analyses of proteins were conducted by the NPPSFA in an effort to contribute to future drug discovery.

Project Achievements

During the project, the structure of 4,517 proteins (4,187 in terms of basic structure) was determined, and 3,923 of them were registered in the Protein Databank (PDB).* The number of structure-determined proteins greatly exceeded the initial objective. 403 patent applications were filed and 4,195 research papers presented. Moreover, the relationship between structure and functions was defined for several proteins with functions crucial to living things - a significant achievement in both biological and qualitative terms.

Progress was also made in the development of technologies for structural analysis. Many technologies relating to protein production (26), crystallization (16), X-ray analysis (26), NMR analysis (24) and other areas (26) were developed. These technical development achievements have made crystal structure analysis of proteins comparatively easy, even for researchers with no experience in this field.

A system enabling the use of 99 NMR systems and 226 X-ray diffractometers at research institutions throughout Japan was also established. Further progress was made on the beamlines of the Photon Factory at the High Energy Accelerator Research Organization (KEK) and the Super Photon ring-8 GeV (SPring-8) at the Institute of Physical and Chemical Research (RIKEN), greatly enhancing the research infrastructure for protein structural analysis.

Since 800 researchers participated in the project, it also made a significant contribution to the training of protein structure researchers.

* Data bank containing 3D structural data on proteins and nucleic acids. Operated by a research institution in the US, it has data entries from around the world.

Achievements of the Project in Numbers

Proteins whose structure was determined	**4517**
in terms of basic structure	4187
Proteins recorded in the PDB	**3923**
Patent applications filed	**403**
Research papers presented	**4195**

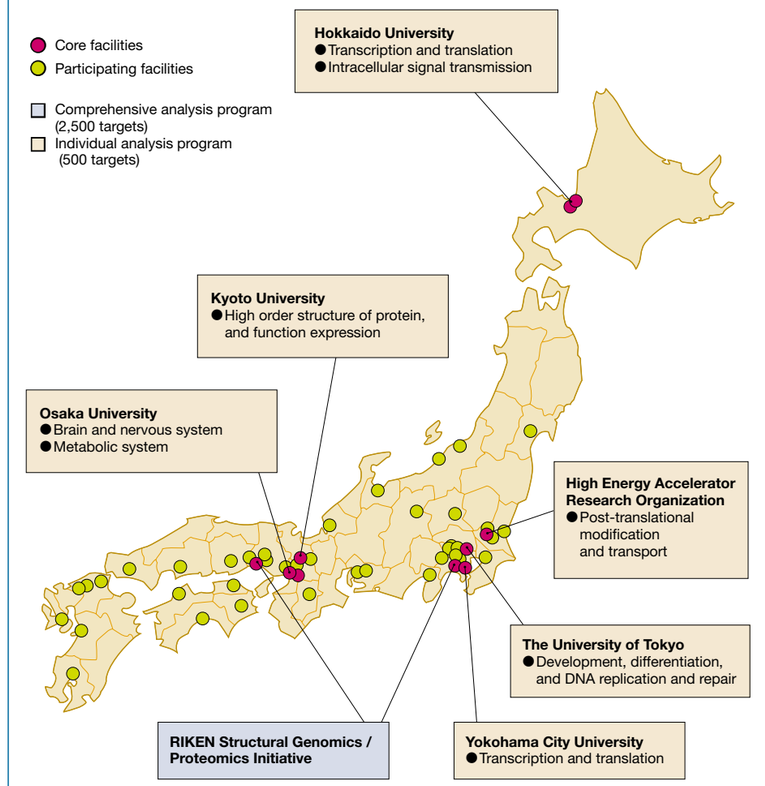
As of March 31, 2007

Project Structure and Promotional System

The project was made up of a comprehensive analysis program and individual analysis programs. The comprehensive analysis program was conducted by RIKEN, and aimed to rapidly analyze the structure and functions of over 2,500 proteins. In the individual analysis programs, eight core institutions and their individual affiliates took up seven biological issues with a goal of analyzing the structure and functions of 500 proteins. The participating institutions were located throughout Japan.

A committee to promote the NPPSFA was also set up in order to manage project progress, conduct overall supervision, and draft basic policy. The committee was led by Tairo Oshima, then a professor at Tokyo University of Pharmacy and Life Sciences, currently Prof. Emeritus of that university, Prof. Emeritus of Tokyo Institute of Technology. In FY 2003, an evaluation committee for the NPPSFA (headed by Teruhiko Beppu, professor at Nihon University and Prof. Emeritus of the University of Tokyo) was established. It conducted assessments and made recommendations and proposals relating to research content and research promotion methods, which were taken into consideration when coordinating the execution of the project.

System for implementation



Examples of Technical Development

TERA, a fully automated robot that can search for crystallization conditions and observe crystal formation

SPring-8 Precise Automatic Cryo-sample Exchanger (SPACE), a fully automated sample unit installed on the SPring-8 beamline BL26B2

→ See the next page for an outline of the infrastructure facilities developed (including the technical developments shown at left).

Research Budget

FY2002 - 2006

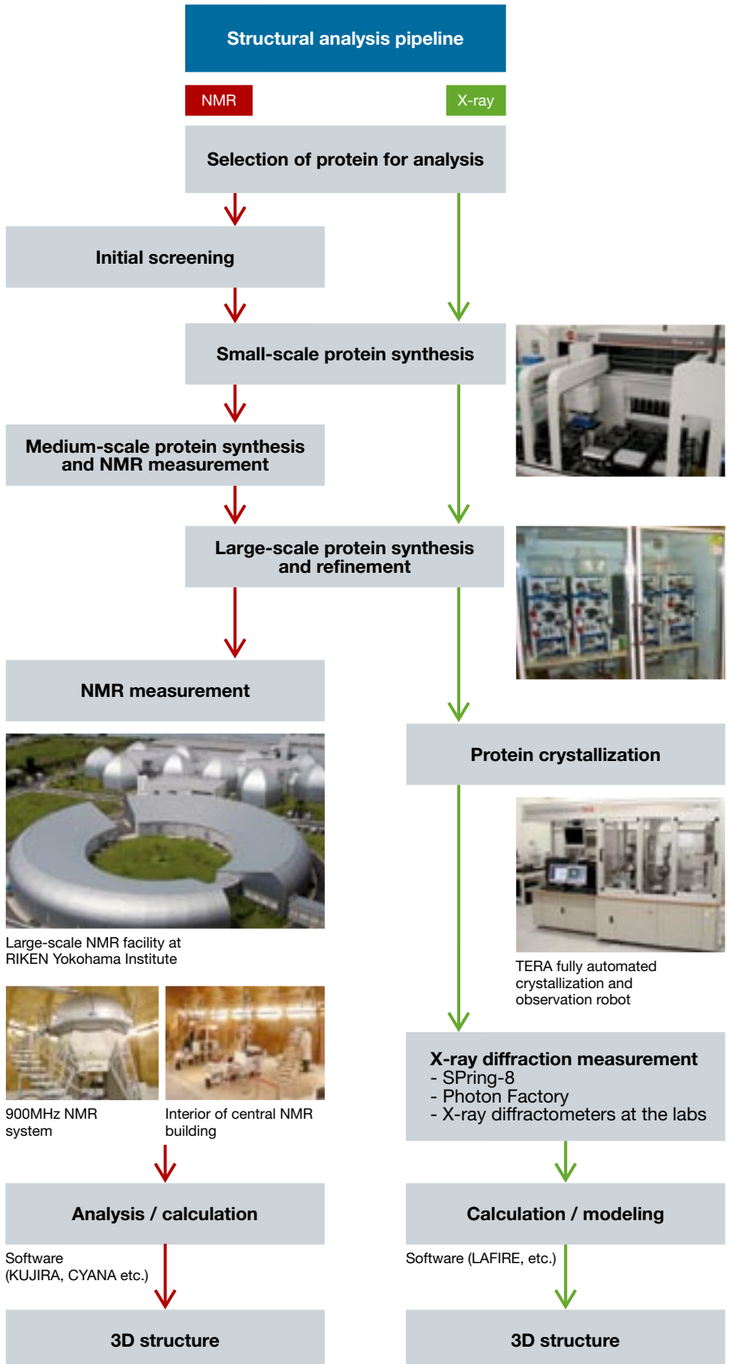
FY 2002	FY 2002 supplementary budget	FY 2003	FY 2004	FY 2005	FY 2006	Total
11.8 billion yen	9.1 billion yen	9.1 billion yen	9.1 billion yen	9.8 billion yen	8.6 billion yen	58 billion yen

Infrastructure Development: Facilities and Equipment Constructed for Structural Analysis

Construction of Structural Analysis Pipeline

In the structural analysis of proteins, first a small quantity of the protein is synthesized based on DNA. After checking whether it is suitable for analysis, a large quantity of the protein is synthesized. The necessary data is then obtained in an aqueous solution (in the case of NMR) or crystallized form (in the case of X-ray analysis). By analyzing the data, the protein's 3D structure is determined.

RIKEN tried to automate and optimize each stage of this process and assembled them into a "pipeline". The pipeline enabled the operation to be conducted efficiently, and greatly increased the speed of the process from protein synthesis through determination of the structure. After the completion of the project, the NMR pipeline was made available to outside parties, and private companies began using it through an open application system.



Large-scale NMR facility at RIKEN Yokohama Institute

900MHz NMR system

Interior of central NMR building

TERA fully automated crystallization and observation robot

Enhancement of NMR Systems

The RIKEN Yokohama Institute has about 40 NMR systems. In order to pursue structural analysis of proteins using these systems, a new highly sensitive measurement method was developed and automation of the analysis technology pursued. In addition, a system capable of measurement with higher magnetic fields was developed with the National Institute for Materials Science (NIMS).

These enhancements made it possible to determine the structure of proteins with various functions, such as the protein groups involved in transcription and translation, the processes through which proteins are created based on DNA within cells.

Enhancement of Beamline for X-ray Analysis of Crystalline Structure

Powerful X-rays are needed to analyze the 3D structure of large proteins by means of X-ray crystalline structure analysis. The radiant beams obtained when electrons accelerated to several billion volts using an accelerator are bent by a powerful magnet contain extremely powerful X-rays. These are extracted and introduced to the beamline and used for the structural analysis of proteins. Such beamlines are present on two large synchrotron radiation facilities, the SPring-8 at RIKEN and the Photon Factory (PF) at KEK.

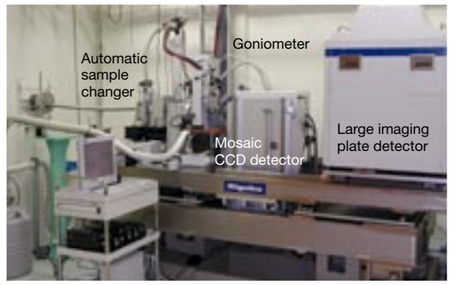
In the NPPSFA, experimental equipments have been developed to realize automatic and efficient operations, making these beamlines exert full capability. This enabled structural analysis to be performed more quickly, and by non-specialist researchers.

Enhancement of SPring-8 Beamline

Protein crystallization is time- and labor-intensive, and since this made structural analysis difficult, the TERA robot was improved using technologies developed during the project. TERA is a fully automated robot that can search for conditions that will allow crystallization, and observe the process of crystal formation. It was one of the key technologies in the structural analysis pipeline (see page at left).

Automatic sample changers were introduced on the two beamlines used to analyze the crystalline structure of proteins, and these sample changers were configured with the beamline control software to automate the process from sample changing through measurement of diffraction data. An effort was also made to automate the process of analyzing the diffraction data and determining the protein structure.

SPring-8

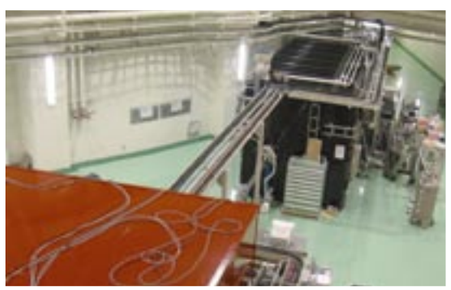


Sample measurement system for BL26B2 (one of the dedicated beamlines for analysis of protein structure)

Enhancement of Photon Factory Beamline

Four beamlines from the two rings (PF and PF-AR) were made available to the many researchers participating in the project.

The world's fastest crystallizing robot was developed, and a large capacity sample changing robot was introduced and improved. Hardware and software were put in place to control the beamlines, and the large 315 mm square CCD detector (then the largest in the world) introduced on beamline BL-5A speeded up the collection of diffraction data and enabled large-volume processing of many samples.



AR-NW12 (one of the dedicated beamlines for analysis of protein structure)

Large-area CCD detector and ultra-high precision diffractometer provided on BL-5