List of research subjects

Key technology developments			The Targeted Proteins Research Program is a national project pron
Research subject	Principal investigator	Representative organization	The program aims to reveal the structure and function of proteins the
Development of advanced production technologies for target proteins	Shigeyuki Yokoyama	RIKEN	program's study of the targeted proteins selected will deepen our u
Development of novel affinity tag system for the high-quality production of extracellular and membrane proteins	Junichi Takagi	Osaka University	and pharmaceutical sciences, and be industrially applied in various a proteins are difficult to study structurally and functionally, the projection
Development of innovative methods to support membrane protein crystallization	Hiroaki Kato	Kyoto University	structural analysis, and function regulation using chemicals, as well
Antibody production for membrane protein crystallization	So Iwata	Kyoto University	with a budget of 5.5 billion yen, and more than 150 scientists are now
Development of the synchrotron beamlines dedicated to the measurement of micron-size protein crystals	Soichi Wakatsuki	High Energy Accelerator Research Organization (KEK)	
Structural analysis of membrane protein complexes by solid-state NMR	Toshimichi Fujiwara	Osaka University	Development of advanced production technologies for
Diversification of protein structural analysis technologies by the SAIL method	Masatsune Kainosho	Nagoya University	target proteins Shigeyuki Yokoyama
Establishment of chemical library and development of protein regulation technology	Tetsuo Nagano	The University of Tokyo	Overview and objectives of the project
Creation and management of information platform in Targeted Proteins Research Program	Hideaki Sugawara	National Institute of Genetics	Overview In life science research, structural analyses of important targets (macromolecular complexes and membrane proteins etc.) frequent encounter severe bottlenecks, because the sample preparation steps are so difficult. Corresponding to the functions of the samples in vivo, the may undergo membrane embedding, complex formation, or post-translational processing or modification (glycosylation, phosphorylation etc.)
Structural bioinformatics for modeling protein complex structures	Kei Yura	Ochanomizu University	They might have multiple states that enable the regulation of the activity, making the lifetime of the functional structures short. For these reason the sample preparation is difficult. In our project, we have developed advanced fundamental technologies that enable the preparation of difficults.

nvestigations of fundamental biological phenomena				
Research subject	Principal investigator	Representative organization		
Structural and functional studies of bacterial type III and type IV protein export systems	Katsumi Imada	Osaka University		
Structural basis for dynamic formation and mechanistic actions of huge and complicated proteolytic machinery	Keiji Tanaka	The Tokyo Metropolitan Institute of Medical Science		
Structural basis of Atg proteins essential for autophagy	Fuyuhiko Inagaki	Hokkaido University		
Structural biology of transcription factors and histone modification factors	Yoshifumi Nishimura	Yokohama City University		
Structure and function of voltage-sensor domain proteins	Yasushi Okamura	Osaka University		
Structure-function analysis of protein complexes that regulate vesicular traffic	Soichi Wakatsuki	High Energy Accelerator Research Organization (KEK)		
Structural basis of Atg proteins essential for autophagy	Toshiaki Sakisaka	Kobe University		
Structural basis of sensor system for cytoprotective gene expression responding to carcinogens and oxidative stress	Masayuki Yamamoto	Tohoku University		
Solid-state NMR investigation on functional and irregular structures of H ⁺ -ATPsynthase F _o	Hideo Akutsu	Osaka University		
3D structural and functional analyses for elucidation of the mechanism of mitochondrial respiration	Shinya Yoshikawa	University of Hyogo		
Towards structure-based design of novel inhibitors for V-ATPase	So Iwata	Kyoto University		
Elucidation of the mechanism of high-order cellular functions achieved by non-coding RNAs	Osamu Nureki	The University of Tokyo		

Research subject	Principal investigator	Representative organiza
Structural analysis of molecules related to the innate immune system	Shizuo Akira	Osaka University
Drug discovery-oriented analysis for structure and function of DOCK2 signaling molecules	Yoshinori Fukui	Kyushu University
Study on reactive oxygen-producing systems involved in neural cell death and structure-based discovery of their inhibitors	Hideki Sumimoto	Kyushu University
Structural analysis of γ -secretase complex for the development of the treatment for Alzheimer's disease	Taisuke Tomita	The University of Tokyo
Development of anti-trypanosome drugs targeting nucleotides biosynthesis and red-ox regulatory pathway	Kiyoshi Kita	The University of Tokyo
Structure-based functional analyses and development of drug intervention in metabolic syndrome and diabetes - AdipoR/AMPK/ACC as key targets -	Takashi Kadowaki	The University of Tokyo
Structural and functional analysis of a molecular target FROUNT for drug discovery	Koji Matsushima	The University of Tokyo
Development of new compounds for fibrosis therapy	Junn Yanagisawa	University of Tsukuba
Structure and drug development of NPP family members involved in cancer and various diseases	Junken Aoki	Tohoku University
Structural and functional analysis of semaphorins and their receptors	Atsushi Kumanogoh	Osaka University

Applications to food production and bioremediation, etc					
Research subject	Principal investigator	Representative organization			
Structural and functional analyses of signaling proteins for insect control	Koji Nagata	The University of Tokyo			
Structure-based functional analysis of key enzymes that can be applied to production of antibiotics and other useful compounds	Sueharu Horinouchi	The University of Tokyo			
Structural and functional analyses of transcriptional regulatory proteins useful for breading of drought and heat stress tolerant crops	Masaru Tanokura	The University of Tokyo			
Structural and functional analyses of regulatory proteins in plant growth and stress resistance, that is useful for improvement of the stress-resistant crop	Makoto Matsuoka	Nagoya University			
Structural biology on efflux transport machineries to understand multi-drug resistance	Satoshi Murakami	Tokyo Institute of Technology			
Structural and functional analyses of the rodent ESP family	Hiroaki Terasawa	Kumamoto University			
Structural and functional analysis of taste receptors applicable to development of new taste substances and taste evaluation systems	Atsuko Yamashita	RIKEN			
Structural biology of bacterial super-biosystem for import and degradation of polysaccharides and its application to food and environmental areas	Wataru Hashimoto	Kyoto University			
Structural and functional analyses of enzymes involved in a new carbon dioxide fixation system and their functional improvement	Kunio Miki	Kyoto University			
Structure-function analyses and improvement of useful enzymes for chiral compound prodcution	Sakayu Shimizu	Kyoto University			
Structure and function of environmentally-responsive proteins	Ko Shimamoto	Nara Institute of Science and Technology			

Targeted Proteins Research Program

Construction of the "protein expression library"

multiple-times membrane-spanning proteins, macromolecular complexes, multi-state proteins, proteins with short lifetimes, etc.).

Enables high-throughput screening of expression conditions for many Evaluate whether the samples have sufficient quality for structural and

Fundamental information and resources for protein production

Membrane proteins and Supramoleclar complexes

In the project, we will not handle all of the proteins but will focus on the important one

Important membrane proteins (e.g. GPCRs, transporters) were selected from the human

genome sequences, on the basis of the total number of membrane proteins and the social

systematic investigation of the expression conditions, we will unveil the rules governing

complexes of signal transduction molecules, and nucleosomes and their complexes with

Systematic use of lipids enabled the regulation of the

Q_{II}:cubic liquid crystal

Increased chemical stability and resistance to the degradation enzymes

ype II cubic liquid crystal that is stable below 0 °C is available.

Systematic use of lipids enabled regulation of the characteristics and t

Production of membrane proteins by cell-free protein synthesis systems

structures of the matrices for the first time.

(medical) significance (under the supervision of Profs. Takashi Tsuruo and So Iwata). By

Important protein families were selected from the large-scale gene resources.

lucleosome binding proteins have also been selected for investigation.

E. coli cell-free system for functional and structural analyses-

Cell-free protein synthesis with lipids and detergents

Synthesize active membrane proteins in liposomes 0% of 30 tested membrane proteins are synthesized as the

(DnaKJ/GrpE, Trigger Factor, GroE)

membrane and removing unwanted materials from the

cultured cells by centrifugation,

and (ii) adding enzymes that

enhance the protein production.

crystal" or "sponge phage," have

GPCRs. As the technology is new,

many problems still remain. We

are developing a strategical and

systematic crystallization method or membrane proteins by (i) using novel lipid-based "cubic liquid

and methodologies specialized fo

investigating the crystallization

systematization

Development of novel lipid matrices for crystallization

Systematic use of lipids enabled the regulation of the

Development of devices for cubic liquid crystal method

an example of the novel lipids

The cell-free protein synthesis system is a reaction system consisting of a cell extract, a template DNA or RNA, and substrates. It has an advantage in (i) high-throughput

Over-expression of membrane proteins in a conventional cellular expression system is often limited by their inhibitory effects on host cell physiology. Several cell-free

systems have been used for membrane protein production. For the preparation of membrane proteins in functional states, multiple technologies are adapted; for example

membrane proteins synthesized in insoluble forms were re-folded by detergents or membrane proteins were synthesized in soluble forms by the addition of detergents.

96-well glass cell for crystal screening

Crystal in the glass cell: crystals under

normal light (left)and polarized light (right)

-Development of a membrane protein production system

based on the wheat cell-free system for functional and

a. Production of functional PEP/phosphate translocator by the wheat cell-free system in the presence of liposomes

Fransport activity (nmol/mg protein/min)

plut 0 500 1000 1500 2000 2500 3000

system based on the wheat cell-free system

ensity gradient ultracentrifugation

density gradient centrifugation

Fractionation of liposomes by sucrose or Accudent

unnatural amino acids in vivo and i

Accumulation of innovative and state-of-the-art fundamental technologies that are generally useful for the expression and the structura

The Targeted Proteins Research Program is a national project promoted by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan. The program aims to reveal the structure and function of proteins that have great importance in both academic research and industrial application. The results of the program's study of the targeted proteins selected will deepen our understanding of fundamental biological phenomena, contribute to the advancement of medical and pharmaceutical sciences, and be industrially applied in various areas, such as the enhancement of food production and bioremediation. As most of the targeted proteins are difficult to study structurally and functionally, the project also promotes the development of basic and innovative technology for protein production, structural analysis, and function regulation using chemicals, as well as the construction of an information platform. The project started as a five-year plan in FY 2007 with a budget of 5.5 billion yen, and more than 150 scientists are now conducting research on 43 subjects. The budget for FY2008 is 5.2 billion yen.

Development of a micro-beam

will be also developed.

analysis with crystals of less than 10 microns will be

evelopment of a beamline dedicated

A beamline dedicated for sulfur SAD experiment is under

development at the Photon Factory. The beamline (BL-1A

for lower energy SAD experiment

enhance anomalous signals from light atoms.

Optimized beam delivery for lower energy

higher precision and higher stiffness

short period in-vacuum undulator

2nd: to measure the rest of images

3.K-B mirror system with large demagnification factor

4.Photon flux density 1014 photons/sec/mm2 @ 100 μ m2

Development of beamline components

New data collection method to avoid

Total amount of dose can be decrease

Development of the synchrotron beamlines dedicated

data collection method

method to avoid radiation damage_____

Micro-beam enables micro-crystallography

Diffraction measurement from micro-sized crystals

current goal X-ray microprobe

15000 20000

Energy spectrum of the short gap undulator. The lower

nergy (4-5 keV) beam gives higher anomalous signals fron

light atoms. The 3rd harmonics covers the energy range for

SPring-8 BL32XU design concept

K-B mirror system with large demagnification facto

3.Photon flux density 1016 photons/sec/mm2 @ 1 µ m

Right: a cassette currently used at SPring-8

evelopment of sample cassette

to the measurement of micron-size protein crystals











