Research Activities from Individual Analysis Program of the National Project on Protein Structural and Functional Analyses (Protein 3000 Project)

A structural genomics project in Japan, the National Project on Protein Structural and Functional Analyses (Protein 3000 Project) funded by MEXT (Ministry of Education, Culture, Sports, Science and Technology, Japan) was established in the fiscal year 2002 and completed in March 2007. This project was comprised of two separate programs. One is focusing on the comprehensive analysis of the fundamental structures of proteins where RSGI (RIKEN Structural Genomics/Proteomics Initiative) takes a leading part. Another program called the "Individual analysis program" involves individual analysis efforts mainly performed by the members of the Japanese university community. In both programs, the structural biology beamlines of the synchrotron radiation facilities played an important role in determining the three-dimensional structures of the target proteins by means of X-ray crystallography. In this report, research activities obtained from the individual analysis program of this project by the use of SPring-8 beamlines are briefly summarized.

The aim of the individual analysis program by the university community is to study both structures and functions of proteins in a selected target field of biological systems. In the individual analysis program, a target of determining more than 500 three-dimensional structures of proteins and their complexes was set as an initial plan. For seven targeting research fields, eight consortiums mainly composed of the university community members were established as presented below:

1) "Development and Differentiation of Organisms and Replication and Repair of DNA" directed by Prof. Masaru Tanokura, the University of Tokyo
2) "Transcription and Translation (Part I)" directed by Prof. Isao Tanaka, Hokkaido University
3) "Transcription and Translation (Part II)" directed by Prof. Yoshifumi Nishimura, Yokohama City University
4) "Posttranslational Modification and Transport"

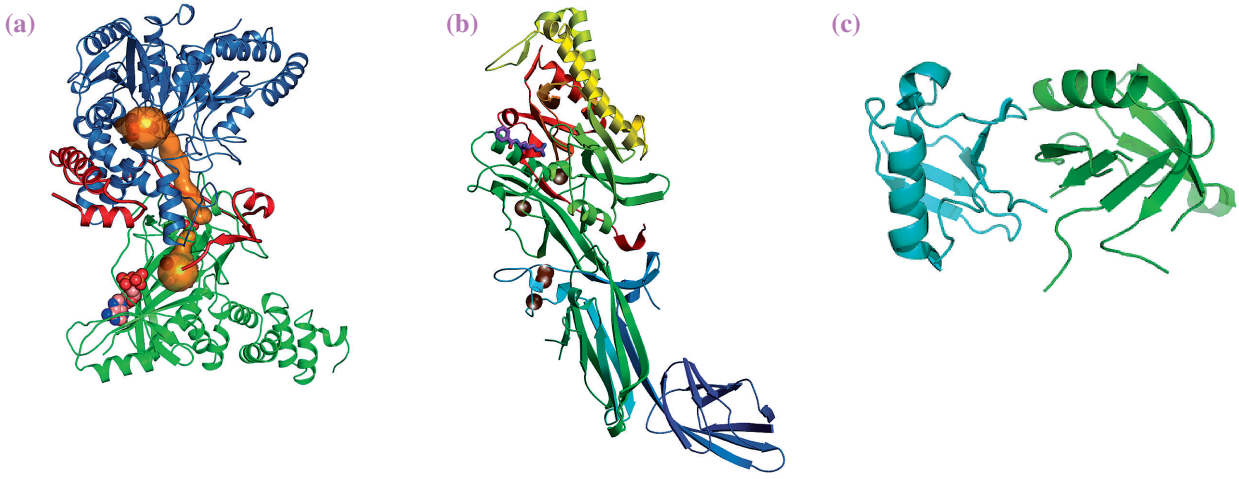
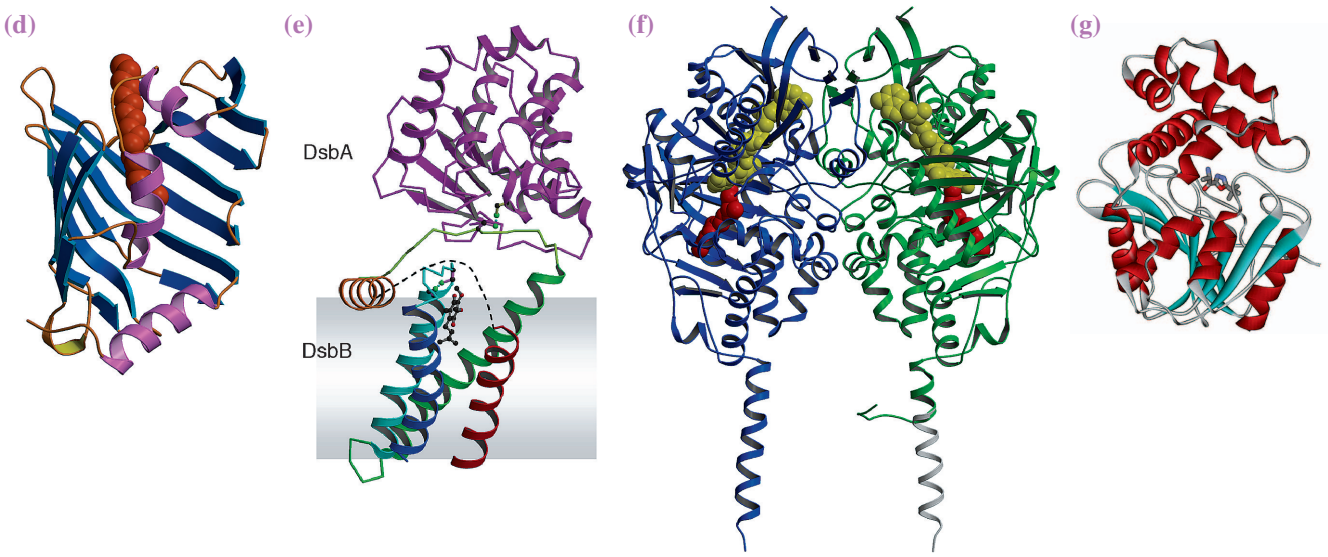


Fig. 1. **(a)** GatCAB (PDB ID: 2F2A) [1]. GatCAB converts Glu-tRNA^{Gln} into Gln-tRNA^{Gln}. In this reaction, initially Glu-tRNA^{Gln} is activated using ATP in GatB (green), and subsequently activated Glu-tRNA^{Gln} is transamidated into Gln-tRNA^{Gln} using ammonia generated by hydrolysis of glutamine in GatA (blue). Two identified reaction centers in GatA and GatB are markedly distant but connected by a long channel (orange), which transfers generated ammonia from GatA to GatB.
(b) Peptidylarginine deiminase 4 (PDB ID: 1WDA) [2]. Peptidylarginine deiminase 4 (PAD4) is a post-translational coregulator that catalyzes the calcium-dependent conversion of specific arginine residues in histone to citrulline. Significant association between rheumatoid arthritis (RA) and functional variants of the gene encoding PAD4 in the Japanese population indicates that human PAD4 is associated with RA and PAD4 inactivators represent potential lead compounds for the treatment of RA.
(c) GLUE-domain of murine EAP45 (PDB ID: 2DX5) [3]. ESCRT-II, a complex that sorts ubiquitinated membrane proteins to lysosomes, localizes to endosomes through interaction between the Eap45 subunit's GLUE domain and phosphatidylinositides. The structure of GLUE domain of mammalian Eap45 complexed with ubiquitin showed that they interact directly, while the corresponding domain of yeast homologue, Vps36, interacts with ubiquitin through inserted Zn-finger.



(d) LolB (PDB ID: 1IWN) [4]. The Lol system in Gram negative bacteria exports insoluble outer membrane lipoproteins across the periplasm. LolB is a lipoprotein receptor on the outer membrane. The structure of LolB comprises an antiparallel β-sheet covered by three α-helices. PEGMME used for crystallization is observed in the internal cavity, which might show a plausible binding mode of the lipid moiety of the lipoprotein.
(e) DsbB-DsbA complex (PDB ID: 2HI7) [5]. DsbB generates a disulfide bond in conjunction with ubiquinone, to be relayed by DsbA to client proteins. The crystal structure of a DsbB-DsbA complex reveals the ubiquinone-DsbB reaction center as well as a cysteine relocation mechanism that allows DsbB to oxidize the extremely oxidizing DsbA enzyme.
(f) Monoamine oxidase A (PDB ID: 1O5W) [6]. Monoamine oxidase (MAO), a mitochondrial outer membrane enzyme, catalyzes the degradation of neurotransmitters in the central nervous system and is the target for anti-depression drug design.
(g) Prolyl aminopeptidase (PDB ID: 1X2E) [7]. Prolyl aminopeptidase from *Serratia marcescens* is a pathogenic enzyme causing the collagen degradation of the human skin. The structure predicted the extra space in the active site that can accommodate the acetyloxyproline moiety of collagen. High reactivity toward acetyloxyproline substrate suggested the necessity of acetylation step for efficient degradation of collagen.

directed by Prof. Soichi Wakatsuki, Institute of Materials Structure Science, KEK
5) "Protein Higher-Order Structure Formation" directed by Prof. Kunio Miki, Kyoto University
6) "Intracellular Signal Transduction" directed by Prof. Fuyuhiko Inagaki, Hokkaido University
7) "Brain and Nervous System" directed by Prof. Atsushi Nakagawa, Osaka University
8) "Metabolic Systems" directed by Prof. Seiki Kuramitsu, Osaka University

In the individual analysis program for five years of the project, a total of more than 1,800 protein structures were determined (more than 1,300 structures were deposited with the Protein Data Bank, PDB) by both X-ray crystallography and NMR. The structural biology beamlines of SPring-8 were extensively used by the members of eight consortiums of the individual analysis program. For this purpose, some part of beamtimes is preferentially assigned in the public beamlines such as **BL41XU**, **BL40B2**, and **BL38B1**. Consequently, numbers of crystal structures of the target proteins were determined in this program. Several examples of the protein structures determined by X-ray crystallography using the SPring-8 beamlines are summarized in the Fig. 1(a-g).

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