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Proteins: Structure, Function, Applications, and Changes in Relation to Disease

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I. EXECUTIVE SUMMARY

This grant is the second phase of a Senior Research Scholar Award (2004-2007) awarded to Professor Jisnuson Svasti. As previously, the grant focuses on studying proteins and developing capablility in protein research. This is essential, since proteins perform biological actions in all living systems (see Section II for Overview). Thus, the completion of the Human Genome project in 2001, establishing the DNA sequence of human beings, has shown clearly that further studies are needed to understand the structure, action, synthesis, and localisation of proteins which are synthesised from this DNA, if we wish to understand living systems. Unfortunately, protein science in Thailand, although started earlier than DNA research, underwent a period of slow growth due to the lack of expensive major equipment necessary for modern protein research. However, during the first phase of this Senior Research Scholar Award (2001-2004), various agencies recognised the importance of protein research, and provided major equipment items, enabling us to delve deeper into more advanced research in protein science. Thus, the second phase of the Senior Research Scholar Award continues the studies intiated in the first phase, allowing significant advances in the field. In addition, apart from obtaining research results of interest, we have placed emphasis in the development of manpower in protein research, and encouraging other researchers to be more active in protein research. These various aspects are discussed below.

Our research group has two major focal points. First, the core of the research group is represented by the Center for Excellence in Protein Structure and Function (CPSF), which has 6 members: Professor Jisnuson Svasti, Dr. Jirundon Yuvaniyama, Dr. Palangpon Kongsaeree, Dr. Pimchai Chaiyen, Dr. Pramvadee Wongsaengchantra, and Dr. Kittisak Yokthongwattana. CPSF has X-ray crystallographic equipment for determining three-dimensional structure of a protein, as well as a stop-flow spectrophotometer for studying reaction intermediates in enzyme reactions at the pre-steady state phase. The other focal point is the Laboratory of Biochemistry, Chulabhorn Research Institute (CRI), which has instrumentation for amino acid analysis, protein sequencing, proteomic and mass spectrometry facilities. These facilities complement each other, and allow a broad range of research activities for studying proteins, which are made available to collaborators in the project.

The present grant now has 16 co-investigators from nine universities, namely Mahidol University, Kasetsart University, Suranaree University of Technology, Khon Kaen University, Prince of Songkhla University, Srinakarintwirot Prasarnmitr University, Naresuan University, Mahasarakham University, and Mae Fah Luang University. This networking between investigators throughout Thailand is important, because researchers in provincial universities, especially in departments which are still developing their research, often feel isolated. The connections that we have established with this grant have allowed researchers to contact each other, in addition to linking with the Senior Research Scholar. This has brought researchers with complementary expertise together, so that they can form collaborations that take advantage of this complementarity. In addition, since 12 several researchers in the group are under the age of

40, the networking and linkages supported by this grant have helped these develop their research capabilities and establish their laboratories.

The involvement of many co-investigators has allowed the grant to cover a broad range of research areas. Indeed, a major policy of the principal investigator has been to give younger colleagues freedom of choice in selecting or developing a research theme, since this has been an important component in the success of the MRC Laboratory of Cambridge, UK, where he was trained. Nevertheless, the projects can be broadly grouped into three main themes: a) Protein Structure-Function Relationships; b) Protein Changes in Disease, Therapy and Healing; c) Applications of Enzymes in Biotechnology (see Section III for details). Study of structure-function relationships is still a major focus of the grant, including the original areas of glycosidase enzymes (now including the chitinases) which hydrolyse carbohydrates, enzymes involved in the synthesis of penicillin derivatives, flavoprotein oxygenases, and synthetic compounds with proteinase activity. New areas in terms of protein structure and function include lysozyme and other antibacterial peptides in reptiles, structure-function relationships in acetyl-coenzyme A carboxylase, an enzyme involved in CO_2 fixation, and proteins in algae responsible for adaptation to irradiance-stress.

Although the structure-function studies represent basic research, carried out to increase the knowledge of mankind by publication of research articles in international journals, many of the topics have obvious implications in terms of future applications. Thus, the β-glucosidase enzymes studied from a basic science viewpoint have resulted in discovery of isoflavonoid β-glucosidases, that may be useful in the food industry for enhancing phytoestrogens. Cassava β-glucosidase enzyme has potential for stereospecific synthesis of chiral glycosides, while suitably mutated rice β-glucosidase may be used for oligosaccharide synthesis. Studies of structure-function relationships in bacterial chitinase provides ideas for improving the enzymatic degradation of a major biomass source, chitin from shellfish, insects, and fungal cell walls, for example for production of biofuels and raw materials for synthezising a host of compounds. In addition, our studies of glycosidase enzymes in rice have revealed that many such enzymes are present and are yet poorly understood, and if we could understand their importance, novel ideas for improvement of rice production and/or quality may emerge.

Study of the enzymes involved in penicillin modification have obvious important applications for the synthesis of many common penicillin and cephalosporin derivatives locally, rather than importing them from abroad at greater cost. In terms of flavoprotein oxygenases, some enzymes have been shown to be able to cleave aromatic rings and have potential application in terms of bioremediation of toxic aromatic waste. Another enzyme, luciferase, which emits light, is being used commercially for analytical measurements of drugs and environmental pollutants. Study of antibacterial compounds from reptiles also has potential application: for example our discovery of antibacterial substances in crocodile blood may allow this commodity to be sold, like snake blood, in addition to the skin and meat of the crocodile. Other projects with potential applications include proteomic studies of moulting in shrimp, which may have future benefits in shrimp culture, and study of the response of algae to high levels of light radiation, which may help improve the commercial cultivation of such algae as food supplements.

In terms of the second theme "Proteins in Health Disease and Wound Healing", a major component involves study of genetic diseases. Thalassemia is an important genetic disease found in a high proportion of the population. Apart from characterisation of novel abnormal hemoglobins, we have attempted to devise an immunological test for detection of thalassemia, based on Hb Barts. We have also started to work on inborn errors of metabolism, which can cause developmental disorders or poor mental development of the newborn. Although each of these diseases is relatively rare, there are as many as 500-600 such diseases, so they have significant impact to the population. In addition, in a limited number of diseases, early diagnosis can allows suitable treatments to alleviate the deleterious effects. Our studies have focused on methylmalonic acidemia and several mucopolysaccharide disorders. Although these diseases have been studied for many years in developed countries, molecular studies in Thailand have only started recently, and there are still only a handful of clinicians specialising in these diseases throughout the country. Interestingly, our studies showed that many mutations found in Thailand are unique, and have not been found in other populations, such as USA, Europe or Japan. Thus, it is still important to study the mutations occurring in Thailand, so that we can develop proper diagnostic techniques and provide genetic counseling to parents. This research naturally requires collaboration with many physicians who provide clinical specimens, highlighting another aspect of networking, namely bringing together the clinicians who have the research problems and the scientists who have the technologies to solve those problems.

Another disease, due to mutations causing changes in the level and/or activity of certain proteins is cancer, which is a major cause of death within the country. One area of research has been the use of proteomic technologies to compare normal and cancerous tissues, for example of the thyroid, with a view to finding possible biomarkers for improved diagnosis. Liver cancer is particularly prevalent in Thailand, in the form of cholangiocarcinoma (cancer of the bile duct) in the Northeast and hepatocellular carcinoma in the North. We are therefore using cultured cell models of these diseases, HuCCA-1 cells for cholangiocarcinoma and HCC-S102 for hepatocellular carcinoma, both of which are derived from Thai patients. We have not only studied the proteomic patterns of these cells under normal conditions, but also after treatment of cytotoxic agents to understand the mechanism of action of these compounds. We have also searched for anti-cancer agents from medicinal plants and marine compounds. This includes using cultured cells to screen for cytotoxic agents, which kill cancer cells. However, such cytotoxic agents also have some toxic effects to normal cells, and can only be used for short intermittent periods of time. So, we have developed screening methods for detecting compounds which are not cytotoxic, but which can inhibit the invasion capability of cancer cells, and therefore can be used for prolonged periods of time to inhibit cancer metastasis (or spreading of cancer cells to other tissues), which is a major cause of death. Such studies have obvious applications in the discovery of novel compounds that can be used as lead compounds for anti-cancer drugs of various types. We have also developed cell models for screening for substances which can improve wound healing, which again can provide useful lead compounds.

Finally, three projects aim at developing the biotechnology applications of enzymes. The first aims to improve the degumming of silk, where the protective silk gum or sericin protein is removed, leaving the long extended fibroin protein, thereby improving the texture, sheen, color, and dyeing capability of the silk. Our group is searching for a sericin-specific protease for

developing a more environmentally friendly process for degumming silk to replace the present methods which require boiling in alkaline solution. A second project aims to screen for the enzyme naringinase to use for removing bitter glycosides from fruit juice. The third aims to develop a novel enzyme-based approach for purifying silica from rice husk waste, which would not only be more environmentally friendly than burning the husk, but may also yield silica in a more useable form.

However, as noted above, in addition to these three biotechnology-related applications, several of the basic research projects and disease-related projects also have potential applications. Thus basic research must be supported by funding agencies, since the applications of tomorrow depend on the basic research findings of today. To support only applied research would be to deny our country the platform for development of novel products and technologies. Moreover, basic research is essential in building up scientific and technical manpower, as well as the infrastructure for research and development.

Another noteworthy consideration in our work is that much of it makes use of indigenous materials found in Thailand. Thus our β-glucosidase enzymes are derived from Thai plants, such as rice, cassava, Thai rosewood, and frangipani. Luciferase enzyme was isolated from bacteria found in the sea water near Phuket. Enzymes for synthesis of penicillin were isolated from bacteria found in Thailand. Antibacterial agents from reptiles are being isolated from turtle eggs and crocodile blood. Sericin-specific proteinases are being sought from soil in the neighborhood of silk factories. Cancer cell lines being studied were originally derived from Thai patients, and are being used to screen for bioactive natural products from Thai plants and marine organisms. This emphasis on indigneous starting materials aims to make use of our natural resources, and provide added value to these resources by scientific research, in accordance with the principles of "sufficiency economy".

The major output of the research in this grant has been in terms of publications in international journals (Section VI). Some projects have undoubtedly been more successful than others. However, overall the grant produced 47 papers in international journals, more than 50% greater than the 30 international papers projected in the original proposal. Of the 47 publications, 37 papers had journal impact factors for 2006 (JIF2006) listed in the ISI-Web of Science database, and the remaining 10 publications included 5 papers in overseas journals listed in ISI-WOS, Scopus, or Pubmed, and another 5 papers in international journals published in Thailand and listed in Scopus. Average JIF2006 for the 37 listed publications was 2.818, and individual papers included 1 paper with JIF2006 >9.50; 3 papers with JIF2006 of 5.01-6.00, 3 with JIF2006 of 4.01-5.00, and 5 papers with JIF2006 of 3.01-4.00, indicating that overall, research showed novelty and quality. In addition, the group also published 3 research articles in International Proceedings volumes.

The principal investigator also published 8 non-research articles in international journals, including 2 special articles in *IUBMB Life* with JIF2006 of 2.116, and another 6 Editorials in *ScienceAsia* (Section VII). Other types of output included deposition of 4 sequences in databases, and one patent application. The group also made 53 presentations in international meetings over the period of the grant (Section VIII), greatly exceeding that projected. Students and staff also

made total of 74 presentations in national meetings over the period of the grant (Section IX), again more than planned. Such presentations are particularly important for young staff and students in enabling them to attend meetings and exchanging ideas with others.

Another important aspect of the grant was the training of graduate students (Section X). The number of Ph.D.s produced during the grant period was only seven, fewer than expected. However, another 37 students are in the process of studying for their Ph.D. degrees, so it is hoped that several more Ph.D.s will graduate in the next few years. In addition, another 12 M.Sc. graduates were produced during the grant period, and another 20 are currently studying for their M.Sc. degrees. Finally, some 40 B.Sc. students undertook senior year research with staff members in the grant. In addition, 12 students, mainly Ph.D. students, had the opportunity for research and/or training activities abroad, broadening their outlook.

The development of young staff is another important consideration. Its success can be demonstrated by the ability of staff to publish good articles in international journals, as described above. The ability of staff to attract research funding is another indicator of their ability to compete with others. Thus, in addition, to the present grant, and grants provided to the Center for Excellence in Protein Structure and Function and the Chulabhorn Research Institute, many young staff also had their own competitive research grants (Section XI). Finally, staff won various awards, promotions, and research fellowships (Section XII). These included two notable awards to Professor Jisnuson Svasti, and 4 other research awards to young members of the team, as well as promotions of four staff to Assistant Professor or Associate Professor.

An equally important development was the founding of the Protein Society of Thailand in January 2006 by Professor Jisnuson Svasti and his group (Section V). The society promotes protein research, exchange of expertise, and provides liaison with other scientific organizations and the general public. The society now has about 100 members, including various companies as supporting members. It has co-sponsored a Workshop on Protein Crystallography, organised by the National Synchrotron Research Center, Nakhon Ratchasima and a Workshop on Proteomics organised by BIOTEC. It also arranged the First Annual Symposium of the Protein Society of Thailand on "Challenges in Protein Research in Thailand" on 24-25 October 2006. These Annual Symposia of the Protein Society are now well established and are well attended by more than 300 registered participants. An important feature is that these meetings have continued to encourage young Ph.D. researchers, by providing them with the opportunity to make oral presentations, as well as poster presentations, thereby helping them to make contacts and see what research is being done in protein science in Thailand.

In addition to the formation of the Protein Society of Thailand, Professor Svasti represents the society as a Council Member of the Asian Oceanic Human Proteome Organisation (AOHUPO), linking protein scientists in Thailand with others in the region. Professor Svasti also plays other roles in international organisation, such as being an Honorary Member of the Federation of Asian and Oceanian Biochemists and Governing Council Member of the Asian-Pacific International Molecular Biology Network. This interaction with international organisations is important in providing Thai scientists with the opportunity of

interacting with leading researchers of the world, and increasing the visibility of Thailand in the international arena. In addition, as a Senior Research Scholar, Professor Svasti is conscious of his role in promoting science in Thailand, for example by serving as Editor of *ScienceAsia*, the Journal of the Science Society of Thailand, since this provides a platform for publication of articles of international quality in a local setting.

These various activities will contribute to the understanding of the relationship between the protein structure and function, the roles of proteins in disease and treatment, and the potential applications of proteins in biotechnology. Various sophisticated techniques, such as X-ray crystallography, protein sequencing, proteomics, and stop-flow spectrophotometry have been established, expanding the technical capability of Thailand for protein research. Numerous protein scientists are also being trained at both postdoctoral and predoctoral level, and efforts are being made to promote the interaction of these researchers both locally within Thailand, and with overseas researchers. This should lead to development of protein science in Thailand, which should in turn help to develop Thailand.

II INTRODUCTION

1.1 Overview of the Structure and Function of Proteins

The word *protein* derives from the Greek word **πρώτα** ("prota"), which means "of primary importance" and is said to have been coined in 1838 by the Swedish chemist Jons Jakob Berzelius, one of the fathers of modern chemistry. However, it nearly one century before the importance of proteins was established when J.B. Sumner first purified and crytstallized an enzyme, urease, in 1925. Thirty years later, Frederick Sanger first established the complete structure of a protein, bovine insulin, in 1955, showing that proteins consists of amino acids linked together in a specific sequence by peptide bonds to form a polypeptide chain (Figure 1a).

However, proteins do not exist as linear polymers but fold to form the three-dimensional structure of the protein, which is essential for its function. Kaj Linderstrom-Lang first proposed the terms *primary structure*, *secondary structure*, *tertiary structure* and *quaternary structure* to describe the various levels of folding. The simplest level, the *primary structure*, is the amino acid sequence of the polypeptide chain. The next level, the *secondary structure*, results from folding of local regions into more stable structures linked by hydrogen bonds: two such structures are particularly common, the α-helix and the β-pleated sheet, as predicted by Linus Pauling in 1951 (Figure 1b). In so-called fibrous proteins, which form various structures in the body, polypeptide chains are often extended and consist of one type of secondary structure. However, in globular proteins, which perform the dynamic functions of the body, the whole polypeptide chain tends to fold up to give a compact structure, which has an active site to perform the protein's function, such as catalysis. This overall conformation is termed the *tertiary structure* of the protein (Figure 1c), and may consist of organized regions containing various secondary structures, as well as less organized structure. In some cases, the proper functioning and/or stability of the protein requires more than one polypeptide chain, and the term *quaternary structure* is used to describe the manner in which the polypeptide chains are organized to form the polymeric molecule (Fgure 1d). This interaction of subunits often adds additional interesting capabilities to the protein.

Max Perutz and John C. Kendrew were the first to perform to determine the three-dimensional structures of proteins, hemoglobin and myoglobin respectively in 1959. This pioneering work showed clearly that the three-dimensional structure of a protein determines how it acts at molecular level, which is what we now call structure-function relationships in proteins. Understanding structure-function relationships is a major theme of the research in this grant. In this connection, proteins fulfil myriad functions in the body, since they are the molecules which perform the functions encoded in the DNA of the genome. These functions include forming various structures in the cell and body, enzymatic catalysis of reactions, transport of less soluble molecules, regulation of metabolism and other processes, nutrient storage, contraction and motility, and defence mechanisms.

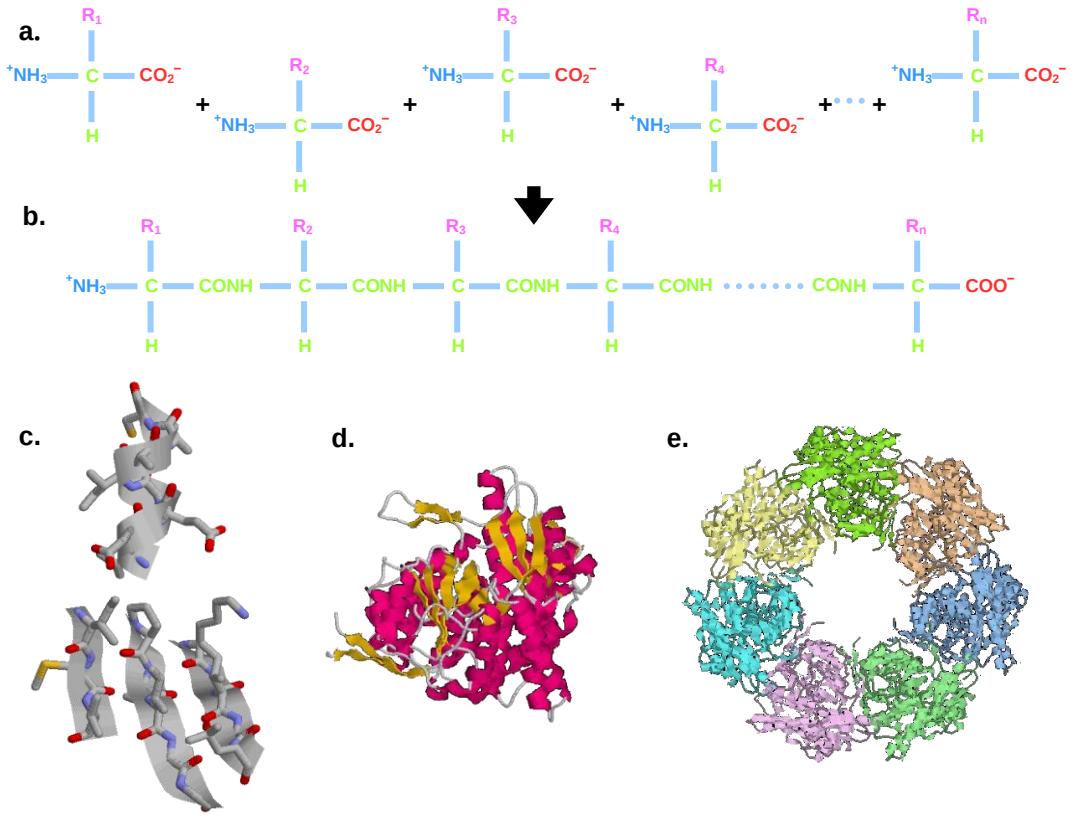


Figure 1. Levels of protein structure: a) linkage of amino acids by peptide bonds; b) *primary structure* or amino acid sequence of protein; c) local folding of peptide chain to form organised *secondary structure*, such as α-helix and β-pleated sheet; d) *tertiary* structure or overall three-dimensional structure of protein; e) *quaternary structure* resulting from the combination of polypeptide chains into oligomers.

Hallmarks of most proteins involved in dynamic processes, such as catalysis, are their specificity, efficiency and ability to work at moderate temperatures. This arises from their specific three-dimensional structures which allow them to bind tightly to their substrates (or reactants) and which bring the catalytic groups close together to react to provide highly efficient catalysis. Moreover, protein molecules are designed to work at the moderate temperatures found in living organisms, since their function depends on the integrity of the three-dimensional structure, which is stabilized by weak forces and may be destroyed at high temperatures. These three properties, namely specificity, high efficiency and ability to work at moderate temperatures distinguish proteins and enzymes from other chemical catalysts, which tend to be less specific and efficient, and require higher temperatures. For this reason, enzymes have been used in many biotechnology applications, and part of our project is involved in the discovery of novel enzymes of potential use in biotechnology. Moreover, studies of structure-function relationships may allow existing enzymes to be improved to change their specificity or increase efficiency.

Because of their importance in almost all biological processes, deficiencies in proteins and enzymes can disease to diseases. Such changes result from mutations at the genetic level, which decrease the amount of protein being expressed or which change the structure so that the protein is less effective in its action. For example, the hemogloblinopathies consist of two types, the thalassemias which result from decreases in the synthesis of the hemoglobin polypeptide chains and the abnormal hemoglobins which result from changes in the structure of the polypeptide chain. Indeed, our understanding of the molecular basis of disease was initiated by the discovery by Vernon M. Ingram that a single amino acid interchange was the cause of sickle cell anemia. Later studies of amino acid interchanges in the hemoglobin by Perutz's group provided important insights in understanding structure-function relationships, snce the disease severity is correlated to the role of the amino acid in the function of the protein and on how the characteristics of the amino acid are changed. Such defects in the functional level of proteins lead to genetic diseases, such as thalassemia and inborn errors of metabolism.

In addition, mutations can also occur at the somatic level, leading to cancer, due to increased expression of oncogenes or decreased expression of tumor-suppressor genes, making cancer cells divide continuously and invade to other parts of the body. The present grant also studies these changes in the proteins which lead to disease, including abnormal hemoglobins, inborn levels, and changes of the proteins occurring in cancer.

1.2 Importance of Protein Research Viewed from Nobel Prizes

Nobel Prizes are awarded for research of outstanding impact. Many were awarded for research involving proteins, indicating the importance of protein research to science and medicine. A list of Nobel Prizes awarded for research relating to proteins is summarized in Table 1: this is somewhat subjective in that it is often difficult to gauge whether a particular award involves proteins as the main thrust. In addition, attempts have also been made to classify whether each discovery reflects a) development of new technology for studying proteins (shown as "Technique"); b) discovery of new principles relating to proteins ("Principles"); c) roles of different proteins in systems or different tissues ("Systems"); d) applications for proteins for

other purposes ("Applications"): again such classifications are rather subjective. Moreover, some discoveries may have involved more than one element, for example both novel technique and new principle (Technique/Principle), or understanding proteins in a system leading to applications (System/Application),

Since 1945, some 16 Nobel Prizes in Chemistry (Table 1A) and another 16 Nobel Prizes in Physiology or Medicine (Table 1B) have been awarded for research relating to proteins. In initial years, the development of novel techniques, such as protein/purification, chromatography, ultracentrifugation, played an important role, sometimes leading to important concepts of protein structure, such as the sequencing of insulin by Sanger and the use of X-ray crystallography for studying myglobin and hemoglobin by Kendrew and Perutz. Other important techniques for study of proteins included development of protein NMR by Wuthrich and ionization techniques for mass spectrometry of proteins by Fenn and Tanaka. Novel principles developed later include allosteric enzyme control by Jacob and Monod, the concept of signals for localization of proteins by Blobel, and the Prusiner's discovery of the prion as a new infective principle, dependent on protein conformation. Since proteins are involved in almost all biological processes, study of proteins in many systems have provided important insights, such as study of antibody structure and diversity by Edelman and Porter, the photosynthetic reaction center by Diesendorfer, Huber and Michel, and ubiquitin-mediated protein degradation by Ciechanover, Herschko and Rose. Importantly, profoundly important applications have been developed through the use of proteins, such as the di-deoxy DNA sequencing technology of Sanger, the monoclonal antibody technique by Kohler and Milstein for making specific antibodies, and the polymerase chain reaction for amplifying DNA by Mullis.

1.3 Protein Research in Thailand in Relation to the Present Grant

Protein research in Thailand appears to have had two origins. The first was the genetic disease work being performed at the Division of Hematology, Department of Medicine, Faculty Medicine Siriraj, Mahidol University, with research work on the characterization of abnormal hemoglobins by Dr. Sagna Pootrakul as early as 1968, followed by work on glucose-6-phosphate dehydrogenase variants found in Thailand by Dr. Vicharn Panich from 1972. The other origin of protein research in Thailand was the Department of Biochemistry, Faculty of Science, Mahidol University, where several proteins were purified and characterised relating to various systems, including carotene 15,15'-dioxygenase by Olson in 1972, seminal plasma acidic proteinase by Ruenwongsa and Chulavatnatol in 1973, human sperm protamine by Panyim in 1975, and human testis-specific lactate dehydrogenase X by Svasti in 1975. Since that time, protein research expanded and covered various aspects screening for novel enzymes, purification and characterisation, exploration of the role of specific proteins in various systems, and applications in biotechnology. However, work on structural analysis at primary and tertiary structural could not be accomplished to to limitations of the major equipment required. On the other hand recombinant DNA research introduced to Thailand by Panyim in the early 1980's progressed well, since it not require such sophisiticated equipment, and several researchers joined the field placing more emphasis on related to the genome.

Table 1A. Nobel Prizes in Chemistry Awarded (1945-2006) for Research Related to Proteins*

Year	Discovery	Laureates	Type of Advance
1946	Purification/Crystallisation	J Northrop, WM Stanley, JB Sumner	Technique/Principle
1948	Ultracentrifugation	Arne Tiselius	Technique
1952	Chromatography	AJP Martin, RLM Synge	Technique
1958	Amino acid sequence	Frederick Sanger	Principle/Technique
1962	Three-dimensional structure	JC Kendrew, MF Perutz	Principle/Technique
1972	Structure, conformation, catalysis	C Anfinsen, S Moore, WH Stein	Principle
1980	Nucleic acid structure	P Berg, W Gilbert, F Sanger	Principle/Application
1982	Virus structure	Aaron Klug	Technique/System
1984	Peptide synthesis	Bruce Merrifield	Technique
1990	Photosynthetic reaction center	J Deisenhofer, R Huber, H Michel	System
1993	DNA technology	KB Mullis, M Smith	Application
1997	ATP synthesis	PD Boyer, JC Skou, JE Walker	System
2002	Mass spectrometry/NMR	JB Fenn, K Tanaka, K Wüthrich	Technique
2003	Water and Ion Channels	P Agre, R MacKinnon	System
2004	Protein degradation	A Ciechanover, A Hershko, I Rose	System
2006	Eucaryotic transcription	RD Kornberg	System

*Awards have been classified as development of new technology for studying proteins (shown as "Technique"; discovery of new principles relating to proteins (shown as "Principles"); elucidating roles of various proteins in different systems or different tissues (shown as "Systems"); or applications for proteins for other purposes (shown as "Applications"); or a combination of the above.

Table 1B. Nobel Prizes in Physiology or Medicine Awarded in 1945-2006 for Research Related to Proteins*

Year	Discovery	Laureates	Type of Advance
1947	Conversion of glycogen	C Cori, G Cori, B Houssay	System
1953	Citric Acid Cycle and Coenzyme A	H Krebs, F Lipmann	System
1955	Oxidation Enzymes	H Theorell	System
1964	Cholesterol and Fatty Acid Biosynthesis	K Bloch and F Lynen	System
1965	Control of Enzyme and Virus Synthesis	F Jacob. A Lwoff, J Monod	Principle/System
1971	Action of Hormones	EW. Sutherland, Jr.	System
1972	Antibody Structure	GM. Edelman , RR. Porter	System
1975	Interaction of Tumour Viruses with Cell	D Baltimore, R Dulbecco, HM. Temin	System/Application
1977	Peptide Hormone Action and Determination	R Guillemin, AV. Schally, R Yalow	System/Application
1978	Discovery & Applications of Restriction Enzymes	W Arber, D Nathans , HO Smith	Application
1984	Immune System and Monoclonal Antibodies	NK. Jerne, GJF Köhler, C Milstein	System/Application
1992	Protein Phosphorylation as Control Mechanism	EH. Fischer. EG. Krebs	Principle/System
1994	G-proteins and Role in Signalling Transduction	AG. Gilman. M Rodbell	System
1997	Prions as New Infective Principle	Stanley B. Prusiner	Principle/System
1999	Protein Signals for Localisation	G Blobel	Principle
2001	Regulators of Cell Cycle	LH. Hartwell, T Hunt, P Nurse	System

*Awards have been classified as development of new technology for studying proteins (shown as "Technique"; discovery of new principles relating to proteins (shown as "Principles"); elucidating roles of various proteins in different systems or different tissues (shown as "Systems"); or applications for proteins for other purposes (shown as "Applications"); or a combination of the above.

However, it became clear that molecular genetics needs to be complemented by study of proteins. Thus, the completion of the Human Genome in 2001 provides information on the genes present in the human species, but is not enough to allow us to understand living processes. This is because the DNA sequences in the genome must be expressed as proteins before they can perform their designated functions. Indeed, the estimated 30,000 genes in the human genome can give rise to more than 200,000 different proteins. This arises due to variations in splicing of the mRNA template used for protein synthesis and subsequent chemical modifications of amino acids in the protein in various ways. In addition, proteins must fold into their specific three-dimensional structures that determine how they function, or be converted from inactive to active forms. Moreover, different proteins may be synthesized in different tissues, or at different stages of development or in disease states. Thus, the complete understanding of living processes requires understanding of structure and function relationships in proteins.

This was the rationale with which the Faculty of Science, Mahidol University established our group at the Center of Excellence for Protein Structure and Function in 2001. As part of the faculty's support, we were able acquire Thailand's first X-ray diffractometer for protein crystallography for determining three-dimensional structure and stopped-flow spectrophometer for following reaction intermediates in enzyme reactions. Another focal point has been the Laboratory of Biochemistry, Chulabhorn Research Institute, which has facilties for protein chemistry and proteomics, later acquiring a dedicated LC/MS/MS mass spectrometer for protein identification. To complement these facilities, a Senior Research Scholar Grant was received from the Thailand Research Fund in 2001 and later extended in 2004 to enable the team to undertake protein research covering three major aspects: a) structure-function relationships; b) proteins changes in disease; c) potential applications of proteins.

III. FINAL REPORT ON RESEARCH PROJECTS

The research in this grant focuses on understanding the structure, function, evolution and applications of proteins, as well as on changes of proteins in disease. This report covers the research activities of the Senior Research Scholar and 16 principal investigators located in 8 universities. Many different topics are covered, since prinicipal investigators have been allowed to develop projects based on their interests and expertise. Nevertheless, the topics may be linked to three major themes: A) Protein Structure-Function Relationships; B) Protein Changes in Disease and Healing; C) Applications of Proteins and Enzymes in Biotechnology. Particular interest has been paid to studying proteins and enzymes found locally in Thailand or studying problems of relevance to the country. The present report focuses on the major findings, without technical details, since these can be found in the publications shown in parenthesis, which are numbered according to publications (section IV). Some collaboration, leading to published work (14), but involving our investigators in minor roles, are not described.

A) Protein Structure-Function Relationships

Protein function depends on three-dimensional structure, arising from the folding of the polypeptide chain. This folding creates the active site for binding substrate specifically and mobilizing the catalytic groups to fulfil their designated functions. Projects in this theme explore the relationship between the functional properties of a protein with its structure. Although such projects aim for basic knowledge, their results have potential future applications.

1. Structure-Function Relationships in Plant Glycosidase Enzymes

1.1 Background on Glycosidases and Glucosidases

Glycosidase enzymes (E.C. 3.2) are enzymes which hydrolyse the glycosidic bond between a sugar and an aglycone or another sugar (Figure 2). For retaining enzymes, this reaction occurs by cleavage of a donor sugar to yield aglycosyl enzyme intermediate (Figure 2 a), which typically will be hydrolysed by water to yield a smaller sugar or monosaccharide (Figure 2b). However, in the presence of an alcohol, the glycosyl group can be transferred to alcohol to yield an alkyl glucoside (Figure 2c). Or in the presence of large concentrations of monosaccharide, the reaction can be reversed leading to synthesis of larger oligosaccharides (Figure 2d). Thus these enzymes not only have potential applications in hydrolysing particular glycosides or polysaccharides of interest, but they might potentially be used for synthesis of oligosaccharides or glycosides by reverse hydrolysis with high monosaccharide concentrations or by transfer reactions using suitable donors and acceptors. The ability to synthesize such compounds is of much interest, since oligosaccharides can fulfil many functions in biological systems, such as cellular recognition, secretion and clearance of glycoproteins, and modulation of cell growth and differentiation. Moreover, certain glycosides also have potential applications in the food and drug industry, or may be useful as biodegradable detergents.

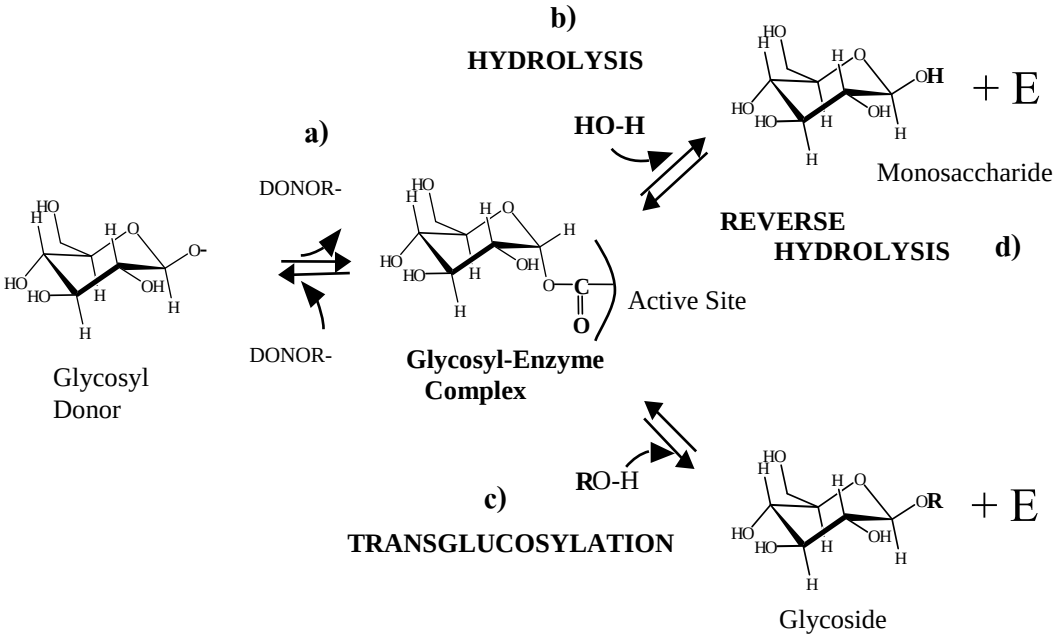


Figure 2. Reactions catalysed by glycosidase enzymes. Typically, the enzyme cleaves a glycosyl-donor to form a glycosyl-enzyme intermediate (a), which is normally hydrolysed with water to form the monosaccharide (b). However, if alcohol is present, the alcohol can accept the glycosyl group from the glycosyl-enzyme, leading to formation of a glycoside (d). In addition, at high concentrations of monosaccharide reversal of hydrolysis can occur (c), eventually leading to formation of glycosyl-enzyme and then oligosaccharide (d).

The glycosidases fall into several families of evolutionarily related proteins, which differ in glycone (or sugar) specificity. Several years ago, we screened for novel glycosidase enzymes from Thai plant seeds, and found several interesting enzymes, such as β-glucosidase from *Dalbergia cochinchinesis* Pierre (Thai Rosewood), β-D-galactosidase from *Hibiscus sabdariffa* var *altissima* (Thai Jute), and α-D-mannosidase from *Albizzia procera* Benth. The present grant extends our previous studies on β-glucosidases and α-D-mannosidases from Thai plants, as well as chitinase from bacterial sources. It covers study of the hydrolytic reaction at molecular level and the effects of site directed mutagenesis of residues important to binding and catalysis, as well as exploring the applications of these enzymes in hydrolyse substrates of commercial interest and for synthesis of various compounds. We also collaborate with Professor Atsuo Kimura, University of Hokkaido, Japan on α-glucosidase enzymes from honeybee (1, 24, 37).

1.2 Research on β-Glucosidases

1.2.1 Background information on β-glucosidases

The β-glucosidases (EC 3.2.1.21) are a heterogeneous group of enzymes with various functions, including the hydrolysis of cyanogenic glycosides, cellobiose, saponin glycosides, and naphthoquinone glycosides. β-Glucosidases involved in defense generally produce toxic compounds, such as hydrogen cyanide, saponins, coumarins and naphthoquinones by deglycosylation of their substrates. Other β-glucosidases are thought to regulate plant growth by releasing cytokinin growth factors from their glucosides and to act in stress response. Although these enzymes are closely related in evolution and catalyse the same basic reaction, their substrate specificities are distinct.

The active site of enzymes in this family are well studied. The results show that catalysis is conducted by two conserved glutamic acid residues, one acting as the catalytic acid/base and the other, the catalytic nucleophile. Though many amino acid residues found in the active site pocket are well conserved in plant beta-glucosidases, consistent with their roles in catalysis and glucose binding, the remaining residues show many substitutions, consistent with their adaptation to many divergent functions. So, it is difficult to predict enzyme function from the sequences of these enzymes, and to define the biological function of plant beta-glucosidases, sequence and structural studies of these enzymes must be combined with studies of protein expression, cellular location, and substrate specificity.

We have discovered several novel β-D-glucosidase enzymes from Thai plants, some with unusual substrate specificity. Thus we have characterized the kinetic properties and amino acid sequence of *Dalbergia cochinchinesis* Pierre (Thai Rosewood) β-glucosidase (dalcochinase) and *Dalbergia nigrescens* (Blackwood) β-glucosidase, which have different isoflavonoid glycosides as natural substrates, differing both in the glycone and aglycone moeity. Important food crops also contain β-glucosidase enzymes: thus cassava (*Manihot esculenta* Crantz) contains a β-glucosidase enzyme specific for its cyanogenic β-

glucoside substrate. More recently, James Ketudat-Cairns at Suranaree University of Technology has characterized a β-glucosidase enzyme from rice (*Oryza sativa)*, which hydrolyzes cello-oligosaccharides.

Thus, different plant species may have different enzyme-substrate combinations, which can be envisaged as being metabolic units (or metabolomes), that fulfil their respective functions in each plant. In addition to this difference in substrate specificity, different enzymes have may differ in the catalytic activity and specificity for the reverse hydrolysis and transglucosylation reactions. So they serve as excellent models for studying structure-function relationships in proteins, which is a major objective of this grant. The research groups of Drs. Jisnuson Svasti, James Ketudat-Cairns, and Prachumporn Kongsaeree, aim not only to understand these structure-function relationships, but also to explore the potential applications of these enzymes in hydrolysis and glycoside synthesis.

1.2.2 Structure and mechanism of Thai rosewood, blackwood and cassava β-glucosidases

Prachumporn Kongsaeree's group at Kasetsart University have tried to identify the amino acid residues in the aglycone binding pocket that are responsible for the differences in substrate specificity and transglucosylation reaction between beta-glucosidases from Thai rosewood and cassava (*Manihot esculenta* Crantz). Apart from differences in their natural substrates, Thai rosewood beta-glucosidase can perform reverse hydrolysis well, but cannot catalyse transglucosylation to tertiary alcohol acceptors, while cassava beta-glucosidase has high effeciency in catalyzing transglucosylation using primary, secondary and tertiary alcohols as acceptors, but is poor in catalyzing reverse hydrolysis. First, a good expression system had to be devised for *D. cochinchinensis* enzyme, involving attachment the N-terminal His-tag directly to an N-terminally truncated enzyme, allowing its purification from *Pichia* media after expression (19). Ten single mutations were made to replace amino acid residues in the aglycone binding pocket of Thai rosewood beta-glucosidase with the corresponding residues of cassava beta-glucosidase. Hydrolytic efficiency toward pNP-glucoside was greatly improved in some mutants, but drastically reduced in others. However, none of the mutants could hydrolyze linamarin, the natural substrate of cassava beta-glucosidase. Some mutant enzymes showed improved transglucosylation activities to long-chain primary alcohol and secondary alcohol acceptors, but none could catalyze the transfer of glucose to tertiary alcohol acceptor. Thus, mutations at more than one position may generate new dalcochinase mutants which show greater similarity in function to linamarase.

Characterization of natural substrates for the *D. nigrescens* β-glucosidase showed that the two most readily identified substrates in *D. nigrescens* seeds were isoflavonoid-7-O-β-D-apiofuranosyl-6-O-β-D-glucopyranosides (7), from which the enzyme removed

the disaccharide, 6-O-β-D-apiosyl-D-glucose (also called acuminose), indicating the enzyme can act as a diglycosidase and resulting in the designation of a new E.C. number to describe this activity, 3.2.1.161. Thai Rosewood dalcochinase also has low acitivity for the *D. nigrescens* substrates, but is much less efficient than the *D. nigrescens* enzyme and much more efficient at hydrolyzing its natural rotenoid β-glucoside substrate, dalcochinin β-D-glucoside (30). James Ketudat-Cairns's group was interested to see if Thai rosewood dalcochinase enzyme could be mutated to be more effective for the *D. nigrescens* substrates. Inspection of the amino acid residues previously described to be important for substrate recognition and molecular modeling suggested that the two residues in the active site differing between Dnbglu2, the recombinantly expressed *D. nigrescens* enzyme, and dalcochinase were residues A454/S454 (dalcochinase/Dnbglu2) and E455/G455. When either of these residues was mutated from dalcochinase to Dnbglu2 sequence individually, little change was seen. However, when the double mutant was made, the ability to hydrolyze isoflavonoid apiosylglucosides was increased 4-7 fold, suggesting the two residues were acting cooperatively in allowing productive binding of these substrates, though little effect was seen on the hydrolysis of dalcochinin β-glucoside and p-nitrophenyl glycosides (39). On the other hand, the activity toward the isoflavonoid diglycosides was still 200-fold less than the Dnbglu2 enzyme, indicating other factors are important for productive binding and hydrolysis of these substrates.

1.2.3 Potential applications of *D. nigrescens* β-glucosidase in the food industry

It occurred to Dr. Prachumporn and Dr. James that the *Dalbergia* enzymes might be of use in hydrolysis of soybean isoflavonoid glycosides to release the antioxidant, phytoestrogen isoflavonoids daidzin, genistin and glycitin. This could increase the functional nutrition of soy foods by providing these compounds, which are thought to be anti-carcinogenic in their more readily absorbed aglycone form. Thus the relative activities of the two *Dalbergia* enzymes towards these substrates in crude soy flour extracts was investigated (30). The initial results indicated that *D. nigrescens* was more efficient in hydrolyzing these substrates, consistent with its natural substrates being structurally similar to the soy isoflavone glycosides, so it was further studied for potential application to soy products. These studies showed that the enzyme could hydrolyze these glycosides in soy flour suspended in aqueous solution - even the glycosides associated with the flour particles (30). This indicates the *D. nigrescens* β-glucosidase could be used for soy food processing to improve its phytoestrogen availability. Thus, starting from a basic question about functional and structural differences in related enzymes, an enzyme could be identified with potential application to the food industry.

1.2.4 Rice β-glycosidases

Dr. James Ketudat-Cairns has a major research interest in rice glycosidases, since rice is the most important crop for Thailand. His group have cloned and expressed the protein for a rice glycosyl hydrolase family 1 (GH1) β-glucosidase gene highly expressed in flowers and seedling shoots, *BGlu1*, also designated *Os3Bglu7* (Opassiri et al., 2003; 2006). The protein had high activity toward laminaribiose and cello-oligosaccharides with degrees of polymerization (DP) of 3 or more, with at least 6 binding sites for β-1,4-linked glucosyl residues. The enzyme also had high transglycosylation activity for oligosaccharides and pyridoxine. Rice BGlu1 protein is at around 67% identical to barley BGQ60/β-II β-glucosidase.

BGlu1 (*Os3BGlu7*) was just one of 39 GH1 genes discovered in japonica and indica rice genome databases, 34 of which look likely to be active and may produce β-glucosidases or related enzymes (28). These genes have been annotated for the TIGR annotated rice genome database, and the gene analysis published. Rice GH1 genes could be seen to fall into 8 groups that had members in both rice and *Arabidopsis*. We have endeavored to express proteins for representative members of the 8 groups, plus all the proteins in the rice BGlu1/barley BGQ60/β-mannosidase group. To date, members of four of the groups have been successfully expressed, while 4 out of 5 of the BGlu1-related group have been expressed, in addition to barley BGQ60. The activities of the recombinantly expressed rice proteins have been characterized against available natural and artificial substrates, and in some cases, plant extracts. Os4BGl12, which is wounding induced, was also found to hydrolyze the oligosaccharides released from rice cell wall extracts by a wounding induced endoglucanase.

A similar analysis was performed with GH family 35 (GH35) in rice, resulting in 15 genes, conserved between indica and japonica subspecies. RT-PCR showed that found that many genes were expressed in multiple tissues, but differed in expression patterns, with some genes predominantly expressed in vegetative and others in reproductive tissues. Several proteins were expressed in *E. coli* and two, Osbgal1 and Osbgal2, were characterized for activity toward natural and artificial substrates. Both enzymes were specific for β-galactoside, but had no activity toward other *p*NP-glycosides. Osbgal1 was purified and found to hydrolyze β-1,3-, 1,4- and 1,6-linked galactooligosaccharides and slowly released galactose from rice cell wall alcohol insoluble fraction. In addition, specific anti-peptide antibodies for these proteins stained the embryo and emerging shoot and root in germinating seeds and seedlings, especially in rapidly growing areas, although the staining patterns were unique for the two isozymes.

A GH family 5 (GH5) exoglucanase was also characterized with similar activity, designated as GH5BG (41). This enzyme comes from a unique subgroup or subfamily of GH5, which has a fascin domain inserted after the first beta-strand of the catalytic domain β-barrel, and has greatly diminished an extended loop seen to act in substrate binding in fungal β-1,3-exoglucanases.

The work done has provided a greater understanding of carbohydrate metabolism in rice, and shown how complex this is, involving several enzymes, many of which have yet to be characterized. It has also yielded enzymes with potential for biomass conversion. Since the cell walls of plants represent a major source of biomass on earth, releasing sugars from these polymers for use in ethanol or polymer production is of keen interest. It is hoped that some of the enzymes identified will be useful for the conversion, in guiding the engineering of other enzymes for biomass conversion, or for genetic manipulation to generate rice plants more amenable to biomass conversion.

1.2.5 Three dimensional structure of rice β-glucosidase *BGlu1*

Dr. James Ketudat-Cairns' group has determined the three-dimensional structure of rice BGlu1 β-glucosidase and its covalent intermediate with 2-deoxy-2-fluoroglucoside at 2.2 and 1.55 Å resolution respectively (20, 42). The structures (Figure 3) were similar to previous glycosyl hydrolase family 1 (GH1) β-glucosidases, but showed several differences in the loops around the active site, which lead to an open active site with a narrow slot at the bottom, compatible with the hydrolysis of long β-1,4-linked oligosaccharides. Though this shape is somewhat similar to that of the *Paenibacillus polymyxa* β-glucosidase B, which hydrolyzes similar oligosaccharides, molecular docking studies indicate that the residues interacting with the substrate beyond the conserved -1 site are completely different, reflecting the independent evolution of plant and microbial GH1 exo-β-glucanase/β-glucosidases. The complex with the 2-fluoroglucoside included a glycerol molecule, which appears to be positioned to make a nucleophilic attack on the anomeric carbon in a transglycosylation reaction. The coordination of the hydroxyl groups suggests sugars are positioned as acceptors for transglycosylation by their interactions with E176, the catalytic acid/base, and Tyr131, which is conserved in barley BGQ60/β-II β-glucosidase, which has similar oligosaccharide hydrolysis and transglycosylation activity to rice BGlu1. As the rice and barley enzymes have different preferences for cellobiose and cellotriose, residues that appeared to interact with docked oligosaccharides were mutated to those of the barley enzyme to see if the relative activities of rice BGlu1 toward these substrates could be changed to those of BGQ60. Though no single residue appeared to be responsible for these differences, I179, N190 and N245 did appear to interact with the substrates and play a role in catalysis.

1.2.6 Development of rice glycosynthase for oligosaccharide synthesis

James Ketudat-Cairns has identified the catalytic acid/base and catalytic nucleophile residues by mutation studies. Mutation of E176 to Q, D or A caused decreases in hydrolysis of *p*NP-β-glucoside of 50-, 180- and 360-fold, respectively, while mutations of E386 to D and Q caused decreases of 3,000- and 60,000-fold, respectively. Further mutagenesis of the catalytic nucleophile to A, S and G was performed to produce a glycosynthase enzyme (34). Jisnuson Svasti's group showed that the E386A, E386S and E386G mutants had activity too low for detection, but they could be rescued by formate and azide, which resulted in the alpha-azo-glucoside product expected for

these mutations. In addition, if α-F-glucoside was used as a donor substrate, the glucose could be transferred to *p*NP-cellobioside or cellotriose to form long chains of β-1,4-linked glucosyl residues of up to 11 residues, which is unusually long for a glycosynthase derived from an exoglycosidase. The E386G mutant was the most rapid for synthesizing these products and the lengths of the major products could be manipulated by varying the relative amounts of donor and acceptor substrates. This suggests that this glycosynthase enzyme could be useful for producing cellooligosaccharides with labels (ie *p*NP) on their non-reducing ends for the study of cell wall transfer reactions or perhaps for incorporation in specialized paper products.

1.2.7 Transglucosylation of alcohols by cassava linamarase

We have shown that cassava β-glucosidase is more effective in transglucosylation of secondary and tertiary alcohols than any other enzyme described (15). Thus, Dr. Palangpon Kongsaeree at Mahidol University is exploring the use of the enzyme for transglucosylating chiral alcohols. Five racemic chiral alcohols, including 2-butanol, 3-methyl-2-butanol, 1-phenylethanol, 1-phenyl-2-propanol, and 4-phenyl-2-butanol, were used to see whether the enzyme exhibited enantioselective properties. The chemical structures of β-glucoside products were elucidated by ^{1}H-NMR and high-resolution mass spectrometry. The stereochemical configuration at the anomeric carbon of isolated glucoside products were established to be β-glucoside form and the % de values of the reaction product mixtures were established based on the difference of the ^{1}H-NMR signals of the CH_3 group next to the carbinol carbon of the aglycone moiety.

In all reactions, the *R*-enantiomer of each alcohols was selectively recognized by linamarase to react with the oxonium glucose intermediate from *p*-nitrophenyl-β-D-glucopyranoside (D-Glcβ-O-pNP), yielding the (*R*)-alcoholic glucosides with diastereomeric excess (% de) as high as 91% for 1-phenylethanol. The enantioselectivity (*E*) of the linamarase-catalyzed reaction was also determined, where the *E* value indicates the efficiency of the enzyme to utilize one enantiomeric substrate over the other isomer. In the best case of 1-phenyl-1-ethanol, the *E*-value of 30 was observed with the conversion of about 30% analyzed by gas-chromatography-mass spectrometer (GC-MS).

However, isolated yields are still low, since the presence of water can result in hydrolysis of the alkyl glucoside product. Thus, a novel approach is being tried by using ionic liquids as solvent. Ionic liquids, such as $[BMIM]^{+}[PF6]^{-}$ and $[BMIM]^{+}[BF4]^{-}$ are ionic compounds that are liquid at room temperature while most ionic compounds are solid. In recent years, ionic liquids have received a significant research interest in industry as "green solvents" since they can be recycled and have low vapor pressure. The possibility of immobilizing cassava linamarase on a solid support is also being investigated to improve reuse. If such strategies are successful, cassava linamarase should become a valuable as stereospecific biocatalyst, helping to add value to cassava.

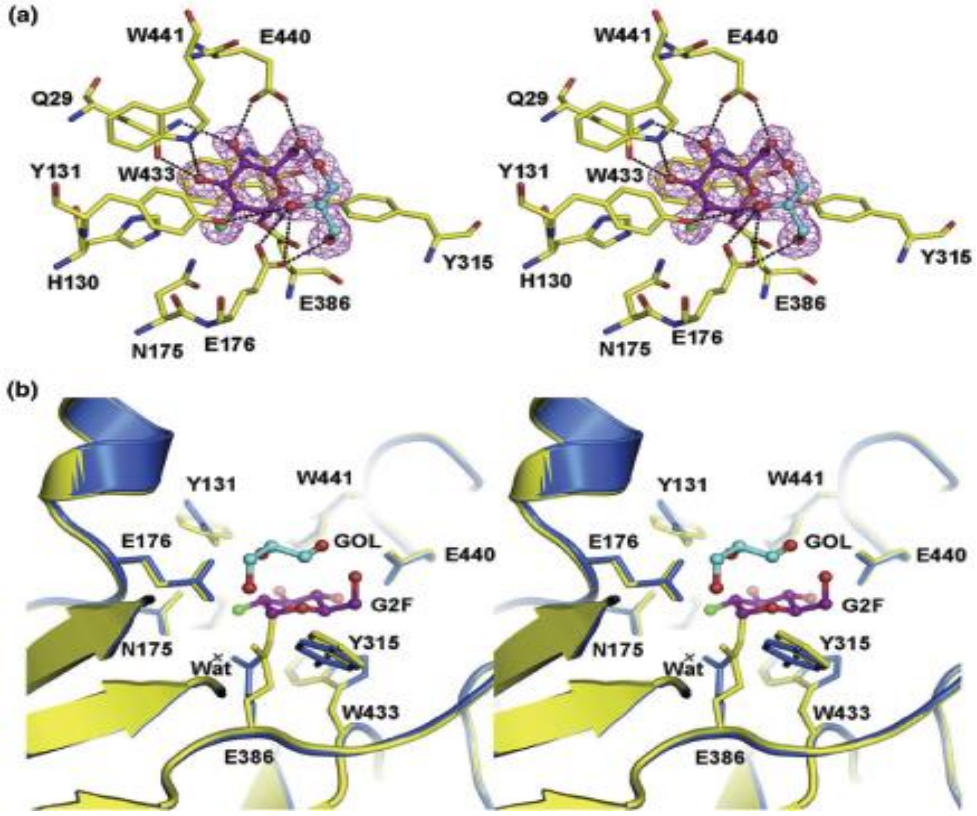


Figure 3. Stereoview of the active site of rice BGlu1. (a) Protein–ligand interactions in the active site of the BGlu1–G2F complex. The amino acid residues surrounding G2F are presented in stick representation, with carbon yellow, nitrogen blue, oxygen red, and fluoride green. G2F and glycerol (GOL) are drawn in ball and stick representation in the same colors, except carbon atoms are purple and cyan, respectively. Hydrogen bonds between the protein and the glycone at subsite –1 and glycerol at subsite +1 are drawn as broken black lines. (b) Superimposition of subsite –1 of the active site of free rice BGlu1 (blue) and covalently bound G2F complex (yellow). The covalently bound G2F and solvent glycerol from the G2F complex are drawn as ball and stick with carbon colored purple and cyan, respectively. The water A1755 from the G2F complex (Wat), which was hydrogen bonded with E386 (the catalytic nucleophile) and N175, is displayed as a cross.

[From Chuenchor, W. et al., Structural Insights into Rice BGlu1 β-Glucosidase Oligosaccharide Hydrolysis and Transglycosylation, J. Mol. Biol. (2008), doi:10.1016/j.jmb.2008.01.076]

1.2.8 Isolation of Novel β-Glucosidases and β-Glucosides from Thai Plants

Professor Jisnuson Svasti and Dr. Dumrongkiet Arthan from the Faculty of Tropical Medicine, Mahidol University have also characterized other enzyme-substrate combinations in other plants: thus *Solanum torvum* has a β-glucosidase enzyme in the leaves (16), which is specific for a steroidal β-glucoside (torvoside) in the fruit, while *Plumeria obtusa* flowers contain both the plumieride coumarate glucoside substrate and its specific β-glucosidase enzyme. Another β-glucosidases which can hydrolyse iridoid glucosides, a group of glucosyl-linked monoterpenoids, is being purified from *Ligustrum confusum* leaves, which could hydrolyze its natural substrate, an iridoid β-glucoside, identified to be a known secoiridoid β-glucoside, namely ligustroloside A.

The steroidal β-glucosidase (torvosidase) was purified to homogeneity from the young leaves of *Solanum torvum* (16). The enzyme was highly specific for cleavage of the glucose unit attached to the C-26 hydroxyl of furostanol glycosides from the same plant, namely torvosides A and H. Purified torvosidase is a monomeric glycoprotein, with a native molecular weight of 87 kDa by gel filtration and a pI of 8.8 by native agarose IEF. Optimum pH of the enzyme for p-nitrophenyl-β-glucoside and torvoside H was 5.0. Kinetic studies showed that K_m values for torvoside A (0.063 mM) and torvoside H (0.068 mM) were much lower than those for synthetic substrates, pNP-β-glucoside (1.03 mM) and 4-methylumbelliferyl-β-glucoside (0.78 mM). The enzyme showed strict specificity for the β-D-glucosyl bond when tested for glycone specificity. Torvosidase hydrolyses only torvosides and dalcochinin-8'-β-glucoside, which is the natural substrate of Thai rosewood b-glucosidase, but does not hydrolyse other natural substrates of the GH1 β-glucosidases or of the GH3 β-glucosidase families. Torvosidase also hydrolyses C5–C10 alkyl-β-glucosides, with a rate of hydrolysis increasing with longer alkyl chain length. The internal peptide sequence of Solanum β-glucosidase shows high similarity to the sequences of family GH3 glycosyl hydrolases.

The iridoid β-glucoside, namely plumieride coumarate glucoside, was isolated from the *Plumeria obtusa* (white frangipani) flower. A β-glucosidase, purified to homogeneity from *P. obtusa*, could hydrolyze plumieride coumarate glucoside to its corresponding 13-O-coumarylplumieride. *Plumeria* β-glucosidase (22) is a monomeric glycoprotein with a molecular weight of 60.6 kDa and an isoelectric point of 4.90. The purified β-glucosidase had an optimum pH of 5.5 for p-nitrophenol (pNP)-β-*D*-glucoside and for its natural substrate.The K_m values for pNP-β-*D*-glucoside and *Plumeria* β-glucoside were 5.04±0.36 mM and 1.02±0.06 mM, respectively. The enzyme had higher hydrolytic activity towards pNP-β-*D*-fucoside than pNP-β-*D*-glucoside.No activity was found for other pNP-glycosides. Interestingly, the enzyme showed a high specificity for the glucosyl group attached to the C-7" position of the coumaryl moiety of plumieride coumarate glucoside. The enzyme showed poor hydrolysis of 4-methylumbelliferyl-β-glucoside and esculin, and did not hydrolyze alkyl-β-glucosides, glucobioses, cyanogenic-β-glucosides, steroid β-glucosides, nor other iridoid -glucosides. In conclusion, the *Plumeria* β-glucosidase shows high specificity for its natural substrate, plumieride coumarate glucoside.

In addition, the biological activities of the glycoside and its aglycone derivatives were tested against *Trichomonas vaginalis*, in comparison to other glycosides in our laboratory (46). Trichomoniasis is now an important health problem in developing countries. Although metronidazole is being used to treat this disease, metronidazole-resistant protozoa have been found, as well as unpleasant adverse effects. The results showed that compared to metronidazole, which has a minimum inhibitory concentrations (MIC) of 0.05 μM against *Trichomonas vaginalis*, several glycosides (such gonocaryoside, kingiside, torvoside A) had MIC of 6.25 μM, while others (ligustraloside A and dalcochinin-8′-O-β-glucoside) had MIC of 12.5 μM. Although the MIC values for our glycosides was 10-20 fold worse than the standard drug metronidazole, it may be worthwhile to screen isoflavonoid, iridoid and steroid glycosides for anti-trichomonal activity.

1.3 α-Mannosidases from Thai Plants

α-Mannosidases (EC.3.2.24) are glycoside hydrolases involved in the cleavage of α-glycosidic bonds to nonreducing mannosyl residues. They are involved in the maturation and the degradation of Asn-linked oligosaccharides in all eukaryotes, and are traditionally grouped into two classes (I and II) based on functional characteristics, sequence homology and their cellular compartmentation. Class I α-mannosidases, belong to family 47 of glycosyl hydrolases, are involved in the maturation of Asn-linked oligosaccharides by trimming of $Man_9GlcNAc_2$ and only hydrolyse α-1,2 mannose bonds. Class II α-mannosidases catalyse the degradation of Asn-linked oligosaccharides, and tend to be less specific, possessing α-1,3, α-1,6, and α-1,2 hydrolytic activity. α-Mannosidases can be used in carbohydrate bioengineering by using their specific hydrolytic activity and capability for synthesising mannose-containing oligosaccharides.

Dr. Sujint Anguravirutt at Mahasarakham University has screened for α-mannosidase from Thai plant seeds, and although several species had α-mannosidase, the species with the highest level of activity was *Albizzia procera* Benth. The α-mannosidase from *Albizzia procera* Benth has now been purified to 95% purity, using the following techniques: 35-75% ammonium sulfate precipitation, followed by ANX Sepharose 4 Fast Flow-ion-exchange chromatography, Sephacryl S-300 HR gel filtration chromatography, and hydrophobic interaction chromatography using Butyl Sepharose 4 Fast Flow. The enzyme had MW of about 320 kDa, and showed maximal activity at pH 4.0 and 60 ^{0}C. It showed highest activity for *p*-NP-α-D-mannoside, and had K_m of 1.2 mM for *p*-NP-α-D-mannoside. The enzyme can also be used for transmannosylation of alcohols using *p*-NP-α-D-mannoside as the mannosyl-donor, and can catalyze synthesis of oligosaccharides by reverse hydrolysis.

1.4 Study of the 3D-structure and the substrate specificity of the active site residues of chitinase A from *Vibrio carchariae*

Dr. Wipa Suginta at Suranaree University of Technology is interested in the potential use of chitin, a fibrous polysaccharide widely distributed in the shells of crustaceans, the cuticles of insects, the shells and skeletons of mollusks, and the cell walls of fungi. Chitin degradation is of considerable interest because its degradation products have important potential applications in the fields of biomedicine, agriculture, nutrition, and biotechnology. However, chitin is a highly stable homopolysaccharide of β(1→4)-linked *N*-acetyl-*D*-glucosamine (GlcNAc or NAG). Several strategies have been developed for converting chitin into small soluble derivatives, but enzymatic degradation using chitinases (EC 3.2.1.14) seems to be the method of choice since the reaction occurs quickly and completely under mild conditions without causing environmental pollution, and the type, quantity and quality of oligomeric products can be controlled.

The marine bacterium *V. carchariae* produces high levels of chitinases A precursor, which can be cleaved to a 63-kDa mature enzyme that can be cloned and functionally expressed in *Escherichia coli*. This enzyme is a member of GH family 18 and acts as an endochitinase, showing greater affinity towards higher M_r chito-oligosaccharides, suggesting that the substrate-binding cleft probably comprises of six GlcNAc-binding sites (10). Mutation of the catalytic residue Glu315 to Met/Gln leads to loss of activity, indicating that this residue is essential. To understand the mechanism of chitinase, the three dimensional (3D) structures of the wild-type chitinase A and its catalytically inactive mutant E315M were studied in the presence and absence of chito-oligosaccharide substrates.

The overall structure of *V. carchariae* chitinase A comprises three domains, a) the *N*-terminal chitin-binding domain (residues 22-138), consisting mainly of β-strands and connecting through a hinge region (residues 139-159), b) the main β/α barrel catalytic domain (residues 160-460 and 548-588), c) the α+β small insertion domain (residues 461-547). The structures of E315M-NAG6 complex show that the sugar substrates are firmly embedded within the long, deep substrate binding groove, and that the substrate binding site of comprises many conserved aromatic residues stacking against the heterocyclic rings of the sugars extending from subsite -4 to subsite +2. These residues include Tyr164, Trp168, Tyr171, Phe192, Trp275, Phe316, Tyr391, Trp397, Tyr435, Trp461, Trp497 and Trp570.

Site-directed mutagenesis of these binding residues resulted in the change of cleavage patterns towards G3-G6 substrates (33). Thus mutation of Trp168 to Gly enhanced the transglycosylation of the enzyme. Mutation of Tyr171 caused less effect on the hydrolytic activity, since its location near to the edge has less on the bound sugar. Mutation of Trp570 caused a severe loss in the hydrolytic activity, since this residue interacts with the most crucial NAG (-1 NAG). Mutation of Trp275 to Gly led to an equal access to the second and the third bonds of G6, indicating that this residue is important for the binding selectivity around the cleavage site. Mutation of Trp397 to Phe entirely changed the cleavage pattern by releasing a series of smaller chito-oligosaccharide intermediates from particularly G5 and G6 substrates, indicating that Trp397 helps to define the primary binding subsite for the incoming sugar. Such

data help us to understand the role of the different amino acids in binding and catalysis, opening up the possibility of engineering the chitinase enzyme to increase catalytic activity

2. Enzymes Involved in Synthesis of Penicillin Derivatives

The β-lactam antibiotics are a very important class of antibiotics, which inhibit bacterial cell wall synthesis, and are frequently used to treat bacterial infections. These include the penicillins, based on the two-ringed structure of 6-aminopenicillinic acid (6-APA), and the cephalosporins, based on the two-ringed 7-aminocephalosporanic acid (7-ACA) structure (Figure 4a). These core structures are typically produced by cleavage of benzyl-penicillin (also called penicillin G) and cephalosporin C respectively. Part of the success of the β-lactam antibiotics has been due to the ability of synthesise derivatives of the penicillin and cephalosporin structures having greater potency from the core 6-APA and 7-ACA structures. Since such antibiotics are typically imported from overseas, the ability to synthesise these derivatives locally would lead to considerable cost savings. The use of enzymes for various steps in the synthesis of penicillin and cephalosporin derivatives provides an important alternative for chemical synthesis, due to the high specificity and moderate conditions of enzyme reactions. Thus the enzymes to be studied here are not only of potential value in terms of applications, but will provide interesting lessons in understanding protein structure and function relationships. Two projects are being undertaken on penicillin synthetic enzymes in the Centre for Protein Structure and Function at the Faculty of Science, Mahidol University.

2.1 Conversion of Bacterial Penicillin G Acylase to Cephalosporin C Acylase by Protein Engineering

Currently, 7ACA is produced from cephalosporin C (CC) through a multi-step biochemical process, leading to the high cost of such semi-synthetic cephalosporins. Thus, a single-step enzymatic conversion of CC to 7ACA using cephalosporin C acylase (CCA) is of great interest in terms of cost effectiveness and environmental safety (Figure 4a). Unfortunately, all identified CCAs from various sources have very low activity with respect to CC. On the other hand, a similar conversion of penicillin G to 6-APA can be efficiently produced using the enzyme penicillin G acylase (PGA, EC 3.5.1.11). The aim of Dr. Jirundon Yuvaniyama is therefore to use protein engineering to mutate PGA to have the catalytic properties of CCA.

In the first instance, it is necessary to know the three-dimensional structure and active site residues in PGA. Thus, *Bacillus megaterium* PGA (Bm-PGA) has been purified, crystallized and its structure studied by X-ray diffraction. The crystal structure, which is now in the final stage of refinement, provides information about the folding, as well as the active site. Based on the initial partially refined structure at 2.6 Å resolution, two mutants were designed, aiming to alter the substrate specificity: PGA-M1 and PGA-M2, which contain three and four mutated amino-acid residues respectively. The mutant PGA-M1 enzyme could be expressed and purified, and gave similar but not identical circular dichroism (CD) spectra to wildtype enzyme, suggesting a similar composition of secondary structure. Crystallization of both proteins yielded some single crystals under slightly different conditions.

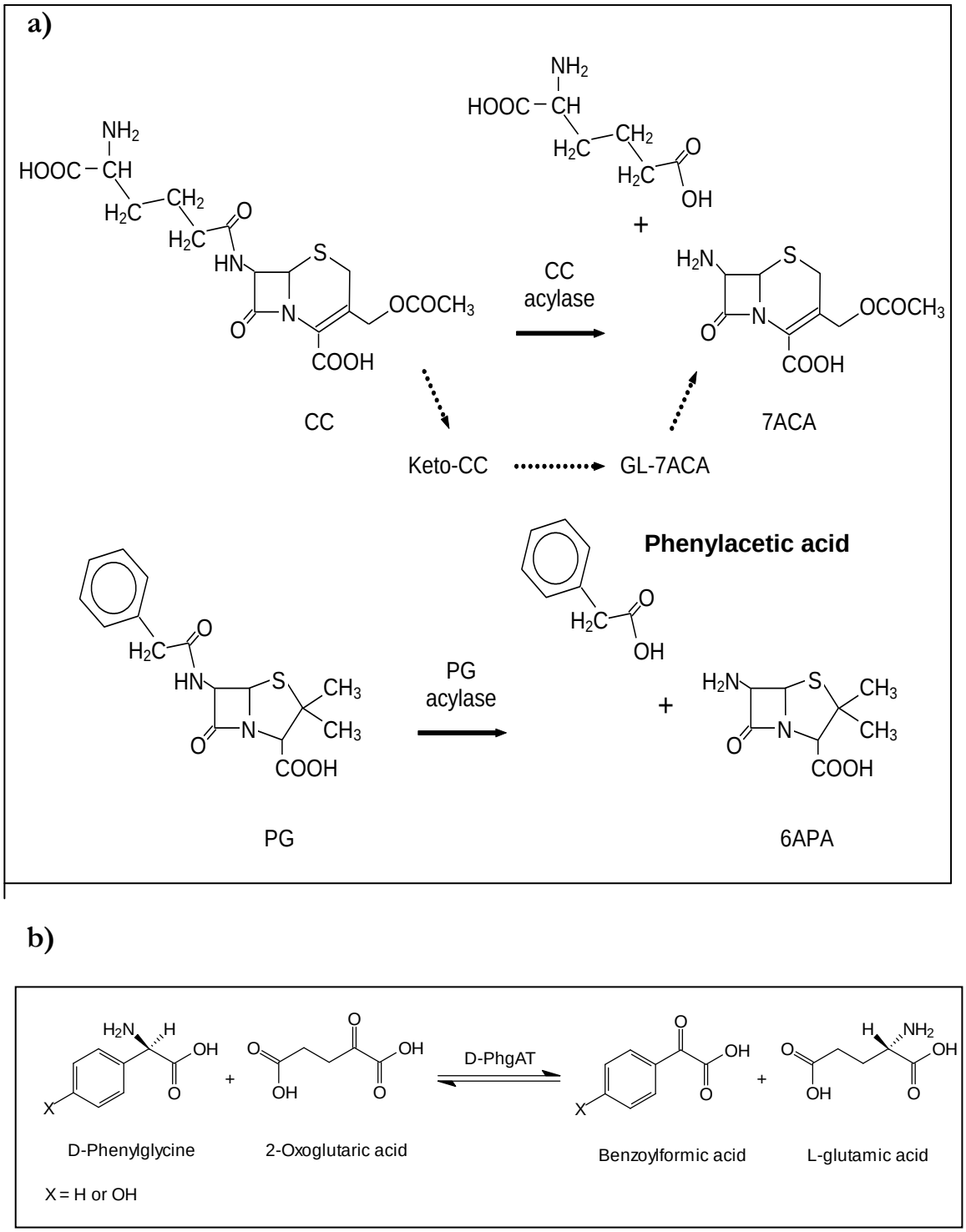


Figure 4: Enzymatic reactions related to synthesis of penicillin derivatives. a) Deamidation of cephalosporin C (CC) to 7-aminocephalosporanic acid (7ACA) and penicillin G (PG) to 6-aminopenicillanic acid (6APA). Dashed arrows show an alternative pathway of CC hydrolysis. **b)** Conversion of benzoylformic acid to D-phenylglycine, catalysed by D-phenylglycine aminotransferase. Benzoylformate may be formed from phenylacetic acid produced in the deamidation of PG.

X-ray diffraction data were collected from PGA-M1 at the National Synchrotron Radiation Research Center (NSRRC), Taiwan to the resolution of 1.92 Å resolution. This new three-dimensional structure of PGA-M1, which is now at the final stage of refinement at 2.6 Å resolution and has the same overall folding as the wildtype enzyme. Thus, replacement of nonpolar amino acids in the active site did not interfere with folding, and the mutated residues were placed at the designated positions as planned. As the Pm-PGA and the PGA-M1 structures become more accurate, two new mutants (PGA-M3 and PGA-M4) were designed, which are expected to show improved properties with the desired CCA activity. This remains to be proven by further enzyme characterization.

2.2 3-Dimensional Structure of *Pseudomonas stutzeri* D-Phenylglycine Aminotransferase

Industrial scale production of 6-APA by enzymatic hydrolysis of penicillin G produces a large amount of phenylacetic acid (Figure 4a). So it would be useful to convert the by-product phenylacetic acid into more useful structurally related compounds, such as D-α-phenylglycine and D-hydroxy-α-phenylglycine. These two compounds are useful as precursors for production of many semi-synthetic penicillins and cephalosporins, D-α-phenylglycine for synthesis of ampicillin, cephalexin, and D-hydroxy-α-phenylglycine for amoxycillin synthesis. Synthesis of D-phenylglycine from phenylacetic acid requires three steps, oxidation at the α-carbon to form an α-hydroxy acid (mandelic acid) and then a second oxidation at the α-carbon to convert mandelic acid to a 2-oxo acid (benzoylformic acid). In the third step, the benzoylformic acid produced is then converted to D-α-phenylglycine by a stereospecific transamination (Figure 4b), catalysed by the enzyme D-phenylglycine aminotransferase.

D-phenylglycine aminotransferase (D-PhgAT) from the soil bacteria *Pseudomonas stutzeri* ST-201 is a dimeric protein of 92 kDa, containing the coenzyme pyridoxal phosphate. It uses D-phenylglycine or D-4-hydroxy-phenylglycine as amino group donor and converts 2-oxoglutarate, the amino group acceptor, to L-glutamic acid. Thus the enzyme may be considered as causing stereoinversion, changing D-phenylglycine to L-glutamate. This is very unusual since most amino acid aminotransferases retain the configuration of the amino acid, and have been used for enantioselective synthesis of L- and D-amino acids from α-ketoacids. Thus, the mechanism of stereoinversion in *Pseudomonas stutzeri* D-PhgAT is of additional interest.

Dr. Palangpon Kongsaeree's laboratory, in collaboration with Prof. Vithaya Meevootisom, have expressed recombinant D-PhgAT in *E. coli* BL21 (DE3) at a level of 20% of total protein.The enzyme was purified using ammonium sulfate fractionation, phenyl-agarose chromatography, and DEAE-cellulose chromatography, and suitable conditions for crystallisation were found. Initial data collection on crystals of native D-PhgAT in cryoprotectant (10-30% glycerol) was performed at the National Synchotron Light Source, Brookhaven, New York, U.S.A. Attempts to solve the crystal structure of D-PhgAT, by using the modified X-ray crystal structure coordinates of glutamate-1-semialdehye aminomutase as a model, yielded over

90% of the structure, but the active site region could not be finished, since the data showed some degrees of disorder in the crystal structure.

Much effort has been made to improve the resolution limit of native crystal by growing large crystals and analyzing the freshly-prepared crystals at the Center for Excellence in Protein Structure and Function. The best resolution obtained was about 2.0-2.1 Å, slightly higher than the original synchrotron data set. The structure of D-PhgAT complexed with its inhibitor/co-factor/substrate was also started, by soaking the native crystal of D-PhgAT and PLP complex in a stabilizing solution containing gabaculine, an aminotransferase inhibitor. Several X-ray data sets of native D-PhgAT crystals and its complex crystals were measured, including several higher resolution data sets at the Spring-8 synchrotron on the beam line BL442B in Japan.

To obtain the structure of external aldimine complex between PLP-cofactor and the amino group donor, D-PhgAT crystals were gradually transferred to higher concentration of ammonium sulphate solutions containing D-phenylglycine or D-4-hydroxy-phenylglycine substrates. In a separate experiment, $NaBH_4$ was added to stabilizing solution to reduce double bond of internal aldimine between the catalytic lysine-269 in the active site of enzyme and cofactor to a single bond. Several high X-ray data sets were taken at X-ray diffraction system at beamline 13B1, NSRRC, Taiwan. The complex structures were solved at 1.9-2.2 Å resolution, and, for the first time, the electron density map around the active site of enzyme was clearly observed and proposed to involve the phosphate group of the cofactor. The results are promising, and it is hoped that the complete three-dimensional structures of the enzyme in several forms will shed light on the stereo-inverting mechanism of D-phenylglycine aminotransferase from the soil bacteria *Pseudomnas atutzeri* ST-201 in the near future.

3. Flavin-Containing Oxygenase Enzymes: Bacterial Luciferase

Dr. Pimchai Chaiyen's laboratory at the Department of Biochemistry, Faculty of Science, Mahidol University focuses on studying the mechanistic enzymology of some selected oxygenase enzymes, especially those having flavins as cofactors. This investigation uses a variety of techniques and approaches, including spectroscopic techniques and kinetics. Indeed her laboratory is the only one in Thailand, to use stopped flow spectrophotometry for making absorbance and fluorescence measurements in milliseconds after the start of the reaction, allowing her to study pre-steady state kinetics and observe changes in the reaction intermediates.

Her work mainly involves the flavoprotein oxygenase enzymes, which are present in all aerobic organisms and are not only crucial for endogenous metabolic pathways, but also participate in the degradation of xenobiotic compounds (2, 4, 8, 21, 29, 35). Flavoprotein oxygenases degrade many aromatic compounds in soil, so they may be useful for environmental remediation. This report describes work on her recent interest, the enzyme luciferase, which is involved in the biological emission of light from many organisms, such as fireflies. The reaction of luciferase typically involves the reaction of oxygen with $FMNH_2$ (or $FMNH^-$) and an aldehyde to form oxidized FMN and a carboxylic acid (Figure 5). The $FMNH_2$ is produced by a flavin reductase (Fre) that derives reducing equivalents from NADH.

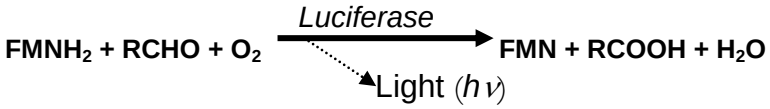


Figure 5A. The reaction of bacterial luciferase.

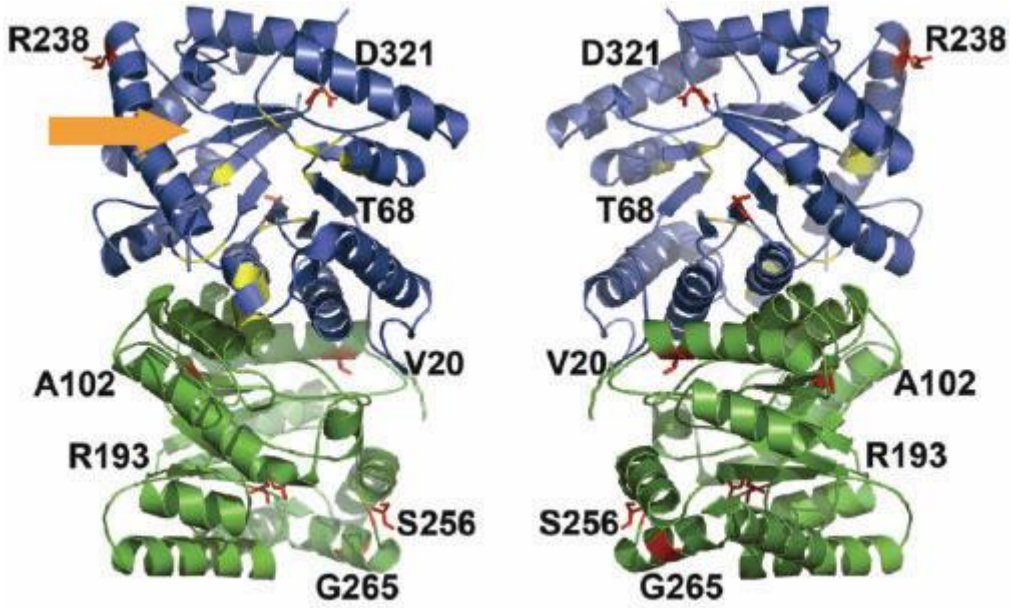


Figure 5b. The structure of luciferase from V. harveyi (1LUC) with locations of the amino acid residue differences in Lux_Vc. The structure of Lux_Vh was generated based on the X-ray structure of Lux_Vh at 1.5A resolution in the Brookhaven Protein Data Bank (PDB entry 1LUC). The "**a**" subunit is shown in blue and the "**b**" subunit in green. The arrow indicates the proposed active site of the enzyme, the C-terminal part of the TIM barrel of the "**a**" subunit. The left picture shows the active site while the right picture was obtained from 1808 anti-clockwise rotation of the left. The eight amino acid differences in Lux_Vc are represented in red. The three amino acid differences on the "**a**" subunit are 68TVh/KVc, 238RVh/KVc, and 321DVh/NVc. The five amino acid differences on the "**b**" subunit are 20VVh/IVc, 102AVh/VVc, 193RVh/KVc, 256SVh/PVc, 265GVh/VVc. Yellow colour indicates the positions of amino acids that have been identified from site-directed mutagenesis studies (18, 26, 40–46, 48–49) and a model of the Lux_Vh-flavin complex as important for luciferase reaction and FMNH_ binding.

[From: Suadee (2007) J. Biochem. 142, 539–552]

Luciferase has long used for many important applications, ranging from very sensitive visualization of gene expression to analytical measurements of drugs, metabolites, and environmental pollutants. Understanding how luciferase works will not only provide interesting basic information, but will also help maximize its potential applications. Since previous studies by other groups have been unable to answer some of the mechanistic questions, Dr. Pimchai's group tried to isolate and characterize luciferase from a novel microorganism. Several bacteria have luminous characteristics, and proteins and their genes of luciferase have been isolated from 4 marine species *Vibrio (Photobacterium) fischeri*, *Vibrio (Beneckea) harveyi*, *Photobacterium phosphoreum*, *Photobacterium leiognathi*, and one terrestial species *Photorhabdus (Xenorhabdus) luminescens*.

The first step involved isolation of suitable bacteria from Thai sources, such as fish and seawater in Phuket. A new luciferase enzyme was isolated from a microorganism identified as *Vibrio campbellii*. The luciferase gene (Lux_Vc) was cloned and expressed in *E. coli* and purified to homogeneity. The amino acid sequences and the catalytic reactions of Lux_Vc were highly similar to those of the luciferase from *V. harhveyi* (Lux_Vh), showing 8 amino acid differences (Figure 5). However, the two enzymes have different affinities toward reduced FMN ($FMNH^-$). The catalytic reactions of Lux_Vc and Lux_Vh were monitored by stopped-flow absorbance and luminescence spectroscopy at 48°C and pH 8. The measured K_d at 4°C for the binding of FMNH_ to Lux_Vc was 1.8 μM whereas to Lux_Vh, it was 11 μM. Another difference between the two enzymes is that Lux_Vc is more stable than Lux_Vh over a range of temperatures; Lux_Vc has $t_{1/2}$ of 1020 min while Lux_Vh has $t_{1/2}$ of 201 min at 37°C.

The superior thermostability and tighter binding of FMNH_ make Lux_Vc a more tractable luciferase than Lux_Vh for further structural and functional studies, as well as a more suitable enzyme for some applications. The kinetics results reported here reveal transient states in the reaction of luciferase that have not been documented before. Thus the present studies (38, 40), not only provide additional basic information concerning the mechanism of the luciferase reaction, but also yielded a better enzyme for potential applicarions.

4. Design of Chemical Proteinases

Enzymatic catalysis requires specific binding of enzyme and substrate followed by catalytic action by reactive groups in the active site of the enzyme. Dr. Apinya Chaivisuthangkura's laboratory at Srinakarintwirot University, Prasarnmit has focused on mimicking enzymatic action with small molecules by developing photochemical reagents to cleave proteins with a high specificity. Such reagents are of intense interest due to their potential for applications in molecular biology, protein-protein interactions, protein adsorption to solids as well as for understanding biomolecular recognition with small ligands. Several photochemical probes are capable of cleaving the protein backbone with high selectivity or at a single site.

The design of probes in the present study exploits the well-known concept of the three-point recognition, consisting of a hydrophobic chromophore, an amino acid or a short peptide fragment, and an ionic functional group. The reagents described here have (a) high affinities for selected sites of proteins, (b) strong absorption bands in the near visible region, (c) long-lived

singlet excited states that are convenient to initiate photoreactions, and (d) a chromophore that can be used to probe microenvironments. This modular approach has resulted in a family of chiral probes with excellent molecular recognition, high to medium affinities, and probes that cleave proteins with high efficiencies. In our studies, we have synthesized three new bifunctional pyrenyl probes, L-phenylalanine-4(1-pyrenyl)methyramine chloride (PMA-L-Phe) and D-phenylalanine-4(1-pyrenyl)methyramine chloride (PMA-D-Phe) (Figure 6), and D-biotinyl-4(1-pyrenyl)methyramine chloride (Py-biotin)

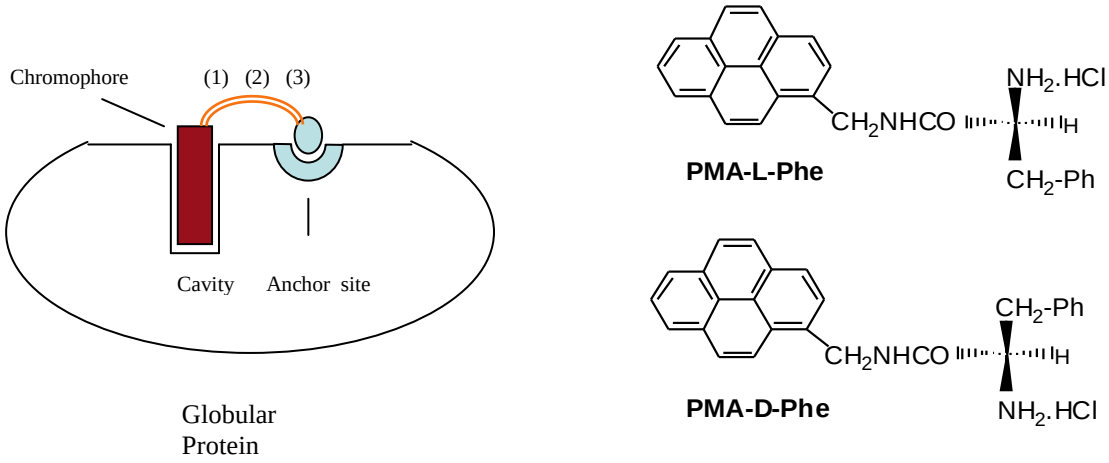


Figure 6: (Left) Schematic diagram of probe design. As the chromophore (1) binds in a hydrophobic cavity at the surface, the polar group (3) anchors the probe, and the linker (2) connects the units. (Right) Structures of L- and D-forms of phenylalanine-4(1-pyrene)methyramine hydrochloride, namely PMA-L-Phe and PMA-D-Phe.

L-phenylalanine-1(1-pyrene)methylamide (PMA-L-Phe) and D-phenylalanine-1(1-pyrene)methylamide (PMA-D-Phe) carrying a free amino terminus, were synthesized by coupling L-phenylalanine or D-phenylalanine to 1(1-pyrene)methylamine. The probes showed characteristic circular dichroism bands, with the spectra indicating a mirror-image relationship (280-370 nm region). Binding of the probes to lysozyme and bovine serum albumin (BSA) could be shown by UV-Vis, fluorescence, and circular dichroism spectral methods. Binding constants indicated large chiral discrimination. Thus, PMA-L-Phe (5) binds to both lysozyme and BSA with nearly equal affinity constants (K_b) of 3.3×10^5 M^{-1} and 3.8×10^5 M^{-1}, respectively. In contrast, its optical isomer, PMA-D-Phe binds to both lysozyme and BSA with much higher affinity constants 1.0×10^6 M^{-1} and 1.0×10^7 M^{-1}, respectively [47]. Chiral selectivities were 3 to 30 fold with BSA and lysozyme, respectively, illustrating the strong role of the chiral center in ligand-protein interactions. These probes also served as chiral protein scissors. Thus, irradiation of the probe-protein complexes, in the presence of Co(III)hexammine (CoHA), resulted in the facile cleavage of the protein backbone. Photocleavage yields were the highest with lysozyme (~ 57%) and these are highest values reported for protein photocleavage. Photocleavage was observed only in the presence of CoHA, and no reaction occurred in the dark. The photofragments have been isolated, purified and subjected to amino acid sequencing, with the results showing that lysozyme

was cleaved between Trp108-Val109. These results are consistent with earlier studies with a related pyrenyl probe, but present yields of the photocleavage werere significantly higher.

This methodology has been developed further for the design of a new chemical reagent, D-biotinyl-4(1-pyrenyl)methyramine chloride (Py-biotin), to target specific binding site on avidin. The absorption spectra of Py-biotin undergo changes upon binding to avidin with red shifts (2 nm) of the absorption peak positions. The fluorescence of Py-biotin is enhanced by the addition of avidin (10 μM), indicating protection of Pyrene chromophore from aqueous solution by the protein matrix. Irradiation of the probe-protein complexes, in the presence of hexaammine chloride Co(III) complex, resulted in cleavage of the protein backbone. At least three new fragments were observed with molecular weights of approximately 14 kDa, 8 kDa and 4 kDa. The N-terminal sequencing of the 14kDa and 8 kDa fragments indicated the residues ARKCS. Therefore, the major cleavage site occurs between Ser24-Ala25. However, a secondary cleavage site was observed with Py-biotin in addition to the above cleavage site, resulting to a smaller fragment (8 kDa) with the same N-terminal sequence.

5. Structure, Function and Molecular Evolution of Reptile Antimicrobial Agents

Antimicrobial peptides and antimicrobial proteins represent innate immunity to respond to microbial infection. Dr. Sompong Thammasirirak's laboratory at Khonkaen University has been studying antimicrobial peptides and proteins from reptiles, amphibians and birds.

Lysozyme is a well known anti-microbial protein. It is a unique glycosidase enzyme, which can lyse certain bacteria by hydrolyzing the β-1,4-glycosidic linkage between *N*-acetylmuramic acid and *N*-acetylglucosamine of the peptidoglycan in the bacterial cell wall. Lysozymes are considered to be self-defense enzymes, which are produced in serum, mucus and many organs of vertebrates. Four lysozymes were purified rom the soft-shelled turtle (SST, *Trionyx sinensis tiwanese*), green sea turtle (GST, *Chelonia mydas*) and Asiatic soft-shelled turtle (AST, *Amyda cartilaginea*). The reptile lysozymes showed lytic activity against several species of bacteria, such as *Micrococcus luteus* and *Vibrio cholerae*, but showed only weak activity to *Pseudomonas aeruginosa*. Then, the primary structures of three reptile lysozymes (SSTL A, SSTL B and ASTL) were determined by peptide mapping, amino acid analysis and determination of amino acid sequence (18). Interestingly, the mature SSTL lysozymes show an extra Gly residue at the N-terminus, which was previously found in soft-shelled turtle lysozyme.

Two lysozymes were also purified from Japanese quail eggs (*Coturnix coturnix* japonica) (32). Compared to hen eggwhite lysozyme (HEWL), these lysozymes showed six amino acid substitutions at positions 3 (Phe to Tyr), 19 (Asn to Lys), 21 (Arg to Gln), 102 (Gly to Val) 103 (Asn to His) and 121 (Gln to Asn) compared to hen egg white lysozyme. QEWL A and QEWL B showed one substitution, at the position 21, Gln replaced by Lys, plus an insertion of Leu between position 20 and 21. The amino acid differences between two lysozymes did not seem to affect antigenic determinants detected by polyclonal anti-hen egg white lysozyme, but caused them to separate well from each other by ion exchange chromatography.

Dr. Sompong Thammasirirak's laboratory is the first research group in Thailand to study antimicrobial compounds from the blood of the crocodile (*Crocodylus siamensis*). Several microbial peptides have been identified in the hemolymph of insects, spiders, scorpions, and frog, and even in humans. To our knowledge, no information is available on the presence of antimicrobial peptides in crocodiles. Crocodile species have separated a long time ago in evolution and are located between amphibians and birds in the phylogenetic tree. Athough the immune system of crocodilians has not been well characterised, several reports have described the efficacy of alligator serum to combat bacteria, virus and amoeba. So far, several anti-microbial compounds have been found in crocodile blood, and they are being purified for characterization. If the antibacterial agent in crocodile blood can be characterized, this would not only of considerable interest in terms of basic science, but it would also be valuable for the crocodile farms, since this would provide added value to crocodile blood, in addition to crocodile skin and crocodile meat.

6. Proteins Involved in Adaptation of Green Algae to Irradiance-Stress

Dr. Kittisak Yokthongwattana's laboratory at the Department of Biochemistry, Faculty of Science, Mahidol University focuses on studying plant responses to irradiance stress, using unicellular green algae as model organisms. The alga being studied, *Dunaliella salina*, is halophilic and often found in sea salt fields. It can survive in highly saline conditions such as salt evaporation ponds, because it has high concentrations of β-carotene to protect against the intense light and high concentrations of glycerol to provide protect against osmotic pressure. *D. salina* has strong anti-oxidant activity, due to its high content of carotenoids, and is used in cosmetics and dietary supplements. The commercial cultivation of Dunaliella for the production of β-carotene throughout has been an important success story of halophile biotechnology.

Although light is essential for photosynthesis, too much light is harmful to photosynthetic organisms. Light energy in excess of that required to saturate photosynthesis can bring about photoinhibition and photo-oxidative damage of protein in the chloroplast. Plants have evolved photoprotective mechanisms to counteract the drastic effects of excess light absorption during irradiance stress. At the molecular level, photoprotection includes scavenging of reactive oxygen species by antioxidant molecules, non-photochemical dissipation of excessive energy as heat and modification of photosynthetic machineries to enhance photon usage and reduce light absorption. Non-photochemical quenching (NPQ) is a process by which excess energy from singlet-excited chlorophyll is quenched and safely dissipated at heat. Generally, NPQ can be distinguished by observing a decrease in Chl fluorescence emission. Three different types of NPQ can be distinguished by their relaxation kinetics following dark incubation as well as their esponses to various inhibitors. Temporally, energy- or ΔpH-dependent quenching (qE) relaxes within a few minutes, followed by state-transition quencing (qT) in many minutes, and then by photoinhibition quenching (qI), which relaxes in hours.

In general, there is a correlation between the amount of zeaxanthin accumulated within the cell and the degree of NPQ, especially the qE type. Although constitutive accumulation of zeaxanthin helps protect plants from photooxidative stress, organisms with such a phenotype were reported to have an altered level and rate of NPQ induction. The *npq2* mutant of

Chlamydomonas reinhardtii showed a slower and smaller degree of NPQ while the *aba1* mutant of *Arabidopsis* has faster induction kinetics when compared to their respective WT. In *Dunaliella salina*, however, constitutive accumulation of zeaxanthin in the *zea1* mutant neither protects the alga from photooxidative damage nor helps in its recovery from photoinhibition. It is therefore of further interest to investigate the effects of the constitutively accumulated zeaxanthin on nonphotochemical energy dissipation in the *zea1* mutant of *D. salina*, in comparison to the WT strain. In the present study, quantitative kinetic analyses of excitation energy quenching were undertaken in both WT and *zea1* mutant of *D. salina*.

We have found that in *D. salina* WT and the *zea1* mutant, unlike *Chlamydomonas reinhardtii* and *Arabidopsis thaliana*, the constitutive accumulation of zeaxanthin within the cell did not result in significant alteration in the rate of NPQ induction kinetics. In depth analyses revealed that most of the quenching components among the WT and the mutant are ΔpH-independent. We concluded that the photoinhibition-type of NPQ (qI) is the major mechanism employed by the unicellular green alga, *Dunaliella salina*, for photoprotection.

7. Acetyl-Coenzyme A Carboxylase from a Thermophilic Photosynthetic Bacterium

Acetyl-Coenzyme A Carboxylase (ACC) is crucial for the biosynthesis of long-chain fatty acids in all animals, plants and eubacteria but it does not function for this purpose in archaea. A similar enzyme in some thermoacidophilic archaea acts as a key carboxylating enzyme in the CO_2 fixation pathway. ACC catalyzes the biotin-dependent carboxylation of acetyl-CoA to produce malonyl-CoA. The enzyme is composed of three different components, Biotin carboxylase (BC), Biotin carboxyl carrier protein (BCCP) and Carboxyltransferase (CT). Although the mechanisms of the enzymatic reaction are quite similar, the arrangements of the subunit structures are diverse among different groups of organisms. In addition, the kinetic properties and structure have been used to define a new group of acyl-CoA carboxylases found in Streptomyces. ACCs are important targets for the development of therapeutics against obesity, diabetes and several herbicides function by inhibiting the CT domain. Therefore, ACCs are the enzymes of interest in terms of molecular characterization, particularly in terms of the subunit interactions.

Dr. Songkran Chuakrut of Naresuan University is interested in producing recombinant thermostable ACC in *E. coli*, with the aim of determining its three-dimensional structure, to improve understanding of the mechanism and specificity of the enzyme. A purple sulfur bacterium isolate number TWC 12 is a moderately thermophilic photosynthetic bacterium, isolated from mud and water samples around hot well in Phrae province. The isolated strain, capable of rapid growth at temperature ranging from 50 to 60 °C, was obtained after the enrichment culture in the medium containing pyruvate as a carbon source and sulfide as an electron donor source. Cell masses of the thermophilic photosynthetic bacterium TWC12, grown under anaerobic-light condition at 55 °C for 36-48 hours using pyruvate as a carbon source, were used for purification of ACC enzyme, but so far without success. In addition, the genomic DNA of TWC 12 has been isolated and used for gene cloning. Molecular cloning of the genes encoding acetyl-CoA carboxylase in the thermophilic photosynthetic bacterium strain TWC12 has been carried out using the conserved regions of BC and BCCP subunits from many bacteria and archaea for primer design. Results are in progress.

8. Expression proteomics study of molt-related proteins in the epidermis of the black tiger shrimp, *Penaeus monodon*.

In crustaceans, the cycle of post-embryonic growth and development is characterized by a unique molting cycle. The progression within each molting cycle is regulated by the balance of the molt-stimulating ecdysteroid hormones, which are secreted by pairs of Y-organs and the mandibular organs located in the oral region, and the molt-inhibiting hormone, which is secreted from the X-organ, sinus gland complex located in the eyestalk. The main purpose of the molting process is to shed the cuticle to allow for an increase in body mass before the newly synthesized cuticle becomes fully calcified during the postmolt period. During the premolt stages the cuticle is gradually degraded by the resorption of organic and inorganic materials, deposited in the cuticle and returned to the shrimp's body for reuse after ecydysis. After ecdysis, organic and inorganic materials from the shrimp's body are secreted back to the newly synthesized cuticle. A variety of organs, including epidermis, are responsible for this crucial task. All findings so far have led to a better understanding of the regulatory mechanisms involved in molting, but this knowledge has been obtained using various crustacean species, and the finer details of any one mechanism are still unknown.

Since shrimps are a major export of Thailand, Dr. Warapon Promwikorn at Prince of Songkla University aims to investigate changes that occur in the protein expression profiles of the integument during the molting cycle of the black tiger shrimp, *Penaeus monodon*. This will lead to the identification of biomarkers, which are expressed in relation to the molting cycle, so as to achieve a better understanding of the molting mechanisms in this species.

To accomplish this goal, black tiger shrimps were cultured, determined for molting stages and examined for hepatopancreatic parvovirus (HPV) and monodon baculovirus (MBV) infection by PCR. The shrimps that were at the right molting stages and free from HPV and MBV infection, were then sacrificed. Epidermis from the carapace area of each shrimp was extracted for proteins and separated by 2D SDS-PAGE in pH range 3 - 10, 4 -7, and 6 -11 followed with silver staining.

Epidermal protein expression profiles of shrimps at 8 molting stages (D0-early premolt, D1, D2- mid premolt, D3, D4- late premolt, A- early postmolt, and B1, B2- late postmolt) around the molting cycle are shown in two dimensional (2D) polyacrylamide gels (Figure 7). The intensity of protein spots in the 2D gels from 4 independent experiments was measured using the analysis software, Imagemaster 2D Platinum. Some 489 protein spots of each gel were matched among these gels. The intensity of any protein spot was next normalized with the total intensity of all 629 spots in the same gel. The mean intensity of each protein spot was analyzed from 4 replicates experiments, and compared to the others of 7 molting stages. Non-parametric statistics (Kruskal-Wallis) were used to determine statistical differences among means of the 8 molting stages at 95% confidence. The significant pairs were further determined with non-parametric statistic Wilcoxon-Mann-Whitney test at 95% confidence.

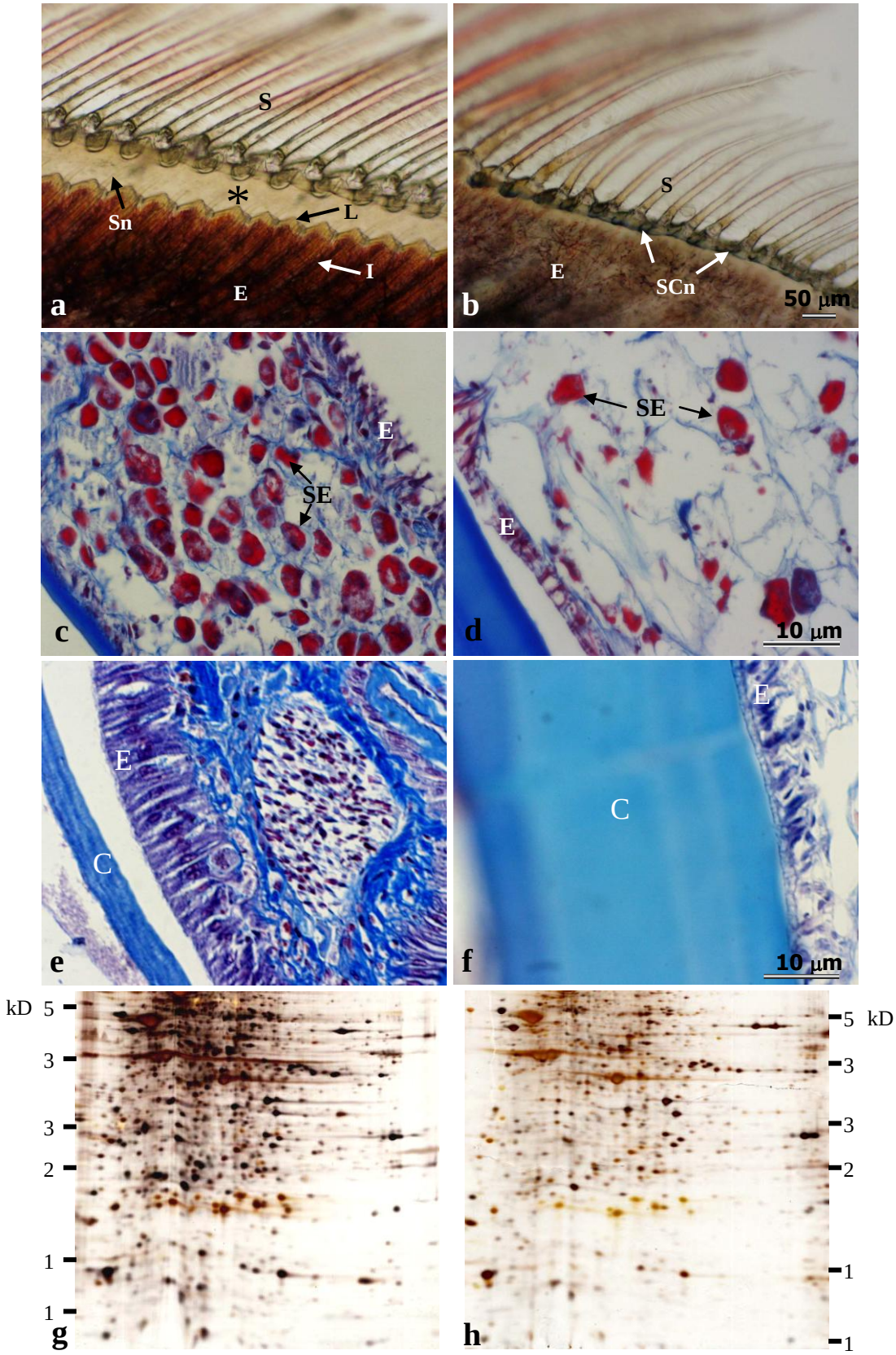
Figure 7

Physical criteria of the shrimp at a stage before ecdysis (a) and inter-molting stage (b). Uropods of the black tiger shrimps were examined and photographed under a light microscope (Olympus BX51). Bar = 50 μm. E = epidermis, I = indent pattern of the epidermis, L = white layer at the edge of the epidermis, S = setae, SC = seta cone, SCn = newly-formed setal cones, Sn = newly-formed seta, * = clear zone between cuticle and epidermis.

Protein deposition in the sub-epidermis at a stage before ecdysis (c) and inter-molting stage (d). Cuticular tissues in each stage were routinely processed for histological study and stained with modified trichrome. E, epidermis; SE, sub-epidermal cell.

Epidermal morphology at a stage before ecdysis (e) and inter-molting stage (f). Intact cuticular tissue from the trunk in various molting stages were routinely processed for histological study and stained with modified Masson's trichrome. C, cuticle; E, epidermis.

2D gel map of integument tissue at a stage before ecdysis (g) and late postmolt stage (h) stained with silver staining.


S
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1
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1
h

Figure 7

There were 46 spots that showed statistic significant differences in expression in both Kruskal-Wallis and Wilcoxon-Mann-Whitney test. From the data, the proteins can be categorized into eight groups based on the stage of maximal level of protein spot intensity. Each group shows specifically higher expression in one molting stage, but less expression in the others. The number of protein spots that is at highest expression in D0 stage is 5, D1 stage is 4, D2 stage is 1, D3 stage is 4, D4 stage is 14, A stage is 4, B1 stage is 3, and B2 stage is 5. This indicates that different sets of proteins take turn to be expressed in a particular molting stage, and it is highly possible that the epidermal proteins are most highly active at D4 stage. This is in accord with the information that each molting stage is responsible for different crucial tasks in the molting cycle progression. Degeneration and absorption of old cuticle occurs in the D0-2 stage, the generation of new epicuticle, exocuticle, and endocuticle occurs in the D3, D4 and B1-2 stages, respectively. Thus these differentially expressed proteins could possibly act as molting cycle-regulatory proteins, end product-proteins incorporated into the newly secreted cuticle, or else in promotion of growth. We plan to specifically focus on the identification and function of proteins that are highly expressed in D4 stage.

These spots and selected proteins showing no change in expression have been selected, and are being analysed by LC/MS/MS in order to obtain identification and delineate function. The forthcoming result will lead to a better understanding of regulatory mechanisms in the molting process, and the development of biomarkers for regulating the molting cycle and cell growth. It may also lead to an understanding of relationship between growth rates and other factors e.g. genetics, environment, and diseases. This will be beneficial for the improvement of shrimp culture, *in vitro* fertilization and shrimp genetics.

B) Proteins Changes in Disease, Therapy and Wound Healing

Since proteins are involved in almost all biological processes, defects in the quality or quantity of proteins can lead to disease states. Studies on the structure and expression of proteins have provided major advances in the understanding of human diseases. In addition, characterization of mutations in many diseases has helped to improve understanding of the relationships between protein structure and function. Prof. Jisnuson Svasti's laboratory at the Chulabhorn Research Institute, together with Dr. Chantragan Srisomsap, Dr. Rudee Surarit, and Dr. James Ketudat-Cairns, is interested in study of protein changes in various human diseases. Current areas of interest are the abnormal hemoglobins, inborn errors of metabolism and protein changes in cancer. Our studies provide information on the defects occuring in the Thai population, and offer medical practitioners the opportunity of collaborating to study diseases of their interest at the molecular level.

Apart from study of the protein changes in disease, the Laboratory of Biochemistry at the Chulabhorn Research Institute is interested in searching for plant natural products, which may have therapeutic potential, such as anti-cancer activity. Such products presumably affect proteins in the cancer cell, and some attempts are being made to study the proteins affected. Finally, another physiological function dependent on proteins is that of wound-healing, and cell-

based assays have been established in Dr. Rudee Surarit's laboratory to study the effects of medicinal plants on wound-healing.

9. Characterization of Hemoglobinopathies Found In Thailand

9.1 Background on Hemoglobinopathies

Genetic diseases of hemoglobin occur with rather high frequency in Thailand, and consist of two types, thalassemia and abnormal hemoglobins. Thalassemia results from lack or decreased synthesis of one or more of the hemoglobin chains. α-Thalassemia results from absence or decreased levels of α chains and occurs with a frequency of about 20% in Thailand, while β-thalassemia is due to absence or decreased levels of β-chains and occurs with a frequency of about 10% in Thailand. Abnormal hemoglobin or have mutations, that alter the structure of one of the globin chains, typically α or β chains. If the mutation occurs in a region of functional importance, this can affect the physiological function or the stability of the hemoglobin, so studies on abnormal hemoglobins provided important information protein structure-function relationships. Nearly 1,000 abnormal hemoglobins have now been characterized world-wide, and more than 30 abnormal Hbs have been detected in Thailand. We have collaborated with Professor Suthat Fucharoen at the Thalassemia Research Centre, Institute of Science and Technology, Mahidol University for some 20 years, and have characterised 10 of these variants. Of the variants found in Thailand, 9 are point mutations in α chains, 15 are point mutations in β chains, 3 are C-terminal elongations, 1 is a deletion, 1 is a frameshift mutation, and 1 is a δ-β hybrid hemoglobin resulting from crossing-over. Most abnormal Hb are rare except for Hb E [β26 Glu-Lys], which can rise to a frequency of 53% in the Northeast, and Hb Constant Spring, an α-chain extension occurring with a frequency of 1-8 %. Moreover, since Thailand has high incidence of Hb E [β26 Glu-Lys], α-thalassemia and β-thalassemia, abnormal Hb can often be found in compound heterozygosity with these conditions in Thailand unlike in most countries, so that the effect of such associations on the hematological and clinical profile is of interest.

9.2 Characterization of Abnormal Hemoglobins

During this phase of the project, few samples were received, and the only notable hemoglobin characterized was Hb Kurosaki in a 30 year old Thai male (3). The mutation could be shown by DNA sequence to be [α7(A5)Lys-Glu (*A*ag-*G*ag)], located in the α2 gene. The patient was normal had rather high content of Hb Kurosaki at 28%, higher than a previous case described in Japan with 18%, suggesting that the latter might have been α1 gene mutation. Due to lack novel hemoglobin variants, our collaboration with Prof. Suthat Fuchareon switched to other aspects, as described below.

9.3 Anti-Barts Antibodies for Thalassemia Diagnosis

An area of potential application involved collaboration with Professor Suthat Fucharoen, Dr. Watchara Kasinrerk (Chiangmai University), and Dr. Ruchaneekorn Kalapravidh (Faculty of Medicine Siriraj) to develop anti-Hb Bart's antibodies for detection of thalassemia (17). Hb Bart's is a tetramer of gamma chains. These chains are also found in Hb F ($\alpha_2\gamma_2$), which is the fetal hemoglobin, synthesized during fetal development and decreasing rapidly after birth, so that it is normally present at low levels <1% in adults. However, Hb F may be elevated in beta-thalassemia, especially where there is no synthesis of normal beta chain.

So, Hb Barts was purified at the Chulabhorn Research Institute, and used to immunize an egg-laying chicken at Dr. Watchara's laboratory in Chiangmai University. This is a novel technology for antibody production, where antibodies are produced in the egg yolk and can be readily isolated. Specificity was improved by immunadsorbent removal of anti-Hb A antibodies. Then, the antibodies were used to develop an ELISA method for detection of Hb F in the blood of thalassemic subjects. Tests were made of blood from thalassemic subjects collected by Dr. Suthat Fuchareon's group. Results showed that thalassemia could be detected, but the approach was not as useful as hoped for, since 4% of normal individuals were positive and α- and β-thalassemia syndromes could not be distinguished. The immunoassay may be more useful when used with other systems, such as specific anti-Hb F Abs, in determining whether high Hb Bart's or high Hb F levels are present. In addition, this assay will be useful for rapid screening of thalassemia and for quantifying Hb F in conditions associated with high values for Hb F.

10. Inborn Errors of Metabolism

10.1 Background Information on Inborn Errors of Metabolism

There are many inborn errors of metabolism, which can cause severe clinical manifestations, such as mental retardation or developmental abnormalities. In general, inborn errors arise from deficiencies in enzymes of various metabolic pathways, such as the urea cycle (12), pathways for synthesis or degradation of specific amino acids, or mucopolysaccharide degradation. Such disorders may be due to mutations leading to ootthese enzymes. Such enzyme deficiencies are typically detected by an accumulation of the substrate of the enzyme reaction and/or a decreased level of metabolites, which occur after the enzyme reaction. Typically, each inborn error of metabolism occurs with low frequency, but there are 500-600 such defects, so cumulatively, inborn errors of metabolism are significant problems. In many cases, the devastating effects can be avoided through proper treatment, e.g. in phenylketonuria, which may be treated with diets low in phenylalanine. So it is important to detect inborn errors early, and in many countries, newborn screening is routine for selected inborn errors of metabolism.

Many inborn errors of metabolism have also been found in Thailand (9). We have studied selected cases of inborn errors of metabolism in collaboration with Professor Pornswan Wasant (Siriraj Hospital), Dr. Duangrurdee Wattanasirichaigoon (Ramathibodi Hospital), Dr. Suthipong Pangkanon (Queen Sirikit National Institute of Child Health), Dr. Vorasak Shotelersuk (King Chulalongkorn Memorial Hospital), and Dr. Pranoot Tanpaiboon (Suan Dok

Hospital, Chiangmai). Typically, this involves analysing the levels of enzymes suspected of being deficient using leukocytes or cultured fibroblasts from patients. Then, we design primers for preparing cDNA or genomic fragments by RT-PCR or PCR, and then perform automated sequencing of the cDNA to determine the mutation. The diseases for which we have studied the molecular basis include methylmalonic acidemia (MMA), mucopolysaccharidosis type I (Hurler syndrome), mucopolysaccharidosis type II (Hunter syndrome), Fabry disease, and Gaucher disease. As described below, our work on protein and gene defects in inborn errors of metabolism has led to a greater understanding of population genetics in Thai populations, in addition to identifying some residues critical for the function of the enzymes involved. It has also allowed the doctors involved to give more knowledgeable advice to the families of the patients, and, in some cases, suggested which treatments would be most appropriate. Therefore, the interest in the structure function relationships in metabolic enzymes has led to some direct clinical benefits, in addition to knowledge of critical residues in the protein sequences.

10.2 Methylmalonic academia

Methylmalonic acidemia (MMA) is an inborn error of organic acid metabolism caused by the functional defect of L-methylmalonyl-CoA mutase (MCM, EC 5.4.99.2). MCM catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA, an intermediate metabolite of the Kreb's cycle, with deoxyadenosylcobalamin (AdoCbl) acting as a required cofactor. Inherited deficiency of MCM activity results from defects either in the gene encoding the MCM apoenzyme (*mut* MMA or vitamin B_{12}-unresponsive MMA, Online Mendelian Inheritance in Man (OMIM) number 251000) or in genes required for th e conversion of cobalimin to AdoCbl (*cbl* MMA or vitamin B_{12}-responsive MMA). MCM structural gene defects are classified into two subtypes on the basis of residual enzyme activity, mut^0, which results from complete mutase deficiency and *mut*, which results from partial deficiency of the enzyme. Recently, the *MMAA* and *MMAB* genes were identified as being responsible for the *cblA* and *cblB* defects in production AdoCbl, respectively. The MMAA protein is speculated to be a transporter or accessory protein involved in Cbl transport into mitochondria, while the MMAB protein is an ATP: cob(I)alamin adenosyltransferase enzyme (ATR, EC 2.5.1.7).

Molecular genetic analysis was performed on three patients diagnosed with methylmalonic acidemia (MMA) (31). One patient had mut^0 MMA, with homozygous for a novel nonsense mutation p.R31X (c.167C>T) in the *MUT* gene encoding the L-methylmalonyl-CoA mutase (MCM .). The other two had *cblB* MMA, due to a new *MMAB* mutation, p.E152X (c.454G>T), found to be homozygous in one *cblB* patient and heterozygous in the other patient . These mutations respresent the first identification of a mutation in MMAB in Southeast Asia, and show that production of cofactors must be considered in enzyme defects. In addition, they suggested which patients might be effectively treated with vitamin supplements.

10.3 Mucopolysaccharidosis type I

Mucopolysaccharidosis type I (MPS I or Hurler syndrome) is a lysosomal storage disease caused by the deficiency of α-L-iduronidase (E.C. 3.2.1.76), which results in the build up of dermatan sulfate and heparin sulfate in the lysozymes, since they cannot be properly degraded.

Many mutations have been identified in the α-L-iduronidase gene, which is located on chromosome 4 at 4p16.3 and contains 14 exons spanning approximately 19 kb, encoding a 653 amino acid mature protein (Human Gene Mutation Database: http://www.hgmd.org). Mutations that cause severe MPS I (Hurler syndrome) include nonsense, missense and splice-site mutations, while milder MPS I defects (such as Scheie Syndrome) include mainly missense mutations and small deletions that leave most of the protein intact. Both severe and mild mutations have previously been reported in Western and Japanese populations, but no reports on the molecular characterization of this defect in Thailand had appeared before our work.

Several patients in Thai hospitals have been diagnosed as having Hurler Syndrome, but we could only confirm this by enzyme analysis for 2 patients, who had undetectable levels of enzyme activity in their leukocytes. One patient was found to be heterozygous for the mutations A75T and S633L, known to cause MPSI. The other patient had the mutation c252insertC, leading to a frameshift in the gene in one chromosome, and the nonsense mutation E299X on the other (13). These studies suggested that functional assays and molecular analysis of α-iduronidase may be critical to the diagnosis of Hurler syndrome, since other defects seem to cause similar symptoms.

10.4 Mucopolysaccharidosis type II

Mucopolysaccharidosis II (Hunter syndrome) is a lysosomal storage disorder caused by a deficiency of iduronate-2-sulfatase (IDS). This deficiency leads to the accumulation of glycosaminoglycans, dermatan sulphate and heparan sulphate. The intra and extracellular accumulation of these substances leads to multisystemic organ abnormality. Hunter syndrome has a range of clinical severity ranging from mild (late onset and normal intelligence) to severe (mental retardation and early death) (Neufeld and Muenzer 1995), presumably due to the variety of mutations that occur in the *IDS* gene. The human *IDS* gene contains 9 exons and spans about 24 kb in Xq28. A highly homologous pseudogene (*IDS-2*, sequences related to exons 2 and 3 and introns 2, 3 and 7 of the *IDS* gene) is located 20 kb telomeric to the active gene.

We have performed mutation analysis of 20 patients (from 18 families) with Hunter disease from Siriraj, Ramathibodi, Children's and Chiang Mai University Hospitals (45). From these cases, we have identified 19 different mutations, including 8 novel mutations and 11 previously reported mutations. These included 9 missense mutations, 3 nonsense mutations, 3 splice site alterations, 1 deletion, 2 indels, and 1 rearrangement. Evaluation of the IDS activity of two hemizygous variants identified in the same patient, p.R101C and p.R468Q, by expression of IDS with the individual mutations in COS 7 cells indicated that only the p.R468Q mutation affected IDS protein activity. Two exonic mutations, c.257C>T (p.P86L) and c.418G>A, were found to activate multiple cryptic splice sites, resulting in aberrantly spliced transcripts. These results indicated that some mutations previously thought to cause aberrations in the protein structure actually interfered with protein production at the splicing level, while other mutations did seem to act at the protein level. Another benefit of this work is we were able to screen the amniotic fluid of one unborn patient, thereby advising the parents on the likely health status of their unborn child.

10.5 Fabry Disease

Fabry is another lysosomal storage disease caused by a deficiency in lysosomal α-galactosidase (ceramide trihexosidase, α-Gal A). This deficiency results in progressive accumulation of the ceramide globotrioacylamide (G3) in the vascular endothelium and visceral tissues, leading to a range of symptoms, including angiokeratoma, corneal opacity, and attacks of severe pain in the muscles. The patients generally succumb to renal failure in their 30s or 40s, if not treated. Fabry is X-linked, but both hemizygous males (70%) and heterozygous females (10-35%) can be affected to some degree.

Our work helped Dr. Duangrurdee Wattanasirichaigoon to characterize the defect in a family with several members afflicted with Fabry disease (23). The mutation responsible for the defect was p.L106R. When the α-Gal A from the normal GLA gene cDNA was expressed in *Pichia pastoris*, it gave high α-galactosidase activity, while no activity could be detected when the GLA cDNA containing the p.L106R mutation was expressed in the same manner. Thus, we were able to show that this mutation was indeed responsible for the defect in the protein in this family. This mutation was found to be in a loop peripheral to the active site, and the replacement of the nonpolar Leu with the positively charged Arg, which is near another Arg in the protein, was likely to destabilize this region of the protein (Figure 8). In addition to this knowledge of the protein structural defect, the work provided a marker to allow assessment of members of this family as to whether they are carriers of the disease, thereby allowing them to more knowledgeably plan their families.

10.6 Gaucher Disease

Gaucher disease is a recessive defect in the lysosomal β-glucocerebrosidase, the product of the acid β-glucosidase gene *GBA* on chromosome 1. The defects in this protein lead to build-up of cerebrosides, which can cause disorders with both neuronal and nonneuronal symptoms.

We have investigated defects in the *GBA* gene leading to defective proteins in patients from Siriraj and Ramathibodhi Hospitals. A defect in the acid β-glucosidase was confirmed by enzyme assays 4-methylumelliferyl glucoside, and then the exons and surrounding regions of the *GBA* gene were sequenced. A few patients were found to have splice-site mutations resulting in truncated, nonfunctional mutations (36). Other patients were found to have missense mutations, including F213I and L444P, which are common mutations in Caucasian and Taiwanese Chinese populations. The disorder seen in the patient with the L44P mutation (homozygous) was more similar to that seen in a Swedish population from Norrbotten than to the symptoms seen in Taiwan Chinese with this mutation, so Gaucher modifying genes may be different in some Thais than in some Chinese populations. Analysis of more independent cases with this mutation in Thailand will be needed to confirm this.

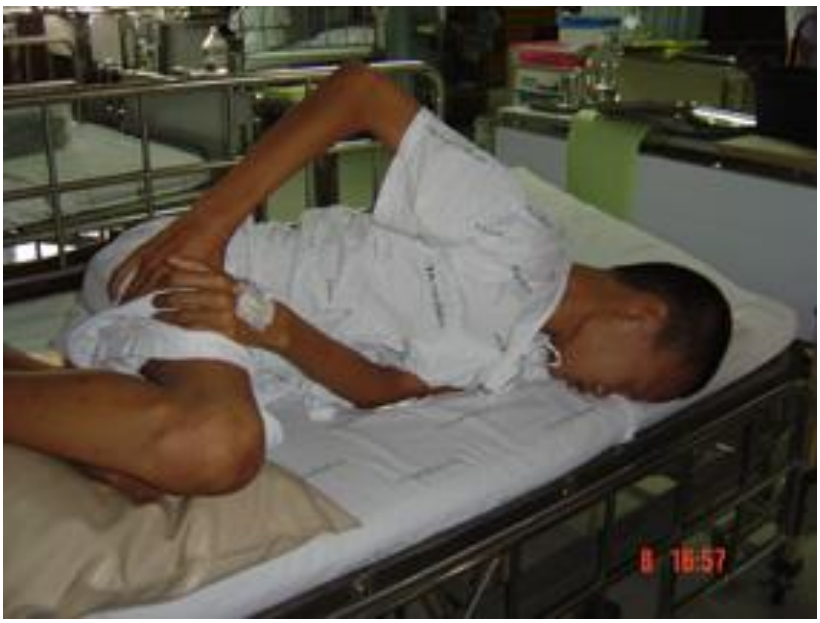

Figure 8 a) The proband, 16-year-old male, had agonizing pain in calf and thigh, burning sensations on palm and sole, unrelated to physical activity. The pain attacks were unpredictable, beocming more frequent and severe. Blood pressure was uncontrolled especially in severe pain, and hypertensive, atherosclerotic, retinopathy symptoms were found.The patient quit school, becoming depressive, even expressing ideas of committing suicide. He was periodically put on narcotics and anesthesia.

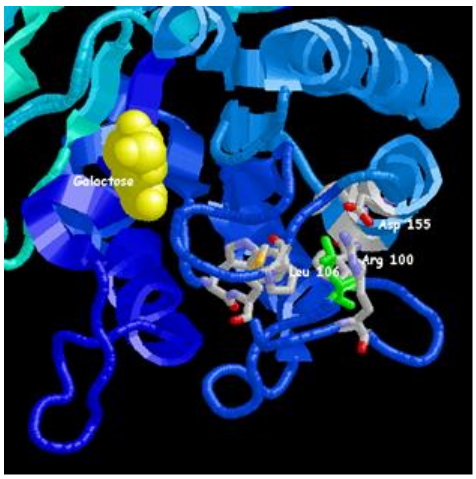

Figure 8 b) Mutation in proband, Leu106Arg in the human alpha-galactosidase structure. Leu 106 is shown in stick representation in green with other neighboring sidechains shown in stick representation with CPK colors. Its nearest neighbor, Arg 100 makes a salt bridge with Asp 155, which may be disrupted by the L106R mutation. Other neighboring sidechains re mostly hydrophobic, e.g. Trp 162, Phe 159, Trp 95, Met 96 and Pro 146. The active site, 15 Å away, is indicated by the position of the galactose residue shown in yellow spacefill representation.

11. Protein Changes in Human Cancer and Cancer Cells

11.1 Background Information

Cancer is a disorder resulting from uncontrolled cell growth and differentiation, and with malignant behavior, is capable of invasion and metastasis. Carcinogenesis is initiated by non-lethal genetic damage, followed by a multistep process at both the phenotypic and genetic levels. Regulatory genes such as the proto-oncogenes, the tumor suppressor genes, and genes regulating apoptosis are important targets of genetic damage, as are the DNA repair genes. Mutational damage to these genes will result in activation or inactivation of the functions of their gene products, resulting in uncontrolled proliferation with abnormal differentiation and acquisition of the capability for invasion or metastasis. In addition, tumors undergo various interactions involving adhesion molecules for detachment and attachment, such as the E-cadherins, laminin or the integrin family; the cytoskeletal proteins such as catenins; and the proteases and anti-proteases such as matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs).

The proteomic approach has gained popularity in the last 10 years for studying differences in protein expression under different conditions, for example under normal condition and disease states, such as cancer. Our group is the first research group in Thailand to use the proteomic approach, where the proteome patterns (or the total protein present at any tissue at any time) are studied, typically by 2-dimensional electrophoresis (2-DE). Initially, we used 2-DE to compare the proteomic patterns of thyroid tissues from neoplastic and non-neoplastic diseases, and showed a distinct up-regulation of cathepsin B in neoplastic conditions.

11.2 Galectin-3 Expression in Thyroid Tissues

Since galectin-3 was in thyroid tissues by 2-DE, we followed its expression in thyroid diseases by 1-DE immunoblotting. Expression was markedly elevated in thyroid papillary carcinoma, compared to follicular adenoma, follicular carcinoma or non-neoplastic diseases (25). Inflammation can result in weak bands of galectin-3 being occasionally found in other thyroid diseases, but these tend to be much fainter. Galectin-3 expression was also elevated in malignant cancers of bone, breast, colon, esophagus, larynx, lung and ovary. Four cases of thyroid papillary carcinoma with metastasis gave 2-3 bands on 1-DE immunoblotting. 2-DE immunoblotting of galectin-3 showed 3 dark spots with MW/pI 32.9/8.29, 31.0/8.40 and 30.0/8.40 and 2 light spots. 1-DE immunoblotting for galectin-3 allows thyroid PC to be readily distinguished from FA and FC, as well as from non-neoplastic diseases. This will be useful for improving diagnosis of PC, since there are many variant forms of PC, including those containing follicular structures. In addition, multiple bands may be found in metastasis.

In order to better understand this protein, galectin-3 from papillary thyroid carcinoma was partially purified by affinity chromatography on lactosyl-agarose (43). Proteins eluted from the column were separated by SDS-PAGE, and galectin-3 was detected with antibodies against the N-terminus and C-terminus of galectin-3. Some protein bands from the lactose binding fraction were also selected for identification by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). Seven protein bands, with molecular weights ranging from 16 kDa

to 31 kDa, were identified as galectin-3. The antibody raised against the C-terminus of galectin-3 gave a strong band for one of the bands detected by the N-terminal antibody and weak bands for the other three. One additional dark immunoreactive band with an approximate molecular weight of 20 kDa, was also detected by the C-terminal galectin-3 antibody. To determine the structural differences of each protein band, N-terminal amino acid sequencing of the seven protein bands was conducted. The three upper bands were N-terminally blocked, while the other bands had N-terminal amino acid sequences starting at positions Gly35, Gly65 (2 bands) and Ala100, respectively. Further studies are necessary to determine whether these are due to nonspecific proteolysis or post-translation modification galectin-1 and galectin-3 were found in the eluted fraction purified from the α-lactose agarose affinity column which is useful for enrichment of low abundance proteins such as galectin-3 in thyroid tissues, which cannot be identified from 1-DE or 2-DE Coomassie blue stained gel. The results suggested cleavage at sites in front of a small amino acid (Gly or Ala) preceeded by a proline five residues earlier and followed by proline two residues later (PX1X2 X3 X4*G/A X5P, where X1 and X2 were small residues, G, A or S). The novel protease that is responsible for this action appeared to be active only in papillary thyroid carcinoma, especially at the metastasis level.

11.3 Comparison of membrane-associated proteins in human cholangiocarcinoma and hepatocellular carcinoma cell lines

Cholangiocarcinoma (CCA), found predominantly in the Northeast, and hepatocellular carcinoma (HCC), with major occurrence in the North, have relatively high incidence in Thailand. Cell line models, originating from Thai patients, are available for both diseases, including the human bile duct epithelial carcinoma cell line HuCCA-1 and the human hepatocellular carcinoma cell line HCC-S102. We have previously studied the proteomic patterns of these cells. Here, we have prepared subproteomes enriched in membrane proteins or in cytosolic proteins from the HuCCA-1 and the HCC-S102 cell lines (27). Study of differential protein expression by 2-DE and LC/MS/MS showed 195 proteins expressed in the two cell lines, including both membrane-associated and cytosolic proteins. Eighteen proteins were found in both membrane and cytosolic fractions of HuCCA-1, but not in HCC-S102, while nine proteins were found in both membrane and cytosolic fractions of HCC-S102, but not in HuCCA-1. Ten membrane proteins were found in HuCCA-1 but not in HCC-S102, including integrin alpha-6 precursor, ezrin, hippocalcin-like protein 1, mitogen-activated protein kinase kinase kinase 2 (MAPK/ERK kinase kinase 2), and calgizzarin. Proteins showing increased expression in the membrane fraction of HuCCA-1 were mainly cytoskeletal proteins (40.9%), while proteins showing increased expression in the membrane fraction of HCC-S102 were mainly metabolic proteins (39.4%). The subproteomic approach used here facilitates detection of potential biomarkers undetected by regular proteomic fractionation. This differential expression of proteins found between the cholangiocarcinoma cell line and the hepatocellular cell line may be useful for developing possible biomarkers for early detection of the different types of cancer.

11.4 Use of Proteomics for Studying Action of an Anti-Cancer Agent Pomiferin

We have used the proteomic approach to study the effect of an anti-cancer agent, pomiferin, on cancer cells (11). Pomiferin (Figure. 9) is a prenylated isoflavonoid, 3-(3,4-dihydroxy-phenyl)-5-hydroxy-8,8-dimethyl-6-(3-methylbut-3-enyl)-4H,8H-pyrano-[2,3-h]-chromen-4-one, ($C_{25}H_{24}O_6$), isolated from the chloroform extract of the leaves of *Derris malaccensis* by Dr. Supanna Techasakul (Kasetsart University). This compound has strong anti-fungal and anti-oxidant activities, and showed cytotoxic activity towards human cholangiocarcinoma cells (HuCCA-1), with IC_{50} of 0.9 μg/mL.

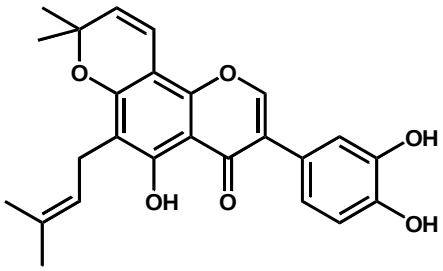

Figure 9: Structure of Pomiferin

Treatment of the human cholangiocarcinoma cell line HuCCA-1 with pomiferin at a final concentration of 3.2 μg/mL for 3 days caused cells to float from the culture dish. DNA from floating cells showed a ladder pattern, indicating apoptosis, but this was not observed with DNA from adherent cells. Floating cells also showed changes in protein expression compared to untreated cells, but adherent cells did not differ in protein pattern to untreated cells (Figure 10a). 2-DE showed increased expression of 12 proteins in pmoferin-treated cells, namely glucose-regulated protein 75 (grp 75), calcyclin (S100A6), degraded cytokeratin 19, ATP synthase D, ribosomal protein P0, degraded cytokeratin 18 (two spots pI/MW 6.03/29.9 and pI/MW 4.66/21.5), cofilin, annexin A1, triose phosphate isomerase, peroxiredoxin-1, calgizzarin, and profilin.

In contrast, cytokeratins (CK) 7, 18 and 19 were down-regulated, and were shown by 1-DE immunodetection to be degraded (Figure 10b). Interestingly, two spots of CK18 fragments (spots d and k) were detected in addition to CK18 (47 kDa/5.25, 5.16, 5.19). Closer examination of the sequence coverage of spots "d" and "k" indicated that they derive from the N-terminal and C-terminal fragments of CK18 suggesting cleavage at the VEVD/A consensus sequence in the L1-2 linker region, presumably by caspases. The induction of apoptosis is interesting, since it is a potentially efficient strategy for cancer therapy.

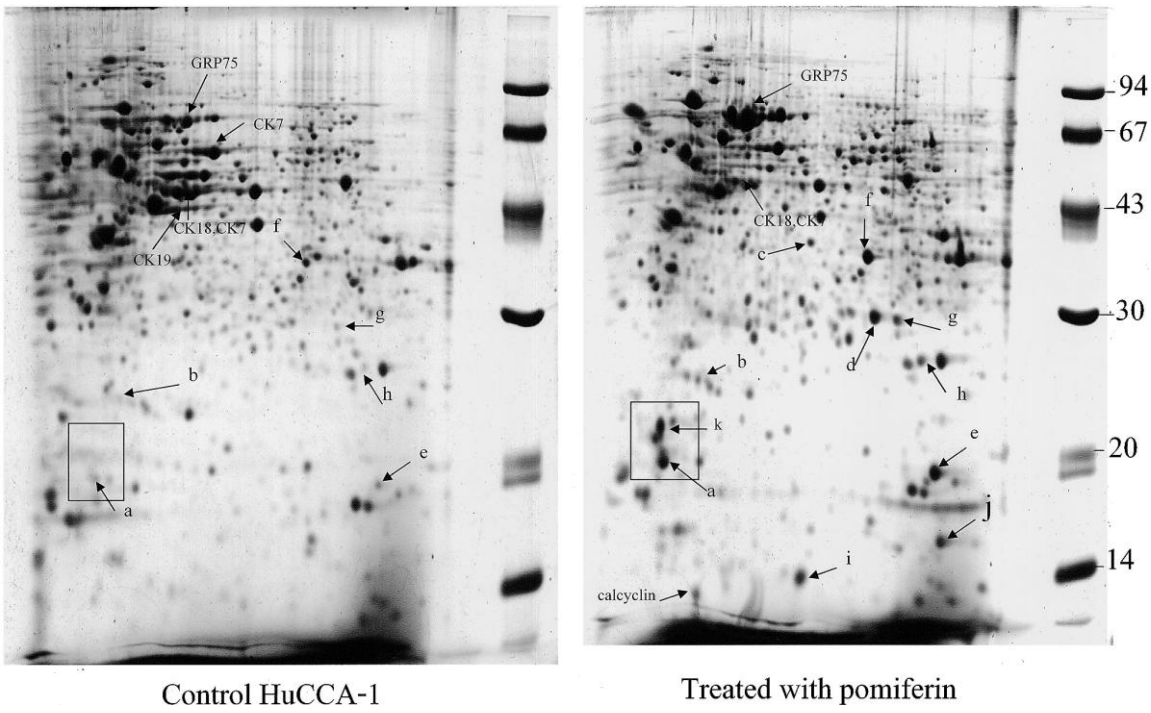

Figure 10a. 2-DE patterns of HuCCA-1 cells cultured for three days in the presence or absence of pomiferin. Left: Control untreated cells. Right: Floating cells from culture treated with pomiferin for 3 days/

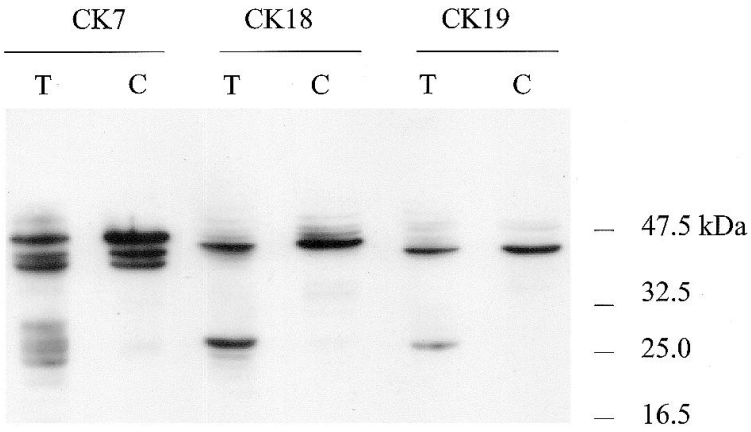

Figure 10b. HuCCA-1 cells were treated with pomiferin for 24 h, and extracts of floating cells were tested by 1-DE immunoblotting using antibodies to cytokeratins (CK7, CK18, CK19).

12. Plant Natural Products as Sources of Potential Therapeutic Agents

12.1 Background

Thailand has abundant resources of medicinal plants and is rich in knowledge of folk medicine, employing such plants. Plant extracts can have various biological activities (e.g. 26). Indeed, many important anti-cancer drugs, such as camptothecin, taxol, podophyllotoxin, vinblastine and vincristine, originate from plants. These compounds have also been used as lead compounds for the development of novel anticancer chemotherapeutic drugs. As part of the Chulabhorn Research Institute's comprehensive research program on cancer, we are also screening medicinal plants for anti-cancer activities in collaboration with Dr. Somsak Ruchirawat (Chulabhorn Research Institute and Mahidol University) and Dr. Supanna Techasakul (Chulabhorn Research Institute and Kasetsart University). Part of the work involves screening for cytotoxic activities using Thai cholangiocarcinoma (HuCCA-1) and hepatocellular carcinoma (HCC-S102) cell lines. However, we also perform functional screening tests, such as inhibition of the *in vitro* invasion of extracellular matrix by cancer cells and inhibition of telomerase enzyme activity. As described earlier, the effect of bioactive compounds is also being studied by proteomics to identify the proteins changed as a result of treatment by the bioactive compound. Thus, overall, the work will involve not only screening for anti-cancer agents, but also studying how potential therapeutic compounds function

12.2 *Eclipta prostrata* Juice Inhibits Cell Migration i*n vitro* and Exhibits Anti-Angiogenic Activity *in vivo*

Invasion of cancer cells is critical for metastasis. In the previous phase of the grant, we reported that extracts of several Thai plants could inhibit cancer invasion. We have collaborated with a group in Germany through the RGJ Ph.D. scholarship program to perform an *in vivo* test for angiogenesis, since the formation of blood vessels is a hallmark of tumour growth, and its inhibition has potential anti-cancer effect. This *in vivo* anti-angiogenic activity involves using the chick chorioallantoic membrane (CAM) assay, where blood vessel formation in the 3-4 day chick embyo is studied. The effects of *Eclipta prostrata*, a Thai medicinal plant, on invasion, migration and adhesion of cancer cells *in vitro* were investigated and the anti-angiogenic activity *in vivo* was evaluated (44). *Eclipta prostrata* juice inhibited cancer invasion and migration, without affecting cell adhesion. Cell migration was inhibited in a variety of cancer cell types and in endothelial cells, with IC_{50} values of 31-70 μg/ml, much lower than the IC_{50} values for cytotoxicity of 203-1,217 μg/ml for cancer cells and >4,000 μg/ml for endothelial cells. Fifty percent inhibition of angiogenesis by *E. prostrata* juice was also observed at 200 μg/egg. These inhibitory activities make *Eclipta prostrata* juice very interesting, and efforts are under way to try to isolate the active compounds in the juice, which may act as lead compounds for future anti-cancer agents.

12.3 Anti-metastatic Effect of Vanillin

Vanillin, a food flavoring agent, has been reported to show anti-mutagenic activity and to inhibit chemical carcinogenesis. We have examined the effect of vanillin on the growth and metastasis of 4T1 mammary adenocarcinoma cells in BALB/c mice in collaboration with a Japanese group (6). Mice orally administered with vanillin showed significantly reduced numbers of lung metastasized colonies compared to controls. *In vitro* studies revealed that vanillin, at concentrations that were not cytotoxic, inhibited invasion and migration of cancer cells and inhibited enzymatic activity of MMP-9 secreted by the cancer cells. Vanillin also showed growth inhibitory effect towards cancer cells in vitro. However, vanillic acid, a major metabolic product of vanillin in human and rat, was not active in these in vitro activity assays. Our findings suggest that vanillin has anti-metastatic potential by decreasing invasiveness of cancer cells. Since vanillin is generally regarded as safe, it may be of value in the development of anti-metastatic drugs for cancer treatment.

13. Effect of Thai Natural Products on Proteins Involved in Wound Healing

Dr. Rudee Surarit's laboratory at the Faculty of Dentistry, Mahidol University is interested in wound healing. Wound healing is a complex biologic event involving inflammation, cellular proliferation, and differentiation that involves the regeneration of both epithelial and connective tissue components. Generally, the wound healing process is composed of two phenomena, firstly clotting and epithelialization, and secondly connective tissue healing. During wound healing process, cells must proliferate and migrate into the wound site.

Various natural products, which have been claimed to give anti-inflammatory effect and promote wound healing process, based on traditional Thai folklore medicine. The present work aims to develop in vitro methods for study of wound healing. Representative cells used in this study are human gingival fibroblast and human oral keratinocyte. After the non-toxic concentration was identified, the effect of natural products on cell proliferation and cell migration were performed on a monolayer culture of cells. In addition, migration was also studied on a 3-dimensional wound healing model, so-called the dermal equivalent. Work so far has demonstrated the suitability of the techniques, using two compounds, plaunotal and propolis. Plaunotol is an acyclic diterpene alcohol, extracted from the leaves of *Croton stellatopilosus* or **Plau-noi**, a Thai medicinal plant, used for treatment of gastric ulcer and claimed to promote wound healing. Purified plaunotol extract was kindly provided by Dr. Amorn Petsom, Faculty of Science, Chulalongkorn University. Another substance, propolis is a product from honey bee, also been claimed to promote wound healing and shown to have anti-bacterial and anti-inflammatory activity, was kindly provided by Dr. Ratna Japa, Mee Fah Luang University. The results showed that the system can be used successfully to test wound healing activity. Plaunotol at the concentration of 0.01 μg/ml can promote wound healing using this test system. Thus, plaunotol could induce migration of cells into the wound at a higher rate than the induction by epithelial growth factor, used as positive control. Propolis collected in Thailand also showed promotion of *in vitro* wound healing, but showed lower promotion of healing

activity than propolis collected in Nepal. This may due to the differences in the plants that the honey bee used for making propolis. The purified extract from **Plau-yai** (*Croton oblongifolius*) was also tested and the wound healing promoting activity was found to be lower than plaunotol.

C. Potential Applications of Enzymes in Biotechnology

Although, most of the previous projects described in the first theme have potential applications, their main focus is on providing better understanding of protein structure and function relationships. The projects in the third theme of this section are more geared towards exploring the potential applications of enzymes, as described below.

14. Isolation and Characterization of a Sericin-specific Protease for Use in Degumming of Silk

Silk consists mainly of fibroin, the silk fibers, and sericin, the gummy proteins holding the fibers together. Sericin is responsible for the hardness of silk-fiber texture and causes difficulties in the dyeing of silk. Therefore, it is necessary to remove sericin using the process called silk degumming. Degumming of silk traditionally requires alkaline solutions, consumes much water and energy, and pollutes environment. In addition, control of the process is difficult and depends largely on workers' skills. Enzymatic degumming of silk is milder and more specific for sericin than fibroin, depending on selection of enzymes and conditions. However, available enzymes are not efficiently used or willingly accepted by Thai-silk weavers.

Dr. Pramvadee Y. Wongsaengchantra at the Faculty of Science, Mahidol University, is trying to isolate a protease to specifically digest sericin, without digesting fibroin. This protease may form the basis of alternative methods for degumming of Thai silk using milder, less destructive conditions than the traditional methods. Her work started by screening of bacteria from various sources for their ability to change the appearance of raw silk. Soil enrichment and selection of bacteria were performed and several enzyme test protocols established including a comparative radial diffusion assay, thin-layer enzyme assay, native- and SDS-submerged zymographic techniques, and finally quantitative assessment of degumming efficiency of silk.

One of the bacteria isolated and screened from an area of silk industrial waste in Thailand, the strain CRC_6NB produced enzymes suitable for silk degumming. It could change the appearance of raw silk from hard threads to become spherical cotton ball-like forms. Its extracellular enzyme could silk sericin better than silk fibroin based on cRD+TEA (Figure 11). Such crude enzyme was also used for degumming efficiency test both qualitatively and quantitatively. Tests of degumming efficiency by scanning electron microscopy (SEM), followed by dyeing with a sericin-sepcific dye showed that the CRC_6NB enzyme could remove sericin just like Alcalase®, a commercial crude enzyme, but did not cause surface fibrillation on the silk threads (Figure 11). A protease with greater activity towards sericin than fibroin has been purified, and its amino acid sequence is being determined. In addition, efforts are being made to explore enzymes from endophytes, as well as the enzyme cocoonase from the silkworm moth *Bombyx mori*. These studies should enable us to obtain suitable enzyme(s) for silk degumming.

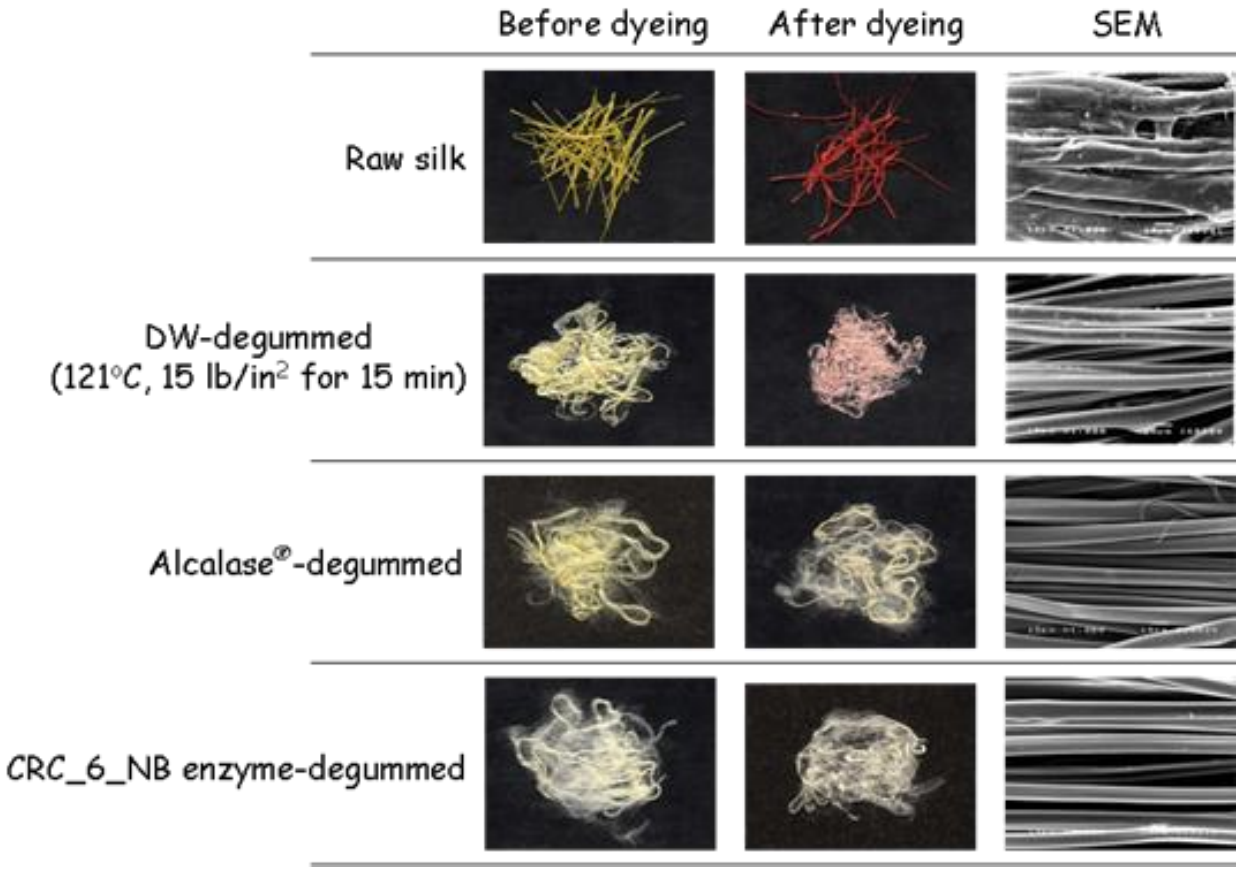

Figure 11: Qualitative Degumming Efficiency Test: Evaluation of the efficiency of degumming was based on the resulting fiber morphology when differentially dyed for remaining sericin and by scanning electron microscope (SEM). Enzymatic degumming conditions (for Alcalase® and crude enzyme CRC_6_NB) had equal protein concentration (200 μg/ml BSA equivalent based on Bradford Assay) and incubation for 4 days at pH 7, 37°C.

15. Screening and Purification of Naringinase from Fungi

Dr. Patjaraporn Wongvithoonyaporn's laboratory at the Department of Biochemistry, Faculty of Liberal Arts and Science, Kasetsart University, Kamphangsaen Campus focuses on studying the enzymology, production, and application of fungal naringinase, which can hydrolyse naringin, one of the bitter substances, mostly found in citrus fruit. Citrus fruits contain high amounts of the limonoid, naringin and neohesperidin, bitter flavonoids, found in the immature stage, which decreases during ripening. Naringin, 4′, 5′, 7′-trihydroxyflavonone-7-rhamnoglucoside, is the major compound causing bitterness in fruit juices, especially in grapefruit. These effects cause industrial fruit juice manufacturers to use various methods, usually chemical ones, to remove bitterness in their fruit products. Removal of naringin may be performed by a combination of methods, both chemical and enzymatic.

Naringinase is a key enzyme which hydrolyses naringin to prunin and naringenin, which are less bitter. This enzyme has two activities, α-rhamnosidase (EC 3.2.1.40) and flavonoid β-glucosidase (EC 3.2.1.21), which act consecutively to hydrolyse naringin in 2 steps. First, naringin will be hydrolysed by α-rhamnosidase into prunin, 4′, 5′, 7′-trihydroxyflavonone-7-glucoside, and rhamnose. Then the flavonoid β-glucosidase will hydrolyse prunin into narigenin, 4′, 5′, 7′-trihydroxy-flavonone, and glucose. From primary and secondary screening of 708 fungi, isolated from 340 host samples collected from 11 provinces in Thailand and a district in China, 73 isolates were obtained**.** Enzyme activities of naringinase, α-rhamnosidase and β-glucosidase were measured at pH 3.0, 4.0 and temperature at 50°C, 55°C and 60°C. Nine fungi, were selected. Four fungal isolates, were already taxonomically identified, and these isolates were kept at the National Center for Genetic Engineering and Biotechnology (BIOTEC) for long term and safe preservation. The other 5 fungal isolates, were sent to identify their species by base sequence analyses at BIOTEC. The nine fungal isolates are being optimized in temrs of growth conditions and production of naringinase enzyme.

16. Preparation of Amorphous Silica from Rice Husk Using Enzymatic Approach

The Materials Science and Ceramic Technology Research Groups of Mae Fah Luang University is focused on finding new uses for agricultural waste, which are appropriate for local development and for ceramic applications. Rice husk ash is of interest, since it is an excellent source of high purity active silica. In Thailand alone, over 5 million tons of rice husk are generated annually as a by-product of rice production. The main constituents of rice husk are cellulose, hemicellulose and lignin, making up approximately 80-85% of the total mass. Burning in open air is the simplest method to obtain silica from rice husk, but this can make silica lose its desirable properties, such as high surface area and amorphous characteristics, if conditions are uncontrolled or temperature is too high. As firing temperature increases, the amount of crystalline silica in rice husk ash also tends to increase and the nanoporous structure of silica collapses. Crystalline silica is not only less active as a starting material in many ceramic applications, but it can also be more harmful to health than amorphous silica. High purity silica can also be prepared from rice husk ash by chemical extraction methods. However, strong,

corrosive acid and base such as HCl and NaOH must be used in the process, which is time consuming and very tedious to completely eliminate the NaCl residue.

Dr. Suthee Wattanasiriwech has been interested to develop an alternative method involving digestion of the organic components of rice husk using cellulase enzymes. There have been many reports on the saccharification of wood, paper, and agricultural waste using fungi and bacteria producing celllulase. Thus, cellulase enzymes secreted from fungi such as *Trichloderma* and *Aspergillus* may be useful digesting for the polysaccharides leaving insoluble amorphous silica with the highly porous nanostructure preserved. Thus, spores of *Trichoderma reesei* were cultured on rice husk or rice straw for 10 days, leading to loss of weight of rice husk by 8% and rice straw by 37%. Concomittantly, % ash content of the remains increased, up to 22%. However, cellulase treatment alone may not be enough, since rice husk and rice straw also contain lignin, which is not degraded by cellulase. However, delignification method can be found in local mushroom farms, where mushrooms often have enzymes capable of delignification. Accordingly, rice straw waste from mushroom farms was used as raw material instead of untreated rice straw, and this led to an increase in the % ash content recovered.

IV OTHER ACTIVITIES

1. Senior Research Scholar Meetings

1.1 Joint Senior Research Scholar Meeting, September 14-15, 2004

On September 14-15, 2004, Professor Jisnuson Svasti held a joint Senior Research Scholar Meeting on "Integration of Biological Science, Protein Chemistry, and Medicine", together with Professor Suthat Fucharoen and Professor Siriwat Wongsiri. Both investigators are collaborators of Professor Jisnuson Svasti. This meeting was attended by more than 120 participants. The meeting covered a broad range of topincs from biological science to protein chemistry to medical science. Each participant had something to learn from participants in other fields, and developed a broader outlook on research.

1.2 Second Protein Research Network Symposium, 22-23 September 2005

Professor Jisnuson Svasti held the second Senior Research Scholar Meeting on his own, and expanded it into the Second Protein Research Network Symposium, held at the Chulabhorn Research Center, 22-23 September 2005. This meeting brought together protein scientists in Thailand, and is a follow up of the first symposium in the series, which was held in 2001. Apart from the budget of 60,000 baht allocated from the TRF grant, additional support was provided to the present meeting by the Chulabhorn Research Institute (60,000 baht), the Faculty of Science, Mahidol University (costs of abstract book, equivalent to 40,000 baht), and Biochemical Section of the Science Society of Thailand (20,000 baht). As result, registration was free.

The Second Protein Research Network Symposium exceeded expectations, and was attended by as many as 396 registered participants (including staff and students), almost double the 201 participants, attending the first meeting in 2001. These participants came from more 16 institutions, including Mahidol University, Kasetsart University, NSTDA/BIOTEC, Chulalongkorn University, Thammasat University, Suranaree University of Technology, Khon Kaen University, Prince of Songkhla University, Chulabhorn Research Institute. Naresuan University, King Mongkut's University of Technology Thonburi, National Institute of Technology, Srinakarintrwirot University, Silpakorn University, as well as from companies.

The scientific program covered three broad areas: Structure & Function of Proteins and Enzymes; Proteins and Enzymes in Systemic Biochemistry; Proteomic Research in Thailand. Oral presentations included consisted of 2 Keynote Lectures (Drs. Suthat Fucharoen and Wanpen Chaicumpa), 3 Special Lectures (Drs. Jisnuson Svasti, Supa Hannongbua, and Pornswan Wasant), as well as 22 Invited Lectures. Of the Invited Lecturers, two overseas were from Austria and four were young Ph.D.s who graduated less than 2-3 years ago. Other important activities included the Poster Session and Exhibition. Some 60 posters were presented, and several prizes were awarded to students. Twelve companies supported the meeting by exhibiting equipment and supplies related to protein science.

2. Protein Society of Thailand

The excellent attendance at the Second Protein Research Network Symposium indicated that there is much nationwide interest in protein science throughout Thailand. Indeed, one of sessions in the meeting discussed the possibility of forming a Protein Society. Accordingly, the Protein Society of Thailand was formed in January 2006 (although still written as a club according to official Thai regulations). The goals of the society are to:

- Strengthen capability in protein research in Thailand
- Exchange expertise and transfer of new technologies among members
- Initiate activities with novelty and creativity in field of protein research
- Liaise with local and international scientific organizations
- Publicize basic knowledge of protein biochemistry and protein technologies to the scientific community

The Committee Members are M.R. Jisnuson Svasti (Chairman), Worachart Sirawaraporn (Deputy-Chairman), Dumrongkiet Arthan (Secretary-General), Chantragan Phiphobmongkol (Treasurer), Kittisak Yokthongwattana (Deputy-Secretary), Rudee Surarit, James-Ketudat Cairns, Chartchai Krittanai, Visith Thongboonkerd, Pimchai Chaiyen, Jirundon Yuvaniyama.

There are four classes of members: a) Ordinary: b) Student: c) Honorary: d) Company. Membership fees for Ordinary Members are 500 baht/year or 2,500 baht for life, while fees for student members are only 100 baht/year. An innovation was the initiation of company membership, where companies can support activities in protein science. So we now have company members at various levels: Gold, Silver and Regular, which have different membership fees and privileges. A website has been established, which will play an important role in the activities of the society: http://www.cbag2.sc.mahidol.ac.th/protein/. This provides information about the society, its activities, links to sites of interest, members, as well as a web board for communication among members.

The Protein Society of Thailand has organised and co-sponsored various meetings. In particular, Professor Jisnuson Svasti's third Senior Research Scholar meeting was held as the First Annual Meeting of the Protein Society of Thailand, at the held 24-25 October 2006 at the Chulabhorn Research Institute Conference Center, Bangkok (Figure 12). The program included Keynote Lecures (two from overseas), 10 invited lectures (one from overseas), 7 Oral presentations, 2 Luncheon Lectures, given by companies and 65 poster presentations. The highlight was the Opening Lecture by Professor Richard Simpson of the Ludwig Institute, Australia on membrane proteomics. In addition two overseas speakers came from the National Synchrotron facilility in Taipei. The meeting was well attended by more than 300 registered persons, including several younger researchers, as well as Ph.D. and M.Sc. students. An important feature of our meeting is to provide opportunities for young Ph.D.s, who have recently returned to Thailand. The most promising are invited to deliver lectures, and others are encouraged to participate and present posters. We believe that this provides an important introduction to young overseas Ph.D. graduates to familirize themselves with the local scientific scene in Thailand, and to make contacts.

Figure 12. First Annual Symposium of the Protein Society of Thailand, held at the Chulabhorn Research Center Conference Center, 24-25 October 2006

In addition, the Protein Society of Thailand has co-sponsored two workshops on important and novel protein technologies. Firstly, the Protein Society of Thailand co-sponsored a Workhop on Protein Crystallography at the Siam Photon Laboratory, National Synchrotron Research Center, Nakhon Rachasima, July 20-23, 2006. The workshop included overseas speakers such as Malcolm Walkinshaw (Edinburgh), Rob Robinson (Singapore), and Chung-Jung Jun (Taiwan). It was attended by some 50 participants. The National Synchrotron Research Center plans to upgrade its facilities to hard X-rays and establish a beamline for protein crystallography, and we are hopeful that protein structure analysis using synchrotron radiation will soon be available in Thailand. Another important event was the Protein Society of Thailand's co-sponsorship of a Proteomics Workshop with BIOTEC, in May 2007. The lecture was attended by over 150 participants, so that there were so many participants at the hands-on workshop, that a second session had to be organised later. These two workshops demonstrate the intense interest in novel protein technologies, and the role that the Protein Society of Thailand can play in improving knowledge and skills in such technologies.

3. Asian-Oceanian Human Proteome Organisation (AOHUPO)

The Human Proteome Organisation (HUPO) was launched on 9th February 2001 to foster international proteomic initiatives to better understand human disease, in analogy to the genomic initiatives of the Human Genome Organisation (HUGO). The Asian and Oceania Human Proteome Organization (AOHUPO) (http://www.aohupo.org) was founded on 7 June 2001 as a regional satellite of HUPO. Professor Jisnuson Svasti was invited to become a member of the AOHUPO Council, representing Thai protein scientists and the Protein Society of Thailand. AOHUPO now has 15 member societies and holds conferences every two years. It has also started a Membrane Proteome Initiative for standardisation of proteomic analysis of membrane proteins, by sending a standard membrane preparation for analysis in laboratories of several countries, including Professor Svasti's laboratory at the Chulabhorn Research Institute.

4. Asia-Pacific International Molecular Biology Network (A-IMBN)

The Asia-Pacific International Molecular Biology Network (A-IMBN) (http://www.a-imbn.org) is an international organisation having outstanding scientists from the Asia-Pacific region as its members. It aims to promote biology at the molecular level in the the region, similar to that done in Europe by the European Molecular Biology Organisation (EMBO). Professor Svasti represents Thailand on the Governing Council of A-IMBN. Although A-IMBN is not focussed solely on proteins, the aim of promoting understanding of biology at the molecular level undoubtedly requires understanding of protein structure, function, expression, and site of action.

5. Editor, *ScienceAsia*

In his role as a senior scientist in Thailand, Professor Jisnuson Svasti has tried to help the development of Thai journals of international quality, by assuming the office of Editor of *ScienceAsia*. This research journal was originally published by the Science Society of Thailand since 1975 as the Journal of the Science Society of Thailand, and was renamed *ScienceAsia* in 1998. He is helped in this effort by Worachart Sirawaraporn, James Ketudat-Cairns and Prachumporn Kongsaeree as Associate Editors. The journal fulfils the requirements of an international journal, as defined by the Thailand Research Fund, Commission for Higher Education, and National Science and Technology Agency. It is listed in various international databases, such as SciFinder CA Plus, Biological Abstracts, and most recently SCOPUS. *ScienceAsia* helps to promote scientific research in Thailand as international journal of publishing articles of quality from Thai and overseas authors.

Professor Svasti has been editor of the journal twice for three years in 1985-1987 and recently seven years in 2001-2008. In this second term, efforts have been made to improve scientific quality of the journal and make it more international. This has met with good success, since 20-25% of submitted papers come from overseas, and the remaining are drawn from some universities and institutions within the country. In addition, in recent years, Professor Svasti has written several Editorials in *ScienceAsia*, highlighting several various important issues concerning science in Thailand, including the scientific output of Thai scientists in international journals, the quality of journals published in Thailand, and the relationship between teaching and research, as shown in the list of publications shown below. In addition, a Special Issue was published on the Occasion of the 80th Birthday Anniversity of His Majesty King Bhumibol Adulyadej, containing articles on a variety of scientific issues, by Outstanding Scientists of Thailand, Senior Scholars of the Thailand Research Fund, and Senior Administrators of scientific organisations in Thailand.

6. Dean, Faculty of Graduate Studies, Mahidol University

During the last 2 years of the grant, Professor Jisnuson Svasti became Dean of the Faculty of Graduate Studies at Mahidol. University, but was permitted to continue to complete the grant, as a special consideration. In this role, Professor Svasti has been able to promote and strengthen graduate education, which is a key to producing the badly needed researchers of the future, not only in protein science, but also in other fields. Although this position has taken much time, this grant has able to fulfil and even exceed the expectations listed in the grant proposal in terms of publications in international journals, as shown below.

V. Output

As seen above, the present grant is focused on proteins, but covers a range of projects in the areas of protein structure and function, roles in disease and potential applications. This broad coverage results from the fact that the principal investigator has not imposed any particular research area, but has allowed young investigators to have freedom of choice in the selection of projects. Some projects were more successful than others, and output is shown below in terms of publications in international journals, presentations in international and local meetings, and production of M.Sc. and Ph.D. graduates.

Publication in international journals is accepted as the most critical of the quality of research. This grant produced 47 papers in international journals (Section VI), more than 50% greater than the 30 international papers projected in the original proposal. Of the 47 publications, 37 papers had journal impact factors in 2006 (JIF2006) (Figure 13): of these, 4 had JIF2006 of 0.01-1.00, 12 had JIF2006 of 1.01-2.00, 9 had JIF2006 of 2.01-3.00, 5 had JIF2006 of 3.01-4.00, 3 had JIF2006 of 4.01-5.00, 3 had JIF2006 of 5.01-6.00, and 1 had JIF2006 of 9.643. Average JIF2006 of these 37 papers was 2.818. Of the other 10 papers in international journals, about half were in international journals published in Thailand and listed in Scopus, but the other half were in newly published overseas journals, which are likely to have impact factors in future. In addition to the above, the group also published 3 research articles in International Proceedings volumes.

The principal investigator also published 8 non-research articles in international journals, including 2 special articles in *IUBMB Life* with JIF2006 of 2.116, and another 6 Editorials in *ScienceAsia*. Other types of output included deposition of 4 sequences in databases, and one patent application.

The group also made several presentations in international meetings (Section VIII), with 19 presentations in the first year, 12 presenstations in the second year, and 22 presentations in the third, amounting to 53 presentations over the period of the grant. Students and staff also made many presentations at national level (Section IX), with 6 presentations in the first year, 32 presentations in the second year, and 36 presentations in the third year, amounting to a total of 74 presentations over the period of the grant.

The number of Ph.D.s produced (Section X) during the grant period was only seven, fewer than expected. However, another 37 students are in the process of studying for their Ph.D. degrees, so it is hoped that several more Ph.D.s will graduate in the next few years. In addition, another 12 M.Sc. graduates were produced during the grant period, and another 20 are currently studying for their M.Sc. degrees. Finally, 40 B.Sc. students undertook senior year research with staff members in the grant. In addition, 12 students, mainly Ph.D. students, had the opportunity for research and/or training activities abroad, broadening their outlook.

The development of young staff is another important consideration. Its success can be demonstrated by the ability of staff to publish good articles in international journals, as described above. The ability of staff to attract research funding is another indicator of their ability to compete with others. In addition, to the present grant, and grants provided to the Center for

Protein Structure and the Chulabhorn Research Institute, many young staff also had their own competitive research grants, as shown in Section XI. Finally, staff won various awards, promotions, and research fellowships (Section XII). Thus, staff won 6 awards for research or academic activity, including the award of the prestigious Dushi Mala medal to Professor Svasti. Four staff received promotion to Assistant Professor or Associate Professor, and another two staff received overseas research fellowships.

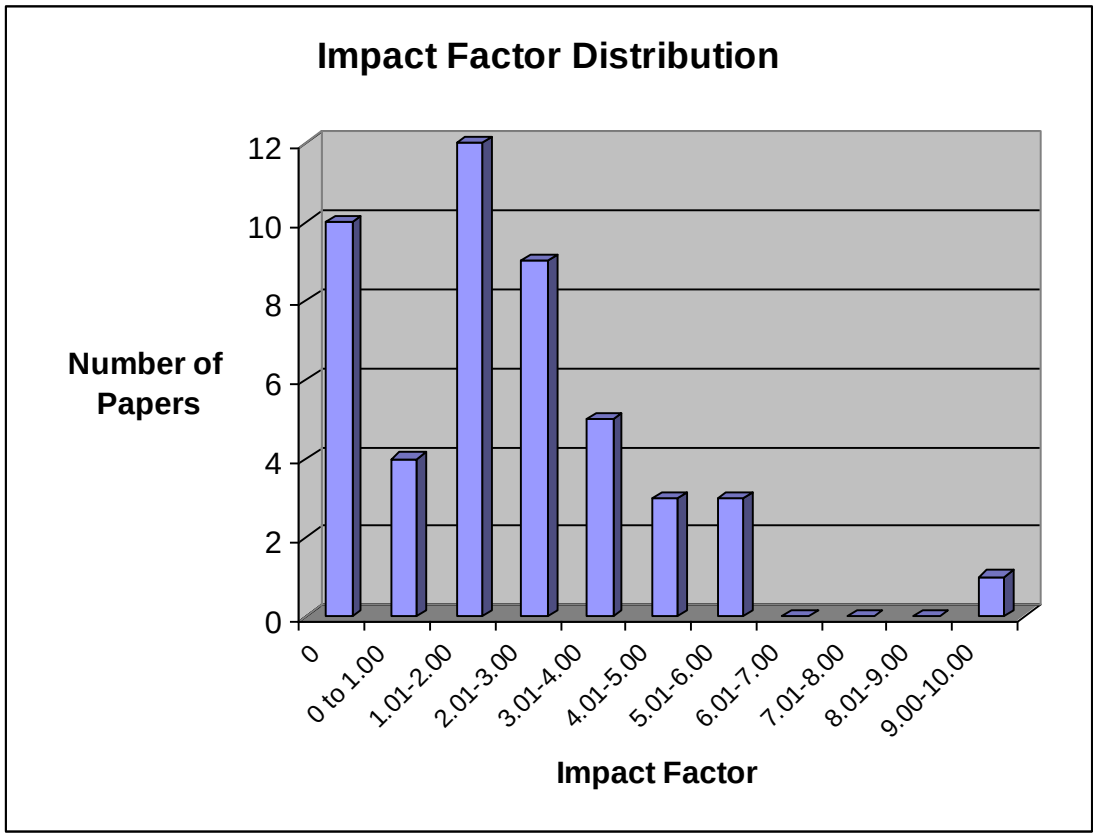

Figure 13. Impact Factor Distribution of 47 research papers obtained from the grant. Of these, 37 were published in ISI-WOS journals having impact factors (JIF2006), and the remaining 10 were published in international journals, printed overseas or in Thailand, and listed in international databases, such as Scopus or Pubmed. In addition to these, another 3 research papers were published in international proceedings volumes. Apart from the research papers, another 8 non-research papers were published in international journals, including two in a journal with impact factor of 2.116.

VI. Research Articles in International Journals and Proceedings

1. Publications in International Journals

YEAR 1

1. Kubota, M., Tsuji, M., Nishimoto, M., Wongchawalit, J., Okuyama, M., Mori, H., Matsui, M., Surarit, R., *Svasti, J.*, Kimura, A. and Chiba, A. (2004) Localization of α-Glucosidases in Organs of European Honeybees, *Apis mellifera* L., and the Origin of α-Glucosidase in Honey. *Biosci. Biotechnol. Biochem.* **68**, 2346-2352. (Impact Factor 2006 = 1.256)

2. Thotsaporn, K., Sucharitakul, J., Wongratana, J., Suadee, C., *Chaiyen, P.* (2004) Cloning and expression of p-hydroxyphenylacetate 3-hydroxylase from *Acinetobacter baumannii*: evidence of the divergence of enzymes in the class of two-protein component aromatic hydroxylases. *Biochim Biophys Acta* **1680**(1), 60-6. (Impact Factor 2006 = 2.293)

3. Ngiwsara, L., Srisomsap, C., Winichagoon, P., Fucharoen, S., Sae-Ngow, B., and *Svasti, J.* (2005) Hb Kurosaki [α7(A5)Lys-Glu (*A*ag-*G*ag)]: an α2-Globin Gene Mutation Found In Thailand. *Hemoglobin,* **29**(2), 155-9. (Impact Factor 2006 = 0.516)

4. Oonanant, W., Sucharitakul, J., *Yuvaniyama, J.*, and *Chaiyen, P.* Crystallization and preliminary X-ray crystallographic analysis of 2-methyl-3-hydroxypyridine-5-carboxylic acid (MHPC) oxygenase from *Pseudomonas sp. MA-1*. *Acta Crystallographica* **F61**, 312-314. (Impact Factor 2006= -)

5. *Buranaprapuk, A.,* Chaivisuthangkura, P., *Svasti, J.,* and Kumar, C. V. (2005) Efficient Photocleavage of Lysozyme by a new Chiral Probe. *Letters in Organic Chemistry* **2**(6), 554-558. (Impact Factor 2006 = 1.004)

6. Lirdprapamongkol, K., Sakurai, H., Kawasaki[a], N., Choo, M.-K., Saitoh, Y., Aozuka, Y., Singhirunnusorn, P., Ruchirawat, S., *Svasti, J.* and Saiki, I. (2005) Vanillin Suppresses *in vitro* Invasion and *in vivo* Metastasis of Mouse Breast Cancer Cells. *Europ. J. Pharmaceut. Sci.* **25**(1), 57-65. (Impact Factor 2006= 2.482)

7. Chuankhayan P., Hua Y., *Svasti J.*, Sakdarat S., Sullivan PA., and *Ketudat Cairns JR.* (2005) Purification of an Isoflavonoid 7-O- β-glycoside β-glycosidase and its substrates from *Dalbergia nigrescens* Kurz. *Phytochemistry*, **66**, 1880-1889. (Impact Factor 2006= 2.417)

8. Sucharitakul, J., *Chaiyen, P.,* Entsch, B., and Ballou, D.P. (2005) The reductase of p-hydroxyphenylacetate 3-hydroxylase from *Acinetobacter baumannii* requires p-hydroxyphenylacetate to control activity. *Biochemistry* **44**, 10434-42. (Impact Factor 2006=3.633)

9. Wasant, P., Vatanavichien, N., Srisomsap, C., Sawangareetrakul, P., Liammongkolkul, S. and *Svasti, J.* (2005) Retrospective Study of Patients with Suspected Inborn Errors of Metabolism at Siriraj Hospital, Bangkok, Thailand (1997-2001) *J. Med. Assoc. Thai* **88**, 746-753. (Impact Factor 2006 = -)

10. *Suginta, W.,* Vongsuwan, A., Songsiriritthigul, C., *Svasti, J.*, and Prinz, H. (2005) Enzymatic Properties of Chitinase A from *Vibrio carchariae* and the Active Site Mutants as Revealed by HPLC-Mass Spectrometry. *FEBS Journal* **272**, 3376-3386. (Impact Factor 2006 =3.033)

11. *Svasti, J.*, Srisomsap, C., Subhasitanont, P., Keeratichamroen, S., Chokchaichamnankit, D., Ngiwsara, L., Chimnoi, N., Pisutjaroenpong, S., Techasakul, S., and Chen, S.T. (2005) Proteomic Profiling of Cholangiocarcinoma Cell Line Treated with Pomiferin from *Derris malaccensis*. *Proteomics* **5** (17), 4504-9. (Impact Factor 2006 =5.735)

12. Wasant, P., Viprakasit, V., Srisomsap, C., Liammongkolkul, S., Ratanarak, P., Sathienkijakanchai, and *Svasti, J.* (2005) Argininosuccinate Synthetase Deficiency: Mutation Analysis in 3 Thai patients. *Southeast Asian J. Trop. Med. Pub. Health,* **36** (3):757-61. (Impact Factor 2006= -)

YEAR 2

13. *Ketudat Cairns JR*, Keeratichamroen S, Sukcharoen S, Champattanachai V, Ngiwsara L, Lirdprapamongkol K, Liammongkolkul S, Srisomsap C, *Surarit R*, Wasant P and *Svasti J.* (2005). The molecular basis of mucopolysaccharidosis Type 1 in two Thai patients. *Southeast Asian Journal of Tropical Medicine and Public Health* **36,** 1308-1312. (Impact Factor 2006= -)

14. Mahakhan, P., Chobvijuk, C., Ngmjarearnwong, M., Trakulnalermsai, S., Bucke, C., *Svasti, J.*, Kanlayakrit, W. and Chitradon, L. (2005) Molecular hydrogen production by a thermotolerant *Rubrivivax gelatinosus* using raw cassava starch as an electron donor. *ScienceAsia* **31 (4),** 415-24. (Impact Factor 2006= -)

15. Hommalai, G., *Chaiyen, P.,* and *Svasti, J.* (2005) Studies on the Transglucosylation Reactions of Cassava and Thai Rosewood β-Glucosidases using 2-Deoxy-2-fluoro-glycosyl Enzyme Intermediates. *Arch. Biochem. Biophys.* **442** (1), 11-20. (Impact Factor 2006=2.969)

16. *Arthan, D.,* Kittakoop, P., Esen, A., and *Svasti, J.* (2006) Furostanol Glycoside 26-O-β-Glucosidase from the Leaves of *Solanum torvum*. *Phytochemistry,* **67** (1), 27-33. (Impact Factor 2006=2.417)

17. Jintaridth, P., Srisomsap, C., Vichittumaros, K. Kalpravidh, R.W., Winichagoon, P. Fucharoen, S., Svasti, M.R.J. and Kasinrerk, W. (2006) Chicken Egg Yolk Antibodies Specific for the Gamma (γ) Chain of Human Hemoglobin for Diagnosis of Thalassemia. Int. J. Hematol. **83**, 408-414. (Impact Factor 2006=1.295)

18. *Thammasirirak, S.*, Ponkham, P., Preecharram, S., Khanchanuan, R., Phonyothee, P., Daduang, S., Araki, T. and *Svasti, J.* (2006) Purification, characterization and comparison of reptile lysozyme. *Comparative Biochemistry and Physiology*, *Part C,* **143**, 209–217. (Impact Factor 2006=1.991)

19. *Toonkool, P.*, Metheenukul, P., Sujiwattanarat, P., Paiboon, P., Tongtubtim, N., Ketudat-Cairns, M., *Ketudat-Cairns, J.* and *Svasti, J.* (2006) Expression and purification of dalcochinase, a β-glucosidase from *Dalbergia cochinchinensis* Pierre, in yeast and bacterial hosts. *Protein Expression and Purification* **48,** 195–204. (Impact Factor 2006=1.867)

20. Chuenchor W, Pengthaisong S, *Yuvaniyama J*, Opassiri R, *Svasti J* and *Ketudat Cairns JR*. 2006. Purification, Crystallization and Preliminary X-ray Analysis of Rice BGlu1 β-Glucosidase with and without 2-Deoxy-2-fluoro-β-D-glucoside Inhibitor. *Acta Crystallographica* **62** *Section **F***, 798-801. (Impact Factor 2006= -)

21. Sucharitakul, J., *Chaiyen, P.*, Entsch, B., Ballou, D.P. (2006) Kinetic Mechanisms of the Oxygenase from a Two-component Enzyme, p-hydroxyphenyl acetate 3-Hydroxylase from *Acinetobacter baumannii*. *J Biol Chem.* 281(25), 17044-53. (Impact Factor 2006=5.808)

YEAR 3

22. Boonclarm, D., Sornwatana, T., *Arthan, D.*, *Kongsaeree, P.* and *Svasti, J.* (2006) A β-glucosidase catalyzing specific hydrolysis of an iridoid β-glucoside from *Plumeria obtusa* flowers. *Arch. Biochim. Biophys. Sinica* **38**, 563-570. (Impact Factor 2006=0.931)

23. Wattanasirichaigoon, D., *Svasti, J.*, *Cairns, J.R.*, Tangnararatchakit, K., Visudtibhan, A., Keeratichamroen, S., Ngiwsara, L., Khowsathit, P., Onkoksoong, T., Lekskul, A., Mongkolsiri, D., Jariengprasert. C., Thawil, C., and Ruencharoen, S. (2006) Clinical and molecular characterization of an extended family with Fabry disease. *J. Med. Assoc. Thailand* **89** (9): 1528-1535. (Impact Factor 2006 = -)

24. Wongchawalit, J., Yamamoto, T., Nakai, H., Kim, Y.M., Sato, N., Nishimoto, M., Okuyama, M., Mori, H., Saji, O., Chanchao, C., Wongsiri, S., *Surarit, R.*, *Svasti, J.,* Chiba, S., and Kimura. A. (2006) Purification and Characterization of alpha-Glucosidase I from Japanese Honeybee (*Apis cerana* japonica) and Molecular Cloning of Its cDNA. *Biosci. Biotechnol. Biochem.* **70**, 2889-2898. (Impact Factor 2006=1.256)

25. Subhasitanont, P., Srisomsap, C., Punyarit, P., and *Svasti, J.* (2006) Proteomic Studies of Galectin-3 Expression in Human Thyroid Diseases by Immunodetection. *Cancer Genomics and Proteomics* **3**, 389-394. (Impact Factor 2006 = -)

26. Opassiri R, Pomthong B, Okoksoong T, Akiyama T, Esen A, *Ketudat Cairns JR*. (2006). Analysis of Rice Glycosyl Hydrolase Family 1 and Expression of *Os4bglu12* β-Glucosidase. *BMC Plant Biology* **6**, 33. (Impact Factor 2006= -)

27. Sangvanich, P., Kaeothip, S., Srisomsap, C., Thiptara, P., Petsom, A., Boonmee, A., *Svasti, J.* (2007) Hemagglutinating activity of Curcuma plants. *Fitoterapia* **78**, 29-31. (Impact Factor 2006=0.908)

28. Srisomsap, C., Subhasitanont, P., Sawangareetrakul, P., Chokchaichamnankit, D., Ngiwsara, L., Chiablaem, K., and *Svasti, J.* (2007) Comparison of Membrane-Associated Proteins in Human Cholangiocarcinoma and Hepatocellular Carcinoma Cell lines. *Proteomics: Clinical Applications*. **1**, 89-106. (Impact Factor 2006=5.735)

29. Alfieri, A., Fersini, F., Ruangchan, N., Prongjit, M., *Chaiyen*, *P.*, and Mattevi, A. (2007). Structure of the monooxygenase component of a two-component flavoprotein monooxygenase. *Proc. Natl. Acad. Sci. U.S.A.* 104, 1177-1182. (Impact Factor 2006=9.643)

30. Chuankhayan, P., Rimlumduan, T., *Svasti, J.* and *Cairns, J.R.K.* (2007) Hydrolysis of Soybean Isoflavonoid Glycosides by *Dalbergia* β-Glucosidases. *J. Agr. Food Chem.* **55**, 2407-2412. (Impact Factor 2006=2.322)

31. Keeratichamroen, S., *Cairns, J.R.K.*, Sawangareetrakul, P., Liammongkolkul, S., Champattanachai, V., Srisomsap, C., Kamolsilp, M., Wasant, P., and *Svasti, J.* (2007) Novel mutations found in two genes of Thai patients with isolated methylmalonic acidemia. *Biochem. Genet.* **45**, 421-430. (Impact Factor 2006= 0.876)

32. *Thammasirirak, S*., Preecharram, S., Ponkham, P., Daduang, S., Araki, T., and *Svasti, J.* (2007) New variant of quail egg white lysozyme identified by peptide mapping. *Comp. Biochem. Biophys. B Biochem. Mol. Biol.* Feb **147,** 314-324. (Impact Factor 2006=1.532)

33. *Suginta, W*., Kobdej, A., Opassiri, R., *Svasti, J*., and Songsiriritthigul, C. (2007) Mutation of Tryp275 and Trp397 altered the binding selectivity of *Vibrio carchariae* chitinase A. *Biochim. Biophys. Acta*. **1770**, 1151-1160. (Impact Factor 2006=2.293)

34. Hommalai, G., Withers, S.G., Chuenchor, W., *Cairns, J.R.K* and *Svasti, J.* (2007) Enzymatic synthesis of cello-oligosaccharides by rice BGlu1 β-glucosidase glycosynthase mutants. *Glycobiology* **17**, 744-753. (Impact Factor 2006=3.668)

35. Sucharitakul, J., Phongsak, T., Entsch, B., *Svasti, J., Chaiyen, P*., and Ballou, D.P. (2007) Kinetics of a Two-Component p-Hydroxyphenylacetate Hydroxylase Explain How Reduced Flavin Is Transferred from the Reductase to the Oxygenase. *Biochemistry* **46**, 8611-8623. (Impact Factor 2006=3.633)

36. Suwannarat, P., Keeratichamroen, S., Wattanasirichaigoon, D., Ngiwsara, L., *Ketudat Cairns, J. R., Svasti, J*., Visudtibhan, A., and Pangkanon, S. (2007) Molecular characterization of type 3 (neuronopathic) Gaucher disease in Thai patients. *Blood Cells, Molecules & Diseases.* **39**, 348-352. (Impact Factor 2006=2.678)

37. Nishimoto M., Mori, H., Moteki, T., Takamura, Y., Iwai, G., Miyaguchi, Y., Okuyama, M., Wongchawalit, J., *Surarit, R*., *Svasti, J*., Kimura, A., Chiba, S. (2007) Molecular Cloning of cDNAs and Genes for Three alpha-Glucosidases from European Honeybees, *Apis mellifera* L., and Heterologous Production of Recombinant Enzymes in *Pichia pastoris. Biosci. Biotechnol. Biochem.* **71**, 1703-1716. (Impact Factor 2006=1.256)

38. Suadee, C., Nijvipakul, S., *Svasti, J*., Entsch, B., Ballou, D.P., and *Chaiyen, P.* (2007) Luciferase from *Vibrio campbellii* is more thermostable and binds reduced FMN better than its homologues. *J. Biochem.(Tokyo)* **142**, 539-552. (Impact Factor 2006=1.963)

39. Chuankhayan, P., Rimlumduan, T., Tantanuch, W., Mothong, N., *Kongsaeree, P.T*., Metheenukul, P., *Svasti, J*., Jensen, O.N. and *Cairns, J.R.K.* (2007) Functional and Structural Differences Between Isoflavonoid β-Glycosidase from *Dalbergia sp. Arch. Biochem. Biophys.* **468**, 205-216. (Impact Factor 2006=2.969)

40. Opassiri, R., Pomthong, B., Akiyama, T., Nakphaichit, M., Onkoksoong, T., Ketudat Cairns M, *Ketudat Cairns JR* (2007). A stress-induced rice (*Oryza sativa* L.) beta-glucosidase represents a new subfamily of glycosyl hydrolase family 5 containing a fascin-like domain. *Biochem J.* **408**(2): 241-9. (Impact Factor 2006=4.100)

41. Nijvipakul, S., Wongratana, J., Suadee, C., Entsch, B., Ballou, D.P., and *Chaiyen, P.* (2008) LuxG Is a Functioning Flavin Reductase for Bacterial Luminescence. *J. Bacteriol.* **190**(5), 1531–1538. (Impact Factor=3.993)

42. Chuenchor, W., Pengthaisong, S., Robinson, R. C., *Yuvaniyama, J*., Oonanant, W., Bevan, D. R., Esen, A., Chen, C. J., Opassiri, R., *Svasti, J*., *Cairns, J. R.* (2008) Structural Insights into Rice BGlu1 beta-Glucosidase Oligosaccharide Hydrolysis and Transglycosylation. *J Mol Biol.* **377**(4), 1200-15. (Impact Factor 2006=4.890)

43. Sawangareetrakul, P., Srisomsap, C., Chokchaichamnankit, D. and *Svasti, J.* (2008) Galectin-3 Expression in Human Papillary Thyroid Carcinoma. *Cancer Genomics and Proteomics.* 5, no.2, 117-122. (Impact Factor 2006=-)

44. Lirdprapamongkol, K., Kramb, J.-P., Chokchaichamnankit, D., Srisomsap, C., Surarit, R., Sila-asna, M., Bunyaratvej, A., Dannhardt, G. and *Svasti, J.* (2008) Juice of *Eclipta prostrata* Inhibits Cell Migration *in vitro* and Exhibits Anti-angiogenic Activity i*n vivo. In Vivo.* In Press. (Impact Factor 2006=1.273)

45. Keeratichamroen, S., *Ketudat Cairns, J.R,* Wattanasirichaigoon, D., Wasant, P., Ngiwsara, L., Suwannarat, P., Pangkanon, S., Tanpaiboon, P., Rujirawat, T.and *Svasti, J.* (2008) molecular analysis of the iduronate-2-sulfatase gene in Thai Patients with Hunter syndrome. *J. Inher. Metab. Dis.* In Press. (Impact Factor 2006=1.574)

46. *Arthan, D.*, Sithiprom, S., Thima, K., Limmatvatirat C., Chavalitshewinkoon-Petmitr, P., *Svasti, J.* (2008) Inhibitory effects of Thai plants β-glycosides on *Trichomonas vaginalis*. *Parasitol Res.* In Press. (Impact Factor 2006=1.140)

47. *Buranaprapuk, A.*, Malaikaew, Y., *Svasti, J.* and Kumar, C. (2008) Chiral protein scissors activated by light: Recognition and protein photocleavage by a new pyrenyl probe. *J. Phys. Chem.B* In Press. (Impact Factor 2006=)

2. Publications in International Proceedings Volumes

1. *Svasti, J.,* Srisomsap, C., *Surarit, R* ., *Ketudat-Cairns, J* ., Techasakul, S. and *Toonkool, P* . (2004) Structure and properties of Thai plant β-glucosidases. In "*Protein Structure-Function Relationship*". (A. Abbasi and S.A. Ali, eds.), BCC&T, University of Karachi, Karachi, pp 221-240.

2. Oonanant W, Sucharitakul J, *Chaiyen P.* , and Yuvaniyama J. (2005) X-ray crystallographic analysis of 2-methyl-3-hydroxypyridine-carboxylic acid (MHPC) oxygenase from *Pseudomonas* sp. MA-1. in ***Flavins & Flavoproteins***, (Eds : Nishino, T., Miura, R., Tanokura, M. and Fukui, K.), ARchiTect inc, Tokyo, pp. 167-171.

3. Suadee, C., *Chaiyen, P.***,** *Svasti, J.*, Entsch, B., and Ballou, D. P. (2005) Luciferase from *Vibrio cambellii*-just a few Changes in Amino Acids Shine New Light on the problem, in ***Flavins & Flavoproteins***, (Eds : Nishino, T., Miura, R., Tanokura, M. and Fukui, K.), ARchiTect inc, Tokyo, pp. 611-616.

VII. Non-research Articles in International Journals and Other Output

1. Non-Research Publications in International Journals

1. *Svasti, M.R.J.* (2005) Thirty Years of *ScienceAsia, Journal of the Science Society of Thailand. ScienceAsia* **31**, 1-3.
2. *Svasti, J.* (2005) My Experience as an IUB Travel Fellow. *IUBMB Life* **57,** 255. (Impact Factor 2006 = 2.116).
3. *Svasti, J.* and Sawyer, W.H. (2006) FAOBMB Inc.: a Brief History. *IUBMB Life* **58**, 280 – 282. (Impact Factor 2006 = 2.116).
4. *Svasti, M.R. J.* and Asavisanu, R. (2006) Update on Thai Publications in ISI Databases (1999-2005). *ScienceAsia* **32**, 101-106.
5. *Svasti, M.R.J.* and Asavisanu, R. (2006) Don't Forget the Name of Your University and How It Is Spelt: another look at ISI databases. *ScienceAsia* **32**, 207-213.
6. *Svasti, M.R. J.* (2006) Teaching and Research: opposite faces of the same coin? *ScienceAsia* **32**, 333-335.
7. *Svasti, M.R. J.* and Asavisanu, R. (2007) Aspects of Quality in Academic Journals: A Consideration of the Journals Published in Thailand. *ScienceAsia* **33**, 137-143.
8. *Svasti, M.R. J.* (2007) Graduate Training, Research and Excellence: a view from Mahidol University. *ScienceAsia* **33**, 253-256.

2. Thai Patent Application

1. *Sompong Thammasrirak*, Patent application # 100769, "Method for extraction of lysozyme using heat and ascorbic acid, submitted 29 April 2005.

3. International Data base Entries

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2. Chantarangsee M, Mizuno K, Fujimura T, Ketudat Cairns J. (2002) *Oryza sativa* (japonica cultivar-group) beta-galactosidase mRNA, complete cds. Genbank accession: AF508799
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VIII. Presentations at International Meetings

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1. Svasti, M.R. J., Srisomsap, C., Ketudat-Cairns, J. and Surarit, C. (2004) Protein Changes in Human Disease. Invited Lecture, *The Fifth Princess Chulabhorn International Science Congress*, Bangkok, Thailand, 16-20 August 2004.
2. Subhasitanont, P., Srisomsap, C., Punyarit, P. and Svasti, J. (2004) Proteomic Analysis of the Expression of Galectin-3 in Human Thyroid Diseases. *The Fifth Princess Chulabhorn International Science Congress,* Bangkok, Thailand, 16-20 August 2004.
3. Sawangareetrakul, P., Srisomsap, C., Subhasitanont, P. and Svasti, J.(2004) Proteomic studies of biomarker from cholangiocarcinoma cell line. *The Fifth Princess Chulabhorn International Science Congress*, Bangkok, Thailand, 16-20 August 2004.
4. Srisomsap, C., Subhasitanont, P., Keeratichamroen, S., Chokchaichamnankit, D., Ngiwsara, L., Chimnoi, N., Pisutjaroenpong, S., Techasakul, S. and Svasti, J. (2004) Proteomic Studies of Apoptosis Induced in Cholangiocarcinoma Cell Line by Osajin from *Derris malaccensis*. The *Fifth Princess Chulabhorn International Science Congress*, Bangkok, Thailand, 16-20 August 2004.
5. Keeratichamroen, S., Ketudat-Cairns J.R., Sukcharoen S., Champattanachai, V., Ngiwsara L., Lirdprapamongkol K, Liammongkolkul S., Srisomsap, C., Surarit R., Wasant, P. and Svasti, M.R.J. (2004) Identification of the Molecular Basis of Mucopolysaccharidosis I in Thai Patients. *The Fifth Princess Chulabhorn International Science Congress on Evolving Genetics and Its Global Impact*, Bangkok, Thailand, 16-20 August 2004.
6. Ngiwsara, L., Sae-Ngow B., Srisomsap, C., Fucharoen, P., Fucharoen, S. and Svasti, M.R.J. (2004) Hemoglobin Kurosaki [α7 (A5) LYS (AAG) -> GLU (GAG)], an alpha-2 globin gene mutation found in Thailand. *The Fifth Princess Chulabhorn International Science Congress on Evolving Genetics and Its Global Impact*, Bangkok, Thailand, 16-20 August 2004.
7. Ketudat-Cairns JR, Opassiri R, Ketudat-Cairns M, Chantrarangsee M, Onkoksoong T, Chuenchor W, Akiyama T, *Svasti J* (2004) Functional genomics of rice beta-glycosidase genes. *The Fifth Princess Chulabhorn International Science Congress: Evolving Genetics and its Global Impact*, Bangkok, Thailand 16-20 August, 2004.
8. Ketudat-Cairns JR, Opassiri R, Ketudat-Cairns M, Chantarangsee M, Cheunchor W, Onkoksoong T, Pomthong B (2004) Investigation of rice beta-glycosidase gene functions. *Invited Lecture, First International Conference on Rice for the Future*, Kasetsart University, Bangkok, Thailand 31 August-3, Sept., 2004.
9. Onkoksoong T, Opassiri R, Pomthong B, Ketudat-Cairns JR (2004) Optimization of recombinant expression of a rice glycosyl hydrolase family 1 beta-glucosidase. *First International Conference on Rice for the Future* , Kasetsart University, Bangkok, Thailand 31 August-3, Sept., 2004.

10. Pomthong B, Opassiri R, Onkoksoong T, Akiyama T, Ketudat-Carins JR. (2004) Recombinant protein expression and functional characterization of a putatie cell wall-bound β-glucosidase from rice. *First International Conference on Rice for the Future*, Kasetsart University, Bangkok, Thailand 31 August-3, Sept., 2004.

11. Svasti J, Ketudat-Cairns J, Srisomsap C, Surarit R, Toonkool P (2004) Structure and catalytic activity of Thai plant β-glucosidases. (invited lecture) *17th FAOBMB Symposium / 2nd IUBMB Special Meeting /A-IMBN Meeting on Genomics and Health in the 21st Century*, Bangkok, Thailand, 22-26 November, 2004.

12. Ketudat-Cairns JR, Opassiri R, Chantarangsee M, Cheunchor W, Onkoksoong T, Pomthong B, Akiyama T, Ketudat-Cairns M, Svasti J (2004) Molecular and enzymatic characterization of β-glycosidases from rice, *Oryza sativa L. I*nvited Lecture, *17th FAOBMB Symposium / 2nd IUBMB Special Meeting /A-IMBN Meeting on Genomics and Health in the 21st Century*, Bangkok, Thailand, 22-26 November, 2004.

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16. Anguravirutt, S. and Svasti, J. (2004) Purification and Catalytic Properties of Alpha-Mannosidase from *Albizzia procera* Benth. *17th FAOBMB Symposium / 2nd IUBMB Special Meeting /A-IMBN Meeting on Genomics and Health in the 21st Century*, Bangkok, Thailand, 22-26 November, 2004.

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19. Chantarangsee M. (2005) Recombinant expression and characterization of substrate hydrolysis of rice beta-galactosidases. The Scotland Cell Wall Meeting, Glassgow, UK, June 2005.

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1. Srisomsap, C., Subhasitanont, P., Chokchaichamnankit, D., Sawangareetrakul, P. and J. Svasti. (2005) Enrichment of galectin-3 in thyroid papillary carcinoma using microscale solution-phase isoelectric focusing and LC/MS/MS. HUPO 4th Annual World Congress, Munich, Germany, 28 August-1 September, 2005.
2. Kantaputra, P.Mm, Limwongsee, Cm, van den Ouwelan, A.M.W., Ketudat Cairns, J. (2006) 4821delA Mutation of *NF1* underlies severe facial manifestation of neurofibromatosis. Craniofacial Morphogenesis & Tissue Regeneration, January 22-27, 2006, Four Points Sheraton: Harbortown, Ventura, CA, USA.
3. Srisomsap C, Chokchaichamnankit D, Subhasitanont P, Ngiwsara L, Chiablem K and Svasti J. (2006) Differential expression of membrane proteins in human cholangiocarcinoma cell lines treated with bisdemethoxycurcumin from *globba malaccensis* ridl. The 11th International Charles Heidelberger Symposium on Cancer Research, Naresuan University, Phitsanulok Thailand, 26-29 January 2006.
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8. Chuankhayan P, Limlumduan S, Mothong N, Svasti J, Ketudat Cairns JR. (2006) Molecular and enzymatic characterization of beta-glucosidase from *Dalbergia nigrescens* Kurz. 20th IUBMB International Congress of Biochemistry and Molecular Biology & 11th FAOBMB Congress. Kyoto, Japan, 18-23 June, 2006. 1P-B-188.

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11. Pornpimol Ponkham, Sakda Daduang, Jisnuson Svasti, Tomohiro Araki and Sompong Thammasirirak. The amino acid sequence of three reptile lysozymes. 20th IUBMB International Congress of Biochemistry and Molecular Biology and 11th FAOBMB Congress in conjunction with 79th Annual Meeting of the Japanese Biochemical Society. Kyoto International Conference Hall, Kyoto, Japan.

12. Sompong Thammasirirak, Supawadee Pata, Supansa Kitthaisong, Sutthidech Preecharram, Sakda Daduang, Nisachon Jangpromma and Anupong Tankrathok. Electrophoretic Pattern of Reptile Egg White Proteins. 20th IUBMB International Congress of Biochemistry and Molecular Biology and 11th FAOBMB Congress in conjunction with 79th Annual Meeting of the Japanese Biochemical Society. Kyoto International Conference Hall, Kyoto, Japan

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14. Ponkham, P., Daduang, S., *Svasti, J.*, Araki, T, and Thammasirirak, S. (2006) The Amino Acid Sequence of Three Reptile Lysozymes. 20th IUBMB International Congress of Biochemistry and Molecular Biology and 11th FAOBMB Congress in conjunction with 79th Annual Meeting of the Japanese Biochemical Society. Kyoto International Conference Hall, Kyoto, Japan.

15. *Svasti, J.* (2006) Internationalisation and Its Many Facets. Invited Lecture, QS APPLE Conference, Singapore, 12-14 July 2006.

16. *Svasti, J.,* Ketudat-Cairns, J., Hommalai, G., Phongsak, T., and Opassiri, O. (2006) Synthesis of Alkyl Glucosides and Oligosaccharides by Transglucosylation Using Cassava and Rice Beta-Glucosidases, 9th A-IMBN Conference, Kuala Lumpur, Malaysia. 4-5 September 2006.

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19. Suwannarat, P., Wattansirichaigoon, D., Keeratichamroen, S., Ngiwsara, L., Cairns, J.R.K., Srisomsap, C. and *Svasti, J.* (2006) Two novel splice site mutations in Thai siblings with neuronopathic Gaucher disease. J. Inherited Metab. Dis. **29**, 134-134.

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21. Khunthongpan, S., Makathan, A., *Svasti, J.*, Promwikorn, W. (2006) Epidermal protein expression profiling during the molting cycle of the black tiger shrimp, *Penaeus monodon*, studied by 2-DE. *Proceeding of Joint 3rd AOHUPO and 4th Structural Biology and Functional Genomics Conference.* Singapore, 4-7 December 2006.

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IX. Abstracts in National Meetings

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1. Prasertanan, T. and Yuvaniyama, J. Effect of codon usage alteration on expression of plasmepsin II in *Escherichia coli* system. (2004) 30*th Annual Congress on Science and Technology of Thailand*, Bangkok, 19-21 October 2004.
2. Chaivisuthangkura, A., Chaivisuthangkura, P., Kumar, C. V., and Svasti, J. (2004) Photochemical Studies of Novel Pyrenyl Probe on Specific Cleavage of Proteins 30*th Annual Congress on Science and Technology of Thailand*, Bangkok, 19-21 October 2004.
3. Aimon Tongpenyai, Samran Prabpai, Palangpon Kongsaeree. Molecular Packing Study of D-Phenylglycine in the Cationic and Zwitterionic Forms. Oral presentation. Congress on Science and Technology of Thailand 2004. 19-21 October 2004. Impact Exhibition and Convention Center, Muang Thong Thani, Bangkok.
4. Sompong Thammasirirak, Pornpimon Polkham, Sutthidech Preecharram, Sakda Daduang, Tomohiro Araki, Chantragan Srisomsap Phiphobmongkol and Jisnuson Svasti. Primary structure of reptile lysozymes. *Joint Senior Research Scholar Meeting* "Integation of Biological Science, Protein Chemistry and Medicine" M.R.Jisnuson Svasti, Suthat Fucharoen and Siriwat Wongsiri. September 14-15, 2004 N101, Faculty of Science, Mahidol University.
5. Sompong Thammasirirak, Pornpimon Polkham, Sutthidech Preecharram, Sakda Daduang, Tomohiro Araki and Jisnuson Svasti. Purification and characterization of reptile lysozymes. Young researchers meet TRF Senior Research Scholars, Kanchanaburi, 14-16 January
6. Wongsaengchantra, P. Y., Kaeyanon, C., and Senatham, D. (2004) Screening of a protease Preferentially Degrading Sericin. *Joint Senior Research Scholar Meeting "Integration of Biological Science, Protein Chemistry, and Medicine", M.R. Jisnuson Svasti, Suthat Fucharoen, and Siriwat Wongsiri*, Faculty of Science, Mahidol University, 14-15 September 2004.

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1. Lirdprapamongkol, K., Sakurai, H., Kawasaki, N., Choo, M.K., Saitoh, Y., Aozuka, Y., Singhirunnusorn, P., Ruchirawat, S., *Svasti, J.,* Saiki, I. (2005) Vanillin Suppresses In Vitro Invasion and In Vivo Metastasis of Mouse Breast Cancer Cells. The 8th National Cancer Symposium, Bangkok, Thailand, 7-9 September 2005.
2. Udomkitpipat, E., Intanakom, J., Liwporncharoenvong, T., *Chaiwisuthangkura, A.* (2005) Site Specific Cleavage of Lysozyme by $(NH_4)_6Mo_7O_{24}.4H_2O$. *31st Congress on Science and Technology of Thailand*, Suranaree University of Technology, 18-20 October 2005
3. Thammawat, K. and Wongvithoonyaporn, P. (2005) Screening of fungal naringinase. In Abstracts of the Second Protein Research Network Symposium on "Proteins: Structure, Function, and Proteomics". Chulabhorn Research Institute Conference Center, Bangkok, Thailand, 22-23 September, 2005.

4. Chuankhayan P, Hua Y, Mothong N, Toonkool P, Svasti J, Ketudat-Cairns JR. (2005) Molecular characterization of an isoflavonoid beta-glycosidase that releases a disaccharide from the glycoside. Invited Lecture, Second Protein Research Network Symposium on "Proteins: Structure, Function, and Proteomics" Bangkok, Thailand, 22-23 Sept., 2005.

5. Chuenchor W, *Ketudat Cairns JR.* (2005) Mutageneisis of rice BGlu1 beta-glucosidase and nucleophilic rescue of acid/base mutants. *Second Protein Research Network Symposium on "Proteins: Structure, Function, and Proteomics"* Bangkok, Thailand, 22-23 Sept., 2005.

6. Keeratichamroen S, Ngiwsara L, Wattanasirichaigoon D, *Cairns JRK,* Visudtibhan A, Tangnararatchakit K, Khowsathit P, Lekskul A, Jariengprasert C, Thawil C, Ruencharoen S, Onkoksoong T, Mongkolsiri D, *Svasti J.* (2005) Molecular characterization of a family with severe Fabry disease. Poster 15, *Second Protein Research Network Symposium on "Proteins: Structure, Function, and Proteomics"* Bangkok, Thailand, 22-23 September, 2005.

7. Metheenukul P, *Ketudat-Cairns JR.* (2005) Recombinant expression and characterization of Thai rosewood beta-glucosidases in *Pichia pastoris*. Poster 21, *Second Protein Research Network Symposium on "Proteins: Structure, Function, and Proteomics"* Bangkok, Thailand, 22-23 Sept., 2005.

8. Sujiwattanarat P, Thongtubtim N, *Ketudat-Cairns JR, Svasti J, Toonkool P.* (2005) Production and site-directed mutagenesis of recombinant β-glucosidase from Thai rosewood. Poster 45, *Second Protein Research Network Symposium on "Proteins: Structure, Function, and Proteomics"* Bangkok, Thailand, 22-23 Sept., 2005.

9. Srisomsap, C., Sawangareetrakul, P., Subhasitanont, P., Chokchaichamnankit, D., Ngiwsara, L., Chiablam, K. and J. Svasti. (2005) Comparison of human cholangiocarinoma and hepatocellular carcinoma cell lines by subproteomic analysis and LC/MS/MS. Second Protein Research Network Symposium on "Proteins: Structure, Function, and Proteomics" Bangkok, 22nd -23rd September.

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11. *Toonkool, P.* and Tongtubtim, N. (2006) Nucleotide and derived amino acid sequences of the cyanogenic beta-glucosidase (linamarase) from cassava (*Manihot esculenta* Crantz). Proceedings of *the 44th Kasetsart University Conference.* Bangkok, Thailand. 30 Jan -2 Feb, 2006.

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13. Kaeyanon, C. and *Wongsaengchantra, P. Y.* Selection and Isolation of a Protease Preferentially Degrading Sericin. (2005) *Protein Research Network Symposium 2005 on Proteins: Structure, Function, and Proteomics*, Conference Center, Chulabhorn Research Institute, Vibhavadee Rangsit Road, Bangkok, 22-23 September 2005.

14. Senatham, D. and *Wongsaengchantra, P. Y.* Preliminary characterization of proteases from bacteria GR_5_4, 4TS9-001, and H1C_13_8. (2005) *31st Annual Congress on Science and Technology of Thailand*, 18 - 20 October 2005 at Technopolis, Suranaree University of Technology, Nakhon Ratchasima.

15. Kaeyanon, C. and *Wongsaengchantra, P. Y.* Degumming of Thai Silk with Bacterial Proteases. (2005) *31st Annual Congress on Science and Technology of Thailand*, 18 - 20 October 2005, at Technopolis, Suranaree University of Technology, Nakhon Ratchasima.

16. Rojviriya, C., Prathumrat, T., Panbangred, W., Meevootisom, V., and *Yuvaniyama, J.* (2005) Crystallographic Study of *Bacillus megaterium* Penicillin-G Acylase and Its Protein Engineering. *Second Protein Research Network Symposium on Proteins: Structure, Function, and Proteomics*, Bangkok, 22-23 September, 2005.

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21. Siritapetawee, J., *Thammasirirak, S.,* and *Yuvaniyama, J.* (2005) Crystallization and Preliminary X-ray Structure Analysis of the Egg-White Lysozyme from a Taiwan Soft-Shelled Turtle (*Trionyx sinensis* Wiegmann) 31*st Annual Congress on Science and Technology of Thailand*, Nakornrachasima, 18-20 October 2005.

22. Songsiriritthigul, C., Vongsuwan, A., Krittanai, C. & *Suginta, W.* (2005) A study of substrate specificity of chitinases A from *Vibrio carchariae. 31st Congress on Science and Technology of Thailand* at Suranaree University of Technology, Nakhon Ratchasima, 18 -20 October 2005. P327.

23. Songsiriritthigul, C., *Yuvaniyama, J.,* Robinson, R.C., Vongsuwan, A. & *Suginta, W.* (2005) Expression, purification, crystallization, and preliminary crystallographic analysis of chitinases A from *Vibrio carchariae. 31st Congress on Science and Technology of Thailand* at Suranaree University of Technology, Nakhon Ratchasima, 18 – 20 October 2005. P335.

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26. Preecharram, S., *Thammasirirak, S.,* Jearranaiprepame, P., Daduang, S., *Svasti, J.* and Temsiripong, Y. (2005) Purification and characterization of antimicrobial peptide in

crocodile (*Crocodylus siamensis*) plasma. 31st Congress on Science and Technology of Thailand. 18 - 20 October 2005 at Technopolis, Suranaree University of Technology, Nakhon Ratchasima.

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28. Boonclarm, D., Sornwatana, T., *Arthan, D., Kongsaeree, P.,* and *Svasti, J.* A β-glucosidase catalyzes the hydrolysis of an iridoid β-glucoside from *Plumeria obtusa* Linn flowers. 31st Congress on Science and Technology of Thailand 18 - 20 October 2005 at Technopolis, Suranaree University of Technology, Nakhon Ratchasima.

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32. Lirdprapamongkol K, Ngiwsara L, Chiablem K, Srisomsap C, and Svasti J. (2005) Antitumor properties of vanilla: A structure-activity relationship study. 31st Congress on Science and Technology of Thailand, Suranaree University of Technology, 18-20 October 2005

YEAR 3

1. Ketudat-Cairns JR, Opassiri R, Chuenchor W, Pengthaisong S, Yuvaniyama J, Luang S, Kuntothom T, Svasti J. (2006) Structure and Function of Rice β-Glucosidases. First Annual Symposium of the Proteins Society of Thailand, Bangkok, Thailand, 23-24 October, 2006.

2. Hommalai G, Chuenchor W, Ketudat Cairns JR, Withers SG, Svasti J. (2006) Enzymatic Synthesis of β-(1,4)-D-Oligosaccharides using Mutated Rice β-Glucosidases. First Annual Symposium of the Proteins Society of Thailand, Bangkok, Thailand, 23-24 October, 2006.

3. Pengthaisong S, Chuenchor W, Yuvaniyama J, Opassiri R, Chen C-J, Svasti J, Ketudat Cairns JR. (2006) Crystallization and Preliminary X-ray Analysis of Rice BGlu1 β-Glucosidase with 2,4-Dinitrophenyl-2-Deoxy-2-Fluoro-β-D-Glucopyranoside. First Annual Symposium of the Proteins Society of Thailand, Bangkok, Thailand, 23-24 October, 2006.

4. Chantarangsee M, Ketudat-Cairns JR. (2006) Purification and Some Characteristics of a Recombinant Rice β-Galactosidase. First Annual Symposium of the Proteins Society of Thailand, Bangkok, Thailand, 23-24 October, 2006.
5. Chuankhayan P, Rimlumduan T, Svasti J, Ketudat Cairns JR. (2006) The Ability of *Dalbergia* β-glycosidase Towards Isoflavonoid Glycosides from Soybean. First Annual Symposium of the Proteins Society of Thailand, Bangkok, Thailand, 23-24 October, 2006.
6. Luang S., Ketudat Cairns JR. Expression and Characterization of β-Glucosidase (Bglu31) of Rice. First Annual Symposium of the Proteins Society of Thailand, Bangkok, Thailand, 23-24 October, 2006.
7. Rimlumduan T, Chuankhayan P, Ketudat Cairns JR. Mutagenesis of Aglycone Binding Residues of β-Glucosidase from Thai Rosewood (*Dalbergia cochinchinensis*). First Annual Symposium of the Proteins Society of Thailand, Bangkok, Thailand, 23-24 October, 2006.
8. Payakapong W, Ketudat-Cairns J. (2006) Expression Analysis of β-Galactosidases from Rice (Oryza sativa L., Cv. KDML105). First Annual Symposium of the Proteins Society of Thailand, Bangkok, Thailand, 23-24 October, 2006.
9. Ngiwsara L, Keeratichamroen S, Ketudat-Cairns JR, Srisomsap C, Wasant P, Svasti J. (2006) Identification of Mutations in Glucocerebrosidase Gene in Thai Patients Causing Gaucher Disease. First Annual Symposium of the Proteins Society of Thailand, Bangkok, Thailand, 23-24 October, 2006.
10. Maneesan J, Ketudat Cairns JR, Akiyama T, Opassiri R. (2006) Expression and Isolation of Natural Substrates of Os4BGlu12, a Rice β-Glucosidase Expressed in Response to Wounding Stress. First Annual Symposium of the Proteins Society of Thailand, Bangkok, Thailand, 23-24 October, 2006.
11. Keeratichamroen S, Ngiwsara L, Cairns JRK, Srisomsap C, Wattanasirichaigoon D, Svasti J. (2006) Mutation Analysis of the Iduronate-2-Sulfatase Gene in Thai Patients with Mucopolysaccharidosis Type II. First Annual Symposium of the Proteins Society of Thailand, Bangkok, Thailand, 23-24 October, 2006.
12. Payakapong W, Chantarangsee M, and Ketudat-Cairns J. (2006). Gene structure and expression analysis of rice *(Oryza sativa* L., CV. KDML105) glycosyl hydrolase family 35. The 18th Annual Meeting of the ThaiSociety for Biotechnology. Bangkok, Thailand, November 2-3, 2006.
13. Promwikorn, W., Khunthongpan, S., Makathan, A., Boonyoung, P., Withyachumnarnkul, B., Svasti, J. (2006) Proteomic Study of Epidermal Tissue of the Black Tiger Shrimp, *Penaeus Monodon*, During the Molting Cycle. *1st Annual meeting on the Protein Society of Thailand*, Bangkok, 24-25 October 2006.

14. Onpium, A., Svasti, J., Kongsaeree, P. (2006) Site-directed mutagenesis in the aglycone binding pocket of Thai rosewood beta-glucosidase (*Dalbergia cochinchinensis* Pierre). *Proceedings of the 32nd Congress on Science and Technology of Thailand* Bangkok, Thailand 10-12 Oct. 2006.

15. Porncharoennop, C., Svasti, J., Kongsaeree, P. (2006) Production and Characterization of mutant forms of Thai rosewood β-glucosidase. *Proceedings of the 32nd Congress on Science and Technology of Thailand,* Bangkok, Thailand 10-12 Oct. 2006.

16. Sujiwattanarat, P., Svasti, J., Kongsaeree, P. (2006) Effects of mutation in the aglycone binding pocket of Thai rosewood β-glucosidase. *Proceedings of the 32nd Congress on Science and Technology of Thailand* Bangkok, Thailand 10-12 Oct. 2006.

17. Chaivisuthangkura, A., Svasti, J., Malaikeaw, Y., and Kumar, C. V. (2007) Chiral Recognition and Protein Photocleavage by a New Pyrenyl Probe *Second Annual Symposium of Protein Society of Thailand*, Bangkok, 20-21 September 2007.

18. Malaikeaw, Y., Chaivisuthangkura, A., Svasti, J., and Kumar, C. V. (2007) Chiral Protein Photocleavage of Avidin by a New Pyrenyl Probe *Second Annual Symposium of Protein Society of Thailand*, Bangkok, 20-21 September 2007.

19. Ketudat-Cairns JR, Opassiri R, Ketudat-Cairns M, Kongsaeree P, Yuvaniyama J, Svasti J. (2007). From Thai rosewood to structural and functional genomics of rice β-glucosidases. Second Annual Symposium of the Protein Society of Thailand: Odysseys in Protein Research. Chulabhorn Research Institute, Bangkok, Thailand 20-21 September, 2007.

20. Opassiri R, Pomthong B, Akiyama T, Onkoksoong T, Ketudat Cairns JR. (2007). Second Annual Symposium of the Protein Society of Thailand: Odysseys in Protein Research. Chulabhorn Research Institute, Bangkok, Thailand 20-21 September, 2007.

21. Kunthotom T, Opassiri R, Hrmova M, Ketudat-Cairns JR. (2007). Cloning and characterization of barley beta-glucosidase/beta-mannosidase. Second Annual Symposium of the Protein Society of Thailand: Odysseys in Protein Research. Chulabhorn Research Institute, Bangkok, Thailand 20-21 September, 2007.

22. Luang S, Opassiri R, Hrmova M, Ketudat-Cairns JR. (2007). Substrate specificity of rice Os7Bglu26 β-glycosidase. Second Annual Symposium of the Protein Society of Thailand: Odysseys in Protein Research. Chulabhorn Research Institute, Bangkok, Thailand 20-21 September, 2007.

23. Seshadri S, Opassiri R, Akyama T, Ketudat Cairns JR. (2007). Recombinant expression, purification, and characterization of rice (Oryza sativa) Os3Bglu6 β-glucosidase. Second Annual Symposium of the Protein Society of Thailand: Odysseys in Protein Research. Chulabhorn Research Institute, Bangkok, Thailand 20-21 September, 2007.

24. Pengthaisong S, Chuenchor W, Chen C-J, Ketudat Cairns JR. (2007). Crystlalization of rice Bglu1 E176Q β-glucosidase with and without olisgosaccharide substrates. Second Annual Symposium of the Protein Society of Thailand: Odysseys in Protein Research. Chulabhorn Research Institute, Bangkok, Thailand 20-21 September, 2007.

25. Chantarangsee M, Tanthanuch W, Fujimura T, Fry SC, Ketudat-Cairns JR. (2007). Characterization of β-galactosidases from rice (*Oryza sativa* L.) Second Annual Symposium of the Protein Society of Thailand: Odysseys in Protein Research, Chulabhorn Research Institute, Bangkok, Thailand 20-21 September, 2007.

26. Tanthanuch W, Chantarangsee M, Ketudat-Cairns JR. (2007). Molecular cloning, expressino and purification of a rice β-galactosidase C-terminal domian for crystal structure analysis. Second Annual Symposium of the Protein Society of Thailand: Odysseys in Protein Research. Chulabhorn Research Institute, Bangkok, Thailand 20-21 September, 2007.

27. Maneesan J, Ketudat Cairns JR, Akiyama T, Opassiri R. (2007). Rice Os4Bglu12 β-glucosidase: its expression in response to stresses and isolation of its natural substrates. Second Annual Symposium of the Protein Society of Thailand: Odysseys in Protein Research. Chulabhorn Research Institute, Bangkok, Thailand 20-21 September, 2007.

28. Supawadee Pata, Sakda Daduang, Jisnuson Svasti and Sompong Thammasirirak. 2007. Study of antibacterial and antifungal from crocodile leukocytes extract *(Crocodylus siamensis*). Second Annual Symposium of Protein Society of Thailand: Odysseys of protein research. Chulabhorn Research Institute Research Conference Center Bangkok Thailand 20-21 September 2007.

29. Laoo Shawsuan, Sukda Daduang and Sompong Thammasirirak. 2007. Study of reptile egg white proteins by electrophoresis methods. (2007). Study of antibacterial and antifungal from crocodile leukocytes extract *(Crocodylus siamensis*). Second Annual Symposium of Protein Society of Thailand: Odysseys of protein research. Chulabhorn Research Institute Research Conference Center Bangkok Thailand 20-21 September 2007.

30. Rodbumrer, P. and Wongsaengchantra, P. Y. Potentiality of Cocoonase from Bombyx Mori for Silk Degumming. (2007) Second Annual Symposium of Protein Society of Thailand: Odysseys of protein research. Chulabhorn Research Institute Research Conference Center Bangkok Thailand 20-21 September 2007.

31. Suthangkornkul, S. and Wongsaengchantra, P. Y. Purification of Protease Enzyme for Silk Degumming from Bacterial Strain CRC_6NB. (2007) Second Annual Symposium of Protein Society of Thailand: Odysseys of protein research. Chulabhorn Research Institute Research Conference Center Bangkok Thailand 20-21 September 2007.

32. Sitthikool, S., Rimpeekool, W., Worapong, J., and Wongsaengchantra, P. Y. Screening of Sericin-specific Protease from Bacteria and Endophytes for Silk Degumming Industry. (2007) Second Annual Symposium of Protein Society of Thailand: Odysseys of protein research. Chulabhorn Research Institute Research Conference Center Bangkok Thailand 20-21 September 2007.

33. Arthan, D., Kiitakoop, P., Esen, A., Kimura, A. and Svasti, J. (2007). Furostanol glycoside-26-O-β-glucosidase from the leaves of *Solanum torvum*. Second Annual Symposium of the Protein Society of Thailand "Odysseys in Protein Research", Chulabhorn Reserch Institute Conference Center, Bangkok, September 20th-21st, 2007.

34. Suginta W. (2007). Identification of chitin binding proteins and characterization of two chitinase isoforms from *Vibrio alginolyticus* 283. Second Annual Symposium of the Protein Society of Thailand "Odysseys in Protein Research", Chulabhorn Reserch Institute Conference Center, Bangkok, September 20th-21st, 2007.

35. Songsiriritthigul C, Kobdaj A and Suginta W. (2007). The active site residues Trp275 and Trp397 are important for the binding selectivity of chitinase A to soluble substrates. Second Annual Symposium of the Protein Society of Thailand "Odysseys in Protein Research", Chulabhorn Reserch Institute Conference Center, Bangkok, September 20th-21st, 2007.

36. Pantoom S, Songsiriritthigul C and Suginta W. The influence of the surface-exposed residues on the binding and hydrolytic activities of *Vibrio carchariae* chitinase A. Second Annual Symposium of the Protein Society of Thailand "Odysseys in Protein Research", Chulabhorn Reserch Institute Conference Center, Bangkok, September 20th-21st, 2007.

X. Students Completing or Undertaking Training

1. Ph.D. Students Graduated

1. Rakrudee Sarntima, Ph.D. in Biochemistry, Mahidol University, September 2004; Thesis titled: Study of their specificity in glycoside synthesis and crystallisation of Thai rosewood β-glucosidase.
2. Phimonphan Chuankhayan, Ph.D. in Biochemistry, Suranaree University of Technology, March, 2005 *Dalbergia nigrescens* β-glucosidase.
3. Jeerus Sucharitakul, Ph.D. in Biochemistry, Mahidol University, June 2005; Thesis entitled: Mechanistic studies of *p*-hydroxyphenylacetate hydroxylase from *Acinetobacter baumannii*.
4. Pornphimon Metheenukul, Ph.D. in Biochemistry, Suranaree University of Technology, Expression of *Dalbergia cochinchinensis* Pierre β-glucosidase in yeast. December, 2005.
5. Greanggrai Hommalai, Ph.D. in Biochemistry, Mahidol University; Thesis entitled: Structure and Activity Relationships in Thai plant β-glucosidase, December, 2006.
6. Chutintorn Suadee, Ph.D. in Biochemistry, Mahidol University, Thesis entitled: A Luciferase enzyme isolated from marine organism, August, 2007.
7. Chomphunuch Songsiriritthigul, Ph.D. in Biochemistry, Suranaree University of Technology Thesis entitled: "Functional and structural characterization of Chitinase A from *Vibrio carchariae*"

2. Ph.D. Students Currently Undertaking Training

1. Kriengsak Lirdprapamongkol, Ph.D. study, Biochemistry, Mahidol University, Research area: Coparative proteomic profile of SK-Hep 1 and HCC-SIOZ cell lines
2. Thanawat Phongsak, Ph.D. study, Biochemistry, Mahidol University, Research area: Study of protein-protein interaction between reductase and oxygenase of *p*-hydroxyphenyl acetate hydroxylase.
3. Preeyachan Laourthai, Ph.D. study, Biochemistry, Mahidol University, Research area: Compounds Stimulating Hb F Synthesis.
4. Catleya Rojviriya, Ph.D. study, Biochemistry, Mahidol University, Research area: X–ray Structure Determination and Protein Engineering of *Bacillus megaterium* Penicillin-G Acylase.
5. Worapoj Oonanant, Ph.D. study, Biochemistry, Mahidol University, Research area: Crystallization and Structure Determination of Flavin-Containing Enzymes.
6. Chamaiporn Budriang, Ph.D. study, Biochemistry, Mahidol University, Research area: Expression, Purification, and X–ray Structure Determination of Cytochrome P-450 CYP6AA3 from Deltamethrin-resistant *Anopheles minimus*.
7. Jarunee Vanichtanankul, Ph.D. study, Biochemistry, Mahidol University, Research area: Crystallization and X-ray Structure Determination of *Trypanosoma brucei* Dihydrofolate Reductase-Thymidylate Synthase.
8. Sirisak Lolupiman, Ph.D. study, Biochemistry, Mahidol University, Research area: Gene Shuffling of *Plasmodium falciparum* Plasmepsins I and II.

9. Sarayuth Nijvipakul, Ph.D. study, Biochemistry, Mahidol University, Research area: Investigation of the role of LuxG in bacterial bioluminescence.
10. Methinee Prongjit, Ph.D. study, Biochemistry, Mahidol University, Research area: Study of Pyranose Oxidase from *Tramates multicolor*.
11. Somchart Maenpuen, Ph.D. study, Biochemistry, Mahidol University, Research area: Study of serine hydroxymethyl transferase from *Plasmodium falciparum*.
12. Janewit Wongrattana, Ph.D. study, Biochemistry, Mahidol University, Research area: Studies of Bacterial luciferase from *Photobacterium sp*.
13. Prangprapai Rodbumrer, Ph.D. study, Biotechnology, Mahidol University, Research area: Cloning and characterization of cocoonase from Bombyx mori for silk degumming process.
14. Chariwat Samanchat, Ph.D. study, Chemistry, Mahidol University, Research area: X-ray crystallographic analysis of *Pseudomonas stutzeri* D-phenylglycine aminotransferase and transgenic abnormal hemoglobins.
15. Puttapol Khongsuk, Ph.D. study, Chemistry, Mahidol University, Research area: Crystallographic investigation of *Plasmodium vivax* dihydrofolate reductase-thymidylate synthase.
16. Atchara Nuam-in, Ph.D. study, Chemistry, Mahidol University, Research area: Synthesis of small peptides and their three-dimensional nanoscale arrangements.
17. Khomsan Tiensomjitr, Ph.D. study, Chemistry, Mahidol University, Research area: Mass spectrometric analysis of hemoglobin modification by artemisinin
18. Srisuda Trirakarn, Ph.D. study, Chemistry, Mahidol University, Research area: Dihydrofolate reductase-thymidylate synthase from *Plasmodium malariae* and *Plasmodium ovale*.
19. Samran Prabpai, Ph.D. study, Chemistry, Mahidol University, Research area: Drug target identification of artemisinin in Malaria.
20. Mallika Chantarangsee, Ph.D. Biochemistry, Suranaree University of Technology, Research area: Expression and characterization of β-galactosidases from rice (*Oryza sativa* L.).
21. Watchalee Cheunchor, Ph.D. Biochemistry, Suranaree University of Technology, Research area: Structure and Functional Relationships in Rice BGlu1 β-glucosidase (x-ray crystallography & mutagenesis).
22. Teerachai Kunthothom, Ph.D. Biochemistry, Suranaree University of Technology, Research area: Characterization of the basis for substrate specificity in new rice β-glucosidase isozyme related to BGlu1.
23. Sukanya Luang, Ph.D., Biochemistry, Suranaree University of Technology, Research area: Rice Glycosyl Hydrolase family 1 glycosidases.
24. Khakhanang Ratananikom, Ph.D. Bochemistry, Kasetsart University, Research area: Structural and functional characterization in the glycone-binding site of Thai rosewood β-glucosidase.
25. Yaowaluk Malaikaew, Ph.D. study, Chemistry, Srinakharinwirot University, Research area: Development of novel probes for site-specific cleavage of proteins.

26. Rutanachai Thaipratum, Ph.D. study, Biochemistry, Mahidol University, Research Area: Response of unicellular green algae to irradiance stress.

27. Phichaya Khamai, Ph.D. study, Biochemistry, Mahidol University, Research Area: Photosystem II damage and repair cycle: dynamic configuration of photosystem II damage in *Dunaliella salina*.

28. Supriya C.S., Ph.D. study, Biochemsitry Suranaree University of Techology, Research Area: Structure and function of rice beta-glucosidase isozymes.

29. Kittisak Thotsaporn, Ph.D. study, Biochemistry, Mahidol University, Research Area: Site-directed mutagenesis studies of the oxygenase component of *p*-hydroxyphenylacetate hydroxylase.

30. Pirom Chenprakhon, Ph.D. study, Science and Technology education, Research Area: Investigation on the hydroxylation mechanism of the oxygenase component of p-hydroxyphenylacetate hydroxylase.

32. Sutthidech Preecharram, Ph.D.study, Biochemistry, Khon Kaen University, Research Area: Primary structure and function of antimicrobial peptides by Mass spectrometry and Edman degradation

33. Warintra Pitsawong, Ph.D. study, Biochemistry, Mahidol University, Research area: Probing Interaction between Bacterial Luciferase and Lux G Protein.

34. Preeyanuch Tongpoo, Ph.D. study, Bochemistry, Kasetsart University, Research area: Screening and characterization of novel β-glucosidase for cellulose degradation.

35. Supansa Pantoom, Ph.D. in Biochemistry, Suranaree University of Technology; Research area: "Functional study and Structures of *Vibrio carchariae* chitinase A complexed with potential chitinase inhibitors"

36. Salila Pengthaisong, Ph.D. study, Biochemistry, Suranaree University of Technology; Research area: To be decided.

37. Thipwarin Rimlumduan, Ph.D. study, Biochemistry, Suranaree University of Technology; Research area: To be decided.

2.1 M.Sc. Students Graduated

1. Duangrudee Tanramluk, M.Sc. in Biochemistry Mahidol University, September 2004; Thesis entitled: Crystal Structure of *Plasmodium falciparum* Dihydrofolate reductase–Thymidylate synthase in complex with trimethoprim

2. Worapoj Oonanant, M.Sc. in Biochemistry, Mahidol University, April 2005; Thesis entitled: Crystallization of 2-Methyl-3-hydroxypyridine-5-carboxylic Acid Oxygenase from Soil Bacterium *Pseudomonas* sp. MA-1.

3. Anothai Supanphong, M.Sc. in Chemistry, Mahidol University, graduated April 2006; Thesis title: Spectroscopic and x-ray crystallographic studies of Metacycloprodigrosin complexed with DNA.

4. Supreeya Cheewarat, M.Sc. in Chemistry, Mahidol University, graduated April 2006; Thesis title: Bioactive secondary metabolites of microorganism.

5. Penporn Sujiwattanarat, M.Sc. in Genetic Engineering, Kasetsart University, May 2006; Thesis entitled: Production and Site-directed Mutagenesis of Recombinant β-Glucosidase from Thai Rosewood.

6. Kittipat Sopitthumakun, M.Sc. in Biochemistry, Mahidol University, graduated July 2006, Thesis title: Purification and Characterization of Pyridoxal-5-Phosphate dependent serine hydroxymethytransferase from *Plasmodium vivax*.
7. Kornkamol Kalapakornchai, M.Sc. in Organic Chemistry, Mahidol University, graduated November 2006; Thesis title: Cassava linamarase in glycoside synthesis: enantioselectivity, non-aqueous synthesis and enzyme immobilization.
8. Duangkamol Senatham, M.Sc. in Biotechnology, Mahidol University, February 2007; Thesis entitled: Screening for sericin-specific protease from microorganisms
9. Chantima Kaeyanon, M.Sc. in Biotechnology, Mahidol University, April 2007, Thesis entitled: Screening and Characterization of a protease preferentially degrading sericin
10. Kanokpan Thammawat, M.Sc. in Biochemistry, Kasetsart University, Research area: Optimization of Fungal Naringinase Production.
11. Chompoonuth Porncharoennop, M.Sc. in Genetic Engineering, Kasetsart University, May 2007; Thesis entitled: Production and Characterization of mutant forms of Thai Rosewood beta-Glucosidase.
12. Amornrat Onpium, M.Sc. in Biochemistry, Kasetsart University, May 2007; Thesis entitled: Site-directed Mutagenesis in the Aglycone Binding Pocket of Thai Roeswood Beta-Glucosidase.

2.2 M.Sc. Students Currently Undertaking Training

1. Patcharapan Siriwat, M.Sc. study, Biochemistry, Mahidol University, Research area: Machanism of vanillin for suppression of cancer metastasis.
2. Krittikorn Kumpornsin, M.Sc. study, Biochemistry, Mahidol University, Research area: Protein Engineering of *Plasmodium falciparum* Plasmepsin I.
3. Theerawat Prasertanan, M.Sc. study, Biochemistry, Mahidol University, Research area: Optimization of Codon Usage for Expression of *Plasmodium falciparum* Plasmepsin II in *Escherichia coli*.
4. Aritsara Jaruwat, M.Sc. study, Biochemistry, Mahidol University, Research area: Crystallization of *Plasmodium falciparum* Plasmepsins.
5. Wilailuk Koinueng, M.Sc. study, Biochemistry, Mahidol University, Research area: Crystallization of Flavin-Containing Enzymes.
6. Kwancharus Cheangpunya, M.Sc. study, Genetic Engineering, Kasetsart University, Research area: Site-directed mutagenesis in beta-glucosidase.
7. Nusra Tongtubtim, M.Sc. study, Genetic Engineering, Kasetsart University, Research area: Site-directed mutagenesis in beta-glucosidase.
8. Kanokpan Thammawat, M.Sc.student, Faculty of Graduate Study, Kasetsart University, Research area: Optimization of Fungal Naringinase Production.
9. Suwannee Khunthongpan, M.Sc. in Biotechnology, Prince of Songkla University, Research area: Study of protein expression in the epidermis during the molting cycle of the black tiger shrimp (*Penaeus monodon*) by 2D SDS-PAGE.
10. Nantidaporn Ruangchan, MSc. study, Biochemistry, Mahidol University, Research area: Studies of reactions of p-hydroxyphenylacetate hydroxylase with substrate analogues at various pH.

11. Nantaporn Kamolsuthipaichit, M.Sc. study, Organic Chemistry, Mahidol University, Research area: Enantioselectivity of cassava linamarase in non-aqueous medium and enzyme immobilization
12. Pailin Srisuratsiri, M.Sc. study, Organic Chemistry, Mahidol University, Research area: Molecular dynamics of enzyme-ligand complexes and co-crystals of active pharmaceutical ingredients.
13. Amporn Saekee, M.Sc. study, Organic Chemistry, Mahidol University, Research area: To be decided.
14. Ketsarin Chantarasunthon, M.Sc. study, Organic Chemistry, Mahidol University, Research area: To be decided.
15. Apinya Thienchumphan, M.Sc. study, Biotechnology, Mahidol University, Research area: Cloning of a high value silk-degumming efficiency protease from bacteria strain CRC_6_NB.
16. Bancha Mahong, M.Sc. study, Biochemistry, Mahidol University, Research area: Proteomic analysis of differential protein expression upon exposure to irradiance stress of unicellular green algae.
17. Suwannee Khunthongpan, M.Sc. in Biotechnology, Prince of Songkla University, Research area: Study of protein expression in the epidermis during the molting cycle of the black tiger shrimp (*Penaeus monodon*) by 2D SDS-PAGE.
18. Monmanus Booncimolrathoo, M.Sc. study, Biochemistry, Kasetsart University, Research area: To be decided.
19. Jirasuda Sampoa, M.Sc. study, Genetic Engineering, Kasetsart University, Research area: To be decided.

3. B.Sc. Students Currently Who Have Undertaken Training

1. Rossukon Thongwichian, B.Sc. study, Chemistry, Mahidol University, Research area: DNA Shuffling of *Plasmodium falciparum* Plasmepsins I and II.
2. Sirisak Lolupiman, B.Sc. study, Biology, Mahidol University, Research area: Generation of Fusion Protein of Green Fluorescent Protein (GFP) and *Plasmodium falciparum* Plasmepsin I.
3. Kanlaya Prapainop, B.Sc. student, Chemistry, Mahidol University, Research area: Cloning and Expression of flavin oxidoreductase from luminous bacteria
4. Yutthaphong Phongbunchoo, B.Sc. study, Biology, Mahidol University, Research area: Generation of Fusion Protein of Green Fluorescent Protein (GFP) and *Plasmodium falciparum* Plasmepsin II.
5. Theerawat Prasertanan, B.Sc. study, Chemistry, Mahidol University, Research area: Optimization of Codon Usage for Expression of *Plasmodium falciparum* Plasmepsin II in *Escherichia coli*.
6. Aimon Thongpenyai, B. Sc. study, Chemistry, Mahidol University, Research area: Enzymatic synthesis of short peptides based on phenylglycine amino acid and its conformational studies.
7. Mingmas Chantarakitti, B.Sc. study, Biochemistry, Kasetsart University, Research area: Expression of cassava linamarase in *E. coli*.

8. Patcharee Paiboon, B.Sc. study Biochemistry, Kasetsart University, Research area: Optimisation of dalcochinase production in *P. pastoris*
9. Weerachai Jaikaew, B.Sc. study, Chemistry, Mahasarakham University, Research area: Partial purification of alpha-mannosidase from *Albizia procera* Benth.
10. Kornkanok Boonserm, B.Sc. study, Chemistry, Mahasarakham University, Research area: Partial purification of alpha-mannosidase from *Albizia procera* Benth.
11. Dungdien Mokpar, B.Sc. study, Chemistry, Mahasarakham University, Research area: Screening for the natural substrates of alpha-mannosidase from *Albizia procera* Benth. by using thin-layer chromatography
12. Rapiporn Pindon, B.Sc. study, Chemistry, Mahasarakham University, Research area: Screening for the natural substrates of alpha-mannosidase from *Albizia procera* Benth. by using thin-layer chromatography
13. Miss Jurairat Jongthawil, B.Sc. study, Chemistry, Mahasarakham University, Research area: Testing the ability of alpha-mannosidase from *Albizia procera* Benth in oligosaccharide synthesis
14. Miss Kannika Soikam, B.Sc. study, Chemistry, Mahasarakham University, Research area: Testing the ability of alpha-mannosidase from *Albizia procera* Benth in oligosaccharide synthesis
15. Miss Juthamas Likhitcholatan, B.Sc. study, Chemistry, Srinakharintwirot University, Prasarnmit; Project title: Photochemical studies of novel pyrenyl probe on specific cleavage of proteins
16. Miss Natpatsa Sirikanchanawanich, B.Sc. study, Chemistry, Srinakharintwirot University, Prasarnmit; Project title: Photochemical studies of novel pyrenyl probe on specific cleavage of proteins
17. Miss Nisa Viriyasitaporn, B.Sc. study, Chemistry, Srinakharintwirot University, Prasarnmit; Project title: Footprint metal binding sites on proteins
18. Mr. Prachya Ponpanichrasamee, B.Sc. study, Chemistry, Srinakharintwirot University, Prasarnmit; Project title: Footprint metal binding sites on proteins
19. Theerawat Prasertanan, B.Sc. study, Chemistry, Mahidol University, Research area: Optimization of Codon Usage for Expression of *Plasmodium falciparum* Plasmepsin II in *Escherichia coli*.
20. Miss Wimolrat Simapattanapong, B.Sc. study, Chemistry, Srinakharintwirot University; Project title: Footprint metal binding sites on DNA
21. Miss Duangjai Yingcharoenluk, B.Sc. study, Chemistry, Srinakharintwirot University; Project title: Footprint metal binding sites on proteins
22. Miss Phunrapee Akaravorakulchai, B.Sc. study, Chemistry, Srinakharintwirot University; Project title: Footprint metal binding sites on proteins
23. Ratanon Suntivith, B. Sc. Study, Chemistry, Mahidol University, Project title: Study of flavin reductase from luminous bacteria.
24. Orathai Sawatdichaikul, B.Sc. study, Biochemistry, Kasetsart University, Research area: Isolation of isoflavonoid β-glucosides in soy beans.
25. Yoawalak Jindanuruk, B.Sc. study, Biochemistry, Kasetsart University, Research area: Substrate specificity in β-glucosidase.

26. Patcharee Paiboon, B.Sc. study Biochemistry, Kasetsart University, Research area: Optimisation of dalcochinase production in *P. Pastoris*
27. Jurairat Jongthawil, B.Sc. study, Chemistry, Mahasarakham University, Research area: Enzymatic synthesis of manno-oligosaccharides by reverse hydrolysis using partially purified alpha-mannosidase from *Albizia procera* Benth.
28. Kannika Soikam, B.Sc. study, Chemistry, Mahasarakham University, Research area: Enzymatic synthesis of manno-oligosaccharides by reverse hydrolysis using partially purified alpha-mannosidase from *Albizia procera* Benth.
29. Miss Juthamas Langkrod, B.Sc. study, Chemistry, Srinakharinwirot University; Project title: Spectroscopic Studies of Py-biotin with Avidin
30. Miss Uriawan Singkam, B.Sc. study, Chemistry, Srinakharinwirot University; Project title: Spectroscopic Studies of Py-biotin with Avidin
31. Chanya Thapmontian, B.Sc. student, Faculty of Liberal Arts and Science, Kasetsart University, Kamphang Saen Campus, Research area: Application of naringinase in debitterness of orange juices.
32. Chonnipa Koysakul, B.Sc. student, Faculty of Liberal Arts and Science, Kasetsart University, Kamphang Saen Campus, Research area: Micro-organism database system for internet.
33. Thanyaporn Wongnate, B.Sc. student, Chemistry, Mahidol University. Research area: Overexpression and crystallization of *Plasmodium vivax* dihydrofolate reductase-thymidylate synthase.
34. Ratanon Suntivith, B. Sc. Study, Chemistry, Mahidol University, Project title: Study of flavin reductase from luminous bacteria.
35. Chuthakarn Swatsuk, B.Sc. student, Biotechnology, Mahidol University, Research area: The Application of Sericin for Bacterial Preservation
36. Yanin Rukchanyanon, B.Sc. study, Biotechnology, Mahidol University, Research area: Silk Ice Cream
37. Pornwit Anunsap, B.Sc. study, Chemistry, Mahidol University, Research area: Synthesis of dipeptides and their molecular arrangement in solid-state.
38. Jiraporn Yukolpanichkit, B.Sc. student, Faculty of Liberal Arts and Science, Kasetsart University, Kamphang Saen Campus, Research area: Investigation of anti-bacterial-growth properties against *Salmonell typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* of fragrance plants extracts.
39. Wananya Kaewkaewparn, B.Sc. student, Faculty of Liberal Arts and Science, Kasetsart University, Kamphang Saen Campus, Research area: Investigation of the fragrance compound groups from fragrance plants extracts.
40. Waraporn Yooreum, B.Sc. student, Faculty of Liberal Arts and Science, Kasetsart University, Kamphang Saen Campus, Research area: Selection of limonoate dehydrogenase-producing soil bacteria.

4. Graduate Students Performing Research Overseas

1. Chutintorn Suadee has done research at the laboratory of Professor David P. Ballou, Dept of Biological Chemistry, University of Michigan, Ann Arbor, MI, USA., June 2004 - August 2005

2. Greanggrai Hommalai has done research at the laboratory of Professor Stephen G. Withers, University of British Columbia, Canada between October 2004-September 2005
3. Preeyachan Lourthai has done research at the Murdoch Children's Research Institute, Royal Children's Hospital Victoria Australia, January 2004 – September 2006.
4. Chomphunuch Songsiriritthigul has taken six-month research training at Assoc. Prof. Bob Robinson's laboratory, Institute of Cell and Molecular Biology, Singapore, starting from October, 2005 ending March, 2006.
5. Watchalee Chuenchor has worked with Prof. Asim Esen and Assoc. Prof. Dr. David Bevan at Virginia Tech, USA with partial funding from the RGJ program from February-August, 2006.
6. Sarayut Nijvipakul has done research at the laboratory of Professor David P. Ballou, Dept of Biological Chemistry, University of Michigan, Ann Arbor, MI, USA., February 2006 – February 2007
7. Teerachai Kunthothom has done training in the University of Adelaide Centre for Plant Genomics, under the supervision of Assoc. Prof. Dr. Maria Hrmova and Prof. Dr. Geoffrey Fincher from March, 2006-March, 2007.
8. Sutthidech Preecharam has done six-week research training at the Prof. Tamo Fukamizo laboratory at Kinki University, Japan, from 1 June to 10 July 2006.
9. Phichaya Khamai has done conducting his doctoral research at the laboratory of Professor Anastasios Melis at the Department of Plant and Microbial, University of California, Berkeley, USA, from July 2006-June 2007.
10. Sukanya Luang has done training in the University of Adelaide Centre for Plant Genomics, under the supervision of Assoc. Prof. Dr. Maria Hrmova and Prof. Dr. Geoffrey Fincher from Dec., 2006-March, 2007. Funding is from the RGJ program.
11. Catleya Rojviriya has done training in the laboratory of Professor Mark A. Saper, Dept of Biological Chemistry, University of Michigan, Ann Arbor, MI, USA., October 2006 - April 2007.
12. Sutthidech Preecharram has done a four-week research training period at the Kyushu Tokai University, Kumamoto, Japan, from 1 April to 30 April 2007.

XI. Grants Received by Researchers

Most investigators have some funding support for their research activities. In particular, the Center for Excellence in Protein Structure and Function has a research grant from the government through the Mahidol University budget. This not only supports the central facilities of the center, but also supports one project for each of the five investigators in the center. Research on human disease at the Laboratory of Biochemistry, Chulabhorn Research Institute, is supported by intramural funding from the institute. In addition, many younger investigators are receiving various research grants, which show that they are competitive with other young researchers in Thailand. Some of these grants are listed below,

1. James Ketudat Cairns Thailand Research Fund Basic Research Grant BRG4780020: Structure Function Relationships in Plant β-Glucosidases. 2,000,000 baht, August, 2004-August, 2007.
2. Palangpon Kongsaeree: Thailand Research Fund: Research Career Development grant. 1,600,000 Baht/3 years
3. Apinya Chaivisuthangkura "Photochemical and mechanistic studies on selectivity of protein photocleavage" from Srinakharinwirot University, 259,000 baht, March 2004 - Febuary 2005
4. Apinya Chaivisuthangkura "Phytochemicals from mangosteen and the use as standards" from Srinakharinwirot University, 240,680 baht, October 2004 -September 2005
5. Prachumporn Toonkool "Protein engineering of plant β-glucosidases and its potential industrial applications"; 200,000 baht from Kasetsart University Research and Development Institute during October 2004-Sepetember 2005.
6. Palangpon Kongsaeree: National Synchrotron Research Center: Crystallographic investigation of transgenic abnormal hemoglobins: 1,500,000 Baht/2 years
7. Apinya Chaivisuthangkura "Development of mangosteen extract for using as standards and HPLC fingerprints" from Office of the National Research Council of Thailand (NRCT), 659,000 baht, June 2005 -May 2006
8. Patjaraporn Wonvithoonyaporn "Application of enzymes on debitterness of products from citrus fruit". Three-branched Research Project supported by Kasetsart University Fund. Code no. V-D 31.48. May 2005-Apr 2008. First year 212,500 Baht.
9. Pimchai Chaiyen: Thailand Research Fund RGJ Grant to support Miss Methinee Prongjit's Ph.D. study.
10. Songran Chuakrut "Selection, production and extraction of antimycobacterial substances from rare actinomycetes for growth inhibition of pathogenic Mycobacteria and drug resistant *Mycobacterium tuberculosis*", Naresuan University, 450,000 baht/year, October 2005 - September 2006.
11. Wipa Suginta "A study of three-dimensional structure of chitinases A from *Vibrio carchariae*", National Synchrotron Research Center: 840,000 baht, January 2005 ending December 2006.

12. Sompong Thammasirirak., "Purification and study of antimicrobial peptide from G-type lysozyme", Thailand Research Fund Master Research Grant 200,000 baht, November 2005-April 2007
13. Prachumporn Kongsaeree: Thailand Research Fund Master Research Grant to support Miss Penporn Sujiwattanarat's research project: 200,000 Baht/1 year.
14. Prachumporn Kongsaeree "Structure-function relationships in beta-glucosidases from Thai plants", Thailand Research Fund's New Researcher Grant, 480,000 Baht/2 years, August 2006-July 2008.
15. Jirundon Yuvaniyama: Thailand Research Fund RGJ Grant to support Miss Yupaporn Osiriphun's Ph.D. study.
16. Wipa Suginta, "Investigation of structural and enzymatic properties in chitin utilization of chitinase A mutants from *Vibrio carchariae*", Mid-Career University Staff grant from Thailand Research Fund. Duration 3 years ; amount: 1,200,000.00 baht, August 2006-July 2009
17. Sompong Thammasirirak, "Study of antibacterial peptide in crocodile (*Crocodylus siamensis*) blood)", , Mid-Career University Staff grant; Duration 3 years; amount: 1,200,000.00 baht, August 2006-July 2009
18. Waraporn Promwikorn., "Study of expression proteomics of molt-related proteins in the epidermis of the black tiger shrimp, *Penaeus monodon*", Thailand Research Fund 480,000 baht, 2006-2008.
19. Jame R. Ketudat Cairns "Structural Studies of Carbohydrate Active Enzymes from Rice" National Synchrotron Research Center. 2 years, 1,829,962 baht; October, 2006-October, 2008.
20. Pimchai Chaiyen, "Enzymology of Flavin-dependent and Pyridoxal Phosphate-dependent Enzymes", Faculty of Science, Mahidol University; 5,000,000 baht per 3 years.
21. Jirundon Yuvaniyama: "X-ray crystallographic study of oxygenase enzymes capable of degrading aromatic compounds", National Synchrotron Research Center, 2,405,176 Baht/2 years, November 2006–October 2008.
22. Prachumporn Kongsaeree "Structure-function relationships in cassava linamarase", from National Science and Technology Development Agency, 250,000 Baht, September 2006-August 2007.
23. Prachumporn Kongsaeree "Protein engineering of plant □ -glucosidases and its potential industrial applications"; 200,000 baht from Kasetsart University Research and Development Institute during October 2005-Sepetember 2006.
24. Waraporn Promwikorn "Study of expression proteomics of molt-related proteins in the epidermis of the black tiger shrimp, *Penaeus monodon*", Thailand Research Fund 480,000 baht, Dec. 2006 - Nov. 2008.
25. Sompong Thammasirirak," Production of lysozyme, ovalbumin and ovotransferrin from hen egg white 649,000 Baht/year, January 2006 - January 2007.
26. Sompong Thammasirirak, "Study of antimicrobial peptides from crocodile blood" 220,000 Baht/year, Khon Kaen University, October 2006-September 2007.

XII. Awards, Fellowships and Promotions

1. Professor Jisnuson Svasti received the Dushi Mala Medal from H.M. The King, 2005.
2. Professor Jisnuson Svasti, Mahidol University, received Outstanding Lecturer Award from the Council of the University Faculty Senates of Thailand, 2004.
3. Dr. Jirundon Yuvaniyama, Mahidol University, received the 2004 Mahidol University Prize for Excellence in Research, Mahidol University, Thailand.
4. Dr. Pimchai Chaiyen received the Young Scientist Award from the Foundation for the 2005 Promotion of Science and Technology under the Patronage of His Majesty the King
5. Dr. Wipa Suginta, Suranaree University of Technology, received the L'Oreal-UNESCO Women in Science Award, 2005.
6. Dr. Sompong Thammasirirak received the Outstanding Research Project Award from Khon Kaen University, 2005.
7. Dr. Pimchai Chaiyen, promoted to Associate Professor, Mahidol University, .
8. Dr. James Ketudat-Cairns, promoted to Associate Professor, Suranaree University of Technology, 2005.
9. Dr. Apinya Chaivisuthangkura, promoted to Assistant Professor, Srinakarintwirot University Prasarnmit
10. Dr. Prachumporn Kongsaeree, promoted to Assistant Professor, Kasetsart University, 2006.
11. Dr. Prachumporn Toonkool, Kasetsart University, received the Wood-Whelan fellowship to work at the Karolinska Institutte, Sweden, October-November 2004.
12. Dr. Dumrongkiet Arthan, Mahidol University, received the Matsumae International Foundation fellowship to work at the Hokkaido University, Japan, August 2006-January 2007.