出國報告（出國類別：開會）

第五屆APPA國際亞太蛋白質協會研討會

服務機關：台灣中油股份有限公司煉製研究所
姓名職稱：吳柏龍 化學工程師
派赴國家：泰國
出國期間：106年7月10日~106年7月14日
報告日期：106年8月8日

摘要

APPA, Asia-Pacific Protein Association 是亞洲與大洋洲之蛋白質研究組織，每隔三年會定期舉辦會員大會進行蛋白質結構與功能之學術交流。本次會議是第五次的會議，於 7 月 11-14 日在泰國春武里府（Chonburi）市舉辦。此次會議共有超過七百多人報名與會，多半是泰國當地之學者與學生，主辦單位也邀請了多位國際知名學者前來與會，包括了 2009 年諾貝爾化學獎得主之一美國科學家 Tomas Steitz 前來演講。

本次會議議程涵蓋國際最新蛋白質功能與結構研究、蛋白質於疾病所扮演的角色、蛋白質於醫學上運用等內容，活動包含邀請世界各專家的口頭演講、供應商演講還有包括海報參展發表三個部分，議程共計四天時間，由於時間限制，職此行參加了 7 月 11-13 日等三天的研討會議程，同時參加了海報參觀與討論，會中認識了許多國內外的新朋友，同時拓展了職於蛋白質領域的知識，將來能運用於中油生技原料-利用酵素生產幾丁寡糖製程的研究開發。

目次

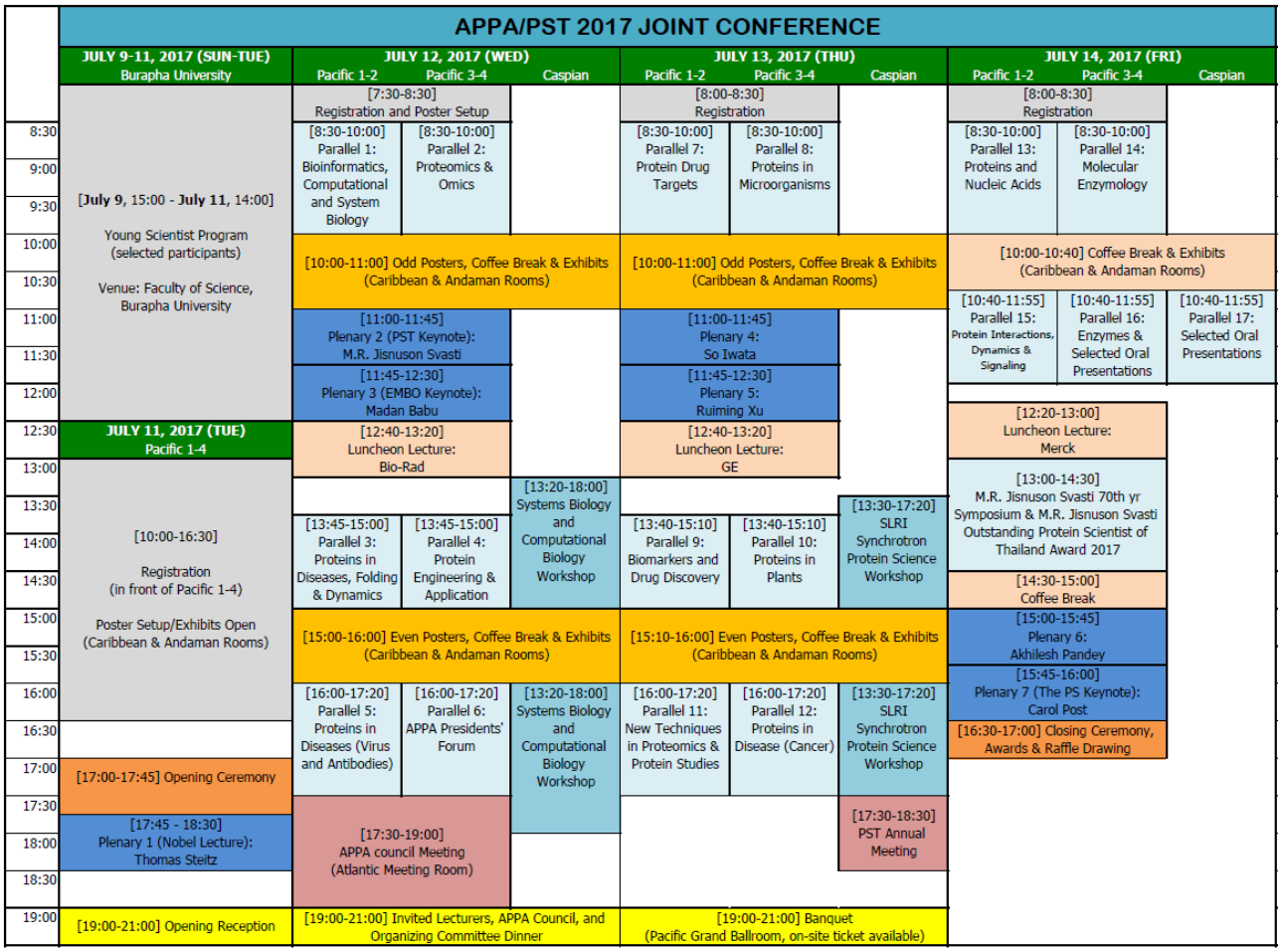
(一)目的

第五屆 APPA 國際亞太蛋白質協會研討會議程涵蓋國際最新蛋白質基因表現及調控、蛋白質結構與功能研究、蛋白質於疾病上的診斷及於藥物開發的運用，與生物技術中心目前進行幾丁質酶蛋白質酶表現研究相關。藉由參加此研討會，與國內外專家學者交流，尋求合作的契機，同時瞭解最新國際上蛋白質研究的方法與運用，將來能運用於中油生技原料-利用酵素生產幾丁寡糖製程的研究開發。

(二)過程

APPA, Asia-Pacific Protein Association 是亞洲與大洋洲之蛋白質研究組織，每隔三年會定期舉辦會員大會進行蛋白質結構與功能之學術交流。第一屆的會議於 2004 年在日本舉行，隨後分別於 2008 年在澳洲、2011 年在中國、2014 年在南韓進行會議。本次會議是第五次的會議於 7 月 11-14 日在泰國春武里府（Chonburi）市舉辦。此次會議共有超過七百多人報名與會，雖然參與的多半是泰國當地之學者與學生，主辦單位也邀請了多位國際知名學者前來與會。包括了 2009 年諾貝爾化學獎得主之一美國科學家 Tomas Steitz 前來演講。

本次會議議程涵蓋國際最新蛋白質功能與結構研究、蛋白質於疾病所扮演的角色、蛋白質於醫學上運用等內容，活動包含邀請世界各專家的口頭演講、供應商演講還有包括海報參展發表三個部分，議程共計四天時間，於泰國的 Bangsaen 舉行，詳細的演講細節如表一，會場場地一覽圖如圖二。由於時間限制，職此行參加了 7 月 11-13 日等三天的研討會議程，同時參加了海報參觀與討論，依照研討會日期分別詳述如下:


APPA/PST 2017 JOINT CONFERENCE
JULY 9-11, 2017 (SUN-TUE)
Burapha University
JULY 12, 2017 (WED)
Pacific 1-2
Pacific 3-4
Caspian
JULY 13, 2017 (THU)
Pacific 1-2
Pacific 3-4
Caspian
JULY 14, 2017 (FRI)
Pacific 1-2
Pacific 3-4
Caspian
8:30
9:00
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10:00
10:30
11:00
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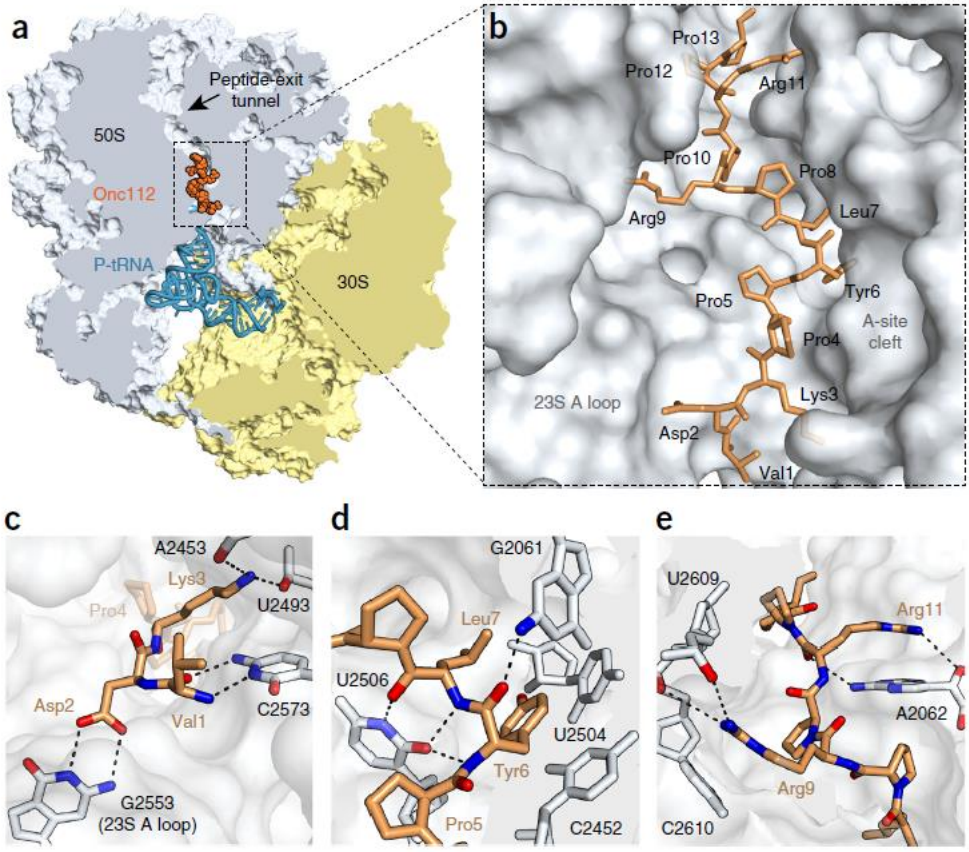
16:00
16:30
17:00
17:30
18:00
18:30
19:00
[July 9, 15:00 - July 11, 14:00]
Young Scientist Program
(selected participants)
Venue: Faculty of Science,
Burapha University
[7:30-8:30]
Registration and Poster Setup
[8:30-10:00]
Parallel 1:
Bioinformatics,
Computational
and System
Biology
[8:30-10:00]
Parallel 2:
Proteomics &
Omics
[10:00-11:00] Odd Posters, Coffee Break & Exhibits
(Caribbean & Andaman Rooms)
[11:00-11:45]
Plenary 2 (PST Keynote):
M.R. Jisnuson Svasti
[11:45-12:30]
Plenary 3 (EMBO Keynote):
Madan Babu
[12:40-13:20]
Luncheon Lecture:
Bio-Rad
[13:20-18:00]
Systems Biology
and
Computational
Biology
Workshop
[13:45-15:00]
Parallel 3:
Proteins in
Diseases, Folding
& Dynamics
[13:45-15:00]
Parallel 4:
Protein
Engineering &
Application
[15:00-16:00] Even Posters, Coffee Break & Exhibits
(Caribbean & Andaman Rooms)
[16:00-17:20]
Parallel 5:
Proteins in
Diseases (Virus
and Antibodies)
[16:00-17:20]
Parallel 6:
APPA Presidents'
Forum
[17:30-19:00]
APPA council Meeting
(Atlantic Meeting Room)
[8:00-8:30]
Registration
[8:30-10:00]
Parallel 7:
Protein Drug
Targets
[8:30-10:00]
Parallel 8:
Proteins in
Microorganisms
[10:00-11:00] Odd Posters, Coffee Break & Exhibits
(Caribbean & Andaman Rooms)
[11:00-11:45]
Plenary 4:
So Iwata
[11:45-12:30]
Plenary 5:
Ruiming Xu
[12:40-13:20]
Luncheon Lecture:
GE
[13:30-17:20]
SLRI
Synchrotron
Protein Science
Workshop
[13:40-15:10]
Parallel 9:
Biomarkers and
Drug Discovery
[13:40-15:10]
Parallel 10:
Proteins in
Plants
[15:10-16:00] Even Posters, Coffee Break & Exhibits
(Caribbean & Andaman Rooms)
[16:00-17:20]
Parallel 11:
New Techniques
in Proteomics &
Protein Studies
[16:00-17:20]
Parallel 12:
Proteins in
Disease (Cancer)
[13:30-17:20]
SLRI
Synchrotron
Protein Science
Workshop
[17:30-18:30]
PST Annual
Meeting
[19:00-21:00] Invited Lecturers, APPA Council, and
Organizing Committee Dinner
[19:00-21:00] Banquet
(Pacific Grand Ballroom, on-site ticket available)
[8:00-8:30]
Registration
[8:30-10:00]
Parallel 13:
Proteins and
Nucleic Acids
[8:30-10:00]
Parallel 14:
Molecular
Enzymology
[10:00-10:40] Coffee Break & Exhibits
(Caribbean & Andaman Rooms)
[10:40-11:55]
Parallel 15:
Protein Interactions,
Dynamics &
Signaling
[10:40-11:55]
Parallel 16:
Enzymes &
Selected Oral
Presentations
[10:40-11:55]
Parallel 17:
Selected Oral
Presentations
[12:20-13:00]
Luncheon Lecture:
Merck
[13:00-14:30]
M.R. Jisnuson Svasti 70th yr
Symposium & M.R. Jisnuson Svasti
Outstanding Protein Scientist of
Thailand Award 2017
[14:30-15:00]
Coffee Break
[15:00-15:45]
Plenary 6:
Akhilesh Pandey
[15:45-16:00]
Plenary 7 (The PS Keynote):
Carol Post
[16:30-17:00] Closing Ceremony,
Awards & Raffle Drawing
JULY 11, 2017 (TUE)
Pacific 1-4
[10:00-16:30]
Registration
(in front of Pacific 1-4)
Poster Setup/Exhibits Open
(Caribbean & Andaman Rooms)
[17:00-17:45] Opening Ceremony
[17:45 - 18:30]
Plenary 1 (Nobel Lecture):
Thomas Steitz
[19:00-21:00] Opening Reception

表一、會議議程及時間表。

7 月 11 日

研討會的第一天安排了 2009 年榮獲諾貝爾化學獎得主 Tomas A. Steitz 演講，Tomas 過去致力於核糖體結構與功能的研究，此次演講的內容為其過往研究核糖體如何與 tRNAs and EF-G 作用與延伸合成蛋白質，同時亦講解蛋白質合成過程如何作用於 P 與 A site，最後以細菌核糖體結構來設計藥物抑制蛋白質合成(如下圖一、為細菌核糖體與小分子胜肽藥物 oncocin 的共結晶蛋白質結構)，而成為一個有用的抗生素，目前正進行 phase II 的臨床人體實驗，其研究於 2009 年榮獲諾貝爾化學獎，詳細摘要如下。



圖一、細菌核糖體與小分子藥物 oncocin(棕色)的共結晶蛋白質結構。oncocin 為 proline-rich peptide，[Nature structural & molecular biology (2015)]

PL-1

The structure and function of the ribosome complexes with various protein factors and antibiotics

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ABSTRACT

We have obtained many insights into the structural basis of ribosome function in protein synthesis from our structural studies of the large ribosomal subunit as well as the 70S bacterial ribosome, and their complexes with substrates, protein factors or antibiotics. These have elucidated the mechanism by which this ribozyme catalyzes peptide bond formation and the specificity and mode of its inhibition by antibiotics.

We have obtained the structure of the complex of the 70S ribosome with tRNAs and EF-G in a previously unseen compact conformation. This compact conformation of EF-G, unlike the elongated one, allows the simultaneous binding of a tRNA in the A site and EF-G. We propose that the conversion of the compact to the elongated conformations of EF-G is responsible for tRNA translocation. The structures of the 70S ribosome with the factor EF4 (LepA) with tRNA bound in the P site or in the A and P sites provide the first insights into EF4's possible role in protein synthesis. Our structure of the 70S ribosome bound with a ribosome rescue protein (yaeJ) shows how it rescues stalled ribosomes.

The structures of some of our antibiotic complexes have been used by Rib-X Pharmaceuticals, Inc. (now Melinta Therapeutics) of New Haven to develop new potential antibiotic compounds that are effective against MRSA, one of which has successfully completed phase II clinical trials. Recently, we have obtained the structures of the 70S ribosome complexed with various oligopeptides that bind in the peptide tunnel, but in the opposite direction from peptides being synthesized; these structures might enable the creation of other new antibiotics.

This research was supported the Howard Hughes Medical Institute and by a grant to T.A. Steitz from the National Institutes of Health.

會場照片

圖二、會場照片一覽圖。

圖三、上圖為研討會晚宴與會清華大學學者合影照、下圖為海報論文場地。

7 月 12 日

參加了生物資訊、電腦及系統生物學會議(Bioinformatics, Computational and System Biology sessions)

生物資訊學（bioinformatics）是利用應用數學、資訊學、統計學和計算機科學的方法研究生物學的問題。雖然目前蛋白質 X 光繞射實驗發展已經接近成熟，但還是有許多的限制導致蛋白質結構無法得到，如細胞膜蛋白不易取得、蛋白質中間態(intermediate state)從蛋白質 X 光繞射實驗無法得到訊息、有些蛋白質無法順利長出晶體、或酵素有結晶但無法取得與受質共結晶的結構，這時科學家就必須仰賴電腦模擬來解決問題了，目前網路上已有許多免費的軟體可供使用下載，大概的邏輯為先找出同源蛋白質三維結構，作為建構蛋白質三維結構的模板，然後將蛋白質結構模版送入 NAMD CUDA 版動力學軟體以 Amber F99 分子動力學力場與水進行結構優化，其後將優化的結構送入 PROCHECK 軟體分析模擬出來的蛋白質結構是否合理。此方法可以得到非實際的蛋白質結構，但基於眾多數據的基礎下可靠性也很高。

Mitsunori 利用電腦分子動力模擬技術，來模擬粒線體上負責合成 ATP 的蛋白質聚合物的結構及解釋 F1-ATPase 與 V1-ATPase 合成 ATP 的可能詳細機制，，詳細摘要如下。

S-1

Molecular dynamics simulations of molecular rotary motors

Mitsunori Ikeguchi

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ABSTRACT

F_1-ATPase and V_1-ATPase are molecular rotary motors driven by ATP hydrolysis. We have investigated the rotation mechanism of F_1-ATPase using molecular dynamics (MD) simulations [1]. Recently, the crystal structure of V_1-ATPase was determined, and the rotation of V_1-ATPase was measured using single-molecule experiments. The crystal structure of V_1-ATPase indicates that the conformational changes of subunits in V_1-ATPase are somewhat different from those of F_1-ATPase. Single-molecule experiments revealed that the 120-degree rotation of V_1-ATPase without substeps, in contrast to F_1-ATPase exhibiting substeps in the 120-degree rotation. To elucidate the rotation mechanism of V_1-ATPase on the basis of the structures, we conducted multiscale MD simulations of V_1-ATPase [2]. First, all-atom MD simulations were carried out to analyze the thermal fluctuations in the hydrolysis-waiting state. The correlated motions in the thermal fluctuations were consistent to conformational changes in V_1-ATPase. Then, to simulate the 120-degree rotation of V_1-ATPase, the coarse-grained (CG) MD simulations were performed. The parameters of the CG MD simulations were determined on the basis of results of all-atom MD simulations using the fluctuation matching method. The 120-degree rotation of V_1-ATPase was successfully observed in the CG MD simulations. The two key events during the rotation were identified: First, the "bindable-like structure" spontaneously emerges just before the rotation. The bindable-like structure is the wide-open structure that is capable of ATP binding. Second, the C-terminal domain of the B subunit was detached from the adjacent subunits, and acts like a gate open for the rotation of the central stalk. After the rotation of the central stalk, the C-terminal domain of the B subunit was attached again just like a gate close preventing the reverse rotation.

韓國 Jooyoung 學者以電腦模擬來進行蛋白質結構的預測及發展新的演算法解決 x-ray 結構決定時的軟體

S-2

Protein structure prediction/determination by global optimization

Jooyoung Lee

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ABSTRACT

First, I will discuss our recent progresses on the protein structure prediction using the methodology of global optimization as illustrated in CASP11&12 competitions held in 2014 and 2016. We will demonstrate that this method can be applied to difficult MR (molecular replacement) targets to determine X-ray crystallography structures of proteins and protein complexes, which could not be solved using conventional MR methods. We will also discuss the potential application of our method to the high throughput NMR protein structure determination including large proteins (over 20 kDa) and membrane proteins.

另一場由 Pivanat 所主講的研究工作與生物技術中心目前進行幾丁質酶蛋白質酶表現研究最為相關(摘要如下)，此作者利用蛋白質繞射技術來解析幾丁質酶的立體結構，同時也解析了幾丁質酶與醣類的立體結構，另外作者也利用小分子藥物資料庫來篩選可能的抑制劑，來解決 GlcNAcase 所導致的代謝疾病:如有些種類的癌症或 Gaucher 等疾病。是將來我們研究幾丁質酶可以的參考方向之一。

O-18

Structural, kinetic and thermodynamic insights into reaction intermediate analogues binding to a GH20 *β-N*-acetylglucosaminidase (GlcNAcase) from *Vibrio harveyi*

Piyanat Meekrathok[1], Arthur T. Porfetye[2], Marco Bürger[2], Ingrid R. Vetter[2], Keith A. Stubbs[3] and Wipa Suginta[1,*]

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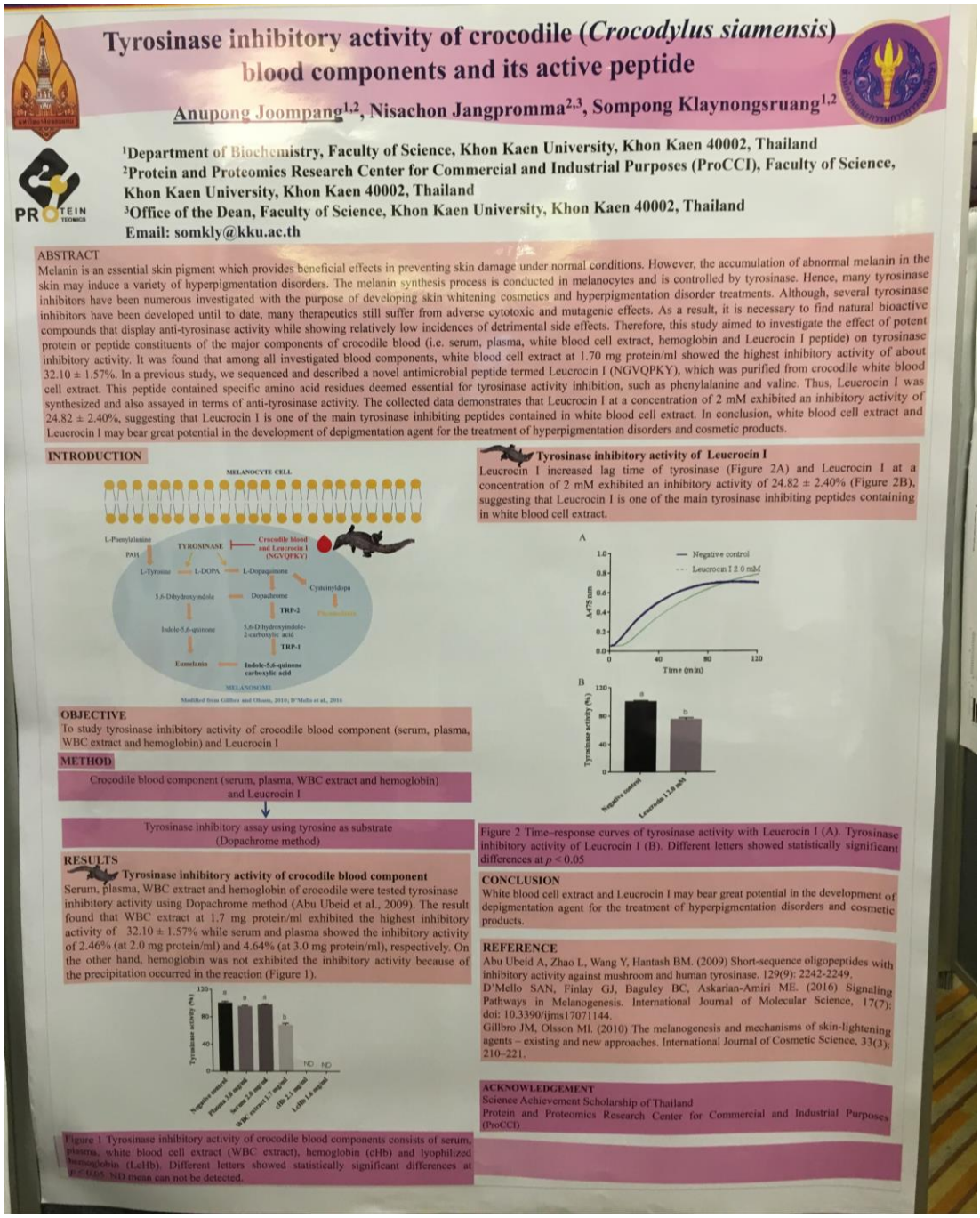
ABSTRACT

Vibrio harveyi β-N-acetylglucosaminidase (*Vh*GlcNAcase) is a new member of the family 20 glycoside hydrolase (GH20) responsible for a successive degradation of chitin biomaterials, generating *N*-acetylglucosamine (GlcNAc) as the final product. In this study, crystal structures of wild-type *Vh*GlcNAcase with and without sugar ligand and its catalytically inactive mutant D437A were solved. The overall structures of *Vh*GlcNAcase variants are identical, which consists of three distinct domains, designated as the *N*-terminal carbohydrate-binding (CBD) domain, the α+β topology domain, and the *C*-terminal TIM-barrel catalytic domain. The active site of *Vh*GlcNAcase has a pocket-like structure, suitable for accommodating a short chain of chitooligosaccharides with 2-4 GlcNAc units. *Vh*GlcNAcase was further exposed to four reaction intermediate analogues and dose-response curves showed that PUGNAc was the most potent inhibitor for *Vh*GlcNAcase (IC_{50} = 1.2 μM), followed by NAG-thiazoline, NHAc-CAS, and NHAc-DNJ, respectively. Evaluation of binding affinity obtained using ITC suggested that *Vh*GlcNAcase bound to the reaction intermediate analogues with K_d values between 0.19 and 25.6 μM and the orders of binding strength for the four inhibitors were in a good agreement with the IC_{50} values. Thermodynamic analysis indicated that *Vh*GlcNAcase binding to all the inhibitors was enthalpy-driven reactions, reflecting that hydrogen bonds and electrostatic forces essentially dominated the protein-inhibitor interactions. The results obtained from this study provide some implications that these inhibitors may be used as a potential drug target for GlcNAcase-induced metabolic diseases, such as some types of cancers and Gaucher's disease.

This research was supported by Suranaree University of Technology (SUT) and by Office of the Higher Education Commission under NRU Project of Thailand (FtR.33/2559) and the German Academic Exchange Service (DAAD) (Code number: A/10/98020).

會議中也參觀了**海報展覽**，有趣的是泰國當地對於鱷魚的紅血球水解產物或短鏈的胜肽研究相當有興趣，它們發現了鱷魚的血液成分或短鏈的胜肽對於美白效果(酪胺酸脢抑制;tyrosinase inhibtion; PP069)、鱷魚的白血球萃取物具有抑菌效果(PP070)、鱷魚的紅血球水解產物具有抗發炎或抗氧化的功效等(PP071)。



PP069-39

Tyrosinase inhibitory activity of crocodile (*Crocodylus siamensis*) blood components and its active peptide

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ABSTRACT

Melanin is an essential skin pigment which provides beneficial effects in preventing skin damage under normal conditions. However, the accumulation of abnormal melanin in the skin may induce a variety of hyperpigmentation disorders. The melanin synthesis process is conducted in melanocytes and is controlled by tyrosinase. Hence, many tyrosinase inhibitors have been numerous investigated with the purpose of developing skin whitening cosmetics and hyperpigmentation disorder treatments. Although, several tyrosinase inhibitors have been developed until to date, many therapeutics still suffer from adverse cytotoxic and mutagenic effects. As a result, it is necessary to find natural bioactive compounds that display anti-tyrosinase activity while showing relatively low incidences of detrimental side effects. Therefore, this study aimed to investigate the effect of potent protein or peptide constituents of the major components of crocodile blood (i.e. serum, plasma, white blood cell extract, hemoglobin and Leucrocin I peptide) on tyrosinase inhibitory activity. It was found that among all investigated blood components, white blood cell extract at 1.70 mg protein/ml showed the highest inhibitory activity of about 32.10 ± 1.57%. In a previous study, we sequenced and described a novel antimicrobial peptide termed Leucrocin I (NGVQPKY), which was purified from crocodile white blood cell extract. This peptide contained specific amino acid residues deemed essential for tyrosinase activity inhibition, such as phenylalanine and valine. Thus, Leucrocin I was synthesized and also assayed in terms of anti-tyrosinase activity. The collected data demonstrates that Leucrocin I at a concentration of 2 mM exhibited an inhibitory activity off 24.82 ± 2.40%, suggesting that Leucrocin I is one of the main tyrosinase inhibiting peptides contained in white blood cell extract. In conclusion, white blood cell extract and Leucrocin I may bear great potential in the development of depigmentation agent for the treatment of hyperpigmentation disorders and cosmetic products.

This research was supported by Science Achievement Scholarship of Thailand.

PP070-40

Antimicrobial activity of Crocodile (*Crocodylus siamensis*) leukocyte extract against *Propionibacterium acnes*: Application for Acne treatment

Weerava Phupiewkham[1,2], Nisachon Jangpromma[2,4], Sakda Daduang[2,3], Threeranan Temsiripong[5] and Sompong Klaynongsruang[1,2]

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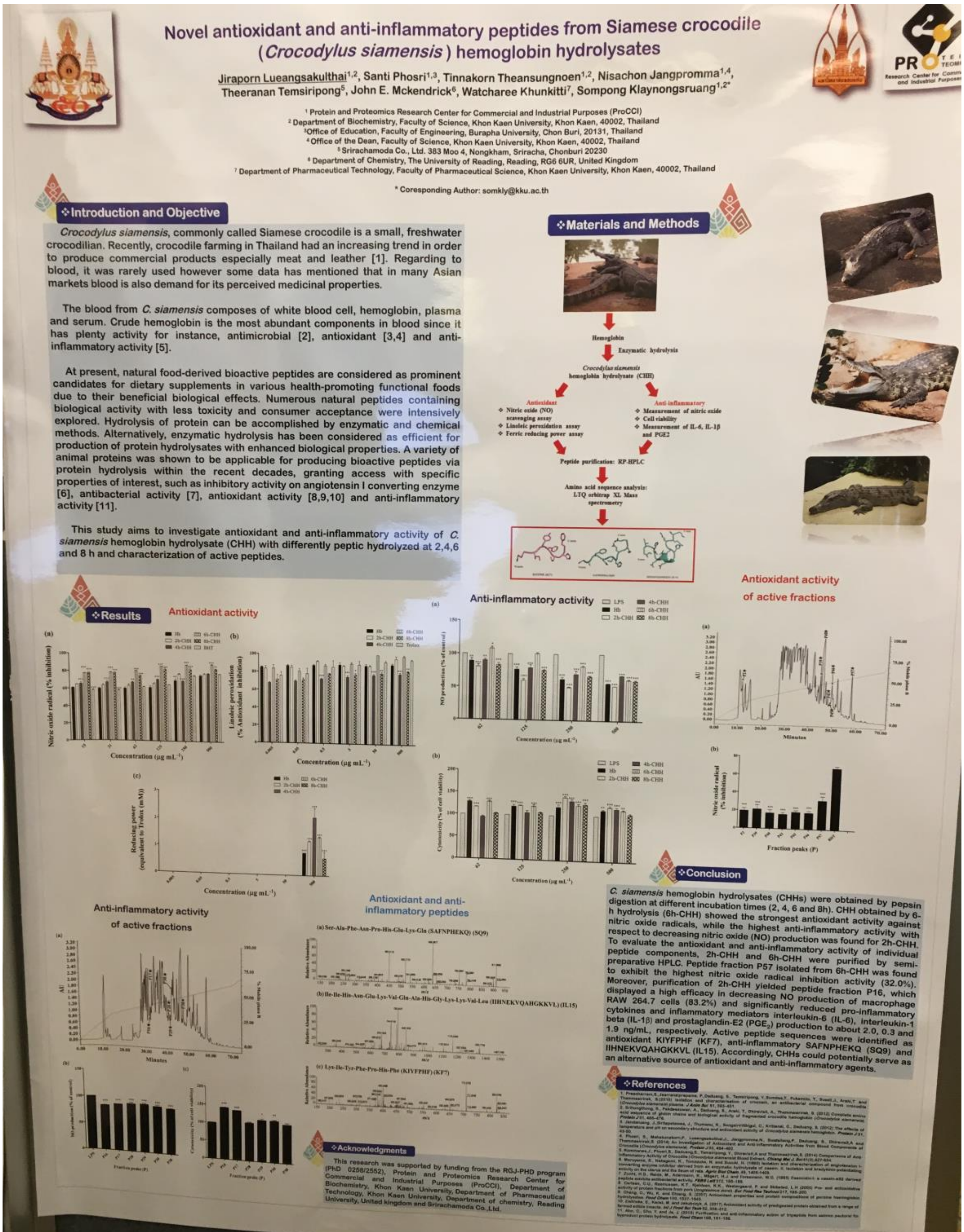
ABSTRACT

Acne vulgaris is a skin disorder of the sebaceous follicles that may be caused by various factors, in particular bacterial infections with *Propionibacterium acnes*, which constitute the main cause of inflammatory lesions in acne. Treatment strategies against acne have therefore focused on killing *P. acnes* using antibiotics; however, such approaches are often complicated by the occurrence of detrimental side effects and emerging antibacterial drug resistance. Hence, this research was aimed at investigating the anti-acne activity of a *C. siamensis* leukocyte extract and isolating potential novel antimicrobial proteins or peptides contained therein. Finally, the feasibility to formulate a commercial product based on crocodile leukocyte extract as anti-acne gel was critically elucidated. Therefore, the antibacterial activity of crocodile leukocyte extract was screened against 8 strains of aerobic bacteria and anaerobic *P. acnes*, revealing a broad spectrum antibacterial activity with MICs of 100 μg/ml and 50 μg/ml against aerobic and anaerobic bacteria, respectively. The inhibitory ability was further found to significantly increase in a concentration dependent manner. In addition, the effects of leukocyte extract treatment on bacterial cells were observed via scanning electron microscopy (SEM). The results showed the concentration-dependent induction of distinct abnormalities for *P. acnes* cells treated with leukocyte extract, such as cell shrinking or blebbing, membrane peeling and cell fragmentation or extensive cellular damage. Of note, leukocyte extract was not found to be toxic against human keratinocyte cells and effectively decreased inflammation in an animal model. Finally, it could be shown that the formulated anti-acne product still exhibited a high potential to kill *P. acnes* bacteria and scavenge DPPH radicals after the gel formulation process.

This research was supported by the Royal Golden Jubilee (RGJ) Ph.D. Program of Thailand Research Fund [grant number PHD/0141/2553] and BEDO [Biodiversity-Based Economy Development Office (Public Organization)].


Novel antioxidant and anti-inflammatory peptides from Siamese crocodile (Crocodylus siamensis) hemoglobin hydrolysates
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Introduction and Objective
Crocodylus siamensis, commonly called Siamese crocodile is a small, freshwater crocodilian. Recently, crocodile farming in Thailand had an increasing trend in order to produce commercial products especially meat and leather [1]. Regarding to blood, it was rarely used however some data has mentioned that in many Asian markets blood is also demand for its perceived medicinal properties.
The blood from C. siamensis composes of white blood cell, hemoglobin, plasma and serum. Crude hemoglobin is the most abundant components in blood since it has plenty activity for instance, antimicrobial [2], antioxidant [3,4] and anti-inflammatory activity [5].
At present, natural food-derived bioactive peptides are considered as prominent candidates for dietary supplements in various health-promoting functional foods due to their beneficial biological effects. Numerous natural peptides containing biological activity with less toxicity and consumer acceptance were intensively explored. Hydrolysis of protein can be accomplished by enzymatic and chemical methods. Alternatively, enzymatic hydrolysis has been considered as efficient for production of protein hydrolysates with enhanced biological properties. A variety of animal proteins was shown to be applicable for producing bioactive peptides via protein hydrolysis within the recent decades, granting access with specific properties of interest, such as inhibitory activity on angiotensin I converting enzyme [6], antibacterial activity [7], antioxidant activity [8,9,10] and anti-inflammatory activity [11].
This study aims to investigate antioxidant and anti-inflammatory activity of C. siamensis hemoglobin hydrolysate (CHH) with differently peptic hydrolyzed at 2,4,6 and 8 h and characterization of active peptides.
Materials and Methods
Hemoglobin
Enzymatic hydrolysis
Crocodylus siamensis hemoglobin hydrolysate (CHH)
Antioxidant
Nitric oxide (NO) scavenging assay
Linoleic peroxidation assay
Ferric reducing power assay
Anti-inflammatory
Measurement of nitric oxide
Cell viability
Measurement of IL-6, IL-1β and PGE2
Peptide purification: RP-HPLC
Amino acid sequence analysis: LTQ orbitrap XL Mass spectrometry
Results
Antioxidant activity
Anti-inflammatory activity
Antioxidant activity of active fractions
Anti-inflammatory activity of active fractions
Antioxidant and anti-inflammatory peptides
(a) Ser-Ala-Phe-Asn-Pro-His-Glu-Lys-Gln (SAFNPHEKQ) (SQ9)
(b) Ile-Ile-His-Asn-Glu-Lys-Val-Gln-Ala-His-Gly-Lys-Lys-Val-Leu (IIHNEKVQAHGKKVL) (IL15)
(c) Lys-Ile-Tyr-Phe-Pro-His-Phe (KIYFPHF) (KF7)
Concentration (µg mL-1)
Fraction peaks (P)
Minutes
Conclusion
C. siamensis hemoglobin hydrolysates (CHHs) were obtained by pepsin digestion at different incubation times (2, 4, 6 and 8h). CHH obtained by 6-h hydrolysis (6h-CHH) showed the strongest antioxidant activity against nitric oxide radicals, while the highest anti-inflammatory activity with respect to decreasing nitric oxide (NO) production was found for 2h-CHH. To evaluate the antioxidant and anti-inflammatory activity of individual peptide components, 2h-CHH and 6h-CHH were purified by semi-preparative HPLC. Peptide fraction P57 isolated from 6h-CHH was found to exhibit the highest nitric oxide radical inhibition activity (32.0%). Moreover, purification of 2h-CHH yielded peptide fraction P16, which displayed a high efficacy in decreasing NO production of macrophage RAW 264.7 cells (83.2%) and significantly reduced pro-inflammatory cytokines and inflammatory mediators interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and prostaglandin-E2 (PGE2) production to about 2.0, 0.3 and 1.9 ng/mL, respectively. Active peptide sequences were identified as antioxidant KIYFPHF (KF7), anti-inflammatory SAFNPHEKQ (SQ9) and IIHNEKVQAHGKKVL (IL15). Accordingly, CHHs could potentially serve as an alternative source of antioxidant and anti-inflammatory agents.
Acknowledgments
This research was supported by funding from the RGJ-PHD program (PhD 0258/2552), Protein and Proteomics Research Center for Commercial and Industrial Purposes (ProCCI), Department of Biochemistry, Khon Kaen University, Department of Pharmaceutical Technology, Khon Kaen University, Department of chemistry, Reading University, United kingdom and Srirachamoda Co.,Ltd.
References

PP071-41

Novel antioxidant and anti-inflammatory peptides from Siamese crocodile (*Crocodylus siamensis*) hemoglobin hydrolysates

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ABSTRACT

Oxidants and inflammation are well-known to be common causes for numerous diseases in the human body. The use of natural protein hydrolysates has become increasingly popular for treating oxidative-associated and inflammatory diseases. *Crocodylus siamensis* hemoglobin hydrolysates (CHHs) were obtained by pepsin digestion at different incubation times (2, 4, 6 and 8h). CHH obtained by 6-h hydrolysis (6h-CHH) showed the strongest antioxidant activity against nitric oxide radicals, while the highest anti-inflammatory activity with respect to decreasing nitric oxide (NO) production was found for 2h-CHH. To evaluate the antioxidant and anti-inflammatory activity of individual peptide components, 2h-CHH and 6h-CHH were purified by semi-preparative HPLC. Peptide fraction P57 isolated from 6h-CHH was found to exhibit the highest nitric oxide radical inhibition activity (32.0%). Moreover, purification of 2h-CHH yielded peptide fraction P16, which displayed a high efficacy in decreasing NO production of macrophage RAW 264.7 cells (83.2%) and significantly reduced pro-inflammatory cytokines and inflammatory mediators interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and prostaglandin-E2 (PGE_2) production to about 2.0, 0.3 and 1.9 ng/mL, respectively. Active peptide sequences were identified as antioxidant KIYFPHF (KF7), anti-inflammatory SAFNPHEKQ (SQ9) and IIHNEKVQAHGKKVL (IL15). Accordingly, CHHs could potentially serve as an alternative source of antioxidant and anti-inflammatory agents.

This research was supported by Royal Golden Jubilee Ph.D. program (RGJ-PHD program), Grant No. PhD 0258/2552.

參加了蛋白質工程及運用會議(Protein engineering & application sessions)

在全球酵素市場的總銷售值裡，工業用酵素約佔 70%，工業用途中又以清潔劑用酵素的市場最大宗(27%)，脂解酶(Lipase)就是其中一項代表，為了讓脂解酶可以在洗劑中發揮功效，從自然中尋求耐熱性、高廣度酸鹼度容忍的脂解酶研究工作就顯得格外重要，Raja 從 *Antarctic Pseudomonas* 發現了一個全新耐熱性、高酸鹼度範圍容忍的脂解酶，利用分子生物學表現了脂解酶蛋白質，並研究其結構與功能。

S-11

A thermotolerant lipase with broad pH isolated from Antarctic *Pseudomonas sp.* AMS3

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ABSTRACT

A gene encoding a thermotolerant lipase with broad pH was isolated from an Antarctic *Pseudomonas* strain AMS3. The recombinant lipase AMS3 was purified by single-step purification using affinity chromatography, yielding a purification fold of approximately 1.52 and a recovery of 50 %. The molecular weight was approximately ~60 kDa including the strep and affinity tags. Interestingly, the purified Antarctic AMS3 lipase exhibited broad temperature profile from 10-70 °C and stable over a broad pH range from 5.0 to pH 10.0. Various mono and divalent metal ions increased the activity of the AMS3 lipase, but Ni^{2+} decreased its activity. The purified lipase exhibited the highest activity in the presence of sunflower oil. In addition, the enzyme activity in 25 % v/v solvents at 50 °C particularly to n-hexane, DMSO and methanol could be useful for catalysis reaction in organic solvent and at broad temperature. The crystal of truncated AMS3 lipase diffracted to approximately 2.77 Å resolution using an in-house diffractometer.

7 月 13 日

參加了蛋白質藥物設計標的會議(Protein Drug target sessions)

瞭解蛋白質的三維結構有助於探討蛋白質在分子層次上的功能及可能作用機制，應用層面包括酵素在分子層次功能之探討、蛋白質與蛋白質間作用及電腦輔助藥物設計之開發等。蛋白質三度結構可由 X-光晶體繞射法測定。首先需純化蛋白質、培養蛋白晶體，分析時 X-光照射在晶體中規則排列的蛋白分子，晶體中的電子對 X 射線產生繞射作用，這些繞射點經由電子偵測器來收集，且分析每一點強度，來推論電子密度的分布情況，從中獲得原子排列位置資料，便可推估此一晶體結構之原子模型，而建立蛋白質的 3D 結構。清華大學呂老師研究與睡眠物質(melatonin)合成有關的重要酵素 dopamine N-acetyltransferase 的結構，藉由結構的細節資訊將可發展出幫助睡眠的小分子藥物的潛力。

S-16

Structural insights into the catalytic mechanism of dopamine N-acetyltransferase

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ABSTRACT

The daily cycle of melatonin biosynthesis in mammals is regulated by arylalkylamine *N*-acetyltransferase (EC 2.3.1.87, AANAT), making it an attractive target for therapeutic control of abnormal melatonin production in mood and sleep disorders. *Drosophila melanogaster* dopamine *N*-acetyltransferase (Dat) catalyzes arylalkylamine *N*-acetylation which transfers acetyl group of acetyl-CoA (Ac-CoA) to arylalkylamine to generate *N*-acetyl-arylalkylamine and CoA. We have solved the high-resolution crystal structure for a ternary complex for *D. melanogaster* Dat/ tryptamine /acetyl coenzyme A (AcCoA) obtained using one-edge (Selenium) single-wavelength anomalous diffraction. Examination of the complex structure indicated that Dat contained a novel AANAT catalytic triad. Site-directed mutagenesis and kinetic study confirmed that Glu47, Ser182, and Ser186 were critical for catalysis. According to the ternary complex structure, we also proposed that three aromatic residues (F43, Y64, and F114) in a hydrophobic substrate-binding pocket of Dat may play key roles in substrate specificity. The binding study by isothermal titration calorimetry suggested that Dat obeyed an ordered sequential mechanism: all the substrates involved are bound to the enzyme before catalysis of the reaction takes place; and AcCoA binds to the enzyme before dopamine binding. To confirm the ordered sequential mechanism, sequential titration of cofactors and substrates was performed and monitored by NMR. This study provides the structural insights into the enzyme activity and the substrate binding selectivity in Drosophila AANAT.

參加了蛋白質體與蛋白質研究新技術的會議(New techniques in proteomics & protein studies sessions)

韓國的學者 Yeon Gyu Yu，題目是利用 *E. coli* 表現與純化 GPCRs 穿膜蛋白。以往 GPCRs 都是利用昆蟲細胞 Insect cell 或哺乳類細胞 293T 進行表現，而無法使用 *E.coli* 表現。其原因普遍學者認為 *E.coli* 之表現系統沒有複雜糖基化或 Chaperone 幫助 GPCRs 表現與摺疊。然而，該學者利用一段序列取自 *Pseudomonas phage phi6*（P9） 連接在 GPCRs 之 N 端，使其可以幫助 *E.coli* 表現出 GPCRs 之序列，並利 amphipathic poly-r-glutamic acid 形成的 micelle 幫助 GPCRs 進行摺疊，並成功的純化到 GPCRs。該學者聲稱其進行了八種的 GPCRs（包含了七個與四個穿膜 Helix）的表現並成功的取得 GPCRs。該方法若可行，將會簡化過往的膜蛋白製備過程，並提升膜蛋白被研究的速度。

S-29

A novel method for the preparation and stabilization of membrane proteins and its application for the biochemical analysis of GPCRs

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ABSTRACT

Difficulties in the production, extraction of membrane proteins from cell membrane and their solubilization in native conformations have hindered their structural and biochemical analysis. We used high-level expression system for membrane proteins in *E. coli* using a fusion protein with a single transmembrane protein (P9) from Phi6 bacteriophage. Various membrane proteins including human GPCRs such as endothelin receptor type A or lysophosphatidic receptors were overexpressed in the plasma membrane fraction of *E. coli*. These overexpressed GPCRs in the plasma membrane were solubilized and purified as by single step-affinity chromatography. We also developed amphipathic polymer by conjugation of octyl, diethylaminopropyl and glucosyl groups to poly-γ-glutamic acid. This amphipathic polypeptide, named as APG, self-assembles as mono-disperse oligomers with a low critical micelle concentration, and stabilizes GPCRs in their active conformation.

Antibodies targeting to disease-related GPCRs have been great interests for the development of potential therapeutic antibody. We have tested whether a GPCR-targeting antibody can be screened using the purified GPCRs. A single chain antibody (scFv) constructed by the connection of two variable regions from heavy and light chains of IgG were successfully screened from a M13 phage library displaying randomized scFvs using the purified GPCRs. These results implied that the overexpression and stabilization techniques of membrane proteins could apply for the biophysical and biochemical analyses as well as for the development of therapeutic agents.

參加了 SLRI 同步輻射光源於蛋白質科學運用工作坊(Synchrotron protein science workshop)

來自台灣同步輻射研究中心陳俊榮博士所介紹的 NSRRC 同步輻射光源利用於蛋白質結構的探討報告，最近剛建立好的 TPS 提供 X 光具有高亮度、能量可調與高穩定度的特性，是目前台灣研究蛋白質結構最重要的利器。未來將可透過合作，申請光束線時間收集中油生技所研究的蛋白質晶體結構。同時同步輻射也提供免費的教育訓練課程，供想要進行蛋白質結構研究單位申請。

W-5

New opportunities of protein research at Taiwan Photon Source (TPS) of NSRRC

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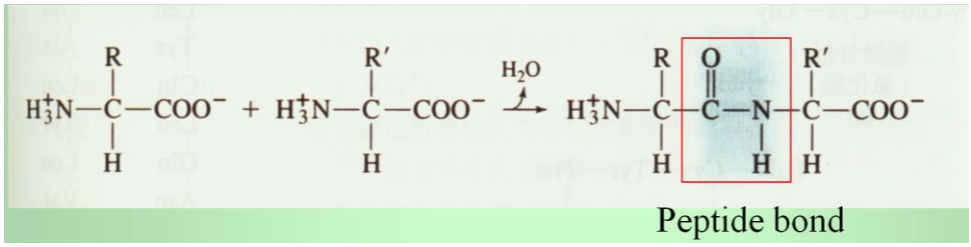
ABSTRACT

In addition to the Taiwan Light Source (TLS), NSRRC has recently successfully constructed a low-emittance 3 GeV synchrotron light source, the Taiwan Photon Source (TPS). This new TPS facility will provide great opportunities for advanced research on the various fields of life sciences. Synchrotron protein crystallography (PX) has been effective for structure determination of biological macromolecules, especially the membrane proteins and large molecular assemblies, such as viruses. The highly bright X-ray provides the special needs for these relatively small or weak-diffraction crystals toward their atomic resolution structures. With the new TPS, a new micro-beam protein crystallography beamline has been open to the domestic and international user community of structural biology since September of 2016. The focused beam size at the crystal sample is 50 μm (H) x 20 μm (V) with photon flux of 6 x10^{12} photons/s. Apertures are used to collimate the beam size to a range of 50–5 μm. The beam divergence at the sample is less than 500 μrad (H) and 100 μrad (V), and the energy range is from 5.7 to 20 keV (wavelength 2.175–0.62 Å). Other beamlines related to protein research and some application examples will also be briefly introduced. Combining this advanced PX beamline at TPS with other operated and planned PX and bio-related beamlines at NSRRC, more complicated and challenging structures of biological macromolecules can be resolved at high resolution in the near future.

另外也將此次研討會收集到的相關資訊及未來可能利用於蛋白質研究技術整理於下

蛋白質(protein; polypeptides)

蛋白質是高度聚合的生物分子，或高分子，是人體中佔最高比例的有機物(約佔15%)，如果生物體無法合成蛋白質就無法生存，可見蛋白質的重要性，蛋白質由一個或多個由胺基酸(Amino acid)殘基組成的長鏈條組成。胺基酸分子呈線性排列，相鄰胺基酸殘基的羧基和氨基通過胜肽鍵(peptide bond)連接在一起(圖四)。蛋白質的胺基酸序列是由對應基因所編碼。除了遺傳密碼所編碼的 20 種常見的胺基酸(圖五)，在蛋白質中，某些胺基酸殘基還可以被改變原子的排序而發生化學結構的變化，從而對蛋白質進行激活或調控。多個蛋白質可以一起，往往是通過結合在一起形成穩定的蛋白質複合物，發揮某一特定功能。

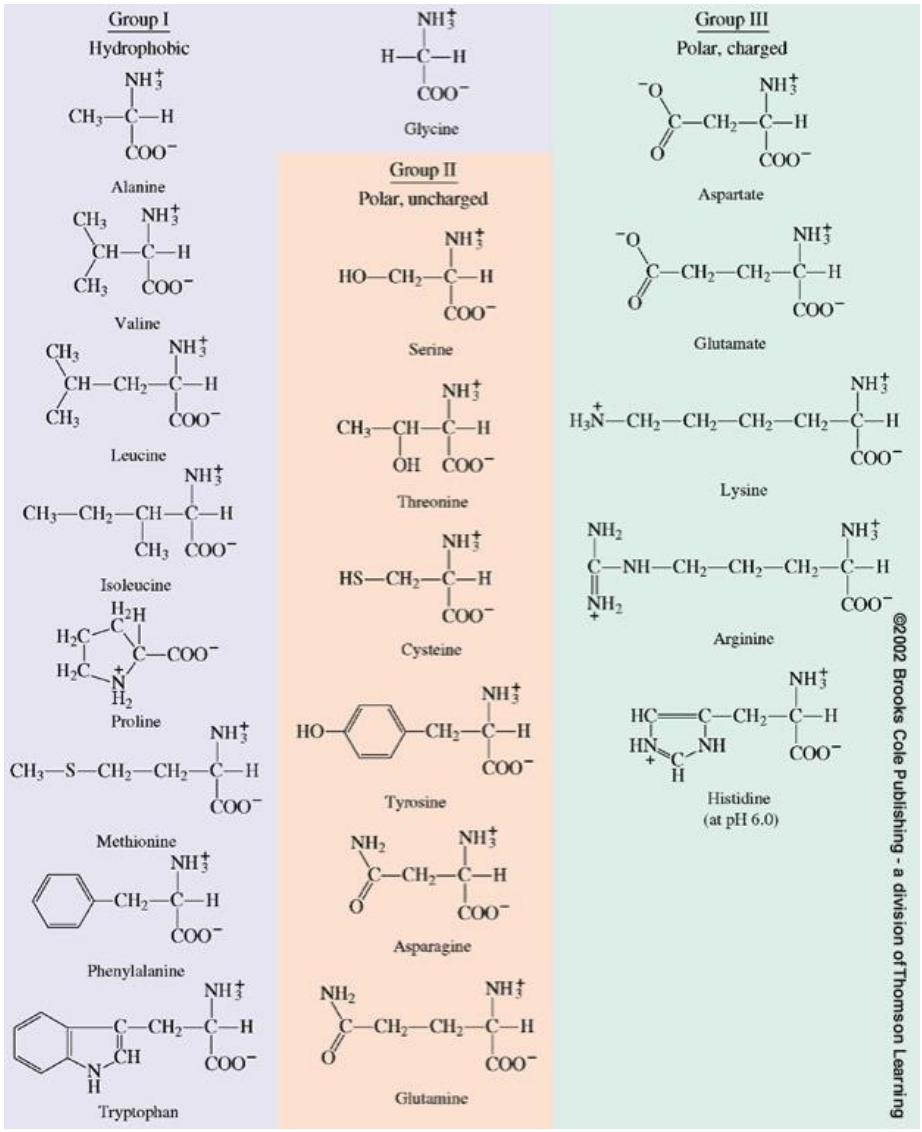


圖四、胺基酸形成胜肽鍵(peptide bond)，聚合成蛋白質。

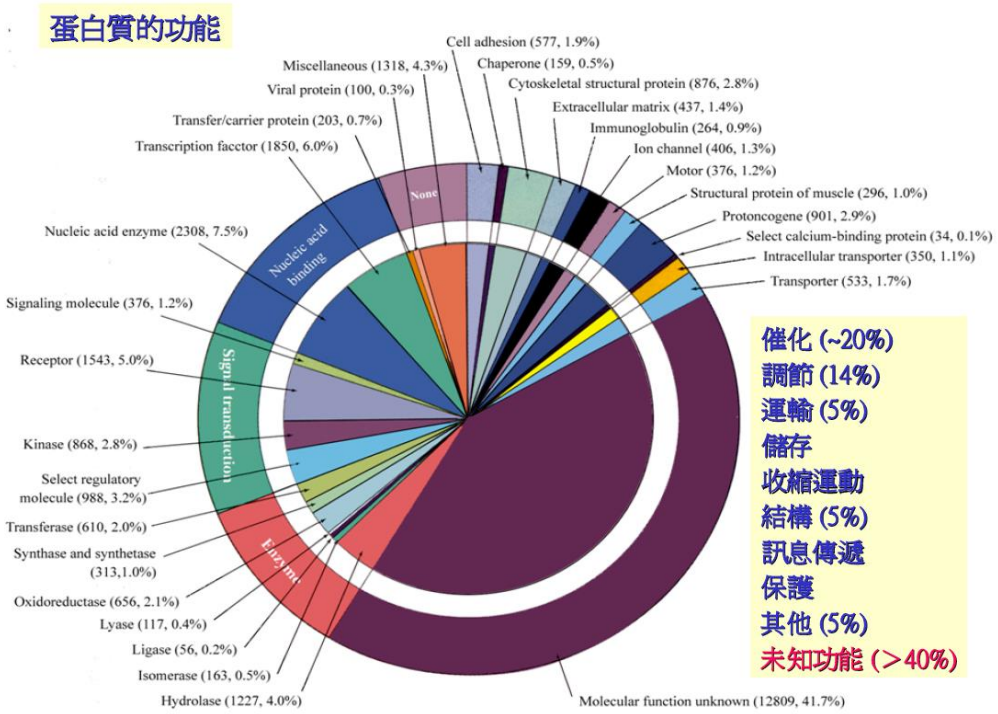
蛋白質的功能

蛋白質與其他生物大分子（如多糖和核酸）一樣，蛋白質是地球上生物體中的必要組成成分，參與了細胞生命活動的每一個進程。酶(或稱酵素)是最常見的一類蛋白質，它們催化生物化學反應，尤其對於生物體的代謝至關重要。除了酶之外，還有許多結構性或機械性蛋白質，如肌肉中的肌動蛋白和肌球蛋白，以及細胞骨架中的微管蛋白（參與形成細胞內的支撐網絡以維持細胞外形）。另外一些蛋白質則參與細胞信號傳導、免疫反應、細胞黏附和細胞周期調控等。蛋白質也構成抗體或干擾素負責對抗外敵入侵的防禦功能。同時，蛋白質也是動物飲食中必需的營養物質，這是因為動物自身無法合成所有胺基酸，動物需要和必須從食物中獲取必需胺基酸。通過消化過

程將蛋白質降解為自由胺基酸，動物就可以將它們用於自身的代謝。人類基因體計畫執行至今，大約還有 40%蛋白質的功能尚未釐清，可見蛋白質研究的重要性(圖六)。



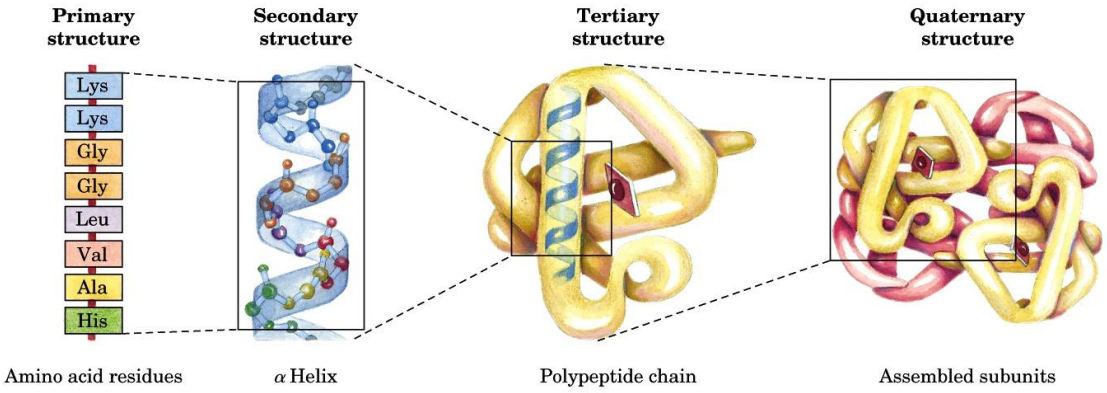
圖五、生物體常見的 20 種胺基酸名稱與化學式。



圖六、人類基因所含有蛋白質的功能比例圖。

蛋白質的結構

蛋白質由胺基酸以胜肽鍵形成長鏈的聚合物後，需要適度的折疊成具有 3D 立體結構的規則排列，才能展現出特定的功能性。蛋白質結構可以分為四類;一級結構為胺基酸殘基的排列序列，二級結構為一級結構中某個特定範圍摺疊的規律性排列，如 alpha-helix, beta-sheet，三級結構為由二級結構堆疊成的三度空間結構，四級結構為兩條或多條 polypeptide 相互結合的蛋白質分子外型(圖七)。一般來說蛋白質具有三級結構才具有特定的功能，所以研究一個有功能的蛋白質要先研究他的蛋白質結構。目前研究蛋白質結構的方法包括 NMR、蛋白質結晶 X 光繞射實驗兩種，NMR 可以量測蛋白質於水溶液下的結構，但僅限於研究分子量小的蛋白質; 反之、蛋白質結晶 X 光繞射實驗是目前最具有解析蛋白質結構的利器，在此次研討會重要的蛋白質研究報告幾乎都有蛋白質 X 光繞射實驗來輔助解釋蛋白質的功能研究。



圖七、蛋白質一級、二級、三級及四級結構。

(三)心得及建議

蛋白質為構成生命要素的巨分子之一，為實現生命主要的執行者，蛋白質的應用也非常廣泛、包括工業用酵素、食品用酵素、疾病的診斷與治療、更可成為藥物設計的標的，所以中油生技中心長遠的規劃不應在蛋白質這研究領域缺席。

此次會議主要的心得為了解世界上各國於蛋白質研究的進展及常用的工具，由其是蛋白質繞射技術來解析蛋白質的立體結構，是目前研究蛋白質不可或缺的重要技術，幾乎此次所有與會蛋白質研究學者都有觸及到的部分。蛋白質的立體結構也可以運用於藥物設計，也是目前台灣或世界上蛋白質藥物開發公司所極力發展的重要領域，有鑑於此世界流行趨勢，蛋白質表現、純化及結構探討是將來生技中心研究的重要參考方向之一。

位於新竹科學園區的同步輻射光源，具有高亮度、能量可調與高穩定度的特性，是目前台灣研究蛋白質結構最重要的利器。職於蛋白質結構領域雖曾有接觸但並非熟悉，未來將可透過與國家同步輻射研究中心合作，申請光束線時間，收集中油生技所研究的蛋白質晶體結構，讓中油生技所研究的蛋白質能力與世界接軌。