財團法人明日醫學基金會專題研究計畫申請書

一、基本資料： 申請條碼：

本申請案所需經費(單選)	■A 類(研究主持費及執行計畫所需經費) ☐B 類(研究主持費，限人文處計畫，不須填寫表 C002 及 C004 至 C009)				
計畫類別(單選)	■一般型研究計畫 ☐新進人員研究計畫 ☐其他				
研究型別	■個別型計畫 ☐整合型計畫				
計畫歸屬	☐自然處 ☐工程處 ■生物處 ☐人文處 ☐科教處 ☐永續會				
申請機構/系所（單位）	清華大學生命科學系暨分子與細胞研究所				
本計畫主持人姓名	王雯靜	職稱	教授	身分證號碼	
本計畫名稱	中文	胃幽門螺旋菌合成 glycosylated 膽固醇之 cholesterol-α-glucosyltransferase 證實與細菌的外觀及致病性相關之研究			
	英文	Investigation of *Helicobacter pylori* cholesterol-α-glucosyltransferase that synthesizes glycosylated cholesterols to support bacterial morphology and pathogenicity			
整合型總計畫名稱					
整合型總計畫主持人				身分證號碼	
全程執行期限	自民國 97 年 01 月 01 日起至民國 97 年 12 月 31 日				
研究學門(請參考本申請書所附之學門專長分類表填寫)	學門代碼	名稱(如為其他類，請自行填寫學門)			
	基礎醫學				
研究性質	■純基礎研究 ☐導向性基礎研究 ☐應用研究 ☐技術發展				
本年度申請主持國科會各類研究計畫(含預核案)共_____件。(共同主持之計畫不予計入) 本件在本年度所申請之計畫中優先順序(不得重複)為第_____。					
本計畫是否為國際合作計畫 ■否； ☐是，合作國家：_____，請加填表 I001～I003					
本計畫是否申請海洋研究船	■否； ☐是，請務必填寫表 C014。				
本計畫是否有進行下列實驗：(勾選下列任一項，須附相關實驗之同意文件) ☐人體實驗/人體檢體 ■基因重組實驗 ☐動物實驗					
計畫連絡人	姓名：王雯靜 電話：(公) 03-5715131 x 42766 (宅/手機) 04-22617849				
通訊地址	台中市南區南和路 203 號 11 樓之二				
傳真號碼	03-5742766	E-MAIL	wcwang@life.nthu.edu.tw		

計畫主持人(申請人)簽章：_____ 日期：_____

二、研究計畫中英文摘要：請就本計畫要點作一概述，並依本計畫性質自訂關鍵詞。

（一） 計畫中文摘要。(五百字以內)

胃幽門螺旋菌（*Helicobacter pylori, H. pylori*） 的感染會導致慢性胃炎，並可能演變成消化性潰瘍（peptic ulcer）、淋巴瘤（lymphoma），甚至是胃癌（gastric carcinoma）。細菌藉由獨特躲避宿主免疫反應的能力，使保護細菌能持續生存及感染。在胃幽門螺旋菌中，cholesterol-α-glucosyltransferase 酵素能催化膽固醇進行 glycosylation 作用，在細菌細胞壁脂質層上形成 cholesterol glucosides，重要是它可避免細胞吞噬作用、T 細胞的活化及細菌的生長。與黏液相關的 O-glycans 為天然的抗生素，可抑制 cholesterol-α-glucopyranoside 的生合成，抵抗胃幽門螺旋菌的感染。我們已成功在大腸桿菌系統中表現及純化出 cholesterol-α-glucosyltransferase 重組蛋白。我們初步結果發現，此酵素在電子顯微鏡下呈現出 oligomeric 狀態。此計畫將針對 cholesterol-α-glucosyltransferase 進行研究，決定 cholesterol-α-glucosyltransferase 之蛋白質結構及研究探討 cholesterol-α-glucosyltransferase 如何由宿主膜上直接吸收膽固醇。我們亦將研究 cholesterol-α-glucopyranosides 如何促進巨噬細胞主導訊息傳達。

（二）計畫英文摘要。（五百字以內）

Helicobacter pylori infection causes gastric diseases including gastritis, peptic ulcers, lymphoma, and/or carcinoma. The capability to evade host immune response by this peculiar microbe ensures its persistent colonization. *H. pylori* cholesterol-α-glucosyltransferase catalyzes the glycosylation of cholesterol to form the bacteria cell wall lipids cholesterol glucosides that are important for escaping phagocytosis, T cell activation, and bacterial growth. Mucus-associated O-glycans that inhibits the biosynthesis of cholesterol-α-glucopyranoside are natural antibiotics against *H. pylori* infection. We have expressed and purified the recombinant cholesterol-α-glucosyltransferase in *Escherichia coli* expression system. Our preliminary results reveal an oligomeric state of this enzyme under electron microscopy. The goal of this proposal is to determine the structures of cholesterol-α-glucosyltransferase and to investigate whether cholesterol-α-glucosyltransferase directly assimilates cholesterol from host membranes. We also aim to investigate whether cholesterol-α-glucopyranosides induce macrophage-mediated response.

Keywords: *Helicobacter pylori*; cholesterol α-glucosyltransferase; *O*-linked glycan.

三、研究計畫內容：

（一）近五年之研究計畫內容與主要研究成果說明。（連續性計畫應同時檢附上年度研究進度報告）

（二）研究計畫之背景及目的。請詳述本研究計畫之背景、目的、重要性及國內外有關本計畫之研究情況、重要參考文獻之評述等。本計畫如為整合型研究計畫之子計畫，請就以上各點分別述明與其他子計畫之相關性。

Background and Significance

Enduring infection of *Helicobacter pylori* (*H. pylori*) in the human stomach causes gasto-intestinal diseases including chronic gastritis, peptic ulcers, lymphoma and/or carcinoma [1, 2]. *H. pylori* colonizes the superficial layer of gastric mucosa and then induces chronic gastritis that is believed to associate with glandular atrophy [1]. Prolonged infection with *H. pylori* is also accounted for the complex inflammatory responses, which are followed by intestinal metaplasia, dysplasia, and eventually gastric adenocarcinoma [2].

Surface adhesion molecules including BabA and SabA that binds to *Lewis b* and *Lewis X* glycolipids, respectively, expressed on the surface of gastric mucous cells are crucial for the successful colonization of *H. pylori* [5, 6]. Virulent strains are frequently found to contain a type IV secretion system (TFSS) encoded by *cag* pathogenicity island (*cag* PAI) and an exotoxin, vacuolating toxin (VacA) [7, 8]. Infection of these strains is closely associated with ulcerations and carcinoma [9-12]. Additionally, *cag* PAI-positive strains delay phagocytosis by macrophages in vitro and are killed less efficiently than those without the PAI element [11, 13]. VacA displays multiple cytotoxic effects in epithelial cells and also inhibits T-cell proliferation [8]. Other factors known to interact directly with immune cells and modulate immune responses to *H. pylori* include neutrophil-activating protein (HP-NAP or NapA), arginase, urease, and Hsp60 (a GroEL heat shock protein) [3]. Recently, cholesterol alpha-transferase that transforms cellular cholesterol into glucosylated derivatives is found to be important to evade phagocytosis during infection [4]. Alternation in the total amount of steryl glucosides and the relative proportions of cholesteryl-α-D-glucopyranoside (αCG), cholesteryl-6'-*O*-tetradecanoyl-α-D-glucopyranoside (αCAG), and cholesteryl-6'-*O*-phosphatidyl-α-D-glucopyranoside (αCPG) are found to cause morphological changes of the bacterium or changes in colony variants [5]. Additionally, inhibition of the cholesterol glucosyltransferase by *O*-glycans of the human gastric mucosa is found to suppress growth of the bacterium [6]. Thus, cholesteryl-α-glucosides synthesized by cholesterol glucosyltransferase are important components of bacterial membranes to support the cellular morphology as well as pathogenicity of this organism.

In this proposal, we focus on the structure-function relationship of cholesterol-α-glucosyltransferase and the molecular mechanisms that lead to immune evasion. It will be also of interest to delineate the role of this enzyme in disrupting host rafts and whether an inhibitor of this enzyme can be used as a novel therapeutic lead.

Specific Aims:

Aim 1. To determine the structure of cholesterol-α-glucosyltransferase by x-ray crystallography or electron microscopy;

Aim 2. To investigate whether cholesterol-α-glucosyltransferase is required to absorb cholesterol and other constituents from rafts of the host-cell membrane.

（三）研究方法、進行步驟及執行進度。請分年列述：1.本計畫採用之研究方法與原因。2.預計可能遭遇之困難及解決途徑。3.重要儀器之配合使用情形。4.如為整合型研究計畫，請就以上各點分別說明與其他子計畫之相關性。5.如為須赴國外或大陸地區研究，請詳述其必要性以及預期成果等。

Research design

Aim 1. To determine the structure of cholesterol-α-glucosyltransferase by x-ray crystallography or electron microscopy

Cholesterol alpha-transferase catalyzes the glycosylation of cholesterol to produce cholesteryl-alpha-D-glucopyranoside and other derivatives, which may help to evade host phagocytosis and subsequent clearance [7, 8]. Thus far, no structural information is available for this enzyme. The first aim is to investigate structure-function of this enzyme with a straightforward structural and biochemical approach as follows.

1.1. Cloning, expression and purification of cholesterol alpha-transferase.

This task is rather straightforward in our laboratory given the established *E. coli* expression system: (1) The cloned gene obtained by polymerase chain reaction (PCR) amplification experiments will be inserted into pQE30 for initial expression trial. (2) Other over-expression vectors will be tested to obtain the optimized production of soluble forms. (3) The expressed protein will be purified by affinity chromatography plus other columns. Indeed, we have cloned, expressed and purified cholesterol alpha-transferase. As illustrated in Fig. 1, purified cholesterol alpha-transferase has been obtained.

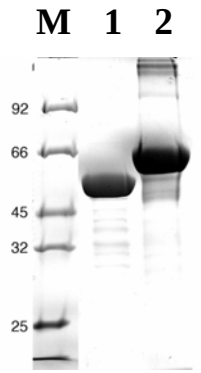

Fig. 1. SDS-PAGE analysis of the purified cholesterol α-transferase recombinant proteins. Lane 1: protein size markers. M: size markers; lane 1: purified cholesterol α-transferase; lane 2, 10 μg of bovine serum albumin.

1.2. Determination of structures.

The structural information at the atomic level will contribute significantly to the understanding of crucial residues and catalytic mechanism as well as provide a basis to investigate intricate biological processes in the *H. pylori* pathogenesis. We have previously determined AmiE, AmiF, and a few enzyme structures in the shikimate pathway as well structures of a few biocatalysts [9-15]. As an extension of our previous research, structures of arginase and cholesterol alpha-transferase will be determined. Indeed, crystallization trials of cholesterol alpha-transferase have yielded single crystals (Fig. 2). We currently attempt to improve the crystal quality. Once a good-diffracting crystal form is obtained, single/multiple anomalous dispersion (SAD/MAD) methods will be utilized to solve the structure owing to the lack of a homologous structure.

In collaboration with Dr. Liu GK in National Health Research Institutue, cryo-negative staining developed as a complementary technique will be utilized. This technique allows imaging biological samples in a comparable state of structural preservation to conventional cryo-EM but the staining produces better contrast in accessible areas and allows data recording at lower defocus values. Cryo-negative staining vitrifies biological particles in the presence of a concentrated ammonium molybdate solution at neutral pH. It was successfully used to study the structure and dynamics of several macromolecules. Imaging macromolecular complexes with cryo-negative staining has been established previously to better than 2 nm detail. Our preliminary results indeed showed oligomeric structure of cholesterol α-transferase (data not shown). We are now under the process to reconstruct the image.

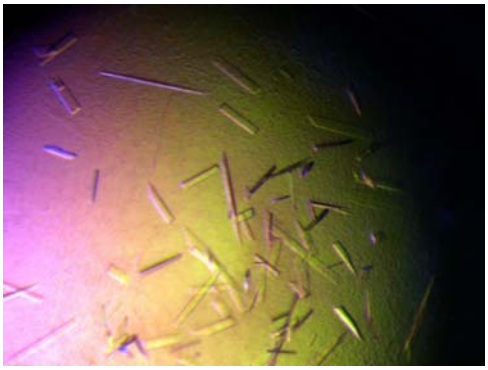

Fig. 2. Crystals of cholesterol alpha-transferase. Crystals were obtained from mother solution containing 45% MPD, 100 mM imidazole, pH8.0, 200 mM $MgCl_2$ with protein solution of ~8 mg ml^{-1}.

1.3. Investigation of structure-function relationship.

To investigate the structure-function relationship, the enzymatic assays will be set up. Enzymatic kinetics will then be investigated. Furthermore, structures of the enzyme-substrate/inhibitor complex will be determined, allowing the detailed analysis of the crucial residues involved in binding and catalysis. Structure-function analysis with other related enzymes will be conducted to investigate the evolution of enzymatic function, which will not only provide fundamental biological understanding but also shed light into the potential biotechnological applications. Site-directed mutagenesis of crucial residues will be conducted to truly understand the role of individual residues. Our next focus will be to evaluate whether a potent inhibitor of these enzymes can be discovered based on structural information. We will further delineate whether cholesterol and its derivatives have any effects on host cells such as to alter gastric functioning in particular directions that affect local inflammation or energy homeostasis.

Aim 2. To investigate whether cholesterol-α-glucosyltransferase is required to absorb cholesterol and other constituents from rafts of the host-cell membrane.

2.1. To generate mutants by allelic exchange mutagenesis.

We will investigate whether cholesterol-α-glucosyltransferase is engaged in absorbing cholesterol from rafts. To approach this issue, we have established a means of allelic-exchange mutagenesis to generate knock-out strains that encodes cholesterol-α-glucosyltransferase from the bacterial genome. The knock-out mutants will be generated respectively by insertion of the *cat* fragment derived from pUOA20 [16] into *capJ* that encodes cholesterol-α-glucosyltransferase through allelic replacement and selection of chloramphenicol-resistant clones [17]. The *capJ* gene fragment was amplified from strain 26695 chromosome by PCR and cloned into plasmid pGEMT (Promega), yielding the plasmid pGEM-*capJ*. The *cat* fragment is amplified by PCR and is inserted into the pGEM-*capJ* plasmid. The *capJ*::*cat* mutant is then obtained by transforming the resultant plasmid into the parental strain *H. pylori* 26695 through allelic replacement and selection of chloramphenicol-resistant clones using a natural transformation method described in Wang *et al*. [17].

2.2 Confocal microscopic analysis of cholesterol/GM1 on AGS cells infected with wild-type *H. pylori* or knock-out capJ *H. pylori*

AGS cells are infected with wild-type H. pylori or knock-out CapJ mutant [multiplicity of infection (MOI) = 50] at 37 °C for different time intervals (0 min, 15 min, 30 min, 1 h, 4 h), followed by confocal microscopic visualization. Non-infected AGS cells are used as a negative control. Cells are then stained with cholera toxin subunit B (CTB) to visualize the distribution of GM1 with or without infection.

Alternatively, AGS cells are stained by FITC-labeled CTB that binds to GM1 on rafts at 4 °C [or Macrophage cells] (at that temperature, membrane is rigid). The labeled cells are then infected with wild-type H. pylori or knock-out CapJ mutant [multiplicity of infection (MOI) = 50] at 37 °C for different time intervals (0 min, 15 min, 30 min, 1 h, 4 h), followed by confocal microscopic visualization. Non-infected AGS cells are used as a negative control. Similarly, the distribution of NBD-cholesterol for NBD-cholesterol-loaded AGS cells that are infected with wild-type bacteria or knock-out capJ mutant will be investigated.

2.3. Electron microscopic analysis of cholesterol α-glucosyltransferase

The recent development of electron cryotomography provides a new and more powerful way to investigate biological structures. In collaboration with Dr. Liu, we will visualize whether cholesterol α-glucosyltransferase is also present as an oligomeric form in *H. pylori*. It will be also of interest to investigate the distribution of cholesterol α-glucosyltransferase upon challenge with AGS cells or macrophages.

Materials and Methods

H. pylori culture—Clinical isolates were recovered from frozen stocks on Wilkins-Chalgren agar with 10% sheep blood, 0.2% β-cyclodextrin, 1% IsoVitaleX, 10 mg/L nalidixic acid and 8 U/ml polymixin B in a microaerophilic atmosphere for 3–7 days at 37°C until colonies were visible. After passages onto fresh plates, the bacteria were routinely cultured on Brucella agar plates (Becton Dickinson, NY, USA) with 6 mg/L vancomycin and 2 mg/L amphotericin B. For liquid culture, the bacteria harvested from plates were cultured in Brucella broth containing 2% fetal bovine serum, 0.2% β-cyclodextrin, 1% IsoVitaleX, 6 mg/L vancomycin and 2 mg/L amphotericin B for 2 days.

Construction of H. pylori isogenic mutants—An isogenic mutant *H. pylori* is generated by insertion of the *cat* fragment into the gene of *H. pylori* through allelic replacement and selection of chloramphenicol-resistant clones [17]. A *vacA*::*cat* mutant is then generated using a natural transformation method described in Wang et al. [17].

Cholesterol-α-glucosyltransferase assay—To assay the cholesterol-α-glucosyltransferase activity, the assay mixture contained in a total volume of 100μl: 5–30 μl (40–240 μg of protein) of *H. pylori* or appropriate amount of the recombinant enzyme, either 5 μl of a solution of 4 mM cholesterol in ethanol (200 μM final concentration) or 4 μl of 270,000 dpm [4-^{14}C]cholesterol in ethanol (final concentration 44 μM, specific activity 2.1 GBq/mmol) and either 100,000 dpm of UDP-[U-^{14}C]glucose (final concentration 1.5 μM, specific activity 12.2 GBq/mmol) or 10 μl of 5 mM unlabeled UDPglucose (final concentration 0.5 mM). After incubating for 30 min at 30 °C the reaction is terminated by the addition of 0.7 ml of 0.45% NaCl solution and either 2 ml of ethyl acetate or 3 ml of chloroform/methanol 2:1. After vortexing and phase separation by centrifugation, the radioactivity in the organic phase is determined by scintillation counting or the extracted lipids are separated by thin-layer chromatography (TLC) or high performance TLC (HPTLC). The radioactivity on the silica gel plate is detected by radioscanning with a BAS-1000 Bio Imaging Analyser (Raytest, Straubenhardt, Germany).

To detect cholesteryl-α-D-glucopyranoside (CGL), reaction mixture (100 μl) that contains appropriate amounts (80 μl) of the recombinant enzyme, 5 μl l of 8 mM cholesterol in ethanol, 5 μl of 7.2 μM UDP-glucose, 1 μl of Triton CF-54, and 9 μl of reaction buffer reacts at 30°C for 3 hours. The reaction is terminated by adding 900 μl of a 0.45% NaCl solution and then 4 ml of a chloroform-methanol mixture (2:1). The lower phase of the resultant mixtures is filtered and dried under a nitrogen gas stream. The lower phase of the resultant mixture is dried by a nitrogen gas stream and treated with 1 ml of 0.5 N NaOH in methanol at 50°C for 1 hour. After neutralization with 6 N HCl, 1 ml of petroleum ether is added to the reaction tubes. After removal of the upper phase, 2 ml of petroleum ether are further added to the lower phase. The dried lower phase is dissolved in 1 ml of a chloroform-methanol-water (86:14:1) mixture (TLP), and then 0.5 ml of another chloroform-methanol-water (3:48:47) mixture (TUP) is added in the same reaction tubes. Finally, the recovered lower phase is dried by a nitrogen gas stream and dissolved in 50 μl of chloroform. One μl of the samples is then added to 1 μl of 2,5-dihydroxybenzoic acid or 1 μl of *trans*-3-indole acrylic acid used as matrix. Mass spectra of the CGL in positive and negative ion modes are taken by a Voyager-DE STR Biospectrometry Workstation (Applied Biosystems) of DE MALDI-TOF MS in a reflector mode with a laser intensity of 2,500 as described previously [18]. A two-point external calibration is performed.

Preparation of high quality of milligram quantities of target proteins—Molecular cloning experiments are performed by standard methods. For structural studies, expression and purification large amounts of target protein in a soluble, active form are required. During the course of current project, we have produced clones that could express in a soluble form of ArsS with adequate quantities. To optimize the yield of protein expression, various conditions including vectors, hosts, and culture conditions, inducing conditions will be tested. If the protein can be expressed at a sufficiently high level and in soluble form, the clone will be used for large-scale preparation (1-5 liters). The appending tag in the expressed polypeptide makes it useful for an efficient purification of the expressed protein. The tag will then be cleaved by the designed protease and the protein will be further purified by a suitable chromatography to achieve a single homogeneous species. The soluble target proteins would be further assessed using far UV circular dichroism spectroscopy on an AVIV CD spectropolarimeter (model 62A DS) and by size-exclusion chromatography (FPLC). Target proteins, which depict a good backbone fold would be considered as ideal candidates for structure determination.

Crystallization—Extensive crystallization trials will be conducted using the vapor diffusion method with a factorial combination of different precipitating agents. We will rapidly screen for conditions that

produce microcrystals, then fine-tune the conditions to produce large single crystals. If any crystals produced, preliminary diffraction studies will then be performed. Since a good-diffraction crystal is essential for further analysis, several strategies will be used to improve the quality of the diffraction: (i) to produce a smaller fragment, various mutants or various forms of a particular target that may be more amenable to crystallization, (ii) to explore other crystallization methods, and (iii) to collect data using synchrotron source that is a factor of several hundredfold or more intense than in-house source. We will also attempt to set up a robotic system to speed up the efficiency of screening good-quality crystals.

Improvement crystal quality—We will fine-tune the conditions to obtain single crystals for crystallographic studies by several strategies. First, we will modify the crystallization conditions. Second, we will remove the tag or modify the protein fragment when necessary. Finally, we will try to add various cofactors or ligands to improve the crystal quality. Various cryo-conditions will be tested for the best diffraction condition. If any well-diffracting crystals are obtained, molecular replacement, SAD, MAD or MIR methods will then be used to solve the structures.

Determination of crystal structures—With the significant advances in the structure determination of proteins, particularly in the x-ray crystallography, structure-based research is entering a new era. These include overexpression recombinant systems for producing proteins, systematic methods for crystallization, screening, cryo-crystallography, brilliant synchrotron radiation, image plate detectors, CCD detectors, MAD phasing, and software for automatic generation of protein structures from MAD data [19, 20]. In solving D-NCAase structure, we have collected native data and xenon derivative data collected at SSRL, utilized the MIRAS method and the program wARP for automatic model tracing [21]. In this proposal, we will fully utilize the advanced methodology and technology in solving crystal structures. Our centralized state-of-art x-ray in-house facility will greatly aid in the crystal screening, preliminary diffraction experiments and data collection using rotating-anode source. The nearby SSRC synchrotron source will be useful to collect high-resolution data. The SPring8 or SSRL synchrotron source will be invaluable for MAD data collection. Data are processed and scaled with DENZO and SCALEPACK [22] or MOSFLM [23] and the CCP4 [24] suite of program. The phases are obtained by the program CCP4 or SHARP [25] and refined and extended to using the solvent flipping procedure in the program SOLOMON [26]. The electron-density map is illustrated by the program O version 6.2 [27]. Crystallographic refinement is carried out using the maximum-likelihood program REFMAC [28] coupled to ARP [29], which is used to add water molecules automatically.

Analysis of crystal structures—Structure comparisons will be carried out with the program DALI [30].The structures will be computationally analyzed by computer simulation and/or molecular docking. Since the molecular (biochemical and biophysical) function of a gene product is closely linked to its three-dimensional structure, finding the structure may provide important insight into the molecular function of the gene product and may subsequently help in understanding its cellular function. When the structures implicate possible molecular functions, they will be tested experimentally. These molecular functions and structural information will be correlated with the cellular function of the gene products. On the other hand, the three-dimensional structural information of virulence factors would greatly facility other biochemical studies for their roles in function and pathogenesis.

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（四）預期完成之工作項目及成果。請分年列述：1.預期完成之工作項目。2.對於學術研究、國家發展及其他應用方面預期之貢獻。3.對於參與之工作人員，預期可獲之訓練。4.本計畫如為整合型研究計畫之子計畫，請就以上各點分別說明與其他子計畫之相關性。

Anticipated results

We have previously worked on *H. pylori* pathogenesis among Taiwanese patients and characterized several important virulence factors. Moreover, we have determined a few protein structures using our functional X-ray macromolecular crystallographic facility, which allows us to delineate their catalytic and binding mechanism. By use of the newly genomic/structural pathogenomic forces as well as our established approach, we are confident in the execution of this proposal. We anticipate obtain research results as in the following.

1. One major aim of this proposal essentially involves in the structure study of *H. pylori* cholesterolα-transferase. The approach in this study is straightforward and feasible based on our previous experiences. The gene encoding these proteins has been cloned, expressed, and purified respectively by the standardized working protocol. We anticipate obtain milligram quantities of recombinant proteins. Proteins will be further purified by various purification procedures and crystallized.
2. As purified cholesterol α-transferase can be obtained, the enzymatic properties of the

recombinant protein will be clarified. We anticipate improve the crystal quality of cholesterol α-transferase for structural determination.

3. We anticipate obtain the EM reconstruction image of the oligomeric cholesterolα-transferase structure.
4. By use of the genetic and cell biological approach, the role of cholesterol α-transferase in disruption the host rafts will be established. These results will shed light into how this factor controls the bacterial morphology and how it influences and inactivates macrophage. As a result, we can learn from those results to improve prevention, diagnosis and treatment.

一、作者主要學歷 請填學士級以上之學歷或其他最高學歷均可，若仍在學者，請在學位欄填「肄業」。

畢／肄業學校	國別	主修學門系所	學位	起訖年月
California Institute of Technology	美國	化學系	博士	1988/09 至 1992/06
University of California, Santa Barbara	美國	化學系	碩士	1983/09 至 1985/12
國立台灣大學	中華民國	農業化學系	學士	1979/09 至 1983/06

二、現職及與專長相關之經歷 指與研究相關之專任職務，請依任職之時間先後順序由最近者往前追溯。

服務機關	服務部門／系所	職稱	起訖年月
現職：國立清華大學	生命科學系	教授	2002/08 至 現在
國立交通大學	生物科技學系	合聘教授	2006/08 至 現在
經歷：國立清華大學	生命科學系	講 師	1992/08 至 1995/07
國立清華大學	生命科學系	副教授	1995/08 至 2002/07
國立清華大學	生命科學系&分子與細胞生物研究所	教 授	2002/08 至 現在
國立交通大學	生物科技學系	合聘教授	2006/08 至 現在

三、專長 請自行填寫與研究方向有關之專長學門。

1. 細菌毒素及酵素研究	2. 生物晶體結構	3.	4.

四、近五年內曾獲得之國內外學術、科技獎項或榮譽

（請列舉出獎項名稱、授予國家及年月日。如有必要，可簡述內容）

1. NSC Excellence Award (89 年度國科會甲等獎助)
2. Wu Da-Yeu Memorial Award (91 年度吳大猷先生紀念獎)
3. Veterans General Hospital-National Tsing Hua University-National Yang Ming University Best Thesis Award ((90 年度及 92 年度榮清陽論文獎第一名)
4. NSC Outstanding Scholar Award (92 年度國科會傑出獎)
5. 國立交通大學生物科技學系合聘教授(95–96 年度)

六、論文著述：

A. 期刊論文(*為通信作者)

1. Hung CL, Liu JH, Chiu WC, Huang SW, Hwang JK, **and *Wang WC**. Crystal structure of *Helicobacter pylori* formamidase AmiF reveals a cysteine-glutamate-lysine catalytic triad (2007). **J. Biol. Chem.** 282, 12220–12229. NSC95-2313-B-007-001, NSC95-3112-B-007-002, NSC94-2313-B-007-002, and NSC94-3112-B-007-005
2. Chi MC, Liu JS, ***Wang WC***, Lin LL*, Hsien-Ben Hwang (2007). Site-directed mutagenesis of the conserved Ala348 and Gly350 residues at the putative active site of *Bacillus kaustophilus* leucine aminopeptidase. **Biochimie.** *in press.*
3. Lin LL, Liu JS, ***Wang WC***, Chen SH, Huang CC and Lo HF. Glutamic acid 219 is critical for the thermostability of a truncated alpha-amylase from alkaliphilic and thermophilic *Bacillus* sp. Strain TS-23 (2007). **World. J. Microbiol. Biotechnol.** *in press.*
4. Lee YC, Wu HM, Chang YN, ***Wang WC***, and Hsu WH (2007). The central cavity from the $(alpha/alpha)_6$ barrel structure of *Anabaena* sp. CH1 N-acetyl-D-glucosamine 2-epimerase contains two key histidines for reversible conversion. **J. Mol. Biol.** 367, 895–908. *SCI.* NSC93-2313-B-007-001, NSC94-2313-B-005-015, NSC93-2313-B-005-051, and NSC94-2313-B-007-002.
5. Chiu WC, You JY, Liu JS, Hsu SK, Hsu WH, Shih CH, Hwang JK, ***Wang WC**** Structure-stability-activity relationship in covalently cross-linked *N*-carbamoyl D-amino acid

amidohydrolase and *N*-acylamino acid racemase (2006). **J. Mol. Biol.** 359, 741–753. *SCI.* NSC93-2313-B-007-001 and NSC94-2313-B-007-002.

6. Lai CH, Kuo CH, Chen PY, Poon SK, Chang CS and ***Wang WC**** (2006). Association of antibiotic resistance and higher internalization activity in resistant *Helicobacter pylori* isolates. **J. Antimicrob. Chemother.** *57*, 466–471. NSC93-3112-B-007-013, NSC94-3112-B-007-005, and VGHUST93-P6-29.

7. Lin LL, Chen PI, Liu JS, ***Wang WC***, Lo HF (2006). Identification of glutamate residues important for catalytic activity or thermostability of a truncated *Bacillus* sp. strain TS-23 α-amylase by site-directed mutagenesis. **Protein J.** 25, 232–239. *SCI.*

8. Chi MC, Huang HB, Liu JS, ***Wang WC***, Liang WC & Lin LL. Residues threonine 346 and leucine 352 are critical for the proper function of *Bacillus kaustophilus* leucine aminopeptidase (2006). **FEMS Microbiol. Lett.** 260, 156–161. *SCI.*

9. Cheng WC, Chang YN, and ***Wang WC**** (2005). Structural basis for shikimate-binding specificity of *Helicobacter pylori* shikimate kinase. **J. Bact.** *187*, 8156–8163. *SCI.* NSC94-3112-B-007-005 and NSC93-3112-B007-013.

10. Lai CH, Poon SK, Chang YJ, Chen YC, Chang CS, and ***Wang WC**** (2005). Lower prevalence of *Helicobacter pylori* infection with *vacAs1a*, *cagA*-positive, and *babA2*-positive genotype in erosive reflux esophagitis disease in Taiwan. ***Helicobacter*** *10*, 577–585. *SCI.* NSC93-3112-B-007-013, NSC93-2313-B-008-001, and VGHUST93-P6-29.

11. ***Wang WC**** (2005). *Helicobacter pylori*, a crafty microbe hiding inside the human stomach. Scientific America (Chinese). *37*, 76–79.

12. ***Wang WC****, Chiu WC, Hsu SK, Wu CL, Chen CY, Liu JS, and Hsu WH (2004). Structural basis for catalytic racemization and substrate specificity of an *N*-acylamino acid racemase homologue from *Deinococcus radiodurans*. ***J. Mol. Biol***. *342*, 155–169. *SCI.* NSC92-2313-B-007-003 and NSC92-3112-B-007-005.

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17. Lai CH, Kuo CH, Chen YC, Chao FY, Poon SK, Cang CS, and ***Wang WC**** (2002). High Prevalence of the *cagA*-positive and *babA2 Helicobacter pylori* Clinical Isolates in Taiwan. **J. Clin. Microbiol.** *40,* 3860–3862. *SCI.* VTY89-P4-27, VTY90-P4-23, NSC90-2313-B-007-003, and a Program for Promoting Academic Excellence of Universities Grant 89-B-FA04-1-4

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20. Chen KC, Lai CH, and ***Wang WC*** (2002). Virulence factors of *Campylobacter jejuni*. **Med. Today** *29,* 1023–1029.

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22. Chien HC, Lin LL, Chao SH, Chen CC, ***Wang WC***, Shaw CY, Tsai YC, Hu HY, Hsu WH (2002). Purification, characterization, and genetic analysis of a leucine aminopeptidase from *Aspergillus sojae*. **Biochim. Biophys. Acta** *1576*, 119–26. *SCI*

B. Patent

1. **Wang WC*** and You JU (2005). Thermostable N-carbamoyl-D-amino acid Amidohydrolase. 中華民國專利公開編號 200528469. 公開日：2005 年 9 月 1 日.

C. Conference papers

1. ***Wang WC***, Hung CL, Chen CY, and Hua YW (2007). Crystal structures of two aliphatic amidases AmiE and AmiF from *Helicobacter pylori* reveal a conserved catalytic triad. AsCA'07/Taipei. The 8th Conference of the Asian Crystallographic Association, November 4–7, 2007, Taipei, Taiwan R.O.C.

2. Lai CH, Tang CH, Hung CH and ***Wang WC*** (2007). *Helicobacter pylori* induce epithelial cell inflammation requires cell membrane rafts. **NZ Society for Biochemistry & Molecular Biology: From Molecules to Complex Systems**, Wellington, New Zealand, Nov 27-30, 2007. Poster and abstract.

3. Lai CH, Fang SH, Hung CH, Tang CH and ***Wang WC*** (2007). *Helicobacter pylori* invasion of epithelial cells requires cholesterol and results in CagA associated with cellular lipid rafts. **The 12th Conference on Bacteriology**, Hsinchu, Aug 22-24, 2007. Oral presentation.

4. Lai CH, Chang YC, Kuo CH, Fang SH, Hung CH, Tang CH and ***Wang WC*** (2007). Lipid rafts are required for inflammatory responses by *Helicobacter pylori* CagA protein，**5th NHRI Conference on Bacterial Gene Regulation and Pathogenesis**, April 20-21, 2007. Poster and abstract.

5. Lai CH, Kuo CH, Cang CS, Chan YC, Poon SK, and ***Wang WC*** (2007). Study of the antibiotic resistance and internalization activity into gastric epithelial cells in refractory *Helicobacter pylori* clinical isolates. **榮總台灣聯合大學合作研究計畫(第三期)成果發表會,** June 23, 2007. Poster and abstract.

6. Hung CL, Chen WC, Lai CH, Chang YC, ***Wang WC*** (2007). Internalization of *Helicobacter pylori* by epithelial cells via a cholesterol-dependent pathway. **Experimental Biology**. San Francisco, CA, April 1-5, 2006. Poster and abstract.

7. Cheng WC, Chen YF, Wang HJ, Yang JM and ***Wang WC*** (2007). Structure-based discovery of Helicobacter pylori shikimate kinase (HpSK) inhibitors from database screening and molecular docking. AsCA'07/Taipei. The 8th Conference of the Asian Crystallographic Association,

November 4–7, 2007, Taipei, Taiwan R.O.C.

8. ***Wang WC***, Wu HM, Lee YC, Chang YN and Hsu WH (2006). Crystal structure and site-directed mutagenesis studies of *Anabaena* sp. CH1 N-acetyl-D-glucosamine 2-epimerase reveal two key histidines for reversible conversion. Joint Conference of the Asian Crystallographic Association and the Crystallographic Society of Japan, AsCA '06/CrSJ. November 20-23, 2006. Poster and abstract.

9. ***Wang WC,*** *Helicobacter pylori* formamidase AmiF contains a fine-tuned cysteine-glutamate-lysine catalytic site. The 8th R.O.C.-Japan Joint Seminar on Crystallography. September 26-27, 2006. Oral presentation.

10. Kuo CH, Lai CH, Poon SK, Cang CS, ***Wang WC***. Multiple infection and high genetic heterogeneity of *Helicobacter pylori* isolates in Taiwan. **榮總台灣聯合大學合作研究計畫(第二期)成果發表會**. 24 June, 2006. Oral presentation.

11. Wang HJ, Poon SK, Cang CS, Lai CH, ***Wang WC***. Phosphorylation site polymorphism of CagA; a preliminary investigation of allelic diversity in 3' region of *cagA* from selected clinical isolates. **4th NHRI Conference on Bacterial Gene Regulation and Pathogenesis**. April 21-22, 2006. Poster and abstract.

12. ***Wang WC.*** Phosphorylation site polymorphism of CagA; a preliminary investigation of allelic diversity in 3' region of *cagA* from selected clinical isolates. The 4th NHRI Conference on Bacterial Gene Regulation and Pathogenesis. April 21-22, 2006. Poster and abstract.

13. ***Wang WC.*** Structure basis of *Helicobacter pylori* formamidase (AmiF). The 4th NHRI Conference on Bacterial Gene Regulation and Pathogenesis. April 21-22, 2006. Poster and abstract.

14. ***Wang WC.*** Structureof shikimate kinase from *Helicobacter pylori*. The 4th NHRI Conference on Bacterial Gene Regulation and Pathogenesis. April 21-22, 2006. Poster and abstract.

15. ***Wang WC.*** Internalization of *Helicobacter pylori* by epithelial cells via a cholesterol-dependent pathway. Experimental Biology. San Francisco, CA, April 1-5, 2006. Poster and abstract.

16. ***Wang WC.*** Shikimate binding in *Helicoabcter pylori* Shikimate kinase. Experimental Biology. San Francisco, CA, April 1-5, 2006. Poster and abstract.

17. ***Wang WC.*** Structure of an N-acylamino acid racemase homologue from *Deinococcus radiodurans*—an example of Hg-MAD method. Invited speaker. The Tenth Symposium on Recent Advances in Biophysics, May 24-28, 2005. Invited speaker.

18. ***Wang WC.*** Refractory *Helicobacter pylori* strains demonstrate elevated internalization into gastric epithelial cells mediated by lipid rafts. The third NHRI Conference on Bacterial Gene Regulation and Pathogenesis. April 30-May 1, 2005. Oral presentation.

19. ***Wang WC.*** Structural basis for enzymatic catalysis and protein engineering of N-carbamoyl-D-amino acid amidohydrolase. Invited speaker. 2004 Annual Meeting of Chemical Society, Taichung. November 20-21, 2004.

20. Lai CH, Kuo CH, and ***Wang WC.*** Uptake of VacA-expressing Helicobacter pylori into epithelial cells via raft-associated trafficking pathway. 2nd NHRI Conference on Bacterial Gene Regulation and Pathogenesis. April 24-25, 2004.

21. ***Wang WC.*** Crystal structure of *N*-carbamoyl-D-amino acid amidohydrolase: insights into enzymatic catalysis and substrate specificity (2003). Invited speaker. NSRRC Tenth Users' Meeting & Workshops on the New Scientific Opportunities, October 30-31, 2003. 國家同步輻射研究中心『光源啟用十週年回顧』.

22. ***Wang WC.*** *Helicobacter pylori* vacuolating toxin: genetic polymorphism and multiple binding modes. (2002). The Seventh Nagasaki-Singapore Symposium on Medical Sciences: Infections Diseases and Host Immune Responses. October, 2002. Invited speaker.

23. ***Wang WC,*** **and Chen CY.** Crystal structure of N-carbamoyl-D-amino-acid amidohydrolase-substrate complexes: structural insights into the binding pocket. (2002). The 19th Congress and General Assembly of the International Union of Crystallography, Geneva, Switzerland, August 6-15, 2002. Poster and abstract.

24. ***Wang WC.*** Primary resistance to antibiotics and its clinical impact on the efficacy of *Helicobacter pylori* lansoprazole-based triple therapies. (2002). June 1, 2002. Oral presentation. 榮清陽九十年度成果發表會.

25. ***Wang WC.*** Two Distinctive Cell Binding Patterns by Vacuolating Toxin. (2002). April 26-27, 2002. Oral presentation. 國家衛生研究院細菌基因調控與致病機轉研討會.

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D. 專書及專書論文

1. 王政光，江意芳，洪小芳，楊舒如，廖芳足，賴麗娟，張芸潔，蕭欣杰，賴志河。***王雯靜*** 總校閱: 醫護微生物學實驗，第二版。文京圖書公司，台北，2006。

2. 王政光，李慶孝，洪小芳，張弘志，張芸潔，賴志河 編譯。***王雯靜*** 總校閱: Lesk *et. al.*, Introduction of Bioinformatics。九州圖書文物公司，台北，2002。**(2008二月更新第二版)**。

3. 張芸潔，賴志河。***王雯靜*** 總校閱:微生物及免疫學，第二版。文京圖書公司，台北，2002。**(2008二月更新第二版)**。

生物處研究人員近五年研究成果統計、獲研究計畫補助及獎勵情形（表A）

（修正：96/05/27）

研究人員姓名：王雯靜　　任職機關系所：清華大學生命科學系暨分子與細胞研究所

（一）請依照個人資料表所列之研究成果提供五年內**(2002.1.1 迄今)**已發表或被接受發表於 SCI、SSCI、EI 或其他之**期刊論文**(發表在**有編輯委員會且有同儕審查制度定期發行之學術期刊**)、研討會論文(有刊載出論文全文者)、專書及專書論文、專利及技術移轉等資料填寫於下表：

研究成果*	總篇(件)數	作者順序
SCI 期刊論文		1. 第一作者論文 3 篇 2. 非第一作者之通訊作者論文 9 篇 3. 非第一或通訊作者之其他序位作者論文 4 篇
SSCI 期刊論文		1. 第一作者論文＿＿篇 2. 非第一作者之通訊作者論文＿＿篇 3. 非第一或通訊作者之其他序位作者論文＿＿篇
EI 期刊論文		1. 第一作者論文＿＿篇 2. 非第一作者之通訊作者論文＿＿篇 3. 非第一或通訊作者之其他序位作者論文＿＿篇
加重計分之國內非 SCI 優良期刊論文 (見填表說明六、(一)、2-1,2-2)		1. 第一作者論文＿＿篇 2. 非第一作者之通訊作者論文＿＿篇 3. 非第一或通訊作者之其他序位作者論文＿＿篇
其他學術期刊論文 (上述四類以外之期刊論文)		1. 第一作者論文＿＿篇 2. 非第一作者之通訊作者論文＿＿篇 3. 非第一或通訊作者之其他序位作者論文 5 篇
其他著作	1.研討會論文(有刊載出論文全文者)＿＿篇　2.專書、專書論文 4 篇 3.專利或技轉＿＿件　4.其他＿＿篇(件)	

*SCI (Science Citation Index)、SSCI (Social Science Citation Index)、及 EI (Engineering Index)期刊目錄，可就近至各大學醫學院圖書館、國科會科學技術資料中心或上網(網址:http://www.isinet.com)檢索或查詢。上述 SCI、SSCI 及 EI 期刊目錄以 2004 年之 JCR 版本為準。

（二）請填寫近五年來獲得**本會**研究補助及獎勵情形。

主持本會之專題研究計畫件數 (共同及協同主持人不列入計算)	獲得本會之研究獎勵費類別（請選填下列獎別名稱） ○傑出 ○甲種 ○乙種 ○吳大猷 ○其他（填名稱）
1. 90 年度(90.8.1~91.7.31) 2 件	1. 90 年度(90.8.1~91.7.31)獲得＿＿＿＿
2. 91 年度(91.8.1~92.7.31) 2 件	2. 91 年度(91.8.1~92.7.31)獲得 吳大猷
3. 92 年度(92.8.1~93.7.31) 2 件	3. 92 年度(92.8.1~93.7.31)獲得 傑出研究獎
4. 93 年度(93.8.1~93.7.31) 2 件	4. 93 年度(93.8.1~93.7.31)獲得＿＿＿＿
5. 94 年度(94.8.1~迄今) 2 件	5. 94 年度(93.8.1 迄今) 獲得＿＿＿＿

註： 國科會一般甲、乙種研究獎勵費於 91 年度停辦。傑出獎於 94 年度停辦。

生物處研究人員研究表現指數(RPI)統計表（表 B)

（修正：96/07/25）

研究人員姓名：__王雯靜__ 任職機關系所：清華大學生命科學系暨分子與細胞研究所

研究年資(請打✓)：　☑滿五年以上(至多 10 篇)　□滿四年(至多 7 篇)　□滿三年(至多 5 篇)
□滿二年(至多 3 篇)　□滿一年(至多 2 篇)　□未滿一年(至多 1 篇)

五年內(2002.1.1 迄今)代表性研究成果一欄表(填表前請先詳閱填表說明及範例)
「刊登雜誌分類排名」以 ISI 之 2005 年版本之 SCI、SSCI 及 EI 期刊目錄為準。

序號/研究成果分類	五年內代表性研究成果名稱及相關發表資料 (期刊名稱及發表年代;卷數:起迄頁數)	論文性質分類加權分數 (C)	刊登雜誌分類排名加權分數 (J)	作者排名加權分數 (A)	分數 C×J×A
1./1	Hung, CL, Liu, J H, Chiu, W C, Huang, SW, Hwang, JK, ***Wang, W C**** Crystal structure of Helicobacter pylori formamidase AmiF reveals a cysteine-glutamate-lysine catalytic triad. **Journal of Biological Chemistry. 2007: 282, 12220-9. (Impact factor: 5.854**)	3	5.854x2=11.708	5	S01 175.62
2./1	Chang, YN, ***Wang, WC****, Hsu, W. H. The central cavity from the (alpha/alpha)6 barrel structure of Anabaena sp. CH1 N-acetyl-D-glucosamine 2-epimerase contains two key histidine residues for reversible conversion. **Journal of Molecular Biology.** 2007: 367, 895-908. **(Impact factor: 5.229**)	3	5.229x2=10.458	5	S02 156.87
3./1	WC Chiu, JY You, JS Liu, SK Hsu, WH Hsu, CH Shih, JK Hwang, ***WC Wang**** Structure-stability-activity relationship in covalently cross-linked *N*-carbamoyl D-amino acid amidohydrolase and *N*-acylamino acid racemase. **Journal of Molecular Biology.** 2006: 359, 741-753. **(Impact factor: 5.229**)	3	5.229x2=10.458	5	S03 156.87
4./1	Lai CH, Kuo CH, Chen PY, Poon SK, Chang CS and ***Wang WC****. Association of antibiotic resistance and higher internalization activity in resistant *Helicobacter pylori* isolates. **Journal of Antimicrobial Chemotherapy** 2006: *57*, 466-471. **(Impact factor: 3.886; Ranking: 47/274**)	3	5	5	S04 75
5./1	Cheng WC, Chang YN, and ***Wang WC****. Structural basis for shikimate-binding specificity of *Helicobacter pylori* shikimate kinase. **Journal of Bacteriology** 2005: 187, 8156–8163. **(Impact factor: 4.168; Ranking: 16/86**)	3	5	5	S05 75
6./1	***Wang WC****, Chiu WC, Hsu SK, Wu CL, Chen CY, Liu JS, and Hsu WH. Structural basis for catalytic racemization and substrate specificity of an *N*-acylamino acid racemase homologue from *Deinococcus radiodurans*. **Journal of Molecular Biology.** 2004: 342, 155–169. **(Impact factor: 5.229**)	3	5.229x2=10.458	5	S06 156.87
7./	Chen CY, Chiu WC, Liu JS, Hsu WH, ***Wang WC****. Structural basis for catalysis and substrate specificity of *Agrobacterium radiobacter*	3	5.854x2=11.708	5	S07 175.62

1	N-carbamoyl-D-amino-acid amidohydrolase. **Journal of Biological Chemistry.** 2004: 342, 155–169. **(Impact factor: 5.854**)				
8. / 1	Kuo CH, ***Wang WC****. Binding and internalization of *Helicobacter pylori* VacA via cellular lipid rafts in epithelial cells. **Biochemical and Biophysical Research Communications.** 2003: 303, 640–644. (**Impact factor: 3.000; Ranking: 97/261**)	**3**	**4**	**5**	**S08 60**
9. / 1	Lai CH, Kuo CH, Chen YC, Chao FY, Poon SK, Cang CS, and ***Wang WC****. High Prevalence of the *cagA*-positive and *babA2 Helicobacter pylori* Clinical Isolates in Taiwan. **Journal of Clinical Microbiology** 2002: 40*,* 3860–3862. (**Impact factor: 3.537; Ranking: 20/86**)	**3**	**4**	**5**	**S09 60**
10. / 1	Chien HR, Hsu CL, ***Wang WC****, and Hsu WH*. Enhancing oxidative resistance of *Agrobacterium radiobacter* N-carbamoyl D-amino acid amidohydrolase by engineering solvent-accessible methionine residues. **Biochemical and Biophysical Research Communications.** 2002: 297, 272–287. (**Impact factor: 3.000; Ranking: 97/261**)	**3**	**4**	**5**	**S10 60**
積　分 (S11=S01+S02+S03+.....+S10)					**S11 1151.85**
研究表現指數(RPI)【(積分×100)/指標上限滿分 =(S11×100)/指標上限滿分】					**153.58**

附表 1.國內 SCI 期刊、三年內曾獲本會選為傑出期刊或國科會彙刊之排名加權分數(J)

	期　刊　名　稱	出　版　者	獲獎年度	SCI期刊	加權分數(J)
1.	Journal of Biomedical Science (National Science Council, Taipei)	國科會		SCI	2
2.	台灣醫學會雜誌 (Journal of the Formosan Medical Association)	中華民國台灣醫學會	85-93	SCI	2
3.	中國化學會誌 (Journal of the Chinese Chemical Society)	中國化學會		SCI	2
4.	動物研究學刊 (Zoological Studies)	中央研究院動物研究所	84-93	SCI	2
5.	中國生理學雜誌 (The Chinese Journal of Physiology)	中國生理學會	84-93	SCI	2
6.	中央研究院植物學彙刊 (Botanical Bulletin of Academia Sinica)	中央研究院植物研究所	84-93	SCI	2
7.	藥物食品分析 (Journal of Food & Drug Analysis)	行政院衛生署藥物食品檢驗局	85-93	SCI	4
8.	Proceeding of the National Science Council, ROC, Part A: Physical Science and Engineering (出版至2002,12)	國科會			1.5
9.	Proceeding of the National Science Council, ROC, Part B: Life Sciences (出版至2002,12)	國科會			1.5
10.	Proceeding of the National Science Council, ROC, Part C: Humanities and Social Sciences (出版至2002,12)	國科會			1.5

※　上表所列國內 SCI 雜誌依據 2004 JCR 資料。
※　國內 SCI 雜誌按實際加權分數加 1 分。
※　Proceeding of the N. S. C., Part A, B, C 均於 91 年 12 月以後停刊。
※　94 年停止辦理國內傑出及優良學術期刊評選。

附表 2、最近一年獲本會選定、近三年內曾二次以上或近五年內曾三次以上年獲本會選定為優等（或甲等）之優良期刊排名加權分數(J)。

	期　刊　名　稱	出　版　者	獲獎年度	加權分數(J)
1.	Chinese Medical Journal (Taipei) (中華醫學雜誌)	中華醫學會	84-93	1.0
2.	Taiwania	國立台灣大學植物學系	85-91,93	1.0
3.	臺灣農業化學與食品科學(Food Science and Agricultural Chemistry)	中國農業化學會及中華民國	90-93	1.0

		食品科學技術學會 中國農業化學會 中華民國食品科學技術學會		

4.	台灣兒科醫學會雜誌	台灣兒科醫學會	84-93	1.0
5.	台灣獸醫學會雜誌	中華民國獸醫學會	84-90,92-93	1.0
6.	台灣水產學會刊	台灣省水產學會	84-93	1.0
7.	The Kaohsiung Journal of Medical Sciences	高雄醫學院	84-93	1.0
8.	Chang Gung Medical Journal(長庚醫學雜誌)	財團法人長庚紀念醫院	85-93	1.0
9.	Acta Anesthesiologica Sinica麻醉學雜誌	中華民國麻醉醫學會	84,85,88-93	1.0
10.	微免與感染雜誌	中華民國微生物學會 中華民國免疫學會 中華民國感染症學會	88,90-93	1.0
11.	The Journal of Nursing Research	中華民國護理學會	86-88,91-93	1.0
12.	台灣昆蟲	台灣昆蟲學會	84-86,90-91,93	1.0

※ 國內獲選為優良期刊之非 SCI 期刊按實際加權分數加 0.5 分。

※ 94 年停止辦理國內傑出及優良學術期刊評選。