2024 第十五屆

桃園四校生命科技領域暨

中原生科專題成果聯合學術研討會

活動時間：2024/06/14（五）9:00 - 16:00

活動地點：中原大學張靜愚紀念圖書館 1F 秀德廳

主辦單位：中原大學生物科技學系

協辦單位：中央大學生命科學系、中央大學生醫科學與工程學系

元智大學生物科技與工程研究所、長庚大學生物醫學系

補助單位：教育部高教深耕計畫、中原大學理學院、中原大學研發處

活動流程：

09:00 ~ 09:10	報到與張貼海報
09:10 ~ 09:20	開幕式
09:20 ~ 09:50	專題演講 主講人：吳宗遠 教授
講題：New baculovirus : constructed in lab and found in wild	
09:55 ~ 10:25	專題演講 主講人：邱冠雄 教授
講題：Microalgae biotechnology : Views in upstream and downstream processing	
10:30 ~ 11:00	專題演講 主講人：Farhan Azhwin Maulana
講題：Leveraging The Activation of Aryl Hydrocarbon Receptor in Neuroblastoma Therapy	
11:00 ~ 12:30	海報評選
12:30 ~ 13:10	午餐
13:10 ~ 13:30	評審委員會議 (會議後公布得獎名單，並準備口頭發表)
13:30 ~ 15:00	各組前三名演講 (每人 10 分鐘, 含 3-5 分鐘提問)
15:00 ~ 15:30	頒獎、大合照、閉幕式

講員摘要

ALGAE BIOSEPARATION RESEARCH

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Microalgae biotechnology: Views in upstream and downstream processing

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Abstract
Microalgae are third-generation biomass contributing to various application including biofuels production, wastewater treatment and environmental bioremediation due to its renewability and regenerative characteristics. In recent years, the research transformation of microalgae biotechnology has evolved contributing to carbon neutrality as deem by its photosynthetic ability to assimilate atmospheric CO_2 and even organic compound (e.g. carbon, nitrogen and phosphorus) derived from waste effluent. Throughout the cultivation process of microalgae, biological macromolecules such as protein, lipids, carbohydrates and carotenoids are biosynthesized and developed within the organelles of microalgae that represented as “biomass” for further conversion process. The following talk will share views on (1) topic of upcycling food waste for the cultivation process of microalgae, and also present some recent insight on (2) IoT-technologies, (3) machine- and deep learning-based classification model for the prediction, monitoring and controlling of microalgae cultivation.

Keyword: Microalgae Biotechnology; Upcycling Food Waste; IoT-Technologies; Machine learning; Deep-learning; Classification Model

Acknowledgement: Kuan Shiong Khoo would like to gratefully acknowledge the work supported and financially funded by National Science and Technology Council, Taiwan (Project number: NSTC112-2222-E-155-005) and Department of Chemical Engineering and Material Science, Yuan Ze University, Taiwan under New Faculty Research Start-Up Fund Scheme (Project no: 303014-1 and 303014-2).

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Title	**Leveraging The Activation of Aryl Hydrocarbon Receptor in Neuroblastoma Therapy**
Author	Farhan Azhwin Maulana[,1], Pei-Yi Wu[1]
Affiliation	Department of Life Sciences, National Central University, Taoyuan, Taiwan

Abstract

Neuroblastoma is a highly malignant pediatric cancer derived from the sympathoadrenal lineage of the neural crest during development. Patients were divided into risk groups (low, moderate, and high-risk groups) according to the disease progression. The current treatment for NB is risk-adapted multimodal therapy, including combinations of chemotherapy, radiotherapy, high-dose autologous peripheral blood stem cell (PBSC) transplantation, and surgery. However, the 5-year survival rate of high-risk group NB patients about 40% of total NB patients, is still less than 40% even with advanced therapy. Given that our previous findings have demonstrated the tumor suppressing effect of AHR in NB, we thus seek to determine whether activation of AHR by agonists can be a viable therapeutic strategy for NB. To this end, we identified an AHR endogenous ligand tetrahydrocortisone (THB) as a candidate. In this study, we would like to investigate the unexplored therapeutic role of THB isomers in restricting tumor progression of NB. Currently, we have optimized the protocol for THB isomer synthesis and the production scale has reached the milligram level. The synthetic THB isomers have been utilized in the experiments examining their limiting effects on the NB progression. We found that compound Y significantly inhibits cell migration and invasion, but enhances cell adhesion. In the in-vivo analysis, oral administration of this compound shrinks the tumor formation. All these data illustrate the novel indication of compound Y in the NB treatment.

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生化生醫工程組

Performance Comparison of Five Methods Available in ImageJ for Bird Counting and Detection from Video Datasets

Kevin Adi Kurnia [1] and Chung-Der Hsiao [1,2,3]

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[3] Department of Biomedical Engineering, Chung Yuan Christian University, Taiwan

In recent years, climate change, habitat loss, and human activity have been prime threat to biodiversity. Birds specifically are being threatened due to these external conditions, altering their habitats and migratory behavior. Researchers monitored the conditions of birds all year round to study their diversity and abundance, especially during migration, as it can provide core data for bird conservation purposes. The previous methods for bird monitoring through are mostly based on manual counting due to high specialized software prices, which suffers from low throughput and a high error rate. In this study, we aimed to provide an alternative bird-counting method from video datasets by using five available ImageJ methods: Particle Analyzer, Find Maxima, Watershed segmentation, TrackMate, and trainable WEKA segmentation. The numbers of birds and their XY-coordinates were extracted from videos to conduct a side-by-side comparison with manual counting results, and the three important criteria of the sensitivity, precision, and F1 score were calculated for performance evaluation. From the tests, which we conducted for four different cases with different bird numbers or flying patterns, TrackMate had the best overall performance for counting birds and pinpointing their locations, followed by Particle Analyzer and Find Maxima, which both showed respectable results as two of the simplest method for bird counting in ImageJ, then Trainable WEKA segmentation that showed similar result to Particle Analyzer, but has problem in creating pseudo-connection between birds and lastly, Watershed, which showed low precision due to unsuitable segmentation for birds. In summary, five ImageJ-based counting methods were compared in this study, and we found TrackMate to show the best performance for bird counting and detection.

Keywords: ImageJ, Bird, Counting, TrackMate, Image analysis

OpenCV Based methods to measure cardiac rhythm, blood flow and 3D locomotion behavior in zebrafish using High Speed CCD videos dataset

Ali Farhan [1] and Chung-Der Hsiao [1,2,3]

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Abstract

Computer vision techniques are well established to analyze imaging and videos datasets. Recent era is transforming with Artificial Intelligence (AI) using OpenCV to track and measure optical flow dynamics using animal models. It is subjected that most of the image processing aspects are computable with advanced visualization to track the objects. Zebrafish is recognized as useful animal model for studying cardiac rhythm, neurodegenerative diseases and blood flow due to it's translucent skin. The molecular incidences exhibited that genome sequence of zebrafish has 70% similarity to humans that provides huge advantage of using zebrafish as compared to other mammalian models. In this study we developed multiple tools to measure cardiac rhythm, blood flow and 3D locomotion behavior of zebrafish. The first tool provides user-friendly environment to get video input of 3 days post fertilization (3dpf) of zebrafish larvae to detect the greyscale pixels changing pattern in waveforms showing cardiac rhythm (AV-VA) intervals. By selecting atrium and ventricle of zebrafish heart chambers the tool would automatically provide the results with heart rate beat per minute. Next study provides comprehensive analysis of blood flow velocity and blood cells count measurement in multiple development; stages of zebrafish larvae as (2 dpf, 3 dpf, 4dpf and 5dpf). The software is comprised to select region of interest (ROI) automatically on dorsal aorta of zebrafish videos and finally user will get blood cells count measurement and blood flow velocity in given dataset. The third study is based on deep learning methods using unsupervised learning approach to analyze 3D trajectories of adult zebrafish providing locomotion behaviror understanding. The 3D trajectories data incorporated to software with easy-handling process of big data (x, y, z).

Keywords: OpenCV, Zebrafish, Cardiac rhythm, blood flow, 3D locomotion, deep learning

Optimization of Laser-Based Method to Conduct Skin Ablation in Zebrafish and Development of Deep Learning-Based Method for Skin Wound-Size Measurement

Petrus Siregar (江次宏) [1,2], Chung-Der Hsiao (蕭崇德) [1,2]

1. Department of Chemistry, Chung Yuan Christian University, Taoyuan, Taiwan
2. Department of Bioscience Technology, Chung Yuan Christian University, Taoyuan, Taiwan

Abstract

Skin plays an important role as a defense mechanism against environmental pathogens in organisms such as humans or animals. It is important for the skin to regenerate quickly upon injury to regain its protective barrier function. Traditionally, scientists use rodents or mammals as experimental animals to study skin wound healing. However, due to concerns about animal welfare and increasing costs of laboratory animals such as rodents, scientists consider alternative methods of implementing replace, reduce, and refine (3R's) in experimentation. Thus, this study aimed to develop a new alternative model for skin wound healing by utilizing zebrafish together with a new rapid and efficient method as an alternative in investigating skin wound healing. First, in order to fulfill the 3Rs concept, the pain in the tested zebrafish was evaluated by using a 3D locomotion assay. According to the test, 3 watts was chosen as effective power for laser ablation since the wound generated did not alter zebrafish swimming behaviors. Wound closure of tested zebrafish was presented in 25%, 50%, and 75% of wound closure percentages to examine the early, middle, and late wound healing progress. After inflicting the wound to zebrafish's skin, wound images were collected and used for deep learning training by convolutional neural networks to measure the area of wounds in an automatic manner. Using ImageJ manual counting as the standard, we found U-Net performance for zebrafish skin wound measurement where it can recognize a wound area in zebrafish skin. Validation of the method was done by examine the effect of temperature and antioxidant on wound healing. The result showed a consistent result with previous study on temperature and antioxidant. Taken together, the laser-based skin ablation and deep learning-based wound size measurement methods reported in this study provide a faster, reliable, and less suffering protocol to conduct skin wound healing in zebrafish for the first time.

Keywords: skin; wound healing; zebrafish; medicine; laser ablation; deep learning; ImageJ

Development of Field-Deployable Rapid Detection of Bat Coronaviruses Using Loop-Mediated Isothermal Amplification Assay

Undarmaa Tsengel[1] (恩達瑪), Tzong-Yuan Wu[2] (吳宗遠), and Yi-Ning Chen[2*] (陳怡寧)

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Abstract

The global impact of the COVID-19 pandemic has emphasized the critical need for effective viral diagnostics. Although polymerase chain reaction (PCR) is a well-established nucleotide amplification technique, its limitations, such as the need for expensive equipment and skilled technicians, have led to the exploration of alternative methods, including loop-mediated isothermal amplification (LAMP). Bats, the second largest mammalian order, are a crucial natural reservoir of CoVs, from these *Scotophilus* bat coronavirus 512 (Sco bat-CoV 512) has highest detection rate among Taiwan's bat population and this study aimed to detect Sco bat-CoV 512 from bats in the field. We investigated the isothermal reaction and visual interpretability characteristics of the LAMP assay in the field for the real-time detection of bat-carrying Sco bat-CoV 512. Therefore, our study delves into the specificity of the LAMP reaction, emphasizing the careful design of primers to prevent false positive results. A specificity test involving eight different microorganisms, including closely related bat CoVs and two bacterial species typically found in feces, revealed that the LAMP assay uniquely detected Sco bat-CoV 512. The developed colorimetric RT-LAMP assay was optimized for the primers targeting nucleocapsid (*N*) gene, and the sensitivity test revealed a detection limit of 2.4×10^3 copies/μL. The findings indicate the potential of the LAMP assay for on-site detection in the field and subsequent laboratory analysis for comprehensive sampling and further research on CoV infection mechanisms. The surveillance and monitoring of bat CoVs contribute substantially to mitigate human threats, particularly concerning the emergence of new pandemic variants.

Keywords: Bat Coronavirus, Loop-mediated isothermal amplification, Nucleocapsid gene

Determination of Baculovirus Host-Range Extension Factor Employing CRISPR-Cas9-Coupled Lambda Red Recombineering System

INDAH PERMATA SARI[1,2], MING-HSIANG CHEN[1,2], TZONG-YUAN WU[1]

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Baculovirus, a double-stranded DNA virus primarily infecting Lepidoptera, has been extensively utilized in biotechnology for protein expression in insect cells, gene delivery in mammalian cells (BacMam), and agricultural biocontrol. Our laboratory developed a hybrid baculovirus, ABM, with a broader host range through homologous recombination of *Autographa californ*ica nucleopolyhedrovirus (AcMNPV), *Bombyx mori* nucleopolyhedrovirus (BmNPV), and *Maruca vitrata* nucleopolyhedrovirus (MaviNPV) genomes. Unlike the parental viruses, which infect Sf21, BmN, and Mv532 cell lines respectively, the ABM baculovirus infects all three. This study identifies the open reading frames (ORFs) responsible for ABM's expanded host range.

Traditional lambda-red recombineering for baculovirus ORF investigation is time-consuming due to multiple steps required for marker-less genome editing. We addressed this by coupling lambda-red recombineering with CRISPR-Cas9, enabling single-step marker-less baculovirus bacmid editing. To our knowledge, this is the first application of this combined system for baculovirus bacmid editing. We initially targeted non-essential ORFs, *p10* and *chiA/cath*, to validate the approach, successfully achieving individual and simultaneous knockouts. The edited bacmid maintained its ability to generate recombinant baculovirus capable of expressing functional exogenous proteins. Additionally, we successfully performed a knock-in of the *egfp* gene using this method.

Subsequently, the identification of the host-range determinants was carried out. Through comprehensive whole-genome sequence comparison between the parental baculoviruses and the hybrid ABM baculovirus, several ORFs were identified as potential host-range determinants, including *helicase*, *pe38*, *orf154* (*cds57*), hr1, *ptp*, *orf4* (*cds58*), *orf5* (*cds59*), and *lef2*. The study identified the helicase ORF as the host-range determinant for BmN cells, consistent with previous findings. However, replacing the AcMNPV's helicase ORF with ABM-derived helicase did not extend the host range to Mv532 cells.

The ABM baculovirus' *pe38*, *orf154* (*cds57*), hr1, *ptp*, *orf4* (*cds58*), *orf5* (*cds59*), and *lef2*, all derived from the MaviNPV genome, were investigated as well. Replacing all the AcMNPV counterparts with these ABM-derived ORFs showed that they are the host-range determinants for Mv532 cells. However, individually edited AcMNPV's *pe38*, *orf154* (*cds57*), *ptp*, *orf4* (*cds58*), *orf5* (*cds59*), and *lef2* failed to extend the host range to Mv532 cells, leaving the *hr1* as the last feature that has to be individually investigated. Testing the combinations of two ORFs (*pe38* and *lef2*, *pe38* and *ptp*) also failed to extend the host range to Mv532 cells. Future studies will explore different ORF combinations for definitive results.

Enhancement of Anthocyanin and Syringin Production by Manipulating the Phenylpropanoid Pathway in *Saussurea involucrate*

陳家尉(Jia-Wei Chen)[1]，薛立詩(Li-Shih Hsueh)[2]，劉裕國(Yu Kuo Liu)[2]，黃麗芬(Li-Fen Huang)[1]

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Abstract

Saussurea involucrate, commonly known as snow lotus, is rich in flavonoids and phenolic acids, including anthocyanin and syringin. Both compounds possess anti-inflammatory properties and reduce free radicals. The snow lotus is primarily distributed in the Tian Shan mountains in Tibet. Due to overharvesting, it has become an endangered species.

This study aims to enhance the yields of flavonoids and phenolic acids, specifically anthocyanin and syringin in snow lotus via plant tissue culture technology. By combining blue light and low temperature treatments, the cultivation of snow lotus calli with high levels of anthocyanin and syringin under sterile conditions was achieved. Additionally, *Agrobacterium*-mediated gene transfer technology is effectively used to deliver recombinant DNA to plant cells, making it widely applicable in crop improvement, medicinal compound production, and research. The MYB transcription factor, *LFMYB19831* from Taiwan Lily, was expressed using *Agrobacterium*-mediated gene transfer technology, which also led to increased accumulation of anthocyanin and syringin. The integration of plant tissue culture technology with specific culture conditions and genetic engineering has the potential to enhance the production of valuable compounds in endangered plant species like snow lotus.

Keyword : *Saussurea involucrate*, Flavonoids, Phenolic acids, Anthocyanin, Syringin

Production of Human Bone Morphogenetic Factor in Rice Seeds Using CRISPR/Cas9-Mediated Gene Editing

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Human bone morphogenic factor (hBMP2) is the strongest osteo-inductive factor essential for maintaining bone homeostasis and promoting bone differentiation during bone damage. However, recombinant BMP2 faces challenges with biosafety, low yield, or protein folding in current commercial expression systems. This study demonstrates the potential of rice recombinant protein expression system for hBMP2 production in rice seeds by the CRISPR/Cas9 gene editing system.

Glutelin is a major type of storage protein, comprising 60-80% of the endosperm in rice seeds. GluB-4 from the rice glutelin gene family has high expression during the early stages of endosperm development but peaks during the mid to late stages. Recombinant gene expression can occur in rice seed through CRISPR/Cas9 mediated knock-in the *GluB-4* intron1 region, under the control of both the *GluB-4* promoter and signal peptide. Using particle bombardment, the CRISPR vector and the *hBMP2* donor vector were co-transformed to rice calli and inserted into *GluB-4* intron1 region. The recombinant DNA transformed calli resulted in the regeneration of 33 transgenic plants. The high expression level of *hBMP2 mRNA* was detected using RT-PCR in the CRISPR-editing immature rice seeds. About 18 kDa of *rhBMP2* monomer protein were detected by Western blot analysis using anti-BMP2 antibodies. The endogenous *GluB-4* promoter driving hBMP2 via CRISPR/Cas9 strategy successfully demonstrated of hBMP2 in rice seeds.

Keywords : Recombinant Bone Morphogenic Factor 2, CRISPR/Cas9, *GluB-4*.

生命科學組

Adaptation of a eukaryote-like prolyl-tRNA synthetase to a prokaryote-like tRNA^{Pro}

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Abstract

Prolyl-tRNA synthetases (ProRSs) are unique among aminoacyl-tRNA synthetases (aaRSs) in having two distinct structural architectures across different organisms: prokaryote-like (P-type) and eukaryote/archaeon-like (E-type). Interestingly, *Bacillus thuringiensis* harbors both types, with P-type (BtProRS1) and E-type ProRS (BtProRS2) coexisting. Despite their differences, both enzymes are constitutively expressed and functional in vivo. Similar to BtProRS1, BtProRS2 selectively charges the P-type tRNA^{Pro} and displays higher halofuginone tolerance than canonical E-type ProRS. However, these two isozymes recognize the primary identity elements of the P-type tRNA^{Pro} G72 and A73 in the acceptor stem through distinct mechanisms. Moreover, BtProRS2 exhibits significantly higher tolerance to stresses (such as heat, hydrogen peroxide, and dithiothreitol) than BtProRS1 does. This study underscores how an E-type ProRS adapts to a P-type tRNA^{Pro} and how it may contribute to the bacterium's survival under stress conditions.

Keywords: aminoacyl-tRNA synthetase / halofuginone / identity element / protein synthesis / soil bacterium

Adaptive Evolution: Eukaryotic Enzyme's Specificity Shift to a Bacterial Substrate

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Analysis of prolyl-tRNA synthetase (ProRS) from three domains of life uncovers two separate architectures: a eukaryote/archaeon-like (E-type) ProRS, distinguished by a C-terminal extension domain, and a prokaryote-like (P-type) ProRS, distinguished by an insertion domain. Diversity also appears in $tRNA^{Pro}$ as the substrate of ProRS. While the anticodon elements of $tRNA^{Pro}$ are highly conserved among all organisms and important for aminoacylation, the acceptor stem elements have diverged, with G72/A73 conserved in bacteria and C72/C73 conserved in eukaryotes. In *E. coli* $tRNA^{Pro}$, G72/A73 are major determinants, whereas in humans, C72/C73 are dispensable. Paradoxically, several bacteria have been revealed to possess an E-type ProRS, with one example being *Thermus thermophilus* ProRS (TtProRS). This bacterial E-type ProRS has been reported to selectively charge the P-type $tRNA^{Pro}$ with G72/A73. This investigation reveals that despite the structural similarity with human ProRS, TtProRS maintains a strong recognition towards the anticodon elements G35/G36 and the acceptor-stem elements G72/A73, similar to *E. coli* ProRS. However, instead of relying on the strictly conserved R residue in the motif 2 loop of P-type ProRS to recognize G72/A73, TtProRS accomplishes this using RTR, a divergent sequence found within the E-type ProRS motif 2 loop. Here we also demonstrate TtProRS's relatively resistance to halofuginone, a synthetic inhibitor of eukaryote-type ProRS derived from febrifugine mimicking Pro-A76, a characteristic similar to that of bacterium-type ProRS. This study highlights the adaptability of a usually conserved essential enzyme to adjust to a new substrate.

Keywords: aminoacyl-tRNA synthetase / halofuginone / identity element / protein synthesis / thermophilic bacterium / tRNA

胡承恩、唐蒂琪、吳家榮、黃冠瑄、張瑞德、吳少傑*

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Identification of genetic determinants involved in HIT4-dependent heat tolerance in Arabidopsis

尋找阿拉伯芥參與 HIT4 媒介耐熱能力之遺傳因子

Abstract—Plants, unable to move freely, must evolve corresponding survival abilities to cope with environmental adversities. To investigate genes related to heat stress in Arabidopsis, our laboratory previously utilized a forward genetic approach to screen for heat-sensitive mutant strains, identifying *heat intolerance 4* (*hit4*). Subsequent studies revealed that the HIT4 protein is localized in the chromocenters of the nucleus at normal temperatures (23°C). Upon heat stress, HIT4 translocate from the chromocenters to the nucleolus before decondensing, and upon cessation of heat stress, it returns to the chromocenters as they reform. Conversely, the hit4 mutant protein, while retaining the ability to translocate to the nucleolus under high temperatures (37°C), does not allow the decondensation of chromocenters in mutant plant nuclei under heat stress. These results suggest the involvement of other molecules in HIT4-mediated heat-induced chromocenter decondensation and plant thermotolerance. To identify these potential molecules and fully elucidate the role of HIT4 in Arabidopsis, this study employed TurbolD to label proteins near HIT4 and utilized pull-down assays and mass spectrometry to identify proteins that may interact with HIT4. Subsequently, mutant strains of these candidate proteins were screened for thermal sensitivity, and candidate proteins were analyzed using techniques such as bimolecular fluorescence complementation (BiFC). Among the candidate proteins, PUB49 (PLANT U-BOX 49) was found to be uniformly distributed throughout the nucleus at normal temperatures but aggregates at the chromocenters upon heat stress, similar to HIT4, and relocates to the nucleolus over time, interacting with HIT4 as indicated by BiFC experiments. Importantly, *pub49* mutant plants also lose their tolerance to high-temperature stress. These results collectively indicate that PUB49 is one of the molecules involved in regulating plant thermotolerance together with HIT4 in Arabidopsis.

Keywords: Heat Intolerance 4 (HIT4), Heat stress, Chromocenter, Arabidopsis, PLANT U-BOX 49 (PUB49)

Functional characterization of VirB2 variants to study the role of T-pilus in *Agrobacterium*-mediated transformation

Sin-Sin Hsu[1,2], Teng-Kuei Huang[1], Shaw-Jye Wu [2], Erh-Min Lai[1]
許馨心 [1,2],黃登魁 [1],吳少傑 [2],賴爾珉 [1]

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Agrobacterium tumefaciens is a phytopathogenic bacterium known to cause crown gall disease in plants. *A. tumefaciens* is capable of sensing plant signals to trigger infection process, in which the effector protein substrates and transferred DNA (T-DNA) located on the tumor-inducing plasmid (Ti-plasmid) are transferred into the plant cells via the type IV secretion system (T4SS). T-DNA is imported into the nucleus merely for transient expression (transient transformation) or further integrated into plant genome (stable transformation). T4SS consists of 11 VirB proteins (B1-B11) and a VirD4 protein. VirB proteins are the components of the T4SS structure, including the translocation channel and the extracellular T-pilus, while VirD4 functions for recruiting substrates. VirB2 protein, as the major subunit of T-pilus, is essential for virulence. Interestingly, previous study showed that some VirB2 variants deficient in T-pilus still possess wild-type levels of tumorigenesis on potato tuber discs as well as tomato stems but have highly attenuated transient transformation efficiency in *Arabidopsis* seedlings. Evidence suggests that T-pilus is not essential for T-DNA transfer and virulence but may enhance *Agrobacterium*-mediated transformation (AMT) efficiency. However, the underlying mechanisms of T-pilus involved in AMT await future investigation. In this study, we employed two uncoupling mutants (T-pilus^{-}/Vir^{+}) VirB2^{L94A} and VirB2^{A110G} that retained virulence without detectable extracellular VirB2/T-pilus to study the role of T-pilus in AMT efficiency. At the same time, we also investigated the phenotype of the uncoupling mutants in bacterial growth, VirB2 protein expression level, and plant colonization in *Arabidopsis* seedlings. The results show that although the transient transformation efficiency of the uncoupling mutants was attenuated, the uncoupling mutants retained similar growth rate and colonization efficiency to the wild-type. The uncoupling mutants exhibited similar VirB2 protein levels to the wild-type in bacterial culture but their protein levels are reduced when co-cultured with *Arabidopsis* seedlings. The uncoupling mutants show similar stable transformation efficiency by *Arabidopsis* floral dip transformation. In summary, the findings suggest that the existence of T-pilus does not affect the stable transformation efficiency, and the bacterial growth and plant colonization efficiency of *Agrobacterium* VirB2 uncoupling mutants is not the cause of reduced transient transformation efficiency.

Keywords: *Agrobacterium*, *Agrobacterium*-mediated transformation (AMT), T4SS, T-pilus, VirB2

Exposure to incense burning in mice triggered amygdala dysfunction and social impairment

盧俊諺(Jyun-Yan Lu)、詹于萱(Yu-Hsuan Chan)、黃佳瑜*(Chia-Yu Huang)

Department of Life Sciences, National Central University, Chung Li, Taiwan
中央生科系

Abstract- Incense burning holds significant cultural and religious importance in East Asia , including Taiwan. However, burning incense releases harmful small particles in the atmosphere, resulting in lung injury. Whether and how these small particles cause brain dysfunction and neurobehavioral deficits remain unclear. In our study, mice were exposed to incense burning via intratracheal instillation for seven weeks, inducing lung inflammation. A battery of behavioral tests revealed that mice with incense burning showed social impairment, although their object recognition memory remained normal. We analyzed several brain regions associated with social behavior and found that the mRNA and protein expression of neuronal pentraxin II (Nptx2) in the amygdala were significantly decreased in the incense-burning mice compared to control mice. Interestingly, the serum Nptx2 level was elevated in the incense-burning mice. Additionally, aberrant AKT-mTOR signaling was observed in the amygdala of the incense- burning mice. Our results suggest that exposure to Incense burning in mice leads to social deficit and amygdala dysregulation.

Keyword: Incense burning, social impairment, neuronal pentraxin II (Nptx2), AKT-mTOR signaling, amygdala

Application of deep-learning method and automated tool to assess the freshwater crayfish behavior as a simple and sensitive aquatic model

Michael Edbert Suryanto (蘇麥克)[1,2], and Chung-Der Hsiao (蕭崇德)[1,2]

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The freshwater crayfish, *Procambarus clarkii* is an excellent aquatic animal model that is highly adaptable and tolerant. *P. clarkii* is widely used as an aquatic toxicity model to study various pharmaceutical exposure and environmental relevant studies. This animal model has complex behavioral traits and is considered sensitive to environmental changes, making it an excellent candidate to study psychoactive drugs based on a behavioral approach. However, up to now, most behavioral studies on crayfish use manual observation and scoring that require panelists. In this study, we aim to develop an automation pipeline to analyze crayfish behavior. We use DeepLabCut™, a deep neural network to label body parts in multiple crayfish, and based on the trajectory results, the intra- or inter-individual crayfish were calculated. Reliable and fast results of several behavior endpoints in multiple crayfish were retrieved. In addition, this study also evaluates other behavior endpoints, such as color preference and circadian rhythm. Total three different assessments were conducted to evaluate this animal model. In their habitat where they are collected, *P. clarkii* exists in different color variants, including white, blue, and red, colors which are commonly sold in aquarium stores. Using the optimized setup, the blue color of *P. clarkii* display more vigorous behavior compared to white and red color variants. Based on the circadian rhythm activity, blue color has significantly higher average velocity ($p<0.0001$) than other colors during both day and night cycles. Meanwhile, it showed no significant difference in color preference between red, blue, and white crayfish with their color ranking: red > green > blue > yellow. However, compared to red and white, the blue crayfish has strong significant difference of choice index in each color combination. Utilizing crayfish with complicated habits as animal models for behavioral studies might provide important information about their environment and behavior. Furthermore, determining such behaviors of the different variants of *P. clarkii* provides additional profile for crayfish which may lead crayfish farmers, crayfish shop owners and future researchers to an efficient propagation without adversely affecting the growth and development of the crayfish.

Keywords: freshwater crayfish, *Procambarus clarkii*, animal behavior, fighting, circadian rhythm, color preference

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探討柑橘果皮抗肥胖之影響

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國科會計畫編號：112-2813-C-197-013-B

摘要

肥胖的主要成因是人體攝取的熱量大於消耗的熱量。研究指出肥胖程度與死亡率成正比，罹患慢性病與癌症的機率大幅上升。雖然市面上已有藥物可以幫助人們減肥，但始終會給人體帶來副作用，因此希望能找到其他方法降低肥胖發生。我們發現芸香科植物之代謝物—類黃酮，能有效抑制肥胖和相關的代謝紊亂。柑橘類就是芸香科的一種水果，它擁有豐富的類黃酮，其中果皮的類黃酮含量比果肉更高。四季柑之活性成分含有 nobiletin、tangeretin、3′,5′-di-C-β-glucopyranosylphloretin (DGPP)；扁實檸檬則有 3’,4’,5,7-tetramethoxyflavone、3’,4’-dinethoxyflavone、sinensetin 等。使用扁實檸檬與四季柑果皮萃取物 (CDCM) 餵養已誘導肥胖之 C57BL/6J 小鼠 4 週，分三組為正常飲食、高脂飲食(HFD)、HFD+ CDCM。結果顯示與 HFD 組相比，HFD+ CDCM 組體重、腎周脂肪及腹股溝脂肪重量、AST 都有顯著降低，顯示扁實檸檬果皮與四季柑果皮萃取物可能可以降低體重、脂肪重量，且對肝臟不具毒性。

關鍵字：四季柑、扁實檸檬、高脂飲食、肥胖

台灣香檬葉萃取物提升細胞抗氧化能力與可能機制探討

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摘要

許多疾病 (如癌症) 的發生常與細胞內氧化壓力過高和慢性發炎反應有很大的關連性，所幸陸續研究發現可透過飲食減緩或預防某些慢性疾病之進展，此「預防勝於治療」的觀念倍受重視。故本研究擬評估台灣香檬 (*Citrus depressa* Hayata) 葉片 (leaves, 簡稱為 CDL) 萃取物提升小鼠 JB6 P+ 正常皮膚細胞之抗氧化能力，進而抑制促癌物 12-*O*-tetradecanoylphorbol-13-acetate (TPA) 誘導 JB6 P+ 細胞癌化作用之化學預防潛力與機制。首先製備台灣香檬葉不同溶劑萃取物，發現葉子中富含柑橘水果主要的黃酮類化合物。進一步針對台灣香檬葉熱水萃取物 (water extract, 簡稱為 CDL-WE) 與 95% 乙醇萃取物 (95% ethanolic extract, 簡稱為 CDL-95EE) 進行生物活性分析，結果發現 CDL-WE 與 CDL-95EE (5-40 μg/mL) 在對 JB6 P+ 細胞低毒性的情況下，能有效抑制細胞非貼覆性生長 (anchorage-independent growth)，其中又以 CDL-95EE 效果更佳。再者，CDL-95EE 能提高 JB6 P+ 細胞內抗氧化/解毒酵素 UGT1A 和 HO-1 蛋白表現量，且增加 Nrf2 途徑相關 mRNA 表現量，並降低 TPA 所誘發的 ROS 上升，故推測 CDL-95EE 可能經由活化 Nrf2 抗氧化途徑以保護 JB6 P+ 細胞免受到 TPA 誘導氧化壓力所引起的癌化作用；另 CDL-95EE 還能降低 DNA methyltransferases (DNMTs) 與 histone deacetylases (HDACs) 蛋白質表現量，故推測其活化 Nrf2 基因表達可能與表觀遺傳學調控機制有關。因此，台灣香檬葉具有癌症化學預防潛力，可作為農業副產品研發策略之參考。

關鍵字：台灣香檬、JB6 P+、抗氧化、癌症化學預防

探討臺灣香檬萃出物和黃酮類化合物抑制人類大腸直腸癌 HT29 癌類幹細胞生長之作用機制

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大腸直腸癌 (colorectal cancer, CRC) 在全球及臺灣的癌症死亡率分別位居第二位及第三位，而癌症化學作用預防和降低癌症治療後復發率的策略上，可針對癌症幹細胞生長進行抑制。本研究室先前的研究成果發現，臺灣香檬榨汁後果渣乙酸乙酯萃出物 (*Citrus depressa* Hayata pomace- ethyl acetate extract, CD-EAE) 具有抑制大腸直腸癌生長之潛力，故本研究即深入探討 CD-EAE 與其富含的主要黃酮類化合物，包括 nobiletin (Nob)、tangeretin (Tan)、hesperidin (Hes)、sinensetin (Sin)、3',4'-dimethoxyflavone (DMF) 、 5-hydroxy-3',4',6,7,8-penta-methoxyflavone (PMF5) 、 3',4',5',5,6,7-hexamethoxyflavone (PMF7)，以及其混合物，包括 Mixture A (Nob + Tan)、Mixture B (Nob + Tan + Hes)、Mixture C (Nob + Tan + Hes + Sin)、Mixture D (Nob + Tan + Hes + Sin + DMF) 等，抑制大腸直腸癌化生成之作用機制。結果發現 CD-EAE 抑制大腸直腸癌細胞 HT29 生長效果佳 (IC_{50} 為 30.74 μg/mL)，且 CD-EAE (25 μg/mL) 對 HT29 細胞非貼覆性生長的抑制率高達 73.47%，而 Nob、Mixture A 和 Mixture B (20 μM) 亦對細胞非貼覆性生長有高於 50% 的抑制率。此外，CD-EAE、Nob 和 Tan 能顯著抑制 HT29 癌類幹細胞 (cancer stem-like cells) 腫瘤球體生成，且降低腫瘤球體生物標記如 CD133、CD166、CD44、Nanog、Oct4 等 mRNA 表現量，而透過 RNA 定序結果更進一步發現 CD-EAE、Nob 和 Tan 可能透過不同路徑以抑制癌類幹細胞生長。從以上結果可得知，台灣香檬榨汁後果渣具有可經回收再利用以製備成抗癌植物藥物之潛力。

關鍵詞：台灣香檬、大腸直腸癌、黃酮類化合物、癌類幹細胞、癌症化學預防

透過抑制人類大腸直腸 HCT116 癌類幹細胞生長之作用機制以瞭解台灣香檬果渣之抗癌潛力

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大腸直腸癌 (colorectal cancer, CRC) 在全世界的癌症死亡率高居第二位，而柑橘類農產品廢棄物 (如榨汁後果渣) 中仍富含許多植化素，若能被開發成具癌症化學預防作用的保健食品或植物藥，藉此可望提升農業附加價值。本研究室針對臺灣香檬果渣乙酸乙酯萃出物 (*Citrus depressa* Hayata ethyl acetate extract, CD-EAE)，以及該萃出物中含量較多的黃酮類 nobiletin (Nob)、tangeretin (Tan)、hesperidin (Hes) 、 sinensetin (Sin) 、 3',4'-dimethoxyflavone (DMF) 、 5-hydroxy-3',4',6,7,8-pentamethoxyflavone (PMF5) 、3',4',5',5,6,7-hexamethoxyflavone (PMF7) 等化合物，探討其抑制 HCT116 人類大腸直腸癌類幹細胞 (cancer stem-like cells, CSLCs) 之能力與機制。結果發現，CD-EAE 可有效抑制 HCT116 細胞生長 (IC_{50} 為 33.59 μg/mL) 外，CD-EAE、Nob、Tan 和混合物 A (Nob + Tan) 皆能減少 HCT116 細胞非貼覆性生長數量，以及抑制 HCT116 腫瘤球體的形成和生物標記 (包括 CD133、CD166、CD44、Nanog、Oct4 等) mRNA 表現量；RNA 定序結果更進一步揭露這些化合物抑制 CSLCs 的可能路徑。根據目前本研究結果可推測，CD-EAE、Nob 和 Tan 可透過抑制癌幹細胞生長的機制，進而抑制大腸直腸癌症生成作用。

關鍵字：臺灣香檬、大腸直腸癌、黃酮類化合物、癌類幹細胞

探討柑橘多甲氧基黃酮類化合物抑制肝癌生長之機制

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摘要- 肝癌在全球與臺灣一直是高發生率的癌症，而有文獻指出柑橘水果中富含多甲氧基黃酮類化合物具有抗癌活性，但可能受限於化合物結構差異性。本研究針對 5 種多甲氧基黃酮類化合物，包括 4’,5-dihydroxy-6,7,8-trimethoxyflavone (PMF1)、4’-hydroxy-5,6,7,8-tetramethoxyflavone (PMF2)、4’,5,6,7,8-penta-methoxyflavone (PMF3)、5-hydroxy-4’,6,7,8-tetramethoxyflavone (PMF4) 和 5-acetoxy-4’,6,7,8- tetramethoxy-flavone (5AC Tan) 等，探討其對肝癌細胞生長的抑制作用與機制。實驗結果發現，在人類肝癌 HepG2 和 HA 22T/VGH 抑制生長的效果上，PMF1 皆展現最佳的抑制活性，IC50 分別為 26.8 和 29.0 μM。進一步探討 PMF1 抑制 HepG2 和 HA 22T/VGH 細胞生長的機制，由流式細胞儀的分析結果發現，PMF1 在處理濃度 30 μM 下，可顯著誘導 HepG2 和 HA 22T/VGH 細胞凋亡 ($p < 0.05$)，PMF1 能減少兩株細胞內促細胞凋亡 pro-caspase-3 和 pro-caspase-9 的蛋白質表現量，以及降低調控表觀遺傳學相關酵素 Histone acetylases 的蛋白質表現量。根據上述研究結果可推測，PMF1 是具有抗肝癌潛力的多甲氧基黃酮類化合物。

關鍵字：肝癌、細胞凋亡、表觀遺傳

Embryotoxic effects and regulatory mechanisms of erianin on mouse embryo development

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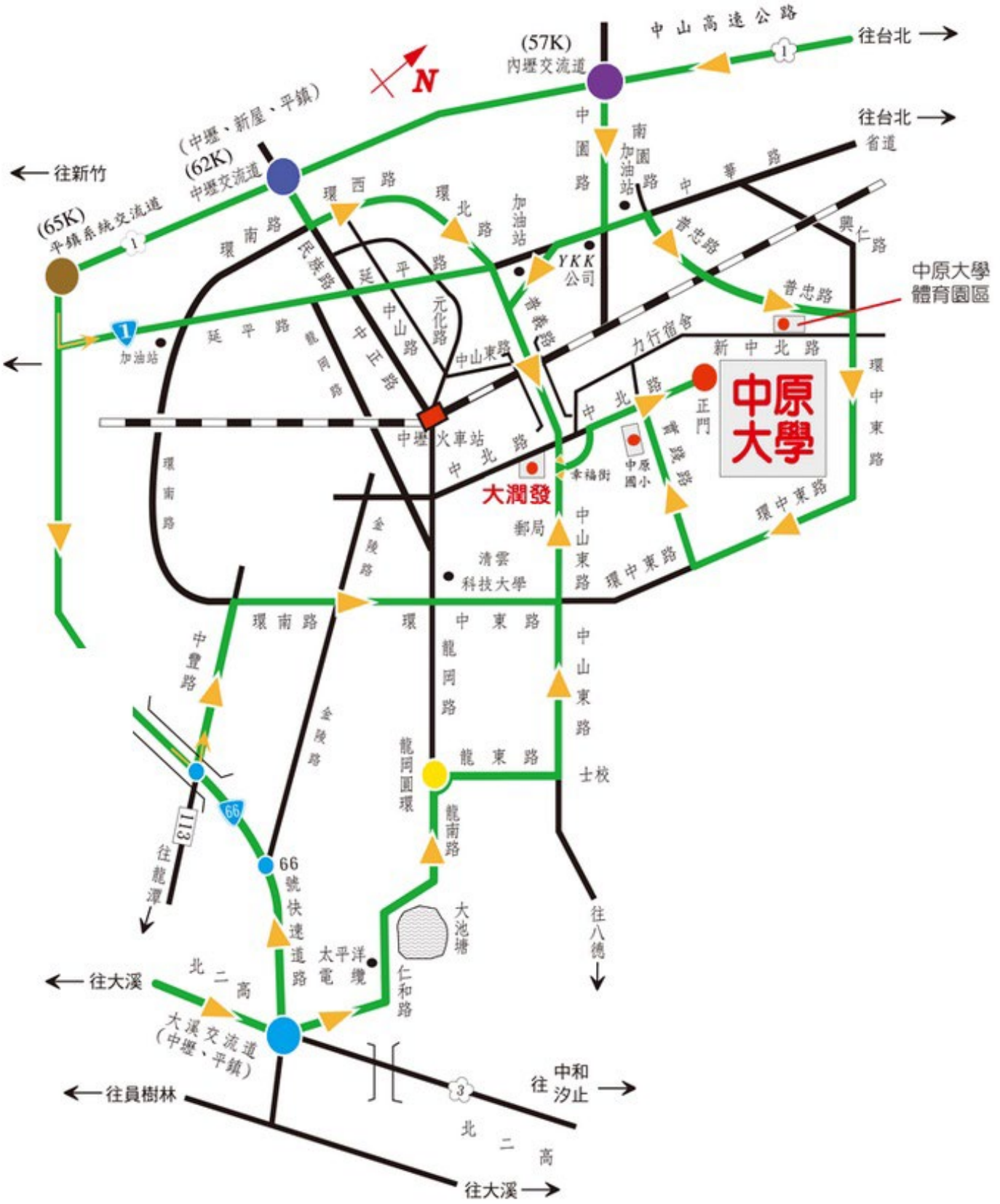
Erianin, a natural product extracted from *Dendrobium chrysotoxum Lindl*, can inhibit tumor growth and angiogenesis both in vitro and in vivo. However, to our knowledge, no related studies to date have investigated the potential cytotoxic impact of the erianin n on embryonic development. Its effects and regulatory mechanisms on pre- and post-implantation embryonic development will be clarification in this study. Hoechst staining showed that under the influence of Erianin, the degree of embryonic development significantly slowed down with increasing concentrations compared to the control group. In the Tunel assay detecting apoptosis, the group treated with Erianin exhibited a significantly higher proportion of apoptosis compared to the control group. Our results indicate that Erianin affects the development of mouse embryos in vitro and induces apoptosis in the embryos.

關鍵字 (Keywords)
Erianin、embryo、apoptosis、cytotoxicity

附件：中原大學地理位置圖



中原大學校區平面圖

Campus Map

索引 Key

1.校門 Main Gate
2.舊校門 Old Gate
3.噴水池廣場 Fountain Square
4.懷恩樓 Huai En Hall
5.維澈樓 Dickson Lee Hall
6.行政大樓 Administration Building
7.鐘塔 Cross Tower
8.產學大樓 Industry-Academia Cooperation Building
9.真知教學大樓 Chen Chih Hall
10.篤信大樓 Tu Hsin Hall
11.電學大樓 Electrical Engineering and Computer Science Building
12.中正樓 Chung Cheng Hall
13.恩慈樓 En Tzu Hall
14.良善樓 Lian Shan Hall
15.建築館 Architecture Building
16.祐生建築中心 Yu Sheng Hall
17.機車停車場 Motorcycle Parking Lot
18.設計學院 Design Building
19.景觀館 Landscape Architecture Building
20.望樓 Wang Hall
21.室設館 Interior Design Building
22.土木館 Civil Engineering Building
23.莊敬大樓 Chuang Ching Hall
24.工學館 Engineering Building
25.商設館 Commercial Design Building
26.資管樓 Information Management Building
27.管理大樓 Management Building
28.自強商學大樓 Tzu Chiang Building
29.恩惠堂 Grace & Mercy Baptist Church
30.化學館 Chemistry Building
31.理學大樓 Science Building I
32.科學館 Science Building II
33.張靜愚紀念圖書館 Chang Ching Yu Memorial Library
34.全人教育村 Holistic Education Village
35.學生活動中心 Student Center
36.生物科技館 Bio-science and Technology Building
37.室內游泳池 Indoor Swimming Pool
38.力行大樓 Li Hsing Hall
39.體育館 Gymnasium
40.喜樂樓 Hsi Le Hall
41.幼稚園 Chung Yuan Kindergarten
42.忍耐樓 Jen Nai Hall
43.和平樓 Ho Ping Hall
44.仁愛樓 Jen Ai Hall
45.溜冰場 Skating Rink
46.體育園區 Sports Park
47.薄膜中心(1～3館) Membrane Research Center
48.信實樓 Hsin Shih Hall
49.電力中心 Electric Power Supply Center
50.第一停車場 Parking Lot No.1
51.污水處理場 Waste Water Treament
52.新中北淨水場 Hsin Chung Pei Water Purification Site
53.薄膜中心(4館) Membrane Research Center