



*R&D Laboratory for Testing of Chinese Medicines*  
中藥研發實驗室

**Prof. Chi-Ming Che**

支志明教授

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香港賽馬會慈善信託基金

The Hong Kong Jockey Club Charities Trust

# Objectives 目標

Modernization of Chinese medicines  
via evidence-based research 通過循証  
研究中藥之現代化

To explore the potential **medicinal uses** of Chinese medicines such as *Anemarrhena asphodeloides*  
發掘中藥的潛在功用如知母

To act as a **bridge** linking both academia and industry and leverage the intellectual and financial resources  
作為連接學術界和產業的橋樑、撬動高學歷人才和財富的杠杆

## 中藥研發

To improve the **efficacy** of current drug leads such as timosaponins, berberine and artemisinin 提高現有中藥的藥效，如知母皂苷、黃連素以及青蒿素

To strengthen the **public confidence** in using Chinese medicines 加強公眾對使用中藥的信心

To explore **new analytical methods** for testing Chinese medicines in particular precious herbs such as Cordyceps (冬蟲夏草) and Cervi Cornu Pantotrichum (鹿茸) and solve problems of adulteration, counterfeits and etc. 開發新的分析方法以檢測中藥，特別是珍貴藥物如冬蟲夏草、鹿茸，以解決摻假、仿品等問題

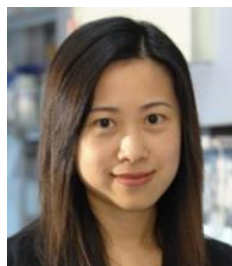
# Core members 重要研究人員

Dr. Kwan-Ming Ng  
(Analytical chemistry)

Prof. Chi-Ming Che  
(Chemistry)



Dr. Yibin Feng  
(Chinese medicine)

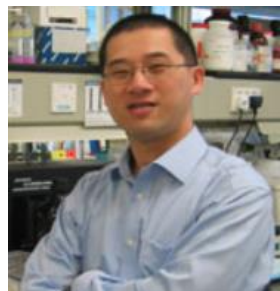


Prof. Alice Wong  
(Anticancer)

Prof. Mingfu Wang  
(Function of food)

## 中藥研發

Dr. Raymond Chang  
(Alzheimer's disease)



Dr. Yungen Liu  
(Organic synthesis)



Dr. Ning Wang  
(Chinese medicine)

Dr. Chun-Nam Lok  
(Chemical biology)



Dr. Chang Xiaoyong  
(X-ray diffractometry)

Dr. Eva Fung  
(Mass spectrometry)

# Other research personnel

## 其他參與中藥之研究人員

Dr. Anna Chan  
Dr. Eva Fung  
Dr. Kwong Wai Lun  
Dr. Jun Li  
Dr. Vanessa Lo  
Ms. Juanyu Wang  
Ms. Kiki Wan  
Ms. Pinky Lee  
Ms. Hu Di  
Mr. Fuping Liu



# Established Collaborations 合作伙伴



FACULTY OF SCIENCE  
THE UNIVERSITY OF HONG KONG  
香港大學 理學院

- ◆ Department of Chemistry 香港大學化學系
- ◆ School of Biological Science 香港大學生物科學學院

## Li Ka Shing Faculty of Medicine HKU



香港大學醫學院

- ◆ School of Chinese Medicine  
香港大學中醫藥學院
- ◆ Department of Anatomy  
香港大學解剖學系

## 中藥研發

### National Research

#### Institutes/Centers: 國際科研機構

- ◆ Professor John R. Yates III, Scripps Research Institute, California
- ◆ Prof Tommy Cheng, Department of Pharmacology, Yale. School of Medicine
- ◆ Centre for Drug Safety Evaluation and Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences 中國科學學院上海藥物研究所
- ◆ Prof. G.-Q Lin, Shanghai Institute of Organic Chemistry 上海有機所
- ◆ Dr. Z.-J. Xu, Shanghai-HK Joint Laboratory in Chemical synthesis and State key Laboratory of Synthetic Chemistry

### Local stakeholders: 本地合作機構

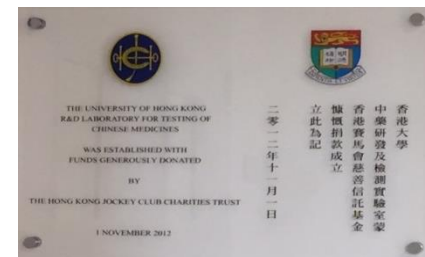
- ◆ Chinese medicines information and research section of Department of Health, HKSAR  
衛生署中藥信息及研究組衛生署
- ◆ SGS Hong Kong Ltd.  
香港通用公證行有限公司
- ◆ Healthy Pharmaceutical Ltd.  
康怡藥業

### International Collaboration: 國際產業合作



# Research Infrastructures and Facilities at HKU for R&D of Chinese Medicines 香港大學中藥研究基礎設施與設備

**R&D Laboratory for Testing of Chinese Medicines 中藥研發實驗室**



**Chemical Biology Centre at The HKJC Building for Interdisciplinary Research 化學生物中心**



**Open Laboratory of Chemical Biology of the Institute of Molecular Technology for Drug Discovery and Synthesis 分子科技研究院及化學生物學開放實驗室**



**State Key Laboratory of Synthetic Chemistry 合成化學國家重點實驗室**



# Chinese medicines and targeting disease investigated at HKU

## 香港大學研究的中藥及其針對疾病

Targeted diseases	Chinese medicines compounds	Experimental model	HKU team members
Anti-cancer 抗癌症 Anti-Alzheimer's disease 阿茲海默氏症	Steroidal saponins 甾體皂苷	Different cancer nude mice xenograft  Transgenic mice	Prof. Chi-Ming Che Chemistry (化學系 支志明)
Neuroprotection against Parkinson's disease 抗帕金森症	Chrysin and derivatives 白楊素及其衍生物	Dopaminergic neurons in mice receiving MPTP	Dr. Raymond Chang, Anatomy (解剖學 鄭傳忠)
Anti-metastasis 抗癌症轉移	Ginsenosides 人參皂苷	Ovarian cancer model with metastatic phenotypes	Dr. Alice Wong, Biological Sciences (生命科學 黃思齊)
Anti-hepatocarcinoma 抗肝癌	Coptis rhizoma 黃連 Berberine 黃連素	Hepatoma cell lines and nude mice xenograft	Dr. Yibin Feng, Chinese Medicine (中醫藥 馮奕斌)
Anti-obesity 抗癩肥	Saponins, phenolics 皂苷, 酚	3T3-L1 adipocyte and Sprague-Dawley rat models	Dr. Mingfu Wang, Biological Sciences (生命科學 王明福)

# Multidisciplinary projects - industrial

## 跨學科研究 - 產業



**RESEARCH COLLABORATION AND LICENSING AGREEMENT IN OSTEOGENESIS**

**ORIGINAL MEMORANDUM OF UNDERSTANDING**

Made this June 7<sup>th</sup>, 2010 (hereinafter the "Effective Date")

**BY AND BETWEEN :**

**THE UNIVERSITY OF HONG-KONG**  
A Hong Kong Special Administration Region entity duly established under the laws of Hong Kong Special Administrative Region, China, having its registered office at Pokfulam Road Hong Kong

and

**VERSITECH LIMITED**  
A wholly owned subsidiary and the technology transfer company incorporated in Hong Kong, having its business address at: RM 405A, Cyberport 4, 100 Cyberport Road, Hong Kong

(The UNIVERSITY OF HONG KONG and VERSITECH LIMITED referred to as "HKU")

ON THE

**AND**

**LES LABORATOIRES SERVIER**  
A corporation duly organized and existing under the laws of France having its registered office at 22 Rue Garnier 92200 Neuilly Sur Seine France

on the first part

**AND**

**THE UNIVERSITY OF HONG-KONG**  
A Hong Kong Special Administration Region entity duly established and validly existing under the laws of Hong Kong Special Administrative Region, China having its registered office at: Pokfulam Road Hong Kong

**THIS MEMORANDUM OF UNDERSTANDING is made this 12<sup>th</sup> of March, ("Effective Date").**

**BY AND BETWEEN :**

**LES LABORATOIRES SERVIER**  
A corporation duly organized and existing under the laws of France having its registered office at: 22 Rue Garnier 92200 Neuilly Sur Seine France

and

**INSTITUT de RECHERCHES SERVIER (IdRS)**  
A corporation duly organized and existing under the laws of France having its registered office at: 3 Rue de la République 92150 Suresnes France

(LES LABORATOIRES SERVIER and INSTITUT de RECHERCHES SERVIER hereinafter referred to as "SERVIER")

台灣葛迪夫生物科技股份有限公司  
台北市中山區敬業一路162號6樓  
電子信箱: simon139@ms71.hinet.net  
公司網址: <http://www.goldbvt.com>  
Goldbvt Biotech Ltd  
6F, No 162, Jing-Yeh 1<sup>st</sup> Road  
Taipei, Taiwan  
Tel: +886 (0)28509-8011  
E-mail: [simon139@ms71.hinet.net](mailto:simon139@ms71.hinet.net)  
Web: <http://www.goldbvt.com>

06 May, 2010

**Prof Chi-Ming Che,**  
Department of Chemistry,  
The University of Hong Kong



Dear Prof Che,

It is with delight that we, the Goldbvt Biotech Ltd, Taiwan, are able to offer sponsorship for your ITF project entitled "Discovery and Pre-Clinical Evaluation of Promising Metal-Based Anti-Cancer Drug Leads". We envision that this project would make great impact in the medical field including the discovery of new metal-based anti-cancer agents. We are willing to provide cash sponsorship amounting \$HKD 200,000 to support this project.

We are looking forward for the success of your project.

Yours sincerely

**Simon Chang**  
President


关于“通往临床应用之路：对金属类抗癌先导药物的开发与预临床评估”项目研究经费赞助

啟者：

本公司愿意为香港大学的创新科技基金项目“通往临床应用之路：对金属类抗癌先导药物的开发与预临床评估”提供现金赞助。本次支持经费共港币二十万五千元，给与香港大学支志明教授领导的研究小组，祈望该项目能得到丰盛的成果。

公司名称: 北京阿格蕾雅科技发展有限公司

日期: 2010年1月21日





# R&D of Chinese medicines 中藥研發



New analytical  
testing methods  
新分析測試方法



Synthesis and  
chemical modification  
of Chinese medicines  
drug leads 藥物合成與修飾



中藥研發  
之  
五大元素

Phytochemical  
analysis of  
Chinese medicines  
中藥的植物化學分析



QSTAR XL™  
LC/MALDI/MS/MS

Bioinformatics of  
Chinese medicines  
中藥的生物信息學  
(collaborating with HKUST)

Chemical biology  
and product  
development  
化學生物學與產品研發

# Highlighted and Significant Projects

## 重要科研項目

# I. Imaging mass spectrometry (IMS) for authentication of precious Chinese medicines 成像質譜於鑒定珍貴中藥的使用

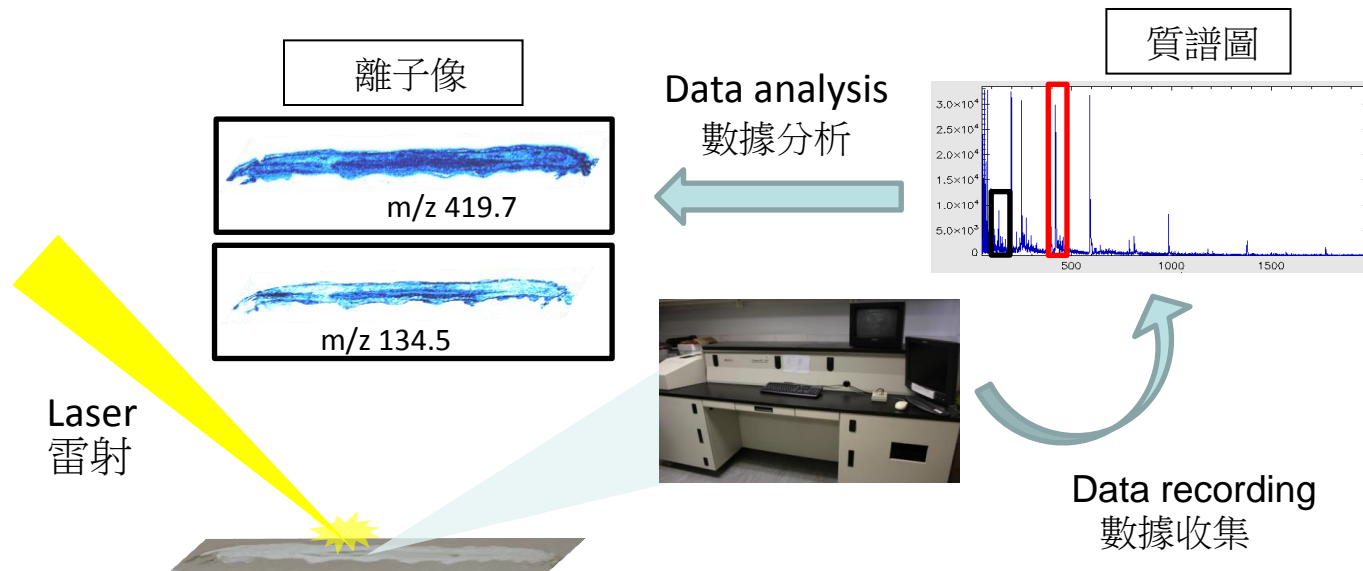
To visualize the special distribution of chemical compositions

## 原理

- ◆ 利用 MALDI-TOF MS 質譜儀於樣本一些特定的點上施加激光直至完全掃描整個樣本表面並於個定點收集質譜
- ◆ 在這些收集得來的質譜中提取離子信號，根據其信號強度及空間分佈用顏色通過軟件建立離子圖像
- ◆ 在離子圖像中，離子於樣本某個位置的信號越強，會用越深的顏色顯示



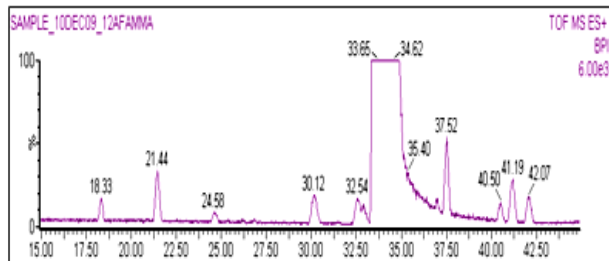
Voyager-DE STR MALDI Time-of-Flight Mass Spectrometer



Nitrogen laser system

# 我們對IMS的新應用

MS spectrum 質譜



摻假  
 仿品  
 偽品  
 不確定性  
 安全性



品質管制



現時質量控制的方法 — 形態學研究

限制:

無化學信息可得到  
 難以分辨類似形態的中草藥  
 需要相關專業知識



IMS 的質量控制

好處:

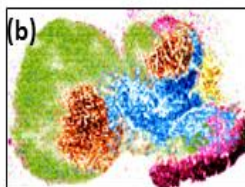
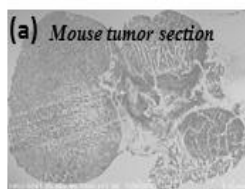
可提供形態及化學信息  
 提供可靠和可重複的結果



發展成像質譜 (IMS)  
 作珍貴的中國藥品認證



ABI 4000QTRAP  
LC/MS/MS System



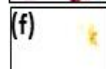
m/z 357



m/z 183



m/z 112



m/z 661



m/z 340

成像質譜



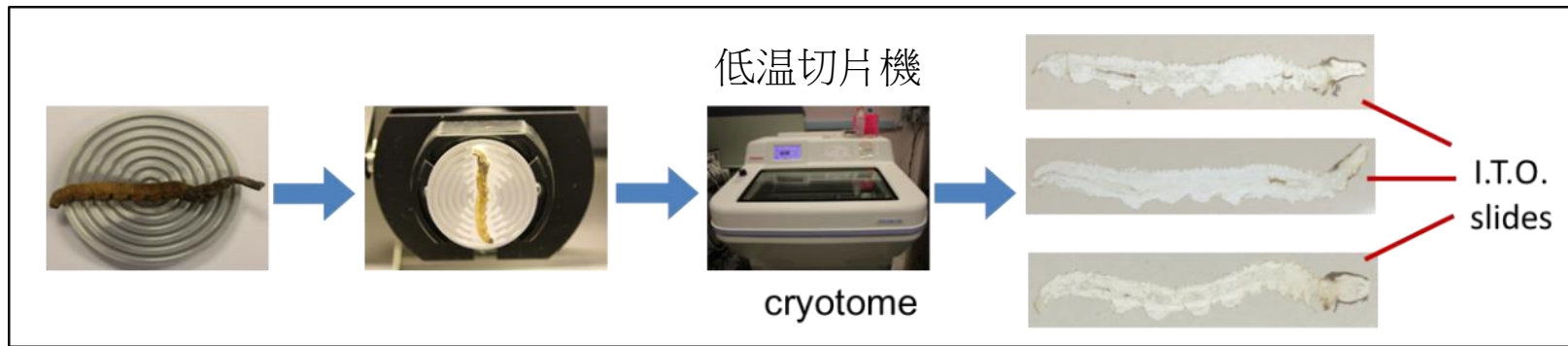
LTQ Orbitrap Velos  
LC/MS/MS system

# IMS的應用 1 - 冬蟲夏草

## 步驟： 冷凍切片

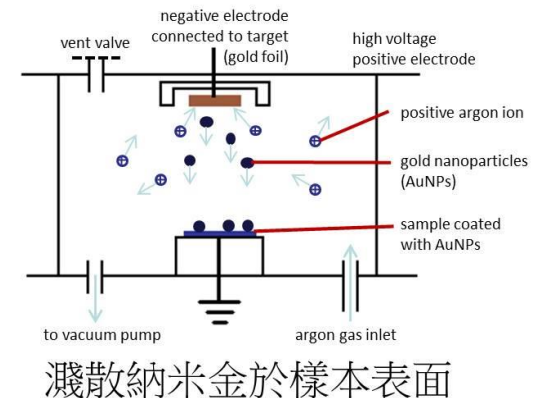


- ◆ 利用低温切片機縱切冬蟲夏草（12  $\mu\text{m}$  厚）
- ◆ 把冬蟲夏草片附在膠片上 (on the conductive side of a indium-tin-oxide slide)



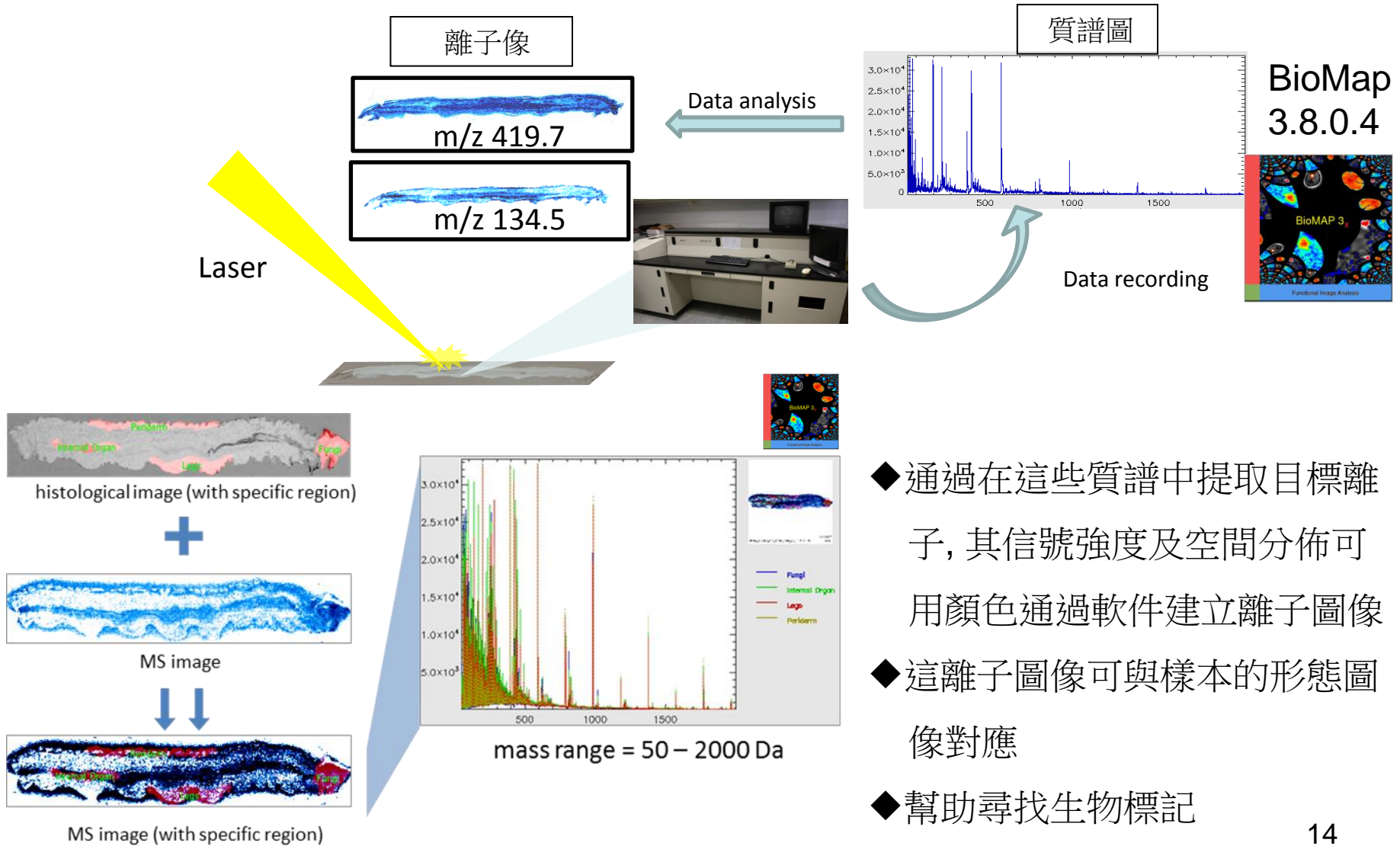
## 塗加基質

- ◆ 利用濺散機把納米金散於樣本表面
- ◆ 利用導電膠紙把塗了納米金的樣本貼於質譜儀的目標上



# 建立成像質譜

◆在樣本表面施加激光及在樣本表面各處收集質譜



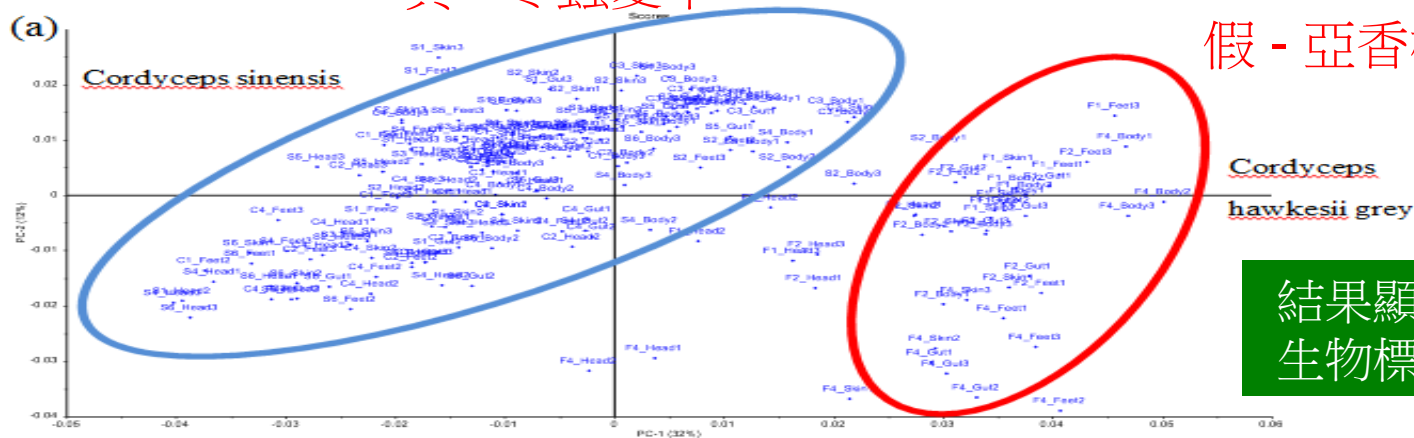
- ◆通過在這些質譜中提取目標離子，其信號強度及空間分佈可用顏色通過軟件建立離子圖像
- ◆這離子圖像可與樣本的形態圖像對應
- ◆幫助尋找生物標記

# 質量控制結果

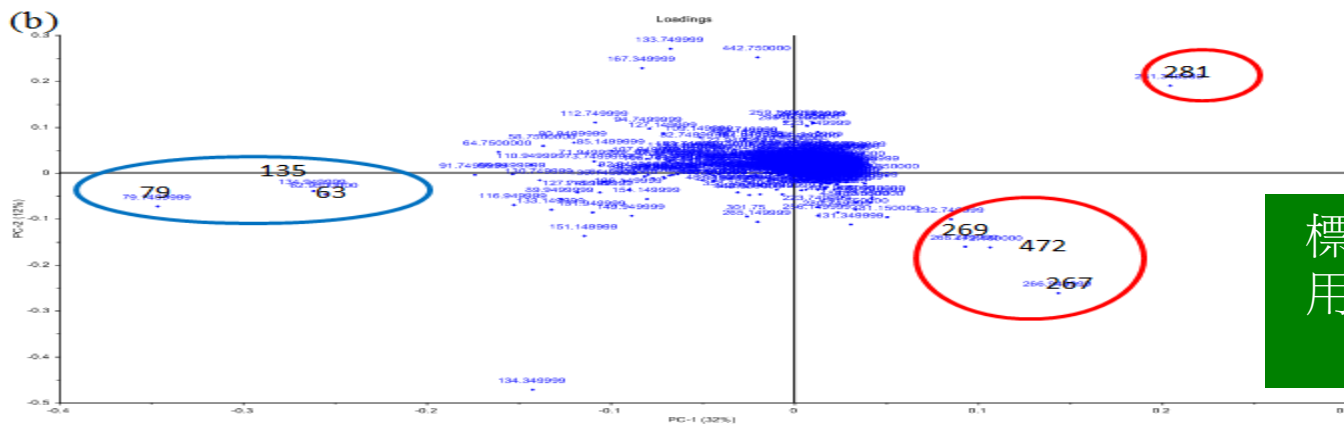
◆ 通過比對真及假的冬蟲夏草的生物標記，可識別標誌性的生物標記作真假冬蟲夏草的區分

真 - 冬蟲夏草

假 - 亞香棒



結果顯示真假蟲草的生物標記是可分辨的



標誌性的生物標記用高分辨率質譜儀或MS/MS確認

Figure 1. PCA (主成分分析) of the mass spectrometric data sets of *Cordyceps sinensis* and *Cordyceps hawkesii grey* obtained using IMS analysis. (a) Score plot result of the analysis revealed that the ion intensities of characteristic ions of the two herbs were distinguishable along the PC1. (b) Loading plot results of the analysis revealed that the characteristic ions being responsible for the differentiation were the ions at  $m/z$  63, 79, 135, 267, 269, 281 and 472. Ions at  $m/z$  135 was confirmed to be a deprotonated hypoxanthine and with higher abundance in *Cordyceps sinensis*.

## 優勢

1. 省卻複雜及費時的樣本提取過程
2. 不需要標籤便可研究樣本中的生物分子
3. 可於同一時間內研究多個生物分子
4. 得到的生物分子離子圖像可以與樣品的形態學圖像重疊, 從而提供化學和形態學的資料
5. 尋找新的生物標記
6. 研究標記在樣本表面的空間分佈

### 參考:

*Analytical Chem.* **2010**, 82, 1589-1593

*Rapid Commun. Mass Spectrom.* **2011**, 25, 3690-3696

*Analytical Chem.* **2011**, 83, 453-458



# IMS的應用2-鹿茸

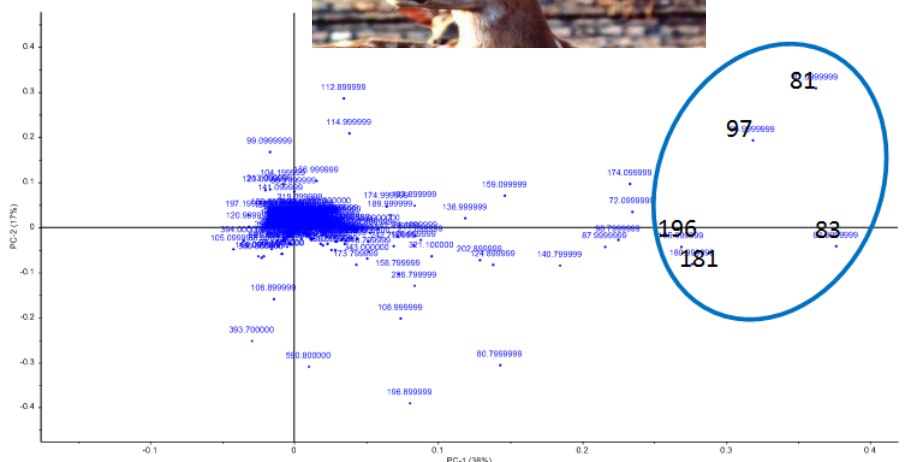
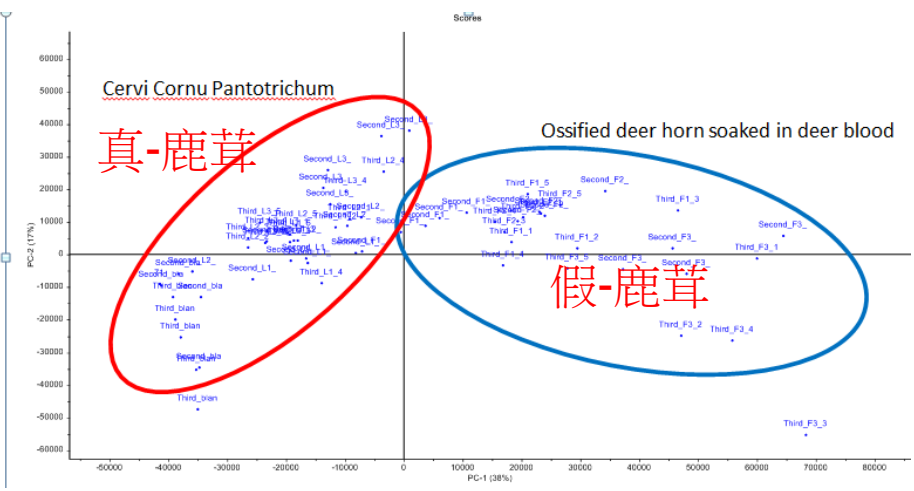


Figure 1: PCA (主成分分析) of the mass spectrometric data sets of Cervi Cornu Pantotrichum and Cervi Cornu soaked with deer blood obtained using MALDI-TOF mass spectrometric analysis. (a) Score plot results of the analysis revealed that the ion intensities of characteristic ions of the two herbs were distinguishable along the PC1. (b) Loading plot results of the analysis revealed that the characteristic ions being responsible for the differentiation were the ions of  $m/z$  at 81, 83, 97, 181 and 196.

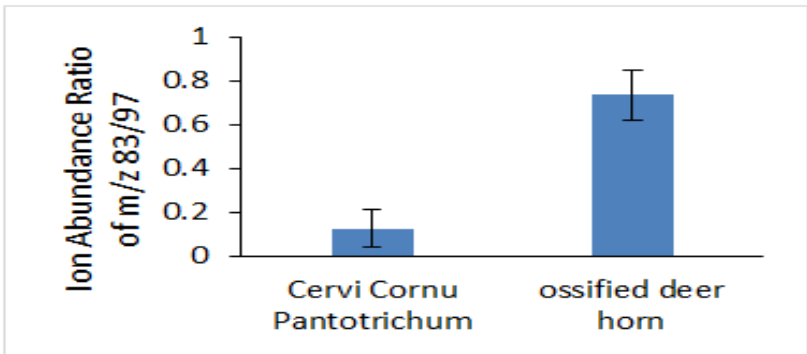
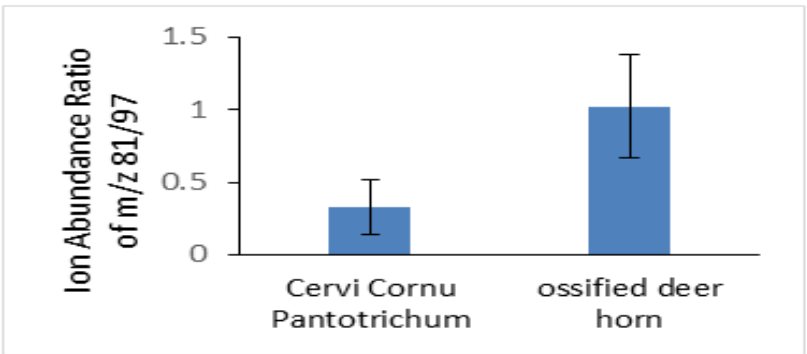


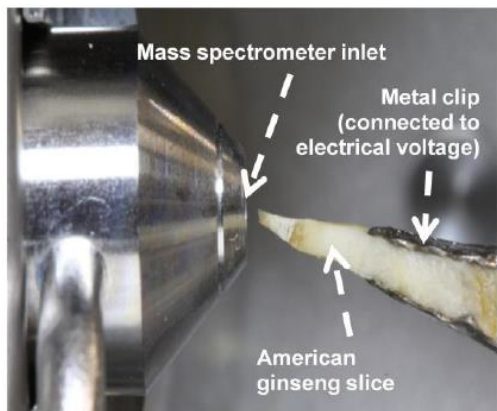
Figure 2. The differentiation of Cervi Cornu Pantotrichum and fake Cervi Cornu Pantotrichum on the ion abundance ratios of  $m/z$  81/97 and  $m/z$  83/97.

# II. Tissue spray ionization mass spectrometric method for direct and rapid analysis of raw herbs

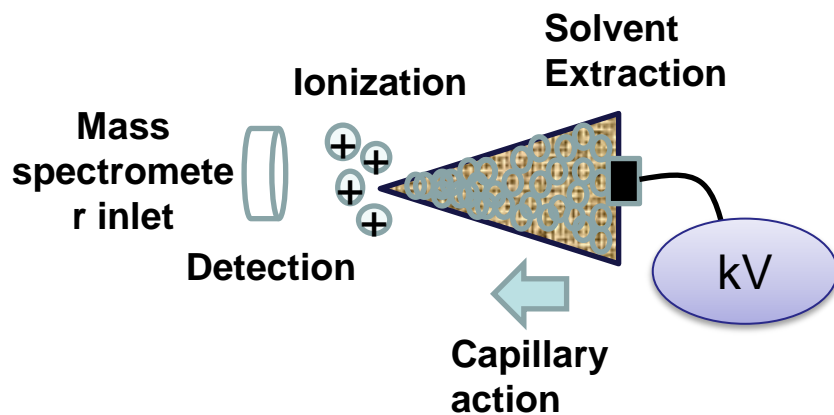
組織噴霧電離質譜法 - 直接快速分析應用於未加工中藥材

## —原理—

- ◆ 把固態狀的中藥材放近質譜儀的入口, 加入少量提取溶劑並進行通電
- ◆ 直接對樣本內的組織物進行化學分析

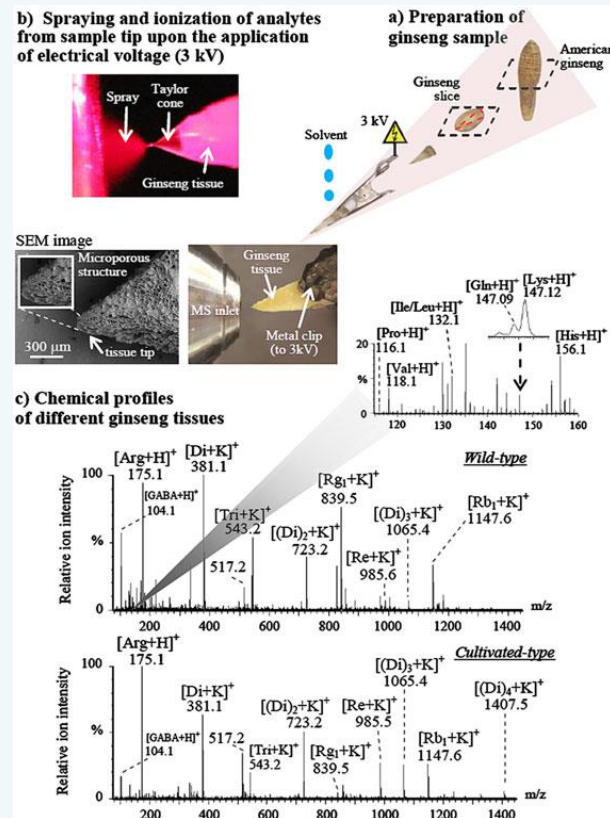


The herbal samples are placed in front of the ion inlet of a mass spectrometer, and analyte molecules can be directly sprayed and ionized from the solvent-wetted tissues upon the application of a high electrical voltage.



Tissue-spray ionization MS 的應用:

## 分辨真假美國蔘



參考:

Rapid Commun. Mass Spectrom. 18  
2011, 25, 2837-2843.

# 組織噴霧電離質譜法的應用1 - 洋金花及鬧羊花



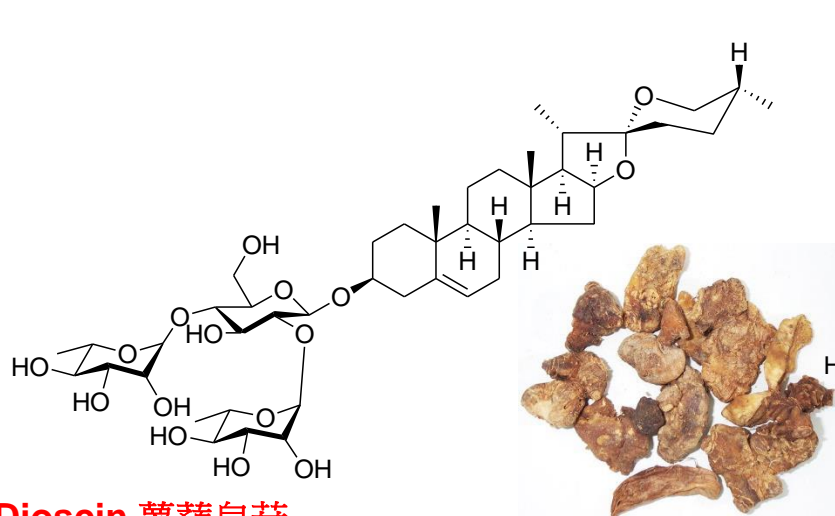
# 組織噴霧電離質譜法的應用2 - 威靈仙, 龍膽草及鬼臼



## Advantages 優勢

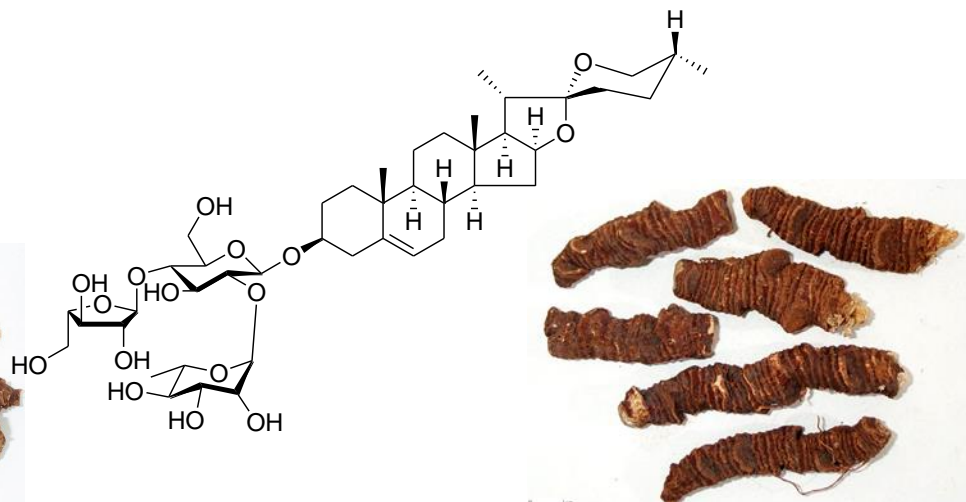
- ◆ Rapid differentiation of morphologically similar herbs  
快速區分形態相似之中藥材
- ◆ In situ analysis of sample, no sample treatment and separation  
樣品原生態分析，不需進行處理與分離

### III. Discovery of steroidal saponins from Chinese medicines for treatment of cancer 中藥中甾體皂苷的發現及其對癌症治療



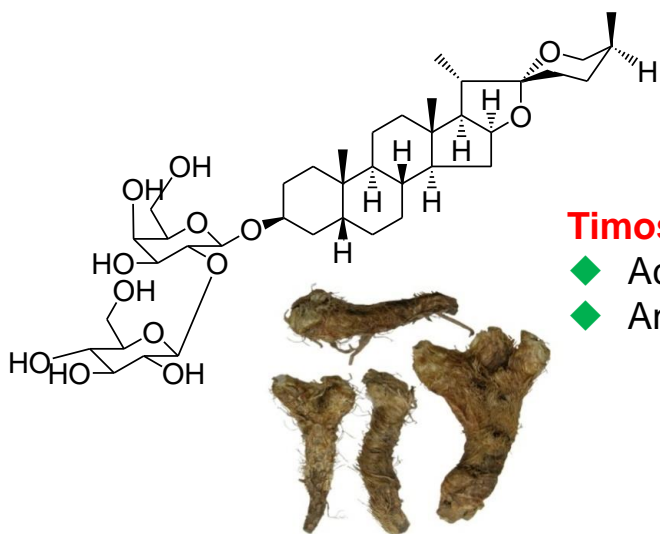
#### Dioscin 薯蕷皂苷

- ◆ Active component from *Polygonatum zanlanscianense* (湖北黃精)
- ◆ Anticancer activities



#### Polyphyllin D (PD) 重樓皂苷

- ◆ Active component from *Paris Polyphylla* (重樓)
- ◆ Anticancer activities



#### Timosaponin AIII (TAIII) 知母皂苷AIII

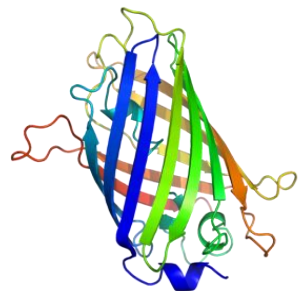
- ◆ Active component from *A. asphodeloides* (知母)
- ◆ Anticancer activities (heptacarcinoma)

《神農本草經》：  
知母，主消渴熱中，除  
邪氣，肢體浮腫，下  
水，補不足益氣。

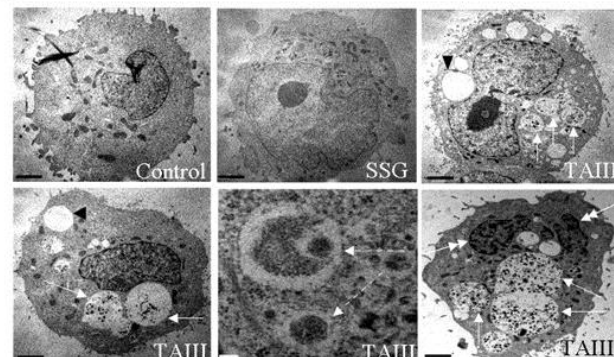
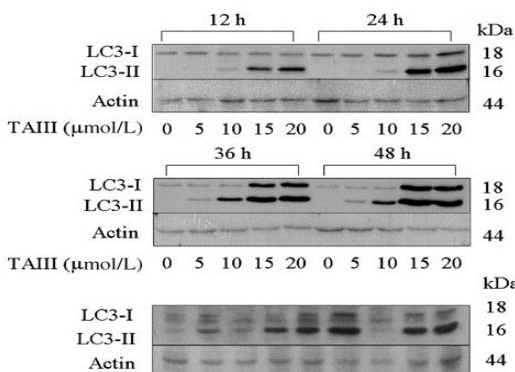
Che et al. *J. Proteome. Res.*, 2007  
Che et al. *Proteomics*, 2006  
Che et al. *Org Lett.*, 2005

# Saponin TAIII enhances autophagy 知母皂苷TAIII促進細胞自噬作用

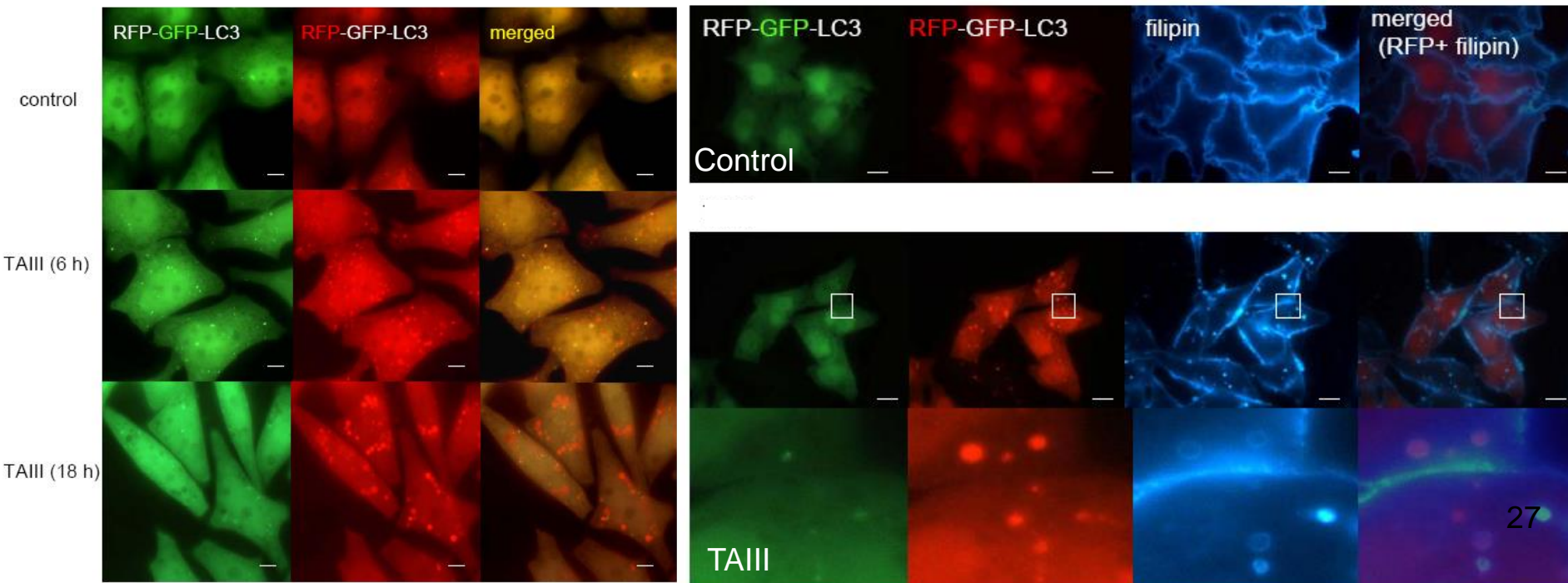
TAIII引起的細胞自噬作用 (利用綠色熒光蛋白和活細胞染色技術研究)



GFP 熒光蛋白標記



TAIII induces LC3-associated autophagic structures (TAIII誘導LC3關聯的自噬性結構)

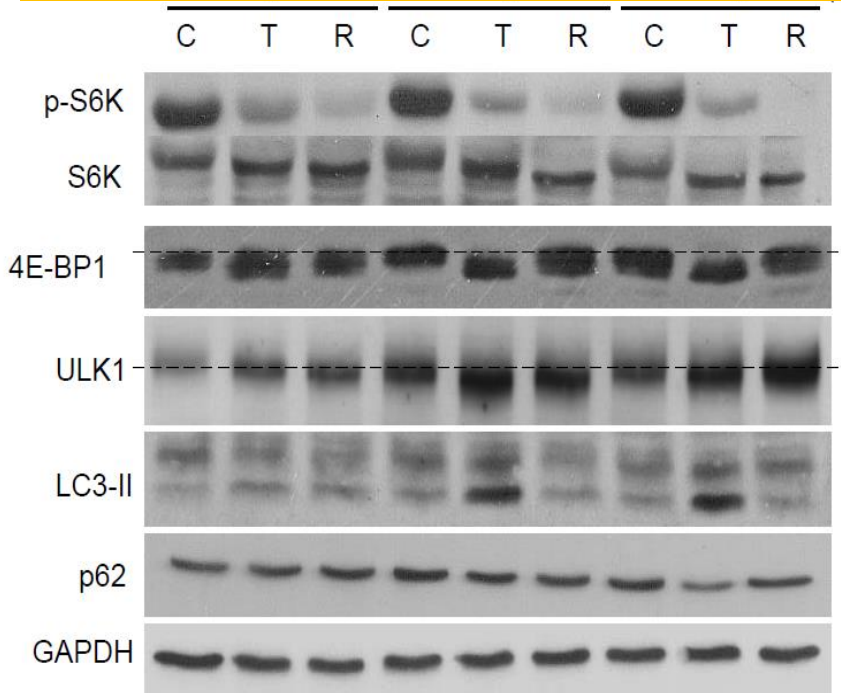


# Mechanism of saponin TAIII-induced autophagy

知母皂苷AIII於細胞自噬作用下誘發的機制

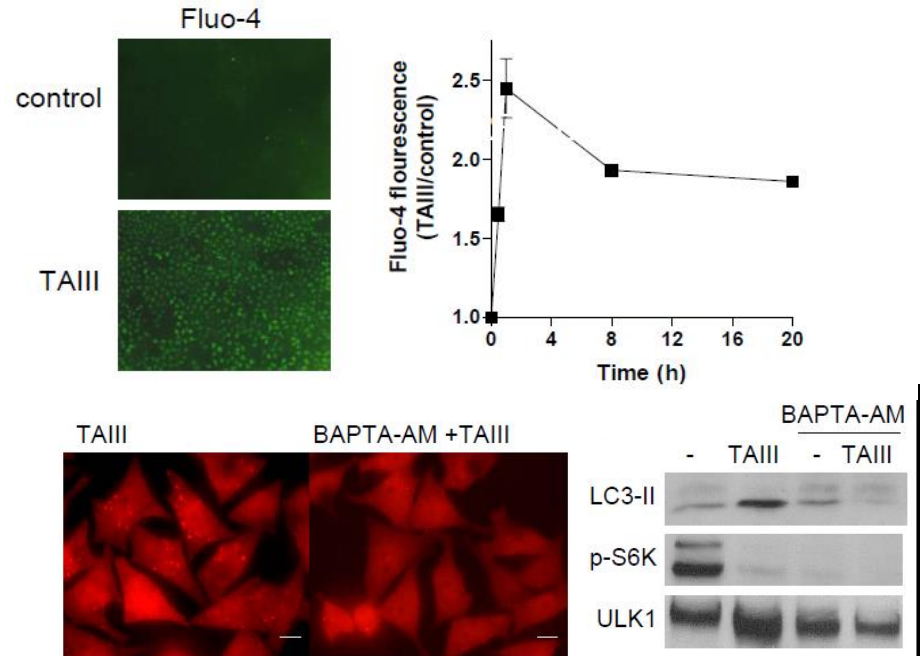
**TAIII inhibits mammalian target of rapamycin (mTOR)**

TAIII抑制哺乳動物雷帕霉素靶點 (mTOR)



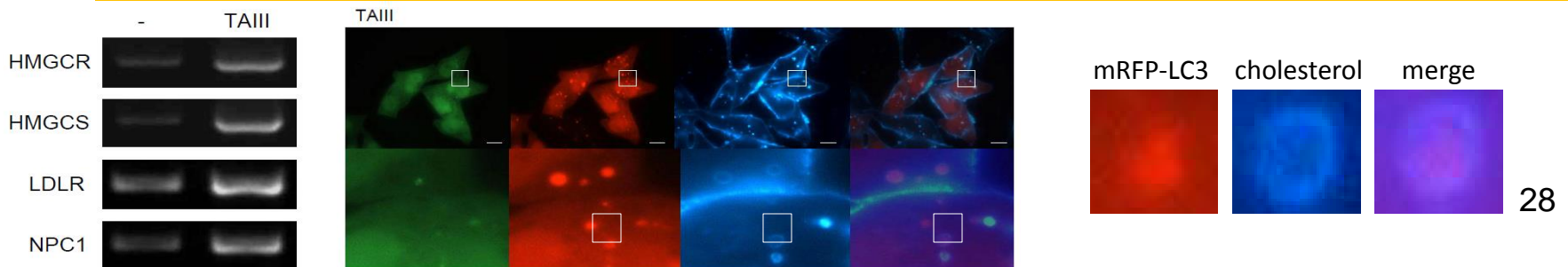
**TAIII increases cytosolic free calcium**

TAIII增加細胞內的遊離鈣



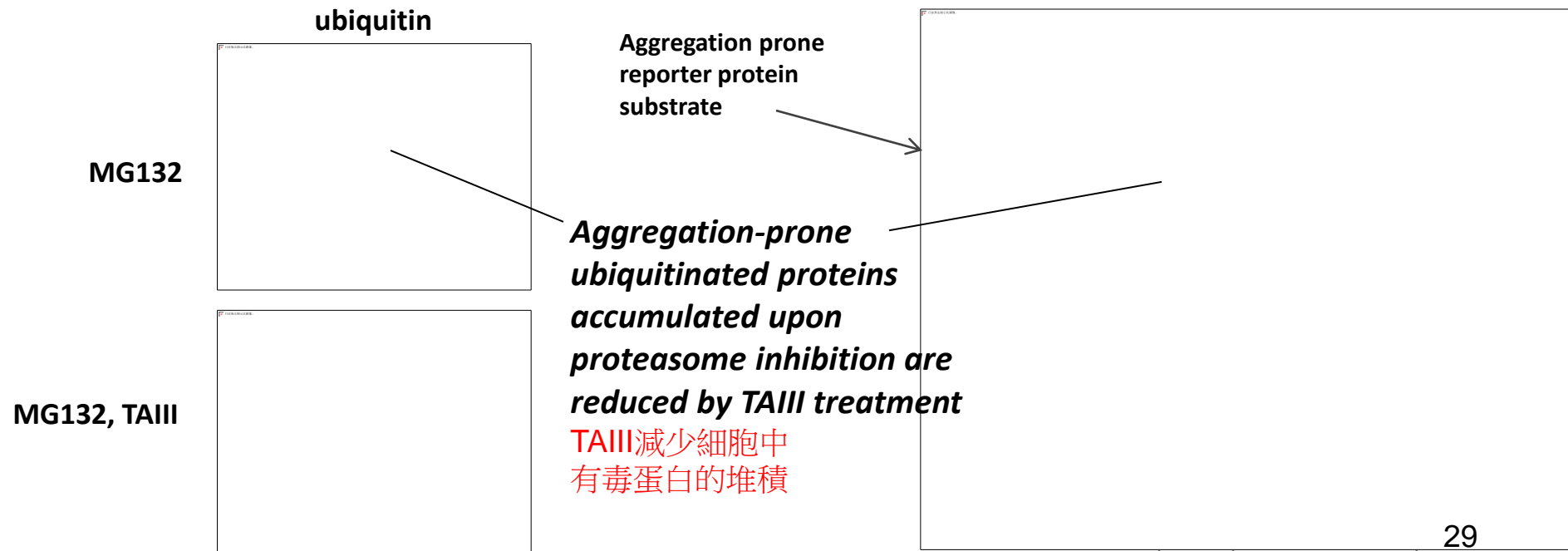
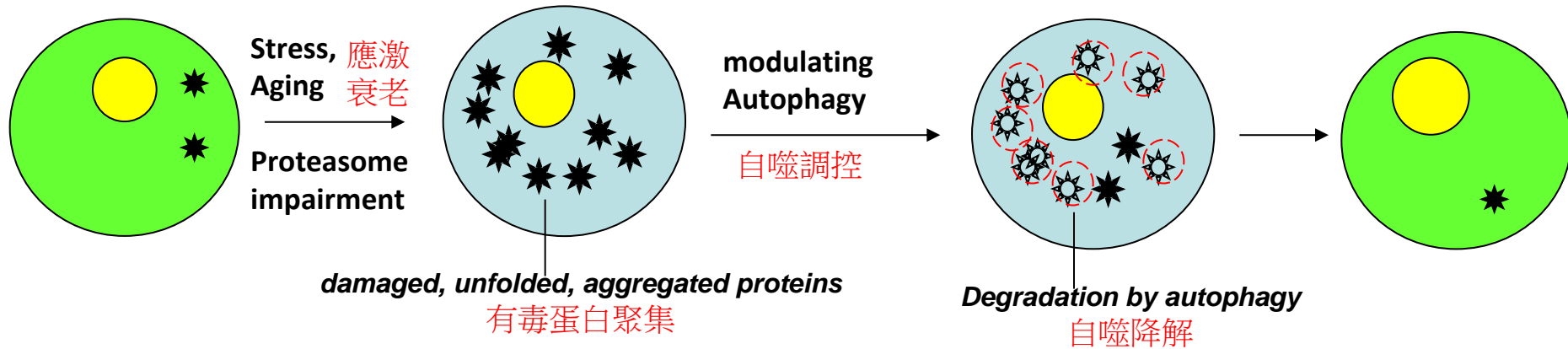
**TAIII stimulates synthesis of cholesterol which is incorporated in autophagic membranes**

TAIII刺激自噬膜中膽固醇的合成



# Saponin TAIII activates autophagic clearance of disease-associated protein aggregates

知母皂苷AIII的細胞自噬作用有助於清除蛋白質的聚集





# Saponin TAIII as an autophagy enhancers with potential for treatment of cancer 知母皂昔AIII之細胞自噬作具備抗癌性質

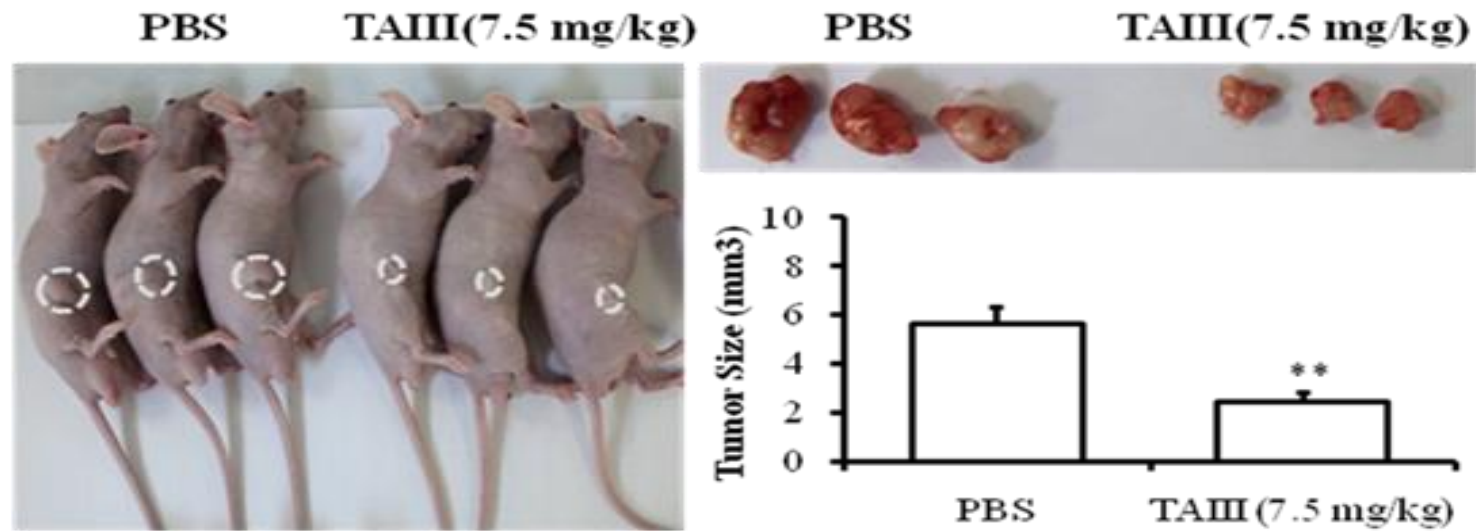


Figure 1: TAIII exhibit anti-tumor activities in nude mice xenograft model. The *in vivo* anti-tumor activity of TAIII was examined by xenograft model of HCC. In tumor bearing nude mice generated by subcutaneous injection of MHCC97L HCC cells, no remarkable body weight loss could be observed in mice treated with TAIII (7.5 mg/kg/2 days) while the tumor growth was significantly restricted. 知母皂昔TAIII有效抑制老鼠肝腫瘤

# Project IV: Discovery of steroidal saponins from Chinese medicines for treatment of neurodegeneration diseases

## 中藥中甾體皂苷的發現及其對神經退行性變疾病的治療

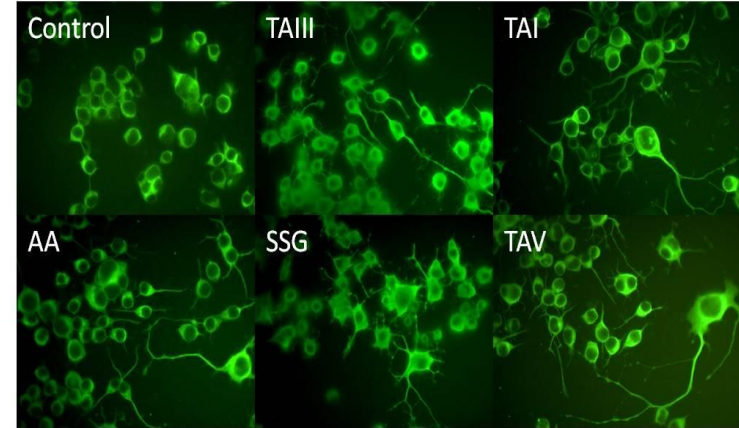
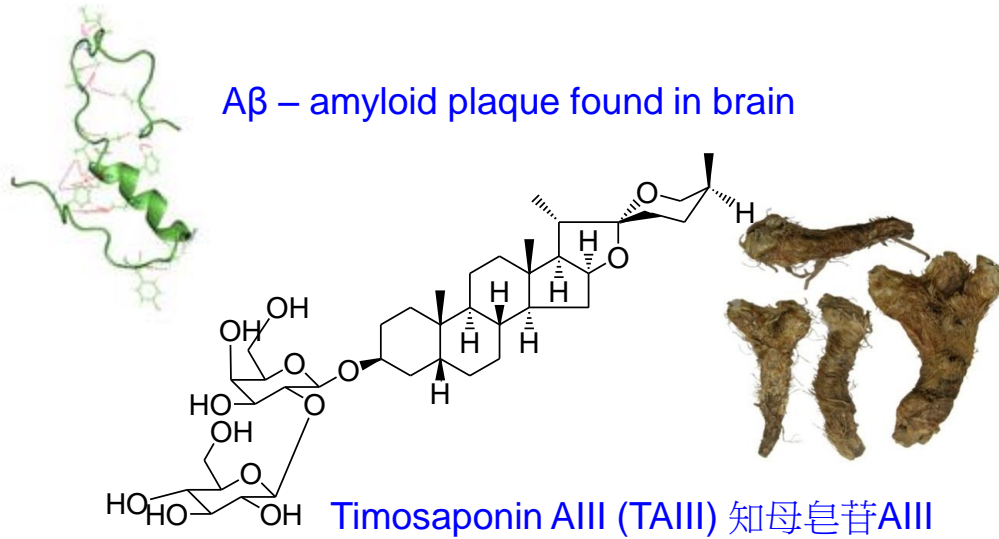


Fig. 1 Effects of timosaponins and SSG on neurite outgrowth of Neuro-2A cells. Cells were treated with DMSO (Control), TAIII (5  $\mu$ M), TAI (10  $\mu$ M), AA (10  $\mu$ M), SSG (25  $\mu$ M) and TAV (10  $\mu$ M) for 18 h, stained with a monoclonal antibody raised against type III  $\beta$ -tubulin and examined by fluorescence microscopy.

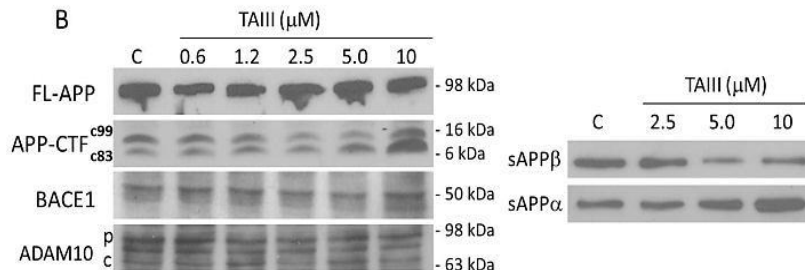
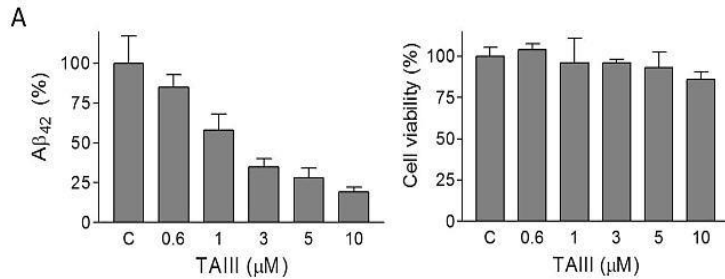
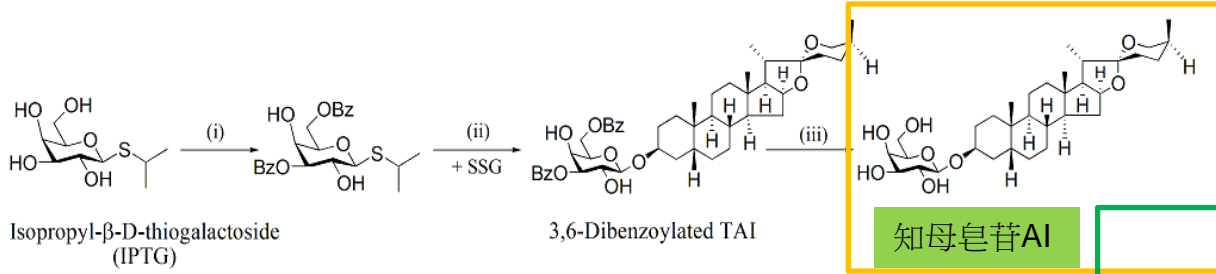


Fig. 2 Effects of TAIII on  $A\beta$ <sub>42</sub> production and APP processing in N2A-APPsw cells. Cells were treated with various concentrations of TAIII or DMSO vehicle as control (C) for 18 h. A, The  $A\beta$ <sub>42</sub> concentrations in the conditioned medium were determined by ELISA. The cell viability was determined by MTT assay. Data represents mean  $\pm$  standard deviation; n = 3. B, The expression of full length (FL), CTF (C99, C83), BACE1 and ADAM10 (p, precursor form; c, cleaved form) in cell lysates and secreted APP (sAPP $\alpha$  and sAPP $\beta$ ) in the conditioned medium were examined by immunoblot.

# Timosaponins derivatives are also effective

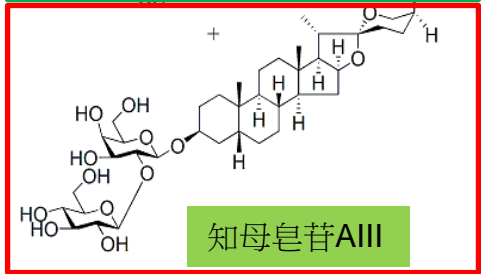
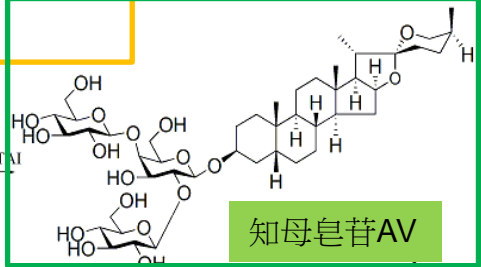
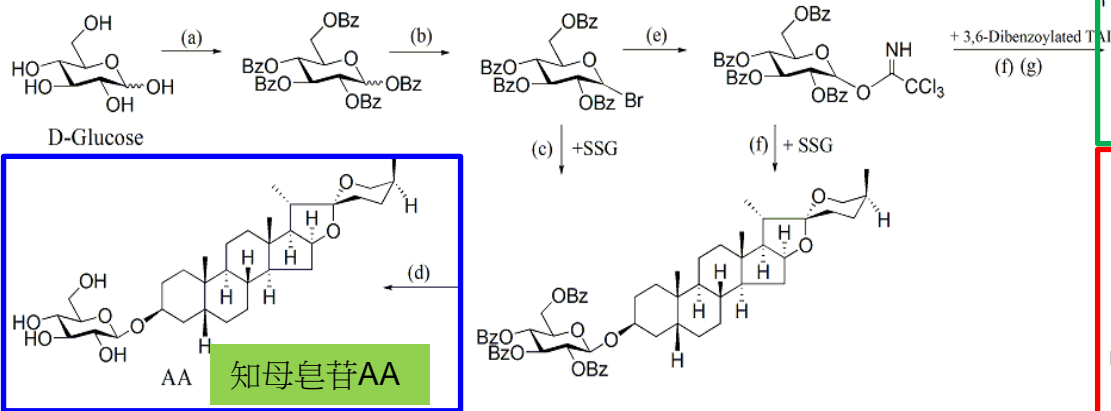
Synthesis of new timosaponin derivatives: confirmed that sugar chain is essential and beneficial to the anticancer activities  
知母皂苷的糖鏈有助於提升細胞的抗癌活性

A



知母皂苷AI

B



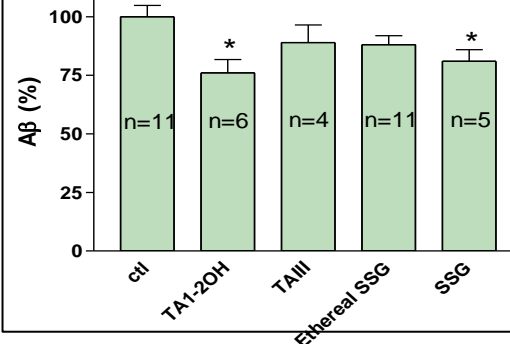
The IC<sub>50</sub> of timosaponins and SSG in lowering Aβ<sub>42</sub> production in N2A-APP<sub>swe</sub> cells

知母皂苷及知母皂苷元降低N2A-APP<sub>swe</sub>細胞中Aβ<sub>42</sub>的生產

Compound	IC <sub>50</sub> (μM)
SSG	50
TAIII	2
TAI	6
AA	6
TAV	4

Timosaponin and derivatives lower Aβ activities in mice

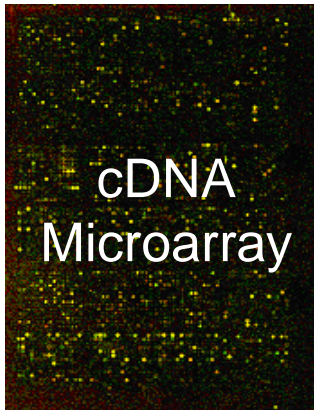
知母皂苷及衍生物降低老鼠中Aβ活性



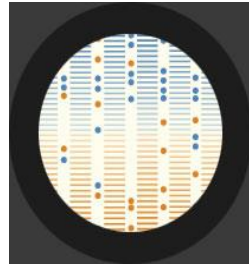
# V. Using chemo- and bio-informatics approach to study anticancer mechanism of steroidal saponins

## 用化學、生物信息學方法研究甾體皂苷的抗癌機制

Identification of endoplasmic reticulum stress pathway as the anticancer mechanisms of polyphyllin D (PD) 重樓皂苷



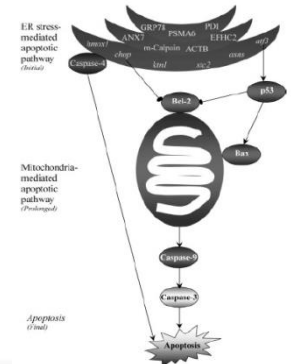
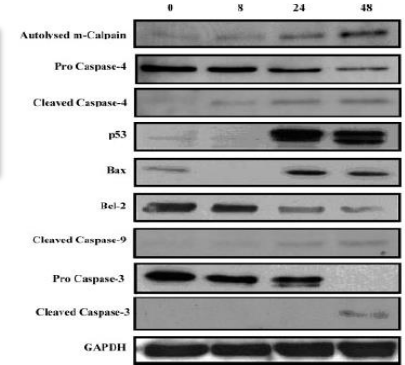
Genomic data obtained from PD treatment



Connectivity Map - Computational pattern mapping with existing drug libraries (Broad Institute, USA)

Name	Rank	Score	Inhibitor	ER stress-related response
MG-132	1	0.993	Proteasome	Unfolded protein
Celastrol	2	0.987	Proteasome	Unfolded protein
2-deoxy-D-glucose	4	0.996	Glycosylation	Glucose deprivation
Calmidazolium	8	0.752	Ca <sup>2+</sup> -calmodulin/Ca <sup>2+</sup> -ATPase	Ca <sup>2+</sup> homeostasis
Clotrimazole	10	0.724	Ca <sup>2+</sup> regulator	Ca <sup>2+</sup> homeostasis
Geldanamycin	11	0.717	HSP90	Unfolded protein
Jonamycin	13	0.685	Ca <sup>2+</sup> ionophore	Ca <sup>2+</sup> homeostasis
Rottlerin	14	0.638	Protein Kinase	Ca <sup>2+</sup> homeostasis
Trifluoperazine	16	0.612	Ca <sup>2+</sup> -calmodulin/Ca <sup>2+</sup> -ATPase	Ca <sup>2+</sup> homeostasis

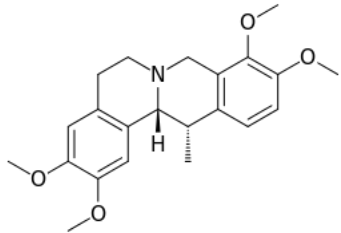
Identification of drugs with similar actions – prediction of the drug target



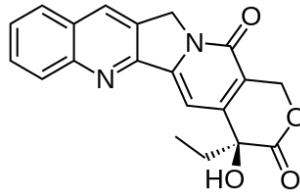
Experimental validation of hypothesis

# VI. Potential Targets Mining of Active Component from Herbal Medicines using Quantitative Proteomics Pathway Analysis 量化蛋白組

By using proteomics expression data from lysate of drug-treated or untreated cells to deduce the potential drug targets with the help of bioinformatics databases.



Corydaline 延胡索甲素

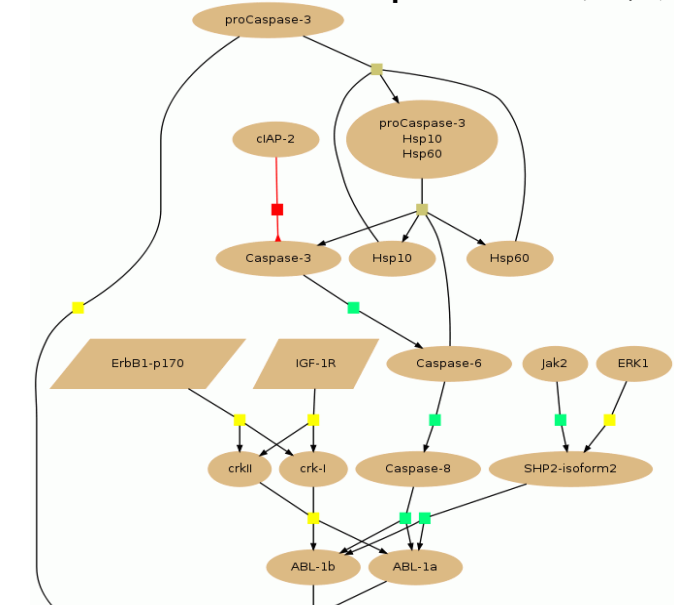
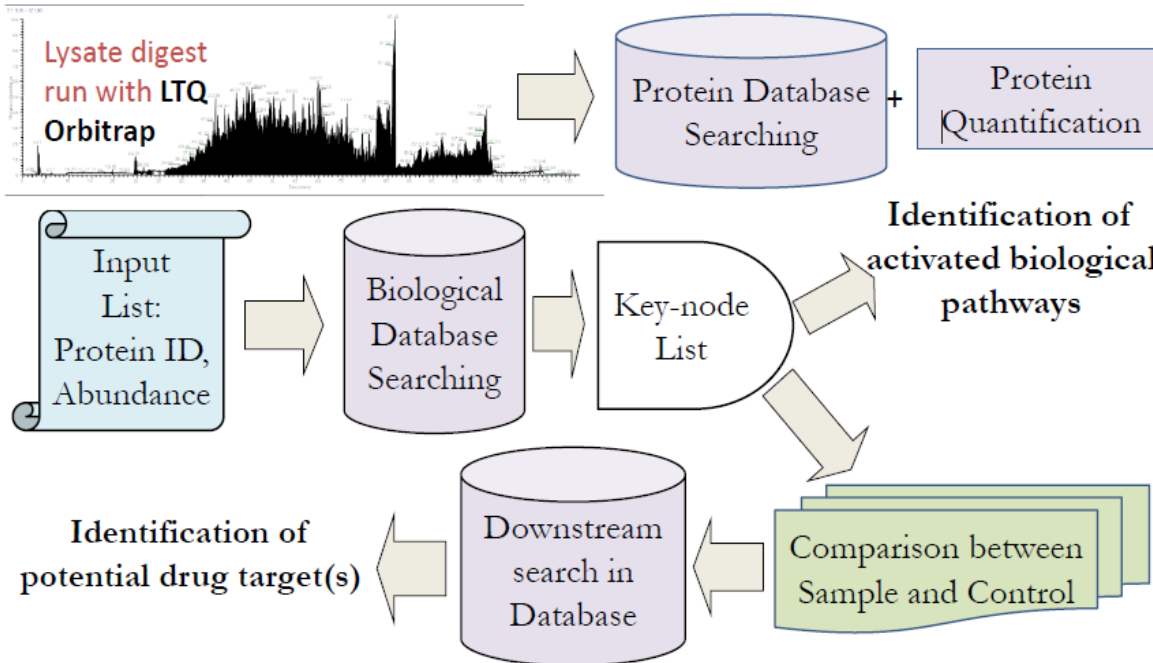


Camptothecin 喜樹鹼



Camptotheca 喜樹

## Workflow of Pathway Mapping through Key-node Mediated Approach

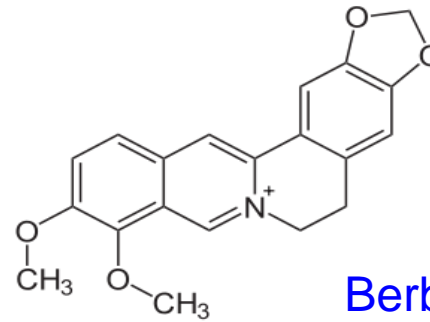


A network displaying the connectivities between the drug target and the top-scoring keynode molecules found in keynode analysis of proteomics data from camptothecin-treated cell lysate

# VII. Development of Coptidis Rhizoma for treatment of liver cancer 黃連能有效抑制老鼠肝腫瘤



黃連



Berberine 黃連素

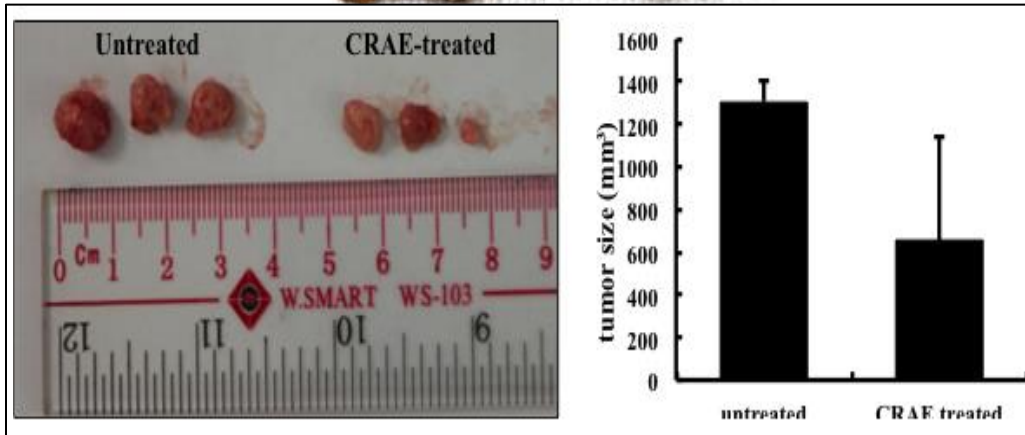


Fig. 2 The excised tumors were measured and established in average of mm<sup>3</sup> ±SD. Significant reduction of tumor volume was observed in CRAE-treated mice

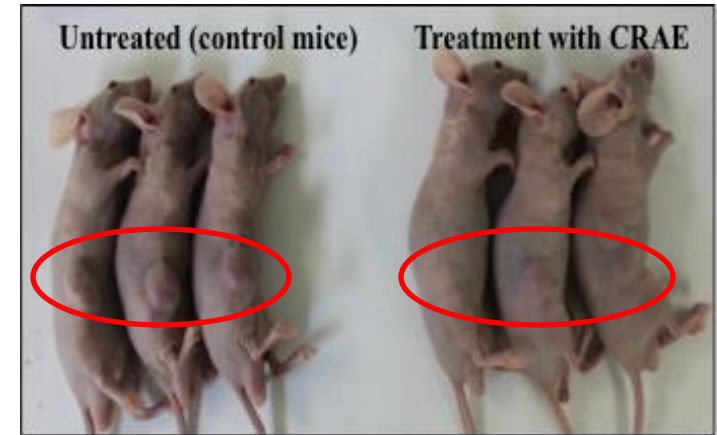
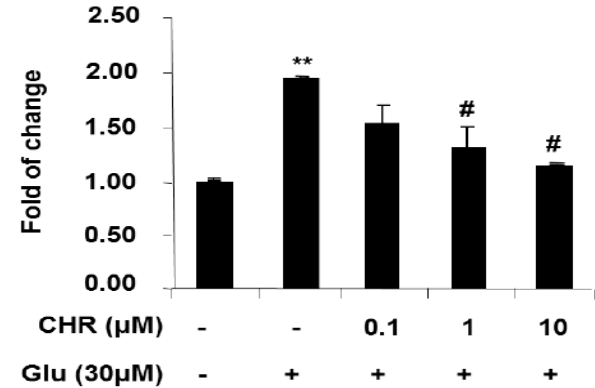
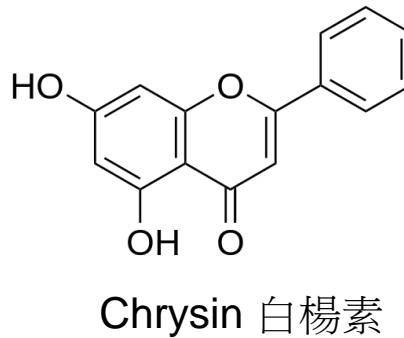


Fig.1 CRAE-treated mice have decreased tumor volume compared with the untreated mice. Two groups of mice were treated with or without CRAE for 3 weeks (n = 3).

# VIII. Neuroprotective effects of Chrysin against glutamate excitotoxicity by *in vitro* and *in vivo* models 白楊素的神經保護作用

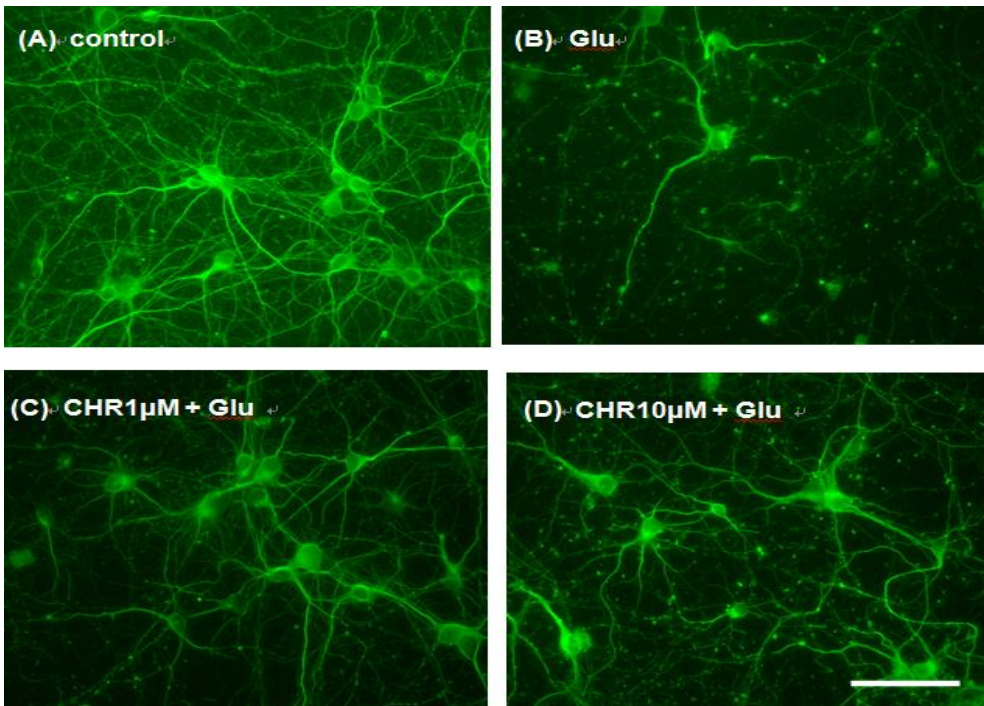
Excitotoxicity is the pathological process by which nerve cells are damaged or killed by excessive stimulation by neurotransmitters such as glutamate and similar substances.



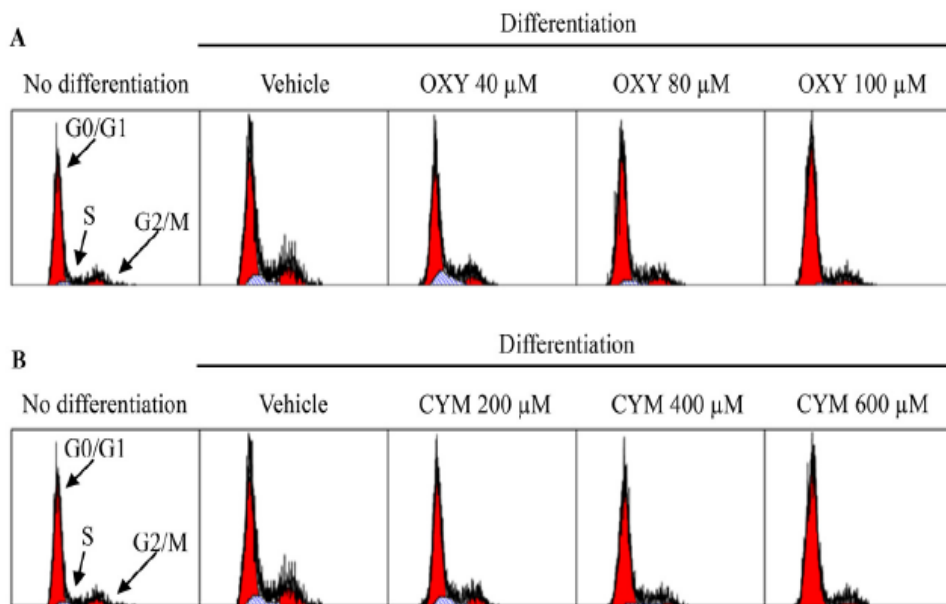
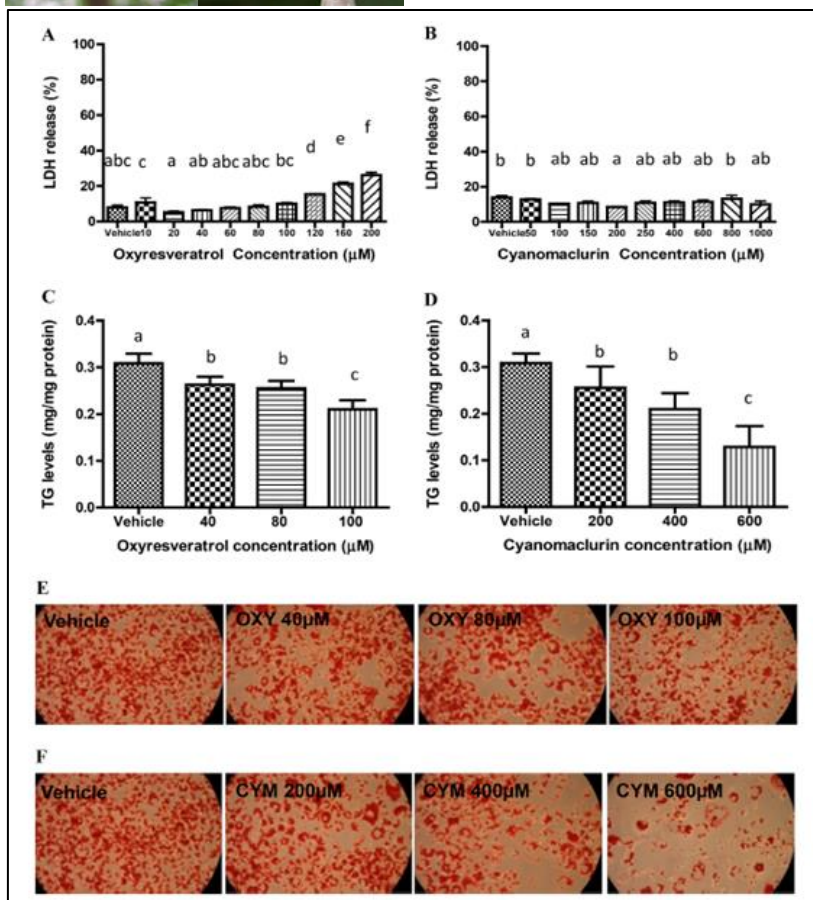
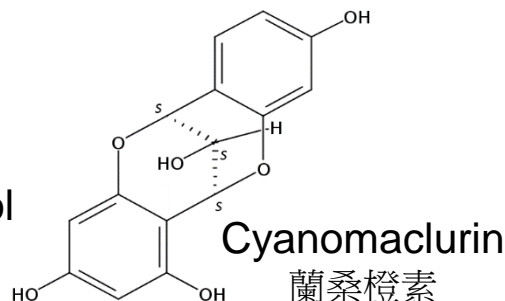
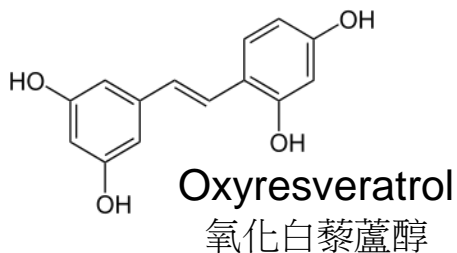
Chrysin attenuated the generation of intracellular ROS 活性氧類 induced by glutamate.

Chrysin preserved the fasciculation of neurites in glutamate-treated cultured neurons.

Immunocytochemical analysis of α-tubulin in neurons treated with chrysin. Neurons were treated with chrysin for 1 h, followed by exposure to 30 μM glutamate for 24 h. (A) control, (B) glutamate stimulation for 24 h alone, (C) neurons treated with chrysin 1 μM, and (D) 10 μM for 24 h and then together with glutamate 30 μM for 24 h. Scale bar represents 50 μm.



# IX. Development of anti-adipogenic agents from Chinese Medicines (抗脂肪積聚)



Cell cycle assay of oxyresveratrol and cyanomaclurin on 3T3-L1 cells. Postconfluent 3T3-L1 preadipocytes were differentiated with IDI plus (A) oxyresveratrol, (B) cyanomaclurin or vehicle for 24 hours. The harvested cells were stained with Vindelov's reagent and analysed by FACS.

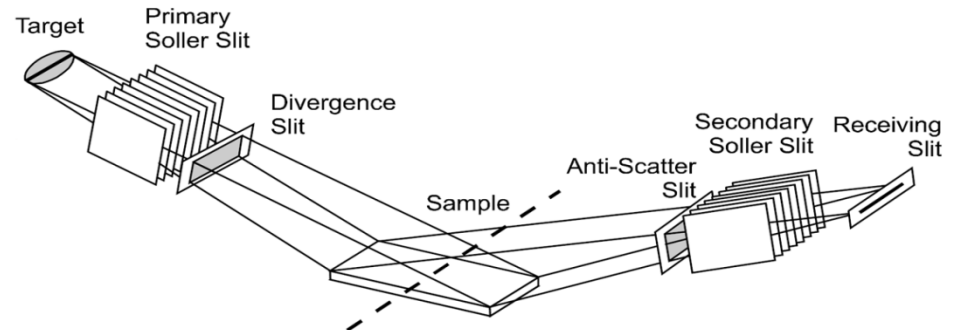
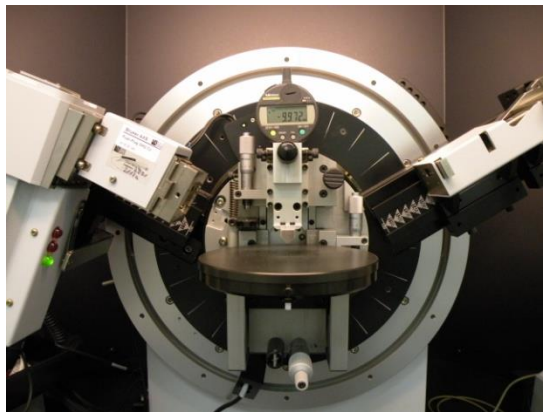
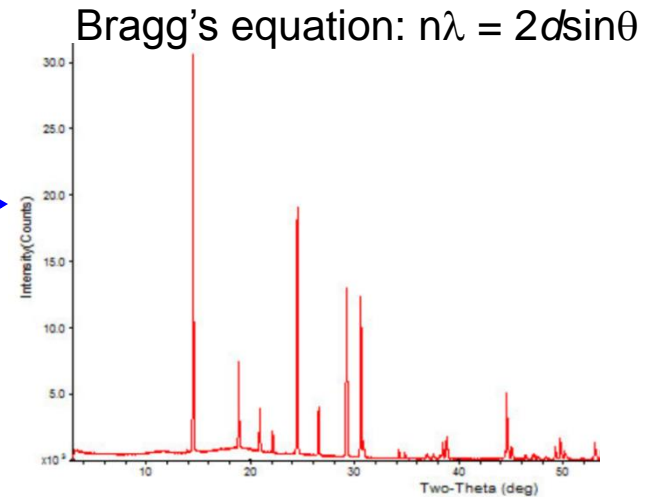
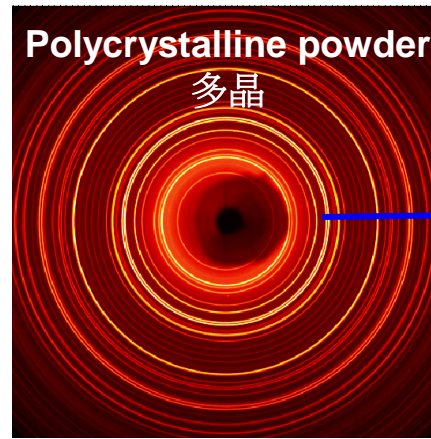
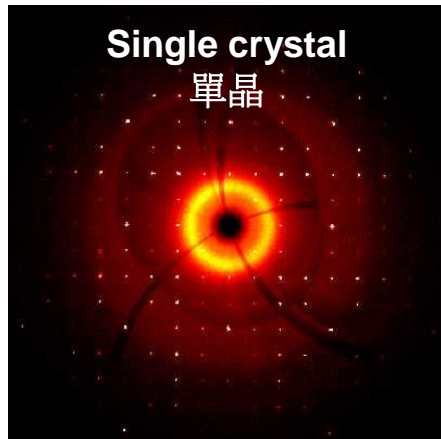
Oxyresveratrol and cyanomaclurin inhibit lipid accumulation in 3T3-L1 cells. 防脂質積聚



# X. X-ray Powder Diffraction (XRPD)

## X-射線粉末衍射法

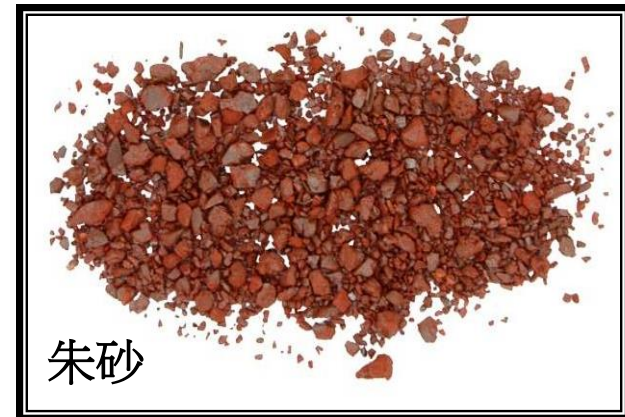
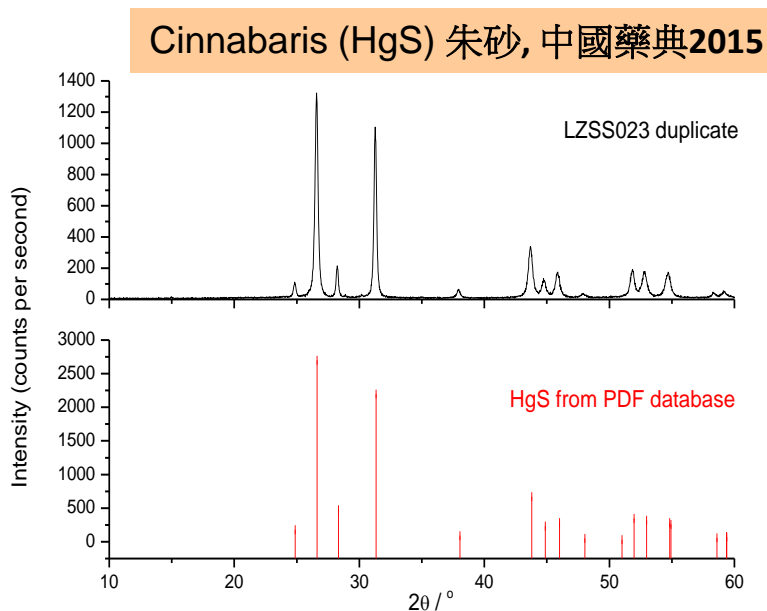
XRPD is a rapid and non-destructive analytical technique primarily used for phase identification of powder or a microcrystalline material and provides structural characterization. X-射線粉末衍射是一種快速、不具破壞性的分析技術，主要應用於粉末或微晶材料的物相鑒定和結構鑒定



# Qualitative XRPD 粉末衍射定性分析 (Phase Identification 物相鑒定)

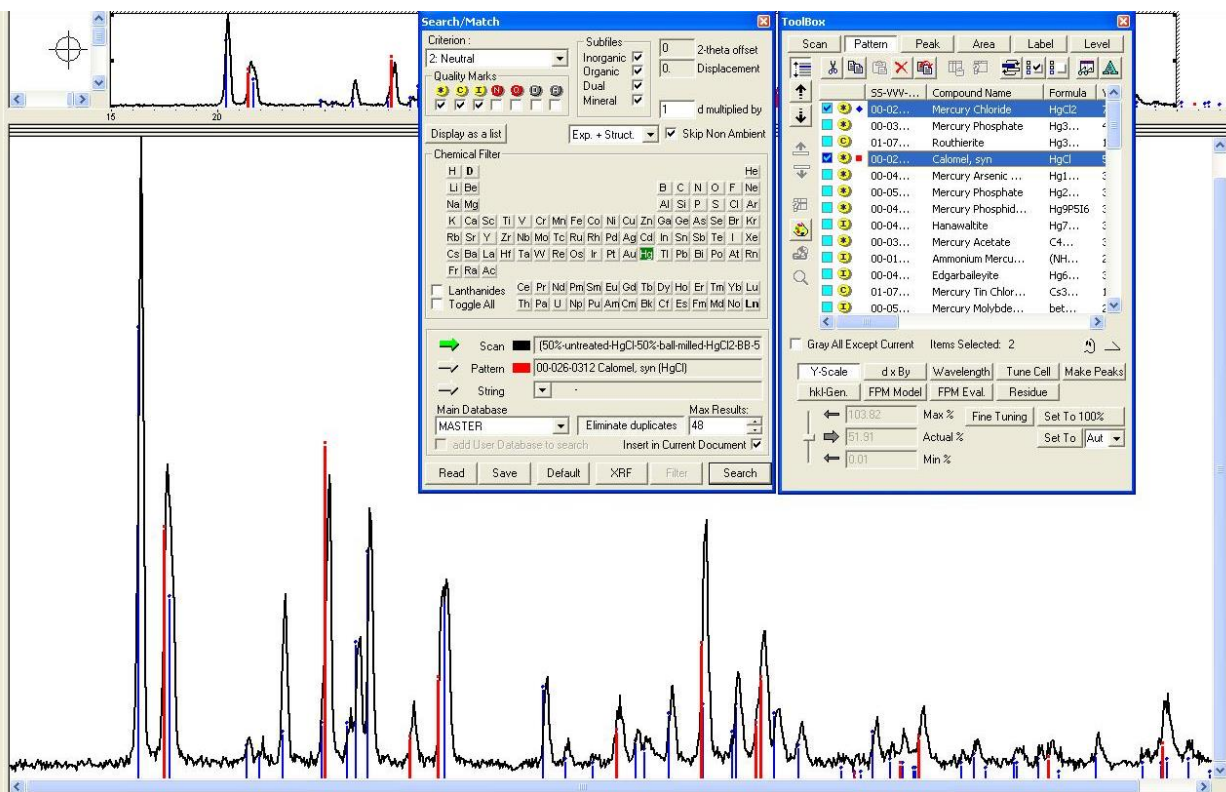
International Centre for Diffraction Data Powder Diffraction Files  
(ICDD PDF-2 or abbreviated as PDF)

- ◆ The diffraction pattern of every phase is as unique as **fingerprint** (指紋圖譜)
- ◆ Phases with the same chemical composition can have different diffraction patterns
- ◆ Identification: comparing the position ( $2\theta$ ) and relative intensity of sample peaks to the reference patterns in the database.



# Quantitative XRPD (Q-XRPD) 粉末衍射定量分析

- ◆ Determine the content of each phase in a mixture 混合物中各個物相的定量
- ◆ The ratio of peak intensities varies linearly as a function of weight of any two phases in a mixture.
- ◆ **Relative Intensity Ratio (RIR, 相對強度比)** method is fast and gives semi-quantitative results.
- ◆ Whole pattern **fitting/Rietveld refinement (擬合/Rietveld精修)** is an accurate but complicated analysis



00-026-0312 Calomel, syn  
 ----- HgCl  
 FWHM(30): 0.209°  
 Crystallite Size (Scherrer): 388.8 Å  
 I/lor: 5.  
 Space group: I4/mmm (139)  
 Cell param.: a=4.48010 c=10.90600

00-026-0315 Mercury Chloride  
 ----- HgCl<sub>2</sub>  
 FWHM(30): 0.233°  
 Crystallite Size (Scherrer): 348.8 Å  
 I/lor: 3.2  
 Space group: Pmnb (62)  
 Cell param.: a=5.97560 b=12.76800  
 c=4.33470

Number of steps: 20  
 R/R0: 3.42  
 RWP: 22.7  
 Concentrations:

-----  
 00-026-0315 56.8% HgCl<sub>2</sub>  
 Mercury Chloride  
 00-026-0312 43.2% HgCl  
 Calomel, syn

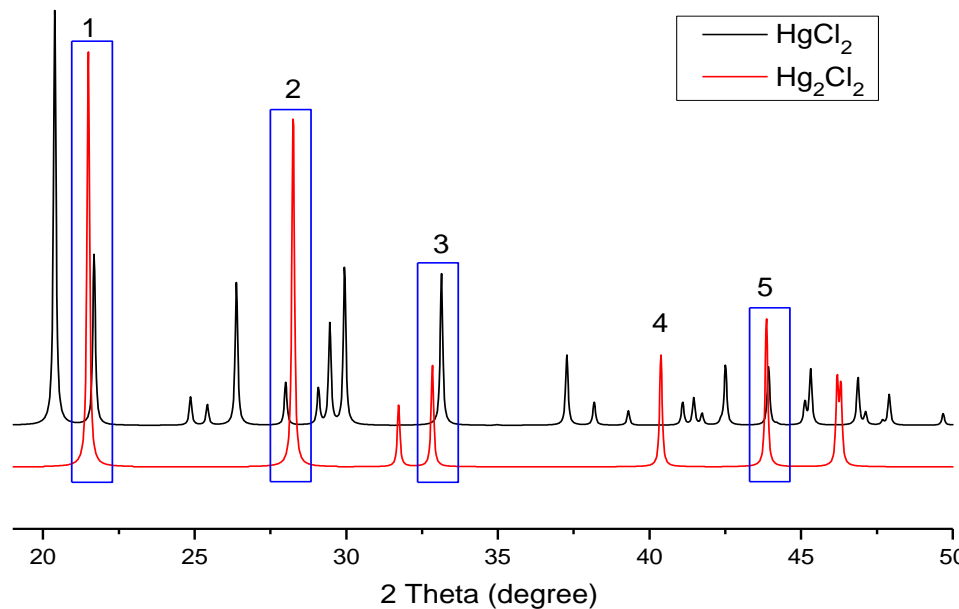


Figure 1: Stack of simulated XRPD pattern of  $\text{HgCl}_2$  (raised curve) and  $\text{Hg}_2\text{Cl}_2$  (lowered curve) showing the peak overlaps in the  $2\theta$  range of  $19 - 50^\circ$ .

### Quantified results

Sample	$R_{\text{HgCl}_2} : R_{\text{Hg}_2\text{Cl}_2}$ (from TOPAS)	$R_{\text{wp}}[\%]$
BJDS001	99.54:0.46	10.9
BJDS003	0:100	1.7
BJDS004	100:0	10.1
BJDS005	99.74:0.26	10.6
BJDS006	99.69:0.31	10.4
BJDS007	99.86:0.14	9.73
LBJDS00166	99.20:0.8	10.6
LBJDS00170	99.79:0.21	10.4
LBJDS00172	99.28:0.72	10.6
LBJDS00174	99.55:0.45	10.1 <sup>46</sup>

- ◆ The severe peak overlaps coupled with the potential preferred orientation effect make the Reference Intensity Ratio (RIR) method unreliable in the QPA of HCC samples.
- ◆ Comparing with the single peak intensity strategy adopted in the RIR method, the whole pattern analysis method gives more accurate results.
- ◆ Refinement of crystallite size, strain, background and preferred orientation correction (March Dollase model) in Rietveld method can efficiently minimize the sample related effects and hence improve the accuracy.





US 20150050367A1

(19) **United States**

(12) **Patent Application Publication**

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(43) **Pub. Date: Feb. 19, 2015**

(54) **TIMOSAPONIN COMPOUNDS**

**Publication Classification**

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**Chunnam Lok**, Hong Kong (CN)

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*A61K 45/06* (2006.01)  
*A61K 31/58* (2006.01)  
*A61K 31/7048* (2006.01)

(72) Inventors: **Chiming Che**, Hong Kong (CN);  
**Laiking Sy**, Hong Kong (CN);  
**Chunnam Lok**, Hong Kong (CN)

(52) **U.S. Cl.**  
CPC ..... *C07J 71/0031* (2013.01); *A61K 31/7048*  
(2013.01); *A61K 45/06* (2013.01); *A61K 31/58*  
(2013.01)  
USPC . **424/752**; 536/6.1; 514/26; 540/48; 514/172;  
514/171; 540/17; 514/173

(21) Appl. No.: **14/390,400**

(22) PCT Filed: **Apr. 2, 2013**

(86) PCT No.: **PCT/CN2013/073666**

§ 371 (c)(1),  
(2) Date: **Oct. 3, 2014**

(57) **ABSTRACT**

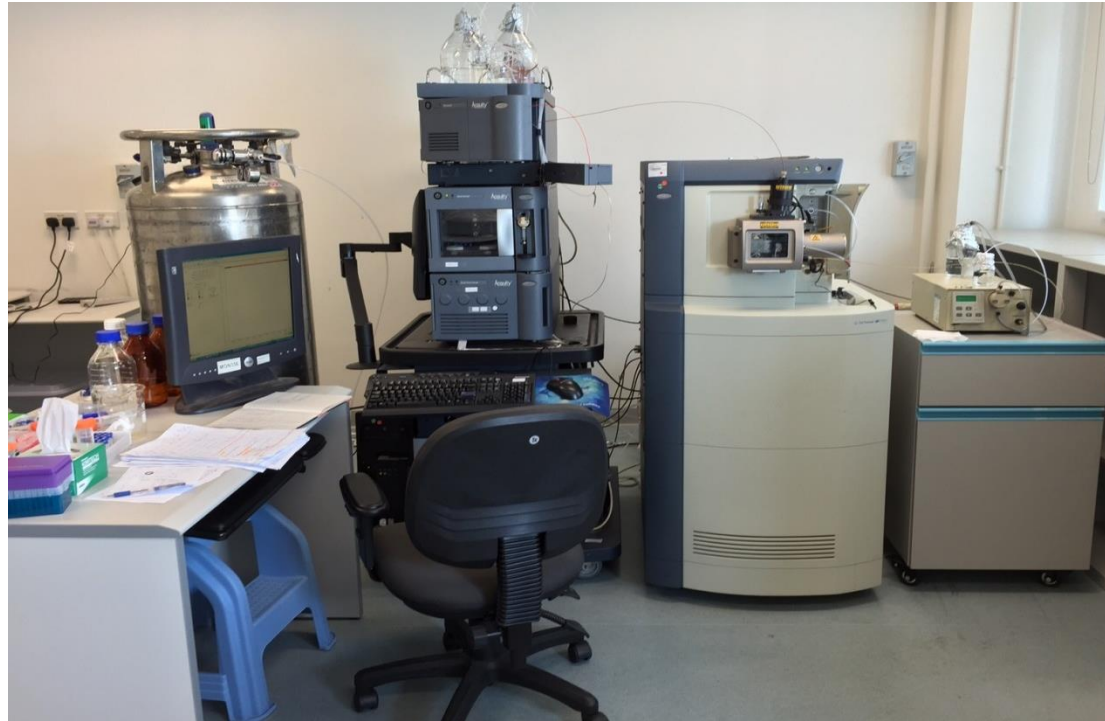
**Related U.S. Application Data**

(60) Provisional application No. 61/619,439, filed on Apr. 3, 2012.

Provided herein are timosaponin compounds of Formula I, II, III, I', II' and III', pharmaceutical compositions comprising the compounds, and processes of preparation thereof. Also provided are uses of said timosaponin compounds for preparing medicament for the treatment of diseases associated with beta-amyloid in hosts or subjects in need thereof.

# Waters UPLC-QTOF Donated by HKJCCT

## 馬會捐贈之液相色譜法-質譜聯用



- (a) Chemical profiling comparison of easily-confused Chinese medicine  
易混淆中藥之化學分析
- (b) Metabolite analysis 代謝物分析
- (c) Pharmacokinetics 藥物代謝動力學
- (d) Bio-distribution study of intriguing drug leads and 前體藥物體內分佈
- (e) A teaching tool for promoting collaborative research 教育工具鼓勵合作性科研

**Chemical markers are available from the following website**

中藥對照品可循下述網站查詢

[www.hktlcm.com](http://www.hktlcm.com)



**Testing Laboratory**  
*for Chinese Medicine* **TLCM**

ABOUT US  
關於我們


PRODUCTS  
產品資料

R & D  
研究成果

CONTACT US  
聯絡我們

 香港科技大學  
THE HONG KONG UNIVERSITY OF  
SCIENCE AND TECHNOLOGY

 香港大學  
THE UNIVERSITY OF HONG KONG

資助機構  
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The Hong Kong Jockey Club Charities Trust



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The Hong Kong Jockey Club Charities Trust



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The Government of the Hong Kong Special Administrative Region  
香港創新科技署



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