

## 謝 誌

在交大六年，實驗室四年，研究生的日子終於要在此時畫下句點。在這段充滿挑戰的日子，謝謝每一位在我研究上給予指教和幫助的人，讓我能順利踏出研究所的門檻。首先感謝指導教授林志生老師，四年來不厭其煩地對學生諄諄教誨，除了在科學研究上訓練學生有嚴謹的態度、縝密的思考及追求完美之外，在做人處事上以身作則，並且讓我有不少機會去面對挫折和挑戰，在過程中學習並成長茁壯，於此獻上最真摯的感謝。另外，也非常感謝口試委員的溫曉薇老師與林岳暉博士，在百忙之中仍撥冗來參加論文口試，對學生的研究仔細的審閱，並且給予許多精闢的建議，使這本論文更加完善。

除了老師的指導，也非常感謝實驗室所有人在這段期間的支持與幫助。感謝已經成家立業的思豪學長，畢業後仍常見面的宜貞學姊與榕均學姊，去年畢業的庭好學姊和郡誼學姊，謝謝你們不論是在實驗室時耐心的教導和討論，或是在平時生活上的建議與幫助，都讓我能更有自信和方向的去接受挑戰。謝謝向科技農夫邁進的曜禎學長，你總是有許多創新的想法和多面向的思考，與你討論後讓我對研究有許多新的啟發。感謝好好先生的棠青學長，不管是生活上的大小事或是實驗上的問題，你都很包容和耐心地跟我討論，讓人安心。謝謝電腦高手的聖壹學長，從研究期刊論文到每周的精神糧食，以及凡舉 3C 產品的問題，你都像超人一樣，沒有變不出來的或修不好的，讓大家在電子資源上不會枯竭。感謝非常熱情的千雅學姊，妳總是會提醒大家要吃飯，也都會顧及到每個人的需求，讓實驗室氣氛溫馨。謝謝熱心的佳蓉，妳都有許多食物、藥材和笑點，讓實驗室充滿歡笑。謝謝聰明的俞任，你都能給予不錯的意見，讓人能更果斷的下決定。碩一活潑的三人組-品萱、睦元和子庭，與大學部的怡儒、孟融、俊昇、苑之、佩衡、孟哲和暄維，感謝你們在生活事物和實驗上的幫忙，讓我的實驗室生活更加豐富。也謝謝戴樂仔細的向我講解投資的訊息和技巧，讓我對未來有更明確的理財方向。另外，也歡迎剛進入實驗室的一帆、琳岡、芳沅、燕秋、一華和伯任，你們的加入讓實驗室又注入了新的氣息，努力的學習一定能讓你們收穫不少。

此外，非常感謝熱心和耐心的曾信華 老師、裕國學長、輔宣學長、中書學長、士傑學長，因為你們在不論是在儀器使用上或是實驗上的大力幫助，我才能有機會做出更完整的研究。

另外，我由衷地感謝一路上走來陪伴的朋友，博翔、均泓、妙慧、卿瑜、筱雯，和交大幫的朝彥、富生、惠晴、修兆、竣璋、志源、子歡、偉賢、昌翰，因為你們的包容和照顧，我才能在枯燥的研究生生活中，嘗試一些瘋狂的舉動和獲得許多歡笑。在低潮的時候，也因為你們的鼓勵，我才能很快地再次站起來迎接挑戰。

最後，我誠摯地感謝爸爸、媽媽、哥哥、嫂嫂、小子芯和其他家人以及你，有你們默默堅定地支持鼓勵，我才能有機會專心地學習，增廣我的視野和知識並取得碩士學位。在此，僅獻上本論文和這份榮耀與你們分享！

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中華民國 100 年 7 月

# 建構以奈米金球為基礎的平台用於胜肽酶活性的檢測與藥物之裝載

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## 中文摘要

奈米金球 (gold nanoparticles; AuNPs) 具有獨特的光學特性和良好的穩定性，因此在生物醫學領域中有前瞻性的發展與應用。在本研究中，我們利用 AuNPs 的表面電漿共振 (surface plasmon resonance; SPR) 的特性，建構光學與螢光生物檢測系統，應用於檢測胜肽酶 (peptidase) 之活性。當 AuNPs 的大小改變或聚集時，其 SPR 光譜會改變，基於這個特性，AuNPs 可被發展成特定生物分子的光學檢測平台。另外，藉由 AuNPs 的表面修飾技術，AuNPs 可以應用於藥物的裝載與傳輸。

在本研究中，我們在 AuNPs 修飾上特定胜肽 (peptide) 後，peptide 除了是基質金屬蛋白酶-2 (matrix metalloproteinase-2; MMP-2) 的受質之外，也會在 AuNPs 表面提供足夠的排斥力，避免 AuNPs 在緩衝液中聚集。當加入 MMP-2 於 AuNPs/peptide 後，MMP-2 會將 AuNPs 表面上 peptide 降解，使 AuNPs 失去保護進而產生聚集現象。據此，AuNPs/peptide 聚集後和聚集前的  $A_{625\text{ nm}}$  與  $A_{530\text{ nm}}$  吸收峰比值 ( $A_{625\text{ nm}}/A_{530\text{ nm}}$ ) 可用於測定 MMP-2 活性。實驗結果顯示，所建立的 AuNPs 可見光學檢測平台用於 MMP-2 活性的檢測低限為 100 ng/mL，而當 MMP-2 活性位於 100 - 1,500 ng/mL 之間時，量測結果呈一線性相關 ( $R^2 = 0.9703$ )。

為了提升本 AuNPs/peptide 之檢測靈敏度，我們將 AuNPs/peptide 上的 peptide 置換

成 peptide-FITC，以建構一 AuNPs 為基礎的螢光檢測平台。當 peptide-FITC 修飾在 AuNPs 時，FITC 的螢光會被 AuNPs 遮蔽；然而，加入 MMP-2 於 AuNPs/peptide-FITC 後，peptide 會被 MMP-2 降解並釋放出 FITC 以顯現螢光，透過螢光強度偵測便可量化 MMP-2 的活性。此 AuNPs 螢光檢測平台用於 MMP-2 活性檢測的低限為 0.01 ng/mL，而當 MMP-2 活性位於 0.01 – 2 ng/mL 之間時，量測結果呈一線性相關 ( $R^2 = 0.9759$ )。由於我們所建立的 AuNPs 螢光檢測平台靈敏度是 AuNPs 可見光學檢測平台的 10,000 倍，因此我們已將之運用於單一細胞中 peptidase 活性的測定。

另一方面，由於 AuNPs 擁有生物相容性與表面修飾的特性，因此可以利用 AuNPs 建立多功能藥物裝載與傳輸平台。據此，我們將人類生長荷爾蒙 (human growth hormone; hGH) 與有拉曼 (Raman) 報導功能的孔雀綠 (malachite green isothiocyanate; MGITC) 修飾在 AuNPs 上，使 AuNPs 同時具有標靶和報導的功能。因此，我們以 AuNPs/hGH-MGITC 處理人類肝癌細胞 HepG2 後，可以用表面增強拉曼散射來偵測 AuNPs 被傳輸至細胞內的位置。此外，AuNPs 也透過修飾上抗癌藥物阿黴素 (doxorubicin) 與 hGH 後，具有成為癌症標靶藥物的可行性，因為利用 AuNPs/hGH-doxorubicin 來處理 HepG2，其對 HepG2 的毒殺性顯著高於單獨使用 doxorubicin 的處理結果。

**關鍵字：**奈米金球，光學檢測平台，基質金屬蛋白酶，螢光，表面修飾

# Fabrication of Gold Nanoparticles-Based Platforms for Assaying Peptidase Activity and Loading Drugs

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## Abstract

Gold nanoparticles (AuNPs) have received interests due to their characteristics, especially optical and physical properties. In this research, the optical biosensing and fluorescent platforms were developed. Both AuNPs-based biosensing platforms were set up based on the surface plasmon resonance (SPR) property of AuNPs to detect certain peptidase activity. Additionally, the surface-modified techniques of AuNPs were also utilized on the drug load and delivery.

The AuNPs-based optical biosensing system was established by means of the varied SPR spectra of AuNPs, while AuNPs changed their sizes, included aggregation or modified with functional molecules. According to the mechanism, AuNPs modified with peptide (AuNP/peptide) that was used as a peptidase (matrix metalloprotease-2; MMP-2) substrate and also a shelter to protect AuNPs from aggregation. After MMP-2 digested, AuNPs became aggregation because of decreasing the steric repulsion among AuNPs. The aggregation of AuNPs was measured via the red-shift of SPR absorption. Therefore, the MMP-2 activity could be quantitatively estimated by the absorption ratio,  $A_{625\text{ nm}}/A_{530\text{ nm}}$ . The results show that the detection limit of the established platform was 100 ng/mL, a linear correlation between MMP-2 was ranging from 100 to 1,500 ng/mL, and the changes of  $A_{625\text{ nm}}/A_{530\text{ nm}}$  was



observed ( $R^2 = 0.9703$ ).

For improving the sensitivity of AuNPs-based platform, the peptide was exchanged with peptide-FITC as substrate modified on AuNPs to establish AuNPs-based fluorescence platform. The FITC would be quenched by AuNPs when the peptide-FITC modified on AuNPs surface. The fluorescence intensity of FITC was detected after MMP-2 digested the peptide leading peptide-FITC released from AuNPs surface. According to the concept, the MMP-2 activity could be analyzed by measuring the change of fluorescence intensity (at emission of FITC, 515 nm). The AuNPs-based fluorescence platform performed a detection limit as 0.01 ng/mL, with a linear correlation ranging from 0.01 to 2 ng/mL of MMP-2 ( $R^2 = 0.9759$ ). Additionally, both AuNPs-based optical and fluorescence platforms showed the ability to assay the efficiency of MMPs inhibitors with high specificity. Especially, the AuNPs-based fluorescence platform could apply in cellular peptidase activity analysis through bio-image (confocal) that revealed a promising potential to utilize in *in vivo* peptidase detection.

On the other hand, the AuNPs-based delivery platform was fabricated due to the biocomplementary and various surface modifications of AuNPs through special molecules with their functional groups. Based on the concept, the AuNPs were conjugated with human growth hormone protein (hGH) used to target the hGH receptor of HepG2 cells, and Raman reporter (malachite green isothiocyanate; MGITC) as tags. After incubating the AuNPs-complexes with HepG2 cells, the AuNPs specifically targeted to the cells and could be traced in cells by surface-enhanced Raman scattering through Raman confocal. In addition, AuNPs were also used as drug delivery carrier by modifying AuNPs with hGH and anticancer drug, doxorubicin. Using AuNPs/hGH-doxorubicin could bind HepG2 cells precisely and inhibit the growth of cancer cells at the same time. This result indicates that the multifunctional AuNPs improved the effective of the medicine *in vitro* according to selective

targeting and treating drug to the objective in once.

**Keywords:** Gold nanoparticles, Optical biosensing platform, Matrix metalloproteinase, Fluorescence, Surface modification



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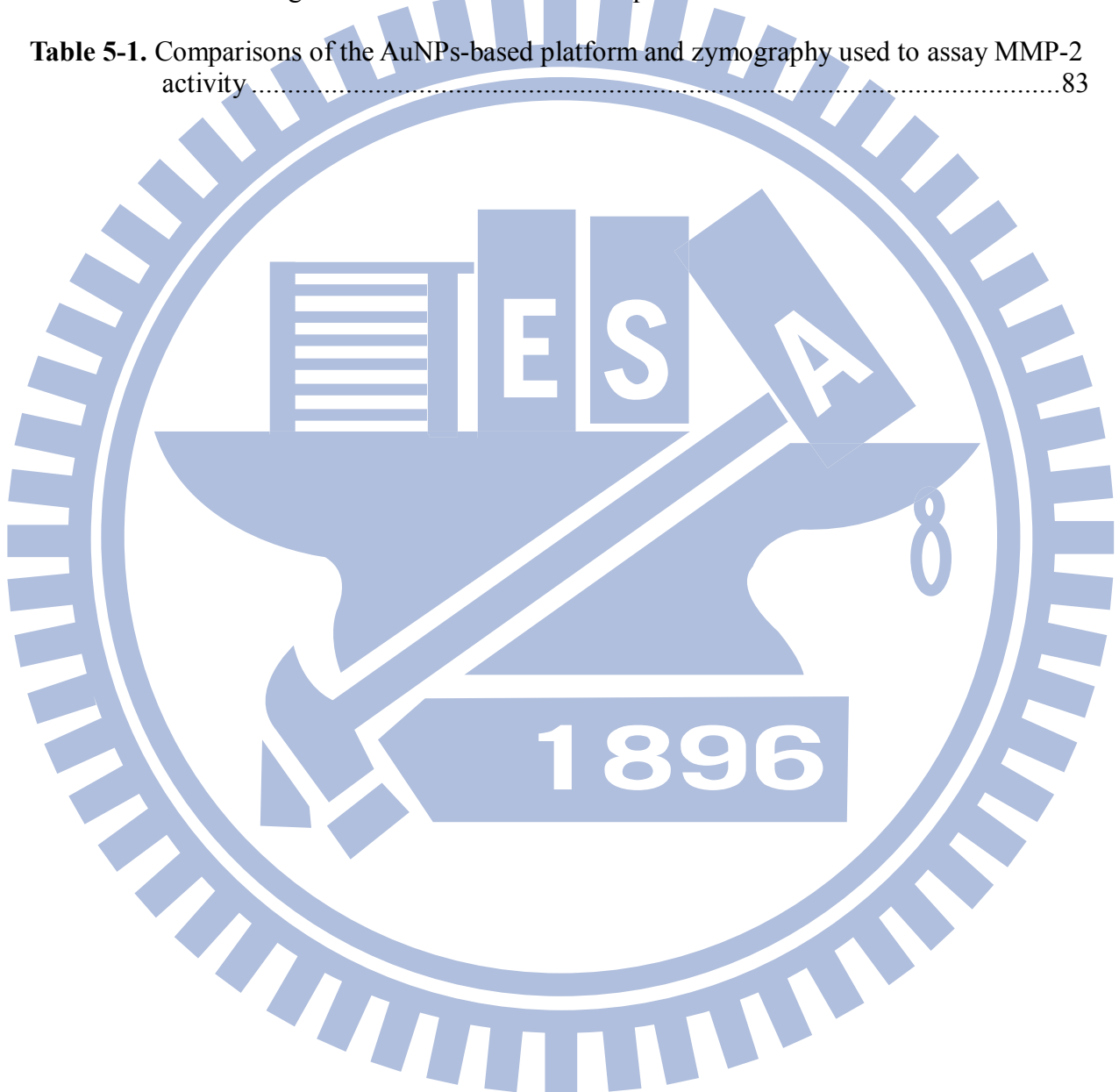
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