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贵金属纳米模拟酶在即时检测中的应用

The Applications of Noble Metal Nanoparticles as Enzyme Mimics in Point-of-Care Testing

刘 芳

指导教师姓名：周雷激 副教授

杨朝勇 教 授

专 业 名 称：分 析 化 学

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The Applications of Noble Metal Nanoparticles as Enzyme Mimics in Point-of-Care Testing

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by

Fang Liu

Supervisor: Associate professor Leiji Zhou

Professor Chaoyong Yang

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Department of Chemistry, Xiamen University

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摘 要

酶联免疫吸附反应（Enzyme-Linked Immunosorbent Assay，ELISA）由于其高特异性、规范化操作及普适性等优点在免疫诊断、食品安全、毒品检测等方面得到广泛应用。目前，基于免疫反应的临床诊断等主要依赖于光学、电学、磁学等分析方法。但是这些检测手段一般均需大型仪器和专业操作人员，操作复杂，耗时久且费用昂贵。在发病率极高的今天，有必要发展一种操作简单、省时且成本低廉的便携检测技术。即时检测（point-of-care testing，POCT）是一种顺应时代需求的检测技术，它能够现场进行检测并快速得到结果，指导医生对患者进行疾病早期诊断与治疗。

由于自然界中天然酶的生物源本性限制了酶的广泛应用，寻求新型高效稳定的纳米模拟酶作为信号放大分子对于生物传感、食品工程、制药等具有重大意义。基于此，本文以酶联免疫反应为基本反应单元，以贵金属纳米粒子模拟酶为信号放大分子，构建准确、可靠、便携、灵敏的即时检测新方法，用于生物靶标如蛋白、病毒及细胞的高灵敏定量检测。本论文的工作主要分为三个部分：

1、 基于 Pt 纳米模拟酶的即时检测方法

压力传感是日常生活中常见的信号输出方式，且市场上有商品化的便携式压力检测装置。但是据我们所知，目前几乎没有文献利用气压计进行生物分子检测。我们课题组通过巧妙设计，将分子识别信号与压力信号建立联系，发展了一种基于气压的即时检测新方法，成功进行了蛋白、病毒的检测。在此基础上，我们以 CD4 细胞为模型，以贵金属纳米模拟酶 PtNPs 为信号放大分子，结合酶联免疫反应，构建了一种操作简单、快速、灵敏的 CD4 细胞便携式即时检测新方法，实现了 CD4 细胞的高特异性检测。

2、 基于 Au@Ag/Pt 纳米模拟酶的即时检测方法

由于自然界中天然酶的生物源本性，天然酶活性受环境影响严重，且提纯工艺复杂，不易保存。近年来，研究学者一直迫切寻求新型稳定的高效催化剂。Fe_3O_4 纳米粒子固有酶活性的发现引发了人们对基于纳米粒子（特别是贵金属纳米粒子）模拟酶的研究热潮。但是传统方法中将纳米模拟酶用于免疫检测时需要对酶进行修饰，操作繁琐，严重影响酶活性且非特异性吸附较高。AuNPs 合成方法成熟，比表面积大，且易于功能化，便于生物分析应用。本文中，我们在 AuNPs

表面进行生物修饰取代不易修饰的纳米酶，进行免疫反应后再进行银铂染避免直接进行酶修饰引起的酶活性降低问题且非特异性吸附较低，利用其过氧化氢酶活性基于气压计实现蛋白、病毒等的高灵敏定量即时检测。

3、 基于 Au@Ag/Pt 纳米模拟酶的可视化定量检测方法

基于辣根过氧化物酶 HRP 的可视化免疫技术广泛应用于小分子、蛋白质和细胞等检测，但 HRP 催化活性易受环境影响、不稳定且提纯工艺复杂，成本较高，限制了其商业化应用。本文在上述工作基础上，以 Au@Ag/Pt 新型纳米粒子模拟酶代替传统天然酶 HRP，利用其过氧化物酶活性，催化底物产色，基于比色法发展一种高灵敏、高特异性的可视化定量检测新方法，满足不同检测需求。

关键词：即时检测；贵金属纳米模拟酶 ；酶联免疫反应

Abstract

Enzyme-linked immunosorbent assay (ELISA) is widely used in immunodiagnosis, food safety and drug testing because of its high specificity, the standardized operation and the universality. Currently, optical, electrochemical and magnetic signal based ELISA methods are the “gold standard” readout for bioanalysis. However, most of them suffer from the limitation of application in developing countries since they are very expensive and time-consuming, and also need bulky instruments and professional operators. Therefore, it is highly important to develop simple, time-saving and cost-effective detection methods. Point-of-care testing (POCT) technologies which are cheap、simple and equipment-free meet the demands for on-site and in-home detection and are helpful for disease prediagnosis and theapy.

Natural enzymes are easily environmental-affected and only effective under mild conditions because of their native protein property. Therefore, a lot of efforts should be made to look for stable and efficient enzyme mimetics which are very useful in many applications such as biosensor, food industry and pharmaceutical processes. Herein, we utilized these novel nanaoparticles enzyme mimics to develope new POCT techniques intergreted ELISA for the rapid and simple detection of viruses, proteins and cells .

The paper mainly includes three parts of work as follows :

(1) PtNPs enzyme mimics-based point-of-care testing

Pressure sensors are widely used in our daily life and there are portable devices available in commercial for pressure testing. However, to our best knowledge, there is few literature about using pressure meter for bioananlysis. In our previous work, a novel POCT method translating molecular recognition into pressure signal was developed for protein and virus analysis. On the basis of this work, we describe a new POCT method based PtNPs enzyme mimic intergreting ELISA for CD4 cell specific detection.

(2) Au@Ag/Pt nanoparticle enzyme mimics-based point-of-care testing

Although natural protein-based enzymes are widely used in bioanalysis, they suffer from several limiatations such as the environment-sensitive activity, expensive and time-consuming preparation process and long-term stability in room temperature. In recent years, researchers have been looking for novel stable materials that can overcome the disadvantages of natural enzymes. Fortunately, an important discovery of the intrinsic enzyme mimetic activity of Fe_3O_4 was made in 2007 by Gao and coworkers which attracted people's interest to study on the nanoparticles (especially the noble metal nanoparticles) enzyme-like activity. But in traditional methods, the nanomaterials enzyme mimics need to be modified in bioanalysis which may affect the catalytic activity of enzymes. AuNPs is one of the most widely used noble metal nanoparticles which have large surface areas and is easily to be functionalized with biomolucules. For this part, the AuNPs are used for biomolecular adsorption and then for the deposition of Ag/Pt after immunoassay to avoid the decrease of catalytic activity due to biomodification. Based on the catalase-like activity of Au@Ag/Pt nanoparticles, portable and sensitive detection of protein or virus can be realized using pressure meter.

(3) Au@Ag/Pt nanoparticle enzyme mimics-based visual quantitative detection

Horseradish peroxidase (HRP)-based visual quantitative detection methods are widely used for small molecular、protein and cell detection. However, HRP is easily denatured by environment change which restricts the wide use of HRP in commercial. Here, on the basis of the previous work, the Au@Ag/Pt nanoparticle enzyme mimic was used to replace the traditional HRP for biomolecular visual quantitative detection based on colorimetric method.

Keywords: Point-of-care testing; Noble metal nanoparticle enzyme mimics; ELISA