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厦门大学

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基于纳米催化产气和液滴微流控的便携与数字化检测新方法

Portable and Digital Analysis based on Nano-catalytic Gas Generation and Droplet Microfluidics

刘　丹

指导教师姓名：杨朝勇　教授

专　业　名　称：化学生物学

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Portable and Digital Analysis based on Nano-catalytic Gas Generation and Droplet Microfluidics

A Thesis Presented

by

Dan Liu

Supervisor: Professor Chaoyong Yang

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摘 要

体外诊断是当前医学领域发展最为活跃的部分之一，从技术层面上来讲，体外诊断正在朝着两极发展。一种是简单、快速便于普及的即时检测方向，以满足门急诊、社区保健站范围内的现场快速检测以及家庭慢性病持续监测等需求，可大大简化医疗保健中所花费的人力物力，改善病人的生活质量。鉴于生物样品存在成分复杂，靶标浓度低，时效性强等特点，在无法采用大型仪器情况下，如何建立特异、灵敏、简单、便携的检测方法依然是即时检测领域面临的主要科学问题。体外诊断的另一个重要方向是发展高度集成、自动化的仪器诊断，大大提高诊断检测的工作效率和灵敏度，实现疾病的精准诊断，其中单分子数字化检测技术将引领未来精准诊断超痕量分析领域的发展。由于单个分子靶标信号低，且受体系背景信号干扰，目前分析手段又面临研究成本高、通量低，难以对多个靶标有效分析等挑战，因此如何发展简单、高效、高通量的单分子数字化检测技术是亟待解决的关键科学问题。基于以上，本论文从体外诊断技术的现状与需求出发，围绕体外诊断发展的两个重要方向——即时检测和精准诊断，主要开展了以下两个方面的工作。

第一部分 基于纳米催化产气的便携检测新方法

首先提出了基于气压的生物检测新原理，将催化产气与免疫分子识别相结合，以气压信号作为输出信号，发展了一种基于气压传感的便携检测新装置和新方法，实现了肿瘤蛋白等靶标的超高灵敏定量检测。通过目前广泛使用的金标准方法酶联免疫方法作为识别体系，将铂纳米粒子催化剂对检测靶标分子进行标记，催化剂催化底物过氧化氢生成大量气体，实现分子信号向气压信号的转换和放大。在密闭体系中，通过气压传感器对体系气压变化进行监控检测，来实现对靶标分子的超高灵敏度检测。鉴于气压计成本低廉，检测快速，用户友好以及广泛可用的优势，基于气压检测的可视化定量分析方法有潜力发展成为公众用于广泛靶标定量的检测工具。之后进一步拓展简单、便携气压计的应用范围，发展了一种C-反应蛋白（C-reactive protein, CRP）高灵敏、便携检测新方法，利用气压计进行信号读取，实现靶标CRP的高灵敏定量分析检测。该方法对有效快速鉴别病毒、细菌感染、监控抗生素治疗、防止抗生素滥用等具有非常重要的意义。

基于免疫分子识别的体外诊断仍然是目前体外诊断的核心主力，但免疫实验

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步骤繁琐、耗时，需要专业的操作人员。我们进一步发展了一种将繁琐的免疫实验步骤、清洗步骤、距离信号输出集成到一块微流控气动芯片上的简单集成化检测方法，用于蛋白等多种靶标的高灵敏定量即时检测。利用油水互不相容的原理，将反应的水相试剂以及磁珠物理隔开，再通过磁铁拉动磁珠完成每一步反应，从而实现免疫反应的集成；利用铂纳米粒子催化底物生成大量气体，实现信号的转化与放大，气体推动有色染料前进，并最终通过读取染料移动的距离，实现靶标的高灵敏定量检测。该方法无需额外仪器和复杂操作，在即时检测领域具有潜在的应用价值。

第二部分 基于液滴微流控技术的单分子蛋白数字化检测

结合微流控技术和流体力学原理，设计了一种可用于微珠高效捕获并能对捕获微珠形成独立液滴腔体的微流控装置，发展了单分子 ELISA 数字化定量检测新方法。该方法对前列腺特异性抗原（PSA）的理论检测灵敏度可达到 10^{-16} M，大大低于传统 ELISA 的检测限。该方法具有微球捕获不依赖泊松分布、液滴形成快速、所需样品量少、避免试剂浪费、通量高、检测限低、灵敏度高、制作简单、成本低等优势，在基础研究和商业应用等领域将有广阔的前景。

关键词：即时检测；催化产气；数字化检测；液滴微流控

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Abstract

In vitro diagnostics (IVDs) technology is active and important in the medical field and is developing towards two different technical directions. One is fast, accurate and simple point-of-care (POC) testing to meet the clinical rapid detection needs and chronic disease monitoring. Due to complex biological samples, low target concentration, and strong timeliness, the development of simple, affordable, yet highly sensitive and specific POC technologies is a still great challenge in science and engineering today. The other is the development of highly integrated and automated instrumentation to achieve precise diagnosis of the disease with extremely high efficiency and sensitivity. The single-molecule counting digital detection technology enables the detection of ultra-trace levels of analytes. As low signal of single target, and background signal interference, the development of simple, efficient, high-throughput single-molecule counting digital detection technology is the key scientific issues. Pursuing point-of-care testing and precise diagnosis, the major works of the thesis are listed as following:

Part 1. Portable Analysis based on Nano-catalytic Gas Generation

We firstly demonstrate that a very familiar, yet underutilized, physical parameter—gas pressure—can serve as signal readout for highly sensitive bioanalysis. Integration of a catalyzed gas generation reaction with an immune molecular recognition component leads to significant pressure changes, which can be measured with high sensitivity using a low-cost and portable pressure meter. In particular, we have combined the decomposition of H_2O_2 catalyzed by Pt nanoparticles with an antibody-based sandwich assay for detection of various protein analytes. Introduction of the substrate initiates a rapid catalytic gas-generation reaction, leading to a significant pressure increase in the closed reaction chamber allowing a sensitive readout by using a portable pressure meter. This new signaling strategy opens up a new way for simple, portable, yet highly sensitive biomedical analysis in a variety of settings. To further expand the application of the simple and hand-held pressuremeter, a portable method for the rapid detection of the disease biomarker C-reactive protein (CRP) was

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developed. The pressure-based method with a hand-held pressuremeter could facilitate CRP measurements in POCT scenarios, which can provide valuable information for identification of bacterial infection and timely administration of antibiotics.

Enzyme-linked immunosorbent assay (ELISA) is a popular laboratory technique for detection of disease-specific protein biomarkers. However, ELISA requires labor-intensive and time-consuming procedures with skilled operators and spectroscopic instrumentation. Simplification of the procedures and miniaturization of the devices are crucial for ELISA-based POC testing in resource-limited settings. Here, we present a fully integrated, instrument-free, low-cost and portable POC platform which integrates the process of ELISA and the distance readout into a single microfluidic chip. Based on manipulations using a permanent magnet, the process is initiated by moving magnetic beads with capture antibody through different aqueous phases containing ELISA reagents, and finally converts the molecular recognition signal into a highly sensitive distance readout with a catalyzed gas generation reaction for visual quantitative bioanalysis. Without additional equipment and complicated operations, our integrated ELISA-Chip with distance readout allows ultrasensitive quantitation of disease biomarkers, which shows great potential for quantitative POCT in resource-limited settings.

Part 2. Single Protein Digital Analysis based on Droplet Microfluidics

We propose a microchannel design that accomplishes the functions of microbeads trapped efficiently and droplet generation rapidly, and apply it to single-molecule enzyme-linked immunosorbent assay (digital ELISA). The digital ELISA approach detected as few as 10^{-16} M prostate specific antigen (PSA) much lower than conventional ELISA. Beads are encapsulated in generated droplets to achieve one-bead-to-one-trap in the array, which do not rely on Poisson distribution. Our microfluidic chip design with low reagent consumption, low cost, and high-throughput is an ideal device for single-molecule counting digital detection applications.

Key Words: Point of Care Testing; Catalytic Gas Generation; Digital Detection; Droplet Microfluidics.

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