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3.07.5.1 Disposable Imniunosensing 9 h 48 Microscope glass slides are widely used for the fabrication of disposable optical	3.07.5.1 Cảm biến miễn dịch dùng một lần Các tấm vi kính (tấm kính mang vật đặt dưới kính hiển vi) được sử dụng rộng rãi để chuẩn bị

immunosensors.	(điều chế) các cảm biến miễn dịch quang học dùng một lần.
Velev et al.61 proposed a simple and low-cost NP-labeled immunoassay for antigen detection. The disposable immunosensor was prepared on a microscope glass slide, which was activated with aldehyde-terminated silanes using standard coupling chemistry. The silane ends of the molecules attach to the glass substrate leaving the aldehyde groups available to react with amine groups on the proteins.	Velev và các cộng sự 61 đưa ra phương pháp dùng xét nghiệm miễn dịch gắn NP giá thành thấp và đơn giản để phát hiện kháng nguyên. Cảm biến miễn dịch dùng một lần được chế tạo trên một tấm vi kính, cảm biến này được kích hoạt bằng aldehyde-terminated silanes (silanes có aldehyde ở cuối mạch) bằng phương pháp gắn kết hóa học tiêu chuẩn. Các đầu silane của các phân tử gắn vào đế thủy tinh tạo điều kiện để các nhóm aldehyde hiện có phản ứng với các nhóm amine trên các protein.
Primary antibodies are irreversibly attached to the aldehydes on the substrate to bind the target antigens in solution. An optical signal was measured by secondary labeling of antigens with gold NPs and their enhancement by silver nucleation. The opacity of the silver-enhanced spots was quantified by densitometry. The selectivity of the sandwich immunoassays was adequately high, and total antigen concen- trations as low as 4 ng were detected reproducibly.	Các kháng thể chính được gắn cố định với aldehydes trên đế để liên kết các kháng nguyên mục tiêu trong dung dịch. Tín hiệu quang học được ghi nhận bằng cách tiếp tục dán nhãn các kháng nguyên bằng các hạt nao vàng và tăng cường bằng cách tạo mầm bạc. Độ mờ đục của các điểm tăng cường bằng bạc được đánh giá định lượng thông qua
The immunotesting strip, combining with colorimetric or electrochemical measurements, is another well-known disposable immunoassay system. Gandhi et al.62 proposed a strip-based immunochromatographic assay for rapid detection of morphine in urine samples. In this assay,	

specific egg yolk antibodies (IgY) were used, and the antibody was labeled with Au NPs to act as an immunoprobe in the dipstick format for the visual detection of morphine. The dipstick was developed using three membranes: an application pad made of glass fiber membrane to hold the tracer, a signal generation test line on a nitrocellulose membrane (detection zone), and a cellulose membrane used as an absorption pad. Analytes added to the sample well dissolved the labeled antibody (tracer), and the antigen-antibody complex formed was transported by the flow caused by capillary action to the test line. The color signal of the test line was in proportion to the morphine concentration in urine samples. Based on QDs and a lateral flow test strip (LFTS), Lin's group63 recently fabricated a portable fluorescence immunosensor for rapid and sensitive detection of a protein biomarker, nitrated ceruloplasmin. The superior signal brightness and high photostability of QDs combined with the promising advantages of an LFTS resulted in high sensitivity and selectivity and speed for protein detection. As shown in Figure 8, the QD-based fluorescence LFTS was composed of a sample application pad, a conjugation pad, a nitrocellulose membrane, an absorption pad, and a backing card. Under a sandwich immunoassay, QDs were bound

to the surface of the testing line by the formation of an immunocomplex. Quantitative detection of nitrated ceruloplasmin was realized by recording the fluorescence intensity of QDs captured on the test line. Under optimal conditions, this portable fluorescence biosensor displayed rapid responses for nitrated ceruloplasmin with a concentration as low as 1 ng ml_1. Although the immunostrip devices have the advantage of low cost, robust nature, and ease of use, they suffer from the drawback of a general lack of sensitivity and, at best, they are semi-quantitative.

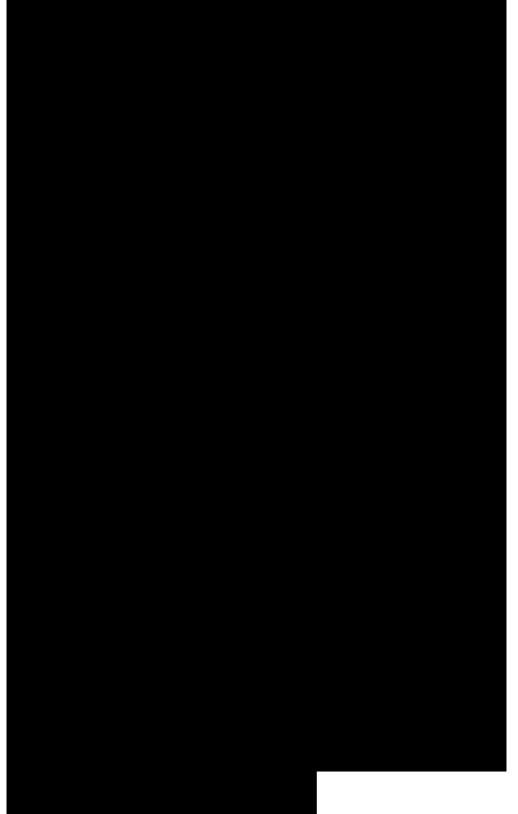
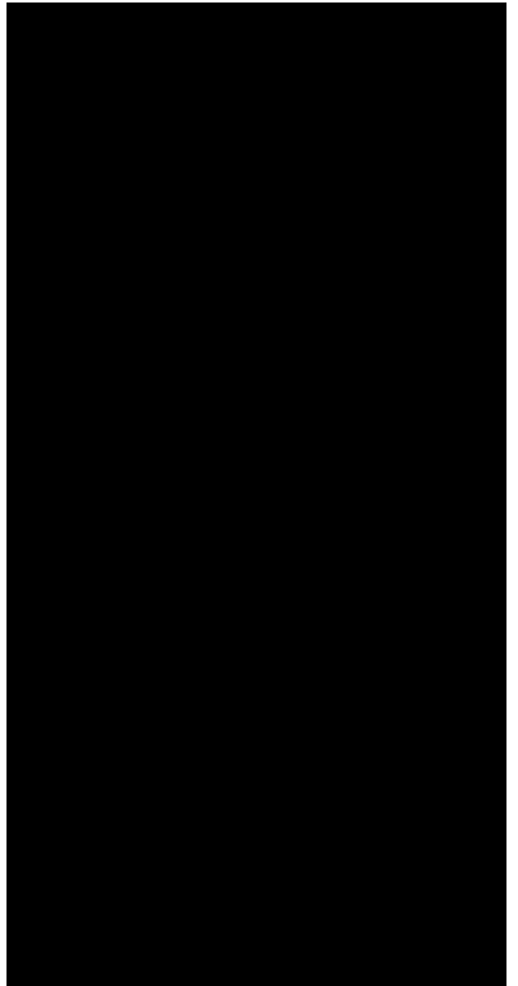


Figure 8 (a) Schematic illustration of the teststrip and (b1—b4) the detection of nitrated ceruloplasmin using fluorescent QD-based LFTS. (b1) Aqueous sample containing nitrated ceruloplasmin is applied to the sample pad. (b2) Nitrated ceruloplasmin combines with QD-antinitrotyrosine conjugate and migrates along the porous membrane by capillary action. (b3) Nitrated ceruloplasmin is captured by anticeruloplasmin antibodies immobilized on thetest line. The excess QD conjugates continue to migrate toward the absorption pad. (b4) Fluorescence signal of QD is detected using a test strip reader (solid line). Asacontrol, ceruloplasmin without nitration cannot be recognized by QD—antinitrotyrosine conjugates, so



nofluorescence signal can be seen on the test strip (dotted line). Reprinted from Li, z. H.; Wang, Y.; Wang, J.; Tang, Z.; Gounds, J. G.; Lin, Y.Anal. Chem. 2010, 82, 7008; with permission © 2010, American Chemical Society.

Screen-printing technology has attracted increasing interest in the past few years. This technology allows the mass production of reproducible yet inexpensive and mechanically robust strip solid electrodes. Immunosensors based on screen-printed electrodes challenge conventional electrochemical immunosensors for disposability and portability. Guan et al.64 introduced an AFP immunosensor using Prussian Blue deposited on a screen-printed carbon electrode to catalyze the electrochemical reduction of H2O2 produced from the enzymatic reaction of glucose oxidase (GOD). Based on a one-step sandwich ELISA, a detection range of 5-500 ng ml_1 for AFP was sufficient to measure clinically relevant AFP levels (>10 ng l_1). When real serum sample testing was carried out using both this method and a typical ELISA, they showed similar results. Yu et al.65 proposed another strategy for preparing a disposable amperometric immunosensor for AFP based on an enzyme-labeled antibody/CHIT membrane-modified screen-printed carbon electrode. The immunosensor was

prepared by entrapping HRP-labeled AFP antibody in a CHIT membrane to modify the screen-printed carbon electrode.

An important feature of screen-printing technology is related to the automation or miniaturization of the corresponding devices along with their ease of handling and manipulation in a disposable manner. Based on CEA/colloid Au/CHIT membrane-modified screen-printed carbon electrode, Wu et al.66 developed a disposable CEA immunosensor coupled with a flow-injection system. The immunosensor was inserted in a flow system with an injection of sample and HRP-labeled CEA antibody. The CEA immobilized on the immunosensor trapped the labeled antibody to produce detectable current signal upon injection of substrates.

Since the system was capable of continuously carrying out all steps, including incubation, washing, enzymatic reaction and determination, this method had the advantages of miniaturization, portability, and programmable operation without the need of skilled operators, so it appears to have commercial potential. Integrating the screen-printing fabrication with the immunochromatographic strip tech- nique, Lin's group67 designed a disposable electrochemical immunosensor diagnostic device for the

detection of IgG and the cancer biomarker, PSA. In this assay, a CdS@ZnS QD was exploited as labels for ampliíying signal output. The device takes advantage of the speed and low cost of the conventional immunochromatographic strip test and the high sensitivity of the NP-based electro- chemical immunoassay. A sandwich immunoreaction was performed on the immunochromatographic strip, and the captured QD labels in the test zone were determined by highly sensitive stripping voltammetric measurement of the dissolved metallic component (cadmium) with a disposable screen-printed electrode, which is embedded underneath the membrane on the test zone. Such a disposable device offered an LOD of 30 pg mp1 for IgG in association with a 7-min immunoreaction time, and 20 pg mp1 for PSA in a human serum sample. This device coupled with a portable electrochemical analyzer provided a new platform for in-field and point-of-care quantitative testing of disease-related protein biomarkers.