DEVELOPMENT OF SIGNAL AMPLIFIED IMMUNOASSAY FOR ADVANCED VIRUS DETECTION

(Doctoral qualification by coursework, Form 7)

Abstract of Dissertation

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Abstract：

Currently, the diagnostic of disease agents is mainly relying on the RT-PCR as the gold standard and rapid test with lateral flow immunoassay and Enzyme-linked Immunosorbent Assay (ELISA) technologies as the frontline surveillance. One suffers from a long detection time, high cost, and highly specialized handling and equipment requirements. The latter is vulnerable to false-negative response due to the concentration of biomarkers usually ultralow in the early stages of diseases that limited the application. It is urgent to overcome the drawback of the insensitive immunoassay in ELISA technologies. Answering the challenge of advanced immunoassay, the rapid development of nanotechnology over the past decade has allowed us to amplify signal transduction using a new class of applied nanomaterial, such as **(1) nanomaterial-based artificial enzymes (nanozymes) and (2) nanomaterial-based carrier (nanocarrier).**

To achieve higher immunoassay sensitivity, nanomaterials that exhibit enzyme-like activity can substitute HRP to improve immunoassay performance. This nanomaterial has been known as **nanozyme.** However, it reduces its activity significantly after functionalization with protein due to the blockage of active sites on the surface. I designed a highly active peroxidase-like activity nanozyme from embedded platinum nanoparticles (PtNPs)-activated Co_3O_4 nanocages to overcome this issue. **The hollow nanostructure of the Co_3O_4 nanocages (NCs) was anchoring the platinum nanoparticles (PtNPs)** enclosed by the exposed oxides framework and formed PtNPs@Co_3O_4 NCs. The embodiment of PtNPs was considered an ideal hybrid nanozyme that efficiently catalyzed the oxidation of the substrate molecules with enhanced activity. Based on their catalytic activity, these nanocages have been demonstrated to enable highly selective signals in the capture-detect immunoassay format, with detection limit of the prepared immunoassay achieving 33.52 viral norovirus (NoV) copies/mL, which is 321-folds lower magnitude of the commercial ELISA.

In the existing biosensor, the signal generation is limited to single virus detection in the reaction chamber. By associating the multiple responses in a single readout, it translates **the recognition into multiple viruses** at a single time. The strategy centralized on the **enrichment of signal molecules in a single nanovesicle** with conjugated biorecognition to achieve this purpose. We investigated two strategies in developing the nanocarrier to deliver the signal molecules in the biosensor: **(1) polymeric nanovesicle with preceding nanoprecipitation of the signal molecules**

as a double enrichment approach, **(2) solvent-dependent co-precipitation of signal molecules and polymer-forming nano-cargo encapsulation**, **(3) embodiment of co-catalyst in nanocomposites** and **(4) 3D-nanoassembly of sandwich aptamer as redox species chelating interface in an electrochemical system.**

Inspired by the self-assembly approach in the study, the chromogen was successfully co-precipitated in an aqueous solution to **form collective nanoparticles (NPs)**. Utilizing poly(lactide-*co*-glycolide) (PLGA) in the molecular delivery approach and functional bioconjugation, the formed **emulsion nanovesicle (chromogen@PLGA or dye@PLGA)** exhibits an enrichment of the collective signal molecules in a single antibody-antigen conjugation. To release the cargo, the addition of external stimuli, either solvent solubility or physical factor of the environment, can dissolve the polymeric membrane, releasing the encapsulated chromogens and sequentially generating the signal for the immunoassay reason. The **developed immunoassay using TMB-NPs@PLGA** demonstrates high sensitivity for influenza virus A (IV/A) with a limit of detection (LOD) as low as **32.37 fg mL^{-1} and 54.97 fg mL^{-1}** in buffer and serum, respectively. Furthermore, another strategy using **pH-dependent dye@PLGA-based immunoassay** shows a feasible one-pot and single detection on two subtypes IV/A with excellent visual colorimetric specificities of **PP@PLGAs on IV/A/H1N1 and TP@PLGAs on IV/A/H3N2** with LODs of **27.56 and 28.38 fg mL^{-1}**, respectively.

To bring that concept of signal enrichment to the more analytically sensitive platform, here **is an electrochemical interface**; the system was adopted for post-cargo loading instead of the pre-loading approach. The study utilized two different **aptamers**: One conjugated to gold nanoparticles (AuNPs), forming AuNPs-Apt$_1$, and its complementary sequence aptamer, forming AuNPs-Apt$_2$. **The unbound Apt$_1$ of AuNPs-Apt$_1$ by DENV-NS1 hybridized to AuNPs-Apt$_2$ and induced a 3D-nanoassembled formation that intercalated a high number of MB molecules within the duplex structure of aptamers**. The complex was captured on the Apt$_2$-conjugated **disposable gold electrode (DGE).** The developed aptamer-based biosensor showed high sensitivity with colorimetric response down to 30 fg/mL of dengue virus nonstructural protein NS1 (DENV-NS1) with good selectivity.