Supporting Information

An ultrasensitive electrochemical immune platform with double signal amplification for assay of indole-3-acetic acid in plant seeds

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Detection strategy

As a way to circumvent these limitations, a non-sandwich electrochemical immunosensor was developed for the selective detection of IAA based on 4-aminophenylboronic acid (4-APBA), horseradish peroxidase conjugated goat anti-rabbit immunoglobulin G functionalized magnetic nanopartilces (HRP-IgG-Fe_3O_4) and rat monoclonal antibody against IAA immuned gold nanoparticles (Anti-IAA-AuNPs). A schematic representation of the fabrication of the immunosensor with five steps was shown in Scheme 1. (1) Gold nanoparticles (AuNPs) were electrodeposited on the glassy carbon electrode (GCE) and then 11-mercaptoundecanoic acid (MUA) was self-assembled on the modified electrode surface via Au-S bond. Then, after the carboxyl in MUA was activated by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxylsuccinimide (NHS), 4-APBA was further covalently assembled on the electrode surface between the reaction of -NH_2 and the activated -COOH. In this step, MUA was not only used to immobilize 4-APBA, but also to provide a stretched spacer for 4-APBA. In addition, coverage of the electrode surface with alkyl acid self-assembled monolayers has made it possible to drastically reduce nonspecific adsorption of proteins.[1, 2] (2) The obtained modified electrode was further incubated with HRP-IgG-Fe_3O_4 to generate the HRP-IgG-Fe_3O_4 modified electrode using the property of 4-APBA that it could react with sugar and glycoprotein (through their carbohydrate moiety) to form boronate complexes.[3, 4] More importantly, the immobilization of glycoprotein using this strategy do not affect the bioactivity of

glycoprotein because the carbohydrate region is generally located in areas that are not involved in glycoprotein activity.[5] In addition, magnetic Fe_3O_4 nanopartilces has good biocompatibility and can be rapidly separated from the substrate solution, which can facilitate the synthesis of HRP-IgG-Fe_3O_4 and maintain the bioactivity of HRP-IgG.[6-9] (3) Through the immunoreaction between goat anti-rat IgG and rat monoclonal antibody against IAA, Anti-IAA-AuNPs were immobilized on the electrode surface. (4) Finally, IAA was captured on the electrode surface by the specific immunoreaction between anti-IAA antibody and IAA. The decrease of the reduction peak current of $Fe(CN)_6^{3-}$ was used to monitor the innmuoreaction. In addition, for testifying the applicability of the fabricated immunosensor, IAA extracted from different plant seeds was detected.

Synthesis of carboxyl functionalized magnetic Fe_3O_4 nanoparticles

Carboxyl functionalized Fe_3O_4 nanoparticles were synthesized by the chemical co-precipitation using oleic acid as the source of carboxyl group according to previous report with some modifications.[10] In brief, 4.3 g $FeCl_2$•$6H_2O$ and 11.6 g $FeCl_3$•$6H_2O$ was dissolved in 20 and 340 mL double distilled deionized water, respectively. After the solution of $FeCl_3$•$6H_2O$ was heated to 70 °C with vigorous stirring, the 20 mL of $FeCl_2$•$6H_2O$ solution was added. Subsequently, 20 ml of 25 wt% NH_4OH was added rapidly into the solution. The resulting suspension was vigorously stirred for 5 min. Following that, 4 mL oleic acid was added into the suspension. The reaction was maintained at 70 °C for 1 h with vigorously stirring. The upper solution was colorless and the tar-like black magnetic gel precipitated and was isolated by the magnet. After

the magnetic gel was washed several times by ethanol to remove the excess oleic acid, they were further washed repeatedly by deionized water until the pH of dispersion solution was 7.0. After separated by magent, the precipitation was re-dispersed in 160 mL of 10 mg/mL $KMnO_4$ solution to oxidize the oleic acid, which was modified on Fe_3O_4 surface. The oxidation process was performed for 8 h with the help of ultrasonication. After separated by magent, the precipitation washed repeatedly by deionized water until the pH of dispersion solution was 7.0. Finally, these particles were dried in vacuum drying chamber.

Photo of Fe_3O_4 dispersion solution

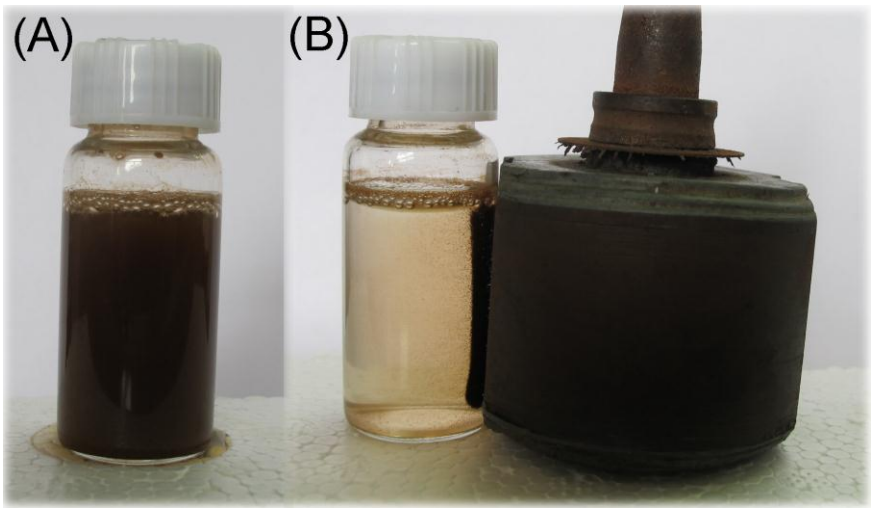

Figure S1. (A) The photograph of carboxyl functionalized Fe_3O_4 nanoparticles dispersed in water. (B) The responsiveness of carboxyl functionalized Fe_3O_4 nanoparticles in an external magnetic field.

As shown in Figure S1A, the carboxyl functionalized Fe_3O_4 nanoparticles could be easily dispersed into the water by ultrasonic treatment. Also, as shown in Figure S1B, Fe_3O_4 nanoparticles can be conveniently removed from the suspension system by magnetic field application.

TEM images

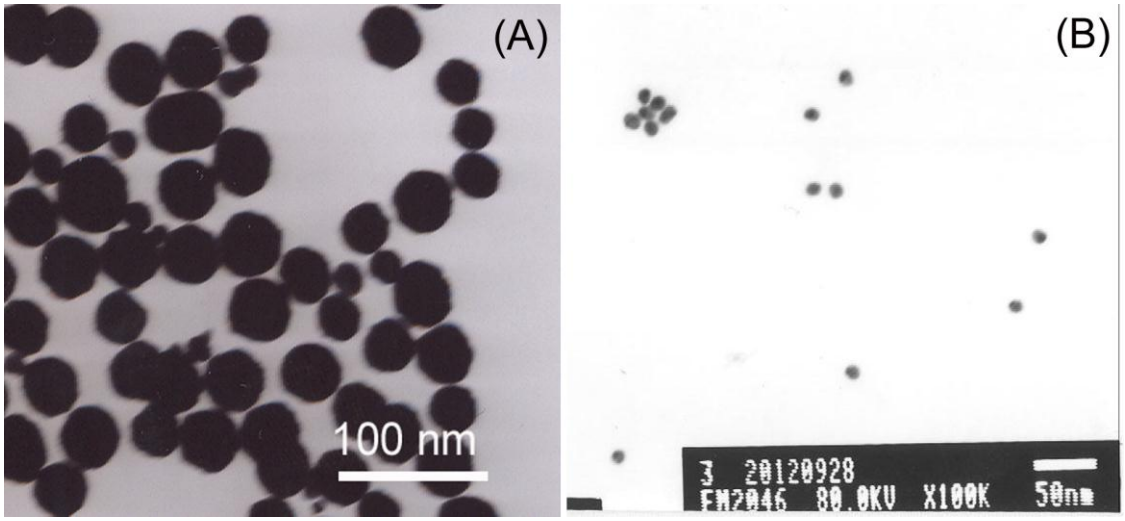

Figure S2. The TEM images of carboxyl functionalized Fe_3O_4 nanoparticles (A) and AuNPs (B).

References

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