A supersandwich electrochemiluminescence immunosensor based on mimic-intramolecular interaction for sensitive detection of proteins

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UV−vis absorption spectra of different nanomaterials

UV-visible absorption spectrum was used to evaluate the surface functionalization by PDA and AuNPs (Fig. S1). The absorbance of MWCNTs aqueous solutions showed a broad absorption band appeared at 250 nm in accordance with the results reported in other studies.1 The absorption spectrum of MWCNTs@PDA at 282 nm decreased significantly compared with DA, which could prove the spontaneous oxidative polymerizing of DA on MWCNTs. After treated by HAuCl₄, there was a new absorption peak appeared at 522 nm, which was attributed to the presence of AuNPs.

The effect of concentration ratio of Ab₂-PAMAM-A₁ to the ECL signal

We did an optimization to gain the optimal concentration proportion of Ab₂ and A₁ labeled on PAMAM and the optimal proportion was 1:7. Owing to the multiple functional sites available for reaction on its surface, the carboxyl-terminated PAMAM

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dendrimer could facilitate high-density immobilization of A_1 with a relatively low content of Ab_2 by controlling the concentration proportion of A_1 and Ab_2. When a relatively low content of Ab_2 labeled on PAMAM, more A_1 were immobilized on PAMAM, which could initiate a continuous hybridization reactions and result in stronger ECL intensity. As shown in Fig. S2, the ECL intensity of the proposed immunosensor increased with the increasing concentration proportion of Ab_2 and A_1 on PAMAM with the maximum response at the proportion of 1:7, which was selected as an optimal condition for the preparation of Ab_2-PAMAM-A_1. When the proportion become higher than 1:7, the ECL intensity began to decrease which may be attributed to the fact that low bind ability of the Ab_2-PAMAM-A_1 at the low amount of Ab_2 in the sandwich-type immunoreactions.

**Optimization of detection conditions**

We had considered the possible experimental variables that mediated the ECL inhibition effect, including solution pH, immunoreaction time as well as DNA hybridization reaction time. In the double antibody sandwich-type immunoassays, temperature and time for the antigen-antibody interaction greatly influenced the sensitivity of the developed immunoassay. Considering the practical application of the proposed immunosensor, all experiments were carried out at room temperature. Following that, the study of pH influence on the ECL detection was conducted in the range of 5.5-10. As shown in the supplemental information (Fig. S3 A), the ECL response gradually increased from pH 5.5 to 7.4 and then changed steadily during 7.4 to 9.0, subsequently the ECL response rapidly decreased at pH values higher than 9.0.
Comprehensive consideration, the optimum pH was chosen as 7.4. The reason may be that the highly acidic or alkaline surroundings would damage the immobilized protein and not favorable to the ECL reaction. The immunoreaction time was investigated between 5 and 40 min. As shown in the supplemental information (Fig. S3 B), the ECL response increased with the increment of incubation time, and tended to level off after 20 min. Longer incubation time had no significantly effect on the ECL response. Therefore, the optimum interaction time was considered as 20 min.

The comparison of different Ab2 bioconjugates

In order to explore the effect of PAMAM dendrimer, histidine and supersandwich structure on signal amplification, we designed some simple comparative studies. As shown in Fig. S4 A, the ECL response obtained from Ab2-PAMAM-A1-Ru-A2-His-A1 (curve a) was much higher than that of Ab2-PAMAM-A1-Ru-A2-A1 (curve b), which indicated the obvious amplification efficiency of histidine. From Fig. S4 B and C, it was also clear that the ECL response of curve a and curve b was much higher than that of Ab2-GA-A1-Ru-A2-His-A1 (curve c) and Ab2-GA-A1-Ru-A2-A1 (curve d), respectively. These were due to that PAMAM dendrimer could not only immobilize many antibodies and probe DNA, but also amplify the ECL signal. As can be seen from Fig. S4 D, the supersandwich DNA structure (curve d) achieved a significantly higher gain than traditional sandwich DNA structure (curve e) in signal amplification. Therefore, we choose Ab2-PAMAM-A1-Ru-A2-His-A1 as the Ab2 bioconjugate for signal amplification in the whole experiments. (In Ab2-GA-A1, glutaraldehyde (GA) was served as crosslinking agent to link Ab2 with A1 by the
Characteristics of the ECL immunosensor

To gain a better understanding of the fabrication process of the ECL immunosensor, we performed the electrochemical impedance spectroscopy (EIS) at different modified electrodes in 5 mM [Fe(CN)₆]³⁻/⁴⁻. The impedance spectrum included a semicircular portion and a linear part. The increase of the semicircle diameter at higher frequencies reflected the increase in the interfacial charge transfer resistance. The bare GCE exhibited a small semi-circle in the high frequency section (Fig. S5, curve a), indicating a very fast electron-transfer process of [Fe(CN)₆]³⁻/⁴⁻. When MWCNTs@PDA-AuNPs was coated on the electrode, the $R_{et}$ increased a little (curve b) due to the inhibition effect of PDA for electron transfer. The $R_{et}$ value increased after Ab₁ (curve c), BSA (curve d), PSA (curve e) and supersandwich Ab₂ bioconjugates (curve f) were successively adsorbed onto the electrode surface, which suggested that big molecular protein blocked the electron transport and hindered the diffusion of ferricyanide toward the electrode surface.

Supporting Figures
**Fig. S1** UV-vis absorption spectra of Carboxylic MWCNTs, DA, MWCNTs@PDA and MWCNTs@PDA-AuNPs

![Graph showing absorption spectra](image)

**Fig. S2** The effect of concentration ratio to the ECL signal between Ab₂ and A₁ in the preparation of Ab₂-PAMAM-A₁.

![Graph showing ECL intensity vs. concentration ratio](image)

**Fig. S3** Effects of (A) pH of buffer solution (B) incubation time with PSA antigen on the ECL intensity.

![Graph showing ECL intensity vs. pH](image)

![Graph showing ECL intensity vs. incubation time](image)
Fig. S4 The comparison of different Ab₂ bioconjugates: (a) Ab₂-PAMAM-A₁-Ru-A₂-His-A₁, (b) Ab₂-PAMAM-A₁-Ru-A₂-A₁, (c) Ab₂-GA-A₁-Ru-A₂-His-A₁, (d) Ab₂-GA-A₁-Ru-A₂-A₁, (e) Ab₂-GA-A₁-Ru-A₂. All working buffer was PBS (0.1 M, pH 7.4).

Fig. S5 Nyquist diagrams for (a) the bare GCE; (b) GCE/MWCNTs@PDA-AuNPs; (c) GCE/MWCNTs@PDA-AuNPs/Ab₁; (d) GCE/MWCNTs@PDA-AuNPs/Ab₁/PSA; (e) GCE/MWCNTs@PDA-AuNPs/Ab₁/BSA/PSA; (f) GCE/MWCNTs@PDA-AuNPs
/Ab1/BSA/PSA/PSA/Ab2-PAMAM-A1-Ru-A2-His-A1 in 0.1 M KCl solution containing 5 mM (1 : 1) [Fe(CN)6]3−/4− with the range from 100 kHz to 10 MHz and an alternate voltage of 5 mV.

References