Supporting information

Electrochemiluminescence Enhancement and Particle Structure Stabilization of Polymer Nanoparticle by Doping Anionic Polyelectrolyte and Cationic Polymer Containing Tertiary Amine Groups and Its Highly Sensitive Immunoanalysis

Noor Ul Ain †, Tian-Yu Wang †, Xiao-Ning Wu, Tong-Hong Wei, Jing-Shuo Zhang * and Hong-Ping Xie *

College of Pharmaceutical Sciences, Soochow University, Suzhou 215123, China; noorulain22@yahoo.com (N.U.A.); 20195226022@stu.suda.edu.cn (T.-Y.W.); xiaoning_wu123@163.com (X.-N.W.); piers.wei@abbott.com (T.-H.W.)

* Correspondence: zhangjingshuo@suda.edu.cn (J.-S.Z.); hpxie@suda.edu.cn (H.-P.X.)

† Who had the equal contribution to this paper.

S1. Materials and Chemical Reagent

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC•HCl, 99%) and 1-hydroxypyrrolidine-2,5-dione (Sulfo-NHS, > 98%) were purchased from Sigma-Aldrich. Tripropylamine (TPA, ≥ 98%) was purchased from Sinopharm Chemical Reagent Co., Ltd. Dialysis bag (MW = 3.5 KD) was purchased from the Sangon Biotech (Shanghai) Co., Ltd.

S2. Synthesis of ECL Polymer

Firstly, according to the method in Reference [S1], the ECL complex bis(2,2-bipyridine)-(5,6-epoxy-5,6-dihydro-[1,10] phenanthroline) ruthenium (epo-Ru, Fig.S1) was synthesized. After that, based on the chemical connection between the epoxy group and the carboxyl group, the PAA (Mw = 100 KD) was used as the carrier, the epo-Ru grafted PAA (PAA-Ru) was synthesized in the DMF solvent as described below. 6 mL DMF was added in a 10 mL single neck round-bottom flask, and 10.16 mg epo-Ru and 20.90 mg PAA were added respectively. Shaking to dissolve, and kept in nitrogen environment, and oil bath at 110 ºC with magnetic stirring 24 h in the dark conditions. After cooling to room temperature, a dialysis bag with a cut-off molecular weight of 3.5 KD and dialysate DMF were used to dialyze the synthesized PAA-Ru solution for 48 h, changing dialysate every other 12 h. Ultimately, the dialysis solution of PAA-Ru was fixed to 12 mL and stored for next use.

S3. ECL Detection of the Doped Polymer Nanoparticle

200 μL dPNP solution was added to 800 μL H_2O and 1 mL TPA solution (0.05 mM TPA in PBS buffer of 0.3 mol L^{-1} and pH 6.8). After mixed evenly, took 1.6 mL of the mixed solution into ECL detection cell. The corresponding detection conditions were: initial potential 0.2 v, sampling rate 10 T s^{-1}, sampling amplification series 3, and photomultiplier voltage 600 V, scanning rate 0.1 v s^{-1}, high potential 1.25 v, low potential 0.2 v, scanning interval 200, scanning sampling interval 0.001, sensitivity 1×10^{-4}.

S4. Carboxyl Activation of the Nanoparticle dPNP

1 mL (45 mg mL^{-1}) EDC solution and 1.5 mL (75 mg mL^{-1}) NHS solution were added to 3 mL dPNP solution separately, incubating at 37 ºC for 30 min. After that, a dialysis bag with cut-off molecular weight 3.5 KD and dialysate of deionized water were used to dialysis the above-mentioned nanoparticle solution. The dialysate was changed every 6 h, then the dialysis bag was dehydrated and concentrated on the dry

silica gel. 0.1 M NaOH solution was used to regulate pH to 7.4. The total volume was controlled to 4.6 mL, and then the carboxyl activated dPNP solution was obtained.

S5. Optimization of Labeled Antibody Amount

Based on immune-reaction between the detector antibody gAb and the second antibody gIgG, the detector antibody was indirectly labelled. In order to achieve good labeling of gAb, two key points needed to be concerned: the immobilized amount of gIgG on dPNP and the immunoreactivity of the immobilized gIgG. In this paper, the magnetic bead-conjugated goat anti-rabbit IgG (rIgG-MB) was used as a tool to form the immune complex dPNP-gIgG···rIgG-MB by the immune reaction of goat anti-rabbit rIgG and rabbit anti-goat gIgG. After separating the immune complex dPNP-gIgG···rIgG-MB and the unreacted rIgG-MB in the reaction solution via magnetic separation,_we may easily obtain the supernatant including the unreacted components dPNP, gIgG and dPNP-gIgG. So the optimization of the immobilized amount of gIgG and the investigation on the immunoreactivity of gIgG immobilized on the dPNP can be realized by determining characteristic fluorescence of dPNP in the supernatant.

Since dPNP-gIgG has been blocked by BSA, here gIgG became the only site to take immunoreaction with other antibodies on the nanoparticle. When the rIgG-MB was added, the immunocomplex dPNP-gIgG···rIgG-MB formed. Different concentrations of gIgG were added into the equal amount of dPNP and rIgG-MB, and the fluorescence intensity of the supernatant was linearly correlated with the amount of the formed dPNP-gIgG···rIgG-MB. At the excitation wavelength of 450 nm, the fluorescence intensity at 601 nm of the characteristic emission peak of the dPNP was used. As the added amount of gIgG increased, the number of the formed dPNP-gIgG increased, and the further immune complex dPNP-gIgG···rIgG-MB has also increased, while the remaining and the unlabeled dPNP decreased. After magnetic separation, the fluorescence intensity of the supernatant also decreased (Fig.S4). The above fact "fluorescence decrease" also demonstrated that the gIgG immobilized on the dPNP had still a good immune-reaction activity, which provided feasibility for further immune-reaction with the detecting antibody gAg. We could still find in Fig.S4 that the fluorescence intensity of the supernatant decreased to the minimum when the gIgG increased up to 50 μg mL^{-1}. But when gIgG increased continuously and reached 70 μg mL^{-1}, it increased instead of continuous decreasing. So 50 μg mL^{-1} of gIgG was an optimal immobilized amount of gIgG on the nanoparticle dPNP. The reason should be from competitive immune-reaction of rIgG-MB with the immobilized gIgG (dPNP-gIgG) and the excessive free gIgG.

S6. Supporting Figures and Table

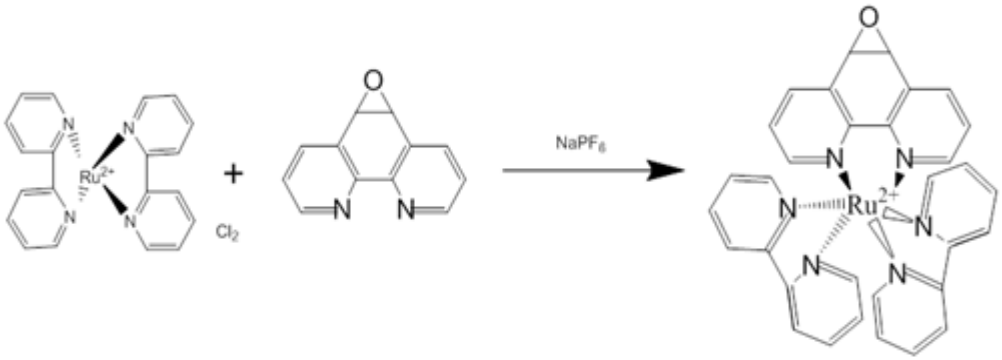

Figure S1. Synthesizing diagram and structure of $Ru^{2+}(bpy)_2$(phenepoxide).

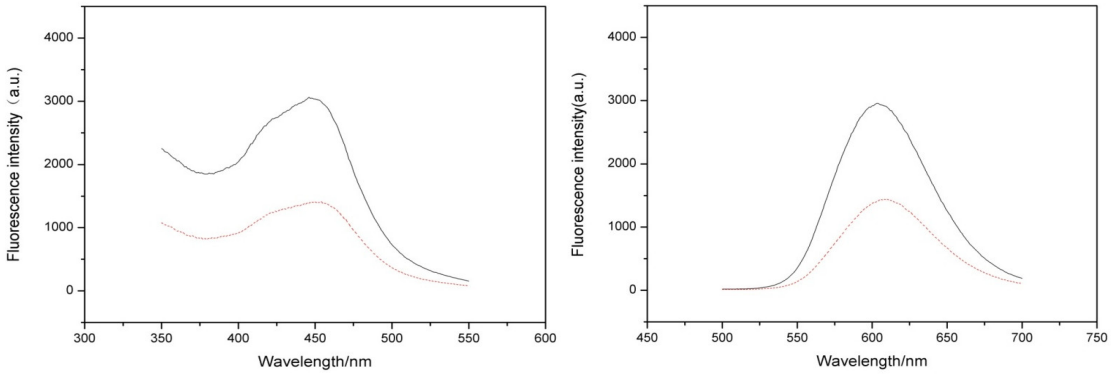

Figure S2. Fluorescent excitation (Upper) and emission (Lower) spectra of epo-Ru (solid line) and PAA-Ru (dash line).

Table S1. Recovery of HBsAg determined by the built ECL immunoassay (n = 3).

Added (pg mL^{-1})	Mean of ECL intensity (a.u.)	RSD	Found (pg mL^{-1})	Recovery (%)
75	99.325	0.167	70.527	94.04
25	71.825	0.058	23.647	94.59

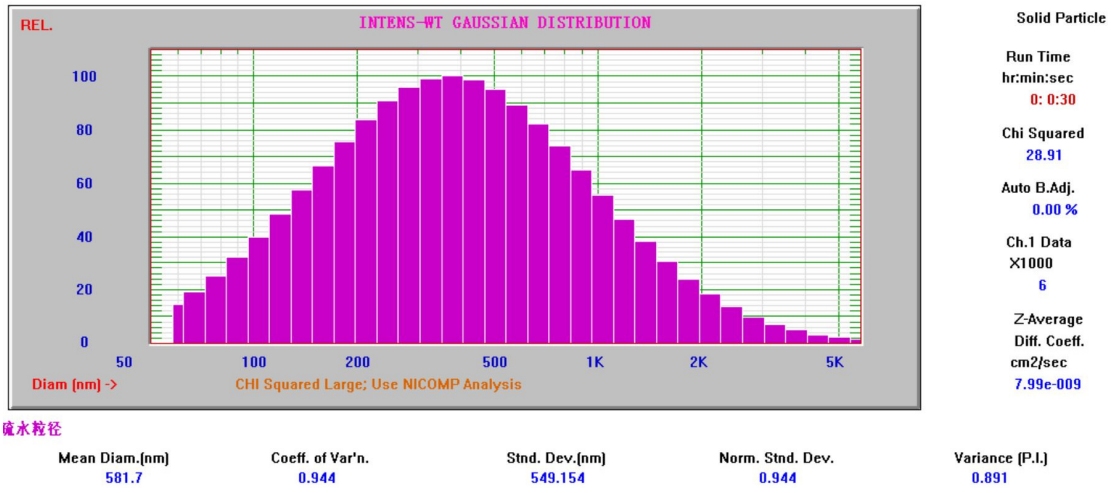

Figure S3. Hydrated size distribution of the polymer nanoparticle PNP.

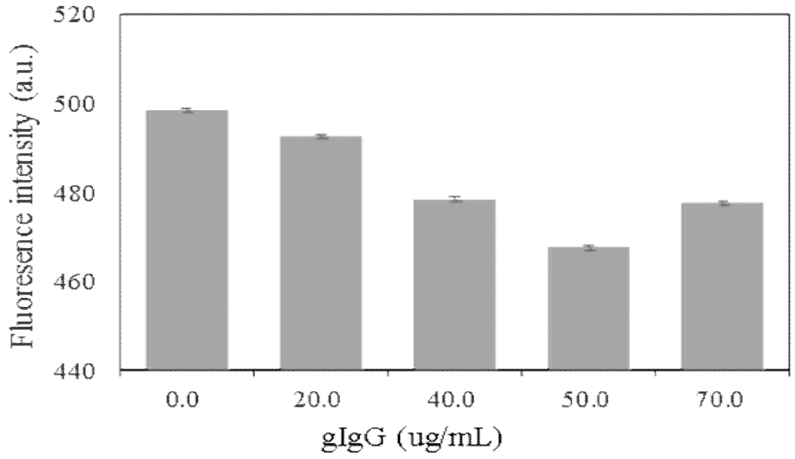

Figure S4. Fluorescence intensity of the supernatant after magnetic separation when adding gIgG at the concentrations of 0 μg mL^{-1}, 20 μg mL^{-1}, 40 μg mL^{-1}, 50 μg mL^{-1}, 70 μg mL^{-1}, respectively, in the immune-reaction system.

Reference

[S1] Wei, H.; Yin, J.Y.; Wang, E.K. Bis(2,2-bipyridine) (5,6-epoxy-5,6-dihydro-[1,10] phenanthroline) ruthenium: Synthesis and Electrochemical and Electrochemiluminescence Characterization. *Anal. Chem.* **2008**, *80*, 5635-5639.