Electronic Supplementary Material (ESI) for Analytical Methods.
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Electronic Supplementary Information (ESI)

A highly sensitive and label-free electrochemiluminescence immunosensor for beta 2-microglobulin

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Supplementary Materials and Methods

Reagents

Anti- β2M antibody produced in goat and β2M antigen from human urine, C-reactive protein (CRP), dehydroepiandrosterone (DHEA), cortisol, uric acid, leptin, alpha fetoprotein (AFP), haptoglobin, carcinoembryonic antigen (CEA), ascorbic acid, bovine serum albumin (BSA), Tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate ($[Ru(bpy)_3]Cl_2.6H_2O$) and tripropylamine (98% purity), sodium azide (NaN_3), Tris, disodium phosphate (Na_2HPO_4), monosodium phosphate ($NaH_2PO_4.H_2O$), potassium ferrocyanide, and potassium ferricyanide were all purchased from Sigma-Aldrich (Saint Louis, USA). Chitosan (85% deacetylated) was purchased from Alfa Aesar (Ward Hill, M.A). Carbon nano-onions was purchased from Carbon Allotropes (Kensington, Australia). Colloidal solutions of gold nanoparticles (10, 20 and 40 nm) were purchased from BBI Solutions (Cardiff, UK). For all experiments, samples were diluted in 20 mM Tris-buffer, pH 7.4. All buffer, ECL probe and redox probe solutions were prepared in-house. PBS-buffer was used for preparation of redox couple ($Fe(CN)_6]^{3-}$ /$[Fe(CN)_6]^{4-}$) for electrochemical analysis. All chemicals and reagents were of ≥ 95% purity and used as received. All solutions were prepared using freshly obtained Milli-Q water (deionized with specific resistance ~ 18MΩ/cm).

Apparatus

For the purpose of ECL measurement, MPI-A Capillary Electrophoresis Electrochemiluminescence Analyzer system was purchased from Xi′an Yima Opto-Electrical Technology Co., Ltd. (Xian, Shaanxi, Mainland China). An in-house working ECL cell was used to receive the light emitted from the ECL reactions (**Figure S1A)** and to conduct it to an ultra-sensitive single photon-counting module or photomultiplier tube (PMT) (**Figure S1B**) connected to MPI-A software. Fabricated immunosensor containing $[Ru(bpy)_3]Cl_2$-TPrA was immersed in the ECL cell (diameter 1.5 cm and height 5 cm) and mounted over PMT to receive the ECL intensity signal. To study the electrochemical layer-by-layer fabrication, kinetic study and charge analysis of the immunosensor, cyclic voltammetry (CV) and chronocoulometry (CC) techniques were performed using an Autolab PGSTAT101 III potentiostat/galvanostat (Metrohm, Netherlands) in combination with the Autolab Nova 1.10 software. Absorption spectra was recorded using Implen NanoPhotometer (CA, USA). The disposable working ceramic CdSe QDs-SPEs were obtained from DropSens (Asturias, Spain). Each of these comprise of a carbon counter electrode, a silver reference electrode and

a 4 mm diameter working electrode, with a maximum working volume of 50 µL. They are of dimensions L33 × W10 × H0.5 mm. To study the microscopic layer-by-layer fabrication of the immunosensor, field-emission electron microscopy (FE-SEM) JEOL, JSM-7610F (Tokyo, Japan) was used. Before analysis, each sample was carbon coated for 30 s. All analyses were performed at room temperature and atmospheric pressure (21±1 °C, air-conditioned laboratory). All experimental points are an average of three replicates obtained with three different fabricated immunosensors.

Blood serum and urine analysis

Blood was obtained from a 30-year healthy adult male (voluntary donor), who had not taken any prescription drug nor received antibiotic therapy for the past year prior to the collection date of the serum. Serum was kindly separated from the collected blood by RIPAS Hospital, Bandar Seri Begawan, Brunei. 10 mL of blood sample was taken from the antecubital vein of the donor using serum separation tube. The tube containing blood was immediately placed on ice to initiate clotting. Afterwards, the blood sample was centrifuged at 2500 rpm at 20°C for 10 min and serum was collected carefully from the upper phase. The collected serum was aliquoted into multiple tubes and stored at -80 °C until the time of analysis to prevent protein degradation. On the same day, urine sample was collected from the same donor as follows: the donor was asked to drink a glass of water and to void his urinary bladder. Urine specimen was collected within 1 h.

Figure S1

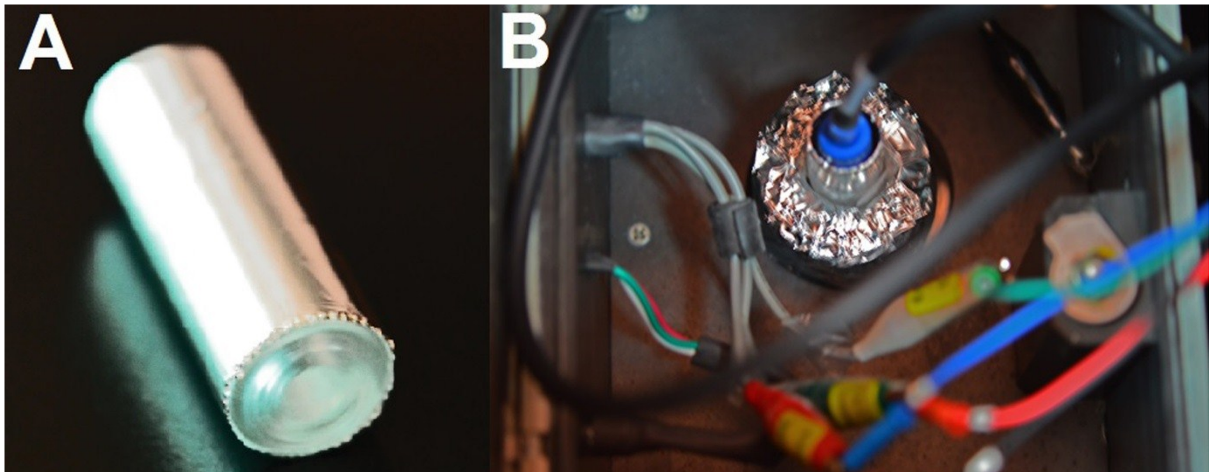

Figure S1 ECL Setup**. A:** In-house-made ECL Cell. **B:** ECL Cell with in-house-made holder over photomultiplier tube (PMT) inside the black box.

Figure S2

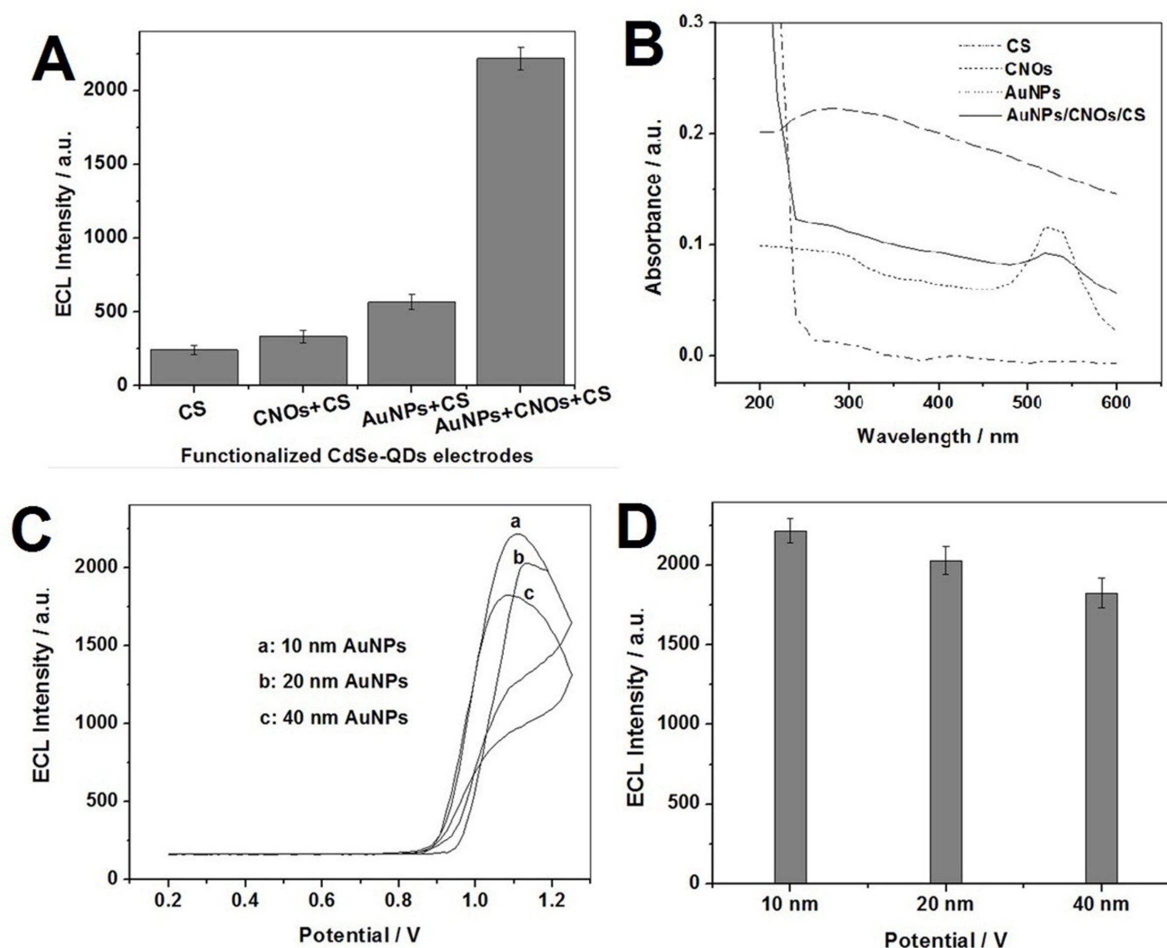

Figure S2: Comparison of ECL signals and absorption spectra of CdSe-QDs electrodes of different material combination. A: CdSe-QDs were modified with chitosan (CS), carbon nano-onions (CNOs), gold nanoparticles (AuNPs) or a combination of the different interface materials; **B:** Absorption spectra of the components of the nanocomposite were characterised using a nanophotometer, separately and in combination; **C and D:** Different sizes of AuNPs at the same concentration and under the same experimental condition were examined for the ECL intensity signal produced (n=3).

Figure S3

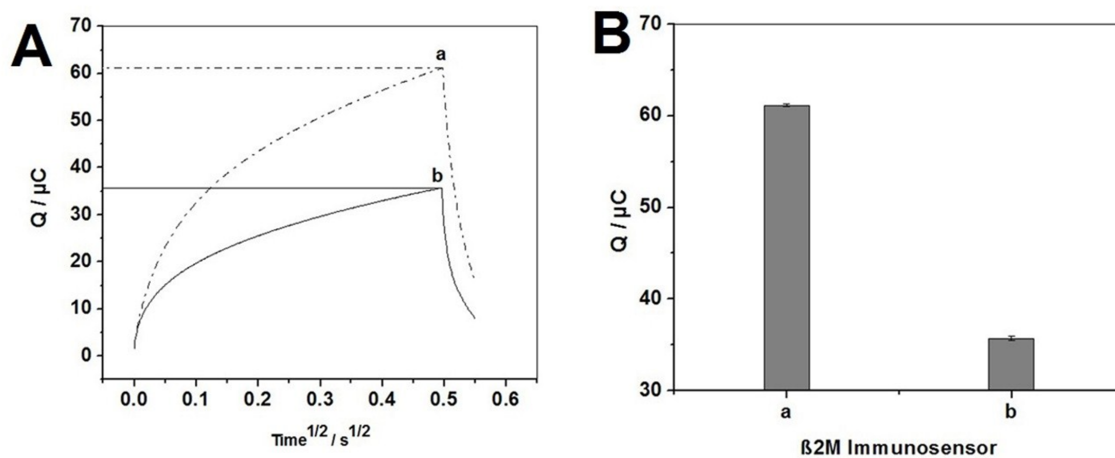

Figure S3: Chronocoulometry analysis to confirm for $[Ru(bpy)_3]Cl_2$-TPrA diffusion-controlled electrochemical process. A: Chronocoulometry curve with 1 ng/mL β2M (dashed line) and without β2M (solid line); and **B:** bar diagram with β2M (1ng/mL) and without β2M. Error bars indicate the standard deviations of at least three replicates (n = 3);

Figure S4

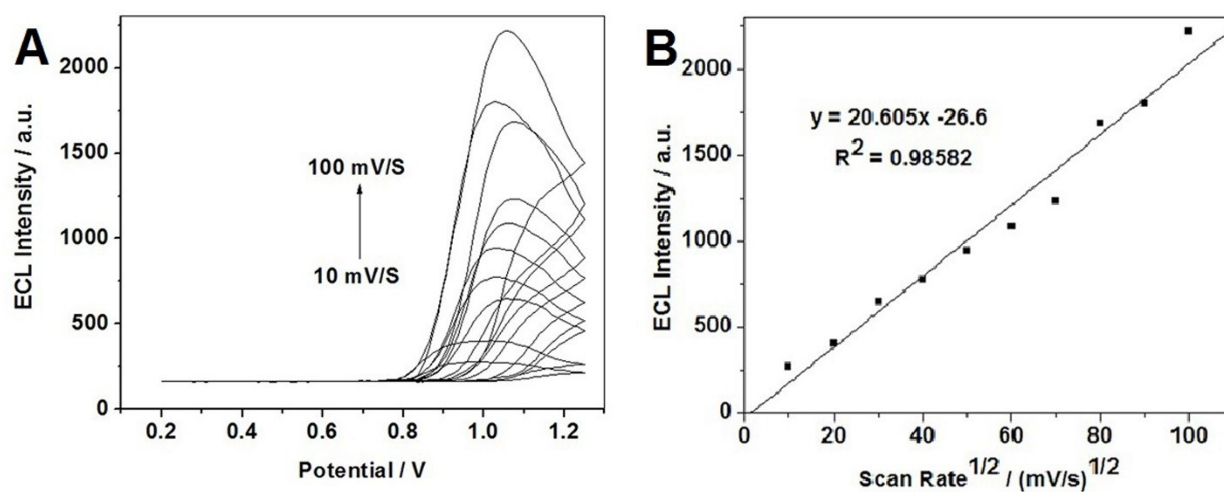

Figure S4: Diffusion kinetic analysis as assessed by ECL intensity. A: ECL intensity of the fabricated immunosensor from 10 mV/s to 100 mV/s scan rate at an interval of 10 (from inner to outer cycle); and **B:** Relationship between scan rate and ECL intensity peak.

Figure S5

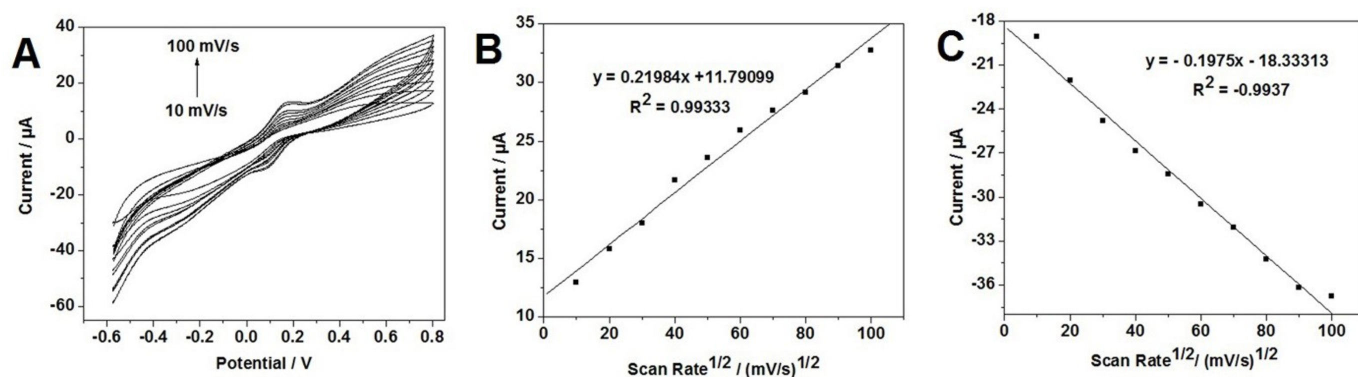

Figure S5: Diffusion kinetic analysis as assessed by cyclic voltammetry. Cyclic voltammetry reading at the surface of the immunosensor was obtained using 5 mM $[Fe(CN)_6]^{3-}$/ $[Fe(CN)_6]^{4-}$ redox probe containing 0.1 M KCl in solution of 10 mM PBS, pH 7.4, ΔES = 0.00244 V. **A:** Cyclic voltammograms of β2M immunosensor with a scan rate from 10 mV/s to 100 mV/s at an interval of 10 (from inner to outer cycle); **B:** Dependence of oxidation peak currents on the square root of the scan rates; and **C:** dependence of reduction peak currents on the square root of the scan rates.