Supporting Information

Signal Amplification Cytosensor for Evaluation of Drug-Induced Cancer Cell Apoptosis

Yafeng Wu\textsuperscript{a}, Wei Wei, Hao Zhou\textsuperscript{b}, Xin Hua\textsuperscript{a}, Lixin Wang\textsuperscript{b,*,} Zhenxian Zhou\textsuperscript{c}, Songqin Liu\textsuperscript{a,*}

\textsuperscript{a} State Key Laboratory of Bioelectronics, School of Chemistry and Chemical Engineering, Jiangning District 211189, Nanjing, Jiangsu Province, P.R. China
\textsuperscript{b} Medical School, Southeast University, Nanjing, 210096, P. R. China
\textsuperscript{c} Nanjing Second Hospital, Nanjing, 210003, PR China
**Materials and Reagents.** Glassy carbon electrodes (GCE) were purchased from CHI Instrument. Arginine glycine aspartic acid serine (RGDS) tetrapeptide was obtained from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). Glass slide, MWCNTs were purchased from Shenzhen Nanotech Port Ltd. Co. (Shenzhen, China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), Tris(2,2’-bipyridyl)dichlororuthenium(II) hexahydrate (Ru(bpy)_3Cl_2 · 6H_2O), Bis(2,2’-bipyridine)-(5-aminophenanthroline) ruthenium bis(hexafluorophosphate) (NH_2-Ru(bpy)_3^{2+}), Triton X-100 (TX-100), 2-(diisopropylamino)ethylamine (DPEA), 3-aminopropyl triethoxysilane (APTS) and glutaraldehyde (25 % in water) were purchased from Sigma-Aldrich (Shanghai, China). Tetraethyl orthosilicate (TEOS) was obtained from Beijing Yili Chemical Reagent Factory (Beijing, China). γ-Glycidoxypropyltrimethoxysilane (GPMS) was from Jintan Huadong Coupling Agent Co., Ltd. (Jiangsu, China). 1-Hexanol, cyclohexane, ammonium hydroxide (25 wt.%) and microscope slides were purchased from Beijing Chemical Reagent Factory (Beijing, China). Apoptosis Inducers Kit (C0005) and human anti-phosphatidyl serine antibody (APSA) were purchased from Beyotime Institute of Biotechnology (Shanghai, China). Rabbit antibody to CEA (anti-CEA) was purchased from Biodesign (Saco, MA). Anticancer drugs such as *cis*-Diamminedichloroplatinum (CDDP), 5-Fluorouracil, Gemcitabine hydrochloride, Paclitaxel and antibiotic drugs, such as Benzylpenicillin, Amoxicillin, Cefalexin, Norfloxacin were provided by the Affiliated Secondary Hospital of Southeast University (Nanjing, China). The buffer
solution was 0.01 M pH 7.4 phosphate-buffered saline (PBS) containing 136.7 mM NaCl, 2.7 mM KCl, 87 mM Na₂HPO₄, and 14 mM NaH₂PO₄. All other reagents were of analytical grade. Aqueous solutions were prepared with doubly distilled water.

MWCNTs of a certain mass were dispersed in 30% HNO₃ and then refluxed for 24 h at 140 °C to obtain carboxylic-functionalized MWCNTs. The resulting suspension was centrifuged, and the sediment was washed with deionized water until the pH reached 7.0. Then, the oxidized MWCNTs were dispersed in deionized water to a concentration of 1 mg mL⁻¹.

**Apparatus.** Electrochemiluminescent (ECL) measurements were carried out on a MPI-E multifunctional electrochemical and electrochemiluminescent analytical system (Xi’an Remex Analytical Instrument Ltd. Co., China). All ECL measurements were performed in a 5 mL glass cell composing of a modified glassy carbon electrode (GCE) working electrode, a platinum counter electrode, and an Ag/AgCl (saturated KCl solution) reference electrode. The potential range applied on the working electrode for ECL measurements was from 0.5 to 1.3 V at 50 mV s⁻¹. The ECL emission intensity was recorded by the MPI-E multifunctional chemiluminescence analyzer. The emission window was placed in front of the photomultiplier tube biased at 700 V. The electrolyte was 0.1 mM DPEA in 0.01 M phosphate solution (pH 7.5).

The morphology and size of the SiO₂@Ru nanoparticles were analyzed with a scanning electron microscope (SEM, LEO 1530 VP, Germany) and a transmission electron microscope (TEM, S-3400N, HITACHI, Japan).
Photoluminescence (PL) spectra were carried out on a RF-540 spectrophotometer (Shimadzu, Japan), which was excited at 460 nm. For PL detection, GCE was replaced by glass slide, other procedures were the same.

Microscopy and Image. Excitations were 488 nm, GCE was replaced by glass slide, other procedures were the same, the glass slide was mounted on the microscopy stage. Phase-contrast and fluorescent images were acquired by a Nikon inverted microscopy (ECLIPSE-TE-2000U, Nikon Corporation, Japan) equipped with a video camera (DS-U1, Nikon Corporation, Japan) and a self-made environment chamber that maintained the temperature at 37 °C. After images were taken, the glass slide was put back to the incubator and the treatment with anticancer drugs was continued. The collected data were analyzed using the Nikon imaging software NIS Elements (Nikon Corporation, Japan). Fluorescent intensity of individual cell was estimated by the program in the region of interest (ROI). For each image, the areas of each interested cell were manually defined three times over time, which gives an average intensity value ± standard deviation.

The Preparation of SiO₂@Ru Nanoparticles. The SiO₂@Ru nanoparticles were synthesized as previously described, but with an additional modification. The W/O microemulsion was prepared first by mixing 1.77 mL of TX-100, 7.5 mL of cyclohexane, 1.8 mL of 1-hexanol, and 340 μL of water. Then, 80 μL 0.1 M Ru(bpy)₃²⁺ aqueous solution was added into the mixture. In the presence of 100 μL of TEOS, the hydrolysation was initiated by the addition of 60 μL of NH₄OH (25%).
The reaction was kept stirred for 24 h. After removal of the surfactant, SiO\textsubscript{2}@Ru nanoparticles were isolated by acetone. They were then centrifuged and washed with ethanol and water several times. At last, some completely dry orange SiO\textsubscript{2}@Ru nanoparticles, with diameter of about 50 nm, determined by TEM (SI Figure 1), were obtained. The resultant SiO\textsubscript{2}@Ru nanoparticles were dispersed with ethanol for the following use.

**HepG2 Cell and Culture.** The HepG2 cell was kindly provided by Medical School of Southeast University, Nanjing, China. HepG2 cells were cultured in a flask with RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 µg mL\textsuperscript{-1}), and streptomycin (100 µg mL\textsuperscript{-1}). The cells were incubated at 37 °C, in a humidified atmosphere containing 5% CO\textsubscript{2}. During the logarithmic growth phase, the cells were trypsinized and washed twice with sterile 0.01 M pH 7.4 PBS by centrifugation at 1000 g for 10 min. The sediment was then resuspended, in 0.01 M pH 7.4 PBS containing 1 mM Ca\textsuperscript{2+} and 1 mM Mg\textsuperscript{2+}, to obtain a homogeneous cell suspension. The presence of divalent cations ensured the effective binding between cell surface integrins and RGDS on the cytosensor. The cell number was determined using a Petroff Hauser cell counter (USA). Apoptosis Inducers Kit and four clinically available anticancer drugs: cis-diaminedichloroplatinum (CDDP), 5-Fluorouracil, Gemcitabine hydrochloride and Paclitaxel, were selected as the model for induced apoptosis of HepG2 cells, respectively. Antibiotic drugs such as Benzylpenicillin, Amoxicillin, Cefalexin, and Norfloxacain were used as control.
**Characterization of RGDS-MWNTs/GCE.** The covalent modification of MWCNTs with RGDS tetrapeptide was confirmed by Raman spectra (Fig. S4). Both spectra of oxidized MWCNTs and RGDS-MWCNTs showed the characteristic peaks of MWCNTs at ~1552 cm\(^{-1}\) (tangential modes) and ~1354 cm\(^{-1}\) (disorder mode). The relatively intensity of the disorder mode is increased. The disorder mode does not increase upon noncovalent functionalization unless processing causes new defect sites on the sidewalls of the nanotubes.\(^{S2-S4}\)

**Reference**


**Scheme S1.** Reaction mechanism of Ru(bpy)$_3^{2+}$/TA ECL system.

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\text{Ru(bpy)}_3^{2+} \rightarrow \text{Ru(bpy)}_3^{3+} + e \quad (1)
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\[
\text{TA} \rightarrow \text{TA}^{**} + e \quad \text{(oxidized on electrode surface directly)} \quad (2a)
\]

\[
\text{Ru(bpy)}_3^{3+} + \text{TA} \rightarrow \text{TA}^{**} + \text{Ru(bpy)}_3^{2+} \quad \text{(oxidized by Ru(bpy)}_3^{3+}) \quad (2b)
\]

\[
\text{TPA}^{**} \rightarrow \text{TPA}^{*} + \text{H}^+ \quad \text{(radical rests on a carbon atom)} \quad (3)
\]

\[
\text{Ru(bpy)}_3^{3+} + \text{TPA}^{*} \rightarrow [\text{Ru(bpy)}_3^{2+}]^* + \text{TPA fragment} \quad (4)
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[\text{Ru(bpy)}_3^{2+}]^* \rightarrow \text{Ru(bpy)}_3^{2+} + h\nu \quad (5)
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Fig. S1. The TEM image of the as-synthesized SiO$_2$@Ru.
Fig. S2 Dependence of ECL intensity on (A) the incubation time with Apoptosis Inducers Kit (C0005) and HepG2 cells, (B) incubation time in $10^5$ cells mL$^{-1}$ of HepG2 cell suspension, (C) incubation time in APSA-SiO$_2$@Ru solution, (D) pH of the electrolyte, when one parameter changes while the others are under their optimal conditions.
Fig. S3. (A) PL intensity of SiO$_2$@Ru/HepG2/RGDS MWNTs/GCE obtained with HepG2 cell concentrations of $800, 1 \times 10^3, 1 \times 10^4, 1 \times 10^5, 1 \times 10^6, 5 \times 10^6,$ and $1 \times 10^7$ cells mL$^{-1}$. (B) Plot of PL intensity vs logarithm of HepG2 cell concentrations.
Fig. S4. Raman spectra of oxidized MWCNTs and RGDS-MWCNTs.