### **Supporting information**

## Integration of DNA bio-gate and duplex-specific nuclease signal amplification: towards electrochemiluminescence detection of survivin mRNA

Jing Wang<sup>ab</sup>, Xiang-Ling Li<sup>a</sup>, Jia-Dong Zhang<sup>a</sup>, Nan Hao<sup>a</sup>, Jing-Juan Xu<sup>\*a</sup> and Hong-Yuan Chen<sup>\*a</sup>

<sup>a</sup> State Key Laboratory of Analytical Chemistry for Life Science and Collaborative Innovation Center of Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China

<sup>b</sup> Department of Analytical Chemistry, School of Science, China Pharmaceutical University, Nanjing 210009, China.

### **EXPERIMENTAL SECTION**

### **Reagents:**

N-cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (3-aminopropyl)triethoxysilane (APTES, 99%). Tri(2.2'-(TEOS). bipyridyl) dichlororuthenium (II) hexahydrate, tripropylamine (TPA) were obtained from Sigma-Aldrich Chem. Co. (St. Louis, MO, USA). Duplex-specific nuclease (DSN) was purchased from Evrogen Joint Stock Company (Moscow, Russia). TRIzol reagent purchased from Invitrogen (Carlsbad, CA, USA). Indium-tin oxide (ITO)-coated (thickness: 100 nm; resistance: 10  $\Omega$  sqr<sup>-1</sup>) aluminosilicate glass slides were purchased from CSG (Shenzhen, China). Sylgard 184 (including poly(dimethylsiloxane) (PDMS) monomer and curing agent) was from Dow Corning (Midland, MI). Other chemicals used in this work were of analytical grade and directly used without further purification. Millipore ultrapure water (resistivity  $\geq 18.2 \text{ M}\Omega \text{cm}$ ) treated with 0.1% DEPC was used throughout the experiment.

DNA oligonucleotide was purchased from Sangon Biotech Co. Ltd.

(Shanghai, China), and RNA oligonucleotides were ordered from GenScript Co., Ltd. (Nanjing, China). Their base sequences were illustrated in Table S1.

Table S1. Sequences Used in This Work:

Name	Sequences (5'-3')
ssDNA	CCCAGCCTTCCAGCTCCTTGAAAA
ssRNA (complementary)	AAAUCAAGGAGCUGGAAGGCUGGG
ssRNA (non-complementary)	UAGCAGCACAUAAUGGUUUGUGCA

### Apparatus.

The electrochemical and ECL emission measurements were conducted on a MPI-A multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remax Electronic Science &Technology Co. Ltd., Xi'an, China) at room temperature. The ECL emission measurements were carried out in 0.1 M pH 7.4 PBS using 25 mM TPA as a coreactant. The spectral width of the photomultiplier tube (PMT) was 350–650 nm, and the voltage of the PMT biased at -750 V in the process of detection.

Transmission electron micrographs (TEM) were obtained on JEM-2100 transmission electron microscope (JEOL Ltd., Japan). The Ru(bpy)<sub>3</sub><sup>2+</sup> content of the samples were measured by inductive coupled plasma atomic emission spectrometer (ICP-AES, Optima5300DV). The UV–vis absorption spectra were recorded on a Shimadzu UV-3600 UV–vis–NIR photospectrometer (Shimadzu Co.). The Zeta-potential was acquired with a Malvern (Nano-Z, Malvern Instruments Ltd., Britain) instrument. N<sub>2</sub> adsorption/desorption measurement was performed through an automated physisorption analyzer (ASAP 2020, Micromeritic, USA). The polyacrylamide gel electrophoresis was performed on the Bio-Rad

electrophoresis analyzer and imaged on Biorad ChemDoc XRS (Bio-Rad, USA).

### Synthesis of MSNs and Ru(bpy)<sub>3</sub><sup>2+</sup> loaded MSNs:

The MSNs were synthesized with a described procedure in the previous report.<sup>1</sup> Briefly, 1.0 g of n-cetyltrimethylammoniumbromide (CTAB, 2.74 mmol) was first dissolved in 480 mL of ultrapure water. 7.0 mL of NaOH aqueous solution (2.0 M) was added to adjust the pH followed by heating to 90 °C. Under continuous stirring, 5 mL of TEOS was then added dropwise to the surfactant solution. The mixture was vigorously stirred for 3 h to generate white precipitate. Then the precipitate was collected by centrifugation and washed thoroughly with ultrapure water and ethanol, and dried in vacuum at 60 °C overnight. To prepare the final porous structure, the final products were calcined at 550 °C for 5 h in order to remove the structure-directing agent.

To loading with ECL luminophore, 0.1 g of calcined MSNs and 1 mL  $Ru(bpy)_3^{2+}$  (5.0 mM) were dispersed in 20 mL of anhydrous ethanol. The mixture was then stirred for 24 h at 37°C in order to achieve the maximum loading in the pores of the MSNs scaffolding. Afterward, the mixture was added with an excess of APTES (0.3 mL) and stirred continuously for 6 h at 37°C to immobilize the amine group on the surface. Finally, the orange NH<sub>2</sub>-MSNs were then collected by centrifugation, washed thoroughly with methanol, and dried in vacuum.

# Preparation of ssDNA capped NH<sub>2</sub>-MSNs and optimization of ssDNA amount for sealing mesopores of MSNs:

Portions of 0.2 mg NH<sub>2</sub>-MSNs were suspended in 100  $\mu$ L of hybridization buffer (10 mM Tris-HCl, 50 mM NaCl, and 10 mM MgCl<sub>2</sub>, pH 7.4), followed by adding a series of volumes of 100  $\mu$ M ssDNA (5, 10,

15, 20, 25, and 30  $\mu$ L), and then adjusted the total volume to 200  $\mu$ L with hybridization buffer. Each suspension was shaken at 37 °C for 1 h. The unreacted gated materials were removed by three centrifugation/washing cycles. Finally, the resulting solids DNA-RuMSNs were collected carefully, dispersed in hybridization buffer and stored at 4 °C for further use.

The surface densities of capped ssDNA on MSN could be measured from the comparison of the ssDNA in the supernatant and in the stock solutions by UV-vis spectra. Following this procedure, 25  $\mu$ L (12.5  $\mu$ mol·L<sup>-1</sup>) ssDNA was selected as the optimum added amount. The results estimated from this capping procedure are showed in Table S2.

surface of NH <sub>2</sub> -MSN:						
sample	DNA added	remanent	% retained	DNA capped on		
	(µmol·L⁻¹)	(µmol·L⁻¹)		NH2-MSN (µmol·g <sup>-1</sup> )		
1	2.5	0.1092	95.63	2.3908		
2	5.0	0.4840	90.32	4.5160		

87.03

84.07

80.75

67.25

6.5275

8.4072

10.0936

10.0876

Table S2. Estimation of content of the DNA ( $\mu$ mol·g<sup>-1</sup>) capped on the surface of NH<sub>2</sub>-MSN:

### Cell Culture and total RNA isolation from cancer cell:

0.9725

1.5928

2.4064

4.9124

3

4

5

6

7.5

10.0

12.5

15.0

Human cervical cells (HeLa), human breast cancer (MCF-7) cells and normal immortalized mammary epithelial cell line (MCF-10A) were cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100  $\mu$ g mL<sup>-1</sup>), and streptomycin (100  $\mu$ g mL<sup>-1</sup>), and the cells were maintained at 37°C in a humidified atmosphere (95% air and 5% CO<sub>2</sub>). Petroff-Hausser cell counter was used for Cell number determination.

In order to create and maintain an RNase-free environment, all the solution and appliances were treated with 0.1% DEPC and autoclaved. The total RNA was extracted from Hela cells by using Invitrogen's TRIzol reagent according to the manufacturer's instructions. The extracted RNA was dissolved in 50  $\mu$ L of DEPC water for detection.

### Polyacrylamide Gel Electrophoresis (PAGE) Analysis:

The 20% native polyacrylamide gel was prepared using  $1 \times \text{TBE}$  buffer. The loading sample was the mixture of 10 µL of samples, 2 µL of  $6 \times$  loading buffer, and 1.5 µL of GelRed and kept for 3 min so that the dye could integrate with sample completely. Then the loading sample was injected into polyacrylamide hydrogel. The gel electrophoresis was run at 120 V for 60 min. The resulting board was illuminated with UV light and scanned with a Molecular Imager Biorad ChemDoc XRS.

#### Fabrication of ECL microreactor and ECL detection:

The fabrication process was similar to our previous work<sup>2</sup>. As illustrated in scheme S1: First, an ITO glass slice  $(30 \times 30 \text{ mm})$  was washed by sonification in water and ethanol each for 15 min and then immersed in a boiling solution of 2 mol L<sup>-1</sup> KOH in 2-propanol for 20 min. The cleaned ITO glass was rinsed thoroughly with ultrapure water and dried under nitrogen flow. As a ground layer, the ITO glass was etched by the same method to the previous work<sup>2</sup>. Afterwards, PDMS was made by the ratio of monomer and curing agent with 10:1 and heated over 1h at 70°C. A PDMS thin-film (thickness 0.15 mm) with three holes (3.0 mm in diameter) was reversibly bonded with the patterned ITO glass

by the plasma generator to form three independent electrodes. A PDMS top frame with a cell (length 15.0 mm, width 5.0 mm, thickness 5.0 mm) was constructed by a man-made hole puncher, and irreversibly joined above PDMS film after the plasma treatment. Finally, the ECL microreactor was successful fabricated.



Scheme S1. Schematic illustration of the fabrication of ECL microreactor

For ECL detection, firstly, 10  $\mu$ l ssDNA wapped MSNs was hybridized with target survivin mRNA in a 1 ml centrifuge tube at 37°C for 30 min, then incubated with 0.2 U DSN at 50 °C for 35 min. This mixture was added into the ECL microreactor, which contained ECL detection buffer (0.1 M PBS (pH 7.4) + 25 mM TPA). Finally, this microreactor was placed in the cassette of MPI-A multifunctional electrochemical and chemiluminescent analytical system at room temperature. The potential was scanned towards the positive direction from 0 V to +1.25 V with a scan rate of 100 mVs<sup>-1</sup>, and the voltage of the PMT biased at -750 V in the process of detection.

Inductive coupled plasma atomic emission spectroscopy (ICP-AES) analysis:

The samples were completely dissolved in hydrofluoric acid before analysis. From the ICP-AES detection, the content of  $Ru(bpy)_3^{2+}$  in Ru-MSNs, NH<sub>2</sub>-MSNs and ssDNA wapped MSNs were 8.19 wt%, 8.12 wt% and 8.08 wt%, respectively.

### **Comparative experiment between MCF-7 cells and MCF-10A cells:**

To assess the specificity and the practicability of the proposed assay, the total RNA was respectively extracted from MCF-7 cells and MCF-10A cells by using TRIzol reagent. The cancer cell extracts were serially diluted with buffer solution and the relative ECL intensities of 100 cells was about 4360, while very small ECL response in MCF-10A cells of the same cell count.



Fig. S1 The ECL response of total RNA extracted from 100 cells (A) MCF-7 cell and (B) MCF-10A cell, incubated with 0.2 U DSN for 35 min.

### References

- 1. Lai, J.; Shah, B. P.; Garfunkel, E.; Lee, K.-B., ACS Nano 2013, 7 (3), 2741-2750.
- Zhang, J.-D.; Xiong, M.; Hao, N.; Xu, J.-J.; Chen, H.-Y., *Electrochemistry Communications* 2014, 47, 54-57.