

Supporting Information

An Ultrasensitive Electrochemiluminescent Sensor for MicroRNA with Multinary Zn-Ag-In-S/ZnS Nanocrystals as Tags

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Contents

(a)	Chemicals and reagents	S-2
(b)	Apparatus and measurements.....	S-2
(c)	PL Life time parameters of ZAIS NCs and ZAIS/ZnS NCs.....	S-2
(d)	HRTEM of ZAIS/ZnS NCs.....	S-3
(e)	PL and ECL spectra characterization.....	S-3
(f)	LOD of different sensor for determining miRNA	S-4

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(a) Chemicals and reagents

All chemical reagents were of analytical grade or better and used as received. P-aminobenzoic acid (ABA) was purchased from Kermel Chemical Reagent Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), and indium acetate ($\text{In}(\text{CH}_3\text{COO})_3$) were purchased from Sigma-Aldrich Chemicals Co. LLC. (St. Louis, MO, U.S.A.). Tris(hydroxymethyl)aminomethane (Tris), sodium chloride (NaCl), magnesium chloride (MgCl_2), potassium phosphate monobasic (KH_2PO_4), dipotassium hydrogen phosphate trihydrate ($\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$), potassium nitrate (KNO_3), sodium hydroxide (NaOH), silver nitrate (AgNO_3), 1-ethyl-3-(3-dimethyl-amino-propyl) carbodiimide hydrochloride (EDC), and *N*-hydroxysuccinimide (NHS) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$), potassium hexacyanoferrate ($\text{K}_4[\text{Fe}(\text{CN})_6]$), and ethylenediaminetetraacetic acid (EDTA) were purchased from Xilong Chemical Co., Ltd. (Shantou, China). Magnesium acetate ($\text{Mg}(\text{CH}_3\text{COO})_2$), tri-*n*-propylamine (TPrA), and L-cysteine were purchased Aladdin Industrial Co., Ltd. (Shanghai, China). Acetic acid glacial (CH_3COOH) was purchased from Yongda Chemical Reagent Co., Ltd. (Tianjin, China). Zinc acetate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) was purchased from Guangcheng Chemical Reagent Co., Ltd. (Tianjin, China). Thioacetamide (TAA) was obtained from Shanghai Macklin Biochemical Co., Ltd (Shanghai, China). Phosphate buffer (PB, pH 7.4, 10 mmol/L) was used as washing buffer.

(b) Apparatus and measurements

Ultraviolet-visible (UV-vis) absorption spectra were recorded on a TU-1901 UV-vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd.). Photoluminescence (PL) spectra were recorded on an F-320 spectrofluorometer (Tianjin Gangdong Sci & Tech Development Co., Ltd.). The fluorescence lifetime decays and PL quantum yields were recorded on a fluorescence spectrometer (model FLS920, Edinburgh Instruments). High-resolution transmission electron microscopic (HRTEM) images were taken on a TecnaiG2 F30 transmission electron microscope with an acceleration voltage of 300 kV (Thermo Fisher Scientific Co.). Energy dispersive spectrometer (EDS) spectra were recorded by using a scanning electron microscope was performed with a SUPRA 55 field emission scanning electron microscope (Carl Zeiss AG, Germany). X-ray diffraction (XRD) pattern was recorded using an X-ray diffractometer (Bruker AXS D8 Advance, Germany) with Cu K α radiation ($\lambda=1.5418\text{\AA}$). ECL and cyclic voltammetric (CV) measurements were carried out on an MPI-EII ECL analyzer (Xi'an Remex Analytical Instrument Co., Ltd., China) using a three-electrode system, including a 5 mm diameter glassy carbon work electrode (GCE), an Ag/AgCl (saturated KCl) reference electrode, and a Pt counter electrode. Differential pulse voltammetry (DPV) was recorded on a CHI 1040 electrochemical analyzer (Shanghai, China). ECL spectra acquisition were accomplished with a homemade ECL spectro-meter consisting of an Acton SP2300i monochromator equipped with a liquid N₂ cooled PyLoN 400BR-eXcelon digital CCD detector (Princeton Instruments, U.S.A) and a VersaSTAT 3 electrochemical analyzer (Princeton Applied Research, U.S.A.).¹⁻³ ECL emission at the electrode surface was collected with an objective lens before it was delivered to the ECL spectrum system.

(c) PL Life time parameters of ZAIS NCs and ZAIS/ZnS NCs

Table S1. PL Decay Constants Obtained from $I(t) = y_0 + B_1 \exp(-t/\tau_1) + B_2 \exp(-t/\tau_2)$

samples	Lifetime (ns)	τ_1 (ns)	B_1	τ_2 (ns)	B_2
ZAIS	325	127.73	0.65	485.11	0.21
ZAIS/ZnS	438	205.54	0.55	719.12	0.13

The PL decay curves of ZAIS NCs and ZAIS/ZnS NCs could be well-fitted by a biexponential function $I(t) = y_0 + B_1 \exp(-t/\tau_1) + B_2 \exp(-t/\tau_2)$, where $I(t)$ is the PL intensity at delay time (t), B_1 and B_2 represent the relative weights of the decay components at $t = 0$, t is time, and τ_1 and τ_2 are the lifetimes for the exponential components. The average lifetime constant (τ^*) could be calculated using the equation $\tau^* = (B_1\tau_1^2 + B_2\tau_2^2)/(B_1\tau_1 + B_2\tau_2)$, are listed in Table S1.

(d) HRTEM of ZAIS/ZnS NCs

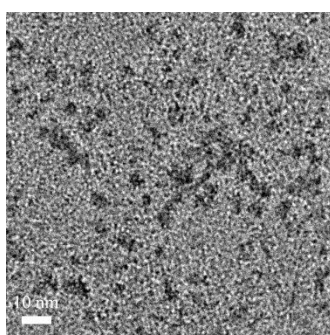


Figure S1. HRTEM pattern of ZAIS/ZnS NCs.

(e) PL and ECL spectra characterization

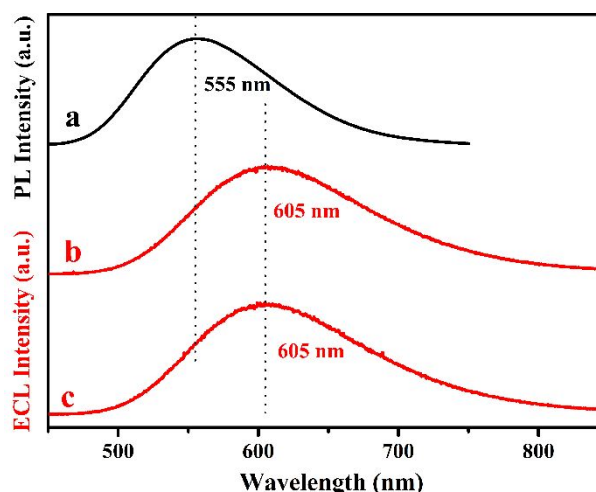


Figure S2. (a) PL spectrum of ZAIS/ZnS NCs; ECL spectra of (b) 0.50 mg/mL ZAIS/ZnS NCs and (c) GCE-ABA-tDNA<miRNA-141> pDNA-ZAIS/ZnS in PBS (0.1 mol/L, pH 7.4) containing 10 mmol/L TPrA. ECL spectra resulted from the total photons generated by scanning the potential from 0 to + 1.6 V for one cycle at 50 mV/s.

(f) LOD of different sensor for determining miRNA

Table S2. LOD of different MiRNA sensors

No.	Method	Signal Tags	Target	LOD	Ref.
1	electrochemistry	$[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$	miRNA-107	10 fM	⁴
2	fluorescence		miRNA-21	10 fM	⁵
3	fluorescence		let-7a	58 fM	⁶
4	ECL-RET	$\text{CdSe@ZnS NCs-Ru}(\text{dcbpy})_3^{2+}$	miRNA-141	33 aM	⁷
5	ECL-RET	$\text{C}_3\text{N}_4\text{-Ru}(\text{bpy})_3^{2+}$	miRNA-21	0.5 fM	⁸
6	ECL	CdS NCs	miRNA-21	0.16 fM	⁹
7	ECL	CdS:Mn NCs	miRNA-141	3.3 fM	¹⁰
8	ECL	ZAIS/ZnS NCs	CdS:Mn	50 aM	herein

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