Supporting information

Ultrasensitive Nucleic Acid Assay Based on AIE-active Polymer Dots with Excellent Electrochemiluminescence Stability

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1. Experimental Section

Materials and Reagents.

6-mercapto-1-hexanol(MCH), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), Tri-n-propylamine (TPrA), triethylamine (TEA), N-butyldiethanolamine (BDEA)**,** Poly(styrene-co-maleic anhydride) (PSMA) were obtained from Sigma-Aldrich. Co., Ltd. (Shanghai, China). 4,4'-(2,2-bis(4-(octyloxy)phenyl)ethene-1,1-diyl)bis(bromobenzene), 4,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[c][1,2,5]thiadiazole, $Pd(PPh_3)_4$, bromobenzene and phenylboronic acid were obtained from J&K Chemical Ltd (Shanghai, China). Potassium Carbonate (K_2CO_3), Toluene (PhMe) and methanol were purchased from Sinopharm Chemical Reagent Co. Ltd. DSN and 10× DSN master buffer (500 mM Tris-HCl, 50 mM $MgCl_2$, 10 mM D,L-dithiothreitol (DTT), pH 8.0) were purchased from Evrogen (Moscow, Russia). HPLC-purified, synthetic DNA and miRNAs were purchased from Sangon Biotech Co. Ltd. (Shanghai, China), detailed sequence information is listed in Table S1. Unless specifically mentioned, other reagents were of analytical-grade quality and used as received without further purification. Aqueous solutions were prepared using ultrapure water. Ultrapure water obtained from Millipore water purification system (≥18 MΩ cm^{-1}, Milli-Q, Millipore, MA) was used throughout the experiments.

Apparatus.

The transmission electron microscopy (TEM) image was acquired on a JEM-2100 (JEOL Ltd., Japan) Instrument. The NMR spectra were obtained from a Bruker Advance 400 spectrometer (Bruker, German) with 400 MHz for 1H NMR reported as parts per million (ppm) from the internal standard tetramethylsilane (TMS). The UV-vis spectrum were recorded on a Model UV-3600 spectrophotometer (Shimadzu, Kyoto, Japan). Fluorescence measurements were obtained on a F-7000 fluorescence spectrometer (Hitachi Co., Japan). Dynamic light scattering (DLS) measurement was performed on a 90 Plus/BI-MAS equipment (Brookhaven Instruments Co., USA). Electrochemical impedance spectroscopic (EIS) measurements were performed on a PGSTAT30/FRA2 system (Autolab, the Netherlands) in a solution containing 0.1 M KCl and 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1). Electrochemical experiments were performed on a CHI 760E electrochemical workstation (CHI instruments Inc., China). ECL experiments were carried out on a MPI-E multifunctional electrochemical and chemiluminescent analytical system (Xi'an Remex Analytical Instrument Co., Ltd. China). ECL transient experiments were performed by our self-built instrument combining a PGSTAT30/FRA2 system (Autolab, the Netherlands) and an ECL analytical system.

ECL spectra measurement

The ECL spectra were obtained on an F-7000 fluorescence spectrophotometer

which in the closed state of lamp and triggered by a CHI 660D electrochemical workstation. The Pdots modified indium tin oxide (ITO) electrodes were inserted in 0.1 M PBS containing 25 mM TPrA to apply a constant potential of +1.5 V for 9 s to get the ECL spectra.

Experiment of ECL imaging

The ECL image was obtained with a self-made ECL imaging system equipped with focus lens (EF 50mm f/1.2L USM, Canon) and Retiga R6 color scientific CCD camera (QImaging, Canada) in a dark box. The Pdots modified indium tin oxide (ITO) electrodes were reacted with 25 mM TPrA contained in 0.1 M PBS solution under a cyclic voltammetry scanning from 0-1.5 V with a scan rate of 0.3 V/s and the exposure time was 30 s.

2. Supplementary Figures

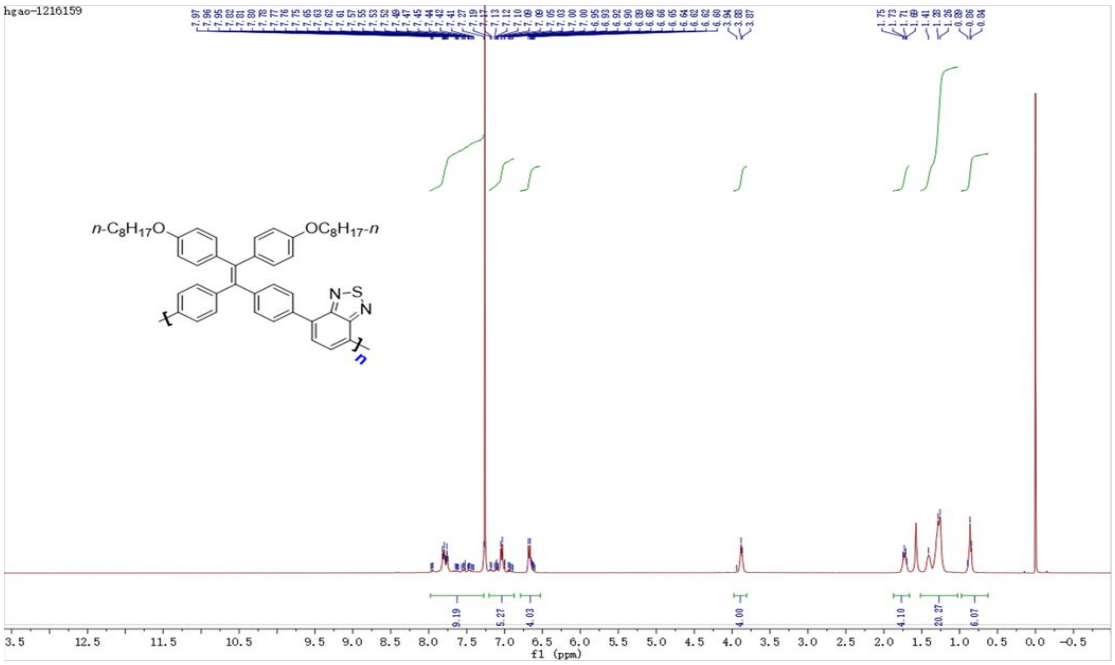

Figure S1. Nuclear magnetic resonance spectroscopy (^{1}H NMR) of polymer.
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.97 - 7.27 (m, 9H), 7.19 - 6.89 (m, 5H), 6.68 - 6.60 (m, 4H), 3.94 - 3.87 (m, 4H), 1.75 - 1.69 (m, 4H), 1.41 - 1.26(m, 20H), 0.89 - 0.84(m, 6H)
GPC: M_n = 23155, M_w = 35026 , PDI = 1.51.

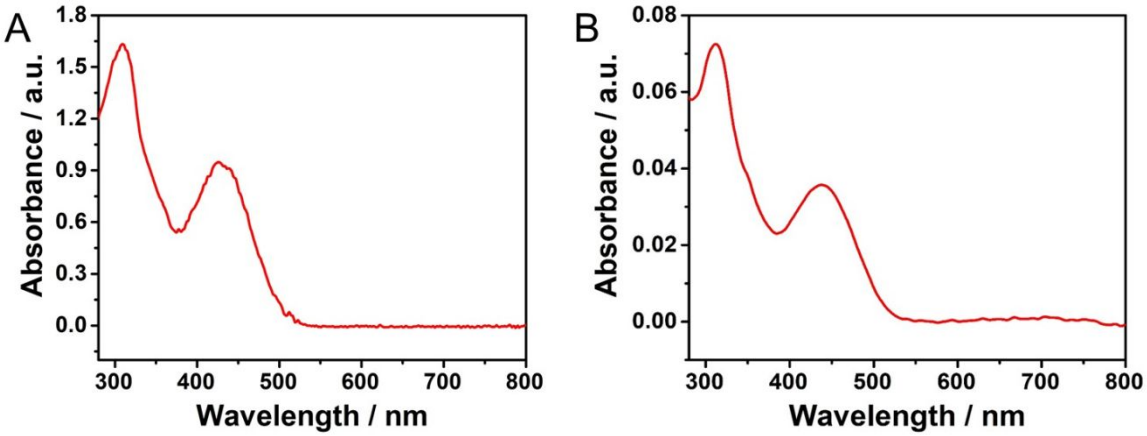

Figure S2. Absorbance spectra of (A) polymer in THF and (B) Pdots.

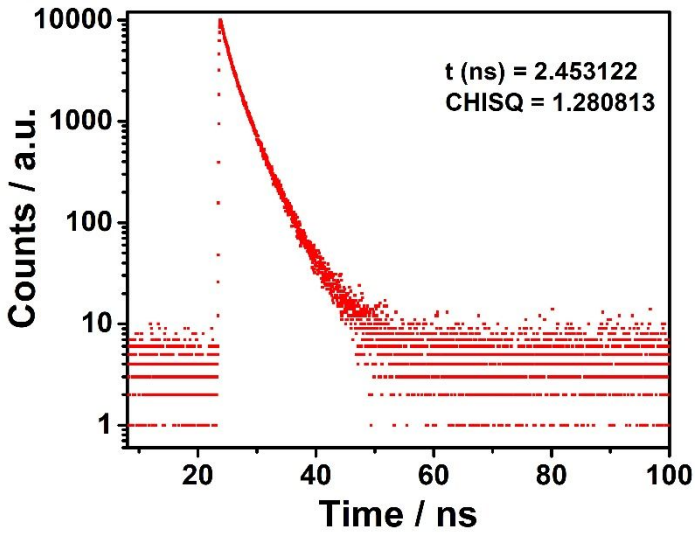

Figure S3. Phosphorescent lifetime of Pdots, which was recorded at excitation wavelength of 365 nm.

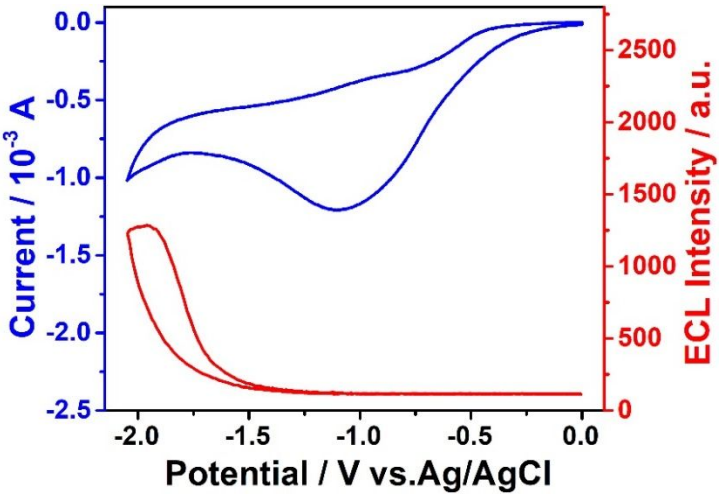

Figure S4. Cyclic voltammetry curve (blue line) and ECL profile (red line) of 5 μg/mL Pdots containing 0.1 M $K_2S_2O_8$. (voltage of PMT, 800 V)

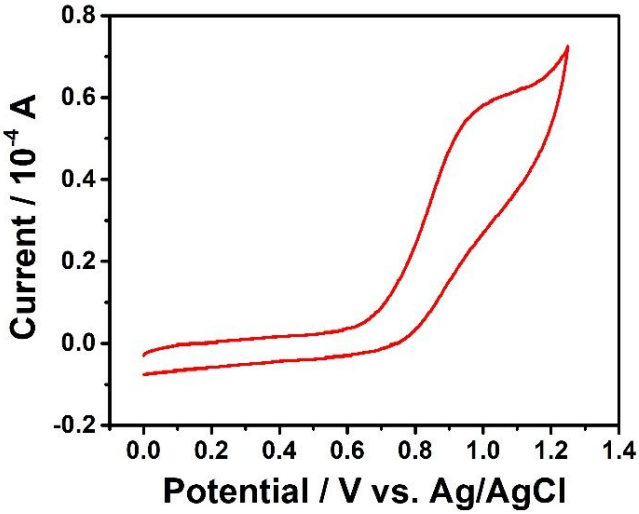

Figure S5. Cyclic voltammogram of 25 mM TPrA in PBS solution (scan rate, 100 mV/s).

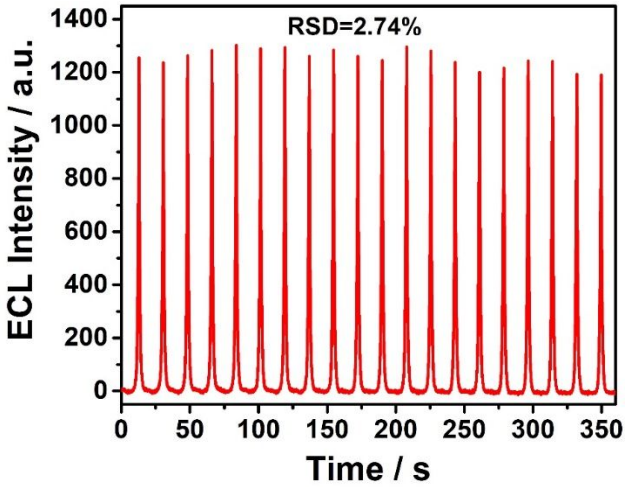

Figure S6. ECL stability test of Pdots modified GCE with 0.1 M $K_2S_2O_8$ under 20 cyclic potential scans. (voltage of PMT, 800 V)

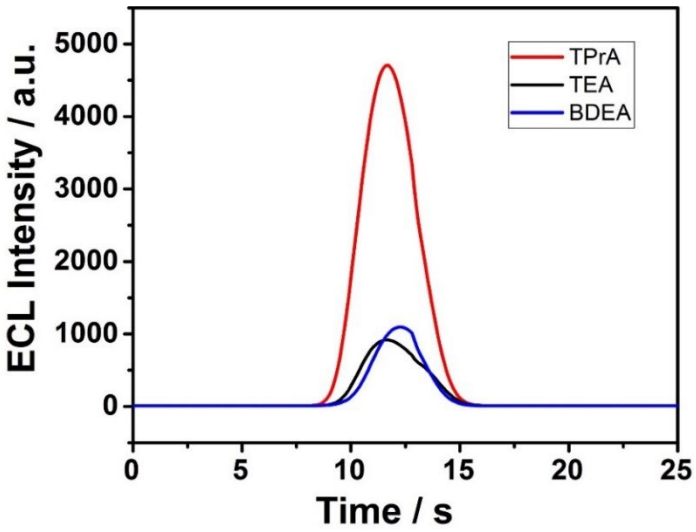

Figure S7. ECL intensity comparision of Pdots with different coreactants.

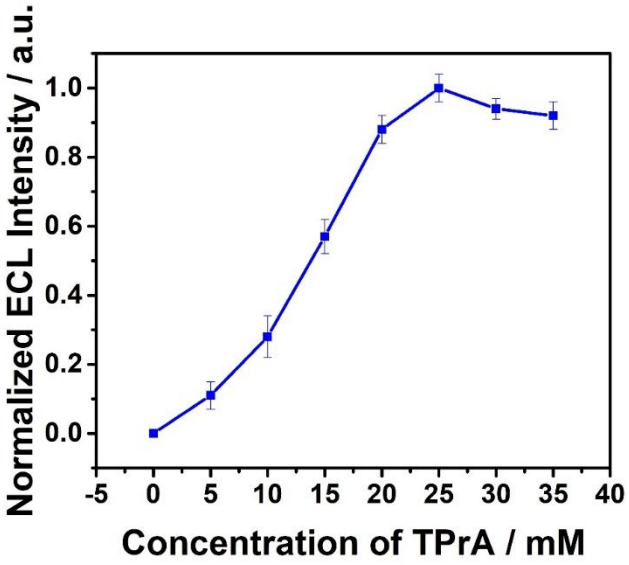

Figure S8. Optimization of the TPrA concentration.

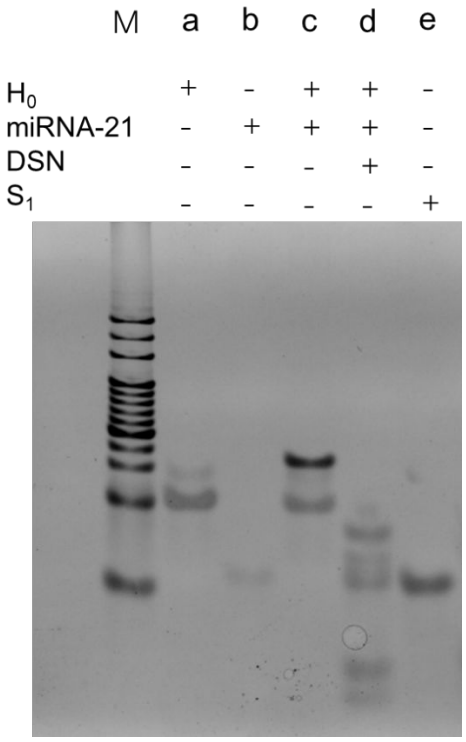

Figure S9. Polyacrylamide gel electrophoresis analysis. Lane M: DNA ladder; lanes a–e: as indicated above.

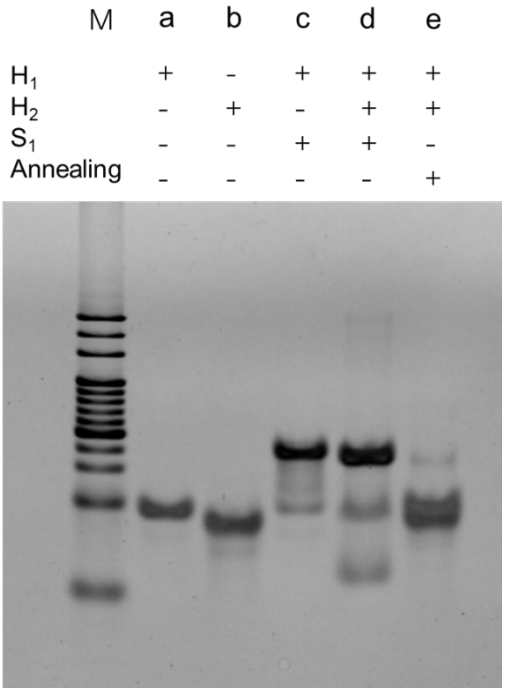

Figure S10. Polyacrylamide gel electrophoresis analysis. Lane M: DNA ladder; lanes a–e: as indicated above.

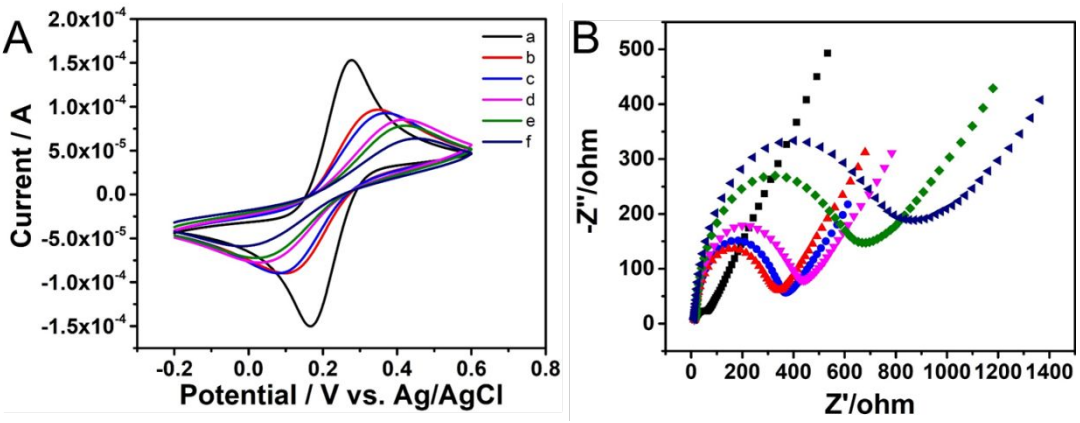

Figure S11. (A) Cyclic voltammograms and (B) EIS profiles of the stepwise fabrications of the ECL immunosensor: (a) bare GCE, (b) Pdots/a, (c) H_1/b, (d) MCH/c, (e) S1/d, (f) H_2-BHQ/e.

3. Supplementary Tables

Table S1. DNA and miRNA sequences used in this work.

name	Sequences (5′ to 3′)
H_0	TTTTCAACATCAGTCTGATAAGCTATTTGATCCCATTA TCAGACTTGCCTCT
H_1	NH_2-$(CH_2)_6$TTTTTTTAGAGGCAAGTCTGATAATGGG ATCATGCCTCTAACCTGATCCCATTATCAGAC
H_2	TTTTATGGGATCAGGTTAGAGGCATGATCCCATTATC AGACATGCCTCTAACCT-BHQ_2
Report DNA	TTTGATCCCATTATCAGACTTGCCTCT
miRNA-21	UAGCUUAUCAGACUGAUGUUGA
miRNA-107	AGCAGCAUUGUACAGGGCUAU
miRNA-122	UGGAGUGUGACAAUGGUGUUUG
miRNA-141	UAACACUGUCUGGUAAAGAUGG

Table S2. Summarization of the experimental findings for Pdots.

	Abs (nm)	PL (nm)	T (ns)	PLQY (%)	Zeta potential (mV)
Pdots	312, 438	573	2.45	25.43	-15.8

Table S3. Comparison of the ECL efficiency of different nanomaterials.

Material	coreactants	$Ø_{ECL}$ (%)	Ref.
o@CdInS	TPrA	2.1	1
TPE nanocrystals	TEA	1.35	2

TPE-CMP	TPrA	1.72	3
CN-PPV Pdots	$K_2S_2O_8$	11.22	4
	TPrA	1.84	
PFBT Pdots	TPrA	1.2	5
	TPrA	0.5	6
TPE-based Pdots	TPrA	11.8	7
	TEA	11	8
Pdots	TPrA	10	This work

Table S4. Comparison of the linear range and detection limits of different methods for detection of miRNA-21.

Methods	Linear range	Detection limit	Ref.
Square Wave Voltammetry (SWV)	1.0 pM-10.0 nM	0.26 pM	9
Chronoamperometry (CA)	0.1 pM-70 pM	0.06 pM	10
Amperometry (I-t)	1 pM-5000 pM	0.4 pM	11
Amperometry (I-t)	5 pM-5000 pM	3.96 pM	12
Differential Pulse Voltammetry (DPV)	200 pM-388 nM	100 pM	13
Differential Pulse Voltammetry (DPV)	100 aM-1 nM	78 aM	14
Fluorescent (FL)	1 fM-0.5 pM	0.27 fM	15
Fluorescent (FL)	0.001 nM-10 nM	300 fM	16
Electrochemical Impedance Spectroscopy (EIS)	1 pM-1000 pM	0.3 pM	17
Electrochemical Impedance Spectroscopy (EIS)	10 fM-50 pM	4.63 fM	18
Photoelectrochemistry (PEC)	1 fM-500 fM	0.56 fM	19
Photoelectrochemistry (PEC)	1 fM-1 nM	0.26 fM	20

Electrochemiluminescence (ECL)	1 fM-100 pM	600 aM	21
Electrochemiluminescence (ECL)	0.5 fM-10 pM	0.17 fM	22
Electrochemiluminescence (ECL)	100 aM-100 pM	33 aM	23
Electrochemiluminescence (ECL)	1 fM-100 pM	0.28 fM	24
Electrochemiluminescence (ECL)	**0.1 fM-100 pM**	32 aM	**This work**

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