Electronic Supplementary Information

A novel Electrochemiluminescent Reagent of Cyclometalated Iridium Complex-Based DNA Biosensor and its Application in Cancer Cell Detection

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**Reagents.** IrCl$_3$·3H$_2$O, 2-phenylpyridine (ppy), 2,2'-bipyridine-4,4'-dicarboxylic acid (dcbpy), tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were ordered from Sigma-Aldrich. 6-Mercapto-1-hexanol (MCH) was obtained from Fluka. Cysteamine was purchased from TCI Development Co., Ltd. All the reagents were analytical grade and used without further purification. Double-distilled, deionized water was used throughout the experiments.

All of synthetic oligonucleotides were purchased from SBS Genetech Co. Ltd. (China), their base sequences are listed in Table S1. Klenow fragment of *E. coli* DNA polymerase (5 IU $\mu$L$^{-1}$, denoted as “Klenow” for short), the buffer for Klenow-catalyzed polymerization (denoted as “Klenow buffer” for short) and the mixture of four dNTPs (2.5 mM for each component) were purchased from TaKaRa Bio Inc. Nicking endonuclease Nb.BbvCI (10 IU $\mu$L$^{-1}$, potential nicking site: CCTCA▲GC) and its buffer NEBuffer were obtained from New England Biolabs Inc.

<table>
<thead>
<tr>
<th>Table S1. DNA Sequence Used in This Work</th>
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<tr>
<td>oligonucleotides name</td>
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<tr>
<td>Capture DNA</td>
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<tr>
<td>Target DNA-1</td>
</tr>
<tr>
<td>Signal probe DNA-1</td>
</tr>
<tr>
<td>Amino-modified aptamer for Ramos cell</td>
</tr>
<tr>
<td>Target DNA-2</td>
</tr>
<tr>
<td>Hairpin probe</td>
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<td>Signal probe-2</td>
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**Apparatus.** $^1$H NMR spectra were measured on Bruker AV 500 spectrometer (500
Spectrometer, using TMS as internal standard. Chemical shifts are expressed in ppm and $J$ values are given in Hz. IR spectra were recorded with a Nicolet FT-IR Magna750 spectrometer. Elemental analyses were performed with a Vario-EL III equipment. The electrochemical measurements for cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were carried out on a CHI 660C electrochemical working station (CH Instrument Co.) using a three-electrode system that consisted of a platinum wire as auxiliary electrode, an Ag/AgCl electrode as reference electrode, and a 3-mm-diameter Pt disk electrode as working electrode. The ECL emission was detected using a model MPI-A electrochemiluminescence analyzer (Xi’an Remex Analysis Instrument Co. Ltd. Xi’an, China) with a three electrode system consisting of a platinum wire as an auxiliary electrode, an Ag/AgCl electrode as reference electrode, and a 2-mm-diameter Au disk electrode as working electrode. Fluorescence spectra were recorded by Perkin-Elmer LS 50B luminescence spectrophotometer. UV-visible spectra were carried out on a Cary 50 UV-vis-NIR spectrophotometer (Varian). Transmission electron microscopy (TEM) image was taken with a JEOL JSM-6700F instrument (Hitachi).

**Synthesis of [(ppy)$_2$Ir(dcbpy)]$^+$PF$_6^-$ and (ppy)$_2$Ir(dcbpy)-NHS Ester** The iridium complex was synthesized according to the literature procedure with a slight modification.$^1$ The mixture of 2-phenylpyridine (0.34 g, 2.2 mmol), IrCl$_3$·3H$_2$O (0.34 g, 1 mmol) in a mixed solvent of 2-ethoxyethanol (10 mL) and water (3 mL) was stirred under N$_2$ at 120 ºC for 24 h. Cooled to room temperature, the precipitate was collected by filtration and washed with water, ethanol and acetone successively, and
then dried in vacuum to give [(ppy)₂Ir(μ-Cl)]₂ dimer. [(ppy)₂Ir(μ-Cl)]₂ (0.21 g, 0.2 mmol) as a solution in dichloromethane (25 mL) was added to a suspension of 2,2'-bipyridine-4,4'-dicarboxylic acid (0.098 g, 0.4 mmol) in methanol (25 mL). The reaction mixture was heated to reflux with stirring for 2 h, and then sodium acetate (excess) in methanol (5 mL) was added, and the mixture was stirred for a further 30 min. The solvent was then removed under reduced pressure, hydrochloric acid (1 M, 10 mL) was added, and the suspension was stirred for 10 min. The product was then filtered, washed with water (2 × 25 mL), and extracted into methanol. A saturated solution of ammonium hexafluorophosphate in methanol (5 mL) was added, and the mixture was stirred for a further 30 min. The solvent was removed under reduced pressure, and the residue was extracted into dichloromethane and filtered. The solvent was removed under reduced pressure to give [(ppy)₂Ir(dcbpy)]⁺PF₆⁻ as a dark-red powder. The crude product was flash chromatographed on silica gel using CH₂Cl₂ as eluent to afford the desired Ir(III) complex. (0.12 g, 65%).

¹H NMR (500 MHz, CDCl₃) δ: 6.21 (d, J = 6.0 Hz, 2H), 6.56 (t, J = 7.50 Hz, 2H), 6.74 (m, 2H), 6.88 (t, J = 7.50 Hz, 2H), 7.33 (t, J = 7.50 Hz, 2H), 7.48 (d, J = 7.50 Hz, 2H), 7.54 (t, J = 7.50 Hz, 2H), 7.74 (t, J = 7.50 Hz, 2H), 7.87 (m, 2H), 8.58 (d, J = 5.50 Hz, 2H), 9.24 (s, 2 H). Mp (°C): 194.5–196. Calcd for C₃₄H₂₄F₆N₄O₄IrP: C, 45.87; H, 2.69; N, 6.31. Found: C, 46.30; H, 3.13; N, 5.96. IR: ν 2923 (OH), 1719 (CO), 843 (PF) cm⁻¹.

To synthesize activated (ppy)₂Ir(dcbpy)−NHS ester, EDC (0.17 g, 1.11 mmol) and NHS (0.119 g, 1.035 mmol) were dissolved in acetonitrile (1.5 mL) with stirring and
cooled in an ice bath. \((ppy)_2\text{Ir(dcbpy)}\)\(^+\) PF\(_6^−\) solution in acetonitrile (100 µL, 4.3 × 10\(^{-4}\) M) was added to. The mixture was stirred for 2 h at room temperature to form

\((ppy)_2\text{Ir(dcbpy)}\)-NHS ester. The formed \((ppy)_2\text{Ir(dcbpy)}\)-NHS ester (2.69 × 10\(^{-5}\) M) was kept in freezer (4 ºC) for further use.

**UV-Visible Absorption, Fluorescence and ECL Emission Spectra of**

\[(ppy)_2\text{Ir(dcbpy)}\]\(^+\) PF\(_6^−\). The UV-visible absorption, fluorescence and ECL emission spectra of \[(ppy)_2\text{Ir(dcbpy)}\]\(^+\) PF\(_6^−\) were depicted in Figure S1(A). The most intense absorption band at 258 nm of UV region displays the ligand-centered (LC) \(\pi \rightarrow \pi^∗\) transitions. The shoulder appearing at 369 nm is likely due to spin-allowed metal-to-ligand charge-transfer (MLCT) transitions according to the previous reports\(^2\) and the calculations of Hay.\(^3\)

**Figure S1** UV-vis absorption (a), ECL spectra in phosphate buffer (pH = 7.4) (b) and photoluminescence spectra (c) of 10 µM iridium complexes in CH\(_3\)CN solution at room temperature, the excitation wavelength is 508 nm (A) and cyclic voltammogram (B) containing 1 mM iridium complexes and 0.1 M Bu\(_4\)NPF\(_6\) in CH\(_3\)CN solution, scan rate = 0.1 V/s.

On irradiation with light of 508 nm, the complex shows strong photoluminescence in CH\(_3\)CN with \(λ_{\text{max}} = 588\) nm. It is attributed to the presence of an excited state resulting from the mixing of comparable percentages of \(^3\)LC and \(^3\)MLCT states, which
is in agreement with Bandini’s\textsuperscript{4} work concerning analogous iridium complexes.

Strong spin-orbit coupling of central metal atoms facilitates the spin-forbidden $^3\text{MLCT}$ transitions of the metal complexes.

The study of the electrochemical properties of the new iridium(III) complex was conducted at a Au electrode in acetonitrile solution (0.1 M Bu$_4$NPF$_6$) by cyclic voltammetry. Half-wave potentials ($E_{1/2}$) were determined by cyclic voltammetry as the average of anodic and cathodic peak potentials ($E_{1/2} = (E_{pa} + E_{pc})/2$). As Figure S1(B) shown, the new iridium(III) complex showed $E_{1/2}$ at ca. +1.20 V vs. Ag/AgCl which was assigned as the oxidation of iridium(III), similar to other related iridium(III) complexes.\textsuperscript{5} The oxidation couple was lower than the Ru$^{2+/3+}$ redox couple of [Ru(bpy)$_3$]$^{2+}$ ($E_{1/2} = 1.26$ V vs. Ag/AgCl).\textsuperscript{6} The peak current ratio ($i_{pa}/i_{pc}$) and peak separation ($\Delta E_p$) were 1.45 and 88 mV at a scan rate of 0.1 V/s, which indicated a quasi-reversible one-electron-transfer system.

The ECL spectrum of the complex is obtained in PBS, with TPA as an oxidative-reductive coreactant. The spectrum consists of a single peak with maximum emission at about 596 nm, consistent with the fluorescence of the parent molecule, indicating that the ECL emission is due to the MLCT transitions. On the basis of the ECL experiments of Ru(bpy)$_2^{2+/TPA}$ and the homologous iridium species,\textsuperscript{5} we speculate that the ECL from [(ppy)$_2$Ir(dcbpy)]$^+$PF$_6^-$ in the presence of TPA can be expressed as follows:

\begin{align*}
\text{Ir} &\rightarrow \text{Ir}^+ + e^- \quad (1) \\
\text{TPA} &\rightarrow \text{TPA}^{2+} + e^- \quad (2)
\end{align*}
\[
\begin{align*}
1 & \quad \text{Ir}^{\text{III}} + \text{TPA} \rightarrow \text{Ir} + \text{TPA}^{\text{IV}} \\
2 & \quad \text{TPA}^{\text{IV}} \rightarrow \text{TPA}^{\text{II}} + \text{H}^+ \\
3 & \quad \text{TPA}^{\text{II}} + \text{Ir}^{\text{II}} \rightarrow \text{Ir}^{\text{III}} + \text{TPA} \\
4 & \quad \text{Ir}^{\text{III}} \rightarrow \text{Ir} + \hbar \nu
\end{align*}
\]

Where Ir represents \([(ppy)_2\text{Ir(dcbpy)}]^+ \text{PF}_6^-\) complex. In the system, the generation of cation radicals of TPA\(^{\text{IV}}\) (eq 2 and 3) plays key roles in light emission since the deprotonation process of TPA\(^{\text{IV}}\) to TPA\(^{\text{II}}\) (eq 4) can be taken at an extremely rapid and oxidation of the emitter (reaction 1) could occur relatively fast. Direct oxidation of TPA on electrode (eq 2) usually occurs relatively slowly in a positive applied potential (0.80 V vs Ag/AgCl). Therefore, the electron-transfer reaction (eq 3) is an important factor for the effective formation of excited-state \(\text{Ir}^{\text{III}}\) in this ECL system. The oxidation potential of the emitter (\(E^\circ(\text{Ir}^{\text{II}}/\text{Ir})\)) should be positive enough for an efficient generation of TPA\(^{\text{IV}}\).

In the presence of TPA as co-reactant, the ECL intensity-potential curve and corresponding cyclic voltammograms in phosphate buffer (pH = 7.4) of \([(ppy)_2\text{Ir(dcbpy)}]^+ \text{PF}_6^-\) were recorded as shown in Figure S2 (A) and Figure S2 (B), respectively. The onset of luminescence occurred at approximately 1.2 V vs. Ag/AgCl.
Figure S2  ECL intensity-potential curve (A) and corresponding cyclic voltammograms (B) of 10 μM [(ppy)$_2$Ir(dcbpy)]$^+$ PF$_6^-$ in buffer (pH = 7.4) in the presence of 0.025 M TPA at room temperature (scan rate = 0.1 V/s)

Preparation of AuNPs. AuNPs were prepared according to the method reported previously with a slight modification. HAuCl$_4$ and tri-sodium citrate solutions were filtered through a 0.22-μm microporous membrane filter prior to use, and then 10 mL of 38.8 mM trisodium citrate in H$_2$O was added to 100 mL of boiling 1.0 mM HAuCl$_4$ solution and stirred for 10 min under refluxing. The color of the solution changed from purple to blue and to wine red in the end, which was an indication for the formulation of the AuNPs. The nanoparticle solution was allowed to cool down to room temperature with continuously stirring. The final AuNPs prepared by this method have an average diameter of ~20 nm as measured by TEM (Figure S3). According to our group’s previous calculation, the Au ion concentration in a solution of AuNPs is ~6.77 × 10$^{-10}$ M.

Figure S3 The TEM image of AuNPs
**Preparation of ECL Nanoprobes.** ECL nanoprobe was constructed through two facile steps. Briefly, the first step was to load cysteamine and thiolated signal probe DNA on AuNPs and then thiols modified AuNPs and [(ppy)_2Ir(dcbpy)]^+PF_6^-NHS ester were mixed and incubate for 2 h. The resulting ECL nanoprobes are separated by centrifugation. The success of the biobarcode amplification is closely related to the loading quantity of the cysteamine conjugated with [(ppy)_2Ir(dcbpy)]^+PF_6^-NHS. Therefore, to construct the optimal ECL nanoprobes, the effect of the different molar ratios of cysteamine–signal probe DNA from 1:1, up to 80:1 on ECL intensities of ECL nanoprobes was investigated (Figure S4). The ECL intensity increases with the increase of ratios of cysteamine–signal probe DNA, and reach the maximum at the ratio of 10:1, then decline. So we found that the optimal ratio of cysteamine to probe DNA was 10:1.

![Figure S4](image_url)

**Figure S4** The ECL intensities of different molar ratio of cysteamine to signal probe DNA

The UV-visible spectra of AuNPs, Ir complex and nonoprobe were recorded by the spectrophotometer as shown in Figure S5. The curve of nonoprobe exhibited both the characteristic absorbance of Ir complex (258 nm and 369 nm) and the characteristic absorbance of AuNPs (~510 nm). The results indicated that the nonoprobe had been successfully fabricated.
The concrete procedure of ECL nanoprobe preparation according to the method reported previously with a slight modification. 1.5 μL of 500 mM tris-HCl (pH = 8.2), 6 μL of 10 mM TCEP, 7.2 μL of 100 μM signal probe DNA-1, and 7.2 mL of 100 μM cysteamine were mixed and incubated for 30 min at room temperature. Then add 1 mL above prepared AuNPs to the mixture and incubate for 6 h at room temperature. Subsequently, 120 mL of 0.1 M sodium boracic acid buffer (pH 9.0) and 330 μL of above prepared (ppy)$_2$Ir(dcbpy)-NHS ester was added, and the tube was wrapped in foil and placed on an orbital shaker for 10 h. Then the solution was centrifuged at 4 °C for 30 min at 12000 rpm and resuspended in 800 μL of washing buffer (100 mM NaCl, 25 mM tris acetate, pH 8.2), repeat 4 times.

Preparation of ECL biosensor of Scheme 2B (in main text) A gold electrode was polished carefully with alumina slurries (1, 0.3, 0.05 μm) and washed ultrasonically with deionized and doubly distilled water. Then it was electrochemically cleaned in 0.5 M H$_2$SO$_4$ solution by cyclic potential scanning between 0.3 and 1.5 V until a standard CV was obtained. Subsequently, the gold electrode was rinsed with deionized and doubly distilled water and absolute ethanol in turn. The electrode was
immersed in 100 μL of 0.1 M PBS buffer containing $1 \times 10^{-7}$ M capture DNA-1 and incubated for 4 h at 37 °C. In order to avoid consequent nonspecific adsorption in the following hybridization steps, the modified electrode was immersed in the sodium phosphate solution containing 200 μL of 1 mM MCH for 1 h to block the uncovered gold surface. The modified electrode with capture DNA-1 was immersed into 200 μL of 0.1 M PBS buffer containing target DNA-1 of different concentrations at 37 °C. Two hours later, the modified electrode was taken out and immersed into 200 μL of 0.1 M PBS buffer containing signal probe DNA-1 functionalized with AuNPs for 2 h. Rinsing the electrode surface with 0.1 M phosphate buffer after each step of the fabrication process is very important to remove nonspecifically adsorbed sequences.

**Preparation of ECL sensor of Scheme 3 (in main text).** The processes of fabrication include two steps: (I) The modification of Au electrodes. The Au working electrodes was cleaned according to the above method. AuNPs were deposited on the working electrodes through electrochemical reduction, which was conducted in a solution containing 6.0 mM chloroauric acid and 0.1 M potassium nitrate under –0.4 V (vs. Ag/AgCl) for 400 s. 40 μL solution of hairpin DNA ($10^{-7}$ M) was cast on the working electrodes, which were placed in petri dishes for 14~16 h to allow the absorption to proceed without excessive evaporation, and then washed with 200 μL TE buffer. At last they were treated with 60 μL MCH solution (1 mM) for 2 h to block any unoccupied site on the electrode surface. (II) Strand-replacement DNA polymerization and strand scission cycle. The Au electrodes with hairpin probes were then dipped into the mixture containing the ECL Nanoprobes (65 μL) with primer, certain
concentration of target DNA (10 μL), Klenow (2 μL), dNTP (10 μL, 12 μM for each component), Nb.BbvCL (0.5 μL), NEBuffer (10 μL), and the system was allowed for incubating for further 3 h. then electrode was taken out and washed them thoroughly to carry out the following measurement. The result was shown in Figure S6.

Figure S6. The calibration curve of ECL Intensities vs. the concentration of target DNA from $2.0 \times 10^{-15}$ to $10^{-12}$ M (Scheme 2B). Inset is the amplification of the linear range from $4.0 \times 10^{-15}$ to $10^{-14}$ M for target DNA determination. The Blank was subtracted for each value.

Cancer cell detection of Scheme 4 (in main text). The process is as follows: (I) The construction of the magnetic beads probe attached with aptamer and messenger (its sequence is the same as target DNA-2). 150 μL solution of the aptamer sequence ($10^{-7}$ M) was mixed with 200 μL solution of imidazole-HCl solution (pH 7.0, 0.1 M) in a 1.5 mL Eppendorf tube, and was left to stand for 30 min to form the activated aptamers. In another Eppendorf tube, 50 μL carboxyl-modified magnetic beads suspension (5mg/mL) was mixed with 1 mL EDC solution (0.8 M), and left to stand for 60 min to obtain the activated magnetic beads. When both were activated, the aptamer and magnetic beads were mixed, and the reaction was allowed to proceed for 12 h to form the aptamer- magnetic beads, which were washed thrice with 200 μL of Tris-HCl buffer each time, and then magnetically separated on an Affimag magnetic stand. The aptamer-magnetic beads were then re-dispersed in 500 μL Tris-HCl buffer
(pH 4.8) to form a suspension of 0.5 mg/mL aptamer-magnetic beads. (II) 200 μL suspension described above was mixed with 200 μL solution of messenger sequence (10⁻⁷ M). After 2 h of hybridization, the magnetic beads with aptamer-messenger hybridization complex were magnetically separated, and washed thrice with Tris-HCl, and then re-dispersed in 500 μL Tris-HCl buffer (pH 4.8) to form a suspension of 0.2 mg/mL magnetic beads. (III) 100 μL suspension of aptamer-messenger-magnetic beads conjugates (0.2 mg magnet beads/mL) were added to 200 μL Ramos cell suspension of certain concentration, and was incubated under 37 °C for 1 h. The suspension was then magnetically separated, and 50 μL supernatant was sampled for the following analysis.

**Specificity of Scheme 4 (in main text).**

The assay was initiated by the aptamer recognition of cancer cell, whose specificity endowed the whole reaction network with selectivity. To demonstrate this, Ramos cell and two control analytes of CEM and Hela with the same number were introduced into this network using the same method. The result showed in Figure S6, as expected, the ECL signals of the sensor have little changes in the presence of Hela and CEM cells compared to the blank, while the ECL intensity obtained in the presence of Ramos cell is much higher than that of the ECL intensities of the above.
Figure S7. Specificity for the detection of Ramos cell against CEM and Hela of scheme 4.
Numbers of Hela, CEM and Ramos cells are all 40 cells. The Blank was subtracted for each value.

Real blood sample detection. Different volume ratios of real blood was added to the
PBS suffer containing a desired amount of Ramos cells (1000 cells in the experiment),
and the total volume was 200 μL, the mixture solution was used to incubate with
aptamer-target-magnetic beads conjugates. Other process is the same as cell detection.
The effect of volume ratios of real blood versus ECL intensity is illustrated in Figure
S6. It was found there was no matrix effect on the responses of detection when added
blood is less than 6% in volume (see Figure S6 in SI). However, a significant decrease
of the signal was observed after adding more than 8% of blood. Herefore, 6% of
human blood in volume was used in the following experiments. The resulting
calibration curve is shown in Figure S7.
Figure S8. The curve of ECL intensity vs. volumes of human blood added in the sample solution.

Figure S9. The calibration curve for Ramos cell determination in samples containing 6% real human blood in volume. The number of cells detected in the assay is 0; 8; 12; 16; 20; 40; 80 and 120 cells.

ECL Measurement. The finally hybridized electrode for determination of target DNA and cancer cell were performed in 2.0 mL of 0.1 M PBS containing 0.025 M TPA by Cyclic Voltammetry. The potential scan was from 0.2 to 1.25 V (vs Ag/AgCl) and the scan rate was 0.1 V/s. Each measurement was repeated for at least five times. The average of the three closest readouts was adopted as the result. The concentration of target DNA (or Ramos cell content) was quantified by an increase of ECL peak height $\Delta I$ ($\Delta I = I - I_0$), where $I$ is the ECL peak height after hybridization with signal probe and $I_0$ is the ECL peak height in absence of target.
Cancer Cell Culture. Ramos cells (target cells), CEM and Hela cells (control cells) were cultured in cell flasks according to the instructions from the American Type Culture Collection. The cell line was grown to 90% confluence in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS) and 100 IU/mL penicillin-streptomycin at 37 ºC, followed by culturing cells in a humidified atmosphere with 5% CO₂. The cell density was counted on a hemocytometer prior to each experiment. Then, a 1.0 mL suspension of ~8.0 × 10⁶ cells dispersed in RPMI 1640 cell media buffer was centrifuged at 3500 rpm for 5 min and washed with phosphate-buffered saline (18.6 mM phosphate, 4.2 mM KCl, and 154.0 mM NaCl) five times and resuspended in 1.0 mL cell media buffer.

Control experiment

Preparation of [(bpy)₂(dcbpy)Ru]²⁺(PF₆)⁻₂ and [(bpy)₂(dcbpy)Ru]²⁺(PF₆)⁻₂-NHS. Synthetic procedure of Ru bis(2,2'-bipyridine) (2,2'-bipyridine-4,4'-dicarboxylic acid) bis(hexafluoropropylene phosphor) ([(bpy)₂(dcbpy)Ru]²⁺(PF₆)⁻₂) was performed according to the procedure reported previously. The Ru(bpy)₂Cl₂ (0.16 g, 0.328 mmol), NaHCO₃(0.16 g), and 2, 2'-bipyridine-4, 4'-dicarboxylic acid (0.12 g, 0.491 mmol) were added to methanol-H₂O solution (10 mL, methanol : H₂O (V) = 4:1) and the mixture was heated at 80 ºC for 4 h. The solution was cooled in ice bath for 2 h, and pH of the mixing solution was adjusted with hydrochloric acid solution to pH 4.4. The formed precipitate was filtrated and washed with methanol. NaPF₆ (2.0 g) in water (14 mL) was added in the resulting filtrate, and then cooled in ice bath. The latter formed precipitate was collected by filtration, and dried.
To synthesize [(bpy)$_2$(dcbpy)Ru]$^{2+}$$(PF_6)^{-2}$-NHS ester, EDC (0.17 g, 1.11 mmol) and NHS (0.119 g, 1.035 mmol) were dissolved in N’, N-dimethylformamide (DMF, 1.5 mL) with stirring and cooled in ice bath. [(bpy)$_2$(dcbpy)Ru]$^{2+}$$(PF_6)^{-2}$ (0.15 g, 0.16 mmol) in DMF (1 mL) was added, and the mixture was stirred for 2 h. The formed [(bpy)$_2$(dcbpy)Ru]$^{2+}$$(PF_6)^{-2}$-NHS ester was diluted to $1 \times 10^{-5}$ M in PBS buffer solution (pH 7.4) and kept in freezer (4 ºC) for further use.

Method. The processes of fabrication ECL nanoprobe labeled with [(bpy)$_2$(dcbpy)Ru]$^{2+}$$(PF_6)^{-2}$ and fabrication of sensor (see Figure S1) for detection of DNA were the same as Ir complex above.

![Scheme S1 Process of ECL nanoprobe preparation labeled with Ru complex (A) and the determination of target DNA (B).](image)

The resulting calibration plot of the ECL intensity vs. the concentration of target DNA is illustrated in Figure S7 (Scheme 2B, based on Ru complex). The results showed that the ECL intensities increased with the increase of concentration of the target DNA ranging from $8.0 \times 10^{-13}$ to $10^{-11}$ M with the equation of $\Delta I_{ECL} = 8.535C + 36.780$ ($\Delta I_{ECL}$ is the ECL intensity subtracted the blank; $C$ is the concentration of target DNA, $10^{-12}$ M; N = 8; $R = 0.99809$). The detection limit of $3.2 \times 10^{-13}$ M target DNA could be estimated using $3\sigma$. 
**Figure S10** (A) The ECL response of different target DNA concentration. (a) Blank; (b) 0.8 pM; (c) 1 pM; (d) 5 pM; (e) 10 pM; (f) 30 pM; (g) 50 pM; (h) 80 pM; (i) 100 pM. (B) The calibration curve of ECL responses vs. the concentration of target DNA from $8.0 \times 10^{-13}$ to $10^{-11}$ M. The Blank was subtracted for each value.

The processes of constructing of cycle based on strand-displacement DNA polymerization and nicking endonuclease (see Scheme S2) were also the same as Ir complex.

**Scheme S2** Schematic representation of strategy labeled with Ru complex for DNA detection based on DNA polymerization and nicking endonuclease assisted circular amplification process.

The resulting curve of target DNA detection based on DNA polymerization and nicking endonuclease assisted circular amplification is illustrated in Figure S8. The fitting equation is $\Delta I_{ECL} = -0.039C^2 + 9.947C + 12.849$ ($\Delta I_{ECL}$ is the ECL intensity subtracted the blank; C is the concentration of target DNA, $10^{-14}$ M; N = 9, R =
0.9947) ranging from $3 \times 10^{-14}$ to $1 \times 10^{-12}$, and the detection limit of $9.7 \times 10^{-15}$ M could be estimated by using $3\sigma$ ruler.

**Figure S11** The calibration curve of ECL Intensities vs. the concentration of target DNA from $3.0 \times 10^{-14}$ to $1.0 \times 10^{-12}$ M based on DNA polymerization and nicking endonuclease assisted circular amplification process. The concentration of target DNA detected to fit the curve is 0; $3.0 \times 10^{-14}$ M; $5.0 \times 10^{-14}$ M; $8.0 \times 10^{-14}$ M; $1.0 \times 10^{-13}$ M; $3.0 \times 10^{-13}$ M; $5.0 \times 10^{-13}$ M; $8.0 \times 10^{-13}$ M; $1.0 \times 10^{-12}$ M.

The processes of the strategy based on Ru complex for cell detection is illustrated in **Scheme S3** as the same as that based on Ir complex.

**Scheme S3** Schematic representation of strategy labeled with Ru complex for Ramos cell detection based on DNA polymerization and nicking endonuclease assisted circular amplification process.
**Figure S12** The calibration curve for Ramos cell determination (Scheme 3) from 120 to 2000 cells target cells. The Blank was subtracted for each value. The number of cells detected in the assay is 0; 120; 160; 200; 400; 800; 1200; 1600 and 2000 cells.

The resulting curve of Ramos cell based on DNA polymerization and nicking endonuclease assisted circular amplification is illustrated in Figure S9. The ECL intensities increased with the increase of number of Ramos cell ranging from 120 to 2000 cells with the equation is

\[
\Delta I_{ECL} = -1.993 \times 10^{-4} C^2 + 0.790 C - 15.189 \quad (\Delta I_{ECL} \text{ is the ECL intensity subtracted the blank; } C \text{ is the number of Romos cells; } N = 9, \ R = 0.9921),
\]

and the detection limit of 87 cells could be estimated by using 3σ ruler.

**Notes and References**

4. M. Bandini, M. Bianchi, G. Valenti, F. Piccinelli, F. Paolucci, M. Monari, A. Umani-Ronchi, M.


