Supporting Information:

Linear $Ru(bpy)_3^{2+}$−Polymer as a Universal Probe for Sensitive Detection of Biomarkers with Controllable Electrochemiluminescence Signal-Amplifying Ratio

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Contents

1. SUPPORTING DATA FOR THE MANUSCRIPT

1.1 Synthesis and Characterization of $Ru(bpy)_3^{2+}$-NHS ester

Synthesis route of $Ru(bpy)_3^{2+}$-NHS ester is constituted by the synthetic procedure of $Ru(bpy)_2(dcbpy)(PF_6)_2$ and the activation steps. The $Ru(bpy)_2(dcbpy)(PF_6)_2$ is synthetized by refluxing the reactant of cis-dichlorobis(2,2′-bipyridine)ruthenium(II) with a excess of the corresponding 2,2′-bipyridine-4,4′-dicarboxylic acid (1:1.2) in an ethanol/water (80%) mixture. Then, the mixture was incubated at 80 ºC for one night. The solution was subsequently cooled and acidified (pH=4.4) to crystallize out the unreacted 2,2′-bipyridine-4,4′-dicarboxylic acid. The sediment was washed and eliminated by the qualitative filter paper based suction filter. This process was crucial and indispensable for eliminating the dissociative 2,2′-bipyridine-4,4′-dicarboxylic acid. It was because of 2,2′-bipyridine-4,4′-dicarboxylic acid can participate in the subsequent reactions.

Subsequently, the pre-cooled solution of sodium hexafluorophosphate (NaPF6) was added to the resultant filtrate isolates the ruthenium complexes as PF6 salts. The mixture was cooled with ice-water mixture. In this step, ice water mixture was regarded as the cold source. After three hours' reaction, the replacement was sufficiently accomplished. Then, a programmed gradient of pH (pH=4, 3, 2, 1) was executed to get the satisfactory yields and purity without recrystallization. The crystal products were treated with the freeze drying process to eliminate the influence of volatile solvent. Hereto, the synthetic procedure of $Ru(bpy)_2(dcbpy)(PF_6)_2$ terminated. The powder of $Ru(bpy)_2(dcbpy)(PF_6)_2$ can be stored at -20 ºC refrigerator for the long-term preservation.

Definitively, the product of $Ru(bpy)_2(dcbpy)(PF_6)_2$ was activated by reacting with N-Hydroxysuccinimide (NHS) and N,N′-dicyclohexylcarbodiimide (DCC) in organic solvent. This reaction requires an anhydrous organic solvent (N,N′-dimethyl formamide (DMF) or dimethylsulfoxide (DMSO) to prevent the hydrolysis of the NHS ester. This reaction was processed in an airtight and anhydrous system at 37 ºC for one night. Then, the precipitation generated in reaction process was eliminated through the centrifugal process. The supernatant liquid was the final products of

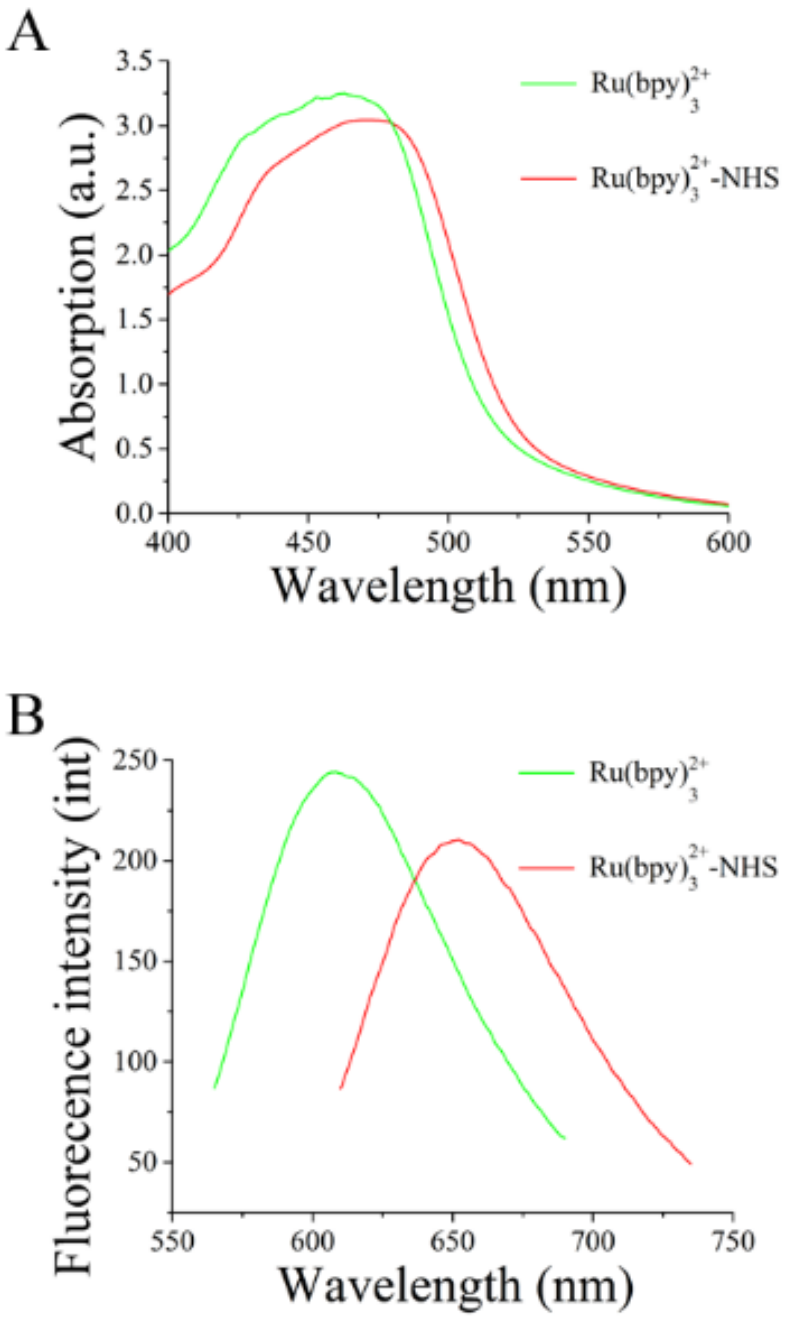

Figure S1. The spectral characterization of the $Ru(bpy)_3^{2+}$ and $Ru(bpy)_3^{2+}$-NHS ester A. Absorption spectrum of the $Ru(bpy)_3^{2+}$ and $Ru(bpy)_3^{2+}$-NHS ester. B. Fluorescence spectrum of the $Ru(bpy)_3^{2+}$ and $Ru(bpy)_3^{2+}$-NHS ester.

$Ru(bpy)_3^{2+}$-NHS Ester which could be employed as the directly reactant for amino-labeled DNA. For quality evaluations of the products, the spectral characterization of the $Ru(bpy)_3^{2+}$ and $Ru(bpy)_3^{2+}$-NHS ester was shown in Figure S1. Obvious changes of the absorption and emission spectrum were produced in absorption and emission spectrum were produced in the synthesis process.

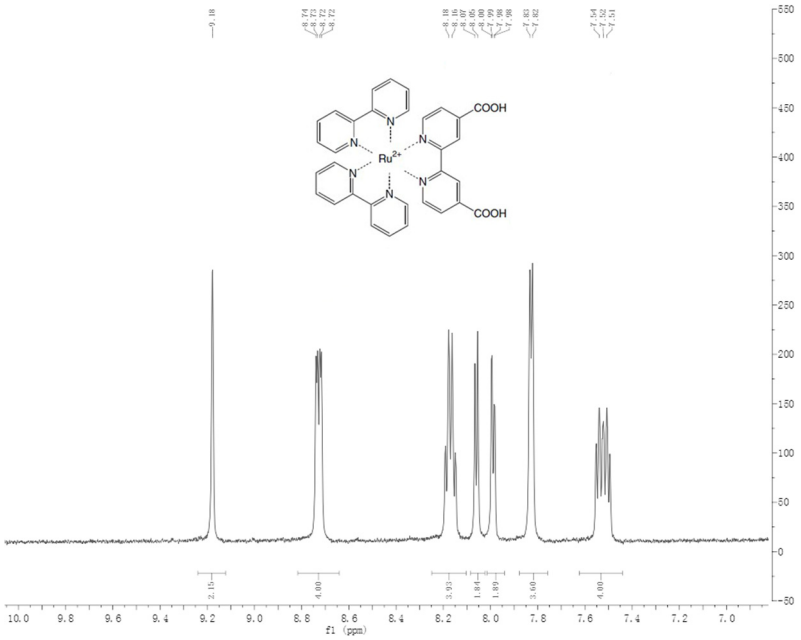

Figure S2. The characterization of ^{1}H nuclear magnetic resonance(NMR) for $Ru(bpy)_3^{2+}$ in d6-DMSO at 500 MHz.

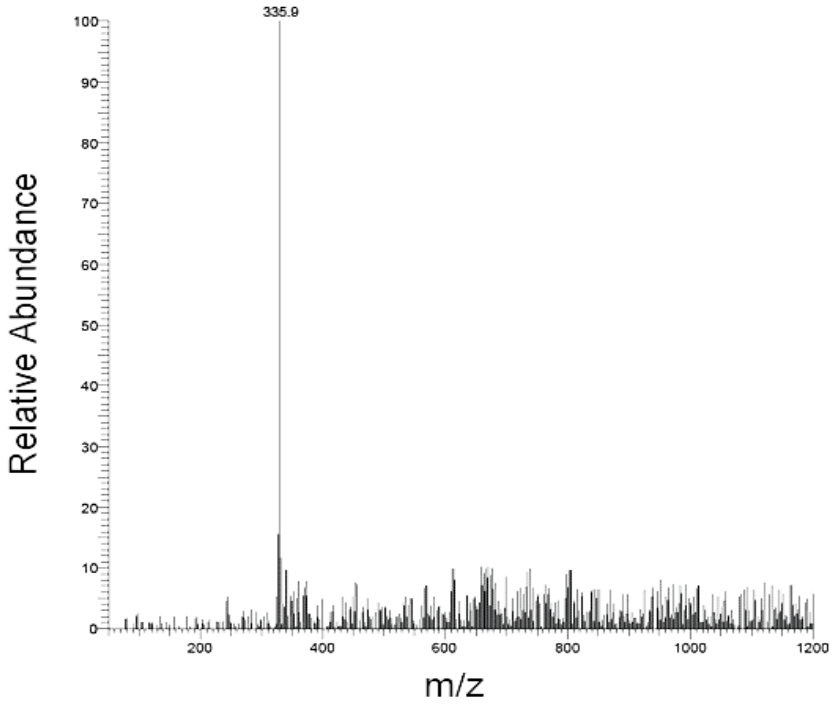

Figure S3. The characterization of electrospray ionization mass spectrometry(ESI-MS) for $Ru(bpy)_3^{2+}$.

We also examined the characterization of ^{1}H nuclear magnetic resonance (NMR) for $Ru(bpy)_3^{2+}$ in d6-DMSO at 500 MHz. The results recorded in Figure S2 indicated that the NMR of $Ru(bpy)_3^{2+}$ accorded with the to the theoretical prediction.

Meanwhile, the characterization of electrospray ionization mass spectrometry (ESI-MS) for $Ru(bpy)_3^{2+}$ was simultaneously executed. The result in Figure S3 showed that the synthesis of $Ru(bpy)_3^{2+}$ was feasible.

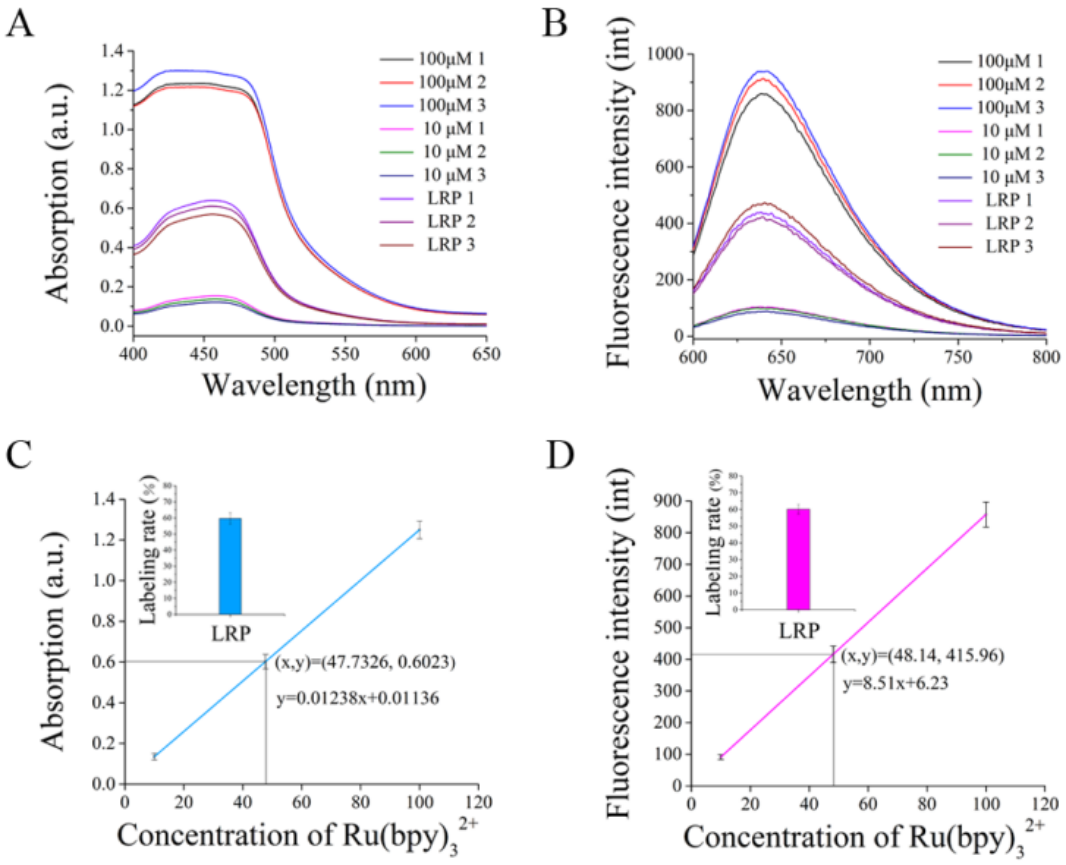

Figure S4. A. Absorption spectrogram of standard $Ru(bpy)_3^{2+}$ with different concentrations. B. Fluorescence spectrogram of standard $Ru(bpy)_3^{2+}$ with different concentrations. C. Standard curves of absorption for $Ru(bpy)_3^{2+}$ and the corresponding labeling rate of $Ru(bpy)_3^{2+}$ on PLL. D. Standard curves of fluorescence for $Ru(bpy)_3^{2+}$ and the corresponding labeling rate of $Ru(bpy)_3^{2+}$ on PLL.

1.2 Synthesis process and characterization data of linear $Ru(bpy)_3^{2+}$-polymer

As shown in Figure 1, linear $Ru(bpy)_3^{2+}$-polymer was synthesized by employing PLL as the binding-skeleton, the repetitive amino group of each monomer on PLL was employed as the binding site of $Ru(bpy)_3^{2+}$. The powder of PLL was obtained from Sigma Aldrich, and the solution of PLL showed colorless and transparent. The PLL (25mg in 1.5mL water) was mixed with excess $Ru(bpy)_3^{2+}$, reached a molar ratio

of 1:10 between the amino and $Ru(bpy)_3^{2+}$. The solution of the mixture turned to crimson. Then, the mixture was sealed and incubated in dimethylformamide (20 percent) for 12 hours at 37 ºC. The insoluble substance was eliminated by centrifuge (12000r/min for 1min). Finally, the products were purified by the Nanosep 10K OMEGA tubular ultrafiltration membrane from Pall Corporation. The purified linear $Ru(bpy)_3^{2+}$-polymer was freeze-dried for 12 hours. And the powder showed brown.

The absorption spectrum of standard $Ru(bpy)_3^{2+}$ is shown in Figure S4A. The fluorescence spectrum of standard $Ru(bpy)_3^{2+}$ was also recorded, as shown in Figure S4B. The standard absorption and fluorescence curves for $Ru(bpy)_3^{2+}$ were simultaneously recorded and are shown in Figure S4C and D, respectively. Additionally, the absorption and fluorescence spectra of the linear $Ru(bpy)_3^{2+}$-polymer were recorded to calculate the extent of labeling via comparison with the standard curves. Both the absorption and the fluorescence spectra indicated that a labeling rate of approximately 60% $Ru(bpy)_3^{2+}$ on PLL was achieved. This labeling rate was achieved in an excess of $Ru(bpy)_3^{2+}$ and thus represents the saturated labeling amount of $Ru(bpy)_3^{2+}$ on the linear $Ru(bpy)_3^{2+}$-polymer.

1.3 Frequency optimization of magnetic separation

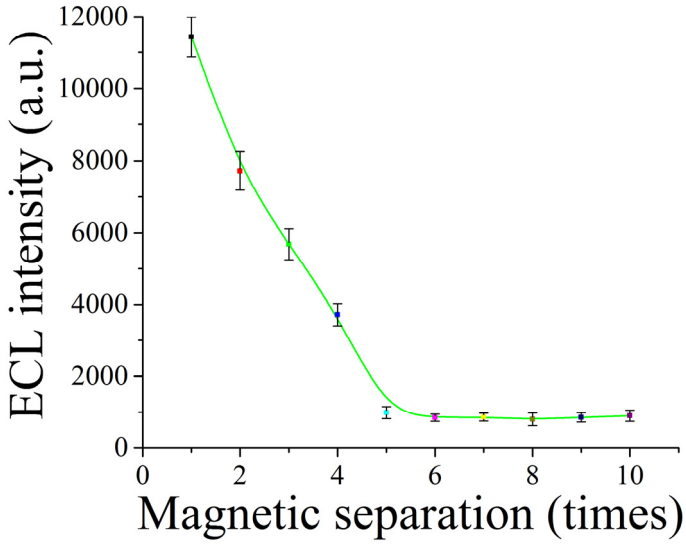

Figure S5. Frequency optimization of magnetic separation and washing step.

The optimization of magnetic separation frequency was executed to prevent the influence from the non-specific adsorption of linear $Ru(bpy)_3^{2+}$-polymer. In this assay, the ECL intensity of control group was detected after each magnetic separation process. The result was shown in Figure S5. It indicated that the ECL intensity of control group was decreased with the magnetic separation process. After five times magnetic separation, the ECL intensity tended to stable. More magnetic separation could not get lower background signal of ECL. Thus, five times magnetic separation was chosen as the optimal frequency.

1.4 Sequence

Table S1. Sequence of polymer-amplified ECL assay for nucleic acid.

Note	Sequence(5'-3')	Modification	Annealing temperature
Target	ACTAGTAAACTGAGCATACTGGC CAGGACACGTGGGTGC	label-free	68.3 °C
Capture probe	GCTCAGTTTACTAGTGCCATTT	3'-biotin	64.3 °C
Signal probe	GGAGCAGGAGCACCCACGTGTC CTGGCC	5'-NH_2C_6	64.3 °C
Random sequence 1	CACCAGCTACATCGGATCCGAGC TCAT ACGAATATCCAC	label-free	65.7 °C
Random sequence 2	CATCAGATGATGAGTTGAACGGG TGAGGCGGTAGTCCTA	label-free	66.6 °C

Table S2. Sequence of polymer-amplified ECL assay for 16sRNA.

Note	Sequence(5'-3')	Modification	Annealing temperature
Capture probe	ACTATCCATTGTAGCACGTGAAAAAA	3'-biotin	52.2 °C
Signal probe	AAAAAATGGGATTAGCTCCACCTCGC	5'-NH_2C_6	50.3 °C

Table S3. Sequence of polymer-amplified ECL assay for thrombin.

Note	Sequence(5'-3')	Modification	Annealing temperature
Capture probe	AAAAAAGTCCGTGGTAGGGCAGGTT GGGGTGACT	5'-biotin	68.5 °C
Signal probe	GGTTGGTGTGGTTGGAAAAAA	3'-NH_2C_6	50.3 °C

1.5 Comparison of linear $Ru(bpy)_3^{2+}$-polymer for immunoassay and nucleic acid

In this assay, we found that the ECL intensity of polymer amplified immunoassay was higher than the linear $Ru(bpy)_3^{2+}$-polymer for nucleic acids. We compared the probe of polymer amplified assay for immunoassay and nucleic acids with equal concentration, the results showed that the ECL intensity of immunoassay is twice or three times higher than the probes for nucleic acids (shown in Figure S6). This phenomenon is attributed to the different amount of amino on antibody and nucleic acids. The probe used in the polymer amplified assay for nucleic acids was modified with single amino. Inversely, the antibody used in the polymer amplified assay for immunoassay is endowed with several amino that is from the lysine or other amino

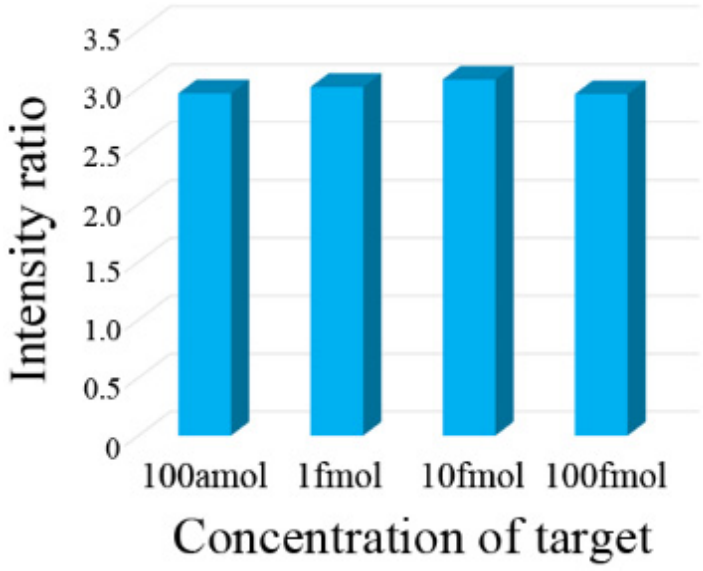

Figure S6. Comparison of linear $Ru(bpy)_3^{2+}$-polymer for nucleic acid and immunoassay.

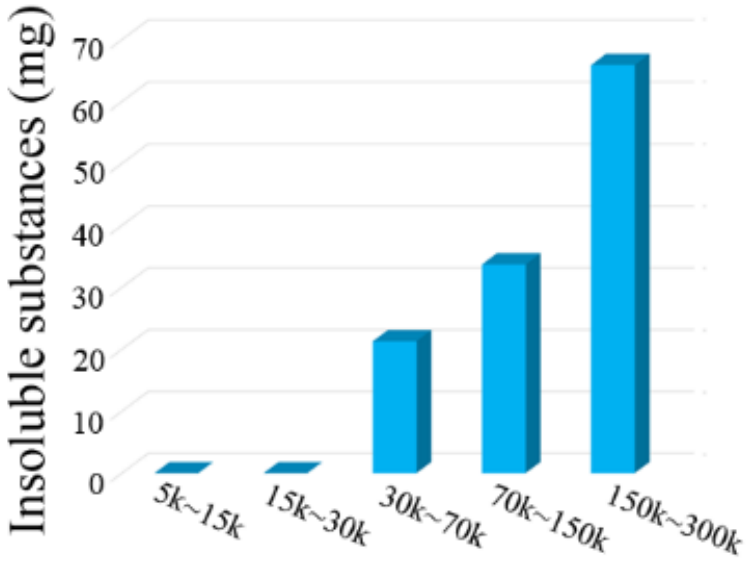

Figure S7. Quantity of insoluble substance in the synthesis process of linear $Ru(bpy)_3^{2+}$-polymer.

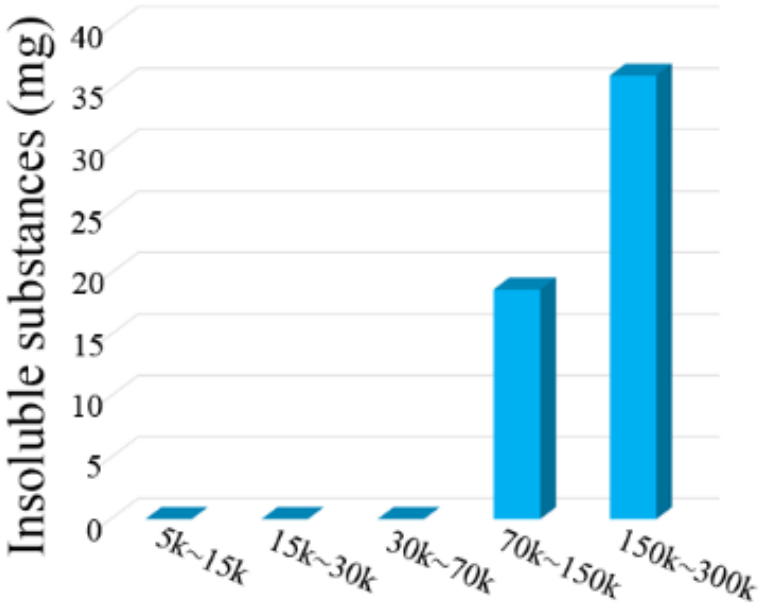

Figure S8. The weight of insoluble substance in the synthesis process of linear sulfo-$Ru(bpy)_3^{2+}$-polymer.

acids with amino.

1.6 Quantity of insoluble substance in the synthesis process of linear $Ru(bpy)_3^{2+}$-polymer

Commercial PLL products with the molecular weight of 5k~15k Dalton (Da), 15k to 30kDa, 30k~70kDa, 70k~150kDa, 150k~300kDa were employed to investigate the

influence of different degree of polymerization. With the increase of molecular weight, the group of 30k~70kDa, 70k~150kDa, 150k~300kDa diversely emerged the red insoluble substance which may be caused by the huge molecular structure of linear $Ru(bpy)_3^{2+}$-polymer while $Ru(bpy)_3^{2+}$ was linked on PLL. The ECL intensities of the linear $Ru(bpy)_3^{2+}$-polymers were also detected in the identical condition. The results showed that the linear $Ru(bpy)_3^{2+}$-polymer with the PLL skeleton of 15k~30kDa achieved most intense ECL signal, higher degree of polymerization could not give out accordingly higher ECL intensity. Meanwhile, we recorded the weight of the insoluble substances which increased with the molecular weight of PLL (Shown in Figure S7).

To improve the solubility, the hydrophilia- improved $Ru(bpy)_3^{2+}$ was designed as the ECL luminophore for larger linear $Ru(bpy)_3^{2+}$-polymer. The sulfo-group (a strong hydrophilic group) was integrated to the hydrophobic domain of $Ru(bpy)_3^{2+}$. With this hydrophilic sulfo-$Ru(bpy)_3^{2+}$, we synthesized the solubility-improved linear $Ru(bpy)_3^{2+}$-polymer. Compared with the linear $Ru(bpy)_3^{2+}$-polymer, the sulfo-modified $Ru(bpy)_3^{2+}$-polymer obviously reduced the weight of the insoluble substances in the experimental group of 70k~150kDa, 150k~300kDa (shown in Figure S8). It presented the preferable solubility of the sulfo-modified assay. Finally, we compared the ECL intensity of linear sulfo-$Ru(bpy)_3^{2+}$-polymer with the linear

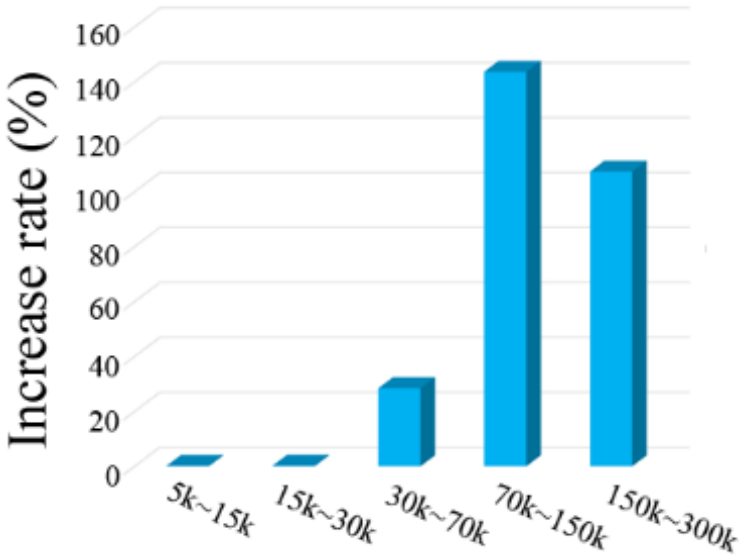

Figure S9. ECL intensity increase rate of linear sulfo-$Ru(bpy)_3^{2+}$-polymer.

$Ru(bpy)_3^{2+}$-polymer, the increasing rate of each group was calculated and recorded in Figure S9. It revealed the enhancement of ECL comparing with the modify-free linear $Ru(bpy)_3^{2+}$-polymer.

2. SUPPORTING EXPERIMENTAL DETAILS

2.1 Synthesis process of linear $Ru(bpy)_3^{2+}$-polymer and polymer-amplified ECL probe

Linear $Ru(bpy)_3^{2+}$-polymer was synthesized by employing PLL as the binding-skeleton, the repetitive amino group of each monomer on PLL was employed as the binding site of $Ru(bpy)_3^{2+}$. The powder of PLL was obtained from Sigma Aldrich, and the solution of PLL showed colorless and transparent. The PLL (25mg in 1.5mL water) was mixed with excess $Ru(bpy)_3^{2+}$, reached a molar ratio of 1:10 between the amino and $Ru(bpy)_3^{2+}$. The solution of the mixture turned to crimson. Then, the mixture was sealed and incubated in dimethylformamide (DMF, 20 percent) for 12 hours at 37 ºC. The insoluble substance was eliminated by centrifuge (12000r/min for 1min). Finally, the products were purified by the Nanosep 10K OMEGA tubular ultrafiltration membrane from Pall Corporation for five times cleaning and concentrating. The concentrated ratio should been set as 10:1. The purified linear $Ru(bpy)_3^{2+}$-polymer was freeze-dried for 12 hours. And the powder showed brown, can be dissolved by water or other solvents (dimethylsulfoxide, DMSO; dimethylformamide, DMF; Methanol, MET).

2.2 Total RNA extraction

Listeria monocytogenes (CMCC54007), *Salmonella enterica* (CMCC50040), and *Escherichia coli O157:H7* (GW1.2020) were obtained from Guangzhou Institute of Microbiology, Guangzhou, China. The Total RNA extraction of *Listeria monocytogenes* (CMCC54007), *Salmonella enterica* (CMCC50040), and *Escherichia coli O157:H7* (GW1.2020) was extracted according to the manufacturer's protocol from the RNAplus Kit (Takara, Dalian, China). The extractions were quantified by measuring the optical density at 260 nm with a spectrophotometer (Eppendorf BioPhotometer, Eppendorf AG, Hamburg, Germany).