Supporting Information

Bipolar Electrode based Multi-Color Electrochemiluminescence Biosensor

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Experimental Section

Materials. All the chemicals were used as received without further purification. *fac*-(Tris(2-phenylpyridinato-C^2,N)iridium(III)($Ir(ppy)_3$, Tetrabutylammonium hexafluorophosph- ate($TBAPF_6$), Tris(2,2'-bipyridyl)dichlororuthenium(II)hexahydrate($[Ru(bpy)_3]^{2+}$), tripropylamine (TPrA), 3-Aminopropyltriethoxysilane(APTES), N-hydroxysuccinimide(NHS), Bovine serum albumin (BSA), 1-Ethyl-3-(3-dimethyl-amino-propyl)carbodiimide-hydrochloride (EDC), L-cysteine and silver enhancer kit (solution A and solution B) were purchased from Sigma-Aldrich. Acetonitrile anhydrous and ferrocene were purchased from Sinopharm chemical reagent company (Shanghai, China). Total prostate-specific antigen (PSA), mouse monoclonal capture and signal antibodies to total PSA (clone: P27A10 and P27B1) were obtained from Shanghai Linc-Bio Science Co. LTD. The clinical serum samples were from Jiangsu Cancer Hospital. ITO-coated (thickness ~100 nm, resistance ~10 Ω/square) aluminosilicate glass slides were purchased from CSG (Shenzhen, China). N-formySG-2506 borosilicate glass (with 145 nm thick chrome and 570 nm thick anodic S-1805 type photoresist) was purchased from Shaoguang Chrome Blank Co. Ltd. Sylgard 184 (including poly (dimethylsiloxane) (PDMS) monomer and curing agent) was from Dow Corning (Midland, MI). Resistances were purchased from Turbine electronic components.

Apparatus. An Olympus DP71 cooled CCD camera (15 s exposure) was applied for chip imaging, results of which were analyzed by Image-Pro Plus (IPP) 6.0 software.

Scanning electron microscopy (SEM) images were obtained using an S-3000 N scanning electron microscope (Hitachi, Japan). Electrochemical experiments were performed with CHI 660C potentiostat. Three-electrode ECL measurements were performed with an ITO working electrode (3 mm in diameter), an $Ag/AgNO_3$ (20 mM) non-aqueous reference electrode, and a platinum wire counter electrode, and potentials were referenced to the Fc/Fc^+ redox couple. Continuous sampling was conducted with Pump 11 Elite (Havard Apparatus, USA). Spooling ECL spectra were recorded with F700 fluorescence spectrometer (Hitachi, Japan). The resistances of BPE were recorded using digital multimeter (VC890D, VICTOR, China).

Synthesis of Au nanoparticles (NPs) labeled antibody 2. AuNPs (5 nm diameter) were synthesized through the reduction of $HAuCl_4$ by sodium borohydride ($NaBH_4$) according to the method described in previous literature. Briefly, 0.6 mL of ice-cold 0.1 M $NaBH_4$ was added to 20 mL of 0.25mM $HAuCl_4$ aqueous solution under constant stirring. The solution was kept stirring in an ice bath for 10 min and then reacted at room temperature for 3 h. The prepared AuNPs were stored at 4 ˚C for further use.

Au NPs-Ab2 was prepared according to the literature with minor modification. 1 mL of 0.175 M L-cysteine was added into 1 mL AuNPs solutions and incubated for approximately 10 h allowing the formation of carboxyl group on Au surface. 7.0 mg EDC and 21.0 mg NHS were added to the carboxyl-modified nanoparticles and kept for 1 h. Then secondary anti-PSA antibody (Ab2, 20 mg/mL, 1 mL) was added into

the mixture and kept overnight followed by blocking with Tris-HCl (pH 7.4). The prepared Au NPs-antibodies were stored at 4 ˚C for further use.

Supplementary Figures

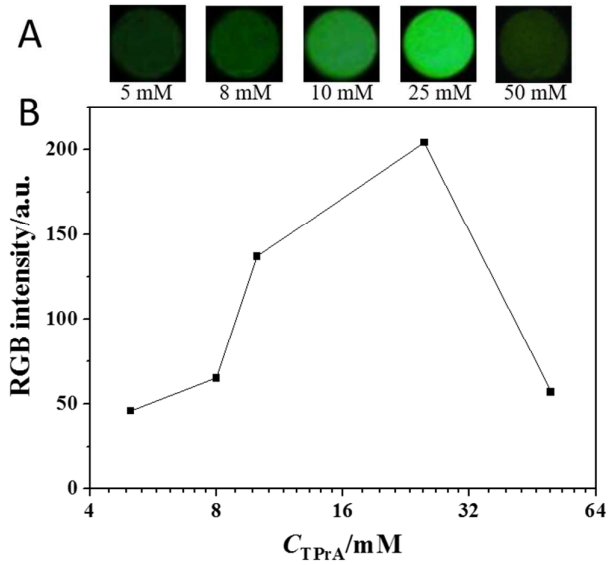

Figure S1. ECL images (A) and corresponding RGB intensities (B) of 0.5 mM $Ir(ppy)_3$ with different concentrations of TPrA. Photographs were taken at potential of 1.1 V vs. ferrocene/ferrocenium couple.

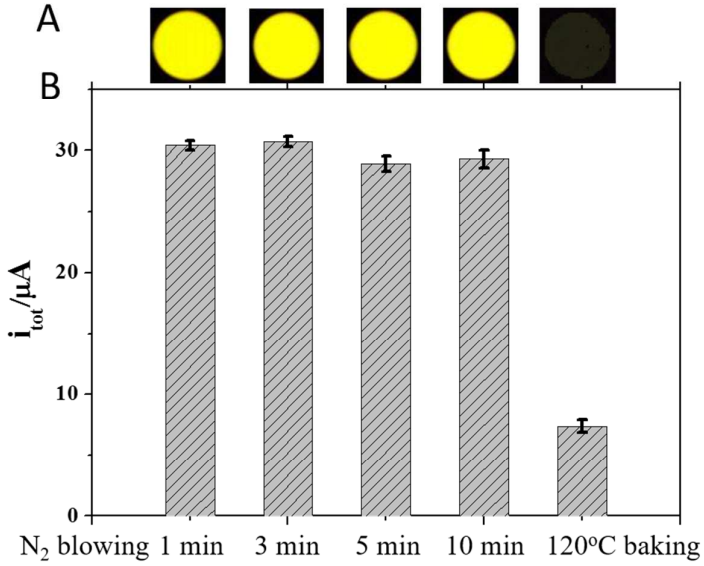

Figure S2. ECL images (A) and corresponding i_{tot} (B) of the system with 4.0 ng/ml

PSA guided silver deposition. The images and i_{tot} were taken using a mixture of 0.5 mM [Ir(ppy)$_3$] and 0.75 mM [Ru(bpy)$_3$]$^{2+}$ with 25 mM TPrA in acetonitrile/0.1 M TBAPF$_6$ added in the anodic reservoir at 5.5 V.

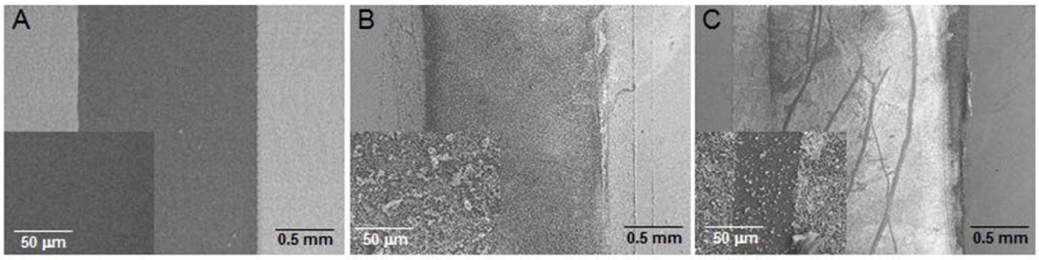

Figure S3. SEM images of the gap between two BPEs before (A) and after 4.0 ng/ml PSA guided silver deposition with N_2 blowing (B) and 120^{o}C baking (C), inset images are the enlarged morphologies of the gaps.

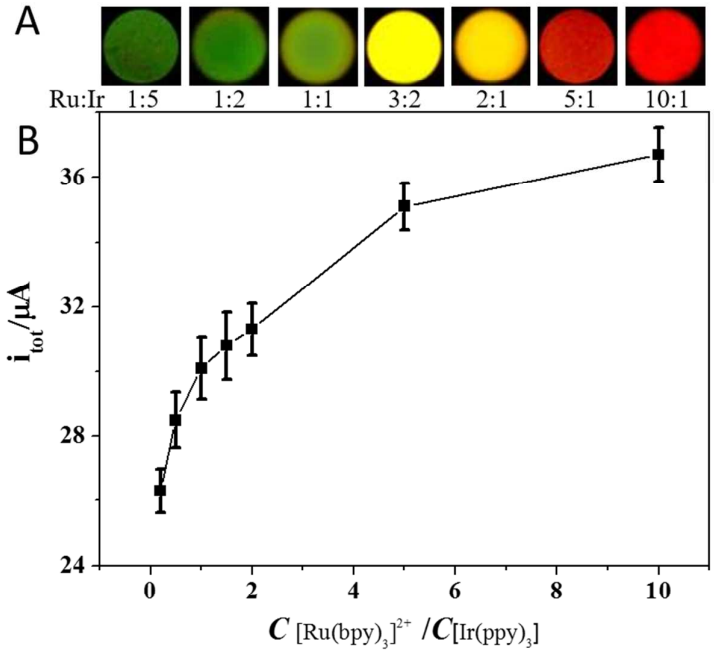

Figure S4. ECL images (A) and i_{tot} (B) of the closed BPE system with 4.0 ng/ml PSA guided silver deposition vs. the ratio of [Ru(bpy)$_3$]$^{2+}$ to [Ir(ppy)$_3$]. The images and i_{tot} were taken using a 0.5 mM [Ir(ppy)$_3$] with different concentrations of [Ru(bpy)$_3$]$^{2+}$ from 0.1 mM to 5.0 mM, with 25 mM TPrA in acetonitrile/0.1 M TBAPF$_6$ added in the anodic reservoir at 5.5 V.

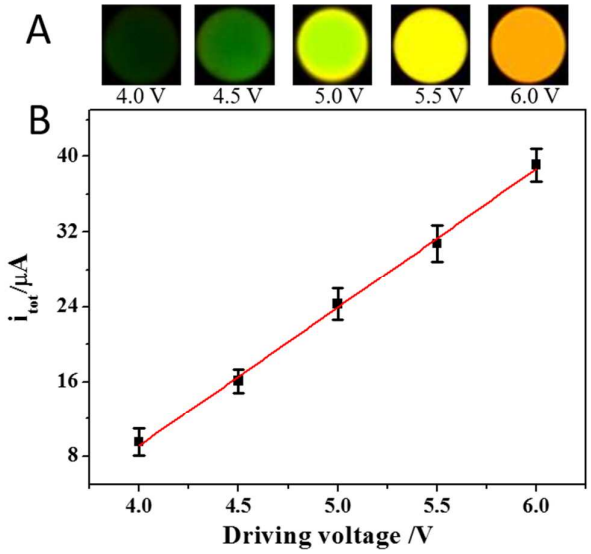

Figure S5. ECL images (A) and i_{tot} (B) of the closed BPE system with 4.0 ng/ml PSA guided silver deposition vs. different driving voltages from 4.0 to 6.0 V. The images and i_{tot} were taken using a 0.5 mM $[Ir(ppy)_3]$ and 0.75 mM $[Ru(bpy)_3]^{2+}$ with 25 mM TPrA in acetonitrile/0.1 M $TBAPF_6$ added in the anodic reservoir.

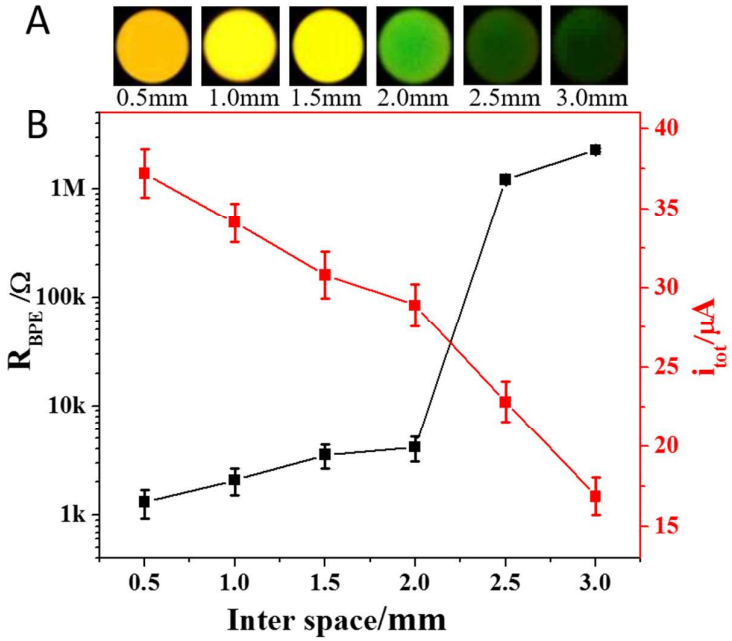

Figure S6. ECL images (A) and resistance of the BPE and i_{tot} (B) of the closed BPE system with 4.0 ng/ml PSA guided silver deposition vs. different inter space in between the BPEs from 0.5 mm to 3.0 mm. The resistances were measured with multimeter. The images and i_{tot} were taken using a 0.5 mM $[Ir(ppy)_3]$ and 0.75 mM $[Ru(bpy)_3]^{2+}$ with 25 mM TPrA in acetonitrile/0.1 M $TBAPF_6$ added in the anodic

reservoir.

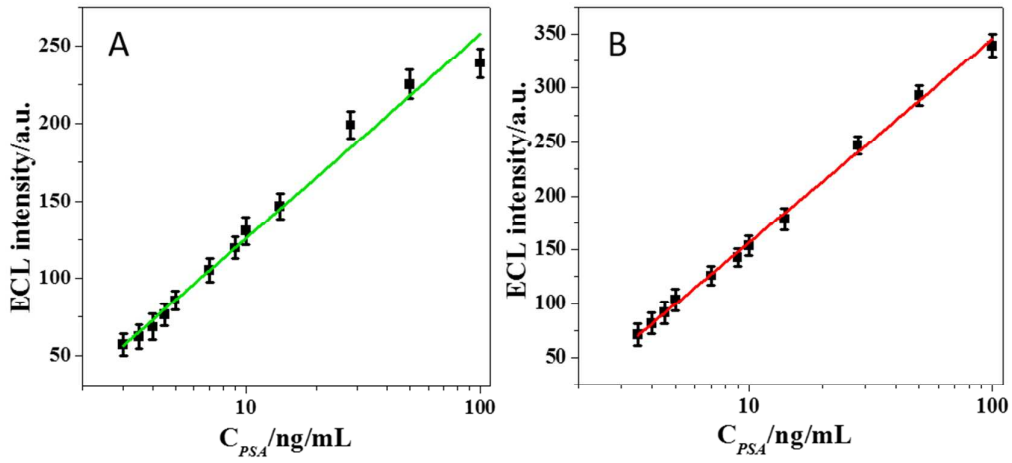

Figure S7. ECL intensities of $[Ir(ppy)_3]$ (A) and $[Ru(bpy)_3]^{2+}$ (B) obtained from the spooling ECL spectra according to concentrations of PSA.

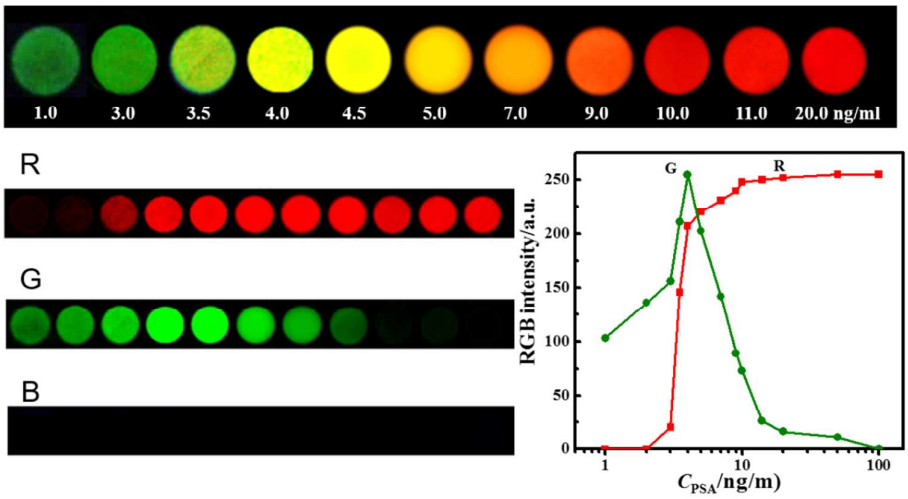

Figure S8. ECL intensities obtained from the ECL images in the R and G channels vs the concentration of PSA.

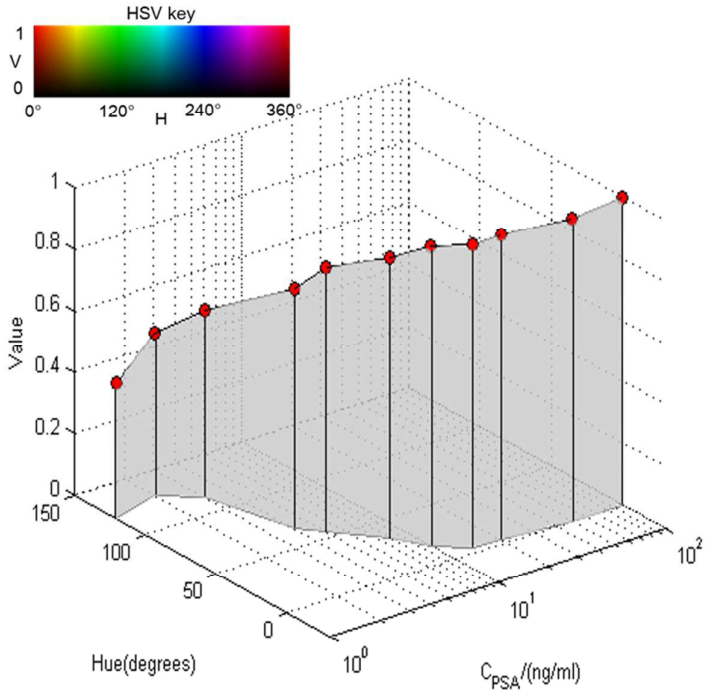

Figure S9. HSV representation of spectral changes obtained from the ECL images, characterizing the change in observed color according to the concentration of PSA. Values of H (hue) and V (value) were derived from images.

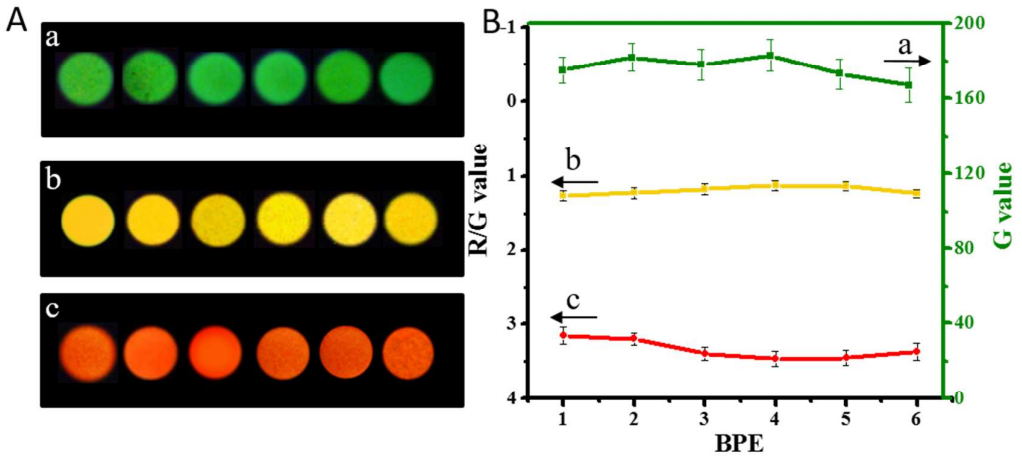

Figure S10. ECL images at the anode of BPE with different concentrations (a: 3.0 ng/ml, b: 5.0 ng/ml and c: 9.0 ng/ml) of PSA (A), and corresponding G value (a) and R/G value (b, c) (B).