

Electronic Supplementary Material (ESI) for Chemical Communication.
This journal is © The Royal Society of Chemistry 2023

Supplementary Material

Regulating the Work Function and Surface Hydrophobicity of Indium Tin Oxide Electrodes for Enhanced Electrochemiluminescence Analysis

Tengyu Li,^a Jialian Ding,^a Yafeng Wang^b and Bin Su^{*a,c}

a. Department of Chemistry, Zhejiang University, Hangzhou 310058, China. E-mail:
subin@zju.edu.cn

b. Department of Clinical Laboratory, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, China

c. General Surgery Department, Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, Hangzhou 310052, China

Contents

S1. Reagents and materials	S2
S2. Instruments and measurements	S2
S3. Preparation of Ru-PSBs	S3
S4. ECL routes	S4
S5. Preparation and characterization of modified ITO Electrodes	S5
S6. Calculation of the standard electrochemical oxidation rate constants	S7
S7. Calibration of the bias voltages of PMT	S9
S8. Finite element simulations	S10
References	S12

S1. Reagents and materials

All chemicals of analytical grade or higher were used as received without further purification. Ultrapure water (18.2 M Ω cm) was employed to prepare aqueous solutions. Sodium dihydrogen phosphate dihydrate (NaH₂PO₄·2H₂O, 99.0%), disodium hydrogen phosphate dodecahydrate (Na₂HPO₄·12H₂O, 99.0%) and potassium chloride (KCl,99.0%) were bought from Sinopharm Chemical Reagent. Tri-*n*-propylamine (TPA,99%) was purchased from Aladdin. Tris(2,2'-bipyridyl)ruthenium dichloride hexahydrate (Ru(bpy)₃Cl₂·6H₂O, 99.95%) was obtained from Sigma-Aldrich. Indium tin oxide (ITO) coated glass (surface resistivity <17 Ω /cm², thickness 100 \pm 20 nm) were purchased from Zhuhai Kaivo Optoelectronic Technology. (Ru(bpy)₂(cpbpy)(PF₆)₂) was obtained from Suzhou Refor Biomedical Co.,Ltd. Amino-functionalized polystyrene bead (3.8 μ m, 2.5% w/v, PSB) was purchased from Tianjin Baseline Chrom Tech Research Centre.

S2. Instruments and measurements

All electrochemical measurements were conducted on an electrochemical workstation (CHI920, Shanghai) in a classic three-electrode configuration. Synchronous acquisition of ECL intensity and electrochemical current was performed on a commercial MPI-E ECL analytical system or a custom system consisting of a photon-counting detector and counting unit (Hamamatsu Photonics Co., Ltd., China). ECL and photoluminescence (PL) imaging was performed on a Nikon ECLIPSE microscope (LV100ND) equipped with a matching objective lens (Nikon CFI Apo 40 \times , N.A. 0.8) and an EMCCD camera (Andor iXon Ultra 897). In a typical experiment, 100 μ L of Ru-PSB suspension was firstly diluted with 900 μ L of ultrapure water and then 20 μ L of the suspension was deposited onto the electrode. The exposure time for PL imaging of single Ru-PSBs was 0.2 seconds. For ECL imaging, the exposure time was 5 seconds, during which a constant potential of +1.5 V (vs. Ag/AgCl) was applied.

The X-ray photoelectron spectra (XPS) were measured on Thermo Scientific ESCALAB 250Xi, utilizing an Al K α light source at 1486.6 eV. The relative surface potential was measured using Kelvin probe force microscopy (KPFM) from Bruker.

S3. Preparation of Ru-PSBs

100 μ L of PSB suspension was washed with 900 μ L of 0.05 M Tris buffer (pH 7.4, Tris), followed by centrifugation at 12000 rpm for 5 minutes. The washed PSBs were then re-suspended in an additional 900 μ L of the same Tris buffer. Subsequently, a mixture containing 19.17 mg of EDC and 10 mg of Ru(bpy)₂(cpbpy)(PF₆)₂ dissolved in 100 μ L of DMSO was added to the PSB suspension. This mixture was incubated at room temperature for six hours under magnetic stirring at a speed of 400 rpm. After incubation, the PSBs were washed sequentially with 1 mL of PB (0.2 M, pH 7.4) solution and ultrapure water for ten and three times, respectively. The beads were separated from the solution via centrifugation at a speed of 12000 rpm for five minutes. Finally, Ru(bpy)₃²⁺-labeled PSB (Ru-PSB) was suspended in one milliliter of ultrapure water and stored at 4 °C.

S4. ECL routes

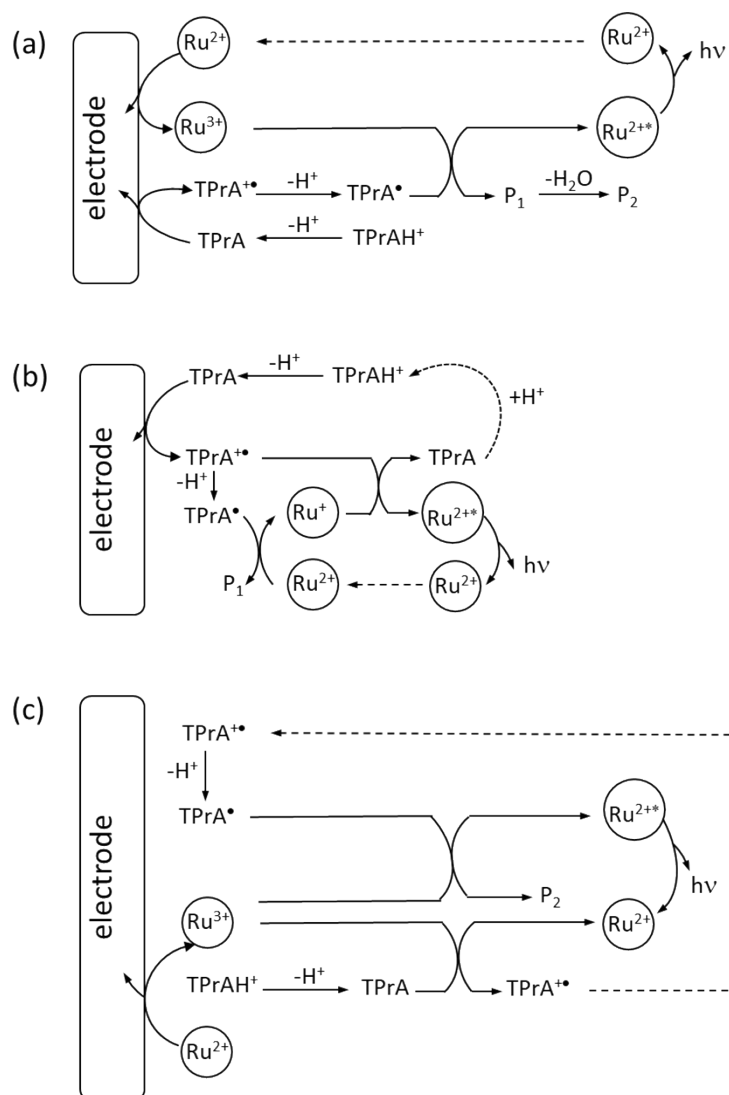


Fig. S1. Different pathways for ECL generation of the $\text{Ru}(\text{bpy})_3^{2+}/\text{TPrA}$ system: (a) the oxidative-reduction route, (b) the low oxidation potential (LOP) route and (c) the catalytic route. Ru^{2+} , Ru^{3+} and Ru^{2+**} represent $\text{Ru}(\text{bpy})_3^{2+}$, $\text{Ru}(\text{bpy})_3^{3+}$ and $\text{Ru}(\text{bpy})_3^{2+**}$, respectively.

S5. Preparation and characterization of modified ITO Electrodes

Preparation of Cl-ITO electrode: The ITO electrode was immersed in a mixture of methanol (12 mL) and trichloromethane (28 mL) containing 1 mM ethephon for 16 h. The ITO electrode was then rinsed with a mixture of methanol and trichloromethane (7:3 v/v) and dried with N₂ stream.

Preparation of PDMS-ITO electrode: As reported previously,^{S1, S2} The ITO electrode was positioned on top of a PDMS monolith with a distance of ca. 2 mm using glass spacers. The assembly was then heated at 100 °C for different durations to deposit PDMS oligomers on the surface of ITO electrode.

Preparation of CIP-ITO electrode:

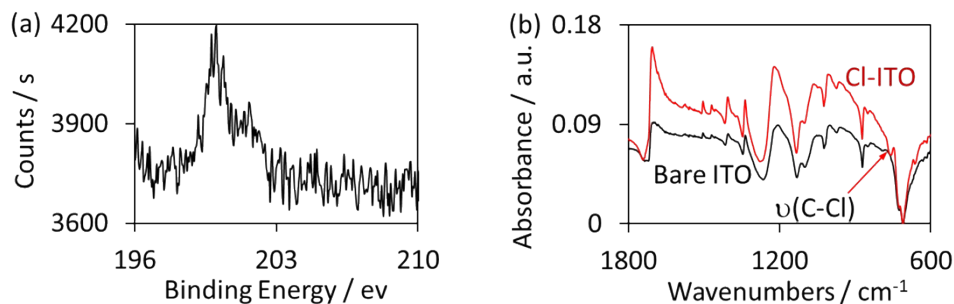


Fig. S2. XPS (a) and FT-IR (b) characterization of Cl-ITO electrode.

Table S1. Relative surface potential ($\Delta V = V_{\text{sample}} - V_{\text{bare ITO}}$) of Cl-ITO and PDMS-ITO electrode

	Cl-ITO	PDMS-ITO	CIP-ITO
$\Delta V / \text{mV}$	160.5	- 15.4	96.5

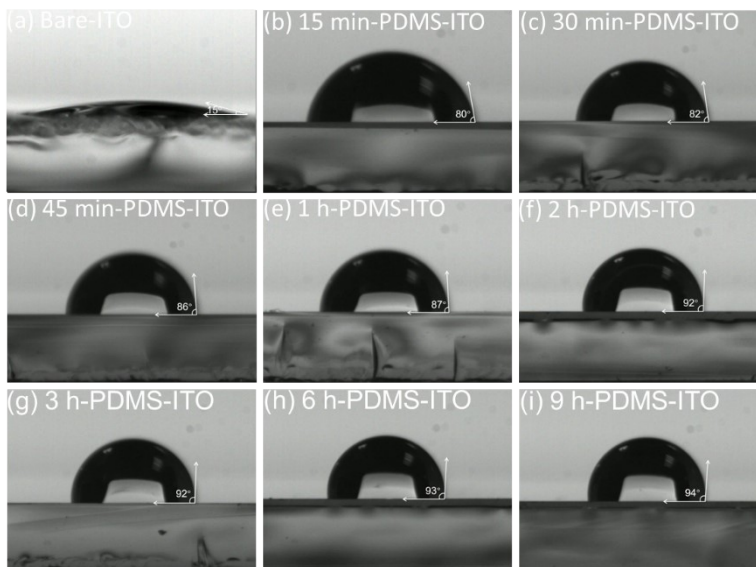


Fig. S3. Variation of the contact angle of ITO electrode with the duration of PDMS modification.

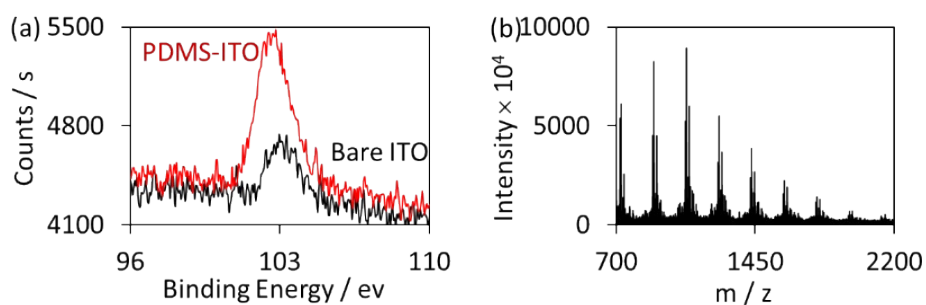


Fig. S4. XPS (a) and MALDI-TOF (b) characterization of PDMS-ITO electrode.

S6. Calculation of the standard electrochemical oxidation rate constants

The electrochemical oxidation of TPrA on the ITO electrode is too slow to show a current peak. In this case, k_{TPrA}^0 can be estimated using the following equations according to the Tafel plot method,^{S3, S4}

$$i_a = i^0 \exp \left[(1 - \alpha) \frac{nF}{RT} (E - E^0) \right] \quad (\text{S1})$$

$$\ln(i_a) = \ln(i^0) + (1 - \alpha) \frac{nF}{RT} (E - E^0) \quad (\text{S2})$$

$$i^0 = nFk^0[\text{TPrA}] \quad (\text{S3})$$

where i_a and i^0 represent the anodic current density and exchange current density. F , R , T , and α are the Faraday constant, gas constant, temperature, and charge transfer coefficient, respectively. k^0 and E^0 are the standard rate constant of electrochemical oxidation and the standard redox potential. Considering the acid-base equilibrium of TPrA, the molecular concentration in the actual electrochemical reaction (deprotonated TPrA) was calculated in terms of the relationship between pK_a (10.4) and experimental pH (7.4). In this case, the amount of electroactive TPrA was roughly 0.1% of the analytical concentration. In the case of $\text{Ru}(\text{bpy})_3^{2+}$, the standard rate constant of electrochemical oxidation (k_{Ru}^0) can be derived according to the Nicholson equation,

$$k^0 = \psi \left(\frac{\pi D n F v}{RT} \right)^{\frac{1}{2}} \quad (\text{S4})$$

where D ($5.9 \times 10^{-6} \text{ cm}^2/\text{s}$) is the diffusion coefficient of $\text{Ru}(\text{bpy})_3^{2+}$ and v is the potential scan rate. The value of ψ refers to the table given by Nicholson.^{S5}

Table S2. k_{TPrA}^0 at different electrodes

Electrodes	Bare ITO	Cl-ITO	PDMS-ITO
$k_{\text{TPrA}}^0 \times 10^6 / \text{cm} \cdot \text{s}^{-1}$	4.31 ± 0.34	9.99 ± 1.54	73.23 ± 7.53

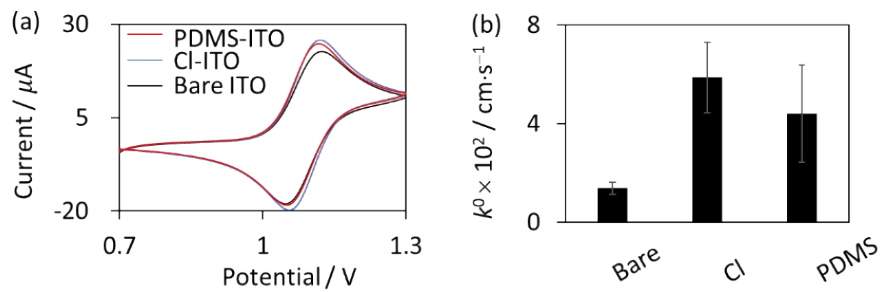


Fig. S5. (a) CVs of different electrodes in 0.2 M PB (pH 7.4) solution containing 0.5 mM $\text{Ru}(\text{bpy})_3^{2+}$
(b) A comparison of k^0_{Ru} for three different electrodes. Standard deviation, $n = 3$

S7. Calibration of the bias voltages of PMT

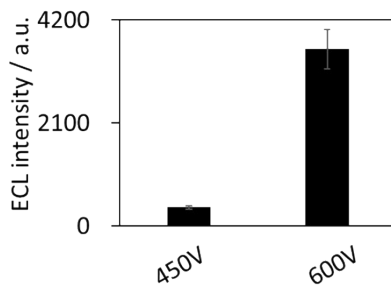


Fig. S6. The calibration of bias voltages of PMT (450 V and 600 V) was conducted using ITO electrode in 0.2 M PB (pH 7.4) solution containing 5 μM $\text{Ru}(\text{bpy})_3^{2+}$ and 5 mM TPrA.

S8. Finite element simulations

If applying a sufficiently positive potential, we can assume that the ECL process is dominated by the “oxidative reduction route” and “catalytic route”, which include the following elementary steps, where k_i represents the rate constant of each step and P is $(\text{CH}_3\text{CH}_2\text{CH}_2)_2\text{N}^+ = \text{CHCH}_2\text{CH}_3$.^{S6-S8}

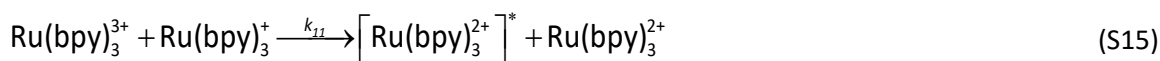
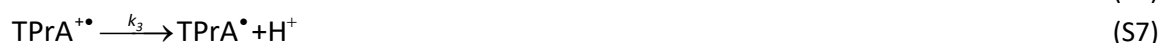


Table S3. Definitions of simulation parameters

Name	Value	Description
r_e	250 μm	Length of the radial electrode
L	200 μm	Length of the axial solution boundary
$c_{0_Ru^{2+}}$	500 μM , 50 μM , 1 μM	Initial concentration of $\text{Ru}(\text{bpy})_3^{2+}$
c_{0_TPrA}	(1 or 200) \times 0.001 mM	Initial concentration of TPrA
$c_{0_TPrAH^+}$	(1 or 200) \times 0.999 mM	Initial concentration of TPrAH^+
c_{0_H}	$10^{-7.4}$ M	Initial concentration of H^+
D_{Ru}	5×10^{-6} (cm^2/s)	Diffusion coefficients of $\text{Ru}(\text{bpy})_3^{2+}$, $\text{Ru}(\text{bpy})_3^{3+}$ and $\text{Ru}(\text{bpy})_3^{2+*}$
D_{TPrA}	5×10^{-6} (cm^2/s)	Diffusion coefficient of TPrA, TPrAH^+ , TPrA^{*+} and TPrA^*
D_H	5×10^{-5} (cm^2/s)	Diffusion coefficient of H^+
k_1	$k_{0r} \times e^{38.92 \times (1-\alpha_1) \times (E-1.05)}$	Forward rate constant of eq (S5)
k_{-1}	$k_{0r} \times e^{38.92 \times (-\alpha_1) \times (E-1.05)}$	Backward rate constant of eq (S5)
k_2	0.01, 0.2 (cm/s)	Rate constant of eq (S6)
k_3	3500 (s^{-1})	Rate constant of eq (S7)
k_4	k_2	Rate constant of eq (S8)
k_5	300 $\text{m}^3/(\text{s}\cdot\text{mol})$	Rate constant of eq (S9)
k_6	300 $\text{m}^3/(\text{s}\cdot\text{mol})$	Rate constant of eq (S10)
k_7	3.3×10^6 (s^{-1})	Rate constant of eq (S11)
k_8	0.1, 0.3 m/s	Rate constant of eq (S12)
k_9	1000 $\text{m}^3/(\text{s}\cdot\text{mol})$	Rate constant of eq (S13)
k_{10}	1×10^6 $\text{m}^3/(\text{s}\cdot\text{mol})$	Rate constant of eq (S14)
k_{11}	10000 $\text{m}^3/(\text{s}\cdot\text{mol})$	Rate constant of eq (S15)

References

- S1. Q. Yang, X. Lin, Y. Wang and B. Su, *Nanoscale*, 2017, **9**, 18523-18528.
- S2. X. Lin, B. Zhang, Q. Yang, F. Yan, X. Hua and B. Su, *Anal. Chem.*, 2016, **88**, 7821-7827.
- S3. V. J. González, *Electroanalysis*, 1997, **9**, 880-882.
- S4. M. Chen, C. Xu, W. Zhao, H. Chen and J. Xu, *Chem. Commun.*, 2020, **56**, 3413-3416.
- S5. R. S. Nicholson, *Anal. Chem.*, 1965, **37**, 1351-1355.
- S6. Y. Zu and A. J. Bard, *Anal. Chem.*, 2000, **72**, 3223-3232.
- S7. F. Kanoufi, Y. Zu and A. J. Bard, *J. Phys. Chem. B*, 2001, **105**, 210-216.
- S8. Z. Chen and Y. Zu, *J. Phys. Chem. C*, 2009, **113**, 21877-21882.