Supplementary Materials

H_2O_2 activated independently bidirectional regulation for sensitive and accurate electrochemiluminescence ratiometric analysis

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1. Materials and reagents

Melamine, 3-aminopropyltriethoxysilane and SiO_2 nanopaticls (99.5%, 30 nm) were obtained from Aladdin Industrial Co., Ltd. (Shanghai, China). Tris(2,2'-bipyridyl) ruthenium(II) chloride hexahydrate and tripropylamine were bought from Sigma-Aldrich (USA). $K_2S_2O_8$, chitosan, glutaraldehyde, $Zn(NO_3)_2$ and HCl was from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). N,N-dimethylformamide was from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Oxalic acid dehydrate was purchased from Tianjin Yongda Chemical Reagent Development Center. (Tianjin, China). Myoglobin (MyO) (L4C00401), anti-MyO antibody (L3C00303) and human immunoglobulin G (hIgG) were from Shanghai Linc-Bio Science Co., Ltd. (Shanghai, China). Human serum albumin (HSA), glucose oxidase (GOx) and bovine serum albumin (BSA) were provided by Sangon Biotech Co., Ltd (Shanghai, China). Human serum samples were supplied from Xinyang Central Hospital (Xinyang, China). All chemicals were of analytical grade.

2. Apparatus

ECL signals were measured by MPI-B ECL analyzer coupled with a multifunctional chemiluminescence detector (Xi'an Remax Eletronic Science & Technology Co., Ltd., Xi'an, China). A three-electrode system was used with a glassy carbon electrode with the two physically separated interfaces (WEs) (3 mm in diameter) as the working electrode, an Ag/AgCl (saturated KCl) as the reference electrode and a platinum electrode as the auxiliary electrode. Scanning electron microscopy (SEM) image was obtained by S4800 cold field emission SEM (Hitachi, Tokyo, Japan). Transmission electron microscope (TEM) image was recorded on Tecnai G2 F20 TEM system (FEI Co., Ltd., USA). Electrochemical impedance spectroscopy was performed on a RST5200 electrochemical workstation (Zhengzhou Shiruisi Technology Co., Ltd., China). Fourier transform infrared spectrum were obtained from Bruker TENZOR 27 spectrophotometer (Bruker Optics, Germany).

3. Optimization of experimental conditions

To obtain an excellent detection performance, some vital factors such as the concentration of co-reactant ($K_2S_2O_8$ and TPA), the pH of detection solution, the concentration of glucose and the catalytic time between GOx and glucose were optimized. As exhibited in Fig. S1A, the ratio value of $ECL_{cathode}/ECL_{anode}$ increased followed by the increasing of the concentration of $K_2S_2O_8$, and it reached to maximum at 0.1 M. After the same operation, it was found that the ECL ratio increased with the increase of TPA concentration and reached a platform at a concentration of 2 mM from the Fig. S1B. Herein, the 0.1 M $K_2S_2O_8$ and 2 mM TPA were selected as the optimal co-reactant concentrations for the following experimentation. The pH is an important factor for the biological activity. It can be seen from Fig. S1C that the ECL intensity achieved a peak value when the pH value at 7.4. Hence, 7.4 was chosen as the optimal pH.

In our design, the presence of H_2O_2 has a great influence on the ECL signal of the sensor. Therefore, the produced amount of H_2O_2 needs to be optimized. H_2O_2 is produced by in-situ oxidation of glucose via the immobilized GOx on the GCE surface in the work. So indirectly, the concentration of glucose and the catalytic time between glucose and GOx were optimized. The concentration of glucose was optimized from 20 mM to 100 mM. As can be seen in Fig.S1D, the ratio value increased followed by the increase of glucose concentration and decreased slightly when the glucose concentration exceeded 100 mM. So, 100 mM glucose was selected. Fig. S1E showed the relationship between the ratio value and reaction time. The ratio value achieved the maximum at catalytic time at 30 min. Therefor, 30 min was chosen as the catalytic time.

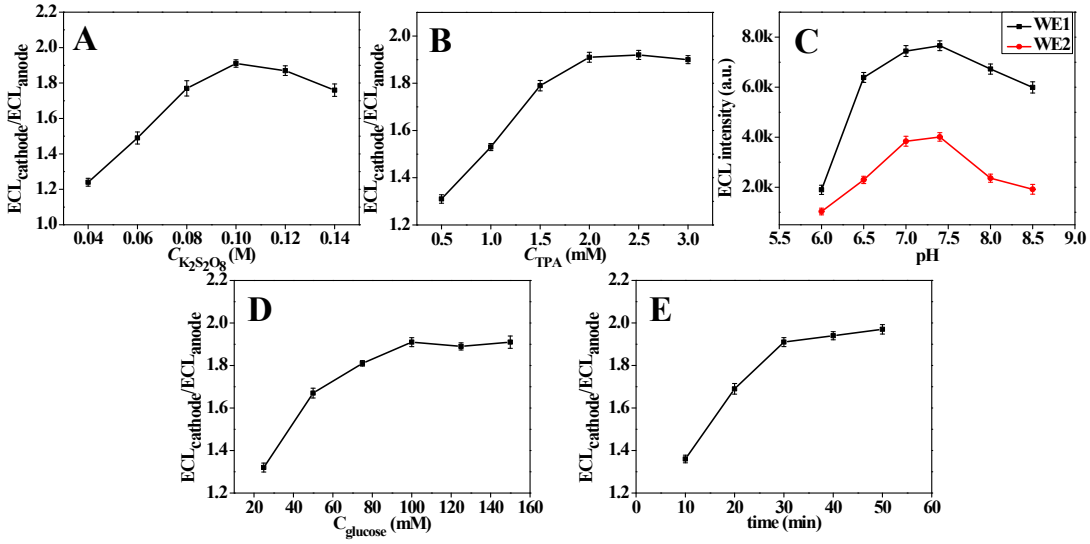

Fig. S1. Effects of (A) the $K_2S_2O_8$ concentration, (B) the TPA concentration, (C) the pH of detection solution, (D) the glucose concentration, and (E) catalytic time between GOx and glucose on the ratio values.

Table S1. Comparison of this method to MyO with other methods.

Detection methods	Linear ranges (g mL^{-1})	Detection limits (g mL^{-1})	References
Photoelectrochemistry	$1.0 \times 10^{-13} – 5.0 \times 10^{-8}$	4.2×10^{-13}	[1]
Fluorescence	$5.0 \times 10^{-11} – 1.0 \times 10^{-7}$	2.0×10^{-11}	[2]
Electrochemistry	$1.0 \times 10^{-9} – 1.4 \times 10^{-6}$	6.7×10^{-10}	[3]
Surface-enhanced Raman spectroscopy	$1.0 \times 10^{-8} – 5.0 \times 10^{-6}$	1.0×10^{-8}	[4]
Electrochemiluminescence (ECL)	$3.0 \times 10^{-9} – 3.2 \times 10^{-7}$	1.1×10^{-10}	[5]
ECL Ratiometry	$1.0 \times 10^{-13} – 1.0 \times 10^{-7}$	4.0×10^{-14}	this work

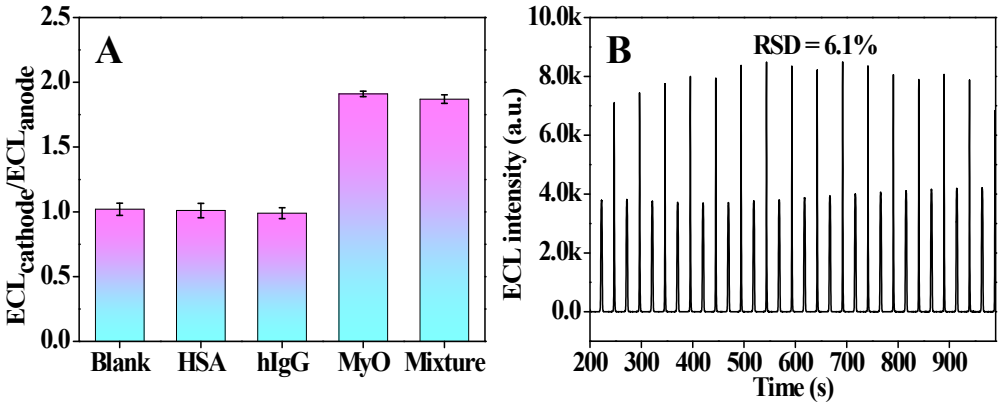

Fig. S2. The (A) selectivity and (B) stability of the ECL biosensor.

References

1 Y. Ma, Y. X. Dong, B. Wang, S. W. Ren, J. T. Cao and Y. M. Liu, *Talanta*, 2020, **207**, 120288.

2 J. S. Chen, F. Y. Ran, Q. H. Chen, D. Luo, W. D. Ma, T. Han, C. M. Wang and C. X. Wang, *RSC Adv.*, 2019, **9**, 4463-4468.

3 S. Singh, S. K. Tuteja, D. Sillu1, A. Deep and C. R. Suri, *Microchim. Acta*, 2016, **183**, 1729-1738.

4 W. A. El-Said, D. M. Fouad and S. A. El-Safty, *Sens. Actuators B Chem.*, 2016, **228**, 401-409.

5 B. Zou, H. Y. Cheng and Y. F. Tu, *Microchim. Acta*, 2019, **186**, 59.