# Supporting Information

## A cheaper substitute for HRP: Ultra-small Cu-Au bimetallic enzyme mimics with infinitesimal steric hindrance to promote catalytic lateral flow immunodetection of clenbuterol

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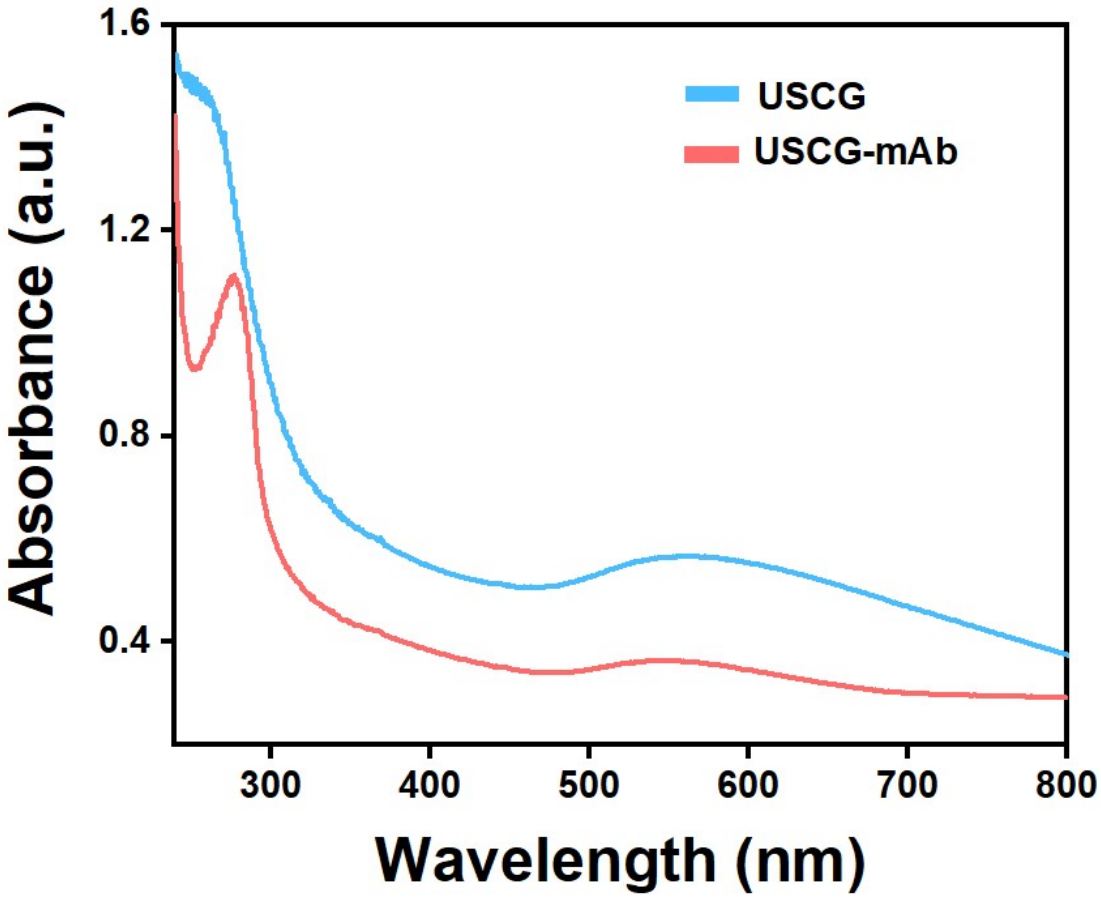


**Figure S1.** UV-vis absorption spectra of the USCG, and USCG-mAb.

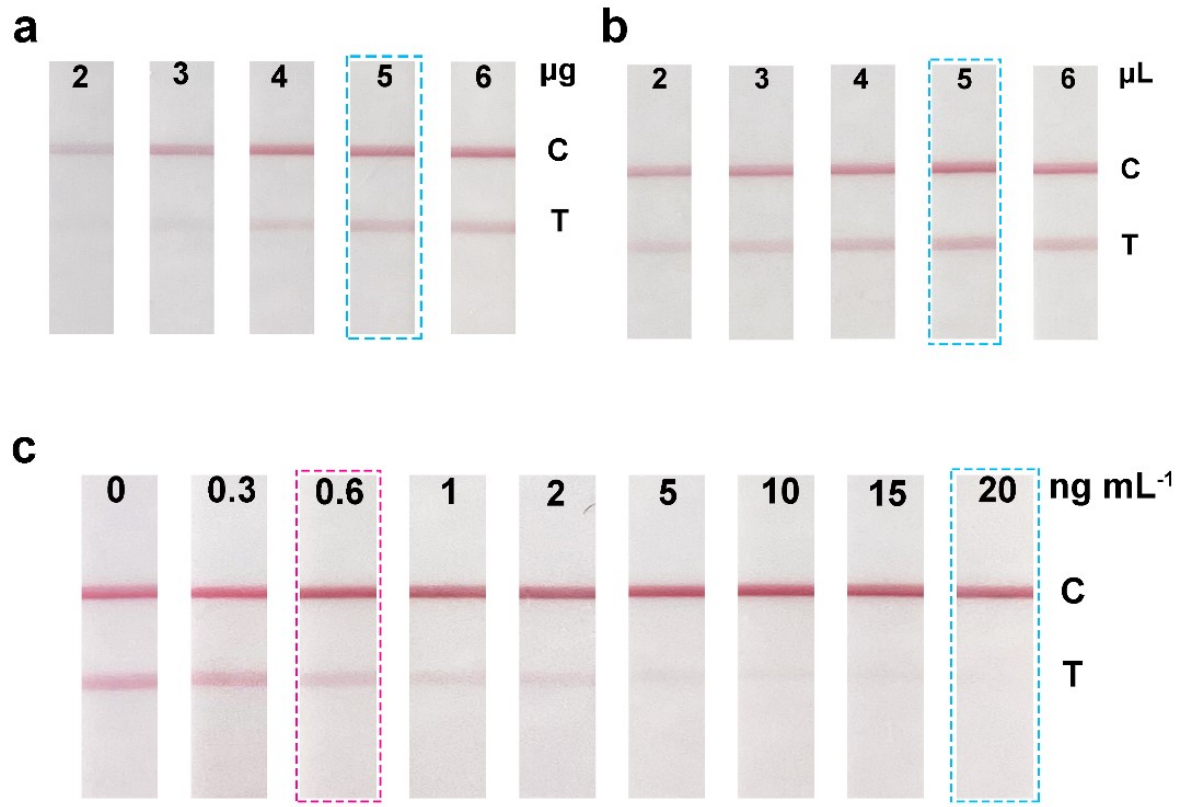


**Figure S2.** Optimization results of (a) the amount of anti-CLE mAb and (b) the volume of Au NPs-mAb probe. Detection results of (c) the CLE standard solution by the Au NPs-LFIA.

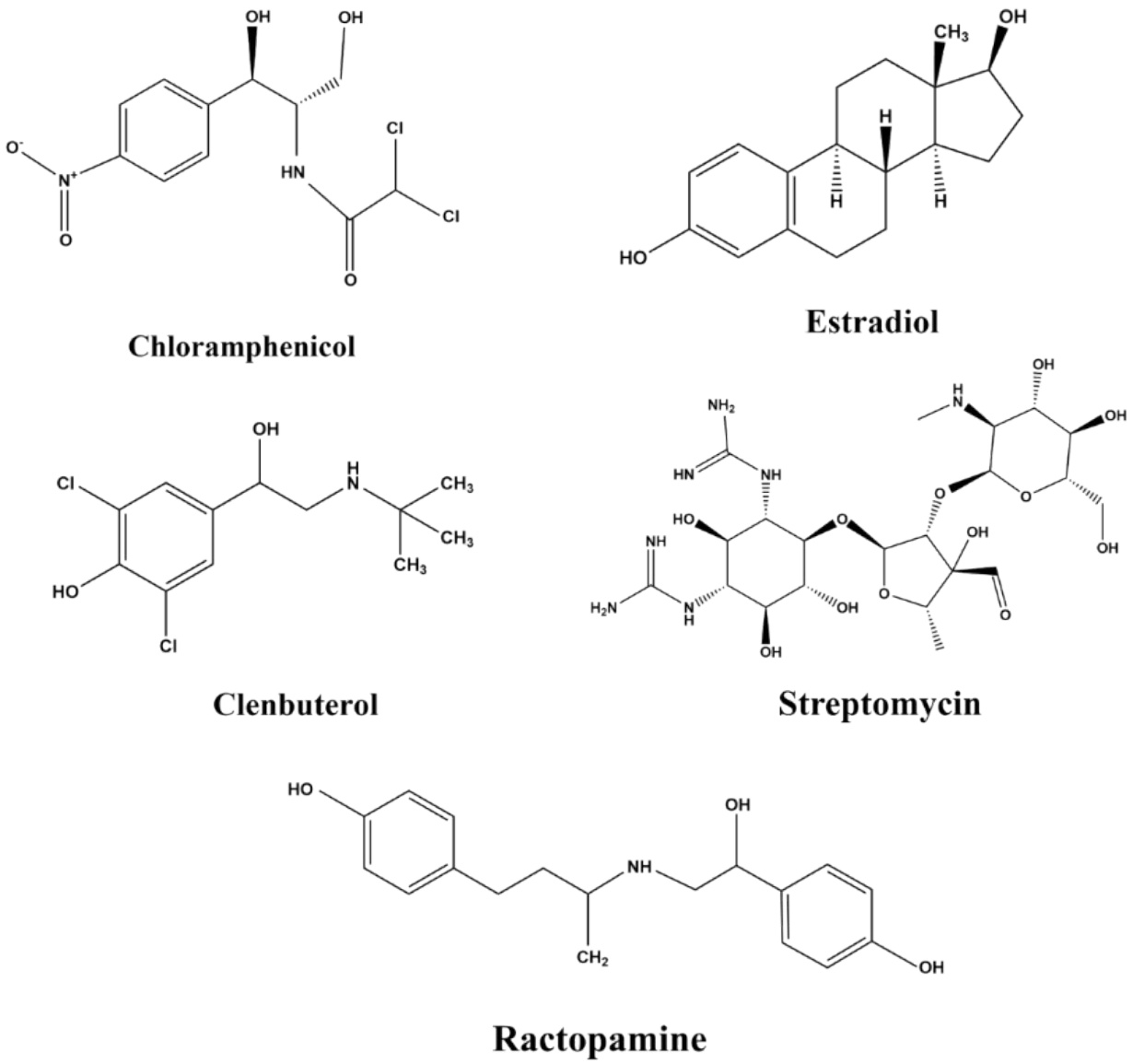


**Figure S3.** Chemical structures of the target analyte and tested interfering substances.

**Table S1.** Recovery analysis of CLE in actual samples

| Sample | Spiked (μg $L^{-1}$/μg $kg^{-1}$) | Found (μg $L^{-1}$/μg $kg^{-1}$) | Recovery (%) | SD (%) |
|---|---|---|---|---|
| Milk | 0.5 | 0.54 ± 0.02 | 107.21 | 1.66 |
| | 1 | 1.06 ± 0.01 | 106.32 | 0.91 |
| | 2 | 2.02 ± 0.05 | 100.99 | 5.31 |
| Mutton | 0.5 | 0.57 ± 0.05 | 115.07 | 5.29 |
| | 1 | 1.09 ± 0.03 | 109.55 | 2.56 |
| | 2 | 1.97 ± 0.05 | 98.53 | 5.16 |
| Pork | 0.5 | 0.58 ± 0.05 | 117.79 | 5.42 |
| | 1 | 1.01 ± 0.01 | 100.54 | 1.15 |
| | 2 | 1.98 ± 0.01 | 99.04 | 0.51 |

**Table S2.** Comparison of Michaelis-Menten constants (Km) and maximum initial reaction rates (Vmax) of the peroxidation reaction catalyzed by USCG, HRP, and other peroxidase-like nanozymes

| Materials | Km (mM) | | Vmax ($10^{-8}$ M $s^{-1}$) | | Reference |
|---|---|---|---|---|---|
| | TMB | $H_2O_2$ | TMB | $H_2O_2$ | |
| HRP | 0.432 | 3.702 | 10.00 | 8.71 | [1] |
| Au@Pt | 0.020 | 76.000 | 2.91 | 0.75 | [2] |
| Cu-hemin MOFs | 1.420 | 2.180 | 26.22 | 116.00 | [3] |
| $Cu(OH)_2$ SCs | 2.448 | 0.199 | 44.80 | 42.50 | [4] |
| $CuMnO_2$ | 0.577 | 27.653 | 8.15 | 27.65 | [5] |
| CuO | 0.025 | 0.400 | 10.49 | 16.10 | [6] |
| RhNPs | 0.198 | 0.380 | 6.78 | 24.10 | [7] |
| CuS-PDA-Au | 0.1082 | 0.229 | 102.9 | 16.56 | [8] |
| USCG | 0.013 | 0.107 | 81.32 | 20.75 | This work |

**Table S3.** Comparison of analytical performances for CLE detection by different methods immunoassays

| Detection method | Linear range (ng $mL^{-1}$) | cLOD (ng $mL^{-1}$) | Reference |
|---|---|---|---|
| Molecularly imprinted polymer-lateral flow immunoassay | 1–50 | 0.3 | 9 |
| Integrated immunomagnetic separation-fluorescence lateral flow immunoassay | 0.25–5 | 0.16 | 10 |
| Streptavidin magnetic particles-fluorometric immunoassay | 0.5–40 | 0.167 | 11 |
| GO/AuNPs-surface-enhanced Raman spectroscopy | 0.5–20 | 0.5 | 12 |
| SeNPs-lateral flow immunoassay | - | 3 | 13 |
| MoS2-Au-polyethylenimine modified-glassy carbon electrode | 10–2,000 | 1.92 | 14 |
| Molecularly imprinted polymers@Upconversion particles-fluorescence analysis | 5–100 | 0.12 | 15 |
| S/N-doped carbon quantum dots-fluorometric immunoassay | 0.07–1.7 | 0.023 | 16 |
| $Fe_3O_4$ microspheres-immunoassay | 0.1-10 | 0.02 | 17 |
| CdSe quantum dots-electrochemiluminescent immunosensor | 0.05-1000 | 0.02 | 18 |
| AuNPs-rhodamine 6G-fluorescence inner filtration immunoassay | 0.03–5.0 | 0.01 | 19 |
| Gold nanoparticles-surface-enhanced Raman spectroscopy | 0.0001–0.1 | 0.0001 | 20 |
| $Fe_3O_4$@AuNPs- surface-enhanced Raman spectroscopy | - | $2.2*10^{-7}$ | 21 |
| USCA-based LFIA | 0.05-1 | 0.03 | This work |

**Table S4.** Optimal assay conditions for the USCG-based LFIA

| Item | Composition | Optimized condition |
|---|---|---|
| C line | Goat anti-mouse IgG | 2 mg $mL^{-1}$, 0.5 μL $cm^{-1}$ |
| T line | CLE-BSA | 0.4 mg $mL^{-1}$, 0.5 μL $cm^{-1}$ |
| Blocking buffer for sample pad | Phosphate buffer saline | 2% BSA in PBS |

## References


1. L. Luo, L. Huang, X. Liu, W. Zhang, X. Yao, L. Dou, X. Zhang, Y. Nian, J. Sun and J. J. I. c. Wang, 2019, 58, 11382-11388.
2. X. Hu, A. Saran, S. Hou, T. Wen, Y. Ji, W. Liu, H. Zhang, W. He, J.-J. Yin and X. J. R. a. Wu, 2013, 3, 6095-6105.
3. F. Liu, J. He, M. Zeng, J. Hao, Q. Guo, Y. Song and L. J. J. o. N. R. Wang, 2016, 18, 1-9.
4. R. Cai, D. Yang, S. Peng, X. Chen, Y. Huang, Y. Liu, W. Hou, S. Yang, Z. Liu and W. J. J. o. t. A. C. S. Tan, 2015, 137, 13957-13963.
5. J. Hu, R. Huang, Y. Sun, X. Wei, Y. Wang, C. Jiang, Y. Geng, X. Sun, J. Jing, H. Gao, Z. Wang and C. Dong, J. Microbiol. Methods, 2019, 158, 25-32.
6. A. P. Nagvenkar, A. J. A. a. m. Gedanken and interfaces, 2016, 8, 22301-22308.
7. T. G. Choleva, V. A. Gatselou, G. Z. Tsogas and D. L. J. M. A. Giokas, 2018, 185, 1-9.
8. Y. Wang, Y. Liu, F. Ding, X. Zhu, L. Yang, P. Zou, H. Rao, Q. Zhao, X. J. A. Wang and b. chemistry, 2018, 410, 4805-4813.
9. H. Zhou, Z. Zhang, D. He, Y. Hu, Y. Huang and D. J. A. c. a. Chen, 2004, 523, 237-242. √
10. Z. Huang, Z. Xiong, Y. Chen, S. Hu, W. J. J. o. a. Lai and f. chemistry, 2019, 67, 3028-3036.
11. T. Peng, J. Wang, S. Zhao, S. Xie, K. Yao, P. Zheng, S. Wang, Y. Ke and H. Jiang, Mikrochim Acta, 2018, 185, 366. √
12. J. Cheng, X.-O. Su, S. Wang and Y. J. S. r. Zhao, 2016, 6, 1-10.
13. Z. Wang, J. Jing, Y. Ren, Y. Guo, N. Tao, Q. Zhou, H. Zhang, Y. Ma and Y. J. M. L. Wang, 2019, 234, 212-215.
14. Y. Yang, H. Zhang, C. Huang, D. Yang, N. J. B. Jia and Bioelectronics, 2017, 89, 461-467. √
15. Y. Tang, Z. Gao, S. Wang, X. Gao, J. Gao, Y. Ma, X. Liu, J. J. B. Li and Bioelectronics, 2015, 71, 44-50.
16. D. Yao, A. Liang and Z. Jiang, Microchimica Acta, 2019, 186, 1-9.
17. K. Fan, D. Li, H. Liu and J. Wu, Instrumentation Science & Technology, 2015, 43, 5, 524-535.
18. X. Yao, P. Yan, Q. Tang, A. Deng and J. Li, Analytica Chimica Acta, 2013, 798, 82-88.
19. F. Peng, B. Li, S. Sun, F. Mi, Y. Wang, C. Hu, P. Geng, L. Pang, M. Guan and J. Li, European Food Research and Technology, 2022, 1-12.
20. G. Zhu, Y. Hu, J. Gao and L. Zhong, Analytica Chimica Acta, 2011, 697, 1-2, 61-66.
21. C. Wei, C. Zhang, M. Xu, Y. Yuan and J. Yao, Journal of Raman Spectroscopy, 2017, 48, 1307-1317.