

## Electronic Supplementary Information (ESI)

### **Cu<sup>2+</sup>-doped zeolitic imidazolate frameworks and gold nanoparticles (AuNPs@ZIF-8/Cu) nanocomposites enable label-free and highly sensitive electrochemical detection of oral cancer-related biomarkers**

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## Experimental details

### Materials and Regents

Zinc acetate dihydrate  $[(\text{CH}_3\text{COO})_2\text{Zn}\cdot 2\text{H}_2\text{O}]$ , Copper nitrate trihydrate  $\text{Cu}(\text{NO}_3)_2\cdot 3\text{H}_2\text{O}$ , methanol ( $\text{CH}_3\text{OH}$ ), chloroauric acid ( $\text{HAuCl}_4$ ), sodium borohydride ( $\text{NaBH}_4$ ), tris (hydroxymethyl) aminomethane (Tris), boric acid ( $\text{H}_3\text{BO}_3$ ), Ethylene Diamine Tetraacetic Acid (EDTA), hydrochloric acid (HCl), sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ), disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ), sodium chloride (NaCl), potassium chloride (KCl), potassium ferricyanide ( $\text{K}_3[\text{Fe}(\text{CN})_6]$ ), potassium ferrocyanide ( $\text{K}_4[\text{Fe}(\text{CN})_6]$ ) were obtained from Sinopharm Chemical Reagent Company Ltd. (Shanghai, China). 2-methylimidazole and bovine serum albumin (BSA) were provided by Aladdin Chemistry Co., Ltd. (Shanghai, China). Ammonium persulphate (APS), 6 $\times$ loading buffer, N, N, N', N'-tetramethylethylenediamine (TEMED) and 30% acrylamide-methylenebisacrylamide [30% Acr-Bis (29:1)] were obtained from Shanghai Beyotime Biotech Inc. SYBR-Gold were obtained from Thermo Fisher Scientific Inc. Glassy carbon electrodes (GCE,  $\Phi=3$  mm) were purchased from Gaoss Union Technology Co., LTD (Wuhan, China). 0.01 M PBS (0.01 M  $\text{Na}_2\text{HPO}_4$ , 0.01 M  $\text{NaH}_2\text{PO}_4$ , 0.01 M NaCl and 0.01 M KCl, pH =7.4) and 0.1 M PBS (0.1 M  $\text{Na}_2\text{HPO}_4$ , 0.1 M  $\text{NaH}_2\text{PO}_4$ , 137 mM NaCl and 0.1 M KCl, pH =7.4) were used to rinse electrode and measure electrochemical signal, respectively. 1 $\times$ TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH=8.0) was chose as electrophoresis buffer. HPLC purified DNAs were synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China) and the sequences were listed in **Table S1**.

### Instrument

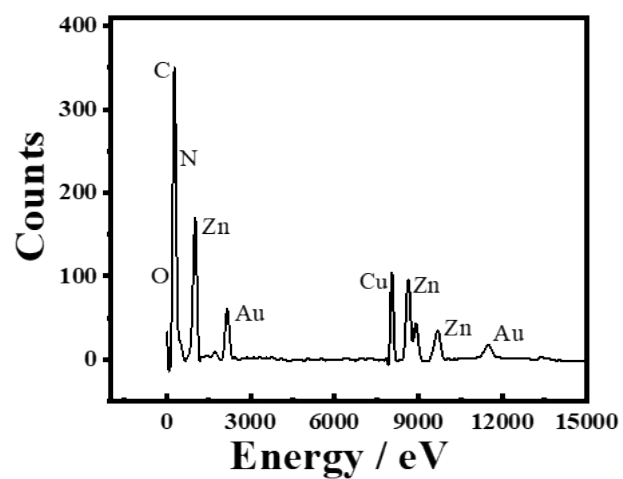
The scanning electron microscopy (SEM) images were taken on a field emission scanning electron microscope (Zeiss Supra 55, room temperature, atmospheric pressure and humidity of 50%). The transmission electron microscopy (TEM) images were taken on a transmission electron microscope (Tecnai 12, room temperature, atmospheric pressure and humidity of 50%). The high-angle annular dark field scanning transmission electron microscopy (HAADF-STEM) and energy dispersive X-ray (EDX) elemental mappings were taken on a field emission transmission electron microscope (Tecnai G2 F30 S-TWIN, The temperature is maintained at 16-30°C and the voltage is 220 $\pm$ 5V). Ultraviolet-visible (UV-vis) absorption spectra were carried out with LAMBDA 650 spectrometer (PerkinElmer, USA). The X-ray diffraction (XRD) were performed on a D8 ADVANCE diffractometer (Conventional wide angle 5-90°, small angle 0.5-10°. Conventional test

rates are 10°/min, 5°/min, 1°/min, and small Angle test rates are 1°/min and 0.5°/min). The zeta potential measurements were accomplished with Zetasizer Nano ZS (25°C). Nucleic acid electrophoresis apparatus (BG-VerMINI, run at 95 V for 2 h in 1×TBE) was applied for 12% native polyacrylamide gel electrophoresis (PAGE). All the electrochemical measurements, including differential pulse voltammetry (DPV, scan from -0.1 V to 0.5 V with amplitude of 0.05 V, pulse width of 0.05 V and pulse period of 0.5 s), electrochemical impedance (EIS, scan from 0.01 Hz to 100 kHz with 5 mV amplitude at 0.213 V potential) and cyclic voltammetry (CV, scan from -0.2 to 0.6 V at 50 mV/s) and square wave voltammetry (SWV, scan from -0.4 to 0.2 V in 0.1 M PBS with amplitude of 0.025 V and frequency of 15 Hz) were measured on an electrochemical workstation (CHI 660D). A standard three-electrode system containing a glassy carbon electrode (GCE), a platinum electrode and an Ag/AgCl (saturated KCl) electrode acted as the working, counter and reference electrode, respectively. Centrifugation in a high-speed centrifuge (H1850) and dry in an oven system (DZF-6002).

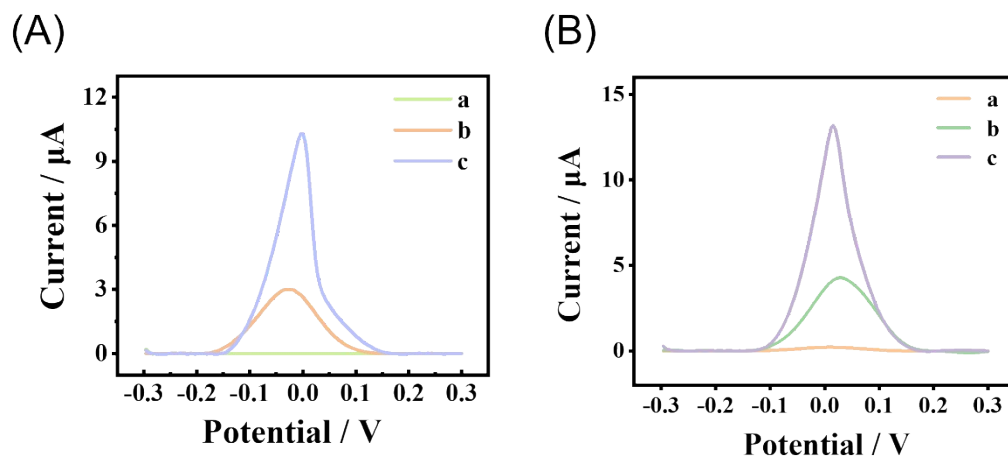
**Table S1.** All oligonucleotides used in this work

Name	5' to 3'
S	CTCTCGTTCTTTATCCCTCGACTGTTTT
L	AAAGAACGAGAGTCTTTCTG
T	CAGAAAGACTCTCGTTCTTT
P1	AAAGAACGAGAG-TTTTT-SH
P2	NH <sub>2</sub> -TTTTT-ACAGTCGAGGGAT
sm DNA	CAGAAAGACT <u>G</u> TCGTTCTTT
dm DNA	CAGAAAGAC <u>GG</u> TCGTTCTTT
non DNA	GCTCCCTTCAGAGCAATCCC

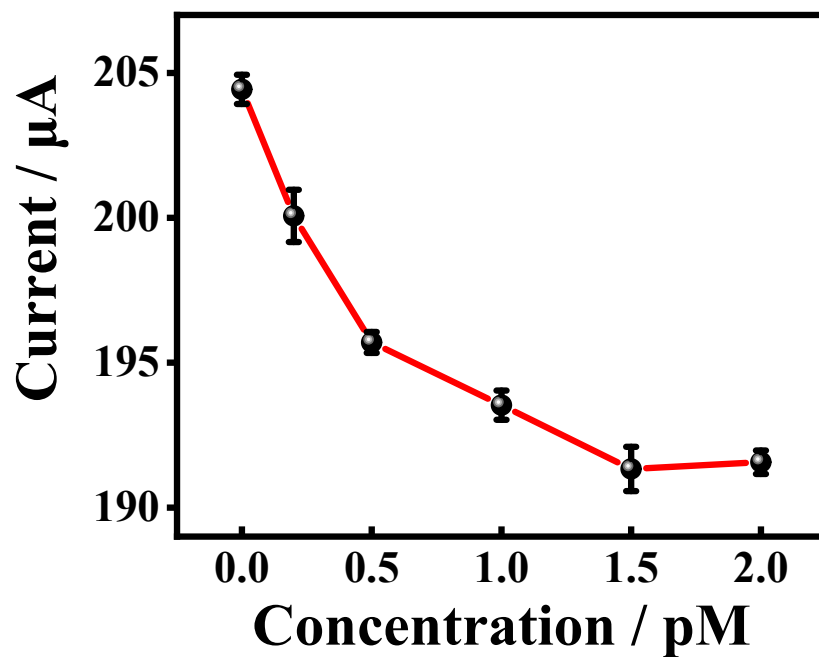
**Note:** The red font represent toehold; the bold font of the underline represents the single-mismatched base and double-mismatched bases; non-DNA denotes the non-complementary DNA.



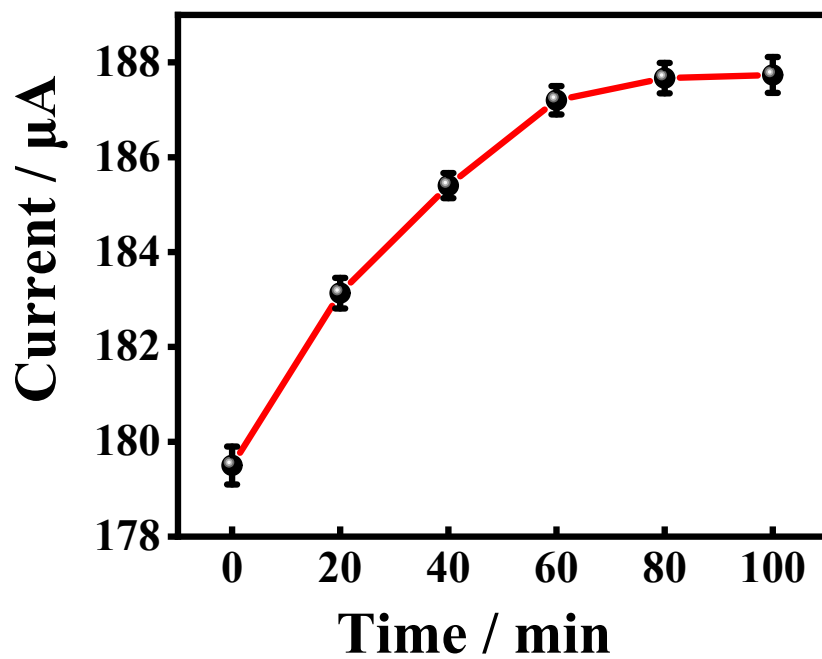
**Fig. S1** The EDX spectrum of AuNPs@ZIF-8/Cu nanocomposites. To avoid the influence of copper mesh, AuNPs@ZIF-8/Cu materials are dropped on tinfoil to obtain the EDX result.



**Fig. S2** The square wave voltammetry (SWV) (A) and differential pulse voltammetry (DPV) measurements (B) of AuNPs@ZIF-8 (a), AuNPs@ZIF-8/Cu nanocomposites (b) and Cu-MOF (c) in 0.1 M PBS (pH 7.4).

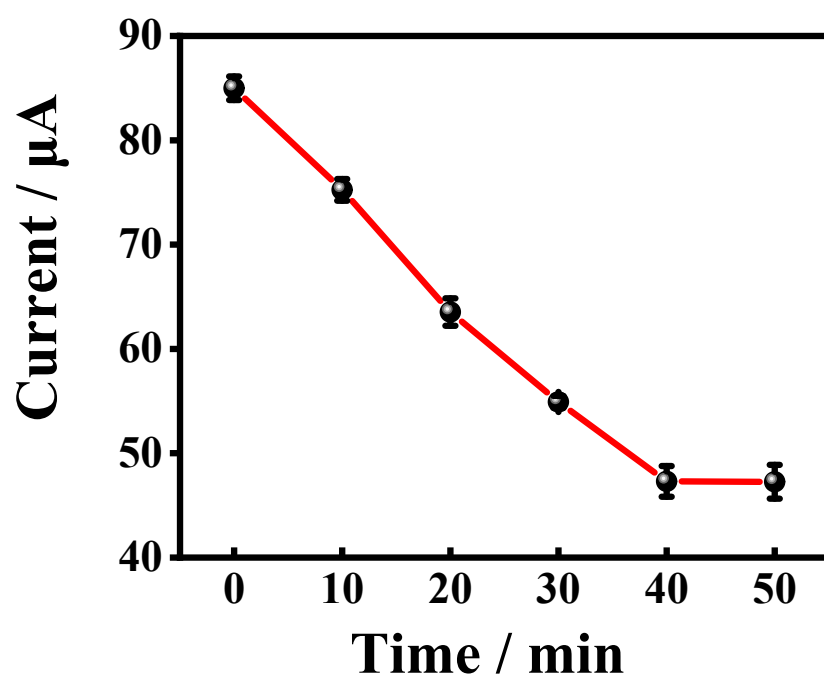


**Fig. S3** The peak current change of newly developed electrochemical sensor as a function of P1 concentration in 0.1 M PBS (pH 7.4) containing 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ . The error bar represents the standard deviation of the three parallel experiments.

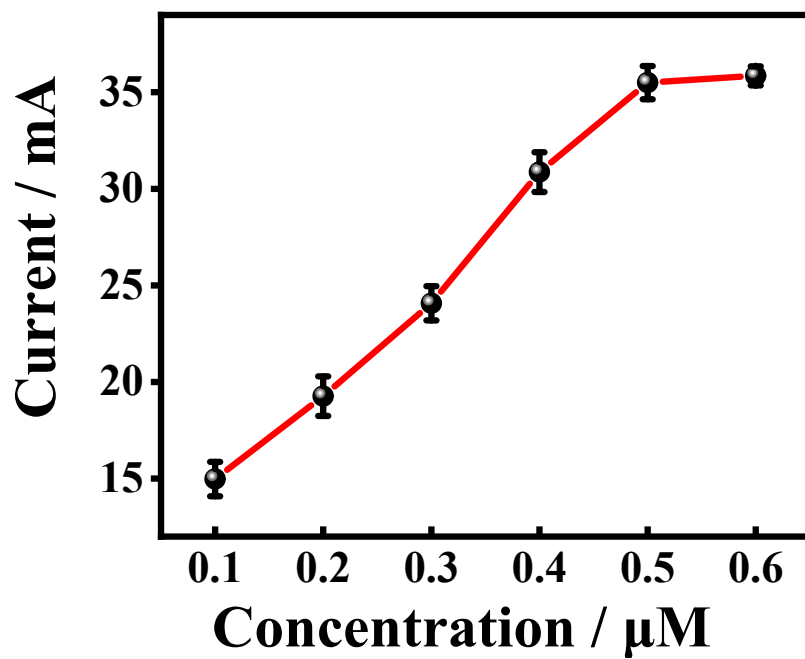


**Fig. S4** The peak current change of newly developed electrochemical sensor as a function of the incubation time of P1 in 0.1 M PBS (pH 7.4) containing 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ . The error bar represents the standard deviation of the three parallel experiments.

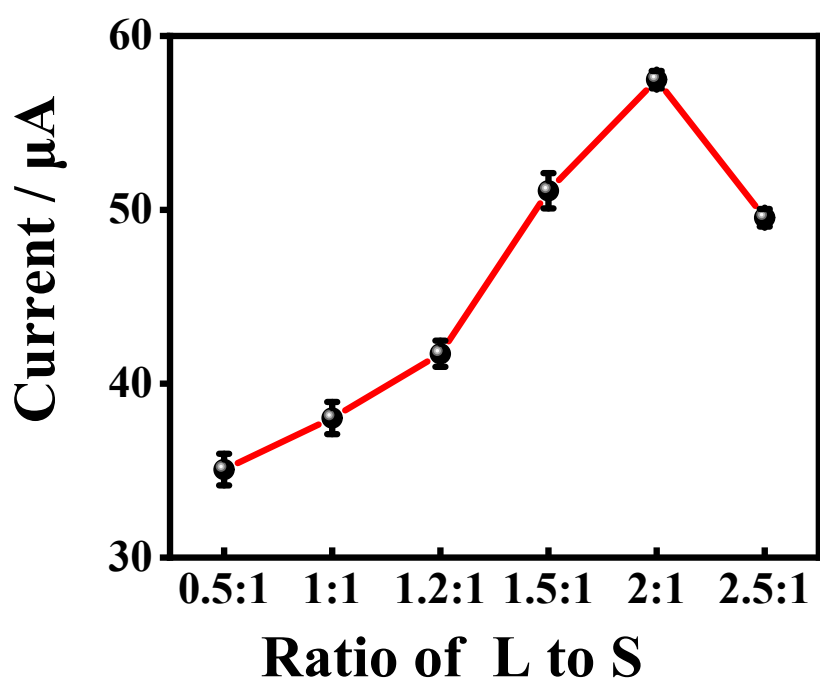




**Fig. S5** The peak current change of newly developed electrochemical sensor as a function of the reaction time of BSA in 0.1 M PBS (pH 7.4) containing 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ . The error bar represents the standard deviation of the three parallel experiments.



**Fig. S6** Study on the effect of P2 concentration on electrochemical signal in 0.1 M PBS (pH 7.4) containing 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ . The error bar represents the standard deviation of the three parallel experiments.



**Fig. S7** Optimization of the hybridization ratio of L to S in 0.1 M PBS (pH 7.4) containing 5 mM  $[\text{Fe}(\text{CN})_6]^{3-/4-}$ . T was incubated with different L+S duplex in 10 mM PBS for 1 h at 37°C. The error bar represents the standard deviation of the three parallel experiments.

**Table S2.** Comparison of different electrochemical biosensors for ORAOV 1 detection.

Method	Design	Reaction time	Linear range	LOD	Reference
EC	Complex (Nt·BstNBI)	14-15 h	10 fM-10 nM	3 fM	[1]
ECL	Complex (Nb.BbvCl)	4-6 h	10 fM-1 nM	3.3 fM	[2]
EC	Complex (exonuclease III)	6-7 h	0.02 pM-2 nM	12.8 fM	[3]
EC	Complex (Nt·BstNBI)	3-5 h	1 pM-10 pM	350 fM	[4]
EC	Simple (enzyme-free)	2-3 h	0.1 pM-10 nM	63 fM	This work

Note: EC denotes Electrochemical, ECL represents Electrochemiluminescence.

## Reference

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