## Construction of a point-of-care electrochemical biosensor for

## Escherichia coli 16S rRNA analysis based on MoS<sub>2</sub> nanoprobes

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## 1. Reagents and Materials

All the reagents are listed in the Supporting Information. N-butyl lithium was purchased from Amethyst. Polyvinylpyrrolidone (PVP·K30, molecular weight = 30 000-40 000), chloroauric acid (HAuCl<sub>4</sub>·3H<sub>2</sub>O) were purchased from Aladdin industries. molybdenum (IV) sulfide powders (<2 µm, 99%), polyacrylic acid(PAA), polyethyleneimine ethoxylated(PEI), potassium ferricyanide, potassium hexacyanoferrate(II), potassium chloride, Tris (2-carboxyethyl) phosphine (TCEP) and 6-mercapto-1-hexanol (MCH) were purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). Thionine (Thi), sodium phosphate dibasic dodecahydrate (Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O, ≥99.0%) and sodium phosphate monobasic dihydrate (NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, ≥99.0%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Phosphate buffer solution (PB) was prepared by 0.2 M sodium phosphate dibasic dodecahydrate and 0.2 M sodium phosphate monobasic dihydrate. Aqueous solutions were prepared by using ultrapure water (> 18 MΩ·cm) obtained from a Millipore water purification system.



**Fig. S1.** CV curves of (a) HFGNs-SPCE, (b) DNA1/HFGNs-SPCE, (c) MCH/DNA1/HFGNs-SPCE, (d) 16S rRNA/MCH/DNA1/HFGNs-SPCE, (e) nanoprobes/16S rRNA/MCH/DNA1/HFGNs-SPCE.



**Fig. S2.** The detection conditions of this electrochemical biosensor were optimized. Signal intensities are obtained from Thi at -0.38 V. (A) Effect of MCH concentration, (B) effect of SH-DNA1 concentration, (C) DNA2-MoS<sub>2</sub>-Thi-AuNPs concentration and (D) incubation time of DNA2-MoS<sub>2</sub>-Thi-AuNPs on the detection performance of this biosensor.

Analytical method	Linear range (fM)	Detection limit (fM)	Reference
Electrochemistry	10 - 106	5	[1]
Electrochemistry	1 - 10 <sup>3</sup>	10	[2]
Electrochemistry	$1 \times 10^{4}$ - 3 × 10 <sup>9</sup>	$10^{4}$	[3]
Chemiluminescent	-	8	[4]
Surface plasmon resonance	-	0.45	[5]
Surface plasmon resonance	$5 \times 10^6$ - $5 \times 10^8$	2×10 <sup>6</sup>	[6]
Fluorescence	$10^{6} - 10^{7}$	$1.7  imes 10^5$	[7]
Fluorescence	$5 \times 10^{3} - 1.6 \times 10^{4}$	$6  imes 10^2$	[8]
Electrochemistry	$10^{1} - 10^{5}$	2.8	This work

**Table S1.** Comparison of analytical performance for 16S rRNA detection.

Table S2. The performance of this electrochemical sensor for 16S rRNA detection in milk

		samples.		
Sample	Add	Found	Recovery (%)	RSD (%)
	(fM)	(fM)		
1	$1.0 \times 10^{1}$	10.8	108.0	2.2
2	$1.0 \times 10^{2}$	105.0	105.0	1.8
3	1.0×10 <sup>3</sup>	1019.1	101.9	1.6
4	$1.0 \times 10^{4}$	9893.6	98.9	1.4
5	1.0×10 <sup>5</sup>	96047.8	96.0	3.0

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