Supplementary Material

High-flux smartphone-integrated lateral flow assay based on chrysanthemum-like Au@Polydopamine for sensitive detection of enrofloxacin in milk

Ganggang Zhang,[a,1] Xiaocui Lai,[b,1] Weihua He,[a] Liu Su,[a] Gan Zhang,[b] Weihua Lai,[b] Shengliang Deng[a,*]

[a]*Institute of Microbiology, Jiangxi Academy of Sciences, Nanchang 330096, China*

[b]*State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, China*

*Corresponding author:

Shengliang Deng, Ph.D., Professor

Institute of Microbiology, Jiangxi Academy of Sciences

E-mail: dslzdy@hotmail.com

Address: 7777 Changdong Avenue, Nanchang 330096, China

1 These authors contributed equally to this work

Outline:

Materials

The glass fiber sample pad (SB08) and the cellulose fiber absorption pad (CH37) were provided by Shanghai Kinbio Tech. Co., Ltd. (Shanghai, China). The nitrocellulose (NC) membrane (CN 140) was purchased from Sartorius AG. (Göttingen, Germany). The commercial AuNP-lateral flow test strips (vvAuNP-LFA) and corresponding colloidal gold-labeled antibody (vvAuNP-mAb″) were purchased from Beijing WDWK Biotechnology Co., Ltd. (Beijing, China). The actual milk samples were obtained from local Rainbow Supermarket (Nanchang, China).

Buffer preparation

Tris–HCl buffer (0.01 M, pH 8.5): 1.576 g Tris–HCl was dissolved in 950 mL ultrapure water. The pH level was adjusted to 8.5 with 1 M NaOH, and the buffer was diluted with ultrapure water to 1 L.

PBS (0.01 M, pH 7.4): 0.24 g KH_2PO_4, 1.44 g Na_2HPO_4, 8 g NaCl, and 0.2 g KCl were dissolved in 800 mL ultrapure water. The pH level was adjusted to 7.4 with NaOH and HCl. The buffer was diluted with ultrapure water to 1 L.

Boric acid buffer (0.2 M): 19.07 g $Na_2B_4O_7 \cdot 10H_2O$ and 12.37 g H_3BO_3 were dissolved in 800 mL ultrapure water. The pH level was adjusted to 8 with 1 M NaOH, and the buffer was diluted with ultrapure water to 1 L.

Equipment

The UV-Vis absorption spectrum was obtained by using Thermo Scientific™ Varioskan™ LUX (Thermo Scientific, USA). The TEM and HRTEM images were taken by JEOL JEM-2100 (JEOL, Japan). The hydrodynamic diameter and zeta data

were accessed from Zetasizer Nano ZS90 (Malvern Instruments, UK). The energy-dispersive X-ray (EDX) spectroscopy was determined EDX spectroscopy via in STEM mode. Atomic force microscopy (AFM) was taken by BRUKER Dimension Icon, and X-ray Diffraction (XRD) was measured by Thermo Fisher ARL EQUINOX 3000. The BioDot XYZ platform, which combined motion control with BioJet Quanti3050k and AirJet Quanti3050k dispensers, was acquired from BioDot (Irvine, CA). The conventional test strip reader was purchased from obtained from Hangzhou Hemai Technology Co., Ltd. (China). The smartphone-integrated device and multichannel strip plate were "printed" by a 3D printer (WeNext Technology Co., Ltd., China) and assembled in our laboratory. The photos were taken by Mi 11 Smartphone (Xiaomi Technology Co., Ltd., China) and processed by ImageJ software (version 1.51j8; National Institutes of Health, USA).

Synthesis of AuNP

AuNP was prepared by a classical sodium citrate reduction method [1]. Under stirring at 500 r/min, 1 mL of $HAuCl_4$ solution (1%, *w/v*) was added into 99 mL of ultrapure water, then heated to boiling for 30 min, followed by 1.45 mL of sodium citrate solution (1%, *w/v*) was added into the above-mixed solution. Finally, the solution was gradually cooled to room temperature to obtain AuNP and stored at 4 °C.

Conjugating efficiency of AuNC@PDA or AuNP with mAb

The conjugating efficiency of AuNC@PDA or AuNP with mAb was evaluated as follows. First, 0.2 mL of mAb solution (25, 50, 75, 100 or 125 μg/mL) was added to 2 mL of AuNC@PDA or AuNP solution and incubated for 1 h. The resulting solution was

centrifuged at 7000 r/min (4 °C) for 15 min and the supernatant was collected. Then 100 μL of supernatant and a series of concentrations of mAb solution were added to the 96-well ELISA plate and incubated at 37 °C for 2 h. After being washed thrice, each well was blocked with 200 μL of blocking buffer at 37 °C for 2 h. After being washed thrice, 100 μL of HRP-labeled sAb solution was added to each well. After being incubated at 37 °C for 30 min, the plates were washed thrice and added with 100 μL of TMB substrate to react at 37 °C for 15 min. Then, the enzymatic reaction was stopped with 50 μL of H_2SO_4 (2 M). Finally, the absorbance at 450 nm (OD_{450}) of solution in the 96-well ELISA plate was measured. Each experiment was repeated thrice. The OD_{450} of supernatant was used to calculate the concentration of the unconjugated antibody with the established standard curve of a series of concentrations of mAb solution. The conjugating efficiency was calculated by the following equation. Conjugating efficiency = (total added mAb − unconjugated mAb) / total added mAb × 100%.

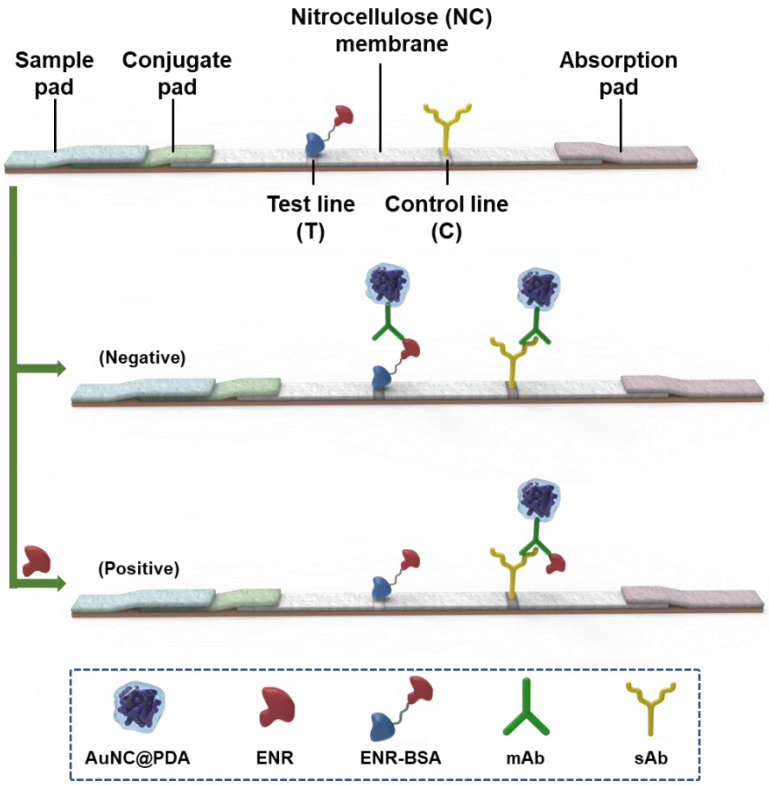


Fig. S1. The principle of AuNC@PDA-based LFA for detection of enrofloxacin (ENR).

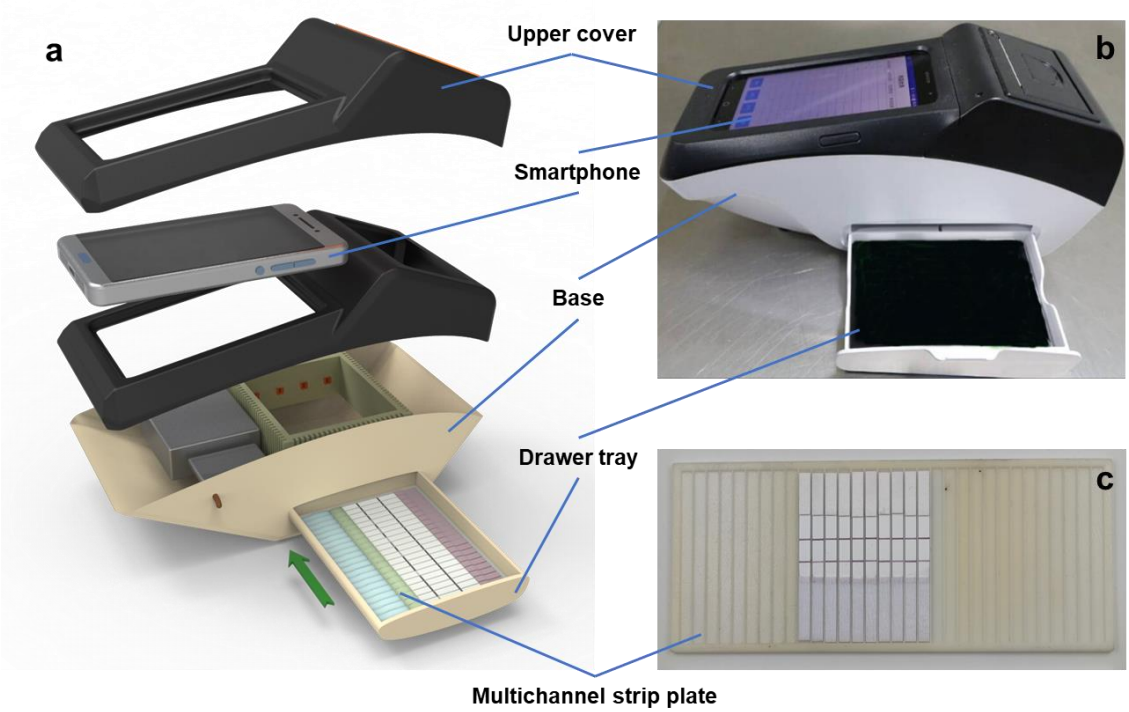


Fig. S2. The structure of smartphone-integrated device. (a) 3D model; (b) Actual picture; (c) Multichannel strip plate.

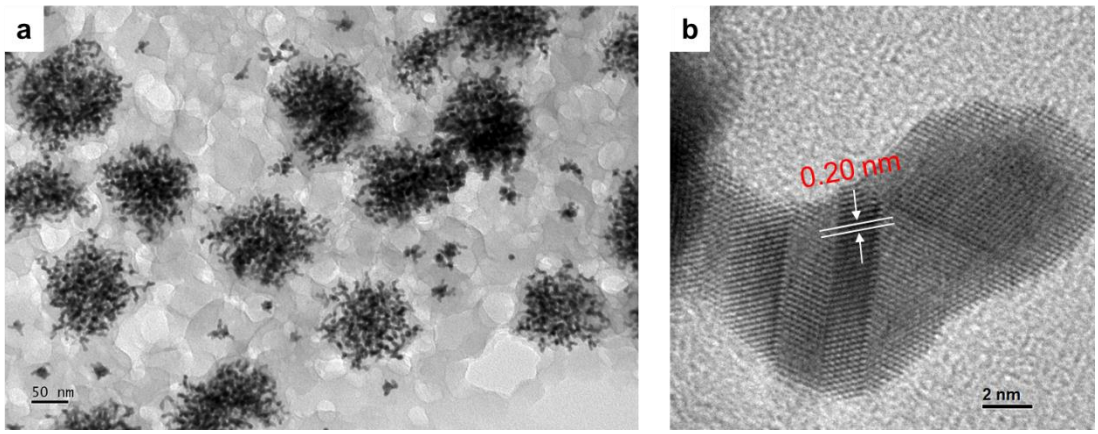


Fig. S3. (a) TEM and (b) HRTEM of AuNC@PDA.

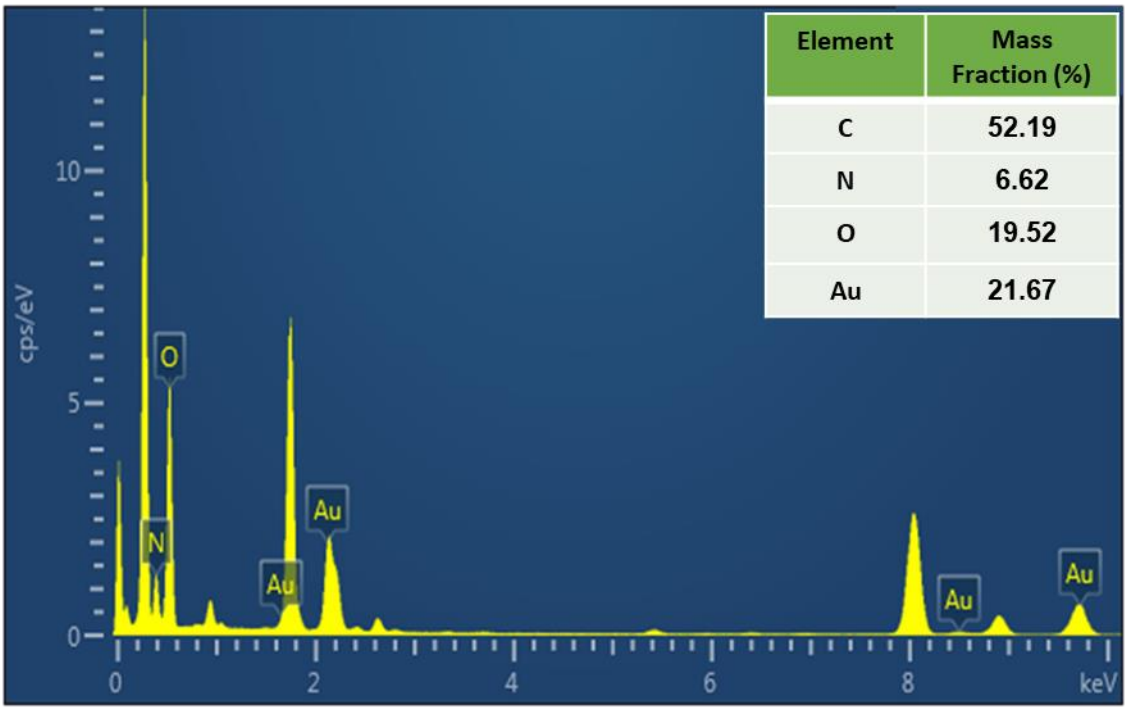
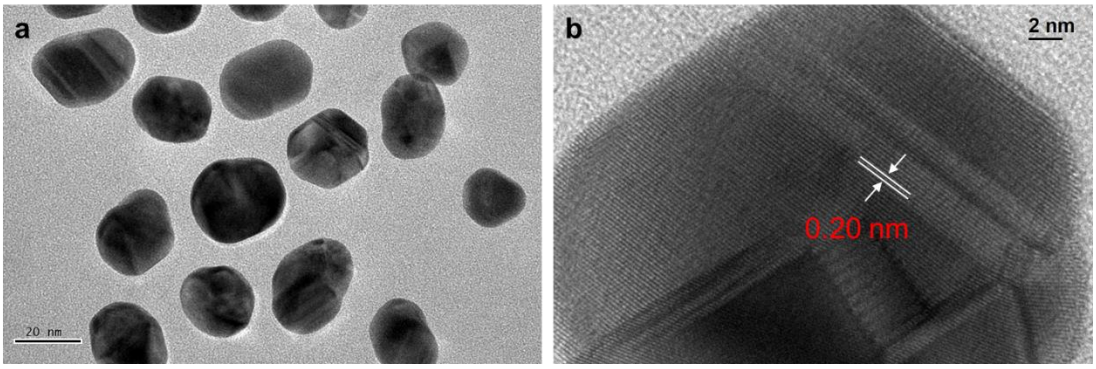


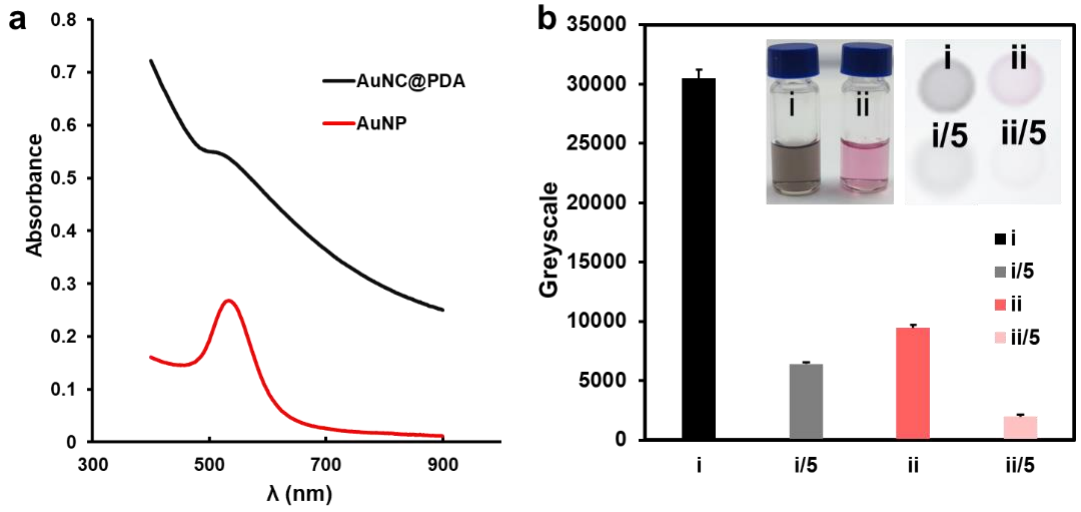
Fig. S4. EDX spectroscopy of AuNC@PDA.



145

146 **Fig. S5.** (a) TEM and (b) HRTEM of AuNP.

147



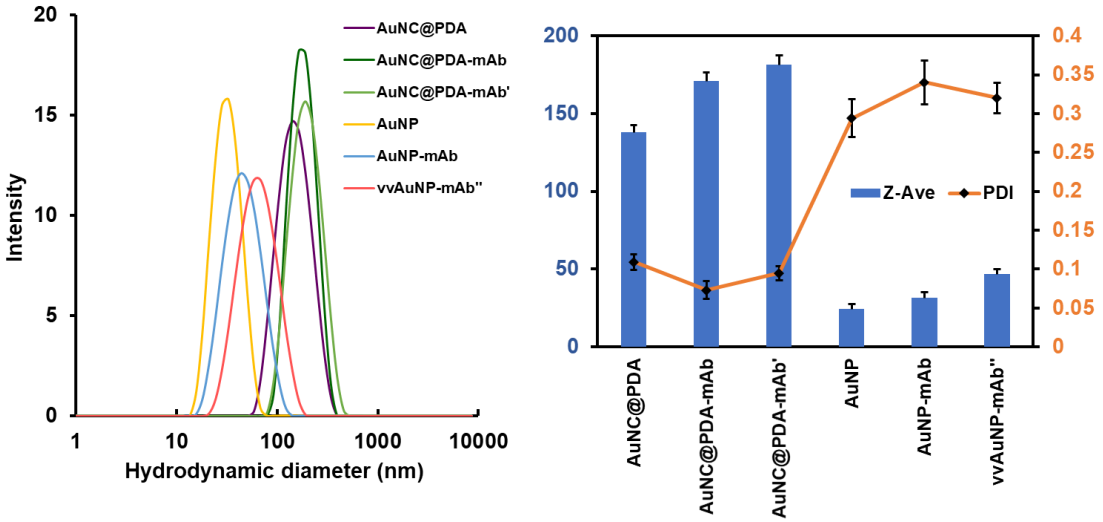
148

149 **Fig. S6.** Optical properties of AuNC@PDA and AuNP. (a) UV–vis absorption spectrum:

150 The concentrations of two nanoparticles were 0.1 mg/mL; (b) The grayscale value on

151 NC membrane. i: 0.1 mg/mL AuNC@PDA; ii: 0.1 mg/mL AuNP; i/5: 0.02 mg/mL

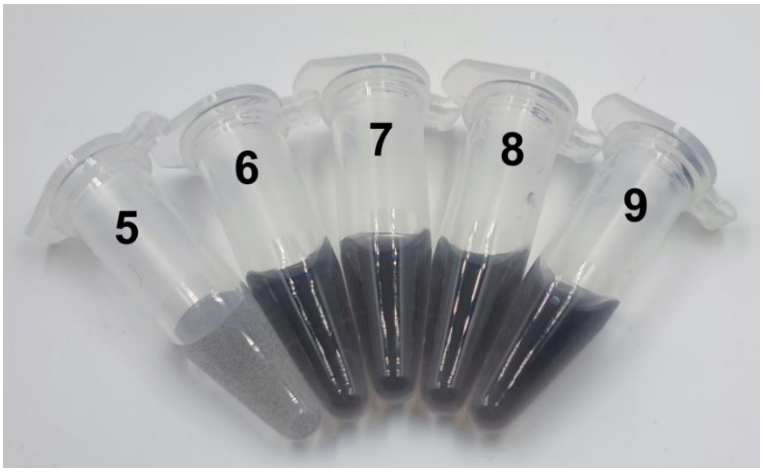
152 AuNC@PDA; ii/5: 0.02 mg/mL AuNP. Their volumes were 3 μL.



153

154 **Fig. S7.** (a) Hydration size distribution and (b)average size–PDI of nanoparticles.

155



156

157 **Fig. S8.** The actual picture of AuNC@PDA-mAb labeling results at different pH (5-9).

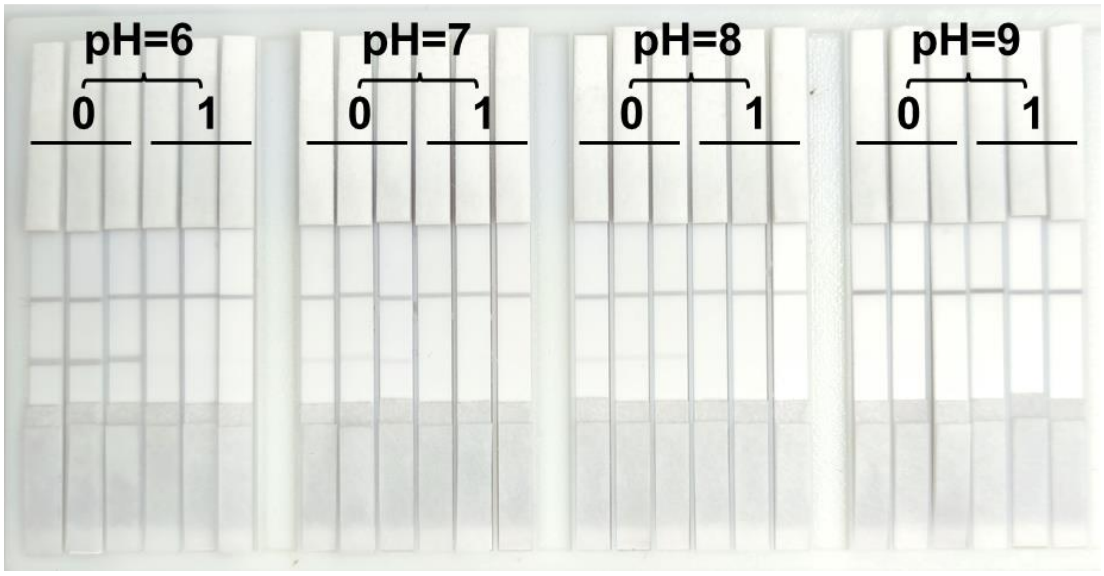


Fig. S9. Photo of pH (6-9) optimization experiments of AuNC@PDA-mAb labeling. The concentration of added mAb was 50 μg/mL. The ENR concentrations of spiked samples were 0 and 1 ng/mL, respectively.

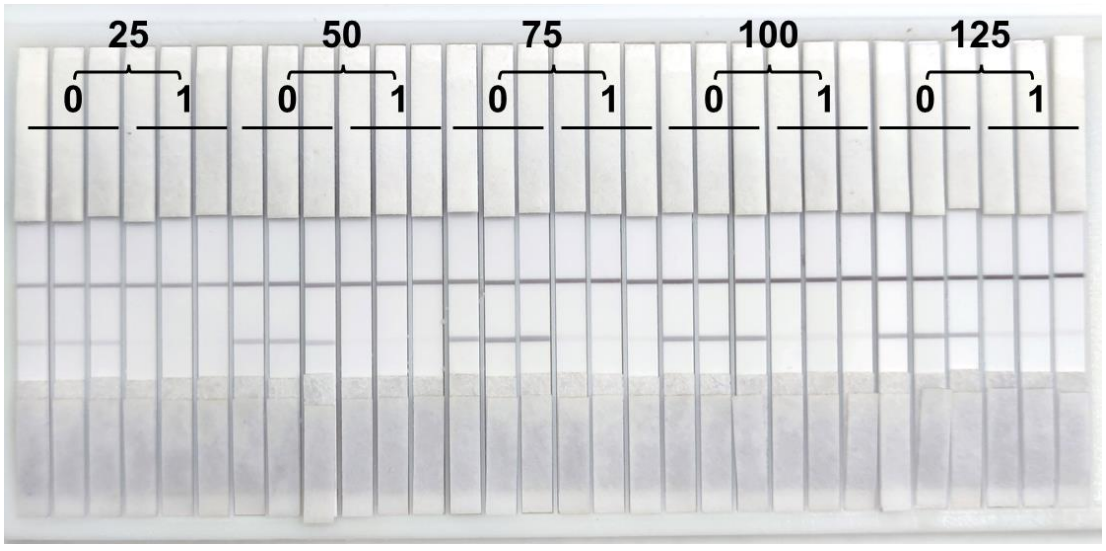


Fig. S10. Photo of concentration of added mAb (25-125 μg/mL) optimization experiments of AuNC@PDA-mAb labeling. The pH was 6. The ENR concentrations of spiked samples were 0 and 1 ng/mL, respectively.

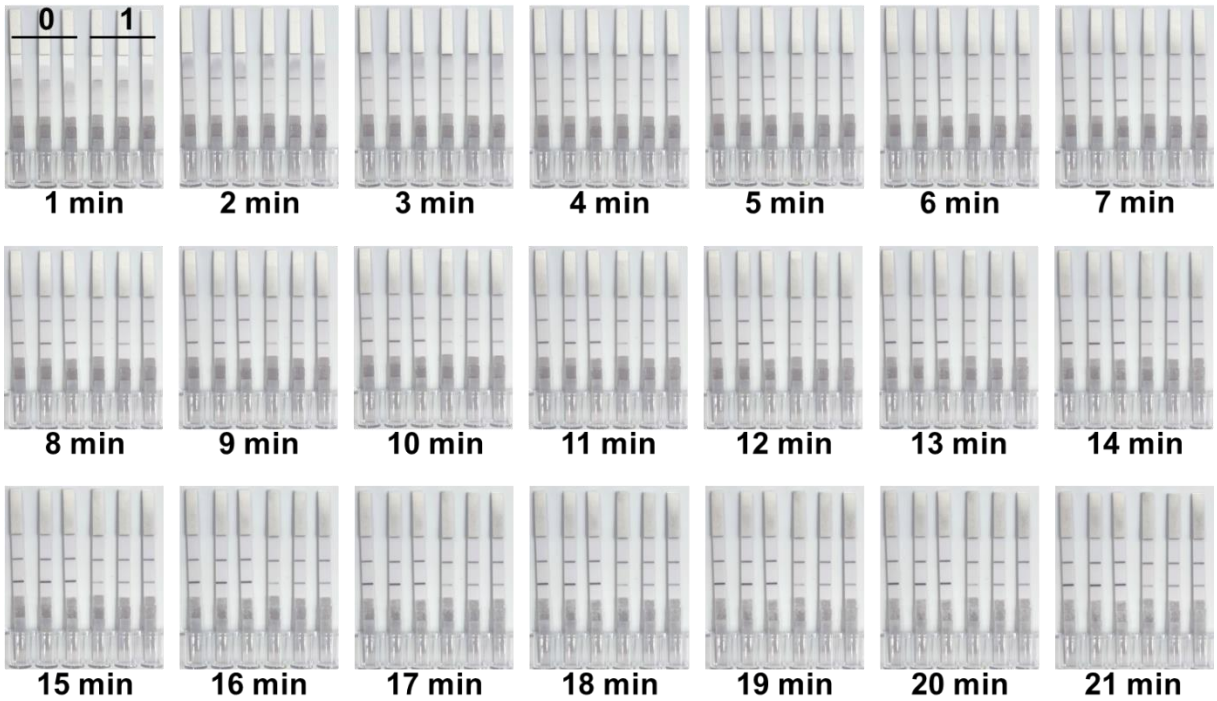


Fig. S11. Photo of test results of AuNC@PDA-LFA with time (1-21 min). The ENR concentrations of spiked samples were 0 and 1 ng/mL, respectively.

Fig. S12. The actual picture of AuNP-mAb labeling results at different pH (5-9).

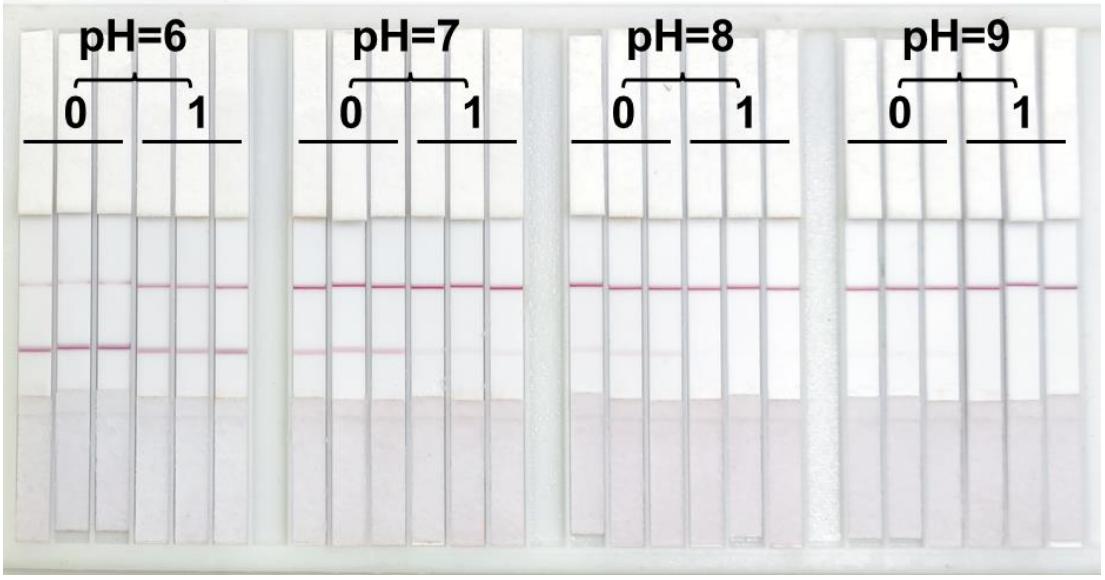
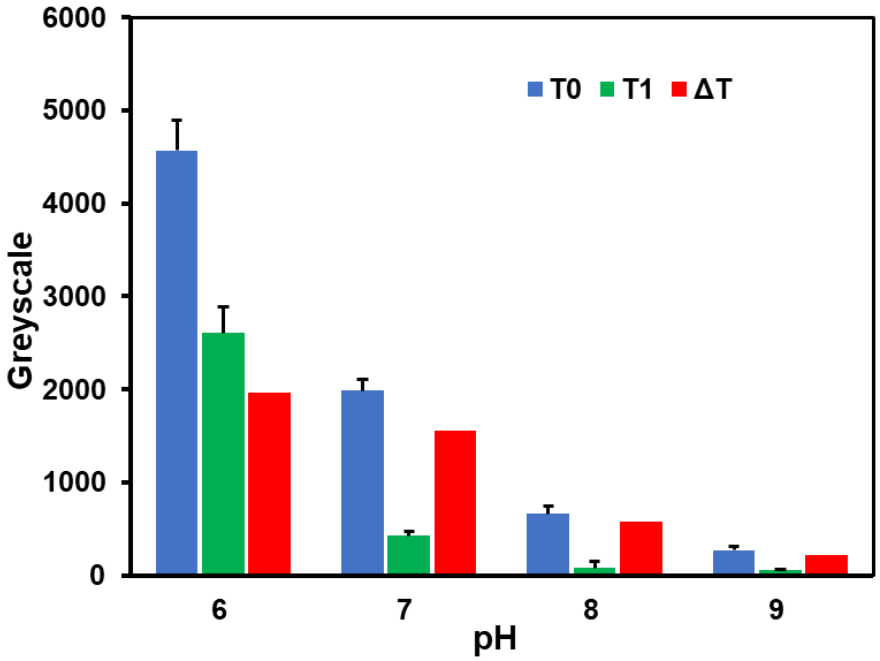


Fig. S13. Photo of pH (6-9) optimization experiments of AuNP-mAb labeling. The concentration of added mAb was 75 μg/mL. The ENR concentrations of spiked samples were 0 and 1 ng/mL, respectively.

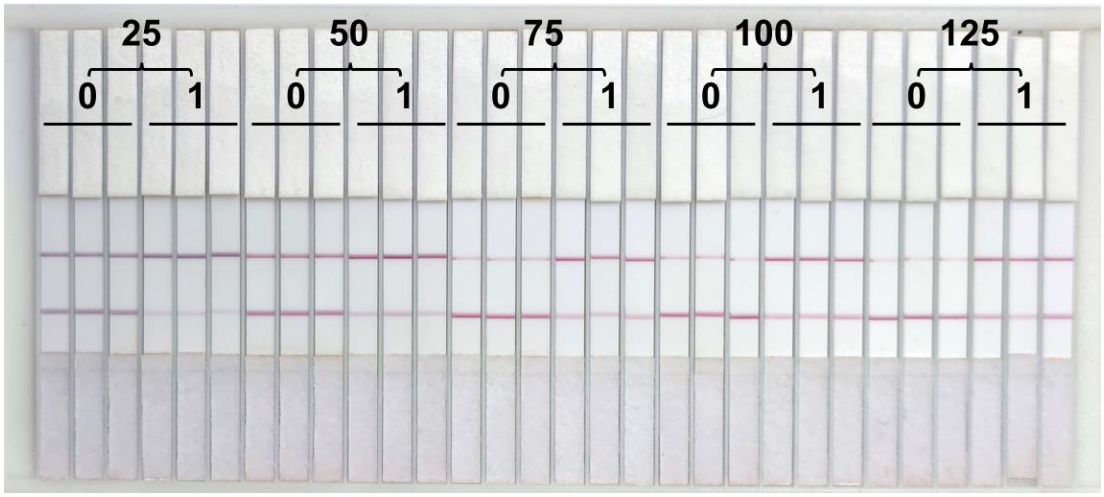


177

178 **Fig. S14.** The pH (6-9) optimization results of AuNP. The ENR concentrations of spiked

179 samples were 0 and 1 ng/mL, respectively.

180



181

182 **Fig. S15.** Photo of concentration of adding mAb (25-125 μg/mL) optimization

183 experiments of AuNP-mAb labeling. The pH was 6. The ENR concentrations of spiked

184 samples were 0 and 1 ng/mL, respectively.

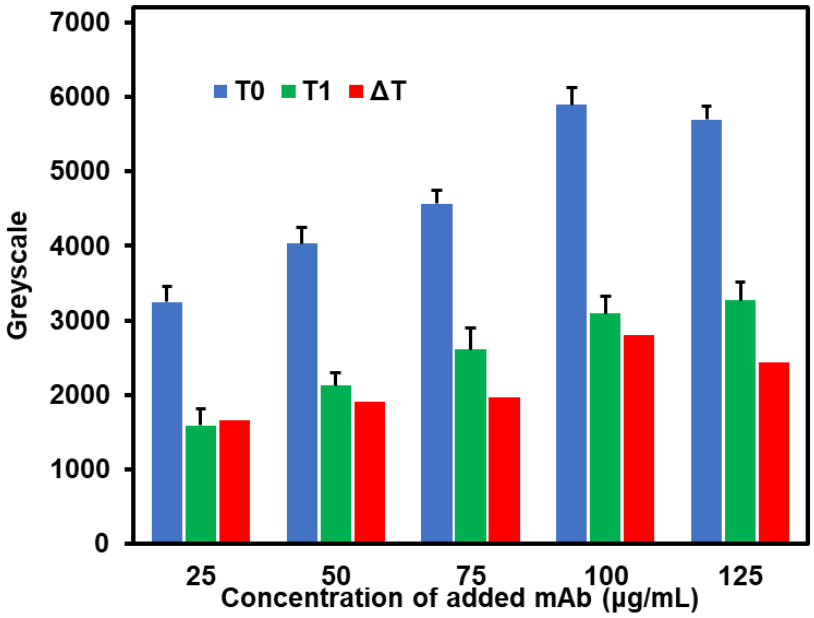


Fig. S16. The concentration of added mAb (25-125 μg/mL) optimization results of AuNP-mAb labeling. The ENR concentrations of spiked samples were 0 and 1 ng/mL, respectively.

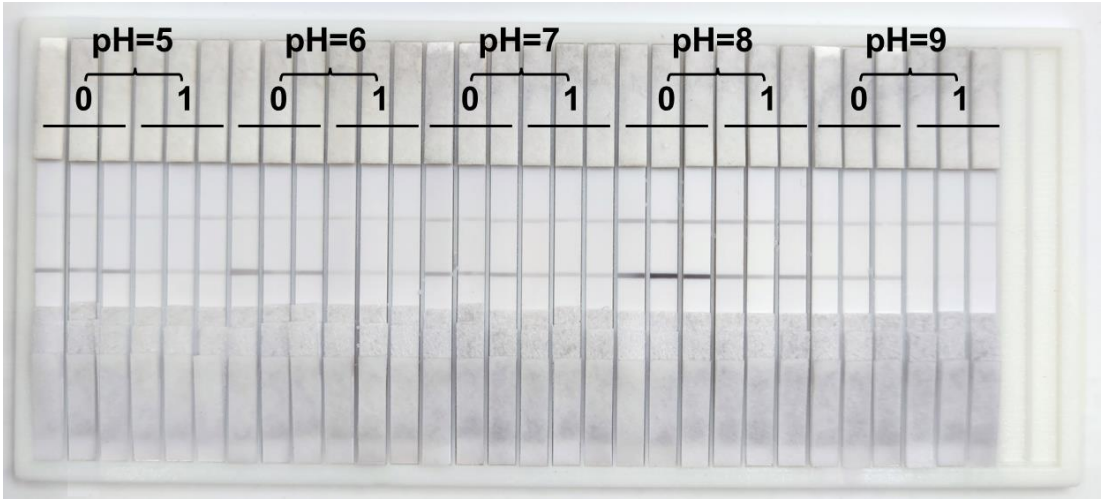


Fig. S17. Photo of pH (5-9) optimization experiments of AuNC@PDA-mAb' labeling. The concentration of added mAb' was 100 μg/mL. The ENR concentrations of spiked samples were 0 and 1 ng/mL, respectively.

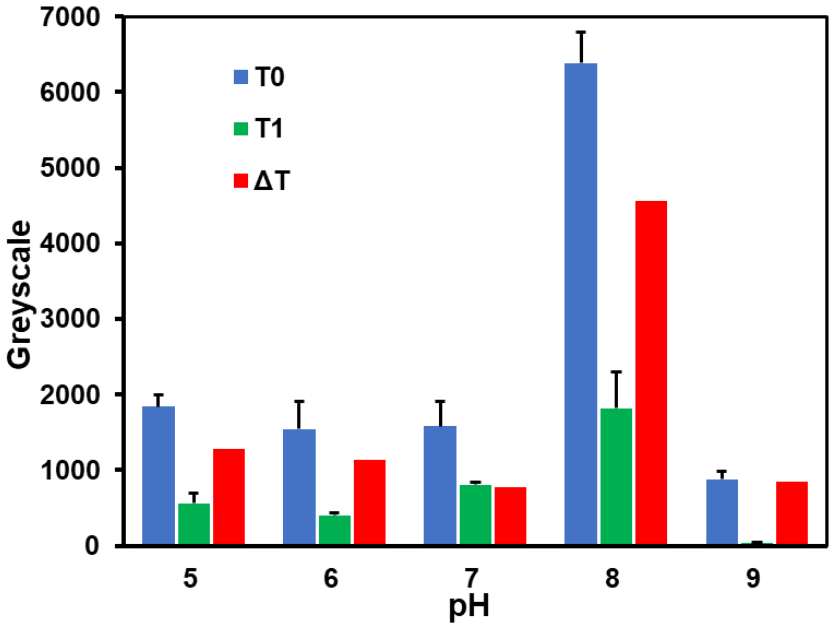


Fig. S18. The pH (5-9) optimization results of AuNC@PDA-mAb' labeling. The ENR concentrations of spiked samples were 0 and 1 ng/mL, respectively.

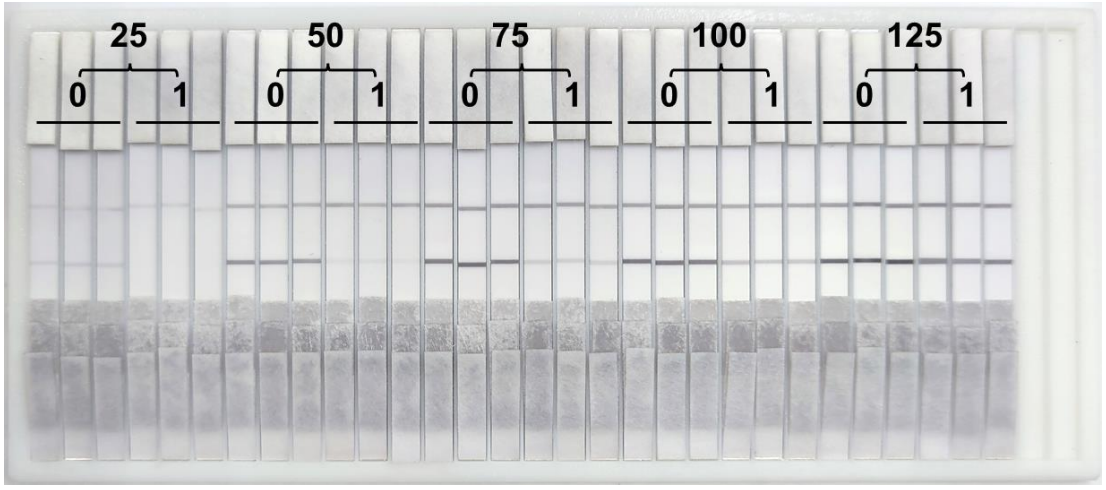
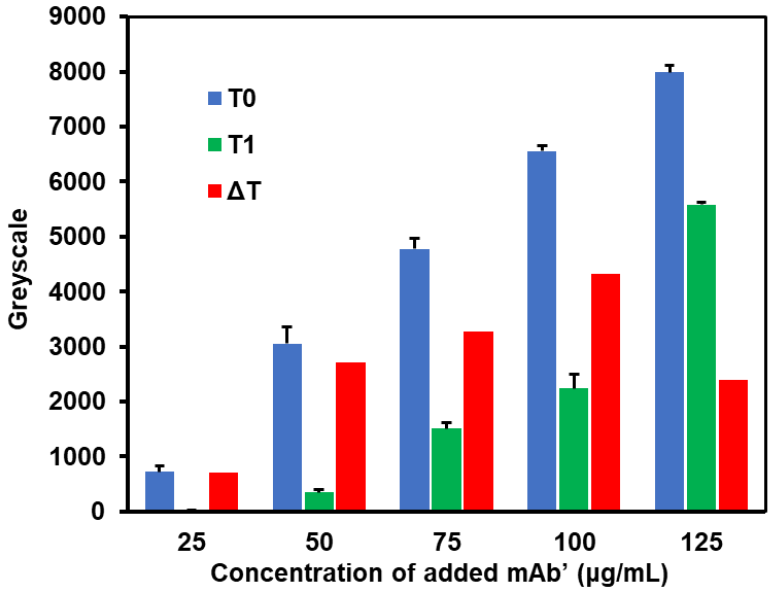


Fig. S19. Photo of concentration of added mAb' (25-125 μg/mL) optimization experiments of AuNC@PDA-mAb' labeling. The pH was 8. The ENR concentrations of spiked samples were 0 and 1 ng/mL, respectively.



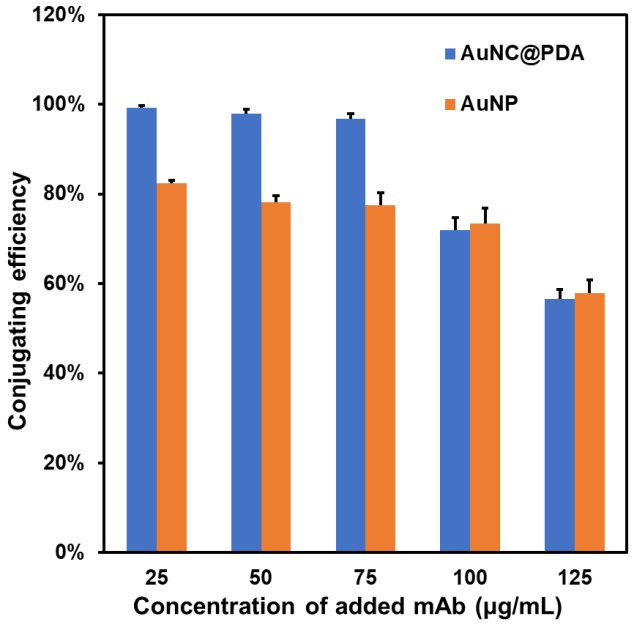
202

203 **Fig. S20.** The concentration of added mAb' (25-125 μg/mL) optimization results of

204 AuNC@PDA-mAb' labeling. The ENR concentrations of spiked samples were 0 and 1

205 ng/mL, respectively.

206



207

208 **Fig. S21.** The conjugating efficiency of AuNC@PDA and AuNP at different

209 concentrations of added mAb.

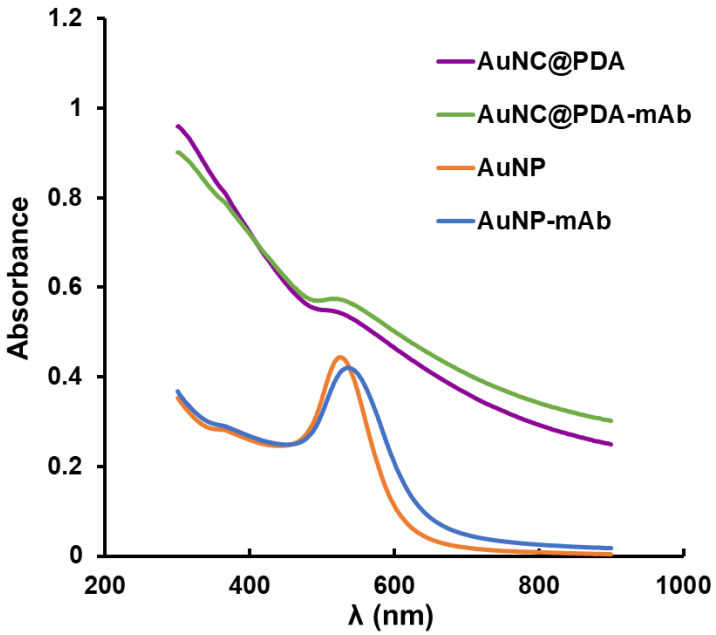


Fig. S22. UV-vis absorption spectroscopy of AuNC@PDA, AuNC@PDA-mAb, AuNP, and AuNP-mAb.

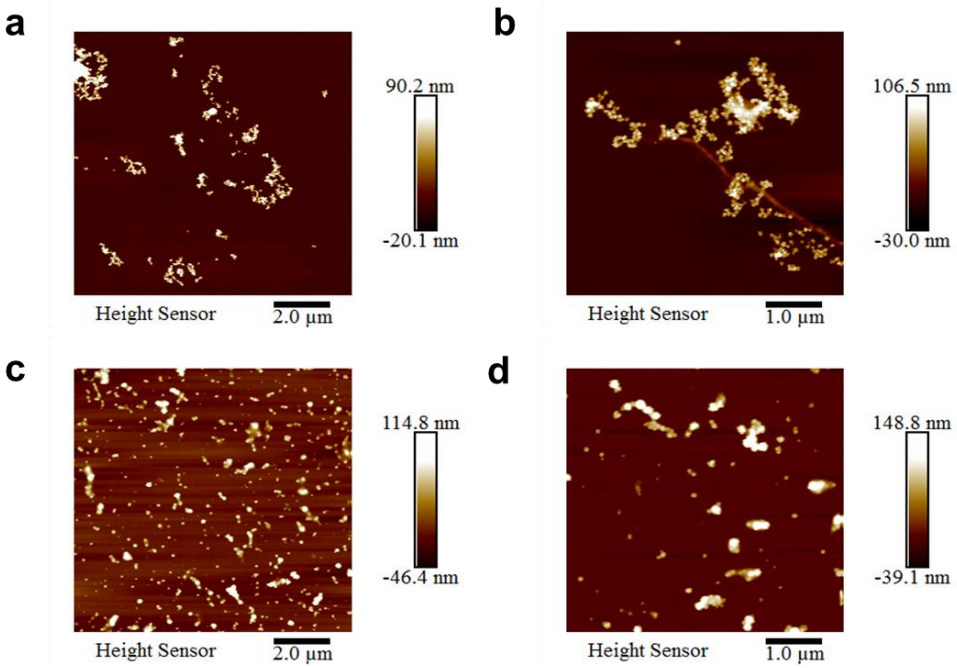


Fig. S23. AFM of AuNC@PDA and AuNC@PDA-mAb. (a, b) AuNC@PDA and (c, d) AuNC@PDA-mAb were analyzed at different magnifications.

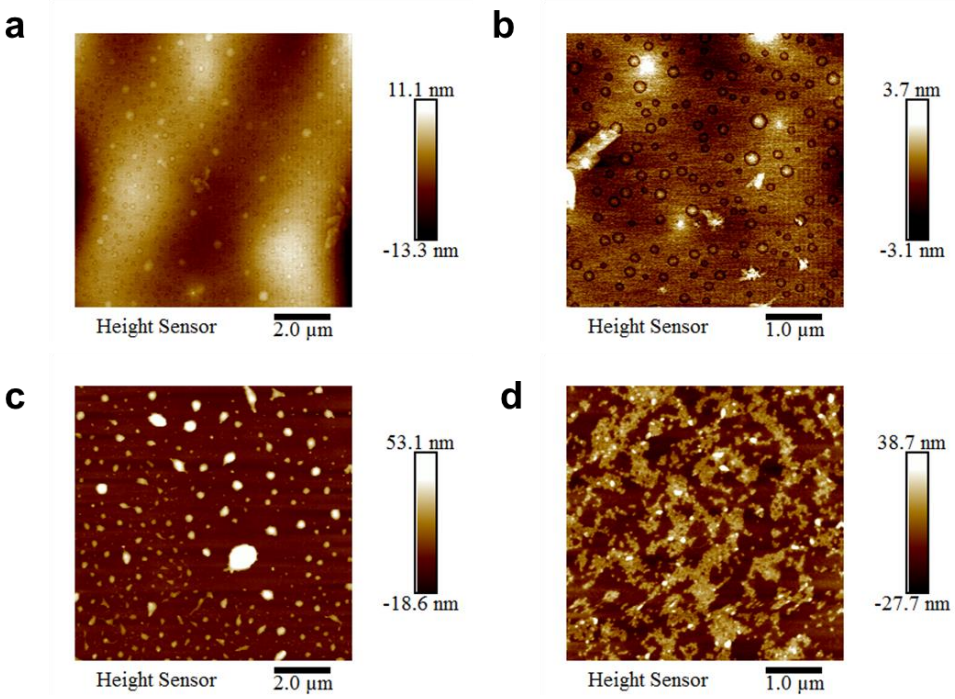


Fig. S24. AFM of AuNP and AuNP-mAb. (a, b) AuNP and (c, d) AuNP-mAb were analyzed at different magnifications.

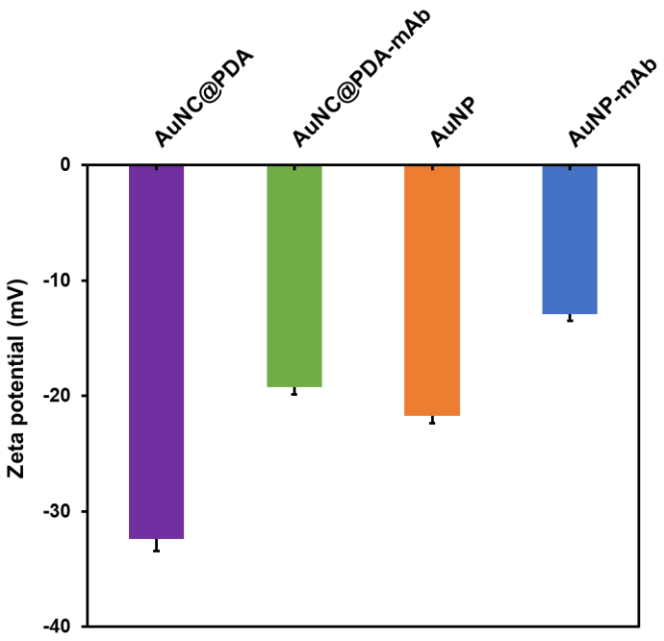
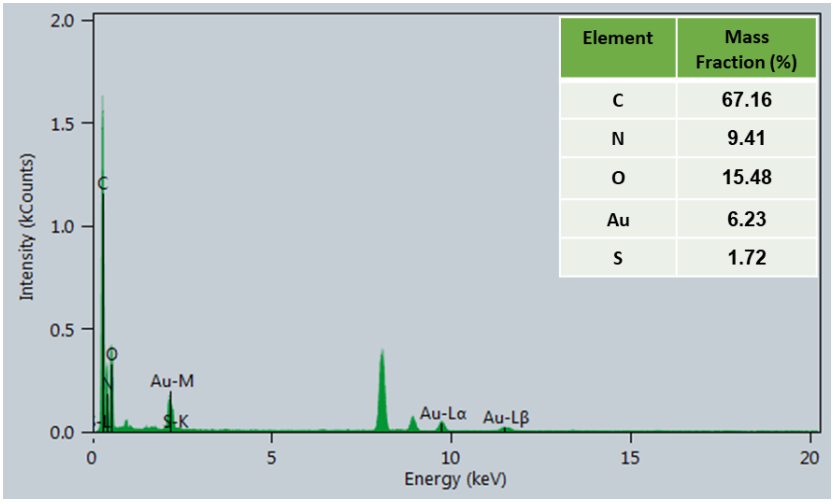
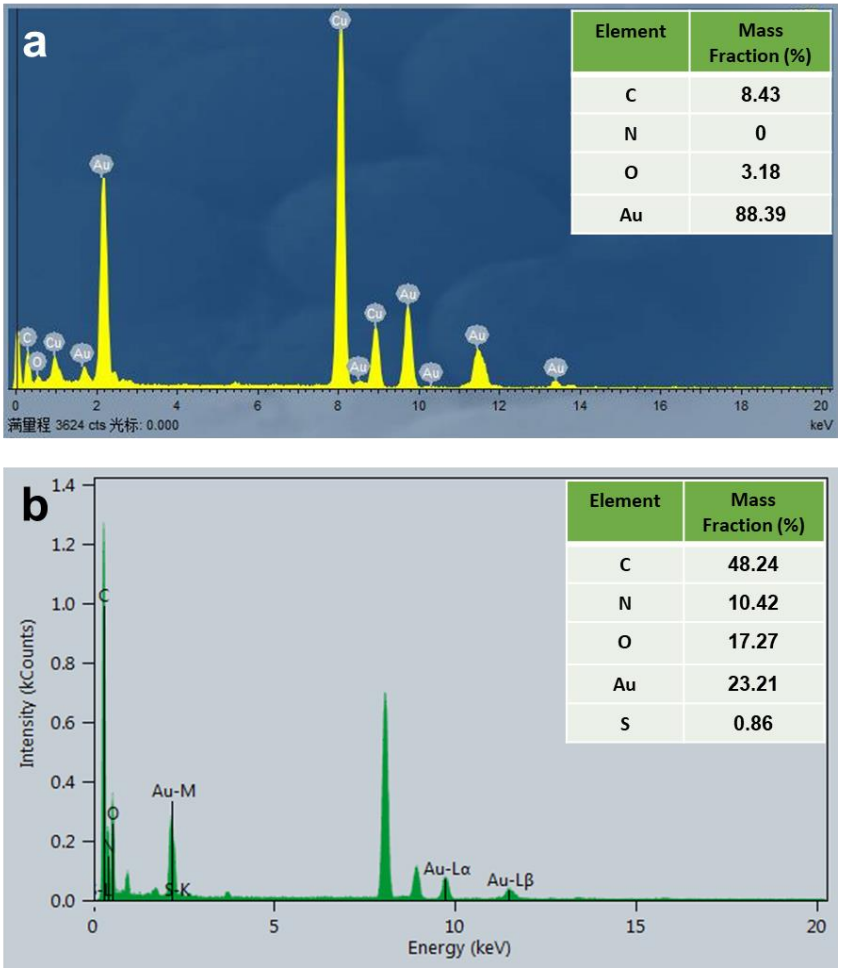


Fig. S25. Zeta potential values of AuNC@PDA, AuNC@PDA-mAb, AuNP, and AuNP-mAb.



224

225 **Fig. S26.** EDX spectroscopy of AuNC@PDA-mAb.



226

227 **Fig. S27.** EDX spectroscopy of (a) AuNP and (b) AuNP-mAb.

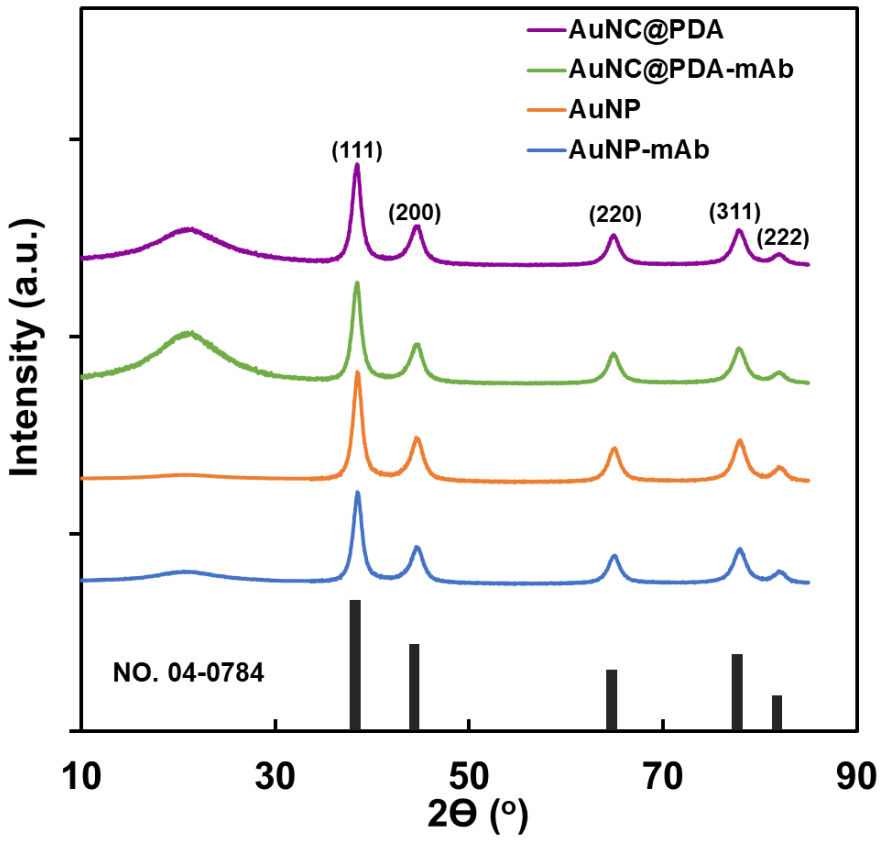


Fig. S28. XRD of AuNP, AuNC@PDA, AuNP-mAb, and AuNC@PDA-mAb.

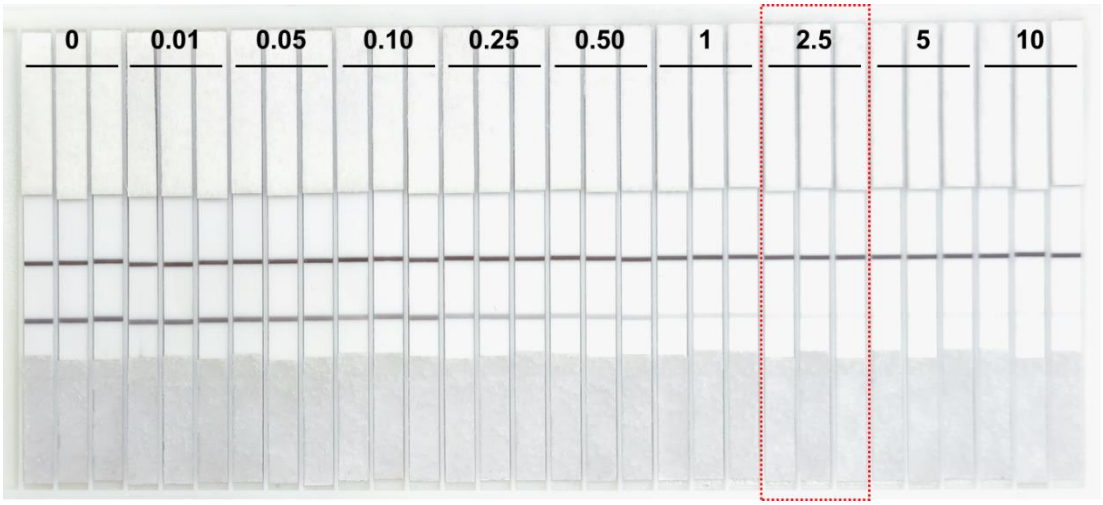


Fig. S29. Photo of AuNC@PDA-sLFA detection of ENR. The ENR concentrations were 0, 0.01, 0.05, 0.10, 0.25, 0.50, 1, 2.5, 5, and 10 ng/mL, respectively.

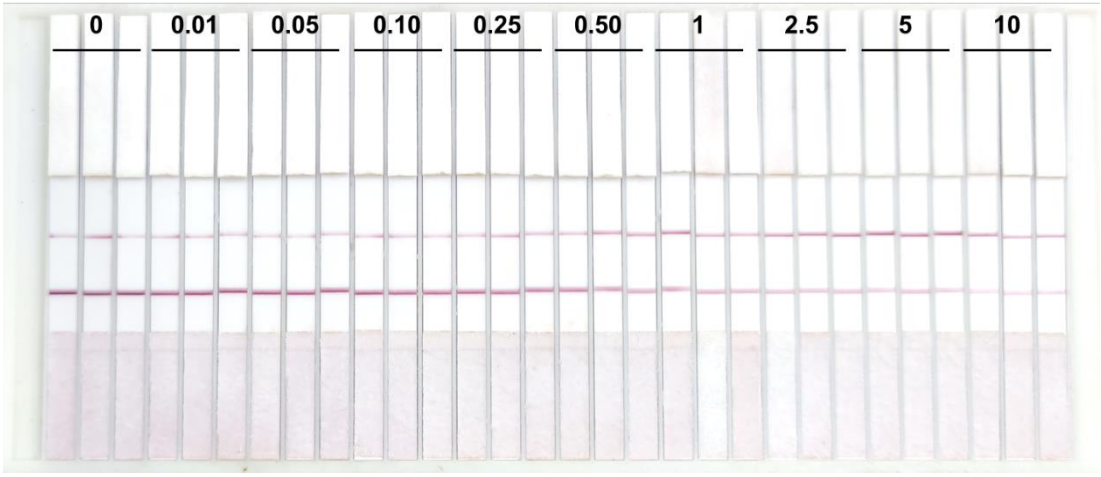


Fig. S30. Photo of AuNP-sLFA detection of ENR. The ENR concentrations were 0, 0.01, 0.05, 0.10, 0.25, 0.50, 1, 2.5, 5, and 10 ng/mL, respectively.

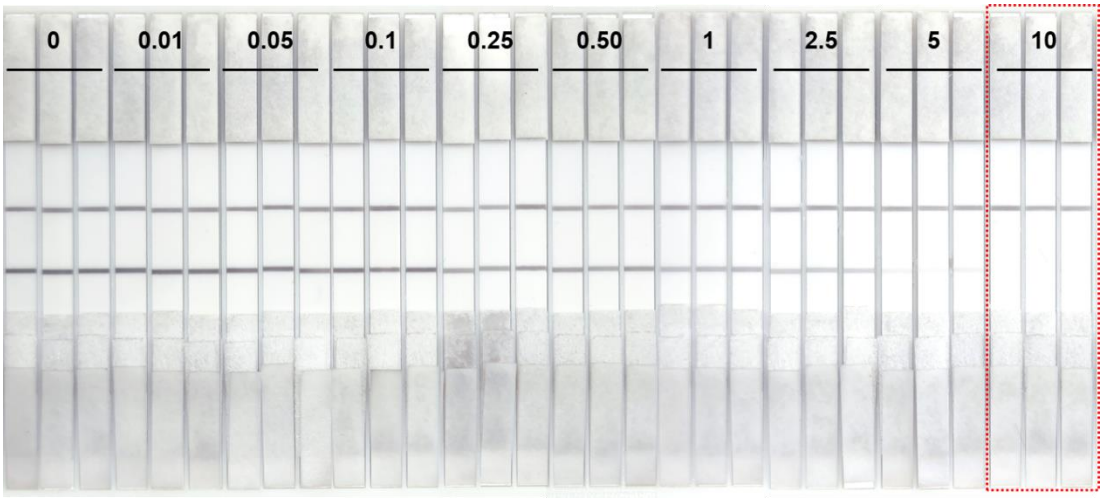


Fig. S31. Photo of AuNC@PDA-sLFA detection of ENR based mAb'. The ENR concentrations were 0, 0.01, 0.05, 0.10, 0.25, 0.50, 1, 2.5, 5, and 10 ng/mL, respectively.

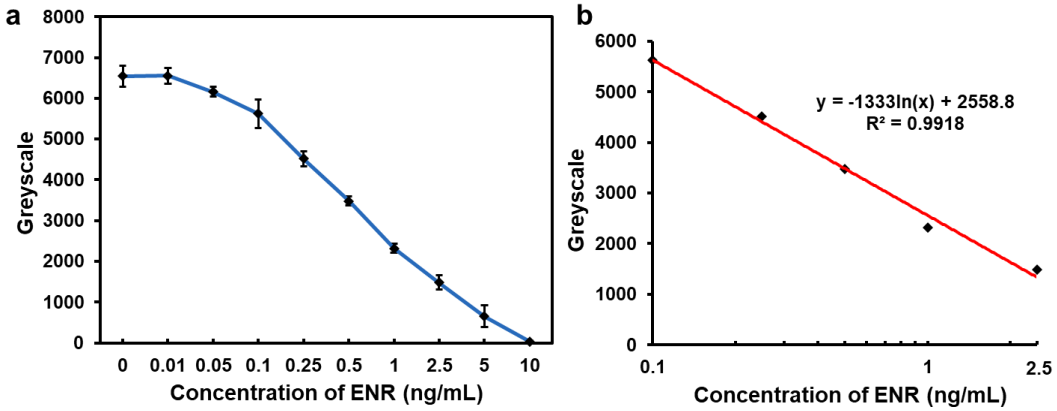


Fig. S32. Test results of AuNC@PDA-sLFA based mAb'. (a) Detection of ENR based on AuNC@PDA-sLFA with mAb' (n=3); (b) Standard curve in linear range.

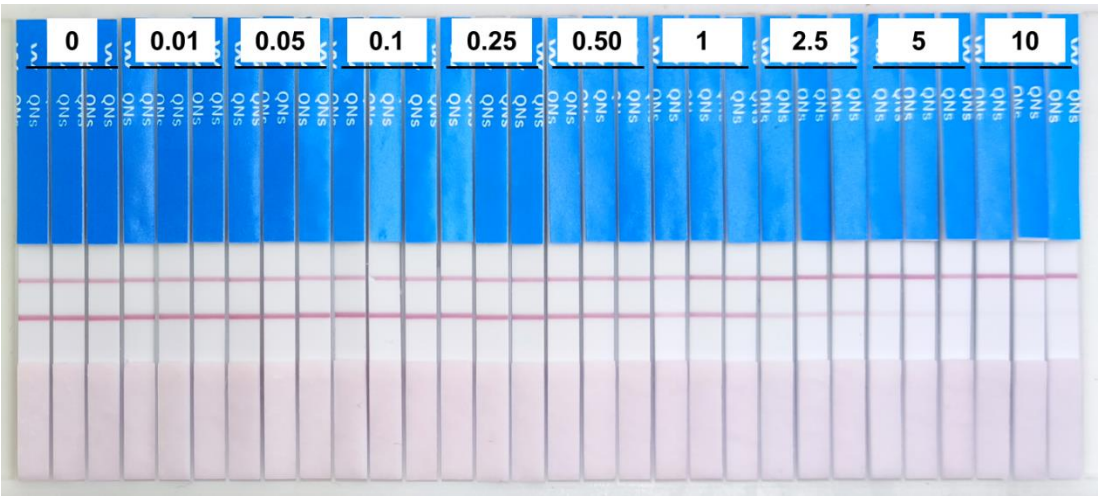


Fig. S33. Photo of vvAuNP-sLFA detection of ENR. The ENR concentrations were 0, 0.01, 0.05, 0.10, 0.25, 0.50, 1, 2.5, 5, and 10 ng/mL, respectively.

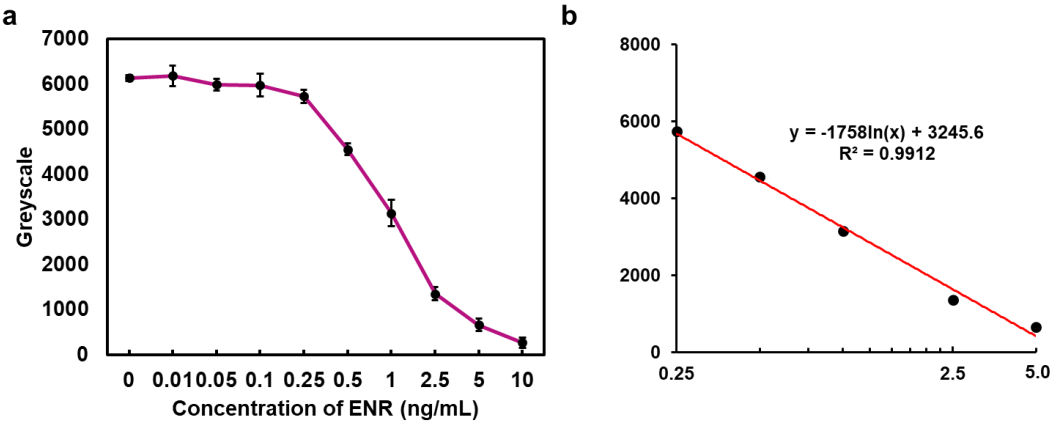


Fig. S34. Test results of vvAuNP-sLFA. (a) Detection of ENR based on vvAuNP-sLFA (n=3); (b) Standard curve in linear range.

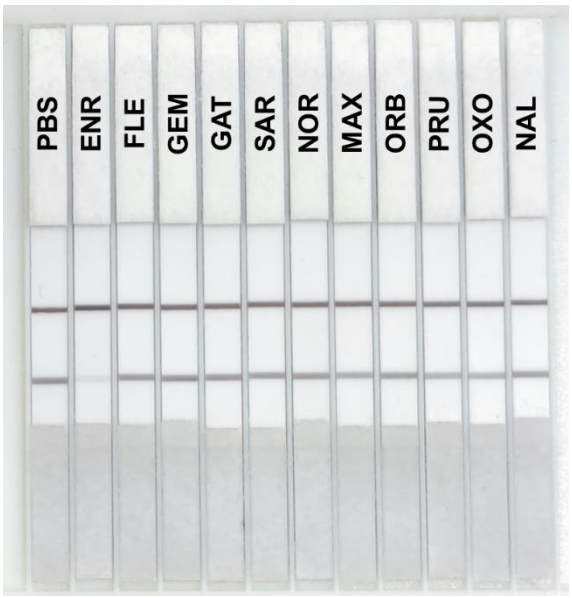


Fig. S35. Photo of specificity results of AuNC@PDA-sLFA. The concentrations of Fleroxacin (FLE), Gemifloxacin (GEM), Gatifloxacin (GAT), Sarafloxacin (SAR), Norfloxacin (NOR), Moxifloxacin (MOX), Orbifloxacin (ORB), Prulifloxacin (PRU), Oxolinic acid (OXO), and Nalidixic acid (NAL) were all 10 ng/mL. In contrast, the concentration of ENR was 1 ng/mL.

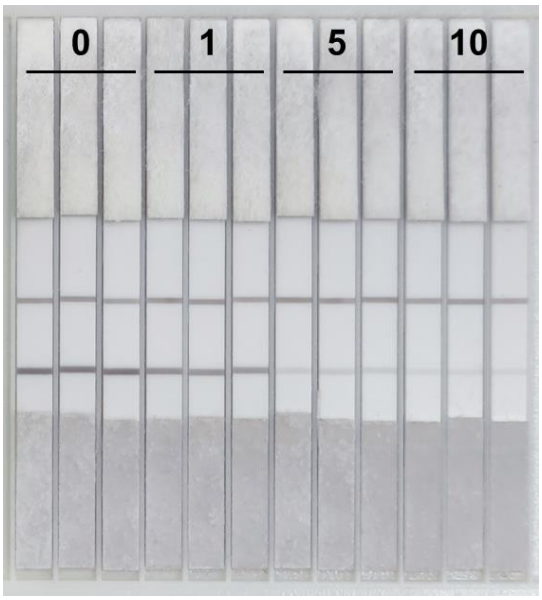


Fig. S36. Photo of recovery results of AuNC@PDA-sLFA. The concentrations of ENR in milk were 0, 1, 5, and 10 ng/mL, respectively.

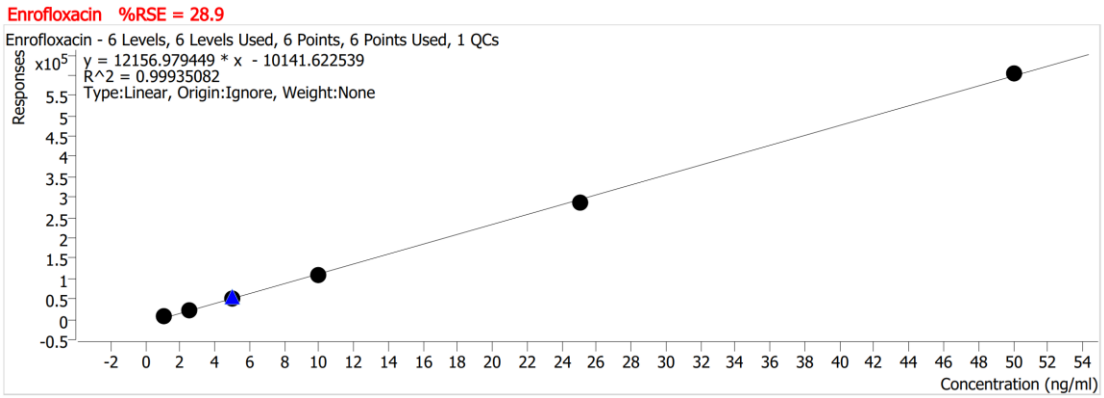


Fig. S37. Calibration report of detecting ENR by LC-MS. The concentrations of ENR were 1, 2.5, 5, 10, 25, and 50 ng/mL, respectively.

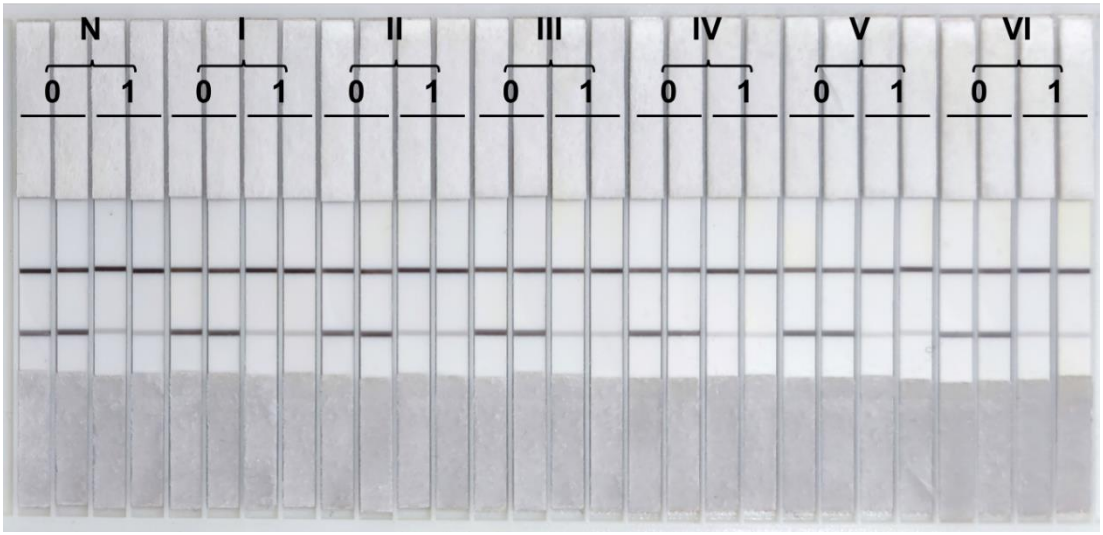


Fig. S38. Photo of high temperature (60 ℃) accelerated aging test results of AuNC@PDA-sLFA. N: for 0 day; I: for 1 day; II: for 2 days; III: for 3 days; IV: for 4 days; V: for 5 days; VI: for 6 days. The concentrations of ENR were 0 and 1 ng/mL.

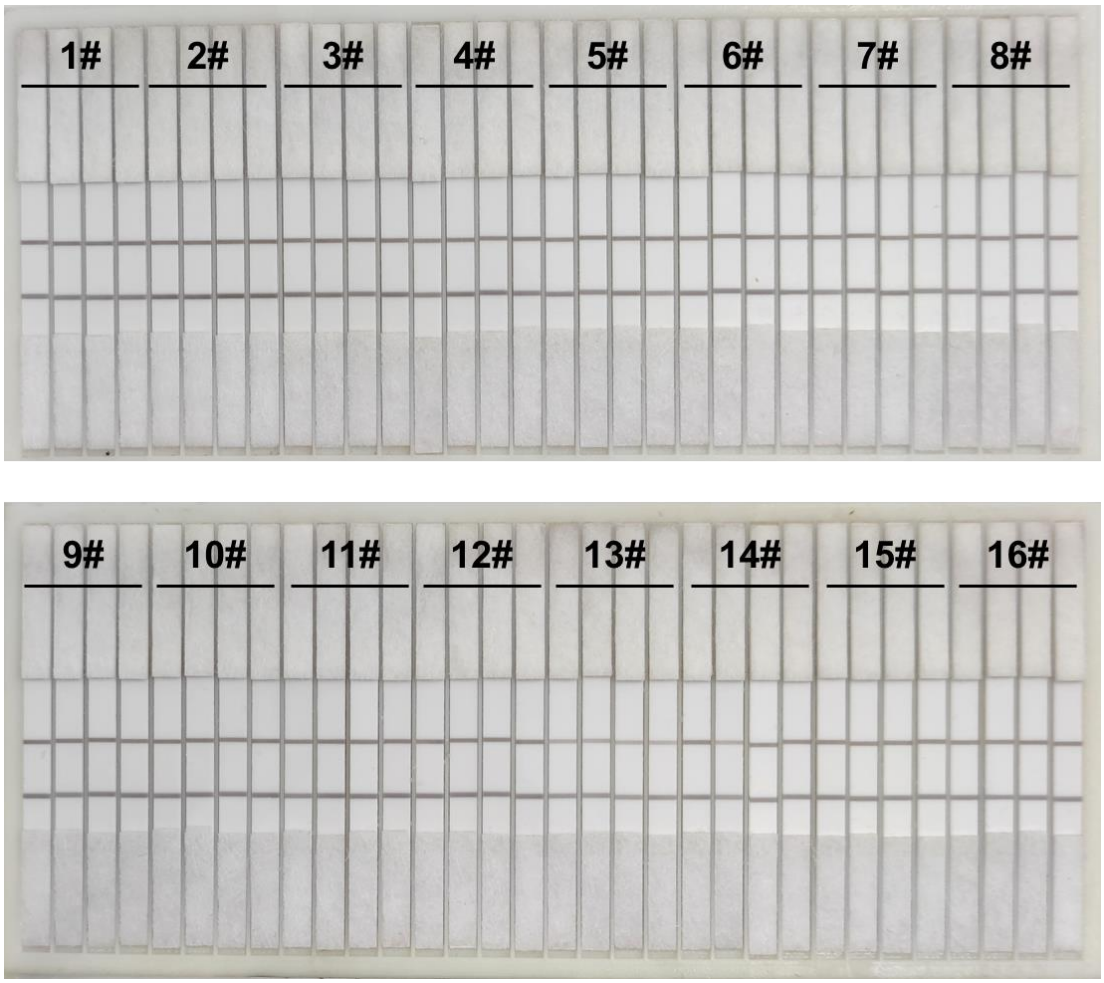


Fig. S39. Photo of actual samples detection results of AuNC@PDA-sLFA.

273 **Table S1** Comparison of present study with other reported works for ENR detection.

Method	Probes	High-flux	LOD (ng/mL)	Time cost	Reference
Bacterial respiration	CO_2 gas	No	10	2.5 h	[2]
	Glucose consumed	No	5	2 h	[3]
Photoelectrochemical assay	$CuInS_2$/3DNG	No	3.3×10^{-3}	70 min	[4]
	Bi/CV-PCN	No	3.3×10^{-6}	70 min	[5]
Solid-Phase Extraction	Fe_3O_4/MIL-100(Fe)/GO	No	0.65	78 min	[6]
	UiO-66	No	15.6	/	[7]
ELISA	Sarafloxacin-BSA	Yes	IC_{50}=0.13	>6 h	[8]
	Microarray chip	Yes	3.3	/	[9]
LFA	AuNPs	No	0.42	20 min	[10]
	AuNPs and quantum dot	No	0.25	30 min	[11]
	AgNP and carbon dot	No	0.1	30 min	
	AuNP	No	10	20 min	
	AgNP	No	5	20 min	
	Dyed polymer microspheres	No	1	20 min	[12]
	Quantum dots	No	1	20 min	
sLFA	AuNC@PDA	Yes	3.378×10^{-2}	15 min	This work

274 / Not mentioned.

Table S2 Actual samples detection results of AuNC@PDA-sLFA.

Number	Test results (ng/mL)	CV (%) *	Number	Test results (ng/mL)	CV (%)
1#	<0.5	4.50	9#	<0.5	5.84
2#	<0.5	4.54	10#	<0.5	4.16
3#	<0.5	7.02	11#	<0.5	1.09
4#	<0.5	6.98	12#	<0.5	2.70
5#	<0.5	5.26	13#	0.852	3.44
6#	<0.5	2.79	14#	0.534	3.21
7#	<0.5	7.89	15#	<0.5	1.90
8#	<0.5	4.74	16#	0.651	6.25

***** Coefficient of variation = Standard deviation / Average

References

1. G. Zhang, Y. Huang, J. Peng, J. Han, P. Guo, L. Zhang, J. Zhang, W. Lai and T. Chen, *Food Analytical Methods*, 2019, **12**, 1666-1673.
2. H. Lee, S. Lee, D. Kwon, C. Yim and S. Jeon, *Sensors and Actuators B: Chemical*, 2017, **244**, 559-564.
3. D. Kwon, H. Lee, H. Yoo, J. Hwang, D. Lee and S. Jeon, *Sensors and Actuators B: Chemical*, 2018, **254**, 935-939.
4. Z. Zhang, Q. Liu, M. Zhang, F. You, N. Hao, C. Ding and K. Wang, *Journal of Hazardous Materials*, 2021, **416**, 125988.
5. Y. Xu, D. Jiang, M. Zhang, Z. Zhang, J. Qian, N. Hao, C. Ding and K. Wang, *Sensors and Actuators B: Chemical*, 2020, **316**, 128142.
6. Z. Li, M. Qi, C. Tu, W. Wang, J. Chen and A.-J. Wang, *Food Analytical Methods*, 2017, **10**, 4094-4103.
7. Z. Sun, H. Liu, Y. Zhou, S. Zhao, J. Li, X. Wang and B. Gong, *RSC Advances*, 2019, **9**, 27953-27960.
8. S. Hu, B. Fang, Z. Huang, Y. Chen, D. Liu, K. Xing, J. Peng and W. Lai, *J. Dairy Sci.*, 2019, **102**, 6037-6046.
9. B. Du, F. Wen, X. Guo, N. Zheng, Y. Zhang, S. Li, S. Zhao, H. Liu, L. Meng, Q. Xu, M. Li, F. Li and J. Wang, *Food Control*, 2019, **106**, 106713.
10. M. Han, L. Gong, J. Wang, X. Zhang, Y. Jin, R. Zhao, C. Yang, L. He, X. Feng and Y. Chen, *Sensors and Actuators B: Chemical*, 2019, **292**, 94-104.
11. S. Li, Y. Wang, X. Mu, W. Sheng, J. Wang and S. Wang, *Analytical Methods*, 2019, **11**, 2378-2384.

12. W. Sheng, S. Li, Y. Liu, J. Wang, Y. Zhang and S. Wang, *Microchimica Acta*, 2017, **184**, 4313-4321.