Electronic Supplementary Material (ESI) for Analyst.

Ratiometric fluorescence determination of alkaline phosphatase activity based on dual emission of bovine serum albumin-stabilized gold nanoclusters and the inner filter effect

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1. Chemicals

ALP was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. BSA, lysozyme (Lys), thrombin (Thr), L-lysine, glucose, glycine, arginine, and histidine (His) were purchased from Sigma-Aldrich. Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was purchased from Aladdin. Horseradish peroxidase (HRP) was purchased from Sangon Biotech (Shanghai) Co., Ltd. Sodium chloride, magnesium chloride, ferric chloride, ferrous chloride, calcium chloride, potassium chloride, zinc chloride, and sodium hydroxide were purchased from Sinopharm Chemical Reagent Co., Ltd. The other chemicals used were analytical grade.

2. Instrumentation

Fluorescence spectra were recorded using a Fluoromax-4 spectrometer (HORIBA Jobin, USA) with an excitation wavelength of 340 nm and excitation and emission slit width of 3 nm. The morphological characterization of the synthesized BSA-AuNCs was carried out using a JEM-2100 transmission electron microscope (Jeol, Ltd., Japan). UV absorbance measurements were carried out on a Perkin–Elmer Lambda 35 UV–vis spectrophotometer. The infrared spectra of the composites were recorded with a Bruker Tensor 27 spectrometer. Fluorescence lifetimes were measured on spectrofluorimeter (FLS1000; Edinburgh Instruments Ltd., UK). Quantum yield was measured by a C9920-02G quantum yield spectrometer (Hamamatsu Co., Japan).

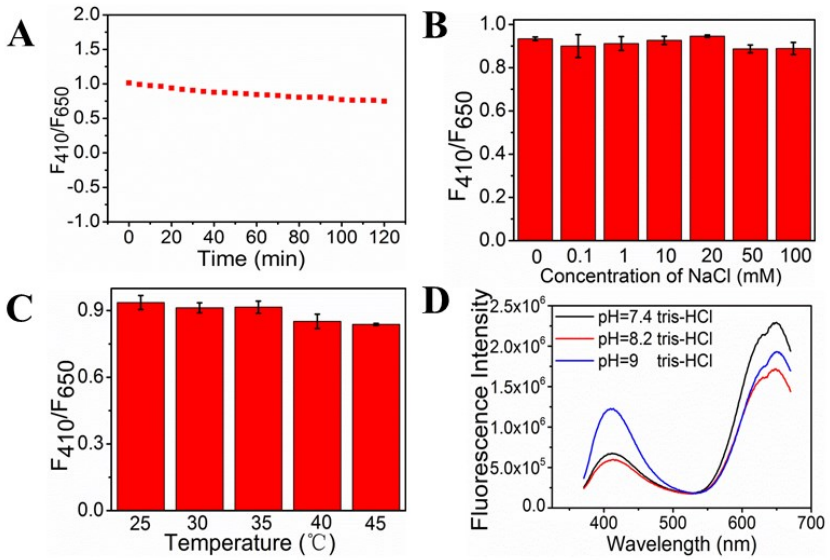

Fig. S1(A) The photostability of the the BSA-AuNCs in Tris-HCl at pH9 measured by fluorescence spectrophotometer every 5 min (Ex=340 nm) (B) FL intensity of the BSA-AuNCs containing various concentrations of NaCl. (C) FL intensity of the BSA-AuNCs at different temperatures. (D) FL intensity of the BSA-AuNCs at different pH values.

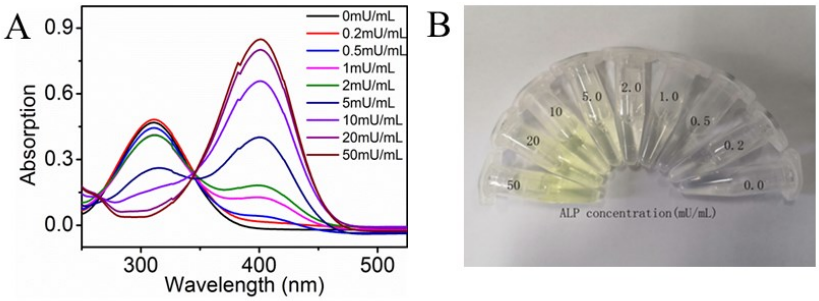

Fig. S2 (A) Absorption spectral changes of the enzymatic reaction solution upon addition of ALP with various activities from 0 to 50 mU/mL with 50μM PNPP and 0.1 μM $MgCl_2$ incubated at 37 °C for 50 min.(B) The photograph of the enzymatic reaction solutions with various levels of ALP activity.

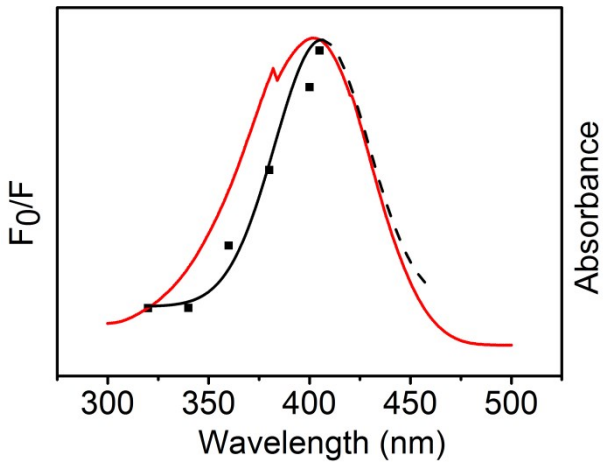

Fig. S3 Comparison of the excitation wavelength-dependent fluorescence quenching spectrum of BSA-AuNCs at 410 nm (black line) with UV-vis absorption spectrum of PNP (red line), in which F and F_0 represent fluorescence intensity at 410nm of BSA-AuNCs in the presence and absence of 10μM PNP, respectively.

Table S1. Influence of PNPP ,PNPP+ALP and PNP on fluorescence lifetime at 410 nm of BSA-AuNCs

System	A_1	$<\tau_1>$(ns)	$<\tau>$(ns)
BSA-Au NCs	599.71	4.28	4.28
BSA-Au NCs+PNPP	200.51	4.21	4.21
BSA-Au NCs+PNPP+ALP	340.54	4.32	4.32
BSA-Au NCs+PNP	380.89	4.22	4.22

Table S2. The developed method compared with the reported ALP assay method

Material	Method	Linear range (mU/mL)	LOD (mU/mL)	Ref.
DNA probes	Electrochemistry	1-20	0.1	1
phosphate ions	Electrochemiluminescence	1-500	0.5	2
Au@Ag nanoparticles	Colorimetry	0.01-6	0.009	3
GSH–AuNCs	Fluorescence	0.01-7	0.003	4
Cerium coordination polymer nanoparticles	Fluorescence	0.1-10	0.09	5
Nanoparticles and MnO_2 Nanosheets	Fluorescence	1-80	0.34	6
4-MPBA-Au@AgNPs	SERS	0.5-10	0.1	7
BSA-AuNCs	Fluorescence	0.2-5	0.03	This work

References

1 P. Miao, L. Ning, X. Li, Y. Shu, G. Li, Biosens. Bioelectron., 2011,27, 178-182.

2 C. Shen, X. Li, A. Rasooly, L. Guo, K. Zhang and M. Yang, Biosens. Bioelectron., 2016, 85, 220-225.

3 C. Chen, G. Zhang, P. Ni, Y. Jiang, Y. Lu, Z. Lu, Microchim. Acta, 2019,186, 348-357.

4 S. Qi, H. Zheng, H. Qin, H. Zhai, Analyst,2020,145,3871-3877.

5 C. Chen, Q. Yuan, P. Ni, Y. Jiang, Z. Zhao, Y. Lu, Analyst,2018,143, 3821-3828.

6 T. Xiao, J. Sun, J. Zhao, S. Wang, G. Liu, X. Yang, ACS Appl. Mater. Interfaces,2018,10, 6560-6569.

7 J. Zhang, L. He, X. Zhang, J. Wang, L. Yang, B. Liu, et al., Sens. Actuators B-Chem. ,2017,253, 839-845.