Supporting Information

Fluorometric and colorimetric dual-mode sensing of α-glucosidase based on aggregation-induced emission enhancement of AuNCs

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Experimental Section

1. Chemicals and Reagents

$HAuCl_4 \cdot 3H_2O$, $Ce(NO_3)_3 \cdot 6H_2O$, HCl, KCl, $CaCl_2$, NaCl, $MgCl_2$, NH_4Cl, 2,6-dichlorophenolindophenol (DCIP), and trometamol (Tris) were purchased from Aladdin Reagent Company (China). L-ascorbic acid-2-O-α-D-glucopyranosyl (AA2G), α-glucosidase (α-Glu), bovine serum albumin (BSA), human serum albumin (HSA), glucose (Glu), threonine (Thr), histidine (His), trypsin (Try), acetylcholinesterase (AChE), glucose oxidase (GOx), and β-glucosidase (β-Glu), alkaline phosphatase (ALP), and acid phosphatase (ACP) were obtained from Yuanye Biotechnology Co., Ltd. (Shanghai, China). Glutathione (GSH), luteolin, apigenin, and hesperidin were purchased from Sigma-Aldrich Company (USA). All of these reagents were of analytical grade and used without further purification. Ultrapure water was obtained through a Millipore Milli-Q water purification system (Taihe, China) with an electric resistance of >18.2 MΩ.

2. Apparatus and Measurements

Transmission electron microscopy (TEM) measurements were carried out on a JEM-2100F field emission electron microscope (JEOL, Japan) under an accelerating voltage of 100 kV. Energy-dispersive X-ray spectroscopy (EDX) elemental mapping and line scanning were determined on a JEM-ARM200F field-emission electron microscope operated at 200 kV (JEOL, Japan). Zeta potential and dynamic light scattering (DLS) data were obtained from a Zetasizer Nano ZS instrument (Malvern, England). Fourier transform infrared (FTIR) spectra were recorded by using a Thermo Nicolet 670 FT-IR instrument (Thermo Nicolet Corporation, USA) with the recorded range of 4000-500 cm^{-1}. X-ray photoelectron spectroscopy (XPS) results were obtained with an X-ray photoelectron spectrometer (Thermo Electron, USA). The fluorescence lifetime and absolute fluorescence quantum yield were acquired on an FLS920

fluorescence spectrophotometer (Edinburgh Instruments, England). All fluorescence measurements were carried out on an RF5301 fluorescence spectrophotometer (Shimadzu, Japan). UV-Vis spectra were recorded on a UV-2450 spectrophotometer (Shimadzu, Japan).

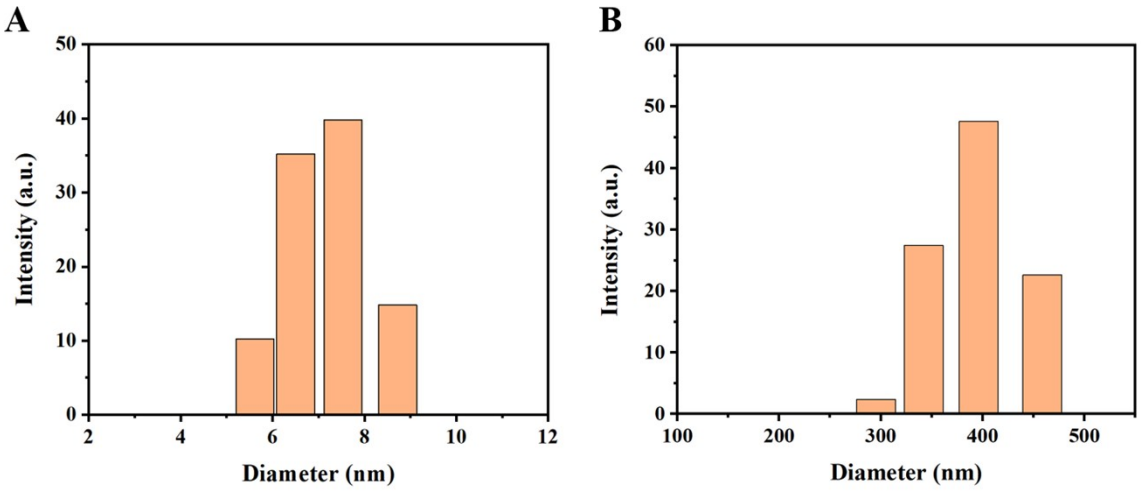


Fig. S1. Hydrodynamic diameters of (A) AuNCs and (B) Ce@AuNCs.

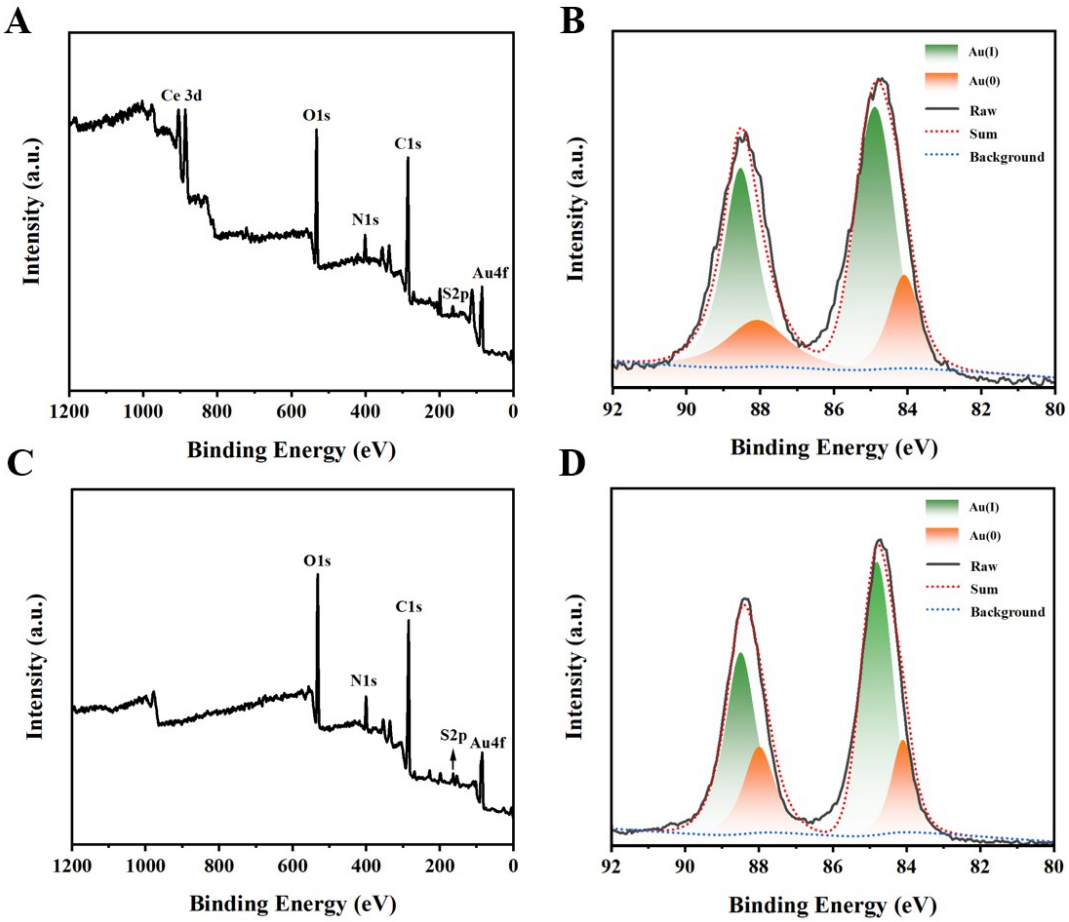


Fig. S2. (A) XPS survey and (B) high resolution Au4f peaks of Ce@AuNCs; (C) XPS survey and (D) high resolution Au4f peaks of AuNCs.

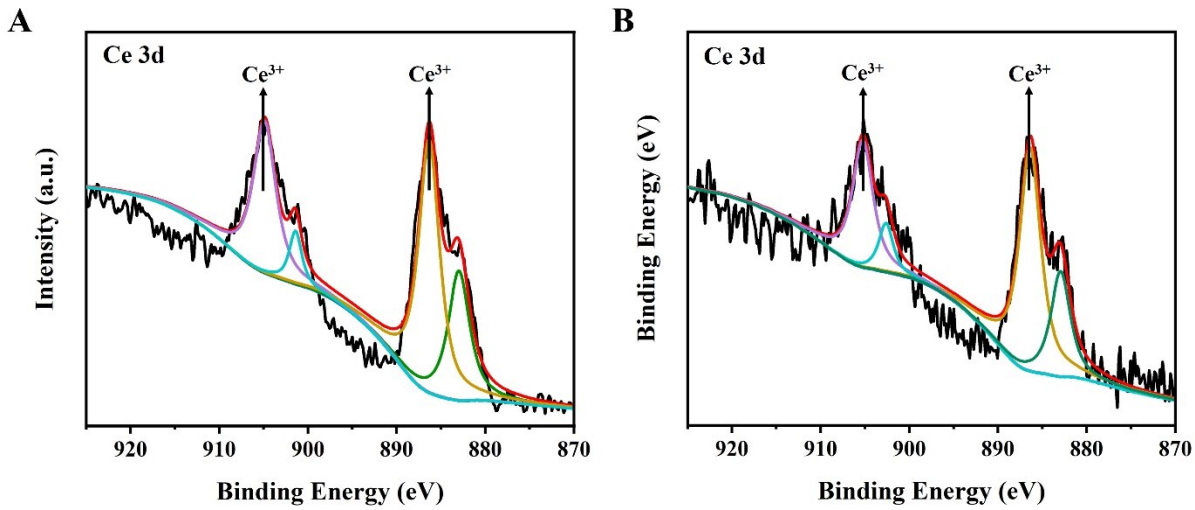


Fig. S3. XPS patterns of Ce 3d (A) before and (B) after inducing the aggregation of AuNCs.

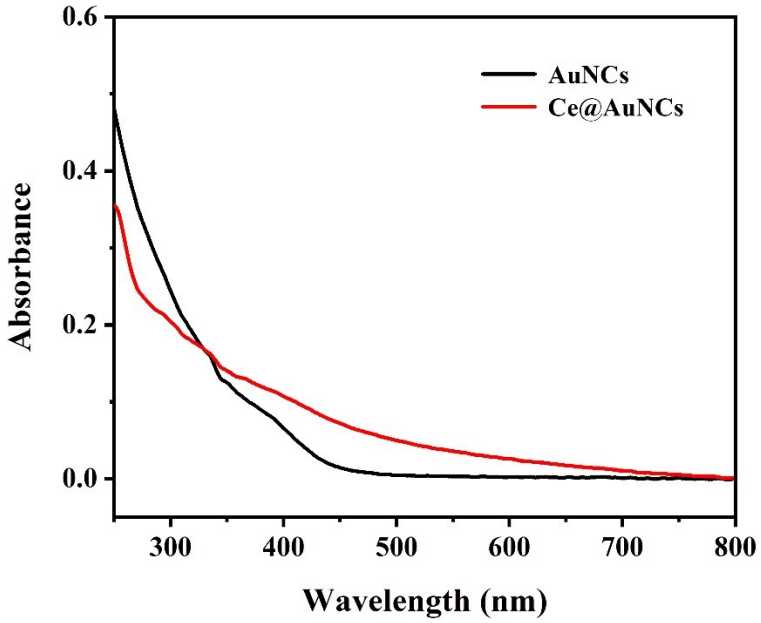


Fig. S4. UV-vis absorption spectra of AuNCs and Ce@AuNCs.

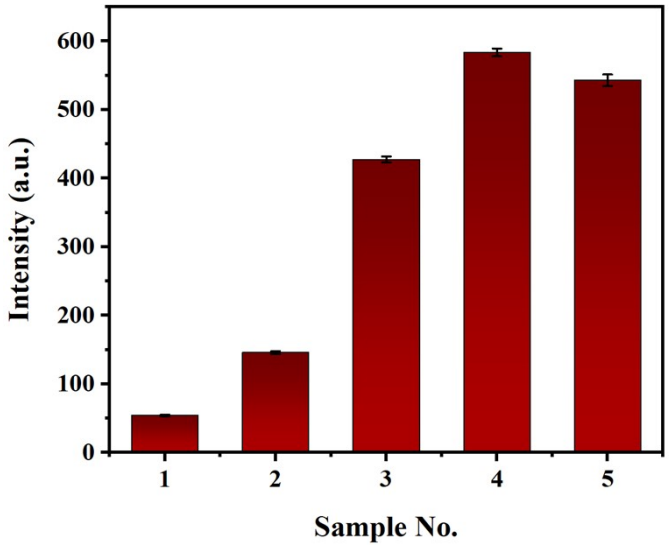


Fig. S5. Fluorescence intensities of Ce@AuNCs prepared at various concentrations of Ce^{3+} (0, 0.05, 0.2, 0.3, and 0.35 mM for Sample 1 to 5).

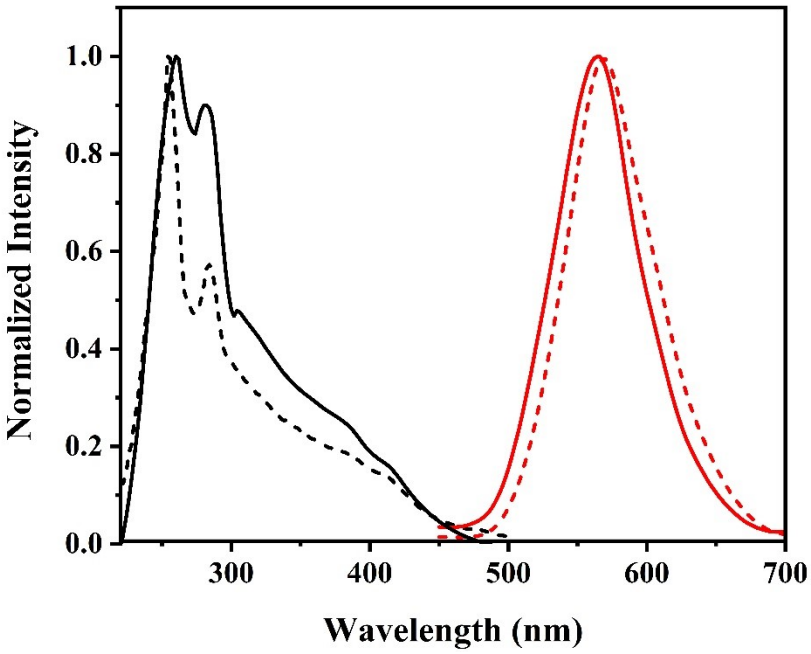


Fig. S6. Normalized fluorescence excitation (black) and emission (red) spectra of AuNCs (dash line) and Ce@AuNCs (solid line).

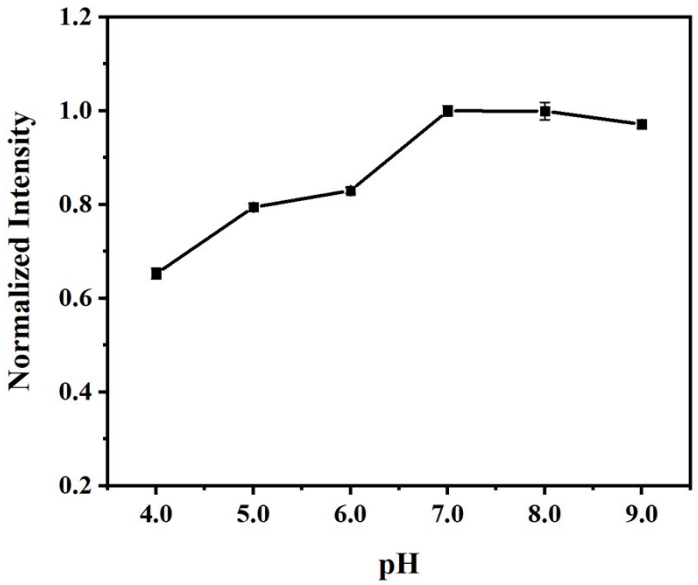


Fig. S7. Effect of pH on the fluorescence intensity of Ce@AuNCs.

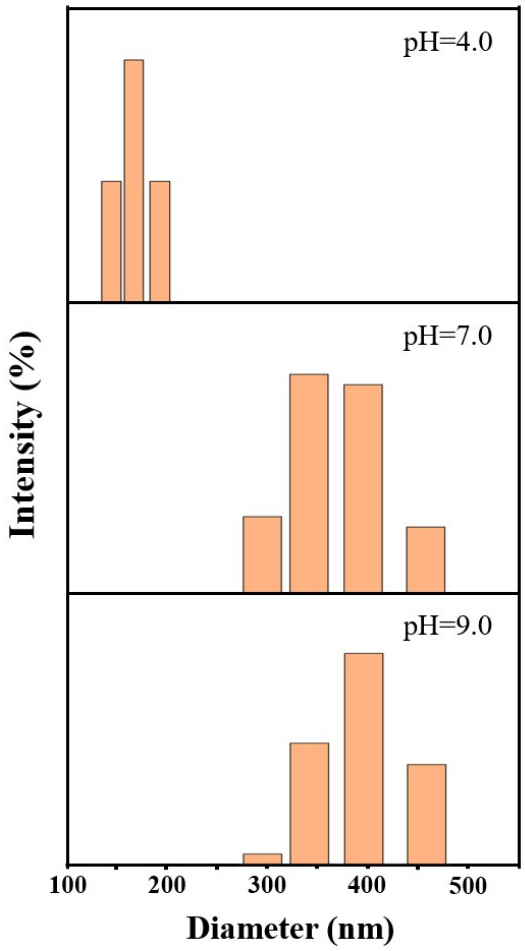


Fig. S8. Effect of pH on the hydrodynamic diameter of Ce@AuNCs.

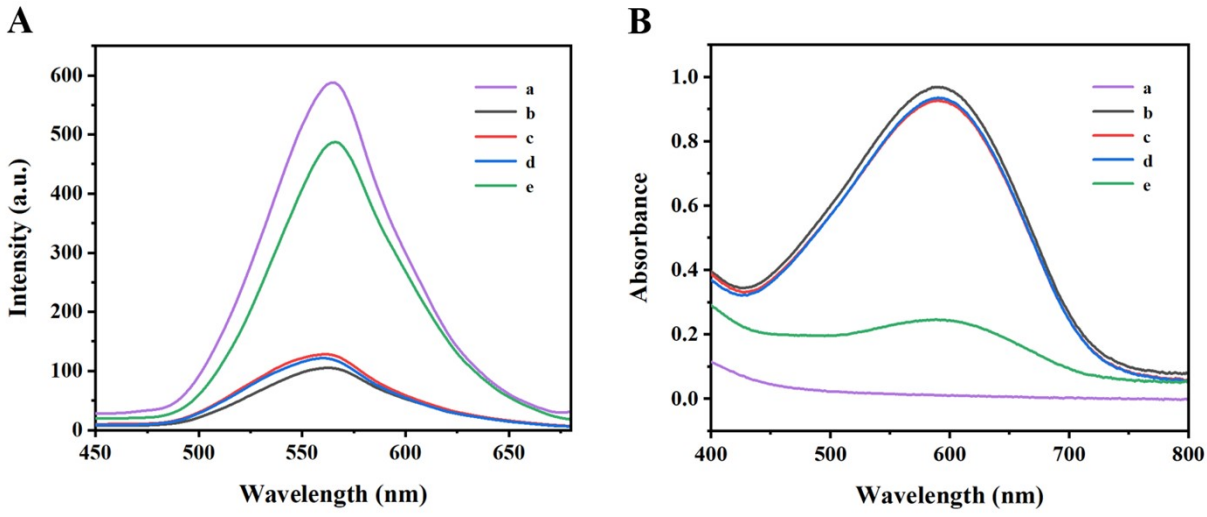


Fig. S9. (A) Fluorescence and (B) absorption spectra of (a) Ce@AuNCs, (b) Ce@AuNCs +DCIP, (c) Ce@AuNCs +DCIP +AA2G, (d) Ce@AuNCs +DCIP +α-Glu, and (e) Ce@AuNCs +DCIP+ AA2G + α-Glu.

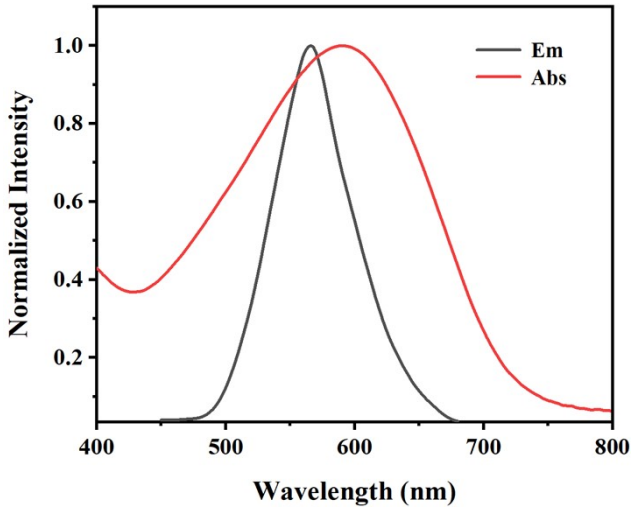


Fig. S10. UV-vis absorption spectrum of DCIP (red line) and fluorescence emission spectrum of Ce@AuNCs (black line, λ_{ex}=350 nm).

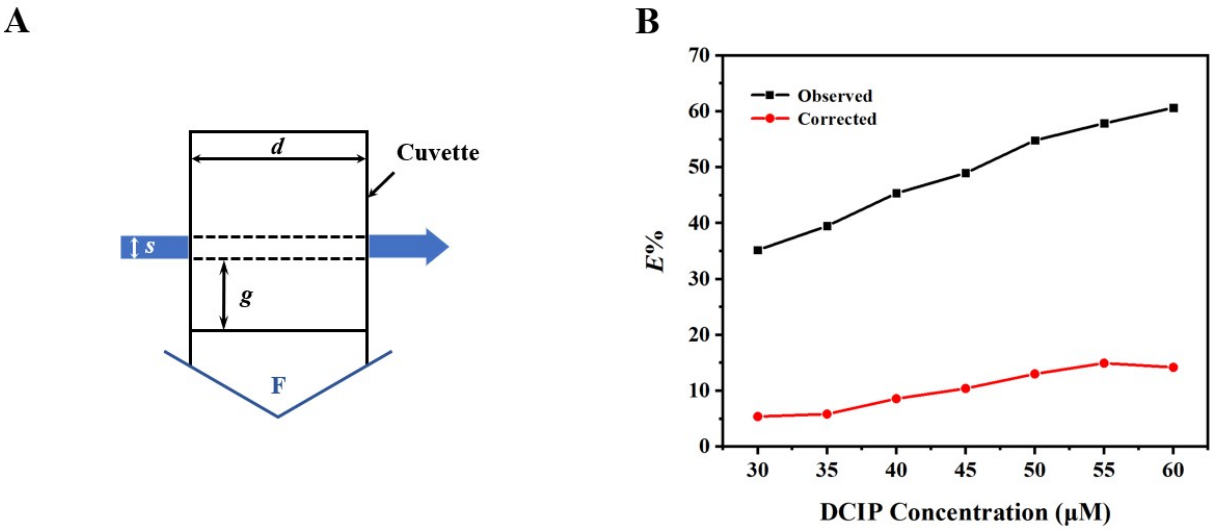


Fig. S11. (A) Parameters used in eq (1). (B) Suppressed efficiency (*E*%) of the observed and corrected measurements for Ce@AuNCs after addition of DCIP with different concentrations. E = 1- I/I_0, where I_0 and I are the fluorescence intensities of Ce@AuNCs in the absence and presence of DCIP.

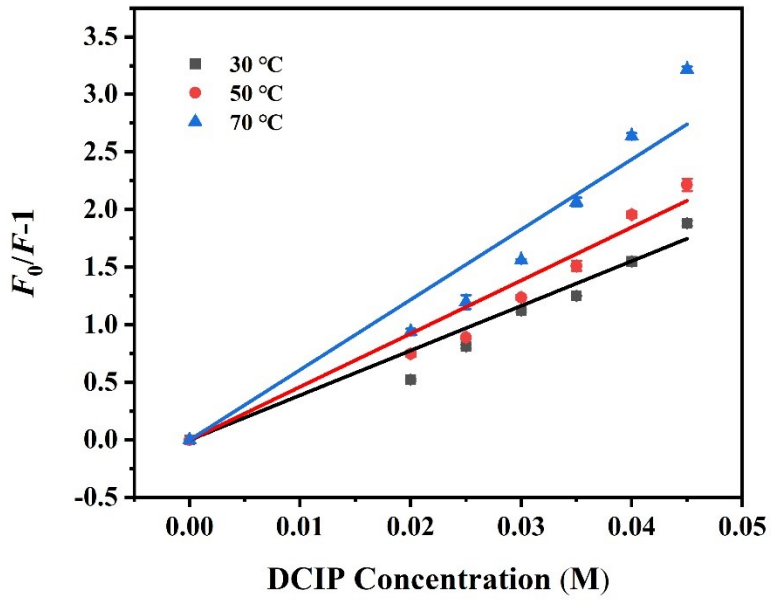


Fig. S12. Stern-Volmer curves for fluorescence quenching of Ce@AuNCs by DCIP.

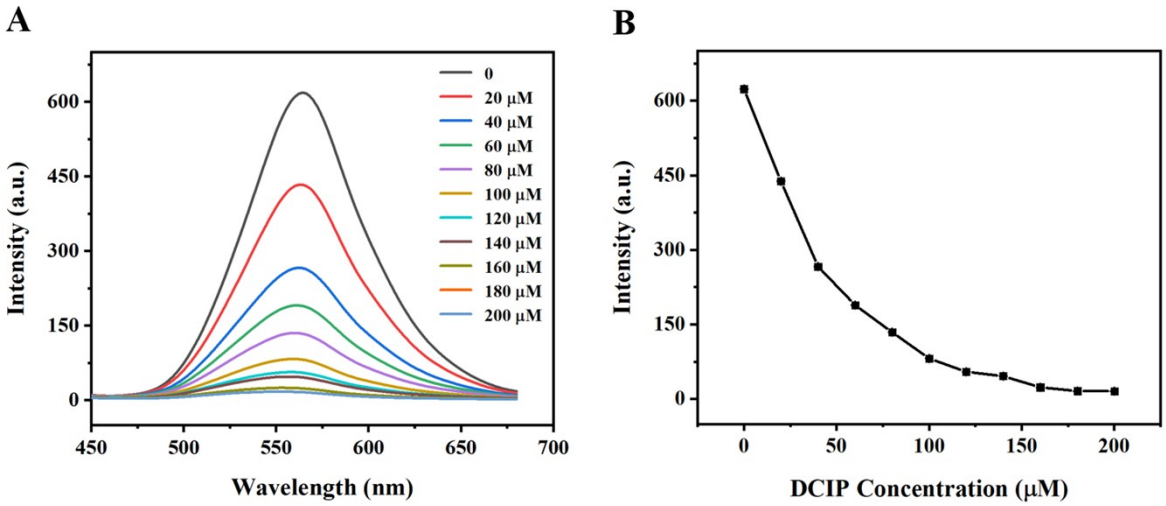


Fig. S13. Effect of DCIP concentration on the fluorescent intensity of Ce@AuNCs.

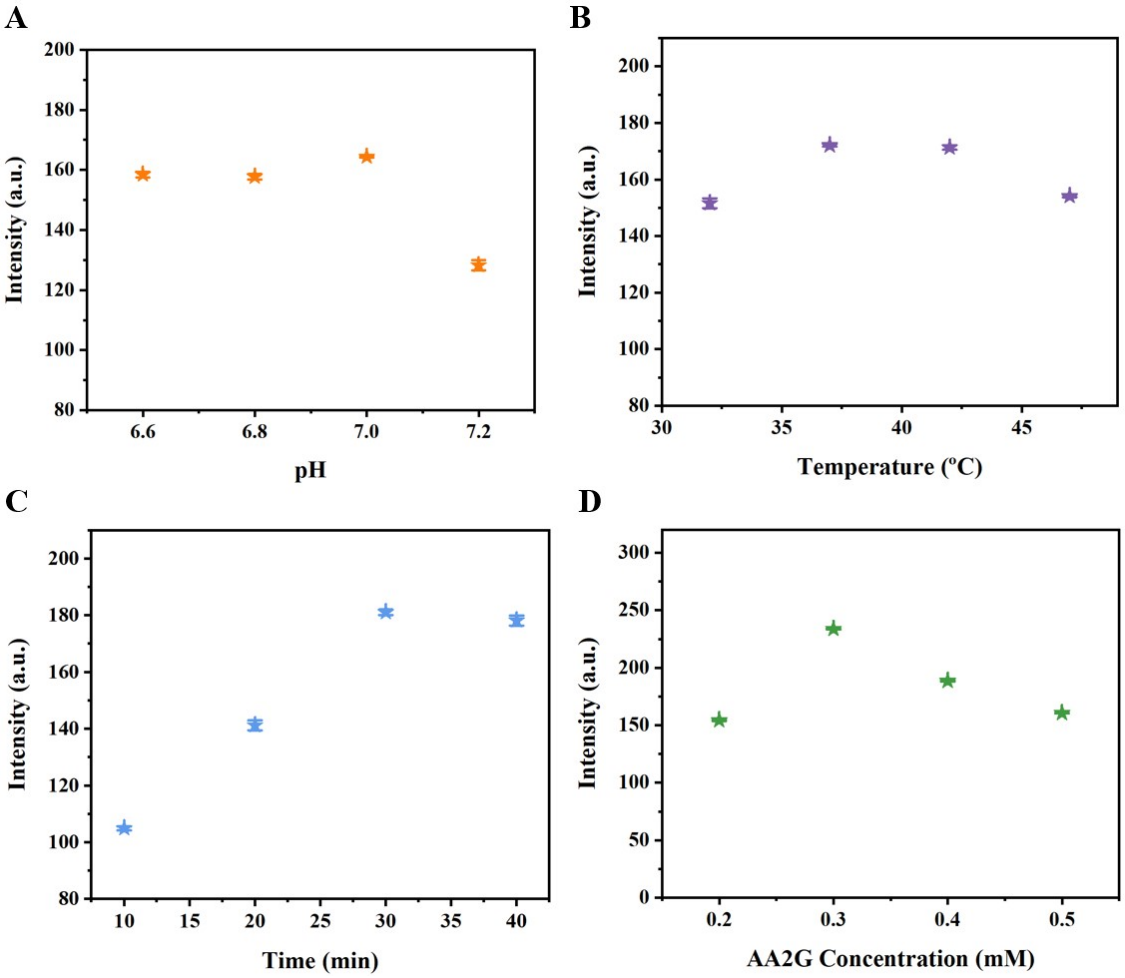


Fig. S14. Optimization of *α*-Glu assay including (A) pH, (B) incubation temperature, (C) incubation time, and (D) AA2G concentration.

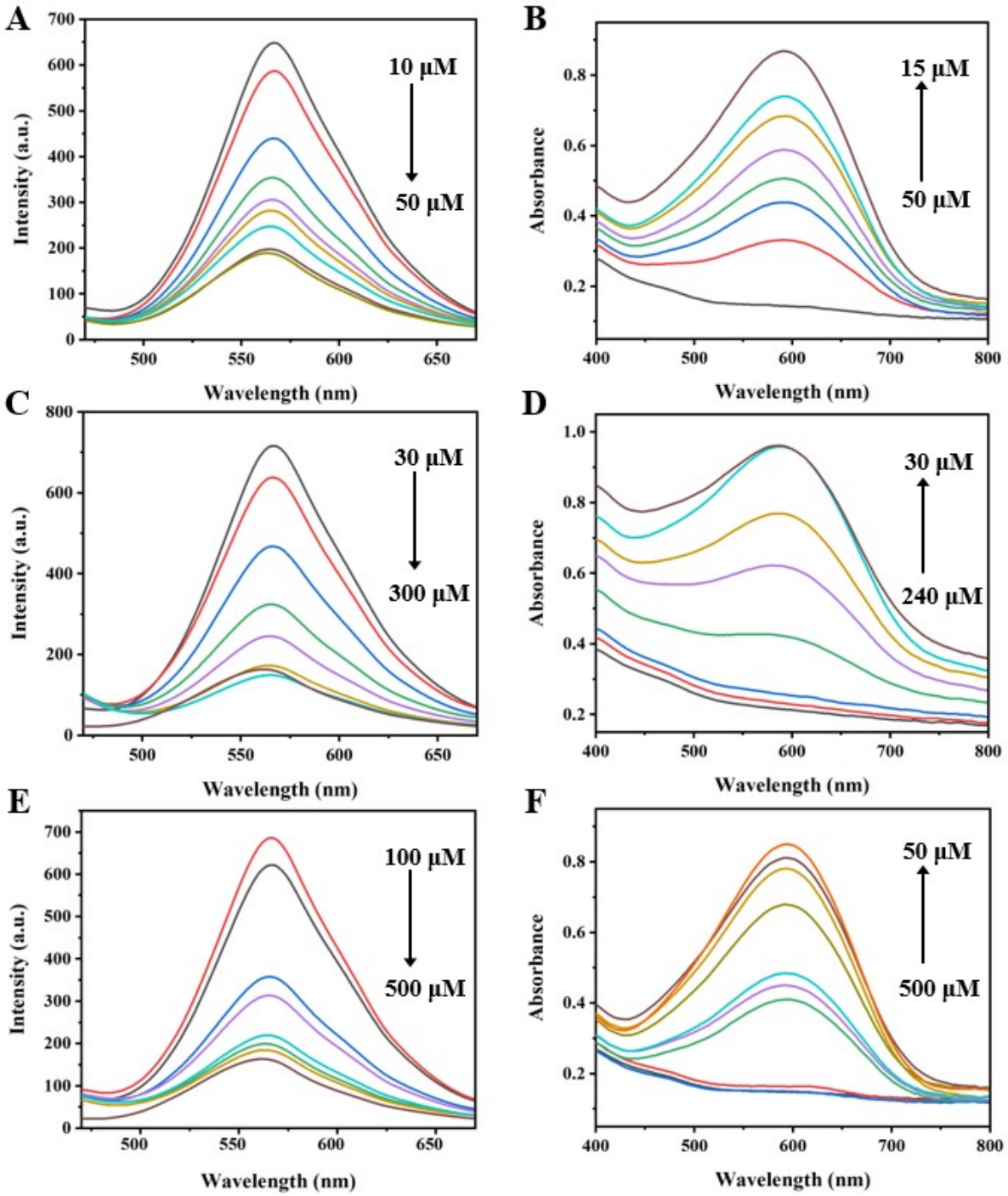


Fig. S15. Fluorescence and UV-vis spectra upon the addition of (A, B) luteolin, (C, D) apigenin, and (E, F) hesperidin with different concentrations.

Table S1

The fitting of fluorescence decay data of AuNCs, Ce^{3+}@AuNCs, and Ce^{3+}@AuNCs + DCIP.

Sample	τ_i (μs)	A_i (%)	τ (μs)	χ^2
AuNCs	0.249	18.23	2.19	1.18
	1.278	46.47		
	4.395	35.30		
Ce@AuNCs	1.305	23.58	6.33	1.28
	7.879	76.42		
Ce@AuNCs + DCIP	0.835	30.59	4.23	1.37
	5.730	69.41		

Herein, a three-exponential function was used to fit the fluorescence decay curves of AuNCs and the double-exponential function was suitable for Ce@AuNCs and Ce@AuNCs + DCIP. The decay in the fluorescence intensity (I) with time (t) was fitted by the following functions.

Three-exponential fit:

$$I = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2} + A_3 e^{-t/\tau_3}$$

Double-exponential fit:

$$I = A_1 e^{-t/\tau_1} + A_2 e^{-t/\tau_2}$$

The weighted mean lifetime $\langle\tau\rangle$ was calculated according to the following equation:

$$\langle\tau\rangle = \frac{A_1\tau_1 + A_2\tau_2 + A_3\tau_3}{A_1 + A_2 + A_3}$$

where τ_i (i = 1, 2, and 3) are the lifetimes of shorter- or longer- lived species; A_i (i = 1, 2, 3) are their respective amplitudes; and χ^2 indicates the goodness of the fit.

Table S2

IFE of DCIP on the fluorescence of Ce@AuNCs.

DCIP (μM)	A_{ex}[a]	A_{em}[b]	CF[c]	I_{obsd}[d]	I_{cor}[e]	$I_{cor,0}/I_{cor}$[f]
0	0.176	0.012	1.23	623.61	766.39	1
30	0.294	0.257	1.79	404.15	724.94	1.057
35	0.316	0.298	1.91	377.38	721.67	1.062
40	0.341	0.343	2.06	340.74	700.49	1.094
45	0.355	0.377	2.16	318.29	686.48	1.116
50	0.388	0.435	2.37	281.71	666.52	1.150
55	0.401	0.467	2.48	262.85	651.68	1.176
60	0.429	0.516	2.68	245.34	657.57	1.165

[c]Corrected factor (CF) was calculated as I_{cor}/I_{obsd}. The maximum value of CF could not exceed 3 to guarantee the correction was convincing. $I_{cor,0}$ is the corrected fluorescence intensity of Ce@AuNCs in the absence of DCIP.

Table S3

Stern-Volmer equations and correlation coefficients at different temperatures.

Temperatures/°C	S-V equation	R^2	K_{sv}/(L/mol)	K_q/[L/(mol·s)]
30	F_0/F-1=38.83[Q]	0.9939	38.83	6.13×10^6
50	F_0/F-1=46.16[Q]	0.9960	46.16	7.29×10^6
70	F_0/F-1=60.91[Q]	0.9890	60.91	9.62×10^6

Table S4

Comparison of different detection methods for α-Glu.

Detection method	Linear range (U/mL)	LOD (U/mL)	Reference
Electrochemical assay	0.01-0.5	0.04	[1]
Fluorometric assay	0.2-8	0.03	[2]
Fluorescence assay	0.1-0.5	0.01	[3]
Fluorescence assay	0.13-6.7	0.036	[4]
Fluorescence assay	0.01-0.16	0.0073	[5]
Colorimetry assay	0.0025-0.05	0.001	[6]
Fluorescence/ Colorimetry assay	0.25-1.10/ 0.25-0.95	0.012/ 0.021	This work

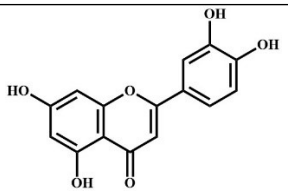
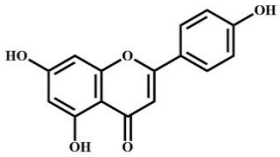
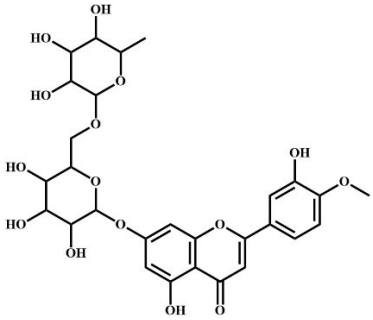
Table S5

Results of α-Glu determination in human serum samples.

Sample	Added	Fluorescence method			Colorimetry method		
	(U/mL)	Founded (U/mL)	Recovery (%, n = 3)	RSD (%, n = 3)	Founded (U/mL)	Recovery (%, n = 3)	RSD (%, n = 3)
1	0.45	0.47	104.4	1.2	0.46	102.2	1.6
2	0.65	0.62	95.4	1.9	0.64	98.5	1.1
3	0.85	0.84	98.8	2.9	0.83	97.6	2.7

Table S6

Structures of three natural inhibitors.

Inhibitor	Structure
Luteolin	
Apigenin	
Hesperidin	

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