Electronic Supplementary Material

Enzyme@bismuth-ellagic acid: a versatile platform for enzyme immobilization with enhanced acid-base stability

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Experimental

1. Characterization

The morphology of Bi-EA and enzyme@Bi-EA was observed using scanning electron microscopy (FEI NanoSEM450) and transmission electron microscope (JEOL-2100F). The crystal structure of samples was detected by X-ray powder diffractometer (Bruker AXS D8). The nitrogen adsorption and desorption isotherms were determined using Micromeritics ASAP 2020 system at 77 K and the pore size distribution was analyzed by BJH model. The confocal microscopy images of FITC-enzyme@Bi-EA were obtained using the confocal microscope (HC PL APO CS2 63×/1.40 OIL). The zeta-potential was measured using Nano laser particle size analyzer (Nano-ZS90).

2. Synthesis of the other enzymes@Bi-EA

To synthesize of the other enzymes@Bi-EA, the enzyme was dissolved in corresponding buffer solution, followed by the successive addition of bismuth acetate and ellagic acid. The complex was obtained after 5 h. The buffer solutions used in the preparation of enzyme@Bi-EA were MES (0.2 M, pH=6) for GOx and HRP, and

HEPES (0.2 M, pH=7) for CALB, OPH and GDH. The protein concentration of the enzyme solution was about 0.15 mg/mL for GDH, and 1 mg/mL for the other enzymes.

3. Determination of enzyme activity and kinetic parameters

For CALB, the enzyme activity was calculated by catalyzing the hydrolysis of p-nitrophenyl palmitate (p-NPP) at room temperature. p-NPP (20 μL), 20 μL of CALB or CALB@Bi-EA dispersion, and 960 μL of PBS solution (50 mM, pH 8) were pipetted to the cuvette, shaken rapidly and placed in a UV spectrophotometer. The change of absorbance values at 410 nm with time was monitored in a kinetic mode. Monitoring duration was set to 10 min with an interval of 20 s. The initial catalytic velocities of free CALB and CALB@Bi-EA were determined after 1min of reaction at different p-NPP concentrations (0.66 to 15.89 mM) and the Michaelis constant were calculated via nonlinear fitting.

For OPH, the enzyme activity was calculated using the hydrolysis of methyl parathion under room temperature conditions. OPH or OPH@Bi-EA dispersion (50 μL), 5 μL of methyl parathion (38 mM), and 945 μL of HEPES (0.2 M, pH 8) were pipetted to the cuvette, and the changes in OD_{410} nm were measured after being shaken quickly. The monitoring duration was set to 5 min with an interval of 20 s. The Michaelis constants were determined in the range of 76-1140 μM of methyl parathion.

For GDH, the enzyme activity was measured by assaying the amount of NADH production. At room temperature, 10 μL of enzyme dispersion, 100 μL of glucose solution (1 M), 50 μL of NAD^+ (20 mM) and 840 μL of Tris-HCl buffer were quickly added to the cuvette after dispersing well and then the changes of OD_{340} were detected via kinetic mode. The monitoring duration was set to 5 min with an interval of 20 s. The Michaelis constants of GDH and GDH@Bi-EA were measured via changing the concentration of NAD^+ from 0.1 to 1.0 mM. The concentration of glucose was constant at 0.1 M.

For GOx and HRP, 2.7 mL of PBS (50mM, pH 7.0) and 100 μL of enzyme dispersion (1:1 mass ratio of GOx to HRP) were incubated at 37 °C for 10 minutes and then 100μL of glucose (0.25 M) and 100 μL 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS, 0.02 M) were pipetted and reacted for 30 min. The OD_{415} values of the supernatant was measured to calculate the enzyme activity.

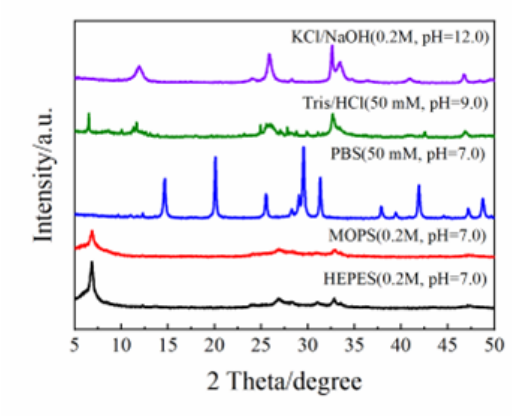

Fig. S1 Effect of different buffers on the synthesis of Bi-EA.

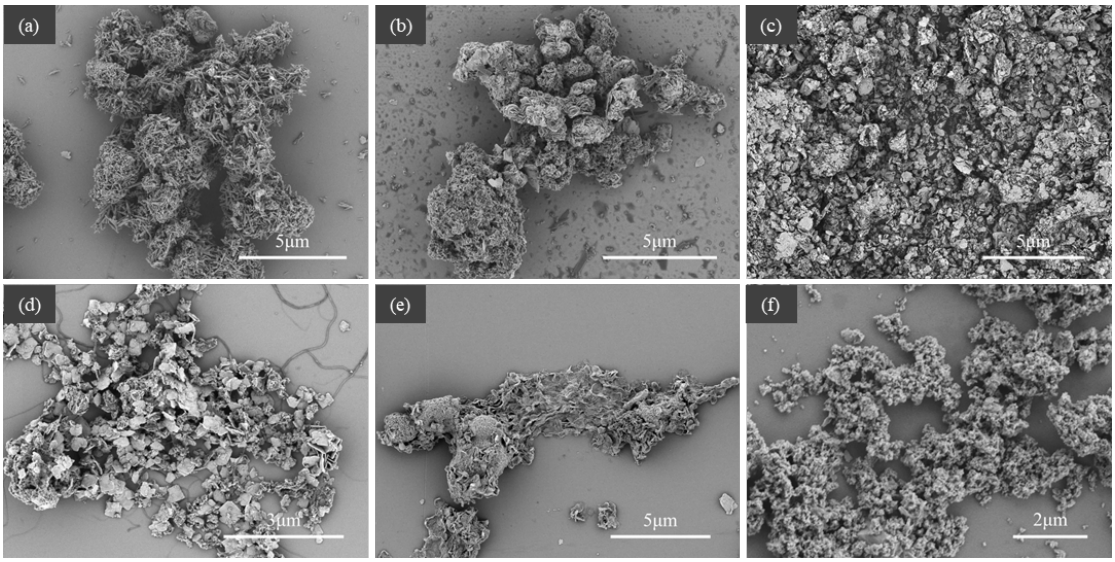

Fig. S2 SEM images of (a)Bi-EA-3, (b)Bi-EA-4, (c)Bi-EA-5, (d)Bi-EA-6, (e)Bi-EA-7, and (f)Bi-EA-8.

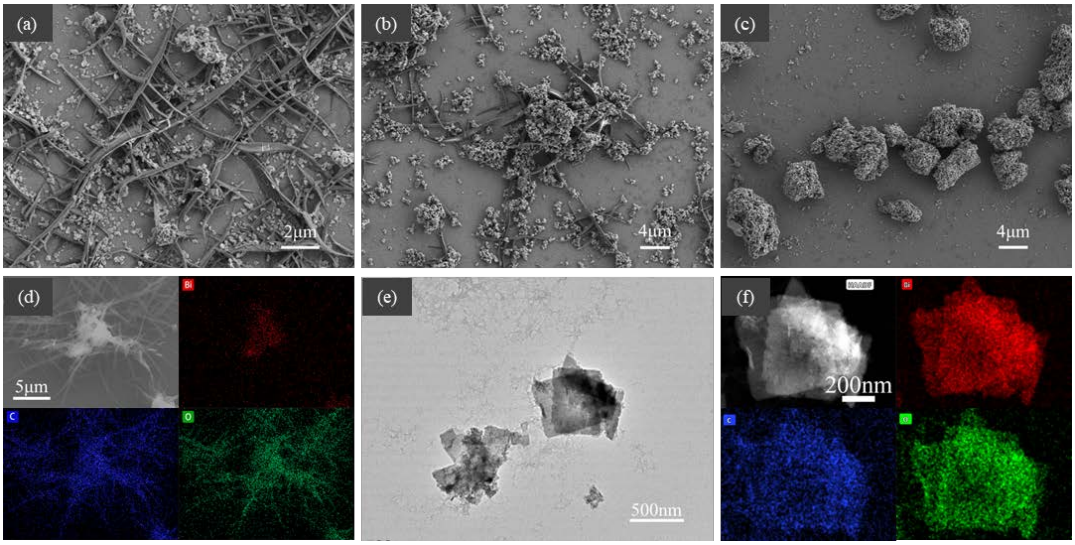

Fig. S3 SEM images of Bi-EA at different reaction times: (a) 1H, (b) 3H, (c) 5H. (d) SEM image and its corresponding elemental mapping of Bi-EA after reaction for 1 hour. (e-f) TEM image and its corresponding elemental mapping of Bi-EA after reaction for 5 hours.

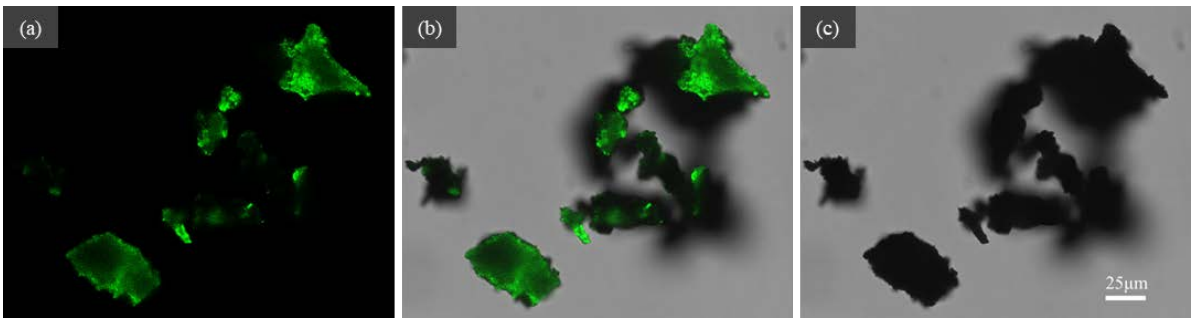

Fig. S4 Confocal laser scanning micrographs showing (a) fluorescence, (b) overlay and (c) bright field images of AMG@Bi-EA.

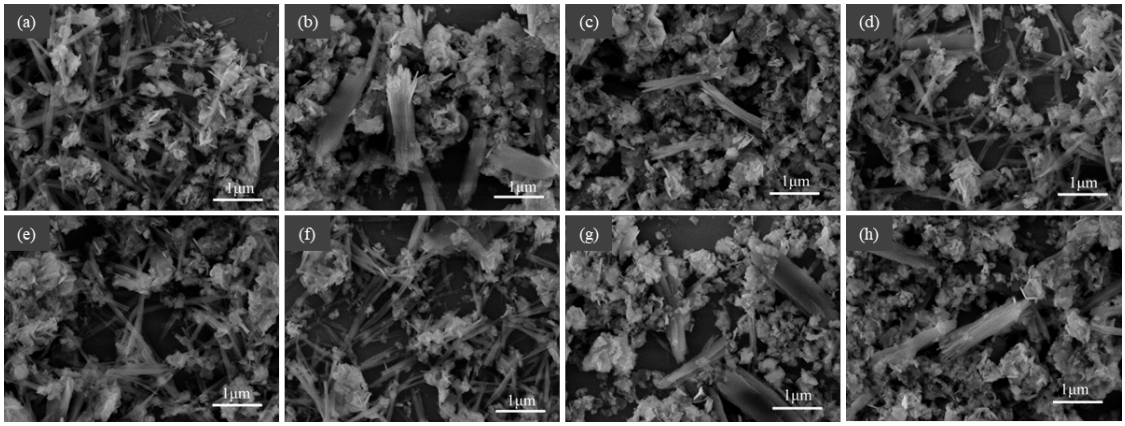

Fig. S5 SEM images of AMG@Bi-EA (a) before and (b-h) after incubating in buffers with different pH: (b) Glycine/HCl(50 mM, pH 2), (c) NaAc/Ac(0.2 M, pH 4), (d) MES(0.2 M, pH 6), (e) PBS(50 mM, pH 7), (f) HEPES(0.2 M, pH 8), (g) Glycine/NaOH(50 mM, pH 10), (h) KCl/NaOH(0.2 M, pH 12).

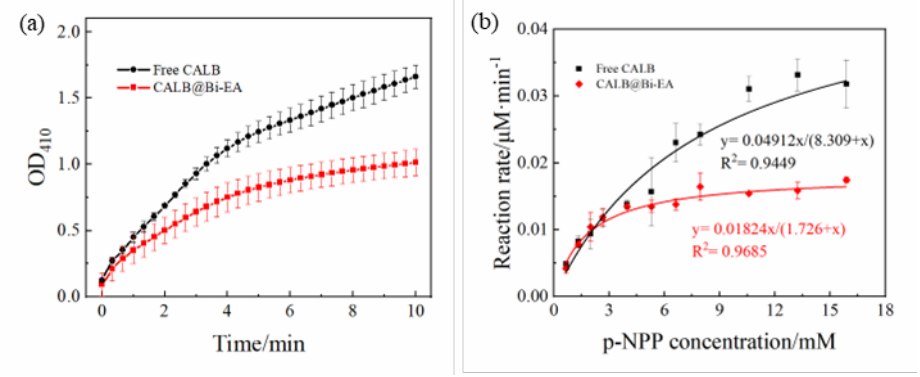

Fig. S6 The catalytic activity and enzymatic kinetics analysis of CALB and CALB@Bi-EA.

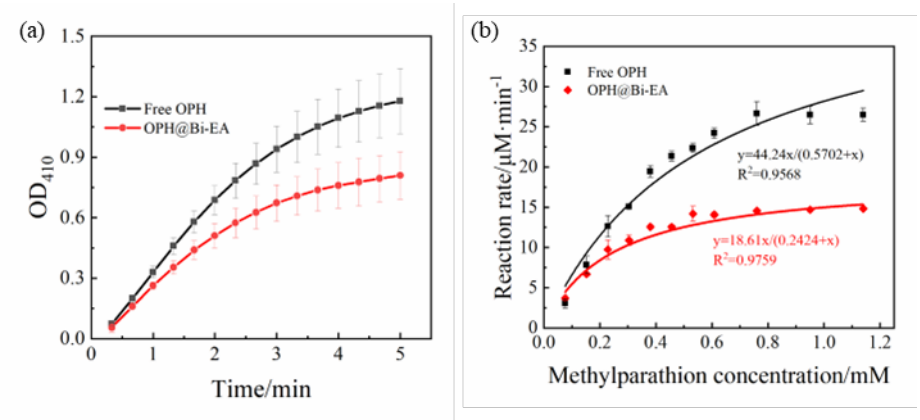

Fig. S7 The catalytic activity and enzymatic kinetics analysis of OPH and OPH@Bi-EA.

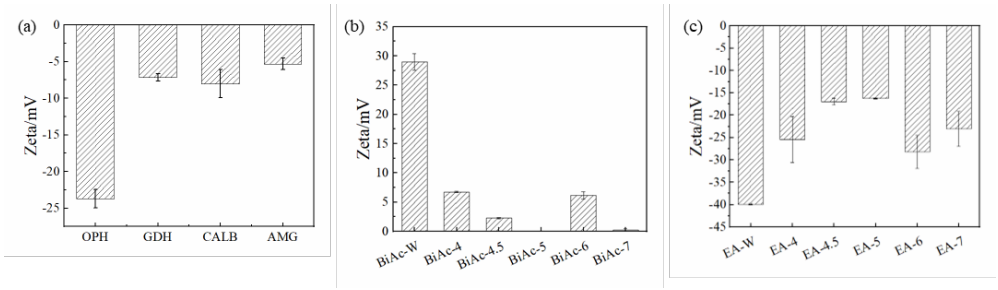

Fig. S8 (a) Surface zeta-potential analysis of enzymes and (b-c) Bi-EA precursors. In Figure a, OPH, GDH, and CALB were dispersed in HEPES (0.2 M, pH 7), and AMG was in sodium acetate buffer (0.2 M, pH 5). In Figure b and c, BiAc and EA were dispersed in water and buffers with different pH values (Sodium acetate buffer, pH 4-5, MES buffer, pH 6, and HEPES buffer, pH 7).

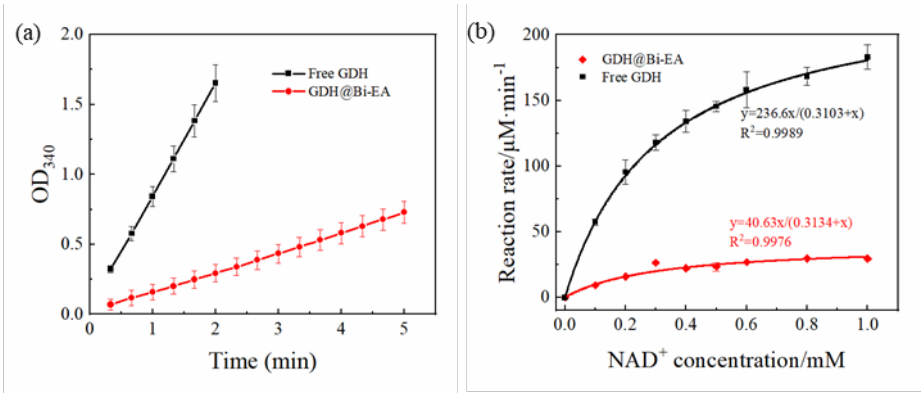

Fig. S9 The catalytic activity and enzymatic kinetics analysis of GDH and GDH@Bi-EA.

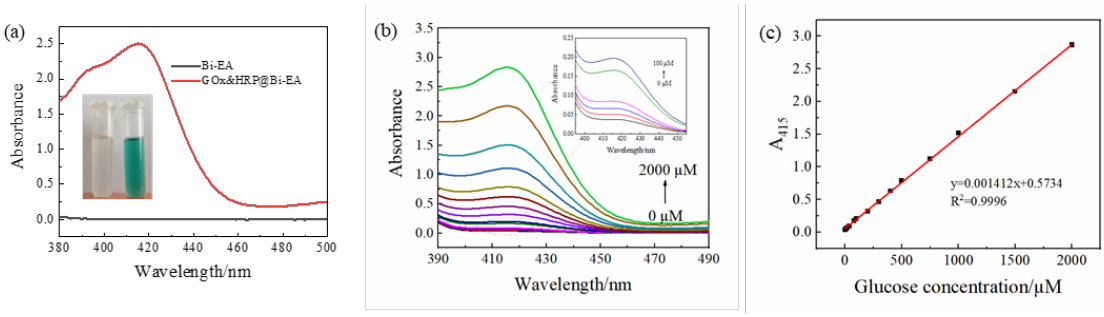

Fig. S10 (a) Biocatalysis activity of GOx&HRP@Bi-EA and (b-c) application of glucose detection.

Tab. S1 Information on several model enzymes in this work

Enzyme	Source	Dalton/kDa	Isoelectric point	Substrate	Optimal pH	Space size/nm^3
AMG	*aspergillus niger*	80 ~ 97	3.6	dextrinized starch	4-5	4×5×6
CALB	*Candida antarctic*	~33	6.0	p-NNP	7-8	3×4×5
OPH	*Plesiomonas sp.*	~72	n.d.	methyl parathion	8-9	4×6×9
GDH	*Bacillus*	~105	4.5	glucose /NAD^+	8-9	5×6×8
GOx	*Aspergillus niger*	~80	4.2	glucose	5-6	5.2 × 6.0 × 7.7
HRP	*Horseradish*	~44	3-9	H_2O_2	~7	4.0 × 4.4 × 6.8

Tab. S2 Kinetic parameters of free enzyme and enzyme@Bi-EA.

	Km/mM	Vmax/μM·min^{-1}
Free CALB	8.309±2.450	0.04912±0.009051
CALB@Bi-EA	1.726±0.2255	0.0182±0.004454
Free OPH	0.5701±0.1085	44.24±4.833
OPH@Bi-EA	0.2424±0.03135	18.61±0.7064
Free GDH	0.3103±0.02488	236.6±8.621
GDH@Bi-EA	0.3134±0.02794	40.63±1.516