Electronic Supplementary Information (ESI)

One-pot HTST synthesis of responsive fluorescent ZnO@apo-enzyme composite microgels for intracellular glucometry

Ruyue Lan,[a] Huijiao Liu,[a] Lin Zhu,[a] Fan Lu,[a] Qingshi Wu,[b] and Weitai Wu*[a]

[a] State Key Laboratory for Physical Chemistry of Solid Surfaces, Collaborative Innovation Center of Chemistry for Energy Materials, The Key Laboratory for Chemical Biology of Fujian Province, and Department of Chemistry, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, Fujian 361005, China. E-mail: wuwtxmu@xmu.edu.cn
[b] College of Chemical Engineering and Materials Science, Quanzhou Normal University, Quanzhou, Fujian 362000, China

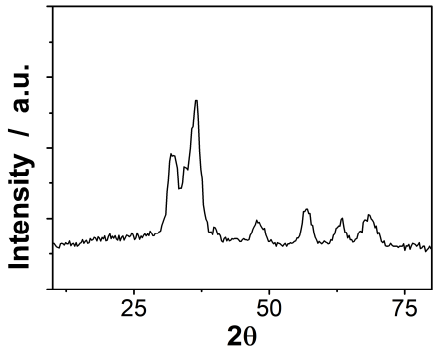

Fig. S1 XRD spectrum of ZnO@apo-GOx composite microgels.

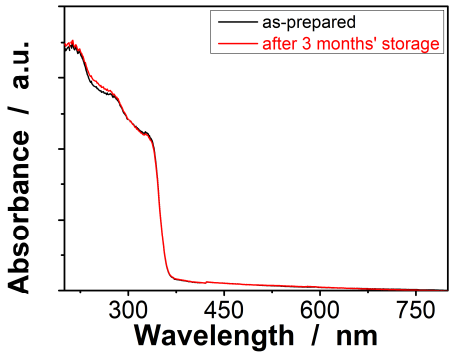

Fig. S2 UV-vis absorption spectra showing stability of ZnO@apo-GOx composite microgels.

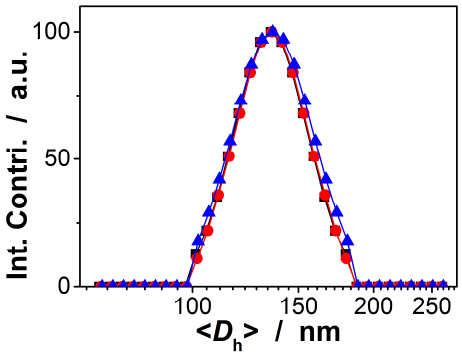

Fig. S3 DLS size distribution of the controlled composite microgels, which were obtained upon the continuous heat treatment at 70.0 °C under otherwise the same conditions for the synthesis of the ZnO@apo-enzyme composite microgels, showing that the controlled composite microgels were insensitive to glucose. The measurements were made in 5.0 mM PBS of pH = 7.4 at 37.0 °C with glucose concentrations [Glu] = 0.0 (■), 5.0 (●), and 20.0 mM (▲).

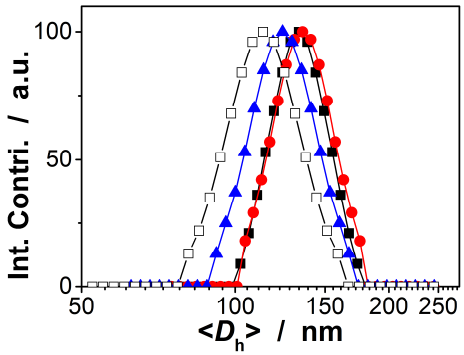

Fig. S4 DLS size distribution of ZnO@apo-enzyme composite microgels upon adding 20.0 mM of fructose (■), mannose (●), and galactose (▲). The result without any saccharides (□) is given for comparison. All measurements were made in 5.0 mM PBS of pH = 7.4 at 37.0 °C.

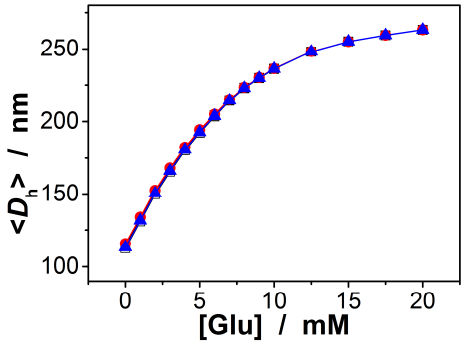

Fig. S5 Glucose-dependent <D_h> values of ZnO@apo-enzyme composite microgels dispersed in PBS with in the presence of 1.0 mM of fructose (■), mannose (●), or galactose (▲). All measurements were made in 5.0 mM PBS of pH = 7.4 at 37.0 °C.

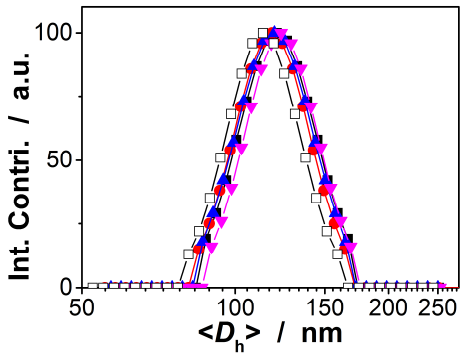

Fig. S6 DLS size distribution of ZnO@apo-enzyme composite microgels dispersed in PBS with in the presence of absorbed dextran (M_r ~ 6,000) (■), dextran (M_r ~ 40,000) (●), dextran (M_r ~ 100,000) (▲), RNase B (▼) and HSA (♦).The result without any saccharides (□) is given for comparison. All measurements were made in 5.0 mM PBS of pH = 7.4 at 37.0 °C.

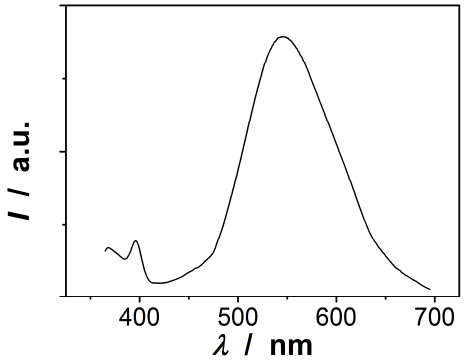

Fig. S7 A typical PL spectrum of ZnO@apo-enzyme composite microgels, excited at 340 nm.

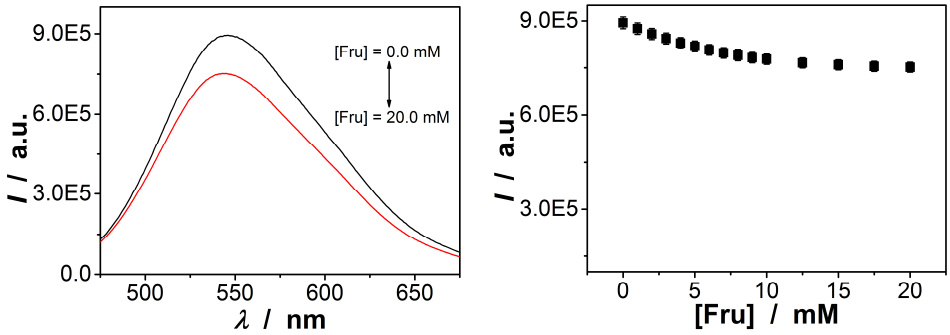

Fig. S8 [Fru]-dependent (a) PL spectra and (b) *I* at 546 nm of ZnO@apo-enzyme composite microgels dispersed in PBS. All measurements were made in 5.0 mM PBS of pH = 7.4 at 37.0 °C.

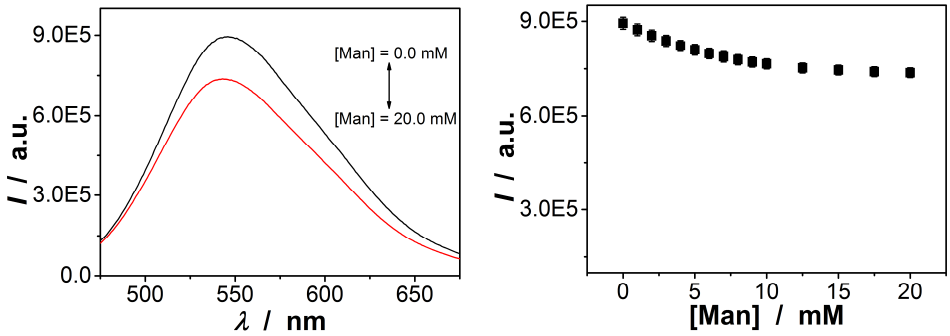

Fig. S9 [Man]-dependent (a) PL spectra and (b) *I* at 546 nm of ZnO@apo-enzyme composite microgels dispersed in PBS. All measurements were made in 5.0 mM PBS of pH = 7.4 at 37.0 °C.

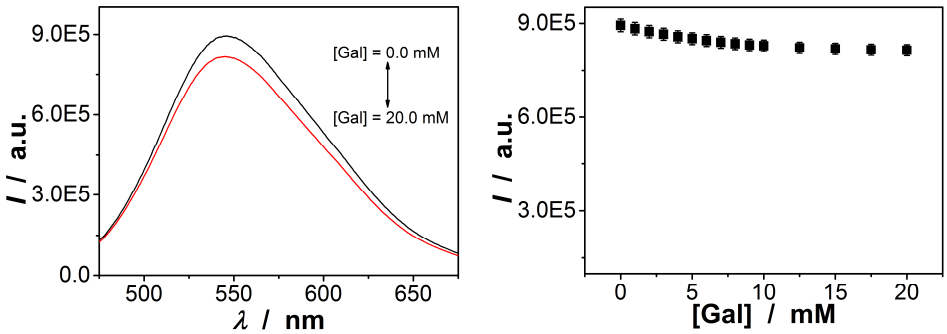

Fig. S10 [Gal]-dependent (a) PL spectra and (b) *I* at 546 nm of ZnO@apo-enzyme composite microgels dispersed in PBS. All measurements were made in 5.0 mM PBS of pH = 7.4 at 37.0 °C.

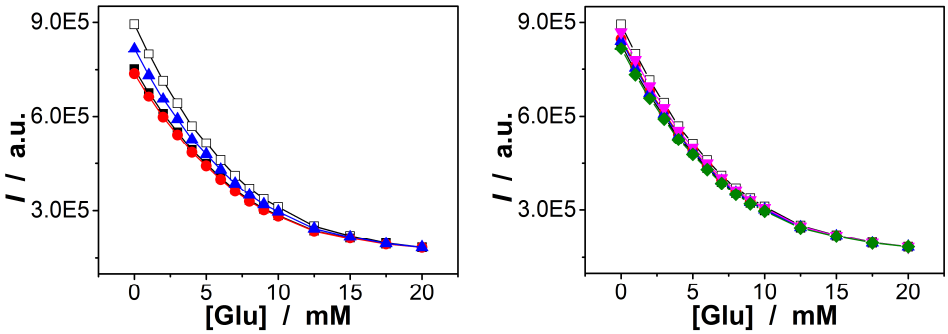

Fig. S11 [Glu]-dependent *I* at 546 nm of ZnO@apo-enzyme composite microgels dispersed in PBS with in the presence of (a) 20.0 mM of fructose (■), mannose (●), or galactose (▲), and (b) absorbed dextran (M_r ~ 6,000) (■), dextran (M_r ~ 40,000) (●), dextran (M_r ~ 100,000) (▲), RNase B (▼) and HSA (♦). The results in the absence of those non-glucose constituents (□) are given for comparison. All measurements were made in 5.0 mM PBS of pH = 7.4 at 37.0 °C.

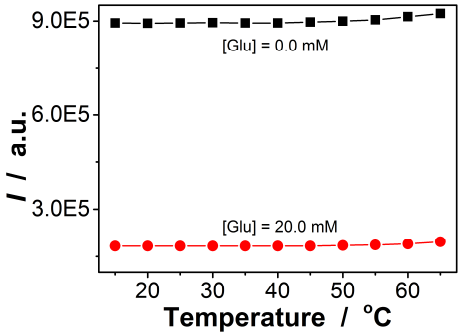

Fig. S12 The effect of solution temperature on the [Glu]-dependent *I* at 546 nm of ZnO@apo-enzyme composite microgels dispersed in PBS. All measurements were made in 5.0 mM PBS of pH = 7.4.

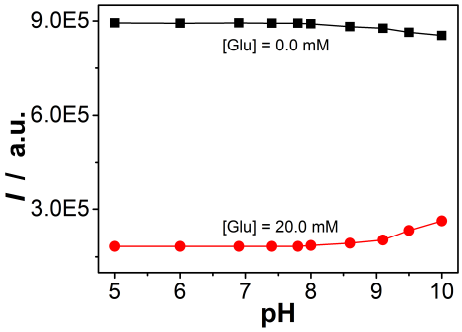

Fig. S13 The effect of solution pH value on the [Glu]-dependent *I* at 546 nm of ZnO@apo-enzyme composite microgels dispersed in PBS. All measurements were made in 5.0 mM PBS at 37.0 °C.

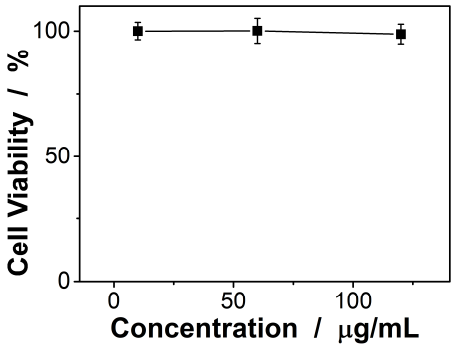

Fig. S14 B16F10 cell viability following treatments with ZnO@apo-enzyme composite microgels.

For cell viability evaluation, B16F10 cells (6×10^4 cell well^{-1}) were cultured in DMEM containing 10% FBS and 1% penicillin-streptomycin in a 96-well plate, and exposed to ZnO@apo-enzyme composite microgels. The plate was incubated at 37 °C for 24 h. The medium was then aspirated, and these wells were washed three times using fresh serum-free DMEM. After that, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) solution (25 μL, 5 mg mL^{-1} in PBS) were added. After incubation for 2 h, the solution was aspirated and DMSO (100 μL) was added to each well, and the plate was sealed and incubated overnight at 37 ºC with gentle mixing. Three portions of the solution obtained from each well were transferred to three respective wells of a 96-well plate. Cell viability was measured using a microplate reader at 570 nm. Positive controls contained no composite microgels, and negative controls contained MTT. Parallel wells (in triplicate) also contained only medium (no cells) and the same concentrations of composite microgels.

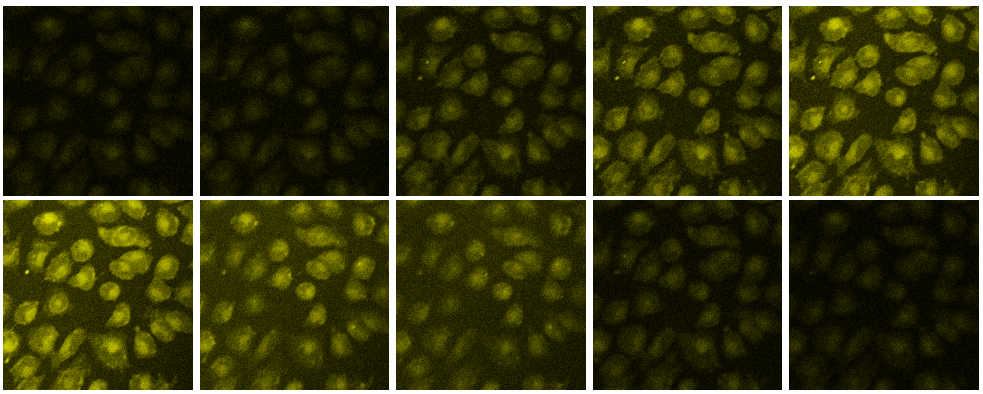

Fig. S15 Top-to-bottom Z-scanning confocal fluorescence images of mouse melanoma cells B16F10 incubated with ZnO@apo-enzyme composite microgels.

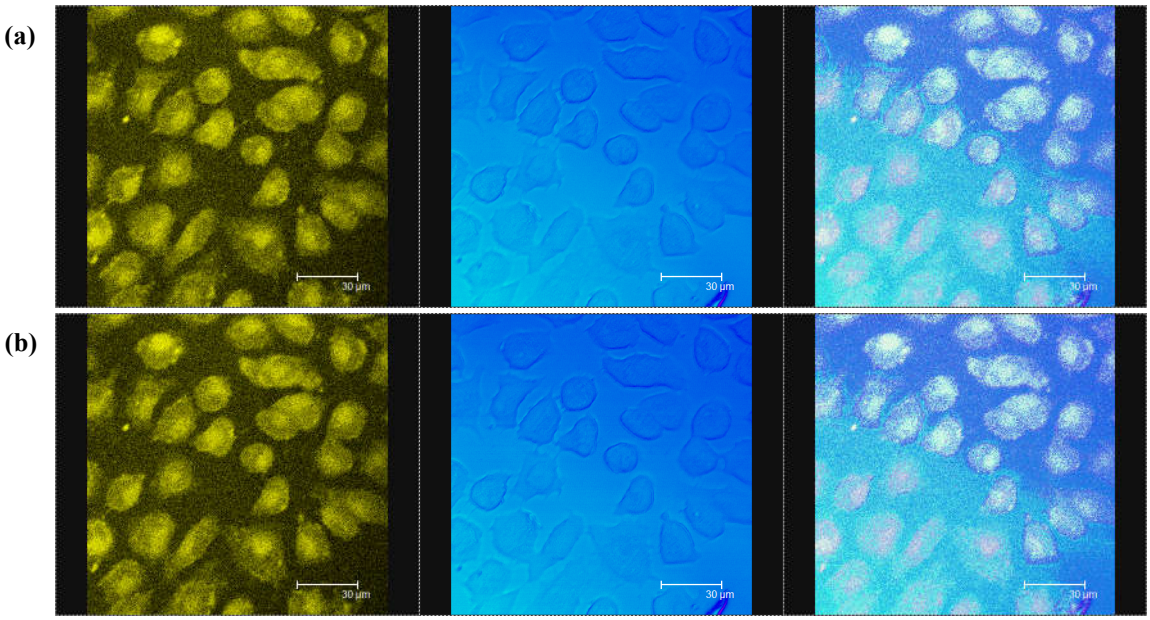

Fig. S16 Scanning confocal fluorescence (left), transmission (centre), and overlaid images (right) of B16F10 cells loaded with ZnO@apo-enzyme composite microgels, before (a) and after (b) continuously illuminated for 60 min.

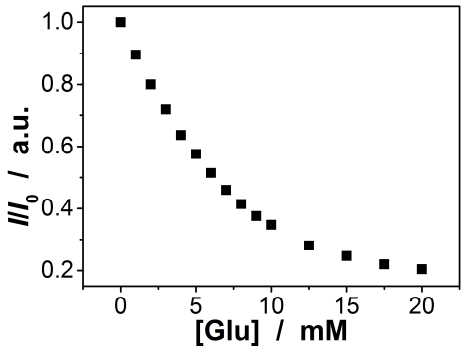

Fig. S17 [Glu]-dependent I/I_0 at 546 nm of ZnO@apo-enzyme composite microgels dispersed in PBS, where I_0 is the PL intensity at [Glu] = 0.0 mM. All measurements were made in 5.0 mM PBS of pH = 7.4 at 37.0 °C.

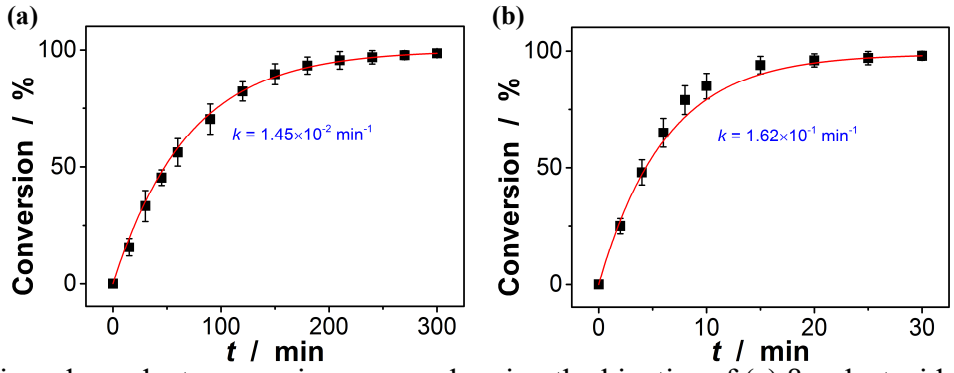

Fig. S18 Time-dependent conversion curves showing the kinetics of (a) β-galactosidase catalytic hydrolysis reaction of lactose and (b) GOx catalysed oxidation reaction of glucose, respectively. The conversion was calculated by changes in glucose concentration, which were determined by the oxygen rate method making use of a Beckman glucose analyzer. Solid lines: 1st-order kinetic fits. All measurements were made in 5.0 mM PBS of pH = 7.4 at 37.0 °C.

Table S1. Interference tests on glucose level reading by using ZnO@apo-enzyme composite microgels

Constituents	Concentration	Relative error[a]	Constituents	Concentration	Relative error[a]
L-lactate	20.0 mM	+2.3%	urea	5.0×10^{-2} mM	–0.2%
pyruvic acid	0.1 mM	+0.1%	citric acid	5.0×10^{-2} mM	+0.3%
K^+	2.0 mM	–0.1%	vitamin C	5.0×10^{-2} mM	+0.2%
Na^+	2.0 mM	–0.2%	γ-globulins	5.0×10^{-2} mM	+0.9%
Ca^{2+}	20.0 mM	–3.1%	lysozyme	5.0×10^{-2} mM	+0.5%
Mg^{2+}	0.5 mM	–0.3%	glycine	0.1 mM	–1.0%
Ba^{2+}	2.0 mM	–0.7%	arginine	0.1 mM	–1.3%
Al^{3+}	5.0×10^{-2} mM	–0.3%	L-phenylalanine	0.1 mM	+2.5%
Cu^{2+}	2.0×10^{-3} mM	–1.5%	lysine	0.1 mM	+1.2%
Zn^{2+}	5.0×10^{-2} mM	–0.9%	L-cystenine	0.5 mM	+2.8%
Co^{2+}	5.0×10^{-3} mM	–0.7%	tyrosine	0.5 mM	–1.9%
Fe^{3+}	2.0×10^{-3} mM	–1.3%	cholesterol	1.0×10^{-2} mM	+2.1%

[a] "+" and "–" indicate an increase and decrease, respectively, in glucose-induced PL intensity quenching at 546 nm.