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Encapsulation of Ionic Nanoparticle Produced Reactive Oxygen Species (ROS)-Responsive Microgel Useful for Molecular Detection

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Supporting Information

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1. Experimental Section

Materials. N-isopropylacrylamide (NIPAM), acrylic acid (AA), bis-acrylamide (BIS), potassium persulfate (KPS), $Zn(NO_3)_2$, $Cu(NO_3)_2$, 3-mercaptopropionic acid, mercaptoacetic acid, Na_2S, N-(aminobutyl)-N-(ethylisoluminol) (ABEI), H_2O_2, NaClO, $FeCl_2$, $FeCl_3$, KO_2, D-glucose, cholesterol, glucose oxidase (GOx), cholesterol oxidase (ChOx) and cholesterol esterase (ChEx) were purchased from Sigma-Aldrich, Inc. (Saint Louis, MO). $Ag(NO_3)$ and Sodium dodecyl sulfate (SDS) were obtained from Fisher Scientific (Waltham, MA). Human serum samples were received from Discovery Life Sciences Inc. and stored at -80 ºC until use (Los Osos, CA). All chemicals were analytical reagent grade and used without further purification. Ultrapure water with electric resistance > 18.2 MΩ was produced by the Milipore Milli-Q water purification system (Billerica, MA).

Synthesis of microgels. Microgels were synthesized according to literature[1] with modifications. Briefly, 0.305 g NIPAM, 0.097 g AA, 0.03 g SDS and 4% (of total monomer) BIS were dissolved in 45 mL ultrapure water inside a three-neck flask. The solution was purged with high purity nitrogen for 30 mins at room temperature and then the temperature was increased to 70 ºC. Five milliliters of the freshly prepared KPS solution (0.002 g/mL) was added quickly to the flask and the solution was stirred for 4 hours at 70 ºC. A milk colored solution appeared after 4 hours, indicating the successful formation of the hydrogel particles. The solution was dialyzed against ultrapure water with a 12-14 kDa Spectra/Por molecular porous membrane tubing (Spectrum labs Inc., CA) to remove the non-reacted monomers and residual SDS for 2 days, replacing the dialysis solution with ultrapure water every 4 hours. The gel solution was kept at room temperature until use.

Synthesis of ZnS or ZnSe nanoparticles.

Briefly, for ZnS nanoparticle, 7.5 mL 0.1 M $Zn(NO3)_2$ and 0.209 mL 3-mercaptopropionic acid (MPA) were mixed and water was added to bring the volume to 42.5 mL. pH of above mixture was then adjusted to 10 with 1 M NaOH. After 30 mins of nitrogen purge, 7.5 mL 0.1 M Na_2S was added and allowed to react for 20 mins. The temperature was increased to 50 ºC and kept for another 2 hours. Products were collected and cleaned by ethanol precipitation. ZnSe was prepared by heating 1.1 g Se and 7.6 g Na_2SO_3 in 40 mL water at 90 ºC for 2 hours with nitrogen bubbling to obtain Na_2SeSO_3. Then 0.35 g $ZnCl_2$ and 0.75 mL mercaptoacetic acid (MAA) were mixed in 50 mL water and pH was adjusted to 9-10 by NaOH. 5.7 mL of Na_2SeSO_3 solution was added.

The mixture was allowed to react at 100 ºC for 1 hour. The product was precipitated with isopropanol and dried under vacuum.

Synthesis of ZnS- and ZnS/CuS-enclosed microgel. The number of $COOH^-$ groups in each microgel preparation was first determined by acid-base titration. Then the microgel solution was diluted with ultrapure water to achieve a final [$COOH^-$] of ~3 mM, and $Zn(NO_3)_2$ and $Cu(NO_3)_2$ were with rapid stirring. The final molar ratio of Zn^{2+}: $COOH^-$ = 1:2, with varying Cu^{2+} content (from 0 to 50% of Zn^{2+} in moles) but the same total cation concentration of 1.5 mM. The pH was adjusted with 1 M KOH to 6.8 to ensure better coordination. After overnight incubation, the above solution was dialyzed with water for 24 hours to remove the unbounded metal ions. On the following day, Na_2S was added with a final concentration of 3 mM and the reaction continued for 2 hours at room temperature. The final product was dialyzed again for 24 hours to remove the unreacted Na_2S and stored at room temperature. The ZnS- or ZnS/CuS-enclosed microgels were characterized by TEM and NTA (Nanoparticles Tracking Analysis). In addition, the ZnS and ZnSe NPs coated by mercaptoacetic acid (MAA) without protection of hydrogels were also fabricated according to our previous work[2,3] and the size was determined by DLS. They were used in NP stability assessment.

Transmission electron microscopy (TEM) and electron spin resonance (ESR). Morphology of ZnS microgels were characterized by Transmission electron microscopy (TEM) in Philips TECNAI 12 by operating at an accelerating voltage of 22 kV. The electron spin resonance (ESR) spectra were recorded on an X-Band ESR Spectrometer (Bruker, MA, U.S.A.) following the protocol recommended by manufacturer and the Analytical Chemistry Instrumentation Facility.

ABEI (N-(4-Aminobutyl)-N-ethylisoluminol) - H_2O_2 chemiluminescence detection. One hundred μM ABEI and different microgels (ZnS and different ratios of ZnS/CuS microgel) were first mixed in 0.1 mM NaOH. The chemiluminescence signal was acquired by a Promega GloMax-Multi+ Microplate Multimode Reader with an online injection system. CL was measured immediately upon injection of H_2O_2. The injection rate is kept at 500 μL/s for all measurements.

ICP-AES analysis. Inductively Coupled Plasma - Atomic Emission Spectrometer (ICP-AES) (Norwalk, CT) was employed to verify the quantities of zinc and copper in microgels. The samples were treated with 10% HNO_3 before analysis. The instrument was first rinsed with 10% HNO_3 before injection. Standard solutions and all samples were measured in triplicate.

Measurement of ROS. Firstly, 10 μL of the ZnS- or ZnS/CuS-enclosed microgel was added into a well of the 96-well microplate; then, 70 μL of H_2O_2 and 20 μL of Fluozin-3 with or without EDTA were added to a final concentration of 3 μM. The solutions were mixed for 15 minutes on a plate shaker, before examined by a plate reader (Perkin Elmer Wallac 1420 Victor 2) with the Ex/Em wavelengths at 485/530 nm. Measurement of the responsivity to other ROS was carried out similarly, by substituting H_2O_2 with NaClO, KO_2, and Fenton reagents (Fe^{2+}/H_2O_2).

Measurement of glucose and cholesterol. Detection of glucose in solution was done as a one-pot assay, by mixing the ZnS/CuS-enclosed microgel, glucose (stock prepared in 1×PBS at 50 mM) and 2 μg/mL GOx in one well and recording the fluorescence every 3 minutes on a plate reader. Cholesterol stock solution was prepared by adding solid cholesterol into the TX-100 solution (5%, v/v) to obtain a concentration of 5 mM. A 5-minute incubation in 70 ºC water bath was necessary for complete dissolvation. Then cholesterol measurement was done in the similar way as the glucose assay, with 20 μg/mL ChOx and 0.5% TX-100 added to keep cholesterol from precipitation. For detection in serum, serum was first diluted 50 times by 1×PBS. The diluted serum was incubated with 20 μg/mL GOx (glucose detection) or 20 μg/mL ChOx/ChEx (20 μg/mL) (cholesterol detection) for 30 minutes. Then the solution was added to a 3 kDa ultra centrifugation filter (Amicon, Millipore) and centrifuged with 10,000×g for 15 minutes. The filtrate was tested by ZnS/CuS-enclosed microgel ($[Zn^{2+}]$ = 1.5 μM).

2. Supporting Figures

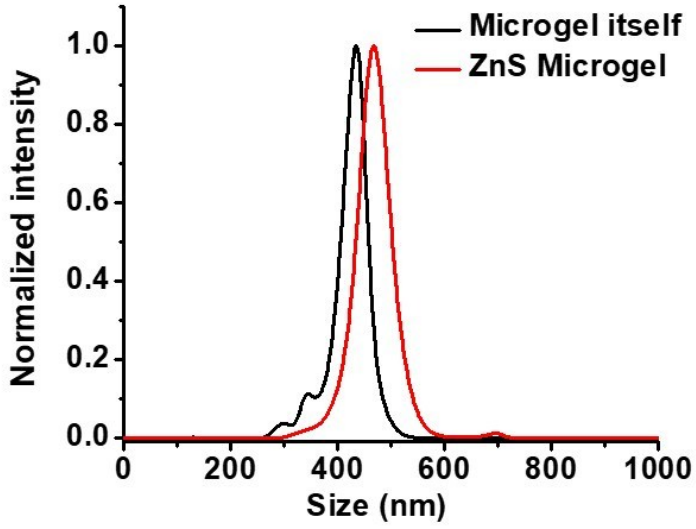


Figure S1. NTA measurements of size of microgel itself and ZnS-enclosed microgel.

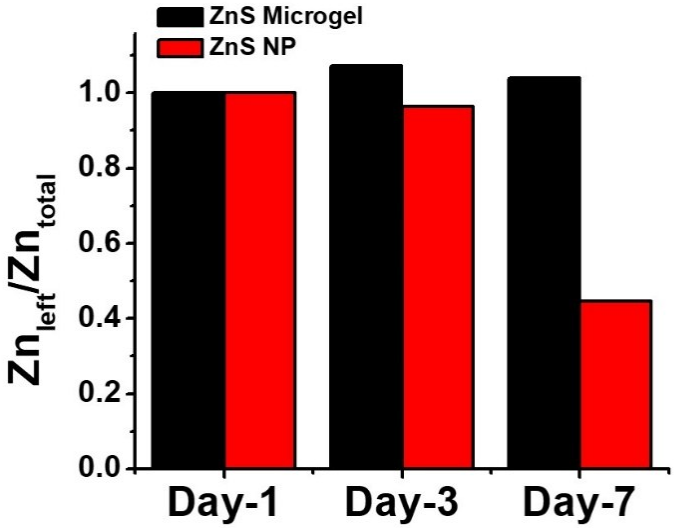


Figure S2. Stability of ZnS-enclosed microgel and ZnS Nanoparticle. Samples were kept in 1× PBS buffer at room temperature and measurements were made on day 1, day 3 and day 7.

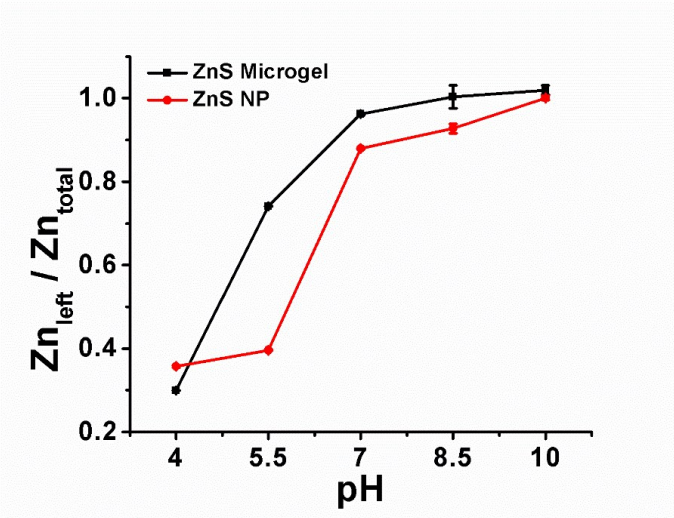
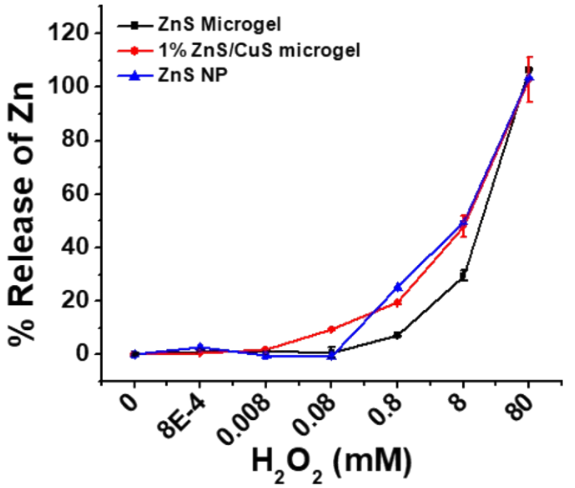


Figure S3. Stability of ZnS-enclosed microgel and ZnS nanoparticles in a range of pHs after 12 hours incubation.



Metal	Total	Cation exchange (100 μM Ag^+)	Efficiency
Zn^{2+}	27.41 μM	26.10 μM	95.2%

Figure S4. % Release of Zn from ZnS NPs or ZnS-enclosed microgel with addition of H_2O_2 (incubate for 15 mins). Table: Cation exchange efficiency of ZnS microgel with addition of $AgNO_3$ and detection was done with fluorometer within 5 mins. Results obtained by ICP-AES.

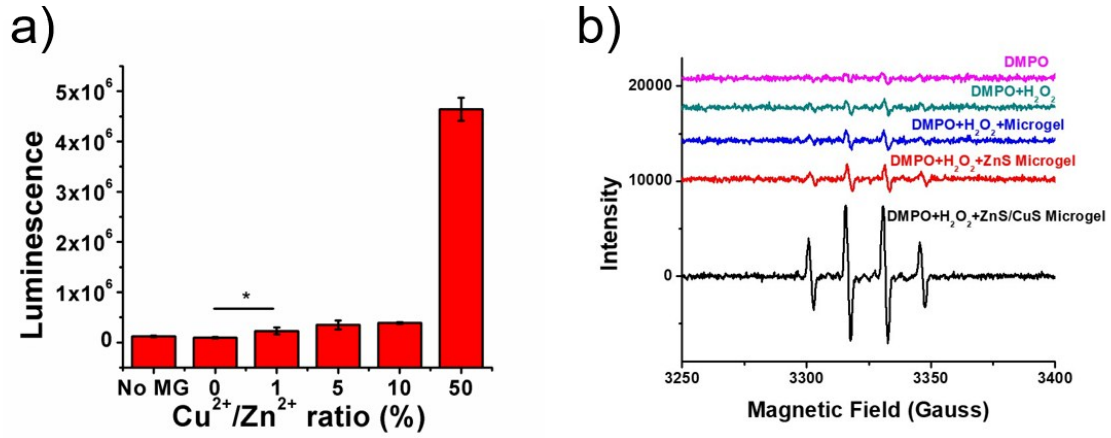


Figure S5. a) Chemiluminescence of different microgels with H_2O_2 and ABEI. [H_2O_2] = 1 mM, [ABEI] = 0.1 mM. b) ESR spectra of different reaction systems with DMPO as the spin trap.1% Cu^{2+} was used here.

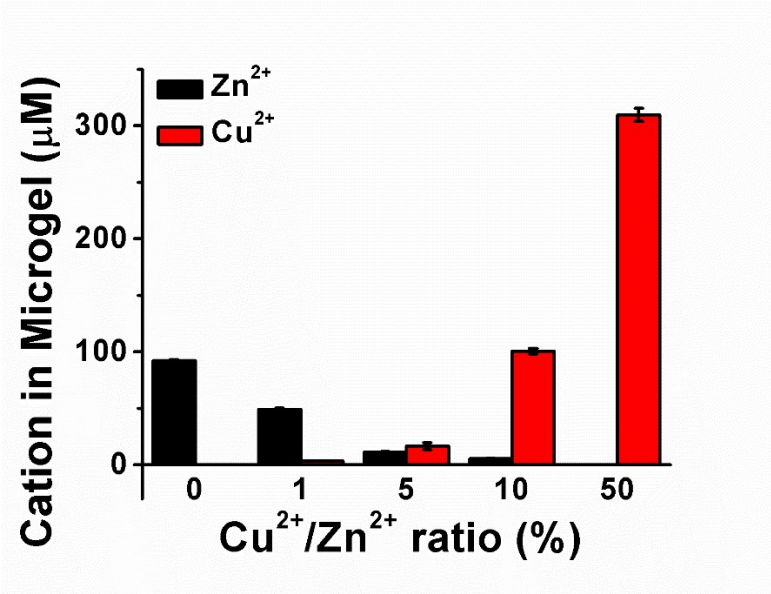


Figure S6. ICP-AES quantification of metal concentration in ZnS/CuS-enclosed microgel with different Cu^{2+}/Zn^{2+} ratio.

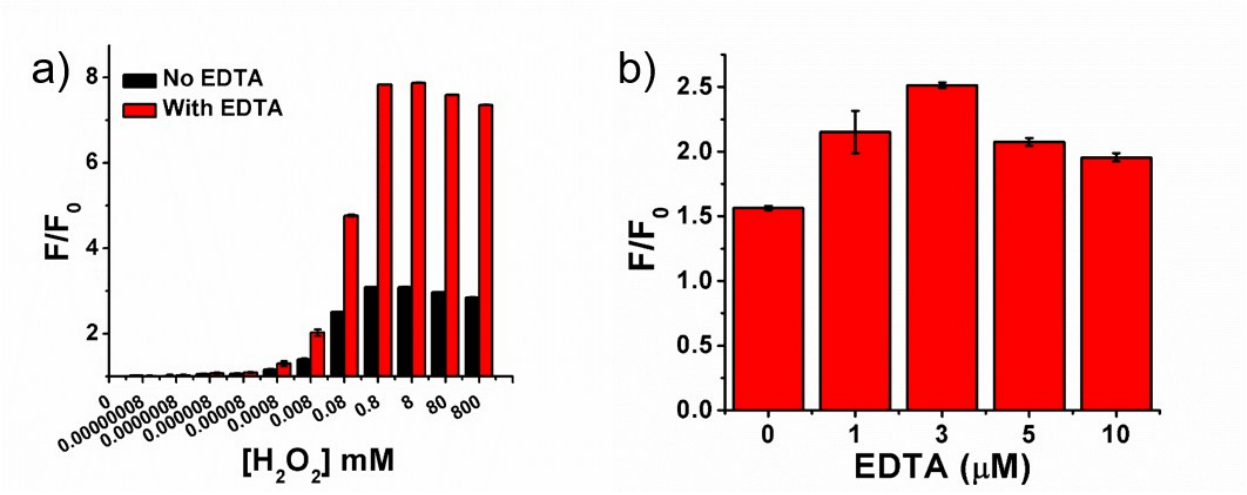


Figure S7. a) Fluorescence ratio change of 1% ZnS/CuS-enclosed microgel upon adding H_2O_2 with and without the presence of EDTA. [EDTA] = 1 μM. b) Fluorescence enhancement ratio (F/F_0) in response of H_2O_2 with different EDTA concentration. [H_2O_2] = 80 μM.

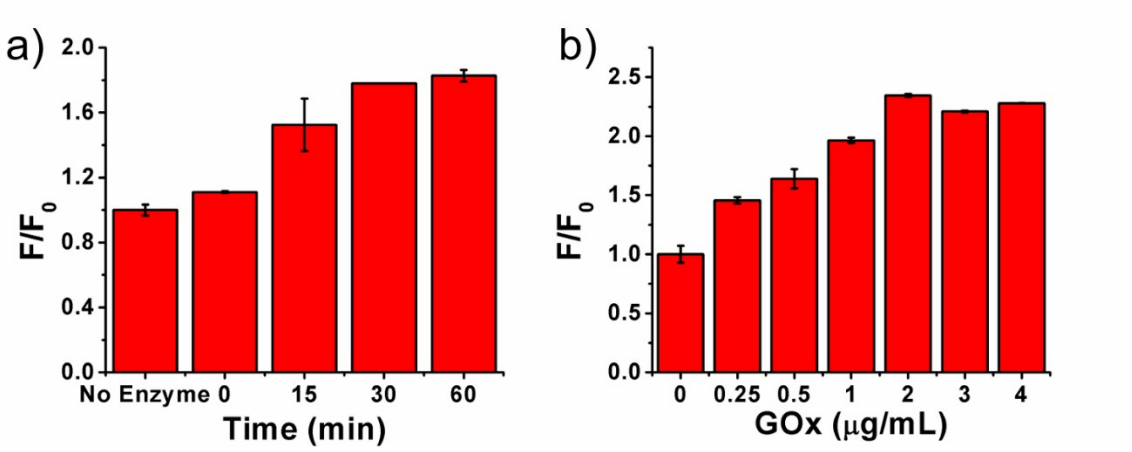


Figure S8. Time and enzyme concentration optimization using 100 μM glucose. a) Fluorescence ratio change of 1% ZnS/CuS-enclosed microgel with different enzyme reaction time. [GOx] = 1 μg/mL b) Fluorescence ratio change of 1% ZnS/CuS microgel with different GOx concentration, reaction time = 30 minutes.

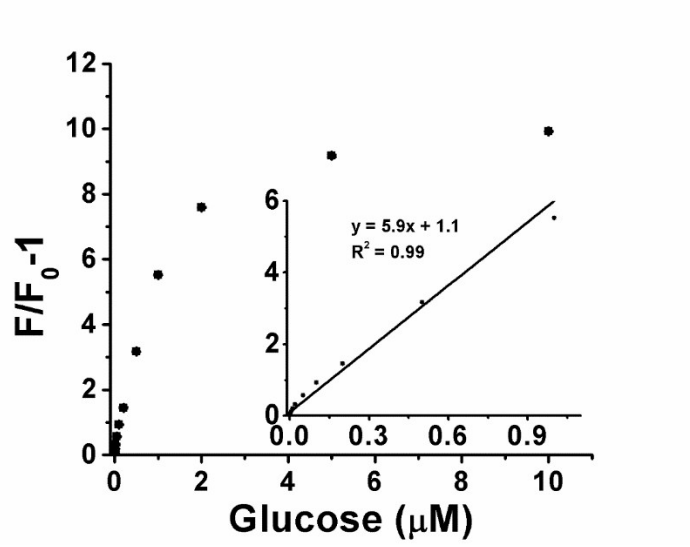


Figure S9. Calibration curve of glucose. Total [Zn^{2+}] in both microgel and ZnS NP = 1.5 μM, [Fluozin-3] = 3 μM, 1×PBS buffer, pH = 7.4. F_0 is the fluorescence before adding H_2O_2, F is the fluorescence after adding H_2O_2.

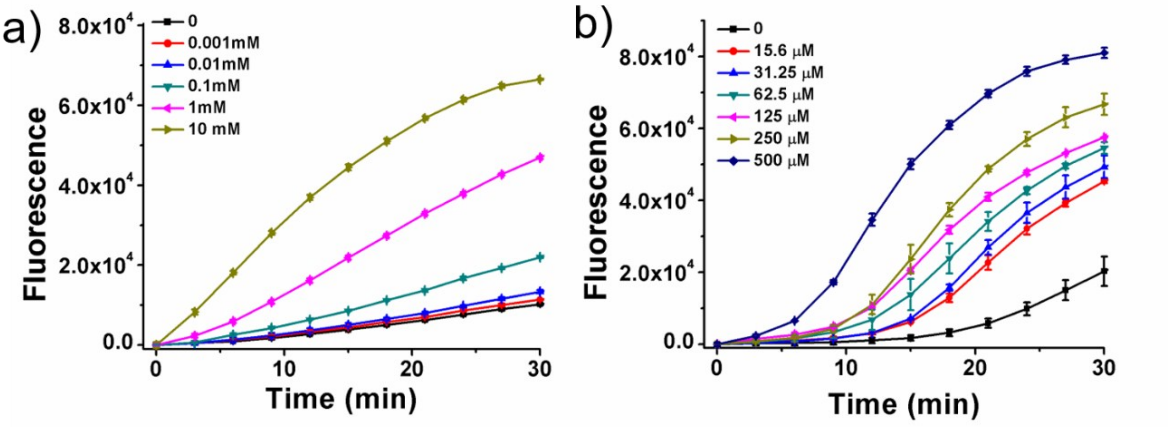


Figure S10. Continuous monitoring of GOx activity with different concentrations of (a) glucose, from 0 to 10 mM and (b) cholesterol, from 0 to 500 μM.

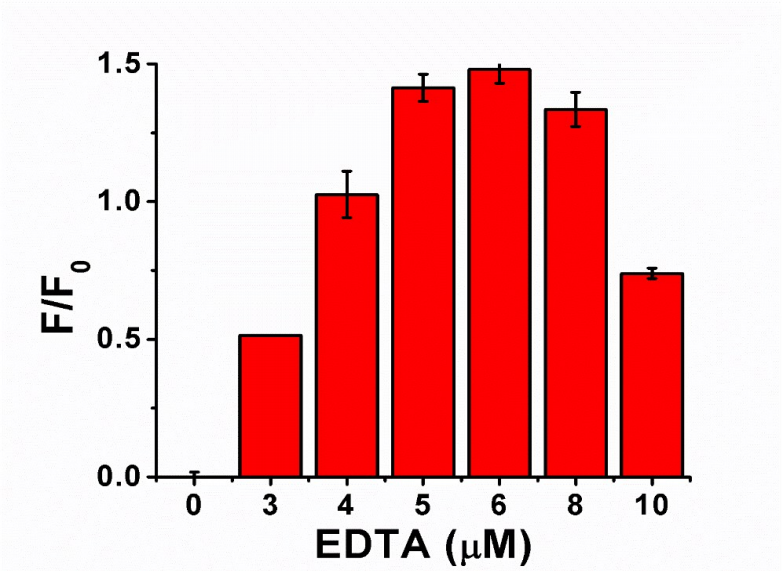


Figure S11. EDTA optimization for cholesterol detection. 5% TX-100 was used to dissolve the cholesterol.

3. Supporting Tables

Table S1. Comparison of our method to other methods using fluorescence as signal output.

Methods	Dynamic Range (μM)	LOD (μM)	Reference
graphene quantum dots	1.11-300	0.32	4
Carbon dots	0-400	0.01	5
MnO_2 nanosheet	0-150	0.9	6
Polymeric nanoprobe	0-500	0.76	7
Gold Nanocluster	1-100	0.8	8
ZnS/CuS Microgel	0-8,000	0.08	Our work

Table S2. Real serum sample tests. Cholesterol esterase was added to hydrolyze cholesterol ester in serum and produce cholesterol before measurement with the microgel and ChOx.

Sample	Product Info. (Glucose mM)	This method (Glucose mM)	RSD
1	5.83	6.93	4.44%
2	25.4	25.86	1.79%
3	2.94	3.74	6.53%
4	16.11	17.61	4.07%
5	10.39	11.44	6.57%
Sample	Product Info. (Cholesterol mM)	This method (Cholesterol mM)	RSD
6	1.94	2.03	1.79%
7	3.37	3.87	1.08%
8	6.14	5.45	8.55%
9	10.98	11.08	1.08%
10	8.8	8.13	6.92%

4. References

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