Supporting Information

Endogenous H_2S-activated Ag nanoparticle embedded in programmed DNA-cube for specific visualization of colorectal cancer cells

Wenhui Chen,[a] Wei Mao,[a,b] Yuqi Yin,[a] Ziyu Ma,[a] Meiqi Song,[a] Zixiao Ma,[a] Tingting Li,[a] Jia Zhu,[a] Chang Liu,[c] Hui Yu,[d] Sheng Tang, [*a,e] Wei Shen[*a]

[a]School of Environment and Chemical Engineering, Jiangsu University of Science and Technology, Zhenjiang 212003, Jiangsu Province, PR China

[b]Central-southern Safety&Environmental Technology Institute Co. Ltd., Wuhan 430071, China

[c]School of Grain Science and Technology, Jiangsu University of Science and Technology, Zhenjiang 212003, Jiangsu Province, PR China

[d]Department of Thoracic Surgery, Affiliated Hospital of Jiangsu University, No. 438, Jiefang Road, Zhenjiang 212000, Jiangsu Province, PR China

[e]College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, China

1. Reagents, materials and instruments

The sequence of the designed oligonucleotide is shown in Table S1, which was ordered from Shanghai Sangon Co., Ltd. (Shanghai, China) and purified by high-performance liquid chromatography. Ethylenediamine tetraacetic acid disodium salt (EDTA•2Na), Tris (hydroxymethyl) aminomethane (Tris), magnesium chloride ($MgCl_2$) were purchased from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Sodium sulfide (Na_2S) and sodium citrate were purchased from Maclin Biochemical Technology Co., Ltd. (Shanghai, China). Sodium borohydride ($NaBH_4$) and silver nitrate ($AgNO_3$) are purchased from Yonghua Chemical Technology Co., Ltd. (Suzhou, China). Hydrochloric acid (HCl) was purchased from Suyi Chemical Reagent Co., Ltd. (Shanghai, China). Agarose was purchased from Gene Biotechnology International Trade Shanghai Co., Ltd. (Shanghai, China). phosphate buffered saline (PBS) and RPMI-1640 medium were purchased from Jiangsu Kaiji Technology Co., Ltd. (Jiangsu, China). The reagents used in the experiment are all analytical grade, and all solutions are prepared by deionized water with resistance of 18.2 MΩ·cm.

The magnetic stirrer (LC-DMS-H) was purchased from Lichen Instrument (Technology Co., Ltd. Shenzhen, China). The pH meter (SX-610) was purchased from Shanghai Sanxin Equipment Co., Ltd. (Shanghai, China). Real-time fluorescence quantitative Polymerase chain Reaction instrument (Gentier 96, Tianlong Technology Co., Ltd. Xi'an, China) for DNA-cube annealing. Fluorescence spectrophotometer (Nanodrop 3300, Thermo Fisher Technology Co., Ltd. Shanghai, China) was used to record changes in fluorescence in solution systems. The inverted fluorescence microscope (IX73, Olympus Trading Co., Ltd. Shanghai, China) was used to record fluorescence images of various cell. Microplate reader (SpetraMax i3, Meigu Molecular Instrument Co., Ltd. Shanghai, China) was used to obtain cytotoxicity data. Gel electrophoresis Instrument (JY300, Junyi Dongfang Electrophoresis Equipment Co., Ltd. Beijing, China) was used to verify the formation of DNA-cube. The morphologies of the nanomaterials were characterized by a Tecnai 12 transmission electron microscope (TEM) (Philips, Amsterdam, The Netherlands).

2. Synthesis of Ag NP@DNA-cube

2.1 Synthesis of silver nanoparticles (Ag NPs)

The synthesis method of 25 nm Ag NPs was referred to previous reports. In brief, Ag seeds were first synthesized. A mixture of 20 mL 1% sodium citrate solution and 75 mL deionized water was heated to 70 ℃. Subsequently, under vigorous stirring, 1.7 mL of 1% $AgNO_3$ and 2 mL of cold 0.1% $NaBH_4$ solution were added to the mixture. The temperature was maintained at 70 ℃ for 1 h. The resulting Ag seeds solution was then utilized in the subsequent step. A mixture of 2 mL 1% sodium citrate and 75 mL deionized water was heated to boiling, followed by the addition of 10 mL Ag seeds solution and further supplemented with an additional amount of $AgNO_3$ (1.7 mL). The reaction continued for another hour before introducing another portion of $AgNO_3$ (1.7 mL) and sodium citrate (2 mL). The entire reaction process concluded after 1 h.

2.2 Preparation of Ag NP/ssDNA composite

First of all, 200 μL of the Ag NPs solution was centrifuged to remove the supernatant and keep its precipitate. Secondly, 50 μL 2 μM of Ag-1, Ag-2, Ag-3 and Ag-4 (collectively referred to as ssDNA) were evenly mixed in a microcentrifuge tube. After that, the precipitate was uniformly dispersed in the solution of ssDNA and incubated at room temperature for 2 h. During the incubation process, the sulfhydryl group modified at one end of ssDNA forms a strong Ag-S bond with Ag NPs to form Ag NP/ssDNA complex with four ssDNA attached to its surface. Finally, the reactants were centrifuged at 5000 rpm for 5 min to remove the ssDNA without Ag NPs in the supernatant, and the obtained Ag NP/ssDNA composite was used in the next experiment.

2.3 Formation of DNA-cube

DNA-cube was prepared in a brown centrifuge tube. A total of 30 oligonucleotides were designed to form DNA-cube, and each side was composed of 4-6 oligonucleotides partially paired with complementary bases. Each oligonucleotide was prepared with TE buffer solution (1 mM EDTA•2Na, 12.5 mM $MgCl_2$, 40 mM Tris-HCl, pH = 8), mixed in equal molar ratio, and then annealized by the following procedures: 95 ℃-70 ℃,

when the temperature drops by 5 °C, keep it there for 5 min; 70 °C-50 °C, when the temperature drops by 2 °C, keep it there for 10 min; at 50 °C to 20 °C, when the temperature drops by 1 °C, keep it there for 15 min.

2.4 Preparation of Ag NP@DNA-cube

Ag NP/ssDNA composite was redispersed in 50 μL DNA-cube solution, and it was evenly distributed in the solution by gentle shaking. In order to ensure that the four kinds of ssDNA on the surface of Ag NPs were completely complementary to the sticky ends on the DNA-cube, the brown centrifuge tube containing the reaction solution was incubated overnight in a water bath at 37 °C, and finally the target structure is formed.

3. Gel electrophoresis validation of DNA-cube

DNA-cube formation was verified using a 1% agarose gel. The programmed 30 oligonucleotides were configured into 200 nM solution using tris(hydroxymethyl)methyl aminomethane-ethylene diamine tetraacetic acid buffer solution. Take the top surface of the DNA-cube, U1+U2, U1+U2+U3, U1+U2+U3+U4 were added to the three centrifuge tubes respectively, and the volume of each oligonucleotide was maintained at 20 μL. Then the three centrifuge tubes were homogenized and incubated in a 37 °C water bath for 2 h to form the designed structure. Similarly, oligonucleotides forming other faces of DNA-cube were added successively to different centrifuge tubes for incubation.

4. Fluorescence imaging in living cells

After adding Ag NP@DNA-cube to the cell culture dish, gently shake and incubate with cells for 12 h. After incubation, slowly aspirate the medium from the dish along the edges using a pipette, and carefully wash HCT 116 cells once with 1 mL of PBS before discarding the washing solution. Finally, add 1 mL of PBS to the dish to maintain cell morphology before observing under an inverted fluorescence microscope.

5. Cytotoxic activities experiment

A 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit was

used to investigate the effect of materials at different stages on cell activity in this experiment. Ag NPs, Ag NP/ssDNA and Ag NP@DNA-cube were incubated with HCT 116 cells at 37 ℃ for 24 h, then 0.5 mg/mL of MTT reagent was added and incubated for 4 h. After the reaction, the medium was carefully absorbed and 100 μL Formazan solution was added. Then, the medium was slowly shaken for 10 min, so that the Formazan solution evenly covered the bottom of the holes in the 96-well plate. Finally, the absorbance value at 570 nm was recorded using an enzyme-labeled instrument.

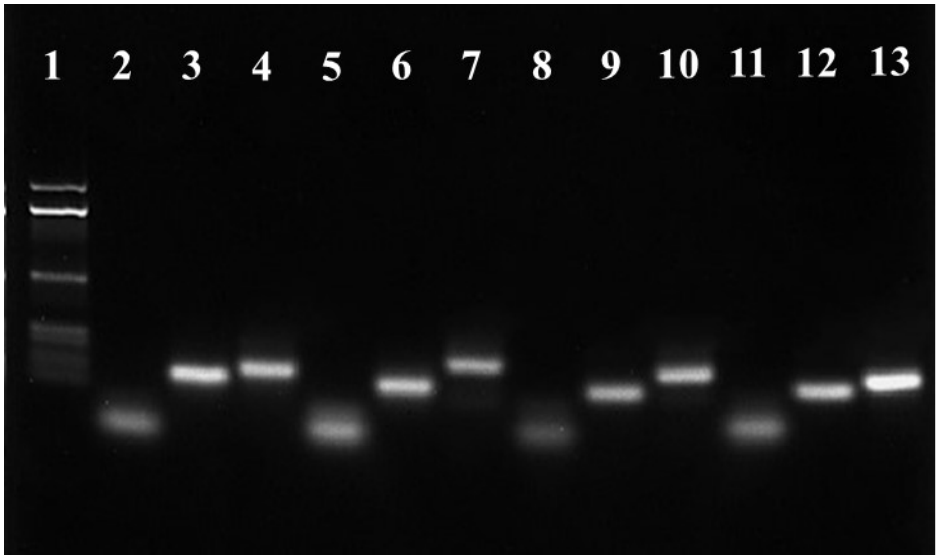

Fig. S1. Agarose gel electrophoresis. Lane 1: DNA Ladder Marker, Lane 2: Ag-1, Lane 3: D5, Lane 4: Ag-1+D5, Lane 5: Ag-2, Lane 6: F5, Lane 7: Ag-2+F5, Lane 8: Ag-3, Lane 9: R6, Lane 10: Ag-3+R6, Lane 11: Ag-4, Lane 12: L5, Lane 13: Ag-4+L5

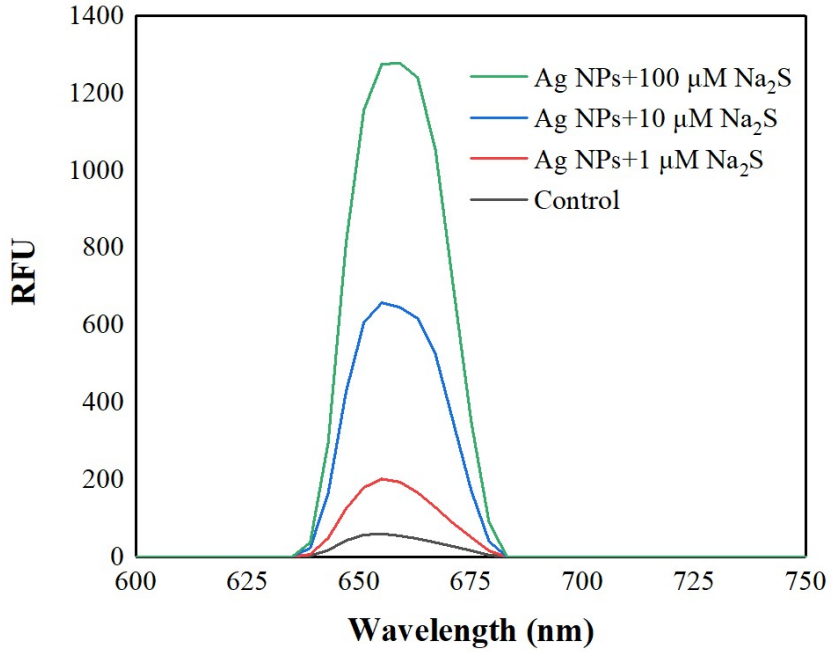

Fig. S2. Fluorescence spectrum of Ag NPs incubated with (h) 0 µM, (i) 1 µM, (j) 10 µM and (k) 100 µM Na_2S (H_2S mimic).

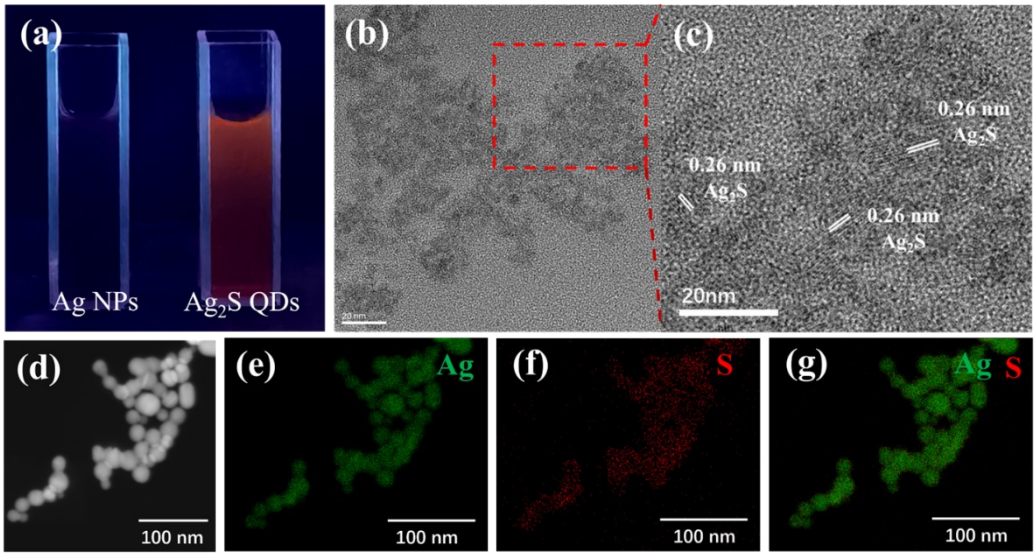

Fig. S3. (a) Images of Ag NPs (left) and Ag NPs etched by 100 μM Na_2S under ultraviolet light irradiation (right), (b and c) HR-TEM image of Ag NPs etched by 1 mM Na_2S, (e)-(g) Elemental mapping of Ag NPs etched by 100 μM Na_2S.

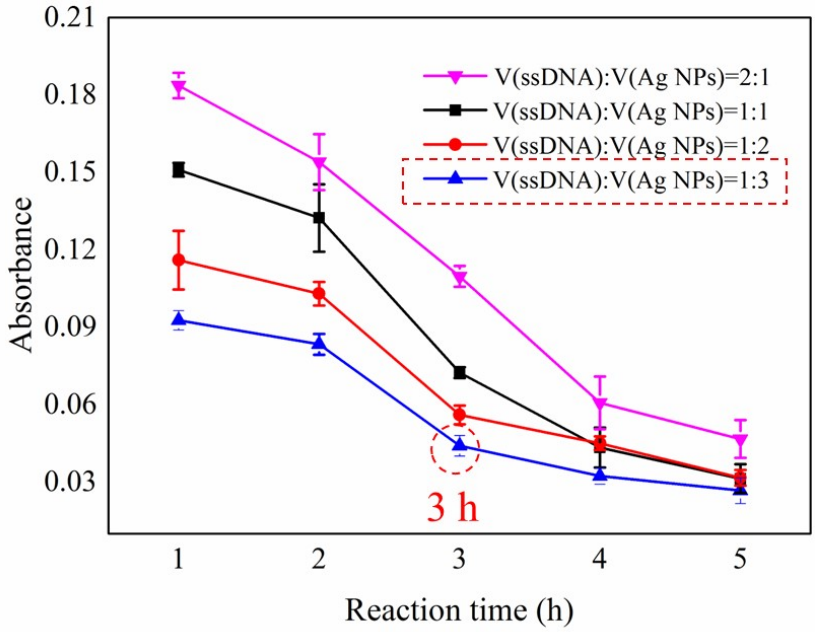

Fig. S4. Optimization of Ag NPs and ssDNA volume ratio and reaction time. The error bars represent the standard deviations (n = 3).

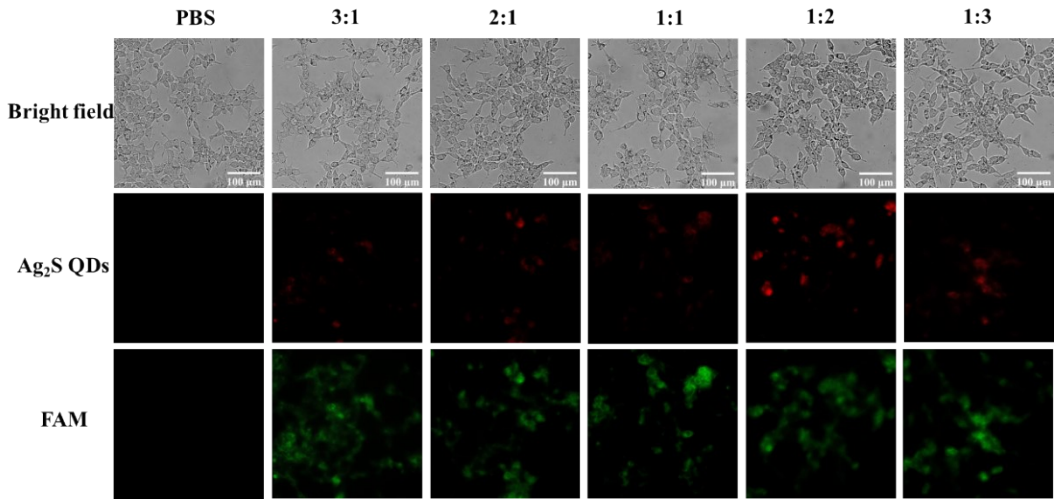

Fig. S5. Optimization of DNA-cube and Ag NP/ssDNA volume ratio. (Scale bar = 100 μm)

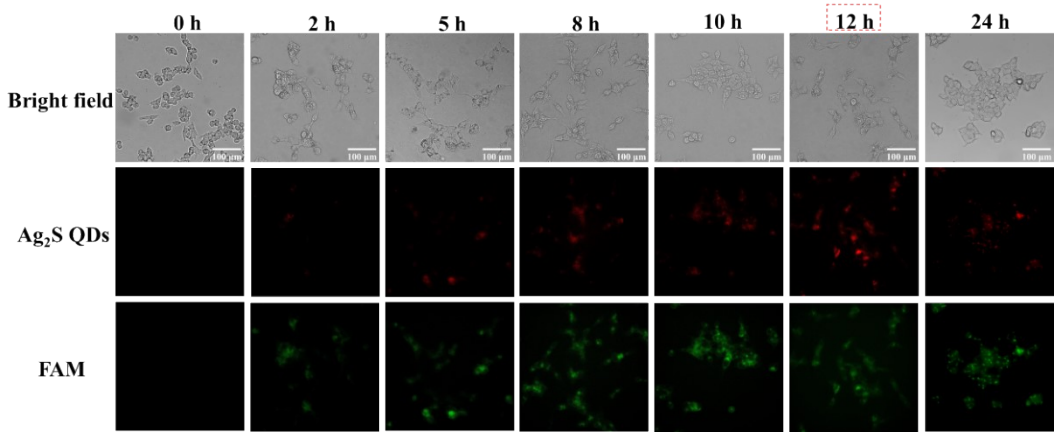

Fig. S6. Optimization of incubation time between Ag NP@DNA-cube and HCT 116 cells from 2 to 24 h. (Scale bar = 100 μm)

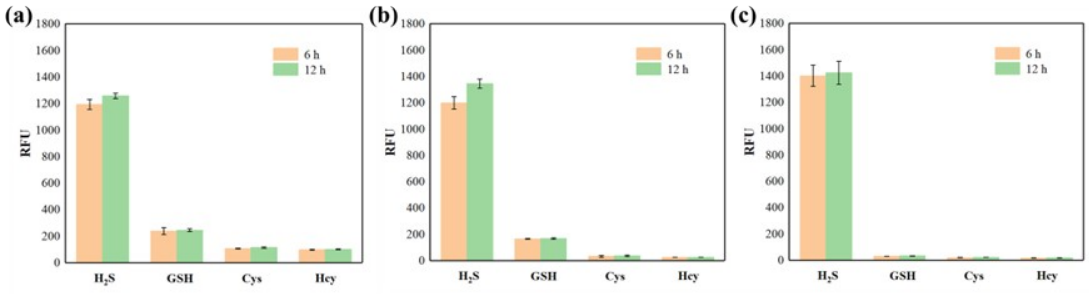

Fig. S7. Fluorescence intensity of (a) Ag NP, (b) Ag NP@DNA-cube and (c) H_2S-specific probe (WSP-5) induced by H_2S, glutathione (GSH), cysteine (Cys) and homocysteine (Hcy) (100 μM) at different reaction times.

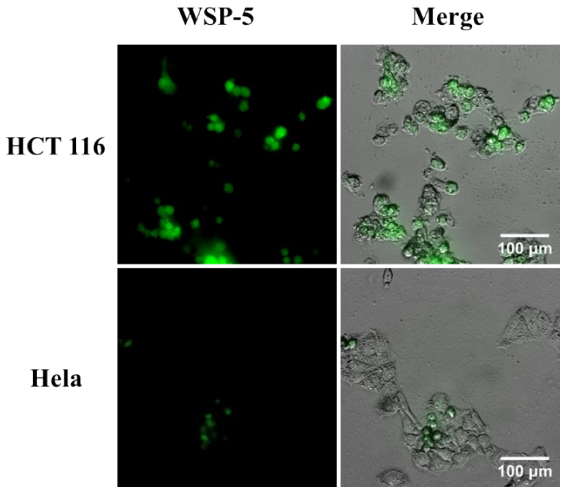

Fig. S8. Fluorescence imaging of WSP-5 probe in HCT 116 cells and Hela cells.

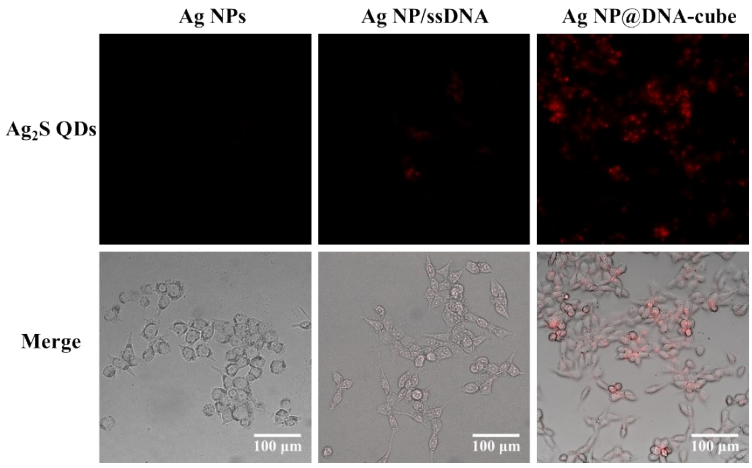

Fig. S9. Fluorescence images of HCT 116 cells after incubation with Ag NPs, Ag NP/ssDNA, and Ag NP@DNA-cube for 12 h, respectively. Scale bar = 100 μm.

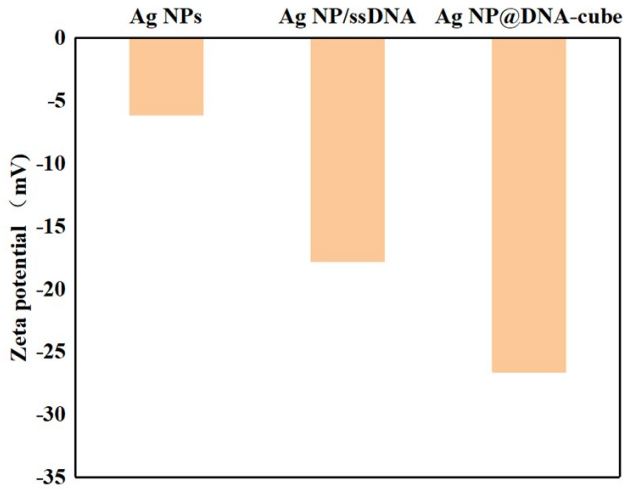

Fig. S10. Zeta potential of Ag NPs, Ag NP/ssDNA, Ag NP@DNA-cube.

Table S1. All nucleic acid sequences in the paper

Oligonucleotides	Sequence (from 5' to 3')
L1	FAMCCATGTTCAAGCTGCATTAAACCAATCATGATTACAATCTGACTTGGGAATCTACGCAATTATCACAACTCTCGCG
L2	TGCAGCTTGAACATGGAACCGTTGACTCCTAACTATTATAAGTGTACATTAGGTCGCTCATTCGAGGGTCTTGCAA
L3	CAGTGAACCTGTGATCAAAATTGCAAGACCCTCGAA
L4	GATCACAGGTTCACTGAAGGTTCCAGTCTCACTTATTGGAACGTATTTACCAGTATCGATCCCAT
L5	AGCGCTAGTACATTGGCCTAGTTACGGCATATCTGACTTGGGAATCTCGGATCATGGGATCGATACTGG
L6	TACCTAAGCTCAGGCTATACCACACTTATTGGAACGTCGCGAGAGTTGTGATA
R1	ATACGGTAACGGCATCTAACGTCATTATGCGTTCGAATCCGTATGATAACGGCAGGTCGATTCTAGCACT
R2	GATGCCGTTACCGTATTTGCTAGATTTCAGATTTTAAAGCGGCTGATTCTATTGGCAGCTGCATAG
R3	AAGCTTGCGACTGAGACCCCAGTGCTAGAATCGACC
R4	TCTCAGTCGCAAGCTTAGGATCGGCAATTCGACTCAGGAGGTTTGCTCGCATGGAATCACGT
R5	ATGAGCTACCATGGATAGGGTAATGCACGTGATTCCATGCGA
R6	GGTCCAAATGTCGATGCCTAGTTACGGCATATAAATGGCAAGGTCATTCGCGAACTTCCTATGCAGCTGCCAAT
U1	CCTTGATTGCCAACTGTCGTTCCAATAAGTGTGCTGGAGATAAGGTCTTGGTCACAGACTTCGCATACGGATTTCG
U2	CGGTAGTCCTTTAACGGCATTACCCTATCCATGGTAGCTCATTGAACGCTTATGCGTTCGCGAAATCCGTATGCGA
U3	CGTTAAAGGACTACCGGCAAACCTCCTGAGTCGGAACGCTTATGCGTTCGCAGAATTGGCTTGATCTCCGAACTGC
U4	CAGTTGGCAATCAAGGATTGCGTAGATTCCCAAGTCAGATTGTATCTGGAGATAAGGTCTGCAGTTCGGAGATCAA
D1	ATTCACGGTTAGCCACTTGTGGAGTTAAAAGGCAGTTATAAATAATCTGAAATCTAGCAAGAATCGTCGTTAGGTC
D2	GTGGCTAACCGTGAATTTACCAAGTATAAGCCTACTGGAACCTTCGGCAGCAATGTTGCACGTGTCCAAGAGGATT
D3	AGCTGGCTTACCGTTAATAGAAAGGTACAACTCAGCTAGCGTACATGTAATCCAGCGAGTAATCCTCTTGGACA

	CG
D4	TTACCGTAAGCCAGCTAGCTACTTTACATTCCAATGC TCTGAGCCGTATCAAC
D5	AACGCTGGTACTTTCGCCTAGTTACGGCATATACGGA TTCGAACGCATAATGACGTTAGACCTAACGACGATTC
F1	AAACTGGAACCCTCGTCGGAGCAAGGCCGATCCTAG CCGTTAACTTGCGAATACGCAAGCTAGGGCACATACC GAA
F2	ACGAGGGTTCCAGTTTATGCGGTGCCGTTATCATGGA ATGTAAAGTAGCTCCTTTCTATGAAGCCG
F3	TGTACTGGCTCGAGTTAATTTGGTTAGTACTAATAGA CCTCTATTCCAGACGGTTAAGACTTCGGTATGTGCCC TA
F4	AACTCGAGCCAGTACATTGGCAACTGAGGATTGATA ATATTCAATGCTCTGAGCCGTATCAAC
F5	TGATCGATGATCTCGGCCTAGTTACGGCATATACGCT AGCTGAGTTGTACCTTTCTATCGGCTTCATAGAAAGG
B1	GAACTGTTTGCGTGCAAGAATCAGCCGCTTTAAATTT ATAACTGCCTTTTAACTCCACAACTGAACTAAGTGCC GT
B2	TGCACGCAAACAGTTCGGAGTTCGCGAATGACCTTGC CATTTACTTGCGAATACGCAAGCGTGGATCATGCGTG AA
B3	CTTGAACCGGTACTGTGAGATTCCCAAGTCAGAGACC TCTATTCCAGAACCAGTGTCTGATTCACGCATGATCC AC
B4	ACAGTACCGGTTCAAGAATCTAAATACGTTCCAATAA GTGAGCTAATCCGAGAAGCGCTGACGGCACTTAGTTC AG
Ag-1	CGAAAGTACCAGCGTTAGCAA-SH C3
Ag-2	CCGAGATCATCGATCAAGCAA-SH C3
Ag-3	CATCGACATTTGGACCAGCAA-SH C3
Ag-4	CCAATGTACTAGCGCTAGCAA-SH C3

Table S2. Comparison of fluorescence imaging methods for colorectal cancer cells

Target	Probe[a]	Imaging methods	Application	Ref.
H_2S	NIR-II@Si	H_2S-activated ratiometric fluorescence and light-up NIR-II emission	Cell/mouse imaging	1
Mitochondrial membrane potential difference	TPE-IQ-2O	Aggregation-induced emission luminogen	Tissue section	2
β-catenin/anti-E-cadherin	AuNPs	Targeted cancer contrast agent based on antibody/AuNPs	Cell	3
TF antigen	Lectin-immobilized fluorescent nanospheres	Lectin immobilized nanospheres loaded with coumarin 6	Cell/mouse	4
Human β-gal	SGG	A dual-recognition unit for discriminating of human colorectal neoplasms	Cell imaging	5
microRNA-21	AuHCNs-HA	Fluorescence resonance energy transfer of gold nanoparticles activated by miRNA-21	Cell	6
β-glucuronidase (GLU)	RN-GLU	A lysosome targetable two-photon ratiometric fluorescent probe	Cell	7
H_2S	DBT	H_2S-activated NIR probe	Cell	8
H_2S	Ag NP@DNA-cube	H_2S-activated dual fluorescent nanoplatform	Cell	This work

[a]TPE-IQ-2O: mitochondrion-targeted AIE luminogen; SGG: a dual-recognition two-photon fluorescent probe; AuHCNs-HA: hyaluronic acid-functional gold-nanodot-decorated hollow carbon nanospheres; RN-GLU: enzyme-activated ratiometric two-photon fluorescent probe; DBT: 8-Bromo-10-(4-(((2,4-dinitrophenyl)sulfonyl)oxy)phenyl)-2-(4-(diphenylamino)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,2]diazaborinin-4-ium-5-uide

References for Supporting Information only

1. G. Xu, Q. Yan, X. Lv, Y. Zhu, K. Xin, B. Shi, R. Wang, J. Chen, W. Gao, P. Shi, C. Fan, C. Zhao and H. Tian, *Angew. Chem. Int. Ed.*, 2018, **57**, 3626-3630.
2. J. Shen, K. Tao, P. Gu, C. Gui, D. Wang, Z. Tan, L. Wang, Z. Wang, A. Qin, B. Tang and S. Bao, *Sci. Chin. Chem.*, 2020, **63**, 393-397.
3. K. M. G. Lima, R. F. Araujo, A. A. Araujo, A. Oliveira and L. H. S. Gasparotto, *Sen. Actuators B Chem.*, 2014, **196**, 306-313.
4. S. Sakuma, T. Yano, Y. Masaoka, M. Kataoka, K. Hiwatari, H. Tachikawa, Y. Shoji, R. Kimura, H. Ma, Z. Yang, L. Tang, R. Hoffman and S. Yamashita, *J. Control. Release*, 2009, **134**, 2-10.
5. H. W. Lee, C. S. Lim, H. Choi, M. K. Cho, C. K. Noh, K. Lee, S. J. Shin and H. M. Kim, *Anal. Chem.*, 2019, **91**, 14705-14711.
6. H. Xiao, H. Fan, L. Xu, Z. Pei, S. Lei, J. Xu, J. Xi, G. Wang, L. Wang and Z. Wang, *Chem. Commun.*, 2019, **55**, 12352-12355.
7. X. Wei, J. Li, X. Yang, B. Dong, B. Geng, Z. Li, X. Hu, B. Ding, J. Zhang and M. Yan, *Anal. Chim. Acta*, 2022, **1192**, 339354.
8. Q. Li, Z. Wang, M. Zhao, Y. Hong, Q. Jin, S. Yao, C. Zheng, Y. Quan, X. Ye and Z. Huang, *Sen. Actuators B Chem.*, 2019, **298**, 126898.