

Electronic Supporting Information

Biomaterialized Nanoparticles Enable an Enzyme-assisted DNA

Signal Amplification in Living Cells

Mengyi Xiong,^a Meng Zhang,^a Qin Liu,^a Chan Yang,^a Qingji Xie,^c Guoliang Ke,^{a,*} Hong-Min Meng,^{b,*} Xiao-Bing Zhang^{a,*} and Weihong Tan^a

^aMolecular Science and Biomedicine Laboratory, State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Collaborative Innovation Center for Chemistry and Molecular Medicine, Hunan University, Changsha 410082. P. R. China.

^bInstitute of Chemical Biology and Clinical Application at the First Affiliated Hospital, Henan Joint International Research Laboratory of Green Construction of Functional Molecules and Their Bioanalytical Applications, College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou 450001, P. R. China

^cKey Laboratory of Chemical Biology and Traditional Chinese Medicine (Hunan Normal University), Ministry of Education, Changsha 410082. P. R. China.

* To whom correspondence should be addressed.

E-mail: xbzhang@hnu.edu.cn; glke@hnu.edu.cn; hmmeng2017@zzu.edu.cn

Experimental Section

Chemicals and Materials

All the DNA oligonucleotides (Table S1, Supporting Information) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). 2-methylimidazole (MIM) was purchased from Energy Chemical (China). Exonuclease III and exonuclease I were obtained from Takara Bio (Beijing, China). All solutions used in the experiments were prepared using ultrapure water (resistance >18 MΩ cm), which was obtained through a Millipore Milli-Q ultrapure water system (Billerica, MA, USA). Unless

otherwise specified, all other reagents used in this work were of analytical grade, commercially purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and used without further treatment.

Table S1. The sequences used in this work.

| Name | Sequence 5' to 3' |
|-------------------|--|
| MB-FAM | TTTTTTTTTTGTCAGTGA/iBHQ1dT/GCTAACCTGGGGGAGTC CATGTGTAGAACTCCCCCAGGTTAGC-FAM |
| MB-21 | Cy3-ATCAGAC ACTATTCA GTCTGAT/BHQ2/AA GCT A |
| MB-21-F | FAM-ATCAGAC ACTATTCA GTCTGAT/BHQ1/AA GCT A |
| Target | TAG CTT ATC AGA CTG ATG TTG A |
| SM miR-21 | TAG CTT ATA AGACTG ATG TTG A |
| TM miR-21 | TAG CTT ATA ACCCTG ATG TTG A |
| MB-GFP | Cy3-AAGCGCG CATGTGATCGCGCTT/BHQ2/CTC GTT G |
| Target-GFP | CAA CGA GAA GCG CGA TCA CAT GG |

Preparation of MOF, protein@MOF, EMOF-DNA nanoparticles

All the MOF and protein@MOF nanoparticles were prepared according to published methods with slight modification.¹ 450 μ L MIM (3.5 M) aqueous solution, 0.25 mg BSA, BSA-FITC, Exo III (2000U) doped BSA (0.23 mg) were mixed together and incubated at RT for 10 min. The reaction turned cloudy immediately with the addition of 50 μ L Zn acetate (0.45 M). The above mixture was incubated for another 10 min to allow the growth of protein@ZIF-8 nanoparticles. The products were collected by centrifugation (3500 rpm, 20 min), then washed by methanol, water, and 5% SDS solution, and then resuspended in water for further use.

To fabricate the EMOF-DNA nanosensor, 2 μ L Exo III/BSA@MOF (EMOF)

nanoparticles (10 mg/mL) were incubated with 1 μ M DNA sensors for 30 min.

Characterization of the nanoparticles

After centrifugation, washing, and resuspension, the resulting biomineralized particles were characterized by using JEOL transmission electron microscopy (TEM, JEM-2010, JEOL, Japan) and dynamic light scattering (DLS). The zeta potential was determined by dynamic light scattering (DLS, Nano-ZS90, Malvern). The decomposition of BSA@ZIF-8 NPs was determined by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer Optima 5300DV). Powder X-ray diffraction (PXRD) data was collected on the Rigaku Miniflex II diffractometer with a scan speed of 5°/min and a step size of 0.05° in 2 θ at room temperature.

Fluorescent measurements

The fluorescent data was collected on a fluorometer (Edinburgh Instruments Ltd, FS5, UK) with both excitation and emission slits of 2 nm.

Activity of released Exo III: To test the activity of Exo III released from EMOF, 1 μ L EMOF (10 mg/mL) was incubated in 4 μ L PBS at pH 5.5 or 7.4 for 30 min, then 10 μ L Exo III buffer and MB-FAM with a final concentration of 10 nM were added to make a total volume of 100 μ L. Ex = 488 nm, Em = 500-650 nm.

Fluorescence titration of target: To study the response of this DNA sensor to different concentrations of target, 100 nM MB-21-F and 0.02 U/ μ L Exo III was incubated with various concentrations of target (0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 50.0 nM) for 1 h at 37°C. Then the fluorescence was

measured. Ex = 488 nm, Em = 500-650 nm.

Protein loading efficiency: To study the protein loading efficiency (L_e), 0.23 mg BSA-FITC and 2000 U Exo III was added to 500 μ L PBS solution followed by recording the fluorescence intensity (F_{sol}). And the same amount of protein was also added in the nanosystem of FITC-BSA/Exo III@ZIF-8 preparation, then the FITC-BSA supernatant was collected by centrifugation and measured the fluorescence intensity (F_{sup}).

$$L_e = (F_{sol} - F_{sup}) / (F_{sol}) \times 100\%.$$

$$\text{Loading amount } (L_a) = L_e \times 2000 \text{ U/M}$$

Where M is the total weight of NPs.

DNA loading efficiency: 30 μ L of 10 μ M MB-21 was added to 20 μ L buffer or EMOF (10 mg/mL), then the MB-21 solution and the supernatant were collected by centrifugation. The MB-21 in above solutions was digested with DNase I (1 U/mL) at 37°C for 1 h followed by fluorescent measurement (F'_{sol} and F'_{sup} , respectively). The loading efficiency (L'_e) and loading amount (L'_a) were calculated with following formulas:

$$L'_e = (F'_{sol} - F'_{sup}) / (F'_{sol}) \times 100\%.$$

$$L'_a = L'_e \times 3 \times 10^{-10} \text{ mol/M'}$$

Where M' is the weight of NPs used here.

Protection of EMOF to DNA: To investigate the protection of EMOF to DNA strand, the fluorescent kinetics of MB-21 or EMOF-DNA in the presence of DNase I was studied. DNase I (1 U/mL) was added to 10 nM MB-21 DNA or 2 mg/mL EMOF-DNA followed by recording the kinetics with a time interval of 1 min.

Exo III activity under different pH: To study the catalytic activity of Exo III in buffers with different pH. 40 U Exo III was added to 10 μ L Tris-HCl buffer with various pH (pH 4.5, 5.5, 6.5, 7.5, 8.5, 9.5, and commercial Exo III buffer) for 0.5 h, then above solutions were added to 1 \times Exo III buffer containing 100 nM MB-21 and 50 nM target. After an incubation time of 1 h, the fluorescent intensity was measured, then relative intensity was analyzed by using the sample in commercial Exo III buffer as standard.

Optimization of Exo III concentration: To optimize the concentration of Exo III, various amount of Exo III (0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 U/ μ L) was added to a solution containing 100 nM MB-21-F and 50 nM target. Then the fluorescence was measured after an incubation time of 1 h.

Gel Electrophoresis Analysis.

Reaction mixtures were finally quantified in a volume of 10 μ L for electrophoresis experiments directly with 12% polyacrylamide gel electrophoresis (PAGE). Electrophoresis was carried out in 1X Tris-acetic-EDTA (TAE) buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesium acetate, pH 8.0) at 80 V for 10 min and 120 V for another 30 min. The gels were imaged and analyzed using a

Bio-Rad ChemiDoc XRS System after stained with Gel green.

Cell culture

The HeLa cells (human cervical carcinoma cell line) and L02 (normal human hepatic cell line), obtained from American Type Culture Collection (ATCC), were cultured in DMEM medium with the addition of 10% FBS (fetal bovine serum, Invitrogen, Carlsbad, CA, USA) and 0.5 mg/mL penicillin-streptomycin (KeyGEN Biotech, Nanjing, China) in a 5% CO₂ environment at 37 °C.

Cell transfection

3×10³ HeLa and L02 cells were seeded in optical dishes 24h before transfection. GFP plasmid (Clover2-N1 from Addgene #54537) was transfected into HeLa cells with Lipofectamine 3000 following the protocol provided by the manufacturer.

Confocal laser scanning microscopy (CLSM) analysis

1×10⁵ HeLa or L02 cells were seeded in a 35-mm confocal laser culture dish for 24 h. The cells were washed 3 times with DPBS buffer, then incubated with 200 µL medium containing 100 µg/mL FITC-BSA@ZIF-8 or BSA@ZIF-8-DNA (MOF-DNA) or Exo III/BSA@ZIF-8-DNA (EMOF-DNA) mixture for a certain duration in the incubator. Then the cells were washed for another 3 times with DPBS to remove the excess mixture. The samples were analyzed using a Zeiss LSM 880 confocal microscope (Olympus). Channels for FITC, Cy3, and LysoTracker Red were chosen. Images were analyzed with ZEN (blue edition) software and quantified by ImageJ.

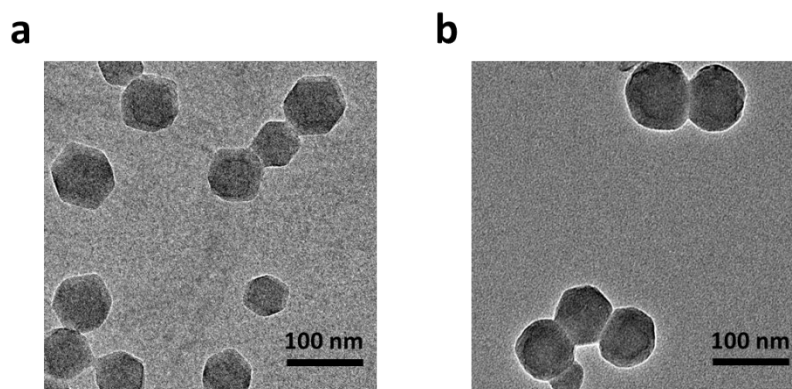


Figure S1. The TEM results of ZIF-8 and BSA@ZIF-8 with an average size of 70.03 ± 8.86 nm and 74.55 ± 4.96 nm correspondingly. Scale bar = 100 nm.

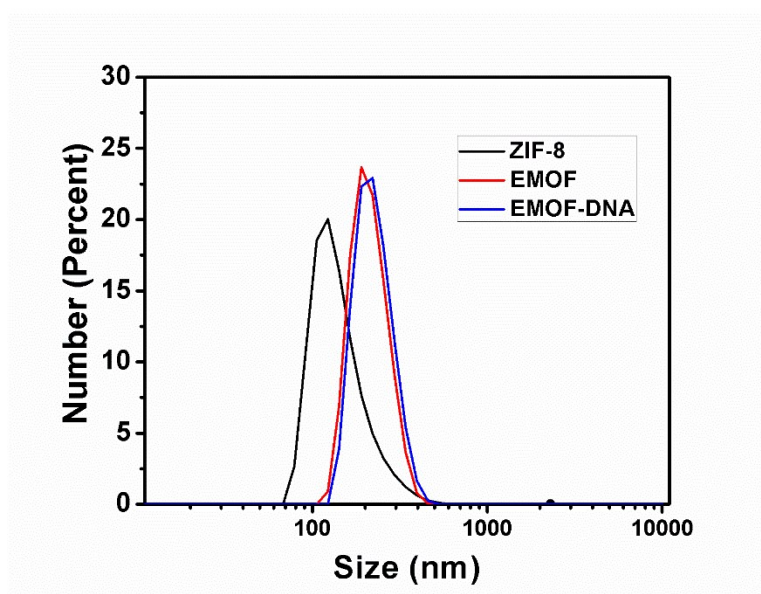


Figure S2. The size distributions of ZIF-8, EMOF, and EMOF-DNA in phosphate buffer saline, were determined by DLS.

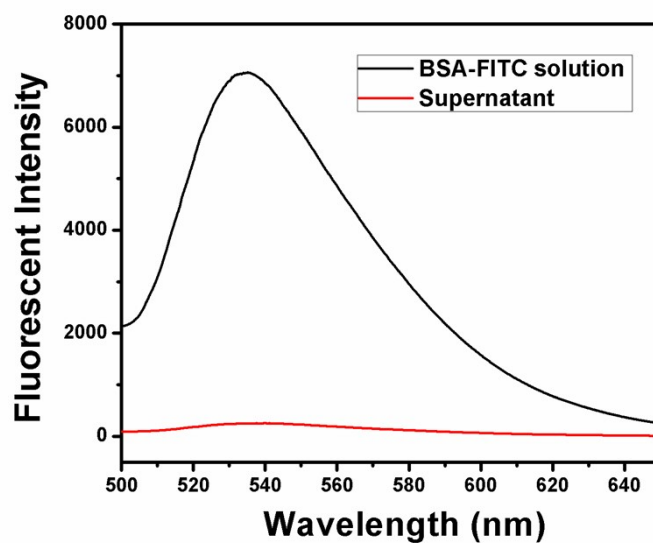


Figure S3. Fluorescent spectra of BSA-FITC/Exo III provided for preparation of BSA-FITC/Exo III@ZIF-8 NPs (black) and supernatant after co-encapsulation of the proteins (red).

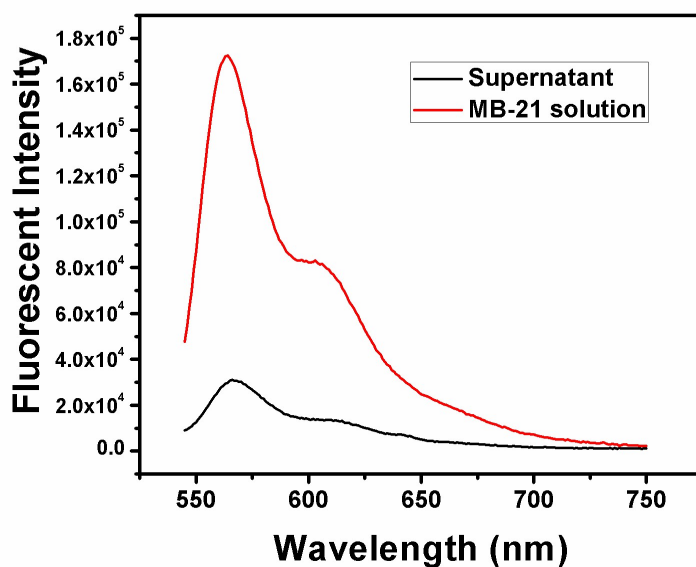


Figure S4. Fluorescent spectra of MB-21 provided for the preparation of EMOF-DNA NPs (black) and supernatant after adsorption (red).

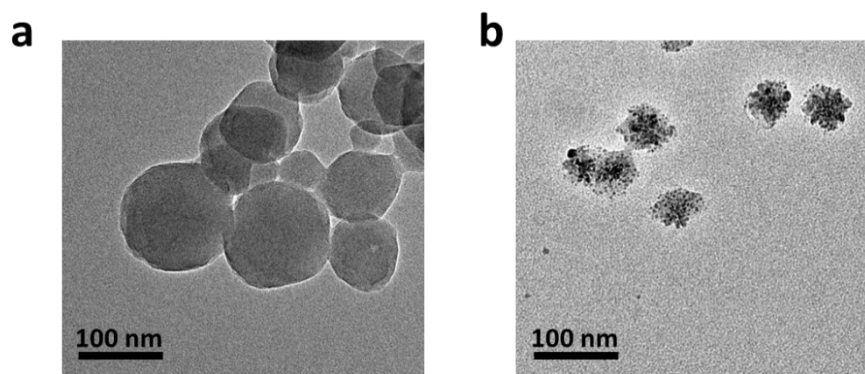


Figure S5. TEM imaging of BSA@ZIF-8 after treatment with PBS buffer at pH 7.4 (a) and pH 5.5 (b) for 1h. scale bar = 100 nm.

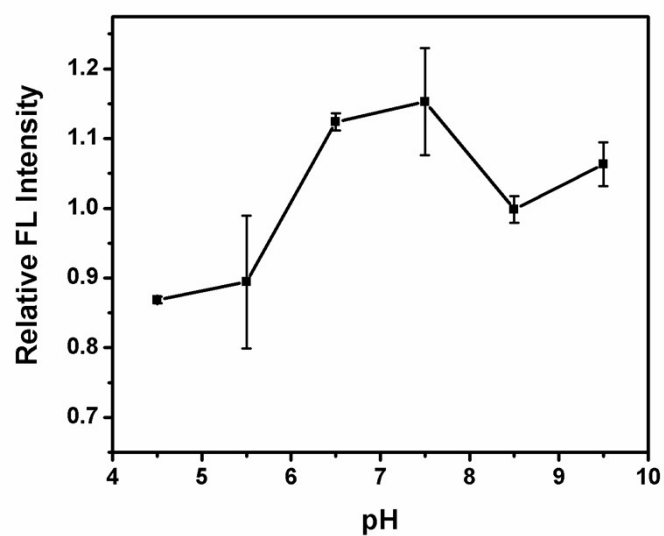


Figure S6. The catalytic activity of Exo III in buffers with different pH.

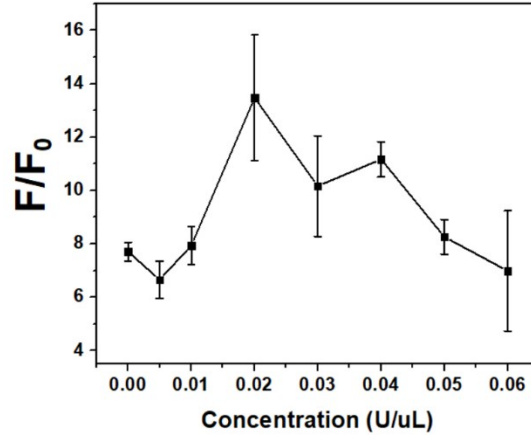


Figure S7. The fluorescent spectrum of the catalytic activity of Exo III at different concentrations.

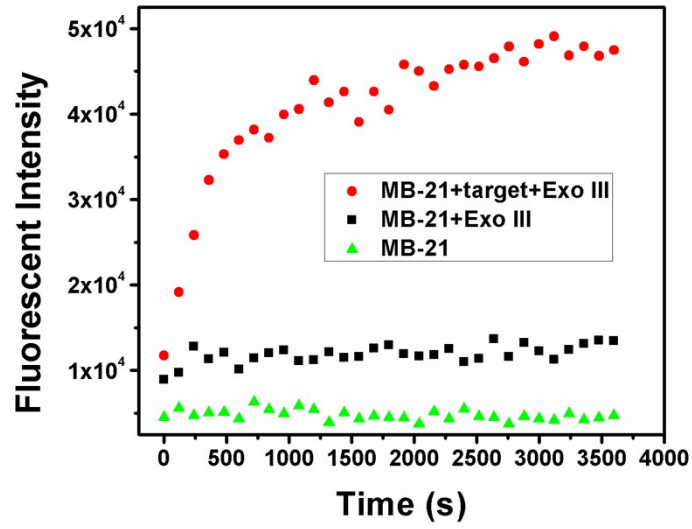


Figure S8. Fluorescent kinetics of MB-21, MB-21 with Exo III, and MB-21 with Exo III in the presence of the target.

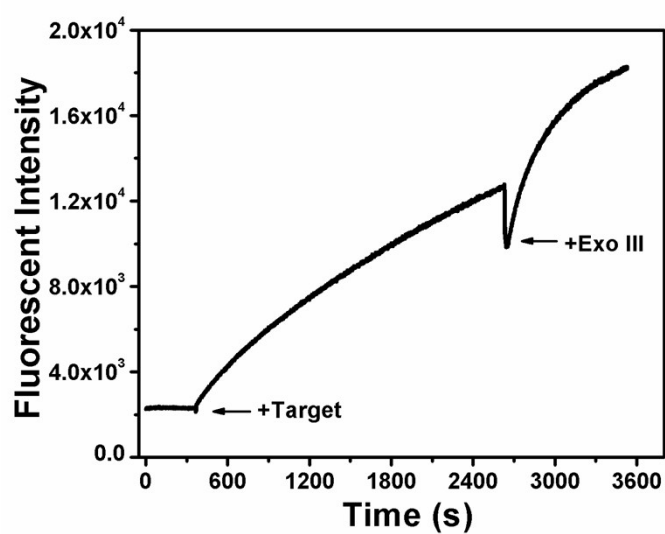


Figure S9. Fluorescent kinetics of MB-21 followed by the addition of target and then Exo III. Initially, the kinetics of 100 nM MB-21 was monitored, then 100 nM target was added after 6 min. Finally, 100U Exo III was added after a total incubation of 44 min.

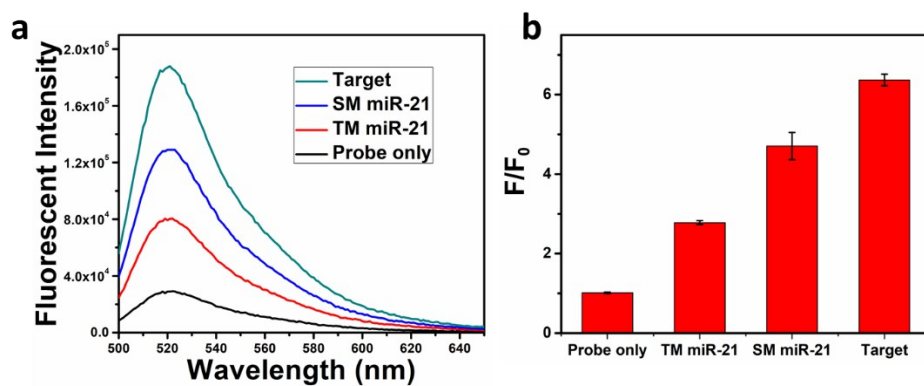


Figure S10. (a) The fluorescent spectrum and (b) relative intensity of the probe in the presence of target, single base mismatch (SM miR-21), and three based mismatch (TM miR-21) sequences. Result was shown with mean \pm S.D.

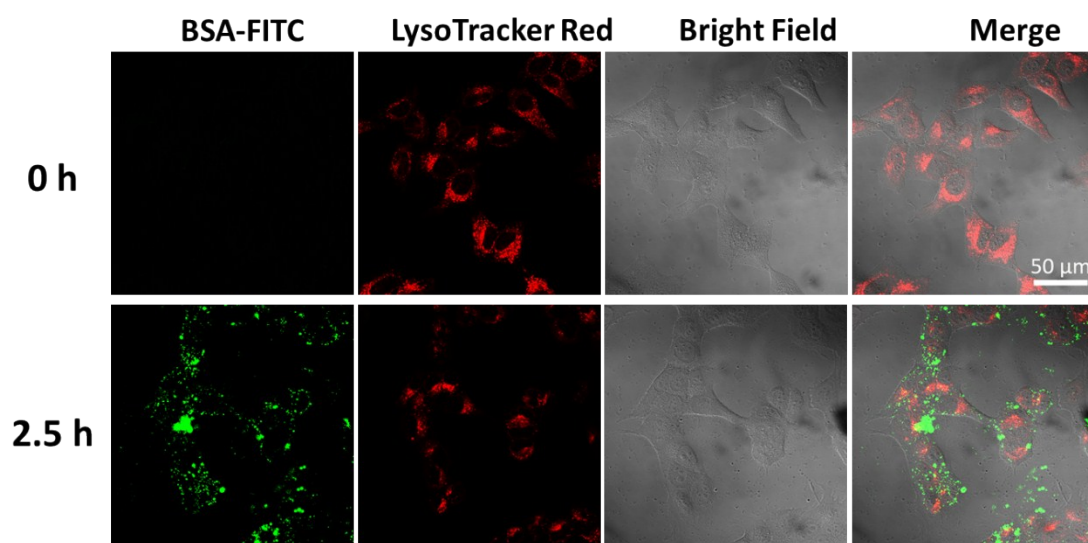


Figure S11. The confocal image of HeLa cells incubated with FITC-BSA@ZIF-8 for 0 or 2.5 h. Scale bar = 50 μ M

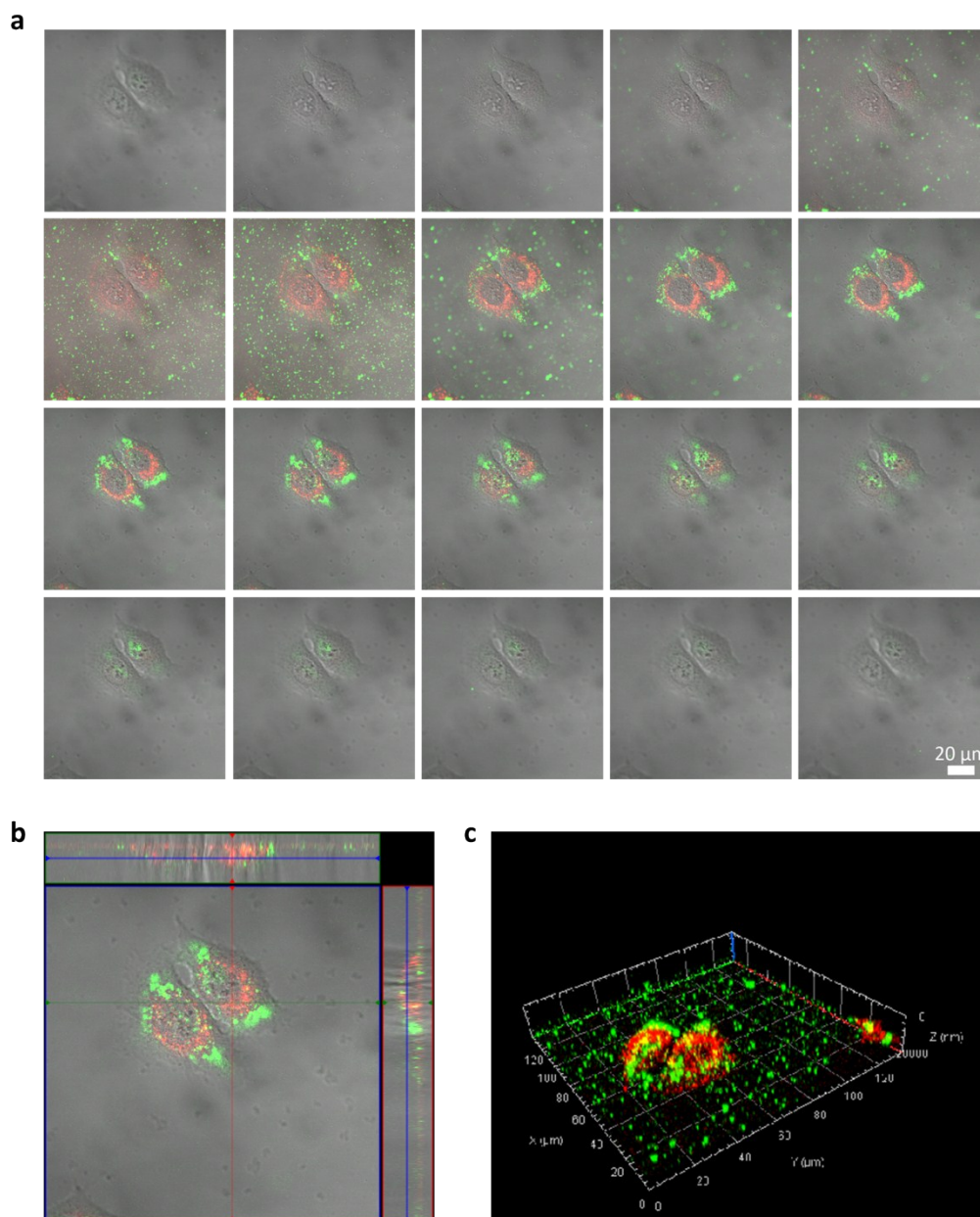


Figure S12. (a) The Z-stack scan, (b) ortho and (c) 3D images of HeLa cells incubated with FITC-BSA@MOF. HeLa cells were incubated with 100 $\mu\text{g/mL}$ FITC-BSA@MOF followed by being imaged at different focus planes through the Z-stack scan model by CLSM. Then the ortho and 3D images were created with ZEN blue software. Scale bar = 20 μm .

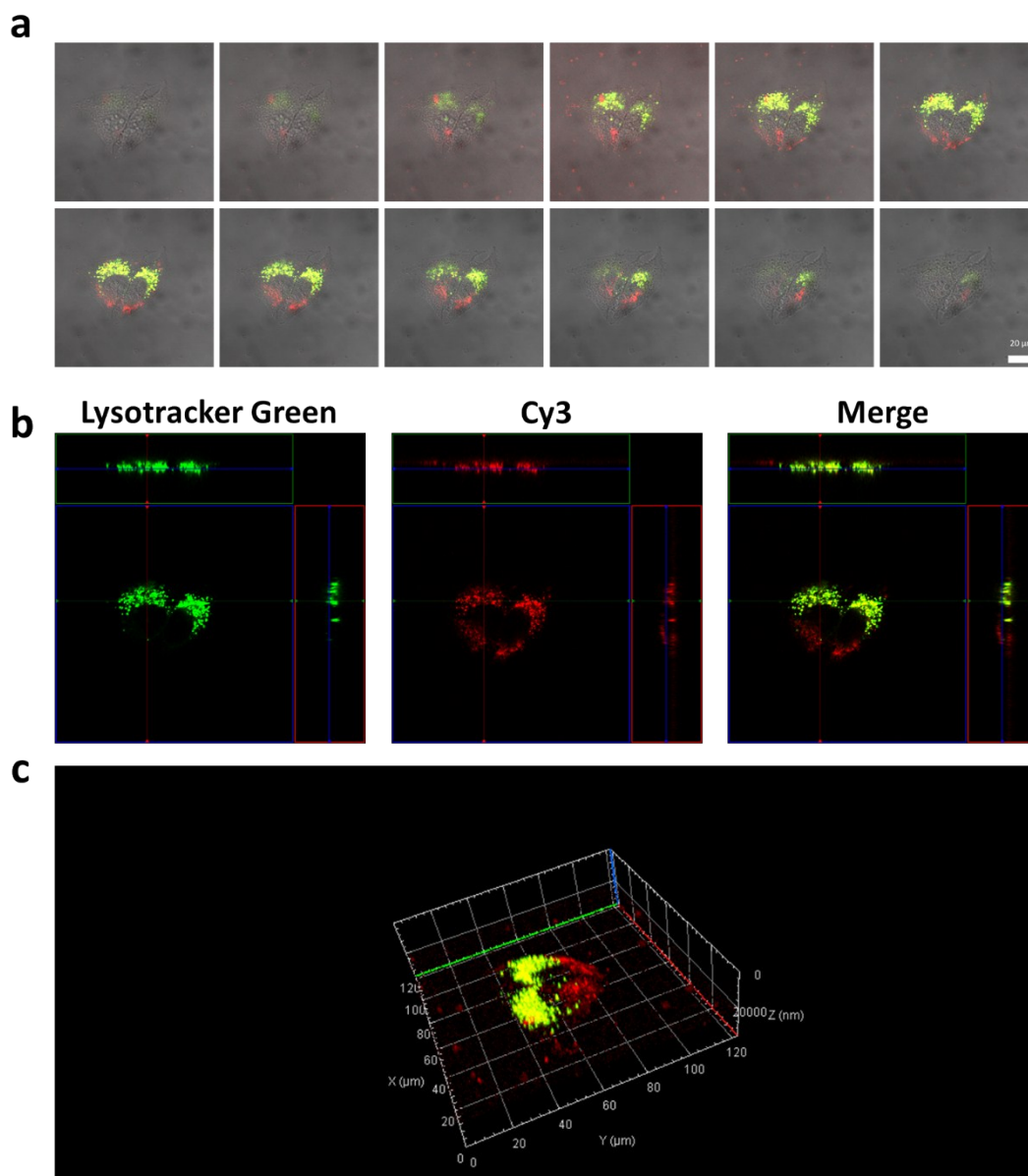


Figure S13. (a) The Z-stack scan, (b) ortho and (c) 3D images of signal HeLa cells incubated with EMOF-DNA. HeLa cells were incubated with 100 ug/mL EMOF-DNA and stained with Lysotracker Green prior to being imaged at different focus planes through the Z-stack scan model by CLSM. Then the ortho and 3D images were created with ZEN blue software. Scale bar = 20 μ M.

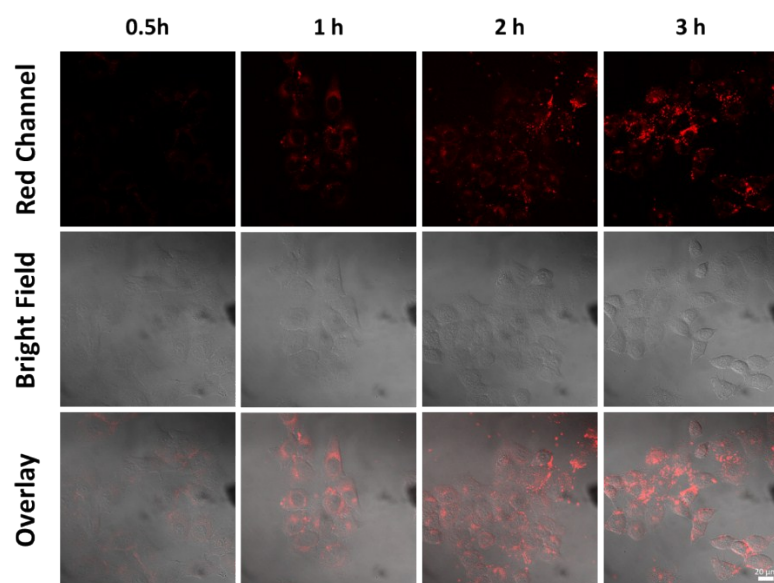


Figure S14. CLSM imaging of HeLa cells incubated with EMOF-DNA for 0.5, 1, 2 h, and 3h. Scale bar = 20 μ M.

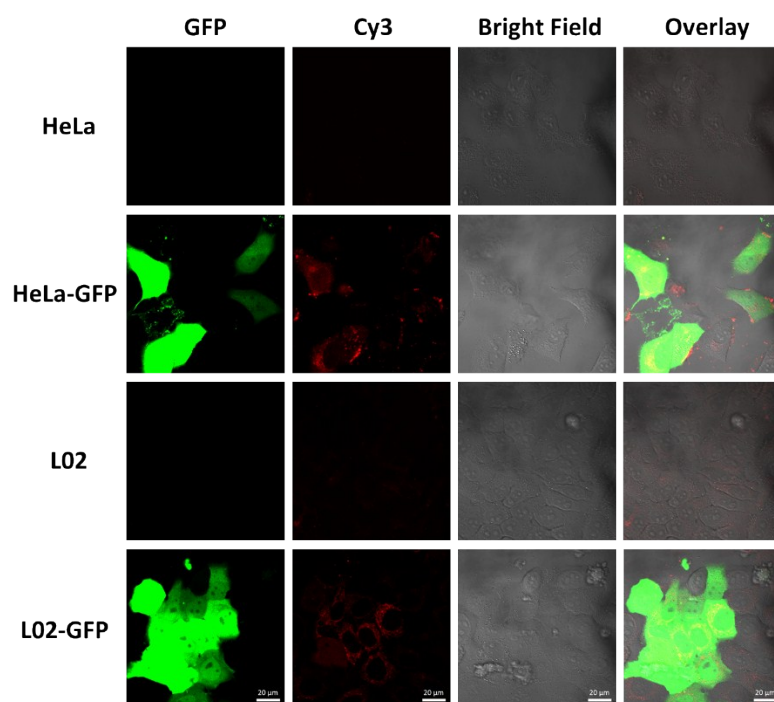


Figure S15. CLSM imaging of HeLa, HeLa-GFP, L02, and L02-GFP cells after incubation with 100 μ g/mL EMOF-MB-GFP nanoprobe for 3h. Scale bar = 20 μ M.

References

1. K. Liang, R. Ricco, C. M. Doherty, M. J. Styles, S. Bell, N. Kirby, S. Mudie, D. Haylock, A. J. Hill, C. J. Doonan and P. Falcro, *Nature Communications*, 2015, **6**, 7240-7248.