

**Nondestructive Analysis of Tumor-Associated Membrane
Protein Integrating Imaging and Amplified Detection *in situ*
Based on Dual-Labeled DNAzyme**

Xiaoxia Chen^{a,#}, Jing Zhao^{a,#}, Tianshu Chen^a, Tao Gao^a, Xiaoli Zhu^{a,*}, Genxi Li^{a,b,*}

^aCenter for Molecular Recognition and Biosensing, School of Life Sciences, Shanghai University, Shanghai 200444, China *E-mail addresses: xiaolizhu@shu.edu.cn

^bState Key Laboratory of Pharmaceutical Biotechnology and Collaborative Innovation Center of Chemistry for Life Sciences, Department of Biochemistry, Nanjing University, Nanjing 210093, China *E-mail addresses: genxili@nju.edu.cn

[#]These authors contribute equally to this work.

Table S1 Drug sensitivity of MCF-7 cells to Doxorubicin, Gemcitabine and Paclitaxel.

DOX								
Concentration(μM)	0	0.1	0.2	0.4	0.8	1.6	3.2	6.4
Cell viability(-)	100.00%	78.94%	68.90%	63.06%	55.45%	41.21%	25.96%	18.68%
Cell viability(+)	100.00%	88.94%	78.40%	68.84%	60.37%	44.67%	30.71%	2.48%
GEM								
Concentration(μM)	0	0.25	0.5	1.0	2.0	4.0	8.0	16.0
Cell viability(-)	100.00%	98.75%	96.93%	97.34%	94.77%	92.63%	92.51%	91.38%
Cell viability(+)	100.00%	96.16%	95.73%	95.39%	93.55%	93.16%	92.07%	91.36%
PXT								
Concentration(μM)	0	0.49	0.99	1.98	3.97	7.94	15.88	31.76
Cell viability(-)	100.00%	52.08%	49.19%	45.35%	45.95%	41.35%	41.05%	40.72%
Cell viability(+)	100.00%	53.02%	51.24%	47.29%	46.75%	44.61%	43.17%	42.62%

Note: "+": Cells with analysis, "-": Cells without analysis, Dox: Doxorubicin, GEM: Gemcitabine, and PTX: Paclitaxel.

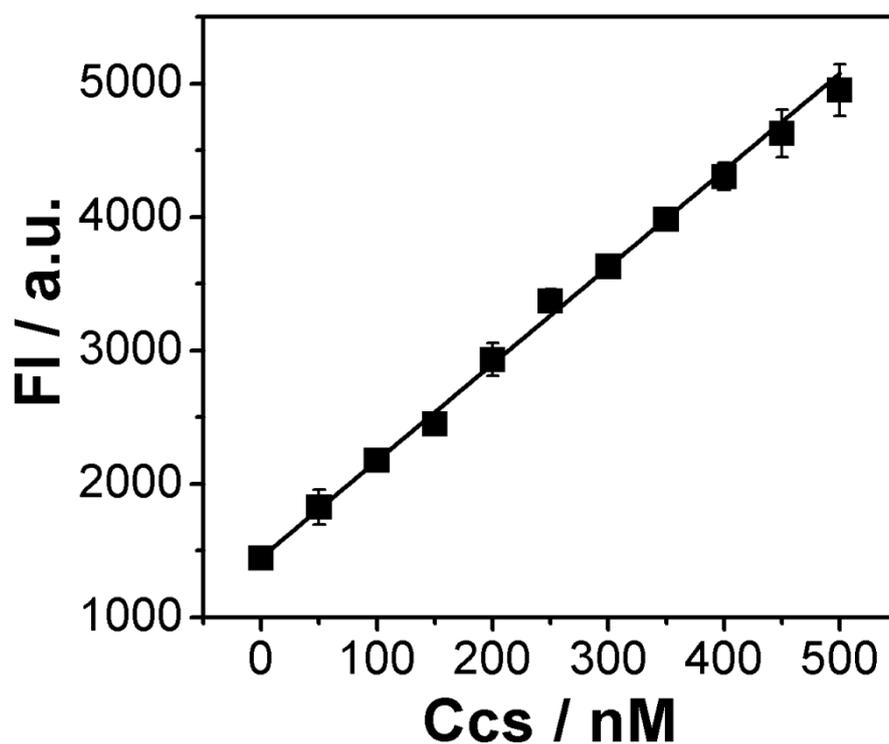


Fig. S1 Standard linear calibration curve of the fluorescent signals of the Cy3-labeled CS1 and BHQ2-labeled CS2 strands with different concentrations ($C_{CS} = C_{CS1} = C_{CS2}$). In the solution of CS, FAM-labeled Enz strand with a fixed concentration of 100 nM, as well as Sub strand with various concentrations ($C_{Sub} + C_{CS} = 500$ nM) is also involved to mimic the products of the DNAzyme-based cleavage.

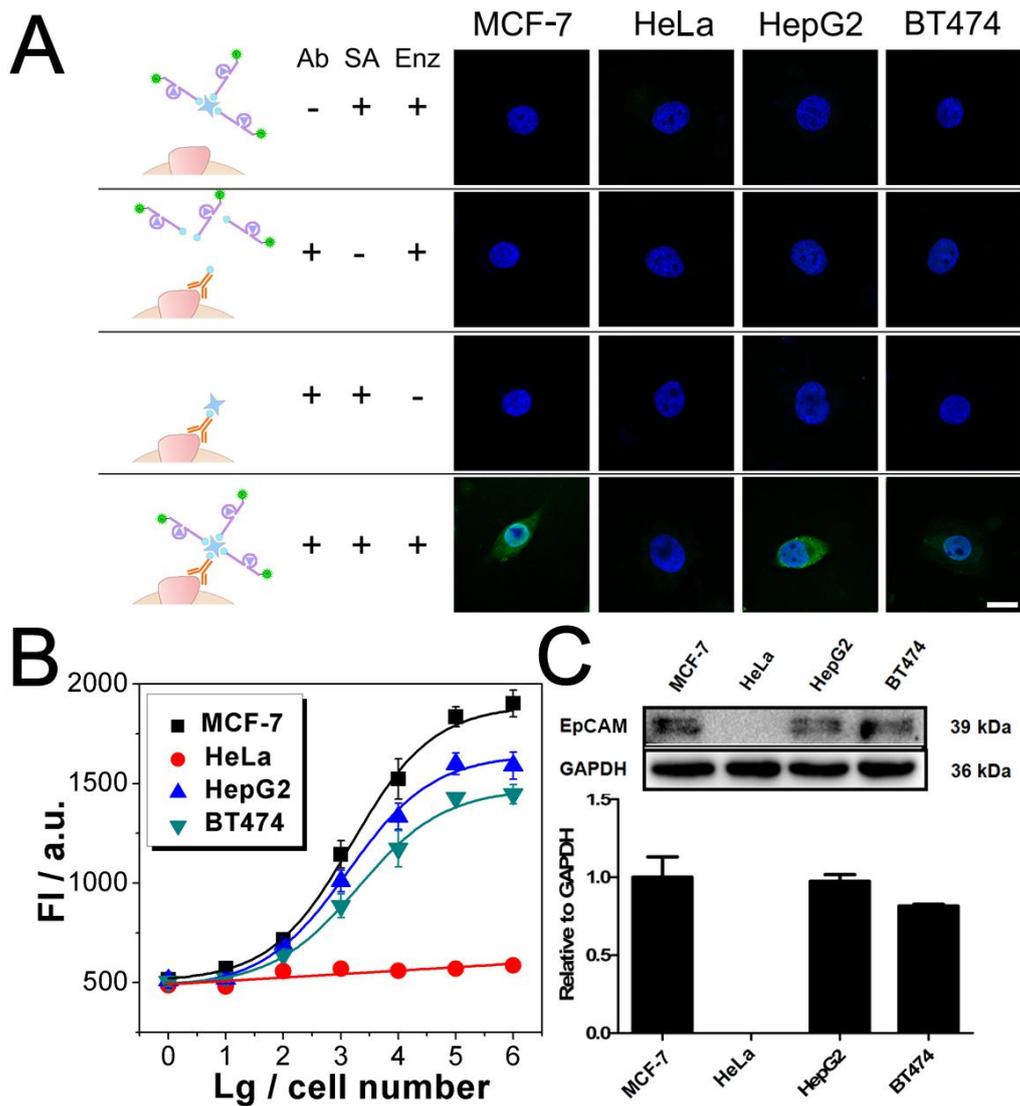


Fig. S2 Imaging and quantitative detection of EpCAM in MCF-7, HeLa, HepG2, and BT474 cells. A) Confocal microscopy images of EpCAM based on FAM labeled DNAzyme. B) Quantitative detection of EpCAM upon the DNAzyme catalysis of substrate. C) Western blot results of EpCAM expression. Scale bar: 20 μ m.

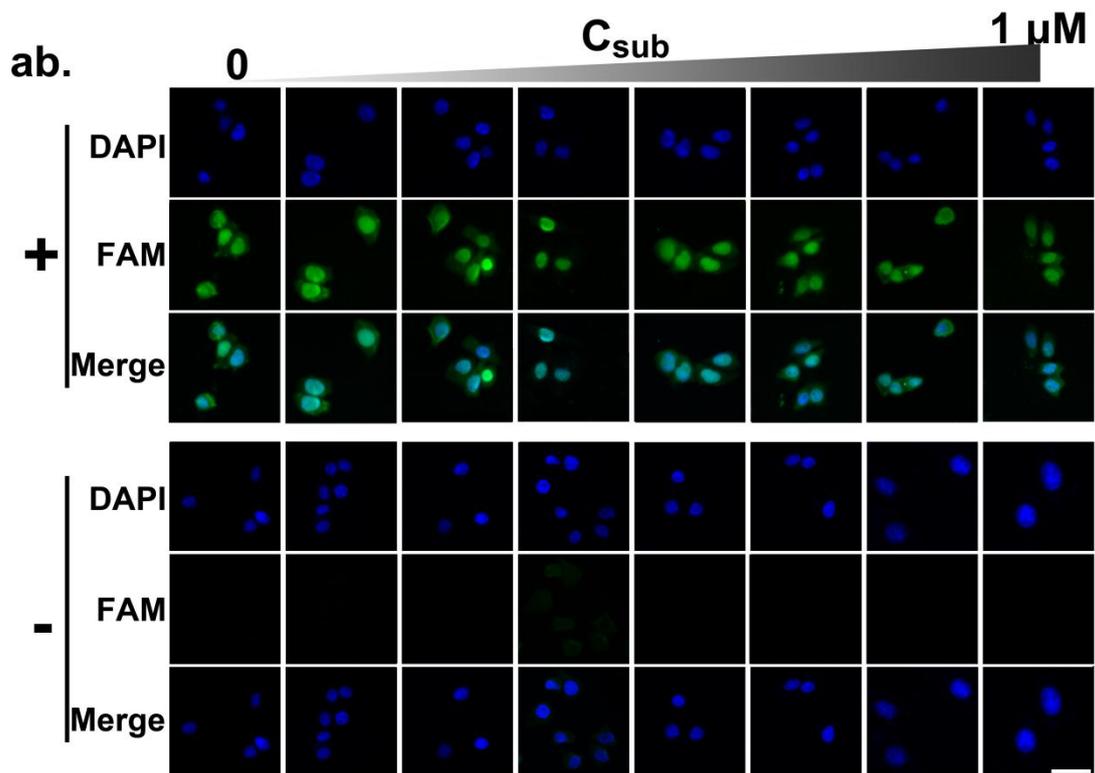


Fig. S3 Confocal microscopy images of MUC1 in MCF-7 with various concentrations of substrate. Scale bar: 50 μm .

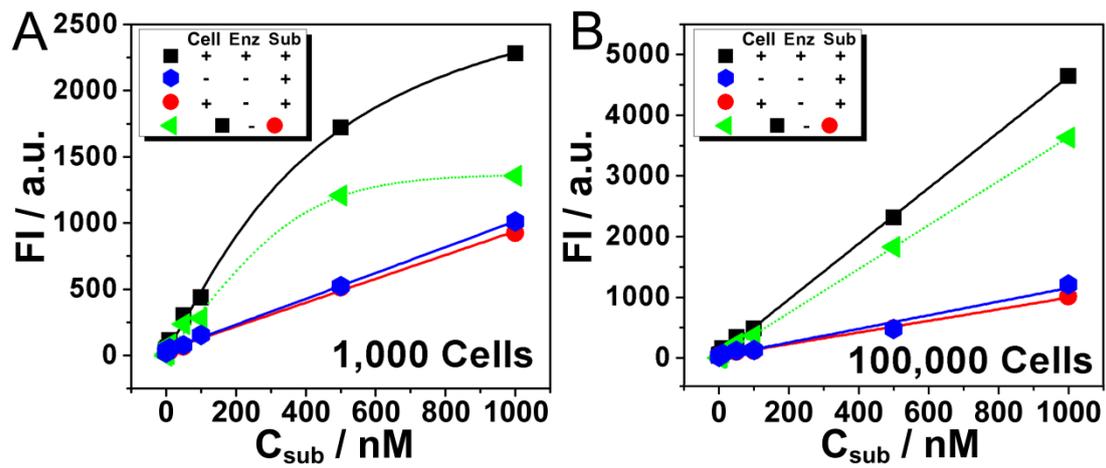


Fig. S4 Effects of different concentration of substrate on TMPs quantification. A) Fluorescence intensity of Cy3 increases in 1 000 cells. B) Fluorescence intensity of Cy3 increases in 100 000 cells.

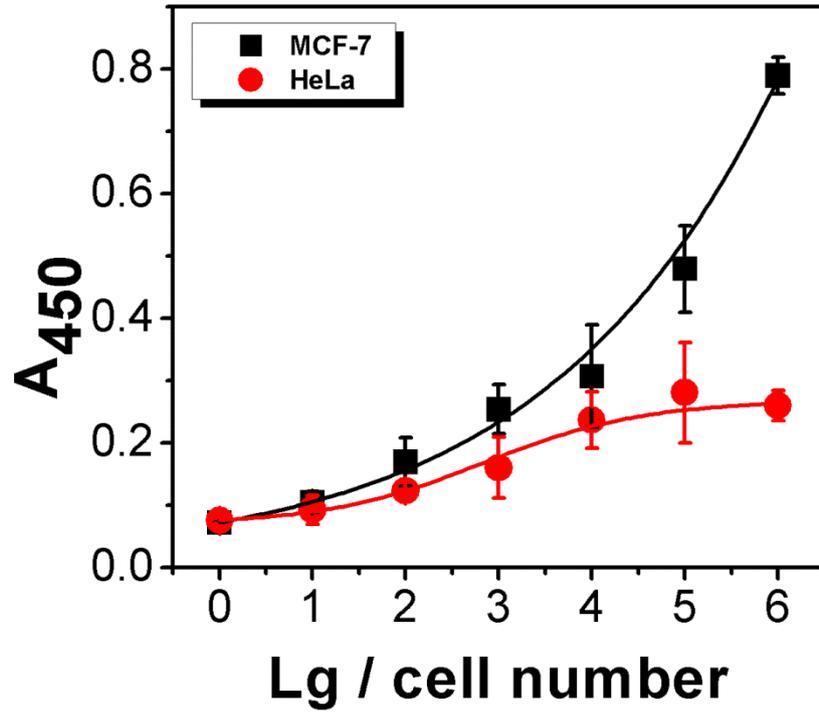


Fig. S5 Detection of EpCAM on MCF-7 and HeLa cell lines by cell-based ELISA.

The absorbance is determined at 450 nm.

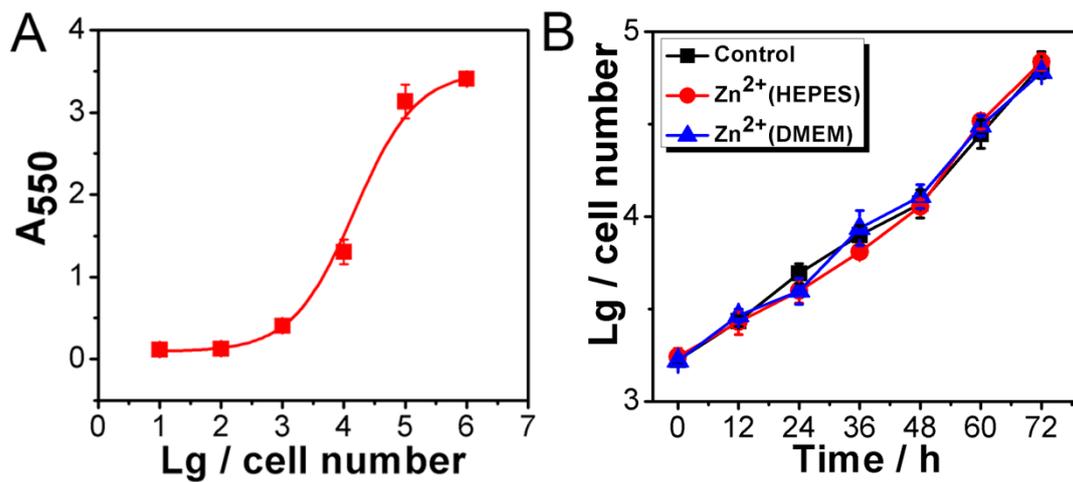


Fig. S6 Investigation of the effects of Zn²⁺ on the physiological activity of the cells. A) Standard cell growth curve of MCF-7. B) Cell growth curve of MCF-7 with and without analysis. The absorbance is determined at 550 nm.

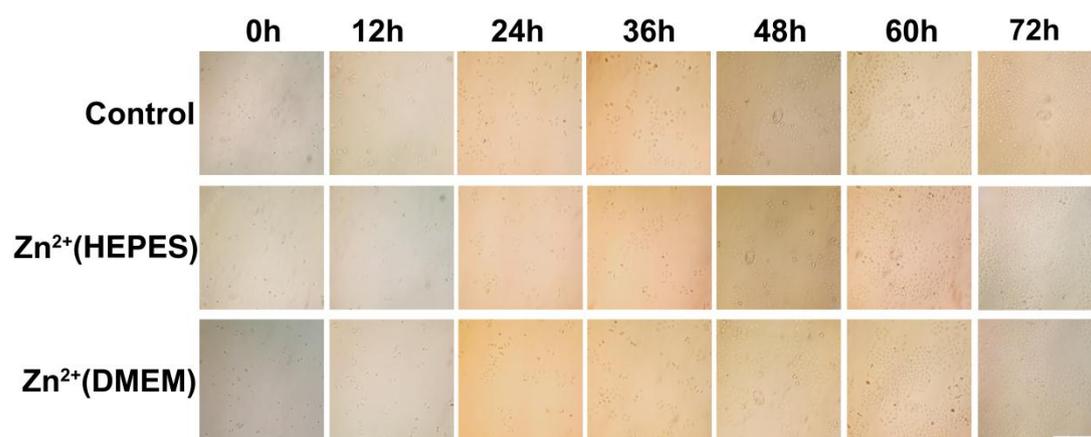


Fig. S7 Light microscopic imaging of MCF-7 with and without analysis. Scale bar: 400 μm .

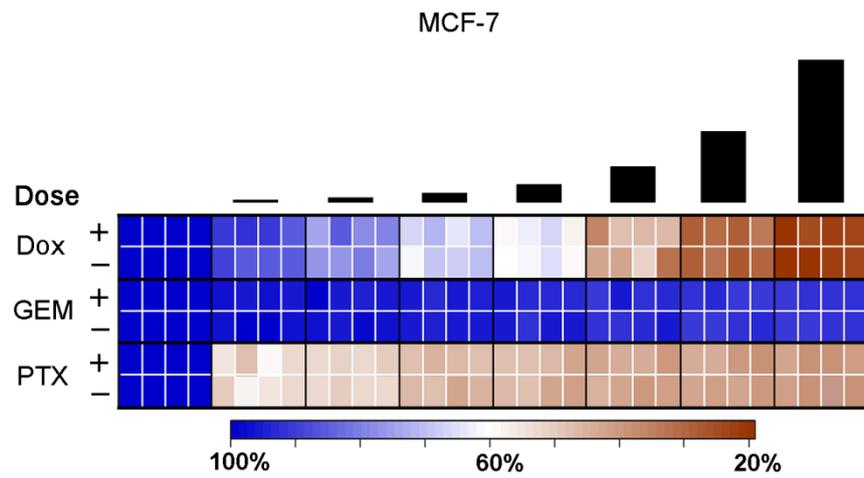


Fig. S8 Drug sensitivity of MCF-7 after treatment with various concentrations of anticancer drugs, Doxorubicin, Gemcitabine and Paclitaxel.