Supporting Information

Fluorescence imaging of a potential diagnostic biomarker for breast cancer cells using a peptide-functionalized fluorogenic 2D material

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S1. Additional figures

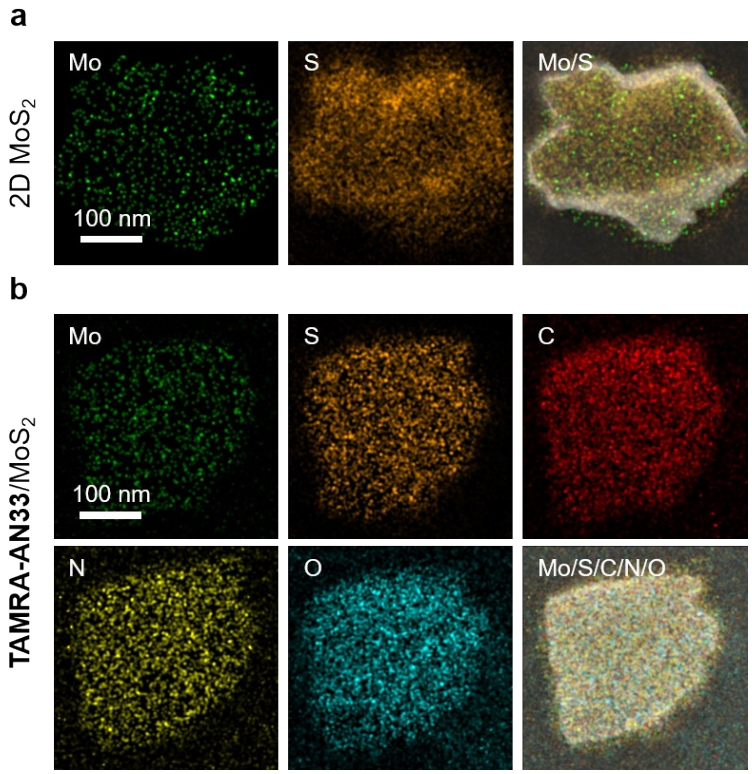


Figure S1. Energy dispersive X-ray spectrometry mapping of (a) 2D MoS_2 and (b) **TAMRA-AN33**/MoS_2.

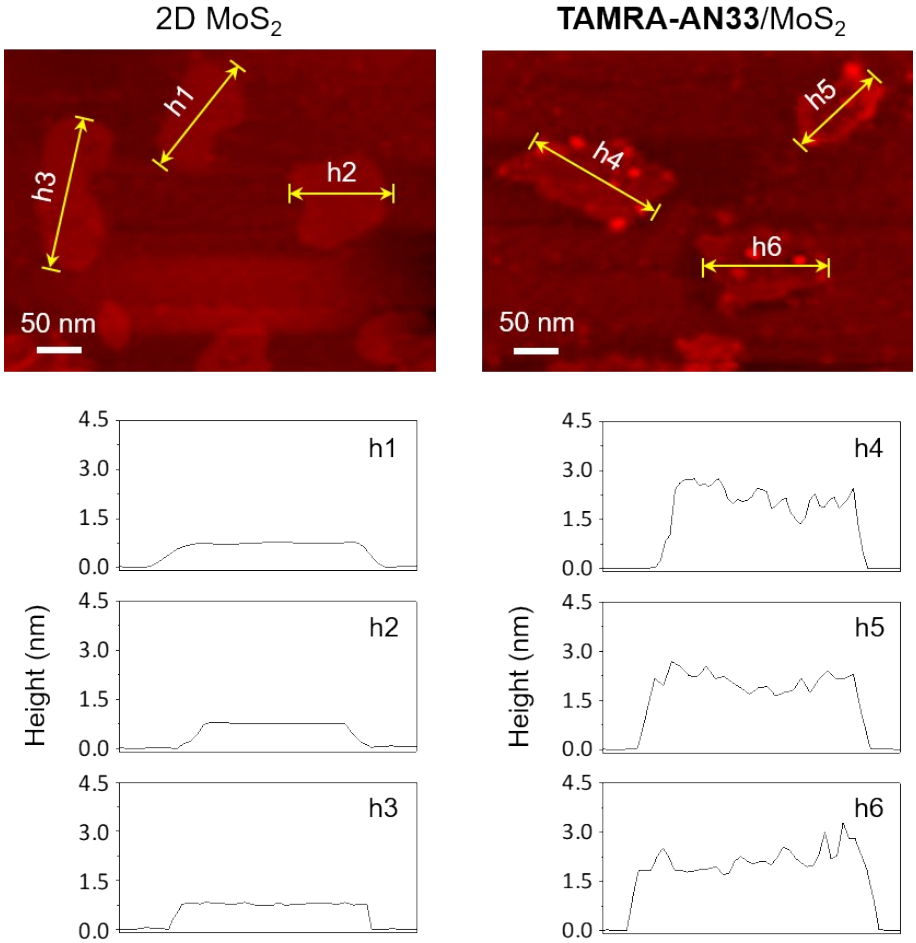


Figure S2. Representative atomic force microscopic images of 2D MoS_2 (20 μg mL^{-1}) and **TAMRA-AN33**/MoS_2 (10 μM/20 μg mL^{-1}).

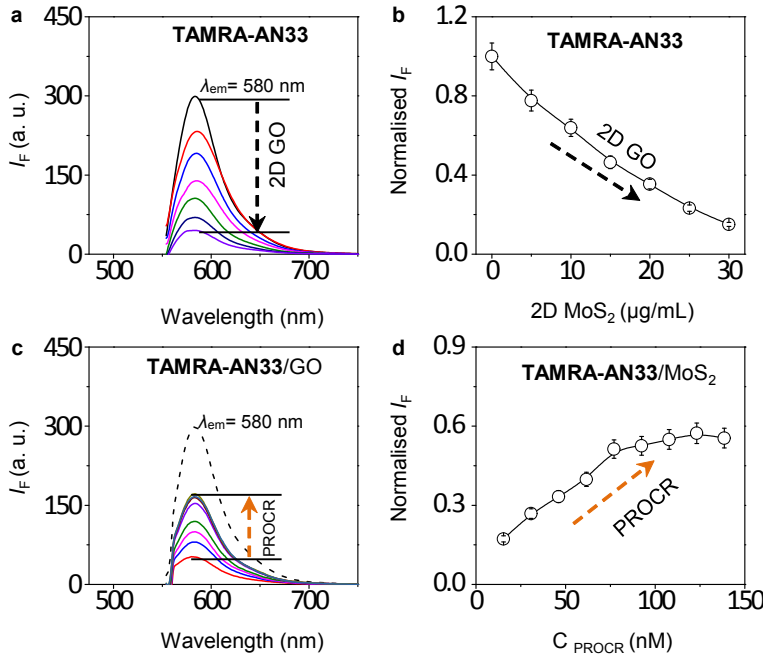


Figure S3. Fluorescence spectra (a) and titration (b) of **TAMRA-AN33** (0.25 μM) with increasing graphene oxide (GO) (0-30 μg mL^{-1}); Fluorescent spectra (c) and titration (d) of **TAMRA-AN33** (0.25 μM) complexed with GO (30 μg mL^{-1}) with increasing PROCR recombinant protein (0-170 nM); PBS (0.01 M, pH 7.40) was used as the buffer solution. Excitation wavelength = 530 nm.

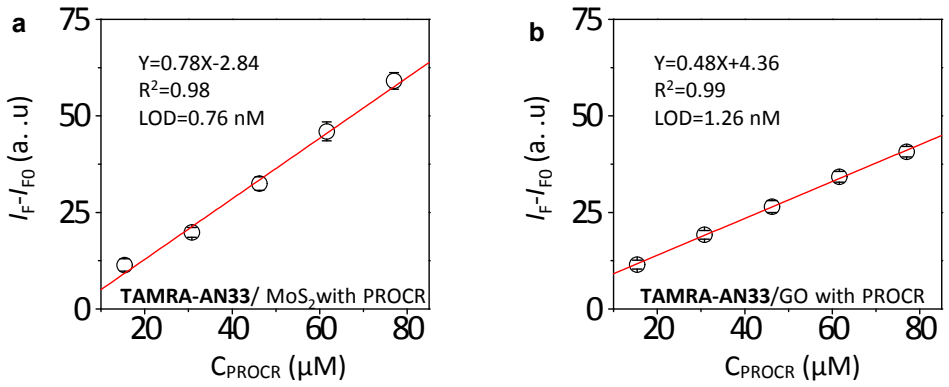


Figure S4. Plotting the fluorescence intensity change of **TAMRA-AN33** (0.25 μM) complexed with (a) 2D MoS_2 (30 μg mL^{-1}) and (b) GO (30 μg mL^{-1}) in the presence of PROCR recombinant protein in PBS (0.01 M, pH 7.40). Excited wavelength = 530 nm.

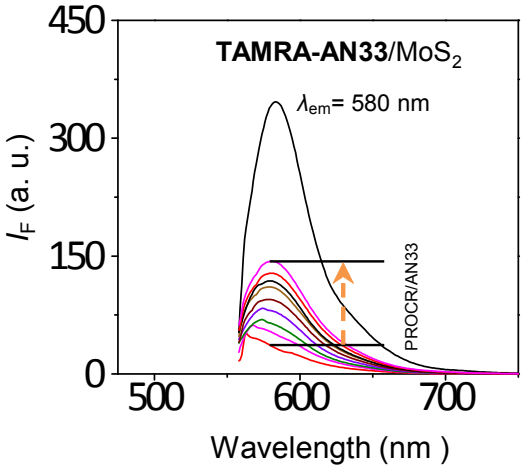


Figure S5. Fluorescence spectra of **TAMRA-AN33** (0.25 μM) complexed with 2D MoS_2 (30 μg mL^{-1}) with increasing PROCR in the presence of **AN33** peptide as a competing agent in PBS (0.01 M, pH 7.40). Excitation wavelength = 530 nm.

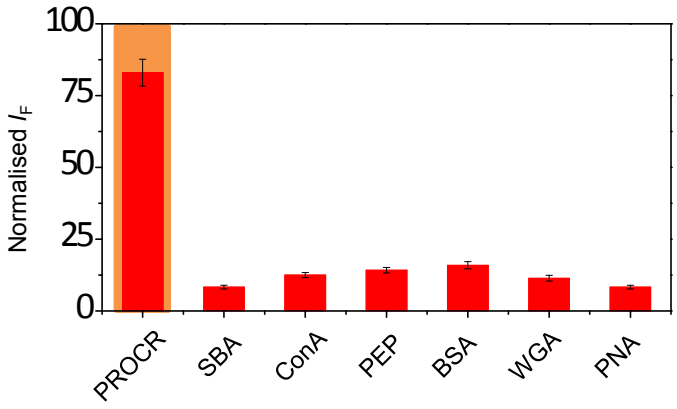


Figure S6. Histogram of **TAMRA-AN33**/MoS_2 in the presence of different proteins (the concentration of PROCR is110 nM, and that of others proteins are 10 μM) in PBS (0.01 M, pH 7.40). Abbr.: Soybean agglutinin (SBA), concanavalin A (ConA), pepsin (PEP), bovine serum albumin (BSA), wheat germ agglutinin (WGA), peanut agglutini (PNA). Excitation wavelength = 530 nm.

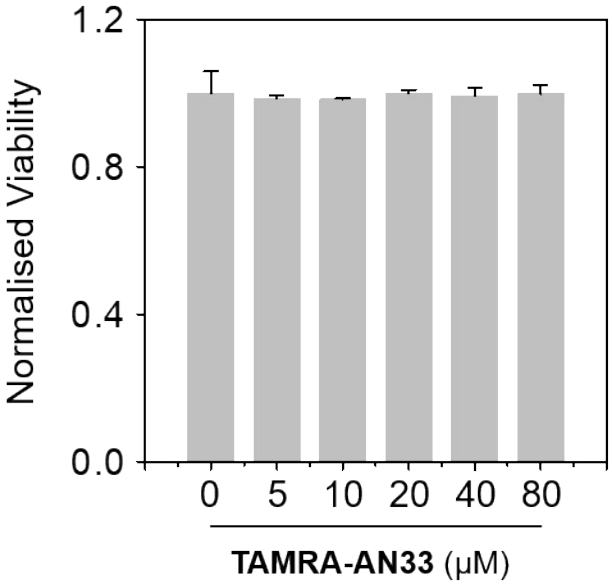


Figure S7. Cell viability of MDA-MB-231(human triple-negative breast cancer) after 24 h treatment without or with **TAMRA-AN33** (0-80 μM).

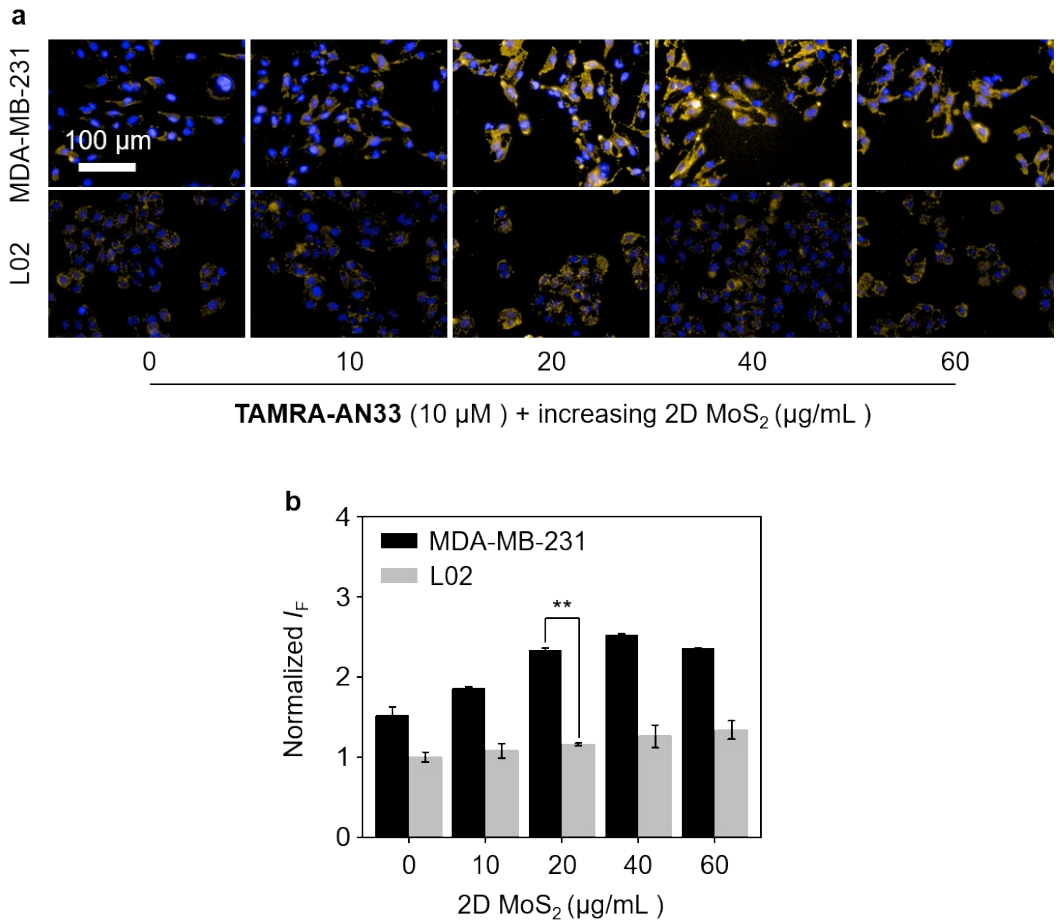


Figure S8. (a) Fluorescence imaging and (b) quantification (**P < 0.01) of MDA-MB-231 and L02 cells incubated with **TAMRA-AN33** (10 μM) with increasing 2D MoS_2 (0, 10, 20, 40, 60 μg mL^{-1}). Scale bar = 100 μm; the excitation and emission channels used are 460−500 nm and 560−630 nm, respectively. The cell nuclei were stained by Hoechst 33342.

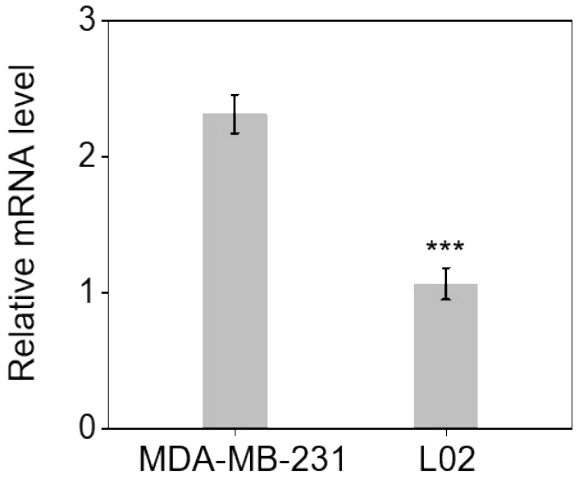


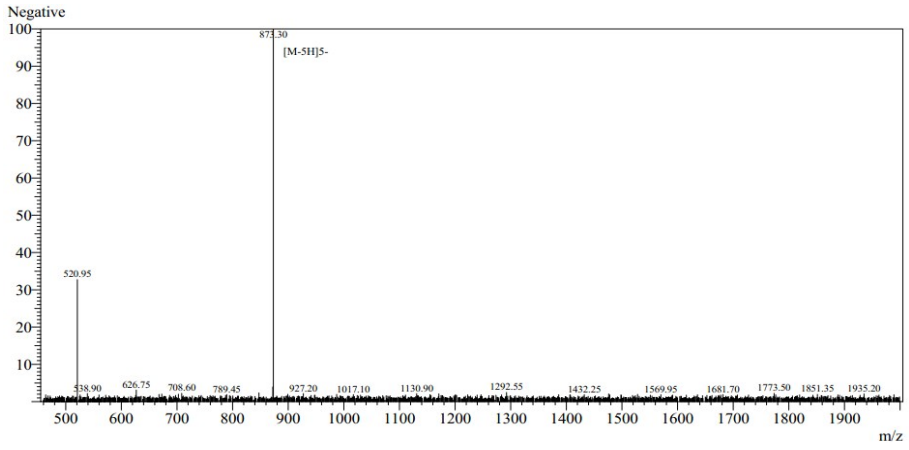
Figure S9. Measuring the relative mRNA level of PROCR in MDA-MB-231 and L02 cells by real-time quantitative polymerase chain reaction (***P < 0.001).

S2. Experimental section

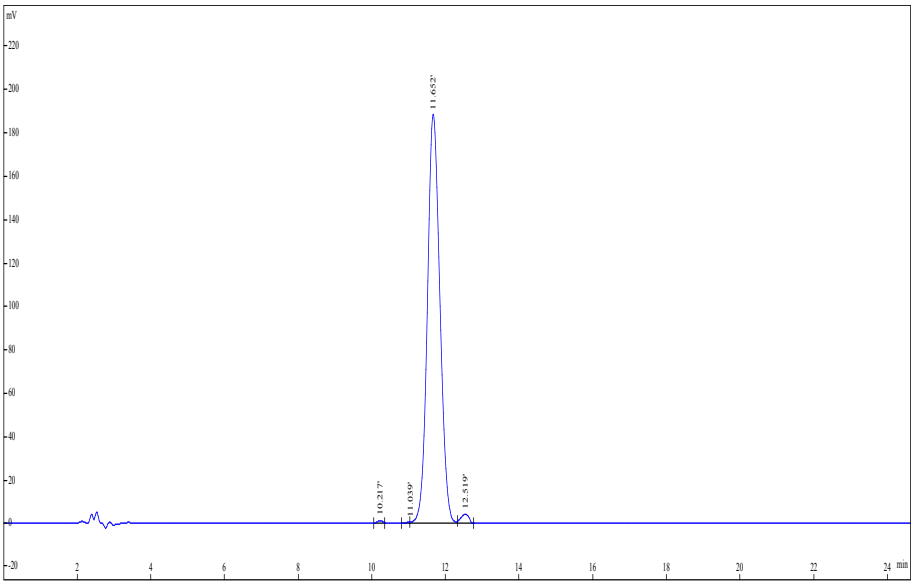
General. All purchased chemicals and reagents were of analytical grade. Proteins (SBA, ConA, PEP, BSA, WGA and PNA) were purchased from Shanghai Yuanye Biotechnology Co., Ltd. Graphene dispersion was purchased from Nanjing XFNANO Co., Ltd. The synthesis of peptides (**TAMRA-AN33** and **AN33**) was commissioned by Shanghai Ziyu Biotechnology Co., Ltd. High-resolution transmission electron microscope (HRTEM) images were obtained with JEOL 2100 equipped with a Gatan Orius charged-coupled device camera and Tridiem energy filter operating at 200 Kv. Dynamic light scattering (DLS) and zeta potential were carried out on a Horiba LB-550 Nano-Analyzer. The Raman scattering was excited by an argon ion laser (I = 514.5 nm). Energy dispersive X-ray spectrometry mapping was obtained with TEAMEDS (EDAX, China). Atomic force microscope (AFM) was carried out on Solver P47-Pro (NT-MDT, Russia). 2D MoS_2 dispersion was prepared according to a previous published literature.[1]

TAMRA-AN33: MS (MALDI-TOF): calcd for $[M-5H]^{5-}$ 873.29, found 873.30. HPLC: 98.2% (0-25 min 17% A and 83% B; 25-30 min 42% A and 58% B; 1.0 mL min^{-1}). Solvent A contains 0.1% trifluoroacetic acid in acetonitrile and solvent B contains 0.1% trifluoroacetic acid in water.

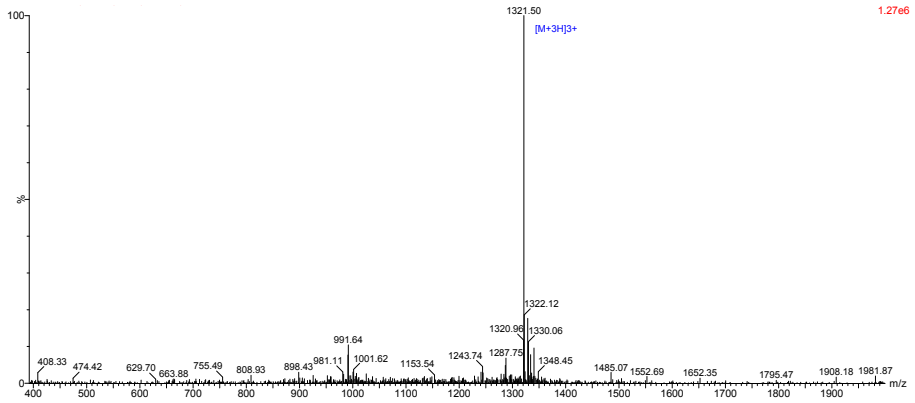
AN33: MS (MALDI-TOF): calcd for $[M+3H]^{3+}$ 1320.76, found 1321.50. HPLC: 95.8% (0-25 min 33% A and 67% B; 25-30 min 58% A and 42% B; 1.0 mL min^{-1}), Solvent A contains 0.1% trifluoroacetic acid in acetonitrile and solvent B contains 0.1% trifluoroacetic acid in water.



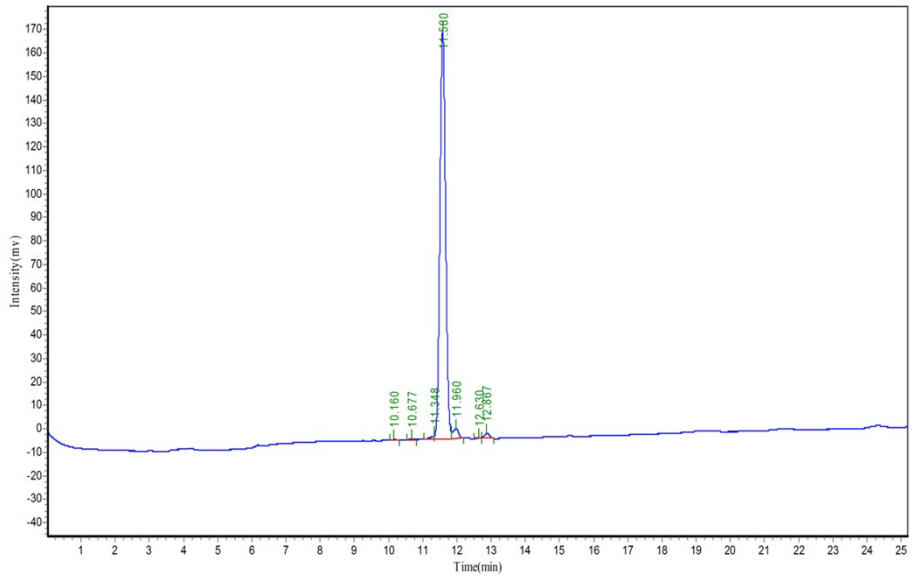
Mass spectrum of **TAMRA-AN33**



HPLC of **TAMRA-AN33**



Mass spectrum of **AN33**



HPLC of **AN33**

Atomic force microscope (AFM). The samples (2D MoS_2 and **TAMRA-AN33**/MoS_2) used were prepared by dispersion on freshly cleaved mica plate and dried at room temperature. Then, the morphology of the samples was recorded on Solver P47-Pro (NT-MDT, Russia).

Scanning Electron Microscope-Energy Dispersive Spectrometer (SEM-EDS). The samples (2D MoS_2 and **TAMRA-AN33**/MoS_2) used were prepared by dispersion on freshly cleaved mica plate and dried at room temperature. Then, element distribution mapping of the samples was performed on TEAMEDS (EDAX, China).

Cell culture. MDA-MB-231 cells were cultured in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 5% fetal bovine serum (Gibco, Gland Island, NY, USA). MCF-7 cells were maintained in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco, Gland Island, NY, USA). L02 cells were cultured in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco, Gland Island, NY, USA) in a humidified atmosphere of 5% CO_2 and 95% air at 37 ℃ and split when the cells reached 90% confluency.

Cell viability assay. Cells were plated on 96-well plates at 15000 cells per well in growth medium overnight. After seeding, cells were treated with different concentrations of **AN33-TAMRA** for 24 h. After that, 10 μL per well of MTS/PMS (20:1, Promega Corp) mixture solution was added to each well, followed by shaking gently. After incubation at 37 °C under 5% CO_2 for 2-4 h, the absorbance of the solutions was measured at 490 nm by an M5 microplate reader (Molecular Device, USA).

Fluorescence imaging of cells. Cells (MDA-MB-231: 20000 cells/well; MCF-7: 21000 cells/well; L02 cells: 25000 cells/well) were separately cultured in corresponding growth medium and seeded on a black 96-well microplate (Greiner bio-one, Germany) overnight. Then, the cells were incubated with different concentrations of **TAMRA-AN33** (2.5, 5, 10 and 20 μM) for 20 min. For MoS_2-dependent fluorescence imaging assay, **TAMRA-AN33** (10 μM) was first mixed with different concentrations of 2D MoS_2 (0, 10, 20, 40 and 60 μg mL^{-1}). Cells were incubated with **TAMRA-AN33**/MoS_2 for 20 min. Then, the cells were washed gently with phosphate buffered saline (PBS) three times, and the cell nuclei were stained with Hoechst 33342 (5 μg mL^{-1}) at 37 °C for 5 min. Cells were washed with PBS three times. The fluorescence images were recorded using an Operetta high content imaging system and quantified by the Columbus image data analysis system (Perkinelmer, US).

Real-time quantitative polymerase chain reaction (RT qPCR). Total RNA was isolated from cells using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. Complementary DNA generated using a PrimeScript® RT reagent kit (TaKaRa, Dalian, China) was analyzed by quantitative PCR using SYBR® Premix Ex TaqTM. qPCR was performed using a 7300 Real-Time PCR system (Applied Biosystems, CA, USA). GAPDH was detected as the housekeeping gene. Primers for qPCR were as follows:

GAPDH forward, 5'-ATCACTGCCACCCAGAAGAC-3'

and reverse, 5'-ATGAGGTCCACCACCCTGTT-3'

Protein C receptor forward, 5′- AACACCAAAGGGAGCCAAACAAG-3′

and reverse, 5′- CTACAGCCACACCAGCAATCATG-3′.

S3. Additional references

1. J. N. Coleman, M. Lotya, A. O'Neill, S. D. Bergin, P. J. King, K. Young, A. Gaucher, S. De, R. J. Smith, I. V. Shvets, S. K. Arora, G. Stanton, H.-Y. Kim, K. Lee, G. T. Kim, G. S. Duesberg, T. Hallam, J. J. Boland, J. J. Wang, J. F. Donegan, J. C. Grunlan, G. Moriarty, A. Shmeliov, R. J. Nicholls, J. M. Perkins, E. M. Grieveson, K. Theuwissen, D. W. McComb, P. D. Nellist, V. Nicolosi, *Science,* 2011, **311**, 568.