Supplementary information for

Graphene oxide-enhanced cytoskeleton imaging and mitosis tracking

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Fig. S1. High-resolution transmission electron microscopy (TEM) of graphene oxide (a representative field) taken on a JEM-2100 TEM system.



Fig. S2. (a) Plotting the fluorescence change (where I and I₀ are the fluorescence intensity of material composite in the presence and absence of microtubule, respectively) of **VAR**/GO (0.5 μ M/9.1 μ g mL⁻¹) as a function of microtubule concentration. (b) Plotting the fluorescence intensity of **VAR**/GO (0.5 μ M/9.1 μ g mL⁻¹) with 10 nM microtubule as a function of time. (c) Fluorescence spectra of **VAR**/GO (0.5 μ M/9.1 μ g mL⁻¹) in the presence of microtubule (29.56 nM) and other proteins (30 nM) (BSA = bovine serum albumin; PSA = Pisum Sativum Agglutinin; IgG = Immunoglobulin G). All fluorescence measurements were carried out in phosphate buffered saline (0.01 M, pH 7.4) at room temperature with an excitation wavelength of 510 nm.



Fig. S3. Fluorescence spectra of **VAR**/GO (0.5 μ M/9.1 μ g mL⁻¹) with microtubule (29.56 nM) in the presence of increasing free VAR (sequence: VARVGSPPD; from top to bottom: 0, 0.5, 1, 2, 4, 8, 32 nM). All fluorescence measurements were carried out in phosphate buffered saline (0.01 M, pH 7.4) at room temperature with an excitation wavelength of 510 nm.



Fig. S4. Fluorescence imaging of increasing **VAR** probe for HeLa cells stably expressing EGFP- α tubulin. Excitation channels for EGFP and TAMRA are 460-490 and 520-550 nm, and emission channels for EGFP and TAMRA are 500-540 and 560-630 nm, respectively. Scale bar = 50 μ m (applicable to all images).



Fig. S5. Viability of HeLa cells in the presence of increasing **VAR** probe, GO or **VAR**/GO determined by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)- 2*H*-tetrazolium) cell proliferation assay.



Fig. S6. Fluorescence imaging (a) and quantification (b) of **VAR** probe (4 μ M), GO (81 μ g mL⁻¹) and **VAR**/GO (4 μ M/81 μ g mL⁻¹) for human embryonic kidney 293T cells expressing EGFP- α tubulin. Excitation channels for EGFP and TAMRA are 460-490 and 520-550 nm, and emission channels for EGFP and TAMRA are 500-540 and 560-630 nm, respectively. Scale bar = 50 μ m (applicable to all images). The 293T cells were transfected with pEGFP- α tubulin for 12 h, and then seeded on poly-L-lysine-coated 96-well plate (Greiner) for another 24 h. After incubation for 30 min with **VAR** probe (4 μ M), GO (81 μ g ML⁻¹) or **VAR**/GO (4 μ M/81 μ g ML⁻¹), cells were washed with PBS three times. Then, images were taken and fluorescence intensity was quantified.

S2. Experimental section

General remarks. All purchased chemicals and reagents are of analytical grade. The peptide ligand **VAR** probe (VARVGSPPDK-5-TAMRA) and free ligand VAR (VARVGSPPD) were purchased from Ningbo KareBay Biochem, Inc.. Graphene oxide was purchased from Nanjing XFNANO Materials Tech Co., Ltd. Taxol stabilized microtubule was purchased from Cytoskeleton, Inc. Dynamic light scattering (DLS) was carried out on a Horiba LB-550 Dynamic Light Scattering Nano-Analyzer. Raman spectra were obtained using a Renishaw InVia Reflex Raman system (Renishaw plc, Wotton-under-Edge, UK) employing a grating spectrometer with a Peltier-cooled charge-coupled device (CCD) detector coupled to a confocal microscope, which were then processed with Renishaw WiRE 3.2 software. The Raman scattering was excited by an argon ion laser (I = 514.5 nm). Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. Zeta potential was measured with a Horiba LB-550 DLS Nano-Analyzer.

Dose and time-dependent fluorescence imaging of HeLa cells by VAR/GO. HeLa cells stably expressing EGFP- α tubulin were seeded on a 96-well plate (Greiner). After 24 h, cells were incubated with VAR probe, GO or VAR/GO. The composition ratios of VAR (μ M) to GO (μ g mL⁻¹) were 0/0, 1/19.7, 2/34.5, 4/66.9 and 5/80.56. For dose dependent experiments, cells were incubated with VAR, GO or VAR/GO for 40 min at indicated concentrations. For time dependent experiments, cells were incubated with 4 μ M VAR probe, 80.56 μ g mL⁻¹ GO or 4 μ M/80.56 μ g mL⁻¹ VAR/GO for 0, 10, 20, 30 and 60 min. Then, cells were washed with phosphate buffered saline (PBS) three times, and images were taken using an Operetta high content imaging system (PerkinElmer). The fluorescence quantification was carried out by the Columbus image data analysis system (PerkinElmer). The mean fluorescence intensity of cell regions (A) and that of background (B) was calculated separately by Columbus image data analysis system (PerkinElmer). The quantification value results from subtracting B from A. More than 500 cells were quantified in each condition. Excitation channels for EGFP and TAMRA are 460-490 and 520-550 nm, and emission channels for EGFP and TAMRA are 500-540 and 560-630 nm, respectively.

Cell viability assay. HeLa cells stably expressing EGFP- α tubulin were seeded on a 96-well plate. After 24 h, cells were incubated with **VAR** probe, GO or **VAR**/GO with indicated concentrations. After incubation for 40 min, cells were washed by PBS and cultured for another 48 or 72 h in fresh DMEM. Then, the cell viabilities were determined through the MTS cell proliferation assay. MTS = (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)- 2*H*-tetrazolium).

Confocal laser scanning microscopy (CLSM). HeLa cells stably expressing EGFP- α tubulin were seeded on poly-L-lysine-coated 12-mm glass coverslips. After 24 h, cells were incubated with

VAR/GO (4 µM/80.56 µg mL⁻¹) for 40 min. Then, cells were washed with PBS three times and fixed in 3.7% paraformaldehyde for 10 min. Coverslips were mounted in mounting medium (Dako), and cells undergoing mitosis were analyzed. Images were obtained by CLSM (Leica SP 8, Plan Apochromat 63X, NA 1.4 oil immersion objective) and subjected to a subsequent deconvolution process (Huygens). The fluorescence of EGFP and that of VAR probe was co-localized by Image Pro Plus 6.0. Excitation channels for DNA (stained by Hoechst), EGFP and TAMRA are 405, 488 and 525 nm, and emission channels for DNA, EGFP and TAMRA are 430-480, 490-540 and 580-620 nm, respectively.





Signal Peak	# RT(min)	Area%	Area(mAU*s)	Height (mAU)	
1	7.666	0. 5861	37213	2581	
2	8. 367	0. 1098	6973	630	
3	8. 985	0.07107	4513	1689	
4	9.347	99.01	6287110	327537	
5	12.172	0. 2197	13950	1414	
Total		100	6349759	333851	

HPLC trace of **VAR** probe (VARVGSPPD-K-5-TAMRA).