Supporting information

Materials and apparatus

1-Pyrenebutyric acid (PBA), *N*,*N*-Diisopropylethylamine (DIEA), Dipyrrolidino(*N*-succinimidyloxy)carbenium hexafluorophosphate (HSPyU), *N,N*-dimethylformamide (DMF), acetone, Tris-HCl, Sodium chloride, calcium chloride, magnesium chloride, manganese chloride, bovine serum albumin (BSA), anti-beta actin antibody, dextrose, glucose, Rnase, are all purchased from Sigma-Aldrich. All chemicals were highest purification grade, and used upon receiving without further purification. Purified integrin αvβ3 was purchased from Millipore, Inc. Cell culture medium, Dulbecco's Modified Eagle's Medium, was from Gibco, Inc. (Grand Island NY), and fetal bovine serum (FBS) was supplied by Hyclone, Co. Human breast cancer cell lines, MDA-MB-435 and MCF-7, were purchased from American type culture collection (ATCC, Manassas, VA).

Synthesis of RGD-pyrene conjugate.

Appropriate amount of PBA and HSPyU at molar ratio of 1:2 was dissolved in anhydrous DMF before charging into 50ml flask with magnetic stirrer. 2% DIEA was also introduced into the flask to catalyze the reaction. The reaction was monitored by HPLC (Ultimate 3000, Dionex, Inc. US) equipped with C18, 4.6X 250mm, column (Dionex, Inc). Upon complete reaction of PBA, the mixture was purified by preparation HPLC. All purified products were confirmed by analytical HPLC and LC/MS. Figure S1 shows the synthetic route and representative HPLC and LC/MS analytical results.

Nanoscale graphene oxide (GO) was kindly provide by Professor Zhuang Liu (Soochow University, P.R.China). This nanoscale graphene oxide shows good water solubility and stability over months. To meet our purpose to prepare biosensor for cell marker detection, we further processed this nanoscale GO with sulfuric acid, followed by extensive washing with distill water. After final filtration, the harvested GO was oven-dried at 60^{o}C before usage.

Preparation of RGD-pyrene/GO complex

The processed GO was suspended in distill water at final concentration of 3mg/ml. The water suspended GO was diluted to 1mg/ml with distill water, followed by water bath sonication for 10 min. The resultant GO was further diluted into working concentration with water. The synthesized cRGdyK-pyrene conjugate was dissolved in distill water at 100 μM as the stock solution before series dilution into various working concentrations. Equal volume of peptide solution and GO water solution were combined, followed by brief vortexing. The resulted complex was kept on bench-top for 30min at room temperature before any analytical measurements.

Atomic Force Microscope (AFM) analysis of GO and RGD-pyrene/GO complex

Briefly, AFM imaging of the graphene oxide samples were carried out under a range of deposition conditions in fluid, rinsed with deionized water and dried under a gentle flow of N2 or Ar gas, using gentle tapping-mode and mostly with a PicoForce Multimode AFM (Bruker, CA) consisting of a Nanoscope® V controller, a type E scanner head, and a sharpened TESP-SS (Bruker, CA), or similar AFM cantilever. For either GO or RGD-pyrene/GO sample visualization, suitable surface attachment of the respective sample was achieved readily by five minute incubation of 5 μl of 6 μg/ml fluid suspension on freshly-peeled and slightly tilted mica on metal disks of 12 mm diameters. The excess amount of fluid

was blown away by an inert gas gush, followed by a complete drying of the sample surface under gentler gas flow. The sample was then sealed into the instrument compartment dehumidified by Drierite® particles and imaged via standard optimizations and a range of imaging settings to enhance reliability. For fuller comparison, the sample surfaces were often rinsed up to twice each with 30-50 µl of deionized water to remove salt deposits, dried under gentle air flow, and re-imaged similarly. AFM images were evaluated within the Nanoscope software (version 7.3, Bruker, CA), and exported to Image J (version 1.4x, NIH, Bethesda, MD) for further analyses and display.

Fluorescence measurement of RGD-pyrene and GO quenching effect evaluation

All fluorescent spectra in this study were recorded on Hitachi F-7000 fluorescence spectrometry. The excitation was set to be 380nm, and emission was scanned from 340nm to 650nm. To evaluate the GO quenching ability to RGD-pyrene at 2 µM, GO stock solution was series diluted from 0.1 µg/ml to 10 µg/ml. After mixing for 30min, the fluorescence was recorded.

Purified integrin αvβ3 recovery fluorescence signal of RGD-pyrene/GO complex

The GO-peptide complex was prepared as described above. Integrin at various concentrations were prepared in modified integrin binding buffer (20mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 1mM $MgCl_2$, 1 mM $MnCl_2$ and 0.05% Tween 20), and added into the complex with gentle mixing. Then, the mixture was kept on water bath (37^{o}C) for 1 h before making fluorescence measurement.

Binding specificity analysis

To investigate the binding specificity, the RGD-pyrene/GO complex (2 µM RGD-pyrene, 2 µg/ml GO) was prepared in modified integrin binding buffer (pH 7.4). Meanwhile, different protein or enzymes at fixed concentration were also prepared: integrin (50 nM), anti-actin antibody (1:2000 dilution), FBS (10% in PBS), BSA (1 µM), Rnase (1000 Units), dextrose (10 mM), glucose (10 mM). The measurement was conducted at the same condition as described above.

Cell surface marker detection

MDA-MB-435 and MCF-7 cell lines were cultured in DMEM supplemented with 10% FBS. One day before the detection assay, cells were harvested and re-cultured in the glass bottom 8 well chamber (Lab-tech, Germany). The RGD-pyrene (2 µM) and GO (2 µg/ml) were pre-mixed prior to the incubation with cells for 2 h. Then, cells were immediately washed with ice-cold PBS 3 times before fluorescent microscope (Olympus, Japan) imaging. For the free RGD blocking assay, 0.1 mM free c(RGDyK) peptide was incubated with MDA-MB-435 cells for 1 h before adding the RGD-pyrene and GO mixture for additional 2 h incubation. Free cRGDyK-pyrene at equivalent concentration was also incubation MDA-MB-435 cells for 2 h as comparison.

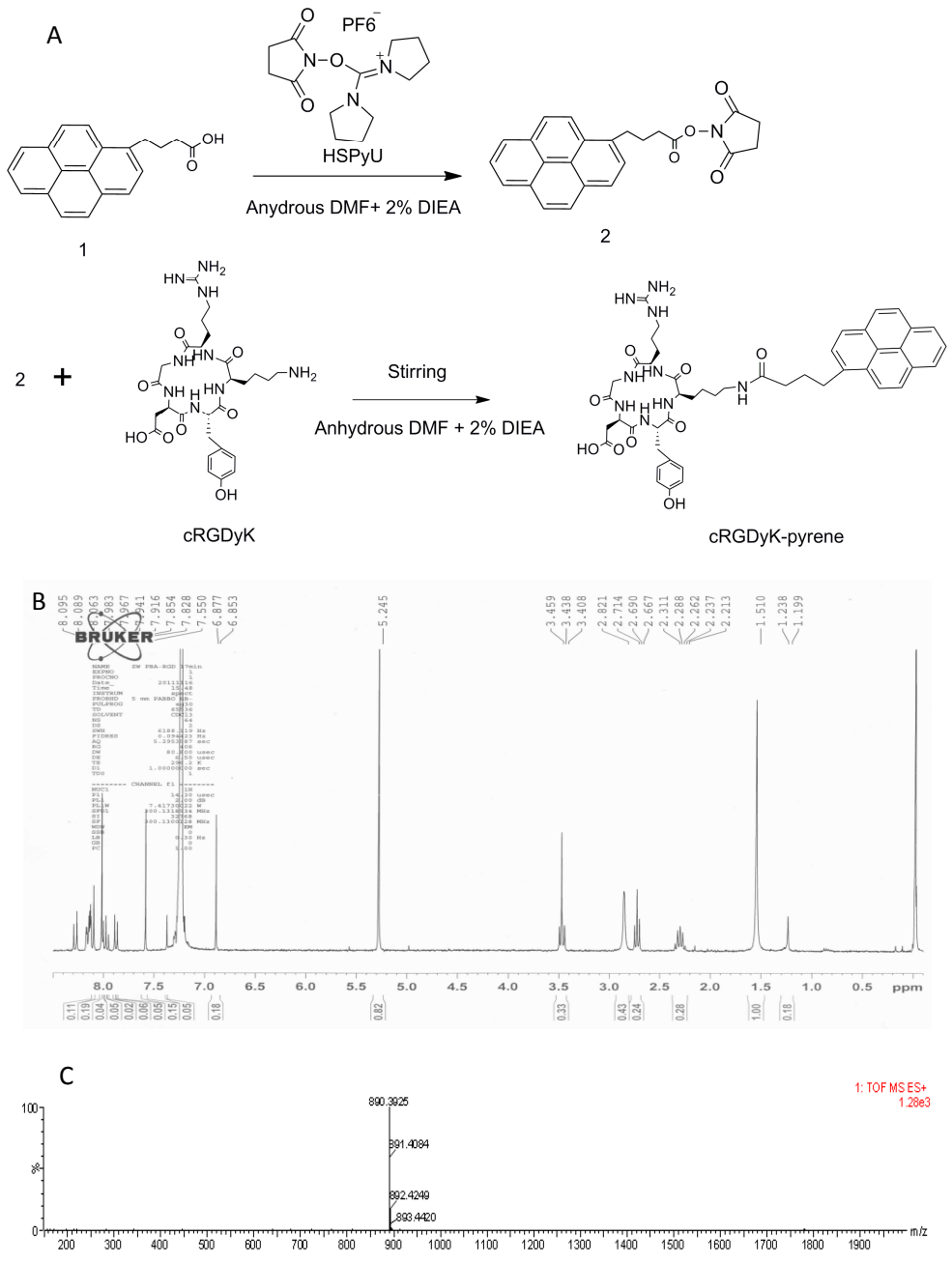

Figure S1. (a) Synthetic scheme of RGD-pyrene conjugate, (b) NMR and (c) mass spectrometry of RGD-pyrene. Abbreviations: DMF, *N,N*-dimethylformamide; DIEA, *N,N*-diisopropylethylamine; HSPyU, dipyrrolidino(*N*-succinimidyloxy)carbenium hexafluorophosphate; c(RGDyK), cyclic Arg-Gly-Asp-D-Tyr-Lys.

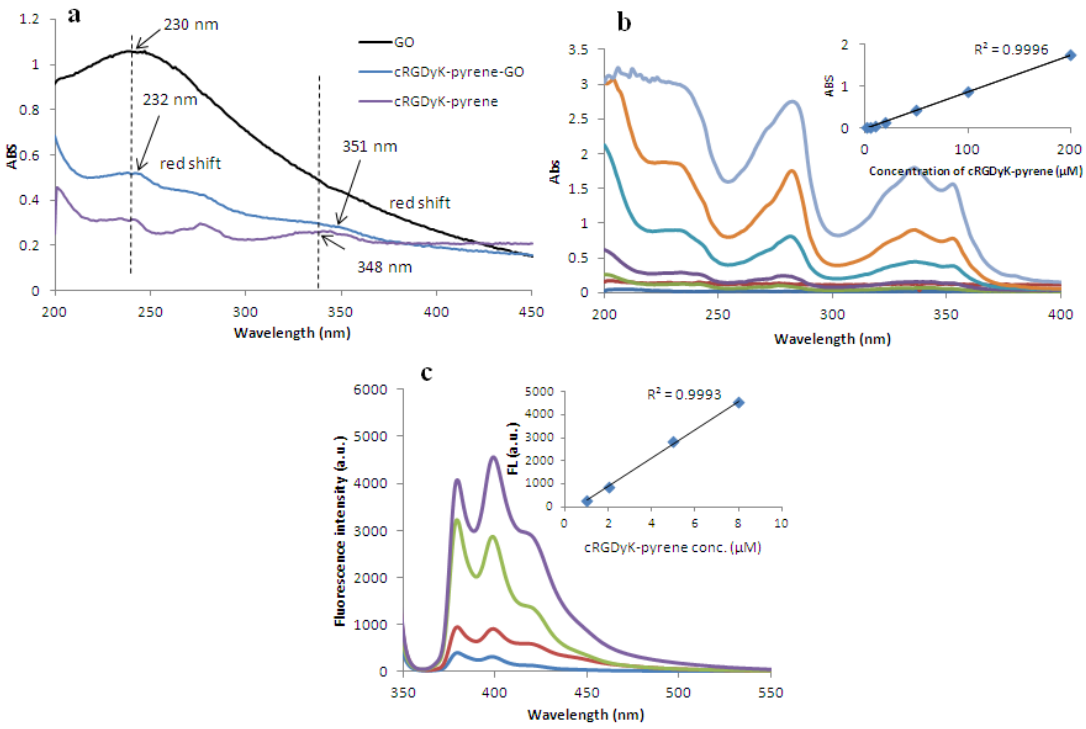

Figure S2. UV/Vis absorption and fluorescent emission spectra. a) UV/vis absorption of RGD-pyrene, RGD-pyrene/GO and GO alone; b) UV/Vis absorption of RGD-pyrene with increased concentration from 2 μM to 200 μM; c) Fluorescence spectra of RGD-pyrene from 1 μM to 8 μM.

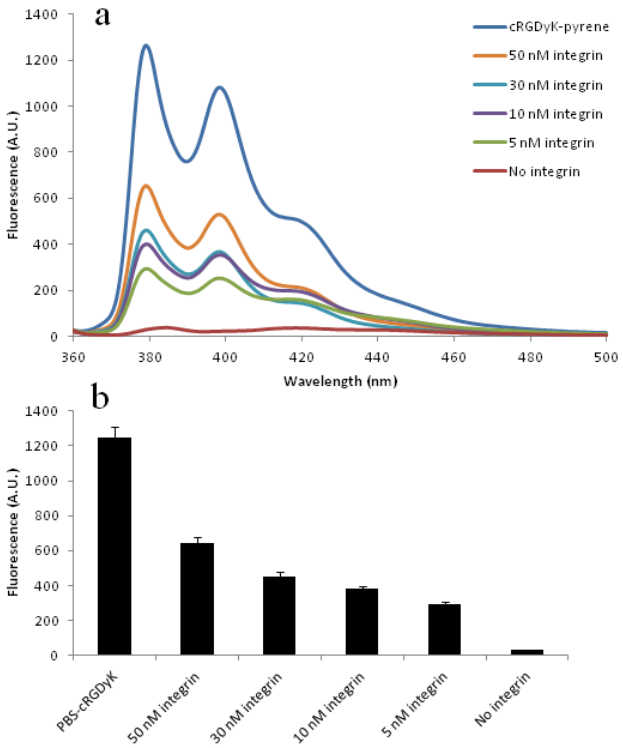

Figure S3. Fluorescence recovery of RGD-pyrene at different concentrations of integrin: a) fluorescence spectra of recovered pyrene fluorescence with ascending concentrations of integrin; and b) fluorescence peak values of pyrene at 380 nm at different concentrations of integrin. Excitation wavelength: 338 nm

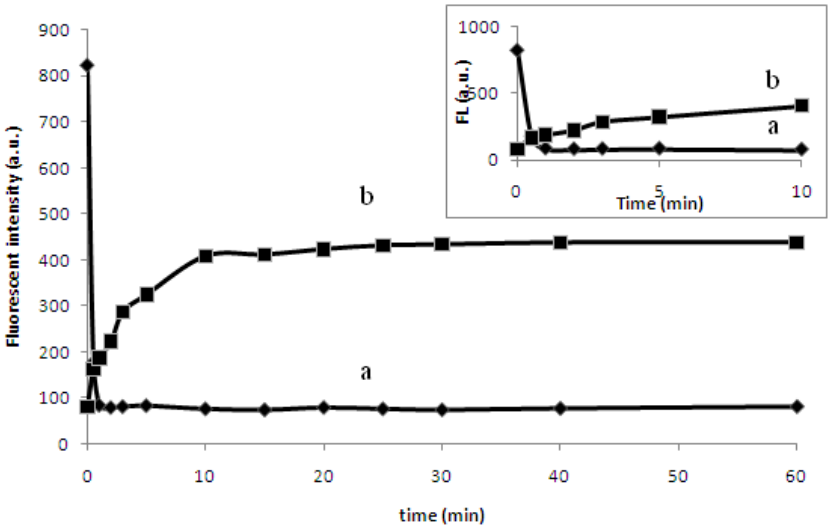

Figure S4. Kinetic studies of quenching effect of GO to RGD-pyrene (curve a) and fluorescence recovery by introduction of integrin in solution (curve b). Insert presents the data points in the first 10 min.

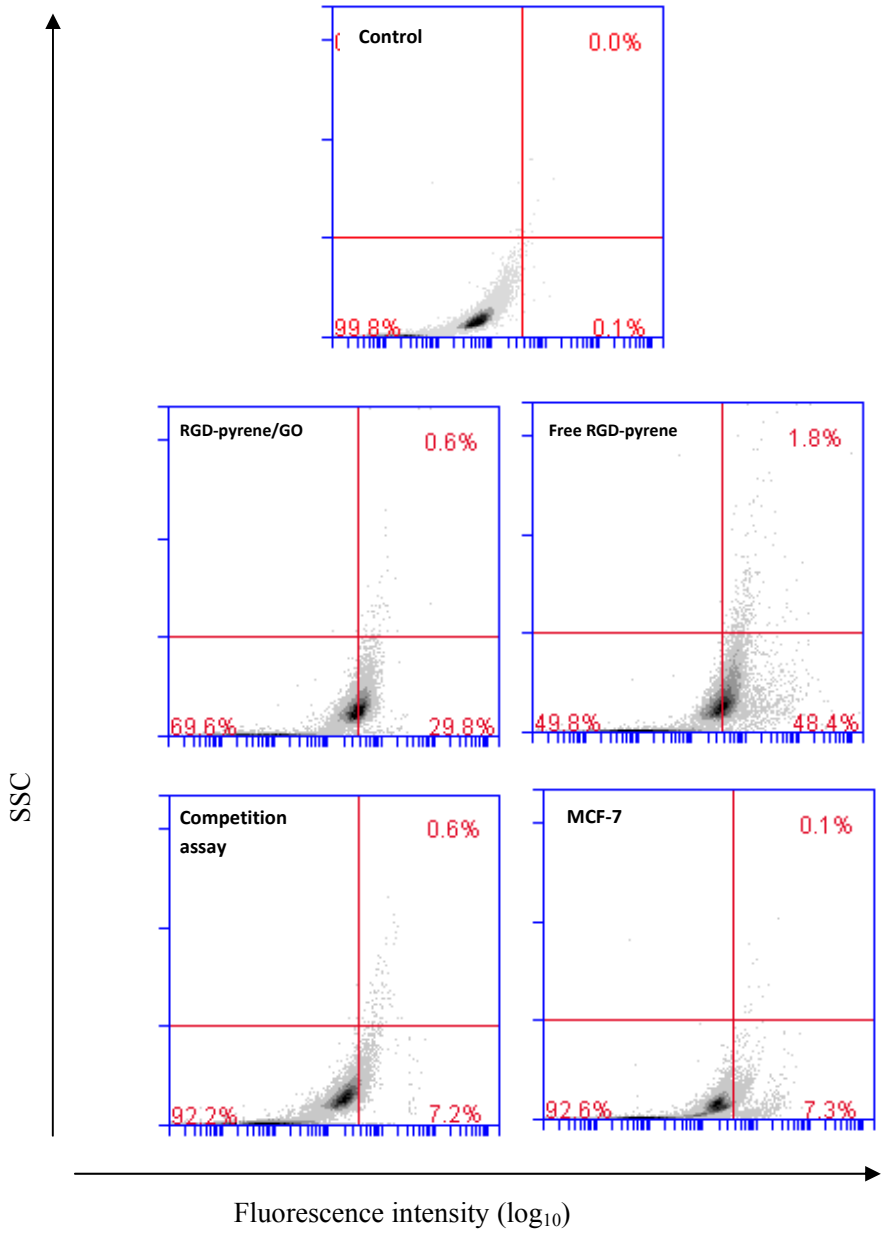

Figure S5. Flow cytometry analysis of cell surface integrin αvβ3 level by RGD-pyrene/GO probe.

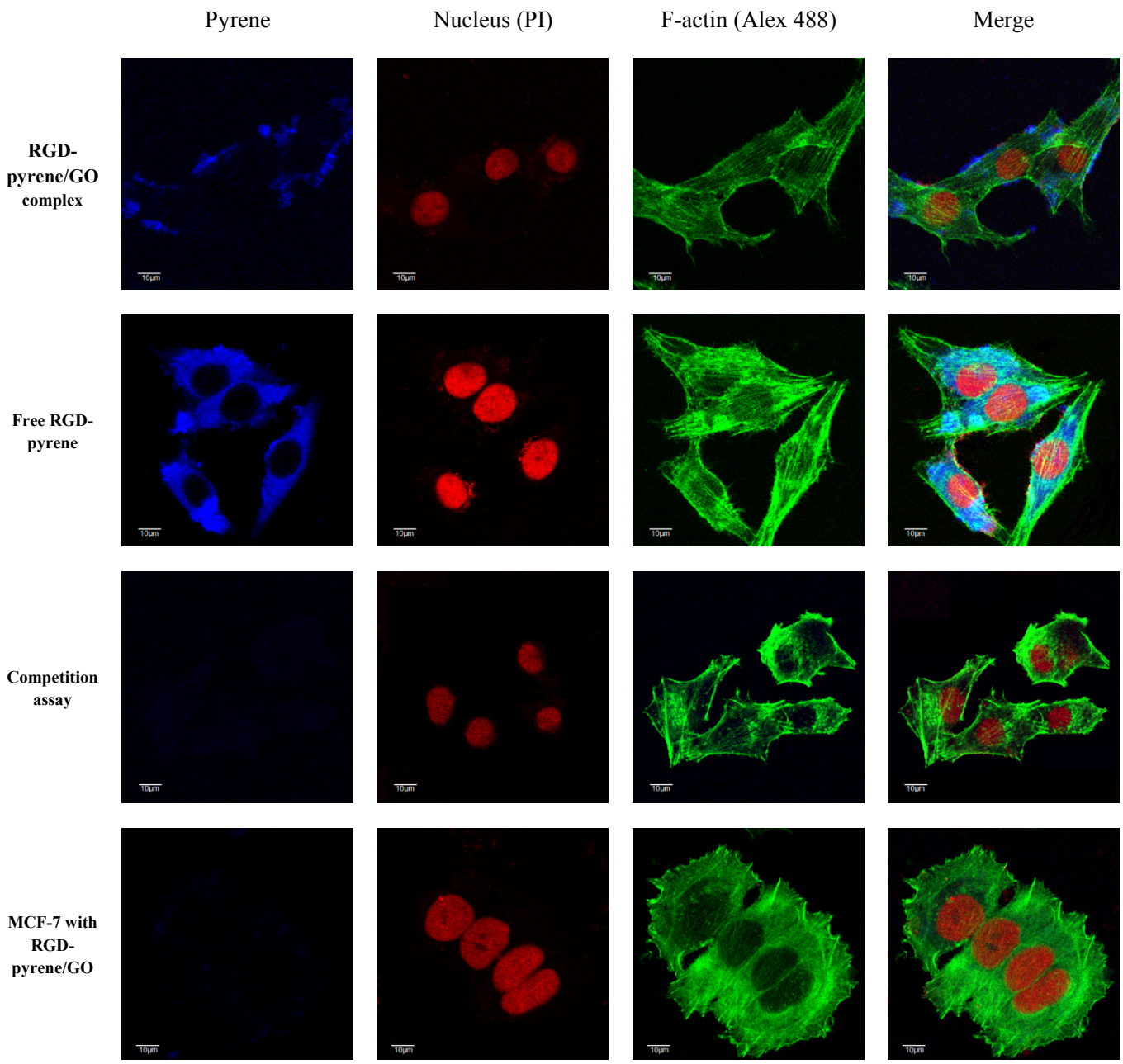

Figure S6. Detection of breast cancer cell surface integrin expression level by RGD-pyrene/GO probe. The cells (MDA-MB-435 and MCF-7) were fixed after specific binding with probes. The RGD-pyrene/GO complex shows preferred binding of RGD-pyrene probe on cell membrane, whilst free RGD-pyrene conjugate demonstrates significant accumulation in cytosol upon cellular incubation (MDA-MB-435). In both competition assay and low surface integrin αvβ3 expression level cells (MCF-7) incubation, the RGD-pyrene/GO probe fluorescence could hardly be recovered. The results confirm the real-time live

cell imaging data in Fig. 4 for the high specificity and binding ability of RGD-pyrene/GO probe in cell surface integrin detection.

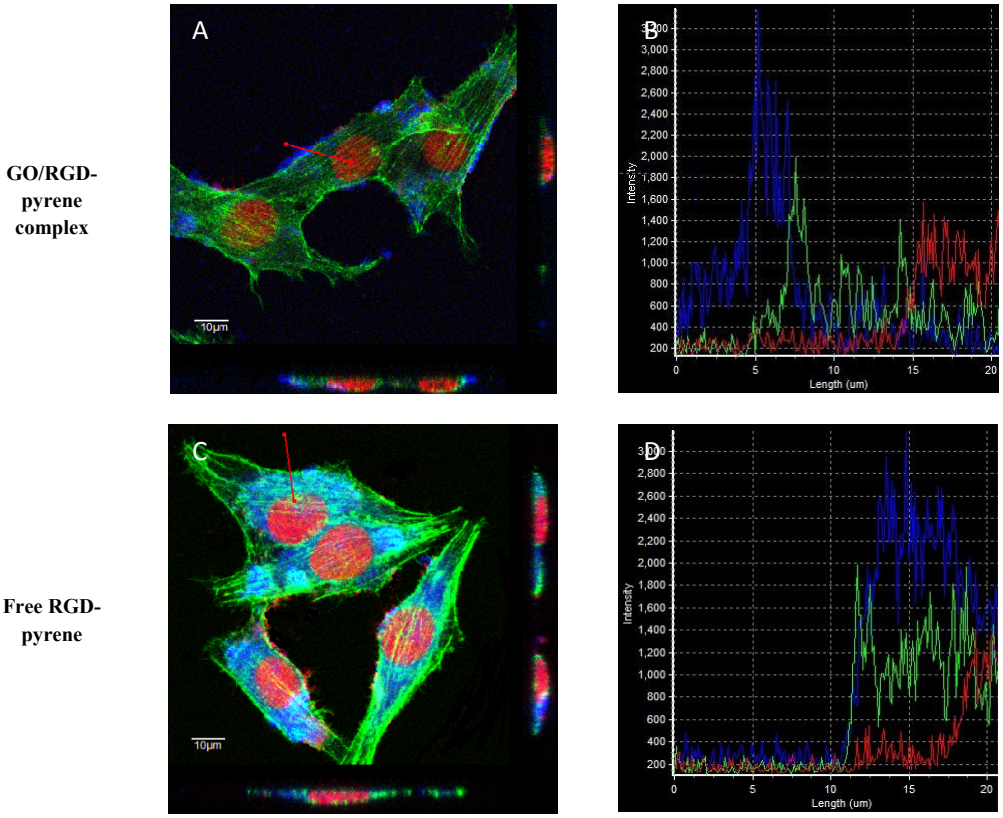

Figure S7. Z-stack imaging (A and C) and fluorescence distribution analysis (B and D) of RGD-pyrene/GO probe (A and B) and free RGD-pyrene conjugate (C and D) association with high integrin expression level of MDA-MB-435 cells. The red lines on A and C indicate the fluorescence distribution analysis areas as represented in B and D, respectively. The Z-stack images and fluorescence distribution analysis data demonstrate the RGD-pyrene is likely resided on cell membrane after binding with integrin (A and B) when GO/RGD-pyrene probe was incubated with cells. In contrast, the RGD-pyrene presents extensive cellular uptake into cytoplasm upon incubation with cells (C and D). The results are consistent with the observation from live cell imaging.

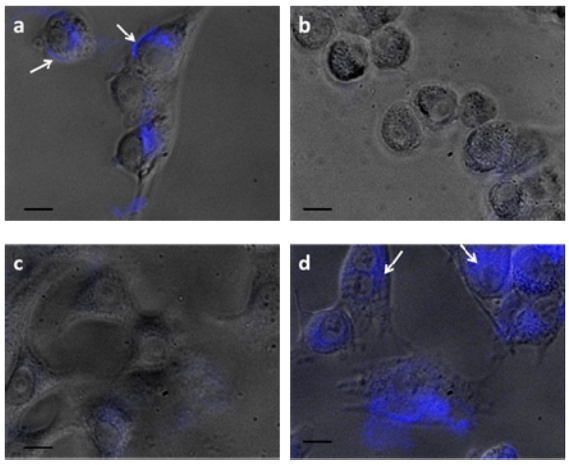

Figure S8. Live cell in situ detection of breast cancer cell surface integrin expression by RGD-pyrene/GO probe; a) probe fluorescence recovery by MDA-MB-435 cancer cells which overexpress integrin αvβ3 on cell surface. The recovered fluorescence is mainly detected on cell membrane as indicated by white arrow; b) MCF-7 cancer cells with very low integrin αvβ3 expression can hardly recover probe fluorescence; c) blocking assay of MDA-MB-435 with free cRGDyK inhibits the probe fluorescence recovery by integrin αvβ3; d) equivalent concentration of free RGD-pyrene incubated with MDA-MB-435 at the same condition demonstrates significant endocytosis as indicated by white arrow. Scale bar=5μm

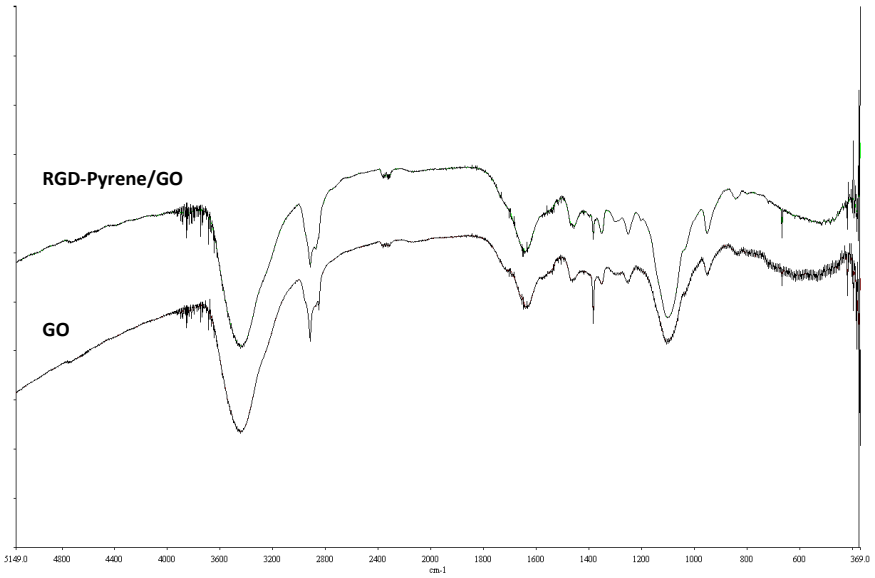

Figure S9. The new bands at 1320 cm^{-1} (C-N stretch of amide) and 1208 cm cm^{-1} (C-O stretch of phenol from tyrosine residue of RGDyK peptide) indicate the introduction of RGD-pyrene conjugate on the GO surface. The intensified peaks at 2400 cm^{-1} are attributed to the strong π-π interaction between pyrene and GO.