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Electronic Supplementary Information

Protein-directed Reduction of Graphene Oxide and Intracellular Imaging

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Chemicals and Materials. Graphite powder was purchased from Alfa Aesar. Herceptin was a generous gift from Genentech Inc. (San Francisco, CA). RPMI-1640 media was purchased from Invitrogen (Carlsbad, CA).

Synthesis of GO. Aqueous dispersed GO was synthesized from graphite powder according to a modified Hummer's method.[1-3] The as-prepared GO was exfoliated by ultrasonication (100 W) of the graphite powder dispersion in water bath for 40 min. Finally, a homogeneous GO aqueous dispersion (2.5 mg/mL) was obtained as a stock solution for further functionalization.

Synthesis of RGO-herceptin. 10 μL of GO aqueous dispersion (1.0 mg/ mL), 50 μL of ddH_2O, and 40 μL of herceptin solution (50 mg/mL) were mixed and vigorously stirred for 2 min. Then, 5 μL of NaOH (1.0 M) was added and followed with another 2 min of vigorous stirring. The initial light yellow-brown solution of GO-herceptin mixture was incubated at different temperatures for several hours until the color of the solution is stable, indicating the successful formation of the RGO-herceptin biocomposites. The products were then purified by centrifugation at 14000 rpm for 15 min. The final purified RGO-herceptin was then redispersed in ddH_2O or phosphate buffered solution (PBS) for further characterization and cellular imaging. RGO-BSA as a control was synthesized and purified using a similar method as mentioned above.

Cell Culture and RGO-herceptin Uptake. SK-BR-3 breast cancer cells were grown in RPMI-1640 media with phenol red, L-glutamine, and HEPES. RPMI-1640 media was supplemented with 10% fetal bovine serum and 1.5% penicillin/streptomycin. The cells were grown in T-25 flasks at 37 ˚C with 5 % carbon dioxide to 70-80% confluency and then split. For splitting, the cells were washed with Hank's solution and then 0.25% trypsin-EDTA was added for 3 minutes. Once the cells were completely detached, 5 mL of RPMI-1640 media was added to dilute the trypsin-EDTA. Trypsin-EDTA was then further diluted by reconstitution of 1 mL of the cells into 5 mL of fresh RPMI-1640 media.

For imaging purposes, the cells were seeded onto circular glass slides with a diameter of 18 mm and a thickness of 1 mm and placed in a 12-well plate. After the cells reached 80% confluency, 1mL MEM buffer with graphene samples (0.1 mg/mL, 100 μL) were added to each of the wells to replace the RPMI-1640 media. The cells were further incubated for the desired time. Before imaging, the cells were washed three times with PBS and then imaged in the presence of 200 μL of PBS.

Instrumentation. UV-vis measurement was carried out using a Jasco V570 UV/Vis-NIR spectrometer (Jasco, Inc., Easton, MD). Atomic force microscopy (AFM) images were recorded using a MFP-3D-BIO Atomic Force Microscope (Asylum Research, USA). The obtained images were processed by using WSxM software.[4] GO or RGO-herceptin dispersions were dropped onto a freshly cleaved mica surface, followed by drying at room temperature for AFM measurement.

Confocal fluorescence images were taken using a MicroTime 200 Fluorescence Lifetime Microscope (Picoquant GmbH, Berlin, Germany) equipped with a Chameleon XR two-photon pulsed laser (Coherent Inc.).[5] The laser excitation was tunable with a range from 700 nm to 980 nm with a frequency of 90 MHz. Laser power used in this study was approximately 22 mW. The emitted laser beam was directed through a beam expander and the set of mirrors and then focused on the sample through a 100× water immersion objective. The objective had a numerical aperture of 0.75. The emitted fluorescence was captured through the same objective and passed into a single photon avalanche photo diode (SPAD) where the signal was filtered in the 500 to 540 nm range. Fluorescence intensity and lifetime values were detected through a 60 second raster scan of 1 μm in depth and 80 μm by 80 μm in area.

To determine the fluorescent characteristics of RGO-herceptin, the dispersed RGO-herceptin solution was dried on a 1 mm thick glass coverslip and then imaged with a confocal fluorescence microscopy.

Of the different excitation wavelengths tested, the 780 nm excitation was found to be the optimal excitation wavelength for the two-photon photoluminescence imaging and the emission signal was detected in the 500-540 nm range.

To determine its fluorescent lifetime, three dried RGO-herceptin were constructed and each sample was measured twice.

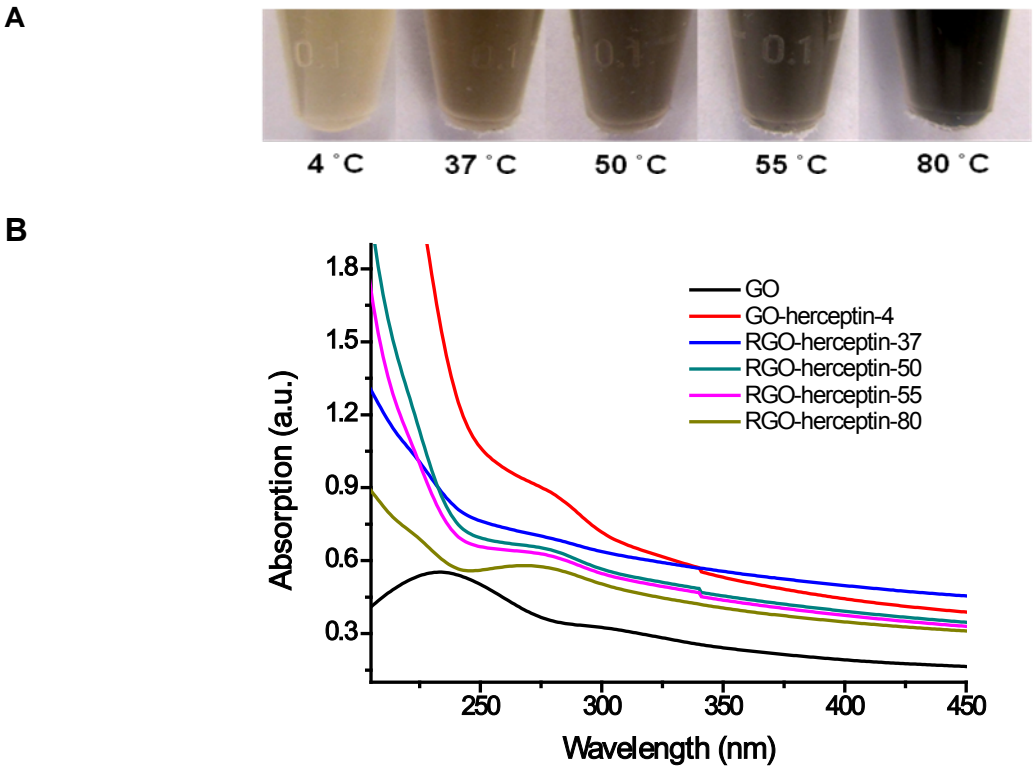

Figure S1. (A) Photographs of GO and herceptin mixture incubated at 4 ˚C and RGO-herceptin composites formed under alkaline condition at different temperature, respectively. (B) Corresponding UV-Vis absorption spectra of GO, mixture of GO and herceptin, and RGO-herceptin composites formed at different temperature. The samples were diluted 10 times before absorbance measurements.

The reduction process could be easily monitored by the color change of the reaction solution, as shown in Fig. S1, compared with the initial light yellow-brown color of the GO-herceptin mixture stored under neutral condition at 4˚C. T he colors of the GO-herceptin mixture incubated under alkaline conditions were darker in hue. The color change indicated the reduction of GO to RGO by herceptin. As the incubation temperature increased, the color of products became darker gradually, suggesting that increasing the temperature could result in higher reduction efficiency. UV-Vis absorption spectra were measured to further verify these results (Figure 1B). GO showed a peak centered at 233 nm, which could be assigned to the π-π* transitions of the aromatic C=C bonds of GO.[6] The mixture of GO and herceptin without NaOH, showed only a weak and broad absorption peak around 280 nm, due to the absorption of the protein, herceptin. While after incubation under the alkaline condition, a peak around 268 nm, characteristic peak of RGO,[7] appeared and increased gradually as the temperature increased, which confirmed that GO was reduced to RGO by herceptin.

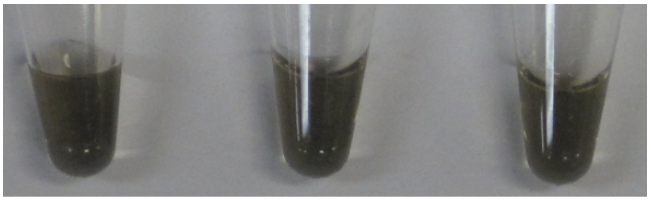

Figure S2. Photograph of the RGO-herceptin composites under different concentrations of NaCl.

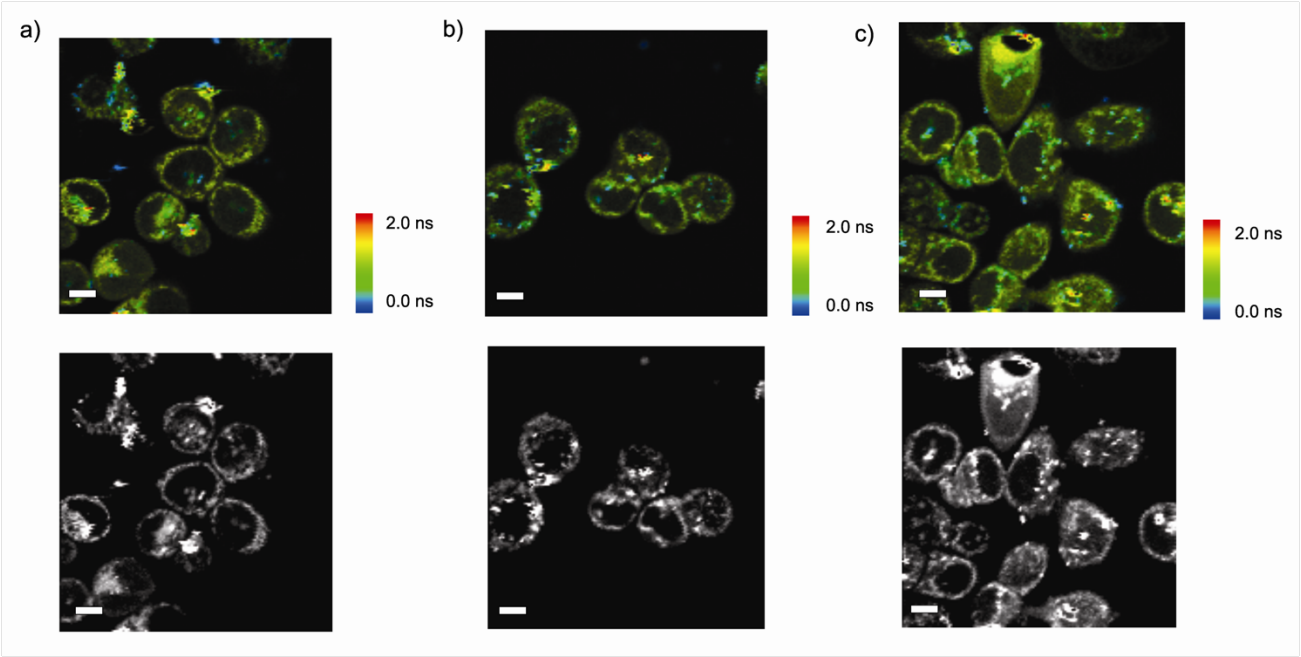

Figure S3. Confocal fluorescence lifetime images (top) and the corresponding fluorescence intensity images (bottom) of SK-BR-3 cell treated with RGO-herceptin formed under alkaline condition at temperatures of: (A) 37 ˚C, (B) 50 ˚C, and (C) 70 ˚C. Scale bar is 10 μm.

The cellular uptake of the RGO-herceptin formed at different temperatures was also investigated to assess the effect of temperature on biomaterial formation. Variations in temperature did not show a significant difference in uptake efficiency as observed in Fig. S3 and 4. Since there was no apparent difference between RGO-herceptin composites synthesized at different temperatures, RGO-herceptin prepared by incubation with the reducing agent at 55˚C was used the cellular uptake experiments.

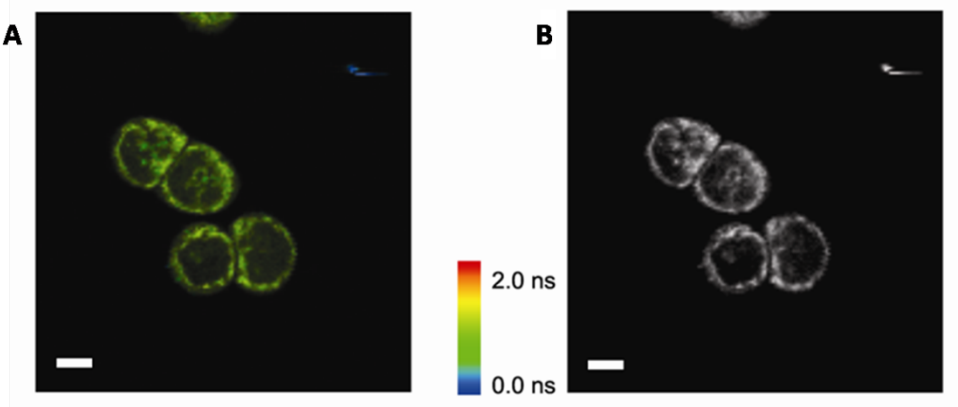

Figure S4. Confocal fluorescence lifetime image (A) and the corresponding fluorescence intensity image (B) of SK-BR-3 cells after 4 hours of incubation with RGO-BSA formed at 50 ˚C. Scale bar is 10 μm.

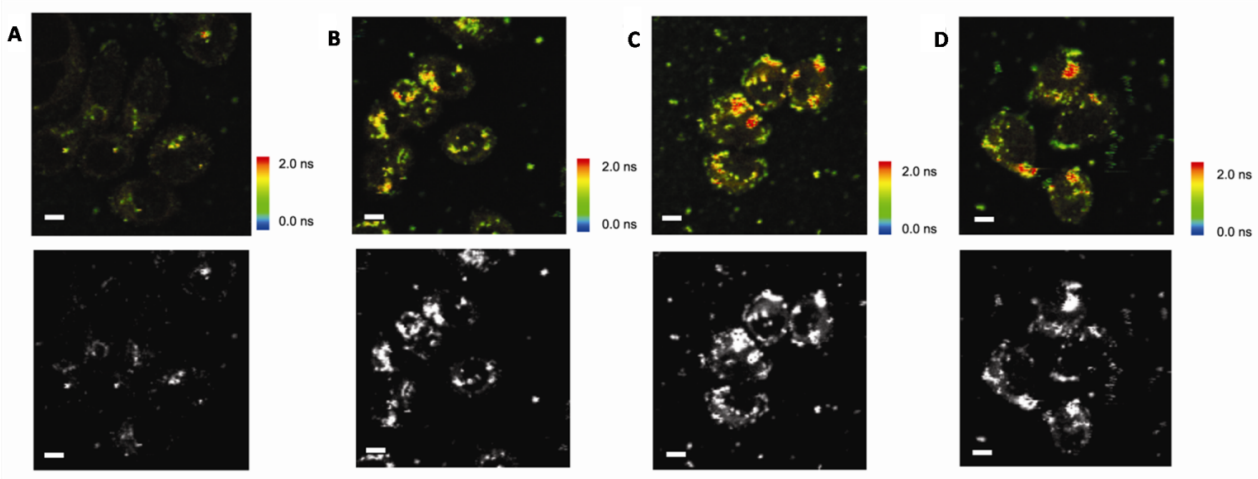

Figure S5. Confocal fluorescence lifetime images (top) and the corresponding fluorescence intensity images (bottom) of RGO-herceptin formed at 55 ˚C in SK-BR-3 cells under incubation conditions of: (A) 4 hours, (B) 8 hours, (C) 16 hours, and (D) 28 hours. Scale bar is 10 μm.

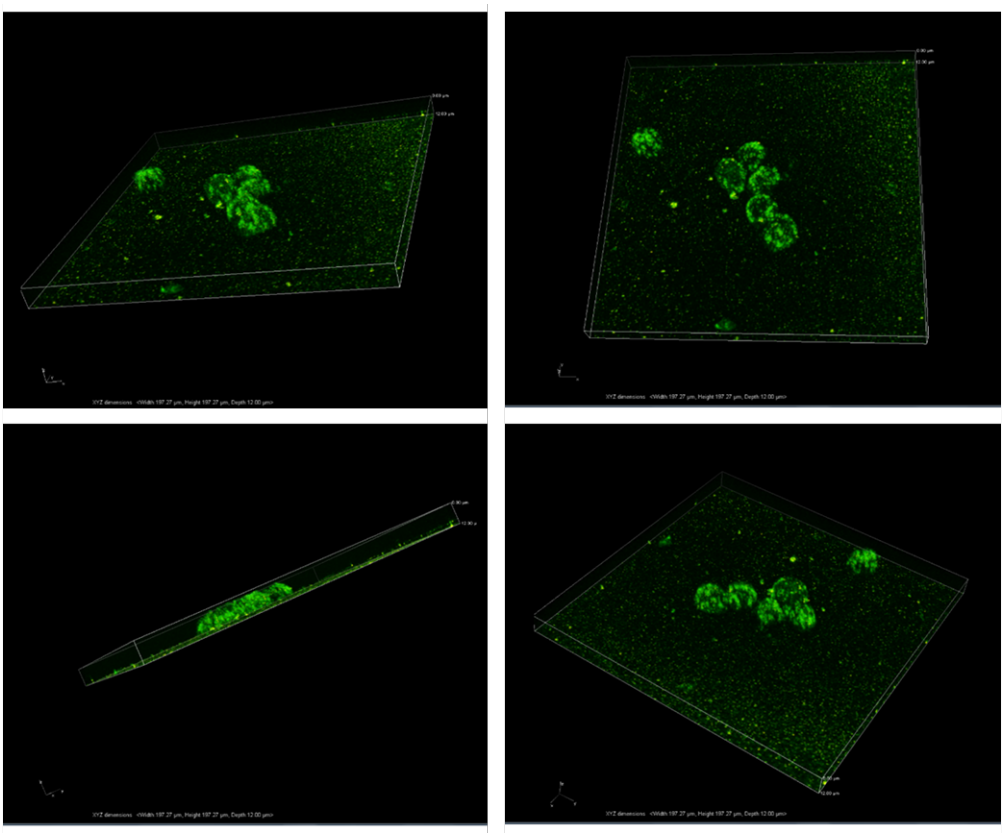

Figure S6. The 3-dimensional reconstructed z-stack confocal fluorescence image indicating the localization of RGO-herceptin in SK-BR-3 cells after 14 hrs of incubation.

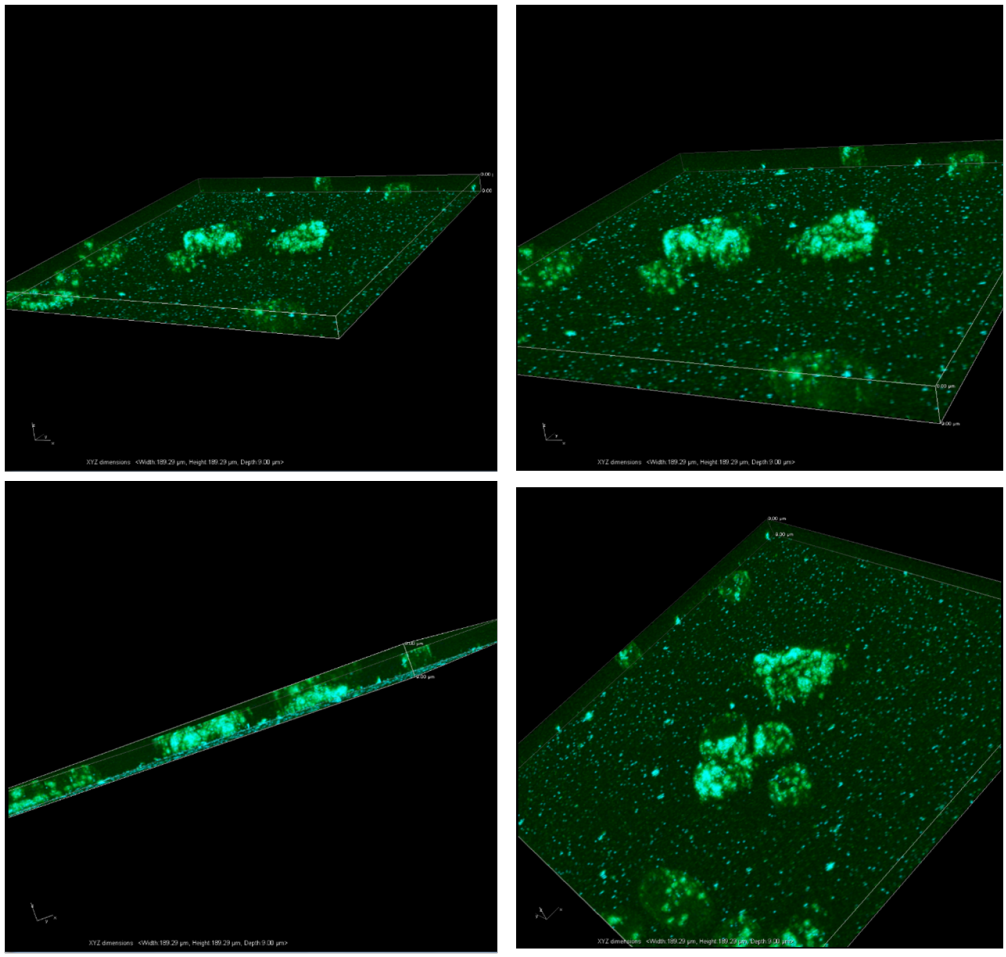

Figure S7. The 3-dimensional reconstructed z-stack confocal fluorescence image indicating the localization of RGO-herceptin in SK-BR-3 cells after 16 hrs of incubation.

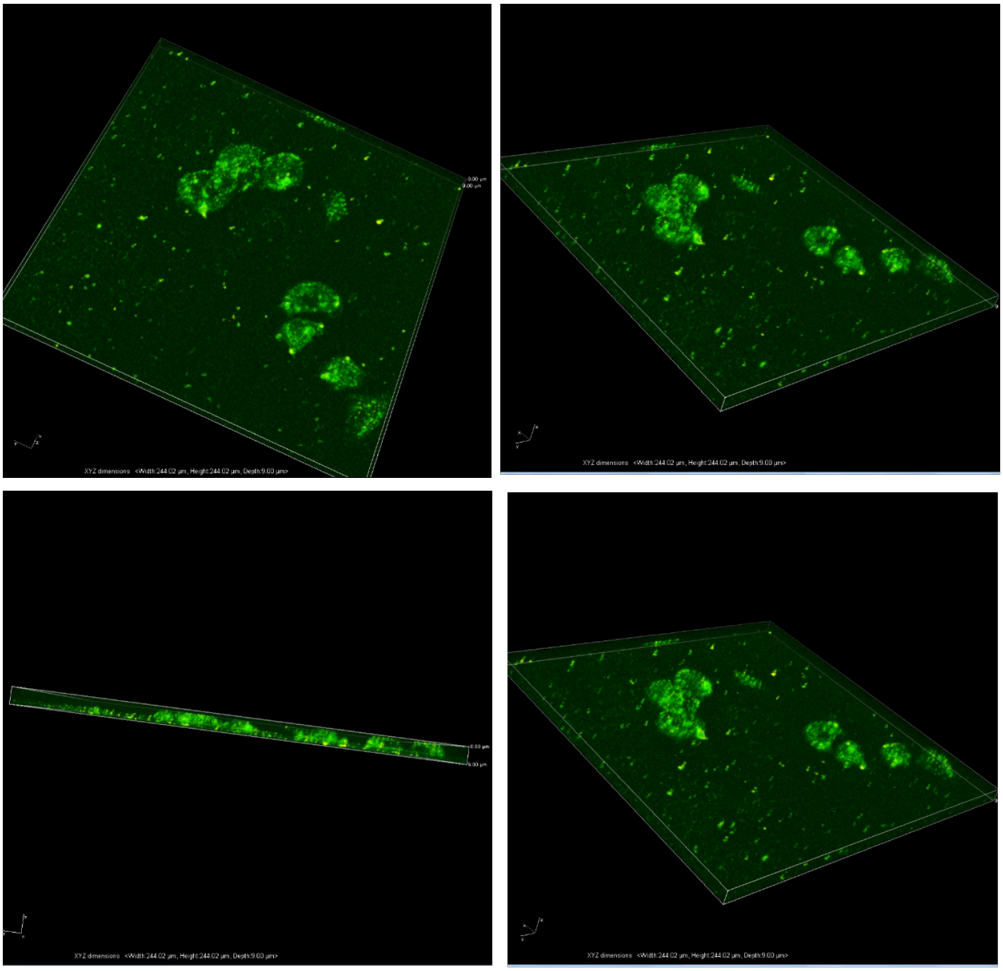

Figure S8. The 3-dimensional reconstructed z-stack confocal fluorescence image indicating the localization of RGO-herceptin in SK-BR-3 cells after 24 hrs of incubation.

References

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