Electronic Supplementary Information

Label-Free Tracking of Nanosized Graphene Oxide Cellular Uptake by Confocal Raman Microscopy

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Contents

S1 Preparation of GO

A modified Hummers method[1] was employed for preparation of GO from the expanded graphite. The average size of the expanded graphite particles was 5 μm and was used as delivered (SGL Carbon group, Germany). For GO oxidation, we used chemicals of analytical grade purity. After drying, the final product was dispersed in UPW (ultra pure water, $\rho \approx 18$ MΩ.cm) and centrifuged (Sigma 3–30 K, Sigma Centrifuges, UK) at 10 000 g for 40 min to remove the unexfoliated GO flakes. In the second step, the supernatant was further refined by centrifugation at 60 000 g for 120 minutes. The resulting supernatant was collected. The final solution contained approximately 0.05 $mg.ml^{-1}$ of GO with the average lateral flake size of less than 100 nm with more than 95% of flakes being smaller than 200 nm. More than 90% GO flakes were monolayer as confirmed by the AFM measurements.

S2 Cell viability assay

To evaluate the toxicity of GO on various cell lines the Cell Titer Blue viability assay (Promega Madison, WI) was used. The assay was performed according to the manufacturer's instructions. Briefly, the cells were plated in 96-well plates (10,000 cells/well) and allowed to grow for 24 h. Tested GO samples were suspended in the culture medium at concentrations of 10 and 100 μg/ml and added to the wells. The cells cultured in the medium without GO served as controls (100%). After 24, 48 and 120 h, 20μl of the CellTiter-Blue solution was directly added to the wells and incubated for another 4 h at 37 °C. The fluorescence was recorded with 530 nm/590 nm (excitation/emission) filter set using Bio-Tek Synergy HT microplatereader. The samples were tested three times for each concentration of GO nanoparticles.

Human cervical cancer cells C33a, cervical carcinoma HeLa cells, colon carcinoma RKO cells, renal carcinoma ACHN cells, lung adenocarcinoma A549 cells, and HEK 293 human embryonic kidney cells, were used for in vitro experiments. The cells were routinely cultivated in DMEM medium with 10% FCS (BioWhittaker) at 37 °C in 5% CO_2 in air.

S3 Raman spectra of the intracellular matrix near internalized GO flakes

Tryptophan (Trp), the amino acid with aromatic side group present in lysozyme, gives rise to a strong Raman line[2, 3] near 760 cm^{-1} (Trp breathing mode). The Raman spectrum of the intracellular matrix near GO flakes (Fig. S1) shows a significant increase of Trp signal when compared to the averaged intracellular matrix.

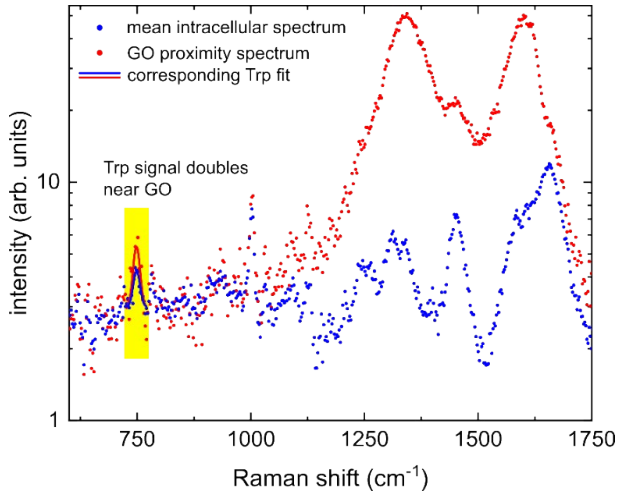

Figure S1 – The Raman spectra of the averaged intracellular matrix (blue) and the intracellular matrix in the proximity of GO flakes (red).

References

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