Electronic Supplementary Material (ESI) for Nanoscale.

Graphene oxide size and oxidation degree govern its supramolecular interactions with siRNA

Giacomo Reina,[a] Ngoc Do Quyen Chau,[a] Yuta Nishina,[b,c] Alberto Bianco*[a]*

[a]University of Strasbourg, CNRS, Immunopathology and therapeutic chemistry, UPR 3572, 67000 Strasbourg, France. E-mail : a.bianco@ibmc-cnrs.unistra.fr, g.reina@ibmc-cnrs.unistra.fr
[b]Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan.
[c]Research Core for Interdisciplinary Sciences, Okayama University, Tsushimanaka, Kita-ku, Okayama 700-8530, Japan

Electronic Supplementary Information

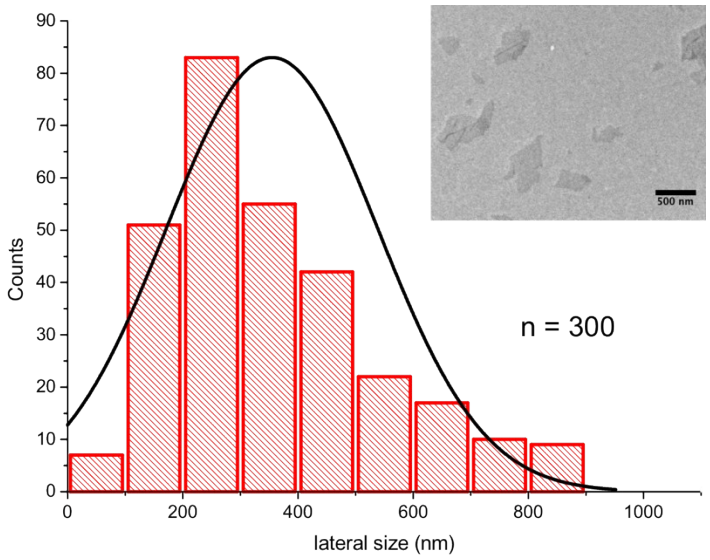

Figure S1. Lateral size distribution calculated by TEM, on the inset TEM image of GO_S.

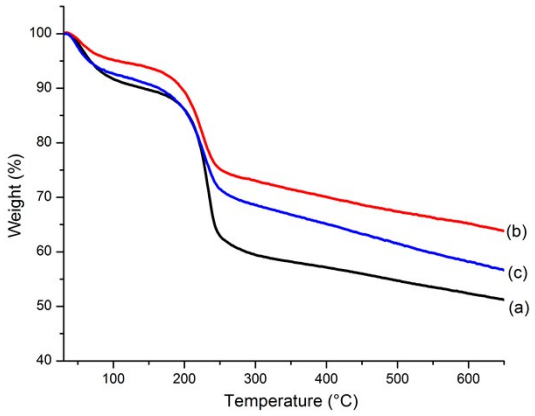

Figure S2. TGA spectra of the different graphene derivatives of GO_S. GO_S in black (a), rGO_S in red (b), and rGO_S-O_3 in blue (c).

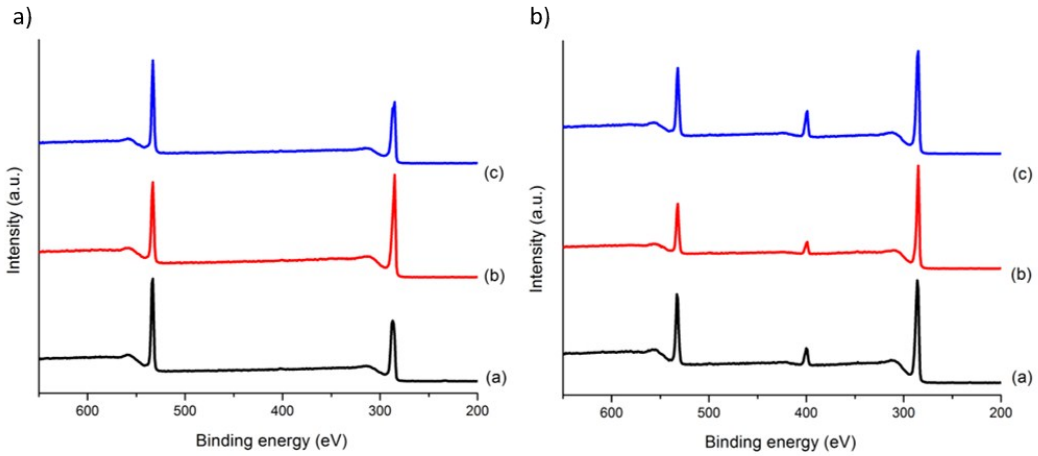

Figure S3. XPS spectra of different graphene derivatives of GO_S and their corresponding PEI functionalization: a) GO_S in black (a), rGO_S in red (b), and rGO_S-O_3 in blue (c); b) GO_S-PEI in black (a), rGO_S-PEI in red (b), and rGO_S-O_3-PEI in blue (c) The C(1s) photoelectron binding energy was set at 284.5 ± 0.2 eV and used as reference for calibrating the other peak positions.

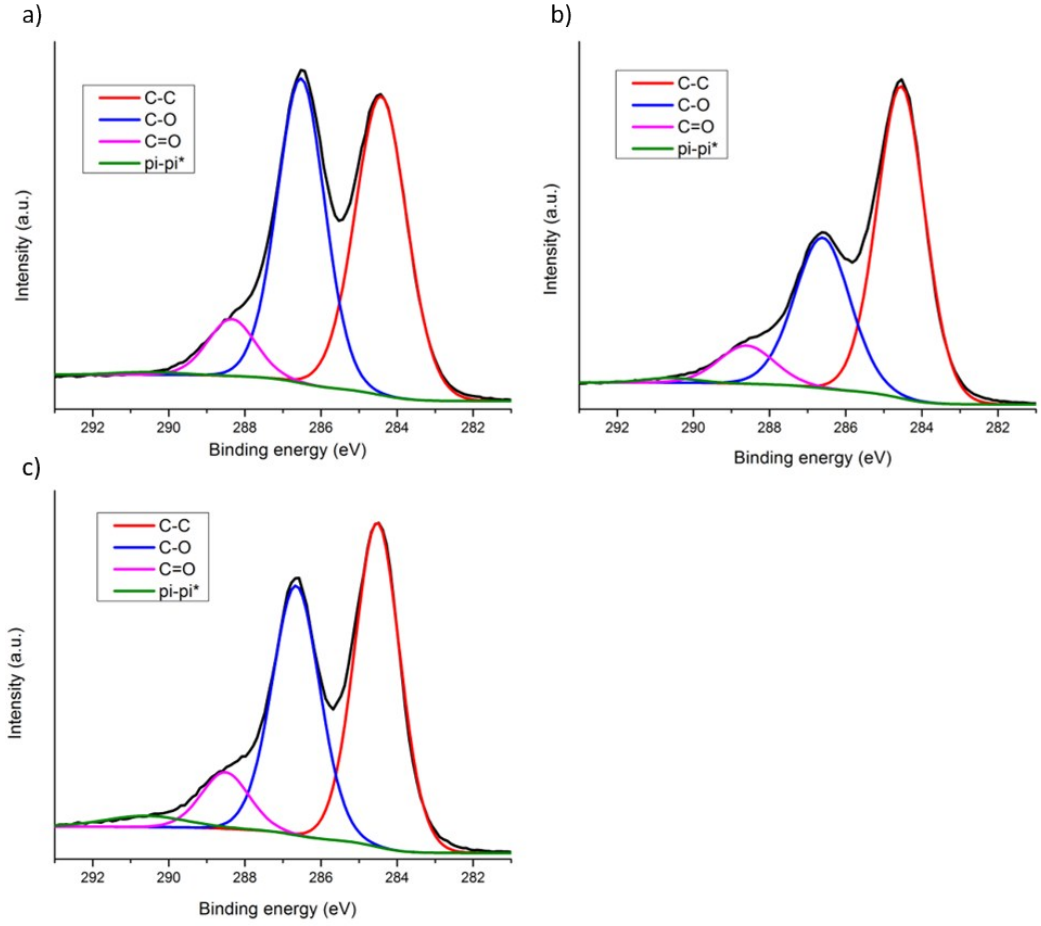

Figure S4. Deconvolution of the C(1s) peak for GO_S (a), rGO_S (b), and rGO_S-O_3 (c).

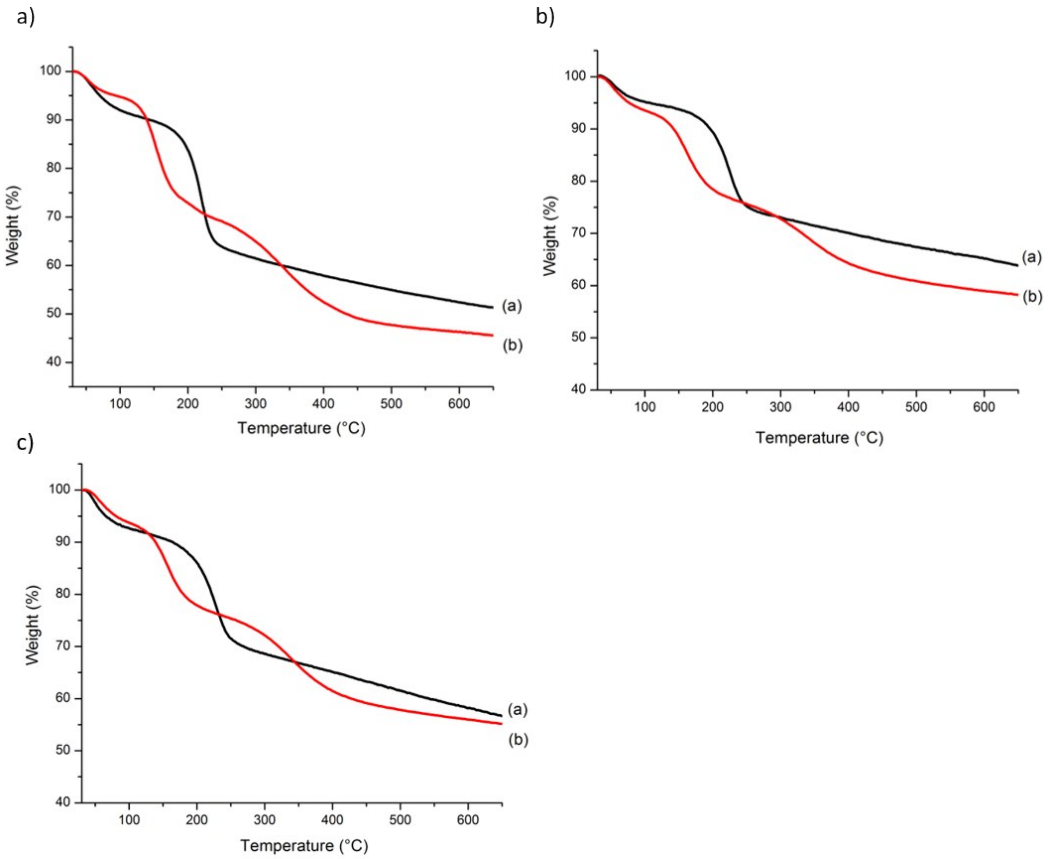

Figure S5. TGA spectra of PEI functionalized GO: a) GO_S in black (a), GO_S-PEI in red (b); b) rGO_S in black (a), rGO_S-PEI in red (b); c) rGO_S-O_3 in black (a), rGO_S-O_3-PEI in red (b). GO displays a typical thermogram with degradation in three steps. The significant weight loss below 100 °C is ascribed to mainly the desorption water. PEI decomposition was recorded above 300 °C. We estimate a functionalization between 6 and 10% in weight of PEI for the GO_L, similar to that estimated for GO_S [1].

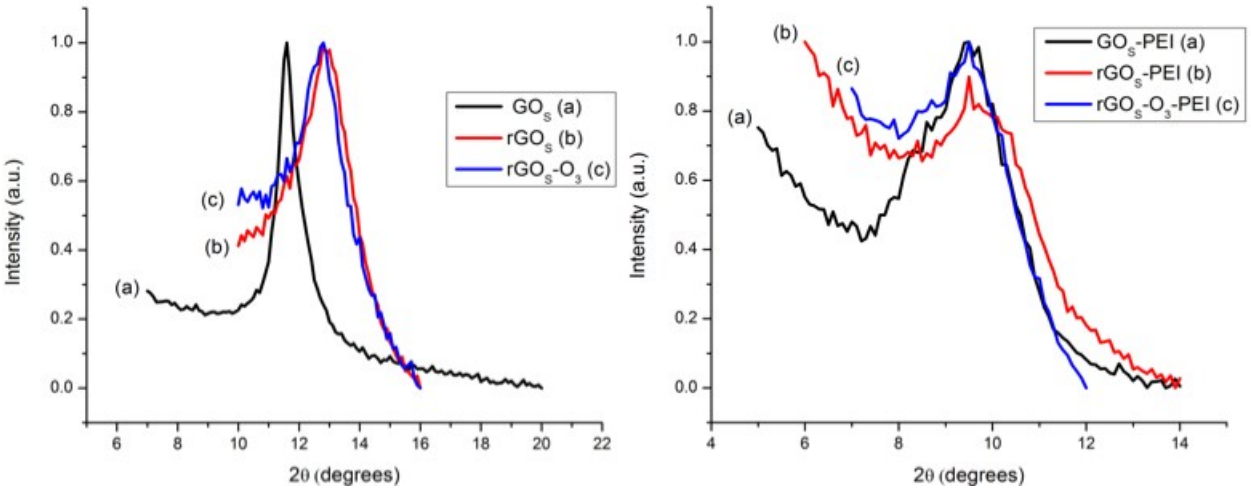

Figure S6. High resolution XRD spectra of the GO_S series. On the left: a) GO_S (black line), b) rGO_S (red line), and (c) rGO_S-O_3 (blue line); on the right: a) GO_S-PEI (black line), b) rGO_S-PEI (red line), and (c) rGO_S-O_3-PEI (blue line).

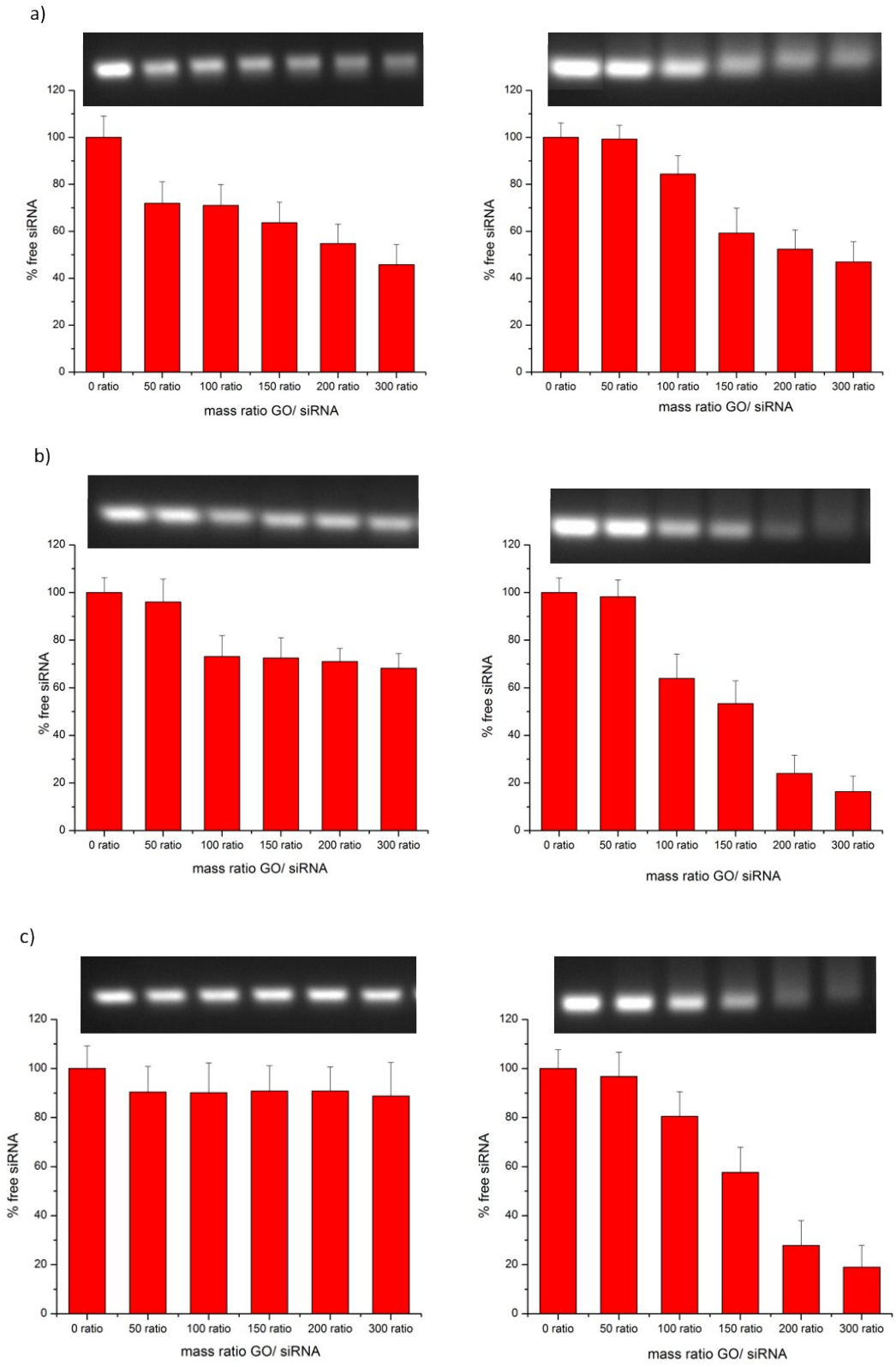

Figure S7. Complexation of different graphene derivatives of GO_S (first column) and their corresponding PEI functionalized (second column): row a) GO_S, row b) rGO_S, row c) rGO_S-O_3. Top: image of the electrophoresis gel; bottom: histograms showing the free siRNA signal at different GO/siRNA mass ratios.

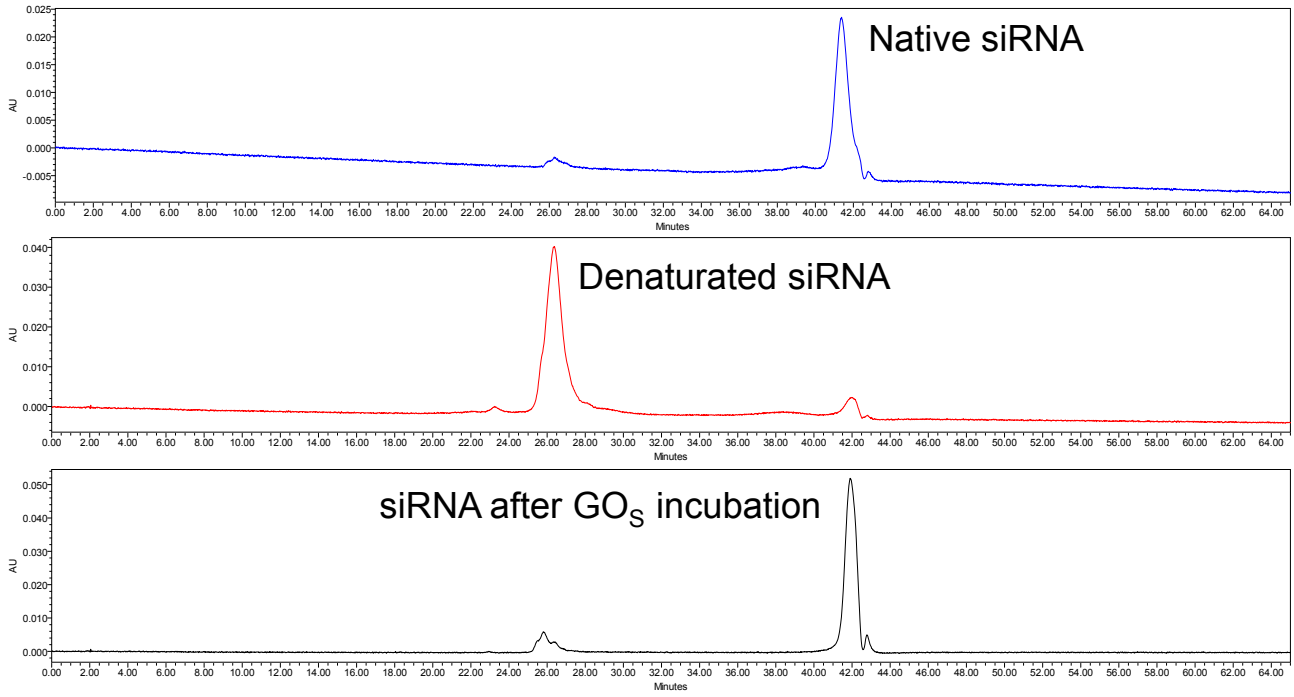

Figure S8. HPLC analysis of Alexa Fluor®647 labelled siRNA: blue curve corresponds to native siRNA, red curve correspond to the siRNA denaturated by formaldehyde, and black curve corresponds to siRNA after incubation with GO_S.

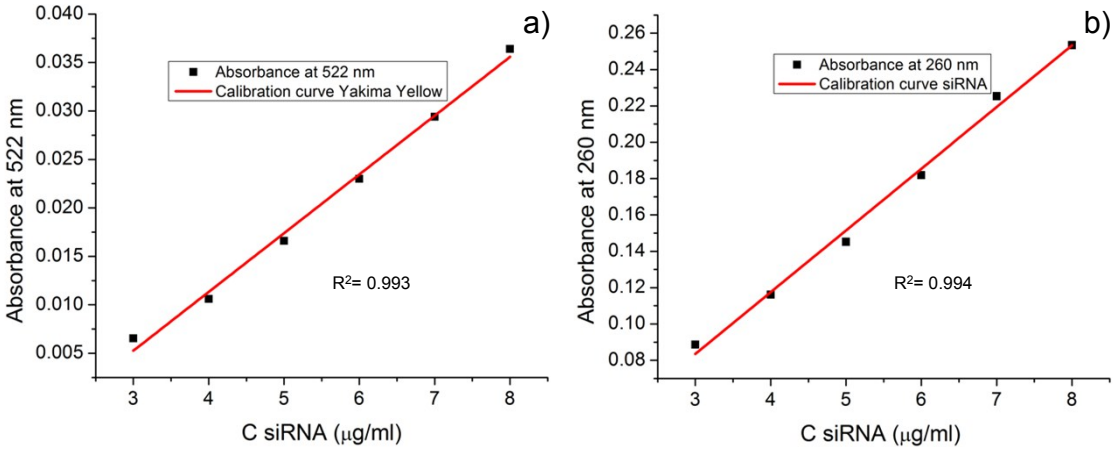

Figure S9. a) Calibration curve of Yakima Yellow signal at 522 nm; b) calibration curve of siRNA signal a 260 nm.

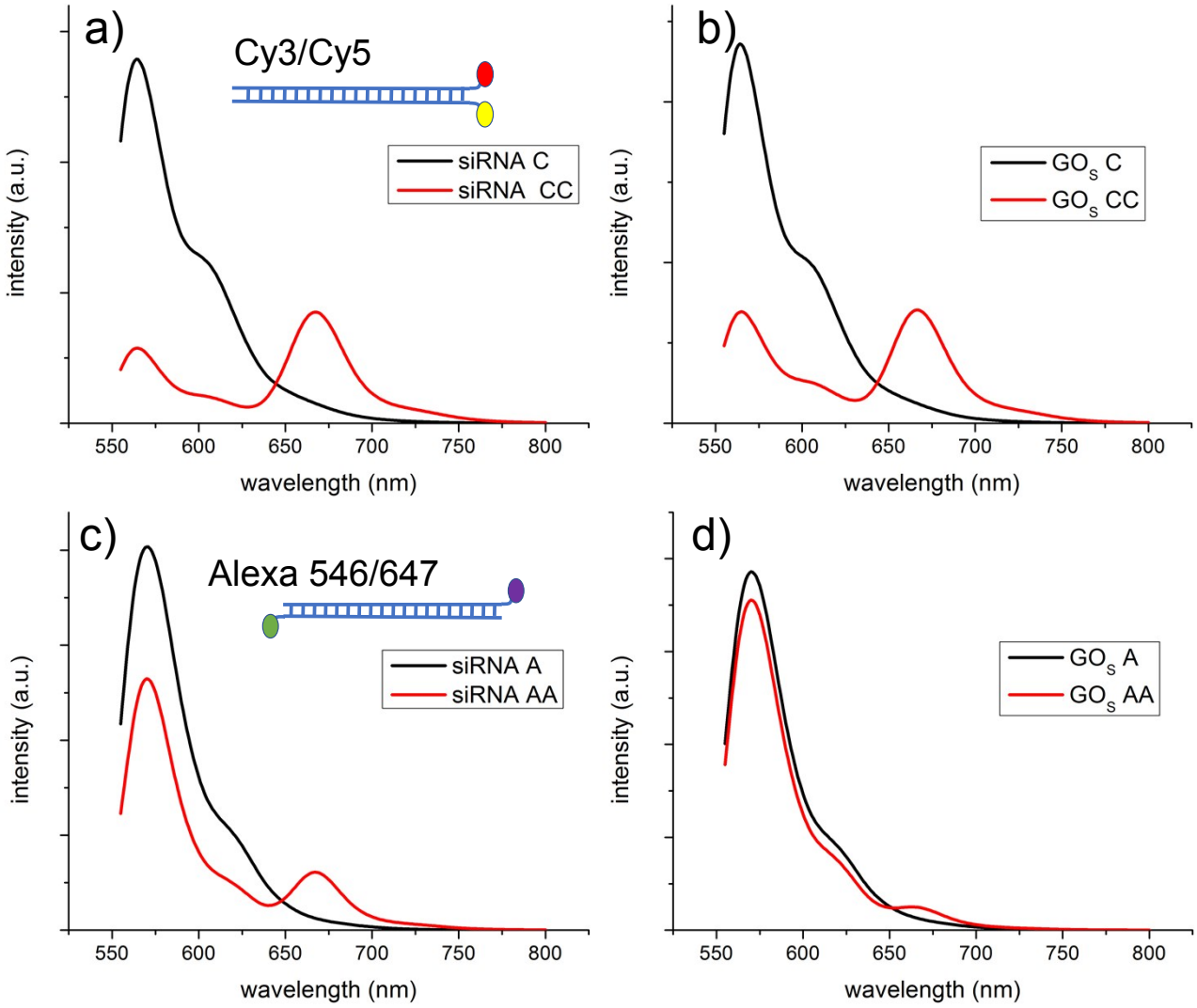

Figure S10 Emission spectra of a) 3'-Cy3 (black line) and 3'-5'-Cy3/Cy5 (red line) labelled untouched siRNA and b) after incubation with GO_S. c) Emission spectra of 5'-Alexa Fluor®546 (black line) and 5'-5'-Alexa Fluor®546/ Alexa Fluor®647 (red line) labelled untouched siRNA and d) after incubation with GO_S. Mass ratio siRNA/GO corresponds to 1:20. Excitation wavelegth 523 nm.

Table S1. N atomic % of PEI functionalized GO_S calculated from the XPS peak N1s at 400 eV.

	Small GO		
	Starting	Hydrothermal	Re-epoxidation
PEI-functionalized	GO_S-PEI	rGO_S-PEI	rGO_S-O_3-PEI
N atomic %	7.4 ± 0.1	5.9 ± 0.1	10.1 ± 0.1

NOTE: The values for large GO_L are reported in our previous publication [1].

Table S2. [002] Calculated distance from XRD using Bragg law, λ = 1.541Å.

Sample	XRD max. (2θ°)	Distance (Å)
GO_S	11.6	7.6
rGO_S	12.9	6.8
rGO_S-O_3	12.8	6.9
GO_S-PEI	9.5	9.3
rGO_S-PEI	9.5	9.3
rGO_SO_3-PEI	9.5	9.3

Equations (1) and (2):

$$E = 1 - \frac{A_D\, I_{DA}}{A_{DA}\, I_D} \quad (1) \qquad\qquad E = \frac{R_0^6}{R^6 + R_0^6} \quad (2)$$

A_D and A_{DA} are the UV absorbance at the excitation wavelength of the donor (535 nm) alone and of the donor in the presence of the acceptor, respectively, while I_D and I_{DA} are the fluorescence intensities of the solution of the donor alone and the donor in the presence of the acceptor at the maximum (570 nm for Alexa Fluor®546, and 565 nm for Cy®3). The FRET distances (R) have been calculated following equation (2), where R is the distance between the donor and the acceptor, and R_0 is the Förster radius distance.

References

[1] N. D. Q. Chau, G. Reina, J. Raya, I. A. Vacchi, C. Ménard-Moyon, Y. Nishina and A. Bianco, *Carbon*, 2017, **122**, 643–652.