Electronic Supplementary Information (ESI)

Tracking graphene by fluorescence imaging: a tool for characterizing graphene in solution

G.Guidetti,[a] A. Cantelli,[a] R. Mazzaro, [a,b] L. Ortolani,[b] V. Morandi [b] and M. Montalti [a]

a. Department of Chemistry G.Ciamician, University of Bologna, Italy, b. CNR- IMM, Bologna, Italy

1 Reagents and materials

All reagents were purchased from Sigma-Aldrich and used as received. In particular exfoliation was performed in Millipore water using graphite flakes **G** (332461 ALDRICH) and Pluronic® P-123 (**P**) surfactant purchased from Sigma–Aldrich (product code: 435465 ALDRICH). To obtain fluorescent surfactants the fluorophores: tetraethyl rhodamine-5-(and 6) Isothiocyanate (**R**; RBITC; Product: 283924 Aldrich) and fluorescein isothiocyanate isomer I (**F**; FITC; Product: F7250 Sigma) were purchased from Sigma–Aldrich.

2 Synthesis

2.1 Fluorescent surfactants

Fluorescent surfactants **PF** and **PR** were prepared using a solvent-free approach. In particular, for synthesizing **PF** the surfactant **P** (2000 mg, 0.35 mmol) was mixed with **F** (0.7 mg, 0.002 mmol) in a mortar for 10 minutes. Mixture was then introduced in an oven at 120 °C for 1 hour. The product was purified by size-exclusion chromatography to remove the unreacted fluorophores.

PR was synthesized following the same procedure reported above for **PF** starting from **P** (2000 mg, 0.35 mmol) and **R** (0.9 mg, 0.002 mol).

2.2 Graphite exfoliation procedure

Graphene water dispersions (10 mL sample volume in cylindrical vials) were prepared according to the method proposed by Guardia et al.[1] In particular 10 mg of graphite **G** were suspended in 10 ml ultrapure water in the presence of the surfactant (0.5%w/v). The mixture was first vortexed at 500 rpm for 30 minutes and then sonicated for 4 hours (ELMA TRANSSONIC T 460/H – 35 KHz frequency Elma GmbH & Co KG) at 40 °C. Additionally, the mixture was handy-stirred every hour. After settling overnight (15 hours) the upper 70% of the suspension was collected and analyzed.

3 UV-Vis and Fluorescence spectra

3.1 Absorption

UV-VIS absorption spectra were recorded at 25°C by means of Cary 300 UV-Vis spectrophotometer (Agilent Technologies).

3.2.1 Steady state fluorescence spectra

The fluorescence spectra were recorded with a Horiba Fluoromax-4 spectrofluorimeter and with an Edinburgh FLS920 fluorimeter equipped with a photomultiplier Hamamatsu R928P. Disposable polystyrene cuvettes with optical path length of 1 cm were used for both absorbance and emission measurements.

3.2.2Fluorescence anisotropy spectra

All fluorescence anisotropy measurements were performed on an Edinburgh FLS920 equipped with Glan-Thompson polarizers. Anisotropy measurements were collected using an L-format configuration, and all data were corrected for polarization bias using the G-factor.

In particular four different spectra were acquired for each sample combining different orientations of the excitation and emission polarizers: I_{VV}, I_{VH}, I_{HH}, I_{HV} (where V stands for vertical and H for horizontal with respect to the plane including the excitation beam and the detection direction;

and the first subscript refers to the excitation and the second subscript refers to the emission). The spectra were used to calculate the G-factor and the anisotropy r: $G=I_{HV}/I_{HH}$ and $r=I_{VV}-GI_{VH}/I_{VV}+2GI_{VH}$.

Details about fluorescence anisotropy are available in PRINCIPLES OF FLUORESCENCE SPECTROSCOPY 2006, EDITORS Joseph R. Lakowicz DOI; 10.1007/978-0-387-46312-4

3.3 Calculation of the absorption spectra expected in the case of no ground state interaction

For a solution containing two species which are not interacting at the electronic ground state the absorption spectrum is expected to be the sum of the spectra that the two species would have individually (in the absence of the other component), in the same conditions.

As individual spectra for the dye A_{Dye} (Dye=F, R or their Pluronic derivatives PF, PR) we chose the absorption spectrum of the solution used for graphite exfoliation before graphite is added. As reference spectrum for exfoliated graphite we chose the extinction spectrum of the suspension obtained by exfoliating graphite with the pristine pluronic (sample G-P). This spectrum was scaled at 750 nm, where the dyes do not absorb light, to match the absorbance of each of the two component system to give spectrum A_G. Calculated spectra of figures 2 and 4 are hence: $A_{calculated}=A_{dye}+A_G$

4 Fluorescence microscopy measurements

The fluorescence images were obtained with an inverted microscope (Olympus IX71) equipped with a Xenon lamp for excitation. Excitation, dichroic and emission filters were purchased from Chroma and Thorlabs. Fluorescence images were acquired with an Electron Multiplying Charge Coupled Device EMCCD Camera (Princeton Instruments, Photon Max 512). Acquisition time was 30

ms per frame at the maximum amplification gain using a 100x oil immersion objective for fluorescence (Olympus UPLFLN100XO2).

4.1 Particle tracking

Trajectories were tracked by analyzing sequences of images acquired with an integration time τ of 30 ms per frame. The particles were localized and tracked by using the plug-in MOSAIC for the software ImageJ.[2] The displacement distribution was processed with the software Sigmaplot to obtain histograms that were fitted with Gaussian peaks. The Einstein-Smoluchowski equation was used to obtain the particle diameter *d*.

5 Transmission Electron Microscopy (TEM)

TEM samples were prepared by casting 3 drops of 1 µL of sample onto holey carbon grids (400 mesh), or lacey carbon grids. Samples were washed with a few drops of water and isopropyl alcohol and then dried at 150 °C to allow for the evaporation of the solvent, to avoid contamination, and the adhesion of the sample to the grid. Bright-field TEM images were taken at the Electron Microscopy Laboratory of the CNR-IMM Institute of Bologna, with a FEI Tecnai F20T HR-TEM instrument.

5.1 Data processing

Data were processed by using ImageJ and Sigma Plot software

5.1.1 1D Graphene flakes size

As reported in Figure S1, each bar represents the number of flakes up to 50nm 1-dimension size. Sigma Plot Weibull, 4 Parameter fits the curve of the data displaying a regression coefficient R =0.98, and, therefore, it is possible to say that this curve fits the histogram trend. The average length value of x0 is 0.22 ±0.01 µm.

5.1.2 Number of layers(N)

To obtain a reliable value of the flake thickness, 86 TEM images were collected and analyzed. The Sigma Plot Log Normal-3Parameter equation was used to fit the curve. A regression coefficient R equal to 0.99, allows us to to say that this curve fits the histogram trend. The calculated Parameters display an average value (N) of of 2-3 layers per flakes.

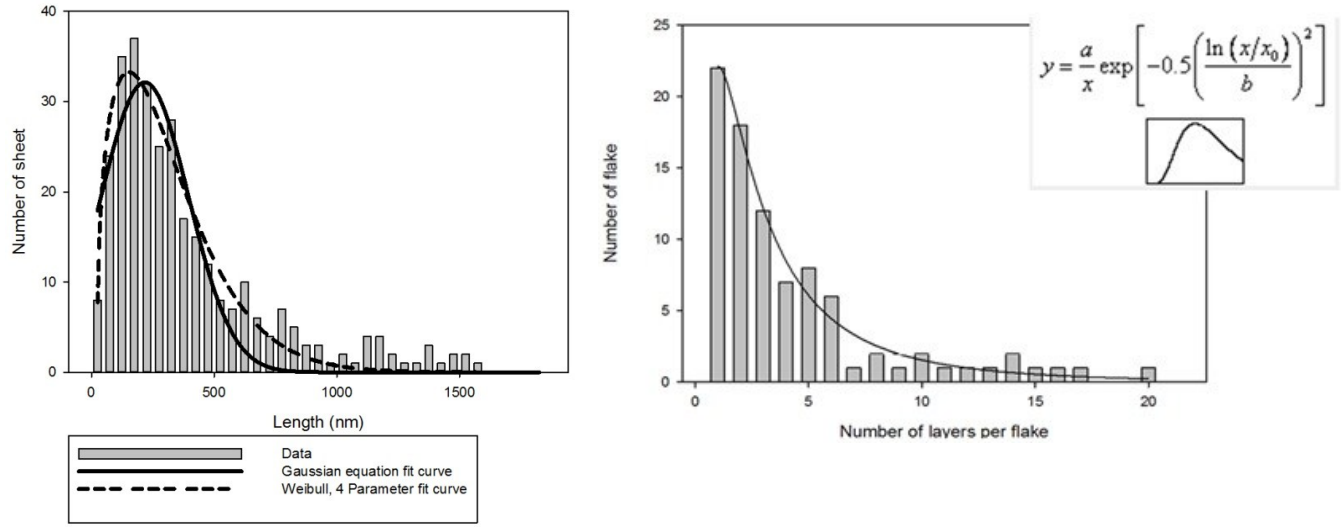

Figure S1 *(Left) Calculation of the average side size of* **G-PF***. Two curves show the different fits performed. Continuous line is Gaussian Equation fit. Dashed line is Weibull Equation fit. (Right) Number of layers detected for the flakes present in the 86 images analyzed. In the inset, the Sigma Plot Log Normal-3Parameter equation is reported.*

5.1.3 Graphene aggregates lateral size

In general, graphene sheets have a rectangular shape, with a long side and a short one, as shown in Figure S2 (left). We tried to discriminate between these two dimensions of the aggregates. 190 different flakes were measured, obtaining an average value for the long side of 2.14 ±0.07 µm, as shown in Figure S2 (right). By studying the same images, we then measured then the short dimension of the flakes, obtaining an average value of 0.72 ±0.02 µm.

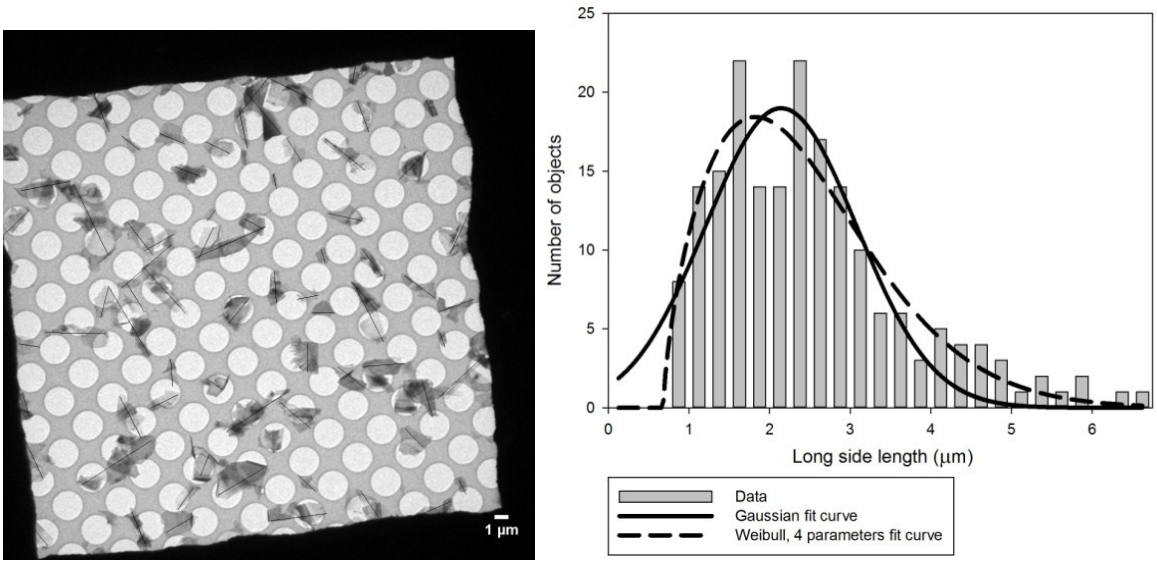

Figure S2 *(Left) TEM grid covered with* ***G-PF****. The reported lines highlight the measurements performed on each flake. (Right) Calculation of the average value of the long side. The different fits performed are reported. Solid line is the Gaussian fit, while the dashed line is the Weibull fit. Results on 190 different measurements.*

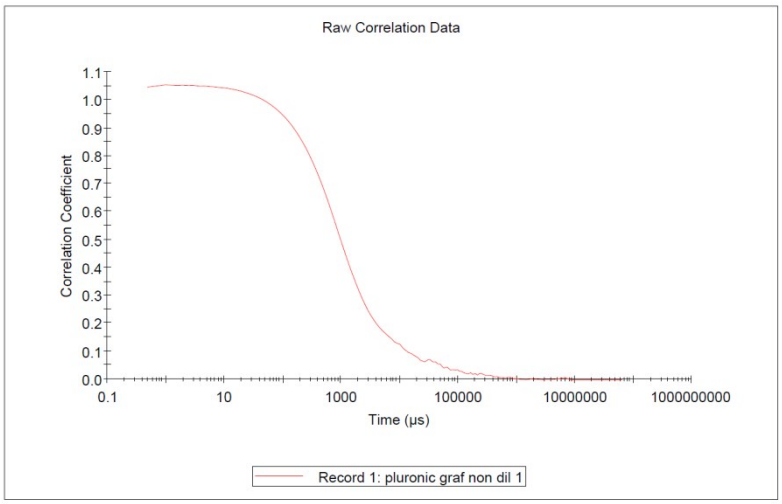

Figure S3 Raw correlation data measured by DLS for sample **G-PF**

6 Dynamic light scattering

Light Scattering measurements were performed using a Malvern Nano ZS instrument equipped with a 633 nm laser diode. Samples were housed in disposable polystyrene cuvettes of 1 cm optical path length, using water as solvent.

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	720.8	Peak 1:	466.2	100.0	110.5
PdI:	0.697	Peak 2:	0.000	0.0	0.000
Intercept:	1.01	Peak 3:	0.000	0.0	0.000
Result quality	Refer to quality report				

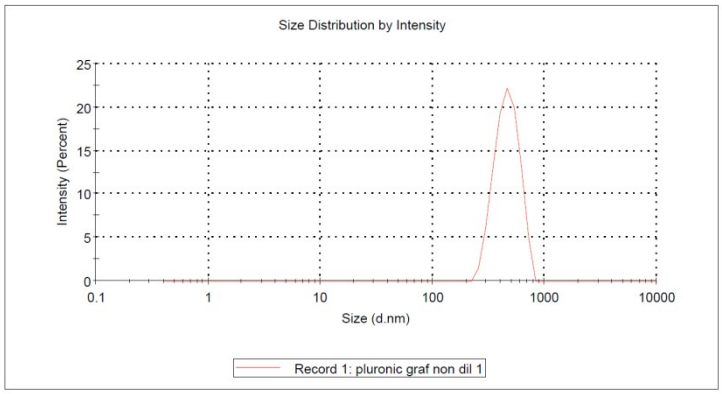

Figure S4 Size distribution plot and results obtained by DLS for sample **G-PF**

[1] L. Guardia, M. J. Fernández-Merino, J. I. Paredes, P. Solís-Fernández, S. Villar-Rodil, A. Martínez-Alonso, J. M. D. Tascón, *Carbon* **2011**, *49*, 1653-1662.

[2] a) N. Chenouard, e. Al., *Nat Meth* **2014**, *11*, 281-289; b) I. F. Sbalzarini, P. Koumoutsakos, *J. Struct. Biol.* **2005**, *151*, 182-195.