Electronic Supplementary Information

On-surface derivatisation of aromatic molecules on graphene: The importance of packing density

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Synthesis, Functionalisation and Transfer of Graphene

Graphene was grown on copper foil by CVD, using methane as the carbon source at a temperature of 1035°C, as described previously.[1] The graphene was subsequently transferred to a Si/SiO_2 (300nm) substrate using the established PMMA-assisted method. Figure S1a depicts an optical image of the transferred graphene on SiO_2. The red box indicates the area of the Raman scan. Corresponding Raman maps of the D/G and 2D/G ratio are also shown. The maximum value of the D/G ratio is 0.2, indicating a low level of defects in the film. The regions with higher D peak intensity indicate grain boundaries and folds. The map of the 2D/G ratio is very homogeneous, with a maximum value of 3, indicating high quality single layer graphene. The dark regions in the map correspond to double layer regions within the film. An average Raman spectrum of the standard graphene produced by this process is shown in Figure S1b, with the D, G and 2D bands indicated. The low intensity D band at 1350 cm^{-1} and sharp, symmetric 2D band at 2700 cm^{-1} are indicative of high quality CVD graphene.

To produce high packing density samples using FLaT, **1** (0.1 % w/v, 0.1 M sodium phosphate, pH 7) was dropcast onto the graphene on copper foil and rinsed with water prior to application of the PMMA layer. The presence of **1** on graphene was confirmed with Raman spectroscopy, as shown in Figure S1c (inset). The copper was etched in a 1 M ammonium persulfate solution. The remaining PMMA/**1**/graphene layer was transferred into deionised water and left to rinse for 1 hour. The film was then fished onto the substrates and left to dry in air. The PMMA was removed by immersion in acetone overnight, then rinsed in isopropyl alcohol (IPA) and dried with N_2. A full Raman spectrum of the sample after this process including the 2D region is shown in Figure S1c (red line). The **1**:G peak intensity ratio is 1.2, as stated in the main text. The additional peaks around the 2D band are associated with the –COOH groups of **1**.[2] High packing density samples were achieved previously by annealing the graphene film prior to deposition, resulting in a **1**:G ratio of 1.5, as can be seen in the sample spectrum shown in Figure S1c (green line).

Low packing density samples were produced by dropcasting **1** after graphene transfer. The samples were then rinsed in water followed by IPA, as described previously.[3]

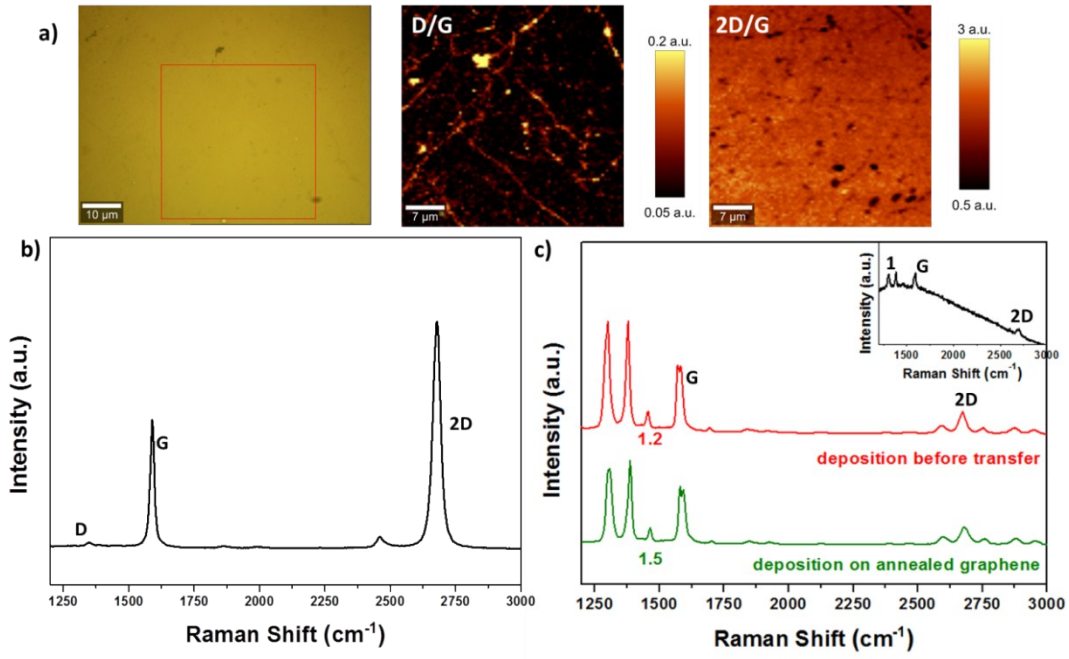

Figure S1: a) Optical image of graphene transferred to SiO_2. Red box indicates Raman scan area. Raman maps of D/G and 2D/G intensity ratios. b) Average Raman spectrum of graphene in scan area. c) Average Raman spectrum of HPD **1** sfter FLaT (red) and HPD after deposition on annealed graphene (green). Inset: Raman spectrum of **1** on graphene on copper growth substrate. The large sloping background is due to heating of the metallic substrate.

Raman Spectroscopy

Raman spectra and maps were acquired using a WiTec alpha 300 confocal Raman microscope with a 532 nm excitation wavelength. Maps were acquired over a 20 x 20 µm area with a spectrum taken every 400 nm. Averaged spectra are obtained from these maps corresponding to 2,500 point spectra.

AFM characterisation

AFM images were acquired using an Asylum MFP-3D atomic force microscope with Tap-300G silicon tips (Budget Sensors). The tips have a resonant frequency of approximately 300 Hz and a tip radius of 50 nm.

Figure S2 shows AFM images of a) LPD and b) HPD monolayer films produced by FLaT on the graphene surface. Wrinkles in the graphene can be observed, along with round features associated with PMMA residue. This PMMA residue is produced after the functionalisation process for the FLaT sample. There are no significant differences between the images as the molecule layer is too thin to be observed in the presence of higher features such as the residue and wrinkles.

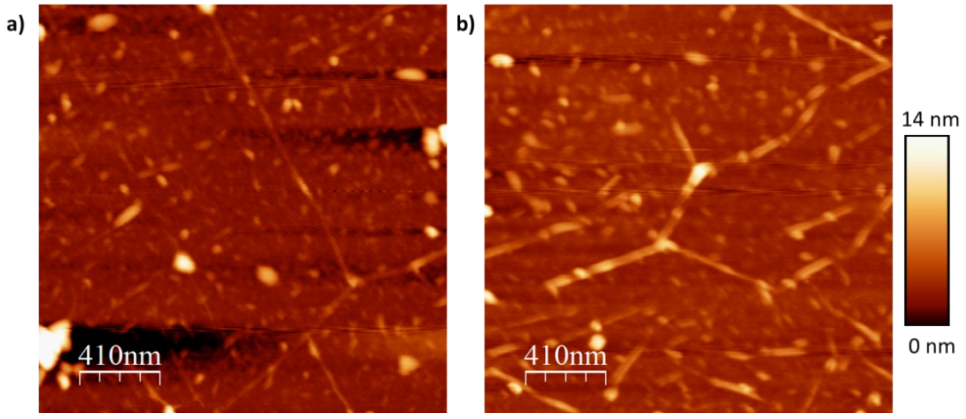

Figure S2: AFM images of a) low packing density and b) high packing density films of **1** on graphene.

Low density perylene film before EDA-derivatisation

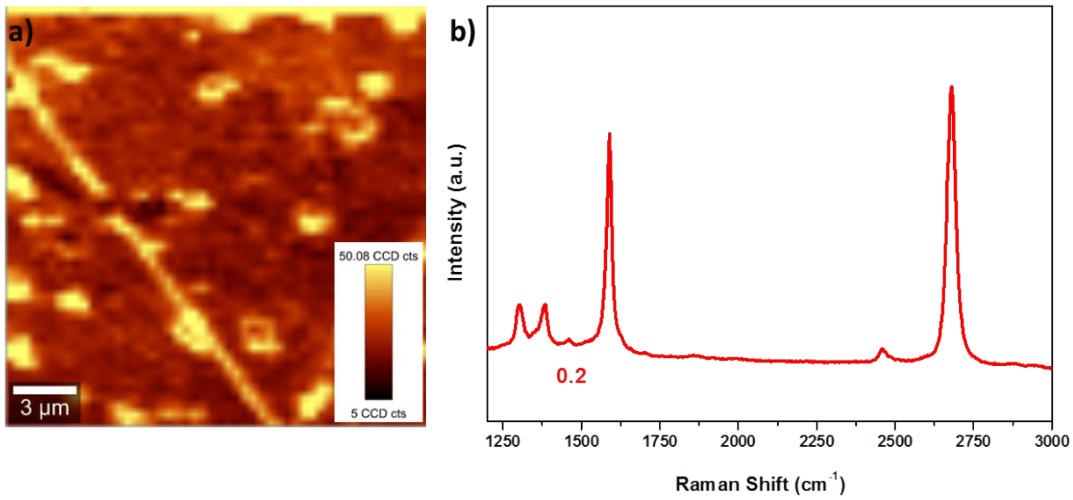

Figure S3: (a) Raman map of **1** peaks of low packing density sample before EDA-derivatisation. (b) Average Raman spectrum of the low density film. The **1**:G peak ratio is 0.2

EDA-derivatisation

The process for coupling of EDA with carboxylic acid groups was adapted from that described by Mohanty and Berry. [4] Graphene samples functionalised with **1** were immersed in 20 ml of EDA with 5 mg of HATU on an incubator shaker at room temperature for 7 hours. Following the reaction they were immersed in methanol for 30 minutes, rinsed with deionised water and dried with N_2.

XPS

X-ray photoelectron spectroscopy was performed using a monochromated 1486.6 eV Al Kα X-ray source (Omicron XM1000 MkII) and an Omicron EA125 energy analyser under ultra-high vacuum conditions. The position of the sample during measurement was kept constant with an accuracy of around 15% after extraction and re-insertion into the vacuum chamber, which made comparisons of the absolute peak intensities possible within this error margin.

XPS survey scans of **1** before and after derivatisation are shown in Figure S3. The C 1s and O 1s peaks are clearly visible in the survey scans. The Si 2s and 2p peaks from the substrate are also present. The N 1s peak intensity is too low in comparison to the C 1s and O 1s peaks to show in the surveys. This can be contributed to two factors: the percentage of nitrogen present is very low in comparison to the other elements and in addition, the N 1s peak has a lower absorption cross section, resulting in a lower signal. However, when repeatedly scanning the area of the N1s peak we obtain a clear signal as shown and analysed in Figure 4 in the main manuscript.

High resolution C 1s spectra are shown below the survey scans. The peak is dominated by the asymmetric component that corresponds to sp^2 carbon from the graphene and perylene cores. There is a smaller component which corresponds to sp^3 carbon which can be attributed to the dendritic chains of the perylene. After derivatisation, there is a trend towards an increase in the C-N bond contribution, indicating the presence of amide and amine bonds. While the accurate deconvolution of the C 1s peak is challenging due to the similarity of the binding energy values for C-N and C-O bonds, the lack of increase in the O 1s signal as well as the analysis of the N 1s region as shown in Figure 4 in the main manuscript still leads us to this conclusion. The C-N contribution remains after the 220°C anneal, indicating that the derivatisation was successful.

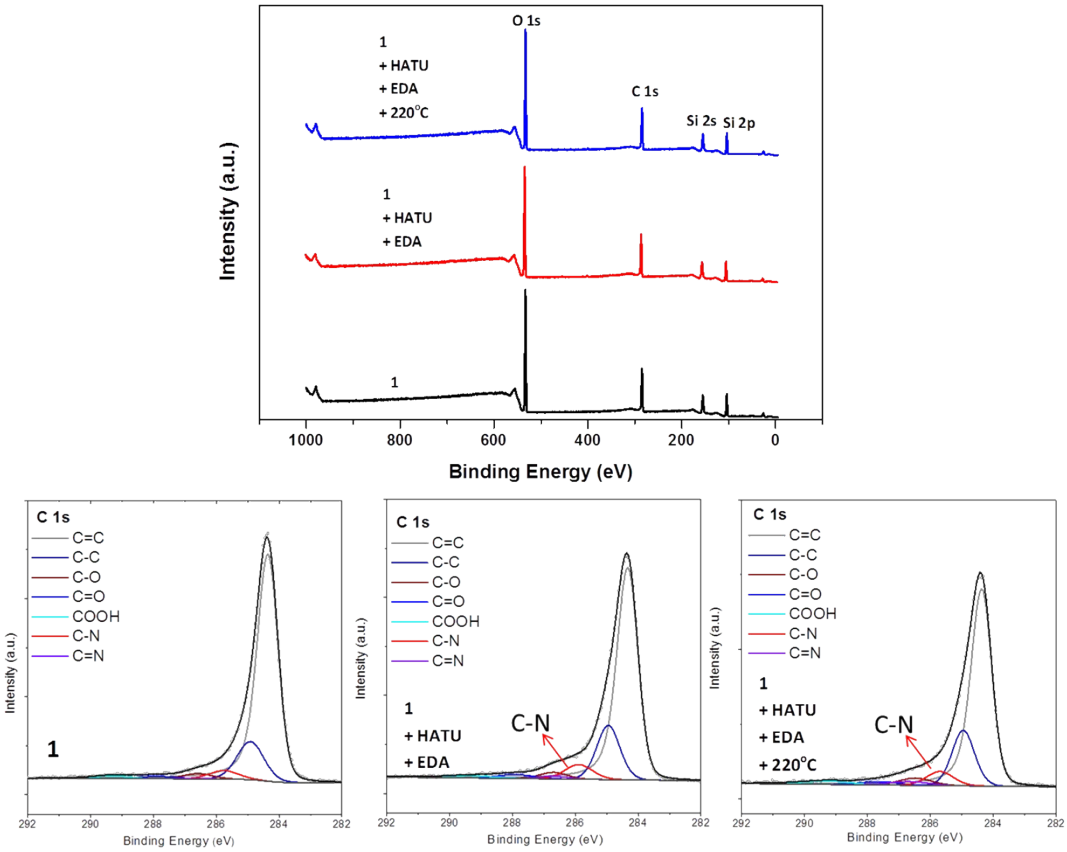

Figure S4: XPS survey scans and high resolution C 1s spectrum of **1** on graphene before and after EDA derivitisation and a 220 °C anneal.

Bacteria adhesion tests

For bacteria adhesion experiments, *Escherichia coli* (*E. coli)* CIP 53.126 were obtained from Collection de l'Institut Pasteur (Paris, France). The bacteria were cultured in lysogeny broth medium at 37 °C using standard protocols and extracted in the logarithmic growth phase for immobilization experiments. A polymethyldisiloxane (PDMS) fluid cell was designed to bring the graphene surface in contact with the bacteria under incubation conditions to avoid sample drying and maintain a favourable environment for the bacteria. After 20 minute incubation, the sample was subsequently rinsed with 0.85 % NaCl solution and the PDMS cell was removed to enable the bacteria to be stained for fluorescence imaging. Propidium iodide fluorescent dye was applied to the bacteria and incubated for 15 minutes. A cover slip was then placed over the sample to obtain fluorescence images. Image area was 23,364 μm^2.

Fluorescence images were collected using an Olympus BX51M fluorescence microscope equipped with an Olympus XM10 camera.

References

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2. N. V. Kozhemyakina, J. M. Englert, G. Yang, E. Spiecker, C. D. Schmidt, F. Hauke and A. Hirsch, *Adv Mater*, 2010, **22**, 5483-5487.
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4. N. Mohanty and V. Berry, *Nano Lett*, 2008, **8**, 4469-4476.