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Nitrene addition to exfoliated graphene: a one-step route to highly functionalized graphene†

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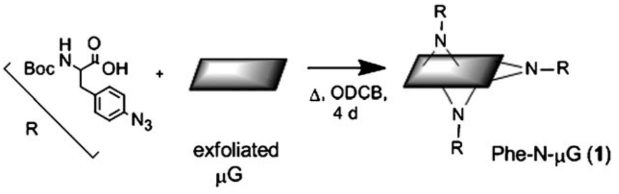
We demonstrate a high yield method of functionalizing graphene nanosheets through nitrene addition of azido-phenylalanine [Phe(N_3)] to exfoliated micro-crystalline graphite (μG). This method provides a direct route to highly functionalized graphene sheets. TEM analysis of the product shows few layer (n < 5) graphene sheets. The product was determined to have 1 phenylalanine substituent per 13 carbons.

Recent years have brought about a renewed interest in graphite or specifically individual or few-layer (n < 5) sheets of graphite called graphene. The unique physical and electronic properties of graphene make it attractive as a substitute for other more costly carbon nanostructures.[1] Due to its similarities and differences with other carbon nanostructures, graphene (2 dimensional, 2D) is no longer the underappreciated parent of fullerenes (0D) and carbon nanotubes (1D). The ability to individualize sheets of graphene has brought the material to the forefront of carbon research.[2] And while graphene does provide similar scale, properties, and reaction pathways as fullerenes and CNTs, it does so at a fraction of the cost. Regardless of the novel electronic properties of graphene, it also presents an inexpensive, large surface area scaffold for the covalent attachment of organic molecules. Covalent functionalization provides a robust material for use as a scaffold and as a means of incorporating additional functionality. Incorporation of functionalized graphene sheets into composite materials may enhance mechanical and electronic properties. The majority of reports of covalently functionalized graphene use graphene oxide (GO) as the starting material.[3] While the structure of GO has been shown to include regions of unoxidized benzene rings, the presence of islands of epoxides and hydroxyl groups on the basal plane and carboxylic acids around the perimeter of the sheet make GO extremely hydrophilic. In addition, graphene oxide contains a large percentage of water requiring more than four weeks to dry post synthesis.[4] While this method does result in highly functionalized graphene sheets, the drawbacks are that reactions using GO as the starting material are limited to aqueous or polar conditions and more important a second reduction step is necessary to remove the excess oxides still present.[3] A simpler route to widespread functionalization is therefore desirable. In this regard, we report a new, high yield method of covalently functionalizing graphene sheets.

It has been shown that both CNTs and fullerenes are easily functionalized by nitrene addition, generated *in situ* by thermal decomposition of azido starting materials.[5] The result is high density functionalization of the nanostructure surface through a [2 + 1] cycloaddition to the C–C double bonds forming an aziridino-ring linkage, however; this methodology has only been applied to curved or strained (*i.e.* reactive) carbon nanostructures. The alkyl and aryl azido starting materials are easily synthesized on the gram scale and azides offer a wide range of functional group tolerance.[6] For our purposes, the azido moiety resides on the R side chain of the amino acid phenylalanine (Phe) allowing incorporation of two new chemical handles on the graphene scaffold for subsequent reaction. In this regard, the graphene sheet could be used as a solid support for solid phase peptide synthesis (SPPS) of a sequence on the graphene surface. The addition of sequences of amino acids to the graphene surface should impart water solubility and some level of biocompatibility.

In a typical reaction,‡ microcrystalline graphite (μG, <20 μm, 50 mg) was exfoliated without homogenization in *o*-dichlorobenzene (ODCB, 20 mL) to make a clear gray solution of graphene sheets without the use of harsh oxidizing conditions.[7] It is worth noting that this reaction was not reproducible using thermally expanded graphite intercalation compounds (GIC)[8] as the starting material. Given that GICs use a variety of reactive molecules to intercalate between graphene layers,[4] side reactions with reactants are inevitable, thus limiting their use as graphitic starting materials as well. The exfoliated μG was reacted with Boc–Phe(4-N_3)–OH§ at refluxing temperatures. The reaction was filtered over a 0.2 μm PTFE filter paper, and washed to remove any unreacted Phe(N_3) (Scheme 1, yield **1** 110 mg). The product **1** was found to make an amber colored solution in DMSO, averaging 0.06 mg mL^{-1} after 10 min of probe sonication and centrifugation (3400 rpms for 15 min). The product also proved sparingly soluble in other organic solvents such as DMF, toluene, and chloroform in comparison to the μG starting material. To confirm the chemical modification FTIR spectroscopy, Raman spectroscopy, and XPS were utilized.



Scheme 1 Nitrene addition to exfoliated μG in refluxing ODCB.

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The elemental composition of **1** was determined by XPS, confirming the presence of the phenylalanine (Fig. S1, ESI†). The oxygen (16.1%) and nitrogen (13.5%) content are consistent with the presence of the phenylalanine substituent with the expected loss of the Boc protecting group which can occur at 180 °C.[9]¶ However, the FTIR of **1** (Fig. S3, ESI†) showed the characteristic absorption of a secondary carbamate carbonyl stretch at 1655 cm^{-1}, indicating there was only partial loss of the Boc protecting group. In addition, the spectrum shows absorption in the carboxylic acid ($\sim$3200 cm^{-1} stretch and 1438 cm^{-1} bend) and the amide C–N regions (1319 cm^{-1}) relative to the μG starting material. Also present in the spectrum are the NH stretch (3426 cm^{-1}) of the Boc amino protecting group and the prominent C–N amine stretch at 1022 cm^{-1} of the aziridino linkage.

High resolution TEM images show graphene sheets layered over each other, with an average length of 300–500 nm and with varying widths (Fig. 1). This is in agreement with STEM measurements (Fig. S6, ESI†), in which large, few-layer graphitic domains are visible. The formation of few- or multi-layer sheets is further supported by selected area electron diffraction (SAED, Fig. 1 inset and Fig. S4, ESI†) and fast Fourier transform (FFT, Fig. S4, ESI†) in selected areas.

The AFM analysis confirmed the presence of graphene sheets with a wide variety of lateral dimensions from 150 nm to >5 μm. The thickness of the observed sheets ranged from 0.5 nm to $\sim$2.5 nm (Fig. 2). The theoretical height of our Phe functional group is $\sim$0.75 nm, correlating to the addition of 1.5 nm to the thickness of the graphene sheet if functionalization occurs on both sides. Several groups have reported a height of $\sim$1 nm for GO sheets with some variability.[10–12] Our analysis of **1** shows a variety of sheet thicknesses, however, we surmise that the thinnest sheets, 0.5 nm, are only edge functionalized while the thickest at 2.5 nm are basal plane functionalized on both sides. Based on both the TEM and AFM characterization, we conclude that few-layer ($n < 5$) graphene sheets are produced by this reaction method.

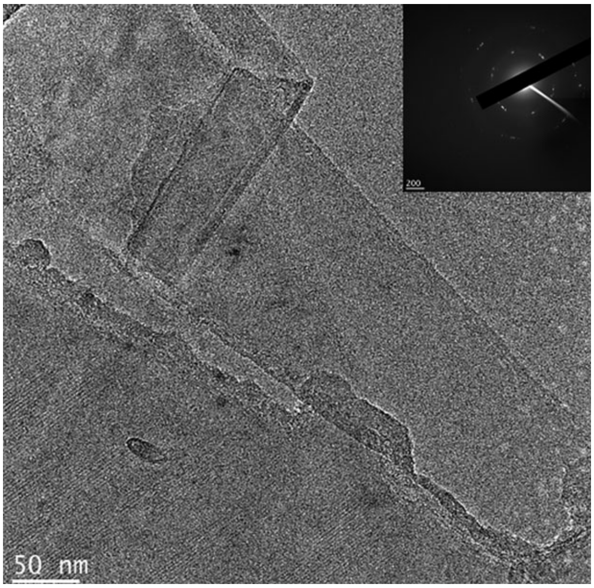

Fig. 1 TEM image of Phe–N–μG (**1**) showing stacked, few layer, sheets with SAED inset confirming few-layer graphene sheets.

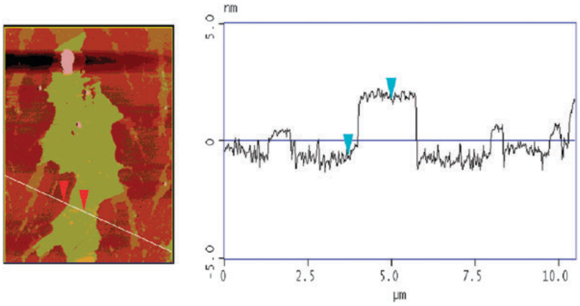

Fig. 2 AFM image and associated height profiles of Phe–N–μG (**1**), spin coated on a cleaved mica substrate from CHCl$_3$, showing a large graphene sheet surrounded by smaller graphene flakes.

Scanning TEM (STEM) images of **1** (Fig. S6, ESI†) deposited from DMSO on a lacey carbon grid show few-layer sheets, the majority of which had lengths of over 1 μm and widths of about 500 nm. However, some sheets were as small as 150 × 300 nm, in agreement with TEM and AFM measurements.

The extent of functionalization is obtained from thermogravimetric analysis (TGA) over the temperature range of 130–850 °C in agreement with the decomposition of phenylalanine (Fig. S7, ESI†). Assuming no mass loss due to μG and taking into account the carbonaceous material remaining from phenylalanine (Fig. S7–S8 and discussion, ESI†), the final product **1** was determined to be 69% phenylalanine (wt%). This corresponds to one Boc–Phe substituent per 10 μG carbons (XPS data show 1 : 13 C : Phe, Fig. S1, ESI†). It should be noted, however, that each Phe substituent is bonded to two carbon atoms (Scheme 1). It has been shown previously that graphene is preferentially functionalized along its edges.[11] However, even with the smallest sheets of **1** (150 × 300 nm by STEM), if the functionalization is limited to the edges, only 2–3% by mass of the sample is attributable to Phe. Assuming the entire mass loss by TGA is due to covalently bonded Phe, to achieve 69% by mass functionalization of **1**, the nitrene addition could not occur solely at the edges of the sheets. STEM images (Fig. S6, ESI†) show dark spots along the basal plane of the sheets but EDX analysis of the spots could not definitively confirm that they are Phe. We conclude, therefore, that by our route, functionalization is not limited to the edges and the resulting structure is analogous to that of GO.[4]

Raman spectroscopy (514 nm excitation) of **1** (Fig. 3) showed a D : G ratio of 0.78 in comparison to that of exfoliated μG at 0.66 (Fig. 3a and b). While there is not a significant change, this could be due to the distribution of functional groups on the surface and/or an effect of the size of the sheets.[12] The most noticeable feature in the spectrum of **1** is the loss of the 2D peak (*ca.* 2700 cm^{-1}). Most previous reports of functionalized graphene omit mention of this peak.[11,13] The only material reported to show a similar loss of the 2D peak is graphene oxide (GO),[14] in which the graphitic structure is almost completely oxidized with some unoxidized regions remaining. Based on the similar spectra of **1** to GO (Fig. 3b and c), the absence of the 2D peak indicates the lack of sp^2 carbons present and, therefore, the high degree of functionalization. Thermolysis of **1** at 600 °C for 12 h produced a black powder with a D : G ratio of 0.67 and a partially restored 2D peak (Fig. 3d) similar to that of the

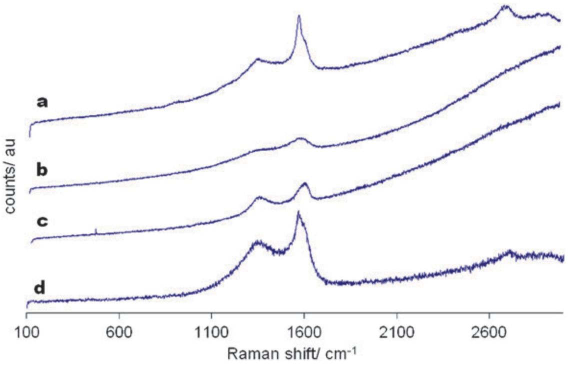

Fig. 3 Raman spectra of (a) exfoliated μG, D : G 0.66; (b) **1**, D : G 0.78; (c) graphene oxide, D : G 0.77 and (d) annealed **1**, D : G 0.67.

exfoliated μG sample. It should be noted that the bulk μG material prior to exfoliation had a D : G ratio of 0.25 indicating possible modification to the electronic framework simply through sonication in ODCB.[15]

In conclusion, we have reported a high yield method of covalently functionalizing exfoliated graphene by nitrene addition in ODCB. The addition results in few-layer, DMSO soluble graphene sheets. Given the extensive range of organic azides accessible by a simple diazo-transfer reaction,[6] this method offers a route to a wide variety of functional addends. The advantages of our method are as follows: (1) no final reduction step is required, (2) a wide variety of functional addends are accessible, (3) reactions are not limited to aqueous/polar conditions, and (4) highly functionalized, few-layer graphene sheets are produced. The control over the extent of functionalization is currently under investigation, as well as the feasibility of **1** as a solid phase support for peptide synthesis on the surface of a graphene scaffold.

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Notes and references

‡ Boc–Phe(4-N_3)–OH (250 mg in ODCB) was added to the exfoliated μG (Sigma Aldrich) solution in a 100 mL round bottom flask fitted with a condenser and a stir bar. Refluxing temperatures were maintained for 4 days after which the brown suspension was filtered over a 0.2 μm PTFE filter paper. The filter cake was washed copiously with MeOH, $CHCl_3$, and DMF to remove any unreacted Phe(N_3). The fine brown powder was dried in an oven overnight at 125 °C. Final yield: 110 mg.

§ In a 500 mL Schlenk flask, imidazole-1-sulfonylazide·HCl (1.02 g, 6 mmol), Boc–Phe(4-NH_2)–OH (1.40 g, 5 mmol), and $ZnCl_2$ (27.3 mg, 0.20 mmol) was dissolved in MeOH : MeCN (3 : 2, 300 mL) to this was added NEt_3 (1.39 mL, 10.0 mmol). After stirring at room temperature for 12 h, it was diluted with Et_2O (200 mL) and washed with AcOH (10%, 3×) and brine (1×). Drying with $MgSO_4$ and concentration, followed by flash chromatography yielded the pure product. Yield = 1.2 g, 80%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.07 [2H, d, J(H–H) = 8.97 Hz, CH], 6.97 [2H, d, J(H–H) = 8.97 Hz, CH], 4.93 [1H, d, J(H–H) = 8.63 Hz, NH], 4.58 [1H, dd, J(H–H) = 6.64 Hz, J(H–H) = 14.92 Hz, CH], 3.12 [1H, dd, J(H–H) = 5.12 Hz, J(H–H) = 13.92 Hz, CH_2], 3.05 [1H, dd, J(H–H) = 6.41 Hz, J(H–H) = 13.64 Hz, CH_2], 1.42 [9H, s, ^{t}Bu]. IR (cm^{-1}): 2115 (ν_{N3}).

¶ To determine if polymerization of Phe on the graphene surface was occurring, we repeated the reaction with a methyl ester of Boc–azido–Phe. Protection at both carboxy and amino terminus should eliminate the possibility of peptide formation. Details of the reaction and characterization are included in the ESI.†

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