We describe a new approach to attach organic dyes to graphene oxide (GO) sheets with high loading and minimal perturbation of the electronic and optical properties of the dye. The dye unit used (a pH-sensitive terthiophene) is grafted to GO using a new modular synthetic approach, passing through a C6-aminic linker which makes GO more soluble in different organic solvents and allows straightforward attachment at high yield not only of terthiophene but of many commercially available amino-reactive dyes. The covalent engraftment to GO does not perturb the absorption and emission properties of the dye, and in particular the pH sensing capability through amidic group reversible protonation. This approach can allow (i) high solubility of the GO intermediate in organic solvents, (ii) convenient coupling with commercial, stable amino-reactive dyes under mild conditions, (iii) easy control of the spacer length between the GO and oligothiophene dye and finally (iv) high (up to 5 wt%) dye functionalization loadings.

Introduction

Graphene oxide (GO) is rapidly emerging as a viable scaffold to realize new hybrid materials with potential applications in organic electronics, catalysis, light harvesting and biology.1

The GO sp² carbon network together with its chemically rich and flat 2D surface can indeed be exploited for chemical functionalization with nanoscopic “functional” moieties (optically active molecules, polymers, nanoparticles, to mention just a few) using covalent and/or non-covalent approaches. In this way, GO can be made a useful platform for drug delivery,2 catalysis,3 photovoltaics,4 and sensing application.5

In particular, the covalent grafting of functional organic materials to the GO surface represents a valuable strategy to enhance the material performance in thin film transistors (TFTs) or solar cells, where the molecular surface organization and packing are key parameters for improving the final device performance. If devices with improved performance can be conveniently achieved by attaching small molecules to 1D scaffolds such as linear, long polymeric chains,6–9 the use of a 2D
scaffold, such as graphene sheets, is expected to provide even more original materials (Fig. 1). Indeed, 2D substrates allow molecules to be positioned over flat but highly flexible (and solution processable) sheets. In addition, regiochemically defined grafting on the 2D surface can be achieved by exploiting the hydroxyl, carboxylic, epoxide groups present on the basal sheets or the edge sides as anchoring groups, allowing us to tune the functional properties of the final hybrids by changing substitution pathways, the type of linker and spatial positioning on the sheet.

Several optically active organic molecules have been covalently grafted on the GO by different chemical approaches. The peculiar structures of GO and graphene, where every atom is exposed to the environment and thus available for functionalization and sensing, make them perfect materials for sensor applications; fluorescence quenching and modification of electronic properties have been already demonstrated for graphene-based devices. However, it is still not clear how the properties of the molecule are influenced by the covalent attachment to the 2D material as well as the type of linker employed. The molecules can freely float in the solution or be fixed covalently on a rigid substrate. In GO, we have a hybrid situation in which the molecule is “tethered” to a macroscopic substrate, which is nonetheless highly flexible and dynamically moving in the solvent. Indeed, grafted molecules shall either lie on the GO basal plane or float freely in the solvent, depending on the flexibility of the linker and the solvent used. According to that, the optoelectronic properties can also be influenced, given that the GO is an excellent fluorescence quencher. It has been recently shown that the length of the linker between organic dyes and the GO surface strongly affects the optical properties of the attached molecules and the degree of quenching. Even the graphene properties can be deeply influenced by the presence of the molecule, due to charge-transfer and doping effects.

To assess this issue, we studied the optical properties of a test molecule when isolated or when grafted onto GO sheets, either in solution or after deposition on a solid substrate. For this, we used as a test molecule a fluorescent dye, a terthiophene (T3) bearing a conjugated end amidic moiety. In oligothiophene dyes, fluorescence mainly depends on the size of the π-conjugated backbone or on the type of thiophene substituents (using push-pull, donor acceptor configurations). Here we demonstrate by experimental and computational analysis that in our T3, thanks to the protonation of the amidic moiety, the fluorescence is capable of a strong and reversible emission color switch that depends on pH. The T3 unit is grafted to the GO using a new, modular approach, passing through a C6-diaminonic linker which makes the GO more soluble in different organic solvents, and allows straightforward attachment at high yield not only of T3 but of many commercially available aminoreactive dyes.

The obtained GOC₆T₃ material has been characterized by Absorption and Emission spectroscopy (UV-Vis, PL), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy-dispersive X-ray spectroscopy (EDX) and Laser Scanning Confocal Microscopy (LSCM), and its optical and morphological properties have been compared to those of the pristine GO and the T3 precursors.

### Results

#### 1. Synthesis

The newly synthesized dye consists of a β-dimethyl functionalized terthiophene having a terminal N-hydroxysuccinimide (NHS) activated carboxylic acid (T3NHS – 2). Starting from this dye, the amide functionalized analogue T3NBut (4) was straightforwardly prepared by reaction with n-butylamine 3. This compound, analogous to GOC₆T₃, is characterized by an amide moiety between the thiophene ring and the butyl chain and was used in this work as a “free dye” reference molecular system for optical measurements.

The two-step synthesis of GOC₆T₃ is outlined in Scheme 1. As a first step, the NH₂-ended hexyl chain was covalently grafted to the –COOH groups of the GO. Afterwards, the NH₂-modified GO was reacted with T3NHS. The functionalization of GO is achieved by attaching the dye to already existing chemical “defects” (i.e. COOH groups), without creating additional defects on the GO sheets. Both reaction products (GOC₆NH₂ and GOC₆T₃) underwent workup procedures consisting of several washing/centrifugation steps; water and ethanol (EtOH) were used in the first step while dichloromethane (DCM) was employed on the final T3-labeled material.

The solubility of GOC₆NH₂ and GOC₆T₃ was tested in a series of solvents, including toluene (Tol), tetrahydrofuran (THF), DCM, acetonitrile (ACN), EtOH, orthodichlorobenzene (o-DCB) and water. As shown in Fig. 2a and b, both samples showed good solubility in water, acid water, ACN, DCM, EtOH and toluene providing clear and homogeneous solutions stable for days.

#### 2. Materials characterization

Absorption and emission spectra of GOC₆T₃ compared to T3NHS and T3NBut in dichloromethane (DCM) are shown in Fig. 3a. T3NHS shows the maximum absorption at λmax = 374 nm and emission at λem = 493 nm, while GOC₆T₃ shows the maximum absorption wavelength at λmax = 351 nm and emission at λem = 467 nm. These latter values are very close to those observed for T3NBut (λmax = 352 nm and emission at λem = 457 nm), which confirms the conversion of the succinimidyl ester (COON–) into the amidic moiety (CONH–) upon reaction with GOC₆NH₂, with minimal perturbation of the emission spectra of the T3 dye, similarly to what is observed with amidation of SWNTs with naphthalimide.

![Scheme 1](image) Synthetic route to GOC₆T₃ and the model compound 4.
A comparison of emission spectra of GOC6T3 and T3NBut (reported in Fig. S1 of ESI†) allowed us to estimate a quenching of 16% of the fluorescence intensity for GOC6T3 compared to the dye alone. Even if uncertainty due to absorption by the GO of part of the exciting and emitted light can affect this value, we observed a much lower quenching for T3 attached to GO than what was observed in previous work (ref. 10, see ESI† for details). The low quenching can be explained by comparing the linker used in ref. 10 (a C3 alkyl chain) with the much longer linker used here (a C6 alkyl chain), which gives much more freedom, allowing the dye to float freely at a larger distance from GO (11 Å, as compared to ~7 Å for the method in ref. 10 and ~2 Å for the method in ref. 20). This, together with the smaller size and better solubility of T3 compared to T4, gives a much weaker GO-dye interaction as compared to other functionalization techniques.

FT-IR spectroscopy (Fig. 3b) shows that the typical GO bands at 3425 cm$^{-1}$ (stretching of OH groups on the edge of GO basal planes) and at 1729 cm$^{-1}$ (stretching vibrational modes of the carbonyl groups) disappeared upon modification with T3NHS. Indeed, in GOC6T3 the amide NH stretching bands at 3300 cm$^{-1}$ and the amide C=O stretching at 1630 cm$^{-1}$ were observed. Moreover, bands typical for C–H stretching vibrations of the thiophene rings at 3070 cm$^{-1}$ (aromatic C=C stretching) and at 2930 cm$^{-1}$ (aromatic C=C bending) were also found.

A rough estimation of the dye functionalization loading in the GOC6T3 sample was obtained by comparing the Thermal Gravimetric Analysis profile (TGA) of the NH$_2$-decorated GO (GOC6NH$_2$) with that of the dye decorated material (Fig. 4); a 5.30% weight loss was measured in the 40–800 °C temperature range and entirely attributed to the covalently grafted T3 moiety. Accordingly, one may roughly conclude that 0.17 mmol of T3 per 1 g of GOC6T3 are covalently grafted to the final material. At higher temperatures, only a gradual weight loss (for both samples) due to partial oxidative decomposition of the bulk material was observed.

GOC6T3 films on silicon substrates and on copper grids were analysed by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) respectively. The TEM image in Fig. 5b shows a flake on a copper grid. Selected Area Electron Diffraction (SAED) analyses, on the region highlighted with the yellow circle, revealed the hexagonal honeycomb symmetry expected for graphite-like lattice, thus confirming that the functionalization process does not destroy the hexagonal backbone of graphene (Fig. 5c).
X-ray spectroscopy (EDX, not shown) revealed the presence of nitrogen and sulphur atoms on the flakes surface confirming the chemical functionalization. Noteworthily, other oligothiophene–GO hybrids reported in the literature showed closely related morphologies to that of the unfunctionalized GO materials (flat layered nanosheets).\(^7\) In the present case, where a different linker (C6 spacer-amido bridge) was used, a significant change in the final material morphology was observed. Indeed, morphology of the GOC\(_6\)T\(_3\) samples consisted of highly crumpled GO materials (Fig. 5a) rather than flat layered sheets. A similar morphology trend was reported by Compton and co-workers for graphene–polystyrene nanocomposites consisting of graphene nanosheets dispersed in a polymeric matrix and prepared by reduction of chemically modified GO in the presence of polystyrene.\(^{21}\)

Such composites, due to their crumpled morphology, allow the fabrication of films with low O\(_2\) permeability and effective reduction of transparency, crucial issues for packaging purposes. In our case, a possible explanation for the crumpled morphology observed (that we did not fully explore in the present work) can be ascribed to the occurrence of a partial interlayer cross-linking of the diaminoalkyl reagent. However, in order for crosslinking to change the morphology so drastically, the cross-linking should be effectively widespread, and this would likely impair dye loading. Even if we cannot exclude that the latter reactivity partially takes place, the successful T3 grafting on GOC\(_6\)NH\(_2\) suggests that most of the free amino groups are still available for post-grafting.

3. Comparative study of the optical properties and pH induced fluorescence switch

To assess how the chemical engraftment to the graphene scaffold influences the functionality of the active molecule, we compared the optical properties of the GOC\(_6\)T\(_3\) with the ones of both T3NHS and T3NBuT.

Fig. 6 shows the PL spectra at neutral and acid pH of GOC\(_6\)T\(_3\), T3NHS and T3NBuT in EtOH solution (the latter two molecules being poorly soluble in water). Interestingly, GOC\(_6\)T\(_3\) showed a reversible red shift from \(\lambda_{\text{max}} = 462\ \text{nm}\) to \(\lambda_{\text{max}} = 534\ \text{nm}\) upon treatment with HCl (pH = 1) in EtOH (Fig. 6d). DFT/TDDFT calculations performed on T3NBuT, see below, show that the bathochromic effect is due to the protonation of the amide linker between the T3 and the GO framework. Accordingly, T3NBuT shows a similar pH dependent emission (\(\lambda_{\text{max}} = 454\ \text{nm},\ \text{pH}\ 7 \to 531\ \text{nm},\ \text{pH}\ 1\), Fig. 6b).
experiments on the model system T3NHS (without the amidic linker) show that there are no effects on the dye fluorescence associated with the pH environment; the optical features of T3NHS were only slightly affected by pH changes (Fig. 6c), indicating that the pH sensitivity is imparted uniquely by the amidic moiety. For T3NHS, only a slight blue shift was observed at acidic pH ($\lambda_{\text{max}} = 500 \text{ nm} \rightarrow 479 \text{ nm}$) (upon base treatment, hydrolysis of the succinimidyl group can occur).

The red-shift upon acidification was observed also at sheet level by spectral Laser Scanning Confocal Microscopy (LSCM), which revealed the spectral changes for cast films of GO_{6}T3 upon acidification. Fig. 7a shows the LSCM image of a cast film of GO_{6}T3 on silicon. Emitting aggregates of tens of micron size lying in a non-fluorescent background can be seen. The spectrum recorded in the region of the observed aggregate shows the maximum emission wavelength at about 470 nm (Fig. 7b, blue curve), compatible with the emission of the T3 dye (see Materials characterization section). Conversely, no signal was detected in the background area, which confirms the absence of free dye both in the solution and on the substrate (Fig. 7b, red curve). Fig. 7b also shows the spectra obtained with fluorescence microscopy images after the acidic treatment (green curve). It can be clearly seen that upon protonation, the spectrum becomes a mixture of the blue and green emission.

To gain insight into the origin of the emission red shift measured in acidic media for T3NBHut, we performed DFT/ TDDFT calculations on the neutral T3NBHut species and on the corresponding species protonated at the oxygen and nitrogen amidic sites. The results, summarized in Table 1 and Fig. 8, show in all cases an almost isolated intense transition, giving rise to the emission with mainly LUMO $\rightarrow$ HOMO character.

Although slightly underestimated with respect to the experimental data (0.23 eV vs. 0.41 eV), calculations reproduce overall the emission red-shift observed upon acidification of the T3NBHut solution. The origin of the red-shifted emission can be understood by looking at the data in Table 1 and in Fig. 8.

It can be noticed that O- and N-protonation induces a substantial stabilization of both the HOMO and LUMO compared to the neutral T3NBHut species. However, since the LUMO extends over the amidic group more than the HOMO, the effect of protonation on the LUMO is larger than for the HOMO, leading to the observed emission red-shift. We also notice that both O- and N-protonation lead to a sizable polarization of the LUMO towards the amidic moiety, which recalls electron density from the thiophene $\pi$ system.

**Discussion and conclusions**

For the development of an extensive, reliable chemistry of graphene, chemical functionalization procedures should be optimized to allow high versatility, treatment in both polar or apolar media and reliability.

Here, we give a brief discussion on how this chemical functionalization procedure compares to other approaches already reported by us and other groups.

<table>
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<tr>
<th>$E_{\text{HOMO}}$ (eV)</th>
<th>$E_{\text{LUMO}}$ (eV)</th>
<th>$E_g$ (eV)</th>
<th>$\lambda_{\text{em, DFT}}$</th>
<th>$\lambda_{\text{em, exp}}$</th>
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<td>No proton</td>
<td>$-6.07$</td>
<td>$-1.27$</td>
<td>4.80</td>
<td>498 nm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>454 nm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2.488 eV)</td>
</tr>
<tr>
<td>O-proton</td>
<td>$-6.59$</td>
<td>$-2.08$</td>
<td>4.51</td>
<td>551 nm</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>534 nm</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>(2.249 eV)</td>
</tr>
<tr>
<td>N-proton</td>
<td>$-6.60$</td>
<td>$-2.09$</td>
<td>4.51</td>
<td>544 nm</td>
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<tr>
<td></td>
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* Calculated in EtOH.

**Fig. 7** (a) Confocal fluorescence microscopy images of GO_{6}T3 on silicon and (b) spectra of a GO_{6}T3 flake at pH = 7 (blue curve), at pH = 1 (green curve) and of the background (red curve), recorded in the 420-620 nm emission range.

**Fig. 8** Isodensity plots of the HOMOs and LUMOs for T3NBHut and for the corresponding O- and N-protonated species.
The interest in GO–oligothiophene (OT) hybrids stems from the outstanding chemical, physical and functional properties of both components. We recently reported on the fluorescent quenching between GO nanosheets and an oligothiophene self-assembled monolayer allowing for the high contrast visualization of GO on quartz, silicon and ITO. Similar effects were observed in solution for oligothiophenes modified GO. Liu et al. reported the synthesis of a GO-sexithiophene (T6) material with interesting optical limiting properties originated by the spatial proximity, and comparing them with and without GO.

Liu’s synthetic approach consisted of a direct grafting of a sexithiophene amino end-capped framework to the –COOH moieties of the GO (Scheme 2a). On the contrary, our group reported on the MW-assisted synthesis of a quaterthiophene modified GO (GOT4) by reacting triethoxysilane T4-derivatives at the –OH groups of the GO (Scheme 2b).

However, both these synthetic approaches presented important drawbacks and limitations. The poor stability and/or non-trivial synthesis of the amino-functionalized oligothiophenes (in the former case) as well as the occurrence of undesired triethoxysilane polymerizations (in the latter case) represented important limiting factors.

The main advantages of our protocol (compared to those already reported in the literature) can be summarized as follows: (i) high solubility of the amino ended GO intermediate in organic solvents which allows the convenient coupling with dyes under mild conditions, (ii) easy access to several commercial, stable succinimidyl ester dye derivatives, (iii) easy control of the spacer length between the GO and oligothiophene dye, and (iv) high dye functionalization loadings (up to 5 wt%).

We observe high loading but low level of quenching and no significant change in the shape of the emission spectrum, which is observed, instead, in the case of solid films where intermolecular interactions are relevant.

Overall, the GO–C6T3 sheets studied here could be easily synthesized, processed and studied in different ways; pH dependent fluorescent emission could be observed even at a single-sheet level by confocal microscopy.

The main advantage of the molecule we chose is the ability to monitor chemical activity (pH dependence, solubility, concentration, etc.) by watching at the emission properties of the molecule, and comparing them with and without GO.

Changes in the dye structure can be exploited to tune the emission features of the resulting GO hybrid material over the entire visible range, paving the way towards new 2D low cost and highly processable fluorescent platforms for optoelectronic and imaging applications.

Main conclusions that shall be drawn from experimental and computational data presented here are:

- GO functionalized with C6-diamine can be effectively processed in several organic solvents, allowing its combination with the T3NHS dye, which is not soluble in water.

- The covalent engraftment to GO does not perturb the absorption and emission properties of the dye, and in particular the strong T3 interaction with the amidic group, which is the one giving pH sensitivity. GO sheets can be made photoluminescent and sensible to pH switching, obtaining the same properties of the free dye.

- It is noteworthy that the pH switching is observed even when the functionalized sheets are deposited on a substrate, indicating that the majority of the grafted molecules is accessible to the solvent and can feel the pH change, with no observable differences as compared to the free dye in solution.

- Thanks to this approach, GO can be loaded with high fraction (>5%) of T3, higher than the ones reported using other approaches.

Developing new techniques to attach molecules to a GO scaffold while maintaining high solubility and minimal perturbation of the properties of the molecules would allow the development of new materials and devices, with predictable properties, merging together the advantages of two or more different nanoscopic building blocks.

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