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# Graphene oxide functionalization with aminocoumarin nanosheet fluorescent dye: Preparation, electrochemistry, spectroscopy and imaging in the living cells

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ABSTRACT

A new nanosheet fluorescent chemosensor (GO–NH–COUR) based on graphite oxide (GO) covalently functionalized with a 4-methyl-7-aminocoumarin ($NH_2$-COUR) has been prepared. The GO were prepared by a modified Hummers method. The products were characterized by TEM, XRD, and FT-IR. The photophysical properties of GO, $NH_2$–COUR and GO–NH–COUR in aqueous and ethanol solution have been investigated by UV/vis spectrophotometry, steady-state and time-resolved fluorometry. They reflect a large effect of the $NH_2$–COUR substituent on the fluorescence characteristics of GO. In aqueous solution, GO–NH–COUR probes undergo protonation–deprotonation in the acid to basic pH range, producing intensity increases with acid to near-neutral pH range. The advantages of the newly prepared nanoparticles are that they offer good dispersion in aqueous solution and optical properties. Confocal microscopy experiments showed that GO–NH–COUR can be transfected into the living cells and applied for fluorescence imaging.



## 1. Introduction

Carbon-based nanomaterials, such as carbon nanotubes, nanodiamond, and carbon dots (C-dots), has been widely researched for years in optoelectronic devices, biological labeling and sensing [1–4] owing to their excellent properties like chemical-stability, tunable surface functionalities and electrical conductivity. Among these materials, graphene and graphene oxide (GO) have acquired much attention since the isolation of graphene nanosheets in 2004. GO, a two-dimensional (2D) oxidized derivative of graphene, is of great interest for its unusual properties such as good dispersion in water, unique optical properties, the large specific surface area, high mechanical strength, and superior electrical conductivity [5]. What's more, it has been shown that graphene induces no obvious toxic effects in vivo. It is believed that manipulation of the size, shape and relative fraction of the $sp^2$-hybridized domains of GO provides opportunities for tailoring its optical properties. Regarding GO exhibit fluorescence in a broad range including visible and near-infrared ranges, which have been used in chemical sensing and biosensing [6–8].

Since the first application of GO as biosensor [9], much GO-based fluorescent sensors based on fluorescence resonance energy transfer (FRET) have been elaborately designed for DNA [10–13], protein [14], ATP [15], glucose [16,17], RNA [18] and metal ions [19]. Great attention has been focused on the research of GO-based nanoparticles such as GO-Au nanocomposites [20], GO-n-butylamine complex [21] and graphene oxide/hydroxyapatite (RGO/HA) [5]. When it comes to the synthetic aspect, a lot of methodologies to synthesize graphene or reduced graphene oxide (RGO) have been reported, such as electrochemical, thermal methods and reduction of the precursor graphene oxide (GO) solutions using reagents. These reactions typically are more graphitic and conductive, which include the reduction of the functional groups such as epoxides on the surface of GO, and the resulting suspensions of the RGO.

Amino-coumarin dyes fluoresce in the blue-green spectral region. These dyes have high extinction coefficients and large Stokes shifts [22]. Fundamental studies of photophysical properties (quantum yield [23–25] lifetime [26] and solvent effects [26]) of amino-coumarin dyes in solution have been reported. One of the attractive features of these dyes is that they have high fluorescence quantum yields (typically 0.5–0.75) [26]. In general, GO shows very weak fluorescence due to the isolated $sp^2$ domains generated by oxidation [27]. Combining the fluorescent amino-coumarin with

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the GO can yield hybrid materials which combine good dispersion in water and optical responses on a single platform. Information about the photophysical properties of aminocoumarin dyes covalently functionalized with GO is not available in the literature.

In this paper, we investigate the photophysical properties of GO, GO –NH–COUR in aqueous and ethanol solution by UV/Vis absorption and steady-state and time-resolved fluorescence techniques. pH is an important factor that is closely related to physiological activity [28]. It is of great significance to reveal how the vis-NIR fluorescence of GO responds to pH in order to further explore GO for imaging and optical chemo/biosensing. However, to the best of our knowledge, no such study has been reported so far. So, the design of fluorescent chemosensors for the detection of pH in this work is the unique research. Here we report that the fluorescence was sensitive response of pH value. So the GO–NH–COUR has potential applications in bioimaging and pH detection devices.

## 2. Experimental

### 2.1. Instrument and reagent

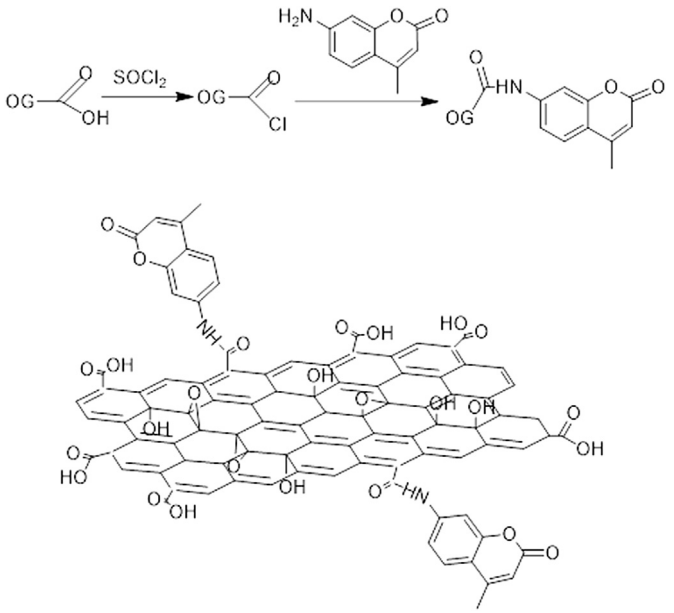
All reagents (Aldrich, Sigma–Aldrich, Acros, or Riedel-Dehaën) were reagent grade quality and were used without further purification. The 4-Methyl-7-aminocoumarin was purchased from Bai Ling Wei and was used as received without further purification.

### 2.2. Synthesis of GO–NH–COUR

**Synthesis of GO**: Graphite oxide was prepared from graphite by a modified Hummers method according to the reference [29,30].

**Synthesis of GO–NH–COUR nanoparticles**: GO–NH–COUR was prepared according with the following and shown in Scheme 1.

20 mg GO sheets were mixed with 5 mL DMF and Sonicated for 20 min and then transferred in 100 mL flask, 20 mL thionyl chloride were added to the suspension and stirred at 80 °C for 14 h. The reaction mixture was centrifugated and washed by centrifugation with THF for 2–3 times. Then the product and 0.2 g $NH_2$–COUR were mixed with 10 mL THF in 50 mL flask and stirred at 30 °C for 15 h Under the protection of argon.




**Scheme 1.** Synthesis of GO–NH–COUR.

### 2.3. Instrument

XRD measurements were performed on X-ray diffraction (D/max-2400pc, Rigaku, Japan) with Cu Kα radiation ($\lambda = 1.54178$ Å), with the operation voltage and current at 40 kV and 60 mA, respectively. The $2\theta$ range was from 0 to 50° in steps of 0.02°. The transmission electron microscopy (TEM) was obtained on a JEM-2100 transmission electron microscope at an acceleration voltage of 120 kV. Samples were prepared by placing a drop of a dilute alcohol dispersion of the products on the surface of a copper grid. Fourier transform infrared (FTIR) spectra were conducted within the 4000-400 $cm^{-1}$ wavenumber range using a Nicolet 360 FTIR spectrometer with the KBr pellet technique.

### 2.4. Electrochemistry

Electrochemical data were obtained using a CHI660B potentiostat and a standard three-electrode cell. All electrochemical tests were carried out in a conventional three-electrode electrochemical cell [glassy carbon (GC) working and platinum foil counter electrodes, and a Hg/saturated calomel electrode (SCE) reference] at a scan rate of 50 mV/s. The voltammograms were recorded at room temperature using a solution of 0.1 M NaOH aqueous solution. All solutions were purged with argon prior to measurement.

### 2.5. Steady-state UV–vis absorption and fluorescence spectroscopy

UV–vis absorption spectra were recorded on a Varian UV-Cary100 spectrophotometer. For the corrected steady-state excitation and emission spectra, a Hitachi F-4500 spectrofluorometer was employed. Freshly prepared samples in 1 cm quartz cells were used to perform all UV–vis absorption and emission measurements. For the determination of the fluorescence quantum yields $\phi_f$ of 1, only dilute solutions with an absorbance below 0.1 at the excitation wavelength ($\lambda_{ex} = 365$ or 340 nm) were used. Quinine sulfate/0.5 M $H_2SO_4$ ($\Phi = 0.55$) was used as fluorescence standard [31]. The $\phi_f$ values reported in this work are the averages of multiple independent measurements. The titration experiments with pH were carried out by adding small quantities of a stock solution of HCl or NaOH aqueous solution to a much larger volume (25 mL) of solutions of GO–NH–COUR.

### 2.6. Time-resolved fluorescence spectroscopy

Fluorescence lifetimes were measured by means of an Edinburgh Instruments FLS920 equipped with a light emitting diode (excitation wavelength 360 nm), using the time-correlated single photon counting technique [32,33] in 2048 channels at room temperature. The samples were dissolved in water or ethanol and the concentrations were adjusted to have optical densities at the excitation wavelength <0.1. The monitored wavelength was 460 nm and 480 nm for GO and GO–NH–COUR in solvents when excite the samples at 360 nm.

Histograms of the instrument response functions (using a LUDOX scatter) and sample decays were recorded until they typically reached $5.0 \times 10^3$ counts in the peak channel. Obtained histograms were fitted as sums of the exponential, using Gaussian-weighted nonlinear least squares fitting based on Marquardt–Levenberg minimization implemented in the software package of the instrument. The fitting parameters (decay times and preexponential factors) were determined by minimizing the reduced chi-square $\chi^2$. An additional graphical method was used to judge the quality of the fit that included plots of surfaces ("carpets") of the weighted residuals vs channel number. All curve fittings presented here had $\chi^2$ values <1.1. A global fitting of fluorescence