



# Theranostic Nanoplatforms of Thiolated Reduced Graphene Oxide Nanosheets and Gold Nanoparticles

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Received: 14 July 2020; Accepted: 3 August 2020; Published: 10 August 2020



# Featured Application: Spherical and rod-shaped gold nanoparticles grown on graphene-based nanosheets provide tunable plasmonic features as well as cytotoxic effects on human neuroblastoma cells.

Abstract: In this study, graphene oxide (GO) and reduced-thiolated GO (rGOSH) were used as 2D substrate to fabricate nanocomposites with nanoparticles of gold nanospheres (AuNS) or nanorods (AuNR), via in situ reduction of the metal salt precursor and seed-mediated growth processes. The plasmonic sensing capability of the gold-decorated nanosheets were scrutinized by UV-visible (UV-VIS) spectroscopy. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), thermogravimetric analyses (TGA), and atomic force microscopy (AFM) were performed in order to prove the actual reduction that occurred concomitantly with the thiolation of GO, the increase in the hydrophobic character as well as the size, and preferential gathering of the gold nanoparticles onto the nanosheet substrates, respectively. Moreover, the theoretical electronic and infrared absorption (UV-VIS and IR) spectra were calculated within a time-dependent approach of density functional theory (DFT). Eventually, in vitro cellular experiments on human neuroblastoma cells (SH-SY5Y line) were carried out in order to evaluate the nanotoxicity of the nanocomposites by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium reduction (MTT) colorimetric assay. Results pointed out the promising potential of these hybrids as plasmonic theranostic platforms with different hydrophilic or hydrophobic features as well as cytotoxic effects against cancer cells.

**Keywords:** plasmonics; nanorods; nanospheres; graphene toxicity; atomic force microscopy; thermogravimetry; ATR-FTIR; hydrophobicity; neuroblastoma

# 1. Introduction

Theranostics has a high potential to develop personalized medicine, since it combines diagnostic and therapeutic capabilities into a multicomponent platform, thus offering enhanced specific targeting, therapeutic efficacy, and real-time imaging of the therapeutic process [1]. Several classes of



nanomaterials and hybrid nanocomposites, owing to their unique physicochemical properties, are currently exploited as theranostic nanoformulation, especially for cancer diagnosis and treatment [2,3].

Graphene, made up of sp<sup>2</sup>-hybridized carbon atoms monolayers arranged into hexagonal crystal lattice [4], has shown intriguing properties such as ultra-high specific area [5], high thermal [6] and electrical [7] conductivity, low charge-transfer resistance [8], high tensile strength [9], and elasticity [10]. Despite such promising outlook, so far, a wide use of graphene in nanomedicine has been limited by its superhydrophobic character, i.e., the poor solubility in aqueous solvent. The development of chemically or physically modified graphene-based materials, including graphene oxide (GO) and reduced graphene oxide (rGO), allows for a more versatile use of graphene with tailored properties for applications in biosensing, imaging, therapeutic, and drug delivery [11–15].

The main oxygen-containing functional groups on GO nanosheets are epoxides (C–O–C), phenolic hydroxyl (–OH), carboxylic (–COOH), and carbonyl groups (C=O), which provide polar properties to the graphene-based material. Indeed, due to these functionalities, GO nanosheets are hydrophilic and stable in aqueous media. It is commonly accepted that in GO the –COOH groups are mainly located at the edges, while –OH and C–O–C groups are located at the basal plane of the nanosheets [16].

The introduction of heteroatoms on GO, including nitrogen, sulfur, and boron moieties [17,18], as well as polymers [19], enzymes [20], semiconductor quantum-dots [21], and magnetic and metal nanoparticles [22–24] has been successfully demonstrated either via covalent or noncovalent interactions [25]. To note, the reduction of GO after its chemical modification provides the possibility to improve the electrical, physical, chemical, and biological properties of the nanosheets [26–30] and, at the same time, to modulate their stability in aqueous dispersion by preventing aggregation caused by Van der Waals and  $\pi$ - $\pi$  stacking interactions [31]. In particular, the functionalization of GO and rGO with sulfur-containing moieties, either directly linked to the graphitic sp<sup>2</sup> carbon lattice or via sulfur-containing organic molecules, e.g., cysteine, has been exploited extensively for application in catalysis, electrochemistry, imaging, and biosensing and for the implementation of hydrophobic drug nanocarriers [32–36]. To this respect, great attention has been given to the high stability of the covalent bond between gold and sulfur, usually mediated by thiol (–SH) groups [37].

Along with the applications of GO in nanomedicine and theranostics devices, contrasting results were obtained from different authors for its cytotoxicity. Indeed, the different cytotoxic effects of GO in several cellular lines (both in vivo and in vitro) [38-41] are generally attributed to the internalization of the nanosheets inside the cells' matrix with the subsequent induction of reactive oxygen species' (ROS) production. Moreover, it was demonstrated that either GO or rGO cause loss of cell adhesion, can induce apoptosis and subcellular organelles morphology modification, destruction of the membranes' integrity, and DNA mutation [42,43]. To this regard, the cytotoxic effects of graphene oxide strongly depend on the physical-chemical characteristics of the nanosheets surface. Interestingly, rGO were found to be less toxic then bare GO, as tested with in vitro models [44] as well as with in vivo experiments, which did not induce thrombus formation caused by blood coagulation [45]. It has been reported that GO cytotoxicity is strictly dependent on the solution concentration, the ionic strength of the solvent, the absorption of plasma corona proteins, the nanoplates' lateral size, and, most importantly, the number of oxygenated groups present on its surface [46–48]. Noteworthy, a direct correlation between the reduction degree of rGO and the cells' viability was highlighted as result of lower interferences with the electron transport chain with oxygen surface groups of GO [49], which in turn prevents the donation of electrons to site I/II of the electron transport chain and thereby slows down the generation of ROS [50].

To note, during oxidation of the graphene layers of graphite to obtain GO, defects are generated, and oxidation occurs at these defect sites. The main possible oxygen-containing functionalities at defect sites are mainly ketone- and quinone-type groups; however, depending on the size of the defects, other functional groups can also be formed. The oxidation process also activates a side product formation during GO synthesis, referred to as oxidative debris (OD), which acts as a natural surfactant of the

GO nanosheets in dispersions and affects agglomeration of graphene oxide layers, thus influencing significantly the stability of GO nanosheets in the suspension [51].

Plasmonic properties of gold nanoparticles (AuNPs) are commonly exploited in biomedical imaging and biosensing [24,52], in biochemistry [53], photothermal therapy [54] and anti-angiogenic applications [55–58] as a result of the easily tunability of the surface plasmon (SP) band by varying size, shape, or by gold surface modification [59–61].

Specifically, gold nanospheres (AuNS) and nanorods (AuNR) have attracted a great interest in nano-biotechnology because of their easily adjustable cytotoxicity by size control, dose, or the capping agent and stabilizer used, facile way of synthesis, and high sensitivity to the microenvironment change [62–64]. The integration of GO layers with gold(0) nanostructures, to obtain hybrids GO@Au nanocomposite biosensors, exhibits the dual advantage of increasing the sensing performance, due to the localized surface plasmon resonance (LSPR) property [65], and owning the intrinsic biological activities of nanogold [24,66].

Previous studies have shown that the presence of GO substrates negatively affects the stability of gold nanospheres and nanorods, the main mechanism proposed for the nanoparticle destabilization involving the disruption and stripping of the protective capping ligands by the GO through strong electrostatic interactions [67].

In this study, we proposed a new theranostic platform based on thiolated reduced graphene oxide and integrated with plasmonic spherical and rod-like gold nanoparticles. To the best of our knowledge, although several examples exist in literature on AuNP-decorated GO nanocomposites obtained by in situ growth methods, including green approaches [24,68,69], only few studies have focused on the functionalization of thiolated GO with gold nanoparticles [70].

To avoid uncontrolled reactions and to modulate the hydrophobic character, the OD was removed from the as-received pristine GO by a treatment referred to as base-washing of GO (bwGO) [71]. Reduced monothiolated GO (rGOSH) was synthetized by a selective substitution of epoxydic and hydroxylic groups with thiourea at high temperature (70 °C), after a brominating treatment. Meanwhile, the surface decoration with AuNS and AuNR was performed by in situ reduction of the metal salt precursor and seed-mediated growth processes, by using the GO dispersion as solvent, either bwGO or rGOSH.

The plasmonic-sensing capability of the nanohybrids, in comparison to bare AuNP and GO, were investigated by UV-visible (UV-VIS) spectroscopy. The physicochemical characterization of the nanocomposites was performed by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), thermogravimetric analyses (TGA) in nitrogen environment, and atomic force microscopy (AFM). The experimental spectroscopic studies were complemented by density functional theory (DFT)-based calculations. *In vitro* cellular experiments on human neuroblastoma cancer cells (SH-SY5Y line) were carried out to test the nanotoxicity of the GO@AuNP hybrids in the comparison with the respective single components of AuNP, bwGO, and rGOSH.

#### 2. Materials and Methods

#### 2.1. Chemicals

Graphene oxide (chemically exfoliated) in aqueous dispersion (0.4 wt.%) was purchased from Graphenea Inc., US. Sodium hydroxide (NaOH,  $\geq$ 97%, pellet), hydrochloric acid (HCl, 37% in H<sub>2</sub>O), Thiourea ( $\geq$ 99%), hydrobromic acid (HBr, 48% volume ratio in H<sub>2</sub>O), ascorbic acid, gold(III) chloride trihydrate, cetyl-trimethylammonium bromide (CTAB), trisodium citrated dihydrate and silver nitrate (AgNO<sub>3</sub>) were purchased from Sigma-Aldrich (St. Louis, MO, USA). For all experiments, ultrapure water was used (18.2 m $\Omega$ ·cm a 25 °C, total organic carbon (TOC) lower than 5 parts per billion (ppb), UltrapureMillipore<sup>®</sup> Water Type) and the glassware was cleaned with aqua regia (HCl: HNO<sub>3</sub> 1:3 volume ratio) and rinsed with ultrapure water before each use. For cellular experiments, Dulbecco's Modified Eagle Medium (DMEM)-F12, penicillin-streptomycin, L-glutamine, fetal bovine serum (FBS), dimethyl sulfoxide (DMSO), Dulbecco's phosphate-buffered saline (PBS), paraformaldehyde and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich.

#### 2.2. Preparation of Base-Washed Graphene Oxide

The OD stripping from as-received GO was performed according to the procedure described by Thomas et al. [71]. Briefly, GO suspension was diluted with ultrapure ultrapure water to reach a concentration of 0.56 mg/mL. NaOH was added to the solution to a final concentration of  $14 \times 10^{-3}$  mM and then heated at 70 °C for 1 h under constant stirring. The obtained solution was centrifuged (30 min at 15,260× *g* Relative Centrifugal Force, RCF, room temperature, RT) (Eppendorf Centrifuge 5424) and the collected pellet was resuspended in ultrapure water and recentrifuged. The resultant dispersion was reprotonated with 14 mM HCl solution (1 h at 70 °C, under stirring) and, therefore, the pellet was collected and washed as before. The wet solid was lyophilized overnight to give bwGO powders. The bwGO was suspended in ultrapure water to reach a final concentration of 0.4 mg/mL with 120 min sonication (59 kHz, 25 °C) and then ultra-sonicated 2 h with a titanium cup-horn sonicator (Hielscher UP200Ht) at 200 W and 24 kHz to obtain bwGO120. The as-prepared suspension was centrifuged (20 min at 15,871× *g* RCF, RT) and the supernatant (bwGO120S), containing nanosheets of 200-nm average lateral size [72], and the pellet (bwGO120P) were lyophilized overnight.

#### 2.3. Synthesis of Thiolated Reduced Graphene Oxide

The functionalization of GO was performed by a one-pot thiolation-reduction mechanism [34]. In summary, bwGO120S (3.25 mg) was suspended in ultrapure water to give a 1-mg/mL solution after sonication (120 min, 59 kHz, 25 °C) and then 220  $\mu$ L of HBr were added under stirring (2 h at 30 °C). Subsequently, 220 mg of thiourea were added and the solution left to react for 24 h at 80 °C and then cooled at RT. After that, 2.2 mL of NaOH 4 mol dm<sup>-3</sup> were added, and the mixture stirred for 30 min, past which the mixture was filtered and washed with water over a 0.2  $\mu$ m polytetrafluoroethylene (PTFE) membrane. The solid collected was dried at 30 °C for 5 h to provide rGOSH.

#### 2.4. Synthesis of Gold Nanospheres (AuNS)

Aqueous dispersions of AuNS were prepared by a modified Turkevich method [73]. Briefly, 2 mL of 1 mM gold(III) chloride dihydrate solution in ultrapure water were brought to boiling point on a hotplate under stirring, then 200  $\mu$ L of trisodium citrate dihydrate solution (1% w/v) were quickly added and the mixture was vigorously stirred until it turned from yellow to deep red. The obtained colloidal dispersion was washed with ultrapure water through two centrifugation steps (15 min at 9766× *g* RCF, RT), in order to remove the excess of reactants. The average core diameter size (D) of citrate-capped AuNS was estimated by UV-VIS spectra, according to Equation (1):

$$\lambda_{\rm max} = 515.04 + 0.3647 \, \rm D, \tag{1}$$

where  $\lambda_{max}$  is the maximum absorption peak [74]. Therefore, the actual concentration of AuNS dispersion was calculated according to Equation (2):

$$\varepsilon = AD^{\gamma},$$
 (2)

where  $\varepsilon$  (M<sup>-1</sup> cm<sup>-1</sup>) is the extinction coefficient, D is the core diameter of the nanoparticles previously calculated,  $\gamma$  and A (in mol<sup>-1</sup> dm<sup>3</sup> cm<sup>-1</sup>) are experimental constants ( $\gamma = 3.30$ , A = 4.7 × 10<sup>4</sup> or  $\gamma = 1.47$ , A = 1.6 · 10<sup>8</sup> for D ≤ 85 nm or D > 85 nm, respectively) [75]. Assuming the size monodispersity of spherical nanoparticles' dispersion and uniform face-centered cubic (fcc) structure, the average number of gold atoms (N) for each nanosphere was calculated By Equation (3):

$$N = (\pi \rho D^3)/(6M),$$
 (3)

#### 2.5. Synthesis of Gold Nanorods (AuNR)

Aqueous AuNR dispersions were obtained by seed-mediated surfactant-capped synthesis [77]. Cetyl trimethylammonium bromide-capped seeds (Seed@CTAB) were prepared as follow: 25  $\mu$ L of 50 mM HAuCl<sub>4</sub> were added to 4.7 mL of 0.1 CTAB solution, in water-bath kept to 30.5 °C, and gently stirred for 5 min. Subsequently, 300  $\mu$ L of freshly prepared 10 mM sodium borohydride were added and vigorously stirred for 5 min, until color turned to brown-yellow. The growth solution was prepared by mixing 25  $\mu$ L of 50 mM HAuCl<sub>4</sub> to 2.5 mL of 0.1 M CTAB solution under stirring for 10 min, in 27.5 °C water-bath. Afterwards, 19  $\mu$ L of 100 mM ascorbic acid (solution turned colorless) and 20  $\mu$ L of 5 mM AgNO<sub>3</sub> were added. Lastly, 30  $\mu$ L of Seed@CTAB were added and the solution left undisturbed under vigorous stirring at 27.5 °C for 15 min. Turn in color was observed at the end of this time. The obtained colloidal dispersion was washed by reactants' excess through two centrifugation steps (9766× *g* RCF, 15 min), with water washings, by keeping the temperature just above 27 °C. The actual concentration of AuNR dispersion was calculated using molar extinction coefficient  $\epsilon$  (nM<sup>-1</sup> cm<sup>-1</sup>) by Equation (4):

$$\varepsilon = 1.2\lambda_{\max} - 4.8,\tag{4}$$

where  $\lambda_{max}$  is the maximum absorption of longitudinal plasmon resonance SPR peak [78], which position is affected, with a linear dependence, by the aspect ratio value [79], as shown in Equation (5):

$$\lambda_{\max} = 95R + 420. \tag{5}$$

#### 2.6. Synthesis of Hybrid GO@AuNS and GO@AuNR

For the synthesis of hybrid GO@AuNS nanocomposites, a one-pot reduction/decoration method was used [24]. Briefly, 2 mL of 1mM HAuCl<sub>4</sub> were heated to the boiling point under stirring. Then, 200  $\mu$ L of trisodium citrate dihydrate solution (1% *w/v*), prepared in GO aqueous dispersion (0.3 mg/mL, both for bwGO120S and rGOSH), were quickly added and the mixture was vigorously stirred until it turned in color. The resultant dispersions were washed with two centrifugation steps (9766× g RCF, 15 min, RT) to remove reactants in excess.

For the synthesis of GO@AuNR nanohybrids, an in situ seed-mediated growth method was used, as described. Then, 70  $\mu$ L of 1 mg/mL bwGO120S or rGOSH aqueous dispersions were mixed to 2.43 mL of 0.1 M CTAB solution under stirring for 5 min, in 27.5 °C water-bath. Afterwards, 25  $\mu$ L of 50 mM HAuCl<sub>4</sub> were added and stirred for 5 min. Next, 19  $\mu$ L of 100 mM ascorbic acid (solution turned colorless) and 20  $\mu$ L of 5 mM AgNO<sub>3</sub>, and finally 30  $\mu$ L of Seed@CTAB solution were added to the mixture. The solution was maintained for 15 min under vigorous stirring at 27.5 °C. Turn in color was observed at the end of this time. To remove the excess of reactants, the resultant dispersions were purified by means of two centrifugation (9766× g RCF, 15 min, T ≥ 27 °C) and water-washing steps.

#### 2.7. Physicochemical Characterization

#### 2.7.1. UV-Visible (UV-VIS) and Attenuated Total Reflectance FTIR (ATR-FTIR) Spectroscopies

UV-VIS spectra of the aqueous dispersions were recorded using Perkins Elmer 2S spectrometer and quartz cuvettes with an optical path length of 0.1 cm. ATR-FTIR spectra were recorded in the range of 4000–400 cm<sup>-1</sup>, using Perkins Elmer 100 FTIR spectrometer equipped with ATR system and zinc selenide (ZnSe) crystal. In order to prepare the samples for FTIR, the pellets recovered after the procedure of purification of the nanoparticle dispersions described above were lyophilized by a freeze-drying process. Pure trisodium citrate and CTAB spectra were obtained by analyzing the as-purchased solid. Thermogravimetric analyses were carried out on lyophilized powders by using a PerkinElmer Pyris TGA7 instrument. Experiments were run in the temperature range of 50–800 °C, with a heating rate of 10 °C min<sup>-1</sup> and under nitrogen flow (60 mL min<sup>-1</sup>). The samples to test were prepared as lyophilized powders of the dispersions purified as described above.

#### 2.7.3. Atomic Force Microscopy (AFM)

Topographic AFM images were acquired using a Cypher S instrument (Asylum Research, Oxford Instruments, Santa Barbara, CA) operating in dynamic contact mode (also called intermittent contact, AC mode or tapping mode) tapping AC mode and equipped with XY scanner with scan range of 30/40 µm (closed/open loop). Silicon tetrahedral tips, mounted on rectangular 30 µm long cantilevers, were purchased from Olympus (AC240TS, Oxford Instruments). The probes had nominal spring constant of 2 N/m and driving frequencies of 70 kHz. Samples were prepared by dropping 10 µL of bwGO, rGOSH, AuNS, AuNR, and hybrids' aqueous dispersion on a freshly cleaved muscovite mica (Ted Pella, Inc.). After 5 min of incubation at RT, samples were washed with 1 mL of Millipore water and dried under a gentle nitrogen flow.

#### 2.8. Computational Studies

The surface of graphene oxide was built by following the analyses of Klinowski et al. [80], with a random distribution of hydroxyl and epoxy groups on both planar sides of the GO sheet. The rGOSH was obtained by substituting oxygen atoms with sulphur atoms. A fcc gold unit cell was built by replicating it  $3 \times 3 \times 3$  along the unit cell edge length (*a b c*) in the three crystallographic directions. From the supercell, we obtained a rod-shaped Au nanoparticle with diameter of 1 nm and length of 2.5 nm and 135 atoms that was generated by using the build nanostructure tools included in the MS Materials Studio package.

All the calculations were performed by Gaussian 09 software at Density Functional Theory (DFT) level. The dispersion-corrected functional M06L [81] was used together with the 6-31G (d) basis set for GO and reduced monothiolated GO, whereas AuNR was described at the semi-empirical (SE) PM6 level [82]. UV-VIS and IR spectra were calculated within the time-dependent DFT approach.

#### 2.9. Cell Cultures

SH-SY5Y tumoral neuroblastoma cells were cultured in DMEM-F12 supplemented with 10% (v/v)FBS, glutamine 2 mM, 100 UI penicillin/ 0.1 mg/mL streptomycin. Cells were grown in tissue culture-treated Corning<sup>®</sup> flasks (Sigma-Aldrich, St. Louis, MO) under a humidified atmosphere of air/CO<sub>2</sub> (95:5) at 37 °C in an incubator (Heraeus Hera Cell 150C incubator). The cytotoxicity of the samples was tested on neuroblastoma tumor cells (SH-SY5Y). The cells were plated into a 96-multiwell plate in complete medium at a density of  $15 \cdot 10^3$  cells per well. Afterward, cells were treated for 24 h, in medium supplemented with 1% (v/v) FBS, with bwGO120S, rGOSH at the two concentrations of 1.35 µg/mL and 0.54 µg/mL, respectively, and the nanosystems, namely, AuNS, AuNR, the hybrids bwGO@NS, bwGO@NR, rGOSH@NS, and rGOSH@NR, by diluting 20 and 50 times the lyophilized samples resuspended at the concentration of 1 mg/mL in MilliQ water. Cytotoxicity was determined at 37 °C, by using the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) [83]. After 90 min of incubation, the enzymatic reduction of MTT to the insoluble purple formazan product was detected by dissolving the crystals with 100  $\mu$ L of dimethyl sulfoxide and thus measuring the absorbance at 570 nm by Varioscan spectrophotometer (Varioskan® Flash Spectral Scanning Multimode Readers, Thermo Scientific). The experiments were performed in triplicate and the results are presented as the means  $\pm$  standard error of mean (SEM). The statistical analysis was performed by student's *t*-test.

#### 3. Results and Discussions

# 3.1. Physicochemical Characterisation UV-Visible Characterization of GO-Based Nanosheet Dispersions and Plasmonic Nanoplatforms

From all UV-VIS spectroscopic studies, carried out in the comparison of thiolated reduced graphene oxide with graphene oxide, it can be inferred that the optical absorption of GO is dominated by the  $\pi \rightarrow \pi^*$  plasmon peak near 230 nm (Figure 1).



**Figure 1.** (a) Experimental UV-VIS spectra normalized to the  $\pi \rightarrow \pi^*$  absorbance peak of as-received graphene oxide (arGO, in water, solid line), base-washed GO (bwGO, in water, dashed line), and thiolated reduced GO (rGOSH, in dimethyl sulfoxide DMSO, dotted line). (b) Theoretical UV-VIS spectra of GO and rGOSH from semi-empirical computational calculations.

Figure 1a displays the experimental spectra of as-received pristine GO (arGO) as well as of GO after the base-wash treatment (bwGO) and the functionalization with thiol groups (rGOSH). The as-received GO exhibits an absorption peak centered at 232 nm, attributed to  $\pi \rightarrow \pi^*$  transition of aromatic C=C bonds and a shoulder at 300 nm, attributed to  $n\rightarrow\pi^*$  transition of carboxyl and other carbonyl and hydroxyl auxochromes [84]. The base-wash treatment on GO was expected to increase the hydrophobic character of the GO by the desorption of OD from the nanosheets' surface [71], which decreased the number of oxygen-containing groups and produced a partial restoration of the  $\pi$ -onjugated systems. Accordingly, the spectrum of bwGO displayed a redshift to 236 nm of the  $\pi\rightarrow\pi^*$  transition band accompanied by an increased broadening. No significant differences were observed in the  $n\rightarrow\pi^*$  absorption shoulder, except in the relative intensity increase (from about 50% to about 70%) compared to the peak of the  $\pi\rightarrow\pi^*$  transition. Since the latter depends on two kinds of conjugative effects, related, respectively, to nanometer-scale sp<sup>2</sup> clusters and linking chromophore units such as C=C, C=O and C-O bonds [85], it was inferred that the change of the UV-VIS absorption relative intensity of  $n\rightarrow\pi^*$  to  $\pi\rightarrow\pi^*$  transition in bwGO in comparison with arGO was caused by the decreased conjugative effect of chromophore aggregation, which mostly influenced the  $\pi\rightarrow\pi^*$  plasmon peak [17].

Finally, the UV-VIS spectrum of rGOSH sample showed the disappearance of the shoulder at around 300 nm and the absorption peak associated with the aromatic bonds significantly redshifted to 282 nm. The bathochromic effect, which is characteristic of the electrical continuity of the nanosheets and enhanced restoration of  $\pi$ -aromatic bonds [86], was a result of the brominating treatment at

elevated temperature, which provided the reduction of the bwGO [87]. Indeed, it was demonstrated that the thiolation reaction produced hydrosulfide byproduct ( $H_2S$ ), which, in turn, reduced GO partially to rGO, inducing a redshift in the UV-VIS absorption [32].

Noteworthy, the lower solubility of rGOSH than hydrophilic bwGO, and the tendency of the dispersion to aggregate in water (which required the samples to be dispersed in DMSO), ruled out the increased hydrophobicity of the nanosheets as further mark of the graphene oxide reduction.

Within the limits determined by the semi-empirical approach, the calculated UV-visible spectra, reported in Figure 1b, were in good agreement with experimental data. In particular, the theoretical UV-VIS spectrum of GO showed the peak related to the  $\pi \rightarrow \pi^*$  transitions at 240 nm, which was slightly redshifted than the experimental finding (at 236 nm), while the expected  $n \rightarrow \pi^*$  absorption near 300 nm was calculated at 370 nm. Additionally, theoretical data showed a  $\varepsilon_{\text{shoulder}}/\varepsilon_{\text{peak}}$  ratio of about 0.6. As to the calculated spectrum of rGOSH, an absorption peak of the  $\pi \rightarrow \pi^*$  transitions was found at 250 nm, but again no significant spectral evidence of  $n \rightarrow \pi^*$  transitions.

Table 1, which gives the molar extinction coefficients at the peak and shoulder wavelengths' positions as estimated from the experimental spectra and calculated by the computational method, confirms the qualitative trend discussed above.

**Table 1.** Calculated molar extinction coefficients from the experimental UV-visible spectra of as-received GO ( $140 \times 10^{-3}$  mg/mL in water), base-washed GO ( $20 \times 10^{-3}$  mg/mL in water), and thiolated reduced GO ( $10 \times 10^{-3}$  mg/mL in DMSO) and from the computational analysis.

|        | Experimental   |   | Theoretical   |   |  |
|--------|--|---|---|---|--|
| Sample | $\epsilon_{peak}$ ( $\pi \rightarrow \pi^*$ )<br>(mg <sup>-1</sup> mL cm <sup>-1</sup> ) | $\epsilon_{ m shoulder}~(n{ ightarrow}\pi^*)$ (mg $^{-1}$ mL cm $^{-1}$ ) | ε <sub>peak</sub> (π→π*)<br>(mg <sup>-1</sup> mL cm <sup>-1</sup> ) | ε <sub>shoulder</sub> (n→π*)<br>(mg <sup>-1</sup> mL cm <sup>-1</sup> ) |  |
| arGO   | 71.7   | 37.0  | -   | -   |  |
| bwGO   | 24.5   | 18.2  | 7.1   | 5.1   |  |
| rGO-SH | 47.0   | -   | 6.7   | -   |  |

Thermogravimetric studies confirmed the substantial structural modification of bwGO and rGOSH compared to the pristine GO. Indeed, the TGA curves reported in Figure 2 show different ranges of temperature and weight loss in the typical three steps, namely: (1) the low-temperature region (first step, from 50 to 110 °C), corresponding to the loss of physiosorbed water; (2) the intermediate-temperature interval (second step, from 110 to 350 °C), due to the loss of the unstable oxygen moieties (i.e., carboxylic groups); (3) the high-temperature region (third step, above 360 °C and up to 800 °C), assigned to degradation of the most stable functionalities (such as residual oxygenated groups and thiolated moieties) (see the quantitative analysis in Table 2.



**Figure 2.** TGA curves recorded under nitrogen flow for the lyophilized powers of as-received GO (solid line), base-washed GO (dashed line), and thiolated reduced GO (dotted line).

**Table 2.** Characteristic weight loss (%) as function of temperature steps calculated from TGA analyses for as-received GO, base-washed GO, and thiolated reduced GO.

|        | Step 1  |                    | Step 2  |                    | Step 3  |                    |                          |
|--------|---------|--------------------|---------|--------------------|---------|--------------------|--------------------------|
| Sample | (Δ ° C) | Weight<br>Loss (%) | (Δ ° C) | Weight<br>Loss (%) | (Δ ° C) | Weight<br>Loss (%) | Residue<br>at 800 °C (%) |
| arGO   | 50-110  | 4.2                | 111-300 | 44.5               | 360-450 | 10.7               | 34.0                     |
| bwGO   | 50-110  | 7.6                | 120-300 | 22.8               | 460-610 | 11.6               | 40.6                     |
| rGO-SH | 50-110  | 2.9                | 150-340 | 7.6                | 430-800 | 51.0               | 34.5                     |

The TGA curve of pristine GO exhibited a loss of physiosorbed water corresponding to 4.2% of weight loss. The structural modification expected in GO due to the base-washing procedure was confirmed by the higher release of physiosorbed water (7.6% weight loss during the first step), which suggested a weaker interaction of the water molecules on the bwGO (more hydrophobic) purely driven by hydrogen bonds and hence a dominant role of the attractive Van der Waals dispersive interactions than arGO. Moreover, the efficient stripping of OD can be ruled out by the lower weight loss during the second step (about 22.8% and 44.5% for bwGO and arGO, respectively). As to the third degradation step, it disclosed comparable weight losses both in arGO (10.7%) and bwGO (11.6%). To note, the first step of the TGA curve for rGOSH exhibited the lowest weight loss in the adsorbed water content ('only' 2.9% desorbed mass), thus evidencing a completely different binding mode of the water molecules onto the reduced GO in comparison with arGO and bwGO samples.

Also, as expected by the reduction that occurred upon the hydrobromic treatment of GO during the one-pot synthesis of thiolated GO, the second degradation step in the TGA curve for rGOSH sample was barely visible (7.6%). On the other hand, because of the presence of the sulfhydryl groups onto the reduced GO sheets, rGOSH exhibited the highest weight loss in the third degradation step (51.0%, i.e., considerably higher than the corresponding weight loss measured for arGO and bwGO), leaving a residue of 34.5%.

The structural changes of bwGO and rGOSH in the comparison with arGO were confirmed by infrared spectroscopy analyses (Figure 3).



**Figure 3.** Infrared spectra of rGOSH in the comparison with GO: (**a**) Experimental attenuated total reflection-FTIR (ATR-FTIR) spectra of pristine GO, base-washed GO, and thiolated reduced GO; (**b**) calculated IR spectra of GO and thiolated reduced GO.

In Figure 3a, the experimental ATR-FTIR spectra of arGO, bwGO, and rGOSH samples are shown. The IR spectrum of pristine GO demonstrates the presence of –OH (at 3392 cm<sup>-1</sup>), carboxyl –C=O and –C–O (stretching modes at 1737 cm<sup>-1</sup> and 1425 cm<sup>-1</sup>, respectively), aromatic –C=C (at 1622 cm<sup>-1</sup>), epoxy O–C–O (bending modes at 874 cm<sup>-1</sup>), and C-OH phenolic stretching (at 1050 cm<sup>-1</sup>), indicating the existence of oxygen-containing groups on the GO sheets [88]. The C-H stretching modes at 3222 cm<sup>-1</sup> are also visible.

Upon the base-wash treatment of GO, a diminished intensity in both the epoxy (at 874 cm<sup>-1</sup>) and the hydroxy (at 1050 cm<sup>-1</sup>) vibrational mode bands were observed, concomitantly with the appearance of a band at 1228 cm<sup>-1</sup>, attributed to the C–O–C stretching, and a redshift to 1580 cm<sup>-1</sup> and to 1716 cm<sup>-1</sup> of the aromatic –C=C and of C=O stretching modes, respectively. These findings are characteristic of the deoxygenated surface, which occurred with the removal of OD and subsequent restoration of conjugation in the  $\pi$ -aromatic system [89].

After the brominating and thiolation treatments, it was expected that the hydroxyl (–OH) and carboxylic (–COOH) functional groups of GO would be transformed into sulphur-containing moieties, including thiol (–SH), for the most part, but also thiocarboxylic (–COSH) or dithiocarbonxylic (–CSSH) groups [32]. Accordingly, for rGOSH sample, the absorption broadband in the wavenumber region around 3400 cm<sup>-1</sup> disappeared, which revealed the absence of OH group after reduction. In general, all C–O vibrational modes were no longer observed in the rGOSH spectrum and the peak belonging the –C=C vibrational mode was redshifted to 1565 cm<sup>-1</sup> [90]. The weak S-H stretching band was barely visible at 2667 cm<sup>-1</sup> [89].

The calculated IR spectra in Figure 3b confirmed the trend observed in the experimental spectra, i.e., the disappearance in rGOSH sample of the peaks due to the O-H stretch modes ( $3600-3400 \text{ cm}^{-1}$  region), the appearance of S-H stretching at 2770 cm<sup>-1</sup>, and the redshift of C=C stretching mode ( $1570 \text{ cm}^{-1} \text{ vs.} 1580 \text{ cm}^{-1}$  in GO sample). Moreover, in the fingerprint region, 2000–800 cm<sup>-1</sup>, where several convoluted bands were superimposed on a broader absorbance, significant changes in

the spectral feature were visible for rGOSH compared to GO, including the decrease of epoxide stretch at 1260 cm<sup>-1</sup> [91] and a significant absorption by C–S stretching modes at 720 cm<sup>-1</sup> [92].

The hybrid graphene-plasmonic gold nanoparticle nanocomposites were fabricated by a one-pot synthetic process through the reduction of the precursor metal salt in the aqueous dispersions of base-washed GO or thiolated reduced GO nanosheets.

Figure 4 shows the UV-VIS spectra of graphene-based hybrids with gold nanosphere (AuNS, Figure 4a) and nanorods (AuNR, Figure 4b), in the comparison with the spectra of the corresponding nanoparticles alone. To note, for all hybrid samples, the optical absorption of GO was buried by the spectral features of the gold nanoparticles, which instead maintained or even enhanced their plasmonic-sensing capability in the AuNS@GO and AuNR@GO nanocomposites.



**Figure 4.** UV-VIS spectra for as-prepared samples of: (**a**) citrate-capped gold nanospheres (AuNS, wine line) and hybrids with base-washed GO (bwGO@AuNS, red line) or thiolated reduced GO (rGOSH@AuNS, orange line); (**b**) cetyl trimethylammonium bromide-capped gold nanorods (AuNR, cyan line) and bwGO@AuNR (light blue line) or rGOSH@AuNR (navy line) hybrids.

The absorption spectrum of metal nanoparticles with diameters between 3–100 nm is characterized by a strong band caused by collective excitation of free electrons, which is usually ascribed to the surface plasmon resonance phenomenon. This absorption band is very sensitive to the structure of the particle and the nature of the surrounding environment, namely the size and shape of the nanosystem, and the presence of the ligands as well as the solvent in which the particles are dispersed [93].

Spherical gold nanoparticles having a diameter between 12 and 40 nm show an absorption peak in the visible region, at 520–530 nm, which gradually redshifts as the size of the nanoparticle increases [74]. Accordingly, Figure 4a shows that citrate capped AuNS exhibited a plasmon peak centered at 525 nm, which corresponds to spherical nanoparticles with an optical diameter of about 27 nm (Table 3). As to the NPs grown in the presence of bwGO or rGOSH, in both cases the SPR band of the hybrid was slightly broader and redshifted by about 5 nm, suggesting an effective interaction of the AuNS with the GO sheets, which provided new relaxation pathways to the free electrons on the metal nanosystems [67].

| Sample     | $\lambda_{max}$ (nm) | FWHM (nm) | D (nm)/ | R    |
|------------|----------------------|-----------|---------|------|
| AuNS       | 525                  | 53        | 27      |      |
| bwGO@AuNS  | 530                  | 63        | 41      |      |
| rGOSH@AuNS | 530                  | 62        | 41      |      |
| AuNR       | 716                  | 111       |         | 3.12 |
| bwGO@AuNR  | 689                  | 86        |         | 2.83 |
| rGOSH@AuNR | 687                  | -         |         | 2.81 |

**Table 3.** Optical parameters of wavelength at the maximum of absorbance ( $\lambda_{max}$ ) full width at half maximum (FWHM), optical diameter (D) and aspect ratio (R) calculated from the surface plasmon (SP) bands of AuNS, AuNR, and their hybrids with bwGO and rGOSH, according to Equations (1)–(5) in Materials and Methods section.

When the shape of AuNPs changed from spheres to rods (Figure 4b), the SP signal was split into two bands: A strong peak in the near-infrared (NIR) region (716 nm), corresponding to electron oscillations along the long axis, referred to as longitudinal band, and a weak band in the visible region at a wavelength similar to that of spherical gold (512 nm) due to the transverse electronic oscillation [94]. Interestingly, a blueshift of about 28 nm and 29 nm for the longitudinal plasmon band, respectively, of bwGO@AuNR and rGOSH@AuNR, indicated that the aspect ratio of AuNRs was significantly reduced when the seed-mediated growth process occurred in the presence of GO nanosheets. To note, for the rGOSH@AuNR hybrid the SP band at ~512 nm almost completely disappeared and the longitudinal plasmon band decreased in intensity, thus evidencing electronic and optical interference of GO on the plasmonic nanostructures [67].

In order to remove the excess of reactant, especially the CTAB used for the growth of nanorods, the nanocomposite systems were purified by centrifugation and water-washing steps (see Materials and Methods section) and characterized by UV-VIS spectroscopy (Figure 5).



**Figure 5.** UV-VIS spectra of the different nanocomposite dispersions, before and after the two centrifugation steps for (**a**) AuNS (wine), (**b**) bwGO@AuNS (red), (**c**) rGOSH@AuNS (orange), (**d**) AuNR (cyan), (**e**) bwGO@AuNR (light blue), (f) rGOSH@AuNR (navy).

The spectra in Figure 5 demonstrate: (1) The efficiency of the methodology, which collected most of the plasmonic particles in the pellets, while the supernatants were optically transparent; (2) the general stability of the prepared particles upon aggregation, with minor shifts (bathochromic for AuNS and GO@AuNS, hypsochromic for AuNR and GO@AuNR, respectively) in the plasmon wavelengths measured for most of the tested conditions.

Figure 6 illustrates the morphology of GO@AuNP nanocomposites, with spherical and rod-like nanoparticles detected most preferentially at the basal plane and at the edge of the nanosheets, respectively. The cross-sectional view of the typical AFM height images indicated a length over the longitudinal and transversal axes of AuNRs, respectively, of about 70 and 28 nm, while for AuNS a diameter of about 30 nm was measured, according to the electronic spectroscopy analyses. Moreover, substrate-dependent effects in the size and circularity of the nanoparticles grown in the presence of bwGO or rGOSH were disclosed by the quantitative study of the AFM height micrographs, as estimated by the values of particle average height, *z*, and aspect ratio, *r*, respectively (Table 4).



**Figure 6.** AFM micrographs of amplitude (top panels), height (middle panels, z scale = 15 nm), and section analysis curves (bottom panels) of: (a) AuNS, (b) bwGO@AuNS, (c) rGOSH@AuNS, (d) AuNR, (e) bwGO@AuNR, (f) rGOSH@AuNR. Arrows point to surfactant residues adsorbed on the mica substrates.

| Sample          | z (nm)     | r               |
|-----------------|------------|-----------------|
| AuNS            | $14 \pm 2$ | $0.75 \pm 0.02$ |
| bwGO@AuNS       | $12 \pm 2$ | $0.98 \pm 0.02$ |
| rGOSH@AuNS      | $15 \pm 2$ | $0.88 \pm 0.02$ |
| 4 NID           | 6 ± 2      | $0.60 \pm 0.02$ |
| Aunk            | 6 ± 2      | $0.88 \pm 0.02$ |
| hter<br>CO@AuNR | $8 \pm 2$  | $0.60 \pm 0.02$ |
| DwGO@Aunk       | $7 \pm 2$  | $0.84 \pm 0.02$ |
| rGOSH@AuNR      | $7 \pm 2$  | $0.98 \pm 0.02$ |
|                 |            |                 |

**Table 4.** Average height (*z*, in nm) and aspect ratio (r) values calculated from the atomic force microscopy (AFM) micrographs images recorded in AC mode in air for the different samples.

In particular, an opposite size change trend but a similar increase in circularity were displayed both by bwGO@AuNS (Figure 6b) and rGOSH@AuNS (Figure 6c), compared to the citrate-capped gold nanospheres (Figure 6a). On the other hand, a bimodal distribution of circularity was found for AuNRs with both rod-like and spherical-grown seeds (Figure 6d), which was maintained in bwGO@AuNR (Figure 6e) but not on rGOSH@AuNR (Figure 6f), where mostly spherical nanoparticles were detected. To note, residues of the CTAB used to assist the seed-mediated growth of the nanorods were clearly detected, especially for the sample of AuNR (see arrows in Figure 6d), since the cationic surfactant strongly interacted.

The infrared spectra analyses also confirmed the strong association of the gold nanoparticles to the GO substrates in the GO@AuNP nanocomposites (Figure 7).



**Figure 7.** Infrared spectra of rGOSH in the comparison with GO: (**a**) Experimental ATR-FTIR spectra of citrate-capped AuNS and hybrid GO@AuNS samples in the comparison with trisodium citrate; (**b**) experimental and calculated IR spectra of AuNR and GO@AuNR hybrids in the comparison with cetyl trimethylammonium bromide (CTAB) surfactant.

As to the hybrids of GO and AuNS, the experimental spectra in Figure 7a disclose significant shifts in the bands associated to the asymmetric and symmetric stretching modes of the citrate carboxylate (-COO<sup>-</sup>) moieties, interpreted as due to the unidentate covalent bond formation between oxygen atom of citrate -COO<sup>-</sup> moieties and gold atoms on the nanoparticle surface [95]. In particular, in the ATR-FTIR spectra of citrate-capped AuNS, bwGO@AuNS, and rGOSH@AuNS, the peaks for the -COO<sup>-</sup> asymmetric stretching modes were visible, respectively, at 1560, 1569 and 1556 cm<sup>-1</sup>, largely redshifted in comparison to the original peak position in the citrate at 1582 cm<sup>-1</sup> [96]. On the other hand, the peak for carboxylate symmetric stretching at 1388 cm<sup>-1</sup> in the citrate reference sample was found at 1398 cm<sup>-1</sup> for both AuNS and bwGO@AuNS, while it was slightly shifted to 1391 cm<sup>-1</sup> in the rGOSH@AuNS spectrum. Noteworthy, a weak absorption at 676 cm<sup>-1</sup>, attributed to the O-Au bond stretching mode, was visible in the AuNS spectrum [97]. Also, the characteristic features of C=O carboxylic and carbonylic vibrational modes of GO were discernable in the bwGO@AuNS and rGOSH@AuNS spectra at 1702 and 1701 cm<sup>-1</sup>, respectively. Moreover, characteristic C-O and C-H vibrational modes were quite visible in the GO@AuNS spectra below 1200 cm<sup>-1</sup> as well as the weak absorption attributed to S-H stretching mode (at 2664 cm<sup>-1</sup>) in the rGOSH spectrum [98]. The experimental IR spectra of CTAB-capped AuNR and of the hybrids with GO showed the characteristic absorption peaks of CTAB, with no significant shifts compared to CTAB alone, indicating the likely presence of unbound CTAB on the gold surface (Figure 7b). Specifically, the spectrum of CTAB showed the strong absorption bands attributed to the symmetric and asymmetric C–H stretching modes at 2916 and 2849 cm<sup>-1</sup>, respectively. Furthermore, the asymmetric and symmetric C-H scissoring of CH<sub>2</sub> of the hydrocarbon tail of CTAB were visible at 3017 and 1488 cm<sup>-1</sup> [99]. The peaks at 1463 and 1473 cm<sup>-1</sup> were characteristic of CH<sub>3</sub>–N<sup>+</sup> scissoring modes [100], while the C–N stretching mode exhibited a peak at 960 cm<sup>-1</sup>, as well as the vibrational mode at 719 cm<sup>-1</sup> corresponding to the bending of more than four adjacent CH<sub>2</sub> groups [101].

As to the calculated IR spectra, Figure 7b shows that the presence of AuNR on the surface of GO or rGOSH induced both hyperchromic and bathochromic shifts for the stretching modes of O-H (at 3490 cm<sup>-1</sup>) or S-H (at 2730 cm<sup>-1</sup>), respectively. To note, the computational approach unravels enhanced vibrational modes for the GO@AuNP hybrids that are commonly observed in surface enhanced Raman scattering (SERS) [102], such as graphitic band (at 1570 cm<sup>-1</sup>), representative of the sp<sup>2</sup> hybridized network of carbon in GO and C=C bonds at 1510 cm<sup>-1</sup> in rGOSH, respectively.

### 3.2. Biological Characterization of GO and GO@AuNP Nanocomposite Platforms

The theranostic capabilities of the fabricated GO@AuNP were tested in terms of cytotoxicity on human neuroblastoma cells (SH-SY5Y line).

The cell viability results from MTT assays demonstrated that the cells' treatment with bwGO and rGOSH at the tested conditions did not induce cytotoxic effects (Figure 8). Such results are in agreement with literature data, which evidence only a slight cytotoxicity on SH-SY5Y cells for treatments with GO at concentrations lower than 80  $\mu$ g/mL and a decrease (about 20% than control untreated) in the cell viability after 96-h treatment with 100  $\mu$ g/mL GO [24,103].

As to the cellular treatments with the nanoparticles, after 24 h of cells' incubation with AuNS or hybrid GO@AuNS, still no significant toxicity was detected (Figure 8a). On the contrary, the cellular treatments with AuNR or GO@AuNR induced a viability decrease of about 80% with respect to the control untreated cells (Figure 8b).

To note, the size, shape, and surface functional groups are pivotal to drive bioavailability, uptake, subcellular distribution, metabolism, and degradation of the nanoparticle. In particular, according to many reports, gold nanoparticles in the size range of 4–18 nm, upon capping with agents like citrate, cysteine, glucose, and biotin, are nontoxic in several cell lines, including leukemia cells (nanoparticle concentration up to 200 micromolar ( $\mu$ M), neuroblastoma and brain glioblastoma (nanoparticle concentration lower than 10 nanomolar [57,58]), and human immune system cells (nanoparticle concentration up to 100 micromolar [104]).

On the other hand, the synthesis of rod-like gold nanoparticles used in the present work required a seed-mediated approach based on the use of CTAB cationic surfactant [105], which formed a bilayer at the nanoparticle surface, which can be toxic to cells even at sub-micromolar dose [106]. Moreover, CTAB drives the growth and the formation of the rod [107] and, simultaneously, inferring also a positive charge to the nanoparticle [108]. However, the bilayer structure of CTAB on the AuNRs has been demonstrated being able to generate defects on the cell membrane, inducing cell death [109]. Moreover, as a cationic surfactant, CTAB itself can easily pass through the cell membrane and target mitochondria, thus causing apoptosis [110]. It has been demonstrated that the cellular toxicity of CTAB-capped gold nanoparticles is significantly reduced when the nanoparticles are centrifuged and washed with water, to remove unbound CTAB [111].

Although all the dispersions with rod-like gold nanoparticles used in this study were purified by centrifugation and water-washing (repeated a limited number of times for avoiding excessive aggregation) to remove the CTAB in excess (see Materials and Methods), the AFM and FTIR results discussed above rule out the presence of a significant residual amount of unbound CTAB. Hence, even if the concentrations of GO samples, nanorods, and related hybrid nanocomposites were scaled down for the cellular treatments, in comparison with the corresponding cellular experiments with AuNS, the cellular treatments either with bwGO@AuNR or rGOSH@AuNR nanocomposites resulted as still highly cytotoxic against the tumor cell line used in the present experiments.



**Figure 8.** Cell viability of SH-SY5Y cells after 24-h incubation with the dispersion of gold nanospheres (**a**) or nanorods (**b**) as well as the corresponding nanocomposite with bwGO and rGOSH. The (\*) p < 0.05, (\*\*) p < 0.01 (\*\*\*) p < 0.001, vs. untreated (CTRL), (#) p < 0.05, (##) p < 0.01, (###) p < 0.001, (####) p < 0.001 vs. bwGO and rGOSH (student's t-test).

## 4. Conclusions

In this work, nanocomposite 2D theranostic platforms based on graphene and gold nanoparticles were scrutinized by a combination of experimental techniques (including electronic and vibrational spectroscopies, thermogravimetric analyses, and atomic force microscopy) and computational methods. The surface functional groups on the graphene oxide and thiolated reduced graphene oxide substrates, and the resulting hydrophobic character, were demonstrated to drive the growth and the optical

response of plasmonic gold nanosphere or nanorods fabricated by a simple, one-pot, chemical reduction process from the metal salt precursor. The cytotoxic effects tested on a human neuroblastoma cell line showed the promising potential of these systems as theranostic platforms in cancer therapy.

**Author Contributions:** Conceptualization, P.T. and C.S.; experimental investigation, P.T., V.S., C.B., L.M.C., G.C., A.N., and P.G.M.; theoretical investigation, G.F.; data curation, P.T., V.S., and L.M.C.; writing—original draft preparation, P.T. and V.S.; writing—review and editing, C.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was partially funded by the Italian ministry of Education, Universities and Research (MIUR) (PRIN call, project code: 2017WBZFHL), the ERA-NET Cofund "M-ERA-NET 2" call (Project name "SmartHyCAR", number: 4274), and University of Catania (call: Piano della Ricerca di Ateneo, Linea di Intervento 2, 2018–2020 and GRABIO projects).

Acknowledgments: C.S. acknowledges the Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici (CIRCMSB), Bari, Italy.

Conflicts of Interest: The authors declare no conflict of interest.

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