Hybrid Structures for Surface-Enhanced Raman Scattering: DNA Origami/Gold Nanoparticle Dimer/Graphene

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A combination of three innovative materials within one hybrid structure to explore the synergistic interaction of their individual properties is presented. The unique electronic, mechanical, and thermal properties of graphene are combined with the plasmonic properties of gold nanoparticle (AuNP) dimers, which are assembled using DNA origami nanostructures. This novel hybrid structure is characterized by means of correlated atomic force microscopy and surface-enhanced Raman scattering (SERS). It is demonstrated that strong interactions between graphene and AuNPs result in superior SERS performance of the hybrid structure compared to their individual components. This is particularly evident in efficient fluorescence quenching, reduced background, and a decrease of the photobleaching rate up to one order of magnitude. The versatility of DNA origami structures to serve as interface for complex and precise arrangements of nanoparticles and other functional entities provides the basis to further exploit the potential of the here presented DNA origami–AuNP dimer–graphene hybrid structures.

1. Introduction

With the introduction of the DNA origami technique by Paul Rothemund in 2006[1] a versatile tool for the folding of DNA into almost any desired shapes and patterns was created. Initially applied for the fabrication of 2D structures the technique was rapidly extended to construct 3D objects.[2]

Due to the addressability of every single DNA staple strand being part of the self-assembly process, functional units, e.g., gold nanoparticles (AuNPs), can be attached to DNA origami templates with nm precision. Such hybrid structures have been used for the study of plasmonic effects[3–6] or surface-enhanced spectroscopies such as fluorescence enhancement[7] or quenching,[8] surface-enhanced Raman scattering (SERS),[9–12] and even for single-molecule SERS.[13] The basis of surface-enhanced spectroscopy methods is a metal surface—in most cases a metal nanoparticle (NP)—which is brought in close vicinity to the analyte molecule(s). Caused by the excitation of the surface plasmon resonance the electromagnetic field surrounding the NP is enhanced resulting in an increase of the detected signal, that is, fluorescence or Raman scattering. Dimers of AuNPs turned out to be superior over single AuNPs regarding their performances in electromagnetic field enhancements due to hot spot formation.[14,15]

Graphene is a 2D material consisting of sp²-hybridized carbon atoms which are arranged in a honeycomb lattice. It exhibits a unique combination of electronical, mechanical, and optical properties including high electronic[16] and thermal conductivities,[17] impermeability to any gases,[18] as well as high optical transparency.[19] Although graphene flatly
adsorbs on various substrates, it has been shown to replicate the shape of underlying macromolecules such as double-stranded plasmid DNA,\textsuperscript{[20]} 1D five-helix ribbon structures and 2D double-crossover lattices,\textsuperscript{[21]} as well as DNA origami structures\textsuperscript{[22]} very well due to its flexible nature. Recently, it has been reported that the structural stability of triangular DNA origami substrates toward mechanical forces or prolonged exposure to deionized water can be enhanced by graphene encapsulation.\textsuperscript{[22]}

Combining graphene and metal NPs within one hybrid structure is especially advantageous due to the resulting synergy of unique electrical, mechanical, and optical properties introduced by both initial materials. Especially within the fields of biomedicine\textsuperscript{[23,24]} and biosensing\textsuperscript{[25,26]} various types of hybrid materials based on graphene and NPs have been created. Principally, graphene–NP hybrid structures can be obtained either by decorating graphene sheets using metal NPs\textsuperscript{[27–29]} or by wrapping\textsuperscript{[30,31]} or covering\textsuperscript{[32,33]} NPs with graphene. Furthermore, graphene has been reported to remarkably suppress the photobleaching of dye molecules during continuous laser exposure. In shell-isolated SERS experiments using metal nanoparticles encapsulated by few-layer graphene (FLG), the SERS signal intensity of cobalt phthalocyanine (CoP) has been shown to be constant over a time range of 160 s.\textsuperscript{[30]} In another study, Zhao et al. presented R6G molecules being sandwiched between a Ag surface and a monolayer of graphene leading to enhanced photostability of the R6G molecules due to isolation from ambient oxygen.\textsuperscript{[34]}

Here, we introduce a novel kind of multifunctional hybrid material in which the programmability of DNA origami structures, the optical properties of AuNPs, as well as the protective properties of graphene are efficiently merged. The synergistic properties of these hybrid structures are investigated with respect to their SERS performance, polarization dependence of the SERS signal, and photostability of the dyes.

2. Results and Discussion


An overview of the two-step synthesis of the AuDG hybrid structures is depicted in Figure 1a. Initially, triangular DNA origami substrates are folded with a set of 208 staple strands using the genome of the bacteriophage M13mp18 as scaffold. Each DNA origami substrate contains four capture sequences for each AuNP (5′-(AAA)₈T₄-3′) protruding from opposite sides of the DNA origami triangle. Furthermore, 40 nm AuNPs are coated with dye-modified ssDNA (5′-(TTT)₄TX-SH-3′ or 5′-X-(TTT)₄T-SH-3′; X = carboxyethylrhodamine (TAMRA)). During a DNA hybridization process between the capture strands of the DNA origami substrates and the coating strands of the AuNPs the initial dimetric structures are created. Subsequently, the dimers are adsorbed on Si wafers covered with a layer of 290 nm thick dry thermal oxide (Si/SiO₂), which enhances the optical contrast within graphene and therefore allows for its identification.\textsuperscript{[35]} Since the mixture is not further purified after DNA hybridization AuNP dimers coexist with unbound AuNPs. In a second step graphene is deposited on top of the immobilized structures following the classical micromechanical exfoliation process\textsuperscript{[16]} resulting in AuDG hybrid structures.

Subsequent correlation of AFM and SERS images allows for a direct assignment of SERS signals to defined structures which is illustrated in Figure 1b (left: AFM image; right: SERS image). Usually, in AFM images of AuDG

**Figure 1.** Scheme of sample preparation and correlated AFM-SERS imaging. a) AuNP dimers attached to DNA origami substrates coexisting with single 40 nm TAMRA-modified AuNPs are adsorbed on an Si/SiO₂ substrate. In a second step graphene is exfoliated on top of the aforementioned structures by mechanical exfoliation resulting in numerous variations of structures: AuNP dimers can be covered by single-layer graphene (SLG), bilayer graphene (BLG), trilayer graphene (TLG), few-layer graphene (FLG), or graphite. b) Large-size AFM image of one graphene flake exfoliated on top of AuNPs (left) as well as the corresponding Raman map of a selected area (right). c) AFM image of single AuNPs as well as AuNP dimers (marked with white circles) covered by SLG. d) High-resolution AFM images of AuDG hybrid structures with two 40 nm AuNPs attached to one side of the triangle in which a part of the DNA origami triangle is visible (white arrows). The folds of the SLG flake surrounding the hybrid structure are also clearly apparent.
hybrid structures solely the AuNP dimers are visible (Figure 1c, white circles) due to the geometrical arrangement of the AuNPs with respect to the DNA origami substrate preventing the DNA origami from flat adsorption.[13] However, when the two AuNPs are attached to one side of the DNA origami substrate and when the structures are located in close proximity to the edge of the graphene flake the apex of the DNA origami triangle might become visible (Figure 1d, white arrows). Due to the larger gap size between the two AuNPs and the related reduced SERS signal this design of hybrid structure is not further investigated.

2.2. Dye-Modified Single AuNPs Covered by SLG

Basically, the TAMRA modification might be introduced at any position within the DNA coating strands of the AuNPs. Upon covering the structures with SLG some TAMRA molecules surrounding the AuNPs are sandwiched in between the Au surface and the graphene layer. Since both materials are known to influence the optical properties of dye molecules such as fluorescence[7,8,36] and Raman scattering[37,38] it is crucial to investigate the SERS performance of individual structures in dependence of the relative dye position. To disentangle additional effects due to hot spot formation within dimeric structures, also very basic SERS experiments on differently functionalized AuNPs are performed.

In the first case, the TAMRA dye is positioned in close vicinity to the AuNP surface using the 5′-(TTT)4T-SH-3′ (X = TAMRA) sequence as coating strands (Figure 2a). The same type of AuNPs is studied with SLG on top (Figure 2b). In the second case, the TAMRA dye is positioned further away from the AuNP surface by coating the particles with the 5′-X-(TTT)4T-SH-3′ sequence and thereby introducing a DNA spacer of 13 bases between dye and Au surface. In order to reduce the concentration of TAMRA molecules per particle, coating strands without dye are introduced (Figure 2c). Again, the same type of AuNPs covered by SLG is studied (Figure 2d). In all cases SERS measurements on single AuNPs are accompanied by AFM measurements in order to confirm the origin of each signal. In every SERS series (Figure 2a–d) the characteristic TAMRA bands[13] are marked with a green star whereby the peaks at 1361 and 1654 cm−1 are visible in all spectra and additional peaks at 1222, 1509, 1538, and 1570 cm−1 only appear in some cases. For AuNPs coated by DNA with TAMRA at the 5′-end (Figure 2c,d) a slight red-shift of 4 cm−1 for the two most prominent TAMRA bands is observed resulting in spectral positions of 1357 and 1650 cm−1, which is attributed to distance-dependent interactions between the dye molecules and the gold surface. Upon graphene encapsulation no further shift of the spectral positions of the TAMRA bands is noticed.

Furthermore, in the two cases with SLG (Figure 2b,d) the two characteristic bands arising from graphene (G band (1586 cm−1) and 2D band (2680 cm−1)) are highlighted. Both bands are highly sensitive to doping[39–41] and strain[42–44] which can cause peak shifts of several cm−1 even for pristine graphene without underlying AuNPs. Therefore, the slight variations in peak positions for the G and the 2D band which can be observed over the whole area of the graphene flake cannot be directly correlated to doping or strain caused by the AuNPs. For all SERS spectra of each series the noise within the spectral range of 1800–2200 cm−1 is determined and stated next to each spectrum (in units of counts). Figure 2a–d). Comparing the two cases without SLG (Figure 2a,c) it is obvious that the fluorescence background due to the resonant excitation of the TAMRA molecules (λem = 582 nm Δ 1610 cm−1) is more pronounced when the dyes are located at the 5′-end and thereby separated from...
the AuNP surface (Figure 2c). This is in good agreement with the known distance dependence of fluorescence enhancement \(^7\) and fluorescence quenching. \(^8\) In both cases without graphene a broad distribution of SERS intensities can be observed which is attributed to slight variations in AuNP sizes, numbers of TAMRA molecules per particle, as well as different photobleaching rates for individual AuNPs. The absolute SERS intensities obtained from AuNPs coated by DNA with TAMRA at the 3′-end or 5′-end cannot directly be compared since in the latter case a mixture of dye-modified and non-modified sequences was used as coating strands. However, both the noise averaged over all spectra and the maximum noise among all spectra are higher in the case of TAMRA being at the 5′-end (average: 6.5 counts; maximum: 13.0 counts) (Figure 2c) compared to TAMRA at the 3′-end (average: 3.4 counts; maximum: 6.6 counts) (Figure 2a).

For both types of AuNPs (with TAMRA being close to the AuNP surface (Figure 2a) or further apart from it and thus closer to graphene (Figure 2c)) the appearance of the SERS spectra is dramatically influenced by graphene encapsulation (Figure 2b,d). In the case of TAMRA being at the 3′-end (Figure 2b) the fluorescence background appears to be efficiently quenched resulting in a cleaner baseline compared to the analogue non-covered AuNPs (Figure 2a). The function of graphene as a potential quencher of fluorescence from dye (R6G) molecules was first described by Xie et al. \(^36\) and afterward also confirmed for the photoluminescence arising from a gold surface. \(^45\) In the first case the quenching process was postulated to be caused by a resonance energy transfer from dyes to graphene enabled by considerable π–π interactions. \(^36,46\) However, within the here presented structures direct π–π interactions between the TAMRA molecules and the graphene layer may not occur since both are separated by the DNA coating whose thickness is estimated to be 2.5 nm. \(^10\) Nevertheless, a remarkable quenching of the fluorescence background is observed. Although the nature of this effect is not resolved here, two possible explanations might be considered. On the one hand, based on the distance between TAMRA molecules and graphene an energy transfer from excited dye molecules to SLG is conceivable. Typical energy transfer processes such as Förster resonance energy transfer (FRET) can be observed for distances between 1 and 10 nm \(^47\) which is in good agreement with the here considered system. On the other hand, it cannot be excluded that a DNA-mediated charge transfer mechanism from the AuNPs to SLG is involved in the fluorescence quenching process similar to observations by Fritzsche and co-workers. \(^48,49\) They found that the excitation of a silver nanoparticle can be transferred via a dsDNA nanowire over a distance of several micrometers resulting in photobleaching of intercalating dyes. In the case of the here presented structures, also a combination of both, energy transfer and charge transfer, is conceivable.

Compared to their non-covered analogues the values for the average noise and the maximum noise are reduced for both, TAMRA located at the 3′-end (average: 2.6 counts; maximum: 3.5 counts; Figure 2b) as well as at the 5′-end (average: 3.0 counts; maximum: 5.7 counts; Figure 2d). Furthermore, the distribution of SERS intensities arising from the TAMRA signals is much narrower compared to the three other cases (Figure 2a,c,d).

The aforementioned π–π interactions responsible for an effective resonance energy transfer between dye molecules and graphene should be enabled if the TAMRA modification is located at the 5′-end of the AuNP coating strands. Interestingly, the SERS spectra arising from those types of structures seem to be categorized in two classes (Figure 2d). On the one hand, the three upper SERS spectra clearly display the characteristic fluorescence background of TAMRA with a maximum at \(\approx 1610\,\text{cm}^{-1}\). However, the different appearance of the backgrounds in the following three spectra can be assigned to the typical photoluminescence background characteristic for AuNPs with diameters less than 150 nm. \(^27\) The occurring differences are most probably due to a different extent of contact between SLG and the underlying AuNPs. Although at least some of the TAMRA molecules get in direct contact to the SLG their fluorescence is not significantly quenched in the case of the upper three spectra. For the following three AuNPs the interactions between graphene and the TAMRA molecules are more pronounced resulting in efficient fluorescence quenching and a remaining background whose appearance is defined by the photoluminescence of the AuNPs. This type of luminescence in turn is not quenched due to an insufficient contact between the AuNPs and SLG. Phase AFM images of all AuNPs corresponding to the SERS spectra presented in Figure 2b are shown in Figure S1 (Supporting Information). Additionally, analogue measurements are performed using cyanine 3 (Cy3) as Raman reporter molecule instead of TAMRA (see Figure S2, Supporting Information).

In summary, these experiments show that the best reproducible TAMRA signals as well as the cleanest baselines are obtained under two conditions: (a) the TAMRA molecules have to be located in close proximity to the AuNP surface in order to experience efficient fluorescence quenching and (b) the dye-modified AuNPs have to be covered by SLG to benefit from an additional fluorescence quenching, a reduced noise level, as well as a narrow distribution of TAMRA signal intensities. As a consequence, the following discussions are based on AuDG hybrid structures containing AuNPs with TAMRA at the 3′-end.

### 2.3. Comparison of AuNP Dimers and AuDG Hybrid Structures

In the next step AuNP dimers attached to one DNA origami substrate are investigated. In Figure 3a typical Raman maps of a surface area covered by different types of graphene (SLG, FLG, and graphite) are presented. The four maps exhibit the SERS intensity distributions of the four most important bands arising from AuNP dimers/AuDG hybrid structures, that is, (i) the G band (1586 cm\(^{-1}\)), (ii) the 2D band (2670 cm\(^{-1}\)), (iii) the TAMRA band at 1361 cm\(^{-1}\), and (iv) the TAMRA band at 1654 cm\(^{-1}\). It has to be mentioned that the TAMRA band at 1361 cm\(^{-1}\) can be spectrally overlapped by the D band of graphene at 1338 cm\(^{-1}\). For this reason Raman map (iii) exhibits the SERS intensity distribution of both peaks. Especially Raman map (iv) shows that there
are three different types of AuNP dimers: some covered by SLG, some covered by FLG, and also non-covered structures. In Figure 3b phase AFM images of representative AuDG hybrid structures (A, B, C; first row) as well as topography AFM images of non-covered AuNP dimers (a, b, c; second row) are shown. In the case of the AuDG hybrid structures it is obvious that the graphene layer covering the hybrid structures is differently folded and therefore the extent of contact between AuNPs and SLG differs for each individual structure. For hybrid A several folds within the graphene layer can be observed as a result of AuNP replication. This is not the case for hybrid B, and for hybrid C only a few folds are visible. In Figure 3c correlated SERS spectra arising from the AuNP dimers (red spectra) and the AuDG hybrid structures (blue spectra) shown in the AFM images in Figure 3b are presented. Additionally, for comparison typical SERS spectra obtained from SLG, FLG, and graphite are shown in gray and black. A comparison of SERS spectra from AuNP dimers and AuDG hybrid structures reveals that in both cases the characteristic TAMRA bands[13] at 1219, 1361, 1509, 1538, 1570 (superimposed by the G band for hybrids; blue spectra), and 1654 cm$^{-1}$ (spectral overlap with the TAMRA band at 1361 cm$^{-1}$) (ii), and the most characteristic TAMRA band at 1654 cm$^{-1}$ (iv). b) AFM images of three AuDG hybrid structures (first row; phase images) and three AuNP dimers (second row; topography images). Scale bars: 200 nm. c) Typical SERS spectra of SLG, FLG, and graphite (gray and black spectra). Extended SERS spectra including the 2D band at 1586 cm$^{-1}$ can be detected which coincide with the recorded bands for the single AuNPs (Figure S3 (Supporting Information)). Additionally, SERS spectra of the AuNP dimers (red spectra) and the hybrid structures (blue spectra) originating from the structures shown in (b) are presented. Laser wavelength: 532 nm, laser power: 80 µW, integration time: 10 s.

Figure 3. AFM–SERS correlation of AuNP dimers and AuDG hybrid structures. a) Raman maps visualizing the SERS intensity distribution of the G band at 1586 cm$^{-1}$ (i), the 2D band at 2670 cm$^{-1}$ (ii), the D band at 1338 cm$^{-1}$ (spectral overlap with the TAMRA band at 1361 cm$^{-1}$) (iii), and the most characteristic TAMRA band at 1654 cm$^{-1}$ (iv). b) AFM images of three AuDG hybrid structures (first row; phase images) and three AuNP dimers (second row; topography images). Scale bars: 200 nm. c) Typical SERS spectra of SLG, FLG, and graphite (gray and black spectra). Extended SERS spectra including the 2D band at 1586 cm$^{-1}$ which has not been used for any analysis is shown in Figure S3 (Supporting Information). Additionally, SERS spectra of the AuNP dimers (red spectra) and the hybrid structures (blue spectra) originating from the structures shown in (b) are presented. Laser wavelength: 532 nm, laser power: 80 µW, integration time: 10 s.

2.4. Polarization-Dependent SERS Measurements on AuDG Hybrid Structures

To test the extent of contact between SLG and underlying AuNP dimers which is related to induced strain within graphene, polarization-dependent SERS measurements are performed. In Figure 4 the obtained results for one representative AuDG hybrid are presented. Figure 4a shows two AFM images (left: topography; right: phase) of the investigated structure clearly exhibiting the folds within the covering SLG flake. In the right image the extension of folds is marked by yellow dashed lines and the position of the dimer axis (0°) relative to the angle of polarization is shown. SERS measurements are performed by using polarized laser light for excitation (parallel to the dimer axis; 0°; $\lambda = 532$ nm) and by changing the angle of the detection plane in steps of 15° for each measurement starting with +90°. In Figure 4b the corresponding SERS spectra for different polarization angles are presented. Both, TAMRA signals (green stars) as well as SLG signals (G and 2D band) arising from the AuDG hybrid structure in Figure 4a are polarization-dependent.

On the one hand, highest intensities for the TAMRA signals are detected for the polarization angle parallel to the axis of the AuNP dimer due to the optimal geometry for plasmon coupling of the two individual AuNPs.[14] Moving
Figure 4. Polarization-dependent SERS measurements of one individual AuDG hybrid structure. a) AFM images (left: topography; right: phase) of one representative AuDG hybrid structure for which polarization-dependent SERS measurements are performed. Scale bars: 100 nm. b) SERS spectra obtained for different polarization angles in steps of 15°. The most characteristic bands for TAMRA (green stars) and SLG (G and 2D band) are highlighted. c) Lorentzian fit curves for the 2D band in dependence of the polarization angle. A splitting of the 2D band is observed for polarization angles of +60°, −60°, −75°, and −90°.

away from this optimal polarization angle for plasmon coupling toward −90°/+90° leads to a decrease of the TAMRA signal intensities. The remaining signal is due to the detection of scattered light, which is not or only to a small extent affected by plasmon coupling of the two individual AuNPs.

On the other hand, in the case of SLG a polarization-dependence of the 2D band at ≈2670 cm⁻¹ can be observed. This is clarified in Figure 4c where Lorentzian fit curves for the 2D band under different polarization angles are presented. Nine of the 13 different SERS spectra can be described by a single Lorentzian function with a maximum at around 2670 cm⁻¹ (+90°, +75°, +45°, +30°, +15°, 0°, −15°, −30°, −45°). However, in the case of polarization angles of +60°, −60°, −75°, and −90° a second peak at ≈2630 cm⁻¹ arises resulting in fit curves consisting of two Lorentzian functions (blue and cyan bands in Figure 4c). From previous reports it is known that the G band as well as the 2D band in SLG can split in two components upon uniaxial strain. However, under the currently applied conditions the G band at 1586 cm⁻¹ is not clearly resolved due to low laser intensities and arising luminescence from the TAMRA dyes, especially for polarization along the axis of the dimer. Therefore, the following considerations concerning strain-induced observations are only based on the 2D band. Generally, splitting of the 2D band is induced by a change in symmetry of the graphene lattice upon uniaxial strain. As a consequence, the Dirac cone—representing a scheme of the electronic dispersion—is displaced from its original position resulting in altered interactions with its three nearest neighbors. This should result in the appearance of three contributions to the 2D band, however, the third peak is usually difficult to observe.

In the case of the AuDG hybrid structure in Figure 4a a clear correlation between the direction of folds surrounding the underlying AuNP dimer (yellow dashed lines in Figure 4a, right) and the polarization-dependent SERS signal is observed. The relative orientation of the polarization angles for which a splitting of the 2D band appears (Figure 4c; +60°, −60°, −75°, and −90°) to the AuNP dimer is in excellent agreement with the direction of folds and therefore with the direction of uniaxial strain.

It has to be mentioned that the laser spot size (≈1.3 µm in diameter) is much larger compared to the size of the AuDG hybrid structure. The detected SERS signals arising from SLG therefore contain information about several influences such as folding, doping or strain summed up over the graphene area irradiated by the laser. Therefore, the main proportion of the band at 2670 cm⁻¹ is arising from the area of SLG which is surrounding the AuDG hybrid structure and therefore only weakly influenced by the induced strain. The 2D⁺ mode which is expected to appear red-shifted upon uniaxial strain is most probably superimposed by the band at 2670 cm⁻¹. Nevertheless, confirmed by the appearance of the 2D⁺ band at 2630 cm⁻¹ the strain caused by the underlying AuNP dimer is obviously high enough to induce a mode splitting of the 2D band which is on the order of ≈40 cm⁻¹. Based on results obtained by applying controlled strain to SLG we estimate that the underlying structure in Figure 4a
induces an uniaxial strain component within the covering SLG of about 1%–2%.

2.5. Suppression of Photobleaching due to SLG

Photobleaching is an undesired side effect which often accompanies SERS measurements. It arises from irreversible decomposition of the analyte molecules caused by photochemical reactions.[51] Under the here applied conditions we expect heating effects,[52] as well as reactions with ambient oxygen[53,54] to be the two main sources for damages of the dye molecules.

In order to investigate the potential of graphene in terms of suppressing the photobleaching of the TAMRA dyes SERS time series measurements under continuous laser exposure for 800 s are performed for individual AuNP dimers and AuDG hybrid structures (Figure 5). Therefore, SERS spectra are recorded in time intervals of 10 s and for every spectrum the signal-to-noise (S/N) ratio for the most intense TAMRA band at 1654 cm\(^{-1}\) is calculated (the noise level is determined within the spectral range of 1800–2200 cm\(^{-1}\) of each spectrum). As can be seen in Figure 5a the S/N ratio is continuously decreasing for all three AuNP dimers with increasing laser exposure time. Since the amount of the aforementioned processes resulting in damages of the TAMRA molecules differ for each individual structure different photobleaching rates are obtained for dimers i–iii (inset of Figure 5a). More precisely, for dimers i (black data) and ii (dark red data) photobleaching rates (with regards to the S/N ratios) of 3.6 \(\times 10^{-2}\) and 1.7 \(\times 10^{-2}\) s\(^{-1}\) are determined. On the contrary, the S/N ratio arising from dimer iii (light red data) is decreasing following two different photobleaching rates: 4.7 \(\times 10^{-2}\) s\(^{-1}\) within the first 250 s of laser exposure and 1.0 \(\times 10^{-2}\) s\(^{-1}\) within the subsequent time interval. In Figure 5b the time evolution of the associated SERS spectra for the three investigated AuNP dimers are shown (the two most characteristic TAMRA bands are marked with a green star). The SERS spectra are presented in time intervals of 50 s starting with 10 s and ending with 800 s of laser exposure. Again, the first spectrum of each dimer recorded after 10 s of integration exhibits different overall SERS intensities, which is consistent with the aforementioned diversity for individual structures. In all three SERS time series measurements a significant photobleaching during laser exposure for 800 s can be observed. Interestingly, not only the SERS intensities of the TAMRA bands but also the noise level is reduced over time for all AuNP dimers, confirming the correlation between high SERS intensities and high noise levels (see Figure S4, Supporting Information for independent time evolutions of the signal and the noise level).

In contrast to the AuNP dimers a different photobleaching behavior is observed for the AuDG hybrid structures (Figure 5c,d). For hybrid I and II (Figure 5c, black and light blue data) the photobleaching rate of the S/N ratio is nearly identical (5.9 \(\times 10^{-3}\) and 4.2 \(\times 10^{-3}\) s\(^{-1}\)). Thus, compared to the non-covered dimers (Figure 5a,b) the photobleaching rate is reduced by approximately one order of magnitude.

In the case of AuDG hybrid I (black data) the time evolution of the SERS signal (Figure S4c, Supporting Information) can be divided in two parts: (1) an initial rapid decrease of the TAMRA band at 1654 cm\(^{-1}\) within the first 60 s of laser exposure and (2) in between the time interval of 60 and 800 s the decrease of signal intensity is significantly slowed down.

Figure 5. Photobleaching behavior of AuNP dimers and AuDG hybrid structures. Time series SERS measurements of a,b) individual non-covered AuNP dimers as well as of c,d) AuDG hybrid structures. SERS spectra are recorded in time intervals of 10 s using 532 nm laser excitation, a laser power of 25 µW, and integration times of 10 s. The temporal evolution of the S/N ratio of the TAMRA band at 1654 cm\(^{-1}\) is presented as a function of continuous laser exposure for three AuNP dimers (a) as well as for three AuDG hybrid structures (c). The corresponding SERS spectra recorded during 800 s of laser exposure are presented in steps of 50 s exposure time for AuNP dimers (b) and AuDG hybrid structures (d).
This behavior is attributed to different extents of interactions between the TAMRA molecules and the graphene layer. During the first 60 s of laser exposure the observed photobleaching is probably caused by those TAMRA dyes which are not or only weakly interacting with the SLG. Subsequently, the signal is more stable since the remaining intact TAMRA dyes efficiently interact with the SLG. On the contrary, after a slow decrease within the first 200 s the SERS signal of AuDG hybrid II (Figure S4c, Supporting Information, light blue data) stays constant until the end of the laser exposure confirming that SLG can provide extensive protection against photobleaching if the dye molecules interact strongly with the graphene layer. A damage of the SLG upon laser exposure can be excluded since no Raman modes characteristic for graphene damage such as D band (1338 cm\(^{-1}\)), D’ band (≈1620 cm\(^{-1}\)),\(^{55}\) or the combination band D+D’ (≈2940 cm\(^{-1}\))\(^{55}\) are observed.

However, AuDG hybrid III (blue data in Figure 5c,d) also exhibits a time evolution of the TAMRA signal which can be divided in two different parts, similar to the non-covered dimer iii (light red data in Figure 5a,b). The initial time interval (0–450 s of laser exposure) is defined by a rapid decrease of the S/N ratio with a photobleaching rate of 3.0 × 10\(^{-2}\) s\(^{-1}\) which is of the same order as for AuNP dimers. Subsequently, the rate is dramatically reduced to 7.3 × 10\(^{-4}\) s\(^{-1}\) within the time interval between 450 and 800 s of laser exposure.

The fact that for some structures two successive photobleaching rates can be determined allows for the assumption that the observed decrease in SERS signal intensity is based on at least two different contributions. This is also confirmed by the time evolutions of the corresponding noise levels (Figure S4b,d, Supporting Information) and attributed to (a) heating effects caused by the laser exposure which should be especially pronounced within the hot spot\(^{52,56}\) and (b) reactions with ambient oxygen. In the cases of AuNP dimers both should significantly contribute to the total photobleaching rate. However, for AuDG hybrid structures the SLG is expected to have an effect on both contributions for two reasons: (1) Since SLG has been demonstrated to be impermeable to any kind of gases\(^{18}\) the reaction with ambient oxygen can be excluded as possible photobleaching mechanism. (2) Graphene is known to be an excellent heat conductor exhibiting values of ≈5 × 10\(^3\) W mK\(^{-1}\).\(^{17}\) Thus, the plasmonically generated heat can be dissipated by the graphene.

The stronger the coupling of the surface plasmon resonances of the two individual AuNPs within a dimer the higher is the resulting electromagnetic field enhancement and thus the expected heating.\(^{52}\) This postulation is in good agreement with the experimentally observed behavior for different structures. In the case of AuNP dimers (Figure 5a,b) the highest initial SERS signal has been recorded for dimer iii (light red data) which is attributed to a hot spot providing the highest field enhancements among the three investigated structures (i–iii). As a consequence, the initial photobleaching is more significant compared to the other two AuNP dimers (black and red data) due to higher heat induced damage.

A similar behavior is observed for AuDG hybrid structure III (Figure 5c,d; Figure S4c,d, Supporting Information; blue data). In this case the contact between SLG and the underlying AuNPs is probably not strong enough to enable efficient heat conduction. On the contrary, AuDG hybrid II exhibits a relatively low initial SERS signal correlated with a low noise level which both remain constant over the whole period of 800 s (Figure 5c,d; Figure S4c,d, Supporting Information; light blue data). This is caused by a less pronounced electromagnetic field enhancement within the hot spot resulting in lower heating effects.

3. Conclusion

In summary, the synthesis of novel AuDG hybrid structures is reported and their optical properties with regard to SERS are carefully characterized. We find that AuNPs and graphene have a competing influence on the appearance of the fluorescence background obtained by sandwiched dye molecules. Moreover, a deeper understanding of the hybrid material is gained by polarization-dependent SERS measurements which allows for precise correlation of visible disorder within the graphene layer and spectral mode splitting of the 2D band. Furthermore, an improved photostability due to graphene encapsulation resulting in significantly lower photobleaching rates is clearly demonstrated. This is attributed to the efficient protection of the dye molecules from reactions with ambient oxygen by graphene and heat dissipation from the SERS hot spots.

The novel AuDG hybrid structures combine unique properties of three different building blocks within one material. Since every component can be independently tuned, hybrid structures represent a multifunctional tool in fields such as biosensing and bioelectronics. In this context, the addressability of the system introduced by the DNA origami template is highly beneficial since it allows for the precise arrangement of nanoparticles and other functional entities such as dyes, conducting polymers,\(^{57}\) etc. On the other hand, the structures might be suitable for promoting investigations in the field of graphene-enhanced Raman scattering (GERS)\(^{37,58}\) and correlated mechanisms. The main profit in this context is the possibility of using the DNA origami substrate as mediator in order to precisely tune the interaction between NPs and graphene.

4. Experimental Section

Preparation of AuDG Hybrid Structures: DNA origami structures, DNA-coated AuNPs, as well as AuNP dimers were prepared following a previously published procedure.\(^{13}\) The synthesis of DNA origami structures is based on the M13mp18 virus strand (New England Biolabs) used as scaffold which is folded to triangular shape by the addition of 208 short ssDNA staple strands.\(^{11}\) Eight staple strands (t-1s6e, t1s6i, t-1s8g, t1s8i and t-2s5f, t-2s7f, t2s5f, t2s7f) were extended at the 5’-end by the capture sequence 5’-(AAA)_4T_4-3’ in order to realize the attachment of two AuNPs per DNA origami substrate. Extended ssDNA strands were purchased...
from metabion, non-extended from Integrated DNA Technologies. Citrate-capped 40 nm AuNPs from BBI solutions were coated with two different types of TAMRA-modified ssDNA (5′-TTT4′, TX5′H′SH-3′ or 5′-X(TT)3′, T5′SH-3′; X = TAMRA) or Cy3-modified ssDNA (5′-(ATT)4′, T5′XSH-3′; X = Cy3) (Metabion) similar to the protocol of Ding et al.[9] using adjusted concentrations, reaction times, and 0.02% sodium dodecyl sulfate (SDS, Sigma Aldrich). DNA-coated AuNPs were attached to DNA origami substrates via DNA hybridization. Therefore, a temperature program was applied to a mixture of DNA origami structures and AuNPs (ratio 1:1). After hybridization the solution was used without further purification resulting in a coexistence of AuNP dimers and unbound AuNPs.

**Preparation of Si Substrates:** Si wafers covered with 290 nm thick dry thermal oxide (SiO2/ Si) (IDB Technologies Ltd.) were cleaned with acetone (absolute), heated to 250 °C for 10 min, and finally cleaned using an ozone cleaner (Diener electronic, ZEPTO) for 10 min. Immediately after ozone cleaning the AuNP dimers (coexisting with unbound AuNPs) were immobilized by transferring a 0.5–1.0 µL drop of the hybridization solution to the Si substrate and adding 40 µL of 10× TAE with 100 × 10–3 M MgCl2. After an incubation time of 60 min Si wafers were rinsed with ethanol (absolute)/Millipore water (1:1) and dried with compressed air.

**Mechanical Exfoliation of Graphene:** Graphene was deposited by the well-established process of micromechanical cleavage.[10] Flakes of Kish graphite (Naturgrafite GmbH) were repeatedly cleaved using sticky tape (Nitto Denko ELB PT150ECM) and afterward deposited on top of the AuNP dimers immobilized on Si substrates. As-prepared Si wafers were stored in methyl isobutyl ketone (MIBK, Sigma Aldrich) at room temperature for 3 h. After removal of the sticky tape, wafers were washed with MIBK and 2-propanol and dried with compressed air. Finally, exfoliated flakes were cleaved another time using sticky tape and flakes of SLG were identified using an optical microscope.

**AFM Imaging:** AFM images were recorded in tapping mode with a Nanosurf flexAFM using Tap150Al-G cantilevers (force constant 5 N m–1) (Budget Sensors). For analysis of AFM images the software Gwyddion 2.34 (freesware) was used.

**Raman Imaging:** For SERS measurements a confocal Raman microscope (WITec alpha300) with an upright optical microscope was used. The 532 nm excitation laser was coupled into a single-microscope (WITec alph a300) with an upright optical microscope on the sample by passing a 100× objective (Olympus MPlanFL N, NA = 0.9). Raman images in Figure 3a were performed using a laser power of 900–1000 µW and an integration time of 2 s. The SERS spectra of SLG, FLG, and graphite (Figure 3c) were extracted from these Raman images. Further Raman images (including the image in Figure 1b) were recorded with a laser power of 80 µW and an integration time of 10 s, and the SERS spectra a-c and A-C in Figure 3c as well as the spectra in Figure 2 were extracted from these Raman images. All other SERS spectra were obtained by recording single spectra on predefined positions. The laser power was further decreased to 25 µW for polarization-dependent measurements (Figure 4) and time series measurements (Figure 5). Before the beginning of the polarization series (Figure 4) it was ensured that the SERS signal stayed constant over three unpolarized measurements. In all cases, the grating of the spectrograph was set to 600 g mm–1. SERS spectra were vertically shifted for better visualization.

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**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

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