Hybrids Based on Graphene Oxide and Porphyrin as Tools for Detection and Stabilization of DNA G-Quadruplexes

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ABSTRACT: Telomerase inhibition has been an important strategy in cancer therapies, but for which effective drugs are still required. Here, noncovalent hybrid nanoplatforms containing the tetracationic 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin (TMPyP) and graphene oxide (GO) were prepared for promoting telomerase inhibition through the selective detection and stabilization of DNA guanine-quadruplex (G-Q) structures. Upon binding TMPyP to the GO sheets, the typical absorption bands of porphyrin have been red-shifted and the fluorescence emission was quenched. Raman mapping was used for the first time to provide new insights into the role of the electrostatic and π−π stacking interactions in the formation of such hybrids. The selective recovery of fluorescence observed during the titration of TMPyP@GO with G-Q, resembles a selective “turn-off—on” fluorescence sensor for the detection of G-Q, paving the way for a new class of antitumor drugs.

INTRODUCTION

Guanine-Quadruplexes (G-Q) are high-order structures of DNA formed by the self-assembly of guanine (G) bases, via Hoogsteen hydrogen bonding into planar G-quartets, and subsequent stacking of the G-quartets on top of each other, via π−π interactions.1 The stabilization of G-Q structures in telomeres has been considered as a promising strategy to achieve antitumor activity through the inhibition of telomerase. This enzyme plays a key role in the maintenance of telomeres length in roughly 90% of human tumor cell populations and prevents their senescence.2 Quadruplex folding in telomeric ends inhibits telomerase activity because this enzyme acts only on single-stranded DNA (ssDNA) and cannot recognize G-Q as substrates.3 Human telomeric DNA comprises tandem repeats of an overhanging single-stranded sequence 5′-TTAGGG-3′, which are able to form G-Q structures in physiological solutions and in the presence of suitable cationic ligands.4 To assess the potential of a compound as a telomerase inhibitor, selectivity for DNA G-Q over duplex DNA (dsDNA) is a fundamental attribute. This is because the drug must be able to recognize DNA G-Q in the presence of a large amount of duplex DNA, in the cellular nucleus.5 Early reports showed that cationic porphyrins have ideal features to inhibit telomerase through the stabilization of G-Q structures, namely due to their size, positive charge, and aromatic structure.6–9 5,10,15,20-Tetrakis(1-methylpyridinium-4-yl)-porphyrin (TMPyP, Figure S1) is easily bound to several DNA structures, establishing electrostatic interactions between its positively charged groups and the negatively charged phosphate backbone from DNA, as well as π−π stacking interactions between the porphyrin macrocycle and DNA aromatic nucleobases. However, a major drawback in TMPyP as G-Q ligands is the lack of selectivity against the more common dsDNA.10,11

As such, new strategies that circumvent this limitation are useful to improve the efficacy of therapies based on telomerase inhibition. As a striking example, the functionalization of graphene oxide (GO) with porphyrins has been pointed out as a strategy to develop selective and effective cancer therapies.12,13 To the best of our knowledge, the research reported here is the first example addressing the selective stabilization of DNA G-quadruplexes through hybrid nanoplatforms comprising cationic porphyrins and GO. Unlike graphene, GO contains both sp2 and random sp3-hybridized carbons with a large number of oxygen-containing functional groups on its surface, such as residual carboxylic acid, epoxide, hydroxyl, and carbonyl groups.14 Although the structure of GO is still under debate, it is commonly accepted that the majority of carboxylic groups are located at the peripheral edges, whereas the remaining groups are distributed over the basal planes of the GO sheets.15–17 In water suspensions, the carboxylic groups of GO are deprotonated above pH 4.3, thus, conferring to GO a negative surface charge at physiological medium (pH 7.4).18 Therefore, in addition to the π−π stacking interactions through the nonoxide domains, the negatively charged GO is able to establish electrostatic interactions with the positively charged molecules, such as cationic porphyrins. Here, noncovalent hybrid materials comprising GO sheets and TMPyP have been prepared and investigated as selective G-Q stabilizers. The porphyrin

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TMPyP was selected for this work based on previous studies with both GO-like sheets\textsuperscript{19−21} and G-Q structures.\textsuperscript{22−24} Nevertheless, other studies on DNA sensing using GO-based systems have been described in the literature.\textsuperscript{25} Examples include research on DNA adsorption/desorption mechanism on GO,\textsuperscript{26,27} the use of hybrid structures comprising metal oxide nanoparticles and GO,\textsuperscript{28} and the development of a GO-based system for sequence-specific identification of DNA molecules generated by polymerase chain reaction amplification.\textsuperscript{29}

\section*{RESULTS AND DISCUSSION}

Free TMPyP exhibits an intense Soret band at 423 nm in the absorption spectrum, corresponding to $S_0 \rightarrow S_2$ transitions (Figure 1). Significant spectral changes are observed upon successive additions of GO to TMPyP: the band originally peaked at 423 nm is red-shifted to 437 nm and its intensity decreases, indicating interaction between the porphyrin and GO.\textsuperscript{19} These are noncovalent interactions that occur mostly through: (i) electrostatic attraction between the positive pyridyl groups of porphyrin and the ionized oxygen moieties of GO; (ii) $\pi-\pi$ stacking promoted by the aromatic structures in both components of TMPyP@GO. Earlier reports have explained similar red-shifts to the molecular flattening of TMPyP molecules onto GO sheets, thus, the four cationic methylpyridinium moieties located at the meso-position of the TMPyP are rotated to lie flat in its plane.\textsuperscript{19−21} An effect that causes an increase in the energy of the ground state.\textsuperscript{25} Furthermore, the intensity of the strong emission band of TMPyP at 653 nm (inset on Figure 1) decreases upon the addition of GO. The interaction enlarges the $\pi$-conjugation of the system and contributes to the electron transfer from the excited state of TMPyP to GO, thus, decreasing its fluorescence emission compared to the free porphyrin.\textsuperscript{19,30,31} The potential of free TMPyP to promote telomerase inhibition has been explored because it has suitable molecular dimensions to match, induce, and stabilize G-Q structures.\textsuperscript{22−24} As mentioned above, the free porphyrin lacks selectivity, and therefore, the TMPyP@GO hybrids described here were evaluated as alternative G-Q stabilizing agents. First, a DNA oligonucleotide containing the telomeric sequence T$_2$AG$_3$ was selected because it is able to form tetramolecular G-Q structures (abbreviated as $(T_2A_3G_3)_4$) in physiological conditions, in the presence of suitable ions (e.g., K$^+$), and it allows the formation of complexes with positively charged ligands.\textsuperscript{3,24} Furthermore, the selectivity of TMPyP for G-Q was assessed by comparing the behavior of such hybrids in the presence of a DNA oligonucleotide containing the 5'-GC GCCGCGC-3' sequence that is able to fold into duplex DNA (termed as 4GC) in physiological medium. Hence, the association of free TMPyP and TMPyP@GO hybrids with the aforementioned oligonucleotides was monitored by spectrofluorimetric titrations. Upon addition of 4GC or G-Q to immobilized TMPyP in a physiological solution, the fluorescence intensity of the porphyrin decreased and the emission maximum wavelength shifted from 653 to 663 nm or to 659 nm, respectively (Figure 2A,B). These spectroscopic alterations are indicative of interactions between the positively charged porphyrin and the negatively charged DNA, mostly through electrostatic and stacking interactions involving the nucleobases. It has already been reported that TMPyP fluorescence may be quenched upon G-quadruplex binding by photon-induced electron transfer from guanine residues.\textsuperscript{24} In the present study, a more pronounced decrease in intensity was observed for the titration with the duplex 4GC when compared to G-Q. This result suggests that TMPyP has a higher affinity for the common duplex DNA than to induce and stabilize G-Q structures. This behavior limits the practical

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{UV−vis absorption spectra (350−550 nm) for the titration of TMPyP (1 mL, 2 μM) with GO (1 mg/mL), in aqueous solution. The inset corresponds to the fluorescence emission spectra (550−750 nm), at 423 nm excitation.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Spectrofluorimetric titrations (550−750 nm, excitation at 423 nm) of TMPyP (1 mL, 2 μM) with DNA oligonucleotides: (A) 4GC (able to form duplex structures), (B) G-Q $(T_2A_3G_3)_4$.}
\end{figure}

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applications of TMPyP as a selective G-Q stabilizer. To overcome this selectivity issue, the previous studies were extended to TMPyP@GO hybrids using both oligonucleotides for similar experimental conditions. It should be noted that the TMPyP fluorescence in these hybrids is quenched due to interactions with the GO sheets. The addition of 4GC (able to fold into dsDNA) to the TMPyP@GO hybrids causes a shift of the emission maximum from 653 to 664 nm (11 nm), while the band intensity undergoes a small increase (Figure 3A). Conversely, the addition of the G-Q (T2AG3)4 to the TMPyP@GO hybrid causes a shift from 653 to 660 nm (7 nm) and a prominent increase in the fluorescence intensity (Figure 3B).

**Figure 3.** Spectrofluorimetric titrations (550–750 nm, excitation at 423 nm) of a typical TMPyP@GO solution (1 mL, 2 μM) with DNA oligonucleotides: (A) 4GC; (B) G-Q (T2AG3)4.

GO interacts strongly with ssDNA through π-stacking between the aromatic hexagonal lattice of GO and the ring structures from the nucleobases and, possibly, hydrogen bonding between the nitrogenuous bases and the oxygen groups of GO.32,33 On the other hand, the adsorption of dsDNA onto the GO sheets is hampered due to shielding of the nucleobases by the negative charges from the phosphate backbone, the reason why GO displays lower affinities towards dsDNA.52,53 In this context, it is reasonable to assume that the TMPyP@GO hybrids show higher affinity towards the G-Q formed from the ssDNA sequence T2AG3 than to the duplex DNA 4GC.

The recorded results resemble the optical behavior that operates in a reversible "turn-off—on" fluorescent sensor for the detection of G-Q structures. G-Q structures are able to get closer to the TMPyP@GO hybrid, causing the release of TMPyP from the GO surface, which gives rise to an increase in the fluorescence emission (turn-on state). Thereby, the folding and stabilization of G-Q complexes are enhanced due to the TMPyP release. The selectivity of the hybrid nanoparticles towards G-Q structures was further tested through the simultaneous addition of both oligonucleotides to the TMPyP@GO hybrids (Figure S2). As expected, the TMPyP fluorescence was enhanced significantly and a red-shift from 653 to 657 nm was observed. This observation suggests that the release of TMPyP from TMPyP@GO towards the G-Q structure is still favored, even in the presence of the duplex 4GC. To assure that the fluorescence recovery was not related to a buffer effect, aliquots of phosphate-buffered saline (PBS) were added to the hybrid materials and no significant changes were observed (Figure S3). Besides, fluorescence was not observed in the 653–664 nm range, when the oligonucleotides were added to a physiological solution of pure GO (Figure S4). These control experiments demonstrate that the fluorescence recovery is not due to nonspecific interactions of oligonucleotides with buffer or GO, thus, supporting the hypothesis that the fluorescence turn-on state is a result of desorption of porphyrin from GO with subsequent G-Q stabilization. Furthermore, a comparison of Figures 2 and 3 above shows that the red-shifts observed in the emission band during the titrations of TMPyP@GO with the individual oligonucleotides are similar to those observed in the titration of free porphyrin. This suggests that the final species responsible for the fluorescence enhancements correspond to porphyrin and G-Q or 4GC DNA, i.e., without contribution of GO. However, the results presented above also indicate that the GO sheets in the hybrids do not have an innocent role in promoting, or hampering, the interaction between the TMPyP and the G-Q (T2AG3)4 or dsDNA 4GC, respectively.

This raises the question about the type of the hybrid structure present, in particular, by considering that the nanoscale distribution of the TMPyP over the GO sheets is certainly a relevant parameter.

The transmission electron microscopy (TEM) images of TMPyP@GO (Figure S5) have revealed GO sheets decorated with darker flakes that we ascribe to TMPyP aggregates. These aggregates are not evenly distributed over the GO sheets, rather accumulate in certain regions of the substrate, likely corresponding to over-stacked porphyrin layers. Indeed, scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM) images of the same regions in the hybrid have confirmed that even the less dark areas of the hybrid sheets were covered with porphyrin molecules (Figure S6). To have a better insight into the distribution of TMPyP over the GO sheets, we carried out a Raman imaging study on these samples. En route with this objective, we also demonstrate here for the first time the potential of this approach to get information about the spatial distribution of porphyrinic fluorophores on graphene-based materials and the interactions involved. Figure 4 presents the Raman spectra of TMPyP@GO collected on different surface regions of the sample (positions highlighted in the optical image in Figure 4A), together with the Raman spectra of GO and pure TMPyP. The most important vibrational modes and respective band assignments have been performed based on the literature.34–37 Here, we have monitored the diagnostic bands of GO at 1350 cm⁻¹ (D-band) and at 1600 cm⁻¹ (G-band), assigned respectively to the sp²-carbon containing...
moieties due to the defects introduced to the graphene lattice and to the sp\(^2\)-carbons of the two-dimensional hexagonal lattice.\(^{34,35}\) Although a detailed characterization of the vibrational modes of free TMPyP can be found elsewhere,\(^{36,37}\) the band at 978 cm\(^{-1}\), which has been assigned to vibrational modes of the pyridine groups, and the band at 1006 cm\(^{-1}\), which is closely related to the vibration of the porphyrin core, have been used here as diagnostic bands. It should be noted that for the hybrid material the latter bands exhibit intensities that are dependent on the analyzed region (Figure 4). This is most clear for the band at 978 cm\(^{-1}\), which is enhanced in relation to the free porphyrin, but whose intensity decreases as the Raman analysis progressively moves on to the inner surface of GO (Figure 4, from regions 1–3). This band is assigned to the in-plane bending deformation of the pyridine rings, thus having a strong contribution of electrostatic attractions through the positively charged pyridine groups and the negative charges on oxygen moieties, that are mostly located on the edges of GO sheets. The Raman band at 1006 cm\(^{-1}\) was observed in all the regions analyzed of the hybrid sample, thus suggesting that a layer of flattened TMPyP may be paving the inner region of GO sheets (with fewer negative charges), mostly through van der Waals and π−π stacking interactions.

Taking advantage of the Raman signatures associated with the two types of chemical interactions involved between the GO sheets and the porphyrin, the distribution of TMPyP on the GO sheets was mapped by Raman confocal microscopy. The Raman images shown in Figure 5 show brighter colors for the regions with the stronger Raman signal for the porphyrin (Figure 5A,B) and for the GO sheets (Figure 5C). Figure 5A shows the Raman image that results from the integration of the area of the band at 978 cm\(^{-1}\), corresponding to the vibrational mode of the positively charged pyridine groups of TMPyP, exhibiting a strong Raman signal predominantly around the GO sheets. This result supports the above interpretation that TMPyP molecules are mostly located at the edges of GO through electrostatic attractions.

On the other hand, the Raman image corresponding to the integration of the band at 1006 cm\(^{-1}\), assigned to the stretching mode involving the α- and meso-carbons of the porphyrin core, indicates an even distribution of the porphyrin over the GO surface. This result is interpreted by assuming a flattened orientation of the TMPyP molecules on the surface of the GO through van der Waals and π−π stacking interactions. Thus, the combined Raman image (Figure 5D) based on the spectra displayed in Figure 5E, shows the overall mapping of the TMPyP molecules that are interacting with the GO sheets via electrostatic (blue area) or by van der Waals and π−π stacking interactions (red area). Topography atomic force microscopy (AFM) measurements performed on the TMPyP@GO hybrids are consistent with the presence of GO sheets covered with TMPyP aggregates (Figure S7), as previously indicated by electron microscopy. In addition, the Raman mapping performed in the same region analyzed by AFM, by integrating the intensity of the Raman band corresponding to the stretching mode of the porphyrin core (at 1006 cm\(^{-1}\)), also indicates that the porphyrin is distributed over the GO surface but aggregates are also present. For comparative purposes, a Raman map is also shown for that region of the sample obtained by monitoring the D-band of GO (at 1350 cm\(^{-1}\)).

To obtain deeper insights into the turn-off–on model described above, Raman spectroscopic studies have been carried out using GO substrates treated with solutions of TMPyP@GO containing each of the DNA oligonucleotides, after the spectrofluorimetric titrations. In this case, no significant changes were observed in the average Raman spectra collected in different regions of the substrate, in the 1850–850 cm\(^{-1}\) range (Figure S8). In both cases, the band at 978 cm\(^{-1}\), which has been used here to monitor the TMPyP@GO electrostatic interaction is not distinguishable from the background noise. This result suggests that the porphyrin molecules linked through electrostatic interactions have been more easily detached from the surface of the hybrids, giving rise to the fluorescence enhancement described above.

However, in some regions of the substrate TMPyP@GO containing G-Q (T\(^{2}\)A\(^{3}\)G\(_{3}\))\(_{4}\) the absence of the band at 1006 cm\(^{-1}\), assigned to the vibration of the pyridine core, can be observed. This result suggests that the TMPyP molecules are more selective for the G-Q than to the GO sheets, in which they can migrate more easily from the GO surface and unbound the van der Waals and π−π stacking interactions to stabilize G-Q structures.

### CONCLUSIONS

Overall, the results achieved throughout this work provide a new basis to explore functionalized GO nanoplatorms that selectively stabilize G-quadruplex structures. Indeed, the noncovalent hybrids comprising TMPyP and GO have been successfully obtained and their complexation induced bathochromic shifts of the Soret absorption band and significant
fluorescence quenching. The hypothesis advanced in this research considers that the electrostatic interactions are the major driving forces to attract porphyrins to GO sheets, while π−π stacking interactions will assure a homogeneous distribution of porphyrins over the GO sheets. The stabilization of G-Q seems to occur through a turn-off–on fluorescence process. Upon the addition of oligonucleotide sequences that are able to form G-Q structures, the porphyrin is detached from the GO surfaces leading to fluorescence recovery. These findings open new avenues in antitumor drug design that envisages selective anticancer therapies based on the detection and stabilization of G-quadruplexes.

**EXPERIMENTAL SECTION**

**General Remarks and Synthesis.** All reagents were used as purchased from Sigma-Aldrich due to their high purity. Solvents were used as received or distilled and dried by using standard procedures according to the literature.59

Commercial graphene oxide aqueous suspensions (2 mg/mL, Nanocs) were centrifuged twice at 13 300 rpm for 5 min, and the solid was redispersed in deionized water to a final concentration of 1 mg/mL. The neutral precursor of TMPyP, 5,10,15,20-tetra-(4-pyridyl)porphyrin (TPyP, Figure S1) was synthesized through the reaction of 4-pyridinecarboxaldehyde with pyrrole in refluxing acetic acid and nitrobenzene for 1 h at 120 °C.40,41 After chromatographic purification, the quaternization of the pyridine units in TPyP was performed in the presence of iodomethane in dimethylformamide for 24 h at 40 °C, affording the desired cationic porphyrin TMPyP. The structure of all the synthesized compounds (Figure S1) was confirmed by 1H NMR (Bruker DRX 300, 300.13 MHz) and ESI-MS (Micromass Q-Tof-2).

A stock aqueous solution of TMPyP 50 μM was prepared and then diluted to 2 μM. The preparation of the TMPyP@GO hybrid was monitored by UV–vis spectroscopy and spectrofluorimetric titrations. Briefly, these titrations were performed at room temperature by the successive addition of 10 μL aliquots of the GO aqueous suspension (1 mg/mL) to 1 mL of an aqueous solution of the porphyrin (2 μM). After adding each aliquot, the mixture was allowed to rest for 1 min, to assure a higher reproducibility in the results. The interactions between GO and the porphyrin were monitored

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**Figure 5.** Raman images of TMPyP@GO hybrids, in which the vertical bar shows the color profile with the relative intensity scale. The images were obtained by using the integrated intensity of the Raman bands of TMPyP at 978 cm⁻¹ (A) and at 1006 cm⁻¹ (B), and the Raman band of GO at 1350 cm⁻¹ (C). The combined Raman image (D) was obtained by using two different Raman spectra for the TMPyP decorating GO sheets, as shown in (E).
through the red-shift in the position of the Soret band and by the quenching of porphyrin fluorescence. GO was continuously added to the porphyrin solutions until obtaining three consecutive and similar measurement values of red-shift and fluorescence, meaning that optimal interactions were achieved. In total, a volume of 50 μL of GO was added to each 1 mL of porphyrin solution.

**Assembly Assays Using DNA Oligonucleotides.** To test the selectivity of the hybrids towards G-Q structures versus duplex DNA, the selected sequences were: S′-TTAGGG-3′, able to fold into a tetramolecular G-Q structure (abbreviated as (T₄A₄G₄C₄)), and S′-GCGCGGC-3′, which folds as duplex DNA (abbreviated as 4G). The DNA oligonucleotides were purchased from Thermo Fisher Scientific (Germany). A phosphate-buffered saline (PBS) solution, containing 20 mM of phosphate buffer (10 mM of KH₂PO₄, 1 M and 200 μM of K₂HPO₄, 1 M), 0.1 mM of ethylenediaminetetraacetic acid, and 100 mM of KCl was prepared and the final pH was adjusted to 6.8. PBS solution was used as the solvent for TMPyP, GO, and oligonucleotides during the titrations. Each oligonucleotide was heated up to 85 °C for 10 min and left to cool overnight to assure the correct folding into duplex or G-Q structures. The concentrations of oligonucleotides in a single chain was determined by absorption spectrophotometry at 260 nm after the heating process described above and the final oligonucleotide had a concentration of ca. 15 μM. To ensure better reproducibility in the behavior of the hybrids in the presence of DNA in the spectrofluorimetric titrations, an excess of negatively charged GO (5 μL of GO 3 mg/mL) was added to each 1 mL of TMPyP@GO (porphyrin concentration 2 μM) before adding the DNA aliquots. During the spectrofluorimetric titrations, all the compounds were dissolved in PBS to mimic the physiological conditions. The emission spectra were recorded after excitation at 423 nm (TMPyP Soret band). The fluorescence values were corrected according to the dilution effect and the same amounts of oligonucleotides were added to the compounds to facilitate the comparison.

**Instrumentation.** All the UV–vis absorption spectra were recorded using a Jasco V-560 spectrophotometer, by placing the samples in quartz cells of 1 cm length. All the spectra were analyzed in the 195–850 nm range but the analysis was more detailed in the Soret band (350–500 nm) absorption region due to porphyrin absorption: Soret (350–500 nm) and Q-band (500–700) regions. Fluorescence emission spectra were measured with a Jasco FP-8300 spectrophuorometer. These spectra were obtained in the range of 433–750 nm by exciting the sample at the porphyrin Soret absorption maxima (423 nm). Excitation and emission slits were set at 10 and 5 nm, respectively. All the spectra were mathematically corrected for the dilution effect.

Raman studies were performed in a combined Raman-AFM confocal microscope WITec alpha300 RAS+. Drops of each hybrid solution and free GO were left to dry on glass substrates and then excited with a 532 nm line of a Nd:YAG laser (2 s, 10 acquisitions, 1 mW laser power). For Raman analyses of the hybrids with DNA oligonucleotides, a drop of each sample was collected and left to dry for 3 days before the measurements. To avoid the interference of PBS signals, these studies were performed by analyzing the interaction of TMPyP@GO in water with each oligonucleotide in PBS, instead of having the whole system in PBS (Raman data not shown). All spectra were normalized with respect to the intensity of the GO G-band at 1600 cm⁻¹ and corrected with respect to the baseline.

The solid spectrum of the free porphyrin TMPyP was also performed for comparison. Raman mappings were performed by raster-scanning the laser beam over the hybrid samples, acquiring a full Raman spectrum at each pixel of the optical image and finally integrating them to generate false color images based on the absolute area underneath specific bands at each pixel. The images were created by the WITec software, WITec Project 2.0 with 150 × 150 points per grid in a 50 × 50 μm area for small areas and 175 × 175 points per grid in a 175 × 175 μm area for large areas. The vertical bar in each Raman image corresponds to the relative color intensity of each selected Raman band. A Hitachi H-9000 electronic microscope operating at 300 kV was employed to collect the TEM images of a drop of the aqueous hybrid solutions when placed on a carbon-coated Cu grid and left to dry at room temperature. The SEM and STEM images were acquired by using a JEOL 2200FS instrument using the same sample preparation procedure.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01366.

Experimental details, chemical structures of porphyrins, fluorescence spectra of several titrations, TEM images, SEM and STEM images, average Raman spectrum of different regions of the hybrid samples after the titrations with oligonucleotides (PDF), AFM images.

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**Notes**

The authors declare no competing financial interest.

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**ABBREVIATIONS**

DNA, deoxyribonucleic acid; dsDNA, double-stranded DNA; GO, graphene oxide; G-Q, G-Quadruplex; QD, quantum dots; ssDNA, single-stranded DNA; SEM, scanning electron
microscopy; STEM, scanning transmission electron microscopy; TEM, transmission electron microscopy; TMPyP, 5,10,15,20-tetrakis(1-methyl-4-pyridinium)porphyrin

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