

# PROCEEDINGS OF SPIE

[SPIDigitalLibrary.org/conference-proceedings-of-spie](https://spiedigitallibrary.org/conference-proceedings-of-spie)

## Interactions of DNA coated upconversion nanoparticles with 2D materials

Davide Giust, María Isabel Lucío, Otto L. Muskens,  
Antonios G. Kanaras

Davide Giust, María Isabel Lucío, Otto L. Muskens, Antonios G. Kanaras,  
"Interactions of DNA coated upconversion nanoparticles with 2D materials,"  
Proc. SPIE 10507, Colloidal Nanoparticles for Biomedical Applications XIII,  
105070U (23 February 2018); doi: 10.1117/12.2282413

**SPIE.**

Event: SPIE BiOS, 2018, San Francisco, California, United States

# Interactions of DNA coated upconversion nanoparticles with 2D materials

Davide Giust<sup>a</sup>, María Isabel Lucío<sup>a</sup> Otto L. Muskens<sup>a,b</sup> and Antonios G. Kanaras<sup>a,b</sup>

<sup>a</sup>School of Physics and Astronomy, University of Southampton, Southampton, UK, SO17 1BJ

<sup>b</sup>Institute for Life Sciences, University of Southampton, Southampton, UK, SO17 1BJ

## ABSTRACT

In this work we investigated the nature of the quenching that occurs between different types of 2D materials (WS<sub>2</sub>, MoS<sub>2</sub> and graphene oxide) and oligonucleotide coated-upconversion nanoparticles. This study contributes towards the efficient design of bio-sensors based on 2D materials and DNA-coated upconversion nanoparticles.

**Keywords:** upconversion nanoparticles, DNA, dichalcogenides, Graphene oxide, 2D materials.

## 1. INTRODUCTION

In recent years, there has been a growing interest to explore the potential of upconversion nanoparticles for various applications<sup>1-3</sup> with a focus in biomedicine.<sup>4</sup> Moreover, platforms based on upconversion nanoparticles have been developed for the detection of specific biological targets.<sup>5</sup> Upconversion nanoparticles consist of a host matrix doped with lanthanides. These particles, when excited with near-IR light (low energies), emit at the visible region of the electromagnetic spectrum (high energies). Their optical properties offer various advantages compared to the use of standard emitters, or fluorescent organic dyes, such as the photochemical stability, large Stokes shift, absence of photobleaching and, most important, the avoidance of background autofluorescence.<sup>6</sup>

Various types of sensors based on the interactions between a fluorescent dye and a quencher have been recently reported for biomolecular detection.<sup>7-8</sup> Typical examples involve the use of 2D materials as quenchers and organic dyes or nanoparticles as fluorescence sources.<sup>9-12</sup> In particular, nucleic acids, among other biomolecules, have been reported to interact with various 2D materials and this property has been used to design relevant biosensors.<sup>13-14</sup> The working principle of this kind of fluorescent DNA sensors is based on the different interaction of dye modified single-stranded DNA (ssDNA) and dye modified double-stranded DNA (dsDNA) with the 2D materials. While the fluorescence of the dye modified single-stranded DNA (ssDNA) is quenched by the 2D materials, once in close proximity, the dye modified double-stranded DNA (dsDNA) does not interact with the 2D materials, and thus, its fluorescence is not quenched. These differences allow the detection of target oligonucleotides. Within this context, our group recently reported the design of a specific sensor for oligonucleotide detection based on the interactions occurring between UCNPs coated with single stranded DNA (UCNPS-ssDNA) and graphene oxide (GO).<sup>15-16</sup> The efficiency of the quenching is correlated with the adsorption of the oligonucleotides onto the surface of the 2D materials. The nature of the interactions taking place between the ssDNA and 2D material are pivotal to define as to whether quenching will efficiently occur or not.<sup>17-18</sup>

In this work we studied the nature of the interactions taking place between UCNPs-ssDNA and the 2D materials WS<sub>2</sub>, MoS<sub>2</sub> and GO. Initially, hexagonal core-shell UCNPs, which possess enhanced optical properties compared to the parental particles, were synthesized. Then, the surface of these particles was coated with a poly-acrylic acid polymer (PAA) in order to render them water-soluble. The end carboxylic groups on the nanoparticles were further functionalized with different amine-modified oligonucleotides using EDC coupling chemistry.<sup>5</sup> In particular, ssDNA sequences with two different lengths (30 or 40 bases) and different GC content (30% and 40%) were chosen to be used in this study. Then, the oligonucleotide coated nanoparticles were incubated with various concentrations of 2D materials and

subsequently in the presence of a DNA denaturing agent. After the analysis by fluorescence of the different experiments, the kind of interaction taking place between the UCNP-ssDNA particles and the different 2D materials was elucidated. This information could be used to get a more precise design of biosensors.

## 2. EXPERIMENTAL PART

### 2.1 Materials and methods

All chemicals in the experiment were of analytic grade purity and were used without further processing. Erbium(III) chloride hexahydrate (99.9%), ytterbium(III) chloride hexahydrate (99.9%), yttrium(III) chloride hexahydrate (98%), ammonium fluoride (98%), methanol (99.9%), n-hexane (95%), Poly(acrylic acid) (PAA) (MW 1.8 kDa), phosphate buffered saline (PBS) tablets, 2-(N-Morpholino)ethanesulfonic acid, 4-Morpholineethanesulfonic acid (MES), Sodium Borate, Sodium Chloride, 1-Octadecene (90%), Oleic Acid, N-(3-(dimethylamino)propyl)-N'-ethylcarbodiimide hydrochloride (EDC) (99%), Urea and N-hydroxysulfosuccinimide sodium salt (Sulfo-NHS) (98%) were purchased from Sigma-Aldrich (St. Louis, MO). Tetrahydrofuran (THF), ethanol and hexane were purchased from Fisher Scientific (Loughborough, UK) in laboratory grade. GO (powder, flake size: 0.5-5 $\mu$ m, flake thickness: 1 atomic layer – at least 80%) was purchased from Graphene Supermarket, Inc. (New York, NY). MoS<sub>2</sub> and WS<sub>2</sub> were purchased from ACS Material (Medford, MA) as powder of 0.2-5 $\mu$ m diameter, 1nm thickness and 90% of monolayer ratio for MoS<sub>2</sub> and 0.1-4 $\mu$ m diameter, 1nm thickness and 90% of monolayer ratio for WS<sub>2</sub>. All oligonucleotide sequences were obtained in collaboration with ATDBio Ltd (Southampton, UK). TEM images were obtained with a Hitachi H7000 Transmission electron microscope operating at a voltage of 75 kV. Milli-Q water was used in all experiments for PBS preparation. PBS (tablet, Sigma Aldrich, UK) was used to prepare the buffer accordingly to manufactures' instructions.

### 2.2 UCNPs nanoparticle synthesis

#### 2.2.1 Synthesis of core UCNPs (NaYF<sub>4</sub>: Yb<sup>+3</sup> (25%), Er<sup>+3</sup> (3%)).

The synthesis was performed according to previously reported methods with some modifications.<sup>19</sup> Briefly, the salts YCl<sub>3</sub>·6H<sub>2</sub>O (245mg, 0.81mmol), YbCl<sub>3</sub>·6H<sub>2</sub>O (107mg, 0.28mmol) and ErCl<sub>3</sub>·6H<sub>2</sub>O (15mg, 0.04mmol) were placed in a 100mL round bottom flask. Oleic acid (6mL, 19nmol) and 1-octadecene (15mL, 2.5mmol) were added to the flask and the solution was stirred under vacuum at 100°C for 1 h. Then, the mixture was left to cool down and a solution of NaOH (100mg, 2.5mmol) and NH<sub>4</sub>F (150mg, 4mmol) dissolved in 10mL of dry MeOH was added dropwise. The mixture was then stirred for 30 min at RT and it was heated under an argon flow gradually to 130°C to ensure the complete evaporation of MeOH or water. The temperature was raised to 305°C at 15°C/min rate and kept for 1h10min before to cool down the solution at room temperature overnight. The particles were precipitated with EtOH and centrifuged at 2200 rpm for 15min. This process was repeated for three times. After purification, the pellet of the particles (white pale powder) was left to dry at 80°C for several hours and stored.

#### 2.2.2 Synthesis of core-shell UCNPs (NaYF<sub>4</sub>: Yb<sup>+3</sup> (25%), Er<sup>+3</sup> (3%) @NaYF<sub>4</sub>).

The synthesis of core-shell nanocrystals was performed following previously published protocols with some modifications.<sup>5</sup> Briefly, YCl<sub>3</sub>·6H<sub>2</sub>O (150mg, 0.8mmol) was dissolved in a solution of 15mL of 1-octadecene and 6mL of oleic acid and stirred for 1h under Ar flow at 120°C. The mixture was cooled down to 40°C and after that a CHCl<sub>3</sub> (10mL) solution of the nanoparticles formed in section 2.2.1. (150mg) was added dropwise and the mixture heated under an Ar flow to 80°C (45min) to completely evaporate the CHCl<sub>3</sub>. Then, the mixture was cooled to room temperature under an Ar flux and NaOH (100mg, 2.5mmol) and NH<sub>4</sub>F(150mg, 4mmol) dissolved in 10mL dry MeOH was added dropwise. The temperature was increased gradually to 130°C to ensure evaporation of the MeOH or water. Then the mixture was brought to 305°C at 15°C/min rate under a steady Ar flow, left there for 1h 20min and then it was cooled at room temperature overnight. The particles were precipitated with dry EtOH and centrifuged at 2200rpm for 15min. This process was repeated three times. Then the core-shell nanoparticles were collected as white powder, left to dry, and stored as suspension in CHCl<sub>3</sub>.

## 2.3 Functionalization of UCNPs with ssDNA

### 2.3.1 Synthesis of UCNPs-PAA particles

Core-shell upconversion nanoparticles were coated with PAA to render them water soluble and appropriate for further coupling with ssDNAs. The experiment was carried out using a well-established ligand exchange method occurring between the oleic acid (OA) and the PAA.<sup>20</sup> Thus in a typical reaction 21mg of UCNPs-OA dissolved in 7mL of THF were added to PAA (0.25g) dissolved in 3mL of THF. The sample was left under stirring for 48h and then centrifuged and washed with EtOH to extract the nanoparticles (16400 rpm and 4°C for 10 min, 3 times). The particles were re-suspended in sterile DNase/RNase free Milli-Q water and stored at 4°C.

### 2.3.2 Synthesis of UCNPs-ssDNA

The carboxylic groups on PAA coated UCNP were coupled with amine modified oligonucleotides. In a typical reaction 0.5mg/mL of UCNPs-PAA suspended in borate buffer (pH 8.5) were added to 10µl EDC (2.9mg, 0.3M) and 20µl NHS (3.5mg, 0.3M) both dissolved in MES buffer (pH 5.5). The solution was sonicated for 10 min and then 21µl of ssDNA (8.4µM final conc.) were added and the mixture was left under stirring at room temperature overnight. Then the particles were purified via centrifugation (16400rpm and 4°C for 10 min, 3 times). The UCNPs-ssDNA were resuspended in sterile DNase/RNase free Milli-Q water and stored at -20°C for further use.

### 2.3.3 Design and Synthesis of ssDNA sequences

Specific sequences were found in the BLAST database <https://blast.ncbi.nlm.nih.gov/Blast.cgi> under the Genebank accession number AY266290 (Osmtpl), NM\_179953.3 (AtACT1), AY145451.1 (HvACT1). To the sense strands a poly-A (10 bases) sequence was added to impart rigidity to the sequence once attached on the particles to minimize the interaction of the single strands with the surface of the particles. Appropriate sequences were then chosen with the following criteria: Length of sense strand: 20-30 bases, melting temperature ~50 °C, GC content ≤ 50 %, E value ≤ 0.05, E value of nearest match > 1. The complete sequences as used for the synthesis of UCNP-ssDNA are reported in the table 1.

**Table 1.** DNA sequences used for the synthesis of UCNPs-ssDNA

Name	ssDNA sequences and modification
Osmtpl	5'-aminohexyl-AAAAAAAAAACTGATGGAGAGCACGCCCCG-3'
AtACT1	5'-aminohexyl- AAAAAAAAAAACTGTTTGTGGCTTGACG-3'
HvACT1	5'-aminohexyl AAAAAAAAAAAACCCAAAAGCCAACAGAGAGAAG-3'

## 2.4 Design of the experiment

### 2.4.1 Interactions of 2D materials and UCNPs-ssDNA.

UCNPs-ssDNA were sonicated in 1mL of PBS (0.5mg/mL). Then, they were added to known concentrations of 2D materials and the fluorescence was monitored. The upconversion fluorescence and photon measurements were

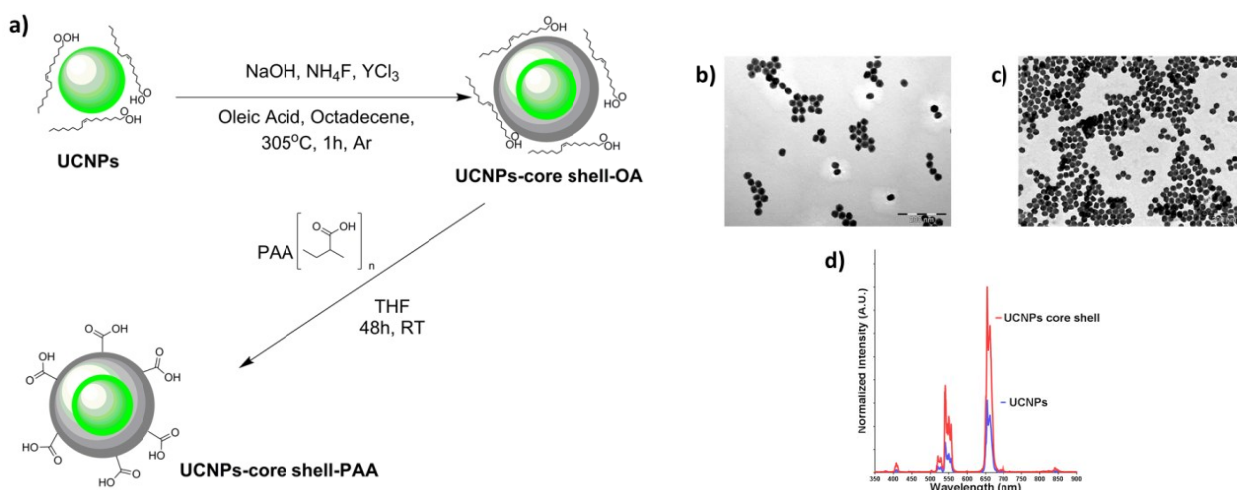
performed in a manually aligned setup with a continuous wave 980 nm 300mW diode laser (Thorlabs) as excitation source, and an SpectraSuite Spectrometer (OceanOptics, USA) and a single photon counting avalanche detector (id100, ID-Quantique, Switzerland). The emitted fluorescence was collected at an angle of 90° to the excitation beam using a 35mm focal length lens, and was analyzed using a fiber-coupled grating spectrometer (USB4000, OceanOptics, USA). Alternatively, fluorescence was detected by the id100 avalanche detector coupled to a PMS-400 photon counter card (Becker & Hickl GmbH). A short pass IR-blocking filter (FGS900) and a narrowband optical filter centered around 545nm (Thorlabs) were used to suppress scattered excitation light and select only the fluorescence emission. A cuvette with the corresponding solvent was measured under illumination with the 980 nm laser beam and set as the blank for each measurement. All fluorescence spectroscopy measurements were performed with 500 ms of integration time and 100 scans to average and were repeated at least three times with independent measurements. All photon counting measurements were performed at a wavelength of 545 nm with 10.000 ms of integration time, and each measurement was repeated 10 times.

### 3. RESULTS AND DISCUSSION

#### 3.1 Synthesis of functionalized nanoparticles

##### 3.1.1 Synthesis of PAA coated UCNPs

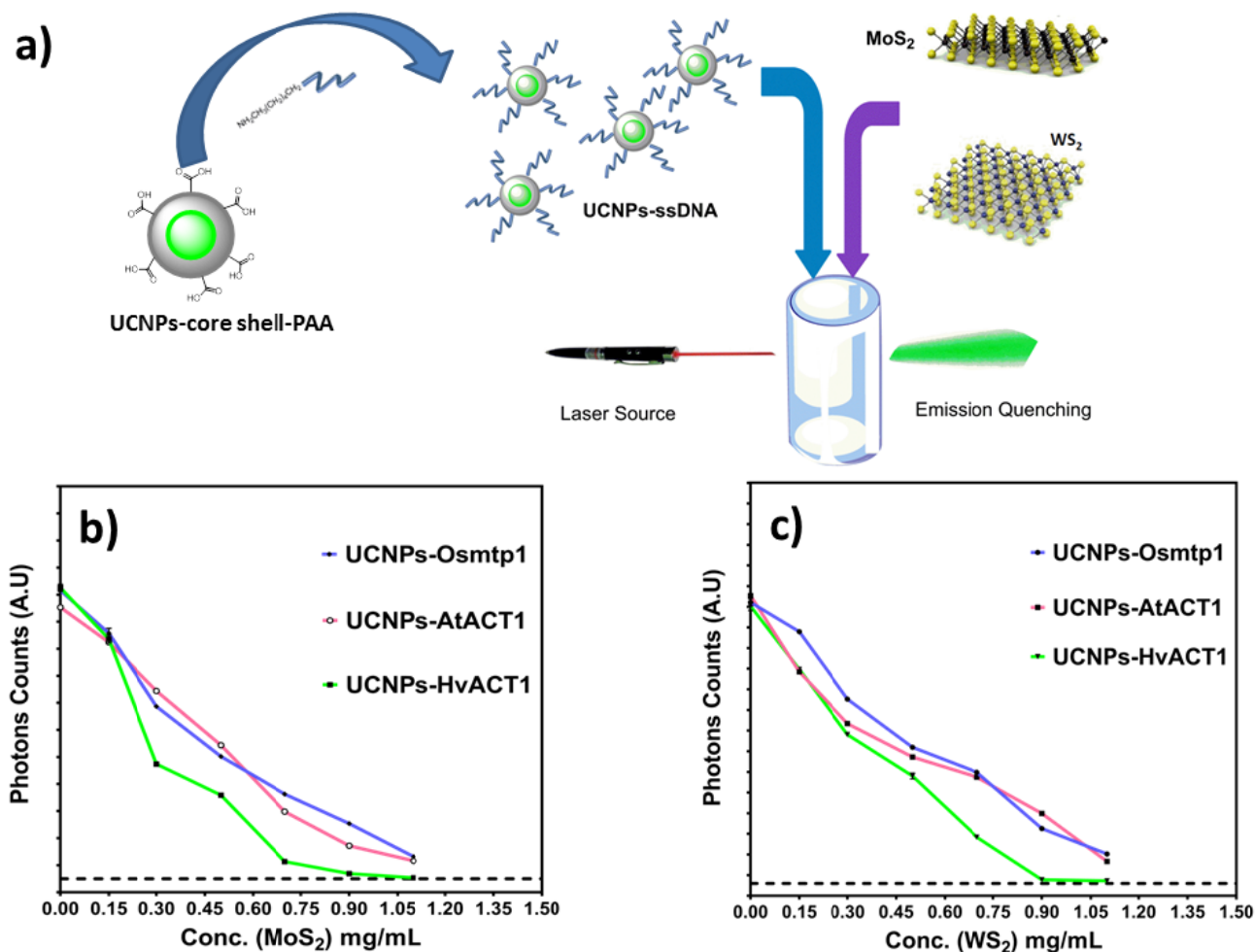
Core shell UCNPs were synthesized following methods reported in the literature with slight modifications.<sup>5,19</sup> In order to transfer UCNPs in water, a ligand exchange approach was utilized where the oleic acid was replaced by PAA (Figure 1a). This ligand exchange strategy offers various advantages compared to other common capping strategies, such as silica coating. The PAA polymer does not influence the emission properties of the nanoparticles and offers the functional carboxylic groups, which can be functionalized with amine modified molecules, here with the amino groups of ssDNA.<sup>20</sup> The core and core shell UCNPs were analysed by transmission electron microscopy (TEM) (Figure 1b and 1c). The images showed that the shell thickness of the shell was 7nm, leading to the formation of nanoparticles with an average size of 41nm diameter. The formation of the core shell also resulted in a considerable increase of the fluorescence emission of the UCNPs as shown in the normalized graph of nanoparticle fluorescence signatures under a 300mW excitation continuous power laser diode at 980nm (Figure 1d).



**Figure 1.** (a) A schematic illustration showing the formation of core-shell UCNPs and the subsequent ligand exchange to obtain PAA-coated UCNPs; Transmission electron microscopy images of (b) as synthesized pristine UCNPs and (c) after core-shell formation (scale bar on TEM pictures is 200nm); (d) normalized fluorescence emission of pristine UCNPs (blue line) and core shell UCNPs (red line). Fluorescence spectra were collected in hexane with the concentration of both UCNPs set at 1mg/mL.

### 3.1.2 Synthesis of core-shell UCNPs-ssDNA and quenching with 2D materials

Following PAA coating, the core-shell UCNPs displayed excellent solubility in water. These particles were functionalized with single strand DNAs (ssDNA) following an amino coupling procedure involving the formation of an amide bond between the amino group on the DNA strand and the carboxylic moieties of the PAA (Figure 2a). The fluorescence emission of the UCNPs-ssDNA was investigated in presence of various concentrations of 2D materials, and the quenching was analysed as a function of the decreased photons count (Figure 2b and 2c). The fluorescence of the UCNPs-ssDNA is affected by the ability of WS<sub>2</sub> and MoS<sub>2</sub> to quench the fluorescence of excited UCNPs-ssDNA after their adsorption onto the surface of these materials.



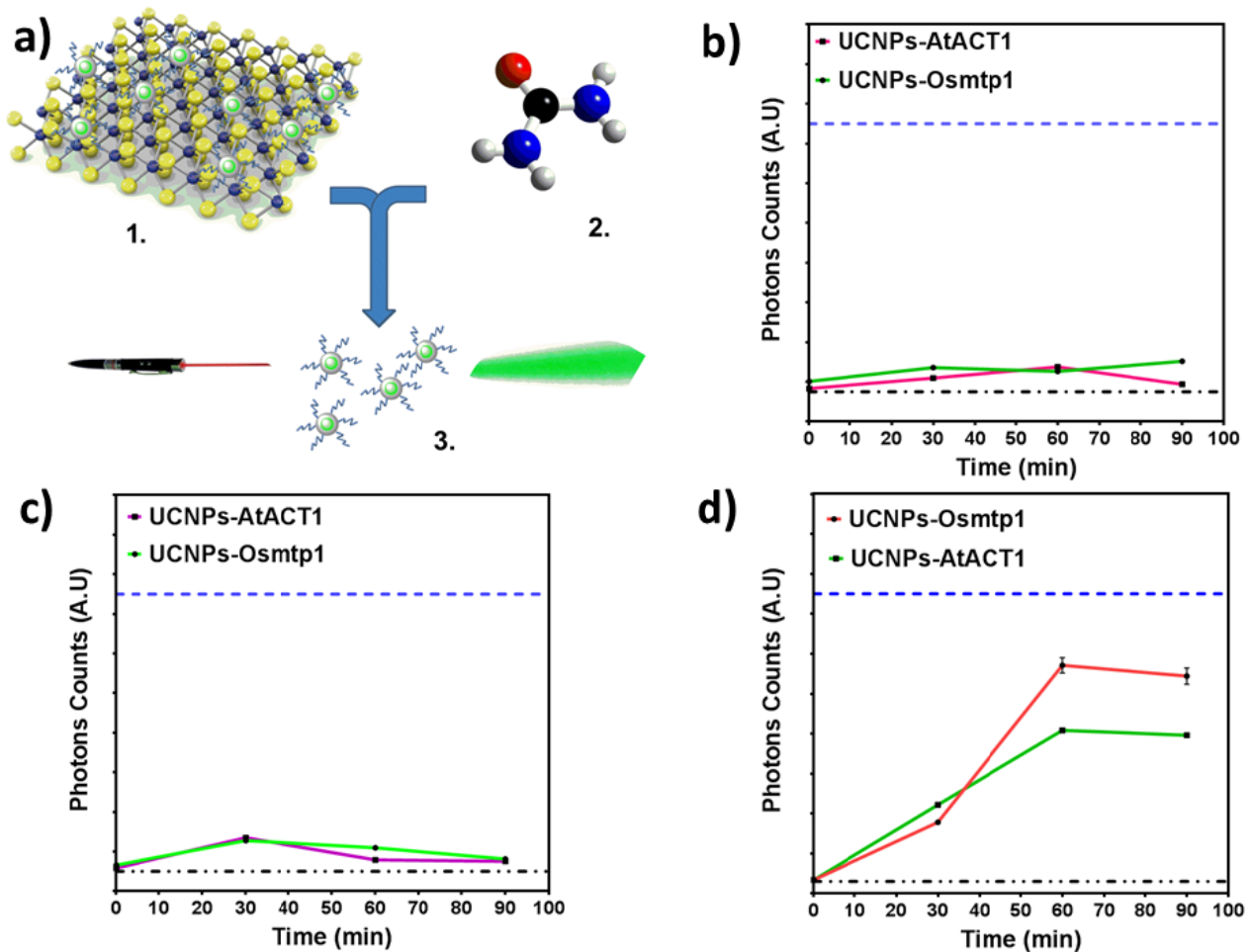
**Figure 2.** (a) A schematic illustration of the coupling between (a) amino-modified ssDNA and PAA-coated core-shell UCNPs; the UCNPs-ssDNA were incubated with increasing concentrations of 2D materials, and the photon counts were monitored in the presence of increasing concentrations of (b) MoS<sub>2</sub> and (c) WS<sub>2</sub> with a fixed concentration of UCNPs-ssDNA of 0.5mg/mL in PBS.

The collected photons' emission of UCNPs-ssDNA in the presence of increasing concentrations of 2D materials showed different quenching profiles related to the different oligonucleotides used. While for both, MoS<sub>2</sub> and WS<sub>2</sub>, similar trends were observed for all the investigated UCNPs-ssDNA, a difference can be observed and related with the length of the oligonucleotide sequences. In the case of UCNPs-HvACT1, which have the longest ssDNA sequences, a more efficient quenching by both MoS<sub>2</sub> and WS<sub>2</sub> was observed in comparison with the other samples (Figure 2b and 2c). This was in agreement with previous studies, which reported that the oligonucleotide length played an important role towards its

adsorption onto 2D materials.<sup>21</sup> On the contrary, the different length of the ssDNA oligonucleotides on UCNPs did not affect their quenching profile when the particles were incubated with graphene oxide, which also exhibited a better quenching activity.<sup>17</sup>

### 3.1.3 Effect of hydrogen bonding on the quenching activity

In order to further elucidate the mechanisms underneath the quenching effect of the 2D materials, we monitored the fluorescence recovery after the addition of urea in quenched samples (Figure 3). Urea is well known for its ability to break hydrogen bonds,<sup>22,23</sup> thus we employed it to elucidate the nature of oligonucleotide adsorption. To carry out the experiment we used the AtACT1 and Osmtp1 sequences, which possess the same length in nucleobases but different GC content (30 % for AtACT1 and 46.7% for Osmtp1).



**Figure 3.** (a) A schematic illustration of the experiment. Urea was added to the adsorbed nanoparticles on the 2D material surface and the emission collected over time. Photons' emission collected over 90 minutes after the addition of 5M urea to UCNPs-ssDNA with (b) WS<sub>2</sub>, (c) MoS<sub>2</sub> or (d) GO respectively at 1.1mg/mL or 0.5mg/mL. The blue dotted line represents UCNPs-ssDNA fluorescence before the addition of the 2D material.

Figure 3d shows a significant increase of nanoparticle fluorescence quenched by GO after the interaction with urea, which implies that the interaction of the oligonucleotides with the graphene oxide surface was greatly due to hydrogen bonds. On the other hand, as expected, the fluorescence of the particles interacting with MoS<sub>2</sub> and WS<sub>2</sub> was not enhanced after the interaction urea, which indicated that the interactions of the oligonucleotides with the 2D materials are probably driven by van der Waals interactions (Figure 3b-c).

## 4. CONCLUSIONS

In conclusion, we investigated the interactions between UCNPs-ssDNA and different 2D materials. In particular, we confirmed that the quenching effect between GO and UCNPs-ssDNA is driven by the formation of hydrogen bonds, while the UCNPs-ssDNA do not interact with MoS<sub>2</sub> and WS<sub>2</sub> through hydrogen bonding but probably through van der Waals forces. Also, it is evident that the different length of DNA or the percentage of GC content may influence the strength of interactions between nanoparticles and 2D materials.

## ACKNOWLEDGEMENTS

The authors would like to acknowledge ATDBio for the supply of the relevant DNA sequences. MILB, OLM and AGK would like to thank BBSRC (BB/N021150/1) for funding of this project.

## REFERENCES

- [1] Frangioni, J.V., "In vivo near-infrared fluorescence imaging," *Curr. Opin. Chem. Biol.*, 7, 626–634 (2003).
- [2] Chen, Z., Chen, H., Hu, H., Yu, M., Li, F., Zhang, Q., Zhou, Z., Yi, T. and Huang, C., "Versatile Synthesis Strategy for Carboxylic Acid-functionalized Upconverting Nanophosphors as Biological Labels," *J. Am. Chem. Soc.*, 130, 3023–3029 (2008).
- [3] Wang, X., Valiev, R. R., Ohulchanskyy, T. Y., Ågren, H., Yanga, C. and Chen, G., "Dye-sensitized lanthanide-doped upconversion nanoparticles," *Chem. Soc. Rev.*, 46, 4150–4167 (2017).
- [4] Zhu, X., Su, Q., Feng W. and Li, F., "Anti-Stokes shift luminescent materials for bio-applications," *Chem. Soc. Rev.*, 46, 1025–1039 (2017).
- [5] Jiang, G., Pichaandi, J., Johnson, N. J. J., Burke, R. D., van Veggel, F. C. J. M., "An Effective Polymer Cross-Linking Strategy To Obtain Stable Dispersions of Upconverting NaYF<sub>4</sub> Nanoparticles in Buffers and Biological Growth Media for Biolabeling Applications," *Langmuir*, 28(6), 3239–3247 (2012).
- [6] Wang, M., Abbineni, G., Clevenger, A., Mao, C. and Xu, S., "Upconversion nanoparticles: synthesis, surface modification and biological applications," *Nanomedicine*, 7(6), 710–729 (2011).
- [7] Li, H., Fang, X., Cao, H. and Kong, J., "Paper-based fluorescence resonance energy transfer assay for directly detecting nucleic acids and proteins," *Biosensors and Bioelectronics*, 80, 79–83 (2016).
- [8] Zhu, D., Wang, L., Xu, X. and Jiang, W., "Label-free and enzyme-free detection of transcription factors with graphene oxide fluorescence switch-based multifunctional G-quadruplex-hairpin probe," *Biosensors and Bioelectronics*, 75(11), 155–160 (2016).
- [9] Xuan, W., Yao, A. and Schultz, P. G., "Genetically Encoded Fluorescent Probe for Detecting Sirtuins in Living Cells," *J. Am. Chem. Soc.*, 139 (36), 12350–12353 (2017).
- [10] Xia, N., Wang X. and Liu, L., "A Graphene Oxide-Based Fluorescent Method for the Detection of Human Chorionic Gonadotropin," *Sensors*, 16(10), 1699 (2016).
- [11] Li, Z. and Wong, S. L., "Functionalization of 2D transition metal dichalcogenides for biomedical applications," *Materials Science and Engineering C*, 70, 1095–1106 (2017).
- [12] Loo, A. H., Sofer, Z., Bouša, D., Ulbrich, P., Bonanni, A. and Pumera, M., "Carboxylic Carbon Quantum Dots as a Fluorescent Sensing Platform for DNA Detection," *ACS Appl. Mater. Interfaces*, 8(3), 1951–1957 (2016).



- [13] Hu, R., Liu, T., Zhang, X.-B., Huan, S.-Y., Wu, C., Fu, T. and Tan, W., "Multicolor Fluorescent Biosensor for Multiplexed Detection of DNA," *Anal. Chem.*, 86 (10), 5009–5016 (2014).
- [14] He, H.-Z., Leung, K.-H., Wang, W., Chan, D. S.-H., Leung C.-H. and Ma, D.-L., "Label-free luminescence switch-on detection of T4 polynucleotide kinase activity using a G-quadruplex-selective probe," *Chem. Commun.*, 50, 5313-5315 (2014).
- [15] Liu, B., Salgado, S., Maheshwari, V. and Liu, J., "DNA adsorbed on graphene and graphene oxide: Fundamental interactions, desorption and applications," *Current Opinion in Colloid & Interface Science*, 26, 41-49 (2016).
- [16] Wu, M., Kempaiah, R., Huang, P.-J. J., Maheshwari, V. and Liu, J., "Adsorption and Desorption of DNA on Graphene Oxide Studied by Fluorescently Labeled Oligonucleotides," *Langmuir*, 27(6), 2731–2738 (2011).
- [17] Alonso-Cristobal, P., Vilela, P., El-Sagheer, A., Lopez-Cabarcos, E., Brown, T., Muskens, O. L., Rubio-Retama, J. and Kanaras, A. G., "Highly Sensitive DNA Sensor Based on Upconversion Nanoparticles and Graphene Oxide," *ACS Appl. Mater. Interfaces*, 7 (23), 12422–12429 (2015).
- [18] Vilela, P., El-Sagheer, A., Millar, T. M., Brown, T., Muskens, O. L. and Kanaras, A. G., "Graphene Oxide-Upconversion Nanoparticle Based Optical Sensors for Targeted Detection of mRNA Biomarkers Present in Alzheimer's Disease and Prostate Cancer," *ACS Sensors*, 2 (1), 52–56 (2017).
- [19] Li, Z. Q. and Zhang, Y., "An efficient and user-friendly method for the synthesis of hexagonal-phase NaYF<sub>4</sub>: Yb, Er/Tm nanocrystals with controllable shape and upconversion fluorescence," *Nanotechnology*, 19(34), 345606 (2008).
- [20] Lin, W., Fritz, K., Guerin, G., Bardajee, G. R., Hinds, S., Sukhovatkin, V., Sargent, E. H., Scholes G. D. and Winnik, M. A., "Highly luminescent lead sulfide nanocrystals in organic solvents and water through ligand exchange with poly(acrylic acid)," *Langmuir* 24(15), 8215-8219 (2008).
- [21] Lu, C., Liu, Y., Ying, Y. and Liu, J., "Comparison of MoS<sub>2</sub>, WS<sub>2</sub> and Graphene Oxide for DNA adsorption and Sensing," *Langmuir*, 33, 630–637 (2017).
- [22] Hua, L., Zhou, R., Thirumalai, D. and Berne, B. J., "Urea denaturation by stronger desipersion interactions with proteins than water implies a 2-stage unfolding," *PNAS*, 105 (44), 16928-16933 (2008).
- [23] Priyakumar, U. D., Hyeon, C., Thirumalai, D. and MacKerell, A. D., "Urea destabilizes RNA by forming stacking interaction and multiple hydrogen bonds with nucleic acid bases," *J. Am. Chem. Soc.*, 131(49), 17759–17761 (2009).