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Programming the assemblies of DNA-gold nanoparticles on graphene oxide sheets

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We present a new method to program the covalent binding of gold nanoparticles on graphene oxide sheets. The binding selectivity is driven by chemically modified oligonucleotides, grafted onto the surfaces of each nanomaterial. In the presence of a templating complementary DNA strand, nanoparticles are brought near the surface of the graphene oxide. Once in close proximity, the DNA is ligated to create a permanent link between the nanoparticles and graphene oxide. Due to the DNA selectivity and specificity, a second layer of gold nanoparticles of different size can be grafted on the top of the first layer of particles. The ease of this new method allows for its universal applicability when the formation of highly programmable hybrid nanoparticle-graphene oxide structures is a neccesity.

Introduction

Ground-breaking work by Novoselov and Geim in 2004[1] reported for the first time a new crystallographic two-dimensional form of carbon, namely graphene. Very soon, it was realized that graphene possesses intrinsic electronic, optical and thermal properties.[2] These unique properties of graphene in combination with high flexibility, strength and the ease of its chemical modification resulted in an immense rise of scientific interest.[2-4] Lately, a significant amount of research on graphene and its derivatives has been focussed on the development of hybrid systems, where graphene is functionalized with other materials (e.g. nanoparticles (NP), active molecules) with the aim of developing novel nanostructures with enriched properties.[5-8] The applications of such systems span a broad range of research fields ranging from biomedicine to energy harvesting. For example Zhang and co-workers showed that quaternary composites of graphene@Fe_3O_4@SiO_2@polyaniline possess great potential as novel lightweight microwave absorption materials.[7] Moreover, Qian *et al.* demonstrated that aptamer-functionalized quantum dots physisorbed onto the surface of graphene oxide (GO) can act as sensors for the detection of Pb^{2+} ions.[6] Kim and co-workers reported the use of reduced GO-magnetite hybrids for the efficient removal of arsenic from drinking water.[9] Choe *et al.* demonstrated an improved electrocatalytic oxygen reduction reaction using reduced graphene oxide (rGO) modified with PEDOT and MnO_2 nanoparticles.[8] On the other hand, Gilbertson et al. highlighted that the formation of hybrid plasmonic metal-graphene systems is especially attractive due to combined unique optical properties of both materials, resulting in a new class of optical metamaterials.[10] From all the recent studies,it is evident that graphene and graphene derivative hybrid systems display enormous potential for a variety of applications and the requirement for novel strategies to create more complex and advanced graphene oxide-NP hybrid structures in a controlled manner becomes apparent. However, most chemical protocols for the formation of graphene oxide-NP hybrid assemblies, reported to date, lack the element of specificity and programmability and in many cases these protocols describe the uncontrollable adsorption of NPs onto the surface of graphene. This chemical approach is advantageous because of its simplicity, but results in limitations with respect to the advancement of the final hybrid structure. We suggest the employment of DNA as an excellent tool to create programmable GO-nanoparticle hybrid structures. DNA has become an established scaffold for the assembly of novel nanomaterials, due to its unique selectivity and versatility.[11-14] Moreover, DNA strands can be easily modified with chemical groups for covalent DNA attachment to surfaces, for introduction of fluorescent dyes, as well as for performing DNA ligation reactions.[15]

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Here we show for the first time that oligonucleotides can be employed to rationally program the binding of gold nanoparticles on graphene oxide templates. Moreover, the utilization of chemically modified oligonucleotides enables the use of copper-free click chemistry for the formation of DNA ligated hybrid structures that remain robust even under DNA denaturing conditions.

Results and discussion

Covalent attachment of oligonucleotides on GO

GO contains a large number of carboxylic groups, which could be readily reacted with amine terminated molecules using an EDC/sulfo-NHS coupling strategy. Therefore, in order to covalently bind DNAs on the GO surface we modified the relevant oligonucleotides (**S1** and **C1**) with an amine group. It is noteworthy to say here that the yield of the coupling reaction is strongly dependent on the experimental conditions chosen. Because in this particular experiment our aim was to maximize the number of DNA strands attached to the GO, we optimized a previously reported coupling protocol developed by our group, which has proven efficient for nanoparticulate systems.[16]

Once the GO was covalently functionalized with oligonucleotides, the samples were characterized using several techniques, including UV-vis spectroscopy and ζ-potential measurements. The characteristic UV spectrum of GO exhibits a maximum peak at 230 nm, owing to π→π* transitions (C=C bonds) as well as a shoulder from 290-350 nm attributed to n→π* transitions (C=O bonds).[17] However, spectral changes can be observed upon conjugation to DNA. **Figure 1A** shows the appearance of the characteristic DNA absorption maximum at 260 nm. Moreover, a decrease of the shoulder at 280-350 nm (GO n→π* transition) is observed. As reported in earlier studies, the conjugation of DNA alters the electronic ground state of GO resulting in this spectral variation.[18] (As a comparison see ESI for UV-vis spectra of DNA simply adsorbed onto GO). The functionalization of GO with the oligonucleotides was also confirmed by ζ-potential measurements, which showed a decrease in the net charge from -25.2 ± 0.4 mV to -40.1 ± 1.4 mV after DNA conjugation (**Fig. 1B**). This decrease in charge can be attributed to the increased negative charge inferred by the DNA phosphate backbone.

Formation of graphene oxide-gold nanoparticle hybrid assemblies

The general assembly strategy is illustrated in **Scheme 1**. In our experiment, a solution of alkyne-oligonucleotide coated 13 nm gold nanoparticles (see ESI Fig. SX) was mixed with a solution of azide-oligonucleotide functionalized GO. Then a third DNA strand (**S3**) partially complementary to both **S1** and **S2** was added to the solution, catalysing the reaction. Once hybridized, the reactive alkyne and azide groups on **S1** and **S2** are brought into close proximity and spontaneously 'click' (see ESI scheme 1). Using a strained cyclooctyne as the reactive

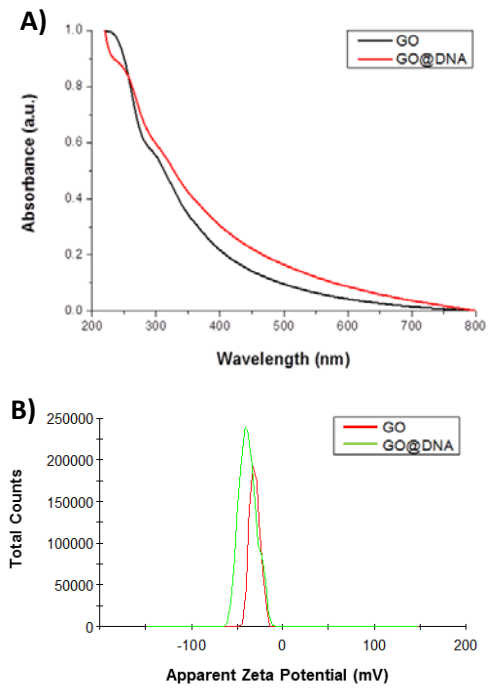
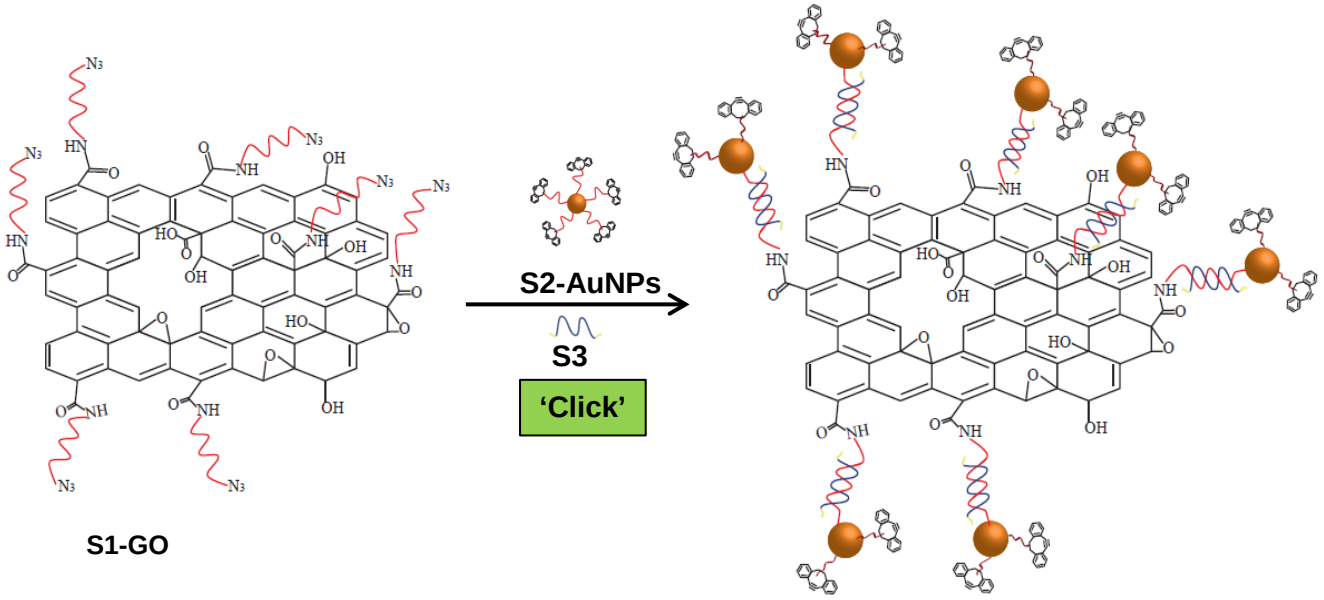


Figure 1 **A**) Normalized UV-vis spectra of GO (black) and DNA-modified GO (red). **B**) ζ-potential graphs of GO (red) and GO@DNA (green).

alkyne group allows for a spontaneous, copper-free click reaction with an azide group via the ring-strain promoted alkyne-azide [3+2] cycloaddition.[19] This simple and high yielding type of chemistry has been previously employed by us to successfully link gold nanoparticles to each other.[15] The selectivity of the proposed method enables a multi-step programming of the desired materials as presented here.

To purify the GO-nanoparticles from non-specifically attached nanoparticles, we made use of their large size difference. Agarose gel electrophoresis is widely employed to separate molecules according to their size and charge. Here we used this technique to purify GO-AuNP hybrid conjugates from unconjugated AuNPs. This effective purification method has been reported by others to isolate graphene oxide hybrid materials. [10, 20] A representative gel is depicted in **Figure 2A**. The gel shows two distinct bands: At the top, a red band can be seen, corresponding to unconjugated DNA-AuNPs.The small pore size of the agarose gel matrix allowed only the comparatively small non-conjugated DNA-AuNPs to enter the gel. However, large GO-AuNP assemblies remained in the wells and were visible as a distinct brown band.[21] This accumulation in the gel well allowed for facile removal of purified hybrid assemblies. Different techniques were then employed to show that AuNPs were successfully conjugated to GO. Owing to their optical properties, UV-vis spectroscopy represents a quick and simple tool for the characterisation of hybrid assemblies. **Figure 2B** displays the UV-vis spectra of GO modified with DNA (black) and pure GO-AuNP hybrids (red). As expected, the spectrum of the hybrid assemblies shows the appearance of the distinct gold nanoparticle plasmon peak at 520 nm, indicating the presence of gold nanoparticles. [18]



S1: 5'-(**X**)AAAAAAAACGAGTGCTAAGGATCCGAA**R**$_1$	**R**$_1$: Azide
S2: 5'-**R**$_2$TTCACTGCAGATATCCATTCGAAAAAAAA(**X**)	**R**$_2$: Alkyne
S3 :5'-GTGTGTCGAGTGCTAAGGATCCGAATTCACTGCACATATCCATTCGGTGTGT	**X**: $C_6H_{12}NH_2$ OR $C_6H_{12}SH$

Scheme 1. Illustration of the formation of programmed GO-NP hybrid assemblies using DNA as a scaffold. **S1**-oligonucleotide modified GO is reacted with **S2**-AuNPs and a complementary splint strand **S3**, allowing for hybrid assemblies to form. Due to the close proximity of the azide and alkyne functional groups on **S1** and **S2** respectively – brought together by **S3**- click ligation occurs instantaneously.

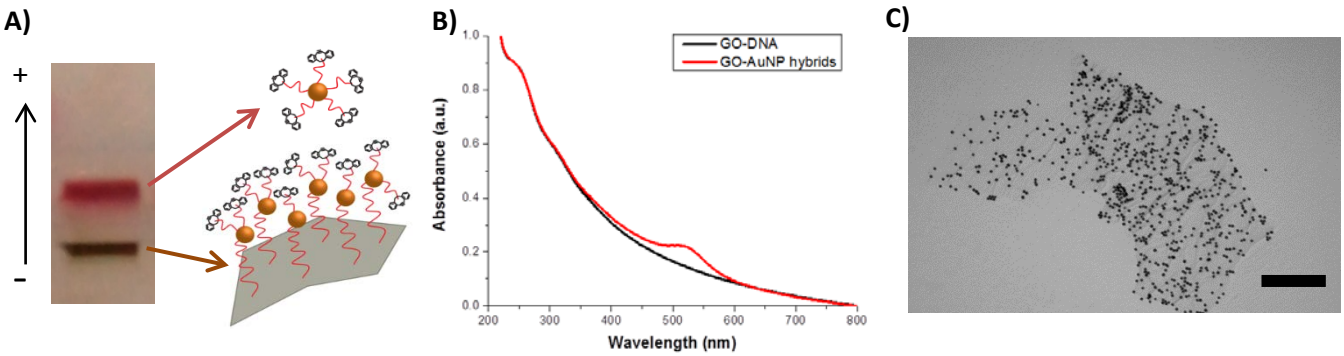


Figure 2 **A)** Purification of unconjugated AuNPs (red band) from GO-AuNP hybrids (brown band); **B)** Normalized UV-vis spectra of GO-DNA (black) and GO-AuNP hybrids (red); Representative TEM image of GO-AuNP hybrids after purification. Scale bar is 500 nm.

Further evidence that the nanoparticles were successfully grafted onto GO was obtained by transmission electron microscopy. As desired, **Figure 2C** shows a sheet of GO decorated with AuNPs (additional images in ESI).

In order to confirm that AuNPs and GO sheets were indeed covalently ligated, a control experiment was performed (see ESI scheme S2). In this case assemblies were formed as before, but with GO-DNA conjugates lacking the azide functional group required for the click reaction (**C1**-GO conjugates). Thus assemblies could still form in the presence of **S3**. As **S3** was designed to contain non-complementary overhangs, it can be selectively removed using a complementary DNA strand **S4** via competitive hybridization.[15] (See ESI scheme S1). Hence, after treatment with the splint complement **S4**, non-ligated assemblies should dissociate.

The success of the click ligation was confirmed by various means. One of these is agarose gel electrophoresis, where any GO containing structures should remain in the wells of the gel, whilst smaller DNA-AuNP conjugates should enter the gel, if not covalently linked to GO. **Figure 3A** displays a representative gel of ligated and non-ligated assemblies after removal of the templating splint strand **S3**.

Lane 1 shows the clicked assemblies as a brown band, which remained in the well of the agarose gel due to their large size. As no further bands were observed, this suggested that GO-AuNP hybrid structures remained intact and were indeed covalently linked. In lane 2, once again a brown band

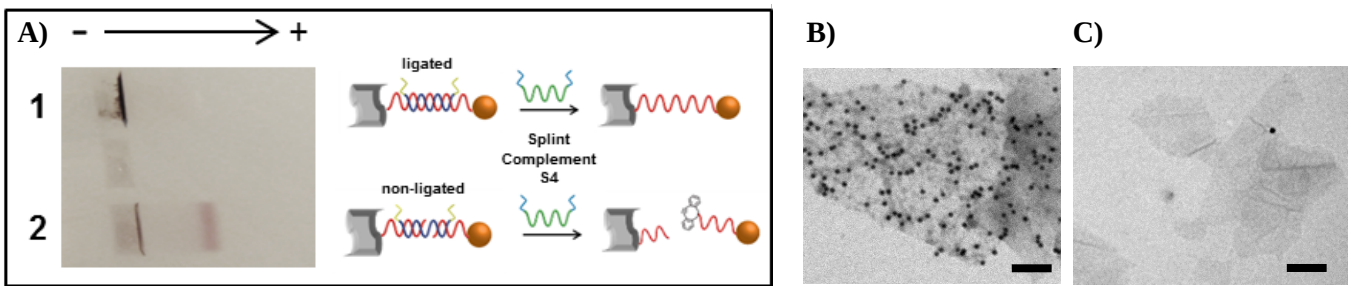


Figure 3 **A)** Agarose gel of ligated (lane 1) and non-ligated (lane 2) hybrid assemblies after removal of the template strand **S3** using the splint complement **S4**. Ligated assemblies remain intact, whereas non-ligated assemblies dissociate. **B)** Representative TEM micrograph of ligated GO-AuNP assemblies recovered from lane 1 in A. **C)** Representative TEM micrograph of 'non-ligated GO-AuNP assemblies' recovered from lane 2 in A). Scale bars are 50 nm.

could be seen in the well of the gel, accompanied by an additional red band.

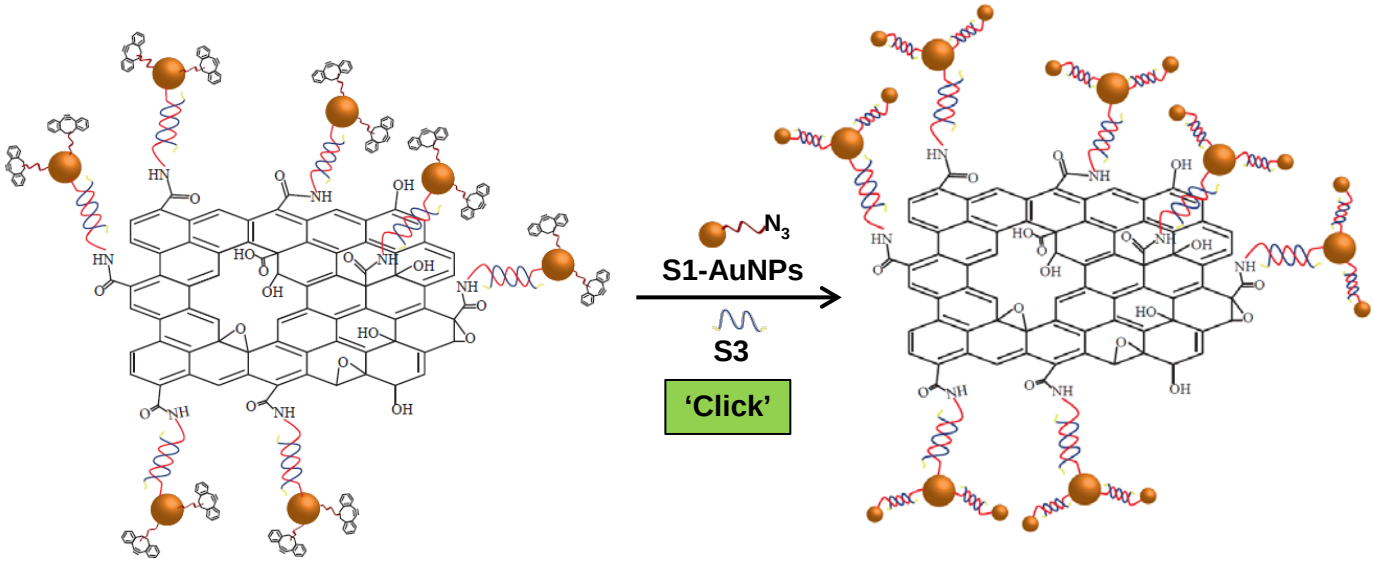
This appearance of the red band can be attributed to dehybridised **S2**-AuNP conjugates.Due to the lack of a clicking group on the **C1**-GO conjugates, assemblies dissociated after removal of the splint strand, thus allowing the DNA-AuNP conjugates to enter the gel, whilst GO-DNA conjugates remained in the well. Subsequent analysis of the GO containing products from lanes 1 and 2 further confirmed these findings. **Figure 3B** shows GO-AuNP hybrid assemblies as recovered from lane 1. As expected, the image shows gold nanoparticles grafted onto GO sheets. Contrastingly, **Figure 3C** depicts mostly bare GO sheets, confirming that assemblies had dissociated and DNA-AuNP conjugates had moved into the gel. Further evidence was obtained from UV-vis analysis (see ESI Fig. SX), showing the disappearance of the gold nanoparticle plasmon peak in the spectrum of non-ligated assemblies. From these results it can be concluded that covalent functionalization of GO with AuNPs was indeed achieved successfully.

Further functionalization of GO-AuNP hybrid assemblies

In order to demonstrate that the formation of more complex hybrid assemblies can be programmed using our universal strategy, we performed a second click ligation step utilizing smaller, 5 nm, AuNPs. These were functionalized with thiolated DNA **S1**, thus being able to form assemblies only with **S2**-AuNPs grafted on the GO surface. The assembly strategy is outlined in **scheme 2**.

As prepared GO-AuNP hybrids, with attached AuNPs bearing free **S2**-DNA, were mixed with an excess of 5 nm AuNPs bearing one strand of **S1** (see ESI Fig, SX). These particles were then brought into close proximity *via* **S3**, allowing for the click ligation to occur. Subsequent purification by agarose gel electrophoresis finally yielded the pure hybrid structures of GO-AuNP(13 nm)-AuNP (5 nm). The success of this additional grafting step was visualised by UV-vis spectroscopy (**Fig. 4A**) and transmission electron microscopy (**Fig. 4B**).

As expected, the UV spectrum for GO-AuNP(13 nm)-AuNP(5 nm) hybrid assemblies shows similarities to that of



Scheme 2 Schematic illustration of the prgrammed assembly of GO-13 nm AuNPs- 5 nm AuNPs hybrids using DNA click ligation. **S2**-AuNPs (13 nm) conjugated to GO were assembled with **S1**-AuNPs (5 nm) *via* **S3** resulting in a covalently linked structures.

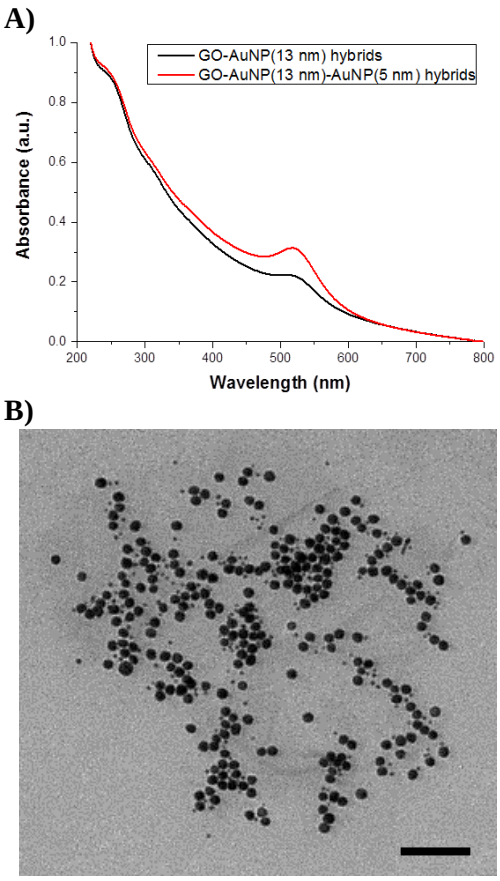


Figure 4 **A)** Normalized UV-vis spectra of GO-AuNP(13 nm) (black) and GO-AuNP (13 nm)-AuNP(5 nm) (red) hybrid assemblies; **B)** TEM micrograph of GO-AuNP (13 nm) – AuNP (5 nm) hybrid assemblies. Scale bar is 100 nm.

GO-AuNP(13 nm) assemblies. However, a clear increase in the absorbance of the characteristic AuNP plasmon peak can be observed, giving evidence for successful ligation of 5 nm AuNP-DNA conjugates. Ultimately, transmission electron microscopy validated these results. **Figure 4B** shows a GO sheet decorated with both 13 and 5 nm AuNPs (additional images in ESI Fig. SX). One can see that the majority of 5 nm AuNPs is located in the close vicinity of a 13 nm AuNP, owing to their covalent DNA link. This shows that further modification of hybrid assemblies is easily achievable, opening up new possibilities of multifunctional assembly formation.

Conclusions

Graphene/nanoparticle hybrids have seen a great rise in interest in the past years, yet there is a lack of specificity and programmability in many reported assembly strategies. In this study we have shown that graphene oxide sheets can successfully be modified with DNA strands by employing well-established EDC chemistry. Gold nanoparticles bearing non-complementary DNA strands can further be grafted onto the graphene oxide surface and covalently linked *via* DNA click chemistry by using a templating splint strand. The programmability and specificity of our assembly strategy was successfully demonstrated by incorporation of a second degree of functionalization with 5 nm gold nanoparticle-DNA conjugates. The described protocol represents a new and straightforward way to create programmable GO hybrid structures that are covalently linked. Due to the simple nature of the method, it will be universally applicable for the formation of even more complex advanced hybrid systems, unraveling new era of functional materials.

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References

1. K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, Y. Zhang, S. V. Dubonos, I. V. Grigorieva and A. A. Firsov, *Science*, 2004, **306**, 666-669.
2. A. K. Geim and K. S. Novoselov, *Nat Mater*, 2007, **6**, 183-191.
3. S. Roy, N. Soin, R. Bajpai, D. S. Misra, J. A. McLaughlin and S. S. Roy, *Journal of Materials Chemistry*, 2011, **21**, 14725-14731.
4. Y. Zhu, S. Murali, W. Cai, X. Li, J. W. Suk, J. R. Potts and R. S. Ruoff, *Adv Mater*, 2010, **22**, 3906-3924.
5. C. Y. Li, Y.; Zhang, B.; Chen, G.; Wang, Z.; Li, G., *Part Part Syst Char*, 2013, **31**, 201-208.
6. Z. S. Qian, X. Y. Shan, L. J. Chai, J. R. Chen and H. Feng, *Biosensors and Bioelectronics*, 2015, **68**, 225-231.
7. L. Wang, J. Zhu, H. Yang, F. Wang, Y. Qin, T. Zhao and P. Zhang, *Journal of Alloys and Compounds*, 2015, **634**, 232-238.
8. J. E. Choe, J. M. You, M. Yun, K. Lee, M. S. Ahmed, Z. Ustundag and S. Jeon, *J Nanosci Nanotechno*, 2015, **15**, 5684-5690.
9. V. Chandra, J. Park, Y. Chun, J. W. Lee, I. C. Hwang and K. S. Kim, *ACS nano*, 2010, **4**, 3979-3986.
10. A. M. Gilbertson, Y. Francescato, T. Roschuk, V. Shautsova, Y. Chen, T. P. H. Sidiropoulos, M. Hong, V. Giannini, S. A. Maier, L. F. Cohen and R. F. Oulton, *Nano Lett*, 2015, **15**, 3458-3464.
11. D. Nykypanchuk, M. M. Maye, D. van der Lelie and O. Gang, *Nature*, 2008, **451**, 549-552.
12. R. J. Macfarlane, B. Lee, M. R. Jones, N. Harris, G. C. Schatz and C. A. Mirkin, *Science*, 2011, **334**, 204-208.
13. S. A. Claridge, A. J. Mastroianni, Y. B. Au, H. W. Liang, C. M. Micheel, J. M. J. Frechet and A. P. Alivisatos, *Journal of the American Chemical Society*, 2008, **130**, 9598-9605.
14. C. J. Loweth, W. B. Caldwell, X. G. Peng, A. P. Alivisatos and P. G. Schultz, *Angew Chem Int Edit*, 1999, **38**, 1808-1812.
15. A. Heuer-Jungemann, R. Kirkwood, A. H. El-Sagheer, T. Brown and A. G. Kanaras, *Nanoscale*, 2013, **5**, 7209-7212.
16. D. Bartczak and A. G. Kanaras, *Langmuir : the ACS journal of surfaces and colloids*, 2011, **27**, 10119-10123.
17. Q. Yang, X. Pan, K. Clarke and K. Li, *Industrial & Engineering Chemistry Research*, 2011, **51**, 310-317.

18. F. Liu, J. Y. Choi and T. S. Seo, *Biosens Bioelectron*, 2010, **25**, 2361-2365.
19. M. Shelbourne, X. Chen, T. Brown and A. H. El-Sagheer, *Chem Commun*, 2011, **47**, 6257-6259.
20. F. L. Bei, X. L. Hou, S. L. Y. Chang, G. P. Simon and D. Li, *Chem-Eur J*, 2011, **17**, 5958-5964.
21. Z. H. Wang, Z. L. Ge, X. X. Zheng, N. Chen, C. Peng, C. H. Fan and Q. Huang, *Nanoscale*, 2012, **4**, 394-399.