

Supporting Information for

Programming the assembly of gold nanoparticles on graphene oxide sheets using DNA

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S1 Experimental procedures

Materials and methods

Commercially available reagents and solvents were used from the following suppliers without further purification unless stated otherwise: sodium tetrachloroaurate (III) dehydrate, trisodium citrate, bis(p-sulfonatophenyl)phenyl phosphine dehydrate dipotassium salt (BSPP) and sodium borohydride were obtained from Sigma Aldrich. Potassium carbonate was obtained from Fisher Scientific. UV-visible spectra were recorded on a Cary 100 UV-vis spectrophotometer over a range of 200-800 nm. TEM images were obtained on a Hitachi H7000 transmission electron microscope operating at a bias voltage of 75 kV. Dynamic light scattering and zeta potential measurements were carried out on a Malvern Zetasizer Nano ZS.

Oligonucleotide synthesis and purification

Standard DNA phosphoramidites, solid supports, and additional reagents were purchased from Link Technologies and Applied Biosystems Ltd. All oligonucleotides were synthesized on an Applied Biosystems 394 automated DNA/ RNA synthesizer using a standard 1.0 μ mole phosphoramidite cycle of acid-catalyzed detritylation, coupling, capping, and iodine oxidation. Stepwise coupling efficiencies and overall yields were determined by the automated trityl cation conductivity monitoring facility and in all cases were >98.0%. All β -cyanoethyl phosphoramidite monomers were dissolved in anhydrous acetonitrile to a concentration of 0.1 M immediately prior to use. The coupling time for normal A, G, C, and T monomers was 35 s. Aminolink C7 columns (Link Technologies) were used for the introduction of the 3'-aminoethyl moiety into oligonucleotides. Cleavage of the oligonucleotides from the solid support and deprotection was achieved by exposure to concentrated aqueous ammonia solution for 60 min at room temperature followed by heating in a sealed tube for 5 h at 55 °C. The oligonucleotides were purified by reversed-phase HPLC on a Gilson system using an XBridge™ BEH300 Prep C18 10 μ M 10x250 mm column (Waters) with a gradient of acetonitrile in ammonium acetate (0% to 50% buffer B over 30 min, flow rate 4 mL/min), buffer A: 0.1 M ammonium acetate, pH 7.0, buffer B: 0.1 M ammonium acetate, pH 7.0, with 50% acetonitrile. Elution was monitored by UV absorption at 300 nm. After HPLC purification, oligonucleotides were desalted using NAP-10 Sephadex columns (GE Healthcare) and analysed by gel electrophoresis. All oligonucleotides were characterised by electrospray mass spectrometry and capillary electrophoresis (CE). Mass spectra of oligonucleotides were recorded in ES- mode, and in all cases confirmed the integrity of the sequences. For azide labelling the oligonucleotide was

synthesized with a 3'-hexyl amino linker for post-synthetic labelling. The freeze-dried oligonucleotide was dissolved in $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer (80 μL , 0.5 M, pH 8.75). 6-azidohexanoic acid NHS ester¹ (1 mg) was dissolved in DMSO (80 μL) and added to the oligonucleotide. After 4 h, the now fully labelled oligonucleotide Oligo 1 was desalted using a NAP-10 column (GE Healthcare) and purified by RP-HPLC. For alkyne labelling, the oligonucleotide was synthesised with a 5'-hexyl amino linker for post-synthetic labelling. After freeze-drying, it was dissolved in $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ buffer (160 μL , 0.5 M, pH 8.75). DIBO active ester (1.8 mg) was dissolved in DMF (160 μL), added to the oligonucleotide and the mixture incubated at 55 °C for 4h. The labelled oligonucleotide Oligo 2 was then desalted using a NAP-10 column, followed by purification by RP-HPLC.

Synthesis of graphene oxide

Well-dispersed sheets of graphene oxide were prepared from graphite powder (Alfa Aesar. ~200 mesh) according to a modified Hummers' method². In detail, graphite powder (0.5 g) was placed into a mixture of H_2SO_4 (40 mL, 98%) and NaNO_3 (0.375 g). The mixture was then stirred and cooled in an ice bath. While maintaining vigorous stirring, KMnO_4 (3.0 g) was then added in portions over a period of 2 h. The reaction mixture was left for 4 h in order to reach room temperature before being heated to 35 °C for 30 min. It was then poured into a flask containing deionized water (50 mL) and further heated to 70 °C for 15 min. The mixture was then decanted into 250 mL of deionized water and the unreacted KMnO_4 was removed by adding 3% H_2O_2 . The reaction mixture was then allowed to settle and decanted. The graphite oxide obtained was then purified by repeated centrifugation and redispersed in deionized water until neutralized pH was achieved. Finally, the resulting GO was dried at 60 °C in a vacuum oven for 48 h before use.

Synthesis of gold nanoparticles (AuNPs)

Preparation of 13 nm AuNPs

13 ± 1 nm AuNPs were synthesised according to established literature protocols.³⁻⁵ In detail, an aqueous solution of sodium tetrachloroaurate (100 mL, 1 mM) was brought to the boil under stirring. Once boiling, a hot aqueous solution of trisodium citrate (5 mL, 2% wt/V) was added to the gold solution. A colour change from yellow to colourless to finally deep red could be observed, indicating the formation of nanoparticles. The solution was then stirred under boiling for an additional 15 min and subsequently allowed to cool to room temperature under stirring. To exchange the citrate ligand,

BSPP (20 mg) was added to the solution.⁶⁻⁹ After 4 h of stirring, brine was used to induce particle aggregation via charge screening. Following two rounds of centrifugation (5000 rpm, 10 min), decantation/re-dispersion and sonication, particles were finally re-dispersed in Milli-Q water and purified by filtration (0.2 μm syringe filter, VWR). Particles were stored at 4 °C prior to use.

Preparation of 5 nm AuNPs

5 \pm 2 nm AuNPs were synthesised in an aqueous solution according to a published literature procedure.¹⁰ In detail, solutions of sodium tetrachloroaurate (375 μL , 4% wt/V) and potassium carbonate (500 μL , 0.2 M) in ice-cold water (100 mL) were stirred in an ice bath. To this solution, a freshly prepared solution of sodium borohydride in water (0.5 mg/mL) was added in 5 \times 1 mL aliquots with rapid stirring. A colour change from dark purple to reddish orange was observed indicating the formation of AuNPs. After stirring for a further 5 min, the solution was centrifuged (12000 rpm, 10 min) to remove larger particles. The supernatant was collected and BSPP (20 mg) was added whilst stirring. After 15 h, NaCl was added to the solution to induce particle aggregation. Particles were then centrifuged (5000 rpm, 30 min) and re-suspended in Milli-Q water. After filtration (0.2 μm syringe filter, VWR), the purified particles with final concentrations of about 600 nM were stored at 4 °C prior to use.

DNA functionalization of graphene oxide via EDC/sulfo-NHS coupling

Amine-functionalized DNA was covalently attached to GO sheets according to a slightly modified literature protocol.¹¹ To an aqueous solution of GO (600 μL , 0.2 mg/mL) were added aqueous solutions of EDC (10 μL , 0.5 M) and sulfo-NHS (20 μL , 0.5 M), followed by amine-terminated DNA Oligo 1 or Oligo 5 (3 nmol, typically between 15-20 μL) (Sequence in table S1). The mixture was left to incubate overnight. Excess DNA was removed by triple centrifugation at 16400 rpm for 10 min and re-dispersion in ultra-pure water (600 μL). It was noted that DNA functionalised GO precipitated down more readily than the equivalent pure GO solution.

DNA functionalization of AuNPs

Functionalization of 13 nm AuNPs

A solution of 13 nm gold nanoparticles (30 pmol, 500 μL) in phosphate buffered silane (PBS) (20 mM phosphate, 5 mM NaCl, pH 7.4) was incubated with a 10 \times excess of thiolated, azide-functionalized DNA Oligo 2 (300 pmol) (sequence in table S1) and a solution of BSPP (1 mg/20 μL , 10 μL), to reduce the disulfide on the oligonucleotide to the reactive thiol form,¹² for 1 h whilst being

gently shaken at 300 rpm. Afterwards conjugates were purified by 3× centrifugation/decantation and re-dispersion in PBS (pH 7.4, 80 mM phosphate, 6 mM NaCl). Conjugates were stored at 4°C prior to use.

Functionalization of 5 nm AuNPs

AuNP-DNA monoconjugates (i.e. particles modified with one DNA strand) were prepared according to modified literature procedures.¹² In detail, equimolar amounts (100 pmol) of 5 nm BSPP-coated AuNPs and thiolated oligonucleotides bearing alkyne moieties (Oligo 1) were incubated in PBS (20 mM phosphate, 6 mM NaCl). Typical reaction volume ~500 µL). To this, an aqueous solution of BSPP (1 mg/20 µL, 1/10 of total reaction volume) was added. After 1 h incubation, functionalised AuNPs were purified by agarose gel electrophoresis (3% agarose gel, 9 V/cm, 90 min). Respective bands in the gel were extracted, cut into small pieces and stored in 0.5 × TBE buffer at 37 °C with gentle shaking overnight to allow conjugates to diffuse out of the gel. The resulting solution was collected, purified by triple high speed centrifugation (16400rpm, 6 °C, 45 min), decantation/re-dispersion and sonication. Conjugates were finally re-dispersed in hybridisation buffer (5 mM phosphate, 80 mM NaCl).

Click-ligation of GO@DNA and DNA-AuNPs

To the as-prepared sample of Oligo 1-functionalised GO (or Oligo 5-functionalized GO for control experiment) were added equimolar amounts of Oligo 2 functionalised AuNPs (30 pmol, 500 µL) in PBS buffer (pH 7.4, 20 mM phosphate, 80 mM NaCl) and the splint strand Oligo 3 (30 pmol, 0.53 µL). The reaction mixture was then carefully incubated at ~ 70 °C for 5 min and was then slowly left to cool to room temperature. Excess nanoparticles could then be removed by loading the sample into a 3% agarose gel. When put into a gel, the nanoparticles would pass into the gel but the assemblies would not, allowing them to be carefully recovered.¹³ Recovered assemblies were then purified by centrifugation and re-dispersed in PBS buffer (600 µL, 80mM phosphate, 6mM NaCl, pH 7.4). Optionally assemblies were further functionalised with differently sized DNA-AuNPs (5 nm). For this equimolar amounts of Oligo 1 functionalized 5 nm AuNPs (30 pmol, 200 µL) and Oligo 3 (30 pmol, 0.53 µL) were added to the GO-AuNP assemblies. The reaction mixture was then hybridised and gel purified as before.

S2 Sequences of oligonucleotides

Table S1 Oligonucleotide Sequences

Abbreviation	DNA sequence and modifications
Oligo 1 (azide derivative)	5' - X TTTTTTTTTCGAGTGCTAAGGATCCGAAR ₁
Oligo 2 (alkyne derivative)	5' - R ₂ TTCACTGCAGATATCCATTTCGAAAAAAAA X
Oligo 3 (Splint)	5' - ACACACCGAATGGATATCTGCAGTGAATTCGGATCCTTAGCACTCGACACAC
Oligo 4 (Splint complement)	5' - GTGTGTCGAGTGCTAAGGATCCGAATTCCTAGCACTCGACACAC
Oligo 5	5' - X TTTTTTTTTCGAGTGCTAAGGATCCGAA

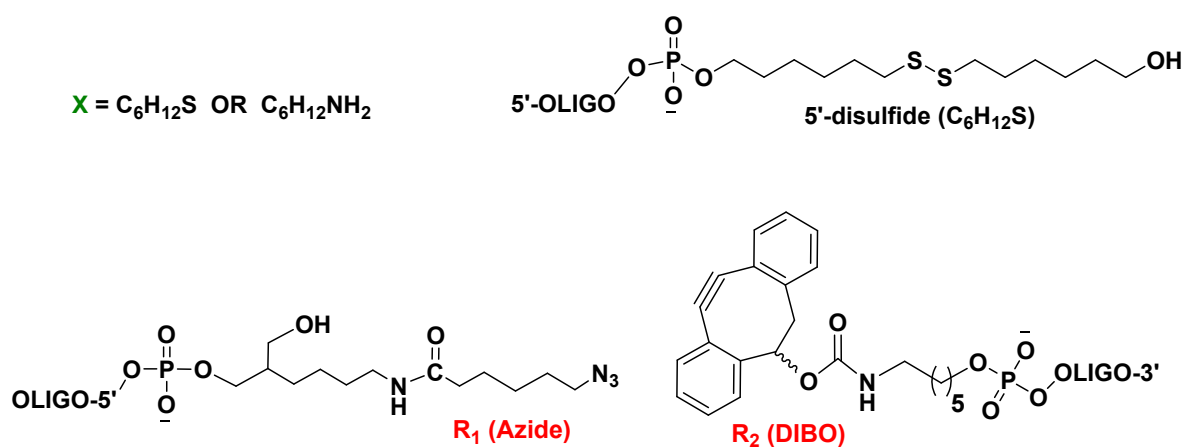


Figure S1 Chemical modifications of oligonucleotides.

S3 Characterization of AuNPs and DNA-AuNP conjugates

13 nm AuNPs

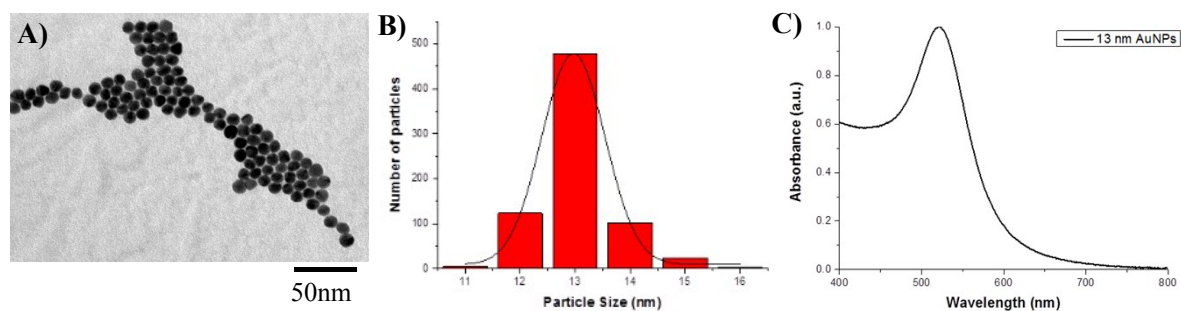


Figure S2 A) Transmission electron micrograph, B) corresponding size distribution histogram and C) normalized UV-vis spectrum of 13 nm AuNPs.

13nm AuNP-DNA conjugates

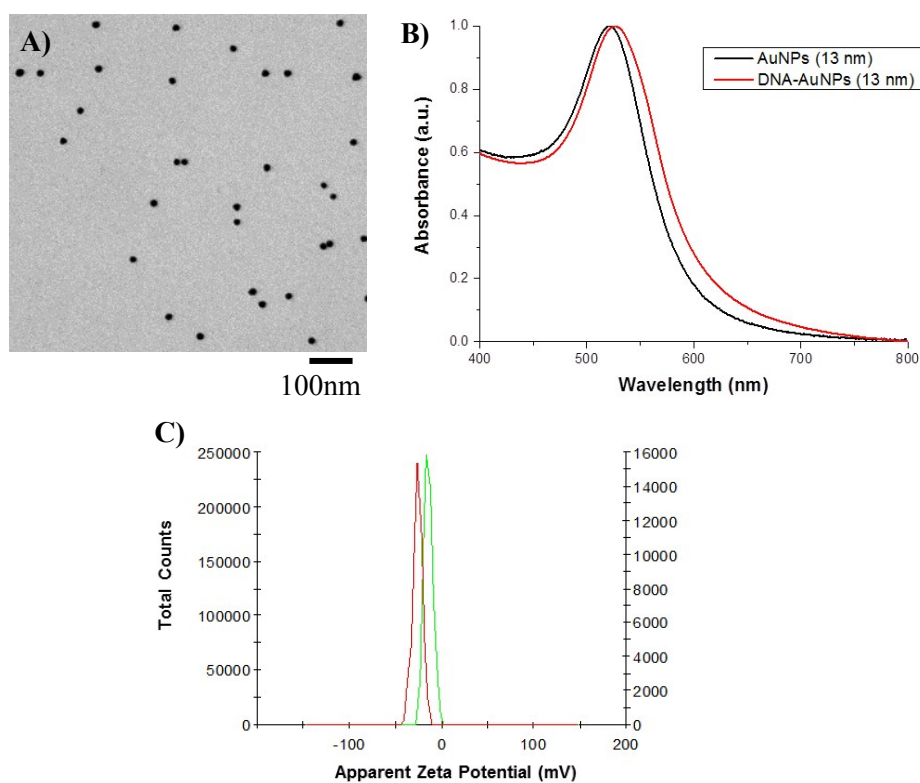


Figure S3 A) TEM micrograph of DNA-AuNPs. B) Normalized UV-vis spectra of BSPP- (black) and DNA-coated (red) AuNPs. C) Corresponding ζ -potential measurements of BSPP-coated AuNPs (green) and DNA-AuNPs (red).

5 nm AuNPs

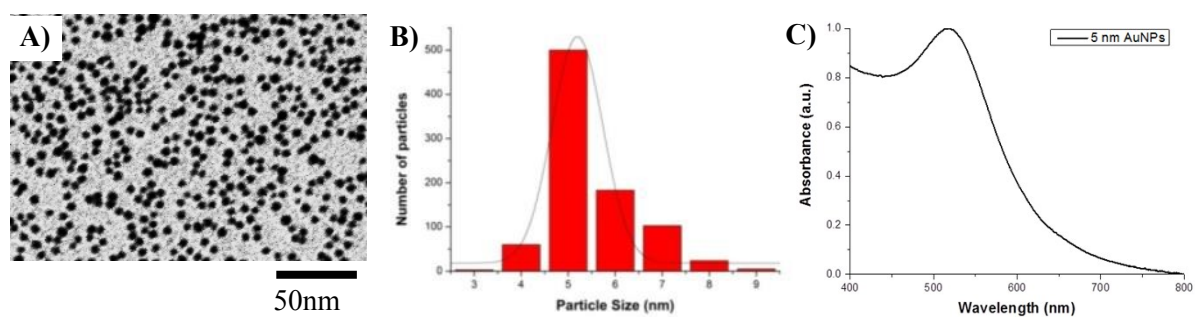


Figure S4 Transmission electron micrograph **A)** and corresponding size distribution histogram **B)** as well as a normalized UV-vis spectrum of 5 nm AuNPs.

5 nm AuNP-DNA monoconjugates

5 nm AuNPs modified with only one DNA strand were obtained by stoichiometric mixing and purification by agarose gel electrophoresis according to well-established procedures.^{14, 15}

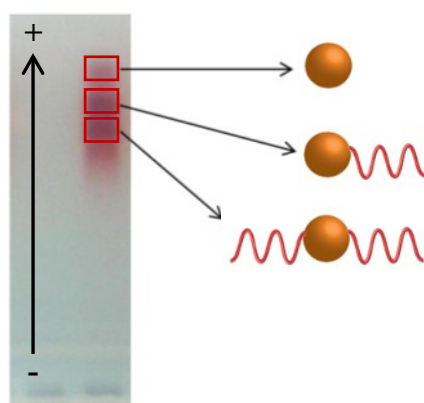
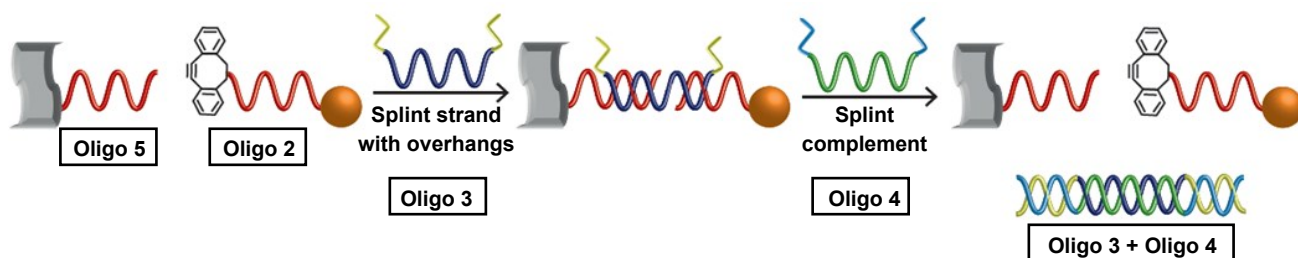


Figure S5 Agarose gel for the separation of 5 nm AuNP mono- and diconjugates.

S4 Assemblies formed by DNA hybridisation as a control experiment



Scheme S1 Schematic illustration of the control experiment. Oligo 5-modified GO and Oligo 2-modified AuNPs are brought together *via* Oligo 3. Upon treatment with Oligo 4, assemblies dissociate as they were not able to ligate. (N.B.: Only one DNA strand is shown per material for ease of visualisation).

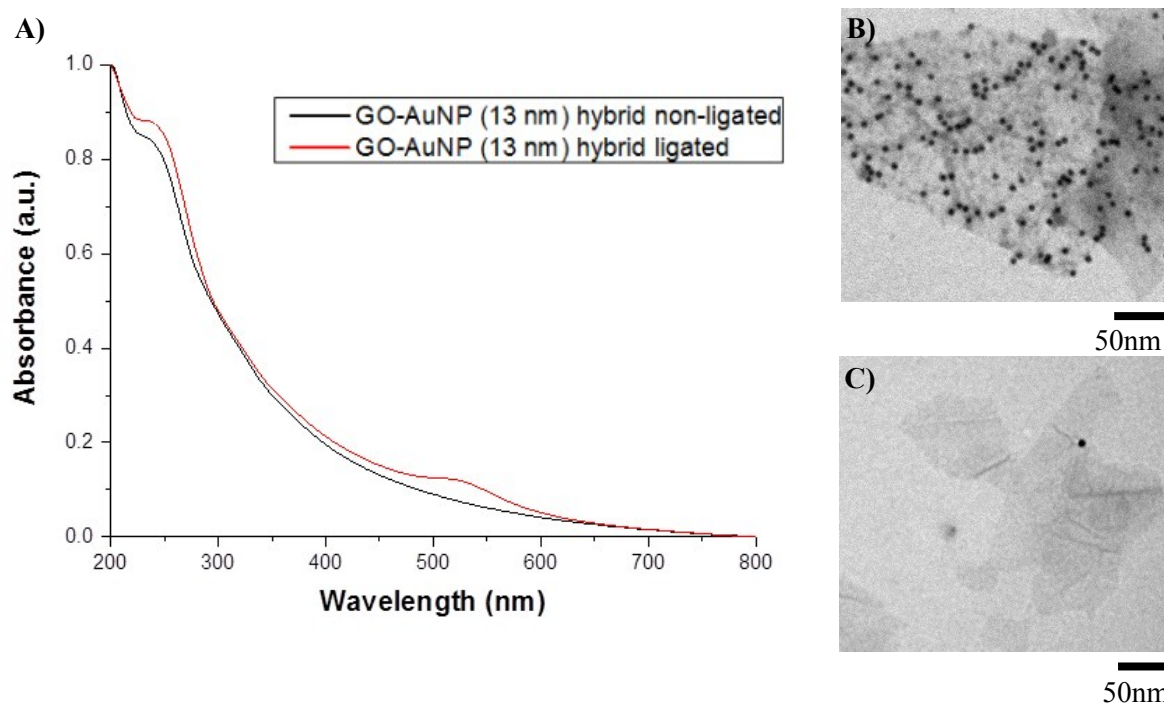


Figure S6 A) Normalized UV-vis spectra of ligated GO/AuNP hybrid assemblies and non-ligated assemblies after treatment with Oligo 4 and agarose gel purification (cf. Fig.3A) Representative TEM images of B) ligated GO-AuNP hybrid assemblies and C) non-ligated assemblies after removal of the splint strand Oligo 3 and recovery from the agarose gel (cf. Fig.3A).

S5 Additional characterization of GO-DNA and GO-AuNP conjugates

UV-vis spectra of DNA-GO conjugates

For comparison, UV-vis spectra of GO (black), GO mixed with DNA (blue) and DNA-GO conjugates after EDC coupling (red) were recorded. (N.B.: All spectra were recorded after three purification steps by centrifugation/decantation and re-dispersion in water).

The spectra clearly show that only if DNA is covalently linked to GO *via* EDC coupling, the characteristic DNA absorption peak at 260 nm is present.

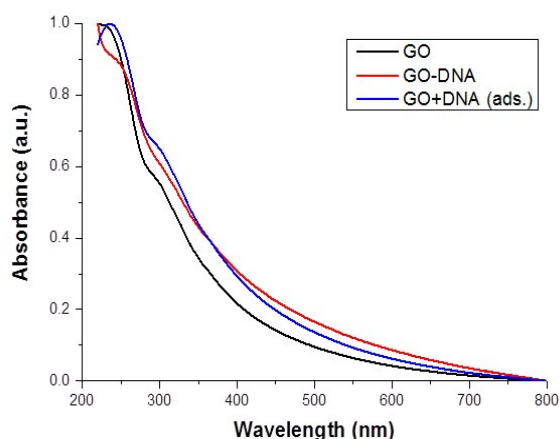


Figure S7 Normalized UV-vis spectra of GO (black), GO simply mixed with DNA (blue) and DNA-GO (red) conjugates after EDC coupling. All spectra were obtained after purification.

FT-IR spectra of GO and GO-DNA

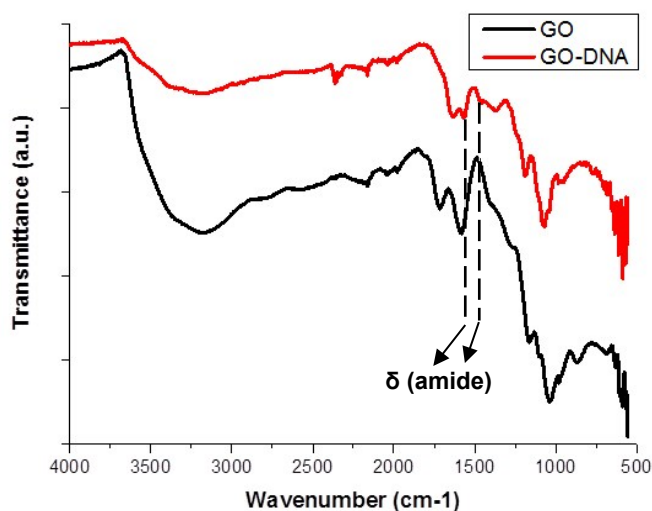


Figure S8 FT-IR spectra of GO (black) and GO-DNA (red) after EDC coupling. All spectra were obtained after purification.

The FT-IR spectrum of GO (Fig. S8, black graph) shows distinct peaks at 3200, 1716, 1583 and 1039 cm^{-1} corresponding to O-H stretch, C=O stretch, O-H bend/ aromatic C=C stretch and alkoxy C-O stretch respectively.¹⁶ Spectral changes were observed after the conjugation of GO to DNA (Fig. S8, red graph) with the appearance of characteristic amide absorption bands at 1637 and 1425 cm^{-1} corresponding to amide C=O stretching and 2nd amide N-H bending.¹⁷ Furthermore a strong peak at 1071 cm^{-1} can be observed, arising from the DNA backbone P-O/C-O stretch¹⁸, suggesting that covalent conjugation of DNA to GO was achieved successfully.

Raman spectra of GO-DNA and GO-AuNP hybrids

The samples were characterized by Raman spectroscopy at room temperature using a Nicolet Almega XR Raman spectrometer (Thermo Scientific) with a 473 nm blue laser as an excitation source. Raman spectra recorded from GO-DNA and GO-AuNP sheets (Figure S8) showed the characteristic broad peaks at 1360, 1594 and 2795 cm^{-1} , of GO, corresponding to D, G and 2D bands, respectively. We have observed a small, but systematic enhancement of the D and G bands intensity for the GO-AuNP conjugate that may be attributed to SERS effects previously reported for gold-graphene derivatives.¹⁹ The low enhancement factor for GO-AuNP conjugate indicates the presence of a chemical interaction or bond between Au NP and GO.

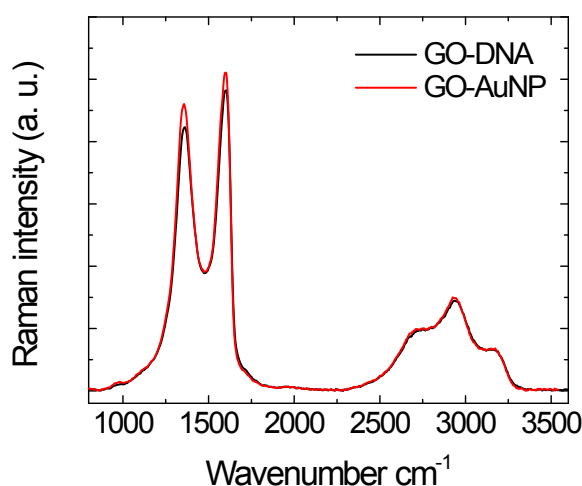
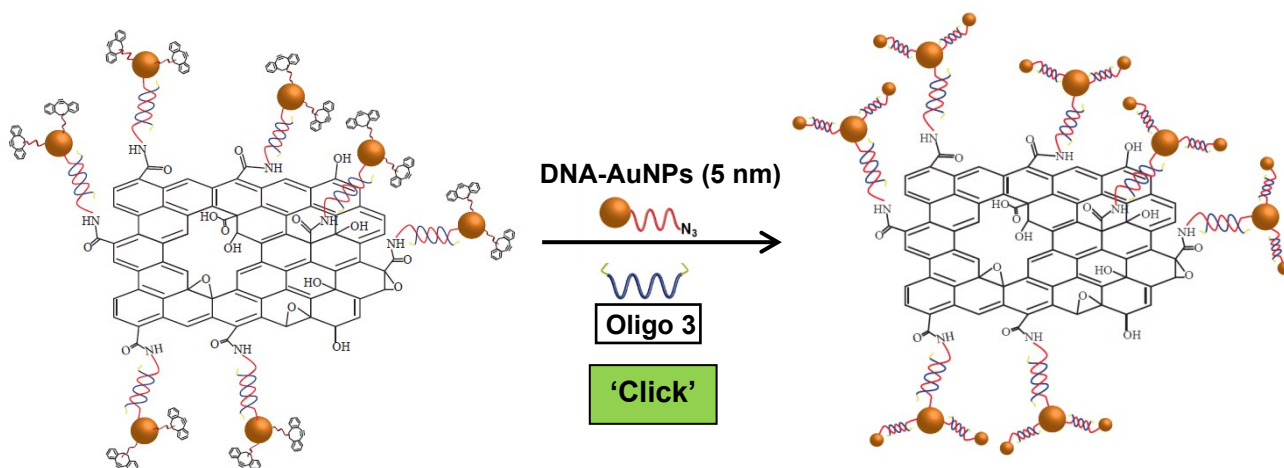


Figure S9 Normalized Raman spectra of GO-DNA (black), GO-AuNP (red) conjugates

S6 Formation of GO-AuNP(13 nm) – AuNP (5 nm) hybrid assemblies



Scheme S2 Schematic illustration of the programmed assembly of GO-13 nm AuNPs- 5 nm AuNPs hybrids using DNA click ligation. Oligo 2-AuNPs (13 nm) conjugated to GO were assembled with Oligo 1-AuNPs (5 nm) *via* the splint strand Oligo 3 resulting in a covalently linked structures.

S7 Additional TEM images of hybrid assemblies

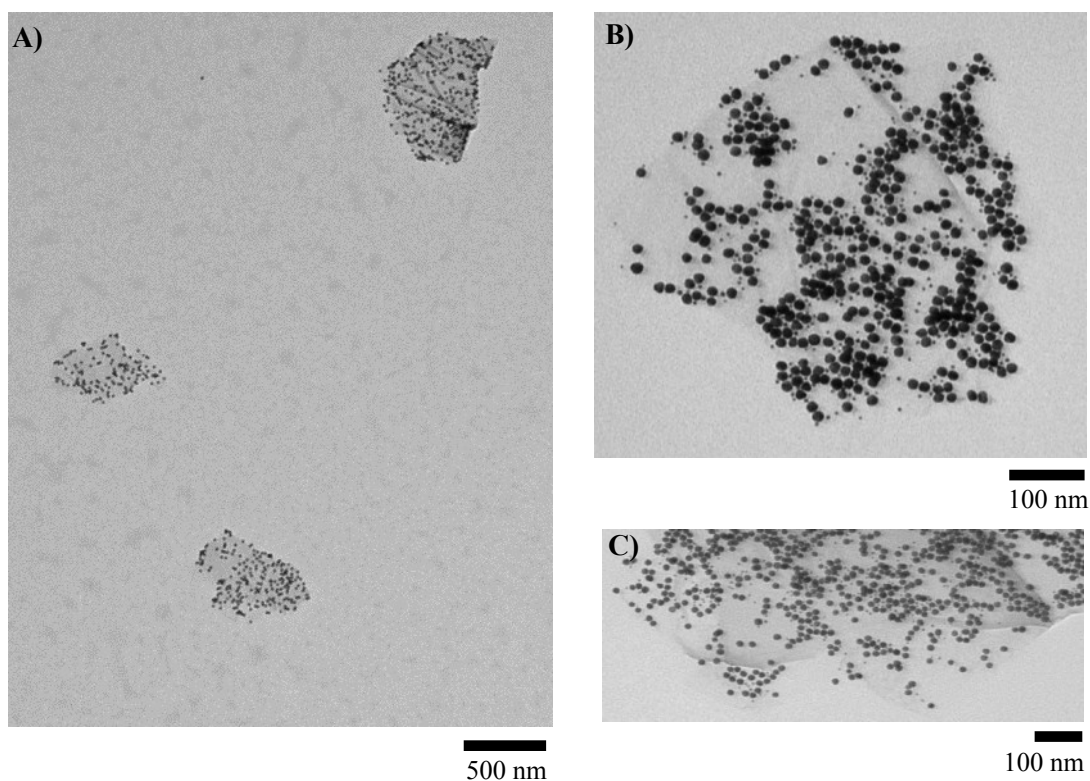


Figure S10 Additional TEM images of GO-AuNP hybrid assemblies. **A)** GO-AuNP (13 nm) **B)** and **C)** GO-AuNP (13 nm) – AuNP (5 nm).

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