Supporting Information

Copper-free Click Chemistry as an Emerging Tool for the Programmed Ligation of DNA-functionalised Gold Nanoparticles

Amelie Heuer-Jungemann,[a] Robert Kirkwood,[a] Afaf H. El-Sagheer,[b,c] Tom Brown[b,d] and Antonios G. Kanaras*[a,d]

[a] *Physics and Astronomy, Faculty of Physical Sciences and Engineering, University of Southampton, Southampton, SO17 1BJ, UK Fax: +44 (0) 2380593910; Tel: +44 (0) 2380592466; E-mail: A.Kanaras@soton.ac.uk*
[b] *Chemistry, Faculty of Natural and Environmental Sciences, University of Southampton, Southampton, SO17 1BJ, UK.*
[c] *Chemistry Branch, Department of Science and Mathematics, Faculty of Petroleum and Mining Engineering, Suez University, Suez 43721, Egypt.*
[d] *Institute for Life Sciences, University of Southampton, Southampton, SO17 1BJ, UK*

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S1. Materials and Methods

Commercially available reagents and solvents were used from the following suppliers throughout the experiments without further purification unless stated otherwise: Sodium tetrachloroaurate (III) dihydrate, trisodium citrate, sodium phosphate monobasic, sodium phosphate dibasic, Bis(p-sulfonatophenyl)phenyl phosphine dihydrate dipotassium salt (BSPP) and agarose were purchased from Sigma-Aldrich. Potassium Carbonate was purchased from Fisher Scientific. All reactions were carried out using Milli-Q water unless stated otherwise.

UV – visible spectra were recorded on a Cary 100 UV- Vis spectrophotometer over a range of 400–800 nm. TEM images were obtained on a FEI Tecnai12 Transmission Electron Microscope operating at a bias voltage of 80 or 120 kV. Sample preparation involved deposition and evaporation of a specimen droplet onto a grid (carbon-copper coated 400 mesh). Solvent evaporation was carried on an Eppendorf Concentrator. Polyacrylamide gels were visualised under short-wave UV irradiation using a Syngene G:Box gel imager and GeneSnap software. All glassware and stirrer bars were rinsed with Aqua Regia followed by Milli-Q water (≥18.2 MΩ/cm).

S2. 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 μmole 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, and the coupling time for the modified phosphoramidite monomers (C6-disulfide and aminolink C6, (Link Technologies)) was extended to 360 s. Aminolink C7 columns (Link Technologies) were used for the introduction of the 3′-aminohexyl 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

XBridgeTM BEH300 Prep C18 10 µM 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.

Labelling with Azide[1]

The oligonucleotide was synthesized with a 3'-hexyl amino linker for post-synthetic labelling. The freeze-dried oligonucleotide was dissolved in Na_2CO_3/$NaHCO_3$ buffer (80 µL, 0.5 M, pH 8.75). 6-Azidohexanoic acid NHS ester[2] (1 mg) was dissolved in DMSO (80 µL) and added to the oligonucleotide. After 4 h, the now fully labelled oligonucleotide **S1** was desalted using a NAP-10 Sephadex column (GE Healthcare) and purified by RP-HPLC.

Labelling with DIBO[1]

The oligonucleotide was synthesised with a 5'-hexyl amino linker for post-synthetic labelling. After freeze-drying, it was dissolved in Na_2CO_3/$NaHCO_3$ buffer (160 µL, 0.5 M, pH 8.75). DIBO active ester **1** (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 **S2** was then desalted using a NAP-10 Sephadex column, followed by purification by RP-HPLC.

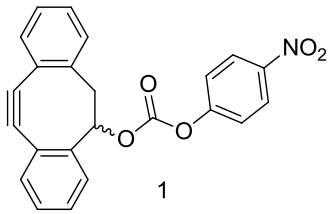

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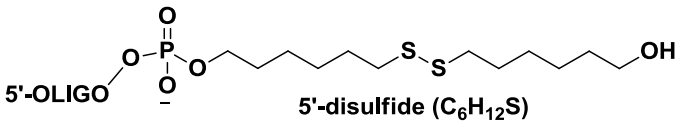
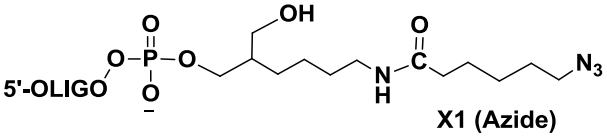
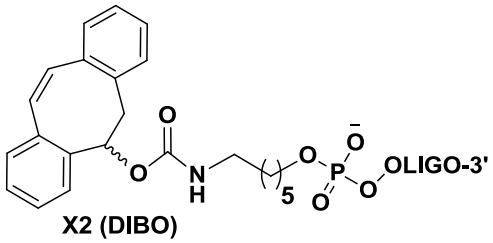
Figure SI 1. DIBO active ester

Table SI 2. Oligonucleotide sequences.

Abbreviation	DNA sequence and modifications
S1 (azide derivative)	**5' – ($C_6H_{12}S$)AAAAAAAACGAGTGCTAAGGATCCGAAX_1**
C1 (unmodified)	**5' – ($C_6H_{12}S$)AAAAAAAACGAGTGCTAAGGATCCGAA**
S2 (alkyne derivative)	**3' – ($C_6H_{12}S$) AAAAAAAAGCTTACCTATAGACGTCACTTX_2**
S3 (Splint)	**5' – ACACACCGAATGGATATCTGCAGTGAATTCGGATCCTTAGCACTCGACACAC**
S4 (Splint complement)	**5' – GTGTGTCGAGTGCTAAGGATCCGAATTCACTGCAGATATCCATTCGGTGTGT**







S3. Denaturing Polyacrylamide Gel of Clicked DNA

Clicking was first tested on the free DNA. Equimolar amounts of **S1**, **S2** and **S3** were incubated for 1h at room temperature (in 0.2 M NaCl) and afterwards denatured (addition of 50% formamide, heating to 90 °C for 5 min and immediate cooling in ice after). Samples were run in a 10% polyacrylamide gel (20 W, 1.5 h).

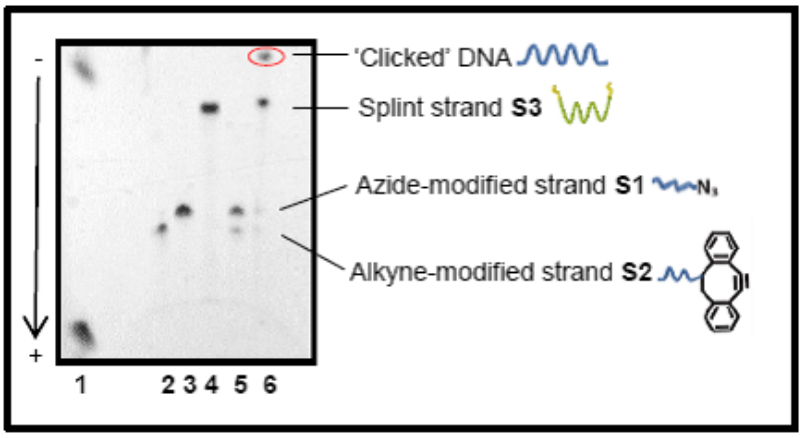


Figure SI 3. Denaturing polyacrylamide gel of clicked DNA. Lane 1 is a reference dye; Lane 2, 3 and 4 show the individual DNA strands (S1, S2 and S3); Lane 5 shows a mixture of S1 and S2 and Lane 6 shows the clicked DNA as well as S3.

S4. Triazole Linkage of Clicked DNA

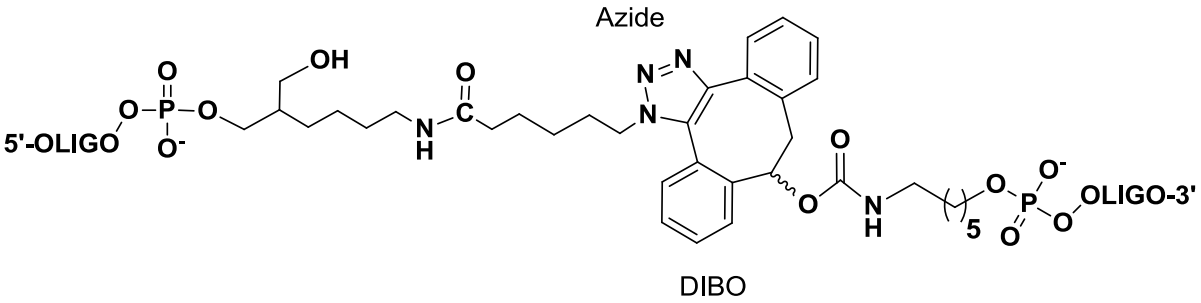


Figure SI 4. Chemical structure of the triazole linkage formed upon successful clicking of **S1** and **S2**. The linker is a mixture of stereoisomers at the cyclooctyne linkage and a mixture of regioisomers at the triazole ring.

S5. Synthesis and DNA Mono-functionalization of 5 nm Gold Nanoparticles

Synthesis

5 ± 1 nm AuNPs were synthesised according to published literature procedures.[3] In detail, $NaAuCl_4$ solution (375 µL, 4% wt) and K_2CO_3 (500 µL, 0.2 M) in water were stirred in an ice bath. To this, a freshly prepared $NaBH_4$ solution (0.5 mg/mL) was added in 5 × 1 mL aliquots with rapid stirring. A colour change from dark purple to reddish orange could be observed. After stirring for a further 5min, the solution was centrifuged at 12000 rpm for 10 min to remove larger particles. The supernatant was collected and BSPP (5 mg) was added whilst stirring. After 15 h, NaCl was added to the solution until a colour change from wine red to light purple was observed indicating particle aggregation. Particles were then centrifuged at 3000 rpm for 30 min and re-suspended in Milli-Q water. The purified particles with final concentrations of about 600 nM were stored at 4 °C.

Mono-conjugation of DNA to 5 nm AuNPs

AuNP-DNA conjugates were prepared according to modified literature procedures.[4] In detail, AuNPs in PBS (20 mM) were incubated with DNA bearing alkyne or azide moieties to achieve an AuNP to DNA ratio of ~1:1. Then BSPP (1 mg/ 20 µL) was added (1/10 of total reaction volume). After 1h, the functionalised AuNPs were purified by agarose gel electrophoresis (3 % agarose gel, 90 V, 90 min). Respective bands in the gel were cut out and stored in 0.5 ×TBE buffer overnight. Conjugates which had diffused out of the gel were purified by high speed centrifugation (16,400 rpm, 6 °C, 45 min) and re-dispersed in hybridisation buffer (5 mM phosphate, 80 mM NaCl).

S6. Programmed Assembly and Click Ligation (5 nm AuNPs)

In a typical dimer formation, 15 pmol of mono-conjugates bearing 3'-azide (or unmodified for control) and 5'-alkyne functional groups respectively were incubated in hybridisation buffer (5 mM phosphate, 80 mM NaCl). Typical reaction volume (V_{total}): 200 µL. Slow increase of salt concentration with simultaneous reduction of V_{total} was achieved in an Eppendorf Concentrator. After ~2h, V_{total} was reduced to 10 µL. **S3** (15 pmol) was then added and the reaction was incubated at 65 °C for 5 min followed by slow cooling overnight. Prepared assemblies were then purified using agarose gel electrophoresis (3%, 90 V, 45 min).

The dimer band was extracted from the gel as before and used in further experiments.

S7. Additional TEM images of 5 nm AuNP Dimers

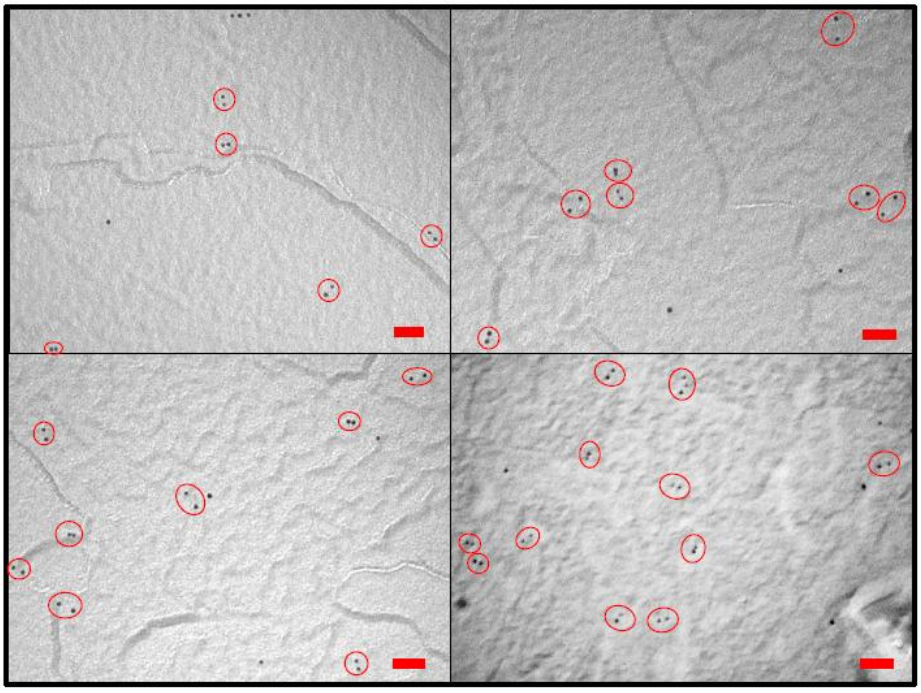
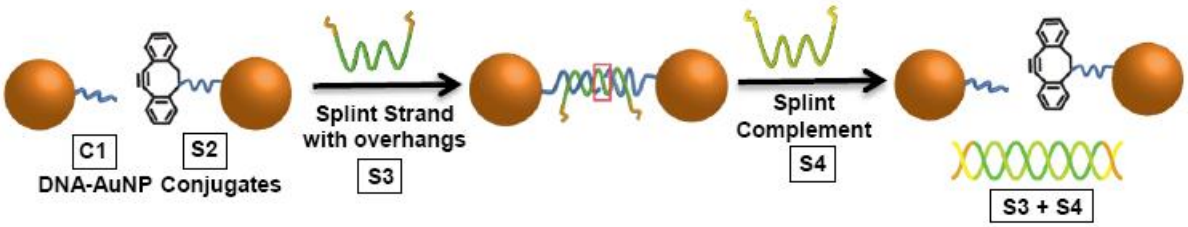


Figure SI 5. Additional TEM images for 5 nm AuNP dimers connected via single strand of clicked ssDNA. (Scale bars are 40 nm).

S8. Gold Nanoparticle Assembly using 'nicked' DNA



Scheme SI 6. Schematic representation of the gold nanoparticle assembly using 'nicked' DNA. Due to the lack of an azide group on **C1**, dimer formation is only possible in the presence of the splint strand **S3.** Upon treatment with **S4** the system breaks apart into mono-conjugates as the DNA strands were unable to undergo the click reaction.

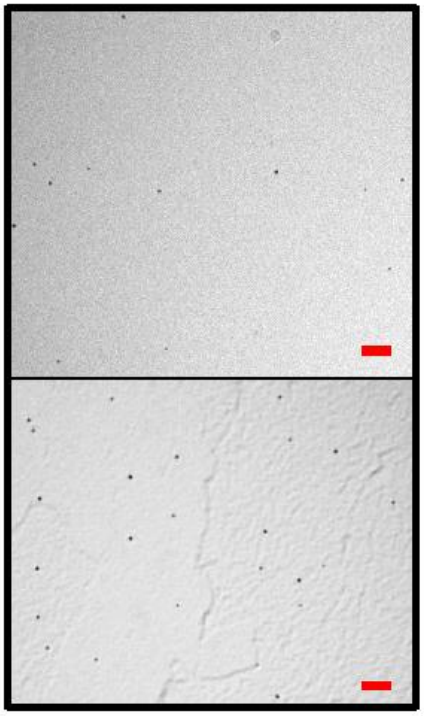


Figure SI 7. TEM images displaying mostly single particles (mono-conjugates) after removal of the splint strand **S3.** Scale bars are 40 nm.

S9. Gel Image Analysis of Click-Ligation

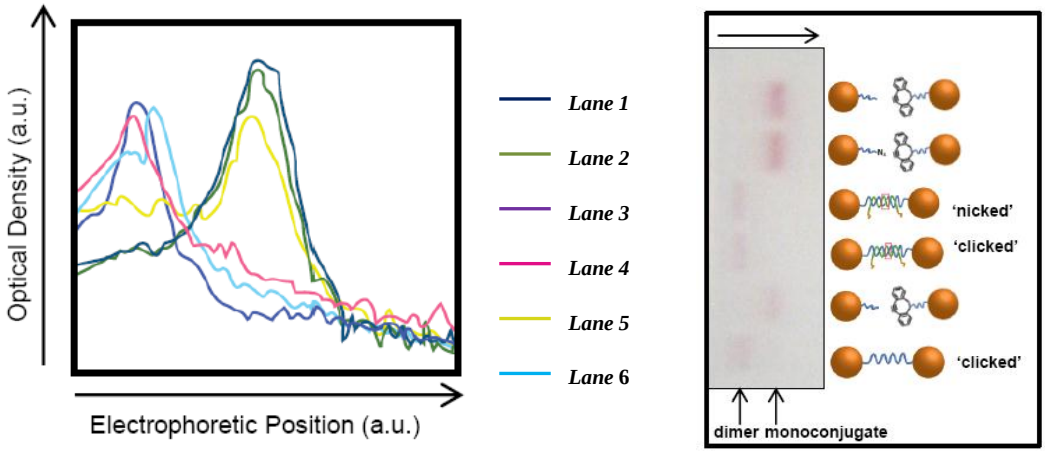


Figure SI 8. Agarose gel and corresponding gel analysis (using Image J software) for dimer formation and removal of **S3** through competitive hybridisation. Lanes 1, 2, 3 and 5 show control experiments. Lanes 4 and 6 show the clicked product. Lanes 3 shows dimers, unable to click due to the lack of an azide group. Once treated with **S4**, they break apart into mono-conjugates (lane 5). Lane 6 shows the success of the click reaction. Clicked dimers connected via ssDNA (light blue curve) display greater mobility and thus travel further in the gel than dimers still connected via dsDNA (pink curve).

S10. Synthesis of 13 nm AuNPs

Synthesis

13 ± 2 nm spherical AuNPs were synthesised by the citrate reduction method.[5] In detail: A solution of sodium tetrachloroaurate (100 mL, 1 mM) was brought to the boil (250 °C) under stirring. Once boiling, sodium citrate solution (5 mL, 2% wt) was added to the gold solution. A colour change from yellow to colourless to finally deep red could be observed. Once the colour was stable, the solution was left to stir for an additional 15 min. Afterwards the reaction mixture was left to cool to room temperature under stirring. These citrate capped particles were then treated with BSPP as before. After 4 h, NaCl was used to induce particle aggregation. Following two rounds of centrifugation at 5000 rpm for 10 min, particles were re-dispersed in Milli-Q water to final concentrations of ~ 300 nM.

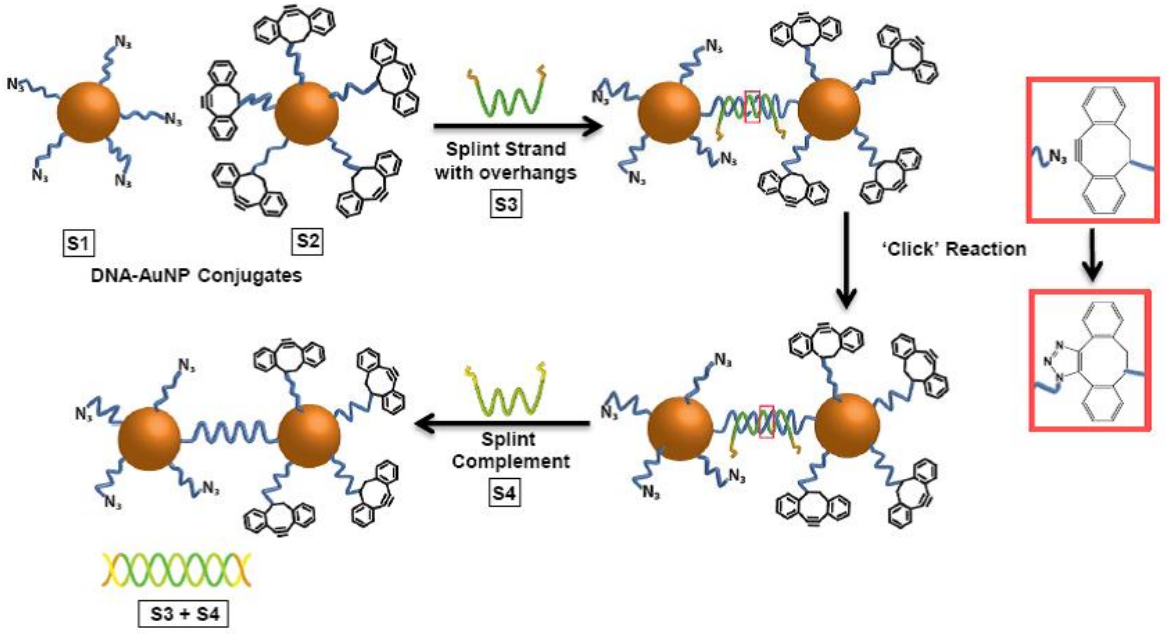
DNA Modification of 13 nm AuNPs

AuNPs were modified with multiple oligonucleotide strands according to literature procedures.[6] In detail, AuNPs (500 µL, 6 nM) in Milli-Q water were incubated with DNA **S1** or **S2** (25 µL, 40 µM) overnight. The reaction volume was then increased to 1 mL with final concentrations of NaCl and phosphine of 0.5 M and 2 mM respectively. After 2 h the volume was then slowly reduced to ~500 µL by vacuum centrifugation at 35 °C, ensuring a gradual increase in ionic strength and DNA concentration. The resulting DNA-coated AuNPs were then purified by two subsequent centrifugation steps and stored in a NaCl/phosphate buffer (0.2 M and 6 mM respectively) at 4°C.

S11. Programmed Assembly and Click-Ligation (13 nm AuNPs)

Dimer Formation

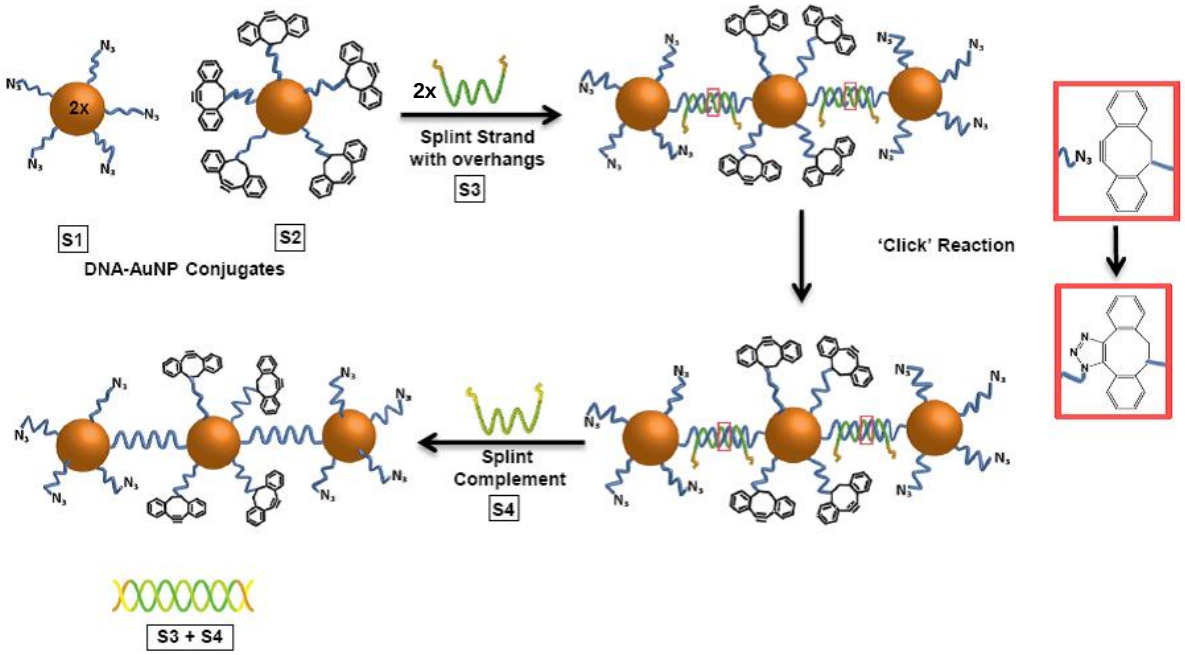
AuNPs functionalized with multiple strands of **S1** and **S2** were mixed with equimolar quantities of **S3** in hybridisation buffer (0.1 M NaCl, 6 mM phosphate, pH 7.4). The system was briefly heated to 65 °C and then left to cool to room temperature overnight. Gel electrophoresis was employed to purify dimers. They were then extracted from the gel via diffusion and purified by two rounds of centrifugation (14,000 rpm, 10 min) and re-dispersion in buffer as before. Treatment with an excess of **S4** then resulted in dimers connected via a single strand of ssDNA (see SI 9 and 11).



Scheme SI 9. Schematic illustration of dimer formation for 13 nm AuNPs functionalised with multiple strands of **S1** or **S2**.

Trimer Formation

For trimer formation AuNPs functionalized with multiple strands of **S1** and **S2** were mixed with **S3** in a 2:1:2 ratio (two azide-DNA modified AuNPs to one alkyne-DNA modified AuNP with two splint strands to link) in hybridisation buffer (0.1 M NaCl, 6 mM phosphate, pH 7.4). The system was briefly heated to 65 °C and then left to cool to room temperature overnight. Gel electrophoresis was employed to purify trimers. They were then extracted from the gel via diffusion and purified by two rounds of centrifugation (14,000 rpm, 10 min) and re-dispersion in buffer as before. Treatment with an excess of **S4** then resulted in trimers connected via a single strand of ssDNA (see SI 10 and 11).



Scheme SI 10. Schematic illustration of trimer formation for 13 nm AuNPs functionalised with multiple strands of **S1** or **S2**.

S12. Additional TEM images of 13 nm AuNP Dimers and Trimers

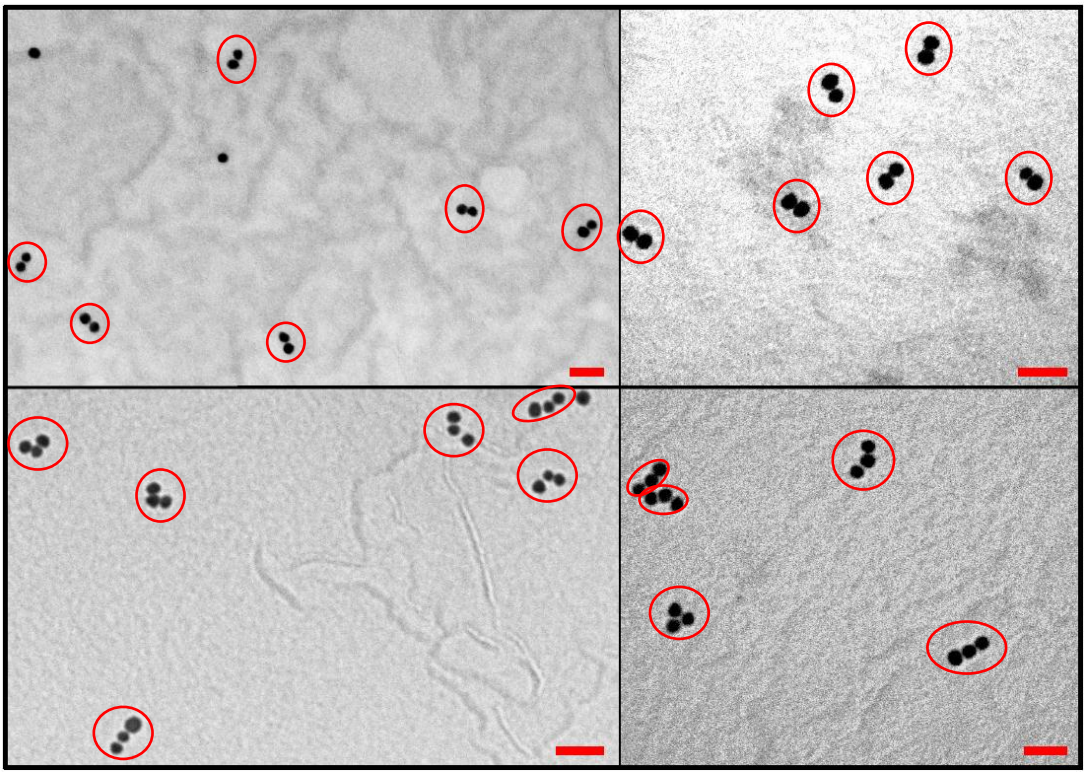


Figure SI 11. Additional TEM images of 13 nm AuNP dimers and trimers connected via a single strand of clicked ssDNA. (Scale bars are 50 nm).

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