Dynamic DNA Templates for Discrete Gold Nanoparticle Assemblies: Control of Geometry, Modularity, Write/Erase and Structural Switching

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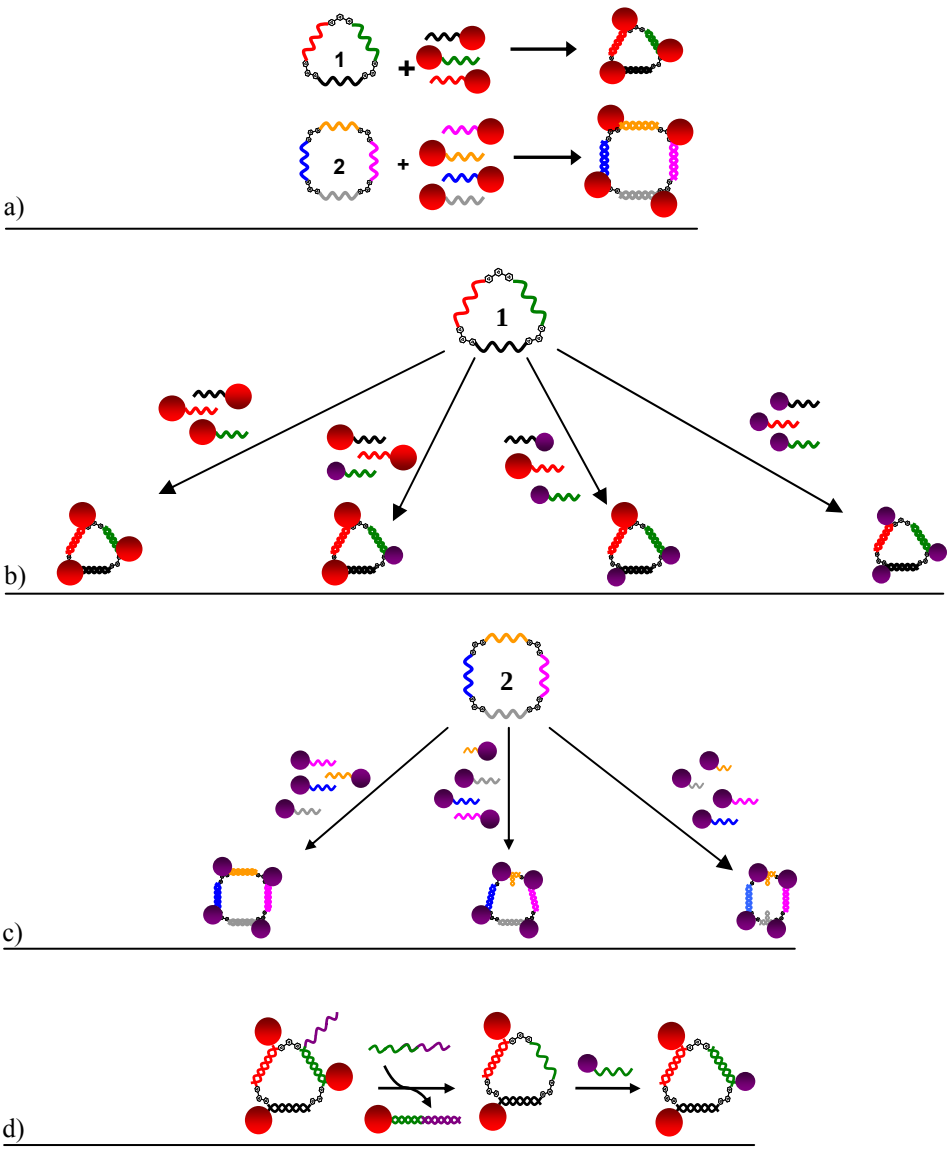

A method for the ready access to a large number of discrete nanoparticle assemblies using a small number of single-stranded and cyclic DNA templates that are dynamic is presented. (a) Triangle template **1** and square template **2** are used to generate gold nanoparticle assemblies with geometrical control by the simple tagging of each particle with a DNA sequence that serves to dictate its final position within the construct. (b) The same template **1** is used to access all the possible triangular combinations that two gold nanoparticles of different sizes may be organized in (i.e. three larger, two larger / one smaller, one larger/ two smaller, all smaller). (c) The same square template **2** is used to generate nanoparticle assemblies in which four gold nanoparticles are organized into square, trapezoidal and rectangular arrangements. (d) Post-assembly addressability is demonstrated by a write/erase experiment in which three gold nanoparticles of a single size are assembled into a triangular arrangement, a specific particle is erased using an external eraser strand, and the empty position is re-written with a smaller sized particle. Our approach could be generalized to easily generate large sets of nanoparticle groupings with control over the position, size, type and *addressability* of each nanoparticle within the construct.

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I. General

Tris(hydroxymethyl)-aminomethane (Tris), boric acid, acetic acid, ethylenediaminetetraacetic acid, NaCl, $MgCl_2 \cdot 6H_2O$, DL-dithiothreitol (DTT), StainsAll®, formamide, and ethidium bromide were used as purchased from Aldrich. 5' phosphate modification reagent {2-[2-(4,4'-dimethoxytrityloxy)ethylsulfonyl] ethyl-(2-cyanoethyl)-(N,N'-diisopropyl)-phosphoramidite}, 5' thiol modification reagent [dimethoxy trityl protected amidite derivative of bis(6-hydroxyhexyl)disulfide], guanidine derivatized 1000Å LCAA-CPG solid support with a loading density of 28 μmol/g, 5-ethylthiotetrazole, and reagents used for automated DNA synthesis were purchased from ChemGenes. Sephadex G-25 (super fine DNA grade), T4 DNA ligase (source: recombinant), and Mung Bean Nuclease (source: Mung Bean Sprouts) were purchased from Amersham Biosciences. 10 bp molecular weight DNA ladder (1.0 μg/μL) was purchased from Invitrogen Life Technologies. Microcon® size-exclusion centrifugal filter devices were purchased from Millipore. Citrate coated gold nanoparticles were either made in-house or purchased from Ted Pella. The ligand bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt was purchased from Strem Chemicals. 400 mesh carbon coated copper grids for transmission electron microscopy imaging were purchased from Electron Microscopy Sciences.

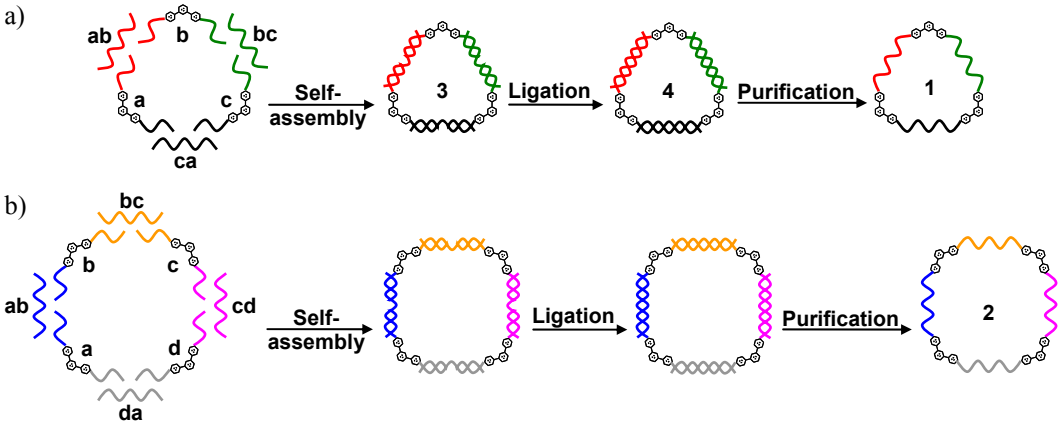
II. Instrumentation

Standard automated oligonucleotide solid-phase synthesis was performed on a Perspective Biosystems Expedite 8900 DNA synthesizer. UV-vis experiments were conducted on a Varian Cary 300 biospectrophotometer. Gel electrophoresis experiments were carried out on an acrylamide 20 X 20 cm vertical Hoefer 600 electrophoresis unit, and an agarose 7 X 8 cm horizontal Owl separation minigel system. Electroelution was performed using a Centrilutor® electroeluter from Millipore. Ethidium bromide gels were visualized on a Fischer Scientific variable wavelength transilluminator. Matrix assisted laser desorption time-of-flight (MALDI-TOF) spectra were obtained using a KOMPACT MALDI III mass spectrometer. Transmission electron microscopy (TEM) images were obtained using a JOEL 2000FX electron microscope.

III. Synthesis of building blocks a, b, c, d and linker strands ab, bc, cd, da, ca

Triangular single stranded template **1** is constructed from the stepwise ligation of the self-assembly product of building blocks **a**, **b**, **c** with linker strands **ab**, **bc**, **ca** (Scheme S1a), while square template **2** is constructed from the ligation of the assembly product of building blocks **a**, **b**, **c**, **d** with linker strands **ab**, **bc**, **cd**, **da** (Scheme S1b).

Scheme S1 Synthesis of templates 1 and 2. Construction of (a) single stranded triangular template **1** using building blocks **a**, **b**, **c**, and linker strands **ab**, **bc**, **ca**, and of (b) square template **2** using building blocks **a**, **b**, **c**, **d**, and linker strands **ab**, **bc**, **cd**, **da**.



The synthesis of building blocks **a**, **b**, **c**, and **d** is performed on 1000Å LCAA-CPG solid support with a loading density of 28 μmol/g. For each respective building block, the first DNA arm is grown using standard automated oligonucleotide synthetic protocols, and is followed by the incorporation of the trityl protected amidite derivative of the organic vertex 1,3-bis(4-hydroxyphenyl)benzene using a modified protocol (extended coupling/deprotection times of 10/2 min). The synthesis of this vertex has already been reported.[S1] The synthesis of each building block is then terminated following the addition of the second DNA arm of appropriate sequence and the conversion of the final 5' OH group into a phosphate using the reagent 2-[2-(4,4'-dimethoxytrityloxy)ethylsulfonyl]ethyl-(2-cyanoethyl)-(N,N'-diisopropyl)-phosphoramidite. The chemically incorporated 5' phosphate will facilitate the enzymatic ligation of the assembled building blocks to one another. Finally, the building blocks are cleaved and deprotected into a concentrated solution of ammonium hydroxide (12 hrs, 55^{o}C), and are directly purified from 24% TBE denaturing polyacrylamide

gels. The desired bands are excised, extracted into 3 mL of water (16 hrs, 37°C), and purified using Sephadex G-25 column chromatography. Quantification is carried by UV-vis analysis using Beer's law ($A_{total} = A_{vertex} + A_{DNA}$), in which the extinction coefficient of the vertex at 260 nm is calculated to be 2.30 X 10^5 L mol^{-1} cm^{-1}. Table S1 summarizes the sequences of building blocks **a**, **b**, **c**, **d** along with their expected and experimentally obtained molecular masses (MM). MALDI-TOF MS is performed using a co-matrix composed of 6-aza-2-thiothymine and fucose, and with the additive spermine.[S2]

Table S1 Building blocks a, b, c, d: sequences and calculated/experimentally obtained MM

Name	Sequence (5' → 3')	MW (g/mol)	
		Calculated	Obtained
a	**Phosphate**-TTTGCTAACTGGTAGAGTTC-**Vertex**-GACCAATAACACAAATCGGG	12,740.13	12,764.47 [M+ Na^+]
b	**Phosphate**-GCAATACTATTTCGATCTGG-**Vertex**-ACATGGTAGAAGGAGGAAAG	12,869.16	12,869.74
c	**Phosphate**-CCTGCTCATACTGCAATCTG-**Vertex**-CCAGAATGACATCACCTTGG	12,540.07	12,537.30
d	**Phosphate**-ACGCCCAAACCTTTCAACTT-**Vertex**-CCCAGCCTTTCGACATCTCG	12,444.06	12,445.40

The synthesis of linker strands **ab**, **bc**, **cd**, **da** and **ca** is also performed on 1000Å LCAA-CPG solid support with a loading density of 28 μmol/g. In each case, forty base long DNA strands of appropriate sequences are grown using standard automated oligonucleotide synthetic protocols, and are capped with the 5' thiol modifying reagent [dimethoxy trityl protected amidite derivative of bis(6-hydroxyhexyl)disulfide] to allow for their subsequent conjugation to gold nanoparticles (extended coupling/deprotection times of 10/2 min). The use of a dimethoxy trityl protected 5' thiol modification reagent allows for the real time monitoring of the coupling efficiency, which in all cases is found to be greater than 95%. Cleavage and deprotection of the linker strands and the simultaneous reduction of the 5' disulfide into a primary thiol, is performed in a 0.05 M solution of DTT in concentrated ammonium hydroxide (16 hrs, 55°C). The desired products are directly isolated from 24% TBE polyacrylamide denaturing gels, purified using Sephadex G-25 column chromatography, quantified using UV-vis analysis, and characterized using MALDI-TOF MS. Table S2 summarizes the sequences of linker strands **ab**, **bc**, **cd**, **da**, and **ca** along with their calculated and experimentally obtained molecular masses.

Table S2 Linker strands ab, bc, cd, ca, da: sequences and calculated/experimentally obtained MM

Name	Sequence (5' → 3')	MW (g/mol)	
		Calculated	Obtained
ab	HS-CCAGATCGAAATAGTATTGCCCCGATTTGTGTTATTGGTC	12,482.08	12,505.23 [M+ Na^+]
bc	HS-CAGATTGCAGTATGAGCAGGCTTTCCTCCTTCTACCATGT	12,405.07	12,401.05
cd	HS-AGGTTGAAAGGTTTGGGCGTCCAAGGTGATGTCAGGCTGG	12,718.12	12,719.47
ca	HS-GAACTCTACCAGTTAGCAAACCAAGGTGATGTCAGGCTGG	12,544.13	12,543.75
da	HS-GAACTCTACCAGTTAGCAAACGAGATGTCGAAAGGCTGGG	12,593.15	12,626.17 [M+ Na^+]

IV. Self-assembly of building blocks a, b, c, and d into triangular and square constructs using linker strands ab, bc, cd, da, and ca.

The construction of the triangular assembly is achieved using building blocks **a**, **b**, **c** and linker strands **ab**, **bc**, **ca** (Scheme 1). The process is sequentially monitored using 10% native polyacrylamide gel electrophoresis, and is visualized following staining for 2 hrs in a solution of StainsAll® (12.5 mg StainsAll® in 125 mL of distilled water and 125 mL of formamide). As seen in Figure S1, building block **a** alone results in a single band of relatively high electrophoretic mobility (lane 1), while the assembly of the linear construct of two building blocks ($\mathbf{a^{ab}b}$; generated by building the linking of building blocks **a** and **b** with strand **ab**) and three building blocks ($\mathbf{a^{ab}b^{bc}c}$) show well-defined single bands of steadily decreasing mobility (lanes 2 and 3, respectively). These assignments also correlate well with the 10-base pair linear DNA ladder in lane 6 of Figure S1. The addition of linker strand **ca** to the linear assembly $\mathbf{a^{ab}b^{bc}c}$ results in both duplex formation and cyclization. A single band of reduced mobility relative to that of

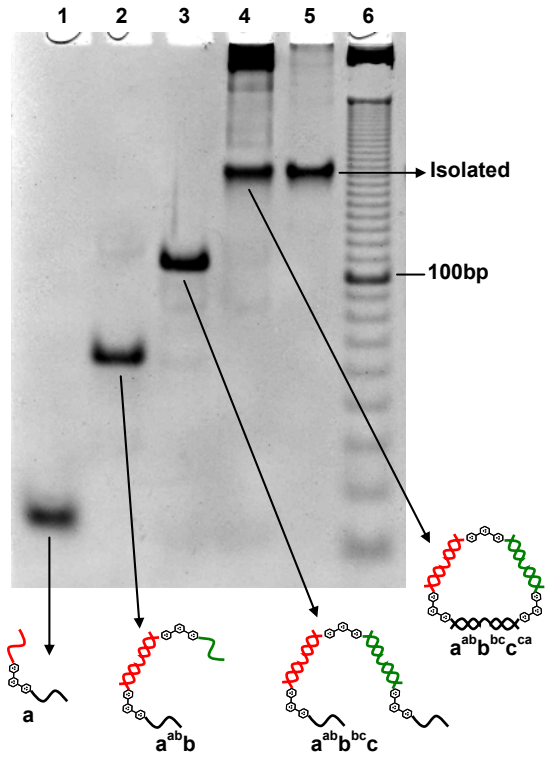


Figure S1 Sequential self-assembly of triangular template. 10% Native polyacrylamide gel of the sequential self-assembly of the triangular construct via the step-wise addition of linker strands **ab**, **bc**, **ca** to building blocks **a**, **b**, **c**, forming **a**, $\mathbf{a^{ab}b}$, $\mathbf{a^{ab}b^{bc}c}$, $\mathbf{a^{ab}b^{bc}c^{ca}}$ (lanes 1-4, respectively). The triangular assembly $\mathbf{a^{ab}b^{bc}c^{ca}}$ is directly isolated using electroelution and is characterized in lane 5. 10 bp molecular weight marker (lane 6).

$\mathbf{a}^{ab}\mathbf{b}^{bc}\mathbf{c}$ is observed (lane 4) and is assigned to the triangular construct $\mathbf{a}^{ab}\mathbf{b}^{bc}\mathbf{c}^{ca}$. Although the cyclized triangular system contains sixty base pairs, it is found to move as a 185mer when compared to the linear base pair marker. This is consistent with the fact that cyclic structures travel anomalously when compared to their linear counterparts.[S3] A second band of extremely low electrophoretic mobility is also detected in lane 4 of Figure S1, suggesting the concomitant formation of higher order oligomeric species. The clean isolation of the cyclic triangular assembly is performed using electroelution and is shown in lane 5.

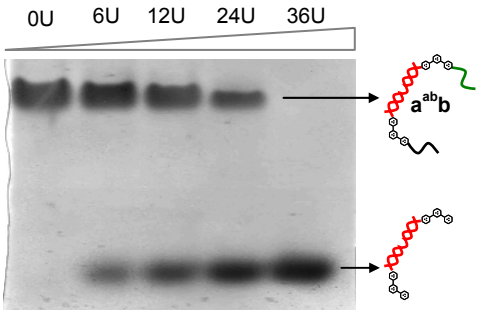


Figure S2 Protocol for the selective digestion of single-stranded DNA. Enzymatic digestion study determining that the use of 36 U of MBN, at 0°C and 3 hrs, is selective for single strand digestion.

The cyclic nature of the isolated triangular assembly in lane 5 of Figure S1 is confirmed using enzymatic digestion assays with Mung Bean nuclease (MBN). Under optimized conditions, this enzyme is selective for the digestion of single stranded DNA over that of double stranded DNA by a factor of 30,000:1.[S4,S5] MBN is thus expected to degrade linear open structures that contain single stranded DNA, but is not expected to degrade cyclic closed structures that contain only double stranded DNA. In an attempt to ensure that the enzyme is active on DNA to which our vertex is attached and to find the optimal conditions under which single stranded DNA is preferentially digested, initial work was directed towards subjecting the linear construct of two building blocks ($\mathbf{a}^{ab}\mathbf{b}$) to varying amounts of the enzyme. Digestions are performed in an ice bath for a period of 3 hrs. In all these cases, 1.2 X 10^{-10} moles of $\mathbf{a}^{ab}\mathbf{b}$ in 10 μL of TAE buffer are subjected to varying units of enzyme. The addition of 36

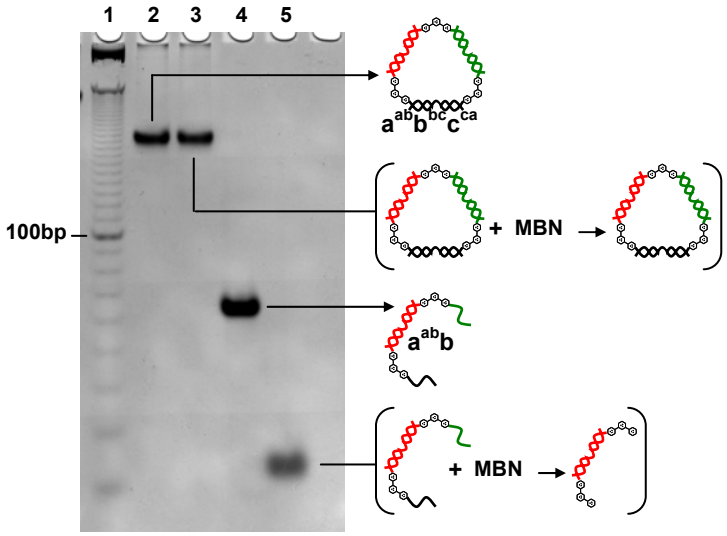


Figure S3 Enzymatic digestion of triangular assembly. MBN enzymatic digestion confirms the double stranded cyclic nature of the isolated triangular assembly $\mathbf{a}^{ab}\mathbf{b}^{bc}\mathbf{c}^{ca}$. Lanes 2 and 3, respectively, contain isolated $\mathbf{a}^{ab}\mathbf{b}^{bc}\mathbf{c}^{ca}$ before and after treatment with MBN, while lanes 4 and 5, respectively, contain the single and double stranded control assembly $\mathbf{a}^{ab}\mathbf{b}$ before and after treatment with MBN. Lane 1 contains the 10-bp linear molecular weight DNA ladder.

units of enzyme results in the selective, and complete digestion of the single stranded portion of $\mathbf{a^{ab}b}$ (Figure S2). As seen in Figure S3, treatment of the purified triangular assembly (Lane 2) with the enzyme MBN using the optimized conditions (lane 3), confirms the cyclic nature of triangle **1**.

The assembly of the square construct is similarly achieved using building blocks **a**, **b**, **c**, **d** and linker strands **ab**, **bc**, **cd**, **da**. The sequential assembly is also monitored using 10% native PAGE (Figure S4a), in which lanes 1, 2, 3, and 4 correspond to linear groupings of one (**a**), two ($\mathbf{a^{ab}b}$), three ($\mathbf{a^{ab}b^{bc}c}$) and four ($\mathbf{a^{ab}b^{bc}c^{cd}d}$) building blocks, lane 5 corresponds to the formation of the cyclic square construct in the presence of oligomeric species, and lane 6 corresponds to the isolated square. The cyclic nature of the isolated square assembly in lane 6 of Figure S4a is also confirmed using MBN digestion assays (Figure S4b).

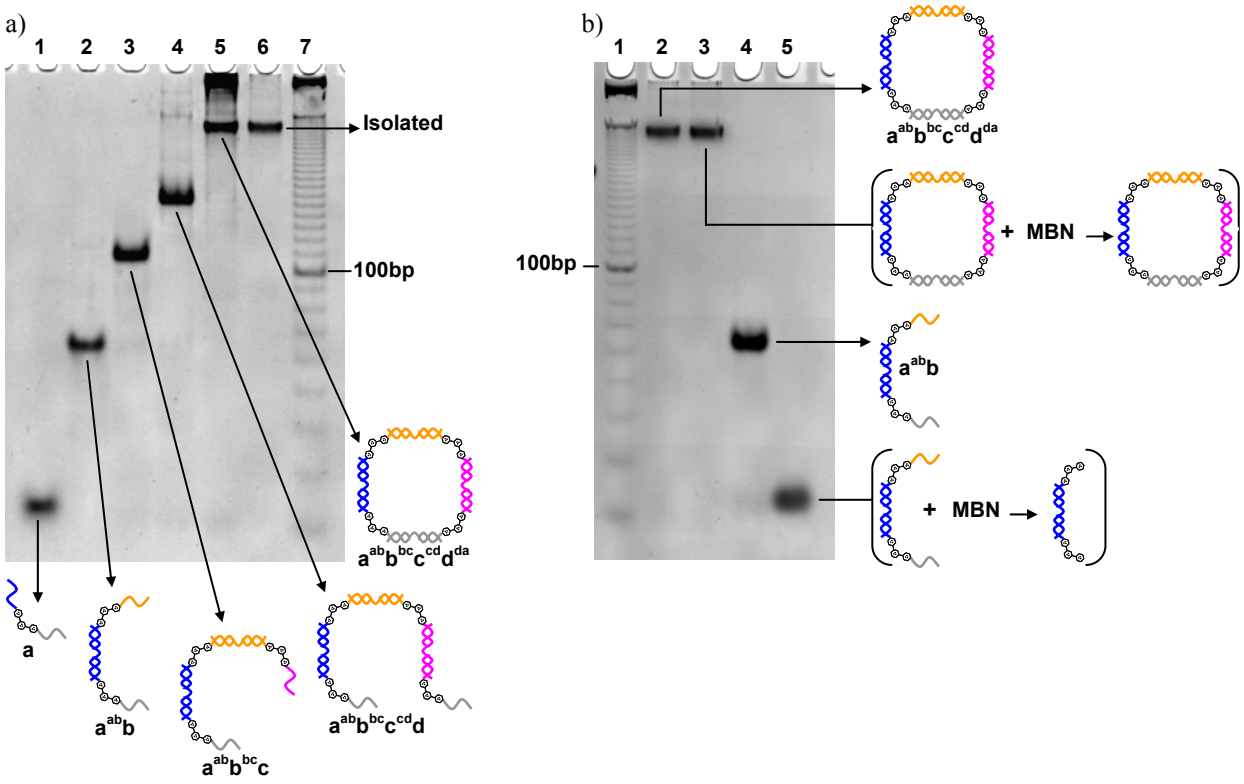


Figure S4 Sequential self-assembly of square template and enzymatic digestion of isolated square . (a) Native polyacrylamide gel of the sequential self-assembly of square $\mathbf{a^{ab}b^{bc}c^{cd}d^{da}}$ via the step-wise addition of linker strands **ab**, **bc**, **cd**, **da**, to building blocks **a**, **b**, **c**, **d,** forming **a**, $\mathbf{a^{ab}b}$, $\mathbf{a^{ab}b^{bc}c}$, $\mathbf{a^{ab}b^{bc}c^{cd}d}$, $\mathbf{a^{ab}b^{bc}c^{cd}d^{da}}$ (lanes 1-5, respectively), and of isolated $\mathbf{a^{ab}b^{bc}c^{cd}d^{da}}$ (lane 6). 10 bp molecular weight marker (lane 7). (b) Native polyacrylamide gel of isolated $\mathbf{a^{ab}b^{bc}c^{cd}d^{da}}$ before and after MBN enzymatic digestion (lanes 2 and 3, respectively) and of single and double stranded control assembly $\mathbf{a^{ab}b}$ before and after treatment with MBN (lanes 4 and 5, respectively). 10 bp molecular weight marker (lane 1).

V. Enzymatic ligation, purification and characterization of templates 1 and 2

The isolated triangular and square assemblies are enzymatically ligated using T4 DNA ligase (Figure S5a and S5b, respectively). The protocol involves incubating each μg of DNA with 0.001 units of ligase at 25°C for 16 hrs, followed by an incubation at 60°C for 10 mins to halt enzymatic activity before analysis and purification using 24% TBE denaturing polyacrylamide gel electrophoresis. The gels are visualized following staining in ethidium bromide for 20 min (concentration of 0.5 μg per mL of distilled water). Lanes 1 in both Figures S5a and S5b contain home-made single stranded internal markers constructed from the ligation of linear groupings of two (**a**^{ab}**b**), three (**a**^{ab}**b**^{bc}**c**) and four (**a**^{ab}**b**^{bc}**c**^{cd}**d**) building blocks (i.e. **ab**, **abc**, **abcd**). The ligation products in lanes 2 of Figures S5a and S5b contain single bands assigned to single stranded triangle **1** and square **2**, respectively. As expected, the fully cyclized triangular and square templates are slightly retarded in mobility when compared to the mobility of their respectively linear counterparts. Both the linear and cyclic analogous of the single-stranded grouping of three building blocks (i.e. linear trimer **abc** and cyclized triangle **1**) and four building blocks (i.e. linear tetramer **abcd** and cyclized square **2**) are directly isolated from the gel via electroelution and quantified using UV-vis analysis.

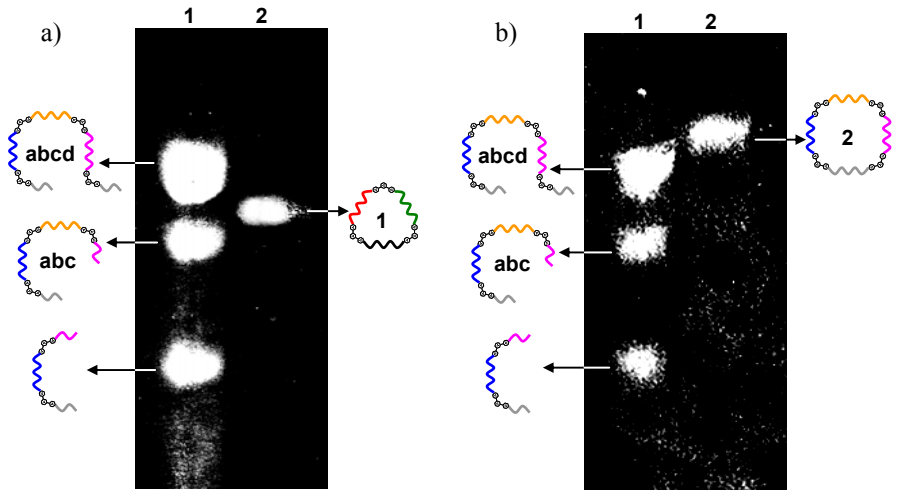


Figure S5 Enzymatic ligation of triangular and square assemblies for the synthesis of triangle 1 and square 2. Enzymatic ligation of triangle **1** (lane 2 in left panel), of square **2** (lane 2 in right panel), and of the linear assemblies of two (**ab**), three (**abc**) and four (**abcd**) building blocks [lane 1 of both panels].

MALDI-TOF MS analysis on all four single-stranded templates is performed. The expected/observed molecular masses of linear trimer **abc**, cyclic triangle **1**, linear tetramer **abcd**, and cyclic square **2** are found to be 38,113.34/38,118.56, 38,095.23/38,132.57 [M+ K^+], 50,539.39/50,546.55, and 50,467.38/50,490.24.

In an attempt to further characterize the single stranded linear trimer **abc**, cyclic triangle **1**, linear tetramer **abcd**, and cyclic square **2** using gel electrophoresis, titration experiments were conducted in which each of the four templates are sequentially incubated with complementary strands of appropriate sequence. Figures S6a and S6b are respective 10% TAE native polyacrylamide gels of the titration of linear trimer **abc** and cyclic triangle **1** with appropriate complementary strands, respectively. Lane 1 in Figure S6a contains a single band with high electrophoretic mobility corresponding to the isolated single stranded trimer **abc**, while lanes 2 and 3 correspond to the single products formed from the titration of **abc** with one and two complementary strands. As expected, the mobility of the fully ligated linear trimer that has been hybridized to two linker strands (lane 3) is identical to that of the unligated $\mathbf{a}^{\mathbf{ab}}\mathbf{b}^{\mathbf{bc}}\mathbf{c}$ (i.e. assembly of building blocks **a**, **b**, **c** with linker strands **ab**, **bc**). The fully cyclized single stranded triangle **1** is similarly titrated with

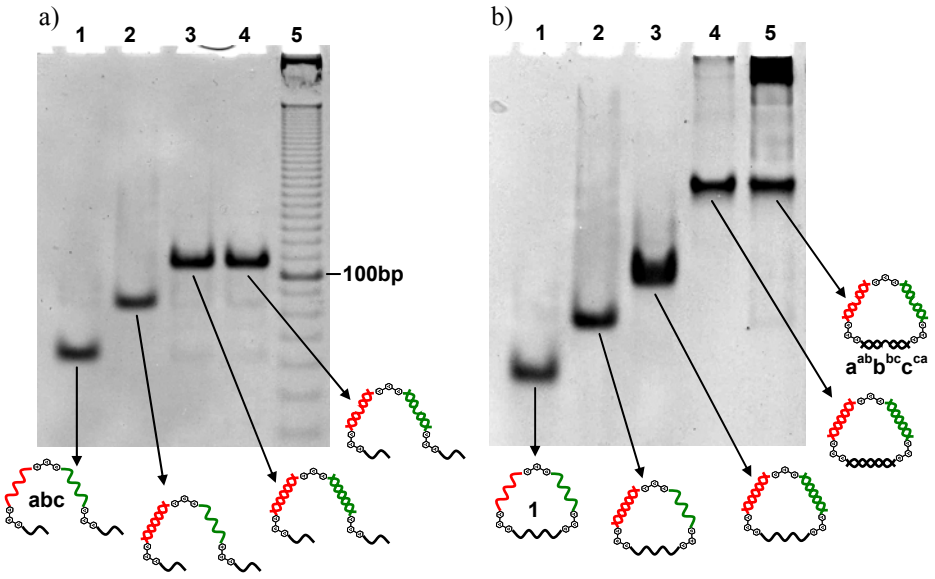


Figure S6 Titration of singles-stranded trimer abc and triangle 1 with complementary strands of DNA. (a) Sequential titration of isolated single-stranded trimmer **abc** (lane 1) with complementary linker strands **ab** (lane 2) and **ab**, **bc** (lane 3). Linear trimer $\mathbf{a}^{\mathbf{ab}}\mathbf{b}^{\mathbf{bc}}\mathbf{c}$ (lane 4). 10 bp molecular weight ladder (lane 5). (b) Sequential titration of isolated triangle **1** (lane 1) with complementary linker strands **ab** (lane 2), **ab**, **bc** (lane 3) and **ab**, **bc**, **ca** (lane 4). Assembly of triangle $\mathbf{a}^{\mathbf{ab}}\mathbf{b}^{\mathbf{bc}}\mathbf{c}^{\mathbf{ca}}$ (lane 5).

complementary linker strands. The band in lane 1 of Figure S6b corresponds to triangle **1**, while the single bands in lanes 2, 3, and 4 correspond to the titration of **1** with 1, 2 and all 3 complementary linker strands, respectively. The mobility of the fully titrated cyclic triangle **1** with linker strands **ab**, **bc**, **ca** is also found to be identical to that of the triangular assembly $\mathbf{a}^{\mathbf{ab}}\mathbf{b}^{\mathbf{bc}}\mathbf{c}^{\mathbf{ca}}$ (i.e assembly of building blocks **a**, **b**, **c** with linker strands **ab**, **bc**, **ca**).

Isolated linear tetramer **abcd** and cyclic square **2** are similarly characterized using titration experiments (Figures S7a and S7b, respectively).

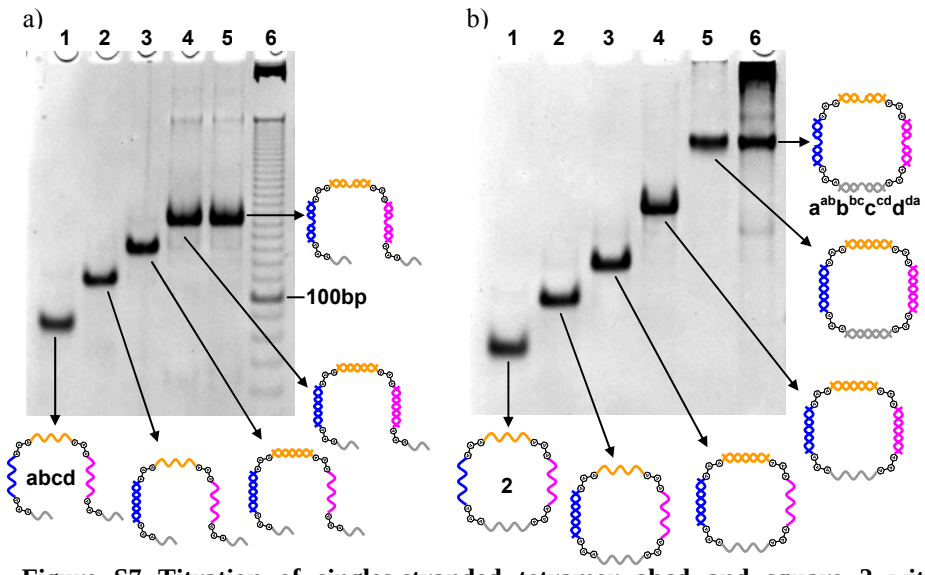


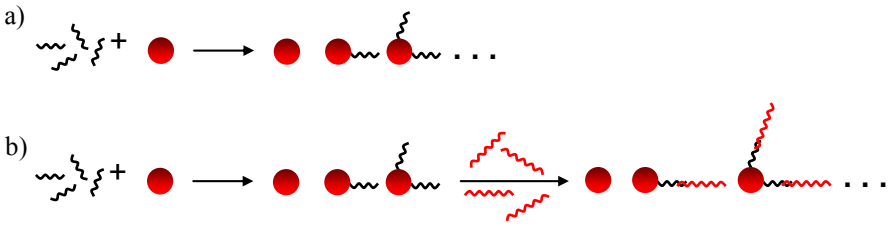
Figure S7 Titration of singles-stranded tetramer abcd and square 2 with complementary strands of DNA. (a) Sequential titration of the isolated single-stranded tetramer **abcd** (lane 1) with complementary linker strands **ab** (lane 2), **ab**, **bc** (lane 3) and **ab**, **bc**, **cd** (lane 4). Linear tetramer $\mathbf{a}^{\mathbf{ab}}\mathbf{b}^{\mathbf{bc}}\mathbf{c}^{\mathbf{cd}}\mathbf{d}$ (lane 5), and 10 bp molecular weight ladder (lane 6). (b) Sequential titration of isolated square **2** (lane 1) with complementary linker strands **ab** (lane 2), **ab**, **bc** (lane 3), **ab**, **bc**, **ca** (lane 4) and **ab**, **bc**, **cd**, **da** (lane 5). Assembly of square $\mathbf{a}^{\mathbf{ab}}\mathbf{b}^{\mathbf{bc}}\mathbf{c}^{\mathbf{cd}}\mathbf{d}^{\mathbf{da}}$ (lane 6).

VI. Gold nanoparticle synthesis

Gold nanoparticles were either prepared in-house using the citrate/tannic acid method[S6] or were directly obtained from Ted Pella. In either case, the citrate coated gold nanoparticles are not stable enough at the salt concentrations required to conjugate DNA, and are thus first exchanged with a negatively charged phosphine shell using the reagent bis(*p*-sulfonatophenyl)phenylphosphine dehydrate dipotassium salt as per Alivisatos *et al.*[S7,S8]

VII. Mono-functionalization of gold nanoparticles with thiolated DNA

Scheme S2 Conjugation of thiolated DNA to gold nanoparticles. (a) Incubation of fourty base DNA strands with five nm gold nanoparticles. (b) Non-covalent extension of the DNA strands within the same mixture by a seventy base run of thymines provides adequate mass/charge for separation using agarose gel.



In order to conjugate thiolated DNA to gold nanoparticles, freshly reduced solutions of each respective thiol DNA are added to gold (1:1 molar ratio) and are left incubating at room temperature for 2 hrs (Scheme S2a). When the resulting mixtures are characterized using 2% agarose gel electrophoresis, adequate separation between the conjugates is not observed (Figure S9a, lane 2). This is due to a lack of the necessary charge to mass ratio between the gold nanoparticles used and the DNA strands. Previous studies have shown that a forty base DNA sequence (i.e. the length of our thiolated linker strands) is not long enough to achieve functional separation between the conjugates when used with five nm gold nanoparticles,[S8] and is certainly not long enough when used with larger sized particles. To overcome this problem, we effectively increased the length of the DNA strands being conjugated using a series of extension strands that serve to non-covalently lengthen each of the strands from forty to 110 bases (Scheme S2b). Addition of the extension strands, of appropriate sequences, to the gold/DNA conjugate mixtures described above, followed by an incubation period of fifteen minutes and analysis using 2% agarose gels electrophoresis revealed adequate separation between the conjugates (Figure S9b, lane 2). Table S3 summarizes the sequences and the

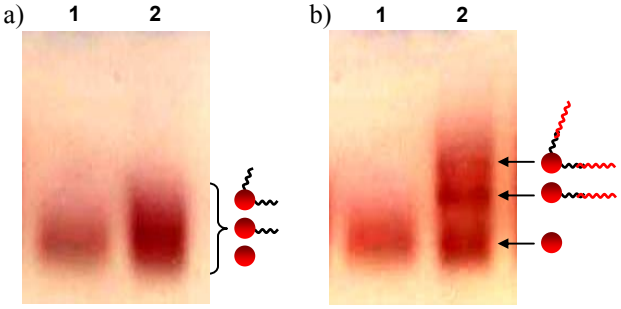


Figure S9 Analysis of gold/DNA mixtures with and without extension strands. (a) When the mixture from the incubation of **ab** to 5 nm Au particles is analyzed using 2% agarose gel electrophoresis, separation between the conjugates is not observed (lane 2). (b) However, when the DNA strands within the same mixture are lengthened by 70 bases using **ab-extension**, adequate separation is obtained (lane 2). Lane 1 contains 5 nm Au particles as a control.

expected and obtained molecular masses of each respective extension strand. The desired DNA/gold conjugate are visualized and isolated from 2% agarose gels directly as per Alivisatos *et al.*[S7,S8]

Table S3 Strands ab-extension, bc-extension, cd-extension, ca-extension, da-extension: Sequences and calculated/experimentally obtained MM

Name	Sequence (5' → 3')	MW (g/mol)	
		Calculated	Obtained
ab-extension	$(T)_{70}$GACCAATAACACAAA	25,828.07	25,854.14 [M+ Na^+]
bc-extension	$(T)_{70}$ACATGGTAGAAGGAG	25,971.07	25,974.32
cd-extension	$(T)_{70}$CCAGCCTGACATCAC	25,763.07	25,764.18
ca-extension	Same as **cd-extension**	"	"
da-extension	$(T)_{70}$CCCAGCCTTTCGACA	25,754.01	25,796.55 [M+ K^+]

Extension strands are not expected to interfere with the hybridization of the isolated mono-functionalized gold/DNA conjugates to any of the templates. This is because the hybridization of all forty bases of the template is expected to occur preferentially over that of just fifteen bases of the extension strand. This is confirmed using 10% TAE native polyacrylamide gel electrophoresis experiments (Figure S10). An experiment was conducted in which the forty base long thiolated DNA strand **ab** (lane 1) is hybridized to its respective extension strand (lane 2) via fifteen bases to yield an assembly that is 110 bases long (lane 3). When the ligated dimer template that contains the fully complementary 40 base sequence to the extended linker strand **ab** is added, the extension strand is fully displaced (lane 4). This confirms our ability to add the isolated mono-functionalized gold/DNA adducts to any of the desired templates without the need to have to remove any of the extension strands that provide the initial separation for purification.

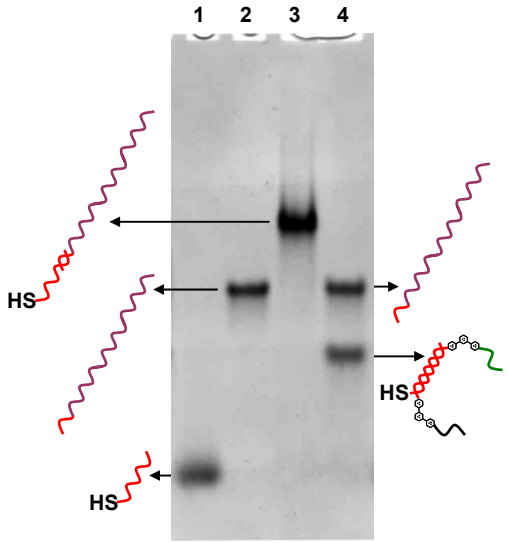


Figure S10 Quantitative replacement of the extension strands with the templates. 10% Native polyacrylamide gel confirming the quantitative hybridization of an extender strand (lane 2) to the partially complementary linker strand **ab** (lane 1) yields a single assembly (lane 3). Quantitative displacement of the extender strand in the presence of a template (lane 4).

VIII. Sequences for the construction of trapezoidal/rectangular assemblies using square 2 and for write/erase experiments using triangle 1.

In order to construct a trapezoidal grouping of four gold nanoparticles using square **2**, it is necessary to shorten the length of one of the sides. Similarly, a rectangle can be constructed using single stranded square **2** if two arms that are parallel to each other are shortened. This is accomplished by the use of continuous DNA strands that are complementary to the first and last ten bases of the forty base long side to be shortened. This effectively introduces a twenty base long single stranded internal loop that shortens the length of the respective side by one half. The thiolated strands **ab-short** (5' HS-CCAGATCGAAGTTATTGGTC) and **cd-short** (5' HS-AGGTTGAAAGGTCAGGCTGG) are synthesized in a manner similar to that of **ab** and **cd**, and are found to have respective calculated/observed masses of 6,325.09/6,324.25 and 6,822.19/3,822.97.

Write erase experiments are conducted using triangle **1**. The DNA strand attached to the particle to be erased is sixty bases long, with the first forty bases being complementary to the respective side, and the remaining twenty bases being a mixed sequence that is not complementary to any part of the template. The twenty base over-hang allows for the selective removal of the gold nanoparticle upon its hybridization to a fully complementary sixty base eraser strand. The sequence of the sixty base long thiolated DNA strand that is conjugated to the gold nanoparticles (**ab-write**) is 5' HS-CCAGATCGAAATAGTATTGCCCCGATTTG TGTTATTGGTCTTGGTTTTTGTTTTGGTTTT, while the sequence of its full complement (**ab-eraser**) is 5' AAAACCAAAACAAAAACCAAGACCAATAACACAAATCGGGGCAATACTATTTCGATCTGG. The respective calculated/observed molecular masses of purified **ab-write** and **ab-eraser** are 18,688.03/18,902.74 [M + Na^+] and 18,432.20/18,432.60.

IX. TEM Sample preparation

Mono-functionalized gold/DNA conjugates that have been freshly isolated are added to the respective templates in a 1.2:1 molar ratio and are left incubating at room temperature for a period of less than 5 min before deposition on the TEM grids. Deposition involves the addition of a 10 μL droplet of the assembled mixture onto the grid, an adsorption period of fifteen minutes, removal of the excess solution using a filter paper, and a single wash step with Milli-Q water. When possible, analysis is performed within 48 hrs to minimize sample degradation.

X. Statistical analysis of the nanoparticle assemblies

Template **1** is used to generate all the triangular assemblies containing two different sizes of gold nanoparticles (i.e. groupings of three larger, two larger / one smaller, one larger / two smaller, and three smaller) (Figure S11a). In order to truly measure the ability of template **1** to direct the programmed organization of gold nanoparticle assemblies, it is necessary to compare and contrast the distribution of all the different triangular groupings that are formed within each system. For example, when attempting to construct assemblies of two larger / one smaller gold nanoparticles, what percentage of the observed triangular constructs are in fact two larger / one smaller? Do we also observe a large number of any of the other combinations (i.e. three larger, one larger / two smaller, or three smaller)? As seen in Figure S11b, when all the triangular assemblies within each case are analyzed for the formation of the desired construct, the yield is found to be essentially quantitative. This confirms our ability to use single-stranded templates to effectively mediate the organization of nanoparticle groupings.

Similarly, square **2** is found to template the generation of the desired polygon with a very high synthetic efficiency (Figures S12a and S12b). Analysis and tabulation of all the quadrilaterals observed within each assembly reveals that each desired gold nanoparticle grouping (i.e. square, trapezoid or rectangle) is constructed in approximately 90 % yield.

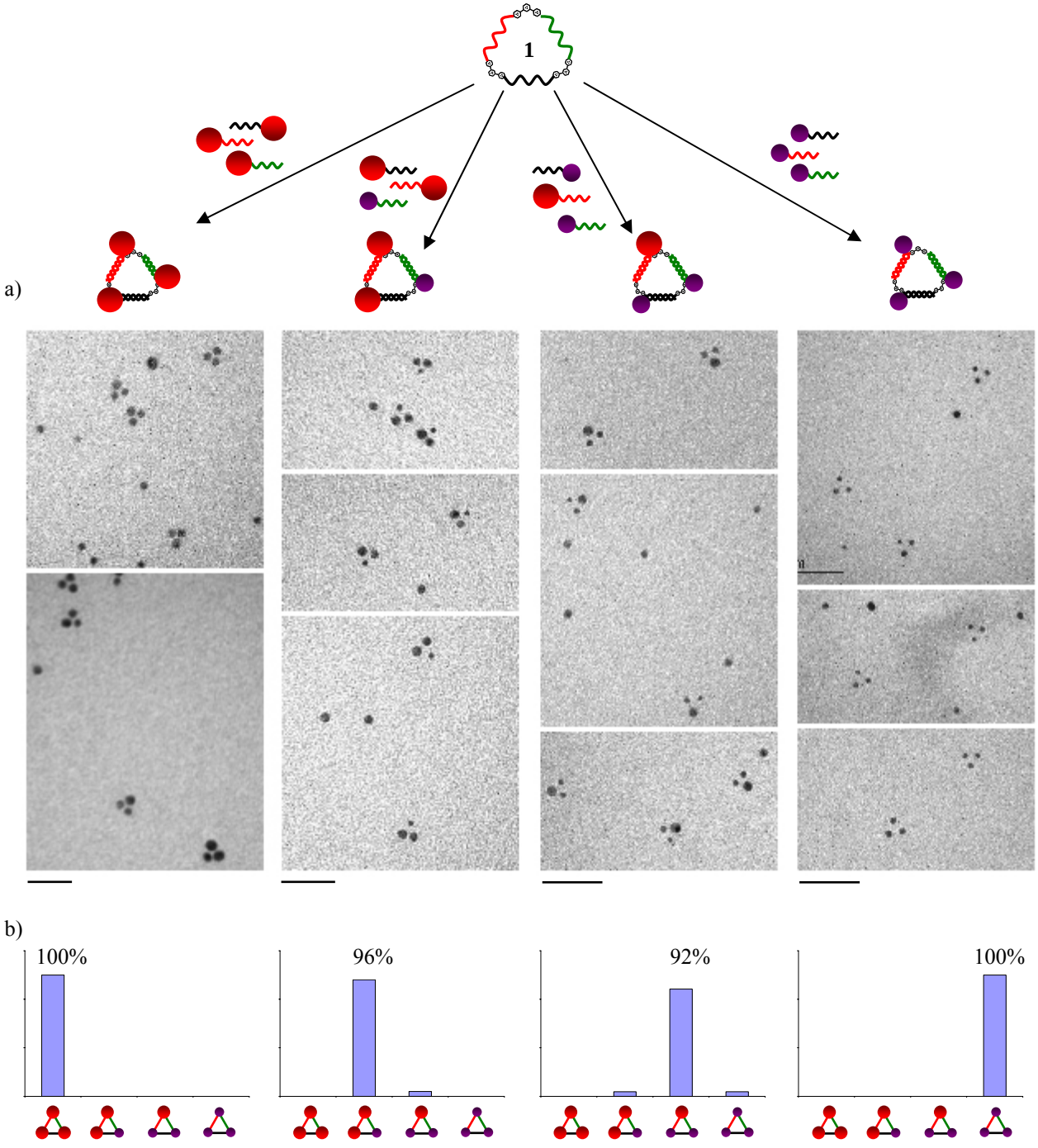


Figure S11 Statistical analysis on the types of triangular assemblies formed using templates 1. (a) Template **1** is used to generate all the possible combinations that two gold nanoparticles of different sizes may be organized in. This is achieved by the simple tagging of each respective gold nanoparticle with a sequence of DNA that serves to dictate its final location within the construct. (b) When the triangular assemblies are further analyzed to determine the percentage of the formation of the desired grouping, the templates were found to mediate the assembly of the desired combination in over 90% yield. Bar corresponds to 50 nm.

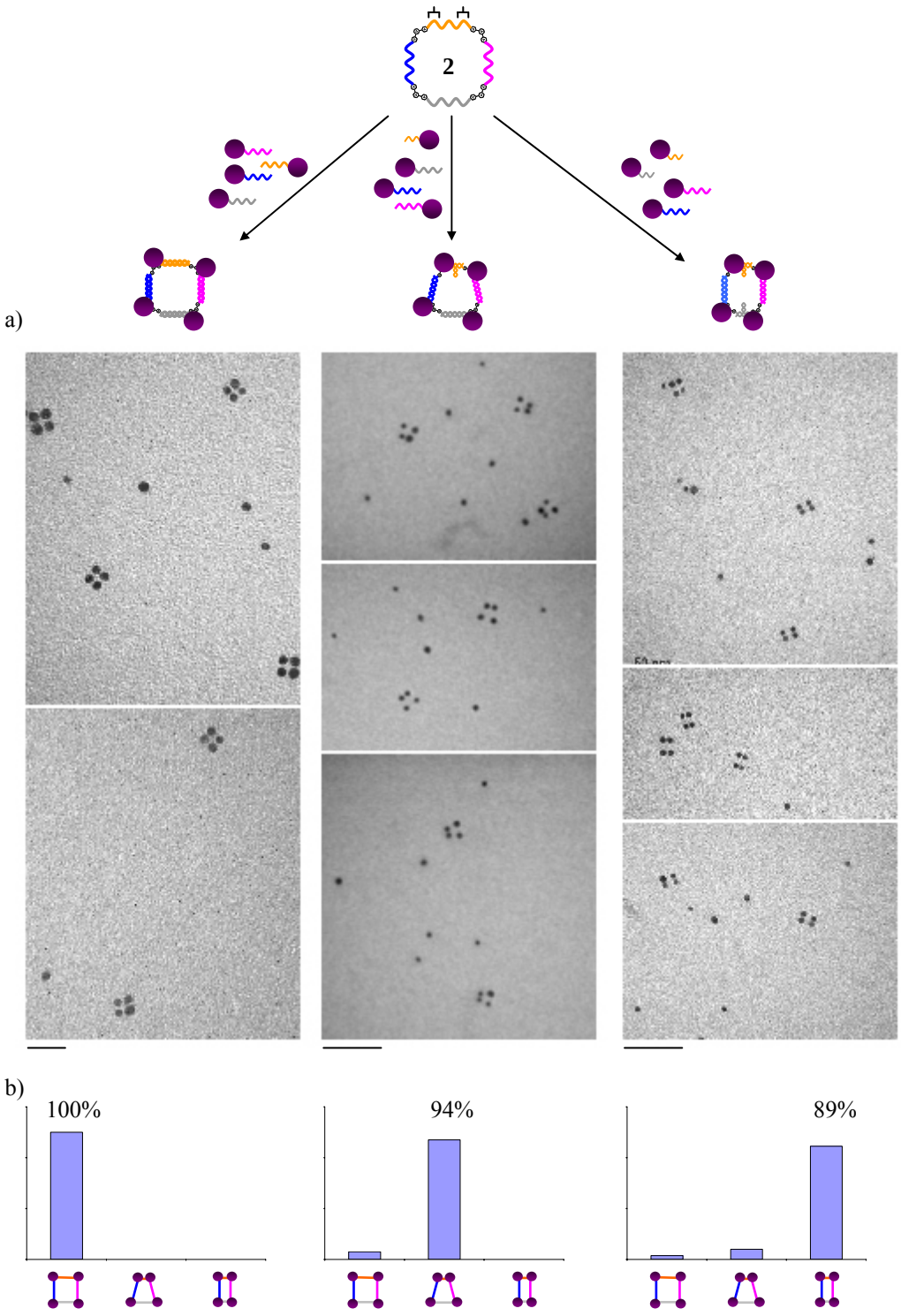


Figure S12 Statistical analysis on the types of quadrilaterals formed using templates 2. (a) Template **2** is used to assemble four gold nanoparticles into three geometrically distinct quadrilaterals. (b) Statistical analysis on the type of quadrilaterals formed using template **2** reveal the ability of our single stranded cyclic template to mediate the assembly of the three different assemblies in ~ 90% yield. Bar corresponds to 50 nm.

Write erase experiments are conducted using template **1**. Initially, three larger sized gold nanoparticles are organized into a triangular arrangement (molar ratio of each particle to template is 1.2:1, incubation period of 5 min) (Figure S13a). One of these particles, however, is mono-functionalized with a DNA strand that provides a twenty base over-hang (i.e. **ab-write**). This twenty base over-hang allows for the selective removal of the gold nanoparticle upon its hybridization to a fully complementary eraser strand (i.e. **ab-eraser**). When the eraser strand is added to the triangular grouping of three larger sized gold nanoparticles (molar ratio of strand to template is 2:1, incubation period of five minutes), an almost quantitative decrease in the amount of the triangular constructs is observed (Figure S13b). The empty position within the template is then re-written with a smaller sized nanoparticle. This is accomplished by the addition of a five nm gold

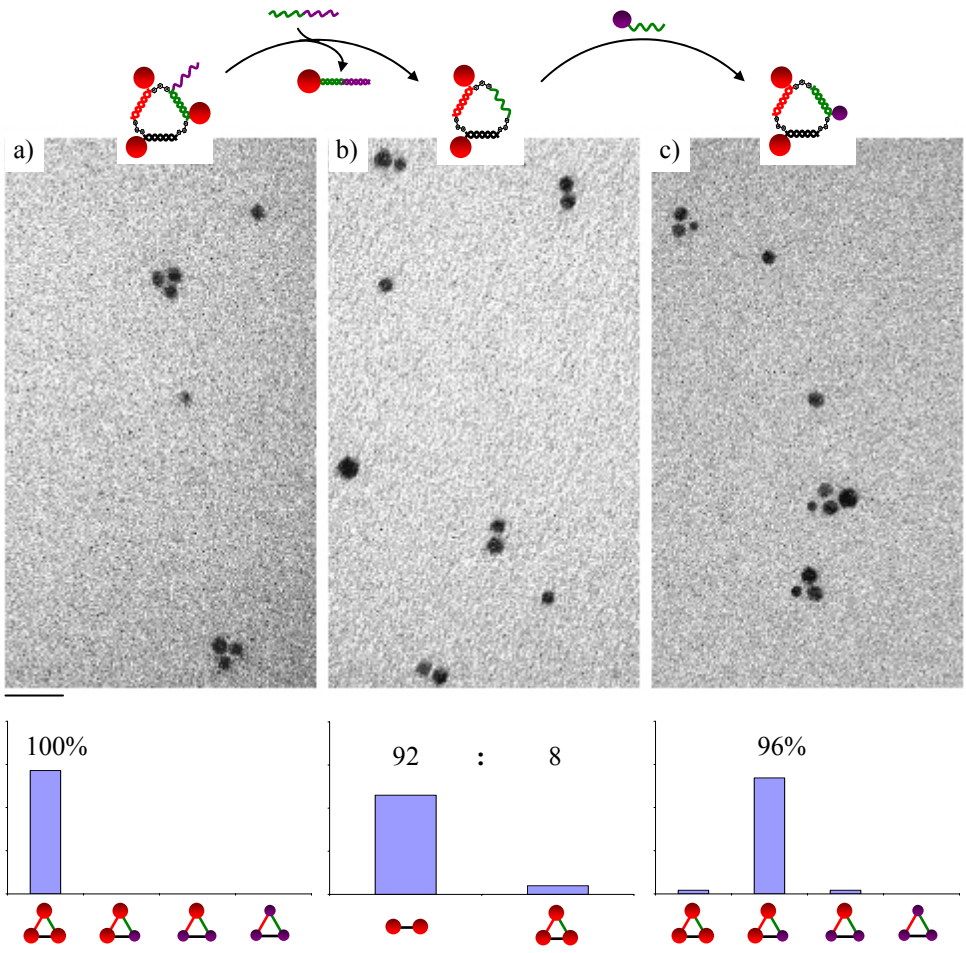


Figure S13 Statistical analysis on the type of triangular groupings observed during a write/erase experiment. (a) Three fifteen nm gold nanoparticles are assembled into a triangle. (b) A specific particle is removed using a DNA eraser strand, and (c) the empty position is replaced with a five nm gold nanoparticle. Bar corresponds to 40 nm.

particle that is mono-functionalized with **ab** (molar ratio of particle to template is 2:1, incubation period of 5 min). TEM analysis revealed the essentially quantitative formation of triangular groupings made up of two larger and one smaller gold nanoparticles (Figure S13c).

A control experiment was conducted to confirm the fidelity of this method to selectively address the nanoparticles within the construct. In this experiment, a triangular grouping of three larger sized gold nanoparticles is also constructed in which one of the particles used is mono-functionalized with a strand that contains a twenty base over-hang. However, in this experiment, the smaller sized gold nanoparticle, which is mono-functionalized with strand **ab,** is added to the mixture prior to the intermediate addition of **ab-eraser** (molar ratio of particle to template is 2:1, incubation period of five minutes). The smaller sized particle is not expected to replace any of the larger sized nanoparticles within the triangular assemblies. This is confirmed following TEM analysis (Figure S14).

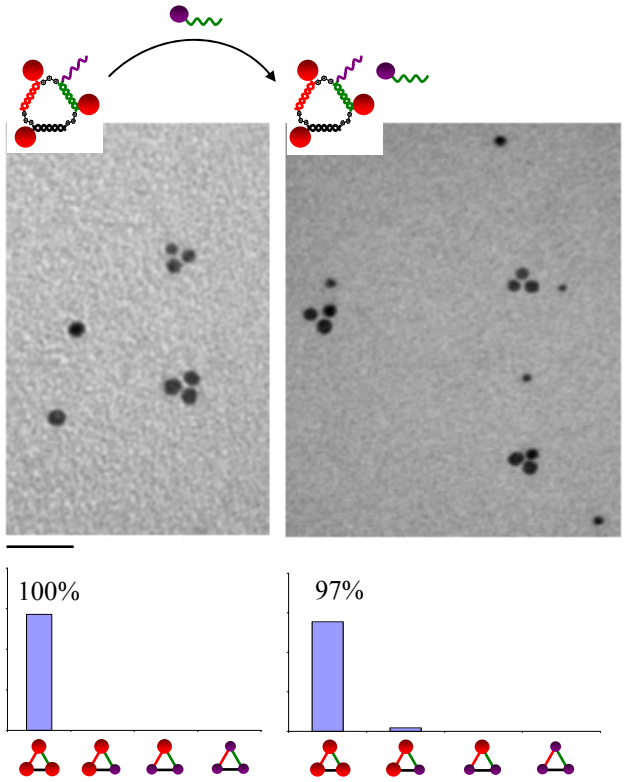


Figure S14 Write/erase experiment in which the intermediate eraser step is omitted. Addition of mono-functionalized five nm gold nanoparticles to the triangular assembly of fifteen nm gold nanoparticles, without an intermediate eraser step, does not result in replacement of either of the nanoparticles on the assembly. Bar corresponds to 40 nm.

It should be noted, however, that statistical analysis of the overall observed yield of the desired assembly, within each system, is in fact found to be ~ 25 %. The instability of the thiol linkage between the gold nanoparticles and the DNA strands[S9,S10] is expected to contribute to the lowering of the overall observed synthetic yields. This is because the isolated mono-functionalized gold/DNA adducts are expected to undergo re-equilibration upon purification. An agarose gel electrophoresis experiment was conducted in which a mono-functionalized gold/DNA adduct is isolated, allowed to sit at room temperature (in our buffer conditions), and is then re-analyzed for the type of conjugates in solution (Figure S15). Within fifteen

minutes, the isolated mono-functionalized gold/DNA adduct has undergone re-equilibration into a mixture that contains gold nanoparticle conjugates with zero, one, two and three DNA strands. Thus, the observed yields on the TEM grids are not a true reflection on the ability of our templates to mediate nanoparticle assemblies. The future development of mono-functionalized gold/DNA conjugates that possess long-term stability is expected to improve the overall observed synthetic yield of any of our assemblies. This is a general problem in the current attempts to generate mono-functionalized gold/DNA adducts, and is a separate problem from the specificity of our approach to generate particle assemblies with control over combination and geometry, which statistical analysis shows to be high.

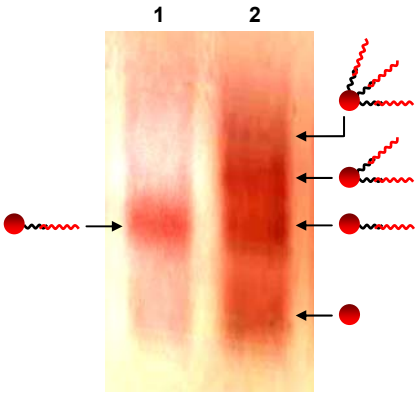


Figure S15 Stability of freshly isolated mono-functionalized gold/DNA conjugates. Mono-functionalized gold/DNA conjugates (using **ab** and **ab-extension**) are characterized using 2% gel electrophoresis within fifteen seconds (lane 1) and fifteen minutes (lane 2) of isolation.

XII. References

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