Supplementary Information

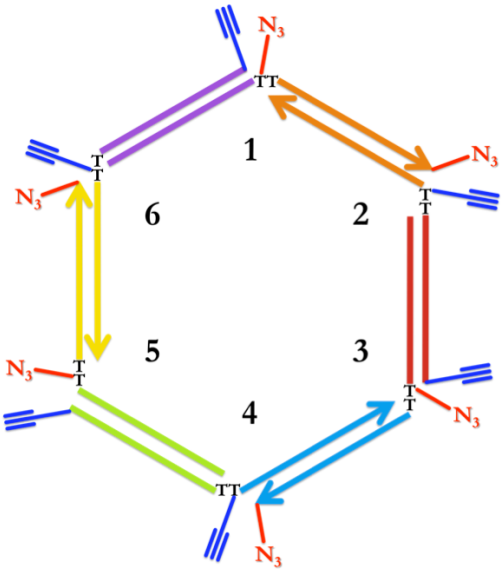
A New Fixation Strategy for Addressable Nano-Network Building Blocks

Erik P. Lundberg[1], Afaf H. El-Sagheer[2,3], Petr Kocalka[2], L. Marcus Wilhelmsson[1], Tom Brown[2] and Bengt Nordén[1]

[1]Department of Chemical and Biological Engineering/Physical Chemistry, Chalmers University of Technology, SE-41296 Gothenburg, Sweden,

[2]School of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, U.K.,

[3]Chemistry Branch, Dept of Science and Mathematics, Faculty of Petroleum and Mining Engineering, Suez Canal University, Suez, Egypt.



1.	5' G ATT AGC GTC $T^{Z}T$ CGA TGG TAT C^{Z} 3'
2.	5' C^{K} TGT AGA GCC TT^{K} GAT ACC ATC G 3'
3.	5' G GCT CTA CAG $T^{Z}T$ GAG GAG GAT G^{Z} 3'
4.	5' G^{K} TAT GTA TGG TT^{K} CAT CCT CCT C 3'
5.	5' C CAT ACA TAC $T^{Z}T$ CCA CAG CAT C^{Z} 3'
6.	5' G^{K} ACG CTA ATC TT^{K} GAT GCT GTG G 3'
1F.	5' G ATT AGC GTC $T^{Z}T$ CGA T^{F}GG TAT C^{Z} 3'
2D.	5' C^{K} TGT AGA GCC TT^{K} GAT ACC AT^{D}C G 3'
1F*.	5' G ATT AGC GTC $T^{Z}T$ CGA T^{F}GG TAT C 3'
2D*.	5' C^{K} TGT AGA GCC TT GAT ACC AT^{D}C G 3'

Figure S1. Base sequences of the oligonucleotides in the system. The color-coding indicates complementary sequences used to build up the hexagonal DNA nanostructure. Superscript Z and K represent azide and alkyne modifications, respectively. Superscript F and D represent FAM and dabcyl modifications, respectively. 5'-alkyne reacts with internal azide and internal alkyne reacts with 3'-azide. See Figure S5 below for precise chemical structures.

DNA sequences

The sequence of the modified 22mer oligonucleotides used in this paper can be seen in Figure S1. Two 10mer sequences on each oligonucleotide are designed to be complementary to only one other sequence in the system, indicated by the color-coding in the figure above. The sequences are designed to ring-close, forming a hexagonal nanostructure with each side being ten bases long. Two unpaired thymines bridge every 10mer sequence creating the necessary hinges in the system. The superscript Z (sequence 1, 3, 5) and K (sequence 2, 4, 6) represent the azide and alkyne modifications respectively. The oligonucleotide 1F* are similar to 1F but lack 3'-azide; 2D* are similar to 2D but lack internal alkyne. These oligonucleotides are used to form the hexagonal construct with only five fixation sites, subject to ring opening. Superscript F and D represent FAM and dabcyl modifications, respectively. The chromophores act as a FRET-pair, FAM being the donor and dabcyl the acceptor. In the experiments, all oligonucleotides are set to 2 μM using the absorbance at 260 nm. The extinction coefficients at 260 nm were calculated by the nearest-neighbor approximation (NNA).

Hybridization reaction

The hybridization reaction was performed by mixing equimolar amounts of all six 22mers in 200 mM NaCl, heating to 85°C for 5 min and slowly cooling to 5.5°C over 6 hours with a linear gradient. Different samples containing sub-structures from monomer to pentamer were also prepared (see Figure S2) to show the step-wise build-up of the hexagon. The build-up was done starting with sequence 1 as monomer (lane 1) followed by sequence 1 and 2 as dimer (lane 2), sequence 1, 2 and 3 as trimer (lane 3), and so on ending with all sequences in one sample creating the hexagon (lane 6). Samples were analyzed using native 10% PAGE (Ready Gels, Bio-Rad) on a Mini-protean 3 Cell system (Bio-Rad). Ficoll 400 (Sigma-Aldrich) was added to each sample as a loading agent, giving a concentration of 5% (w/w). The PAGE was run in 1xTBE at 70 V, giving a field strength of approximately 8 V/cm, for 2 h 45 min. Circulation of the buffer through a heat exchange system was performed to keep the temperature constant at 4°C. The gel was post-stained with SybrGold (Invitrogen) for 5 min and visualized using a Typhoon 9410 (GE Healthcare) with excitation 488 nm and 520 nm band pass filter. Figure S2 show that the hexagon is the major product when all sequences are mixed (lane 6). The faint band above the strong hexagon band in lane 6 most likely corresponds to the ring-closed 12mer (dodecahedron). The two fastest moving bands in lane 4 to 6 both correspond to monomers. The difference in gel-mobility is sequence-dependent

which can be seen in Figure S3 where each 22mer sequence is run separately. In Figure S3, lane 1 contains a hexagon sample followed by the three azide modified monomers: sequence 1 (lane 4), sequence 3 (lane 2) and sequence 5 (lane 3). The three alkyne modified monomers: sequence 2, 4 and 6 are in lane 5, 6 and 7, respectively.

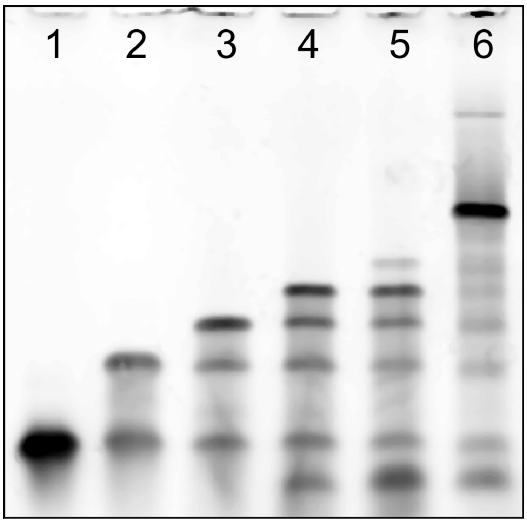


Figure S2. The hybridization reaction analyzed with native 10% PAGE. (Lane 1) Monomer (sequence 1), (lane 2) dimer (sequences 1 & 2), (lane 3) trimer (sequences 1, 2 & 3), (lane 4) tetramer (sequences 1, 2, 3 & 4), (lane 5) pentamer (sequences 1, 2, 3, 4 & 5) and (lane 6) hexagon (all sequences).

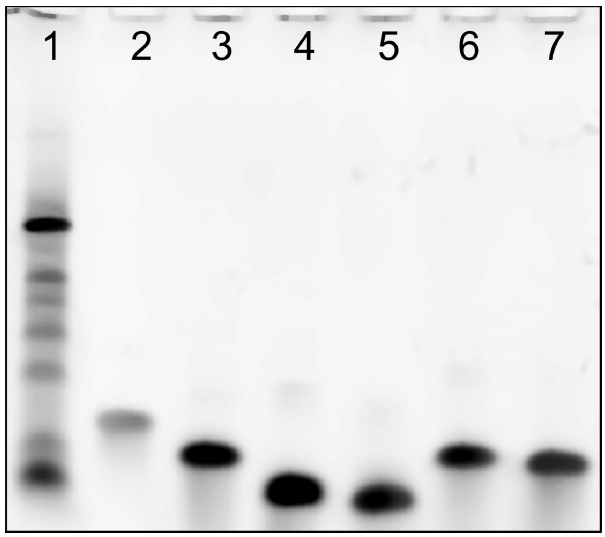


Figure S3. 22mer sequences analyzed separately with native 10% PAGE. (Lane 1) Hexagon sample, (lane 2) sequence 3, (lane 3) sequence 5, (lane 4), sequence 1, (lane 5) sequence 2, (lane 6) sequence 4 and (lane 7) sequence 6.

Looking more closely at the result of the hybridization reaction in Figure S2, the fraction of the different sub-structures where analyzed when all sequences were mixed (lane 6, Fig. S2). To get better statistics of the yield of the hexagon, several identical samples (n=8) were prepared and analyzed with PAGE using the same conditions as described above. The quantification was done using the emission intensity from individual bands analyzed in ImageQuant TL (GE Healthcare).

Structure	Yield
Dodecahedron (12mer)	2.7% (±0.6%)
Hexagon (6mer)	26.9% (±4.0%)
6mer	9.3% (±1.1%)
5mer	12.4% (±1.1%)
4mer	10.0% (±0.8%)
3mer	10.1% (±0.8%)
2mer	7.6% (±1.3%)
Monomer2	7.7% (±1.7%)
Monomer1	13.3% (±2.2%)

The analysis of the hybridization reaction was preformed at 4°C, which is well below the melting temperature of the construct (T_m = 31 °C, ref 9 in main text). Previous studies on the effect of ionic strength on T_m indicated that it is possible to raise the melting temperature of the construct by 8°C using a Na^+ concentration of 500 mM. At higher salt concentration, very little increase in T_m is observed. A major risk in raising the salt concentration higher is that mismatched duplexes will be stabilised and incorrect constructs may form. Actually, the resistance of the linear DNA constructs to bending is a more likely source of the moderate yield of the cyclic nanoconstruct, as implied by the fact that the major impurity is indeed the linear construct. The monomer units left over after the hybridization reaction points towards difficulties in estimating exact 1:1 stoichiometry for the participating DNA strands when preparing the hybridized nanoconstruct.

Click reaction

Typically CuSO4•5H2O (0.45µl 10mM) (Sigma-Aldrich), tris-hydroxypropyl triazole ligand[1] (0.7µl 45mM) and Sodium (L-)Ascorbate (0.9µl 50mM) (Sigma-Aldrich) were mixed before added to a degassed (Argon 5 min) solution of hybridized oligonucleotides (90µl 2µM), see above for details. The reaction mixture was then kept under Argon in room temperature for 24 hours before reagents were removed using a disposable NAP-5 column (GE Healthcare). The eluted volume was freeze-dried to remove excess water and redissolved in MilliQ (MilliPore) water (typically 20 µl) to appropriate concentration for analysis with denaturing PAGE. Prior to loading the samples to the gel, formamide was added to equal volume of sample. The samples were then heated to 90°C for 5 min, then immediately set on ice. This was done to ensure complete denaturation of the DNA samples. The protocol for PAGE was the same as described above for the hybridization reaction, apart from the use of denaturing 10% PAGE (Ready Gels, Bio-Rad).

Extraction procedure

After staining gels with SybrGold (Invitrogen), they were visualized on a UV-table. Specific bands containing desired DNA constructs were then cut out from the gel and placed in a 1.5 mL tube (Eppendorf). After addition of 1 mL MilliQ the sample was vortex-mixed and kept at 37°C, typically for 24 h. Quick vortex-mixing was followed by centrifugation, after which the supernatant was removed. The supernatant was desalted using a disposable NAP-10 column (GE Healthcare) and the eluted volume was freeze-dried and resolved in MilliQ (MilliPore) water (typically 20 µl) to appropriate concentration.

Emission spectroscopy

Two hexagonal samples were prepared as described above: hybridized, click fixated, extracted from gel and re-dissolved in 200 mM NaCl; one construct with all six fixation sites and the other with only five fixation sites, respectively. Both hexagonal samples contained the FRET-pair FAM and dabcyl. Prior to spectroscopic experiments, the samples were rehybridized to form the ring-closed construct. Emission spectra were recorded on a SPEX Fluorolog τ2 spectrofluorimeter. FAM was excited at 490 nm and the emission recorded between 495 nm and 750 nm. The denaturing agent formamide was added to both samples (20 µl in 60 µl original sample volume) and emission spectra were recorded again. The peak intensities (523 nm) were compared after the dilution effect had been taken into account.

Fluorescence melting curves were recorded on a Cary Eclipse (Varian) equipped with a multicell temperature block. The temperature range was 6°C to 65°C with a rate of 2°C/min. Samples were excited at 490 nm and the emission measured at 515 nm. The emission was measured at a temperature interval of 1°C. The melting curves of the hexagonal construct with five fixation sites and the totally fixated hexagon are presented in Figure S4. The increase in emission of the first construct corresponds to the separation of the FRET-pair upon ring-opening of the system (Figure S4, left), with a T_m of 38°C. The almost linear decrease in emission of the totally fixated hexagon corresponds to the change in quantum yield of the fluorophore with higher temperature (Figure S4, right).

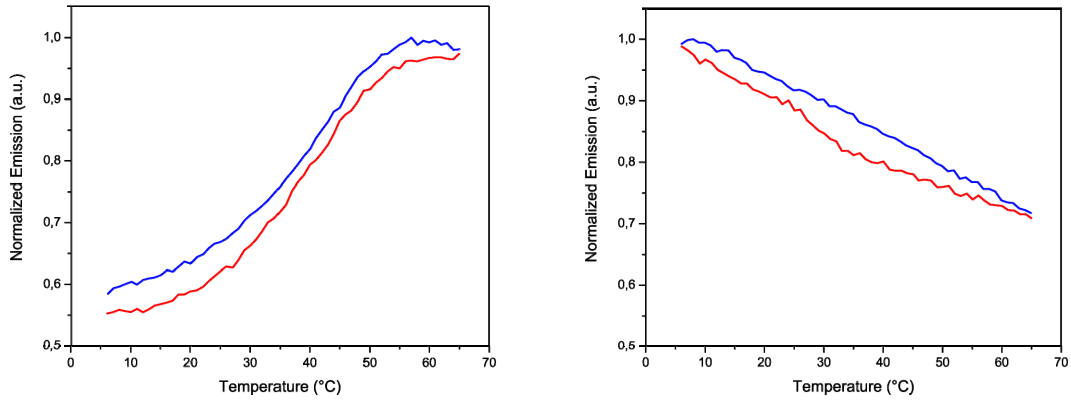


Figure S4. Fluorescence melting curves of the hexagonal construct with five fixation sites (left) and the corresponding construct with six fixation sites (right).

Oligonucleotide synthesis and purification

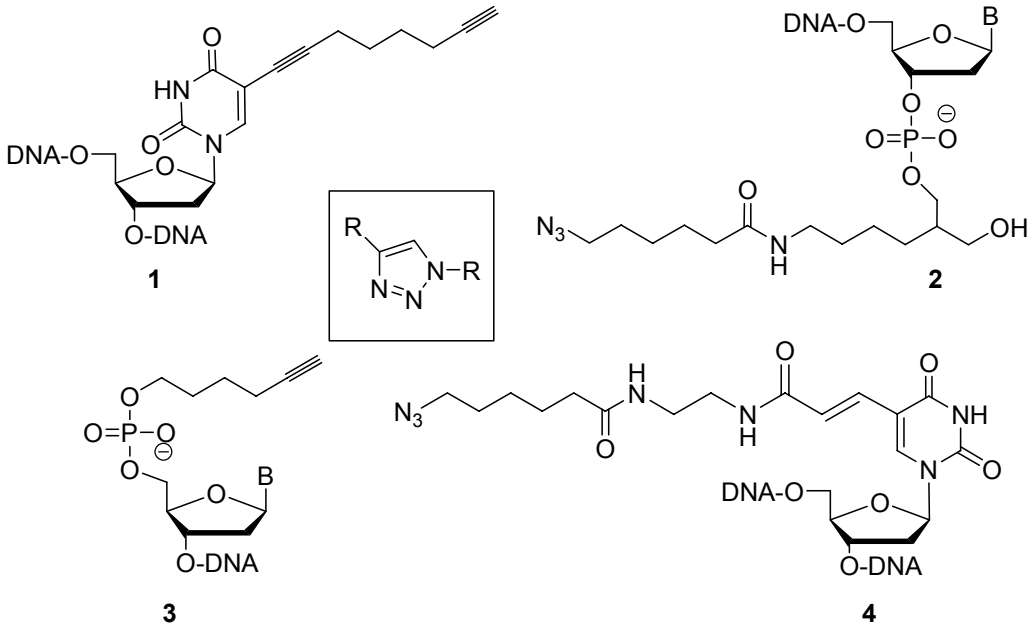


Figure S5. Chemical structures of alkynes and azides used in the formation of the closed hexagon in DNA. Alkyne 1 reacts with azide 2 and alkyne 3 reacts with azide 4. The resultant triazole linkage shown in the box.

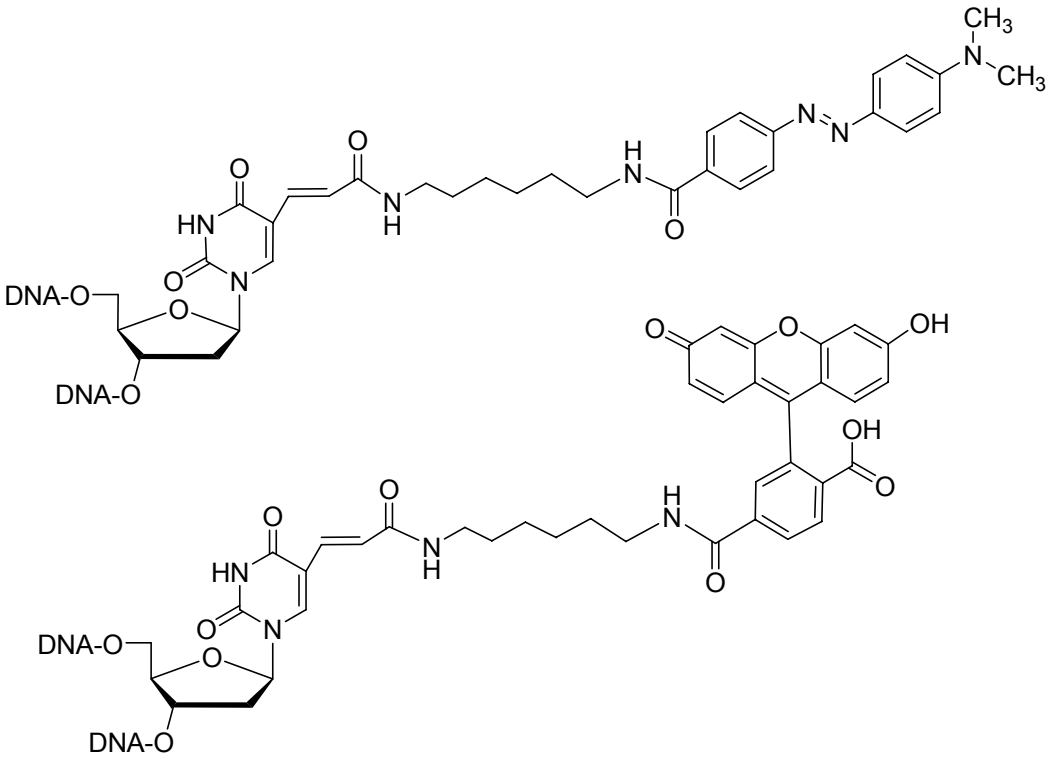


Figure S6. Fluorescein dT (bottom) and dabcyl dT (top) incorporated into DNA.

Oligonucleotide synthesis was carried out 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. 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 this was extended to 10 min for the non-standard phosphoramidite monomers of hexynol,[2] fluorescein dT, amino C2 dT and dabcyl dT. The fluorescein dT, amino C2 dT and dabcyl dT phosphoramidite monomers and amino C7 cpg were purchased from Link Technologies Ltd. Stepwise coupling efficiencies and overall yields were determined by automated trityl cation conductivity monitoring and in all cases were >98.0%. Cleavage of oligonucleotides from the solid support and deprotection were achieved by exposure to concentrated aqueous ammonia for 60 min at room temperature followed by heating in a sealed tube for 5 h at 55 ^{o}C. Amino-modified oligonucleotides were labelled with azidohexanoic acid NHS ester as previously described.[2, 3]

Purification of oligonucleotides was carried out by reversed-phase HPLC on a Gilson system using a Brownlee Aquapore column (C8, 8 mm x 250 mm, 300 Å pore) with a gradient of acetonitrile in ammonium acetate increasing from 0% to 50% buffer B over 30 min with a flow rate of 4 mL/min (buffer A: 0.1 M ammonium acetate, pH 7.0, buffer B: 0.1 M ammonium acetate with 50% acetonitrile pH 7.0). Elution of oligonucleotides was monitored

by ultraviolet absorbtion at 305 nm. After HPLC purification, oligonucleotides were desalted using NAP-10 Sephadex columns (GE Healthcare) according to the manufacturer's instructions.

References

1. T. R. Chan, R. Hilgraf, K. B. Sharpless and V. V. Fokin, *Org. Lett.*, 2004, **6**, 2853-2855.
2. A. H. El-Sagheer, R. Kumar, S. Findlow, J. M. Werner, A. N. Lane and T. Brown, *ChemBioChem*, 2008, **9**, 50-52.
3. P. Kocalka, A. H. El-Sagheer and T. Brown, *ChemBioChem*, 2008, **9**, 1280-1285.