Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Assembly of Dynamic Supramolecular Polymers on a DNA Origami Platform

Jurgen Schill[+], Bas J. H. M. Rosier[+], Berta Gumí Audenis, Eva Magdalena Estirado, Tom F. A. de Greef, and Luc Brunsveld**

anie_202016244_sm_miscellaneous_information.pdf

Contents

Supplementary methods 2
Chemicals and reagents 2
Methods 2
Synthetic procedure 2
Design of the DNA origami rectangle 3
Folding and purification of DNA origami nanostructures 3
Preparation of supramolecular assemblies and incorporation onto DNA origami 4
AFM imaging 4
Gel electrophoresis 4
Preparation of DNA-functionalized gold nanoparticles 4
Supplementary figures 5
Supplementary tables 10
Supplementary references 14

Supplementary methods

Chemicals and reagents

All reagents and solvents were obtained from commercial sources and used without further purification. Analytical thin layer chromatography (TLC) was carried out using Merck pre-coated silica gel using ultraviolet light irradiation at 254 and 365 nm. Manual column chromatography was performed using Merck silica gel (pore size, 60 Å; particle size, 63–200 μm). Monomers **1** and **1a** were synthesized according to literature procedure[1,2]. Sulfo-Cy3-NHS and Sulfo-Cy5-NHS were purchased from Lumiprobe. All oligonucleotides (ODNs) were obtained from Integrated DNA Technologies. Unmodified staple strands were obtained in desalted form and dissolved in DNase/RNase-free water at a stock concentration of 500 μM, while amine- and azide-functionalized ODNs were obtained HPLC-purified and dissolved at 250 μM (see Supplementary Table 1 and Supplementary Table 2). The M13mp18 scaffold was purchased from Eurofins.

Methods

Matrix assisted laser desorption/ionization time-of-flight mass spectra (MALDI-TOF-MS) were measured on a PerSeptive Biosystems Voyager-DE Pro spectrometer with a Biospectrometry workstation using 2-[(2E)-3-(4-t-butylphenyl)-2-methylprop-2-enylidene] malononitrile (DCTB) and α-cyano-4-hydroxycinnamic acid (CHCA) as matrix material and methylene chloride as solvent. Reverse-phase liquid chromatography-mass spectrometry (LC-MS) analysis was performed on an Applied Biosystems Single Quadrupole Electrospray Ionization Mass Spectrometer API-150EX in positive mode employed with a Jupiter SuC4300A, 150×2.00 mm column. H2O and acetonitrile, both enriched with 0.1% formic acid, were used as eluent using a gradient of 5% to 100% acetonitrile in 10 min and a flow rate of 0.2 mL min^{-1}. Fluorescence was measured over time in quartz cuvettes by recording spectra on a Varian Cary Eclipse fluorescence spectrophotometer. The FRET ratio was calculated using

$$\text{FRET ratio} = \frac{I_{\text{DA}}}{I_{\text{DA}}+I_{\text{DD}}} \quad (1)$$

where I_{DD} is the Cy3 signal (ex.: 515 nm, em.: 570 nm) and $I_{\text{DA}}$is the FRET signal (ex.: 515 nm, em.: 670 nm). Visualization by transmission electron microscopy (TEM) was performed on a Technai G2 Sphera (FEI) operating at an acceleration voltage of 80 kV. Samples were prepared by drop-casting a DNA nanostructure solution on a carbon film on a copper grid (400 square mesh) and dried for 1 minute. Samples were negatively stained with 1% aqueous uranyl formate for 30 seconds prior to imaging.

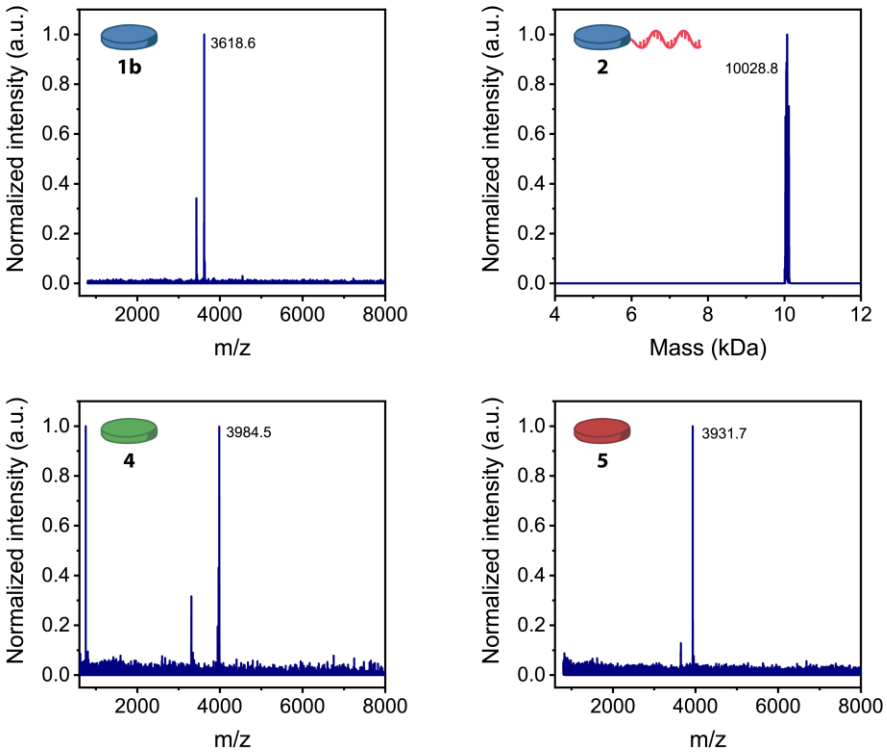
Synthetic procedure

DBCO-disc (**1b**): dibenzocyclooctyne-N-hydroxysuccinimidyl ester (DBCO-NHS, 1.5 mg, 3.8 μmol) was added to a solution of disc **1a** (5.0 mg, 1.5 μmol) and triethylamine (1.5 μL, 6.0 μmol) dissolved in dry methylene chloride (0.2 mL) and the resulting reaction mixture was stirred overnight at room temperature under an inert atmosphere. The crude reaction mixture was purified by size exclusion column chromatography (BioBeads, methylene chloride). Pure fractions were pulled and dried in vacuo to yield DBCO-disc in 89% (4.8 mg, 1.3 μmol). MALDI-ToF (m/z): [M] calc. for $C_{179}H_{256}N_{14}O_{62}$, 3593.7, found $[M+Na]^+$ 3618.6.

DNA-discs (**2**, **3**): oligonucleotides (50 μL, 100 μM; Supplementary Table 7.1) and an excess of DBCO-disc **1b** (50 μL, 250 μM) in DNase/RNase-free water were mixed and shaken at room temperature for 1 hr and subsequently put to react overnight at 4°C. The crude mixture was saturated with 5 μL of a NaCl solution (5 M) and 300 μL ice-cold isopropanol was added. The samples were incubated at -20°C overnight. The resulting suspension was centrifuged at 4°C, 14,000 rpm and the supernatant containing unreacted disc **1b** was discarded, yielding DNA-discs **2** and **3** as a pellet. MS (ESI): **2**, [M] calc. 10031.4, found $[M]^-$ 10028.8.

Dye-discs (**4**, **5**): Sulfo-Cyanine3-N-hydroxysuccinimidyl ester (1.0 mg, 1.4 μmol) was added to a solution of amine-disc **1a** (2.3 mg, 0.7 μmol) and triethylamine (0.7 μL, 2.7 μmol) dissolved in dry methylene chloride (0.3

mL) and the resulting reaction mixtures were stirred overnight at room temperature under an inert atmosphere. The crude reaction mixtures were purified by size-exclusion column chromatography (BioBeads, methylene chloride). Pure fractions were pulled and dried in vacuo to yield Cy3-disc **4** in 86% (2.3 mg, 0.6 µmol) and Cy5-disc **5** in 78% (2.1 mg, 0.5 µmol), respectively. MALDI-ToF (m/z): **4**, [M] calc. for $C_{190}H_{276}N_{15}O_{67}S_2^{2-}$, 3903.8, found $[M+2K]^+$ 3984.5; **5**, [M] calc. for $C_{192}H_{278}N_{15}O_{67}S_2^{2-}$, 3929.8, found $[M]^-$ 3931.7.



Design of the DNA origami rectangle

DNA origami rectangle used in this study was designed using caDNAno v0.2 based on the *tall rectangle* design by Rothemund[3], (Supplementary Figure 2). The 7249-nt single-stranded M13mp18 scaffold strand folds into a single-layer structure of 32 helices using 192 staple strands (Supplementary Table 3). To correct for global twist of the DNA origami rectangle, 3 base pair deletions per helix were introduced[4]. To prevent DNA origami aggregation through blunt-end stacking all 32 edge staples were omitted during folding and not listed here. The modular use of staple pools enables straightforward addition and removal of functionalities in the DNA nanostructure, depending on the experiment. For details on the incorporation of DNA-disc **2**, fluorophores, and AuNPs, see Supplementary Table 2.

Folding and purification of DNA origami nanostructures

Folding reactions were performed at a volume of 50 µL in folding buffer (10 mM Tris, 1 mM EDTA, 10 mM MgOAc, 50 mM NaOAc, pH 8.0), with 25 nM scaffold strand and 250 nM of each staple strand. The reaction mixture was heated to 95°C for 15 min and then slowly cooled to 20°C at a rate of 1°C/min. Excess staple strands were removed using 100 kDa MWCO 0.5 mL Amicon centrifugal filters (Merck Millipore). Briefly, a filter was pre-wetted with 500 µL reaction buffer (10 mM Tris, 1 mM EDTA, 10 mM MgOAc, 100 mM NaOAc, pH 8.0). The folding mixture was diluted to 500 µL with reaction buffer, added to the filter and centrifuged at 20°C for 5 min at 5,000 g. This step was repeated for a total of three washing steps. The concentrate was recovered by inverting the filter and spinning for 2 min at 1,000 g. Samples were stored in DNA LoBind tubes at 4°C for next day use or at -30°C. The DNA origami concentration was determined by measuring the absorption at 260 nm, assuming an extinction coefficient of 1.24×10^8 M^{-1} cm^{-1} (ref. [5,6]).

Preparation of supramolecular polymers and incorporation onto DNA origami

Monomer stock solutions were prepared by dissolving in DNase/RNase-free water to a final concentration of 200 µM, annealed for 5 min at 65°C, and stored at -30°C. Dilutions were made fresh prior to every measurement. Multicomponent supramolecular polymers were prepared by mixing stock solutions in the desired ratio at a total monomer concentration of 10 µM. The solutions were annealed for 5 minutes at 65°C and subsequently incubated for at least 1 hr at room temperature to ensure full mixing of components. For incorporation onto DNA nanostructures, a 10 µL reaction mixture was prepared in reaction buffer (10 mM Tris, 10 mM MgOAc, 100 mM NaOAc, pH 8.0) containing 4 nM purified DNA origami and a 2.5 µM polymeric mixture containing 5% DNA-disc **2**, corresponding to a two-fold molar excess per handle, unless stated otherwise. The reaction mixture was incubated for 1 hr at 18°C and subsequently used without further purification.

AFM imaging

Topographic AFM images were acquired in AC mode at room temperature under liquid conditions using an MFP-3D AFM (Asylum Research) and V-shaped Si_3N_4 cantilevers with sharpened pyramidal tip and a nominal spring constant of 0.04 N m^{-1} (OTR4, Bruker AFM Probes). Circular mica substrates (Ted Pella) were glued to Teflon (VWR) using epoxy-based mounting glue before use. DNA nanostructure solutions were first diluted to 2 nM in imaging buffer (10 mM Tris, 1 mM EDTA, 10 mM $MgCl_2$, pH 8.0) and then 10 µL was incubated for 30 s on a freshly-cleaved mica surface. Subsequently, 50 µL of imaging buffer was used to rinse the sample twice and finally 100 µL of imaging buffer was added to perform the AFM imaging. Topographic images of 1.0×1.0 µm² (512×512 px) were acquired in various regions of the mica substrate, using drive amplitudes within the range of 0.6-1.0 V, optimizing the scanning and feedback parameters for each image. Image processing was performed with Gwyddion (v2.51) software.

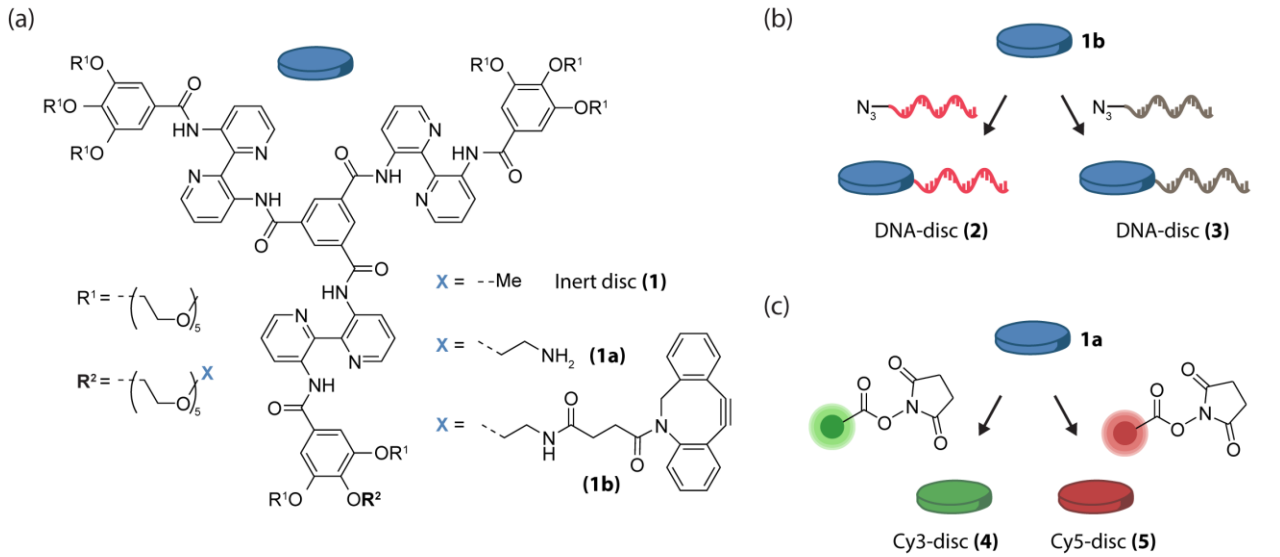
Gel electrophoresis

Agarose gel electrophoresis was used for analysis of functionalized DNA origami nanostructures. In short, 1.5% and 3.5% agarose gels were cast in gel buffer (1×TAE, 10 mM $MgCl_2$, pH 8.0), and supplemented with SYBR Safe if necessary. Gels were run in gel buffer for 90 min at 65 V in an ice bath. DNA origami samples were diluted just before loading to a final concentration of 4 nM and Ficoll-400 (final concentration 1.5% (w/v)) was added. Gel extraction was performed by excising the correct band from the gel upon completion. The band was cut into small pieces and loaded onto a Freeze 'N' Squeeze column (Bio-Rad). After centrifugation for 2 min at 2,000 g, the supernatant was collected and stored on ice for further processing. Imaging was performed on an ImageQuant 400 Digital Imager (GE Healthcare) and analyzed with ImageJ.

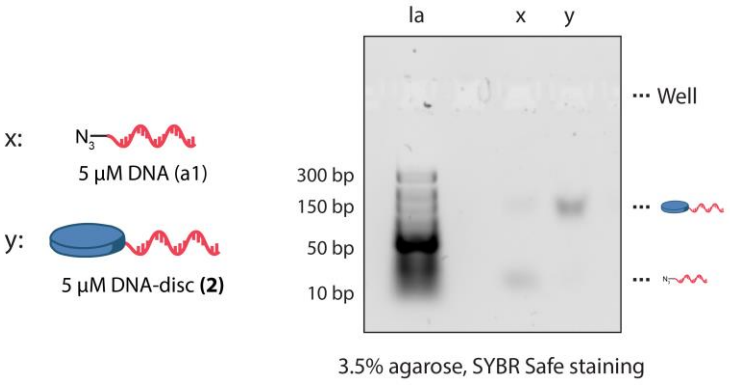
Preparation of DNA-functionalized gold nanoparticles

Functionalization of 10-nm diameter gold nanoparticles (AuNPs) with oligonucleotides was performed using the Cytodiagnostics NHS-Activated Gold Nanoparticle Conjugation kit (Sigma-Aldrich), according to the manufacturer's instructions. In short, the ODN a4 (Supplementary Table 1) was diluted with the kit's resuspension buffer to 20 µM and reacted with NHS-activated AuNPs for 2 hr at room temperature under continuous shaking at 600 rpm. Excess ODN was removed using 100 kDa MWCO 0.5 mL Amicon centrifugal filters, as described for DNA origami purification. AuNP concentration was determined by measuring the absorption at 520 nm, assuming an extinction coefficient of 1.01×10^8 M^{-1} cm^{-1}. The purified DNA-functionalized AuNPs were stored at 4°C until use.

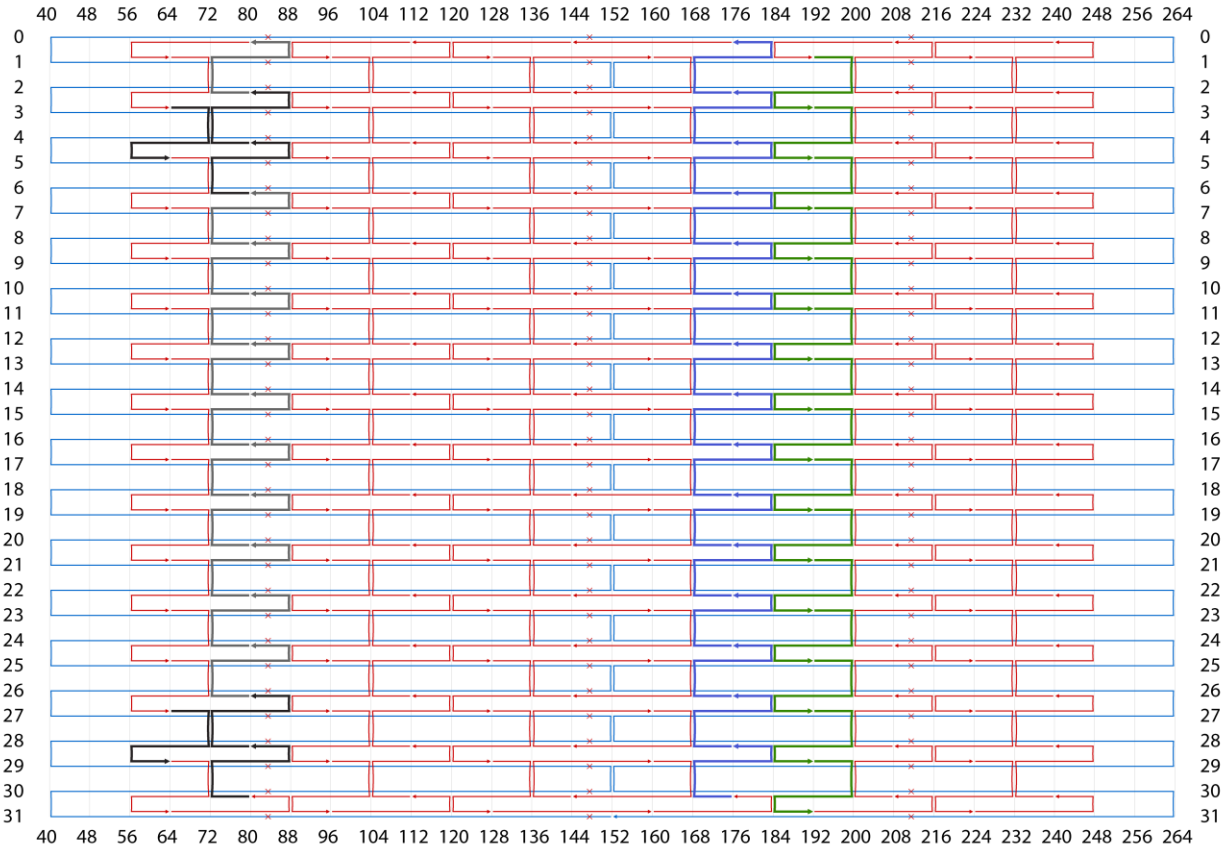
Supplementary figures



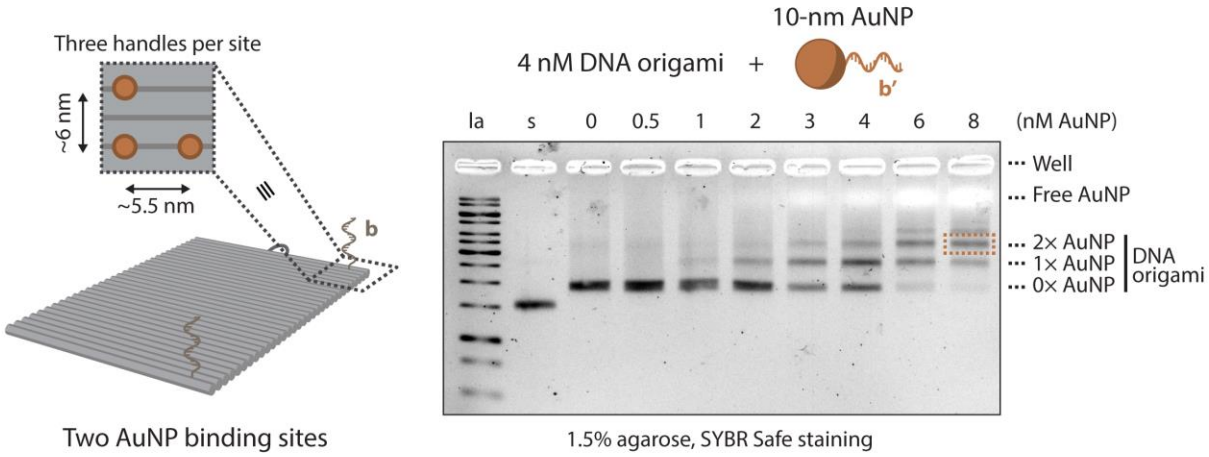
Supplementary Figure 1 | Overview of the bipyridine-based monomers. (a) Molecular structure of inert disc **1**, mono-functionalized amine-disc **1a** and DBCO-functionalized disc **1b**. (b,c) Reaction schemes for the synthesis of monomers **2** – **5**. Conjugation of DBCO-disc **1b** to azide-functionalized ODNs was performed using copper-free click chemistry, affording DNA-discs **2** and **3** (b) (for DNA sequences, see Supplementary Table 1). Conjugation of amine-disc **1a** to fluorophores was performed using the N-hydroxysuccinimide esters of the sulfonated variants of cyanine dyes Cy3 and Cy5, affording Cy3-disc **4** and Cy5-disc **5**, respectively (c).



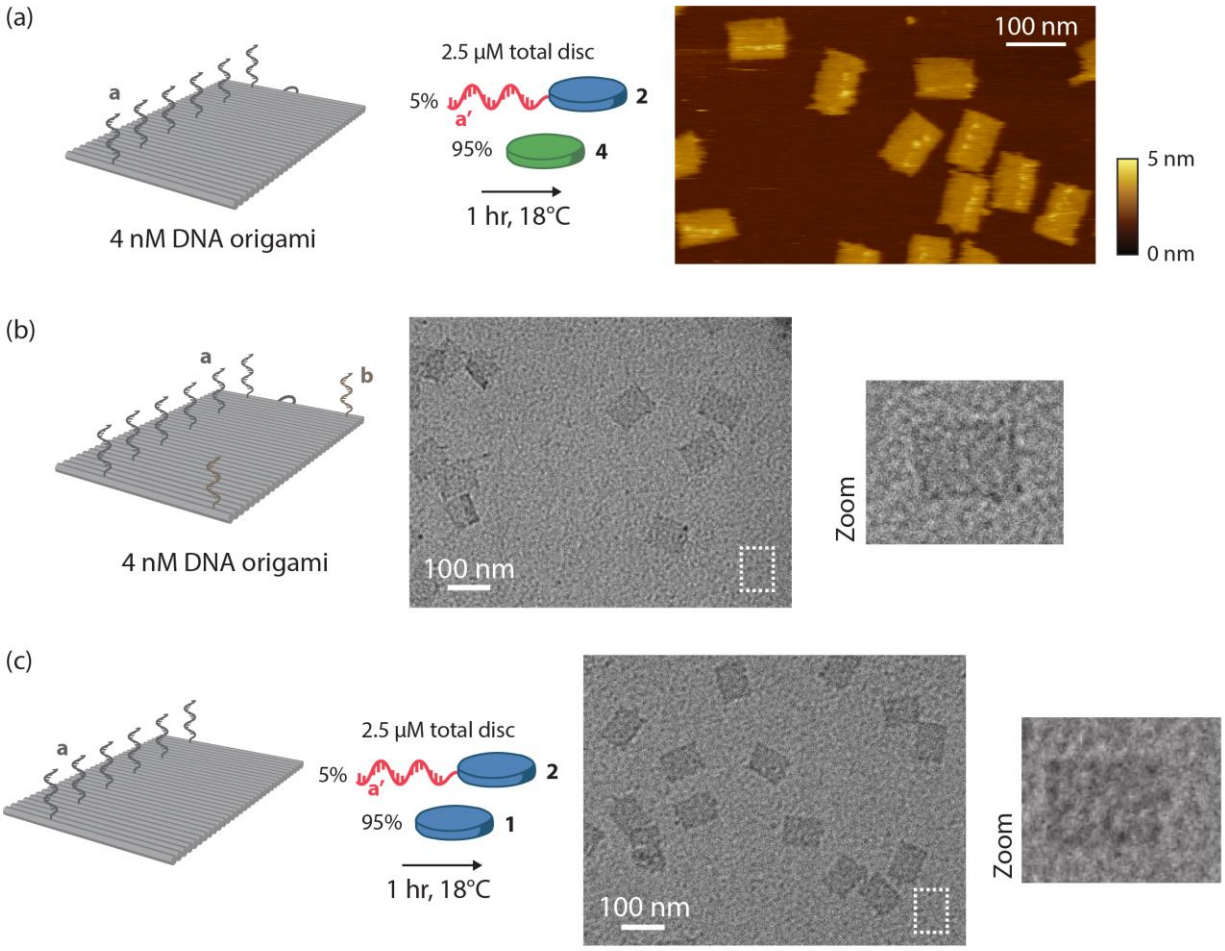
Supplementary Figure 2 | Agarose gel analysis of DNA-disc. Oligonucleotide a1 was conjugated to an excess DBCO-disc **1b**, which afforded DNA-disc **2** after purification by precipitation. Image of a 3.5% agarose gel (stained for DNA with SYBR Safe) of 5 µM oligonucleotide a1 and 5 µM DNA-disc **2**. Gel analysis indicated that the oligonucleotide is conjugated to the disc, as visualized by the decrease in gel mobility, as well as the absence of unconjugated oligonucleotides. Labels: la, reference DNA ladder (GeneRuler Ultra Low Range DNA ladder, Thermo Scientific); x, oligonucleotide a1; y, DNA-disc.



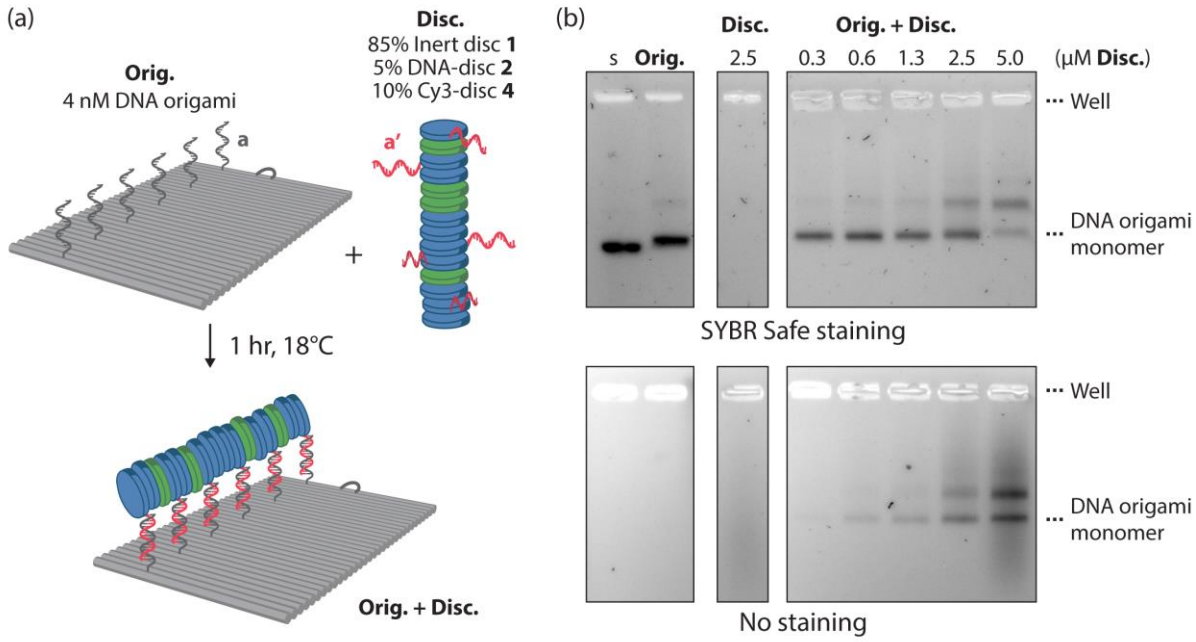
Supplementary Figure 3 | Schematic overview of the DNA origami rectangle design. The scaffold strand is shown in light blue and unmodified staple strands in red. Staples used for DNA-disc **2** incorporation are shown in green (p1-p16), for AuNP incorporation in black (g1-g6), and for fluorophore incorporation at 6 nm and 39 nm in blue (f1-f16) and gray (c1-c16), respectively (Supplementary Table 2). Base-pair deletions to correct for global twist of the structure are indicated by crosses and 3' ends of DNA strands are indicated by arrows. Numbers on left and right indicate the reference helix number, while numbers on top and bottom indicate reference nucleotide position.



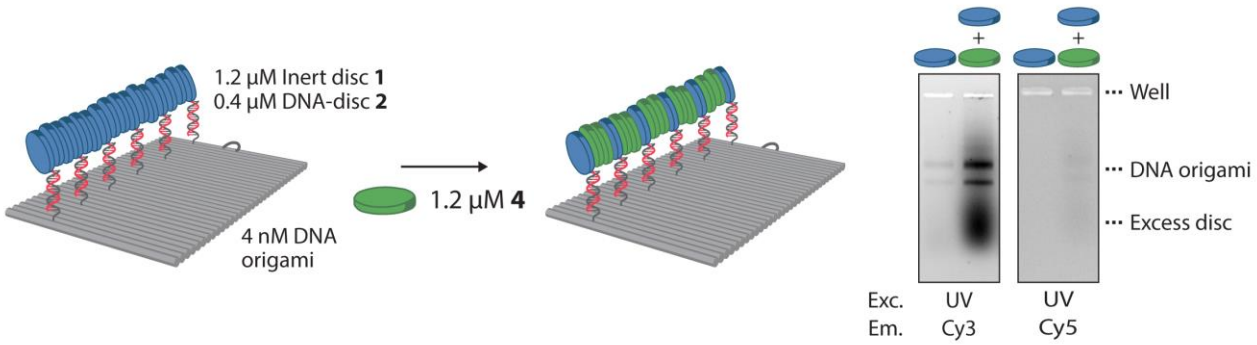
Supplementary Figure 4 | Agarose gel analysis of 10-nm AuNP incorporation onto DNA nanostructures. The DNA origami nanostructure was designed with binding sites for incorporation of DNA-functionalized AuNPs at two corners of the structure. Each binding site contains three handles to increase the incorporation efficiency, but for clarity only a single handle strand per binding site is displayed. Image of a 1.5% agarose gel (stained for DNA with SYBR Safe) of 4 nM DNA origami incubated with varying concentrations of DNA-functionalized AuNP for 1 hr at 18°C. Gel analysis indicated mainly single AuNP incorporation (0×AuNP) up to 4 nM, while at 8 nM predominantly two AuNP per nanostructure (2×AuNP) were observed. For TEM analysis, purification of 2×AuNP-functionalized DNA nanostructures was performed by gel extraction from the region indicated by the dashed rectangle. Gel electrophoresis and extraction was performed as described. Labels: la, reference DNA ladder (GeneRuler 1 kb DNA, Thermo Scientific); s, ssDNA scaffold.



Supplementary Figure 5 | AFM and TEM analysis of DNA origami nanostructures. The rectangular DNA origami structure was designed to incorporate 15 copies of DNA-disc **2** through DNA hybridization of ODN a' and the single-stranded handles a protruding from the surface, following the long side of the nanostructures. (a) The DNA origami structure was incubated with a polymeric mixture of Cy3-disc **4** with 5% DNA-disc **2**. Topographic AFM images revealed successful incorporation of the polymeric mixture onto DNA nanostructures as indicated by spots of high intensity at the programmed positions. The patchy morphology remains clearly visible while Cy3-disc **4** is expected to occupy the room in between the adjacent handle sites. The absence of a continuous high intensity line is attributed to the physical nature of the AFM technique that may disrupt the weak non-covalent interactions. (b) TEM micrograph of DNA origami structures, additionally equipped with protruding single stranded handles for the site-selective recruitment of AuNPs, prior to functionalization with multicomponent polymers and AuNPs. (c) TEM micrograph of DNA origami structures incubated with a polymeric mixture of inert disc **1** with 5% DNA-disc **2**. While the DNA nanostructures can be identified, the polymeric species are not observed due to their inherently low TEM contrast.[7] TEM grids were negatively stained with 1% aqueous uranyl formate for 30 seconds prior to imaging. The expected size of a 75×100-nm^2 nanostructure is indicated as a reference (dashed white rectangle). Scale bars, 100 nm.



Supplementary Figure 6 | DNA-guided assembly of a multicomponent supramolecular polymer onto DNA nanostructures. (a) Schematic overview of the assembly reaction. DNA origami with 15 single-stranded handles (orig., 4 nM) was incubated with varying concentrations of a pre-annealed polymeric mixture (disc.) consisting of 85% inert disc **1**, 5% DNA-disc **2**, and 10% Cy3-disc **4** for 1 hr at 18°C. (b) Reaction mixtures were subjected to gel electrophoresis in parallel on 1.5% agarose gels with (top images) and without (bottom) SYBR Safe staining, to independently visualize DNA and the Cy3-labeled monomer, respectively. Fluorescence from SYBR Safe and Cy3 was separated using emission filters in the gel imager. Label: s, single-stranded scaffold.



Supplementary Figure 7 | Agarose gel analysis of intermixing Cy3-labeled monomers into DNA origami-templated supramolecular polymers. Assembly was performed by incubating 4 nM DNA origami with 15 handles with 1.6 μM pre-annealed polymeric mixture containing 25% DNA-disc **2** and 75% inert disc **1**. After incubation for 1 hr at 18°C, 1.2 μM Cy3-disc **4** was added to the reaction mixture and incubated for 1 hr at 18°C. Agarose gel electrophoresis was performed on 1.5% agarose gels without staining. Images were obtained by illumination with a UV light source (exc.) and emission through either a Cy3 (left) or Cy5 (right) filter (em.).

Supplementary tables

Supplementary Table 1 | Anti-handles used for conjugation. Sequences were based on literature with minimal secondary structure and melting temperatures >40°C[8]. All sequences were tested with NUPACK to detect any possible undesired interactions[9]. IDT modifications /5AzideN/, /5Cy3/, and /5AmMC6/ were used, respectively. Underlined thymine nucleotides were added as a spacer.

ID	Use	Sequence (5' to 3')	MW (g mol^{-1})
a1	conjugation to obtain disc **2**	N_3-CAGTCAGTCAGTCAGTCAGT	6435.3
a2	fluorophore for FRET studies	Cy3-CCCTAGAGTGAGTCGTATGA	6636.6
a3	conjugation to obtain disc **3**	N_3-TTTTCATACGACTCACTC	5702.9
a4	conjugation to AuNP	H_2N-TTTTTGAGTGAGTCGTATGA	6372.2

Supplementary Table 2 | Handle-extended staple strands. To incorporate functional molecules onto DNA origami nanostructures, unmodified staple pools were replaced by staples extended at the 5' end with a handle sequence. Handle-extended staple strands p1-p16 are complementary to anti-handle a1 and were used for DNA-disc **2** incorporation in all experiments. Strands g1-g6 are complementary to a4 and were used for AuNP incorporation in Figure 2. Strands f1-f16 and c1-c16 are complementary to a2 and were used for Cy3 incorporation in the experiments for Figure 4. Underlined thymine nucleotides were added as a spacer. Unmodified staple IDs refer to staples used in Supplementary Table 3. Colors correspond to the ones used in Supplementary Figure 2.

ID	Unmodified staple IDs	Handle sequence (5' to 3')	Color
p1-p16	11,23,35,47,59,71,83,95,107,119,131,143,155,167,179	staple-TACTGACTGACTGACTGACTG	green
f1-f16	16,28,40,52,64,76,88,100,112,124,136,148,160,172,184	staple-TTTCATACGACTCACTC	blue
c1-c16	13,25,37,49,61,73,85,97,109,121,133,145,157,169,181	staple-TTTCATACGACTCACTC	gray
g1-g6	19,25,37,151,169,181	staple-TTCATACGACTCACTCTAGGG	black

Supplementary Table 3 | Sequences of unmodified staple strands. Locations of the 5' and 3' end are indicated using the reference helix number used in Supplementary Figure 2, with the reference nucleotide position denoted in brackets. Staple strands indicated in green, blue, gray, and black can be extended with handle strands to allow for incorporation of other components (see Supplementary Table 2).

Staple ID	Location of 5' end	Location of 3' end	Sequence (5' to 3')
1	0[79]	1[63]	ACTGAGTTTCGTCACCAGTACAAATCATAGTT
2	0[111]	1[95]	GCAAGCCCAATAGGAACCCATGTACGTCTTTC
3	0[143]	1[127]	CCTCAGAGCCACCACCCTCATTTTGTATGGGA
4	0[175]	0[144]	CCCTCAGAACCGCCACCCTCAGAACCGCCAC
5	0[207]	1[191]	TATCACCGTACTCAGGAGGTTTAGATTATTCT
6	0[239]	1[223]	GGGTTGATATAAGTATAGCCCGGATGAGACTC
7	1[64]	3[63]	AGCGTAACAAAAGGCTCCAAAAGGTTCGAGGT
8	1[96]	3[95]	CAGACGTTAATAATTTTTTCACGTCGATAGTT
9	1[128]	3[127]	TTTTGCTAAATAGAAAGGAACAACGCCCACGC
10	1[160]	2[144]	TGCCCCCTAACAGTGCCCGTATAATTTCAGC
11	**1[192]**	**3[191]**	**GAAACATGTAATAAGTTTTAACGGAGGTTGAG**
12	1[224]	3[223]	CTCAAGAGCATGGCTTTTGATGATTATTCACA
13	**2[79]**	**0[80]**	**CTCCAAAAGATCTAAAGTTTTGTCCGTAAC**
14	2[111]	0[112]	TTGCGAATAGTAAATGAATTTTCTCAGGGATA
15	2[143]	1[159]	GGAGTGAGAACAACTTTCAACAGACAGTTAA
16	**2[175]**	**0[176]**	**CCTTGAGTGCCTATTTCGGAACCTTACCGCCA**

17	2[207]	0[208]	TGTACTGGAAAGTATTAAGAGGCATAGGTG
18	2[239]	0[240]	GCGTCATAAAGGATTAGGATTAGCCGTCGAGA
19	**3[64]**	**5[63]**	**GAATTTCTCAACGGCTACAGAGGCTTCCATTA**
20	3[96]	5[95]	GCGCCGACGCAGCGAAAGACAGCACTACGAAG
21	3[128]	5[127]	ATAACCGATAAAGGCCGCTTTTGCAAAAGAAT
22	3[160]	4[144]	AGAGCCGCAGAGCCGCCACCAGAAAGGCTTG
23	**3[192]**	**5[191]**	**GCAGGTCATCAGAACCGCCACCCTTTTGCCTT**
24	3[224]	5[223]	AACAAATAACCGGAACCGCCTCCCTCATCGGC
25	**4[79]**	**2[80]**	**GAGGGTAGTAAACAGCTTGATACTGAAAAT**
26	4[111]	2[112]	CACCCTCAAATGACAACAACCATCTAAAGGAA
27	4[143]	3[159]	CAGGGAGTTATATTCGGTCGCTGCCACCACC
28	**4[175]**	**2[176]**	**CCACCCTCCGCCAGCATTGACAGGGGTCAGTG**
29	4[207]	2[208]	CGCCACCCGACGATTGGCCTTGAACAGGAG
30	4[239]	2[240]	GAGCCACCAATCCTCATTAAAGCCTCCAGTAA
31	5[64]	7[63]	AACGGGTACGACCTGCTCCATGTTATAAGGGA
32	5[96]	7[95]	GCACCAACATCATCGCCTGATAAAAGGACAGA
33	5[128]	7[127]	ACACTAAACAAGCGCGAAACAAAGTAGGCTGG
34	5[160]	6[144]	GTAATCAGAATGAAACCATCGATACCCCAGC
35	**5[192]**	**7[191]**	**TAGCGTCACCATTACCATTAGCAAAGCGCCAA**
36	5[224]	7[223]	ATTTTCGGGGAATTAGAGCCAGCAGATTGAGG
37	**6[79]**	**4[80]**	**GAAATCCGAAATACGTAATGCCATCGGAAC**
38	6[111]	4[112]	AGATTTGTCTAAAACGAAAGAGGCGGGATCGT
39	6[143]	5[159]	GATTATACACACTCATCTTTGACGCAGCACC
40	**6[175]**	**4[176]**	**ACGTCACCTAGCGACAGAATCAAGCAGAGCCA**
41	6[207]	4[208]	CAGTAGCAGACTGTAGCGCGTTTTCAGAGC
42	6[239]	4[240]	GCCATTTGTCATAGCCCCCTTATTCGGAACCA
43	7[64]	9[63]	ACCGAACTAAACACCAGAACGAGTCTTTAATC
44	7[96]	9[95]	TGAACGGTTCATTCAGTGAATAAGTTAAGAAC
45	7[128]	9[127]	CTGACCTTATTCATTACCCAAATCTTGGGAAG
46	7[160]	8[144]	AATAGAAAGAATAAGTTTATTTTGTGACAAG
47	**7[192]**	**9[191]**	**AGACAAAAGCAACATATAAAAGAAAAGTAAGC**
48	7[224]	9[223]	GAGGGAAGAGCAAACGTAGAAAATAAGGAAAC
49	**8[79]**	**6[80]**	**CTGACGAGGACCAACTTTGAAAGTTGTGTC**
50	8[111]	6[112]	AAAGCTGCGTACAGACCAGGCGCATACAACGG
51	8[143]	7[159]	AACCGGATCATCAAGAGTAATCTTCACAATC
52	**8[175]**	**6[176]**	**ACACCACGATTCATATGGTTTACCGGCCGGAA**
53	8[207]	6[208]	TAAAGGTGGGGCGACATTCAACCAAATCAC
54	8[239]	6[240]	AGTATGTTGTAAATATTGACGGAACGACTTGA
55	9[64]	11[63]	ATTGTGAAACATAACGCCAAAAGGTAACCCTC
56	9[96]	11[95]	TGGCTCATTTTAGGAATACCACATCAAAATAG
57	9[128]	11[127]	AAAAATCTTTATTACAGGTAGAAATTGCCAGA
58	9[160]	10[144]	TCTTACCGGAAACAATGAAATAGCACTAACG
59	**9[192]**	**11[191]**	**AGATAGCCAAGAATTGAGTTAAGCGTTTAACG**
60	9[224]	11[223]	CGAGGAAAATTGAGCGCTAATATCTACAGAGA
61	**10[79]**	**8[80]**	**ATGCAGATTTACCTTATGCGATTGCTTGCC**
62	10[111]	8[112]	AGTTGAGATATACCAGTCAGGACGAACGTAAC
63	10[143]	9[159]	GAACAACAACGTTAATAAAACGAAATAGCTA
64	**10[175]**	**8[176]**	**AAGAGCAAAAGCCCTTTTTAAGAAACGCAAAG**
65	10[207]	8[208]	TAACCCACGAACAAAGTTACCAGACATACA
66	10[239]	8[240]	AGAGGGTACGCAATAATAACGGAATATTACGC
67	11[64]	13[63]	GTTTACCAACGAGAATGACCATAAAGTCAGAA
68	11[96]	13[95]	CGAGAGGCTCCCCCTCAAATGCTTATTAAGAG
69	11[128]	13[127]	GGGGGTAAATACTGCGGAATCGTCGCGTTTTA
70	11[160]	12[144]	AATCCAAAATAAACAGCCATATTAACTGGAT

71	11[192]	13[191]	TCAAAAATGTCTTTCCAGAGCCTAAGGCTTAT
72	11[224]	13[223]	GAATAACATTTATCCTGAATCTTAGTTTTAGC
73	12[79]	10[80]	TTCAGAAAGACGACGATAAAAACTCAACTA
74	12[111]	10[112]	TCATTGAATTTTGCAAAAGAAGTTGATTCATC
75	12[143]	11[159]	AGCGTCCATAGTAAAATGTTTAGTTTATCCC
76	12[175]	10[176]	GTTACAAATAAGAAACGATTTTTTCCAATAAT
77	12[207]	10[208]	TAACGAGCGAAAATAGCAGCCTTAGAGAGA
78	12[239]	10[240]	GCTACAATTAAAAACAGGGAAGCGACAAAGTC
79	13[64]	15[63]	GCAAAGCGTGGCTTAGAGCTTAATTAAATATG
80	13[96]	15[95]	GAAGCCCGGCTCCTTTTGATAAGAGTTTCATT
81	13[128]	15[127]	ATTCGAGCCAACAGGTCAGGATTACTGCGAAC
82	13[160]	14[144]	AATAGCAAATCGTAGGAATCATTAACCGGAA
83	13[192]	15[191]	CCGGTATTTCATCGAGAACAAGCAACGCGCCT
84	13[224]	15[223]	GAACCTCCCCAAGAACGGGTATTATGAACAAG
85	14[79]	12[80]	TTTGCGGAGATTGCATCAAAAAGTAAACAG
86	14[111]	12[112]	CTTTAATTAAAGACTTCAAATATCATAAATAT
87	14[143]	13[159]	GCAAACTCTTCAAAGCGAACCAGCCGCGCCC
88	14[175]	12[176]	TTATTTTCGCAAATCAGATATAGAATTTGCCA
89	14[207]	12[208]	TACCGCACCTAAGAACGCGAGGCCCAACGC
90	14[239]	12[240]	TTATCATTCGACTTGCGGGAGGTTTGCACCCA
91	15[64]	17[63]	CAACTAAACTACTAATAGTAGTAGAAAGAATT
92	15[96]	17[95]	CCATATAATGGGGCGCGAGCTGAACAGAGCAT
93	15[128]	17[127]	GAGTAGATTGGTCAATAACCTGTTACATTATG
94	15[160]	16[144]	AACAACATAATTCTGTCCAGACGAGATACAT
95	15[192]	17[191]	GTTTATCAAGAGAATATAAAGTACAAAAGCCT
96	15[224]	17[223]	AAAAATAATTTAGGCAGAGGCATTAAATTCTT
97	16[79]	14[80]	CATCAATTGTACGGTGTCTGGAAGGTCATT
98	16[111]	14[112]	TTTTCATTCAGTTGATTCCCAATTGAGAGTAC
99	16[143]	15[159]	TTCGCAAATTAGTTTGACCATTACGACAATA
100	16[175]	14[176]	GGTAAAGTGTTCAGCTAATGCAGAAGCCGTTT
101	16[207]	14[208]	CAGTAATAACAATAGATAAGTCCAACCAAG
102	16[239]	14[240]	ACATGTAATATCCCATCCTAATTTGTCTTTCC
103	17[64]	19[63]	AGCAAAATGTAAAGATTCAAAAGGCAATATGA
104	17[96]	19[95]	AAAGCTAAATTTTAAATGCAATGCATTAATGC
105	17[128]	19[127]	ACCCTGTACGCAAGGATAAAAATTGATCTACA
106	17[160]	18[144]	AACACCGGTAAATAAGGCGTTAAAAAGCCTT
107	17[192]	19[191]	GTTTAGTAAAATTTAATGGTTTGAGGTCTGAG
108	17[224]	19[223]	ACCAGTATATATATTTTAGTTAATTTAGGTTG
109	18[79]	16[80]	ATGTGTAGTAAGCAATAAAGCCTAAGGTGG
110	18[111]	16[112]	CCTCATATATCGGTTGTACCAAAATAGCTATA
111	18[143]	17[159]	TATTTCAAATACTTTTGCGGGAGTAAGAATA
112	18[175]	16[176]	CCGTGTGAAATCATAATTACTAGACGACAAAA
113	18[207]	16[208]	TCTGACCTTCATATGCGTTATACTTCGAGC
114	18[239]	16[240]	TTTTTCAAAAAGCCAACGCTCAACCAACGCCA
115	19[64]	21[63]	TATTCAACTCAGAAAAGCCCCAAATGTAAACG
116	19[96]	21[95]	CGGAGAGGGCATGTCAATCATATGTTAAATTT
117	19[128]	21[127]	AAGGCTATAAGAGAATCGATGAACCCAATAGG
118	19[160]	20[144]	AATAGTGATAGATTAAGACGCTGAGAGTCTG
119	19[192]	21[191]	AGACTACCCCCTTAGAATCCTTGAGATGAAAC
120	19[224]	21[223]	GGTTATATCTTCTGTAAATCGTCGTTACATTT
121	20[79]	18[80]	GTTGATAACGTTCTAGCTGATAACTGAGTA
122	20[111]	18[112]	TAAAACTAGTAGCTATTTTTGAGATTTAGAAC
123	20[143]	19[159]	GAGCAAACCAGGTCATTGCCTGAGAAGAGTC
124	20[175]	18[176]	CGATAGCTATTTATCAAAATCATAAATACCGA

125	20[207]	18[208]	TTAATTTTTTTTTAACCTCCGGCTTCATCT
126	20[239]	18[240]	TAACCTTGAACTATATGTAAATGCGAGAAAAC
127	21[64]	23[63]	TTAATATTACGGCGGATTGACCGTCATCGTAA
128	21[96]	23[95]	TTGTTAAATAACAACCCGTCGGATGACGACGA
129	21[128]	23[127]	AACGCCATGCCAGCTTTCATCAACACTCCAGC
130	21[160]	22[144]	TCAATTACTCGCGCAGAGGCGAATTCTGGCC
131	**21[192]**	**23[191]**	**AAACATCACGCCTGATTGCTTTGAATAATGGA**
132	21[224]	23[223]	AACAATTTCCTTTTACATCGGGAGAATTATTT
133	**22[79]**	**20[80]**	**GGGAACAATTGTTAAAATTCGCATACCCCG**
134	22[111]	20[112]	TGAGCGAGTCAGCTCATTTTTTAAGGTAATCG
135	22[143]	21[159]	TTCCTGTACAAAAATAATTCGCGTATTCATT
136	**22[175]**	**20[176]**	**TTACAAAACTGAGCAAAAGAAGATAAACATAG**
137	22[207]	20[208]	AACGGATTAGAAAACAAAATTAACTATTAA
138	22[239]	20[240]	TAACAGTACATTTGAATTACCTTTTGAGTGAA
139	23[64]	25[63]	CCGTGCATGCTATTACGCCAGCTGTTGGGTAA
140	23[96]	25[95]	CAGTATCGGTTGGGAAGGGCGATCCGTTGTAA
141	23[128]	25[127]	CAGCTTTCAAAGCGCCATTCGCCAATGCCTGC
142	23[160]	24[144]	TGATTGTTGATGGCAATTCATCAATGCCGGA
143	**23[192]**	**25[191]**	**AGGGTTAGCGGAATTATCATCATAGATAATAC**
144	23[224]	25[223]	GCACGTAATTTTGCGGAACAAAGAACTTTACA
145	**24[79]**	**22[80]**	**GCCTCTTCCTGCCAGTTTGAGGGTCTCCGT**
146	24[111]	22[112]	GCGCAACTGCCTCAGGAAGATCGCATTAAATG
147	24[143]	23[159]	AACCAGGCCGGCACCGCTTCTGGTATAATCC
148	**24[175]**	**22[176]**	**TATCAGATTGGATTATACTTCTGAATACCAAG**
149	24[207]	22[208]	AGAAGGAGAACCTACCATATCAAAAACAAT
150	24[239]	22[240]	CATTATCAAACAGAAATAAAGAAAATATACAG
151	**25[64]**	**27[63]**	**CGCCAGGGCATACGAGCCGGAAGCGAGCTAAC**
152	25[96]	27[95]	AACGACGGGAAATTGTTATCCGCTCTGCCCGC
153	25[128]	27[127]	AGGTCGACTTCGTAATCATGGTCACCAGCTGC
154	25[160]	26[144]	ACTAATAGAAAATATCTTTAGGAGGGTACCG
155	**25[192]**	**27[191]**	**ATTTGAGGCAACAGTTGAAAGGAAAAAACAGA**
156	25[224]	27[223]	AACAATTCCCCTCAATCAATATCTCGCCTGCA
157	**26[79]**	**24[80]**	**CCACACAATTTTCCCAGTCACGAGGTGCGG**
158	26[111]	24[112]	TCCTGTGTCCAGTGCCAAGCTTGCTTCAGGCT
159	26[143]	25[159]	AGCTCGAATCTAGAGGATCCCCGCACTAACA
160	**26[175]**	**24[176]**	**GGTTATCTATTAGAGCCGTCAATATTCCTGAT**
161	26[207]	24[208]	TGGCAAATATTTAGAAGTATTAGAACCACC
162	26[239]	24[240]	ATATCAAAGACAACTCGTATTAAATTGAGTAA
163	27[64]	29[63]	TCACATTACACCGCCTGGCCCTGACCCCAGCA
164	27[96]	29[95]	TTTCCAGTCCAGTGAGACGGGCAAGTTCCGAA
165	27[128]	29[127]	ATTAATGACGTATTGGGCGCCAGGAAAGAATA
166	27[160]	28[144]	CCGAACGACCTAAAACATCGCCATGGGAGAG
167	**27[192]**	**29[191]**	**GGTGAGGCGGCTATTAGTCTTTAATCAATCGT**
168	27[224]	29[223]	ACAGTGCCAGAATACGTGGCACAGCAGATTCA
169	**28[79]**	**26[80]**	**TTGCCCTTATTGCGTTGCGCTCACACAATT**
170	28[111]	26[112]	TCTTTTCACGGGAAACCTGTCGTGTAGCTGTT
171	28[143]	27[159]	GCGGTTTGATCGGCCAACGCGCGTAAAAATA
172	**28[175]**	**26[176]**	**CTGATAGCACCACCAGCAGAAGATTTGAGGAA**
173	28[207]	26[208]	TTTTGAATGGTCAGTATTAACACGGTCAGT
174	28[239]	26[240]	AAAGCGTAACGCTGAGAGCCAGCAAACCTCAA
175	29[64]	31[63]	GGCGAAAAATGGCCCACTACGTGAGGTGCCGT
176	29[96]	31[95]	ATCGGCAAGTCAAAGGGCGAAAAAAGGGAGCC
177	29[128]	31[127]	GCCCGAGAAGAGTCCACTATTAAAAGCCGGCG
178	29[160]	30[144]	ATGGAAATGCCATTGCAACAGGAATTCCAGT

179	**29[192]**	**31[191]**	**CTGAAATGTGCTGGTAATATCCAGGAATCCTG**
180	29[224]	31[223]	CCAGTCACGCCTGAGTAGAAGAACGGCCACCG
181	**30[79]**	**28[80]**	**TCAGGGCGTCCTGTTTGATGGTGCAGCTGA**
182	30[111]	28[112]	ACTCCAACAATCCCTTATAAATCAGTGGTTTT
183	30[143]	29[159]	TTGGAACATAGGGTTGAGTGTTGAAACGCTC
184	**30[175]**	**28[176]**	**TACCGCCAACCTACATTTTGACGCTGCGCGAA**
185	30[207]	28[208]	ATCGGCCTGATTATTTACATTGGACAATAT
186	30[239]	28[240]	CATCACTTACGACCAGTAATAAAACTGACCTG
187	31[64]	30[80]	AAAGCACTAAATCGGAACCCTAACCGTCTA
188	31[96]	30[112]	CCCGATTTAGAGCTTGACGGGGAAGAACGTGG
189	31[128]	31[159]	AACGTGGCGAGAAAGGAAGGGAATTAAAGGG
190	31[160]	30[176]	ATTTTAGACAGGAACGGTACGCCAAACAATAT
191	31[192]	30[208]	AGAAGTGTTTTTATAATCAGTGATCAAACT
192	31[224]	30[240]	AGTAAAAGAGTCTGTCCATCACGCAGTAATAA

Supplementary references

1. Petkau-Milroy, K., Uhlenheuer, D. A., Spiering, A. J. H., Vekemans, J. A. J. M. & Brunsveld, L. Dynamic and bio-orthogonal protein assembly along a supramolecular polymer. *Chem. Sci.* **4**, 2886–2891 (2013).
2. Alemán García, M. Á., Magdalena Estirado, E., Milroy, L. G. & Brunsveld, L. Dual-Input Regulation and Positional Control in Hybrid Oligonucleotide/Discotic Supramolecular Wires. *Angew. Chemie - Int. Ed.* **57**, 4976–4980 (2018).
3. Rothemund, P. W. K. Folding DNA to create nanoscale shapes and patterns. *Nature* vol. 440 297–302 (2006).
4. Schmied, J. J. *et al.* DNA origami-based standards for quantitative fluorescence microscopy. *Nat. Protoc.* **9**, 1367–1391 (2014).
5. Hung, A. M. *et al.* Large-area spatially ordered arrays of gold nanoparticles directed by lithographically confined DNA origami. *Nat. Nanotechnol.* **5**, 121–126 (2010).
6. Kibbe, W. A. OligoCalc: an online oligonucleotide properties calculator. *Nucleic Acids Res.* **35**, W43-6 (2007).
7. Magdalena Estirado, E., Aleman Garcia, M. A., Schill, J. & Brunsveld, L. Multivalent Ultrasensitive Interfacing of Supramolecular 1D Nanoplatforms. *J. Am. Chem. Soc.* **141**, (2019).
8. Hsiao, S. C. *et al.* Direct cell surface modification with DNA for the capture of primary cells and the investigation of myotube formation on defined patterns. *Langmuir* **25**, 6985–6991 (2009).
9. Zadeh, J. N. *et al.* NUPACK: Analysis and design of nucleic acid systems. *J. Comput. Chem.* **32**, 170–173 (2011).