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Supporting Information

Template-directed conjugation of heterogeneous oligonucleotides to a homobifunctional molecule for programmable supramolecular assembly Seham Helmi^{1,2} & Andrew J. Turberfield^{1,2}

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1- Abbreviations:

- CuAAC: copper-catalyzed azide-alkyne cycloaddition
- DMF: dimethylformamide
- DMT-MM: 4-(4, 6-dimethoxy-1, 3, 5-triazin-2-yl)-4-methylmorpholinium chloride
- DSS: disuccinimidyl suberate
- HPLC: high-performance liquid chromatography
- MOPS: N-[3-morpholinopropane]sulfonic acid
- MQ: Milli-Q water
- nt: nucleotide
- NT: non-templated
- T: templated
- PAGE: polyacrylamide gel electrophoresis
- PBS: phosphate-buffered saline
- PEG: polyethylene glycol
- TAE: tris-acetate-EDTA
- TAPS: [tris(hydroxymethyl)methylamino]propanesulfonic acid
- TBE: tris-borate-EDTA
- TCEP: tris(2-carboxyethyl)phosphine hydrochloride
- TEAA: triethylammonium acetate
- THPTA: tris(3-hydroxypropyltriazolylmethyl)amine
- rcf: relative centrifugal force
- rpm: rotations per minute

2- Materials:

Unless noted otherwise, all reagents and solvents were purchased from commercial sources and used as received.

- 2.1 Homobifunctional molecules
 - PEG-N was supplied by Deepak Asthana as part of a collaboration with the laboratory of Professor Richard E. P. Winpenny, School of Chemistry, University of Manchester.
 - PEG-5000, phthaldialdehyde, and biphenyl-4,4'-dicarboxylic acid: purchased from Sigma Aldrich.
 - DSS: purchased from Thermo Scientific.
 - Sulfo-cyanine5 bis-NHS ester: purchased from Lumiprobe
 - N,N'-bis[2(iodoacetamido)ethyl]-N,N'-dimethylrhodamine dihydroiodide: purchased from Santa Cruz Biotechnology.
- 2.2 Other reagents
 - DMT-MM: purchased from Fluka.
 - All other chemicals were purchased from Sigma Aldrich.
- 2.3 DNA oligonucleotides and origami scaffold
 - Oligos were purchased either from Integrated DNA Technologies (IDT) or Biomers, as specified in section (6).
 - Scaffold p8064: purchased from tilibit nanosystems.

3- General Methods:

3.1 <u>Conjugation of DNA adapters</u>

In each reaction the concentration of each DNA adapter was 10 μ M. In templated reactions, the template concentration was 10 μ M. The annotation "n×" indicates the molar excess of the molecule over adapters (n:1:1). In all cases, molar equivalents specified are relative to the concentration of one DNA adapter, i.e. 10 μ M.

CuAAC - PEG-N (Figures 1 and 2- main text):

PEG-N was dissolved in DMF.

For the templated reaction (T): DNA adapters were annealed (using the short annealing program, SI §3.2) to DNA template in TBE buffer pH 8 with 11mM MgCl₂. PEG-N was mixed with the annealed DNA adapters in the stated molar ratios "n×" (2× - 256×) in the presence of 10% DMF.

For the non-templated reaction (NT): PEG-N was mixed with the DNA adapters in the stated molar ratios " $n\times$ " (2x-256x) in TBE pH 8 with 11mM MgCl₂ in the presence of 10% DMF. To both reactions were added sodium ascorbate (600×), THPTA ligand (420×) and CuSO₄ (60×). The reaction was degassed and left covered overnight on a shaker at room temperature.

CuAAC - PEG-5000 (Figure 3 A- main text):

PEG-5000 was dissolved in DMF. DNA adapters were annealed (using the short annealing program, SI §3.2) to DNA template in TBE buffer pH 8 with 11mM MgCl₂. 20 molar equivalents of PEG-5000 was mixed with the annealed DNA adapters in the presence of 10% DMF. To the reaction was added sodium ascorbate (600×), THPTA ligand (420×) and CuSO₄ (60×). The reaction was degassed and left covered on a shaker at room temperature overnight.

Reductive amination - phthaldialdehyde (Figure 3 B- main text):

Phthaldialdehyde was dissolved in DMF. Amine-labeled DNA adapters were annealed (using the short annealing program, SI \S 3.2) to DNA template in TAPS buffer 100 mM pH 8.5 with 1M NaCl. To the annealed DNA adapters was added phthaldialdehyde (40×) then NaBH₃CN to a final concentration of 80 mM. The reaction was carried out in a covered Eppendorf thermomixer at 25°C overnight, 300 rpm.

Amine acylation - Sulfo-Cyanine5 bis-NHS ester (Figure 3 C- main text):

Sulfo-cyanine5 bis-NHS ester was dissolved in MQ water. Amine-labeled DNA adapters were annealed (using the short annealing program, SI §3.2) to DNA template in 100 mM PBS pH 8.75. Sulfo-cyanine5 bis-NHS ester (2×) was added to the annealed DNA. The reaction was carried out in a covered Eppendorf thermomixer at 25°C for 2 h, 300 rpm.

Amine acylation - DSS (Figure 4 D- main text):

DSS was dissolved in DMF. Amine-labeled DNA adapters were annealed (using the short annealing program, SI \$3.2) to DNA template in 100 mM PBS buffer pH 8. DSS ($30\times$) was added to the annealed DNA in the presence of 15% DMF. The reaction was carried out for 30 min at room temperature.

Amine acylation- biphenyl-4,4'-dicarboxylic acid (Figure 3 E- main text):

Biphenyl-4,4'-dicarboxylic acid was dissolved in DMF. Amine-labeled DNA adapters were annealed (using the short annealing program, SI \S 3.2) to DNA template in aqueous MOPS buffer 100mM pH 7.0 with 1M NaCl, 15% DMF. Biphenyl-4,4'-dicarboxylic acid (40×) was

added to the annealed DNA adapters. DMT-MM was added to the reaction mixture to a final concentration of 50mM. The reaction was carried out for 15 h at 25°C.

<u>Alkylation - N,N'-bis[2 (iodoacetamido)ethyl]-N,N'-dimethylrhodamine dihydroiodide (Figure 3 F-main text):</u>

Rhodamine was dissolved in DMF. Thiol-labeled DNA adapters were annealed (using the short annealing program, SI §3.2) to DNA template in 50mM PBS buffer pH 8 with 11mM MgCl₂ and containing 15%DMF. 40mM TCEP was added to a final concentration of 20 nM and left covered at room temperature for 1 h to reduce the templated thiol adapters. N,N'-bis[2 (iodoacetamido)ethyl]-N,N'-dimethylrhodamine dihydroiodide (50×) was added. in equal steps, each with half the final rhodamine volume. Between the two steps the reaction was left covered at room temperature for 30 min. The reaction was then carried out in the dark at 4°C overnight.

3.2 <u>Short annealing program:</u>

- Start: 96°C
- End 15°C
- -1°C/3 sec
- Hold: 15°C

3.3 <u>Reaction product purification:</u>

In cases of Cy5 and rhodamine conjugation, Biorad Micro Bio Spin column Bio-Gel® P-6 in Tris buffer was used to remove the unreacted molecule (unbound dye) following the manufacturer's instructions before HPLC purification. Other reactions were subjected directly to HPLC purification.

HPLC (Agilent 1200):

- Buffer A: 5% MeCN, 0.1 M TEAA
- Buffer B: 70% MeCN, 0.1 M TEAA
- Column: Waters XBridge® Oligonucleotide BEH C18 column, 130 Å, 2.5 μm 4.6×50 mm
- Temperature: 60°C

Time / min	%B	Flow
0.0	0.0	1 mL/min
1.0	0.0	1 mL/min
20.0	100.0	1 mL/min
23.0	100.0	1 mL/min
23.10	0.0	1 mL/min

3.4 Denaturing PAGE gel:

- 20% PAGE (29:1acrylamide:bis-acrylamide) containing 25% formamide, 30% W/V urea in TBE.
- gels were pre-run for 30 min, 300 V.
- samples were heated to 95°C for 10 min then placed on ice before loading.
- 300 V, 50 min after sample loading.
- loading buffer: 80% formamide, 10% tris glycine, 10% urea.

3.5 <u>Native PAGE gel:</u>

- 10% PAGE (19:1acrylamide:bis-acrylamide) in TAE.
- 300 V, 15 min.

3.6 Gel scanning and analysis:

- All gels were scanned in each fluorophore fluorescence channel, then stained with SYBR® gold and scanned in the SYBR® gold channel.
- All gels were scanned using a Typhoon FLA 9500 gel scanner.
- Gel images were analysed using ImageJ.

3.7 <u>Hetero-DNA-Cy5 hybrid integration into 3D origami structure (Figure 5 main text):</u>

The long stem of the T-shaped origami comprises two layers of DNA helices with a gap between four helices (Figure 5 main text, Supporting Figure S13). The two DNA adapters of the hetero-DNA-Cy5 hybrid allow the Cy5 molecule to bridge the gap by hybridizing to the scaffold strand on either side of the gap (Figure 5 main text, Supporting Figure S13). The purified hetero-DNA-Cy5 hybrid was added either with the staple strands during the assembly or by annealing to the assembled origami structure. Samples were analysed on a 2% agarose gel. The coincidence of Cy5 fluorescence and the SYBR® gold-stained origami band confirms the integration of the Cy5 molecule into the assembled origami structure in both cases (Figure 5 main text lanes 3 and 5).

3.7.1 <u>DNA origami Folding & hetero-DNA-Cy5 hybrid incorporation during assembly:</u> To fold the DNA origami template structure, 20 nM of p8064 scaffold was mixed with 200 nM of each unmodified staple oligonucleotide in 10 mM Tris, 1 mM EDTA (pH 8), 16 mM MgCl₂. The folding solution was heated to 65 °C for 15 min to denature all DNA strands and then slowly cooled to 15 °C over a period of 18 h using the origami assembling program.

- Origami assembling program:
 - Start: 65°C for 15 min
 - $65^{\circ}C 59^{\circ}C > -1^{\circ}C/5 \min$
 - $59^{\circ}C 35^{\circ}C > -1^{\circ}C/35 \text{ min}$
 - $35^{\circ}C 30^{\circ}C > -1^{\circ}C/10 \text{ min}$
 - $30^{\circ}C 15^{\circ}C > -1^{\circ}C/5 \min$
 - Hold: 15°C

3.7.2 Origami purification:

Free staples were removed by three rounds of PEG precipitation¹. For each round an equal volume of 12.5 mM MgCl₂, 0.5 M NaCl, 15% PEG (w/v) and TAE was added and the samples were spun at 20,000 rcf for 30 minutes at 20°C.

3.7.3 Agarose gel:

After folding the DNA origami template structures, they were checked via gel electrophoresis. - <u>2% agarose</u>

- 11 mM MgCl₂.
- TAE 40 mM (Tris, 40 mM acetic acid, 1 mM EDTA, pH 8)
- 60V for 2 h on ice.

3.7.4 Integration of Cy5 during the origami assembly:

Supporting Figure S13 D, lane 3: the hetero-DNA-Cy5 hybrid was added to the folding solution to a concentration of 40 nM before folding using the aforementioned origami assembling program.

3.7.5 Integration of Cy5 after origami assembly:

Supporting Figure S13 D, lane 5: the hetero-DNA-Cy5 hybrid was mixed with the PEG-purified origami (15 nM) (sample in lane 4, Figure 5 main text) in a 2:1 molar ratio of hetero-DNA-Cy5 hybrid to DNA origami using the origami post-assembly program.

- Origami post-assembly program
 - Start: 40°C
 - -1°C/15 min
 - Hold: 15°C

3.8 <u>Hetero-DNA-Cy5 hybrid programmable assembly (Figure 5 - main text):</u>

3.8.1 <u>Step 1- first template:</u>

HPLC-purified hetero-DNA-Cy5 hybrid (at a final concentration of 125 nM) was mixed with the 3'-modified Cy3 oligo ($1.5\times$) and the first template ($2\times$)) in TBE buffer pH 8 with 16 mM MgCl₂ and annealed (using the short annealing program) in a total volume of 150 µl.

3.8.2 <u>Step 2- second template:</u>

After 10 min, to the mixture (step 1) we added the 2^{nd} template (2.5× excess) (Supporting Figure S14, A-2).

3.8.3 <u>Step 3- first template complement:</u>

After 10 min, to the mixture (step 2) we added the complement to the 1^{st} template (3× excess) (Supporting Figure S14, A-3).

3.8.4 <u>Step 4- third template:</u>

After 10 min, to the mixture (step 3) we added the complement to the 1st template $(3.5 \times \text{ excess})$ (Supporting Figure S14, A-3).

After each step, the reaction was analysed using an Agilent Cary Eclipse Fluorescence Spectrometer (Supporting Figure S14, middle) using excitation – emission bands centred at the following wavelengths:

- Cy5: 650-670
- Cy3: 550-570

FRET: 550-670

Then 4 μ l were collected for analysis on 10% native PAGE gel (Supporting Figure S14, bottom).

3.8.5 <u>Following the same protocol, we tested two controls:</u>

<u>SUsing a non-fluorescently labeled oligo with the same sequence as the 3' Cy3-modified</u> oligo (Supporting Figure S14, B):

Upon the addition of each of the templates, bands with the same electrophoretic mobilities as in (Supporting Figure S14, A) were obtained. However, none of the assemblies showed any FRET signal, as expected.

<u>§Using a 3'Cy3-modified oligo with a random sequence (Supporting Figure S14, C):</u> No interaction between the Cy3 oligo and other reaction components was observed by either gel analysis or FRET, as expected

4- Calculation of yield:

Yields were estimated from the integrated Cy5 and Cy3 fluorescence intensities of the corresponding gel bands and are expressed relative to the concentration of DNA adapters of each type. The integrated intensity for a given fluorophore in each band was normalized to the total integrated intensity for that fluorophore in the entire lane (Cy3 and Cy5 fluorophore-labeled oligos were present in equal quantities in each reaction). This normalization procedure assumes that the quantum yield of a given fluorophore in all products is approximately the same. For the heterofunctionalized products the yields deduced from normalized intensities in Cy3 or the Cy5 channels were approximately equal, consistent with this assumption. Yields quoted in the main text are averages of these two measures.

Stoichiometry (n:1:1)	Normalized Hetero-PEG-N Cy3 channel	Normalized Hetero-PEG-N Cy5 channel	Average
2	0.584	0.585	0.585
4	0.559	0.564	0.561
8	0.533	0.540	0.537
16	0.442	0.455	0.448
32	0.427	0.427	0.427
64	0.419	0.419	0.419
128	0.413	0.417	0.415
256	0.400	0.402	0.401

For the templated reactions:

For the non-templated reactions:

Stoichiometry	Normalized Hetero-PEG-N	Normalized Hetero-PEG-N	Average
(n:1:1)	Cy3 channel	Cy5 channel	
2	0.198	0.210	0.204
4	0.173	0.175	0.174
8	0.132	0.130	0.131
16	0.112	0.120	0.116
32	0.102	0.102	0.102
64	0.094	0.094	0.094
128	0.067	0.069	0.068
256	0.047	0.048	0.047

5- Supporting Figures:

5.1 PEG-N reaction yields with different PEG-N concentrations:



Supporting Figure S1.Reaction yields, deduced from normalized fluorescence intensities, as functions of molar excess of PEG-N over each adapter. "T" and "NT" indicate templated and non-templated reactions, respectively.

5.2 Single Color-Channel images:

5.2.1 Templated and Non-templated CuAAC - Figure 2 main text:



Supporting Figure S2. Templated and non-templated CuAAC reaction. Left:merged color image of 20% denaturing PAGE gel showing templated (T) and non-templated (NT) functionalization of alkyne-PEG-N-alkyne using two different azide-modified DNA adapters, as Figure 1B. To the right are the single-color-channel images of the same reactions from the Cy3, Cy5 and SYBR gold channels. Refer to the main textFigure 1B for key to the identities of bands.



5.2.2 Reaction yields as functions of PEG-N concentration - Figure 2 main text:

Supporting Figure S3.Reaction yields as functions of PEG-N concentration. For each gel, corresponding to the specified molar excess of PEG-N, the merged color image shown in main text Figure 2A is combined with single-color-channel images of the same reaction from the Cy3, Cy5 and SYBR gold channels.

5.2.3 Templated heterofunctionalization reactions of a wide range of target molecules - Figure 3 main text:



Supporting Figure S4. Templated heterofunctionalization of a wide range of target molecules. For each gel, corresponding to a different conjugation reaction, the merged color image shown in main textFigure 3A-Fis combined with single-color-channel images of the same reaction from the Cy3, Cy5 and SYBR gold channels.

5.2.4 Hybridization reactions demonstrating programmed control of a hetero-DNA-Cy5 hybrid by means of the two oligonucleotide adapters - Figure 5 main text:



Supporting Figure S5. Hybridization reactions demonstrating programmed control of a hetero-DNA-Cy5 hybrid by means of the two oligonucleotide adapters. Top: 10% native PAGE gel showing reaction products imaged in Cy5 (red),Cy3 (green), and FRET (Cyan) fluorescence channels (main textFigure 5B). Yellow bands indicated by * correspond to products containing both fluorophores and FRET signal. Bottom: component single-color-channel images.

5.3 HPLC chromatograms of all tested reactions and molecules:

5.3.1 PEG-N "Figure 1- main text"



Supporting Figure S6.HPLC analysis of templated CuAAC reaction of PEG-N (gel from Figure 1B to the left) showing the 260 nm absorption of DNA in blue, 648 nm Cy5 fluorescence in green and 554 nm Cy3 fluorescence in red. The peak where all the three signals coincide (marked with the yellow arrow) corresponds to the hetero-DNA-functionalized product.



5.3.2 PEG-5000 "Figure 3 A- main text"

Supporting Figure S7. HPLC trace of Templated CuAAC reaction of PEG-5000 molecule (gelfrom Figure 3A to the left)showing the 260nm absorption of DNA in blue, 648nm Cy5 fluorescencein green and 554nm Cy3 fluorescencein red. The peaks where all the three signals coincide (marked with the yellow arrow) correspond to the hetero-DNA-functionalized product.

5.3.3 Phthaldialdehyde "Figure 3 B- main text"



Supporting Figure S8.HPLC trace of templated reductive amination reaction of pthaldialdehyde (gel from Figure 3B to the left) showing the 260 nm absorption of DNA in blue, 648 nm Cy5 fluorescence in green and 554 nm Cy3 fluorescence in red. The peak where all the three signals coincide (marked with the yellow arrow) corresponds to the hetero-DNA-functionalized product.



5.3.4 Sulfo-Cyanine5 bis-NHS ester "Figure 3 C- main text":

Supporting Figure S9. HPLC trace of templated amide bond formation reaction of Sulfo-Cyanine5 bis-NHS ester (gel from Figure 3C to the left) Showing the 260 nm absorption of DNA in blue, 648 nm Cy5 fluorescence in green and 554 nm Cy3 fluorescence in red. The peak where all the three signals coincide (marked with the yellow arrow) corresponds to the hetero-DNA-functionalized product.

5.3.5 DSS "Figure 3 D- main text":



Supporting Figure S10.HPLC trace of templated amide bond formation reaction of DSS molecule (gel from Figure 3D to the left)Showing the 260 nm absorption of DNA in blue, 648 nm Cy5 fluorescence in green and 554 nm Cy3 fluorescence in red. The peak where all the three signals coincide (marked with the yellow arrow) corresponds to the hetero-DNA-functionalized product.





Supporting Figure S11.HPLC trace of templated amide bond formation reaction of Biphenyl-4,4'-dicarboxylicacid molecule (gel from Figure 3E to the left)showing the 260 nm absorption of DNA in blue, 648 nm Cy5 fluorescence in green and 554 nm Cy3 fluorescence in red. The peak where all the three signals coincide (marked with the yellow arrow) corresponds to the hetero-DNA-functionalized product.

5.3.7 <u>N,N'-bis[2(iodoacetamido)ethyl]-N,N'-dimethylrhodamine dihydroiodide "Figure</u> <u>3 F-main text":</u>



Supporting Figure S12.HPLC trace of templated amide bond formation reaction of N,N'-Bis[2 (iodoacetamido)ethyl]-N,N'dimethylrhodamine dihydroiodide (gelfrom Figure 3F to the left)showing the 260nm absorption of DNA in blue, 648nm Cy5 fluorescence in green and 554nm Cy3 fluorescence in red. The peak where all the three signals coincide (marked with the yellow arrow) corresponds to the hetero-DNA-functionalized product.

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Supporting Figure S13. DNA origami design. CaDNAno²image of the DNA origami structuredisplaying scaffold (blue), staple oligonucleotides (black) and oligo adapters conjugated to the Cy5 fluorophore (red). The hetero-functionalized DNA-Cy5 hybrid bridges a gap in the structure: it is held in place by hybridization of the two conjugated DNA strands to scaffold domains on either side.



5.5 Programmable assembly of hetero-Cy5 – Controls:

Supporting Figure S14. Hybridization reactions demonstrating programmed control of a hetero-DNA-Cy5 hybrid by means of the two oligonucleotide adapters. Top: reaction schemes. Bottom: intensity trajectories of Cy5 (red) and Cy3 (green) fluorescence and Cy3-Cy5 FRET (yellow); merged-color image of a 10% native PAGE gel constructed from Cy5 (red), Cy3 (green), and Cy3-Cy5 FRET (cyan) channels for each assembly. Interval between steps: 10 min. A) As Figure 5. B) As Figure 5 but with Cy3 fluorophore omitted. C) As Figure 5 but with random (non-complementary) sequence for Cy3-modified oligo.

6- Oligo Sequences:

- <u>"Figure 1 and 2 main text"</u>: Oligos were purchased from IDT
- 1- /5Cy5/ATCGGATGGATTATTTACATTAAAAGGGACATTCTG/3AzideN/
- 2- /5AzideN/CAACAGAGATAGAAC/3Cy3Sp/
- 3- TCCTGTTCTATCTCTGTTGCCCAGAATGTCCCTTTTAATGTAAATAATCCAT CCGATTCCT
- <u>"Figure 3 A main text"</u>: Oligos were purchased from IDT
- 1- /5Cy5/ATCGGATGGATTATTTACATTAAAAGGGACATTCTG/3AzideN/
- 2- /5AzideN/CAACAGAGATAGAAC/3Cy3Sp/
- 3- TCCTGTTCTATCTCTGTTGCCAGAATGTCCCTTTTAATGTAAATAATCCATC CGATTCCT
- <u>"Figure 3 B main text"</u>: Oligos were purchased from IDT
- 1- /5Cy5/CACCGCTGGTAGCGGTGAGTATTTGGTATCTGCGCTCTGC/3AmMO/
- 2- /5AmMC6/GAAGCCAGATCCGGCAAACAAAC/3Cy3Sp/
- 3- TCTGTTTGTTTGCCGGATCTGGCTTCCGCAGAGCGCAGATACCAAATACTC ACCGCTACCAGCGGTGT
- <u>"Figure 3 C main text"</u>: Oligos were purchased from Biomers
- 1- CTCACCGGAAAGAACGGATAACTTTT/3AmMC3/
- 2- /5AmMC3/TTTTTTTTTTTTCCTGATTATCAG
- 3- TACACTCTGATAATCAGGAAAAAAAAAAACCCAAAAAGTTATCCGTTCTTTC CGGTGAG
- <u>"Figure 3 D main text"</u>: Oligos were purchased from IDT
- 1- /5Cy5/CACCGCTGGTAGCGGTGAGTATTTGGTATCTGCGCTCTGC/3AmMO/
- 2- /5AmMC6/GAAGCCAGATCCGGCAAACAAAC/3Cy3Sp/
- 3- TCTGTTTGTTTGCCGGATCTGGCTTCCCGCAGAGCGCAGATACCAAATACT CACCGCTACCAGCGGTGT
- <u>"Figure 3 E main text"</u>: Oligos were purchased from IDT
- 1- /5Cy5/AACCATCGCCTTTAATTGTATCGG/3AmMO/
- 2- /5AmMC6/ATGGATTATTTACATT/3Cy3Sp/
- 3- TCCTAATGTAAATAATCCATGACCGATACAATTAAAGGCGATGGTTTCCT
- <u>"Figure 3 F main text"</u>: Oligos were purchased from IDT
- 1- /5Cy5/CACCGCTGGTAGCGGTGAGTATTTGGTATCTGCGCTCTGC/3ThioMC6/
- 2- /5ThioMC6/GAAGCCAGATCCGGCAAACAAAC/3Cy3Sp/
- 3- TCTGTTTGTTTGCCGGATCTGGCTTCCCGCAGAGCGCAGATACCAAATACT CACCGCTACCAGCGGTGT

- <u>Hetero-DNA-Cy5 hybrid programmable assembly (Figure 6- main text):</u> Templates were purchased from Biomers, other oligos from IDT
- Figure 4- main text, and Supporting Figure S5& S6 A:
- b) <u>1st template:</u> TACAACTTATCAAAAAGAGTATTGACTTAAAGTCTACAAAAAGTTATCCGT TCTTTCCGGTGAG
- c) <u>2nd template:</u> CTGATAATCAGGAAAAAAAAAAACCGGGTTGGACTCAA
- d) <u>1st template complement:</u> CTCACCGGAAAGAACGGATAACTTTTTGTAGACTTTAAGTCAATACTCTTT TTGATAAGTTGT
- e) <u>Hetero-DNA-Cy5 Hybrid complement:</u> TACACTCTGATAATCAGGAAAAAAAAAAAAAAGTTATCCGTTCTTTC CGGTGAG
- f) <u>3' Cy3-modified oligo:</u> TTGAGTCCAACCCGG/3Cy3Sp/
 - <u>SI 5.5.1 Supporting Figure S6 B (1st control)</u>:
- g) TTGAGTCCAACCCGG
 - <u>SI 5.5.2 Supporting Figure S6 C (2nd control):</u>
- $h) \ CAACAGAGAGATAGAAC/3Cy3Sp/$
- Origami staples: were purchased from IDT

	End		Sequence
[236]	41	[238]	TACACTCAAGAGTCCACTACAGTCAAATCACCATCAATTACACT
[167]	44	[164]	GGGCGATGAAGCACTAATTTAATGAAAACTTTTAAT
[199]	43	[186]	TCCAACGTGCCCGAGACCGGAGAGGGTAGATTTGC
[172]	32	[160]	GGAAAGCCCCCGGGGCGCTGCGCCAATTACCTGAGC
[130]	2	[127]	TACACTCAAGTTTTTTGGGCGAACGTGGCGTACACT
[152]	41	[159]	GTGCCGTAGCCCACTATAAATAAG
[212]	1	[236]	GTTGTTCCAGTTTGGAATACACT
[204]	31	[207]	GTTGTCCGAAATACGCTGGTGTAATGGGGGGGGACGA
[188]	41	[193]	AATACAAAGGGCAAAGATTCAA
[231]	43	[233]	TACACTATCCTGTTTGACAGGTCATCCGTTCTATAAAAATTTTTAGTACACT
[160]	37	[163]	GGGCGCTAATTTAGAGTAACAATTACTATATGTCTG
[192]	33	[195]	AGCGGTCCCGGCAAAACATT
[148]	4	[127]	AGCGACCACACCCGCCGCGCTTAATTACACT
[176]	4	[172]	GCAGAGAGCCTGAAGTGTAG
[127]	32	[130]	TACACTAGAAAGGAAGGGAACAAACATTACACT
[171]	30	[160]	CGGTAGCACGTAGCCAGAAACAATAACG
[207]	29	[207]	GCTGATTGGGGCGCCACACCGCTTGGGAAGGG
[231]	3	[231]	TACACTCCAGTGAGACGAGCAGGCGAAATACACT
[127]	30	[130]	TACACTGCGCCGCTACAGGTTGAATACTACACT
[148]	6	[127]	GTACTTAAAGGGATTTTAGACAGGATACACT
[192]	32	[192]	TGCGTATTCCCTTCACCAGTTTGAATAGGTCA
	 [236] [167] [199] [172] [130] [152] [204] [188] [231] [160] [192] [148] [176] [127] [127] [127] [148] [192] 	End[236]41[167]44[199]43[172]32[130]2[152]41[212]1[204]31[188]41[231]43[160]37[192]33[148]4[176]43[127]32[171]30[207]29[231]3[127]30[148]6[192]32	End[236]41[238][167]44[164][199]43[186][172]32[160][130]2[127][152]41[159][212]1[236][204]31[207][188]41[193][160]37[163][160]37[163][148]4[127][176]4[127][171]30[160][207]29[207][231]3[231][148]6[127][148]6[127][148]6[127][192]32[192]

5	[160]	31	[178]	CTTTGACGCACGCTGCGAATTATTCATTTATCGTA
6	[207]	26	[213]	CAACGCGCTCCAGTCGGATGTGCTCGCCAGGGTTTTCCCAGGT
6	[175]	28	[160]	AGCGGGAGAGTGAGCCCCAGAAATAAAGAAAT
6	[231]	5	[231]	TACACTCTGCATTAATGTTTTCTTTTCATACACT
7	[127]	28	[130]	TACACTACGGTACGCCAGATTTAACGTTACACT
7	[160]	29	[175]	TTATAATCCTAAACAGCTTTTACATCGGGATT
7	[189]	29	[195]	ACTGCCCGCTTGGGGAGAGCAAC
8	[223]	7	[231]	GAGTGAGCTGTCGTGCCAGTACACT
8	[241]	27	[239]	TACACTTGGGGTGCCTAATGTTGTAAAACGACTACACT
8	[173]	27	[174]	GCCACCGAGTAAAATTTGCACGTAAAAC?
8	[143]	26	[113]	CAAATTAATAACATCAAATATAATCCTGATTGTTTGGATTATATACACT
8	[207]	25	[207]	CATTAATTCACAACATCGCCACGGCAATCGGC
9	[189]	8	[189]	TCACAATTCCAGCGTTGCGCTC
9	[116]	27	[136]	TACACTTTCTTTGATTAGTAACCGTTGTATGGAAGGGT
9	[160]	9	[173]	AACTCAAACTATCG
10	[241]	25	[239]	TACACTTTCGTAATCATGGATGTTTACCAGTCTACACT
10	[207]	23	[207]	GTGTGAAACCTCACAGCGTGGTGATCTGCTCA
10	[143]	10	[115]	CCGCCAGCCATTGCAACAGGAAAATACACT
10	[173]	25	[175]	GCCTTGCTGGTAATGAATTATCATCATA??
11	[192]	10	[189]	GTGAGCCTTTGTTATCCGC
11	[115]	11	[138]	TACACTACGCTCATGGAAATACCTA
11	[148]	12	[127]	GCTCATTCACCAGTCACGACCAGTACACT
11	[139]	24	[122]	CATTTAAAGTTTGAGTAACATTATTACACT
11	[160]	24	[160]	GAAATGGACACGCGTGGACAATCCTTTGCCCG
12	[175]	21	[179]	TCTGCCAGGGTGCCCCTGAAGAGCAGATTTGAAGGG
12	[232]	11	[241]	TACACTGTGCTGCGGCCTCCCCGGGTACCGAGCTCGAATACACT
12	[207]	21	[207]	GGCGGGCCGTCACTGCCCCGTAAATGCCGTTC
13	[160]	23	[175]	ATAGAACCTTATTTACACAACTCGTATTAGCA
13	[127]	22	[131]	TACACTTAATAAAAGGGACATTTAGAATACACT
13	[196]	24	[192]	CAGTGTTTTCACCTTAAATTAGGGATAG
13	[148]	14	[127]	TGGCGTAAGAATACGTGGCACAGACTACACT
14	[175]	19	[175]	GCGGTGCCCCTGCGGCGTTGGCAAAAACCCTC
14	[207]	19	[207]	AACGGCATCTCGTCATCCAGAGCAAACGTCAG
14	[232]	13	[232]	TACACTTGTGTTCAGCATGCACTCTGTGTACACT
15	[127]	20	[131]	TACACTAATATTTTGAATAAGGTTATTACACT
15	[163]	22	[160]	GCGAACTTCTGACCAACTAATCGTCAATA
15	[192]	22	[192]	TTTCTTTGCAGATGCCATGCTGATAAAAGCCG
15	[148]	16	[127]	ATTAATCGCCATTAAAAATACCGAATACACT
16	[207]	18	[192]	CCGCCGGGTGTCCAGCGCACCGTCGGTGGTGC
16	[232]	15	[232]	TACACTGGCGCTTTCGCCCTTACACTGGTACACT
16	[175]	18	[160]	ACTGTTGCTCAGTATTGCAACAGTGCCACGCT
17	[127]	18	[131]	TACACTCGAACCACCAGCATGAAAAATTACACT
17	[192]	20	[192]	GCTGGAGGCGCGGTTGACTTGTAGCATCCTCA
17	[160]	20	[160]	TGAGGCGGCTGATAGCCAAATATCATCAACAG
18	[236]	19	[236]	TACACTCAACCGCAAGAATGCCCTGGTCTGGTCAGCAGTACACT
18	[191]	15	[191]	CATCCCACCGTGCCGGCGGTATGAGGTAAAGG
18	[159]	15	[162]	GAGAGCCACTGAACCTCCTAAAACGTCTTTAATGC

19	[131]	17	[159]	TACACTCTAAAGCATCACCTTGGCAGCAAAGAAGATAAAACAGAGG
19	[176]	17	[191]	AATCAATAGCACGCCTAACACACCAGCTTACG
19	[208]	17	[232]	CGTGGTGAACGGCAATCAGCGGGGTCATTGCATACACT
20	[236]	21	[236]	TACACTGTCTCGTCGCTGGCAGCGCGGTCCGTTTTTTCTACACT
20	[159]	13	[159]	TTGAAAGGAGCACTAACTGAAAGCCAACAGAG
20	[191]	13	[195]	TAACGGAAGTTAAACGGGGTTACCGACGATCCAGCG
21	[180]	16	[176]	TAAATCTGGTCATGGTAATGGCCGGGTC
21	[208]	16	[208]	CGGCAAACCTCCGGAAACATCACTCAAT
21	[131]	15	[147]	TACACTCTAAAATATCTTTAGGAATTGAGGGGCT
22	[191]	11	[191]	CACAGGCGGGATCAAAGGTCATACTCGCGTCC
22	[159]	11	[159]	GATAATACACAATTCGATTGGCAGAATCGTCT
22	[236]	23	[236]	TACACTTGTGTACATCGACATACCAGCAGTTGGGCGGTTACACT
23	[208]	14	[208]	TTTGCCGAAAAAATGCGCCTGAATCGTT
23	[131]	13	[147]	TACACTGTATTAGACTTTACAAATTTGAGGATTC
23	[176]	14	[176]	GAAACAGCGCCTTTAGCTGCATCATGCAGCCA
24	[191]	12	[176]	CTCTCACGGAAAAAGACCTGTTCTCGGGGGGTT
24	[239]	25	[219]	TACACTCCGGAATTTGTGAGAGATAGAACAG
24	[159]	9	[159]	AACGTTATAAGGAGCGATCCAGAAAGTAGAAG
25	[220]	9	[241]	CGCCTCATAGCTGGAAGCATAAAGTGTAAAGCCTACACT
25	[208]	12	[208]	GAAACGTCTTTCTCTTGAGGAAGAATGC
25	[122]	11	[147]	TACACTCATTTTGCGGAACAAAGAAACCACCAGTAATTTTATGAC
25	[189]	26	[185]	CTCACCGGAAAGAACGGATAACTTTT
26	[159]	7	[159]	ATGATGGCCAAAATTAGAGTCTGTAAGTGTTT
26	[212]	10	[208]	GGAGCACGAGCCGTTTCCT
26	[181]	26	[160]	TTTTTTTTCCTGATTATCAG
26	[239]	27	[223]	TACACTGGCCAGTGCCAAGCTTTCAGAGTCACGAC
27	[113]	8	[116]	TACACTCTTCTGAATAAGCAATACTACACT
27	[137]	10	[144]	TAGAACCTACCATATAATTCATCCTTGCCTGCAATATTA
27	[189]	28	[189]	AAGTTGGGTAAGCAAGGCGATT
28	[159]	5	[159]	TGCGTAGAAACAGTACGAGGCCGATATGGTTG
28	[239]	29	[239]	TACACTATTACGCCAGCTGGCGTGCGGGCCTCTTCGCTTACACT
29	[176]	5	[191]	CGCCATTCAGGCTGCGGCGTCGTTTTTCCGTT
29	[130]	8	[144]	TACACTCAGATGAATATACAGTTTTTCAGGATCCTGAGCCATCACG
29	[208]	8	[208]	CGATCGGAAAGGGGGGAAACCTAACTCA
29	[196]	3	[191]	TGTTCTGGTGCCGGAAACCAGCATCTGCCGCCTGGCTTGCAGCA
30	[239]	31	[239]	TACACTTCGCACTCCAGCCAGCATCGGCCTCAGGAAGATACACT
30	[159]	3	[159]	GATTCGCCGCAGAGGCGCGTAACCAAAGGAGC
31	[179]	6	[176]	ACCGTGGCAAAGCTAACGTGCAGAATCAG
31	[130]	5	[147]	TACACTCAAGTTACAAAATCGCTGATTGCTGCGC
31	[208]	6	[208]	CGACAGTTTTCCGGGGGTGGTAATCGGC
32	[159]	40	[157]	AAAAGAAGATTACATTCTTGACGGAACGCGAGGTT
32	[239]	33	[239]	TACACTTGGGAACAAACGGCGGCCCGTCGGATTCTCCGTACACT
32	[191]	39	[194]	CGTTGGTGTTCATCAATCCCTTATTTACTATTTT
33	[130]	34	[130]	TACACTCAAGAAAAGAGTGAATAACTACACT
33	[212]	4	[208]	ACAAATTGACCTTGCCCCGGCAACA
33	[196]	48	[192]	AAATGTAAAACTTCATATGTTTAACATCAGCTATATTCGCAAAT
34	[201]	36	[187]	CGTCTGGCCTATTAAATTCGTTAATATTTTG

34	[233]	37	[220]	TACACTATAGGAACGCCATCAGAA
34	[167]	36	[160]	AACAGTACTTAATTTTTAGATTAA
35	[130]	35	[151]	TACACTCTTGCTTCTGTAAATCGTC
35	[208]	35	[233]	ATCAGCTCATTTTTTAACCATACACT
35	[176]	45	[187]	ATCCTTGAAAATTCGCTCCTGTAGCCGGTCAAAGCATGCTGGCA
35	[152]	45	[154]	GCTATTAAATAAATCAATCATAGGTAAATGCTAAA
36	[159]	50	[157]	GACGCTGAAAGAAAACTAATGCAAAACCAAGTAC
36	[238]	47	[233]	TACACTGGAAGATTGTATAAGCAAAGAGCTGAAAAGGTTACACT
36	[186]	57	[187]	TTAACCTGTTTCAATAAATAGCGTGTCGTACGCGTGTTT
37	[134]	3	[147]	TACACTTGAATTTATCAAAATATATGTCAAAATTAATGATGAAAAGAA
37	[164]	48	[160]	AGAGATGTTCAGTAATATCCAATAATCG
37	[221]	45	[233]	AAGAAGAGAATTAAAGCCTCAGAGTACACT
38	[179]	1	[187]	TTGGTATTTTAGAAAAAGGGCCCTATCAAAAG
38	[238]	37	[238]	TACACTCTGGAGCAAACCCCCAAAAACATACACT
39	[134]	1	[151]	TACACTTCGCAAGACAAAGGGAAAGCCGGGTCGAG
39	[195]	49	[196]	GAGAGCATTATGAAAAGAATTCAACTAAATGGAA
40	[179]	0	[168]	CTTCATAATTACGAAAAACCGTCTATCA
40	[156]	53	[164]	TGAAAAGGGCTTAATGCGTTAGCACCCAGCTACA
40	[238]	39	[238]	TACACTATGATATTCAATGCCTGAGAGTTACACT
41	[194]	51	[196]	AAGGGTTGAGTAATAAGCCTTTTTTAATTGTAAGA
41	[160]	51	[162]	AATAAACAAGTATCATATTGAGAACGGGAGGTCCG
41	[134]	0	[130]	TACACTATAAATAAGGCGTCGTGAACCATCACCCAAATTACACT
42	[233]	53	[238]	TACACTAACCCTCATATATCAAAGCGAACCAGACCGGATACACT
42	[175]	1	[171]	GCCTGTTTCCGGAATCTGACCTAAAATC
43	[129]	44	[129]	TACACTAAAGCCAACGCGCCAGTAATAATACACT
43	[148]	40	[134]	CAGTTACCGACCGTGTGTACACT
43	[187]	53	[191]	GGGAGGTGTAGGTGACTTCAA
44	[163]	59	[163]	TTAGAAGTAATTGGAATCATTACCGCACGAGCGCTAAACA
44	[233]	51	[220]	TACACTCATAAAGCTAAATCTTAG
44	[172]	34	[168]	ACATGTTCAAATAGTTATATATCATTTGATTAATGGA
44	[204]	34	[202]	AAAAAATCTACAACGGTAATCGTGAGCGAAATTCG
45	[188]	55	[187]	AGGCCCCTGTAATTTTTTGACTCCTTAGCCAT
45	[212]	1	[211]	GCAACGATGAAAGGCTATTGGTGGTAGT
45	[155]	54	[157]	AGGTAGCAGAGGCAGGCTTATTTTGAAGCAATCCAAAAGC
45	[129]	46	[129]	TACACTGAGAATATAAACTGTTTATCAATACACT
45	[173]	35	[175]	CAGTAAACAACACTACCTTCGATAGCTCCCTTAGA
45	[205]	35	[207]	AATTAGTAGCAACCCCGGTATTGTAAATTTGTTAA
46	[147]	38	[134]	GCGCGTACCGACGATGCAAATCCAATACACT
46	[233]	49	[220]	TACACTGGCATCAATTCTAGTTGA
47	[129]	36	[134]	TACACTCAATAGATAAGTCCTGAACGAAGAGTCAATAGTACACT
47	[176]	32	[176]	TTTACGAGAAACATAGTTTAACCTCCACTTTTATTACGCTTAGATGGG
47	[208]	33	[211]	GGGGCGCTATTTAATGATAATCAAAAATGTA
48	[238]	59	[232]	TACACTAACGAGTAGATTTAGTTTGAAAAACCAAAATATACACT
48	[159]	62	[157]	GCTGTCTTGAGCAAGAATATCAGACGGAATACAGA
48	[191]	58	[188]	GGTCAATACTATCATAGGTA
49	[197]	79	[195]	GTTCCAGAGGGACCCTCGTCATAGTAACACC
49	[134]	46	[148]	TACACTAAGAACGGGTATTGAAC

49	[221]	57	[232]	TTCTAGCTCAAATCGTCATAAATATACACT
50	[238]	49	[238]	TACACTATATAATGCTGCCAATTCTGCGTACACT
50	[156]	63	[155]	CGCGCGGAGAATTGCCTTTACATAA
51	[180]	40	[180]	GGCGTACCAACGTTTAATTTTCAT
51	[134]	43	[147]	TACACTAATCAGATATAGAATTTTCGATCAA
51	[163]	61	[164]	GTATTAAATAGCAAACTGAACGCAAACGTCCAAA
51	[221]	55	[232]	AGCACAGGTCACTATTATAGTCAGTACACT
51	[197]	61	[195]	GGTACAGTTCATGGATAGCAATAAAACAACA
51	[212]	0	[200]	ATGGCGGTTGTGCAAGGAGCTGATACCGGAGATTAAAGAACGTGGAC
52	[238]	51	[238]	TACACTAGCAAACTCCATTAATTGCTGATACACT
53	[165]	64	[164]	ATTCTTTCCAGTAAGAAACAGAC
53	[192]	63	[195]	ATATCGCGAAGAGGAACAAAAATCTAAATTGGGTTT
53	[134]	42	[129]	TACACTTAGTTGCTATTTTACAAATTCTTACCAGTATTACACT
53	[212]	1	[203]	GCTTTTTAAATGCAATGCCGAGAAAGGAATTAATGTAGG
54	[175]	44	[173]	ACGAGCGTTTATCCTGCCGACTTGTCGCCATACCA
54	[156]	68	[152]	CTAATTAAAGGTGCAGTAGCAATCACCGGCGCGTTTT
54	[232]	69	[220]	TACACTAAGCAAAGCGGATCCCAAATCACCAGGCGCGCGA
55	[188]	69	[187]	AAATGCCCGAAACCAAATCTAGAGTTTTCTCA
55	[203]	45	[204]	TCTTTTGCTTTAACATTTTGTAAATATGAGCAA
55	[128]	56	[128]	ΤΑCΑCTCAGCCATATTATAACATAAAAATACACT
55	[171]	45	[172]	TTTTTAAAAATGACTAAGAACTCATCGTACTGTC
56	[232]	64	[220]	TACACTTTCATTGAATCCCGTGAATTAACGA
57	[128]	58	[128]	TACACTCAGGGAAGCGCCCACAAGAATTTACACT
57	[171]	47	[175]	CTGAAGGGTAATTTCATCGAGAACCAATCCATCCTAA
57	[203]	47	[207]	CAATAAAGTTTTGTCATTCCAGATACATTTTTCATTT
57	[188]	71	[188]	AGACGAAAACGATCAAGATGGCTTGTATGCTTT
58	[187]	72	[184]	ATAGCTCAACGGGAACTCTTCATAACCGCAACGGCT
58	[232]	62	[220]	TACACTGCGAGAGGCTTTTGATTCATCCGTT
59	[176]	38	[180]	TAGCTATCCATGTAGAAACAAGCACATGACAAACGACACATAGG
59	[208]	45	[211]	CGACGATACCATTATAACACTAATAGTAA
59	[164]	77	[175]	ATGATAAGAAAAAGGAACCCCTCCTCAATAGCCCGGAATAGGTG
59	[128]	48	[134]	TACACTGAGTTAAGCCCAATAATAATCCTTATCATTCCTACACT
60	[249]	79	[227]	TACACTCATTCAACTAATGCAGATACACCAC
61	[123]	60	[123]	TACACTGAAACCGAGGAAACGCACAAAGTTACCAGAAGTACACT
61	[212]	51	[211]	GAAAGCAAAAGCTGCGGACATGTTTCGG
61	[148]	50	[134]	ATAAGAGATAACATTAGACGCCAATAGCAAGCATACACT
61	[173]	59	[175]	CATGCCCTTTTAATAGCAA
61	[228]	75	[231]	GAGAAAACAGCTGCTTGCTT
61	[180]	51	[179]	AAGATAATCAGACAAAGAATTTTTATTTGCGA
61	[165]	75	[167]	AGATAATAAGTTATTCTGA
61	[196]	75	[199]	ACATATGACAACAAAAGGAG
61	[205]	59	[207]	CAGTTACGAGGTTACCAGA
62	[219]	72	[216]	GGGACAGGGAGTGCAGCGAA
62	[249]	61	[227]	TACACTGGCTCATTATACCAGTCAGGAAGTT
62	[156]	72	[152]	ΑΑΑΤΑΤΑΑGCGTCTCACAAAC
63	[196]	73	[199]	AATTGAGGGTAGATATATTC
63	[229]	73	[243]	TGCCACCCTCATAAAGGCCGCTTTTGCTACACT

63	[212]	53	[211]	CATTCCTCAAAACCCTGAGGATTAGCGA
63	[123]	62	[123]	TACACTAAAGAAACGCAAAGACAAGGTGGCAACATATATACACT
63	[156]	70	[152]	GTTTGGGCGACACGCCAGCACCTCAGAA
63	[180]	42	[176]	AAATGAAACGTCGTTTATGACGAACCTCAATCTTACTAGAAAAA
63	[148]	52	[134]	CGGAAGAGAGAATTTATCCCCTTAAATCAAGATTACACT
64	[163]	73	[167]	AAAAATTTTGTCCTTGATATATACATGG
64	[219]	70	[216]	GAAAAGTTTCCAAGGCACCA
64	[178]	54	[176]	ACCGACTTGAGCAACGCTA
64	[249]	63	[228]	TACACTCAGTGAATAAGGCTTGCCCTGCCTTA
65	[212]	44	[205]	ATTATGCATCAAAAAGATTTTTAATTAGAGTACCATTTCAACACC
65	[123]	70	[118]	TACACTTAAATATTCACCACCCTCAGATACACT
65	[229]	71	[243]	AACCACTACGATTAAACGGGTAAAATATACACT
65	[148]	54	[128]	TCATTTGCCAGTTACAAAATAAATACACT
66	[146]	67	[159]	ACCCATCGATAGCAGCACC
66	[178]	67	[191]	TTAGAATCAAGAGCCGGAA
66	[249]	65	[228]	TACACTGGACAGATGAACGGTGTACAGAACGT
66	[167]	71	[167]	AAAATCACAATTATCAGCCGCCACTTGACAGG
66	[199]	71	[199]	CTTCATCATGACAAGAGGCAAAAGGACTAAAG
67	[192]	69	[199]	CGAGGCGCCTGCTCCATGACCCCC
67	[160]	69	[167]	GTAATCAGGACTGTAGAACCAGAG
67	[141]	66	[123]	AACATTAGCAAGGCCGGAATACACT
67	[123]	68	[118]	TACACTACGTCACCCATAGCCCCCTTATACACT
67	[216]	68	[227]	AGGGAACCGAACTGACTCGCC
68	[207]	66	[200]	TCCGCGACAGACGGTCTGGCTGAC
68	[226]	69	[243]	TGAAAAGTACAACGGAGATTACACT
68	[151]	67	[140]	CATCGGCATTTTCGGTAATGA
68	[175]	66	[168]	TAGCGTCATAGCGACAGAGCCAGC
68	[243]	67	[249]	TACACTTTGTATCACAACTTTGAAAGATACACT
69	[188]	68	[176]	TCTTTGTTACTTTTGCCTT
69	[221]	68	[208]	AACTAAATTGTGTCGAAA
69	[168]	55	[170]	CCACCACCCCCTCAGACCGTCACCAGCGCCAAGAT
69	[118]	70	[140]	TACACTTTAGCGTTTGCCATCTTTTCACTCA
69	[200]	55	[202]	AGCGATTAACGAAAGAACCGGATAACGAGTAGAGG
70	[183]	66	[179]	AAACGCCTGGAACACAGGGAA
70	[215]	67	[215]	ACCTAAATACCAAGCATAGGCAATCATA
70	[151]	66	[147]	CCGCCACCTAATCAAACCATT
70	[243]	65	[249]	TACACTCGTAATGCAAAGCTGCTCATTTACACT
70	[139]	64	[123]	GAGCGACGGAAAATTGAGGGAGGGAAGGTACACT
71	[189]	70	[184]	GAGAATACACT
71	[118]	72	[118]	TACACTGCCGCCACCAGAACCACCACCCCTCATTAAAGCCAGAATGGATACACT
71	[168]	64	[179]	AGGTTGAGGCAGGGAGGGTTT
71	[200]	57	[202]	ACTTTTTCATCGGAACTCAACTTTTCTACGTTGTC
72	[151]	65	[147]	AAATAAATAGAGCCGCTTCAACCGTTAT
72	[243]	63	[249]	TACACTGGGATCGTGATTTTAAGAACTTACACT
72	[215]	65	[211]	AGACAGCATGAGGACACCAGATTC
72	[183]	57	[170]	ACATCAGACGATTGGCACAATCAAGTATGTTAACC
73	[118]	74	[118]	TACACTAAGCGCAGTCTCTGAATTTACCCTTGAGTAACAGTGCCCGTATACACT

73	[200]	61	[204]	GGTCGCTGCGCCGACATATTA
73	[168]	63	[179]	CTTTTGATGATACACGATTACGCATAGA
74	[183]	61	[172]	CCCAGGAGTGTACTGGACTGG
74	[151]	63	[147]	GGTCAGTGCGTTCCAGCATACATAACCA
74	[215]	63	[211]	ATAGTTGAGGCTTGAGAAAAAAA
74	[243]	61	[249]	TACACTAATTTCTTTTAGGAATACCATACACT
75	[232]	79	[247]	TCGAGGTGGAAAGGAAAGCCCTCATAGTTTACACT
75	[118]	79	[140]	TACACTTAAACAGTTAATGCCCCCTGCAGCGGGGTATTTT
75	[168]	78	[168]	AACATGAAGGCTGAGAATGTACCGCCGCCACC
75	[200]	78	[202]	CCTTTAATAATTTTTTCAAACTACGTTAGT
75	[220]	74	[216]	ATCATGATACCG
76	[151]	61	[147]	TTAGGATTCTATTTCGATAGCCGAAATA
76	[186]	61	[179]	TCCAAATAAGAAGTATAAATTACCGAAGATT
76	[215]	61	[211]	GAATAATTGTATCGAAAGGAAGTA
77	[149]	79	[151]	TGATCAGAACCGGATAGCA
77	[208]	75	[219]	AACTTTCTCCAGACAACGCCTGTAGCATTTAACGCCAGTTT
77	[176]	76	[187]	TATCACCGTACTCGTAAAATC
77	[118]	77	[148]	TACACTAGGCGGATAAGTGCCGTCGAGAGGGT
78	[247]	78	[224]	TACACTAGCGTAACGATCTAAAGTTTT
78	[201]	79	[183]	AAATGAATTTTCTAGGAGGTTTAGTATAACACTG
78	[223]	76	[216]	GTCGTCTTAACAGTTTGGAATTGC
78	[167]	76	[152]	CTCAGAACCGCCACCCTATAAGTAGAGAAGGA
79	[152]	74	[152]	AGCCCAATGTAAGCAGGAACCTATTTTAACGG
79	[141]	78	[125]	CAGGCCACCCTCAGAGCCATACACT
79	[228]	77	[239]	AGACCAACTAAACAGCGGAGTGAGAATA
79	[125]	76	[118]	TACACTCCACCCTCTTTGCTCAGTACCTACACT
79	[196]	77	[207]	AGTACACGTTGATGGGATTTTGCTAAAC
79	[184]	74	[184]	AGTTTCGTGAGCAACAAAGGCTCCAACCATCG

7- References

- Stahl E, Martin TG, Praetorius F, Dietz H. Facile and scalable preparation of pure and dense DNA origami solutions. *Angew Chem Int Ed Engl.* 2014;53(47):12735-12740. doi:10.1002/anie.201405991 Castro CE, Kilchherr F, Kim D-N, et al. A primer to scaffolded DNA origami. *Nat Methods*. 2011;8(3):221-229. doi:10.1038/nmeth.1570 1.
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