Supplementary Information

Cellular delivery of enzyme-loaded DNA origami

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1. Materials

All reagents are commercially available and applied without any further purification. In the procedures the water used was either Milli-Q purified or autoclaved. Streptavidin-Lucia (LUC) luciferase enzyme (100 μg/ml, 37 kDa) and coelenterazine-based luminescence assay reagent

(QUANTI-Luc) were purchased from InvivoGen. 10x TAE buffer was purchased from Thermo Fisher Scientific. Ethidium bromide (EthBr) and agarose were purchased from Sigma-Aldrich. 10x TAE buffer Ultrapure (400 mM tris(hydroxymethyl)aminomethane) (Tris), 0.01 M ethylenediaminetetra acetic acid (EDTA) and glacial acetic acid for adjusting the pH to 8.3) was purchased from USB Corporation.

2. DNA origami and Streptavidin-Lucia luciferase enzyme

2.1. DNA origami annealing

DNA origamis were prepared by folding a 7249 bases long single stranded DNA scaffold from virus M13mp18 (Tilibit Nanosystems or New England Biolabs) with a set of short staple strands (Integrated DNA Technologies). The folding solutions were prepared by mixing the following components:

- 20 µl M13mp18 scaffold (100 nM)
- 40 µl staples (500 nM)
- 40 µl folding buffer containing 2.5 x TAE, 12.5 mM NaCl and 50 mM $MgCl_2$

The samples contained biotinylated binding sides for streptavidin functionalization (see the strand list). For transfection studies, the origamis were labeled fluorescently (5 Cy3-tags / 1 origami). For that, 5 binding sites were incorporated to the origami ends. The overhangs of the binding site strands are complementary to the Cy3-modified strand thus enabling a desired attachment (see the strand list).

Folding solution with the total volume of 100 µl was annealed using G-Storm thermocycler with the following ramp:

- 65 °C => 60 °C 15 min/°C
- 60 °C => 40 °C 45 min/0.25°C
- Store at 12 °C.

After the folding procedure, the origamis were stored at 4 °C in the fridge.

2.2. Purification of DNA origami

The excess amount of staples was removed either by PEG-based purification or by non-destructive spin-filtering process using Eppendorf centrifuge 5424R. For filtering, we used Millipore Amicon Ultra 0.5 ml Centrifugal Filters with 100 kDa molecular weight cut-off. Filtration steps are described below.

- 50 µl of DNA solution was mixed with 450 µl of filtration buffer (1x TAE, 5 mM NaCl, 20 mM $MgCl_2$, pH 8.57) and injected into the filter
- Solution was spun with 14,000 rcf, 3 min at room temperature.
- Flowthrough was discarded and 450 µl of buffer was added to the filter.
- Sample was spun in total 4 times repeating the procedure described above, expect for the expect for the last round the buffer was changed to HEPES-NaOH, pH 6.8 and the centrifugation time was set to 5 min.
- After the last spinning the solution was collected from the filter with a pipette and the rest of the solution was recovered by turning the filter upside down in a fresh container and spun 2 min at 1,000 rcf.

After filtration the volume of the solution was typically brought from 500 µl down to 17-24 µl.

PEG-based purification was carried out similarly as reported in Ref. [S1]. The purification steps are listed below:

- 200 µl of DNA solution was diluted four-fold in 1x folding buffer (1x TAE, 5 mM NaCl, 20 mM $MgCl_2$, pH 8.57) to obtain a starting volume of 800 µl.
- Solution was mixed 1:1 with 800 µl of PEG precipitation buffer (15 % PEG 8000 (w/v), 1x TAE, 505 mM NaCl) and mixed thoroughly.
- Sample was spun with 14,000 rcf, 30 minutes at room temperature.
- Supernatant was removed and the pelleted materials were re-dissolved in 1x folding buffer at the initial volume of the starting sample.
- The purified sample was incubated overnight at RT prior to use.

2.3. DNA origami with Streptavidin-Lucia

Streptavidin-Lucia (LUC) luciferase enzyme was added to origamis in 10-fold excess (100 µg/ml stock solution) and incubated at least 6 hours at room temperature. The excess amount of added Streptavidin-Lucia was removed by the same filtering methods as above (2.2.) (see also 2.4.). After filtering the origamis were stored at 4 °C.

2.4. Free enzyme activity

In order to ensure that Streptavidin-Lucia luciferase enzyme (LUC-enzyme) works properly, the activity of free enzyme was checked. The bioluminescence intensity of Streptavidin-Lucia was measured as a function of time. 10 µl of Streptavidin-Lucia enzyme (100 µg/ml) was mixed with ready 50 µl of coelenterazine-based luminescence assay reagent (QUANTI-Luc, InvivoGen). The results shown in Figure S1 demonstrate that the reaction is very fast. Therefore, in each measurement only the luminescence decay is monitored.

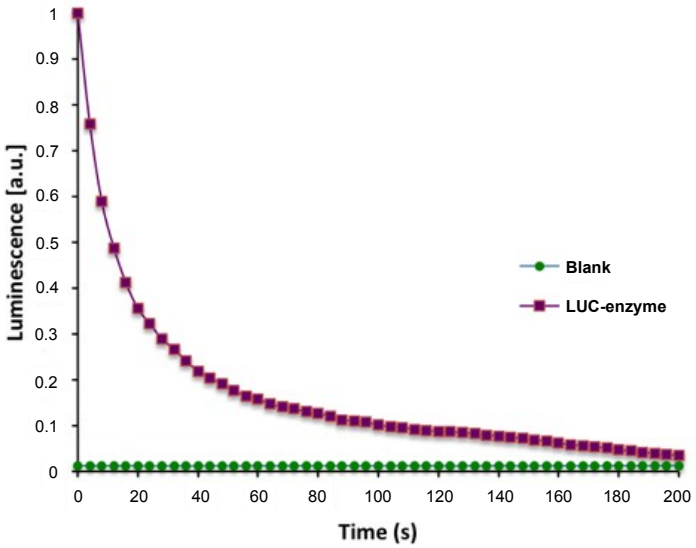

Figure S1. Characteristic luminescence intensity of the free Streptavidin-Lucia enzyme (violet) as a function of time. Here, the maximum bioluminescence intensity for the LUC-enzyme is normalized to 1. As a blank sample (green) we used autoclaved water (10 µl) mixed with the reagent.

In order to ensure that above-mentioned purification procedures (2.2 and 2.3) work efficiently for the LUC-enzymes, maximum luminescence intensities for free and purified enzymes were measured. The concentration of the purified enzyme was brought back to its theoretical initial value after the procedure. The graph below (Figure S2) shows that 4 times repeated spin-filtering removes LUC-enzymes efficiently. It is noteworthy that the first data points are measured around 10 seconds after adding the substrate, and thus the measured maximum intensities do not directly compare to the actual LUC concentrations (in order to get exact maximum intensities one should extrapolate the data). By extrapolating the data, one finds that the maximum intensity of LUC is over 10-fold compared to the filtered LUC.

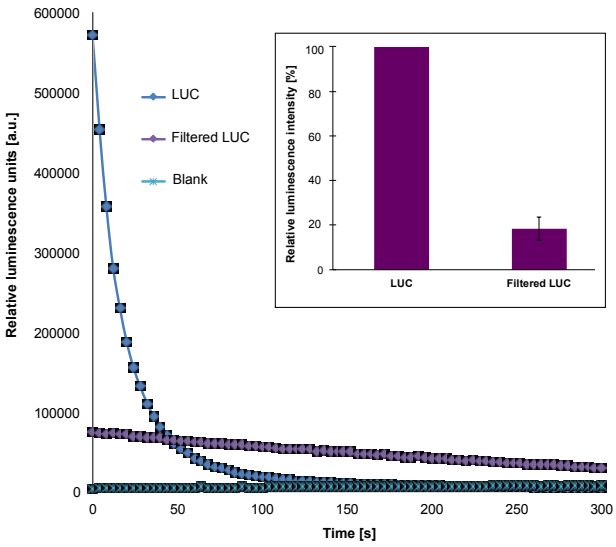

Figure S2. Relative luminescence intensity for the plain Streptavidin-Lucia (LUC) and the spin-filtered Streptavidin-Lucia (Filtered LUC). Free LUC is normalized to 1 in each measurement and the maximum intensity value is compared to corresponding Filtered LUC sample in the inset. Note that the first measurement point is obtained after 10 s of adding the substrate: therefore by extrapolating the data, the difference between LUC and Filtered LUC is actually even larger indicating an efficient purification.

3. Concentration of DNA origami determined by UV/VIS spectroscopy

DNA origami concentration (c_{DNA}) was estimated using Beer-Lambert relation, $A_{260} = \varepsilon_{260} c_{DNA} l$, where A_{260} is absorbance at 260 nm wavelength, ε_{260} is the approximated extinction coefficient (0.9 * 10^8 M^{-1} cm^{-1}) [S2] and l is the length of the light path in centimetres (0.05 cm). Final DNA concentration calculated after one or two filtration steps was 35-97 nM. The concentration was determined using BioTek Eon microplate spectrophotometer.

4. Agarose gel electrophoresis

The quality of origami folding and purification of DNA origami tubes were verified by agarose gel electrophoresis using BIO-RAD Power Pac Basic equipment. 2 % agarose gels were prepared by dissolving 2 g of agarose into 100 ml of 1 x TAE buffer (40 mM tris(hydroxymethyl)aminomethane, 19 mM acetic acid, 1 mM ethylenediaminetetraacetic acid) with 11 mM MgCl stained with 80 µl of ethidium bromide (EthBr) solution (0.625 mg/ml). 1x TAE + 11mM MgCl was used as a running buffer. Samples (10 µl) were stained with 2 µl of 6 X Blue Loading Dye (New England Biolabs). 10 µl of sample solutions were pipetted into the agarose gel

wells. As a reference we used an M13mp18 scaffold strand. The gels were run with a constant voltage of 90 V for 45 minutes.

5. TEM imaging

Transmission electron microscopy (TEM) images were taken with Tecnai 12 Bio Twin instrument. Samples were prepared on Formvar carbon coated or carbon only copper grids (Electron Microscopy Sciences) by placing a 3 µl drop of the sample solution on the grid. The sample drop was left on the grid for 1 min after which the excess solution was blotted away with a piece of filter paper. Samples were negatively stained by applying 3 µl of stain (0.5 % uranyl acetate in Milli-Q water) onto the grid and removing the excess stain with a piece of filter paper. Additional 3-µl drop of uranyl acetate was applied to the grid and excess liquid was blotted away after 20 s. Finally, the samples were dried under ambient conditions for at least 5 min before imaging.

6. Cell culture and treatment with LUC-origami complexes

6.1. Cell culture

HEK human embryonic kidney cells are from ATCC. HEK cells were maintained in growth media containing Dulbecco's modified Eagle's medium (DMEM) with high glucose (4.5 g/l), L-glutamine (4.0 mM), sodium pyruvate (4.0 mM), glutamine and penicillin streptomycin mix (HyClone-Thermo Scientific), supplemented with 10 % (v/v) fetal bovine serum (FBS) (Gibco-Life Technologies). Cells were maintained at 37 °C in a humidified (5 % CO_2) incubator.

6.2. Transfection of HEK cells

Cells were seeded at 50 000–70 000 cells/ml media on the coverslips and incubated overnight to let cells to attach. Cells were seeded at a split ratio 1:6 (a day before). Freshly grown HEK cells were plated on a 24- or 96-well plate, 24 h before transfection. The DNA origami was transfected into HEK cells transiently with or without polyethylimine (PEI) transfection reagent (25 kDa, linear, Polysciences # 23966-2). 1 µg of DNA origami was diluted into 50 µl of 150 mM NaCl and 2 µl of PEI solution (optional) was diluted with 50 µl of 150 mM NaCl and mixed. The formed PEI solution was added to the diluted DNA origami solution and mixed. The complex was kept 20 min at room temperature and finally the mixtures were added drop-wise onto serum containing media. The cells were then let grown at 37 °C for 12–48 h to allow the DNA origami transfection. Finally, cells were washed three times with PBS (each well for 5 minutes) and fixed in 4 % paraformaldehyde (PFA) for 15 min, after which the PBS washing was again repeated.

The cells transfected with DNA origami structures were detected using a fluorescent Cy3 dye for the origamis, Pacific Blue dye (labeled with Molecular Probes' Pacific Blue™ Protein Labeling Kit) for LUC-enzymes, and Cell Mask Deep Red (Invitrogen) for cell membranes. The fluorescent signals were detected with confocal microscope (Leica TCS SP5) with 63× objective. The 405 nm, 561 nm and 633 nm lasers were used to excite Pacific Blue, Cy3 and Cell Mask Deep Red, respectively.

The confocal images (article Fig. 4) show successful transfection of the origami complexes (both enzymes and origamis are located inside the cells). However, in some cases, the Cy3-signal from the HTB (green) is not co-localized with the LUC (blue), as it can be seen, for example, in Fig. S3. The plausible explanation is that the enzymes (especially the unspecifically bound ones) may detach from the origami in the transfection procedure.

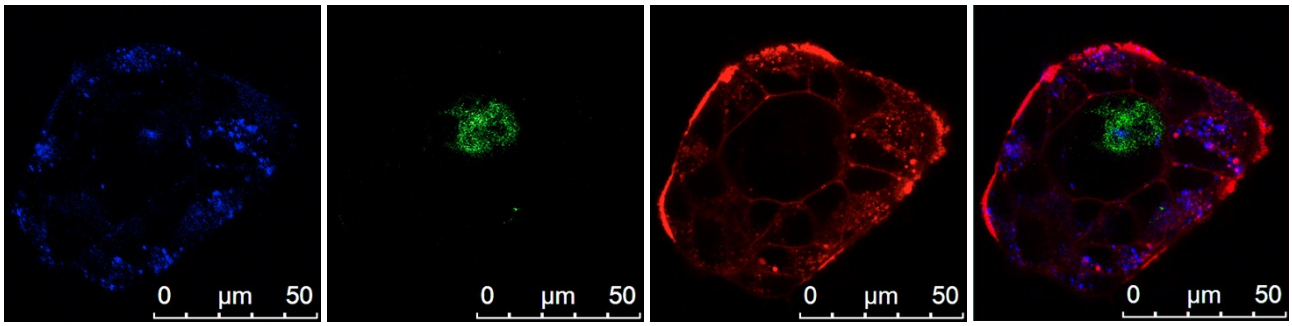

Figure S3. Similar confocal micrographs as in the main article Fig. 4A. Here, Cy3-signal from the HTB (green) is not co-localized with the LUC (blue) indicating that the unbound enzymes can be detached from the origami carrier during the transfection.

A longer transfection test (36 h) further indicates that the majority of the origamis are indeed located inside the cells. Fig. S4 shows that the Cy3-labeled origamis are found in close proximity of the cell nuclei or within the nuclei. Here, all coverslips were incubated with DAPI (Sigma Aldrich) nucleic acid stain (1:10 000) in PBS for 1 min, washed three times in PBS and mounted onto microscope slides using ProLong Gold Antifade Reagent from ThermoFisher. Images were taken by a confocal microscope (Leica TCS SP8, 405 nm violet blue diode laser and 514 nm argon blue laser) using a 63× objective (HC PL APO CS2 with glycerol).

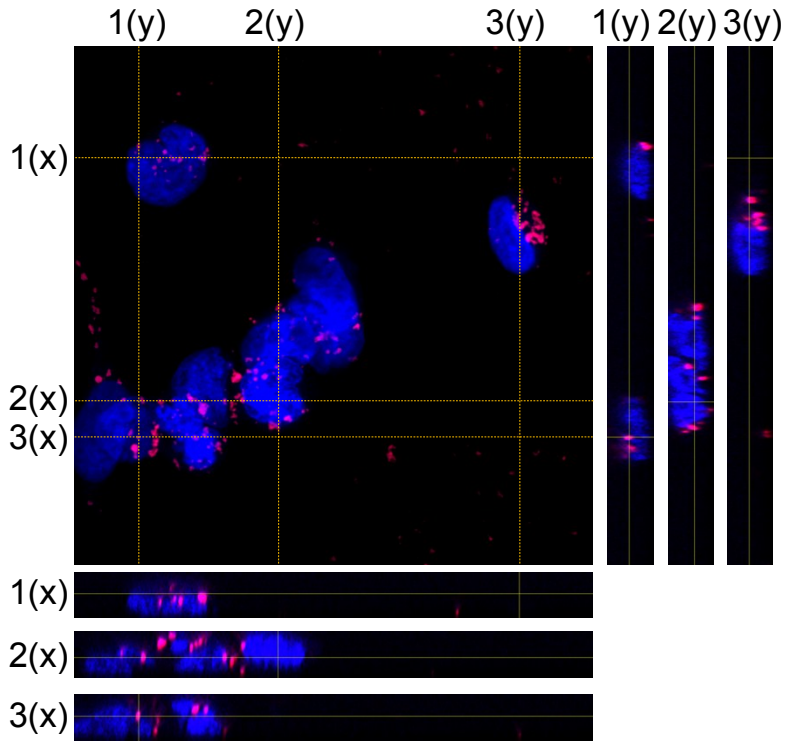

Figure S4. Confocal microscopy image of HTB transfection (z-stack projection) with orthogonal cross sections (x and y directions). Here HTB is labeled with Cy3 (red) and cell nuclei are labeled with DAPI (blue). The Cy3-labeled origamis are in close proximity of the cell nuclei or inside the nuclei, indicating intracellular localization of the origamis.

7. Luminescence assay for LUC-HTBs and controls measured from cell lysates

As mentioned in the main article, we have also tested samples that were not purified and contained unbound enzymes. These samples allow an easy adjustment of LUC in each sample, and these samples act as controls in studying the effect that plain DNA molecules may have in the transfection. We used 4 different sample types: LUC+HTB, LUC+staples (same staples and same amount of staples as in the LUC+HTB sample), LUC+scaffold (same amount of scaffold strand as in the LUC+HTB sample) and bare LUC. Each sample had excess amount of LUC (15× excess to HTB), but importantly, the amount of LUC was same in each sample. We measured the luminescence intensity from the cell lysates after 12 h transfection with or without PEI (transfection procedure was similar as for the other transfected samples). The results can be seen in Fig. S5 and S6 (left-hand side histograms). The results show that LUC+HTB and LUC+staples have similar intensities, whereas LUC+scaffold shows less activity. Importantly, all these samples have higher

activities than bare LUC indicating that plain DNA can increase the transfection. Moreover, PEI as a transfection reagent seems to enhance the transfection as expected. However, PEI does not change much the ratio between the intensities. The same is true for the right-hand side histograms in Fig. S5 and Fig. S6, where the purified LUC+HTB, bare LUC (adjusted), and bare HTB samples have been transfected and their intensities compared with or without PEI.

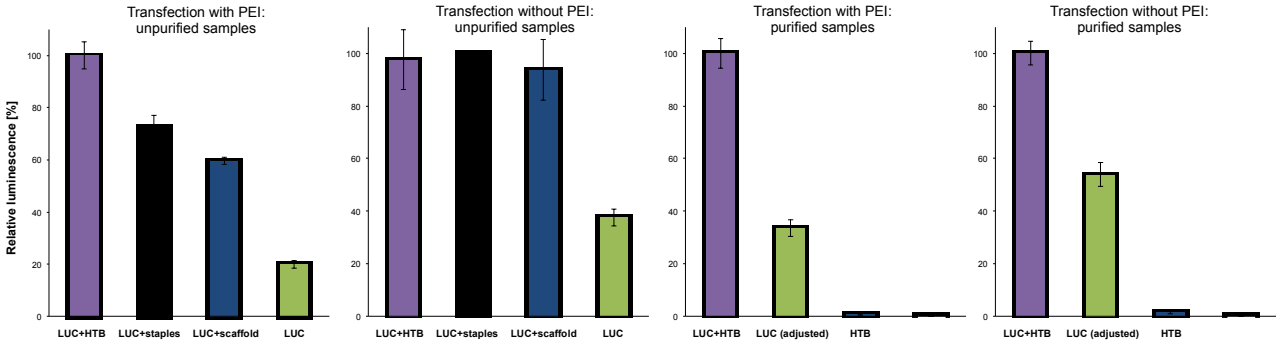

Figure S5. Normalized maximum luminescence intensities measured from cell lysates after 12 h transfection. In each measurement the highest intensity value is presented as 100 % relative luminescence.

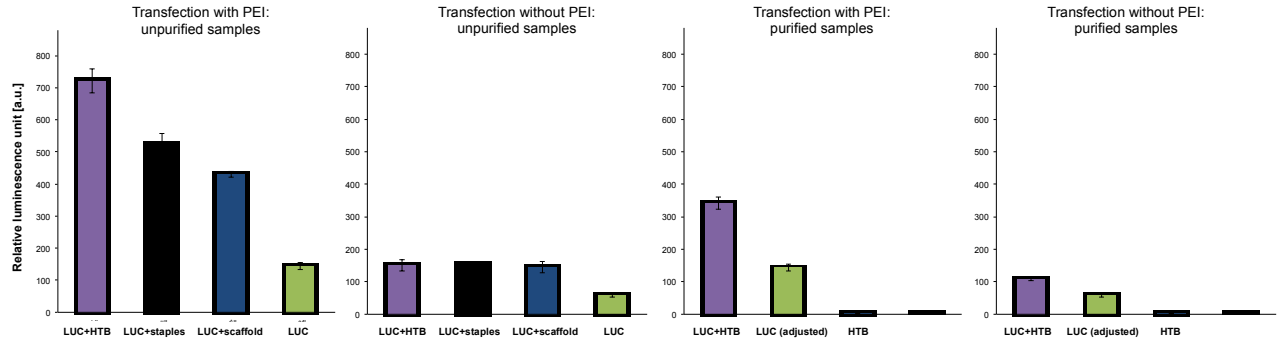

Figure S6. Same data as in Figure S5, but presented in relative luminescence units.

8. Strands for DNA origami

The complete set of the staple strands for DNA origami are listed below. More information available from the Ref. [S3].

Biotinylated strands for HTB (3 strands):

Sequence (5' -> 3')	**Bases**
Biotin-AAACATTAAATTTTGCTCCAACACGTTG	28
Biotin-AGCTTTCAACATTAAATAGTGAATTTGCCAGAATGATTGAC	41
Biotin-	38

ACGAGGCAATTCCAACGAAACGCAAAGACGTTCAGCTA	

Cy3-labeled fluorescent strand, optional (1 strand):

Sequence (5' -> 3')	**Bases**
Cy3-GGGAAAGGAGAAAAAA	16

Binding sites for the Cy3-labeled strand (5 strands):

Sequence (5' -> 3')	**Bases**
CAACTAATCATAACCAGACGACTGGATAGCGTTTTTTTCTCCTTTCCC	48
TGGTCAGTACAGTTGACAGGTCAGTTTTTTCTCCTTTCCC	40
CCAAGCGGCCTGATGAAATCCTGAAAGAGGACATTTTTTCTCCTTTCCC	49
GAATACGAAACCGGATAGCCAAGCCCTTTTTAAGAATTTTTTCTCCTTTCCC	52
AAGCTTGAATCATGGTTTTTTTCTCCTTTCCC	32

Core strands (91 strands):

Sequence (5' -> 3')	**Bases**
CGTAATACATCAACATCTGGCC	22
AGGCAATGCAGCTGATTGCCTTAAACGGGCCTAAAAAGGCGTTGCTTATC	50
CAATCCAATTTATTTACTCATCCAACATATAAAAGAGCATGTAAAACCAA	50
TATATTTAGGATAAATGACCCAAGAATT	28
TTTCACCGCAGCAACCGCGAAAGAC	25
AATTCGGAAAAGCCCTATAGCCCGGAAAATATAATCAATTGATA	44
GAGCTGCTCAGAGAAAATACGTGAGGC	27
AATATGATACAAACTACAAGGTTTCAGGCCACCCTTCTAGGTGT	44
TAGTAAATTTCAACCCGAACCTCAA	25
AATTCACAGAGCCCTGACTATTATAATTATGTA	33
CGCGAGATCTTCTATAAGAACTGTTT	26
CAGCACCTTTTCATGGAAGGGCGCCAT	27
AATGCTTATAAATAAGTAAAATAACGGA	28
TTTCAGAAGATAAAACAGAGCGAACGAATATACGTGG	37
TCAATCACAGGTCAAGAACCGGATAGCA	28
TTGCCCTGACGATAATCATCTAAAGAA	27
CGATTAAGTTGGTGACCTTCAAAAGCTGGCGTTAAGACCTAA	42
ATCATTTTATCAGTTTGGATACGTAAATTTAACG	34
GAAATACGCATTTTCGAACCAGACAGCCAGGTTTGAGG	38
TGTTACTTGGGAACCTAGGCTGGCGTAACGCCAGGG	36
CTTGCTAAAAAAAAGTAGGATGGCTTAGA	29
CACAGACAATAGCCATTACATGGAA	25
TCACCCTCAGCAGAAATCGGCAACATTAGACG	32
TTGAAAACTCTGAGAAGGAGGTTGAAATCAAAATCATAGGATAGCGATAG	50
TTCCTGTAGTTACGAGGCATAAATAGCG	28

CGCCATTCGATCGGAAAGGGGACGTTGTGCAGGTCCGATTGACAAAGAC	49
GAAGCGTTGAGTTAAGCAATAGACGCTGGAGGGTGG	36
AGCCCAATCACCAGTATTCAAAAAGGGT	28
GGTCATTTTTGCGAACCCTCAGAGAAAGGCGGAGTGTCTTTCCAGACGT	49
ATCGGTTATAAAGCAAAAGGTTTAAAGGCCGCTGTTTAGCTATGGGGCGC	50
TGTGATGAAACCATAGCAAGCGCCATAGCATTTT	34
CTTAGATTGAGTGAATAATTTTCGTTGGGTCAATCG	36
GTACCGCTCATCGTAGGAATCCTATTATTTATCC	34
ATCACCGTACTCCACCCTCTTGCCTGGAGATCTACAAAGGCTGTCAGAAG	50
ACATGACATTCAACGACTCTAGAGGAAGACGGTCAATAAACA	42
AAGGTGGGAGAACACTTTCCAGAATCGG	28
GGCAGAGTTTAACAACGCCAAAGCACCAAGTCACGGATGTGCTGCAAGG	49
ACAGTCAAAGCGAAAAACAACTGAATTTTCTGTATGGGAAGG	42
TAATTGCTATAATGAAGTACGGTGTCTAAAGCTAAGCTTAATCATCAC	48
TACATTTGACGCCTGTAGCATTCCACAGTTTTGTC	35
AACGATTACCAGAAGCCAAAAGAACTGCAAGCCGTTATAAGA	42
TATCGGTGAATTACCAAATCTAGGCTTAGCCTTAGAATCC	40
TTCGCGTTAATGCCCCAGAGGAGAGGCTTTTGCAAAACATTAAATTT	47
CACCCTAGCATTGACGACTACCTTTTTCACCCTCCCGGAACGGTTT	46
TAAAGTGTAAACCTGTCGAAGAATACACTAACGCCGGAAGCA	42
CGAAAAACCGTTGGAAATACAACTGAACACCCCGTCAAAGGG	42
ACGCTCACTATCAAGCCATTGCTGACCT	28
TTTTTCTCCAACGCGTTTTTTGTTTAA	27
TTGAGTCACCCTCATATTTAGATTCAAATCACCATC	36
ATAACCGATACCACCAGCTTAAACAGCTTGCATCGCCCACGC	42
TGAAACAAACATCTGAGTAACTATTTCGGAAGGATTAGGATGCGTAG	47
AGTATCGTCACCAATAAATAAGCTCATTC	29
GGACGAACTAACGGAGGGATAGGTCACTCTGCCACTTTCCG	41
GGTCAGTTCTAAAGTGCTGAATCCTTTTGATAAGA	35
AGTTGAGGGAAGAATTATGCGTCAACTTGAAACACACGTAAC	42
GAAAGCGTAAGAATTCGGTCGCAGGGAGGGCATCA	35
TCAGATGGAAACAATGTTTAGACGATAA	28
TAATTTTCAAACAAATATCGCGGAAGCA	28
CGAGCCAGACGACAATCATAAAGCCGGA	28
GGAGAATTCTACATTTTAACGAGCGTATAAAAACAGG	37
TTTTCCCTTACCATTCGATAG	21
ATTCTACAGCAAAATTAAGCAGTACCAA	28
CGGGCAACCAGCTGATAAACAGCCATAAGAACGCGCGAAAG	41
CGGTATTATTACCGGGGTATTGAAACCATCCCATC	35
CAAGACCAGAGCCGCAACCTCCCGTTAATTAGAAAGCGCCAAAAGGAACC	50
AGTGACAACTGTTGCGCGACCG	22
TCAAGAGAGGCGCAGAACTGAAATTCTGTATCAACAATAGA	41
GCCTGTTTCCAGACGTAATAAGCTTAAT	28
GTTTTGCTCAGATATAAGCAAAAACTAGCATG	32
ATTTTCAGGACAGAAATAAAGAAATTTAGCGGG	33

GCCTAAATCAAGATCACTTCACCGCCTGCGAGGGTCTTTTGCGGGATCG	49
GGTGGTTGCGGTCCCTTTTACAGAGAGAATAACCTTTCCAGA	42
AAAAGGGCACCACGTGTTATCGGGTGCC	28
ATTTGACAATATATGTAAGACGCTGAGACATTTAGCAAAAGCACTGATTG	50
CTTTGAATACCATTTCAATCAACACTATGCAGATACATAAATTCATC	47
GAGAATAATTTTTTAAGGAGCGAGGTGAA	29
CGTCAAAACATTAATGTCGGGAAAGCCTGCGCTCACGCTCCCCGGGTACC	50
ATATCAAACCTTTTGCTCCAGACCGTTTTAAGCATCAAATCAGGT	45
TTGAGGACTCAATCTGAAAAA	21
CCCCGGTCCCCCTCACTTTACCACAACATT	30
CAAAATTAATTAAGAGTCTTACAGGAAAACGACGACAG	38
CAAAGCGGATTTTCGAGCAGTATTATAGATAA	32
AGCATCGGAAGCCCTGAGAGAGTTAGTGAGA	31
TCAGGCTGCGCACTCGCCACCAAGAACCGC	30
ATCAGAAGTTTTGCCCTGCCAGTGCCCGTATAAAAAGATGA	41
GGGTAATTTCATTGCTGATTGATGATGGC	29
GAGCTCGGTGAAATGAATAAGATACATA	28
AAACCAAGTAAGAGTACCTGAACAATTTC	29
ATGCAGAACGCCTAATTTCACAAC	24
ATTTAATCGCCTCCTGCCTCAGGAAGATCGATAAGGC	37
AAAATCTAGTTTCAGCCGGAG	21

Left core strands (20 strands):

Sequence (5' -> 3')	**Bases**
CTGCGGCTGAATACATCATA	20
ATTAACAAAACATCTTTTTGAAACCCTTCAACACGACCAGT	41
AAGAAATATCATCCGAAACA	20
TATTTGCTATACTTAATCGTCTAAACAGTTCAGAAAACGA	40
AAAAGCTAAACACCAAATCACAGAACGAGTAGTAAA	36
TGCGGGAGGTTTTGAAGCCTTAATTTGCCAGTTACAAAATGAAAATA	47
ACTAACAACTAATAGATTAGAGCCGTCAAGACTTTAAAAAGT	42
GTTTCATCGTCATTTATTTAGAAATGGTTGAAATGG	36
CAAATCTATATAAGACGTTGATTTAGGAA	29
GACTTCAATTCGACAACT	18
ATCGGGAAATATACTCAAAAT	21
AAGTACAAAACACTCAAGAACCGCCCAATAGCAA	34
GAATGACCATAAACAAAGAACGTTAT	26
TACAATTTTATCCTGAATCTTACCAACGCTGACGCTC	37
ATACGAGGAGATTTGAATAATAATCAATAATCGGCTGT	38
TTCACCAGTCACAGGAAAAAATCGTCCAATAACAGCAACG	40
TGAGAATGCCGGAAACATATGCGTTATACAGTAGGGAGAATATAAAGT	48
ATTAATTAACCTTGCATAAATATTACCT	28
TAAAGTCCAACTTGCGACCTGCTCCA	26
CAACTACTGTAGCCAGCAAAAATATC	26

Right core strands (15 strands):

Sequence (5' -> 3')	**Bases**
TCGATGAACGGTAATCGTAAAACAGGAAGAAATATTTTTATTCT	44
TAATAGTGTTGAGTGTGCTTGCTGAGTGTTCCAGTTTGGAACAAG	45
AGGTAAAGATTCACCGTTCTTTTTTGAAGAGTCTGGAGCAAACAAGAGAA	50
CGTTCCAGTAAGCGTCATACATGGCAAGAAAA	32
TGAGCGAACGGCGGGAAAGCGATAAATC	28
TAATGAGCTGCCCGAGGCATGATTAAGAAAAATACTTTATTTTGT	45
AGTTTTAACGGGGTCAGTGCCTTTTTGATGATTCGCGTTAAATG	44
CTCATTAAGGCAGGTCAGACGATT	24
GTCATAGTAGCGCGGTAATCACACCAGTCACCGTCACCGACTTGA	45
TAACCGTGCAGTTGGTGCCAGAGCCGCCGCCCAGAGCCTCACCGG	45
TTACCAGCGCGGGAGGGGGTGAATTATCATGTAATTTA	38
CTGAGTTTCGTAGGAACGAACCGCAGGAGGTAGGGTTG	38
GCAGCCATCTTACCGGAACAAAGCGGGGAGAGGCGGT	37
AAGAACGTGGACTCCAATGAACAAAGTCAGAGGGTAATTGAGCGCTA	47
TACCAGGGAGACTCCTCAAGAGAACCTATGTTAA	34

9. Supporting information references

[S1] E. Stahl, T. G. Martin, F. Praetorius and H. Dietz, *Angew. Chem. Int. Ed*., 2014, **126**, 12949-12954.

[S2] A. M. Hung, C. M. Micheel, L. D. Bozano, L. W. Osterbur, G. M. Wallraff and J. N. Cha, *Nat. Nanotechnol*., 2010, **5**, 121-126.

[S3] V. Linko, M. Eerikäinen and M. A. Kostiainen, *Chem. Commun.,* 2015, **51**, 5351-5354.