Supplementary Information

Binary Control of Enzymatic Cleavage of DNA Origami by Structural Antideterminants

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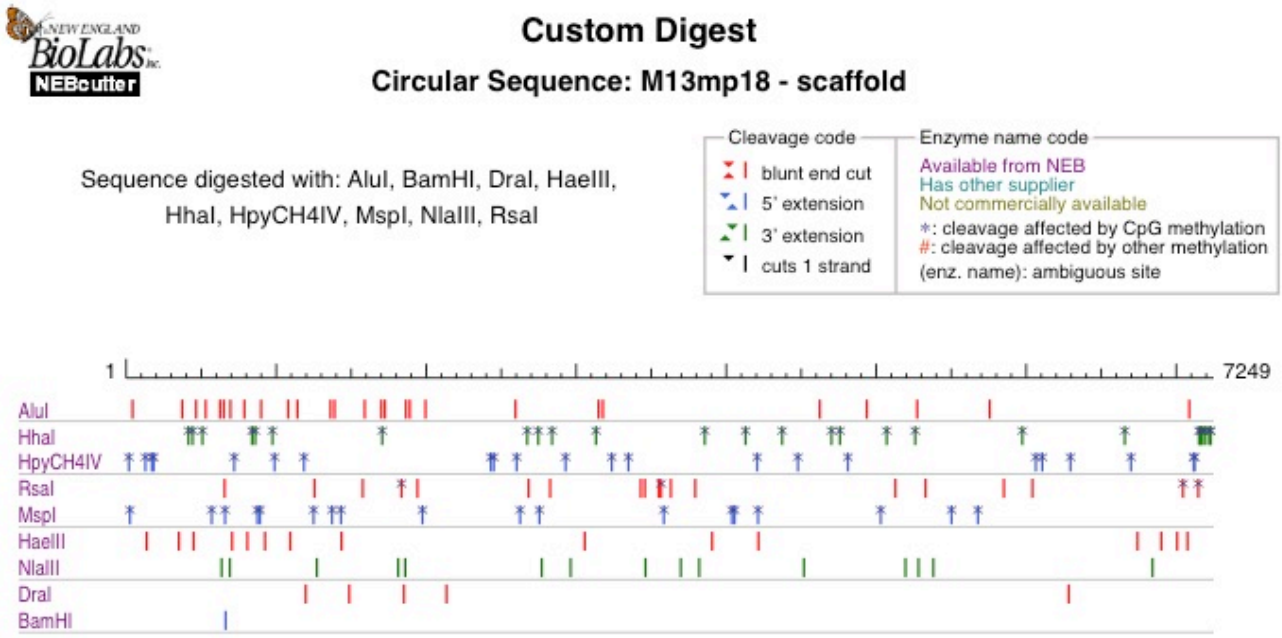
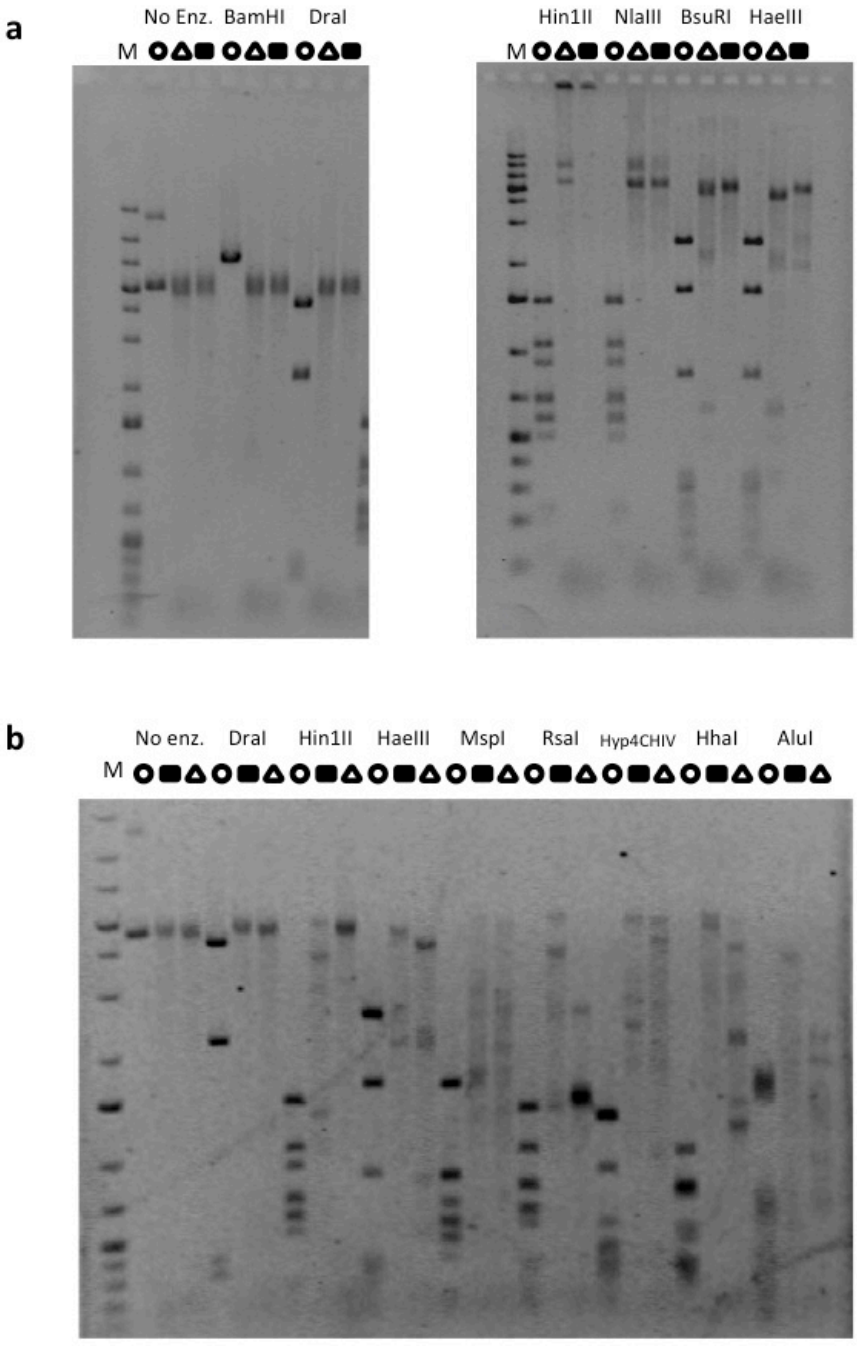
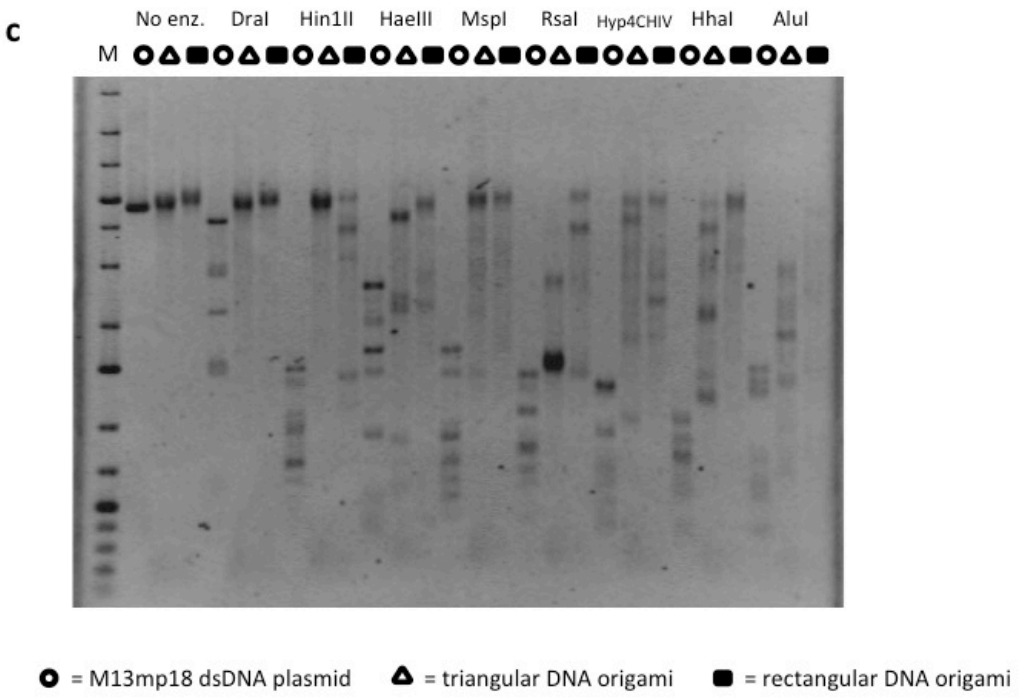


#	Enzyme	Specificity	Cuts
1	AluI	AG▼CT	27
2	BamHI	G▼GATC▲C	1
3	DraI	TTT▼AAA	5
4	HaeIII	GG▼CC	15
5	HhaI	G▲CG▼C	26
6	HpyCH4IV	A▼CG▲T	22
7	MspI	C▼CG▲G	18
8	NlaIII	▲CATG▼	15
9	RsaI	GT▼AC	19

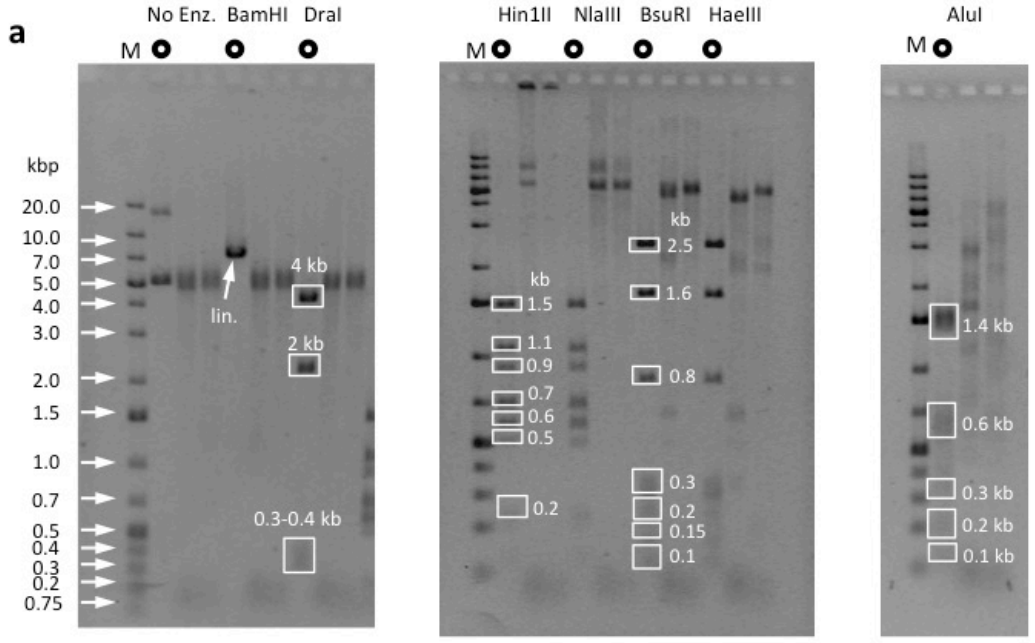
Supplementary Figure 1: linear distribution and number of restriction sites for investigated restriction endonucleases in M13mp18 DNA sequence that is used as scaffold to form DNA origami. The table lists the selected restriction enzymes with the sites sequences recognised, and the total number of sites in the M13 DNA sequence. NlaIII and Hin1II share the restriction site as well as HaeIII and BsuRI (isoschizomers). The shown data were generated with NEBcutter V2.0 (online tool, http://nc2.neb.com/NEBcutter2/) by inputting the M13mp18 DNA sequence (see page 30 of the SI).



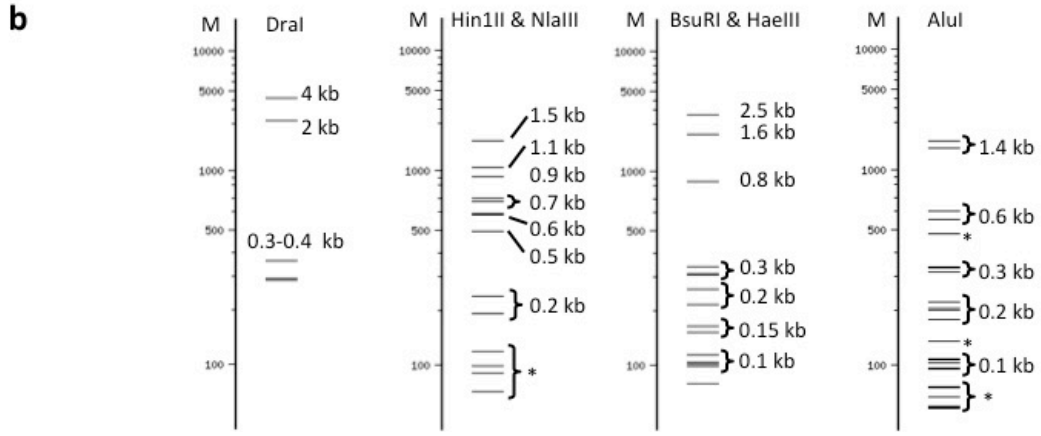
Legend: ○ = M13mp18 dsDNA plasmid △ = triangular DNA origami ■ = rectangular DNA origami

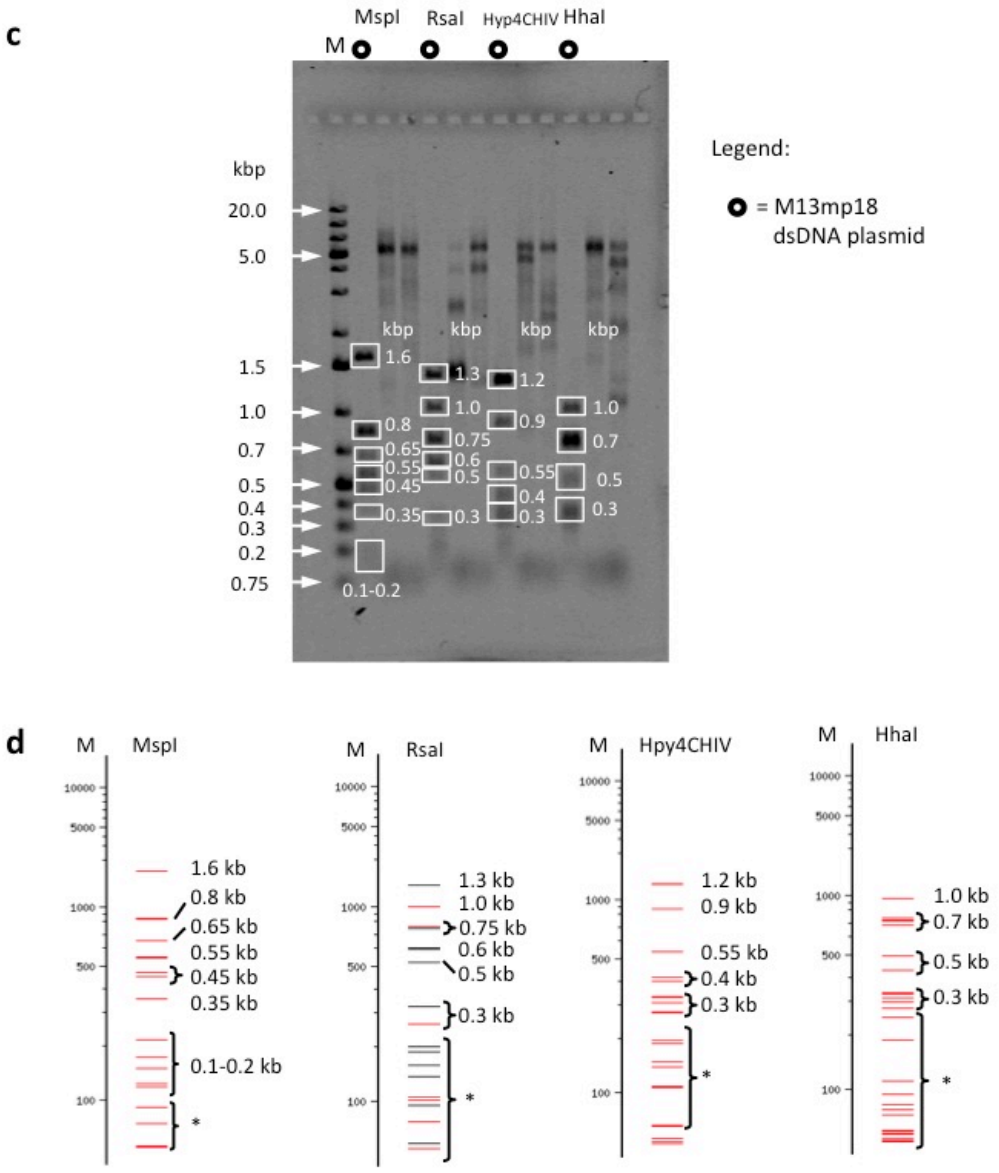


Supplementary Figure 2: Repeats of the experiments showed in Fig. 1d (a-c) Photographs of 1% agarose gels that display digestion products of the action of restriction endonucleases on the sharp triangular DNA origami, rectangular DNA origami and M13 dsDNA plasmid. (a) DNA residuals are present in the gel wells for both nanostructures treated with Hin1II in "G" Buffer. In (b-c), for Hin1II we changed to Tango Buffer. The digestion efficiency can vary on restriction enzymes aging. M, 1 kb molecular weight marker.

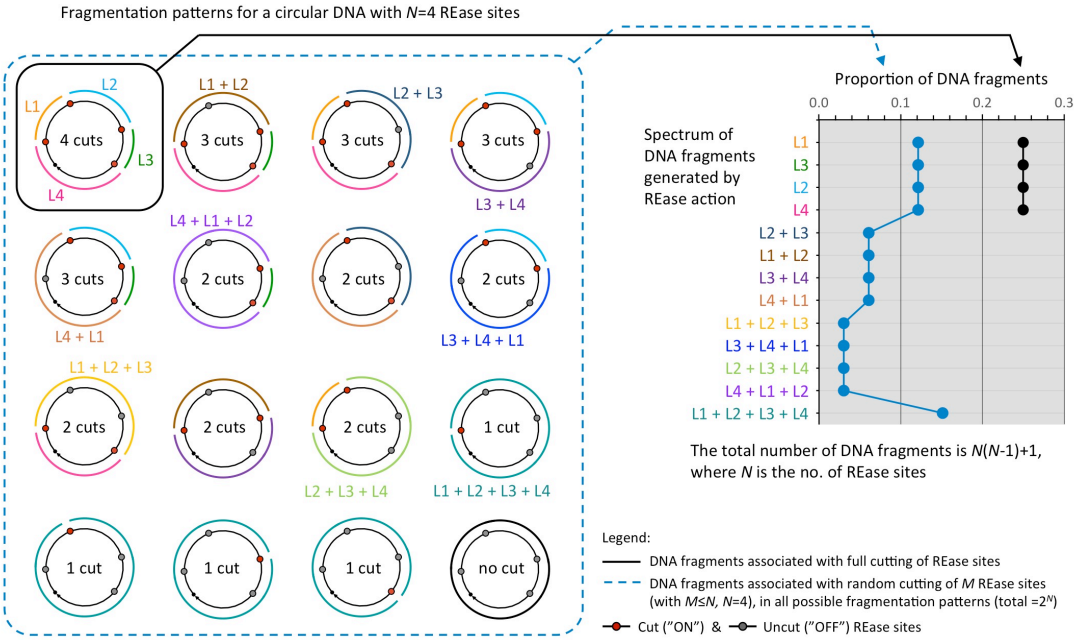


Legend: = M13mp18 dsDNA plasmid

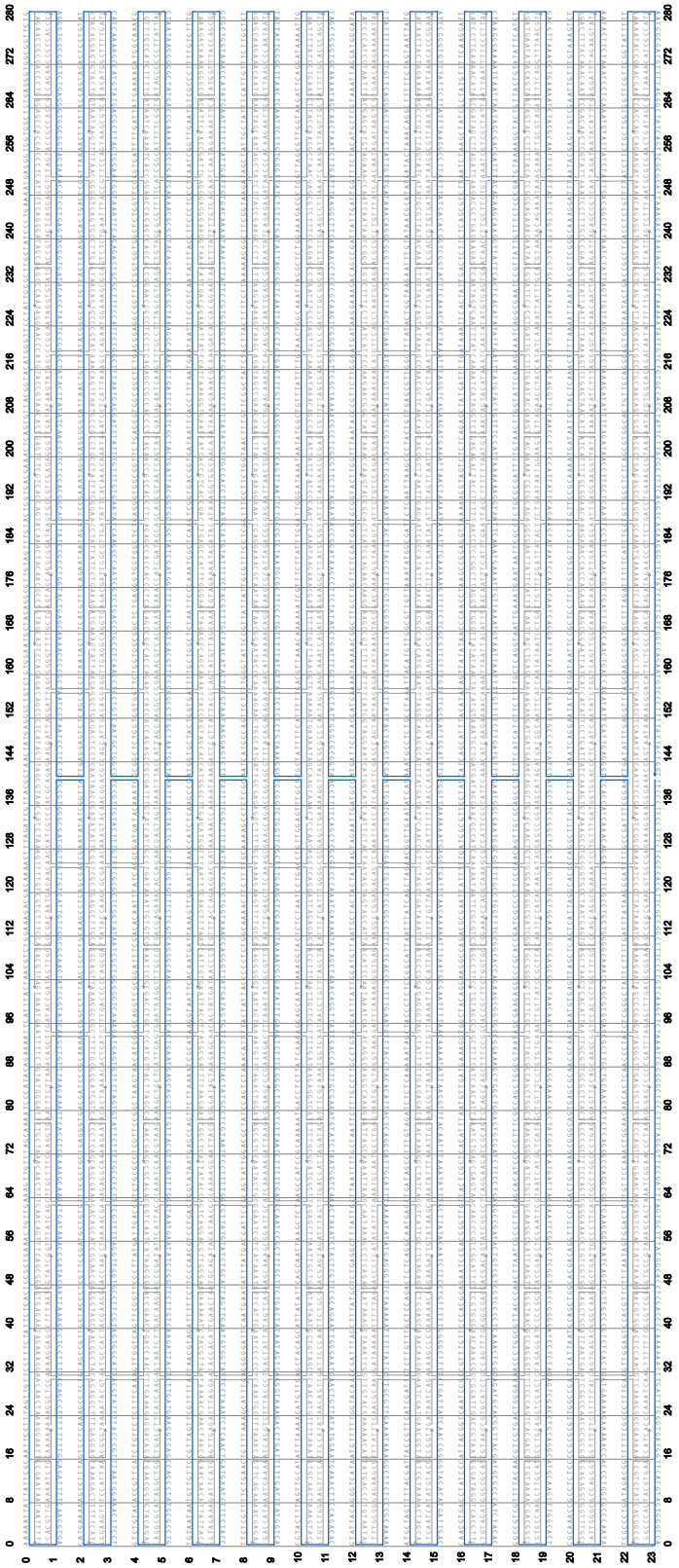




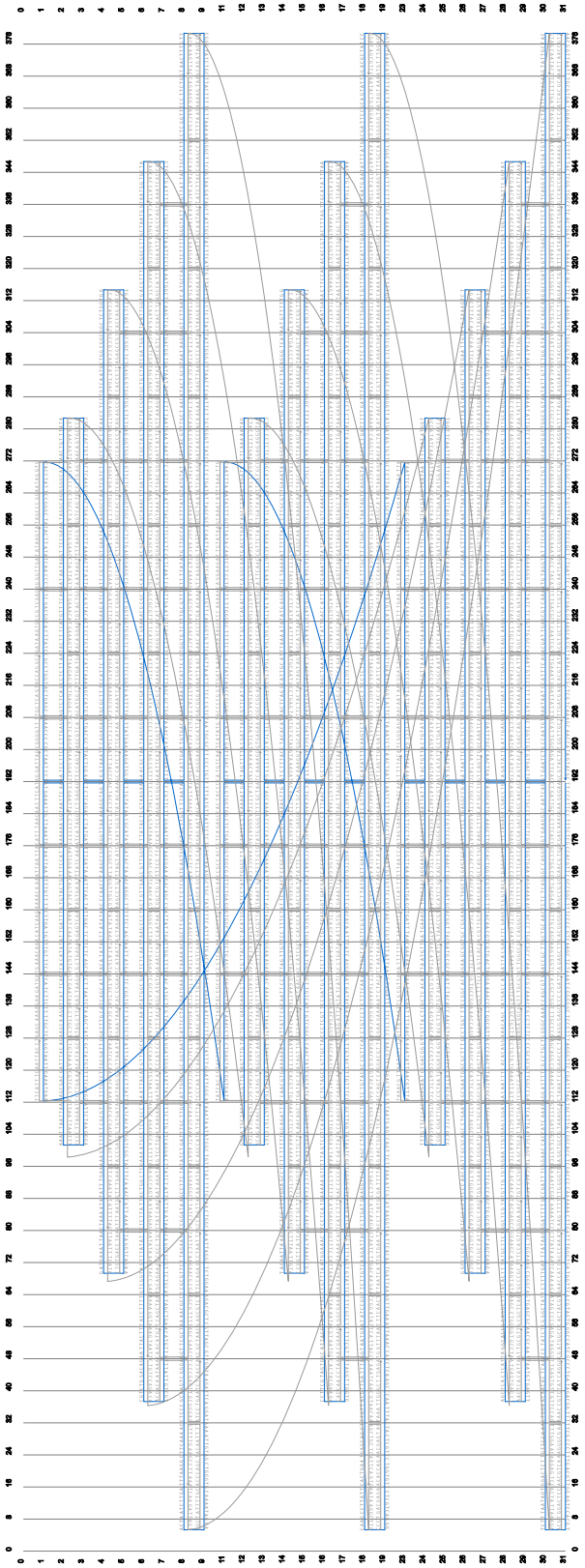
Supplementary Figure 3: Analysis of number and molecular weight (MW) of M13 dsDNA plasmid fragments produced by the action of REases. (a-c) Gel images from Supplementary Figure 2 and Fig. 1 were analysed, and each gel band corresponding to a M13 dsDNA fragment or group of fragments (with similar MW and migration distance) is highlighted (white box) and with an estimation of MW is provided. Isoschizomer REases (Hin1II and NlaIII; BsuRI and HaeIII) share the same restriction sites, and the analysis was performed for only one REase. (b-d) Diagrams representing virtual gels analysis of fragments produced by the complete action of each REase on the M13 dsDNA plasmid, as generated with NEBcutter V2.0 (http://nc2.neb.com/NEBcutter2/). Setting parameters for each REase: "Gel type" = 1% agarose; "L" (the length of the gel) = 90 mm; inputted DNA sequence = M13mp18 (see page 30 of the SI). Fragments with similar MWs are clustered as they cannot be accurately resolved with agarose gel electrophoresis. The good match between the predicted distributions of M13 fragments and the gels bands suggests that the M13 dsDNA plasmid was completely cut by all REases under investigation. *, gel bands difficult to be recognised or that are not visible in experimental gel; M, 1 kb molecular weight marker; lin., linearised form of the M13 dsDNA.



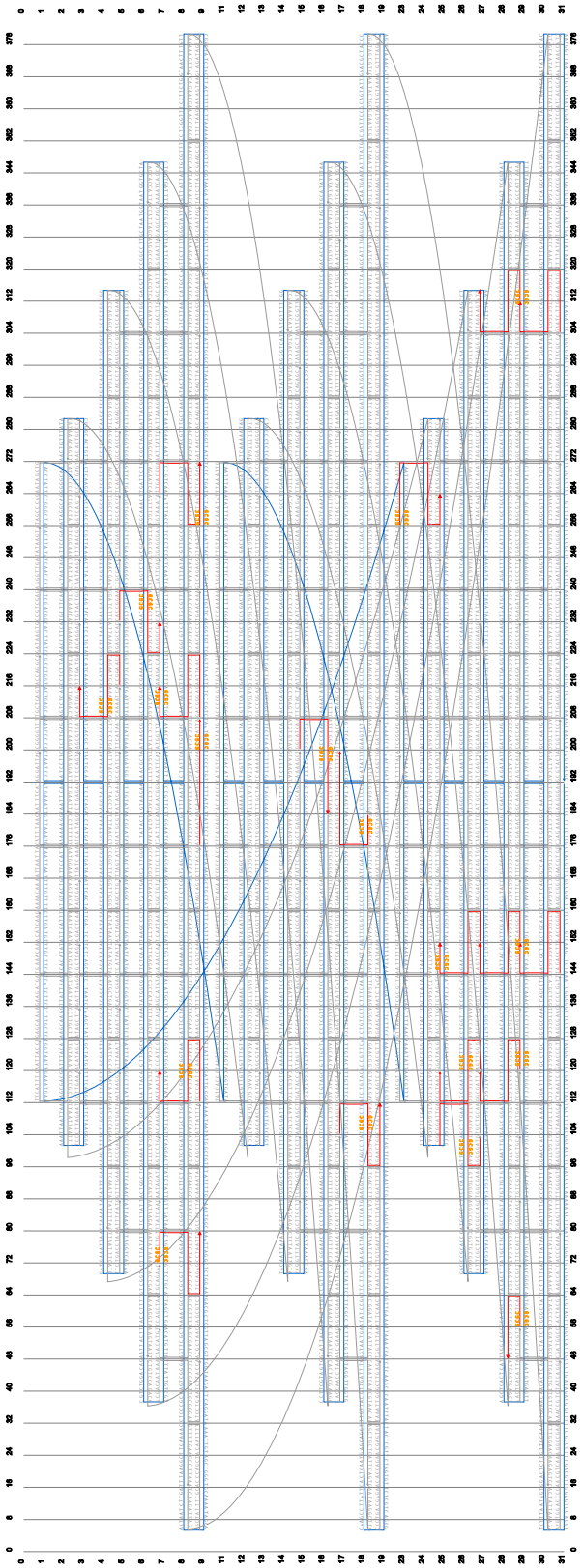
Supplementary Figure 4: (Left) diagrams depicting all the possible fragmentation patterns with which an REase cleaves 4 available sites within a circular DNA molecule (regardless it is unfolded or folded as scaffold within a DNA origami). In the case of a full cleavage (4 cuts), the circular DNA molecule is converted into N=4 fragments (L1, L2, L3, and L4). For partial cleavage figures, max. 3 or less fragments can be generated (M, with $M \leq N$). The total number of ways the REase can chose sites to cleave over the circular DNA is 2^N, while the number of fragments with different sequence content (and therefore molecular weight) that can be generated in this way is $N(N-1)+1$ (i.e. 13 for N=4). (Right) Proportion of DNA fragments produced by the action of a REase either in the case of full cleavage (black) (i.e. with site reactivity = 100% for all sites), or partial cleavage (blue) (i.e. with site reactivity = 50% for all sites). In the first case, gel analysis of the products would lead to max. 4 gel bands, while in the second case, the max. number of gel bands would be 13.



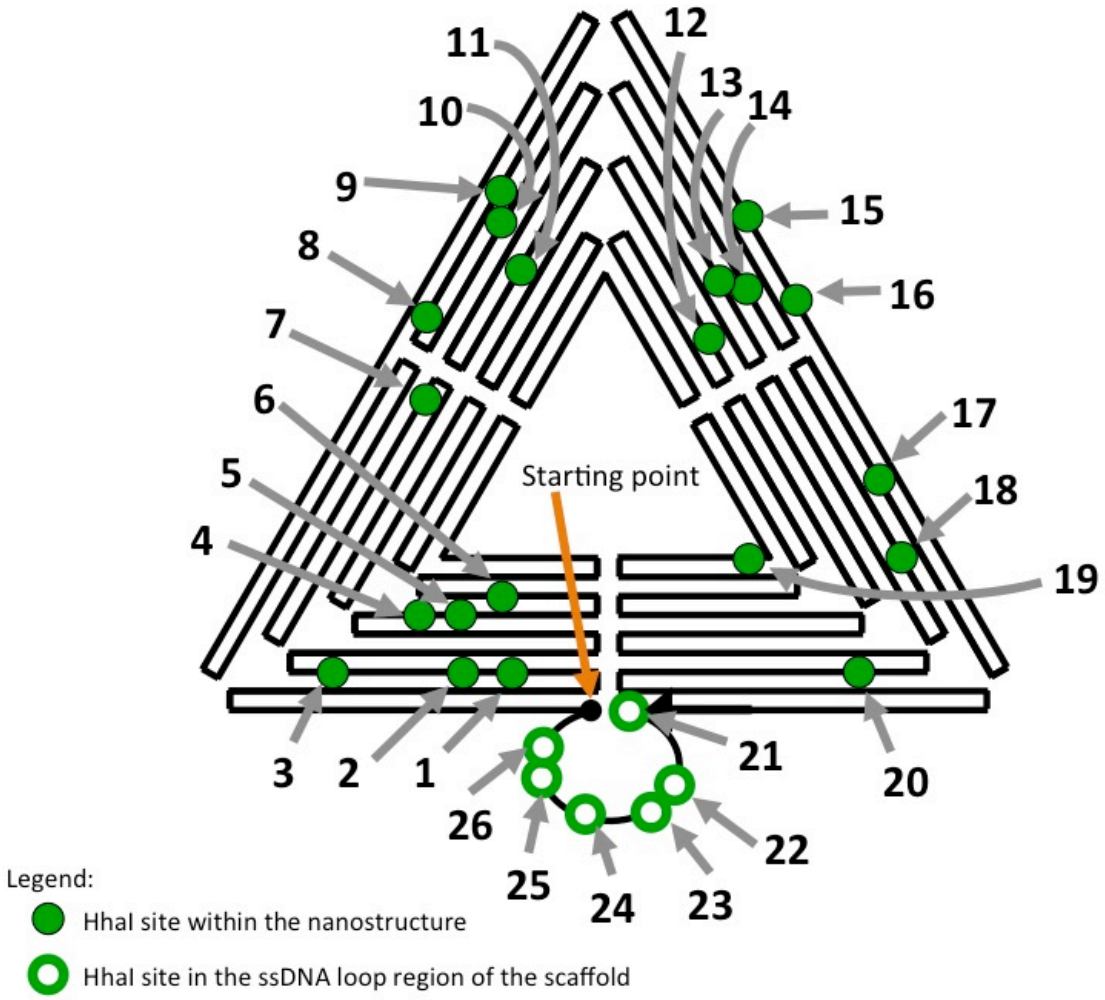
Supplementary Figure 5: Design diagram with scaffold and staple strands of the DNA origami rectangle, as generated with Cadnano V1.0.



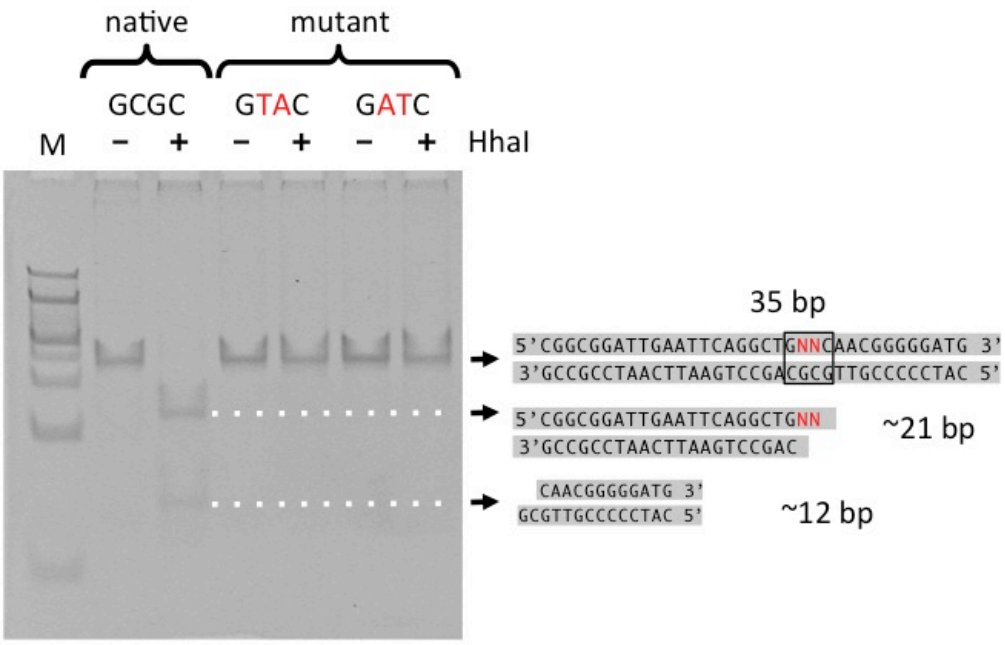
Supplementary Figure 6: Design diagram with scaffold and staple strands of the DNA origami triangle, as generated with Cadnano V1.0.



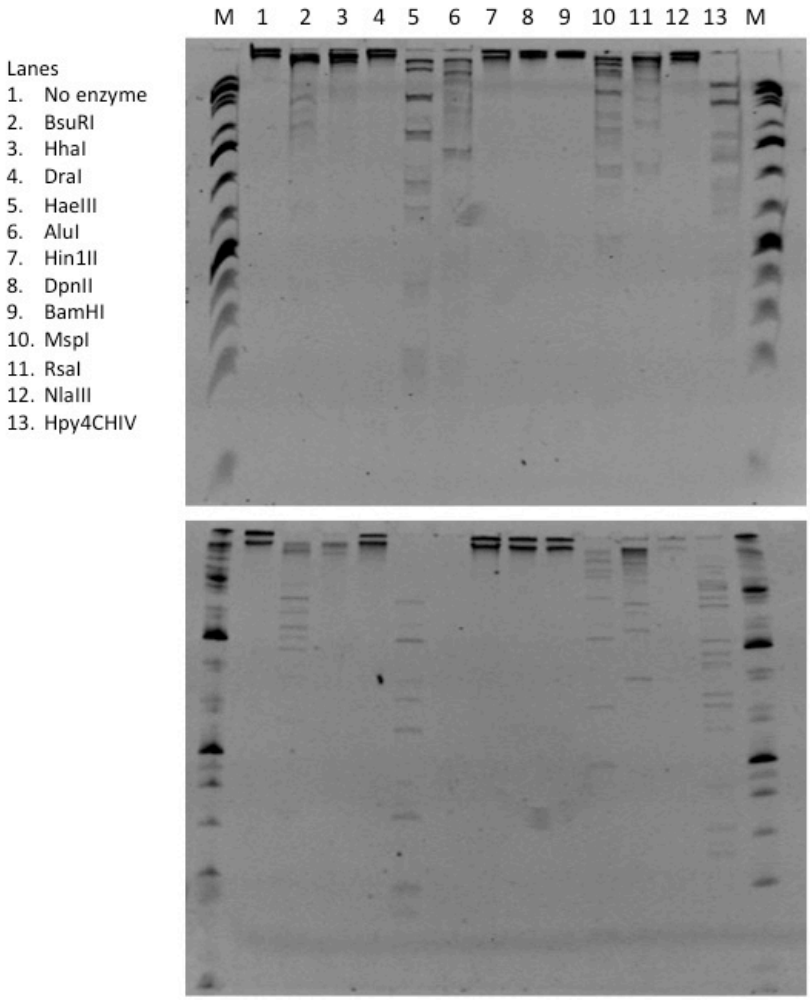
Supplementary Figure 7: Design diagram with scaffold and staple strands of the DNA origami triangle, as generated with Cadnano V1.0. HhaI restriction sites sequences are highlighted in yellow, while staples involved in HhaI restriction site formation are highlighted in red (sequences are indicated below).



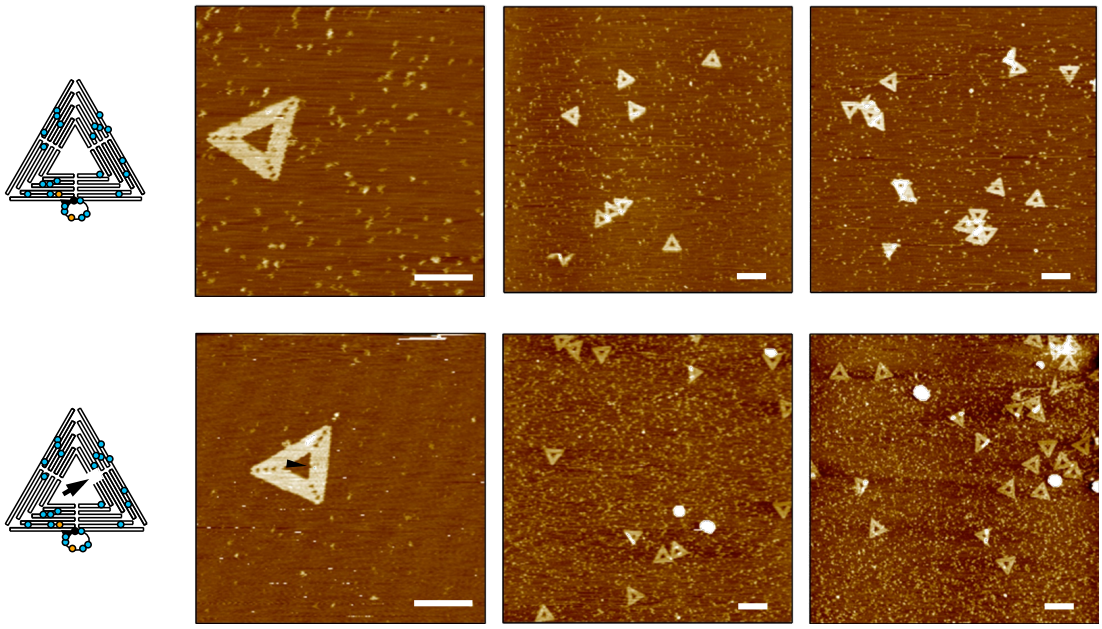
Supplementary Figure 8: Map of HhaI sites within the DNA origami triangle described in Supplementary Figure 7. The M13 scaffold (black line routing throughout the structure) and its HhaI sites (circles) are indicated. Sites are indexed in ascending order according to DNA scaffold 5' to 3' direction, starting from the position indicated with the arrow in orange.



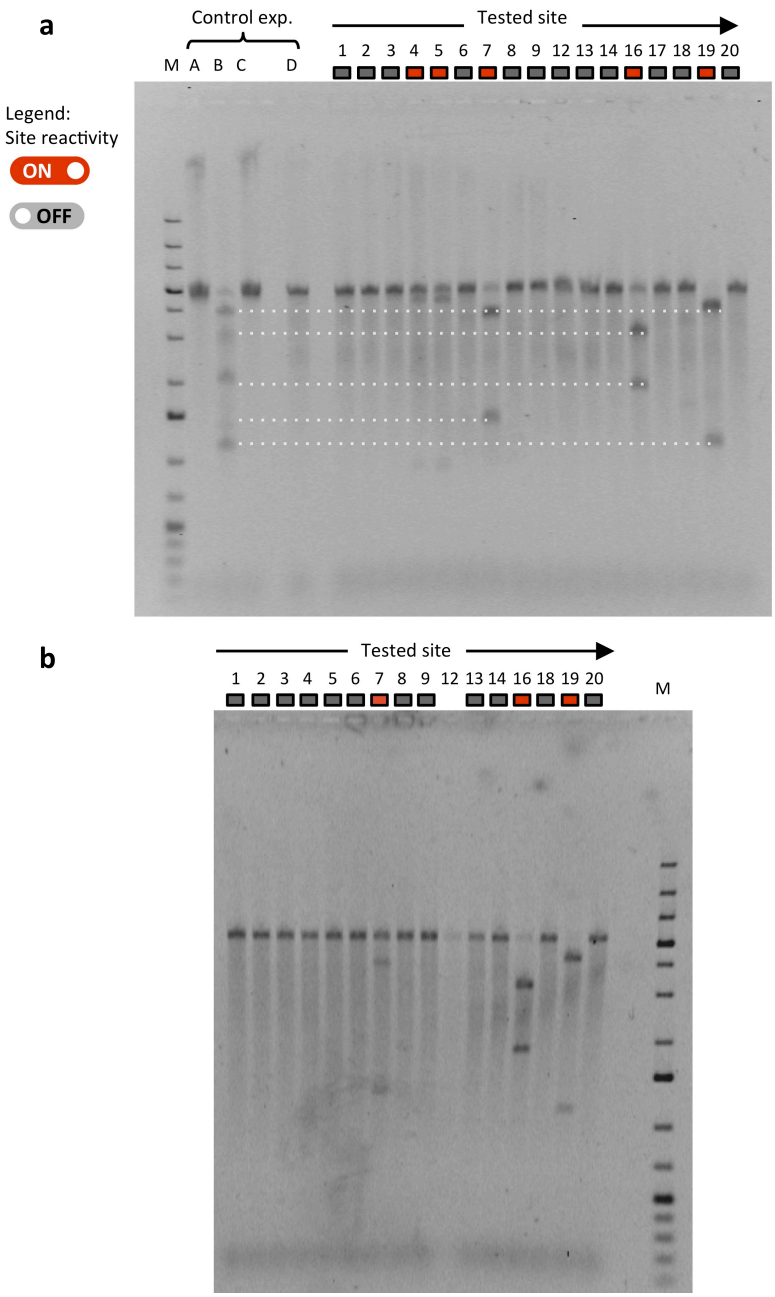
Supplementary Figure 9: Effect of HhaI site mutation on HhaI REase action. (Right) The diagram shows the 35 bp double-stranded DNA segment, with the HhaI site in the middle (box). Two mutants were designed by changing the central nucleotides (in red). For each experiment DNA single strands (see page 39 of the SI) were prior mixed at equimolar concentration to obtain a final dsDNA concentration of 1 µM in 1X Tango buffer. The DNA containing solution was annealed at 80°C and then cooled down to 20°C with a decreasing temperature rate of -2°C/min (using a Thermocycler). REase reaction conditions: 100 nM dsDNA, 10 units of HhaI REase, 1X Tango Buffer, final volume = 50 µL. The reaction was carried out for 1 hour at 37°C (water bath, Julabo GmbH, Seelbach, Germany), and was stopped by freezing the solution at -80˚C for 20 min. (Left) the gel image shows the HhaI REase reaction products relative to three HhaI site mutations (native 20% polyacrylamide gel in TBE 1X). The results demonstrate that an alteration of two nt in the HhaI REase recognition sequence within one of the two strands is sufficient to inhibit HhaI action. M, 10 bp DNA ladder (Thermo Fisher Scientific, Waltham, Massachusetts, USA).



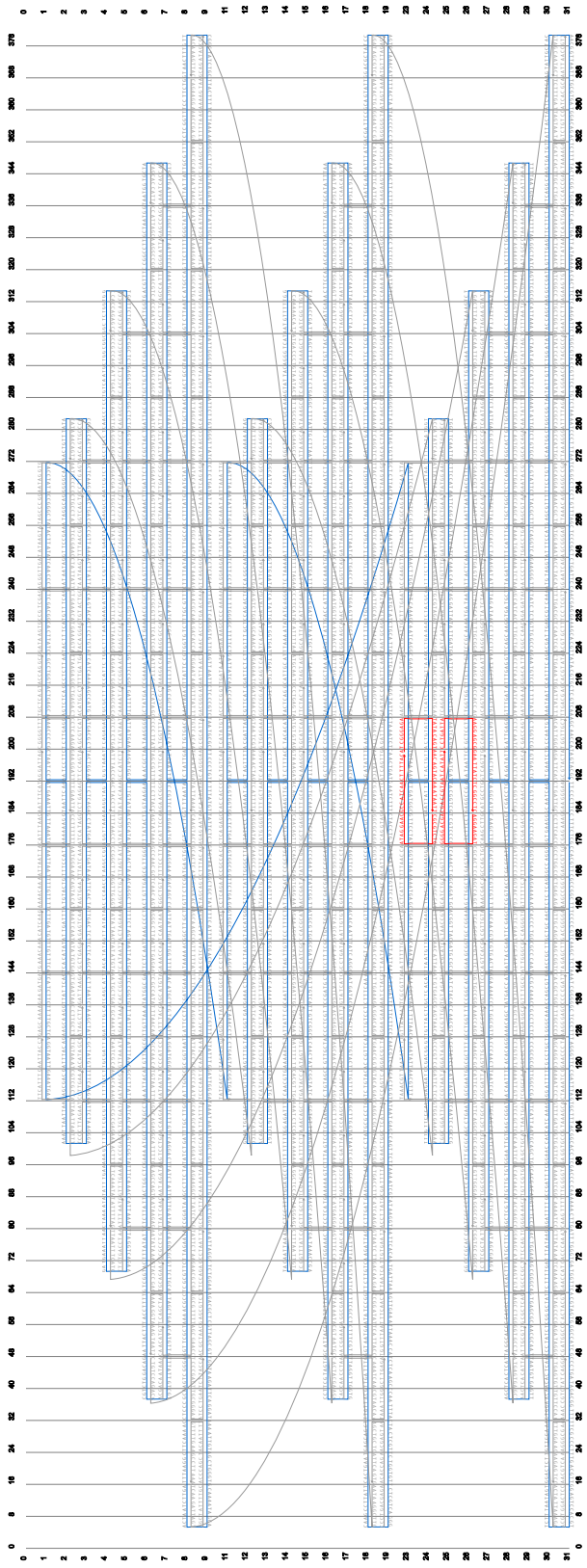
Supplementary Figure 10: Identification of dsDNA-specific REases. The two gel images are relative to two independent experiments. Each gel lane displays the ssDNA M13 reaction products for each REase investigated (legend on the left). The results suggest that BsuRI, HaeIII, AluI, MspI, RsaI, Hpy4CHIV REases are capable of cutting ssDNA M13, and demonstrate that the action of HhaI, DraI, Hin1II, DpnII, BamHI, and NlaIII REases is dsDNA-specific. Enzymatic reaction contained 5 nM M13 ssDNA (the same quantity used in DNA origami experiments), 10 units of REase in either 1X Tango Buffer (for AluI, BsuRI, DraI, Hin1II, HhaI, MspI and RsaI REases) or 1X CutSmart Buffer (for DpnII, BamHI, HaeIII, NlaIII and HypCH4IV REases), in a final volume of 50 µL. Each, reaction was incubated at 37°C for 1 hour in water bath and was stopped with 20 min incubation at -80°C. The reaction products were electrophoresed with a denaturing (8M Urea) 4% polyacrylamide gel in TBE 1X. M, 1 kb DNA molecular weight marker.



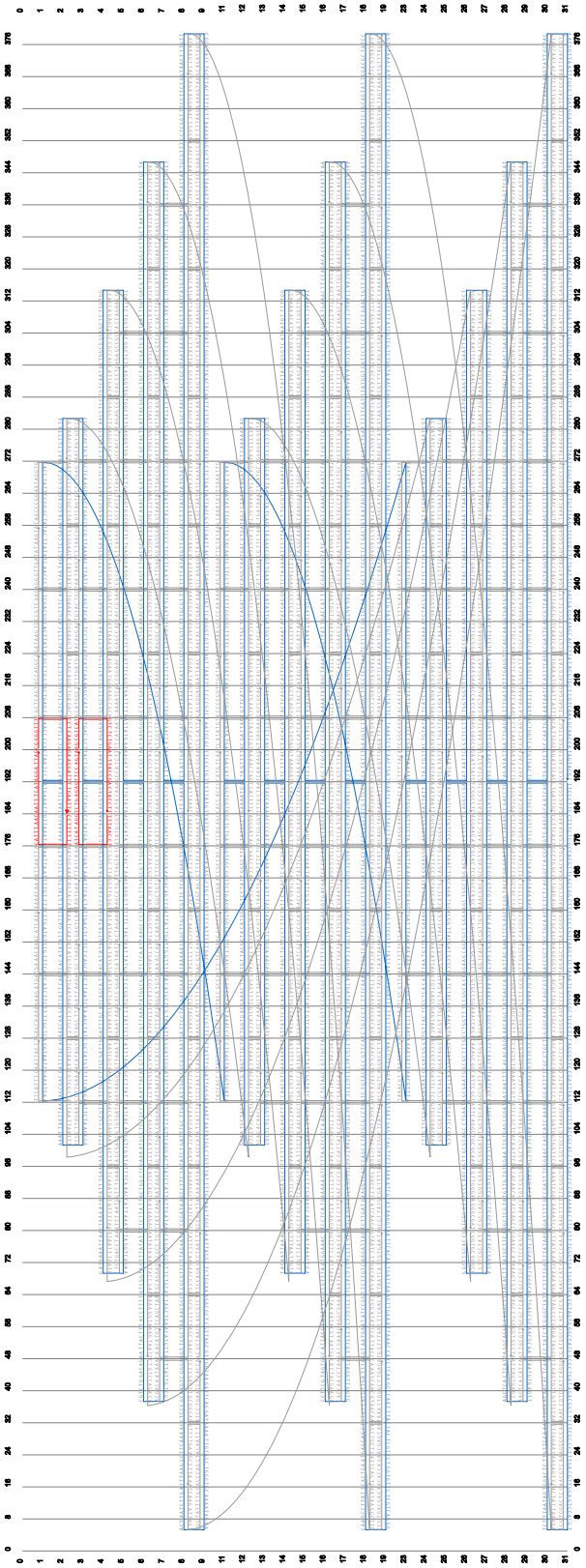
Supplementary Figure 11: Atomic Force Microscopy (AFM) characterization of modified DNA origami triangles. Top left, the scheme depicts the 2SS triangle variant having reactive the HhaI site 1, on the right are shown topographic images obtained for this structure. Bottom left, the scheme depicts the 2SS triangle variant having reactive the HhaI site 1 and a defect in the right trapezoidal element, on the right are shown topographic images obtained for this structure. For high zoom images scale bar is 100 nm and scan size is 0.5 x 0.5 μm^2. For low zoom images scale bar is 200 nm and scan size is 2.0 x 2.0 μm^2. These results show that the modified triangles can form properly either with the presence of small defects (i.e. mismatches) or larger defects (omission of 4 staples). AFM topographic images were obtained in tapping mode in liquid, using a MFP-3D Stand-Alone AFM (Oxford Instruments - Asylum research, Santa Barbara, CA, USA). Following the assembly reaction of the modified triangles, 20 µL of 0.5 nM triangles in the buffering solution of 40 mM Tris, 12.5 mM $MgCl_2$, and 10 mM $NiCl_2$, were deposited on the freshly cleaved mica, fixed over a 100 µl-volume, homemade liquid cell. The physio-adsorption steps lasted for 10 -15 minutes, and then the substrate was rinsed twice with miliQ water. Next, 100 µL of the assembly buffer (40 mM Tris, 12.5 mM $MgCl_2$, pH 8.5) was introduced onto the sample to allow enough solution to cover the homemade liquid cell. Afterward, the sample was transferred onto the baseplate of the XY scanning stage and the following parameters were used to acquire the images; AFM mode: AC mode in liquid, cantilever type: BL-AC40TS-C2 (Olympus, Japan), resonant frequency: 110 kHZ, spring constant: 0.09 N/m (as specified by the manufacturer), scan rate: 1 Hz. We acquired 4 – 5 images, from different locations of each substrate, at least 70 µm apart with respect to each other. The topographic images were processed with 2^{nd} order flattening, and analysed using Igor Pro 6.37 A (Oxford Instruments - Asylum research, Santa Barbara, CA, USA).



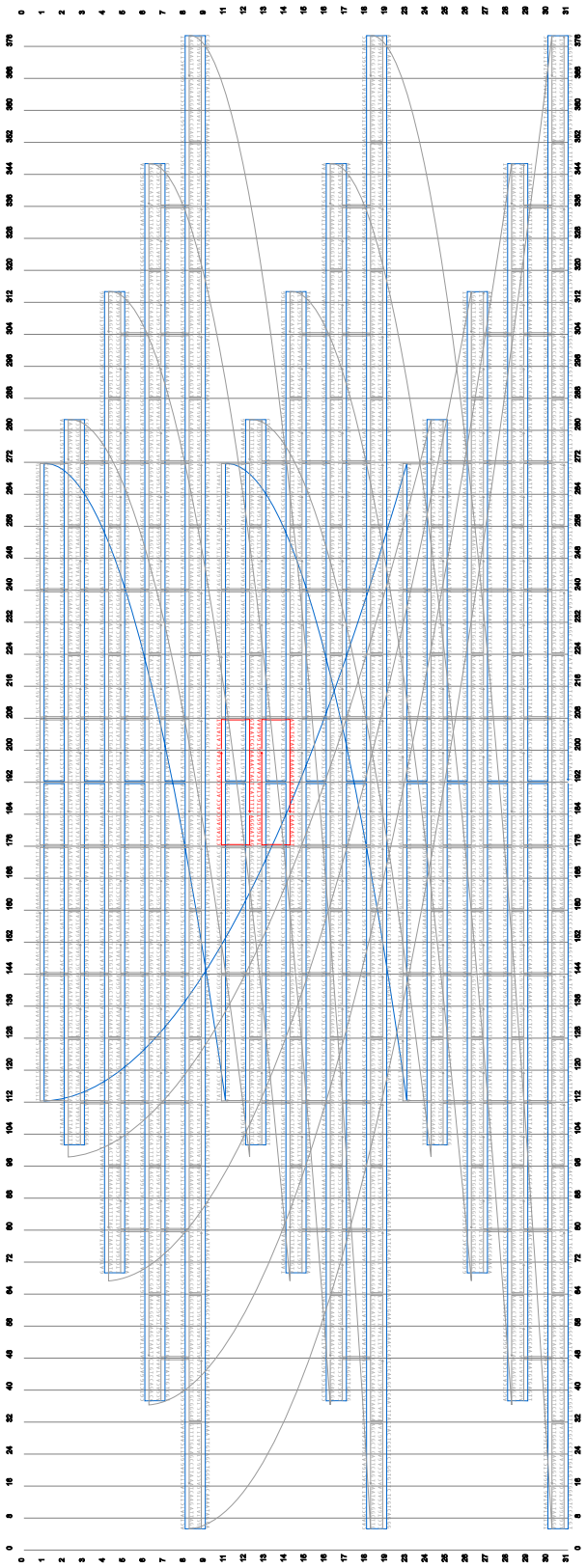
Supplementary Figure 12: HhaI REase reactivity of the sharp triangle at single site resolution. (a, b) Two separate experiments were performed on (2SS) sharp triangle variants as described in Fig. 3a. Each lane of the gels (ethidium-bromide-stained 1% agarose) shows the HhaI reaction products for one 2SS triangle variant (as in Fig. 3b). Visible F_1 and F_2 fragments (see bottom diagram in Fig. 3a) appear in both gels for sharp triangles variants containing sites 7, 16 and 19, and also sites 4 and 5 in one case (b). HhaI REase action of the remaining 2SS triangle variants produced a single gel band compatible with F_0 fragments. Control experiments (gel a, left) were carried out with lacking HhaI enzyme (Lane A); in the presence of HhaI and the unmodified triangle (all sites available; Lane B), the triangle variant having all HhaI sites masked (0SS) (Lane C), and the triangle variant having only site 24 available. F_1 and F_2 fragments associated with sites 7, 16 and 19, match migration mobility of fragments of the reacted unmodified triangle in Lane B (white dashed lines). This suggests that, on average, after 1h of REase reaction with in the unmodified DNA nanostructure, only one reactive restriction site can be actually cleaved. M, 1 kb DNA molecular weight marker.



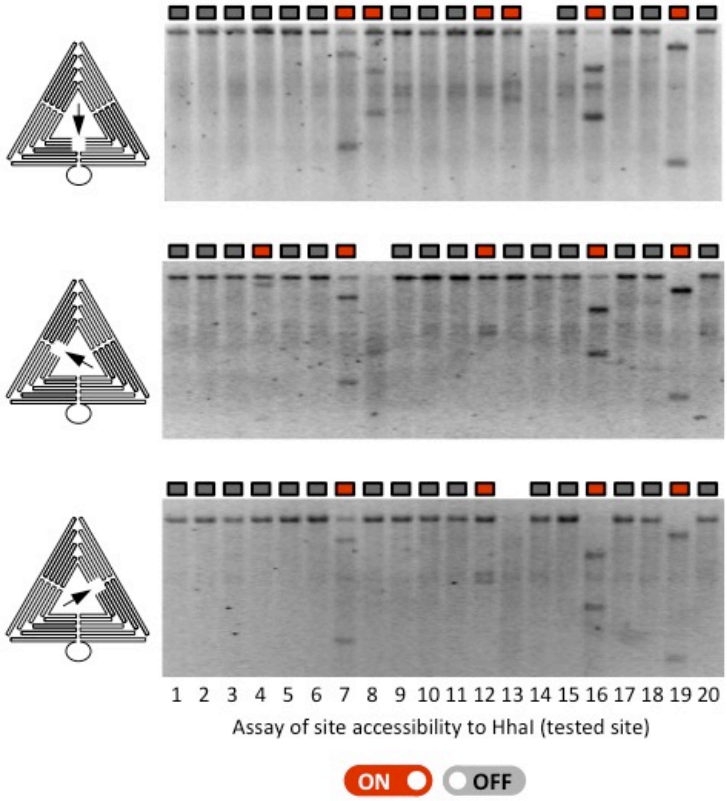
Supplementary Figure 13: Design diagram with scaffold and staple strands of the defective, sharp triangular DNA origami, with the defect in trapezium at the bottom (B). Staples marked in red (B1, B2, B33, B34, see below) were omitted. The diagram was generated with Cadnano V0.2.



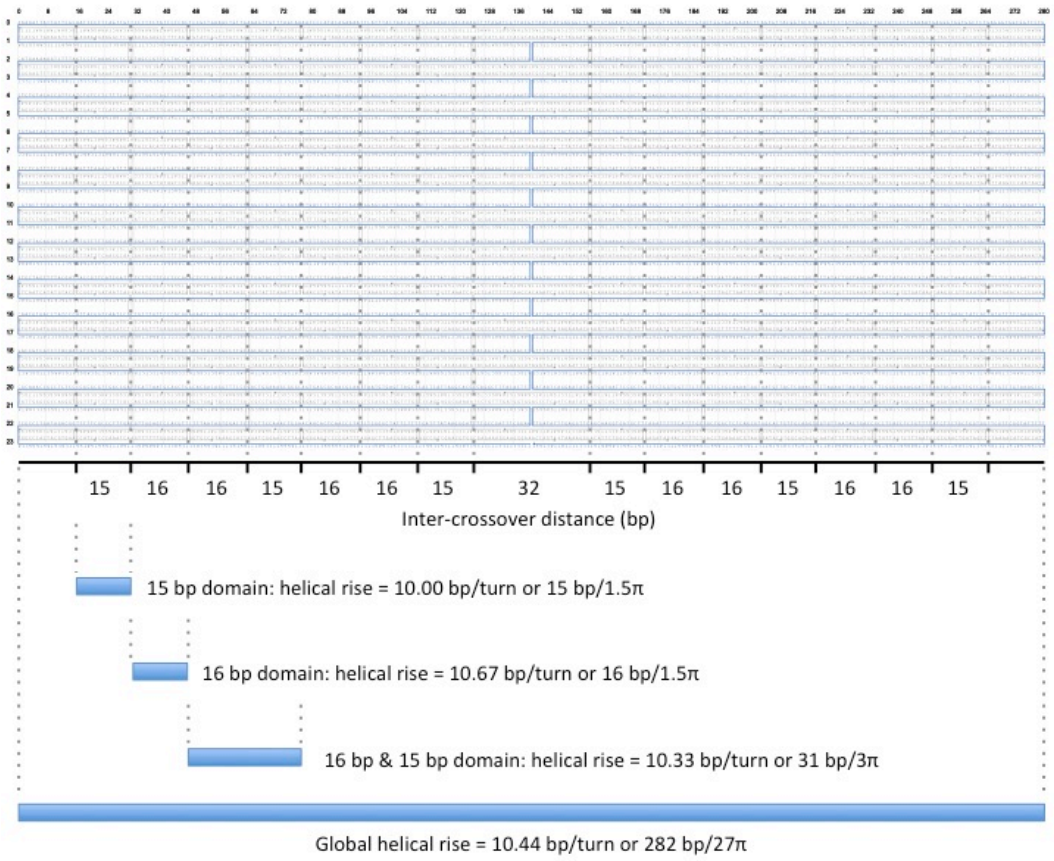
Supplementary Figure 14: Design diagram with scaffold and staple strands of the defective, sharp triangular DNA origami, with the defect in trapezium on the right (R). Staples marked in red (R1, R2, R33, R34, see below) were omitting. The diagram was generated with Cadnano V0.2.



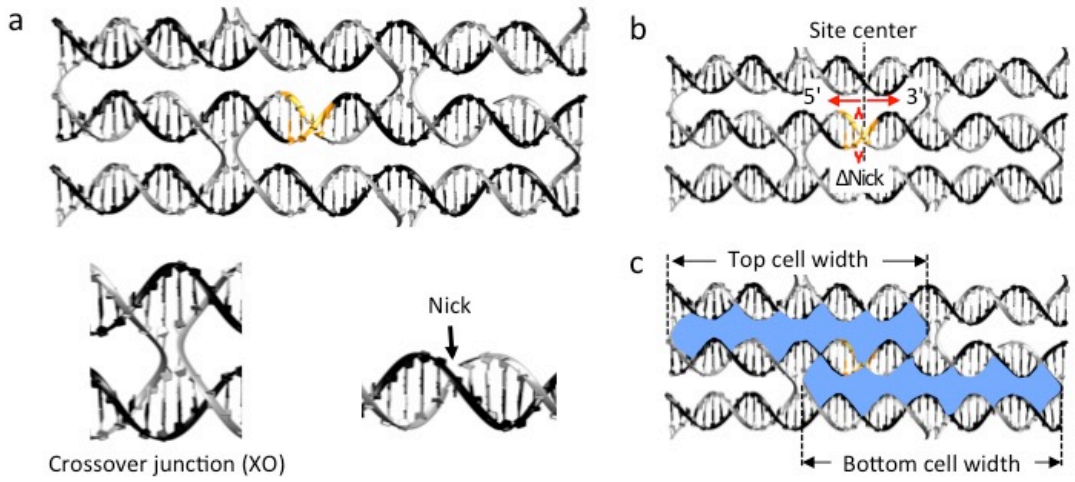
Supplementary Figure 15: Design diagram with scaffold and staple strands of the defective, sharp triangular DNA origami, with the defect in trapezium on the left (L). Staples marked in red (L1, L2, L33, L34, see below) were omitted. The diagram was generated with Cadnano V0.2.



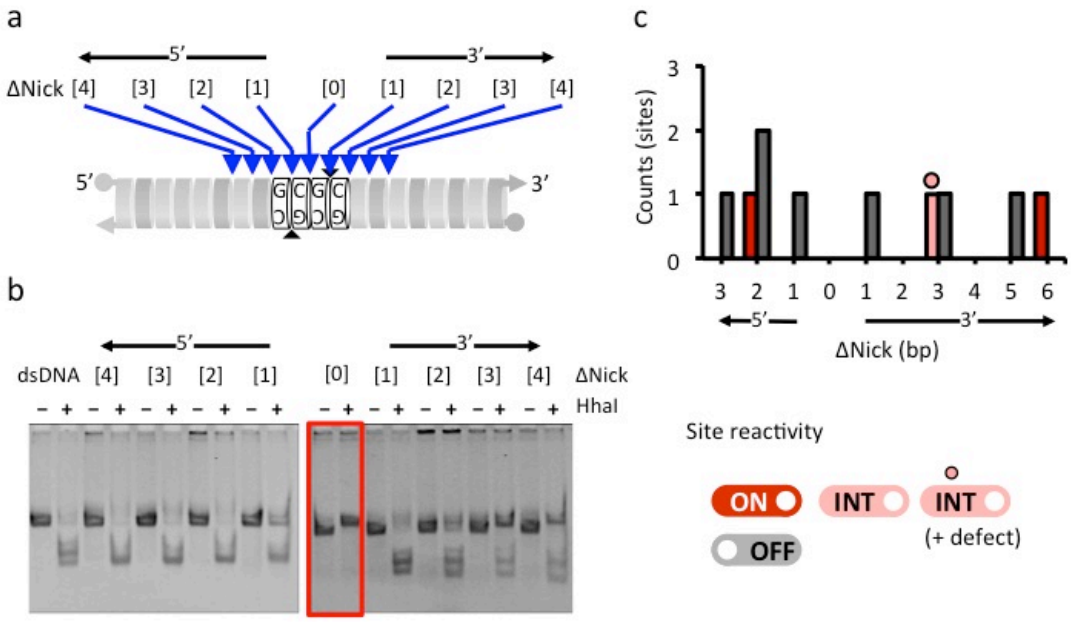
Supplementary Figure 16: Gel analysis (Ethidium-bromide-stained 1% agarose gels) of HhaI reaction products for the three defective, sharp triangular DNA origami. Defect is located in trapezoid B, L and R (from top to bottom). The results reproduce those shown in Fig. 4.



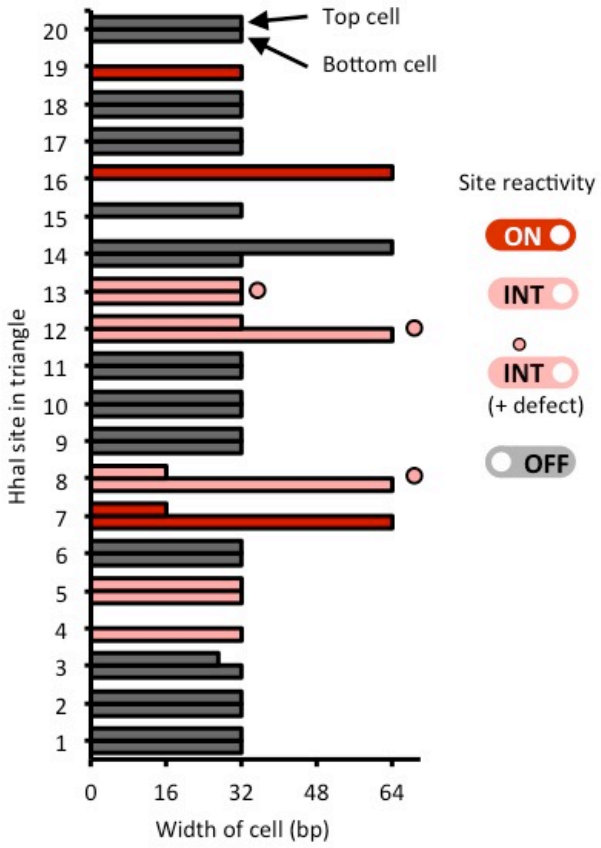
Supplementary Figure 17: Determination of the local DNA helical rise in the "twist-corrected" rectangular DNA origami. The figure on top shows the design diagram of such a rectangle from Supplementary Fig. 5 with a global helix rise of 10.44 bp/turn (1). Rothemund and co-workers reported that such type of structure is more relaxed and "flat" than the corresponding uncorrected rectangle that has a helix rise of 10.66 bp/turn (Woo & Rothemund, Nat. Chem. 2011). Under the top diagram, a ruler marks the position of crossover junctions (only those made from staples). From inter-crossover distances, we calculated the helix rise as a function of its spatial position and found that the helix rise values are position-dependent and vary between 10.00 and 10.67 bp/turn.



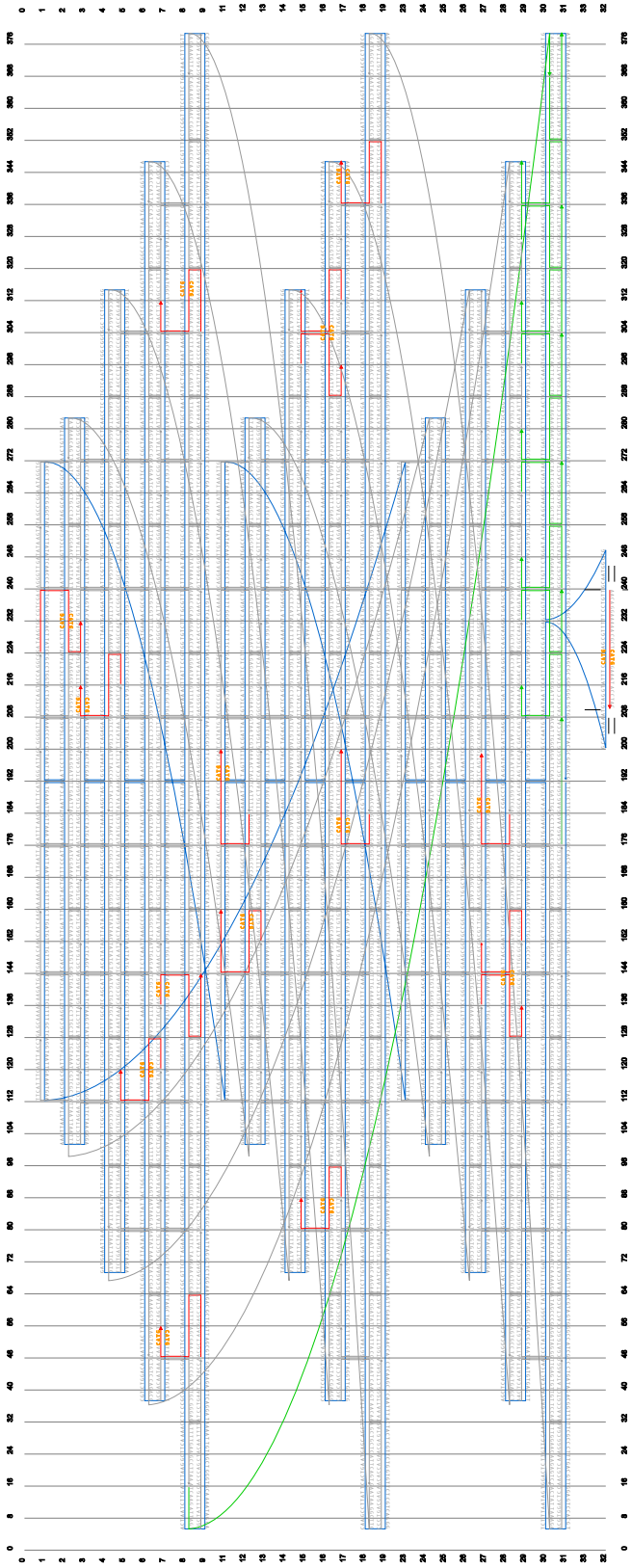
Supplementary Figure 18: Structural constrains potentially affecting REase action. (a) (top) Double helical model of DNA molecules surrounding a 4-bp-long HhaI restriction site (yellow) in the sharp triangle (site 1, Fig. 3a), with staple strands in grey and segments of M13 scaffold in black. (bottom) Key structural constrains affecting REase action such as crossover junction and DNA nick. (b) In our model (Fig. 5), nicks are considered that flank the restriction site and are within the nearest (two) crossover junctions. Nicks, however, can also be within the DNA double helix that contains the REase site. In such case, ΔNick is defined as the distance (in bp) between the nick and the site centre (red arrows), and can be located towards either the 3' or 5' end of the scaffold segment that carries the site. For the illustrated case (site 1 in the DNA sharp triangle) ΔNick = 1 bp → 3'. (c) For each site, the top and bottom "cells" (in blue) are comprised of adjacent, double helical DNA segments (one of which contain the site) and the interconnecting crossover junctions. The "cell width" is defined as the bp distance between these junctions. The Illustrated site 1 in the sharp triangle has two "cells", 32 bp in width each. To obtain the double helical models shown in this figure (as well as those presented in Figs 1 and 5), DNA nanostructure diagrams were generated as Cadnano (2) designs that were then submitted to the online tool Cando (3) (using default settings) to obtain inherent, molecular representations in .pdb format, which were finally visualised and adapted with Chimera (4).



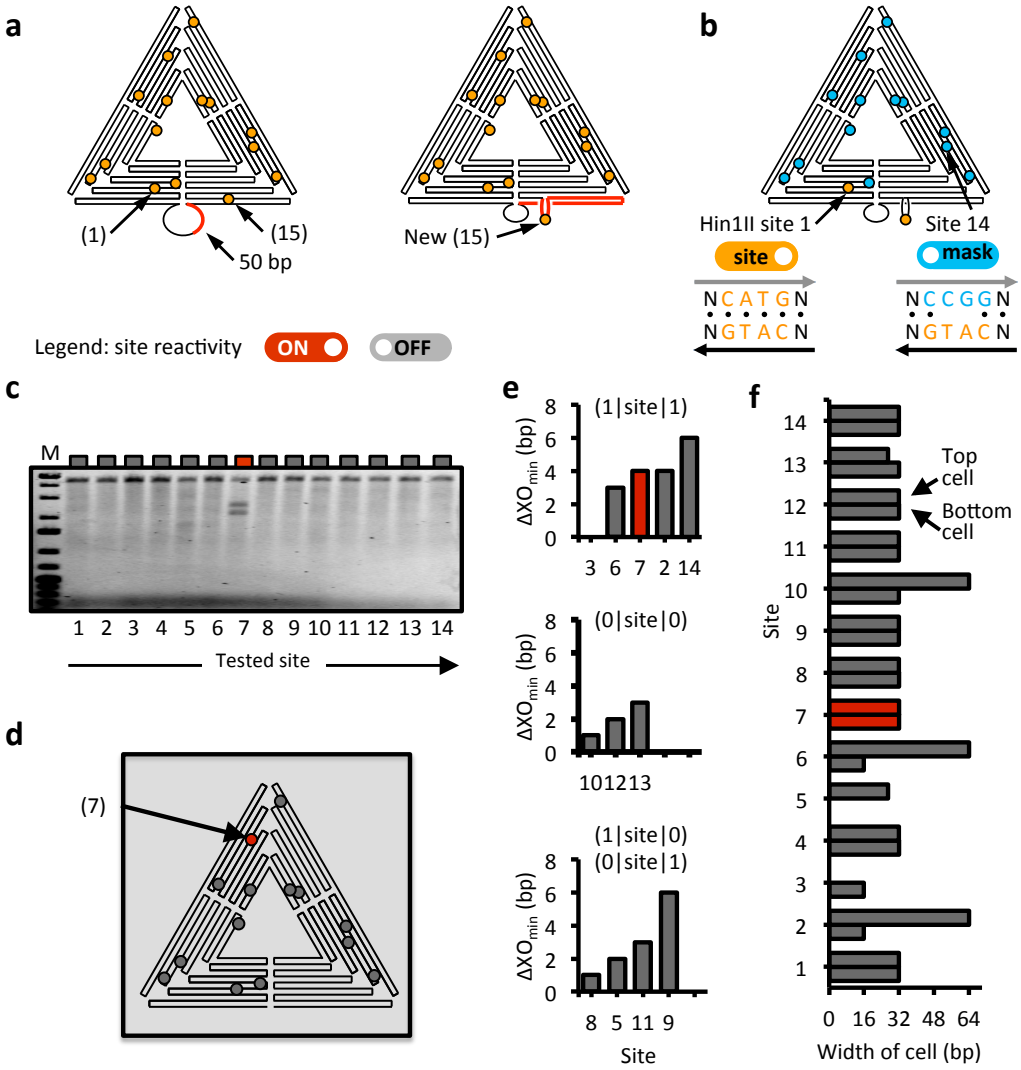
Supplementary Figure 19: Influence of a nick adjacent or within the HhaI site on its reactivity. (a) Diagram of the different, tested dsDNA molecules. Experiments were performed on 64 bp long dsDNAs with a HhaI restriction site in the middle and a nick in different positions (blue arrows). Black triangles indicate the phosphodiester bonds that are cleaved by HhaI. Three ssDNA molecules (see page 40 of the SI) were mixed to form the dsDNA variants, with each strand at 1 µM concentration in 40 mM Tris, 12.5 mM MgCl2, pH 8.5 in a volume of 25 µL. The DNA was annealed by heating at 80°C and then cooling down to 20°C the solution with a decreasing temperature rate of -2°C/min. The reaction was performed in 50 µL containing 100 nM dsDNA and 10 units of HhaI enzyme in 1X Tango Buffer. The reaction was incubated for 1 hour at 37°C in water bath and was stopped by freezing to -80˚C for 20 min. (b) Gel analysis (Ethidium-bromide 18% polyacrylamide in 1X TBE) of the HhaI reaction products. All dsDNA molecules are equally reactive, except that carrying a nick in the site centre (ΔNick=0), which results to be fully resistant. (c) Number of HhaI sites in the sharp DNA triangle as a function of ΔNick (see Supplementary Fig. 18b). While certain HhaI sites are unreactive, none is found with ΔNick=0. Therefore ΔNick does not seem to be an antideterminant of HhaI REase action, and was taken into account in our mechanical model (Fig. 5).



Supplementary Figure 20: Influence on HhaI site reactivity of the length of the double helical segments bounded by crossover junctions that flank the HhaI site in the sharp triangular DNA origami. For each site, the plot illustrates the widths of the upper and bottom "cells" for each HhaI site in the sharp DNA triangle, as defined in Supplementary Fig. 18c. For sites 4, 15, 16 and 19 that are located on a nanostructure edges, a single value is provided (as a single "cell" is definable).



Supplementary Figure 21: Design diagram with scaffold and staple strands of the DNA origami triangle, as generated with Cadnano V1.0. Hin1II restriction sites sequences are highlighted in yellow; staples involved in Hin1II restriction site formation are highlighted in red (sequences are indicated below in page 40-41 of SI); other staples were modified to introduce an unfolded DNA sequence that protrudes from the nanostructure and contains a reference restriction site (in green).



Supplementary Figure 22: Hin1II REase reactivity of the sharp triangle at the single-site level. (a) (Left) diagram of the sharp DNA triangle investigated in Fig. 1, with the routing M13 DNA scaffold (black line) and 15 Hin1II restriction sites (yellow circles). The design on the left lacks Hin1II sites to use as reference. (Right) A sharp DNA triangle variant is designed by varying the folding pattern of the M13 scaffold (red) to allow a Hin1II restriction site (no. 15) to be highly reactive. The repositioned site is located within an unfolded portion of the M13 sequence that protrudes from the nanostructure (see the DNA origami design in Supplementary Fig. 21 and the DNA sequences at page 40-41 of the SI). (b) Fourteen ("two sites states" or "2SS") sharp triangle variants containing only two Hin1II sites (one of which is the reference) were generated following the approach described in Fig. 3 (see the DNA origami design in Supplementary Fig. 21, and the DNA sequences at page 40-41 of the SI). Briefly, the Hin1II recognition sequence (5'-CATG-3', in orange) in all staples except two was changed to 5'-CCGG-3' (in blue) for masking Hin1II sites. (c) Agarose gel analysis (Ethidium-bromide-stained 1% agarose gel) of Hin1II reaction products of 2SS triangle variants. F1 and F2 fragments appear only for the site 7. (d) A sharp triangle diagram shows the position of reactive Hin1II site in the nanostructure, according to the results shown in (c). (e) Parameterisation of site reactivity according to Fig. 5a-c. For each mechanical configuration (see Fig. 5b), the value of ΔXO_{min} associated to Hin1II sites is plotted in ascending order as a function of the site index (in analogy to Fig. 5c for HhaI). (f) Widths of the top and bottom "cells" for each Hin1II site in the sharp DNA triangle (see Supplementary Fig. 18c).

Hin1II (E2)		EXPERIMENT ON	EXPERIMENT OFF
MODEL	ON	True ON 1	False ON 1
	OFF	False OFF 1	True OFF 11

HhaI (E5)		EXPERIMENT ON	EXPERIMENT OFF
MODEL	ON	True ON 3	False ON 1
	OFF	False OFF 0	True OFF 16

(1) $$T_{ON} = \frac{\text{True ON}}{\text{True ON + False OFF}}$$

(2) $$F_{OFF} = \frac{\text{False OFF}}{\text{False OFF + True OFF}} = 1 - T_{ON}$$

(3) $$T_{OFF} = \frac{\text{True OFF}}{\text{True OFF + False ON}}$$

(4) $$F_{ON} = \frac{\text{False ON}}{\text{False ON + True OFF}} = 1 - T_{OFF}$$

(5) $$\text{Accuracy} = \frac{\text{True ON + True OFF}}{\text{Total number of sites}}$$

Supplementary Figure 23: Analysis of model performance in determining site reactivity on a site-by-site basis. (Left) The contingency tables summarise model reactivity testing for Hin1II and HhaI sites in sharp triangular DNA origami, based on site-by-site experiments. Site reactivities extrapolated from experiments (horizontal) are matched with expectations (vertical). "True ON" are all sites that experimentally result to be reactive (and therefore are defined as "ON"), and which are correctly identified by the model. Similarly, "True OFF" are all sites that experimentally result to be unreactive (and therefore are defined as "OFF"), and which are correctly identified by the model. "False OFF" are all sites that experimentally result to be reactive (and therefore are defined as "ON"), but which the model fails to identify as such. Similarly, "False ON" are all sites that experimentally result to be unreactive (and therefore are defined as "OFF"), but which the model fails to identify as such. "True ON rate" (T_{ON}) and "True OFF rate" (T_{OFF}) provide a measure of model's ability to correctly detect REases site reactivity, and were calculated using equations (1) and (2). "False OFF rate" (F_{OFF}) and "False ON rate" (F_{ON}) provide a measure of the model's incompetence in detecting site reactivity, and were calculated using equations (3) and (4). The accuracy of the model was calculated separately for both Hin1II and HhaI REases using equation (5).

Supplementary Table 1: expected length of F_1 and F_2 DNA scaffold fragments generated by the combined cleavage of the two indicated HhaI sites (24 is the reference). F_1 is defined as the longest. nt, (nucleotides).

(Tested site):(Reference site)	F_1 fragment length (nt)	F_2 fragment length (nt)
(4):(24)	6357	892
(5):(24)	6336	913
(7):(24)	5489	1760
(16):(24)	4811	2438
(19):(24)	6027	1222

Supplementary Table 2: Values of ΔXO_{min} and structural states for each restriction site recognised by Hin1II and NlaIII (5'-CATG), HaeIII and BsuRI (5'-GGCC), MspI (5'-CCGG), RsaI (5'-GTAC), Hpy4CHIV (5'- ACGT), and AluI (5'- AGCT), in the sharp triangular DNA origami, as depicted in Fig. 5a-b.

enzyme (isoschizomer)	scaffold region	# site	ΔXOmin	site configuration
Hin1II (NlaIII)	structured	1	-1	(0|site|0)
Hin1II (NlaIII)	structured	2	4	(1|site|1)
Hin1II (NlaIII)	structured	3	0	(1|site|1)
Hin1II (NlaIII)	structured	4	-2	(0|site|0)
Hin1II (NlaIII)	structured	5	2	(0|site|0)
Hin1II (NlaIII)	structured	6	3	(1|site|1)
Hin1II (NlaIII)	structured	7	4	(1|site|1)
Hin1II (NlaIII)	structured	8	1	(0|site|1)
Hin1II (NlaIII)	structured	9	6	(0|site|1)
Hin1II (NlaIII)	structured	10	1	(0|site|0)
Hin1II (NlaIII)	structured	11	3	(1|site|0)
Hin1II (NlaIII)	structured	12	2	(0|site|0)
Hin1II (NlaIII)	structured	13	3	(1|site|1)
Hin1II (NlaIII)	structured	14	6	(0|site|0)
Hin1II (NlaIII)	structured	15	-1	(0|site|0)
HaeIII (BsuRI)	structured	1	8	(0|site|1)
HaeIII (BsuRI)	structured	2	4	(1|site|0)
HaeIII (BsuRI)	structured	3	2	(0|site|0)
HaeIII (BsuRI)	structured	4	5	(0|site|0)
HaeIII (BsuRI)	structured	5	0	(1|site|0)
HaeIII (BsuRI)	structured	6	2	(0|site|0)
HaeIII (BsuRI)	structured	7	-1	(0|site|0)
HaeIII (BsuRI)	structured	8	0	(0|site|0)
HaeIII (BsuRI)	structured	9	4	(0|site|0)
HaeIII (BsuRI)	structured	10	3	(1|site|1)
HaeIII (BsuRI)	structured	11	2	(1|site|1)
HaeIII (BsuRI)	structured	12	1	(0|site|0)
HaeIII (BsuRI)	structured	13	3	(1|site|0)
HaeIII (BsuRI)	structured	14	13	(0|site|1)
HaeIII (BsuRI)	structured	15	10	(0|site|1)

enzyme (isoschizomer)	scaffold region	# site	ΔXOmin	site configuration
MspI	structured	1	3	(0|site|1)
MspI	XO	2	-2	
MspI	structured	3	5	(1|site|1)
MspI	structured	4	4	(1|site|1)
MspI	structured	5	2	(1|site|1)
MspI	structured	6	3	(1|site|0)
MspI	structured	7	-2	(0|site|0)
MspI	structured	8	2	(0|site|0)
MspI	structured	9	-1	(0|site|0)
MspI	structured	10	0	(1|site|0)
MspI	XO	11		
MspI	structured	12	8	(1|site|0)
MspI	structured	13	2	(0|site|0)
MspI	structured	14	4	(0|site|0)
MspI	structured	15	0	(1|site|1)
MspI	structured	16	0	(1|site|0)
MspI	structured	17	2	(0|site|0)
MspI	structured	18	0	(1|site|1)
RsaI	structured	1	3	(1|site|1)
RsaI	structured	2	-2	(1|site|0)
RsaI	XO	3		
RsaI	structured	4	1	(1|site|1)
RsaI	structured	5	4	(0|site|0)
RsaI	structured	6	2	(1|site|0)
RsaI	structured	7	-1	(0|site|0)
RsaI	structured	8	4	(1|site|0)
RsaI	structured	9	5	(0|site|1)
RsaI	structured	10	7	(1|site|0)
RsaI	structured	11	6	(1|site|0)
RsaI	structured	12	3	(1|site|0)
RsaI	structured	13	1	(0|site|0)
RsaI	structured	14	4	(0|site|0)
RsaI	structured	15	2	(1|site|1)
RsaI	structured	16	0	(0|site|1)
RsaI	structured	17	8	(1|site|1)
RsaI	structured	18	26	(0|site|1)
RsaI	loop	19		

enzyme (isoschizomer)	scaffold region	# site	ΔXOmin	DNA segments configuration
Hpy4CHIV	structured	1	10	(0|site|1)
Hpy4CHIV	structured	2	0	(0|site|1)
Hpy4CHIV	structured	3	11	(1|site|1)
Hpy4CHIV	structured	4	0	(1|site|1)
Hpy4CHIV	structured	5	4	(0|site|0)
Hpy4CHIV	structured	6	1	(0|site|0)
Hpy4CHIV	structured	7	3	(1|site|0)
Hpy4CHIV	structured	8	13	(0|site|1)
Hpy4CHIV	structured	9	2	(0|site|1)
Hpy4CHIV	structured	10	5	(1|site|0)
Hpy4CHIV	structured	11	7	(1|site|1)
Hpy4CHIV	structured	12	3	(1|site|1)
Hpy4CHIV	structured	13	2	(0|site|0)
Hpy4CHIV	structured	14	2	(1|site|1)
Hpy4CHIV	structured	15	4	(1|site|0)
Hpy4CHIV	structured	16	11	(0|site|1)
Hpy4CHIV	structured	17	4	(0|site|1)
Hpy4CHIV	structured	18	3	(0|site|1)
Hpy4CHIV	structured	19	3	(1|site|1)
Hpy4CHIV	structured	20	0	(0|site|0)
Hpy4CHIV	structured	21	0	(0|site|0)
Hpy4CHIV	structured	22	3	(0|site|1)
AluI	structured	1	9	(0|site|1)
AluI	XO	2		
AluI	structured	3	1	(0|site|0)
AluI	structured	4	0	(1|site|0)
AluI	structured	5	4	(1|site|1)
AluI	structured	6	-1	(0|site|0)
AluI	structured	7	2	(1|site|1)
AluI	structured	8	5	(0|site|0)
AluI	structured	9	0	(0|site|0)
AluI	structured	10	-2	(0|site|0)
AluI	structured	11	13	(1|site|0)
AluI	XO	12		
AluI	structured	13	-2	(0|site|0)
AluI	structured	14	1	(0|site|0)
AluI	structured	15	0	(1|site|1)
AluI	structured	16	4	(1|site|1)
AluI	structured	17	-2	(1|site|0)
AluI	structured	18	4	(0|site|0)
AluI	structured	19	0	(0|site|0)
AluI	structured	20	0	(1|site|0)
AluI	structured	21	0	(0|site|0)
AluI	structured	22	-1	(1|site|0)
AluI	structured	23	13	(0|site|1)
AluI	structured	24	8	(0|site|1)
AluI	structured	25	2	(0|site|0)
AluI	structured	26	2	(0|site|0)
AluI	structured	27	-2	(0|site|1)

Supplementary Table 3: Estimation of the number of the reactive sites in sharp triangular DNA origami for each investigated restriction endonuclease. Values are obtained by counting the number of gel bands produced by separating scaffold fragments after the reaction with the sharp DNA triangle described in Supplementary Fig. 2. HhaI produces 6 major gel bands and therefore there are 3 HhaI reactive sites in the nanostructure (see the diagram describing scaffold fragmentation depicted at the bottom Fig. 1a).

Restriction endonuclease	Estimated accessible sites	average	Fig. 1d	Suppl. Fig. 2a (right)	Suppl. Fig. 2b	Suppl. Fig. 2c
Hin1II	0	1	1		1	1
NlaIII	0	1		1		
BsuRI	2	3		3		
HaeIII	3	4		4	4	5
MspI	3	5	5		ND	5
RsaI	2	3	4		2	2
Hpy4CHIV	4	7	6		ND	8
HhaI	3	6	6		5	6
AluI	4	8	7		ND	8

List of DNA sequences

M13mp18 DNA sequence scaffold in the sharp triangular and rectangular DNA origami. (5' - 3').

TTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGAT
TTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGA
TAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACA
CTCAACCCTATCTCGGGCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTCGGAACCACCATCAAACAGGATTT
TCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAG
CTGTTGCCCGTCTCGCTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTT
GGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAA
TGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGT
GAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCC
TCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTG
GCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACC
GATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGT
GCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACGGTCGTCGTCCCCTCAAACTGGCAGATGCAC
GGTTACGATGCGCCCATCTACACCAACGTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAAT
CCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTT
TGATGGCGTTCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACG
TTTACAATTTAAATATTTGCTTATACAATCTTCCTGTTTTTGGGGCTTTTCTGATTATCAACCGGGGTACATATGA
TTGACATGCTAGTTTTACGATTACCGTTCATCGATTCTCTTGTTTGCTCCAGACTCTCAGGCAATGACCTGATAGC
CTTTGTAGATCTCTCAAAAATAGCTACCCTCTCCGGCATTAATTTATCAGCTAGAACGGTTGAATATCATATTGAT
GGTGATTTGACTGTCTCCGGCCTTTCTCACCCTTTTGAATCTTTACCTACACATTACTCAGGCATTGCATTTAAAA
TATATGAGGGTTCTAAAAATTTTTATCCTTGCGTTGAAATAAAGGCTTCTCCCGCAAAAGTATTACAGGGTCATA
ATGTTTTTGGTACAACCGATTTAGCTTTATGCTCTGAGGCTTTATTGCTTAATTTTGCTAATTCTTTGCCTTGCCTG
TATGATTTATTGGATGTTAATGCTACTACTATTAGTAGAATTGATGCCACCTTTTCAGCTCGCGCCCCAAATGAA
AATATAGCTAAACAGGTTATTGACCATTTGCGAAATGTATCTAATGGTCAAACTAAATCTACTCGTTCGCAGAAT
TGGGAATCAACTGTTACATGGAATGAAACTTCCAGACACCGTACTTTAGTTGCATATTTAAAACATGTTGAGCTA
CAGCACCAGATTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAAAGGAGCAATTAAAGGT
ACTCTCTAATCCTGACCTGTTGGAGTTTGCTTCCGGTCTGGTTCGCTTTGAAGCTCGAATTAAAACGCGATATTT
GAAGTCTTTCGGGCTTCCTCTTAATCTTTTTGATGCAATCCGCTTTGCTTCTGACTATAATAGTCAGGGTAAAGAC
CTGATTTTTGATTTATGGTCATTCTCGTTTTCTGAACTGTTTAAAGCATTTGAGGGGGATTCAATGAATATTTATG
ACGATTCCGCAGTATTGGACGCTATCCAGTCTAAACATTTTACTATTACCCCCTCTGGCAAAACTTCTTTTGCAAA
AGCCTCTCGCTATTTTGGTTTTTATCGTCGTCTGGTAAACGAGGGTTATGATAGTGTTGCTCTTACTATGCCTCGT
AATTCCTTTTGGCGTTATGTATCTGCATTAGTTGAATGTGGTATTCCTAAATCTCAACTGATGAATCTTTCTACCT
GTAATAATGTTGTTCCGTTAGTTCGTTTTATTAACGTAGATTTTTCTTCCCAACGTCCTGACTGGTATAATGAGCC
AGTTCTTAAAATCGCATAAGGTAATTCACAATGATTAAAGTTGAAATTAAACCATCTCAAGCCCAATTTACTACT
CGTTCTGGTGTTCTCGTCAGGGCAAGCCTTATTCACTGAATGAGCAGCTTTGTTACGTTGATTTGGGTAATGAAT
ATCCGGTTCTTGTCAAGATTACTCTTGATGAAGGTCAGCCAGCCTATGCGCCTGGTCTGTACACCGTTCATCTGT
CCTCTTTCAAAGTTGGTCAGTTCGGTTCCCTTATGATTGACCGTCTGCGCCTCGTTCCGGCTAAGTAACATGGAG
CAGGTCGCGGATTTCGACACAATTTATCAGGCGATGATACAAATCTCCGTTGTACTTTGTTTCGCGCTTGGTATA
ATCGCTGGGGGTCAAAGATGAGTGTTTTAGTGTATTCTTTCGCCTCTTTCGTTTTAGGTTGGTGCCTTCGTAGTG
GCATTACGTATTTTACCCGTTTAATGGAAACTTCCTCATGAAAAAGTCTTTAGTCCTCAAAGCCTCTGTAGCCGTT
GCTACCCTCGTTCCGATGCTGTCTTTCGCTGCTGAGGGTGACGATCCCGCAAAAGCGGCCTTTAACTCCCTGCAA
GCCTCAGCGACCGAATATATCGGTTATGCGTGGGCGATGGTTGTTGTCATTGTCGGCGCAACTATCGGTATCAA
GCTGTTTAAGAAATTCACCTCGAAAGCAAGCTGATAAACCGATACAATTAAAGGCTCCTTTTGGAGCCTTTTTTT
TTGGAGATTTTCAACGTGAAAAAATTATTATTCGCAATTCCTTTAGTTGTTCCTTTCTATTCTCACTCCGCTGAAA
CTGTTGAAAGTTGTTTAGCAAAACCCCATACAGAAAATTCATTTACTAACGTCTGGAAAGACGACAAAACTTTA
GATCGTTACGCTAACTATGAGGGTTGTCTGTGGAATGCTACAGGCGTTGTAGTTTGTACTGGTGACGAAACTCA
GTGTTACGGTACATGGGTTCCTATTGGGCTTGCTATCCCTGAAAATGAGGGTGGTGGCTCTGAGGGTGGCGGT
TCTGAGGGTGGCGGTTCTGAGGGTGGCGGTACTAAACCTCCTGAGTACGGTGATACACCTATTCCGGGCTATA
CTTATATCAACCCTCTCGACGGCACTTATCCGCCTGGTACTGAGCAAAACCCCGCTAATCCTAATCCTTCTCTTGA

GGAGTCTCAGCCTCTTAATACTTTCATGTTTCAGAATAATAGGTTCCGAAATAGGCAGGGGGCATTAACTGTTT
ATACGGGCACTGTTACTCAAGGCACTGACCCCGTTAAAACTTATTACCAGTACACTCCTGTATCATCAAAAGCCA
TGTATGACGCTTACTGGAACGGTAAATTCAGAGACTGCGCTTTCCATTCTGGCTTTAATGAAGATCCATTCGTTT
GTGAATATCAAGGCCAATCGTCTGACCTGCCTCAACCTCCTGTCAATGCTGGCGGCGGCTCTGGTGGTGGTTCT
GGTGGCGGCTCTGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGGCGGTTC
CGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCAAACGCTAATAAGGGGGCTATGACCGAA
AATGCCGATGAAAACGCGCTACAGTCTGACGCTAAAGGCAAACTTGATTCTGTCGCTACTGATTACGGTGCTGC
TATCGATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGTGATTTTGCTGGCTCTAA
TTCCCAAATGGCTCAAGTCGGTGACGGTGATAATTCACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCTC
CCTCAATCGGTTGAATGTCGCCCTTTTGTCTTTAGCGCTGGTAAACCATATGAATTTTCTATTGATTGTGACAAAA
TAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCACCTTTATGTATGTATTTTCTACGTTTGCTAAC
ATACTGCGTAATAAGGAGTCTTAATCATGCCAGTTCTTTTGGGTATTCCGTTATTATTGCGTTTCCTCGGTTTCCT
TCTGGTAACTTTGTTCGGCTATCTGCTTACTTTTCTTAAAAAGGGCTTCGGTAAGATAGCTATTGCTATTTCATTG
TTTCTTGCTCTTATTATTGGGCTTAACTCAATTCTTGTGGGTTATCTCTCTGATATTAGCGCTCAATTACCCTCTGA
CTTTGTTCAGGGTGTTCAGTTAATTCTCCCGTCTAATGCGCTTCCCTGTTTTTATGTTATTCTCTCTGTAAAGGCTG
CTATTTTCATTTTTGACGTTAAACAAAAAATCGTTTCTTATTTGGATTGGGATAAATAATATGGCTGTTTATTTTG
TAACTGGCAAATTAGGCTCTGGAAAGACGCTCGTTAGCGTTGGTAAGATTCAGGATAAAATTGTAGCTGGGTG
CAAAATAGCAACTAATCTTGATTTAAGGCTTCAAAACCTCCCGCAAGTCGGGAGGTTCGCTAAAACGCCTCGCG
TTCTTAGAATACCGGATAAGCCTTCTATATCTGATTTGCTTGCTATTGGGCGCGGTAATGATTCCTACGATGAAA
ATAAAAACGGCTTGCTTGTTCTCGATGAGTGCGGTACTTGGTTTAATACCCGTTCTTGGAATGATAAGGAAAGA
CAGCCGATTATTGATTGGTTTCTACATGCTCGTAAATTAGGATGGGATATTATTTTTCTTGTTCAGGACTTATCTA
TTGTTGATAAACAGGCGCGTTCTGCATTAGCTGAACATGTTGTTTATTGTCGTCGTCTGGACAGAATTACTTTAC
CTTTTGTCGGTACTTTATATTCTCTTATTACTGGCTCGAAAATGCCTCTGCCTAAATTACATGTTGGCGTTGTTAA
ATATGGCGATTCTCAATTAAGCCCTACTGTTGAGCGTTGGCTTTATACTGGTAAGAATTTGTATAACGCATATGA
TACTAAACAGGCTTTTTCTAGTAATTATGATTCCGGTGTTTATTCTTATTTAACGCCTTATTTATCACACGGTCGG
TATTTCAAACCATTAAATTTAGGTCAGAAGATGAAATTAACTAAAATATATTTGAAAAAGTTTTCTCGCGTTCTTT
GTCTTGCGATTGGATTTGCATCAGCATTTACATATAGTTATATAACCCAACCTAAGCCGGAGGTTAAAAAGGTA
GTCTCTCAGACCTATGATTTTGATAAATTCACTATTGACTCTTCTCAGCGTCTTAATCTAAGCTATCGCTATGTTTT
CAAGGATTCTAAGGGAAAATTAATTAATAGCGACGATTTACAGAAGCAAGGTTATTCACTCACATATATTGATT
TATGTACTGTTTCCATTAAAAAAGGTAATTCAAATGAAATTGTTAAATGTAATTAATTTTGTTTTCTTGATGTTTG
TTTCATCATCTTCTTTTGCTCAGGTAATTGAAATGAATAATTCGCCTCTGCGCGATTTTGTAACTTGGTATTCAAA
GCAATCAGGCGAATCCGTTATTGTTTCTCCCGATGTAAAAGGTACTGTTACTGTATATTCATCTGACGTTAAACC
TGAAAATCTACGCAATTTCTTTATTTCTGTTTTACGTGCTAATAATTTTGATATGGTTGGTTCAATTCCTTCCATAA
TTCAGAAGTATAATCCAAACAATCAGGATTATATTGATGAATTGCCATCATCTGATAATCAGGAATATGATGATA
ATTCCGCTCCTTCTGGTGGTTTCTTTGTTCCGCAAAATGATAATGTTACTCAAACTTTTAAAATTAATAACGTTCG
GGCAAAGGATTTAATACGAGTTGTCGAATTGTTTGTAAAGTCTAATACTTCTAAATCCTCAAATGTATTATCTATT
GACGGCTCTAATCTATTAGTTGTTAGTGCACCTAAAGATATTTTAGATAACCTTCCTCAATTCCTTTCTACTGTTG
ATTTGCCAACTGACCAGATATTGATTGAGGGTTTGATATTTGAGGTTCAGCAAGGTGATGCTTTAGATTTTTCAT
TTGCTGCTGGCTCTCAGCGTGGCACTGTTGCAGGCGGTGTTAATACTGACCGCCTCACCTCTGTTTTATCTTCTG
CTGGTGGTTCGTTCGGTATTTTTAATGGCGATGTTTTAGGGCTATCAGTTCGCGCATTAAAGACTAATAGCCATT
CAAAAATATTGTCTGTGCCACGTATTCTTACGCTTTCAGGTCAGAAGGGTTCTATCTCTGTTGGCCAGAATGTCC
CTTTTATTACTGGTCGTGTGACTGGTGAATCTGCCAATGTAAATAATCCATTTCAGACGATTGAGCGTCAAAATG
TAGGTATTTCCATGAGCGTTTTTCCTGTTGCAATGGCTGGCGGTAATATTGTTCTGGATATTACCAGCAAGGCCG
ATAGTTTGAGTTCTTCTACTCAGGCAAGTGATGTTATTACTAATCAAAGAAGTATTGCTACAACGGTTAATTTGC
GTGATGGACAGACTCTTTTACTCGGTGGCCTCACTGATTATAAAAACACTTCTCAAGATTCTGGCGTACCGTTCC
TGTCTAAAATCCCTTTAATCGGCCTCCTGTTTAGCTCCCGCTCTGATTCCAACGAGGAAAGCACGTTATACGTGC
TCGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGC
GTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTC

Oligo name	**DNA sequence of staples for DNA origami triangle (5' - 3').**
R1	CGGGGTTTCCTCAAGAGAAGGATTTTGAATTA
R2	AGCGTCATGTCTCTGAATTTACCGACTACCTT
R3	TTCATAATCCCCTTATTAGCGTTTTTCTTACC
R4	ATGGTTTATGTCACAATCAATAGATATTAAAC

R5 TTTGATGATTAAGAGGCTGAGACTTGCTCAGTACCAGGCG
R6 CCGGAACCCAGAATGGAAAGCGCAACATGGCT
R7 AAAGACAACATTTTCGGTCATAGCCAAAATCA
R8 GACGGGAGAATTAACTCGGAATAAGTTTATTTCCAGCGCT
R9 GATAAGTGCCGTCGAGCTGAAACATGAAAGTATACAGGAG
R10 TGTACTGGGGATCTTCATTAAAGCAGAGCCAC
R11 CACCGGAAAGCGCGTTTTCATCGGAAGGGCGA
R12 CATTCAACAAACGCAAAGACACCAGAACACCCTGAACAAA
R13 TTTAACGGTTCGGAACCTATTATTAGGGTTGATATAAGTA
R14 CTCAGAGCATATTCACAAACGAATTAATAAGT
R15 GGAGGGAATTTAGCGTCAGACTGTCCGCCTCC
R16 GTCAGAGGGTAATTGATGGCAACATATAAAAGCGATTGAG
R17 TAGCCCGGAATAGGTGAATGCCCCCTGCCTATGGTCAGTG
R18 CCTTGAGTCAGACGATTGGCCTTGCGCCACCC
R19 TCAGAACCCAGAATCAAGTTTGCCGGTAAATA
R20 TTGACGGAAATACATACATAAAGGGCGCTAATATCAGAGA
R21 CAGAGCCAGGAGGTTGAGGCAGGTAACAGTGCCCG
R22 ATTAAAGGCCGTAATCAGTAGCGAGCCACCCT
R23 GATAACCCACAAGAATGTTAGCAAACGTAGAAAATTATTC
R24 GCCGCCAGCATTGACACCACCCTC
R25 AGAGCCGCACCATCGATAGCAGCATGAATTAT
R26 CACCGTCACCTTATTACGCAGTATTGAGTTAAGCCCAATA
R27 AGCCATTTAAACGTCACCAATGAACACCAGAACCA
R28 ATAAGAGCAAGAAACATGGCATGATTAAGACTCCGACTTG
R29 CCATTAGCAAGGCCGGGGGAATTA
R30 GAGCCAGCGAATACCCAAAAGAACATGAAATAGCAATAGC
R31 TATCTTACCGAAGCCCAAACGCAATAATAACGAAAATCACCAG
R32 CAGAAGGAAACCGAGGTTTTTAAGAAAAGTAAGCAGATAGCCG
R33 CCTTTTTTCATTTAACAATTTCATAGGATTAG
R34 TTTAACCTATCATAGGTCTGAGAGTTCCAGTA
R35 AGTATAAAATATGCGTTATACAAAGCCATCTT
R36 CAAGTACCTCATTCCAAGAACGGGAAATTCAT
R37 AGAGAATAACATAAAAACAGGGAAGCGCATTA
R38 AAAACAAAATTAATTAAATGGAAACAGTACATTAGTGAAT
R39 TTATCAAACCGGCTTAGGTTGGGTAAGCCTGT
R40 TTAGTATCGCCAACGCTCAACAGTCGGCTGTC
R41 TTTCCTTAGCACTCATCGAGAACAATAGCAGCCTTTACAG
R42 AGAGTCAAAAATCAATATATGTGATGAAACAAACATCAAG
R43 ACTAGAAATATATAACTATATGTACGCTGAGA
R44 TCAATAATAGGGCTTAATTGAGAATCATAATT
R45 AACGTCAAAAATGAAAAGCAAGCCGTTTTTATGAAACCAA
R46 GAGCAAAAGAAGATGAGTGAATAACCTTGCTTATAGCTTA
R47 GATTAAGAAATGCTGATGCAAATCAGAATAAA
R48 CACCGGAATCGCCATATTTAACAAAATTTACG
R49 AGCATGTATTTCATCGTAGGAATCAAACGATTTTTTGTTT
R50 ACATAGCGCTGTAAATCGTCGCTATTCATTTCAATTACCT
R51 GTTAAATACAATCGCAAGACAAAGCCTTGAAA
R52 CCCATCCTCGCCAACATGTAATTTAATAAGGC
R53 TCCCAATCCAAATAAGATTACCGCGCCCAATAAATAATAT
R54 TCCCTTAGAATAACGCGAGAAAACTTTTACCGACC
R55 GTGTGATAAGGCAGAGGCATTTTCAGTCCTGA
R56 ACAAGAAAGCAAGCAAATCAGATAACAGCCATATTATTTA
R57 GTTTGAAATTCAAATATATTTTAG
R58 AATAGATAGAGCCAGTAATAAGAGATTTAATG
R59 GCCAGTTACAAAATAATAGAAGGCTTATCCGGTTATCAAC
R60 TTCTGACCTAAAATATAAAGTACCGACTGCAGAAC
R61 GCGCCTGTTATTCTAAGAACGCGATTCCAGAGCCTAATTT

R62	TCAGCTAAAAAAGGTAAAGTAATT
R63	ACGCTAACGAGCGTCTGGCGTTTTAGCGAACCCAACATGT
R64	ACGACAATAAATCCCGACTTGCGGGAGATCCTGAATCTTACCA
R65	TGCTATTTTGCACCCAGCTACAATTTTGTTTTGAAGCCTTAAA
L1	TCATATGTGTAATCGTAAAACTAGTCATTTTC
L2	GTGAGAAAATGTGTAGGTAAAGATACAACTTT
L3	GGCATCAAATTTGGGGCGCGAGCTGAGTTAAA
L4	TTCGAGCTAAGACTTCAAATATCGCGGAACGA
L5	ACAGTCAAAGAGAATCGATGAACGACCCCGGTTGATAATC
L6	ATAGTAGTATGCAATGCCTGAGTAGGCCGGAG
L7	AACCAGACGTTTAGCTATATTTTCTTCTACTA
L8	GAATACCACATTCAACTTAAGAGGAAGCCCGATCAAAGCG
L9	AGAAAAGCCCCAAAAAGAGTCTGGAGCAAACAATCACCAT
L10	CAATATGACCCTCATATATTTTAAAGCATTAA
L11	CATCCAATAAATGGTCAATAACCTCGGAAGCA
L12	AACTCCAAGATTGCATCAAAAAGATAATGCAGATACATAA
L13	CGTTCTAGTCAGGTCATTGCCTGACAGGAAGATTGTATAA
L14	CAGGCAAGATAAAAATTTTTAGAATATTCAAC
L15	GATTAGAGATTAGATACATTTCGCAAATCATA
L16	CGCCAAAAGGAATTACAGTCAGAAGCAAAGCGCAGGTCAG
L17	GCAAATATTTAAATTGAGATCTACAAAGGCTACTGATAAA
L18	TTAATGCCTTATTTCAACGCAAGGGCAAAGAA
L19	TTAGCAAATAGATTTAGTTTGACCAGTACCTT
L20	TAATTGCTTTACCCTGACTATTATGAGGCATAGTAAGAGC
L21	ATAAAGCCTTTGCGGGAGAAGCCTGGAGAGGGTAG
L22	TAAGAGGTCAATTCTGCGAACGAGATTAAGCA
L23	AACACTATCATAACCCATCAAAAATCAGGTCTCCTTTTGA
L24	ATGACCCTGTAATACTTCAGAGCA
L25	TAAAGCTATGTAACAGTTGATTCCCATTTTTG
L26	CGGATGGCACGAGAATGACCATAATCGTTTACCAGACGAC
L27	TAATTGCTTGGAAGTTTCATTCCAAATCGGTTGTA
L28	GATAAAAACCAAAATATTAAACAGTTCAGAAATTAGAGCT
L29	ACTAAAGTACGGTGTCGAATCTGG
L30	TGCTGTAGATCCCCCTCAAATGCTGCGAGAGGCTTTTGCA
L31	AAAGAAGTTTTGCCAGCATAAATATTCATTGACTCAACATGTT
L32	AATACTGCGGAATCGTAGGGGGTAATAGTAAAATGTTTAGACT
L33	AGGGATAGCTCAGAGCCACCACCCCATGTCAA
L34	CAACAGTTTATGGGGTTTTGCTAATCAAAAGG
L35	GGCCGCTTCGCTGAGGCTTGCAGGGAAAAGGT
L36	GGCGCAGACTCCATGTTACTTAGCCGTTTTAA
L37	ACAGGTAGAAAGATTCATCAGTTGAGATTTAG
L38	CCTCAGAACCGCCACCCAAGCCCAATAGGAACGTAAATGA
L39	ATTTTCTGTCAGCGGAGTGAGAATAACCGATA
L40	TATTCGGTTTGCGGGATCGTCACCCGAAATCC
L41	GCGACCTGCGGTCAATCATAAGGGACGGAACAACATTATT
L42	AGACGTTACCATGTACCGTAACACCCCTCAGAACCGCCAC
L43	CCACGCATAGAAAGGAACAACTAAGTCTTTCC
L44	AATTGTGTCTCAGCAGCGAAAGACACCATCGC
L45	TTAATAAAACGAACTAAACCGAACTGACCAACGCCTGATA
L46	AGGTTTAGTACCGCCATGAGTTTCGTCACCAGGATCTAAA
L47	GTTTTGTCAGGAATTGCGAATAATGCCGACAA
L48	TGACAACAAGCATCGGAACGAGGGGGAGATTT
L49	GTATCATCTTTGAAAGAGGACAGAGGAAGAAAAATCTACG
L50	AGCGTAACTACAAACTACAACGCCTATCACCGTACTCAGG
L51	ATAGTTGCAATTTTTTCACGTTGATCATAGTT
L52	AGTACAACTAGCAACGGCTACAGATGATACCG
L53	ACCAGTCAGGACGTTGTGAACGGTGTACAGACCGAAACAA

L54 ACAGACAACCCAAATCTCCAAAAAAAAAATTTCTT
L55 AAACAGCTGGCTTTGAGGACTAAAGCGATTAT
L56 ACCAAGCGCAGGCGCATAGGCTGGAGAACTGGCTCATTAT
L57 TCGAGGTGAGGCTCCAAAAGGAGC
L58 GACCCCCAGACTTTTTCATGAGGAGCTTGCTT
L59 ACCTTATGCGATTTTACTGACCTTCATCAAGATCATCTTT
L60 TCGGTTTATCAAGTTTCCATTAAACGGGAATACAC
L61 TAAAACACGTAATCTTGACAAGAATTAATCATTGTGAATT
L62 AGGCGAAAGTAAAATACGTAATGC
L63 TGGTTTAATTTCAACTCCGGATATTCATTACCAACGAAAG
L64 CACCAACCTAACAAATCAACGTAACAATAAATTGGGCTTGAGA
L65 CCCTGACGAGAACACCAGAACGAGTAGAGCTGCTCATTCAGTG
B1 ATCGGGAGATATACAGTAACAGTACAAATAATT
B2 CCTGATTAAAGGAGCGGAATTATCTCGGCCTC
B3 GGCAAATCACCTCAATCAATATCTGCAGGTCGA
B4 CGACCAGTACATTGGCAGATTCACCTGATTGC
B5 TGGCAATTTTTAACGTCAGATGAAAACAATAACGGATTCG
B6 AAGGAATTACAAAGAAACCACCAGTCAGATGA
B7 GGACATTCACCTCAAATATCAAACACAGTAGA
B8 TTGACGAGCACGTATACTGAAATGGATTATTTAATAAAAG
B9 CCTGATTGCTTTGAATTGCGTAGATTTTCAGGCATCAATA
B10 TAATCCTGATTATCATTTTGCGGAGAGGAAGG
B11 TTATCTAAAGCATCACCTTGCTGATGGCCAAC
B12 AGAGATAGTTTGACGCTCAATCGTACGTGCTTTCCTCGTT
B13 GATTATACACAGAAATAAAGAAATACCAAGTTACAAAATC
B14 TAGGTGCATAAAAGTTTGAGTAACATTGTTTG
B15 TGACCTGACAAATGAAAAATCTAAAATATCTT
B16 GGAATCAGAGCGGGAGATGGAAATACCTACATAACCCTTC
B17 GCGCAGAGGCGAATTAATTATTAGCACGTAAATTCTGAAT
B18 TATGGAAGCGAACGTTATTAATTTCTAACAAC
B19 TAATAGATCGCTGAGAGCCAGCAGAAGCGTAA
B20 GAATACGTAACAGGAAAAACGCTCCTAAACAGGAGGCCGA
B21 TCAATAGATATTAAATCCTTTGCCGAATTGAACCA
B22 CAATATTTGCCTGCAACAGTGCCATAGAGCCG
B23 TTAAAGGGATTTTAGATACCGCCAGCCATTGCGGCACAGA
B24 ACAATTCGACAACTCGTAATACAT
B25 TTGAGGATGGTCAGTATTAACACCTTGAATGG
B26 CTATTAGTATATCCAGAACAATATCAGGAACGGTACGCCA
B27 CGCGAACTAAAACAGAGGTGAGGCTTAGAAGTATT
B28 GAATCTTGAGAAGTGTATCGGCCTTGCTGGTACTTTAATG
B29 ACCACCAGCAGAAGATGATAGCC
B30 CTAAAACATTAGAAGAACTCAAACTTTTTATAATCAGTGAG
B31 GCCACCGAGTAAAAGAACATCACTTGCCTGAGCGCCATTAAAA
B32 TCTTTGATTAGTAATAGTCTGTCCATCACGCAAATTAACCGTT
B33 CGCGTCTGATAGGAACGCCATCAACTTTTAC
B34 AGGAAGATGGGGACGACGACCGTAATCATATT
B35 CTCTAGAGCAAGCTTGCATGCCTGGTCAGTT
B36 CCTTCACCGCGAGACGGGCAACAGCAGTCACA
B37 CGAGAAAGGAAGGGAAGCGTACTATGGTTGCT
B38 GCTCATTTTTTAACCAGCCTTCCTGTAGCCAGGCATCTGC
B39 CAGTTTGACGCACTCCAGCCAGCTAAACGACG
B40 GCCAGTGCGATCCCCGGGTACCGAGTTTTTCT
B41 TTTCACCAGCCTGGCCCTGAGAGAAAGCCGGCGAACGTGG
B42 GTAACCGTCTTTCATCAACATTAAAATTTTTGTTAAATCA
B43 ACGTTGTATTCCGGCACCGCTTCTGGCGCATC
B44 CCAGGGTGGCTCGAATTCGTAATCCAGTCACG
B45 TAGAGCTTGACGGGGAGTTGCAGCAAGCGGTCATTGGGCG

B46	GTTAAAATTCGCGTTAATGTGAGCGAGTAACATACGTTGG
B47	TGTAGATGGGTGCCGGAAACCAGGAACGCCAG
B48	GGTTTTCCATGGTCATAGCTGTTTGAGAGGCG
B49	GTTTGCGTCACGCTGGTTTGCCCCAAGGGAGCCCCCGATT
B50	GGATAGGTACCCGTCGGATTCTCCTAAACGTTAATATTTT
B51	AGTTGGGTCAAAGCGCCATTCGCCCCGTAATG
B52	CGCGCGGGCCTGTGTGAAATTGTTGGCGATTA
B53	CTAAATCGGAACCCTAAGCAGGCGAAAATCCTTCGGCCAA
B54	CGGCGGATTGAATTCAGGCTGCGCAACGGGGGATG
B55	TGCTGCAAATCCGCTCACAATTCCCAGCTGCA
B56	TTAATGAAGTTTGATGGTGGTTCCGAGGTGCCGTAAAGCA
B57	TGGCGAAATGTTGGGAAGGGCGAT
B58	TGTCGTGCACACAACATACGAGCCACGCCAGC
B59	CAAGTTTTTTGGGGTCGAAATCGGCAAAATCCGGGAAACC
B60	TCTTCGCTATTGGAAGCATAAAGTGTATGCCCGCT
B61	TTCCAGTCCTTATAAATCAAAAGAGAACCATCACCCAAAT
B62	GCGCTCACAAGCCTGGGGTGCCTA
B63	CGATGGCCCACTACGTATAGCCCGAGATAGGGATTGCGTT
B64	AACTCACATTATTGAGTGTTGTTCCAGAAACCGTCTATCAGGG
B65	ACGTGGACTCCAACGTCAAAGGGCGAATTTGGAACAAGAGTCC
LINKER-L1R	TGTAGCATTCCTTTTATAAACAGTT
LINKER-L2R	CTTTAATTGTATTCCACCAGAGCC
LINKER-L3R	CACTACGAAGGTTAGCACCATTA
LINKER-L4R	AATAAGGCTTGAACAAAGTTAC
LINKER-R1B	TTAATTAATTTTTTACCATATCAAA
LINKER-R2B	TTAATTTCATCTTAGACTTTACAA
LINKER-R3B	CTGTCCAGACGTATACCGAACGA
LINKER-R4B	TCAAGATTAGTGTAGCAATACT
LINKER-B1L	GTGGGAACAAATTTCTATTTTTGAG
LINKER-B2L	CGGTGCGGGCCTTCCAAAAACATT
LINKER-B3L	ATGAGTGAGCTTTTAAATATGCA
LINKER-B4L	ACTATTAAAGAGGATAGCGTCC

Oligo name	**DNA sequences of staples for DNA origami rectangle (5' - 3').**
RECT_1	TCACGTTGAAAATCTCGCGAATAATAATTTTT
RECT_2	AGGAAGTTTCCATTAATAAAGACTTTTTCATG
RECT_3	CAGGCGCATAGGCTGGTGAACGGTGTACAGAC
RECT_4	GGTAGAAAGATTCATCGAACAACATTATTACA
RECT_5	TGACCATAAATCAAAAGTTCAGAAAACGAGAA
RECT_6	GTGTCTGGAAGTTTCAATGCAACTAAAGTACG
RECT_7	TTTTGCGGGAGAAGCCTATGACCCTGTAATAC
RECT_8	GTCAATCATATGTACCATCGTAAAACTAGCAT
RECT_9	GTGTAGATGGGCGCATGGGATAGGTCACGTTG
RECT_10	AGTGCCAAGCTTGCATTTGTAAAACGACGGCC
RECT_11	TATTGGGCGCCAGGGTGGAGAGGCGGTTTGCG
RECT_12	TGGCCCACTACGTGAACCGTCTATCAGGGCGA
RECT_13	TCTTTTCACTCAAAGGGCGAAAAACCATCA
RECT_14	AGGTCGACTTCGGCCAACGCGCGGGGTTTT
RECT_15	CGTGCATCTTTCCCAGTCACGACGGCCTGC
RECT_16	GATAATCAGCGGATTGACCGTAATCGTAAC
RECT_17	TCAACGCAAAATCGATGAACGGTACCGGTT
RECT_18	ATAACAGTTTTGTACCAAAAACATTTTATT
RECT_19	TCTTTACCCCAACATGTTTTAAATTTCCAT
RECT_20	GATTTAGGACAAATGCTTTAAACAATCAGG

RECT_21	TTCATCAAGTAAAACGAACTAACGAGTTGA
RECT_22	AAAATACGTTTGAAAGAGGACAGACTGACC
RECT_23	AAAGGCTCCAGAGGCTTTGAGGACACGGGT
RECT_24	AGAAAGGAACAACTAAAGGAATTCAAAAA
RECT_25	CCCAAATCAAGTTTTTTGGGGTCGAAACGTGGA
RECT_26	CTCCAACGCAGTGAGACGGGCAACCAGCTGCA
RECT_27	TTAATGAACTAGAGGATCCCCGGGGGGTAACG
RECT_28	CCAGGGTTGCCAGTTTGAGGGGACCCGTGGGA
RECT_29	ACAAACGGAAAAGCCCCAAAAACACTGGAGCA
RECT_30	AACAAGAGGGATAAAAATTTTTAGCATAAAGC
RECT_31	TAAATCGGGATTCCCAATTCTGCGATATAATG
RECT_32	CTGTAGCTTGACTATTATAGTCAGTTCATTGA
RECT_33	ATCCCCCTATACCACATTCAACTAGAAAAATC
RECT_34	TACGTTAAAGTAATCTTGACAAGAACCGAACT
RECT_35	GACCAACTAATGCCACTACGAAGGGGGTAGCA
RECT_36	ACGGCTACAAAAGGAGCCTTTAATGTGAGAAT
RECT_37	TGCCCTTCAGAGTCCACTATTAAAGGGTGCCG
RECT_38	CTCGAATTCGGGAAACCTGTCGTGCAGCTGAT
RECT_39	GTATCGGCCGCAAGGCGATTAAGTTTACCGAG
RECT_40	TGTATAAGCCAACCCGTCGGATTCTGACGACA
RECT_41	ATATATTTTGTCATTGCCTGAGAGTGGAAGAT
RECT_42	AGATTTAGTCAATAAAGCCTCAGAGAACCCTC
RECT_43	GCGGATTGCAGAGCTTAATTGCTGAAACGAGT
RECT_44	TACATAACGGGAATCGTCATAAATAAAGCAAA
RECT_45	ATTCATTACGTCAGGACGTTGGGAAATGCAGA
RECT_46	CTAAAACGAGGTCAATCATAAGGGAACCGGAT
RECT_47	GTTTATCAGGACAGCATCGGAACGACACCAAC
RECT_48	CAACTTTCAACAGTTTCAGCGGATGTATCG
RECT_49	TAAAGCACTAAATCGGAACCCTAATCCAGTTT
RECT_50	GGAACAACCGCCTGGCCCTGAGGCCCGCTT
RECT_51	TCCAGTCGTAATCATGGTCATAAAAGGGGG
RECT_52	ATGTGCTTCAGGAAGATCGCACAATGTGAG
RECT_53	CGAGTAAAAATATTTAAATTGTTACAAAGG
RECT_54	CTATCAGAAATGCAATGCCTGAATTAGCAA
RECT_55	AATTAAGTTGACCATTAGATACTTTTGCGG
RECT_56	ATGGCTTATCAAAAAGATTAAGAGCGTCCA
RECT_57	ATACTGCCCAAAAGGAATTACGTGGCTCAT
RECT_58	TATACCACCAAATCAACGTAACGAACGAGG
RECT_59	CGCAGACAAGAGGCAAAAGAATCCCTCAGC
RECT_60	AGCGAAACTTGCTTTCGAGGTGTTGCTAAA
RECT_61	CAGCAAGCGTAGGGTTGAGTGTTGTAGGGAGC
RECT_62	CCTGTGTGATTGCGTTGCGCTCACTAGAGTTG
RECT_63	AGCTTTCCGATTACGCCAGCTGGCGGCTGTTT
RECT_64	AATATTTTGGCTTTCATCAACATTATCCAGCC
RECT_65	GTAGGTAAACTATTTTTGAGAGATCAAACGTT
RECT_66	AAATGGTCAACAGGCAAGGCAAAGAGTAATGT
RECT_67	CCGAAAGACTTTGATAAGAGGTCATATTTCGC
RECT_68	GTAAGAGCAAATGTTTAGACTGGATAGGAAGC
RECT_69	CTCATTCAGATGCGATTTTAAGAACAGGCATA
RECT_70	AACACTCATCCATGTTACTTAGCCGAAAGCTG
RECT_71	TAAACAGCTTTTTGCGGGATCGTCAACACTAA
RECT_72	TAAATGAATTTTCTGTATGGGATTAATTTCT
RECT_73	CCCCGATTTAGAGCTTGACGGGGAAAAAGAATA
RECT_74	GCCCGAGAGTCCACGCTGGTTTGCAGCTAACT
RECT_75	CACATTAAAATTGTTATCCGCTCATGCGGGCC
RECT_76	TCTTCGCTGCACCGCTTCTGGTGCGGCCTTCC
RECT_77	TGTAGCCATTAAAATTCGCATTAAATGCCGGA

RECT_78	GAGGGTAGGATTCAAAAGGGTGAGACATCCAA
RECT_79	TAAATCATATAACCTGTTTAGCTAACCTTTAA
RECT_80	TTGCTCCTTTCAAATATCGCGTTTGAGGGGGT
RECT_81	AATAGTAAACACTATCATAACCCTCATTGTGA
RECT_82	ATTACCTTTGAATAAGGCTTGCCCAAATCCGC
RECT_83	GACCTGCTCTTTGACCCCCAGCGAGGGAGTTA
RECT_84	AAGGCCGCTGATACCGATAGTTGCGACGTTAG
RECT_85	AGGCGAAAAATCCCTTATAAATCAAGCCGG
RECT_86	CACACAACAGGTGCCTAATGAGTGCCCAGC
RECT_87	CCAGGCAAAGGGAAGGGCGATCGGCAATTC
RECT_88	GTTAAATCAAAATAATTCGCGTCTCGGAAA
RECT_89	CGGAGACAGCTAGCTGATAAATTAATTTTT
RECT_90	CATTTGGGGATAGTAGTAGCATTAAAAGGC
RECT_91	GAGCTTCAATCAGGATTAGAGAGTTATTTT
RECT_92	CCAGACGACAAAGAAGTTTTGCCATAATTC
RECT_93	GAAACACCAAATTTCAACTTTAATCGTTTA
RECT_94	CAAGCGCGATGATAAATTGTGTCGTGACGA
RECT_95	AATGACAACTCGCTGAGGCTTGCATTATAC
RECT_96	TCTAAAGTTTTGTCGTCTTTCCAGCCGAC
RECT_97	CGAACGTGGCGAGAAAGGAAGGGAAACCAGTA
RECT_98	TCGGCAAATCCTGTTTGATGGTGGACCCTCA
RECT_99	AAGCCTGGTACGAGCCGGAAGCATAGATGAT
RECT_100	CAACTGTTGCGCCATTCGCCATTCAAACATC
RECT_101	GCCATCAAGCTCATTTTTTAACCACAAATCC
RECT_102	CAACCGTTTCAAATCACCATCAATTCGAGCC
RECT_103	TTCTACTACGCGAGCTGAAAAGGTTACCGCG
RECT_104	CCAACAGGAGCGAACCAGACCGGAGCCTTTA
RECT_105	CTTTTGCAGATAAAAACCAAAATAAAGACTC
RECT_106	GATGGTTTGAACGAGTAGTAAATTTACCATT
RECT_107	TCATCGCCAACAAAGTACAACGGACGCCAGC
RECT_108	ATATTCGGAACCATCGCCCACGCAGAGAAGG
RECT_109	ATCAATATCGAACCTCAAATATCAATTCCGAAA
RECT_110	GGCAATTCACATATTCCTGATTATCAAAGTGTA
RECT_111	AAGAAAACAAAGAAGATGATGAAACAGGCTGCG
RECT_112	AATCGCAAGTATGTAAATGCTGATGATAGGAAC
RECT_113	AGTAATAAGTTAGGCAGAGGCATTTATGATATT
RECT_114	CCCAATAGCTCATCGTAGGAATCATGGCATCAA
RECT_115	CAGAGAGAAAAAAATGAAAATAGCAAGCAAACT
RECT_116	CTTATTACGAAGAACTGGCATGATTGCGAGAGG
RECT_117	AGCAAGGCCTCACCAGTAGCACCATGGGCTTGA
RECT_118	ATTGACAGGCCACCACCAGAGCCGCGATTTGTA
RECT_119	ATTAGGATTGGCTGAGACTCCTCAATAACCGAT
RECT_120	TCCACAGACAGCCCTCATAGTTAGCGTAACGA
RECT_121	ATAAAAGGGACATTCTGGCCAACAAAGCATC
RECT_122	ACCTTGCTTGGTCAGTTGGCAAAGAGCGGA
RECT_123	ATTATCATTCAATATAATCCTGACAATTAC
RECT_124	CTGAGCAAAAATTAATTACATTTTGGGTTA
RECT_125	TATAACTAACAAAGAACGCGAGAACGCCAA
RECT_126	CATGTAATAGAATATAAAGTACCAAGCCGT
RECT_127	TTTTATTTAAGCAAATCAGATATTTTTTGT
RECT_128	TTAACGTCTAACATAAAAACAGGTAACGGA
RECT_129	ATACCCAACAGTATGTTAGCAAATTAGAGC
RECT_130	CAGCAAAAGGAAACGTCACCAATGAGCCGC
RECT_131	CACCAGAAAGGTTGAGGCAGGTCATGAAAG
RECT_132	TATTAAGAAGCGGGGTTTTGCTCGTAGCAT
RECT_133	TTGAAAGGAGCAAATGAAAAATCTAGAGATAG
RECT_134	GATTATACTAAGAAACCACCAGAAGTCAACAG

RECT_135	TCATTTGAAGGCGAATTATTCATTTTTGTTTG
RECT_136	TTCAAATATAACCTCCGGCTTAGGTAACAATT
RECT_137	GGTAAAGTAATCGCCATATTTAACAAAACTTT
RECT_138	TTATCCGGTCTCATCGAGAACAAGCGACAAAA
RECT_139	ATTAGACGGCCAAATAAGAAACGATAGAAGGC
RECT_140	AATACATACCGAGGAAACGCAATAAGAAGCGC
RECT_141	TCGATAGCATTGAGCCATTTGGGAACGTAGAA
RECT_142	TGGCCTTGAAGAGCCACCACCCTCAGAAACCA
RECT_143	GGCGGATAACCTATTATTCTGAAACAGACGAT
RECT_144	TCACCAGTACAAACTACAACGCCTAGTACCA
RECT_145	AACCCTTCTGACCTGAAAGCGTAAGACGCTGAG
RECT_146	AGCCAGCAATTGAGGAAGGTTATCATCATTTT
RECT_147	GCGGAACATCTGAATAATGGAAGGTACAAAAT
RECT_148	CGCGCAGATTACCTTTTTTAATGGGAGAGACT
RECT_149	ACCTTTTTATTTTAGTTAATTTCATAGGGCTT
RECT_150	AATTGAGAATTCTGTCCAGACGACTAAACCAA
RECT_151	GTACCGCAATTCTAAGAACGCGAGTATTATTT
RECT_152	ATCCCAATGAGAATTAACTGAACAGTTACCAG
RECT_153	AAGGAAACATAAAGGTGGCAACATTATCACCG
RECT_154	TCACCGACGCACCGTAATCAGTAGCAGAACCG
RECT_155	CCACCCTCTATTCACAAACAAATACCTGCCTA
RECT_156	TTTCGGAAGTGCCGTCGAGAGGGTGAGTTTCG
RECT_157	ATCTTTAGGGCCTGCAACAGTGCCAATACG
RECT_158	ACCTACCATAGTTTGAGTAACATTTAAAAT
RECT_159	TACATAAATCTTTGAATACCAAGTGTTAGA
RECT_160	GACCTAAATCAAAATCATAGGTCTAAACAG
RECT_161	AAACAACATGCCAACGCTCAACAGTCTTCT
RECT_162	TAGCGAACCTCCAAGAACGGGTATGACAAT
RECT_163	ACAAAGTCACAAAATAAACAGCCAGCGTTT
RECT_164	GAAACGCAAAGATAGCCGAACAAACCCTGA
RECT_165	AATCAAGTTTCATTAAAGGTGAATATAAAA
RECT_166	CATTAAAGCCAGAGCCGCCACCCTCGACAG
RECT_167	AAGTATAGCAAACAGTTAATGCCCAATCCT
RECT_168	AGGAACCCATGTACCGTAACACTTGATAT
RECT_169	TGGCACAGACAATATTTTTGAATGGGGTCAGTA
RECT_170	TTAACACCAGCACTAACAACTAATCGTTATTA
RECT_171	ATTTTAAAATCAAAATTATTTGCACGGATTCG
RECT_172	CCTGATTGCAATATATGTGAGTGATCAATAGT
RECT_173	GAATTTATTTAATGGTTTGAAATATTCTTACC
RECT_174	AGTATAAAGTTCAGCTAATGCAGATGTCTTTC
RECT_175	CTTATCATTCCCGACTTGCGGGAGCCTAATTT
RECT_176	GCCAGTTAGAGGGTAATTGAGCGCTTTAAGAA
RECT_177	AAGTAAGCAGACACCACGGAATAATATTGACG
RECT_178	GAAATTATTGCCTTTAGCGTCAGACCGGAACC
RECT_179	GCCTCCCTCAGAATGGAAAGCGCAGTAACAGT
RECT_180	GCCCGTATCCGGAATAGGTGTATCAGCCCAAT
RECT_181	AGCCGTCAAAAAACAGAGGTGAGGCCTATTAG
RECT_182	CAGAAATAAAAATCCTTTGCCCGAAAGATTAG
RECT_183	TGCTTCTGTTCGGGAGAAACAATAACGTAAAA
RECT_184	TGTGATAAAAAGACGCTGAGAAGAGATAACCT
RECT_185	TGTTTATCAATATGCGTTATACAAACCGACCG
RECT_186	AGCCTTAAACCAATCAATAATCGGCACGCGCC
RECT_187	AGAGAGATAGAGCGTCTTTCCAGAGGTTTTGA
RECT_188	TTGTCACAATCTTACCGAAGCCCTTTAATATC
RECT_189	GCGTTTTCAAGGGAGGGAAGGTAAAGTTTATT
RECT_190	AATTTACCGGGAACCAGAGCCACCACTGTAGC
RECT_191	TCAGGAGGTGGGGTCAGTGCCTTGAGTCTCTG

RECT_192	CCACCCTCATTTTCAGGGATAGCAACCGTAC
RECT_193	TCTTTAATGCGCGAACTGATAGCCCCACCAG
RECT_194	CAGAAGATTAGATAATACATTTGTCGACAA
RECT_195	CTCGTATTAGAAATTGCGTAGATACAGTAC
RECT_196	CTTTTACAAAATCGTCGCTATTAGCGATAG
RECT_197	CTTAGATTTAAGGCGTTAAATAAAGCCTGT
RECT_198	TTAGTATCACAATAGATAAGTCCACGAGCA
RECT_199	TGTAGAAATCAAGATTAGTTGCTCTTACCA
RECT_200	ACGCTAACACCCACAAGAATTGAAAATAGC
RECT_201	AATAGCTATCAATAGAAAATTCAACATTCA
RECT_202	ACCGATTGTCGGCATTTTCGGTCATAATCA
RECT_203	AAATCACCTTCCAGTAAGCGTCAGTAATAA
RECT_204	GTTTTAACTTAGTACCGCCACCCAGAGCCA
RECT_205	AAAAATACCGAACGAACTAAAACATCGCCATT
RECT_206	AGACTTTACAAACAATAGGATTTAGAAGTATT
RECT_207	AGATGAATATACAGTATTTCAGGTTTAACGTC
RECT_208	AATCCTTGAAAACATAATTAATTTTCCCTTAG
RECT_209	CATAATTACTAGAAAAGAATAAACACCGGAAT
RECT_210	TATCCCATCCTAATTTTGAACAAGAAAAATAA
RECT_211	CAATTTTATCCTGAATATTTTGCACCCAGCTA
RECT_212	AGAGCAAGAAACAATGGTTAAGCCCAATAATA
RECT_213	CAAAGACAAAAGGGCGTATGGTTTACCAGCGC
RECT_214	CGTTTGCCATCTTTTCATAGCCCCCTTATTAG
RECT_215	ATACAGGAGTGTACTGTACATGGCTTTTGATG
RECT_216	CAGAACCGCCACCCTCTCAGAACCGCCACCCT

DNA sequences for determining an efficient strategy for masking HhaI sites involving site sequence mutation (Supplementary Figure 9).

Oligo name	**Staple sequence (5' - mutation - 3')**
WtCG (B54)	CGGCGGATTGAATTCAGGCT**GCGC**AACGGGGGATG
MutTA	CGGCGGATTGAATTCAGGCT**GTAC**AACGGGGGATG
MutAT	CGGCGGATTGAATTCAGGCT**GATC**AACGGGGGATG
Compl_B54	CATCCCCCGTTGCGCAGCCTGAATTCAATCCGCCG

DNA sequences of staples modified for preparing triangle variants with masked HhaI restriction sites (Figs 3 and 4, Supplementary Figs 11 and 15).

Site #	**Oligo name**	**Sequence of staples with mutated site (5' - 3')**
1	B45_Mut	TAGAGCTTGACGGGGAGTTGCAGCAAGCGGTCATTGGGAT
2	B52_Mut	CGATCGGGCCTGTGTGAAATTGTTGGCGATTA
3	B62_Mut	GATCTCACAAGCCTGGGGTGCCTA
4	B54_Mut	CGGCGGATTGAATTCAGGCTGATCAACGGGGGATG
5	B51_Mut	AGTTGGGTCAAAGATCCATTCGCCCCGTAATG
6	B43_Mut	ACGTTGTATTCCGGCACCGCTTCTGGATCATC
7	L3_Mut	GGCATCAAATTTGGGGATCGAGCTGAGTTAAA
8	L56_Mut8	ACCAAGCGCAGGATCATAGGCTGGAGAACTGGCTCATTAT
9	L36_Mut	GGATCAGACTCCATGTTACTTAGCCGTTTTAA
10	L56_Mut10	ACCAAGATCAGGCGCATAGGCTGGAGAACTGGCTCATTAT
8,10	L56_Mut	ACCAAGATCAGGATCATAGGCTGGAGAACTGGCTCATTAT
11	L47_Mut	GTTTTGTCAGGAATTGCGAATAATTCCGACAA
11	L51_Mut	ATAGTTGAAATTTTTTCACGTTGATCATAGTT
12	R06_Mut	CCGGAACCCAGAATGGAAAGATCAACATGGCT

13	R11_Mut	CACCGGAAAGATCGTTTTCATCGGAAGGGCGA
14	R8_Mut	GACGGGAGAATTAACTCGGAATAAGTTTATTTCCAGATCT
15	R20_Mut	CCTTGAGTCAGACGATTGGCCTTGATCCACCC
16	R37_Mut	AGAGAATAACATAAAAACAGGGAAGATCATTA
17	R53_Mut	TCCCAATCCAAATAAGATTACCGATCCCAATAAATAATAT
18	R61_Mut	GATCCTGTTATTCTAAGAACGCGATTCCAGAGCCTAATTT
19	B17_Mut	GATCAGAGGCGAATTAATTATTAGCACGTAAATTCTGAAT
20	B27_Mut	ATCGAACTAAAACAGAGGTGAGGCTTAGAAGTATT
22, 23	Loop3_Mut	CCACACCCGCCGATCTTAATGATCCGCTACAGG
24	Loop2_Mut	GTAGCGGTCACGCTGATCGTAACCA
24	Loop2_Wt	GTAGCGGTCACGCTGATCGTAACCA
25, 26	Loop1_Mut	AAGCGAAAGGAGCGGGATCTAGGGATCTGGCAAGT

DNA sequences modified for studying the effect of a DNA nick near or overlapping the HhaI restriction site on its reactivity (Supporting Figure 16).

Oligo name	**Sequence (5' - 3')**
dsDNA_Compl	
AGGTAATTGAAATGAATAATTCGCCTCTGCGCGATTTTGTAACTTGGTATTCAAAGCAATCAGG	
dsDNA	
CCTGATTGCTTTGAATACCAAGTTACAAAATCGCGCAGAGGCGAATTATTCATTTCAATTACCT	
I-a	CCTGATTGCTTTGAATACCAAGTTACAAAA
I-b	TCGCGCAGAGGCGAATTATTCATTTCAATTACCT
II-a	CCTGATTGCTTTGAATACCAAGTTACAAAAT
II-b	CGCGCAGAGGCGAATTATTCATTTCAATTACCT
III-a	CCTGATTGCTTTGAATACCAAGTTACAAAATC
III-b	GCGCAGAGGCGAATTATTCATTTCAATTACCT
IV-a	CCTGATTGCTTTGAATACCAAGTTACAAAATCG
IV-b	CGCAGAGGCGAATTATTCATTTCAATTACCT
V-a	CCTGATTGCTTTGAATACCAAGTTACAAAATCGC
V-b	GCAGAGGCGAATTATTCATTTCAATTACCT
VI-a	CCTGATTGCTTTGAATACCAAGTTACAAAATCGCG
VI-b	CAGAGGCGAATTATTCATTTCAATTACCT
VII-a	CCTGATTGCTTTGAATACCAAGTTACAAAATCGCGC
VII-b	AGAGGCGAATTATTCATTTCAATTACCT
VIII-a	CCTGATTGCTTTGAATACCAAGTTACAAAATCGCGCA
VIII-b	GAGGCGAATTATTCATTTCAATTACCT
IX-a	CCTGATTGCTTTGAATACCAAGTTACAAAATCGCGCAG
IX-b	AGGCGAATTATTCATTTCAATTACCT

DNA sequences modified for preparing triangle variants with masked Hin1II restriction sites (Supplementary Figure 20).

Site	**Oligo name**	**Sequence (5' - 3')**
1	B48_MUTHIN	GGTTTTCCCGGGTCATAGCTGTTTGAGAGGCG
2	B35_MUTHIN	CTCTAGAGCAAGCTTGCCGGCCTGGTCAGTT
3	L33_MUTHIN	AGGGATAGCTCAGAGCCACCACCCCCGGTCAA
4A	L25_MUTHIN	TAAAGCTAGGTAACAGTTGATTCCCATTTTTG
4B	L27_MUTHIN	TAATTGCTTGGAAGTTTCATTCCCAATCGGTTGTA
5	L31_MUTHIN	AAAGAAGTTTTGCCAGCATAAATATTCATTGACTCAACCGGTT
6	L36_MUTHIN	GGCGCAGACTCCCGGTTACTTAGCCGTTTTAA
7	L58_MUTHIN	GACCCCCAGACTTTTTCCGGAGGAGCTTGCTT
8	L42_MUTHIN	AGACGTTACCCGGTACCGTAACACCCCTCAGAACCGCCAC

9	R09_MUTHIN	GATAAGTGCCGTCGAGCTGAAACCGGAAAGTATACAGGAG
10	R06_MUTHIN	CCGGAACCCAGAATGGAAAGCGCAACCGGGCT
11	R28_MUTHIN	ATAAGAGCAAGAAACCGGGCATGATTAAGACTCCGACTTG
12	R49_MUTHIN	AGCCGGTATTTCATCGTAGGAATCAAACGATTTTTTGTTT
13	R63_MUTHIN	ACGCTAACGAGCGTCTGGCGTTTTAGCGAACCCAACCGGT
14	R52_MUTHIN	CCCATCCTCGCCAACCGGTAATTTAATAAGGC
	B37_SHIFT	CGAGAAAGGAAGGGAACGCTGCGCGTAACCAC
	B8_SHIFT	CACACCCGCCGCGCTTCTGAAATGGATTATTTAATAAAAG
	B12_ SHIFT	AGAGATAGATTACCGCTCAATCGTAATGCGCCGCTACAGG
	B16_SHIFT	GCGCGTACTATGGTTGTAATATCCAGAACAATAACCCTTC
	B20_SHIFT	GAATACGTCTATCGGCCTTGCTGGCTTTGACGAGCACGTA
	B23_SHIFT	TAACGTGCTTTCCTCGAGTAGAAGAACTCAAAGGCACAGA
	B26_SHIFT	CTATTAGTTAACATCACTTGCCTGTTGGAATCAGAGCGGG
	B28_SHIFT	AGCTAAACAGGAGGCCCTTCTTTGATTAGTAACTTTAATG
	B30_SHIFT	CTAAAACATAACCGTTGTAGCAATAGATTAAAGGGATTTTA
	B31_ SHIFT	GACAGGAACGGTACGCGTCCATCACGCAAATTCGCCATTAAAA
	B32_SHIFT	CCGAGTAAAAGAGTCTCAGAATCTTGAGAAGTGTTTTTATAAT
	LINKER-R4B_SHIFT	TCAAGATTAGTCAGTGAGGCCA
15	LOOP COMPL_S15_SHIFT	AACAGGAAAAACGCTCATGGAAATACCTAC

Bibliography

1. Woo,S. and Rothemund,P.W.K. (2011) Programmable molecular recognition based on the geometry of DNA nanostructures. *Nat. Chem.*, **3**, 620–7.
2. Douglas,S.M., Marblestone,A.H., Teerapittayanon,S., Vazquez,A., Church,G.M. and Shih,W.M. (2009) Rapid prototyping of 3D DNA-origami shapes with caDNAno. *Nucleic Acids Res.*, **37**, 5001–5006.
3. Pan,K., Boulais,E., Yang,L. and Bathe,M. (2014) Structure-based model for light-harvesting properties of nucleic acid nanostructures. *Nucleic Acids Res.*, **42**, 2159–70.
4. Pettersen,E.F., Goddard,T.D., Huang,C.C., Couch,G.S., Greenblatt,D.M., Meng,E.C. and Ferrin,T.E. (2004) UCSF Chimera A visualization system for exploratory research and analysis. *J. Comput. Chem.*, **25**, 1605–1612.

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