Supplementary Information for Unraveling the interaction between doxorubicin and DNA origami nanostructures for customizable chemotherapeutic drug release

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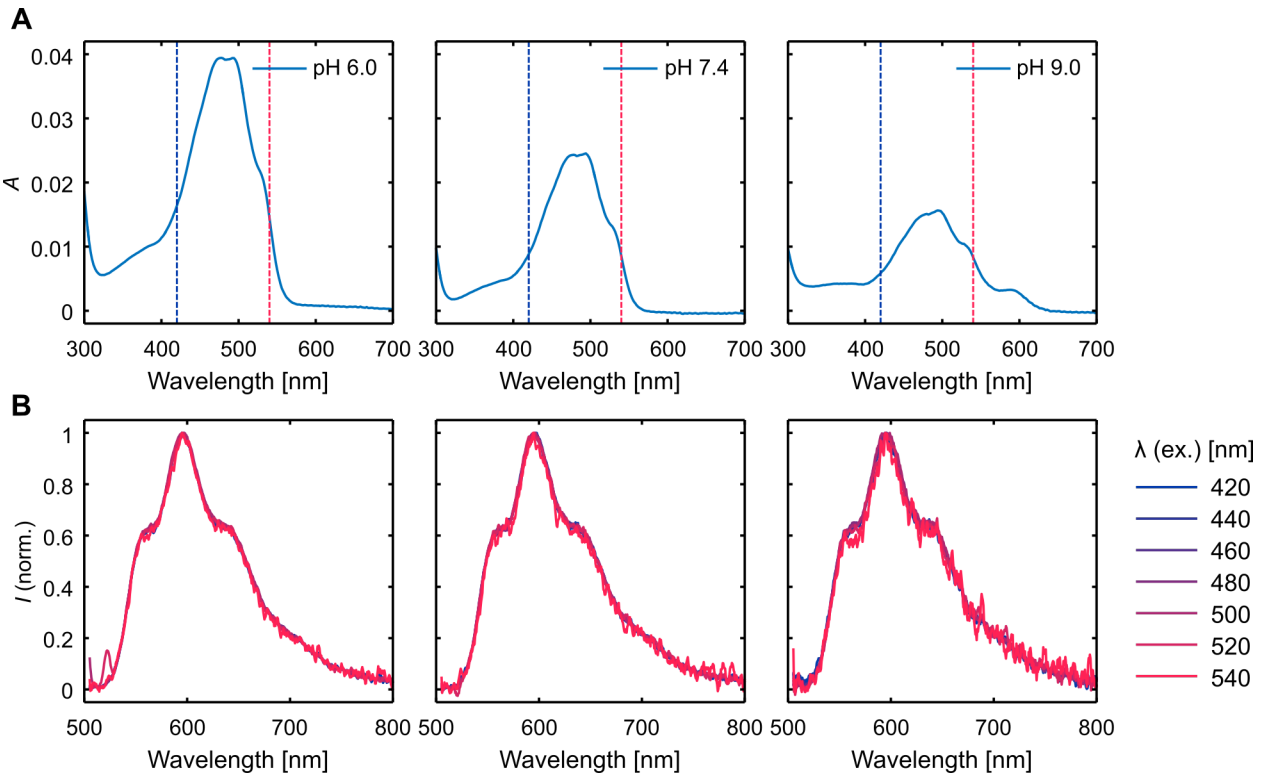
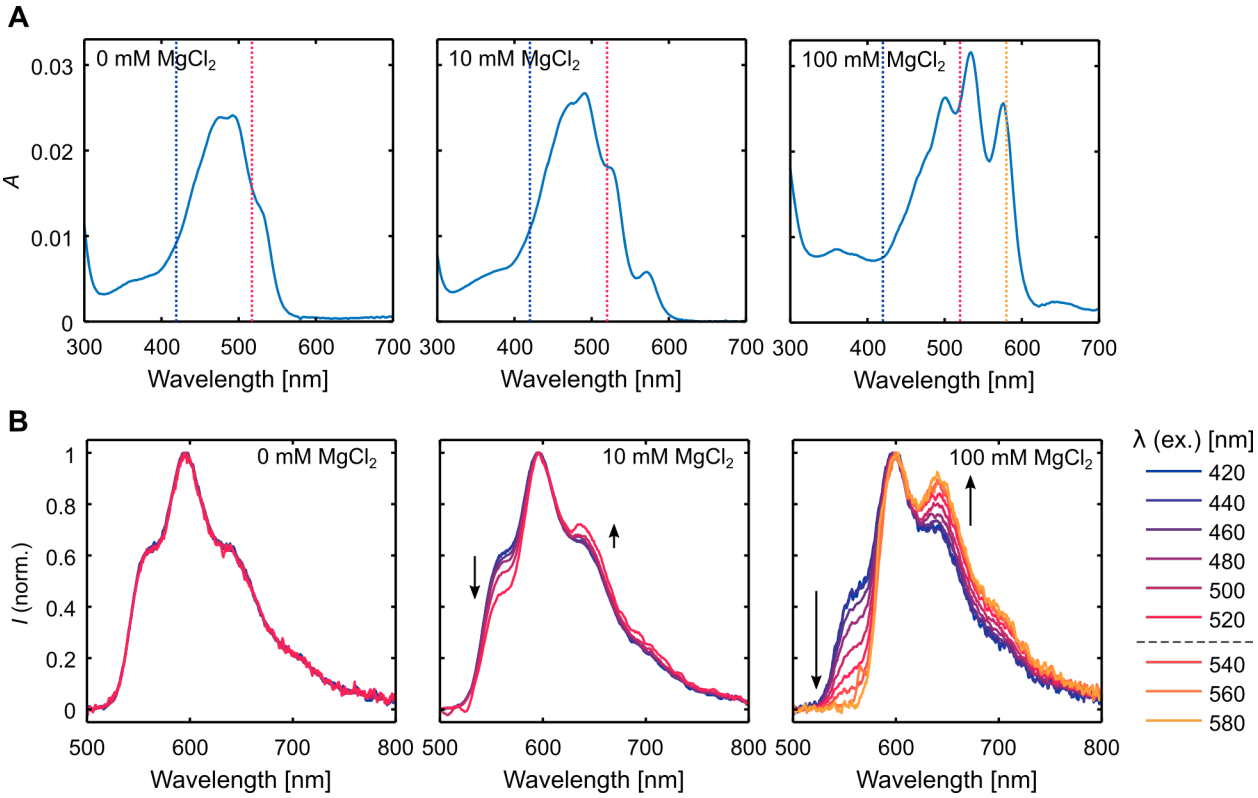
Note S1: DOX fluorescence in different pH buffers



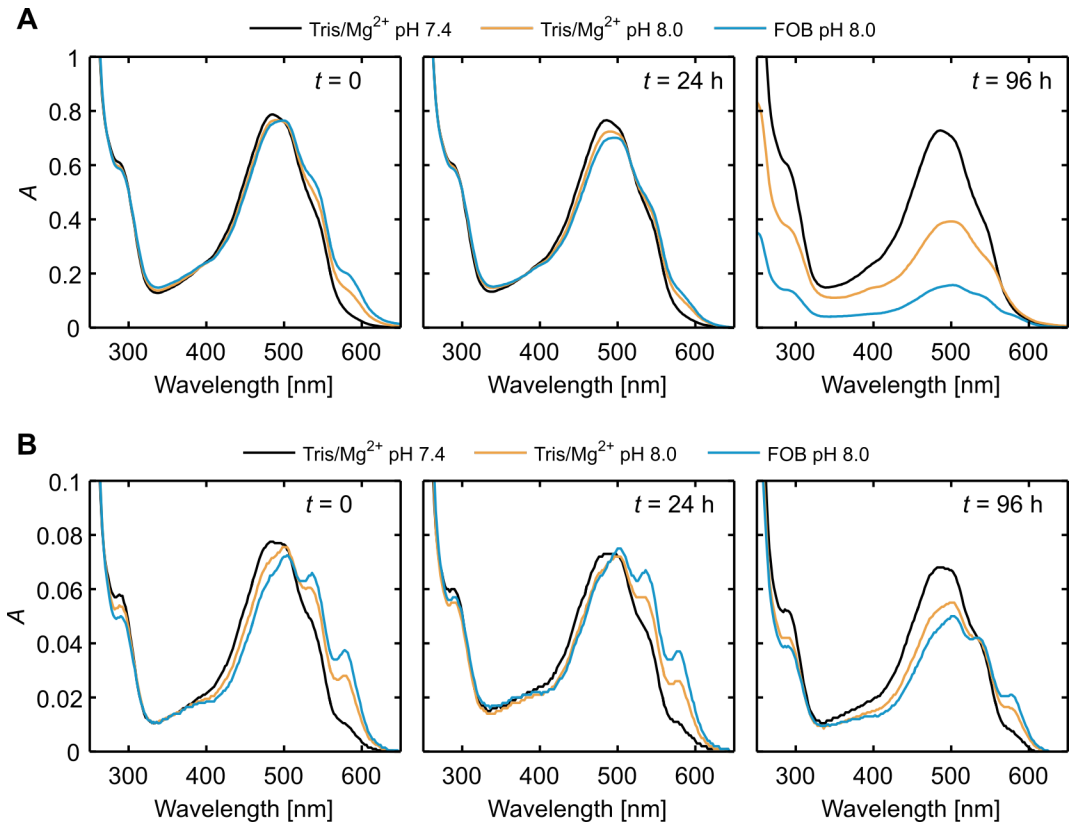
Supplementary Figure 1. Comparison of the shape of the absorption and emission spectra of 3 μM DOX in 40 mM Tris-HCl buffer at pH 6.0, 7.4, and 9.0 prepared without magnesium. (**A**) Absorption spectra at pH 6.0, 7.4, and 9.0. The colored dashed lines indicate the excitation wavelength range applied in Figure B. (**B**) The shape of the emission spectra collected with excitation wavelengths in the range of 420–540 nm. All spectra are normalized to the maximum value. While the emission intensity of DOX depends on the excitation wavelength and the pH of the sample (lower emission intensity at higher pH), the shape of the emission spectrum does not change, indicating that the full emission originates from a homogeneous group of fluorescent molecules.

Note S2: DOX fluorescence spectrum heterogeneity in the presence of different concentrations of $MgCl_2$



Supplementary Figure 2. Absorption and emission spectra of 3 μM DOX in 40 mM Tris pH 7.4 supplemented with different concentrations of $MgCl_2$. (**A**) Absorption spectra measured at 0, 10, or 100 mM $MgCl_2$ concentration (the concentration indicated in the upper-left corner of each figure). The excitation wavelength range applied for the emission spectra in Figure B (420–520 nm, and 520–580 nm) are indicated with the colored dashed lines. (**B**) Heterogeneity of the emission spectrum of DOX caused by Mg^{2+} complexation. The arrows indicate the changes observed in the shape of the spectrum relative to the spectrum collected with 420 nm excitation when the excitation wavelength changes. For 0 mM and 10 mM $MgCl_2$ samples, the shape of the emission spectrum is compared between 420–520 nm excitation. For the 100 mM $MgCl_2$ sample, additional excitation wavelengths 540–580 are included. The emission spectrum collected with 580 nm excitation originates purely from the DOX-Mg^{2+} complexes, as pure DOX (0 mM $MgCl_2$ sample) does not absorb light at this wavelength. All spectra have been normalized to the intensity at emission maximum.

Note S3: Full absorption spectra of 2 mM and 200 μM DOX in the aggregation experiment



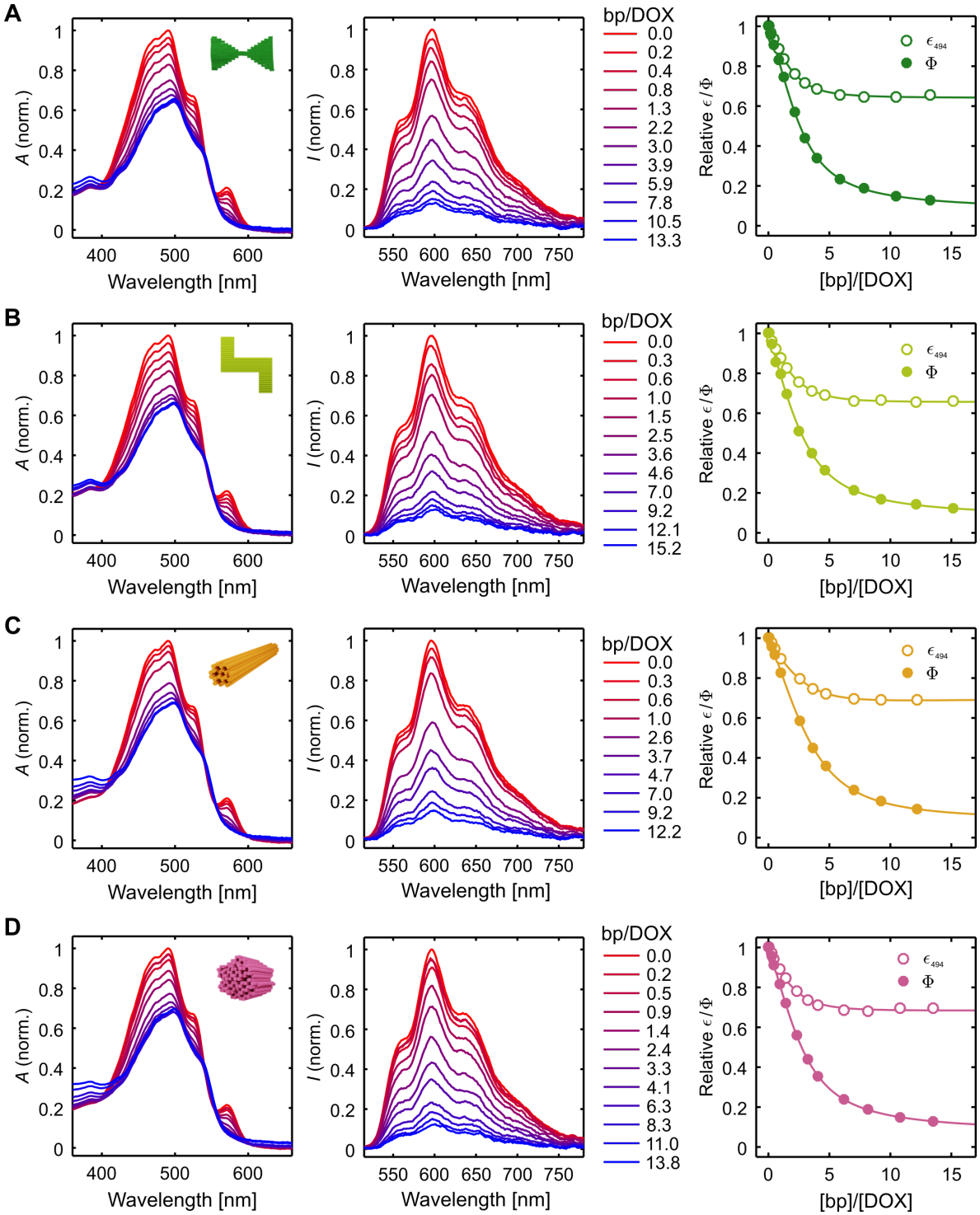
Supplementary Figure 3. Absorption spectra measured from the supernatant of DOX samples containing initially either 2 mM or 200 μM DOX, after incubation at RT for 0, 24, or 96 h in different buffers and centrifugation at 14,000 g for removing the insoluble fraction. Tris/Mg^{2+} denotes 40 mM Tris, 10 mM $MgCl_2$. FOB = 1× TAE, 12.5 mM $MgCl_2$. (**A**) 2 mM DOX. (**B**) 200 μM DOX.

Note S4: Details of the DNA origami designs

Supplementary Table 1. Nucleotide amounts in the studied DNA origami designs (N_{total}) and the fractions of unpaired (N_{ss}) and hybridized (N_{ds}) nucleotides. Molar extinction coefficients per nucleotide at 260 nm (ϵ_{260}/nt) have been calculated for each shape according to Equation 1.

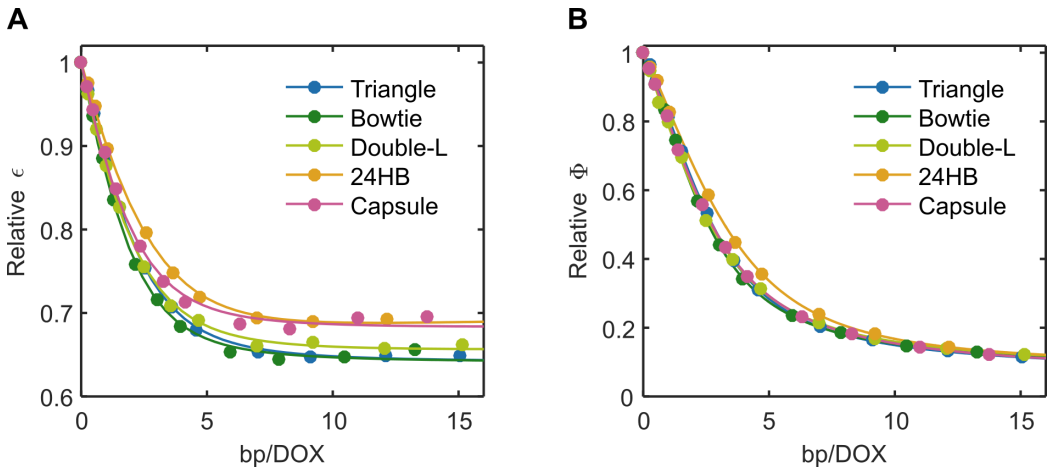
Origami shape	N_{total}	N_{ds}	N_{ss}	$N_{\mathrm{ds}}/N_{\mathrm{total}}$ (%)	ϵ_{260}/nt ($\mathrm{cm^{-1}M^{-1}}$)
Triangle	14,516	14,464	52	99.6	6,700
Bowtie	15,039	13,948	1,091	92.7	6,900
Double-L	15,193	14,324	869	94.3	6,900
24HB	15,504	15,120	384	97.5	6,800
Capsule	16,732	14,704	2,028	87.9	7,000

Note S5: Spectra and titration curves for the bowtie, double-L, 24HB, and capsule origami



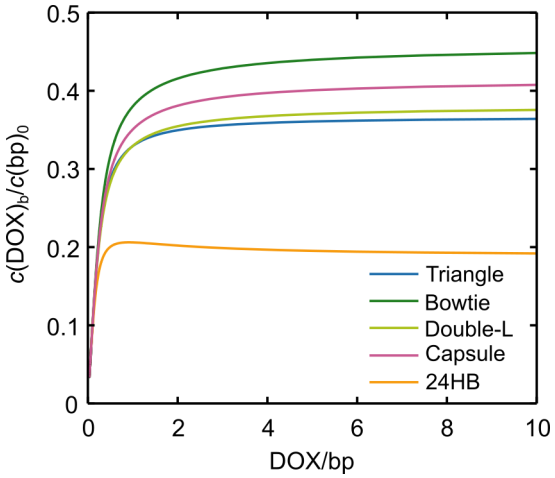
Supplementary Figure 4. DOX absorption spectra (left panel), fluorescence emission spectra (middle panel), and the dependence of DOX extinction coefficient at 494 nm (ϵ_{494}) and fluorescence quantum yield (Φ) on the amount of added DNA (right panel) for all studied DNA origami shapes. For both the absorption and emission spectra, the molar ratio of DNA base pairs and DOX (bp/DOX) is indicated in the legend. The concentration of DOX is 3 μM. The emission spectra have been collected after 494 nm excitation and corrected for the decrease of ϵ_{494}. The titration isotherms on the right panel have been fitted with a 1:2 molecular binding model. The fitting parameters are listed in Supplementary Table 2. (**A**) Bowtie. (**B**) Double-L. (**C**) 24HB. (**D**) Capsule.

Note S6: Comparison of titration isotherms (ϵ and Φ) for all structures



Supplementary Figure 5. Comparison of the effect of titrating DOX with the origami structures in terms of the relative decrease of the molar extinction coefficient at 494 nm (ϵ_{494}) and fluorescence quantum yield (Φ). (**A**) Relative decrease of ϵ_{494} of DOX upon addition of DNA origami. (**B**) Relative decrease of Φ measured with 494 nm excitation.

Note S7: Density of DOX molecules in the DNA origami structures in the titration experiments



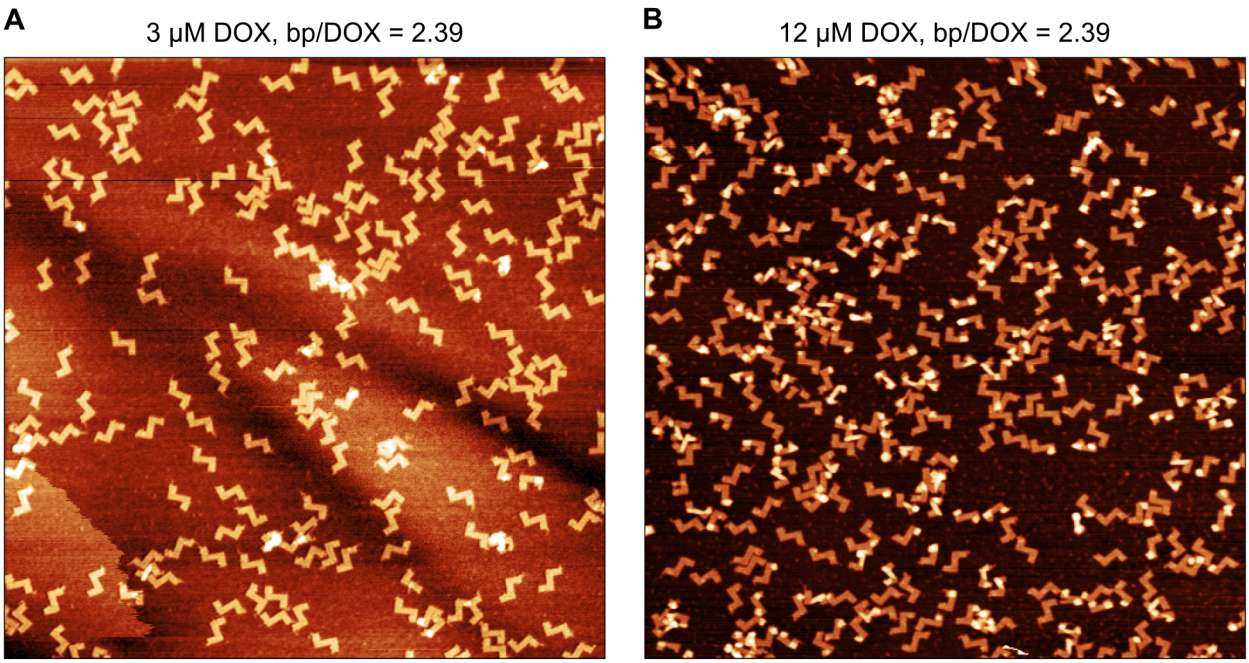
Supplementary Figure 6. Density of DOX molecules in the DNA origami structures in the titration experiments expressed as the number of bound DOX molecules per base pair of DNA (c(DOX)$_b$ / c(bp)$_0$). The molar ratio DOX/bp on the x axis refers to the total concentration of DOX and base pairs in the samples (c(DOX)$_0$ and $(bp)_0$).

Note S8: Fitting parameters K, Φ, and ϵ for all origami shapes obtained from the 1:2 DOX-DNA binding model

Supplementary Table 2. Association constants (K_{11}, K_{12}), fluorescence quantum yields (Φ_{11}, Φ_{12}), and molar extinction coefficients at 494 nm (ϵ_{11}, ϵ_{12}) for the two distinct DOX-DNA complexes. The values have been obtained by fitting the titration data with the 1:2 molecular binding model as described in the Methods section. Φ_{11}, Φ_{12}, ϵ_{11}, and ϵ_{12} are presented relative to the extinction coefficent and quantum yield of free DOX (ϵ_0 and Φ_0.

Origami shape	K_{11} ($\times 10^5 \mathrm{M}^{-1}$)	K_{12} ($\times 10^5 \mathrm{M}^{-1}$)	Φ_{11}/Φ_0 (%)	Φ_{12}/Φ_0 (%)	ϵ_{11}/ϵ_0 (%)	ϵ_{12}/ϵ_0 (%)
Triangle	1.93 ± 0.06	3.07 ± 0.06	62 ± 3	6.2 ± 0.3	62 ± 3	64 ± 3
Bowtie	2.81 ± 0.12	2.95 ± 0.07	64 ± 4	6.9 ± 0.4	64 ± 4	64 ± 4
Double-L	2.0 ± 0.2	2.15 ± 0.14	47 ± 5	7.0 ± 0.7	60 ± 6	66 ± 7
24HB	0.77 ± 0.03	2.59 ± 0.09	27.8 ± 1.2	8.2 ± 0.4	42 ± 2	71 ± 3
Capsule	2.36 ± 0.05	2.26 ± 0.03	57 ± 6	1.3 ± 0.2	64.2 ± 1.5	69 ± 2

Note S9: AFM images of double-L loaded with DOX and DOX-loading estimation

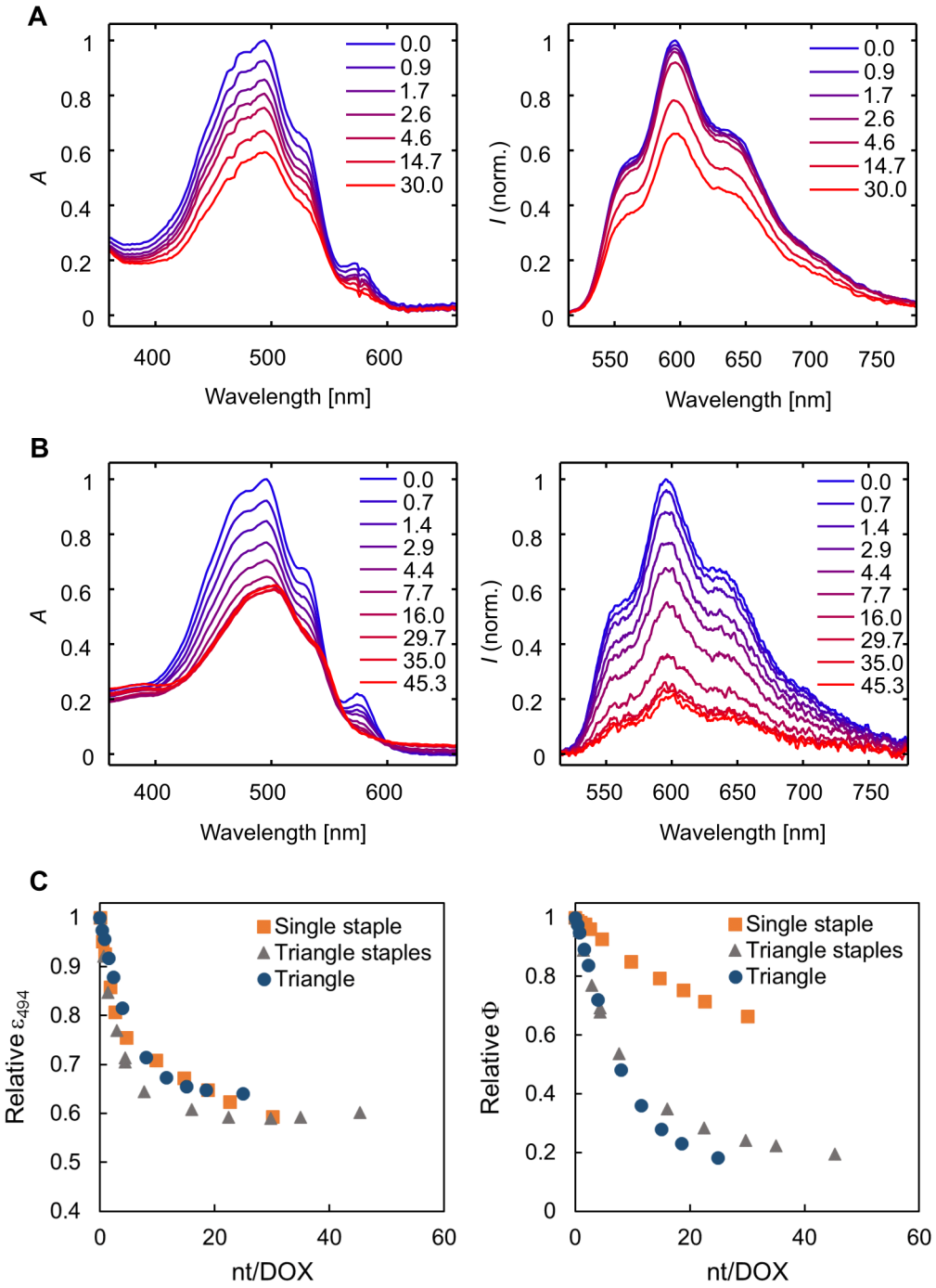


Supplementary Figure 7. AFM images of double-L origami loaded with DOX at two different sample concentrations while keeping the same molar ratio of DNA base pairs (bp) and DOX (bp/DOX). The edge of each figure is 3 µM in length. (**A**) DOX-origami sample prepared with a base pair concentration ($c(\mathrm{bp})_0$) of 7.16 µM and DOX concentration ($c(\mathrm{DOX})_0$) of 3 µM. (**B**) DOX-origami sample prepared at 4 times higher concentration of both DOX and double-L origami than Figure A ($c(\mathrm{bp})_0$ = 28.7 µM, $c(\mathrm{DOX})_0$ = 12 µM). While the bp/DOX ratio is identical in both cases, the higher total concentration of both DNA origami and DOX in Figure B can be seen to lead to an increased fraction of twisted DNA origami shapes, indicating higher DOX binding density through intercalation. The analysis result is in line with the theoretical values predicted by the 1:2 binding model (Supplementary Table 3.

Supplementary Table 3. Theoretical prediction of the density of loaded DOX (number of bound DOX molecules per base pair, $c(\mathrm{DOX})_\mathrm{b}/c(\mathrm{bp})_0$) in the double-L structures shown in Supplementary Figure 7 based on the 1:2 binding model.

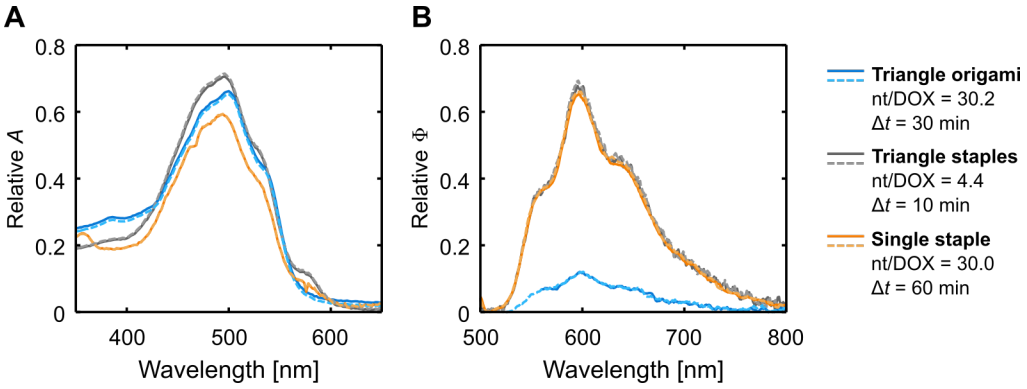
	3 µM DOX	**12 µM DOX**
$c(\mathrm{DOX})_0$ (µM)	3.0	12
$c(\mathrm{bp})_0$ (µM)	7.16	28.7
bp/DOX	2.39	2.39
$c(\mathrm{DOX})_\mathrm{b}$ (µM)	1.90	10.6
$c(\mathrm{DOX})_\mathrm{b}/c(\mathrm{bp})_0$	0.27	0.37

Note S10: Titration of DOX with ssDNA



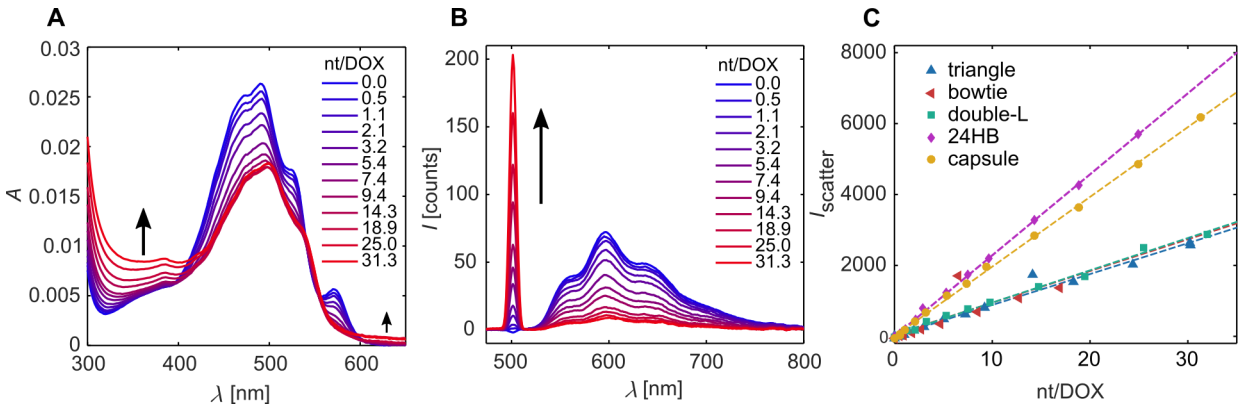
Supplementary Figure 8. Titration of 3 μM DOX with single- and double-stranded DNA. (**A**) Titration with a single oligonucleotide with a low probability of self-hybridization (5'–GAACAACGCTCCAACCATCGC–3'). Absorption spectrum of DOX is shown in the left panel and fluorescence emission in the right panel. The emission intensities have been collected with 494 nm excitation and corrected for the decrease of ϵ_{494} to represent the quantum yield of the DOX molecules (Φ). The legends in both figures denote the molar ratio of nucleotides and DOX (nt/DOX) for each spectrum. The concentration of nucleotides ($c(\mathrm{nt})_0$ has been calculated from the measured A_{260} value and a molar extinction coefficient for 100% single-stranded DNA, ϵ_{260} = 10,000 $\mathrm{M}^{-1}\mathrm{cm}^{-1}$. (**B**) Titration with a mixture of the 232 staple oligonucleotides used for folding the triangle origami, presented as Figure A. While the mixture of staple strands can be expected to contain a high number of hybridized dsDNA regions, the exact fraction of ssDNA and dsDNA nucleotides in the sample is unknown. The nt/DOX ratios presented in the legends have been calculated from the measured A_{260} value and a molar extinction coefficient for 100% single-stranded DNA, ϵ_{260} = 10,000 $\mathrm{M}^{-1}\mathrm{cm}^{-1}$. (**C**) The relative decrease of ϵ_{494} and Φ compared upon titration with a single oligonucleotide (Figure A), the triangle staple oligonucleotide mixture (Figure B), and the folded triangle origami (Figure 3B in the main text).

Note S11: Kinetics of DOX-DNA association – comparison of different incubation times during the titration experiments



Supplementary Figure 9. Comparison of the effect of incubation time on the spectra collected during titration of 3 μM DOX with the triangle origami, triangle origami staple mixture, or a single ssDNA staple oligonucleotide. For each sample, the spectra shown with solid lines have been collected after a 2.5–min incubation. The spectra plotted with dashed lines have been collected after continuing the incubation for a time indicated with the Δt. The DNA content in the samples is shown as the ratio between the total amount of nucleotides and the DOX concentration (nt/DOX). (**A**) Absorption spectra, with A values normalized to the spectrum of free 3 μM DOX. (**B**) Emission spectra depicting the relative fluorescence quantum yield (Φ) relative to free DOX.

Note S12: Scattering intensity of the origami



Supplementary Figure 10. Light scattering caused by the DOX-loaded DNA origami. (**A**) Absorption spectrum of a sample containing DOX and an increasing concentration of the capsule origami. Absorption baseline shift upwards during the titration is indicated with the black arrows and shows the increased light scattering in the sample. The amount of DNA origami is described as the total concentration of nucleotides in the sample, and indicated in the legend as the molar ratio of nucleotides vs. DOX (nt/DOX) (c(DOX) = 3 μM). (**B**) Scattered light detected as an increasing intensity of excitation light (494 nm) in the fluorescence emission measurement (90° detection relative to the excitation beam). The spectra are collected for 3 μM DOX upon titration with the capsule origami. (**C**) Intensity of scattered light during titration of DOX with different origami shapes (increasing nt/DOX ratio) shows the stronger light scattering detected with the 3D structures. The scattering intensities have been obtained from the emission spectra by integrating the excitation light peak.

Note S13: Details of DOX-DNA origami samples in the DNase I digestion experiments

Supplementary Table 4. Composition of the DNA origami-DOX samples in the DNase I digestion experiments before addition of DNase I. The DOX quantum yields relative to free DOX references (Φ/Φ_{ref}) and the base pair/DOX molar ratios (bp/DOX) are based on the measured DNA absorbance and DOX fluorescence intensities. The concentrations of free DOX ($c(\mathrm{DOX})_{\mathrm{ub}}$), bound DOX ($c(\mathrm{DOX})_{\mathrm{b}}$), and bound DOX molecules bound per base pair of DNA ($c(\mathrm{DOX})_{\mathrm{b}}/c(\mathrm{bp})_0$) have been calculated according to Equation 6 and the parameters in Supplementary Table 2.

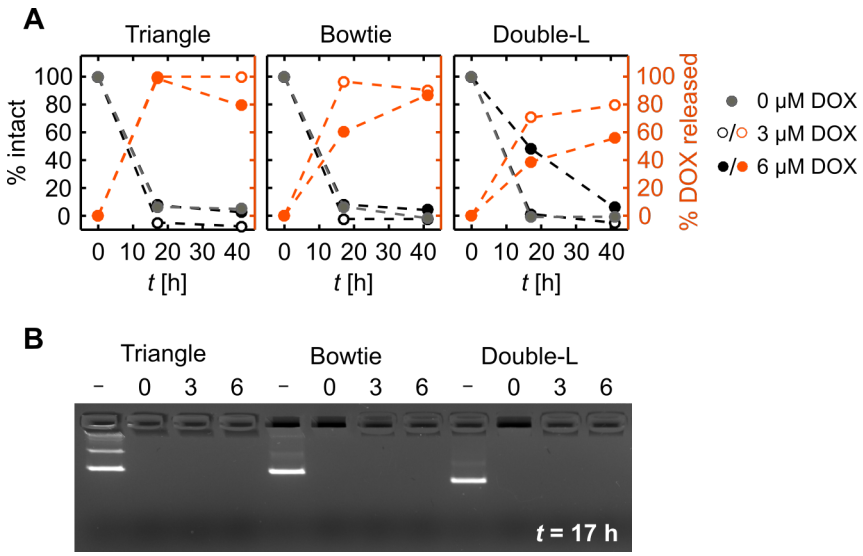
3 μM DOX

	Φ/Φ_{ref}	bp/DOX (μM)	$c(\mathrm{DOX})_{\mathrm{ub}}$ (μM)	$c(\mathrm{DOX})_{\mathrm{b}}$	$c(\mathrm{DOX})_{\mathrm{b}}/c(\mathrm{bp})_0$
Triangle	0.29	3.53	0.62	2.38	0.23
Bowtie	0.26	3.35	0.53	2.47	0.25
Double-L	0.24	3.39	0.71	2.29	0.23
Capsule	0.29	5.75	0.25	2.75	0.16
24HB	0.26	6.40	0.47	2.53	0.13

6 μM DOX

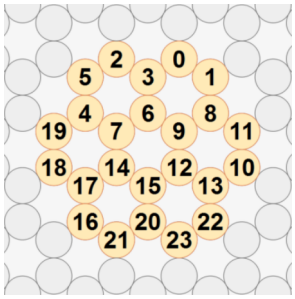
	Φ/Φ_{ref}	bp/DOX (μM)	$c(\mathrm{DOX})_{\mathrm{ub}}$ (μM)	$c(\mathrm{DOX})_{\mathrm{b}}$	$c(\mathrm{DOX})_{\mathrm{b}}/c(\mathrm{bp})_0$
Triangle	0.45	1.76	2.03	3.97	0.38
Bowtie	0.40	1.67	1.87	4.13	0.41
Double-L	0.39	1.70	2.80	3.20	0.31
Capsule	0.39	2.87	0.86	5.14	0.30
24HB	0.41	3.20	1.38	4.62	0.24

Note S14: DNase I digestion and DOX release profiles of the 2D structures over 41 h incubation

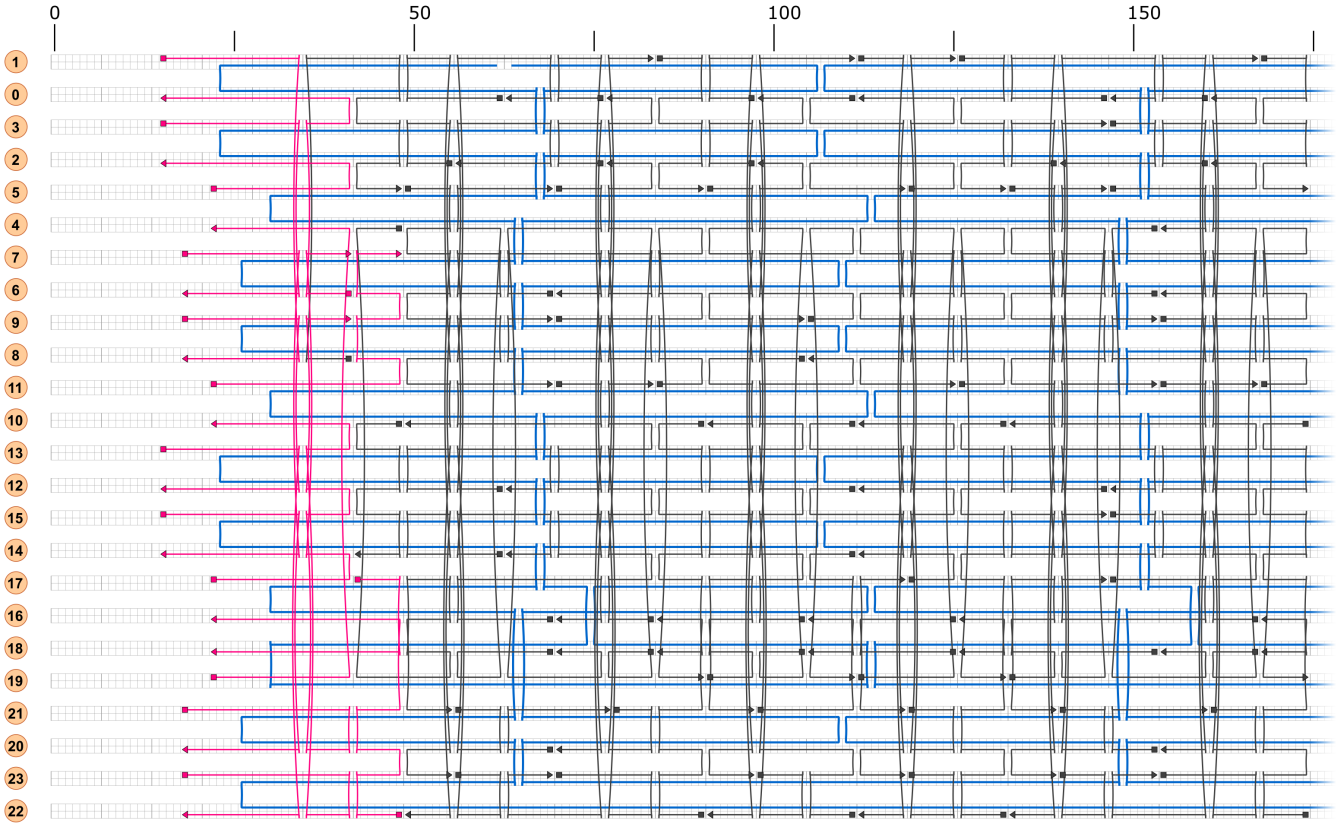


Supplementary Figure 11. DNase I digestion of the 2D origami structures over 41 h incubation in the presence of 28 U/mL DNase I and either 0, 3, or 6 μM DOX. (**A**) Digestion and DOX release profiles based on the A_{260} readings and the quantum yield of DOX relative to free DOX reference at the same concentration. The structural integrity of the DNA origami (% intact), as determined from the increase of the A_{260} signal, is shown with the gray triangle markers for the samples without DOX, and with the empty and filled black markers for the samples containing DOX at either 3 or 6 μM concentration. For the samples with 3 and 6 μM DOX, the orange markers depict the fraction of released DOX molecules relative to the initial concentration of bound DOX molecules. (**B**) Agarose gel electrophoresis (AGE) analysis result of the 2D origami shapes after 17 h incubation with DNase I. The first lane for each sample (-) contains the DNA origami without DNase I or DOX; the lanes marked with 0, 3, and 6 contain the indicated concentration of DOX in μM and 28.2 U/mL of DNase I.

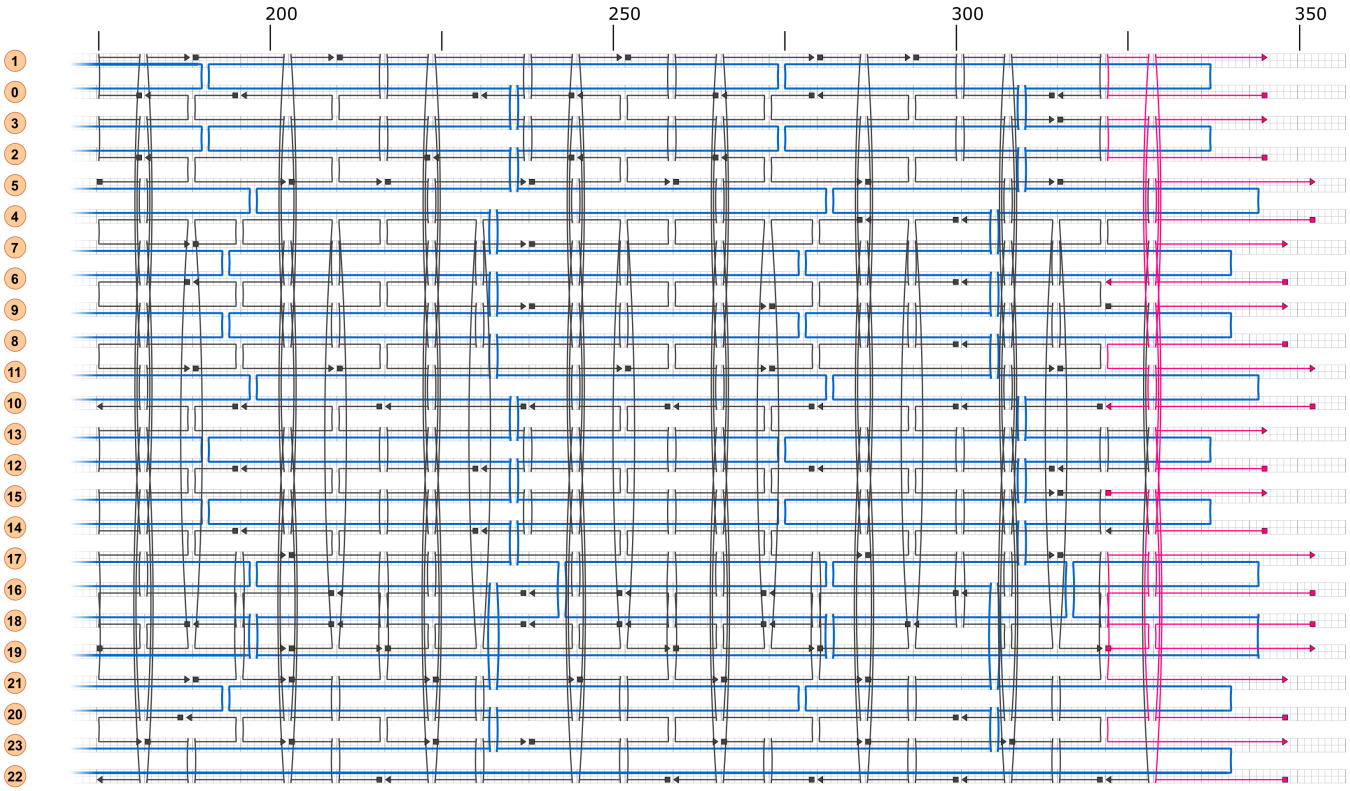
Note S15: 24HB design and staple sequences



Supplementary Figure 12. Cross-section of the 24 helix bundle (24HB) design and the helix numbers.



Supplementary Figure 13. Part 1 (grid positions 15–174) of the 24HB CaDNAno blueprint. The helix numbers are shown on the left side of the figure, and the numbers on top indicate the grid position. The 7,560-nt scaffold is shown in blue, fully complementary scaffold strands in dark gray, and scaffold strands containing 8× poly-T overhang sequences in pink.



Supplementary Figure 14. Part 2 (grid positions 175–352) of the 24HB CaDNAno blueprint.

Supplementary Table 5. Staple strand sequences for the 24HB design. Nucleotide bases complementary to the scaffold strand are written in capital letters. The 8× poly-T overhang sequences in staples #174–202 have been indicated by lowercase letters. Start pos. and end pos. indicate the positions of the staple strand termini in the caDNAno design (helix number[grid position]).

#	Start pos.	End pos.	Sequence
1	3[147]	2[140]	TTGAAATTCAAATCCAATCGC
2	6[153]	20[154]	CTCATTTATCTAATTTACGAGAGAGAGAATTTATCAAAGACA
3	22[279]	9[272]	GACAGTAGTTGGGAGAGGCTTGAGATGGGCAAAAGGGAAGTTAGCTTGA
4	21[78]	1[83]	GAAGGTAGATAATCAGTCACGGATTATATCCTGAGCAAATTTACGTGA
5	1[210]	5[216]	GTACCGCTAAGTATTTTTGCTTTAAGAGGCCCCCT
6	5[238]	18[238]	TTTTGTCGAGAATAGAAAGTACCGGAACGAGGCGCATGAAAC
7	10[48]	14[42]	TATCCGCTCGAATTGTGCTTGGACCTCCTTGAATC
8	22[258]	16[252]	ATCGCACGCTCATTTCAACTTGAAACACACGTAAC
9	5[70]	18[70]	GGCGCTAGCGTACTATGGTCAAATACCGAACGAACGCACGTG
10	10[195]	20[188]	GAAGCCTTTCCAGAGCCACCAGCCATTCGCCATTCGGTTTACAAATACAT
11	5[287]	5[286]	CGGAAGCTTAATTGCTCCTTTTGATAATTCTGTATGGCAGAC
12	7[238]	21[244]	ACTAAAGCGATTATACGAAGGTGTGAATTACCTAGGAATAAGGCTTGCC
13	20[153]	21[160]	CCACGACGCTTAAGACTCCTT
14	17[287]	0[280]	CCAAAAGATAACCCAAGACTTTCAAAAATGCGGATTAGCTCAACATGTT
15	21[140]	15[146]	GATGATGATTATCATACCTTT
16	19[217]	21[223]	TCCATGTATTTGTAGTCACCAAGCAAAACTTGAGC
17	21[189]	7[188]	AATACAGACCGAGGAAACGCACAAAGTTCGTCAAATCGGCTG
18	0[195]	12[196]	GTATCAGAGAGGATTAGGATTCGTCATACAAACAAAAATCAC
19	2[265]	22[259]	ACAGCCCCTACAACAGGCTCCCTTAAACTCCATTACTAAAGACAGGAAG
20	0[181]	10[175]	TTGAGAAGCCAACGCAAGCAAAACCTCCAGATTAG
21	2[181]	22[175]	AGTACCGTCGAGCCAAGAACGTCATCGTAATTTGCGCTAACGGCAACTG
22	16[83]	0[77]	AGTTGGCCAAATGATTCTGACCACGTATAAGGGATCCCGATTCTAAATC
23	18[167]	2[161]	CCCTTTTAAGAATTAGAAAAAACAATAAGTAAAGT
24	1[126]	5[132]	AAAGCCTTAAGGCGTTCTGACAGAACGCATATAAC
25	4[300]	16[301]	TTCGAGCTTGATAATCAGAAATAATCGTGAGGCATTCCCACA
26	21[98]	16[105]	TTAGGAGCACTAGCATATCAA
27	1[252]	5[258]	TCAGAGCATAGGAAGTACAAATCATAGTCAGACGT
28	20[69]	21[77]	AGGATGCTCGAAAGGAATTGAG
29	15[147]	3[146]	TACATTTATAATTATTTGCACTATCAAACCCTTAGTAATGGT
30	9[70]	23[69]	CGCCAGATTACATTATTGCAACCCAGTCACGACGTATTTAGA
31	5[315]	14[322]	AAAGGTGGCATCAAAAATCATAATAAAGGAGAGTC
32	10[111]	22[112]	ACTATCGATTTACAAAATCGCGATGTGC
33	20[300]	15[314]	GGAACAAGATTTAGGAATAGCGTCTGGCCGGATTCGCTTTTG
34	6[300]	20[301]	GATTGCACAAATATCGCGTATAGAGCAAGGCTTTTTCCTAAC
35	17[203]	0[196]	GCAAGGCAGAATCAATGATACCAGTAAGAGCGGGGAGCCCGGAATAGGT
36	10[300]	18[294]	TTCATTGCAATACTTTTGCCAAGCGAGACACTATCGAATTACAAAACTA
37	10[279]	22[280]	TCGTCACATGTTTAGACTGGAGGACGAC
38	16[104]	19[111]	ACCCTCACACCTTGTGGCCAAGTTAGAATCAGATACCACACCGCGGTCA
39	19[91]	21[97]	AAAACAGACAGTGCAAAGCATATCAATAAATATCT
40	0[230]	10[217]	CGAGAGGGTTGATACACCCTCGAGGCAGCCGCCGCTCAGAGC
41	21[266]	16[273]	AGTAGTAAATTGAAGGATATT
42	23[140]	12[147]	GTTTGAGTCGCTATCAATTACCTGAGATTACGGGA
43	11[168]	19[174]	TTTAGCGATCAGATTTTATTTGGTATTAAAACCAACTGAACAGAGTTAA
44	6[69]	20[70]	AGGAATGTTTGCTTTGACGAGCTGAAAGGATTCACACATTTG
45	0[314]	8[301]	ATTAGGCTGAATATAGTTTCATTAGAAT
46	11[84]	19[90]	ATAACATCCATCACGAAGTGTCCGATTAAACGTGCCGCCGCTAGAAGAT
47	19[175]	21[188]	GCCCAATCTATCTTAGCCGAAATAATAATAGCAAACGTAGAA
48	21[224]	14[231]	CATTTGGGGAGGGATCGGTCATAGCCCTACATAGC
49	9[273]	0[266]	TACCGTTAGTCTCCAAAAAAAGCCTGTAGGATAGC
50	23[308]	12[315]	TGGGAACTCGTAACCGTCAATATGATATGAGGGTA

51	11[126]	19[132]	AACAATTACATAAATAAATCGGAAAACAGTCAATATACCTTTGGTAATT
52	4[48]	21[55]	AACAGCTCCCTAAAACATCGCTTTGAATCTGTAAGTCGCCCTAACCCCG
53	22[111]	9[104]	TGCAAGGTTAAATCGAACAACTAATAGAAAGGGACTGACGCTATCAGTG
54	12[195]	23[202]	CGGAACCTTGCGTCAGACTGTATTCATTCAGCGCC
55	14[111]	18[105]	ATTTTTCCTGAACCTAACACC
56	0[265]	10[259]	AAGCCCACACCACCACAATGAGGAGTTAAGCGAAA
57	22[132]	16[126]	GCTGGCGTATTAATGAACAAAATTCCTGCCTGATT
58	12[314]	0[315]	GCTATAATAATACTCATATATTTCCATATAACAGTTTTGACC
59	17[119]	0[112]	GAATAATAAGAAATGAGAAGATAGCGATTTTCATCTTAAATAAGAATAA
60	18[188]	2[182]	GCAATAGAATAAGAACAATAGTAATGCAAATATAA
61	9[154]	23[153]	ACAAGCAACAGCCATTTATCCCGGTGCGGGCCTCTTAGAATA
62	1[280]	10[301]	AACTAAAAATCAGGGGATGCTTTAAACAGTTCAGAAAACGTAATAAATA
63	0[111]	12[112]	ACACCTGGGTATTTTAGTTAAAGCTTAGTAACCTTTTGAATA
64	8[300]	22[301]	GACCATATAGTCAGAAGCATGAAGAAGTGCGGAATCGTGCAT
65	20[187]	6[189]	AAAGGTTTTTTGTAGAGCCTAGGAATCATTACCACACCTTA
66	10[258]	18[252]	GACAGCATTGAGGAAACGGGTCTAAAACTCTTTGAGACCTTCTACAGAC
67	11[315]	19[321]	TGCCTGAGAACCCTTTTGCGGAAAAACAATTAAGCACAGGCAAAAACAG
68	15[315]	3[314]	AGAGATCTGGAGCAAACAATATAGCAAATTATGACTTATACA
69	17[147]	17[146]	ATCAAAACATAAAGGAACTGGCATGAAATTCATCACTACCAT
70	5[259]	23[265]	TAGTAAATTTCAACAATTTTTACACTCAGAAAGAGTTTAATTATACCAG
71	22[174]	16[168]	TTGGGAACAATCAATATAAAAAGTATGTCGGAATA
72	2[223]	22[217]	GAAAGTACAGTACCTGAATTTAAGCCAGTTTGCCACTCAGAGGTGCCGG
73	21[119]	1[125]	TCATCATGAAACCACAGATGAGATTGCTGCTTCTGTCAATATACTAGAA
74	0[97]	10[91]	GGTGCCGCCCAAATAGTCTGTCACTTGCTGGTAAT
75	10[216]	18[210]	CACCACCCGCCTCCTCTTTTCTTTCATCTAGCGACCGGAAACTCATCGC
76	2[139]	22[133]	AAGACAACTAAATTAATCCTTTCGCTATTAACGGATTCATTTTACGCCA
77	14[195]	18[189]	CTTTAAAACCAGAATGAAATA
78	16[272]	19[279]	CATTACCGTAATCTCACTAAATCACGTTGAAAAGATTTTGCTGACCAAC
79	0[160]	9[153]	AACGCCAACAAATTGCTTATCCGGTATTTTCGAGA
80	2[55]	22[49]	AGCAGGCAAAGAATCATTAATGTCGGGACTACGTGCGTAATCCGACGGC
81	2[244]	7[237]	ACGATCTGAGTTTCATTGTATCGGTTCGTTGAACA
82	21[203]	1[209]	ATCACCGGGAAATTAGCGCGTATAATCAATAAATCGATTGGCAGGTTTA
83	23[182]	11[188]	TTCATATAGGCTGCAGCGTCTTAAATCACGACTTG
84	23[98]	8[105]	ACTCGTACGATTAATACCTACGCCTTGCCTGAGTAGAAGAACTGACCGA
85	22[216]	16[210]	AAACCAGAAAGGGCTATTGACTCACCGATCACCAG
86	9[322]	22[322]	GAGAAGCTGCCGGATCAACCGTGTAGAT
87	11[210]	19[216]	ACCAGAGGTCAGACCTCATTAACCGTTCAGGAGTGTTGAGTAGACCTGC
88	1[112]	11[125]	CATAATTATGTGAGTCTTAATTACATTT
89	9[238]	23[237]	GCTTGCTGTAATGCCGGCTACAGCTTTCCGGCACCTGTATGC
90	11[70]	11[69]	TTCTTTGTACCGCCAGGTTTCCTGTGTGTGTAAAGCCAATAC
91	22[300]	23[307]	CTGCCAGTAATAAAACGAACG
92	0[244]	9[237]	ACCGTAACAGAACCCACGCATAACCGCAAGTATCA
93	21[161]	1[167]	ATTACGCGAAACGCCCAATCCAAAATAAAGCCGTTATAGAAGCTTACCA
94	7[189]	19[202]	TCTTTTGTTTATCAGCAAGAAACGTCGA
95	6[188]	0[182]	TCATTCCAGTAATAGGCTTAA
96	23[154]	11[167]	AGTTTATTTTGTCAGGGCGATTGAATCTTTTGCACGAGGCGT
97	23[70]	11[83]	AGTATTAGACTTTAGGGTTTTCAGGAAAACAATATATTAGTA
98	0[76]	9[69]	GGAACCCGGCCCACAACCGTTGTAGCTGCGCGGTA
99	5[133]	23[139]	TATATGTGAGAGACGTGAATTGTAAAACGTAACAGTTTTGCGTTTAAAA
100	1[84]	5[90]	ACCATCATAAAGCATAGAGCTGGAAGGGGGCAAGT

101	23[203]	10[196]	AAAGACAGCAAAGCCCGGAACCTCAGAGCCGCCTT
102	22[90]	16[84]	AACGCCACAAACAACGTCAATTATCTAATCTGGTC
103	12[62]	0[63]	GCTTAAGAACCTGTGGGTGCCTCTATCAGGGCGATTAGGTTG
104	5[203]	5[202]	AGTTAATGCTGAGACTCCTCAAGAGAAGGAACGCGCCTAAAC
105	19[112]	14[112]	GTACAAAGTCAGAGTTAACCTCCGGCCAAGACGCTTGCGTAG
106	21[56]	14[63]	CTTCTAAGCACGACTGTAATGAGTAACACGGGGCA
107	2[160]	6[154]	AATTCTGGTAATTTTACCGCA
108	18[125]	17[118]	TGAACACCCTGAATTCCTTCT
109	3[315]	6[301]	TTTCGCATTTGGGGCGCGAGGATTAGAGAGCTTAACCAAGCG
110	4[286]	18[273]	CGAACGAAGCCCGATCGTTTACCAGATATGACAAGAAAGAGG
111	23[266]	11[272]	TCAGGACTCGGCCTCTTTTTCCCTCAGCAAGGCCG
112	11[189]	1[188]	CGGGAGGTTCGCGCCCAATAGCTCAACA
113	5[49]	23[55]	TTGCAGCGACGGGCGGGTGGTAGGGGCCTGGTTGGTTAAGTGGTGGTTG
114	1[168]	5[174]	GTATAAATCGCCATGGCATTTACAAAAGACAACAT
115	16[237]	16[238]	AGTGAATTAGAGCCATGAAACCATCGCAAGCGCAGCTCATTC
116	5[175]	23[181]	GTTCAGCATAAGTCTCAATAAAATGAAAAAACGATGGCAACATAGAAAA
117	18[209]	17[202]	CTGATAAATTGTAAGGCATTA
118	5[217]	23[223]	GCCTATTCAGTGCCTACTGGTTAATCAGGGCATTTAGGTAAAGACATTC
119	23[224]	12[231]	AACCGATGCTTCTGCCGCCACCCTCAAACACCCTT
120	12[111]	23[118]	CCAAGTTATCAGGTTTAACGTCCAGAAGCTTTGCC
121	17[315]	5[314]	TCATTCGTTAAAGATGAACGGAGCCCCAAGGCAAACAGCTGA
122	9[105]	0[98]	AGGCCAAATGCGGGAGCTAAAGAAAGCCGGGTCGA
123	16[69]	16[70]	CAGTTTATTTACGCCAACTCGTCGGTTAAGAATAGAAATCAA
124	23[287]	10[280]	TCTACGTTTTGAGGTAGCGTCAATCCCCCTCAAGA
125	14[230]	2[224]	AGCACCGAATAAGTCAGTCTCAGGCGGATAAGTCTAAATTCTGAAACAT
126	18[293]	17[286]	GCATGTCAATCATGAATAACG
127	18[104]	2[98]	GCCTGCAAGGTGAGCGCCGCGTCACGCTAACGTGG
128	21[245]	1[251]	CTGACGATAATCATCACCAACAAAATACTTCGAGGCATCGCCGCCACCC
129	1[189]	11[209]	GTACCGTACTCAGGCTTGATATTACCAGAACCACC
130	16[251]	0[245]	AAAGCTGGCTGGCTCCCCCAGGAATTGCGCCTTTAGTCACCACCCATGT
131	10[237]	10[238]	GCGAACCGCCACCCCAGCATTGAATATATTCGGTCGAGGGTA
132	10[132]	18[126]	AAACATCCGAATTATTCGCCTATATACAAGAAATAGGAAGGGAATTAAC
133	16[300]	21[286]	TTCAACTAATGCAGATACACCGATTCAT
134	18[251]	2[245]	CAGGCGCAGACGGTGCGGAGTGTCTTTCTAGCGTA
135	10[174]	18[168]	TTGCTATTACCAACCAGTTACAAATAAGATAGCAGAGCAGATACCGAAG
136	12[146]	0[147]	GAAACAATAATTAATTAATGGATCATATGCGTTATACATACC
137	10[90]	18[84]	ATCCAGAAACGCTCCTGAAATACGACCAAGAACCCAAAATCTCACGCTG
138	19[203]	14[196]	AATCCGCACAGTGCCCGTATGGCTTTTGAGTTTGC
139	18[237]	5[237]	AAAGTACAACGGAGTACTTAGACGGGGTTCGGAACCTATTAG
140	8[104]	1[111]	GTAAAAGCAAGTTTTTGGAAT
141	11[154]	11[153]	AGAACGCCCAGCTACACAAAAGAAGATGAATTACCTTTTCTA
142	16[167]	0[161]	CCCAAAAAAAAAGTACCTTTACCATGTAGAACCAAGAGGCAGAATTTAAC
143	19[280]	4[287]	TTTATGTACCCCGGTTCAAAG
144	11[273]	1[279]	CTTTTGCTCATAGTTGCGCCGCTCATTTTCTATGC
145	12[279]	23[286]	AAAATGAAACGACGATAAAAAACAGGTAAGAAAAA
146	0[279]	12[280]	TTAAAAGGCAGAGGTCATTTTGATTAAGTACCCTGTAATAGT
147	18[83]	2[77]	AGAGCCACACCAGCACAGGGCGGGCGCTAAGAAAG
148	14[62]	2[56]	CGAATATTTTTCTTCCAGCTGAGCCCGAGATAGAAAGCTGGTTTGCCCC
149	10[321]	11[314]	GTCAAATCACCATCAATGCAA
150	11[252]	19[258]	CTTGCAGCAACAACTGAATTTAAAAGGAGAATAATAGTTTCACAATCAT

151	1[294]	4[301]	GTCTGGAAATGCTGGGCTTAGAGTACCTAAACTCCAACAGGTGATTTAA
152	21[287]	1[293]	CAGTTGACATTATTCCAAAATGAGGGGGACTATTAAATCAAAGTACGGT
153	23[56]	12[63]	TGAATTCTGTAAAAATGGTCATAGCTCCGGACAGG
154	23[238]	11[251]	GATTTTAAGAACTGTCCAGCCAGAGGCTTCGGAACGCTGAGG
155	12[230]	0[231]	ATTAGCGAATGGAAGGAGGTTAGAACCGCCACCCTCAGCCGT
156	16[209]	21[202]	TAGCACCATTACAAGTGAATT
157	18[272]	2[266]	ACAGATGCCGAACTAAACAACTGAATTTTCCACAG
158	5[147]	4[154]	GATGCCAGACGACGTAATATC
159	2[97]	22[91]	CGAGAAATGACGGGCAGGAGGTTTTATACAATCGTATGGAAAGTTGGGT
160	16[125]	21[118]	GTTTGGATTATAAAGGAATTA
161	5[91]	23[97]	GTAGCGGCTTAATGTTTCCTCCAGAGATGTAATAATTAGAGCTTCGACA
162	23[119]	10[112]	CGAACGTAAAGGGGGCAGAGGAAGAAAACAAAAAA
163	4[153]	18[154]	CCATCCAGAGAGATAACCCACTAAAACA
164	0[146]	10[133]	GACCGTGTGATAAAGTTTAGTAAACAGTTCATTTGATGAAAC
165	22[321]	17[314]	GGGCGCAAAACGGCAACCCGTCTTCCTGCCATCAAAAATAATAGGAGAA
166	19[133]	21[139]	GAGCGCTACGGGAGTTAGAACATATAATATTATCA
167	2[76]	6[70]	CGAAAGGGGGAGCCTTTAGAC
168	18[69]	5[69]	GCACAGACAATATTCATTAAACCAGTGAAAGCGGTCCACGCG
169	8[41]	5[48]	ACTCACAACGTCAAAGGGCGATTGGAACTAAATCAGAAAATCGAGAGAG
170	19[259]	21[265]	AAGGGAAAACGGTGATCAAGACAAATCACAGAACG
171	5[119]	5[118]	TTGGGTTGAGAAAACTTTTTCAAATACGGCGCGTAACTTAGG
172	0[62]	10[49]	AGTGTTGTTCCAGTAAAACCGTAATGAGCATAAAGAAATTGT
173	18[153]	5[146]	GGGAAGCGCATTAGAATATCATAGGTCTAAATGCT
174	8[348]	1[345]	tttttttGCAAGGATAAAACAATTCTGCttttttt
175	5[22]	2[15]	tttttttCCGCCTGGCCCTCTGTTTGATGGTGGTTCCGttttttt
176	9[18]	12[15]	tttttttTTGCGCTCAGATAAAGACGGAttttttt
177	14[345]	7[348]	tttttttTCATTGCCTCCTCAGAGCATAttttttt
178	1[15]	8[18]	tttttttACGTGGACTCCATTAATTGCGttttttt
179	6[41]	0[15]	CCAACGCTCCCTTAAAGAGTCCACTATTAAAGAttttttt
180	20[348]	15[345]	tttttttTTAAATGTGAGCGCTATCAGGttttttt
181	3[15]	6[18]	tttttttAAATCGGCAAAAGCGGGGAGAttttttt
182	6[348]	3[345]	tttttttAAGCTAAATCGGAATAACCTGttttttt
183	12[345]	9[348]	tttttttATAAATTAACTTTATTTCAACttttttt
184	13[15]	22[18]	tttttttGGATCCCCGGGTCTCAGGAGAttttttt
185	21[18]	9[41]	tttttttTTATGACAATGTCCCGTGCTGAAGAACTCTTTACCTCCTGCCCG
186	15[322]	23[348]	TACAAAGGAGTAACGGATTGACCGTAATGGGATttttttt
187	23[18]	10[22]	tttttttAGCCAGGGTGGATGTTAAGCTTTACCGAGCTCACAATTCCACttttttt
188	11[22]	7[48]	tttttttACAACATACGAGCCGGAAGTGAGCTACTTTCCAGAATCGGGGCGCCA
189	0[345]	10[322]	tttttttGAACGAGTAGATTTAGTGATTCCATTTTTAGTAATGTGGAGACA
190	22[348]	13[345]	tttttttAGGTCACGTTGGTTCTAGCTGttttttt
191	22[48]	16[22]	CAGTGCCCTTCTAATCCTTAGCCAAAATGGAGTGACTCTATGATACCttttttt
192	19[22]	4[22]	tttttttGCGAACTGATAGGATTGCCCTTCAttttttt
193	17[42]	18[22]	CTGCCATGGCTATTAGTCTTTAATGCttttttt
194	7[18]	14[15]	tttttttGGCGGTTTGGCATTTCACATAttttttt
195	19[322]	21[348]	GAAGATTTATTTTGCATTAAAAGGAACGTAGCCAGCTTTCATCAACAttttttt
196	15[15]	20[18]	tttttttAATCATTTCTCCTTGTCAACCttttttt
197	17[22]	7[41]	tttttttGACAGTGCGGCCGGCTGACCGTATTG
198	2[345]	6[322]	tttttttTTTAGCTATATTTTCAAATGGTCTTGTACC
199	18[352]	19[352]	tttttttAAATTGTAAACGTTAAGTATAAGCAAATATTTttttttt
200	10[352]	11[352]	tttttttAAGGGTGAGAAAGGCCGTAGGTAAAGATTCAAttttttt
201	16[352]	17[352]	tttttttTCATTTTTTAACCAATTTTTTGTTAAATCAGCttttttt
202	4[352]	5[352]	tttttttAGCATTAACATCCAATTTCTACTAATAGTAGTttttttt