Electronic Supplementary Material (ESI) for Chemical Communications.

Electronic Supplementary Information

Pandora´s DNA Origami Box for the Site-specific Facet Protection of Gold Nanoparticles: A Building Block for Molecular Plasmonics

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Preparation of AuNS/AuNC and coating with ssDNA

Tween20-stabilized AuNS and AuNC were synthesized according to a reported method.[2] The synthesized and CTAB-stabilized particles were centrifuged and the precipitate was subsequently resuspended two times in 1 % sodium dodecyl sulfate (SDS) followed by three washing steps with 1 % Tween20. After the last centrifugation step, the pellets were collected and the concentration of AuNP was determined by UV-Vis extinction spectroscopy according to a reported method.[3] Coating of the synthesized AuNS and AuNC with ssDNA was carried out following a published protocol.[4]

Agarose gel electrophoresis (AGE)

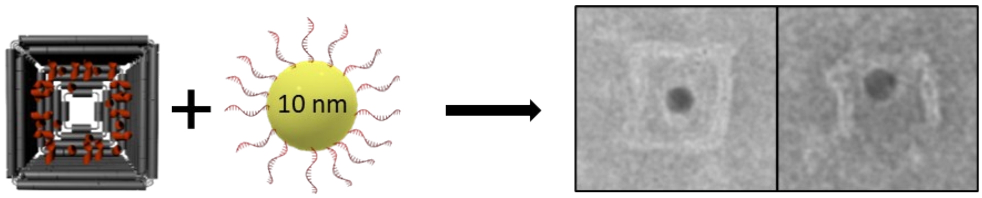
Purification of AuNP/origami box conjugates was achieved by using AGE in a 1 % agarose gel (containing 0.2 % Tween20) with 0.5xTBEMg as running buffer with 0.2 % Tween20 for 2 h at 4°C at 80 V. Isolation of origami band containing AuNP/origami conjugates was achieved by using spin column extraction (Freeze `n Squeeze Quantum prep, BioRad) at 10°C for 10 min and 250 g.

TEM Analysis

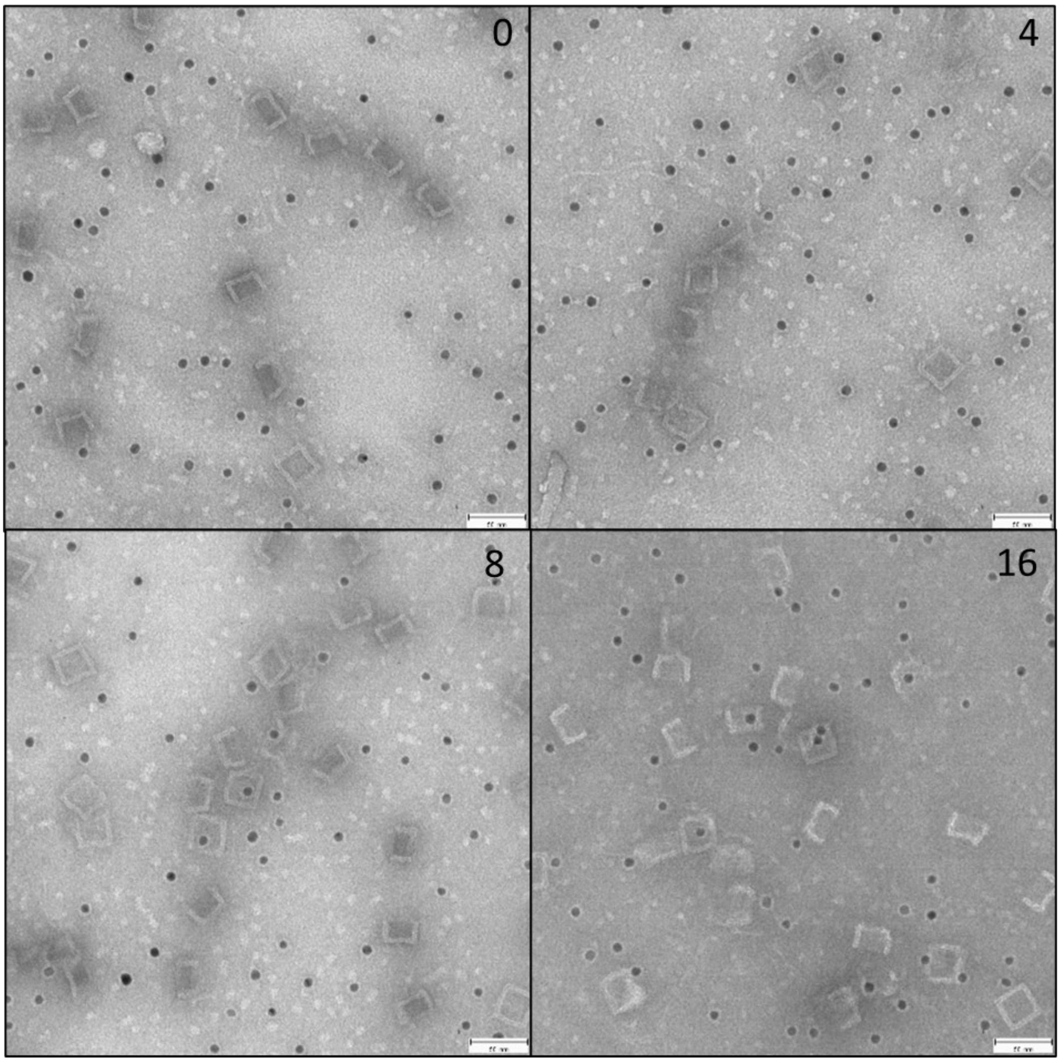
For the TEM analysis 400 mesh carbon-coated copper grids (Quantifoil) were glow charged and samples were drop-casted onto the grid. After incubation (2 min) the origamis were stained with 1 % uranyl formate before drying. The TEM images were obtained using a JEOL JEM 1400 Plus and a ZEISS EM910.

References

1. Y. Ke, S. M. Douglas, M. Liu, J. Sharma, A. Cheng, A. Leung, Y. Liu, W. M. Shih and H. Yan, *J. Am. Chem. Soc.*, 2009, 15903–15908.
2. J. E. Park, Y. Lee and J. M. Nam, *Nano Lett.*, 2018, **18**, 6475-6482.
3. T. Hendel, M. Wuithschick, F. Kettemann, A. Birnbaum, K. Rademann and J. Polte, *Anal. Chem.*, 2014, **86**, 11115-11124.
4. S. Xu, H. Yuan, A. Xu, J. Wang and L. Wu, *Langmuir*, 2011, **27**, 13629-13634.



Binding via Hybridization of ssDNA coated AuNP (10 nm) "gold standard"					
No. of protruding strands inside Pandora	**0**	**4**	**8**	16	**28**
Binging efficiency	0.5%	2%	9%	14%	62%



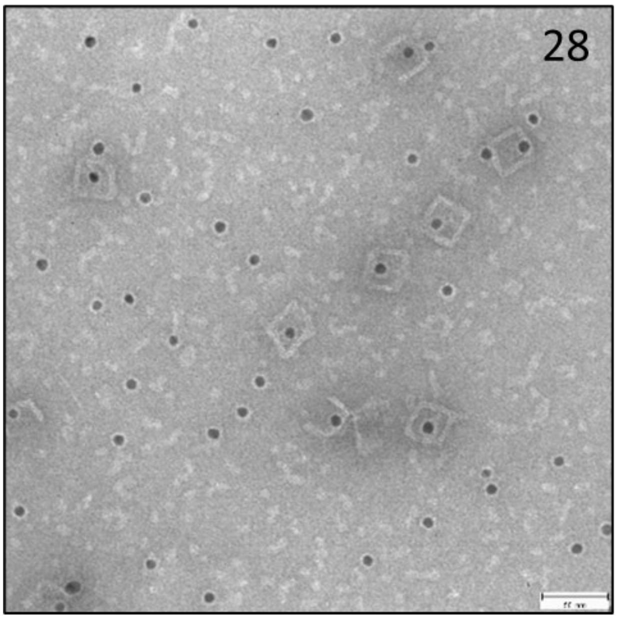


Fig. S 1 Top: **Incorporation of AuNS by hybridization** and representative TEM images. Middle: Binding yields of incorporation by using different numbers of protruding arms inside the cavity. Hybridization was tested in 0.5xTEMg with 0.15 % Tween20 and a 2.5-fold excess of AuNS. Bottom: TEM images (uranyl formate stained) were statistically analyzed by counting of origami structures (n ≥ 130).

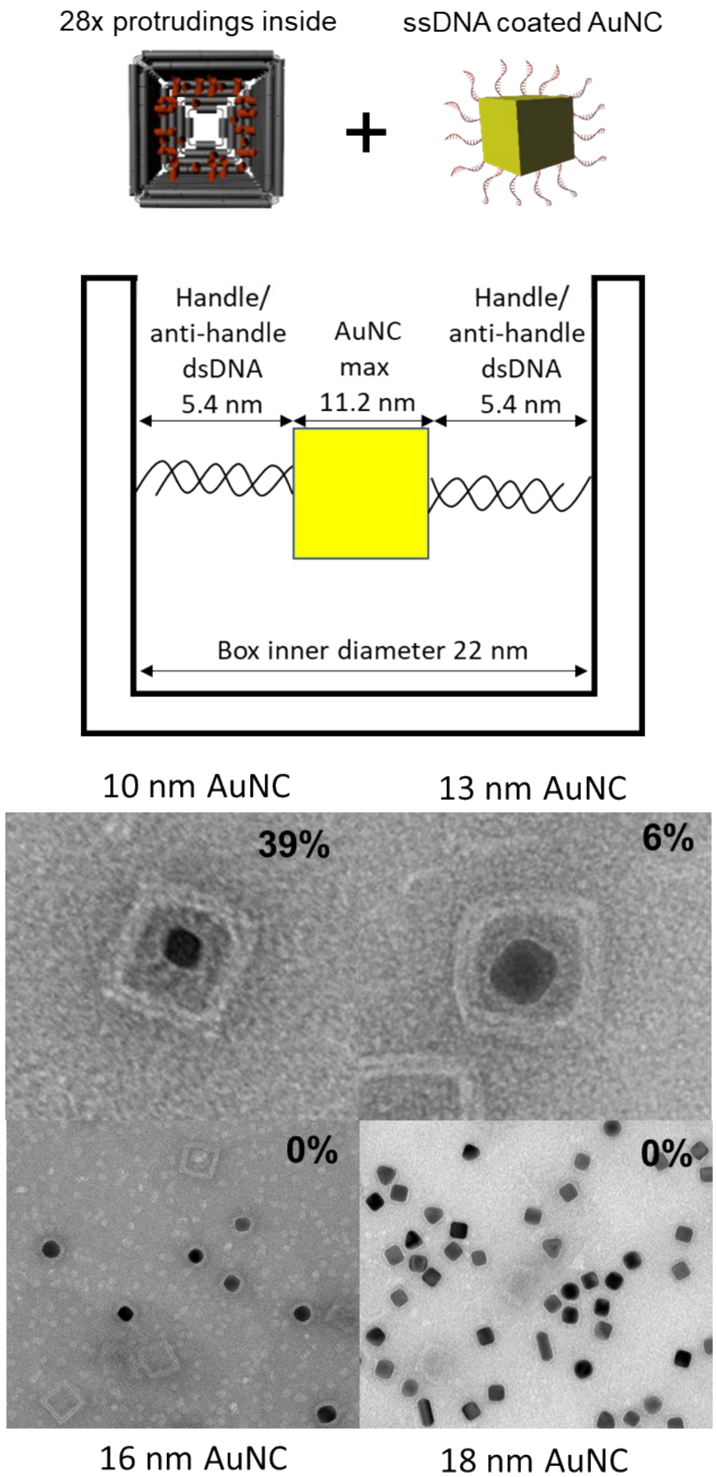
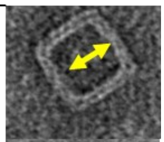
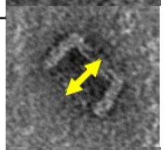
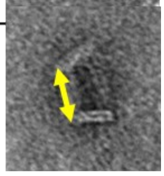


Fig. S 2 Top: **Theoretical and experimentally determined maximum AuNC size.** Top: Hybridization scheme of ssDNA-coated AuNC with maximum dimensions of AuNC fitting in the cavity (dimensions derived by design structure). Bottom: Experimentally determined yield for the incorporation of AuNC with different edge lengths (10-18 nm) into DNA origami boxes by hybridization. The unsuccessful incorporation of the larger 16 nm and 18 nm AuNC is attributed to steric hindrance and electrostatic repulsion.

	Measured [nm]	**Calculated [nm]**	
Inner diameter:	25.4 ± 1.8	21.3	
Height:	26.1 ± 2.5	25.4	
Outer diameter:	34.5 ± 2.9	31.7	

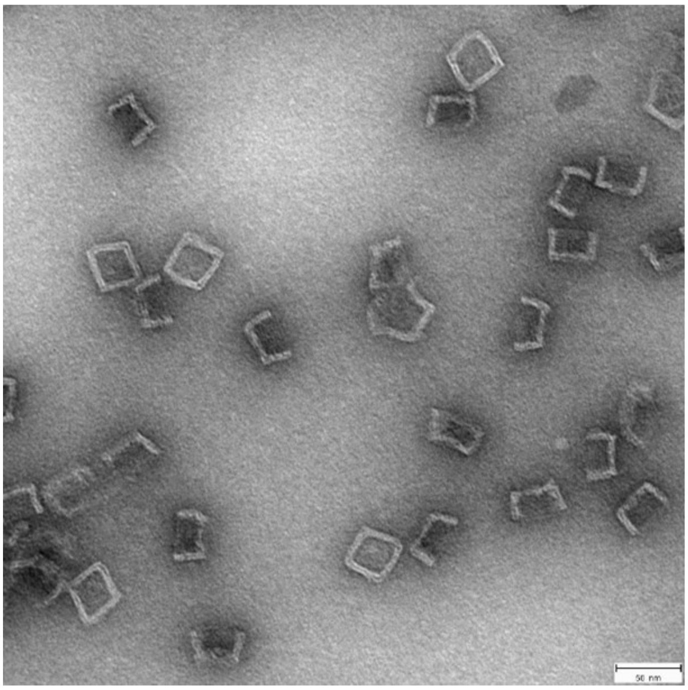


Fig. S 3 **DNA origami box dimensions.** Top: Theoretical values are derived by considering previously reported distance measurements.[1] Bottom: Measured values are derived from statistical analysis of DNA origami box TEM images (n=100).

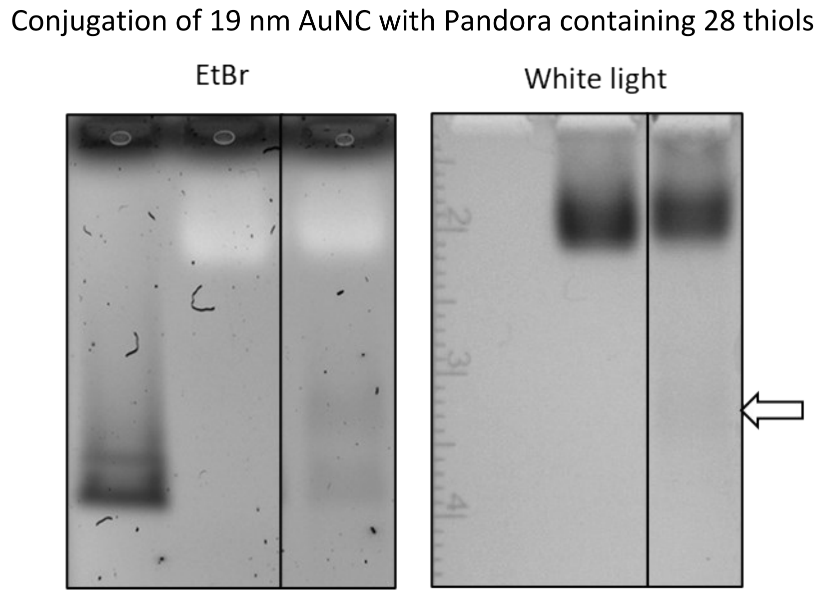


Fig. S 4 **Purification of DNA origami AuNC conjugates by agarose gel electrophoresis.** The analysis under fluorescence illumination allows to identify origami. Left: The origami structures stained with ethidium bromide (EtBr) appear dark in the fluorescence channel, while the AuNC (18 nm) appear bright due to their optical extinction. Right: The AuNC appear dark in the white-light image due to their pale reddish color. The electrophoretic mobility of the AuNC/DNA conjugates is higher than that of single or agglomerated AuNC which leads to a pale red conjugate band visible in the white-light channel indicated with an arrow.

Table S1 Staple sequences for the assembly of Pandora. Sequences include several modified staples, responsible for distinct features of the final construct. Staples are color-coded the same way as in the cadnano design.

Sequence	Length	Description
CACCAGTATCACCGTCTAAACAGTCCACGGAATAAG	36	Connections of bottom plate and side walls
CAAATTTGCACCGGGCAACGTTTGCGTATTGGGCGTCGG	39	
TTCGACTTGTAGAACGTCAGAAATAAATATA	31	
GAGAGGCGAGCTGATTGCCCTTCACCGC	28	
AGCTCAATAGAGGTTTACCCATATTCCTGATTATCTGAG	39	
GATTTAAAACGACAACATTATTACAGGTATAA	32	
ATTTTAACCGTGTTTGAGGGGACGACGAGTGC	32	
AGTGAAAATACTTATACCAAGAGGCAAAAGAATACTTTC	39	
CTGAACGCTGGTAGGCGAAAATCCTGTTGATA	32	
TCCAGCCAGGGTGGTTGCCAGCATCTTTTCTTT	33	
GAAGACTAAAACACTTTATTGGAAACATCTTT	32	
TAGCCCGATGATGGTGGAGTCTGTAGGGTTAGAACC	36	
TGTGATTCCCAATTCTGCGGAAACAATACAT	31	
AAAACGAAAGCGCGAAACAAAGTACAAC	28	
AACGATATTGACCATTAAAGGTGAATTAGCAC	32	
TGTCCGTTGTAGCAATACTTGATTGTTTTCC	31	
ACGTTTCGGAACCTATTATAGGTGGCATAAG	31	
AAGTTAGATGATGGCACCAGCTTGCCATTCATC	33	
TTTTAGCGAGTAACAACGGAAACTAACCCGTCG	33	
CAGATACAGAAAGATGAAGTTTCTTATTACGCAGT	35	
GCTTCTGCAGTATCGACATCCTCAACGTCAGATGA	35	
AGCTTTCAGGCGGATTGACCGTAATGGG	28	
ATTATCATAGCGCCAAAGACAAAAGGGC	28	
CCAACCTTTTAGAACAAACTCAACATTAAATGTGAACCA	39	
CCTGTTTATCA	11	Core Staples, bottom plate
AGAGAATAAACAACTGAAAAGGGATGGGAAGAAAAATCTA	40	
CCGACAAAAAC	11	
TTTTTGAAGCCTTAAATCATCCTAATTCAAGAACGAGCTA	40	
TTATGTGAATAAAAATAAGGCGTTAAATAAGA	32	
TTAGGCGAATTATTCATTTCGATAGCTTTTAACCTCTAAAT	41	
AGAGAAATCAATTCATCGTAGGAATCATTACC	32	
GATTTGTATCATCGCCTGATGAAAT	25	
CCGAAACGCAATAATAACGTAGACGGGATAGCAGCCTTGCG	41	
AAACCAGAGGGTATAGAAGGCTTATCCGGTAT	32	
TTTAATTTTATCCTGAATCTAAGTCCTTCATCGAGTAAAC	40	
TAACCCCTTAGAGAAAAAGCCTGTTTAGTATC	32	
TTTTAATGCTGATGCATTTCCAATCTGTA	29	
AAGAATTGAGTTATAAG	17	
GGTCACGTTGGTGTAGATGGCAACT	25	
TACAGTAACAGTAGTTACAATAAGAACTGGCTCATT	36	
AGGCTGATGATTTAGCACGGTGTACAGACTTTGCGCAT	38	

GTACAAATATATTTTAGTTCATGTAATAGTATAAACCAGA	40	
ACATTTAACAATTTCATTTCCTTGCTTCGCAAGACAGAAA	40	
GAGCGCTAATATAGCCCTTTAAGA	24	
CTGAACACCCTGAACAA	17	
GAGCGTCTTGGATTATTTTTTTAAACCGGATATTCATTAC	40	
TTGAAAACATAGCAATTACCGTAG	24	
CGATTTAATCTTTCAGAATCATTGTGAATTTTCTTATG	38	
TTAGCAAACGTATACCAGAAACGAGGCGCAGACGGT	36	
GGTTGGGTGAAATTGCTGAGCAAAATTGGGCTTGAGATGG	40	
TTTAACAACGCCAAAATTTCATAATTACTAATCC	34	
GCCCTGAGAGAGTTGCAGCAACGTA	25	
GACGCTGAGAAGGAATA	17	
GAGAATATAAA	11	
AGGCATTTTCGAGCTTACCAACTTTTATTTATAT	34	
ATTCAACCGATTGAGGGAGGCAGAT	25	
AAAATAATATCCCAAGATTAGTAATCAGATAATT	34	
ATTTTGTCACAAAAGAAACAGCTGACCTTCATCAAG	36	
GTGAGTGAATAAGAATTACCACTT	24	
ATAGACAGTAA	11	
CGTCGCTATTAAAACAT	17	
CATATCAAAATTGAAAACAAGCCCTGACGAGAAACA	36	
ATGGTAGGTCTGAGAGTTTACCTTTAGAT	29	
CTACGCCAGTTACAAATTTAACAGTAACC	29	
GCCGGAGGAATTTGAGGCGACCTGCTCCATTTTACTTA	38	
AATCCAAAACATATAATTAAGAAAGAACTGACCAACTTTG	40	
AGGCTTAATTTTTTACAAAGCTGCTCATTTTTTGAATA	38	
GGTTGTTTAACGTCAATTTTGAAAAGAAT	29	
AGGGAAGCGCATGAATACCCGCAT	24	
TGTAGAAACCAATCTGTGCGAATTTTAGATAAAC	34	
TCATTTGAAATACCGACCGAATGAGAATAATTAATTAAAG	40	
GAAATAGCTATCTTACCGACAGAGAGACCATATTATGCACC	41	
AGTGCCCTTTTAGAACTCA	19	
ACAGCGGATCATTTTGCAATGCC	23	
GCAGGTCAGCAGCAACTTTTAAA	23	
GAGGCCGTTTTGTCATTGC	19	
CGGCCAGTTTTGCAAATGG	19	
GAGGTGAACAGCAGCGAAAGACAGCATCTTTCCAAAATAGCGA	43	
AACTCGTATTTGCGGAACAAAGAAACCATTTAGCCAGCAAAAT	43	
CGGACGTCGAGAGGGTTTTTGGA	23	**Connections of side walls**
AGGCAGGTGTCCAGCATTTTATTAAAGGGATGCCA	35	
TAGCGTCCAATATTTCAGCTTGCTTTC	27	
GCATTGACAGGTTTTCTGATAGC	23	
ATAGCTGTGCATAAAGTGTAAAGCCTGGTTTAAAACCGT	39	
GAGAAAGGAAGGTTTCGGTCATACCGG	27	
GACTTCAAATATTTTAGACAGCC	23	
GCAGCAAACAACAGTTGAAAGGAATTGATTTAATCACCG	39	
ACGGTGTTTTTCAAGAGAA	19	
AGCAAGGTGGCATCAATTTTAAGTTAAACGACCTC	35	

CTGATAAAAACAAGAGAATCGATGAACGTTTGGCCAGTG	39
TACCTTTTTTTGCCCAATA	19
TCATCAGTTGAGTTTAGGCACCAACCT	27
CCCTCAGAACCGTTTCGCTGAGAGCCA	27
ATACCATTGCAACAGGTTTTATACATGGCTTTAAC	35
CAACTTTCAACATTTCTTTACCCTGAC	27
TCCTCGTTTTTACACTGGT	19
AGCGTTTGCCATTTTAAGGTTATCTAA	27
ATCAATATGATATTTCGGAATTT	23
GCCGTAAAGCACTTTGTAATCATGGTC	27
GAATGACCATAATTTTTCAGCGGAGTG	27
GTTCCGAAATCGTTTCCAACGCGCGGG	27
CCTAATTAGTCTTTAATTTTAGGTTGAGGCAAATG	35
AAGGGTATTTTATAGTAGT	19
TGGGAAGGGCGATTTAACGTTAATATT	27
AACGGCTAAAAATACGTAATGCCACTACTTTCATTCAACTAATG	44
TCCCAGTCACGATTTAATCGTAAAACT	27
AGCATGTCATAAGCAAATATTTAAATTGTTTTTCGCTAT	39
ACGACCAGCGGTGCCGTTTTCCGCCGCGCTTGCTT	35
GCGGGCCGTTTTTTTGAGCGGGC	23
GAGCTAACGCCAGCTGCATTAATGAATCTTTAAATCAAAAGAA	43
AGAATAGAGCCTTTAATTGTATCGGTTTTTTTAAATATTCATT	43
GTGTGCTCGTCATAAATTTTTAGAATCAGAGACAG	35
GCCTCAGGAAGATTTCTTCCTGTAGCC	27
ACGTACAGCGCCTTTCAACCGTTCTAG	27
AAAAAAATTTTGTAATACT	19
GGATATTAAGAGGCTGTTTTCTG	23
GGAACTCATTTTCAGGTTTTAAT	23
ACCGACTTGAGCTTTAGAAGGAGCGGA	27
CTGATGGCCAACAGAGTTTTCAGTCTCTGAACAGT	35
CCTCGTTTACCATTTAACGAGGGTAGC	27
TGAGAACCCTCATATATTTTAACTTAAATTTCCGT	35
TTTTTGCTTTTGTATAGCC	19
TTTGACCAAAAACATTTTTTGCCGCACAGGCGATG	35
AACCACCACACTTTTTGCATCAG	23
AGCAGCACCGTATTTAAACAATTCGAC	27
AAGCGTCTTTTTCATGGAA	19
ACGTGGACTCCATTTTGCCTAATGAGT	27
GAAAGCGTTTTCTTCTGAC	19
GGGTTTCTCCCGGGTACCGAGCTCGAATTTTAAAGGGAG	39
TTGTTAAATCAAAAATAATTCGCGTCTGTTTGCTTTCCGGCACC	44
AACTACATCACTTGCCTTTTGTA	23
CTGCAACAGTGCTTTACCACCCT	23
TCAATTGACCATTAGATTTTAGC	23
CTCACAACGCCTGTAGTTTTTCG	23
CCGAGTATTTTTGCCAACG	19
AATATCTTATTTAGAAGTATTAGACTTTTTTAATCAAGT	39

TGCTTTGACAGTCACATAGACCCACCATCAGCCGTCAATAAACG	44	
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GTCGCCTGTCGTTCACATTAATTGCGTTAACA	32	
CCCCCGATTTAGGTCGAGGT	20	
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CTATCAGGGCGAATTAAAGA	20	
CAGGCAATAAATAAGCTAAATCGGTTGTCGGG	32	
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CTGTGAACGCCAATTCGCATTAAATTTTAAAA	32	
TTATATCCAGAAATATCCTGAGAAG	25	
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AATACCGAACGAACCAGCGCGTACCTGAA	29	
TTGCCTTTAGCGCCATCGAT	20	
GTAATGGCAAATTGAAAAATCTAAAGCATGAGGCGGCCATTAAA	44	
ATAGGAACCCGTGAGCCTCTTATCCGCCTGG	31	
TCGCAGCCGGAATTCCTGTGTGAAATTGCTCA	32	
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TATTATAGGAAAACGACGTTTTAAAGAG	28	
CAAACTCCAAAGCCTCTCAGGGGGCCAGAGAAAAGCCCCATGTT	44	**Core staples, walls**
CGTCAACCAGGCTCGCCATTCAGGCTGCCGAA	32	
CATACCGCCACGACCTCACCGGAAACAACTTTCTCCACGCAGAA	44	
GATCATCACCGTCCTCAGAGCCACCACCCCCA	32	
GCTAGGGCGCTGAACGTGGC	20	
GTGTAGACAGTCAAATCACC	20	
CTAAAGTTTTGTCGTCTTGATATTATAAT	29	
GTGAGAGATAGATCGGCGAA	20	
TCAGTACCATTAGAACGTCACCAATGAAATCAG	33	
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CCACACTCAGGAGGTTTAGTGTACTGGTTTTCCGGACAAGGTCATGAG	48	
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CAGTGAATACGTATATTTTTGAATGGCTAAAC	32	

CACGGGGATCAACAAATGAAAGCCGGCGGCAAGTGTCTGCGCGT	44	
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GCGCCTGCGGCCAGAATGCG	20	
TACGACTCAATCGGGTAAAGGTTTCTTTTCAG	32	
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AAAGCAAAAGAGTTGATAATTAAGCAATAACA	32	
CATCAAGATTGTAATCATATGTACCCCGCTGA	32	
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AGAACTGGAGCATTAATGCCGGAGAGGGAAGGCCGGAGGTAAAG	44	
CGGAAAAGCCAGGGTCAGACGATTGGCCTTTAGAGCCCACCCCA	44	
ATACTGACGCTCAATAAAAGGGACATTCAAGC	32	
TTGCATCAGGGTGAGATAGCTATTCGGCGCAATTCAAAAG	40	
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CATACATACAGGCAAGGCAAATAATGCTCAGAGGCATTACGCTCATT	47	
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GAGGCTATCAGGTCATTGCGTTGATAAAGAG	31	
CCTACCCTCAATCAATATCTAATAGATCGAC	31	
ACTGCCTCGTTCTTTACCAGAACCGCCATCCT	32	
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AAATGTACATTTATTTTGCTCAACA	25	
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TCGCACTTTTTTTTTTTCCAGCCA	24	
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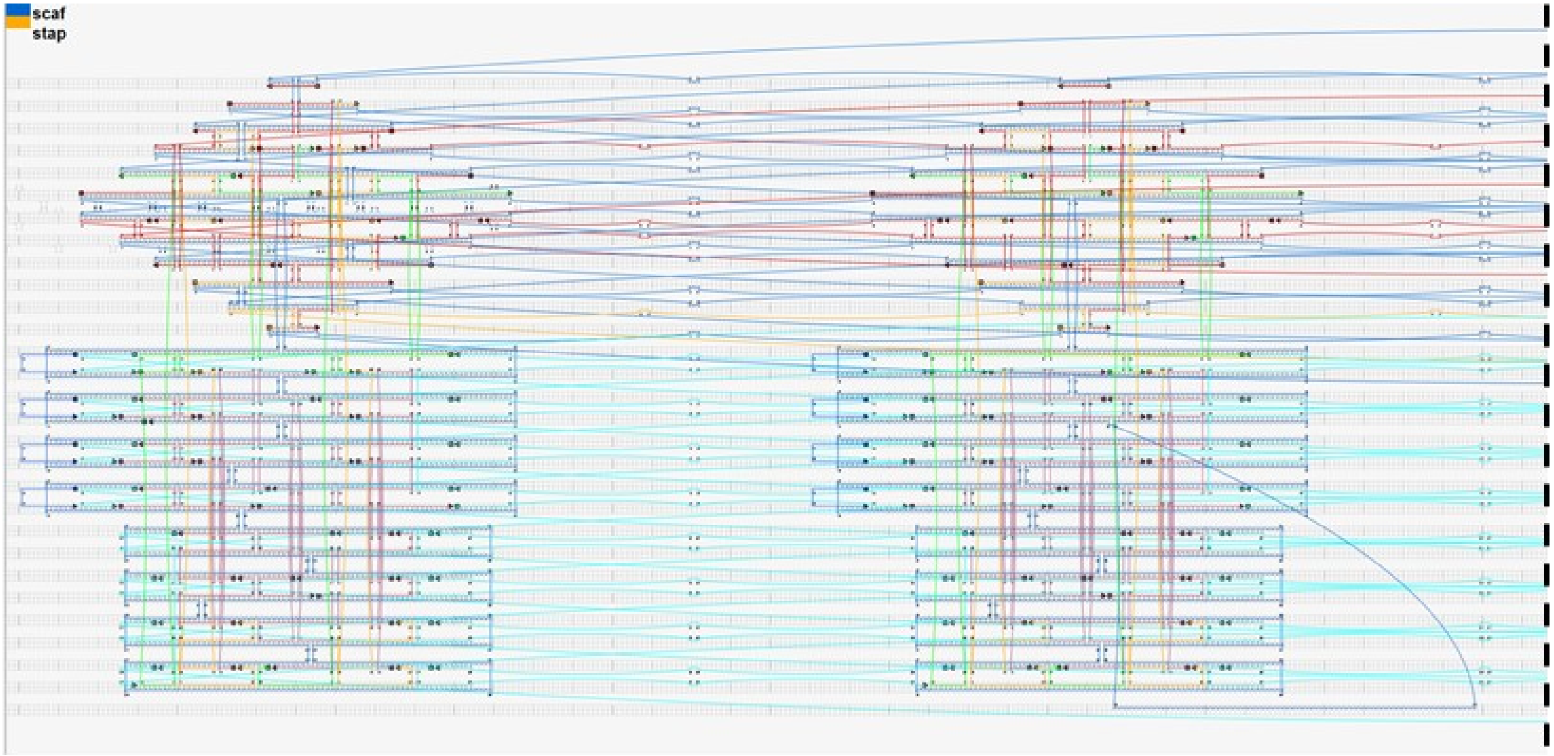
ATCAAAATTTTTTTTTTATCAGGT	24	
GCAAAATTTTTTTTTTTCCCTTAT	24	
CCACCCTTTTTTTTTTTCAGAGCC	24	
CGTTGTATTTTTTTTTTAAACGAC	24	
GACGACGTTTTTTTTTTATAAAAA	24	**Edge staples (prevent stacking)**
GAAGAAATTTTTTTTTTGCGAAAG	24	
TCGGTGCTTTTTTTTTTGGGCCTC	24	
CTTTTCATTTTTTTTTTTAATCAA	24	
CATTTGGTTTTTTTTTTGAATTAG	24	
CTGCGGATTTTTTTTTTATCGTCA	24	
ATCAGTATTTTTTTTTTGCGACAG	24	
TAAATCGTTTTTTTTTTGAACCCT	24	
ATTTAGGTTTTTTTTTTAATACCA	24	
ATTCAAAAAAAAAGATTAACCAGACTTTGA-TAMRA	29	**Fluorophore staple**

GATTCAGGACGTTTCGCCTGATTGCTTTAGTCAATACAAAAGATTGCCACTTTCCAC	57	
ATAGTTAGCTATGGGGCGCGAGCTGAAATTAAGATTGCCACTTTCCAC	48	
CGGAGCTGGCAGTGCTGATTGCCGTTCCGGTAGATTGCCACTTTCCAC	48	
AACACCGGAATCATCTTCTGACCCGGCTTAGATTGCCACTTTCCAC	46	
ATACCAGTCTCCGTGGCATCGGGAAACGAGTAGATTTAGTTAACGATTGCCACTTTCCAC	60	
CATTGCGGGGTTCCAGGCGGATAAGTGCATAGGATTGCCACTTTCCAC	48	
AAGAACGCGAGGCGCCTCCCGACTTTACAGGATTGCCACTTTCCAC	46	
CATTGCCTTGAGTTGATGATACAGGAGTACCGGATTGCCACTTTCCAC	48	
TGCGTTATACCGTGCTACGCGAAAGAAACGATTGCCACTTTCCAC	45	
AGTAATCTTAACATATAAATTTCCTCTTTGATTAGTAATAATCGGATTGCCACTTTCCAC	60	
CGCCTGCAACTAAGCTTAATTGCTGAATAGAAGATTGCCACTTTCCAC	48	
TAACTGCTGGTACAATATTACCGCCAGCCTACGATTGCCACTTTCCAC	48	
AAAGAGGAGAAGGTAACAAAGACATAATGCCCCCTGCCTAGGGGATTGCCACTTTCCAC	59	**Protruding staples (These staples protrude into Pandora and can bind thiolated-oligonucleotides)**
TTCTGTGCCAACGCTCAACATTTGGGCTTCGCCGATTGCCACTTTCCAC	49	
GAAAGTGGTGCCACCAGCTTACGGCTGGGCTTGATTGCCACTTTCCAC	48	
CGTGTGTCAAATTTAAAAGACTCCATTCCATATAACAGTTTTTAGATTGCCACTTTCCAC	60	
ATGTTCGGTATTAAACCAAGTTTCGCACGAACAGATTGCCACTTTCCAC	49	
AATATGACAAGATGGAAACAGTACAATAAAGCCCATCCTAAGATTGCCACTTTCCAC	57	
CCAGAACGCAGTGAGAGTAAAACAGCGTGGTGCTGGTCTGCACCGATTGCCACTTTCCAC	60	
CAATCATACCCAGCGAATACATAATCTGAAACATGAAAGTTAGGATTGCCACTTTCCAC	59	
CAGAGGCGGCTGTCTTTCCTTTTCATTCTACGAGATTGCCACTTTCCAC	49	
CCCAATAGCAAGCATGCTATTTTTTATCCCGATTGCCACTTTCCAC	46	
CGACAAAACAAGCAAGCAAATTTTTACCTTAGGGATTGCCACTTTCCAC	49	
TCACAGTAGTAAAGAAGATGATGAAACATTAATTTTTATATGATTGCCACTTTCCAC	57	
GGGTTCAGTGAGTTTAGACAGGAACGGTTTATGATTGCCACTTTCCAC	48	
CCAAATCAAGCGGTCCATAATGGACCATCACGCAAATTAATTTTGATTGCCACTTTCCAC	60	
TTTAATTTGCGCATCGTCAGGTTTATAACGGAACGTGCCGGTCTGATTGCCACTTTCCAC	60	
GACCAGGGAACCAGTAAGCAGATAGCCGAACAAAGTGATTTGATTGCCACTTTCCAC	57	
Thiol-GTGGAAAGTGGCAATC	16	**Thiol-staple (binds the AuNC)**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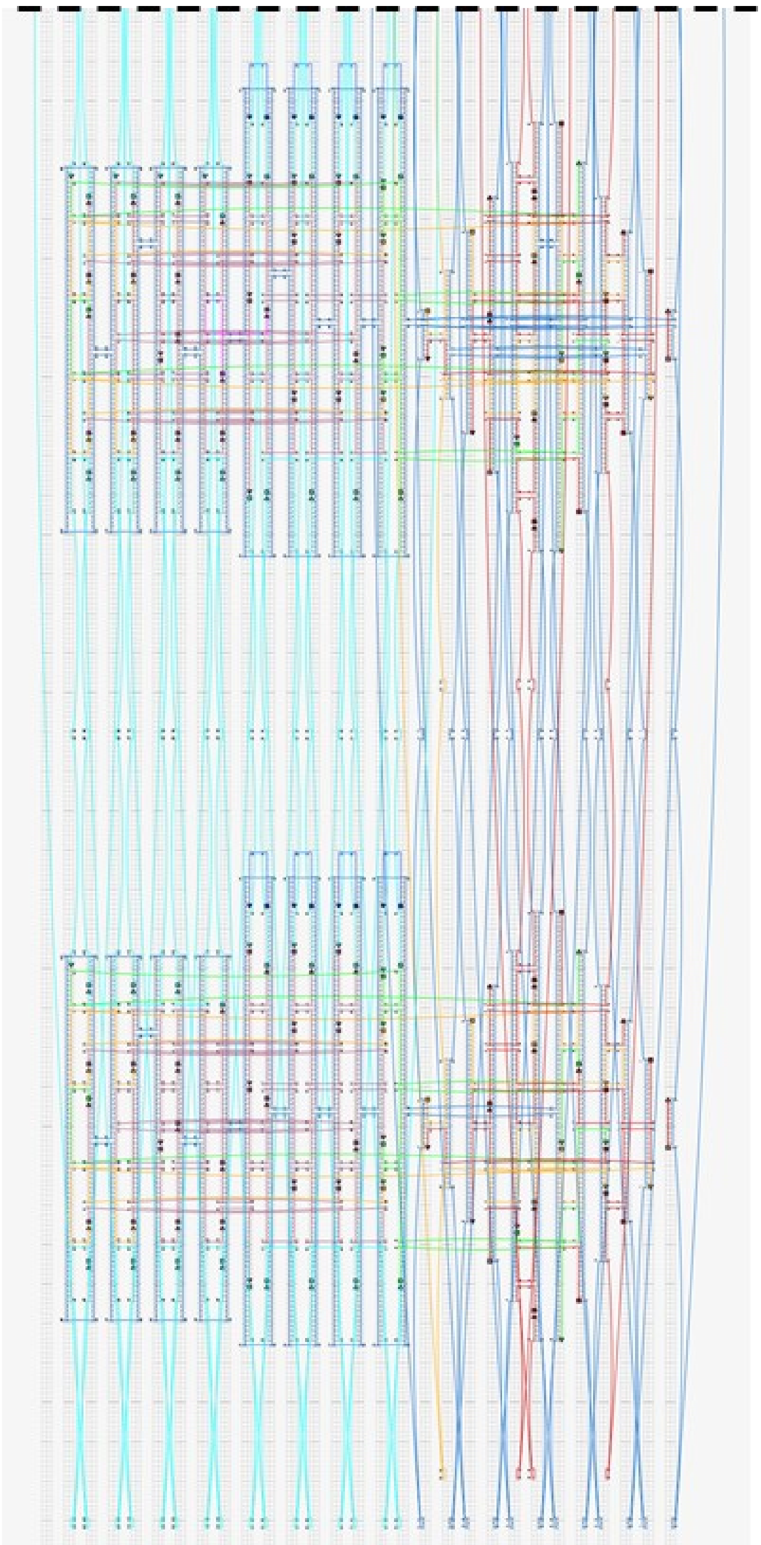

Fig. S 5 **Cadnano design of Pandora.** The structure consists of a bottom plate and four walls. Small single-stranded DNA segments are interspersed in between each face to release possible strain. Staples are color-coded (red: bottom plate core staples; green: Staples connecting bottom plate and walls; purple: wall core staples; cyan: Staples connecting walls; magenta: fluorophore-staple; dark blue: edge staples (prevent stacking); orange: protruding staples.