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Supporting Information

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A Compact DNA Cube with Side Length 10 nm

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Ralf Jungmann, Peng Yin, and Friedrich C. Simmel**

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Supporting Information

A compact DNA cube with side length 10 nm

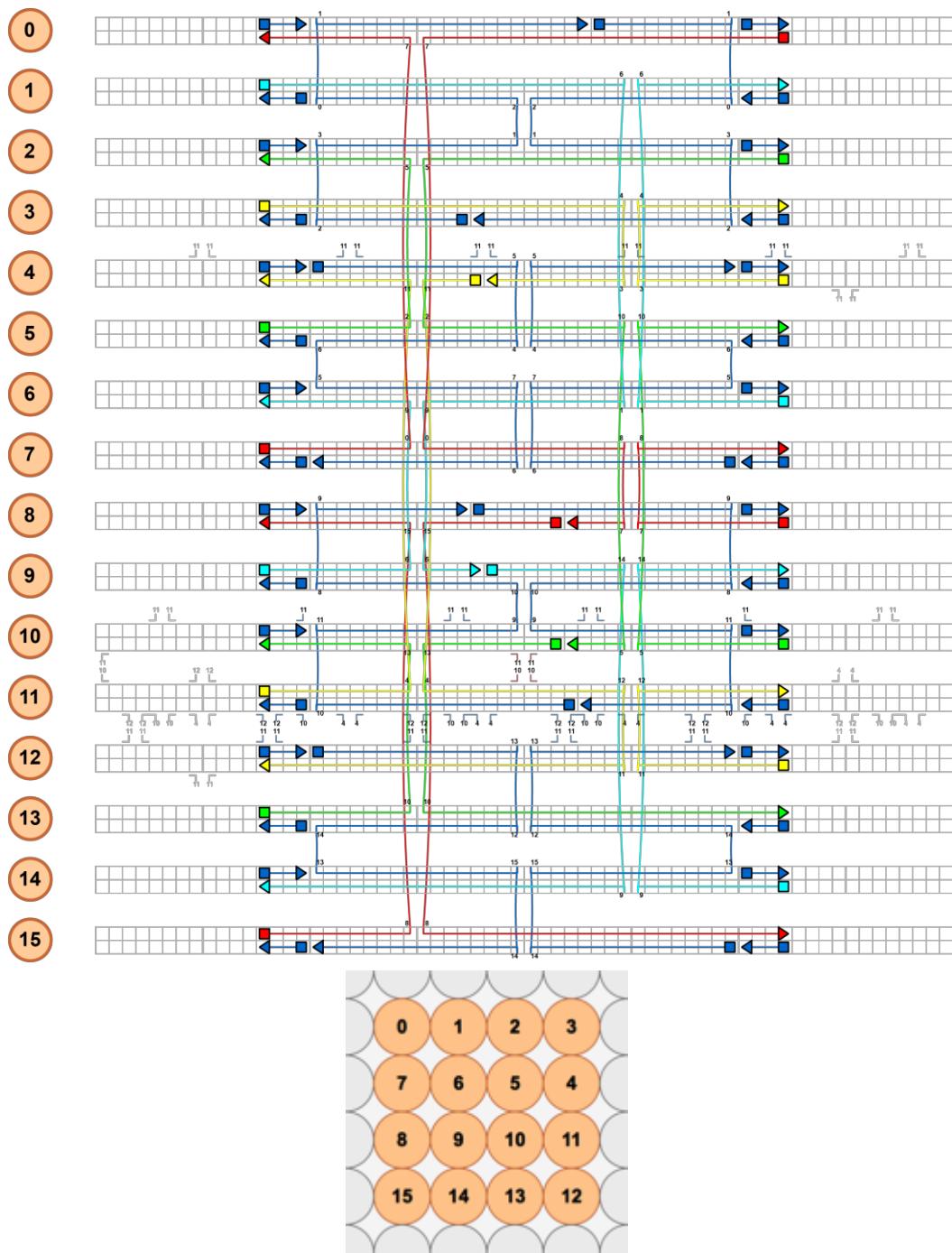
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and Friedrich C. Simmel**

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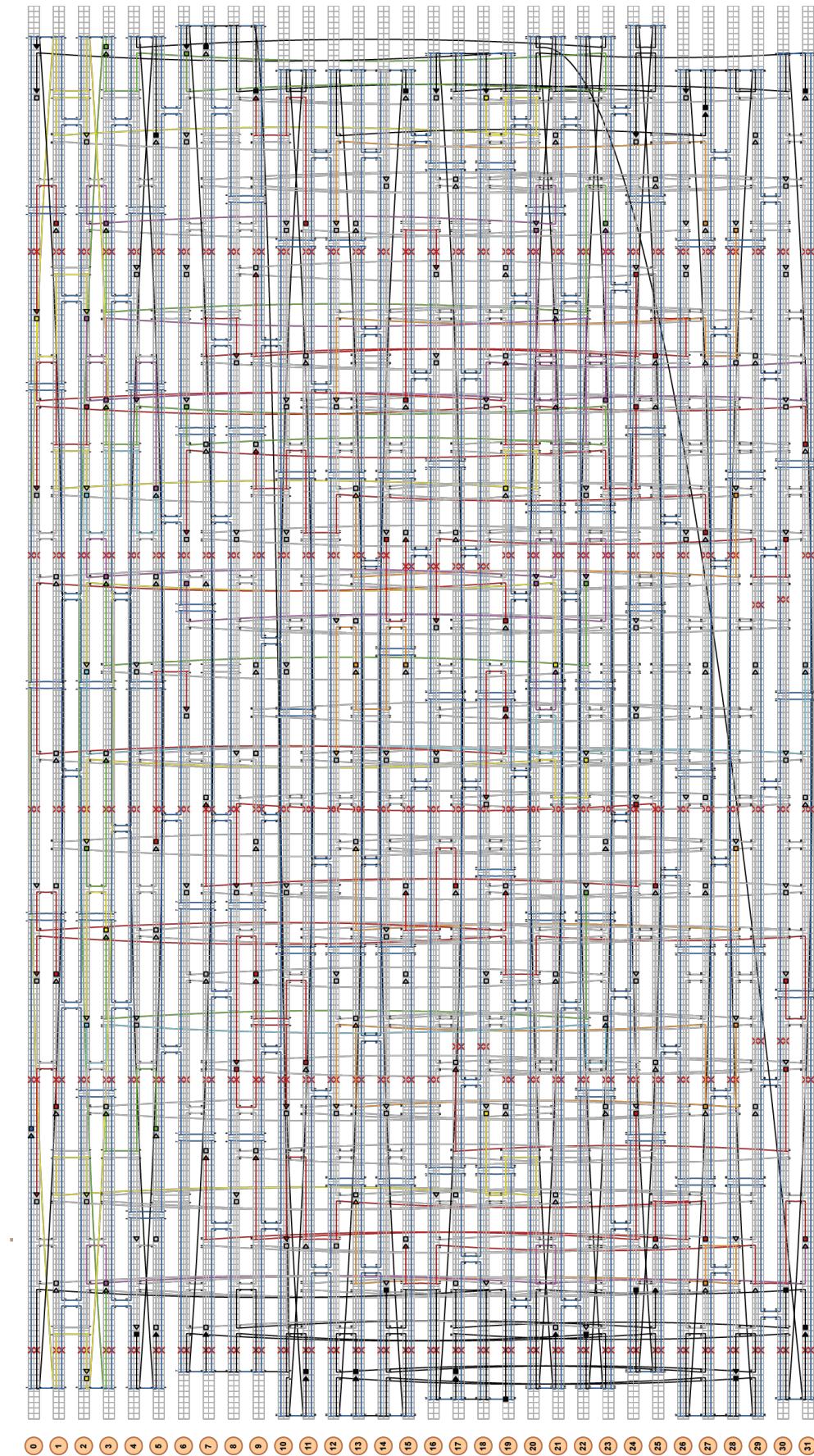
S1. caDNAno design

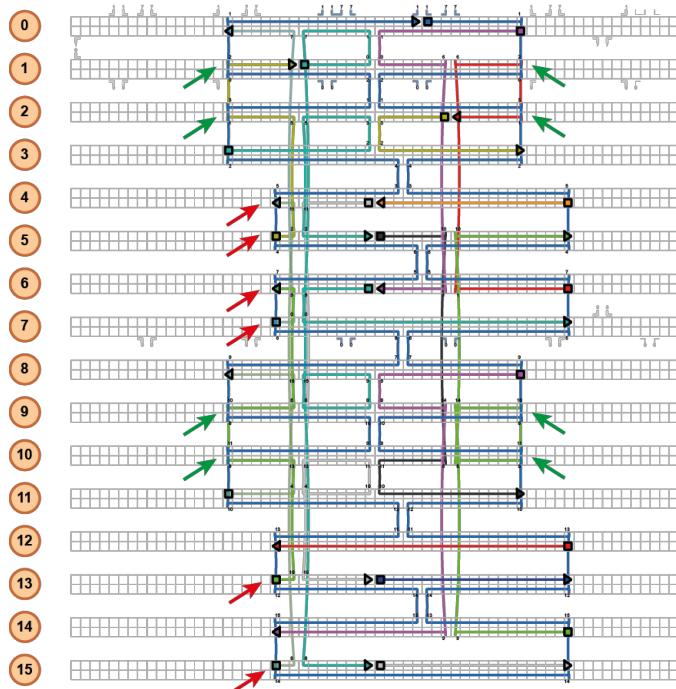
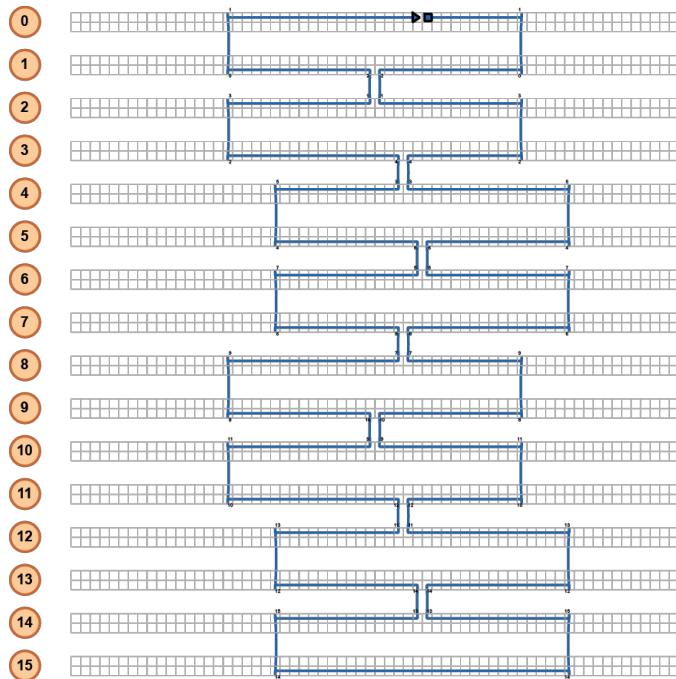
Cube:



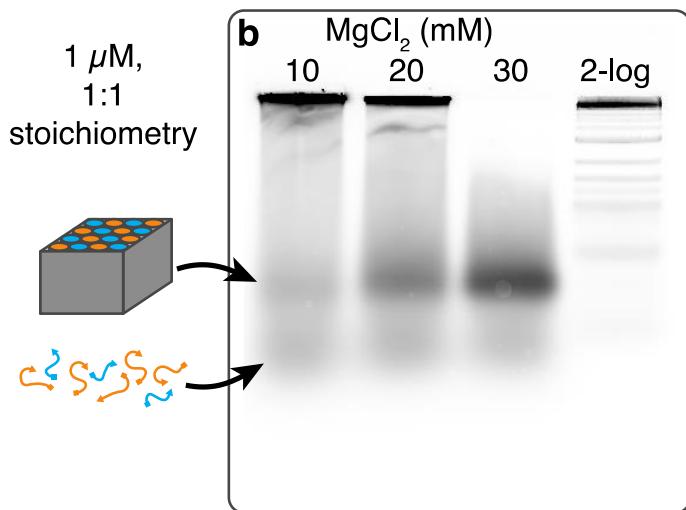
X layers are given in dark blue. Y layers are given in the following color code: layer I: red; layer II: cyan; layer III: green; layer IV: yellow. The additional short blue segments on the left and right side support the design-based labeling of Y strands with four thymine nucleotides to prevent base stacking on the blunt ends.

DNA origami filament monomer (tunnel structure):

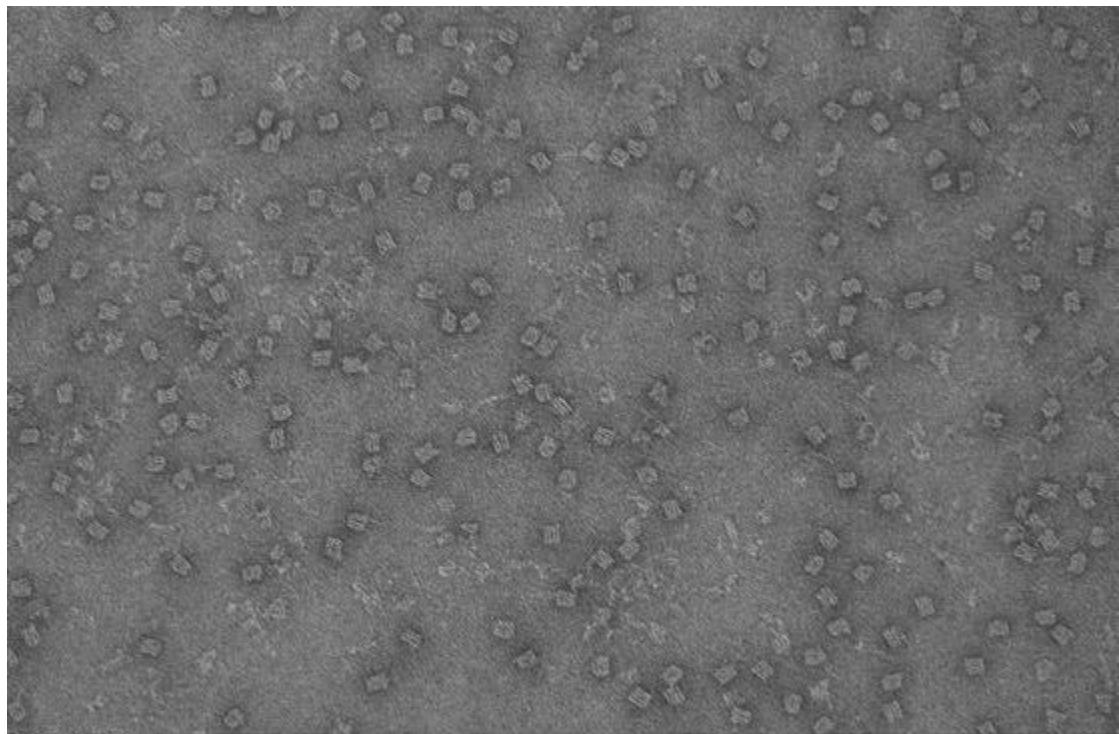


S2. Why a circular scaffold routing of the cube is discarded.

A circular scaffold routing is theoretically possible (upper image), but it requires a shift between adjacent layers and therefore distorts the overall cubic structure. Further, the addition of staple strands reveals design-related problems such as too short and unstable domains (red arrows) or blunt ends which lead to undesirable base stacking interactions (green arrows).

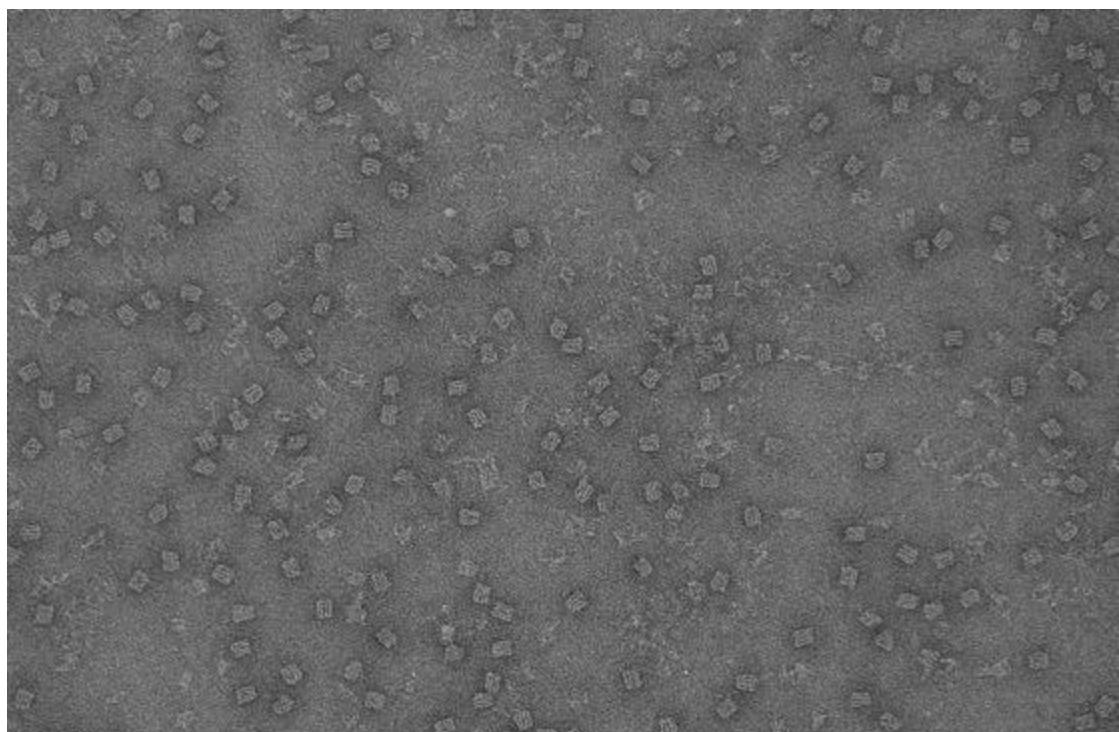
S3. Magnesium screening

S4. Additional TEM images



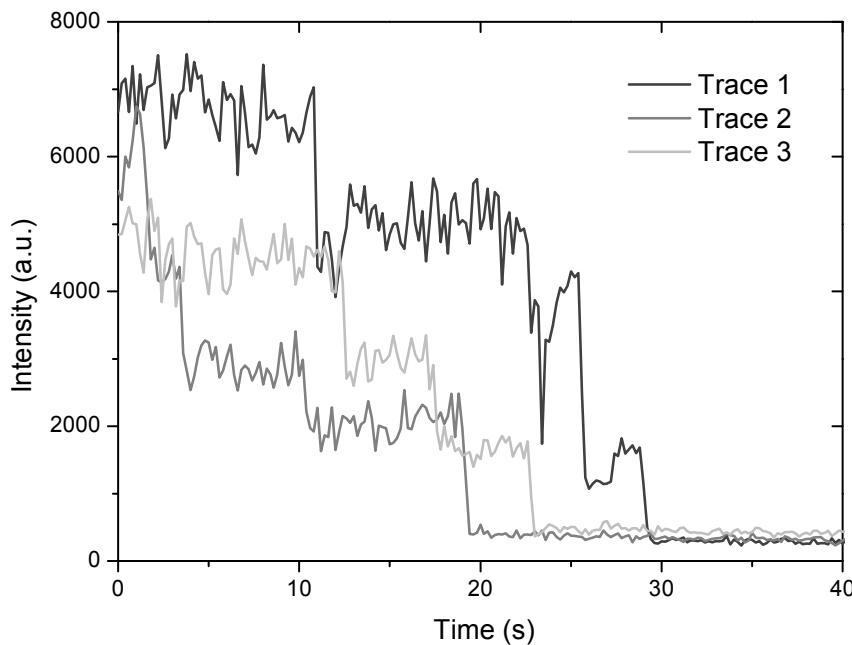
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8:19:08 PM 5/10/2013

100 nm
HV=80.0kV
Direct Mag: 100000x
X: -7.33 Y: -6.49
AMT Camera System



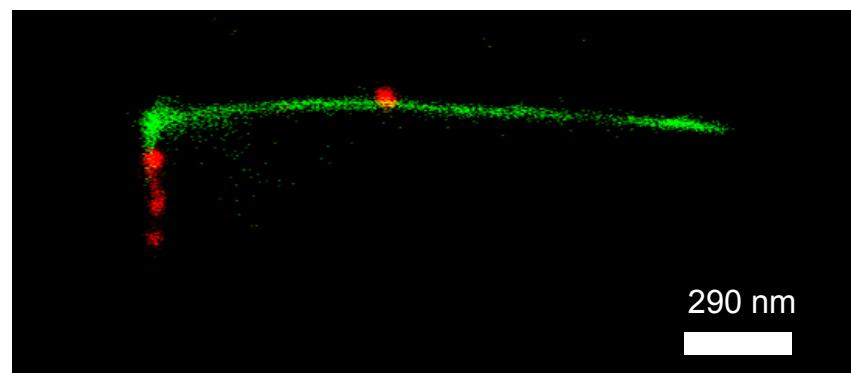
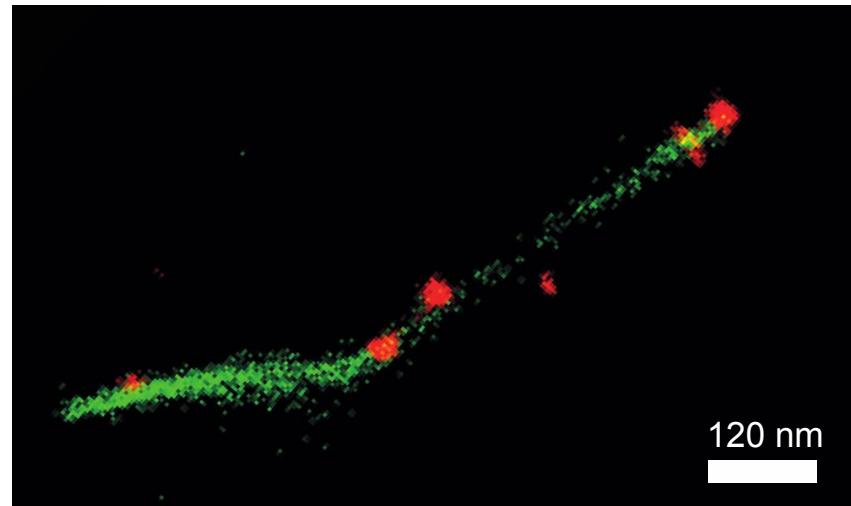
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Print Mag: 9670x # 7 mm
8:19:49 PM 5/10/2013

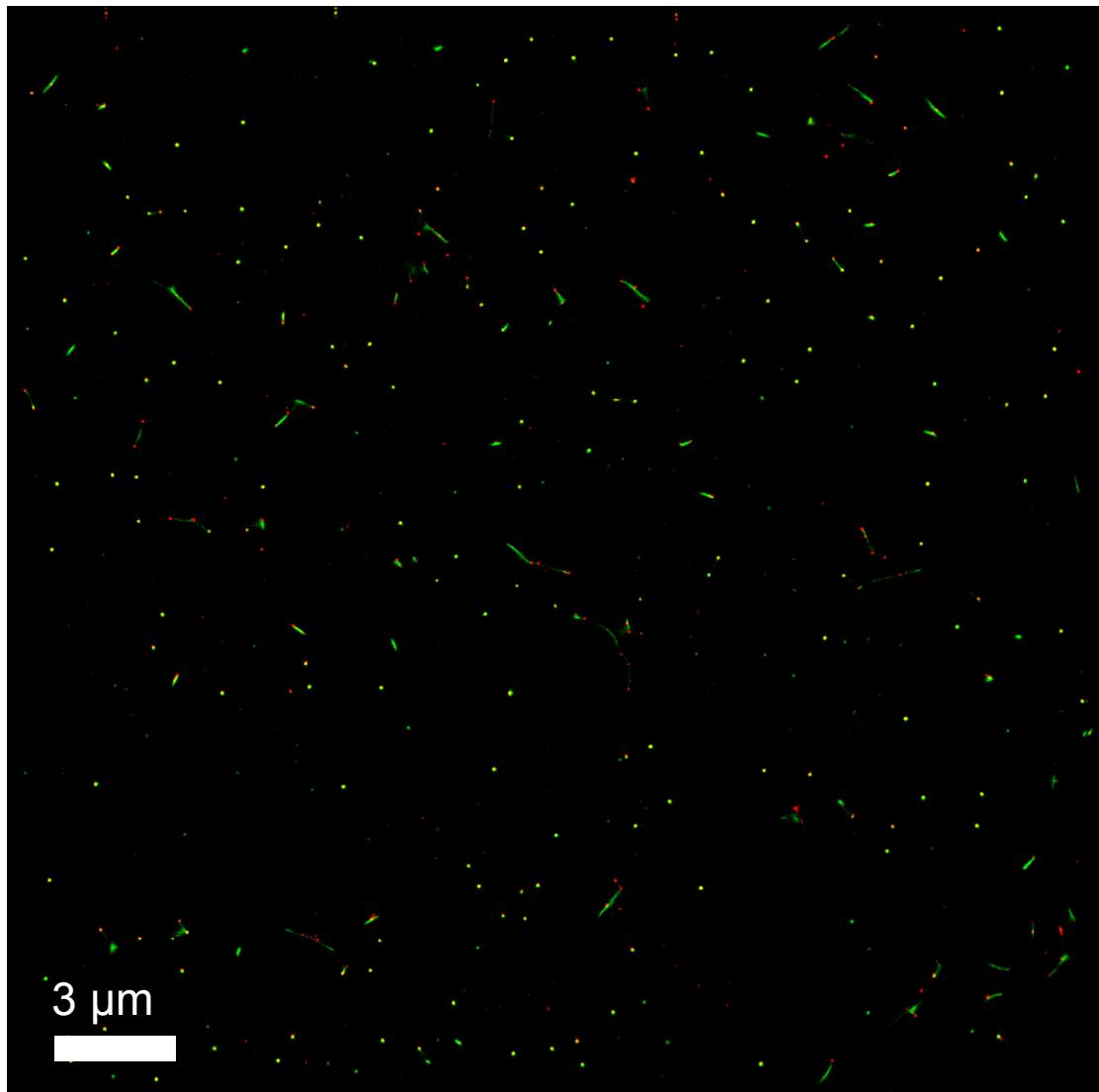
100 nm
HV=80.0kV
Direct Mag: 100000x
X: -7.86 Y: -6.02
AMT Camera System

S5. Additional bleaching steps of Cy3 labeled cubes

Traces are taken from individual and clearly separated point-spread functions (PSFs) from surface immobilized cube structures. Due to single molecule blinking of the fluorophores many traces rather appear noisy and smeared instead of showing clear bleaching steps. This is why a qualitative display of examples is favored over an incomplete quantitative treatment.

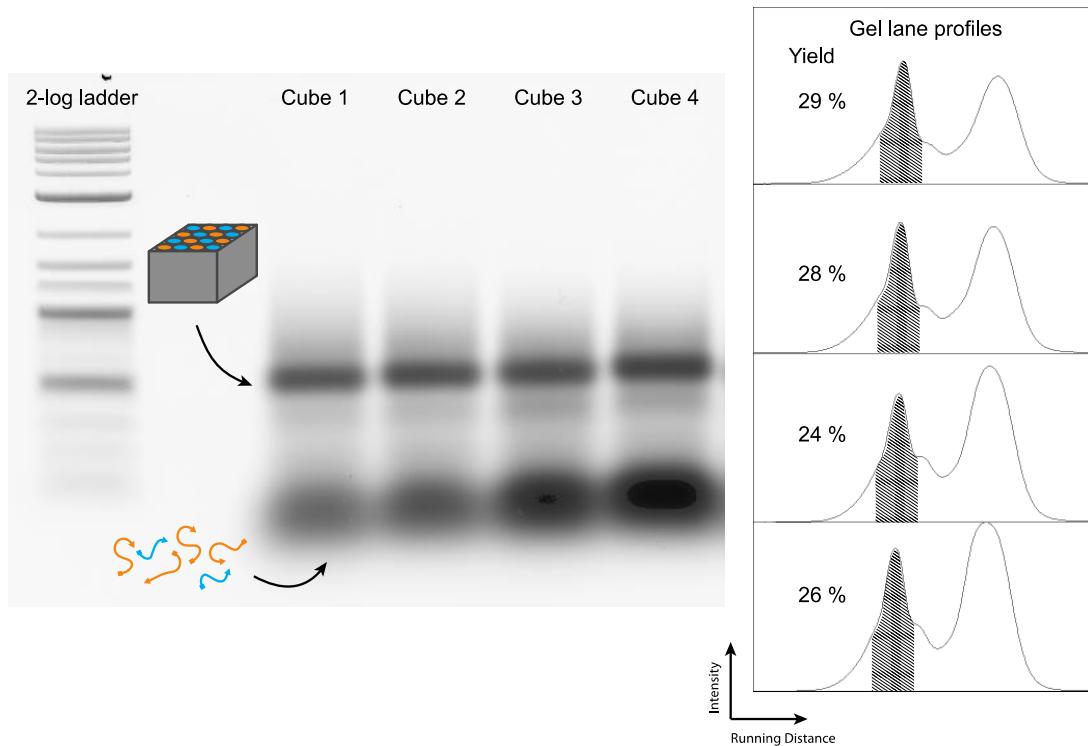
S6. Additional super-resolution images of cube-filament complexes





Zoom in for details. Single green/red/yellow spots depict fiducial markers for colocalization.

S7. Gel lane profile



Gel electrophoresis of four independent cube folding approaches. The profiles of the gel lanes show two peaks for cube structures (hachured) and unfolded single-stranded DNA, respectively. Yields are determined as ratio of the hachured cube area over the total area. The gel is quantified using ImageJ gel analyzer. The folding yield is determined to approx. $(27 \pm 2)\%$.

S8. Sequence list

Cube:

Name	Sequence	Comment
SST_4432_X(0,37)	GTAAGTTGAAGTAGGAAGCTTTCTAGCCATAGCATCGACACTACGACCTGCTTTCGACAC	X Layer 4
SST_4432_X(3,27)	GGACTGCATTCTGGACAGTAAGTGATTAACACTACGTGCTCCAACATAAGTGACGTCCTCAGCAG	X Layer 4
SST_4432_X(4,16)	TTGAAAATTATCTCGATAAGCAGAAGGACCTGTATAACTGGCAAGAGACAAGGCCGTTCAGAA	X Layer 3
SST_4432_X(7,47)	AGGATAGCCGGACCGTATTAATGCCCGCCAACGGTTCCCGACCTAGTGTCTATCAAGTCTA	X Layer 3
SST_4432_X(8,28)	TTCTATGAAACCATTCTCGGGTCGAGCGGGTCACTGTTGACCTACGAGAACGCTATAGATGT	X Layer 2
SST_4432_X(11,35)	TCCCGCGAATAGCTCACAGGCGAACTACGTATGAATTGGTTAACAGCTCCTCGGAATTAAT	X Layer 2
SST_4432_X(12,16)	ACGACAGGGCAAAACCAACCTCCGATGTCAGCGCCGATACCCATTACTGTGAATTCTCACAC	X Layer 1
SST_4432_X(15,47)	CGAGGATTCCGAGGTCCATGGGATTCAACCAAGCTCGTATACACCCGTATTCTCATGGCAGCGC	X Layer 1
SST_4432_Y(3,12)	TTTTAGAATGCACTCCGTGCGAAAAGCATAGACACTCG	Y Layer IV
SST_4432_Y(4,51)	TTTTAGACTTGAGGTGCTATT	Y Layer IV
SST_4432_Y(5,12)	TTTCAGGTCTTACTGCTTTT	Y Layer III
SST_4432_Y(6,51)	TTTTTGGCGCGGTTCTT	Y Layer II
SST_4432_Y(7,12)	TTTTTCTGAAGCACTTATGTTT	Y Layer I
SST_4432_Y(0,51)	TTTCTCAACTTACCTGCTGAGGACGTCGGCCTGACGGTCCGGTT	Y Layer I
SST_4432_Y(1,12)	TTTTTGGGAGCACGTAGTTAGAAAAAGCATTAAATTCTCTGCTAAC	Y Layer II
SST_4432_Y(2,51)	TTTGTGTCGATGCTATGGCAATGCAGTCTGCTTATAGGTCCGGTCA	Y Layer III
SST_4432_Y(8,51)	TTTCCGAGAATGCTACCTTTT	Y Layer I
SST_4432_Y(9,12)	TTTGGAGCGTTCAAGTTATTTT	Y Layer II
SST_4432_Y(10,51)	TTTTTCTCGTAGGAAACGTTT	Y Layer III
SST_4432_Y(11,12)	TTTCTGTGAGCATTCAATT	Y Layer IV
SST_4432_Y(4,28)	AGATATATTGCGCGGAACATATGGAGAACGTTGCCACCTGTCGTTT	Y Layer IV
SST_4432_Y(8,34)	CATAGAAATTAAATTCACAGGACCTCGGAATCCTCGTTT	Y Layer I
SST_4432_Y(9,29)	CAACAGTGACCATCCCATGTGAATGGGTATGCGGCTTT	Y Layer II
SST_4432_Y(10,34)	CAATTCTACCGGGAGGTGTCAGGGGTATACGAGTTT	Y Layer III
SST_4432_Y(12,51)	TTTGCCTGCCCTATACGTTT	Y Layer IV
SST_4432_Y(13,12)	TTTGCTGACATTAGTTCGTTT	Y Layer III
SST_4432_Y(14,51)	TTTCTTGGTACGCTCGACTTT	Y Layer II
SST_4432_Y(15,12)	TTTGTGTTGAAATTCCGATT	Y Layer I
Binding domain	GTTAGGAGTAGGGTAGTAGG	Binding domain for bottom extension
Linker strand	CCTACTACCCTACTCCTAACTTATACATTGA	Linker with P5*
2x P1 including 2x TT spacer	TTATACATCTAGTTATACATCTAG	2x P1 extension for cube
SST_4432_bt_Y(0,51)	TT TTT TCT TCA ACT TAC CTG CTG AGG ACG TCG GCC TTG ACG GTC CGG GTT T	5'-Biotin anchor
SST_4432_bt_Y(4,51)	TT TTT TTA GAC TTG AGG TCG TAT TTT	5'-Biotin anchor
SST_4432_bt_Y(8,51)	TT TTT TCC GAG AAT GCT ATC CTT TTT	5'-Biotin anchor
SST_4432_bt_Y(12,51)	TT TTT TGC GCT GCC CTA TAC GCT TTT	5'-Biotin anchor
Imager P1*	CTAGATGTAT	5'...-3'-ATTO 655
Imager P3*	GTAATGAAGA	5'...-3'-Cy3b
S2	TAACATTCCCTAACTTCTCATA	Top extension for selected Y strands
S2*	TATGAGAAGTTAGGAATGTTA	5'...-3'-Cy3

DNA origami polymer:

The original sequence list including biotin modifications and DNA-PAINT docking sites are published in Ralf Jungmann et al., “Multiplexed 3D Cellular Super-Resolution Imaging with DNA-PAINT and Exchange-PAINT.” *Nature Methods*, February 2, 2014, doi:10.1038/nmeth.2835

Additional sequences:

Name	Sequence	Comment
t2_0[199]2[200]_cP5	CCTTACACAGCAAATCGTTGGGTGGTAAAACCAATTCAATGTAT	filament2 with TT spacer and P5 domain
t2_2[7]2[8]_cP5	GATTAAATCAGCTCCAATAGGAACGAATTAACCGTCTCTTTCAATGTAT	filament2 poly with TT spacer and P5 domain
t2_3[88]2[72]_cP5	TTCTCCGAGTGGTCAGTTGGAACAAGAGACATTCAATGTAT	filament2 with TT spacer and P5 domain
t2_18[55]2[40]_cP5	AATCATAATTACAACAAACGCCTAGCCAACGCCACACGACGCTCAATCTTCAATGTAT	filament2 with TT spacer and P5 domain
t2_18[239]2[232]_cP5	AACCAGACCTTTAACCGTCAATCATTAACATTTACATTCAATGTAT	filament2 with TT spacer and P5 domain
t2_19[168]2[168]_cP5	ACGTTAATTTAGGAATGTCACTGAGCCAGCGTTCAATGTAT	filament2 with TT spacer and P5 domain
t2_21[136]2[136]_cP5	ACCAGAACGAGTATTAGCAGCGTGCCTGTTCTTTCAATGTAT	filament2 with TT spacer and P5 domain
t2_22[119]2[104]_cP5	TTTCACGGCACCAAAGTGGCGAAATCCTTTCAATGTAT	filament2 with TT spacer and P5 domain
t3_2[103]3[119]_cP5	GTTTGATGAATCGGCAAAATTGCGTATTGGTTCAATGTAT	filament3 with TT spacer and P5 domain
t3_3[248]3[23]_cP5	TGTTAGTAATAACATCACCCATTCAATGTAT	filament3 poly with TT spacer and P5 domain
t3_5[52]3[55]_cP5	CGCGAACTGATTGGCACAGACAATATATGGAAATTTCAATGTAT	filament3 with TT spacer and P5 domain
t3_6[183]3[183]_cP5	AGTTTAAGACGATAATCTGGTCACAACCAGCTTCAATGTAT	filament3 with TT spacer and P5 domain
t3_6[247]3[247]_cP5	GCAAATATGCAAAGCGTTTGTATAAATTCTTTCAATGTAT	filament3 poly with TT spacer and P5 domain
t3_22[95]3[87]_cP5	AAAAACAGCTGATACCGATACTTAGCGGGTTGAGTGTGTTCAATGTAT	filament3 with TT spacer and P5 domain
t3_22[151]3[151]_cP5	ATTGTAAATTGGGCTTCCGTGAGCCTCTAGCTTCAATGTAT	filament3 with TT spacer and P5 domain
t3_23[216]3[215]_cP5	ATCCCCCTTAAGAGGTCACTTGCAGGATTACAGCCGGTCAGTGTCAATGTAT	filament3 with TT spacer and P5 domain
t2h_2[71]23[71]	GAGATAGAGACCTGAAAAATCAAGCTATTTG	filament2helper
t2h_2[135]31[135]	TCGTTTCTTTCACCCCTAAAACAAAGAATAAGCACCATTACAGCGT	filament2helper
t2h_2[167]5[167]	GTGCCGGTGTGGTGCCATCCCACGGCAGCAACCGCAAGAAATGACTTG	filament2helper
t2h_31[136]19[143]	CAGACTGTAGCGCTTTCATCGCAGATTACCCAAATCAACGTAACATTCACTGTA	filament2helper
t3h_2[199]23[215]	GGCATCAGGGAGGTGTCGAGGCATATAGCGAGAGGGCTTATTCAATTGA	filament3helper
t3h_3[24]31[31]	GCCATTGCTGGATTATGAACCGAAGGGCTTAGAACAAAG	filament3helper
t3h_3[152]20[152]	ACCGTCGGCCCCCTAGAACTGCCAGTC	filament3helper
t3h_3[184]18[184]	TTACGGCTATGCCGGTACATAACGATTATCGCCAGCATCCAAGGGT	filament3helper
t3h_3[216]20[216]	TTGAGTAAGAGCGCCCTAACAGAGAGGATTATTG	filament3helper
t3h_5[168]4[184]	TAGAACGTCAGCGTGGTGTGAGGTCAATTGG	filament3helper
t3h_6[151]22[152]	TTTCCCGTCAACTTAAATCATTGCG	filament3helper
t3h20[151]21[135]	GGAGCTTGGCTGACCGAGAAGGCAGAAAGAAC	filament3helper
t3h_20[215]21[199]	CTGAATATAATGACAGGTAGAAAGCCAAAAG	filament3helper
t3h_23[184]21[183]	AAACCAAAAGTAAGAGCAACACTAAATGCAGA	filament3helper