Supplementary Information

Cost-efficient folding of functionalized DNA origami nanostructures via staple recycling

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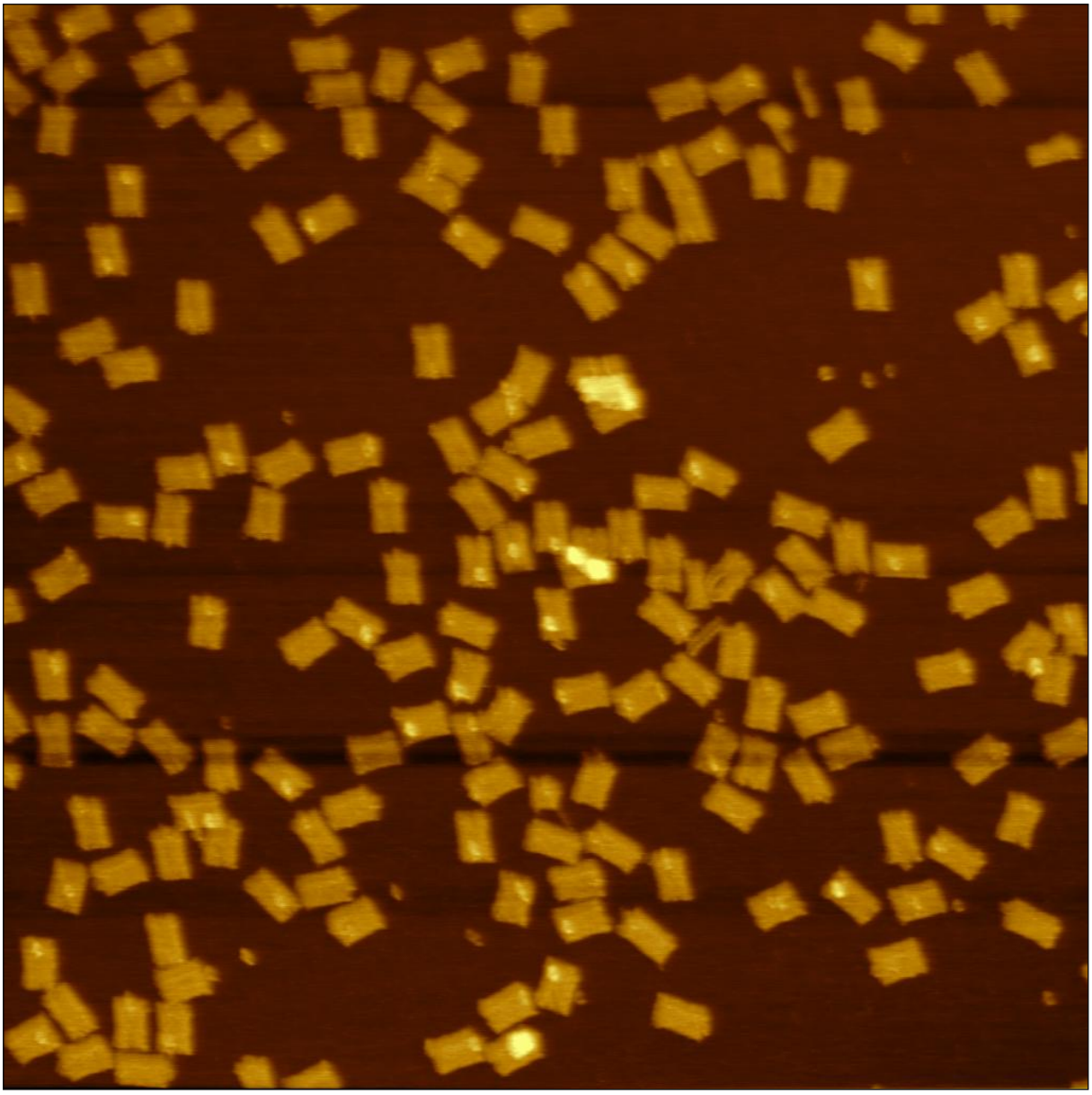

Figure S1. AFM image of rectangular DONs folded with 2-fold staple excess. This image was taken in air using a JPK Nanowizard Ultra Speed (JPK Instruments) operated in intermittent contact mode with HQ:NSC/Al BS cantilevers (75 kHz and 2.8 N/m) from MikroMasch (NanoAndMore). The scan area was 2 µm × 2 µm at a resolution of 1024 × 1024 pixels. The height scale is 11 nm.

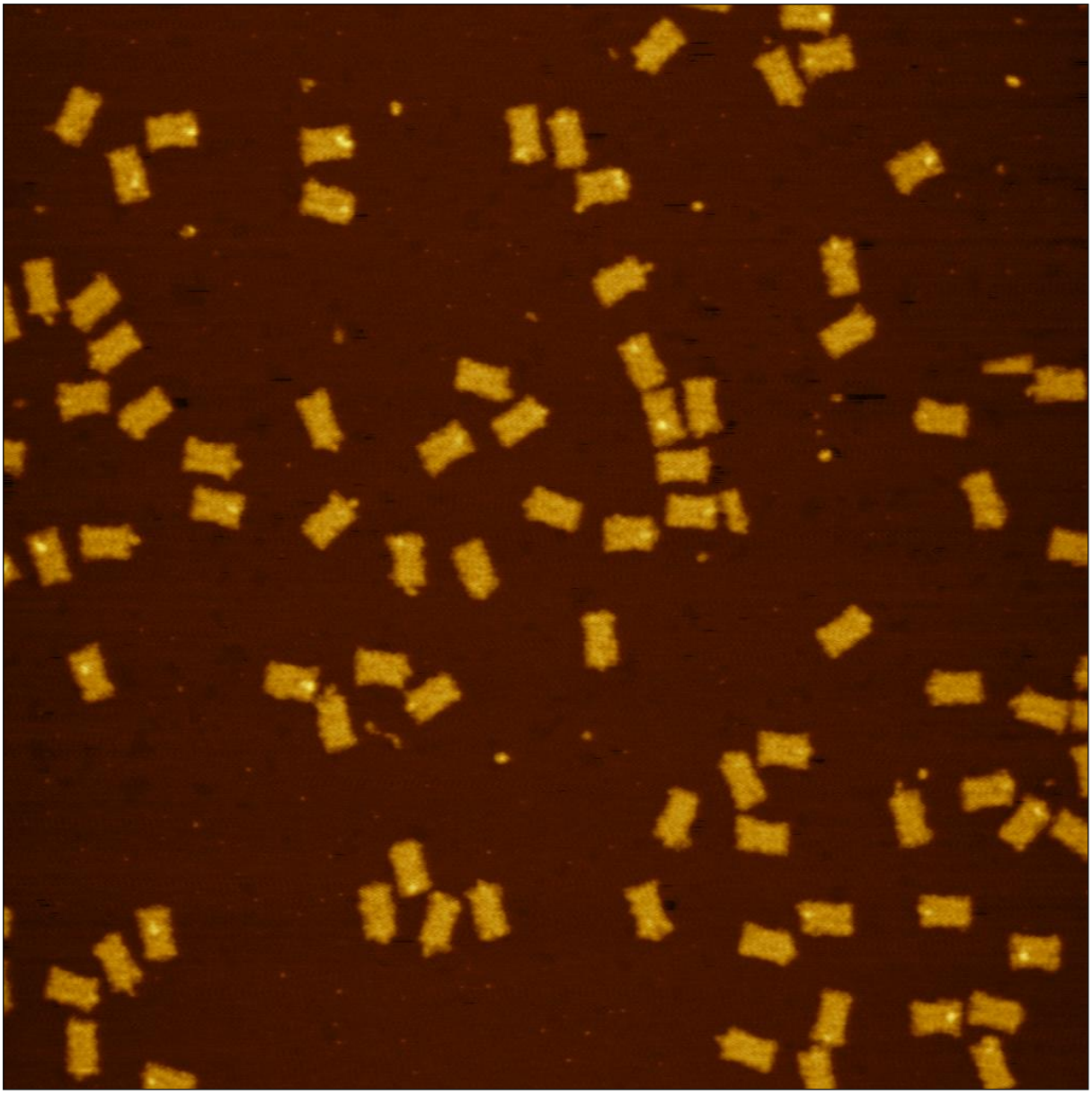

Figure S2. AFM image of rectangular DONs folded with 4-fold staple excess. This image was taken in air using a JPK Nanowizard Ultra Speed (JPK Instruments) operated in intermittent contact mode with HQ:NSC/Al BS cantilevers (75 kHz and 2.8 N/m) from MikroMasch (NanoAndMore). The scan area was 2 µm × 2 µm at a resolution of 1024 × 1024 pixels. The height scale is 4.5 nm.

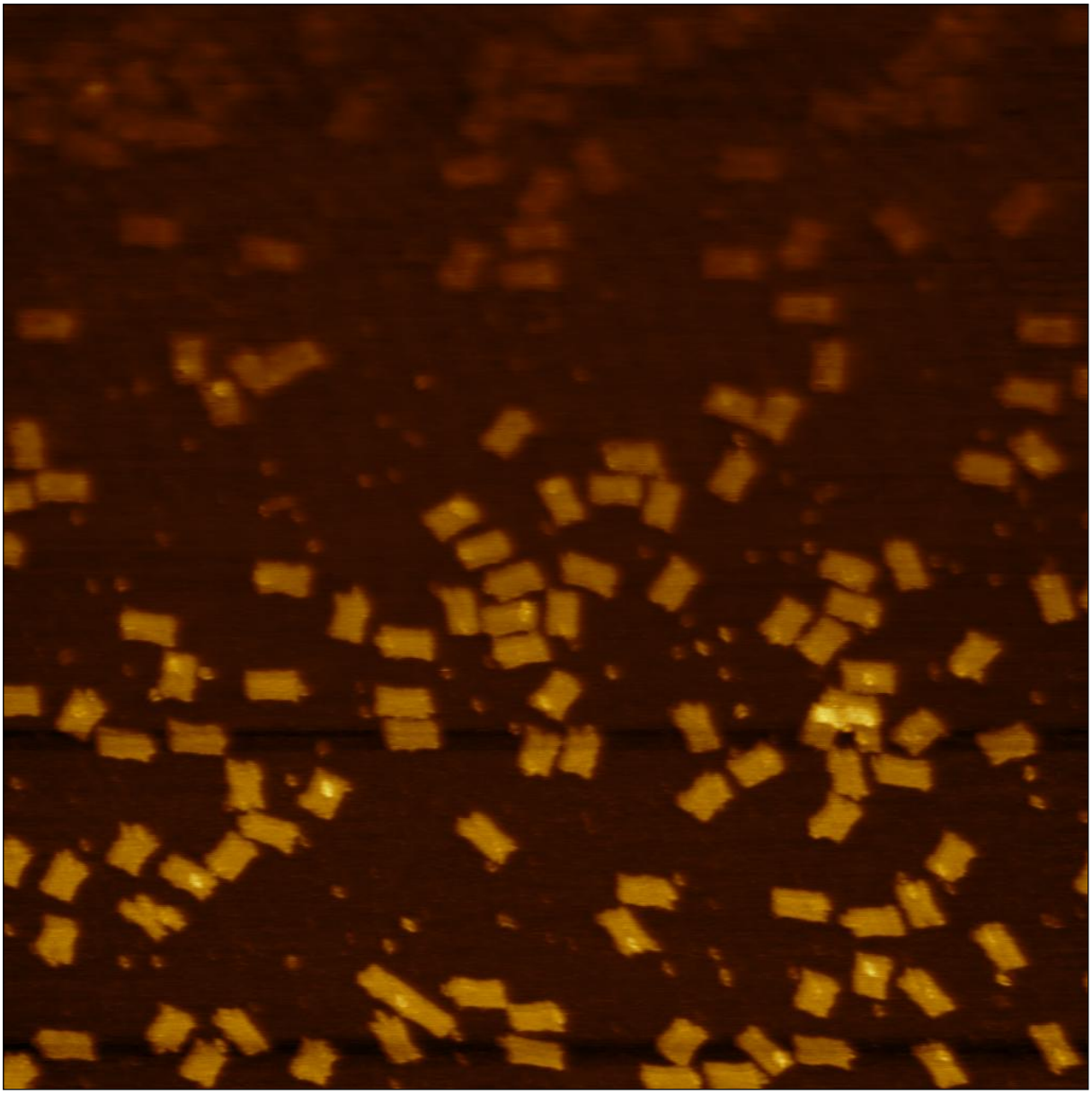

Figure S3. AFM image of rectangular DONs folded with 6-fold staple excess. This image was taken in air using a JPK Nanowizard Ultra Speed (JPK Instruments) operated in intermittent contact mode with HQ:NSC/Al BS cantilevers (75 kHz and 2.8 N/m) from MikroMasch (NanoAndMore). The scan area was 2 μm × 2 μm at a resolution of 1024 × 1024 pixels. The height scale is 4.5 nm.

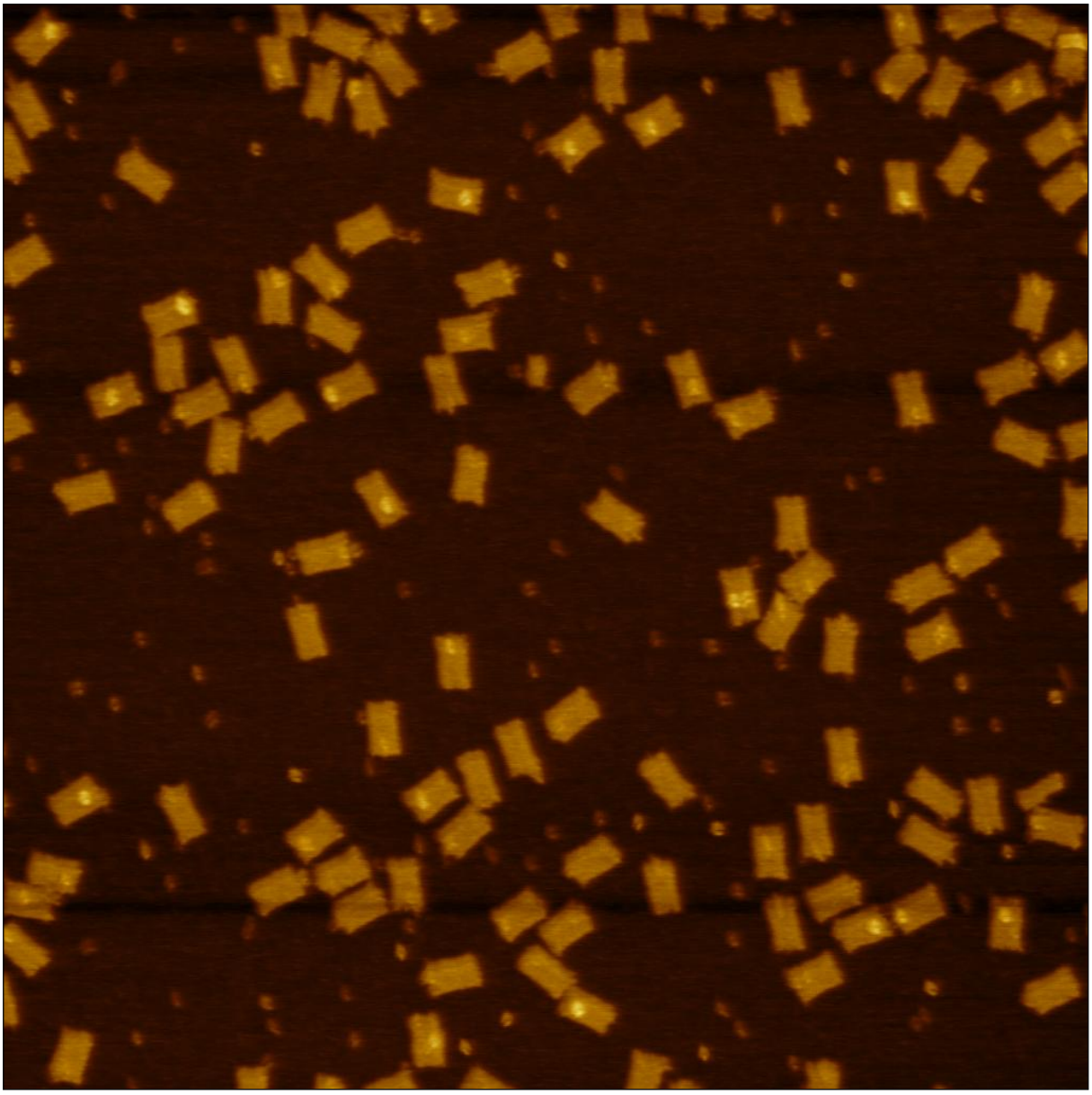

Figure S4. AFM image of rectangular DONs folded with 8-fold staple excess. This image was taken in air using a JPK Nanowizard Ultra Speed (JPK Instruments) operated in intermittent contact mode with HQ:NSC/Al BS cantilevers (75 kHz and 2.8 N/m) from MikroMasch (NanoAndMore). The scan area was 2 µm × 2 µm at a resolution of 1024 × 1024 pixels. The height scale is 4.5 nm.

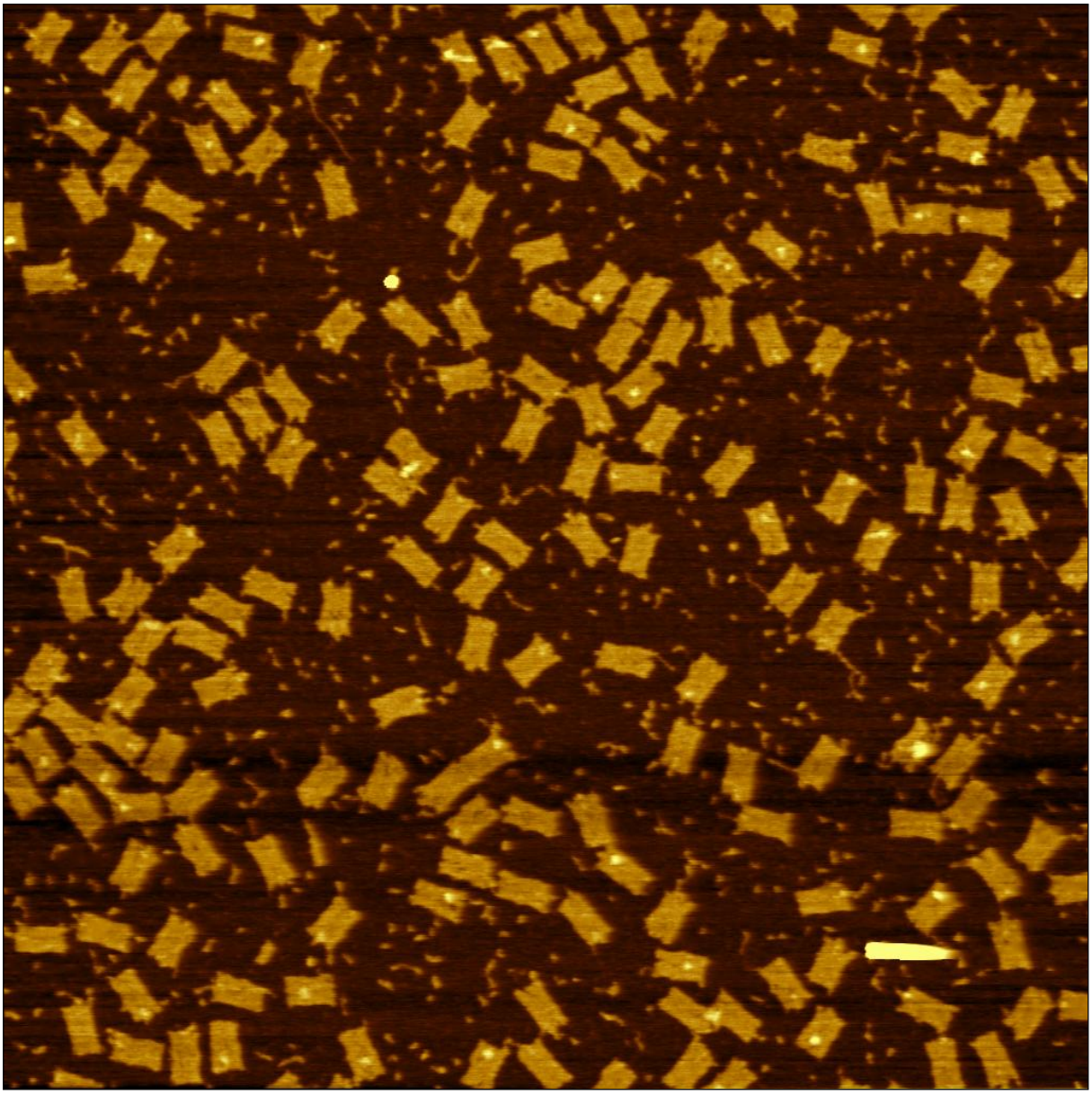

Figure S5. AFM image of rectangular DONs folded with 10-fold staple excess. This image was taken in air using a JPK Nanowizard Ultra Speed (JPK Instruments) operated in intermittent contact mode with HQ:NSC/Al BS cantilevers (75 kHz and 2.8 N/m) from MikroMasch (NanoAndMore). The scan area was 2 µm × 2 µm at a resolution of 1024 × 1024 pixels. The height scale is 14 nm.

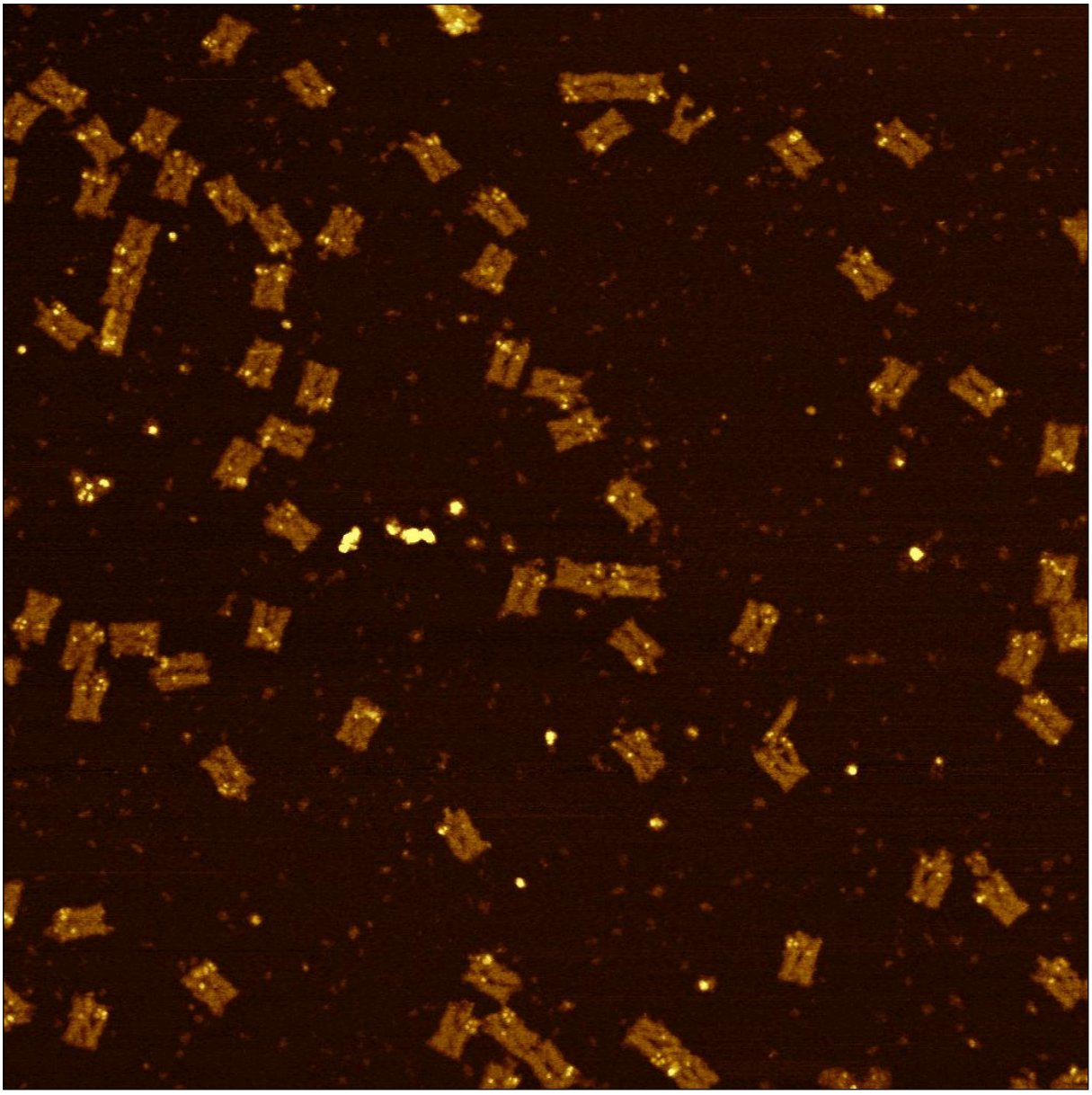

Figure S6. AFM image of biotin-modified rectangular DONs after the first cycle step (first folding) and subsequent SAv exposure. This image was taken in air using PeakForce Tapping mode with ScanAsyst using a Bruker Dimension Icon and SCANASYST-AIR cantilevers (70 kHz and 0.4 N/m). The scan area was 2 μm × 2 μm at a resolution of 1024 × 1024 pixels. The height scale is 5.0 nm.

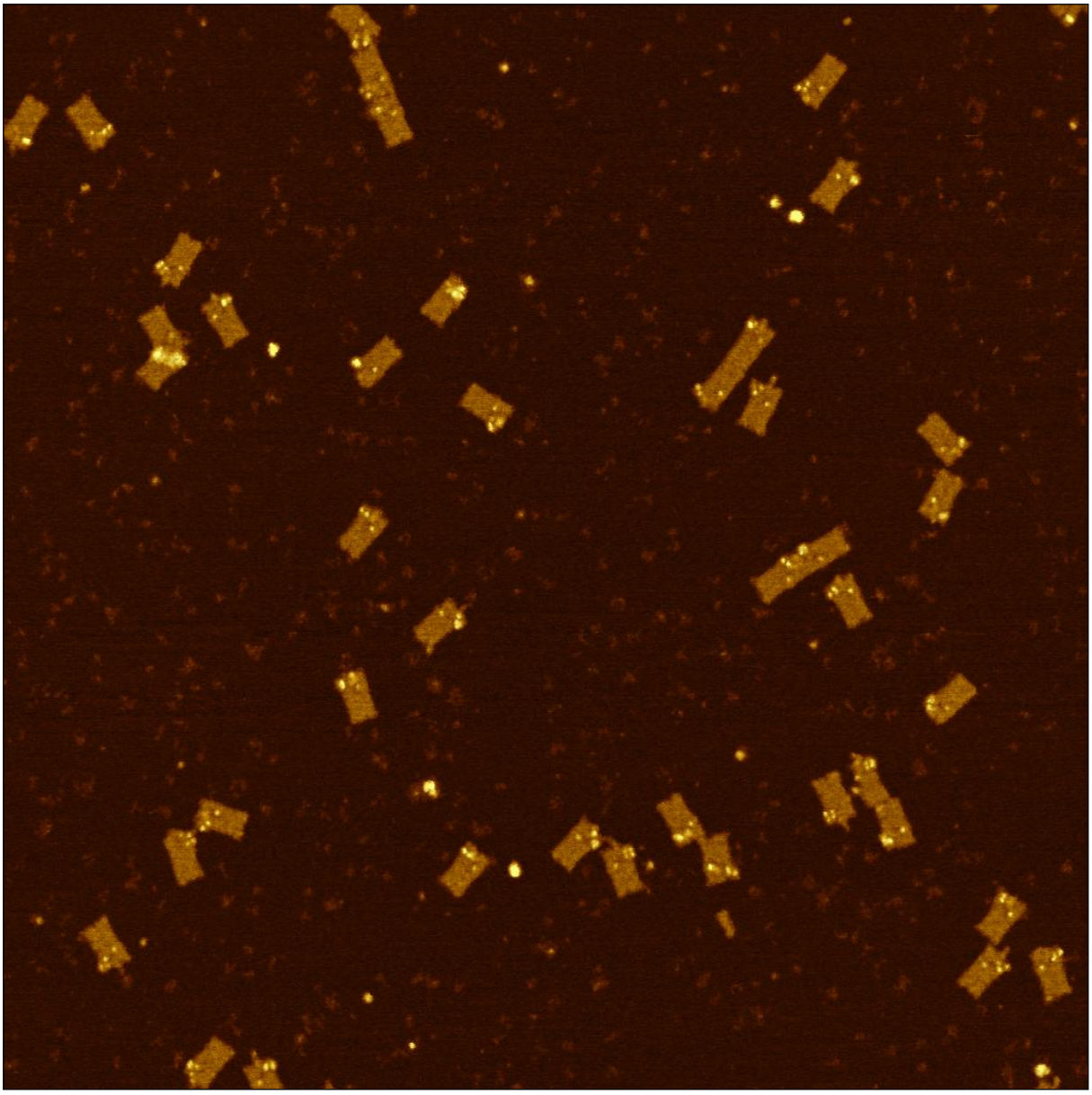

Figure S7. AFM image of biotin-modified rectangular DONs after the second cycle step and subsequent SAv exposure. This image was taken in air using PeakForce Tapping mode with ScanAsyst using a Bruker Dimension Icon and SCANASYST-AIR cantilevers (70 kHz and 0.4 N/m). The scan area was 2 µm × 2 µm at a resolution of 1024 × 1024 pixels. The height scale is 5.0 nm.

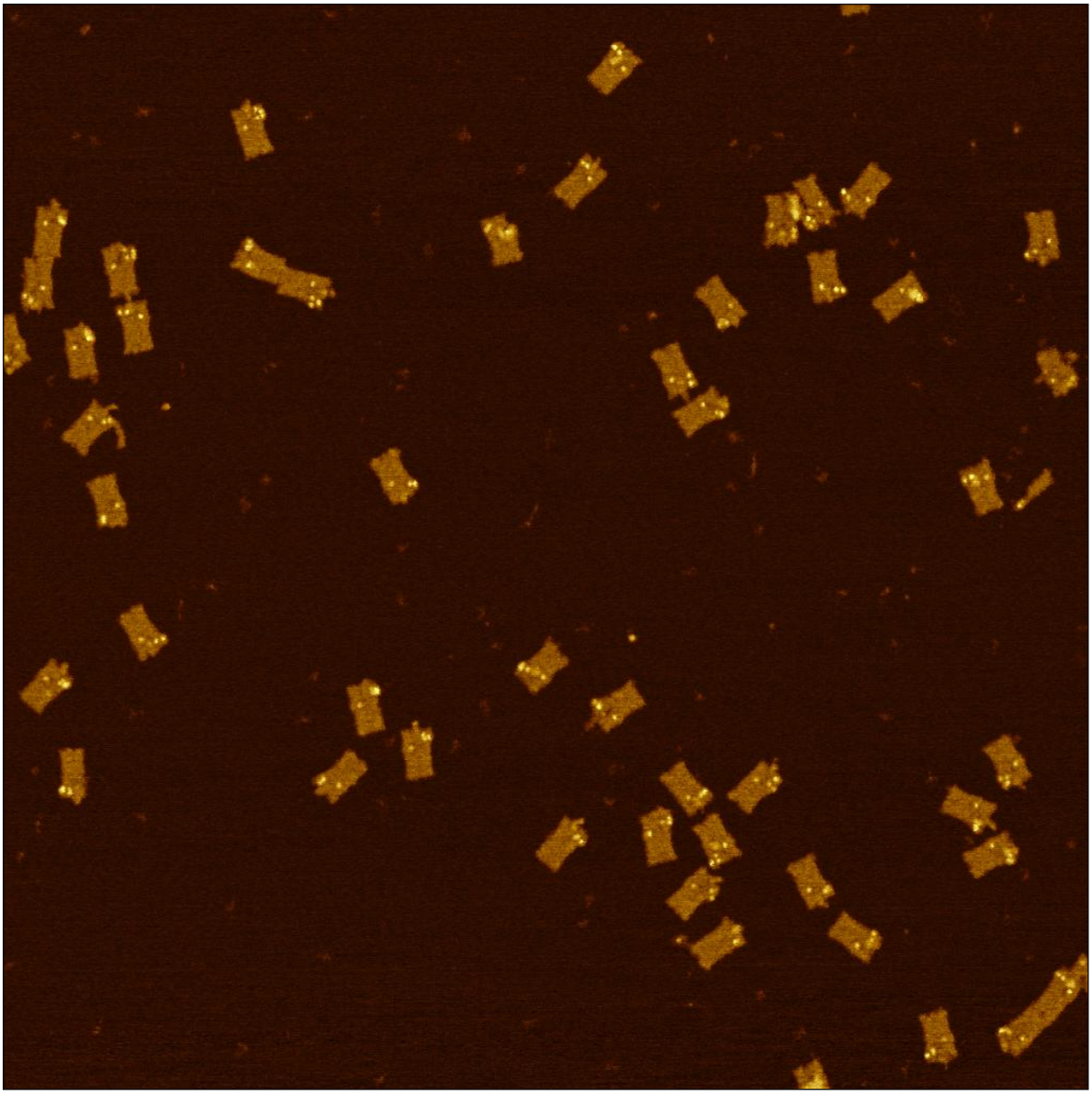

Figure S8. AFM image of biotin-modified rectangular DONs after the third cycle step and subsequent SAv exposure. This image was taken in air using PeakForce Tapping mode with ScanAsyst using a Bruker Dimension Icon and SCANASYST-AIR cantilevers (70 kHz and 0.4 N/m). The scan area was 2 μm × 2 μm at a resolution of 1024 × 1024 pixels. The height scale is 5.0 nm.

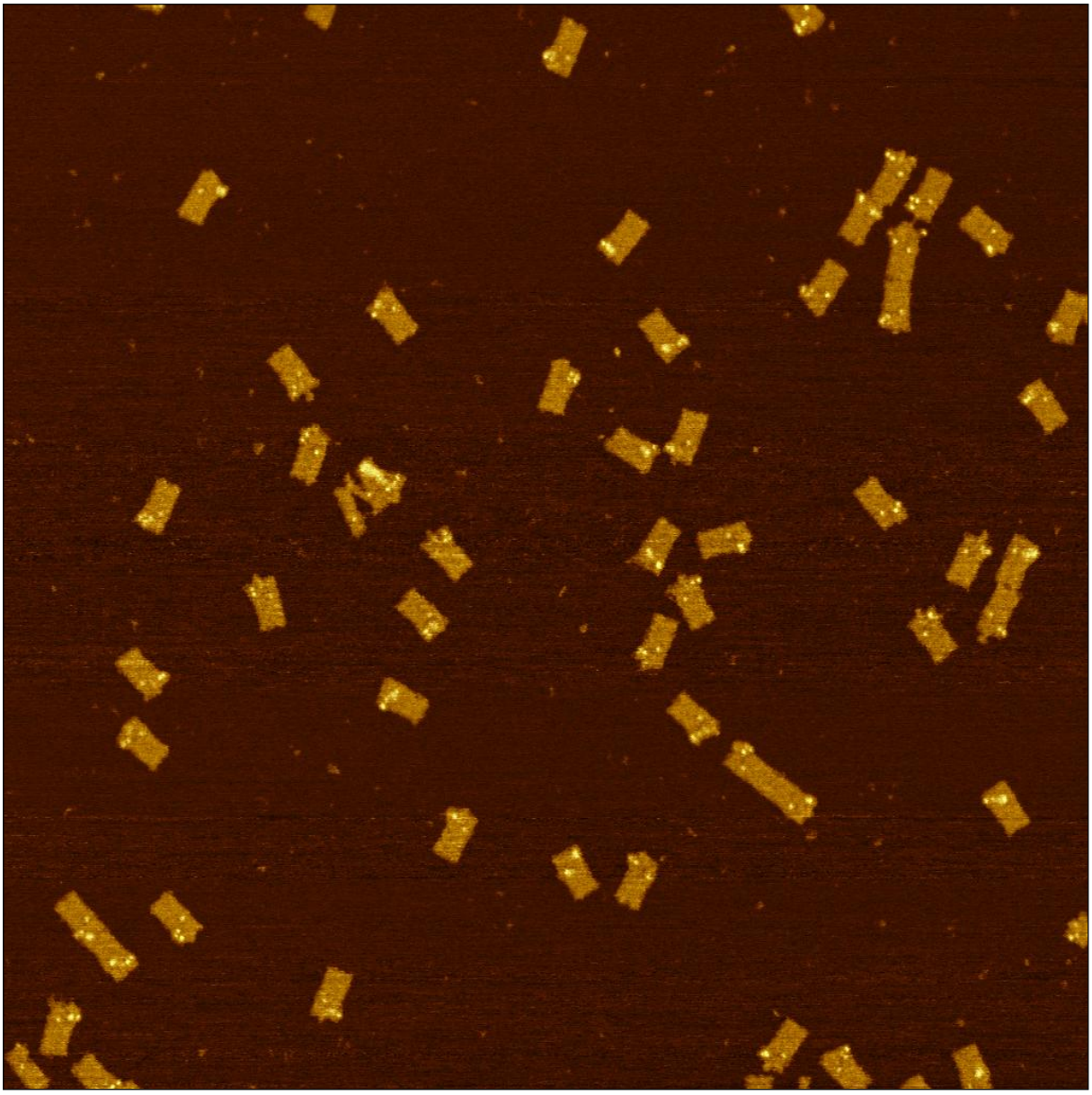

Figure S9. AFM image of biotin-modified rectangular DONs after the fourth cycle step and subsequent SAv exposure. This image was taken in air using PeakForce Tapping mode with ScanAsyst using a Bruker Dimension Icon and SCANASYST-AIR cantilevers (70 kHz and 0.4 N/m). The scan area was 2 μm × 2 μm at a resolution of 1024 × 1024 pixels. The height scale is 5.0 nm.

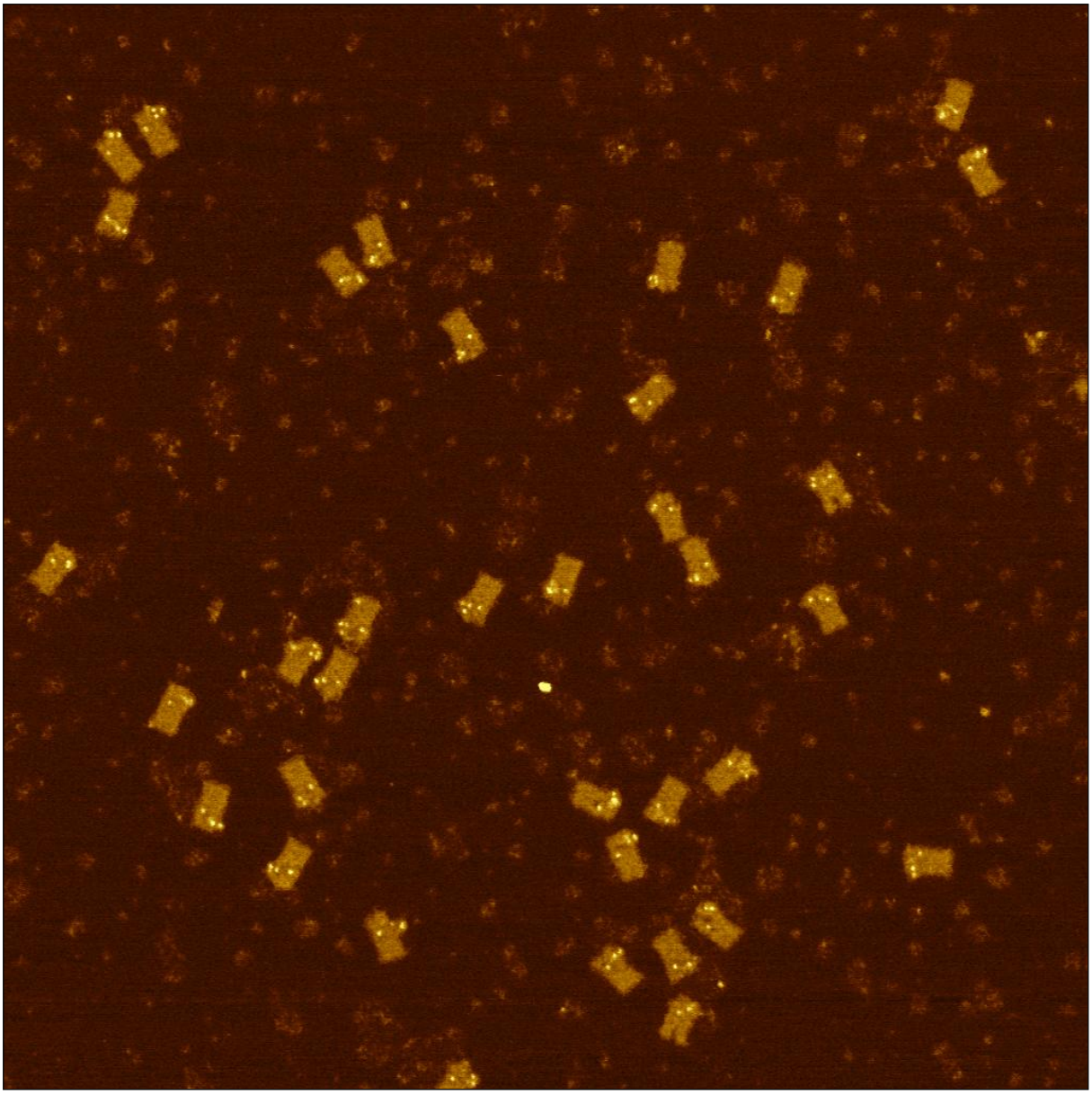

Figure S10. AFM image of biotin-modified rectangular DONs after the fifth cycle step and subsequent SAv exposure. This image was taken in air using PeakForce Tapping mode with ScanAsyst using a Bruker Dimension Icon and SCANASYST-AIR cantilevers (70 kHz and 0.4 N/m). The scan area was 2 μm × 2 μm at a resolution of 1024 × 1024 pixels. The height scale is 5.0 nm.

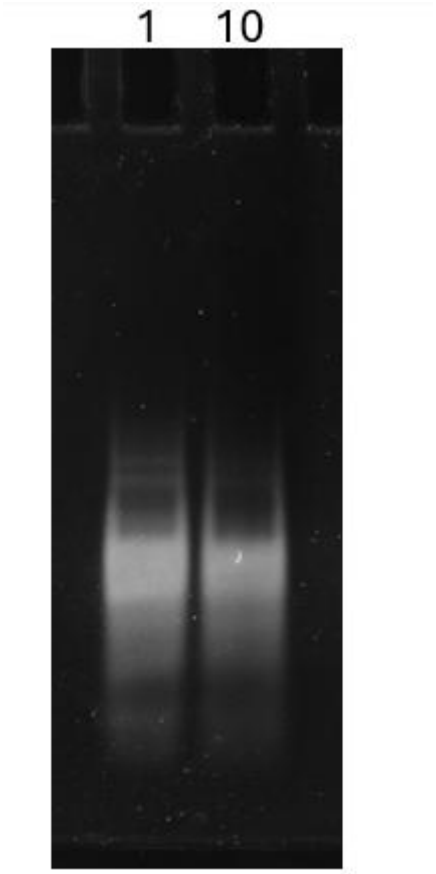

Figure S11. Native PAGE (7.5 %) of the staple strand mix (5 nM) of the 6HBs recovered after folding (cycle step 1) and after cycle step 10. The band patterns appear similar, suggesting that recycling and recovery does not result in notable staple degradation.

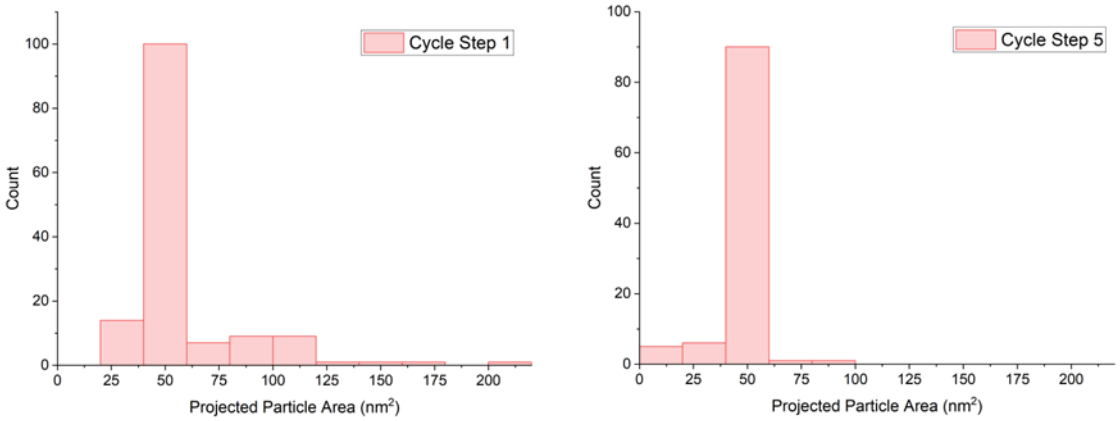

Figure S12. Distributions of the projected areas of the DON rectangles after recycling steps 1 and 5 as determined from the AFM images. The distribution after both steps peaks at 50 nm^2.

Table S1. Staple sequences for the DON rectangle. Biotin-modifications were added to the 5' ends of the non-modified staples with T_8-spacers (orange). ATTO488-modifications were added to the 3' ends of the non-modified staples with T_4-spacers (green).

Sequence (5' → 3')
GCATTAACATCCAATAAATCATAATAAAGCC
GATAGCAAGTTTCGTCACCAGTAAAGGAGC
AAAAGGTGGCATCAATTCTACTAATGTACCAA
CACCCTCATAGCATTCCACAGACACACGTTGA
TTAGCTATATTTTCATTTGGGGCGTTTGCGGG
CCACCCTCGTAACGATCTAAAGTTCAACTAAA
AGATACATTTCGCAAATGGTCAAATAAAAAT
GTGTATCACGTTAGTAAATGAATACAGTTT
TTCTGCGAACGAGTAGATTTAGTTTGACCATT
GAATGGCAACAGTTGAAAGGAACACTAACA
AGTTTCATTCCATATAACAGTTGATCAAATAT
ATAGCCCTCCCTCAATCAATATCTAGATAATA
TTAAATATGCAACTAAAGTACGGCGAACCAG
GAACGAACAGCATCACCTTGCTGAGACTTTAC
TTGCTGAATATAATGCTGTAGCTCCAGGATTA
TGAGGCGGCTGAGAGCCAGCAGATCCTTTG
TCAGAGCGCCGGAGAGGGTAGCCATTGCCT
ATGCCCCCTAACAGTGCCCGTATACCTGCAAC
AAACATTATGATATTCAACCGTTCATCGATGAACGGTAATTAGGAACG
CTGAAACATAATAAGTTTTAACGGGAGCCACC
AGAAGCCTAAGGCCGGAGACAGTCTCAATCAT
CCTCAAGCATGGCTTTTGATGAACCACCAG
TTTTAGAAATGTGTAGGTAAAGAAGCCCCAAAAACAGACGTTAA
TTGCTCAGTCTCTGAATTTACCGTGGTTGAGG
ATTAAGAGAGCAAAGCGGATTGCAAATGCAAT
GGCAAATCTATTAGTCTTTAATGCCATTCTGG
CGCGTTTTTCAGGTCTTTACCCTGCACTATCATAACCCTCGTTGAGAT
ATATCAAAAAAACATCGCCATTATCACCAG
ACCGGAATCAGAAAACGAGAATTAAAAACC
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Bt-TTTTTTTTAAATTAATATAAAGCTAAATCGGTTAGTAGTA
GCCTTGAGTGCCTATTTCGGAACTTTCAGG
Bt-TTTTTTTTCATCAATATGACCCTGTAATACTCGAGCTG
TGTACTGGTGAAAGTATTAAGAGGAGAACCGC
GGGTGAGATTATTTCAACGCAAGGTAACCTGT
GCGTCATAAGAAGGATTAGGATTATAGTACCG
GCCTGAGTACCCTCATATATTTTATCAAAAAG
AAGCGCAGTACCAGGCGGATAAGGGAATAG
TAGTCAGAGAAGCCCGAAAGACTTTCCCAA
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ATCAAAAAAATTCGAGCTTCAAAGTGTCTGGA
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AAACAATTAGCGGAATTATCATCAGATGAATA
ATAAATATCTTTAATTGCTCCTTAGCTTAA

CCCGAACCATTTTGCGGAACAAGGGAGAAACAATAACCAAAATC
GAGAGTCCCTTCCTGTAGCCAGACCCGTCG
CACCCTCACGGAACCGCCTCCCTCGTTTGAGT
ATGTACCCTTTTTGTTAAATCAGCTGTAGATG
ACCCTCAGTAATCAAAATCACCGGTGAGCCATTTGGGAATCATTCAAC
ATTACGAGTGCAGATACATAACGCAATATTTA
AGCCGCCAGCCCCCTTATTAGCACCAGTAG
AAAATAGACAACATTATTACAGACGAGTAG
CAGGTCAGTGTAGCGCGTTTTCATGAAACGTCACCAATGATTTATTTT
GCGTCTGGTGGAGCAAACAAGAGATAGCTGAT
TCCTGATTGATTAGAGCCGTCAATGGTCAGTT
CCATCAAAGGATTGACCGTAATGGGGCTGCGCAACTGTTG
GATTATCAGATTTAGAAGTATTAACCTCAA
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