## **Supplementary Information**

## Toward high-density streptavidin arrays on DNA origami nanostructures

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**Table S1:** Sequences of biotinylated staples used for testing different biotin arrangements.Spacer sequences are indicated in boldface.

Label	position	position	biotinylated staples
T <sub>8</sub> -6	2[111]	0[112]	[biotin]TTTTTTTGGGTGAGATTATTTCAACGCAAGGTAACCTGT
T <sub>8</sub> -6	4[111]	2[112]	[biotin]TTTTTTTGCATTAAACGGTTGATAATCAGAAATTCAAAA
T <sub>8</sub> -6	6[111]	4[112]	[biotin]TTTTTTTTTCTGGTGCCGTAACCGTGCATCTGCTAAAATTC
T <sub>4</sub> -6	2[175]	0[176]	[biotin]TTTTAGTCAGAGAAGCCCGAAAGACTTTCCCAA
T <sub>4</sub> -6	4[175]	2[176]	[biotin]TTTTTCAACTAAGCATAGTAAGAGCAAACTATTA
T4-6	6[175]	4[176]	[biotin]TTTTGACAAGAAAGCTGCTCATTCAGTACCACAT
T <sub>8</sub> -12	12[111]	10[112]	[biotin]TTTTTTTTAATGGATTTAATATCCAGAACAATTCCACGCT
T <sub>8</sub> -12	16[111]	14[112]	[biotin]TTTTTTTTCCAGAAGGCGACAACTCGTATTAACAAATGAA
T <sub>8</sub> -12	20[111]	18[112]	[biotin]TTTTTTTTTTCTTAGGTTCGATAGCTTAGATTAAGCGAATTA
Marker	14[271]	12[272]	[biotin]TTTTAAGCGCAGTACCAGGCGGATAAGGGAATAG
Marker	16[271]	14[272]	CGTCAGACACGATTGGCCTTGATGAATGGATTTT[biotin]
Marker	18[271]	16[272]	[biotin]TTTTGGAATAAGAACCATCGATAGCAGCCTTTAG
Marker	20[271]	18[272]	TGAGCGCTTAGAAAATACATACAACACCACTTTT[biotin]
Marker	22[271]	20[272]	[biotin]TTTTGCGGGAGGAGCGCATTAGACGGGGGGTAAT
Marker	23[256]	22[272]	AGATATAGAAGGCTTATCCGGTACCGACTTTTTT[biotin]

Label	position	position	biotinvlated staples
20001	[start]	[end]	
T <sub>8</sub> -12	4[47]	2[48]	[biotin]TTTTTTTTGCGTCTGGTGGAGCAAACAAGAGATAGCTGAT
T <sub>8</sub> -12	4[79]	2[80]	[biotin]TTTTTTTTTTTAACCAACGTAAAACTAGCATGAAATCAC
T <sub>8</sub> -12	4[111]	2[112]	[biotin]TTTTTTTTGCATTAAACGGTTGATAATCAGAAATTCAAAA
T <sub>8</sub> -12	4[143]	3[159]	[biotin]TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
T <sub>8</sub> -12	8[47]	6[48]	[biotin]TTTTTTTTTGAGTGAGGGTAACGCCAGGGTTTGGAAGGGC
T <sub>8</sub> -12	8[79]	6[80]	[biotin]TTTTTTTGAAGCATAGTAAAACGACGGCCAGCCATTC
T <sub>8</sub> -12	8[111]	6[112]	[biotin]TTTTTTTTCCGCTCACCCTGCAGGTCGACTCTCACCGCTT
T <sub>8</sub> -12	8[143]	7[159]	[biotin]TTTTTTTTGTCATAGCACCGAGCTCGAATTCGAGAGGCAA
T <sub>8</sub> -12	12[47]	10[48]	[biotin]TTTTTTTTCCAACAGAACGTGGACTCCAACGTTCCCTTAT
T <sub>8</sub> -12	12[79]	10[80]	[biotin]TTTTTTTTTTCACACGAGTCTATCAACTCAAACTGTTTG
T <sub>8</sub> -12	12[111]	10[112]	[biotin]TTTTTTTTTTAATGGATTTAATATCCAGAACAATTCCACGCT
T <sub>8</sub> -12	12[143]	11[159]	[biotin]TTTTTTTTTTAATACCTATGCAACAGGAAAAACGTGTACCGT
T8-12	16[47]	14[48]	[biotin]TTTTTTTTTTCCTGATTGATTAGAGCCGTCAATGGTCAGTT
T <sub>8</sub> -12	16[79]	14[80]	[biotin]TTTTTTTGATTATCAGATTTAGAAGTATTAACCTCAA
T <sub>8</sub> -12	16[111]	14[112]	[biotin]TTTTTTTTCCAGAAGGCGACAACTCGTATTAACAAATGAA
T <sub>8</sub> -12	16[143]	15[159]	[biotin]TTTTTTTTTAACATTATGTTATTAATTTTAAAAAAGAGCCGC
T <sub>8</sub> -12	20[47]	18[48]	[biotin]TTTTTTTTGCGAGAAATAACCTTGCTTCTGTAAATTAATT
T <sub>8</sub> -12	20[79]	18[80]	[biotin]TTTTTTTTGCTGATGCTAATTTTCCCTTAGAGAAGATG
T <sub>8</sub> -12	20[111]	18[112]	[biotin]TTTTTTTTTTTTAGGTTCGATAGCTTAGATTAAGCGAATTA
T <sub>8</sub> -12	20[143]	19[159]	[biotin]TTTTTTTAGGTCTGACAATAGTGAATTTATCGATAGCCG

**Table S2:** Sequences of biotinylated staples used for generating the 4 x 5 biotin array. Spacer sequences are indicated in boldface.











**Figure S3:** Bidentate SAv-Bt binding yields of the marker positions under the same conditions as shown in Figure 2. The data are presented as mean values with the standard deviations as error bars. For each condition, 6 AFM images with a total of 70 to 105 DONs have been analyzed.



**Figure S4:** Increase of the SAv concentration over time due to stepwise addition of 1.5  $\mu$ L of 5  $\mu$ M streptavidin solution to 100  $\mu$ L incubation buffer in 5 min increments.



**Figure S5:** AFM images of DON rectangles with  $T_4$ -6,  $T_8$ -6, and  $T_8$ -12 Bt modifications after incubation with 215 nM SAv for 5 min.



**Figure S6:** AFM images of DON rectangles with  $T_4$ -6,  $T_8$ -6, and  $T_8$ -12 Bt modifications after incubation with 215 nM SAv for 15 min.



**Figure S7:** AFM images of DON rectangles with  $T_4$ -6,  $T_8$ -6, and  $T_8$ -12 Bt modifications after incubation with 413 nM SAv for 30 min.



**Figure S8:** AFM images of DON rectangles with  $T_4$ -6,  $T_8$ -6, and  $T_8$ -12 Bt modifications after stepwise SAv addition at a final concentration of 74 nM.

![](_page_7_Picture_0.jpeg)

**Figure S9:** AFM images of DON rectangles with  $T_4$ -6,  $T_8$ -6, and  $T_8$ -12 Bt modifications after stepwise SAv addition at a final concentration of 146 nM.

![](_page_7_Figure_2.jpeg)

**Figure S10:** AFM images of DON rectangles with T<sub>4</sub>-6, T<sub>8</sub>-6, and T<sub>8</sub>-12 Bt modifications after stepwise SAv addition at a final concentration of 215 nM.

![](_page_8_Picture_0.jpeg)

**Figure S11:** AFM images of DON rectangles with  $T_4$ -6,  $T_8$ -6, and  $T_8$ -12 Bt modifications after stepwise SAv addition at a final concentration of 283 nM.

![](_page_8_Figure_2.jpeg)

**Figure S12:** AFM images of DON rectangles with T<sub>4</sub>-6, T<sub>8</sub>-6, and T<sub>8</sub>-12 Bt modifications after stepwise SAv addition at a final concentration of 349 nM.

![](_page_9_Picture_0.jpeg)

**Figure S13:** AFM images of DON rectangles with  $T_4$ -6,  $T_8$ -6, and  $T_8$ -12 Bt modifications after stepwise SAv addition at a final concentration of 413 nM.

![](_page_9_Figure_2.jpeg)

**Figure S14:** AFM images of DON rectangles with the 3 x 4 Bt array after stepwise SAv addition at a final concentration of 215 nM.

![](_page_10_Figure_0.jpeg)

**Figure S15:** AFM images of DON rectangles with the 3 x 4 Bt array after stepwise SAv addition at a final concentration of 283 nM.

![](_page_10_Figure_2.jpeg)

**Figure S16:** AFM images of DON rectangles with the 3 x 4 Bt array after stepwise SAv addition at a final concentration of 349 nM.

![](_page_11_Picture_0.jpeg)

**Figure S17:** AFM images of DON rectangles with the 3 x 4 Bt array after stepwise SAv addition at a final concentration of 413 nM.