Supporting Information for

"**Asymmetric Secondary and Tertiary Streptavidin/DNA Complexes Selectively Formed in a Nanometer-scale DNA Well**"

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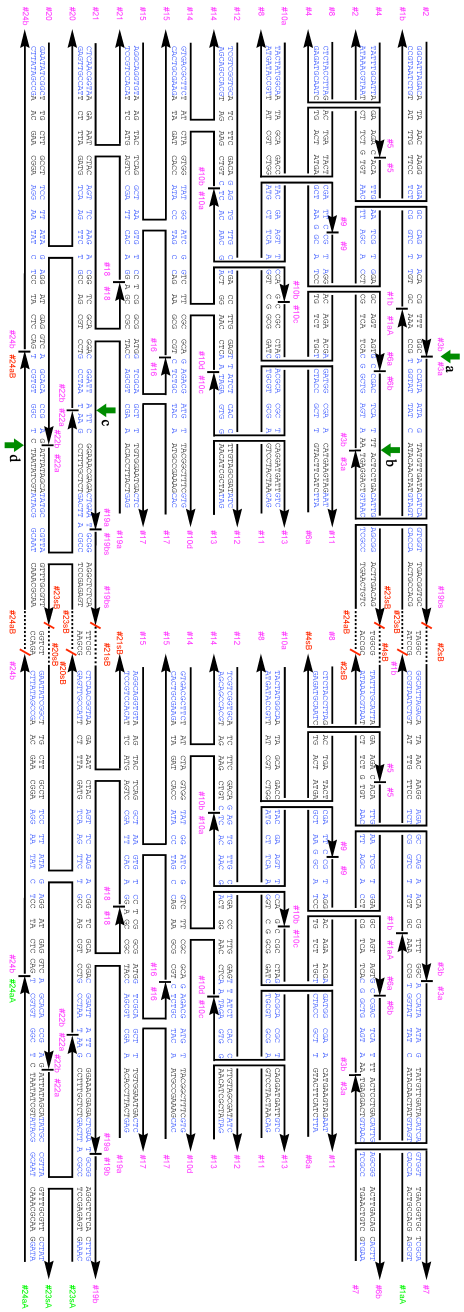

Figure S1. Detailed sequence of the strands making up the DNA motifs (**2a**, **2b**). For constructing **3** or **4**, the nucleotides listed in Table S1 were inserted in the points indicated by the green arrows.

Table S1. Inserted nucleotides in the motifs.

	3	**4**
a	5′-TAGCGAGTGA-3′	5′-TGGACTTTAGGTAGCGAGTGA-3′
	3′-ATCGCTCACT-5′	3′-ACCTGAAATCCATCGCTCACT-5′
b	5′-TGATTACTGT-3′	5′-CACTCTTTCAATGATTACTGT-3′
	3′-ACTAATGACA-5′	3′-GTGAGAAAGTTACTAATGACA-5′
c	5′-CGGTACACTA-3′	5′-CAGTTGTATTGCGGTACACTA-3′
	3′-GCCATGTGAT-5′	3′-GTCAACATAACGCCATGTGAT-5′
d	5′-ACAACTCTCC-3′	5′-CTAAACTCGTAACAACTCTCC-3′
	3′-TGTTGAGAGG-5′	3′-GATTTGAGCATTGTTGAGAGG-5′

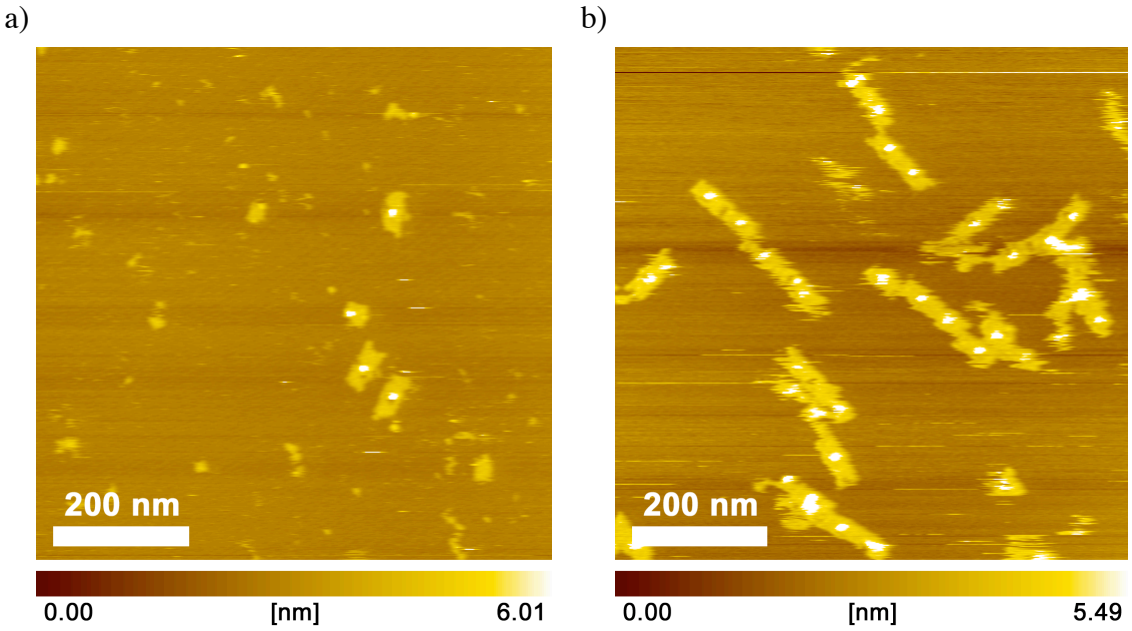

Figure S2. AFM images of (a) SA/**2a** complexes and (b) SA/**2b** complexes.

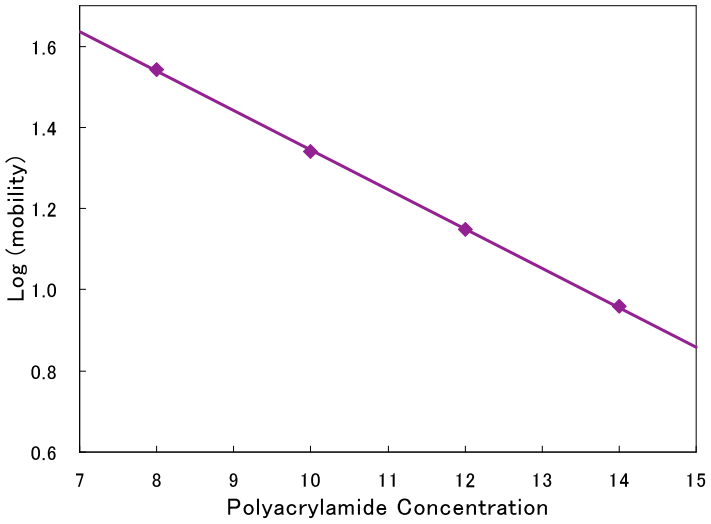

Figure S3. The Ferguson plot of the lower band in Figure 4a. The slope is -0.097 and the intercept is 2.32.

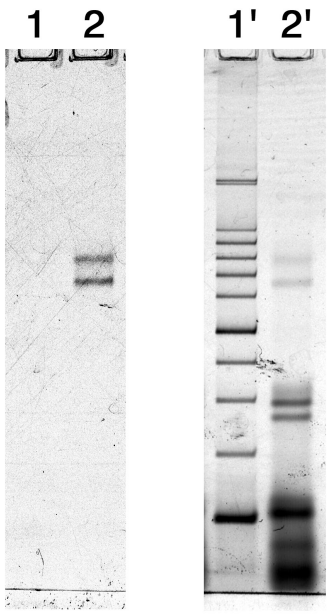

Figure S4. Quantification of the complexes with FAM-labeling. (a) A 12% denaturing PAGE analysis of SA captured in **2a** containing FAM-labeled **14bio**. Lanes 1 and 2 was imaged by the fluorescence of FAM and lanes 1' and 2' was stained with GelStar afterward. Lane 1 and 1', 100-bp dsDNA ladder; lanes 2 and 2', **2a** containing FAM-labeled **14bio** was mixed with SA. Conditions: [SA] = 1.1 μM, [DNA motifs] = 14 nM. Quantified intensities of FAM fluorescence were normalized by expected number of FAM in the complex. The yield of the secondary SA/DNA complex (the lower band) in lane 2 calculated from FAM fluorescence was 38%. (b) The same gel was stained with GelStar. The yield of the secondary SA/DNA complex (the lower band) in lane 2' calculated from GelStar fluorescence was 39%.

a) b)

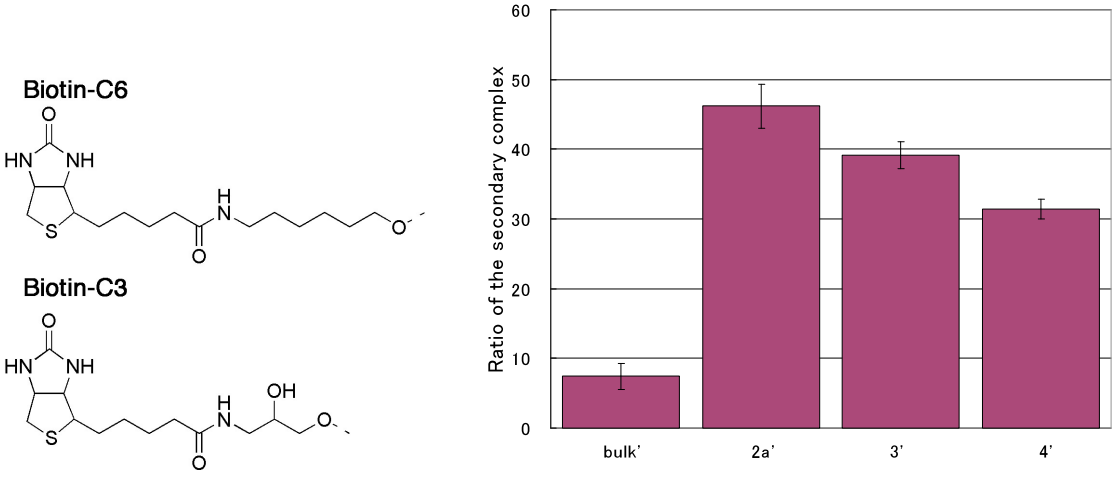

Figure S5. Bidentate capture of SA using shorter linkers between biotins and DNA. (a) Structures of the biotin residues with shorter linkers. The 5' end of **13** was modified with a biotin-C6 residue (**13bio'**), similarly the 3' end of **14** was modified with a biotin-C3 residue (**14bio'**). By using these biotinylated strands, **2a'**, **3'** and **4'** was prepared. (b) The yield of the secondary SA/DNA complex formed in **2a'**, **3'**, and **4'** estimated in the same way as Figure 4a. The yield in **2a'**, **3'** and **4'** was 46 ± 3%, 39 ± 2% and 31 ± 1%, respectively.

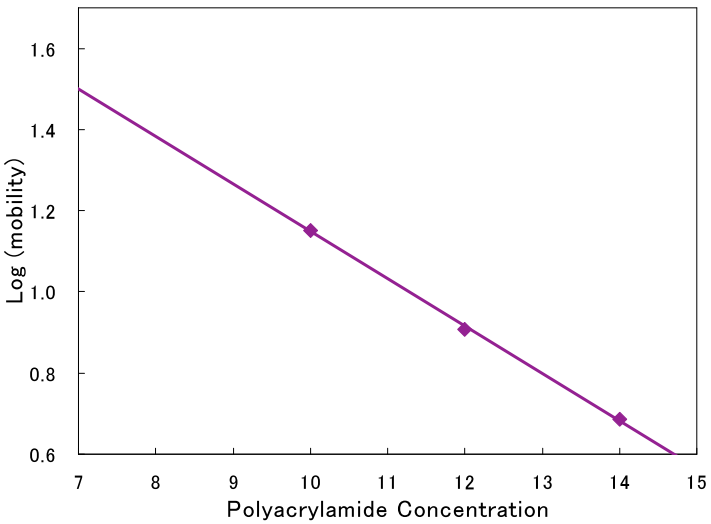

Figure S6. The Ferguson plot of the top band in Figure 5. The slope is -0.117 and the intercept is 2.32.

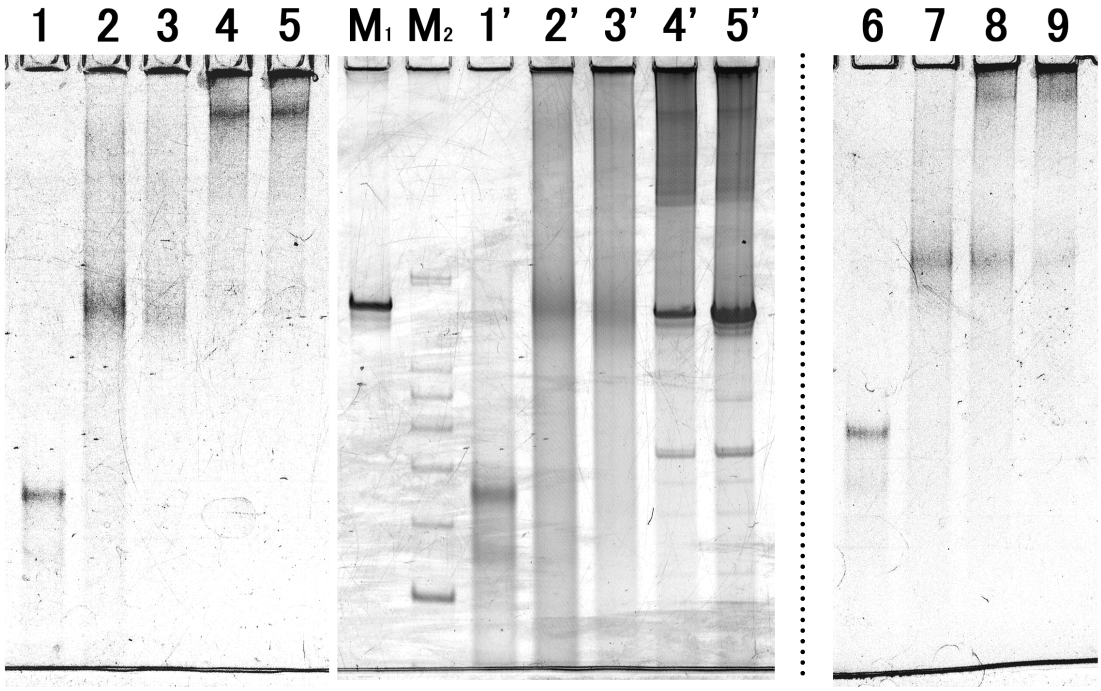

Figure S7. Further modification of SA in a well by biotinylated long dsDNA (1272 bp). Lanes 1–5, the same as Figure 6. Lane M_1, the biotinylated dsDNA; lane M_2, a 100 bp dsDNA ladder. Lanes 1'–5', lanes 1–5 were stained with GelStar. Lanes 6–9, capture of a SA/dsDNA complex in a well. Preformed complex of SA and the biotinylated dsDNA was added to the solution of **2b** containing FAM-labeled **11bio** and **17bio**. The mixture was analyzed by a 4% non-denaturing PAGE. Lane 6, **1** containing FAM-labeled **11bio** and **17bio**; lane 7, **2b** containing FAM-labeled **11bio** and **17bio**; lanes 8 and 9, SA and a biotinylated dsDNA was mixed first and added to the solution of **2b** containing FAM-labeled **11bio** and **17bio**. Conditions: [DNA motifs] = 33 nM, [SA] = [biotinylated dsDNA] = 33 (lane3), 67 nM (lane 4)