Supporting Information:

Electron Microscopic Visualization of Protein Assemblies on Flattened DNA Origami

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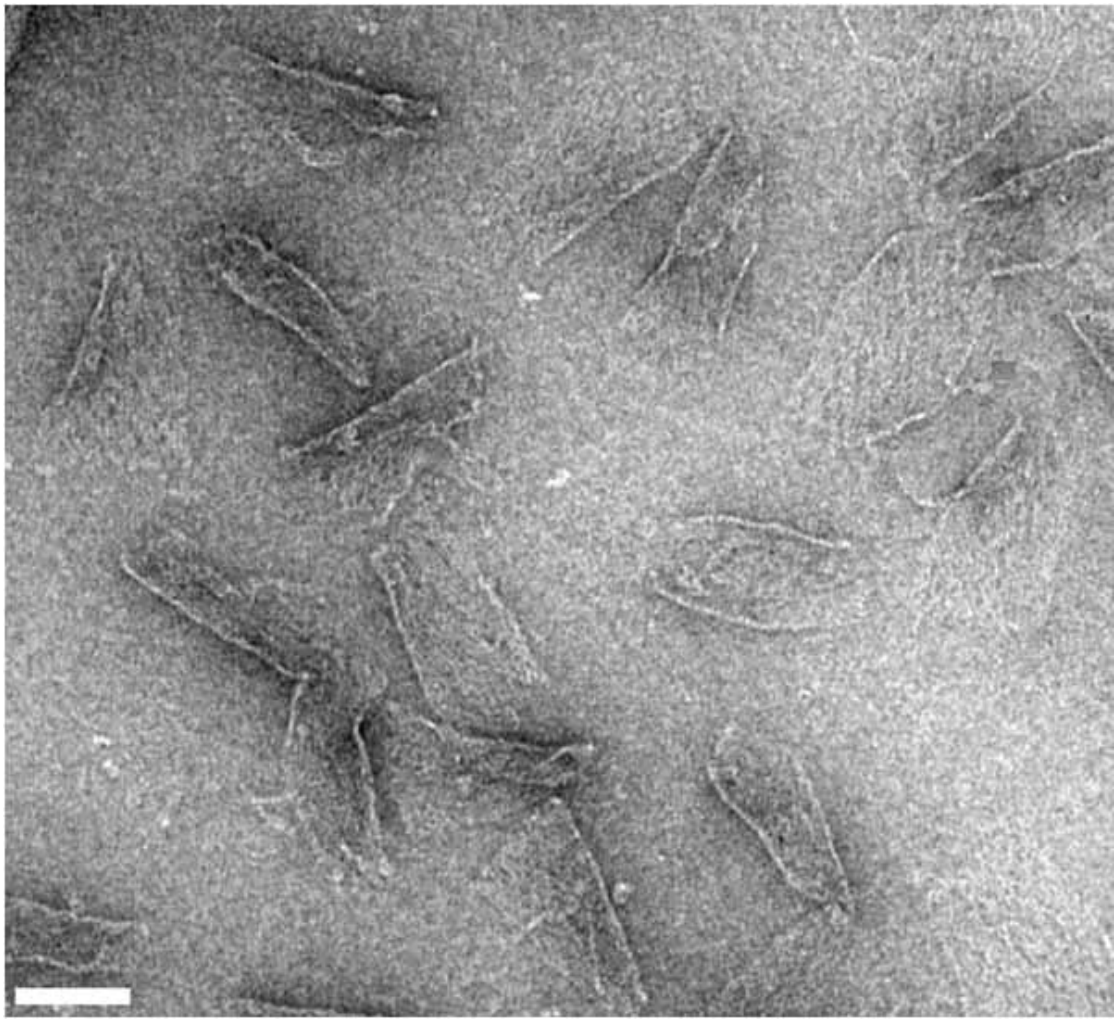


Figure S1. Conformational polymorphism of rectangular 2D origami structures revealed by negative stain EM. Scale bar is 50 nm.

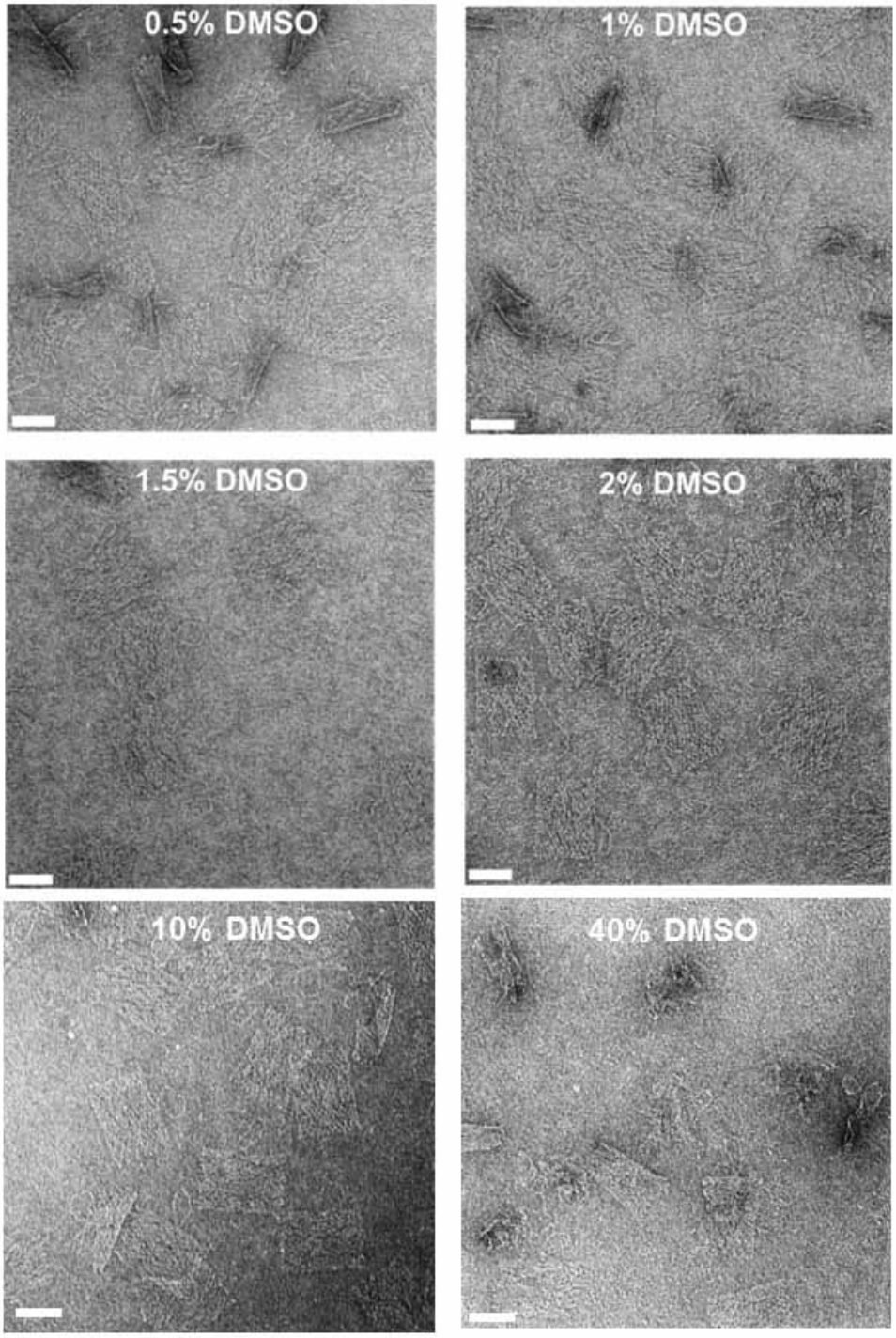


Figure S2. Surface flattening of DNA origami at different concentrations of DMSO. The experiment was performed in 1× TAE-Mg^{2+} buffer. Scale bar is 50 nm.

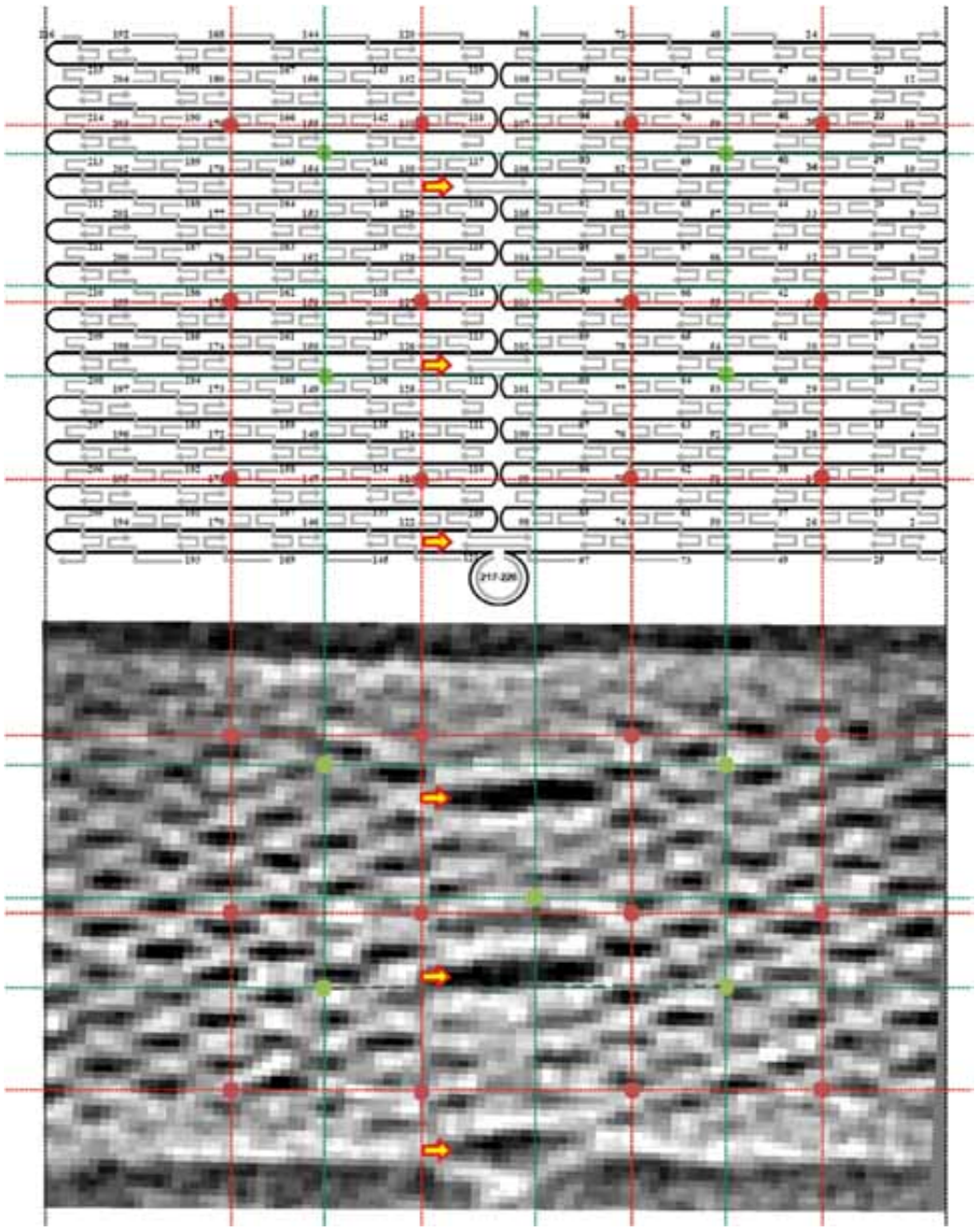


Figure S3. EM mapping of the DNA origami design. Red and green dots represent the position of protruding single stranded capture DNA oligonucleotide and biotins, respectively. The corresponding positions of the three holes that appeared in the class average image are highlighted with arrows.

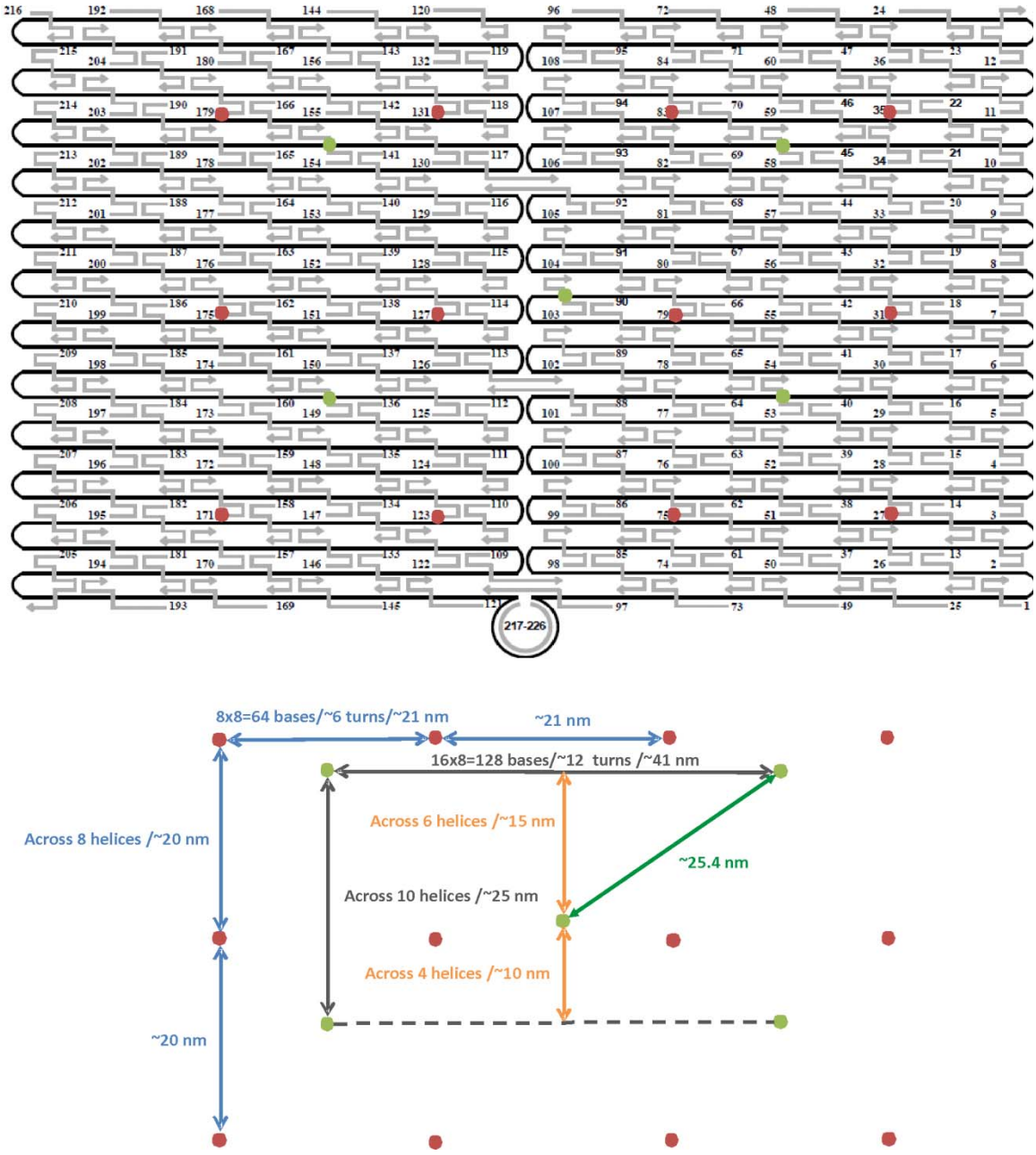


Figure S4. DNA origami map (top) and distance measurements (bottom) between the capture DNA oligonucleotides (red dots) and biotins (green).

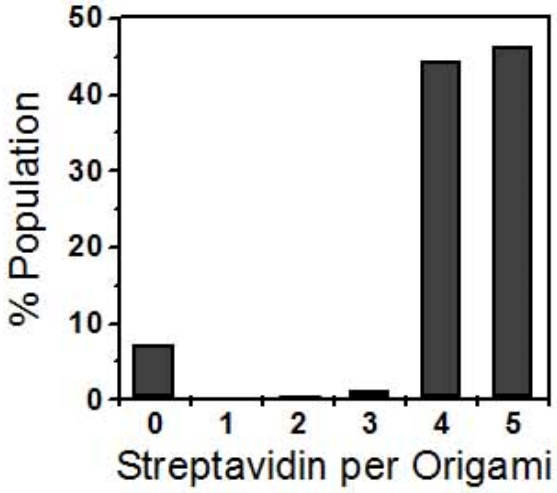
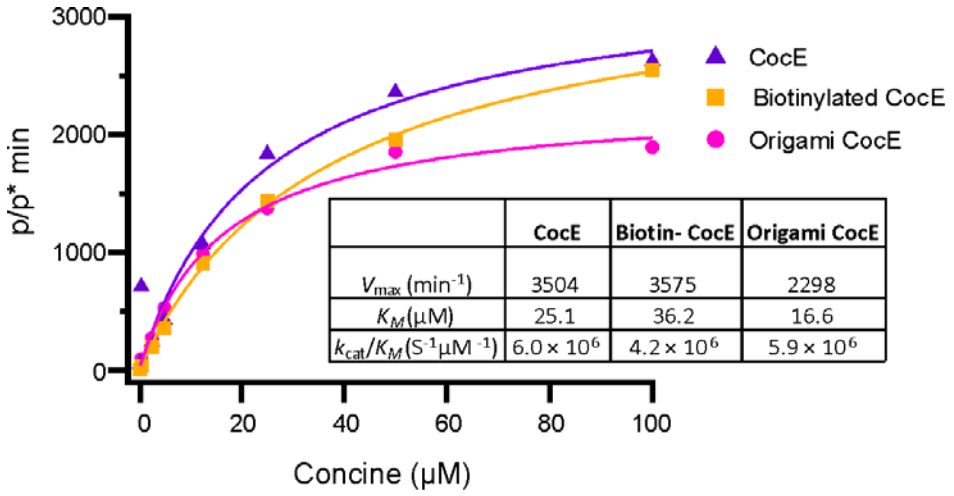


Figure S5. Distribution of streptavidin on 2D DNA origami. Most origami particles show 4 or 5 streptavidin on them and only a small fraction (~7%) show no binding. Total of 304 origami particles were analyzed.



	CocE	Biotin- CocE	Origami CocE
V_{max} (min^{-1})	3504	3575	2298
K_M(μM)	25.1	36.2	16.6
k_{cat}/K_M($S^{-1}μM^{-1}$)	6.0×10^6	4.2×10^6	5.9×10^6

Figure S6. Enzyme activity of origami bound CocE. Data were fitted with Michaelis-Menten kinetics to extract kinetic parameters. The enzyme's catalytic efficiency (k_{cat}/K_M) was compared among wild type, biotin-modified and origami-bound biotin-modified CocE.

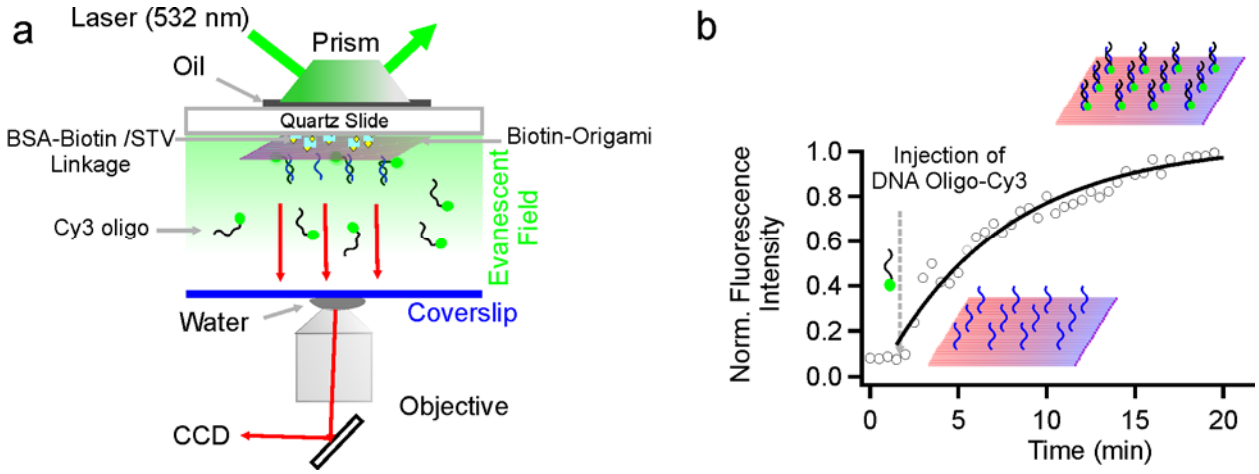


Figure S7. Measuring the capture kinetics of the DNA origami for complementary DNA oligonucleotides. (a) Single-molecule experimental set up to visualize the binding of Cy3-labeled complementary DNA oligonucleotides to the capture DNA oligonucleotides protruding from the DNA origami surface. The origami nanoplatforms were immobilized on the slide surface *via* the biotin-BSA/streptavidin interaction. (b) Observing the hybridization of Cy3-labeled DNA oligonucleotides to the immobilized origami. 300 µL of 30 nM Cy3-labeled DNA oligonucleotides in 1× TAE-Mg^{2+} buffer was injected into the channel and the fluorescence intensity over the entire field of view was monitored using a shuttered illumination scheme with 0.5 s exposures separated by 29.5s dark periods. The fluorescence intensity was integrated, normalized to the maximum, and fitted with a single-exponential function (black curve). The average rate constant for capturing the complementary oligonucleotides was determined to be 0.52 min^{-1} (n =2 experimental replicates). The experiment was carried out at room temperature.

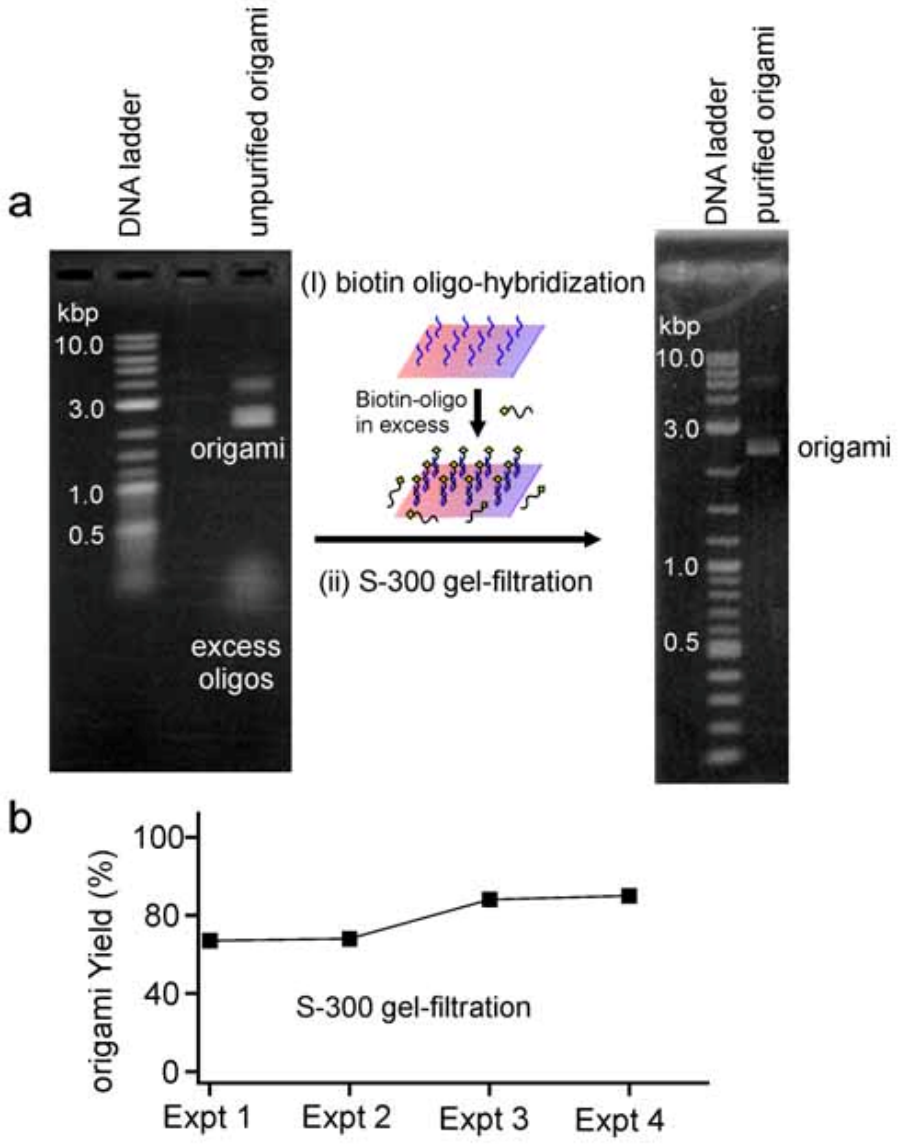


Figure S8. Purification of DNA origami. (a) Agarose gel electrophoresis before ('unpurified origami') and after ('purified origami') removing the excess staple strands or biotin labeled strands by gel filtration. (b) The yield of DNA origami (67-90% yield) determined by UV-Vis absorbance at 260 nm after the gel filtration. Experiments (Expt) 1 to 4 represent the purification yield of four independent batches of DNA origami assembled using our standard origami assembly protocol.

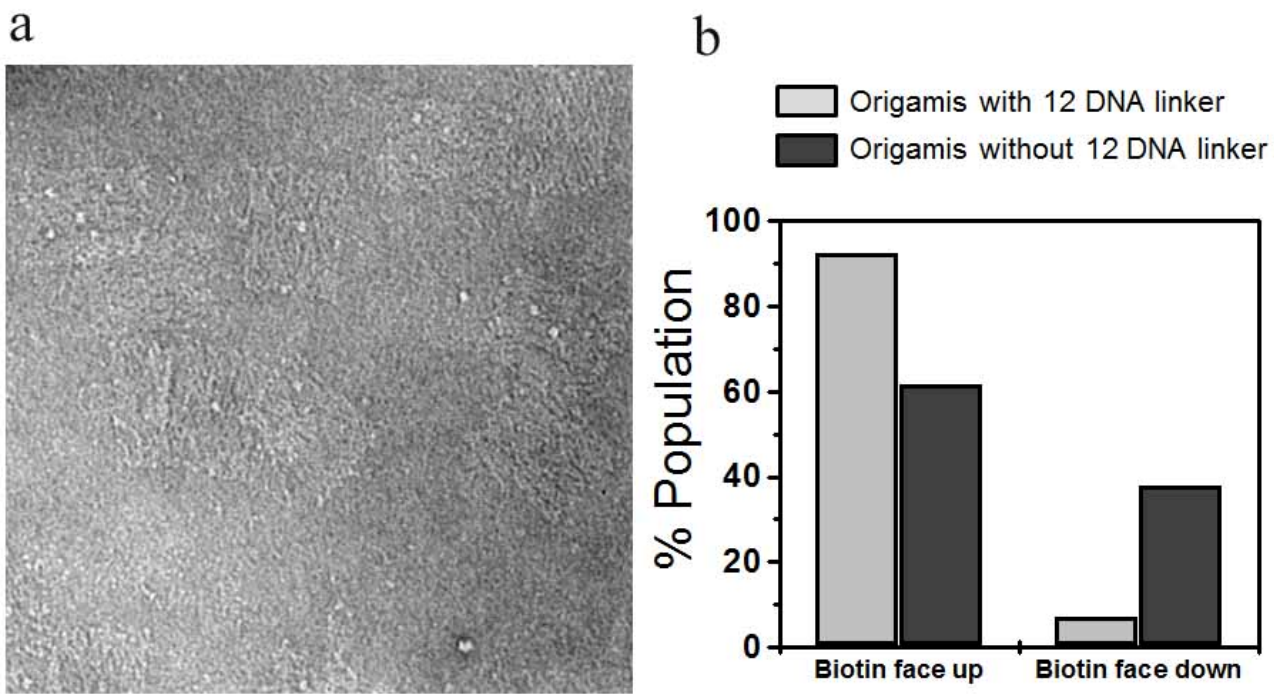


Figure S9. Determining the face up and face down population by streptavidin binding to DNA origami lacking 12 protruding DNA linkers. (a) A representative negative stain image. In this experimental setting, only the face up origami can display streptavidin. (b) Population distribution of face up and face down origami. A total of 304 and 307 origami particles were analyzed for the origami with and without DNA linkers, respectively. The data for the origami with 12 DNA linkers were obtained by analyzing the images as shown in Figure 3a.