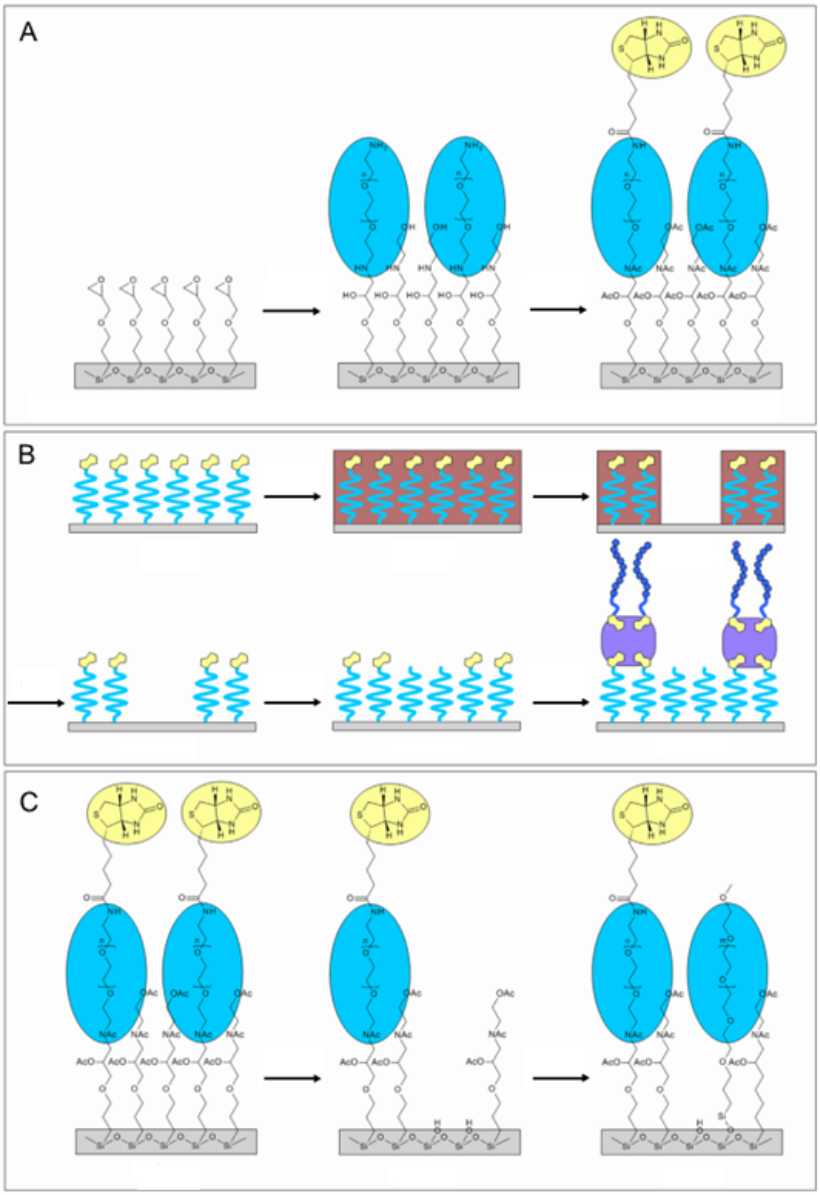

Figure S1. Scheme illustrating the preparation of micropatterned biotin-PEG/PEG films on glass substrates. (A) The preparation of PEG films carrying terminal biotin groups. (B) The micropatterning process and steps leading to micropatterned biotin-PEG films. (C) Surface chemistry before and after micropatterning. PEG is highlighted in light blue, biotin groups in yellow, photoresist in brown, streptavidin in purple, and DNA strands in dark blue.[1]

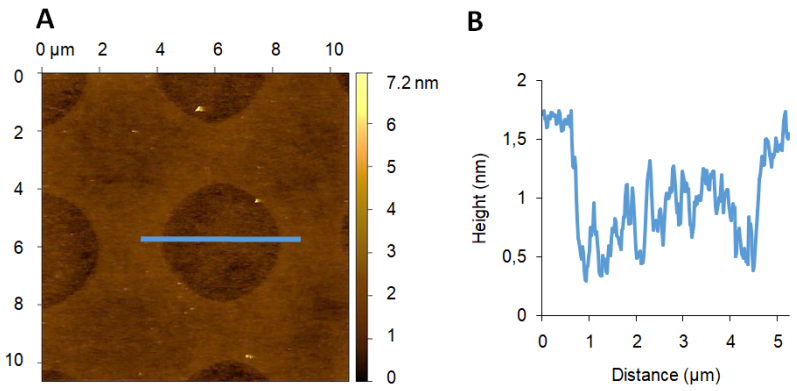

Figure S2. AFM characterization of biotin-PEG micropatterns (A) Contact-mode AFM image of PEG-biotin micropatterns on glass substrates after plasma treatment, and (B) corresponding height profile. Mean height of PEG-biotin film: 0.89 ± 0.33 nm, n = 40.

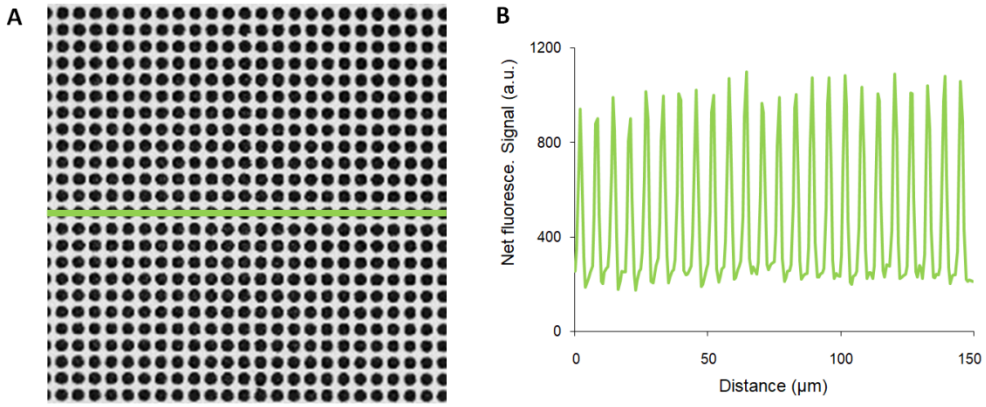

Figure S3. Fluorescence microscopic analysis of biotin-PEG micropatterns. (A) Fluorescence microscopy images of a PEG-biotin microgrid with PEG-covered round features. Biotin tags are decorated with Cy5-labeled streptavidin. (B) Corresponding fluorescence intensity line profile. Image size: 148 x 148 µm.

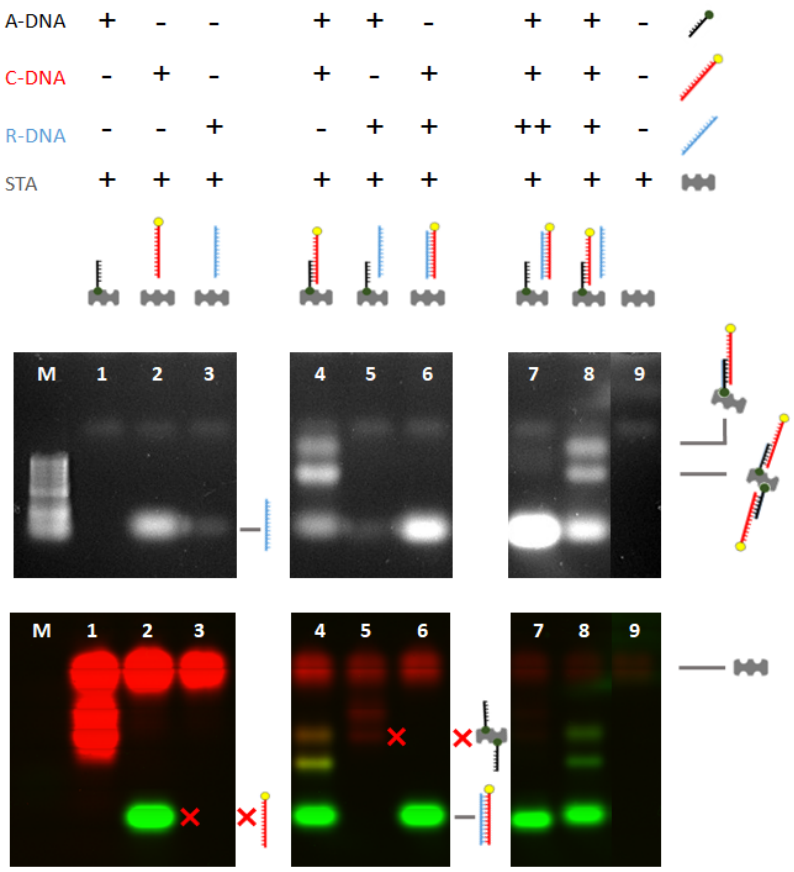

Figure S4. DNA strand displacement in solution visualized by agarose gel electrophoresis. The gels' UV images (top) display DNA, and an overlay of fluorescence scans in the Cy3 and Cy5 channel (bottom) shows Cy3-labeled C-DNA and Cy-5-labeled streptavidin, respectively. Lane M: GeneRuler DNA Ladder mix, 50 bp. Lane 1: streptavidin-Cy5 (11.2 pmol) + A-DNA (10 pmol). Lane 2: streptavidin-Cy5 (11.2 pmol) + Cy3-labeled C-DNA (10 pmol). Lane 3: streptavidin-Cy5 (11.2 pmol) + R-DNA (10 pmol). Lane 4: streptavidin-Cy5 (11.2 pmol) + A-DNA (10 pmol) + C-DNA-Cy3 (10 pmol). Lane 5: streptavidin-Cy5 (11.2 pmol) + A-DNA (10 pmol) + R-DNA (10 pmol). Lane 6: streptavidin-Cy5 (11.2 pmol) + C-DNA-Cy3 (10 pmol) + R-DNA (10 pmol). Lane 7: streptavidin-Cy5 (11.2 pmol) + A-DNA (10 pmol) + C-DNA-Cy3 (10 pmol) + R-DNA (100 pmol). Lane 8: streptavidin-Cy5 (11.2 pmol) + A-DNA (10 pmol) + C-DNA-Cy3 (10 pmol) + R-DNA (10 pmol). Lane 9: streptavidin-Cy5 (11.2 pmol). The decrease in red fluorescence intensity in the gel from left to right could be due to bleaching during the triple scanning the gel in the UV region and at the two wavelengths for the fluorescence read-out.

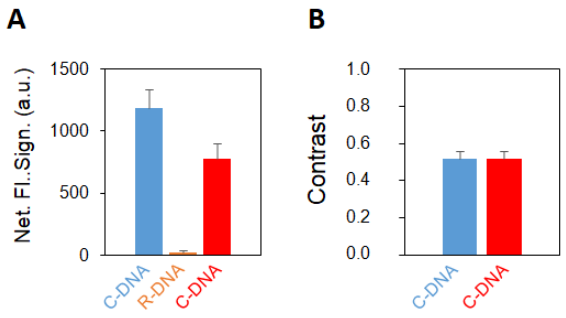

Figure S5. Results of the quantitative fluorescence microscopic analysis on the hybridization of DNA to and release from microstructured surfaces displaying A-DNA patterns. (A) Net maximum fluorescence intensity of microstructured surfaces after incubation of Cy3-labeled C-DNA, strand displacement with R-DNA, and re-hybridization of labeled C-DNA. (B) Contrast of micropatterns before and after strand displacement contrast = $(F_{max} - F_{min}) / (F_{max} - BG)$ whereby F_{max} and F_{min} are fluorescence counts in the bright Cy3-C-DNA areas and in the dim non-DNA areas of the pattern, respectively. BG refers to background which is the glass substrate that had not been exposed to Cy3-C-DNA. The averages and standard deviations were derived from three independent experiments with each three data sets.

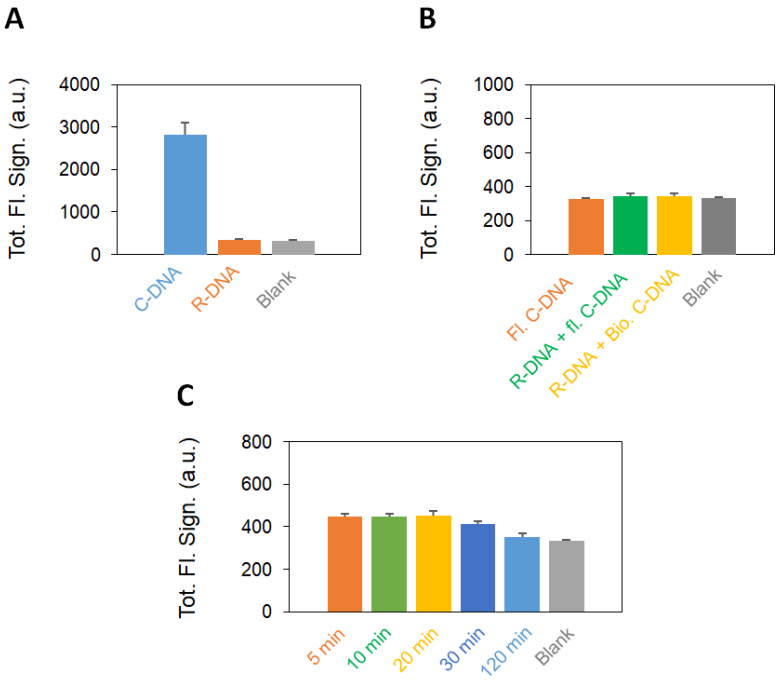

Figure S6. Optimization of release conditions, and control experiments. (A) Fluorescence intensity maxima from microscopic analysis of micropatterned streptavidin surfaces after incubation with biotinylated A-DNA and fluorescence labeled C-DNA incubated (1 h incubation), and after toehold-mediated displacement with R-DNA (2 h)(n = 4). Blank surfaces were not incubated with fluorophore-tagged C-DNA. (B) Fluorescence signals from control experiments with various DNA strands that were not supposed to bind to the micropatterned streptavidin surfaces. (C) Fluorescence intensity after toehold-mediated strand displacement with R-DNA incubated for various durations.

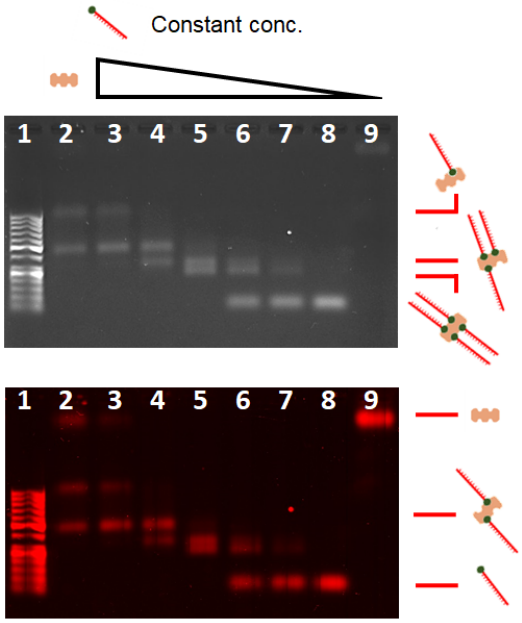

Figure S7. Gel electrophoretic characterization of streptavidin-DNA conjugates. UV image (top) and fluorescence scan (bottom) of the gel. Lane 1: GeneRuler DNA Ladder mix, 50 bp. Lane 2: Atto550-streptavidin (18.8 pmol) + biotinylated C-DNA (10 pmol), ratio ~ 2:1. Lane 3: Atto550-streptavidin (9.4 pmol + biotinylated C-DNA (10 pmol), ratio ~ 1:1. Lane 4: Atto550-streptavidin (4.7 pmol) + biotinylated C-DNA (10 pmol), ratio ~ 1:2. Lane 5: Atto550-streptavidin (2.3 pmol) + biotinylated C-DNA (10 pmol), ratio ~ 1:4. Lane 6: Atto550-streptavidin (0.9 pmol) + biotinylated C-DNA (10 pmol), ratio ~ 1:10. Lane 7: Atto550-streptavidin (0.5 pmol) + biotinylated C-DNA (10 pmol), ratio ~ 1:20. Lane 8: biotinylated C-DNA (10 pmol). Lane 9: Atto550-streptavidin (18.8 pmol).

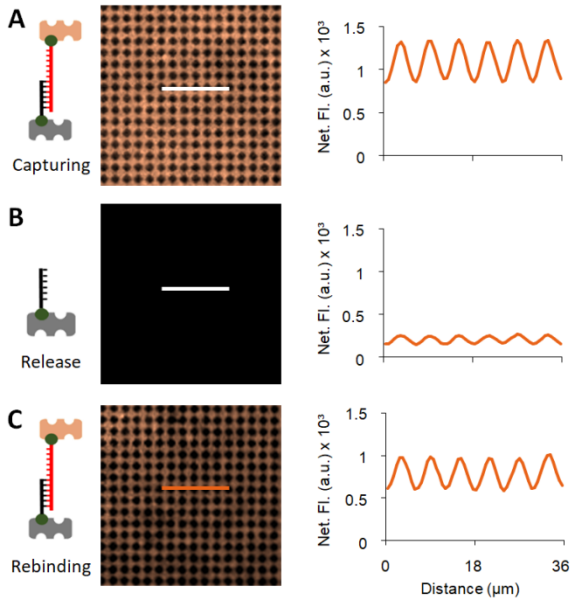

Figure S8. DNA-triggered release of streptavidin-DNA conjugates from microstructured A-DNA surfaces. Schematic overview (left), fluorescence microscopic images of microstructured surfaces (middle) and corresponding line profiles of fluorescence microscopic images (right). (A) After incubation with C-DNA/streptavidin conjugates (molar ratio 1:10; streptavidin labeled with Atto550), (B) after toehold-mediated strand displacement of conjugate with R-DNA, and (C) after re-hybridization of the conjugate. Image size: 96 x 96 µm.

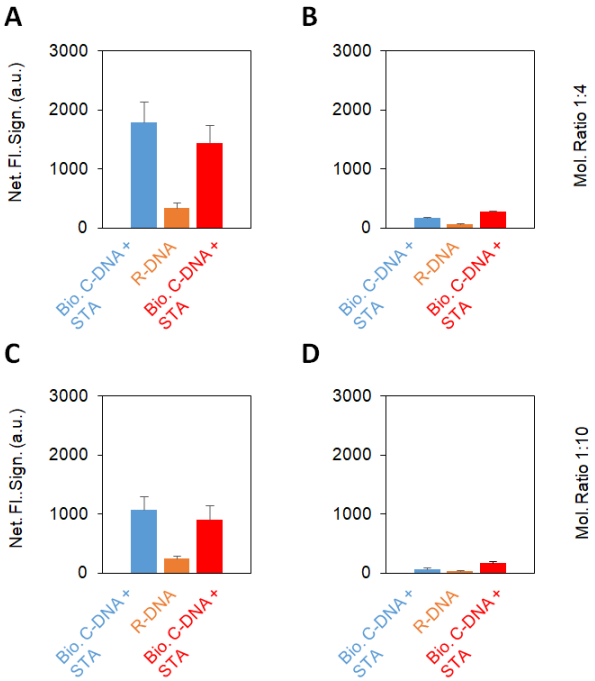

Figure S9. Quantitative analysis on the release from microstructured A-DNA surfaces of conjugates with different molar ratios between biotinylated C-DNA and fluorescence-labeled streptavidin. (A) Net fluorescence intensity of maxima of microstructured surfaces after incubation with conjugates of streptavidin and biotinylated C-DNA conjugates with a molar ratio of 1:4, after strand displacement with R-DNA, and after re-hybridization of the conjugate. (B) Analogous to A but incubated on surfaces that did not display A-DNA and were not supposed to bind the conjugate. (C) and (D) are analogous to A and B but with conjugates of a molar ratio of 1:10. The averages and standard deviations are from four independent experiment with each three data sets.

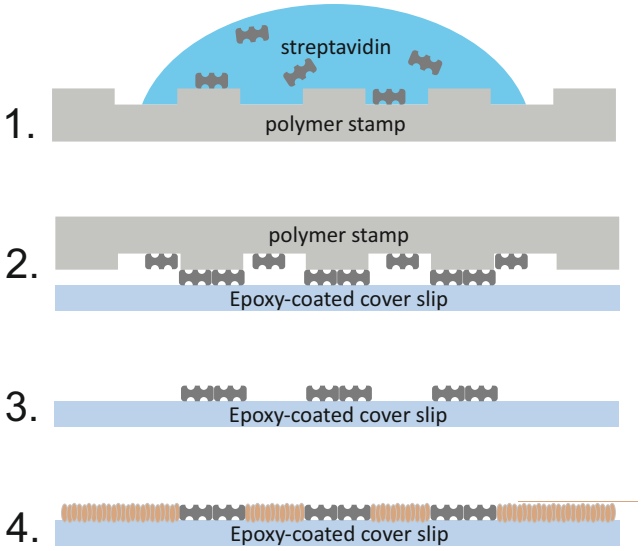

Figure S10. Preparation of streptavidin patterns by microcontact printing. After (1) incubation with streptavidin, (2) polymer stamps were dried under a nitrogen stream and pressed onto epoxy-coated glass cover slips. (3) Removal of the stamp was followed (4) by passivating non-streptavidin-coated surfaces by incubation with BSA.

References

(1) Lipp, A. M.; Ji, B.; Hager, R.; Haas, S.; Schweiggl, S.; Sonnleitner, A.; Haselgrubler, T. Micro-Structured Peptide Surfaces for the Detection of High-Affinity Peptide-Receptor Interactions in Living Cells. *Biosens. Bioelectron.* **2015,** *74*, 757-763.