Electronic Supplementary Information

for

A versatile fluorometric in-situ hybridization method for the quantitation of hairpin conformations in DNA self-assembled monolayers

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Additional experimental data including supplementry DNA sequences, hpDNA stem optimzation, surface activation of PC, gold, and glass; the principle and application of Exo I-assisted electrochemical protocol; the influence of substrate type, DNA surface density, and DNA spacer length on the yield of hairpin conformations in mixed DNA SAMs, the FL intensity variation of ssDNA SAMs before and after hybridization, and the assembly behavior of hpDNA on different substrates.

Table S1 Supplementary oligonucleotide sequences used in this work.

DNA strand	Sequence
hpDNA-8-NH	5’-NH_2–$(CH_2)_6$-GCG AGA TAA AAC GAC GGC CAT ATC TCG C-spacer 9-biotin-3’
ssDNA-28-NH	5’-NH_2–$(CH_2)_6$-GCG AGA GAA CCA GAC GGC CCC TTG TAC G-spacer 9-biotin-3’
Target-8	5’- GCG AGA TAT GGC CGT CGT TTT ATC TCG C 3’
hpDNA-C12-NH	5’-NH_2–$(CH_2)_{12}$-GCG AGG TAA AAC GAC GGC CAG TCC TCG C-spacer 9-biotin-3’
hpDNA-C12-NH -NSP	5’-NH_2–$(CH_2)_{12}$-GCG AGG TAA AAC GAC GGC CAG TCC TCG C-biotin-3’

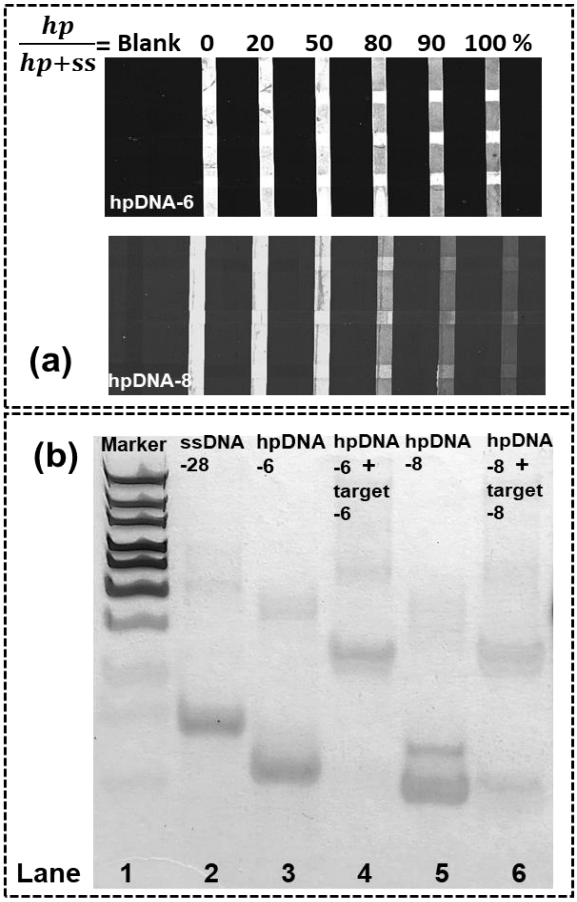

Fig. S1 (a) Fluorescence images of a DNA SAM array prepared with ssDNA-28/hpDNA-6 (top) and ssDNA-28/hpDNA-8 (bottom) on PC after hybridization with targe-6 along the perpendicular direction. The prescence of weak fluorescence signals upon hybridization, indicates that hpDNA-8 probes in the mixed SAMs are not fully opened. (b) Native PAGE to show the hybridization of hpDNA-6/hpDNA-8 with its target DNA at 37 °C. The gel was stained with silver nitrate for visualization. Lane 1: DNA ladder (every 10-mers); Lane 2: 3.5 μM ssDNA-28; Lane 3: 3.5 μM hpDNA-6; Lane 4: 3.5 μM hpDNA-6 + 3.5 μM target-6; Lane 5: 3.5 μM hpDNA-8; Lane 6: 3.5 μM hpDNA-8 + 3.5 μM target-8. The results shown in lane 4 and lane 6 indicate that hpDNA-6 can hybridize fully with its target strands but hpDNA-8 can only partly hybridize with its target strands.

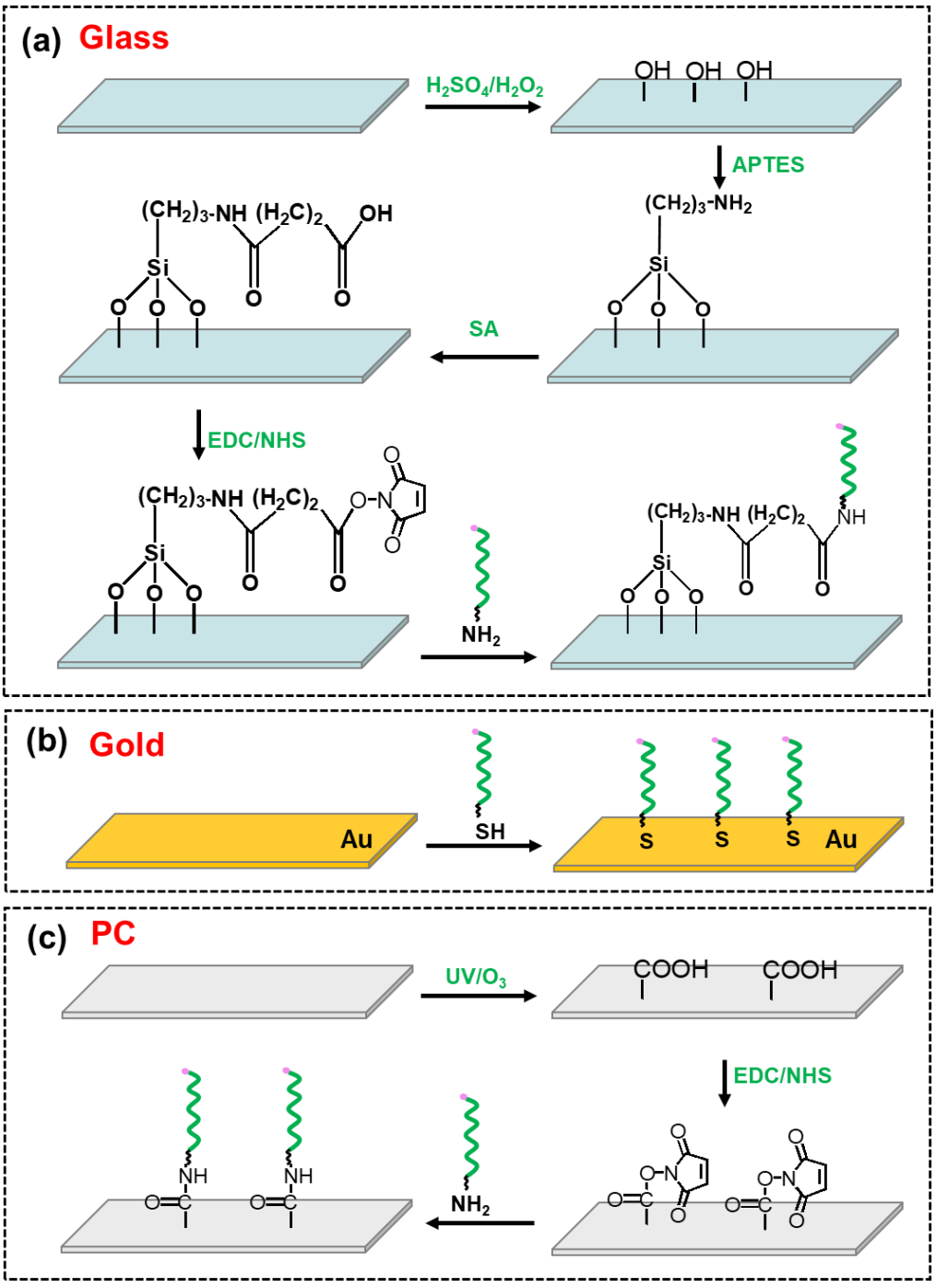

Fig. S2 Schematic view of the surface activation and DNA immobilization reactions for glass (a), gold (b), and PC (c) substrate.

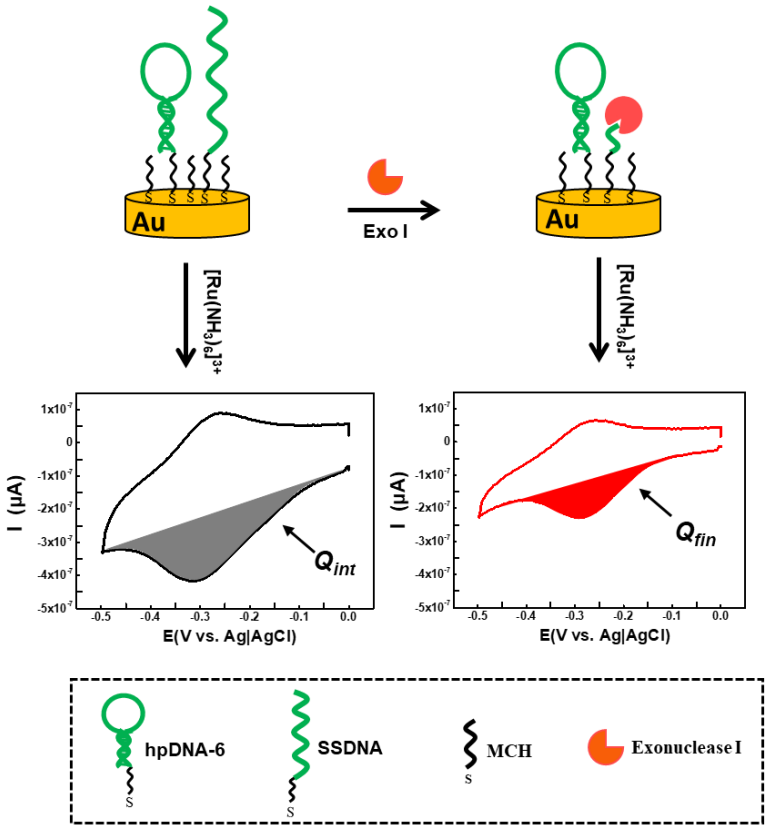

Fig. S3 Exo I-assisted electrochemical protocol (reported in Ref. 22) for quantifying surface-tethered hpDNA on gold via comparision of the integrated charges of their cathodic CV peaks measured before (Q_{int}) and after Exo I hydrolysis (Q_{fin}). The bottom plots show the CV responses of 5.0 μ M $[Ru(NH_3)_6]^{3+}$ on gold electrodes modified with hpDNA/ssDNA SAMs in 10 mM Tris buffer before (left) and after (right) Exo I treatment.

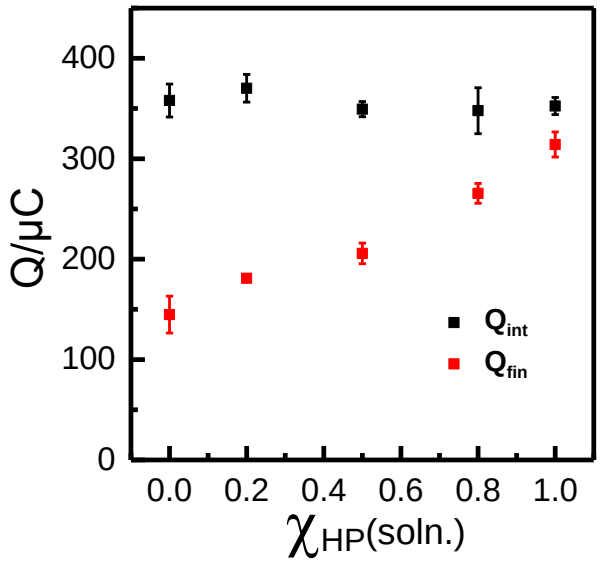

Fig. S4 Integrated charges of the cathodic CV peaks of gold electrodes modified with DNA SAMs of different hpDNA/ssDNA ratios measured with 5.0 μM $[Ru(NH_3)_6]^{3+}$ in 10 mM Tris buffer before (Q_{int}) and after (Q_{fin}) Exo I treatment .

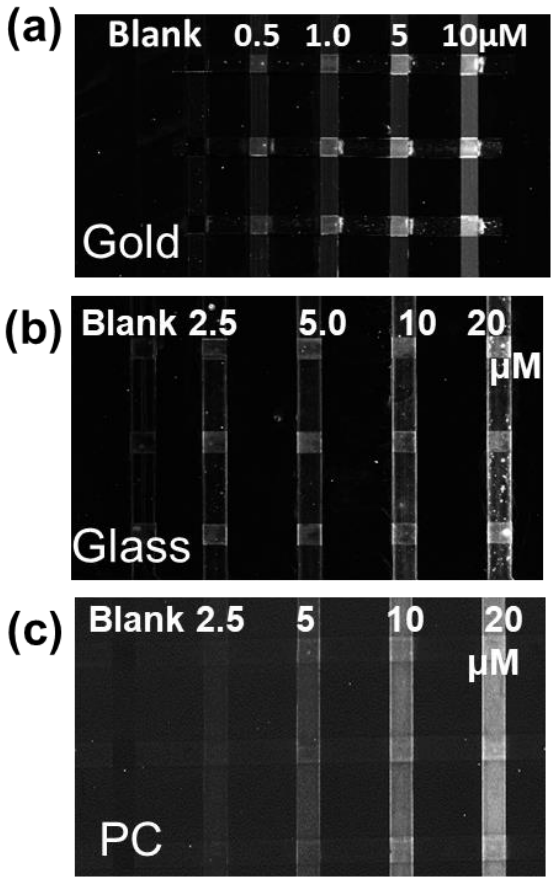

Fig. S5 Fluorescence images of DNA SAM arrays created on gold (a), glass (b), and PC (c) substrate with using different concentrations of hpDNA-6 for probe immobilization and subseqeuent on-chip hybridization with target DNA along the perpendicular direction. The quantatiative analysis of these images allowed us to investigate the influence of substrate type and DNA surface density on the yield of surface-tethered hairpin conformations (as shown in Fig. 5 in the main text and discussed therein).

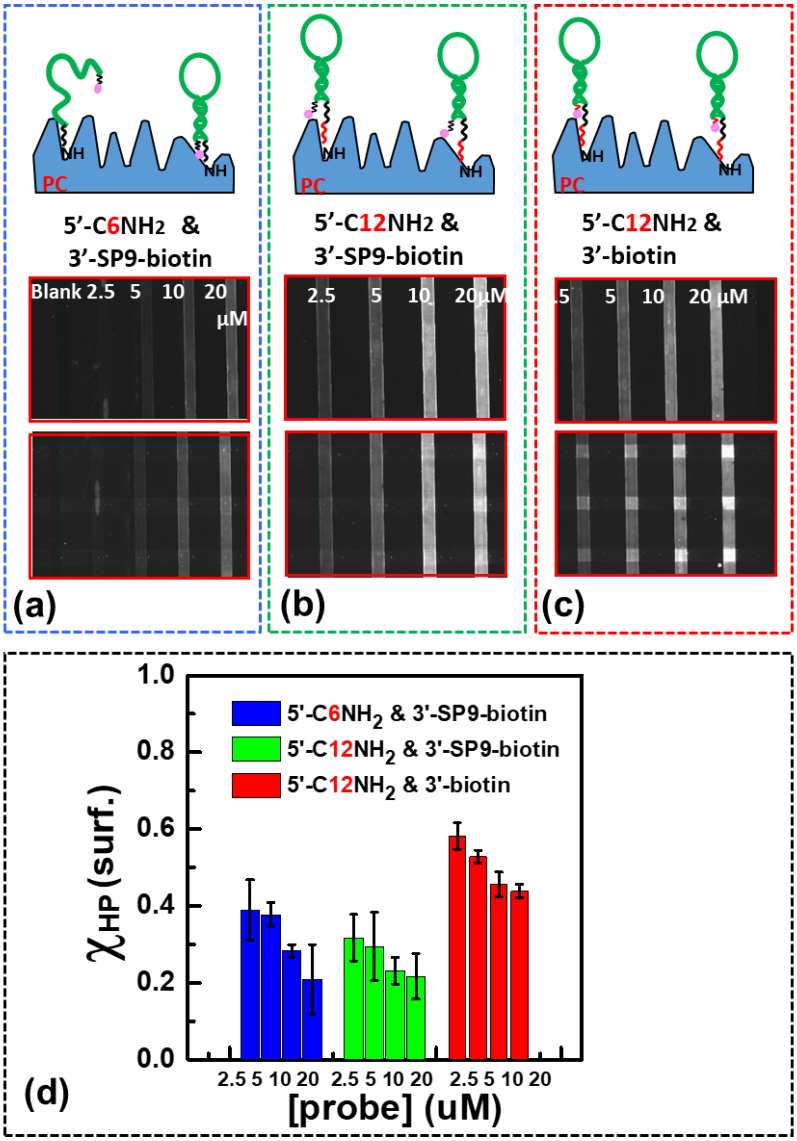

Fig. S6 Influence of the spacer length on the yield of hairpin conformations formed on PC substrate: (a-c) Fluorescence images of a DNA SAM array prepared from hpDNA-6 with a spacer of C6 at 5'- end and SP9 at 3'- end (a) , a spacer of C12 at 5'- end and SP9 at 3'- end (b) and a spacer of C12 at 5'- end but without SP at 3'- end (c); (d) molar fraction of surface-tethered hairpin conformations as function of the concentration of hpDNA-6 with different "spacers" in the deposition solutions. The top insets schemetically show the advantage of using suitable anchoring and biotin linkers to immobilize hpDNA strands on PC.

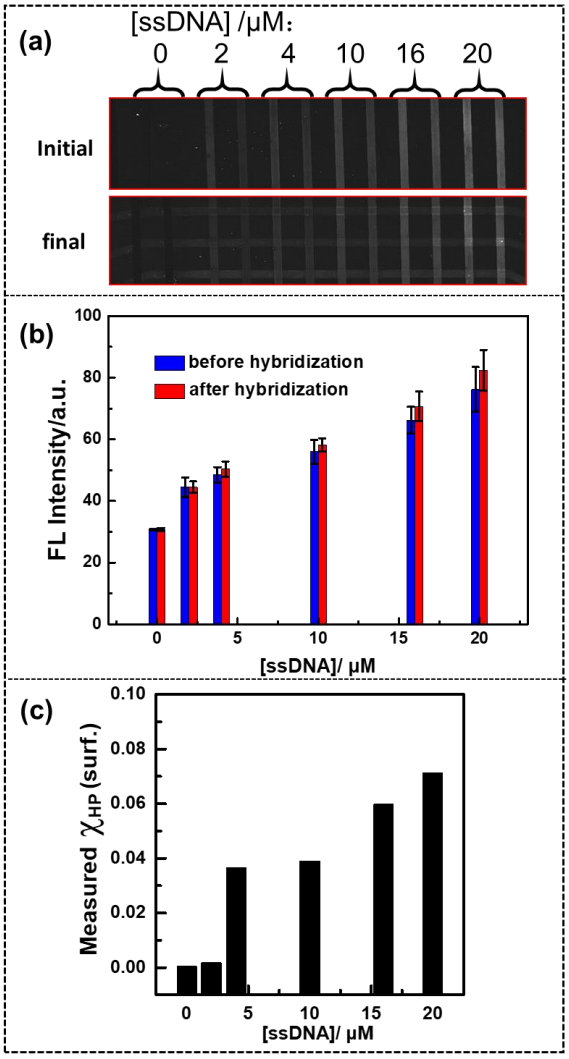

Fig. S7 Fluorescence images (a) and fluorescence intensities (b) of a pure ssDNA SAM array of different surface densities created on a PC slide before and after hybridization. (c) The measured χ_{HP} (surf.) values of this ssDNA SAM array based on the fluorometric in-situ hybridization protocol.

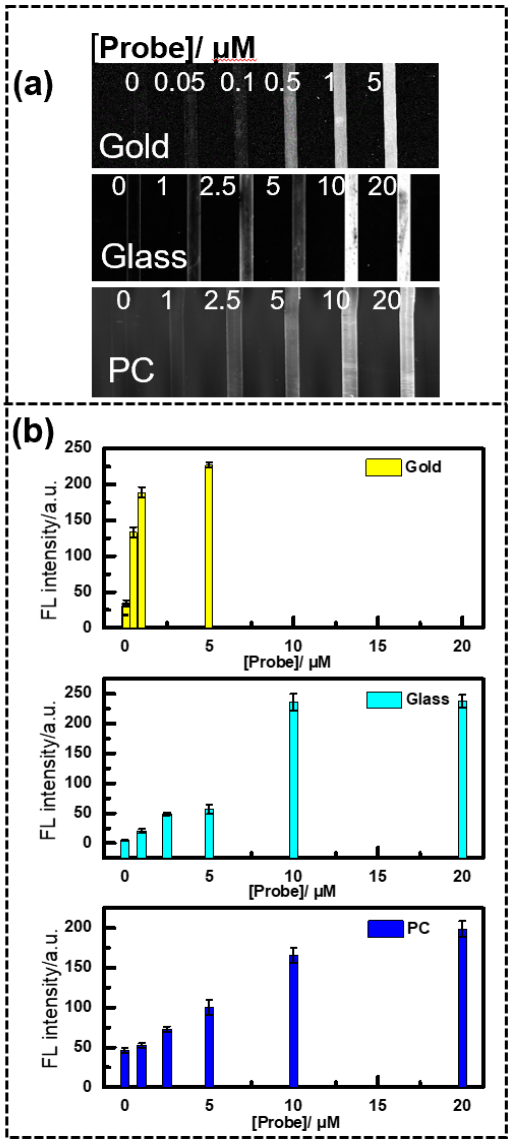

Fig. S8 Investigation of the assembly behavior of hpDNA on different substrates: (a) fluorescent images and (b) corresponding FL intensities of hpDNA SAMs formed thereon.