Supporting Information

One-Step Surface Modification to Graft DNA Codes on Paper: The Method, Mechanism and Its Application

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Supplementary Experimental Details

All oligonucleotides were synthesized and modified by Integrated DNA Technologies (Coralville, IA). The ssDNA sequences are listed in Table S1.

Table S1 All DNA strands used and their sequences

DNA strands	**Sequence**
Cy3-labeled single-strand DNA (ssDNA)	5'-Cy3-CTC GGG ATC AAG ACG ATG AT-3'
Capture DNA probe	5'-CGT CGA TCA GGC ACG GGC CCT CGT ACC AGG GCA TC-3'
Giardia lamblia target DNA	5'-GAT GCC CTG GTA CGA GGG CCC GTG CCT GAT CGA CG–Cy3-3'
Neisseria Meningitis (N. Meningitis) DNA	5'-AAC CTT GAG CAA TCC ATT TAT CCT GAC GTT CT-Cy3-3'

Fabrication of the Patterned Paper Device. Whatman No. 1 chromatography paper was used via a modified protocol using the photolithography method.[1, 2] Briefly, the photoresist (SU-8) was added to chromatography paper and spread evenly on the paper surface. The paper was baked on a hot plate (Thermo Scientific, Rockford, IL) at 130 °C for 20 min (10 min for each side). The paper was then placed below a pre-designed photomask and exposed to a UV exposure system (Intellirary 600 UV curing system, Uvitron international Inc., West Springfield, MA) for 20 s, to polymerize the photoresist. The unexposed portions remained unpolymerized and were washed away with acetone and isopropyl alcohol. The patterned paper was obtained after drying with the diameter of each detection zone of 2.0 mm. The oxygen plasma treatment (for 4 min) was used in this work to treat the patterned paper because of the following reasons. First, plasma treatment was used for removal of residues or contamination on the paper surface, for instance, through oxidization and evaporation. Besides, by treating with plasma, both hydrophilicity and surface roughness of the patterned paper were

increased, which were beneficial for the capillary penetration and interaction between paper surface and chemicals (i.e., APTMS) in aqueous solutions.[3-5]

Grafting DNA Codes on the TA-Modified Paper via Covalent Cross-linking Reactions (Conventional Methods). In this research, we compared two different DNA immobilization methods: ionic adsorption and covalent bonding. The newly proposed one-step surface modification method using APTMS was used to immobilize DNA via ionic adsorption, while a conventional method using APTMS, EDC, Sulfo-NHS, and TA (terephthalic acid) was selected to immobilize DNA through covalent bonding. As shown in Scheme S2, the primary amine groups ($-NH_2$) were first introduced after modifying the substrate with APTMS. Prior to the carboxyl-to-amine reaction between APTMS and TA, the carbodiimide, EDC, was used to active carboxyl groups on TA by forming an active ester, *O*-acylisourea intermediate. This intermediate was easily replaced by nucleophilic attack from primary amine groups on the substrate, forming an amide bond between the amine groups on APTMS and the original carboxyl groups on TA. Additionally, because O-acylisourea intermediate was unstable in aqueous solution and also easily hydrolyzed, the Sulfo-NHS was also used in this coupling reaction by producing a stable amine-reactive intermediate, Sulfo-NHS ester, therefore improving conjugation efficiency. Hence, by using EDC and Sulfo-NHS as carboxyl-to-amine crosslinkers, the substrate was modified with carboxyl groups from TA, which was then used to immobilize amine groups labeled DNA molecules in a similar manner.

Basically, 2 μL of 5% TA in a 1:1 mixture solution of EDC (0.4 M) and Sulfo-NHS (0.1 M) was added on each detection zone of the APTMS-modified paper, and followed by the incubation for 1 hour at room temperature. The TA-modified paper was then rinsed three times with deionized water and dried under ambient temperature. 2 μL of 1 μM Cy3-labeled ssDNA was added onto each detection zone on the TA-modified paper and followed by incubation for 3 hours at 37 °C. The cellulose paper was then rinsed with a washing buffer and dried under ambient temperature. Fluorescence

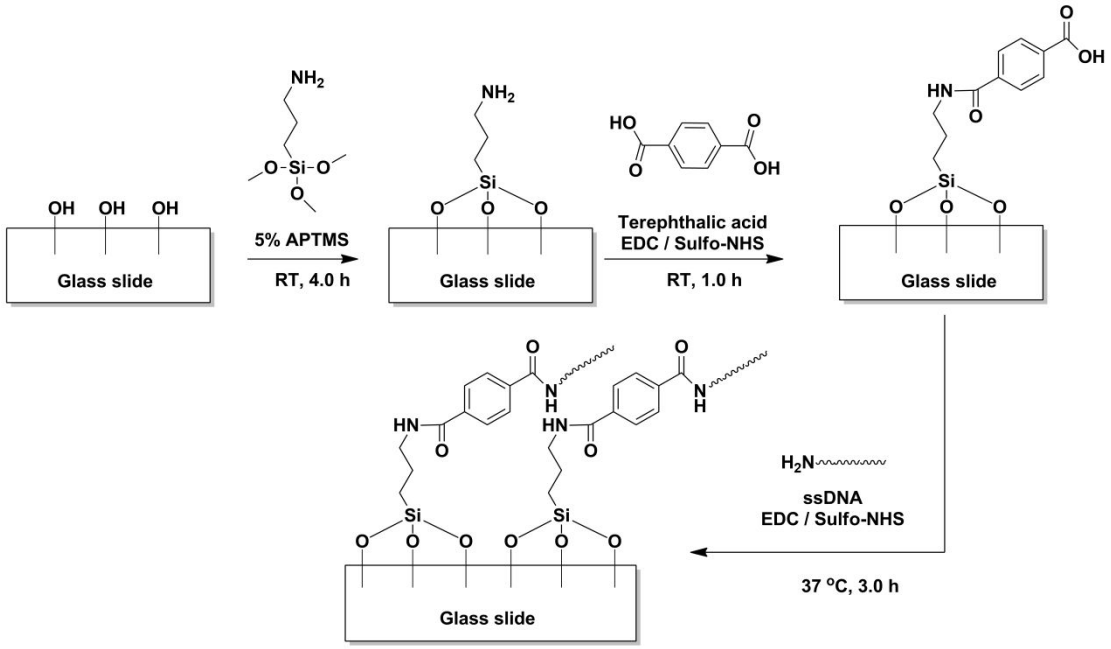
images were captured after each washing step by fluorescence microscopy with the exposure time for 9 ms, and the fluorescence intensities were analyzed by Nikon Element.

Glass Surface Modification with TA. The conventional modification and functionalization of glass slides via covalent cross-linking reactions were conducted using a previously reported procedure (Scheme S1).[6] Briefly, glass slides were cleaned with 10 % NaOH solution for 10 min in a water bath at ~80 °C and rinsed with deionized water. A 50-mL piranha solution (70:30, v/v. sulfuric acid to 30% hydrogen peroxide, highly toxic and corrosive, handling with EXTREME CAUTION) was freshly prepared to treat the above glass slides for 1 hour in a water bath at ~80 °C. The glass slides were then rinsed with deionized water and dried at ambient temperature. The cleaned glass slides were assembled with a drilled PDMS plate (25 mm x 75 mm x 2 mm) to form detection reservoirs in the diameter of 2.0 mm, according to our previous protocol.[7] The glass reservoirs were treated with 5% APTMS in 95% ethanol for 2 hours at room temperature and then rinsed three times with 95% ethanol and deionized water, respectively, to remove excess reagents. After being dried under ambient temperature, then 2 μL of 5% TA in a 1:1 mixture solution of EDC (0.4 M) and Sulfo-NHS (0.1 M) per zone was added on the aminated glass slides, and followed by the incubation for 1 hour at room temperature. The TA-modified paper was then rinsed three times with deionized water, dried under ambient temperature, and stored in dark at 4 °C prior to the probe immobilization.

Grafting DNA Codes on TA-modified Glass Slides via Covalent Cross-linking Reactions (Conventional Methods). 2 μL of 1 μM Cy3-labeled ssDNA was added onto each detection zone on the TA-modified glass slides and followed by incubation for 3 hours at 37 °C. The glass slides were then rinsed with a washing buffer and dried under ambient temperature. Fluorescence images were captured after each washing step by the fluorescence microscopy with the exposure time for 9 ms, and the fluorescence intensities were then analyzed by Nikon Element software.

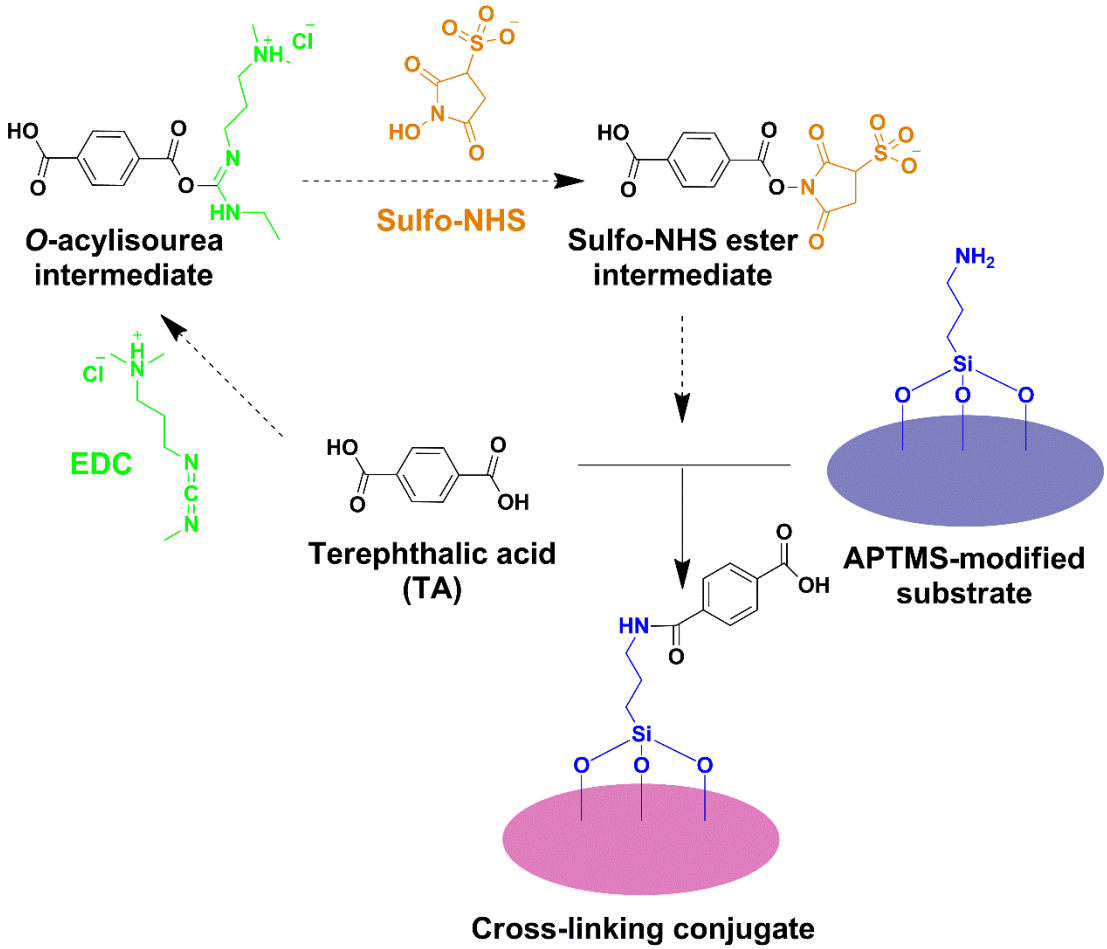
Supplementary Experimental Results

Scheme S1



Scheme S1. Conventional DNA immobilization on terephthalic acid (TA)-modified glass slides via cross-linking reactions.

Scheme S2



Scheme S2. Carboxyl-amine conjugation process between TA and the APTMS-modified substrate using EDC and Sulfo-NHS as cross-linking reagents.

Figure S1

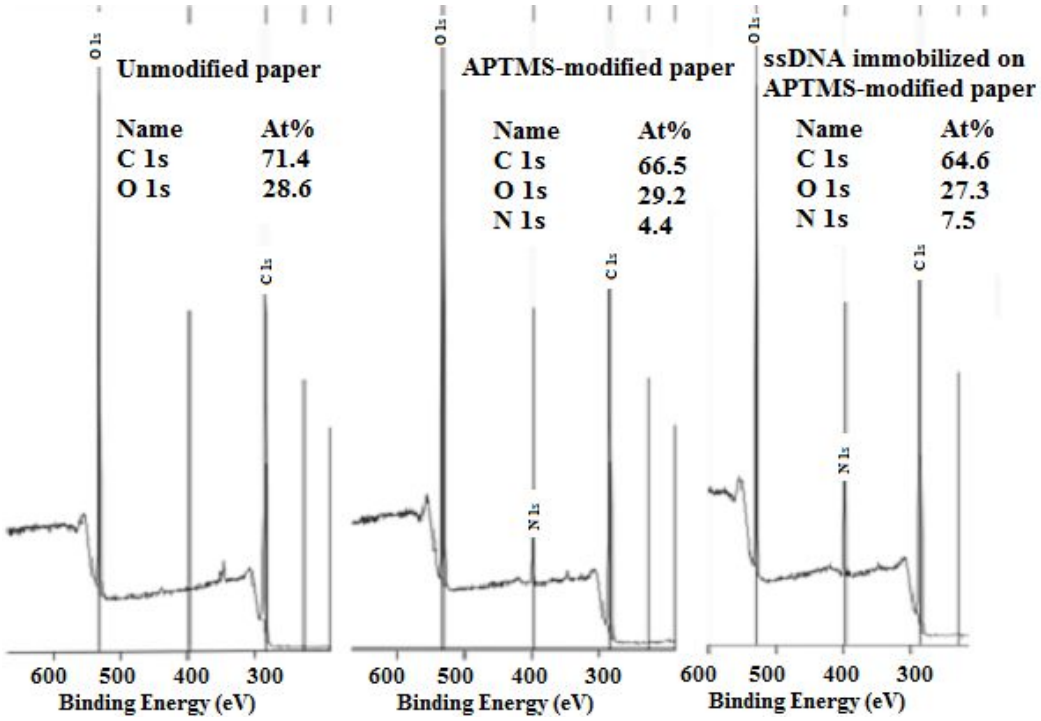


Figure S1. XPS survey scans of unmodified paper, APTMS-modified paper, and DNA-functionalized APTMS-modified paper, containing C 1*s*, O 1*s*, and N 1*s* signals.

Figure S2

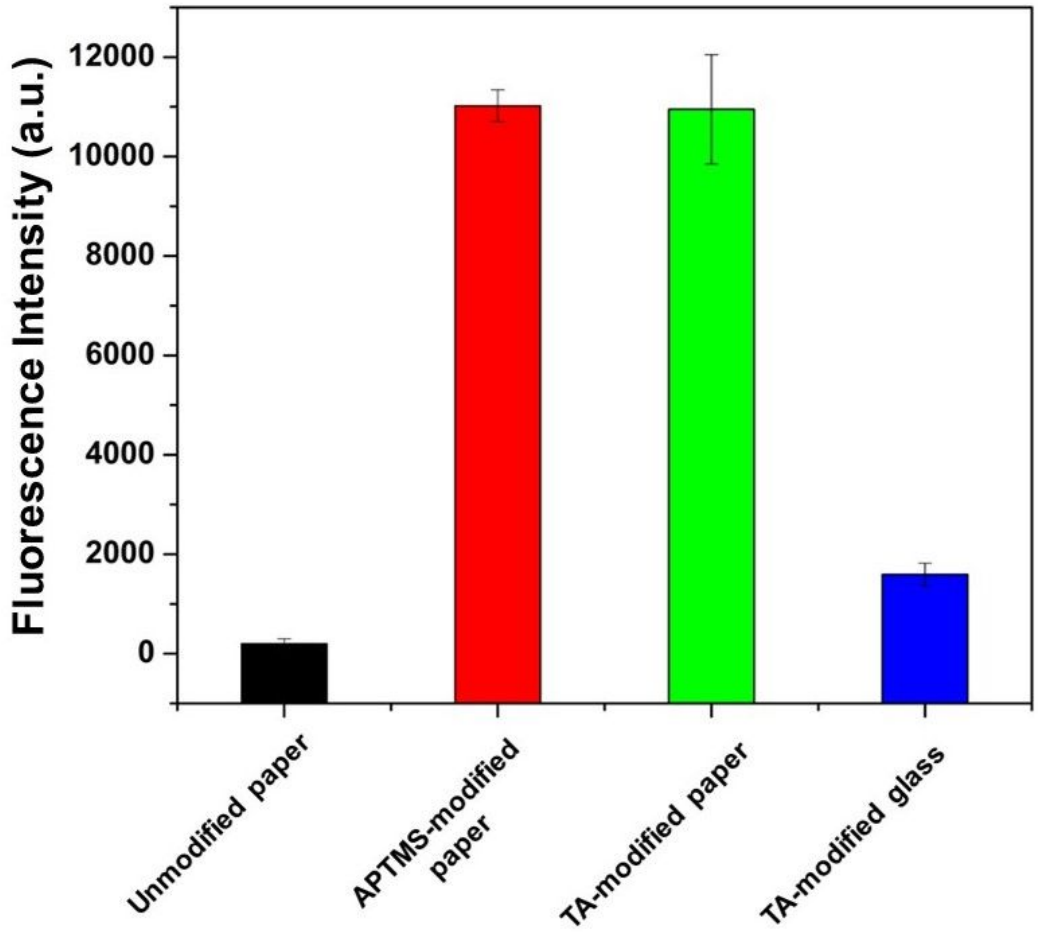


Figure S2. Fluorescence detection of Cy3-labeled ssDNA immobilized on different substrates after 4 times of washing: Unmodified paper (■), APTMS-modified paper (■), TA-modified paper (■), and TA-modified glass (■). Error bars represent standard deviations (n = 6).

References

1. Sanjay, S. T.; Zhou, W.; Dou, M. W.; Tavakoli, H.; Ma, L.; Xu, F.; Li, X. J., Recent advances of controlled drug delivery using microfluidic platforms. *Adv. Drug Deliv. Rev.* **2018,** *128*, 3-28.
2. Tavakoli, H.; Zhou, W.; Ma, L.; Perez, S.; Ibarra, A.; Xu, F.; Zhan, S. H.; Li, X. J., Recent advances in microfluidic platforms for single-cell analysis in cancer biology, diagnosis and therapy. *Trac-Trend in Anal. Chem.* **2019,** *117*, 13-26.
3. Li, X.; Tian, J.; Nguyen, T.; Shen, W., Paper-based microfluidic devices by plasma treatment. *Anal. Chem.* **2008,** *80* (23), 9131-4.
4. Zhao, M.; Li, H.; Liu, W.; Guo, Y.; Chu, W., Plasma treatment of paper for protein immobilization on paper-based chemiluminescence immunodevice. *Biosens. Bioelectron.* **2016,** *79*, 581-8.
5. Vesel, A.; Mozetic, M.; Hladnik, A.; Dolenc, J.; Zule, J.; Milosevic, S.; Krstulovic, N.; Klanjsek-Gunde, M.; Hauptmann, N., Modification of ink-jet paper by oxygen-plasma treatment. *J. Phys. D Appl. Phys.* **2007,** *40* (12), 3689-3696.
6. Park, S.; Pai, J.; Han, E. H.; Jun, C. H.; Shin, I., One-step, acid-mediated method for modification of glass surfaces with N-hydroxysuccinimide esters and its application to the construction of microarrays for studies of biomolecular interactions. *Bioconj. Chem.* **2010,** *21* (7), 1246-1253.
7. Dou, M. W.; Dominguez, D. C.; Li, X. J.; Sanchez, J.; Scott, G., A versatile PDMS/paper hybrid microfluidic platform for sensitive infectious disease diagnosis. *Anal. Chem.* **2014,** *86* (15), 7978-7986.