Supporting Information

# Analytical Devices Based on Direct Synthesis of DNA on Paper

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## EXPERIMENTAL PROCEDURES

### Materials

All the reagents needed for the synthesis of DNA in this study were purchased from Glen Research Co. Ltd, and used without further purification. Oligonucleotides cleaved from paper anchored DNA arrays were separated by reverse-phase high-pressure liquid chromatography (HPLC, Agilent 1200) using a C18 stationary phase (Eclipse-XDB C18, 5 μm, 9.4 × 200 mm) and an acetonitrile/100 mM aqueous triethylammonium acetate gradient. UV spectrometry was measured on a Beckman Coulter DU800 spectrometer. LC-MS experiments were performed on an Alliance 2695 (Waters) HPLC system using a UPLC BEH C18 column (1.7 μm, 2.1 × 50 mm) stationary phase and 6 mM aqueous triethylammonium bicarbonate/methanol mobile phase interfaced to a Q-Tof Micro massspectrometer (Waters).

Chromatography paper (Whatman #1 Chr) was purchased from GE Healthcare (NJ, USA). General Purpose Vinyl Tape (764 Black, 5.0 mil) was purchased from 3M (St. Paul, MN). Polystyrene microtiter plates (UltraCruz ELISA Plate, high binding, 96 well, Flat bottom) were purchased from Santa Cruz Biotech (Dallas, TX, USA). Rabbit IgG, Streptavidin-Cy5, bovine serum albumin (BSA) solution (10 % m/m in DPBS), human serum, and phosphate buffered saline (PBS) pH 7.6 (25 °C) were purchased from Sigma Aldrich (St Louis, MO, USA). Tablets of Tris Buffered Saline Buffer (TBS) were purchased from Utech Products Inc (Schenectady, NY). Goat anti-rabbit IgG antibody labeled with DyLight™ 549 (DL549 anti-rabbit IgG) was purchased from Jackson ImmunoResearch (West Grove, PA, USA). Mouse anti-human CRP (capture antibody, Part 842676), biotinylated mouse anti-human CRP (detection antibody, Part 842677),

recombinant human CRP (Part 842678) were purchased from R&D Systems (Minneapolis, MN).

Absorbance and fluorescence measurements were performed using a microtiter plate reader (model SpectraMax M2, Molecular Devices, Vienna, VA, USA). Fluorescence measurements were performed using a Typhoon FLA 9000 scanner (GE Healthcare, Wilmington, MA, USA).

The DNA sequences (from 5' to 3') used in this study are summarized in Table 1:

**Table 1**: DNA sequences used in this work

| Name | Sequence | Bases | Properties |
|---|---|---|---|
| S1 | CGATCCACTACAAGCTTGCCATCATGTCGATC | 32 | Synthesized on Paper |
| S2 | GTGAAGTGGCAGTATCTTTTTTTTTTTTTTTT | 35 | Synthesized on Paper |
| CS1 | GGCAAGCTTGTAGTGGATCG/3ThioMC3-D/ | 20 | Complementary to S1, thiolated, for conjugation with hCRP |
| CS2 | GATACTGCCACTTCAC/3**Thio**MC3-D/ | 16 | Complementary to S2, thiolated, for conjugation with rabbit IgG |
| F | GGCAAGCTTGTAGTGGATCG/3**Cy5**Sp/ | 20 | Complementary to S1, 3' Cy5 modification |
| Q | /5**IAbRQ**/CGATCCACTACA | 12 | Complementary to F, 5' Iowa Black Quencher modification |

All the purified oligonucleotides (F, Q, CS1, CS2) used in this work were purchased from Integrated DNA Technologies, Inc. (Coralville, IA).

We conjugated proteins (rabbit IgG and mouse anti-human CRP) with thiolated DNA oligomers (IDT DNA, Inc) using sulfosuccinimidyl 4-[*p*-maleimidophenyl]butyrate

(Sulfo-SMPB) from Pierce Biotechnology (Rockford, IL, USA). All chemicals and reagents were used as received without further purification.

**Direct DNA synthesis on paper**

A commercial filter paper (Whatman Chromatography paper No. 1) was cut into ~2 cm × 2 cm squares for convenient handling, scrolled, and inserted into DNA synthesis columns, and subjected to DNA synthesis using a standard 1 μmole DNA synthesis protocol on a PerSeptive Biosystems Expedite DNA synthesizer (model 8909). After synthesis, the paper was removed from the synthesis column and the DNA was deprotected with AMA (1:1 v/v of 30% aqueous ammonium hydroxide: 40% aqueous methylamine) at 65 °C. The paper was rinsed with methanol and water, and then dried. **Figures 1** shows the process used for DNA synthesis on paper.

**Post-synthesis Characterization of Paper-anchored DNA Arrays**

The deprotection of DMT before each coupling step provide a means to assess the success of each coupling step in generating the full-length oligonucleotide on paper support. DNA was synthesized on paper in DMT-on mode (leaving the last DMT protective group on the 5' terminus of the oligonucleotide) and was treated with a 100 μL of 3 % trichloroacetic acid in dichloromethane for 10 minutes, and the UV absorbance (at 495 nm), $A_{495}$, of the resulting solution was measured.

**Yield of Oligonucleotides in Paper-anchored DNA Synthesis**

The target sequence for the DNA synthesized on paper in this assay was 5'-DMT-CGATCCACTACAAGCTTTTS-STTTTTTTTTTTTT-3'; after cleavage with dithiothreitol (DTT), the full-length product forms an oligonucleotide with sequence 5'-DMT-CGATCCACTACAAGCTTTT-SH -3'. The full length and truncation products generated after cleavage oligonucleotides that can be analyzed in the eluent by HPLC and LC-MS.

We used HPLC to determine the yield of the synthesis process, calculated based on the integration of the analytical HPLC signals that correspond to oligonucleotides. The HPLC yield was defined as the ratio of the integration of the peak corresponding to the full-length product (confirmed by LC-MS), divided by the sum of all peak integrations combined.

**Fabrication of simple paper-based devices**

Fenton et al. pioneered the fabrication of microfluidic devices using paper shaped using a knife plotter and encased in tape to serve as backing.[1] Here, simple paper-based devices were fabricated using a similar strategy from strips of paper with DNA-anchored arrays using tape (General Purpose Vinyl Tape, 764 Black, 5.0 mil, from 3M (St. Paul, MN)). as a support (**Figure S2**). We cut paper into disks with a 3-mm diameter using a biopsy punch. We trimmed tape into ~1 cm × 1 cm squares and bored a 2-mm diameter hole at the center using a biopsy punch. The paper was sandwiched between the two layers of tape such that the disk-shaped paper was placed over the holes.

**Formation of dsDNA arrays**

Oligomers F and Q (sequences in Table 1) were purchased from IDT DNA Inc and used as received, without further purification. We prepared stock solutions (100 μM) by dissolving the oligomers in water.

A working solution containing 50 nM of F and 450 nM of Q was prepared in TBS buffer (25mM Tris, 140mM NaCl, and 3 mM KCl, pH 7.6) from AMESCO. The hybridization product FQ was prepared by heating the mixture to 37 °C in a water bath and allowing it to cool to room temperature. The working solution was further diluted in TBS to a series of concentrations of FQ between 25 nM and 0.5 nM.

Microzones in a paper-based device were prepared by cutting strips of paper with anchored DNA arrays into disks with a 3-mm diameter, using a biopsy punch. The device was assembled as described in the previous section (see Figure S2). Blocking was not necessary because the hybridization product FQ does not fluoresce. A solution of FQ (10 μL) was added to each microzone of a paper-based device, and was allowed to incubate at 37 °C for 30 min in a humidity-controlled chamber. The device was allowed to cool to room temperature after this step. The fluorescence produced after the DNA anchored on paper displaces Q to hybridize to F was recorded using a fluorescence scanner.

**Formation of antibody-oligonucleotide conjugates**

Antibody-ssDNA conjugates were synthesized by chemically linking thiol-terminated ssDNA (purchased from IDT DNA, Inc) with a protein (rabbit IgG or mouse anti hCRP2). A water-soluble crosslinker, sulfosuccinimidyl 4-[*p*-maleimidophenyl]butyrate

(Sulfo-SMPB), containing an NHS-ester and a maleimide reactive group connected by a spacer, was added in 20-fold molar excess (1 mM in PBS, pH 7.6) to an equal volume of a 50–μM solution of the protein (in PBS, pH 7.6). The reaction mixture was then incubated for an hour with mixing at 1000 rpm at room temperature, using a vortex mixer (Benchmark Scientific, Inc), according to the manufacturer's instructions. The derivatized protein were desalted by Nap-10 size exclusion columns (GE Healthcare) and diluted in PBS, pH 7.6, to 500 μl. Thiolated ssDNA (25 μM) was added at a 1:1 molar ratio and the mixture was incubated for an hour with mixing at 1000 rpm at room temperature. Unreacted oligomers were removed by ultrafiltration with a 100000 MW cut-off membrane (EMT Millipore).

The volume of the solution containing the protein-ssDNA conjugate was adjusted to 100 μL (10mg/mL concentration, measured using a Thermo Scientific NanoDrop Spectrophotometer based on absorption at 280 nm[2]) and stored in 5-μL aliquots at -20°C. A small portion of the antibody-ssDNA conjugate was subjected to denaturing PAGE. A shift in the position of the bands corresponding to IgG heavy and light chains to higher molecular weights confirmed the conjugation (Figure S1).

**Formation of DNA-directed protein arrays**

The strip of paper-anchored ssDNA was cut into disks with a 3-mm diameter using a biopsy punch. The surface of the disks was blocked with 50 μL of a 1% wt/vol solution of BSA in PBS, pH 7.6, for 30 min. The disks were washed with PBS, and then incubated with 50 μL of a solution of DNA-protein conjugates (100 nM in PBS) at 37° C for 30 min, then at room temperature (23 ± 3 °C) for 30 min. Unbound conjugate was

removed by washing three times with 50-μL volumes of a PBST buffer (0.05% Tween in PBS, pH 7.6).

**Characterization of rabbit IgG arrays on paper**

The rabbit IgG array was incubated with a 50-μL volume of a solution of a fluorescently-labeled anti-IgG antibody, DL549 anti-rabbit IgG (monoclonal goat anti-rabbit IgG antibody conjugated with a proprietary florescent dye, DyLight549) containing 10% vol/vol goat serum for 30 min. Unbound antibody was removed by washing with a total volume of 100 μL of PBST buffer (0.05% Tween in PBS, pH 7.6). The disks were placed in a 96-well black plate with clear bottom (purchased from Corning) and the fluorescence intensity was scanned with excitation and emission wavelengths of 544 nm and 590 nm, respectively, in a microtiter plate reader (SpectraMax M2, Molecular Devices, Sunnyvale, CA).

**Sandwich ELISA for detection of *hCRP***

Mouse anti-human CRP (capture antibody, Part 842676), biotinylated mouse anti-human CRP (detection antibody, Part 842677), recombinant human CRP (Part 842678) were part kit (Part DCP00) of a purchased from R&D Systems (Minneapolis, MN). DNA-directed arrays of capture antibody (Mouse anti-human CRP) were formed on the surface of paper and subsequently used to fabricate paper-based devices as described before (see Figure S2).

The devices were suspended in air, using an empty pipette-tip box, to prevent the vertical flow of reagent solutions away from the test zones. The solutions of recombinant

human C-reactive protein (*h*CRP) were prepared in a 10 % v/v solution of human serum in PBST (0.05% Tween in PBS, pH 7.6). The microzones were incubated with 10 μL per well of solutions of human serum spiked with concentrations of *h*CRP between 16 and 1000 pg $mL^{-1}$, for 20 min. The microzones were then washed three times with 20 μL PBST and incubated with 10 μL of biotinylated mouse anti-human CRP detection antibody (250 ng $mL^{-1}$ in PBS, pH 7.6). After incubation for 20 min, each microzone was washed three times with 20 μL PBST. Detection was completed by adding 10 μL of 100 ng $mL^{-1}$ Streptavidin-Cy5 in PBS buffer, pH 7.6 to each microzone; unbound Streptavidin-Cy5 was removed by washing three times with 20-μL volumes of PBST.

**Immunoassays on untreated hydrophilic paper**

Paper-based devices were assembled as described in Figure S2. We used non-specific adsorption to immobilize rabbit IgG on untreated (used as received from the manufacturer) paper, by adding 5 μL of a solution of rabbit IgG in PBS, pH 7.6, to the test zone, and allowed it to dry for 10 min at room temperature. We blocked microzones by adding 5 μL of a 1% wt/vol solution of BSA in PBS, pH 7.6, and allowing it to dry for 10 min. A volume (5 μL) of a solution containing the DL549 anti-rabbit IgG in a solution of PBS with 10% vol/vol goat serum was added to each zone and allowed to incubate for 5 min. Each microzone was washed three times with 10 μL of PBS. Each microzone was scanned, and the fluorescence intensity was measured using ImageJ.

**Device using paper-anchored ssDNA arrays for the multiplex detection of fluorescently-labeled nucleic acids and antibodies.**

DNA with orthogonal sequences S1 and S2 (see Table 1) was synthesized independently on the surfaces of paper. The strips of paper were cut into disks with a 3-mm diameter using a biopsy punch, then into half-disks using a blade. For the paper with anchored DNA of sequence S2, the surface of the half-disks was blocked with 25 μL of a 1% wt/vol solution of BSA in PBS, pH 7.6, for 30 min, and the half-disks were washed with PBS, and then incubated with 25 μL of a solution of DNA-rabbit IgG conjugates (100 nM in PBS) at 37° C for 30 min, then at room temperature (23 ± 3 °C) for 30 min. Unbound conjugate was removed by washing three times with 25-μL volumes of a PBST buffer (0.05% Tween in PBS, pH 7.6).

Paper-based devices were assembled as described in Figure S2, except that the two half-disks of paper (one half with ssDNA with sequence S1, and another with the DNA-anchored rabbit IgG array described before) were placed in close proximity to form a single microzone. An solution containing 5 nM of FQ (prepared by hybridizing oligonucleotides F and Q in a 1:9 ratio, as described before) and 5 nM of DL549 anti-rabbit IgG in TBS buffer containing 10% vol/vol goat serum, was added to the microzone, incubated for 30 min at 37° and allowed to cool to room temperature. The microzones were then washed three times with PBS, and imaged with a fluorescence scanner. We normalized the average fluorescence of the microarray probe to the average fluorescence intensity of the adjacent control microarray probe (i.e. the ratio of the signal from a probe to the signal related to nonspecific binding or cross-hybridization, in the fluorescence channel and in the same device).

**Immunoassays in standard polystyrene plates**

The wells of a standard polystyrene 96-well plate (Corning) were incubated with a 50-µL volume of a solution of rabbit IgG (10 µg/mL, in PBS pH 7.4) for 2 hr at room temperature. The wells were washed three times using 100-µL volumes of PBST buffer (0.05% Tween in PBS, pH 7.6), and blocked using 50 µL of a 1% wt/vol solution of BSA in PBS, pH 7.6, for 60 min. The wells were washed three times using 100-µL volumes of PBST buffer, then were incubated with a 50-µL volume of a solution of DL549 anti-rabbit IgG (monoclonal goat anti-rabbit IgG antibody, conjugated with DyLight549, from Jackson Immunoresearch, Inc.) containing 10% vol/vol goat serum, for 60 min. The wells were washed five times using 100-µL volumes of a PBST buffer, and the fluorescence intensity was determined at 530 nm in a microtiter plate reader.

**Figure S1:** PAGE gel of non-denatured (left) and denatured (right) IgG-DNA complexes. Arrows indicate the position of the IgG-DNA complexes (left, <160 kDa) and of the heavy (~50 kDa) and light chains (~25 kDa) derived from the IgG-DNA complexes (right).

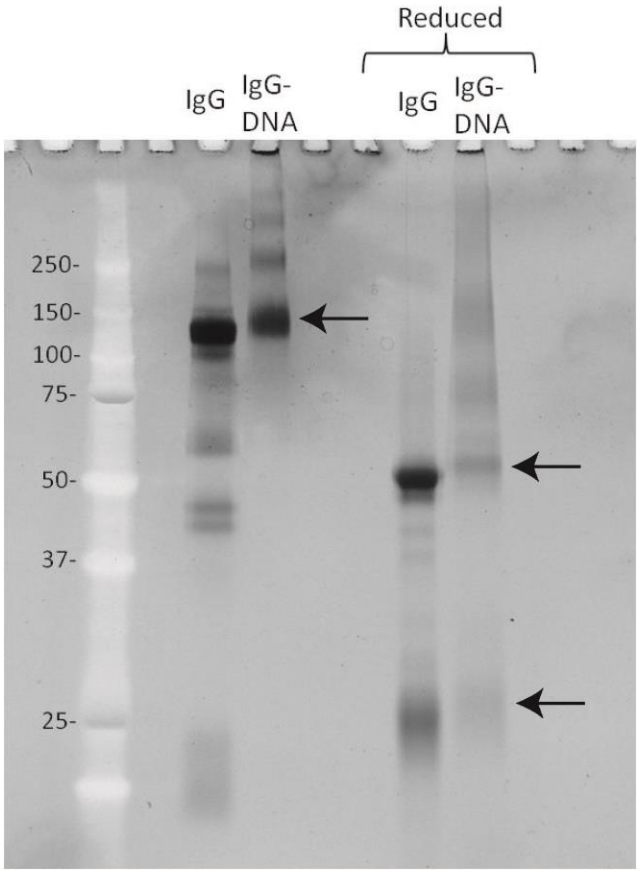
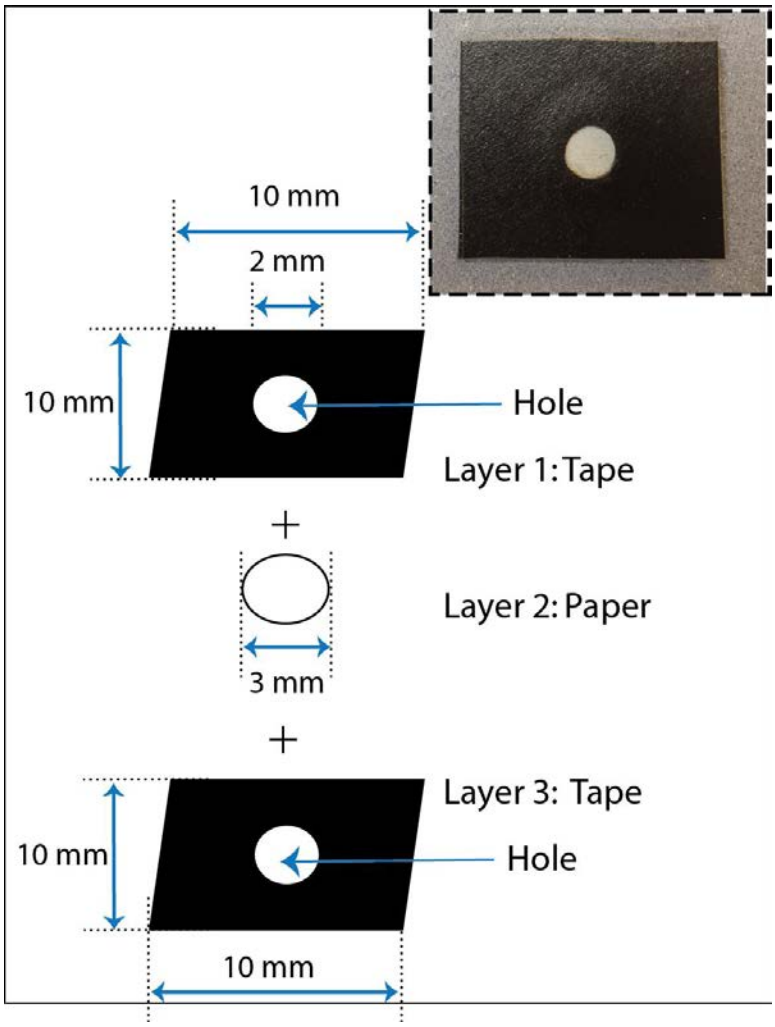


**Figure S2**: Design and fabrication of paper-based devices.




[1] E. M. Fenton, M. R. Mascarenas, G. P. Lopez, S. S. Sibbett, *ACS Appl. Mater. Inter.* **2009**, *1*, 124.
[2] S. R. Gallagher, P. R. Desjardins, *Current Protocols in Human Genetics* **2006**, A. 3D. 1.