Paper-based electrochemical immunosensor for rapid, inexpensive cancer biomarker protein detection

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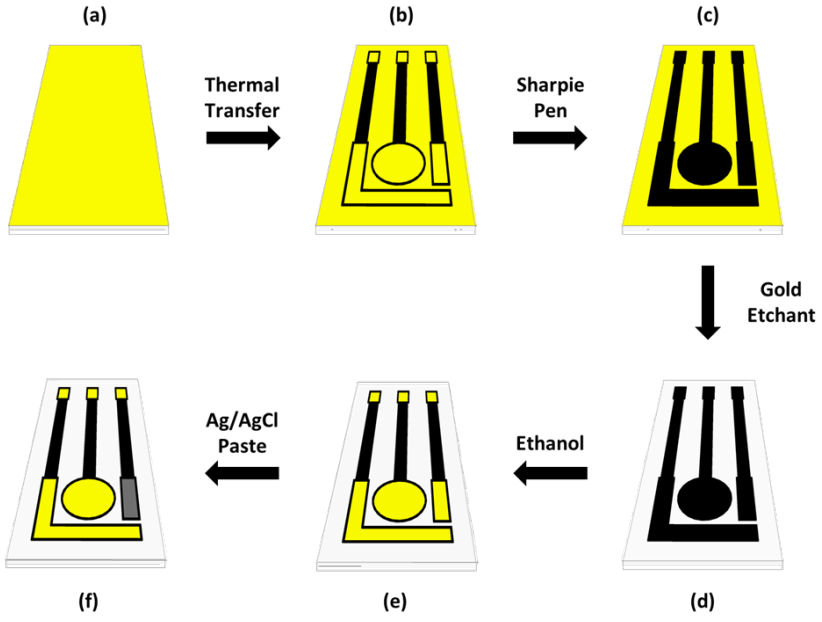
Supplementary Information (ESI)

Experimental Details

Chemicals and Materials. Gold compact discs were Mitsu recordable CD-R, 650 MB MAM-A Inc. Whatman Protran nitrocellulose membranes were from Fisher Scientific. Monoclonal anti-human prostate specific antigen (PSA) antibody (clone no. 181823), biotinylated anti-human PSA antibody and recombinant human PSA (carrier free) were obtained from R&D Systems. Streptavidin Poly-HRP was from Thermo Scientific. Bovine serum albumin (BSA) and Tween-20 were from Sigma Aldrich. Hydrogen peroxide (H_2O_2, 30%) was from Fisher. Immunoreagents were dissolved in pH 7.4 PBS buffer (0.01 M in phosphate, 0.14 M NaCl, 2.7 mM KCl) unless otherwise noted. Protein standards were prepared in calf serum, which has similar properties to human serum but lacks human proteins. The human serum samples were purchased from Capital Biosciences.

Instrumentation. A Hewlett Packet Laserjet 1020n was used to print patterns onto wax paper. A thermal press (Stahls USA, Maxx Press) was used to transfer the pattern onto the gold CD-R at 120°C. A CHI 660 electrochemical workstation connected to the sensor chip was used for differential pulse voltammetry (DPV) at ambient temperature (22±2 ºC) vs. Ag/AgCl reference electrode.



Scheme S1 Steps in the fabrication of gold arrays from commercial gold CD-Rs. (a) Remove the protective layer of the CD-R to expose the gold film; (b) Print the array pattern on wax paper using laserjet printer and transfer onto the gold surface by applying heat and pressure, contacts

are the squares at top of each chip, sensor electrode is the circle in the middle of chip, counter is the L-shape on left, reference is the rectangle on right; (c) Use Sharpie® pen to cover contact pads and electrodes; (d) Remove unprotected gold in etchant solution; (e) Wash with ethanol/water to expose contact pads and electrodes; (f) Manually screen printing of Ag/AgCl reference electrode.

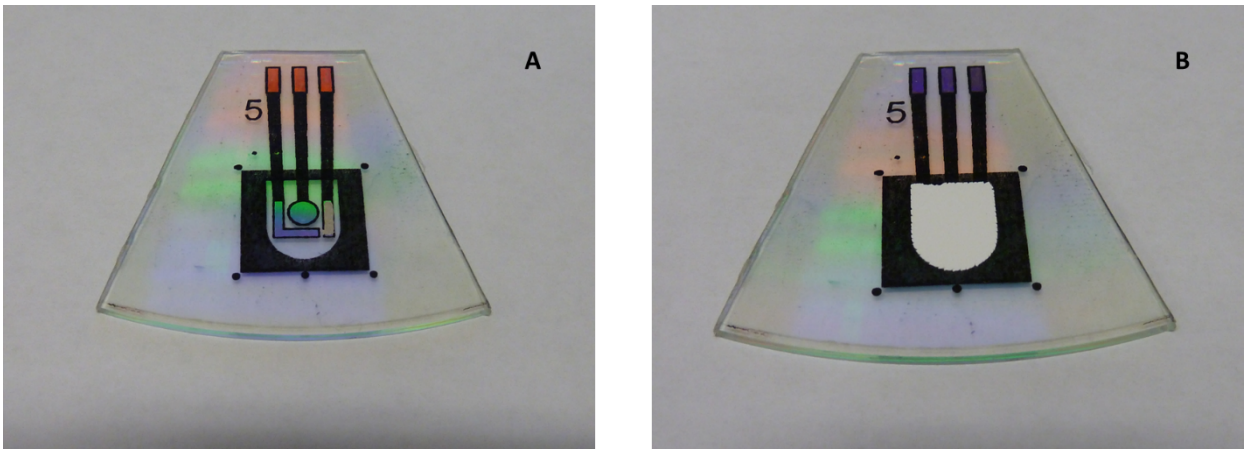


Figure S1 Finished sensor chip. (a) Sensor chip featuring reagent well with gold working electrode and built-in Ag/AgCl reference and gold counter electrode at the center; (b) A paper disk placed onto the chip for sensing applications.

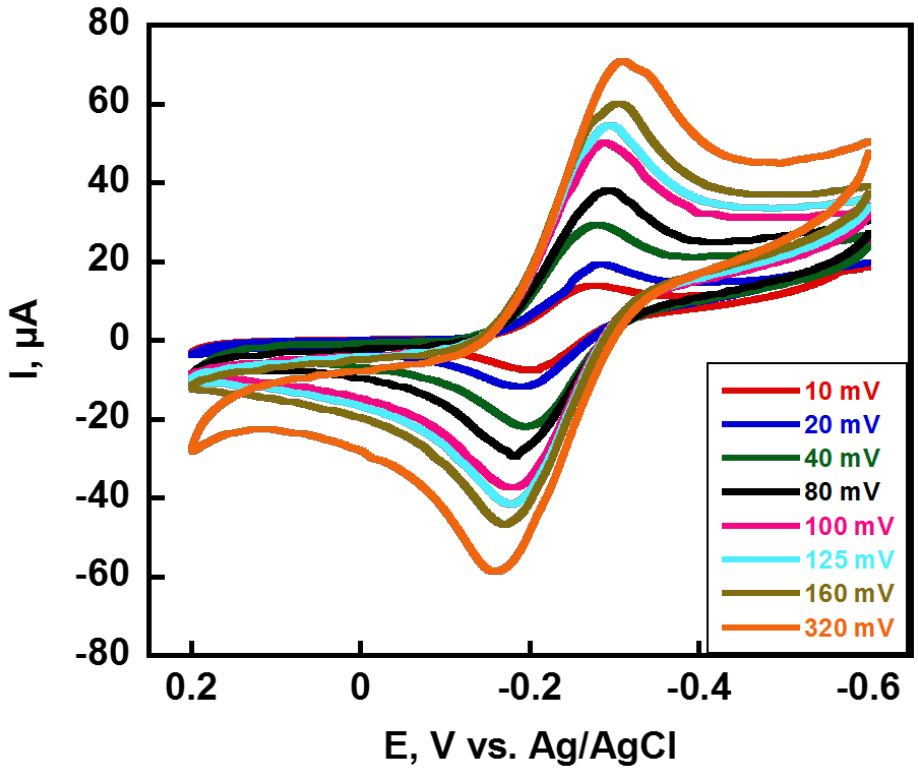


Figure S2 Electrochemical characterization of the paper disk sensor. Cyclic voltammograms of 4 mM ruthenium hexamine chloride (RuHex) in 0.09 M phosphate at various scan rate

demonstrating diffusion control of voltammetric peaks. The halved diffusion coefficient calculated by Randles-Sêvcîk for the paper sensor as compared to that in solution suggests that there's percolation of redox species within the paper to the sensor electrode.

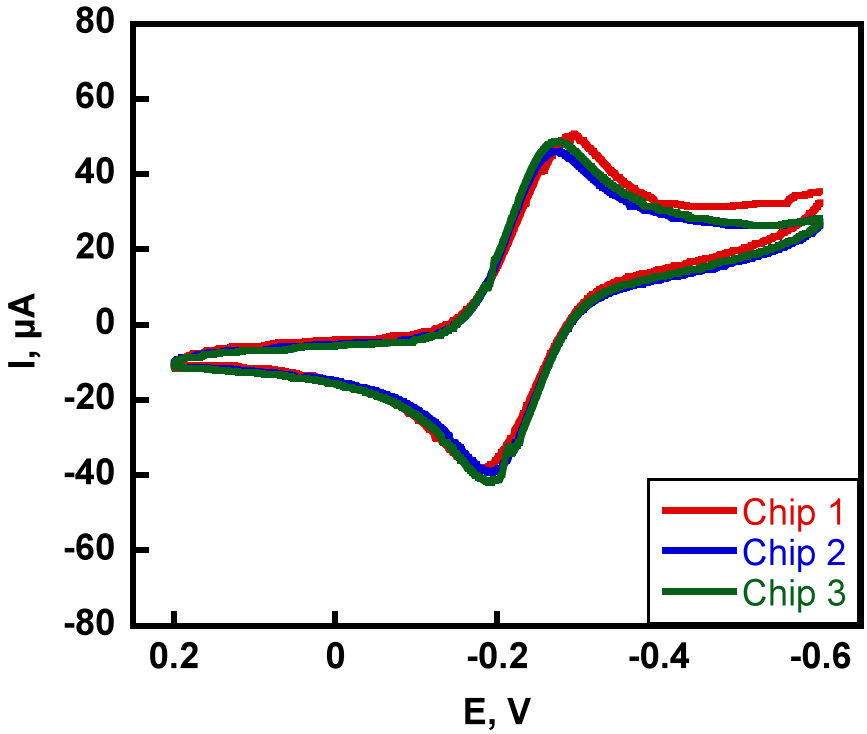
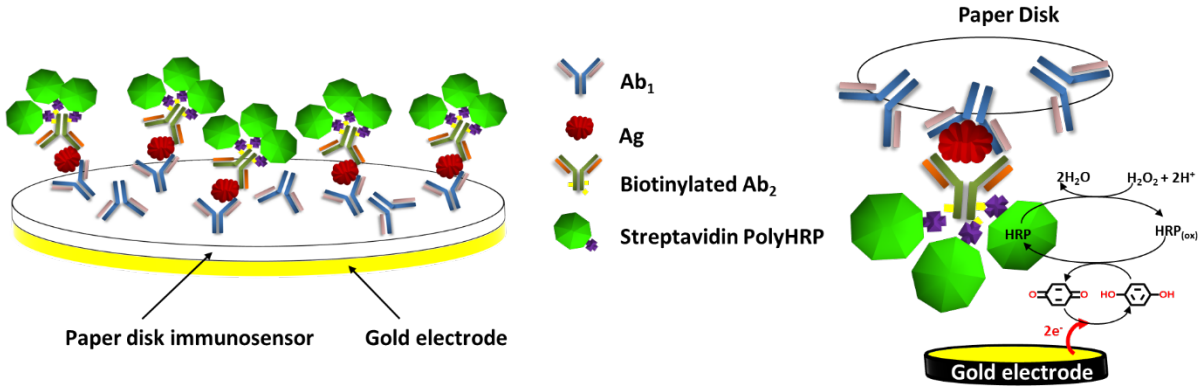


Figure S3 Chip-to-chip Reproducibility. Cyclic voltammograms of three different chips in 4 mM ruthenium hexamine chloride (RuHex) in 0.09 M phosphate. Cathodic peak currents of the three CVs was 52.4 ± 1.4 µA demonstrating good reproducibility.

Fabrication of electrodes. Three-electrode chips were made by wet chemistry etching of gold CDs using previously described protocols(Scheme S1, ESI).[1] In brief, the electrode pattern was designed using the Canvas 11 software on a 1:1 scale. The design was printed onto high gloss paper (backing of Avery labels) using the HP laserjet 1022n at 1200 dots per inch (dpi). The pattern was then cut and placed onto a piece of gold CD-R with the polymer coating removed. The pattern and the CD was sandwiched in a thermal press at 120°C for 110 s. The contact area and working electrodes were manually covered with a Sharpie® permanent marker and immersed into a ferricyanide etching solution to remove all Au that is not protected. The chips were washed with ethanol to expose contacts pads and sensor electrode, then washed with water and dried under nitrogen. Finally, the reference electrode was manually screen printed with Ag/AgCl ink (DuPont 5269) onto the designated area. Before all measurements, the chips were subjected to 5 cycles of a -0.1 to 1.2V potential sweep in 0.18 M sulfuric acid.



Scheme S2. Amplification strategy on the paper disk immunosensor using streptavidin Poly-HRP attached to biotinylated detection antibody. Detailed scheme of the electrochemical reaction is shown on the right. The hydroquinone act as a electron mediator to shuttles electrons to the electrode surface to give a measurable signal.

Preparation of paper disk immunosensors. Paper disk was cut into desired shape using a craft cutter (Cameo®, Silhouette America, Inc.). Cut pattern was designed using a computer equipped with Silhouette Studio®. Each paper disk was first dipped in 500 µL of 100 µg mL^{-1} anti-PSA capture antibody (Ab_1). The disks were washed in PBS-T (PBS + Tween-20) and PBS and then blocked with 1% BSA for 5 mins, followed by the same washing step. For standardization, the paper disks were dipped into vials with respective concentrations of human PSA antigen standards (Ag) in calf serum 5 mins. For validation, the human serum samples were diluted 50X or 2X with PBS and incubated with the disks for 5 mins. After washing, each disks was dipped into 1 µg mL^{-1} of the corresponding biotinylated secondary antibody (Ab_2) for 5 min. Again, disks were washed and dipped into 1:1000 diluted streptavidin PolyHRP to incubate for another 5 mins (Scheme S2). All incubations were done at room temperature unless otherwise noted. After a final wash in PBS, the paper disk immunosensors were stored at 4°C before electrochemical measurements. For assays performed at 37°C, all reagents and paper disks were allowed to equilibrate to 37°C for one hour before assays in Fisher Isotemp Incubator. Incubation times were reduced from 5 mins to 3 mins for all steps for total assay time of 10 mins.

Electrochemical analysis. The paper disks immunosensors were dipped into 1 mM hydroquinone (HQ) and 100µM hydrogen peroxide (H_2O_2) purged with purified nitrogen and placed onto the electrode sensor chip immediately for electrochemical analysis. Differential pulse voltammetry was done at 4 mV step, 25 mV pulse and 15 Hz frequency vs. Ag/AgCl from 0.05 V to -0.4 V.

A calibration was performed at 37 °C. Reagents were equilibrated to 37 °C in Fisher Isotemp Incubator for 1 hour before performing assays. Given that the frequency for biomolecules to collide increases at higher temperature, we expect that there would be considerable effect on the reaction time for the immunoassay to reach equilibrium. Thus, we reduced the incubation times from five minutes to three minutes for each step (Fig. S4). Nearly identical sensitivity and detection limit was achieved for 16 min assays at 25°C and 10 min assays at 37 °C.

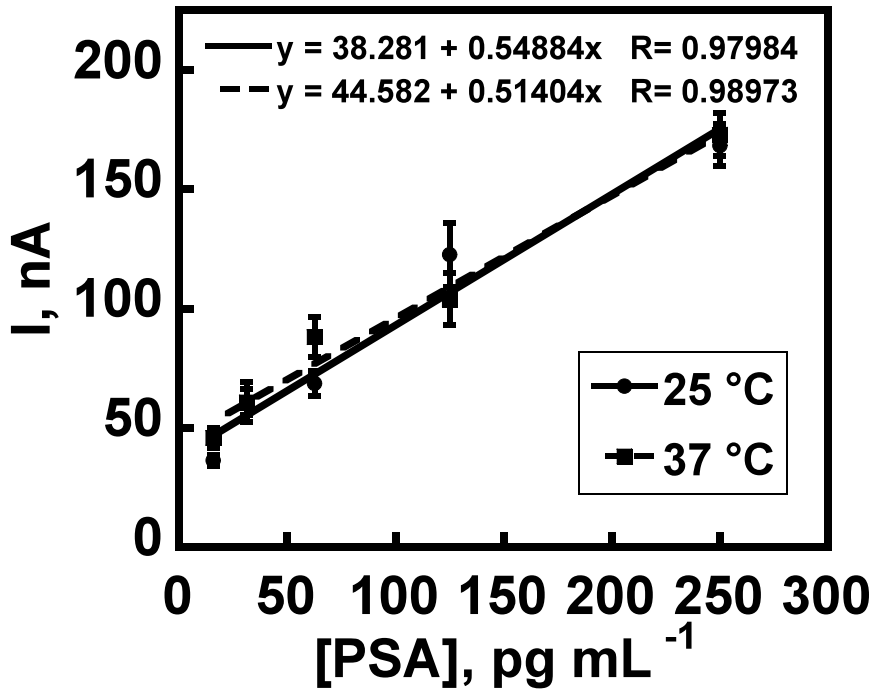


Figure S4 Calibration curve at at 37 °C compared to 25 °C. Similar sensitivity was achieved for 16 minutes assays at 25°C and 10 minutes assay at 37 °C. In the case of assay performed at higher temperature (>35 °C), the incubation times for each step was reduced to 3 mins for a total assay time of 10 minutes. A detection limit of 15 pg mL^{-1} was achieved for the assay at 37°C.

	Paper based electrochemical ELISA (this work)	Magnetic beads based electrochemical ELISA[1] (For rapid assays)	Conventional electrochemical ELISA[2]
	Time (mins)	Time (mins)	Time (mins)
1.Antigen capturing	5	25 (3)	60
2.Washings	1	2 (2)	5
3.Detection antibody	5	N/A	60
4.Signal Amplification	5	N/A	20
5.Beads capturing	N/A	15 (3)	
Total per sensor/array	**16**	**42 (8)**	**145**

Table S1. Comparison of time in paper based electrochemical ELISA in current work with our previous magnetic beads based microfluidic electrochemical assay and conventional electrochemical assay. For the magnetic beads based assay, time can be further reduce to 8 mins by sacrificing ultralow detection limits for speed (Col. 2, Estimated time in parentheses).

References

1 C. E. Krause, B. A. Otieno, A. Latus, R. C. Faria, V. Patel, J. S. Gutkind and J. F. Rusling, *ChemistryOpen*, 2013, **2**, 141–145.

2 C. K. Tang, A. Vaze and J. F. Rusling, *Lab Chip*, 2012, **12**, 281-286.