Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

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Aptamer-Based Origami Paper Analytical Device for Electrochemical Detection of Adenosine**

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Chemicals and materials

All DNA was ordered from Integrated DNA Technologies, Inc. Streptavidin-labeled glucose oxidase (sGOx) was purchased from Rockland Immunochemicals, Inc. (Gilbertsville, PA). Streptavidin-coated polystyrene microbeads and fluorescent polystyrene microbeads with Nile red inside were purchased from Spherotech, Inc. (Lake Forest, IL). Adenosine and Glucose oxidase (GOx) from Aspergillus niger were obtained from Sigma Aldrich. D-glucose and $K_3Fe(CN)_6$ was purchased from Fisher Scientific. All solutions were prepared with deionized water (18.0 MΩ·cm, Milli-Q Gradient System, Millipore). Conductive carbon ink (Creative Materials, Inc. Tyngsboro, MA) was used to fabricate electrodes using a coating kit (RD Specialties, Inc. Webster, NY). All paper fluidic devices were fabricated using Grade 1 Whatman chromatographic paper. Scotch thermal laminating pouches (TP5851-20) and a Jorestech impulse thermal edge sealer were used for thermal lamination of the device. A 2.2 μF electrolytic capacitor, a switch, and a breadboard with jumper wires were obtained from RadioShack. All reagents and materials were used as received.

Experimental procedures

The patterning of paper is based on a slight modification of a wax printing procedure reported previously (Carrilho, E. et al. Anal. Chem. 2009, 81, 7091–7095). Briefly, a Xerox 8570DN inkjet printer was used to print wax-based solid ink on Whatman chromatography paper. The paper was then placed on a hot plate with the wax side up for 15 s at 120 °C, and then cooled to 20 °C. For fabricating electrodes, the patterned paper was placed on a flat glass surface, and then covered with a home-made stencil. An aliquot of carbon ink was

dropcast on the stencil. A coating rod was used to coat the carbon ink uniformly onto the patterned paper through the stencil. To cure the carbon ink, the stencil was removed from the paper, and the paper was placed in an oven at 80 ^{o}C for 30 min.

For preparing GOx-labeled DNA, 12.5 μM biotin-modified DNA (bDNA) solution was mixed with a 100 μM streptavidin-labeled GOx (sGOx) solution to yield a solution containing 1:1 bDNA and sGOx. The sGOx solution also contains 10 mg/mL bovine serum albumin (BSA) as a stabilizer and 0.1 mg/mL sodium azide as a preservative. After reaction for 24 h, an Amicon 50 K centrifugal filter (Millipore) was used to separate the GOx-labeled bDNA from free bDNA by centrifugal filtering. All solutions were diluted to the same volume for UV-vis characterization, which leads to a concentration of 0.46 μM for both bDNA and sGOx solutions. The UV-vis spectra were taken using a UV-vis spectrometer (Hewlett-Packard 8453).

As shown in Figure S3, the characteristic maximum absorptions of protein and DNA are at 280 nm and 260 nm respectively. Note that the absorption of sGOx at 280 nm is significantly higher than that of bDNA at 260 nm at the same concentration due to the difference in extinction coefficients and excess BSA in the sGOx solution. For a solution containing both sGOx and bDNA at a 1:1 molar ratio, the absorption at 260 nm was higher than that of the solution containing only sGOx due to the presence of bDNA in the solution. After filtration using a 50 k cut-off centrifugal filter, all the sGOx-labeled bDNA was maintained in the retentate, but the free bDNA passed the filter into the filtrate. The absorption of the retentate at 260 nm was obviously higher than that of the solution containing only sGOx, which demonstrates the formation of the bDNA-sGOx conjugate that was left in the retentate. The absorption of the retentate overlapped with the absorption of the 1:1 sGOx and bDNA

mixture, which demonstrates that the ratio of sGOx to bDNA in the final product was approximately 1:1. This agrees with the fact that the absorption of the filtrate at 260 nm was very small. Therefore, almost all bDNA binds to sGOx with minimal free bDNA in the filtrate.

To prepare the device for electrochemical sensing, 12 μL of 100 mM glucose and 100 mM $Fe(CN)_6^{3-}$ in 0.01 M PBS buffer (pH 6.0) was loaded into both halves of the electrochemical cell, and dried in air. A 20 μL solution containing 25 μM biotin-DNA strand was mixed with 20 μL 0.5% (w/v) streptavidin-labeled microbeads. After reaction in the dark for 24 h, the resulting solution was mixed with a 40 μL solution containing 8.4 μM GOx-DNA strand and 12 μM aptamer strand. The solution was dropcast in the split channel on the unfolded device, washed with 0.01 M PBS buffer (pH 7.4) containing 0.1% BSA, and dried in air.

To assemble the origami paper analytical devices (*o*PADs), the paper was folded by hand. The folded paper device was placed in a thermal laminating pouch, and the pouch was sealed at the edge of the paper device using the impulse thermal edge sealer. Three holes were punched on one side of the pouch. One of the holes was used as inlet and the other two were used to accommodate ohmic contact of the carbon electrodes to two copper wires using Ag adhesive. Five-minute epoxy was used to reinforce the contact.

To use the device for electrochemical sensing, a 20 μL sample aliquot was loaded at the inlet of the *o*PAD. After 10 min, when the sample fills the whole channel, the *o*PAD was put on a breadboard. The current generated from it was measured by a Sinometer VA18B digital multimeter (DMM), and simultaneously the charge was accumulated on the capacitor. After 60 s, the switch was closed so that the capacitor discharges toward the DMM instantaneously. The

DMM we used has a max/min button. If the button is pressed once, it only displays the maximum value of measurements. Based on this, the magnitude of the maximum discharge current from the capacitor was recorded. The rate at which DMM measures current depends on conversion rate of analog-to-digital converter, which is usually in the range of 1 - 100 s^{-1}. Although there is no way to measure the conversion rate, the time interval between two current measurements for the DMM used in these studies is about 0.1 s based on analysis of Movie 2 in the Supporting Information. This means the conversion rate should be > 10 s^{-1}.

Fluorescence measurements on the paper fluidic devices were made using a Typhoon Trio fluorescence imager (GE Healthcare, Piscataway, NJ). To quantify these results, the images were imported into Adobe Photoshop CS2 and transferred to gray-scale mode. The mean fluorescence intensity was determined from the image histogram for each detection reservoir, and then it was background-corrected by subtracting the average intensity measured at the center of the paper where no adenosine was present.

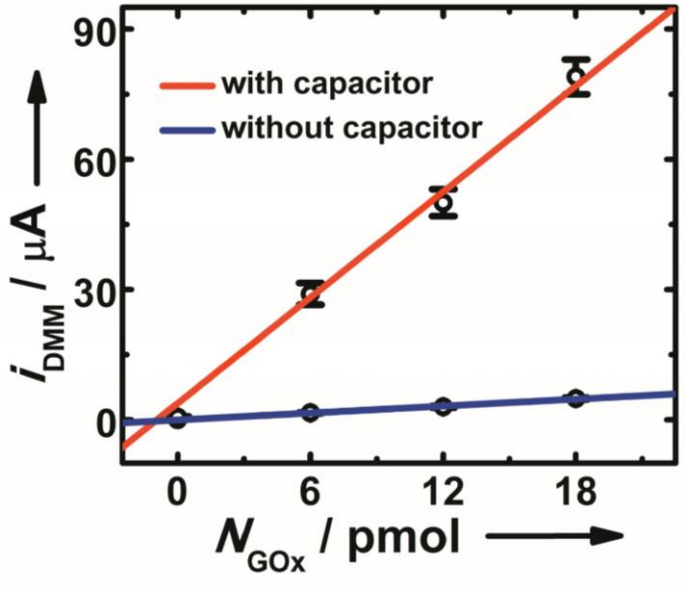

Figure S1. Calibration curve for detection of GOx using the *o*PAD with and without amplification by a capacitor. The error bars represent standard deviation of readings from a DMM for three measurements.

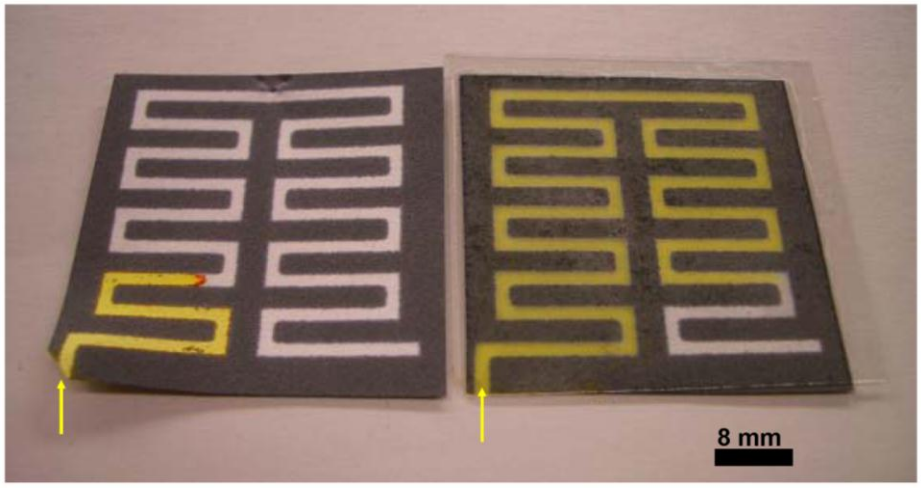

Figure S2. Photograph of two paper fluidic devices. The one on the left was open to the air, and the one on the right was encapsulated by thermal lamination. The devices were placed in an oven at 37 °C, and the openings at the bottom-left corner (marked by the yellow arrows) were dipped into 0.01 M PBS solution (pH 7.4) containing 0.1 mM tartrazine. It took more than 2 h for the solution to fill the entire channel on the right. This experiment shows that lamination prevents evaporation over the time required to carry out the assays reported in the main text.

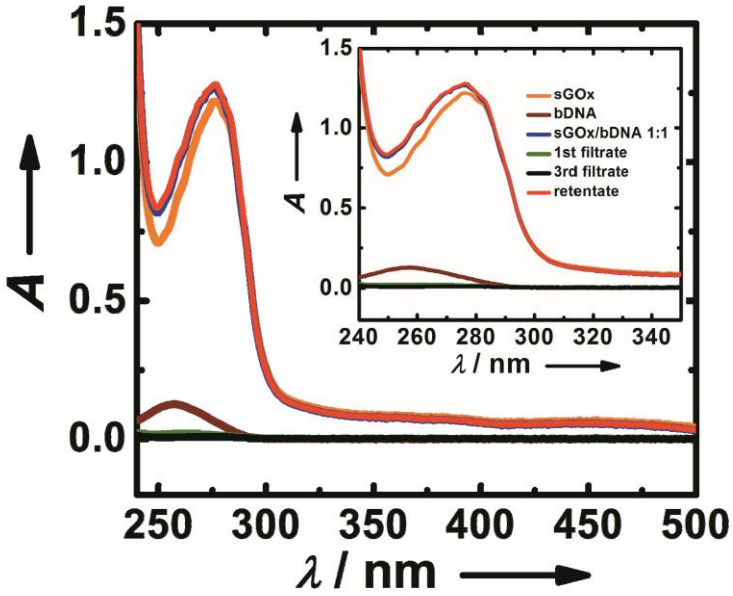

Figure S3. UV-vis spectra of 0.46 μM sGOx solution, 0.46 μM bDNA solution, a mixture of 0.46 μM sGOx and 0.46 μM bDNA, filtrate and retentate of the mixture after reacting for 24 hours obtained by filtering using a 50 K cut-off centrifugal filter. All solutions were diluted to the same volume for comparing the UV-vis spectra. Inset: the UV-vis spectra from 240 nm to 350 nm.

Movies

Movie 1 (mp4 format) is provided showing how the sample solution flows in the channel of the *o*PAD as described in the main text for Scheme 1. The movie plays back at 30 times real time.

Movie 2 (mp4 format) is provided showing the measurement of transient current from the capacitor using the DMM. The movie plays back at real time (30 frames/s). The time internal between two readings (88.8 and 35.8 μA) is 0.1 s (3 frames).