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Faradaic Ion Concentration Polarization on a Paper Fluidic Platform

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Abstract

We describe the design and characteristics of a paper-based analytical device for analyte concentration enrichment. The device, called a hybrid paper-based analytical device (hyPAD), uses faradaic electrochemistry to create an ion depletion zone (IDZ), and hence a local electric field, within a nitrocellulose flow channel. Charged analytes are concentrated near the IDZ when their electrophoretic and electroosmotic velocities balance. This process is called faradaic ion concentration polarization. The hyPAD is simple to construct and uses only low-cost materials. The hyPAD can be tuned for optimal performance by adjusting the applied voltage or changing the electrode design. Moreover, the throughput of hyPAD is 2 orders of magnitude higher than that of conventional, micron-scale microfluidic devices. The hyPAD is able to concentrate a range of analytes, including small molecules, DNA, proteins, and nanoparticles, in the range of 200–500-fold within 5 min.

Abstract

In this paper, we report integration of the method of faradaic ion concentration polarization (FICP) with paper-based analytical devices (PADs).^{1,2} The results show that this new family of PADs provides an inexpensive means for enriching the concentration of fluorescent molecules, nucleic acids, proteins, and nanoparticles by factors ranging from 200 to 500 within 5 min. Because they are fabricated using both nitrocellulose and cellulose paper, we call them hybrid PADs or simply hyPADs (Scheme 1a). There are several desirable characteristics of the hyPAD design. First, hyPADs are easy to fabricate using low-cost

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.anal-chem.7b00365. Details of the hyPAD design, calibration curves for BODIPY²⁻, DNA-Cy5, and BSA-AF, plots of $C_{\max}$ vs t for different C_{init} , a photograph of the hyPAD channel during an FICP experiment, schematic illustration showing how current passing through the BPE and the channel is measured, design of the hyPAD used for measuring the electric field within the channel, quantification of DNACy5 and BSA-AF enrichment on hyPADs, and modification of AuNPs with DNA (PDF)

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materials. Complicated channel modifications, such as membrane patterning,^{3,4} which are usually required for nonfaradaic ICP, are not necessary for fICP. Second, the fICP process can be tuned for optimal performance by adjusting the applied voltage. Third, the use of a bipolar electrode (BPE)^{5–9} to drive faradaic reactions eliminates the need for an external circuit connection and a second power supply. Finally, the throughput of the hyPAD is two orders of magnitude higher than conventional microfluidic channels. All of these points will be discussed in more detail later.

Two- and three-dimensional PADs were first introduced by the Whitesides group in 2007 and 2008, respectively.^{1,10} Since that time, a number of means for detection of analytes on paper platforms have been reported.^{11,12} However, improving the limit of detection (LOD) of PADs remains important in several fields, particularly for point-of-care (POC) applications in medicine.^{12–15} On-chip sample preconcentration is one attractive solution to this problem because it can be implemented with existing detection strategies but still lower LODs. A variety of creative approaches for preconcentration have been reported for use with conventional glass and plastic microfluidic devices,^{16,17} but analogous reports for use with paper-based devices are more limited.^{18–22}

Conventional ICP, which is an electrokinetic molecular trapping technique,^{23,24} has previously been adapted to paper platforms for preconcentrating analytes. For example, in 2014, Gong et al. patterned a nitrocellulose paper channel with a selectively permeable membrane (Nafion) to create a micro-/nanochannel junction.²⁵ Upon application of an electric field across the channel, permselective charge transport through Nafion led to formation of an ion depletion zone (IDZ) and an ion enrichment zone (IEZ) at opposite sides of the membrane. Due to reduction of the charge carrier (e.g., ion) concentration in the IDZ, the local electric field strength increased and blocked ions from entering this region of the channel. For example, negatively charged DNA, transported by electroosmotic flow (EOF) into the vicinity of the IDZ, concentrated at the IDZ boundary where the EOF and electromigration velocities balanced. More recently, there have been additional publications^{26–30} demonstrating the ICP phenomena using PADs having designs similar to that of Gong et al.²⁵

The technique of fICP, which we report here, is similar to conventional ICP, with two major exceptions.^{2,7} First, instead of a micro-/nanochannel junction, the IDZ is created by an electrode (a U-shaped BPE in Scheme 1a) patterned within the channel. Second, instead of selective charge transport, the IDZ in fICP is formed as a result of faradaic processes at the electrode surface. These points are illustrated in Scheme 1b. The external voltage applied to the channel via the two carbon paste electrodes shown in Scheme 1a is sufficient to drive simultaneous water oxidation (eq 1) and reduction (eq 2) reactions at opposite ends of the BPE.^{2,6}

BPE anode:

BPE cathode:

The faradaic reaction (eq 2) at the BPE cathode generates OH^- , which can neutralize cations ($TrisH^+$) present in the buffer (eq 3), resulting in uncharged products (H_2 , $Tris$, and H_2O).² The local loss of charge carrying ions results in formation of an IDZ. Charged analytes, such as DNA, will then be concentrated at the IDZ boundary by the same principles operative in conventional ICP.

In the present article, we show that the hyPAD is capable of concentrating a fluorescent molecule ($BODIPY^{2-}$) up to 513-fold within 5 min. The throughput of hyPAD is ~600-fold higher than that of conventional microfluidic devices. Furthermore, we demonstrate the ability of hyPADs to concentrate DNA, proteins, and gold nanoparticles (AuNPs), which illustrates the potential application of hyPADs for detecting biological molecules and integrating with nanoparticle-based colorimetric assays.

EXPERIMENTAL SECTION

Chemicals and Materials.

A stock solution of 0.5 M $Tris-HClO_4$ (pH 8.1) was prepared from tris(hydroxymethyl)-aminomethane (Sigma-Aldrich, St. Louis, MO) dissolved in water and titrated with perchloric acid (70%, Fisher Scientific, Waltham, MA). 4,4-Difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene-2,6-disulfonic acid ($BODIPY^{2-}$) and albumin from bovine serum (BSA-AF, modified with Alexa Fluor 488) were obtained from Invitrogen (Carlsbad, CA). DNA (DNA-Cy5, 5'-GGA GTA AAT GTT GGA GAA CAG TAT CAA CAA-Cy5-3'; DNA-SH, 5'-SH-C6-AAA AAA AAA ATA CCA CAT CAT CCA T-3') was ordered from Integrated DNA Technologies (Coralville, IA) and purified by HPLC. AuNPs (20 nm diameter) were purchased from Sigma-Aldrich (St. Louis, MO). All solutions were prepared using deionized water (>18.0 M Ω -cm) purified with a Milli-Q Gradient System (Bedford, MA).

Device Fabrication.

The hyPAD comprises three layers and is constructed from both nitrocellulose and cellulose paper (Scheme 1a). Whatman grade 1 cellulose paper (~180 μ m thick, Fisher Scientific) was patterned with wax using a Xerox ColorQube 8750DN inkjet printer.³¹ All three layers of paper were fully covered by wax except for two hydrophilic, cellulose reservoirs present on the second layer (more detailed information about the hyPAD design is provided in the Supporting Information, Figure S1). The wax-patterned paper was placed in an oven at 120 °C for 45 s to melt the wax through the thickness of paper. Next, two driving electrodes

and a BPE were stencil-printed on the first and second layers, respectively, using conductive carbon paste (Engineered Conductive Materials, Delaware, OH).³² The third layer of cellulose paper and an unpatterned piece of nitrocellulose paper (0.45 μm pore size, Thermo Scientific) were then cut to the same size using an Epilog laser engraving system (Zing 16). The nitrocellulose paper was then placed inside the cut-out of the third layer of cellulose paper.³³ Finally, the driving electrodes were connected to copper tape, and the entire paper device was laminated with plastic (3M) that contained two holes as inlet and outlet for the solution.

Device Operation and Microscopic Analysis.

The hyPAD operates as follows. First, 80 μL of the analyte dissolved in 100 mM Tris- HClO_4 (pH 8.1) is added at the inlet to prewet the nitrocellulose channel. Second, a potential bias of 100 V is applied between the two driving electrodes using a Lambda LLS9120 DC power supply. As soon as the voltage is applied, faradaic reactions occur at the BPE, and as we will show later, an IDZ begins to develop near the BPE cathode. This initiates formation of a concentrated band of analyte.

The enrichment process was visualized using a Nikon AZ100 fluorescence microscope. Micrographs were obtained every 1 s using a 100 ms exposure time. The following Nikon filters were used for each analyte: BODIPY²⁻ and BSA-AF, 460–500 nm excitation and 510–560 nm emission; DNA-Cy5, 590–650 nm excitation and 663–738 nm emission. For the AuNP enrichment experiment, a cell phone (Huawei Honor 5X) was used to take photos of the hyPAD at different times under nearly identical lighting conditions. All images were processed using ImageJ software (NIH, Bethesda, MD) to obtain the maximum and integrated fluorescence intensities. These intensity values were then compared to calibration curves of fluorescence intensity vs analyte concentration (Figure S2) to obtain analyte concentrations. The hyPADs are disposable, and therefore, a different device was used for each experiment.

RESULTS AND DISCUSSION

Enrichment of the Fluorescent Tracer BODIPY²⁻ Using hyPADs.

In this section, we examine the performance of hyPADs for the enrichment of the fluorescent tracer BODIPY²⁻.^{34–36} In subsequent sections we will discuss the mechanism of fICP on hyPADs and show that hyPADs are capable of enriching DNA, proteins, and AuNPs.

A detailed description of hyPAD operation is provided in the Experimental Section. Briefly, 80 μL of a solution containing 100 mM Tris- HClO_4 and 20 nM BODIPY²⁻ is added to the hyPAD inlet (Scheme 1a) to wet the channel. Next, a voltage of $E_{\text{tot}} = 100$ V is applied between the two driving electrodes. The evolution of BODIPY²⁻ enrichment can then be followed by fluorescence imaging.

Figure 1a shows representative fluorescence micrographs obtained using a single hyPAD at different times following application of E_{tot} . The thin, vertical, gray lines overlaid onto the image represent the edges of the BPE cathode. This series of images demonstrates, qualitatively, that the fluorescence intensity increases with time after application of E_{tot} .

indicating successful accumulation of BODIPY²⁻ near the BPE cathode. Moreover, the concentrated BODIPY²⁻ plug remains in a nearly constant position just above one edge of the BPE cathode throughout the entire experiment.

The extent of BODIPY²⁻ enrichment can be quantified by plotting the average BODIPY²⁻ concentration (C_{ave} , Figure 1b) and the maximum BODIPY²⁻ concentration (C_{max} , Figure 1c) as a function of time (t). The value of C_{ave} was obtained by integrating the BODIPY²⁻ fluorescence intensity near the BPE cathode, and then comparing that value to a BODIPY²⁻ fluorescence intensity calibration curve (Figure S2). Figure 1b shows that the C_{ave} vs t curve can be divided into three zones. In Zone 1 ($t = 0$ –50 s), C_{ave} is very close to the background value (below the detection limit of the imaging instrument). In Zone 2 ($t = 50$ –140 s), C_{ave} increases linearly with time, and in Zone 3 ($t = 140$ –280 s) it begins to plateau. The average enrichment factor (EF_{ave}) is obtained by dividing C_{ave} by the initial BODIPY²⁻ concentration ($C_{\text{init}} = 20$ nM). At the end of the experiment ($t = 280$ s), $EF_{\text{ave}} = 40$.

C_{max} is determined by comparing the fluorescence intensity recorded at the camera pixel yielding the highest signal to the BODIPY²⁻ calibration curve (Figure S2). The C_{max} data (Figure 1c) increases monotonically with t and exhibits more noise than the analogous C_{ave} plot. The higher noise arises primarily from the stochastic nature of measurements recorded from the single brightest pixel (compared to the averaged data in Figure 1b). As will be discussed later, however, a fraction of this noise is also due to gas bubble formation at the BPE cathode which disrupts BODIPY²⁻ enrichment. The maximum enrichment factor (EF_{max}) for BODIPY²⁻ can be determined from Figure 1c using the maximum C_{max} value ($C_{\text{max}}^{\text{m}}$) divided by the initial concentration ($C_{\text{init}} = 20$ nM). This results in $EF_{\text{max}} = 490$.

Characterization of the Enrichment Process.

To better understand the time-dependent behavior of C_{ave} and C_{max} , a set of C_{init} -dependent experiments was carried out. The conditions for these experiments were the same as in Figure 1, except that C_{init} was varied from 1 to 20 nM.

Figure 2a shows a family of plots of C_{ave} as a function of t for the indicated values of C_{init} . The error bars (shadowing) correspond to the standard deviations for three replicates (three different hyPADs) for each value of C_{init} . The general trends of these curves are similar to the data shown in Figure 1b in that they can be divided into the same three zones: Zone 1 ($t = 0$ –50 s), Zone 2 ($t = 50$ –160 s), and Zone 3 ($t = 160$ –280 s). The enrichment rate (ER) is defined as the slope ($\Delta C_{\text{ave}} / \Delta t$) of each curve, in each of the three zones shown in Figure 2a, divided by C_{init} . The ER is a representation of how quickly BODIPY²⁻ enriches in each zone. The value of ER is plotted as a function of C_{init} in Figure 2b, where the dashed lines represent the average values. The main finding is that ER is independent of C_{init} . The numerical values of ER are Zone 1: -0.04 fold/s; Zone 2: 0.52 fold/s; and Zone 3: 0.05 fold/s.

In Zone 1, ER is slightly negative (-0.04 fold/s) and independent of C , which means BODIPY²⁻ is depleted near the BPE cathode at the start of the experiment. We hypothesized that the slightly lower BODIPY²⁻ concentration in Zone 1 at $t < 50$ s results from formation of the IDZ and designed the following experiment to verify this idea. A detectable

concentration of BODIPY²⁻ (350 nM) was introduced into a hyPAD prior to enrichment so that the formation of the IDZ would be revealed by a local decrease in the BODIPY²⁻ concentration. As shown in Figure 2c, the average BODIPY²⁻ concentration in the region of the channel where we expect the IDZ to form ($C_{\text{ave,IDZ}}$, indicated by the inset) does indeed decrease immediately after application of $E_{\text{tot}} = 100$ V. The value of $C_{\text{ave,IDZ}}$ continues decreasing for ~70 s, and then it reaches a plateau, indicating that the IDZ is fully formed. At $t = 70$ s, the concentration of BODIPY²⁻ in the IDZ drops to only 86% of the original concentration. This result proves our hypothesis that the IDZ forms during the initial stage of the experiment, and it accounts for the ~50 s delay in the onset of BODIPY²⁻ enrichment shown in Figures 1b and 2a.

In Zone 2, BODIPY²⁻ accumulates linearly as a function of t at a rate of 0.52 fold/s (Figure 2b). Because both capillary flow and pressure-driven flow are absent in the hyPAD, we believe that enrichment arises from EOF of BODIPY²⁻ from the reservoir toward the vicinity of the IDZ. If this is true, then the EOF velocity (u_{EOF}) in the hyPAD can be calculated from the value of ER : the result is $u_{\text{EOF}} = 0.78$ mm/s. This value is in agreement with literature reports obtained under similar conditions ($u_{\text{EOF}} \sim 0.5$ mm/s).³⁷ The ER values achieved using the hyPAD are comparable to those obtained using well-defined, conventional channels ($ER = 0.4$ to 1.5 fold/s),³⁴ but importantly the throughput of hyPAD is ~600 times higher due to the much larger channel dimensions (~20 times taller and ~30 times wider than conventional microfluidic devices).

The value of C_{ave} reaches its maximum value in Zone 3, and the ER drops to 0.05 fold/s (Figure 2b). We attribute this behavior to the limited amount of BODIPY²⁻ available in the device (i.e., the limited sample volume).

Figure 3a shows how the C_{max} vs t curves change for different values of C_{init} . These concentration-dependent data are consistent with the results shown in Figure 1c for a single BODIPY²⁻ concentration (20 nM) in that they rise monotonically with t . Note, however, that we have occasionally observed cases in which C_{max} reaches a maximum value and then decreases (Figure S3). This precipitous drop in C_{max} before the end of the experiment is a consequence of gas bubbles that occasionally form within the channel of the hyPAD and disrupt enrichment (Figure S4).

Despite the high level of noise and occasional drop in the C_{max} vs t curves, $C_{\text{max}}^{\text{m}}$ (the maximum value of C_{max} determined from Figures 3a and S3) is linearly correlated to C_{init} (Figure 3b). The slope of this plot is 513, which represents the average EF_{max} for the different values of C_{init} .

Understanding the fICP Mechanism in hyPADs.

In an effort to better understand the fICP mechanism, we examined a number of parameters associated with its performance. Because faradaic reactions occurring at the BPE are essential for establishing the IDZ, this part of the study began with measurements of the current passing through the BPE. It has previously been reported that as much as ~80% of the total current is diverted through the BPE in a conventional microfluidic channel.³⁵ To

compare these earlier results to the hyPAD, ammeters were used to track the current passing through the BPE and the total current through the channel (Figure S5).

The results of this experiment (Figure 4a) indicate that $91 \pm 1\%$ of the total current passes through BPE, which corresponds to a current density of $\sim 10 \text{ mA/cm}^2$. This is a sufficiently high current density that gas bubbles could be produced at the BPE.³⁸ This is consistent with the visual observation of bubbles in Figure S4 and our contention that these bubbles play a role in the occasional unusual enrichment profiles shown in Figure S3.

A fundamental requirement for analyte enrichment by fICP is the generation of a local electric field gradient within the fluidic channel.⁷ The existence of this field is strongly suggested by the presence of the IDZ discussed in the context of Figure 2c, but we wanted to confirm it directly. Accordingly, a modified version of the hyPAD was used to measure this field during enrichment. This device, shown in Figure S6, incorporates a fourth paper layer patterned with multiple electrodes evenly distributed above the channel. The local electric field strength along the channel can then be obtained by measuring the voltage difference between pairs of these electrodes during operation.

When the BPE is absent, the results indicate that the electric field is uniformly distributed across the channel ($\sim 5 \text{ kV/m}$, Figure 4b). In contrast, when a BPE is present, a ~ 3 -fold increase in the local electric field ($\sim 15 \text{ kV/m}$) is observed near the edge of the BPE cathode where analyte enrichment is observed (Figure 1a). In other words, the locations of the IDZ, the local electric field gradient, and the enrichment zone are more-or-less colocated. Another interesting observation from Figure 4b is that the electric field in the area between the poles of the BPE is close to zero. Recall that the current flowing through the channel accounts for only $\sim 9\%$ of the total current, which accounts for this observation.

We also examined the effect of the length of the BPE (L_{BPE}) on the performance of the hyPAD. This is an important parameter, because the potential difference between the two poles of the BPE (E_{BPE}), and hence the rate of the faradaic reaction, depends approximately on L_{BPE} and E_{tot} according to eq 4, where L_{channel} is the length of the channel.⁸

$$E_{\text{BPE}} = E_{\text{tot}} \times \frac{L_{\text{BPE}}}{L_{\text{channel}}} \quad (4)$$

Equation 4 indicates that if E_{tot} and L_{channel} are held constant, then a larger value of L_{BPE} results in higher E_{BPE} .³⁵ Therefore, by fabricating hyPADs with L_{BPE} ranging from 4.0 mm to 8.5 mm, it is possible to determine the effect of E_{BPE} on enrichment. Specifically, we determined the effect of L_{BPE} on $C_{\text{max}}^{\text{m}}$, and found that there is a positive correlation (Figure 4c): BPEs having longer lengths, up to 7 mm, lead to higher $C_{\text{max}}^{\text{m}}$. The increase in $C_{\text{max}}^{\text{m}}$ is likely caused by an increased rate of water reduction, leading to faster formation of the IDZ. $C_{\text{max}}^{\text{m}}$ does not continue to increase when $L_{\text{BPE}} > 7.0 \text{ mm}$ (which corresponds to $E_{\text{BPE}} > 26\text{V}$), possibly due to bubble generation. Accordingly, we settled on $L_{\text{BPE}} = 7.0 \text{ mm}$ as the optimum BPE length for the experiments described in the next section.

Enrichment of DNA, Proteins, and AuNPs Using the hyPAD.

To demonstrate the scope of hyPAD applications, we examined the enrichment of DNA, proteins, and AuNPs. For the enrichment of DNA, a solution containing 20 nM DNA-Cy5 was placed into the inlet of a hyPAD, $E_{\text{tot}} = 100$ V was applied, and then the enrichment process was imaged by fluorescence microscopy. The resulting images are shown in Figure 5a. As for BODIPY²⁻, DNA-Cy5 enriches just above the BPE cathode. A quantitative analysis of these results is provided in Figure S7a, where C_{ave} and C_{max} are plotted as a function of t . As discussed for BODIPY²⁻ (Figure 1b), the C_{ave} vs t curve remains close to zero for the first 50 s, then increases nearly linearly, and finally plateaus. The C_{max} vs t curve is somewhat noisy (probably due to bubble formation), but from the value of C_{max}^m we easily calculate that $EF_{\text{max}} = 264$.

The enrichment of a 10 nM solution of BSA-AF is represented by the fluorescence micrographs shown in Figure 5b. The experimental conditions for this experiment were the same as for DNA-Cy5 enrichment. As for BODIPY²⁻ and DNA-Cy5, BSAAF is enriched at a location just above the BPE cathode. Plots of C_{ave} and C_{max} as a function of t for this experiment are shown in Figure S7b. The characteristics of these data are similar to those of DNA-Cy5, but the value of EF_{max} is a little higher (325 vs 264).

Interestingly, both DNA-Cy5 and BSA-AF are enriched at a location similar to that of BODIPY²⁻, even though the three molecules have different electrophoretic mobilities.^{19,22} This behavior is different from results in conventional ICP or fICP, where molecules having different electrophoretic mobilities concentrate at different locations.^{23,36} This unique behavior is probably a result of the large channel dimensions of the hyPAD.

One final point regarding the fluorescence micrographs shown for DNA-Cy5 and BSA-AF: The region of the channel over which these analytes are enriched is significantly larger than for BODIPY²⁻. We suspect this is because the electrophoretic mobilities of DNA-Cy5 and BSA-AF are lower than BODIPY²⁻.^{19,22} As a result, electromigration in the IDZ, which is proportional to electrophoretic mobility, may be insufficiently strong to completely stop the movement of DNA-Cy5 or BSAAF (i.e., imperfect counterflow forces). Therefore, some DNACy5 and BSA-AF passes through IDZ and spreads over the BPE cathode.

Colorimetric assays based on AuNP labels are becoming commonplace due to their low cost and ability to be observed by simple visual inspection.³⁹ However, the electrokinetic enrichment of nanoparticles on paper is challenging, because in paper matrixes electrophoretic transport of nanoparticles is usually much slower than small molecules.⁴⁰ However, the EOF of hyPADs can be strong enough to transport AuNPs efficiently in the paper channel. Herein, we tested the ability of hyPADs to concentrate AuNPs and imaged the enrichment process using a cell phone camera.

For this experiment, the sample solution contained 1.0 nM AuNPs (determined by UV-vis spectroscopy), but otherwise the experimental conditions were the same as for Figure 1. To avoid aggregation in the relatively high salt solutions required for fICP, the AuNPs were functionalized with DNA using a pH-assisted method reported elsewhere (details provided

in the SI).^{32,41} A cell phone was used to track enrichment of AuNPs as a function of time under nearly identical lighting conditions.

Figure 5c presents photographs of AuNPs concentrating on a hyPAD as a function of time. Even with this primitive imaging technology, it is obvious that AuNPs, like the other analytes, accumulate near the BPE cathode. The purple color of the AuNPs does not change during the experiment, indicating they are stable (no aggregation) throughout the enrichment process. As for DNA-Cy5 and BSA-AF, the enriched AuNP region is broader than for BODIPY²⁻. This finding is consistent with the hypothesis discussed earlier that electromigration in the IDZ may not be strong enough to completely slow down the movement of AuNPs due to their low electrophoretic mobility.⁴⁰

SUMMARY AND CONCLUSIONS

To summarize, we have demonstrated enrichment of four analytes, BODIPY²⁻, DNA-Cy5, BSA-AF, and AuNPs, using fICP implemented on a hybrid (cellulose and nitrocellulose) paper analytical device. The hyPAD is inexpensive and easy to fabricate, and the throughput is two-orders of magnitude higher than that of conventional microfluidic devices.

We carried out several experiments to provide insights into the performance and operating characteristics of the hyPAD. Specifically, time-dependent enrichment studies indicated that no analyte accumulation occurs until ~50 s after application of the driving voltage. During this period, substantial current ($91 \pm 1\%$ of the total) is driven through the BPE, rather than the channel, resulting in formation of the IDZ. Electric field mapping reveals that enrichment is driven by the IDZ and the corresponding local electric field gradient at the edge of BPE cathode. Finally, we demonstrated the versatility of hyPADs by concentrating BODIPY²⁻ ($EF_{\max} = 490$ fold), DNA ($EF_{\max} = 264$ fold), proteins ($EF_{\max} = 325$ fold), and AuNPs (naked eye detection). This broad scope of analytes suggests that the hyPAD is a good candidate for coupling to conventional PADs to lower limits of detection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Enrichment of BODIPY²⁻ using a hyPAD. (a) Fluorescence micrographs of enriched BODIPY²⁻ in a hyPAD. A solution containing 100 mM Tris-HClO₄ and 20 nM BODIPY²⁻ was added at the inlet of hyPAD. A potential bias ($E_{\text{tot}} = 100$ V) was applied at $t = 0$ s. The gray vertical lines indicate the edges of the BPE cathode. (b) Plot of the average BODIPY²⁻ concentration (C_{ave}) vs t . The value of C_{ave} was obtained by integrating the BODIPY²⁻ fluorescence intensity near the BPE cathode of each micrograph in part a and comparing it to a BODIPY²⁻ fluorescence intensity calibration curve (Figure S2). The curve is divided into three distinct zones by dashed lines. (c) Plot of the maximum BODIPY²⁻ concentration (C_{max}) vs t . C_{max} was determined by comparing the camera pixel with the highest intensity (micrographs in part a) to the BODIPY²⁻ calibration curve in Figure S2. The maximum enrichment factor (EF_{max}) was calculated using the maximum value of C_{max} ($C_{\text{max}}^{\text{m}}$) divided by the initial BODIPY²⁻ concentration.

Figure 2.

(a) Plots of C_{ave} vs t for hyPAD enrichment experiments as a function of the initial BODIPY²⁻ concentrations (C_{init}). The solid lines represent the average C_{ave} obtained from three independent measurements. The error bars (shadowing) indicate the standard deviations. (b) Enrichment rate (ER) vs C_{init} for the three zones defined in Figure 1b. The ER is the slope (C_{ave}/t), obtained from the plots in part a divided by C_{init} . The error bars represent the standard deviations of three independent measurements. The dashed lines indicate the average ER values for the different C_{init} . (c) Plot of C_{ave} in the IDZ ($C_{ave, IDZ}$) vs t . The initial BODIPY²⁻ concentration was 350 nM, but other experimental conditions were the same as in Figure 1. The inset indicates the IDZ region from which the data were derived. The dashed line indicates the boundary between Zones 1 and 2.

Figure 3.

(a) Plots of $C_{\max}$ vs t for the indicated values of C_{init} . The experimental conditions were the same as in Figure 2. (b) Plot of $C_{\max}^{\text{m}}$ vs C_{init} . The values of $C_{\max}^{\text{m}}$ were obtained from part a and Figure S3. Error bars indicate the standard deviation from three independent measurements. The dashed line represents the best linear fit to the data points.

Figure 4.

Results obtained during BODIPY²⁻ enrichment experiments. (a) Plots of total current and BPE current vs time obtained using the experimental arrangement shown in Figure S5. The driving voltage ($E_{\text{tot}} = 100$ V) was turned on and off at the times indicated by the black arrows. Other experimental conditions were the same as in Figure 1. (b) Electric field measurements obtained using the modified four-layer hyPAD illustrated in Figure S6. Other experimental conditions were the same as in Figure 1. The blue and red traces show the electric field distribution when the BPE is present and absent, respectively. The orange bars at the bottom of the figure indicate the locations of the BPE cathode and anode. The measurements were made after the electric field profile had achieved steady state. (c) Plot of $C_{\max}^m$ vs BPE length (L_{BPE}). The data were obtained using hyPADs with L_{BPE} ranging from 4.0 mm to 8.5 mm. Other experimental conditions were the same as in Figure 1. The value of $C_{\max}^m$ was analyzed as in Figure 3. The error bars in parts b and c represent

standard deviations obtained from three independent measurements like the one shown in part a.

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Figure 5.

Enrichment of DNA-Cy5, BSA-AF, and AuNPs using hyPADs. (a) Fluorescence micrographs obtained for DNA-Cy5 enrichment as a function of time. The initial DNA-Cy5 concentration was 20 nM, but the other experimental conditions were the same as in Figure 1. (b) Fluorescence micrographs obtained for BSA-AF enrichment as a function of time. The initial BSA-AF concentration was 10 nM, but the other experimental conditions were the same as in Figure 1. (c) Cell phone photographs obtained for AuNP enrichment as a function of time. The initial concentration of DNA-stabilized AuNPs was 1.0 nM, but other experimental conditions were the same as in Figure 1. In all three frames, the vertical gray lines represent the edges of the BPE cathode.

Scheme 1.