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Ion-permeable Membrane for On-chip Preconcentration and Separation of Cancer Marker Proteins

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Abstract

Cancer marker proteins have been electrophoretically concentrated and then separated in a microfluidic device. On-chip preconcentration was achieved using an ion-permeable membrane, consisting of acrylamide, N,N'-methylene-bisacrylamide and 2-(acrylamido)-2-methylpropanesulfonate. This negatively charged membrane was photopolymerized in the microdevice near the injection intersection. Anionic proteins were excluded from the porous membrane based on both size and charge, which concentrated target components in the injection intersection prior to separation by microchip capillary electrophoresis (μ -CE). Bovine serum albumin (BSA) was used in initial characterization of the system and showed a 40-fold enrichment in the μ -CE peak with 4 min of preconcentration. Adjustment of buffer pH enabled baseline resolution of two cancer biomarkers, α -fetoprotein (AFP) and heat shock protein 90 (HSP90), while fine control over preconcentration time limited peak broadening. Our optimized preconcentration and μ -CE approach was applied to AFP and HSP90, where enrichment factors of >10-fold were achieved with just 1 min of preconcentration. Overall, the process was simple and rapid, providing a useful tool for improving detection in microscale systems.

INTRODUCTION

Cancer, a malignant and invasive growth of cells in the body, is the second most common cause of death in the US [1]. Survival rates in some cancer types have improved over the years due, in part, to detection at an early stage when the cancer can be easily treated [2–4]. The development of cost-effective techniques that are sensitive and specific enough to diagnose cancer at an earlier stage than is currently possible will further improve cancer survivability. Cancer biomarkers have been shown to enable early detection, and facilitate the prognosis and monitoring of the response to cancer therapy [2]. However, of the numerous proteins proposed as cancer biomarkers only nine have been approved by the FDA [5], one of which is α -fetoprotein (AFP), a diagnostic marker for hepatocellular carcinoma. AFP is a 67 kDa glycoprotein which has high levels in fetal sera [6]. Its concentration decreases to trace levels soon after birth; therefore, raised AFP levels in adult serum usually indicate a disease state. Another biomarker of interest is heat shock protein 90 (HSP90), a 90 kDa molecular chaperone that oversees the proper folding of newly formed proteins. Many HSP90 clients are proteins whose mutation or overproduction promotes cancer, making HSP90 inhibition a target in cancer therapy [7, 8]. Even though HSP90 is presently not FDA approved, its elevated levels in many human cancer types indicate potential utility for diagnosis and the monitoring of response to treatment [9]. Enhanced detection of these and other biomarkers is thus important in early detection of cancer and follow-up of cancer therapy.

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A diagnostic technique that is applicable to point-of-care (POC) settings needs to be, fast, sensitive and quantitative [10]. The method commonly used for protein detection is ELISA, which is effective for a large number of samples and thus best suited for clinical settings rather than POC analysis [10]. Microfluidic devices are attractive for POC analysis because they offer the benefits of portability, minimal solvent and reagent consumption, and speed [11]. Microchip capillary electrophoresis (μ -CE) in particular has been successfully applied to the analysis of different types of molecules, including cancer biomarkers [12–14]. An appealing aspect of miniaturization is integration: various components can easily be combined in these devices to perform multiple tasks including analyte extraction, control of fluidic movement and sample preconcentration. While microfluidics improves on the slower analysis times offered by ELISA [10], the small volumes and path lengths can lead to reduced sensitivity. Therefore, microfluidic assays could benefit considerably from integrated methods to improve the limits of detection [15].

Several stacking procedures have been successfully used for sample preconcentration in a microfluidic format including field amplified sample stacking [16], field amplified sample injection [17], isotachopheresis (ITP) [18, 19], electric field gradient focusing [20], temperature gradient focusing (TGF) [21], isoelectric focusing (IEF) [22], electrokinetic supercharging [23], and sweeping [24]. Sometimes two of these techniques can be combined for improved results. Munson, et al. [21] combined a form of sample stacking, field amplified continuous sample injection, with TGF. The combination of electrokinetic injection with transient ITP has also been coupled with gel electrophoresis [18]. While methods like ITP and IEF may be compatible with μ -CE, they often require two or three different buffers, which complicate execution in a microchip format. Moreover, the required coupling of many of these techniques with microchip gel electrophoresis renders the overall separation process more challenging. A technique known as electrophoretic exclusion has recently been used to achieve both selectivity and enrichment of proteins by manipulating voltage in combination with hydrodynamic flow [25]. The simplicity of this method promises potential for future application in a microchip format.

Other preconcentration methods for microfluidic systems involve affinity techniques like solid phase extraction (SPE) [26], and exclusion methods based on nanogaps [27, 28] or nanoporous filters [15, 29–35]. In affinity methods the analyte is generally made to adsorb to a column, and a different solution is used to elute the retained components. Affinity techniques are effective but are complicated by the need for different buffers to be used in pretreatment and analysis. Enrichment based on exclusion generally offers a simpler analysis setup, but a more complex device fabrication process. Nanogaps can be formed via photolithography [29] or by applying a high voltage to a PDMS-glass device to cause dielectric breakdown [27, 28]. In nanogap devices, preconcentration without subsequent separation has been done but the process was lengthy (30–60 min) [28]. In contrast, membrane-based preconcentration methods are generally faster than with nanogaps, and the fabrication methods have a range of complexity. Some materials used to fabricate these membranes are polycarbonate (PC) [33], track-etched PC [32, 36], titania [29], nafion [37], and silica gel [31]. These membrane materials were anionic to concentrate analytes based on charge exclusion. For several device arrangements preconcentration only, without protein separation, was done [29, 32, 33, 37]. Long, et al. [36] used a track-etched PC membrane to concentrate rhodamine 123 and FITC-labeled ephedrine samples 1000-fold by SPE- μ CE; however, the technique required careful timing of online injection after the SPE process. Foote, et al. [31] used a porous silica membrane in a glass microchip to obtain signal enhancement of ~600-fold for on-chip preconcentration followed by sodium dodecyl sulfate (SDS) – microchip gel electrophoresis separation. However, the use of glass devices and the coupling of a preconcentration membrane with gel-based separation require a more complicated fabrication process.

Hydrogel membranes involving acrylic monomers offer a special type of preconcentration system and can be either neutral or charged [15, 30, 34, 35]. The pore size of these membranes and their mechanical properties can be controlled by varying the ratio of cross-linking agent to acrylamide. Hatch, et al. [15] integrated two acrylic polymer hydrogel structures in a glass device: one for size-based preconcentration (up to 1000-fold) and the other to separate the preconcentrated sample by SDS-gel electrophoresis. However, the fabrication of two different gels in a microdevice was somewhat complicated, and the proteins analyzed had to be denatured to achieve separation. Addition of an ionic comonomer, such as 2-acrylamido-2-methylpropanesulfonic acid (AMPS), to acrylamide imparts a negative charge and enables the membrane to be ion-selective [38]. This type of polymer membrane has been used for the preconcentration of model proteins [30], but in that work preconcentration was not coupled to separation. In addition, Chun et al. [35] fabricated a glass microchip with a hydrogel made entirely of crosslinked AMPS; however, protein separation was not performed in conjunction with preconcentration. Yamamoto et al. [34] made a polymer membrane combining acrylamide and AMPS, and used it to couple up to 10⁵-fold preconcentration with μ -CE of separate samples of oligosaccharides, α 1-acid glycoprotein, and glycopeptides, but not to analyze a mixture of proteins. Their monomer and crosslinker concentrations were high at 26% T and 20% C; moreover, the standard device layout did not make it easy to remove monomer solution after membrane polymerization. In summary, hydrogel-based microchip preconcentration systems show promise in their ability to be fine-tuned easily, but simplifying fabrication procedures and application to the separation of proteins still needs further effort.

In this work, a polymer membrane was fabricated *in situ* and used for on-line preconcentration prior to separation of cancer marker proteins. The membrane consisted of acrylamide, N,N'-methylene-bisacrylamide and AMPS, and was photopolymerized in the microdevice near the injection intersection region. Negatively charged proteins were excluded from the porous membrane, enabling their enrichment. Initial characterization of the device with bovine serum albumin (BSA) led to a 40-fold enrichment in the μ -CE peak with 4 min of preconcentration time. Fine-tuning of the buffer pH provided baseline resolution of model cancer marker proteins, and careful control of preconcentration time kept peak broadening to a minimum. More than 10-fold enhancement of μ -CE signal in a protein mixture was achieved with just 1 min of preconcentration, and the entire analysis was completed in <5 min. Our approach provides a simple and fast route to the analysis of low-concentration samples.

EXPERIMENTAL SECTION

Reagents and materials

The monomers acrylamide and AMPS, as well as the cross-linking agent N,N'-methylene-bisacrylamide were purchased from Sigma-Aldrich (St. Louis, MO). Riboflavin was obtained from Eastman (Rochester, NY), tetramethylethylenediamine (TEMED) was from Invitrogen (Carlsbad, CA), and ammonium persulfate (APS) was obtained from EMD Chemicals (Gibbstown, NJ). Bovine serum albumin (BSA) was purchased from New England Biolabs (Ipswich, MA), heat shock protein 90 (HSP90) was from Sigma-Aldrich and alpha-fetoprotein (AFP) was from Lee Biosolutions Inc. (St Louis, MO). The proteins were labeled with fluorescein isothiocyanate (FITC) or Alexa Fluor 488 TFP ester purchased from Invitrogen. Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich. Hydroxypropyl cellulose (HPC, average MW 100 000) was from Aldrich (Milwaukee, WI). 10 X PBS buffer (pH 7.4), anhydrous sodium carbonate, sodium bicarbonate, and sodium azide were purchased from EMD Chemicals. Amicon Ultra-0.5 centrifugal filter devices were obtained from Millipore (Billerica, MA).

Device fabrication

The microchips were made from poly(methyl methacrylate), PMMA, using a combination of photolithographic techniques, hot embossing and thermal bonding as previously described [39, 40]. The microchip design, shown in Figure 1A, is similar to an offset-T device, except for the addition of two reservoirs (4 and 6) and the channels leading to them. The channel connected to reservoir 4 helped to empty monomer solution from the channel leading to reservoir 2 after polymerization of the membrane. The channel leading to reservoir 6 provided for cross-injection, as an alternative to offset-T injection. The channels were ~15 μm deep and ~50 μm wide.

Before gel polymerization the microchannels were conditioned sequentially with 0.1 M HCl and 0.1 M NaOH, rinsed with deionized water and dried with vacuum. This made the channel surface more hydrophilic and improved adhesion of the membrane to the channel walls [41, 42]. The pore size of the membrane depends on the total amount of acrylamide present (%T), and the amount of cross-linker (%C), where T is percentage of acrylamide, bisacrylamide and AMPS, expressed in grams per 100 mL of mixture, and C is the percentage of bisacrylamide in the total monomer content. To fabricate the membrane, an 8% T and 5% C monomer solution was prepared. The mass percent of AMPS in the total mixture was 0.5%. The solution was filtered with a 0.2 μm syringe filter and degassed. To initiate polymerization, riboflavin and APS were added to a final mass percent of 0.004% and 0.008%, respectively, followed by addition of 1.5 μL of TEMED per mL of monomer solution. A 10 μL aliquot of the monomer solution was immediately placed in reservoir 3 and left for a few seconds to fill the microchip by capillary action, after which the remaining solution in reservoir 3 was removed to prevent hydrodynamic flow during photopolymerization. A 488 nm laser beam (1 mW) was focused on the membrane location for ~45 s to produce a polymer membrane with diameter of ~60 μm (Figure 1B). The unpolymerized monomer solution was then removed from the chip using vacuum, and the channels were rinsed with deionized water.

Fluorescent labeling

The protein solutions were prepared in carbonate buffer, pH 9.2, at a concentration of 2 mg/mL. 2 mg of FITC was dissolved in 100 μL of anhydrous DMSO. This FITC solution (10 μL) was added to 250 μL of protein sample and incubated in the dark, first for 3 hr at room temperature and then overnight at 4 $^{\circ}\text{C}$. For labeling with Alexa Fluor 488 TFP ester, the dye was dissolved in DMSO to a concentration of 10 mg/mL. Dye solution (5 μL) was added to 250 μL of sample and incubated in the dark for 1 hr at room temperature. The unconjugated dye in each case was separated from the protein samples by diafiltration, using an Amicon Ultra-0.5 centrifugal filter device (30 kDa MWCO) and 10 mM PBS, pH 7.4. The labeled protein was collected, and sodium azide was added to a final concentration of 2 mM. The fluorescently labeled samples were stored in the dark at 4 $^{\circ}\text{C}$ until used.

Electrophoresis experiments

The separation buffer for single-component samples was 10 mM carbonate (pH 9.2) containing 0.1% HPC, while that for the protein mixture was 10 mM phosphate (pH 7.0) containing 0.05% HPC. Buffer solution was filled in the channels and all reservoirs, except reservoir 1 which was filled with the sample solution (20 μL). Platinum electrodes were placed in reservoirs 1, 2, 3 and 5. The electrodes were connected to high voltage power supplies (Stanford Research Systems, Sunnyvale, CA) via a custom-built switch. Electrophoresis involved a standard “pinched injection” with an offset-T layout [43, 44]. For injection, reservoirs 1, 3, and 5 were grounded while 600 V were applied to reservoir 2. For separation, reservoir 3 was grounded, 600 V were applied to reservoirs 1 and 2, and 1600 V were applied to reservoir 5.

The laser-induced fluorescence system used to detect the analytes has been described previously [39]. Briefly, sample excitation was done with a 488 nm laser (Ar ion) focused at a spot in the channel close to reservoir 5 using a 20 $\times$ 0.45 NA objective. Emitted photons were detected (after spectral and spatial filtering) with a photomultiplier tube, then amplified and filtered, and finally recorded on a computer. The data sampling rate was 20 Hz

Data analysis

Calculation of concentrations was based on peak areas, obtained by subtracting the background individual signal values from a given peak and then summing the results. For comparison, the peak height was obtained by subtracting the baseline from the peak maximum. For preconcentration of HSP90 and AFP, control data were obtained from similar chips without a preconcentration membrane. The peak areas or heights obtained after preconcentration were then divided by the corresponding areas or heights before preconcentration to determine the amount of preconcentration that occurred.

RESULTS AND DISCUSSION

The devices used for these experiments were designed to provide a good yield during polymerization of the preconcentration membrane. When a simple offset T design was used, it was difficult to empty the channel arm of the microchip leading to reservoir 2 (Figure 1A) after polymerization of the gel. Therefore, a channel connected to reservoir 4 was added to facilitate the flushing process after polymerization. Our device layout can also be used for either cross-injection (sample in reservoir 6) or offset-T injection (sample in reservoir 1). In the experiments reported here, offset-T injection was used to increase sample plug volume.

The membrane was photopolymerized in the injection channel just beyond the intersection region (Figure 1B). The properties of acrylamide gels were found to depend not only on the monomer composition but also on polymerization conditions. When riboflavin only was used as the initiator, polymerization was much slower (5–10 min), and the resulting membrane was more porous and less stable. Addition of APS decreased polymerization time and made the process more reproducible. In addition, when these two initiators were combined the total amount of initiator was lower, decreasing undesirable side effects caused by excess initiator concentration [45]. The diameter of our membrane was about $\sim 60\text{ }\mu\text{m}$. Larger membranes increased the electrical resistance, leading to a higher voltage drop across the membrane, which negatively affected separation efficiency [15]. The apparent pore radius for a 10.5% T, 5% C gel was reported to be 21 nm [46], so our 8% T, 5% C gel is estimated to have a pore radius somewhat larger than that.

BSA was used initially to test the effectiveness of the preconcentration membrane. The buffer was 10 mM carbonate, pH 9.2 with 0.1% HPC added to suppress electroosmotic flow (EOF). Previous experiments had shown that 0.5% HPC was effective in suppressing EOF to yield reproducible results [40]; however, 0.5% HPC was found to block the membrane, resulting in poor separation. Thus, lower HPC concentrations were used (0.05–0.1%) which did not block the membrane but still provided adequate separation efficiency.

Electropherograms of increasing concentrations of BSA (Figure 2A) show corresponding increases in peak area. Figure 2B shows μ -CE of 5 nM BSA without preconcentration, along with other electropherograms after on-chip preconcentration times of 30 s to 4 min. Preconcentration times of 30 s to 1 min produce a ~ 10 -fold enhancement of signal without compromising the peak shape. A larger, 20-fold enhancement was produced with 2 min preconcentration, but peak tailing was beginning to be evident. By 4 min of preconcentration a significant degree of peak tailing occurred with the ~ 40 -fold enrichment. Thus, enrichment

factors of at least 10 can be obtained quickly and without distortion of peak shape; even higher factors (~40) can be achieved if some peak shape distortion can be tolerated.

Peak tailing at longer preconcentration times could be caused by concentration polarization (CP), which occurs when current traverses an ion-selective membrane [47]. Increased cation and anion concentrations on alternate sides of the membrane make a concentration gradient that increases the electrical resistance across the membrane, resulting in a local voltage drop [48]. This voltage drop reduces the effectiveness of the potentials applied to reservoirs 1 and 2 during separation to prevent sample leakage and pull remaining analyte out from the intersection [49], leading to peak tailing. Since this CP-induced voltage drop increases as a function of preconcentration time [15, 48], most of our experiments were carried out with a preconcentration time of ~1 min to limit these tailing issues.

Quantitation of the BSA samples was done on the basis of peak area, which increased linearly as a function of concentration, as seen in Figure 3A. Figure 3B similarly shows a linear increase in BSA peak area with preconcentration time for the range of 0.5 to 4 min. A 5 min preconcentration time (result not shown) resulted in a peak with severe tailing and a height that was lower than that in the 4 min result. At this point the negative factors associated with a long preconcentration time became apparent. This tailing puts an upper limit of ~4 min on the preconcentration time, to allow for adequate separation performance in μ -CE.

The preconcentration and separation conditions optimized for BSA were next applied to AFP and HSP90. Preconcentration of 5 nM HSP90 having a small amount of FITC (Figure 4A) was done for 4 min, resulting in ~80-fold enrichment of the HSP90 peak in the electropherogram. The enriched peak was symmetrical, but its migration time was somewhat slower than in the separation without preconcentration. The slower migration time was likely due to some CP occurring during this longer preconcentration time, as noted above. The FITC peak in the separation without enrichment was almost undetectable; however, the peak became readily observable after preconcentration, with ~20 fold enhancement. Even though the pore size of the membrane was large enough to allow the passage of FITC, electrostatic repulsion between FITC and the AMPS in the membrane allowed some preconcentration. The preconcentration of 10 nM AFP for 1 min in Figure 4B yielded a symmetrical peak with an enrichment factor of 16. The AFP peak was somewhat broader and migrated slower than the HSP90 peak in the separation without preconcentration [12]. To determine if the peak width of AFP was influenced by the FITC tags, AFP was also labeled with Alexa Fluor 488. The μ -CE peak produced by Alexa Fluor 488-labeled AFP was ~2 s narrower than that of FITC-labeled AFP (Figure 5), most likely due to reduced impacts of multiple site labeling. However, the electrophoretic mobility of Alexa Fluor 488-labeled AFP was slightly faster than FITC-labeled AFP, so when run with HSP90 there was a higher degree of peak overlap.

We found that the resolution of AFP and HSP90 depended on the pH. Poor resolution was obtained when electrophoresis was carried out in 10 mM carbonate buffer (pH 10.5) but resolution improved to near baseline at pH 9.2, with baseline resolution being achieved with a pH of ~7 (Figure 6). Liu, et al. [50] likewise noticed an improvement in protein resolution at lower pH, which they believed was caused by an increase in the charge difference between proteins at pH values near the pI.

Preconcentration and separation of mixtures of different concentrations of HSP90 and AFP (Figure 7) show well-resolved peaks with 1 min preconcentration time. The enrichment factors for HSP90 and AFP in the 10 nM mixture were 10- and 16-fold, respectively, while those for the 20 nM mixture were 7- and 13-fold, respectively. Thus, a slightly higher

enrichment factor was achieved with lower sample concentrations. AFP also showed a higher level of preconcentration compared to HSP90, which could be attributed to factors such as size, charge, or mobility. The diagnostic threshold for AFP is ~0.3 nM (20 ng/mL) [12], while normal serum levels of HSP90 are ~0.2 nM (20 ng/mL) [51, 52]. Our detection limits with this preconcentration technique are ~0.6 nM for AFP and ~0.06 nM for HSP90. Thus, we can readily detect normal HSP90 levels, and we are only a factor of two above the AFP diagnostic threshold. An additional increase in enrichment factors of tenfold or more could be achieved readily by optimizing the pore size and charge of the membrane such that longer preconcentration times could be used without compromising separation efficiency. Moreover, integration of our preconcentration method with on-chip affinity extraction [12] would lower the detection limits to well below the normal serum levels. Importantly, our present results demonstrate a rapid and simple procedure by which multiple cancer biomarkers can be concentrated ~10-fold or more with a straightforward, 1 min process prior to μ -CE analysis.

CONCLUSIONS

Cancer marker proteins have been electrophoretically concentrated and separated in a microdevice using a simple and quick method. On-chip preconcentration was achieved at an ion-permeable membrane formed by *in situ* photopolymerization just beyond the injection intersection in the microchip. Baseline resolution of two cancer marker proteins with similar electrophoretic mobilities was accomplished at pH 7.0. A >10-fold increase in the signal of these proteins was achieved under our optimized conditions with just a 1 min preconcentration time. Such signal enhancement offers improved limits of detection that are essential in clinical diagnosis where target proteins can be present in low concentrations. The preconcentration and separation process carried out with our device is simple, fast, and generalizable. The simplicity and speed of analysis provide good potential for application in POC analysis. The membrane used in this device could easily be coupled to a suitable pretreatment technique, such as affinity extraction [12], for the analysis of clinically significant biomolecules in a complex sample matrix. This technique offers the potential for enhanced analysis of multiple cancer biomarkers, which should facilitate diagnosis and monitoring of response to treatment.

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REFERENCES

1. [Accessed 12/02/2010] Statistic provided by the American Cancer Society. at <http://www.cancer.org/Research/CancerFactsFigures/CancerFactsFigures/cancer-facts-and-figures-2010>
2. Tainsky MA. Biochim. Biophys. Acta. 2009; 1796:176–193. [PubMed: 19406210]
3. Tchagang AB, Tewfik AH, DeRycke MS, Skubitz KM, Skubitz APN. Mol. Cancer Ther. 2008; 7:27–37. [PubMed: 18187805]
4. Gromov P, Gromova I, Bunkenborg J, Cabezon T, Moreira JMA, Timmermans-Wielenga V, Roepstorff P, Rank F, Celis JE. Mol. Oncol. 2010; 4:65–89. [PubMed: 20005186]
5. Polanski M, Anderson NL. Biomark Insights. 2006; 1:1–48. [PubMed: 19690635]
6. Uversky VN, Narizhneva NV, Ivanova TV, Kirkitadze MD, Tomashevski AY. FEBS Lett. 1997; 410:280–284. [PubMed: 9237645]
7. Yi F, Regan L. ACS Chem. Biol. 2008; 3:645–654. [PubMed: 18785742]

8. Biamonte MA, Van de Water R, Arndt JW, Scannevin RH, Perret D, Lee W-C. J. Med. Chem. 2010; 53:3–17. [PubMed: 20055425]
9. Bagatell R, Whitesell L. Mol. Cancer Ther. 2004; 3:1021–1030. [PubMed: 15299085]
10. Heath JR, Davis ME. Annu. Rev. Med. 2008; 59:251–265. [PubMed: 17937588]
11. Zhang JY, Do J, Premasiri WR, Ziegler LD, Klapperich CM. Lab Chip. 2010; 10:3265–3270. [PubMed: 20938505]
12. Yang W, Yu M, Sun X, Woolley AT. Lab Chip. 2010; 10:2527–2533. [PubMed: 20664867]
13. Minarik M, Gassman M, Belsanova B, Pesek M, Schouten J, Chudoba R, Gas B, Benesova L. Electrophoresis. 2010; 31:3518–3524. [PubMed: 20967766]
14. Zhang S, Cao W, Li J, Su M. Electrophoresis. 2009; 30:3427–3435. [PubMed: 19802853]
15. Hatch AV, Herr AE, Throckmorton DJ, Brennan JS, Singh AK. Anal. Chem. 2006; 78:4976–4984. [PubMed: 16841920]
16. Shiddiky MJA, Park H, Shim Y-B. Anal. Chem. 2006; 78:6809–6817. [PubMed: 17007500]
17. Gong M, Wehmeyer KR, Limbach PA, Arias F, Heineman WR. Anal. Chem. 2006; 78:3730–3737. [PubMed: 16737230]
18. Wainright A, Nguyen UT, Bjornson T, Boone TD. Electrophoresis. 2003; 24:3784–3792. [PubMed: 14613206]
19. Wang J, Zhang Y, Mohamadi MR, Kaji N, Tokeshi M, Baba Y. Electrophoresis. 2009; 30:3250–3256. [PubMed: 19722200]
20. Sun X, Farnsworth PB, Woolley AT, Tolley HD, Warnick KF, Lee ML. Anal. Chem. 2008; 80:451–460. [PubMed: 18081261]
21. Munson MS, Danger G, Shackman JG, Ross D. Anal. Chem. 2007; 79:6201–6207. [PubMed: 17616169]
22. Shimura K, Takahashi K, Koyama Y, Sato K, Kitamori T. Anal. Chem. 2008; 80:3818–3823. [PubMed: 18407668]
23. Xu Z, Ando T, Nishine T, Arai A, Hirokawa T. Electrophoresis. 2003; 24:3821–3827. [PubMed: 14613211]
24. Pan Q, Zhao M, Liu S. Anal. Chem. 2009; 81:5333–5341. [PubMed: 19492801]
25. Meighan MM, Vasquez J, Dziubcynski L, Hews S, Hayes MA. Anal. Chem. 2010 Article ASAP, 10.1021/ac1025495.
26. Yu C, Davey MH, Svec F, Fréchet JMJ. Anal. Chem. 2001; 73:5088–5096. [PubMed: 11721904]
27. Kim SM, Burns MA, Hasselbrink EF. Anal. Chem. 2006; 78:4779–4785. [PubMed: 16841895]
28. Lee JH, Chung S, Kim SJ, Han J. Anal. Chem. 2007; 79:6868–6873. [PubMed: 17628080]
29. Hoeman KW, Lange JJ, Roman GT, Higgins DA, Culbertson CT. Electrophoresis. 2009; 30:3160–3167. [PubMed: 19722198]
30. Song S, Singh AK, Kirby BJ. Anal. Chem. 2004; 76:4589–4592. [PubMed: 15283607]
31. Foote RS, Khandurina J, Jacobson SC, Ramsey JM. Anal. Chem. 2005; 77:57–63. [PubMed: 15623278]
32. Long Z, Liu D, Ye N, Qin J, Lin B. Electrophoresis. 2006; 27:4927–4934. [PubMed: 17117457]
33. Wu D, Steckl AJ. Lab Chip. 2009; 9:1890–1896. [PubMed: 19532964]
34. Yamamoto S, Hirakawa S, Suzuki S. Anal. Chem. 2008; 80:8224–8230. [PubMed: 18841941]
35. Chun H, Chung TD, Ramsey JM. Anal. Chem. 2010; 82:6287–6292. [PubMed: 20575520]
36. Long Z, Shen Z, Wu D, Qin J, Lin B. Lab Chip. 2007; 7:1819–1824. [PubMed: 18030406]
37. Shen M, Yang H, Sivagnanam V, Gijs MAM. Anal. Chem. 2010; 82:9989–9997. [PubMed: 20964443]
38. Travas-Sejdic J, Eastal A. Polym. Gels Networks. 1997; 5:481–502.
39. Kelly RT, Woolley AT. Anal. Chem. 2003; 75:1941–1945. [PubMed: 12713054]
40. Sun X, Yang W, Pan T, Woolley AT. Anal. Chem. 2008; 80:5126–5130. [PubMed: 18479142]
41. Diaz-Quijada GA, Peytavi R, Nantel A, Roy E, Bergeron MG, Dumoulin MM, Veres T. Lab Chip. 2007; 7:856–862. [PubMed: 17594004]
42. Chen R, Guo H, Shen Y, Hu Y, Sun Y. Sens Actuators B. 2006; 114:1100–1107.

43. Jacobson SC, Hergenröder R, Koutny LB, Ramsey JM. *Anal. Chem.* 1994; 66:1114–1118.
44. Yang W, Sun X, Pan T, Woolley AT. *Electrophoresis.* 2008; 29:3429–3435. [PubMed: 18702050]
45. Menter, P. *Bio-Rad Bulletin* 1156. Hercules, CA 94547 USA: Bio-Rad Laboratories; 2000.
46. Stellwagen NC. *Electrophoresis.* 1998; 19:1542–1547. [PubMed: 9719523]
47. Nischang I, Reichl U, Seidel-Morgenstern A, Tallarek U. *Langmuir.* 2007; 23:9271–9281. [PubMed: 17683148]
48. Dhopeswarkar R, Crooks RM, Hlushkou D, Tallarek U. *Anal. Chem.* 2008; 80:1039–1048. [PubMed: 18197694]
49. Currie CA, Heineman WR, Halsall HB, Seliskar CJ, Limbach PA, Arias F, Wehmeyer KR. *J. Chromatogr. B.* 2005; 824:201–205.
50. Liu Y, Foote RS, Culbertson CT, Jacobson SC, Ramsey RS, Ramsey JM. *J. Microcolumn Sep.* 2000; 12:407–411.
51. Sun Y, Zang Z, Xu X, Zhang Z, Zhong L, Zan W, Zhao Y, Sun L. *Int. J. Mol. Sci.* 2010; 11:1423–1433. [PubMed: 20480028]
52. Szerafin T, Hoetzenecker K, Hacker S, Horvath A, Pollreisz A, Arpad P, Mangold A, Wlisczak T, Dworschak M, Seitelberger R, Wolner E, Ankersmit HJ. *Ann. Thorac. Surg.* 2008; 85:80–87. [PubMed: 18154785]

Figure 1.

Photograph of a microfluidic device and zoom view of a preconcentration membrane. (A) Photograph of the microfluidic device used for sample preconcentration. Reservoir labels are 1, sample; 2, sample waste; and 3–6, buffer. The channel connected to reservoir 4 helped to empty the monomer solution from the channel leading to reservoir 2 after polymerization of the gel. The channel leading to reservoir 6 provides cross-T injection while the channel connected to reservoir 1 provides offset-T injection. (B) Photomicrograph of microchannel intersection region showing position of the polymerized membrane, indicated by the black arrow.

Figure 2.

Dependence of peak width on sample concentration and preconcentration time. (A) Dependence of peak shape on injected BSA concentration during μ -CE. BSA concentrations are: 1 nM, black; 5 nM, blue; 20 nM, red; 50 nM, green. (B) Effect of preconcentration time on BSA signal during μ -CE; all traces are for 5 nM initial BSA concentration. The preconcentration times for the curves are: 0 min (no preconcentration), black; 0.5 min, blue; 1 min, red; 2 min, green; 4 min, violet. All electropherograms are offset vertically for clarity.

Figure 3.

Dependence of μ -CE peak area on BSA concentration and preconcentration time. (A) Plot of peak area as a function of BSA concentration. The slope is 0.71 ± 0.02 , and the intercept is -0.21 ± 0.16 , with $R^2 = 0.9972$. (B) Plot of peak area as a function of preconcentration time. The slope is 15.10 ± 0.60 , and the intercept is -0.7 ± 1.5 , with $R^2 = 0.9959$.

Figure 4.

Preconcentration and μ -CE of cancer-related proteins. (A) Preconcentration of 5 nM HSP90. The peak at ~23 s is FITC. HSP90 (peak at ~42 s) is concentrated ~80-fold with the 4 min preconcentration time. (B) Preconcentration of 10 nM AFP. AFP is concentrated ~15-fold in 1 min. In both (A) and (B) the trace in black represents μ -CE without preconcentration, while the blue trace denotes preconcentration followed by μ -CE separation.

Figure 5. Electropherograms of 50 nM AFP labeled with FITC (bottom) and Alexa Fluor 488 TFP ester (top). Traces are offset vertically. AFP labeled with FITC was about ~2 s broader than that labeled with Alexa Fluor 488. Peak intensity was ~2-fold higher with Alexa Fluor 488-labeled AFP than the FITC-tagged protein, and this peak also had a higher electrophoretic mobility.

Figure 6.
Effect of pH on resolution of a mixture of HSP90 and AFP. The pHs were from bottom to top: 7.0, 9.2, 10.0, and 10.5. Resolution is improved at lower pHs.

Figure 7.

Preconcentration and μ -CE of cancer-related proteins. Mixture of HSP90 and AFP; (A) 10 nM and (B) 20 nM. Preconcentration time was 1 min. The black traces represent μ -CE without preconcentration, while the blue traces denote preconcentration followed by separation. Enrichment factors are given in the text.