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Western Blotting Using Microchip Electrophoresis Interfaced to a Protein Capture Membrane

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Abstract

Western blotting is a commonly used assay for proteins. Despite the utility of the method, it is also characterized by long analysis times, manual operation, and lack of established miniaturized counterpart. We report a new way to Western blot which addresses these limitations. In the method, sodium dodecyl sulfate (SDS)-protein complexes are separated by sieving electrophoresis in a microfluidic device or chip. The chip is interfaced to a moving membrane so that proteins are captured in discrete zones as they migrate from the chip. Separations of SDS-protein complexes in the molecular weight range of 11 to 155 kDa were completed in 2 min with 4×10^4 theoretical plates at 460 V/cm. Migration time and peak area relative standard deviations were 3–6% and 0.2% respectively. Detection limit for actin was 0.7 nM. Assays for actin, AMP-kinase, carbonic anhydrase, and lysozyme are shown to demonstrate versatility of the method. Total analysis time including immunoassay was 22–32 min for a single sample. Because processing membrane for immunoassay is the slow step of the assay, sequential injections from different reservoirs on the chip and capture in different tracks on the same membrane allow increased throughput. As a demonstration, 9 injections were collected on one membrane and analyzed in 43 min (~5 min/sample). Further improvements in throughput are possible with more reservoirs or parallel channels.

Introduction

Western blotting combines electrophoretic sieving with immunoassay to provide semi-quantitative assay of proteins.^{1–2} The method has become a workhorse in biochemical research and clinical laboratories because it provides high selectivity of both separation and immunoassay. Fractionation prior to immunoassay overcomes interference by non-specific binding of antibodies, even in complex mixtures, and allows probing of multiple isoforms of a protein with a single antibody. Western blotting is also reliable, robust, and easily optimized. The primary weaknesses of the method are that it is slow (often taking 8–20 h), requires significant manual intervention, and has no established microscale counterpart preventing application when sample is limited.

The past few years have seen significant success in improving different aspects of Western blotting.^{3–12} Methods to improve the application of blocking agents and antibody have

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Supporting Information

Supporting information contains figures illustrating effects of stage moving rate on separation resolution, comparison of ladder separation captured on membranes using different separation electric fields as well as electropherograms of different injections showing peak migration time shifts. Relationship of fluorescent signal to protein concentration and line scan from the membrane used to calculate signal to noise ratio are also included in supporting information. An overlaid plot used to estimate the molecular weight of AMPK is also included. This material is available free of charge via the Internet at <http://pubs.acs.org>.

decreased time required for the immunoassay step.^{3, 12} Integrated microfluidic systems in which proteins are sieved on a chip and then directed towards an antibody capture region on-chip have demonstrated Western blot-like results in a rapid, miniaturized format.⁴⁻⁶ While fast and sensitive, these systems require covalent antibody immobilization on the chip and/or use of pre-labeled proteins which may be limiting in some settings and result in single-use chips. Another approach has been to separate proteins by capillary isoelectric focusing (IEF) or chip sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then immobilize them by photo cross-linking to the capillary wall or gel.⁷⁻⁹ Immunoassay is performed by passing primary and secondary antibodies through the separation channel followed by imaging detection. These methods show good performance and replicate the selectivity of Western blot. Further, they allow parallelization (e.g., 48 channels for the sieving example) for good throughput.⁹ These systems are highly promising, but rely on single use chips or capillaries which may increase cost.

We have previously described a hybrid approach in which sieving capillary electrophoresis is interfaced to a blotting membrane so that separated proteins are captured as they exit the column.¹⁰ The resulting immobilized proteins are then detected using a rapid immunoassay. The method uses relatively fast CE separation (40 min) and blotting during separation to improve the speed of the overall assay.

In this work, we extend this approach by using a microfabricated chip for sieving separation. The use of a microfluidic system greatly improves the speed of separation, automates injection, and improves reliability and performance of interface to membrane. Multiple injections and separations using the same channel and capture membrane are demonstrated showing potential of improved throughput and economical use of chips by reuse.

Experimental Section

Materials and Reagents

Fluorescein isothiocyanate (FITC), FITC protein ladder, and Alexa Fluor® 532 goat anti-rabbit secondary antibody were purchased from Invitrogen (Carlsbad, CA). Polyvinylidene fluoride (PVDF) membranes were purchased from Millipore (Billerica, MA). Rabbit anti-lysozyme and anti-actin were purchased from Sigma (St. Louis, MO). Rabbit anti-AMPK was purchased from Cell Signaling (Danvers, MA). Rabbit anti-carbonic anhydrase was from Genway Biotech (San Diego, CA). All solutions were made using Milli-Q (Millipore) 18 M3 deionized water. Borofloat glass slides were purchased from Telic Company (Valencia, CA).

Sample Preparation

FITC-labeled bovine serum albumin (BSA) was prepared by mixing 10 μ L of 24 mg/mL FITC in dimethyl sulfoxide with 300 μ L of 4 mg/mL of BSA in 100 mM Na₂HPO₄ adjusted to pH 7.5 with 100 mM NaH₂PO₄ (PBS) and incubating for 1 h at room temperature. Proteins to be analyzed were denatured by heating at 75° C for 5 min in denaturation buffer consisting of PBS, 3% SDS and 5% β -mercaptoethanol (BME). Denatured protein samples were diluted from stocks with sample buffer (50 mM PBS, 200 mM GABA, and 0.01% Tween 20). FITC-BSA was diluted to 0.5 mg/mL protein for assay. Protein ladder was diluted 1:10 from supplier for assay.

INS-1 cells were grown on cell culture plates in Roswell Park Memorial Institute (RPMI) 1640 culture medium. RPMI was poured off and radio-immunoprecipitation assay buffer (RIPA) from Thermo Fisher Scientific, Inc. (Rockford, IL) was added to lyse cells. Cell lysate was incubated on ice for 30 min before transfer to a tube and centrifuging at 1,000 $\times g$ for 5 min. Supernatant was desalted using Micro Bio-Spin 6 chromatography columns from

Bio-Rad (Hercules, CA) and proteins denatured as described above. Chicken eggs were purchased from a local grocery store. Egg whites were stirred using magnetic stirrers until clear (~40 min). Clarified egg white was diluted in 20 mM Tris buffer and then prepared as described for cell lysate.

Chip Fabrication

Chips were fabricated from borofloat glass slides using photolithography and wet etching methods.^{13–15} Briefly, slides coated with a layer of chrome and photoresist were exposed to UV light (Optical Associates, Inc., Milpitas, CA) for 5 s through a photomask (Fineline-imaging, Colorado Springs, CO) that had the desired channel network patterned in it (see Figure 1). The exposed glass was etched in 17:24:79 (v/v/v) HNO₃/HF/H₂O for 25 min resulting in channels that were 15 μm deep and 50 μm wide except the post-column channel which was 90 μm wide. Access holes (0.4 or 1 mm) were made in the etched glass using drill bits from Kyocera (Costa Mesa, CA). The resulting slides were soaked in piranha solution (H₂SO₄/30% H₂O₂ 3:1 v:v) for 20 min and then RCA solution (NH₄OH/30% H₂O₂/H₂O 1:1:5 v:v:v) for at least 40 min. A borofloat glass slide was thermally bonded, as a cover, to the etched glass slide using a programmable furnace.^{13, 15} The chip was cut using a dicing saw (Diamond Touch Technology, Inc. Golden, CO) to create a point at the post-separation channel as shown in Figure 1. Polyimide-coated fused silica capillaries were connected to the sheath channels using PEEK Nanoports (IDEX Health & Science, Oak Harbor, WA).

Microchip Gel Electrophoresis Preparation and Performance

Prior to use channels were conditioned by sequential rinsing with 0.1 M NaOH, water, 0.1 M HCl, and water for 10, 2, 5, and 2 min respectively, followed by pumping sieving media (an entangled polymer solution of proprietary composition, Beckman-Coulter part 390953, Brea, CA) through channels for 10 min. Reservoirs, except sample reservoir, were filled with the same sieving media. The chip was mounted on a lab jack and positioned over a PVDF membrane that was wetted with a 50:50 v:v mixture of methanol and 100 mM Tris buffer at pH 8.8. The membrane was supported by a grounded Al block mounted on an automated X-Y translational stage (Thomson-Linear, Radford, VA) as shown in Figure 1. The chip, with voltage applied as in Figure 1, was lowered towards the membrane while monitoring current. When current was detected, indicating contact, lowering was stopped and the chip held in position for separation(s). Gated injection with –1400 V applied for 3 s was used to inject samples.^{16–17} During separation, sieving media was pumped through the two sheath channels at 30 nL/min using a syringe pump (Chemyx Inc., Stafford, TX) and the stage was moved at 6 mm/min, unless otherwise stated, so that separated protein bands migrated out of the chip and were captured on membrane. Fluorescent proteins were detected on membrane by direct imaging using a Typhoon 9410 variable mode imager (GE Healthcare). In some cases, separations were detected directly on the chip using an inverted fluorescence microscope (Olympus, Melville, NY) equipped with a Xenon arc lamp (Sutter instrument company, Novato, CA) and an electron multiplier CCD camera (Hamamatsu, Bridgewater, NJ).

Immunoassay

Immunoassay of captured protein was performed using a SNAP i.d. (Millipore) according to manufacturer instructions.³ Membranes with separated proteins were wetted with methanol and placed on a blot holder. Vacuum was used to pull blocking buffer (0.3% dry milk with 0.1% Tween 20), antibody solutions, and wash buffer (TBS buffer with 0.1% Tween 20) sequentially through the membrane. Primary antibodies were diluted to 1:1000, 1:1666, 1:30, and 1:256 for AMPK, lysozyme, carbonic anhydrase II, and actin, respectively.

Secondary antibody, conjugated with Alexa Fluor® 532, was diluted to 1:2000. All antibody solutions were diluted using blocking buffer.

Results and Discussion

Chip Design and Experimental Overview

The microfluidic device used for separation and blotting is illustrated in Figure 1. Protein-SDS complexes are injected and separated by sieving electrophoresis using a tee arrangement.^{16–17} As proteins migrate from the separation channel they are transported via a sheath flow to a membrane that is slid past the exit of the chip to capture separated zones. Blotting occurs as compounds exit, thus saving time relative to a traditional Western blot which has a separate blotting step. Sheath flow of sieving media was found to be necessary to achieve stable current as previously reported for CE Western blot.¹⁰ (The unstable currents without sheath flow may result from migration of methanol from the blotting membrane or hydroxide ion electrochemically formed at the exit into separation channel¹⁸. With a sheath gel, these potentially interferences are prevented from entering the separation channel and instead flow to the membrane.) A significant advantage of microfabrication is that sheath channels are precisely formed and integrated with the device with minimal extra column volume whereas a capillary-based system required manually aligned coaxial capillaries to have a sheath flow¹⁰. As a result, microfabrication increased robustness and reproducibility of operation.

Separation

We first examined separations of fluorescent protein-SDS complexes on the gel-filled chip. Size-based separations of proteins from 11 kDa to 155 kDa were obtained in 5 min at 240 V/cm over a 2 cm length (Figure 2). Separation efficiency ranged from 5×10^4 to 1.8×10^5 theoretical plates and peak capacity was 45 (using the average zone width and resolution = 1 for calculation) when detecting at the exit of the separation channel. Separation times could be reduced to 2 min with 4×10^4 plates (average for ladder) at 460 V/cm, but above this field bubble formation was common. The separations are substantially faster than what has been achieved with capillaries where typical separation times are 20 min.^{19, 20} The shorter time is due to miniaturization of the separation channel and illustrates another advantage of microfabrication for the Western blot.

It may be possible to further improve the separation time. Previous chip-based work using a lower injection volume, pinched injection, narrower channels, and less viscous sieving media achieved 7×10^4 to 1.2×10^5 plates and peak capacity of 55 in 1 min separation on a 1.25 cm long channel.²¹ The larger channels and injection volumes used here were to accommodate a less sensitive detector (Xe-arc lamp source rather than laser) while the sieving media was used because it is commercially available. Thus, use of other media and narrower channels are likely to yield faster separations.

Transfer to Membrane

After migrating from the separation channel, band broadening may occur both in the sheath flow channel and during deposition on the membrane. The post-column length was just 300 μm to minimize broadening during transfer. Sheath flow rate had to be above 20 nL/min to avoid degrading separation efficiency (Figure 3A). Higher flow rates both decreased plates, likely to due flow-induced zone broadening, and diluted zones during transfer. 30 nL/min was chosen as the best value. At this flow rate, little difference could be seen in peaks detected at the end of the capillary compared to end of the sheath flow (Figure 3B).

During protein deposition the membrane velocity also had an effect on separation. It is necessary to move the membrane fast enough to capture resolved proteins in different spots (i.e., a velocity that is too low would allow separated zones to stack together on the membrane); however, increasing the speed also spreads the zone out on membrane and dilutes signal (see Figure S-1). For 240 V/cm, we typically used 6 mm/min, which decreased efficiency by 28% on-membrane relative to on-channel (Figure 3B). As shown in Figure 3C, using these conditions, separated proteins were captured in discrete zones on the membrane. A line scan of the membrane allows comparison to separation detected on column (Figure 3D). The electropherogram retains an average of 5×10^4 theoretical plates; but, the overall separation shows lower resolution than detected on chip, e.g. resolution of the peaks at 2.5 and 2.8 min decreased from 1.8 to 1.7 and for the peaks at 3.2 and 3.4 min it decreased from 1.2 to 1.0. Some differences in spacing and intensity were observed on the membrane relative to the on-column detection, especially for the largest (slowest) protein. These effects were not investigated thoroughly. The intensity effect could relate to quenching on the membrane or transfer and capture efficiency. The spacing effect may be due to an effect of sheath flow speeding up the slowest migrating protein; however, this requires a formal study to reach a firm conclusion. Conditions can be varied to aid sensitivity, separation time or resolution depending on requirements of the assay. For most assays described below we used 460 V/cm and 6 mm/min membrane rate yielding 2 min separation times for the complete protein ladder with 3×10^4 plates on membrane (see Figure S-2).

Figures of Merit

Sensitivity and limits of detection (LOD) were determined for several proteins by Western blot analysis. For these experiments, chips with three sample reservoirs were used and samples at different concentrations injected sequentially onto the separation channel so that separation of samples was recorded in different lanes on a single membrane. The entire membrane was processed for immunoassay at once. Blots for calibration are shown in Figure 4. Using actin as a test protein, we found a linear relationship ($R^2 = 0.98$; see Figure S-3) between concentration (from 2.4 nM to 240 nM) and fluorescent signal (measured as peak area for a line scan through the protein spot). LOD was $0.03 \mu\text{g/mL}$ (0.7 nM) using a S/N of 3 as the lowest detectable signal (see Figure 4 and line scan membrane for 2.4 nM actin in Figure S-3). We estimate 200 pL injected suggesting a 25 fg mass LOD. Using a similar approach, the LOD for carbonic anhydrase II was $0.05 \mu\text{g/mL}$ (2 nM) and lysozyme was $0.06 \mu\text{g/mL}$ (4 nM). These detection limits compare well to other microfluidic Western blots, e.g. 2.5 nM for protein G.⁶

An advantage of the method described here is that any secondary antibody and detection method could be used since the separation and blotting step are decoupled. For these experiments, we used Alexa Fluor® 532 labeled secondary antibodies and fluorescence detection; however, the same approach could utilize other detection methods such as chemifluorescence. Fluorescent antibodies allow the same detector to be used to detect the calibration proteins as illustrated in Figure 4.

For most Western blots, it is desirable to use a standard ladder for size calibration. In this system the ladder could be mixed with the sample or injected and analyzed on a separate lane. Less interference occurred by injecting in a separate lane so this approach was generally used. Reproducibility of migration time is important for sequential injections. For multiple injections the RSD of migration time was 3 to 6% for actin (89 ± 3 , $n = 10$), carbonic anhydrase II (82 ± 2 s, $n = 4$), and lysozyme (75 ± 5 s, $n = 4$). Possible sources of variability are inconsistent moving rate of the stage holding the membrane (it was operated at lower end of its range and resolution), and lack of thermal control.

Analysis of Complex Samples

To assess the utility of this approach for Western blotting in biological samples we measured actin and AMP-activated protein kinase (AMPK) in INS-1 cell lysate and lysozyme in egg white as demonstrations. Actin was readily detected at its expected molecular weight (42 kDa) in cell lysate samples that contained 600 $\mu\text{g/mL}$ protein. Peak area RSD was 0.2% for multiple injections. Using a calibration curve made from actin standards, the actin concentration in INS-1 cell lysate was estimated to be 10 $\mu\text{g/mL}$ or 0.7% of total protein. This value is lower than previously reported estimates of 2–3% of total protein content reported for hamster ovary cells.²² The difference may relate to differences in cells or differences in the response in binding of antibody to bovine (used for standard) and murine (INS-1 cells) actin.

While actin is a commonly used standard protein, we also sought to demonstrate detection of a more typical target, such as a signaling enzyme. AMPK is a “master” regulator of metabolism and of strong interest for its control of β -cell growth, metabolism, and insulin secretion.²³ The α -subunit of AMPK was readily detected as a 66 kDa protein in INS 1 cell lysate (1 and 2 mg/mL total protein) as shown in Figure 5B and Figure S-4. This molecular weight was in agreement with that found by a traditional Western blot (data not shown). The α -subunit of AMPK is nominally a 63 kDa protein suggesting that perhaps a modification affected its migration rate in these samples.²³ Multiple injections from the same reservoir had peak area RSD of 0.4%. Although standards were not readily available for quantification, the good reproducibility and results from assay of different dilutions of cell lysate show the potential for relative quantification.

As another demonstration of the assay, lysozyme was detected as a 14 kDa protein in egg white samples (Figure 5C). Lysozyme is a major protein component of egg white (3.4%). As with the AMPK assay, repetitive samples were reproducible (RSDs of area were 5% and 2% for 1/40 and 1/100 diluted samples). Using comparison to lysozyme standards, the estimated concentration in egg white was 4 mg/mL, which falls in the expected range of 2.2 to 4.5 mg/mL.²⁴

With multiple sample reservoirs and use of a common secondary antibody, it is possible to determine different proteins from different samples on the same membrane. This is illustrated in Figure 6 where AMPK in cell lysate and lysozyme in egg white were both determined by sequentially separating them with capture on different lanes and then processing the membrane for immunoassay. To avoid carry-over between injections, sample was driven towards the injection cross for 30 s to rinse the cross region when switching sample reservoirs.

Throughput and Analysis Time

As shown by the above demonstrations, the microfluidic Western blot described here has good sensitivity and reproducibility. The assay also retains the information content, versatility (different proteins in different sample types) and flexibility of a conventional Western blot (i.e., it uses the same detection methods and antibody treatment steps). Perhaps the most significant advantage is the potential for good throughput. Using this approach a single Western blot requires 22–32 min (2 min separation and another 20–30 min for immunoassay). This time is much faster than conventional gel Westerns; but slower than a fully integrated chip which has 5 min total analysis time⁶. The rapid assays on an integrated chip however used antibodies covalently attached to the chip or pre-labeled proteins whereas the system reported here used unlabeled sample proteins and conventional membranes. (In discussing these times, we are ignoring set-up and sample preparation that is required for all approaches).

The current approach has the potential for considerably higher throughput, when multiple samples are analyzed. The rate limiting step of the method described here is the immunoassay; however, multiple separations can be captured on a single membrane and immunoassay performed on the entire membrane allowing for improved throughput. Several approaches for laying multiple separation tracks on a single membrane can be envisioned, some of which were illustrated here. As shown in Figures 5 and 6, using multiple sample reservoirs, different samples can be separated sequentially through one channel and collected in different tracks on one membrane for subsequent analysis. In Figures 5B, 5C, and 6, nine separations (3 separate samples, including ladder, in triplicate) were collected on a single membrane which was processed in 25 min for a total analysis time of 43 min or ~5 min/sample. In principle it is feasible to scale up the number of sample reservoirs coupled to a single separation channel to further increase the throughput. As shown by the examples here, the samples may be different and may have the same or different protein targets. A related approach would be to use a single sample reservoir that could be rapidly reloaded for sequential separations. The limiting factor on scale up of samples would be the number of separations that can be acquired from a single separation channel. In a pilot study, we found that 60 injections could be achieved through a single channel before the separation times began to drift (Figure S-5). This sequence required 100 min suggesting a potential throughput of 60 samples/130 min or slightly over 2 min/sample. After 60 injections migration time increased 10%. Replacing the gel completely regenerated the separation performance. These results illustrate another important feature which is reusability of the chip. The ability to pump the entangled polymer solution in and out of the chip as needed would also allow extended operation time for many samples. Other methods to improve throughput, such as separation from channels operated in parallel, are also in readily within reach.

Needed Improvements

This work is a significant step forward from the original hybrid method CE interfaced to membrane because it increased the speed of separation 8-fold, demonstrates potential for rapid serial injections through the same sample, increases robustness and reduced band broadening by using a microfabricated sheath-flow, and demonstrates utility for biological sample analysis. Despite these advances, further improvement is essential to make this a practical tool. To achieve the throughput advances described above, it is necessary to integrate more sample reservoirs and parallel channels. Improved separation conditions will allow faster separations (e.g., different gels).²⁵ Detection limits may be improved by better preconcentration. A modest isotachophoretic effect was achieved by adding leading and terminating electrolytes to the sample; but more effective approaches have been described.^{26, 27}

Comparison to Other Approaches

Previous work has recently demonstrated excellent throughput (e.g., 48 samples in 60 min) for a microfluidic Western blot.⁹ Besides demonstrated throughput, the prior work also offers advantages of minimal moving parts and integration within the chip. The system described here, seems to have the potential to achieve comparable levels of throughput. The method here may offer other advantages such as repeated injections from a single chip, simple regeneration of chip (rinse of gel rather than overnight incubation of aggressive solvents), potential for long term operation, capturing protein external to the chip for other processing (e.g., mass spectrometry analysis or stripping and re-blotting of different proteins), and compatibility with existing blotting and immunoassay protocols. These differences suggest that both approaches extend the capability of Western blots and offer new opportunities for protein analysis.

Conclusion

These results demonstrate a new approach to microfluidic Western blot. The microfluidic chip used allows fast size-based separation and direct delivery of proteins onto blotting membranes. Microfabrication also allows well-controlled sheath flow for the blotting step ensuring low loss of separation efficiency and robust performance. The method has good sensitivity and is compatible with analysis of proteins in complex samples. The use of conventional Western blot membranes and immunoassay allow rapid and versatile assay development illustrated by the 4 different assays used in this report. Preservation of protein on separate membrane also offers the potential of other physical and chemical tests on the sample. Because a single membrane can capture multiple rapid separations, potential for good throughput exists.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Microchip overview. Samples are loaded in different sample reservoirs (S). Samples are injected by floating the buffer reservoir (BR) and sample waste (SW) with voltage applied between the desired sample reservoir and the Al block at the exit.^{16–17} During separation, flow from the sample reservoir is gated to the sample waste reservoir (SW) using the voltages as shown. During these operations other sample reservoirs are floating. Size based separated SDS-protein complexes are captured in discrete zones on the PVDF membrane moving beneath the chip outlet to preserve separation information. Sieving media is pumped through the sheath channels to give stable current. Channel lengths are indicated by double arrow lines and direction of flow during separation is indicated by solid, single arrows.

Figure 2.

(A) Size dependent separation of FITC-labeled protein ladder in microchips. Detection window was set at the end of separation channel, 300 μm away from the chip outlet. Electric field during separation was 240 V/cm. (B) Relationship of MW to migration time.

Figure 3.

Transfer of separated proteins to blotting membrane. Electric field was 240 V/cm, separation channel length was 2 cm, sheath flow rate was 30 nL/min, and membrane was moved at 6 mm/min. (A) Effect of sheath flow rate on theoretical plates (N) for SDS-FITC-BSA. Error bar is ± 1 standard deviation (n = 4). (B) Comparison of peak width for on-chip detection (solid line), in post-channel 300 μm beyond the end of separation (dashed line), and on membrane (gray line) for electrophoresis of 0.5 mg/mL SDS-FITC-BSA. (C) Gray scale fluorescent image of membrane that captured size-dependent separation of FITC-labeled protein ladder. (D) Comparison of protein ladder separation on membrane (upper) to detection in channel (lower). Membrane trace is a line scan through membrane with x-axis converted to separation time.

Figure 4.

Microfluidic Western blot of unlabeled actin, carbonic anhydrase II (CA II), and lysozyme at different concentrations. Each trace shows repeated injection of a sample captured on the same membrane. Separations were performed using 460 V/cm electric field. Samples were injected every 90 s. Time axis on right indicates time since injection. Protein ladder was injected separately to provide size calibration. (Line scans of actin injections shown in Figure S-3).

Figure 5.

Western blot assay of biological samples. (A) Assay of actin in 1.5 mg/mL INS-1 cell lysate (duplicates are shown). Standard actin assays used for calibration are shown to the right. Separation field was 460 V/cm. (B) Western blot assay of AMPK from INS 1 cell lysates. Lysate was diluted to 1 mg/mL (Lysate A) and 2 mg/mL (Lysate B) before analysis. (C) Immunoassay of unlabeled lysozyme from egg whites (EW) with initial fluorescent scanning of labeled size standards. Egg white sample A (EW A) had a concentration of 1/100 of original homogenate and sample B (EW B) had a concentration of 1/40.

Figure 6.

Analysis of different samples on one membrane. AMPK from 1.5 mg/mL INS 1 cell lysate and lysozyme (Lys) from egg white (EW) homogenate were immunoassayed at the same time after separations from different sample reservoirs.