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Fabrication of an Open Microfluidic Device for Immunoblotting

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Abstract

Given the wide adoption of polydimethylsiloxane (PDMS) for the rapid fabrication of microfluidic networks and the utility of polyacrylamide gel electrophoresis (PAGE), we develop a technique for fabrication of PAGE molecular sieving gels in PDMS microchannel networks. In developing the fabrication protocol, we trade-off constraints on materials properties of these two polymer materials: PDMS is permeable to O₂ and the presence of O₂ inhibits the polymerization of polyacrylamide. We present a fabrication method compatible with performing PAGE protein separations in a composite PDMS-glass microdevice, that toggles from an “enclosed” microchannel for PAGE and blotting to an “open” PA gel lane for immunoprob ing and readout. To overcome the inhibitory effects of O₂, we coat the PDMS channel with a 10% benzophenone solution, which quenches the inhibiting effect of O₂ when exposed to UV, resulting in a PAGE-in-PDMS device. We then characterize the PAGE separation performance. Using a ladder of small-to-mid mass proteins (Trypsin Inhibitor (TI); Ovalbumin (OVA); Bovine Serum Albumin (BSA)), we observe resolution of the markers in <60 s, with separation resolution exceeding 1.0 and CVs of 8.4% for BSA-OVA and 2.4% for OVA-TI, with comparable reproducibility to glass microdevice PAGE. We show that benzophenone groups incorporated into the gel through methacrylamide can be UV-activated multiple times to photocapture protein. PDMS microchannel network is reversibly bonded to a glass slide allowing direct access to separated proteins and subsequent in situ diffusion-driven immunoprob ing and total protein Sypro red staining. We see this PAGE-in-PDMS fabrication technique as expanding the application and use of microfluidic PAGE without the need for a glass microfabrication infrastructure.

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Supporting Information

The Supporting Information is available free of charge on the [ACS Publications website](https://pubs.acs.org/doi/10.1021/acs.analchem.7b02406) at DOI: 10.1021/acs.analchem.7b02406. Additional materials and methods and results; delamination of the composite microdevices results in a defective PA gel on the silanized glass substrate (PDF)

The authors declare no competing financial interest.

In the 1930s, Arne Tiselius developed an apparatus for electrophoretic analysis of colloidal mixtures.¹ In the 1950s, the technique was adapted to filter paper² or starch³ as supporting media. Since then, electrophoretic methods have expanded to include capillary gel electrophoresis,^{4,5} electrophoretic mobility shift assays,⁶ and isoelectric focusing,⁷ among others. Nevertheless, for over 4 decades, slab polyacrylamide gel electrophoresis (PAGE) has remained ubiquitous for protein analysis.⁸ The polyacrylamide (PA) gel is a sieve that retards protein electromigration on the basis of size and structure, yielding protein separations that address questions in research and clinical laboratories.^{9,10}

In contrast to conventional slab gels, microfluidic chips afford high efficiency protein separations with faster assay times, lower consumption of sample volume, and ready integration with automation systems.^{11,12} Microfluidic PAGE dissipates Joule heating efficiently owing to large surface area-to-volume ratios inherent to microchannels. This favorable thermal transport supports high electric field strength operation (e.g., 1000 V/cm in chip vs 10 V/cm in slab gel PAGE), thus resolving two proteins in a few seconds compared to hours on slab gel PAGE.^{6,13} Microchannel networks (e.g., cross-t) geometrically define sample plugs having minimal injection dispersion,¹⁴ giving rise to exceptional separation efficiencies. Typically, separations with durations of <1 min are completed in 1 mm separation lengths, with plate heights as high as 4.41×10^5 plates/m.^{15–17}

Microfluidic PAGE has been reported in microchannels formed by the thermal bonding of two glass substrates, one side having defined wet-etched trenches.¹⁸ Because of the irreversible nature of the thermal bonding, microchannels cannot be readily opened for protein collection, staining, or in situ mass spectrometry.^{18–21} To address this issue, Duncombe and Herr reported on “mesofluidic” free-standing PA gels, which facilitate rapid prototyping, assay multiplexing, reduced time and costs, and direct access to protein for postseparation manipulation.²² However, in these “free standing” gels, evaporation confounds the benefits of microfluidic-related efficient heat dissipation so much so that encapsulating fluids are often applied.^{6,23}

An advance in chip design would merge the advantages of free-standing formats with the advantages of microfluidics. Consequently, we develop a fabrication and assay protocol that performs PAGE in microchannels cast in a polydimethylsiloxane (PDMS) substrate that is reversibly bonded to a glass slide. PDMS is widely used, because the silicone elastomer is relatively straightforward to fabricate, is robust, and can be readily integrated with unit-function modules (including pneumatic pumps).^{24,25} That said, PDMS is gas permeable, and the O₂ diffusion through PDMS inhibits PA gel polymerization.²⁶ Intriguingly, several studies have incorporated UV-reactive benzophenone (BP) for grafting functional acrylamide groups on the PDMS surface as a means to scavenge O₂.^{26–28} Here, we report on an approach to harness the O₂ quenching effect of BP to perform PAGE in composite PDMS-glass devices. In developing the device and the immunoblotting assay, we assess: (i) the feasibility of reducing O₂ concentration in order to polymerize PA in PDMS and associated PAGE separation performance as compared to enclosed glass microdevices; (ii) the resultant photoimmobilization efficiency of proteins captured in the PA gel for postPAGE processing of proteins; (iii) the feasibility of delaminating the device (by

removing the glass slide) to perform in situ protein staining in the resultant protein-laden PA gel.

MATERIALS AND METHODS

PDMS-Glass Device Fabrication.

Microchannels were fabricated in PDMS substrate using standard soft lithography techniques.²⁹ The PDMS substrate is then assembled to a glass slide prefunctionalized with acrylate monomers as previously described³⁰ to enable gel cross-linking to its surface. A detailed procedure is described in the Supporting Information.

Fabrication of Discontinuous Pore-Size Gel.

First, the microchannels were incubated with a 10% w/v benzophenone solution dissolved in acetone for 10 min and were washed out with methanol and water. Then, a discontinuous gel was fabricated in the channels. This discontinuous pore-size gel consists of a step-change, large-to-small pore-size gel at the start of the separation channel (e.g., stacking gel in slab gel electrophoresis) to reduce injection dispersion for more efficient separations for a given separation length.³¹ The fabrication protocol is fully described in the Supporting Information.

Sample Separation, Protein Blotting, and Antibody Probing.

Electrophoretic separation of the protein ladder was performed in cross-channels consisting of two orthogonal channels, one for the injection and another for protein separation and readout. Such cross-channels design was chosen in order to minimize injection dispersion.¹⁴ Detailed sample separation and blotting are described in the Supporting Information, as well as the diffusion driven probing.

Imaging and Image Analysis.

Micrograph acquisitions as well as calculations for separation resolution (SR) and photocapture efficiency (η) are described in the Supporting Information.

RESULTS AND DISCUSSION

Immunoblotting is a multistage assay comprising electrophoresis (e.g., PA gel electrophoresis or PAGE), protein blotting (immobilization), and immunoprobings with detection antibodies (Figure 1). Accordingly, we scrutinize three performance metrics related to each assay step: (1) PAGE separation performance, (2) blotting efficiency (post-PAGE protein photocapture efficiency), and (3) immunoprobings efficiency.

PAGE Performance: Protein Stacking, SR, and Variability.

First, we assessed the effect of the gel discontinuity on protein band stacking, which minimizes injection dispersion and can enhance PAGE separation performance. We assessed the stacking behavior of each protein individually (Figure 2A). Protein stacking is defined as concentrating a protein sample into a small volume as described by Ornstein.³² When a protein migrates through the large-to-small pore-size gel discontinuity, the average

electrophoretic mobility is reduced, and the protein concentrates into a smaller peak. A stacking factor (SF) for Trypsin Inhibitor (TI), Ovalbumin (OVA) and Bovine Serum Albumin (BSA) was calculated as the ratio of the protein peak width immediately before the gel discontinuity to the protein peak width immediately after the gel discontinuity. For each protein target ($n = 3$), we observed: $SF_{TI} = 7.5$ (CV = 13.6%), $SF_{OVA} = 6.75$ (CV = 9.3%), and $SF_{BSA} = 7.03$ (CV = 7.8%). The advantages of protein stacking are an enhanced assay sensitivity owing to the enriched sample stack and an improved SR owing to reduced injection dispersion.

This effect of pore-size transition can also be observed in the electrophoretic behavior of the proteins. Recall that the protein migration velocity ($dx/dt = \mu E$) is dependent on electrophoretic mobility μ ($\text{cm}^2/(\text{V}\cdot\text{s})$) and the applied electrical field strength E (V/cm). Protein mobility in gels is described by Ferguson³³ and predicted as $\log(\mu) = \log(\mu_0) - K_r T$, where μ_0 is the mobility of the protein in free solution, K_r is a retardation coefficient related to the size of the protein and the sieving quality of the separation matrix, and T is the total acrylamide concentration. Hence, the protein migration along the separation channel follows a linear relationship with time, owing to a constant mobility. The velocity decreases after the pore-size transition owing to the smaller pore size of the gel (larger % T), as described in the Ferguson relationship (Figure 2B). This decrease in mobility is also analyte dependent as K_r is specific to each protein.³⁴ We performed least-squares linear fitting for the electrophoretic migration curves of TI, OVA, and BSA before and after the gel discontinuity, in order to confirm constant velocity in each gel region. The R -squared values exceed 0.95 for all conditions. Electrophoretic mobility was calculated by dividing the protein electrophoretic velocity by E , assuming E is uniform throughout the channel (Figure 2C).

Another essential factor that alters the assay performance is the protein peak width, which in the absence of pore-size transition increases over time according to diffusion as $\sigma = (\sigma_0^2 + 2Dt)^{1/2}$, where σ_0 is the injected peak width, D is the diffusion coefficient, and t is the elapsed separation time. When altering gel pore size, both σ and μ are altered with the electrophoretic migration and diffusion encountering the same drag force.³² Therefore, the calculated ratios between the mobility before and after the gel discontinuity $\mu_{\text{before}}/\mu_{\text{after}}(\text{TI}) = 6.76$ (CV = 9.7%), $\mu_{\text{before}}/\mu_{\text{after}}(\text{OVA}) = 7.3$ (CV = 13.3%), and $\mu_{\text{before}}/\mu_{\text{after}}(\text{BSA}) = 8.75$ (CV = 37.5%) are equal to the above calculated stacking factors (Figure 2D).

Second, we assessed the PAGE separation performance with three ladder proteins (Figure 3A). Proteins are fully resolved starting from the 27 s mark for OVA-TI and 45 s mark for OVA-BSA with a SR > 1 (Figure 3B). SR is a measure of the capability to resolve two species. SR is decreased because of band broadening, which arises from molecular diffusion, dispersion from the injection, Joule-heating-induced dispersion, and the size of the detection region.²⁴

We next compared PAGE separation performance in this composite and enclosed microchannel device to that in freestanding PAGE formats for the same protein ladder.³⁵ In a 15% T free-standing format, BSA-OVA were resolved in 110 s at a separation length of 3.4 mm, while the SR of OVA-TI was still <1 after 380 s and a separation length of 9.1 mm. In contrast, the enclosed PDMS PAGE system reported here resolves BSA-OVA after 45 s at a

separation length of 850 μm and OVA-TI after 27 s at 800 μm . This superior performance of the microchannel system derives from both a lower injection dispersion owing to use of a stacking gel and a higher applied electric field strength¹⁶ (>250 V/cm in enclosed system vs 100 V/cm in freestanding system).

To ascertain the run-to-run and chip-to-chip reproducibility of PAGE in these composite devices, we scrutinized the migration behavior of our well-behaved and well-characterized ladder proteins. Observed run-to-run variation in SR has a $\text{CV} < 5\%$ ($\text{CV}(\text{SR}_{\text{OVA-BSA}}) = 4.2\%$; $\text{CV}(\text{SR}_{\text{TI-OVA}}) = 2.3\%$; $n = 3$ chips). Chip-to-chip variation in SR is $\text{CV} < 9\%$ ($\text{CV}(\text{SR}_{\text{OVA-BSA}}) = 8.4\%$; $\text{CV}(\text{SR}_{\text{TI-OVA}}) = 2.4\%$; $n = 3$ chips). The variability of the SR between BSA-OVA is higher than for OVA-TI, which is comparable to results reported by Shameli and Ren, where 2D protein separations were performed in PA gel-filled microchannels having three PDMS walls.³⁶ On the basis of the protocol reported and personal communication with the authors, the Shameli and Ren study applied high UV intensity excitation through a microscope objective to polymerize the PA gels in situ. The mechanistic basis for the fabrication approach is the assumption that high UV intensity generates radicals at a high enough rate to overcome the rate of oxygen diffusing into the channel and reacting with the generated radicals, resulting in a net positive rate of radical generation, which leads to polymerization. Note that the high UV intensity-based fabrication approach would still see inhibition of radical polymerization at the PDMS interface, which would limit physical attachment of PA gels to the PDMS walls, as reported by others.^{26–28,37} As the present study is concerned with fabrication of PA gels that are attached to several microchannel walls, to avoid electro-osmotic flow and to keep the protein-decorated PA in PDMS channels during delamination, here we report on an alternate fabrication strategy which does graft each PA gel to the PDMS walls. Moreover, the BP coating method allows the PA polymerization in several chips simultaneously and is not restricted by the reported direct write method,³⁶ in which fabrication time does scale linearly with channel length, and consequently could be limiting for more extensive networks of channels and/or for the fabrication of multiple chips.

Blotting: Protein Photocapture Efficiency.

We sought dual functions for BP: (1) to scavenge O_2 at the microchannel walls so as to allow polymerization of PA in the microchannels and (2) to functionalize the bulk PA gel for subsequent protein photocapture after PAGE. To achieve the first function, we coated the PDMS microchannels with a BP solution dissolved in acetone as a means to scavenge O_2 .^{26–28} In order to assess PA gel polymerization, we applied vacuum to the PDMS channels after UV exposure. We interpreted no visible aspiration of liquid polymer out of the channel as a proxy for gel polymerization. At 0% BP, the bulk of the PA solution was immediately aspirated from the channel into the vacuum trap, thus supporting the hypothesis that O_2 diffusion through the PDMS walls inhibits PA polymerization under these conditions. We next assessed polymerization in channels coated with 1%, 10%, and 20% BP, respectively. In all three cases, we did not observe liquid aspiration out of the channels after polymerization conditions were applied. The observation supports the conclusion that the gel polymerized. As a corollary assessment of gel polymerization, we next measured the electrophoretic mobility of the ladder protein OVA through each nominal 15% T gel. For

channels coated with 1% BP, we observed an apparent electrophoretic mobility of $1700 \pm 200 \mu\text{m}^2/\text{V}\cdot\text{s}$ ($n = 3$) for the protein peak. In contrast, for channels coated with either 10% or 20% BP, we observed mobilities of 150 ± 10 and $175 \pm 27 \mu\text{m}^2/\text{V}\cdot\text{s}$ ($n = 3$) respectively. Given the nearly order of magnitude higher observed electrophoretic mobility under the 1% BP condition as compared to either the 10% or 20% BP conditions, we conclude that the gel in the 1% BP conditions is not fully polymerized. The result suggests that inhibition of PA polymerization arises, at least in part, from O_2 diffusion through the PDMS channel walls.

To achieve the second function, we sought UV photocapture of proteins to facilitate in situ blotting (on the molecular sieving gel) in lieu of a post-PAGE transfer of proteins to a hydrophobic blotting membrane as performed in conventional Western blotting.^{11,38} In situ photocapture is a critical step, as capture efficiency dictate the amount of protein available for further processing and analysis. For a UV exposure of 45 s, the photocapture efficiencies for PA-gels functionalized with BPMA are $\eta_{\text{BSA-dimer}} = 49.32 \pm 0.57 \%$, $\eta_{\text{BSA}} = 56.84 \pm 19.20 \%$, and $\eta_{\text{OVA}} = 22.18 \pm 6.67 \%$ ($n = 3$). For PA-gels without functionalizing BPMA, but with only O_2 -scavenging BP at the PDMS microchannel walls, $\eta_{\text{BSA-dimer}} = 15.64 \pm 4.4 \%$, $\eta_{\text{BSA}} = 32.24 \pm 13.85 \%$, and $\eta_{\text{OVA}} = 14.41 \pm 8.67 \%$ ($n = 3$). For PA-gels without functionalizing BPMA, but with only O_2 -scavenging BP at the PDMS microchannel walls, $\eta_{\text{BSA-dimer}} = 15.64 \pm 4.4$, $\eta_{\text{BSA}} = 32.24 \pm 13.85$, and $\eta_{\text{OVA}} = 14.41 \pm 8.67$ ($n = 3$). Previously published data¹¹ in glass chips showed a $\eta_{\text{BSA}} = 51.6\%$ ($\text{CV} = 6.8\%$, $n = 3$) and $\eta_{\text{OVA}} = 48.9\%$ ($\text{CV} = 4.8\%$, $n = 3$). For BSA, the capture efficiency is similar, while for OVA, the capture efficiency is substantially lower. The low OVA capture efficiency can be attributed to photoimmobilization in nondenaturing conditions as opposed to the conditions compared with protein sizing, where SDS was present in the gel.³⁹ Indeed, protein denaturation is thought to promote hydrophobic interactions by exposing protein residues more readily to BP groups present in the gel.³⁹ In cases where BP was used only in the O_2 scavenging function, we observed substantial photoimmobilization of proteins in the bulk PA gel, even with no BPMA present in the gel precursor solution. We hypothesize that BP present initially at the PDMS microchannel walls (for scavenging O_2) diffuses into the precursor solution prior to PA polymerization. During polymerization, that BP would be incorporated into the bulk PA gel and function for protein photocapture. As a corollary, protein molecules located proximal to the PDMS channel wall surface during the second UV exposure step would be immobilized, compounding any nonspecific adsorption of proteins to the PDMS walls.⁴⁰

Immunoprobng: Diffusion of Probe Solution into Exposed PA Gel.

We next sought to determine if immunoprobng after delamination of the composite PDMS-glass device was feasible in the protein-decorated PA gel. In such configuration, diffusion of antibody into the gel would be along the transverse (smaller) gel feature dimension, in contrast to probe transport along the axial feature dimension, as required when the gel remains fully encapsulated in a microchannel. Our previous assay development of in-channel immunoprobng relied on electrophoresis to sweep antibody probe down the separation axis of the gel (i.e., 20 min of probe electromigration, followed by a 20 min antibody washout in the reverse direction to remove background).¹¹

To perform immunoprob­ing via diffusion of reagents to the gel, we performed PAGE on OVA (200 nM) in a composite PDMS-glass device, immunoblotted OVA to the gel via photocapture, delaminated the composite device, and then incubated antibody probe solutions on the exposed gel feature seated on the glass (600 nM Ab; overnight incubation; 1 h wash). With silanized glass slides, we observed damaged gel housing the immunoprob­ed protein peaks as sections of the gel remained attached to the silanized glass (Figure S-1). To reduce or eliminate these gel defects observed with silanized glass substrates, we developed the protocol with nonsilanized glass slides. PA gel is expected to not attach or minimally attach to nonsilanized glass slides. Under the nonsilanized glass conditions, we again immunoprob­ed for OVA in the gel retained in the PDMS trench. We observed well-formed Gaussian protein peaks ($R^2 = 0.98$) after immunoprob­ing in these gels, indicating that the gel was successful delaminated from the glass slide and retained in the PDMS trench as desired (Figure 4). No trace of gel material was observed on nonsilanized glass slides after delamination. Thus, the remaining protein signal from the initial protein peaks after photocapture, PDMS release, and probing was 30.7 ± 5.4 (CV = 17.6%, $n = 3$) BSA-dimer, 32.5 ± 2.8 (CV = 8.6%, $n = 3$) for BSA, and 33.6 ± 6.1 (CV = 18.2%, $n = 3$) for OVA.

In Situ Staining of Immobilized, PAGE-Separated Proteins.

As a corollary to immunoprob­ing as an assay readout, we sought to stain the immobilized protein peaks with uncharged reagents (Sypro red) using diffusion of reagents into the open, protein-decorated PA gel, which would not be feasible in fully enclosed channels (Figure 5). A conventional 15% T slab PA gel has a recommended incubation time with the dye of ~60 min.⁴¹ We assess two shorter duration incubation times in the composite microfluidic assay (Figure 5A). As the Sypro red staining is highly protein selective, especially in nondenaturing conditions,⁴¹ we further sought to reduce protein-to-protein staining variability and enhance detection sensitivity by incubating the protein-decorated PA gel in a 0.05% SDS solution prior to staining (Figure 5B), as recommended by the stain manufacturer. Incubation with SDS prior to Sypro staining nearly doubled the SNR for the SDS-coated BSA protein, as compared to native BSA protein. For a 200 nM BSA sample with SDS treatment, the $\text{SNR}_{\text{BSA}} = 389$ (CV = 0.1%), compared to an $\text{SNR}_{\text{BSA, native}} = 199$ (CV = 0.2%) for BSA under native conditions.

At a starting concentration of 200 nM, neither OVA nor TI was detectable with Sypro red staining in the gel. At 400 nM, both the BSA and OVA peaks were detectable, with the Sypro red treated TI protein not exceeding the lower limits of detection (Figure 5C). TurboGFP (26 kDa) was detectable with this approach (Figure 5D). Surprisingly, at TI concentrations of up to 20 μM , Sypro red staining did not yield a detectable signal for this protein. Given the small molecular mass of TI, as compared to any other protein studied, we observe notable TI band broadening (and dilution) at the completion of even a 30 s PAGE analysis (Figure 5A). Comparison of the SNR of TI (SNR = 143.1, CV = 9.8%) to that of BSA (SNR = 885.7, CV = 6%) or OVA (SNR = 539.4, CV = 5.7%) shows the challenge of probing small or low-abundance species, even with rapid microfluidic formats.

CONCLUSIONS

We report on an approach to harness the O₂ quenching effect of BP to perform PAGE in composite PDMS-glass devices. In developing the device and the immunoblotting assay, we verified the feasibility of BP to reduce O₂ concentration in order to polymerize PA in PDMS microchannels. Subsequent use of the PA gels as PAGE sieving matrix shows a three-protein ladder fully resolved by PAGE in 60 s, with <9% CV in SR performance across devices thus indicating robust and repeatable pore-size control. Further, we successfully photo-immobilized protein to the PA gel after PAGE. We show that use of a nonsilanized glass slide allows ready delamination of the device and, thus, open access to the protein-laden PA gel feature. The dual functionality of the BP means that the molecule can be activated with UV to photocapture protein bands with efficiencies between 20% and 60%. Diffusion-driven immunoprobng of OVA in an “open” PA gel was possible as was similar introduction of protein stains but with limitations associated with the detection of TI. This PAGE-in-PDMS fabrication technique expands the utility of microfluidic PAGE by eliminating the need for a glass chip microfabrication infrastructure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A composite PDMS-glass microdevice for protein immunoblotting. (A) Photograph of a composite PDMS-glass chip (gel is visualized with Allura red stain). Neither the PDMS substrate nor the glass slide are plasma treated, creating a reversible bond to facilitate delamination of the PDMS from the glass slide. After delamination, proteins immobilized in the microgel are accessible for subsequent manipulation (e.g., immunoprobing, staining, collection, tandem analyses). (B) Microchannels are formed by mating a precast PDMS substrate and a glass slide. Channels are imbided with benzophenone, which diffuses into the PDMS to form an O_2 shield and promotes PA polymerization upon UV exposure. (C) The fabrication workflow consists of wicking a polymer precursor solution having a high % T acrylamide concentration into the microchannels. A gel pore-size discontinuity is created by masking areas to avoid photopolymerization in the upper channels. Unpolymerized precursor is suctioned out of the channel; a low % T PA precursor solution is introduced, and the entire chip is exposed to UV. (D) Protein separation and blotting: Proteins are analyzed by PAGE and immobilized to the gel via photocapture (blotting) prior to chip delamination and immunoprobing.

Figure 2.

Native protein PAGE in the composite PDMS and glass device. (A) Inverted fluorescence micrographs of TI, OVA, and BSA exhibit sample stacking at the large-to-small pore-size discontinuous gel interface. Note the reduced peak width at the gel interface ($E = 250 \text{ V/cm}$, discontinuous 5–15% TPA gel, scale bar = $50 \mu\text{m}$). (B) Velocities of TI, OVA, and BSA peaks are affected by local gel pore size. (C) Electrophoretic mobility of TI, OVA, and BSA decreases after migrating from the large-pore gel to the small-pore gel. (D) Stacking factors (SF) for TI, OVA, and BSA calculated as the ratios between the bandwidth before and after gel discontinuity as well as the mobility before and after gel discontinuity: SF is equal in both cases. Error bars report standard deviations.

Figure 3. Protein sizing in a composite PDMS and glass device. (A) Inverted fluorescence micrographs show time evolution of a PAGE analysis of TI, OVA, and BSA in a discontinuous 5–15% T gel (electric field = 250 V/cm, scale bar = 50 μm), with the three protein bands after 60 s of migration. (B) Time evolution of the average SR between OVA-BSA and OVA-TI peak pairs ($n = 3$ chips, errors bars = SD).

Figure 4.

Diffusion-driven immunoprobings of a PA gel housed in a delaminated, “open” PDMS trench. From the left panel to the right panel: (i) Micrographs of PAGE analysis ($E = 250$ V/cm) of OVA and BSA in a discontinuous 5–8% TPA gel; (ii) after photoblotting, PDMS substrate release, and immunoprobings; (iii) immunoprobed OVA; (iv) fluorescence trace of immunoblot.

Figure 5.

Protein staining of a protein ladder in the exposed protein-decorated PA gel. In (A–C), PAGE and blotting of Alexa 488 conjugated BSA, OVA, and TI are completed; then, the protein-decorated gel is subjected to Sypro red staining. (A) Protein staining under native protein conditions, with each ladder component initially at ~200 nM. Comparison of two Sypro red incubation periods shows no appreciable difference in detectable BSA signal. (B, C) Protein staining under 0.05% SDS conditions, with each ladder component initially at ~200 nM (B) and ~400 nM (C). Micrograph and intensity traces are after 30 min of SDS incubation and 10 min of Sypro red incubation. (D) Same conditions as in (C), with the addition of 400 nM turboGFP to the protein ladder. 45s PAGE duration; discontinuous 5–15% TPA gel; $E = 250$ V/cm.