Published in final edited form as:

Anal Chem. 2012 August 21; 84(16): 7057–7063. doi:10.1021/ac301278s.

“Flow valve” microfluidic devices for simple, detectorless and label-free analyte quantitation

Debolina Chatterjee, Danielle S. Mansfield, Neil G. Anderson, Sudeep Subedi, and Adam T. Woolley*

Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah, USA

Abstract

Simplified analysis systems that offer the performance of benchtop instruments but the convenience of portability are highly desirable. We have developed novel, miniature devices that feature visual inspection readout of a target's concentration from a ~1 μ L volume of solution introduced into a microfluidic channel. Microchannels are constructed within an elastomeric material, and channel surfaces are coated with receptors to the target. When a solution is flowed into the channel, the target crosslinks multiple receptors on the surface, resulting in constriction of the first few millimeters of the channel and stopping of flow. Quantitation is performed by measuring the distance traveled by the target solution in the channel before flow stops. A key advantage of our approach is that quantitation is accomplished by simple visual inspection of the channel, without the need for complex detection instrumentation. We have tested these devices using the model system of biotin as a receptor and streptavidin as the target. We have also characterized three factors that influence flow distance: solution viscosity, device thickness, and channel height. We found that solution capillary flow distance scales with the negative logarithm of target concentration and have detected streptavidin concentrations as low as 1 ng/mL. Finally, we have identified and evaluated a plausible mechanism wherein time-dependent channel constriction in the first few millimeters leads to concentration-dependent flow distances. Their simplicity coupled with performance makes these “flow valve” systems especially attractive for a host of analysis applications.

Introduction

Advanced analysis systems have a number of important desired performance characteristics, namely accuracy, reliability, selectivity for target analytes, quantitation, low detection limits, simplicity, speed, cost, multiplexing, and so on. Frequently, there is a tradeoff between these objectives (e.g., low cost vs. high accuracy), requiring a compromise that best meets the analysis requirements. For example, the performance of benchtop analyzers takes precedence over portability, while for point-of-care (POC) systems, versatility and performance are often sacrificed in favor of convenience.

A number of automated and robust laboratory-based systems are available for analyses. Liquid chromatography¹ is widely used and has seen recent progress in stationary phases^{2,3} and with increased pressures.⁴ Mass spectrometry methods^{5,6} have advanced through improved mass analyzers⁷ and sample introduction techniques.⁸ Spectroscopy can provide analyte-specific information from absorbance⁹ or Raman¹⁰ techniques. In addition, clinical diagnostic tools¹¹ such as enzyme-linked immunosorbent assay (ELISA)¹² are broadly used for targeted detection of biomolecules of interest. The above examples nicely illustrate

*corresponding author: atw@byu.edu.

systems with excellent performance, but that capability generally comes at the expense of portability.

On the other hand, portable instruments offer significantly increased analysis convenience. For example, POC diagnostic devices have been implemented in monitoring blood glucose for diabetes¹³ and in home pregnancy testing.¹⁴ Paper-based microfluidic systems^{15–17} offer simplified analysis coupled with low cost. These portable systems are advantageous in terms of simplicity and speed, but this generally comes at the cost of some performance characteristics such as low detection limits, quantitation capabilities, or multiplexing. In this paper we describe a simple, portable and detectorless microdevice system with quantitation capabilities, ~1 ng/mL detection limits and potential for broad applicability, addressing key limitations of both benchtop and portable systems.

Our method involves the capillary flow of target solution through a receptor-coated microchannel in a deformable material, which leads to channel constriction and flow stoppage due to target-receptor interaction. Importantly, in this “flow-valve” method, the distance of capillary flow is correlated with the target’s concentration, and the ability to differentiate between filled and empty channels visually enables detectorless determination of flow distance, and hence target concentration. Our use of flow distance as a quantitative detector is somewhat complementary to work of Zhong et al.,¹⁸ who very recently demonstrated a microfluidic adsorption-length-based approach for quantifying peptides, albeit requiring external mass spectrometry detection. We have fabricated polydimethylsiloxane (PDMS) devices and tested them with the model target-receptor system of streptavidin and biotin. Furthermore, we have studied three factors that affect assay performance: solution viscosity, device material thickness and channel height. We have also measured the concentration dependence of flow distance and assayed streptavidin solutions as dilute as 1 ng/mL. Finally, we have evaluated the mechanism of channel closure in these assays. Notably, the “flow valve” approach should be adaptable to various target-receptor pairs, offering a very broadly applicable analysis method.

Experimental Section

Mold design and preparation

Molds were prepared using a 500 μm thickness, 10 cm diameter glass wafer (Fig. 1A) with spun on AZ50XT positive photoresist (AZ Electronic Materials, Branchburg, NJ) of 5–20 μm thickness (Fig. 1B). Next, photolithography was used to transfer the serpentine design of the mask (Fig. 2A) onto the glass wafer by UV exposure followed by development in AZ400K developer (AZ Electronic Materials), resulting in elevated features of 50 μm width on the wafer (Fig. 1C). Reflowing of photoresist¹⁹ was then done at 150 $^{\circ}\text{C}$ for 5 min to round the edges of the elevated features in the mold (Fig. 1D).

PDMS device fabrication

Devices were fabricated by casting PDMS against the positive relief mold. PDMS (Dow Corning, Centennial, CO) was prepared by mixing the base and curing agent in a 10:1 ratio, pouring it on the mold to a thickness of 0.45–1.1 mm (Fig. 1E), and heating to 80 $^{\circ}\text{C}$ for 45 min for curing. This PDMS was removed from the mold (Fig. 1F) and bonded to an unpatterned PDMS layer (thickness: 0.4–1.1 mm) after exposure to an oxygen plasma for 30 s²⁰ to form a completed device with embedded channel (Fig. 1G). Fabrication reliability prevented the usage of devices with PDMS films thinner than ~0.4 mm. After plasma bonding, devices were stored with water in the channels to ensure that the surface remained hydrophilic.

Procedure for experimentation

Experiments were carried out on a biotin-streptavidin model system using the general protocols given here. The water-filled microchannel was first aspirated and then filled with biotinylated bovine serum albumin (b-BSA, Thermo Scientific, Rockford, IL, 2 mg/mL in 0.14 mM citrate, pH 6.8) or a control solution of BSA (Sigma-Aldrich, St. Louis, MO, 2 mg/mL in phosphate buffered saline, PBS) via capillary action. The b-BSA was allowed to adsorb to the PDMS channel walls for 15 min, leaving exposed biotin groups. Then, the b-BSA solution was removed and the channel was flushed with PBS (10 mM, pH 7.2) to remove unadsorbed material. Finally, PBS was aspirated from the channel and a 1 μ L streptavidin solution (New England Biolabs, Ipswich, MA) of specified concentration in PBS was pipetted into the reservoir (see Fig. 2C–D). The flow distance of streptavidin solution in the microchannel was measured with a ruler, and photographs were obtained with a digital camera. Some flow experiments were also carried out with streptavidin solutions having added glycerol (0–36%) to explore the influence of viscosity.

Flow restriction mechanism

Fluorescein sodium salt (80 ng/mL, Spectrum, Gardena, CA) in PBS was mixed with unlabeled streptavidin or BSA as a control (both 500 μ g/mL in PBS) and allowed to flow in 13 μ m tall biotin-modified microchannels.

Fluorescence signal was monitored using a CCD camera (CoolSNAP HQ2, Photometrics, Tucson, AZ) attached to an upright microscope (Axio Scope, A1, Zeiss, Thornwood, NY). Illumination was provided by a 625 mW LED (MBLED, Thorlabs, Newton, NJ) that passed through a filter cube (FITC-LP01-Clinical-OMF, Semrock, Rochester, NY). Images were acquired using a 400 ms exposure time. Image acquisition and data analysis were performed using Image J software. The fluorescence signal from fluorescein in these images, integrated across the channel at different flow distances, was obtained. From these traces, background subtracted and normalized channel fluorescence signal peak areas (proportional to channel cross-sectional areas) were obtained for flow solutions containing either streptavidin or BSA (control).

Contact angles of streptavidin solution droplets of different concentrations on biotinylated PDMS substrates were measured using a contact angle goniometer (Rame-Hart, Succasunna, NJ).

Results and Discussion

Experiments on a model system, biotin-streptavidin, were conducted to test the devices and enable their optimization. We studied the effects on flow distance of channel height and shape, PDMS cover layer thickness, and solution viscosity. We also probed the mechanism through which channel closure affects flow. Figure 2 shows a few examples of the data resulting from “flow valve” assays. Unfilled flow channels are easily seen in the photographs (e.g., Fig. 2A), and similarly under simple visual inspection. In contrast, microchannels containing liquid, as demonstrated in Figure 2B, are no longer seen readily.

Initial experiments were conducted on 35 mm long, 58 μ m wide channels with a 1.1 mm thick PDMS cover layer. Channels with a height <5 μ m usually became blocked by the flow of only water or during coating with b-BSA, either because of channel deformation due to capillary forces or due to surface crosslinking during BSA adsorption. In a revised device design with slightly taller microchannels (5.2 μ m) and a PDMS cover layer thickness of 0.7 mm, a 1.0 mg/mL streptavidin solution traveled 10 mm, and a 0.88 mg/mL streptavidin solution traveled 15 mm, while solutions lacking streptavidin flowed the full length (35 mm) of the b-BSA coated channel. When glycerol was added to a 28% concentration, a solution

with a streptavidin concentration of 60 $\mu\text{g/mL}$ flowed 30 mm in a 5.2 μm tall channel with a 0.7 mm PDMS cover layer thickness. These experiments identified three assay parameters (channel height, cover layer thickness and solution viscosity) that could be altered to affect the dynamic range and limit of detection for “flow valve” experiments. Reproducible results for these initial device designs were still somewhat difficult to obtain, which we attribute to the above-noted blockage issues associated with relatively shallow channels.

A curved channel cross-section (Fig. 1G) was necessary for channel constriction, probably because pinching shut from the sides towards the center was possible with this geometry (see Fig. 1H, J). We did flow experiments with curved and rectangular cross-section channels coated with biotin (both 58 μm wide and 5.2 μm tall with a 0.7 mm PDMS top layer thickness); 1.0 mg/mL streptavidin solution flowed only 10 mm in the curved cross-section channel before flow stopped, but the same solution flowed the full length of the rectangular cross-section channel. Our results with this channel geometry are also consistent with published work showing that a curved channel is easier to close than a rectangular channel for valves actuated by external pressure.²¹

Building on these initial studies, we further characterized the three factors that affected channel closure: solution viscosity, PDMS cover layer thickness and channel height. We varied one parameter while holding others constant and observed any effects on the flow distance. We also used taller (13–17 μm) channels to avoid some of the issues previously seen with shallower ones. When a higher streptavidin concentration (10 $\mu\text{g/mL}$) was introduced into a biotin-modified channel (Fig. 2C), more rapid cross-linking of the biotin anchored to the surface in the first few millimeters of the channel length led to faster constriction at the start of the channel and a shorter capillary flow distance traveled by the streptavidin solution. On the other hand, when a lower concentration of streptavidin solution (100 ng/mL) was loaded (Fig. 2D), slower cross-linking led to a greater capillary flow distance for the streptavidin solution before constriction in the first few millimeters of the channel stopped flow. A more in-depth discussion of this hypothesized mechanism of channel closure and flow stoppage is provided later.

The effect of solution viscosity on the flow distance was studied with other variables held constant. Added glycerol adjusted the solution viscosity, and control solutions containing glycerol but lacking streptavidin flowed the entire length of the microchannels. Figure 3 shows the effects of solution viscosity (1.0–3.2 cP, corresponding to glycerol concentrations of 0–36%)²² on flow distance for 100 ng/mL and 1.0 $\mu\text{g/mL}$ streptavidin. There was little effect on the flow distance for viscosities less than ~2 cP (24% glycerol), but with further increases in viscosity, the flow distance decreased. Below 2 cP, the solution viscosity also had little effect on flow velocity (10–12 s to flow 80 mm, with or without glycerol). However, above 2 cP, the solution viscosity led to slower solution flow through the channel that increased the time for biotin-streptavidin interaction and closure of the first few millimeters of the channel. Thus, the distance solution traveled before channel constriction restricted flow was shorter for both concentrations of streptavidin. The 100 ng/mL solutions travelled a greater distance than the 1.0 $\mu\text{g/mL}$ ones, in line with expectations. We further found that added glycerol could be used to adjust the linear range for detection for a given microchannel length, although adding glycerol increased the assay complexity compared to flowing solution without viscosity adjustment. Indeed, flow experiments done in duplicate in glycerol-adjusted 3.0 cP solutions in biotin-modified 17 μm tall channels yielded the following results: control solutions lacking streptavidin flowed 95 and 100 mm; solutions containing 1 ng/mL streptavidin flowed 56 mm and 67 mm; and 100 pg/mL streptavidin solutions (a factor of 10 lower concentration than we have been able to detect reliably in 1.0 cP solutions) flowed 71 mm and 84 mm. It is also valuable to understand the viscosity

dependence of flow distance in these devices for possible future work with viscous samples like blood.

The effect of PDMS cover layer thickness on the flow distance was also studied with all other parameters held constant. Figure 4 shows the influence of different PDMS top layer thicknesses on the flow distance for 1.0 $\mu\text{g/mL}$ streptavidin in biotin-modified channels. The flow distance decreases at a slower rate as PDMS layer thickness is reduced, approaching an asymptote around 0.45 mm cover layer thickness. We attribute the shorter flow distances for thinner cover layers to the reduced force needed to deflect the cover layer and constrict the first few millimeters of the channel, resulting in more rapid constriction and hence shorter capillary flow time and distance. The leveling off observed approaching 0.45 mm thickness may occur because forces exerted in the channel by capillary flow may start to contribute to constriction at these shallower depths, leading to similar flow times and distances. From the data obtained, we conclude that cover thickness allows control of the flow distance, and thus this parameter can adjust dynamic range or limit of detection.

We explored the correlation between flow distance and streptavidin concentration for two different channel heights. A plot of flow distance for various streptavidin solution concentrations in biotin-modified 13 μm tall channels is given in Figure 5A. The plot shows a linear relationship between the logarithm of streptavidin concentration and flow distance, along with a good R^2 value of 0.95. A different set of experiments was carried out on biotin-modified 17 μm tall channels (Figure 5B) and likewise showed a linear relationship with an improved R^2 value of 0.98 and less data scatter than in the 13 μm tall channels. Also, improved assay sensitivity was observed for deeper versus shallower channels, with a 40% increase in the magnitude of the calibration curve slope. The linear relationship between logarithm of streptavidin concentration and flow distance across a broad swath of concentrations in different channel heights highlights the wide dynamic range for this method. The lowest quantified streptavidin concentration was 1.0 ng/mL, with flow distances of ~ 100 mm for 13 μm tall channels and ~ 130 mm for 17 μm tall channels, with potential to detect lower streptavidin concentrations using longer channels. This very low detection limit compared to the ~ 0.2 $\mu\text{g/mL}$ protein detection limits in paper-based assays^{17, 23} and excellent quantitation capability marks an important improvement in performance for simple, rapid and inexpensive assays.

We have evaluated and eliminated several plausible explanations for the observed flow behavior that do not involve channel constriction. We measured contact angles of solutions of different streptavidin concentrations (1 ng/mL–100 $\mu\text{g/mL}$) on b-BSA coated PDMS to be 25–26°. Thus, the mechanism of flow stoppage is clearly not linked to concentration-dependent changes in surface wettability or tension. In addition, flow experiments on buffer solutions lacking streptavidin were performed in biotin-modified 13 μm tall PDMS microchannels with 0.45 and 0.5 mm cover layers. These solutions flowed the entire channel length, indicating that the flow stoppage was not due to any pressure drop or channel constriction caused by capillary action. Finally, flow was unaffected by non-specific adsorption, as streptavidin solutions from 1 ng/mL–100 $\mu\text{g/mL}$ in 13 μm tall channels coated with BSA (lacking biotin) flowed the entire channel distance. Thus non-specific adsorption, which is a significant problem for conventional immunoassays, appears not to play a major role in our “flow valve” devices, showing promising potential for extension to other assay systems.

We further explored the mechanism of flow stoppage via channel closure using fluorescent imaging. After capillary flow of a solution containing streptavidin mixed with the unreactive small molecule marker fluorescein in a biotin-coated channel, the fluorescence in the first 10 mm of the microchannel was imaged to observe any differences due to constriction (Fig.

6A–B). Plots of normalized fluorescence signal across the channel at different flow distances demonstrate a significant, 3-fold increase in channel fluorescence (i.e., cross-sectional area) moving away from the solution introduction point until the signal plateaus at around 6 mm flow distance, as shown in Figure 6C. Importantly, control experiments wherein streptavidin was replaced by BSA and similarly flowed with fluorescein (Fig. 6D–F) showed no appreciable change in channel cross-section over the same portion of the flow channel, clearly supporting a channel constriction mechanism that is specific to biotin-streptavidin interaction. We hypothesize that once this initial portion of the channel is constricted to a sufficiently small aperture, flow stops. Thus, the capillary flow distance of the target solution depends on the time needed to close the first few millimeters of channel enough for flow to cease, which will be a function of target concentration. Hence, for future “flow valve” designs, only the first few millimeters of the channel need to be modified with receptor, and deeper channels after the constriction zone could also be used in designing assays without serpentine channels.

A key question regarding “flow valve” assays is the following: how can molecular-scale (~ 10 nm) surface interactions translate into much larger, micrometer-dimension alterations in microchannel cross section that affect flow? We believe that the answer can be found in the data in Figure 6, coupled with the posited channel constriction mechanism illustrated at the bottom of Figure 1. At the edges of the microchannels, where the curved regions meet the flatter bottom segment (see Fig. 1), the channel height is around the ~ 10 nm molecular scale, such that biotin-streptavidin crosslinking of the top surface to the bottom is possible. This interaction would pull the top and bottom surfaces incrementally closer, enabling similar molecular-scale crosslinking to occur moving inward toward the middle of the channel. As this interaction progresses, the edges of the channel would be fully constricted, while the middle would remain partially open (i.e., Fig. 1J–K). Importantly, the data in Figure 6A–B are indicative of exactly this type of change in cross-sectional channel profile induced by streptavidin solution flow, strongly supporting our hypothesized mechanism. In further support of surface intermolecular interactions leading to channel constriction, we have carried out simple force calculations. A typical surface density of b-BSA molecules is $6 \times 10^{16}/\text{m}^2$,²⁴ while the force needed to unbind one biotin-streptavidin molecular pair has been measured as 200–300 pN.^{25, 26} Hence, the force per area exerted by biotin-streptavidin surface interactions would be at least $1.2 \times 10^7 \text{ N/m}^2$, or 1740 psi, which is over a factor of 100 greater than the 5–10 psi needed to completely close similarly shaped PDMS microfluidic valves.²¹ Thus, we conclude that molecular-scale interactions have sufficient force to induce channel constriction and that the occurrence of such interactions from the edges toward the center of a microchannel is both plausible and consistent with the channel imaging data we have obtained.

Several matters must be considered as we look toward extending this assay method beyond biotin-streptavidin. Receptors must recognize at least two distinct sites on the target to crosslink channels. Streptavidin readily meets this criterion with four biotin binding sites. Polyclonal antibodies or two different monoclonal antibodies to a target would recognize different epitopes and should also cause receptor-mediated crosslinking of microchannels in response to an antigen target. Additionally, hybridization of a target nucleic acid sequence to complementary surface-attached single-stranded oligonucleotides should mediate microchannel closure. Are antigen-antibody or base pairing interactions strong enough to develop “flow valve” assays? The unbinding forces for target-receptor pairs have been studied by scanning probe microscopy, and were 200–300 pN^{25, 26} per biotin-streptavidin molecular pair. The measured unbinding force for a single antigen-antibody pair is 50–60 pN,^{27–29} which is less than biotin-streptavidin by a small factor of 3–6 that could likely be accommodated through adjusting device parameters. The unbinding force for hybridized DNA oligonucleotides, depending on the sequence and number of base pairs, ranges from

450 pN³⁰ for 14-mer sequences to 2700 pN for 20-base-long hybridized pairs.³¹ These published unbinding data affirm the likely feasibility of generalization of “flow valve” systems beyond biotin-streptavidin measurements to nucleic acid hybridization and antigen-antibody interactions.

Conclusions

We have demonstrated a detectorless microfluidic approach for quantifying target analytes through simple visual inspection of capillary flow distance in a microchannel. We have identified and characterized three important parameters (solution viscosity, PDMS cover layer thickness and channel height) that affect the flow distance in these assays for the biotin-streptavidin model system. In addition, we found a linear relationship between flow distance in biotin-modified channels and logarithm of streptavidin concentration over a 100,000-fold range of concentrations. Moreover, we identified and studied a plausible mechanism of channel constriction and how this leads to concentration-dependent flow distances. Importantly, we have measured streptavidin concentrations as low as 1 ng/mL using these microsystems, demonstrating low detection limits, with potential for future improvement. “Flow valve” microfluidic devices show great promise for simplified, low cost, but high performance chemical analysis that could be extended to antigen and nucleic acid determinations. “Flow valve” systems are especially promising for POC testing due to their portability, and detectorless and label-free quantitation.

Acknowledgments

We thank Prof. Paul Farnsworth for helpful suggestions about testing the mechanism of flow stoppage and Prof. Matthew Linford for letting us use his goniometer. This work was supported by the National Institutes of Health (R01 EB006124).

References

1. LaCourse WR. *Anal Chem.* 2002; 74:2813–2831. [PubMed: 12090667]
2. Svec F. *J Chromatogr A.* 2010; 1217:902–924. [PubMed: 19828151]
3. Li Y, Lee ML. *J Sep Sci.* 2009; 32:3369–3378. [PubMed: 19824026]
4. Jorgenson JW. *Annu Rev Anal Chem.* 2010; 3:129–150.
5. Aebersold R, Goodlett DR. *Chem Rev.* 2001; 101:269–295. [PubMed: 11712248]
6. Griffiths WJ, Wang Y. *Chem Soc Rev.* 2009; 38:1882–1896. [PubMed: 19551169]
7. Perry RH, Cooks RG, Noll RJ. *Mass Spectrom Rev.* 2008; 27:661–699. [PubMed: 18683895]
8. Harris GA, Galhena AS, Fernandez FM. *Anal Chem.* 2011; 83:4508–4538. [PubMed: 21495690]
9. Gillie JK, Hochlowski J, Arbuckle-Keil GA. *Anal Chem.* 2000; 72:71R–79R.
10. Mulvaney SP, Keating CD. *Anal Chem.* 2000; 72:145R–157R.
11. Kaplan, LA.; Pesce, AJ. *Clinical Chemistry: Theory, Analysis, Correlation.* 5. Elsevier; Amsterdam: 2010.
12. Zhang H, Wang S, Fang G. *J Immunol Meth.* 2011; 368:1–23.
13. Vashist SK, Zheng D, Al-Rubeaan K, Luong JHT, Sheu FS. *Anal Chim Acta.* 2011; 703:124–136. [PubMed: 21889626]
14. Tomlinson C, Marshall J, Ellis JE. *Curr Med Res Opin.* 2008; 24:1645–1649. [PubMed: 18457603]
15. Martinez AW, Phillips ST, Butte MJ, Whitesides GM. *Angew Chem Int Ed.* 2007; 46:1318–1320.
16. Dungchai W, Chailapakul O, Henry CS. *Analyst.* 2011; 136:77–82. [PubMed: 20871884]
17. Liu H, Crooks RM. *J Am Chem Soc.* 2011; 133:17564–17566. [PubMed: 22004329]
18. Zhong M, Lee CY, Croushore CA, Sweedler JV. *Lab Chip.* 2012; 12:2037–2045. [PubMed: 22508372]

19. Barber JP, Lunt EJ, George ZA, Yin D, Schmidt H, Hawkins AR. *IEEE Phot Technol Lett*. 2006; 18:28–30.
20. Duffy DC, McDonald JC, Schueller OJA, Whitesides GM. *Anal Chem*. 1998; 70:4974–4984. [PubMed: 21644679]
21. Unger MA, Chou HP, Thorsen T, Scherer A, Quake SR. *Science*. 2000; 288:113–116. [PubMed: 10753110]
22. Sheely ML. *Indust Eng Chem*. 1932; 24:1060–1064.
23. Martinez AW, Phillips ST, Butte MJ, Whitesides GM. *Angew Chem Int Ed*. 2007; 46:1318–1320.
24. Sun YS, Landry JP, Fei YY, Zhu XD, Luo JT, Wang XB, Lam KS. *Anal Chem*. 2009; 81:5373–5380. [PubMed: 19563213]
25. Wong SS, Joselevich E, Woolley AT, Cheung CL, Lieber CM. *Nature*. 1998; 394:52–55. [PubMed: 9665127]
26. Moy VT, Florin EL, Gaub HE. *Science*. 1994; 266:257–259. [PubMed: 7939660]
27. Allen S, Chen X, Davies J, Davies MC, Dawkes AC, Edwards JC, Roberts CJ, Sefton J, Tendler SJB, Williams PM. *Biochemistry*. 1997; 36:7457–7463. [PubMed: 9200694]
28. Ros R, Schwesinger F, Anselmetti D, Kubon M, Schafer R, Pluckthun A, Tiefenauer L. *Proc Natl Acad Sci USA*. 1998; 95:7402–7405. [PubMed: 9636161]
29. Harada Y, Kuroda M, Ishida A. *Langmuir*. 2000; 16:708–715.
30. Noy A, Vezenov DV, Kayyem JF, Meade TJ, Lieber CM. *Chem Biol*. 1997; 4:519–527. [PubMed: 9263640]
31. Mazzola LT, Frank CW, Fodor SPA, Mosher C, Lartius R, Henderson E. *Biophys J*. 1999; 76:2922–2933. [PubMed: 10354420]

Figure 1.

“Flow valve” device fabrication and channel closure process. Microchannel and PDMS layer dimensions are not shown at scale. (A) Glass wafer (gray). (B) AZ50XT positive photoresist (black) spun on wafer. (C) Photolithographic patterning to form a mold with elevated feature using UV exposure and development with AZ400K developer. (D) Reflowing of photoresist at 150 °C for 5 min to yield a curved feature. (E) PDMS cured (80 °C, 45 min) on mold. (F) Cured PDMS released. (G) Plasma bonding to enclose the microchannel. (H) Cross section zoom view of the open channel in (G) coated with receptors. (I) Zoom view (~1000X) of the boxes in (H), showing receptors on the top and bottom channel walls. (J) Cross section of a partially closed channel. (K) Zoom view (~1000X) of the box in (J), showing receptor-target interaction leading to channel closure.

Figure 2.

Flow-valve assay concept and data, with device schematic (top) and photograph (bottom) in each panel. Devices had biotinylated-BSA coated channels 17 μm tall and 58 μm wide, and a PDMS cover layer thickness of 0.5 mm. White arrows indicate direction of flow. (A) Open and empty channel, visible as no solution is in it. (B) Channel filled with solution lacking streptavidin, which travels the entire length of the channel without stopping, making the channel difficult to distinguish from the surrounding device. (C) 32 mm flow distance for 10 $\mu\text{g/mL}$ streptavidin solution loaded in the channel. (D) 83 mm flow distance for 100 ng/mL streptavidin solution added to the channel.

Figure 3. Effect of solution viscosity on the flow distance of 100 ng/mL (X) and 1.0 μg/mL (O) streptavidin solutions in biotin-modified 17 μm deep and 58 μm wide channels with a 0.5 mm PDMS top layer. Flow distance is only affected above a threshold viscosity of ~2 cP, corresponding to 24% glycerol.

Figure 4. Effect of PDMS layer thickness on the flow distance for 1.0 $\mu\text{g/mL}$ streptavidin in biotin-modified 17 μm deep and 58 μm wide channels. Flow distance decreases asymptotically as PDMS layer thickness is reduced.

Figure 5.

Flow distance traveled as a function of streptavidin concentration in biotin-modified microchannels (58 μm wide) of two different heights, with a 0.5 mm thick PDMS top layer. (A) 13 μm deep channels. (B) 17 μm deep channels.

Figure 6.

Plots of background subtracted, normalized fluorescence signal and peak area in 13 μm deep biotin-modified channels as a function of channel position and flow distance to probe constriction. (A–B) Unlabeled streptavidin (500 $\mu\text{g/mL}$) mixed with fluorescein sodium salt (80 ng/mL); signal (which scales with pathlength or channel height) is lowest near the reservoir, rising gradually to a constant level at about 6 mm flow distance. (C) Normalized peak area of unlabeled streptavidin versus flow distance is lowest near the reservoir, increasing to a constant value at ~6 mm flow distance. (D–E) Unlabeled BSA (500 $\mu\text{g/mL}$) mixed with fluorescein sodium salt (80 ng/mL); signal is essentially constant along the flow distance. (F) Normalized peak area of unlabeled BSA versus flow distance is approximately constant throughout the flow distance.