Microfluidic force spectroscopy for characterization of biomolecular interactions with piconewton resolution

M. Javanmard,^{1,2} F. Babrzadeh,¹ and R. W. Davis^{1,a)}

¹Stanford Genome Technology Center, Stanford University, Palo Alto, California 94304, USA

²Department of Electrical Engineering, Stanford University, Stanford, California 94305, USA

(Received 7 February 2010; accepted 8 July 2010; published online 29 October 2010)

In this paper we present a scalable method based on the use of microfluidics and shear force spectroscopy which can be used for determining the affinity between molecules. Our method involves the use of functionalization of the surface of microfluidic channels with ligand molecules, and the surface of microspheres with receptor molecules. Bound beads are detached from the surface of the microchannels using pressure driven flow. The drag force required to detach the beads is used to determine the affinity of the bond holding the two molecules together. The minimum force we are able to detect is 5 pN. We have used this method to determine the binding force between protein-protein interactions and DNA base-pair interactions. We also have shown the ability of this technique to distinguish between strong and weak protein-protein interactions. Using this approach, it may be possible to multiplex an array of these functionalized channels onto a chip and probe the interactions between large varieties of biomolecules. © 2010 American Institute of Physics. [doi:10.1063/1.3491547]

The ability to quantify the strength of interactions between various ligands and receptor molecules can be useful for various applications in biotechnology such as biomarker detection,^{1–3} drug screening,⁴ and systems biology.⁵ Interactions of interest include protein-protein interactions,⁶ DNA base-pair interactions,⁷ nucleic acid-protein interactions,⁸ antibody-antigen interactions,⁹ and many others. The most common methods used to characterize the forces of various biomolecular interactions include the use of atomic force microscopy (AFM),⁹ optical tweezers,¹⁰ and magnetic tweezers.¹¹ The AFM approach relies on functionalizing the AFM tip with a probe molecule and the surface with a receptor molecule, and then bringing the tip in proximity of the surface such that the probe-receptor interaction takes place. The probe is then pulled away from the surface until the bond is broken and the force of the interaction is thus measured. The optical tweezers approach involves bringing functionalized microparticles in proximity to each other, using the radiation force which the laser applies to the particle, and then measuring the force required to pull them apart. Magnetic tweezers operate on a similar concept with the exception that magnetic beads are used and are actuated via magnetic force. These three techniques are advantageous in that single molecule interactions can be measured. All three suffer the drawback that it is very difficult and costly to multiplex such that multiple reactions can be measured simultaneously. Recently dielectrophoretic tweezers have been presented for use in analyzing molecular bonds and seem promising for multiplex analysis.¹¹ The use of microfluidics for characterizing receptor-ligand interactions has gained interest recently, due to the ability to rapidly perform various assays, and the reduction in cost as a result of reduced reagent usage.^{12–14}

In this paper, we describe the design, construction, and characterization of an inexpensive scalable microfluidic platform based on hydrodynamic shear stress for detecting and quantifying various biomolecular interactions. We examine

protein-protein interactions and DNA base-pair interactions, and we demonstrate the ability to distinguish between interactions of various strengths.

The basic device (Fig. 1) operates as follows. The probe molecule is immobilized onto the surface of the microchannel and the receptor molecule is immobilized onto the surface of a microscopic bead. The microchannel is monitored from above with an optical microscope. The beads are injected into the channel and allowed to come to rest so that the channel is completely packed. Afterwards, a controlled flow is applied to the channel. The flow rate is increased in a controlled manner, and the beads are counted one by one as they are pulled off. The flow rate is proportional to the drag force, which is proportional to the affinity of the probe and receptor molecules. The number of beads which are detached at each particular drag force is recorded.

The computational software, COMSOL (COMSOL, Stockholm, Sweden), was used to develop a two-dimensional (2D) and a three-dimensional (3D) model to emulate a microsphere captured on the surface of the channel. The relationship between the fluid flow rate and the drag force on a sphere attached to the surface of a microchannel was determined.

In the 2D model, we assume, a channel height of 50 μm , and a channel length of 700 μm . In the 3D model, we assume a channel height of 50 μm , a channel length of 700 μm , and a channel width of 200 μm . We assume a no-slip boundary condition at the walls, and a pressure dif-

FIG. 1. (Color online) Microchannel functionalized with probe molecule and beads functionalized with target molecule of interest. Shear force is applied in order to detach bound beads. Drag force required to detach beads correlates with bond affinity.

^{a)}Electronic mail: dbowe@stanford.edu.

FIG. 2. (Color online) Relationship between channel flow rate and drag force applied to bead attached to microchannel surface.

ference at the inlet and outlet. We solve the steady-state Navier–Stokes equation to determine the velocity flow profile inside the channel, based on which we obtain the shear force and pressure force at every point on the immobilized microsphere. We integrate both of these forces over the area of the sphere to determine the drag force applied to the sphere, which is the most accurate description of the binding force holding the probe and receptor molecules together. These simulations were run for 7, 10, and 20 μm diameter spheres, and the relation between flow rate and drag force is plotted in Fig. 2 for all three cases. The 3D model is compared to the 2D model for the 10 μm case, and the results are quite similar. Another comparison is made to the sphere-drag formula of Stokes:¹⁵

$$F = 6\pi\mu Ua, \quad (1)$$

where U is the mean velocity at which the sphere travels and a is the radius of the sphere. The results of the simulation and the sphere-drag formula of Stokes are on the same order of magnitude (Fig. 2).

The devices were fabricated using standard soft-lithography processing. Channel molds were fabricated on a silicon wafer using standard photolithography with SU-8 photoresist. PDMS along with its' primer (10:1 ratio) is then poured onto the mold and allowed to cure at 80°C for two hours, where the PDMS slab is then peeled off. 1 mm holes are then punched through the PDMS slab to create inlets and outlets. The PDMS slab and the glass slide is then treated with oxygen plasma, and the two are brought into contact together to create an irreversible seal. The inlet of our micro-channel is tied to a syringe pump which controls the flow rate at which fluid is injected into the sample. The channel is monitored under an optical microscope in order to quantify bead binding.

The first type of interactions we examined were DNA base-pair interactions. Probe DNA molecules were immobilized onto the glass surface using streptavidin based surface chemistry. Biotinylated bovine serum albumin (BSA) was incubated in the channel for 20 min until the surface was completely saturated. Afterwards streptavidin linker molecules were incubated for 15 min. The surface was then blocked with BSA in order to inhibit nonspecific interactions. Given that the affinity of biotin-streptavidin molecules is several orders of magnitude stronger than the DNA base-pair interactions, it's fair to assume that the double strand will break before the streptavidin-biotin linker does.

FIG. 3. (Color online) Average drag force required to detach beads from channel surface. Perfectly complementary target DNA and DNA with single basepair mismatch are not distinguishable in the case where 39 base pairs are hybridized to each other.

We also immobilized the DNA onto the surface of 10 μm beads using streptavidin chemistry. We link biotinylated DNA to streptavidin pre-functionalized beads. After coating the channel surface with probe DNA, we inject the beads into the surface and incubate them briefly until they settle to the bottom of the channel. Afterwards we apply a flow to the channel. We continuously increase the flow rate and monitor the number of beads getting detached from the surface at each flow rate. Figure 3 shows the force spectroscopy results for a comparison between perfectly matched probe and target DNA strands, a single base pair mismatch, and a completely mismatched strand. The average drag force required for pulling off the beads and the standard error bars have been plotted. The nonspecifically settled beads are pulled off with a minimal force less than 5 pN. This minimal amount of force is necessary to move the beads at rest are due to gravitational forces. As for the perfectly complementary DNA target and the single base pair mismatch, the force required is indistinguishable, which is reasonable for a 40 base pair length DNA strand. In future studies, we will explore the use of shorter DNA probes to see if we can detect single nucleotide polymorphisms.

In order to calibrate our system we measured the rupture forces between streptavidin and biotin, which is a well studied system. According to previous work done in the literature,¹⁶ the rupture force for streptavidin-biotin is roughly 257 ± 25 pN. In appendix Fig. 4, we plot the percentage of beads pulled off for a given drag force. The average force required to detach the streptavidin coated beads from the biotin coated surface is approximately 240 pN, demonstrating that the rupture forces obtained through our

FIG. 4. (Color online) Histogram of the percentage of beads pulled off at a given drag force for three cases: Negative control, Perfectly Complementary DNA, and Streptavidin.

FIG. 5. (Color online) Drag force required to detach bead as a function of DNA hybridized to channel surface.

assay are comparable to those reported with other techniques.

One of the reasons why all of the beads in a single experiment are not pulled off at a single drag force is due to the fact that the force at which the beads are pulled off is dependent on the strength of the interaction and the number of interactions. Most of the bead surface interactions are held together by multiple DNA base-pair interactions, and the numbers of interactions vary from bead to bead, thus the drag force required to pull of a bead varies. In order to explore the effect of the number of interactions and the binding strength, we explored the average binding strength versus target DNA concentration as shown in Fig. 5. Decrease in concentration of target DNA results in a decrease in the average force required to detach a bead. Below 15 nM, the force required to detach the beads is below the specific detection threshold. This means that at 15 nM we can assume that many of the beads are held down only by a single double stranded DNA molecule.

Based on experimental results obtained with AFM, as the rate at which the applied force increases, the detachment force becomes more and more independent of the length of the double stranded DNA. In this case, the force required to detach a double stranded DNA is roughly on the order of 50 pN.⁹ The average force required to detach beads in the case where 15 nM of target DNA was greater than 30 pN. The order of magnitude is the same, however, the reason for the 40% discrepancy could be due to some beads being more weakly bound as a result of nonspecific binding, thus resulting in an average lower than that if all of the beads bound were due to a single DNA molecule holding each bead down.

The second type of interaction we examined was protein-protein interactions (Fig. 6). One example we examined was the hCG antigen and the anti-hCG antibody. In comparison we also examined the interaction between hCG and lactoperoxidase which is glycoprotein.

In both cases, anti-hCG antibody was attached to the surface by physical adsorption. As for the beads, hCG antigens and lactoperoxidase were each separately covalently bound to 9 μ m carboxylic acid functionalized polystyrene beads, in a procedure described in detail previously.¹⁷ Given that hCG and lactoperoxidase are both glycoproteins, they interact with each other, however, with a weaker affinity than the antibody-antigen interaction which attracts hCG with its' antibody.

FIG. 6. (Color online) Drag force required to detach protein functionalized beads from channel surface.

In summary, in this study we have demonstrated the concept of using shear force spectroscopy for studying the binding forces of various types of molecular interactions including DNA base-pair interactions and protein-protein interactions. The force holding down each bead is proportional to the affinity of the probe-receptor interaction, which we demonstrate with our protein-protein interaction experiments. By varying the concentration of target DNA molecules hybridized to the probe, we also show that the drag force required to detach each bead is also proportional to the number of interactions binding each bead to the surface. We have shown the utility of this technique by looking at single channels. However, by fabricating multiple channels in parallel on a single chip and immobilizing a different probe in each channel, one can use this technique for multiplexed analysis.

This research was supported by National Institutes of Health under Grant No. P01 HG000205.

- ¹W. J. Catalona, A. W. Partin, K. M. Slawin, M. K. Brawer, R. C. Flanigan, A. Patel, J. P. Richie, J. B. deKernion, P. C. Walsh, P. T. Scardino, P. H. Lange, E. N. P. Subong, R. E. Parson, G. H. Gasior, K. G. Loveland, and P. C. Southwick, *JAMA, J. Am. Med. Assoc.* **279**, 1542 (1998).
- ²G. MacBeath, *Nat. Genet.* **32**, 526 (2002).
- ³H. Norppa, *Toxicol. Lett.* **149**, 309 (2004).
- ⁴M. R. Arkin and J. A. Wells, *Nat. Rev. Drug Discovery* **3**, 301 (2004).
- ⁵S. J. Maerkl and S. R. Quake, *Science* **315**, 233 (2007).
- ⁶D. Gerber, S. J. Maerkl, and S. R. Quake, *Nat. Methods* **6**, 71 (2009).
- ⁷P. O. Brown and D. Botstein, *Nat. Genet.* **21**, 33 (1999).
- ⁸R. M. T. d. Wildt, C. R. Mundy, B. D. Gorick, and I. M. Tomlinson, *Nat. Biotechnol.* **18**, 989 (2000).
- ⁹T. Strunz, K. Oroszlan, R. Schafer, and H.-J. Gantherodt, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 11277 (1999).
- ¹⁰C. Danilowicz, V. W. Coljee, C. Bouzigues, D. K. Lubensky, D. R. Nelson, and M. Prentiss, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1694 (2003).
- ¹¹A. R. Bausch, W. Möller, and E. Sackmann, *Biophys. J.* **76**, 573 (1999).
- ¹²C. Cozens-Roberts, J. A. Quinn, and D. A. Lauffenburger, *Biophys. J.* **58**, 857 (1990).
- ¹³S. R. Bates and S. R. Quake, *Appl. Phys. Lett.* **95**, 073705 (2009).
- ¹⁴H. Lu, L. Y. Koo, W. M. Wang, D. A. Lauffenburger, L. G. Griffith, and K. F. Jensen, *Anal. Chem.* **76**, 5257 (2004).
- ¹⁵F. M. White, *Viscous Fluid Flow*, 2nd ed. (McGraw-Hill, New York, 1991).
- ¹⁶V. T. Moy, E. L. Florin, and H. E. Gaub, *Science* **266**, 257 (1994).
- ¹⁷M. Javanmard, A. H. Talasaz, M. Nemat-Gorgani, D. E. Huber, F. Pease, M. Ronaghi, and R. W. Davis, *IEEE Sens. J.* **9**, 883 (2009).