

Supplementary Information:

Mass Transfer in the Biomolecular Binding of a Target Against Probe Molecules on the Surface of Microbeads Sequestered in Wells in a Microfluidic Cell

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Experimental

Microfluidic Cell Fabrication: The microfluidic devices used in the experiments were constructed from two layers of polydimethylsiloxane (PDMS) via soft lithography. SU8 photoresist (Microchem) spin-coated (Laurell) on 3" silicon wafers was lithographically patterned using transparency masks (PageWorks, 20000 dpi) printed from a (Adobe Illustrator) drawing and a near-UV flood lamp light source (OAI, $\lambda=350$ nm). The PDMS precursor (Dow Corning Sylgard 184, 10:1 base to curing agent by mass) was mixed and degassed (Thinky), poured over the SU8 molds and further degassed using a vacuum pump. The molds were incubated at 65°C for two hours to polymerize the PDMS before the patterned layers were peeled from the molds. Access ports were added to the upper layer containing the channel using a 1.5 mm biopsy punch (Harris Uni-Core). The two PDMS layers were exposed to an oxygen plasma for 30 s (Harrick Plasma Cleaner) and then contacted together to form the microchannel. Each device was mounted on a microscope slide by an additional plasma exposure step to facilitate observation using a microscope stage. Inlet and side bead entrance ports are connected to plastic and glass syringes (Hamilton) with luer locked needles by polyethylene tubing (1.5 mm OD, 1 mm ID, VWR) and the exit port is connected to a waste beaker by tubing. For the functionalization of the interior surfaces of the microfluidic cell with a polyethylene glycol (PEG) oligomer and for later chemistries described below, aqueous solutions are prepared with 18 M Ω -cm resistivity distilled (DI) water from a Millipore filtration unit, ethanol, chloroform and dimethylformamide (DMF) from Alfa Aesar, hydrochloric acid, ammonium hydroxide and hydrogen peroxide from Fisher and alkyl silanes from Gelest. An oxidizing solution (hydrogen peroxide (4 percent H₂O₂ (w/w)), hydrochloric acid (HCl) (0.4 M) and DI water in a volume ratio of 1:1:5) is flowed through the inlet port by injection from a syringe, to create surface hydroxyl groups on the PDMS polymer surface of the assembled cell. The cell is then flushed with DI water and dried with an N₂ gas stream. A neat solution of a PEG trimethoxy silane (CH₃(OCH₂CH₂)_nSi(OCH₃)₃, n=6-9) is incubated in the cell for 30 min., creating a siloxane linkage between the silane and the surface hydroxyl groups. The microfluidic cells are then flushed with DI water, and ready for use.

Microbead Functionalization: The functionalization of the glass microbead surfaces with the biotin probe is undertaken by using a silane (amino propyl

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trimethoxy silane, $\text{NH}_2(\text{CH}_2)_3\text{Si}(\text{OCH}_3)_3$, APS, to attach amino groups to the hydroxyl groups of the glass surface by a siloxane bond, and then using N-hydroxysuccinimide (NHS) derivative of biotin NHS-PEG₄-Biotin (Pierce)) to link biotin to the surface amines by the formation of an amide bond between the NHS and the amine. The beads were first cleaned in an aqueous solution of 4 percent NH_4OH and 4 percent H_2O_2 (w/w) heated to 70° for 30 minutes. The beads were then washed twice with deionized water, twice with ethanol, centrifuged, washed twice with chloroform and centrifuged. They were then suspended in a 5 mM solution of aminopropyltrimethoxysilane (APS) in chloroform for one hour to graft amine groups to the surface. To remove unbound APS, the beads were washed and sonicated with chloroform. They were then centrifuged, washed twice with ethanol, twice with water and twice with dimethylformamide. The beads were then suspended in a 1 mg/ml (DMF) solution of NHS-PEG-Biotin. To ensure that no photobleaching of the fluorescein occurred, the vial was covered with aluminum foil. The beads were then washed three times with deionized water and stored in a refrigerator at 4°C until used.

Biotin Probe Density: The molecular scale of the binding event involves the biotin ligand inserting into one of the two pockets on the binding face of NeutrAvidin. Biotin is covalently attached to the microbead surface by first conjugating aminopropyl methoxy silane ($\text{NH}_2(\text{CH}_2)_3\text{Si}(\text{OCH}_3)_3$, APS) to the glass microbead surface via a siloxane linkage of the silane to the surface hydroxyl groups of the glass microbead, and then binding NHS-PEG₄-Biotin to the surface amines. Formation of the siloxane linkage involves hydrolysis of the methoxysilane (usually by surface water), followed by a water extraction to form the siloxane linkage to the surface silanol groups. The surface concentration or density of the biotin probe moiety (Γ_p) on the microbead surface using this coupling scheme is determined by several factors. The typical value for the number of silanol ($-\text{OH}$) sites on silica is approximately 5 sites/ nm^2 (see for example Iler (1979)), which provides a maximum packing area per molecule of approximately 20 Å^2 /molecule, equal to the cross sectional area of an alkyl chain in an all trans configuration. Self assembled monolayers of long chain alkyl silanes (e.g. greater than 16 carbons), deposited from an organic solvent, can, depending on deposition conditions (e.g. solvent, bulk and surface water content, temperature and silane concentration and reaction time) assemble into ordered structures with this density due to the strong van der Waals interaction between the chains, However, silanes with alkyl chains of only a few carbons assemble in a much more disordered state with smaller surface densities (for a review, see SchreiberSchreiber (2000)). This is especially true of aminopropyl terminated silanes, in which hydrogen bonding of the amine group to surface silanols, amine catalyzed bulk polymerization. as well as their small hydrocarbon chain length lead to low grafting densities (see, for example, Kanan *et al.* (2002); Zhang and Srinivasan (2004); Horwater and Youngblood (2006); Smith and Chen (2008)). In this study, the important consideration is the surface density of accessible amine groups after silanization to the short chain NHS-PEG₄-Biotin esters. This accessibility has been studied quantitatively using NHS esters bound to fluorescent tags, and measuring the concentration of the

tag after coupling or after release of the coupling group (see for example Wang and Vaughn (2008); Jung *et al.* (2012)). The accessible density depends on the APS silanization conditions, but from an organic solvent, as is the deposition to the microbead surfaces, grafting densities of 0.2 - 0.4 molecules/ nm², or 250 - 500 Å²/site are typical. The cross sectional area of the PEG oligomer (in either helical or extended configuration) is of order 20 Å²/molecule Lee and Laibinis (1998), which is much smaller than this density, as is the breadth of the urieodo and tetrahydrothiophene rings which insert into the binding pocket of the NeutrAvidin DeTitta *et al.* (1976). Hence we can use $\Gamma_p = 0.2 - 0.4$ molecules/ nm² as an estimate for the biotin probe density.

Theoretical

We provide more details of the circulating flow in the wells as obtained from the COMSOL hydrodynamic simulations. The streaming flow above the microbead generates a slow circulating flow in the liquid in the well which surrounds the microbead. The magnitude of this velocity, is very slow. In Fig. 4c of the paper the y-component of this circulation is shown in the lower half of the microbead, and we noted that the values of this component are of order of 10⁻⁴ to 10⁻³ of the average velocity in the channel. The y-component has negative values, as the streaming flow in the channel circulates liquid around the microbead in the clockwise direction. This circulation is shown schematically in the figure below (a), and generates vortex flows in the corners and on the underside of the microbead as indicated by the large values of the magnitude of the vorticity in the vorticity map (b) in the plane $x=0$.

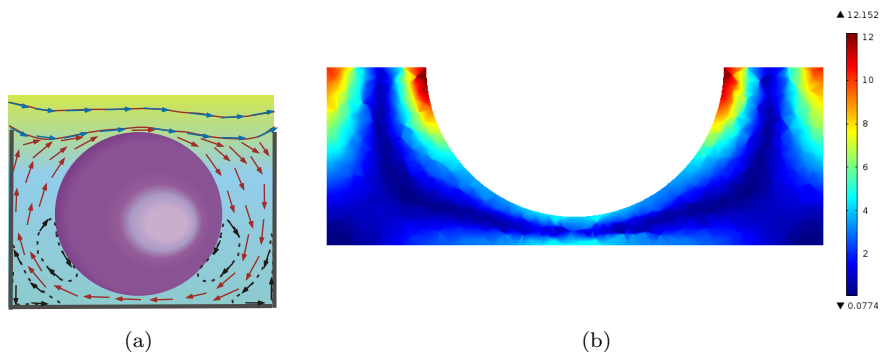


Figure S- 1: Flow in the plane $x=0$ at the bottom half of the microbead in the well (a) Schematic of the streamlines of the circulation flow (b) The magnitude of the vorticity.

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