Transport of biomolecules to binding partners displayed on the surface of microbeads arrayed in traps in a microfluidic cell

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Arrays of probe molecules integrated into a microfluidic cell are utilized as analytical tools to screen the binding interactions of the displayed probes against a target molecule. These assay platforms are useful in enzyme or antibody discovery, clinical diagnostics, and biosensing, as their ultraminiaturized design allows for high sensitivity and reduced consumption of reagents and target. We study here a platform in which the probes are first grafted to microbeads which are then arrayed in the microfluidic cell by capture in a trapping course. We examine a course which consists of V-shaped, half-open enclosures, and study theoretically and experimentally target mass transfer to the surface probes. Target binding is a two step process of diffusion across streamlines which convect the target over the microbead surface, and kinetic conjugation to the surface probes. Finite element simulations are obtained to calculate the target surface concentration as a function of time. For slow convection, large diffusive gradients build around the microbead and the trap, decreasing the overall binding rate. For rapid convection, thin diffusion boundary layers develop along the microbead surface and within the trap, increasing the binding rate to the idealized limit of untrapped microbeads in a channel. Experiments are undertaken using the binding of a target, fluorescently labeled NeutrAvidin, to its binding partner biotin, on the microbead surface. With the simulations as a guide, we identify convective flow rates which minimize diffusion barriers so that the transport rate is only kinetically determined and measure the rate constant. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4973247>]

I. INTRODUCTION

Analytical methods which allow a target protein to be screened against a probe library of potential binding partners (e.g., other proteins or small biomolecular ligands) to determine binding affinities are central to fundamental studies in cell and molecular biology, where they can be used to map the web of interactions by which proteins orchestrate biological activity. Screening tools are also important in applied research, where they are used in drug, antibody, and enzyme discovery,¹ in the identification of disease markers and in the development of biosensors for environmental surveillance and food monitoring.^{2,3} The standard method for screening protein binding interactions is the microtiter well plate, in which the library of probe molecules is spotted into an array of wells, and each well is then incubated with a target and interrogated individually (typically with an enzyme linked sandwich immunoassay) to determine target-probe conjugation. Many applications, particularly those using combinatorial synthesis for the development of new biomolecules, require a large number of probe molecules to be displayed, and for these applications the screening platform should be miniaturized to enable higher library probe densities than can be accommodated with microtiter plates. Flat microarrays (see, for example, the reviews^{4,5}) are one innovation, in which probes are robotically spotted into small areas or patches (a few hundreds of microns in diameter) on a substrate to form a library. The library is then incubated with a target, and binding is detected in a single

(parallel) step usually by fluorescently labeling the target and examining the array for spots which fluoresce upon excitation. In the flat microarray (as with the microtiter plate assay), target first diffuses through a quiescent analyte solution to the surface and subsequently kinetically binds to the surface probe. Binding is detected when enough target binds to the probes to register a fluorescent signal. When transport through the analyte phase is only by diffusion, this assay time can be relatively long even if the kinetic step is fast.⁶ Streaming analyte across the flat microarray can enhance the transport by bringing analyte solution directly to the probe surface in a convective boundary layer. The detection time would then be determined by only the kinetic rate (a property of the probe and target), and would not be limited by a diffusion barrier. With this as a goal, biosensor designs have incorporated a printed microarray of probes (or a single patch of one probe) as the bottom surface of a microfluidic channel, and have convectively screened the array with target (for reviews, see Refs. 7–9).

Microbeads (10–100 μm in diameter) arrayed in a microfluidic flow channel have been used as a more facile method to display probe molecules in a microfluidic format to take advantage of convective transport.^{10–13} Probe biomolecules are first covalently linked to the microbead surface in sets, with each set prepared separately and displaying a single probe on its surface. A microbead suspension is then streamed through the microfluidic flow channel, and patterning techniques are used to position and fix the microbeads in separable positions along the channel to form a probe array. A library of multiple probes is constructed by sequentially streaming the microbeads in sets with each set displaying only one probe. After a set is introduced, the locations of the members of the set in the channel are noted before the next set is introduced so a registry of the probe on each arrayed microbead can be compiled without encoding the beads. Fluorescently labeled target can then be streamed through the cell and over the microbeads to undertake a screening assay, with binding events recognized by identification of fluorescing microbeads, and the binding probe identified by referencing the library registry. Assays for interrogating binding interactions which use trapped microbeads with bound probes in a microfluidic cell are similar to flow cytometric assays in which beads with surface probes are suspended in a medium with a fluorescently labeled target analyte and binding interactions are identified by passing the microbeads through a flow cytometer to identify beads fluorescing with targets.^{4,14–16} The microbead microfluidic format has the advantage of reduced consumptions of analytes and reagents, higher probe densities and the versatility to identify the probes on the probe surface without encoding. Flow cytometry requires encoding the beads with a fluorescent label to identify the probe on the microbead surface.

Two common methods for arraying microbeads in a microfluidic cell are by using wells patterned into the bottom surface of the cell, e.g., Duffy *et al.*¹⁷ and Ramsey *et al.*¹⁸ or traps arranged as a microfluidic obstacle course, e.g., Nehorai *et al.*^{19,20} and our prior study on arraying lipobeads.²¹ Well deposition has also been enhanced by using electric and magnetic fields to assist in the capture,^{22–24} or by using holes placed in the well and connected to a drain to provide fluid suction (see McDevitt *et al.*^{25,26} and Ketterson²⁷). The use of traps to array microbeads follows from the studies of arraying cells, or droplets containing cells (e.g., Refs. 28–33). For capturing microbeads, the traps are typically designed to sequester a single microbead and are usually half-open, “V” shaped retaining structures which span the height of the channel and are oriented with the open part of the cavity facing the flow. These designs have an opening at the back end of the trap, to facilitate flow through the trap enabling easy capture and allowing target to stream over a trapped microbead (e.g., Refs. 19–21).

When retaining structures such as traps and wells are used to array microbeads in fixed positions, the confinement reduces the convective flow around the microbead and decreases the mass transfer rate of target to the surface probes by increasing the size of the diffusion zone or the boundary layer thickness. A reduction in the target transport rate affects the performance of the screening assay, as it increases the time necessary to achieve the particular level of target concentration necessary to detect a signal. The focus of this study is to examine this effect for the case in which microbeads are arrayed by an obstacle course of traps, using the staggered array of “V” shaped enclosures used in our prior study²¹ (see Fig. 1). While the analysis of the binding of targets to surface probes as a screening platform has been studied in detail for the

FIG. 1. Microbeads hosting surface probes are arrayed in a microfluidic cell using a trapping course.

case of probe “patches” situated on a microchannel wall (e.g., Refs. 34–36), the binding of target to microbeads captured in traps has received very limited attention. Bau *et al.*^{37–40} studied the geometry of microbeads sandwiched between the top surface of a flow channel and a shallow well at the bottom of the channel (the microbeads were preassembled in the shallow wells before closing the cell), and obtained solutions for the target concentration on the microbead surface as a function of the stream velocity and target-probe kinetic rate constants. These simulations represent approximately the idealized case of unobstructed microbeads. Our aim is to construct numerical solutions for the hydrodynamic flow and target mass balance equations to determine the surface concentration on the bead surface as a function of time when the microbeads are entrapped. The solutions are compared to the binding rates of target to probes on unconfined microbeads in a channel (as the Bau simulations) to determine the effect of the trap. We will also undertake screening experiments on the microbead array to illustrate how the simulations can be used to select experimental conditions on the imposed flow rate so that the experimentally measured overall binding rate of the target to the surface probes is controlled by the surface conjugation step so the overall binding rate can be easily simulated. For this demonstration, we will use the protein NeutrAvidin as the target, and biotin (its binding partner) as the probe. Biotin will be bound to the surface of the microbeads, and the NeutrAvidin will be fluorescently labeled so that its accumulation on the microbead surface can be quantitatively measured to obtain the overall binding rate.

II. HYDRODYNAMIC AND MASS TRANSFER NUMERICAL SIMULATIONS

A. Formulation

The simulation of the target analyte flow, and the convective diffusion and binding of the target to the probes on the microbeads arrayed in traps in the flow cell is constructed as a three dimensional simulation which models directly the trapping geometry used in our earlier publication on lipobead arraying²¹ (Fig. 1) and the NeutrAvidin-biotin binding assay experiments which are described later on. A schematic of the experimental layout of the trap arrangement in the obstacle course, and the details of an individual trap, is shown in Fig. 2(a). The traps, in a wide channel, are arranged in parallel rows (perpendicular to the flow) which are separated by 200 μm ,

and columns (along the flow) which are separated by $260\text{ }\mu\text{m}$ and staggered symmetrically with an offset of $130\text{ }\mu\text{m}$ (Fig. 2(b)). The channel is of rectangular cross section, with an overall channel width of 3 mm , a length of 6.1 mm , and a height h of $60\text{ }\mu\text{m}$. A total of 144 traps are arranged in a cluster at approximately the center of the flow channel. (The open area upstream of the cluster accommodates an entry port for the introduction of the microbeads, and both ends are connected to channels of smaller width ($300\text{ }\mu\text{m}$) from which the analyte flow enters and exits.) The “V” shaped configuration of the trap contains an aperture at the back end which is $20\text{ }\mu\text{m}$ in width, permitting the microbeads which have a diameter $2a$ equal to $42.3\text{ }\mu\text{m}$ to be captured. The trap height is equal to the channel height. The microbeads, which are made of glass in the experiments, settle to the bottom of the flow cell during the entrapment process, and therefore an approximately $20\text{ }\mu\text{m}$ gap exists between the microbead and the top wall of the microchannel, permitting flow over a microbead when it is localized in the trap. To simplify the calculations, we ignore the edge effects of the side walls of the channel, and undertake calculations on a unit cell (Fig. 2(b)) with symmetry boundary conditions for the flow and mass transfer along the side walls of the unit cell. A cartesian coordinate system is located with an origin at the center of the entrance and the bottom wall of the channel, with y in the flow direction, x perpendicular to the side walls, and z perpendicular to the bottom wall. The unit cell consists of 4 of the twelve rows of the trapping course bounded by inlet and exit cross-section planes at $y=0$ and L , respectively. (Calculations demonstrate that the addition of a further row did not change either the flow or the

mass transfer.) The microbeads in the unit cell are symmetrically placed at the centerline of the traps, and are fixed in a position touching at one point the bottom of the flow cell. While in experiments the analyte flow pushes the microbead directly against the trap walls, in the simulations, the microbeads are separated by a distance of $1\ \mu\text{m}$ to avoid complexities in the mesh construction for the numerical solution. Reducing this separation distance did not change the results. A uniform velocity U (in the y direction) and a uniform target concentration c_o is imposed at the upstream inlet ($y=0$); at the exit ($y=L$), a zero pressure condition is imposed, and the derivative of the concentration in the y direction is set equal to zero. (The distance from the inlet plane to the first row in the periodic cell is equal to $L_1=700\ \mu\text{m}$, and the distance from the back row to the exit plane is $L_2=500\ \mu\text{m}$; increases in these distances do not affect the calculated flow around the microbeads (or mass transfer of solute) and are adequate to achieve far-field conditions.) The symmetry boundary conditions along the side walls ($x=\pm W$) of the unit cell are that the velocity in the x direction is equal to zero, and the derivative, in the x direction, of the velocity in the y and z directions, and the concentration is equal to zero. (Alternatively, we could have used symmetry conditions about $x=0$ in the unit cell to solve for the flow and mass transfer in half of the unit cell domain, but the entire cell computation was pursued here.) At the initial time, the target concentration in the unit cell is assumed to be uniform and equal to c_o .

The target solution is assumed to be an incompressible Newtonian fluid with density ρ and viscosity μ of water ($\rho=10^3\ \text{kg m}^{-3}$ and $\mu=10^{-3}\ \text{kg m}^{-1}\text{s}^{-1}$) independent of the analyte concentration which is assumed to be dilute. The steady hydrodynamic flow is described by the solution of the continuity and Navier-Stokes equations (see [supplementary material](#)) in which the Reynolds number $\text{Re} = \frac{\rho U a}{\mu}$ scales the ratio of inertial to viscous forces. These equations are solved in cartesian coordinates with the inlet and outlet conditions detailed above, and boundary conditions of no slip on the interior walls of the trap and the bead surface and symmetry conditions along the unit cell border $x=\pm W$. The solution is obtained numerically using finite elements implemented with the COMSOL Multiphysics simulation package (4.2), and using both triangular and quadrilateral meshes.

The concentration field of the target is obtained by solving the convective diffusion equation for target mass conservation (see [supplementary material](#)) which in nondimensional form is scaled by the Peclet number defined as $Pe = Ua/\mathcal{D}$, where $\mathcal{D}$ is the molecular diffusion coefficient of the target. Pe is the ratio of the time scale for target to diffusive over the lengthscale of the microbead radius a ($\tau_D = a^2/\mathcal{D}$) to the time required for fluid to convect over the microbead (a/U). At large Pe , the mass transfer to the microbead surface is through a thin boundary layer of characteristic thickness $\delta \ll a$ around the microbead; at lower Pe the diffusion length scales are large and of order of the microbead radius a or the channel height h . The convective diffusion equation is solved with the inlet and outlet conditions detailed above, and assuming zero flux of target on the surfaces of the trap and channel walls and symmetry conditions on the borders ($x=\pm W$) of the unit cell.

The boundary condition for the target concentration on the microbead surface is a surface mass balance which describes the binding of target from the sublayer of liquid immediately adjacent to the microbead surface to the probes on the surface. The binding kinetics is a bimolecular process, which is described generally by a Langmuir kinetic scheme including binding and disassociation steps (see [supplementary material](#) for a general discussion). In the experiments to be reported, the probe density on the microbead surface is low enough relative to the size of the binding area of the target (A_b) that the instantaneous binding rate is proportional to the probe sites that are unoccupied (no blocking of unoccupied probe sites by neighboring bound targets) and the maximum concentration of target Γ_∞ is equal to the probe density, Γ_p . In addition, many biomolecular binding processes are nearly irreversible and disassociation can be neglected. (In our screening experiments on the binding of NeutrAvidin to surface biotin, previous experiments show that this is certainly the case.) The mass conservation boundary conditions on the microbead surface then take the form

$$\{\mathbf{n} \cdot \nabla \tilde{c}\}_{\text{bead}} = Da[\tilde{c}_s\{1 - \tilde{\Gamma}\}], \quad (1)$$

$$\Omega \frac{\partial \tilde{\Gamma}}{\partial \tau} = \{\mathbf{n} \cdot \nabla \tilde{c}\}_{\text{bead}}, \quad (2)$$

where τ is the nondimensional time (scaled by τ_D), $\mathbf{n}$ is the outward normal to the microbead surface, $\tilde{c}_s$ is the nondimensional sublayer concentration (nondimensionalized by the inlet concentration c_o), $\Omega = \frac{\Gamma_\infty}{c_o a}$, Da is the Damkohler number, $Da = \frac{k_a \Gamma_\infty a}{D}$ where k_a is the rate constant for binding or association and $\tilde{\Gamma}$ is the surface concentration scaled by the maximum binding density Γ_∞ . The parameter Ω is the ratio of the adsorption depth Γ_∞/c_o , the distance above the surface which contains (per unit area) enough target to saturate the surface to the microbead radius a . The Damkohler number is the ratio of the characteristic kinetic flux to the surface ($k_a c_o \Gamma_\infty$) to the characteristic diffusive flux on the particle length scale ($D c_o/a$). The surface concentration (Γ) is a function of the position on the bead surface, and we denote by $\bar{\Gamma}$ the average value on the bead surface. $\bar{\Gamma} = \frac{1}{A} \int_A \Gamma dA$ where A is the area of the microbead surface.

The mass transfer equations are integrated using the COMSOL simulation package, with forward marching in time. An adaptive time step is used in which for the earliest times for large Pe the time step is set to one order of magnitude smaller than the time required for formation of the boundary layer around the microbead, δ^2/D and for order one Pe the diffusion time is scaled as a^2/D .

B. Hydrodynamic flow simulations

In a typical microfluidic trapping geometry, channel heights and widths are of order $10^2 \mu\text{m}$ and $10^3 \mu\text{m}$, respectively, and for flow rates of order 10^{-1} – $10^2 \mu\text{l/min}$, the average velocity $U \sim 10$ – $10^4 \mu\text{m/s}$. Hence, Re , based on the microbead radius (typically of order $10 \mu\text{m}$), is usually less than 10^{-1} , and the motion is predominately a Stokes inertialess flow. In these simulations, we choose $\text{Re} = 0.2$, which is the experimental value. The hydrodynamic flow pattern around a microbead situated at the bottom of the downstream trap at the center of the unit cell (“A” in Fig. 2 with center at position $y = Y_A$) is shown in Fig. 3(a). The flow pattern is the magnitude of the velocity (divided by the average velocity U) in the x – z plane

FIG. 3. Simulation of the flow around the upstream center microbead in the unit cell (microbead “A” in Fig. 2) in a trapped (a) and untrapped (b) configuration for a flow Reynolds number $\text{Re} = 0.2$. The magnitude of the velocity (divided by the average velocity U) is plotted in the x – z plane corresponding to the equatorial section of the bead perpendicular to the flow, $y = Y_A$.

($y = Y_A$) perpendicular to the flow and through the bead equator. (The flow is symmetric in x since $x = 0$ is the symmetry plane of the unit cell. The projection of the figure is an oblique view, accounting for the elliptical appearance of the spherical bead.) Due to the staggered configuration of the traps, most of the flow circulates around, rather than through the trap, as the hydrodynamic resistance through an occupied trap is much larger than the resistance in the open space between the traps. Within the trap, the figure makes clear that the maximum velocity, as would be expected, is in the large gap ($\approx 20 \mu\text{m}$) between the top of the channel and the bead ($h > z > 2a$, $y = Y_A$, $x = 0$). Analyte flows through this space, and then out the back of the trap. The lateral gap thickness between the trap sidewalls and the microbead ($L_{\min} - a > x - a > 0$, $y = Y_A$, $z = a$) in the equatorial plane of Fig. 3(a), is much smaller ($L_{\min} = 10 \mu\text{m}$) and the velocity in this gap is less than between the bead and the top wall of the channel. The flow velocity in the same plane around microbead “A” in the untrapped configuration (the same array of microbeads as in Fig. 2 but without traps) is given in Fig. 3(b), and as expected, the flow around the lateral sides of the microbead is larger due to the absence of the trap. In this case, the analyte stream freely flows over most of the microbeads, except the lower half of the bottom hemisphere which is obstructed by contact with the bottom wall. As fluid is no longer directed around the blunt edges of the traps, the fluid velocity in the space occupied between the symmetry plane and the position formerly occupied by the outer trap wall is reduced by half relative to when the trap is present. In fact in this space, spanning a distance of $\approx 2a$ from the microbead to the symmetry plane, approaches two dimensional Poiseuille flow with the maximum velocity at the midplane of the channel in this space equal to $3/2$ of the upstream average velocity U in front of the trap cluster.

As stated earlier, in the regime in which the convective rate of transport of target along the microbead is large relative to the diffusive flux to the surface, the mass transfer is through a thin boundary layer around the particle. The thickness of this boundary layer decreases with the local velocity gradient (or shear rate) at the microbead surface, and the target flux becomes correspondingly larger. Hence, an examination of the surface shear rate is important in understanding the target transport in the convective regime. Detailed profiles of the velocity in the y (stream) direction, as a function of the distance across the gaps at the top and sides of the trapped microbead, are shown in Figs. 4(a) and 4(b), and compared to the profiles for a microbead without a trap. For the trapped microbead, the flow in the lateral gap is very small (velocities are of order $10^{-2} U$), and the shear rate at the wall is also small. For the top gap, the velocity (of order U) and shear rate are much larger, and the target mass transfer to the upper hemisphere of the microbead should be expected to be larger than the lower hemisphere

FIG. 4. Simulation of the flow velocity in the y (streamwise) direction (divided by U) between (a) the top of the channel and the microbead ($0 < z - 2a < h - 2a$, $y = Y_A$, $x = 0$) and (b) the side of the microbead and the channel wall ($0 < x - a < L_{\min}$, $y = Y_A$, $z = a$) and the trap wall and the symmetry plane of the unit cell for the upstream center microbead in the unit cell (microbead “A” in Fig. 2) and comparison to the untrapped microbead for $\text{Re} = 0.2$. The inset shows the distribution in the gap between the microbead and the trap wall.

(see Sec. II C 5). In contrast, in the case of the untrapped microbead, while the shear rate at the top is comparable to that of the trapped microbead, the shear rate along the lateral side is much larger as the trap no longer blocks the flow, and the mass transfer rate should be correspondingly more symmetrical between the top and bottom hemispheres. Interestingly, the flow rate through the top gap is less for the untrapped microbead than the trapped one, as fluid streams more easily laterally around the microbead.

C. Mass transfer simulations

1. Parameter values for Peclet and Damkohler numbers

The objective of the mass transfer calculations is to determine the nondimensional average concentration on the microbead surface ($\bar{\Gamma}/\Gamma_\infty$) as a function of nondimensional time τ . Aside from the geometric parameters of the unit cell, the average concentration is a function of Ω , Da and Pe , and therefore, $\frac{\bar{\Gamma}}{\Gamma_\infty}(\tau; Da, Pe, \Omega)$. In most screening applications, the concentration of the target is low enough or the binding capacity large enough so that $\Gamma_\infty/c_o \gg a$, and hence $\Omega \gg 1$. As a result, the accumulation of target on the surface, as is clear from Eq. (2), occurs in accordance with a slower time scale $\tau^* = \tau/\Omega$, as the larger the value of Ω , the slower is the binding rate. Rescaling Eq. (2) with τ^* reformulates the balance to become independent of Ω , i.e., $\frac{\partial \bar{\Gamma}}{\partial \tau^*} = \{\mathbf{n} \cdot \nabla \tilde{c}\}_{\text{bead}}$. The second boundary condition, Eq. (1), is independent of Ω (as is the zero flux boundary condition on the trap and channel walls), and the convective diffusion equation (see [supplementary material](#)) becomes $\frac{1}{\Omega} \frac{\partial \tilde{c}}{\partial \tau^*} + Pe \tilde{\mathbf{v}} \cdot \nabla \tilde{c} = \nabla^2 \tilde{c}$. Solutions of the rescaled equations are therefore independent of Ω to leading order for $\Omega \gg 1$, as the transport dynamics in the bulk becomes quasi-stationary and dictated in time only by the change in flux on the surface in τ^* . We find that the time derivative term in the bulk is only important in the earliest times in which the diffusion zone around the particle develops from a uniform concentration. Otherwise the bulk time derivative term is negligible, and as such, simulations integrated in τ done for a particular value of Da and Pe , and Ω (assumed much larger than one) can be used for another large value of Ω by just rescaling time in the completed solution by the ratios of the two values of Ω . Therefore for $\Omega \gg 1$, simulations varying Ω are not necessary. In the general set of simulations presented here, Ω is fixed at 32 which is smaller than the experimental value, but is used to accelerate the integration time.

The target surface concentration on the microbead as a function of time ($\Gamma(\tau)$) is determined by a balance between the Peclet ($Pe = Ua/\mathcal{D}$) and Damkohler ($Da = k_a \Gamma_\infty a/\mathcal{D}$) numbers. Pe is the ratio of the time scale for diffusion over a distance a normal to the surface ($a^2/\mathcal{D}$) to the nominal scale for fluid to convect over the microbead surface (estimated as a/U). Target proteins or smaller biomolecular ligands have molecular weights of order 10^3 – 10^4 and corresponding diffusion coefficients of $\sim 10^2 \mu\text{m}^2/\text{s}$; for $U \sim 10^2$ – $10^4 \mu\text{m}/\text{s}$ and a of order $10 \mu\text{m}$, Pe is of order 1 – 10^3 , which encompasses different transport behavior in the analyte stream. For Pe of order 1 – 10 , diffusion zones (regions where the concentration is less than the inlet concentration c_o) develop normal to the microbead surface and are of thickness of order a . However, for increasingly larger Pe (10^1 – 10^3), the nominal convection time is much faster and the diffusion zone takes the form of asymptotically thin boundary layers. In the simulations, we consider an order one value ($Pe = 3.5$) with order a diffusion zones, and $Pe = 3500$, characteristic of thin diffusion zones and representative of our experiments. Values for the Damkohler number Da , the ratio of the characteristic kinetic flux to the surface ($k_a c_o \Gamma_\infty$) to the characteristic diffusive flux on the particle length scale ($\mathcal{D} c_o/a$), are dependent on the surface kinetics. For Da of order 1 or smaller, the kinetic rate step is of the same order or slower than diffusion, and the gradients in concentration in the diffusion zone are small as the zone is nearly at the bulk concentration c_o . In the limit $Da \rightarrow 0$, the bulk concentration around the microbeads becomes equal to c_o , and the transport becomes kinetically limited with the surface concentration independent of the position on the microbead surface and given by the solution to Eqs. (1) and (2) after elimination of the diffusive flux term,

$$\frac{\Gamma^K(\tau)}{\Gamma_\infty} = 1 - e^{-\frac{Da}{\Omega}\tau}. \quad (3)$$

For larger Da , the kinetic step becomes faster and the concentration gradient is large in the diffusion zone. In the simulations, we study the values of $Da = 1$, characteristic of the experiments, and two larger values of 10 and 100 representing large gradients in the diffusion zones.

2. Small Peclet number regime

Consider first a low value of $Pe = 3.5$. The average nondimensional target surface concentration $\bar{\Gamma}$ (scaled by Γ_∞) on microbead “A” in a trap as a function of nondimensional time for $Pe = 3.5$ and $Da = 1, 10$ and 10^2 ($\Omega = 32$) is given in Fig. 5(a) and compared to the kinetic limits, Eq. (3), for each of the values of Da . Due to the fact that the convective and diffusive fluxes are of the same order, large diffusion zones develop around the microbead, and this slows the transport rate of the target to the surface. As a result, for every value of Da in the figure, the binding rate is much slower than the corresponding kinetic limit which assumes the concentration is uniform and equal to c_o around the microbead. The diffusion zones around the microbead are depicted for $Da = 10$ in Fig. 5(b) for three nondimensional times, $\tau = 0.136, 20.4$, and 32.6 in the equatorial plane $z = a$. Diffusion brings target to the surface where it kinetically binds to the receptors, and the bulk concentration decreases in the zone in the direction normal to the microbead surface. As time progresses, target depletion becomes more pronounced and the gradients become sharper. This is especially evident at the downstream end of the microbead, where the concentration drops to zero through the opening at the back of the trap by $\tau = 32.6$. At this time, the region at the back of the trap is also depleted. This significant depletion is due to the reduced convective flow at the back of the microbead, relative to the front, and as a result, the back side of the microbead lags behind the front in adsorption. This is shown in Fig. 5(b) which depicts the nondimensional surface concentration on the microbead surface (Γ/Γ_∞) as a projection on the equatorial plane $z = a$ of the surface concentrations of the top and bottom hemispheres. For longer times (not shown), the saturation of the surface decreases the binding rate and the diffusive flux repopulates target in the diffusion zone. As a result, the concentration gradients in the zone decrease, and eventually fade away. The surface concentration at the back end of the microbead catches up with the front end as the surface concentration over the entire microbead surface approaches Γ_∞ .

The binding curves in Fig. 5(a) show clearly the effect of Da at this low (and fixed) Pe . For the highest value ($Da = 10^2$), the binding rate is fast relative to diffusion and the sublayer concentration at the microbead surface (c_s) falls far below c_o , and sharp concentration gradients develop in the diffusion zone. As a result, the binding rate is much reduced, very far from the kinetic limit where $c_s = c_o$. As Da decreases to 10, the kinetic limit slows down (in accordance with Eq. (3)), as does the simulated binding rate. At $Da = 10$, the binding rate curve is, however, closer to its kinetic limit because the reduced kinetic rate does not deplete the sublayer of target as dramatically as when $Da = 10^2$. Finally when $Da = 1$, the simulated curve is even closer to the kinetic limit, but is still apart due to the diffusion barriers due to the large diffusion zones for $Pe = 3.5$. In fact, a Da less than 0.1 is required for the simulated curves to overlap the kinetic curve for $Pe = 3.5$.

3. Large Peclet number regime

When Pe is increased to 3500, a thin concentration boundary layer develops over the microbead surface due to the higher convective rate relative to the diffusion flux, which decreases the diffusional resistance to the target transport by reducing the thickness of the diffusion zones, and increases the rate of binding (Fig. 6). The diffusion zone is shown in Fig. 6(b), again for $Da = 10$, but for earlier nondimensional times $\tau = 0.012, 2.9$, and 5.4 than those shown for the smaller Pe . The boundary layer is very thin, except at the very backend of the microbead where

(a)

(b)

FIG. 5. The binding of a target to the surface of a microbead in a trap (microbead “A,” $y = Y_A$) for $Pe = 3.5$ ($\Omega = 32$, $\Re = 0.2$): (a) The average nondimensional surface concentration on the surface of the microbead, Γ/Γ_∞ , as a function of τ for $Da = 1, 10$, and 10^2 alongside the kinetic limits for these values of Da . (b) Target concentration diffusion zones for $Da = 10$ in the equatorial plane $z = a$ and the projection of the surface concentration averaged for the upper ($z > a$) and lower ($z < a$) hemispheres of the microbead for $\tau = .136, 20.4$, and 32.6 and $Da = 10$.

the slow convection through the trap’s backend opening leads to some depletion. Because of the thin boundary layer circumscribing most of the microbead, the kinetic binding on the microbead surface is more uniform than in the case of $Pe = 3.5$. The backend adjoining the trap opening lags only slightly behind. The surface concentration on the lateral sides of the microbead which adjoin the trap side walls also lag behind, due to the reduced convection through the side gaps between the trap walls and the microbead which enlarges the boundary

FIG. 6. The binding of a target to the surface of a microbead in a trap (microbead “A,” $y = Y_A$) for $Pe = 3500$ ($\Omega = 32$, $\Re = 0.2$): (a) The average nondimensional surface concentration on the surface of the microbead, $\bar{\Gamma}/\Gamma_\infty$, as a function of τ for $Da = 1, 10$, and 10^2 alongside the kinetic limits for these values of Da . (b) Target concentration boundary layer for $Da = 10$ in the equatorial plane $z = a$ and the projection of the surface concentration averaged between the upper ($z > a$) and lower ($z < a$) hemispheres of the microbead for $\tau = 0.012, 2.9$, and 5.4 and $Da = 10$.

layer. The boundary layer on the underside is also enlarged by the reduced convection between the microbead and the bottom wall of the flow cell. Because of this, and the fact that the gap between the top of the channel and the bead is large ($\approx 20 \mu\text{m}$), and the boundary layer fits within the gap, the top ($z > a$) and bottom hemispheres have different average concentrations as

shown in Fig. 7 for $Pe = 3500$ and $Da = 10^2$, where the more unobstructed flow in the top gap results in a larger binding rate. This difference becomes reduced as Da decreases, and the kinetic limit is approached, since the sublayer concentration becomes uniform. In the case of small Pe (3.5), the difference in binding rates between the hemispheres is not very pronounced because of the large diffusion zone which wraps around the trap creating an overall barrier to transport.

In Fig. 6(a), $\Gamma(\tau)/\Gamma_\infty$ is given for $Da = 1, 10$, and 10^2 and $Pe = 3500$, and it is clear on comparison with Fig. 5(a) for $Pe = 3.5$ that binding rates are much faster for the same values of Da . In this case, because of the reduced diffusional resistance, the binding curve at $Da = 1$ now overlaps the kinetic curve. This overlap represents the case where the binding is controlled solely by the target-probe kinetics, and is a regime which has the particular advantages when these platforms are used to measure binding constants, since the kinetic binding data only has to be regressed against the analytic kinetic curve (Eq. (3)), without the need to model the convective diffusion.

4. Scaling analysis of kinetic behavior at high Peclet number

In the high Peclet number regime, general criteria can be developed through scaling arguments to identify the value of Da below which the transport becomes kinetically limited. When a thin concentration boundary layer develops around the microbead, the boundary layer thickness, δ at the back end can be estimated as the distance the target should be away from the surface for which the time to move along the microbead, a/U_δ (where U_δ is the velocity at a distance δ from the microbead) becomes equal to the time required for the target to diffuse to the surface, δ^2/D . The velocity can be estimated as the average velocity gradient (or shear rate) evaluated at the microbead surface, $\bar{\gamma}$ multiplied by δ , and therefore $\frac{a}{\bar{\gamma}} = \frac{\delta^2}{D}$ or $\frac{\delta}{a} \sim \left[\frac{D}{a^2\bar{\gamma}}\right]^{1/3}$. For the well studied case of a fixed microbead in an infinite medium subject to a uniform flow U_∞ with concentration c_o far from the bead, the average shear rate $\bar{\gamma} \sim U_\infty/a$.⁴¹ The average shear rate over the surface of microbeads in a microchannel either situated in a trap or (ideally) fixed to a surface is a more complicated flow than the unbounded case due to the effect of the

FIG. 7. Asymmetric binding to the top and bottom hemispheres of a trapped microbead ("A") due to reduced convection at the bottom half of the bead which rests on the channel floor. $Pe = 3500$, $Da = 10^2$, $\Omega = 32$, $\Re = 2 \times 10^{-1}$.

channel and trap walls. However, to a first approximation in order to construct a simple criterion, we assume that $\bar{y} \sim U/a$ so that $\frac{\delta}{a} \sim [\frac{1}{Pe}]^{1/3}$ for $Pe \gg 1$. The diffusive flux to the surface, for $Pe \gg 1$, through the boundary layer scales as $\frac{D\{c_o - \bar{c}_s\}}{\delta}$ (where $\bar{c}_s$ is the average sublayer concentration on the microbead surface); equating this flux to the maximum kinetic flux ($k_a \Gamma_\infty$) defines a scale for the (minimum) sublayer concentration at the earliest times, $\frac{\bar{c}_{s,min}}{c_o} \sim \frac{1}{1 + \frac{Da}{Pe^{1/3}}}$. When $Da/Pe^{1/3} \ll 1$, the sublayer concentration is equal to the farfield concentration, the process is kinetically controlled and the binding is given in Eq. (3). To validate this criterion, note first Fig. 8 which plots for microbead “A” the average nondimensional sublayer concentration $\bar{c}_s$ as a function of nondimensional time for $Pe = 3500$ for $Da = 1, 10$, and 10^2 . For the largest value of Da , the large kinetic binding rate relative to diffusion leads to a rapid drop in the sublayer concentration to a value of approximately 0.1, and the sublayer slowly recovers to one as the surface saturates with target and the concentration in the diffusion zone relaxes. As Da decreases, the slowing of the kinetic rate decreases the binding, and the initial reduction in the sublayer concentration in the earliest times is much smaller than the case for $Da = 10^2$. The scaling evaluation for the minimum sublayer concentration, $\frac{\bar{c}_{s,min}}{c_o} \sim \frac{1}{1 + \frac{Da}{Pe^{1/3}}}$ gives 0.9, 0.6, and 0.1 for $Da = 1, 10$, and 10^2 , respectively, which is in very good agreement with the simulation minima as given in Fig. 8. The criteria for kinetic control $Da/Pe^{1/3} \ll 1$ are also validated in the simulations in Fig. 6, where it is clear that for the three values of Da , 1, 10, and 10^2 , $Da/Pe^{1/3} = 0.06, 0.6$, and 6.6 , respectively, and only the $Da = 1$ value satisfies the criteria and is in fact the only curve coincident with the kinetic limit, Eq. (3).

Alternatively when $Da/Pe^{1/3} \gg 1$, the sublayer concentration tends to zero, at least initially, and the target flux to the surface is controlled solely by the diffusive mass transfer. The characteristic time for the target to bind to an equilibrium surface density ($t_{eq,D}$) is given by $t_{eq,D} \frac{Dc_o}{\delta} \sim \Gamma_\infty$ or $\tau_{eq,D} = \frac{t_{eq,D}}{a^2/D} \sim \frac{\Omega}{Pe^{1/3}}$ where $\tau_{eq,D}$ denotes a nondimensional completion time scaled by the diffusion time across the microbead (τ_D). For the case of $Da = 10^2$ in Fig. 6, which is the only value of the Damkohler number large enough to satisfy $Da/Pe^{1/3} \gg 1$, $\tau_{eq,D} \approx 2.2$ which is in excellent agreement with the simulation equilibration time. Bruus

FIG. 8. The average nondimensional sublayer concentration $\bar{c}_s/c_o$ at the surface of a trapped microbead “A,” as a function of nondimensional time for $Pe = 3500$, $Da = 1, 10$, and 10^2 , $\Omega = 32$ and $\Re = 2 \times 10^{-1}$. The inset details the sublayer concentration immediately after the target analyte is introduced for $Da = 100$.

et al.,³⁶ for a patch of probes on one wall of a two dimensional microfluidic channel, have examined, by comparison to numerical simulations, the validity of reproducing the entire binding curve of the target to the patch for $Pe \gg 1$ by a boundary layer approximation in which $\mathcal{D}\{\frac{c_a - \bar{c}_s}{\delta}\} = k_a \Gamma_\infty \bar{c}_s \left\{1 - \frac{\Gamma}{\Gamma_\infty}\right\} - k_d \Gamma$ with the boundary layer thickness given by $Pe^{-1/3}$. Bruus *et al.* found for large Ω , small Da , and large Pe the approximation is most accurate. We find that simulating the binding curves of Fig. 6 for binding of target to microbeads in traps in our parameter space gives only qualitative agreement, although the equilibration is captured accurately. This is probably due to the more complicated nature of the flow in the case of the entrapped microbeads.

5. The effect of the trap on the mass transfer

To assess the effect of the trap on the mass transfer of the target to the microbead probe surface, simulations of the binding are presented for microbeads situated in the same array as in Fig. 2, but without the traps. Consider first the case of $Pe = 3.5$, where the diffusion zone is very large, and in the presence of a trap, extends beyond the trap itself as shown for $Da = 10$ (Fig. 5(b)). For the very same conditions ($Pe = 3.5$, $Da = 10$, $\Omega = 32$, and $\Re = 2.0 \times 10^{-1}$) and the same nondimensional times (0.136, 20.4, and 32.6), Fig. 9(a) shows that the diffusion zone around the unobstructed microbead is more compact. The concentration gradients at the later times are not as sharp, and less depletion is evident at the back end of the microbead. What becomes clear in comparing Figs. 5(a) and 9(a) is that the trap obstructs the transport to the back end completely at low Pe , leading to a complete depletion in the aperture at the back end of the trap. When unobstructed, the transport is like a bead in an infinite medium, where some depletion at the back end would be expected due to the larger boundary layer at the downstream face of the microbead. The nondimensional surface concentration on the microbead surface (again a projection of the surface concentration averaged between the top ($z > a$) and bottom ($z < a$) hemispheres) shows a simple asymmetry with the back end lagging behind the front because of thinner boundary layer at the upstream face of the microbead.

The more compact diffusion zone in the case of open rather than trapped microbeads also leads to a significantly more rapid binding rate, as shown in Fig. 9(a) which compares the two transport geometries for $Pe = 3.5$. In fact, this difference increases with the Da number, as the diffusion limitation becomes more rate determining due to a decrease in the sublayer concentration. In the opposite limit, as $Da \rightarrow 0$ and the sublayer concentration approaches the inlet concentration, both the trapped and untrapped microbeads approach the kinetic limit. For this value of Pe , Da is required to be as small as 0.1 for overlap, so differences exist for Da larger than this value.

When the Pe number is increased to 3500, the diffusion zone around a trapped microbead takes the form of a thin concentration boundary layer which hugs the microbead and lies within the trap except at the back face and bottom part of the microbead where the bead contacts the trap walls. The presence of the enclosing trap is therefore not as important in the high Peclet number regime, and as shown in Fig. 10, for identical conditions ($Pe = 3500$, $\Omega = 32$, and $\Re = 2.0 \times 10^{-1}$), the kinetic binding rate to the unobstructed microbeads is only marginally faster at $Da = 10^2$ (Fig. 10(a)) and becomes equal to the binding rate for the trapped microbead by $Da = 1$ (Fig. 10(b)), at which point both agree with the kinetic equation (Eq. (3)).

From these simulations, we can conclude that if flow rates are large enough, the presence of the trap retaining structure necessary to hold the microbeads in place does not necessarily hinder the target transport to the surface. In the trap geometry used here, the aperture at the back end of the trap (and the gap between the microbead and the channel top) allows flow over the microbeads (see the flow simulation, Figs. 3 and 4). It is this flow, when Pe is large enough, that allows the development of the thin boundary layers which sit inside the trap and around the microbeads and enhance the diffusion rate of the target to the surface. In other studies, wells at the floor of the microchannel are used to sequester and array microbeads to construct the probe library.^{17,18,22–24} In the well geometry, target only streams over the top part of

FIG. 9. The binding of a target to the surface of an untrapped microbead (“A”) for $Pe=3.5$ and $Da=10$ ($\Omega=32$, $\Re = 2.0 \times 10^{-1}$): (a) The average nondimensional surface concentration on the surface of the microbead, $\bar{\Gamma}/\Gamma_\infty$, as a function of τ for $Da=10$ and comparison to a trapped microbead for the same Pe , Da , Ω , and $\Re$. (b) Target concentration boundary layer for $Da=10$ in the equatorial plane $z=a$ and the projection of the surface concentration averaged between the upper ($z > a$) and lower ($z < a$) hemispheres of the microbead for $\tau = 0.136, 20.4$ and 32.6 .

the microbead, and at high Pe thin boundary layers cannot encircle a large portion of the microbead. As a result, diffusive transport is hindered, and one solution which is proposed is to create drains in the wells to enhance convection around the microbead.^{25–27} The trapping geometry studied here uses an aperture at the back end to accomplish the same goal (as well as providing a gap between the microbead and the top surface of the channel), and is much easier to implement. Another method for constructing a microbead array/probe library in a microfluidic cell is to place the functionalized microbeads in shallow wells arrayed at the bottom of the channel, and then place the channel top on the microbead array.^{37–40} The height of the channel

FIG. 10. Comparison of the binding rate for trapped and untrapped microbeads “A” in the array in the high Peclet number regime ($Pe = 3500$) for (a) $Da = 10^2$ and (b) $Da = 1$. ($\Omega = 32$ and $\Re = 2.0 \times 10^{-1}$.)

is the same size as the microbead diameter, so that the channel top holds the microbeads in place. The binding rate in this geometry approaches the idealized case of an unobstructed microbead in the channel for all Pe , but the microbead array assembly is undertaken *ex-situ*, which is more demanding than the *in-situ* hydrodynamic entrapment studied here. It is also important to note that while the thin boundary layers accompanying high flow rates (high Pe) result in enhanced mass transfer which is not hindered by the traps, a trade-off does exist. Although the enhanced mass transfer allows for a shorter time for the completion of the assay, the larger flow rates can result in more analyte used for the assay.³⁵

III. EXPERIMENTS ON THE BINDING OF NEUTRAVIDIN TO BIOTIN FUNCTIONALIZED MICROBEADS ARRAYED IN THE MICROFLUIDIC CELL

The aim of the experiments is to first demonstrate a binding assay with the hydrodynamically assembled microbead library platform of Figs. 1 and 2, using as a prototype the conjugation of fluorescently labeled NeutrAvidin as the target analyte to biotin, its binding partner, displayed on the microbead surface, and second to measure the surface kinetic rate constant for the binding of NeutrAvidin to surface biotin. Using the simulations as a guide, we illustrate how the flow rate of the assay (or equivalently the Pe number) can be selected to be large enough so that the transport of target is through thin diffusion boundary layers around the trapped microbeads. As such, the rate of binding of the target to the surface probes becomes kinetically controlled, and a kinetic rate constant can be obtained by comparing the measurements to a simple kinetic expression (e.g., Eq. (3)) without having to solve the convective-diffusion equation for the transport of target to the microbead surface. We also validate the use of the kinetic expression by a direct simulation incorporating convection and diffusion.

A. Materials and methods

The fluorescently labeled protein, fluorescein conjugated NeutrAvidin (NeutrAvidin-FITC), was obtained from Thermo Fisher Scientific, Inc. NHS-PEG₄-biotin and NHS-PEG₄ (N-hydroxysuccinimide esters linked to four polyethylene glycol (PEG, $-(CH_2CH_2)_4O-$) groups and capped with biotin or a methyl group were purchased from Pierce Corp. Toluene, acetone, isopropanol, methanol (99.8%), and dimethylformamide (DMF) were obtained from Alfa Aesar (HPLC Grade). Phosphate buffered saline buffer (PBS, pH = 7.4), hydrogen peroxide, ammonium hydroxide, and hydrochloric acid were obtained from Fisher Scientific. Aminopropyltrimethoxysilane ($NH_2(CH_2)_3Si(OCH_3)_3$, APS) and PEG silane, 2-(methoxy(poly-ethyleneoxy)propyl trimethoxysilane ($CH_3(CH_2CH_2O)_n(CH_2)_3Si(OCH_3)_3$, $n = 6-9$) were obtained from Gelest. Ultrapure water with a resistivity of 18 M Ω cm was obtained from a Millipore water filtration system. Negative

tone SU8–2050 photoresist and developer were purchased from Microchem. Sylgard 184 elastomer base and curing agent was purchased from Dow Corning as a kit. All chemicals were used as received except for the PBS which was filtered with $0.2\ \mu\text{m}$ syringe filters. Soda lime glass calibration microbeads, $42.3\ \mu\text{m}$ in diameter with a $\pm 1.5\ \mu\text{m}$ standard deviation in the size distribution, were obtained from Thermo Fisher Scientific, Inc. Test grade single crystal polished silicon wafers (3 in. in diameter) were obtained from Silicon Quest Inc. Polyethylene tubing (1.5 mm OD, 1 mm ID) was obtained from VWR Company. Dye removal resin column kits were obtained from Thermo Fisher Scientific (Pierce Protein Biology Products).

B. Functionalization of microbeads with biotin

N-hydroxysuccinimide ester (NHS)-conjugated reagents are commonly used to label proteins, with the NHS reacting with primary amine groups on amino acids to form an amide bond between the protein and the reagent. Here in order to display biotin on the surface of the glass microbeads, we utilize NHS linking chemistry and first functionalize the glass microbeads with amine groups by using silane coupling chemistry. We covalently bind aminopropyltrimethoxysilane ($\text{NH}_2(\text{CH}_2)_3\text{Si}(\text{OCH}_3)_3$, APS) via the silane moiety $-\text{Si}(\text{OCH}_3)_3$ to glass surface hydroxyl groups on the microbeads by a siloxane bond. We then use an NHS conjugated to biotin reagent (NHS-PEG₄-biotin) to covalently link biotin to the amine functionalized microbeads. In the NHS-conjugated to biotin reagent, the NHS is separated from the biotin by four polyethylene glycol groups (NHS-PEG₄-biotin) to allow the biotin probe to extend into solution from the microbead surface and thereby make it more accessible to the NeutrAvidin target. To ensure that the bound NeutrAvidin target does not block adjoining probe sites and the binding kinetics can be described by the Langmuir model used in the computational section, we reduce the surface density of the biotin. During the linkage step to the amine terminated microbeads, NHS-PEG₄-biotin is mixed with NHS-PEG₄, a molecule capped with a methyl group rather than biotin. To determine the mixing ratio, we note that the target NeutrAvidin (MW = 60 000) is a homotetrameric protein (the glycosylated form of Avidin) with four binding pockets for the small molecule biotin (MW = 244).^{42–44} The pockets are arranged with two sites located on one face of the protein and two sites on the opposite face. Crystallographic studies of the binding face of the NeutrAvidin protein measure a value of $3025\ \text{\AA}^2$ (Ref. 42) which we set to be the molecular area of the target $\mathcal{A}_t$. The surface density of the amine groups after silanization to the glass microbead surface determines the density of the displayed biotin. Silanes with alkyl chains of only a few carbons, as the APS silane used here, assemble in a disordered state (for a review, see Ref. 45), and this is especially true of aminopropyl terminated silanes in which the amine hydrogen bonds to surface silanol groups (see, for example, Refs. 46–49). The surface density of amines during silanization to glass surfaces 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, Refs. 50 and 51). The accessible density depends on the APS silanization conditions, but from an organic solvent, as is the deposition to the microbead surfaces used here, grafting densities of $0.2\text{--}0.5\ \text{molecules/nm}^2$ or $200\text{--}500\ \text{\AA}^2/\text{site}$ are typical. Thus, if NHS-PEG₄-biotin reacted with all amine sites, the probe density Γ_p would be large enough that the protein would block surface biotin sites as $\Gamma_p\mathcal{A}_t$ would be greater than one. To reduce the biotin probe density, we mix the NHS-PEG₄-biotin with NHS-PEG₄ in a molar ratio of 1 to 1000, or 1 to 100, so that $\Gamma_p\mathcal{A}_t$ is much less than one, and target blocking of unbound biotin is not important as there is on average much less than a single biotin molecule under the binding face of the target as it attaches to the surface. We also note that the use of the NHS-PEG₄ to reduce the biotin density also inhibits the nonspecific adsorption of the NeutrAvidin to the microbead surface, as polyethylene glycol oligomers have been shown to resist protein adsorption. Thus, only the biomolecular binding of the protein to the surface biotin is measured.

Soda lime glass microbeads ($42.3\ \mu\text{m}$ in diameter) are first washed in an aqueous solution consisting of 4 wt. % hydrogen peroxide and 4 wt. % ammonium hydroxide to remove hydrophobic surface contaminants. The solution is heated for 10 min on a hotplate with vortexing,

decanted, and the beads are resuspended in water. This is repeated three times, and the beads are then washed similarly with an aqueous solution consisting of 4 wt. % hydrogen peroxide and 0.4 M HCl. After resuspension in ultrapure water, the microbeads are dried in a convection oven for 12 h at 85 °C. To form amine-functionalized glass microbeads, the washed and dried microbeads (30 mg) are added to a 5 mM solution of aminopropyltrimethoxysilane (APS) in toluene for 1 h 30 min with stirring. The trimethoxy silane moiety of APS becomes hydrolyzed in the presence of water in the toluene solution or on the microbead surface, and the hydrolyzed APS reacts with surface silanol (hydroxyl) groups on the glass microbead surface to form siloxane bonds. This produces a self-assembled monolayer (SAM) terminated with the amine functionality required to proceed with the NHS reaction. To remove unbound APS, the microbeads are washed and sonicated with toluene. They are then centrifuged and washed twice with dimethylformamide (DMF). The microbeads are suspended in a 1:1000 or 1:100 mixture of NHS-PEG₄-biotin to NHS-PEG₄ in DMF at a total concentration of 1 mg/ml to functionalize the surface with biotin extending from the microbead surface by short PEG arms. The microbeads are then washed three times with ultrapure water and stored in a refrigerator at 4 °C until used.

C. Design and fabrication of microfluidic cell and arraying of microbeads

The microfluidic cell used to array the microbeads consists of an array of “V” shaped enclosures with the exact shape and in the same trapping configuration as modeled, cf. Fig. 2. The cell contains three ports, two opposing ports (inlet and outlet) for flow of target through the trapping array, and one port, used for injecting the microbeads, which is punched through the open area in the cell upstream from the traps. The molded part of the cell is fabricated from the elastomer polydimethylsiloxane (PDMS) using soft lithography to mold features on the faces of a slab of the elastomer using a photoresist master, which is subsequently joined to a glass slide to enclose the cell. A description of the fabrication of the microfluidics device is described in the [supplementary material](#). The inside surfaces of the flow channels and the interior surfaces of the traps are functionalized with PEG silane to prevent nonspecific adsorption of the target protein. The procedure of surface functionalization is also described in the [supplementary material](#), as the procedure for introducing the biotin-functionalized microbeads through the injection port and arraying the microbeads in the traps.

D. Fluorescence imaging of target-probe binding

After the biotinylated microbeads are captured in the microfluidic device, a NeutrAvidin-FITC target solution is flowed through the array using a syringe pump (Harvard Apparatus, PHD). (Before injecting into the array, the solution is first passed through a dye removal resin column to remove free FITC which has become disassociated from the protein.) Epifluorescence images of the FITC labeled NeutrAvidin binding to the microbeads are recorded by mounting the microfluidic cell to the stage of an inverted microscope (Nikon Eclipse TIE) with motorized stage movement to scan different areas of the trapping array. The array is imaged at 10× using a NA=0.3 air objective and a CCD camera (DS-Qi1Mc, DigiSight, Nikon), with a filter cube for epifluorescent measurement of the FITC labeled protein (excitation filter 470 ± 40 nm and emission (barrier) filter 525 ± 50 nm).

E. Binding curves of NeutrAvidin to biotinylated microbeads

The mass transfer simulations make clear that the rate at which target binds to the probes on the microbead surface is controlled by three nondimensional groups, the Peclet number Pe scaling convective to diffusive transport, the Damkohler number Da scaling kinetic to diffusive transport, and Ω scaling the depleted region of target away from the surface to the particle radius. To reduce the effect of bulk diffusion on the mass transfer, the calculations show that large Peclet numbers are necessary, i.e., $Pe = O(10^3)$ or larger, in which case diffusion to the microbead surface proceeds through thin boundary layers which surround the microbeads and

lie within the traps (cf. Figs. 5 and 6). Bulk diffusive transport is rapid because these layers are thin. Even for these regimes of thin boundary layers, for the binding kinetics of the target to the surface probes to be determined by the surface kinetics alone, the surface kinetic rate still has to be slower than the fast diffusion attending large Pe . In the computational section, we showed that the values of Da for which the transport is kinetically limited for large Pe are given by $Da/Pe^{1/3} \ll 1$, and hence the larger Pe the larger is the Da which can still result in kinetically limited transport. As our aim is to arrange conditions for which the assay is kinetically limited, we require as a first step for the Peclet number to be large (order 10^3). For our prototype assay, the diffusion coefficient of NeutrAvidin has been measured⁵² and is equal to $\mathcal{D} = 6 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$, and we set a flow rate of analyte through the device equal to $100 \mu\text{l}/\text{min}$ which gives an average upstream velocity of $U = 10^{-2} \text{ m/s}$ (Fig. 2(a)) and a $Pe = \frac{Ua}{\mathcal{D}}$ of 3500. (The Reynolds number $\mathcal{Re} = 0.24$.) As we have explained, the parameter $\Omega = \frac{\Gamma_\infty}{c_o a}$ controls the influence of bulk concentration on the time scale for the target to completely bind to the probes. Two sets of microbeads are used, the first set with a ratio of 1:1000 in the surface concentration of biotin-terminated PEG to methyl-terminated PEG, and one with a ratio of 1:100. Assuming a value for the grafted amine sites equal to $0.5 \text{ molecules}/\text{nm}^2$ yields a value of $\Gamma_\infty = \Gamma_p = 8.3 \times 10^{-10} \text{ mol}/\text{m}^2$ for the 1:1000 microbeads and one order of magnitude larger for the 1:100 microbeads. In the limit in which surface kinetics is controlling, these estimates for Γ_∞ are not essential to the measurement of the kinetic rate constant k_a because the surface concentration in the kinetic limit (Eq. (3)) is a function of Ω/Da and is independent of Γ_∞ . We choose a value for $c_o = 8.33 \times 10^{-6} \text{ mol m}^{-3}$ which sets $\Omega = 4.2$ (1:1000 biotin ratio) and $\Omega = 42$ (1:100 biotin ratio) and allows equilibration within 1.5 h.

An experiment is started by flowing the NeutrAvidin-FITC solution through the cell, and capturing images of the FITC fluorescence on the microbead surface over sections of the array at 10 min intervals. Typically, approximately 25 microbeads are imaged, and examples of the (false color) fluorescence images over one section of the array is shown in Figs. 11(a) and 11(b) for 600 s and 3600 s, respectively, for microbeads biotinylated with a 1:100 ratio of NHS-PEG₄-biotin to NHS-PEG₄. To quantify the fluorescence, at each time that an image is recorded, the fluorescence intensity on each microbead is obtained by summing the intensities of the pixels enclosed by the circumference of the bead as it appears in the image. As the biotin binding sites on the microbead surface become saturated with NeutrAvidin, the fluorescence intensity on each microbead asymptotes to a constant value, which is obtained by averaging the microbead intensities over the last few minutes of the assay experiment. For each microbead, we divide the value of the intensity at time t by this asymptotic value to obtain a normalized intensity which represents the average surface concentration on the microbead surface to the maximum packing concentration (i.e., $\frac{\bar{\Gamma}}{\Gamma_\infty}$). This normalized intensity is then averaged over all the microbeads quantified in the array, and the results are given in Fig. 11(c) for microbeads biotinylated with a 1:1000 and

FIG. 11. Fluorescence images of bound NeutrAvidin-FITC on the surface of microbeads entrapped in the trapping course at (a) $t = 600 \text{ s}$ and (b) 3600 s for a 1:100 molar ratio of NHS-PEG₄-biotin to NHS-PEG₄. (c) A comparison of simulation and experimental results for the normalized fluorescence intensity of bound NeutrAvidin-FITC bound to 1:1000 (0.1%) and 1:100 (1%) molar ratio of NHS-PEG₄-biotin to NHS-PEG₄ as a function of time. The simulation fit is for a value of $k_a = 1.80 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, and the curve corresponding to the kinetic expression (Eq. (3)) overlies the simulation (not shown).

1:100 ratio of NHS-PEG₄-biotin to NHS-PEG₄. Error bars are constructed from the standard deviation of the individual microbead measurements from the average. The data represent 3–4 experiments on each set of microbeads.

From Fig. 11(c), it is clear that for both sets of microbeads $\frac{\bar{\Gamma}}{\Gamma_{\infty}}$ (the normalized intensities) as a function of time nearly overlap despite the fact that the number of probes on the surface of the microbeads is 10 times larger for the 1:100 ($\Omega=42$) biotinylated microbeads compared to the 1:1000 ($\Omega=4.2$). This is the first indication that the binding is kinetically controlled and that the kinetic constant k_a is such that $Da/Pe^{1/3}$ is smaller than one. If the transport at large Pe were diffusion controlled with the sublayer concentration near zero, then the equilibration time, as we noted earlier, would increase with the number density of surface probes since the target flux is independent of the probe density. Hence, more time is required to bind all the probes (i.e., $\frac{t_{eq,D}}{a^2/D} \sim \frac{\Omega}{Pe^{1/3}}$). However, when the transport is surface kinetically limited, the equilibration time scales as Ω/Da which is independent of the probe density. For the kinetically controlled case, the kinetic rate of binding increases with the number of surface probes which offsets the fact that more probes have to bound for equilibration and hence $\frac{\bar{\Gamma}}{\Gamma_{\infty}}$ is independent of Γ_{∞} (e.g., Eq. (3)). In the regime of probe densities that are used ($\Gamma_p = 8.3 \times 10^{-10}$ mol/m² for 1:1000 and ten times larger for 1:100), $A_t\Gamma_p$ is much less than one and the surface kinetic binding follows the Langmuir equation. Hence, steric hindrance is not a factor, as reflected by the fact that the two binding curves overlap.

To match the experimental measurements, and thereby measure the kinetic rate constant k_a , COMSOL simulations are undertaken for the values of Re , Pe , and Ω specified above, and with the value of k_a varied. The fitting is done for each of the surface densities, and we search an optimal value of k_a which results in a simulation curve close to both experimental curves. We find a value of $k_a = 1.8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ which corresponds to a value of $Da = 0.05$ for the 1:1000 biotin probe density microbeads, and 0.5 for the 1:100 biotin probe density microbeads. Self-consistently, in both cases, $Da/Pe^{1/3}$ is much less than one. The computational fit is shown as the dotted curve in Fig. 11(c), and we note that this curve is identical to the kinetically determined curve (Eq. (3)) as we would expect for this value of Pe (3500) and the two values of Da (0.05 and 0.5).

To compare our value for the kinetic rate constant for the binding of NeutrAvidin to surface biotin, we note first that there have been several studies of the binding of avidin proteins to self assembled monolayers of biotinylated thiols on gold surfaces which is similar to our system (see, for example, Knoll *et al.*,^{52–56} Stayton *et al.*,^{57–59} and Seifert *et al.*⁶⁰). However, none of these have examined quantitatively the kinetics of binding of the protein to the surface. Quantitative measurements using a Langmuir adsorption kinetics were undertaken by Conboy *et al.*⁶¹ using second harmonic generation to measure the binding of Avidin to a bilayer of phospholipid with linked biotin, and Zhao and Reichert⁶² who measured the binding of fluorescently labeled Streptavidin to a Langmuir-Blodgett (LB) monolayer of arachidic acid doped with biotinylated lipid. The value of Conboy *et al.*⁶¹ for k_a ($k_a = 9.8 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$) and values of Zhao and Reichert⁶² ($10^4 \text{ M}^{-1} \text{ s}^{-1} < k_a < 10^5 \text{ M}^{-1} \text{ s}^{-1}$) are less than the value obtained in this study. One reason for the differences is that the biotin probe surfaces of Conboy *et al.* (phospholipid bilayer) and Zhao and Reichert⁶² (LB fatty acid layer) were not identical to the PEG microbead probe surface used here, and neither study accounted for diffusive transport. Another important difference is that the surface densities of the displayed biotin by Conboy *et al.* and Zhao and Reichert⁶² were much larger than the dilute limit used here and $A_t\Gamma_p$ was order one or larger. As such the binding is accompanied by blocking of unbound biotin sites, and their kinetic constants are smaller (“slower”) effective constants which account for the slow-down of the kinetics due to crowding (and possibly the effect of bulk diffusion).

Thompson and Bau³⁷ studied experimentally and theoretically the kinetics of binding of biotin (fluorescently labeled with a quantum dot) to Streptavidin agarose beads arrayed in a microfluidic cell, and obtained a value of $1.65 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$, which is in excellent agreement with our value. Thompson and Bau used a Langmuir formulation for the kinetic binding in which the fraction of sites available for binding was corrected at high enough densities to

account for the covering of the biotin-QD complex to unbound surface protein, and their k_a represents the rate of binding in the dilute limit. The study of Bau *et al.* accounted for convection and diffusion around the microbeads, which were sandwiched between the top and bottom surfaces of the cell. The excellent agreement with our value (also in the dilute limit) suggests that in the heterogeneous binding of target and probe for the NeutrAvidin/biotin pair, which species is displayed on the surface, is not important as long as the biotin ligand and NeutrAvidin binding sites are accessible.

F. Summary

We have examined the mass transfer of a target molecule to a probe library consisting of microbeads functionalized with probes on their surface and captured in a staggered array of traps in a wide channel of a microfluidic cell. The traps are V-shaped, half-open enclosures with the open end facing the flow, and an aperture opening at the back end to allow flow over the surface of the microbead. Assaying the binding interaction of a target with the probe library is undertaken by flowing a fluorescently labeled target (analyte) through the course, and identifying microbeads which are fluorescent.

Our focus is the influence of the retaining structure of the trap on the accessibility of the surface probes to the target analyte streaming over the microbeads. Using numerical simulation, we computed the rate at which target binds to the probes on the microbead surface as a function of the Peclet number, Pe (characteristic rate of target convection across the microbead to the rate of bulk diffusion to the particle surface), and the Damkohler number (Da) (the rate of target-probe surface conjugation to the rate of diffusion to the surface). For fixed Da and order one values of Pe (corresponding to weak convection), large diffusion zones develop around the microbead and envelop the trap, resulting in a binding rate which is much slower than the rate at which target binds to the surface in the idealized case of an unobstructed microbead at the floor of the microchannel. For the same value of Da , and Pe of order 10^3 , the larger convection leads to the development of diffusion zones which take the form of thin boundary layers that encircle the beads within the trap. Because the boundary layers are thin, the diffusion fluxes are large; the overall binding rate increases relative to Pe of order one and approaches the rate for the idealized case of an unobstructed microbead positioned at the bottom of the channel. We find that as Da decreases for a fixed Pe , the reduced kinetic conjugation rate relative to diffusion results in a more uniform surface concentration around the microbead as the diffusion zones disappear. From the simulations and scaling arguments, we constructed a criterion for large Pe , $Da/Pe^{1/3} \ll 1$, for which the surface kinetics controls the overall binding rate.

We verified the regime of kinetically limited transport at large Pe for $Da/Pe^{1/3} \ll 1$ by studying the binding of the target NeutrAvidin, to its binding partner, biotin, grafted to the surface of the microbeads. Binding of the protein to the microbead surface was measured by labeling the NeutrAvidin with a fluorophore (FITC) and quantifying the fluorescence signal on the microbeads as binding proceeds. For experiments at large Pe (3500) and two different probe densities at low surface concentration, we fit the measured binding of protein to the biotin functionalized microbeads as a function of time with the simulations by varying the kinetic rate constant, and find a kinetically controlled regimes which satisfies $Da/Pe^{1/3} \ll 1$. The kinetic rate constant obtained is in agreement with other measurements for the surface binding of biotin and NeutrAvidin, and the value represents the dilute limit binding constant unaffected by steric blocking of unbound sites by target.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for details on the hydrodynamic equations for the fluid flow through the microbead array, the mass transfer conservation relation for the target, and kinetic equations for the binding of target to the probes on the microbead surface, the validations for the numerical solutions of these equations and the experimental procedures for the soft lithography fabrication of the microfluidic device and the methodology for arraying the microbeads in the trapping course.

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