Electronic Supplementary Material (ESI) for Lab on a Chip

Supporting Information

Device Fabrication

The polydimethylsiloxane (PDMS) elastomeric microfluidic device is fabricated using soft lithography, in which the elastomer is molded from a photoresist master template. A brief description of the fabrication of the photoresist master is as follows: A three inch polished single crystal silicon wafer is cleaned by rinsing with acetone, isopropyl alcohol (IPA) and DI water. The excess water is removed with a nitrogen gas stream, and finally the wafer is dried at 150°C on a hotplate for 10 min. in order to remove any remaining water. A negative master for molding the cell is made by spin coating (Laurell Technologies) a negative tone epoxy photoresist (SU-8, Microchem) to a prescribed thickness on the polished side of the cleaned silicon wafer. The spun resist layer is then soft baked on a hot plate at 65°C for 2 min. and then at 95°C for 6 min. to remove a part of the solvent in the photoresist. A computer drawing program (Adobe Illustator) is used to draw a negative of the features to be molded on the surface and write the image to a file, and a brightfield mask of the image is made by using a high resolution (20000 dpi) printer to print the image onto a transparency (PageWorks). The mask is placed on the resist, and the resist is then exposed to the illumination of a UV flood lamp system (OAI, λ=350 nm) with an intensity of 20 mW/cm^2 to polymerize areas of the resist under the transparent regions of the mask; masters for several cells are made on the single wafer. The exposed resist is then post baked on a hotplate at 65°C for 2 min. and then at 95°C for 6 min., to remove the last part of the solvent in the photoresist. An SU-8 developer (ethylene glycol monomethyl ether acetate) is used to remove the unpolymerized parts of the resist, by immersing the wafer in the developer for 6 min. with vigorous agitation.

The resist is then washed with IPA and finally dried with nitrogen and hard baked at 150^{o}C for 5 minutes to remove resist cracks.

The fluidic cell, made of PDMS, is molded from this master using the Dow Corning Sylgard 184 two component heat curing system; the base is mixed with a curing agent in a mixer (Thinky) in the ratio of 10:1 by weight. The silicon wafer containing the master is placed at the center of a Petri dish, and the base-curing agent mixture is poured over the master, and allowed to sit under vacuum for 30 min. to release bubbles trapped between the master and the PDMS. The mixture is then cured by heating in a convection oven at 65^{o}C for one hour. The resulting elastomer is subsequently peeled off of the master, and cut into individual cells. The cut elastomer and glass slide are sealed by first oxygen plasma treating their open faces (Harrick Plasma Cleaner) to create silanol groups and then bringing the faces in conformal contact to form siloxane linkages between the faces and seal the device.

After the devices have been heated at 65°C for several hours in a convection oven to insure a strong seal, the inside surfaces of the flow channels and the interior surfaces of the traps are functionalized with PEG silane. This functionalization follows the following steps[1]: A mixture solution of hydrogen peroxide/hydrochloric acid/distilled water (in a volume ratio of 1:1:5) is flowed through the cell (by injection from a syringe through the inlet port) for several minutes at room temperature to oxidize the interior PDMS surfaces which become rapidly hydrophobic after the plasma treatment. The cell is then flushed with water and dried by passing N_2 through the cell. The treatment oxidizes the PDMS surface into silanol groups. A neat solution of a PEG trimethoxysilane ($CH_3(OCH_2CH_2)_nSi(OCH_2)_3$, n=6-9) is flushed through the cell, and the cell interior is

then incubated with the silane for 30 min. Hydrolysis of the silane by residual water on the cell surfaces, and reaction of the hydrolysis product with the surface silanol groups forms siloxane bonds which functionalizes the surface with PEG. The cells are then flushed with DI water, and are ready to use.

Optical and Fluorescence Imaging Microscopy

An inverted, confocal laser scanning microscope (CLSM; Leica TCS SP2, employing an acoustic-optical beam splitter (AOBS) and a prism spectrophotometer detector), 63× (oil) NA = 1.4 and 10x (air) NA=0.3 objectives are used for the epifluorescent detection of the emission fluorescence of the DHPE-TRITC, Neutravidin-FITC, Alexa Fluro 488 labeled cholera toxin subunit and the DiD dyes. Settings for the fluorescent labels are as follows: FITC, Alexa Fluor 488 ($\lambda_{ex(max)}$=494 nm, $\lambda_{em(max)}$=518 nm; 488 nm laser excitation; collection window of 510-530 nm) (false color labeled green), TRITC ($\lambda_{ex(max)}$=555 nm, $\lambda_{em(max)}$=580 nm; 543 nm laser excitation; collection window of 565-620 nm) (false color labeled red), DiD ($\lambda_{ex(max)}$=630 nm, $\lambda_{em(max)}$=670 nm; 633 nm laser excitation; collection window of 650-690 nm) (false color labeled red). For the confocal measurements the pinhole diameter is set to 1 AU (Airy unit) for both objectives. Optical z section thicknesses of the voxel size for 10x is 4.8 μm and for 63x is 0.22 μm. Through a transmission mode, the CLSM is also used to obtain phase contrast optical images. Fast video recordings of the entrapment are imaged in brightfield mode using an upright Nikon Eclipse E600 POL microscope, 10x/NA=0.3 air objective and a Photron SAI (Tech Imaging) fast video recording camera. For the study of the binding kinetics, epifluorescence measurements of the luminescence were undertaken with an electron multiplying CCD camera (Evolve 512, Photometrics) using a Nikon Eclipse TIE inverted

microscope with a motorized stage for scanning different areas of the trapping array, a 20x/NA=0.5 air objectives and filter cubes for epifluorescent measurements of FITC (excitation filter 470±40 nm, emission (barrier) filter 525±50 nm) and DiD (excitation filter 620±60 nm, emission (barrier) filter 700±75 nm).

Reference Fluorescence Images of Lipobead Bilayer Stability

In Fig. S1(b) is shown a 63X (zoomed in) confocal image at the equatorial cross section of the TRITC fluorescence from a lipobead containing DHPE-TRITC as given in the schematic of Fig. S1(a). The lipobead imaged was part of an aqueous suspension of

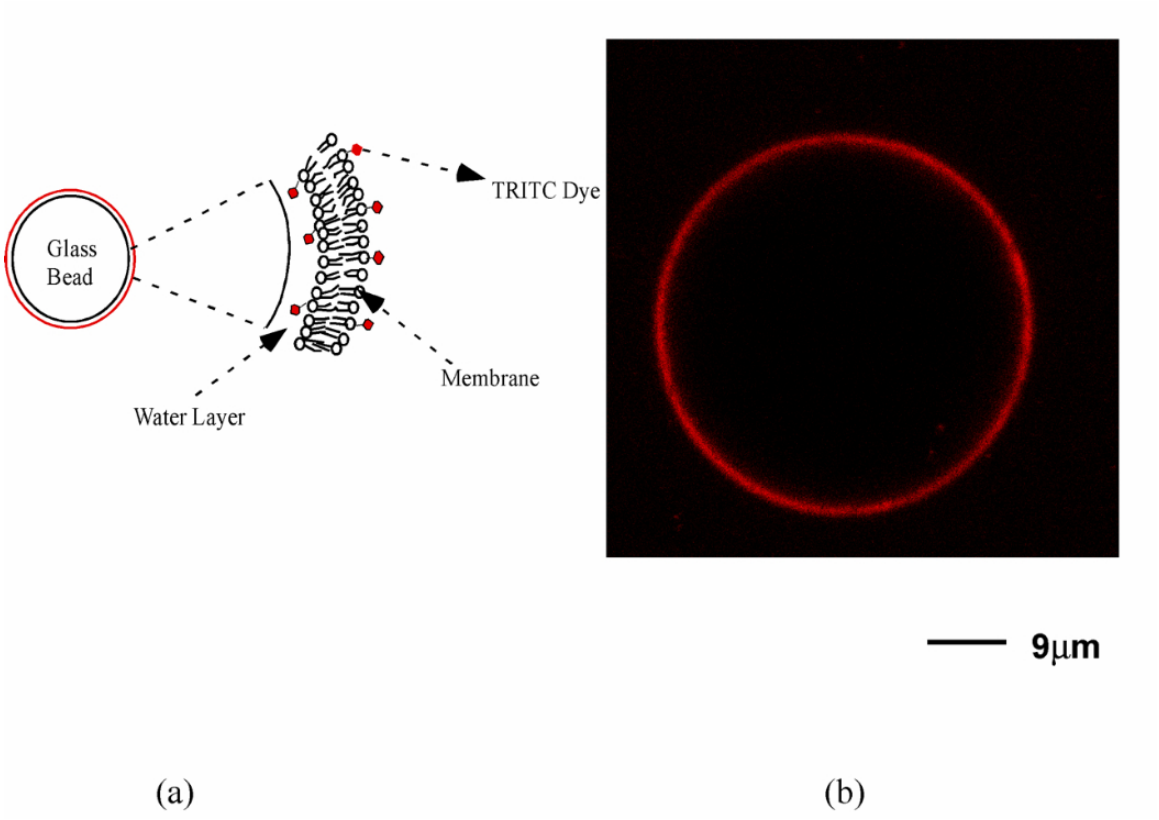

Figure S1: DMPC lipobeads with one mole percent of the lipid headgroup labeled probe DHPE-TRITC: (a) A schematic of a DHPE-TRITC lipobead with a supported lipid bilayer. (b) CLSM fluorescence image of an equatorial section of a DHPE-TRITC labeled lipobead deposited directly on a glass slide under 63x magnification and zoom.

several

lipobeads, placed directly onto the glass slide and before evaporation of the suspending aqueous phase. The fluorescence appears as a halo or circle because of the imaging of the equatorial section. The uniformity of the fluorescence suggests an intact bilayer.

Reference Fluorescence Images of Fluorescently Labeled Neutravidin Binding to Biotinylated Lipobeads

Figure S2(a) shows a reference binding assay undertaken in solution in a centrifuge tube in which the biotinylated lipobeads are first incubated with a Neutravidin-FITC analyte solution at a concentration of 20 µg/ml in PBS buffer for several minutes, and subsequently washed 3 times in PBS buffer. The wash, which is necessary to remove the

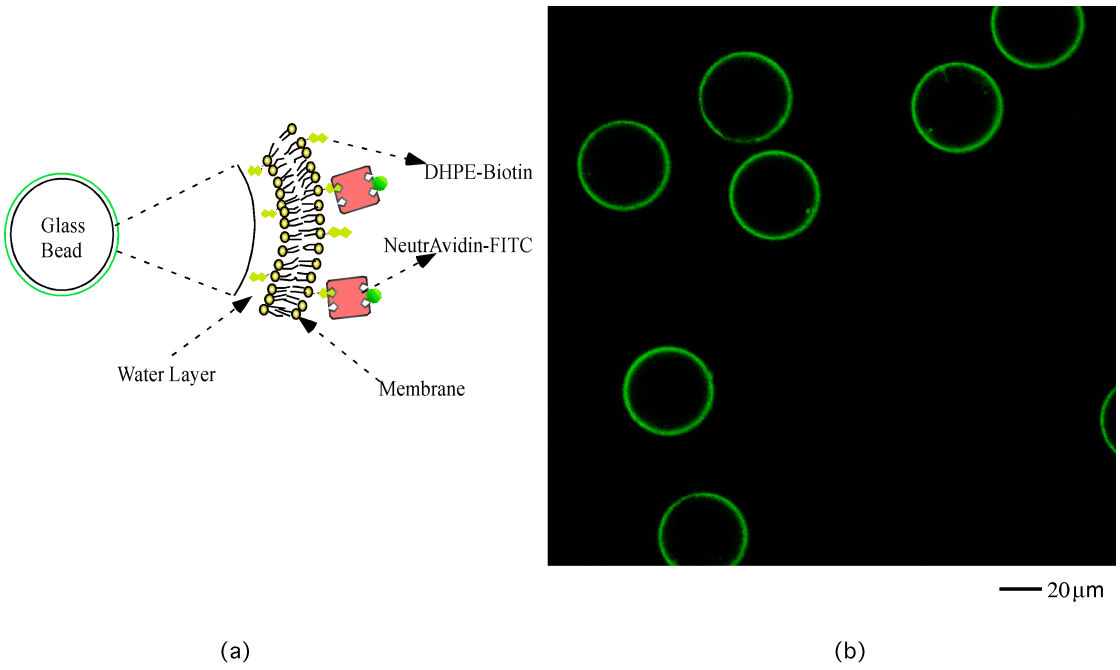

Figure S2: Binding of DMPC lipobeads displaying DHPE-Biotin (5 mole percent) to solution phase Neutravidin-FITC. (a) Schematic of biotin attached to the headgroup of lipids in the supported bilayer on the lipobead surface, and binding to Neutravidin-FITC in solution. (b) CLSM image of an equatorial section of DMPC lipobeads incubated with Neutravidin-FITC analyte, washed and spread on a glass side at 63x magnification and equatorial cross section.

unbound Neutravidin-FITC from the suspending solution, is undertaken by repeatedly suspending the lipobeads in the buffer, allowing them to settle, decanting the buffer and resuspending the lipobeads. After the wash and final resuspension in PBS, an aliquot is placed on a glass slide and imaged at 63x magnification with the confocal laser scanning microscope for epifluorescent detection of the FITC fluorescence (Fig. S2(b)). The uniform fluorescence indicates that the bilayer sequestered DHPE-Biotin is functional (i.e. able to bind Neutravidin), and distributed uniformly around the microbead.

COMSOL Numerical Simulation

The COMSOL numerical solution of the flow through the trapping array (a finite element solution of the Navier Stokes equations with triangular and tetrahedral mesh domain discretization) is undertaken on a unit cell. The unit cell is an approximation of the real flow domain in which the trapping array is assumed to be infinite in width (number of columns) and therefore reproduces the flow in unit cells (Figure S3). The unit cell approximation simplifies the calculation, and describes accurately the flow except along the columns of the array immediately next to the walls of the device. In addition, only four rows (rather than 12) are considered in the unit cell, again to simplify the calculations; we have found that the inclusion of additional rows does not change appreciably the flow in the preceding upstream traps. An upstream uniform flow is imposed (with velocity U) at the entrance plane of the domain (y=0), zero pressure imposed at the exit plane (y=L), no-slip conditions imposed on the trap surfaces, and the upper (z=H, the channel height) and lower walls (z=0) of the channel (causing the imposed uniform velocity at y=0 to develop into a Poiseuille further downstream), and

symmetry conditions imposed on the symmetry planes parallel to the channel walls (x=W and x=-W, cf. Figure S3). The dimensions of the traps and their spatial arrangement are exactly as depicted in Fig. 2 in the manuscript. The height of the channel is 60 μm and $U=10^{-2}$ m/sec, as in the experiments. The distance from the inlet plane to the first row in the periodic cell is equal to L_1=700 μm, and the distance from the back row to the exit plane is L_2= 500 μm; increases in these distances does not affect the calculated flow around or drag force on the lipobeads. The physical parameters of the streaming flow are set to those of water: Newtonian viscosity $\mu = 10^{-3}$ Kg $m^{-1}sec^{-1}$, density $\rho=10^3$ Kg m^{-3}. For these parameters, and screening assay perfusion flow rates of $10^2 - 10^3$ μl/min (average velocity $U = 10^{-2} - 10^{-1}$ m/sec) for which the bilayers were stable, the Reynolds number Re based on the channel height H ($Re=\rho UH/\mu$) is equal to 0.6-6, indicating that the flow is relatively inertialess, especially at the lowest flow rate.

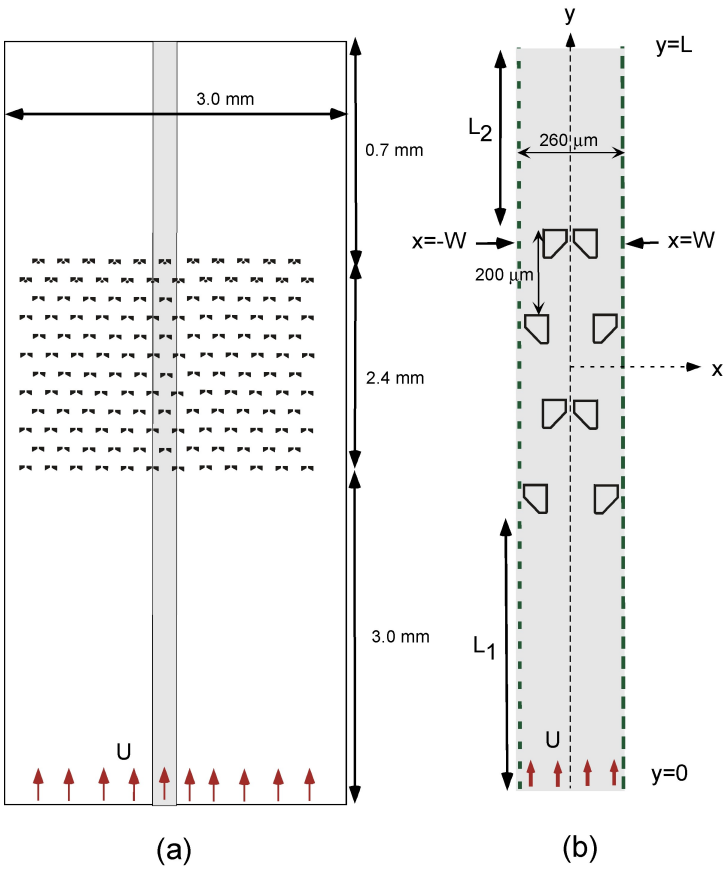

Figure S3: Real and computational unit cell domain for the COMSOL calculations.

The numerical computations are first verified by computing the drag force on one isolated (test) bead positioned in y and x at the center of the unit cell with the traps

removed, and with the symmetry planes (x=±W) at a distance far enough from the test sphere (approximately 150 bead diameters) so that the presence of the planes does not affect the drag. The inlet flow is fixed to a two dimensional Poiseuille flow, with an average velocity of 10^{-2} m sec^{-1} (Re=.6) so that inertial effects can be neglected (Stokes flow). For Stokes flow (Re=0) of a spherical particle entrained in a Poiseuille flow between two plane walls, Feuillebois et al [2] et all present correlating formulae (based on numerical simulations using a multipole expansion method) of the drag in two dimensional channels as a function of the ratio of the sphere radius to the height of the channel, and the ratio of the radius to the position Z of the sphere center relative to the bottom wall. By adjusting the discretization of the mesh to finer values, the drag force calculated from the COMSOL calculations was is within five percent of the Feuillebois et al correlation, for several values of a/H and a/Z including the value for when the test sphere touches the wall. This mesh refinement is then used for all the remaining simulations. Note that finite values for the hydrodynamic drag are obtained for beads touching the wall because, with the sphere immobilized, there is no relative motion between the bead and the wall, and hence no hydrodynamic singularity is encountered as the gap distance between the sphere and the wall tends to zero.

1. I. Wong and C. Ho, *Microfluidics and Nanofluidics*, 2009, **7**, 291-306.
2. L. Pasol, M. Martin, M. L. Ekiel-Je≈ºewska, E. Wajnryb, J. B≈Åawzdziewicz and F. Feuillebois, *Chemical Engineering Science*, **66**, 4078-4089.