Electronic Supplementary Information

Highly fluorescent, monolithic semiconductor nanorod clusters for ultrasensitive biodetection

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Table of Contents

1. Experimental Details

Reagents and Chemicals: Cadmium oxide (CdO, 99.5%), cadmium acetylacetonate (Cd(acac)2, ≥99.99%), sulfur (S, reagent grade), 1-hexadecylamine (HDA, 90%), 1,2-hexadecanediol (HDDO, 90%), selenium shot (Se, 99.99%), trichloro (1H,1H,2H,2H-perfluoro-octyl)silane (TCPOS, 97%), Diisooctylphosphinic acid (DIPA, 90%), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, BioXtra), Bovine Serum Albumin (BSA, ≥98%) were purchased from Sigma Aldrich. Trioctylphosphine (TOP, 97%) n-octylphosphonic acid (ODPA, 97%), trioctylphosphine oxide (TOPO, 99%), and n-hexylphosphonic acid (HPA, 97%) were purchased from Strem. Streptavidin (SA) and SYBR@Gold were purchased from Life Technologies. EZ-Link Amine-PEG_2-biotin was obtained from Thermo Scientific. Biotin or amine labeled and dye labeled DNA aptamers were produced by IDT Technologies Inc.. 6 µm diameter polystyrene (PS) microbeads with PEG-COOH modified surface were purchased from Micromo Partikeltechnologie GmbH. Amicon® ultra centrifugal filter unit with Ultracel-100 membrane (100 kD MWCO) was purchased from Merck Millipore. 10x phosphate buffered saline (PBS) and 1.0 M Tris buffer were purchased from 1st Base. Unless stated otherwise, all chemicals were used as received without further purification. All the reactions for NRs preparation were conducted in oven-dried glassware under nitrogen atmosphere using standard Schlenk techniques.

Synthesis of CdSe seeded CdS NRs: CdSe cores were synthesized following a previously reported method with slight modifications.[1] Briefly, a mixture of 9 g TOPO, 6 g HDA, and 0.25 mL DIPA was degassed under vacuum at 100 °C for 1.5 h in a 50 mL round bottom flask (RBF). A precursor solution comprising of 317 mg $Cd(acac)_2$ and 567 mg HDDO in 6 mL 1-ODE was degassed at 120 °C for 1.5 h, followed by addition of 4 mL 1.5 M trioctylphosphine selenide (TOPSe) at room temperature. The precursor solution was swiftly injected into the RBF at 360 °C and allowed to cool to 80 °C. The resulting CdSe QDs were subsequently processed by 3 cycles of precipitation/dispersion in a butanol-methanol/hexane mixture and dispersed in toluene for further use. The synthesis of highly fluorescent colloidal CdSe seeded CdS NRs was carried out via the core seeded approach previously reported by Carbone et. al.[2] Briefly, in a 50 mL three-neck RBF, a mixture containing 3 g TOPO, 65 mg CdO, 290 mg ODPA and 80 mg HPA are degassed at 150 °C for about 1.5 h. Separately, the sulfur stock solution was prepared by dissolving 80 mg S in 1.8 mL TOP at 50 °C before adding 200 µL of the prepared CdSe stock

solution. This S stock solution was degassed at 50 °C for 0.5 h. The temperature of the reaction mixture in the three-neck RBF was raised to 360 °C under N_2 atmosphere. Upon reaching the desired temperature, 1.8 mL TOP was added, and the temperature was allowed to recover to 360 °C before the mixture of S, TOP and CdSe was swiftly injected. The growth of the anisotropic CdS shell was complete after 6-8 min reaction at 360 °C. The solution was allowed to cool to 80°C. The NRs were purified by 3 cycles of precipitation in methanol and re-dispersion in toluene. As synthesized NRs are uniform in size and well dispersed in toluene as shown in Fig. S1. The resulting NRs were then dispersed in $CHCl_3$ for subsequent use.

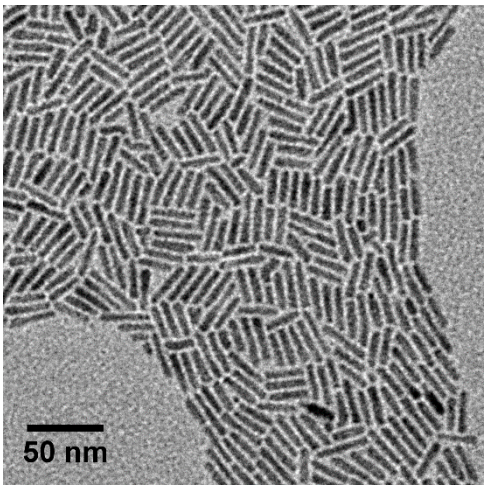

Fig. S1. Low resolution transmission electron microscopy (TEM) data of the as synthesized NR in toluene.

Rendering hydrophobic NRs dispersible in water: An amphiphilic polymer used to coat the hydrophobic NRs and render them dispersible in water was synthesized by coupling a fraction (40%) of the carboxylic acid groups of a 2000 MW polyacrylic acid with octylamine using EDC in N,N-dimethylformamide via a previously published protocol.[3] 10 mg of CdSe seeded CdS NRs and 50 mg of the amphiphilic polymer were mixed in 4 mL chloroform. The solution was stirred for 30 min and the solvent was subsequently removed under vacuum. 5 mL 0.1 M NaOH was then added and the mixture was stirred overnight. Any large aggregations formed during the reaction were removed through a 0.2 µm syringe filter. The NR were further purified with an ultra-centrifugal filter unit (100 kDa MWCO) to remove any excess polymer and reaction reagent. The resulting polymer coated NRs were stored in PBS at 4 °C prior to further use.

Functionalization of microbeads with DNA-aptamers: Amine modified aptamers was conjugated on the surface of PEG-COOH modified polysyrene microbeads via EDC coupling.

Briefly, 20 µL microbeads (50 mg/mL) and 10 µL of capture aptamer (100 µM) were mixed with 170 µL of MES (pH 6.0). 0.6 mg EDC was added and incubated at room temperature for 20 min. Then, another 0.6 mg EDC was added and incubated for 20 min. Finally, 0.6 mg EDC was added and incubated for 80 min. The supernatant was removed and the microbeads were resuspended in TE buffer for 30 min to quench the unreacted EDC. Excess aptamer was removed by 3 times wash with 1×PBS.

Functionalization of NRs with SA: 3 µL of 5 µM polymer coated NRs were mixed with 250 µL 10mM borate buffer (pH 7.4) and 7 µL of 10 mg/mL SA. Subsequently, 5 µL of 10 mg/mL EDC was added and the reaction mixture was shaken on a tube rotator at room temperature for 2 h. Excess SA was removed by washing 5 times with 50 mM borate buffer (pH 8.3) using an ultra-centrifugal filter unit (100 kDa MWCO).

Functionalization of NRs with biotin: The functionalization of biotin on polymer coated NRs by EDC coupling. Briefly, 200 µL of 0.5 µM NRs, 2 µL of 5 mM amine-PEG_2-biotin, and 0.8 µL of 1.25 mM freshly prepared EDC in 0.1 M pH 6.0 MES buffer were mixed with gentle stirring for 2 h. Excess Biotin was removed by washing 5 times with 50 mM borate buffer (pH 8.3) through an ultra-centrifugal filter unit (100 kDa MWCO).

Formation of NR cluster-aptamer complex: 10 µL of biotin functionalized NRs (150 nM in PBS) was mixed with 50 µL streptavidin conjugated NRs (150 nM in PBS). The mixture was left to incubate for 30 min at room temperature. The resulting NR-clusters were subsequently incubated with 3 µL of biotin modified aptamer (100 µM in H_2O) for 30 min. Excess aptamer was removed with an ultra-centrifugal filter unit (100 kDa MWCO).

2. Characterization

Transmission electron microscopy (TEM)*:* A JEOL JEM 1220F (100kV accelerating voltage) microscope was used to obtain bright field TEM images. For TEM sample preparation, a drop of the nanoparticle solution was placed onto a 300 mesh size copper grid covered with a continuous carbon film. Excess solution was removed by an adsorbent paper and the sample was dried at room temperature.

Dynamic light scattering (DLS): Hydrodynamic sizes of NCs were obtained using a DynaPro Plate Reader II at room temperature.

Optical*:* Absorption spectra were obtained using an Agilent 8453 UV-Visible spectrophotometer. Photoluminescence (PL) spectra were collected via a HORIBA Jobin Yvon Fluorolog 3 spectrometer. The quantum yield were taken using a HORIBA Quanta-ϕ integrating sphere equipped with a 150 mm diameter sphere with spectralon coating. A Qubit@2.0 fluorometer was used to measure the concentration of protein or DNA. Fluorescence images of the chip and the eluted beads was captured via a Nikon ECLIPSE Ti-U high resolution inverted fluorescence Microscope equipped with a camera U3 desktop system.

Image and statistical analysis*:* The fluorescence images were analyzed with Image J and the data are presented as mean values with their standard deviation.

3. Determination of the average number of SA and aptamers per NC

To determine the average number of SA per NR, N_x , we use the relation:

$$N_x = \frac{n_0 - n_1}{n_Q}$$

where n_0 is the initial number of moles of SA used for conjugation with n_Q moles of NRs or NR-SA respectively and n_1 is the number of moles of unbound SA at the end of the process. n_0 and n_1 are determined via fluorescence measurements with a Qubit fluorometer. The value of n_Q is obtained by determining the total number of moles of NRs using sample absorbance at 350 nm and the calculated molar extinction coefficient based on NR size.

An example of how the average number of SA and aptamers per NC is determined is as follows: for the conjugation of CdSe seeded CdS NRs with streptavidin (SA), 1.4×10^{-9} mol of SA was added to 2.5×10^{-11} mol of EDC activated NR in a borate buffer. The resulting NR-SA conjugates were subjected to 5 cycles of concentration and dilution using an ultra-centrifugal filter to remove unreacted SA and excess EDC. The amount of SA in the washing buffer was determined by Qubit fluorescence assay to be 8.8×10^{-10} mol. The average number of detection aptamer per NR was therefore 21.

4. Identification and binding affinity assessment of DNA-aptamers.

DNA-aptamers were developed by Base Pair Biotechnologies, Inc. The two aptamer sequences and their binding affinities to tetanus toxoid detection are as follows:

5’-Biotin-TAATACGACTCACTATAGGTTAACTAAAAACACATACACCCTATGTCAACTGACAATGC

GAATTGACCTG-3' (K_d=15.9 ± 9.0 nM in 1× PBS, 1 mM $MgCl_2$ (pH 7.4)).

5'-NH_2- ACCCCCGAATGGCCGCCGCTAAACACGGCGCT -3' (K_d=1.0 ± 0.2 nM in 1× PBS, 1 mM $MgCl_2$ (pH 7.4))

The capture/detection aptamers are individually subjected to native 10% polyacrylamide gel electrophoresis (PAGE) analysis to verify their binding affinity to the tetanus toxoid, which is prepared in a non-reducing, non-denaturing sample buffer in native PAGE to maintain its secondary structure and charge density. The PAGE gel is stained with SYBR@Gold dye that is specific to nucleic acids, therefore only allowing the aptamers to be visualized. Fig. S2 is a representative PAGE gel data reflecting the electrophoretic mobility of the capture and detection aptamers with and without the presence of the tetanus toxoid. We observe that after the addition of the tetanus toxoid, a few very early bands appear as exemplified in Lanes 2 and 5 (in contrast to Lanes 1 and 4), indicative of the formation of the toxoid-aptamer complex. When the amount of toxoid added is increased, the early bands become wider and more intense, which is consistent with an increased presence of the toxoid-aptamer complex.

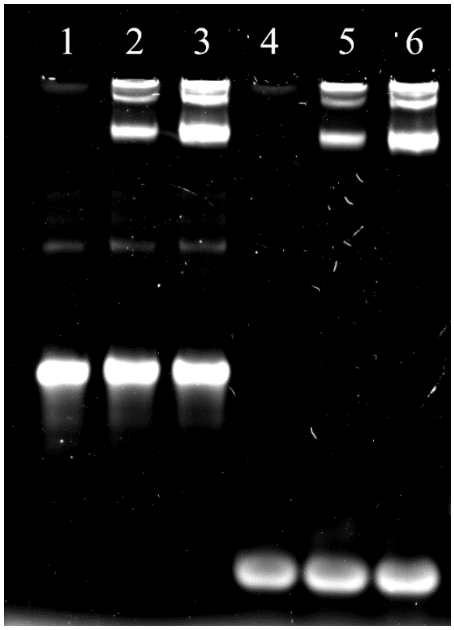

Fig. S2. Native PAGE result of two aptamers with various concentrations of tetanus toxoid. Lane 1, 0.2 M detection aptamer; Lane 2, 0.2 M detection aptamer with 4 g toxoid; Lane 3, 0.2 M detection aptamer with 10 g toxoid; Lane 4, 0.2 M capture aptamer; Lane 5, 0.2 M capture aptamer with 4 g toxoid; Lane 6, 0.2 M capture aptamer with 10 g toxoid. The gel was run at 100 V using a 0.5× TBE buffer for 45 min.

5. Fabrication and manipulation of microfluidic device

Sylgard 184 silicone elastomer kit was obtained from Dow Corning. SU-8 2025 permanent epoxy negative photoresists and SU-8 developer were obtained from Micro-Chem. AZ 50XT photoresist and AZ 400K developer were obtained from AZ Electronic Materials. One-side polished silicon wafers (500-550 µm in thickness, 4 inch in diameter) were purchased from University Wafer.

Molds for flow and control layers were fabricated by photoresist-based photolithography on silicon wafers followed by multilayer soft lithography optimizing previous reported protocols.[4,5] The AutoCAD design (CAD/Art Services) for the microfluidic chip was printed on plastic masks with 20 000 dpi resolution. Molds for flow and control layer were fabricated by photolithography using silicon wafers as substrate (Fig. S3). Briefly, the flow mold for the reaction chamber with a height of about 40 µm was made by spin-coating a layer of SU-8 photoresist on the silicon wafer. Then, AZ-50 XT photoresist was used to obtain round flow channels with a height of about 30 µm where control valves can operate. The control mold was made by spin-coating a single layer of SU-8 photoresist on the silicon wafer to obtain control channels with a height of about 25 µm. The flow and the control channels were ~ 200 µm and ~ 100 µm in width, respectively. Molds were treated once with Trichloro(1H,1H,2H,2H-perfluoro-octyl)silane prior to use. The microfluidic chips were fabricated with PDMS as shown in Fig. S4. The PDMS flow layer was made by pouring uncured PDMS (5:1 elastomer: cross-linker ratio) onto the flow mold. The control layer was made by spin-coating a thin layer of uncured PDMS (20:1 elastomer: cross-linker ratio) onto the control mold. After curing the flow

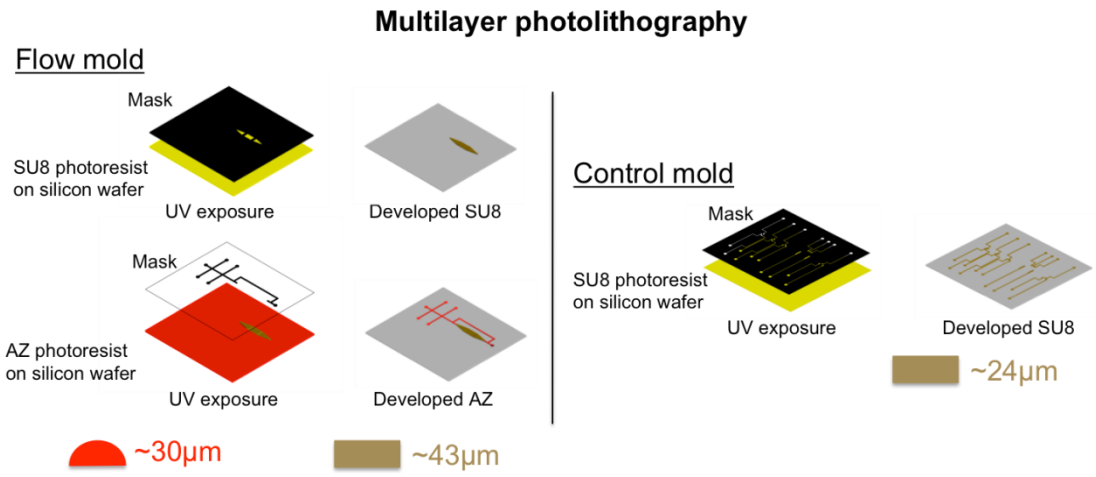

Fig. S3. Scheme of the multilayer photolithography method to fabricate molds on silicon wafers.

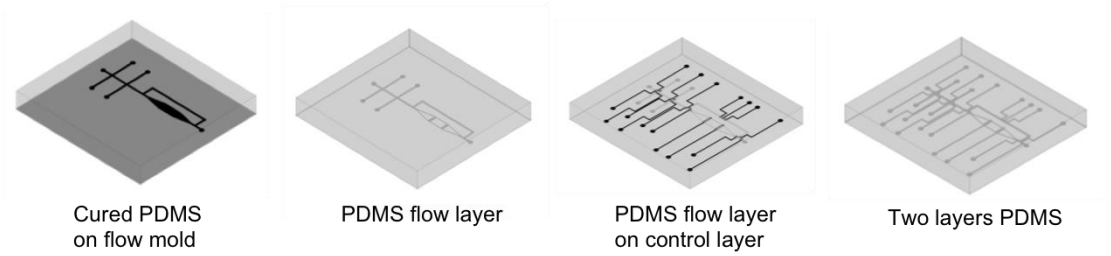

Fig. S4. Illustration of the approach used to fabricate multilayer PDMS-based large scale integrated microfluidic devices.

and channel layers at 80 °C for 1 h and 20 min respectively, the flow layer was peeled off, trimmed and punched for inlet and outlet holes. The flow layer was aligned with the control layer and baked at 80 °C for 1 h. The two layers were peeled off, punched for the control holes and plasma-bonded onto glass slides. The push-up valves within each device were activated by pneumatic solenoid valves actuated by custom electronic circuit and controlled by a custom LabView graphical interface.

The device fabricated includes a control layer that uses pneumatically actuated push-up valves to effect precise control of the fluid flow in the inlets (whose control channels are in yellow in Fig. S5) and the reaction chamber (whose control channels are in red in Fig. S5). Accurate independent control of the five different inlets permits fast injection of buffers and reagents with reduced risk of cross-contamination. Two sieve valves (green control channel in Fig. S5) have been incorporated at each end of the chamber, allowing for microbead trapping

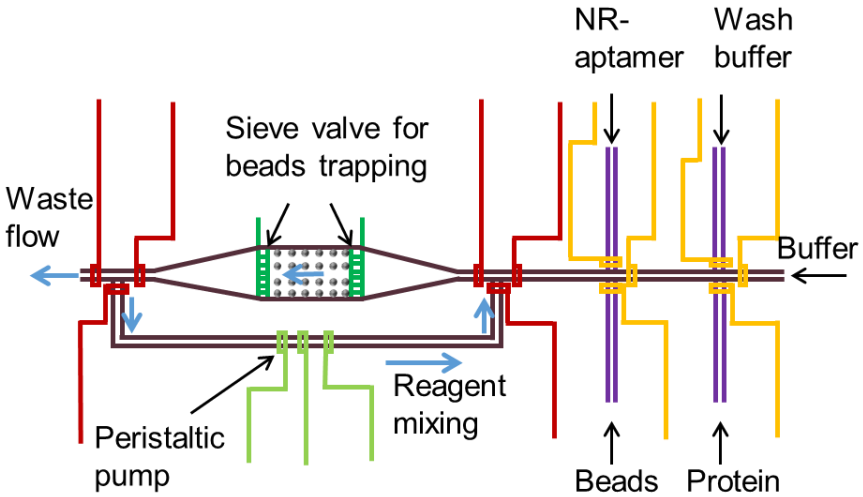

Fig. S5. Schematic of the flow and control channels in the LSI microfluidic device used for tetanus toxoid detection.

while facilitating fluid flow. The process of microbead trapping in this reaction chamber is illustrated in Fig. S6. Additionally, the three parallel push-up valves (light green control channel in Fig. S5) located below the reaction chamber constitute an integrated peristaltic pump when actuated in sequence and enable flow recirculation in the reaction chamber to maximize the pull-down of the toxoid and NRs to the surface of the microbeads. A magnified view of the network of actuating valves observed under an optical microscope is given in Fig S7.

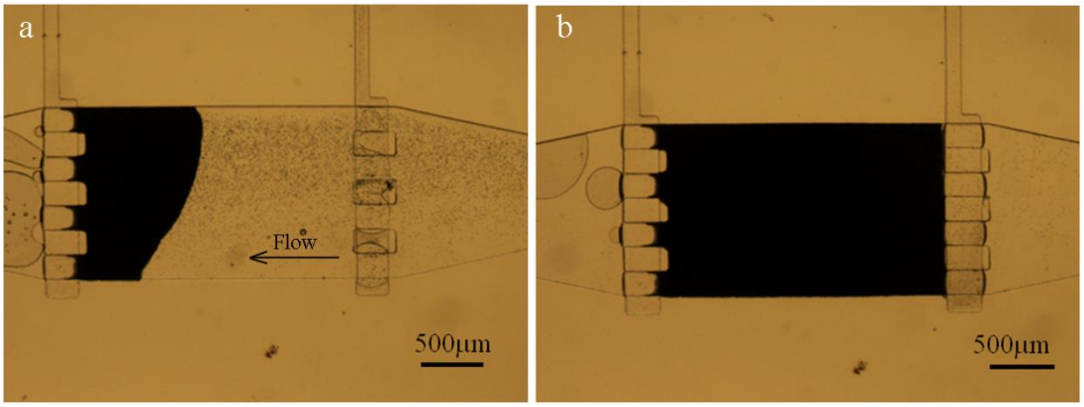

Fig. S6. Microscope image of micron-sized beads being trapped by sieve valves as flow progresses from right to left. (a) Actuating the sieve valve on the left successfully isolates the beads within the chamber. (b) When the entire chamber is fully packed with beads, the sieve valve on the right will also be actuated to trap all the beads within the chamber. The beads were functionalized with aptamers that recognize the tetanus toxoid, and having a packed column of beads provides a large surface area for target capture.

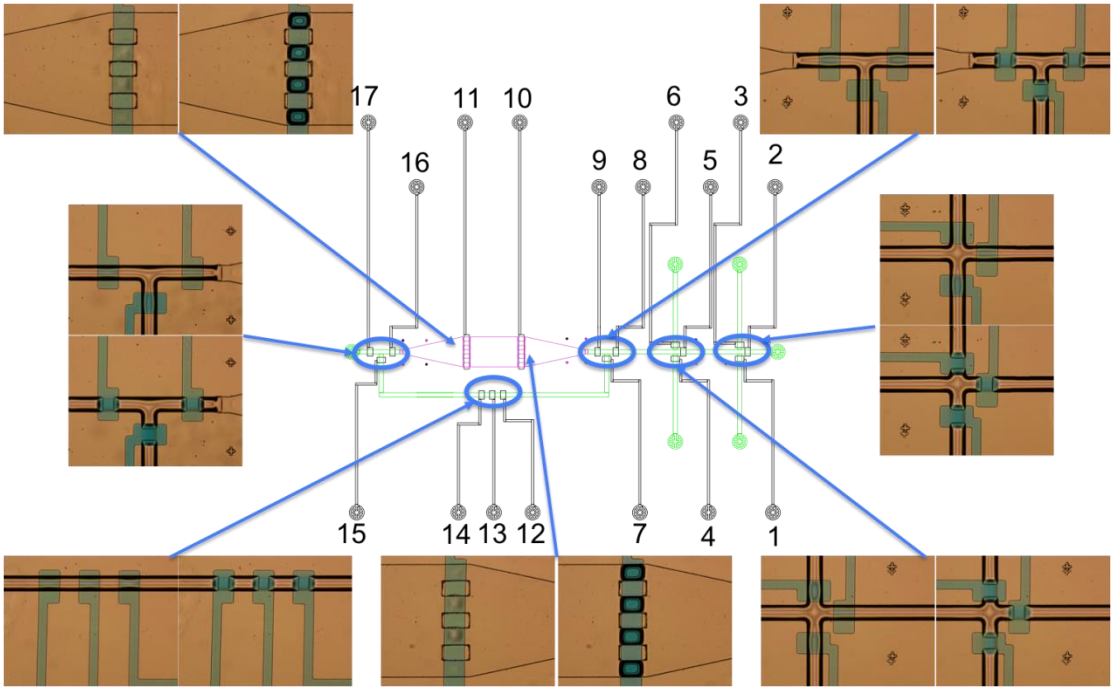

Fig. S7 Scheme of the integrated system of valves and their actuation. In the insets each push-up valve and sieve valve is shown open (left/up) and closed (right/down)

At the beginning of each experiment, 1×PBS was used to wash all the channels and reaction chamber and then 1% BSA in PBS was incubated for 30 min to prevent non-specific adsorption on the channels. After BSA blocking, the channels were washed with PBS and ready to start the detection assay. The packing of micron-sized beads in the reaction chamber was implemented to increase the capture surface area and to reduce the diffusion time to the bead surface, therefore shortening detection times as compared to functionalizing receptors directly onto the surface of the reaction chamber. The sieve valve actuation pressure was set to 15 psi to efficiently (within ~10 mins) trap the 6 µm diameter beads without obstructing fluid flow. The use of smaller bead sizes provides larger total surface areas but results in much longer bead loading times (due to a reduced flow rate) and the need for larger valve actuation pressures. The use of large actuation pressures increases the probability of chip delamination off the glass substrate. Therefore, a trade-off between bead size and actuation pressure is necessary to ensure effective operation of the microfluidic device.

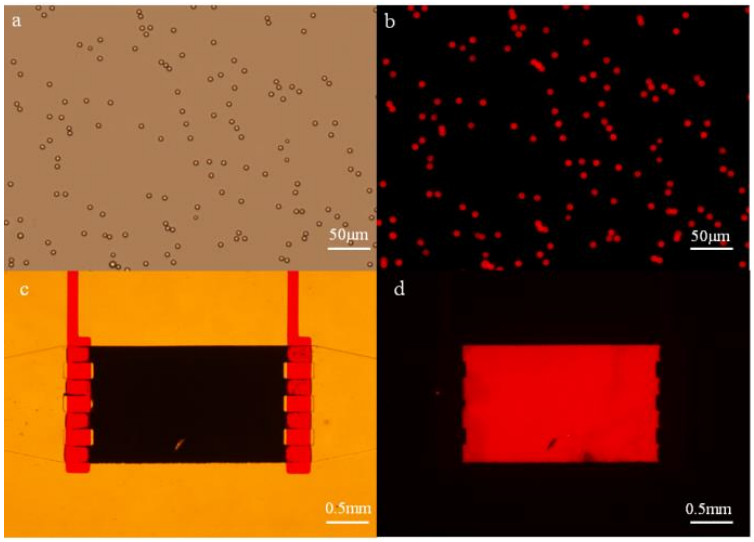

Fig. S8. (a) Bright-field and (b) fluorescence microscope images of Cy5 dye coated PS beads dispersed onto a glass substrate. (c) Bright-field and (d) fluorescence microscope images of Cy5-aptamer coated beads trapped in the reaction chamber of the LSI microfluidic device.

To ascertain the successful immobilization of amine-functionalized capture aptamers to the carboxylic acid groups on the microbead surface, Cy5 labelled capture aptamers with an amine functional group were EDC coupled with carboxylic acid functionalized microbeads. Subsequently, the Cy5-aptamer labelled beads emitted strong red fluorescence signal characteristic of Cy5. When packed into the microfluidics chamber, relatively uniform fluorescence intensity across the device chamber was observed (Fig. S8). This suggests that

the overall detection efficiency should be consistent between samples and would not be significantly affected by bead-to-bead variation.

6. Calculation of the limit of detection (LOD)

The response of the microfluidic sandwich assay to different concentrations of tetanus toxoids within the range where it is linear (i.e. before saturation) is illustrated in the mean fluorescence intensity versus toxoid concentration graph in Fig. S9. Because the background signal is not completely insignificant, the limit of detection (LOD) is determined by 3 times of the standard deviation of the blank sample based on the ICH guideline. The concentration of tetanus at LOD (C_{LOD}) is calculated based on the equation below:

$$C_{LOD} = \frac{3s_{Blank}}{slope}$$

where s_{Blank} is the standard deviation of the blank sample. The C_{LOD} for detection using individual NR and NR clusters is therefore calculated as ~1.0 ng/mL and ~0.7 ng/L, respectively.

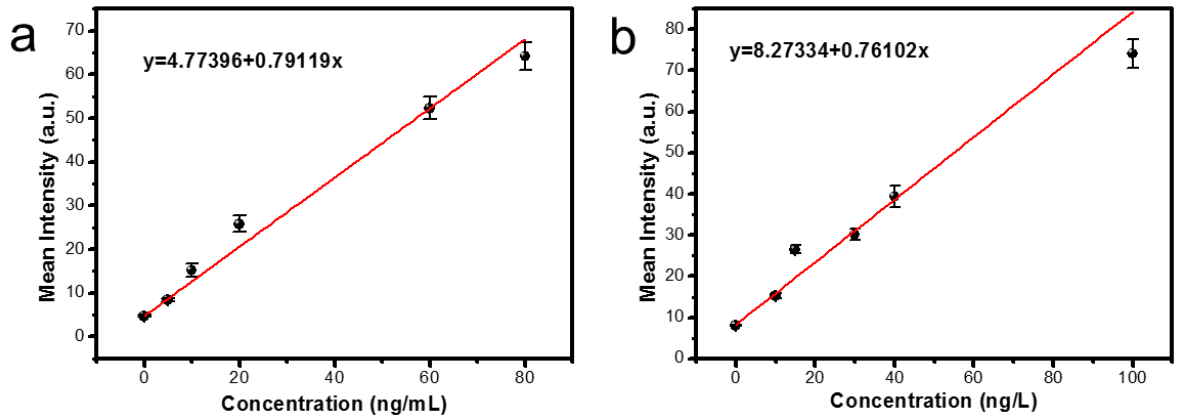

Fig. S9. (a) Linear response of the mean fluorescence intensity versus tetanus toxoid concentration for the sandwich assay using individual NRs. (b) Linear response of the mean fluorescence intensity versus tetanus toxoid concentration for the sandwich assay in 100% fetal bovine serum using NR clusters.

7. Reference

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