Aptamer based dispersion assay using TRPS

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Supporting Information

Materials and methods

Chemicals and reagents

Phosphate Buffered Saline (PBS – P4417), Tween 20 (P1379), MES hydrate (M2933), Bovine Serum Albumin (BSA – A2153), and thrombin (T7513) were sourced from Sigma Aldrich, UK without any further purification unless otherwise stated. EDC (77149) was purchased from Thermo Scientific.

DNA sequences were obtained from Sigma Aldrich's custom oligonucleotides service as lyophilised powders and made up to a stock concentration of 100pmol/µL: 5' GGT TGG TGT GGT GGT TTT TTT TTT[AmC3]3' (Thrombin 15 aptamer with amine terminus) and 5' ACA CCA ACC TTT TTT TTT[AmC3]3' (Complementary sequence to thrombin 15 aptamer with amine terminus). Water purified to a resistivity of 18.2 MΩ cm (Maxima) was used to make all solutions unless otherwise specified and 1xPBST (0.05% Tween) was used as the buffer.

Carboxyl beads of known concentration and diameter were sourced from Izon Sciences (Oxford, UK) to be used both as a substrate for DNA attachment and as a calibrant: SKP400 (mode diameter 350 nm, concentration 7.5×10^{11} beads/mL); CPC800 (mode diameter 750 nm, concentration 9.5×10^{10} beads/mL); and CPC1000 (mode diameter 910 nm, concentration 5.5×10^{10} beads/mL). Superparamagnetic dynabeads® MyOne™ carboxylic acid (650.12) were purchased from Invitrogen provided as 10 mg/mL concentration.

EDC coupling chemistry

Beads were diluted to the required concentration (typically $\times10^{11}$ non-magnetic beads or $\times10^9$ dynabeads MyOne) in MES hydrate buffer adjusted to pH 6 to a total volume of 450 µL. EDC was made up to 10 mg/mL concentration in MES hydrate buffer. 50 µL of 10 mg/mL EDC was then added to the 450 µL of beads. 4.5 nmol of the required DNA sequence was then added and the beads were left on a rotary wheel at room temperature for 2 hours. Following the incubation, the bead solution was removed from the rotary wheel and

centrifuged for 5 minutes at 13 400 rpm. Any liquid was carefully pipetted out and the beads were resuspended in 450 µL of PBST, vortexed and sonicated. This procedure was repeated 3 times. Concentration analysis on the beads was performed via TRPS as described below. For these experiments dynabeads MyOne were coated with thrombin binding aptamer (TBA) and CPC800s or SKP400s with a complementary sequence with a T spacer using the procedure described above.

Aggregate formation

To create aggregates, the dynabeads MyOne coated with TBA and either CPC800 or SKP400 beads coated with a complementary sequence were incubated together in the following concentrations for 30 minutes:

CPC800 assay: 1×10^9 dynabeads MyOne/ mL and 7×10^9 CPC800/mL were diluted in PBST.

SKP400 assay: 1×10^9 dynabeads MyOne/ mL and 5.6×10^{10} SKP400/ mL.

These were then stored upright in a centrifuge tube in the fridge overnight (total time = 24 hours).

Thrombin addition

From stock serial dilutions of thrombin were carried out in PBST such that the same volume of thrombin would be added to each sample so as not to introduce a potential source of bias by diluting the beads. To each 100 µL sample prepared as described in "aggregate formation" 2 µL of thrombin was added to give concentrations ranging from 0 – 10 pM thrombin. As protein-rich control was also conducted in 0.2 mg/mL BSA (3 µM) to test the effect of non-specific protein addition.

Samples were then left to incubate at room temperature on a rotary wheel for 30 minutes prior to analysis. These samples were then purified by magnetic separation on a MagRack (GE life sciences, Buckinghamshire, UK). After 5 minutes on the MagRack a pellet was clearly visible on the side of the sample vial adjacent to the magnet and the supernatant containing any remaining non-magnetic beads was carefully removed to a fresh vial for concentration analysis. Any further dilutions that were required were then carried out in PBST.

Tunable Resistive Pulse Sensing (TRPS) measurements

Measurements were made with the Izon qNano system, (Izon Sciences, Oxford, UK) which incorporates the fluid cell, stretching apparatus, data recording and analysis software (Control Suite V2.2.2.117). Pores used to analyse SKP400 beads were designated "np400" by the manufacturer and are described as most suitable for detecting beads in the range 200 – 800 nm, pores to analyse CPC800 beads, dynabead MyOne beads and aggregates were designated "np1000" by the manufacturer and have a specified range of 500 – 2000 nm. For np400 pores an applied stretch of 47 mm and applied voltage of 0.24 V was selected for np1000 pores a stretch of 47 mm was applied and a voltage of 0.08 V chosen. These conditions ensured that blockade events were clearly visible above the level of noise (<10pA); these conditions were maintained throughout the experiments so that data was comparable. 80µL of electrolyte buffer (PBS) was carefully pipetted into the lower fluid cell, taking care not to introduce bubbles. The upper fluid cell was then attached to the instrument and 40 µL of PBST was placed into the fluid cell to enable confirmation of a stable baseline and clean pore.

To perform a calibration and attain size and concentration data a pressure of greater than 2 cmH_20 was applied so that the additional electrophoretic mobility lent to the beads by the highly anionic DNA did not dominate the signal, which would lead to an overestimate in sample concentration[1]. The application of pressure was also advantageous to increase throughput and decrease analysis run time. Sample concentrations were then extracted for Izon Science's (Oxford, UK) Control Suite V3.1 software. To count aggregates the raw data detailing the blockade magnitudes for each individual peak were extracted as a .csv file. As object volume is proportional to

blockade magnitude the total volume of possible aggregates was then calculated and used to generate the expected magnitude (nA) which were used as bins to sort the data as described in our previous work[2].

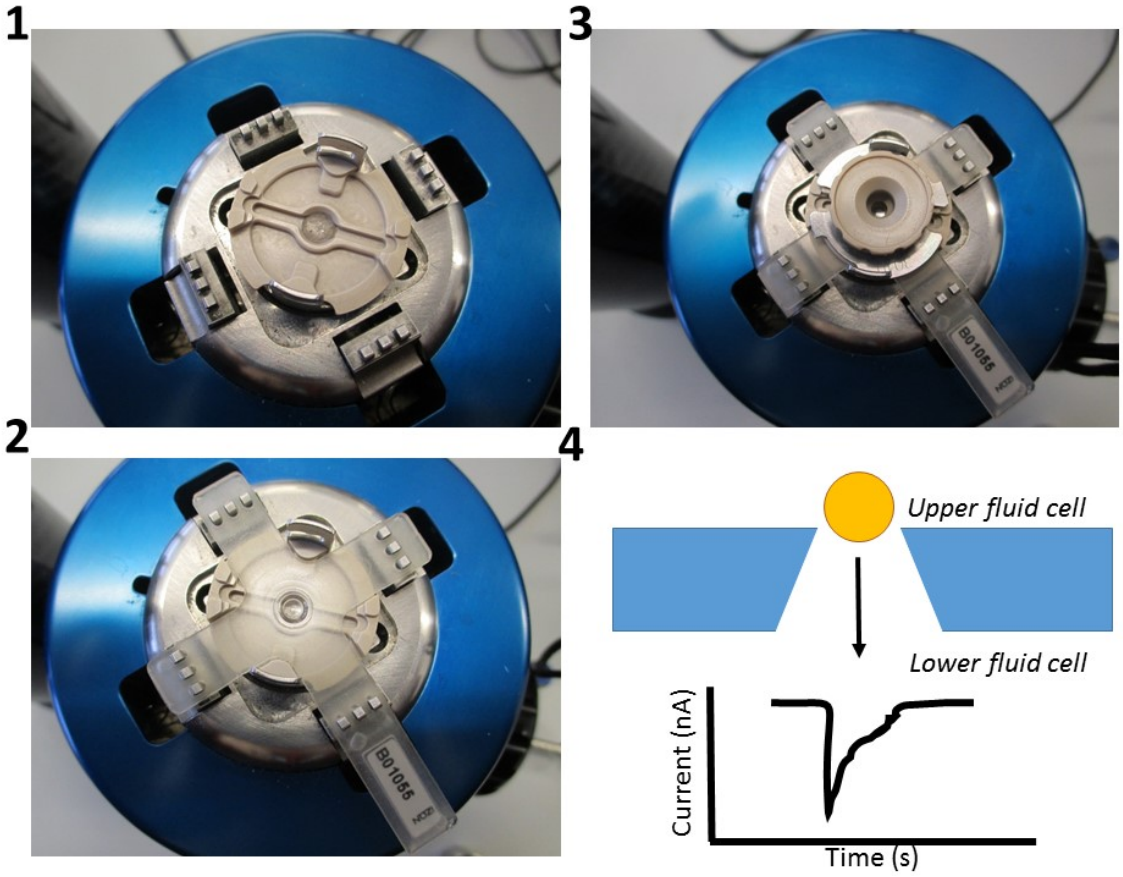

Figure S1: Schematic showing the setup of the TRPS device. **1** birds-eye view of the lower fluid cell, this channel holds 75 – 80 µL of electrolyte buffer and contains an electrode. **2** the pore is mounted laterally to the device by eyelets on the pore being hooked to teeth on the instrument – these teeth are able to be moved apart or closer together, stretching or relaxing the pore respectively. **3** the upper fluid cell is fitted to the instrument securely, and a 40 µL sample is pipetted into it. **4** side-view of a particle traversing the conical pore and an illustration of the resultant asymmetric current trace.

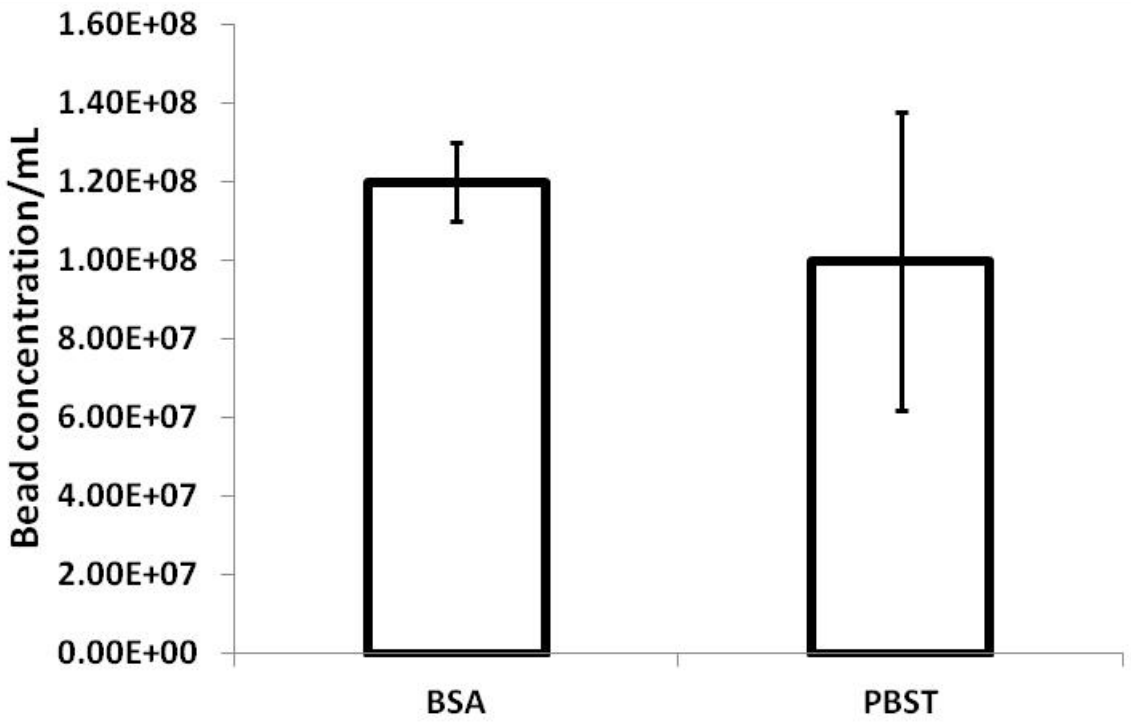

Figure S2: Concentration of free-floating dispersant following aggregate formation in the presence of either blank buffer or BSA.

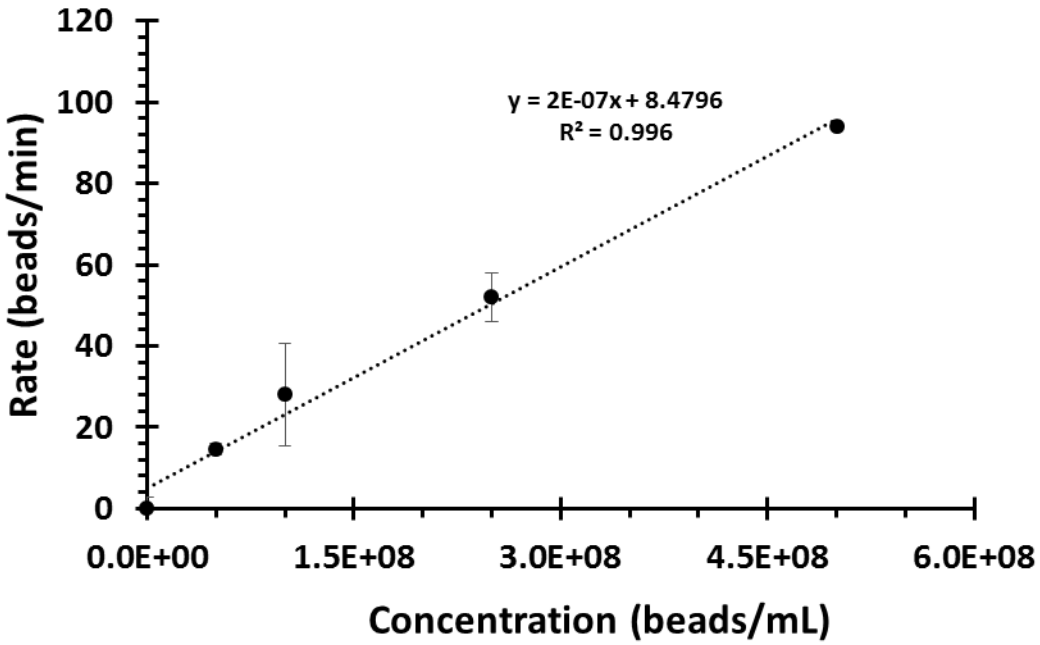

Figure S3: Calibration curve displaying the measured rate against prepared bead concentrations for triplicate measurements. This graph was used to calculate a limit of detection of 3.73×10^7 beads/mL for the pore setup used to measure dispersed 400 nm beads.

Concentration (nM)	Thrombin/bead	% signal
1.4	281	32.77
2.8	562	47.64
14	2810	90.48
70	14051	91.67
140	28103	100

Table S1: Table displaying the calculated number of thrombin proteins per bead and the resultant signal from our previous work[3].

References

1 R. Vogel, W. Anderson, J. J. Eldridge, B. Glossop and G. R. Willmott, *Anal. Chem.*, 2012, **84**, 3125–3131.

2 E. R. Billinge, J. Muzard and M. Platt, *Nanomater. Nanosci.*, 2013, **1**, 1.

3 E. R. Billinge, M. Broom and M. Platt, *Anal. Chem.*, 2014, **86**, 1030–7.