Electronic Supplementary Material (ESI) for Analyst.

Supporting Information for

Vertically encoded tetragonal hydrogel microparticles for multiplexed detection of miRNAs associated with Alzheimer's disease

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Control of Height of Code and Probe regions.

To adjust the code and probe layer thickness, we first defined blocking pressure (P_b) as pressure at which a flow stream of interest would completely block any other flow streams or be blocked by other streams. In our study, we fixed pressure to inlet 2 (P_2) of a rhodamine acrylate solution as 2.35 psi and changed pressure to inlet 1 (P_1) of a green nanobead suspension in order to find blocking pressure (Fig. S1a-c). While we changed P_1 of (translucent pink in Fig. S1b and c) with, we observed a color change at the fluidic intersection between streams 1 and 2 for measurements of P_b. As a result, blocking pressure P_{b1*}, where stream 1 was blocked by stream 2, was 0.5 psi (Fig. S1b). P_{b1**}, where stream 1 blocked stream 2 was 2.8 psi (Fig. S1c). At the pressure of P_{b1*}, only stream 2 traveled to the wide channel for polymerization of tetragonal microparticles incorporated only with the green nanobeads. Similarly, microparticles contained only rhodamine acrylate at the pressure of P_{b1**}. Then, we can set a range of P_1, which enables the stream 1 to travel into the wide channel: $P_{b1*} \leq P_1 \leq P_{b1**}$. Therefore, we expected that a ratio of (P_1 -P_{b1*}) to remaining pressure (P_{b1**} - P_1) would be equal to a ratio of volumetric flow rate of stream 1 (Q_1) to that of stream 2 (Q_2).

$$\frac{Q_1}{Q_2} = \frac{P_1 - P_{b1*}}{P_{b1**} - P_1}$$

Since the oxygen inhibition layer (~5 μm) was much smaller than the height of microparticles, we assumed that the ratio of volumetric flow rates would match with the height of each region. As shown in Fig. S1d, we chose three points of P_1 (1.25, 1.65, and 2.05 psi) to verify that our model showed great agreement with experiments. We expected that the three values of P_1 would permit forming various ratios of each region (1:2, 1:1, and 2:1 respectively).

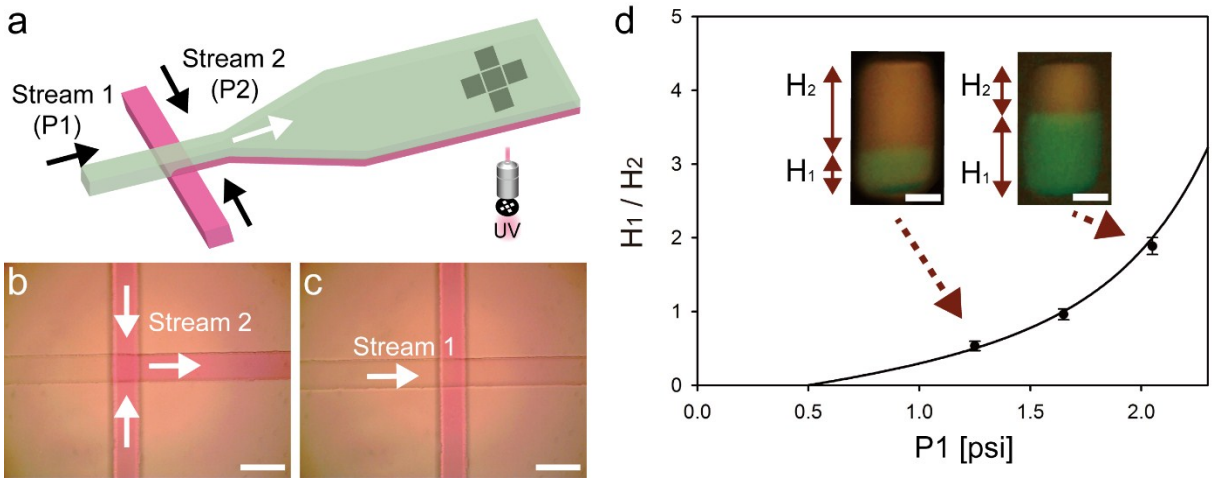

Figure S1. Control of height of code and probe regions in tetragonal hydrogel microparticles. (a) Schematic view of experimental set-up in the two-layered PDMS chip. Green and pink represent a mixture of PEG precursor and green nanobeads, introduced to top stream (i.e., stream 1), and a mixture of PEG precursor and rhodamine acrylate, introduced to bottom stream (i.e., stream 2), respectively. P_1 and P_2 are input pressure applied to streams 1 and 2 respectively. (b) and (c) Optical micrographs showing blockage of stream 2 (b) and stream 2 (c) to measure blocking pressure (P_b). (d) Plot showing height ratios of streams 1 and 2 (H_1/H_2) in tetragonal microparticles. Solid line represents predicted ratios based on our theoretical model. Black circles are ratios measured experimentally. Error bars represent standard deviation. Scale bar is 10 µm.

Table S1. Sequences of AD-miRNA targets, probes, and universal adapter

Name		Sequence
hsa-miR-1306-5p	Target	5’-CCACCUCCCCUGCAAACGUCCA-3’
	Probe	/5Acryd/G**ATATATTTTA**TGGACGTTTGCAGGGGAGGTGG/3InvdT
hsa-miR-342-3p	Target	5’-UCUCACACAGAAAUCGCACCCGU-3’
	Probe	/5Acryd/G**ATATATTTTA**ACGGGTGCGATTTCTGTGTGAGA/3InvdT/
hsa-miR-18b-5p	Target	5’-UAAGGUGCAUCUAGUGCAGUUAG-3’
	Probe	/5Acryd/G**ATATATTTTA**CTAACTGCACTAGATGCACCTTA/3InvdT/
Universal adapter		/5Phos/**TAAAATATAT**AAAAAAAAAAAA/3Bio/

Red color-marked bases represent a part of the universal adapter considering melting temperature as well as hairpin structure.[1]

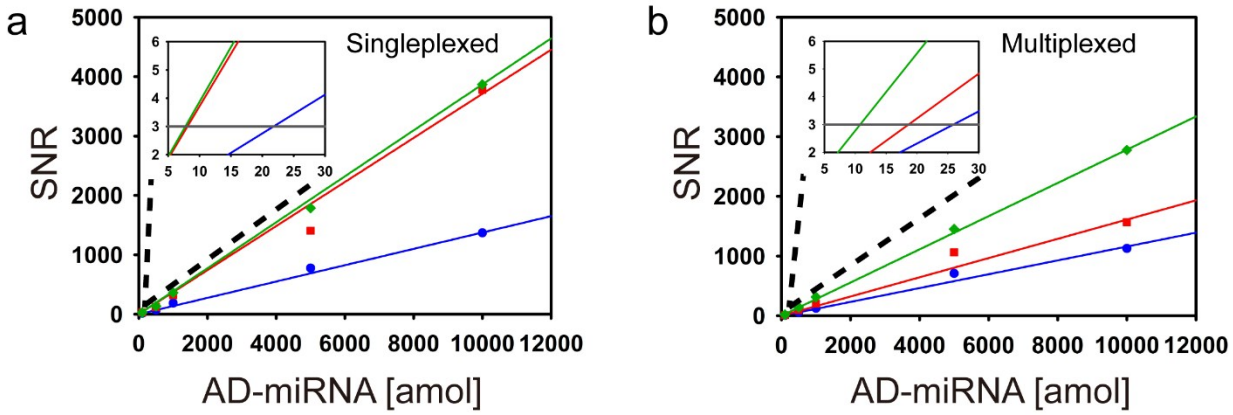

Figure S2. Plots of signal-to-noise ratio (SNR) to estimate limit of detection (LoD) for singleplexed (a) and multiplexed (b) detection of AD-miRNAs. Inset plots show zoomed-in views for extrapolation of SNR. Blue, red, and green lines represent linear regression curves for hsa-miR-18b-5p, hsa-miR-342-3p, and hsa-miR-1306-5p respectively.

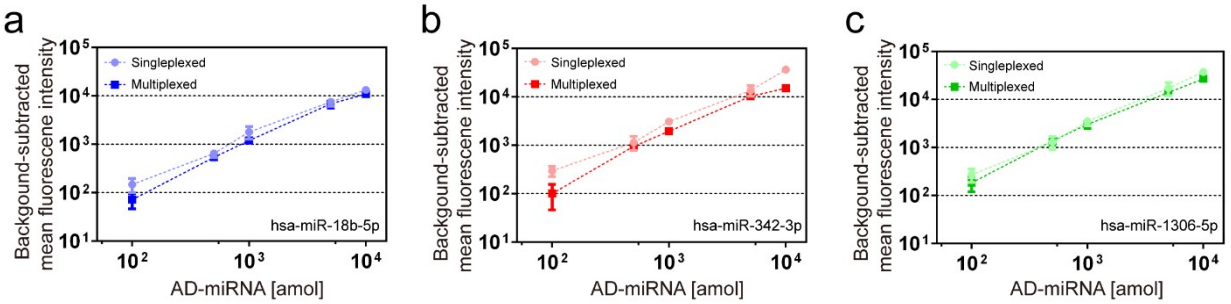

Figure S3. Plots of background-subtracted mean fluorescence intensity for singleplexed and multiplexed detection of hsa-miR-18b-5p (a), hsa-miR-342-3p (b), and hsa-miR-1306-5p (c).

Table S2. Statistical comparisons between singleplexed and multiplexed detection of AD-miRNAs

	100 amol	500 amol	1,000 amol	5,000 amol	10,000 amol
hsa-miR-18b-5p	*	ns	ns	ns	ns
hsa-miR-342-3p	****	ns	****	ns	****
hsa-miR-1306-5p	*	ns	ns	ns	****

ns, *, and **** denote no statistical significance, $p < 0.05$, and $p < 0.0001$.

Reference

1. S. C. Chapin, D. C. Appleyard, D. C. Pregibon and P. S. Doyle, *Angew. Chem., Int. Ed.*, 2011, **50**, 2289-2293.