Supporting Information for

Multiplexed detection of mRNA using porosity-tuned hydrogel microparticles

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Table S-1. Forward and reverse primers used to prepare cDNA templates for *in vitro* transcription of mRNA targets.

Primer type	Sequence	Remark
Forward	GTAATACGACTCACTATA	18 nt from T7 promoter region within pCMV6-XL5 vector
Reverse	CCGCAATCTAGAGTCGAG	18 nt after cDNA insert site within pCMV6-XL5 vector

Tables S-2. ssDNA oligonucleotides used for hydrogel-based mRNA detection. Acryd-CP, CE, BL, and LE are acrydite-modified capture probe, capture extender, blocker, and label extender, respectively.

1. Target: B2M

Name	Sequence
Acryd-CP	/5Acryd/TTTTT TCTCCCGTTTACTTG
CE 1	TCAGGAATGCCCGCCAG TTTTT CAAGTAAACGGGAGA
CE 2	GTAGCGCGAGCACAGCTAAG TTTTT CAAGTAAACGGGAGA
CE 3	TGCTGGATGACGTGAGTAAACCT TTTTT CAAGTAAACGGGAGA
CE 4	GGAATTCATCCAATCCAAATGC TTTTT CAAGTAAACGGGAGA
CE 5	CATTATTATAACCCTACATTTTGTGCA TTTTT CAAGTAAACGGGAGA
CE 6	CCCCACCTCTAAGTTGCCAG TTTTT CAAGTAAACGGGAGA
BL 1	GCCACGGAGCGAGACATCTCGGCCCGAATGCTGTCAGCT
BL 2	AGAAAGACCAGTCCTTGCTGAAAGACAAGTCTGAATGCTCCACTTTT
BL 3	CACTTAACTATCTTGGGCTGTGACAAAGTCACATGGTTCACACGGC
BL 4	TAAAGTGTAAGTGTATAAGCATATCAATATTAAAAAGCAAGCAAGCAGAATTT
BL 5	CCCTCCTAGAGCTACCTGTGGAGCAACCTGCTCAGATACATCAAA
LE 1	ATTTGTCTCACACCGTACTTATCATGAC TTTTT GAATCTTTGGAGTACGCTGGATAGCCTCCAGGCCAGAAAGAGAGA
LE 2	ATTTGTCTCACACCGTACTTATCATGAC TTTTT ATGAAACCCAGACACATAGCAATTCAGGAAATTTGACTTTCCATTCTC
LE 3	ATTTGTCTCACACCGTACTTATCATGAC TTTTT TCAATTCTCTCTCCATTCTTCAGTAAGTCAACTTCAATGTCGGATGG
LE 4	ATTTGTCTCACACCGTACTTATCATGAC TTTTT AGGCATACTCATCTTTTTCAGTGGGGGTGAATTCAGTGTAGTACAAGAGAT
LE 5	ATTTGTCTCACACCGTACTTATCATGAC TTTTT

	GGCATCTTCAAACCTCCATGATGCTGCTTACATGTCTCGATCC
LE 6	ATTTGTCTCACACCGTACTTATCATGAC TTTTT CATGGAGACAGCACTCAAAGTAGAATTATAAAGAAGATCATGTCCATGTTAA

2. Target: HSPA1A

Name	Sequence
Acryd-CP	/5Acryd/TTTTT CACGAGAAGGAAAGA
CE 1	TTTTTGTGATCTTGTTGGCCTTGCCTTTTTTCTTTCCTTCTCGTG
CE 2	TTTTTCTCCTGCACCATGCGCTCTTTTTTCTTTCCTTCTCGTG
CE 3	TTTTTGTTCTTGGCTGACACCCTCTTTTTTTCTTTCCTTCTCGTG
CE 4	TTTTTGAAGGCGTAGGACTCCAGGGCTTTTTTCTTTCCTTCTCGTG
CE 5	TTTTTCTTGTCCAGCACCTTCTTCTTGTTTTTTCTTTCCTTCTCGTG
LE 1	ATTTGTCTCACACCGTACTTATCATGAC TTTTT GGTGCTCTTGTCCGTGGCCGTGACGTTCAGGATGCCGTT
LE 2	ATTTGTCTCACACCGTACTTATCATGAC TTTTT GATCTCCTCCTTGCTCAGGCGGCCCTTGTCGTTGGTGATG
LE 3	ATTTGTCTCACACCGTACTTATCATGAC TTTTT CGCGCTGCACCTCGTCCTCCGCTTTGTACTTCTCCGC
LE 4	ATTTGTCTCACACCGTACTTATCATGAC TTTTT CCCTTGAGCCCCTCATCCTCCACGGCGCTCTTCATGTT
LE 5	ATTTGTCTCACACCGTACTTATCATGAC TTTTT GCCAAGGTGTTGGCGTCCAGCCACGAGATGACCTCTTGACA
LE 6	ATTTGTCTCACACCGTACTTATCATGAC TTTTT GCTCCAGCTCCTTCCTCTTGTGCTCAAACTCGTCCTTCTCG

3. Target: HSPH1

Name	Sequence
Acryd-CP	/5Acryd/TTTTT GAGTGGGGATTGATT
CE 1	TCCTGCTCACATATAAATTTTTCATAT TTTTT AATCAATCCCCACTC
CE 2	CTTCCTGAAACCGAACTTTAAC TTTTT AATCAATCCCCACTC
CE 3	AACATTTTTGGCCGTTCTTCAG TTTTT AATCAATCCCCACTC
CE 4	GCAGCCTCTGTCCTAGTTCTTCA TTTTT AATCAATCCCCACTC
CE 5	TTTGGGTGATTCAATTTTTGGT TTTTT AATCAATCCCCACTC
CE 6	GCCATTTGGAGTTCTTTCCAG TTTTT AATCAATCCCCACTC
BL 1	GAGGAGTCTCAAAAAATTTTGATGA

BL 2	CATACAGCCAGTCTTCAGTTTCTGT
BL 3	TGGAGTGCCAATTTTCATTAATTC
BL 4	CATTTCAGACTCATCAATATGGTTGT
BL 5	TCATTAACAGACTTCTCCACTTTTTT
LE 1	ATTTGTCTCACACCGTACTTATCATGAC TTTTT GGTCCACACAGCTTGTCTCTGAACTCATACACATATTCCTCAACTGCA
LE 2	ATTTGTCTCACACCGTACTTATCATGAC TTTTT TTCCAACTTGTCAACATATGCTTGTTTAGCTTGGTCCTCTCCTTCTT
LE 3	ATTTGTCTCACACCGTACTTATCATGAC TTTTT ATTTCTCATCCTTATTTCTGAAGTCAGCTGCTATCTTGGCATAATGCT
LE 4	ATTTGTCTCACACCGTACTTATCATGAC TTTTT TCTTTTTAGCCTGAGCATTCATGACATTATTCATCCATTCCATCACT
LE 5	ATTTGTCTCACACCGTACTTATCATGAC TTTTT TTTTGTTTTAATTTCCTGAGCACGTACAACTGGATCCTGATCAAGAC
LE 6	ATTTGTCTCACACCGTACTTATCATGAC TTTTT TTCGGTTGTGTTACAACGGGTTCACATGTGTTGTTCAATTCCTTGAT

4. Universal label adapter used to synthesize multi-biotinylated label probe

Name	Sequence
Univ-ADT	GTCATGATAAGTACGGTGTGAGACAAAT

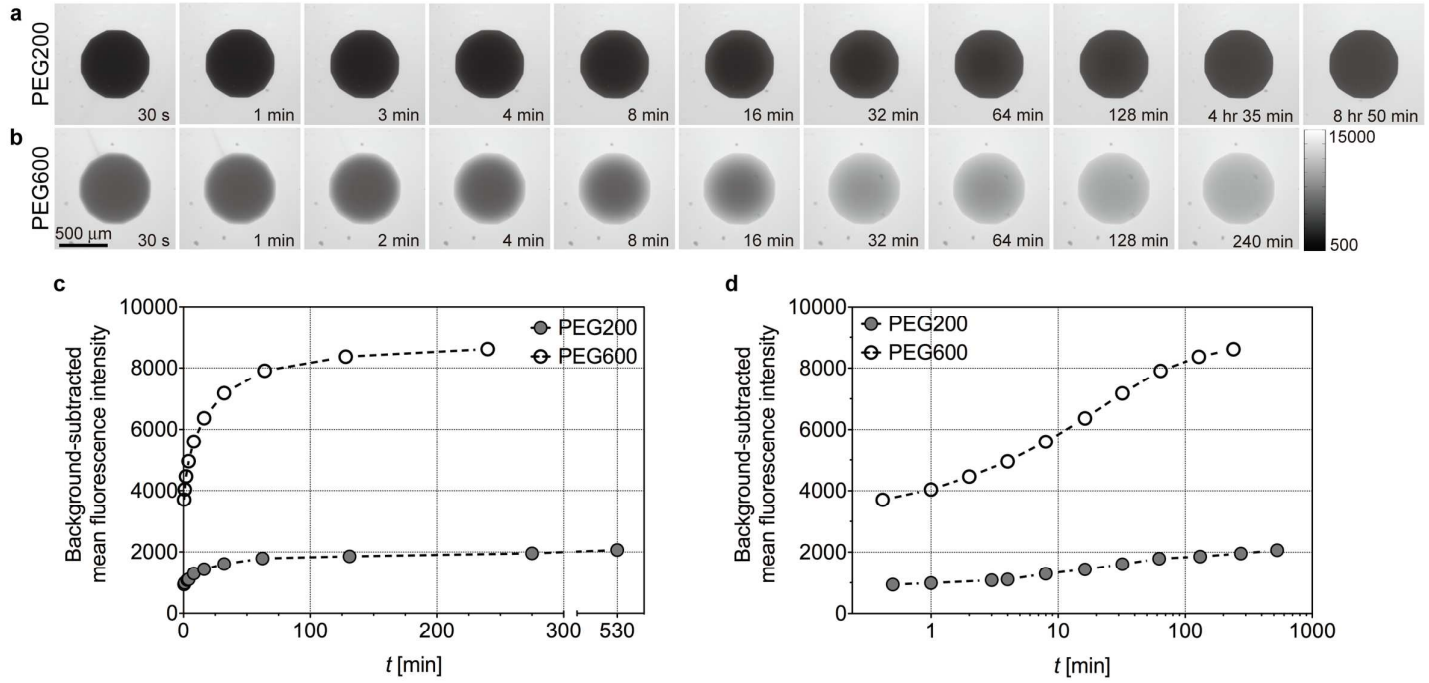

Figure S-1. Transient diffusion of 500 kDa FITC-dextran into 20 % [w/v] PEG700DA hydrogel posts crosslinked with PEG200 and PEG600 porogens. a and b. Fluorescence micrographs at various time points during the transient diffusion into hydrogel crosslinked with PEG200 (a) and PEG600 (b). Images are scaled with the same range (500-15000). **c and d.** Temporal evolution of background-subtracted mean fluorescence intensity within hydrogel post crosslinked with PEG200 (dark gray circle) and PEG600 (white circle), plotted with linear (c) and log (d) time scales.

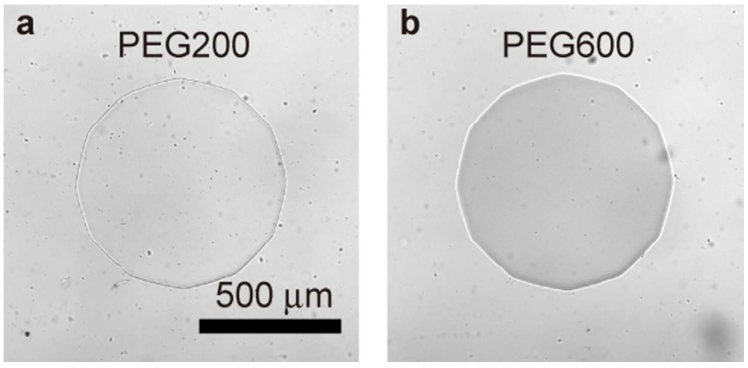

Figure S-2. Optical micrographs of 20 % [v/v] PEG700DA hydrogel posts crosslinked with 40 % [v/v] PEG200 (a) and PEG600 (b) porogens, showing the qualitative difference in opaqueness. Images are scaled with the same range (0-9000).

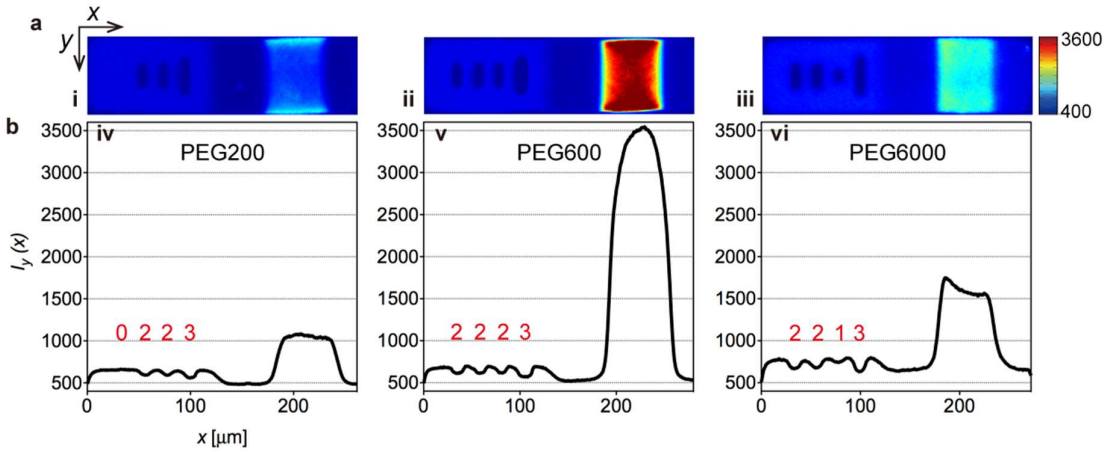

Figure S-3. Comparison among PEG porogens in the detection of biotinylated HSPA1A using barcoded hydrogel microparticles. a. Fluorescence micrographs showng representative barcoded hydrogel microparticles synthesized with PEG200 (i; code 3220), PEG600 (ii, code 3222), and PEG6000 (iii; 3122) porogens, after detecting 50 fmol of biotinylated HSPA1A, labeled with SA-PE. The biotinylated HSPA1A was obtained via *in vitro* transcription similarly as presented in the experimental section, except for the addition of biotinylated uracil. For the PEG6000 porogen, note that 35 % [v/v] PEG700DA and 20 % [v/v] PEG6000 were used to avoid macroscopic phase separation. **b.** Plots of spatially resolved *y*-averaged fluorescence intensity along *x*-direction (iv-vi) for the microparticles shown in frames i-iii.

Video S-1. Synthesis of shape-encoded "intraplex" PEGDA hydrogel microparticles via Stop Flow Lithography (SFL)

Video S-2. Synthesis of barcoded PEGDA hydrogel microparticles vis SFL