Supplementary Information for

Phosphorylcholine-based Encoded Hydrogel Microparticles with Enhanced Fouling Resistance for Multiplex Immunoassays

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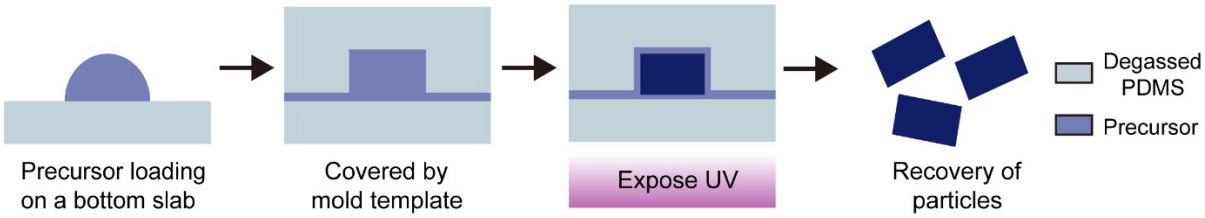

Figure S1. Schematic view of the Degassed Molding Lithography (DML) for the synthesis of encoded hydrogel microparticles. PDMS mold template and bottom slab was first degassed in a vacuum chamber. After taking the degassed PDMS out of the chamber, precursor was introduced on a bottom slab. Then, the precursor was covered by PDMS mold template which contains a large array of desired shape for the mass production of graphically encoded hydrogel particles. Degassed PDMS acted as a suction pump and removed air bubbles, allowing the precursor to be completely filled inside the mold. After few minutes, UV was exposed to the mold to polymerize the particles. Finally, mold template was detached from the bottom slab and particles were recovered by dropping recovery solution onto the mold template.

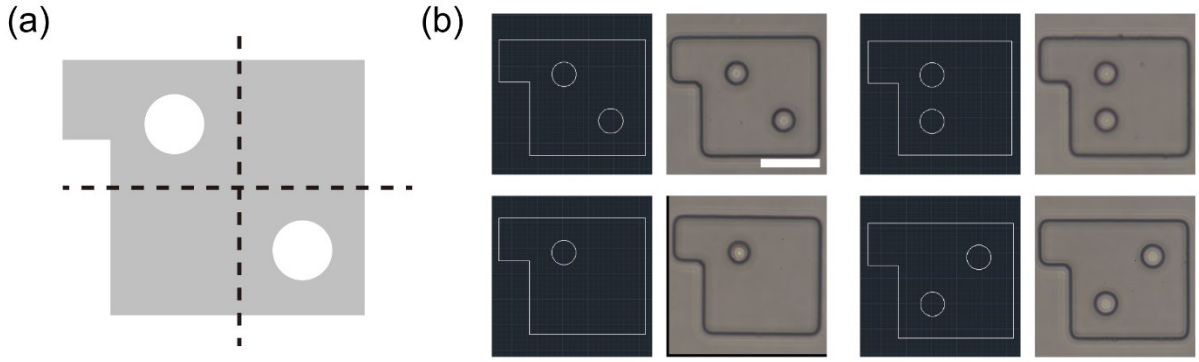

Figure S2. Design of encoded hydrogel microparticles. (a) Region of particles was divided into four sectors and simple shape was arranged in each sector to define identifiable graphical codes. (b) Example of engraving graphical codes in hydrogel microparticles. Images with black background are photomask patterns designed by AutoCAD and images with gray background are micrograph of PDMS mold template synthesized from photomask patterns. Scale bar is 25 μm.

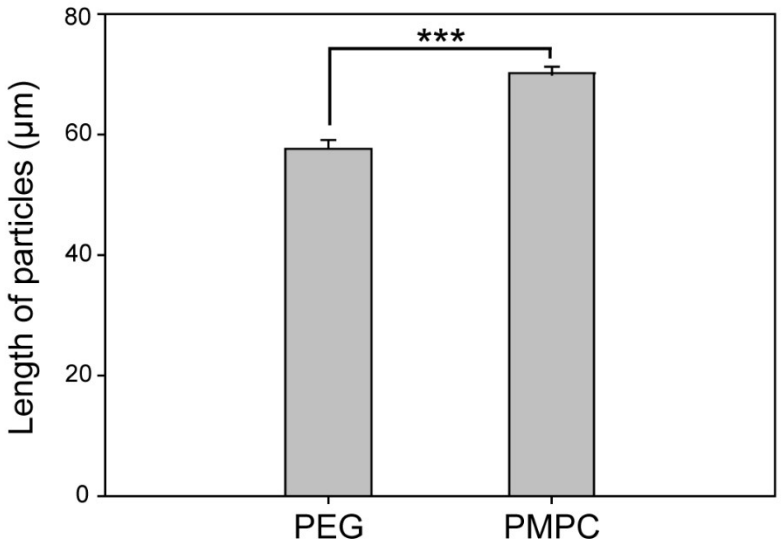

Figure S3. Comparison of the length of particle synthesis. Due to the higher swelling capacity of MPC, length of PMPC particles increased by 20% compared to that of PEG particles. *** denotes $p < 0.001$.

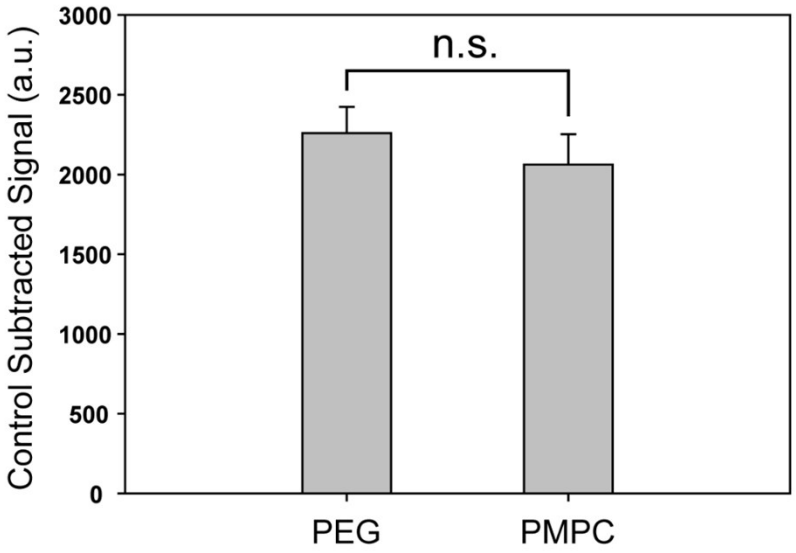

Figure S4. Comparison of the unconverted double bonds after the particle synthesis by measuring the fluorescent intensity of encoded hydrogel microparticles. FITC-PEG-SH was used as a fluorescent probe to quantify remaining unconverted double bonds. Fluorescent intensity of PMPC particles was comparable to that of PEG particles. ns denotes no statistical differences.

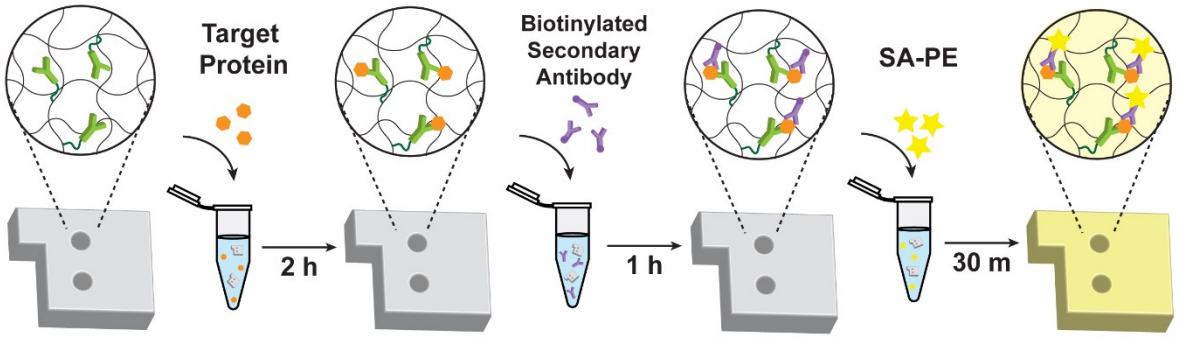

Figure S5. Schematic view of the immunoassay using encoded hydrogel microparticles. Antibody functionalized particles are mixed with target proteins and after the reaction, secondary antibodies are introduced to label biotin at target binding site. Then, streptavidin r-phycoerythrin conjugate (SAPE) are added to fluorescently label target binding site. Thus, fluorescent intensity can be measured from hydrogel particles in the presence of target protein.

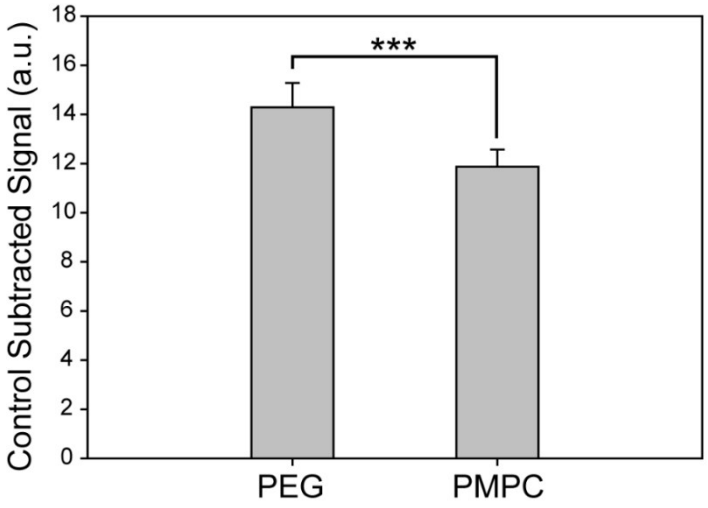

Figure S6. Comparison of the control signal by measuring the fluorescent intensity of encoded hydrogel particles after the immunoassay procedure at the absence of target protein. Due to the high anti-fouling effect of MPC, non-specific adsorption of proteins that causes

undesired fluorescent intensity was clearly reduced compared with that of PEG particles. *** denotes $p < 0.001$.

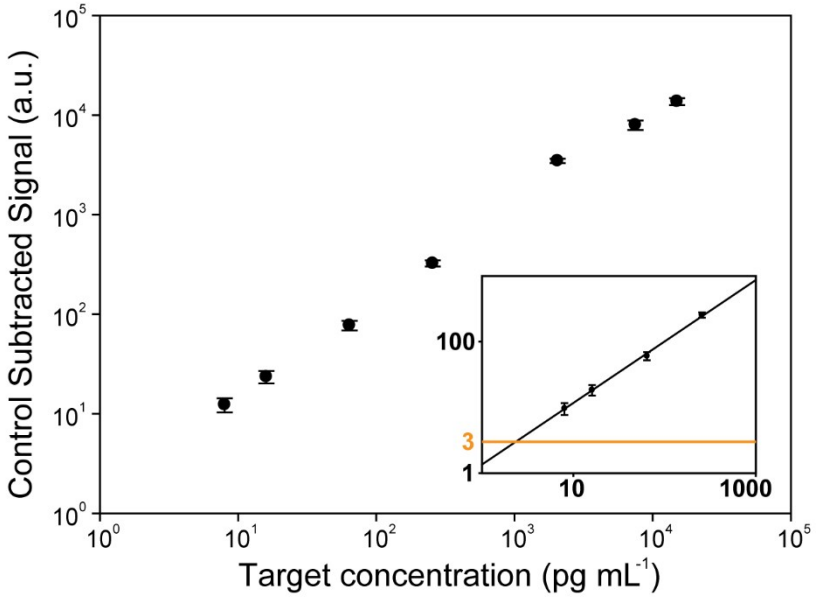

Figure S7. Standard calibration curve for CG beta by performing immunoassay using PMPC particles at various concentrations of CG beta from 8 pg mL^{-1} to 15000 pg mL^{-1} and their signal to noise plots. The limit of detection is defined as the concentration that produces three signal to noise ratios.

Target	Assay	Range [LOD-max.] (log_{10})
PlGF	ELISA	[31.2 – 2,000 pg mL^{-1}] (1.8)
	PMPC particles	[7.8 – 15,000 pg mL^{-1}] (3.3)
CG beta	ELISA	[7.8 – 5,000 pg mL^{-1}] (1.8)
	PMPC particles	[2.39 – 15,000 pg mL^{-1}] (3.8)

Table S1. The limit of detection and dynamic range of ELISA and PMPC particle-based immunoassays

Table S2. Multiplex detection of PlGF and CG beta and their recovery

	Control Subtracted Signal (a.u.)			
Case	**PlGF**		**CG beta**	
1	-	38.45 ± 2.72	-	13.87 ± 2.3
2	+	618.83 ± 64.74	-	14.41 ± 1.84
3	-	42.74 ± 2.89	+	389.45 ± 45.14
4	+	610.95 ± 57.17	+	449.46 ± 59.41

Avg.	614.63 ± 58.71	421.34 ± 60.1
Recov.(%)	122.21	126.42