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Supplementary Information

## Multiplexed immunoassay using post-synthesis functionalized hydrogel microparticles

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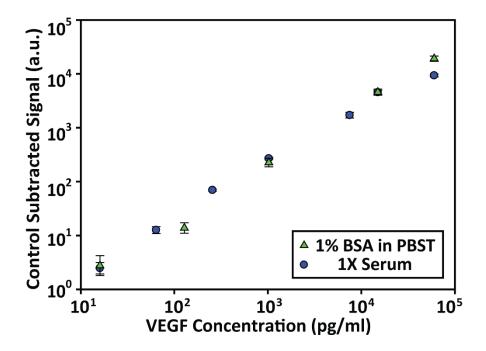
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## I. VEGF assay in animal serum

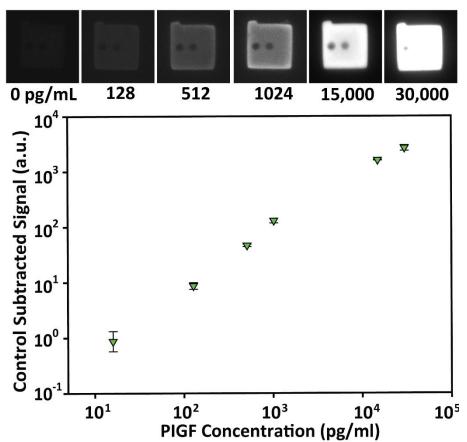
While the determination of the assay performance was carried out in standard ELISA buffer consisted of 1 % BSA in PBST, the compatibility of the PSF particle assay with biological samples was investigated with VEGF detection in animal serum (R&D Systems, cat. no. D6050 part no. 895018). Target VEGF was serially diluted in 1x serum and mixed with 50 PSF particles in 1x serum. The control subtracted signals of detection in the serum did not show significant deviations from the detection signals in the standard buffer (Fig. S1). The assay range was determined to be 9.0- 15,000 pg mL<sup>-1</sup>. This demonstrates the assay's robustness in regard to changes in sample media.



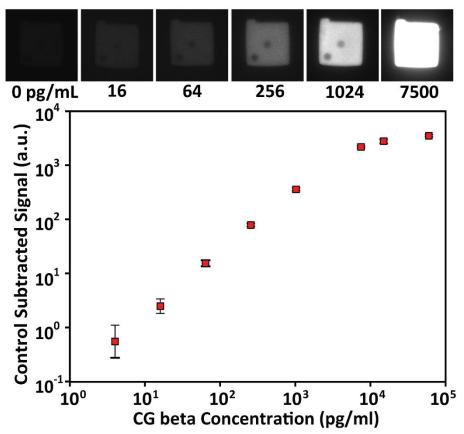
**Fig. S1** IL-6 detection in standard buffer and 1x animal serum. The signals of VEGF detection in 1x serum are plotted along with the signals of detection in standard ELISA buffer (1% BSA in PBST). Each data point and the vertical error bars represent the average signal and standard deviation of >10 particles. The resulting assay range of the detection in serum is 9.0- 15,000 pg mL<sup>-1</sup>.

## II. Single-plex PIGF and CG beta assay

Figure 4 in the article has combined three single-plex assays of VEGF, PIGF, and CG beta. The single-plex fluorescence images and the standard calibration curve for PIGF and CG beta are provided in figure S2 and S3, respectively.



**Fig. S2** PIGF assay and the standard calibration curve. The assay range is 17.7- 30,000 pg mL<sup>-1</sup>. Each data point and the vertical error bars represent the average signal and standard deviation of >10 particles.



**Fig. S3** CG beta assay and the standard calibration curve. The assay range is 4.2- 7500 pg mL<sup>-1</sup>. Each data point and the vertical error bars represent the average signal and standard deviation of >10 particles.

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**III.** Signal invariability with particle orientation

**Fig. S4** Invariability of fluorescent signals with particle orientation. (a) Fluorescent image of particles in singleplex detection of VEGF at 1,024 pg mL<sup>-1</sup>. One of the three particles is on its backside. (b) The average signals of particles facing front and back. Each bar plot represents the average and standard deviation of >5 particles. Scale bar is 100  $\mu$ m.