Supplementary Information for:

# Rapid Capture of Biomolecules from Blood via Stimuli-Responsive Elastomeric Particles for Acoustofluidic Separation

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**Gel electrophoresis characterization of purified ELPs and ELP fusion proteins**

The molecular weight and purity of ELPs were characterized using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A 10 μL solution of polypeptides (100 μM, unless otherwise noted) was diluted with 10 μL Laemmli loading buffer with betamercaptoethanol (BioRad, Inc.) and heated to 95ºC for 5 min. We ran the samples on a 4-20% Tris-HCl polyacrylamide gel (BioRad) and stained the gel for 10 min (Coomassie Brilliant Blue; Thermo Fisher Scientific, Inc.). Figure S1 illustrates the purity of different ELPs. In addition, the bands observed for each ELP in SDS-PAGE was consistent with its theoretical molecular weight (i.e., GFP-ELP-Cys: 45.5 kDa; ELP-40: 17.1 kDa; ELP-Cys: 19.9 kDa; biotin-ELP: 17.6 kDa).

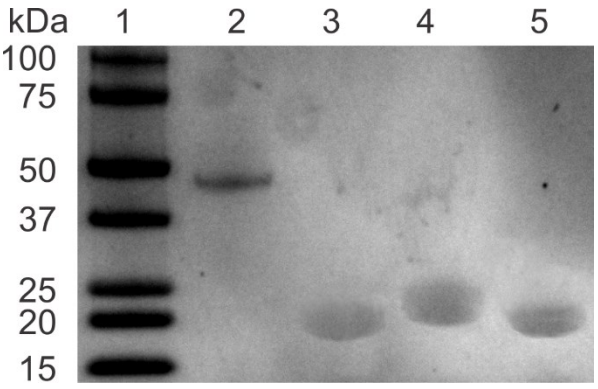


**Figure S1.** SDS-PAGE gel with a molecular weight ladder (lane 1), GFP-ELP-Cys (25 μM) (lane 2), ELP-40 (lane 3), ELP-Cys (lane 4) and biotin-ELP (lane 5).

**Characterization of aqueous phase behavior of purified ELPs and ELP fusion proteins**

To characterize their temperature-dependent aqueous phase behavior, the optical densities at 350 nm ($OD_{350}$) of different ELP solutions (150 μM) were measured as a function of temperature. Samples were heated at 1°C/min in a UV-visible spectrophotometer equipped with a multi-cell thermoelectric temperature controller (Cary 300; Varian, Inc.). Figure S2 reveals the $T_t$ of different

ELP solutions (150 μM in PBS).

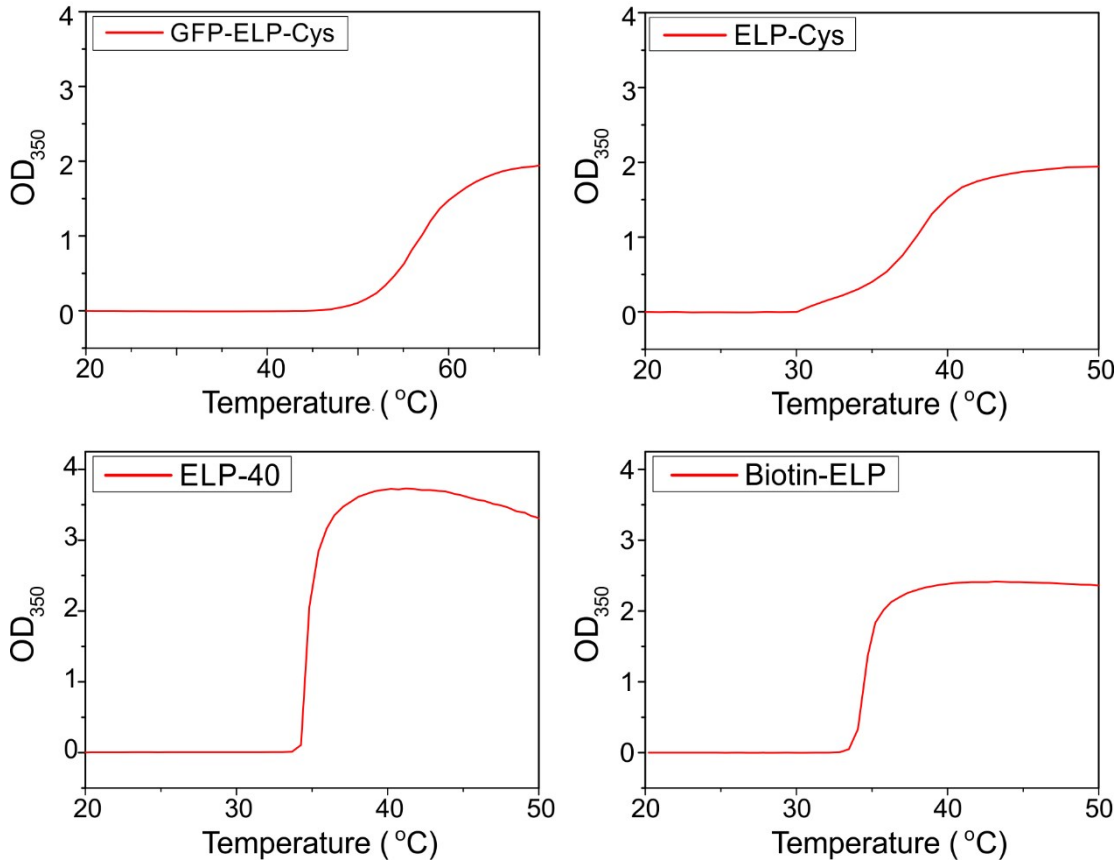


**Figure S2.** Turbidity profile of GFP-ELP-Cys, ELP-Cys, ELP-40 and biotin-ELP at 150 μM as a function of temperature, as determined by spectrophotometry.

**Acoustic separation of biomarker-particle complexes from polystyrene beads**

The acoustofluidic chip was made from silicon and glass components using conventional microfabrication techniques; these details have been described elsewhere.[1] We designed the acoustofluidic chip to contain a trifurcating inlet and outlet, and a main channel with a width of 315 μm (see Figure S3a,c). The etched silicon wafer (thickness: 675±25 μm; Addison Engineering,

Inc.) was fused to a borosilicate glass slide (Nexterion glass B, thickness: 1.0±0.005 mm; Schott AG) with six holes that accommodate inlets and outlets (silicone tube connection sites). The lead zirconate titanate (PZT) transducer (841 WFB, 2.93 MHz resonance frequency; APC International, Ltd.) was soldered to wires and was centered underneath the microchannel, as shown in Figure S3b,d.

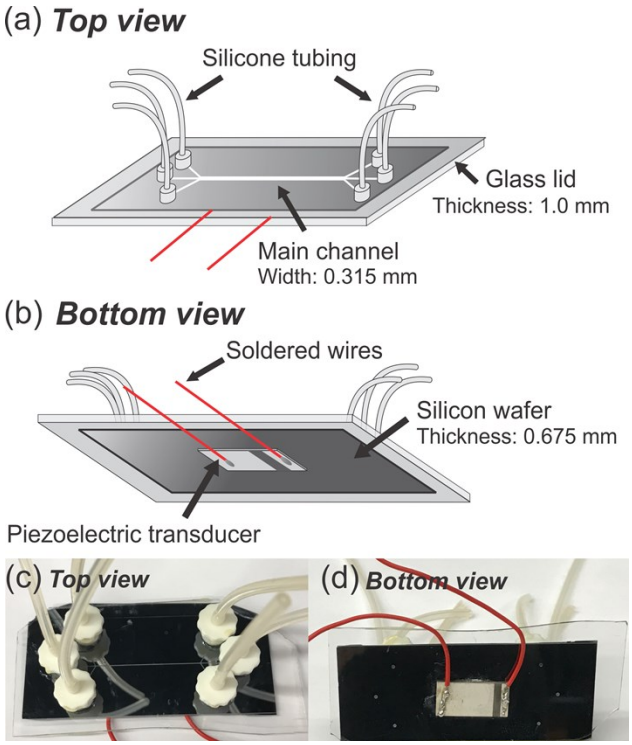


**Figure S3.** Schematic views of the (a) top and (b) bottom of an acoustofluidic device comprised of an etched silicon substrate, a borosilicate glass "lid" and a PZT soldered to wires. Photographs of the (c) top and (d) bottom of the device.

We conducted a pilot test to acoustically separate ELP-modified silicone particles with captured Alexa Fluor 488-labeled streptavidin (SA; Thermo Fisher) from polystyrene (PS) beads (FluoSpheres™, 10 µm, yellow-green fluorescent (505/515); Thermo Fisher). We actuated the

PZT transducer at 2.35 MHz to induce a half-wavelength bulk acoustic standing wave across the width of the microchannel using a waveform generator (33250A; Agilent Technologies, Co.) at 40.0 V peak-to-peak after amplification using a high frequency amplifier (25A250AM6; Amplifier Research, Co.). The central inlet was injected with warm PBS at a flow rate of 75 μL/min, and three syringe pumps regulated the flow of liquid through the outlets using "withdraw mode" (i.e., the peripheral outlets were withdrawn at 25 μL/min and the central outlet was withdrawn at 75 μL/min). The tubing directed to the two side inlets was freely suspended in the sample on a hot plate at 40°C with constant stirring at 100 rpm to ensure that a constant concentration of particles and PS beads was supplied to the chip throughout the experiment. A mixture of biomarker-particle complexes and PS beads in a ratio of 1:1 was injected into an acoustofluidic device (see schematic illustration in Figure S4a). The outputs from the two peripheral outlets consisted of 97.5% silicone particles, and the output from the central outlet consisted of 84.7% PS beads (Figure S4b). The acoustic parameters established from this pilot test were used for the acoustic separation of these particles from blood cells (see Figure 4 in the manuscript).

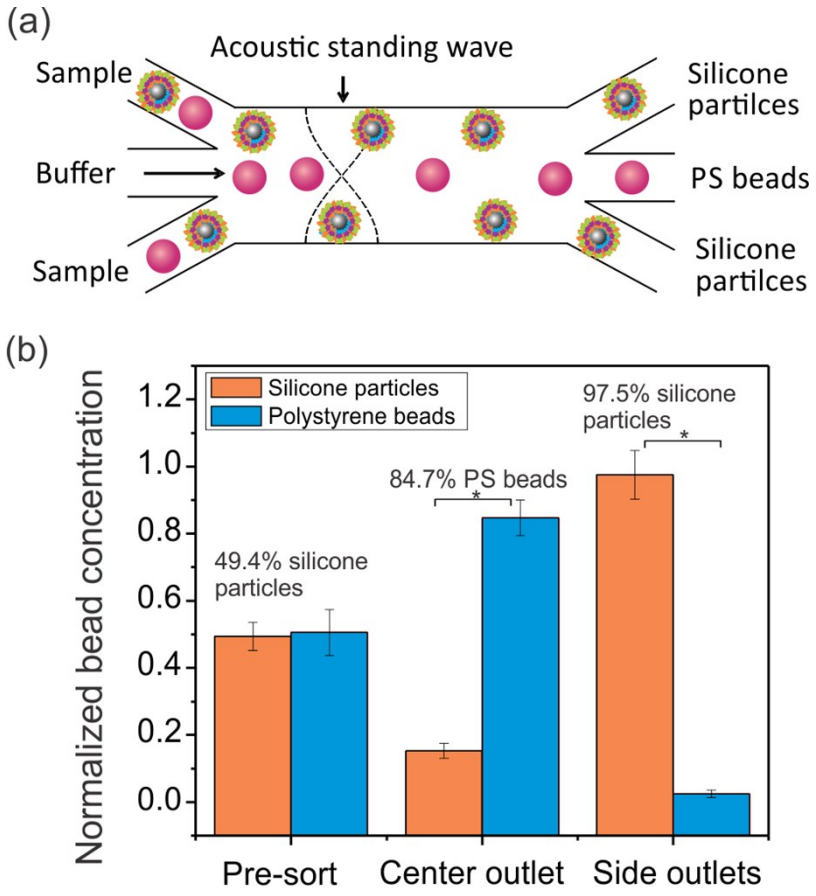


**Figure S4.** (a) Schematic illustration depicts the acoustic separation of ELP-modified silicone particles with captured SA from PS beads (representing blood cells) in an acoustofluidic device (see manuscript for separation results from blood cells). Figure is not to scale. (b) Bar graph shows the relative amount of silicone particles and PS beads in the initial sample and fractions from the side outlets and center outlet after sorting. Asterisks indicate a significant difference between conditions (*$p < 0.05$, $n = 5$).

For experiments involving blood samples, the central inlet was injected with warm PBS at a flow rate of 25 μL/min, and three syringe pumps regulated the flow of liquid through the outlets using "withdraw mode" (i.e., the peripheral outlets were withdrawn at 12.5 μL/min and the central outlet was withdrawn at 25 μL/min).

**Quantification of captured streptavidin on the surfaces of ELP-modified particles before and after acoustic separation**

We used a flow cytometer (Accuri C6; BD Biosciences) to assess the amount of captured SA on ELP-modified silicone particles both before and after acoustic separation. In this test, we performed the capture assay in physiological buffer prior to mixing with blood to quantify the amount of sequestered SA per particle via flow cytometry before and after acoustic separation. No significant difference in the amount of SA per particle was observed ($p > 0.05$, Figure S5), indicating that biomarkers remained stably associated with the particles throughout the acoustic separation process.

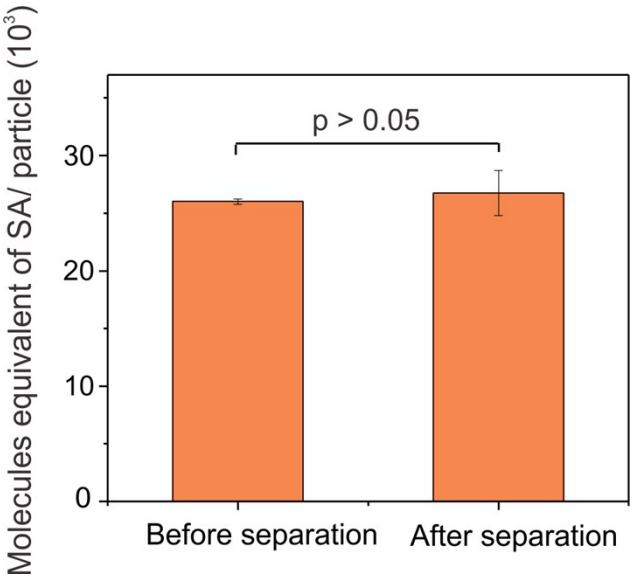


**Figure S5.** The bar graph shows the molecules equivalent of SA per ELP-modified particle both before and after acoustic separation, as measured by flow cytometry ($p > 0.05$, $n = 5$).

**Controls for biomarker capture assay in porcine plasma**

To assess the detection sensitivity of the SA capture assay, we spiked porcine plasma (BioreclamationIVT, LLC) with SA ranging from 0 to 1 μM. The non-specific adsorption of SA to the surfaces of the ELP-modified silicone particles was examined by conducting a series of control experiments using SA pre-incubated with free biotin. We first added 500 μM free biotin (Sigma-Aldrich, Co.) to 100 μL plasma spiked with SA (i.e., 0-1 μM) under gentle shaking for 1 h. We then added 500 μM biotin-ELP to the solution. After 10 min of incubation, we added the ELP-modified particles to the undiluted plasma mixture and raised the temperature above the $T_t$ (i.e., 34°C) to 40°C for 5 min to allow the tethered and untethered ELPs co-aggregate. The amount of SA non-specifically adsorbed to the surfaces of the particles was measured using flow cytometry. Figure S6 shows that the fluorescence from particles with non-specifically adsorbed SA was much lower than particles with sequestration (see Figure 5), confirming that the capture occurs biospecifically between the biotin-ELP and SA.

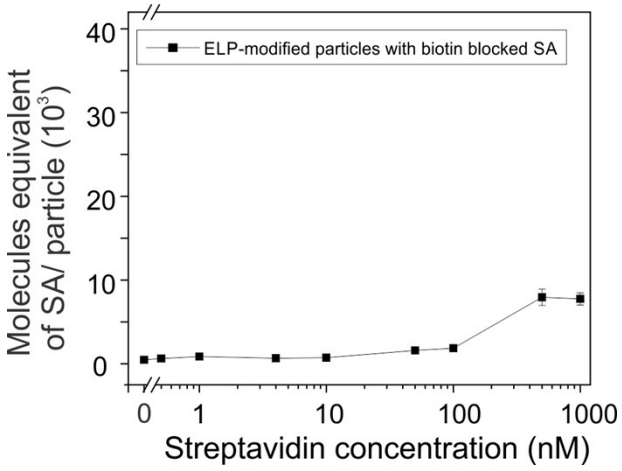


**Figure S6.** The molecules equivalent of SA per ELP-modified silicone particle after incubation with various amounts of biotin-blocked SA (ranging from 0-1 μM; see manuscript for results of

incubation with non-blocked SA). The values for molecules equivalent of SA per particle were normalized by subtracting the average value measured for the autofluorescence signal of the bare silicone particles. Error bars represent the standard error of the mean ($n \geq 3$).

## REFERENCES

(1) Shields, C. W.; Cruz, D. F.; Ohiri, K. A.; Yellen, B. B.; López, G. P. *J. Vis. Exp.* **2016**, No. 109, 1–7.