Supplementary Information for:

Digital Microfluidcs for Immunoprecipitation

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HSA Conformation Experiments

Human serum albumin (HSA) conformation was monitored using UV fluorescence using techniques similar to those described previously (*J. Biol. Chem.*. 2000, 275, **5**, 3042-3050) Briefly, three different buffers were prepared: (1) 25 mM tris-HCl with 750 mM NaCl (2) 100 mM citric acid with 700 mM NaCl, and (3) 100 mM citric acid. The pH of the buffers were measured to be 7.5, 1.7, and 1.7, respectively, using an AR 50 pH meter (Fisher Scientific, Waltham, MA, USA) equipped with a Beckman Coulter (Brea, CA, USA) pH electrode. HSA was then added to each buffer to a final concentration of 9 μM, and the solutions were allowed to incubate for 1 h at room temperature. Solution (3) was subsequently neutralized by addingNa_3PO_4 to 200 mM and then allowed to incubate for an additional hour at room temperature (the pH of the neutralized solution was measured to be 7.1). Blank solutions not containing HSA were also prepared for each condition.

Fluorescence intensities were monitored on a Perkin Elmer LS50B Luminescence spectrometer using an excitation wavelength of λ_{ex} = 295 nm (5.0 nm slit width) and an emission scan range of λ_{em} = 320-360 nm (7.5 nm slit width, 250 nm/min scan rate) in a quartz cuvette with path length of 1 cm (Precision Cells, Inc., New York). Each HSA-containing solution was evaluated in triplicate with 10 scans averaged for each replicate; blank measurements (not containing HSA) were subtracted from each scan. The data were plotted as a function of emission wavelength to determine the wavelength of maximum intensity $\lambda_{em,max}$.

HSA has a “normal” conformation *N* at neutral pH, but adopts “extended” conformation *E* at pH <3 (Theodore, P., *All About Albumin: Biochemistry, Genetics and Medical Applications*, 1995, Academic Press). The native fluorescence of HSA (originating from a single tyrosine residue) reflects this change – the $\lambda_{em,max}$ is blue-shifted for *E* relative to *N* (*J. Biol. Chem.*. 2000,

275, **5**, 3042-3050). This phenomenon was used here to evaluate the effects of 100 mM citric acid on HSA conformation. Three HSA solutions were prepared (with approximately equivalent ionic strength): (1) HSA in neutral buffer (pH 7.5), (2) HSA in citric acid (pH 1.7), and (3) HSA in citric acid (pH 1.7) which was incubated for an hour and then subsequently brought to neutral pH (7.1). Fluorescence spectra of each sample are shown in Figure S1. As expected, reducing the pH from neutral (1) to acidic (2) resulted in a reduction in emission-maximum from $\lambda_{em,max(1)} = 346 \pm 2$ nm to $\lambda_{em,max(2)} = 338 \pm 1$ nm (Figure S1). This suggests that the immunoprecipitation-elution buffer (100 mM citric acid) described in the main text causes a change in conformation from *S* to *E*. But the spectrum of solution (3), which has an emission max of $\lambda_{em,max(3)} = 345 \pm 1$ nm, suggests that the conformation change is not permanent. When evaluated in replicates, these data were statistically indistinguishable from those of solution (1) (Student's *t*-test, $\alpha = 0.05$, $p = 0.840$)

Gel Electrophoresis Experiments

A 1 mg/mL solution of HSA in FBS was prepared and subjected to the eight-step DMF-IP as described in the main text. Three fractions were collected (by pipette) for analysis: fraction 1 is the product-droplet generated after step 8, fraction 2 is the pooled supernatant droplets collected after step 5, and fraction 3 is the supernatant droplet generated after step 3. (Note that fractions 2 and 3 are typically driven to a waste reservoir and discarded, but in these experiments, they were collected for analysis.) The fractions were analyzed by SDS-PAGE using a 10% polyacrylamide gel with beta-mercaptoethanol as a reducing agent. The gel was stained with Coomassie Blue G-250 (Bio-Rad, Hercules CA., USA) as per manufacturer's instructions. The stained gel was visualized using a Gel Doc EZ Gel Documentation System (Bio-Rad). As shown in Figure S2, the serum sample contains a large number of bands representing different

proteins, while the DMF-IP sample shows a single band at ~66 kD, suggesting that the sample has been substantially purified. Note that this test does not distinguish between HSA and bovine serum albumin (in the matrix), which co-elute.

HPLC-MS Experiments

50 ng/mL HSA in FBS samples were prepared and subjected to the P-CLIP-DMF-IP procedure, and then reduced, alkylated, digested and extracted as described in the main text. The samples were then diluted 1:1 in water containing 0.1% formic acid and analyzed by a Shimadzu (Kyoto, Japan) 20AC HPLC operating in-line (via electrospray ionization) with a SCIEX 6500+ QTRAP mass spectrometer (Concord, ON). The HPLC column was a Phenomenex (Torrance, CA) Kinetex (2.1 mm x 100 mm, packed with 2.6 μm dia. particles with 100 Å-thick C18 coating), operated in gradient elution mode at 40°CThe injection volume was 5 μL and the mobile phase was a mixture of solution A (98% water, 2% acetonitrile each with 0.1% formic acid) and B (2% water, 98% acetonitrile each with 0.1% formic acid). The HPLC gradient was a total of 30 minutes with an initial concentration of solution B at 5%, with a linear ramp up to 27% after 15 minutes followed by a second linear ramp up to 97% at 18 minutes where it was held for 5 minutes before linearly ramping down to 5% at 25 minutes where it was held until the end of the run, all at 300 μL/min. The mass spectrometer was operated in MRM mode monitoring the same transitions listed in the main text but with different operating parameters: the source temperature was 400°C and the electrospray voltage was 5500 V. The collision, curtain and sheath gases (GS1 and GS2) were 8, 20, 30 and 40 psi respectively. The collision energy, cell exit potential and declustering potential for the HSA peptide were 25 eV, 14 V and 90 V respectively and for angiotensin I (internal standard) they were 30 eV, 15 V and 90 V. Chromatograms were treated with Gaussian smoothing (0.5-point width) using PeakView 1.2.2

(SCIEX). The retention time for the HSA peptide was found to be 8.8 minutes and, with peak area 1247 (Figure S3) relative to peak area in a blank sample of 13. This result suggests that P-CLIP-DMF-IP in combination with HPLC-MS is suitable for quantitation of low-abundance protein targets.

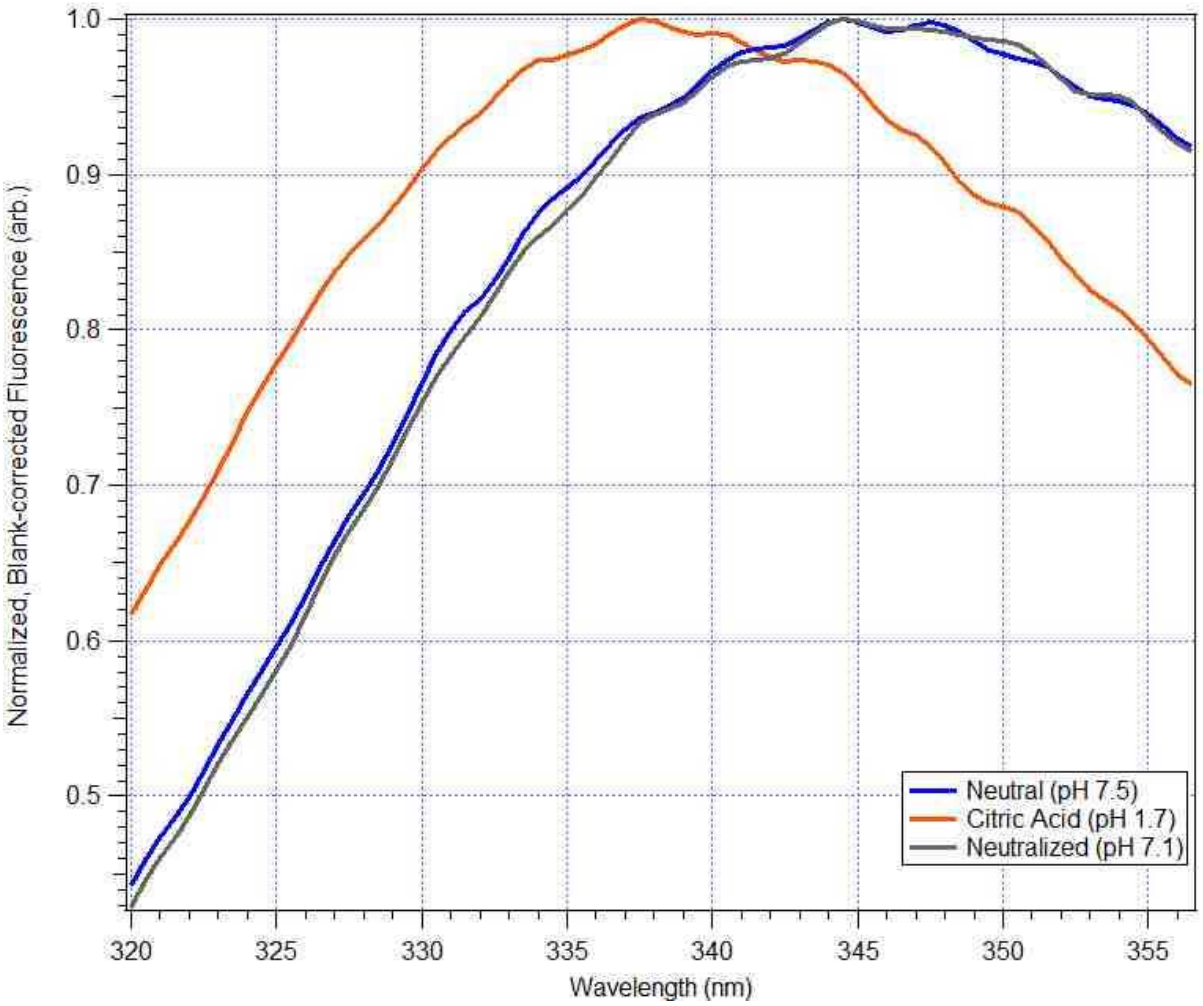

Figure S1: Effects of immunoprecipitation-elution buffer (100 mM citric acid) on protein conformation. Fluorescence spectra of 9 μM HSA in (1) neutral buffer (pH 7.5, blue), in (2) 100 mM citric acid (pH 1.7, orange), and in (3) 100 mM citric acid that was subsequently neutralized (pH 7.1, gray). The wavelengths of maximum emission are $\lambda_{em,max(1)}$ =346 ± 2 nm, $\lambda_{em,max(2)}$ =338 ± 1 nm, and $\lambda_{em,max(3)}$ = 345 ± 1 nm, respectively.

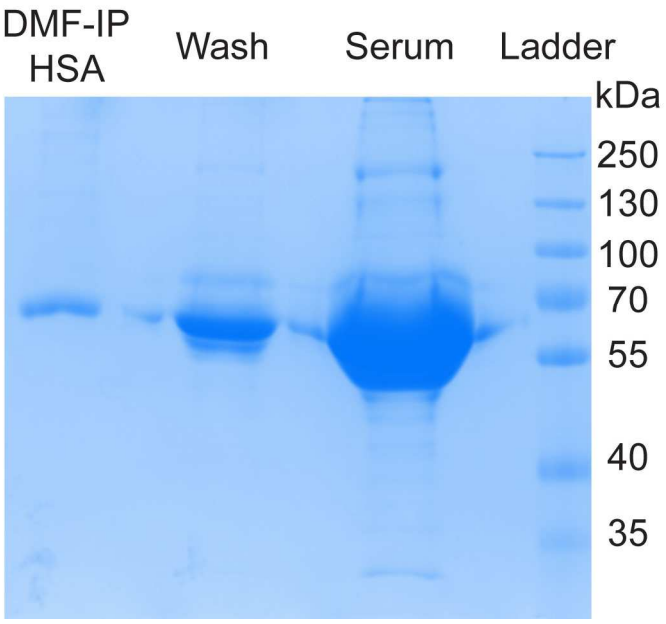

Figure S2: Image of a Coomasie Blue-stained SDS-PAGE gel of human serum albumin (HSA) under reducing conditions. Each lane contains a sample collected from a DMF-IP device at different stages in the eight-step procedure. Lane 1 (left): fully processed sample collected after completing step (8). Lane 2: combined wash buffer droplets collected after step (4). Lane 3: serum sample supernatant droplet collected after being treated with magnetic particles in step (3). Lane 4 (right): protein mass ladder.

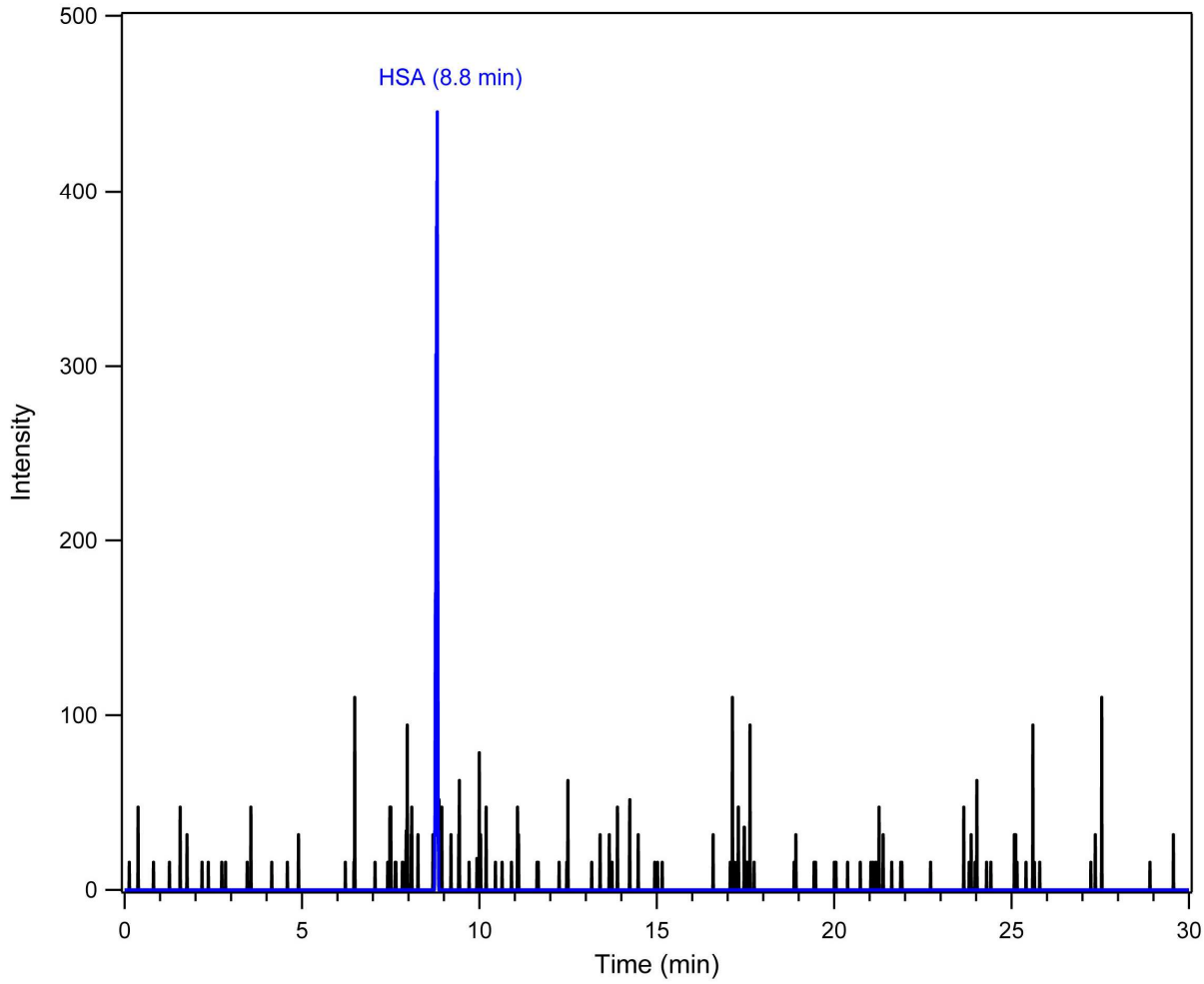

Figure S3: Representative HPLC-MS/MS chromatogram of HSA after P-CLIP-DMF-IP. A sample of FBS containing 50 ng/mL HSA was processed using P-CLIP-DMF-IP and then analyzed, monitoring the same peptide transition (575.3 → 937.6) described in the main text. The retention time of HSA was found to be 8.8 minutes (highlighted in blue). Blank samples showed negligible signal intensity at that retention time.