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Supplemental Information

Protein Corona in Response to Flow: Effect on Protein Concentration and Structure

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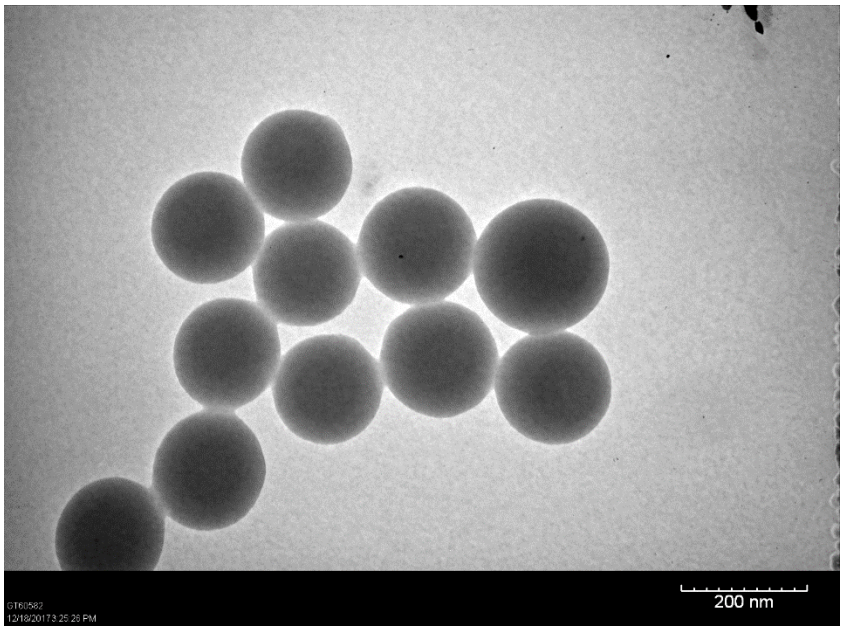

Figure S1. TEM (JEOL 100 CX II, 100 kV with 200 kX magnification) of the polystyrene NPs ("orange," #F8809, 200 nm, Excite: 540 nm/Emit: 560 nm ThermoFisher Scientific, Carlsbad, CA) used in the main text. Diameter = 200 nm ± 15 nm for n= 50 NPs, analyzed with ImageJ.

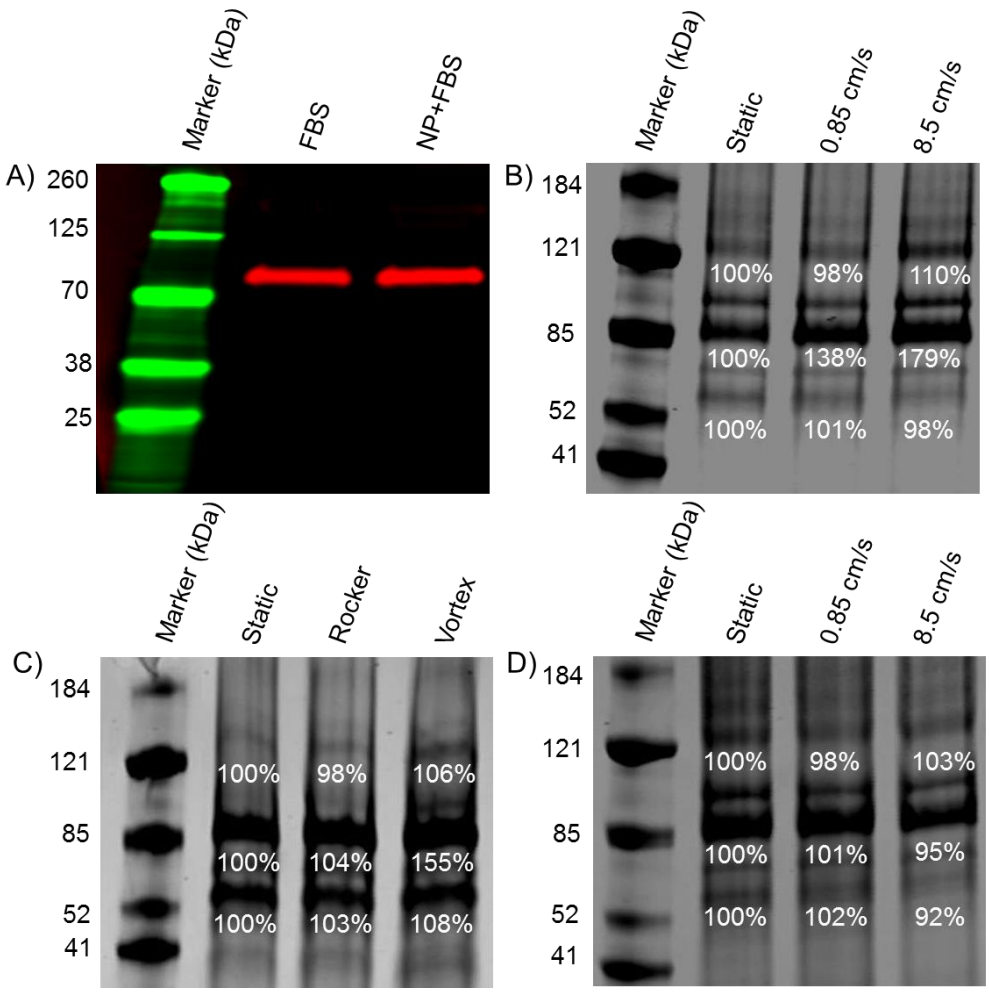

Figure S2. Additional characterization of the protein corona. Representative gels are shown with the values measured for that specific gel. Averages and standard deviations for triplicate experiments are reported in the caption. (A) Western blotting was used to confirm the presence of plasminogen in FBS and the protein corona (NP+FBS). (B) Loading equal concentrations of NPs onto the gel showed a similar enrichment in plasminogen at 88 kDa. (0.85 cm/s = 135% ± 4%, 8.5 cm/s = 178% ± 6%; both n=3) with BSA constant (0.85 cm/s = 103% ± 2%, 8.5 cm/s = 102% ± 4%; both n=3). Static values are set to 100%. (C) The mixing method used affected the final protein corona. Use of a rocker (30 min) was similar to static conditions (104% ± 3%, n=3). Use of a vortexer (30 min) was similar to the use of higher flow speeds (155% ± 4%, n=3). (D) Pre-coating the NPs with a protein corona (30 min, Static) prevented any flow (30 min, 8.5 cm/s)-dependent changes (100% ± 4%, n=3).

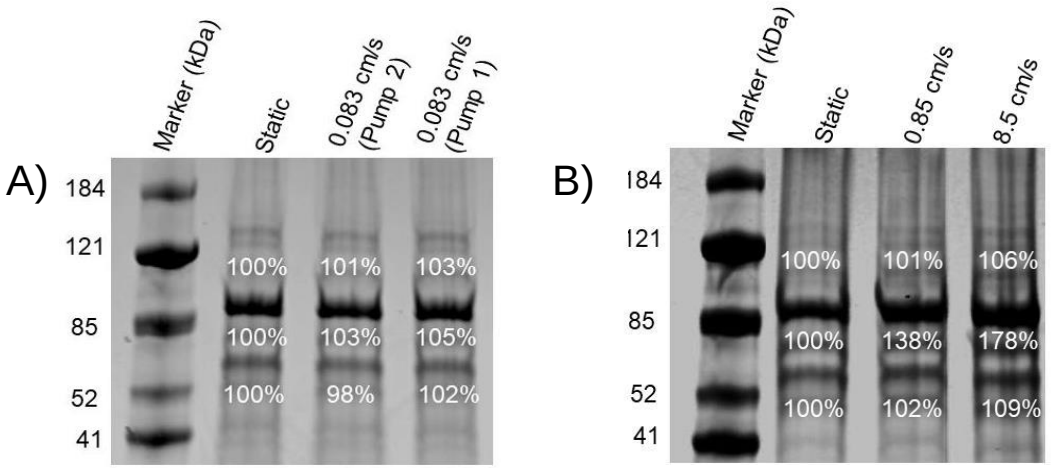

Figure S3. Control experiments to ensure results are independent of specific pump used or batch (lot) of FBS. Representative gels are shown with the values measured for that specific gel. Averages and standard deviations for triplicate experiments are reported in Tables S1 and S2. (A) Two pumps were used for these experiments. For faster flow rates (0.42, 0.85, 8.5 and 42.5 cm/s), a Harvard Apparatus peristaltic pump was used (#55-7777, "Pump 1," Harvard Apparatus, Holliston, MA). Slower flow rates (0.0085, 0.042 and 0.085 cm/s) used a second peristaltic pump (#724048, "Pump 2," Harvard Apparatus, Holliston, MA). As no changes in protein concentration were observed at slower speeds, which were all obtained using Pump 2, we wanted to ensure that Pump 1, rather than the faster speeds, was *not* responsible for the observed changes. Coronas were obtained using Pump 1 and Pump 2 at the same speed (0.083 cm/s) using methods identical to Figure 2. Identical coronas were obtained (representative gel and Table S1) demonstrating that the pump is not responsible for the altered corona. (B) To ensure that the results described in the main text were not specific to that batch of FBS (lot unknown), experiments were repeated with a new batch (Lot # 1927414) of FBS. Although it is possible that this lot is the same as our previous, untracked lot, we find this unlikely as the bottles of FBS were bought ~1 year apart. The same trends were observed with the new lot of FBS (compare to Table 1).

Table S1. Percent change in protein concentration as a function of pump.

Speed	Plasminogen (%)	BSA (%)	125 kDa (%)
Pump 1 (0.083 cm/s)	108 ± 6	103 ± 4	104 ± 2
Pump 2 (0.083 cm/s)	104 ± 5	102 ± 5	101 ± 5

Table S2. Percent change in protein concentration using FBS (Lot #1927414).

Speed	Plasminogen (%)	BSA (%)	125 kDa (%)
0.85 cm/s	136 ± 3	103 ± 2	105 ± 5
8.5 cm/s	181 ± 5	106 ± 3	108 ± 6

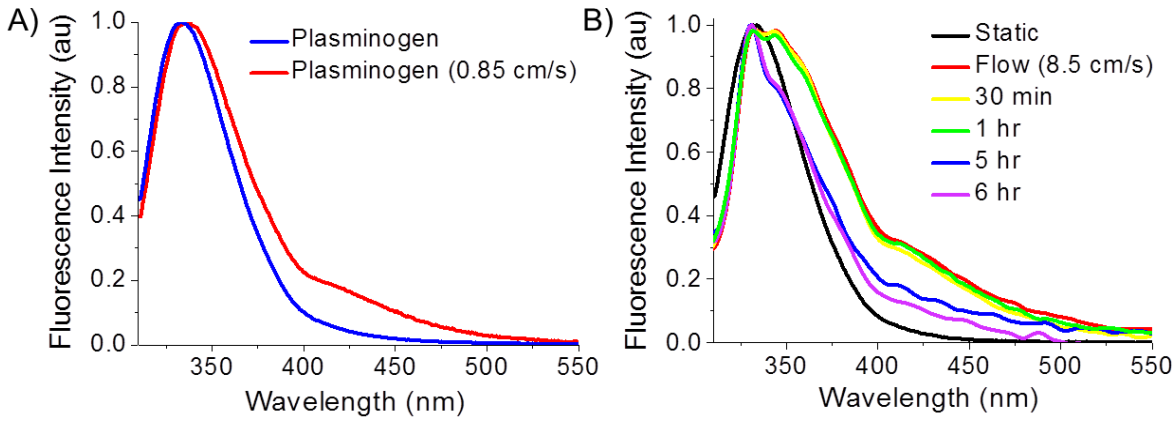

Figure S4. Fluorescence spectra of plasminogen. (A) Slower flow rates (0.85 cm/s) led to changes in fluorescence emission, similar to 8.5 cm/s (Fig. 3). (B) Following flow (8.5 cm/s), the fluorescence spectrum of plasminogen showed incomplete recovery over a 6 hr period (37 °C, PBS).

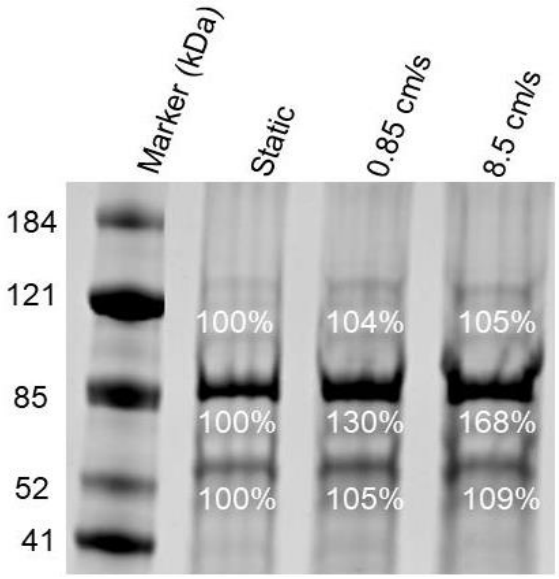

Figure S5. Flowing FBS in the absence of NPs (30 min) then using this FBS solution to form a protein corona under static conditions results in the same enrichment in plasminogen as a corona formed under flow. A representative gel is shown with the values measured for that specific gel. Averages and standard deviations for triplicate experiments are reported in Table S3.

Table S3. Percent change of proteins for a corona formed using FBS subjected to flow with the corona formed under static conditions.

Speed	Plasminogen (%)	BSA (%)	125 kDa (%)
0.85 cm/s	133 ± 6	103 ± 3	103 ± 5
8.5 cm/s	172 ± 7	107 ± 5	105 ± 8

Table S4. Characterization of hard corona-NPs with the corona formed under either static conditions (30 min, room temperature) or with constant vortexing (30 min, room temperature). Vortexing for 30 min results in a corona identical to that of the higher flow speeds (Fig. S2*C*). Hard corona-NPs (20 pM) were resuspended in water for DLS measurements, n=3.

hard corona-NPs	d_h (nm)	PDI	ZP (mV)
Static	312 ± 4	0.12 ± 0.02	-17 ± 3
Vortex	313 ± 4	0.14 ± 0.02	-18 ± 3

Table S5. Characterization of "yellow-green" NPs.

NPs	d_h (nm)	PDI	ZP (mV)	TEM (nm)
#F8811 (Lot: #28609W)	252 ± 4	0.14 ± 0.01	-51 ± 1	190 ± 10, n=50

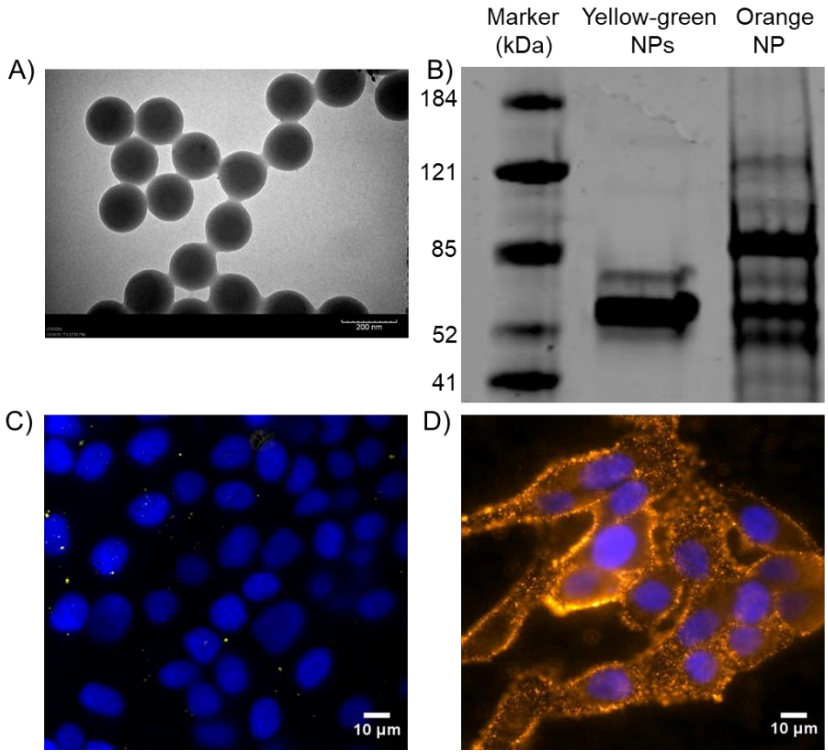

Figure S6. A comparison of carboxylate-modified polystyrene NPs ("orange," #F8809, 200 nm, Excite: 540 nm/Emit: 560 nm, ThermoFisher Scientific, Carlsbad, CA) used in the main text and carboxylate-modified polystyrene NPs ("yellow-green," #F8811, 200 nm, Excite: 505 nm/Emit: 515 nm, ThermoFisher Scientific, Carlsbad, CA) used in previous Payne Lab experiments (Ref. 6, 34, 46), show that small differences in NPs can lead to large differences in protein corona and, subsequently, cellular binding. (A) TEM (JEOL 100 CX II, 100 kV with 200 kX magnification) image of the "yellow-green" polystyrene NPs. (B) Gel electrophoresis shows that "yellow-green" carboxylate-modified polystyrene NPs form a protein corona dominated by albumin (66 kDa). The dominant corona protein on the "orange" NPs used in the current studies is plasminogen (88 kDa). (C) Imaging (IX-71 microscope, 60x water-immersion objective, 1.20 N.A., Olympus, with EMCCD camera, DU-897, Andor) the binding of NPs (20 pM) to HeLa cells confirms that cellular binding of the "yellow-green" NPs is blocked by free serum proteins, as observed previously with BS-C-1 and CHO cells (Ref. 6, 34, 46). NPs and FBS (10%) were added to cells for 20 minutes of cold-binding at 4 °C. Prior to imaging, cells were washed 3 times with PBS to remove excess NPs. Nuclei are stained with DAPI (blue, 27 μM). (D) Binding of the "orange" NPs is not blocked by serum proteins. Both imaging experiments were performed under static conditions in the absence of flow with cells at identical passage number. Future work will examine the NP differences responsible for these differences in protein corona.