Secondary Structure of Corona Proteins Determines the Cell Surface Receptors Used by Nanoparticles

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SUPPORTING INFORMATION

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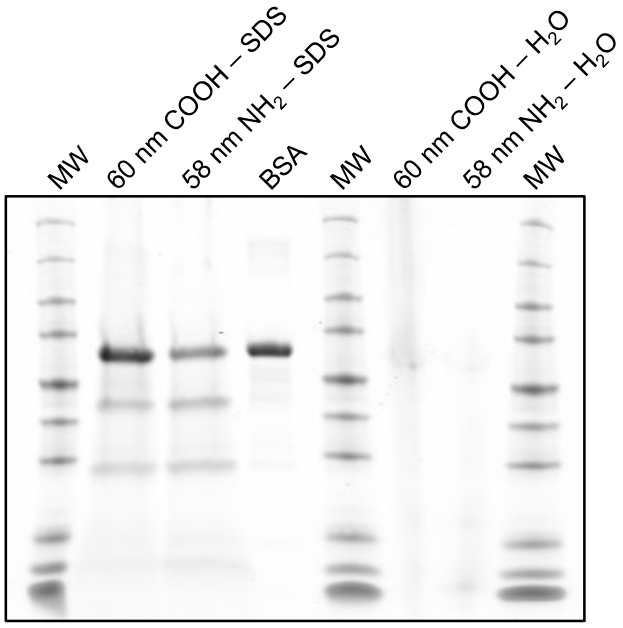

Figure S1. Gel electrophoresis of 60 nm carboxylate-modified (60 nm COOH) and 58 nm amine-modified (58 nm NH_2) NPs following incubation in MEM supplemented in 10 mg·mL^{-1} BSA for 10 minutes. Free BSA was removed from solution with four wash steps consisting of centrifugation, removal of supernatant, and resuspension in water. SDS was then used to remove the protein corona from the NP surface. Incubation of NPs in H_2O, rather than SDS, led to no visible protein. Molecular weight marker (MW) shows 225, 150, 100, 75, 50, 35, 25, 15, 10, and 5 kDa. For both anionic and cationic NPs, a protein band is present at ~66 kDa, the molecular weight of BSA. The protein observed following treatment with SDS is the corona.

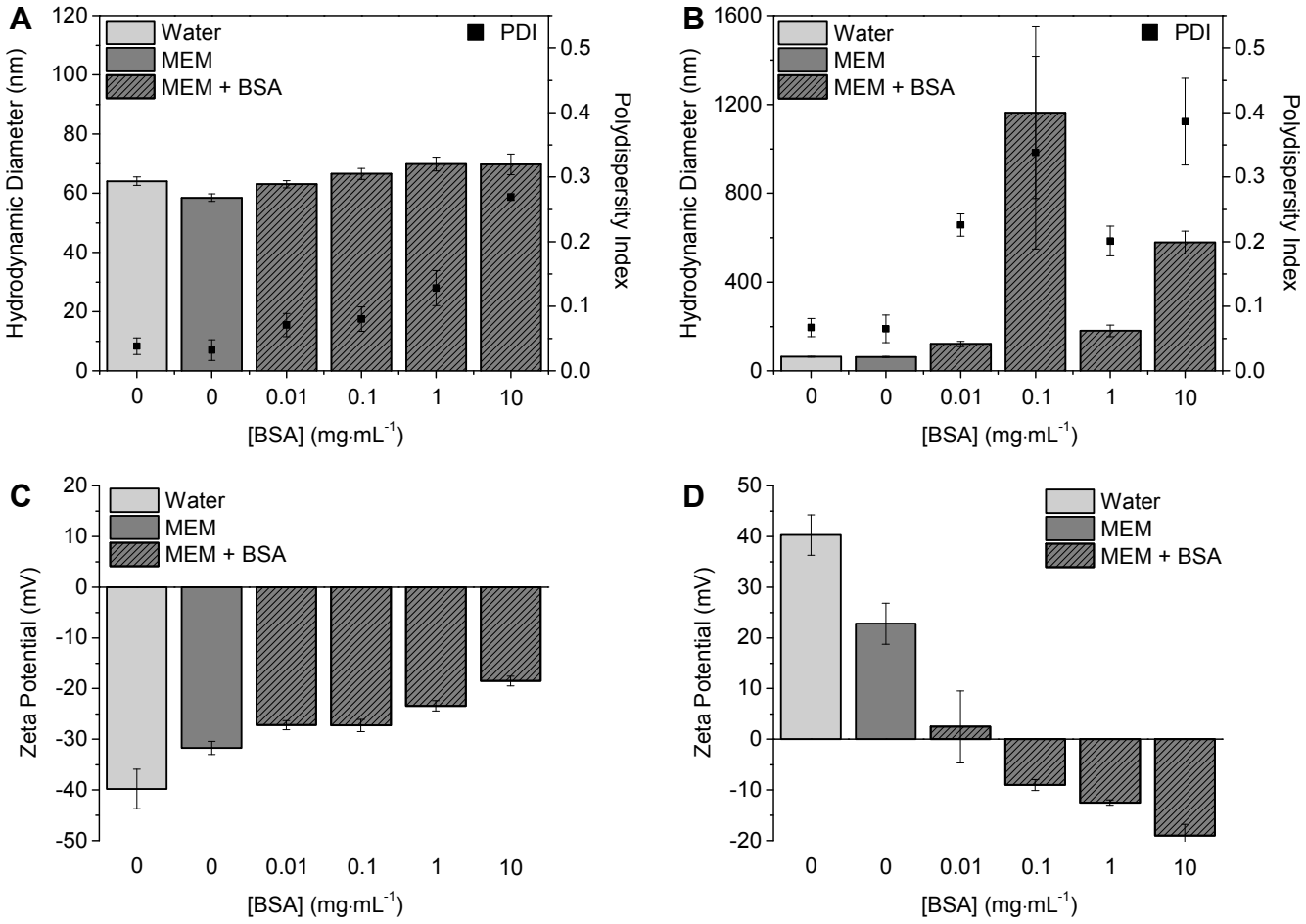

Figure S2. Dynamic light scattering and zeta potential measurements of 60 nm carboxylate-modified and 58 nm amine-modified NPs in the presence of increasing concentrations of BSA. Hydrodynamic diameter and polydispersity index (PDI) of (A) 60 nm carboxylate-modified and (B) 58 nm amine-modified NPs. Zeta potential of (C) 60 nm carboxylate-modified and (D) 58 nm amine-modified NPs. For the 60 nm anionic, carboxylate-modified NPs, increasing concentrations of BSA led to an increase in hydrodynamic diameter and slight aggregation of the NPs, indicated by the increasing PDI (Figure S2A). For the 58 nm cationic, amine-modified NPs, the addition of BSA led to a significantly larger increase in both hydrodynamic diameter and PDI (Figure S2B). These large aggregates are not observed in cell experiments as they are likely removed, along with unbound NPs, during the wash steps prior to analysis. Both anionic and cationic NPs are net anionic in the presence of 10 mg·mL^{-1}. The increased diameter and change in zeta potential support the gel electrophoresis experiments (Figure S1) showing the formation of a BSA corona on both anionic and cationic NPs.

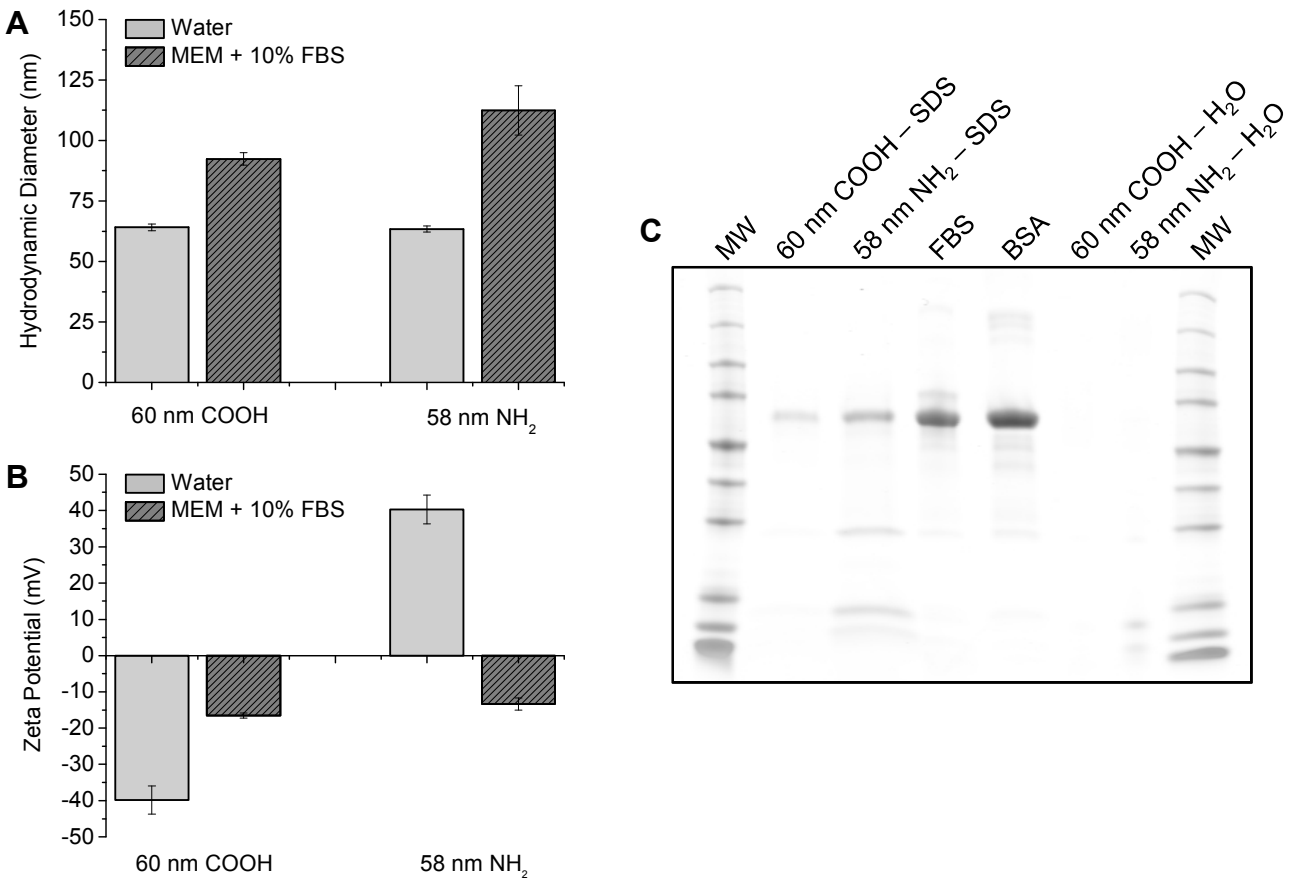

Figure S3. Dynamic light scattering and gel electrophoresis of 60 nm carboxylate-modified (60 nm COOH) and 58 nm amine-modified (58 nm NH_2) NPs in water and following incubation in MEM supplemented with 10% (v/v) FBS. (A) Hydrodynamic diameter. NPs incubated in FBS are larger than NPs incubated in BSA (Figure S2), suggesting that other proteins and components in FBS contribute to an increased hydrodynamic diameter. (B) Zeta potential. (C) SDS-PAGE of 60 nm and 58 nm NPs after four wash steps with centrifugation and resuspension in water. The final pellet was resuspended in either SDS or water. Molecular weight marker (MW) shows 225, 150, 100, 75, 50, 35, 25, 15, 10, and 5 kDa. A protein band is present at ~66 kDa, demonstrating that for FBS, the only protein detected in the corona is BSA. This supports the use of isolated BSA as a representative serum protein.

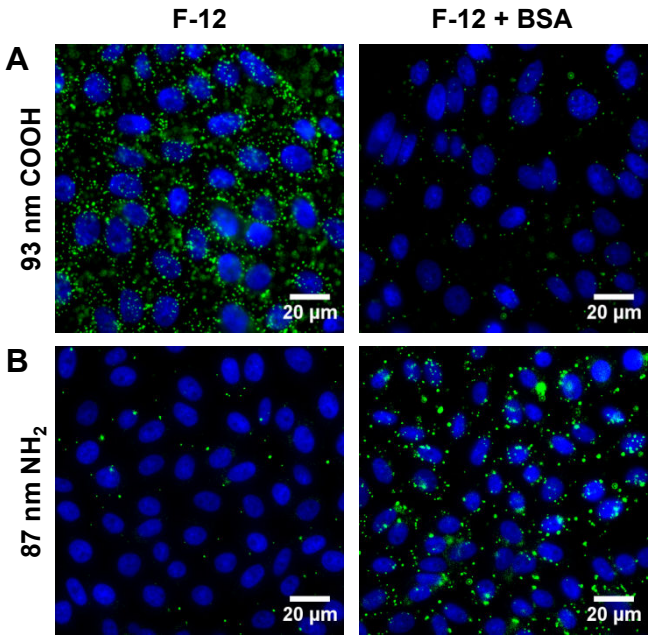

Figure S4. Fluorescence microscopy images show cellular binding of NPs (green) in F-12 media and F-12 media supplemented with 10 mg·mL^{-1} BSA (F-12 + BSA) to Chinese hamster ovary cells (CHO) at 4 °C. F-12 is the medium used to culture CHO cells. (A) 93 nm carboxylate-modified NPs. (B) 87 nm amine-modified NPs. Nuclei are stained with DAPI (blue). Cellular binding is consistent with experiments performed using BS-C-1 cells (Figure 1) indicating that results are not cell type dependent.

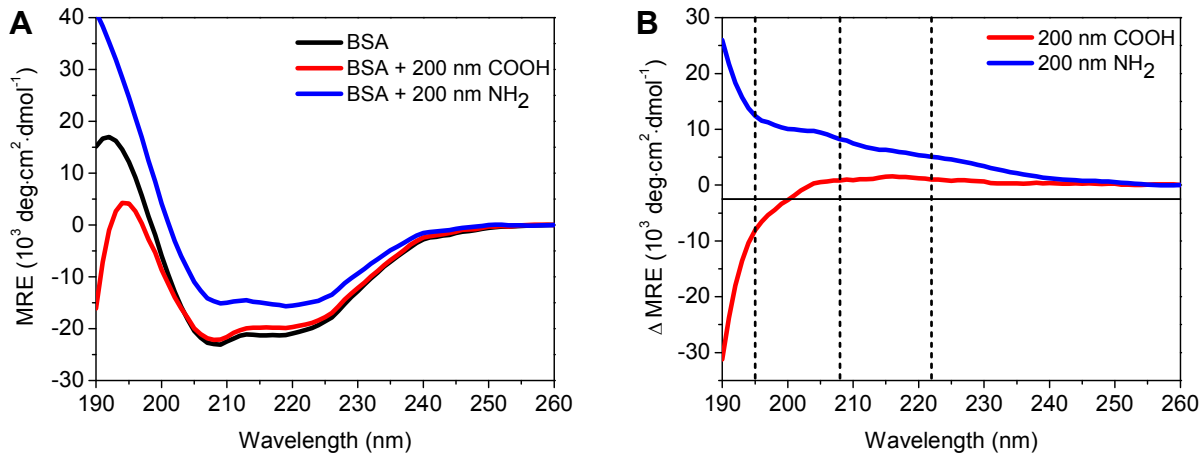

Figure S5. CD spectra of BSA in the presence of 200 nm carboxylate-modified NPs (red), 200 nm amine-modified NPs (blue), and in the absence of NPs (black). Spectra, in units of mean residue ellipticity (MRE), are the average of 10 consecutive scans. (A) Raw CD spectra. (B) CD difference spectra were calculated by subtracting the spectrum of BSA from BSA in the presence of 200 nm carboxylate-modified (red) or 200 nm amine-modified NPs (blue). Black dashed lines correspond to spectral peaks at 195, 208, and 222 nm. Difference spectra demonstrate that the change in BSA secondary structure in response to carboxylate-modified NPs is minimal (red), while amine-modified NPs lead to a greater loss in protein secondary structure (blue). Spectral changes are similar to those observed with 60 nm and 58 nm NPs (Figure 3).

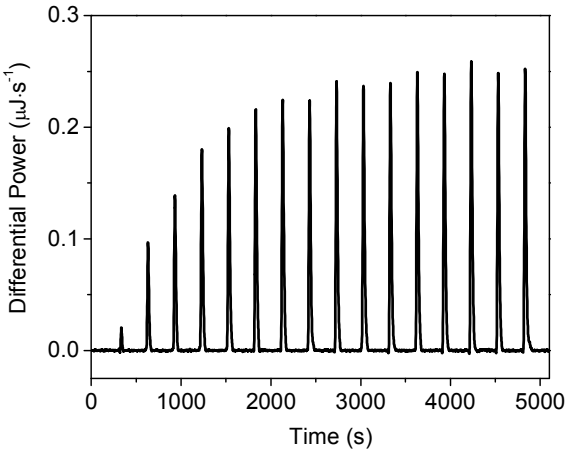

Figure S6. Isothermal titration calorimetry (ITC) plot of BSA into buffer in the absence of NPs. Peaks were subtracted injection-by-injection from the titration of BSA into a solution containing NPs.

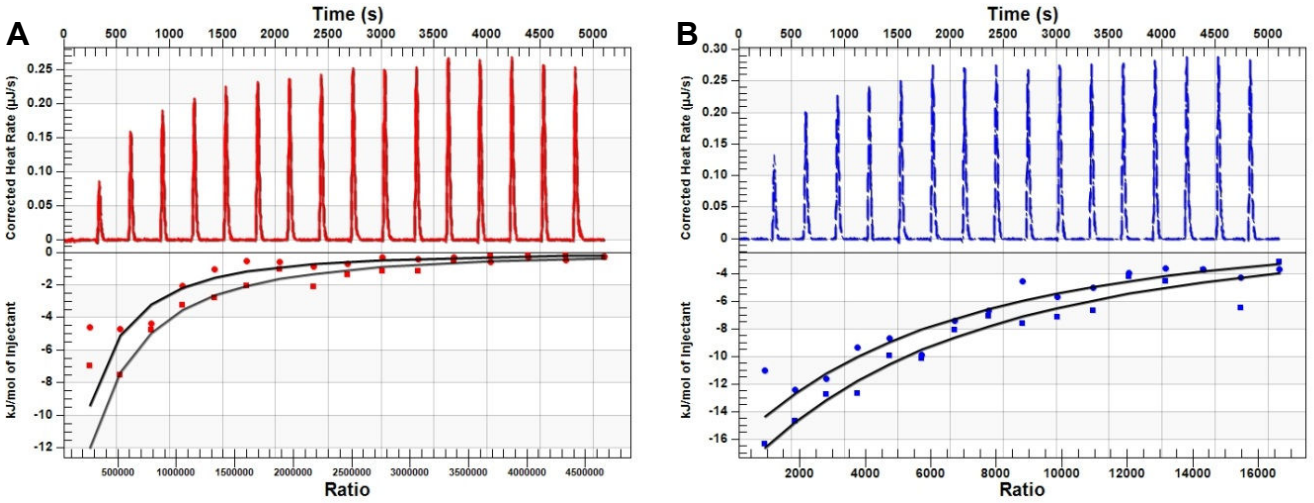

Figure S7. Isothermal titration calorimetry (ITC) plots of differential power throughout the titration (top) and integrated heat as a function of the mole ratio of BSA binding to NPs (bottom) displayed using the TA Instruments software, NanoAnalyze. The heat of dilution of BSA into buffer (Figure S6) was subtracted for each injection from the heat of BSA titrated into a NP solution. (A) BSA titrated into a solution of 60 nm carboxylate-modified NPs. (B) BSA titrated into a solution of 58 nm amine-modified NPs. The titration curve is more evident in the bottom plot of integrated heat values, as the enthalpy of BSA dilution into buffer has been subtracted from the titration of BSA into NPs.

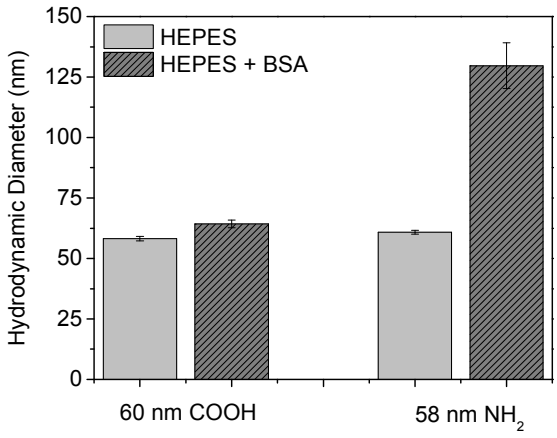

Figure S8. Hydrodynamic diameter of 60 nm carboxylate-modified (60 nm COOH) and 58 nm amine-modified (58 nm NH_2) NPs in 20 mM HEPES buffer and in 20 mM HEPES buffer supplemented with 0.6 mg·mL^{-1} BSA. Experiments were carried out in triplicate. NP solutions were prepared under the same conditions used for isothermal titration calorimetry experiments. The 60 nm carboxylate-modified NPs increased in size by ~6 nm after the addition of BSA. The 58 nm amine-modified NPs doubled in hydrodynamic diameter, indicative of NP dimer formation.