Supporting Information for:

Lipid Adsorbed on the Surface of Nanoparticles Enhances Protein Corona Formation

Morgan M. Heckman,[†] Catherine Cai,[†] Abhishek Kalpattu,[†] Emily M. Simmons,[†] Robert M. Tighe,[§,] and Christine K. Payne[†,*]

[†]Thomas Lord Department of Mechanical Engineering and Materials Science, Duke University, Durham, North Carolina, USA 27708
[§]Department of Medicine, Duke University School of Medicine, Durham, North Carolina, USA 27710

*christine.payne@duke.edu

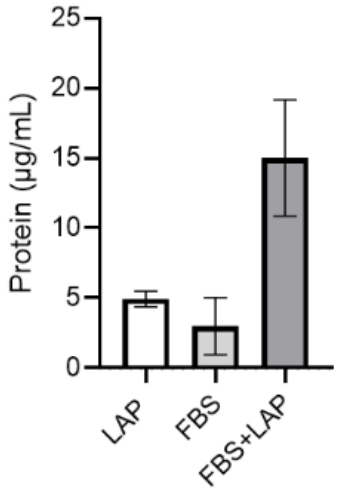

Fig. S1 LAP (1.8 mg/mL), FBS (40 mg/mL), and a mixture of FBS (40 mg/mL) and LAP (1.8 mg/mL) (FBS+LAP), in the absence of nanomaterials, were suspended in PBS and treated identically to samples used to form coronas (centrifugation and resuspension x3). The resulting pellet contains both lipid and protein. We measured the protein concentration of the resulting pellets, including LAP alone, which results in a small false positive (4.9 ± 0.6 μg/mL) just at the limit of detection of the protein assay (5 μg/mL). Centrifugation of FBS alone does not result in a visible pellet. Measuring the solution at the bottom of the tube yields a value (3.0 ± 2 μg/mL) below the limit of detection of the protein assay (5 μg/mL). A larger concentration of FBS is pelleted when LAP is present (FBS+LAP = 15 ± 4 μg/mL). This value includes both the protein concentration and the false positive from LAP.

To remove this centrifugation artifact from the corona data of individual nanomaterials, we define the protein component of the artifact as FBS+LAP (15 ± 4 μg/mL) – LAP (4.9 ± 0.6 μg/mL). This protein value (10 ± 5 μg/mL) is subtracted from the total FBS+LAP signal. We observed that different nanomaterials result in different amounts of lipid pelleted during the wash process. Some of this lipid is likely free lipid, but there may also be lipid associated with nanomaterials in the form of the lipid corona. For this reason, an individual nanomaterial lipid (false positive) was also subtracted from FBS+LAP values (Table S1). The data handling of each sample is described throughout the text.

An identical method is used to remove the centrifugation artifact from the BSA and transferrin data with a protein value of BSA = 4.5 ± 0.7 μg/mL and transferrin = 0.4 ± 0.5 μg/mL and the same lipid signal (false positive) as the FBS experiments (Table S1).

Table S1 The formation of coronas using LAP alone generates a small positive signal from the protein assay. This signal can be due to both LAP pelleted during the centrifugation process and LAP forming a lipid corona on the nanomaterial. This value, for each nanomaterial, was subtracted from the FBS+LAP data in Figure 3 and Figure 4. The limit of detection of the protein assay is 5 μg/mL.

Nanomaterial	LAP-generated protein signal (μg/mL)
TiO_2	1.94 ± 0.16
SiO_2	9.88 ± 0.61
Ag	1.78 ± 0.10
MWCNT	9.34 ± 0.62

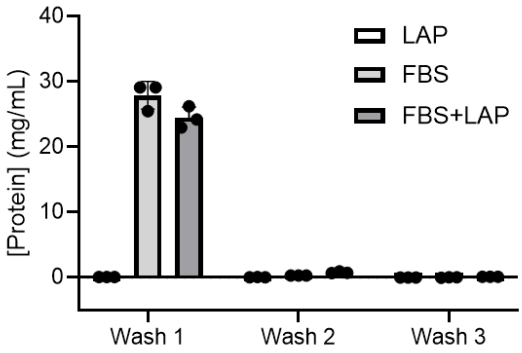

Fig. S2 The protein concentration in the wash (supernatant) was measured during the magnetic separation (pull-down) of unbound biomolecules from biomolecule-magNP complexes. After 3 wash steps, no lipid (false positive) or FBS is detected in the supernatant of coronas made from LAP or FBS. The signal for FBS+LAP is 89 μg/mL, approaching the limit of detection of this assay (50 μg/mL).

Table S2 To determine the concentration of magNPs lost during the pull-down process, we measured the concentration of magNPs remaining in the supernatant following each wash step. These measurements were carried out in the absence of biomolecules. MagNPs were suspended and resuspended in PBS (1 mg/mL) in 1.5 mL centrifuge tubes and placed against a magnet (Grade 12, 10 mm) for 5 min at room temperature to pull-down the magNPs. The concentration of magNPs in the supernatant at each wash step was measured by UV-Vis spectrophotometry (UV7, Mettler-Toledo, Columbus, OH) as described previously.[40] Absorbance of the magNPs was measured at 330 nm and compared to a calibration curve generated from known concentrations of magNPs. We measure a loss of 25 μg/mL from a 1 mg/mL solution of magNPs and use the value of 975 μg/mL to calculate final ratios of protein or lipid to magNPs.

Wash Step	[magNP] (μg/mL)
1	13 ± 3.8
2	6.5 ± 0.4
3	5.8 ± 0.6
Total	25 ± 4.0

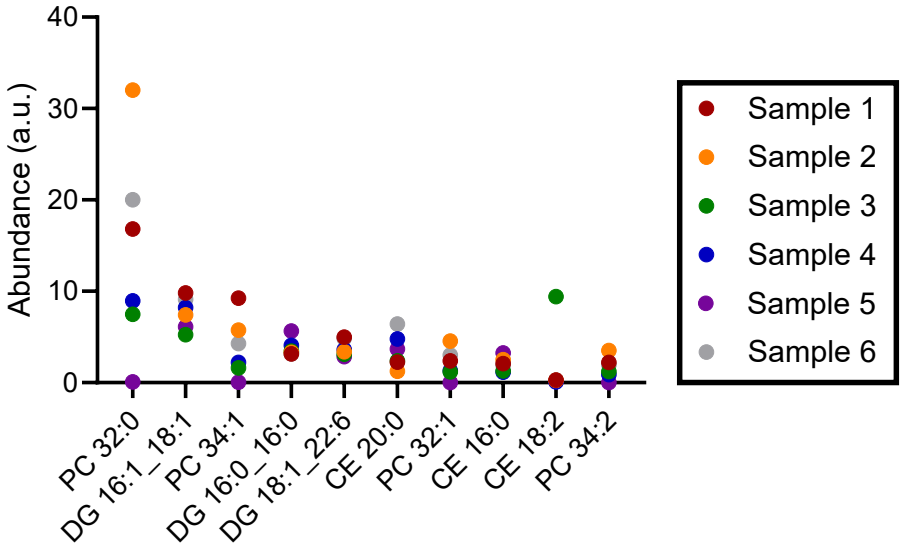

Fig. S3 Human BALF lipid composition. The top 10 most abundant lipids are shown, measured in six distinct male human BALF samples. Phosphatidylcholines (PC), diglycerides (DG), and cholesteryl esters (CE) are the three categories of lipids present in the top ten. Numbers in the lipid name indicate the number of carbon atoms and the number of double bonds, respectively.

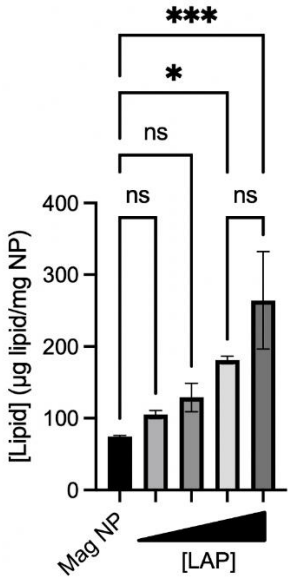

Fig. S4 LAP concentration was measured for bare magNPs, in the absence of lipids, and coronas formed on the magNPs following incubation with LAP (0.45, 0.9, 1.8, and 3.6 mg/mL). At 1.8 mg/mL LAP, a significant difference is observed between the LAP corona and the bare magNPs. No significant increase is observed at the higher concentration of 3.6 mg/mL. The bare magNPs interact with the dye used in the lipid assay resulting in a small positive signal. This signal was not subtracted from the LAP data shown here. The limit of detection for the lipid assay is 50 µg lipid/mg NP. The lipid assay was carried out for n = 3 distinct samples. Significance was determined using a one-way ANOVA. * $p<0.05$, *** $p<0.001$.

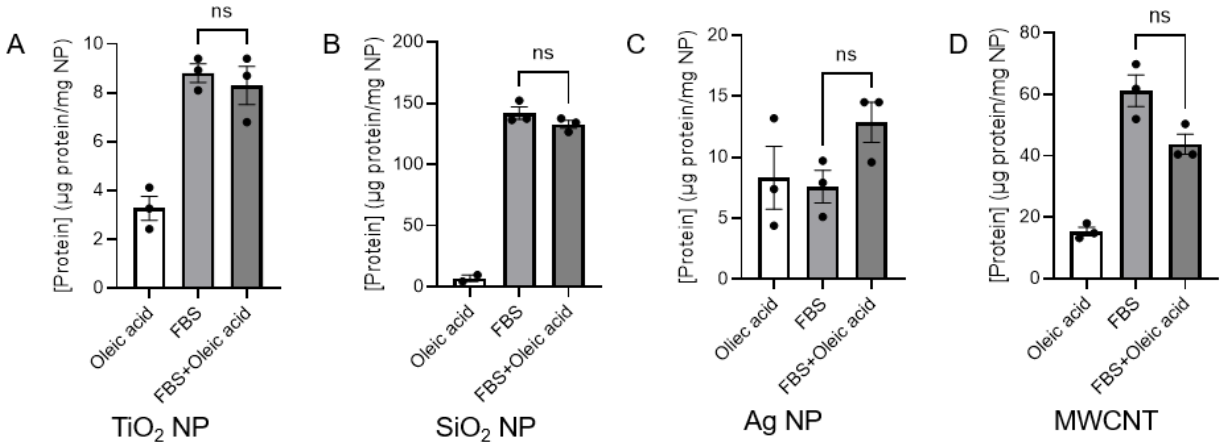

Fig. S5 Concentration of protein (FBS; 40 mg/mL) adsorbed on the surface of nanomaterials with and without oleic acid (1.8 mg/mL) present during corona formation. A corona formed from oleic acid alone (Oleic Acid), in the absence of protein, generates a small positive signal from the protein assay. If the signal from oleic acid was removed from the FBS+Oleic Acid data, it suggests that oleic acid may decrease the protein in the corona. The limit of detection for the protein assay is 5 μg protein/mg NP. Oleic acid does not lead to the pelleting of protein observed with LAP and no values are subtracted. n = 3 distinct samples. Significance between FBS and FBS+Oleic Acid was determined using a one-way ANOVA. ns = non-significant.

Table S3 Concentration of endogenous lipid present in FBS measured with the colorimetric lipid assay described in Materials and Methods. No nanomaterials were present in these samples.

FBS sample	[Lipid] (mg/mL)
1	1.01
2	1.18
3	1.04
Total	1.08 ± 0.09

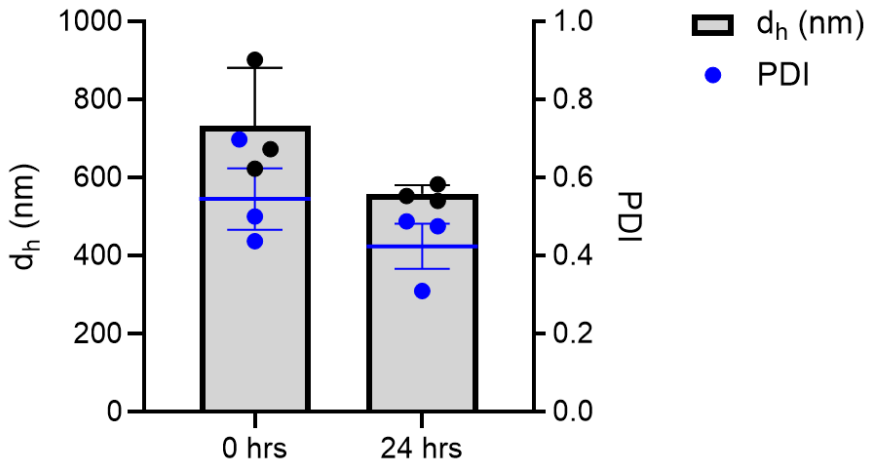

Fig. S6 Dynamic light scattering (DLS) was used to measure the diameter (d_h) and polydispersity index (PDI) of Ag NPs in a freshly made sample (1 mg/mL in water) and after 24 hrs. The decrease in diameter may suggest Ag NP dissolution.