

Supplementary Information:

Interaction of TiO₂ nanoparticles with lung fluid proteins and the resulting macrophage inflammatory response

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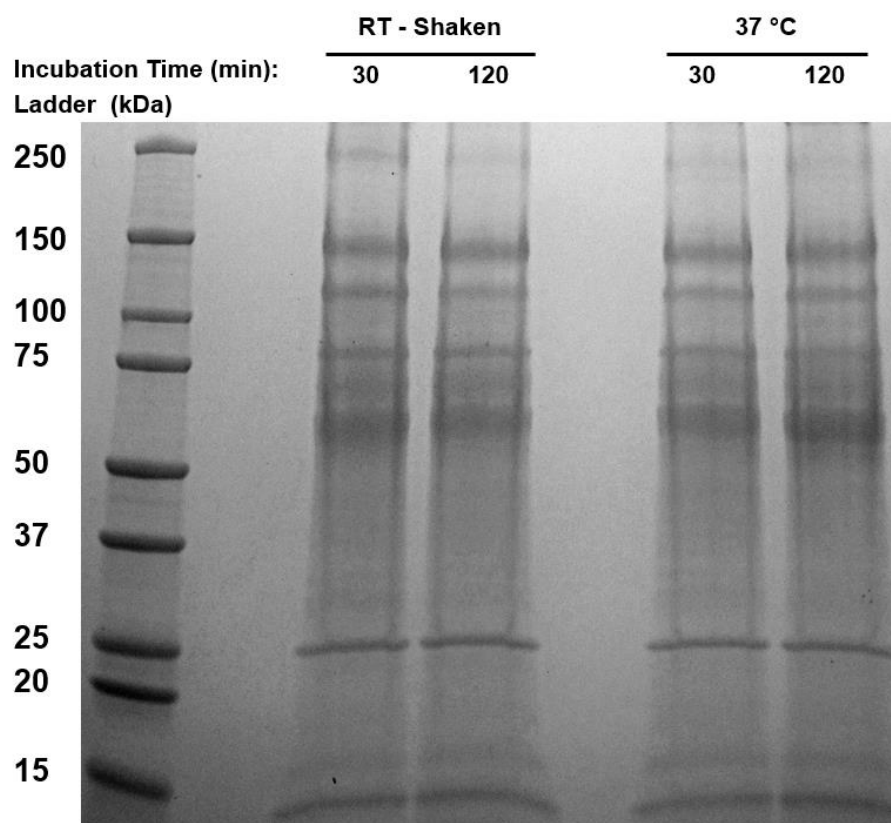


Fig. S1 Gel electrophoresis showing the proteins present in coronas formed from FBS (10%) on TiO₂ NPs at room temperature (RT; ~22 °C) and 37 °C with incubation times of 30 min and 120 min. The samples at RT were incubated on an orbital shaker. The samples incubated at 37 °C were static. The 30 min, RT-shaken condition is identical to the preparation of samples as described in the main text.

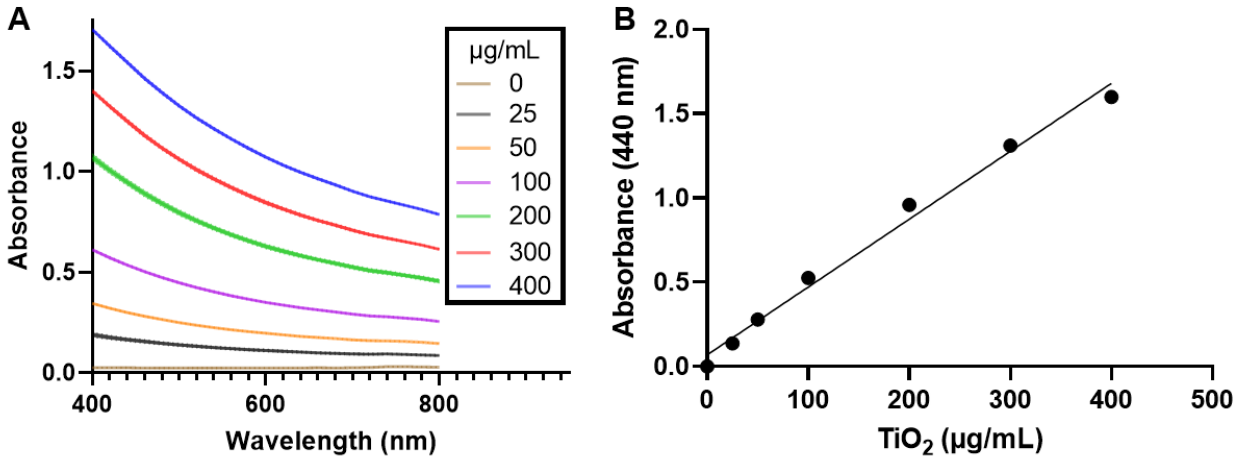


Fig. S2 The concentration of TiO₂ NPs was determined using a calibration curve. (A) Known concentrations of TiO₂ NPs (0-400 µg/mL) were suspended in PBS. Absorption spectra were measured using a plate reader (SpectraMax, iD3, Molecular Devices, San Jose, CA). Solid lines show the mean absorbance and shading shows standard deviation (n=3). (B) TiO₂ NP absorbance was measured at 440 nm. Error bars showing standard deviation are too small to see. Linear regression was performed in GraphPad Prism. R² = 0.989. The limit of detection was determined to be 50 µg/mL. A minimum concentration of 250 µg/mL was used for experiments.

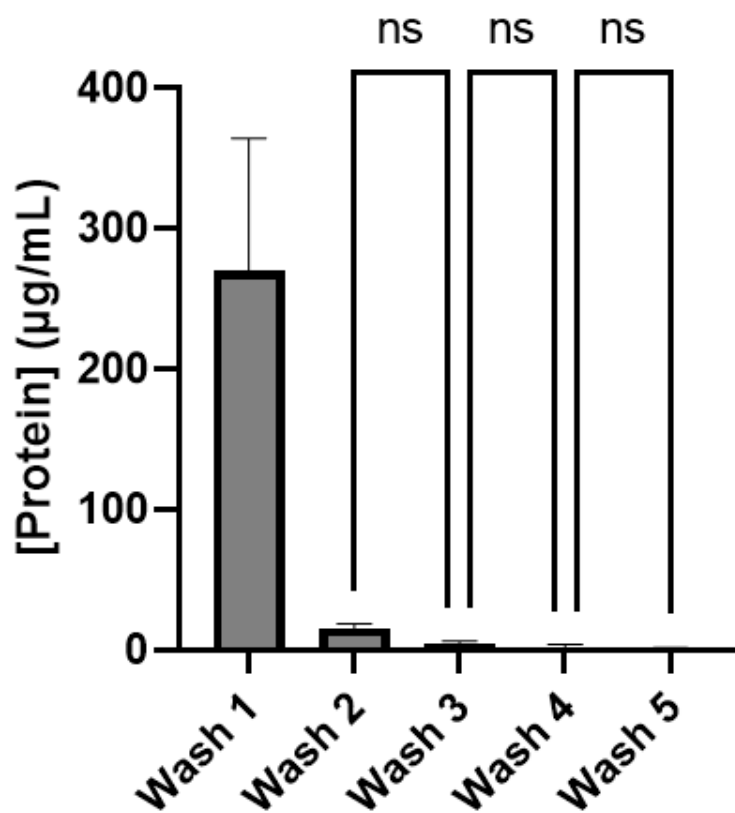


Fig. S3 Concentration of protein present in the supernatant following “washing” of protein-TiO₂ NP complexes incubated with BSA (10 mg/mL). TiO₂ NPs were incubated with BSA for 30 min at RT and washed with PBS (n=3). BSA (10 mg/mL) is used as it is the highest incubation concentration used for protein corona formation. The first supernatant, Wash 0, is not shown because it has much higher protein concentration and is not typically measured. Error bars show standard deviation. The limit of detection of the BCA assay is 69 µg/mL. Significance was determined using a one-way ANOVA with a post hoc Tukey test.

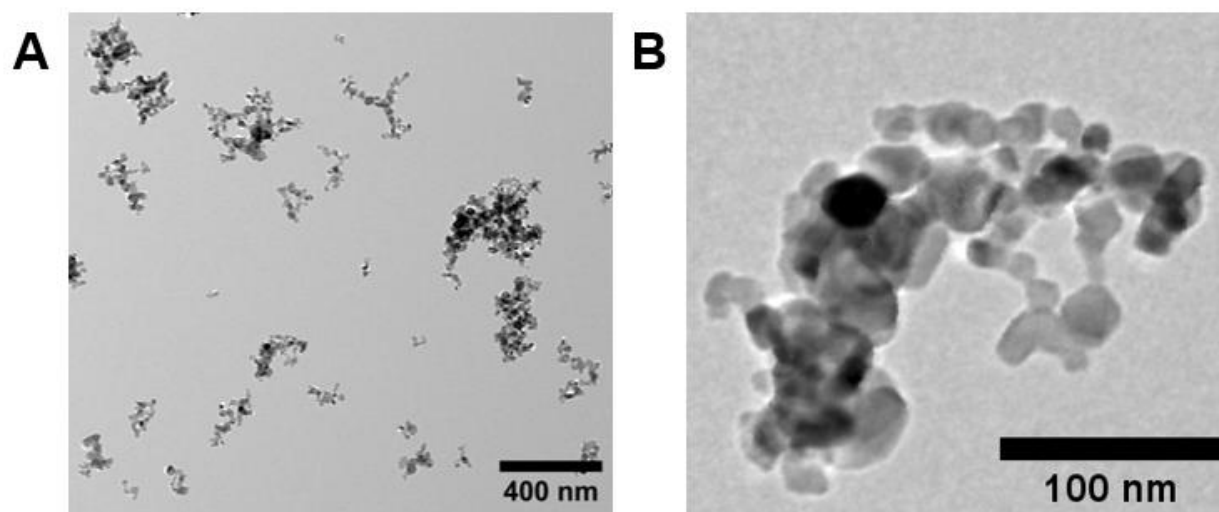


Fig. S4 Representative transmission electron microscopy (TEM) image (FEI Tecnai G2 Twin, 160 kV, 14.5 kX) of the TiO_2 NPs used in these experiments. TiO_2 NPs were suspended in ultrapure water by sonication (5 min, RT; Qsonica) and dried on a 400 mesh copper grid (#CF400-Cu, Electron Microscopy Sciences, Hatfield Township, PA).

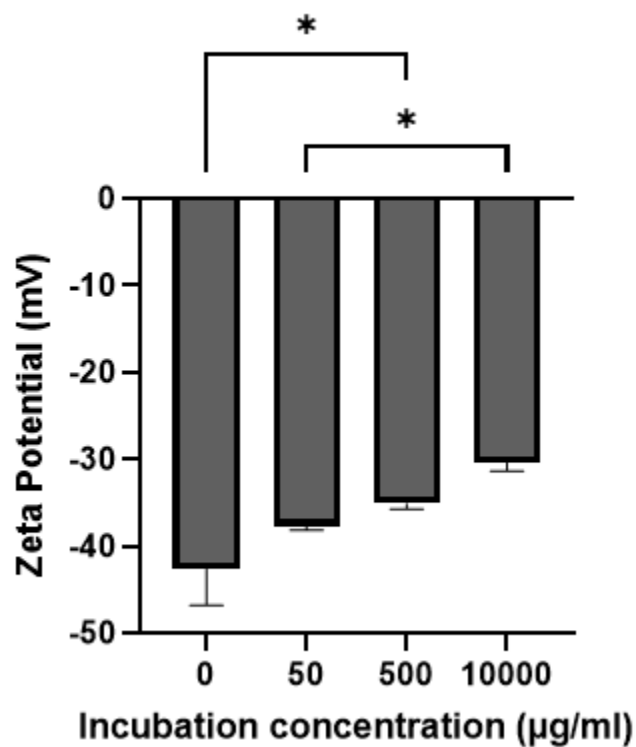


Fig. S5 Increasing the amount of BSA present during the formation of the protein corona increases the zeta potential of the protein-TiO₂ NP complexes. Experiments were carried out in triplicate. Error bars show standard deviation. Significance was determined using a one-way ANOVA with a post hoc Tukey test. *p<0.05.

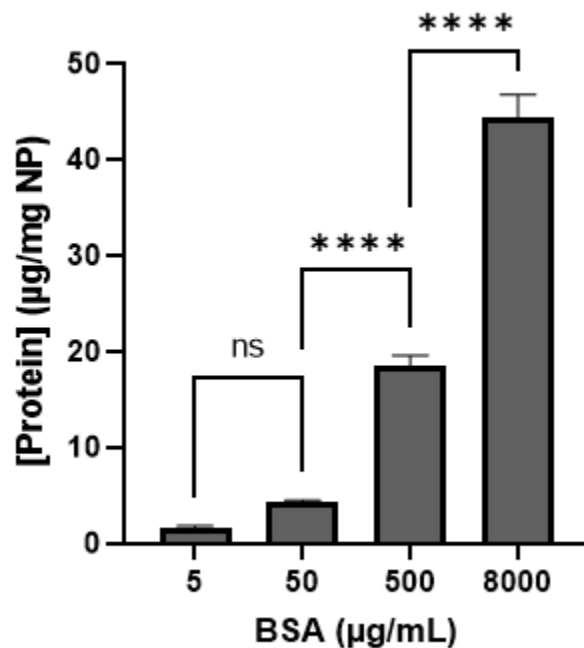


Fig. S6 Increasing the amount of BSA (5 µg/mL – 8000 µg/mL) present during the formation of the protein corona increases the concentration of protein present in the corona formed on TiO₂ NPs. Experiments were carried out in triplicate. Error bars show standard deviation. Significance was determined using a one-way ANOVA with a post hoc Tukey test. ****p<0.0001.

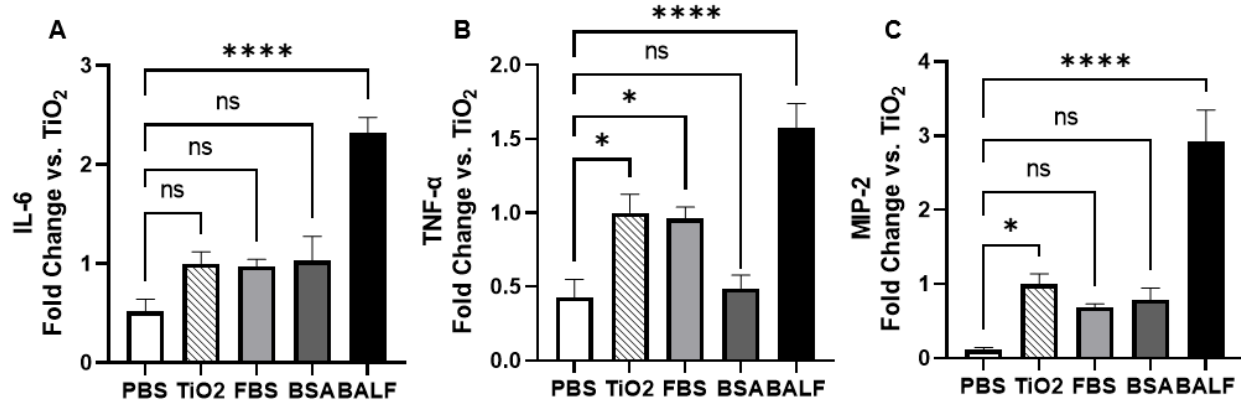


Fig. S7 (A) IL-6, (B) TNF- α , and (C) MIP-2 showed elevated expression levels in response to TiO₂ NPs with and without proteins coronas compared to the use of PBS as vehicle control. Significance was determined using a one-way ANOVA with a post hoc Tukey test. This is the same dataset and one way ANOVA as shown in Fig. 4. * $p < 0.05$, **** $p < 0.0001$.