Electronic Supplementary Information for:

Nanoparticle-induced oxidation of corona proteins initiates an oxidative stress response in cells

Dhanya T. Jayaram,[a,‡] *Sabiha Runa*,[a,‡] *Melissa L. Kemp*[b,c] and *Christine K. Payne*[a,c,*]

[a]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332, USA. [b]The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA 30332, USA. [c]Parker H. Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA 30332, USA.

*e-mail: christine.payne@chemistry.gatech.edu

‡ these authors contributed equally to this work.

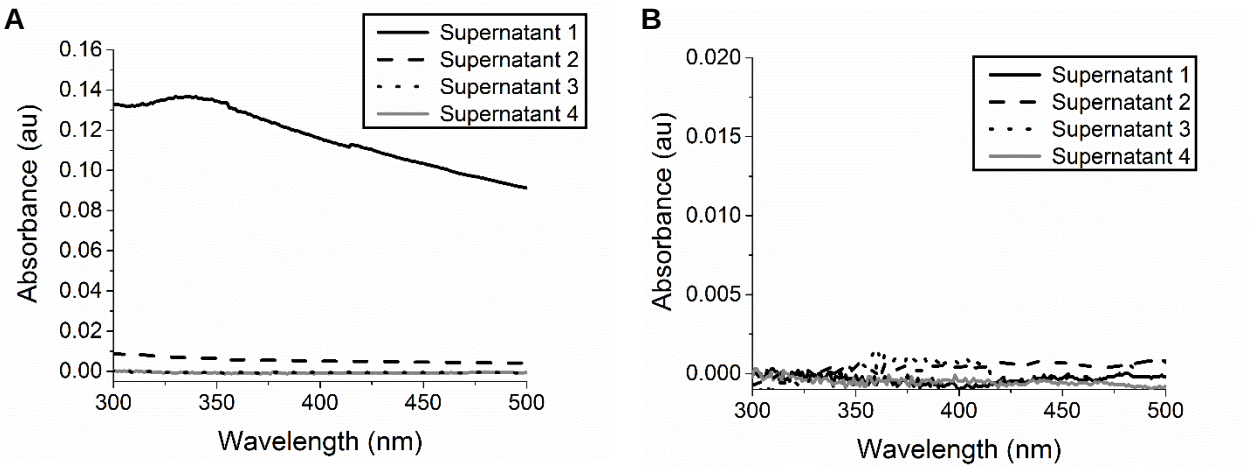

Fig. S1 TiO_2 NP removal by centrifugation and concentration determination. (A) P25 and (B) E171 TiO_2 NPs were removed from solutions prior to light-based measurements using repeated (3x) centrifugation (8,000 rcf, 15 min). Supernatant 1 is the supernatant following the first centrifugation step. After 3 rounds (Supernatant 3) of centrifugation, no NPs are detected. Supernatant 3 was used for measurements. Supernatant 4 is shown for comparison.

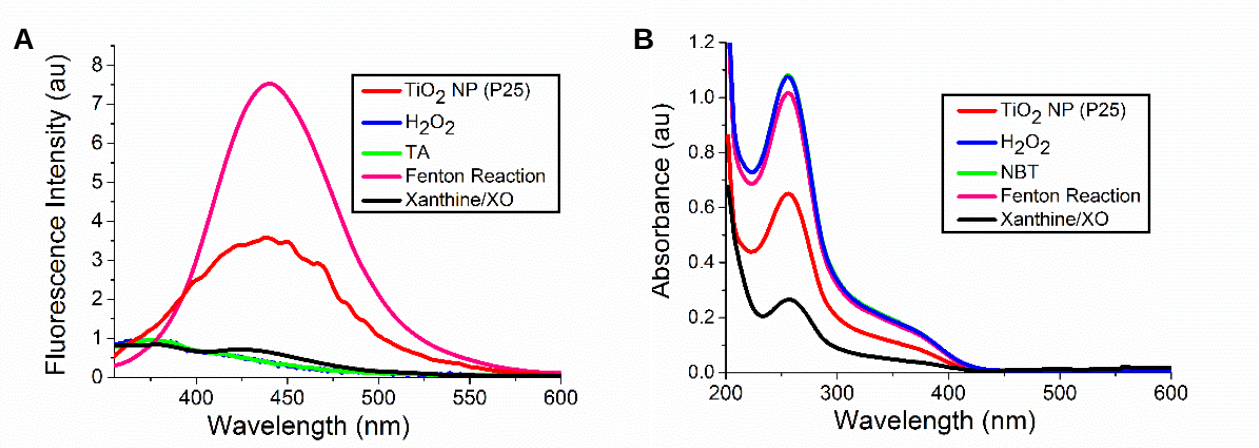

Fig. S2 TA and NBT assays are specific to hydroxyl radicals and superoxide, respectively. (A) The selectivity of TA was confirmed using Fenton-generated hydroxyl radicals ($CuSO_4$ (10 μM) and H_2O_2 (1 mM), pink), H_2O_2 (blue), and xanthine (300 μM)/xanthine oxidase (XO, 0.01 U/mL)-generated superoxide (black). No emission was observed from TA alone (2 mM, green). (B) The selectivity of NBT was confirmed using xanthine (300 μM)/xanthine oxidase (XO, 0.01 U/mL)-generated superoxide (black), Fenton-generated hydroxyl radicals ($CuSO_4$ (10 μM) and H_2O_2 (1 mM), pink), and H_2O_2 (blue). No change was observed from NBT alone (2 mM, green). For both assays, measurements were made after a 1 hr incubation with TiO_2 NPs or the relevant reagent. TiO_2 NPs were removed (8000 rcf for 15 min) from the solution prior to measurement. TiO_2 NP (1 mg/mL, red), TA, and NBT data are replotted from Figure 1 for comparison.

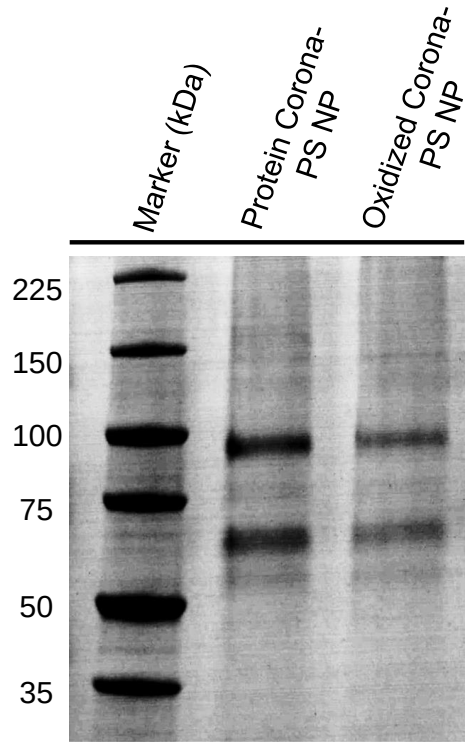

Fig. S3 Gel electrophoresis was used to compare the corona formed on the surface of polystyrene (PS) NPs using FBS (unoxidized) or FBS oxidized by TiO_2 NPs, as described in Section 2.8. To analyze the corona, unbound and weakly bound proteins were first removed from the protein-NP mixture by centrifugation (8,000 rcf, 15 min, x3). A detergent, SDS (Laemmli loading buffer, #BP-110R, Boston BioProducts, Ashland, MA; 5 min, vortexing) was used to remove the hard corona. Protein samples were heated for 5 min at 100 ºC and then loaded onto a gel (tris-glycine SDS gel, #456-1094, Bio-Rad, Hercules, CA) for SDS-PAGE (230 V, 35 min). A 5-225 kDa molecular weight marker (Lonza ProSieve Unstained Protein Marker, #50547, VWR, Rockland, ME) was included. Gels were stained for 1 hr (SimplyBlue Safe Stain, #LC6060, ThermoFisher) and then imaged. Densitometric analysis (Image J; http://rsb.info.nih.gov/ij/) was used to compare the two protein coronas. Analysis was carried out for 3 distinct samples, a representative gel is shown. No differences between the two coronas were observed.

		O 1s		
		O1	O2	O3
TiO_2 NP (P25)	B.E. (eV)	530.0	531.3	533.2
	Area (%)	73.3	11.5	15.2
Plasma-treated	B.E. (eV)	530.6	531.0	536.3
	Area (%)	74.2	23.3	2.5
Passivated	B.E. (eV)	530.2	531.7	532.5
	Area (%)	35.9	0.7	63.3

Table S1. XPS characterization of surface-modified TiO_2 NPs. Binding energies (B.E.) and % area under the curve are shown for unmodified, plasma-treated, and surface passivated TiO_2 NPs.

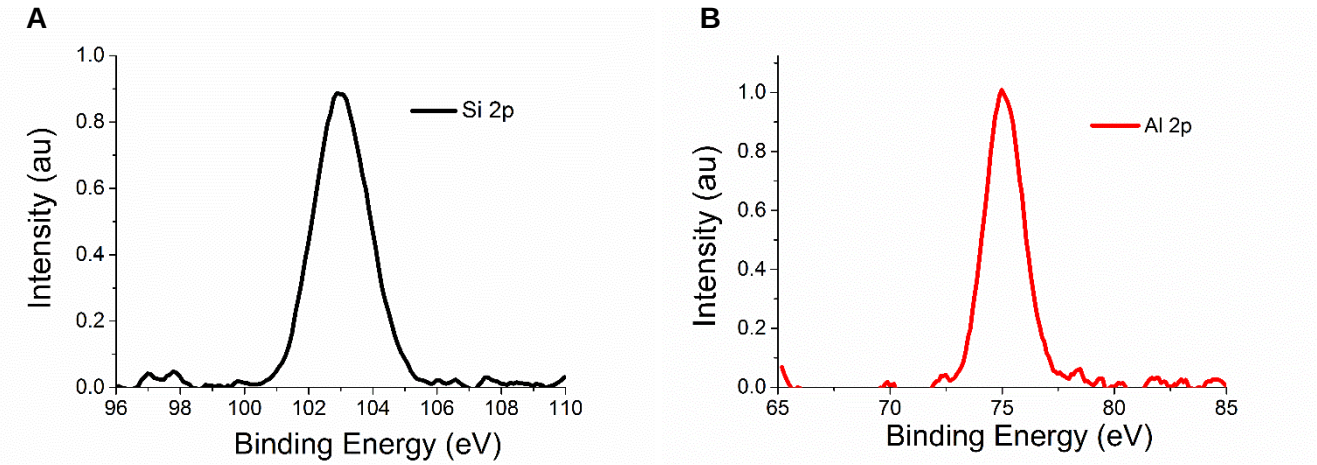

Fig. S4 XPS of surface passivated TiO_2 NPs. XPS measured for (A) Si 2p and (B) Al 2p confirms that TiO_2 NPs (P25) were passivated with a silica-aluminum shell on the NP surface. Measurements were carried on 4 distinct samples (2 mg/mL, dried), representative spectra are shown.

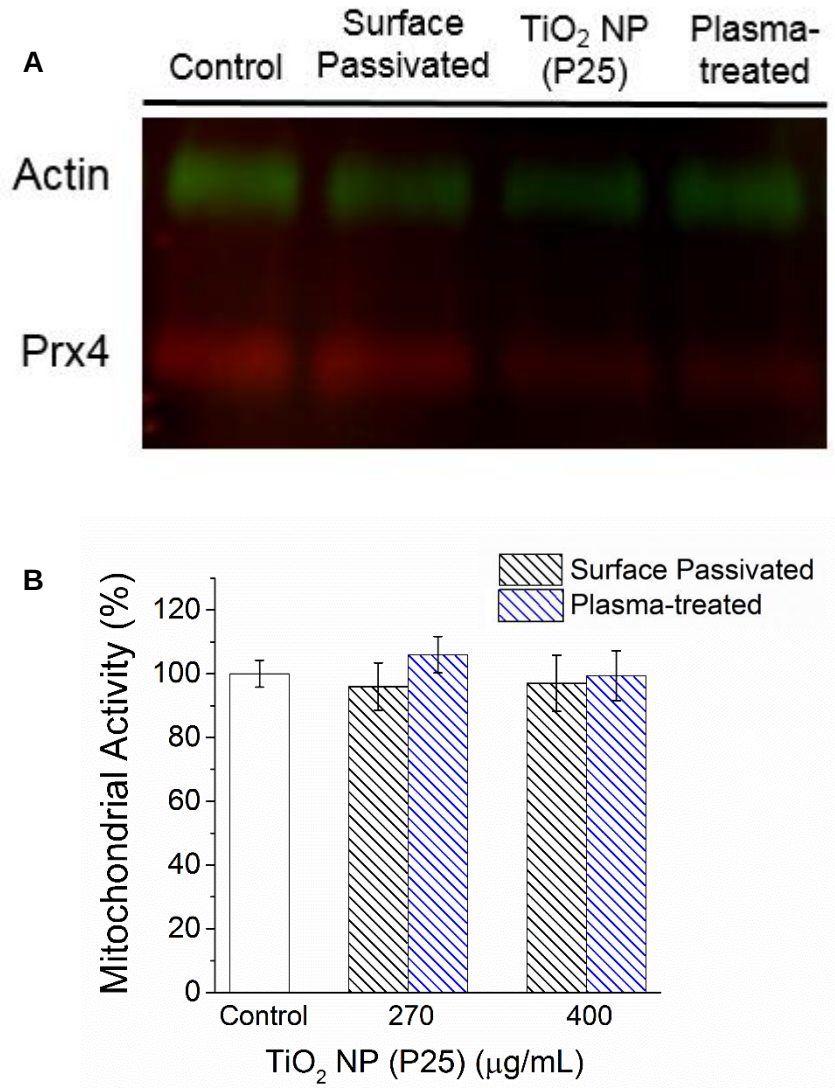

Fig. S5 Cellular response to plasma-treated and surface passivated TiO_2 NPs. (A) Representative western blot of peroxiredoxin 4 (Prx4, red) following incubation with TiO_2 NPs (400 μg/mL 24 hrs, red). Actin (green) was used as a housekeeping control. Cells lacking NPs (Control) were incubated with standard cell culture media (MEM + 10% FBS). This blot is representative of three trials with densitometric analysis shown in Figure 4D. (B) Cytotoxicity. An MTT assay of mitochondrial enzyme activity was used to ensure that the concentration of plasma-treated and surface passivated TiO_2 NPs (270 μg/mL, 12-well plate, 24 h incubation) did not lead to decreased cell health. Measurements are the average of three wells and error bars represent ± standard deviation. MTT activity (%) is normalized against a control in the absence of NPs. There was no significant difference between the control and the concentration of TiO_2 NPs used for experiments (400 μg/mL), which was used to confirm that we were working in a non-cytotoxic regime.

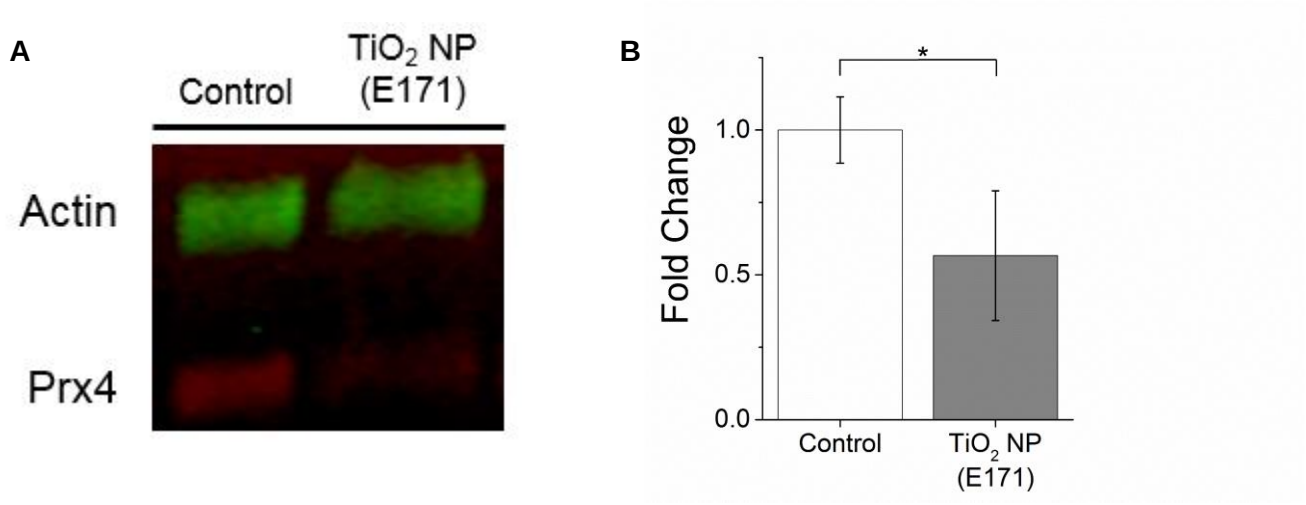

Fig. S6 Food grade E171 TiO_2 NPs induce oxidative stress. (A) Representative western blot of peroxiredoxin 4 (Prx4, red) following incubation with E171 TiO_2 NPs (400 μg/mL 24 hrs, red). Actin (green) was used as a housekeeping control. Cells lacking NPs (Control) were incubated with standard cell culture media (MEM + 10% FBS). This blot is representative of three trials. (B) Densitometric analysis, *$p<0.05$.