Supporting information

Separation and characterisation of cow serum without TiO_2 NPs

To ensure that any unbound cow serum proteins were separated from proteins bound to the TiO_2 NPs, 2 mL of 10% cow serum, treated as a sample containing TiO_2 NPs, was loaded on top of a sucrose gradient in a preparative centrifugation tube and fractions collected after centrifugation. Figure S1 shows absorbance spectra from DCS as a comparison of a sample without NPs (figure S1A) and with 500 µg/mL NPs (S1B). No particles were detected in fractions without TiO_2 NPs. After high speed centrifugation of the collected fractions, no pellet was observed in any of the samples. Samples without NPs were prepared as described in Materials and methods. Figure S2 shows SDS-PAGE gels of the fractions. No proteins are seen in the fractions after centrifugation.

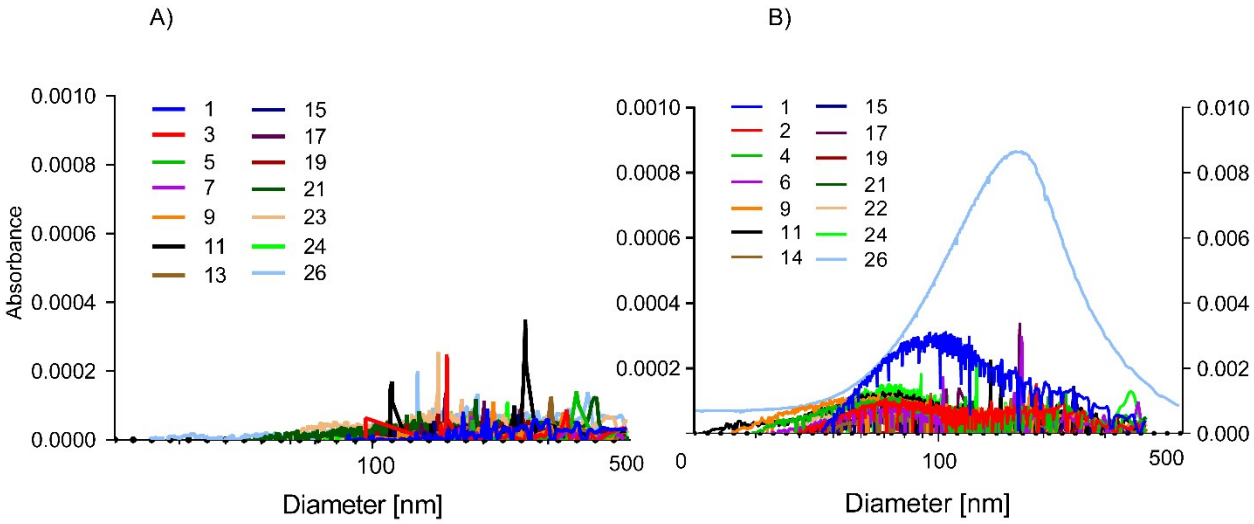

Figure S1. Differential centrifugal sedimentation measurement of fractions collected from preparative centrifugation of A) 10% cow serum, and B) 10% cow serum with 500µg/mL TiO_2 nanoparticles. Legend shows number of fraction, where 1 is the first collected fraction, approximately 1 cm from the bottom of the preparative centrifugation tube, fraction 26 in A) is the

fraction corresponding to the pellet collected from the tube. In B), fraction 26 is plotted on the right y-axis.

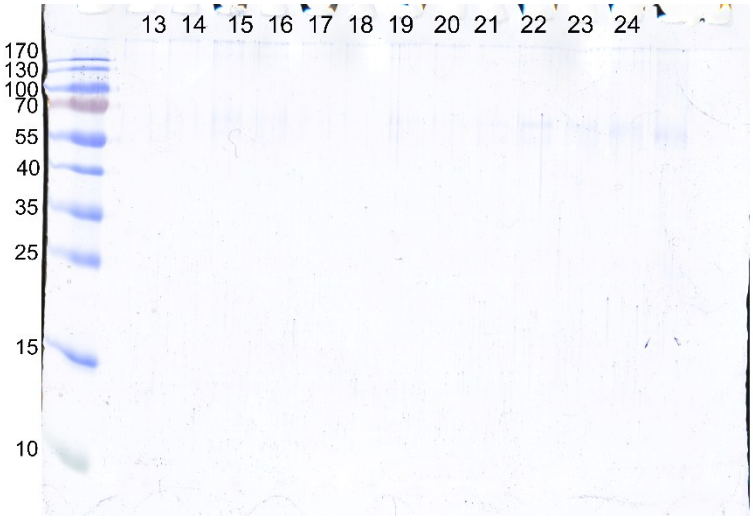

Figure S2. SDS-PAGE gel of fractions collected from preparative centrifugation without NPs.

SDS-PAGE of fractions from bottom half of preparative centrifugation tube

Under the conditions studied, fractions 1-12 were not of particular interest since the slower sedimenting complexes did not sediment into those fractions and no pellet was observed after high speed centrifugation. The conditions chosen were to separate the two main size populations; the pellet and complexes in suspension.

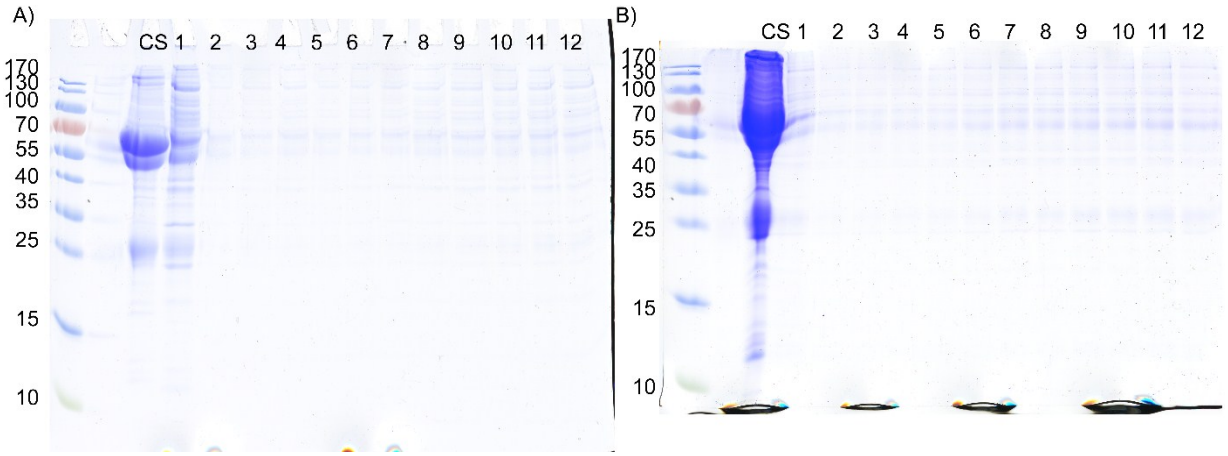

Figure S3. SDS-PAGE gels of fractions 1-12 for the concentration ratios studied. A) 10% cow serum and 500 µg/mL TiO_2 NPs; Lane 1: Protein Ruler, lane 3: cow serum control w.o. TiO_2, followed by fractions 1-12 B) 50% cow serum and 500 µg/mL TiO_2 NPs; Lane 3: cow serum control w.o. TiO_2 followed by fractions 1-12.

Additional duplicates of protein profiles of collected fractions

To further support the analysis of the SDS-PAGE gels shown in the article, each experimental conditions were run at least two more times. For the lower abundant proteins, gelsolin and ApoAI, relative quantification was difficult due to the separation of bands on the gels.

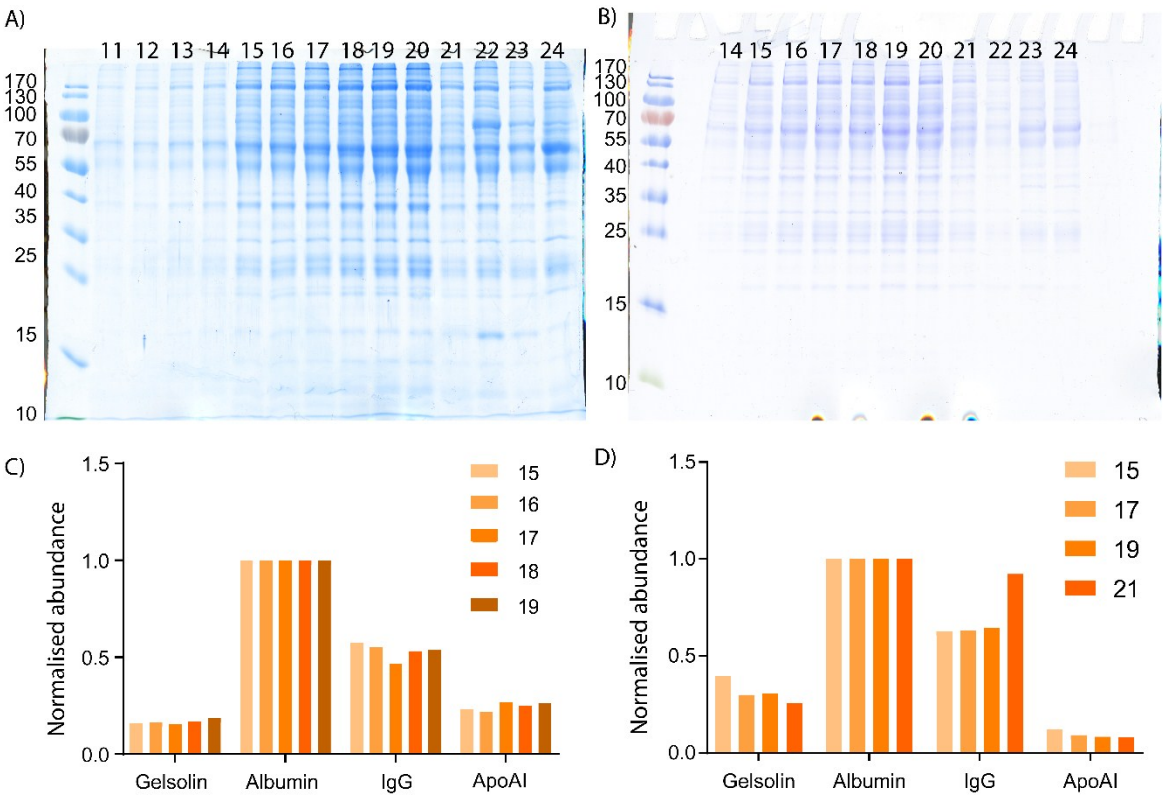

Figure S4. Slow sedimenting fractions of 10% cow serum mixed with 500 µg/mL TiO_2 in duplicate, A) and B) show the SDS-PAGE gels and C) and D) show the abundance of gelsolin, IgG, and ApoAI normalised against the albumin peak.

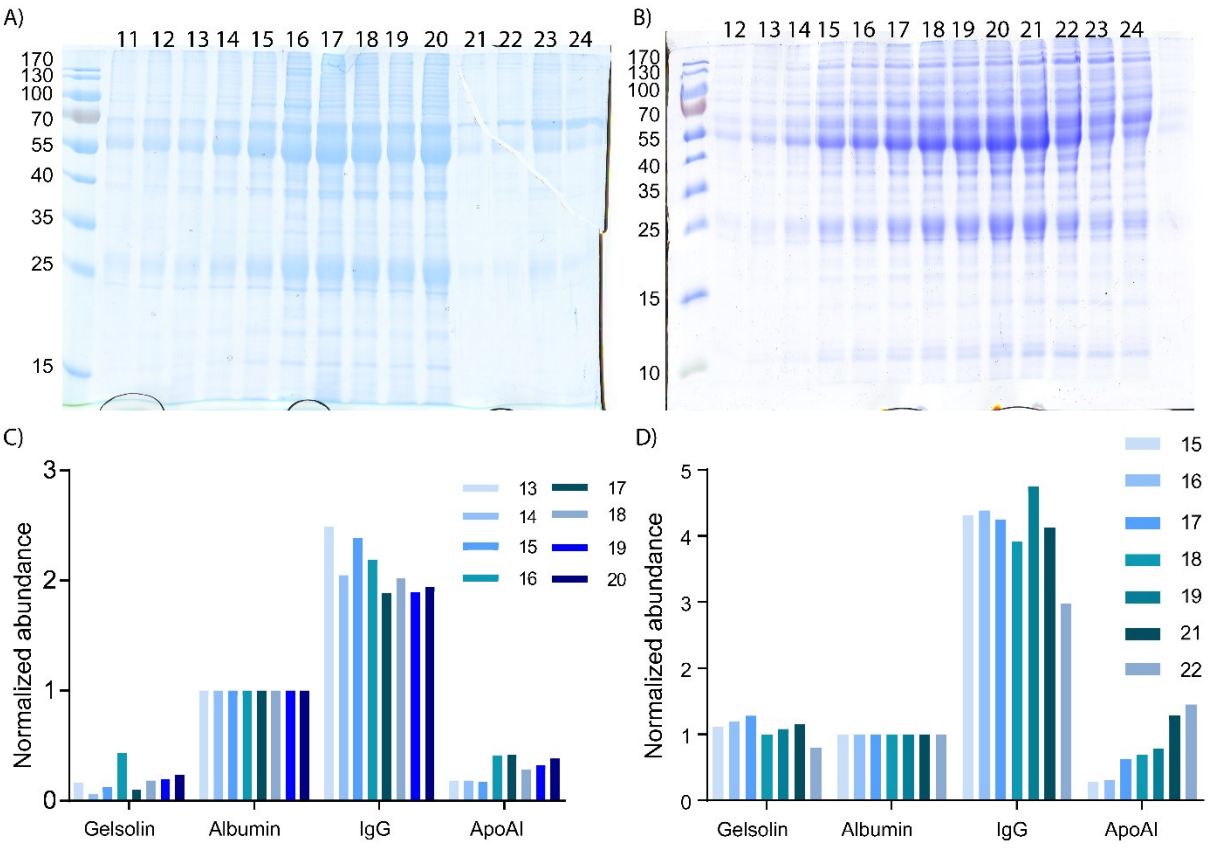

Figure S5. Slow sedimenting fractions of 50% cow serum mixed with 500 µg/mL TiO_2 in duplicate, A) and B) show the SDS-PAGE gels and C) and D) show the abundance of gelsolin, IgG, and ApoAI normalised against the albumin peak.

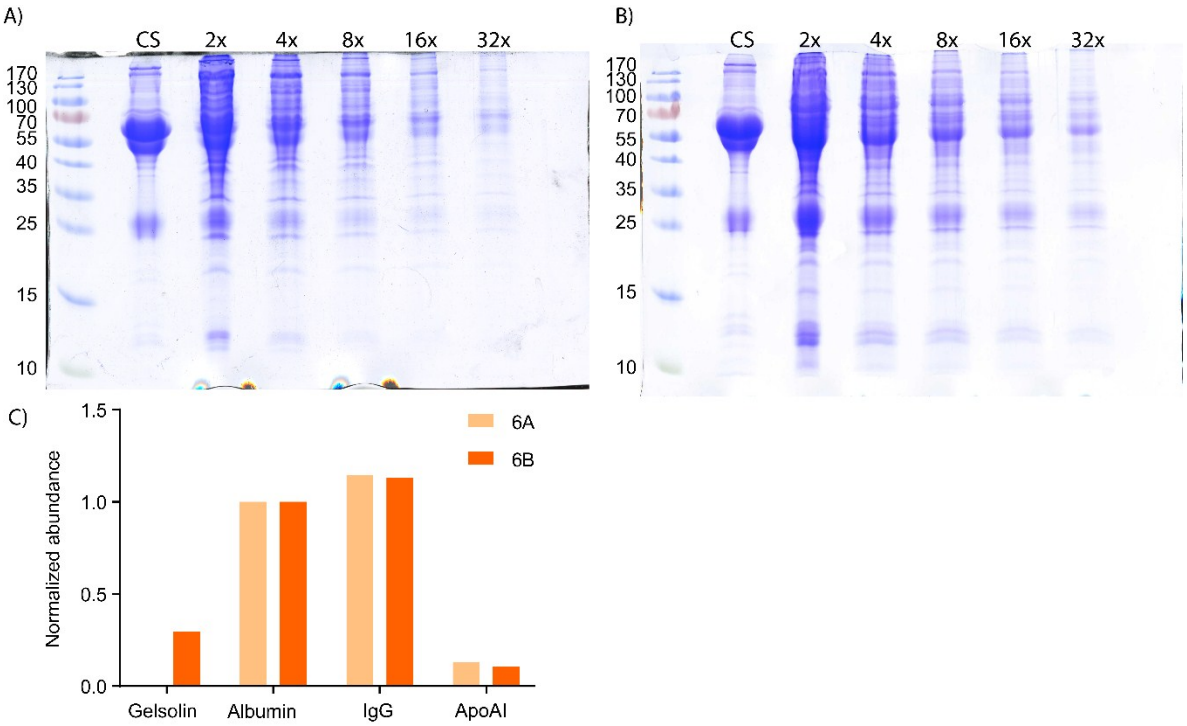

Figure S6. Dilutions of fast sedimenting complexes formed when 10% cow serum was mixed with 500 µg/mL TiO_2. A) and B) show the SDS-PAGE gels, C) shows the abundance of gelsolin, IgG, and ApoAI normalised against the albumin peak.

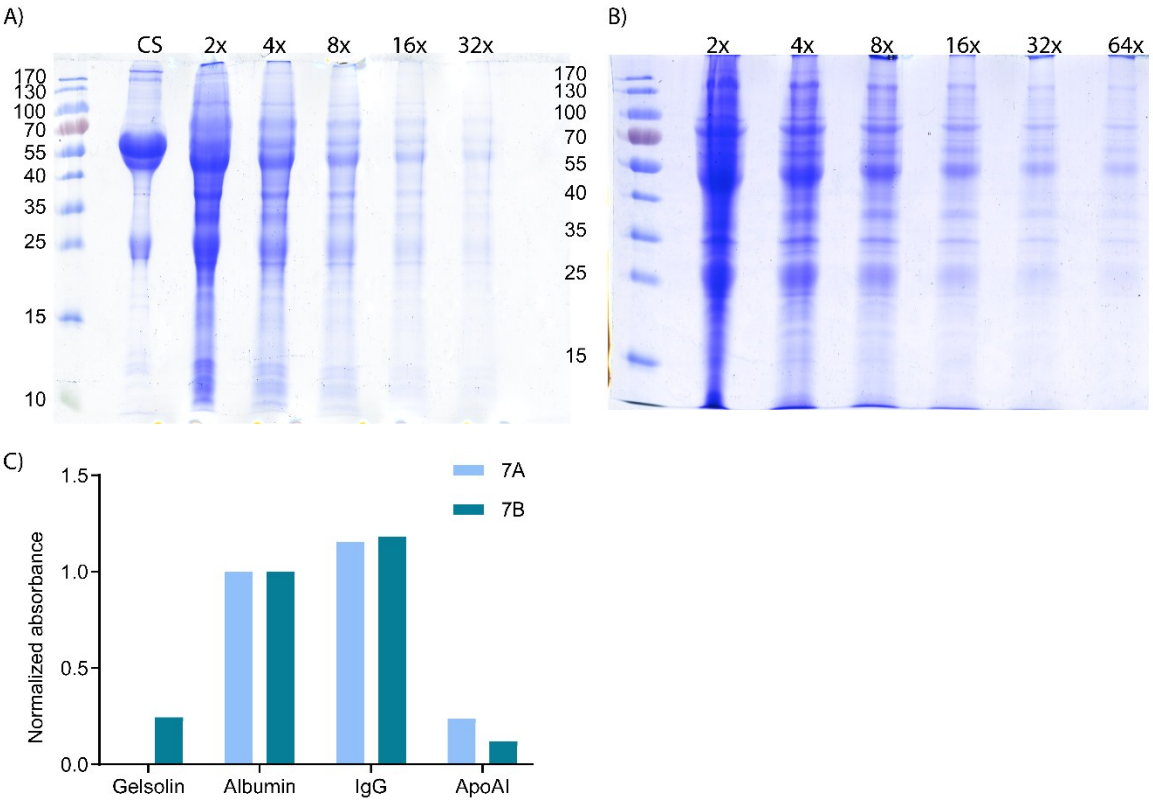

Figure S7. Dilutions of fast sedimenting complexes formed when 50% cow serum was mixed with 500 µg/mL TiO_2. A) and B) show the SDS-PAGE gels, C) shows the abundance of gelsolin, IgG, and ApoAI normalised against the albumin peak.

DLS analysis of cow serum and TiO_2 NPs fractions

Table S1 shows the size distribution and polydispersity (%DP) of the fractions collected after 4 hours of centrifugation at 2,000 RPM, a condition tested while developing the method. The fractions were analysed by DLS without any further manipulation and assuming an average sucrose concentration of 30%. The diameters of fractions with most proteins, fractions 16 to 21, are between 160 and 50 nm. Interestingly the polydispersity is lowest when the amount of proteins is high indicating that a high protein to surface area allows for well-defined complexes to form.

Before preparative centrifugation, the sample was not measureable by DLS, due to broad size distribution and precipitation of the largest complexes.

Table S1. Diameter and % polydispersity of collected fractions measurable by DLS. No size was acquired for fractions not shown.

Fraction	Diameter [nm]	%DP
6	411.3	Multimodal
11	262.0	Multimodal
12	238.0	Multimodal
13	216.5	Multimodal
14	183.4	Multimodal
15	186.9	Multimodal
16	156.4	30.7
17	137.1	23.9
18	102.0	19.3
19	80.0	13.4
20	63.6	11.9
21	51.4	23.8
22	14.8	Multimodal
23	15.7	Multimodal
24	220.2	Multimodal

Since DLS is dependent on the viscosity of the sample solution, absolute size measurement requires known concentration of sucrose. By dialysing the fraction against a known amount of sucrose, accurate size can be obtained for the complexes in each fraction. Dialysing 1.5 mL fractions proved problematic due to dilution of the sample. Therefore 3 mL fractions were

collected and measured by DCS before and after dialysis, and by DLS after dialysis (figure S8). The size as determined by DLS is somewhat larger than before dialysis which is expected if the sucrose concentration, and thereby the viscosity, is estimated to be higher than the actual concentration. In the DCS the size of the complexes is similar before and after dialysis although some material seems to be lost during the dialysis process or the sample is diluted.

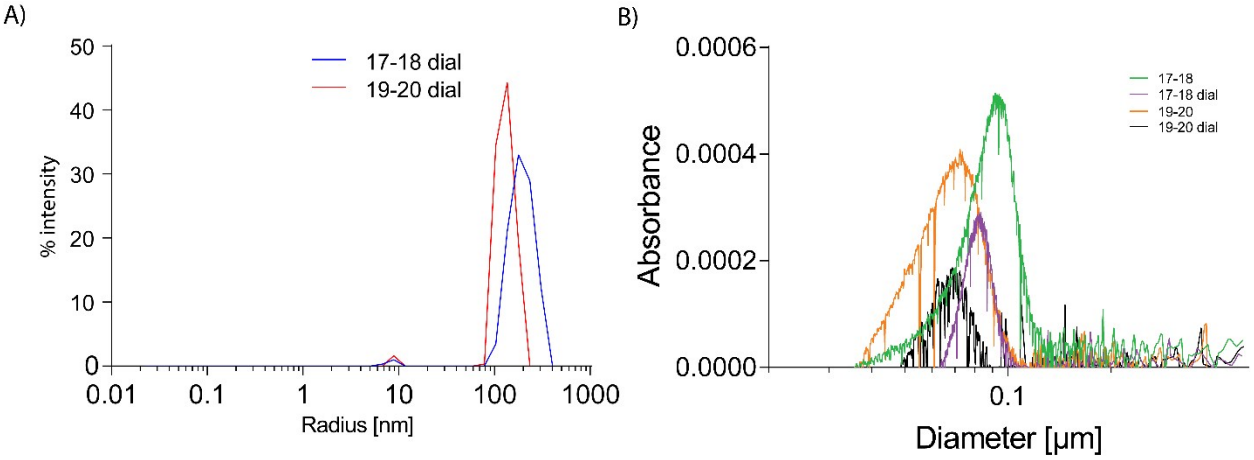

Figure S8. Dialysis of complexes from fractions 17-18 and 19-20. A) DLS data of the dialysed (dial) fractions, and B) DCS of fractions 8 and 9 before and after dialysis.

Protein depletion in high concentration of TiO_2 NPs

To ensure that the observed effect of normalised abundance of proteins was not because of protein depletion from the serum solution, 500 µg/mL of NPs was mixed with 50, 10, and 5% serum, centrifuged at 18,000 RPM (31,514 x g) for 10 minutes and the supernatant analysed by SDS-PAGE. Dilutions of the serum were made to give 2,5% final concentration before mixing with SDS-PAGE sample buffer to ensure the same serum amount loaded onto the gel. No difference in

albumin or IgG is observed, compared to control, showing that the most abundant proteins were not depleted at 500 µg/ml NPs in any of the serum concentrations.

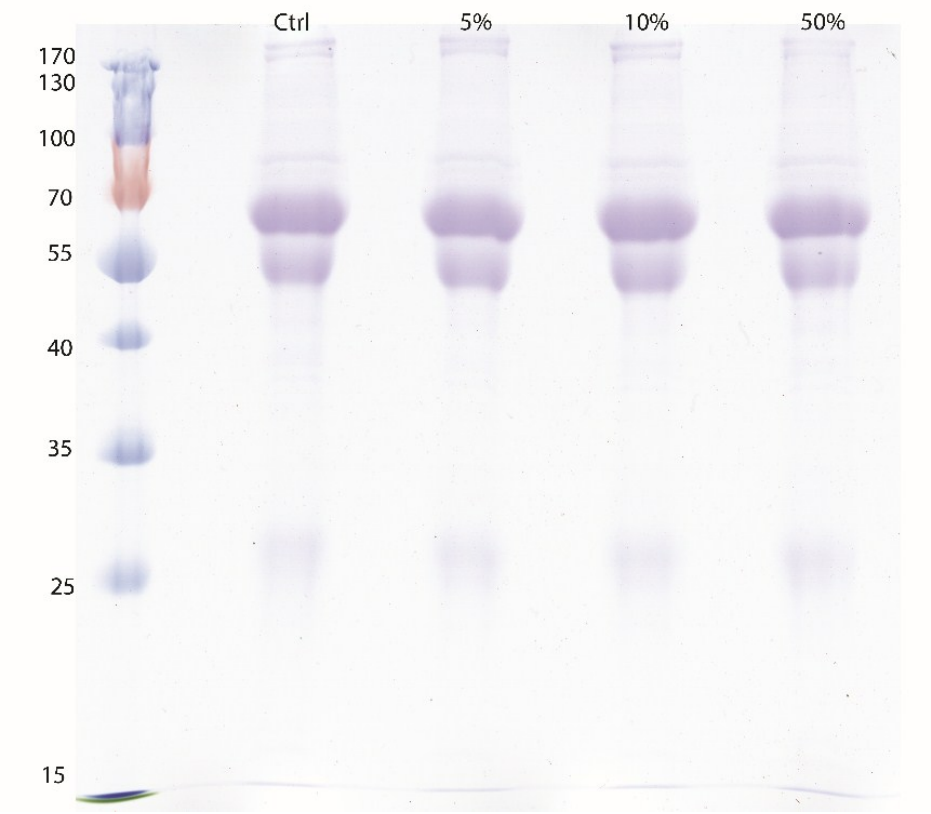

Figure S9. SDS-PAGE of supernatant after high speed centrifugation of TiO_2 NPs 500 µg/mL mixed with different concentrations of serum to ensure that no depletion of proteins happens at the studied NP serum concentration ratios. Lanes: 1. Ruler 26616, 2. 2.5% cow serum control without NPs, 3. 5% serum, 4. 10% serum, 5. 50% serum.

Mass spectrometry

Table S2. Proteins identified by mass spectrometry from excised bands of SDS-PAGE gel for the slow sedimenting fractions of 50% serum, 500 µg/mL TiO_2 nanoparticles. In each band tens of proteins were identified. The table shows proteins in high abundance in each protein band. When

proteins of unexpected size were identified in high abundance, priority was given to proteins of expected molecular weight.

Band	Size [kDa]	Protein(s)
1	130+	Complement C3, Thrombospondin
2	90	Gelsolin, Complement factor B
3	65	Albumin, Complement C9
4	50	Ig gamma chain, Antithrombin-III
5	35-40	Conglutinin, Apolipoprotein E
6	30	Apolipoprotein E
7	25	Ig kappa chain, Apolipoprotein A-I
8	20	Apolipoprotein A-I, Ig kappa chain
9	13	Hemoglobin beta, Hemoglobin alpha
10	11	Histone H4

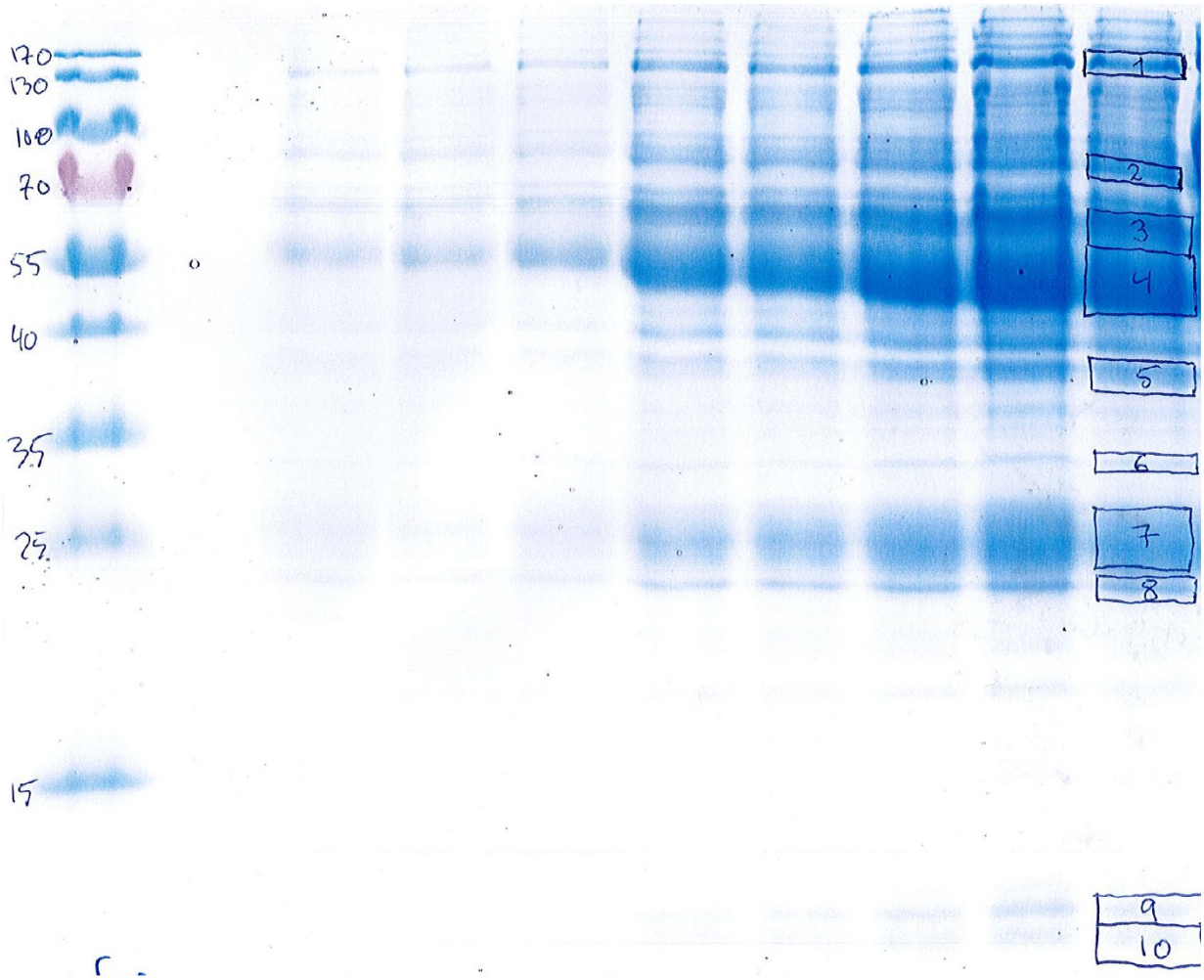

Figure S10. Excised protein bands used for LC-MS analysis and shown in table S2.