Electronic Supplementary Material (ESI) for Environmental Science: Nano.
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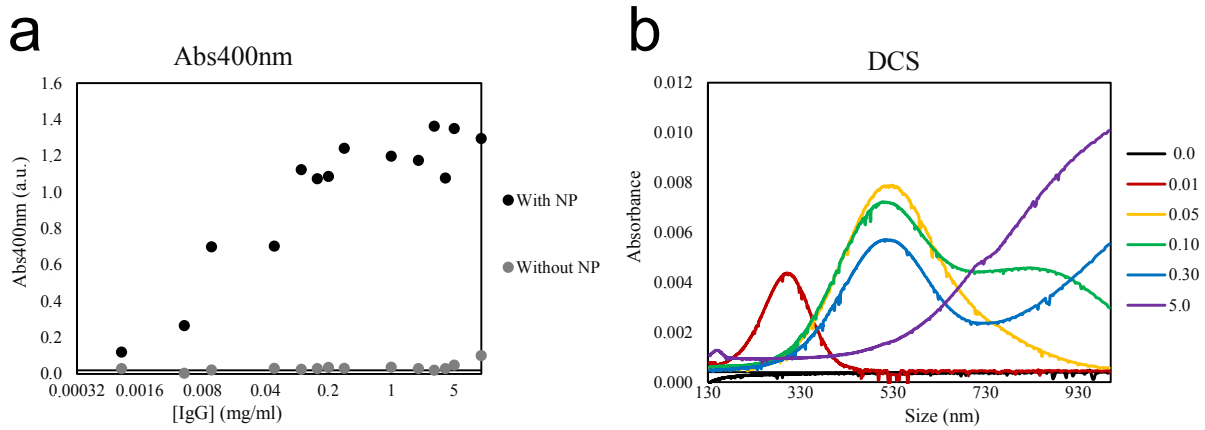
Supplementary information

Conditions for protein particle aggregation

IgG concentration dependency

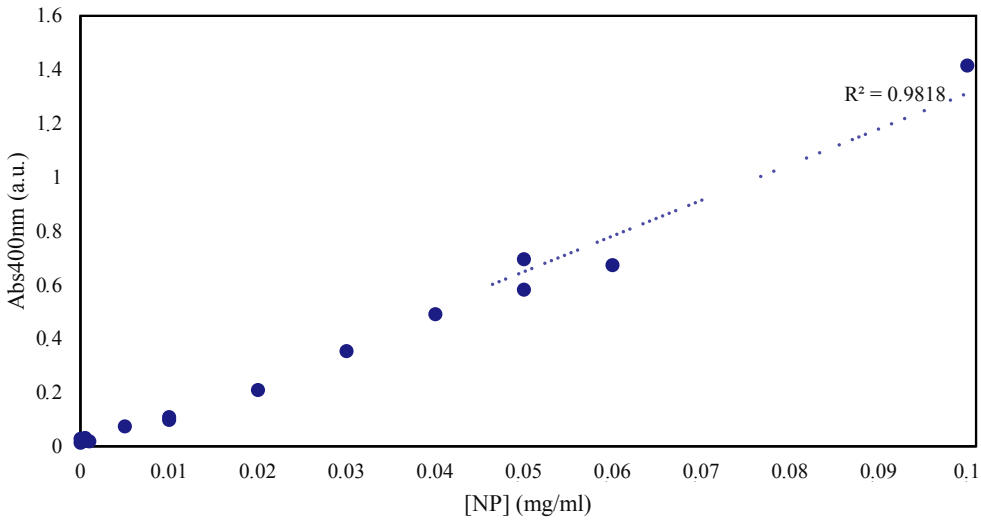
The effect of IgG concentration on NP aggregation was determined by making samples with IgG (0-10 mg/mL), in 10 sodium phosphate buffer, pH 7.5, and 20 mM NaCl, with or without 0.1 mg/mL ($1.2X10^9$ particles/mL) NPs (50 nm). The aggregation was followed by measuring the scattered light at Abs_{400nm}. For selected samples the size was measured by DCS. The absorbance increased with the IgG concentration, indicating the formation of larger aggregates, reaching maximum Abs_{400nm} at ≥0.3 mg/mL IgG, Supplementary Figure 1a. The increase was also shown using DCS, where 0.01 mg/mL IgG gave a peak around 300 nm, and increased IgG concentrations resulted in increased aggregate sizes Supplementary Figure 1B.



Supplementary Figure 1. The Abs_{400nm} (a) and the DCS data (b) obtained for the formed aggregates (in 10 mM sodium phosphate buffer, pH 7.5, 20 mM NaCl) in increasing IgG concentration (0-10 mg/mL). The NP (50 nm) concentration was 0.1 mg/mL for all samples. In (b), the legend describes the IgG concentrations in mg/mL.

NP concentration dependency

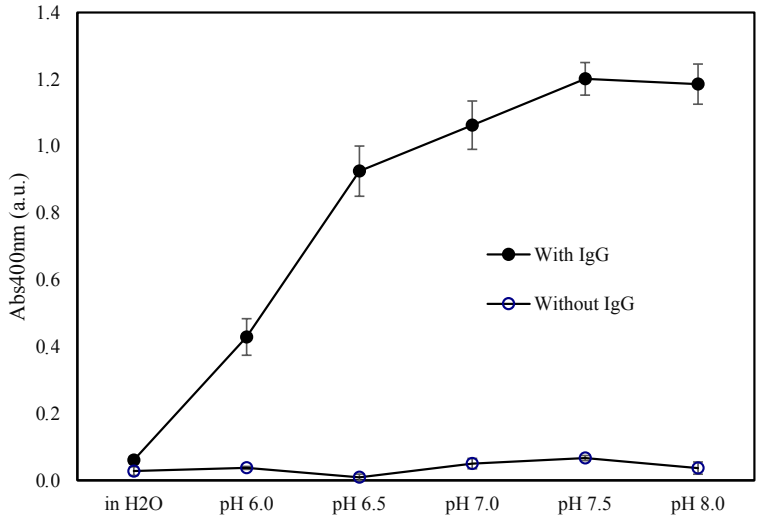
The effect of NP concentration was investigated. The NP concentration ranged between 0-0.1 mg/mL. An IgG concentration of 5.0 mg/mL was used. The IgG did not contribute significantly to the Abs_{400nm} (in the absence of NPs). The results show a linear increase in absorbance with increase in NP concentration (Supplementary Figure 2).



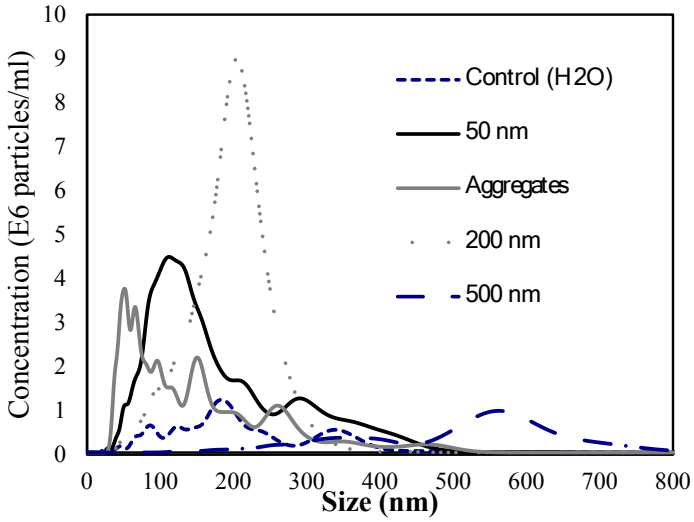
Supplementary Figure 2. The Abs_{400nm} obtained for the created aggregates (in 10 mM sodium phosphate buffer, pH 7.5, 20 mM NaCl) when altering the NP concentration (0-0.1 mg/mL), but having constant IgG concentration of 5.0 mg/mL. The measurements resulted in a linear relationship between NP and IgG concentration, with an R^2-value of 0.9818.

pH dependency

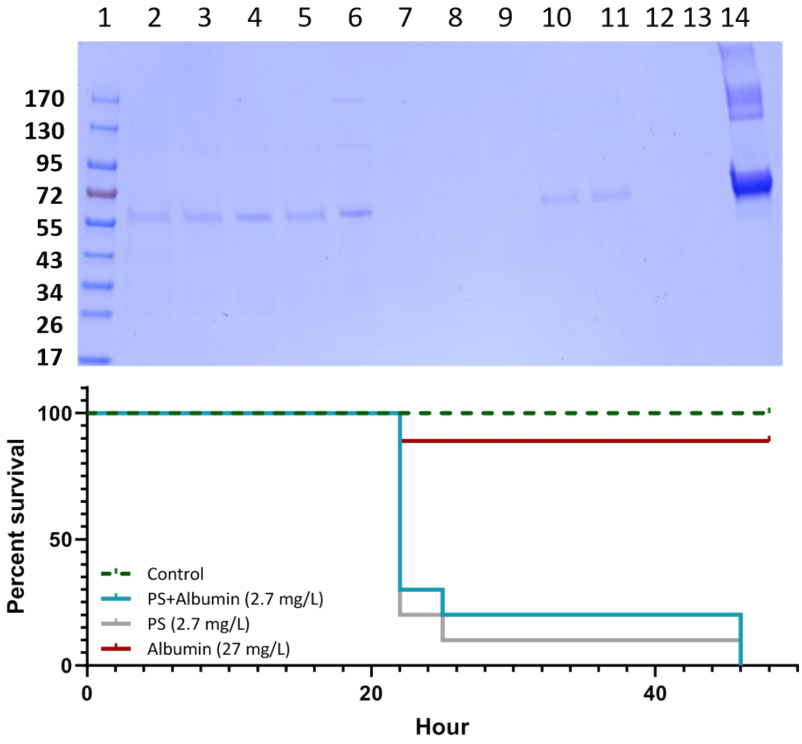
The influence pH for the aggregation was investigated between pH 6.0-8.0. The IgG concentration was 0.3 mg/mL, and the NP concentration 0.1 mg/mL, and the buffer 10 mM sodium phosphate, and 20 mM NaCl. The results show an increase in aggregation with an increase in pH, up to pH 7.5 (Supplementary Figure 3). This increase could either be due to binding sites on the IgG being more readily available or less electrostatic repulsion between the positively charged NPs.



Supplementary Figure 3. The Abs_{400nm} obtained for the created aggregates – with 0.1 mg/mL NP, and, for the brown measurements, 0.3 mg/mL IgG – when altering the buffer pH in 10 mM buffer and 20 mM NaCl. Mean values of three replicates calculated together with standard deviation.



Supplementary Figure 4. The nanoparticle and aggregate size determined by NTA after 24 h exposure of control (H_2O), free particles (50, 200, or 500 nm) or aggregates to *D. magna*.



Supplementary Figure 5. Top panel: Shows the protein content on nanoparticles. BSA is the protein band around 62 kDa. Lines 1. Molecular weight standard (kDa), Lines 2-6, BSA content after 24 h incubation of BSA (27 mg/L) coated 50 nm PS-NH_2 (2.7 mg/L) with 15 *D. magna* individuals, Line 7-9, 24 h incubation of non-coated 50 nm PS-NH_2 (2.7 mg/L) for 24 h with 15 *D. magna* individuals, Lines 10-11 incubation of BSA (27 mg/L) with 15 D. *magna* individuals, Line 12, empty, Line 13, non-coated 50 nm PS-NH2 (2.7 mg/L without *D. magna*, Line 14, BSA.

57