

Supplementary information

**Label-free detection of polystyrene nanoparticles
in *Daphnia magna* using Raman confocal mapping**

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Table S1. Zeta potential and hydrodynamic size in various media at 100 µg/mL.

Samples	Zeta potential (mV)	Size (nm)
COOH PS in Milli-Q®	-37,4 ± 0,72	211 ± 1
COOH PS in tap H ₂ O	-35,7 ± 2,55	187 ± 2
COOH PS in RPMI	-17,1 ± 0,6	428 ± 31
COOH PS in RPMI + FBS	-13,0 ± 1,23	270 ± 3
NH ₂ PS in Milli-Q®	-18,5 ± 1,2	1164 ± 30 ^a
NH ₂ PS in tap H ₂ O	-25,7 ± 0,6	1099 ± 265 ^a
NH ₂ PS in RPMI	-11,6 ± 0,8	811 ± 45
NH ₂ PS in RPMI + FBS	-12,6 ± 0,7	213 ± 23

Measurements were done for triplicate samples and data are shown as mean values ± S.D.

^aTwo intensity peaks were identified in these samples; the average values are reported here.

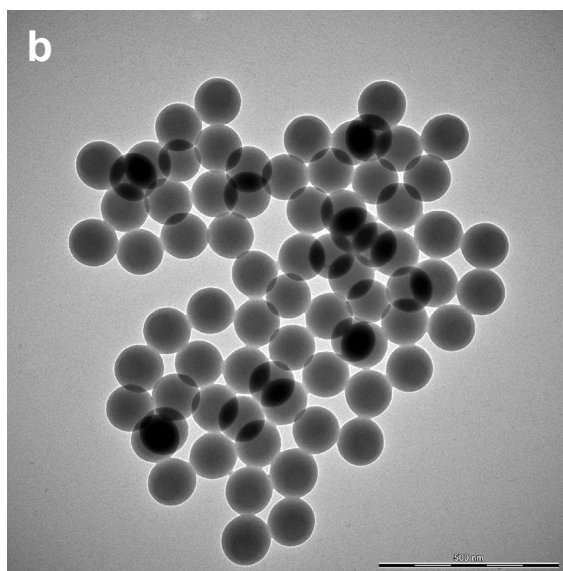
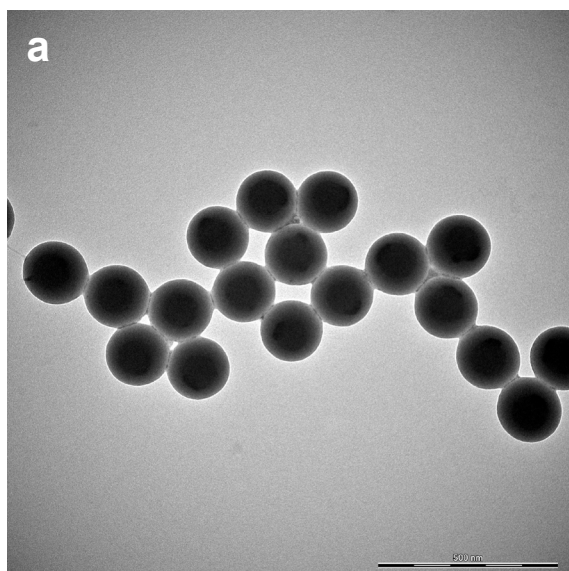


Figure S1. TEM micrographs of (a) PS-COOH NPs (diameter: ~ 170 nm), and (b) PS-NH₂ NPs (diameter: ~ 130 nm) dispersed in tap water. The particles were adhered onto grids and washed once with Milli-Q[®] water (no counterstaining). Some particle necking is seen. Scale bars: 500 nm.

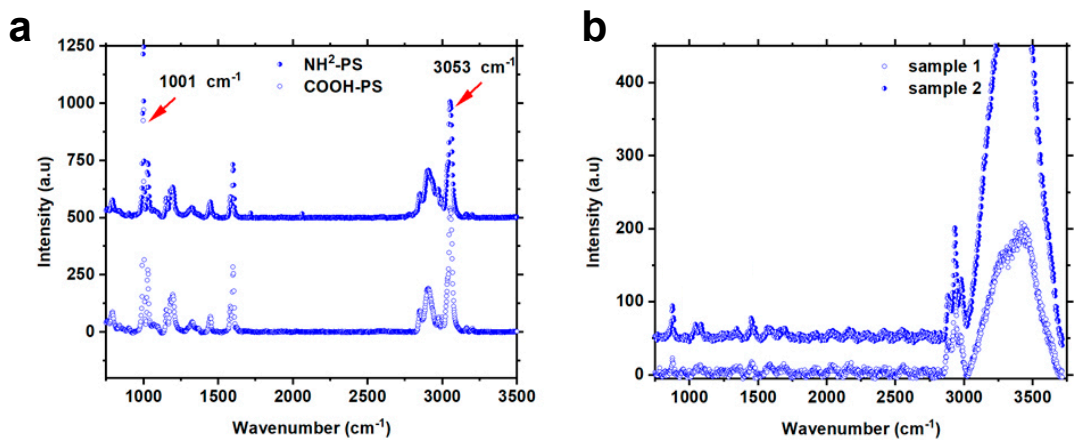


Figure S3. Raman reference spectra measured for PS NPs with COOH (empty circles) and NH₂ (half-filled circles) functional groups are shown in panel (a). Both specific peaks for PS at 1001 cm⁻¹ and 3053 cm⁻¹ are indicated with a red arrow. Spectra measured for control daphnia samples 1 (empty circles) and 2 (half-filled circles) are shown in panel (b). There is no PS specific peak at 1001 cm⁻¹ in these controls.

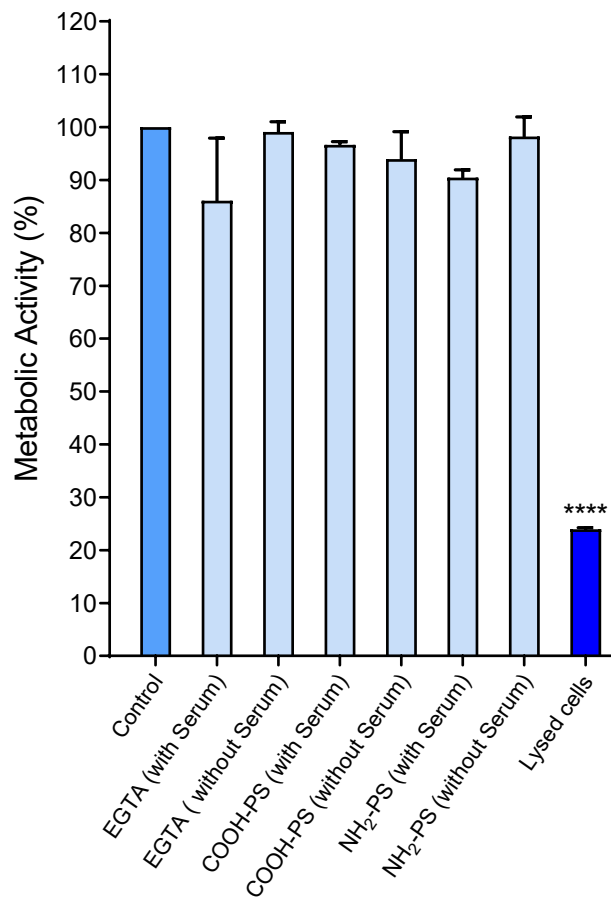


Figure S4. No PS NP-induced cytotoxicity in differentiated HT-29 cells. Human HT-29 cells differentiated for 21 days were exposed for 24 h to 100 mg/mL COOH-PS NPs or NH₂-PS NPs in the presence or absence of serum (10% FBS). Cell viability (metabolic activity) was determined using the Alamar blue assay. EGTA (2.5 mM) was also included (with/without serum). The results shown are mean values \pm S.D. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* analysis. * $p < 0.05$, **** $p < 0.0001$. For TEER measurements under the same conditions, see Figure 6.