

## Supplementary material

### Separation, detection and characterisation of engineered nanoparticles in natural waters using hydrodynamic chromatography and multi-method detection (light scattering, analytical ultracentrifugation and single particle ICP-MS)

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#### Composition of the eluent

The effect of eluent composition was verified for the 20 nm nAg because it was potentially the most difficult ENP to analyse (because of ongoing dissolution/agglomeration). All eluents had a pH of 7.5 ( $\pm 0.2$ ) and a NaNO<sub>3</sub> concentration of 1 mM. Radii obtained by injecting 2 mg L<sup>-1</sup> of nAg had a much greater signal to noise ( $\sim 0.02$  V) and were less accurate than those measured at 20 mg L<sup>-1</sup>.

**Table S1. Hydrodynamic radii obtained for nAg (Sigma) with a nominal radius of 20 nm when analysed with the on-line HDC-DLS**

All eluents contained 1 mM NaNO<sub>3</sub> at pH 7.5 ( $\pm 0.2$ ).

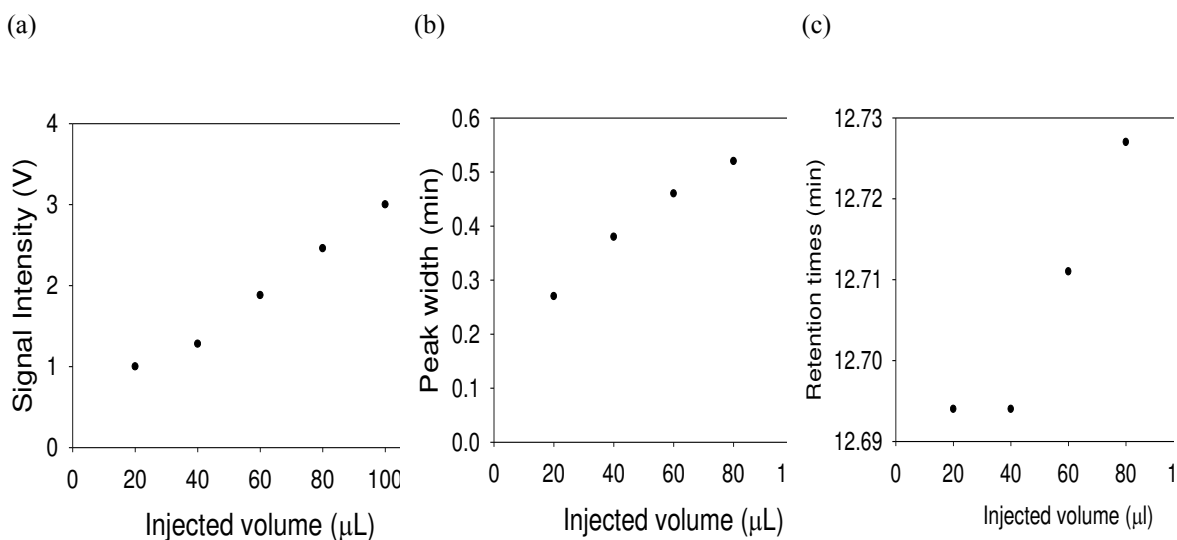
Eluent			Nanoparticles	
SDS concentrations (%w/w)	Triton X-100 concentrations (%w/w)	nAg concentrations (mg L <sup>-1</sup> )	$R_h$ (nm)	Signal intensity (V)
0	0	20	20.8 $\pm$ 1.0	2.01
0.0013	0.0013		20.7 $\pm$ 1.2	2.81
0.0013	0.0013		42.1 $\pm$ 1.1	0.36
0.0025	0.0025	2	30.5 $\pm$ 0.6	0.34
0.01	0.01		36.7 $\pm$ 0.8	0.39
0.05	0.01		34.4 $\pm$ 0.7	0.37

Other preliminary work on the optimisation of the eluent composition showed that the nanoparticles appeared to be stable over the pH range of 6.5 to 8. Furthermore, in the absence of added surfactant, particle agglomeration was observed for eluent NaNO<sub>3</sub> concentrations that exceeded 0.1 mM. In order to ensure minimal perturbation of environmental samples, a pH of 7.5 and ionic strength of 1 mM were selected for general use in order to fairly closely mimic a natural water. Maximum recovery of the ENP was obtained when the surfactants SDS and Triton X-100 were added to the eluent. We assume that the surfactant addition minimised ENP adsorption onto larger particles and colloids. Nonetheless, when the SP-ICP-MS was used as a detector, surfactant

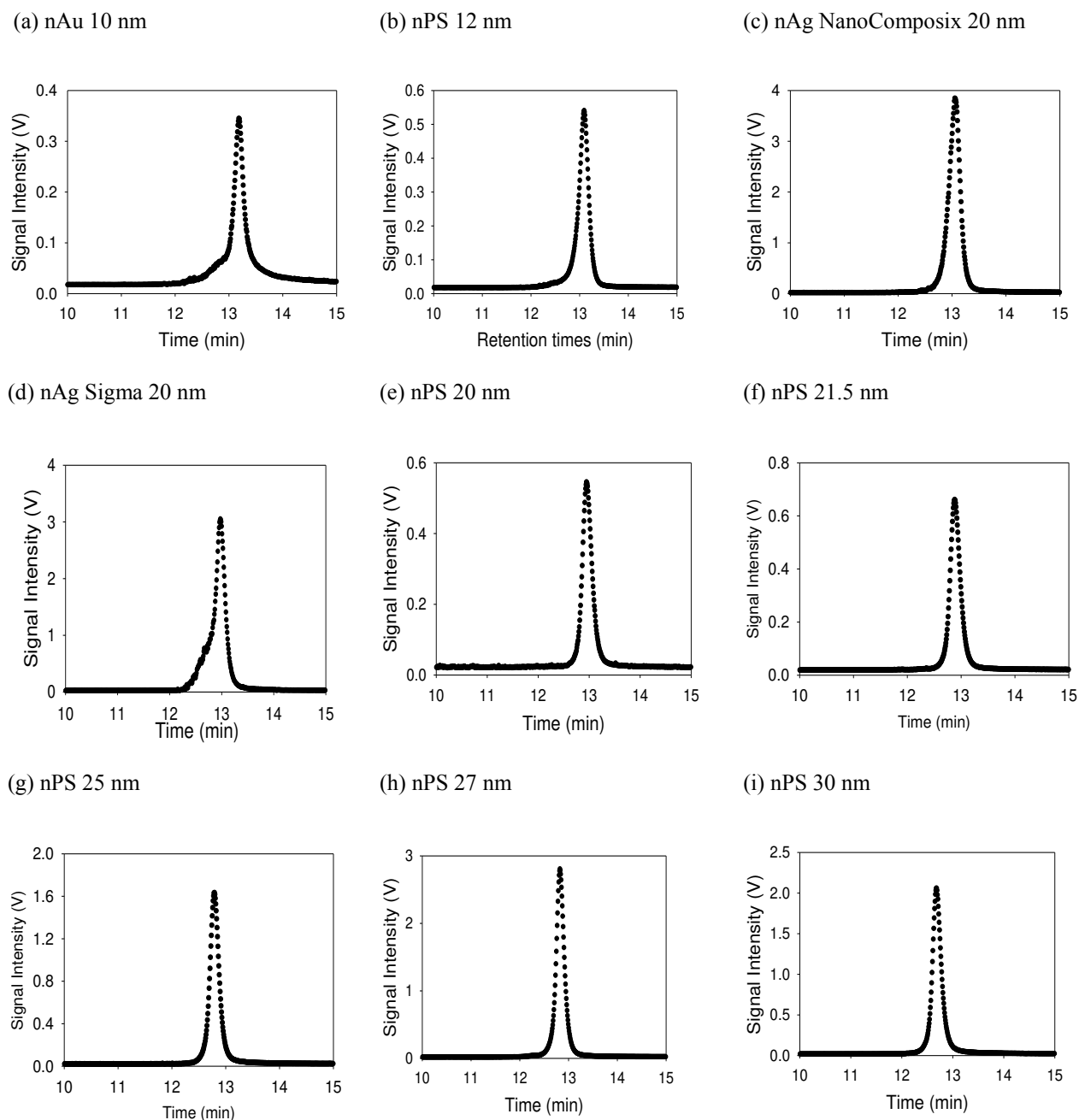
concentrations had to be minimised because of the formation of emulsions during the sample nebulisation step. Based upon all of the previous considerations and the results in Table S1, an eluent composition of 1 mM NaNO<sub>3</sub>, 0.0013 % w/w SDS, 0.0013 % w/w Triton X-100 at a pH of 7.5 was used for all subsequent analyses. Indeed, a further increase in the surfactant concentrations above 0.0013% w/w did not result in a substantial increase to the peak intensity (Table S1).

### Role of injection volume

To some extent, detection limits can be decreased by increasing sample injection volumes. As expected, for 250 mg L<sup>-1</sup> of the 40 nm nPS standard, the height and width of the peaks obtained from the on-line DLS detector increased with increasing injection volume (Fig. S1a,b). In contrast, retention times were also seen to increase for injection volumes above 40 μL. To minimise problems associated with wide peaks and variable retention times, an injection volume of 20 μL was used for all of the reported experiments.

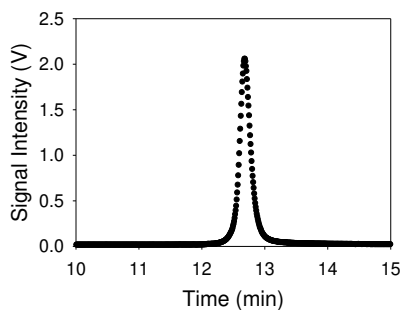


**Fig. S1.** Role of the injected sample volume on: (a) signal intensities; (b) peak widths; and (c) retention times.



**Fig. S2.** Chromatograms of ENP standards obtained using the on-line light scattering signal (DLS) collected at 99°.

(j) nPS 40 nm



(k) nPS 60 nm

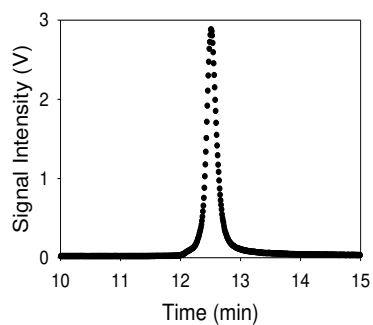


Fig. S2. (Cont.)

### Calibration curves using manufacturers' radii

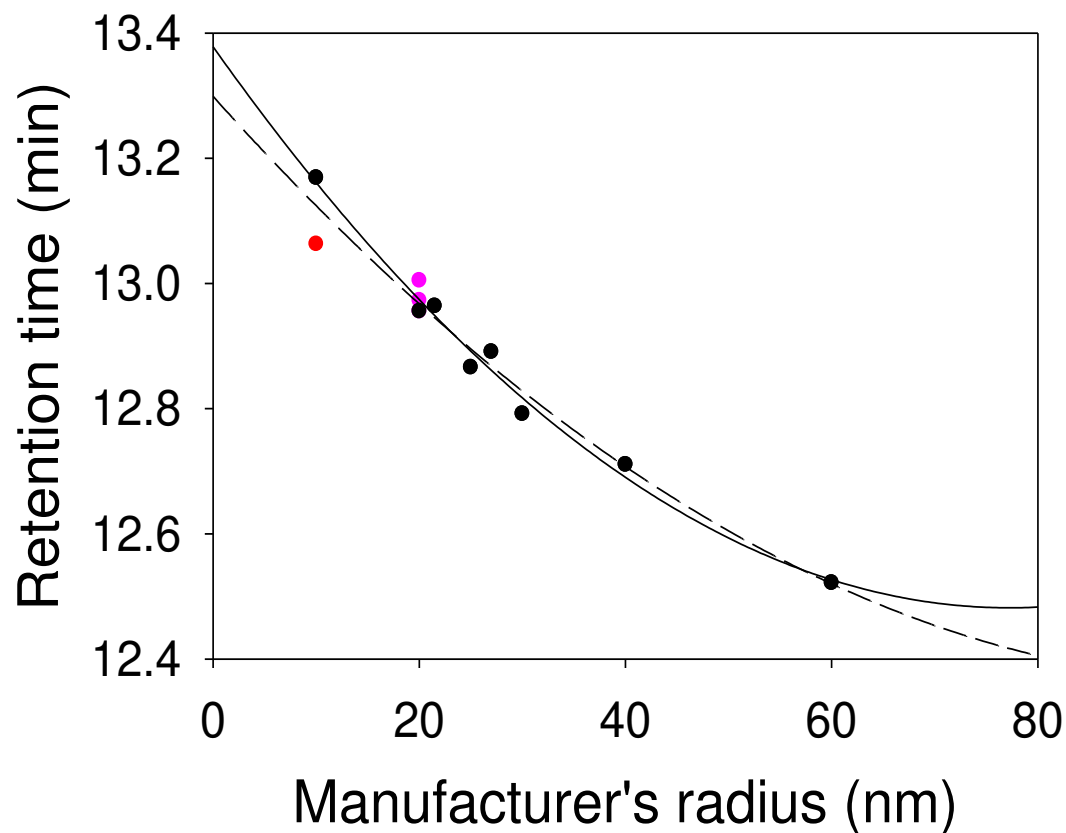


Fig. S3. Calibration curve based on the nPS (black points), nAu (red point) and nAg (pink points) standards. The curve was prepared using the manufacturers' radii for the particle standards (solid line, nPS only; dotted line, all ENP).

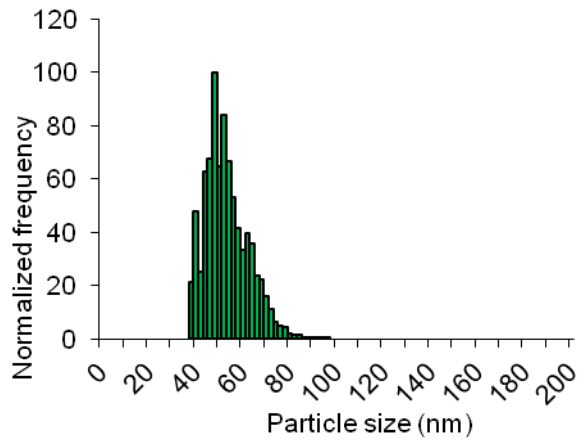
**ENP mixtures**

**Table S2. Separation of an ENP mixture (composition corresponding to the ENP mixture of Fig. 2)**

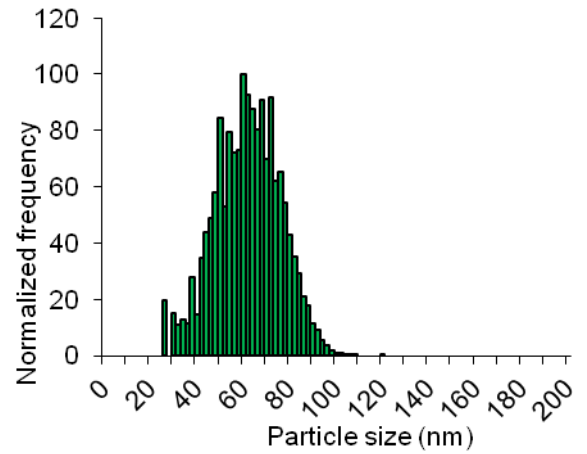
The characterisation of sample fractions was performed using off-line and on-line dynamic light scattering

# Fraction	Retention time (min)	DLS off-line $R_h$ (nm)	DLS on-line $R_h$ (nm)
1	12.0–12.1	61.3 ± 4.0	
2	12.1–12.2	58.3 ± 5.2	
3	12.2–12.3	55.7 ± 6.6	57.1 ± 1.0
4	12.3–12.4	65.4 ± 6.2	45.0 ± 2.0
5	12.4–12.5	69.0 ± 8.1	
6	12.5–12.6	66.6 ± 7.1	
7	12.6–12.7	61.2 ± 15.7	
8	12.7–12.8	40.5 ± 8.4	29.7 ± 0.6
9	12.8–12.9	34.9 ± 7.4	21.8 ± 0.6
10	12.9–13.0	27.8 ± 4.5	22.2 ± 0.5
11	13.0–13.1	21.8 ± 2.6	
12	13.1–13.2	25.0 ± 2.1	
13	13.2–13.3	28.8 ± 6.7	
14	13.3–13.4	24.4 ± 5.0	
15	13.4–13.5	15.5 ± 1.2	

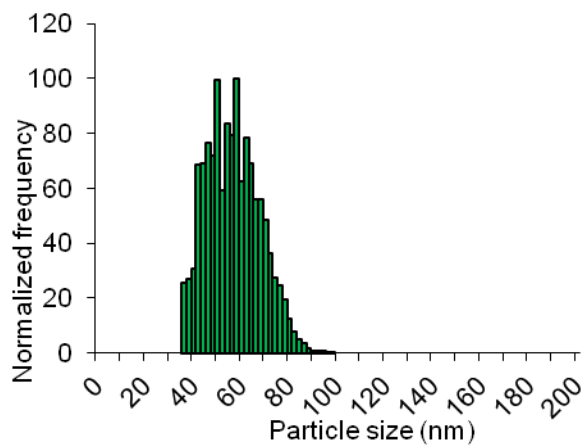
Fraction #9



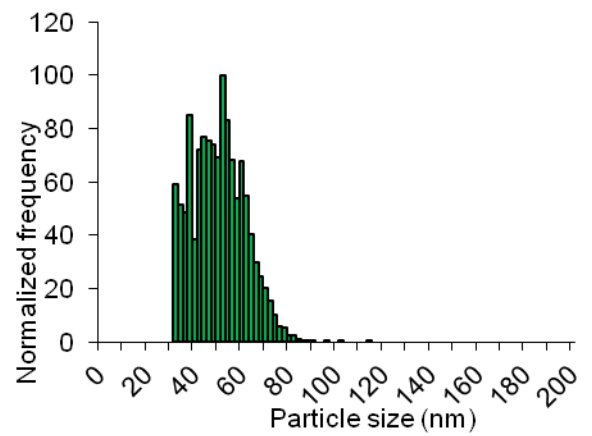
Fraction #10



Fraction #11

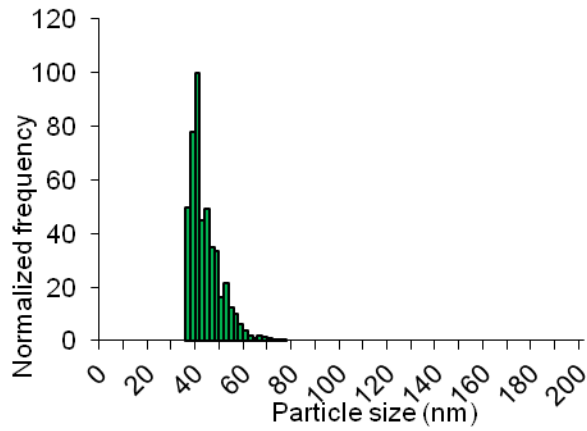


Fraction #12

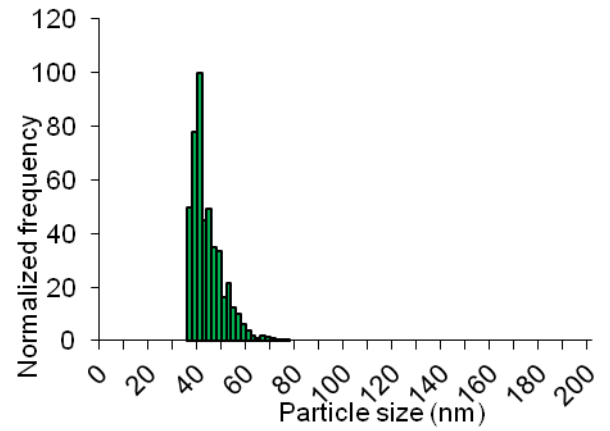


**Fig. S3.** SP-ICP-MS nAg size distributions data of several sample fractions of the ENP mixture detailed in Table 6b.

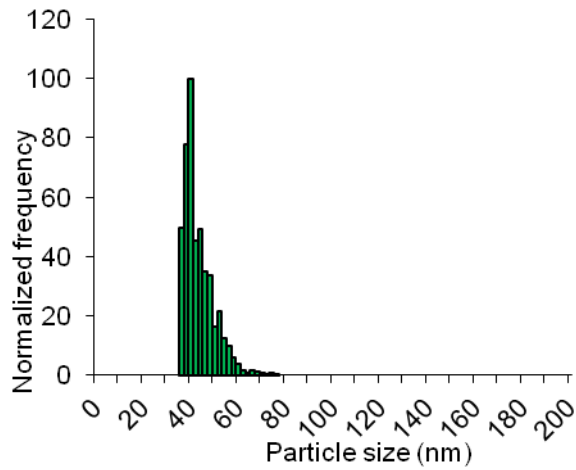
Fraction #8



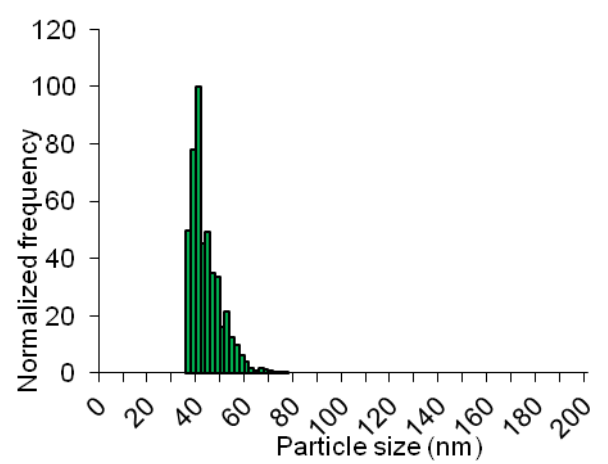
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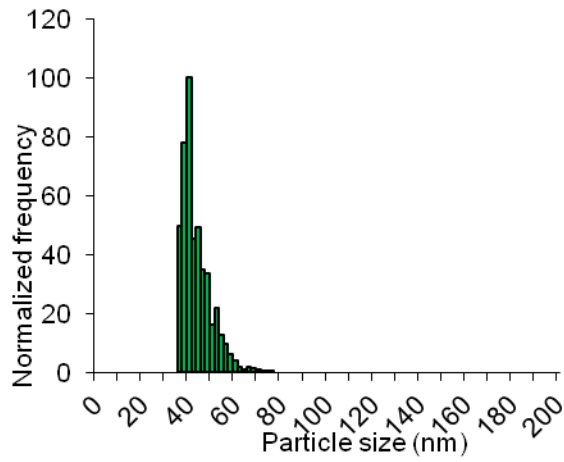


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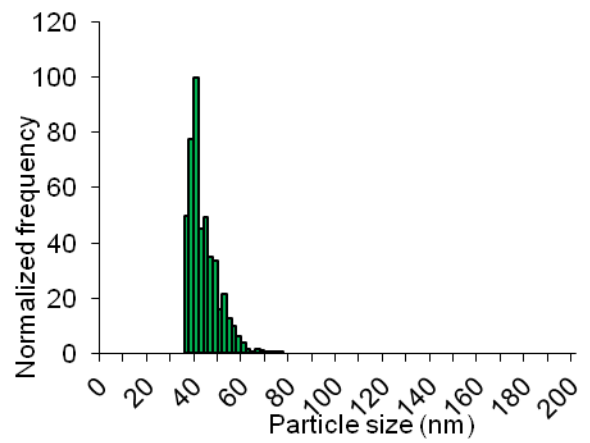


**Fig. S4.** SP-ICP-MS nAg size distributions data of several sample fractions of the ENP mixture in the natural river water (detailed in Table 8).

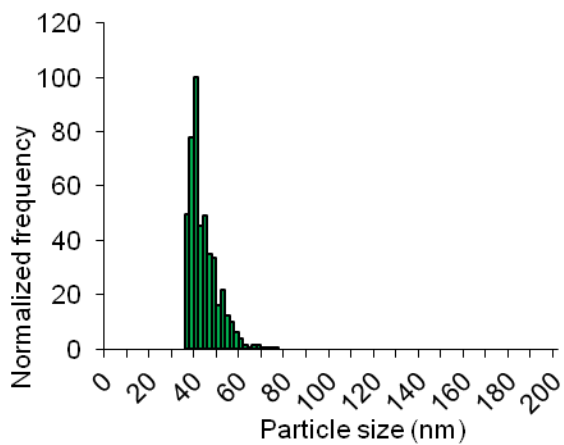
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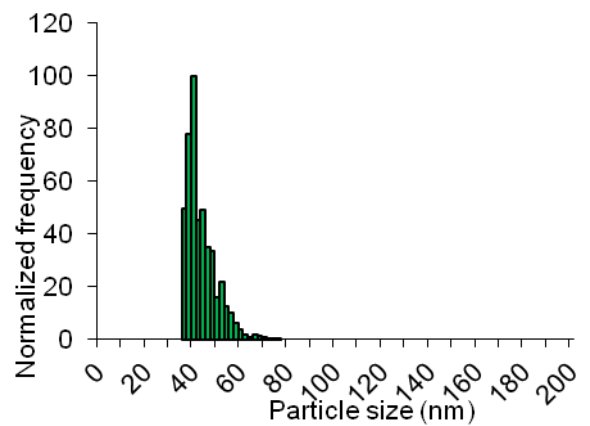
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Fraction #10

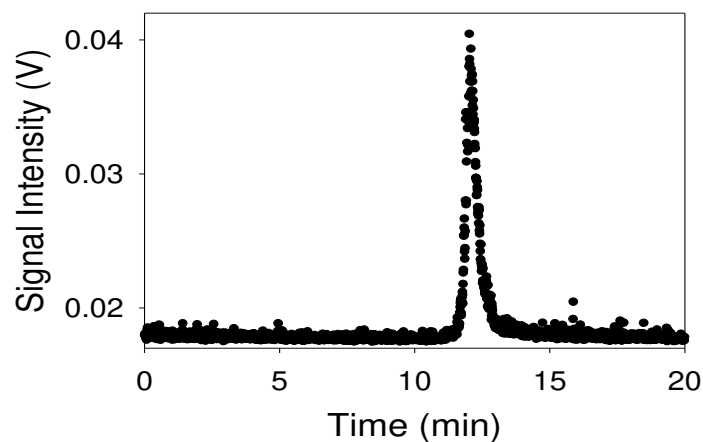


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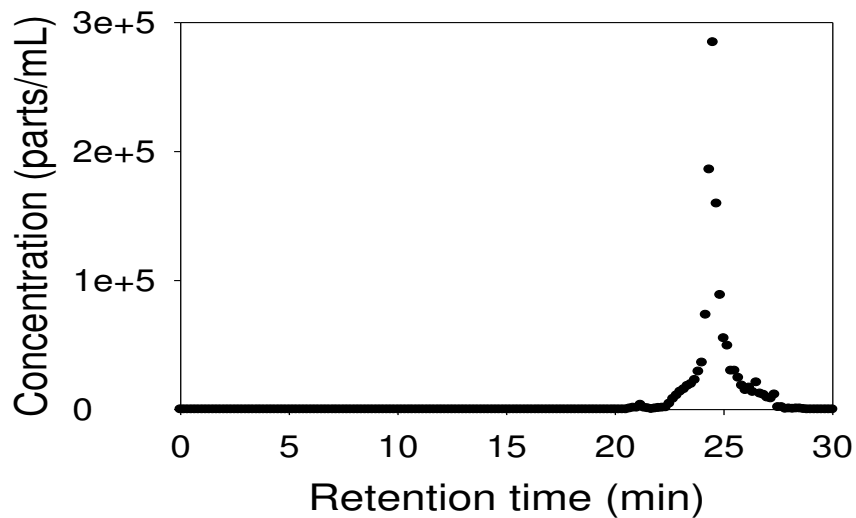


**Fig. S5.** SP-ICP-MS nAg size distributions data of several sample fractions of the ENP mixture in the natural river water (detailed in Table 9).





**Fig. S6.** Chromatogram of river water with no added ENP, obtained using the on-line light scattering signal (DLS) collected at 99°.



**Fig. S7.** Chromatogram of a river water sample spiked with 4  $\mu\text{g L}^{-1}$  of nAg (NanoComposix, nominal radius of 20 nm) following separation by HDC and detection using SP-ICP-MS. A particle radius of  $20.3 \pm 0.6$  nm was determined for the Ag signal detected at 24.7 min using SP-ICP-MS.