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

AF_4-MALS-SP ICP-ToF-MS analysis gives insight into nature of HgSe nanoparticles formed by cetaceans.

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Table S1. Data from AF_4-MALS-ICP-TOF-MS measurement of sperm whale liver. Data processed in open access software created by Lockwood et. al. [16] (Version: spcal_1.1.2). Hg_{mLOD} = 0.04 fg. The 5-minute sub-fractions are shown compared with the larger 15 minute fractions as described in **Figure 1** in the main test.

Fraction (Figure 1)	Sub-fraction	Time/ min	Hg particle count	Hg median mass/ fg
A	1	10-15	108	0.44
	2	15-20	114	0.29
	3	20-25	230	0.23
B	4	25-30	742	0.27
	5	30-35	795	0.37
	6	35-40	515	0.42
C	7	40-45	654	0.41
	8	45-50	133	0.37
	9	50-55	55	0.35

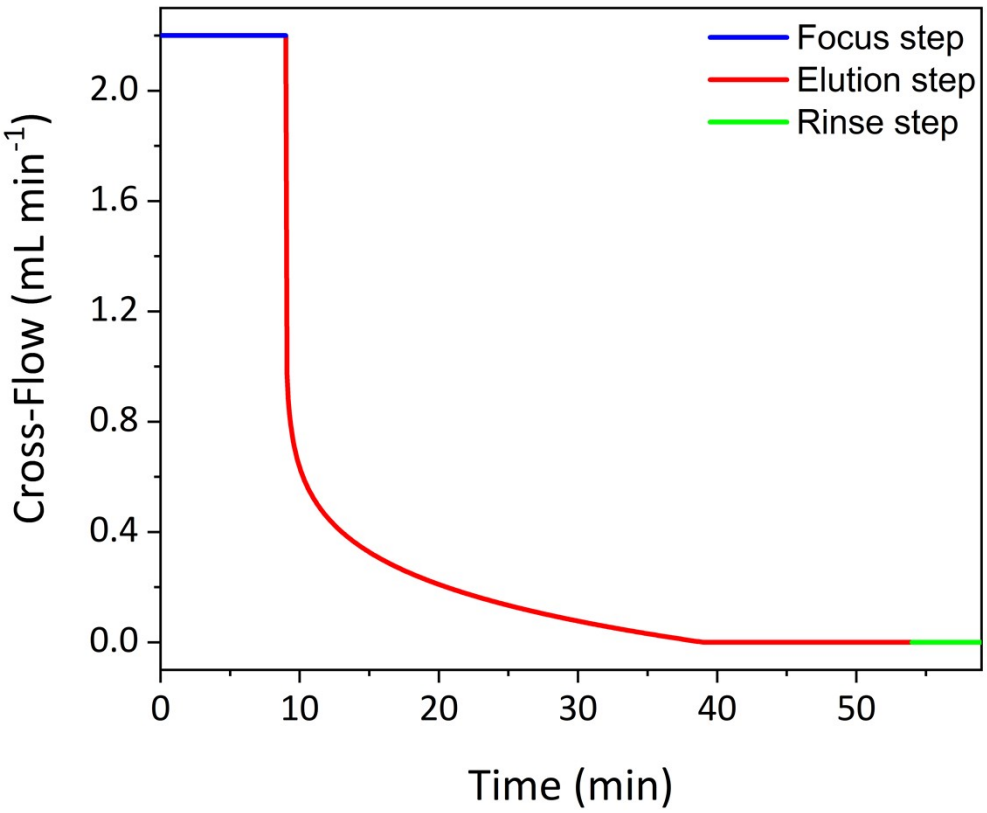

Figure S1. Elution profile used for the fractionation of sperm whale samples. Focus step: focusing time = 8 min, X-Flow = 2.2 mL min^{-1}, injection flow = 0.2 mL min^{-1}. Elution step 1 (Power Decay): elution time = 30 min, X-Flow = 2.2 mL min^{-1}, exponent = 0.1. Elution step 2 (no decay): elution time = 15 min, X-Flow = 0 mL min^{-1}.

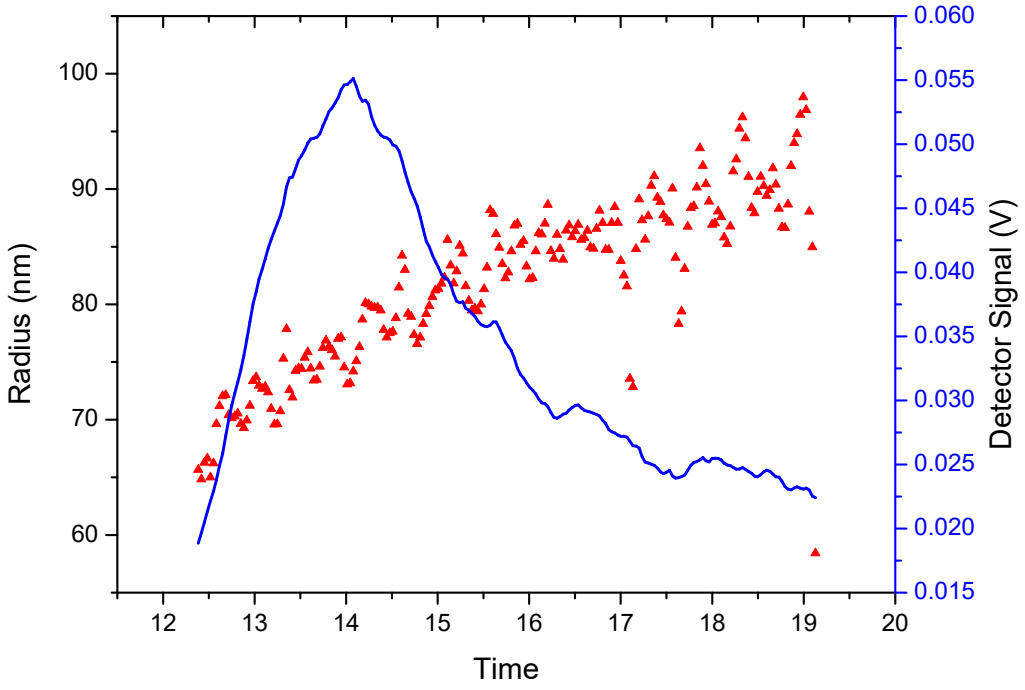

Figure S2. MALS signal corresponding to the first peak shown and discussed in **Figure 1,** coming between 12 and 20 minutes into the separation. The red triangles represent each radius of gyration detected, showing that radius of gyration increases with increasing fractionation time.

Figure S3. Code applied in R for the statistical analysis of data produced by AF_4-MALS-spICP-ToF-MS, data is shown in **Table S1** and **Figure S5**

```
#number of bootstrap samples
iter <- 10^4

#vector for storing bootstrap results
rdif <- rep(NA,iter)

#number of observations, data set 1 (df1)
n1 <- dim(df1)[1]

#number of observations, data set 2 (df2)
n2 <- dim(df2)[1]

#calculation of iter bootstrap samples of differences in slopes
for (i in 1:iter) {

 #ith bootstrap sample of df1 of size n1 with replacement)
 sub1 <- df1[sample(n1, n1,replace = T), ]

 #ith bootstrap sample of df1 of size n2 with replacement)
 sub2 <- df2[sample(n2, n2,replace = T), ]

 #applying linear regression to ith bootstrap sample of df1
 lms1 <- lm(y~x,data=sub1)

 #applying linear regression to ith bootstrap sample of df2
 lms2 <- lm(y~x,data=sub2)

 #calculation and storage of ith slope difference (slopes extracted
 #from lms1 and lms2)
 rdif[i] <- (lms1$coefficients[2] - lms2$coefficients[2])

}

#calculation of bootstrap p-value: p-value = 2*minimum(nr. of slope
#differences greater than zero,  nr. of slope differences less or
#equal than zero)/(nr. of bootstrap iterations)

min(2*sum(rdif > 0, na.rm = T)/iter,2*sum(rdif <= 0, na.rm =
T)/iter)
```

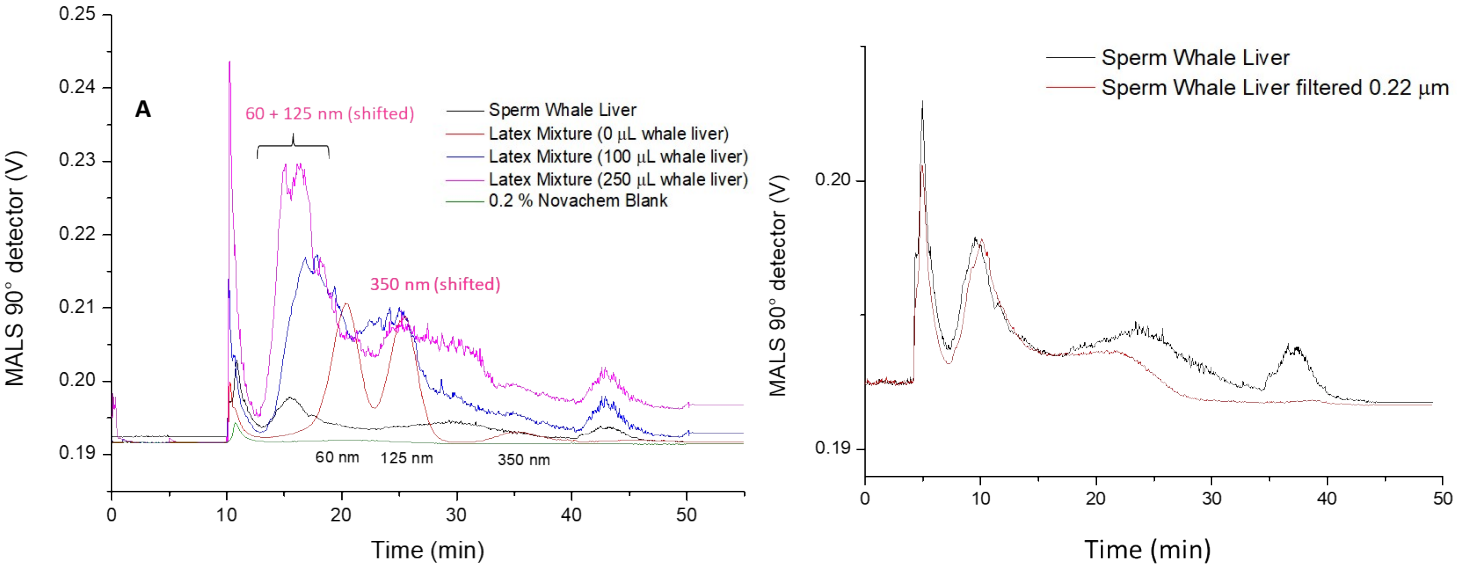

Figure S4. A) Fractograms produced from analysis of: 0.2 % novachem blank (green), sperm whale liver digest (black), latex standard mixture prepared in 0.2 % novachem (red), and two latex mixes which were spiked with whale digest to identify peak shifting caused by the complex matrix (blue, 1:10 whale digest: latex mixture; pink 1:4 whale digest: latex mixture). The final peak eluting after the cross flow was not impacted by the matrix effect. **B)** Comparison of sperm whale liver digest with (red) and without (black) filtering prior to measurement with a 220 nm filter, showing a drop off in signal following the first peak and just prior to the peak identified at around 300 nm in size.

Method validation with pilot whale liver tissue

The analysis was conducted as part of a pilot study project between the University of Aberdeen and TOFWERK Switzerland. Measurements were done in March 2016. The experiments were planned and executed by Dr. Olga Borovinskaya at TOFWERK using an ICP-TOFMS (icpTOF R, TOFWERK, Switzerland) equipped with the standard glass nebulizer and cooled spraychamber configuration. This ToF-MS was equipped with an MCP type detection system providing 6 orders of magnitude signal detection range of proven linearity. The extraction period of this instrument was 30 µs. For interference control the reaction cell was pressurized with H_2.

Samples were prepared from an enzymatic digestion of tissue sample and pre-concentrated using a 50 kDa centrifugal filter unit. Filters were washed using ammonium acetate buffer. The resulting sample solution was diluted with milliQ water prior to the measurements with the ICP-ToF-MS. Quantification of analyte mass per particle was done according to Pace et al. [25] using the following reference materials and wash solutions:

- Standard with 60 nm Au particles diluted in H_2O
- Mixture of Fe, Cd, Te, Se, S, Hg single element solution in H_2O at different concentrations
- Au solutions in H2O
- MilliQ H_2O as blank
- 5% HNO_3 as washing solution

The MS signal was recorded using triggered acquisition and 30 µs integration times (particles) and un-triggered with 1 s integration time for blank and ionic solutions. The sample uptake rate was 0.4 mL min^{-1}. Transport efficiency was determined with Au 60 nm NPs (NIST 8013) and Au solution (using known particle size/mass, not particle number concentration) to find absolute mass of analyte from solution effectively entering the plasma. Particle signals were evaluated using Tofware based on IGOR pro. The algorithms correspond to the ones used in the current icpTOF control software TOFpilot (https://www.tofwerk.com/products/icptof/software/).

Instrumental parameters: RF power = 1550 W, coolant gas flow = 14 L min^{-1}, auxiliary gas flow = 0.8 L min^{-1}, nebuliser gas flow = 1.014 mL min^{-1}, H_2 in reaction cell = 3 mL min^{-1}.

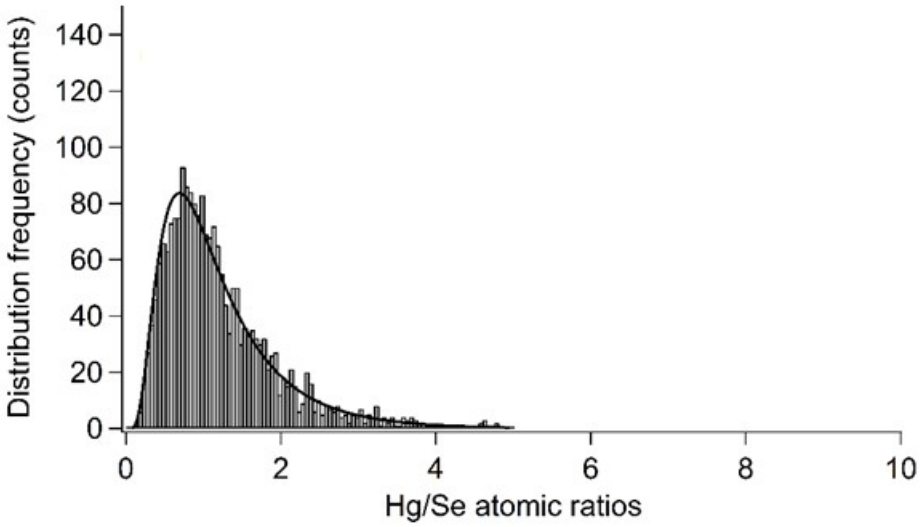

Figure S5. Histogram showing the frequency of particles detected at increasing Hg/Se ratios. The median ratio determined here w8s 0.7:1 (Hg:Se).

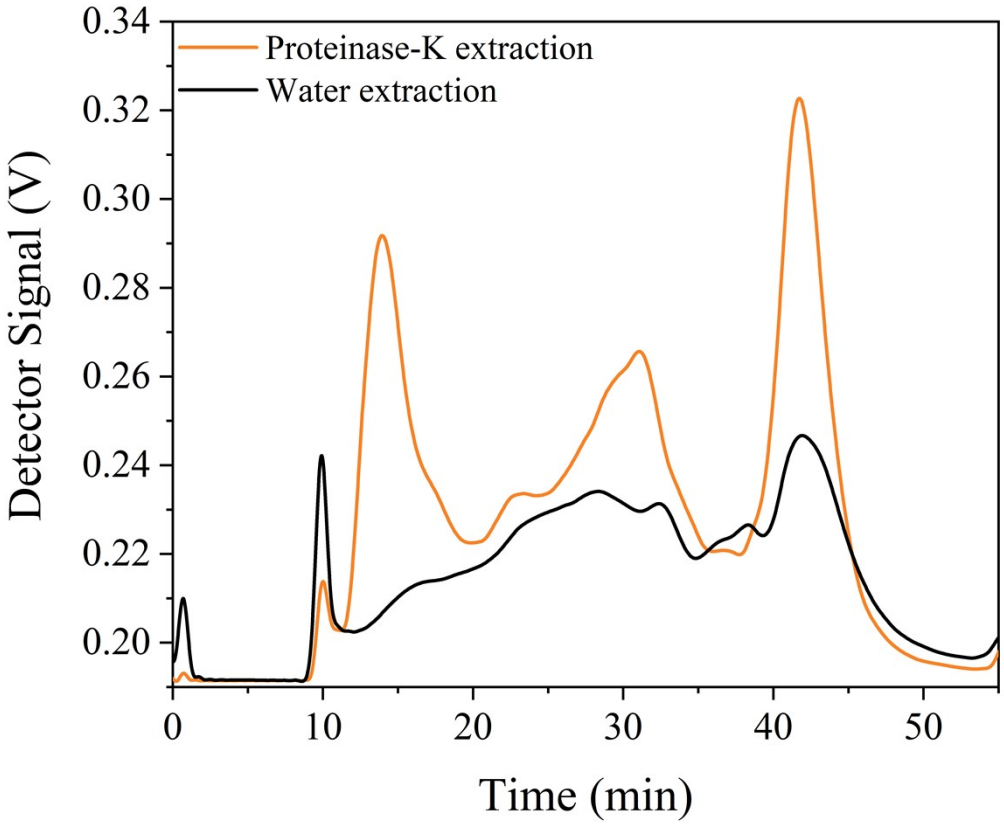

Figure S6. Here a comparison is given between the MALS signals, following separation by AF4, for a water and proteinase K extract. Fractionation was possible without the use of proteinase K, although it would appear many of the smaller particles shifted in retention time with a higher injection peak and a less intense peak at 15 minutes.

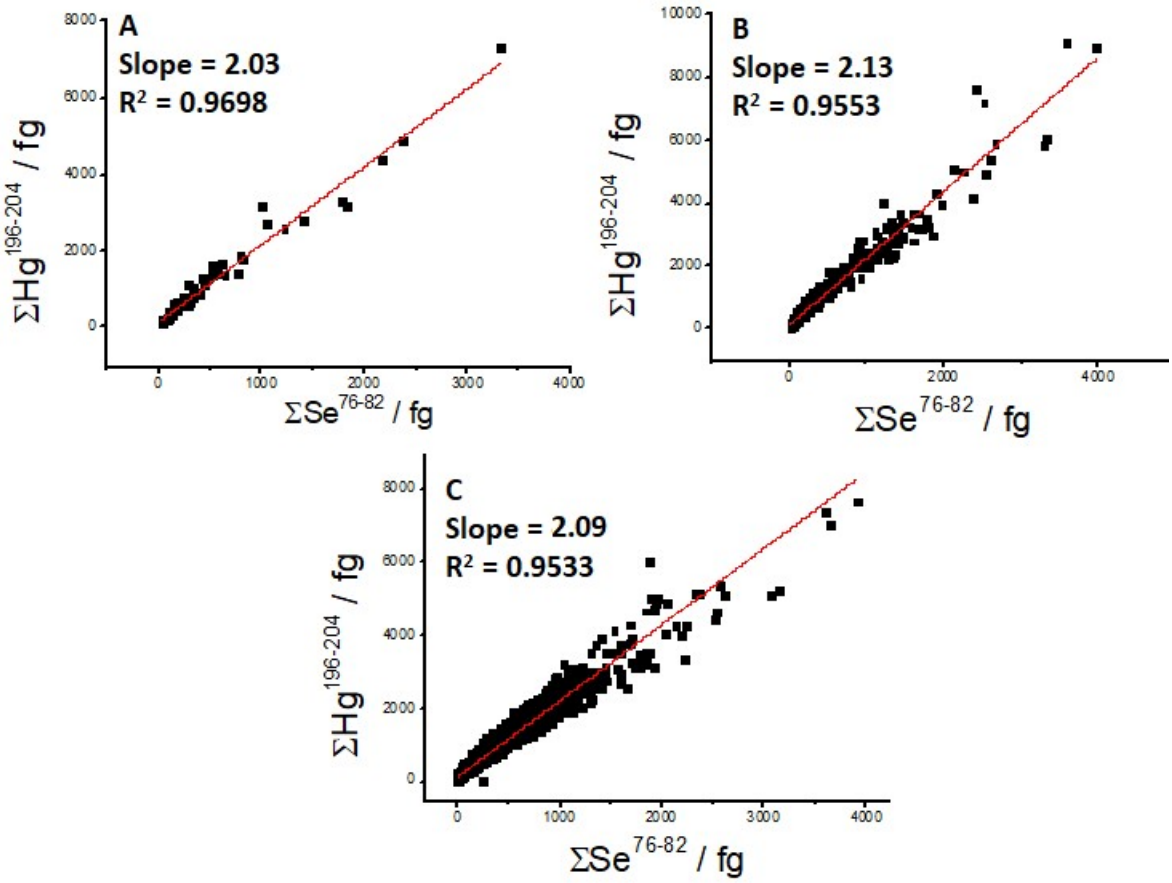

Figure S7. Plot of all particles containing both Hg and Se found across the AF_4-MALS-ICP-ToF-MS measurement. **A)** Hg/Se particles detected in fraction A (10 to 25 minutes, 73 particles) **B)** Hg/Se particles in fraction B (25 to 40 minutes, 416 particles) **C)** Hg/Se particles detected in fraction C (40 to 55 minutes, 3684 particles). Y axis = Hg mass, X axis = Se mass, per particle. For both Hg and Se the masses presented here were determined from the signal produced after summing the responses from all isotopes.

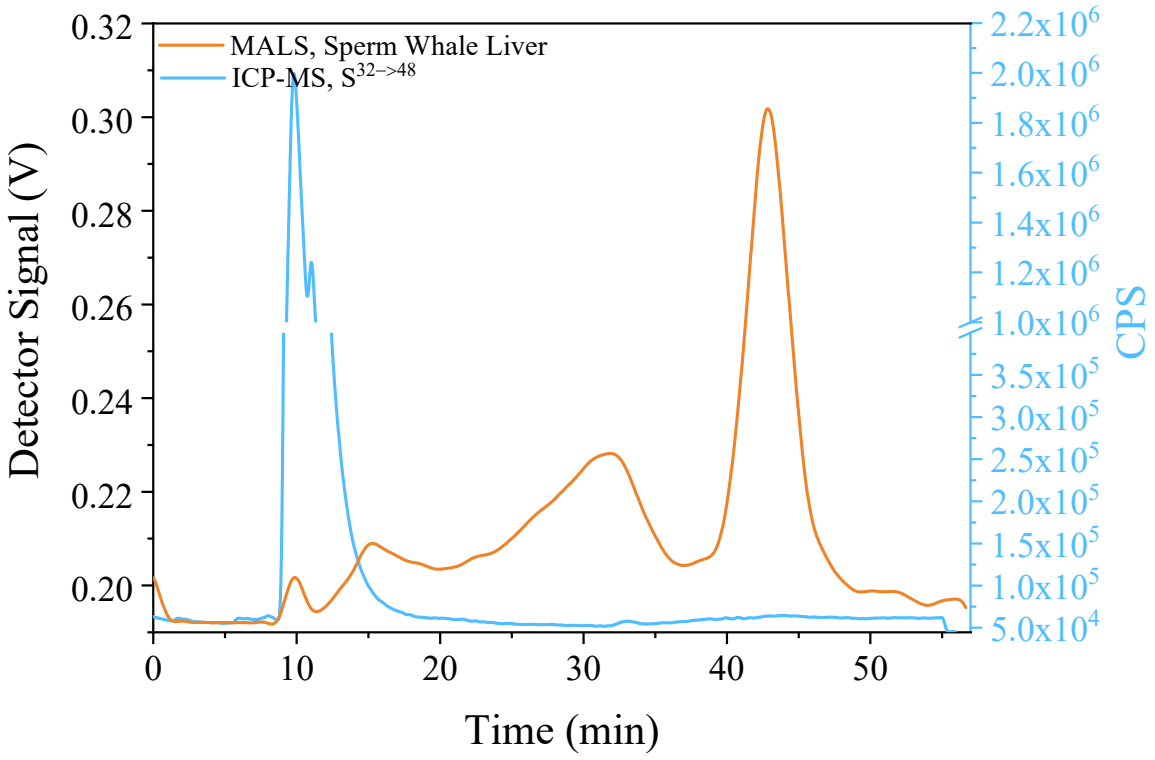

Figure S8. AF_4-MALS-ICP-MS/MS analysis of sperm whale liver sample. The blue line shows the signal form S after mass shifting with oxygen and the orange signature is the result from the MALS signal. Sulphur was found almost entirely In the first five minutes after the end of the focusing time.