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Supplementary Material

Quantification of palladium-labelled nanoplastics algal uptake by single cell and single particle inductively coupled plasma mass spectrometry

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Synthesis of palladium core shell nanoplastic particles

The chemicals used for the synthesis and treatment of the palladium and polyacrylonitrile core shell nanoplastic particles (Pd NPPs) are listed in Table S1. The synthesis was done in a near identical manner to previous work (Mitrano *et al.* 2019), with only minor changes. After the synthesis was complete, the core shell nanoplastic particle suspension was washed and filtered using a stirred cell (MilliporeSigma Amicon Stirred Cell). The suspension was washed and filtered three times. Upon completion, the Pd@polymer core shell particles were stored in an Erlenmeyer flask in the dark at room temperature.

Table S1. Chemicals used in the emulsion polymerisation of Pd-containing polyacrylonitrile core-shell nanoparticles.

Solution	Chemical	Weight or volume	Remarks
A	Distilled Water (PURELAB Option-Q, Type 1, 18 MΩ cm)	20 mL	Mixed and dissolved completely
	Acrylonitrile (MilliporeSigma, ≥ 99.0%)	3.25 g	
	Sodium dodecyl sulfate (SDS) (MilliporeSigma, ≥99.0%)	90 mg	
Initiator	Potassium persulfate (KPS) (MilliporeSigma, ≥99.0%)	62.5 mg	Mixed with 2.5 mL of water and dissolved completely
Stabiliser	Potassium poly(ethylene glycol) 4-nonylphenyl 3-sulfopropylether potassium salt (KPE) (MilliporeSigma, ≥ 99.0%)	45 mg	Mixed with 2.5 mL of water and dissolved completely
Metal	Potassium tetrachloropalladate(II) (abcr Gute Chemie, 99%)	35.4 mg	Mixed with 2.5 mL of water and dissolved completely
B	Distilled Water (PURELAB Option-Q, Type 1, 18 MΩ-cm)	15.25 mL	Mixed and dissolved completely
	Styrene (MilliporeSigma, ≥99.0%)	7.4 g	
	Sodium dodecyl sulfate (SDS) (MilliporeSigma, ≥99.0%)	60 mg	
	Divinylbenzene (MilliporeSigma, ≥60.0%)	22.5 mg	

Cell suspension preparation and analysis

Two freshwater algal cell cultures were used in the exposure experiments, *Cryptomonas ovata* (*C. ovata*) and *Cryptomonas ozolini* (*C. ozolini*). The cells originated from The Culture Collection of Algae at the University of Texas at Austin (UTEX, strain numbers: *C. ovata* UTEX 2783, *C. ozolini* UTEX 2194). *C. ovata* and *C. ozolini* cell cultures were grown in a ThermoFisher incubator at 20°C and a 12-h light:12-h dark cycle until cell concentrations reached ~400,000 cells mL^{-1} (Merrifield *et al.* 2018).

C. ovata and *C. ozolini* cells were grown in Bold 1NV Medium and Modified Bold 3N Medium respectively (UTEX). Once cell suspensions were ready, they were divided into 10-mL aliquots. For each cell type there were four replicates with 0.5 μg L^{-1} Pd NPPs and four replicates without NPPs (blank controls) for a total of 8 samples per cell type. Once the NPPs were added, the samples were stored in the ThermoFisher incubator for 72 hours. After the 72-hour exposure, all sixteen cell suspension samples were prepared for analysis by SC- and SP-ICP-MS.

Figure S1 shows a simplified schematic of the cell preparation steps prior to SC-ICP-MS analysis. Briefly, the 10-mL aliquots of the cell suspension were centrifuged at 500 rpm (*C. ovata*) and 1,000 rpm (*C. ozolini*) three times for fifteen minutes each time. After each centrifugation, the supernatant was removed and collected in separate vials and set aside. The cell pellet was reconstituted twice in fresh growth media and a third time in 1/20 dilute growth media (ultrapure water, 18.2 MΩ cm, Purelab Option-Q). After preparation of the cell suspensions and prior to analysis by SC-ICP-MS, 2.2-mL aliquots of the cell suspensions were collected and preserved with Lugol's iodine solution (VWR) to a final concentration of 2% v/v to count the cells. The fixed cells were maintained in a refrigerator at 4ºC until later analysis (counting). Following the third centrifugation and resuspension of the cell pellet in 1/20 dilute media, the cell suspensions were immediately analysed by SC-ICP-MS. Immediately following SC-ICP-MS analysis of the cells, the supernatant samples that had been set aside were diluted 1/10 (ultrapure water, 18.2 MΩ cm, Purelab Option-Q) and analysed by SP-ICP-MS. Cells were later counted using a Nikon Eclipse TS 100 inverted microscope at 40X power according to the Utermöhl method (Utermöhl 1931). It is known from optimising cell suspension preparation that 80% of cells are lost during the cell suspension preparation steps (centrifugation and reconstitution of the cell pellet). Of the cells that remain, approximately 8% of *C. ovata* and *C. ozolini* cells nebulised into the spray chamber and injector enter the plasma, with the rest going out as waste. Furthermore, 85% of cells that enter the plasma are non-lysed while the other 15% are lysed.

The SC-ICP-MS parameters were modified from those in Merrifield *et al.* (2018). The sample introduction system consisted of a PerkinElmer Asperon spray chamber, high efficiency nebuliser (HEN, Meinhard) and 2-mm quartz injector (Elemental Scientific). Prior to analysis, the ICP-MS was tuned with a 1-ppb multi-element PerkinElmer SmartTune solution. The transport efficiency was determined using a 60-nm citrate-coated gold nanoparticle (nanoComposix, AUCN60) standard diluted to a concentration of 200,000 particles mL^{-1}. The analytical run conditions including the nebuliser gas flow, additional gas flow, sample flow rate, transport efficiency and tune parameters can be found in Table S3.

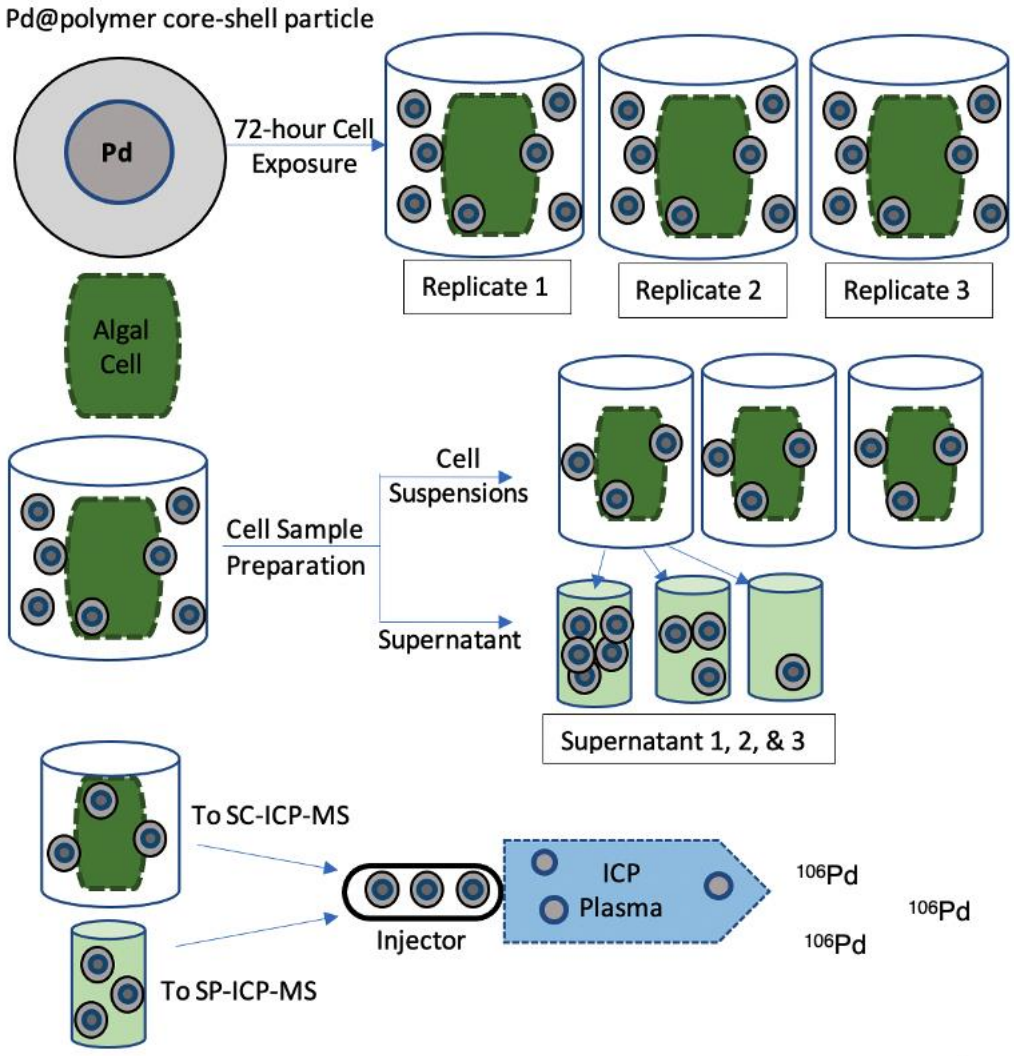

Figure S1. Simplified schematic showing the procedure for exposing triplicate cell suspensions to nanoplastic particles. The triplicate cell suspensions were centrifuged three times and the supernatant collected from each wash step. The cell suspensions were analysed by SC-ICP-MS and the supernatant was analysed by SP-ICP-MS.

Particle characterisation

Figure S2 shows the mean hydrodynamic size of the nanoplastic particles analysed by dynamic light scattering (DLS). Five consecutive measurements were recorded and averaged to calculate a *z*-average size. Additionally, the size of the nanoplastic particles was measured by transmission electron microscopy (TEM). The TEM samples were prepared by partially drying a drop of the cleaned particle suspension on a copper grid (300 mesh) coated with a continuous carbon support film at room temperature. The grid was washed thoroughly with ultrapure water (PURELAB Option-Q, 18.2 MΩ cm) before the original sample dried and was only then fully dried (Tejamaya *et al.* 2012). The morphologies and core-shell structure of the core shell NPs were quantified by analysing images captured by Hitachi H-8000 TEM (Figure S3) and the core shell structure was confirmed with TEM-EDX (Figure S4). Size distributions were collected by measuring greater than 100 nanoplastic particles (NPPs) using *ImageJ* software. The palladium (Pd) nanoplastic core shell particles had a 42-nm total diameter with a 10-nm Pd and polyacrylonitrile (PAN) core. This was in good agreement with the *z* average size by DLS which was 50 nm (Figure S2). Based on a 10-nm diameter of the Pd and PAN core, the total mass of Pd (ag) was calculated using Eqn S1. Equation S1 combines two equations; one is the volume of a sphere using diameter ($V = \frac{1}{6}\pi d^3$) and the second is density ($\rho = \frac{m}{V}$). The density (ρ) of palladium is 12.023 g cm^{-3}.

$$\text{Mass of Pd} = 12.0 \times \frac{1}{6}\pi D^3 \times \frac{1e^{18}}{1} \quad \text{(S1)}$$

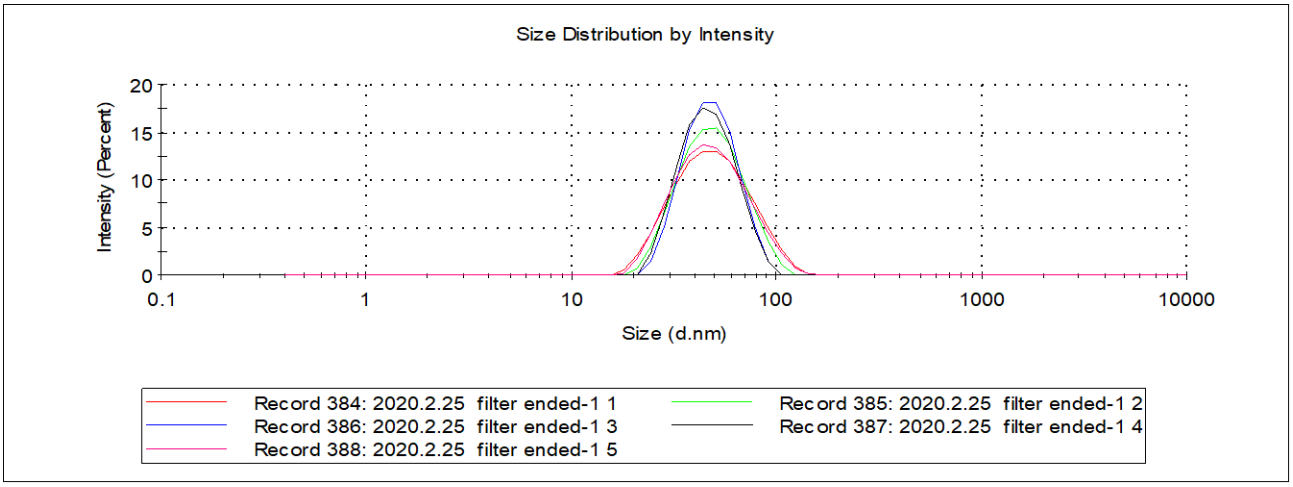

Figure S2. Size distribution of the palladium and PAN nanoplastic core shell particles using dynamic light scattering (DLS).

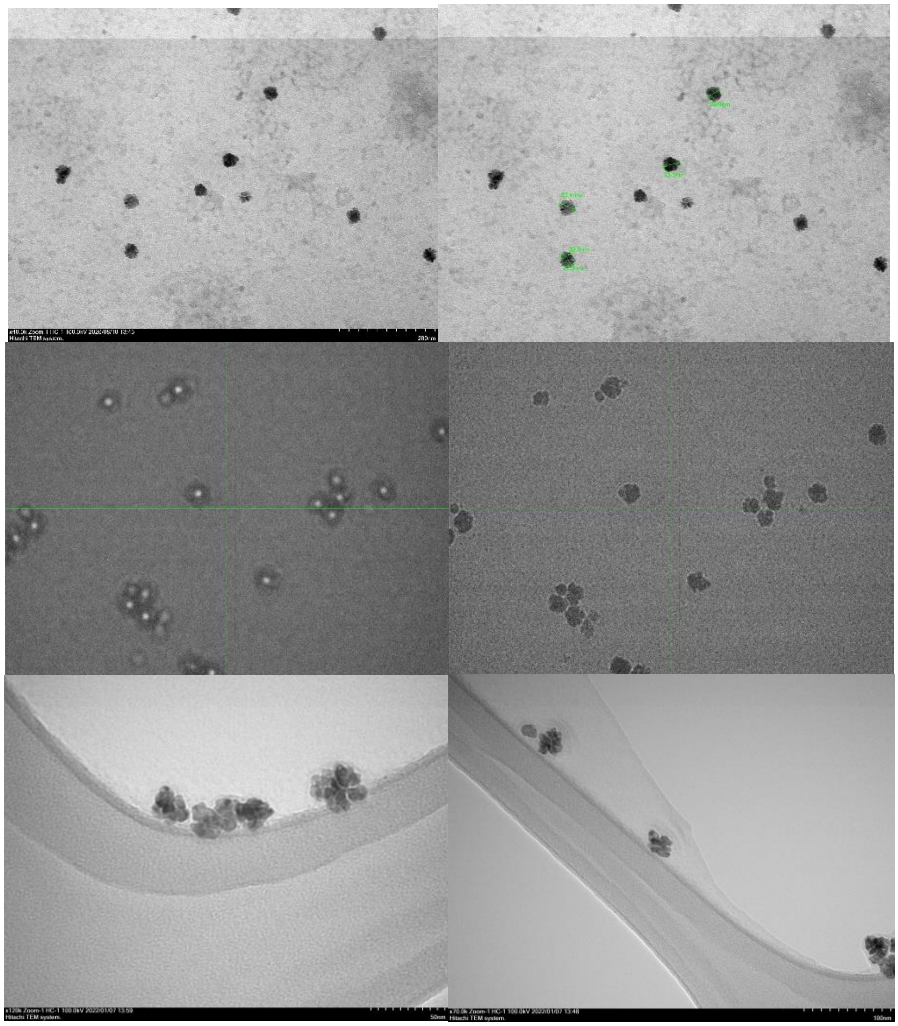

Figure S3. TEM images showing the morphologies of the Pd core shell nanoplastics. The images clearly show clusters of particles.

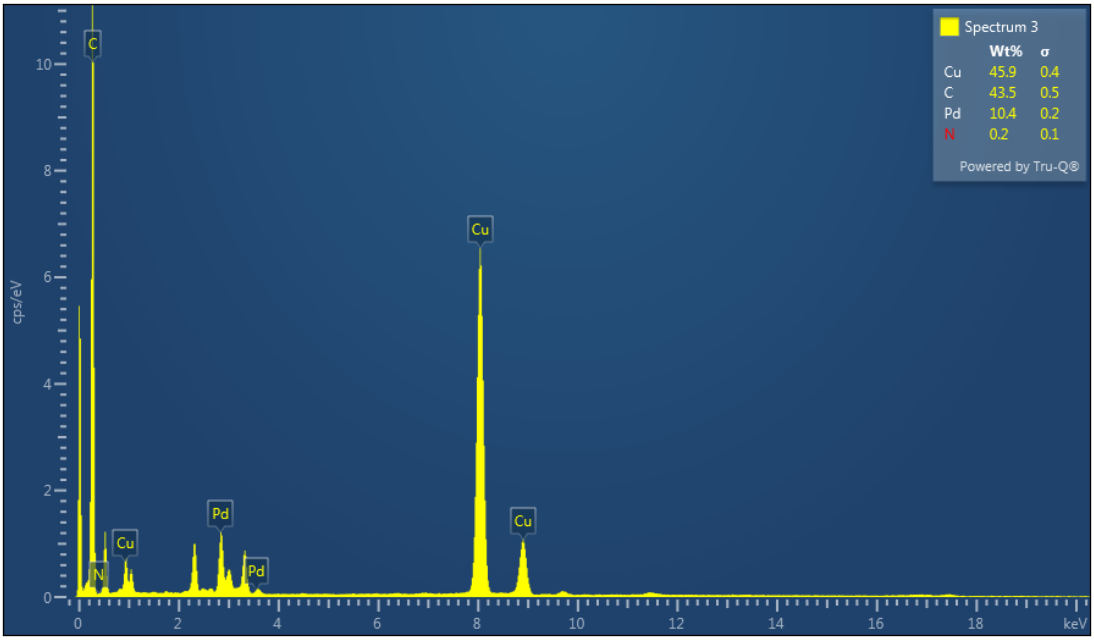

Figure S4. The data of TEM-EDX.

Instrumentation parameters

Prior to analysis by SP-ICP-MS, the ICP-MS was tuned with a 1-ppb multi-element PerkinElmer SmartTune solution. The analytical run conditions including the nebuliser gas flow, sample flow rate, transport efficiency and tune parameters are shown in Table S2. Due to the unavailability of a suitable reference standard for the 10-nm Pd NPP, a 60-nm citrate-coated gold nanoparticle (nanoComposix, AUCN60) was used as both a particle standard (200,000 particles mL^{-1}) and for the transport efficiency.

Table S2. Single Particle ICP-MS analysis parameters including the nebuliser gas flow, sample flow rate, tune parameters, transport efficiency and part numbers.

Parameter	**Setting**
Nebuliser	Meinhard quartz, Type A, 1.0 L min^{-1}
Spray chamber	Quartz cyclonic spray chamber
Injector	2.0 mm of quartz for NexION 350D
Torch	Standard quartz for NexION 350 D
Nebuliser gas flow	1.01 mL min^{-1}
Sample flow rate	0.3 mL min^{-1}
Peristaltic pump speed	24 rpm
Dwell time	100 µs
Scan time	100 s
Transport efficiency	4.0%
Beryllium (9)	6037.68
Indium (115)	99226.78
Uranium (238)	144006.01
Cerium oxide/cerium	0.017
Cerium ++/cerium	0.02

Table S3 shows the SP-ICP-MS data for the NPPs in the supernatant samples collected from the wash steps for both cell types. Shown in Table S3 are the mean and standard deviation for the Pd nanoparticle size (nm), number of peaks and mean intensity (counts). For each parameter, there is a decrease with each wash step for both the *C. ozolini* and *C. ovata* supernatant samples. The SP-ICP-MS data show the successful removal of Pd NPPs in the cell suspension that are not cell associated, either bound to the cell wall or taken up by the cell. As Pd NPPs are washed out of the cell suspension, either the aggregates become increasingly smaller or the Pd NPPs become more dispersed in the supernatant. This is further supported by the decrease in the number of peaks and mean counts. The SP transport efficiency is a ratio of the number of particles detected over the number of particles introduced into the plasma and ionised. The PerkinElmer Syngistix Nano application software calculates TE (%) using Eqn S2.

$$\mathrm{TE} = \frac{\text{Number of Peaks}}{(\text{Flow Rate} \times \text{Particle Concentration})} \times 100 \qquad \text{(S2)}$$

A 60 nm citrate-coated gold nanoparticle (nanoComposix, AUCN60) standard diluted to a concentration of 200,000 particles mL^{-1} was used to determine the TE. Since the TE is not dependent on the type of inorganic nanoparticle used, the TE could be applied to Pd NPPs.

Table S3. Single particle data for two algal cell types, *Cryptomonas ozolini* (*C. ozolini*) and *Cryptomonas ovata* (*C. ovata*) exposed to synthetic palladium@polymer core shell nanoparticles.

Algal cell type	Replicate	Mean size (nm)	Number of peaks	Mean intensity (counts)
Cryptomonas ozolini	Supernatant 1 Mean (±s.d.) *n* = 2	54.9 (0.4)	7,208 (162)	25 (0.6)
	Supernatant 2 Mean (±s.d.) *n* = 3	41.5 (2.1)	2,693 (1,746)	12.3 (3.7)
	Supernatant 3 Mean (±s.d.) *n* = 3	32.7 (5.0)	1,258 (786)	6.1 (2.1)
Cryptomonas ovata	Supernatant 1 Mean (±s.d.) *n* = 3	53.9 (0.4)	6,857 (1,734)	23.6 (0.5)
	Supernatant 2 Mean (±s.d.) *n* = 3	35.0 (0.5)	2,390 (454)	7.3 (0.3)
	Supernatant 3 Mean (±s.d.) *n* = 3	38.7 (6.6)	397 (258)	10.1 (4.2)

The data reflect the measurements made by single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) using the PerkinElmer Syngistix Single particle software application. The data show the mean and standard deviation for the nanoparticle size (nm), number of peaks, and mean intensity (counts). Particles measured in Supernatant 3 are approaching the size detection limit for both cell types, 31 ± 4 nm for *C. ozolini* and 32 ± 1 nm for *C. ovata*.

Table S4 shows the SC-ICP-MS data for the cell-associated NPPs from the 72-hour exposure for both cell types. It includes the mean and standard deviation for the mass of Pd (attograms) per cell, number of cells counted by microscopy (cells mL^{-1}), and cell concentration (particles mL^{-1}). Prior to analysis, the ICP-MS was tuned with a 1-ppb multi-element PerkinElmer SmartTune solution. The analytical run conditions including the nebuliser gas flow, sample flow rate, transport efficiency and tune parameters can be found in Table S5.

Table S4. Single cell inductively coupled plasma mass spectrometry (SC-ICP-MS) data for two algal cell types, *Cryptomonas ozolini* (*C. ozolini*) and *Cryptomonas ovata* (*C. ovata*) exposed to synthetic palladium@polymer core shell nanoparticles.

Algal cell type	Replicate	Mean mass (ag)	Number of cells (cells/mL)	Number of peaks	Concentration (particles/mL)
Cryptomonas ozolini	Mean (±s.d.) *n* = 3	1,785 (176)	52,556 (19,551)	422 (102)	41,312 (9,994)
Cryptomonas ovata	Mean (±s.d.) *n* = 3	1,802 (222)	32,248 (6,134)	344 (99)	33,643 (9,676)

The number of cells (cells mL^{-1}) were determined by counting the cells using an inverted microscope. The particles per cell (particles $cell^{-1}$) were calculated by dividing the cell concentration (particles mL^{-1}) by the number of cells per millilitre (cells mL^{-1}).

Similar to SP-ICP-MS, the transport efficiency (TE) is a measure of the number of cells detected over the number of cells introduced into the plasma and ionised. The PerkinElmer Syngistix Single Cell application software calculates TE (%) the following way:

$$\mathrm{TE} = \frac{\text{Number of Peaks}}{(\text{Flow Rate} \times \text{Cell Concentration})} \times 100 \quad \text{(S3)}$$

The same 60-nm citrate-coated gold nanoparticle (nanoComposix, AUCN60) standard diluted to a concentration of 200,000 particles mL^{-1} was used to determine the TE for SC-ICP-MS.

Table S5. Single Cell ICP-MS analysis parameters including the nebuliser gas flow, additional gas flow, sample flow rate, tune parameters, transport efficiency and part numbers.

Parameter	Setting
Nebuliser	Meinhard Model: HEN-90-A0.2
Spray chamber	PerkinElmer Asperon
Injector	2.0 mm of quartz (ESI)
Torch	Standard quartz for NexION 350 D
Nebuliser gas flow	0.4 mL min^{-1}
Additional gas flow	0.65 mL min^{-1}
Sample flow rate	0.074 mL min^{-1}
Peristaltic pump speed	5 rpm
Dwell time	50 µs
Scan time	60 s
Transport efficiency	13.8%
Analog stage voltage	2800 V
Beryllium (9)	2154.36
Indium (115)	41555.62
Uranium (238)	49082.31
Cerium oxide/cerium	0.019
Cerium ++/cerium	0.09

References

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