Supporting Information

Separation, Sizing, and Quantitation of Engineered Nanoparticles in an Organism Model Using Inductively Coupled Plasma Mass Spectrometry and Image Analysis

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Optimization of TMAH digestion of AuNPs and biological tissue. An organic, base digestion was performed using tetramethylammonium hydroxide (TMAH). Prior to quantitative extraction of intact AuNPs in the presence of dried nematodes, digestion concentrations were initially tested in terms of base strength and digestion efficiency. Concentrations of 1 %, 3 %, 5 %, 7 %, and 10 % (w/w) were assessed for the optimal digestion concentration. A 1 mL portion of each concentration was added to 0.1 mg of dry, un-exposed nematodes, vortexed for 30 s, and left to react ≈ 2 h. After the digestion period, the samples were observed under an optical microscope. A successful digestion of nematode samples, denoted by digest with no visible intact nematodes and minimal debris, was obtained for the 7 % and 10 % (w/w) TMAH concentrations. In an attempt to elicit the effect of solely the TMAH digestion process on the measured PSD and number concentration, the nominal 30 nm and 60 nm AuNPs used in this study were digested with 7 % and 10 % (w/w) TMAH in the complete absence of biological material and then analyzed by spICP-MS. Single particle ICP-MS measurements of both the 7 % and 10 % (w/w) TMAH AuNP digests showed an elevated dissolved Au signal and a loss of 85 % of the expected particle events (data not shown), indicating that pristine AuNPs behave differently from AuNPs in nematodes when exposed to the TMAH concentrations used for the digestion. Such results stressed the need for an alternative strategy to study the effect of the TMAH digestion process on pristine AuNPs versus the AuNPs liberated from *C. elegans*. Using the values calculated from the total Au experiments (See Equations section of the Supporting Information) we found that the number of AuNPs extracted from the dried exposed samples was approximately 100 times greater than the desired optimal spICP-MS particle number concentration (15,000 particles/mL). Therefore, we adjusted the TMAH concentration by 100 fold to 0.07 % (w/w) to test the effect of TMAH digestion on AuNPs absent nematode tissue. A portion of 1 mL 0.07 % (w/w) TMAH was added to the AuNP stock, allowed to digest for 2 h at room temperature, and then was diluted accordingly with water to reach a NP concentration of 15,000 particles/mL. We then investigated whether there was any transformation of the AuNPs by TMAH by monitoring the background signal, the accuracy of size analysis, and the recovery for the AuNPs. The background signal remained low, the size distributions were in agreement with TEM values found in the reports of investigation; and a quantitative recovery for AuNPs of both sizes was obtained. A particle number concentration recovery (average ± SD) of 94.7 % ± 9.8 % and 101.7 % ± 13.6 % was achieved for the 30 nm and 60 nm AuNPs, respectively, used in this study (n = 9 replicate sample digestions). Therefore, the 0.07 % (w/w) and 7 % TMAH (w/w) concentrations were selected as the most appropriate TMAH digestion concentrations for AuNPs and *C. elegans* tissue, respectively.

Equations for the estimation of cuticle-adsorbed AuNPs and ingested AuNPs per *C. elegans*.

$$C_{Au} = \frac{V_{AuNP} * \rho_{Au} * N_{C.\ elegans}}{m_{C.elegans}} \quad (1)$$

C_{Au} = the concentration of Au per *C. elegans* (w/w) as determined by ICP-MS and expressed as in ng per g *C. elegans*

V_{AuNP} = the volume of the exposure AuNP

ρ_{Au} = density of gold in g/cm^3

$N_{C.\ elegans}$ = the number of particles associated with the dry *C. elegans* pellet in particles per g *C. elegans*

$m_{C.\ elegans}$ = approximated mass of 1 *C. elegans,* estimated as the average mass of the dry nematode pellet, in g, in the control experiment divided by a 100,000 nematode exposure population (average number of *C. elegans* per exposure replicate, ≈ 1.65 x10^{-8} g)

Rearranging eq. (1), the number of particles associated with the dry *C. elegans* pellet is then,

$$N_{C.elegans} = \frac{C_{Au} * m_{C.elegans}}{V_{AuNP} * \rho_{Au}} \quad (2)$$

The volume of a spherical particle, V_{AuNP}, is presented as:

$$V_{AuNPs} = \left(4/3\right)\pi(d/2)^3\rho_{Au} \quad (3)$$

where d = the TEM diameter of the nanoparticle. Substituting eq. (3) into eq. (2) gives:

$$N_{C.elegans} = \frac{6 * C_{Au} * m_{C.elegans}}{\pi d^3 \rho_{Au}} \quad (4)$$

Table S1. Relative % Au found in gradient layers following centrifugation of a mixture of 30 nm and 60 nm AuNPs (NIST) at 100 ng mL^{-1}.

	Relative % Au			
Gradient Layer	Sample 1	Sample 2	Sample 3	Sample 4
13	23.8	23.0	20.9	20.1
12	20.3	21.3	20.7	21.5
11	18.7	19.0	19.8	20.4
10	16.3	18.1	18.5	18.4
9	8.8	7.3	7.9	7.9
8	3.6	3.3	3.6	3.5
7	2.5	2.4	2.7	3.0
6	1.6	1.5	1.7	1.6
5	0.9	1.0	0.8	0.9
4	0.8	0.7	0.8	0.6
3	0.6	0.6	0.8	0.5
2	0.5	0.6	0.6	0.2
1	1.5	1.2	1.1	1.4

Table S2. Relative % Au found in gradient layers following a 0 h nematode exposure to a mixture of 30 nm and 60 nm AuNPs (NIST) at 100 ng mL^{-1}. *Indicates the location of nematodes within the gradient after centrifugation.

	Relative % Au			
Gradient Layer	Sample 1	Sample 2	Sample 3	Sample 4
13	23.5	18.7	18.2	17.9
12	23.6	24.8	27.3	26.8
11	20.7	20.3	23.8	21.5
10	15.9	18.1	17.8	17.8
9	6.8	8.2	5.3	7.4
8	2.9	3.3	2.6	3.0
7	2.2	2.3	1.7	1.9
6*	1.6	2.1	1.6	1.8
5*	0.9	0.5	0.5	0.4
4*	0.6	0.4	0.2	0.3
3	0.4	0.4	0.4	0.3
2	0.4	0.3	0.2	0.3
1	0.6	0.7	0.3	0.7

Table S3. Relative % Au found in gradient layers following a 24 h nematode exposure to 60 nm AuNPs (NIST) at 333 ng mL^{-1}. *Indicates the location of nematodes within the gradient after centrifugation.

	Relative % Au		
Gradient Layer	**Sample 1**	**Sample 2**	**Sample 3**
13	**28.5**	**27.3**	**26.2**
12	**24.8**	**25.4**	**25.8**
11	**21.4**	**20.9**	**21.6**
10	**14.0**	**15.4**	**17.2**
9	**7.3**	**7.2**	**5.7**
8*	**1.6**	**1.3**	**1.3**
7*	**0.7**	**0.6**	**0.8**
6*	**0.7**	**0.7**	**0.6**
5	**0.2**	**0.3**	**0.1**
4	**0.1**	**0.1**	**0.2**
3	**0.2**	**0.3**	**0.1**
2	**0.2**	**0.2**	**0.2**
1	**0.3**	**0.4**	**0.3**

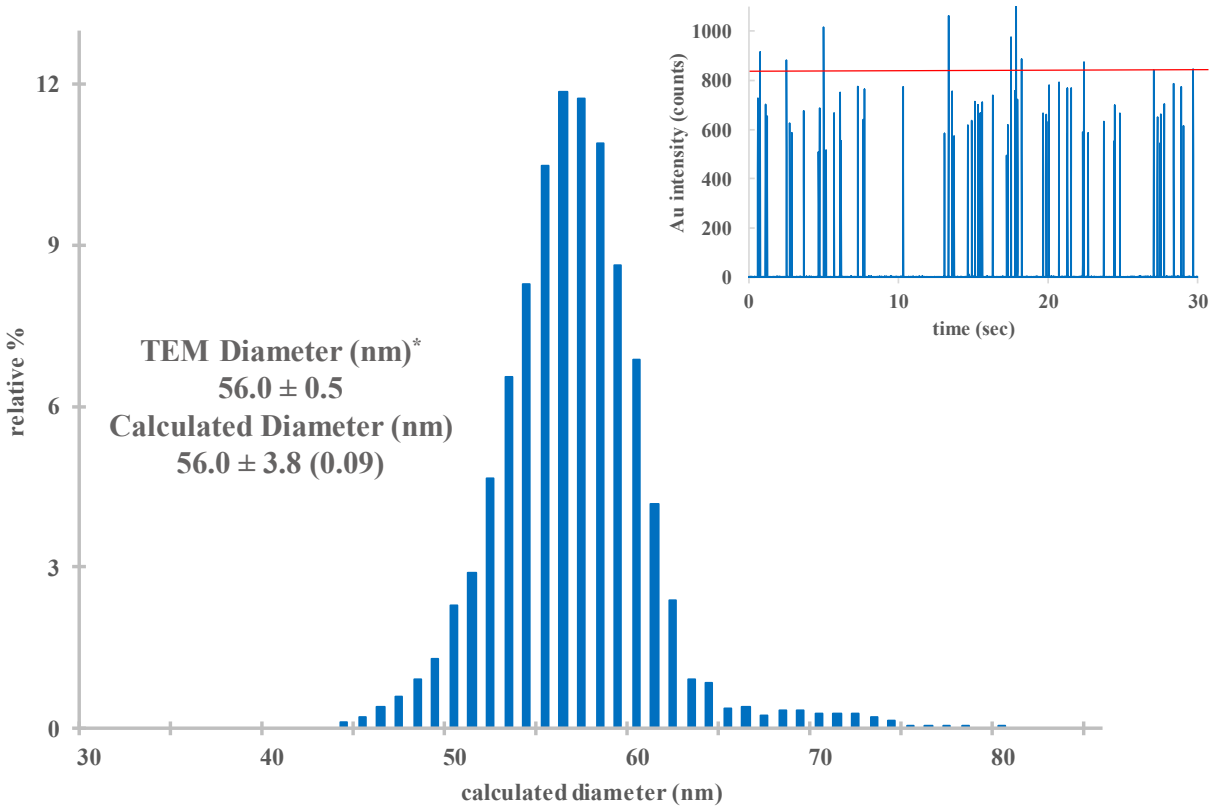

Figure S1. Single particle ICP-MS measured size distribution and representative ^{197}Au time resolved data (inset) of NIST RM 8013 (nominal 60 nm AuNPs). Red bar: average measured particle intensity for 60 nm AuNPs. The uncertainty of the mean value represents one standard deviation. Bin size corresponds to 1 nm. TEM data provided by NIST Report of Investigation for RM 8013. [45]

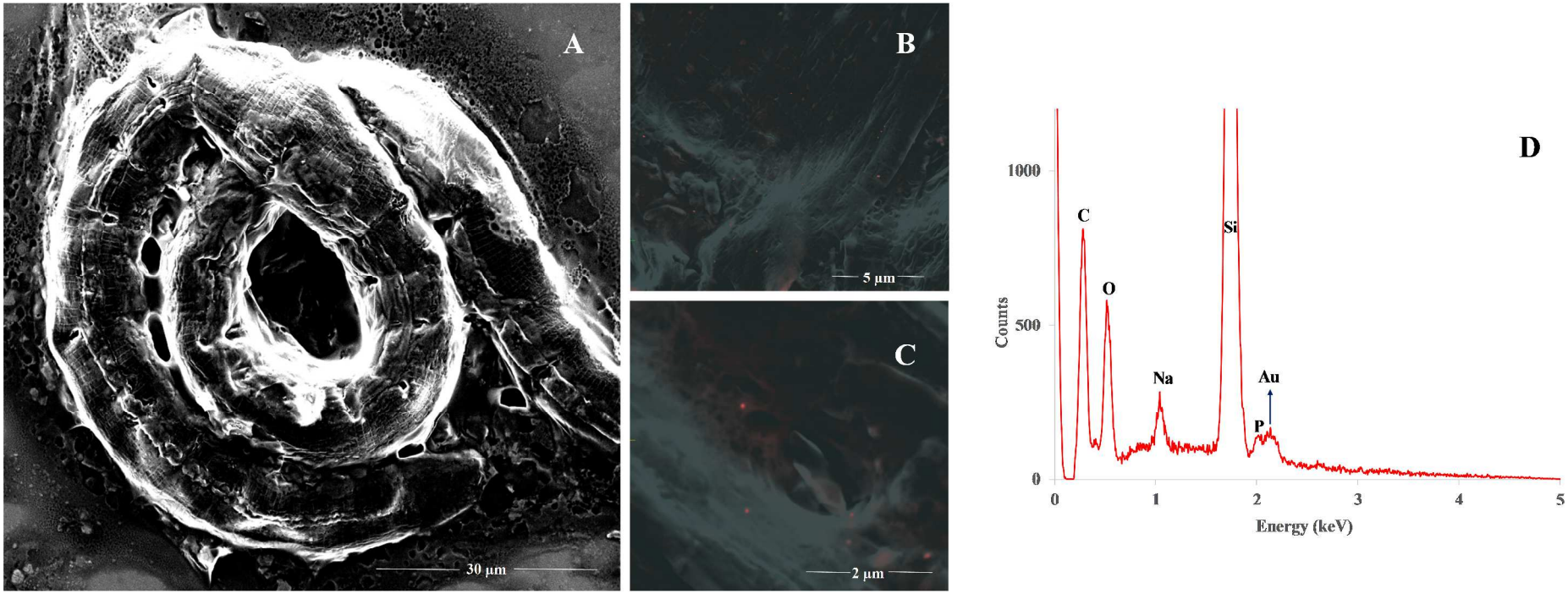

Figure S2. (A) High magnification SEM image of a nematode exposed to 60 nm AuNPs, following by sample processing shown in **Scheme 1. (B,C)**. Bright red spots on cuticle were examined by EDS where the presence of Au was confirmed. **(D)** Energy dispersive X-ray spectrum of cuticle displayed in **(C)**. Note the keV peak associated with the presence of elemental Au at 2.12 keV. *scale bars:* (A) 30 µm, (B) 5 µm, (C) 2 µm.

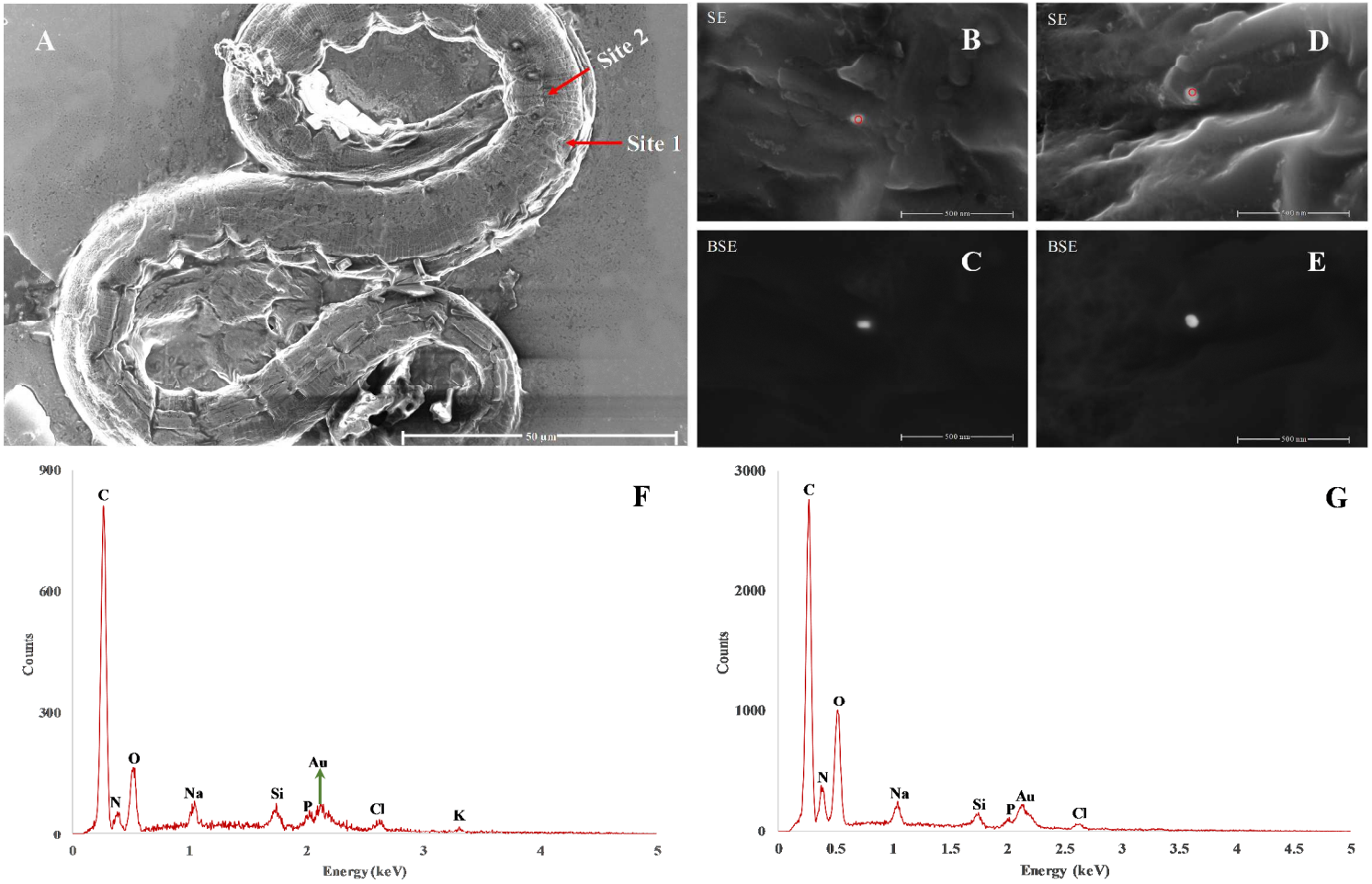

Figure S3. (A) High magnification SEM image of a nematode exposed to 60 nm AuNPs, following by sample processing shown in **Scheme 1.** Site 1 and Site 2 denote areas of exploration in **Figure S3B**, **Figure S3C**, **Figure S3D**, and **Figure S3E**, respectively. Secondary electron (SE) and backscattered electron (BSE) imaging of Site 1 **(B,C)**.and Site 2 **(D,E)** of the nematode cuticle. Imaging of areas of high contrast found on the worm cuticle in Site 1 and 2 was performed at 0 °**(B,C)** and 15 °**(D,E)** stage tilt. High contrast areas on the cuticle were examined by EDS, confirming the presence of elemental Au. **(F,G)** Energy dispersive X-ray spectra from areas under the red circles in **B and D, respectively**. Note the keV peak associated with the presence of elemental Au at 2.12 keV. *scale bars:* **(A)** 50 µm and **(B,C,D,** and **E)** 500 nm.

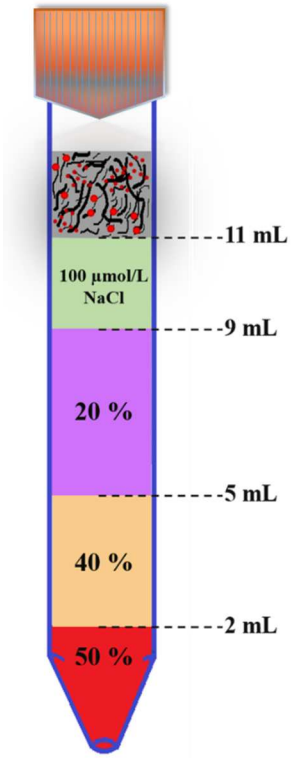

Figure S4. The construction of the layers of the sucrose density gradient within the test tube from top to bottom: 2 mL of AuNP-exposed nematode pellet (gray), 2 mL of 100 µmol/L NaCl (light green) + sucrose [4 mL of 20 % (w/v; purple), 3 mL of 40 % (w/v; orange), and 2 mL of 50 % (w/v; red)].

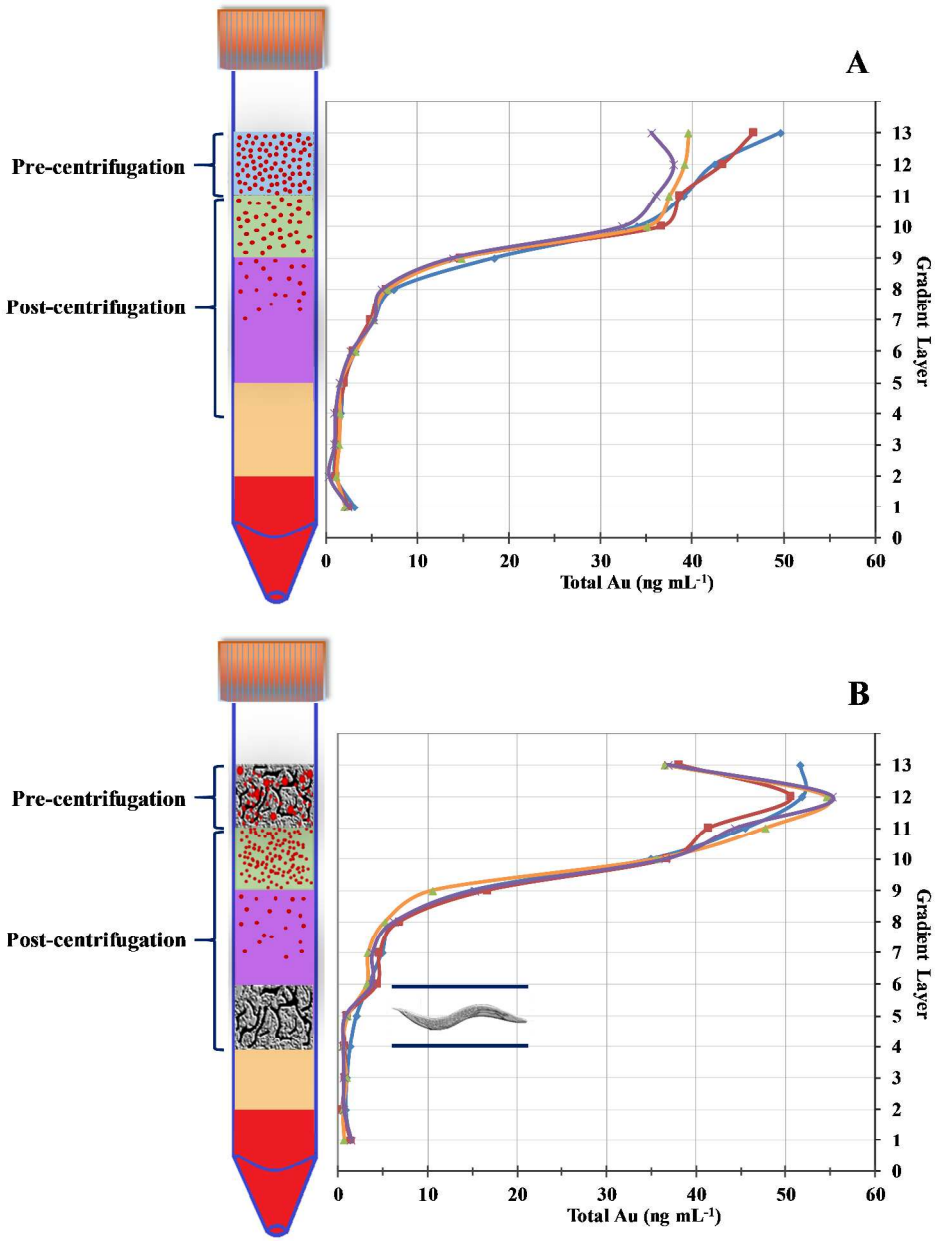

Figure S5. **(A)** Distribution of Au from sucrose density gradient centrifugal separation of a combined mixture of 30 nm and 60 nm AuNPs (NIST). **(B)** Distribution of Au from sucrose density gradient centrifugal separation following nematode exposure to a mixture of 30 nm and 60 nm AuNPs (NIST). The horizontal blue bars represent the layers that nematodes were located in after centrifugation. The color traces represent four sample replicates where: purple = sample 1, blue = sample 2, magenta = sample 3, and orange = sample 4.

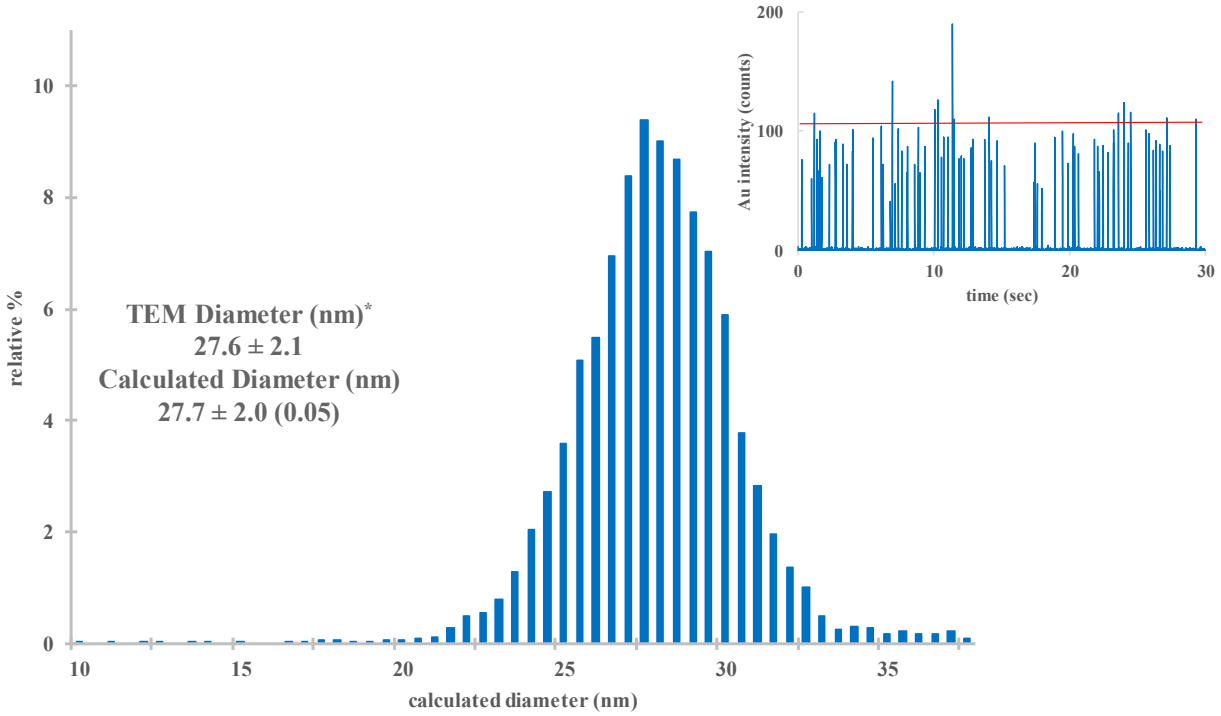

Figure S6. Single particle ICP-MS measured size distribution and representative ^{197}Au time resolved data (inset) of NIST RM 8012 (nominal 30 nm AuNPs). Red bar: average particle intensity for 30 nm AuNPs. The uncertainty of the mean value represents one standard deviation. Bin size corresponds to 0.5 nm. TEM data provided by NIST Report of Investigation for RM 8012.[47]

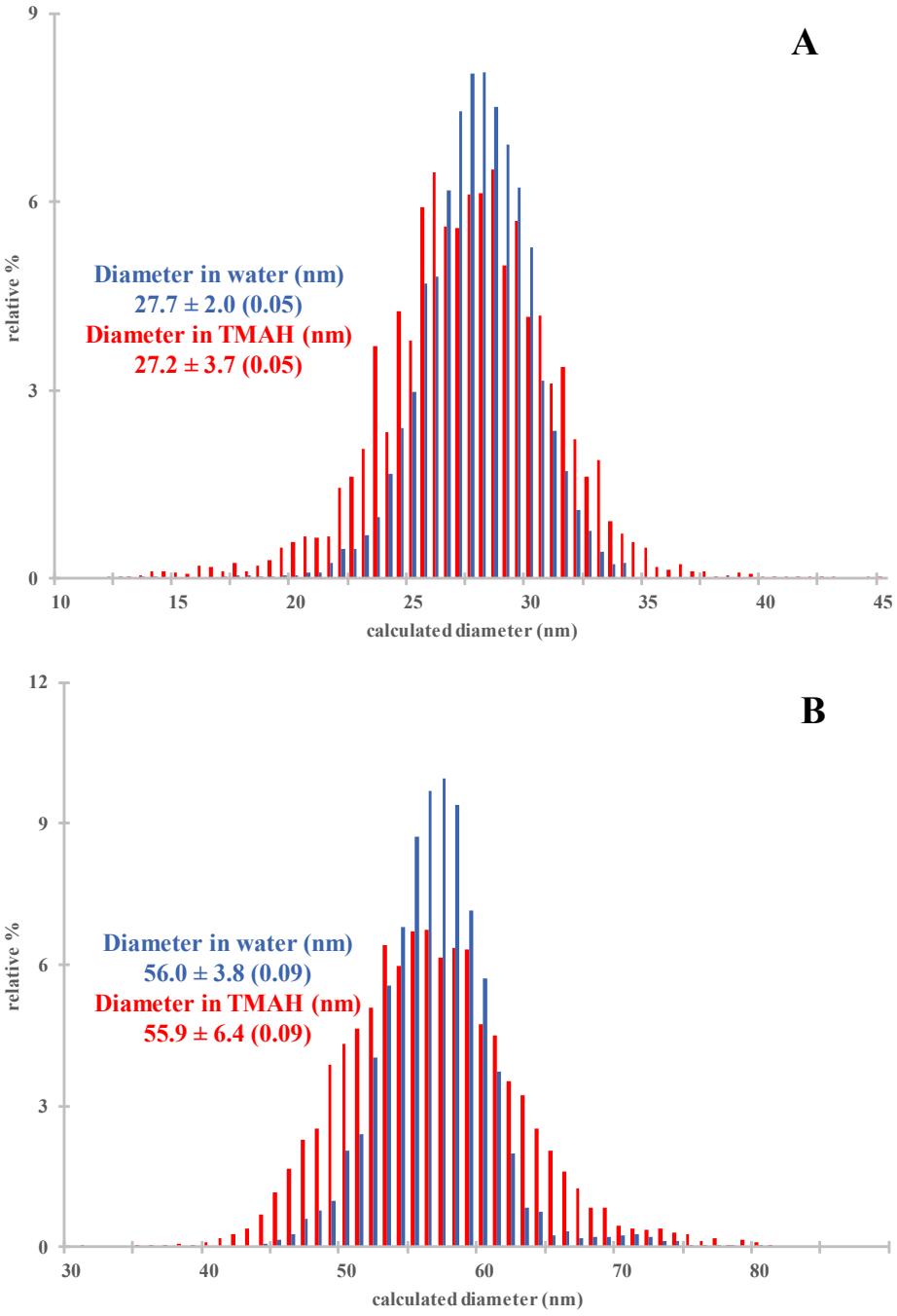

Figure S7. Particle size distributions for NIST 30 nm AuNPs **(A)** and NIST 60 nm AuNPs **(B)** in water and after TMAH digestion (to test for matrix interference; TMAH concentration diluted to > 0.1 % volume fraction). Mean diameter is expressed as the mean of the calculated diameter and uncertainties correspond to one standard deviation. Value in parenthesis corresponds to the expanded uncertainties that account only for measurement replication. Bin size corresponds to 0.5 nm for **(A)** and 1 nm for **(B)**. TEM data provided by NIST Reports of Investigation for RM 8012 and RM 8013.[44, 46]

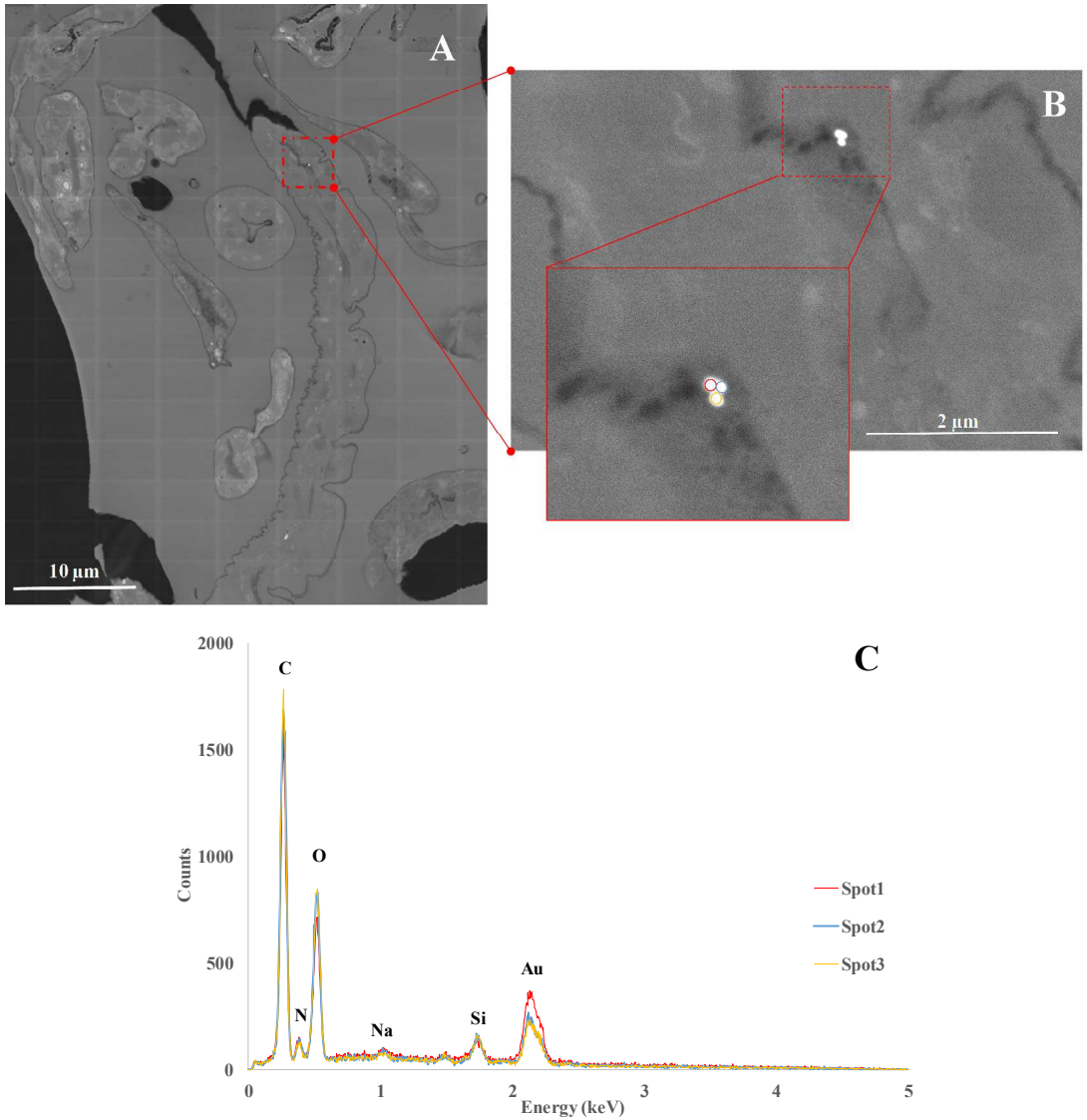

Figure S8. **(A)** Large-area SEM image of nematodes exposed to 60 nm AuNPs (LEx). Sample was processed using **Scheme 2**. **(B)** Higher magnification SEM image of red box inset highlighted in **(A)**. **(C)** Energy dispersive X-ray spectrum of three high contrast areas within the nematode sample. Note the high keV peaks associated with the presence of elemental Au. *scale bars:* (A) 10 µm and (B) 2 µm.

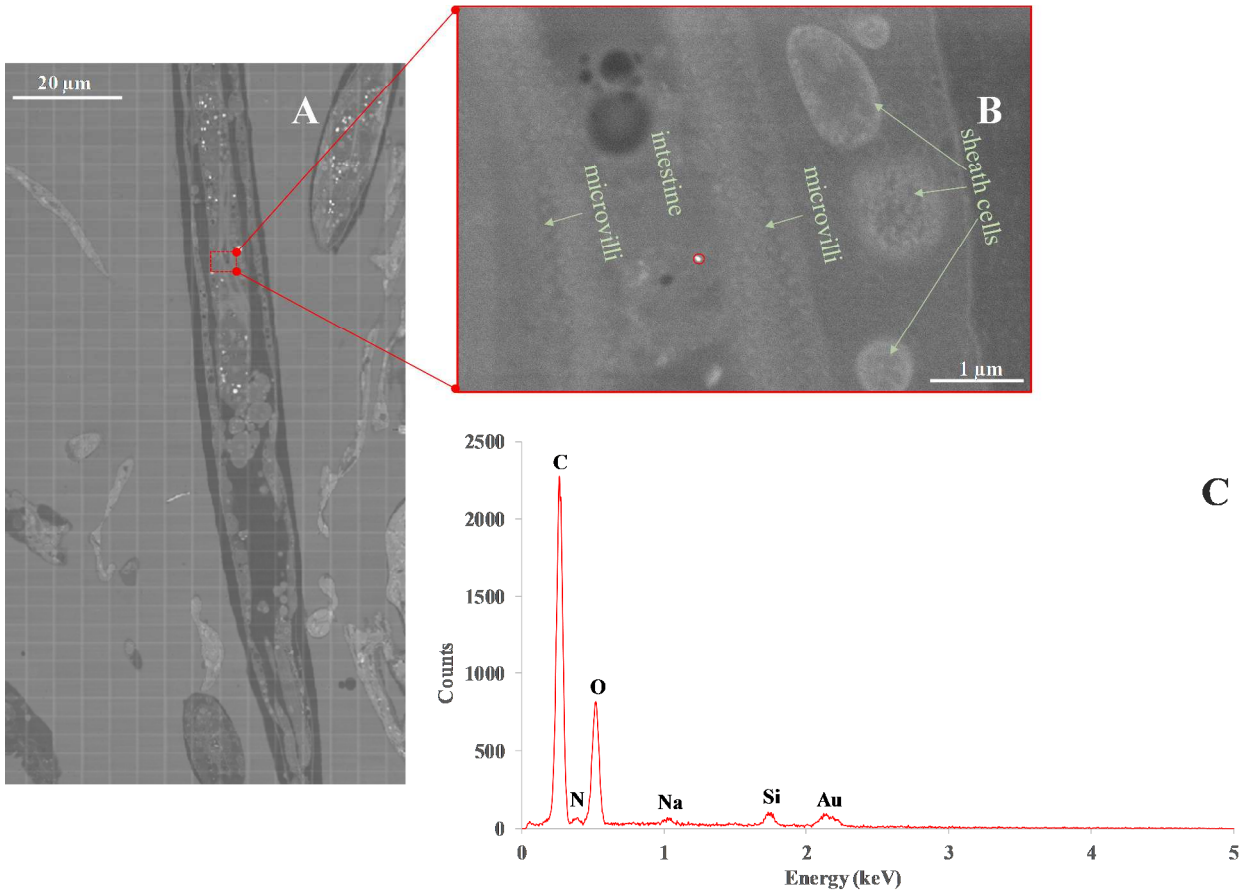

Figure S9. **(A)** Large-area SEM image of nematodes exposed to 30 nm AuNPs (HEx). Sample was processed using **Scheme 2**. **(B)** Higher magnification SEM image of red box inset highlighted in **(A)**. **(C)** Energy dispersive X-ray spectrum of high contrast area highlighted (red) in **(B)**. Note the high keV peaks at 2.12 associated with the presence of elemental Au. *scale bars:* (A) 20 µm and (B) 1 µm.

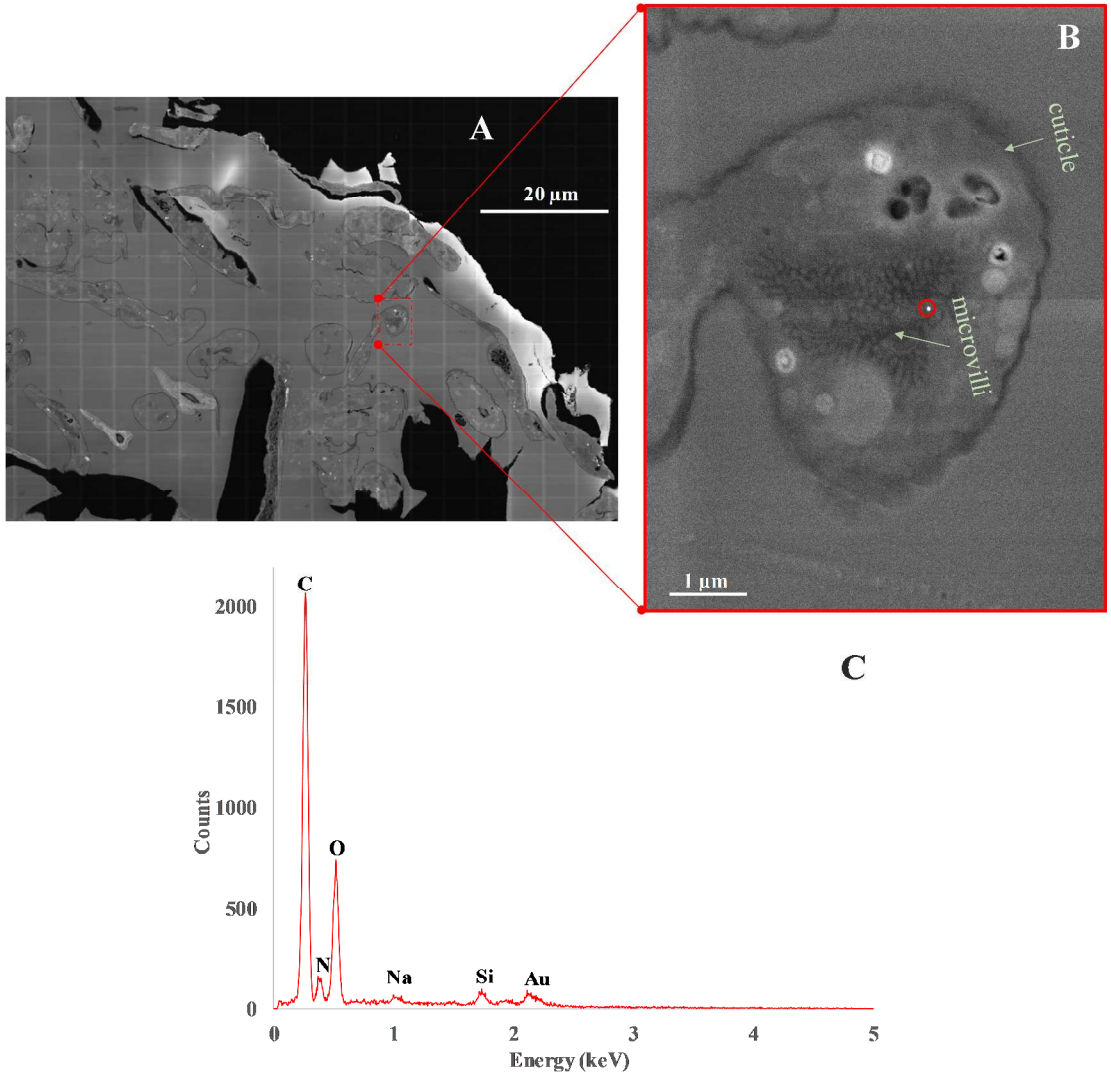

Figure S10. **(A)** Large-area SEM image of nematodes exposed to 60 nm AuNPs (LEx). Sample was processed using **Scheme 2**. **(B)** Higher magnification SEM image of red inset highlighted in **(A)**. **(C)** Energy dispersive X-ray spectrum of high contrast area highlighted in the red circle in **(B)**. Note the high keV peaks at 2.12 associated with the presence of elemental Au. *scale bars:* **(A) 20 µm and (B) 1 µm.**