Supporting information

Quantitative assessment of in vivo distribution of nanoplastics in bivalve *Ruditapes philippinarum* via reliable SERS tag-labeled nanoplastic models

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1. Synthesis of gold nanostar (AuNS).

Au nanostar were synthesized via a seed growth method. Briefly, Au seeds (12 nm) solution was first prepared. Under stirring and refluxing conditions, 15 mL of sodium citrate (1 wt %) was added to 100 mL of boiling $HAuCl_4$ (1 mM) solution. The mixture solution will continue boiling for 15

minutes, and then be cooled and filtered by a 0.22 μm nitrocellulose membrane. For Au nanostar synthesis, under stirring and room temperature, 100 μL of the Au seed solution was added to 10 mL of $HAuCl_4$ (0.25 mM) solution. Then 100 μL $AgNO_3$ (2 mM) and 50 μL AA (0.1 M) were added simultaneously. The mixture solution was stirred for 30 s after 100 μL of CTAB (0.1 M) was added. The color of the solution turned from light red to blue.

2. Synthesis of bare SERS tags.

A 200 μL of Cy7 solution was added to a 20 mL of Au nanostar solution and kept for 0.5 h. The final concentration of Cy7 was 10^{-6} M.

3. SERS@PS NP concentration calculation.

Mass concentration. 150 μL of SERS@PS NP solution was added into a 200 μL centrifugal tube and dried in a vacuum drying oven at 40 °C for 24 h. Then, the mass of SERS@PS samples was weighed. The concentration of SERS@PS NP solution was calculated as 500 mg/L. The concentration of SERS@PS NP solution in exposure experiment was 0.2 mg/L (Take 4 ml of the original SERS@PS NP solution and add it to 10 L sea water, diluted 2500 times).

Particle number concentration. The particle number concentration of SERS@PS can be estimated from the concentration Au nanostar and thus the Au seed NPs by the following formula.

$$C_{Au\ seed} = C_{Au\ element} \times M_{Au\ element} / \rho_{Au} \times V_{single\ Au\ seed}$$

$C_{Au\ seed}$: quantity concentration of Au seeds; $C_{Au\ element}$: molar concentration of $HAuCl_4$ used in the synthesis of Au seeds (assuming the reaction is complete); ρ_{Au}: 19.32 g/cm^3; $V_{single\ Au\ seed}$: volume of single Au seed ($4\pi R^3/3$); R: radius of single Au seed, 6 nm.

$$C_{Au\ element} = \frac{n_{Au\ element}}{V_{Au\ seed\ sulution}} = \frac{0.1\ L\ \times\ 0.001\ M}{0.115\ L} = 8.70 \times 10^{-4}\ M$$

$$M_{Au\ element} = 196.97\ \text{g/mol}$$

$$V_{single\ Au\ core} = \frac{4\pi R^3}{3} = \frac{4 \times \pi \times 6^3}{3} = 9.04 \times 10^{-25}\ m^3$$

$$\therefore C_{Au\ seed} = \frac{C_{Au\ element} \times M_{Au\ element}}{\rho_{Au} \times V_{single\ Au\ seed}} = \frac{8.70 \times 10^{-4}\ M \times 196.97 \frac{g}{mol}}{19.32 \times 10^6 \frac{g}{m^3} \times 9.04 \times 10^{-25}\ m^3} =$$

9.8×10^{15} particles/L

The concentration of Au seed was calculated to be 9.8×10^{15} particles/L, so the concentration of Au nanostar was 9.8×10^{13} particles/L (Au seeds were diluted 100 times), and the concentration of SERS@PS NP was 1.5×10^{15} particles/L (Au nanostar was concentrated 15 times).

4. Au element concentration in SERS@PS NP solution

In order to verify the accuracy of the digestion method, 1.0 mL of HNO_3 (AR) was added to 1 mL 0.5 mg/L SERS@PS NP solution and heated for 12 h at 90 ℃. Clear solutions could be obtained and the volumes of which were fixed to 10.0 mL for ICP-MS analysis of Au. The main operating condition was as follows: the auxiliary flow was 0.025 L/min, and the plasma gas flow was 0.5 L/min. The quantification was performed using a eight-point calibration curve between 1 and 500 ng/mL, and ^{115}In was chosen as an internal standard element.

Theoretical concentration of Au of 1 mL SERS@PS NP solution:

$$C_{Au} = \frac{m_{Au}}{V} = \frac{c_{HAuCl_4} \times V_{AuNS} \times M_{Au\ element}}{V}$$

C_{Au}: theoretical concentration of Au of 1 mL 0.5 mg/L SERS@PS NP solution; V_{AuNS}: volume of AuNS solution used in the synthesis of 1 mL 0.5 mg/L SERS@PS NP solution (assuming the reaction is complete); $M_{Au\ element}$: 196.97 g/mol; V: volume of SERS@PS NP solution for ICP-MS, 10 mL.

$$c_{HAuCl_4} = \frac{\frac{100\ \mu L}{115\ mL} \times 0.1\ L \times 1\ mM + 0.01\ L \times 0.25\ mM}{10\ mL} = 0.26\ mM$$

$$C_{Au} = \frac{m_{Au}}{V} = \frac{c_{HAuCl_4} \times V_{HAuCl_4} \times M_{Au\ element}}{V} = \frac{0.26\ mM \times 15\ \mu L \times 196.97}{10\ mL}$$

The recovery of NPs from the main organs of the bivalves was tested via a standard addition experiment. 3.0 g of the tissues of a blank clam was weighed and added to 1 mL 0.5 mg/L SERS@PS, then, they were soaked in 1.0 mL of HNO_3 (AR) for 1 h, and then heated in an oil bath at 100 ℃ for 10 h to digest. 1 mL 0.5 mg/L SERS@PS sample solution was digested with the same method. Clear solutions could be obtained and the volumes of which were fixed to 10.0 mL for ICP-MS analysis of Au. The main operating condition was as follows: the auxiliary flow was 0.025 L/min, and the plasma gas flow was 0.5 L/min. The quantification was performed using an eight-point calibration curve between 1 and 500 ng/mL, and ^{115}In was chosen as an internal standard element. Each sample was measured 3 times as a parallel, and the Au content was obtained from the average value and standard deviation of 3 parallel measurements.

Table S1 Au account and recovery of SERS@PS (n=3)

	SERS@PS	SERS@PS and tissues
Concentration of Au (ng/mL)	41.95 ± 3.70	36.14 ± 3.55
Recovery (%)	86.2	

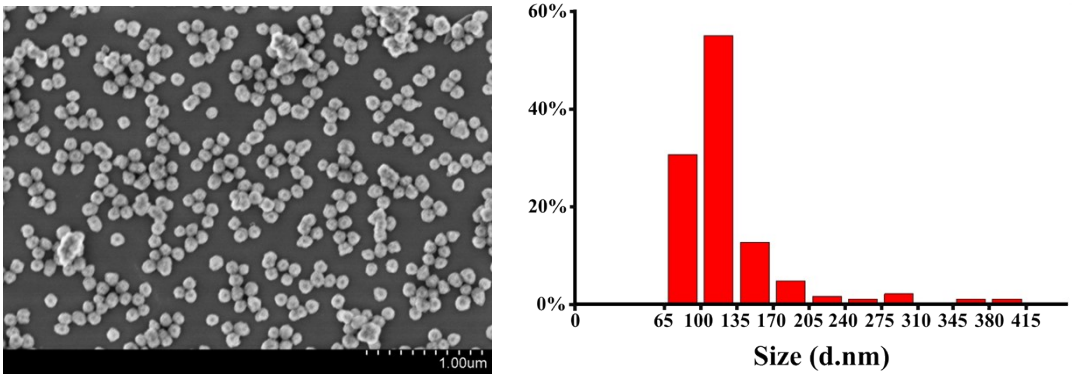

Figure S1. SEM image of SERS@PS NPs and the size distribution by counting 200 NPs.

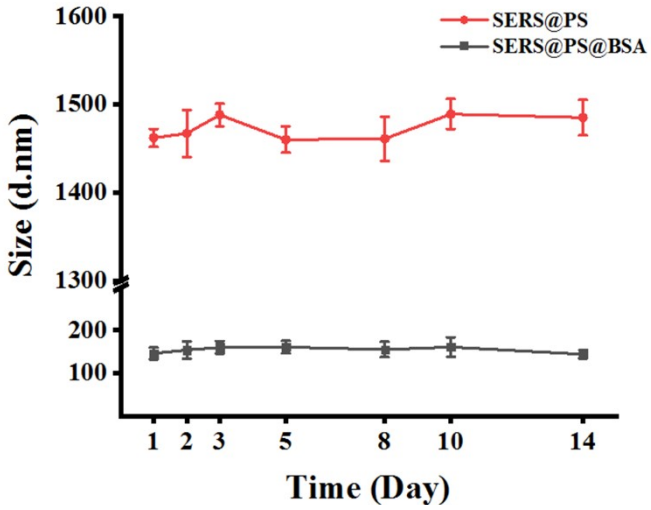

Figure S2. The size distribution of SERS@PS and SERS@PS@BSA NPs in seawater over 14 days.

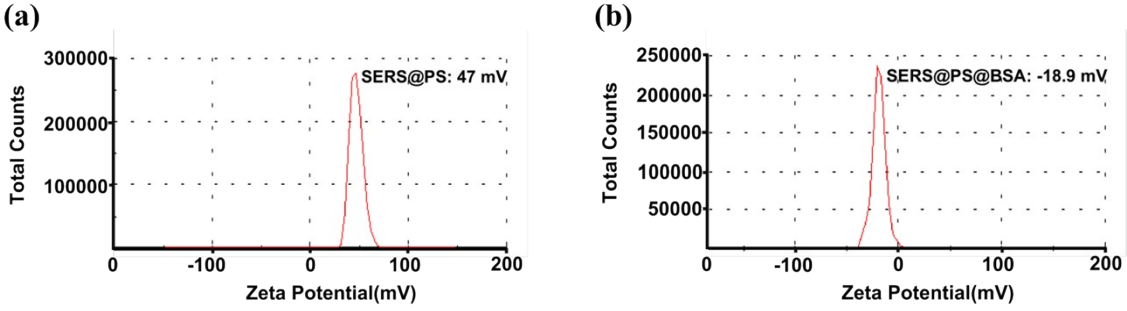

Figure S3. Zeta potential of (a) SERS@PS and (b) SERS@PS@BSA.

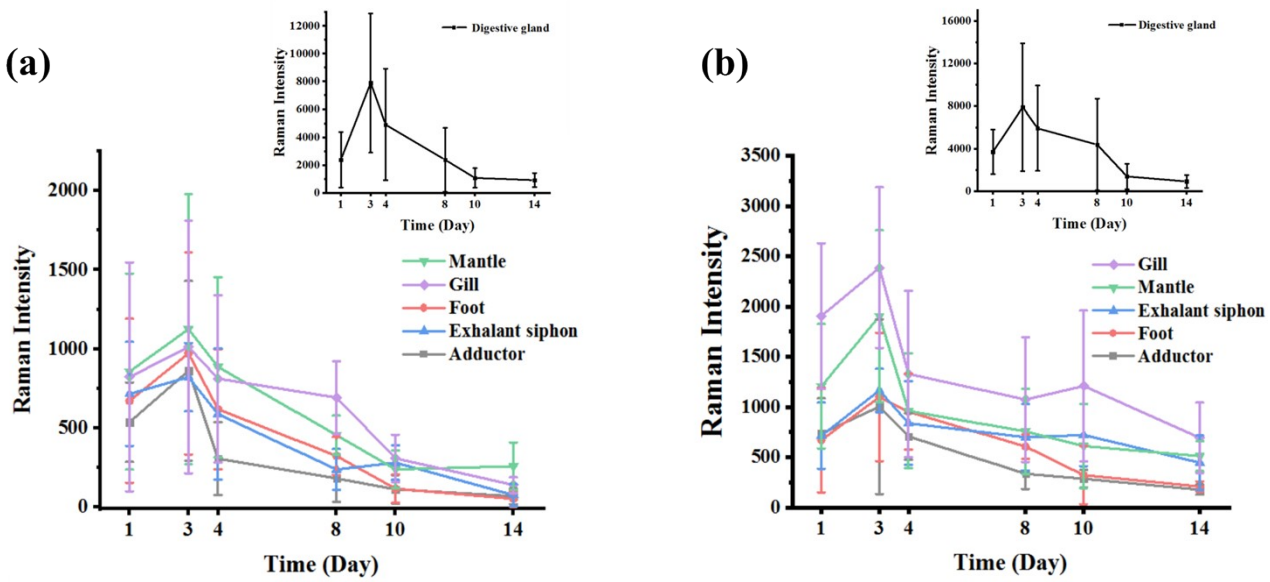

Figure S4. SERS intensities of six organs after exposure to (a) SERS@PS and (b) SERS@PS@BSA (from 1 to 3 day) and depuration (from 4 to 14 day). Each value is the mean of intensities of 15 values (from three bivalves and 5 repeated detections for each bivalve).

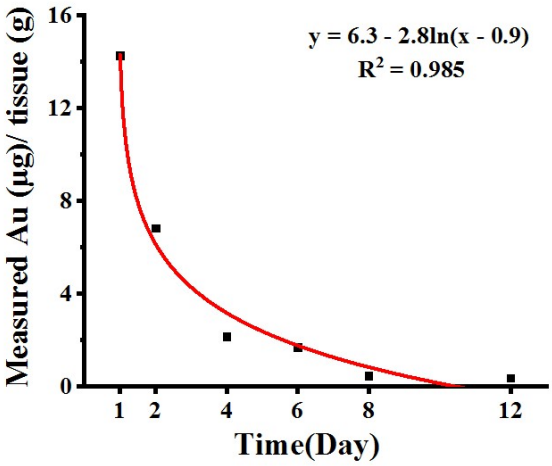

Figure S5. The elimination curve of SERS@PS NPs in digestive gland.