## **STEM-in-SEM high resolution imaging of gold nanoparticles and bivalve tissues in bioaccumulation experiments**

C.A. García-Negrete<sup>a\*</sup>, M.C. Jiménez de Haro<sup>a</sup>, J. Blasco<sup>b</sup>, M. Soto<sup>c</sup>, A. Fernández<sup>a,\*</sup>

<sup>a</sup> Instituto de Ciencia de Materiales de Sevilla (CSIC - Univ. Sevilla), Avda. Américo Vespucio nr. 49, CIC Cartuja, 41092 Sevilla, Spain

<sup>b</sup> Instituto de Ciencias Marinas de Andalucía (ICMAN-CSIC), Campus Universitario Río San Pedro, 11519 Puerto Real (Cádiz), Spain

<sup>c</sup> Zoology and Cell Biology Dept., Science and Technology Faculty & Research Centre in Experimental Marine Biology and Biotechnology (PiE-UPV/EHU) University of the Basque Country, Sarriena auzoa Z/G, 48940 Leioa-Bizkaia, Basque Country, Spain

\* Corresponding authors.

E-mail addresses: asuncion@icmse.csic.es (A. Fernández); carlos.garcia@icmse.csic.es

**Fig. S1** Micrographs of semithin sections (toluidine blue staining) of the gills of control clams (A, B) and clams exposed to Au NPs (C-E). (A) Regular arrangement of filaments. (B) Detail of the distal zone with frontal cilia and ciliary plates between adjacent lamellae. (C) Ciliary plates are nearly absent between lamellae (\*) and basal lamina is enfolded and thickened (#). (D) Lamellae fusion and increased hemocytes (square) in the proximal zone. (E) Detail of the proximal zone with fused lamellae and swollen hemocytes. DZ, distal zone; cp, ciliary plates; hv, haemolymphatic sinuses. Scale bars: A, C, D: 50  $\mu$ m; B,E: 10  $\mu$ m.



Fig. S2 Representative STEM image along with its corresponding EDX spectra of the electron-dense contrasts region (marked area in the left side panel).



**Fig. S3.** STEM-in-SEM image of a control bivalve tissue along with an EDX spectra revealing the presence of Fe in a selected area containing an electrodense contrast (red inset).



**Fig. S4.** STEM-in-SEM image of a control bivalve tissue along with an EDX spectra revealing the presence of Ti in a selected area containing an electrodense contrast (red inset).

