

SUPPLEMENTARY MATERIAL

Two unconventional metallothioneins in the apple snail *Pomacea bridgesii* have lost their metal specificity during adaptation to freshwater habitats

Mario García-Risco^a, Sara Calatayud^b, Michael Niedwerwanger^c, Ricard Albalat^b, Òscar Palacios^a, Mercè Capdevila^a and Reinhard Dallinger^{c,*}

^a Departament de Química, Facultat de Ciències, Universitat Autònoma de Barcelona, E-08193 Cerdanyola del Vallès, Spain.

^b Departament de Genètica, Microbiologia i Estadística and Institut de Recerca de la Biodiversitat (IRBio), Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 643, E-08028, Barcelona, Catalonia, Spain.

^c Institute of Zoology and Center of Molecular Biosciences, University of Innsbruck, Technikerstraße 25, A-6020 Innsbruck, Austria.

Corresponding author email: merce.capdevila@uab.cat

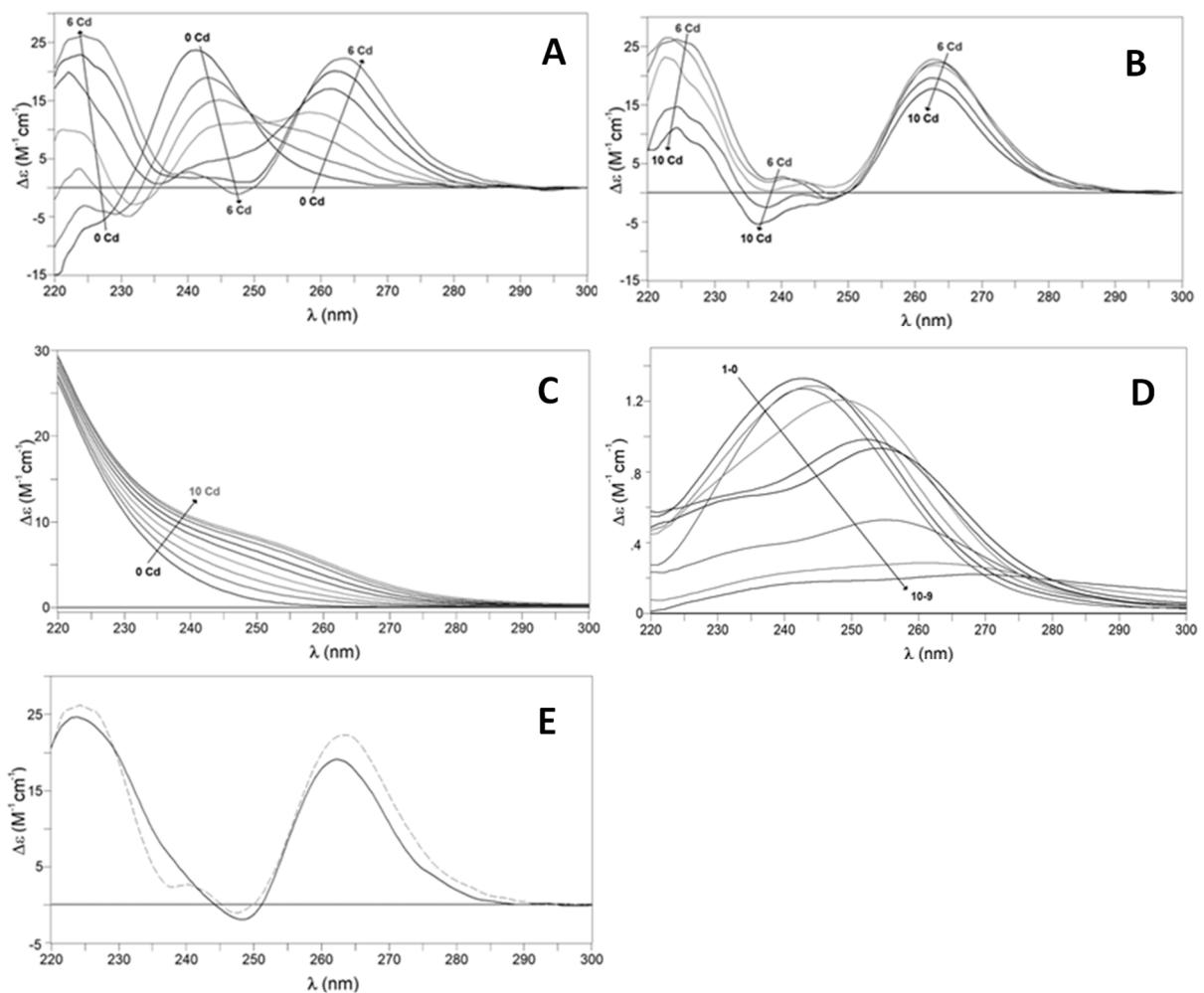


Figure S1. Spectroscopic characterization of the Zn/Cd replacement in the Zn-PbrMT1 preparation followed by (A and B) CD and (C) UV-vis. (D) UV-Vis difference spectra. (E) Comparison of CD spectra of *in vivo* Cd-PbrMT1 preparation (solid line) and the *in vitro* Cd-PbrMT1 spectrum measured after adding 7 equivalents of Cd(II) to the Zn-PbrMT1 preparation (dashed line)

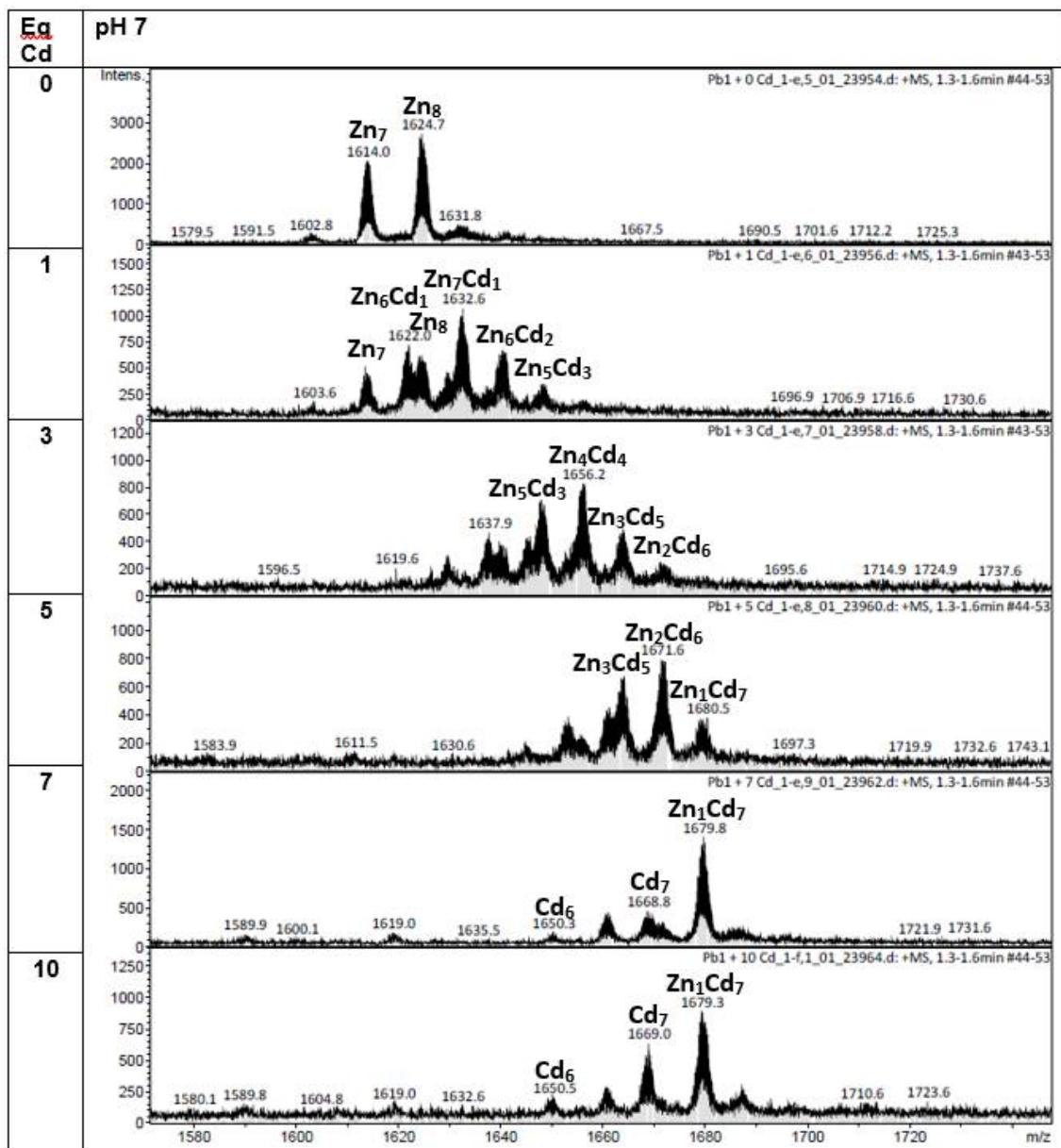


Figure S2. ESI-MS spectra of the Zn/Cd replacement experiment performed on the Zn-PbrMT1 production at pH 7.

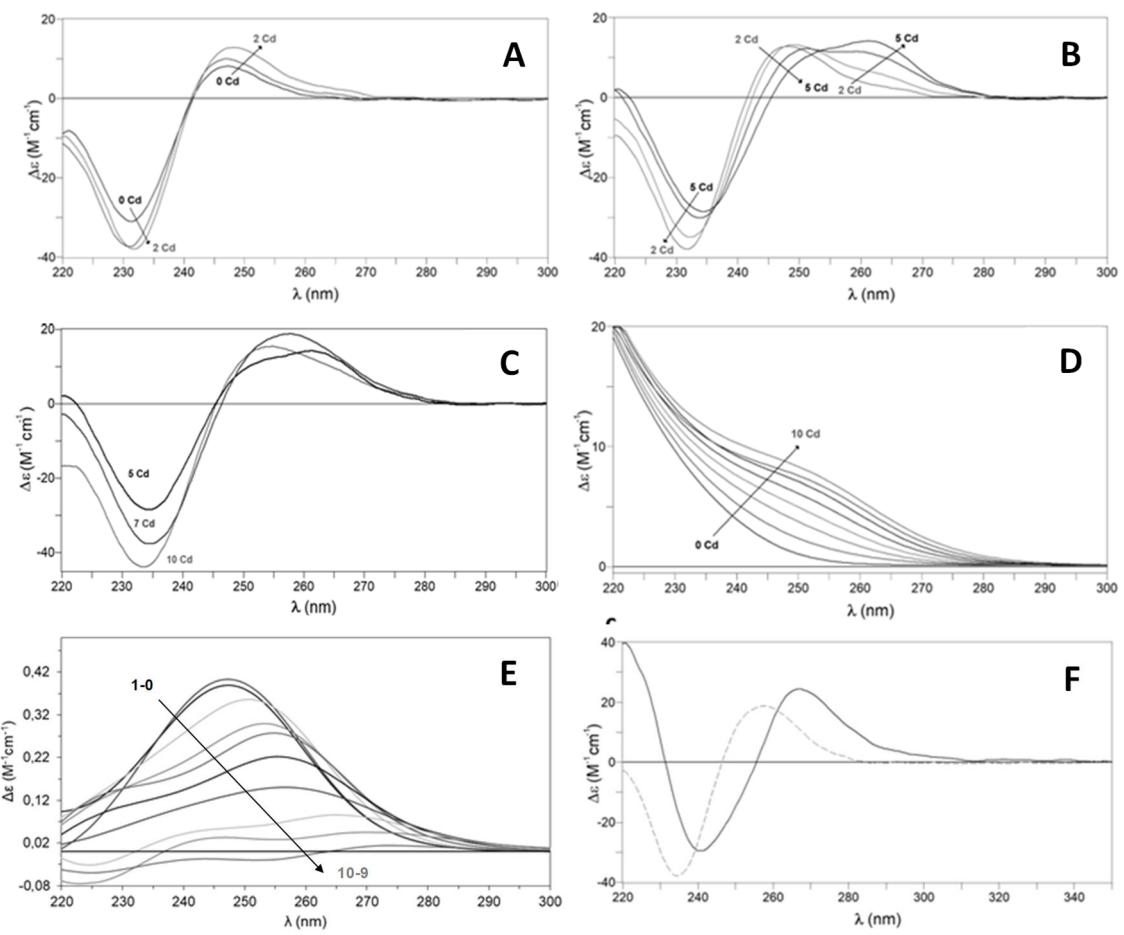


Figure S3. Spectroscopic characterization of the Zn/Cd replacement in the Zn-PbrMT2 preparation followed by (A, B and C) CD and (D) UV-vis. (E) UV-Vis difference spectra. (F) Comparison of CD spectra of *in vivo* Cd-PbrMT2 species (solid line) and the *in vitro* Cd-PbrMT2 species (dashed line) after adding 7 equivalents of Cd(II) to Zn-PbrMT2 production.

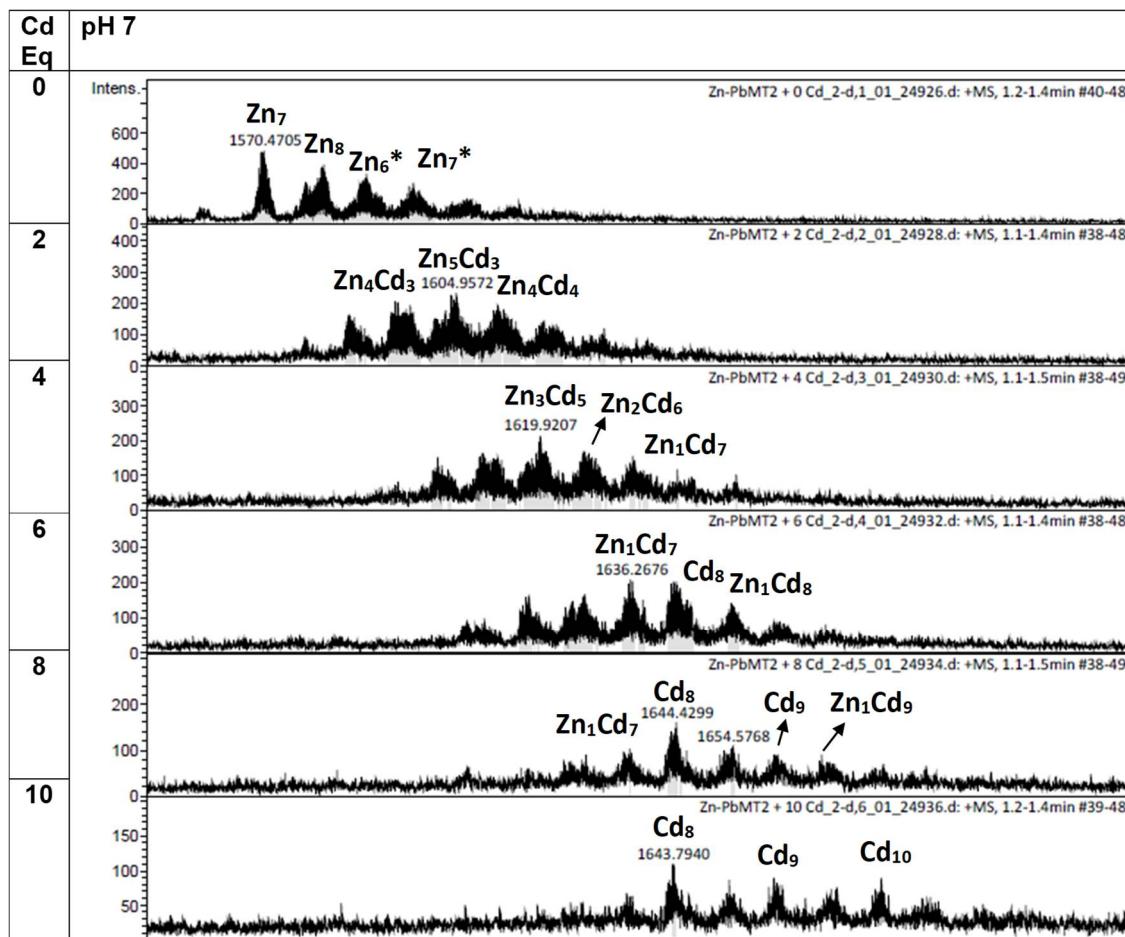


Figure S4. ESI-MS spectra of the Zn/Cd replacement experiment performed on the Zn-PbrMT2 production at pH 7.