Supplementary Information for

Uranium uptake in *Paracentrotus lividus* sea urchin, accumulation and speciation

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protein fractions collected in 346 to 40. These fractions were pooled and then concentrated using Amicon filtration system (100 kDa) cutoff. (S) is the mass ladder. The proteins were migrated in reducing conditions on a 7.5% SDS-page gel.

Figure S5: STXM image of the sea urchin 2 gonad cells at 725 eV, and contrast obtained between the images at 725 eV and 738 eV.

Figure S6: Modulus and imaginary parts of the Fourier transform from the experimental EXAFS spectrum and fit for gonads, toposome and intestinal tract.

Table S1: Description of the measurements performed for each sea urchin specimen (5 specimens).

specimen	**ICP-OES Sea urchin**	**XAS**	**TEM**	**STXM**	**TRLFS**
Sea urchin 1	X				
Sea urchin 2	X	X gonads + intestinal tract	X	X	
Sea urchin 3	X (except gonads)	X gonads			
Sea urchin 4	X				
Sea urchin 5		X test			X test and gonads

Uranium distribution in the aquarium.

Samples were analyzed with ICP-OES (Perkins Elmer Optima 8000) to determine the amount of uranium. Two emission wavelengths were selected for uranium: 409.014 nm and 424.167 nm, to avoid any interference with other elements present in seawater and sea urchin, such as iron and calcium.

Seawater samples: The seawater samples were collected after the contamination experiments with the doped uranium solution and diluted by a factor of 100 prior to analysis.

Sea urchin samples: The sea urchins 1, 2 and 4 were dissected, then the three compartments were separated. Gonads and intestinal tract were freeze-dried for 24h once frozen with liquid nitrogen. The test was dried for 24h in a 50°C oven. The gonads and the intestinal tract were then digested in 10 mL of concentrated nitric acid (14.4 M) using microwaves, for 1 h at 200°C (microwave oven: Ethos EASY Milestone Helping Chemists). The nitric acid was then evaporated, and the dry residue dissolved in diluted nitric acid (~0.1 M), to reach a pH between 1 and 2, as required for ICP-OES analysis. The test was crushed first, then a known mass was dissolved using the same procedure.

Table S2: Distribution of uranium inside the sea urchin organs, and quantity of uranium (elemental) per dry weight.

Description	**Total uranium (%)**	**Mass of uranium (mg) per g of dry weight**
Sea urchin 1		
Test	65.58	0.0122
Gonads	20.15	0.0499
Intestinal tract	14.27	0.1330
Sea urchin 2		
Test	68.46	0.0134
Gonads	18.46	0.0481
Intestinal tract	13.07	0.1280
Sea urchin 4		
Test	85.35	0.0074
Gonads	4.24	0.0076
Intestinal tract	10.41	0.0791

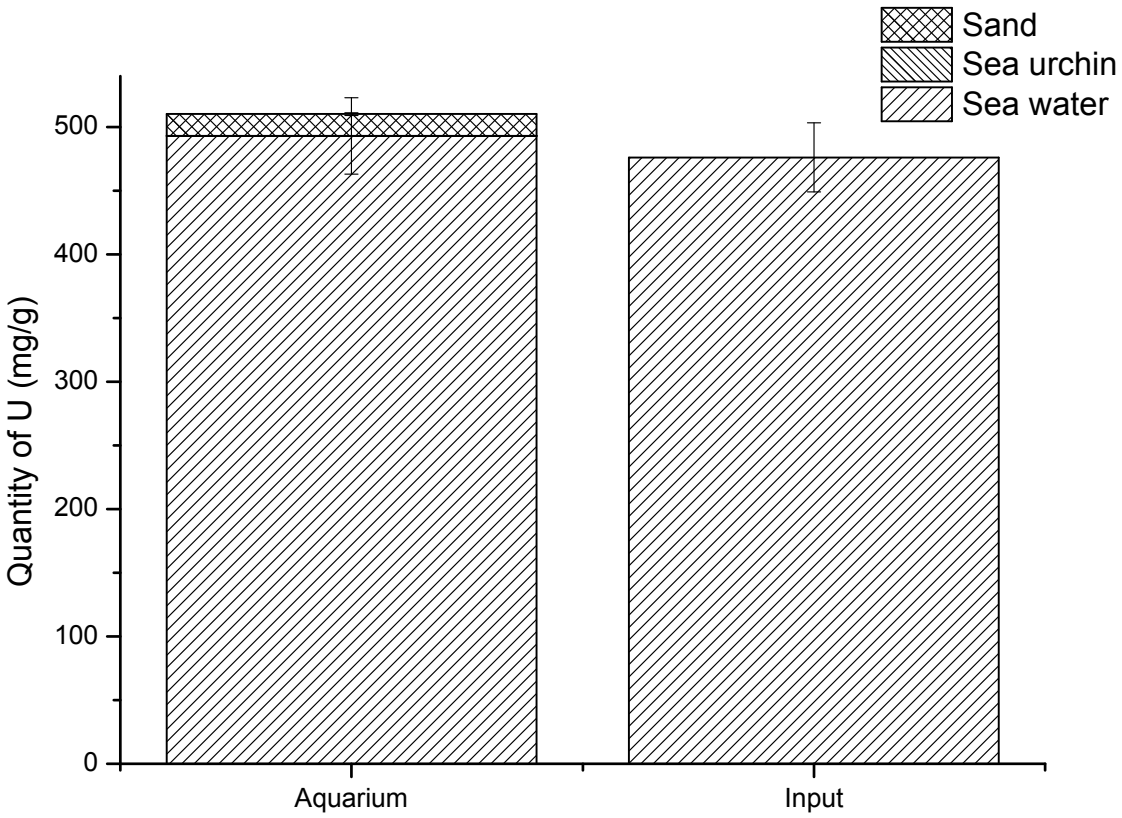


Figure S1: Distribution of uranium in the aquarium with sea urchin (left) 4 (in mg/g, elemental U) and the total uranium input (right) (total of the spikes for 10 days).

Toposome extraction and purification

Three grams of fresh gonads of *Paracentrotus lividus* were recovered in 20 volumes of 10 mM Tris HCl pH 8, 10 mM NaCl, supplemented with 0.1 mM phenylmethylsulphonyl fluoride (PMSF) to avoid proteolysis and ground on ice (using an Ultra Turrax T25, IKA Werke, 2 x 30 s at 9500 rpm separated by 1 min). The sample was centrifuged for 30 minutes at 21000 g at 4°C. The supernatant was collected and placed on crushed ice. The pellet was resuspended in two volumes of lysis buffer (RIPA, G-BIOSCIENCES, 25 mM Tris, 150 mM sodium chloride, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, pH 7.6) supplemented with 1 mM PMSF, Anti protease complete (Sigma Aldrich) and 5 mM EDTA. It was homogenized on ice for 10 minutes (Dounce 7 ml piston tight) and then centrifuged ~21000 g for 20 min at 4°C. The protein content of the supernatant and of the protein fraction obtained after cell lysis were analyzed on SDS-PAA 7.5 % gel in denaturing conditions. The toposome protein was present mainly in the supernatant obtained after gonad crushing (and only in minority, after RIPA extraction). Therefore, we concentrated the supernatant by filtration on an Amicon Ultra (MWCO 30 kD) system. The supernatant was then filtered at 8 µm before injection on a HiPrep 16/60 Sephacryl S-400 HR column equilibrated in Tris 50 mM pH 8, 150 mM NaCl, (2.5 mL injected, flow rate of 0.5 ml/min), as described in Castellano *et al.* in 2018. The elution profile is presented in Figure S2. The column was calibrated using thyroglobulin (660 kD), ferritine (440 kD) and aldolase (158 kDa)(Gel flitration calibration kit HMW from Ge Healthcare). The supernatant and fractions located between the peaks of thyroglobulin and ferritin were collected and analyzed on analytical HPLC Agilent TSK S4000SWXL 7.8 / 30 equilibrated in phosphate buffer 50 mM pH 6.7, 150 mM NaCl (flow rate of 0.5 mL/min, Figure S3). The protein profile of these fractions is shown on denaturating SDS-Page in Figure S4. The fractions with the highest purification yield (fractions 36 to 40) were pooled and concentrated by filtration using an Amicon Ultra MWCO system with a cut off of 100kDa. The buffer was also changed to Tris/HCl (50mM measurements) NaCl (150 mM), pH = 6.5 during the cycles of centrifugation.

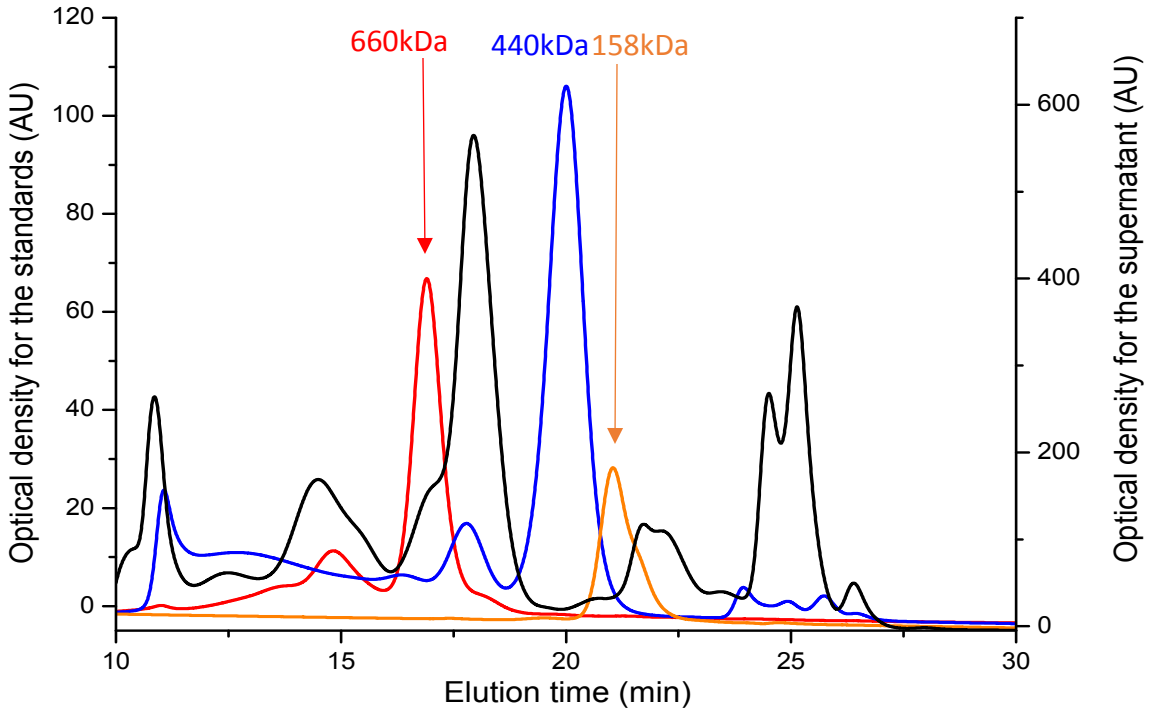


Figure S2: Separation profile of the supernatant (black line) obtained with analytical HPLC Agilent TSK S4000SWXL, with 100 µL, and comparaison with standard proteins (red = thyroglobulin 660kDa, blue = ferritin 440kDa, and orange = aldolase 158kDa).

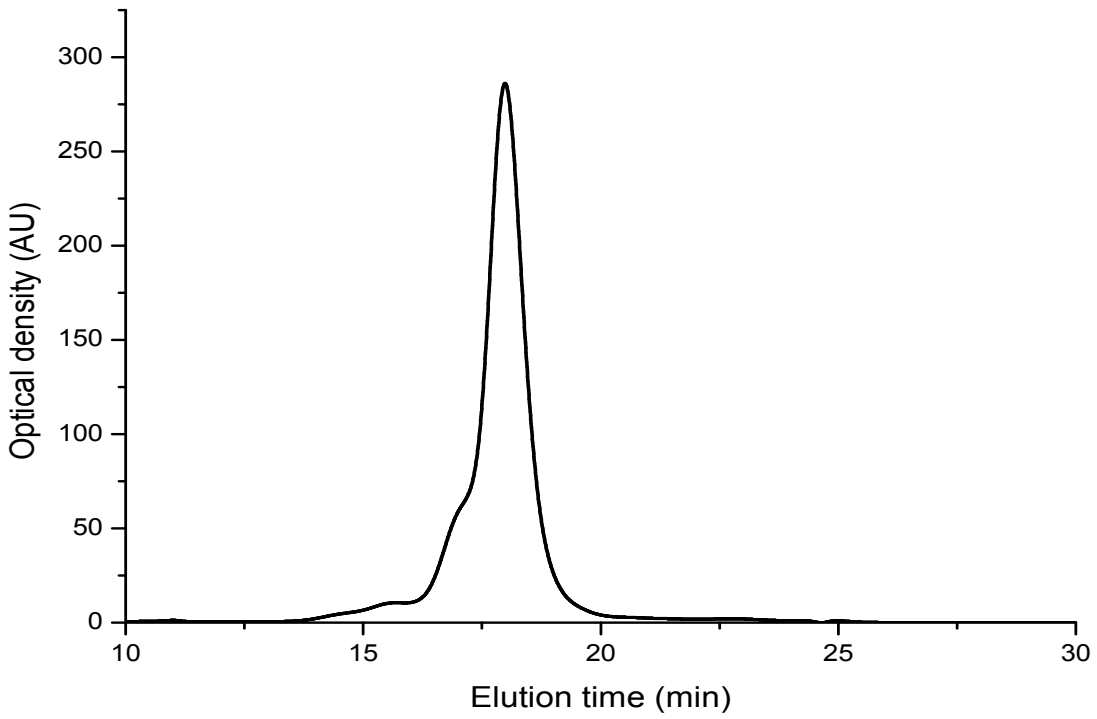


Figure S3: Profile of the pooled fractions (tube 36 to 40) on the analytical Analytical HPLC Agilent TSK S4000SWXL (50 µL injection).

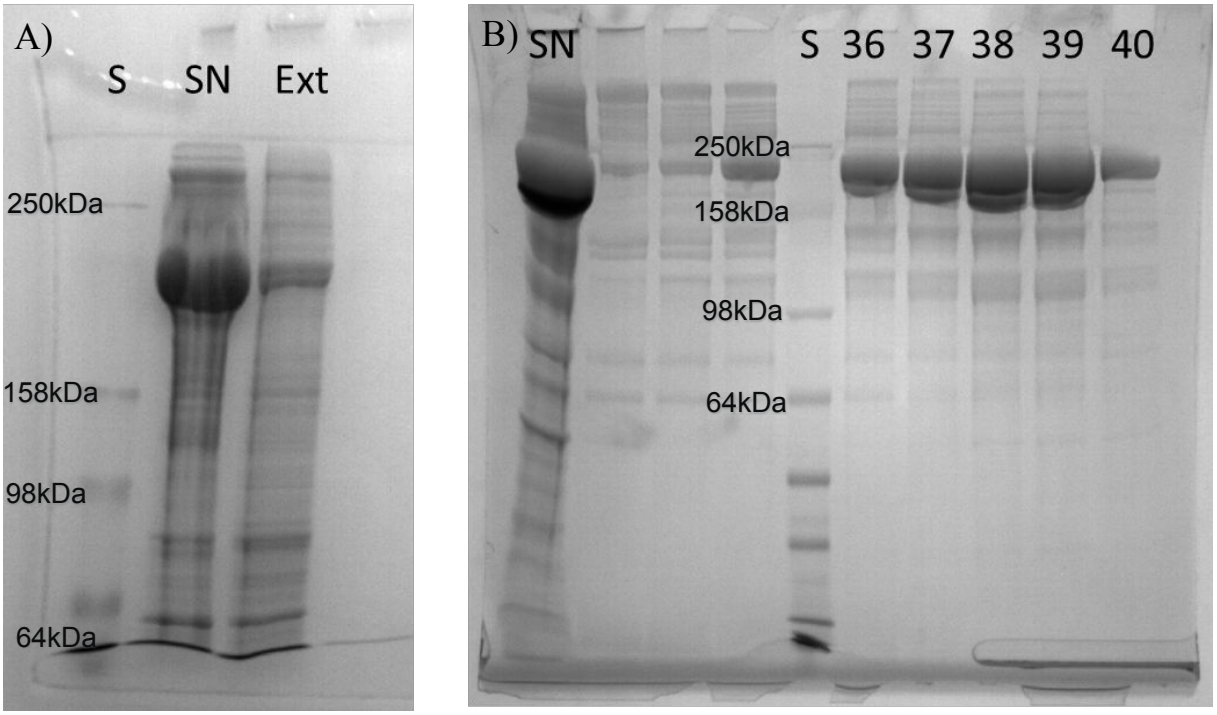


Figure S4: A) comparison of the protein content of the supernatant obtained after gonad grinding and centrifugation (SN) and after cell lysis (Ext). B) comparison of the protein content on the supernatant after concentration using Amicon filtration system (30 kDa cutoff) (SN) and of protein fractions collected in 346 to 40. These fractions were pooled and then concentrated using Amicon filtration system (100 kDa) cutoff. (S) is the mass ladder. The proteins were migrated in reducing conditions on a 7.5% SDS-page gel.

STXM imaging and EXAFS spectra.

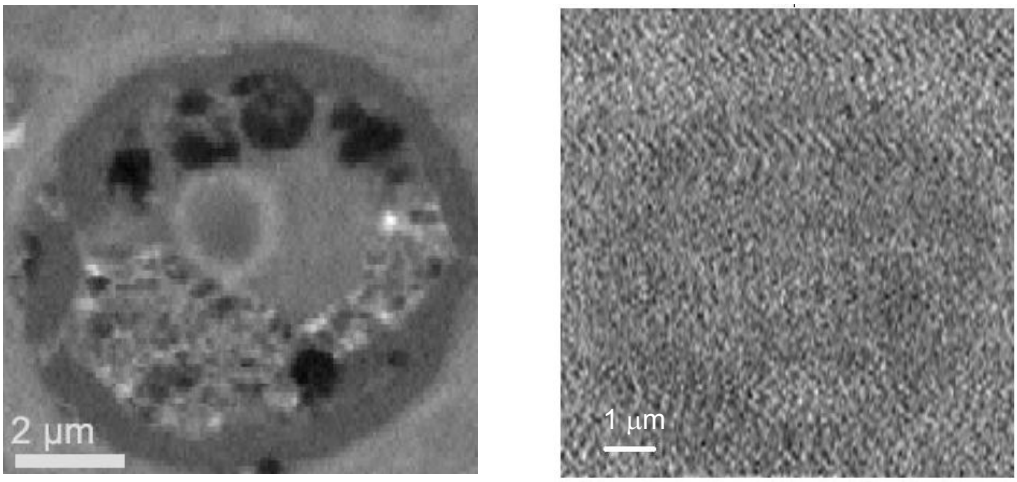
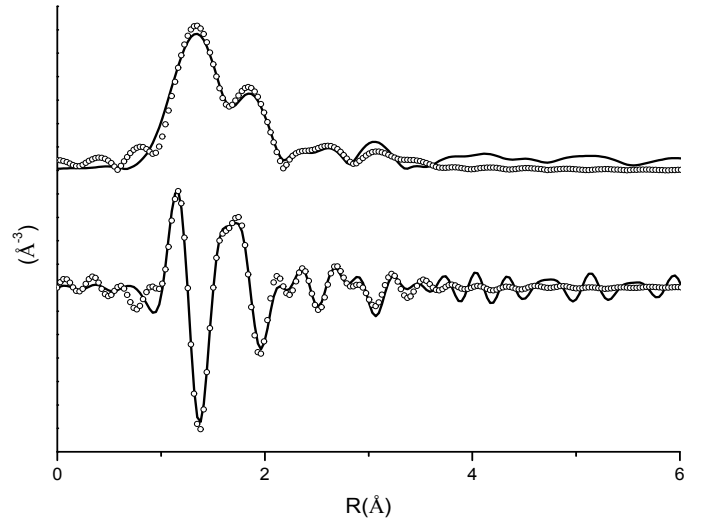


Figure S5: STXM image of sea urchin 2 gonad cells obtained at 725eV (left), and contrast obtained between the images recorded at 725 eV and 738 eV (right).


(Å-3)
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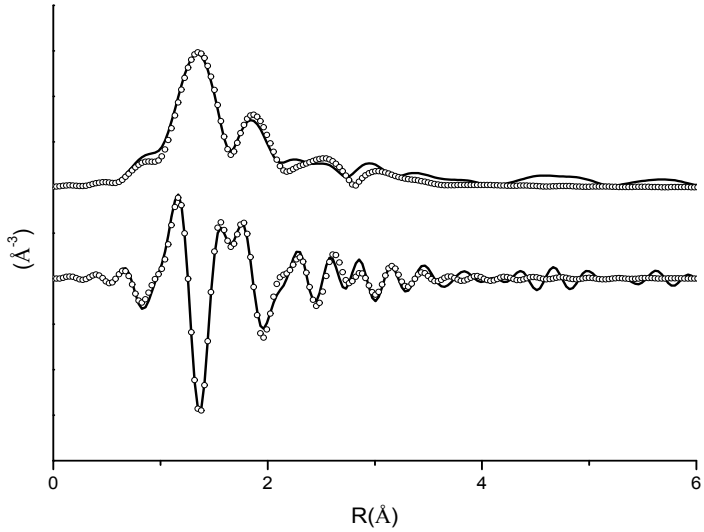
2
4
6
R(Å)


(Å-3)
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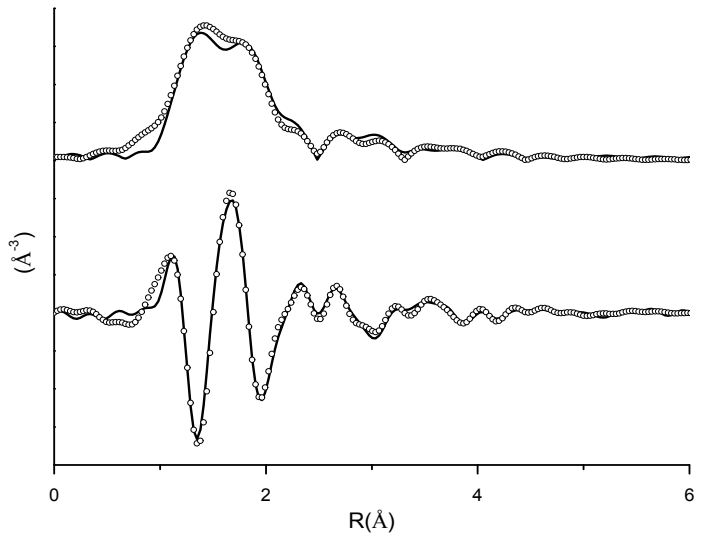
(Å-3)
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Figure S6: Moduli and imaginary part of the Fourier transforms from the experimental EXAFS spectra (straight lines) and fit (dots) obtained for contaminated gonads (top graph) for sea urchin 2, U-toposome complex (second graph) and contaminated intestinal tract (bottom graph) for sea urchin 2.