Supplementary information

Bionics synthesis of magnetic calcite skeletal structure through living foraminifera

Giulia Magnabosco,[a] Hagar Hauzer,[b] Simona Fermani,[a] Matteo Calvaresi,[a] Franco Corticelli,[c] Meganne Christian,[c] Cristiano Albonetti,[d] Vittorio Morandi,[c] Jonathan Erez[b] and Giuseppe Falini*[a]

[a] Dipartimento di chimica "Giacomo Ciamician", Alma Mater Studiorum-Università di Bologna, Italy e-mail: giuseppe.falini@unibo.it
[b] Institute of Earth Sciences, The Hebrew University of Jerusalem, Edmond Safra Campus, Jerusalem 91904, Israel
[c] National Research Council, Institute for Microelectronics and Microsystems, Bologna, 40129, Italy
[d] National Research Council, Institute for Nanostructured Materials (ISMN), via P. Gobetti 101, 40129 Bologna, Italy

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Experimental section

NPs screening

In order to identify the type of particles with the surface functionalization allowing the best occlusion into $CaCO_3$, *in vitro* precipitation of calcite crystals was performed. Superparamagnetic magnetite nanoparticles with a diameter of 100 nm labelled with a fluorescent dye functionalized with i) Polyethylene glycol (2000 Da) having as a functional group phosphate sodium salt (peg-P-nPs), ii) Polyethylene glycol (20000 Da) having amine as a functional group (peg-Am-nPs) and iii) dextran (mds-nPs) provided by Chemicell. 750 μL of a solution of nPs at the desired concentration in 10 mM $CaCl_2 \cdot 2H_2O$ (Fulka) were put in a well of a 24-well microplate covered with aluminium foil, one each well was performed a hole with a needles and it was put into a closed desiccator with a beaker containing 3,5 g of crushed $(NH_4)_2CO_3$ covered with parafilm with 10 holes on it and 16 g of anhydrous $CaCl_2$. After 4 days the wells were washed 3 times with DDW and 1 time with absolute ethanol. Peg-P-nPs and peg-Am-nPs were not stable at the highest concentration used.

Foraminifera culturing

Foraminifera (collected in Eilat-Israel on 7/7/16) with dimension between 250 μm and 350 μm were selected using sieves and *A. lessoni* specimens were selected.
The dead control was prepared washing the forams 2 times with distilled water (DW) and 3 times with double distilled water (DDW), putting them in sodium hypoclorite (NaOCl) (prepared diluting the analytical grade one 1:5 NaOCl:DW) for at least 2 hours and drying them in the oven at 60°C.
The solutions were prepared diluting the dyes directly in seawater to obtain calcein 40 μM and mds-nPs 0,01 mg/mL.

40 specimens were put in a jar and the following experiment were performed:

	Calcein	**mds-nPs**	**Calcein/mds-nPs**
Living specimen	40 specimens in 50 mL	40 specimens in 50 mL	40 specimens in 50 mL
Dead control	10 specimens in 1 mL	10 specimens in 1 mL	10 specimens in 1 mL

The samples were kept under a lamp with a temperature of 23,5 °C.
After 2 days the samples treated with calcein were observed under a binocular and, since the last chamber of all the specimens was labelled, all the incubations were stopped.

Characterization

Optical images were acquired with a Leica light microscope equipped with a Moticam 5.0 camera.
X-ray powder diffraction (XPRD) measurements were carried out using a PanAnalytical X'Pert Pro diffractometer equipped with X'Celerator detector with Cu Kα radiation (range 20°-60°, step size 0.05°, time per step 45 s).
Scanning electron microscopy (SEM) images were collected with a Phenom G2 Pure when detecting backscattered electron without coating the samples and an Hitachi FEG 6400 microscope after coating the samples with 5 nm of gold.
SEM imaging and EDS analysis were performed using Zeiss LEO 1530 on uncoated samples.
SOLVER P47H Scanning Probe Microscope (NT-MDT, Zelenograd, Moscow, RU) was employed to acquire topographic images of the skeleton morphology and electrostatic images of mds-nPs embedded into the skeleton. Topographic images were acquired in Tapping Mode through NT-MDT NSG01 cantilevers (resonant frequency $\omega_0 \sim 150$ kHz, elastic constant $k \sim 5$ N/m), whereas

electrostatic images were acquired by second pass technique with NT-MDT NSG01 cantilevers (ω_0 ~ 140 kHz, k ~ 5 N/m).
Structural and morphological characterization of the nanoparticles was performed using a 200 kV FEI Tecnai F20 ST Transmission Electron Microscope (TEM).
Confocal microscope images were collected using an upright Olympus microscope equipped with laser 488 nm and 561 nm lasers and a spectral detector and analysed using the software imageJ, making a sum of the slides.
Dynamic Light Scattering measurements were performed with a Malvern Nano ZS instrument equipped with a 633 nm laser diode. Samples were diluted in water and then housed in disposable polystyrene cuvettes of 1 cm optical path length. The values were taken averaging three different runs to get the standard deviation.
The concentration of elements was determined by plasma spectrometer (ICP-OES Ametek Spectro, Arcos, Kleve, Germany).

Supplementary data

Table SI1. Nano-particle loading in synthetic calcite crystals determined by ICP-OAS.

	Ca (ppm)	Fe (ppm)	Fe loading (wt.%)
Blank	51.61	0.12	0.22%
0.1 mg/mL mds-nP	129.02	4.07	3.16%
0.1 mg/mL peg-P-nPs	180.04	3.41	1.89%
0.1 mg/mL peg-am-nPs	40.63	0.21	0.52%

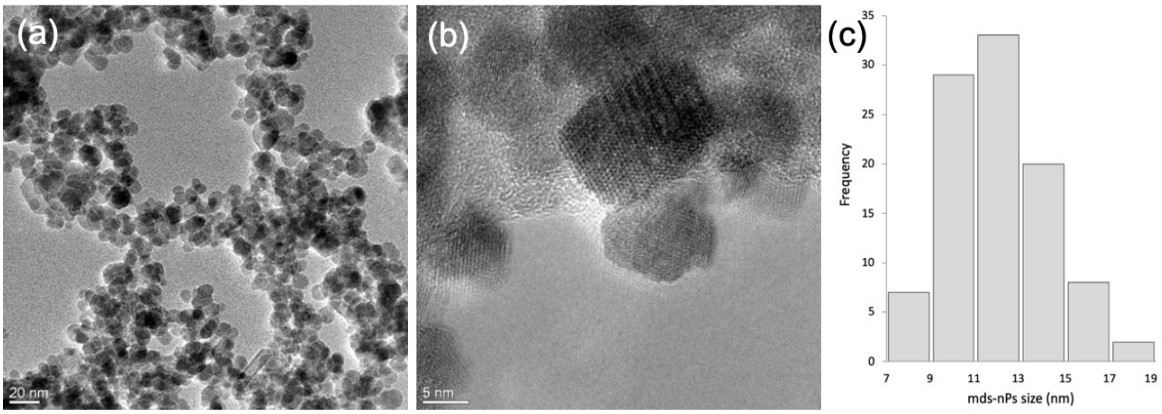

Figure SI1. (a,b) TEM images of mds-nPs and (c) core size distribution (n=100).

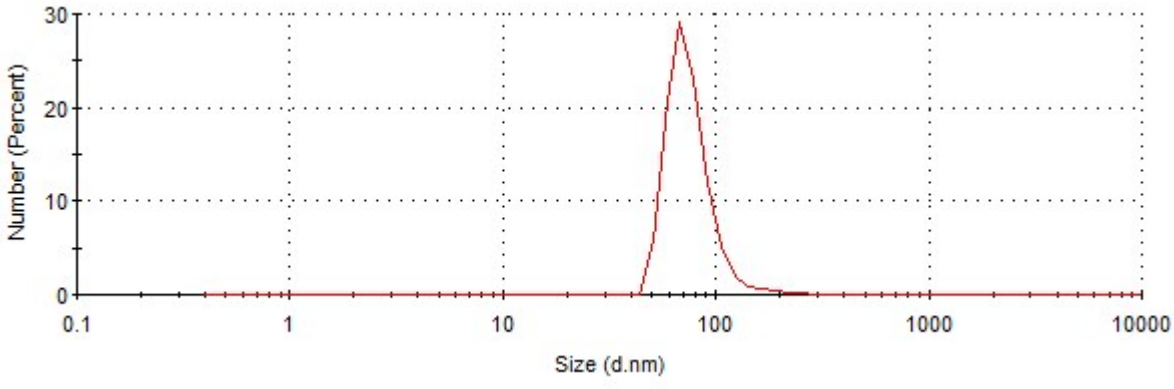

Figure SI2. DLS measurement of the hydrodynamic radius of mds-nPs at a concentration of 0.01 mg/ml.

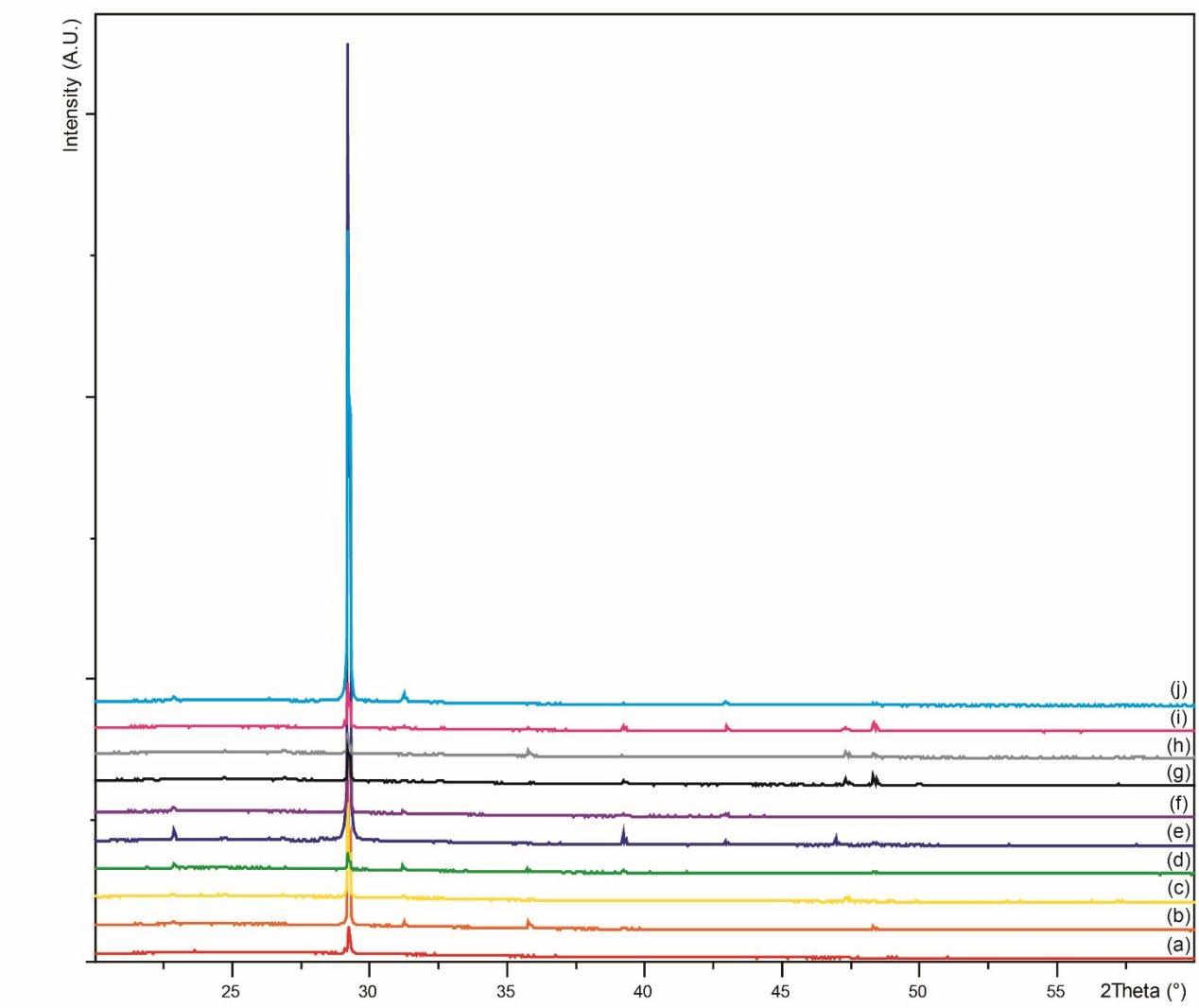

Figure SI3. XRPD pattern of samples prepared (a) without additives and in the presence of (b) 0.1 mg/mL mds-nPs, (c) 0.01 mg/mL mds-nPs, (d) 0.001 mg/mL mds-nPs, (e) 0.1 mg/mL peg-P-nPs, (f) 0.01 mg/mL peg-P-nPs, (g) 0.001 mg/mL peg-P-nPs, (h) 0.1 mg/mL peg-Am-nPs, (i) 0.01 mg/mL peg-Am-nPsand (l) 0.001 mg/mL peg-Am-nPs. Only the diffraction peaks of calcite (JCPDS: 00-005-0586) are present in all the patterns.

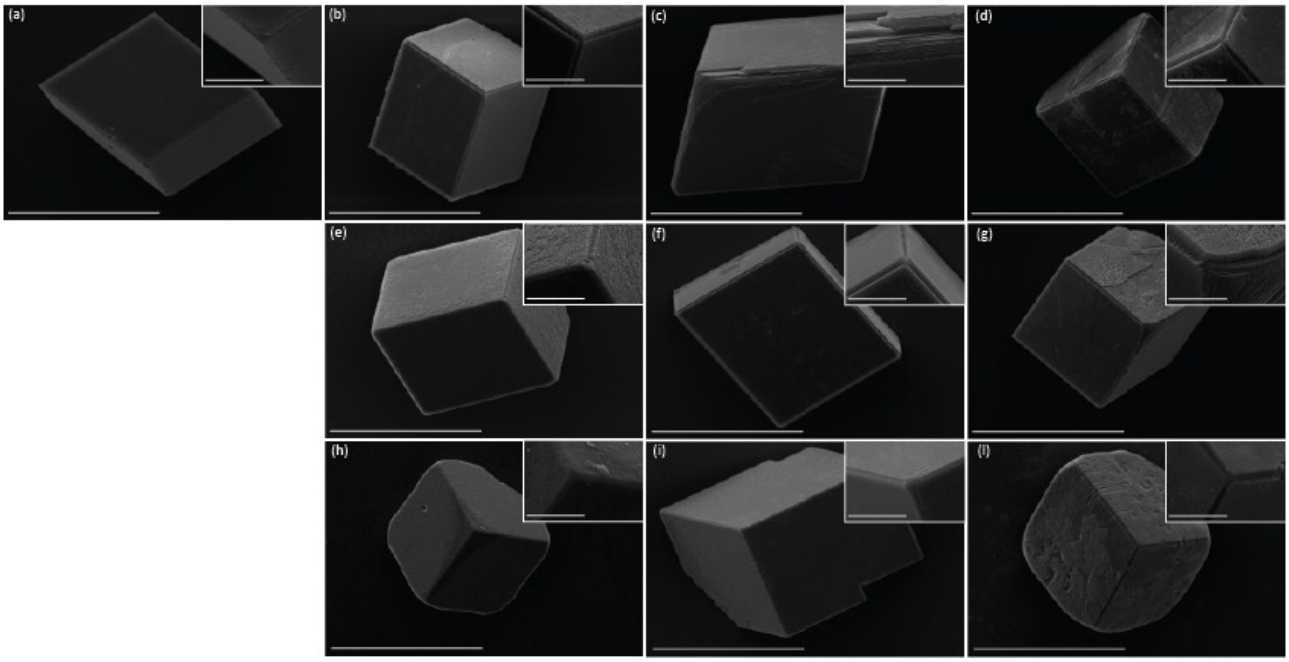

Figure SI4. SEM images of samples prepared (a) without additives and in the presence of (b) 0.1 mg/mL mds-nPs, (e) 0.01 mg/mL mds-nPs, (h) 0.001 mg/mL mds-nPs, (d) 0.1 mg/mL peg-P-nPs, (g) 0.01 mg/mL peg-P-nPs, (l) 0.001 mg/mL peg-P-nPs, (c) 0.1 mg/mL peg-Am-nPs, (f) 0.01 mg/mL peg-Am-nPs and (i) 0.001 mg/mL peg-Am-nPs. The scale bar is 40 µm in the main image and 10 µm in the inset.

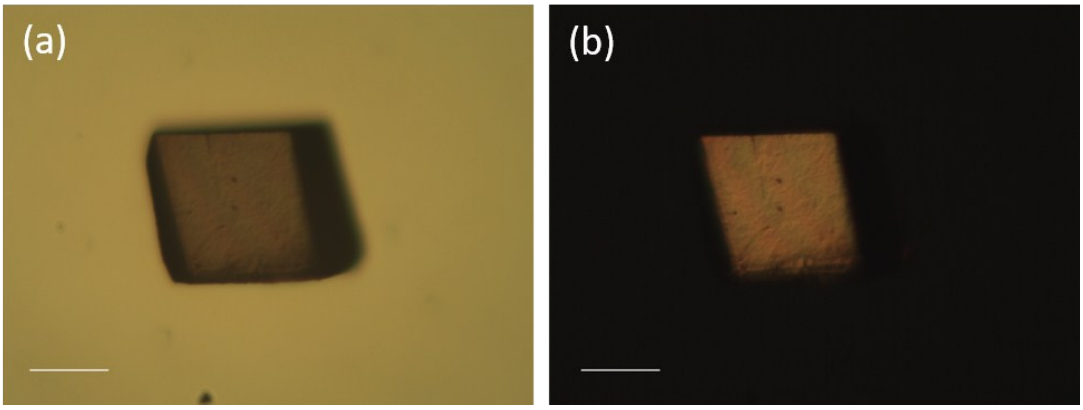

Figure SI5. (a) Optical microscopy image of a crystal of calcite grown in the presence of 0.1 mg/mL mds-nPs. (b) The same image under crossed polars. The fact that all crystal is birefringent confirms that it is a single crystal. Scale bar: 40 µm.

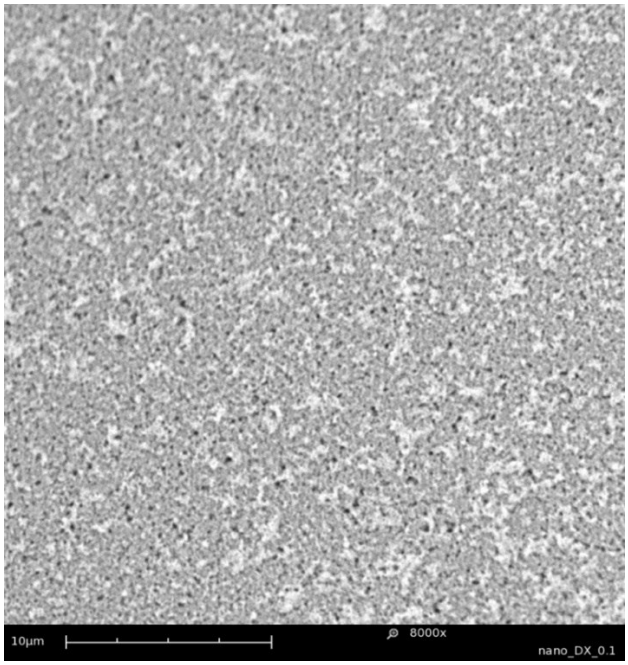

Figure SI6. Surface of a calcite crystal grown in the presence of 0.1 mg/mL of mds-nPs acquired detecting back-scattered electron. The presence of different shades of grey shows the distribution of calcium (darker grey) and iron (lighter grey) atoms.

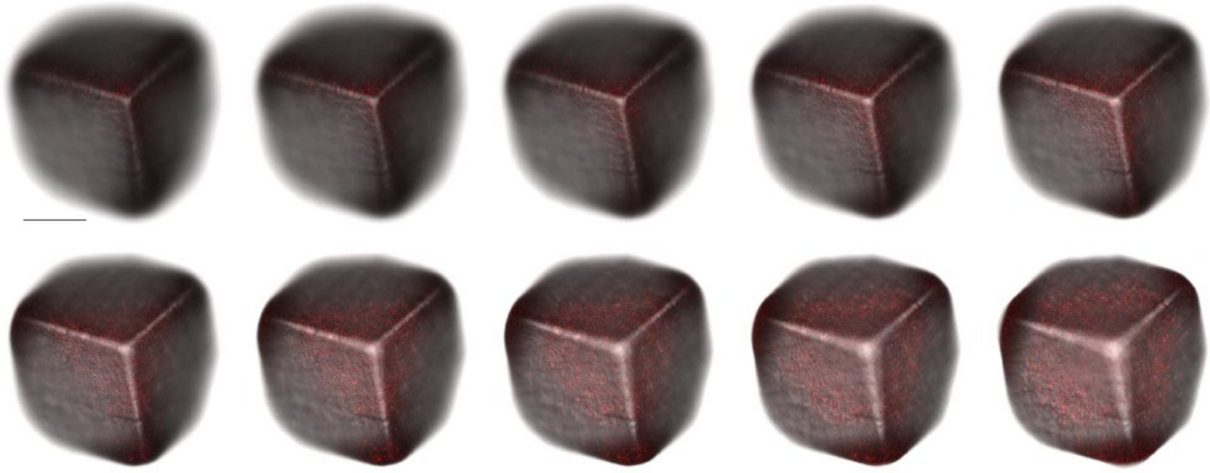

Figure SI7. Confocal fluorescence microscopy images of sections of a calcite crystal grown in the presence of 0.1 mg/mL mds-nPs. Scale bar: 50 µm.

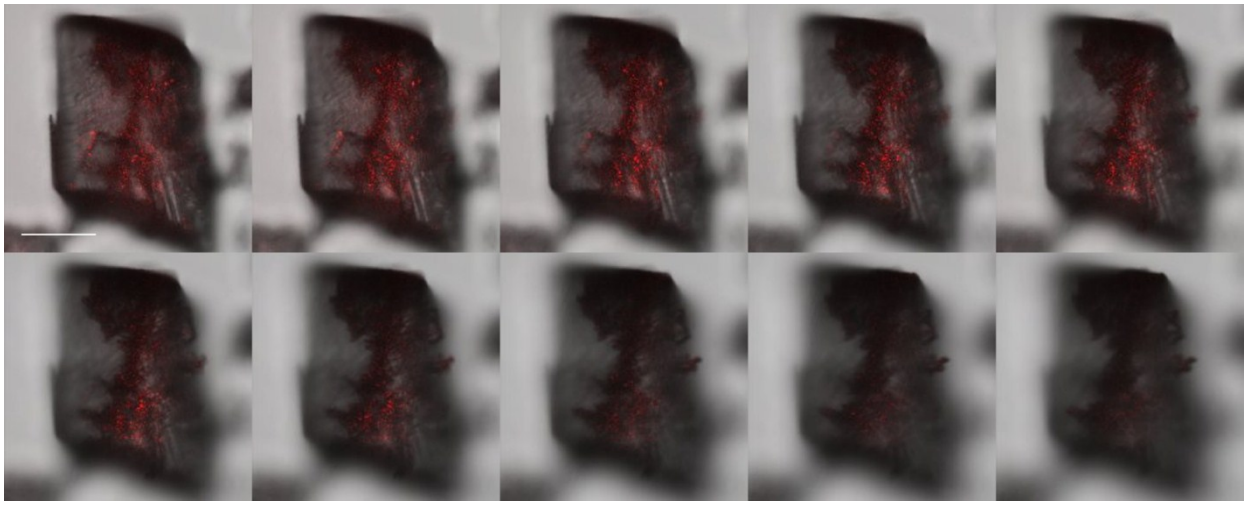

Figure SI8. Confocal fluorescence microscopy images of sections of a bleached and then broken calcite crystal grown in the presence of 0.1 mg/mL mds-nPs. Nanoparticles are homogeneously included inside the crystal. Scale bar: 40 µm.

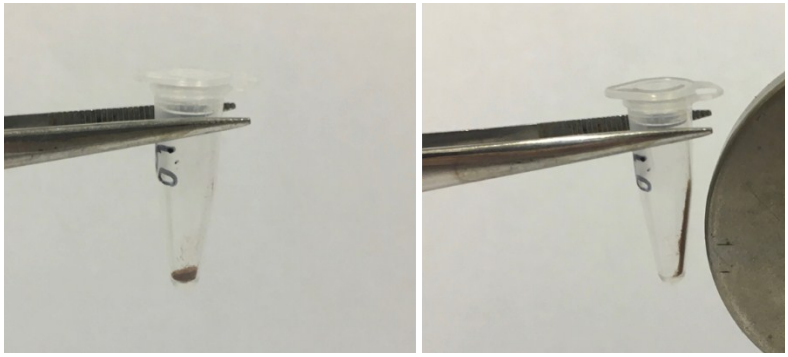

Figure SI9. Crystals prepared in the presence of 0.1 mg/mL mds-nPs respond to an external magnetic field.

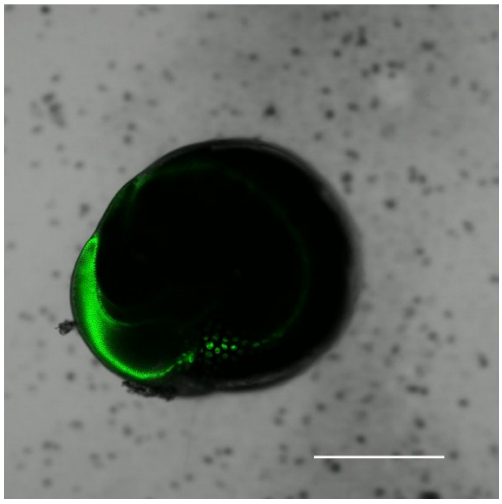

Figure SI10. *A. lessoni* skeleton growth in the presence of calcein. The last grown chamber is labelled. It is visible mainly in the left lower part of the skeleton, but it extends along its periphery. Scale bar: 500 µm.

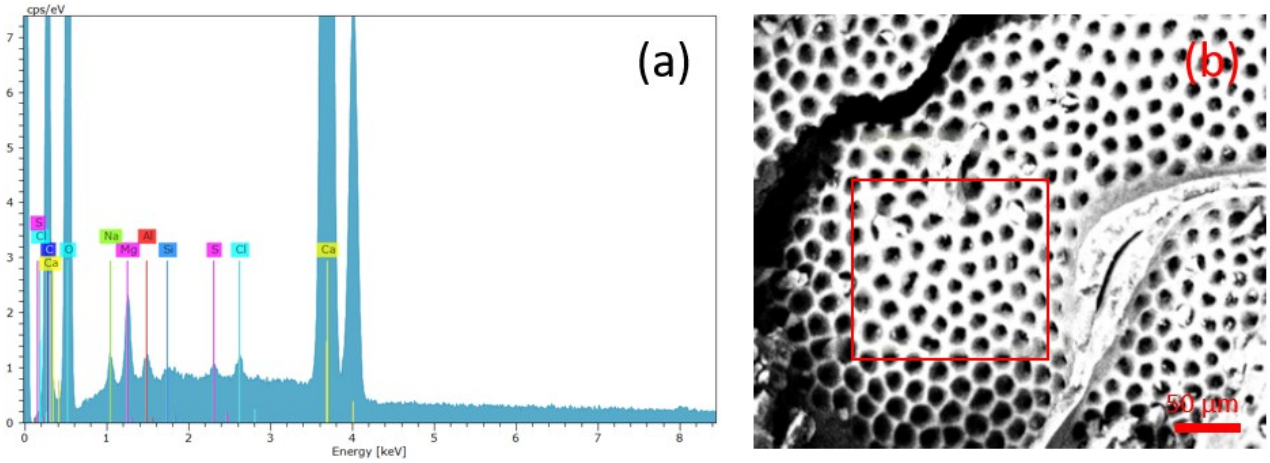

Figure SI11. (a) EDS graph acquired on a dead foraminifera skeleton first incubated in seawater containing nanoparticles and then bleached, no signal due to iron (6.4 KeV) was detected. (b) SEM image of the investigated skeleton, in read perimeter of the area analyzed.

Figure SI12. Optical microscope image of an *A. lessoni* specimen treated with calcein and mds-nPs. Darker spot due to the presence of mds-nPs in the last grown chamber, mainly localized in the periphery of the foraminifer.

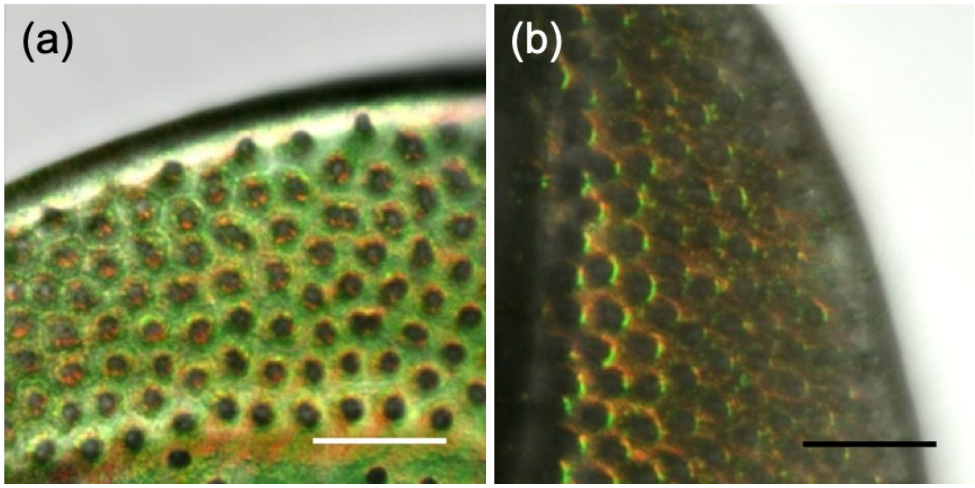

Figure SI13. Confocal microscopy images of (a) shell of *A. lessoni* grown with mds-nPs and calcein, (b) shell of dead control. Scale bar:40 µm.