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Supporting information

# Beyond biotemplating: Multiscale porous inorganic materials with high catalytic efficiency

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## Experimental section

Sea urchin spine section preparation The external layer of *Phyllacantus imperialis* spines was removed by abrasion prior slicing the spine along its axis to obtain 3-5 mm thick cylinders. The slices were then treated with 5% v/v sodium hypochlorite for 24 hours, thoroughly rinsed with water and air dried.

Colloidal particle synthesis PS nPs with diameter of 200 nm were prepared by polymerization of styrene in water emulsion promoted by potassium persulphate, controlling the size by changing the temperature and reaction time. In a typical experiment, 12 g of styrene was added to 100 mL of water in a round bottom flask under vigorous stirring. 500 mg of potassium persulphate was added to the solution and the polymerization was initiated by increasing the temperature until reflux. The reaction was allowed to proceed for 6 hours. The organic phase was then removed and the aqueous one, containing the PS nPs was collected. The PS nPs diameter was determined by DLS measurements (Malvern Nano ZS instrument equipped with a 633 nm laser diode).

Colloidal particles assembly Sea urchin spine slices were placed in 750 µL of as-synthesized 0.1 wt.% 200 nm colloidal particles solution in a 24-well multi-well dish. This was put under vacuum in a desiccator attached to a rotary pump (about $10^{-3}$ Torr) several times to facilitate the wetting of the inner pores. Then, the dish was placed in the oven and kept at 65 °C until water fully evaporated. The process was repeated 2 times.

$TiO_2/SiO_2$ replica fabrication Titania was obtained using titanium tetraisopropoxide (TTIP) as a precursor. Briefly, TTIP was diluted 1:10 with EtOH and poured over the template. The sample was put under vacuum several times to facilitate the wetting of the inner pores

and put in a closed desiccator with water for 2 days. Then, the sample was transferred in an oven and kept at 65 °C until completely dry. The process was repeated 2 times.

Silica was obtained using tetraethylorthosilicate (TEOS) as a precursor. One part of TEOS was mixed with 1 part of 0.1 M HCl and 1.5 parts of EtOH and stirred for 1 hour. Then, the TEOS solution was diluted 1:1 with ethanol and poured over the template. The sample was put under vacuum several times to facilitate the wetting of the inner pores and then placed in the oven and kept at 65 °C until completely dry. The process was repeated 2 times.

All the samples were calcined at 500 °C for 5 hours to remove the nPs and sinter the inorganic matrix.

Template removal The $CaCO_3$ template was removed by placing the calcined sample in 0.1 M acetate buffer (pH 4.5) for 14 days. The sample was then rinsed and freeze-dried to remove the water. Samples were further calcinated at 650°C to improve their mechanical resistance.

Pristine samples Pristine scaffolds were prepared according to the same procedure, with the exception of the step described in the paragraph Colloidal particles assembly, which was omitted.

Scanning Electron Microscopy observations SEM images were collected using a HR-SEM (ULTRA Plus, Zeiss, Oberkochen, Germany or LEO, Zeiss, Oberkochen, Germany) after coating the samples with 3 nm of gold. EDS analyses were performed using Zeiss LEO 1530 on uncoated samples.

Brunauer-Emmett-Teller measurements The BET specific surface area was measured on an ASAP 2020 Micromeritics instrument, using $N_2$ as absorbent at the liquid $N_2$

temperature (77 K). Before measurements, the samples were degassed at 403.15 K for 24 h.

X-Ray Powder Diffraction Analyses X-ray Powder diffraction (XRPD) measurements were performed with a PanAnalytical X'Pert Pro diffractometer equipped with X'Celerator detector with Cu Kα radiation in the range 20-70° 2θ, step size 0.0501 2θ and counting time 40 sec per step.

Photocatalytic performance test The photocatalytic degradation of Rhodamine B (RhB) by scaffolds obtained with and without the addition of colloids was measured using a procedure derived from Huang *et al*[38]. 1 mL of 12 mM RhB solution has been added to 6 wells (2 mL each) containing 5 mg of $SiO_2/TiO_2$ replica in each well. The samples were then irradiated for 1 h with a 300 W solar spectrum simulating lamp (Osram Ultra-vitalux) at a distance of 20 cm. RhB concentration in the solution has been determined by UV-Vis spectroscopy.

## Further characterization

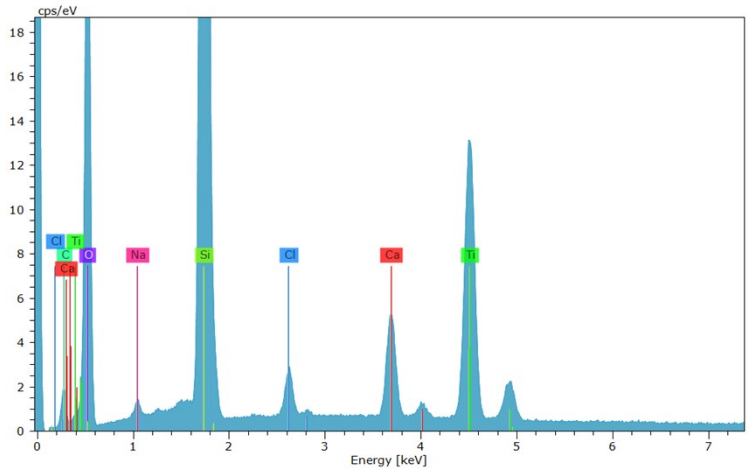


Figure SI1. EDS spectra of the hybrid $SiO_2/TiO_2$ scaffold.

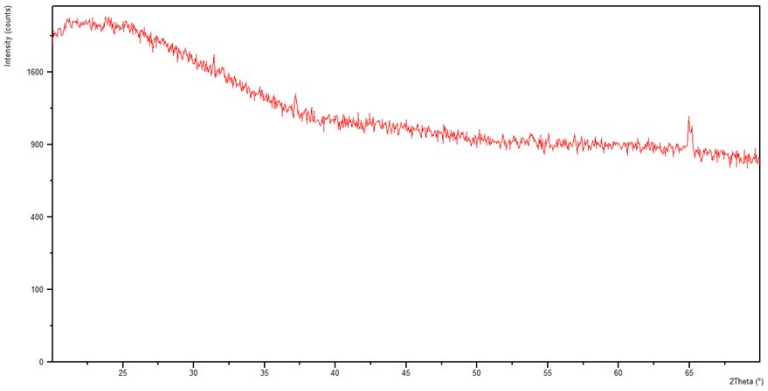


Figure SI2. XRPD of crushed hybrid $SiO_2/TiO_2$ scaffold

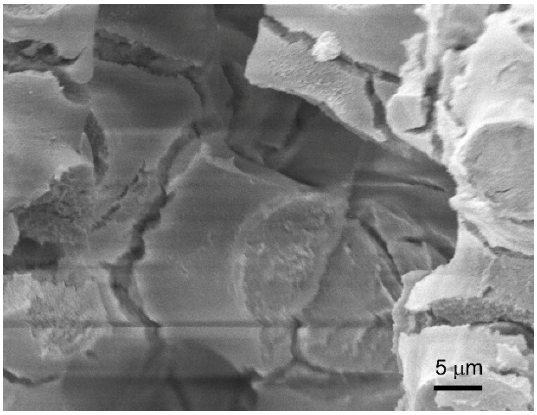


Figure SI3. SEM image of the control hybrid $SiO_2/TiO_2$ scaffold

BET measurements results The surface area of pristine sea urchin spine is 0.1 $m^2/g$, obtained measuring 3.366 g of sample. This value is below the ideal range of the instrument, so we approximated this data as a >1 $m^2/g$ for our further considerations. The surface area measured for the MSP $SiO_2$ samples is 85±2 $m^2/g$, obtained measuring 0.738 g of sample.

## MSP scaffolds for biocatalytic applications

$SiO_2$ replica fabrication The same procedure described in the paragraph "$TiO_2/SiO_2$ replica fabrication" has been used using only tetraethylortosilicate (TEOS) and repeating the deposition process 4 times.

Template removal The $CaCO_3$ template was removed by placing the calcined sample in 0.1 M acetate buffer (pH 4.5) for 14 days. The sample was then rinsed and freeze-dried to remove the water. Samples were further calcinated at 650°C to improve their mechanical resistance.

Enzyme adsorption and activity test $SiO_2$ scaffolds have been submerged in a 7.5 mg/mL *Aspergillus Orzae* α-amylase (Sigma Aldrich-30 U/mg) solution for 24 hours and then rinsed 2 times with DI water.

The enzymatic activity of scaffolds obtained with and without the addition of colloids was tested following the Enzymatic Assay of α-Amylase (EC 3.2.1.1).

Biocatalytic reactions are a powerful tool to perform reactions with high selectivity for a particular substrate and reducing the quantity of waste products even at an industrial level. Among them, enzymes of the family of amylase find applications in a variety of processes, ranging from paper industry to food processing. Amylases are glycoside hydrolases and act on α-1,4-glycosidic bonds, converting starch to sugars. MSP and pristine $SiO_2$ scaffolds have been used as substrates for α-amylase from *Aspergillus orzae*, which has been adsorbed on the $SiO_2$ surface taking advantage of electrostatic interactions. This simple method was chosen over others such as layer by layer deposition or covalent bonding for its easiness and for the minimum number of reagents involved, which lower the chances of clogging the neck between pores and makes the

process greener. We tested the performance of pristine and MSP $SiO_2$ on which α-amylase was adsorbed on the degradation of soluble starch to maltose. The higher surface area of the MSP scaffold should increase the amount of enzyme adsorbed per weight and thus the total catalytic efficiency. As can be seen in figure SI1, MSP scaffolds produce double the amount of maltose than the substrate with just the native spine porosity, thus confirming the efficiency of the MSP platform in improving the reaction outcome. Furthermore, the substrate might potentially be recovered and used or functionalized again after performing the reaction as it deposits at the bottom of the flask.

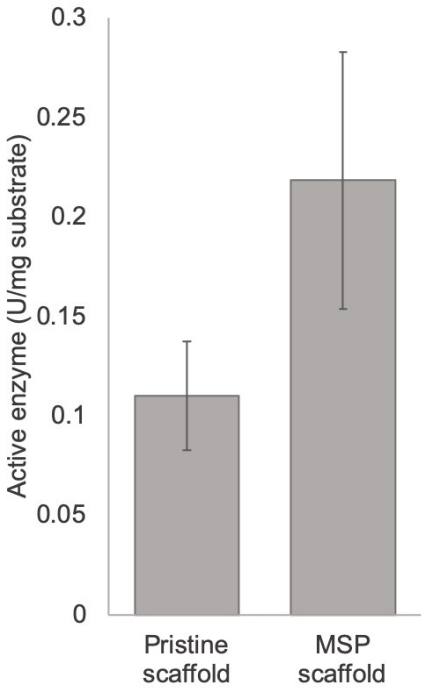


Figure SI4. Enzymatic activity of α-amylase adsorbed onto pristine and MSP saffolds. P-value= 0.056.