Supplementary Information

A liposome-actuated enzyme system and their capability as self-biomineralized silica nanoreactors

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EXPERIMENTAL SECTION

Preparation of urease-containing liposomes. The urease-containing liposomes were prepared by the lipid film hydration and extrusion method:[S1-S3] suitable amounts of dipalmitoylphosphatidylcholine (DPPC) and cholesterol (1:0.2) were dissolved in chloroform and dried to a film under a stream of nitrogen. The dry lipid film was hydrated by adding 2 ml of an aqueous solution of urease (2 mg/ml) and sonicated for 30 minutes (above Tm of the lipids (50 °C) using a bath ultrasonic cleaner (Power = 500 W). Liposomes were sized by multiple extrusion above Tm of the lipids (50 °C) through polycarbonate membrane filters of decreasing pore diameters of 0.8 μm and 0.4 μm (5 and 15 passages respectively). The liposome suspension obtained was a mixture of the urease-containing liposomes and free urease. The sample was centrifugated at 6000 rmp for at least 30 min.[S4-S5] The supernatant containing the free urease was then discarded and the pellet containing encapsulated urease into liposomes was resuspended with Milli-Q H_2O. The mean vesicle size and size distribution of urease-containing liposomes were determined by Dynamic Light Scattering (DLS).

Urease activity assay. A colorimetric assay based on the hydrolysis of urea was used for the activity measure of encapsulated and free urease, as monitored by the pH-sensitive dye bromothymol blue (BTB).[24] A solution containing 50 μM BTB, 0.2 mM EDTA and a known amount of the urease-loaded vesicles or free urease/liposomes system was placed in a UV cell, stirred and annealed to the desired temperature. Then 22 μl of a solution 0.1 M urea was added to the cell and the kinetics was monitored by following the absorption at 617 nm on a Varian Cary 50 UV-Vis Spectrometer (Agilent Technologies).[S6] The increment of the absorbance with time was used to characterize the urease activity in both systems. The specific activity (U $mgenzyme^{-1}$) of urease was calculated from the slope of the linear section of the Product vs time plots.[S7]

Synthesis of mesoporous silica thin film. Mesoporous thin films were produced by combining sol-gel synthesis with evaporation-induced self-assembly and processed under controlled environmental conditions and thermal treatments, following well-established protocols.[S8-S10] The mesoporous SiO_2 films were deposited by spin-coating from alcohol-

water acidic solutions containing the inorganic precursor $Si(OEt)_4$ (TEOS) and Pluronics F127 ($(EO)_{106}(PO)_{70}(EO)_{106}$, where EO and PO represent ethylene oxide and propylene oxide blocks, respectively) as polymeric template. TEOS was prehydrolyzed by refluxing for 1 h in a water/ethanol solution; $[H_2O]/[Si] = 1$; [EtOH]/ [TEOS] = 5. To this prehydrolyzed solution was added surfactant, alcohol, and acidic water in order to prepare the precursor solutions, with final composition TEOS:EtOH:H_2O (0.1 M HCl): F127 equal to 1:40:5:0.0075 mol ratios. The relatively large [F127]/[Si] ratio ensures a high accessible porosity. After deposition by spin-coating, as-prepared films were placed in a 50% RH chamber for 1 h. The films were then subjected to a consolidation thermal treatment, which consisted of heating at 60 °C for 1 h and at 130 °C for another 1 h. Finally, the films were calcined at 350 °C for 2 h in order to remove the template.

Synthesis of silica nanostructures. For the preparation of the silica nanostructures a known amount of a solution containing 3.2 M urea and urease-loaded liposomes (0.4 mg urease/g lipid) was placed onto a mesoporous silica thin film. The sample was then incubated at 25 ºC or 45 ºC for 24 hs in a humidified chamber to favour a slow and controlled evaporation of the droplet. Once the droplet dried, the solid was resuspended with 100 µl of Milli-Q H_2O and then centrifugated at 6000 rpm for 30 min. The obtained precipitate containing the liposomes covered with a silica shell was washed several times with Milli-Q H_2O and stored at 4 ºC. The same protocol was used for the free urease/liposome system.

Characterization. The morphology of the precipitates was studied by field emission scanning electron microscopy (SEM, Carl Zeiss NTS SUPRA 40) and transmission electron microscopy (TEM) using a Philips EM-301 electron microscope. The samples for TEM were prepared by dispersing the final samples in Milli-Q H_2O; this dispersion was then dropped on carbon–copper grids covered by an amorphous carbon film. Liposomes size distribution was measured using a BI-200SM Laser Light Scattering Instruments (DLS, Brookhaven Instruments). Silica particles size analysis was done with software for image analysis (Image J). The Tm of DPPC/cholesterol liposomes was determined by Differential Scanning Calorimetry (DSC) and Turbidity.[S11-S12] DSC measurements were performed on approximately 500 µL of vesicles suspensions with a SetaramLabsis-DSC. The measurements were performed in the temperature interval from 15 to 65 °C, with 5 °C min^{-1} heating. Turbidity measurements were carried out on a Varian Cary 50 UV-Vis Spectrometer (Agilent Technologies) by using Milli-Q H_2O as a reference. Mesoporous film thickness, volume and pore size distribution values were obtained from Environmental Ellipsometric Porosimetry (EEP, SOPRA GES5A).[S8,S9]

S1 P. Walde and S. Ichikawa, *Biomol. Eng*. 2001, 18, 143.
S2 S. Municoy and M. G. Bellino, *ChemistrySelect* 2016, 1, 723.
S3 M.-S. Martina, J.-P. Fortin, C. Ménager, O. Clément, G. Barratt, C. Grabielle-Madelmont, F. Gazeau, V. Cabuil and S. Lesieur, *J. Am. Chem. Soc*. 2005, 127, 10676.
S4 V. M. Madeira, *BBA-Gen. Subjects* 1977, 499, 202.
S5 M. Li, M. J. Hanford, J.-W. Kim and T. L. Peeples, J. Biol. Eng. 2007, 1, 1.
S6 G. P. Gorbenko, *(BBA)-Biomembranes* 1998, 1370, 107.
S7 K. Wilson, J. Walker, Principles and techniques of practical biochemistry, Cambridge University Press, 2000.

S8 M. G. Bellino, I. Tropper, H. Duran, A. E. Regazzoni and G. J. A. A. Soler-Illia, *Small* 2010, 6, 1221.
S9 M. G. Bellino and G. J. A. A. Soler-Illia, *Small* 2014, 10, 2834.
S10 G. Soler-Illia, P. Angelomé, M. Fuertes, A. Calvo, A. Wolosiuk, A. Zelcer, M. Bellino and E. Martínez, *J. Sol-gel Sci. Techn*. 2011, 57, 299.
S11 K. Colbow, *Biochim. Biophys. Acta* 1978, 512, 97-104.
S12 F. Severcan, N. Kazanci, Ü. Baykal, Ş. Süzer, *Biosci. Rep.* 1995, 15, 221.
S13 B. Krajewska, R. Van Eldik and M. Brindell, *J. Biol. Inorg. Chem.*, 2012, **17**, 1123.

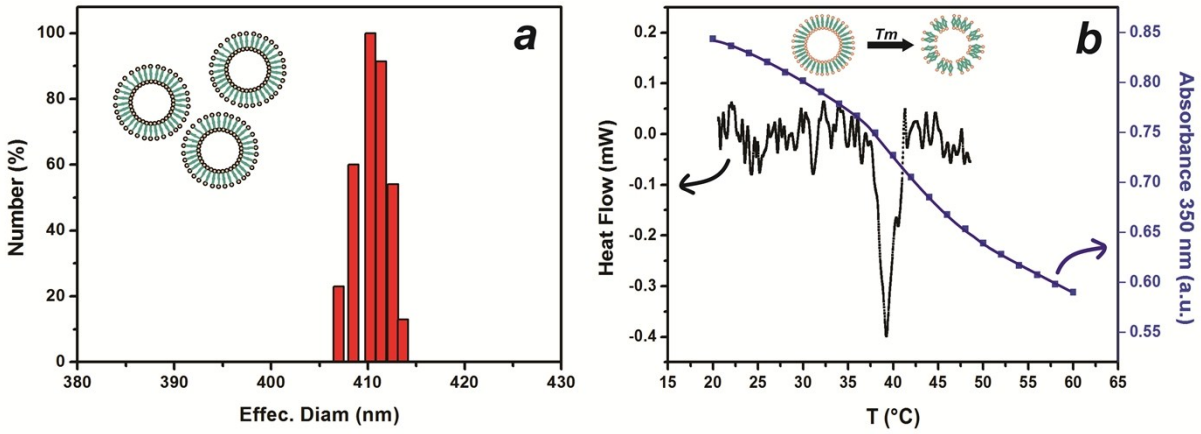

Figure S1. DPPC/Cholesterol liposomes characterization. a) The DLS graph reveals the size distribution of liposome (average size = 410 nm). b) DSC thermogram (black) and Turbidity measurement (blue) of DPPC/Cholesterol liposomes. The sharp black peak at approximately 40 ºC corresponds to the lamellar gel-to-liquid crystalline phase transition (Tm) of lipid bilayer. The midpoint temperature of the transition (blue curve) also indicates that Tm = 40 ºC.

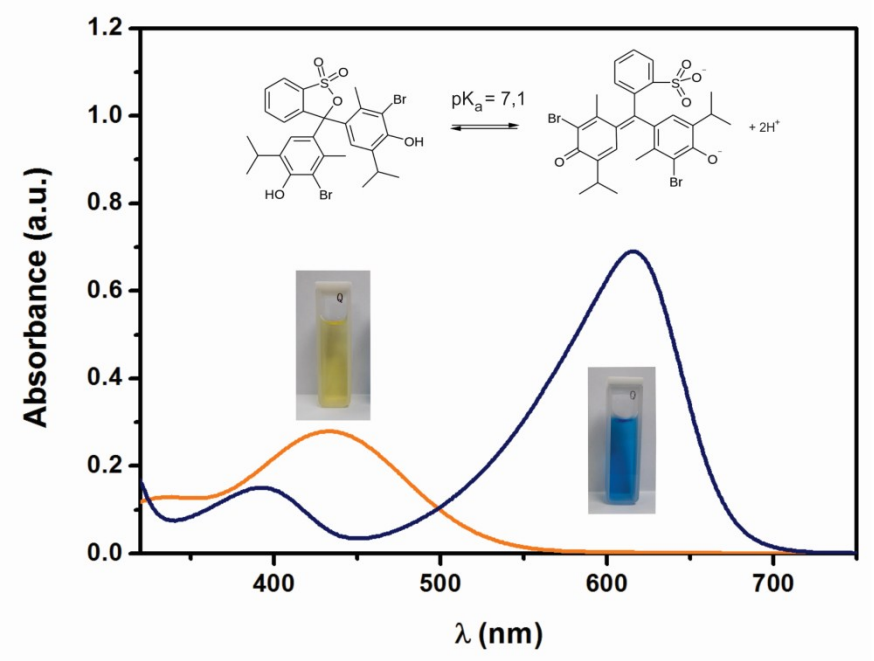

Figure S2. Absorption spectra of dye bromothymol blue: in acidic solutions, it appears yellow while in basic solutions it is blue. The yellow (BTB) and the blue (BTB^-) forms have its maximum absorbance at 432 nm and 617 nm, respectively.

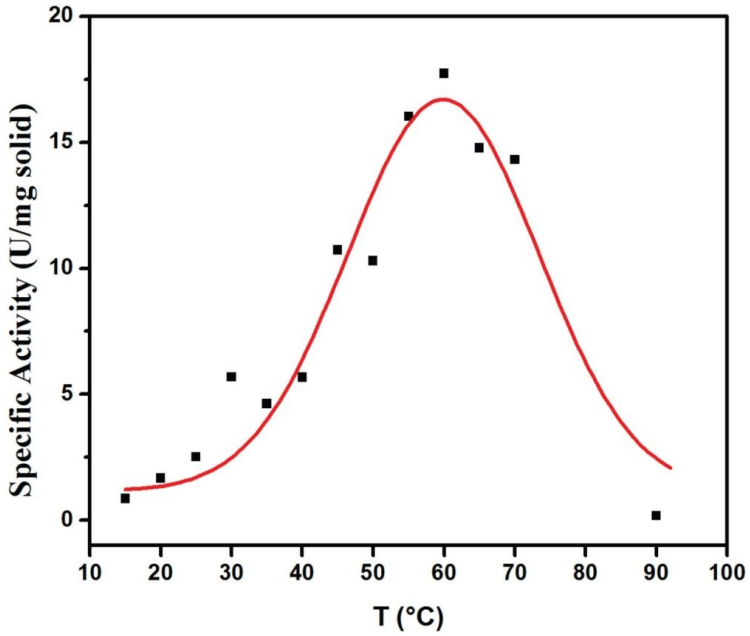

Figure S3. The dependence of urease activity on temperature. The optimum temperature of the urease was found at 60 ºC, in agreement with previous research.[S13]

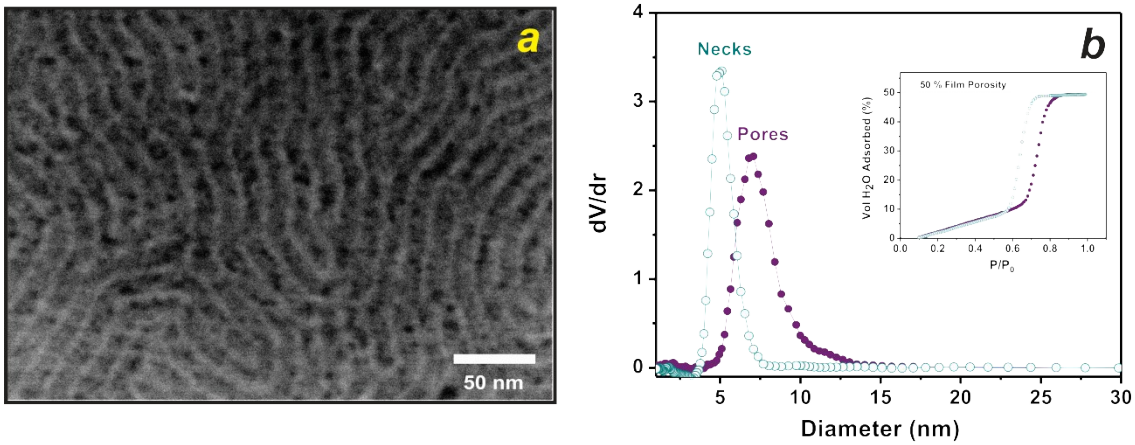

Figure S4. Mesoporous silica thin film characterization. a) Top-view SEM image of the mesoporous thin film. b) Typical pore and neck size distribution of the mesoporous silica thin films used in this work. Inset: water adsorption-desorption isotherms at 298 K. Isotherms were determined by EEP. Film thickness and the real component of the refractive index were obtained from the ellipsometric parameters $\psi(\lambda)$ and $\Delta(\lambda)$ under nitrogen flux containing variable water vapor quantities; P/P_0 was varied from 0 to 1 (P_0 being the saturation water vapor at 298 K); the film refractive index was described according to a modified Cauchy equation. Film pore volume and pore size distribution at each P/P_0 were obtained by modeling the refractive index obtained according to a three-medium Bruggeman effective medium approximation (BEMA); pore size distributions were obtained from the analysis of the refractive index variation, using the WinElli 2 software (SOPRA, Inc.).

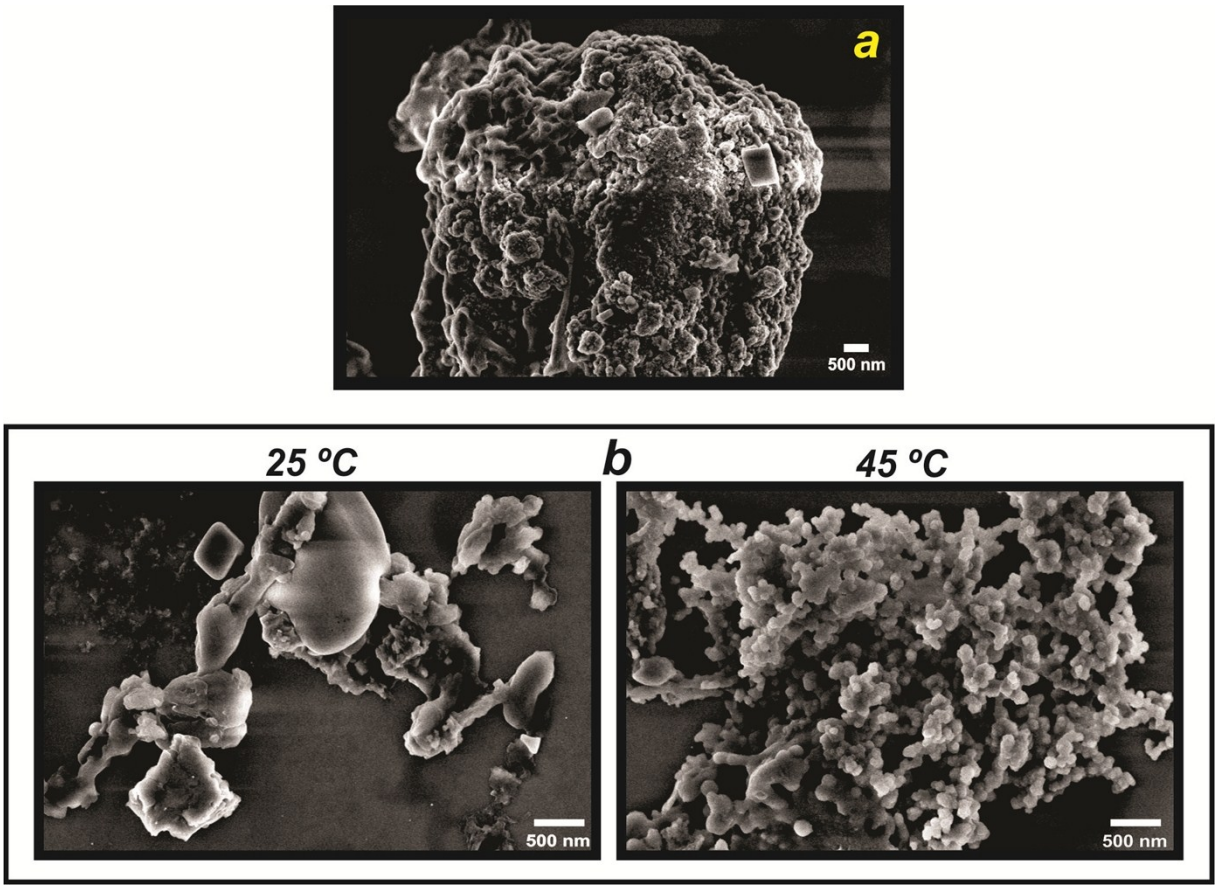

Figure S5. Morphologies of precipitates obtained by enzyme/liposome assembly at 45 ºC (a) and free urease/liposome system at 25 º and at 45 ºC (b), where uncontrolled precipitation is observed.