Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information for:

Immobilizing and de-immobilizing enzymes on mesoporous silica

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Table of Contents

Supplementary figures:

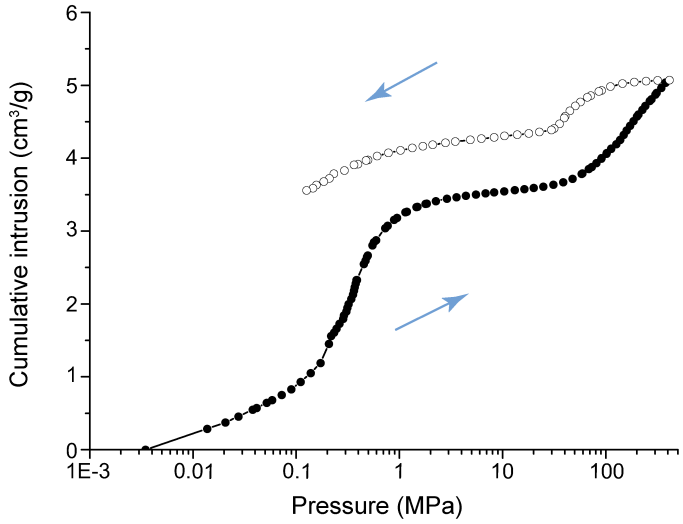


Fig. S1 Cumulative mercury intrusion analysis of the siliceous mesocellular foam (MCF). Solid circles indicate the intrusion whereas the empty counterparts stand for extrusion.

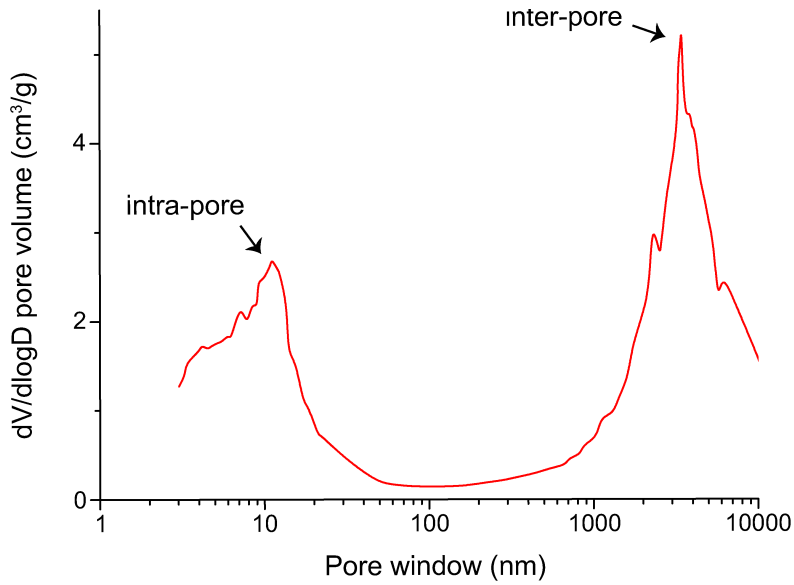


Fig. S2 Size of the pore windows of the MCF cells plotted against the pore volume as extrapolated from the mercury intrusion data.

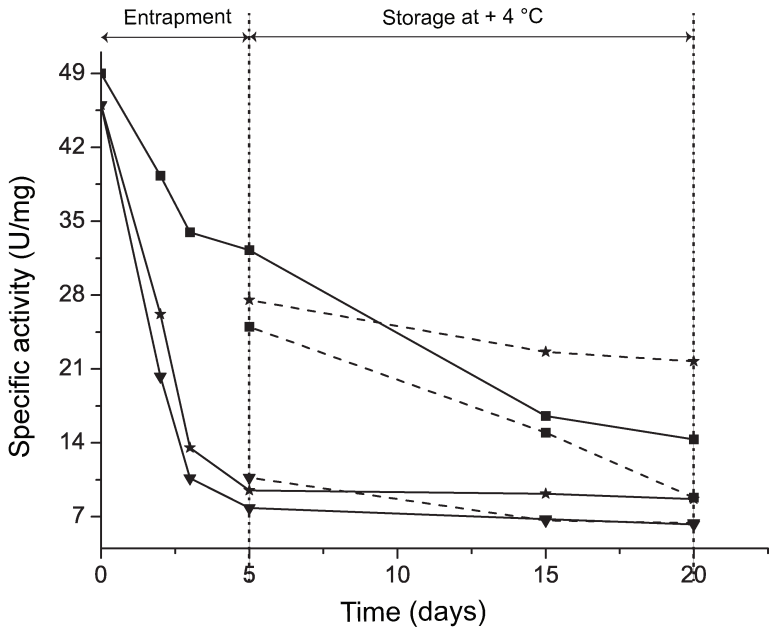


Fig. S3 Influence of the entrapment process and storage on the enzymatic activity of β-glucosidase entrapped (black stars) compared to the β-glucosidase immobilized only (black inverted triangles) and β-glucosidase free in solution (black squares) before (solid lines) and after fluoride buffer support dissolution (dashed lines). No improved activity of the immobilized enzyme after dissolving its support could be observed.

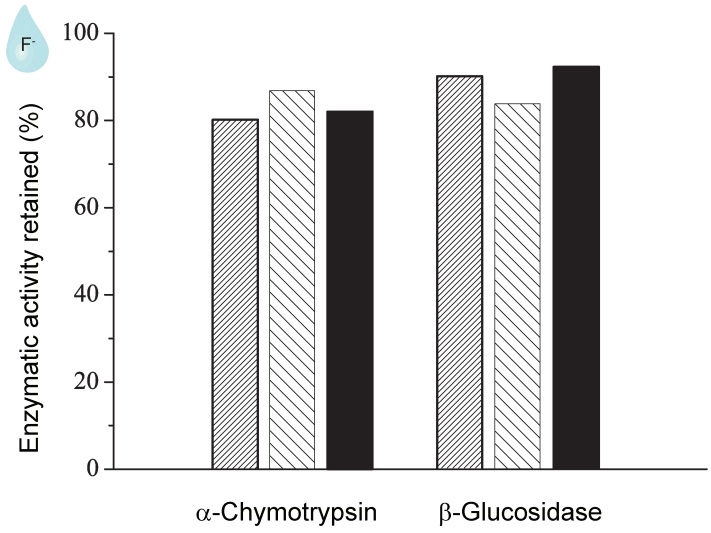
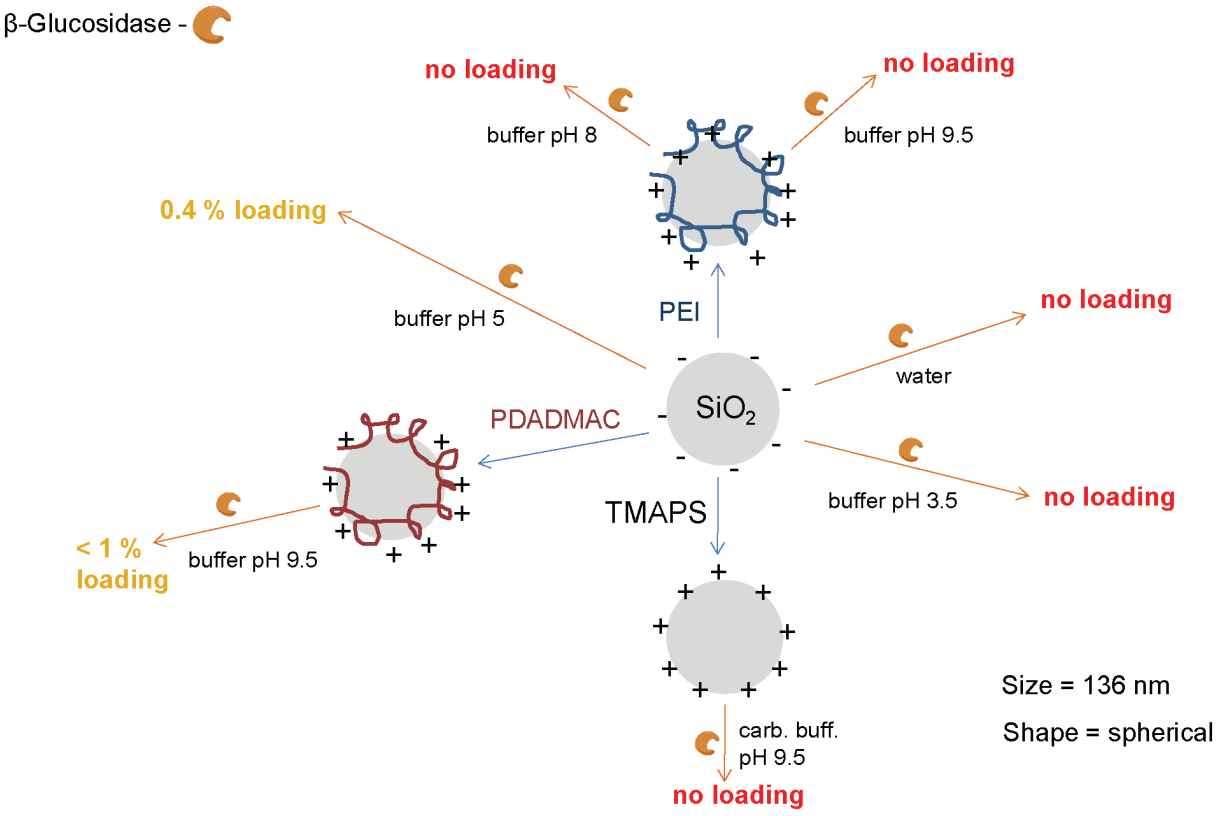


Fig. S4 Influence of the fluoride-buffer on the enzymatic activities of β-glucosidase and α-chymotrypsin. Enzymatic activities of the β-glucosidase free in solution (dense columns) and β-glucosidase released (white columns) after heat treatment; the activities are relative to the corresponding activities at room temperature.

Unsuccessful encapsulation experiments

Silica particles as the support of interest

For comparison, we have tried to immobilize β-glucosidase on spherical silica particles with a size of 136 nm. The spherical particles could be potentially a very interesting support due to the simpler and faster synthesis as well as greater uniformity in particle size. For this purpose we performed an array of adsorption experiments in which we reproduced the binding conditions used in the case of the MCF-β-glucosidase, meaning 20 mg SiO_2 (Microparticles GmbH) and 20 mg β-glucosidase powder, except for exchanging the binding buffer (1 mL) in some cases. This was required after introducing positive charges on the SiO_2 particles surface with the help of either TMAPS (*N*-trimethoxysilylpropyl-*N,N,N*-trimethylammonium chloride) or a positively charged polymer (PDADMAC (Polydiallyldimethylammonium chloride, Aldrich) or PEI (Polyethylenimine, ABCR)) (Scheme S1). All the buffers were prepared at the concentration of 0.1M: sodium carbonate buffer pH 9.5, sodium phosphate buffer pH 8, sodium acetate buffer pH 5 and phosphate-citrate buffer pH 3.5 respectively. As already indicated on Scheme S1, we only managed to obtain very low enzyme loadings on the particles (measured by C, H, N % microelemental analysis) only in the cases where silica particles were incubated in sodium acetate buffer pH 5 and also when the PDADMAC covered particles were incubated with sodium carbonate buffer pH 9.5; no enzyme loading was evident in the rest of the attempts. Consequently, further silica entrapment experiments were not conducted.



Scheme S1 Attempts to immobilize β-glucosidase on spherical silica microparticles. As shown above, very low enzyme bindings were only possible in the case where the silica particles were incubated in sodium acetate buffer pH 5 and also when the PDADMAC covered particles were incubated with sodium carbonate buffer pH 9.5.

Other mesoporous silicas as the support of interest

In order to prove the applicability as a universal enzymatic support, we started immobilizing other enzymes on our MCF and compared the loading and enzymatic activity of the immobilized enzymes to a series of mesoporous silicates of different pore sizes. In the initial experiment, the loadings and the activities retained by the immobilized α-chymotrypsin were assessed. A mixture of the material (2 mg) and 1 mg enzyme powder in PBS pH 6.5 incubated for 3-4 h was the common immobilization way in each case. As seen in Table S1, our MCF showed a very high loading capacity and enzymatic activity of the immobilized enzyme, being a perfect candidate for the support of interest. Current work is underway in which we assess binding of other enzymes as well as their subsequent silica entrapment.

Table S1 Comparison of loading and enzymatic activity of α-chymotrypsin on different mesoporous silicates

Material	mesopore size (nm)	Enzyme loading (mg/g material)	Activity immobilized (%)
SBA-15-A5	5	25	0
SBA-15-D5	5	140	40
SBA-15-VS	6	0	0
USY zeolite	25	150	59
MCF (used in this work)	**23**	**330**	**74**