

Supporting information

Ultrafast enzyme immobilization over large-pore nanoscale mesoporous silica particles

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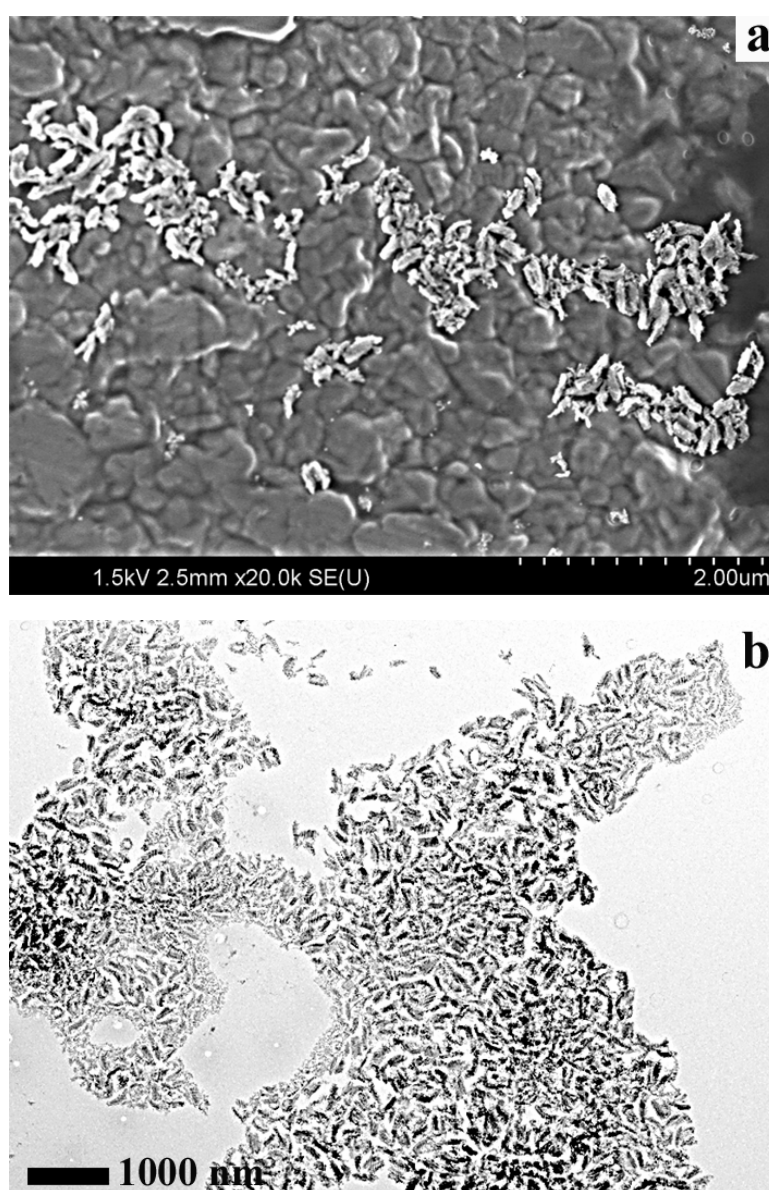


Figure S1. SEM (a) and TEM (b) images give an overview of the nanoscale mesoporous silica particles.

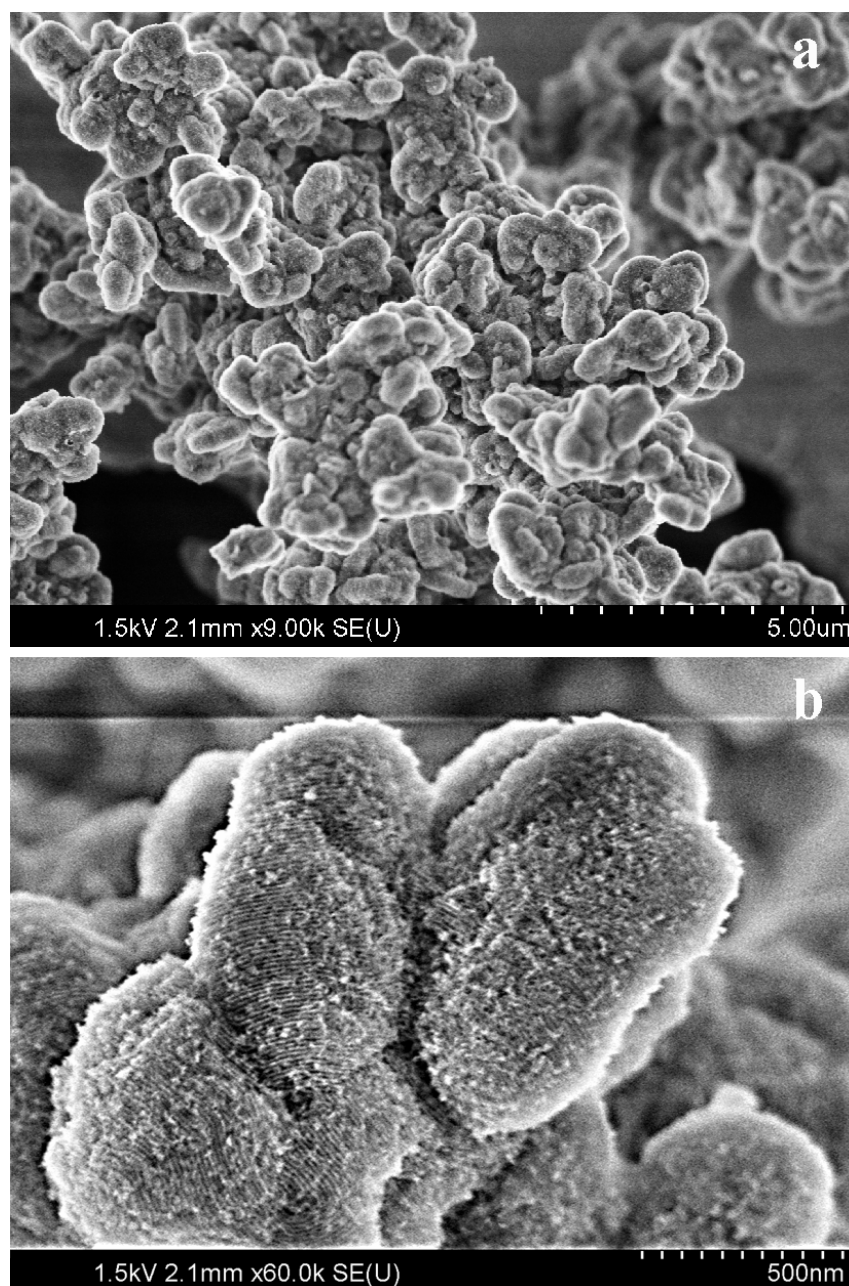


Figure S2. SEM/HRSEM images of the samples prepared with TEOS/P123 ratio of 60 at 298 K

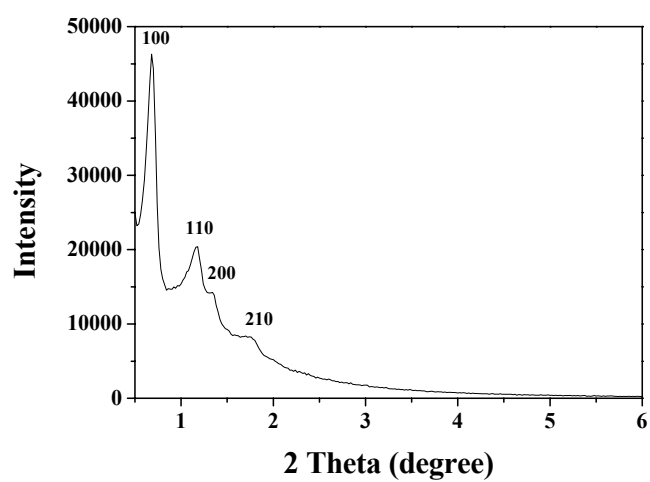


Figure S3. SAXD pattern of the obtained SBA-15 prepared with the molar ratio of TEOS/P123 at 60 at 298 K

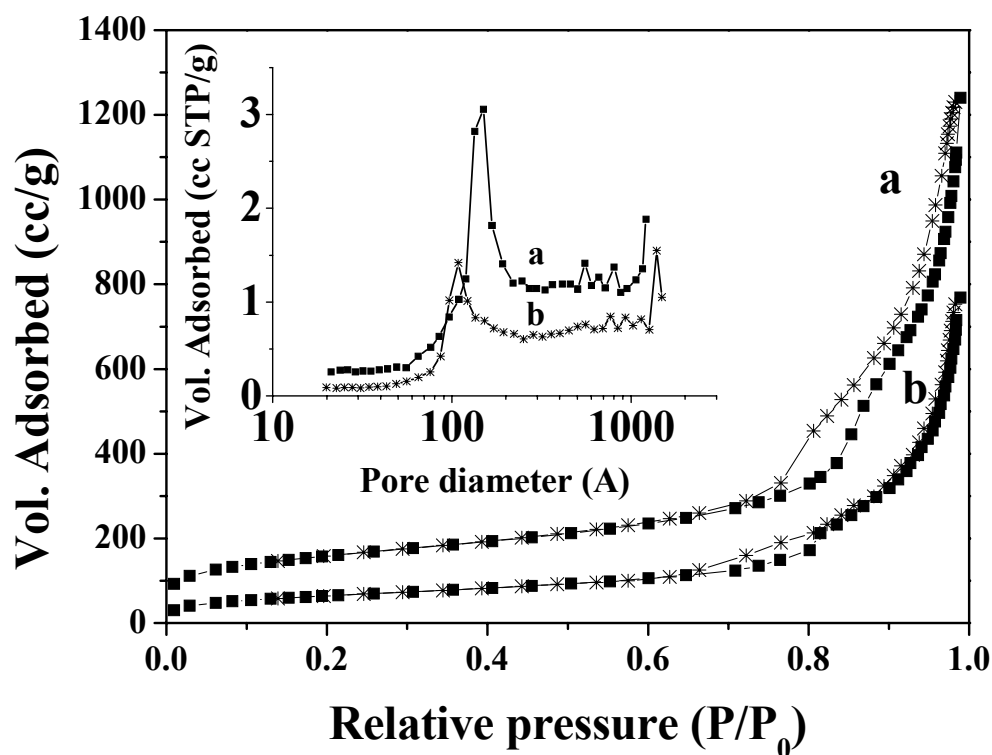
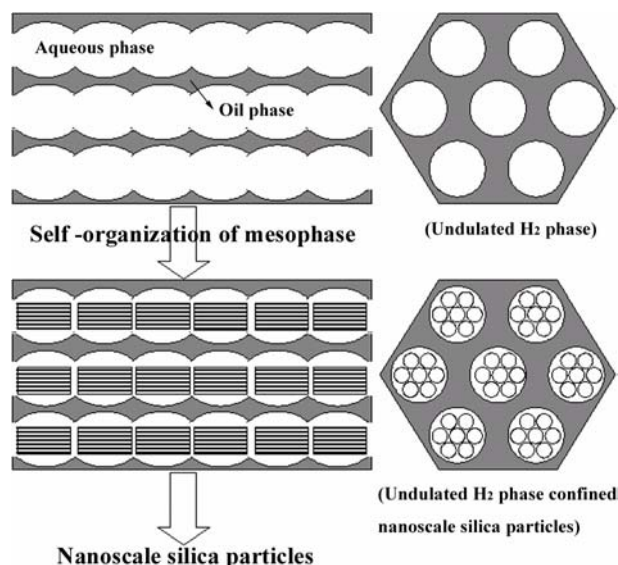


Figure S4 N₂ adsorption-desorption isotherms of nanoscale mesoporous silicas after immobilization of lysozyme (figure S4b). For comparison, the adsorption-desorption isotherms of nanoscale mesoporous silicas are also provided (figure S4a).

** The multipoint BET surface areas, mesopore diameter and BJH cumulative pore volume of nanoscale mesoporous silicas after immobilization of lysozyme are 235 m²/g, 10 nm and 1.11 cc/g respectively, all of which show a significant decrease compared with the nanoscale mesoporous silica itself.



Scheme S1. proposed mechanism for the formation of nanoscale mesoporous silica particles in the octane/water/P123/TEOS emulsion system**

** In the current study, when TEOS/P123 molar ratio is decreased from 60 (the same as that of traditional SBA-15, FigS2, S3 show the HRSEM image and SAXD patterns of the SBA-15 with the molar ratio of TEOS/P123 at 60 at 298 K) to 48, a visible change of the emulsion is that a very viscous synthetic mixture is formed during the synthesis, indicating that an ultrafine emulsion system might be *in situ* formed (Agarwal, V.; Singh, M.; Mcpherson, G.; John, V.; Bose, A. *Langmuir*, **2004**, *20*, 11). It has been reported that the attachment of silicate oligomers to the EO blocks could change the properties of the surfactants (M. Antonietti and C. Göltner, *Angew. Chem. Int. Ed.* 1997, **36**, 910; S. Förster, M. Antonietti, *Adv. Mater.* 1998, **10**, 195; Y.-Y. Fahn, A.-C. Su and P. Shen, *Langmuir* 2005, **21**, 431). On the other hand, TEOS concentration could determine the aggregation numbers of silicate oligomers, and thus the properties of silicate dopped surfactants, which plays the key role in controlling the phase

behavior of emulsion system and therefore the morphologies of the obtained materials.

Moreover, the phase behavior in water/oil/surfactant system has been widely studied and well understood (M. Bourrel, A. Graciaa, R. S. Schechter and W. H. Wade, *J. Colloid Interface Sci.* 1979, **72**, 161; J. L.

Salager, L. Quintero, E. Ramos, J. M. Andérez, *J. Colloid Interface Sci.* 1980, **77**, 287; F. S. Milos, D. T. Wasan, *Colloids Surf. A.* 1982, **4**,

91). Various phases (e.g., H_1 , H_2 and L_α et al.) have been recognized in the block copolymers (e.g., P123, F127)/water/oil (e.g., p-xylene) emulsion systems (P. Holmqvist, P.

Alexandridis, B. Lindman, *Macromolecules* 1997, **30**, 6788; P. Alexandridis, U. Olsson, B. Lindman, *Langmuir* 1997, **13**, 23). We

propose that, under the current conditions, an undulated swollen inverse hexagonal phase (H_2) would form in the octane/water/P123/TEOS quadruple emulsion system,

where the formation of mesoporous silica particles is confined within the separated small spaces and thus, nanoscale silica particles are obtained (Scheme S1). One has to

note that the amount of octane is not sufficient to form a continuous H_2 phase. In this case, w/O/W emulsion might be preferred (J. Allouche, E. Tyrode, V. Sadtler, L. Choplin and J. L. Salager,

Langmuir 2004, **20**, 2134). That is, the undulated H_2 phase is formed locally in the bulk amount

of water while the construction of the nanoscale particles is restricted in the undulated H_2 phase.

Lysozyme activity assay

Lysozyme activity was carried out according to the reported procedure with some modifications. (Agr. Biol. Chem., 1971, 35, 1154-1156). 1 mL Lysozyme (10 mg/mL) and 10 mL chitosan substrate (MW= 500,000-600,000, 0.5 wt %, dissolved by acetic acid at pH =4.5) were incubated at 318 K for 1 h. Immobilized lysozyme was investigated at the same reaction conditions, and the used enzyme was filtered for recycles. For comparison, immobilized enzyme amount is consistent with that in the free lysozyme experiment (24 mg nanoscale silica was added into 12 ml lysozyme (1.0 mg/ml) and stirred for 1h, and obtained immobilized enzyme amount was around *ca.* 10 mg). The produced reductive sugars (glucose) were analyzed by the reported method (M. Lever et al. *Anal. Biochem.* 1972, 47, 373-279). To these solutions (0.4 ml each), 0.3 ml of p-hydroxybenzoic acid hydrazide (PAHBAH) in alkali and 1.2 ml of NaOH (5 wt %) were added. Samples were then heated for 15 min at 393 K and cooled to room temperature. The products adsorption was observed at 410 nm by Uv-vis.

Clearly, immobilized lysozyme shows a little bit reduced activity, and after recycle the activity further decreased. This indicates that the activity of immobilized lysozyme partly lost due to its adsorption on silica. As lysozyme is not covalent bonded to the silica surface, it is easy to understand the enzymatic activity will decrease further after recycle.

Table S1 The Enzymatic study of native and immobilized lysozyme

Samples	Concentration of the produced reductive sugars (mmol/L)	Normalized Enzymatic Activity (referred to the native enzyme)
Native enzyme	0.168	1.00
Immobilized enzyme (IE)	0.149	0.88
Recycled IE	0.131	0.78