

## Electronic supplementary information

# **Fabrication of novel hierarchically ordered porous magnetic nanocomposites for bio-catalysis**

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## ***Experimental***

### *Fabrication of hierarchically ordered porous magnetic nanocomposite:*

- (a) Polystyrene latex template: Non cross linked polystyrene latex spheres were synthesized following the reported protocol using emulsion polymerization technique described in our previous report.<sup>1</sup> Polystyrene latex spheres were packed into a monolithic structure by centrifugation at 6000 rpm for 1 hr and dried at 60<sup>0</sup>C overnight.
- (b) Preparation of silica gel: 20g of tetraethyl orthosilicate (TEOS) was added to a solution containing 0.54g of Pluronic F<sub>127</sub>, 5mL of 0.5M HCl, 10mL of deionised water and 3.4g of n-butanol. The resultant mixture was stirred for 5 minutes before addition of a mixture of 1mL of 1M FeCl<sub>2</sub> and 1mL of 2M FeCl<sub>3</sub>. The final mixture was stirred for further 25 minutes. The resultant gel was yellow in colour.
- (c) Silica gel was repeatedly passed through 4g of polystyrene monolith prepared in step 'a' by vacuum filtration and washed the gel filled polystyrene monolith with 1M NH<sub>4</sub>OH before drying overnight at 60<sup>0</sup>C. The dried gel filled polystyrene monolith was brown in colour.
- (d) Dried material was washed several times with toluene to remove the polystyrene latex and finally calcined at 550<sup>0</sup>C for 10 hr in air with a heating rate of 5<sup>0</sup>C/min.
- (e) The calcined nanocomposite was observed to be light brown in colour indicating the formation of iron oxide silica nanocomposite. Pure silica nanocomposites were observed to be white in colour.<sup>1</sup>
- (f) The calcined material was characterised by XRD, SEM, TEM and nitrogen adsorption before it can be used for surface functionalisation.

*Physiochemical characterization:*

The X-ray diffractograms were recorded on a D8 advanced, Bruker instrument using  $\text{CuK}\alpha$  radiation. All scans were continuous and run between  $2\theta$  values  $1^\circ$  to  $70^\circ$ . The scan rate was  $1^\circ/\text{min}$ . The samples were prepared by, drying and then grinding into a fine powder. The powder was then packed into X-ray sample holder carefully ensuring that the surface was smooth with no visible pits or cracks. Scanning electron micrographs (SEM) were recorded on a FEI Quanta 200 (FEI, USA) instrument. Dried samples were placed onto a carbon coated metal stub. The material was further coated with gold using a gold coating equipment before analyzing on SEM. Transmission electron micrographs were recorded on a Phillips 300 instrument. Samples were suspended in acetone and a drop of the suspension placed onto a carbon coated copper grid using a dropping pipette and dried at RT. The saturation magnetisation and magnetisation curve measurements were carried out using a 6 kOe vibrating sample magnetometer (VSM) at room temperature (RT). Samples of hierarchically ordered porous magnetic nanocomposites (HOPMN) were crushed into a fine power before being packed into plastic tubes of length 10 mm and internal diameter 1.9 mm. This geometry ensures that any demagnetisation effects are kept low.<sup>2</sup>

*Surface functionalisation of hierarchically ordered porous magnetic nanocomposite:*

150mg of calcined nanocomposites were thoroughly wetted in deionised water (50 $\mu\text{g}$ ) and the wetted materials were functionalised using 5% (w/v) 3-aminopropyl triethoxy silane (APTS) in 10mL toluene at  $50^\circ\text{C}$  for 24 hrs in a glass reactor. Aminosilane tend to hydrolysed and self polymerised in deionised water before they can be condensed on to the solid surface hence toluene was used as a solvent. Surface

adsorbed deionised water was used for the hydrolysis and condensation on the surface of the solid for the formation of uniform layer of aminosilane during surface functionalization. We have previously reported the importance of water for surface functionalization using APTS in the case of hydrophilic nanoparticles.<sup>3</sup> Recently, Gurtmann *et al*<sup>4</sup> have reported the similar study on mesoporous silica. The surface amine density of functionalised nanocomposite was determined by established colorimetric assay<sup>5,6</sup> as follows:

Surface functionalised nanocomposites (5 mg) were placed in a 1.5 mL Eppendorf tube and washed ( $\times 4$ ) with 1 mL of coupling solution [0.8% (v/v) glacial acetic acid in dry methanol]. Subsequently, 1 mL of 4-nitrobenzaldehyde solution (7 mg in 10 mL of coupling solution) was added to the nanocomposites and the suspension was allowed to react for 3 h with gentle end-over-end rotation. After removal of the supernatant and washing ( $\times 4$  in 1 mL of coupling solution), 1 mL of hydrolysis solution (75 mL of H<sub>2</sub>O, 75 mL of MeOH, and 0.2 mL of glacial acetic acid) was added to the particles and the tube was shaken for a further hour. The supernatant was then removed from the nanocomposites and its absorbance was measured at 282 nm. The amount of 4-nitrobenzaldehyde in the hydrolysis solution was calculated by interpolation using of a calibration curve constructed from a range of standard solutions of 4-nitrobenzaldehyde prepared separately.

*Conversion of surface amine to aldehyde by treatment with glutaraldehyde:*

The amine functionalised silica nanocomposites were washed with 1 mL (3 times) of coupling buffer (1 $\times$  SSC, pH 7.3) for 2 min at RT. 1 $\times$ SSC buffer was prepared by diluting a stock solution of 20 $\times$ SSC buffer (175.3 g of NaCl, 88.2 g of sodium citrate, and 1 L of H<sub>2</sub>O, pH 7.4) with distilled, deionized water, adjusted to pH 7.4. After

removal of the supernatant, 1 mL of a 5% (w/v) glutaraldehyde solution in coupling buffer was added and the reaction mixture was incubated for 3 h with end-over-end rotation at RT. The material was subsequently washed with 1 mL (3 times) of coupling buffer to remove excess glutaraldehyde. The materials were then washed with 1mL (3 times) of PBS buffer (pH 7.2) and stored in PBS buffer before immobilisation of Lipase.

*Immobilization of lipase (candida rugosa):* 50mg of glutaraldehyde modified nanocomposite was treated with 1mL of lipase (*candida rugosa*, Sigma-Aldrich) solution (2mg/mL) in PBS buffer for 15 hrs with end-over-end rotation at RT. The concentrations of lipase solutions before and after the reaction with nanocomposite were measured by Bradford assay<sup>7</sup> using UV visible spectroscopy (absorption at  $\lambda_{595\text{nm}}$ ). The amount of lipase in the solution was calculated using a calibration curve (see Fig.S3A) constructed from a range of standard solutions of lipase prepared separately in PBS buffer reacting with 1.2mL of Bradford reagent and measuring  $\lambda_{595\text{nm}}$ . A similar reaction was carried out on calcined nanocomposite (non-functionalised) for the immobilisation of lipase.

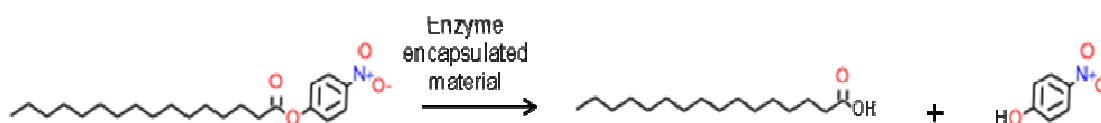
*Bio-catalysis:* Hydrolysis of ester (4-nitro phenyl palmitate) was carried out using 15mg lipase immobilized solid nanocomposites (functionalised and non-functionalised forms) reacting with 1mL of ester solution ( $3.74 \mu\text{mol ml}^{-1}$ ) prepared in a 1:1 mixture of isopropanol and reagent A (0.0667g Gum Arabic + 12mL of 250mM Tris-HCl buffer, pH 7.8 + 48mL of deionized water + 0.267g of sodium deoxycholate) at 20<sup>0</sup>C for 4 hrs in 1.5mL Eppendorf tube by end-over-end rotation.

The products were collected in different time intervals and measured the concentration of 4-nitrophenol following a reported protocol.<sup>8</sup>

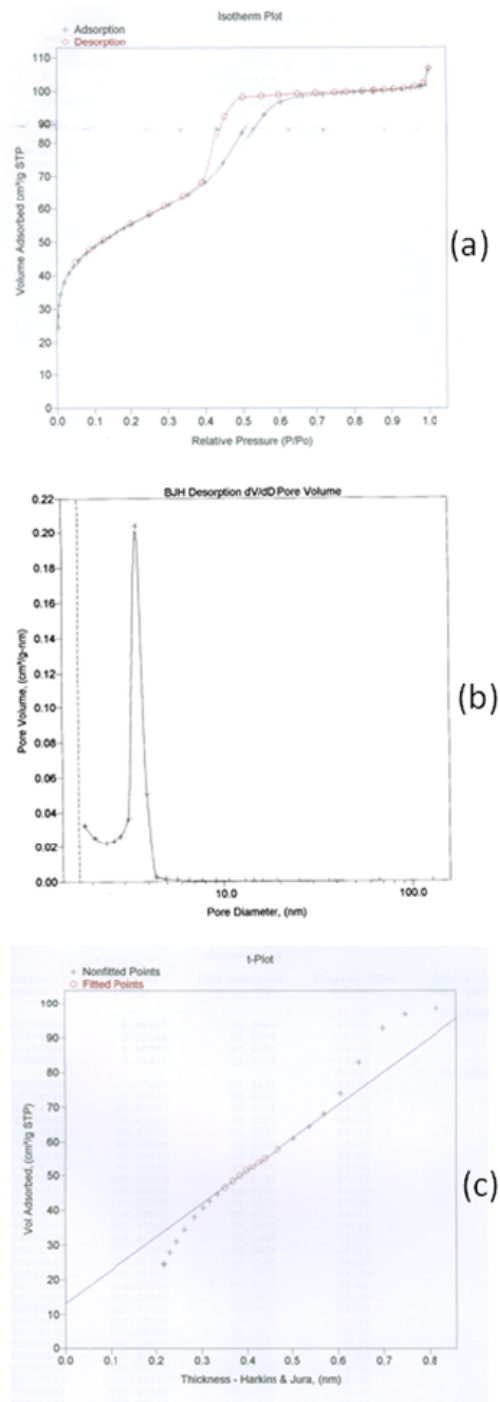
The hydrolysis reaction was monitored by measuring the concentration of 4-nitrophenol in the reaction solution. The concentrations of 4-nitrophenol in the reaction mixture at different time interval were determined by using a calibration curve (Fig.S3B) constructed from a range of standard solutions of 4-nitrophenol prepared separately in a solution containing 1:1 mixture of isopropanol and reagent A by measuring the absorbance at  $\lambda_{410\text{nm}}$ .

Nanocomposites were washed in 1mL (3 times) buffer solution containing 1:1 mixture of isopropanol and reagent A. The washed materials were further used for the hydrolysis of 4-nitrophenyl palmitate under identical condition as above for testing the catalytic efficiency of lipase immobilized nanocomposites in the 2<sup>nd</sup> catalytic cycle.

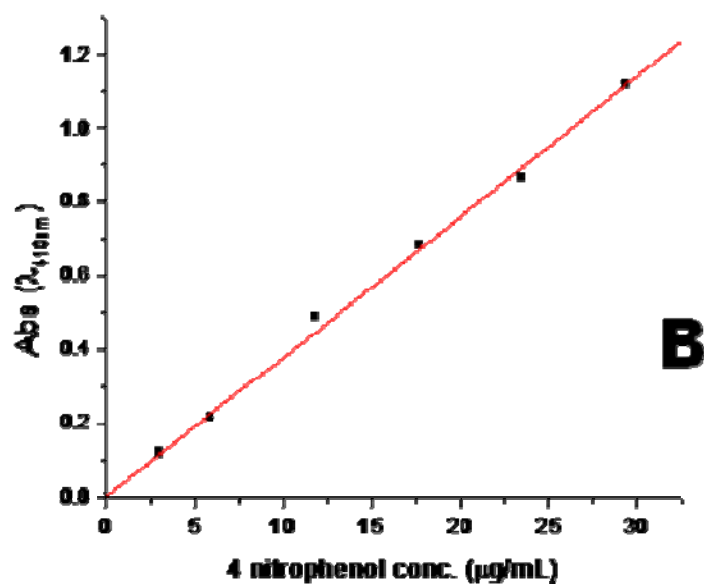
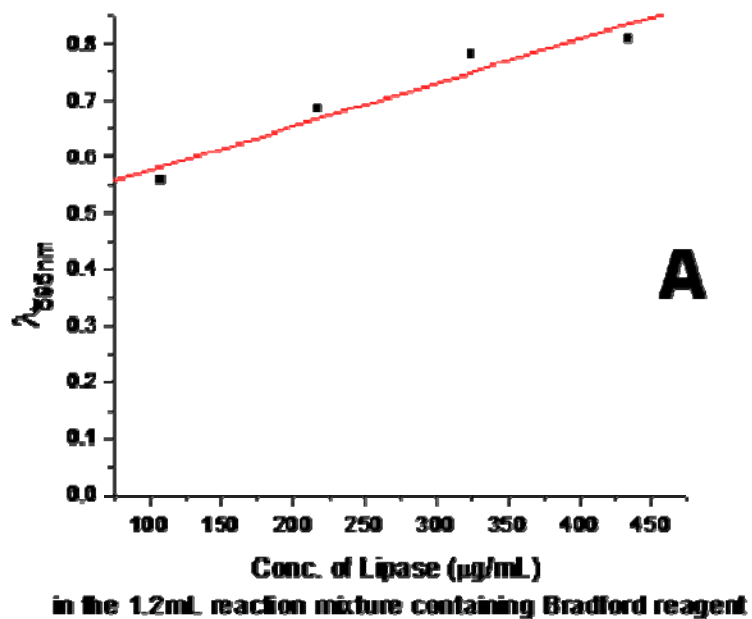
Figure S1 presents the hydrolysis of 4-nitro phenyl palmitate to palmitic acid and 4-nitro phenol.



**Fig S1.** Reaction scheme<sup>9</sup> for the hydrolysis of 4-nitro phenyl palmitate



**Fig S2.**  $N_2$  adsorption isotherm (a), BJH pore size distribution (b) and t-plot analysis (c) of the hierarchically ordered porous magnetic nanocomposite

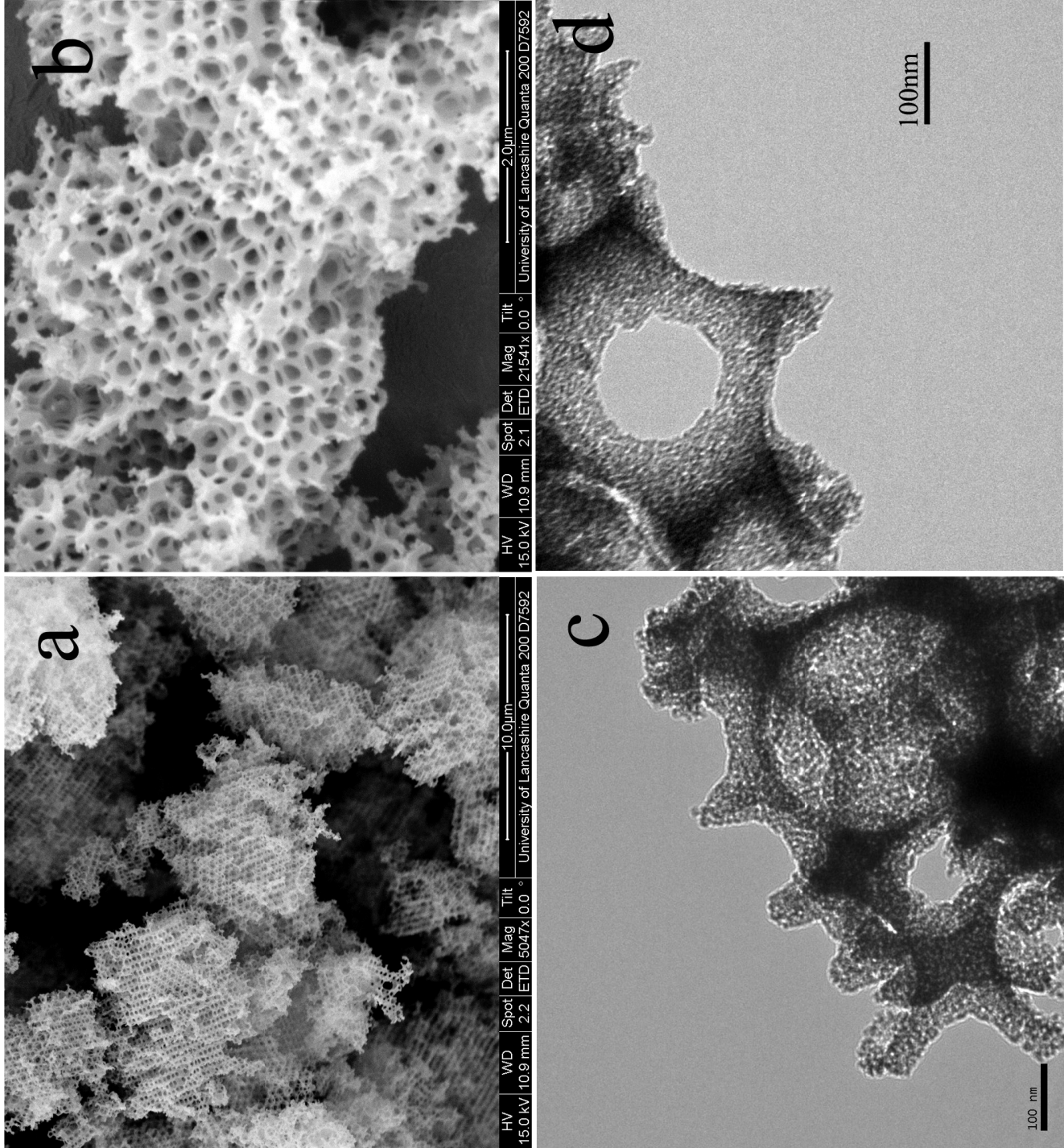


**Fig. S3** Calibration curves of Lipase in PBS buffer (A) and 4-nitrophenol in 1:1 mixture of isopropanol to reagent A (B)



**Table S1** Hydrolysis of 4 nitro phenyl palmitate using the enzyme immobilised hierarchically ordered magnetic nanocomposites (kinetic study at RT)

Sample	Time of reaction (mins)	Conc. ( $\mu\text{g}/\text{mL}$ )	( $\mu\text{g}/\text{min}$ )	( $\mu\text{mol}/\text{min}$ )	( $\mu\text{mol}/\text{min}/\text{mg}$ enzyme) or U/mg	( $\mu\text{mol}/\text{g}$ enzyme)
Non-functionalized	3	0.8	0.27	0.0019	$3.9 \times 10^{-3}$	11.7
	10	2.4	0.24	0.0018	$3.7 \times 10^{-3}$	37.0
	20	6.5	0.32	0.0023	$4.7 \times 10^{-3}$	94.0
	50	14.5	0.29	0.0021	$4.3 \times 10^{-3}$	210
	90	18.2	0.20	0.0014	$2.9 \times 10^{-3}$	261
Functionalized	3	1.4	0.47	0.0034	$5.9 \times 10^{-3}$	17.7
	10	4.7	0.47	0.0034	$5.9 \times 10^{-3}$	59
	20	10.4	0.52	0.0037	$6.5 \times 10^{-3}$	130
	50	27.0	0.54	0.0039	$6.8 \times 10^{-3}$	340
	90	42.8	0.48	0.0035	$6.2 \times 10^{-3}$	558
Lipase	3	112	37.3	0.2683	$2.7 \times 10^{-1}$	810



**Fig S4.** High resolution electron micrographs of hierarchically ordered porous magnetic nanocomposites: SEM (a & b) and TEM (c & d)

## References:

1. T. Sen, J. L. Casci, G. J. T. Tiddy, M. W. Anderson, *Chem. Mater.* 2004, **16**, 2044.
2. D. Jiles, Introduction to Magnetism and Magnetic Materials, Chapman and Hall, London, 1991 (1<sup>st</sup> Ed.)
3. T. Sen, I. J. Bruce, *Chem. Commun.* DOI: 10.1039/b817354K. **2009**.
4. N. Gartmann, C. Schutze, H. Ritter, D. Bruhwiler, *J. Phys.Chem. Lett.* 2010, **1**, 379.
5. J. H. Moon, J. H. Kim, K. Kim, T. H. Kang, B. Kim, C. H. Kim, J. H. Hahn, J. W. Park, *Langmuir* 1997, **13**, 4305.
6. T. Sen, I. J. Bruce, *Langmuir* 2005, **21**, 7029.
7. M. Bradford, *Anal. Biochem.* 1976, **72**, 248.
8. N. Gupta, P. Rathi, R. Gupta, *Anal. Biochem.* 2002, **311**, 98.
9. T. Sen NSTI-Nanoech 2010. ISBN978-1-4398-3401-5 vol. 1