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# Supporting Information

# Enhanced Thermostability of Enzymes Accommodated in Thermo-responsive Nanopores

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**Experimental Section**

*Materials:* Amano lipase PS from *Pseudomonas cepacia* (PCL) and lipase B from *Candida antarctica* (CALB) (lyophilized) were obtained from Sigma-Aldrich and stored at 4 $^{o}$C. Lysozyme (LYZ) was purchased from Dalian Chenyu Biochemical Reagents Co. and also stored at 4 $^{o}$C. [3-(Methacryloxy)propyl]-trimethoxysilane (3-MOP, 98 %) was purchased from ABCR GmbH & Co. KG. P123 (poly (ethylene oxide)-block-poly (propylene oxide)-block-poly (ethylene oxide), $EO_{20}$-$PO_{70}$-$EO_{20}$, $M_w = 5800$) and (*R,S*)-1-phenylethanol was acquired from Sigma-Aldrich. Triacetin is commercially available from Alfa Aesar. *N*-isopropylacrylamide (NIPAM), azobisisobutyronitrile (AIBN), tetraethoxysilane (TEOS), sodium silicate solution (20 % of $SiO_2$, 6 % of $Na_2O$), vinyl acetate and other reagents were obtained from Shanghai Chemical Reagent Inc. of Chinese Medicine Group. All materials are of analytical grade and used as received.

*Synthesis of poly(N-isopropylacrylamide)-organosilanes (PNIPAM-organosilane):* The poly(*N*-isopropylacrylamide)-organosilane (PNIPAM-organosilane) was synthesized by free radical polymerization of [3-(methacryloxy)propyl]-trimethoxysilane (3-MOP) and N-isopropylacrylamide monomer (NIPAM) initiated by azobisisobutyronitrile (AIBN). For polymerization, NIPAM monomer (7.5 g), desired amount of 3-MOP (with 3-MOP/NIPAM molar ratio varied at 1:5, 1:10 and 1:20) and AIBN (67.5 mg) were dissolved in anhydrous THF (200 mL), the reaction flask was deoxygenated and continuously purged with Ar stream. The polymerization took place at 60 $^{o}$C for 8 h with stirring. After removing THF by vacuum distillation, the obtained solid was filtered and thoroughly washed with ether to remove NIPAM monomer residue. The resultant silanes were denoted

as PNIPAM-organosilane-n, where n (n = 5, 10 and 20) is the NIPAM/3-MOP molar ratio in the initial mixture, corresponding to the theoretical degree of polymerization (*DP*) of the obtained silane polymers.

*Synthesis of PNIPAM-functionalized mesoporous silicas (PNIPAM-MS):* PNIPAM-MS was synthesized in NaAc-HAc buffer solution similar to the method we reported previously[9]. In a typical synthesis, P123 (1.0 g) and ethanol (1.69 g) were dissolved in HAc-NaAc buffer solution (28 mL, pH=4.4, HAc: 0.52 M, NaAc: 0.27 M) at 25 °C under vigorous stirring, followed by the addition of sodium silicate (2 mL, 20 % of $SiO_2$, 6 % of $Na_2O$). After stirring at 25 °C for 20 min, the mixture of TEOS and PNIPAM-orgnaosilane (totally 8.62 mmol) in ethanol (1.5 mL) was added. The molar composition of the mixture was sodium silicate : TEOS : PNIPAM-organosilane : P123 : acetic acid : sodium acetate : ethanol : $H_2O$ = 50 : 50-m : m : 1.02 : 85.2 : 44.5 : 367.5 : 8921, where m varied at 11.6, 5.8 and 2.9 with respect to the PNIPAM-organosilane with PNIPAM *DP* of 5, 10 and 20, respectively (therefore the obtained samples with different *DP* of PNIPAM bearing comparable polymer content). The reaction mixture was stirred at 40 °C for 20 h and aged at 100 °C under static condition for an additional 24 h. After filtration, the as-synthesized materials were dried at 25 °C and the surfactant was extracted by refluxing the as-syntheiszed sample (1.0 g) in ethanol (200 mL) for 24 h. The extracted materials were designated PNIPAM-n-MS, where n denotes the *DP* of PNIPAM polymer (n = 5, 10 and 20, respectively) as aforementioned. Mesoporous silica without functionalization (MS) was also synthesized using similar method excluding the addition of PNIPAM-orgnaosilane (the molar ratio of sodium silicate/TEOS was 50 : 50).

*Lysozyme adsorption:* In a typical adsorption process, the solid material (200 mg) was suspended in an aqueous solution of lysozyme (40 mL, 300 μM) which were prepared in sodium carbonate buffer (10 mM, pH =10.8). The resulting mixture was kept under static condition at 20 °C or 45 °C, respectively. Samples (2 mL) were withdrawn periodically, centrifuged to separate the supernatant from the solid material for immediate analysis and then returned to the mixture during the adsorption process. The concentration of lysozyme was measured by an UV spectrophotometer at a wavelength of 280 nm. The amount of lysozyme loaded into the sample was calculated by subtracting the LYZ in supernatant from the total LYZ content.

*Lipase adsorption and activity measurement:* Crude lipase from *Pseudomonas cepacia* (PCL) was purified and immobilized in solid material according to the method as follows: crude PCL (1 g) was dissolved in phosphate buffer solution (PBS, 20 mL, pH=8.0, 50 mM) and shaken (160 rpm) for 3 h at 4 °C. The mixture was centrifuged to remove insoluble components. The protein content in the supernatant was measured by Bradford method. For immobilization process, PCL solution (6 mL) prepared from crude enzyme powder was added to the solid material (30 mg), the suspension was shaken (160 rpm) for 12 h at 4 °C. The resulting product was centrifuged and washed several times with PBS, then dried at 20 °C under vacuum. The amount of PCL in supernatant and washings were also determined by Bradford method, based on which the amount of PCL immobilized in the solid support was calculated. To test the high-temperature tolerance property of lipase, free lipase and lipase-loaded solid samples were separately placed in screw-capped vials and incubated at a certain temperature (ranging from 45 to 90 °C) in a thermostatically controlled water bath

for 2 h prior to the activity measurement. The lipase activity was measured in virtue of the hydrolysis of triacetin: triacetin (250 μL) and PBS (10 mL, pH=8.0, 50 mM) were mixed at 30 $^{o}$C under vigorous stirring for 5 min to prepare a uniform emulsion, free lipase solution (100 μL) or lipase-loaded material (10 mg) was added and mildly stirred for another 10 min, the mixture was titrated with sodium hydroxide solution (0.05 M) in the presence of phenolphthalein indicator. A blank experiment without adding enzyme and carrier was carried out with the same method. From the consumption of sodium hydroxide, the amount of acetic acid produced was obtained and the activity of free lipase or immobilized lipase could be calculated. The activity of lipase B from *Candida antarctica* (CALB) was measured with the same procedure but the enzyme was used as received without purification. The control experiments with the naked nanopore carries without adding lipase were performed and the results indicated that the carries can not impose visible influence on the activity test of lipases (data not shown). The enzyme immobilization yield in terms of protein loading efficiency and activity loading efficiency was listed in Table S3.

*Kinetic resolution of (R,S)-1-phenylethanol:* (*R,S*)-1-phenylethanol (0.5 mmol) and vinyl acetate (2.5 mmol) were dispersed in dry hexane (10 mL) and stirred at 30 $^{o}$C. Desired amount of CALB powder or CALB-loaded solid material (containing identical protein content) was added to the mixture to intrigue the reaction. Aliquots of the samples were periodically withdrawn from the reaction system at fixed time intervals, centrifuged to remove the precipitate and analyzed by the gas chromatograph with a chiral column (Agilent HP-19091G-B233, 30 m×250 μm×0.25 μm). The control experiments with the

naked carries without adding CALB were performed and the results indicated that the influence from the carries was negligible (data not shown).

*Characterization methods:* The nitrogen sorption experiment was performed at 77 K on a Micromeritics ASAP 2020 system. Prior to the measurement, the sample was outgassed at 90 °C for at least 6 h (for the datas presented in Table 1 and Figure S6, the outgas procedure was performed at 30 °C under ultrahigh vacuum for at least 12 h). The Brunauer-Emmett-Teller (BET) specific surface areas ($S_{BET}$) were calculated based on the adsorption isotherm. The pore size distribution ($D_{BJH}$) was calculated from the adsorption branch using the BJH (Barrett-Joyner-Halenda) method. The total pore volumes ($V_p$) were estimated from the amounts adsorbed at a relative pressure ($P/P_0$) of 0.99. FT-IR spectra were recorded on a Thermo Nicolet Nexus 470 Fourier transform infrared (FT-IR) spectrometer using KBr pellets. UV-vis spectra were collected on a SHIMADZU UV-2550 double beam spectrophotometer using 1 cm quartz cell. X-ray diffraction (XRD) patterns were recorded on a Rigaku RINT D/Max-2500 powder diffraction system using Cu Kα radiation of 0.15406 nm wavelength. Transmission electron microscopy (TEM) was performed using a voltage of 120 Kv. Thermogravimetric (TG) analysis was carried out on a Perkin-Elmer Pyris Diamond TG instrument at a temperature range of 20 °C-900 °C with a heating rate of 5 °C min$^{-1}$ under air flow. $^{1}$H NMR ($^{1}$H, 400 MHz) and $^{13}$C NMR ($^{13}$C, 100.6 MHz) spectra were both recorded on a Bruker DRX-400 spectrometer. The peak frequencies were referenced versus an internal standard (TMS) shift at 0 ppm. The water contact angle measurement was conducted using a contact angle measuring system JC 2000A via the sessile drop technique. For each sample, a minimum of four wafers were pressed and

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assayed independently. After the incubation at 45 $^{o}$C for 2 h in an oven, the wafer was measured immediately to make sure its temperature kept around 45 $^{o}$C during the testing operation. The wettability datas below the LCST were obtained by the direct measurement at 20 $^{o}$C using the wafers without any thermal incubation. Molecular weight and molecular weight distribution of PNIPAM polymers were determined with a PL-GPC 220 high temperature chromatograph equipped with the HP 1100 series pump from Agilent Technologies. The GPC columns were eluted with tetrahydrofuran of 1.00 mL min$^{-1}$ at 35 $^{o}$C. The curve was calibrated using monodisperse polystyrene standards covering the molecular weight range from 580 to 460000 Da.

## Supplemental Data

**Table S1.** The molecular characterization of PNIPAM-organosilanes

| Samples | *C* [wt %][a] | *N* [wt %][a] | *PD*[b] | $M_n$ [g mol$^{-1}$][c] | *PD*[d] |
|---|---|---|---|---|---|
| PNIPAM- orgnosilane-5 | 56.75 | 8.28 | 5 | (600)[e] | - |
| PNIPAM-organosilane-10 | 54.71 | 9.34 | 11 | 1714 | 13 |
| PNIPAM-organosilane-20 | 55.81 | 10.02 | 18 | 2579 | 20 |

[a] obtained by elementary analysis. [b] calculated from the results of elementary analysis. [c] number average molecular weight obtained by gel permeation chromatography. [d] based on the results of gel permeation chromatography. [e] the curve used in gel permeation chromatography was calibrated using monodisperse polystyrene standards covering the molecular weight range from 580 to 460000 Da, thus the molecular weight theoretically to be 600 g mol$^{-1}$ was not detected.

The *DP* and molecular weight of PNIPAM-organosilanes are summarized in Table S1 based on elemental analysis and gel permeation chromatography, which well coincides with the NIPAM/3-MOP molar ratio in the initial mixture, suggesting that *DP* and molecular weight of PNIPAM-organosilanes could be willingly monitored by controlling the feed ratio of NIPAM monomer to 3-MOP in the polymerization process.

**Table S2.** Physicochemical properties of prepared mesoporous silicas

| Samples | $S_{BET}$ [$m^2\ g^{-1}$] | $D_{BJH}$ [nm] | $V_p$ [$cm^3\ g^{-1}$] | $d_{100}$ [nm][a] | Polymer content[b] | |
|---|---|---|---|---|---|---|
| | | | | | [$mmol\ g^{-1}$] | [$mg\ g^{-1}$] |
| MS | 452 | 9.8 | 1.02 | 10.6 | - | - |
| PNIPAM-5-MS | 252 | 9.7 | 0.58 | 10.1 | 0.745 | 0.447 |
| PNIPAM-10-MS | 209 | 11.0 | 0.53 | 11.0 | 0.247 | 0.424 |
| PNIPAM-20-MS | 332 | 9.8 | 0.67 | 9.4 | 0.155 | 0.401 |

[a] $d_{100}$ is the spacing value of (100) diffraction obtained by XRD. [b] The polymer content is derived from TG analysis.

**Table S3.** The lipase loading efficiency in mesoporous silicas

| Samples | PCL[a] | | CALB[b] | |
|---|---|---|---|---|
| | Protein loading efficiency | Activity loading efficiency | Protein loading efficiency | Activity loading efficiency |
| MS | 31.5 % | 35.9 % | 48.6 % | 48.3 % |
| PNIPAM-5-MS | 21.3 % | 22.0 % | 28.7 % | 29.8 % |
| PNIPAM-10-MS | 10.1 % | 12.4 % | 20.6 % | 24.3 % |
| PNIPAM-20-MS | 8.2 % | 7.4 % | 16.2 % | 18.3 % |

[a] The concentration and activity of the initial PCL solution are 237.7 μg $mL^{-1}$ and 378.3 U $mg^{-1}$, respectively. [b] The concentration and activity of the initial CALB solution are 58.2 μg $mL^{-1}$ and 31.9 U $mg^{-1}$, respectively.

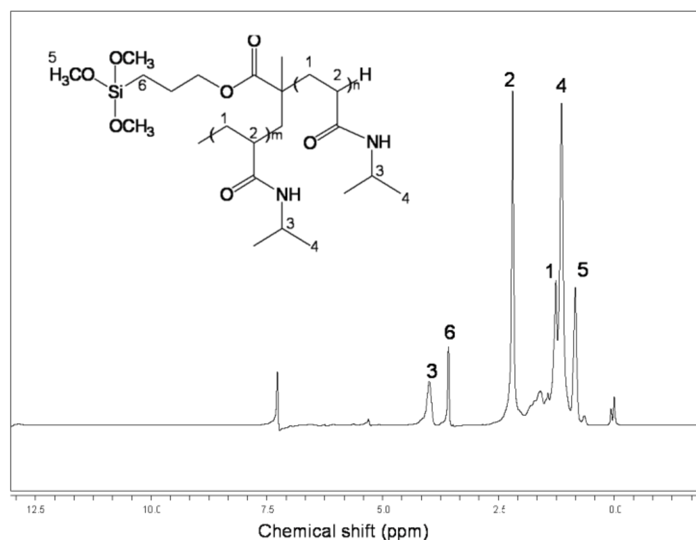


**Fig. 1** $^{1}$H NMR spectrum of PNIPAM-organosilane-10 in $CDCl_3$.

$^{1}$H NMR spectrum confirms the formation of PNIPAM-organosilane in which the resonances at around 4.8-6.5 ppm corresponding to the vinyl groups of NIPAM monomer and 3-MOP cannot be indentified after the polymerization. The proton on the amino group could not be discerned, which is probably due to the fast exchange with the deuteron in $CDCl_3$.

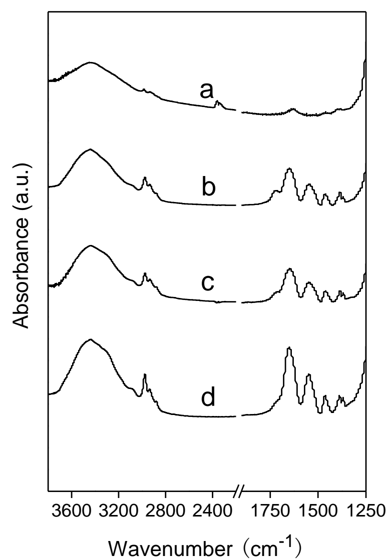


**Fig. S2** FT-IR spectra of a) MS, b) PNIPAM-5-MS, c) PNIPAM-10-MS and d) PNIPAM-20-MS.

The FT-IR spectra of all PNIPAM-MS show two characteristic peaks of PNIPAM at 1644 $cm^{-1}$ and 1545 $cm^{-1}$, arising from C=O stretching and N-H bending vibration, respectively. Additionally, the stretching vibration of C-H at 2800-2900 $cm^{-1}$, the bending vibration of C-H at 1463 $cm^{-1}$ and the bands at 1385 and 1370 $cm^{-1}$ from isopropyl groups were clearly observed.

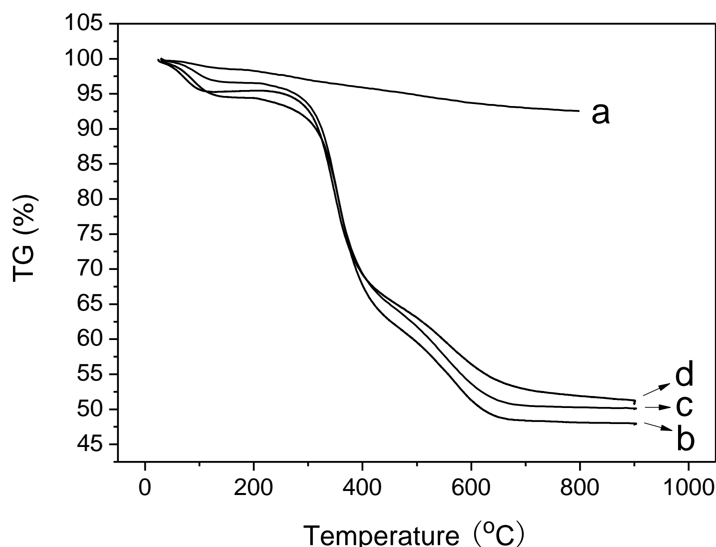


**Fig. S3** TG curves of a) MS, b) PNIPAM-5-MS, (c) PNIPAM-10-MS and (d) PNIPAM-20-MS.

PNIPAM-MS samples are thermally stable up to 300 °C in air atmosphere. The first weight loss of PNIPAM-MS below 150 °C is due to the removal of the physically adsorbed water. The second weight loss occurring in the temperature range between 300 and 460 °C is associated with the degradation of the PNIPAM polymer chains, and the third weight loss observed in the range from 500-650 °C is from the complete decomposition of polymeric chains. The existence of the distinct two-step weight loss from 300 °C to 650 °C suggests that the PNIPAM polymers incorporated in the mesopores of PNIPAM-MS possess relatively uniform molecular mass. Based on TG results, the amount of PNIPAM incorporated in PNIPAM-5-MS, PNIPAM-10-MS and PNIPAM-20-MS correspondingly varied at 44.7, 42.4 and 40.1 wt% (Table S2).

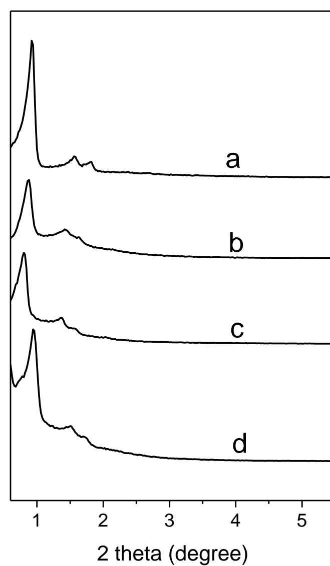


**Fig. S4** XRD patterns of a) MS, b) PNIPAM-5-MS, c) PNIPAM-10-MS and d) PNIPAM-20-MS.

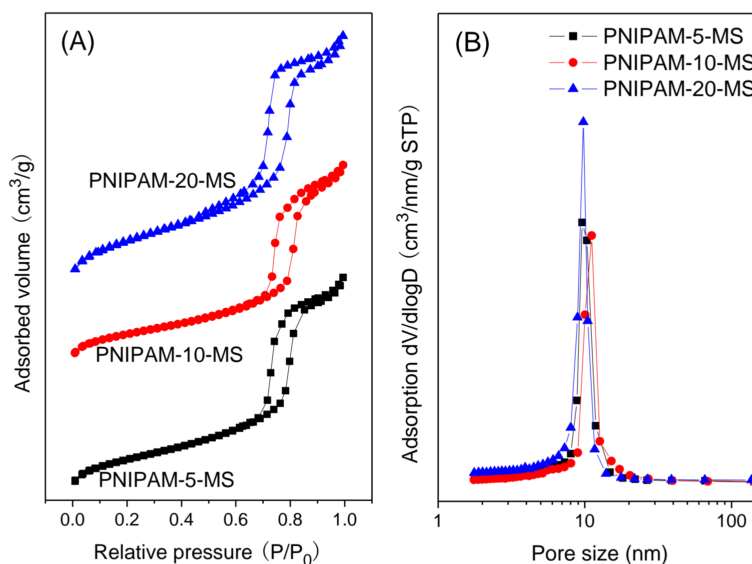


**Fig. S5** (A) $N_2$ adsorption-desorption isotherms and (B) pore size distribution curves of PNIPAM-n-MS (n = 5, 10, 20)

All PNIPAM-MS samples exhibit IV-type $N_2$ adsorption-desorption isotherms with sharp H1-type hysteresis loops, characteristic of the uniform mesopores with open cylindrical geometry. The pore diameter of PNIPAM-MS samples varies from 9.7 to 11.0 nm depending on the amount of PNIPAM incorporated (Table S2), which is much larger than that of the mesosilica/PNIPAM composites reported in literatures, indicating that the co-condensation under mild acidic condition is efficient for the synthesis of polymer functionalized mesoporous materials with large open pores.

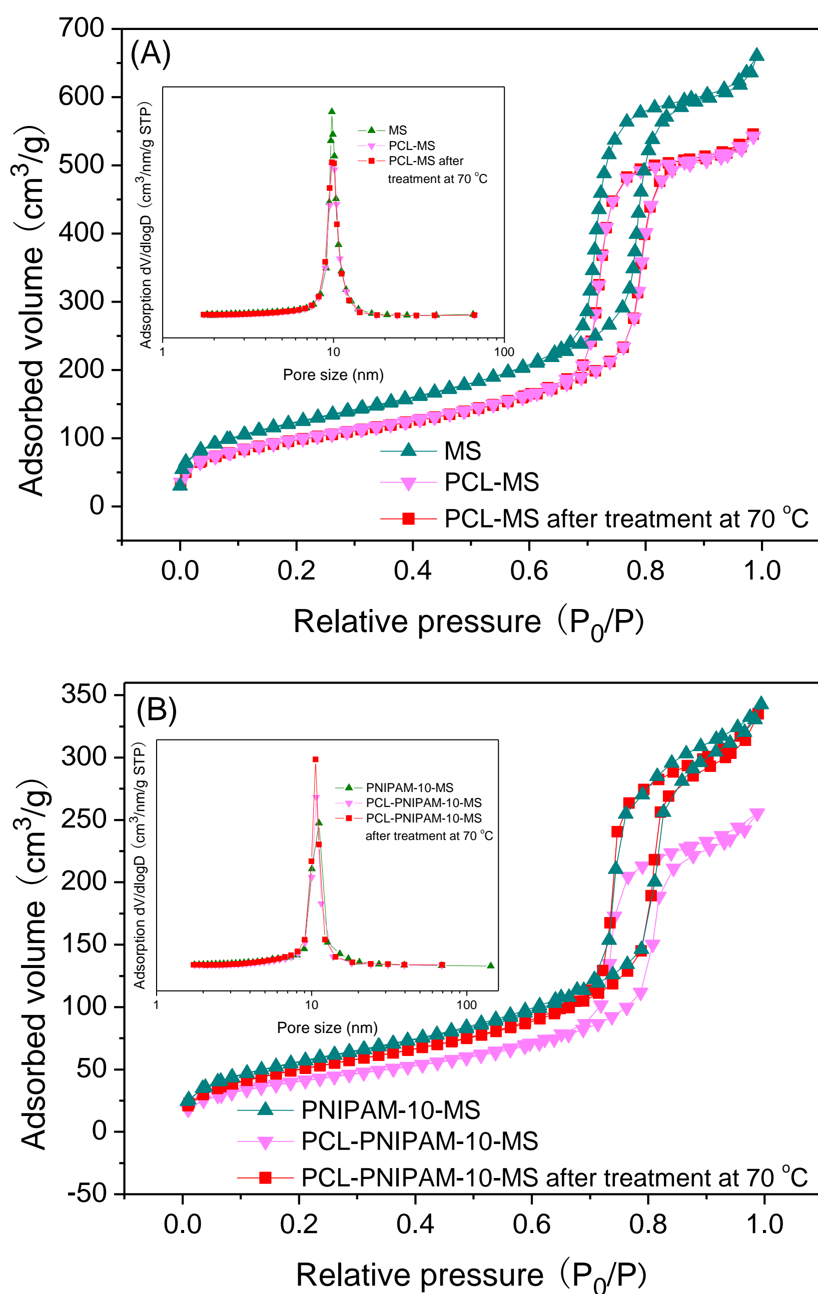


**Fig. S6** $N_2$ adsorption-desorption isotherms and pore size distribution curves (inset) of (A) MS and (B) PNIPAM-10-MS without PCL adsorption (olive), loaded with PCL (pink) and further underwent a thermal treatment (red) at 70 °C for 2 h.

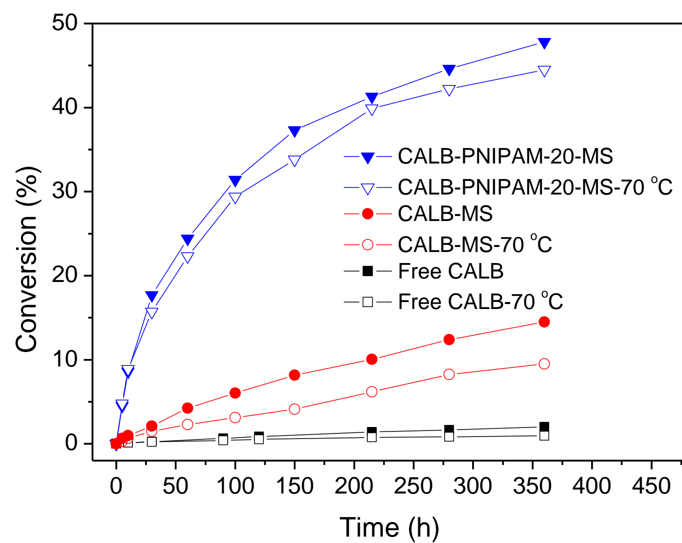


**Fig. S7** Catalytic conversion in the kinetic resolution of (*R*,*S*)-1-phenylethanol catalyzed by the CALB powder and the immobilized CALB before (solid) and after the thermal treatment at 70 $^{o}$C for 2 h (hollow).

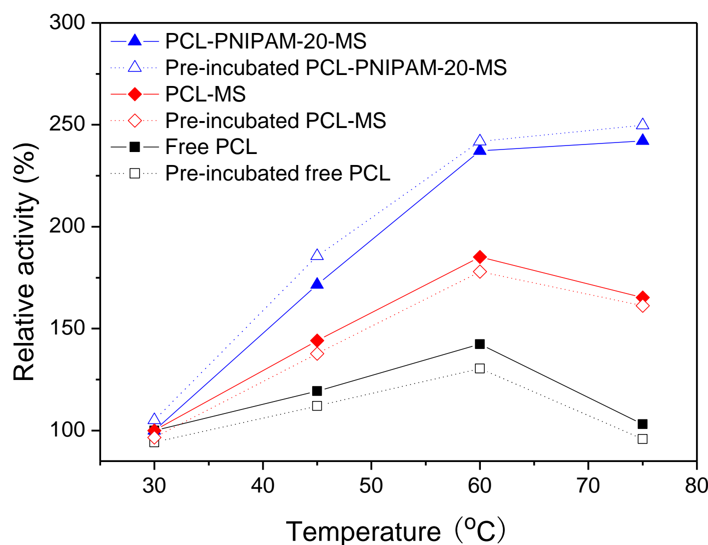


**Fig. S8.** The relative activities of the free PCL and the immobilized PCL at different temperatures before (solid) and after (hollow) the pre-incubation at 40 °C for 20 min. The activity was measured in the hydrolysis of triacetin at different temperatures. The relative activity for each sample was calculated taken its pristine activity (without incubation) at 30 °C as 100 %. The blank experiments without adding enzyme were performed at each temperature to avoid the influence of the auto-hydrolysis of triacetin at high temperatures.

The relative activities of free PCL and MS-PCL firstly increased from 30 to 60 °C, then decreased at 75 °C due to the thermal inactivaion. In contrast, the relative activity of PCL-PNIPAM-20-MS was positively correlated with the temperature in the whole range involved in our experiment. After the short pre-incubation at 40 °C for 20 min, the relative activities of free PCL and MS-PCL both slightly reduced compared with that without incubation, whist a minor increase in relative activity was observed for PCL-PNIPAM-20-MS under the same condition. This modest increase other than the dramatic increase (as presented in Fig. 2(b)) after incubation can ascribed to the low incubation temperature and the insufficient incubation time. The distinctive behaviors of PCL-PNIPAM-20-MS further demonstrated the unique properties of the PCL encapsulated in the thermo-responsive nanopores.