

## Supporting information

# Aldol Reactions Catalyzed by L-Proline Functionalized Polymeric Nanoreactors in Water

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### Methods and materials

**Materials.** Styrene was distilled over CaH<sub>2</sub> and stored at 4 °C. Methylmethacrylate, *tert*-butyl acrylate and *tert*-butyl methacrylate were filtered through an alumina column and stored at 4 °C. The trithiocarbonate RAFT agent dodecyl-1-phenylethyl trithiocarbonate was prepared as reported previously.<sup>1</sup> 4-Nitrobenzaldehyde was purchased from Sigma-Aldrich and filtered through a silica column prior to use. All other reagents were purchased from Sigma-Aldrich and used without further purification.

**Instrumentation.** Both <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded at 400 MHz on a Bruker DPX 400 FT-NMR spectrometer using deuterated solvents. Chemical shifts are reported as  $\delta$  in parts per million relative to CHCl<sub>3</sub> (7.26 ppm for <sup>1</sup>H and 77.2 ppm for <sup>13</sup>C) or d<sub>6</sub>-DMSO (2.50 ppm for <sup>1</sup>H and 39.5 ppm for <sup>13</sup>C) or CD<sub>3</sub>OD (3.31 ppm for <sup>1</sup>H and 49 ppm for <sup>13</sup>C) as the internal standard. Size exclusion chromatography/gel permeation chromatography (SEC/GPC) data for all polymers was obtained in HPLC grade tetrahydrofuran (THF) containing 2% triethylamine (TEA) with a flow rate of 1.0 mL per minute, on a set of two PLgel 5 $\mu$ m Mixed-D columns, plus one guard column. SEC/GPC data was analyzed using Cirrus GPC software based on polystyrene (PS) standards. Dialysis tubing was purchased from Spectrum labs with molecular cut-off of 3.5 and 6-8.0 kDa. Mass spectra were recorded on a Bruker Esquire 2000 ESI spectrometer. High performance liquid chromatography (HPLC) analysis was performed on a Varian 920-LC using an analytical column (reverse phase) Discovery C18 (100 mm $\times$ 4.6 mm $\times$ 5  $\mu$ m) purchased from Sigma-Aldrich, UK and a chiral column Chiralpak IA (150 mm $\times$ 4.6 mm $\times$ 5  $\mu$ m) with guard cartridge (Chiralpak 5  $\mu$ m) purchased from Chiral Technologies Europe. Hydrodynamic diameters ( $D_h$ ) and size distributions of nanoreactor micelles were determined by dynamic light scattering (DLS) on a Malvern Zetasizer Nano ZS instrument operating at 25°C with a 4 mW He-Ne 633-nm laser module. Measurements were made at a detection angle of 173° (back scattering) and the data was analyzed using Malvern DTS 6.20 software. All determinations were made in triplicate (with 12 runs recorded for each measurement). Transmission electron microscopy (TEM) samples were prepared by drop deposition onto copper/carbon grids that had been treated with graphene oxide (GO) and examined with a transmission electron microscope (JEOL TEM-1200), operating at 80 or 200 kV. Micrographs were collected at magnifications varying from 30 K to 100 K and calibrated digitally. Fluorescence emission spectra were recorded on a Perkin Elmer LS55 spectrophotometer and data analyzed using FL WinLab 4.01.

## Experimental Procedure

### *Synthesis of L-proline functionalized methacrylate monomer*<sup>2</sup>

Hydroxy-L-proline (5.0 g, 38 mmol, 1.0 eq) was added in small portions to vigorously stirring trifluoroacetic acid (21.8 g, 191 mmol, 5.0 eq) at 0 °C. To the viscous solution, *p*-toluenesulfonic acid (1.31 g, 7.6 mmol, 0.2 eq) was added and the resulting solution stirred for 5 minutes. Methacryloyl chloride (7.9 g, 76 mmol, 2.0 eq) was then added, resulting in a clear solution. The reaction was stirred for 3 hours at room temperature and subsequently cooled in an ice bath. Diethyl ether was added dropwise to the reaction. The resulting precipitate was filtered off using vacuum filtration, washed twice with additional cold diethyl ether and dried. The monomer was recrystallized in IPA/water (95/5), yielding white crystals (7.7 g, 91%). <sup>1</sup>H NMR (400 MHz, MeOD): δ = 1.86 (3H, t, CH<sub>3</sub>, *J* = 1.3 Hz), 2.30-2.60 (2H, m, CH<sub>2</sub>), 3.41 & 3.46 (2H, m, CH<sub>2</sub>), 4.51 (1H, dd, CH, *J* = 10.5 & 7.8 Hz), 5.40 (1H, m, CH), 5.63 (1H, quin, CH vinyl, *J* = 1.5 Hz), 6.10 (1H, m, CH vinyl).

### *Copolymerizations by RAFT*<sup>3</sup>

Functionalized monomer (5.0 eq), unfunctionalized monomer (60 eq), chain transfer agent (1.0 eq) and AIBN (0.2 eq) were dissolved in DMSO. The styrenic copolymerizations were carried out in bulk and without initiator. The polymerization mixture was then degassed *via* three freeze-pump-thaw cycles, back-filled with N<sub>2</sub> (gas) and put into a pre-heated oil bath at 65 °C (110 °C for styrene polymerizations). Small aliquots were taken to determine degree of conversion by <sup>1</sup>H NMR spectroscopy. All polymerizations were quenched *via* the addition of a small amount of THF and cooling in liquid nitrogen. The functionalized PMMA polymers were precipitated into cold diethyl ether and the functionalized PSt polymers in cold methanol. Molecular weights were determined by <sup>1</sup>H NMR spectroscopy and size exclusion chromatography (SEC). Polymer polydispersities were also determined by SEC analysis.

### *Chain extension of PSt<sub>52</sub>-co-ProSt<sub>3</sub> (1) and PMMA<sub>27</sub>-co-ProMA<sub>2</sub> (2)*

MacroCTA (1.0 eq), AIBN (0.2 eq) and deprotectable hydrophilic monomer *tert*-butyl acrylate or *tert*-butyl methacrylate (100-150 eq, depending on desired DP of hydrophobic block) were dissolved in 1,4-dioxane (styrenic polymer) or DMSO (methacrylate polymer) and put into a dry polymerization ampoule. The polymerization mixture was then degassed *via* three freeze-pump-thaw cycles, back-filled with N<sub>2</sub> and put into a pre-heated oil bath at 75 °C. After 2-3 hours an aliquot was taken to determine the polymerization conversion by <sup>1</sup>H NMR spectroscopy. The polymerization was quenched *via* the addition of THF and cooling in liquid nitrogen. The block copolymers were then precipitated into cold methanol/water (90:10), filtered and dried in the vacuum oven overnight. Molecular weight was determined by <sup>1</sup>H NMR spectroscopy and SEC prior to deprotection of the *tert*-butyl protecting groups. For block copolymer **2** the L-proline acid functionality was protected with a *tert*-butyl group (40 eq *tert*-butanol, 1 eq 4-dimethylaminopyridine, 2 eq 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, in CHCl<sub>3</sub>) prior to SEC analysis.

MWt (<sup>1</sup>H NMR) **1** = 20.0 kDa; MWt (<sup>1</sup>H NMR) **2** = 11.2 kDa; M<sub>n</sub> (GPC, THF, PSt standard) **1** = 23.4 kDa, PDI = 1.18; M<sub>n</sub> (GPC, THF, PMMA standard) **2** = 11.8 kDa, PDI 1.10.

The *tert*-butyl protecting groups were then removed using trifluoroacetic acid in dichloromethane by stirring the solution overnight. The polymer was recovered either *via* precipitation or by dialysis against nanopure water.

### *Micellization of block copolymers (3 and 4)*

The block copolymer (20 mg) was dissolved in DMF (10 mL) and stirred at room temperature. Nanopure water (20 mL) was added dropwise, at a slow rate (1.7 mL/min) to the stirring polymer solution *via* a peristaltic pump. After addition of all the water, the solution was dialyzed against nanopure water using a dialysis membrane with a 6-8 kDa molecular cut-off. Aqueous micelle solutions with concentrations of *ca.* 1.0 mg/mL were obtained. The pH of the micelles **3** and **4** were determined to be *ca.* 5 and *ca.* 6 respectively.

### *Aldol reaction catalyzed by functionalized polymer micelles*

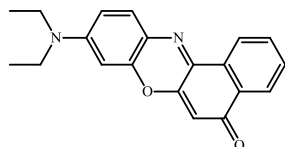
The required volume of micelles was pipetted into a small vial and additional nanopure water was added to make the total volume 2.0 mL (see below for further details). 4-Nitrobenzaldehyde (0.038 g, 0.25 mmol, 1.0 eq) was dissolved in cyclohexanone (0.104 mL, 1.0 mmol, 4.0 eq) and added to the micelle solution. The micelle solution was then shaken and vortexed to give a homogeneous solution. In some cases, some precipitation was observed, which homogenized after stirring for 10 minutes. After 24 hours an aliquot was taken for <sup>1</sup>H NMR analysis to determine the percentage conversion. For reactions carried out at higher catalyst loading, a lower amount of starting materials were used (for 5 mol% 7.6 mg, 0.05 mmol CHO, 20.8 μL, 0.2 mmol ketone, and for 10 mol% 3.8 mg, 0.025 mmol CHO, 10.4 μL, 0.1 mmol ketone).

To effectively remove the product and any remaining starting materials from the core of the micelles at the end of the reaction, DMF was added to break up the micelle. The water/DMF micelle solution was then sonicated for 5 minutes to ensure the breakup of the micelle structure. Ethyl acetate was then added to the solution to extract the product/starting materials from the aqueous phase. The organic phase was then removed and the organic residues analyzed by <sup>1</sup>H NMR spectroscopy.

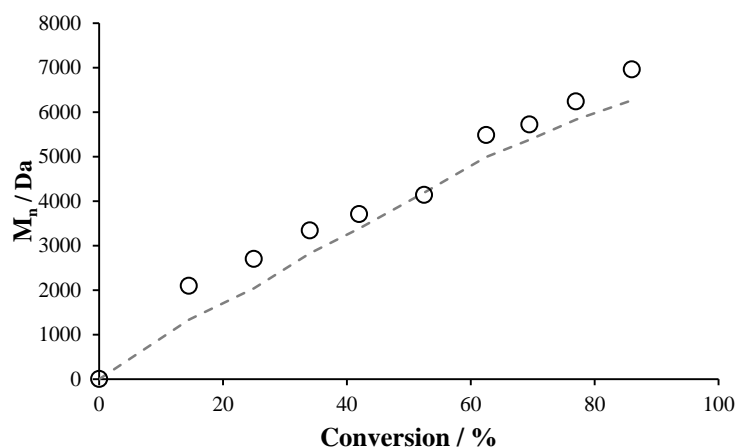
The catalyst loading in the reaction (i.e. mol% catalyst) was determined by calculating the amount of catalyst on each polymer chain and thus in the micelle solution (concentration of micelle solution **3** = 6.15 mg/mL and **4** = 5.07 mg/mL).

Modifications to catalyst loading by changing the amount of micelles (i.e. number of nanoreactors), keeping the amount of reagents constant are detailed below. In each reaction, 4-nitrobenzaldehyde (3.8 mg, 0.25 mmol, 1 eq) and cyclohexanone (0.104 mL, 1.0 mmol, 4 eq) were added to the micelle solution. The volume of micelles **3** and **4** was adjusted to make up the catalyst loadings of 1 and 5 mol% and subsequently diluted to 2.0 mL with nanopure water. After 24 hours only trace amounts of products were observed in all reactions at lower catalyst loading. We attribute the reduced activity to the lower number of nanoreactors present in each reaction, effectively creating fewer hydrophobic pockets for efficient catalysis.

### *Encapsulation of Nile Red (NR) by micelles 3 and 4*



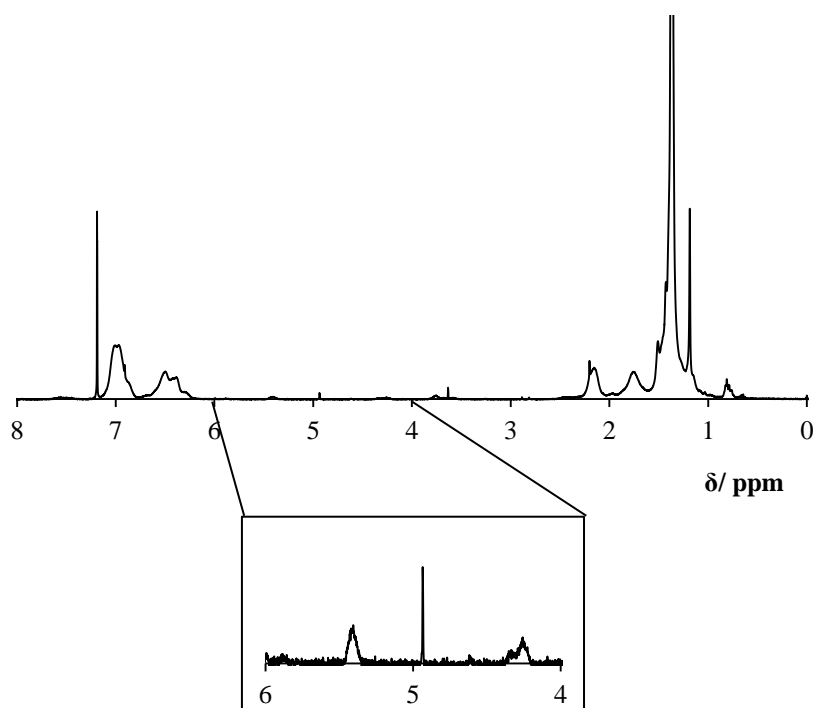
Nile red (1.9 mg,  $6 \times 10^{-3}$  mmol) was dissolved in cyclohexanone (0.052 μL, 0.5 mmol) and added to the micelles **3** and **4** (1.0 mL each). The micelle solutions were first shaken and vortexed for 5 minutes before stirring for 24 hours. The solutions were then diluted with nanopure water to 15 mL. A small aliquot was taken and analyzed by fluorescence spectroscopy after filtering through a 0.45 μm syringe filter to remove the precipitated dye. DMF was then added to the micelles breaking them up, releasing the encapsulated dye which was extracted into the EtOAc organic phase. The organic phase was dried and the dye residue dissolved in THF before further analysis by fluorescence spectroscopy.



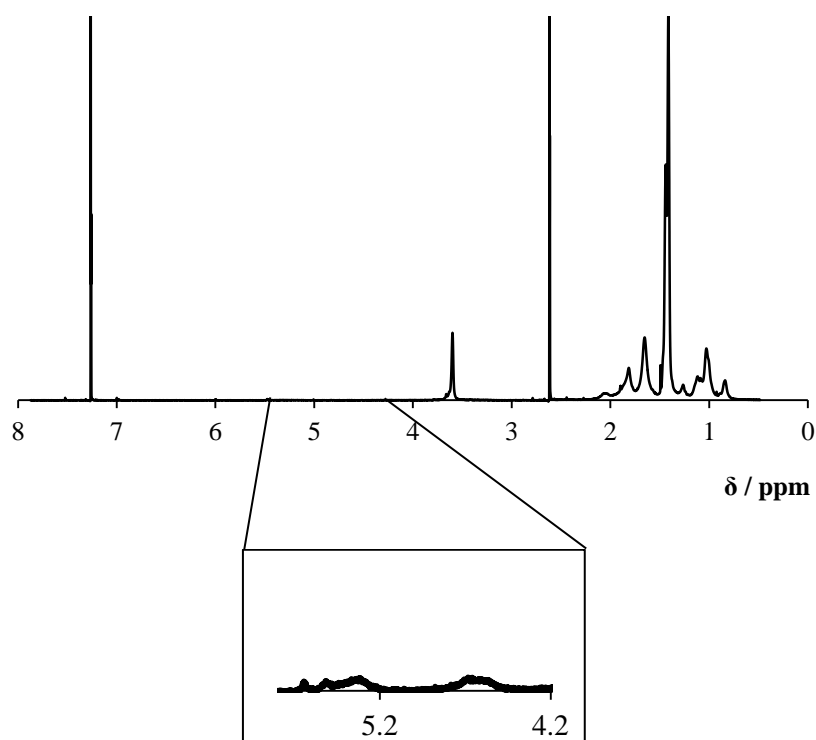
**Fig S1.** Plot of  $M_n$  against conversion for the copolymerization of MMA with L-proline functionalized methacrylate monomer (in the protected form) in DMSO at 65 °C, with  $M_{n,th}$  shown with a broken line.

**Table S1.** Aldol reaction catalyzed by micelles **3** and **4** at different catalyst loadings (1 and 5 mol%), keeping the concentration of reagents the same, changing the amount of micelles in each reaction are detailed.

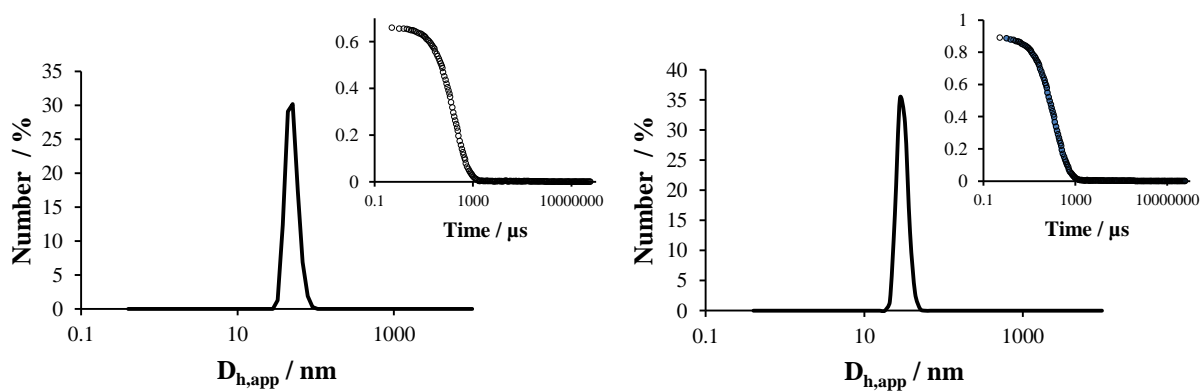
Micelle	mol%	24 h conversion / %	5 day conversion / %
<b>3</b>	1	Trace	31
	5	Trace	23
<b>4</b>	1	Trace	Trace
	5	Trace	13



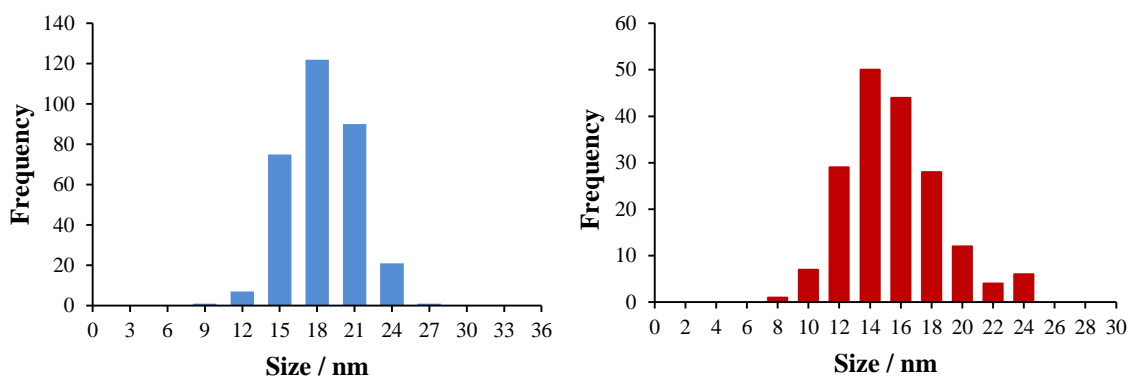
**Fig S2.**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) spectrum of block copolymer **3**, prior to removal of the *tert*-butyl protecting groups: highlighted and expanded are two of the key signals for the functionalized L-proline catalyst.



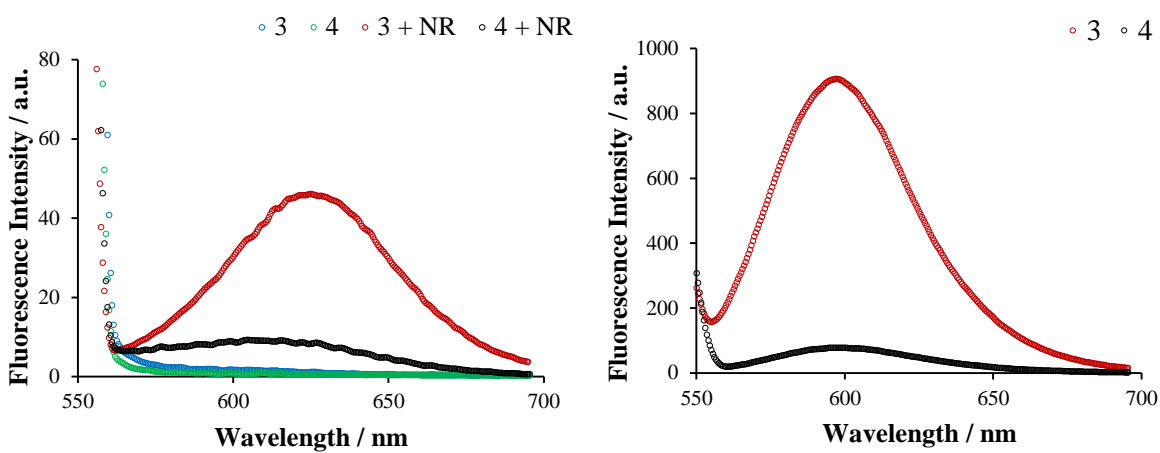
**Fig S3.**  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) spectrum of block copolymer **4**, prior to removal of the *tert*-butyl protecting groups: highlighted and expanded are two of the key signals for the functionalized L-proline catalyst.



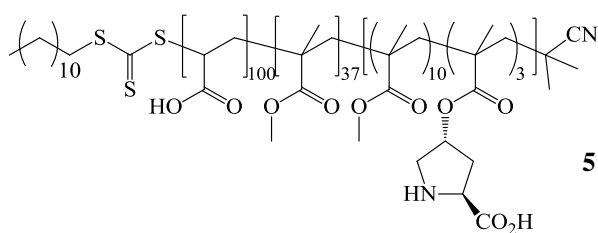
**Fig S4.** Representative DLS traces and correlation coefficients of **3** (left) and **4** (right) at 0.5 mg/mL and 25 °C.



**Fig S5.** Histograms showing size distribution in polymeric nanoreactors **3** (left) and **4** (right), as determined by TEM analysis.



**Fig S6.** Emission spectrum ( $\lambda_{\text{ex}} = 550 \text{ nm}$ ) of micelles **3** and **4** before encapsulation and after stirring the micelles with Nile Red (NR) for 24 hours in water (left) and of the encapsulated dye released from the micelle, in THF (right).



**Fig S7.** Structural representation of triblock-like L-proline functionalized polymer **5**.

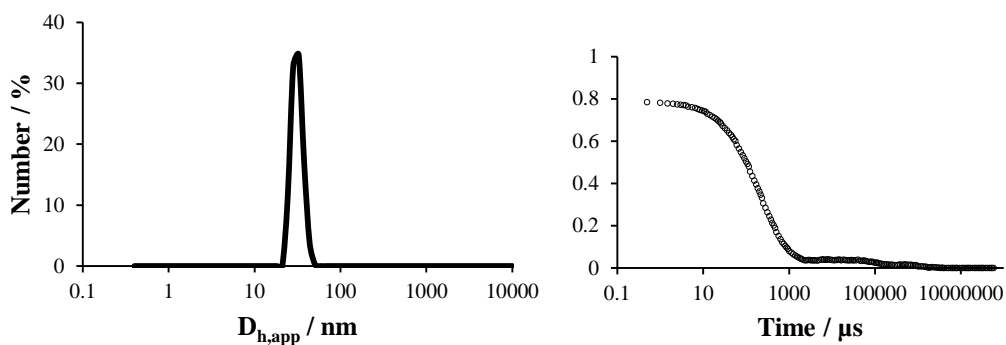


Fig S8. DLS trace of triblock-like functionalized PMMA micelle **5**, at 0.22 mg/mL and 25 °C with the correlation function.

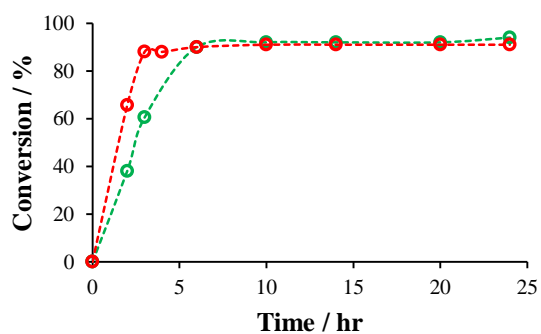


Fig S9. Kinetics of the room temperature aldol reaction catalyzed by **4** (●) and **5** (●) at 1 mol%.

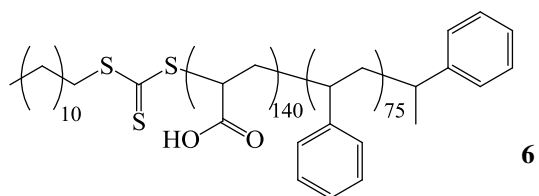


Fig S10. Structural representation of unfunctionalized PST-*b*-PAA block copolymer, assembled to give unfunctionalized micelles **6**.

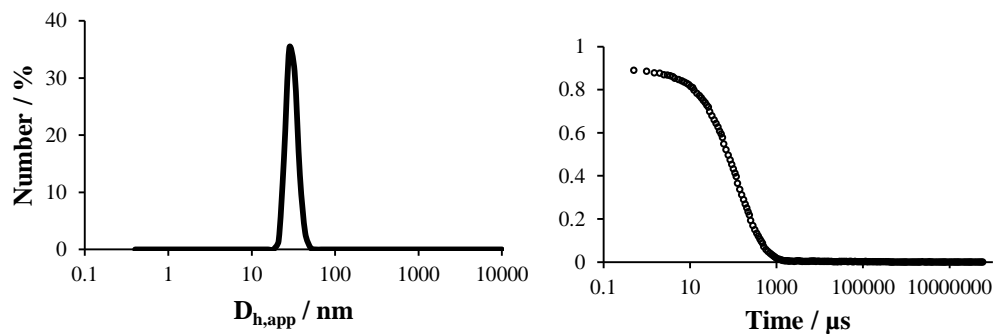


Fig S11. DLS trace of unfunctionalized PST-*b*-PAA micelles **6**, at 0.5 mg/mL and 25 °C with the correlation function.

**Table S2.** Aldol reactions carried out at room temperature in the presence of unfunctionalized styrenic micelles **6** and unsupported L-proline small molecule catalyst.

Catalyst	conversion 24 h / %	anti/syn ratio	ee / %
<b>6</b> <sup>1</sup>	0	-	-
<b>6</b> <sup>2</sup>	0	-	-
<b>6</b> + L-proline <sup>3</sup>	0	-	-

<sup>1</sup>Reaction under same conditions as reactions with **3** and **4** at 1 mol% catalyst loading.

<sup>2</sup>Reaction under same conditions as reactions with **3** and **4** at 10 mol% catalyst loading.

<sup>3</sup>Reaction carried out in the presence of 10 mol% of unsupported L-proline, added to the micelles prior to addition of the organic reagents.

## References

1. A. Lu, T. P. Smart, T. H. Epps, D. A. Longbottom and R. K. O'Reilly, *Macromolecules*, 2011, **44**, 7233.
2. T. E. Kristensen, F. K. Hansen and T. Hansen, *Eur. J. Org. Chem.*, 2009, **2009**, 387.
3. A. C. Evans, A. Lu, C. Ondeck, D. A. Longbottom and R. K. O'Reilly, *Macromolecules*, 2010, **43**, 6374.