

## Supporting Information

Novel nanoaggregates with peripheric superparamagnetic iron oxide nanoparticles and organic cores through self-assembly of tailor-made block copolymers

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## 1. Materials

DMAEMA (Aldrich, 98%), and styrene (Sigma-Aldrich, ReagentPlus<sup>®</sup>, 99%) were passed over a sand/alumina column before use in order to remove radical inhibitors. Copper(I) bromide (CuBr) (Fluka, +98%), copper(II) bromide (CuBr<sub>2</sub>) (Acros, +99% extra pure, anhydrous), deuterated chloroform (CDCl<sub>3</sub>) (Euriso-top, +1%TMS), 2-propanol (IPA) (Fisher Chemical), tetrahydrofuran (THF) (Fisher Chemical), PS standards (Polymer Laboratories) (Acros, 99%), PMDETA (Aldrich, 99%), iron powder (Fe(0)), *n*-bromoisobutyl bromide (BIBB) (Aldrich, 98%), and magnesium sulfate (Sigma-Aldrich, +97%), ethanol (96%; Panreac), diethyl ether (>99.8%; Sigma-Aldrich), methanol (>99.85%; Aldrich), chloroform (99.99%; Fisher Chemical), dimethylformamide (DMF) (+99.8%; Sigma-Aldrich), 2-chloropropionyl chloride (CPC) (97%; Sigma-Aldrich), CuCl<sub>2</sub> (+99%+extra pure, anhydrous; Acros) were used as received. Cu(0) wire (99%; Acros) was activated with nitric acid, washed with acetone and dried before use. Purified water (Milli-Q, Millipore, resistivity >18 MU cm) was obtained by reverse osmosis. Dichloromethane (DCM) (Fisher Scientific, +99.6%) was dried and distilled over calcium hydride, 4-vinylpyridine (4VP) (96%; Fluka), was dried and distilled under reduced pressure, prior to use. Poly(ethylene glycol) methyl ether (mPEG) (Aldrich, Mn 5000 and Mn 2000) was dried by azeotropic distillation with toluene. Triethylamine (TEA) (Sigma-Aldrich, 96%) was distilled and stored over molecular sieves. 4-Dimethylaminopyridine (DMAP) (Sigma-Aldrich, +99%) was recrystallized from toluene. Me<sub>6</sub>TREN was synthesized according to the procedure described in the literature [1].

Iron (III) acetylacetonate (97%, Sigma-Aldrich), oleylamine (70%, Sigma-Aldrich), benzyl ether (98%, Sigma-Aldrich), oleic acid (85-90%, The British Drug House, Ltd.), 1,2-hexadecanediol (>98%, Tokyo Chemical Industry, Co., Ltd.) were used as received.

## 2. Procedures

### 2.1. Preparation of mPEG ATRP's macroinitiators

mPEG<sub>113</sub>-Cl was prepared based on an adaptation of a reported method [2]. DMAP (0.916 g, 7.5 mmol), DCM (20 mL) and TEA (0.7 mL, 5 mmol) were placed into a round-bottom flask. A solution of CPC (1.21 mL, 12.5 mmol) in DCM (20 mL) was then added dropwise and a yellow dispersion was formed. mPEG<sub>113</sub> (10.0 g, 5.0 mmol) and DCM (30 mL) were then added, under a N<sub>2</sub> atmosphere at a temperature of 0°C. After the addition, the temperature was raised to 25°C and the reaction continued under magnetic stirring for 18 h. The obtained dispersion was then filtered, concentrated by solvent evaporation and the product recovered by precipitation in cold diethyl ether. The crude product was purified by recrystallization overnight in absolute ethanol. After being filtered and washed with cold diethyl ether, the macroinitiator was collected and dried for 48 h, under vacuum, at 40°C. A similar procedure was used for the preparation of mPEG<sub>113</sub>-Br and mPEG<sub>45</sub>-Br, but required the use of BIBB instead of CPC.

### 2.2. Synthesis of mPEG-*b*-P4VP block copolymer

mPEG-*b*-P4VP block copolymer synthesis was conducted by ATRP of 4VP using Cu(0) and [CuCl<sub>2</sub>]/[Me<sub>6</sub>TREN]=1/1 catalytic system in IPA 50°C in a N<sub>2</sub> atmosphere for 90 min [2]. mPEG<sub>113</sub>-Cl (2.360 g, 0.46 mmol), CuCl<sub>2</sub> (62.34 mg, 0.46 mmol), Me<sub>6</sub>TREN (106.82 mg, 0.46 mmol) and IPA (5.19 mL) were placed in a Schlenk reactor and immediately frozen in liquid nitrogen. Activated Cu(0) wire was added to the reactor and the system was deoxygenated with four freeze-vacuum-thaw cycles and purged with nitrogen. 4VP (5 mL, 46 mmol) was then added to the Schlenk reaction vessel under a nitrogen atmosphere and the reaction mixture was immediately frozen in liquid nitrogen and deoxygenated by conducting two freeze-vacuum-thaw cycles and purged with nitrogen. The Schlenk reactor was placed in a pre-heated oil bath at 50°C and left reacting under magnetic stirring. After 90 minutes the reaction was stopped. The block copolymer was precipitated in cold diethyl ether. The resulting solid was then dissolved in chloroform and passed through an alumina column to remove the

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copper catalyst. The solution was concentrated by rotary evaporation and the product recovered by precipitation in cold diethyl ether, followed by filtration. Further catalysts removal was achieved by redissolving the product in methanol and submitting it to dialysis for 12 h (molecular weight cut-off (MWCO) = 3500 Da). Finally, the crude product was obtained by precipitation in cold diethyl ether, filtered and then dried, under vacuum, at 40°C for 48 h.

### 2.3. *Synthesis of mPEG-b-PDMAEMA block copolymers*

In a typical procedure, the block copolymer mPEG<sub>113</sub>-b-PDMAEMA<sub>55</sub> was synthesized by ATRP of DMAEMA using Fe(0) and [CuBr<sub>2</sub>]/[PMDETA]=0.1/1.1 catalytic system in IPA/H<sub>2</sub>O (9:1) at 50°C in a N<sub>2</sub> atmosphere for 95 min according to a previously reported methodology [3]. mPEG<sub>113</sub>-Br (1.648 g, 0.33 mmol), IPA (4.5 mL) and Fe(0) (18.4 mg, 0.33 mmol) were placed in a Schlenk reactor. In a vial PMDETA (62.9 mg, 0.36 mmol) was added to DMAEMA (5 mL, 30 mmol), and in another vial CuBr<sub>2</sub> (62.34 mg, 0.03 mmol) was dissolved in H<sub>2</sub>O (0.5 mL). Then both solutions were added to the Schlenk flask and immediately frozen in liquid nitrogen, the system was deoxygenated with four freeze-vacuum-thaw cycles and purged with nitrogen. The Schlenk reactor was then placed in a pre-heated oil bath at 50°C and left reacting under magnetic stirring. After 95 minutes the reaction was stopped. The block copolymer was precipitated in hexane the solid was dissolved in THF and passed through an alumina column to remove the copper and iron catalysts. The solution was concentrated by rotary evaporation and the product recovered by precipitation in hexane, followed by centrifugation. The crude product was dried under vacuum, at 40°C for 48 h.

### 2.4. *Synthesis of mPEG-b-PS block copolymers*

The synthesis of mPEG-b-PS was carried out by ATRP of styrene using a [CuBr]/[PMDETA]=1/1 catalytic system in toluene at 100°C under a N<sub>2</sub> atmosphere for 22 h, based on a previously reported methodology [4]. mPEG<sub>113</sub>-Br (2.240 g, 0.43 mmol), toluene (1.67 mL) and PMDETA (75.4 mg, 0.43 mmol) were placed in a Schlenk reactor and immediately frozen in liquid nitrogen, the system was deoxygenated with four freeze-vacuum-thaw cycles and purged with nitrogen. In a vial, CuBr (24.3 mg, 0.43 mmol) was dissolved in styrene (5 mL, 43 mmol). Then the mixture was added to the Schlenk reactor and immediately frozen in liquid nitrogen; the system was deoxygenated with four freeze-vacuum-thaw cycles and purged with nitrogen. The Schlenk reactor was then placed in a pre-heated oil bath at 100°C and left reacting under magnetic stirring. After 22 hours the reaction was stopped. The block copolymer was precipitated in cold diethyl-ether. The solid was then dissolved in THF and passed through an alumina column to remove the copper catalyst. The solution was concentrated by rotary evaporation and the product recovered by precipitation in diethyl-ether, followed by centrifugation. The crude product was dried under vacuum, at 40°C for 48 h.

### 2.5. *Synthesis of SPION*

The synthesis of SPION followed two procedures, one described by Sun et al. [5], and consisting in a mixture of the iron(III) acetylacetonate precursor (0.5 mmol) with oleic acid (1.5 mmol), oleylamine (1.5 mmol), and 1,2-hexadecanediol (2.5 mmol) in benzyl ether (5 mL, 26 mmol), to produce LOSPION, and the other described by Xu et al. [6] using iron(III) acetylacetonate and oleylamine in benzyl ether, to produce SOSPION.

In both procedures, a few changes were done, which are described herein. For the LOSPION's synthesis, the temperature of the starting mixture was elevated from 20°C to 330°C at 22°C/min and maintained for 30 min, being further elevated to 400°C at rate of 13°C/min, and maintained then for 50 min. The black-brown mixture obtained was left to cool down at room temperature and then diluted in ethanol. The dispersion was then centrifuged at 4000 rpm for 20 min. The precipitated SPION were recovered and dissolved in hexane.

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For the preparation of SOSPION, for 1 mmol of iron(III) acetylacetonate, 4 mL of oleylamine (12 mmol) to 1 mL of benzyl ether (5 mmol) were used. Dehydration of the solution, at 110°C, was carried out for 75 min. and its aging, at 300°C, was carried out for 45 min. The black-brown mixture obtained was then left to cool down at room temperature and purified using the same methodology as for LOSPION.

### 2.6. *Self-assembly of block copolymers and SPION:*

Self-assembly of the block copolymers in the presence of SPION was carried out using a solvent evaporation methodology. In a typical procedure, 500  $\mu\text{L}$  of a 10mg/mL block copolymer solution in THF or methanol, depending on the block copolymers' solubility, was added to 250  $\mu\text{L}$  of a 2mg/mL solution of SPION in THF. Each copolymer/SPION mixture was added dropwise to 5 mL of an aqueous solution at pH 9.0 under vigorous mechanical stirring and then left under stirring for 24 hours to evaporate the organic solvent.

## 3. Characterization:

### 3.1. *Gel permeation chromatography (GPC)*

Gel permeation chromatography (GPC) was carried out using high performance size-exclusion chromatography (HPSEC), with refractive index (RI) (Knauer K-2301) detection. The column set consisted of a PL 10- $\mu\text{L}$  guard column (50 x 7.5 mm<sup>2</sup>), followed by two MIXED-B PL columns (300 x 7.5mm<sup>2</sup>, 10  $\mu\text{L}$ ). The HPLC pump was set with a flow rate of 1 mL/min and the analyses were carried out at 60°C using an Elder CH-150 heater. The eluent was DMF, containing 0.3% of LiBr. Before injection (100  $\mu\text{L}$ ), the samples were filtered through a polytetrafluoroethylene (PTFE) membrane with 0.2  $\mu\text{m}$  pore size. The system was calibrated against PMMA standards.

### 3.2. *<sup>1</sup>H NMR spectroscopy*

400 MHz <sup>1</sup>H NMR spectra of each copolymer, macroinitiator and homopolymer were recorded on a Bruker Avance III 400 MHz spectrometer, with a 5 mm TIX triple resonance detection probe, in CDCl<sub>3</sub> with TMS as an internal standard. Conversion of monomers was determined by integration of the monomer and polymer peaks using the MestRenova software, version 6.0.2-5475.

### 3.3. *Dynamic light scattering (DLS)*

Dynamic light scattering (DLS) measurements were performed on a Malvern Instrument Zetasizer Nano-ZS (Malvern Instruments Ltd., UK). The particle size distribution (in intensity), average hydrodynamic particle size average (z-average) and polydispersity index (PDI) were determined with Zetasizer 6.20 software from measurements at 25°C and at a backward scattering angle of 173°.

### 3.4. *Transmission electron microscopy (TEM)*

Transmission electron microscopy (TEM) was used to observe the size and morphology of the nanoaggregates prepared with the different copolymers. The aqueous dispersion was mounted on 400 mesh copper grid and was examined using a Jeol JEM 1400 transmission electron microscope (Germany). Images were digitally recorded using a Gatan SC 1000 ORIUS CCD camera (Warrendale, USA).

### 3.5. *Thermogravimetric analysis (TGA)*

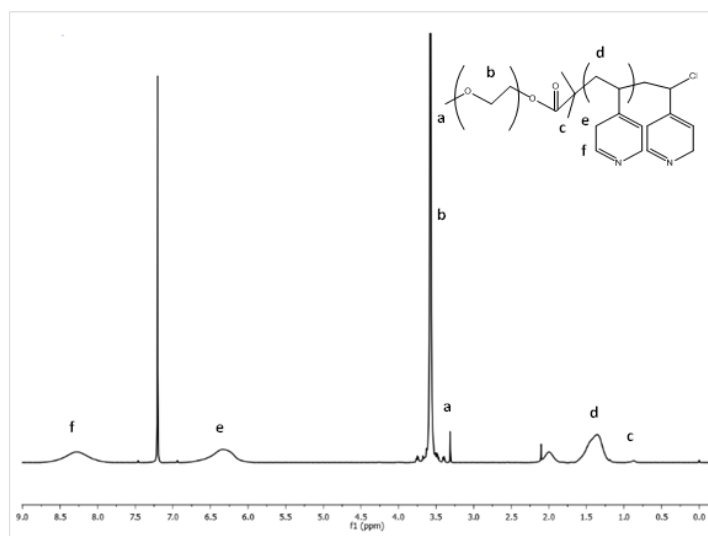
Thermogravimetric analysis (TGA) was carried out to evaluate the percentage of organic coating of SPION samples using a TGA 2920 Thermogravimetric Analyzer (TA Instruments, New Castle, DE, USA). The samples were heated from room temperature to 600 °C at 10 °C/min under a nitrogen atmosphere.

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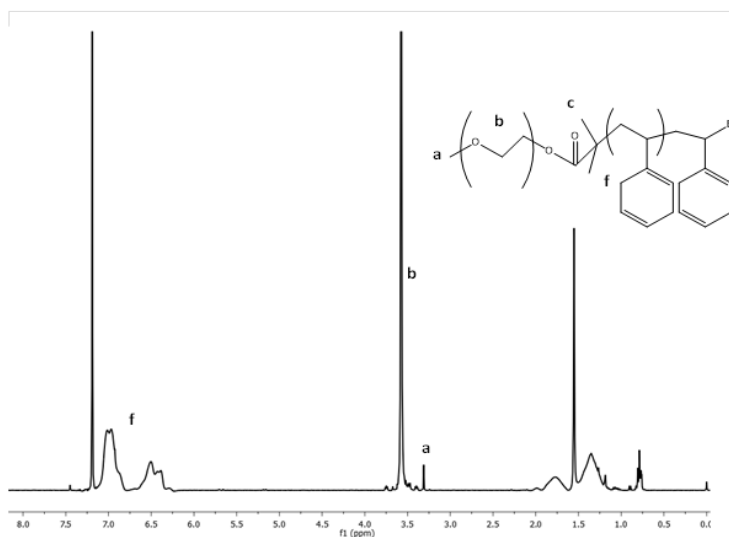
### 3.6. SPION characterization

According to TEM analysis, LOSPION and SOSPION sizes were between 5 and 6 nm, showing a very narrow size distribution. X-Ray Diffraction (XRD) confirmed this result. Based on the XRD analysis, the unit cell parameters for LOSPION and SOSPION were  $a=0.8335$  nm and  $a=0.8369$  nm, respectively. This information supports the conclusion that both SPION are  $\gamma\text{-Fe}_2\text{O}_3$  (maghemite), given the proximity of both values to the unit cell parameter established for this iron oxide [7]. Based on the identification of the iron oxide and its size, we concluded that the SPION were superparamagnetic based on the size below 10 nm [8]. TGA results for LOSPION and SOSPION were used to estimate the composition of the coated nanoparticles, being these constituted by 33% of iron oxide to 67% oleylamine/oleic acid, in the first case, and 55% of iron oxide to 45% oleylamine, in the latter case.

mPEG<sub>113</sub>-b-P4VP<sub>44</sub>



mPEG<sub>113</sub>-b-PS<sub>55</sub>



mPEG<sub>113</sub>-b-PDMAEMA<sub>54</sub>

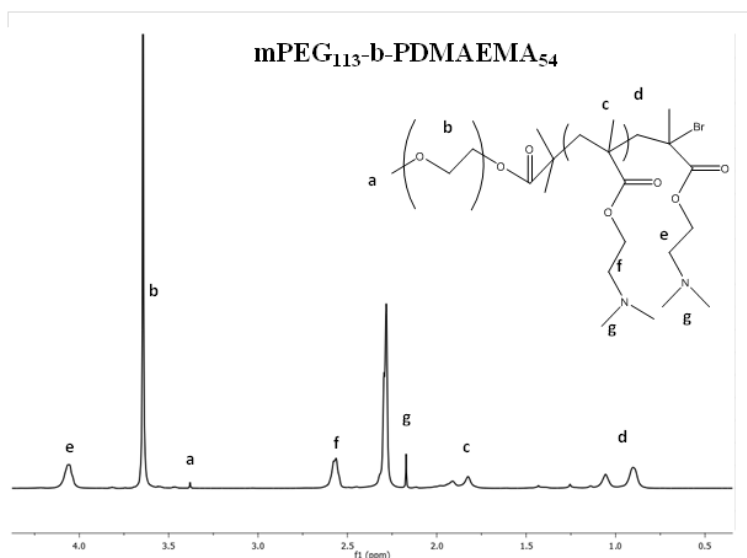


Figure S1 – <sup>1</sup>H NMR spectra of the block copolymers with chemical structure and the proton identification.

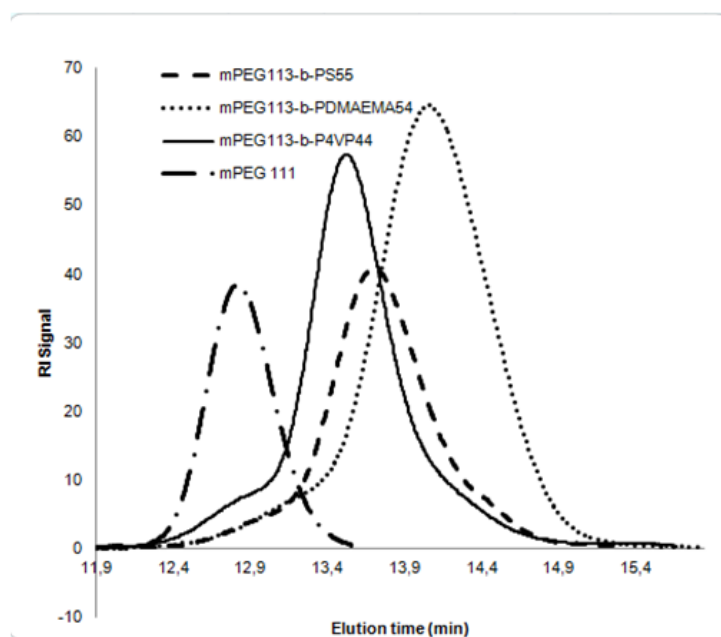
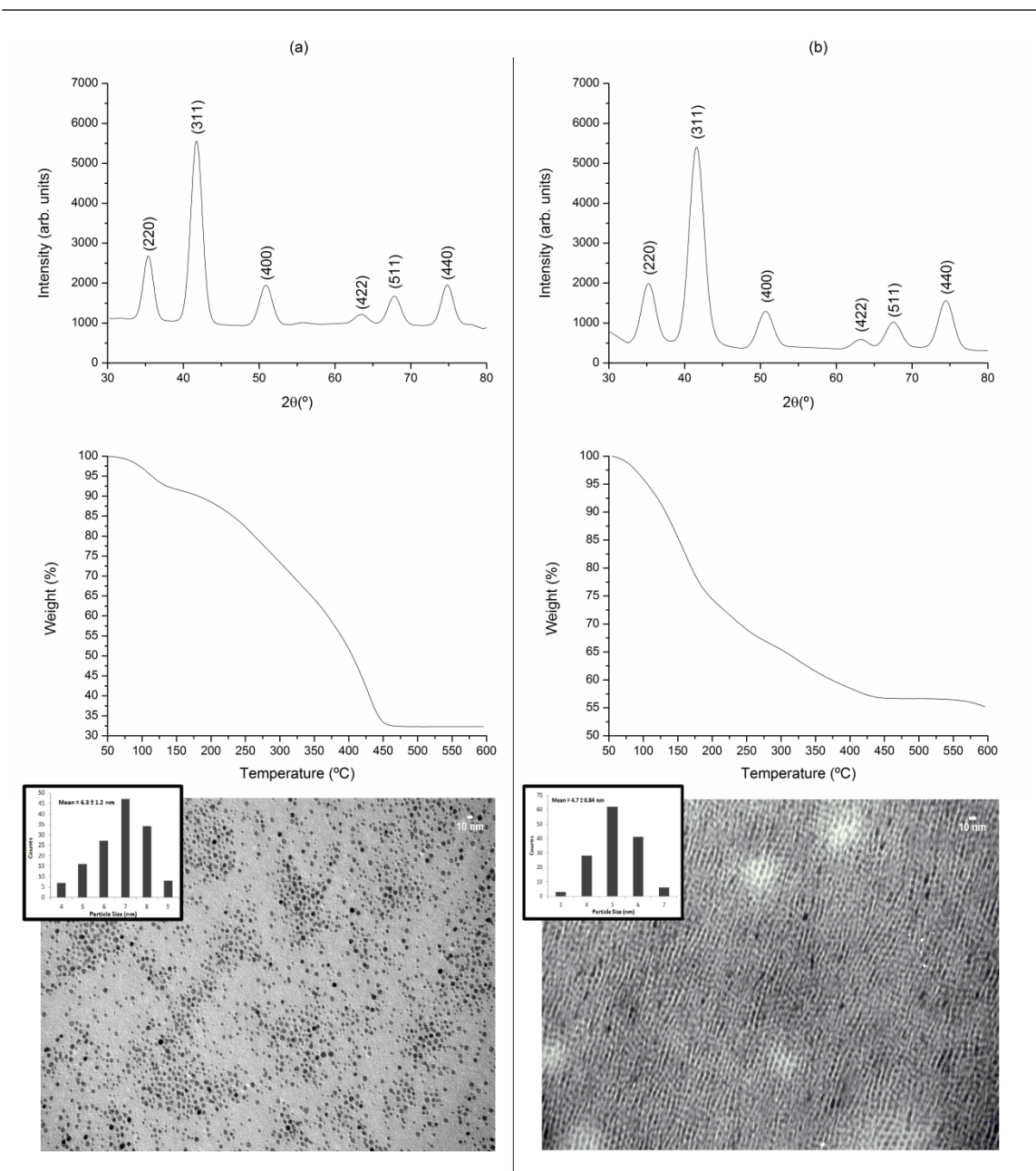


Figure S2 – GPC traces of mPEG<sub>113</sub>-b-P4VP<sub>44</sub>, mPEG<sub>113</sub>-b-PS<sub>54</sub> and mPEG<sub>113</sub>-b-PDMAEMA<sub>55</sub> block copolymers.

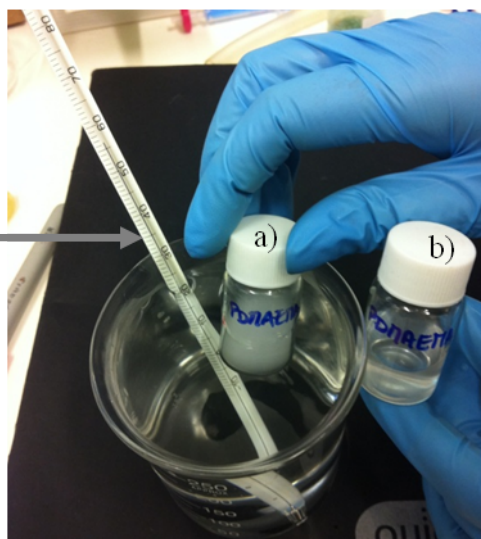


**Figure S3** - X-ray diffractograms, TGA traces and TEM micrographs (including particle size distribution). (a) LOSPION; (b) SOSPION.

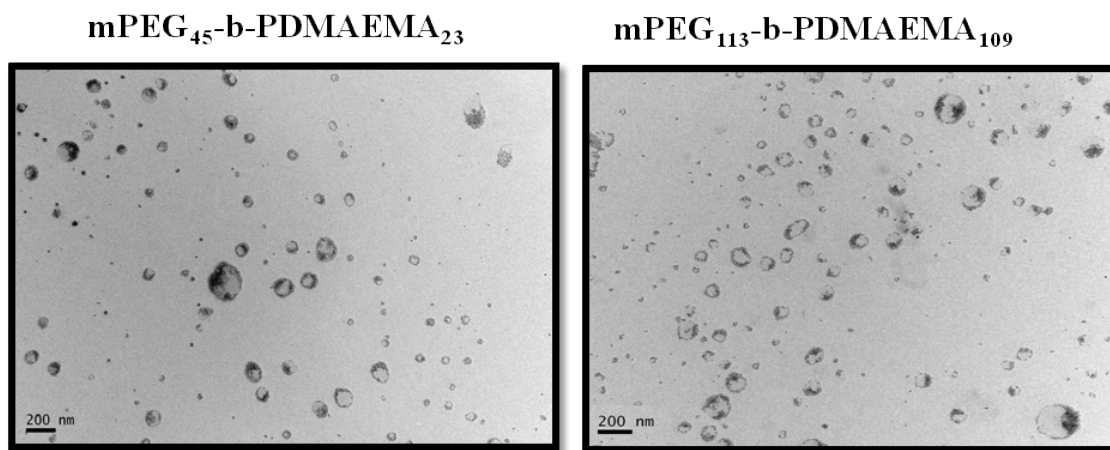


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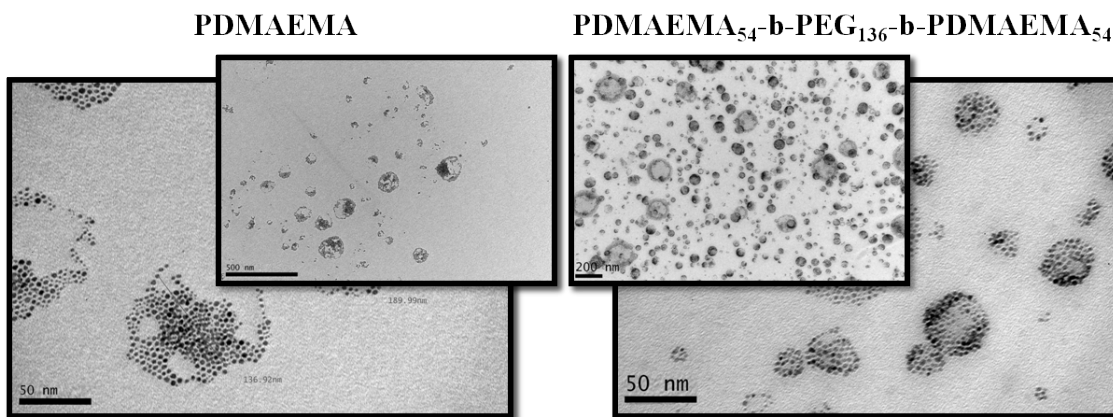
T=57 °C



**Figure S4** – PDMAEMA-Br homopolymer at a concentration of 1 mg/mL and aqueous pH 9 at a temperature a) above LCST and b) below LCST.



**Figure S5** - TEM micrographs of LOSPION self-assembled in the presence of mPEG<sub>45</sub>-b- PDMAEMA<sub>23</sub> and mPEG<sub>113</sub>-b- PDMAEMA<sub>109</sub> block copolymers at a concentration of 1 mg/mL for the block copolymer and 0.1 mg/mL for the LOSPION. Images were taken at magnification x300,000 .



**Figure S6** – TEM micrographs of LOSPION self-assembled in the presence of PDMAEMA homopolymer and PDMAEMA<sub>54</sub>-b-PEG<sub>136</sub>-b- PDMAEMA<sub>54</sub> block copolymer at a concentration of 1 mg/mL for the block copolymer and 0.1 mg/mL for the LOSPION. Images were taken at magnification x50,000 (image detached) and at x300,000 (main image).

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