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Supplementary information for

Modular Construction of Single-Component Polymer Nanocapsules through a One-Step Surfactant-Free Microemulsion Templated Synthesis

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Materials and Methods

4-Cyano-4-(phenylcarbonothioylthio) pentanoic acid (CPADB, 97%, HPLC), 4,4'-Azobis (4-cyanovaleric acid) (ACPA, 98%), isophorone diisocyanate (IPDI, 98%), hexadecane (99%), water (HPLC), deuterium oxide (D_2O), (S)-(+)-Camptothecin (CPT, 90%, HPLC), Folic acid (FA, 97%) and *O*-(2-Aminoethyl)-*O'*-[2-(Boc-amino)ethyl]octaethylene glycol (90%) were purchased from Sigma Aldrich and used without further purification. Cystamine was obtained from protonated cystamine dihydrochloride (Sigma Aldrich, 98%) by adding 3 eq. of triethylamine (Sigma Aldrich, 99%). Miglyol 812 was purchased from SASOL (Germany). Acetone (99.5%) and methanol (99.8%) were purchased from Carlo Erba. Dialysis membranes (Mw cut-off 1000 Da and 300 KDa) were purchased from Spectrum Laboratories, Inc. Other reactants were purchased from Sigma-Aldrich. *N*-[2-(α-D-mannopyranosyloxy)ethyl] methacrylamide) (EMM) was prepared as described in the litterature.[1] The preparation of Poly (*N*-[2-(2-ethoxy)ethyl (α-D-glucopyranoside)]methacrylamide) (PEEGM) has been described in detail in the literature.[2]

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III spectrometer (400 MHz) in $CDCl_3$ or D_2O solution at 300K and referenced to residual solvent peaks.

Acetylated glycopolymer was analyzed with a SEC apparatus running in THF at 25°C (flow rate:1 mL/min) and equipped with a Viscotek VE1121 automatic injector, three columns (Waters HR2, HR1 and HR0.5), and a differential refractive index detector (Viscotek VE3580). The average molar mass of the glycopolymer was derived from a calibration curve based on a series of PS standards.

Particle size measurements were carried out by dynamic light scattering (DLS) using a Malvern Instruments Zetasizer nano series instrument and using the cumulant method. The aqueous solutions were prepared at 1 mg/mL, at least five measurements were made for each sample. Equilibration times of 10 minutes were respected before each measurement.

TEM images were obtained on a Philips CM120 electronic microscope, by observations in transmission at an accelerating voltage of 80 kV. Samples were made by placing a drop of sample (1 mg/mL) onto Formvar-coated copper grid. Excess solution was carefully blotted off using filter paper and samples were dried for a few minutes before analysis.

Fluorescence spectroscopy analyses were performed using a JASCO FP-8000 series spectrofluorometer. All the fluorescent spectra were measured in aqueous solution at room temperature.

Small angle X-ray scattering (SAXS) experiments were performed at the European Synchrotron Radiation Facility (Grenoble, France) on the BM2-D2AM beamline. The incident photon energy was set to 16 keV. We used a 2D CCD X-ray detector from Ropper scientific. The sample-to-detector distance was about 1.15 m and the beam stop had a diameter of 2.5 mm, giving access to a q-range of 6 10^{-3} $Å^{-1}$ to 10^{-1} $Å^{-1}$. The q-calibration ($q = 4\pi \sin(\theta)/\lambda$; 2θ = scattering angle) was realized thanks to a silver behenate powder standard. The SAXS data were fitted with the software SASview using a core-shell model [Guinier, A. and Fournet, G. "Small-Angle Scattering of X-Rays", John Wiley and Sons, New York, (1955)].

Phase diagrams' determination

Glycopolymer/Water/Acetone system

Cloud point boundary. This equilibrium limit corresponds to the solubility limit of the polymer (PEMM as an example) in acetone-water mixtures. The method consists in titrating aqueous solutions of glycopolymer with acetone until the mixtures turn milky (generation of swollen micelles). Compositions at the cloud point line are deduced from the mass of acetone added at the onset of turbidity and at a given glycopolymer initial concentration (see **Figure S1**).

Miglyol/water/acetone system

Binodal curve. This equilibrium limit corresponds to acetone/water compositions for which miglyol 812 is not visually soluble. The method consists in titrating acetonic solutions of miglyol with water until the mixtures turn milky, or even phase separate, through the generation of droplets (see **Figure S1**).

"Ouzo limit". This boundary curve separates the Ouzo domain, where stable miglyol nanodroplets can be formed, from the high concentration region where miglyol phase-separates from the mixture. Special requirements are made here to ensure reproducible measurements of the Ouzo limit: i) a non-ionic surfactant (Brij56) is added in the organic phase to stabilize the miglyol droplets once formed; ii) just after the required content of water, an extra load of water is rapidly poured to dilute the samples and avoid Ostwald ripening. The Ouzo limit is determined by measuring the number of counts by DLS, before and after filtrating the mixture with 1.2 µm polyethersulfone filters. Identical numbers of counts before and after filtration correspond to homogeneous emulsions of miglyol droplets, whereas a decrease indicates a loss of micro-scale miglyol droplets on the filter (**Figure S1**).

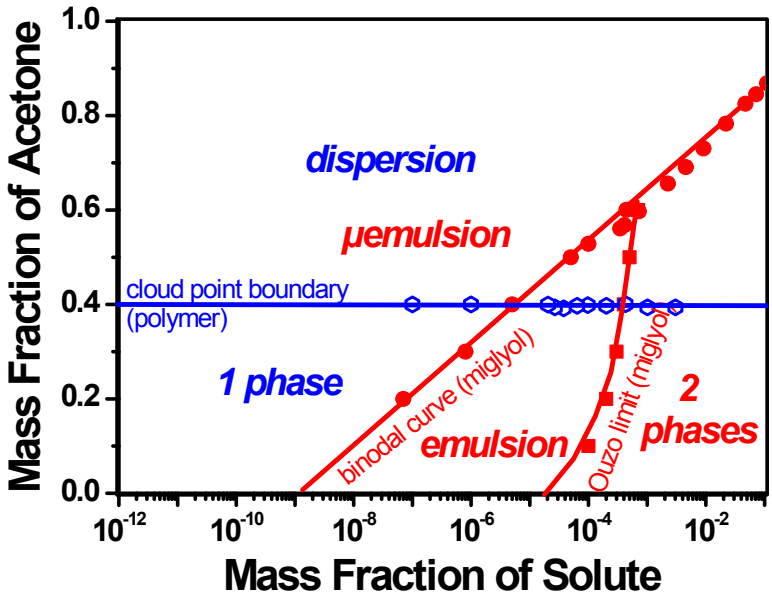


Figure S1: Overlapped phase diagrams of miglyol (in red) and polymer PEMM (in blue) with experimental data. Note that so called '1 phase domain" shown previously in Ouzo work has been replaced by 'microemulsion' to account for the recent discovery of the SFME domain.

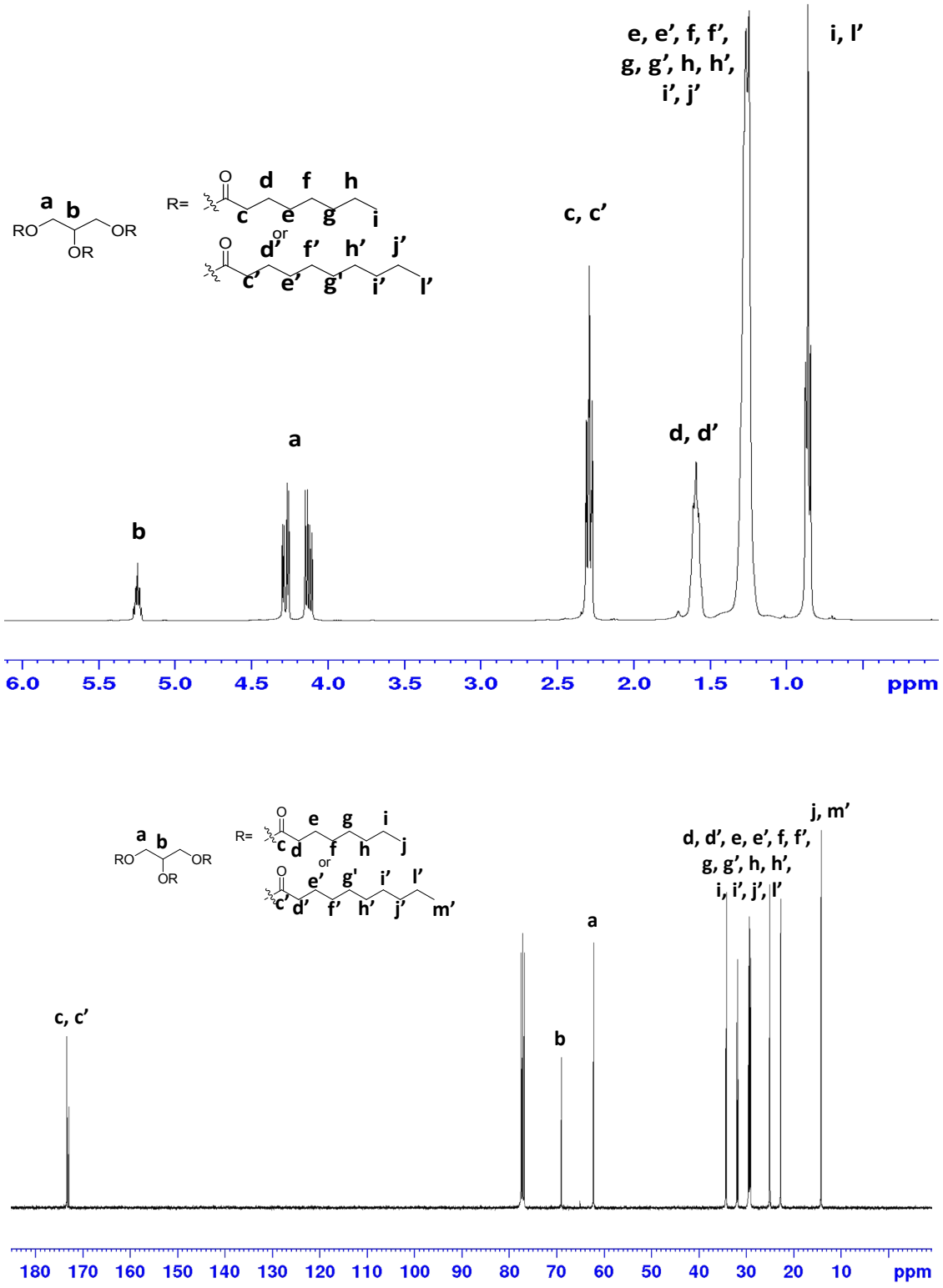
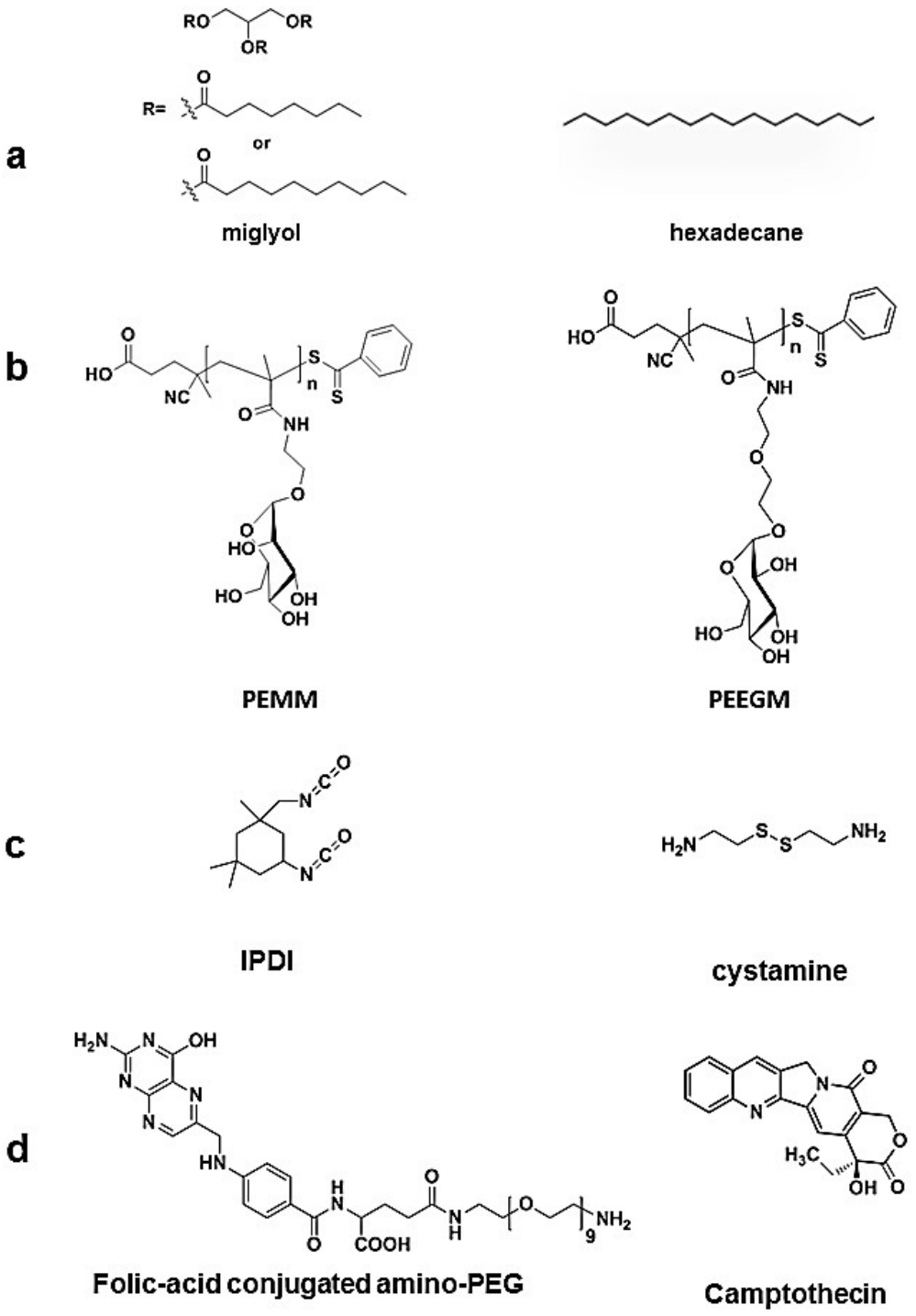


Figure S2: Proton (top) and carbon (bottom) NMR spectra in $CDCl_3$ of miglyol 812 used in this study.



Scheme S1. Structures of: (a) oils; (b) polymers; (c) (co)-crosslinkers and (d) tracers used in this study. Purities for the organic molecules and synthesis of the macromolecules are given in supporting information. Acronyms: PEMM: poly(*N*-[2-(α-D-mannopyranosyloxy)ethyl] methacrylamide); PEEGM: Poly (*N*-[2-(2-ethoxy)ethyl (α-D-glucopyranoside)] methacryl-amide); IPDI: isophorone diisocyanate.

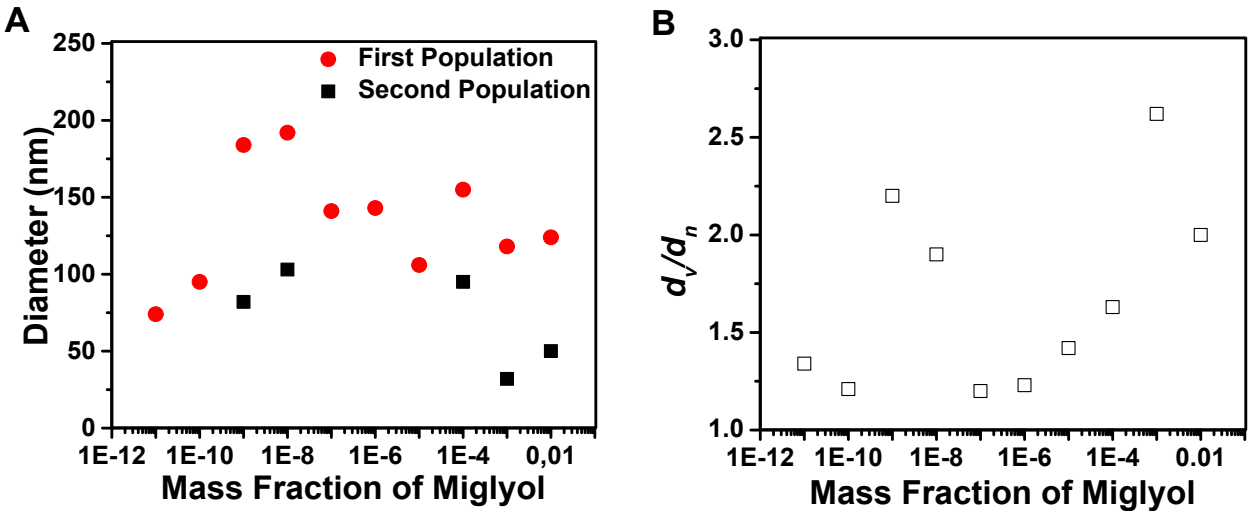


Figure S3: Sizes (A) and size distributions of the main population (B) of SFMEs at acetone mass fraction of 0.9.

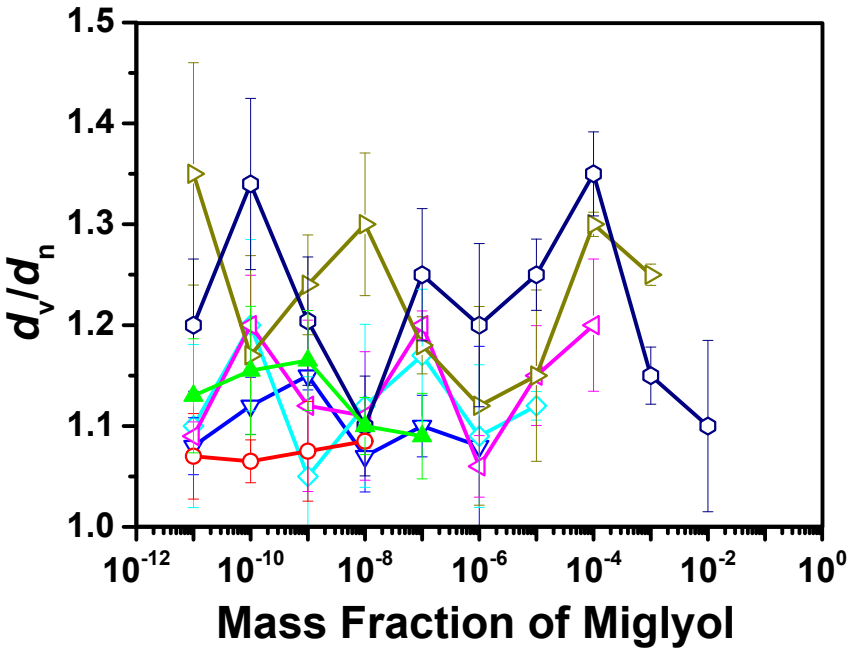


Figure S4: Size distribution of SFMEs as a function of miglyol content. Acetone mass fractions: 0.2 (), 0.3 (), 0.4 (), 0.5 (), 0.6 (), 0.7 (), 0.8 ().

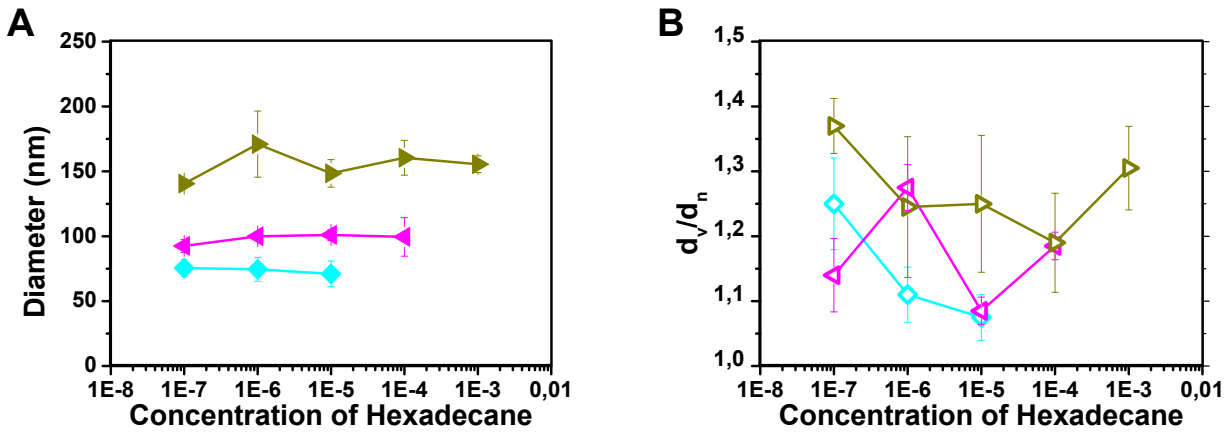


Figure S5: Size (A) and size distribution (B) variations of SFMEs obtained with hexadecane. Acetone mass fractions: 0.5 (🕘), 0.6 (◀), 0.7 (▶).

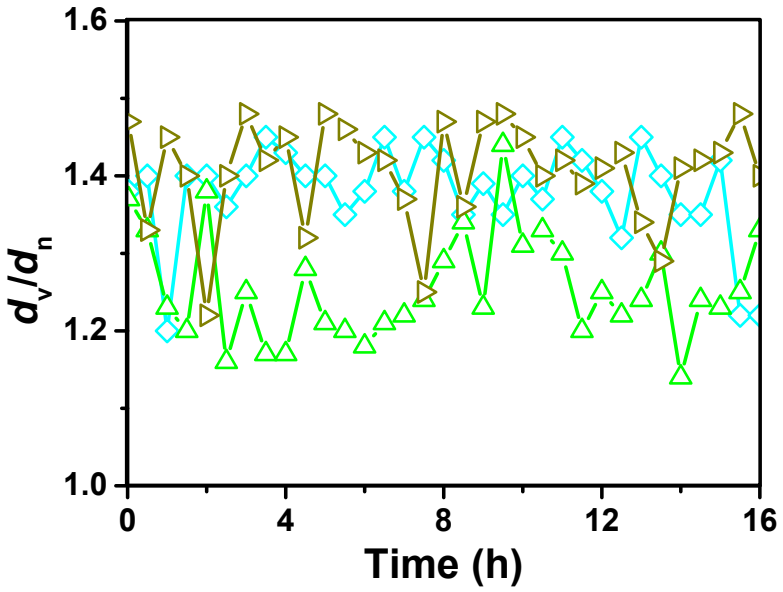


Figure S6: Polydispersities as a function of time for miglyol droplets obtained in the SFME domain: 0.3 (❐), 0.5 (✸), 0.7 (◆).

RAFT Polymerization of EMM

In brief, solution polymerization of EMM (150 mg) was performed in DMSO (0.5 mL) and mediated by 4-cyano-4-(phenylcarbonothioylthio) pentanoic acid using 4,4'-azobis (4-cyanovaleric acid) as initiator ($[M]_0$/[CTA]/[ACPA]=1000/1/0.33). The solution was deoxygenated by three consecutive freeze-pump-thaw cycles and the Schlenk flask was finally filled with nitrogen, allowed to warm to room temperature and finally immersed in an oil bath at 70°C. After polymerization (85% of conversion), the Schlenk flask was plunged into iced water and the solution was then freeze-dried overnight. The crude product was redissolved in a minimal quantity of DMSO and precipitated in an acetone/petroleum ether mixture (volume ratio at 7:3) to remove unreacted monomer and initiator. The powder was dried overnight under vacuum to give 90 mg of PEMM (DP_n = 862, $M_{n,NMR}$ = 251 kg mol^{-1}, $Đ$ =1.1) as a slight pink solid.

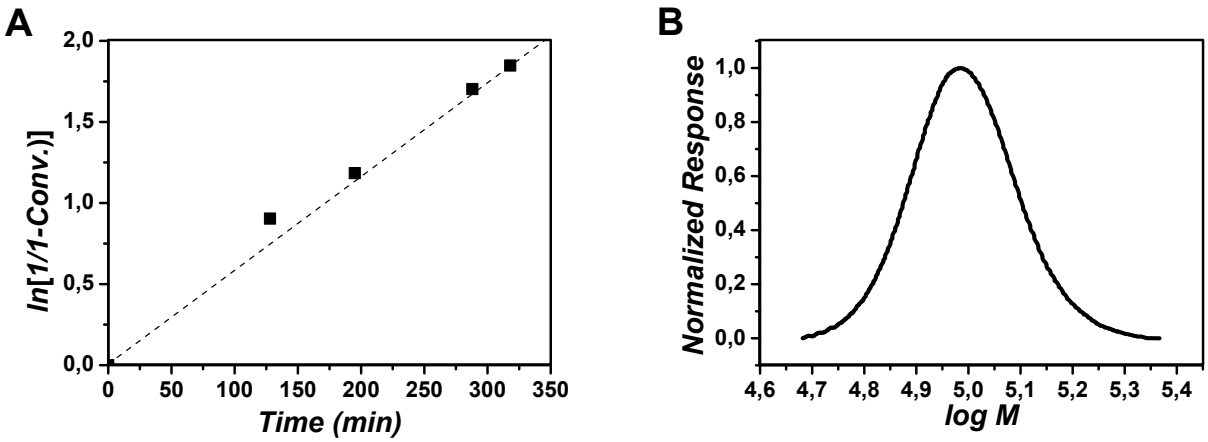


Figure S7. (A) Pseudo first-order kinetic plots for polymerization of EMM. (B) SEC traces of the glycopolymer after acetylation (THF, PS calibration).

Table S1: Glycopolymers obtained by RAFT polymerization

Polymer	$[M]_0$/[CTA]/[Initiator]	Conversion (%)	$M_{n\ th}$ [a] (kg.mol^{-1})	$M_{n\ NMR}$ [b] (kg.mol^{-1})	M_w [c] (kg.mol^{-1})	$Đ$[d]	DP_n[b]
PEMM	1000/1/0.33	85	247	251	101.5	1.1	862
PEEGM	250/1/0.33	92	77.4	81.1	41.8	1.3	242

[a)] calculated from monomer conversion; [b)] determined from relative integration of the aromatic chain end group and polymer backbone peaks; ; [c)] determined from SEC analysis in THF solution after acetylation of the glycopolymers (PS calibration); [d)] $Đ$ from SEC analysis in THF solution after acetylation of the glycopolymers;

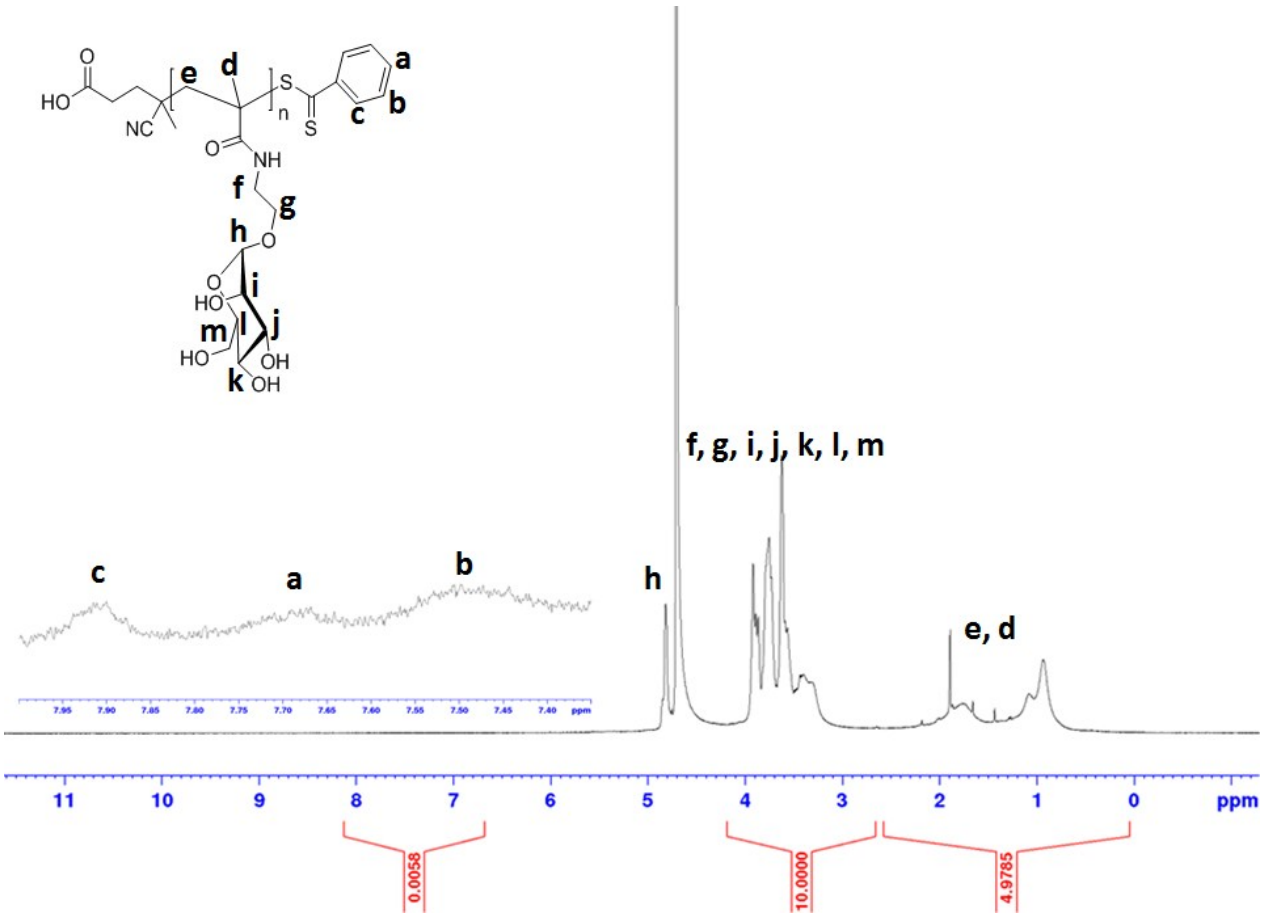


Figure S8. ^{1}H NMR of PEMM in D_2O.

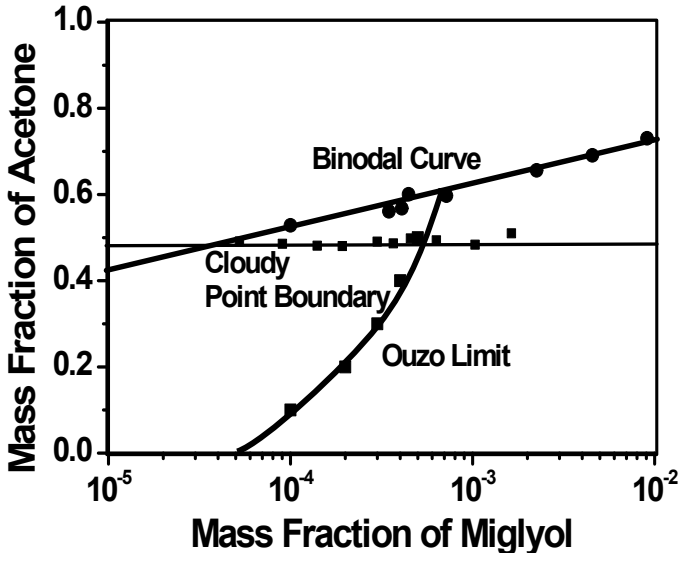


Figure S9: Overlapped phase diagrams of miglyol and polymer PEEGM showing experimental data.

Estimation of shell thickness evolution with time using SAXS and DLS

DLS measurements were performed at certain time on samples taken from the dispersion and after filtration on a 100 nm filter to remove free polymer aggregates.

SAXS measurements were performed on samples prepared right prior to starting the measurement; counting time was set to 50 s and one image was taken every 5 min approximately. The samples were placed and analyzed in silica tubes (external diameter 3 mm, wall thickness 0.2 mm, 60 mm long, from Deutero GmbH) with rubber caps. The scattering contribution of the empty cell and from the solvent (glass tube filled with deionized water:acetone in the appropriate proportions) was subtracted from the scattering intensity of the samples. The images took into account the distortion of the camera and the intensity was corrected by the incident flux, the sample thickness and the dark image. The scattering profiles of the intensity *I* as a function of the scattering wavevector *q* are obtained by azimutally averaging the corrected images. All parameters except for the thickness of the shell were determined on the first data set of the kinetics and were fixed for the following of the fits using a core-shell (model fixed core size, varying shell thickness). Two conditions were tested on a time range of about 2h:

- $w_{miglyol}$: 10^{-5}/ w_{PEMM}: 5.10^{-4}, acetone mass fraction:0.5.
- $w_{miglyol}$: 10^{-4}/ w_{PEMM}: 5.10^{-4}, acetone mass fraction:0.7.

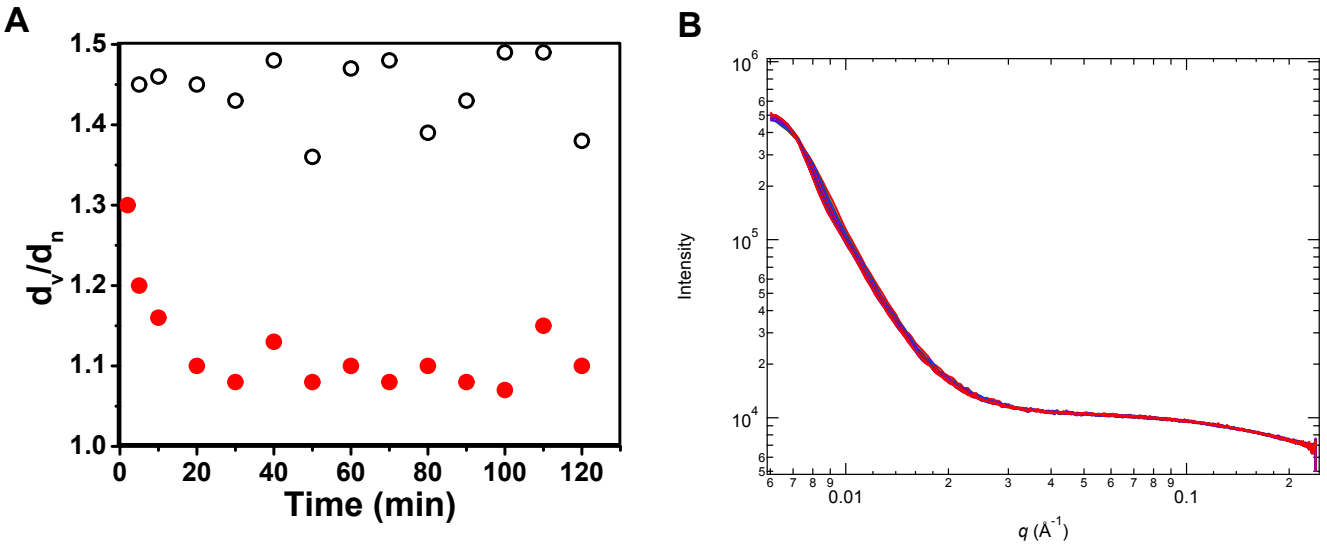


Figure S10. A) d_V/d_N for DLS experiment of non-crosslinking (hollow) and crosslinking (solid) done at $w_{miglyol}$: 10^{-5}/ w_{PEMM}: 5.10^{-4}, acetone mass fraction: 0.5.; b) SAXS curves versus time for nanocapsules built in high supersaturation conditions. The size of the capsule and the thickness of the shell hardly change with time. Conditions: $w_{miglyol}$: 10^{-4}/ w_{PEMM}: 5.10^{-4}, acetone mass fraction: 0.7.

Preparation of miglyol-filled nanocapsules

Typically here, the preparation done at a mass fraction of acetone of 0.5 is used as an example. The glycopolymer PEMM (0.5 mg) was dissolved in 500 mg of water. In a second vial, 0.01 mg of miglyol and 0.066 mg of IPDI were added to 500 mg of acetone. Polymer aqueous solution was then poured into the acetone solution all at once, the transparent solution looked transparent in the beginning, and then turned to be slight milky after few hours. The solutions were left overnight and further characterized by DLS and TEM.

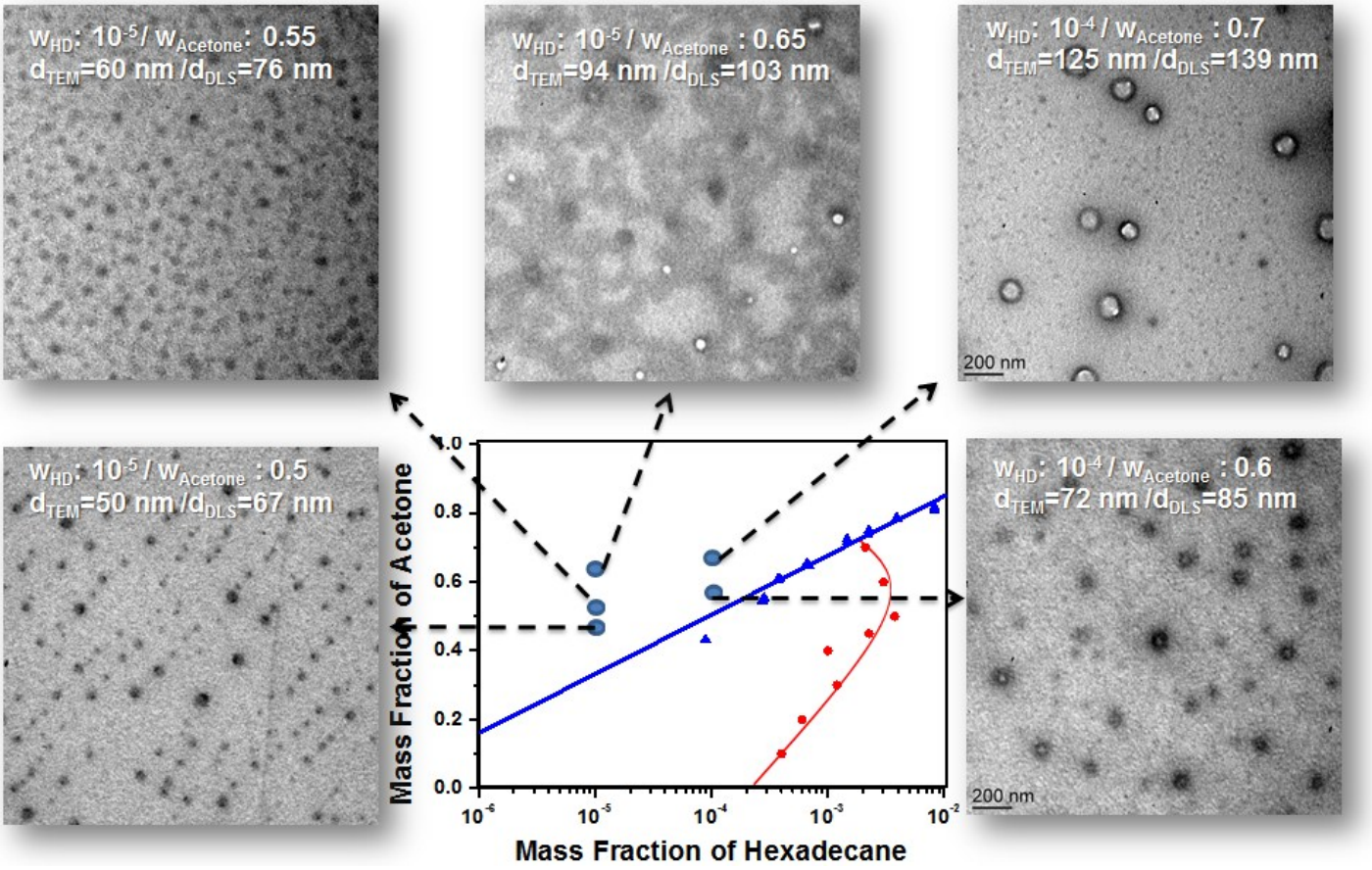


Figure S11. TEM images of PEMM based Hexadecane loaded nanocapsules prepared by the conanoprecipitation / polymer crosslinking process in various places of the SFME domain (see HD and acetone mass fractions given inside the photos). Diameters in TEM were directly calculated by measuring at least 20 capsule sizes using imageJ softawre, whereas DLS values were measured in acetone:water mixtures; both are d_n values.

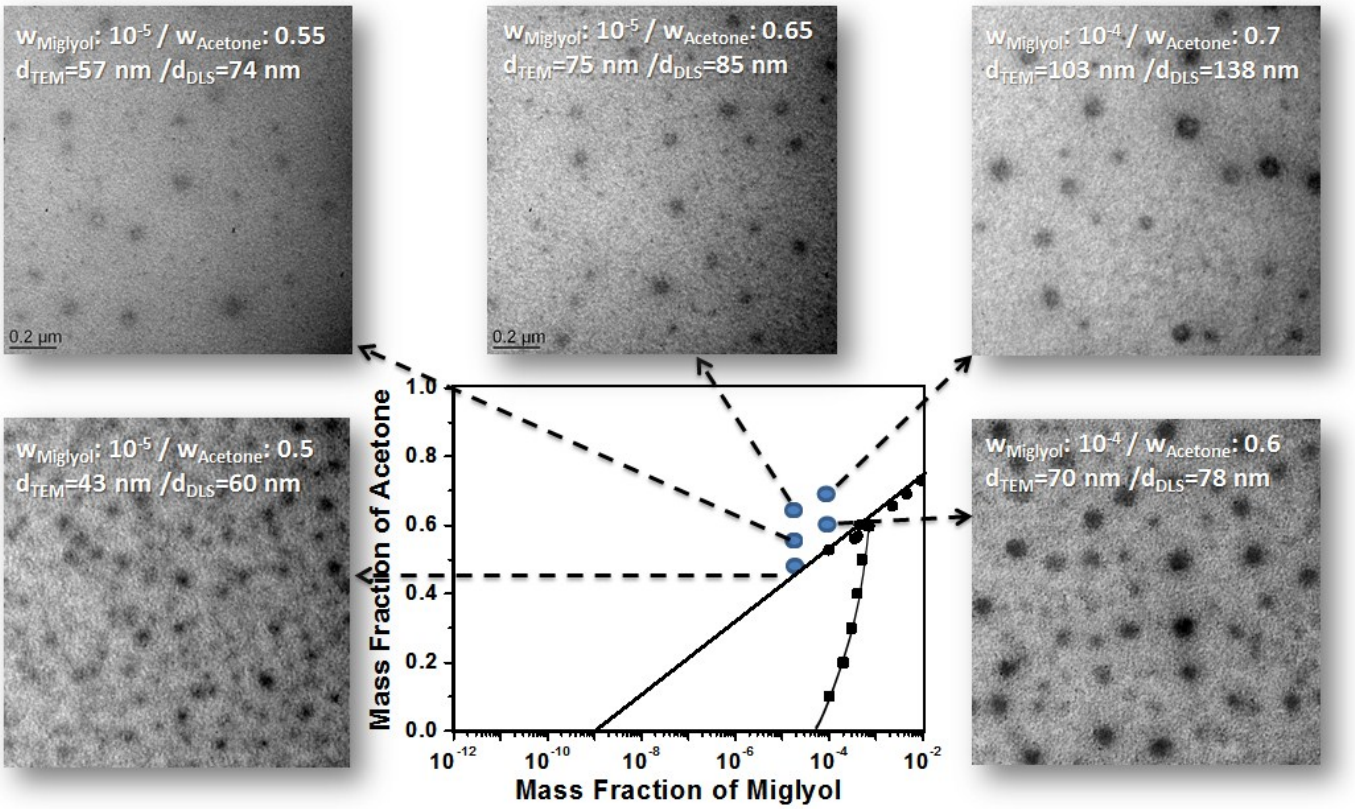


Figure S12. TEM images of PEEGM based nanocapsules prepared by the conanoprecipitation / polymer crosslinking process in various places of the SFME domain (see miglyol and acetone mass fractions given inside the photos). Diameters in TEM were directly calculated by measuring at least 20 capsule sizes using imageJ softawre, whereas DLS values were measured in acetone:water mixtures; both are d_n values.

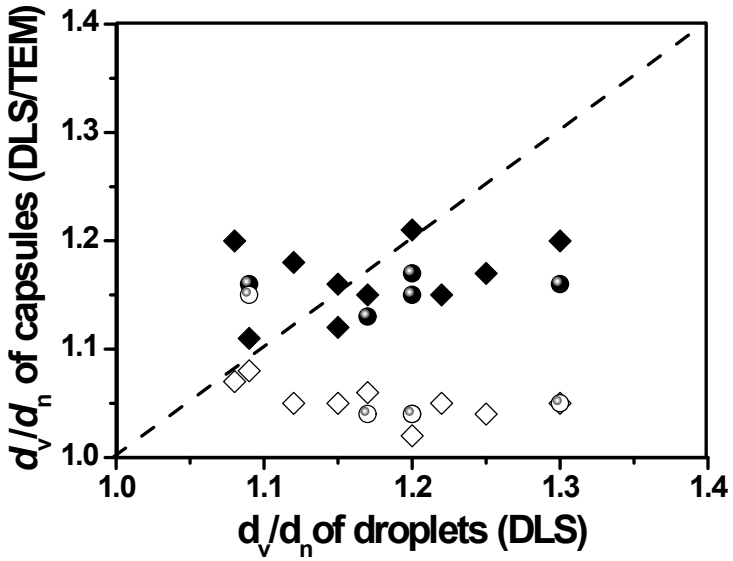


Figure S13: Size distribution of capsules versus droplets in regards with the plot in Figure 3, as obtained from TEM (open symbols) and DLS (plain symbols): (◴) PEMM; (□) PEEGM.

Synthesis of Folic-acid conjugated amino-PEG

The synthesis was performed according to Baier *et al*. with slight modifications.[3]

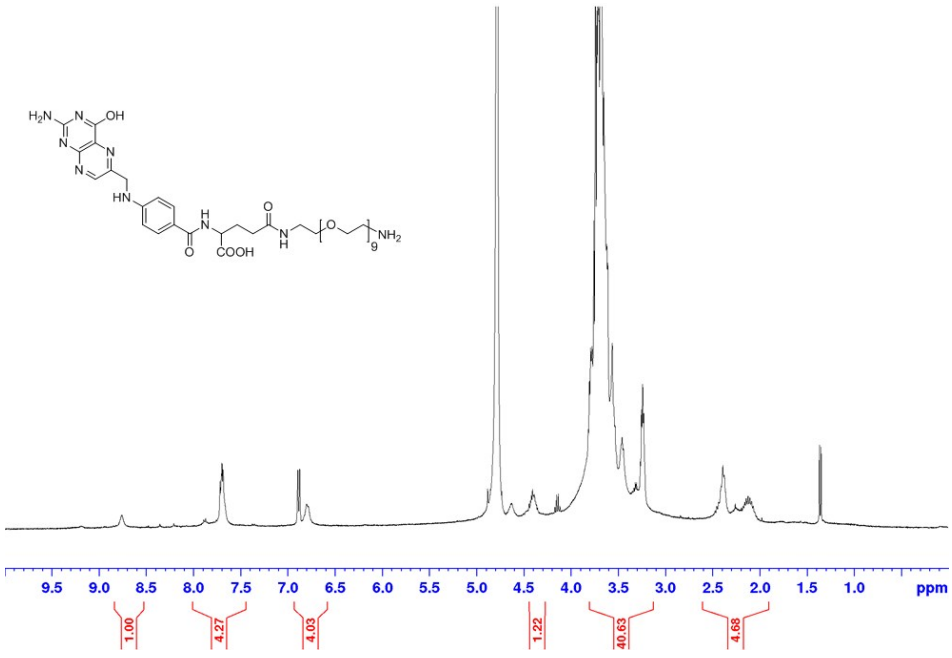
(1) Folic acid (100 mg, 0.23 mmol) was suspended in 7 mL of mixture solution of DMSO/pyridine (v/v, 5/2) under argon atmosphere. Dicyclohexylcarbodiimide (DCC, 94 mg, 0.46 mmol) was then added and the resulting suspension was stirred for 30 min before addition of *O*-(2-Aminoethyl)-*O′*-[2-(Boc-amino)ethyl]octaethylene glycol (126 mg, 0.23 mmol). The mixture was stirred for overnight. The solution was then poured into 200 mL of diethylether, and the resulting crude yellow (200 mg, 90%) precipitate was obtained after filtration.

(2) 200 mg of the crude yellow product were then mixed with 10 mL of trifluoracetic acid (TFA) and stirred for 3 h at 40 °C. Crude brownish gel was collected after removing TFA under reduced pressure. The product was then purified by 2 times cycle of dissolution (10 mL of water) and precipitation (200 mL of diethylether). The yellow solid (100 mg, 55%) was finally obtained after drying under reduced pressure.

^{1}H NMR (400 MHz, D_2O) δ 2.10-2.45 (m, 4H), 3.22-4.17 (m, 40H), 4.38-4.44 (m, 1H), 6.80-6.90 (m, 4H), 7.69-7.87 (m, 4H), 8.76 (s, 1H); ESI-MS: m/z calculated for $[C_{39}H_{62}N_9O_{14}]^+$, 880.4416, found 880.4411.

Preparation of functionalized nanocapsules

The glycopolymer PEMM (0.5 mg) and Folic-acid conjugated amino-PEG (0.01 mg) was dissolved in 500 mg of water. In a second vial, 0.01 mg of miglyol and 0.066 mg of IPDI were added to 500 mg of acetone. Polymer aqueous solution was then poured into the acetone solution all at once, the transparent solution looked transparent in the beginning, and then turned to be slight milky after few hours. After 2 hours, 0.066 mg of IPDI in 240 mg of acetone was added to perform second cross-linking. The solution was left overnight. After 2 times filration with 100 nm filter to remove all polymer aggregates, the solution was evaporated to remove the acetone and then dialyzed and freeze-dried to result in white powder. The functionalization was confirmed by NMR after redispersion of nanocapsules in D_2O.



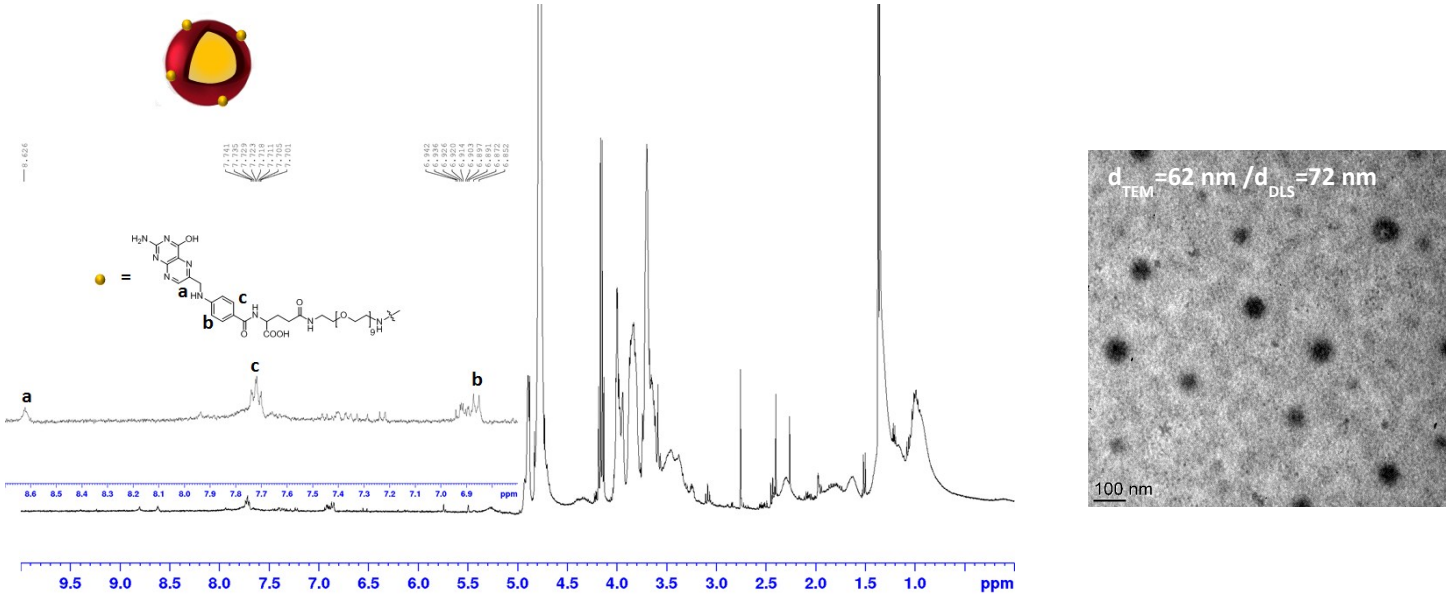
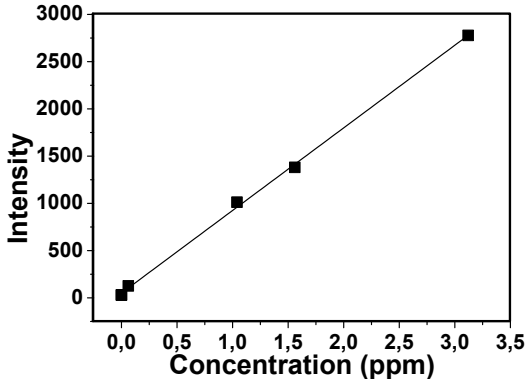


Figure S14: (Top) ^{1}H NMR spectrum of NH_2-terminated Folic Acid conjugated PEG in D_2O. (Bottom) ^{1}H NMR (D_2O) and TEM photo of FA-PEG-functionalized PEMM-based nanocapsules in D_2O.

Encapsulation of camptothecin (CPT)

In order to faciliate their characterizations, nanocapsules shown here were prepared with an acetone mass fraction set at 0.7. The glycopolymer PEMM (0.5 mg) was dissolved in 300 mg of water. In a second vial, 0.1 mg of miglyol (0.01 wt% CPT) and 0.066 mg of IPDI were added to 700 mg of acetone. Polymer aqueous solution was then poured into the acetone solution all at once. After 2 hours, 0.066 mg of IPDI in 240 mg of acetone was added to perform second cross-linking. The solutions were left overnight. The nanocapsules were discarded by passing them 2-times through a 100 nm filter. The content left in the solution was free CPT. After evaporation and freeze drying, the product was redissolved in pure acetone (2 mL). The concentration of CPT in solution could be determined by calibration curve of CPT in acetone.

$$Encapsulation\ Efficiency(\%) = \frac{Total\ CPT - Free\ CPT}{Total\ CPT} \times 100\%$$



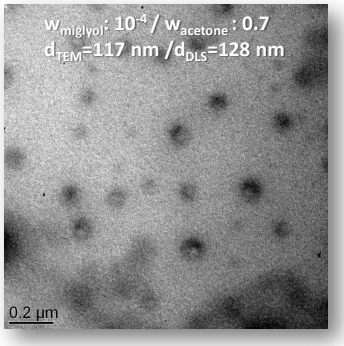


Figure S15. (Left) Calibration curve of CPT in acetone (λ_{ex}=370 nm); (Right) TEM photo of CPT encapsulated PEMM based nanocapsules.

Degradation of nanocapsules

Preparation: In order to facilitate the observation after degradation, the nanocapsules were prepared at an acetone mass fraction of 0.7. The glycopolymer (0.5 mg) and cystamine (0.015 mg) was dissolved in 300 mg of water. In a second vial, 0.01 mg of miglyol and 0.066 mg of IPDI were added to 700 mg of acetone. Polymer aqueous solution was then poured into the acetone solution all at once. After 2 hours, 0.066 mg of IPDI in 240 mg of acetone was added to perform second cross-linking. The solutions were left overnight.

Degradation: Dithiothreitol (DTT, 1 mg) was added into nanocapsules solution after removing acetone by evaporation and dilution with water (final volume is 1mL). After 2 h reaction at room temperature, the degradation was confirmed by DLS (filtration with 0.88 µm filter to remove released miglyol droplet) and TEM.

Ouzo data

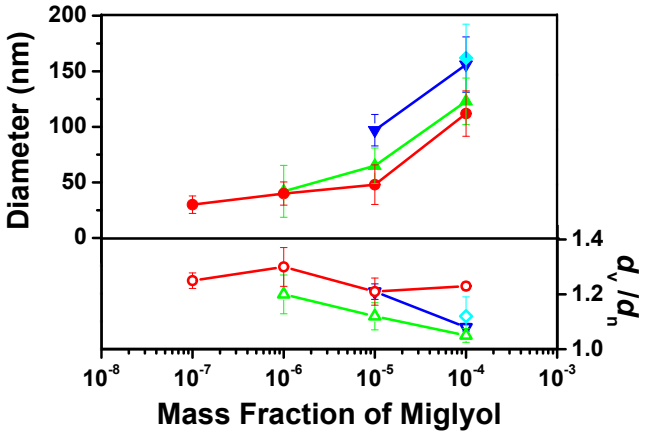


Figure S16: Particle size (plain symbols) and size distribution (open symbols) versus miglyol content as obtained in the Ouzo domain (to be compared with Figure 2a). Note the narrower domain of study, and the fact that quite small sizes can also be reached in this part of the phase diagram.

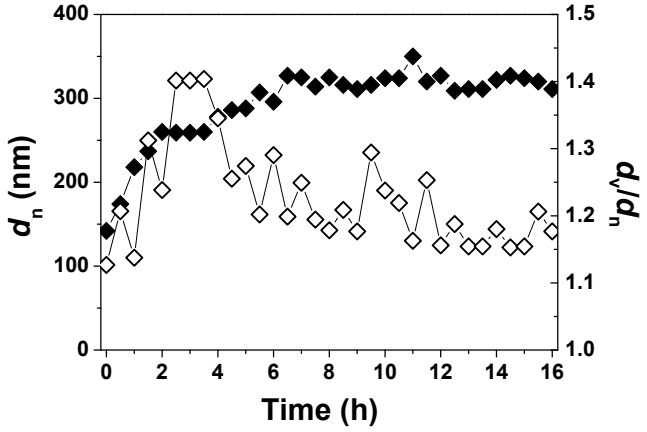


Figure S17: Size (plain) and size distribution (open) variations with time of miglyol droplets generated in the ouzo domain (acetone mass fraction of 0.5). The leveling of the size and the decrease of d_v/d_n with time plead for an Oswald ripening phenomenon.

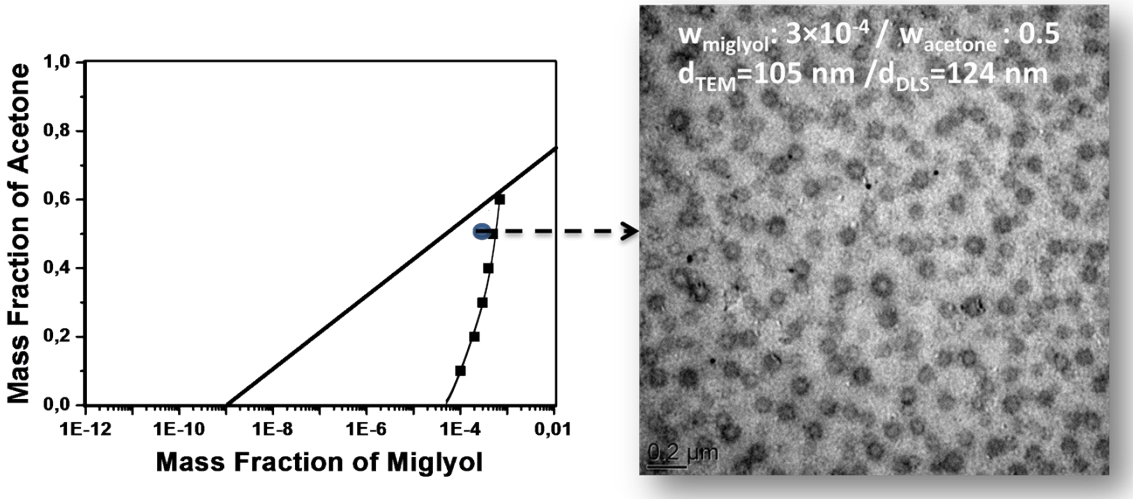


Figure S18. TEM image of PEEGM based nanocapsules prepared by conanoprecipitation / polymer crosslinking in the Ouzo domain (see miglyol and acetone mass fractions given inside the photo). Diameter in TEM was directly calculated by measuring at least 20 capsule sizes using imageJ softawre, whereas DLS value was measured in acetone:water mixtures; both are d_n values.

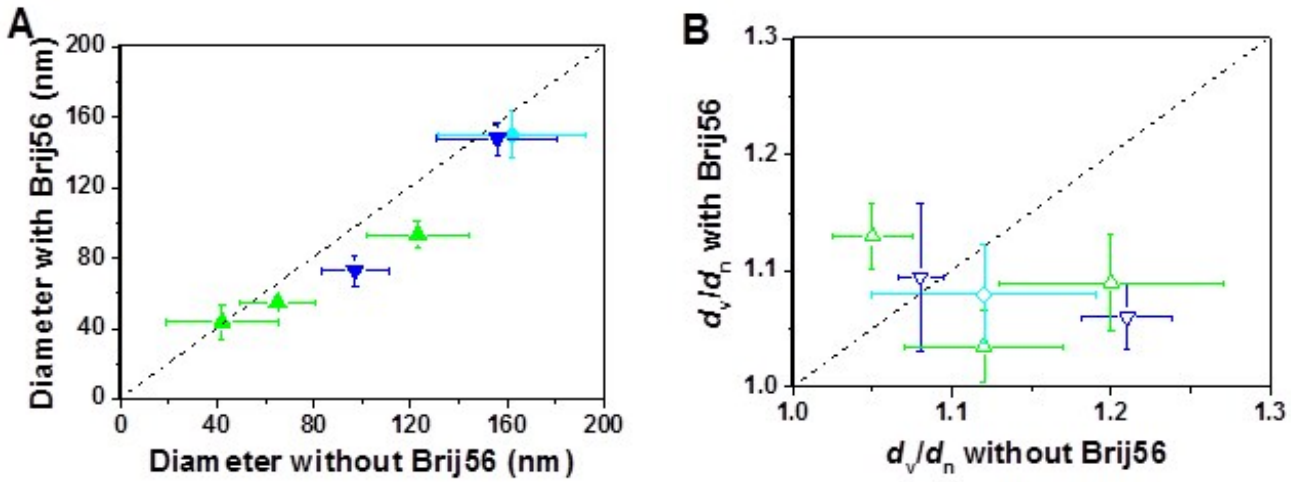


Figure S19: Comparison of (A) diameters and (B) polydispersities obtained in absence or presence of Brij56 that shows that Ouzo droplets are stabilized by the surfactant. Acetone mass fractions: 0.3 (□), 0.4 (❑), 0.5 (◴).

SFME in presence of surfactant

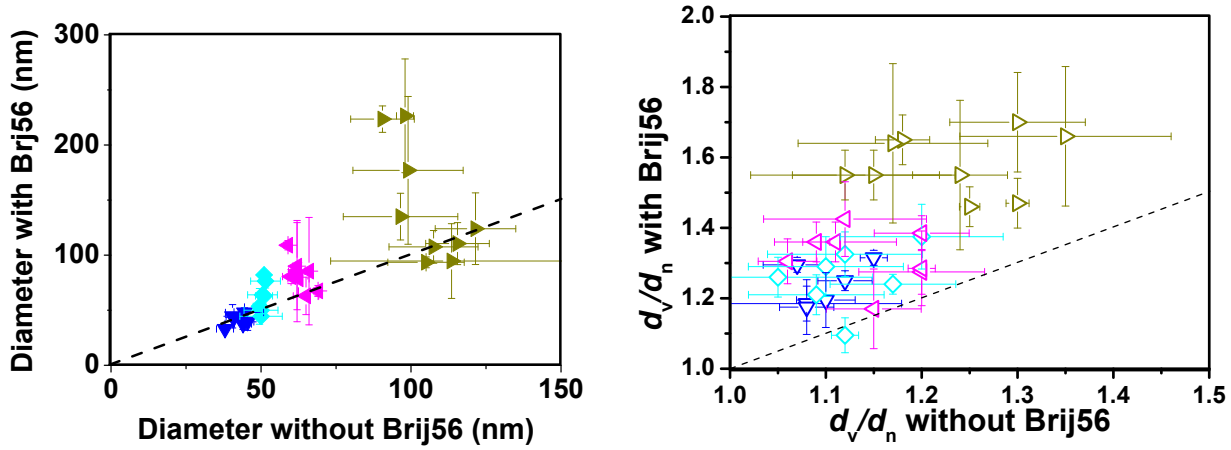


Figure S20: Comparison of a) droplet size (plain symbols) and b) polydispersities (open symbols) obtained in absence or presence of Brij56 that shows that SFME droplets are destabilized by the surfactant. Acetone mass fractions: 0.4 (♦), 0.5 (□), 0.6 (❖), 0.7 (□).

Reference

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