Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting information

In situ Glyco-nanostructures Formulation via Photo-Polymerization Induced Self-Assembly

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MATERIALS AND METHODS

Materials. Dextran T_{40} ($\bar{M}_n$=32 kg.mol^{-1}, Đ=1.4, determined by SEC-MALLS, see below) was purchased from Pharmacia Biotech and dried under reduced pressure at 100 °C for one night prior to use. 1-propanethiol (99%), carbone disulfide (>99%), iodine (99.8%), 4,4'-azobis(4-cyanopentanoic acid) (98%), N,N'-carbonyldiimidazole (CDI) (97%), potassium tert-butoxide (95%), lithium chloride (99%), sodium thiosulfate, sodium sulfate, dimethylsulfoxide (DMSO) (≥99.9%), N,N-dimethylformamide (DMF) (99.8%), diethyl ether (Et_2O), methanol (MeOH) and ethyl acetate (99%) were purchased from Sigma-Aldrich and used as received. 2-Hydroxypropyl methacrylate (HPMA) (98%) was purchased from ABCR, purified by dissolving the monomer in water, then washed with hexane to remove diacrylates. After saturation of the aqueous solution by NaCl, HPMA was extracted by Et_2O to remove acrylic acid and dried over $MgSO_4$ (3 wt %). Finally, Et_2O was evaporated off under reduced pressure at room temperature.

Synthesis of 4-(propylthiocarbonothioylthio)-4-cyanopentanoic acid RAFT agent (TTC) (1, Figure S1).

TTC was synthesized using a modified procedure previously reported [1]. 1-propanethiol (3.4 g, 45 mmol) (1a, Figure S1) was added dropwise under nitrogen atmosphere at 5°C to a suspension of potassium tert-butoxide (5 g, 45 mmol) in anhydrous Et_2O (100 mL) to provide a white slurry of potassium thiopropanate. After stirring the mixture for 20 min, carbon disulfide (3.4 g, 45 mmol) was introduced dropwise to the mixture at 0°C to provide a yellow precipitate of potassium–S-propyl trithiocarbonate, which was collected by filtration and used directly without purification. Afterwards, solid iodine (5.9 g, 23 mmol) was added portion-wise to a stirred suspension of potassium–S-propyl trithiocarbonate (8 g, 42 mmol) in anhydrous Et_2O (200 mL). After stirring the mixture for 2h at room temperature, the white solid of potassium iodide was filtered and the brown filtrate was washed three times with an aqueous solution of sodium thiosulfate to remove excess iodine, then dried over sodium sulfate. After evaporation of Et_2O, a red oil of bis-(propanesulfyanyl thiocarbonyl)disulfide (4 g, 13.2 mmol) (1b, Figure S1) was obtained and characterized by ^{1}H NMR spectroscopy in $CDCl_3$.

A solution of bis-(propanesulfyanyl thiocarbonyl) disulfide (2 g, 7 mmol) and 4,4'-azobis(4-cyanopentanoic acid) (2.8 g, 10 mmol) in ethyl acetate (40 mL) was heated under reflux for 24h.

After removal of solvent, the crude product was solubilized in Et_2O, washed five times with deionized water, and finally dried under vacuum to obtain an oily product. Propyl 2-cyanopropan-2-yl-carbonotrithioate (2.6 g, 9.4 mmol) (1) was analyzed by ^1H NMR in $CDCl_3$ (Figure S1).

Synthesis of the macromolecular Chain Transfer Agent (Dex-TTC) (2, Figure S6).

Dex-TTC containing 5 TTC groups per dextran chain, was prepared via the partial esterification of hydroxyl groups of dextran with carboxylic acid function of TTC in the presence of N,N'-carbonyldiimidazole (CDI). CDI (0.3 g, 1.5 mmol) and TTC (0.4 g, 1.5 mmol) were dissolved in anhydrous DMSO (20 mL) and stirred at 40 °C for 6h to produce the activated acid imidazolide. Afterwards, the acid imidazolide reaction mixture was added under nitrogen atmosphere to a solution of dextran (2g, 12.3 mmol glucopyranosic units) in DMSO previously prepared in a separate flask. The reaction was kept under stirring for 72h at 60°C. Finally, the crude product was precipitated in MeOH. Dex-TTC was collected by filtration, dried under reduced pressure (yield=80%), and then analyzed by ^1H NMR in DMSO-d_6 (Figure S6).

Photoinitiated RAFT homopolymerization of HPMA in DMSO.

In a dried schlenk flask, HPMA (2.5 g, 17.4 mmol) and TTC (16 mg, 0.058 mmol) were dissolved in dried DMSO (5.1 mL). The homogeneous mixture was purged with nitrogen for 10 min, sealed, and then irradiated with a UV lamp (365 nm) using suitable light intensity (4 mW/cm^{-2}, 27 mW/cm^{-2}, 45 mW/cm^{-2} or 53 mW/cm^{-2}) at room temperature. Samples were taken under nitrogen at different time and the polymerization was quenched by exposure to air and addition of a small amount of hydroquinone. Crude product was then analyzed by ^1H NMR in DMSO-d_6 and by SEC-MALLS in DMF/LiCl at 50°C (Figures S2 and S3).

In-situ **preparation of Dex-g^5-PHPMA$_{260}$-based nano-objects via aqueous photo polymerization-induced self-assembly.**

In a typical experiment for the synthesis of Dex-g^5-PHPMA$_{260}$, HPMA (1.18 g, 8.2 mmol) and Dex-TTC (0.2 g, 1.2 mmol glucopyranosic units) were dissolved in a dried schlenk flask using a suitable volume of water to obtain a 5% w/w solids content (26.4 mL). The homogeneous mixture was purged with nitrogen for 10 min, sealed, and then irradiated at room temperature

with a UV lamp (365 nm, light intensity of 27 mW/cm^{-2}) for 1h to achieve a full conversion. A small aliquot of the obtained dispersion was taken. One portion was freeze-dried and analyzed by ^{1}H NMR in DMSO-d_6 (Figure S7) and by SEC-MALLS in DMF/LiCl at 50°C (Figure S9). The other portion was characterized by TEM (Figures S15 to S20).

Kinetics of photoinitiated aqueous RAFT dispersion polymerization of Dex-g^5-PHPMA$_{180}$.

In a typical experiment for the synthesis of Dex-g^5-PHPMA$_{180}$, HPMA (0.8 g, 5.5 mmol) and Dex-TTC (0.2 g, 1.2 mmol glucopyranosic units) were dissolved in a dried schlenk flask and using a suitable volume of D_2O to obtain a 5% w/w solids content (19 mL). The homogeneous mixture was purged with nitrogen for 10 min, sealed, and then irradiated with a UV lamp (365 nm, light intensity of 27 mW/cm^{-2}) at room temperature. Samples were taken under nitrogen at different times and the polymerization was stopped by exposure to air and addition of a small amount of hydroquinone. Crude product was then analyzed by ^{1}H NMR in DMSO-d_6 and by SEC-MALLS in DMF/LiCl at 50°C (Figures S8, S9 and S12).

Cleavage of the PHPMA grafts from Dextran backbone

In a typical experience Dex-g^5-PHPMA$_{180}$, 1 mL of the crude aqueous suspension (5% w/w solids content) was mixed with 2 mL of alkaline solution of NaOH (1 mol.L^{-1}) for 24 hours at 50 °C to hydrolyze the ester functions linking grafts to dextran backbone. The white precipitate (PHPMA) that formed was recovered by centrifugation, washed one time with aqueous solution of HCl (1 mol.L-1) and two times with distillated water, dried overnight at 50 °C under vacuum, then analyzed by SEC-MALLS in DMF at 50°C (Figure S10).

Polymer Analysis.

^{1}H NMR spectra of products were recorded on a Bruker Avance 300 apparatus (300, 13 MHz, 25°C) in $CDCl_3$ or DMSO-d_6.

Size Exclusion chromatography (SEC). The molecular weights were measured using a SEC in DMF/LiCl, [LiCl]=2 g.L^{-1} (flow rate of 1.0 mL min^{-1}) at 50°C, equipped with Multi-Angle Laser Light Scattering (MALLS) detector (Mini Dawn Wyatt), differential refractometer detector (RID 10A, Shimadzu), HPLC pump (LC 20AD, Shimadzu), degazer AF (DGU – 20A3R, Shimadzu), and three PLgel columns (100000, 1000, and 100 Å). Refractive index increments (dn/dc) of 0.01 was

measured for all polymers (PHPMA, Dextran, Dex-TTC and Dex-g-PHPMA) in DMF/LiCl at 50°C using a differential refractometer from WYATT Technology (Optilab rEX and HELEOS-II).

Dynamic Light Scattering (DLS).

Hydrodynamic radii and size distribution of glycopolymers nano-objects were determined at 20°C using Dynamic Light Scattering. Samples were diluted 500-fold with water to produce 0.1 mg.mL^{-1}. Measurements were recorded using an ALV-5000 multitau correlator system in combination with a vertically polarized helium−neon laser with a wavelength of λ = 632.8 nm. The autocorrelation functions were analyzed in terms of relaxation time (t) distribution according to REPES routine.[2] Measurements were done at angles θ varying from 30° to 150° corresponding to scattering wave vectors q ranging from q = 4.6 × 10^{-4} $\dot{A}^{-1}$ to 2.55 × 10^{-3} $\dot{A}^{-1}$. Z-average hydrodynamic radius (R_h) was estimated using the Stokes-Einstein relation (Eq-S1), where D_0 is diffusion coefficient determined from the slope of the q^2 dependence of relaxation rate ($\langle\Gamma\rangle = Dq^2$), k_B is the Boltzmann constant, T is experimental temperature and η_s is the viscosity of the solvent (water).

$$R_h = \frac{K_B \times T}{6 \times \pi \times \eta_s \times D_0} \quad \text{(S1)}$$

Turbidity measurement.

Evolution of the turbidity with conversion was estimated during preparation of glyco-nanostructures via PISA process by measuring transmittance of suspension at predefined time during UV-irradiation. Transmittance was measured at 600 nm using UVikon-XL spectrometer (Bio-Tech instruments) (Figure S11).

Transmission Electron Microscopy (TEM).

The glycopolymers-based nano-objects were investigated by Transmission Electron Microscopy (TEM) using a microscope Philips CM200 operating at an accelerative voltage of 200kV. Images were recorded with a post-column camera Gatan MSC600. Samples were diluted 50-fold with

water to produce 1 mg.mL^{-1}. A drop of the solution was adsorbed onto a copper grid for 30 s, and then blotted with filter paper to remove excess solution. A drop of uranyl acetate solution (0.5 wt%) was soaked on the sample-loaded grid for 30 s, and then blotted with filter paper to remove excess stain.

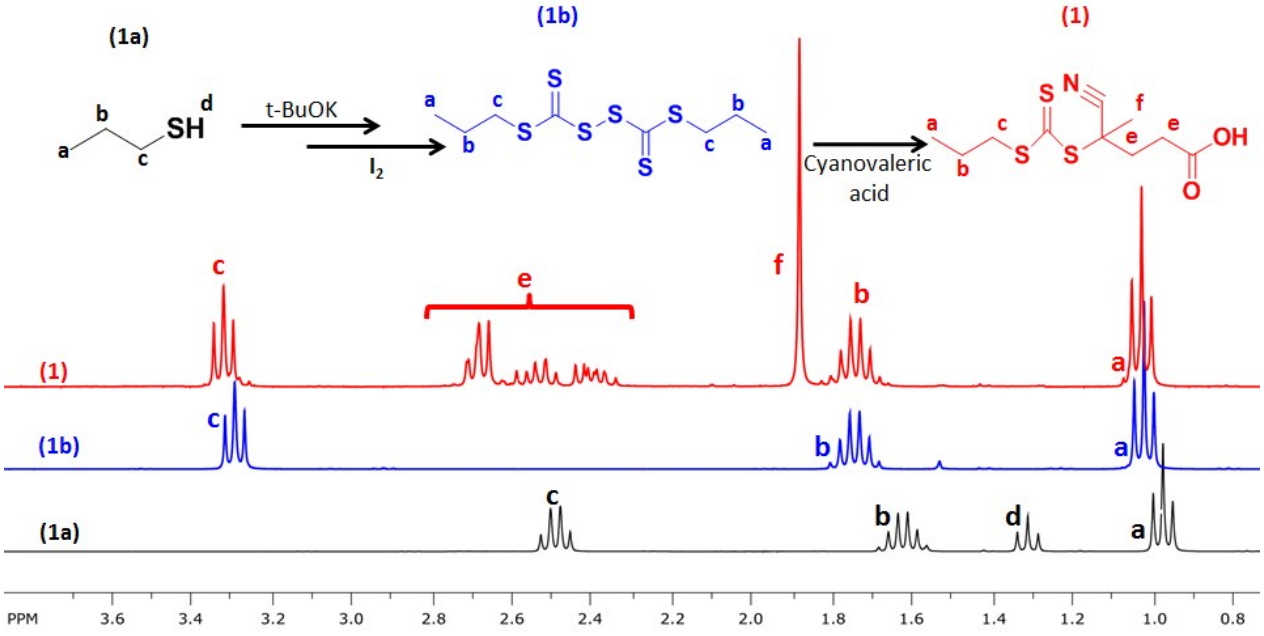

Figure S1. ^{1}H NMR spectra of 1-propanethiol (1a), bis-(propanesulfyanyl thiocarbonyl) disulfide (1b) and 4-(propylthiocarbonothioylthio)-4-cyanopentanoic acid (TTC) (1) in $CDCl_3$.

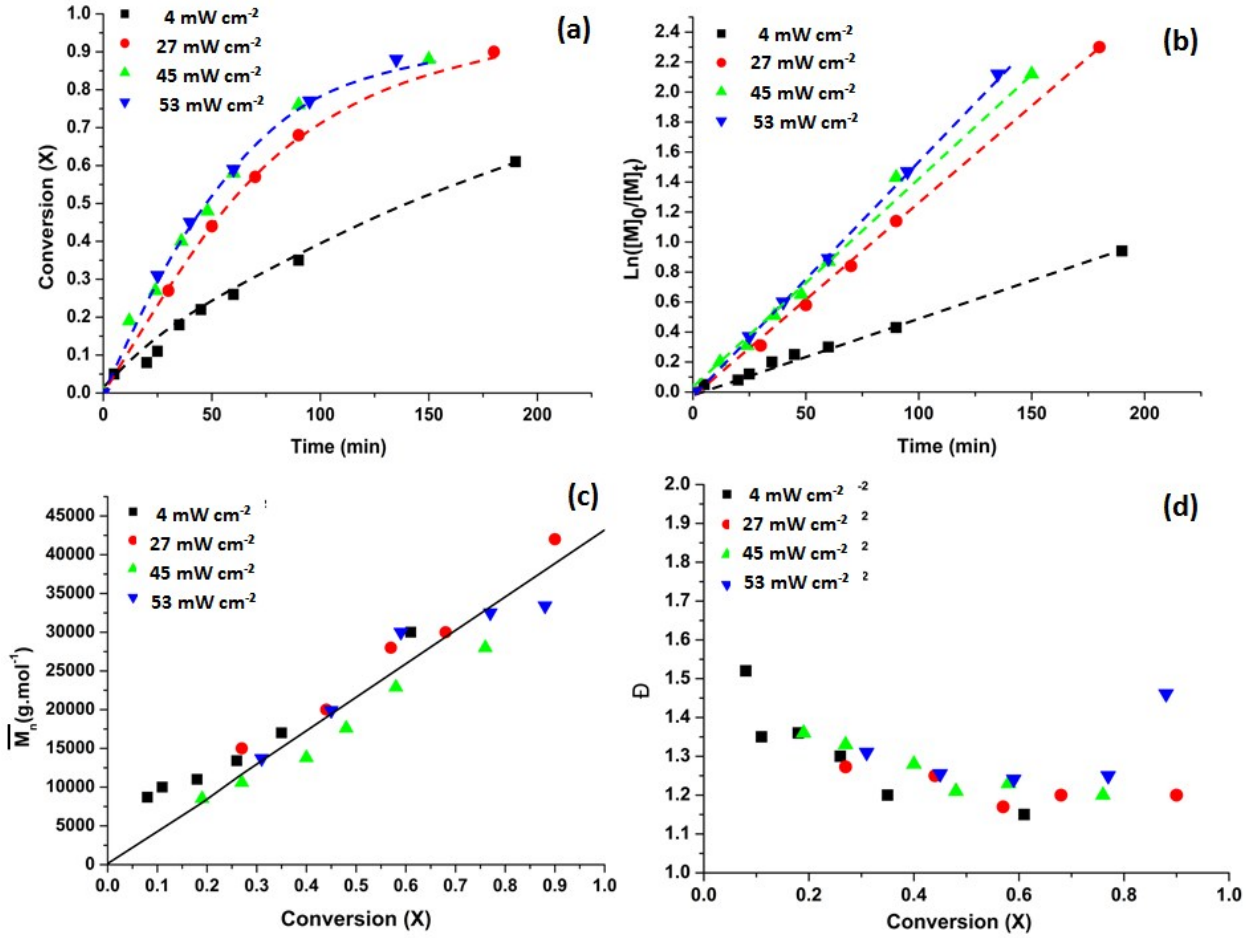

Figure S2. RAFT homopolymerization of HPMA in DMSO at room temperature, using TTC (1) with various UV lamp intensities (4 mW/cm^{-2}, 27 mW/cm^{-2}, 45 mW/cm^{-2} or 53 mW/cm^{-2}). $[HPMA]_0/[TTC]_0$=300/1. Plots of (a) conversion and (b) $\ln[M]_0/[M]_t$ *versus* time. Plots of (c) $\bar{M}_n$ and (d) Đ *versus* conversion.

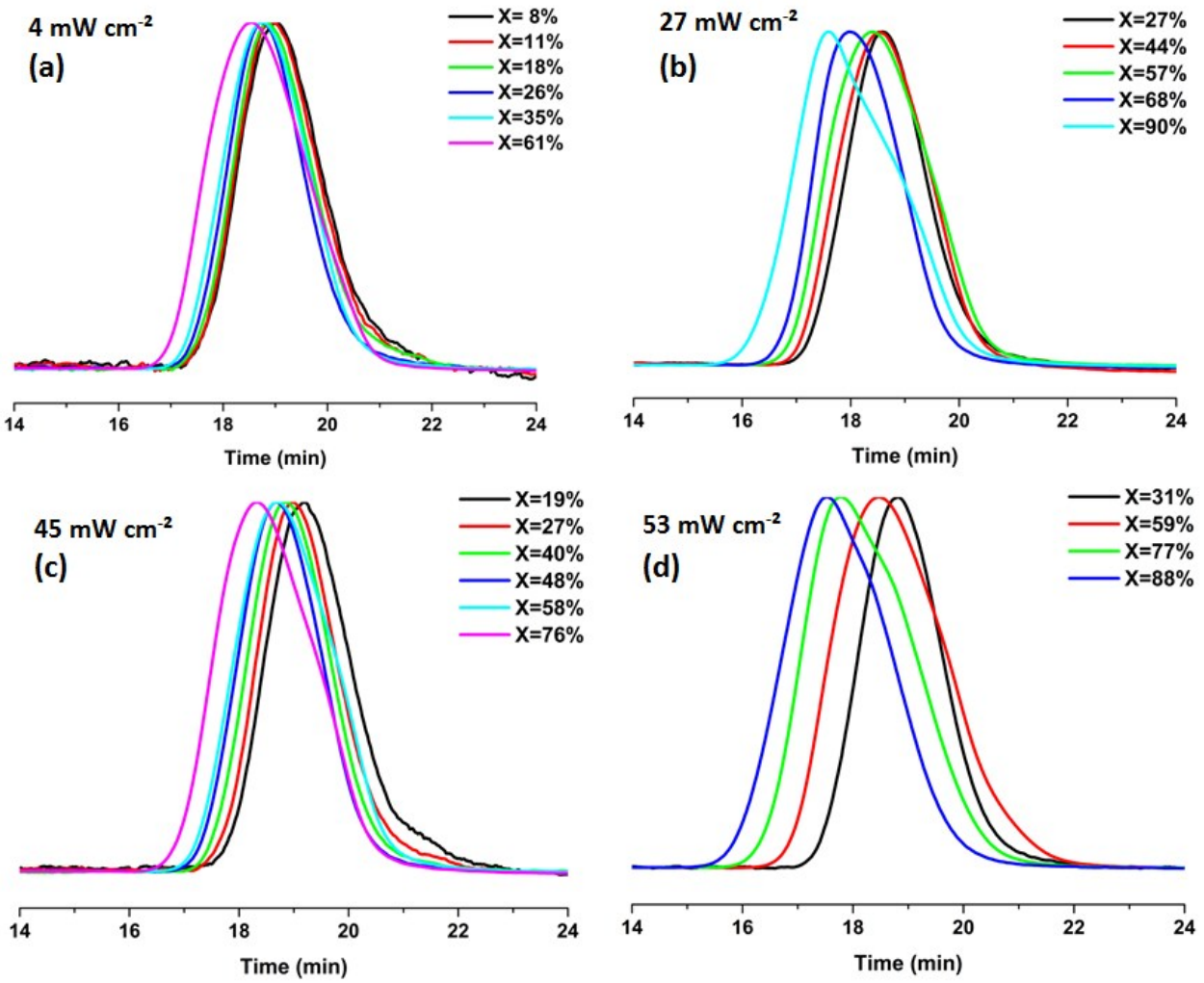

Figure S3. Evolution of PHPMA SEC traces (LS detection, DMF/LiCl at 50°C) with conversion (X). PHPMA were prepared by RAFT polymerization of HPMA at room temperature in DMSO using TTC (1) with various UV lamp intensities: (a) 4 mW/cm^{-2}, (b) 27 mW/cm^{-2}, (c) 45 mW/cm^{-2} and (d) 53 mW/cm^{-2}. $[HPMA]_0/[TTC]_0$=300/1.

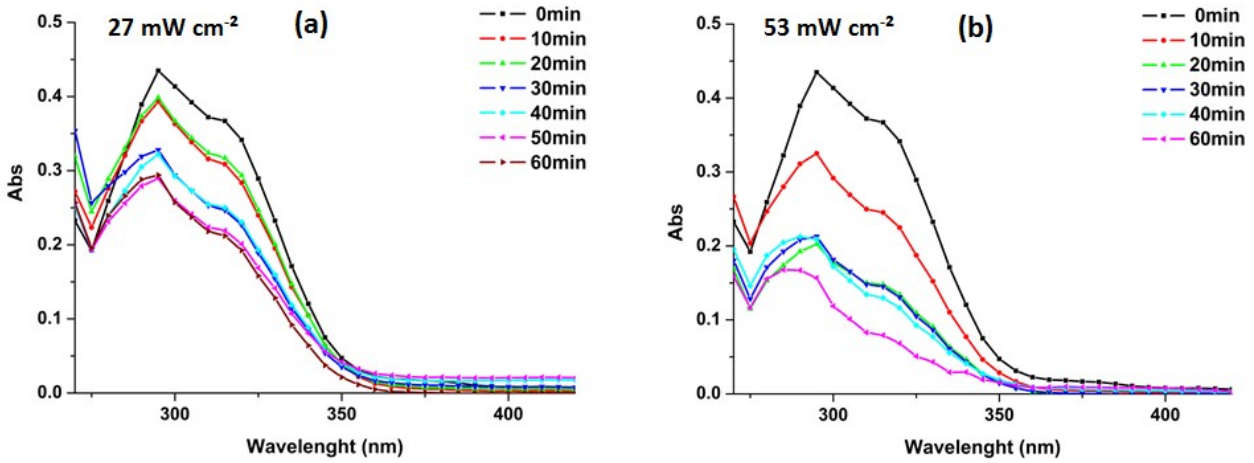

Figure S4. Evolution of UV-Vis absorbance spectra of TTC (1) in DMSO ([TTC]= 0.1 mM) under various UV irradiation intensities. (a)27mW.cm^{-2} and (b) 53mW.cm^{-2}) at different times.

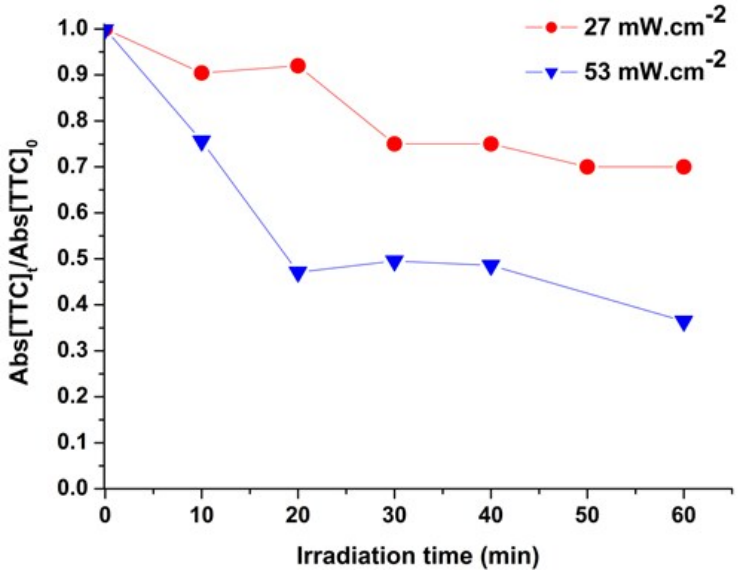

Figure S5. Degradation of TTC (1) in DMSO ([TTC]= 0.1 mM) under various UV irradiation intensities (27mW.cm^{-2} and 53mW.cm^{-2}), using the absorbance peak at 295nm (see figure S4).

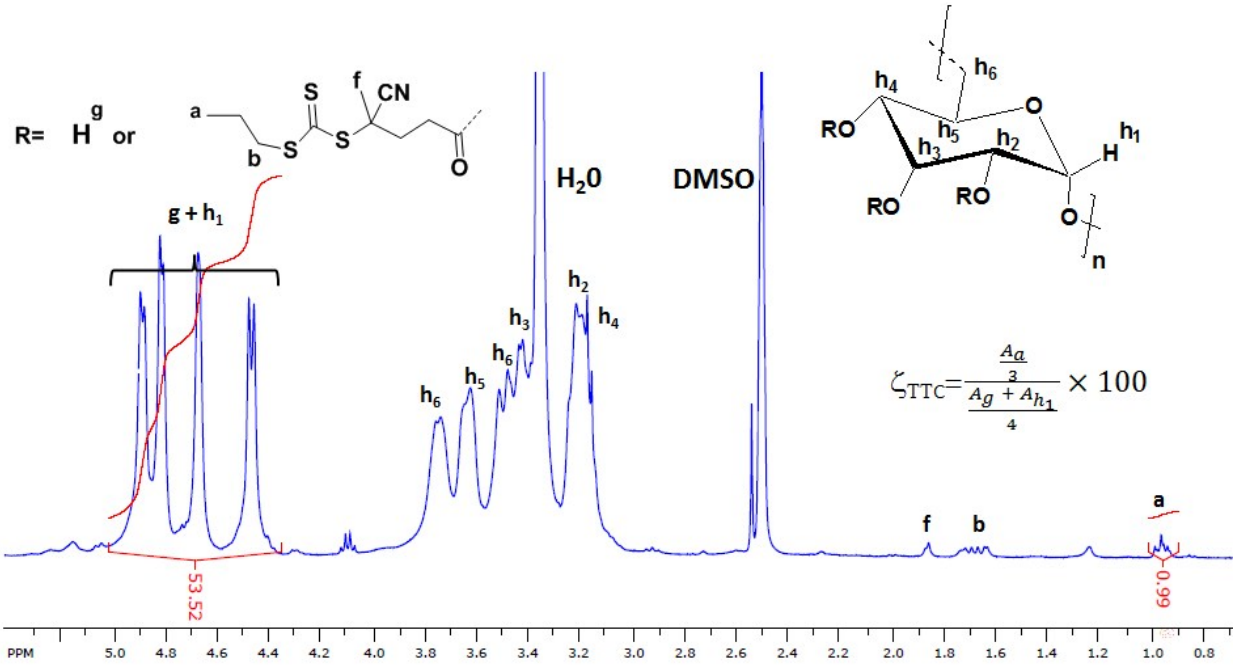

Figure S6. ^{1}H NMR spectrum of Dex-TTC (2). ζ_{TTC} is the number of TTC moieties per 100 glucopyranosic units (here, ζ_{TTC}=2.5).

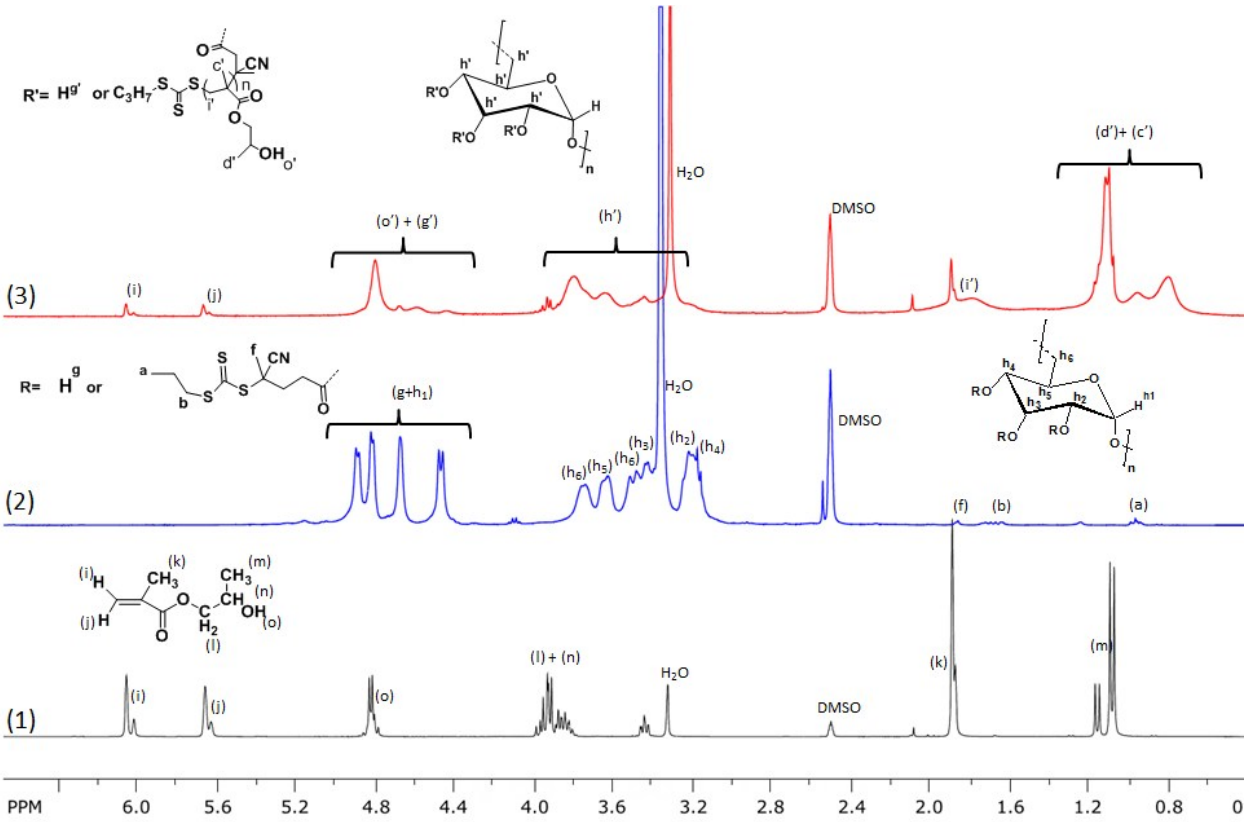

Figure S7. ^{1}H NMR spectra of (1) HPMA monomer, (2) Dex-TTC and crude Dex-g^{5}-PHPMA$_{340}$ glycopolymer prepared by photo-PISA of HPMA in aqueous medium using Dex-TTC as the macro-CTA, at 5% w/w solids content.

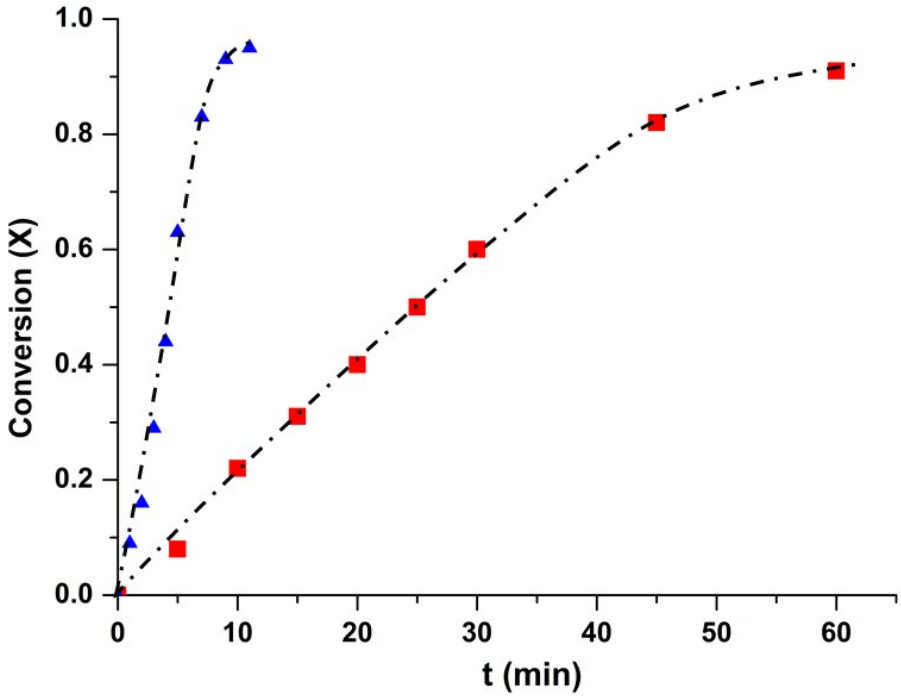

Figure S8. Evolution of conversion with time of aqueous HPMA photo-PISA performed at (■) 5% w/w and (▲)20% w/w solids content using Dex-TTC as the macro-CTA.$[HPMA]_0/[TTC]_0$=190/1.

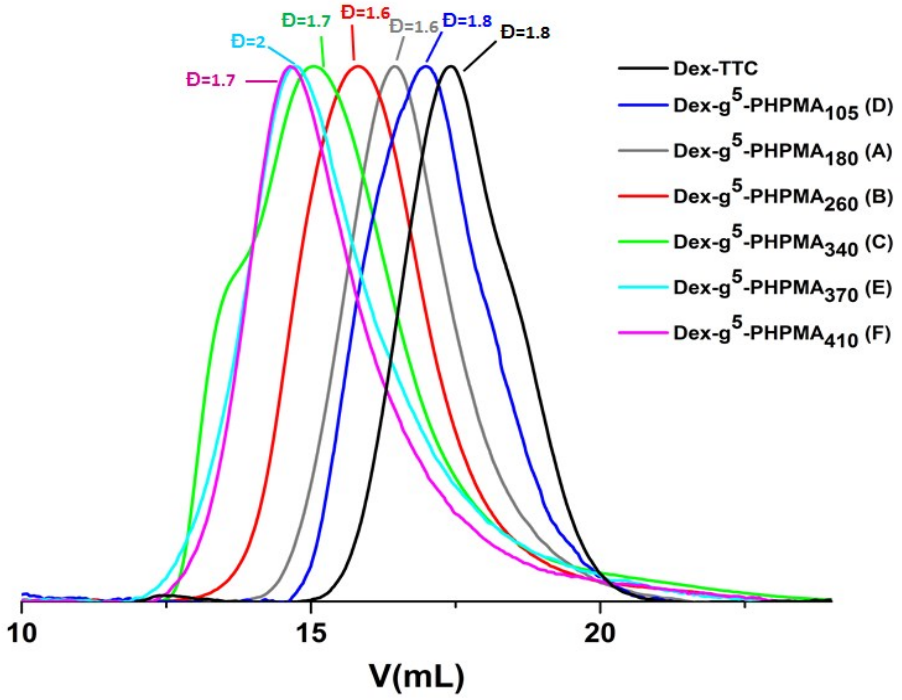

Figure S9. SEC traces of various Dex-g^5-$PHPMA_y$ prepared by aqueous photo-PISA of HPMA using Dex-TTC as the macro-CTA, at a solids concentration of 5% w/w solids content (A, B and C), and at 20% w/w solids content (D, E and F) (table 1).

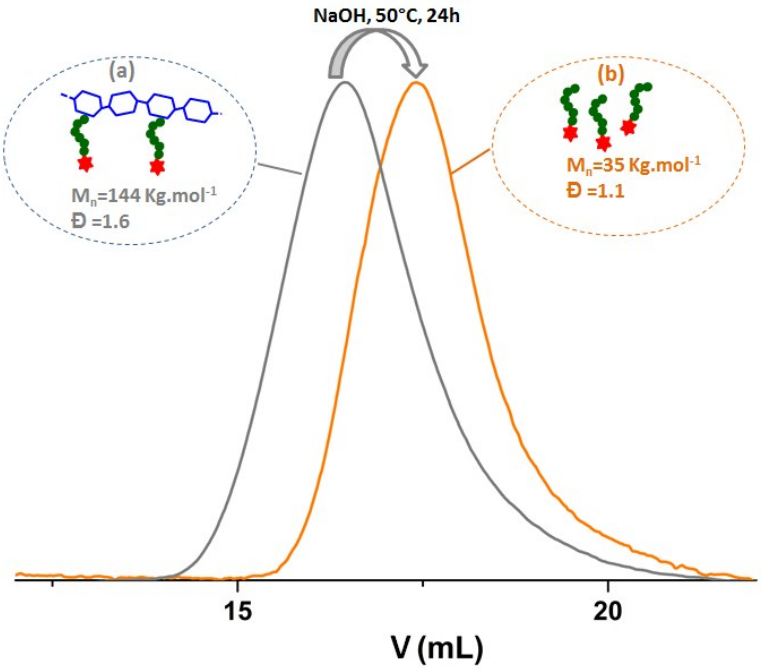

Figure S10. SEC traces of glycopolymers Dex-g^5-PHPMA$_{180}$ (a) and corresponding cleaved PHPMA grafts (b) obtained by treatment in alkaline conditions at 50°C during 24 hours.

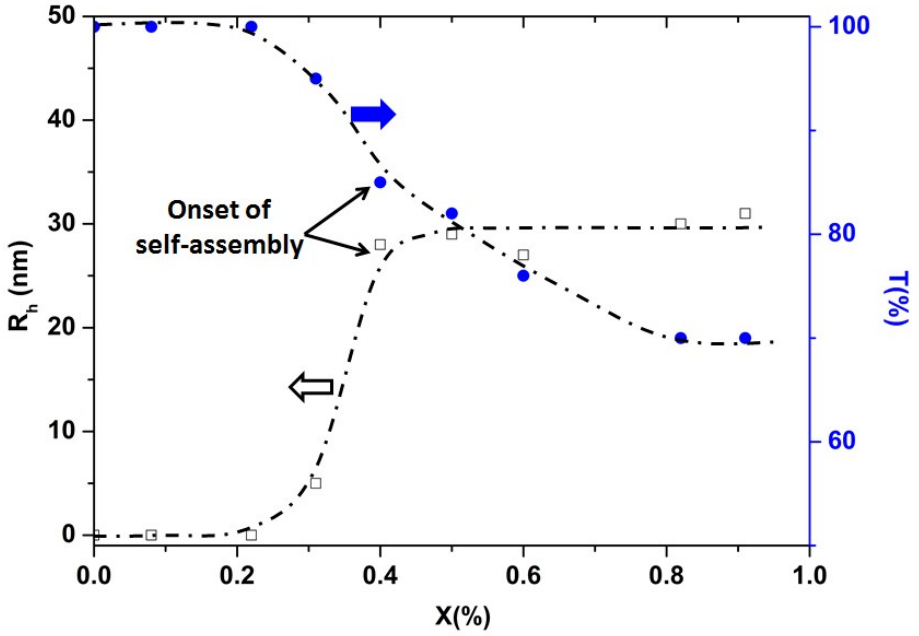

Figure S11. Evolution of (■) hydrodynamic radii (R_h) and (●) transmittance (T%) with time of gluco-nanostructure suspensions prepared via aqueous photo-PISA of HPMA using Dex-TTC as the macro-CTA, at a solids concentration of 5% w/w solids content. $[HPMA]_0/[TTC]_0$=190/1.

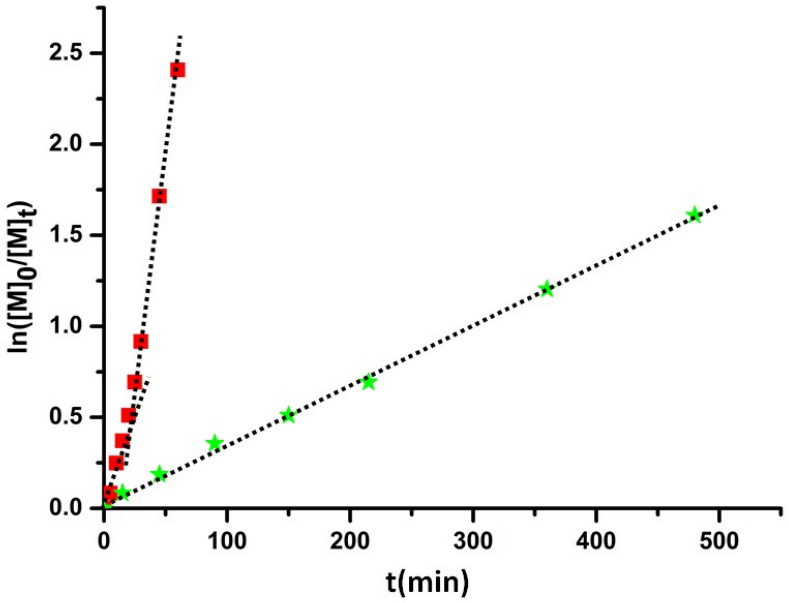

Figure S12. Kinetics of the aqueous HPMA photo-initiated RAFT performed in (■) water and (★) DMSO at 5% w/w solids content using Dex-TTC as the macro-CTA.$[HPMA]_0/[TTC]_0$=190/1.

Table S1. Characteristics of Dex-g^5-$PHPMA_y$ nanostructures prepared by photo-PISA of HPMA in aqueous medium using Dex-TTC as the macro-CTA, at different solids content (HPMA + Dex-TTC).

Entry	Solid Wt(%)	Dex-g^5-$PHPMA_y$	Nano-objects morphologies	R_h[(a)] (nm)	R_{TEM}[(b)] (nm)
A	5 %	Dex-g^5-$PHPMA_{180}$	Micelle	33	21±3
B		Dex-g^5-$PHPMA_{260}$	Mixture of spherical and oblate micelles	45	40±13
C		Dex-g^5-$PHPMA_{340}$	Worm-like micelle	163	225±50[(c)]
D	20 %	Dex-g^5-$PHPMA_{105}$	Micelle	26	16±2
E		Dex-g^5-$PHPMA_{370}$	Worm-like micelle	103	450±50[(c)]
F		Dex-g^5-$PHPMA_{410}$	Vesicle	74	53±7

(a) Hydrodynamic radius obtained from DLS measurements.

(b) Number average radius of nano-objects estimated from TEM images. Statistics were made based on a number of nano-objects between 50 and 100.

(c) Number average length (L) of worm-like nanostructures estimated from TEM images. Statistics were made based on 40 nano-objects.

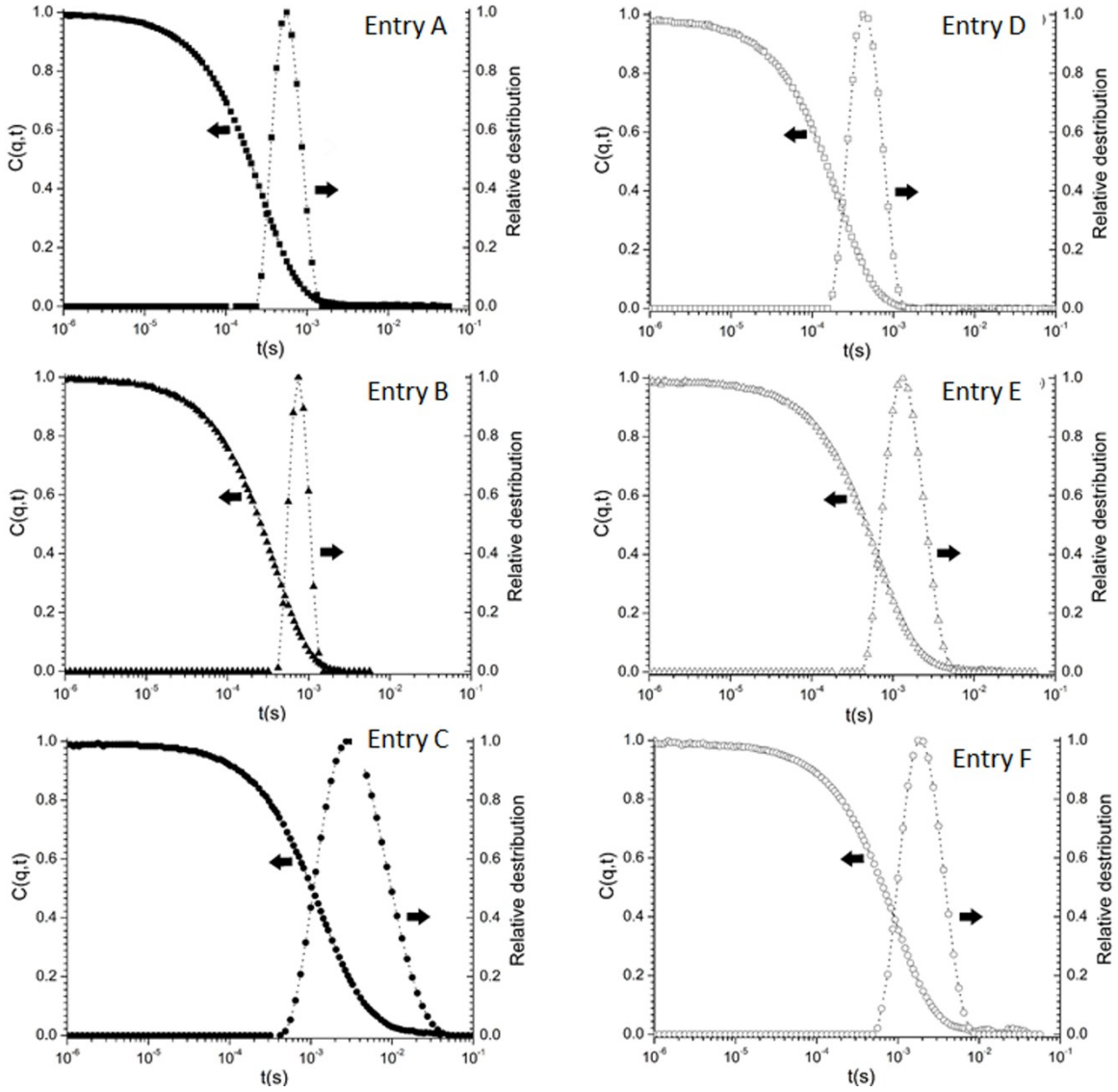

Figure S13. Autocorrelation functions and size distributions recorded at scattering wave vectors q of 1.7×10^7 m^{-1} for different Dex-g^5-$PHPMA_y$ nanostructures prepared by photo-PISA of HPMA in aqueous medium using Dex-TTC as the macro-CTA, at different conditions (see Table S1).

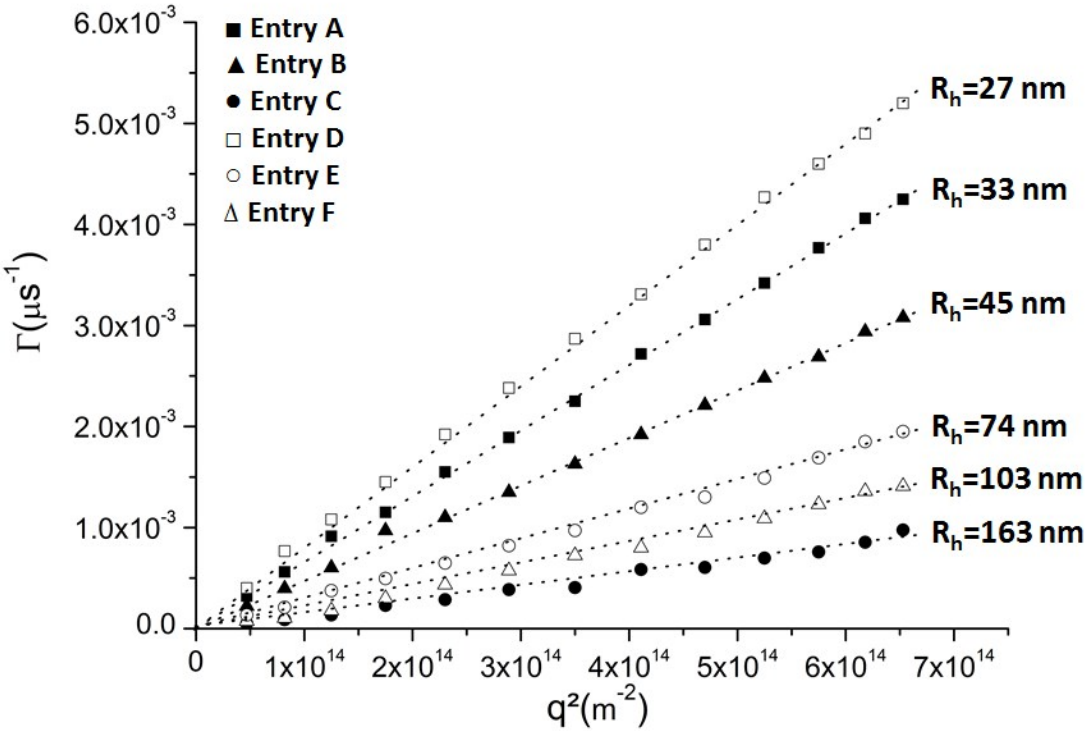

Figure S14. q^2 dependence of the relaxation rate (Γ) for various Dex-g^5-PHPMA$_y$ nanostructures prepared by photo-PISA of HPMA in aqueous medium using Dex-TTC as the macro-CTA, at different conditions (see Table S1).

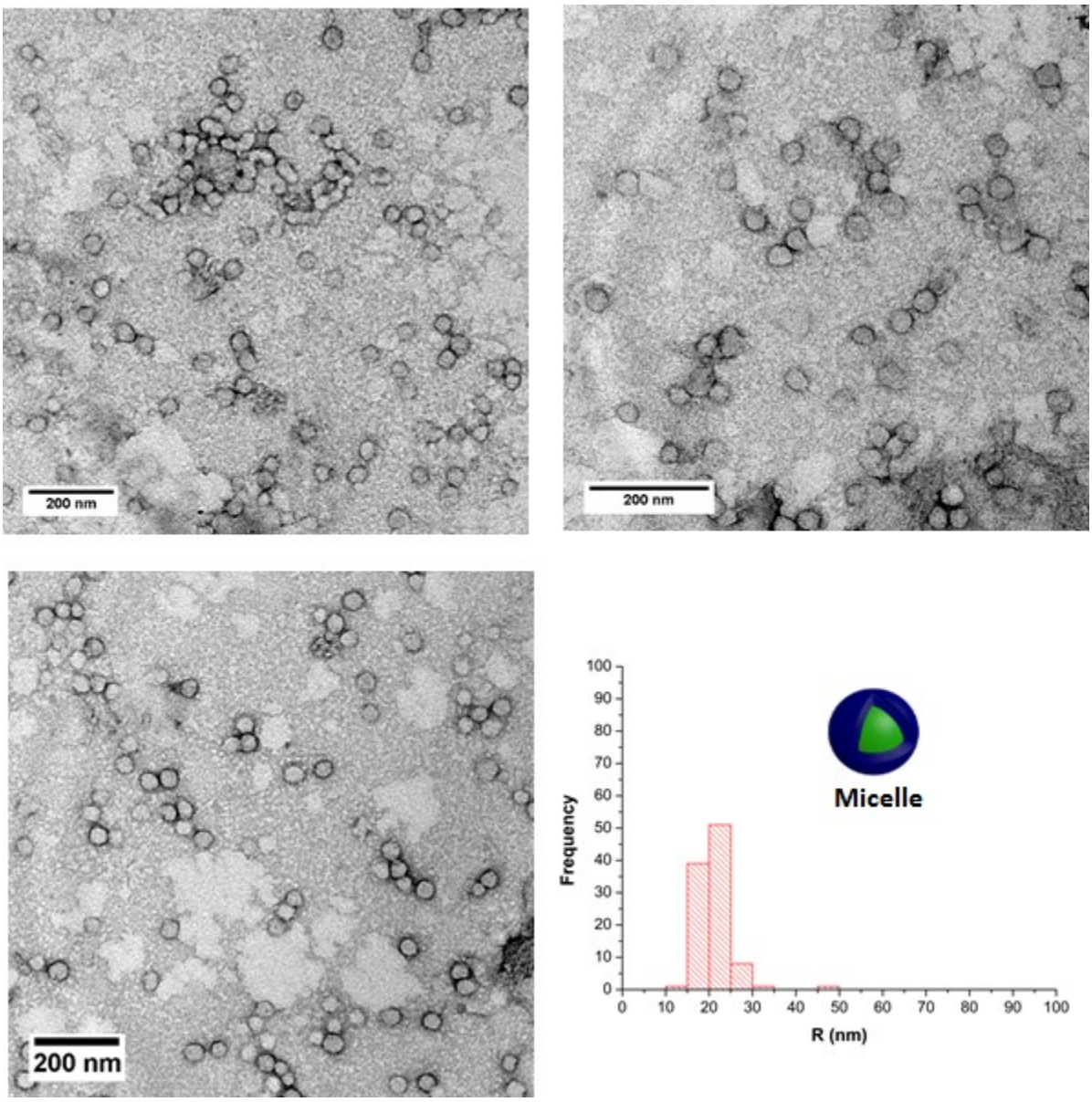

Figure S15. TEM images of Dex-g^5-PHPMA$_{180}$ nanostructures prepared by photo-PISA of HPMA in aqueous medium using Dex-TTC as the macro-CTA, at 5% w/w solids content. The histogram was made from TEM images based on 100 nano-objects.

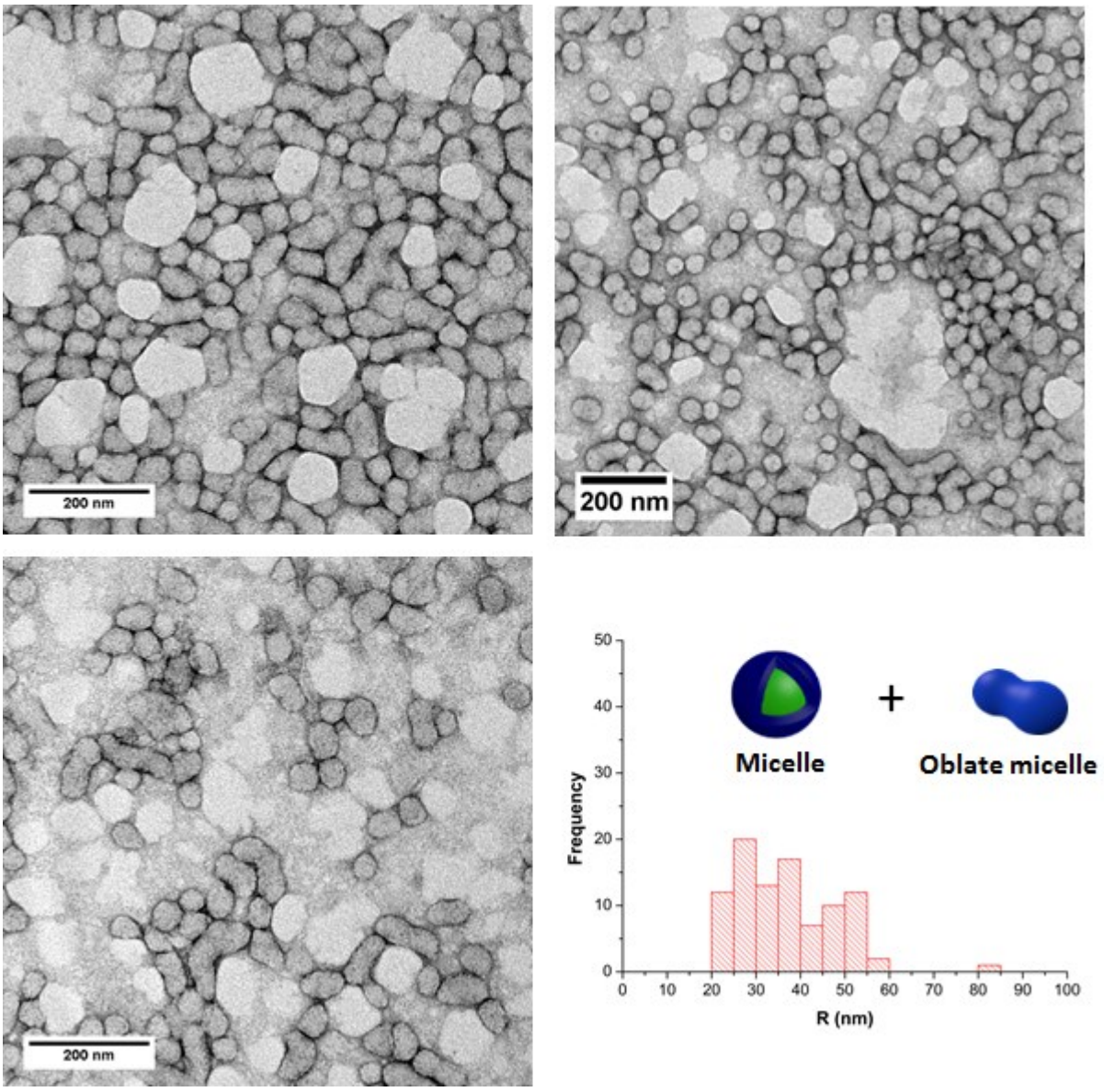

Figure S16. TEM images of Dex-g^5-PHPMA$_{260}$ nanostructures prepared by photo-PISA of HPMA in aqueous medium using Dex-TTC as the macro-CTA, at 5% w/w solids content. The histogram was made from TEM images based on 100 nano-objects.

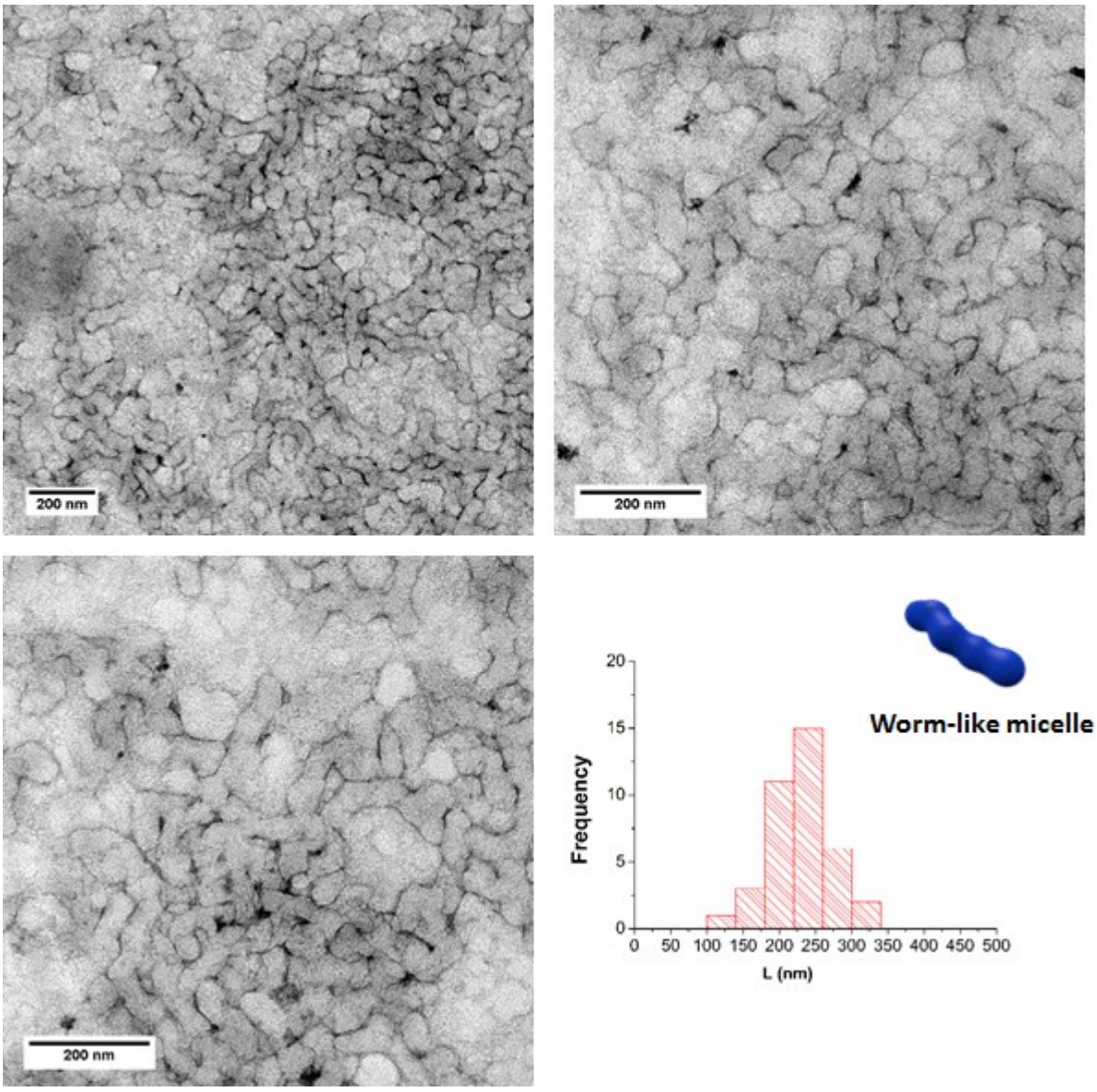

Figure S17. TEM images of Dex-g[5]-PHPMA$_{340}$ nanostructures prepared by photo-PISA of HPMA in aqueous medium using Dex-TTC as the macro-CTA, at 5% w/w solids content.

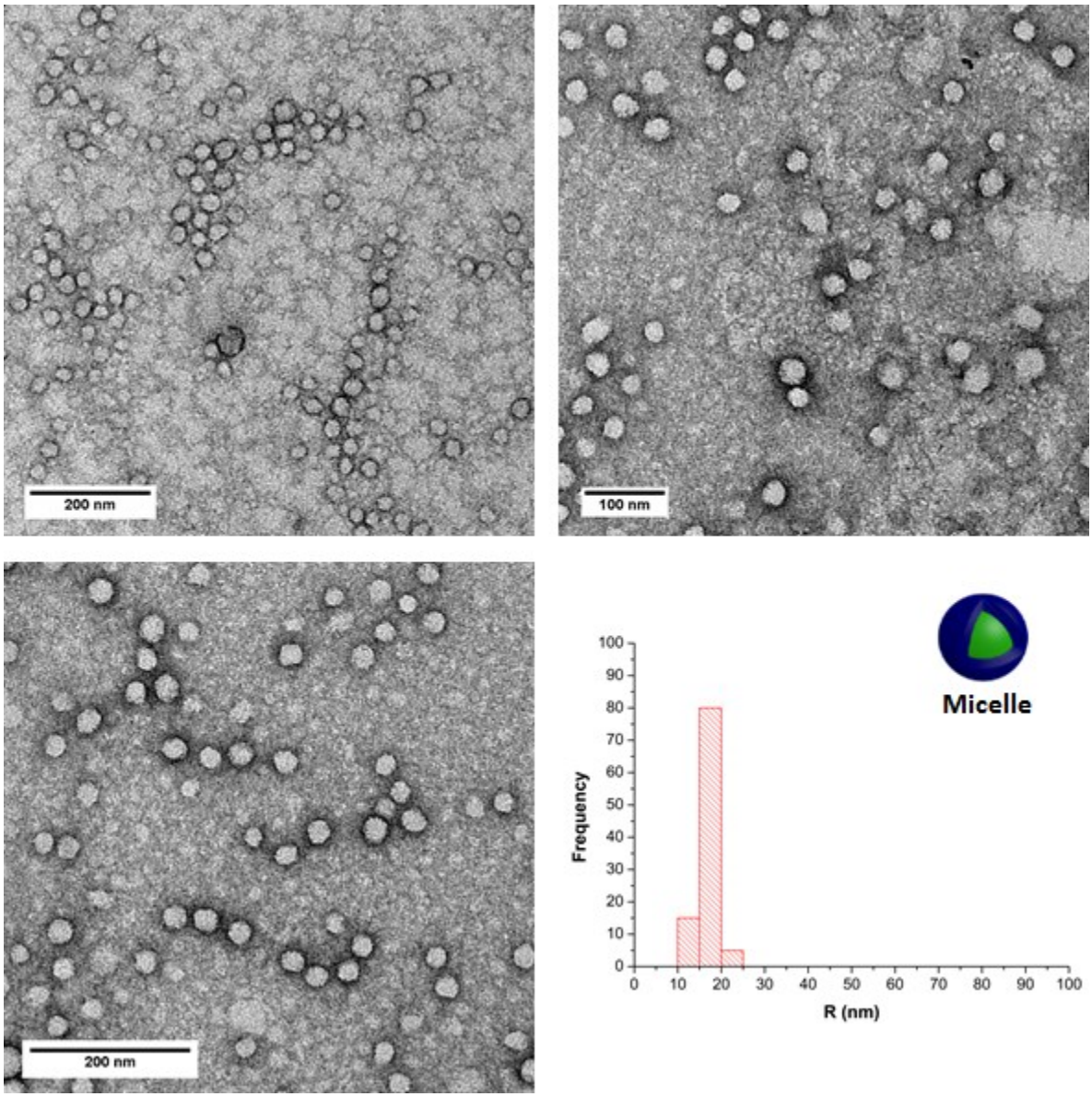

Figure S18. TEM images of Dex-g^{5}-PHPMA$_{105}$ nanostructures prepared by photo-PISA of HPMA in aqueous medium using Dex-TTC as the macro-CTA, at 20% w/w solids content. The histogram was made from TEM images based on 100 nano-objects.

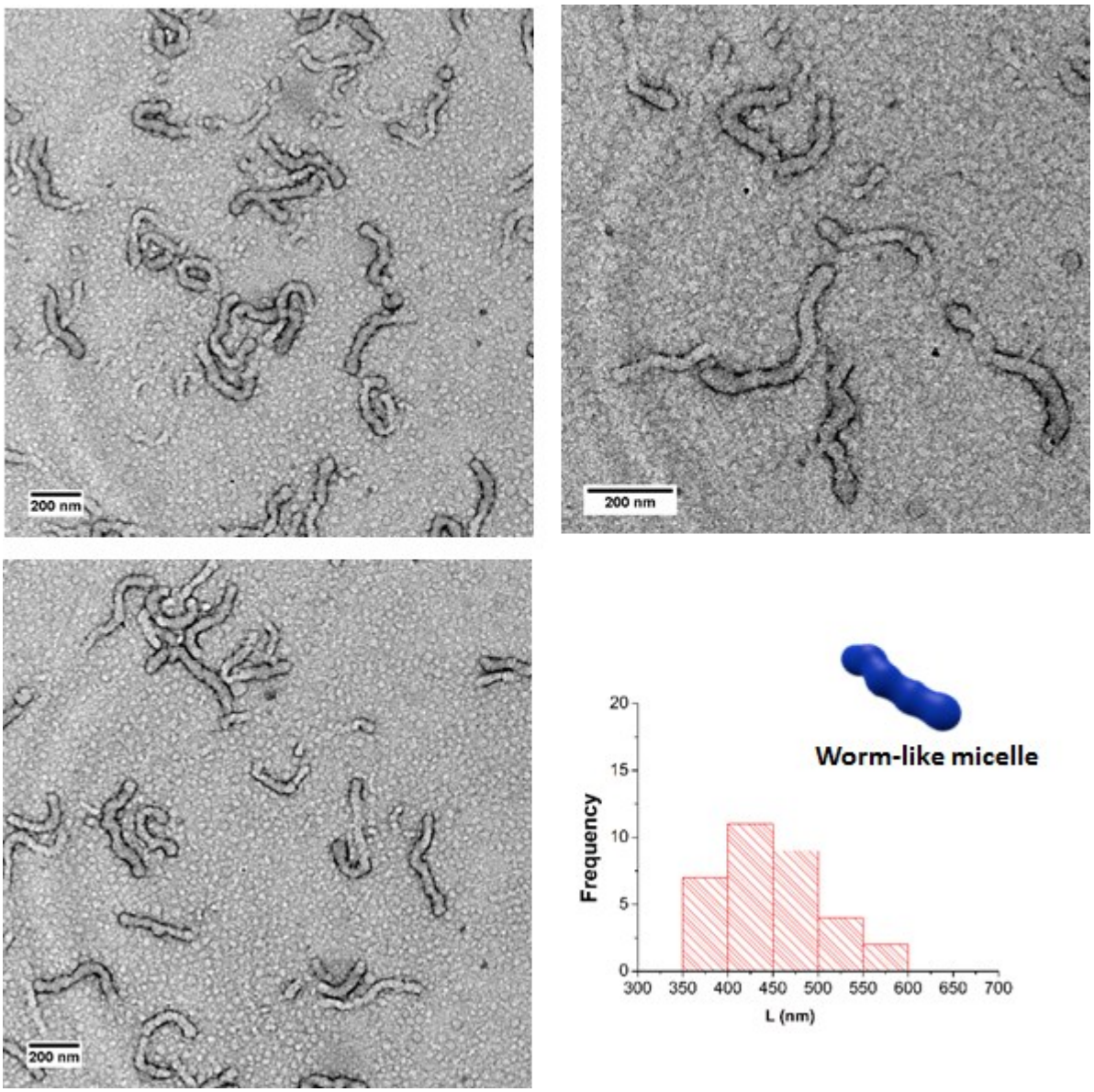

Figure S19. TEM images of Dex-g^{5}-PHPMA$_{370}$ nanostructures prepared by photo-PISA of HPMA in aqueous medium using Dex-TTC as the macro-CTA, at 20% w/w solids content. The histogram was made from TEM images based on length (L) of 40 nano-objects.

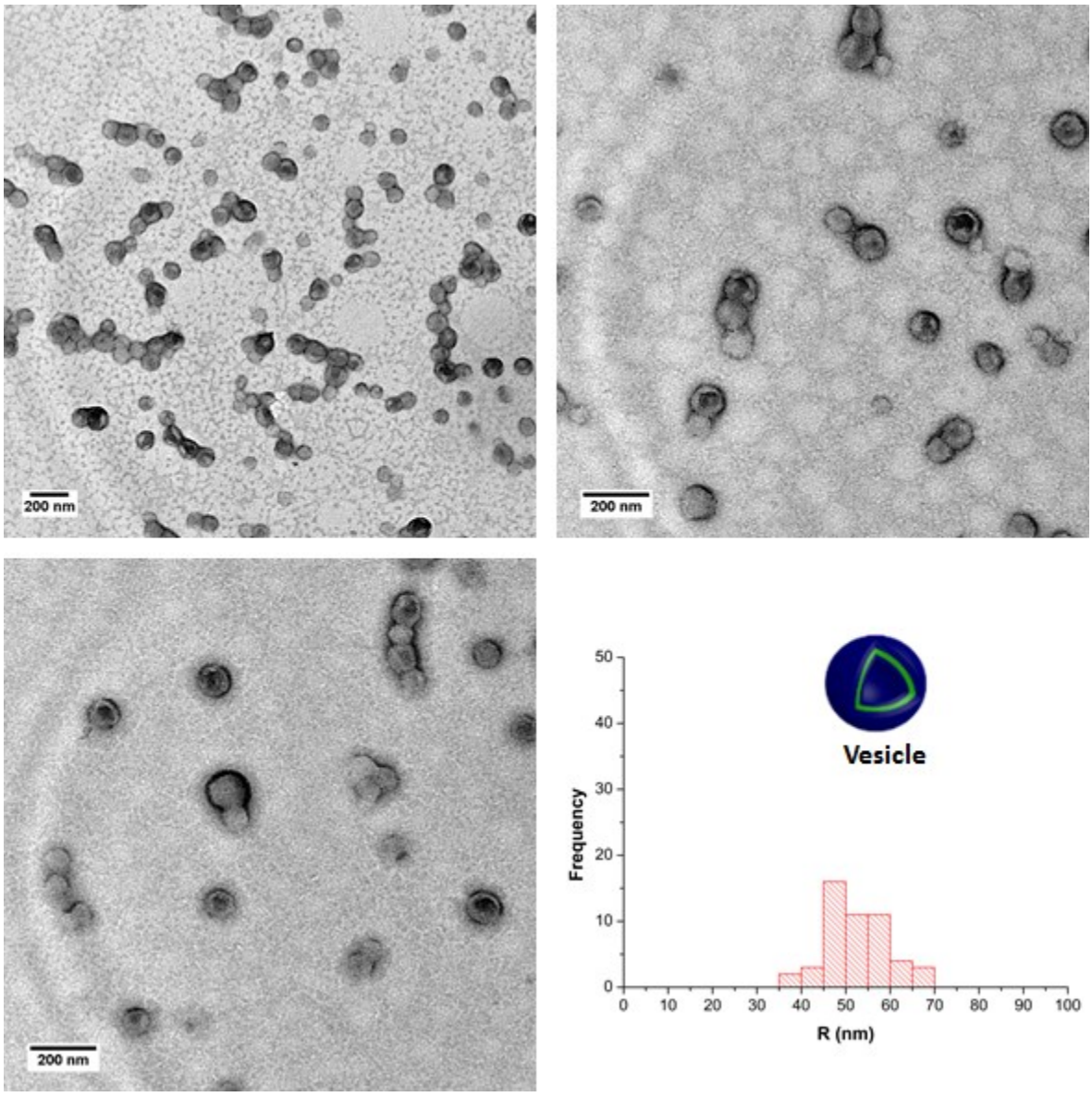

Figure S20. TEM images of Dex-g^5-PHPMA$_{410}$ nanostructures prepared by photo-PISA of HPMA in aqueous medium using Dex-TTC as the macro-CTA, at 20% w/w solids content. The histogram was made from TEM images based on 50 nano-objects.

References

(1) (a) G. Moad, Y. K. Chong, A. Postma, E. Rizzardo, S. H. Thang, *Polymer* 2005**, 46**, 8458; (b) J. A. Alfurhood, H. Sun, P. R. Bachler, B. S. Sumerlin, *Polym. Chem.* 2016, **7**, 2099.

(2) J. Jakes, *J. Collect. Czech. Chem. Commun.* 1995**, 60**, 1781.