Supporting Information

Facile Synthesis of Hybrid Silica Nanocapsules by Interfacial Templating Condensation and Their Application in Fluorescence Imaging

Happy,[a] Nicole Siuyun Liu,[b] Beiping He,[b] Siew Yee Wong,[c] Zhi-Kuan Chen,[c] Xu Li,*[c] and John Wang*[a,d]

[a] NUS Graduate School for Integrative Science and Engineering (NGS), Centre for Life Sciences (CeLS), National University of Singapore (NUS), Singapore 117456.

[b] Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore (NUS), Singapore 117597

[c] Institute of Materials Research and Engineering (IMRE), Singapore 117602

[d] Department of Materials Science and Engineering, National University of Singapore (NUS), Singapore 117574

* To whom correspondence should be addressed.

Experimental

Materials: Block copolymer Pluronic® F127 ($PEO_{106}PPO_{70}PEO_{106}$), tetramethoxysilane (TMOS; 98%), tetrahydrofuran (THF), lipopolysaccharide (LPS), pyrene, indomethacin, phosphotungstic acid (PTA) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Sigma Aldrich. CellTiter 96 AQ_{ueous} Non-Radioactive Cell Proliferation Assay MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) was procured from Promega. 4'-6-Diamidino-2-phenylindole (DAPI) was obtained from Vector Labs. 10 × Phosphate Buffered Saline (PBS) solution was purchased from 1st Base. Fetal Bovine Serum (FBS) was purchased from Hyclone. 1 × PBS was prepared by diluting the 10 × PBS with Milli-Q deionized water. Poly(9,9-dihexylfluorenyl-2,7-diyl) (C6PF), poly(2-(2'-phenyl-4',5'-di(3"-methyl-butoxy)-phenyl-1,4-phenylenevinylene)) (BP-PPV), and poly(2-methoxy-5-(2-ethyl-hexyloxy)-1,4-phenylene vinylene) (MEH-PPV) were synthesized following previous reports [1-3].

Synthesis of hybrid silica nanocapsules: In a typical synthesis, 75 mg of F127 was dissolved into 900 μL THF to form a clear solution. 65 μL of TMOS was then added. The mixture solution was injected into a 10 g of deionized water immersed in water-bath under ultra sonication in three minutes. The solution was further sonicated for ten minutes, followed by stirring at room temperature for four days to evaporate off THF and ensure a complete hydrolysis of TMOS at the interface between the core and corona of the micelles.

Synthesis of fluorescent hybrid silica nanocapsules: A stock solution of MEH-PPV, BP-PPV, and C6PF in THF was prepared by dissolving the respective polymers

in THF to a concentration of 1.0 mg/mL. 75 μL of this stock solution was mixed with 75 mg of F127 in 825 μL of THF to form a clear solution. 65 μL of TMOS was then added. The mixture solution was injected into a 10 g of deionized water immersed in water-bath under ultra sonication in three minutes. The solution was further sonicated for ten minutes, followed by stirring at room temperature for four days to evaporate off THF and ensure a complete hydrolysis of TMOS at the interface between the core and corona of the micelles.

Characterizations: Transmission electron microscopy (TEM, JEM-2010F, 200 kV) was employed to study the morphology of both the as-synthesized hybrid silica nanocapsules and those stained by PTA. Dynamic light scattering (DLS) was performed with Malvern Zetasizer Nano-S using a HeNe laser (633 nm) to measure the nanocapsule size and size distribution. The spectrometer was calibrated by using a standard polystyrene suspension of 60 nm. All measurements were taken using water as the dispersant in glass cuvette. The structure of the hybrid silica nanocapsules was characterized using fourier transform infrared spectroscopy (Perkin-Elmer Spectrum GX). Disc samples were prepared by pressing the mixture of nanocapsules and dried potassium bromide. ^{29}Si NMR characterization was carried out with a Bruker Avance 400 (DRX400) NMR spectrometer at 400 MHz at room temperature. The sample was loaded into 4.0 mm Zirconia PENCIL^{TM} rotors and capped tightly. The ^{29}Si spectrum was recorded at 79.489 MHz with repetition delay 10 s, 35 ms acquisition time, spinning rate of 10,000 rps, and 3000 number of scans. Chemical shift of ^{29}Si NMR was referenced to tetramethylsilane as 0 ppm. Thermogravimetric Analysis was performed by using TGA Q500 Thermal Instrument with a heating rate of 10°C/min to 800°C in air ambient. UV-vis-NIR absorption spectroscopy was measured by using

a Shimadzu UV-3101 PC spectrometer at room temperature. Photoluminescence spectra were measured by using a Shimadzu RF-5301 PC spectrophotometer at room temperature.

Sample preparation for TEM studies: 40 μL of the aqueous suspension containing the as-synthesized hybrid silica nanocapsules was diluted with 200 μL of DI H_2O. A drop of this diluted solution was pipetted directly onto a 200-mesh carbon-coated copper grid. After two minutes, the excess solution was removed by touching the grid edge using a Kimwipe delicate wipe. The sample was finally dried at room temperature.

In order to reveal the PEO chains on the outer surface of the hybrid nanocapsules, the as-synthesized hybrid silica nanocapsules was treated with a phosphotungstic acid (PTA) aqueous solution (1%) for positive staining. The PTA staining has been widely used to improve the contrast of PEO-based block copolymer for TEM observation[4], which otherwise cannot been shown by the conventional TEM as a result of the high transparence to electron beam. Typically, 180 μL of the diluted solution containing hybrid silica nanocapsules was mixed with 20 μL of phosphotungstic acid (PTA) aqueous solution (1%) through a vortex. A drop of this mixture solution was then cast onto a 200-mesh carbon-coated copper grid. After two minutes, the excess solution was removed by touching the grid edge using a Kimwipe delicate wipe. The sample was finally dried at room temperature. As a control, a TEM sample of PTA-stained F127 micelles was also prepared by following the same procedure. The F127 micelles were synthesized in the same way as the hybrid silica nanocapsules without the addition of TMOS.

Antifouling study: F127 micelles and hybrid silica nanocapsules were incubated in PBS containing 10% (v/v) fetal bovine serum (FBS) at 37 °C in water bath, corresponding to physiological condition[5]. FBS was used as a model protein. Dynamic light scattering (DLS) was used to monitor the change in the particle size of the both systems during the incubation period.

Drug release study: A stock solution of indomethacin in THF at the concentration of 8.0 mg/mL was prepared. 937 μL of this stock solution containing 7.5 mg of indomethacin was mixed with 75 mg of F127 to form a clear solution. 65 μL of TMOS was then added. The mixture solution was injected into a 10 g of deionized water immersed in water-bath under ultra sonication in three minutes. The solution was further sonicated for ten minutes, followed by stirring at room temperature for four days to evaporate off THF and ensure a complete hydrolysis of TMOS to synthesize indomethacin loaded hybrid silica nanocapsules. Similarly, indomethacin loaded F127 micelles were prepared in the same way without the addition of TMOS.

The amount of indomethacin loading in the F127 micelles and hybrid silica nanocapsules was determined using UV absorbance at 319 nm. Prior to this, a calibration curve of indomethacin was established by measuring the UV absorbance at 319 nm of different concentrations of indomethacin (0 ~ 260 μg/mL) dissolved in ethanol/THF. The polynomial calibration equation is Concentration (μg/mL) = -0.07488 + 53.23939 × Abs + 0.41169 × Abs^2, R^2 = 0.9999, S.D = 0.04698). The drug loading content was determined to be 8.1 wt%.

In a typical release experiment, a weighed amount of indomethacin loaded F127 micelles or hybrid silica nanocapsules containing 1 mg of indomethacin was sealed inside a dialysis tube (Spectrum, MWCO = 10,000) and placed in a container with 30 mL PBS immersed in water bath at 37°C. 4.0 mL of the release medium was withdrawn at pre-determined time interval and the content of the drug was measured directly with UV absorbance. After the measurement, the release medium was poured back into the container.

Particle stability study: Pyrene loaded F127 micelles and hybrid silica nanocapsules were prepared by the interfacial templating condensation, as described above. The final concentration of pyrene is 1×10^{-5} M. Upon dilution, the fluorescence spectra of pyrene were recorded by using an excitation wavelength (λ_{ex}) of 336 nm. The intensity ratio of the first emission band at 372 nm (I_1) to the third emission band at 384 nm (I_3) is used to determine the location of pyrene in the micelles. A high I_1/I_3 ratio value of ~1.7 indicates that F127 micelles have disintegrated and the pyrene molecules have made contact with water. In contrast, a consistent I_1/I_3 ratio value of 1.4 indicates that F127 micelles are stable and the pyrene molecules are still encapsulated inside the micelles.

Cell culture: BV2 immortalised murine microglial cells were grown in DMEM supplemented with 10% heat inactivated fetal bovine serum (FBS). The cells were routinely subcultured twice a week and incubated at 37 °C in 5% CO_2.

MTS assay for BV2 cell viability: A standard curve was generated by adding 100 μL of BV-2 cells into a 96 well plate with following concentrations: 0.8×10^6, 0.4×10^6,

0.2×10^6, 0.1×10^6, 0.05×10^6, 0.025×10^6. After two hours of settling, 20 μL CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay MTS was added into each well and incubated in 5% CO_2/95% air at 37°C in dark ambient. The optical density of each well was then read and recorded by Tecan Genios. A graph of cell number against optical density was drawn and used as a standard.

BV2 cells were seeded into 96 well plates at a density of 0.07×10^6 cells/mL to monitor the possible toxic effects of BP-PPV hybrid silica nanocapsules. The aqueous suspension of BP-PPV hybrid silica nanocapsules was supplemented with various volumes of 1, 5, 10, 15, and 20 μL into different wells, respectively. The CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay MTS was then added after being cultured for 24 hours to study the changes in cell number.

Cell uptaking of fluorescent hybrid silica nanocapsules: BV2 microglial cells were plated onto cover-slipped 12 well plates at a density of 0.07×10^6 cells/mL for 24 hours. BV2 cells were then stimulated with 1 μg/mL of LPS for another 24 hours prior to the addition of 20 μL aqueous suspension of BP-PPV hybrid silica nanocapsules. After being cultured for 24 hours, the well was washed three times with 1 × PBS, fixed with 4% paraformaldehyde for 15 minutes, and washed another three times with 1 × PBS to remove any trace of paraformaldehyde. Coverslips were then mounted onto glass slides using Vectashield mounting medium with DAPI. The cells were then imaged by a confocal laser scanning microscope (Olympus Fluoview 1000). DAPI fluorescent image was acquired at λ_{ex} = 345 nm and λ_{em} = 458 nm, while BP-PPV hybrid silica nanocapsules fluorescent image was acquired at λ_{ex} = 494 nm

and λ_{em} = 518 nm. The transmitted image of BV-2 cells was taken using differential interference contrast (DIC) technique.

References:

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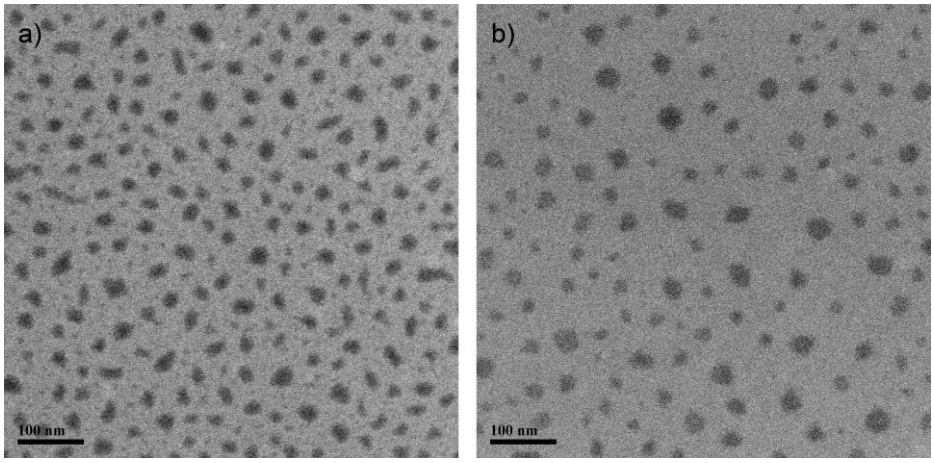

Fig. S1 TEM images of PTA treated: a) pure F127 micelles, and b) hybrid silica nanocapsules. The F127 micelles were prepared in the same way as the hybrid silica nanocapsules without the addition of TMOS. Both samples exhibit similarly spherical morphology, which is the typical micelle morphology. Their particle sizes are in the range of 25 to 30 nm for both F127 micelles and hybrid silica nanocapsules. The enhanced contrast and enlarged particle size in S1(b), as compared to those in Fig 1(a) is due to the positive staining by PTA. Each of the hybrid silica nanocapsules consists of a PPO core, a thin silica shell and an outer layer of PEO chains. Both PPO and PEO are highly transparent to electron beam in the conventional TEM, and therefore, a proper image contrast cannot be generated for the PEO outer layer. In order to reveal the PEO outer layer (PEO chains dangling on the silica shell outer surface), which is hydrophilic, PTA positive staining is used in the present work. Upon staining by PTA, the enhanced contrast shows the overall particle size for both F127 micelles and hybrid silica nanocapsules. Indeed, the two systems show similar sizes, as illustrated above. The size difference between the stained hybrid silica nanocapsules (Fig 1b and S1b) and those of unstained ones (Fig 1a) agrees well with the expected thickness of the PEO outer layer in the hybrid silica nanocapsules. The TEM studies are also consistent with what has been shown by the DLS measurement, where a larger size (29 nm) is measured, which is larger than what was observed by TEM for the as-synthesized hybrid silica nanocapsules (unstained). These size studies confirm that the thin silica shell is formed at the interface between the core and corona of F127 micelles, leaving part of the PEO chains free in the aqueous solution. The existence of the PEO outer layer on the surface of silica shell is clearly demonstrated by the size difference between the unstained hybrid silica nanocapsules and those stained by PTA.

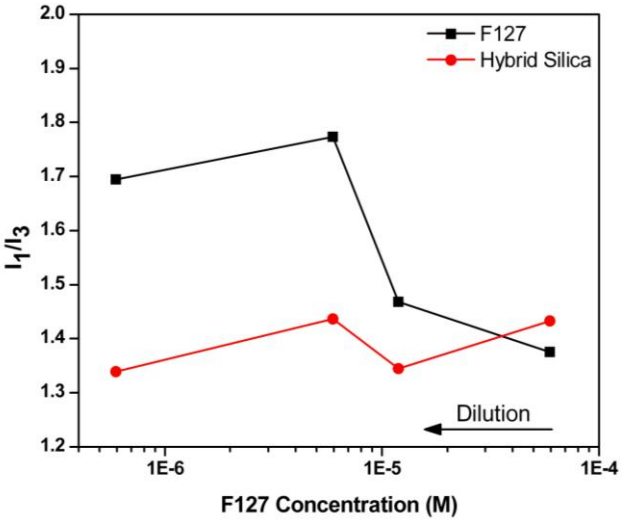

Fig. S2 The fluorescence intensity ratio of pyrene, I_1/I_3, as a function of F127 concentration. The concentration of F127 was adjusted through dilution against DI H_2O. The I_1/I_3 ratio of hybrid silica nanocapsules is relatively stable at about 1.4, while the F127 micelles increases to 1.7 after dilution for 10 times. This result demonstrates that the silica shell has been formed surrounding the core of F127 micelles and it is effective in preventing dissociation of the micelles upon dilution. Otherwise, the I_1/I_3 would increase rapidly as in the case of pure F127 micelles.

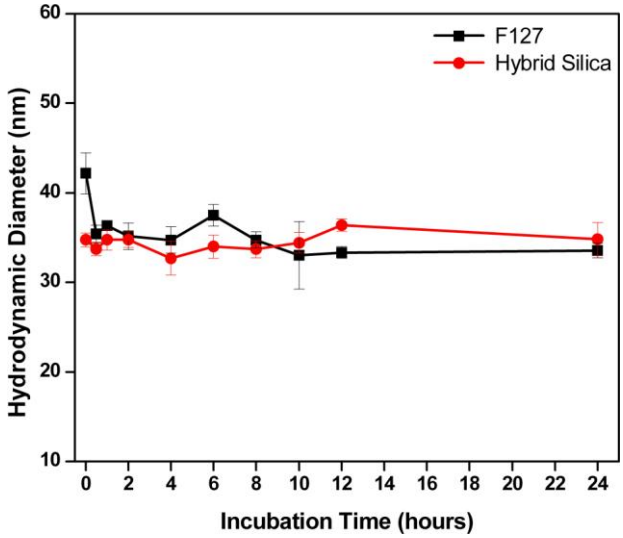

Fig. S3 Change of hydrodynamic diameter of the pure F127 micelles and hybrid silica nanocapsules measured as a function of time upon incubation in PBS containing 10% FBS at 37°C. FBS was used as a model protein. The measured sizes are relatively stable upon incubation up to 24 hours. The excellent stability of the suspension without particle growth indicates the presence of free PEO chains on the surface of the hybrid silica nanocapsules, as in the case of pure F127 micelles.

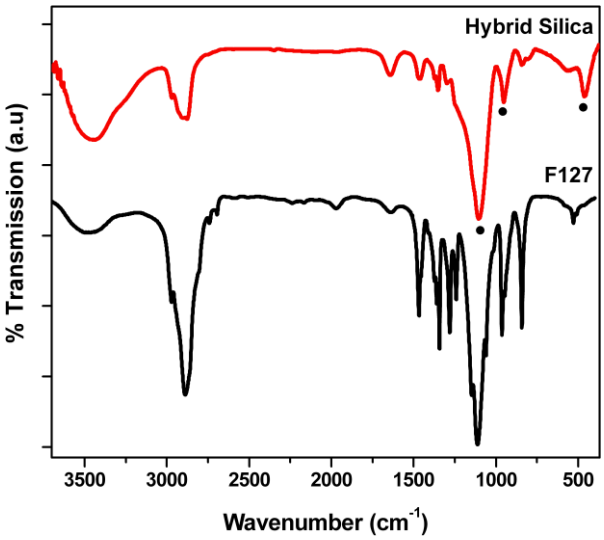

Fig. S4 FT-IR spectrum of pure F127 and hybrid silica nanocapsules. The spectrum of hybrid silica nanocapsules shows the characteristics band of both F127 block copolymers and silica. Filled circle (•) shows the silica vibrational bands at 465 cm^{-1} (Si-O-Si bending), 956 cm^{-1} (Si-OH stretching) and 1100 cm^{-1} (Si-O-Si antisymmetric stretching)[6].

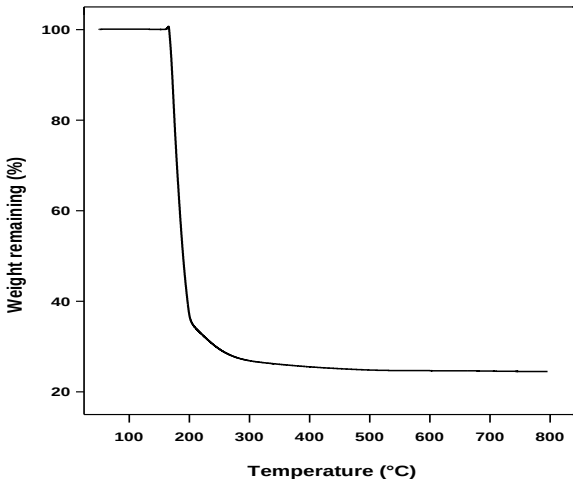

Fig. S5 Thermogravimetric analysis of the hybrid silica nanocapsules showing a residue of weight of 24.5 wt% corresponding to the weight of SiO_2. The weight residue is in agreement with the theoretical calculation of converting TMOS precursor to SiO_2.

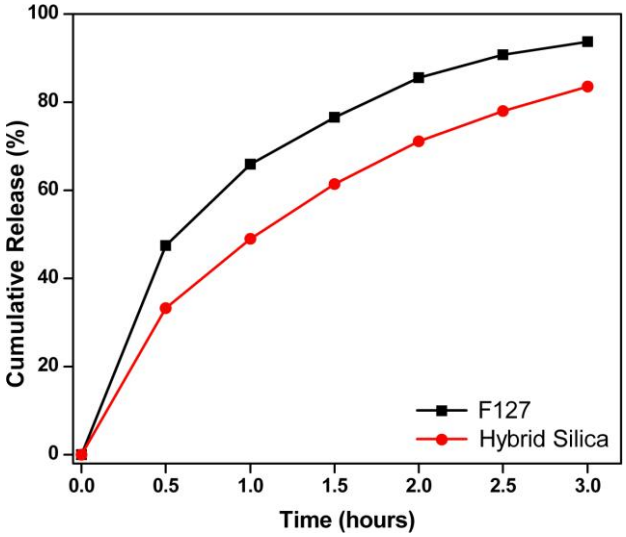

Fig. S6 Cumulative release profiles of indomethacin loaded F127 micelles (■) and hybrid silica nanocapsules (•) in PBS at 37 °C.

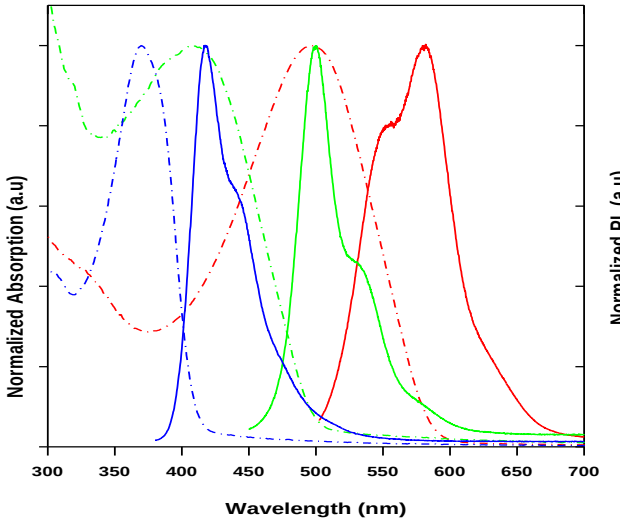

Fig. S7 Normalized UV-Vis absorption (broken lines) and PL (solid lines) spectra of aqueous suspension of the fluorescent hybrid silica nanocapsules encapsulated with C6PF (blue), BP-PPV (green), and MEH-PPV (red).

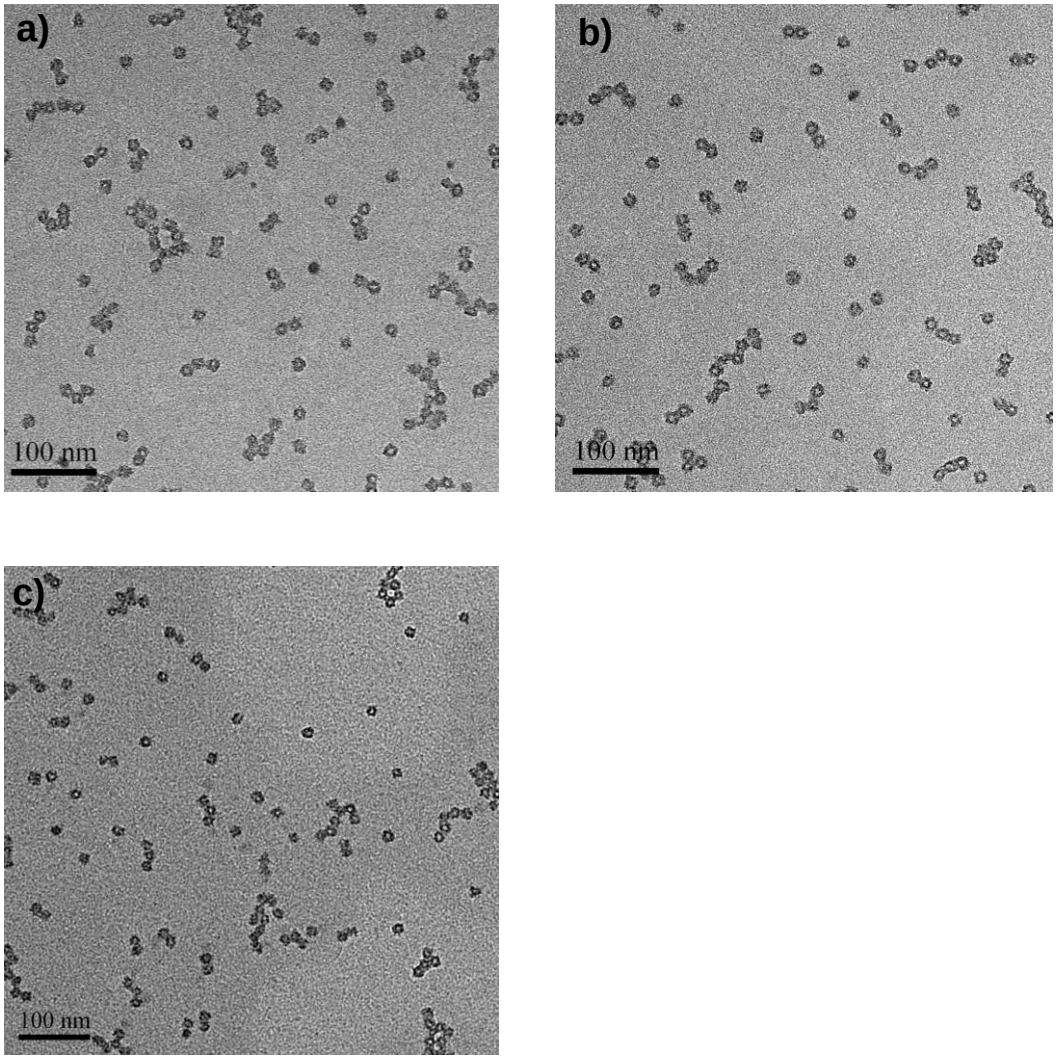

Fig. S8 Transmission electron micrographs of three fluorescent hybrid silica nanocapsules encapsulated with (a) C6PF, (b) BP-PPV, and (c) MEH-PPV, respectively.

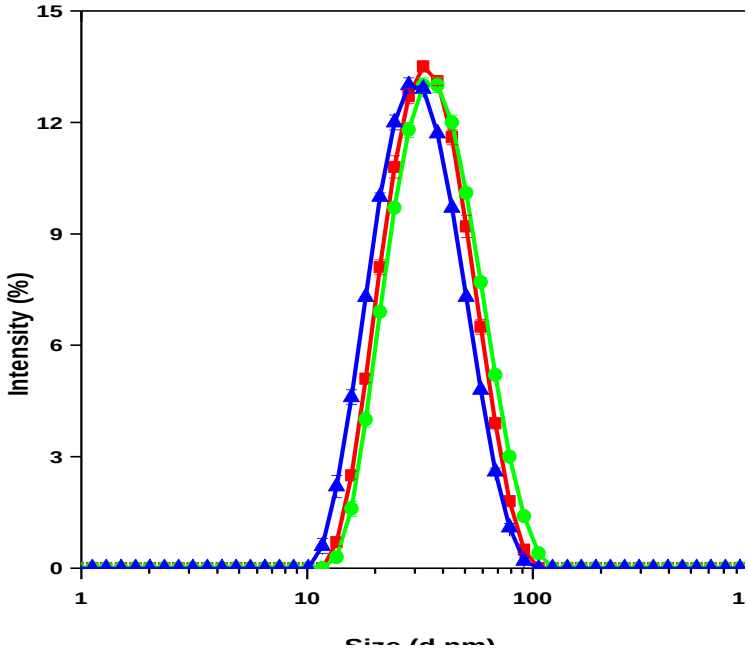

Fig. S9 Particle size distribution of the fluorescent hybrid silica nanocapsules encapsulated with C6PF (blue), BP-PPV (green), and MEH-PPV (red) measured by using dynamic light scattering (DLS), respectively.

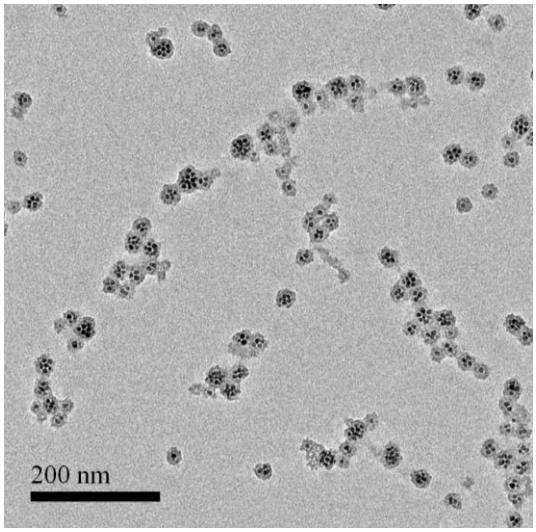

Fig. S10 Transmission electron micrograph of the iron oxide (Fe_3O_4) loaded hybrid silica nanocapsules synthesized by the interfacial templating condensation. The hydrophobic iron oxide nanoparticles were prepared using high temperature non-hydrolytic process and suspended in THF. Iron oxide nanoparticles appear darker since they have higher electron density than silica. Furthermore, the hybrid nanocapsule size varies with the size/number of the iron oxide nanoparticles encapsulated. Since iron oxide nanoparticles are more rigid as compared to fluorescent conjugated polymers, they are generally larger than the fluorescent hybrid silica nanocapsules encapsulated with C6PF, BP-PPV, or MEH-PPV.