Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supporting Information

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Materials:

Amphiphilic polymer PS-PEG-OH (main chain M_w= 6,000, graft chain M_w= 4,000, total chain M_w= 21,000, polydispersity = 1.24) was purchased from Polymer Source (USA), EDC, *N*-hydroxy succinimide, THF and chloroform were obtained from Spectrochem (India), THF was further dried over metallic sodium following the standard protocol. Dulbecco's Modified Eagles Medium (DMEM), Fetal Bovine Serum (FBS), Bovine Serum Albumin (BSA) and folic acid were purchased from Sigma-Aldrich (India), Water used in this study was of MilliQ grade obtained from Millipore purification system.

Instrumentation:
Steady-state absorption and fluorescence measurements:

Steady-state absorption measurement was performed using Cary 5000 Spectrophotometer from Agilent Technologies using 1 cm path length quartz cuvette. All steady-state fluorescence measurements were carried out using HORIBA Jobin Yvon Fluorolog fluorimeter using Origin 8 software provided with the instrument. A dilute solution of the sample was taken for all the measurements to keep the absorption value at such that we can avoid inner filter effect. Fluorescence spectra were recorded using 1 cm path length quartz cuvette and adjusting both excitation and emission slit width accordingly. All the experiments were carried out at room temperature (298 K).

Time-resolved fluorescence measurements:

Time-resolved fluorescence measurements were performed the time-correlated single photon counting (TCSPC) setup from Horiba Jobin Yvon. The instrument response function (IRF) was measured before and after fluorescence lifetime measurement using a dilute suspension of Ludox (purchased from Sigma) colloidal silica. The pico-second pulsed laser was used as the excitation source. The emission polarizer was positioned at magic angle with respect to excitation polarizer. The exponential fitting function was employed by iterative deconvolution method using supplied software DAS v6.2. The quality of the fitted data was judged from the reduced chi-square value (χ^2), calculated using software provided with the instrument. All measurements were carried out at room temperature (298K).

Microscopy:

For live-cell wide-field fluorescence imaging, we have used IX83 inverted fluorescence microscope from Olympus (Japan). The solution of PMIAP doped PDot was drop casted on a glass cover-slip and coated with Gold by sputter coating for 2 min. They were visualized under a scanning electron microscope from Carl Zeiss at a working voltage of 5.0 kV. For AFM, the sample was drop-casted on a freshly cleaned mica surface and air-dried before imaging. The images were obtained by scanning the mica surface in non-contact mode.

Folic acid conjugated polymer synthesis:[1]

Folic acid (4.41 mg, 0.01 mmol) was stirred in 2.5 mL of dry DMSO until it gets dissolved, then EDC (2.0 mg, 0.012 mmol) and *N*-hydroxy succinimide (1.4 mg, 0.012 mmol) were added sequentially to it under stirring condition for 30 minutes at room temperature under inert (N_2 atmosphere) condition. PS-PEG-OH (50 mg) was added to it and stirred for another 24 h at room temperature under inert condition. Resultant solution was placed into a dialysis bag (6 kDa cut off) and dialyzed in 2 L of bicarbonate buffer (pH 9.0) for 12 h (changed over

every 4 h) and then in distilled water for 24 h with change over every 12 h. Finally, the leftover was lyophilized to get the desire FA-conjugated PS-PEG product (PS-PEG-FA).

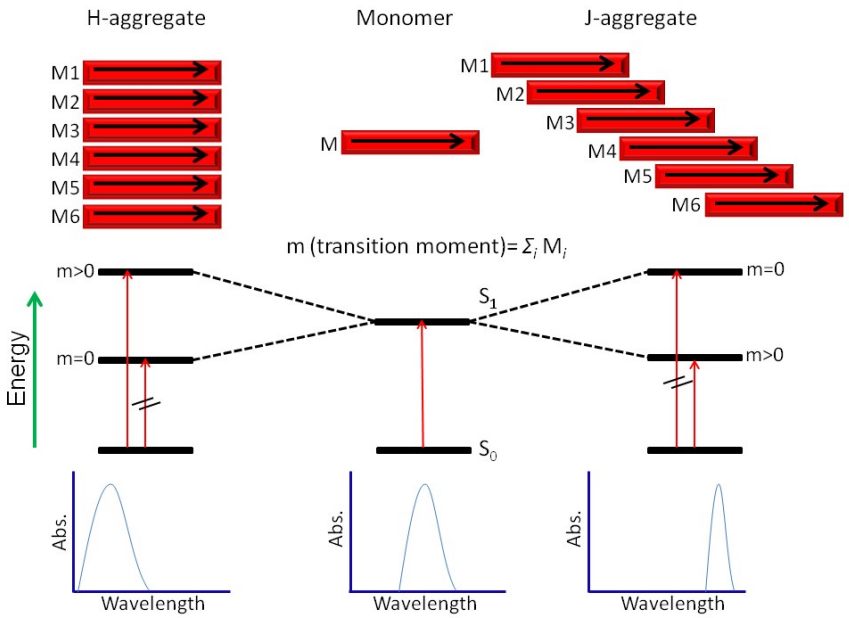
Dye-doped PNP synthesis and characterization:

PMIAP doped PNP was synthesized using nanoprecipitation method.[2] Solution of PS-PEG-OH (PNP)/PS-PEG-FA (PNP-FA) (1 mg/ mL) and dye (0.1 mg/ mL) in dry THF was taken as the stock solution. Then both of them was taken in 2.0 mL of dry THF to give a concentration of polymer 50 μg/ mL and PMIAP was added according to the w/w %. Then the mixture was sonicated at 40 MHz for 5 min in a bath sonicator filled with water. Now, 2.0 mL above mixture was quickly injected into 8.0 mL Milli-Q water under sonication and sonication was continued for another 5-7 min. THF was removed at 25 ^{o}C in the dark using a rotary evaporator. The solution was filtered using a syringe filter of pore size 0.2 μm and concentrated further to 2 mL using a 10 kDa cutoff filter. The synthesized PS-PEG-FA was characterized using FTIR and UV-Vis. spectroscopy.

Cell imaging:

HeLa and MCF-7 cells were grown in a cell culture flask with DMEM with 10% FBS and antibiotic cocktail. In a humidified atmosphere of 5% CO_2 + 95% air at 37 ^{o}C. For the microscopy experiments, cells were grown to 60-70 % confluency in a glass bottom imaging dish. The cells were washed thrice with PBS buffer of pH 7.4 (with 5 mM of $MgCl_2$). For FA competition assay, cells were treated with 10 μM of FA for 1 h before incubated with dye-doped PNP for another 1 h for all cases. Incubation was done inside the CO_2 incubator in PBS buffer of pH 7.4 (with 5 mM of $MgCl_2$). After incubation, cells were washed thrice with same PBS buffer and imaged using a wide-field fluorescent microscope.

Cytotoxicity (MTT assay): Around 10,000 of HeLa cells per well were seeded in a 96 well-plate and grown for 24 h in DMEM medium with 10% serum condition. After that PNP-FA were added to get the desired concentration. After 24 h of incubation 20 μL (5 mg/ mL in PBS buffer) of MTT dye solution was added to each well and incubated for 4 h. The media was removed gently from the well and 200 μL of DMSO to each well and allowed to dissolve the color crystal. Later, the absorption at 570 nm was recorded using microplate reader. All the measurements were performed in triplicate.



Scheme S1. A schematic representation of the orientation of chromophores in monomeric and H and J-aggregated state and their consequence on the energy states, transition probability, and absorption spectra.

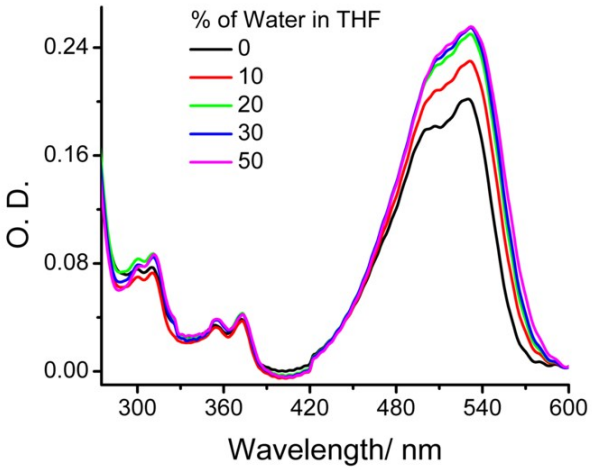


Figure S1. Absorption spectra of 10 μM of PMIAP in THF with increasing amount (v/v %) of water.

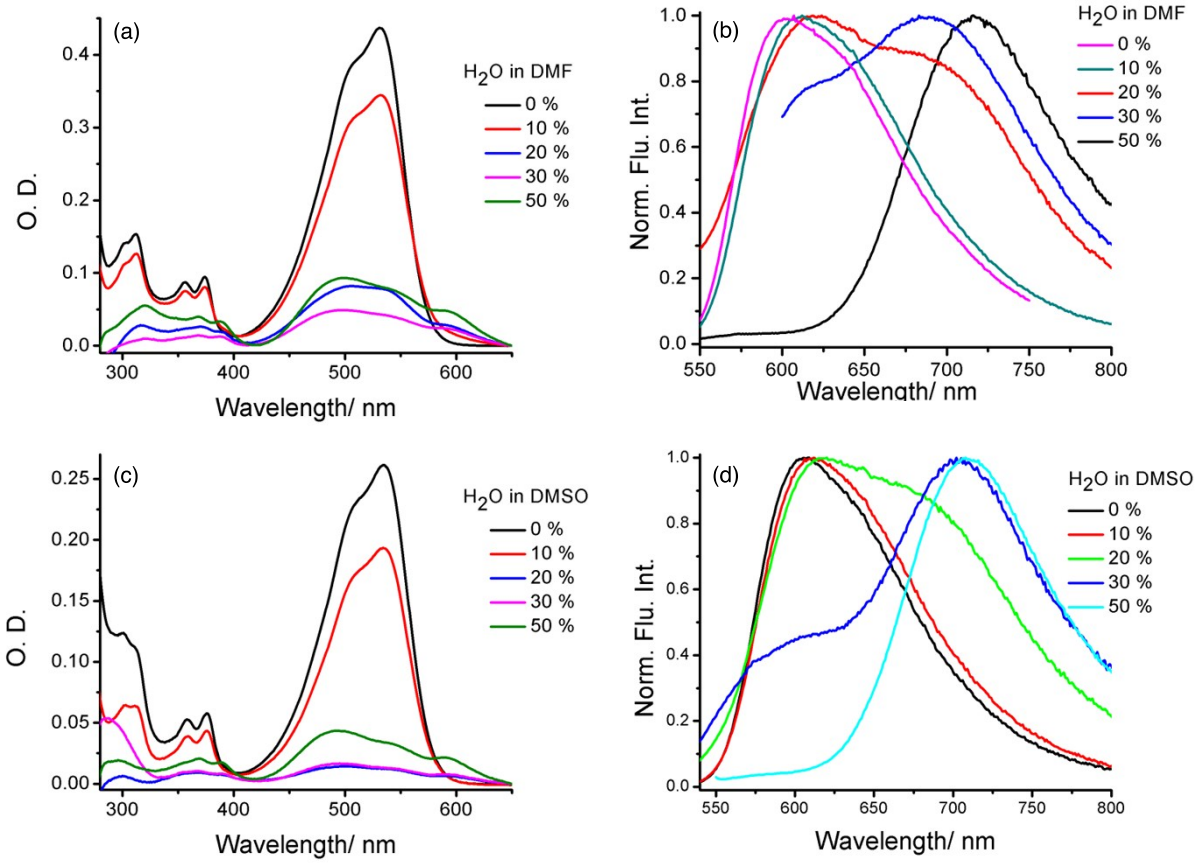


Figure S2. Absorption and emission spectra of 10 μM of PMIAP in DMF (a, b) and DMSO (c, d) with increasing amount (v/v %) of water.

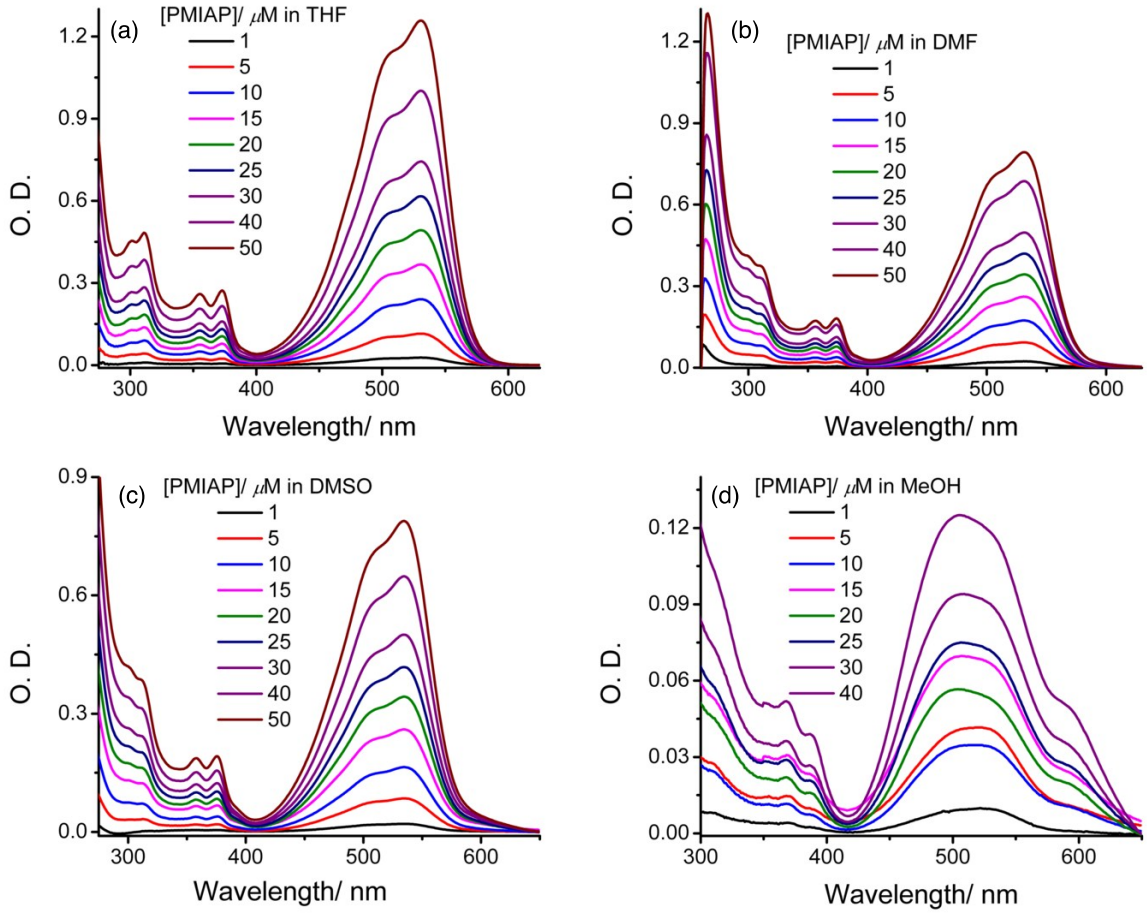


Figure S3: Investigating the concentration effect of PMIAP on the absorption spectra in THF, DMF, DMSO and MeOH

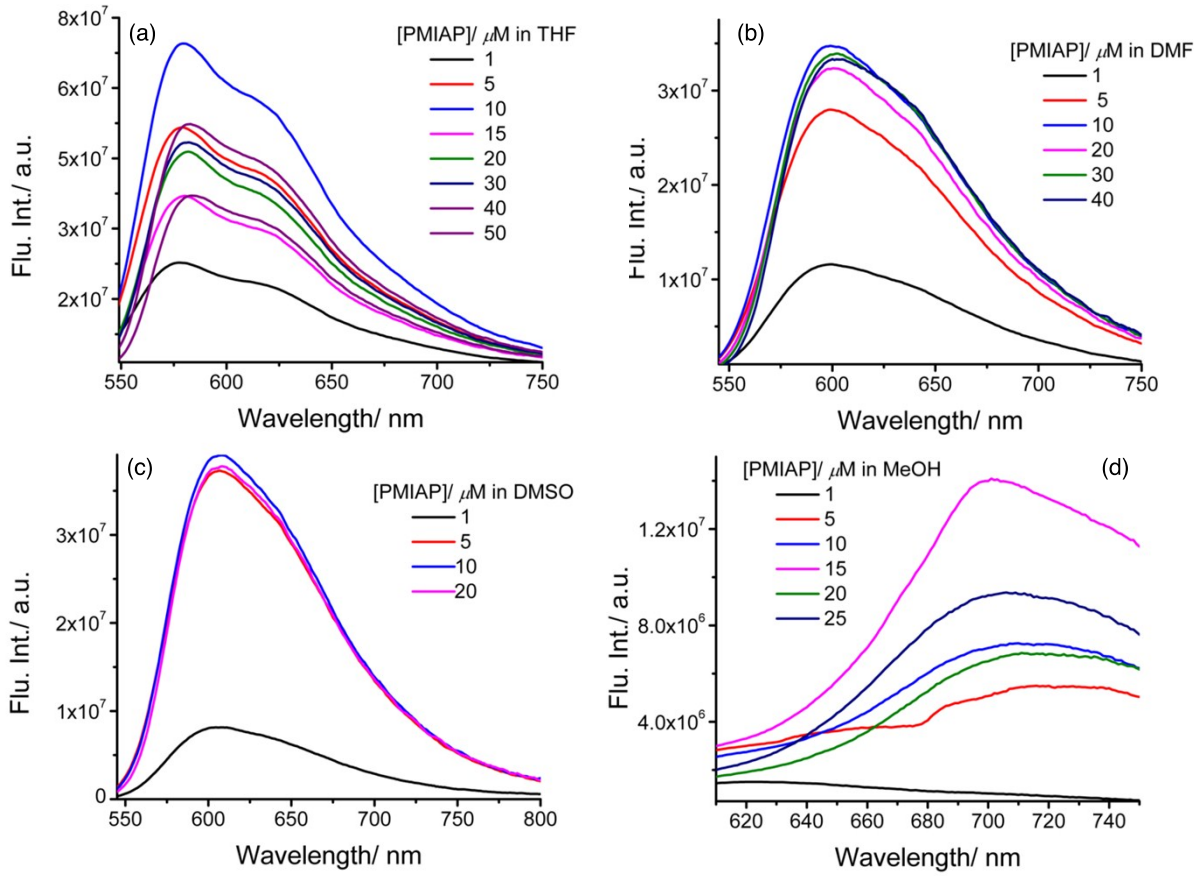


Figure S4: Investigating the concentration effect of PMIAP on the emission spectra in THF, DMF, DMSO and MeOH

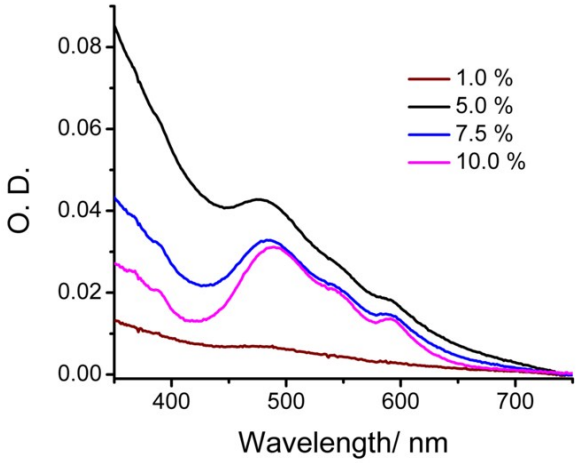


Figure S5. Absorption spectra of PMIAP doped PNP in various loading w/w % in water.

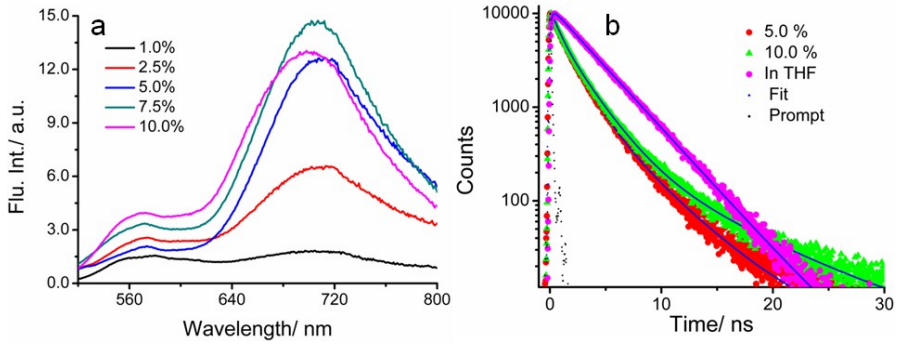
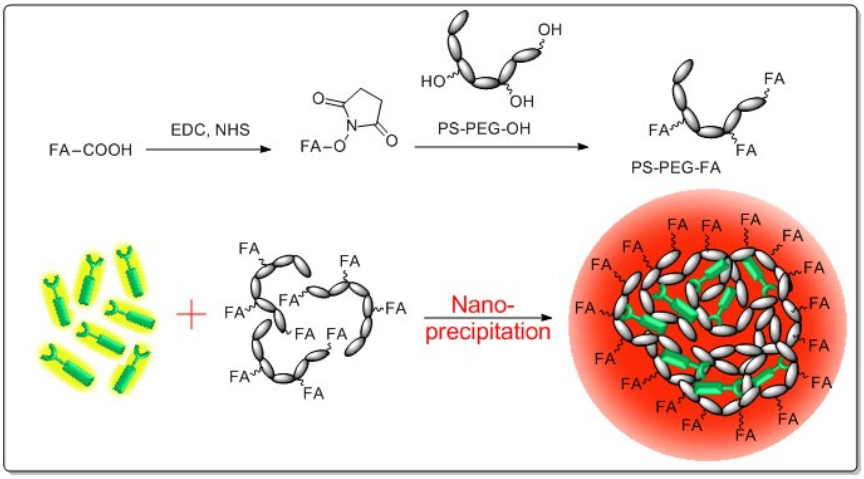


Figure S6. Effect of doping percentage on steady-state and time-resolved fluorescence properties of PMIAP inside PNP (a) Emission spectra with increasing concentration of PMIAP; the ratio between intensity at 710 nm (for aggregate) and 570 nm (for monomer) shows nonlinear behavior and 5.0 % doping appears to be the best in terms of emission at NIR region. (b) Fluorescence lifetime decay monitored at 710 nm where it is shortest with 5.0 % doping and increases gradually upon increasing doping concentration.

Table S1. Effect of doping percentage of PMIAP on the photophysical properties of PNP

w/w /%	τ/ns	I_{710}/I_{570}
1.0	3.2	1.22
2.5	3.1	2.58
5.0	2.3	6.15
7.5	2.5	4.37
10.0	2.9	3.25



Scheme S2. Schematic representation of the synthesis of FA-conjugated polymer PS-PEG-FA and synthesis of PMIAP doped nanoparticles with that *via.* nano-precipitation method.

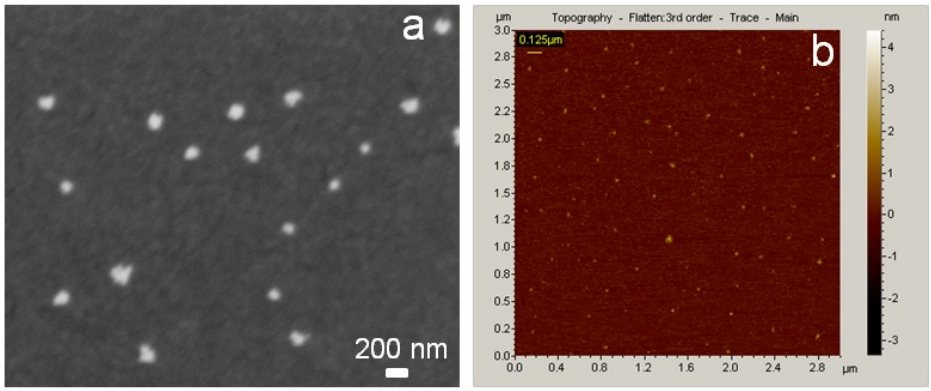


Figure S7. Microscopic characterization of PMIAP doped PNP (a) SEM image of doped PNP shows size distribution 50±10 nm (b) AFM image of PNP in non-contact mode shows size distribution 40±10 nm.

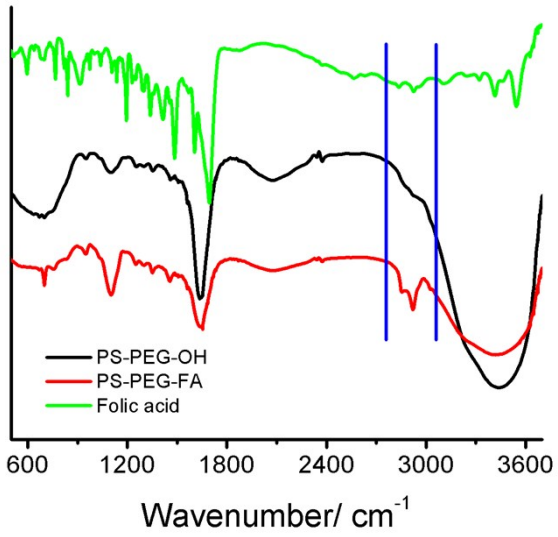


Figure S8: FT-IR spectra of FA, PS-PEG-OH and PS-PEG-FA. The spectrum of PS-PEG-FA showing signal corresponding to characterestic FA signal in the range of 2760-3060 cm^{-1} as shown by lines.

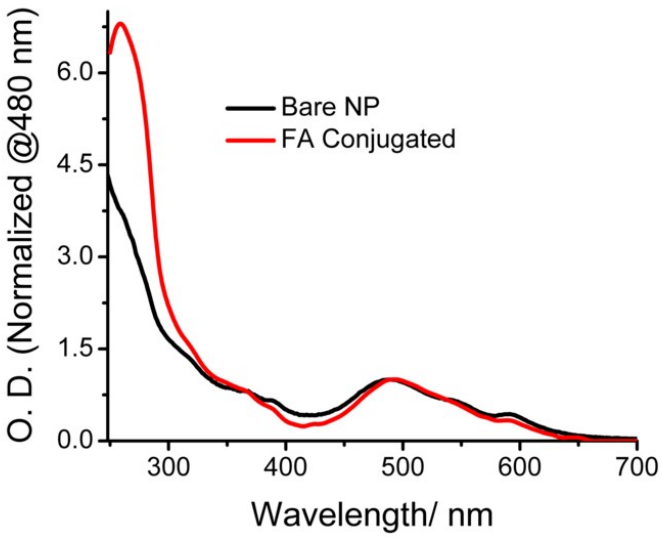


Figure S9. Absorption spectra of PMIAP (w/w 5.0 %) doped PNP made of PS-PEG-OH and PS-PEG-FA, in the latter case a band corresponds to FA centred at 258 nm can be observed.

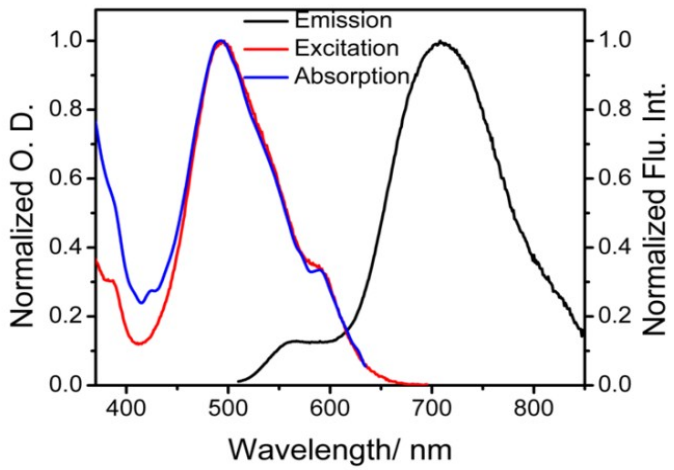


Figure S10. The spectroscopic properties of PNP-FA. Absorption spectrum and excitation spectrum are showing good overlap with each other and absorption maximum is at 490 nm, the emission property is similar to PNP made of PS-PEG-OH with a maximum at 710 nm.

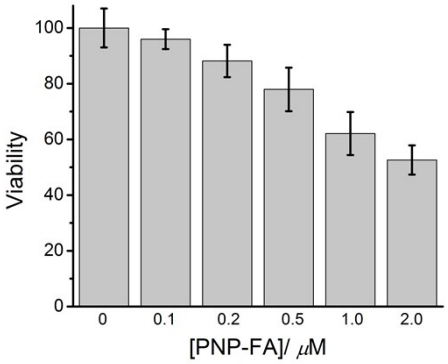


Figure S11: Cytotoxic effect of PMIAP doped PNP-FA in HeLa cells, showing IC_{50} value is way higher than that of concentration required for imaging (0.2 μM).

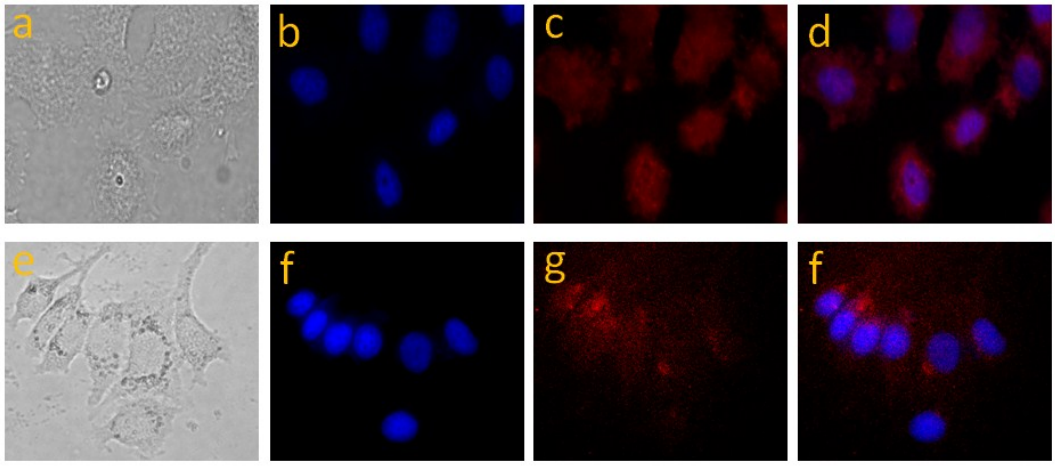


Figure S12: Selective endocytosis of PNP-FA with HeLa. (a-d) cells were incubated with PMIAP doped PNP-FA (0.2 μM) and Hoechst-33342 (2 μM) for 1 h and the images were acquired in bright field, blue channel for nucleus, stained with Hoechst-33342; red channel for PNP and merge of blue and red channel respectively, (e-h) cells were treated with PMIAP doped PNP-OH (0.2 μM) keeping other condition similar.

References:

1. Y. Shi, Z. Su, S. Li, Y. Chen, X. Chen, Y. Xiao, M. Sun, Q. Ping, L. Zong, *Mol. Pharm.* 2013, ***10***, 2479-2489.
2. C. Szymanski, C. Wu, J. Hooper, M. A. Salazar, A. Perdomo, A. Dukes, J. McNeill, *J. Phys. Chem. B* 2005, ***109***, 8543–8546.