Exploiting the Interaction of Pyranine-3 with Poly(*L*-Lysine) to Mediate Nanoparticle–Assembly: Fabrication of Dynamic pH-Responsive Nanocontainers

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Supporting Information

Materials

Poly(*L*-Lysine) hydrobromide (150 kDa), 8-hydroxy pyrene 1,3,6 trisulfonic acid trisodium salt and colloidal silica (LUDOX® HS-40, 40.wt% suspension in water) were procured from Sigma-Aldrich and used as received. All the solutions for synthesis were prepared using de-ionized water (Millipore-18.2 MΩ).

Experimental

HPTS induced microcapsule formation (HPTS@MC):

In a typical process, 3.75mL of PLL (2mg mL^{-1}, 150 kDa, HBr salt; 1Da = 1 g mol^{-1}) was gently vortex mixed for 10 s with 18.75 mL of HPTS solution (2.543 mM). R, The ratio of total negative charges of added HPTS to total positive charges on the polymer was 10. The slightly cloudy polymer/salt solution was aged for 30 min and then vortex mixed with 18.75 mL of colloidal silica for 20 s. There was an immediate increase in turbidity. The cloudy suspension was allowed to age for 2 hr, then centrifuged (4000rpm, 2min) and washed five times with deionised water. The fluorescent green colored precipitate (0.049 gm) was dispersed in 5mL of deionised water, aged for 24 hours and used for further characterisations and studies.

Estimation of the HPTS amount entrapped in HPTS@MC:

The amount of the entrapped HPTS in HPTS@MC was estimated from the amount of dye released from HPTS@MC at high pH (11.5) by adding NaOH. The separated (centrifuged) microcapsules

after treated at this high pH did not exhibit any UV-Vis absorbance, which indicated complete removal of the dye from the microcapsules (Fig. S3).

pH sensitive properties of HPTS@MC:

To evaluate the pH sensitiveness of HPTS@MC, the absorption and emission spectra of HPTS@MC were measured at different pH values ranging 2-12 in Britton-Robinson buffer at room temperature. 100µL of the stock HPTS@MC suspension was mixed with 2.9 mL of the buffer solution of particular pH prepared with deionised water (18.2 Ω). The pH of the suspension with HPTS@MC was again measured to get the actual pH value.

Morphological Characterization:

Scanning Electron Microscopic (SEM) analyses were carried out using Hitachi S-3000N SEM operated at 10kV. The sample was mounted on an aluminum stub and coated with gold for the SEM analyses. To characterise the hollow nature of HPTS@MC, the sample was freeze-fractured on microscope slides at liquid nitrogen temperature and then analysed by SEM. Optical microscopic analyses were done with an Olympus Microscope. Transmission electron microscope (TEM) (JEOL TEM 2010 microscope operating at 200 kV) was used to investigate morphology and size of the particles. The samples for TEM were prepared by dispersing the material in ethanol and drop drying onto a formvar coated copper grid. For Confocal Microscopic studies, the sample was scanned using a 63x1.4NA oil objective at 488nm excitation wavelength. The emission was collected between 500-550 nm on a Leica TCS-SP5 Confocal microscope and analyzed using the Leica software. The slides for these microscopic analyses were prepared by placing a drop of the aqueous suspension of microcapsules on a clean glass slide and covering with a cover slip. Dynamic Light Scattering (DLS) measurements were done with a Zetasizer 3000 HSA (Malvern instruments, UK) using a 90^{o} scattering angle and a laser light of wavelength 633 nm. The hydrodynamic diameters of particles were calculated by using the automated mode. The system was calibrated by using the 199 ± 6 nm NanospheresTM Size Standard (Duke Scientific Corp., Palo Alto, CA, USA) and DTS 0050 standard from Malvern.

Spectroscopic Measurement:

Absorption spectra were measured using a Varian Cary 5000 Spectrophotometer, and the fluorescence measurements were done using the Jobin-Yvon-Horiba Fluorolog III spectrofluorimeter. The excitation source was a 100W Xenon lamp, and the detector used is R-928 operating at a voltage of 950V. Excitation and emission slits width were 5 nm. Fluorescence measurement was carried out in right angle sample geometry. The spectral data was collected using Fluorescence software and OrginPro 6.0 software was used for further data analysis.

The relative fluorescence yield (RFY) was determined as:

RFY = (Fluorescence intensity of HPTS@MC X Absorbance of HPTS) / (Fluorescence intensity of HPTS X Absorbance of HPTS@MC)

Fluorescence Lifetime Measurement:

The fluorescence lifetime measurements were done using Jobin-Yvon-Horiba Fluorolog III spectrofluorimeter, with a pulsed diode laser of excitation wavelength 405 nm. Instrumental response (prompt) for lifetime measurement was carried out using colloidal non-fluorescent particles. The emission and excitation slits width were 5 nm each. The excitation and emission were collected in right angle sample geometry. HPTS@MCs were dispersed in deionised water equilibrated for an hour at room temperature before performing fluorescence lifetime measurement. The decay data were analyzed using Data Analysis Software. To have a good decay fit, the values of $\chi 2$ were in between 0.99 and 1.5 within the acceptable range.

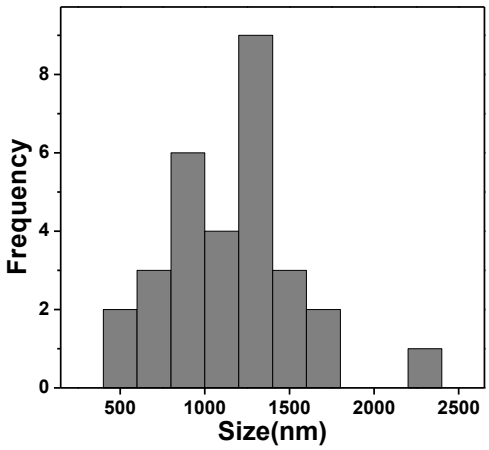

Fig. S1. DLS measurement showing the size distribution of aggregates formed when PLL was added to HPTS (R=10) at 30 min.

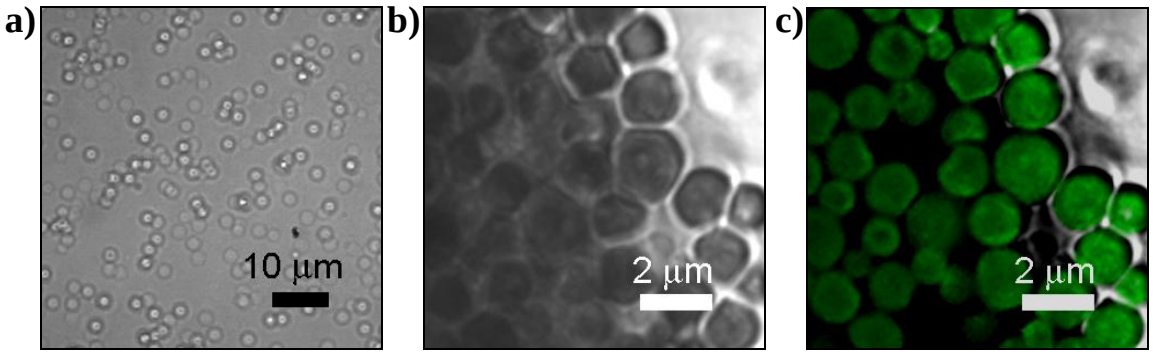

Fig. S2. (a) Optical, (b) Bright field and (c) Merged Confocal and Bright field images of HPTS@MC.

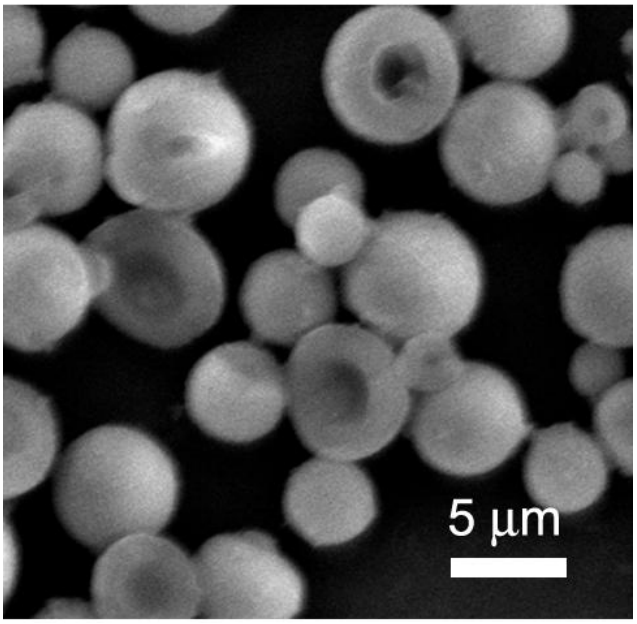

Fig. S3. SEM image of the freeze-fractured HPTS@MC sample illustrating the hollow interior in the broken spheres.

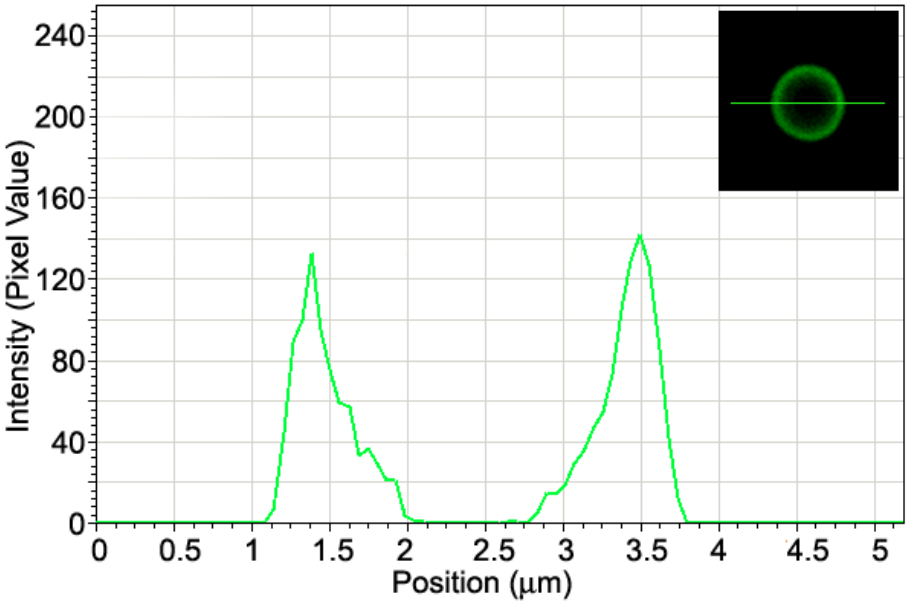

Fig. S4. Line intensity profile across a microcapsule along the green line shown in the (inset) confocal fluorescence image of HPTS@MC.

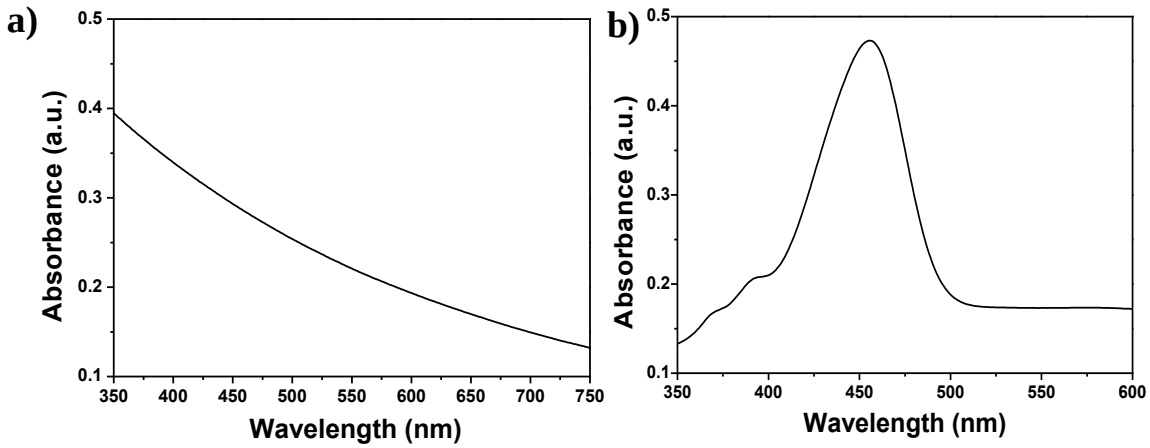

Fig. S5. Absorption spectra of (a) the microcapsules separated from the supernatant, and (b) the supernatant containing the released HPTS from HPTS@MC after it was treated with NaOH at pH = 11.5.

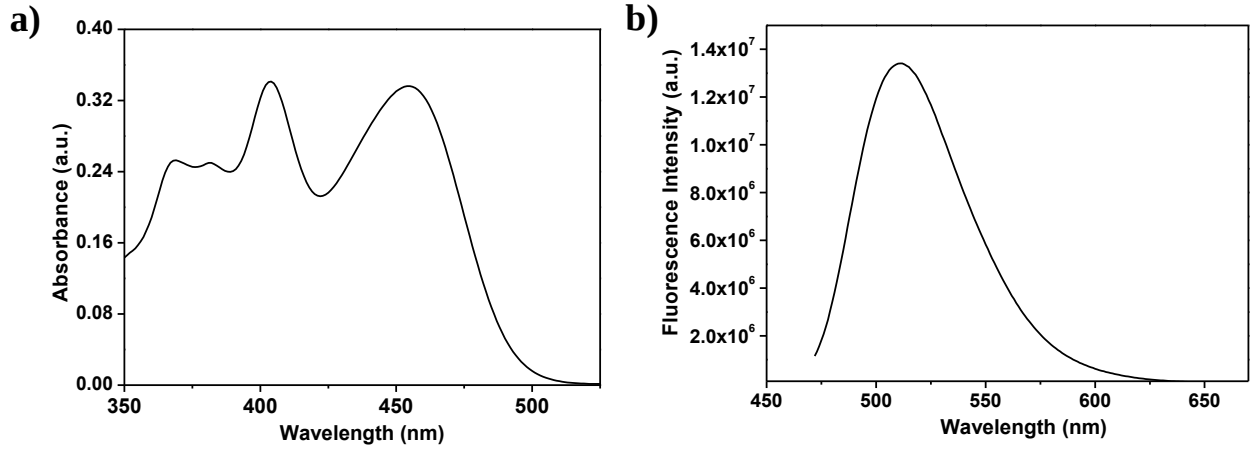

Fig. S6. (a) Absorption and (b) Emission spectra of HPTS at pH 7.51 (Excitation at 454 nm).

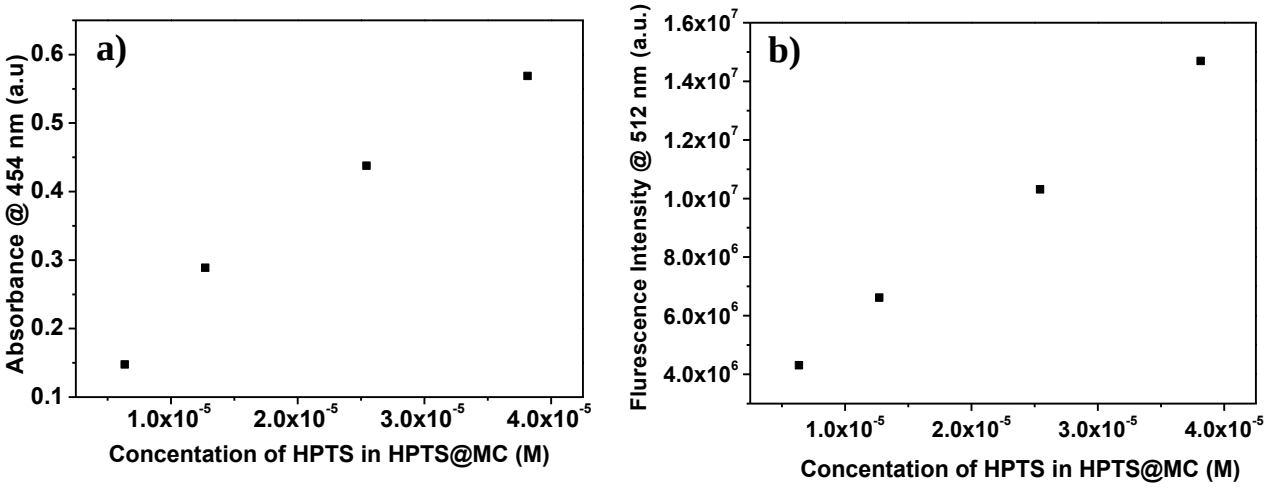

Fig. S7. Change in (a) Absorbance at 454 nm and (b) Fluorescence at 512 nm (excited at 454 nm) with change in concentration of the encapsulated HPTS in HPTS@MC. Measurements were done at pH = 7.51.

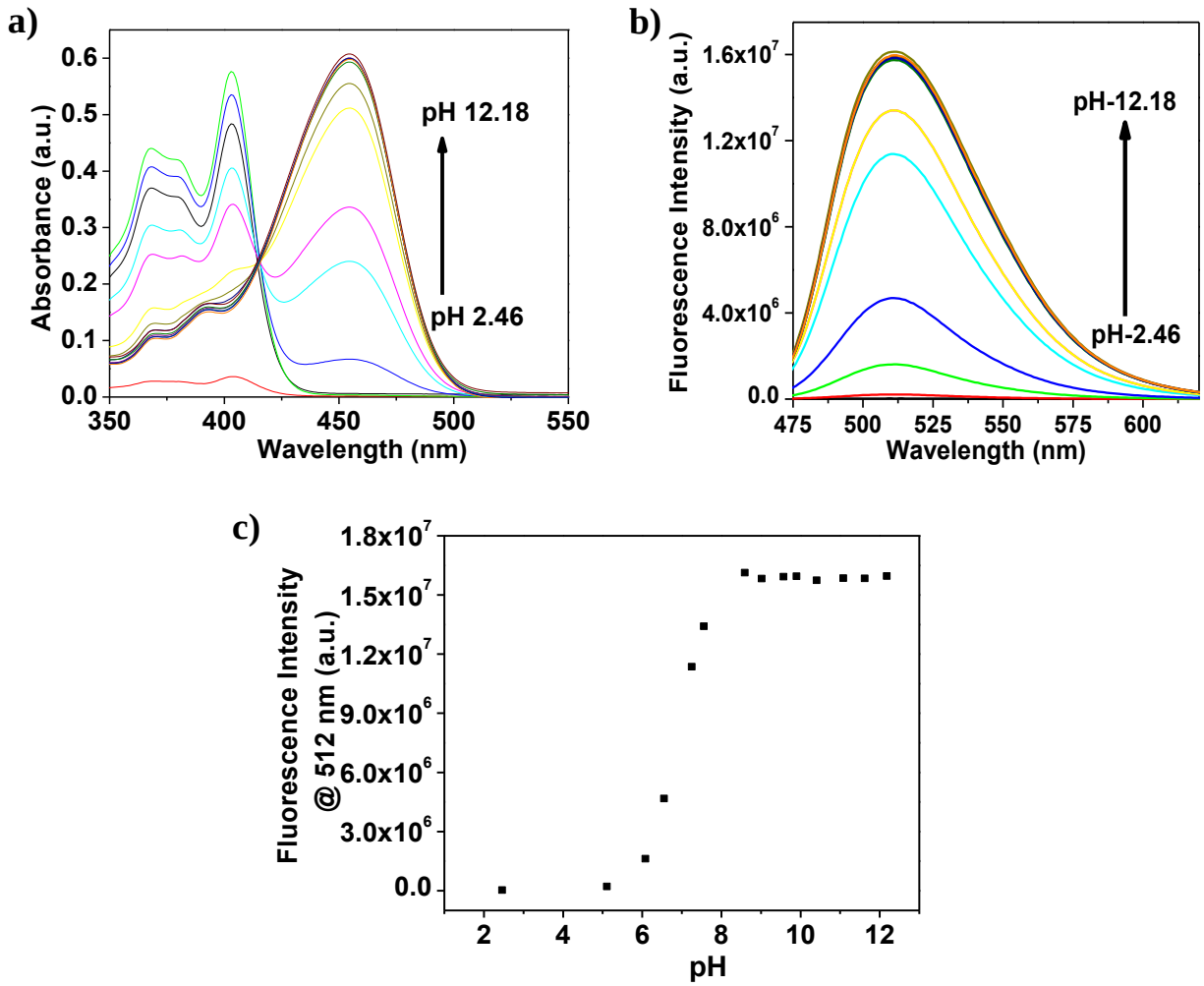

Fig. S8. (a) pH dependent absorption spectra, (b) pH dependent Emission spectra, and (c) the plot of fluorescence intensity at 512 nm *vs* pH for HPTS in Britton-Robinson buffer. Temperature: 25°C. Excitation: 454 nm.

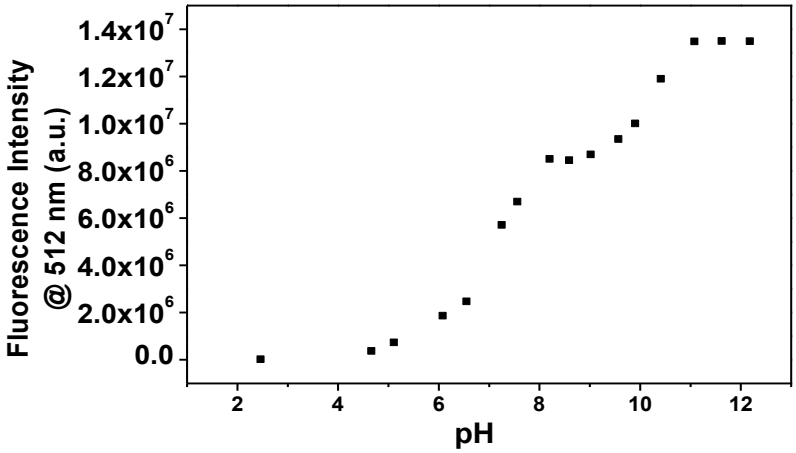

Fig. S9. The plot of fluorescence intensity at 512 nm *vs* pH for HPTS@MC in Britton-Robinson buffer. Temperature: 25°C. Excitation: 454 nm.

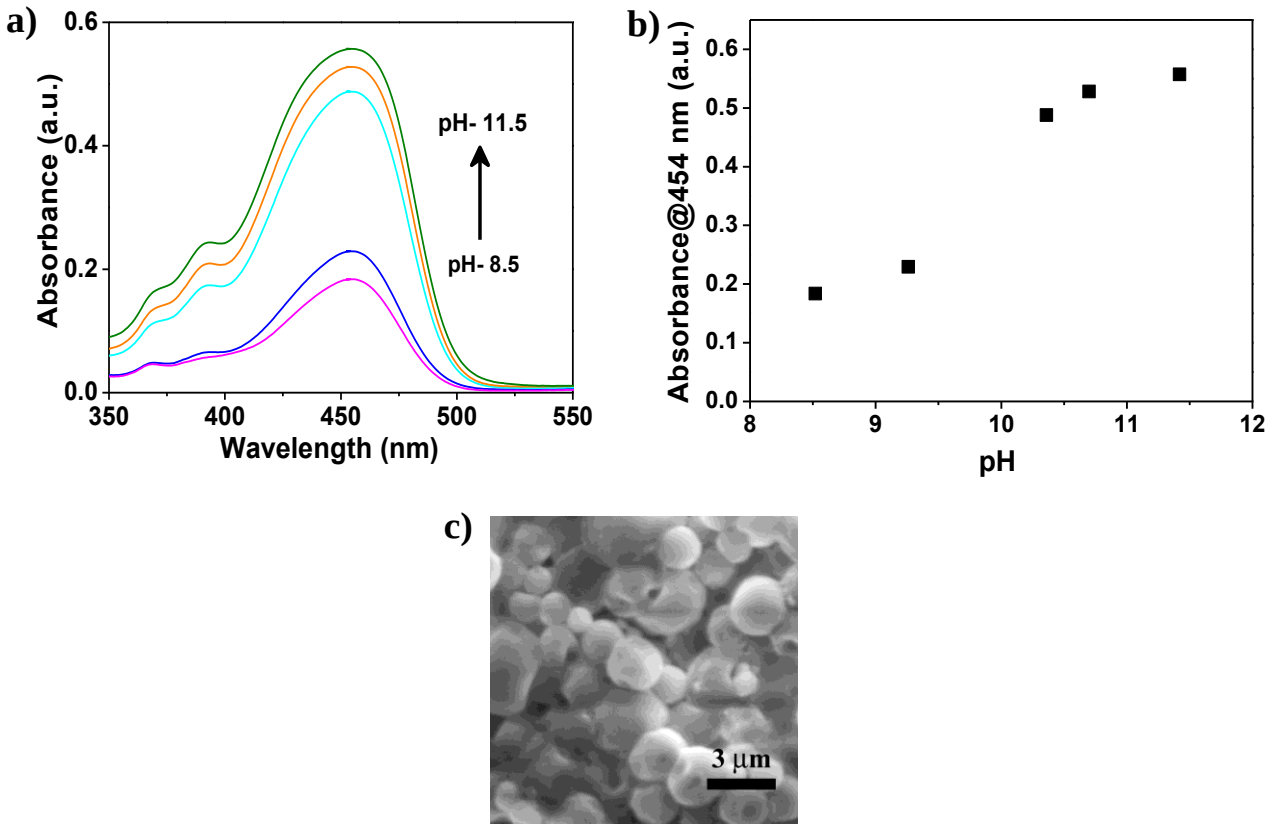

Fig. S10. (a) Absorption spectra of the supernatant obtained from HPTS@MC after treated with the buffer solutions of pH 8.5 –11.5 and (b) Corresponding plot of the Absorption maxima Vs pH; (c) SEM image of the HPTS@MC after treated with the buffer solution of pH-11.5.

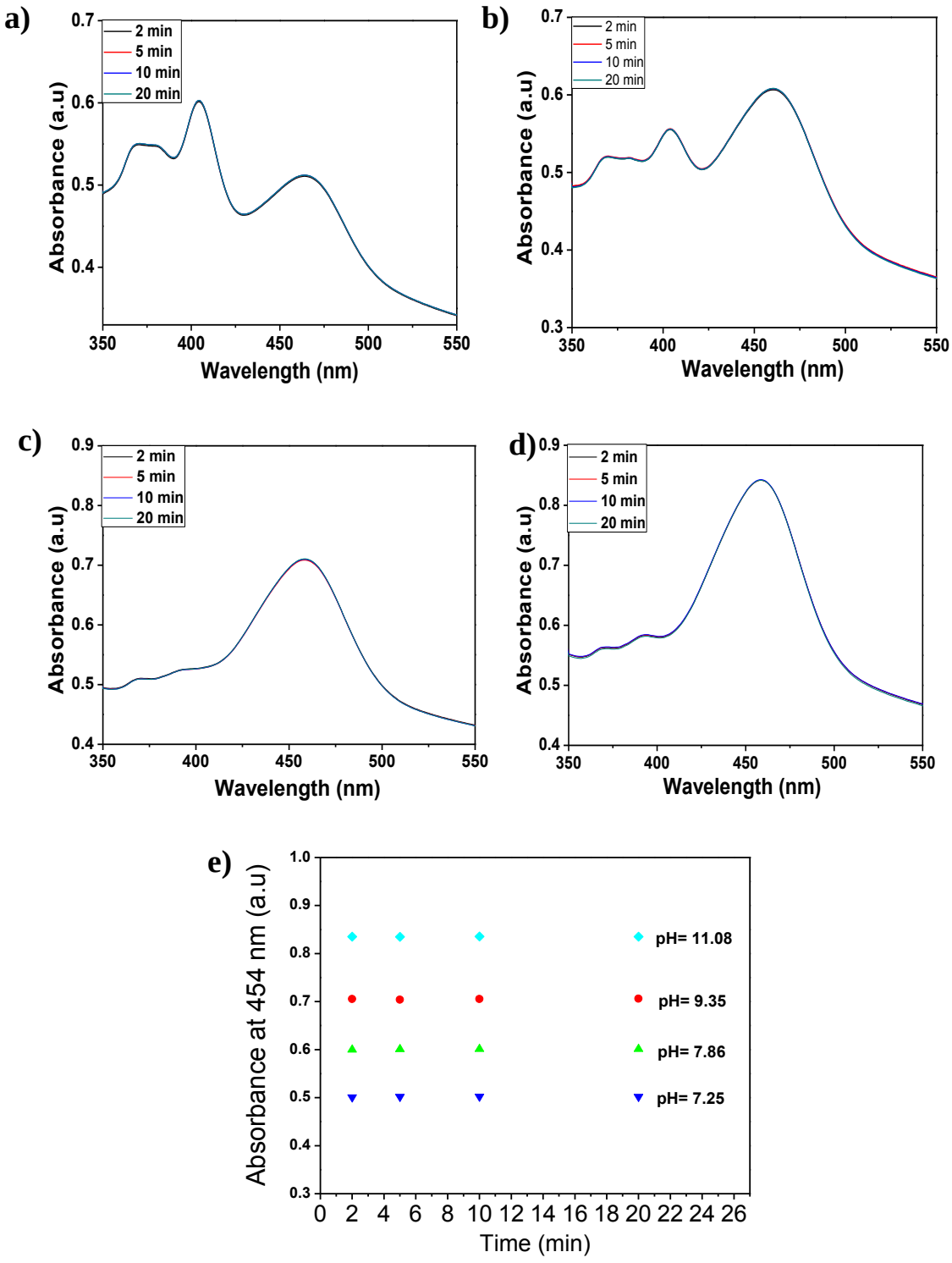

Fig. S11. Absorption spectra of HPTS@MC with time at different pH values. (a) 7.25, (b) 7.86, (c) 9.35, (d) 11.08 and (e) Plot of Absorbance at 454 nm *vs* Time at different pH values.

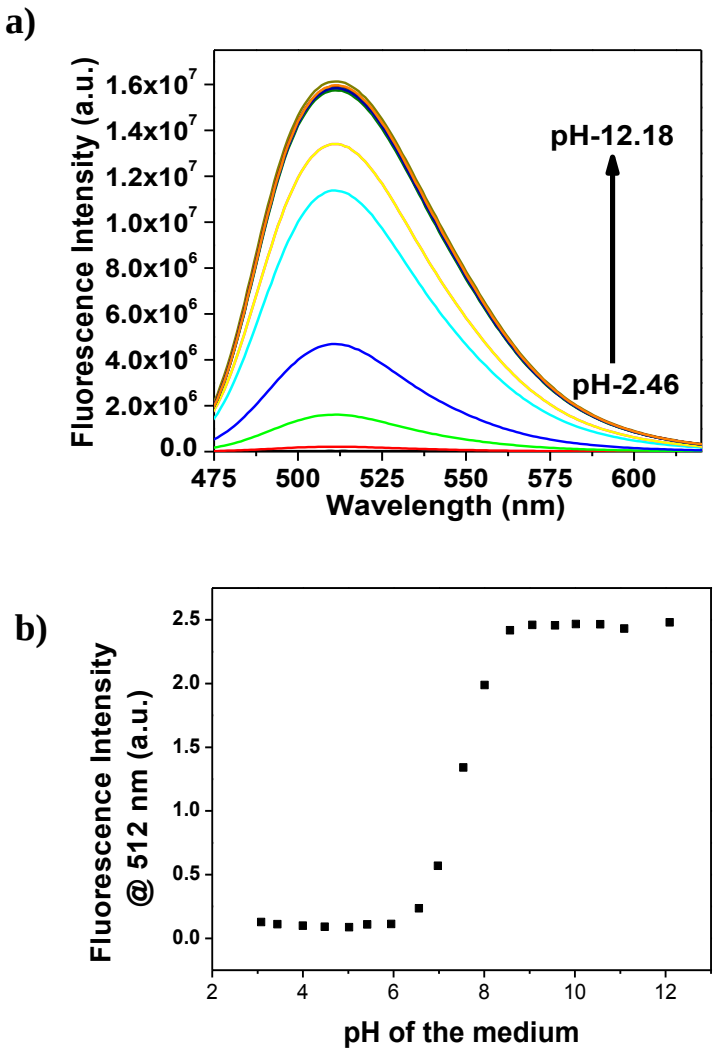

Fig. S12. (a) pH dependent fluorescence spectra and (b) the plot of fluorescence intensity at 512 nm *vs* pH for PLL-HPTS aggregates in Britton-Robinson buffer at 25^{o}C (excited at 454 nm).