Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Dopamine Functionalized Polymeric Nanoparticle for Targeted Drug Delivery

Pradip Das and Nikhil R. Jana*

Centre for Advanced Materials, Indian Association for the Cultivation of Science, Kolkata-700032, India

*Corresponding author. E-mail: *camnrj@iacs.res.in*

Telephone: +91-33-24734971. Fax: +91-33-24732805

Table S1. Average hydrodynamic size of drug encapsulated polymeric nanoparticle with polydispersity index (PDI) measured by dynamic light scattering.

Sample	Average Hydrodynamic Size	PDI
Camptothecin encapsulated ODA-PSI-D (P1)	238 ± 19 nm	0.64
Curcumin encapsulated ODA-PSI-D (P1)	220 ± 16 nm	0.56
Doxorubicin encapsulated ODA-PSI-D (P1)	236 ± 16 nm	0.53
Curcumin encapsulated ODA-PSI-D (P2)	365 ± 34 nm	0.81
Curcumin encapsulated ODA-PSI-D (P3)	317 ± 23 nm	0.72

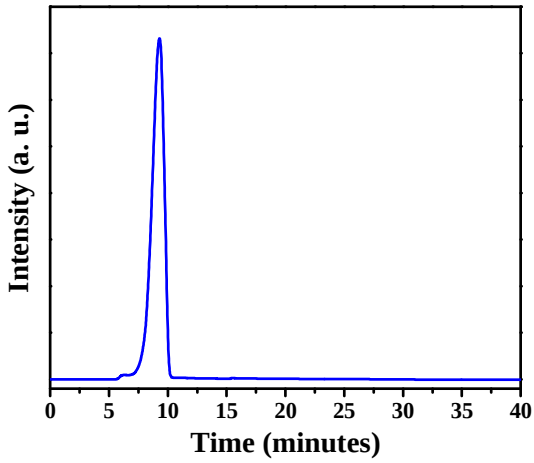

Figure S1. Gel permeation chromatography (GPC) based molecular weight determination of PSI after complete hydrolysis. The number average molecular weight (M_n) and weight average molecular weight (M_w) of polymer were ~ 22500 g/mol and ~ 30800 g/mol with polydispersity index (PDI) ~ 1.37.

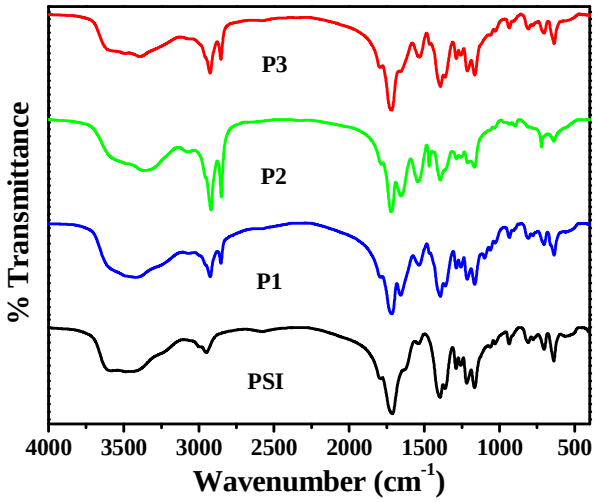

Figure S2. FTIR spectra of polysuccinimide (PSI) and three different ODA-PSI-D (P1, P2, P3) with varied degree of substitution of octadecyl and dopamine. PSI shows characteristic bands at 1713, 1395 and 1218 cm^{-1} for C=O, C-H and C-N functional groups of cyclic imides, respectively. All the ODA-PSI-Ds show two amide bands at 1658 and 1535 cm^{-1}.

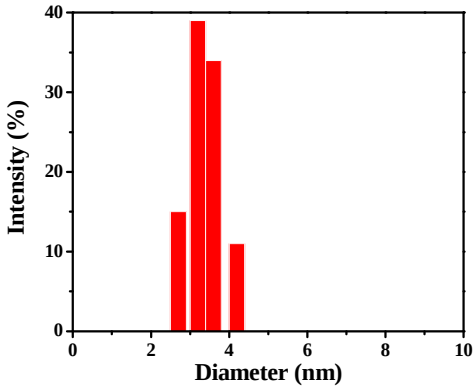

Figure S3. Hydrodynamic size of ODA-PSI-D (P1)-based polymer in dimethylformamide solution as observed by dynamic light scattering study, suggesting that polymer does not self-assemble into micelle form in dimethylformamide. Similar size is also observed for dimethylformamide solution of ODA-PSI-D (P2) and ODA-PSI-D (P3).

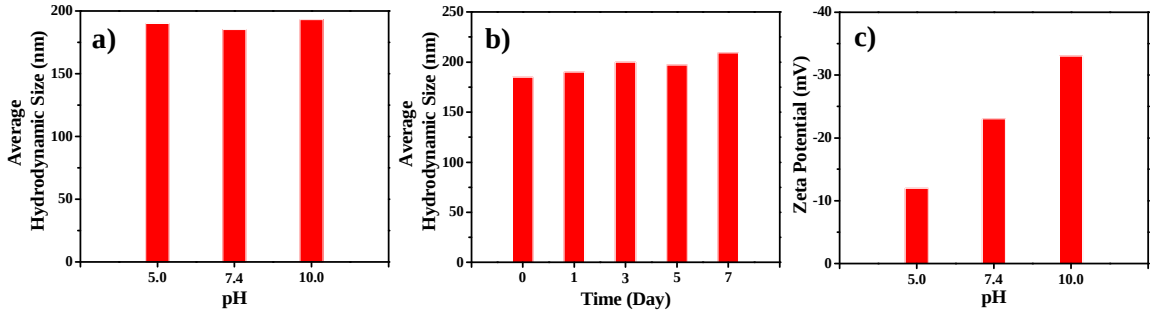

Figure S4. a) Dynamic light scattering-based hydrodynamic size of ODA-PSI-D (P1) polymeric nanoparticle at different pH. b) Dynamic light scattering-based hydrodynamic size of ODA-PSI-D (P1) polymeric nanoparticle at pH 7.4 with changing time. (c) Zeta potential ODA-PSI-D (P1) polymer micelle at different pH.

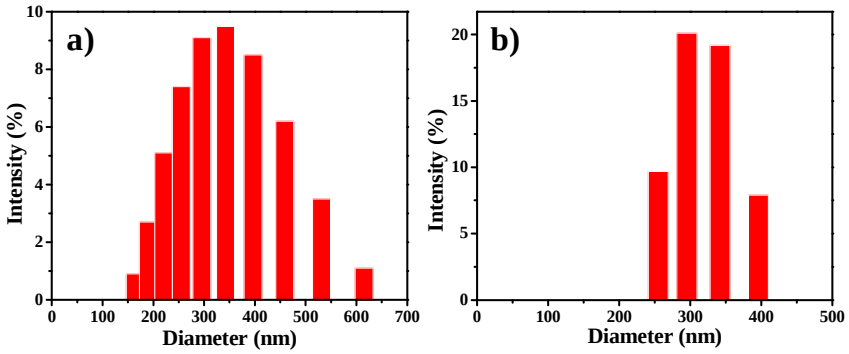

Figure S5. Dynamic light scattering based hydrodynamic size of (a) curcumin encapsulated ODA-PSI-D (P2) polymeric nanoparticle and (b) curcumin encapsulated ODA-PSI-D (P3) polymeric nanoparticle.

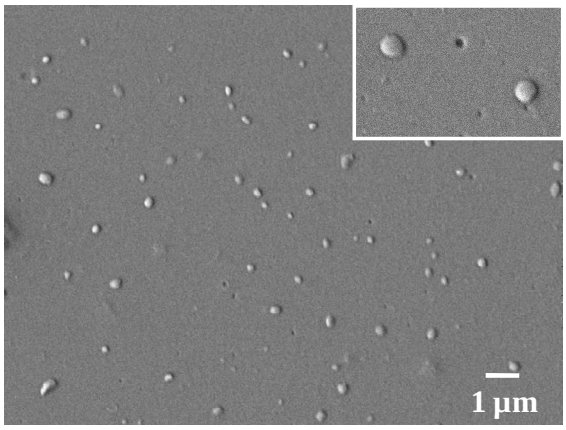

Figure S6. Field emission scanning electron microscopy (FESEM) image of doxorubicin encapsulated ODA-PSI-D (P1) polymeric nanoparticle.

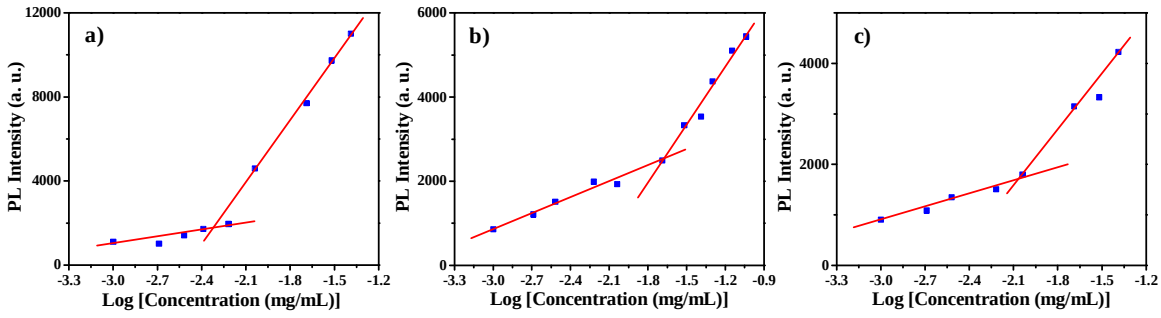

Figure S7. Determination of critical aggregation concentration (CAC) of three different ODA-PSI-D using nile red as fluorescent probe. The PL intensity of nile red at 612 nm was plotted against concentration of ODA-PSI-D and CAC of polymer was determined from inflection point of the plot. (a) ODA-PSI-D (P1), (b) ODA-PSI-D (P2) and (c) ODA-PSI-D (P3)

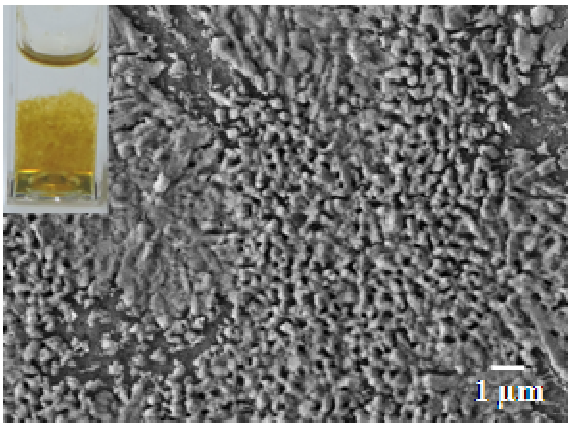

Figure S8. Field emission scanning electron microscopy (FESEM) image of aggregates of curcumin encapsulated ODA-PSI-D (P1) polymeric nanoparticle obtained by adding Fe^{3+} ion. Inset show the digital image of precipitated polymeric nanoparticle.

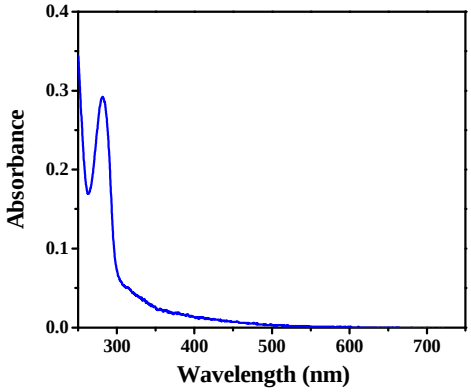

Figure S9. UV-visible absorption spectrum of aqueous solution of ODA-PSI-D (P1) polymeric nanoparticle. The strong absorption peak at 280 nm indicates that the dopamine is present in catechol form without any polymerization.

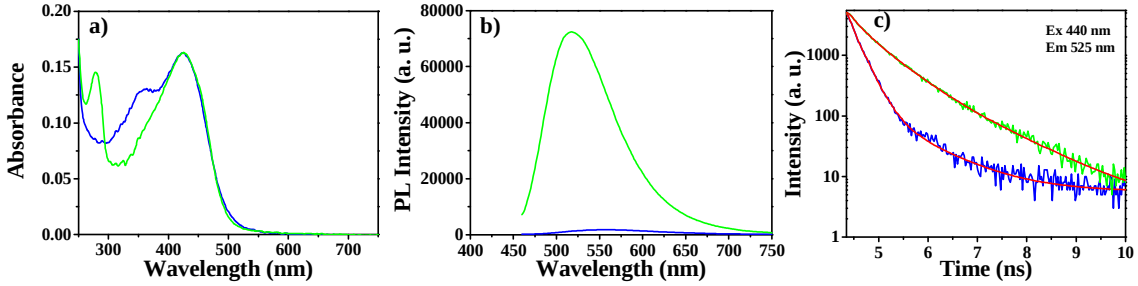

Figure S10. UV-visible absorption spectra (a), fluorescence spectra (b) and fluorescence lifetime decay curve (c) of curcumin in dimethylformamide-water mixture (blue line) or in water after encapsulated into ODA-PSI-D (P1) polymeric nanoparticle (green line). The red line in decay curve corresponds to fitted data. The average lifetime of curcumin is ~ 0.22 ns in dimethylformamide-water mixture and ~ 0.62 ns after encapsulated into polymeric nanoparticle.

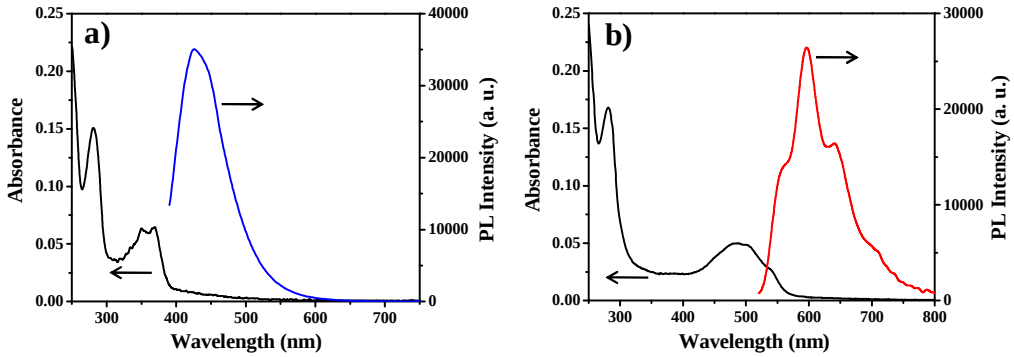

Figure S11. Absorption spectra (black line) and fluorescence spectra (color line) of camptothecin encapsulated ODA-PSI-D (P1) polymeric nanoparticle (a) and doxorubicin encapsulated ODA-PSI-D (P1) polymeric nanoparticle (b) in water.

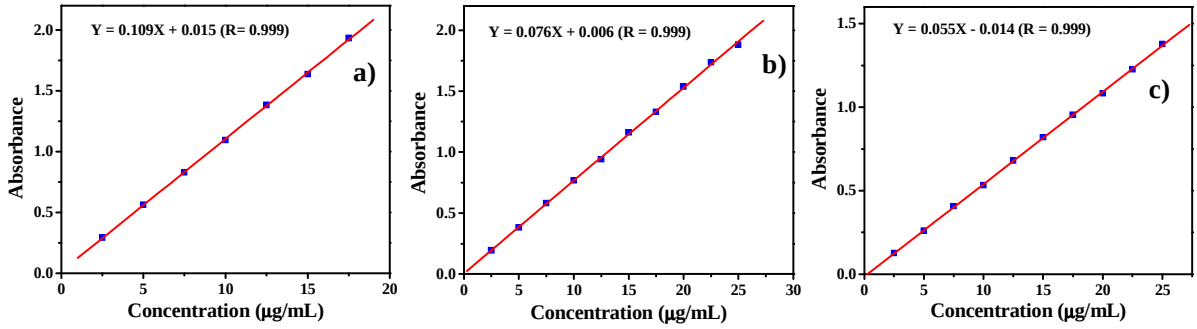

Figure S12. a) Calibration curves prepared and used for quantification of curcumin (a), camptothecin (b) and doxorubicin (c) encapsulated into polymeric nanoparticle. The absorbance has been measured at 425 nm, 366 nm and 490 nm for curcumin, camptothecin and doxorubicin, respectively.

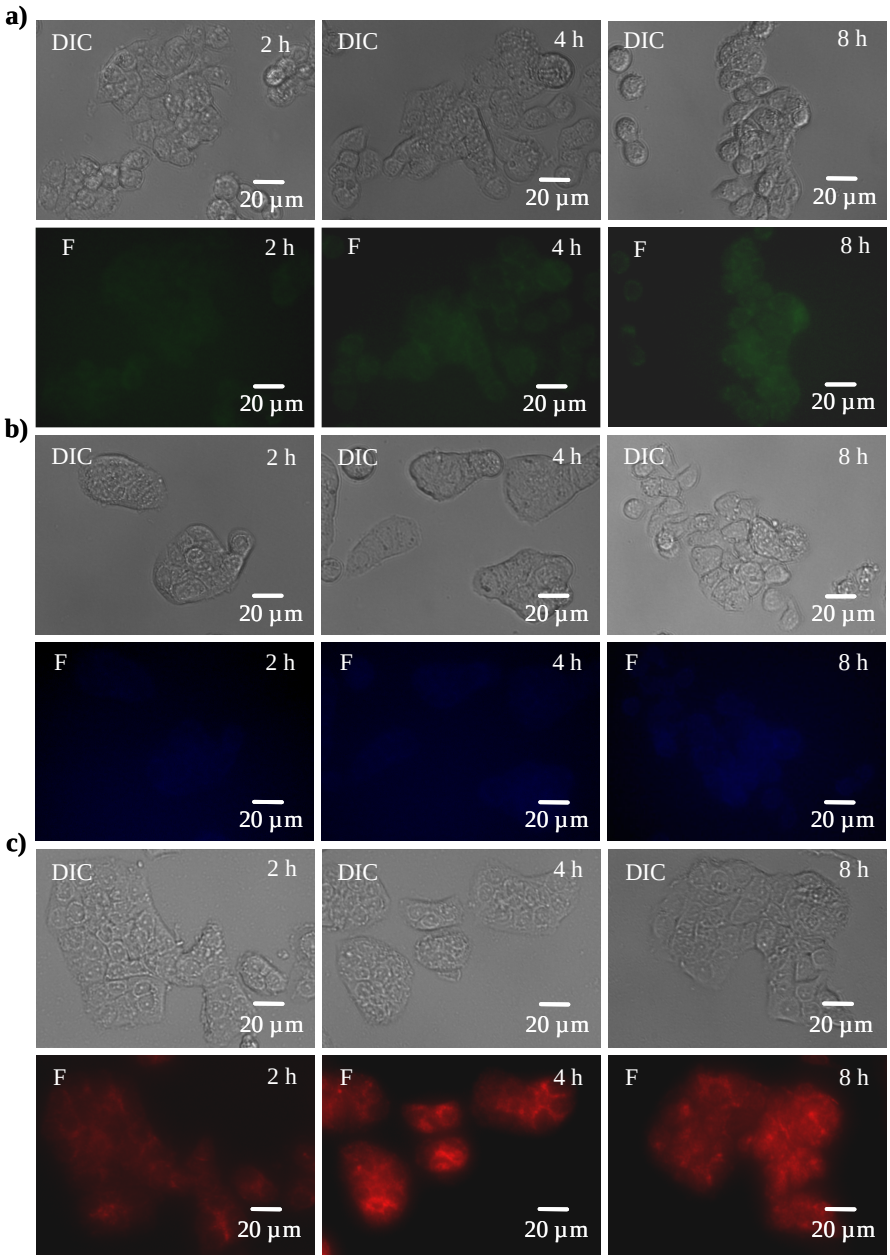

Figure S13. Differential interference contrast (DIC) and fluorescence (F) images of HT-29 cells after different time incubation with curcumin encapsulated ODA-PSI-D (P1) polymeric nanoparticle (a), camptothecin encapsulated ODA-PSI-D (P1) polymeric nanoparticle (b) and doxorubicin encapsulated ODA-PSI-D (P1) polymeric nanoparticle (c). HT-29 cells are incubated with drug encapsulated polymeric nanoparticle for 2, 4 and 8 hrs and then washed cells are imaged under microscope. The fluorescence images were obtained under UV, blue and green excitation for camptothecin, curcumin and doxorubicin, respectively.

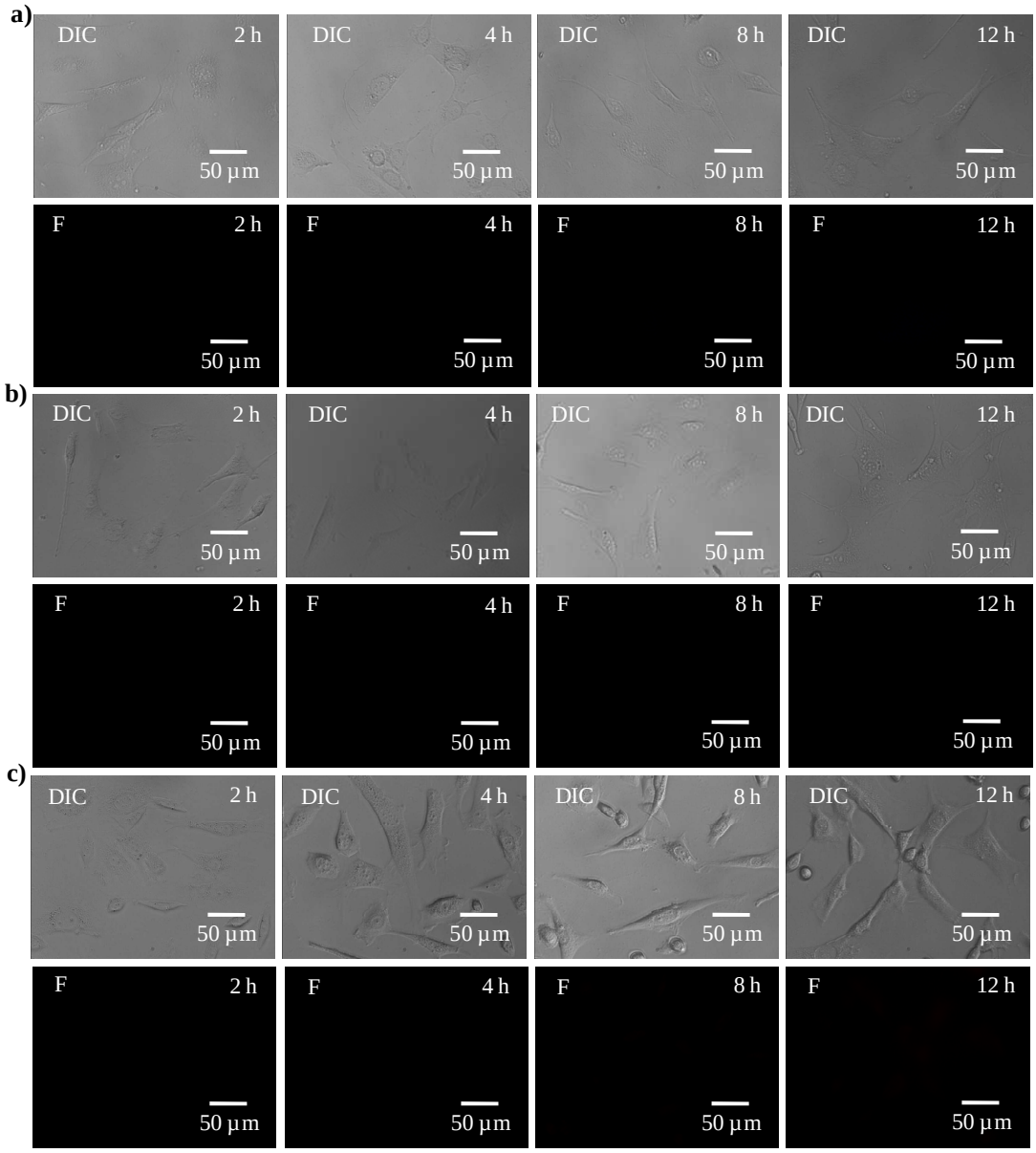

Figure S14. Differential interference contrast (DIC) and fluorescence (F) images of 3T3-L1 cells after different time incubation with curcumin encapsulated ODA-PSI-D (P1) polymeric nanoparticle (a), camptothecin encapsulated ODA-PSI-D (P1) polymeric nanoparticle (b) and doxorubicin encapsulated ODA-PSI-D (P1) polymeric nanoparticle (c). 3T3-L1 cells are incubated with drug encapsulated polymeric nanoparticle for 2, 4, 8 and 12 hrs and then washed cells are measured under microscope. The fluorescence images were obtained under UV, blue and green excitation for camptothecin, curcumin and doxorubicin, respectively.

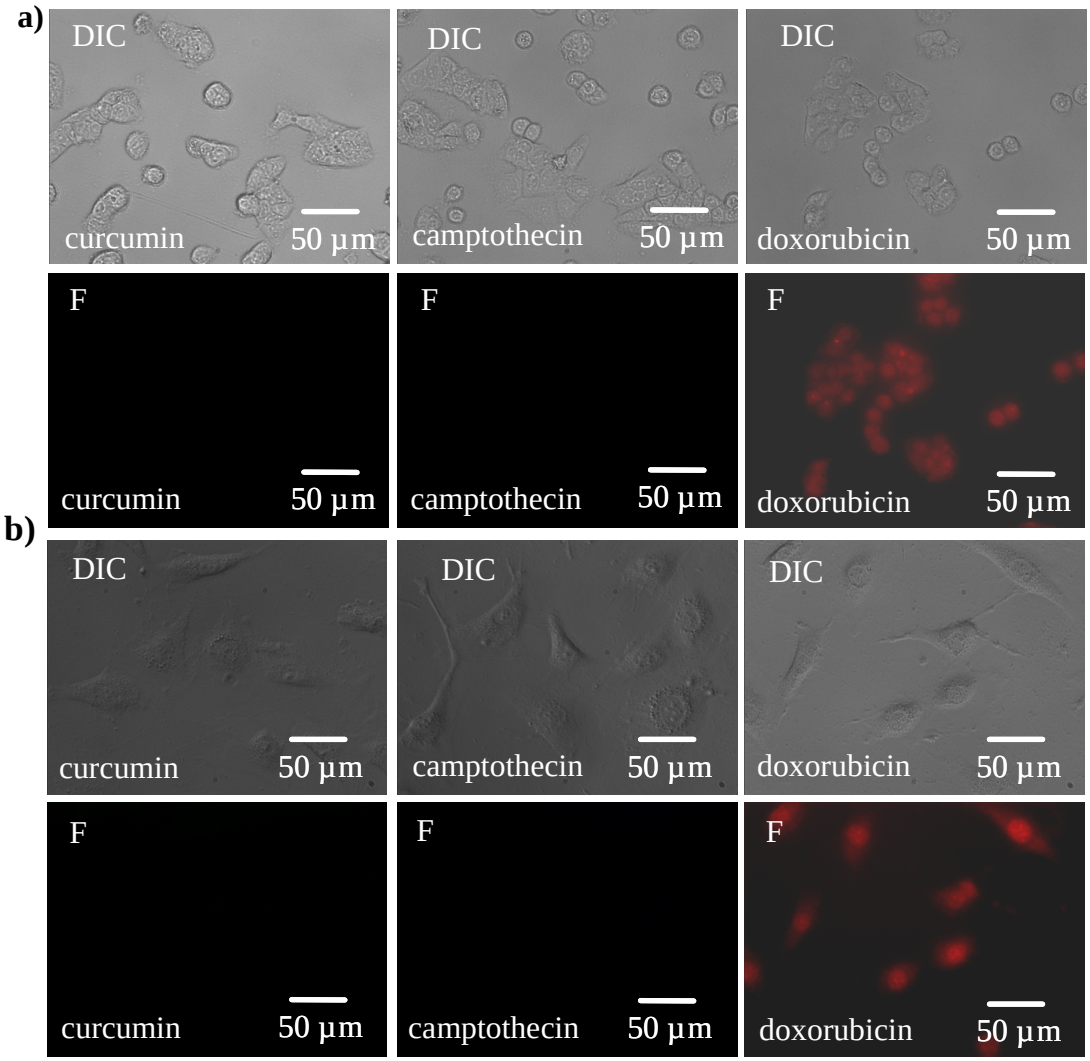

Figure S15. Differential interference contrast (DIC) and fluorescence (F) image of HT-29 (a) and 3T3-L1 cells (b) after 4 hrs incubation of free curcumin, free camptothecin and free doxorubicin. The fluorescence images were obtained under UV, blue and green excitation for camptothecin, curcumin and doxorubicin, respectively.

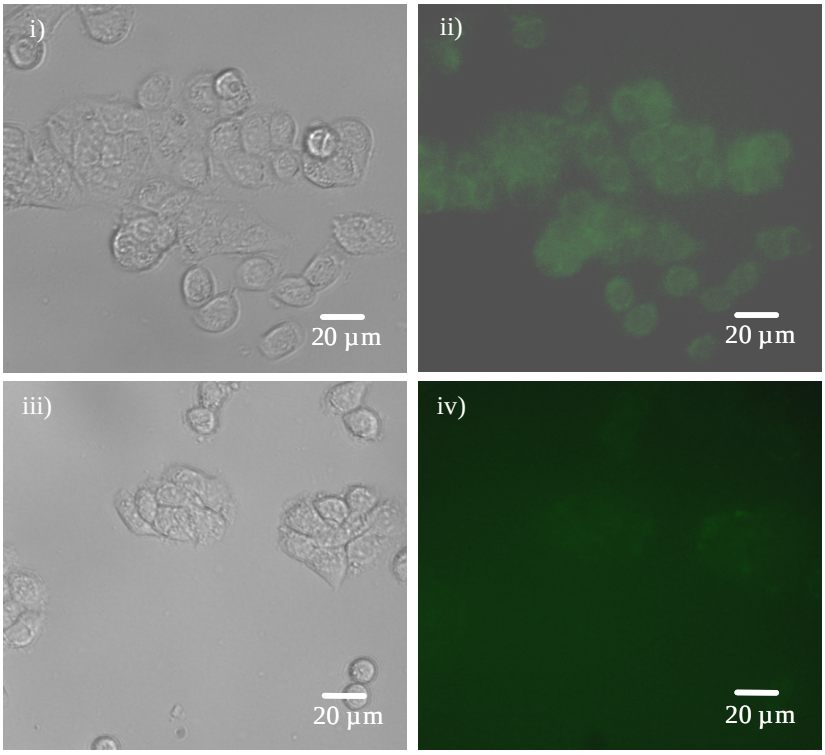

Figure S16. Influence of free dopamine in cellular uptake of curcumin in HT-29 cells. HT-29 cells are incubated with curcumin encapsulated ODA-PSI-D (P1) polymeric nanoparticle for 4 hrs in absence (i, ii) or presence (iii, iv) of free dopamine. Next, cells are washed and imaged under differential contrast mode (i, iii) or fluorescence mode with blue excitation (ii, iv).

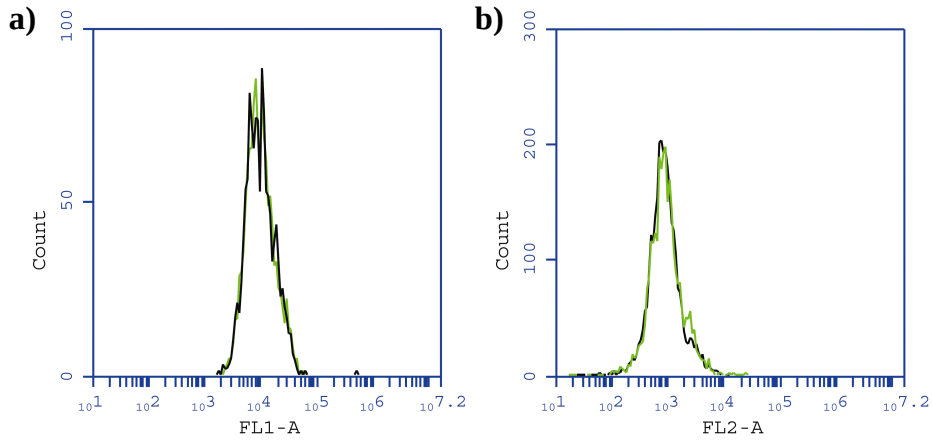

Figure S17. Flow cytometry analysis of 3T3-L1 cells treated with curcumin encapsulated ODA-PSI-D (P1) polymeric nanoparticle (a) and doxorubicin encapsulated ODA-PSI-D (P1) polymeric nanoparticle (b) after 4 hrs incubation (green line) and without any polymeric nanoparticle (black line). FL1-A and FL2-A represent area of fluorescence signal collected by standard optical filters FL1 (533/30 nm) and FL2 (585/40 nm), respectively.

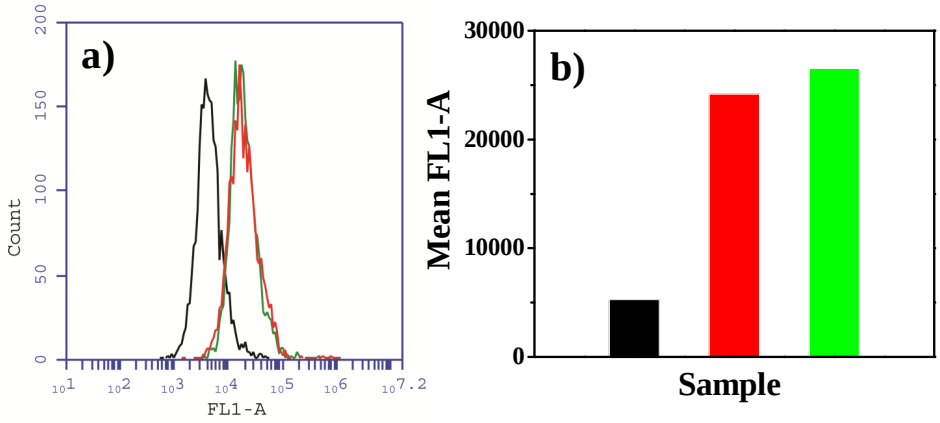

Figure S18. Flow cytometry analysis of HT-29 cells after 4 hrs incubation with curcumin encapsulated ODA-PSI-D (P1) polymeric nanoparticle (red line/bar) and curcumin encapsulated ODA-PSI-D (P3) polymeric nanoparticle (green line/bar) and without any polymeric nanoparticle (black line/bar). FL1-A represent area of fluorescence signal collected by standard optical filters FL1 (533/30 nm).

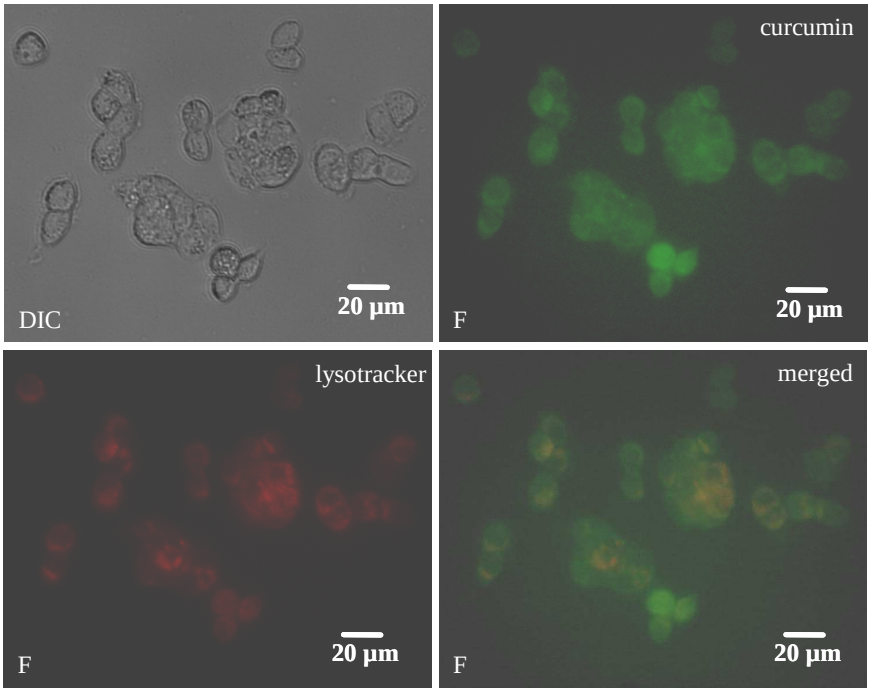

Figure S19. Intracellular distribution of curcumin in HT-29 cells delivered via ODA-PSI-D (P1) polymeric nanoparticle. Cells are incubated with curcumin encapsulated polymeric nanoparticle for 4 hrs followed by further labelling with lysotraker red and imaged under differential contrast mode (DIC) or fluorescence mode (F). Merged fluorescent images indicate little colocalization of curcumin with endosome/lysosome.

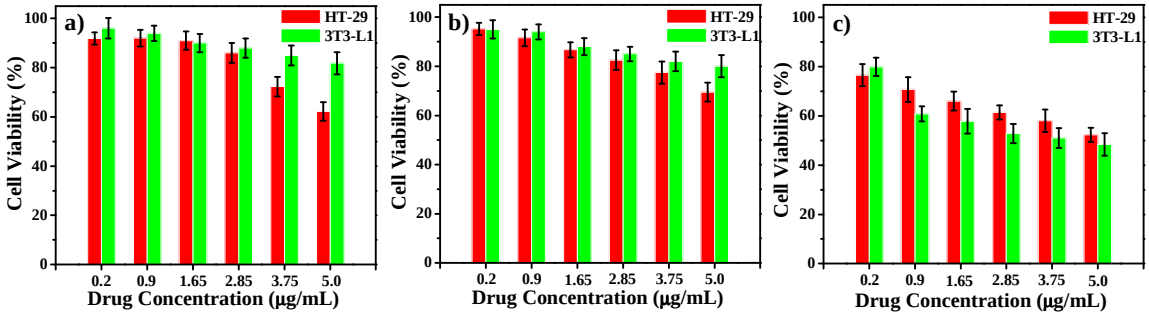

Figure S20. *In vitro* cytotoxicity study of free drugs (a) curcumin, (b) camptothecin and (c) doxorubicin. HT-29 or 3T3-L1 cells are incubated with free drug of different concentration for 24 hrs and then used for MTT based cytotoxicity study. Cell without any drug correspond to 100 % viability.

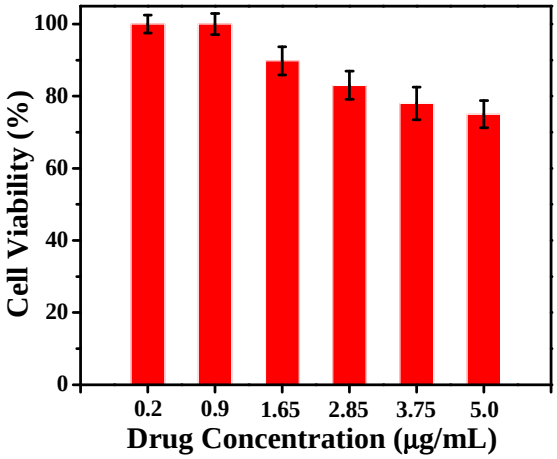

Figure S21. *In vitro* cytotoxicity study of curcumin encapsulated ODA-PSI-D (P1) polymeric nanoparticle in presence of free dopamine. HT-29 cells are incubated with curcumin encapsulated polymeric nanoparticle of different concentration for 24 hrs in presence of 50 µL dopamine (10 mM) and then used for MTT based cytotoxicity study.