Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Ferulic Acid-Loaded Polymeric Nanoparticles Prepared from Nano-emulsion Templates Facilitate Internalisation Across the Blood-Brain Barrier in Model Membranes

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CONTENT

Figure S1. Visual appearance and DLS size distributions of non-loaded and FA-loaded NPs containing FA concentration of 0.1, 0.2, and 0.3 mg·mL^{-1}. A. Non-loaded PLGA NPs; B. FA-loaded PLGA NPs where [FA] = 0.1 mg·mL^{-1}; C. FA-loaded PLGA NPs where [FA] = 0.2 mg·mL^{-1}; D. FA-loaded PLGA NPs where [FA] = 0.3 mg·mL^{-1}

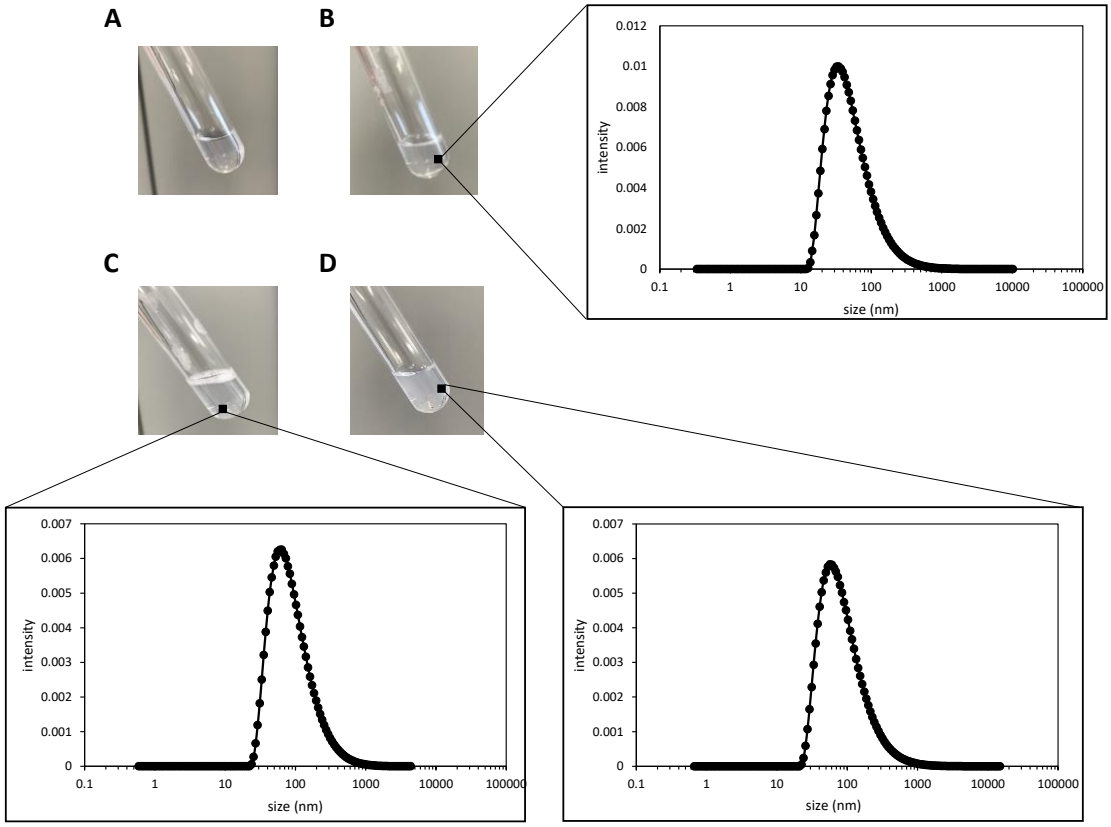

Figure S2. Electron microscopy images of FA-loaded PLGA NPs [FA = 0.1 mg·mL^{-1}]. Scale bar = 2 µm (A) and 200 nm (B). A few few smaller nanosized particles (~ 46 and 23 nm) were also observed.

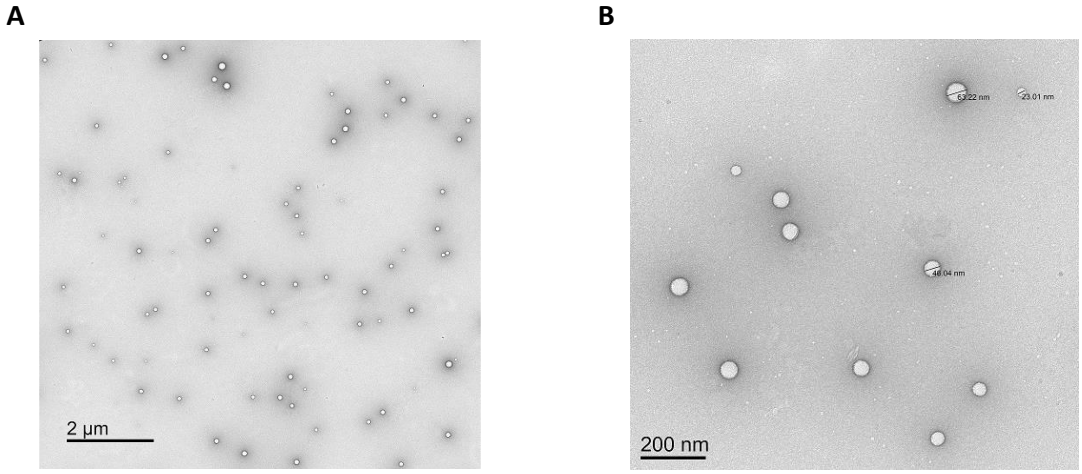

Figure S3. Scattering spectra of PLGA (A), FA (B) and overlay of both scattering spectra (C) from hyperspectral analysis.

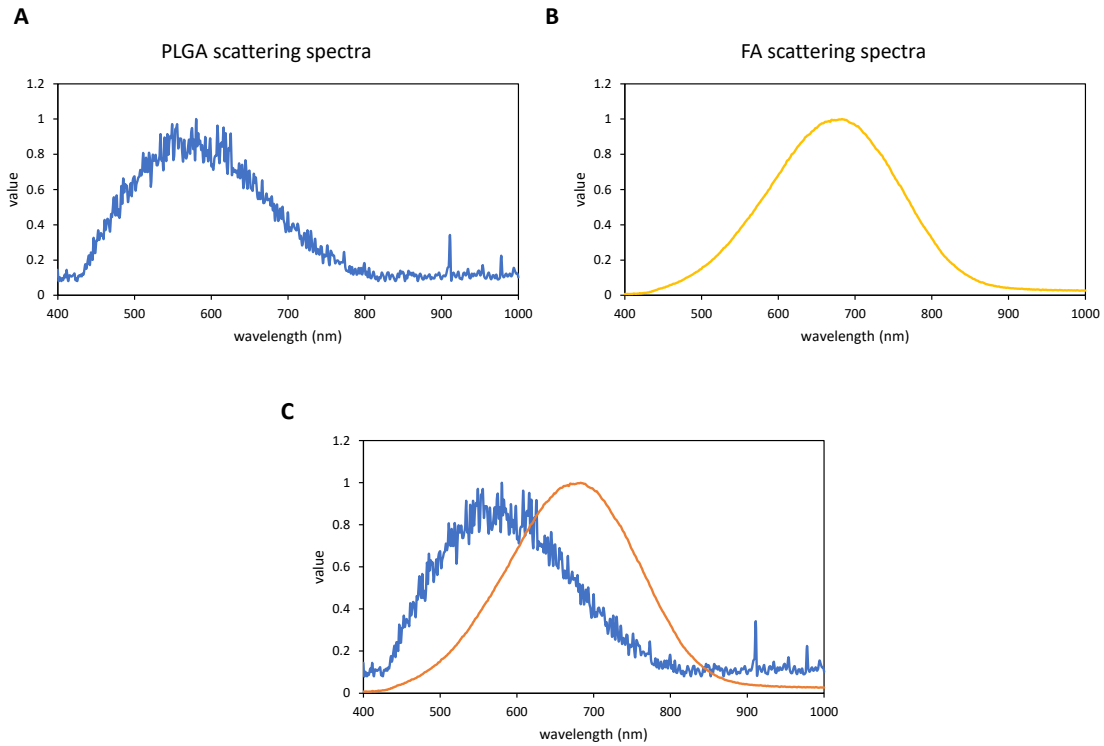

Figure S4. FA calibration curves. (A) Calibration curve used to calculate the EE% for PLGA NPs containing 0.1 mg·mL^{-1} of FA; (B) Calibration curve used to calculate the EE% for PLGA NPs containing 0.3 mg·mL^{-1} of FA; and (C) Calibration curve used to calculate the %cumulative release of FA

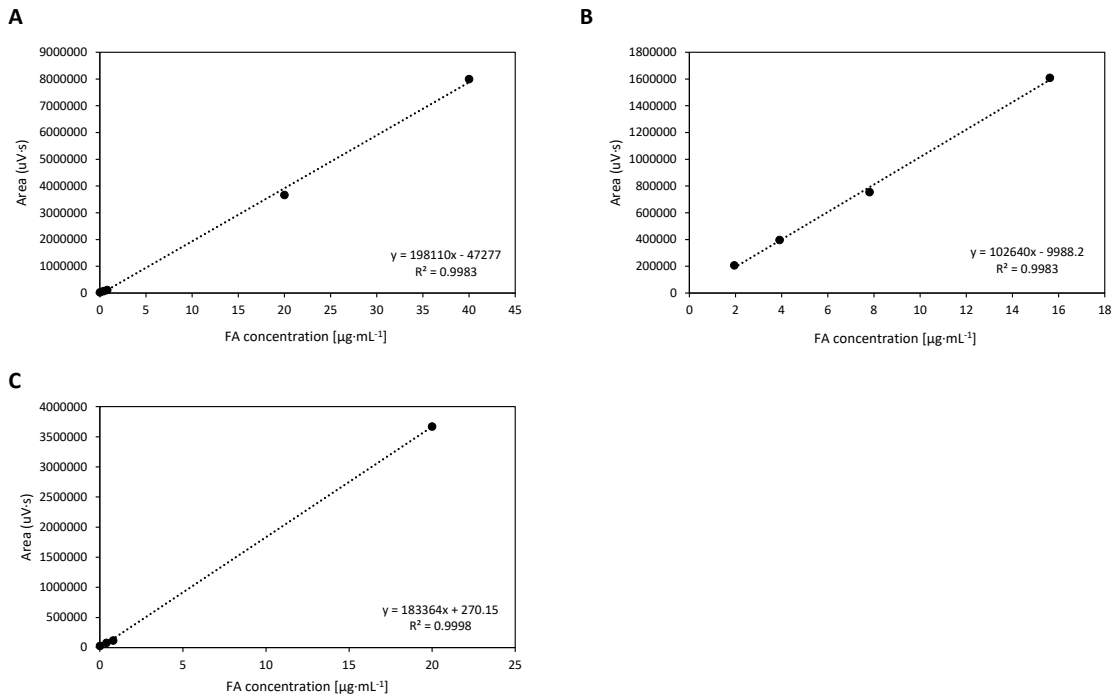

Table S1. Gradient mode for FA quantification

Time (min)	Solvent A (%)	Solvent B (%)
0	100	0
6	90	10
8	70	30
10	60	40
15	100	0

Figure S5. *In vitro* cumulative release of FA from PLGA NPs at 37 ºC monitored over time using two ethanolic receptor solutions: 50% EtOH (blue) and 15% EtOH (orange). Data correspond to the mean of three independent experiments (SD = 3)

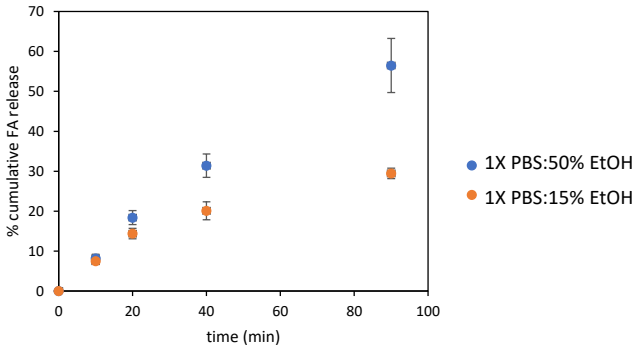

Figure S6. Fitting of FA release data to release kinetic models. A. Zero-order (Z-O) equation; B. First order (F-O) equation; and C. Higuchi kinetic model; D. Korsmeyer-Peppas kinetic model. Inset: log-log plot of FA released versus time and corresponding fitting. Experimental conditions were selected according to Figure 4 without using Tween 80. Statistical parameters and constants are displayed in Table 3

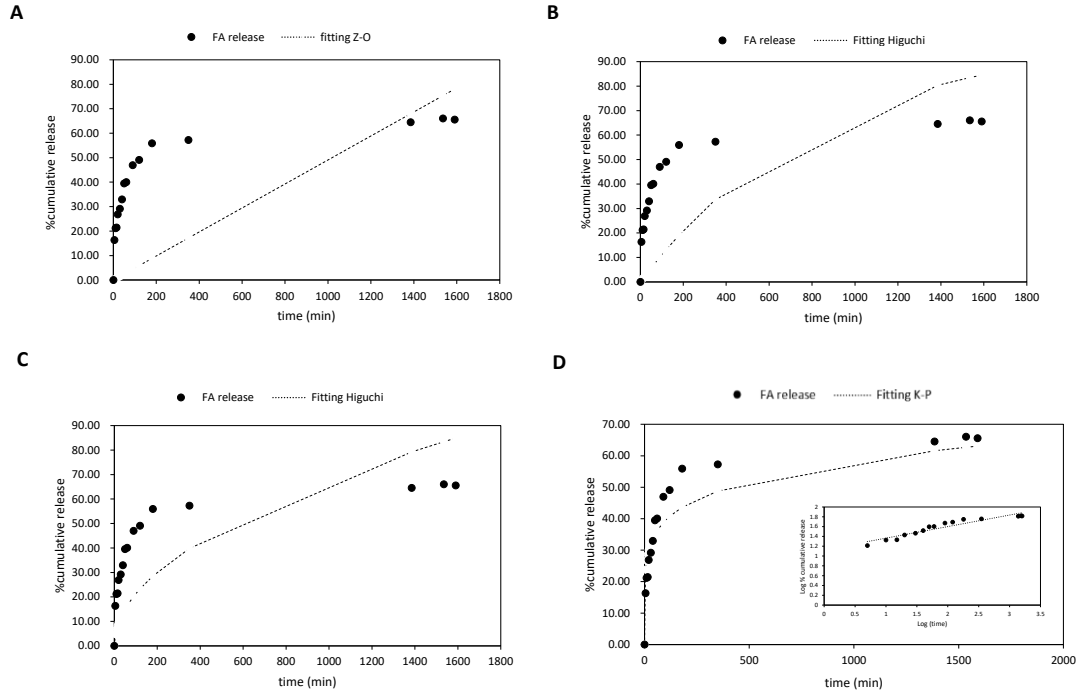

Figure S7. A. Normalised scavenging activities of FA-loaded PLGA NPs using the DPPH· assay. Two FA concentrations were used: 0.2 and 0.1 mg·mL^{-1}. A free FA solution with the same concentration was used as a control. B. Normalised scavenging activities of FA-loaded PLGA NPs using the DPPH· assay at different time intervals (5, 10, and 15 min). The concentration of PLGA in in both experiments was 0.233 mg·mL^{-1}. In (B), the concentration of FA used was 0.2 mg·mL^{-1}.

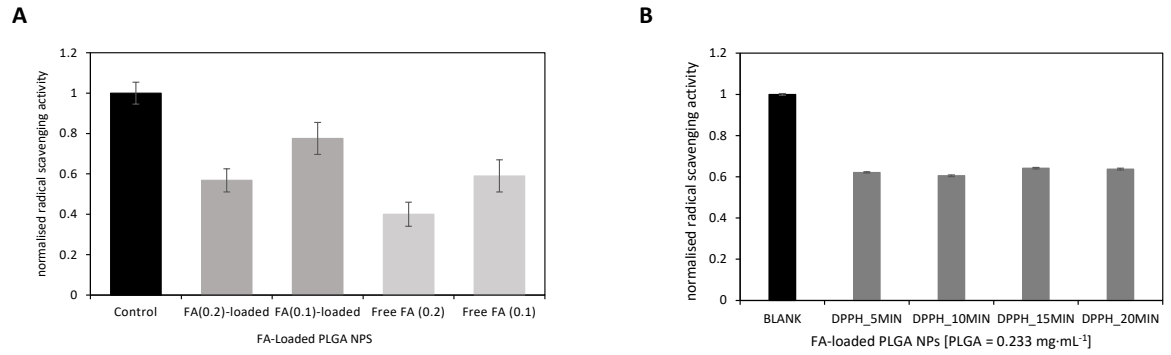

Figure S8. Cytotoxicity. Three FA-loaded NPs were studied. Bare PLGA NPs and free RA in solution were also used as controls. Standard deviation (SD = 3) of 3 independent experiments. B. HA-h, HBVP, and hCMEC/D3 cells viability tested with FA-loaded PLGA_FITC and non-loaded PLGA_FITC NPS. Cells treated with PBS and cells treated with 20% sodium dodecyl sulphate were used as live (CL), vehicle (Cvh) and dead (CD) control, (n=3).

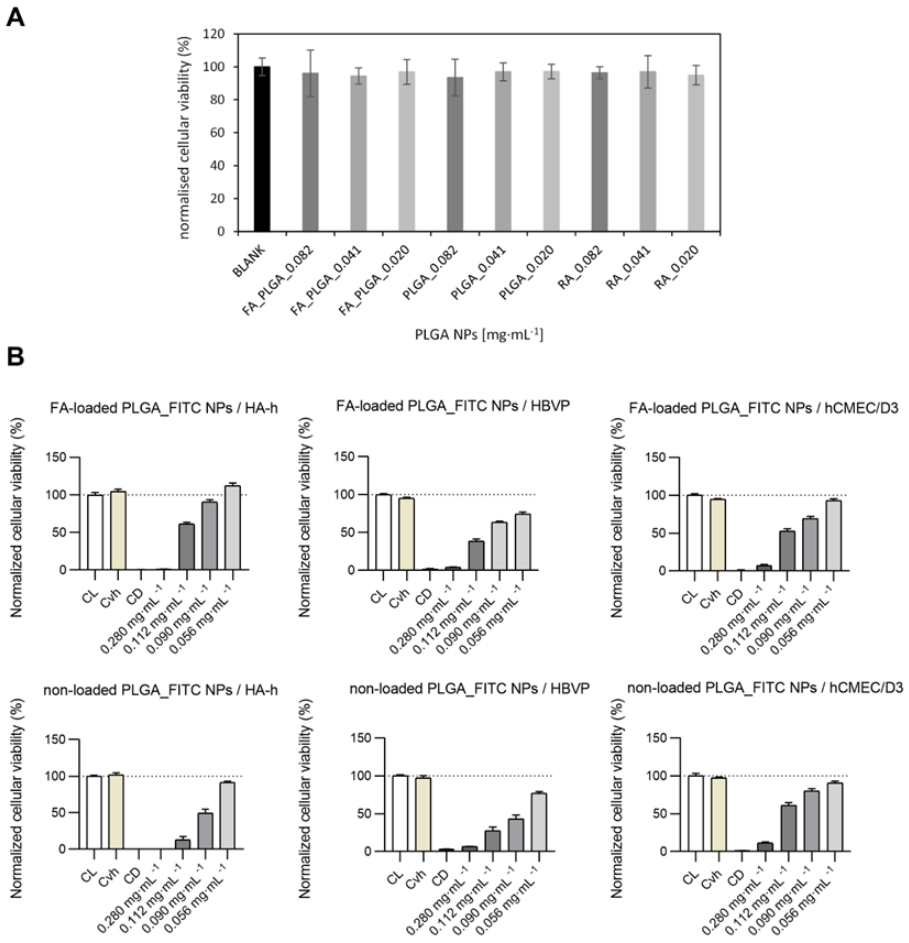

Figure S9. Synthetic strategy to conjugate a fluorescein molecule to FA-loaded PLGA NPs. Inset: A poly(ethylene glycol)diamine molecule was selected as a linker to connect both FA-loaded NPs to fluorescein

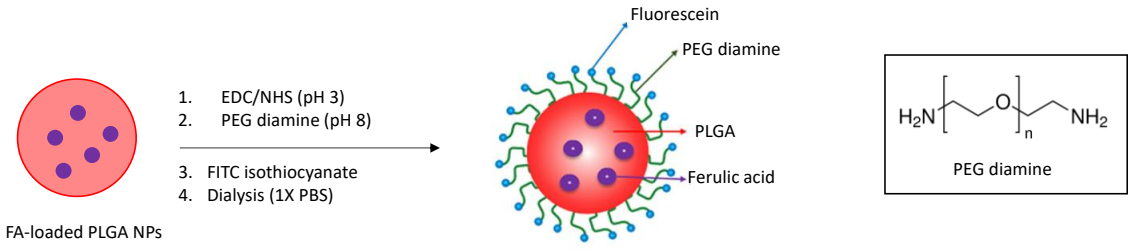

Figure S10. ^{1}H-NMR of lyophilized PEGbisamine-conjugated PLGA nanoparticles in MeOD. The PLGA/PEG molar ratio was obtained from the corresponding CH_2(PLGA) (A) and H_2COCH_2 (PEG) (B) signals.

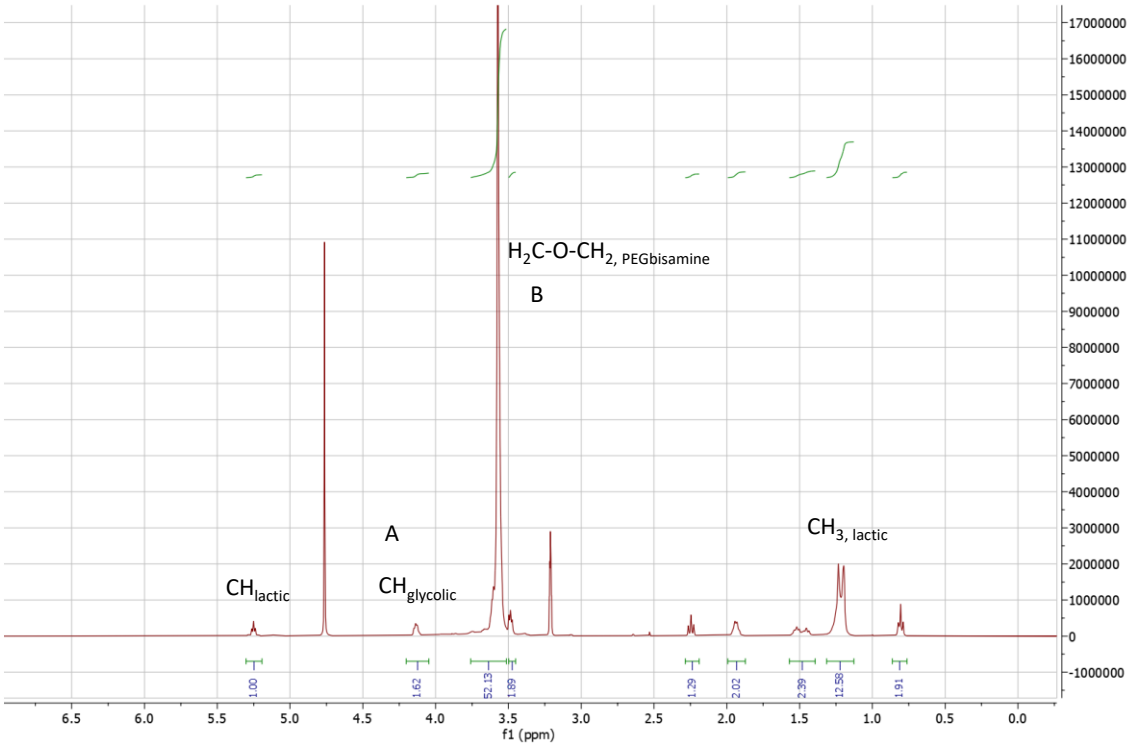

Figure S11. DLS size distributions of unloaded PLGA_Flu (A). (B) Visual appearance and DLS size distribution of FA-loaded PLGA_Flu NPs.

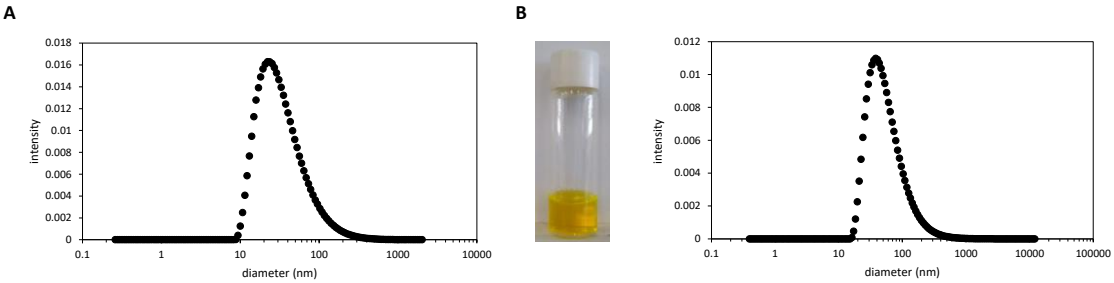

Table S2. Average hydrodynamic diameter and PDI values for PLGA_Flu and FA-loaded PLGA_Flu

Hydrodynamic diameter (nm)		PDI		ζ-potential
PLGA_Flu	**FA-loaded PLGA_Flu**	**PLGA_Flu**	**FA-loaded PLGA_Flu**	**FA-loaded PLGA_Flu**
48.0 ± 4.0	65.3 ± 4.71	0.26 ± 0.06	0.29 ± 0.024	-13.6 ± 0.1 mV

Figure S12. ROS scavenging assay and cellular viability. Two FA-loaded PLGA NPs were evaluated (0.082 and 0.041 mg·mL-1). The viability of cells upon hydrogen peroxide was studied using the MTT assay. Bare PLGA and free FA at the same concentrations were used as controls. Experiment was carried out in triplicate.