Electronic Supplementary Material (ESI) for Nanoscale.

Facile Synthesis of Lactoferrin Conjugated Ultra Small Silica Nanoparticle for Drug Delivery to Brain Tumours

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Supplementary Information

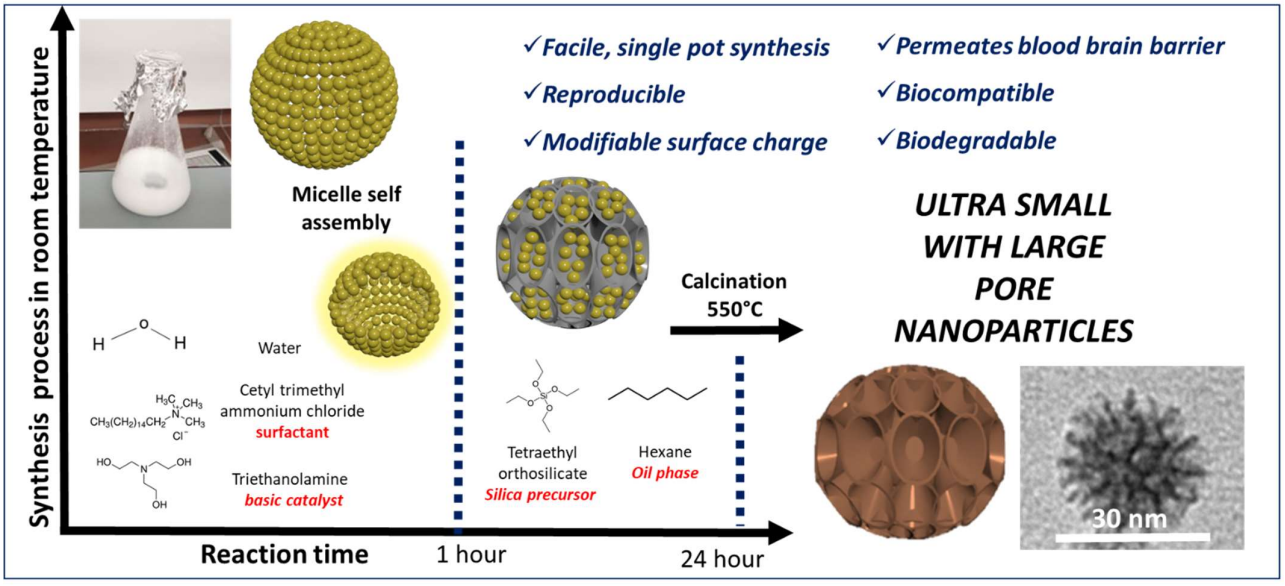


Figure S1 Proposed mechanism for synthesis of ultra small large pore silica nanoparticles.

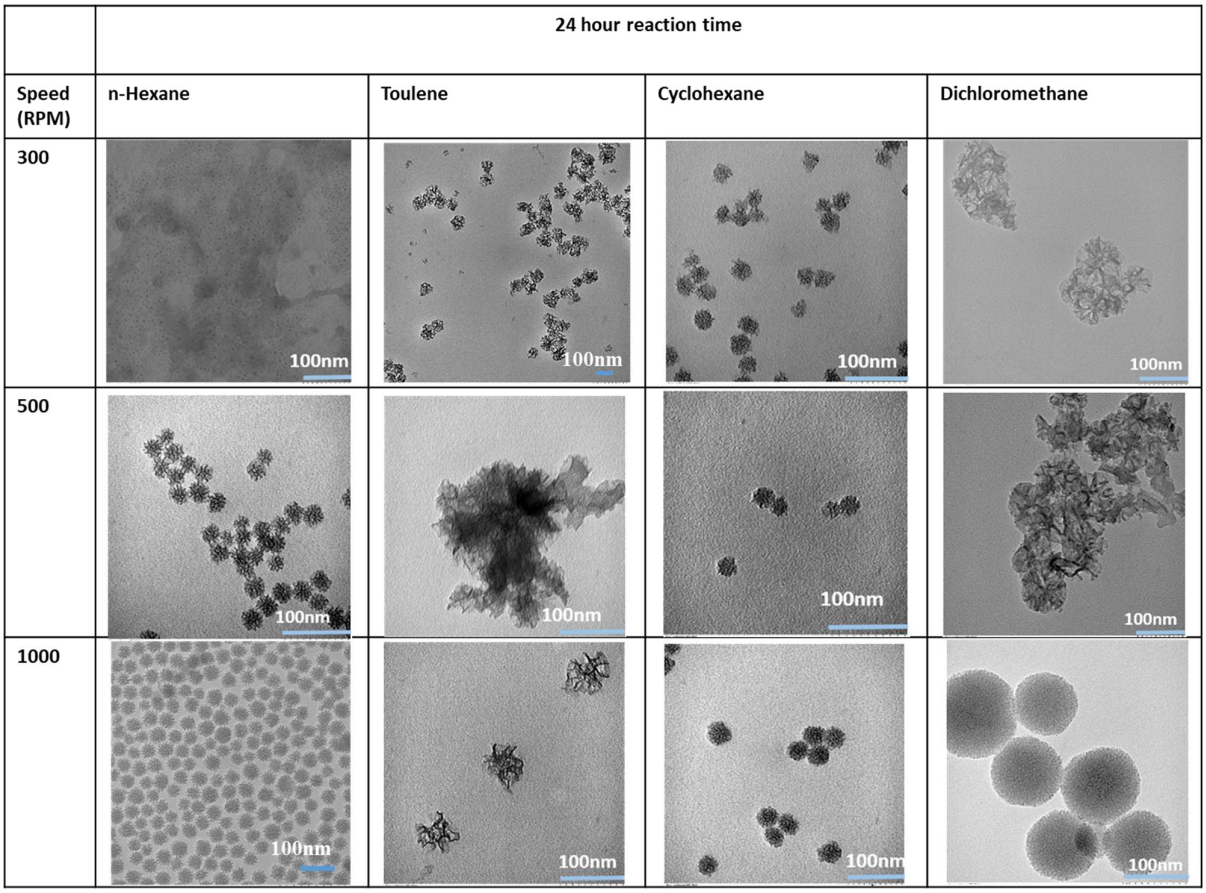


Figure S2. Transmission electron for synthesis optimisation to generate ultra-small silica nanoparticle (USLP). To synthesize these particles, 0.6g TEA was mixed with 18 mL deionised water and 1mL CTAC. The solution was stirred for 1 hour at 1000 rpm in ambient room temperature. Then 2 mL TEOS and 18 mL n-hexane, toluene, cyclohexane or dichloromethane were then added, and this solution was then stirred continuously for a further 24 hours. Nanoparticles were synthesised with different stirring speeds. After calcination, these particles were imaged to characterise both the surface morphology and pore size.

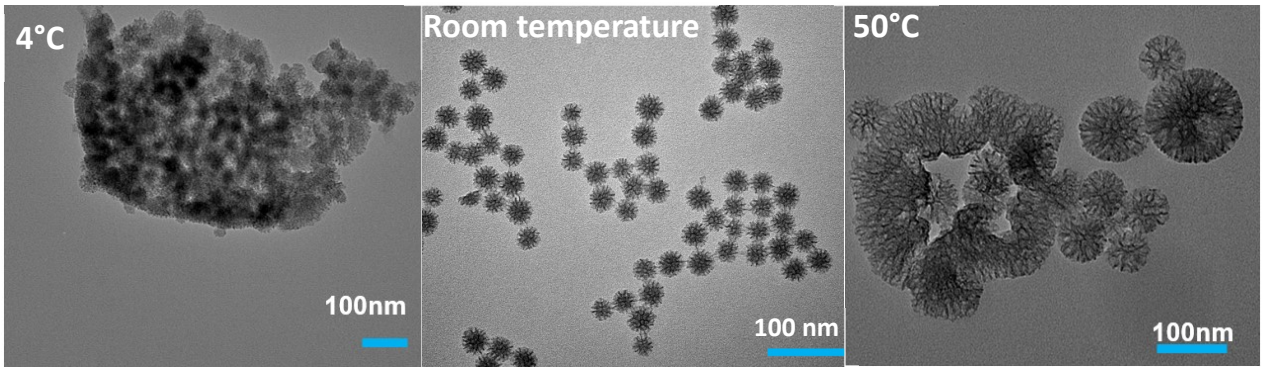


Figure S3 Effect of temperature on synthesis reaction. All parameters for USLP synthesis kept constant except the temperature of synthesis reaction. Note the particle grow in size with increase in temperature to 50°C and do not form at 4°C temperature.

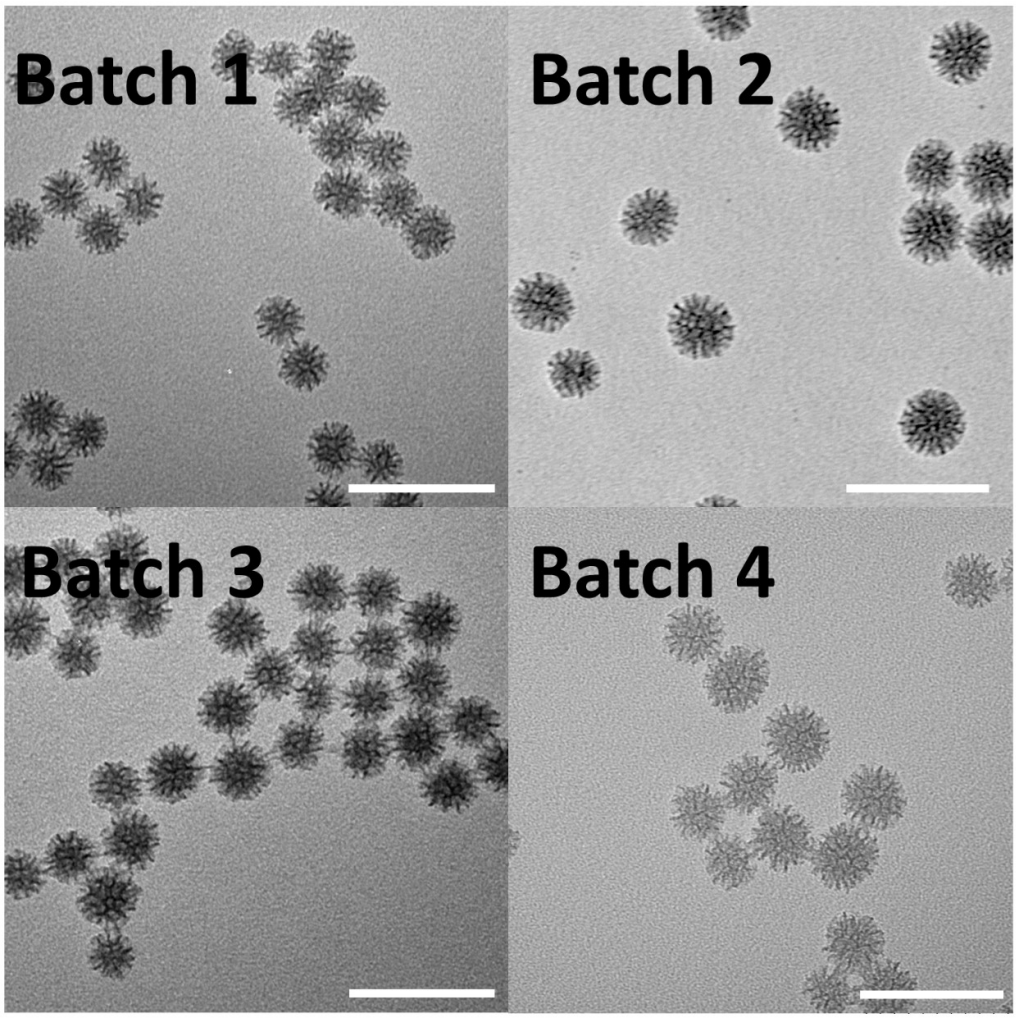


Figure S4. Reproducibility of USLP synthesis. Different batches of USLP were prepared on different days to ensure synthesis protocol is reproducible with nanoparticle morphology. Transmission electron microscope images of different batches. White scale bar 100 nm.

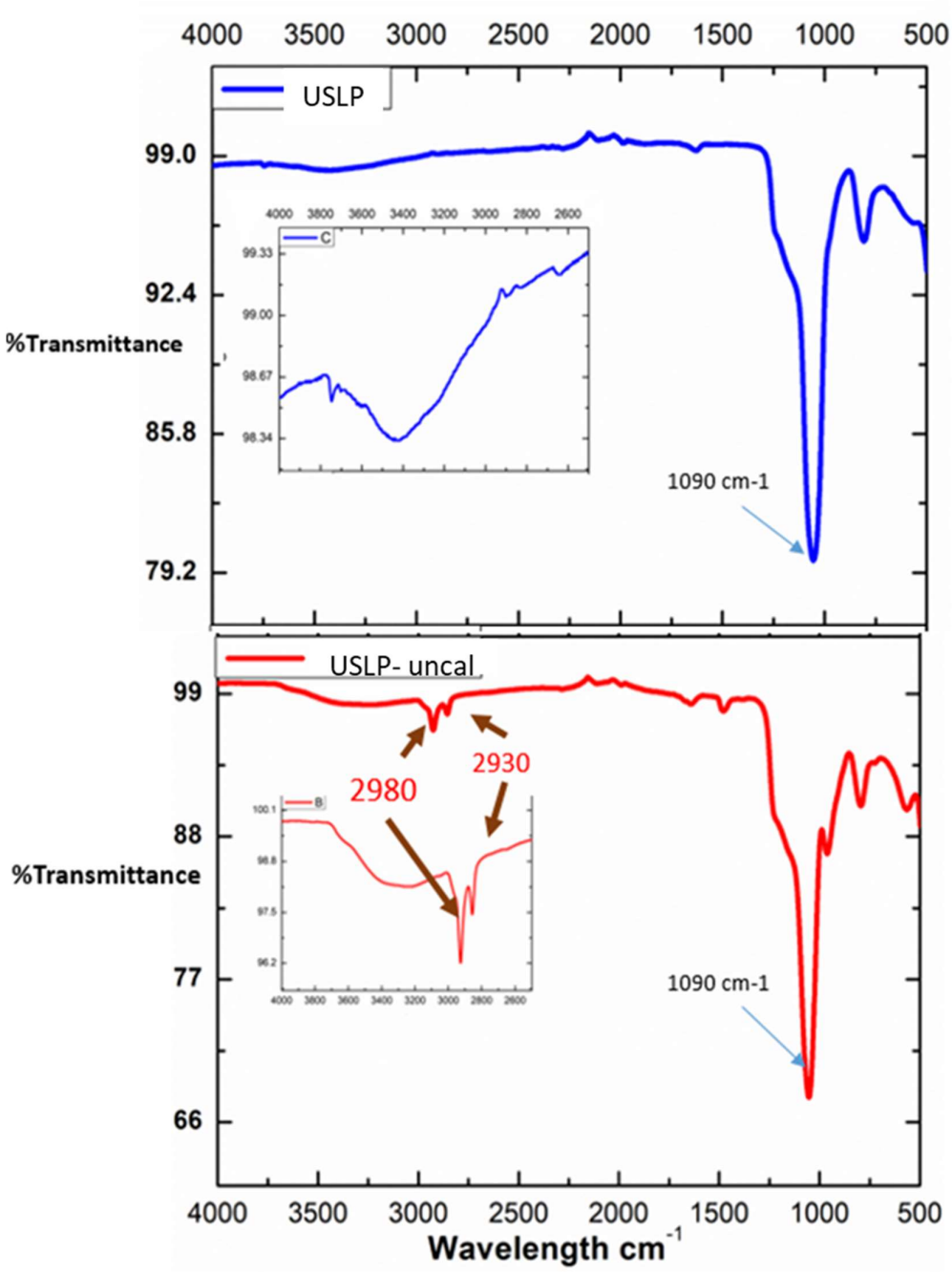


Figure S5. Fourier-transform infrared spectroscopy of USLP after calcination (top) and before calcination (bottom)

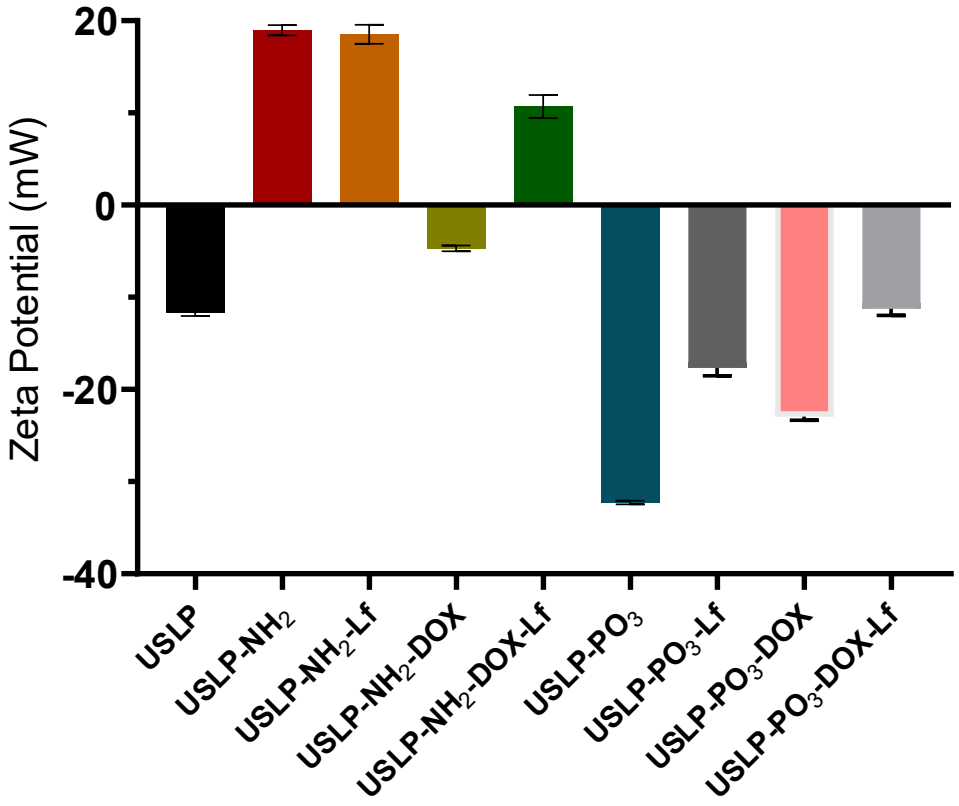


Figure S6. Zeta potential measurements for different nanoparticles with and without surface functionalisation, drug loading and lactoferrin coating onto USLP. To measure the zeta potential, 0.1 mg/mL nanoparticle were suspended in water and sonicated for 10 minutes before analysis (n=3).

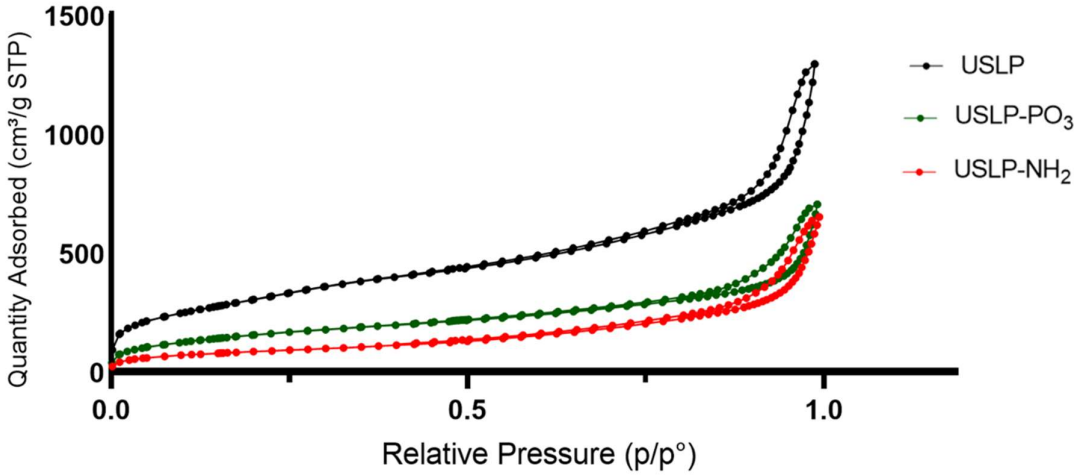


Figure S7. Nitrogen adsoprtion analysis. NanoparticLe weighing between 50-100mg were degassed for 24 hours at 100°C to remove any moisture and air from porous structure. The surface area decreases post-functionalisation, and this effect is more pronounced in USLP-NH_2 compared to USLP-PO_3. The isotherm shape of USLP nanoparticles is type IV and it remains unchanged post functionalisation.

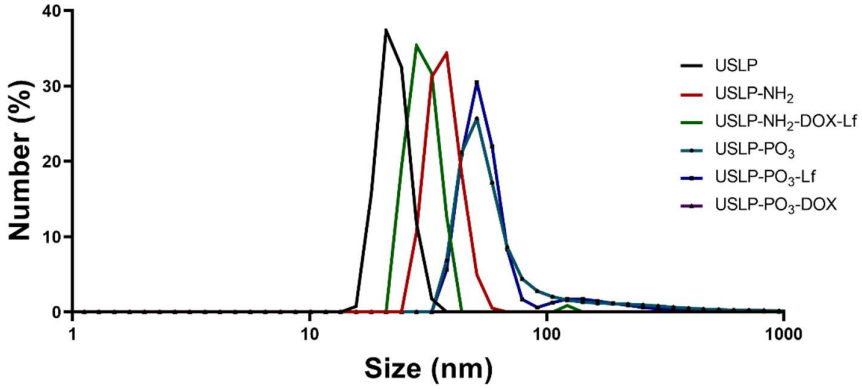


Figure S8. Number mean values for size distribution of nanoparticles using dynamic light scattering method. 0.1 mg/mL nanoparticles were suspended in water and sonicated for 10 minutes before analysis.

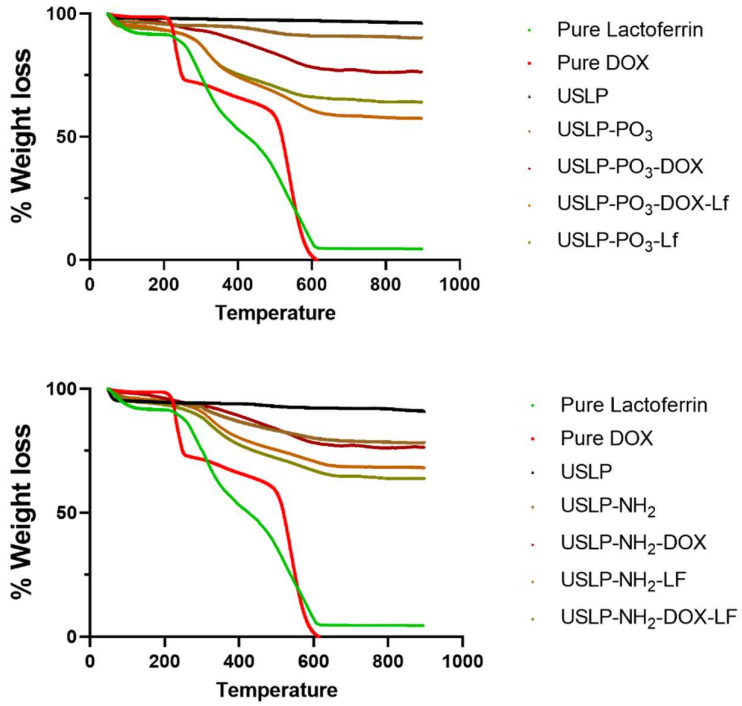
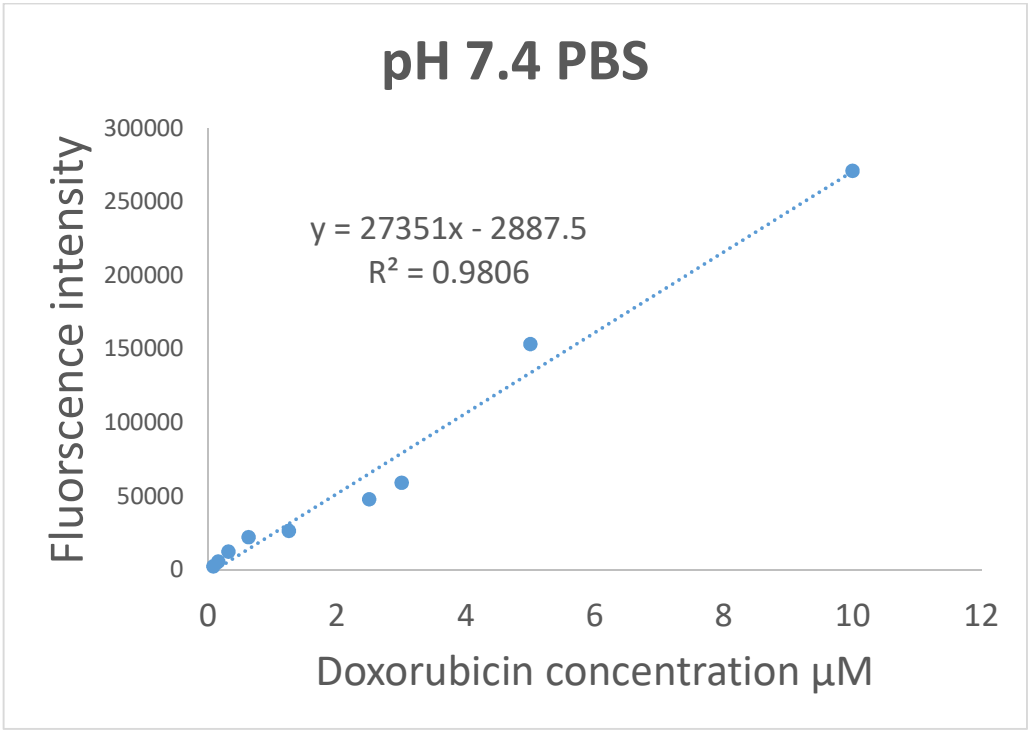


Figure S9 Thermogravimetric analysis to identify loading by measuring weight loss from 100 to 900 °C. Mass grafting of functional group NH_2 and PO_3 , lactoferrin and drug was calculated by weight loss incurred in each type of particle. Doxorubicin loading in USLP-PO_3-DOX 16%, USLP-PO_3-DOX-Lf 11%, USLP-NH_2-DOX 6%, USLP-NH_2-DOX-Lf 2%. The lactoferrin amount loaded on USLP-PO_3 25% w/w and USLP-NH_2 13% w/w



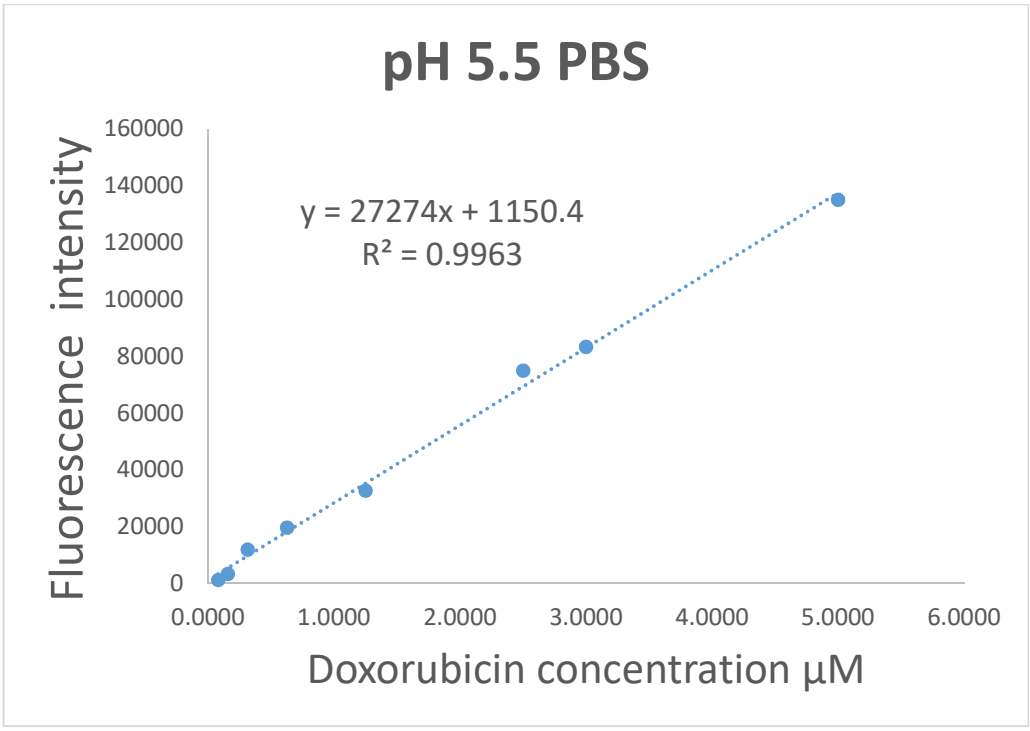


Figure S10 Doxorubicin fluorescence calibration curve in pH 5.5 and pH 7.4 phosphate buffer solution. Measurement taken with Perkin Elmer EnSight Fluorescence Machine at excitation wavelength 470 nm and emission wavelength 560 nm

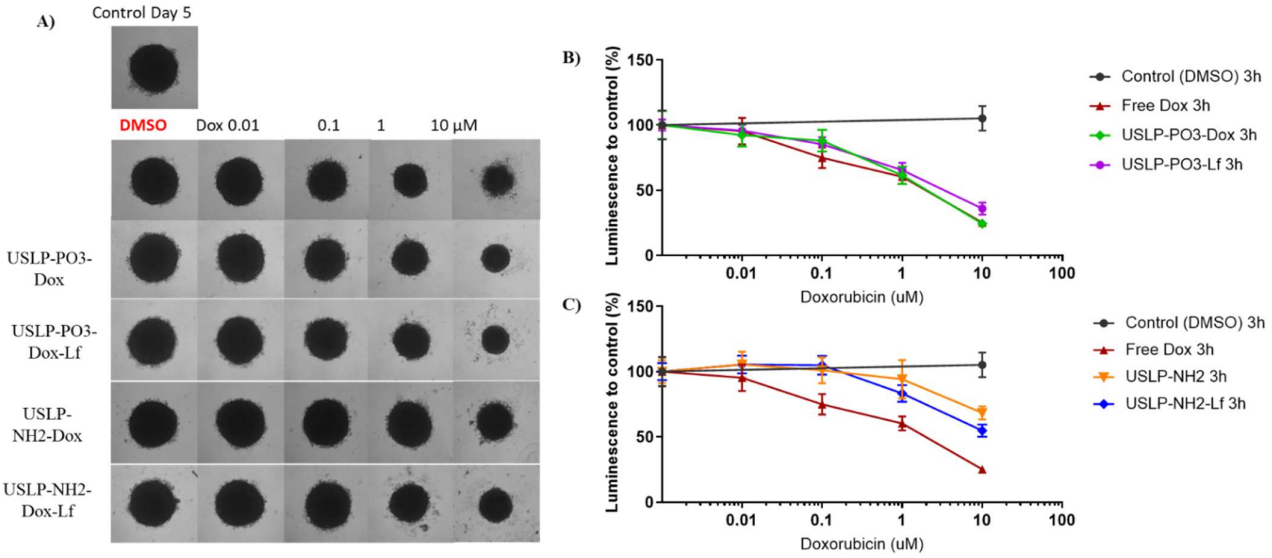


Figure S11 Doxorubicin loaded mesoporous silica nanoparticle (USLP) 3D cytotoxicity data in glioblastoma (U87) tumour spheroids after 3 hours. A) Representative z-stack of U87 spheroids treated with DMSO, USLP_PO3_Dox, USLP_PO3_Dox_Lf, USLP_NH2_Dox and USLP_NH2_Dox_Lf and (B, C) quantified changes in luminesces intensity compared to control with various doxorubicin concentrations after 3 hour treatment. Images acquired using a Zeiss LSM 880 confocal microscope (Fast airy, sequential frame-fast laser excitation at 561 nm and 633 nm, 10X objective).

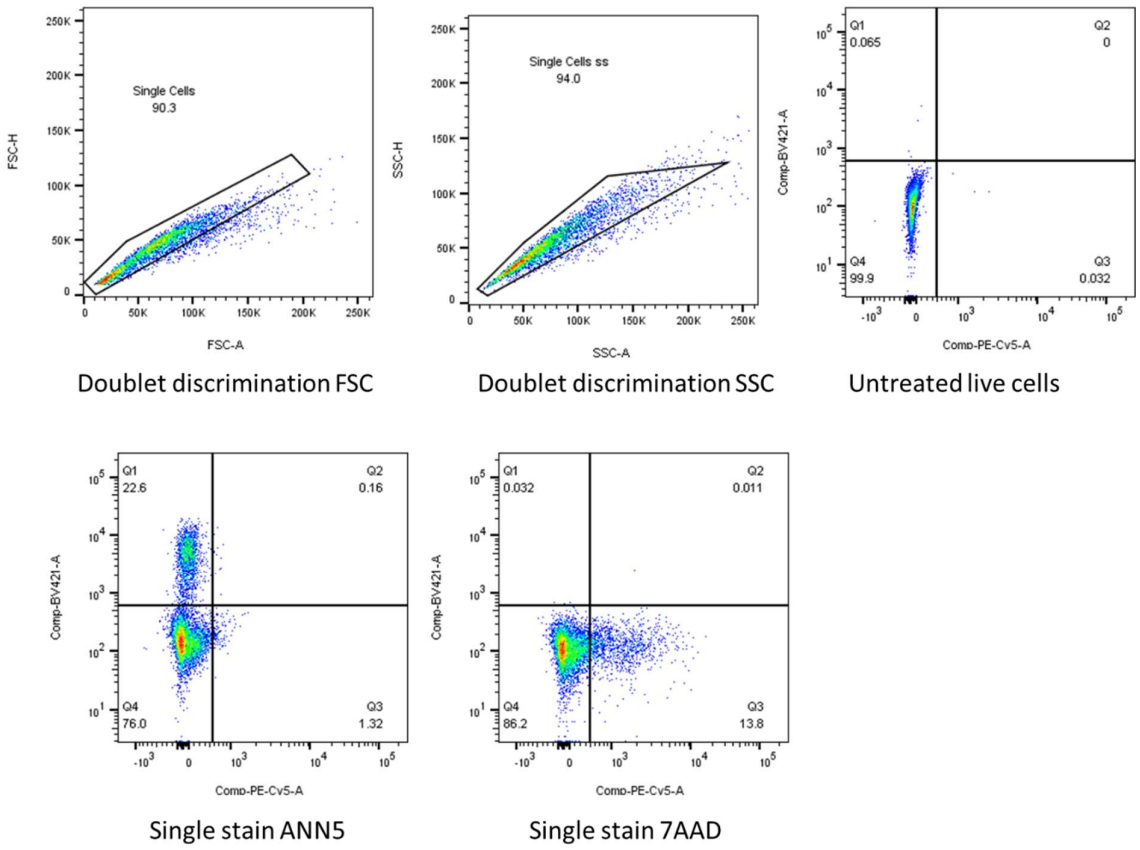


Figure S12 Gating strategy U87 cell line. The cells were doublet discriminated using both forward scattering (FSC) and side scattering (SSC). Unstained untreated control group was used to gate for live cells, single stain annexin 5 and 7AAD were used to gate for positive cell populations