Supporting Information

Improving Anticancer Activity of Chrysin using Tumor Microenvironment pH-Responsive and Self-Assembled Nanoparticles

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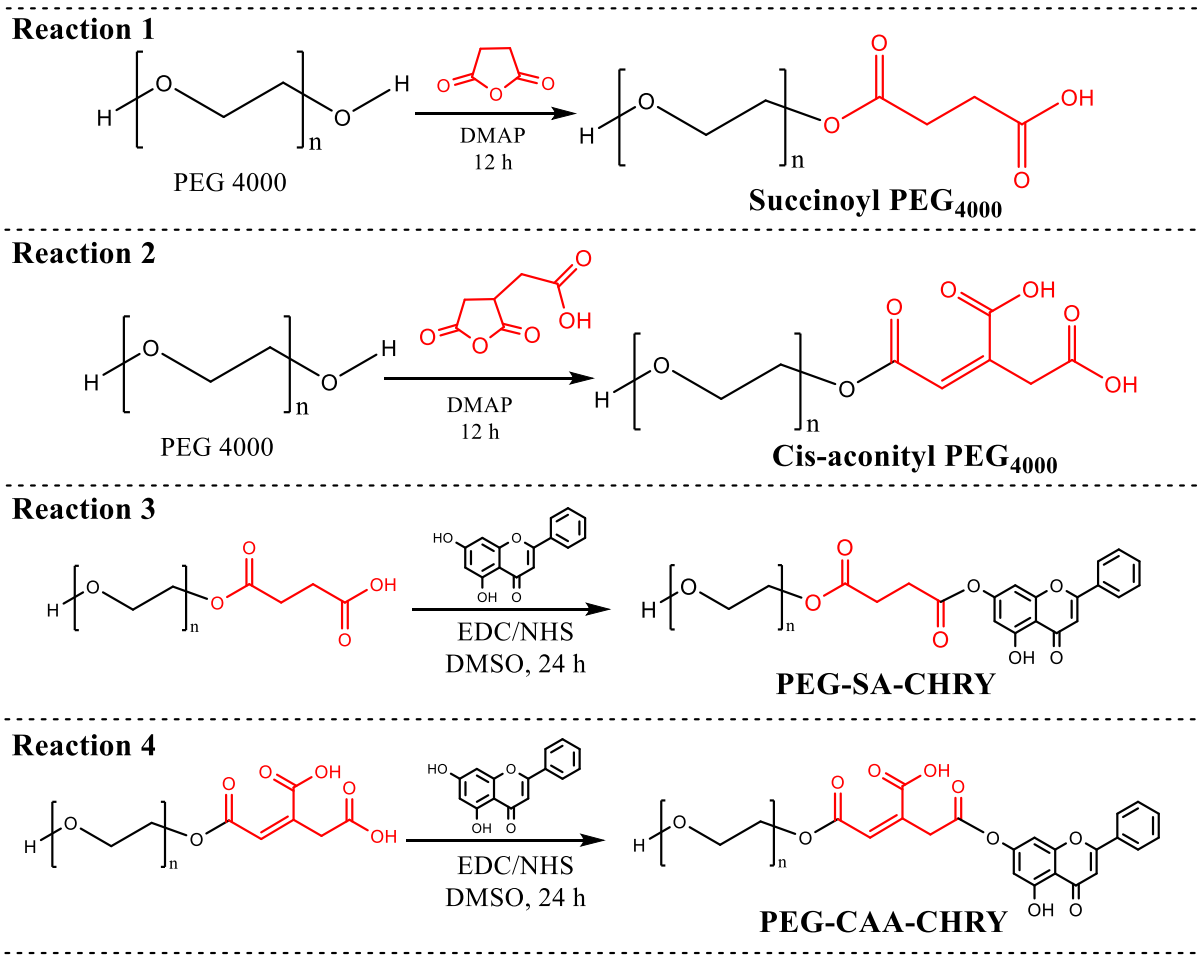

Figure S1. Reaction schemes represent the PEGylation of chrysin *via* succinoyl group (PEG-SA-CHRY) and cis-aconityl group (PEG-CAA-CHRY).

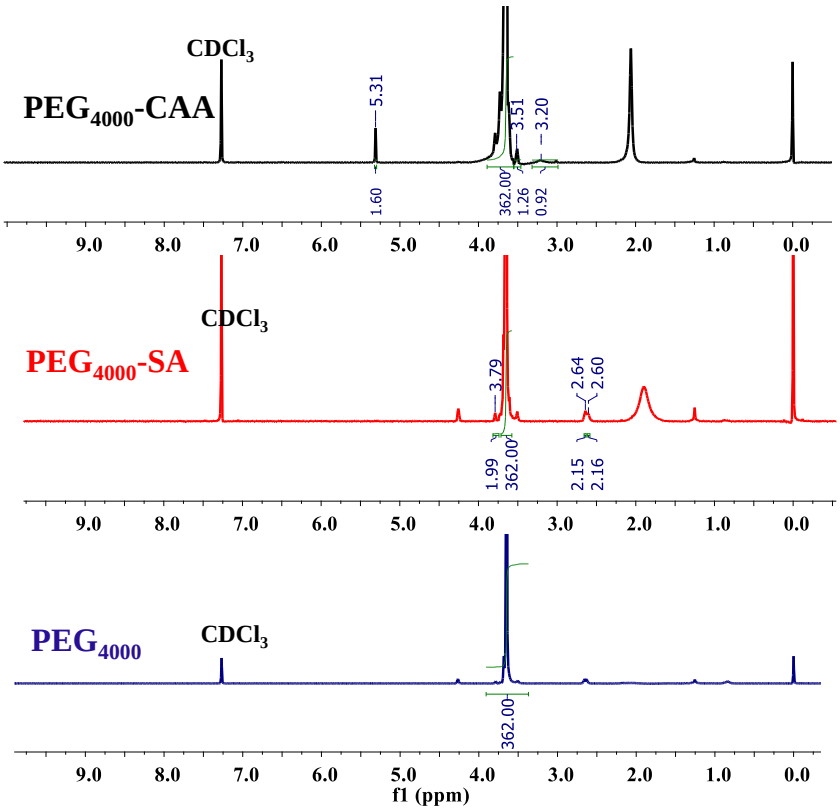

Figure S2. ^{1}H-NMR spectra of pure PEG_{4000}, succinoyl PEG_{4000} (PEG_{4000} -SA) and cis-aconityl PEG_{4000} (PEG_{4000} -CAA). The ^{1}H-NMR spectra were recorded in $CDCl_3$.

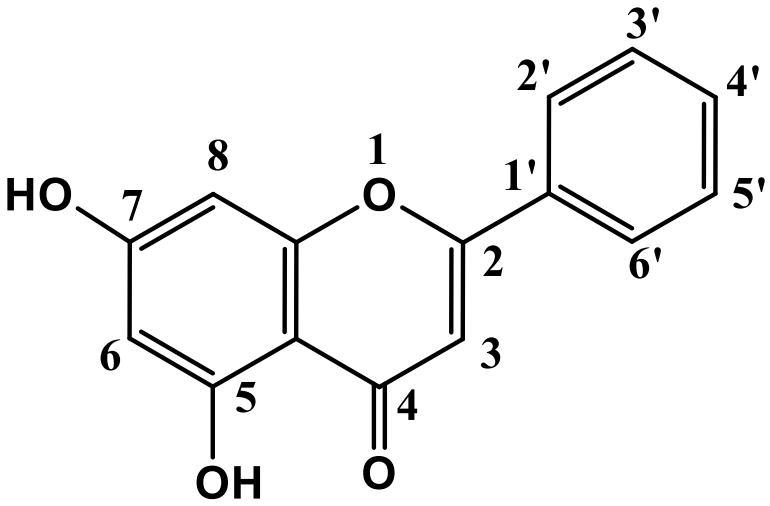

Figure S3. Chemical structure of chrysin.

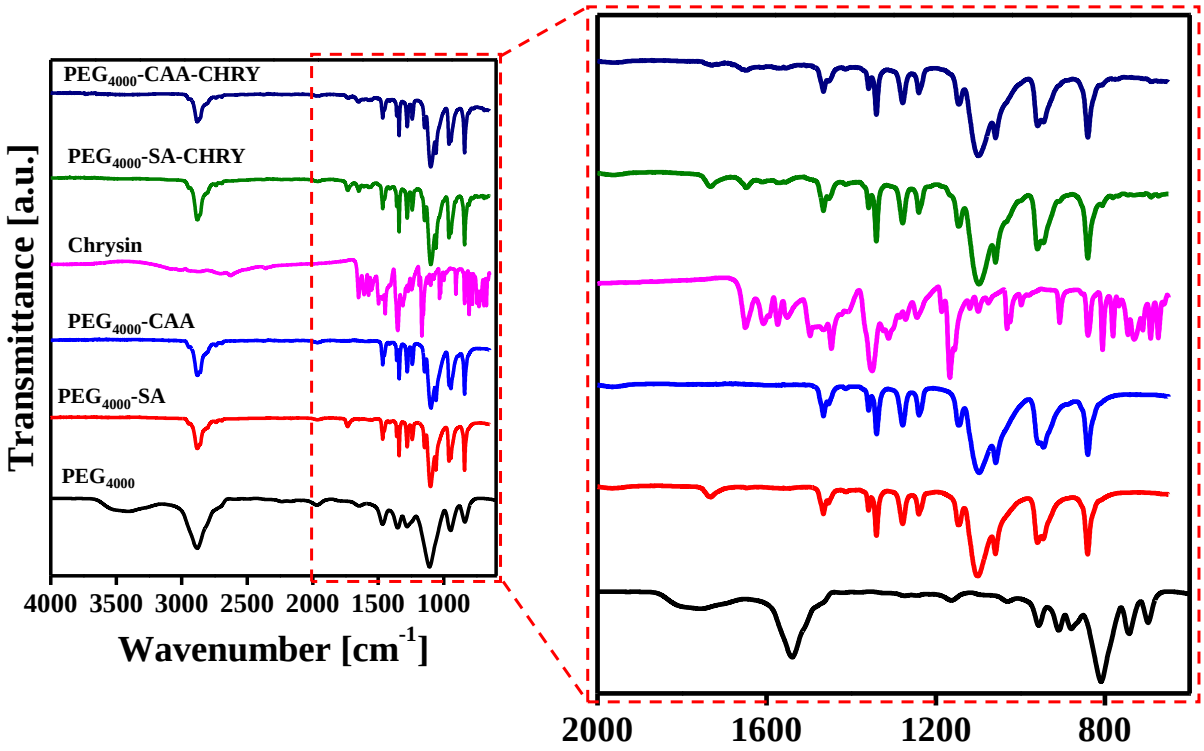

Figure S4. FTIR spectra of pure PEG_{4000}, succinoyl PEG_{4000} (PEG_{4000} -SA), cis-aconityl PEG_{4000} (PEG_{4000} -CAA), chrysin (CHRY), PEG_{4000}–chrysin *via* succinoyl linker (PEG_{4000}-SA-CHRY) and PEG_{4000}–chrysin *via* cis-aconityl linker (PEG_{4000}-CAA-CHRY).

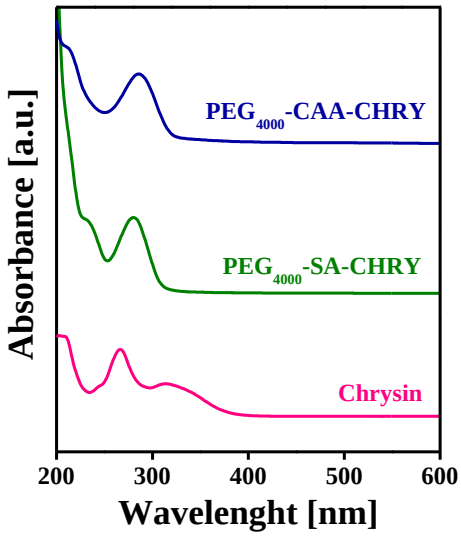

Figure S5. UV-visible spectra of pure chrysin, PEG_{4000}–chrysin *via* succinoyl linker (PEG_{4000}-SA-CHRY) and PEG_{4000}–chrysin *via* cis-aconityl linker (PEG_{4000}-CAA-CHRY).

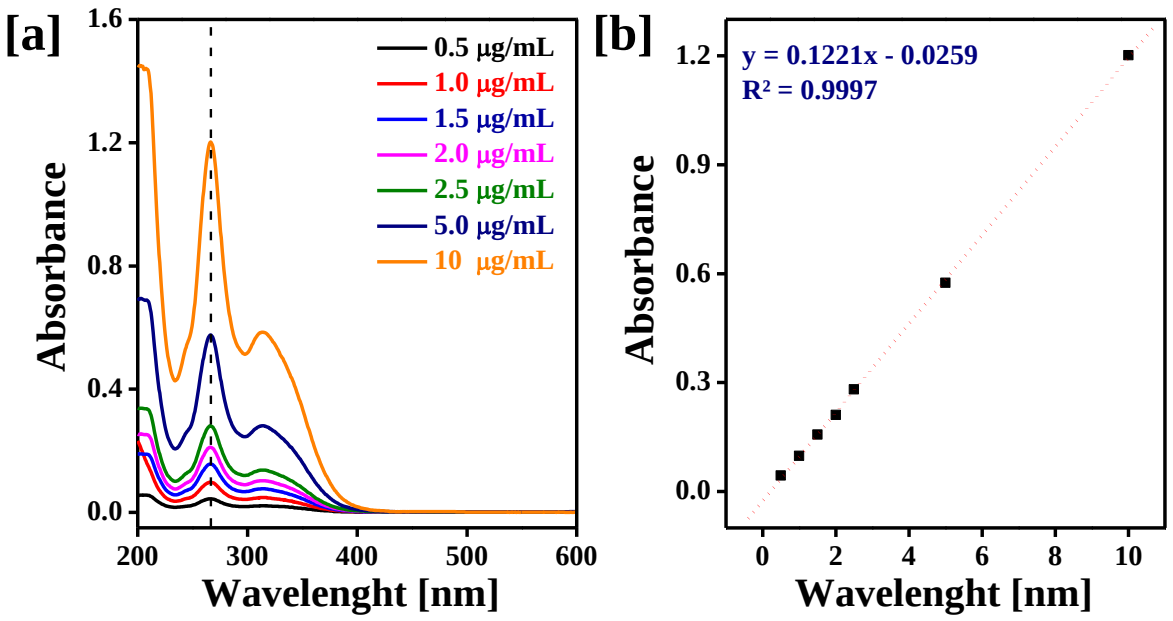

Figure S6. (a) UV-visible spectra of pure chrysin at different concentrations, (b) calibration curve of chrysin at 266 nm.

Sample	Particle size (nm)	PDI	Zeta	%LE
PCNP-1	77.30 ±0.47	0.173 ±0.003	– 4.25	4.05
PCNP-2	75.59 ±0.40	0.185 ±0.040	– 3.47	4.11

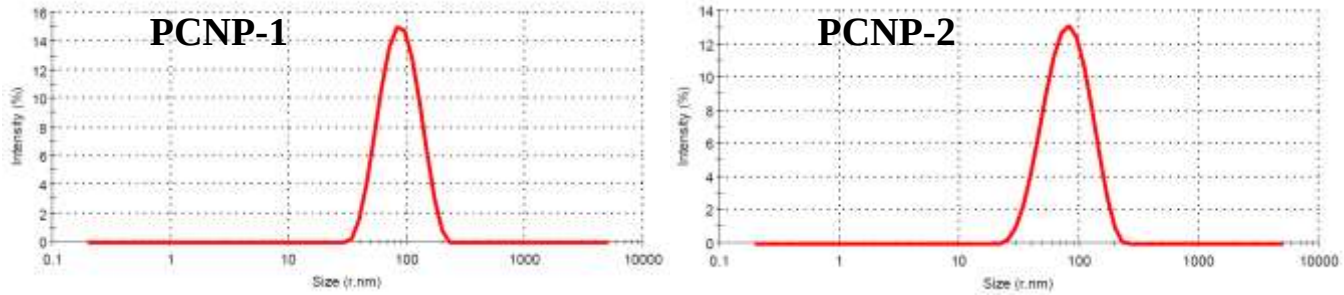

Figure S7. Physicochemical properties of PEGylated chrysin nanoparticles.

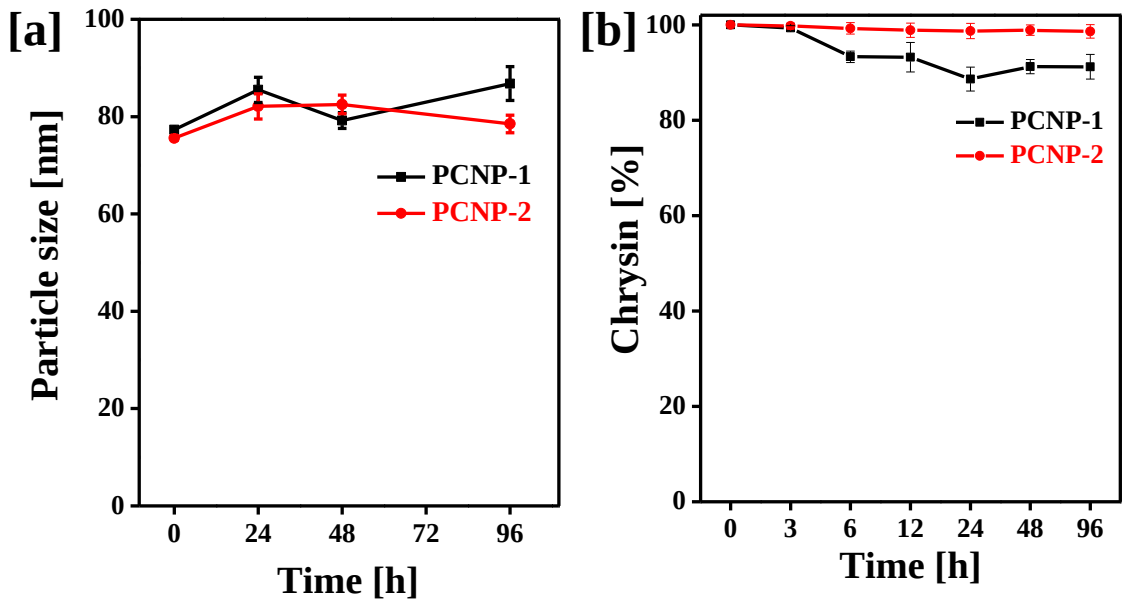

Figure S8. Stability of the PEGylated chrysin nanoparticles (a) colloidal stability and (b) % drug content analysis.

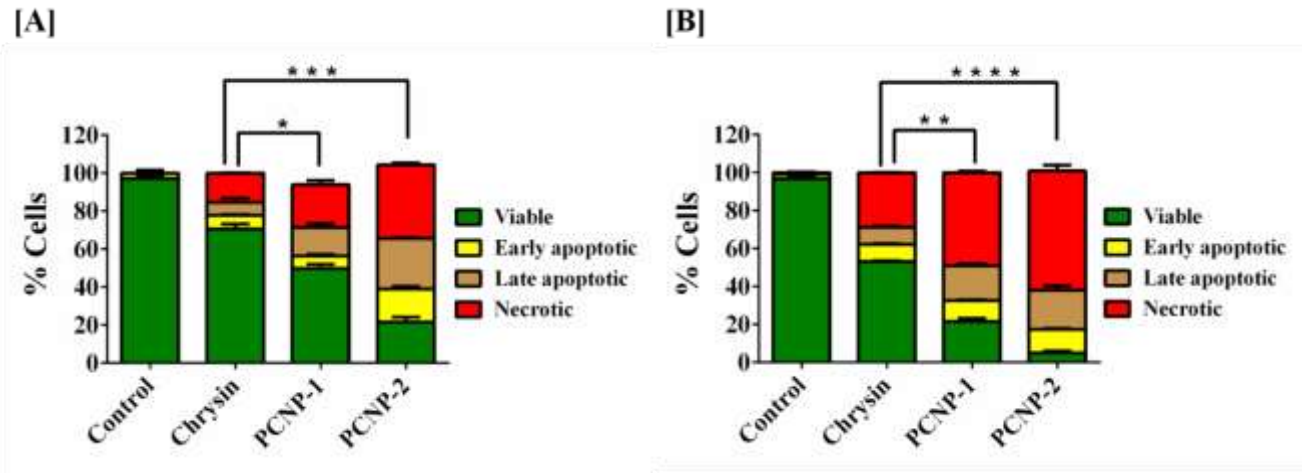

Figure S9. % cells of viable, early and late apoptotic and necrotic cells after treated with chrysin, PCNP-1 and PCNP-2 for (a) 24 h and (b) 48 h. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).