Electronic Supporting Information

Facile design of autogenous stimuli-responsive chitosan/hyaluronic acid nanoparticles for efficient small molecules to protein delivery

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1. $^{13}C\{^{1}H\}$ NMR Analyses of DCS/FCS

Dissolution (DCS) and functionalization (FCS) of chitosan using TKDA was analyzed through ^{13}C NMR. In DCS, the TKDA *C*OOH peak showed a downfield shift from 175.2 ppm to 176.8 ppm. Similarly, the peak from the adjacent *C*H_2 is shifted downfield from 32.8 ppm to 34.3 ppm, while the other TKDA peaks remained consistent. Chemical coupling of TKDA to LCS to yield FCS further shifts the *C*OOH peak downfield to 178.3 ppm and the *C*H_2 peak to 35.7 ppm (Figure S1).

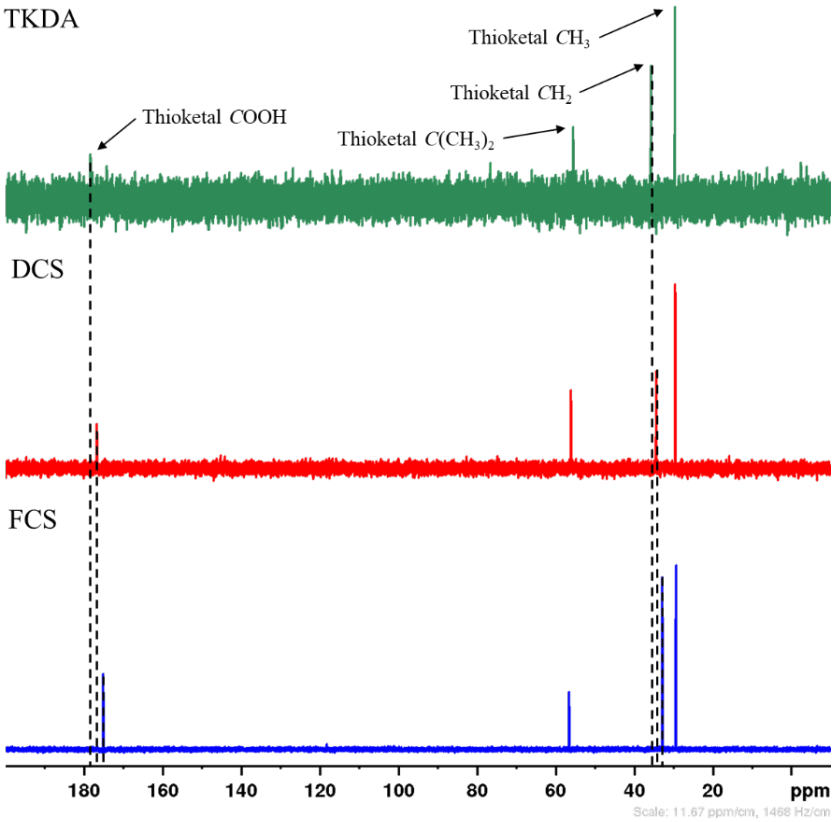


Fig. S1. ^{13}C NMR spectra of TKDA, DCS and FCS prepared by TKDA route.

2. Dynamic light scattering (DLS) analyses

Size distributions and mean hydrodynamic diameters of empty and drug loaded FCS/hyaluronic acid (HA) nanoparticles (NPs) are shown in Fig. S2.

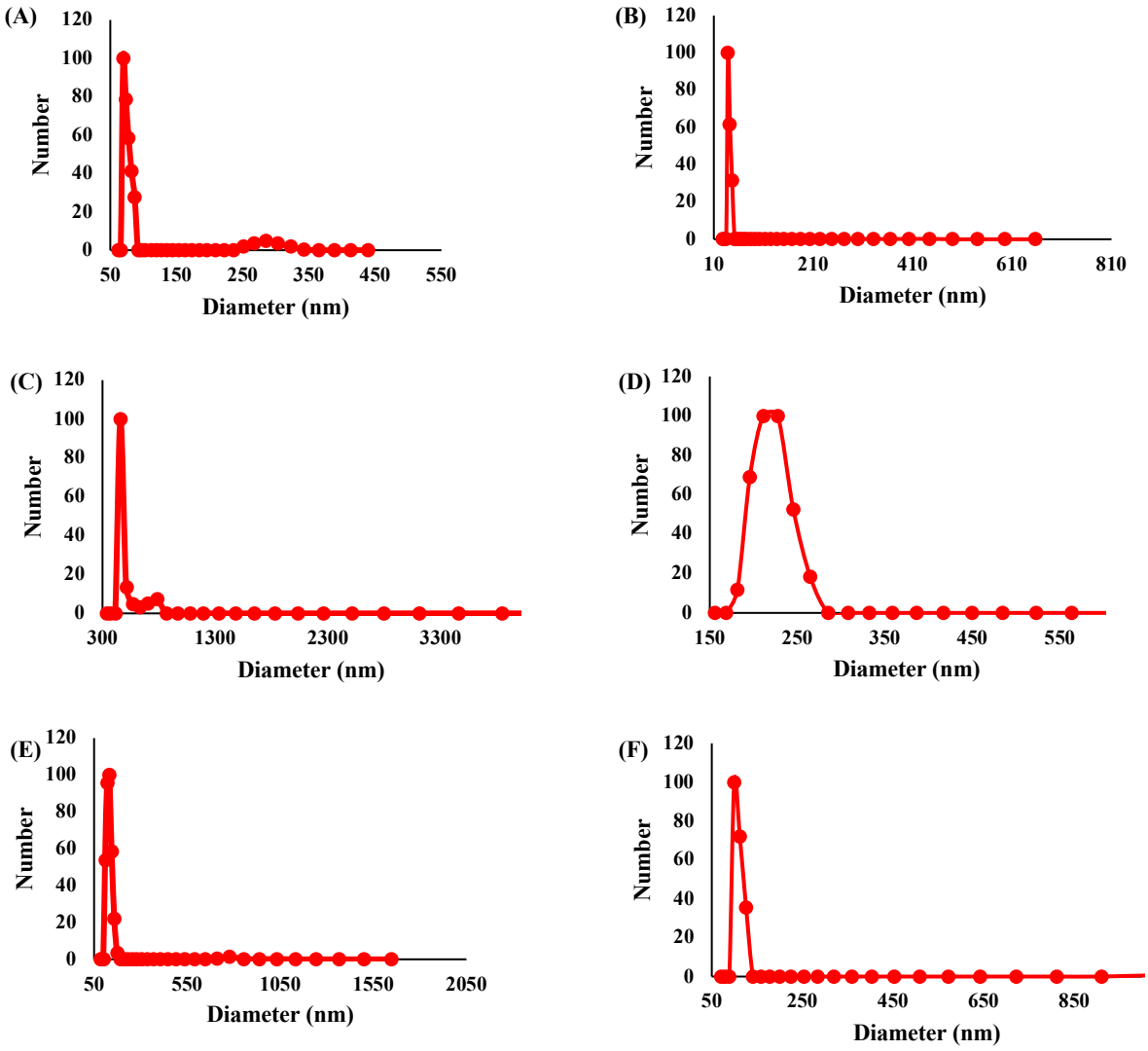


Fig. S2. DLS plots of size distributions for (A) CS/HA, (B) FCS/HA, (C) quercetin loaded CS/HA, (D) quercetin loaded FCS/HA, (E) curcumin loaded FCS/HA and (F) nerve growth factor-fluorescein isothiocyanate (NGF-FITC) loaded FCS/HA NPs.

3. TEM micrograph of porous NGF-FITC FCS/HA NP (Fig. S4)

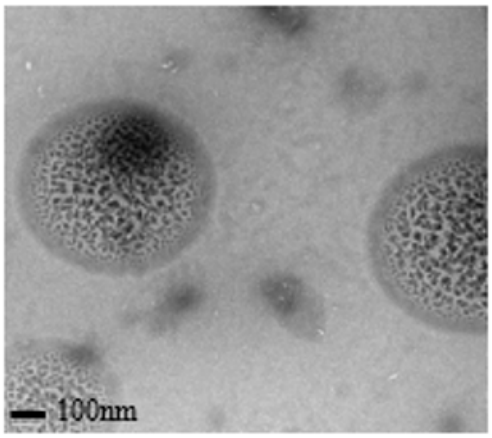


Fig. S3. NGF-FITC loaded FCS/HA porous nanospheres.

4. ROS-responsive behavior of TKDA and FCS

ROS-responsive behavior of the TKDA entity in FCS is exhibited in Fig. S4. The gradually increasing acetone peak over time is shown in top left of each spectrum with red color, as well as formation of SH group was observed between 1.6-1.7 ppm. Time dependent ^{1}H NMR spectra confirmed that TKDA as a ROS-responsive moiety in FCS responded to a small amount of hydrogen peroxide (12.2 µl of 200 mM H_2O_2).

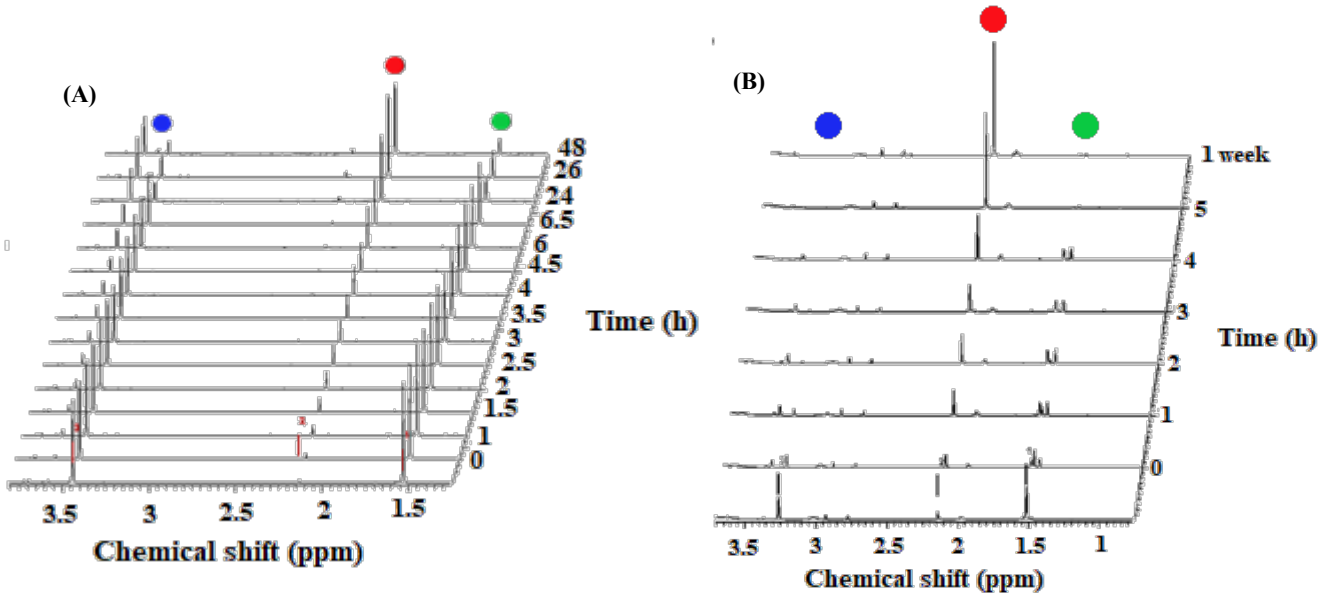


Fig. S4. Time dependent ^{1}H NMR spectra of (A) TKDA and (B) FCS responding to ROS. Green: C*H*$_3$, red: acetone and blue: C*H*$_2$ peaks.

5. Free Drug Release Profiles

As a control for NP-mediated drug release, both quercetin (Fig. S5) and curcumin (Fig. S6) were dissolved in a PEG_{350}/water/dimethyl acetamide mixture with a ratio of 45:40:15 v/v/v, and their drug release profiles were measured.

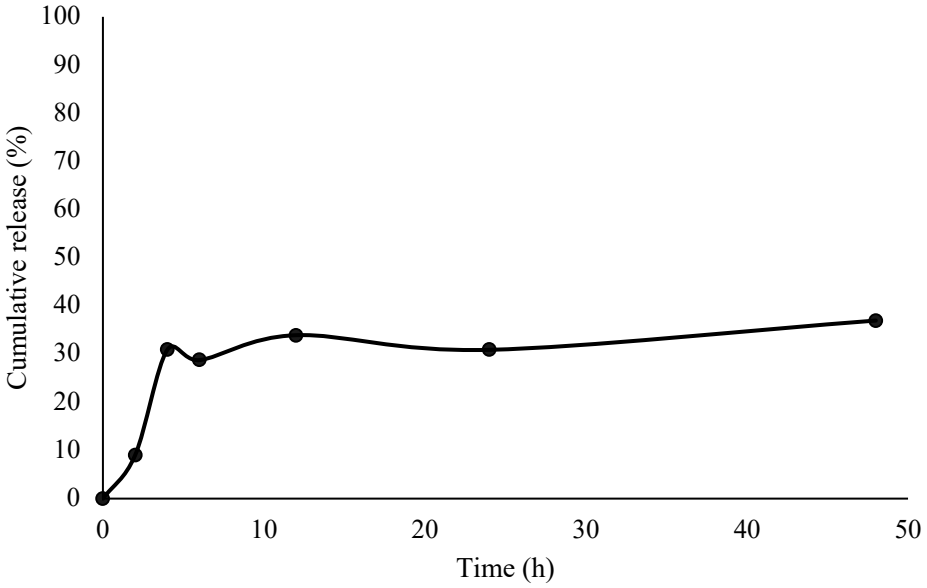


Fig. S5 Free cumulative quercetin release over 48 hours of dialysis time.

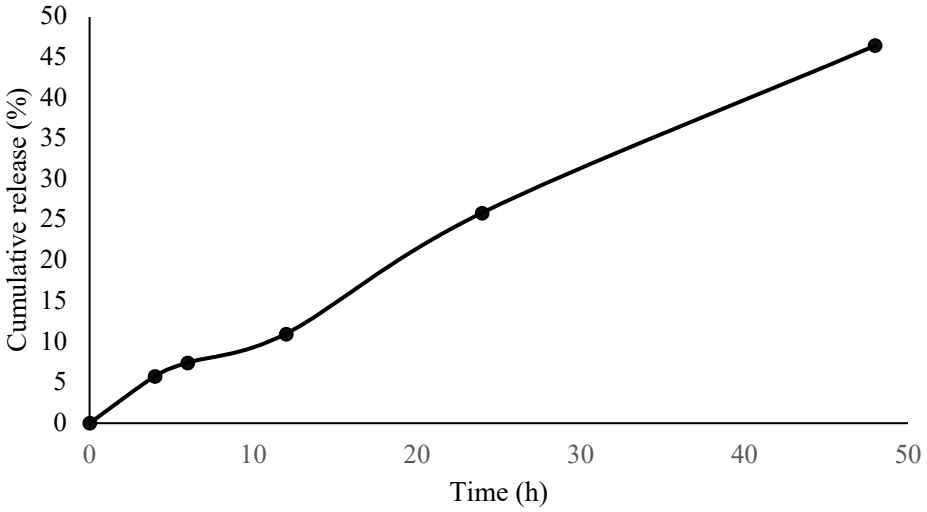


Fig. S6 Free cumulative curcumin release over 48 hours of dialysis time.

6. Evaluation of drug stability in the presence of ROS

To study stability of quercetin in a ROS medium, it was dissolved in methanol at a 0.25 mg/mL concentration. UV-Vis absorption spectra were recorded on a Varian Cary 50 UV-Vis spectrophotometer without H_2O_2, with 100 µM H_2O_2, at zero time and after 24h. Quercetin didn't show any difference in UV absorption peaks (at λ = 255 and 372 nm) after adding 100 µM H_2O_2, but after 24h small changes in UV absorption were observed (Fig. S7).

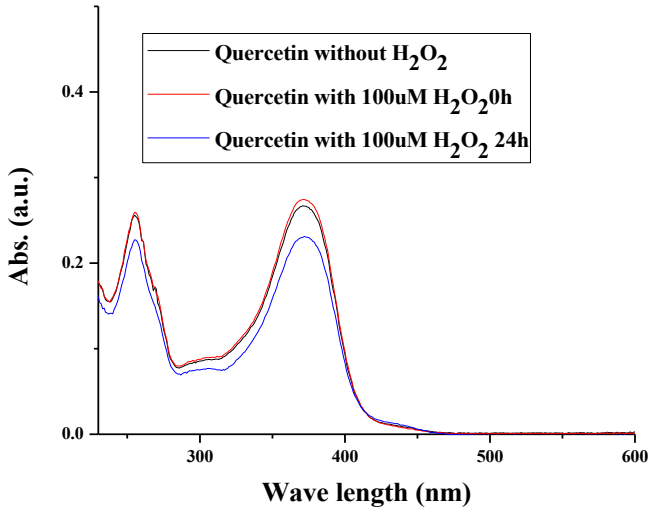
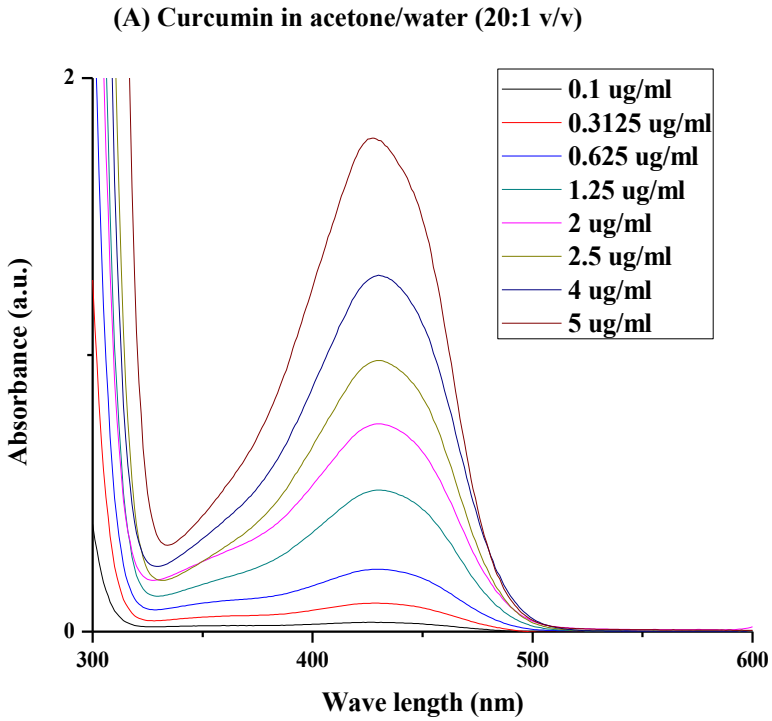


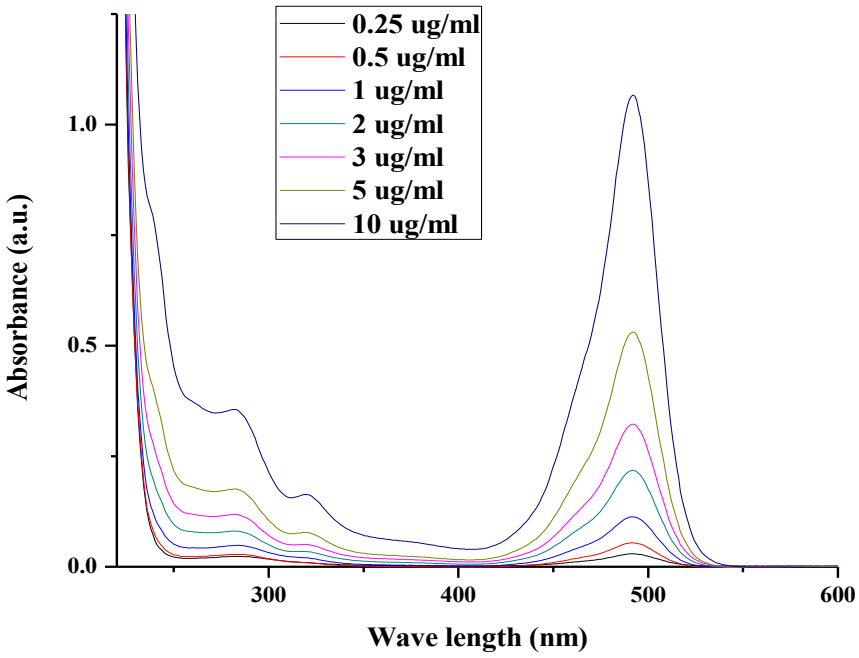
Fig. S7. Stability quercetin in the presence of 100 µM H_2O_2 at zero time and after 24h.

7. UV-visible spectra of cargo compounds and derived standard curves

UV-Vis spectra of different drug molecules and NGF-FITC are shown in Fig. S8. Fig. S9 showed calibration curves of curcumin, NGF-FITC and quercetin solutions with different determined concentrations.



(B) NGF-FITC in water/sodium bicarbonate buffer (20:1 v/v)



(C) Quercetin in methanol/water (20:1 v/v)

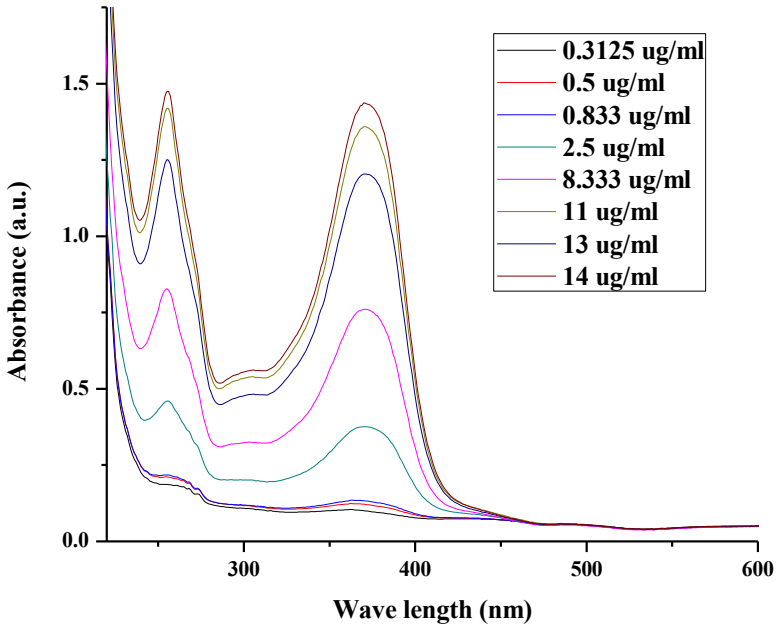


Fig. S8. UV-visible spectra of (A) curcumin, (B) NGF-FITC and (C) quercetin taken to prepare standard curves for each compound

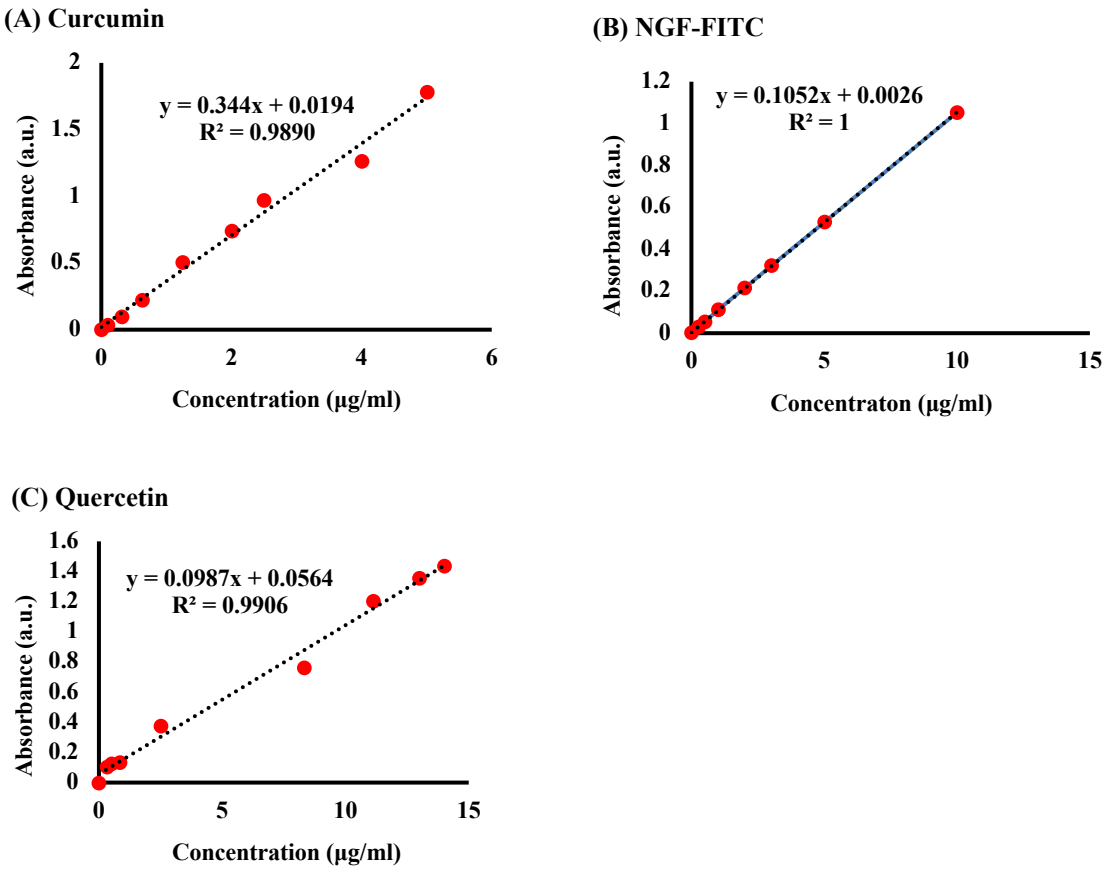


Fig. S9. Calibration curves of (A) curcumin, (B) NGF-FITC and (C) quercetin solutions based on UV-visible spectrophotometry at λ= 437, 494 and 255 nm, respectively.

8. Transcription factor EB (TFEB) nuclear translocation in glioblastoma (GBM) cells

We found comparable TFEB nuclear translocation in GBM treated with curcumin loaded FCS/HA NPs or free curcumin (Fig. S10). Treatment with curcumin loaded FCS/HA NPs showed reduced TFEB nuclear translocation after 1h compared to free curcumin (Fig. S10A-B), although TFEB nuclear translocation became comparable after 8h. Empty FITC-labelled FCS/HA NPs did not induce TFEB nuclear translocation.

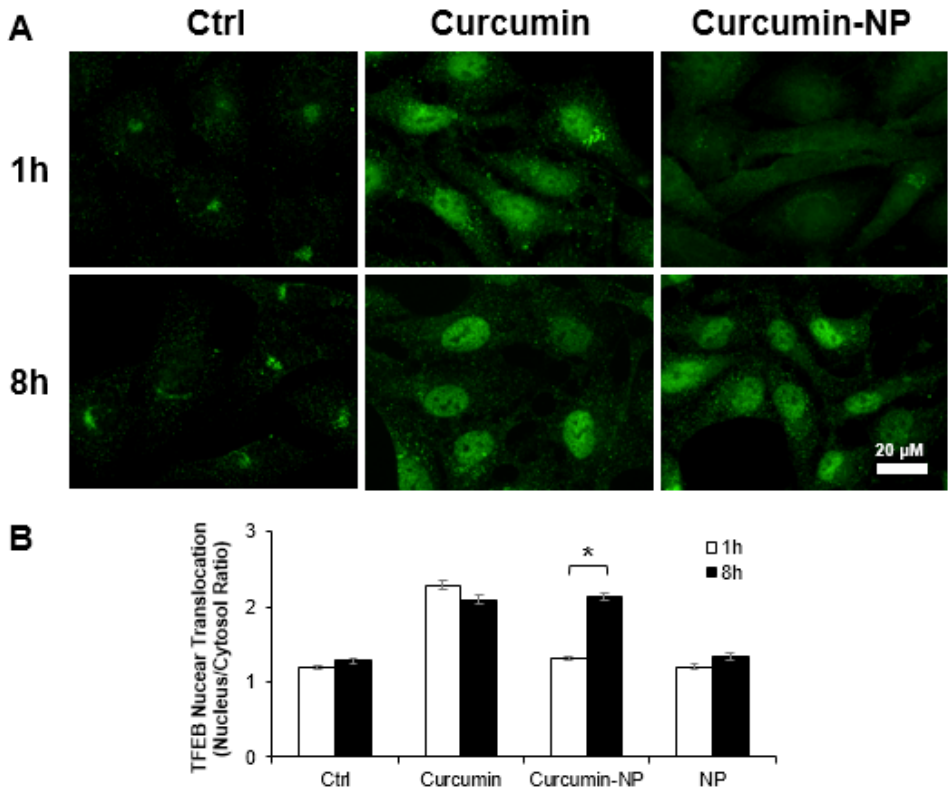


Fig. S10. Intracellular curcumin enhances the fluorescence of nuclear TFEB after 8h. U251 cells were treated with 20 μM free curcumin/curcumin loaded NPs, or equivalent empty FCS/HA NPs for 1h and 8h in serum-free media. (A) Representative photomicrograph of TFEB. (B) Quantification of TFEB nuclear translocation (ratio of average fluorescence of TFEB in the nucleus to cytosol). N>36 cells per condition, error bars = SEM. * denotes significance at $p < 0.05$ comparing 1h to 8h. This highlights the delay in TFEB nuclear translocation in curcumin-NP compared to free curcumin.

9. Nuclear magnetic resonance (NMR) data

CS

^{1}H NMR (400 MHz, D_2O): δ_H (ppm) 1.91 (1059H, s, CH_3), 3.04 (706H, brs, CH), 3.44-3.78 (3530H, m, CH, CH_2), 4.75 (706H, s, CH)

^{13}C NMR (400 MHz, D_2O): δ_C (ppm) 30.19 (353C, CH_3), 59.96 (706C, CH), 69.83 (706C, CH_2), 74.64 (706C, CH), 76.16 (706C, CH), 88.70 (706C, CH), 97.25 (706C, CH), 130.9 (353C, CONH)

TKDA

^{1}H NMR (400 MHz, D_2O): δ_H (ppm) 1.54 (6H, s, CH_3), 3.44 (4H, s, CH_2).

^{13}C NMR (500 MHz, D_2O): δ_C (ppm) 29.4, 32.8, 56.5, 175.2.

DCS

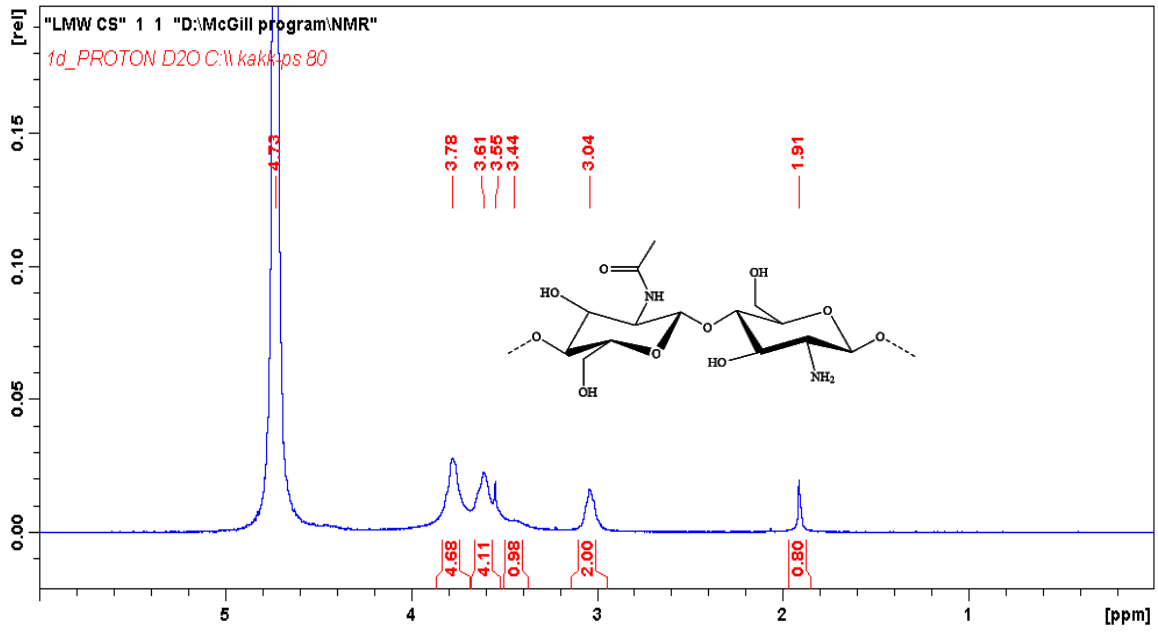
^{1}H NMR (400 MHz, D_2O): δ_H (ppm) 1.53 (6H, s, CH_3), 1.98 (1059H, s, CH_3), 3.09 (706H, brs, CH), 3.35 (4H, s, CH_2), 3.49-3.83 (3530H, m, CH, CH_2), 4.80 (706H, s, CH)

FCS

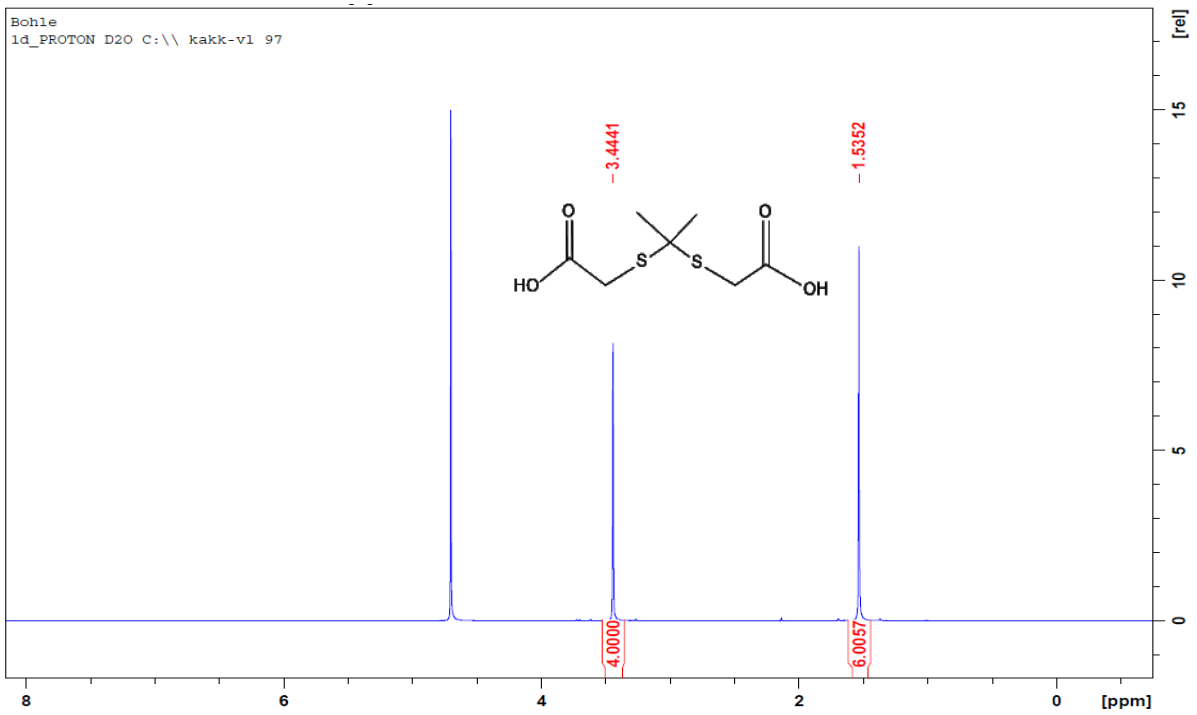
^{1}H NMR (400 MHz, D_2O): δ_H (ppm) 1.52 (6H, s, CH_3), 1.99 (1059H, s, CH_3), 3.00 (706H, brs, CH), 3.27 (4H, s, CH_2), 3.63-3.80 (3530H, m, CH, CH_2), 4.75 (706H, s, CH)

10. ^{1}H NMR Spectra

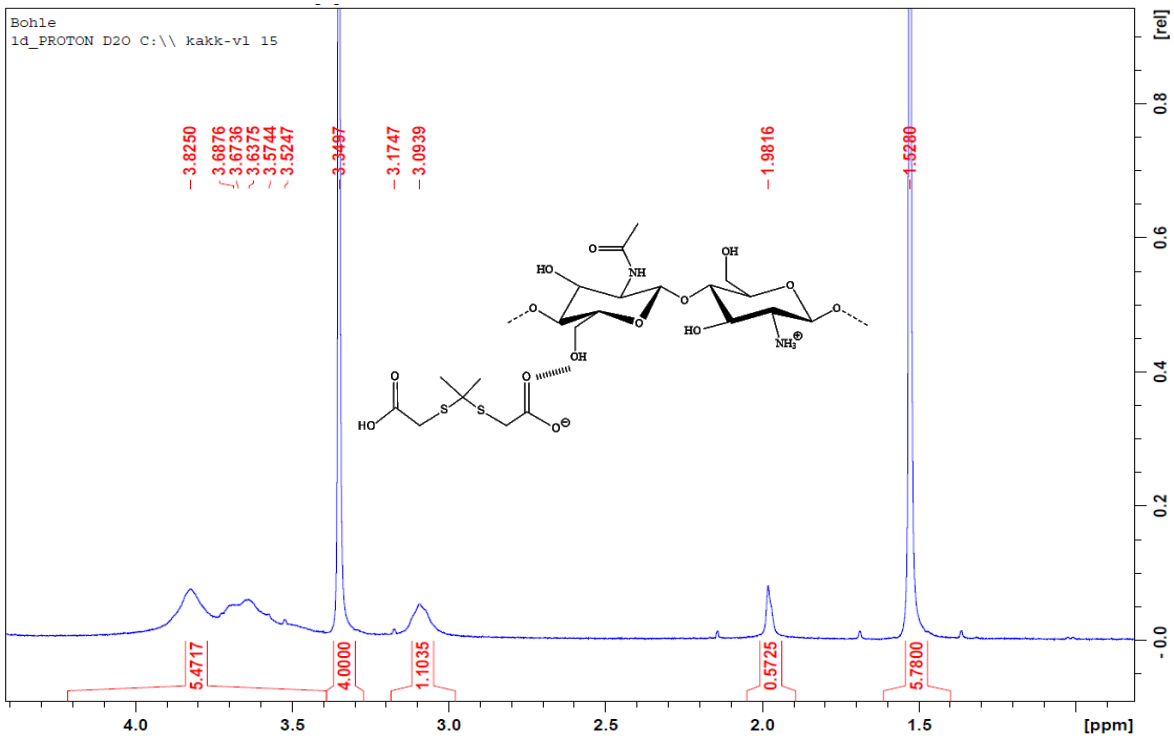
CS: ^{1}H NMR (D_2O)



TKDA: ^{1}H NMR (D_2O)



DCS: ^{1}H NMR (D_2O)



FCS: ^{1}H NMR (D_2O)

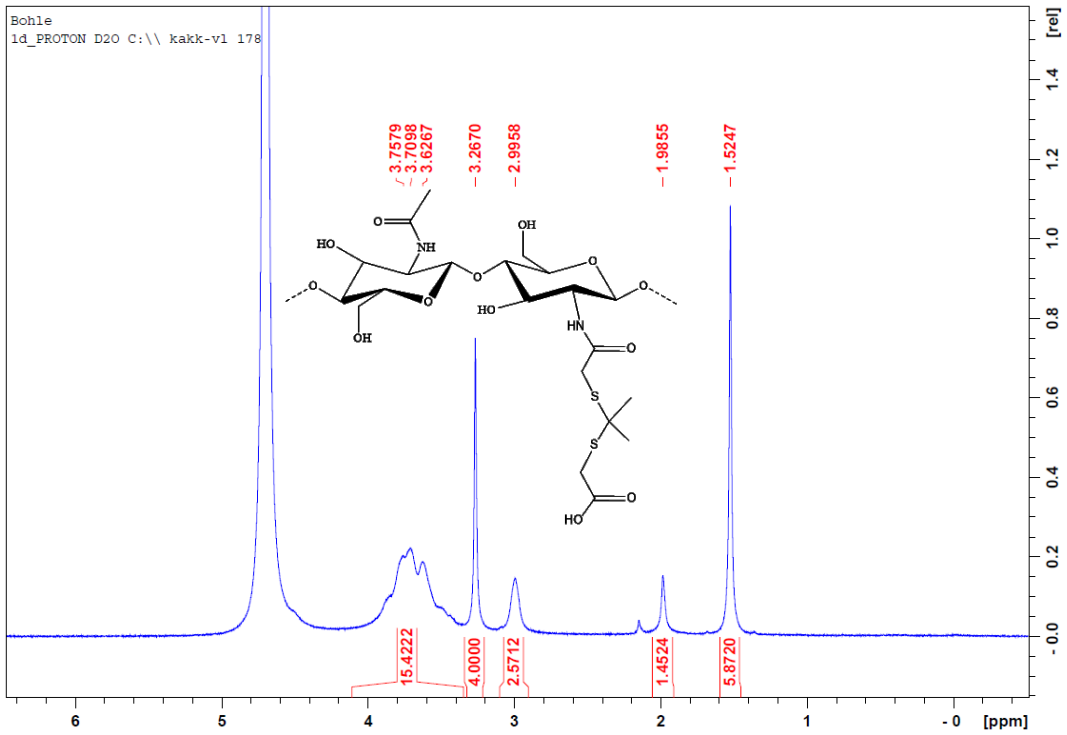


Fig. S11. ^{1}H NMR spectra obtained using Bruker AVIIIHD 400 instrument (400 MHz).