Supplementary Data: Figures S1-S5

Thermally Responsive Nanoparticle-Encapsulated Curcumin and Its Combination with Mild Hyperthermia for Enhanced Cancer Cell Destruction

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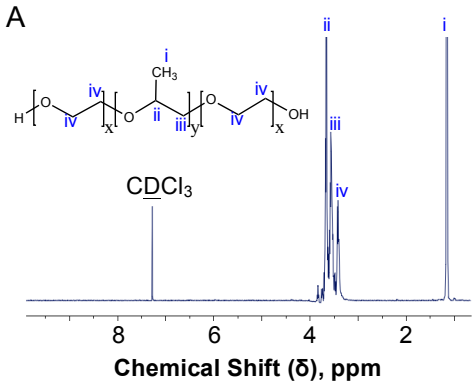
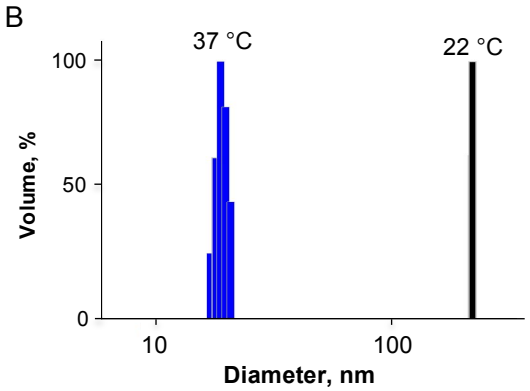
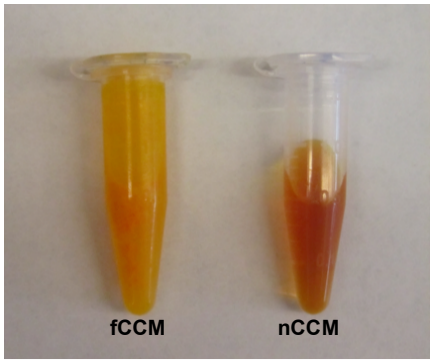
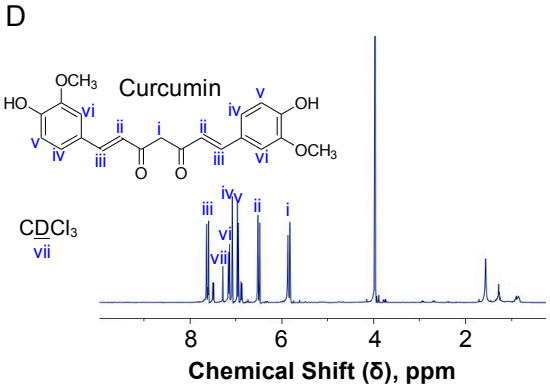
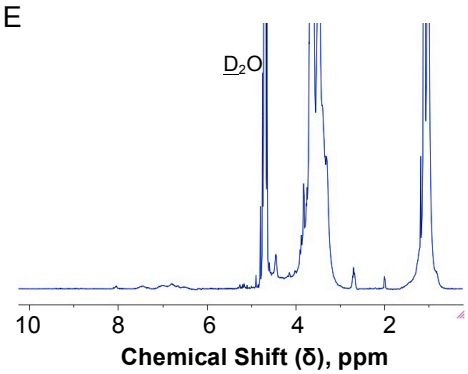
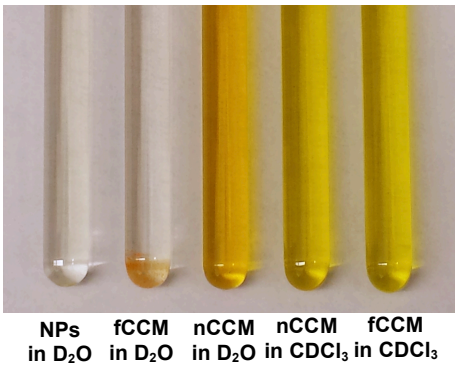

A
i
CH3
iv
H
O
iv
x
O
ii
iii
y
O
iv
x
OH
ii
i
iii
iv
CDCl3
8
6
4
2
Chemical Shift (δ), ppm

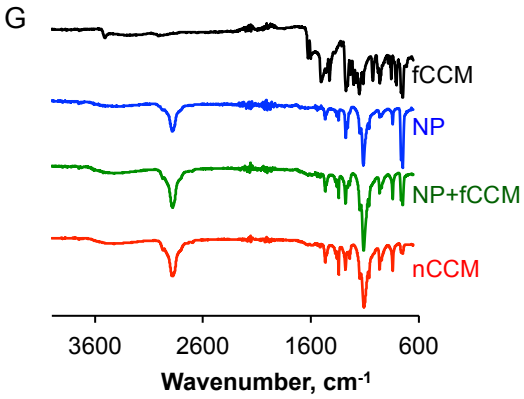

B
37 °C
22 °C
100
50
0
Volume, %
10
100
Diameter, nm


C
fCCM
nCCM


D
OCH3
HO
vi
v
iv
iii
ii
i
ii
iii
iv
v
OH
OCH3
vi
Curcumin
O
O
CDCl3
vii
iii
iv
v
ii
i
vi
vii
8
6
4
2
Chemical Shift (δ), ppm


E
D2O
10
8
6
4
2
Chemical Shift (δ), ppm


F
NPs in D2O
fCCM in D2O
nCCM in D2O
nCCM in CDCl3
fCCM in CDCl3


G
fCCM
NP
NP+fCCM
nCCM
3600
2600
1600
600
Wavenumber, cm-1

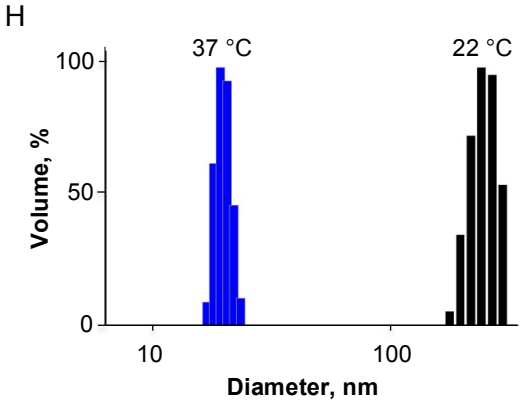

H
37 °C
22 °C
100
50
0
Volume, %
10
100
Diameter, nm

Figure S1. Characterization of nanoparticle (NP) and nanoparticle encapsulated-curcumin (nCCM): (A), ^{1}H NMR spectrum of Pluronic F127 in deuterated chloroform ($CDCl_3$); (B), typical dynamic light scattering (DLS) peaks of Pluronic F127-chitosan nanoparticles; (C), typical image showing aggregate formation of free curcumin (fCCM, 1 mg/ml) in water due to its poor aqueous solubility and clear aqueous solution of nCCM (1 mg/ml); (D), ^{1}H NMR spectrum showing characteristic peaks (i-vi) of fCCM; (E), ^{1}H NMR spectrum of nCCM in deuterated water (D_2O) showing that only tiny peaks (δ: 5.5~8) characteristic of curcumin are observable probably because the curcumin content was dominantly entrapped in the nanoparticles rather than dissolved directly in D_2O; (F), a picture showing 5 mg/ml empty nanoparticles (NPs) dissolved in D_2O with a clear appearance, fCCM (0.17 mg/ml) in D_2O with visible aggregates sunk down at the bottom of the tube, nCCM (0.17 mg/ml encapsulated in 5 mg/ml nanoparticles) dissolved in D_2O with homogeneous brownish appearance, nCCM (0.17 mg/ml, encapsulated in 5 mg/ml nanoparticles before dissolving) dissolved in $CDCl_3$ with homogeneous yellowish appearance, and fCCM (0.17 mg/ml) dissolved in $CDCl_3$ with homogeneous yellowish appearance; (G), whole FTIR spectra of fCCM, NP, simple mixture of NP and fCCM (NP+fCCM), and nCCM; and (H), typical DLS data of thermally responsive nCCM obtained with 1:60 feeding ratio of free curcumin to nanoparticles.

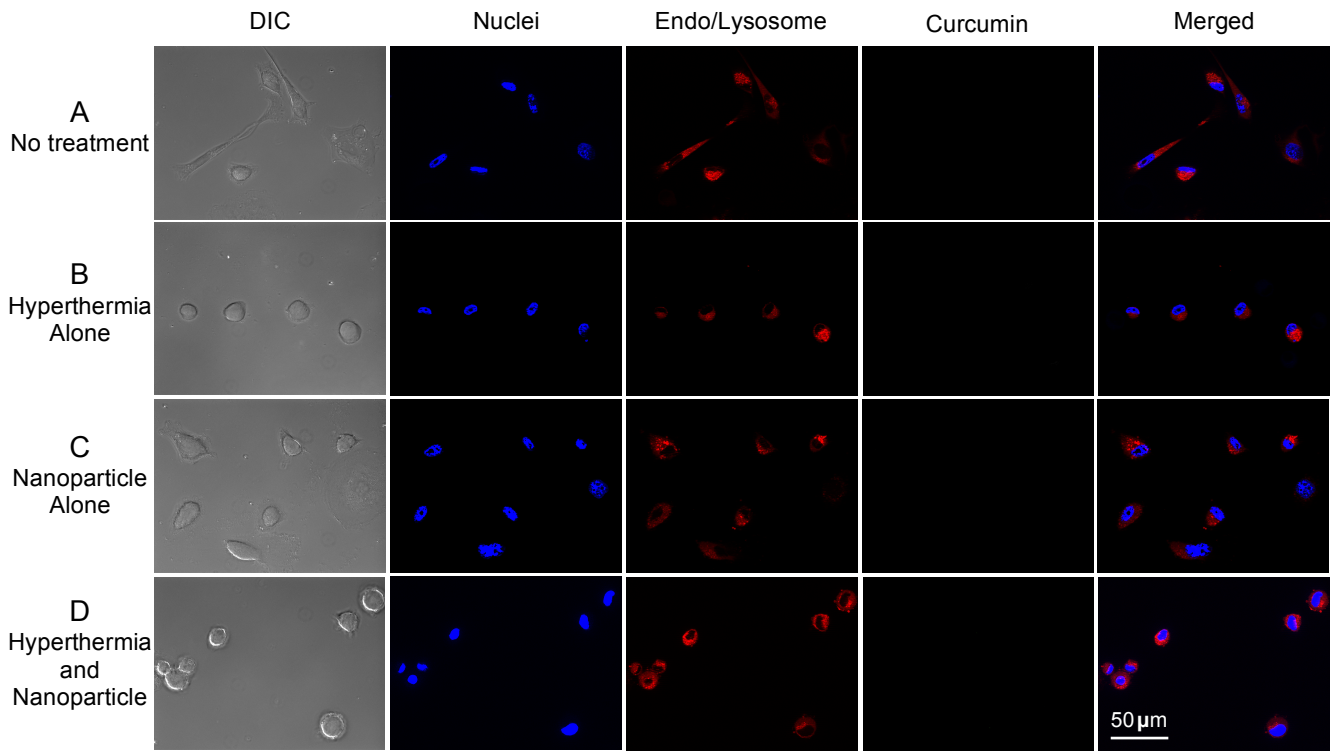


Figure S2. Typical Apotome structured illumination microscopy (SIM) fluorescence images of PC-3 cancer cells: (A) with no treatment, (B) with hyperthermia treatment alone by heating at 43 °C for 30 min, (C) after incubating with empty nanoparticles for 1.5 h, and (D) after incubating with empty nanoparticles for 1.5 h and hyperthermia at 43 °C from 1-1.5 h: the nuclei and lyso/endosomes were stained using Hoechst and LysoTracker Red, respectively; the empty nanoparticle concentration is the highest one (1.845 mg/ml) used in this study; and no clear curcumin fluorescence is observable for all the conditions.

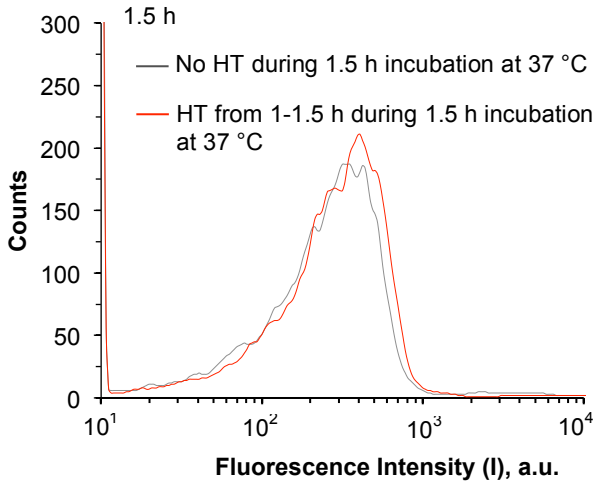


Figure S3. Typical flow cytometry peaks of fluorescence intensity in PC-3 cancer cells showing their uptake of free curcumin (fCCM) either with or without mild hyperthermia (HT) at 43 °C applied between 1 and 1.5 h. The PC-3 cells were treated with 10 μg/ml fCCM for 1 h, then the cells without HT were continued to incubate with fCCM for 0.5 h while cells with HT were heated at 43 °C for 0.5 h. The cells in both groups were then collected and detected for fluorescence at 530/30 nm (excited at 488 nm) using a BD LSR-II Flow Cytometer. The mean curcumin intensity ($I-I_0$) calculated as the difference in mean fluorescence intensity of cells before (I_0) and after (I) curcumin treatment, was 200.7 ± 10.4 and 236.3 ± 24.4 for fCCM treatment without and with HT, respectively. These data are consistent with the microscopy data shown in Figure 5A and B. Both values are much lower than the corresponding values for nCCM at 1.5 h showing in Fig. 4 (1732.4 ± 87.4 and 1453.7 ± 208.0 for without and with HT, respectively). The data are reported as the mean ± standard error of mean of data from three independent runs.

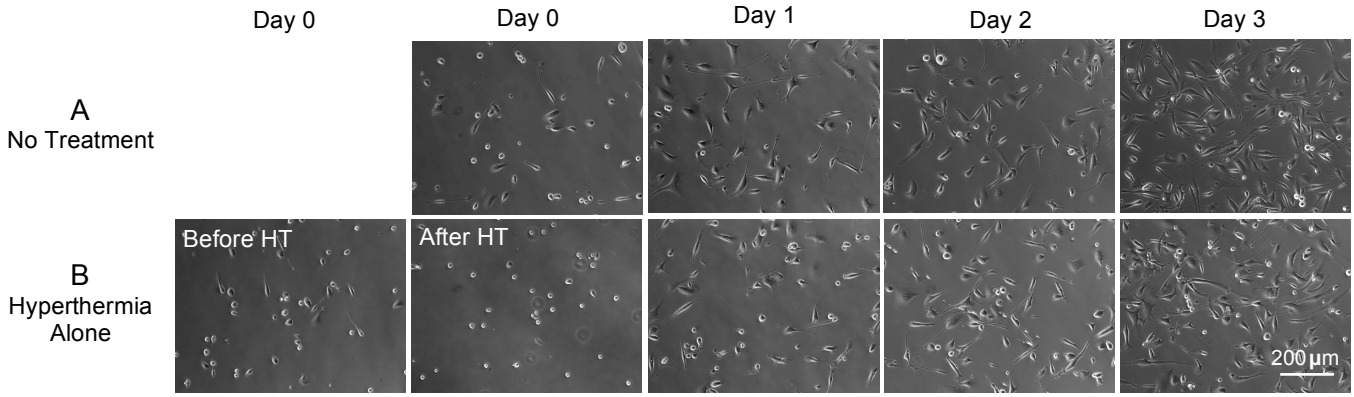


Figure S4. Typical phase contrast micrographs showing the morphology and proliferation of PC-3 cancer cells with (A) no treatment and (B) hyperthermia (HT) treatment alone by heating at 43 °C for 30 min: the cell became rounded temporarily after hyperthermia, which didn't affect the cell proliferation at all in the subsequent days.

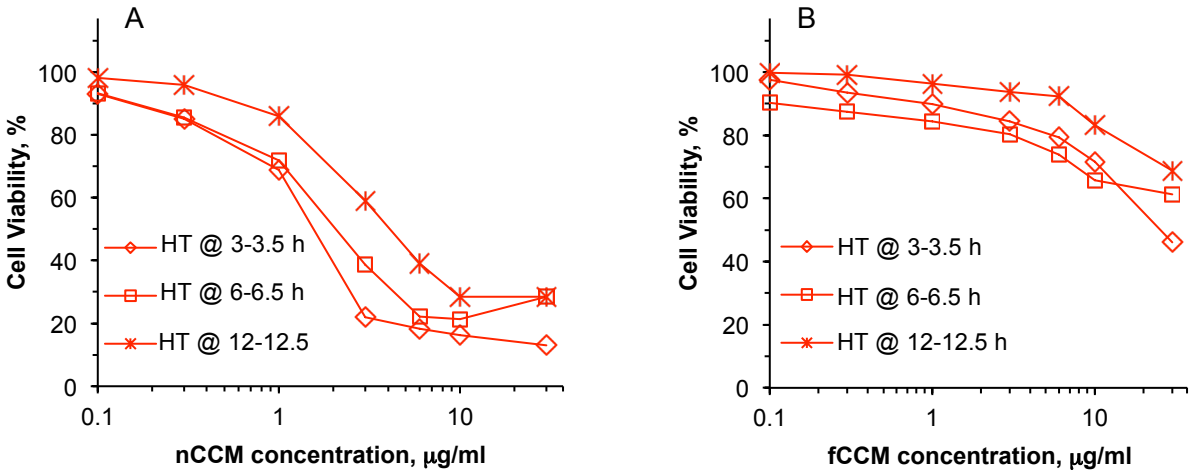


Figure S5. Viability of PC-3 cancer cells after 3-day incubation with: (A), ~ 22 nm nanoparticle-encapsulated curcumin (nCCM) and (B), free curcumin (fCCM) of various concentrations with mild hyperthermia (HT) applied from 3-3.5, 6-6.5, and 12-12.5 h during the incubation. The data are the average of three independent runs. Error bars were omitted for clarity, but the standard error of mean for all the data is within ~ 20% of the average.