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Supplementary Information

Improving photothermal effects of an organic photothermal agent using chitosan hydrogel.

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General Information

Chitosan (low-molecular weight) and pentasodium tripolyphosphate were of analytical grade, obtained from commercial suppliers and used without further purification.

Temperature readings were obtained from thermal images recorded on FLIR E6 WIFI.

Fluorescence spectra were recorded on Agilent Cary Eclipse Fluorescence Spectrophotometer.

UV-Vis absorbance spectra were recorded on Agilent Cary 300 UV-Visible Spectrophotometer.

Photostability measurements were obtained from UV-Vis absorbance readings recorded on Tecan M200 Infinite Monochromator Microplate reader.

Size Characterization were done on Malvern Panalytical Zetasizer Nano ZS.

Synthesis of SQR22

SQR22 was synthesized according to procedures previously reported.[1] The final product was in the form of a dark-blue powder (14% yield) R_f: 0.17 (hexane : ethyl acetate = 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 0.97 (t, J = 7.6 Hz, 6H, -CH_3); 1.33–1.43 (m, 4H, -CH_2-); 1.49 (s, 6H, -CH_3); 1.57–1.65 (m, 4H, -CH_2-); 3.35–3.39 (m, 4H, -CH_2-); 5.83 (s, 1H, =CH-); 6.67 (d, J = 9.2 Hz, 2H, ArH), 7.18–7.35 (m, 4H, ArH); 8.11 (d, J = 9.2 Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$, 400 MHz, δ ppm): 14.00, 20.34, 25.52, 29.59, 49.98, 50.94, 89.73, 111.79, 113.14, 118.73, 122.61, 125.04, 128.69, 130.81, 139.92, 141.46, 151.04, 169.17, 181.17, 181.62, 185.38, 187.27. MS (ESI): found *m/z* $[M+H]^+$ 443.07, calculated for $C_{29}H_{34}N_2O_2$ $[M+H]^+$ 443.61.

Assembly of cs-TPP hydrogel

The introduction of SQR22 into the cs-TPP hydrogel matrix was done according to previous reports[2–6]. Briefly, 2 mL chitosan (2.5 mg/mL) in 1% AcOH at pH 5 was heated to 60 °C. 37 µL ethanolic solution of SQR22 (4.9 mg/mL) was then added to the solution, followed by dropwise addition of 0.4 mL of sodium tripolyphosphate (2.5 mg/mL) in water. The solution was left to stir for 30 minutes at room temperature. Subsequently, the solution was centrifuged at 3,300 g for 2 h. The supernatant was

discarded, and the pellet was resuspended in 1 mL water to obtain cs-TPP-SQR22 hydrogel and further used for analysis.

Photothermal Effect

150 µL of the sample was added into a PCR tube and irradiated under a 640 nm Kessil PR160L lamp (0.5 W cm^{-2}, 10 minutes). Temperature readings were taken at 2-minute intervals.

Photothermal Conversion Efficiency

150 µL of the sample was added into a PCR tube and irradiated under a 640 nm lamp (0.5 W cm^{-2}, 10 minutes). Temperature readings were taken at 30-second intervals.

The data obtained were used to compute the PT conversion efficiency using the following equation:

$$h = \frac{hA(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A_{640}})}$$

where *h* represents the heat transfer coefficient, *A* is the surface area of the PCR tube, T_{max} is the maximum temperature achieved by the laser irradiation of the sample, T_{surr} is the ambient temperature of the environment set at 23 °C, Q_{dis} is the heat dissipation from the light absorption of the solvent and PCR tube, *I* is the incident laser power (0.5 W cm^{-2}), and A_{640} is the absorbance of the sample at 640 nm. The value *hA* was calculated using the following equation:

$$hA = \frac{m_D c_D}{\tau}$$

where τ is the time constant of the sample system, m_D and c_D are the mass (0.15 g) and heat capacity (4.2 Jg^{-1}) of water respectively. The value τ can be derived from the following equation:

$$t = -\tau \ln\theta$$

where t is the time elapsed after laser irradiation ceases and θ is calculated using the following equation:

$$q = \frac{T_{surr} - T_s}{T_{surr} - T_{max}}$$

where T_s is the temperature of the sample at a given time *t*.

Q_{dis} was calculated using the following equation:

$$Q_{dis} = \frac{m_D c_D (T_{\max\,(water)} - T_{surr})}{\tau_{water}}$$

Cell dark cytotoxicity assay

The cell dark cytotoxicity was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The mitochondrial dehydrogenase enzyme in living cell reduces MTT to form formazan crystals. HeLa cells were seeded in a 96-well plate at a density of 2.5 × 10^4 cells/mL in DMEM supplemented with FBS (10%) and penicillin-streptomycin (1%) and grown at 37 °C under 5% CO_2 environment. After 48 hours, the cells were incubated with 100 µL unsupplemented DMEM mixed with cs-TPP-SQR22 at different concentrations (25, 50 and 100 µM) and varying incubation duration (4, 10, 24 hours). Upon completion of incubation with cs-TPP-SQR22, 10 µL MTT solution was added into each well and incubated at 37 °C under 5% CO_2 condition. After 2 hours incubation, 200 µL DMSO was added into each well to dissolve the formed purple formazan crystals. The absorbance of the formazan crystal was then measured at 570 nm while background absorbance was measured at 630 nm using Tecan 200 microplate reader. The corrected signal from each cs-TPP-SQR-treated well was then normalized to that from the control well to obtain relative percentage cell viability.

Cell phototoxicity assay

The cell phototoxicity assay was conducted according to previous studies with some modifications. [6–8] HeLa cells were seeded in a 96-well plate at a density of 2.5 × 10^4 cells/mL in DMEM supplemented with FBS (10%) and penicillin-streptomycin (1%) and grown at 37 °C under 5% CO_2 environment. After 48 hours, the cells were incubated with 100 µL unsupplemented DMEM mixed with cs-TPP-SQR22 at different concentrations (25, 50 and 100 µM). After 4 hours of incubation, the cells were irradiated with 640 nm lamp (0.5 W cm^{-2} ,10 minutes). Following which, the MTT assay was conducted to determine the cell viability.

Supplementary Figures

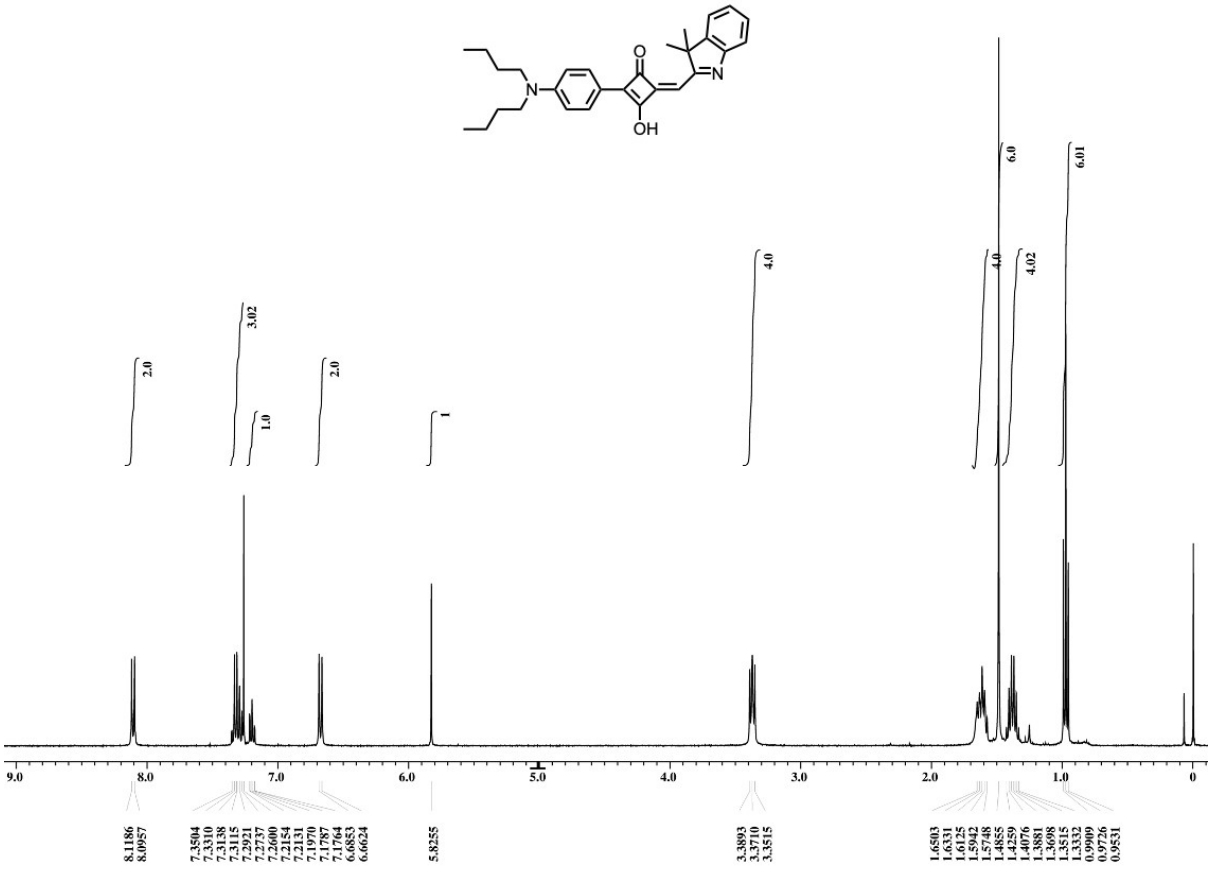

Figure S1. ^{1}H NMR spectrum of SQR22 ($CDCl_3$, 400 MHz)

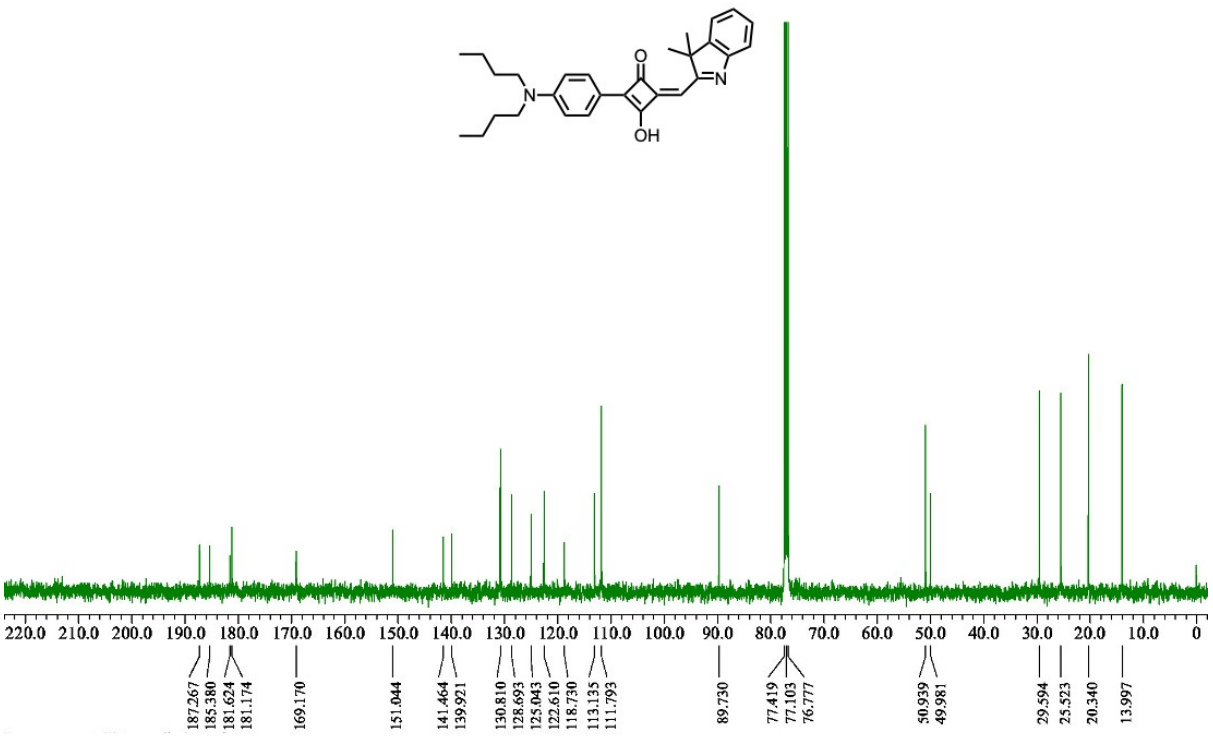

Figure S2. ^{13}C NMR spectrum of SQR22 ($CDCl_3$, 400 MHz)

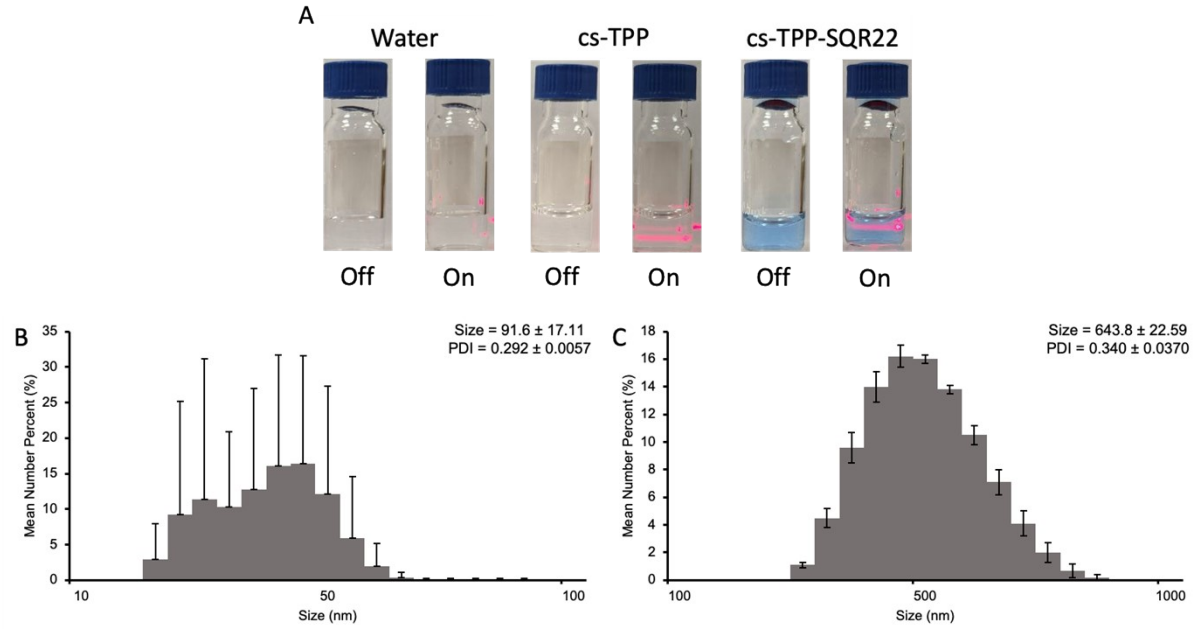

Figure S3. (A) Image of hydrogel solutions displaying Tyndall effect when exposed to a laser pointer. This indicated the successful formation of cs-TPP hydrogel. (B) DLS measurements of the blank cs-TPP hydrogel and (C) that of cs-TPP-SQR22 hydrogel with 3.65 % (w/w) loading.

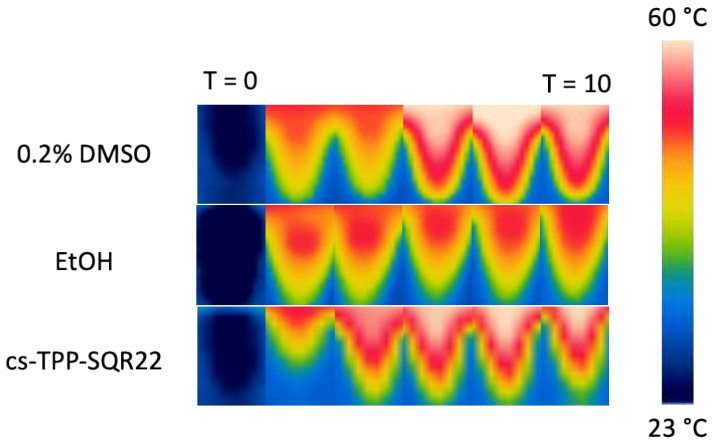

Figure S4. Thermal images recorded on FLIR E6.

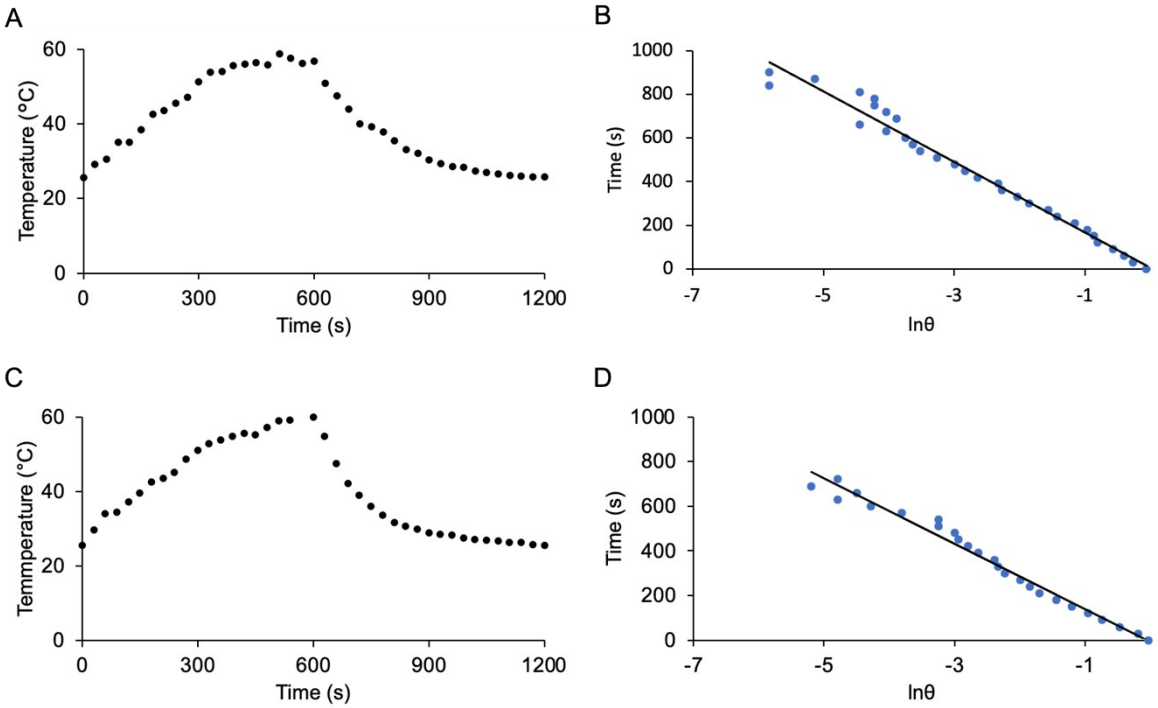

Figure S5. Calculation of photothermal conversion efficiency of SQR22 in (A) 0.2% DMSO and (C) cs-TPP hydrogel. (B and D) Graphs of Time against lnθ for respective environments.

References

1 K. Sou, L. Y. Chan, S. Arai and C. L. K. Lee, *Sci Rep*, 2019, **9**, 1–12.

2 R. Varela-Fernández, X. García-Otero, V. Díaz-Tomé, U. Regueiro, M. López-López, M. González-Barcia, M. I. Lema and F. J. Otero-Espinar, *ACS Appl Mater Interfaces*, 2021, **13**, 3559–3575.

3 A. Rampino, M. Borgogna, P. Blasi, B. Bellich and A. Cesàro, *Int J Pharm*, 2013, **455**, 219–228.

4 N. H. Evans, in *Reactivity in Confined Spaces*, The Royal Society of Chemistry, 2021, pp. 1–28.

5 K. A. M. O'Callaghan and J. P. Kerry, *Food Control*, 2016, **69**, 256–261.

6 G. Chen, Y. Zhao, Y. Xu, C. Zhu, T. Liu and K. Wang, *Int. J. Pharm.*, 2020, **589**, 119763.

7 H. J. Yoon, H. S. Lee, J. Y. Lim and J. H. Park, *ACS Appl. Mater. Interfaces*, 2017, **9**, 5683–5691.

8 S. Macchi, A. Jalihal, N. Hooshmand, M. Zubair, S. Jenkins, N. Alwan, M. El-Sayed, N. Ali, R. J. Griffin and N. Siraj, *J. Mat. Chem. B*, 2022, **10**, 806–816.