Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

A novel facile one-pot synthesis of photothermal-responsive Carbon Polymer Dots as promising drug nanocarriers

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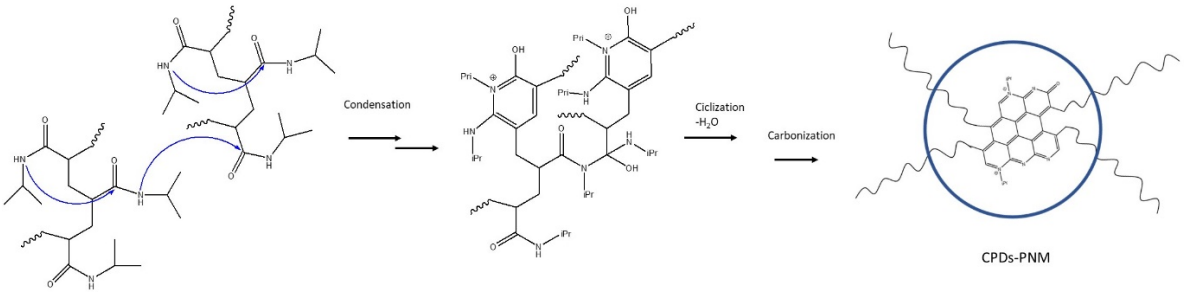
CPDs-PNM preparation. An amount of 50 mg of PNIPAM (M.W.~ 5500 u.m.a) was pyrolyzed for 4 hrs at 200°C in air. The obtained reddish solid product was washed three times with deionized water to remove the excess of unreactive precursor. Then the product was dispersed in a volume of 2 mL of deionized water by ultra-wave process for 5 min. The unwanted solid aggregates were removed by centrifugation (13.000 rpm for 5 min) and then by filtration (pore-size 0.2 um). Finally, the resulting reddish transparent solution was dialyzed using MilliQ-water through a dialysis membrane (10 KDa cut off) for 46 hsr in deionized water. The obtained CPDs-PNM solution was stored at room temperature before the use.

Figure S1. CPDs-PNM (20mg mL^{-1}) in deionized water

Mechanism for the formation of CPDs-PNM. The proposed mechanism is based on intra- and inter-chains condensation reactions, further cyclization with loss of water and aromatization occurs by carbonization processes.



Scheme S1. Proposed mechanism for the formation of a possible CPDs-PNM structure

1D- and 2D-NMR spectra were acquired on a Bruker Avance 400 spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) referring to the solvent signal.

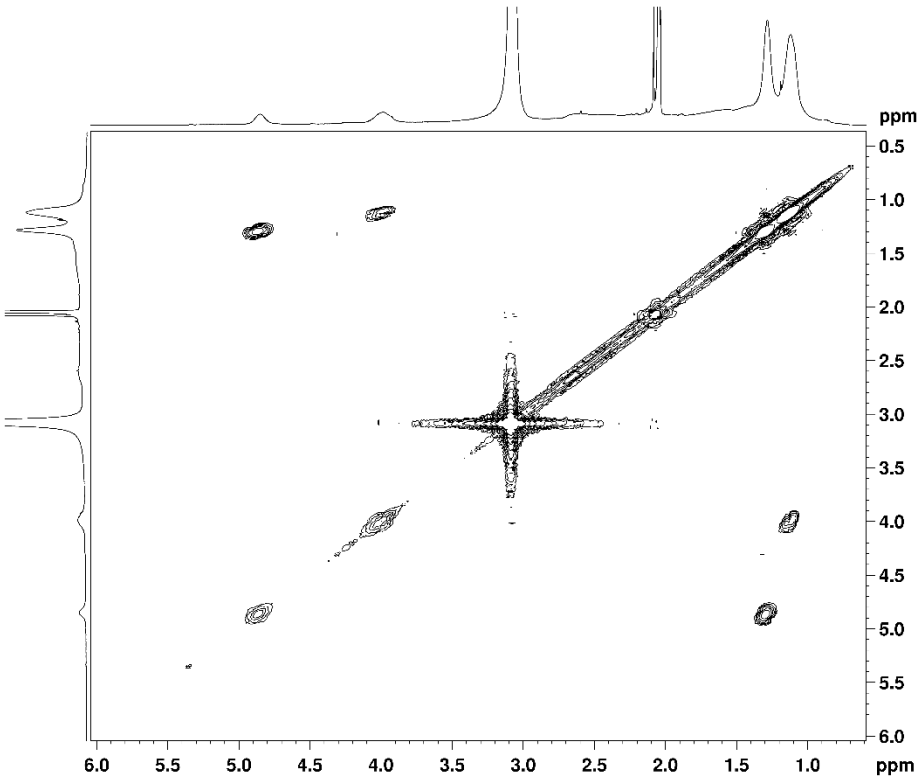


Figure S2. 2D-COSY NMR spectrum (acetone-d6, 297 K) of CDPs-PNM prepared by heating at 300 °C for 4 hrs.

Atomic Force Microscopy. A commercial AFM instrument (Cypher AFM system with equipped with a scanner at an XY scan range of 30/40 µm (closed/open loop), Asylum Research, Oxford Instruments, Santa Barbara, CA) was used to image in AC mode air-dried nanoparticles on freshly cleaved mica surface. Silicon Cantilevers (OMCL-AC240TS, ~ 70 kHz, 2 N/m by Olympus, Japan) were used to acquire height, amplitude error, and phase images of the nanoparticles. The phase and amplitude images produced the clearest 3D visualization of the nanoparticle surface.

An aliquot with a volume of 10 µL of CPDs-PNM (0.6 mg ml-1) sample was dispensed on freshly cleaved muscovite mica (Ted Pella, Inc., Redding, CA, USA) substrate and dried at room temperature. For each sample, various areas on the sample were investigated and statistically relevant images were chosen. AFM images were analyzed using the free tool in the MFP-3DTM offline analysis software.

The AFM height images on a scan size of 2x2 μm^2 (Figure 2-ESI, a-b) clearly show two distributions of surface structures, namely larger structures 100-300 nm sized and much smaller spherical particles. As to the latter, the amplitude (Figure 2-ESI, c) and phase (Figure 2-ESI, d) images produced the clearest 3D visualization of the nanoparticle surface, showing that the particles appeared fluffy, with irregular heights and ruffled edges. The phase images allowed to visualize changes of tip–sample interactions for hard and soft areas of the core-shell CPDs-PNM samples. In particular, regions of the sample with greater elasticity and viscoelasticity (outer polymer shell) generate a weaker signal relative to harder areas because more of the energy associated with the cantilever oscillation is dissipated by the material.

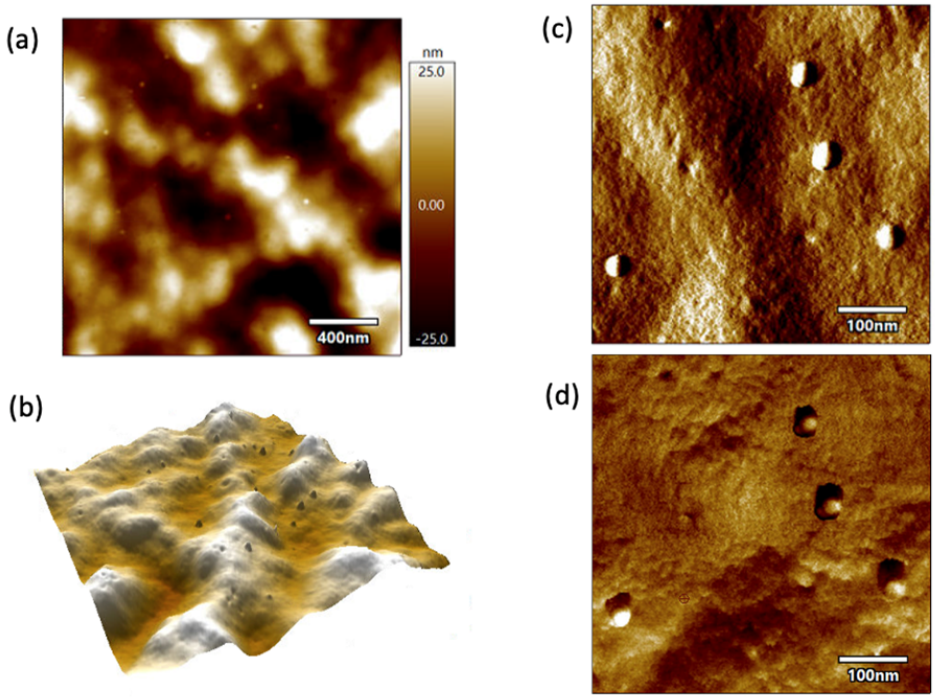


Figure S3. AFM images for CPDs-PNM on a 2 μm (a: 2D height, z scale = 50 nm; b: 3D height) and a 500 nm (c: amplitude; d: phase) scan size area.

Optical absorption spectrum of CPDs-PNM dispersion at various carbonization process time.

The optical absorption spectra were recorded using a Perkin-Elmer 365 and standard quartz cuvettes (l=1cm). The CPDs-PNM samples at various carbonization process time were dispersed in a volume of 2 ml of deionized water and the optical spectra acquired. The spectrum for untreated PNIPAM in water as reference was also recorded (green line).

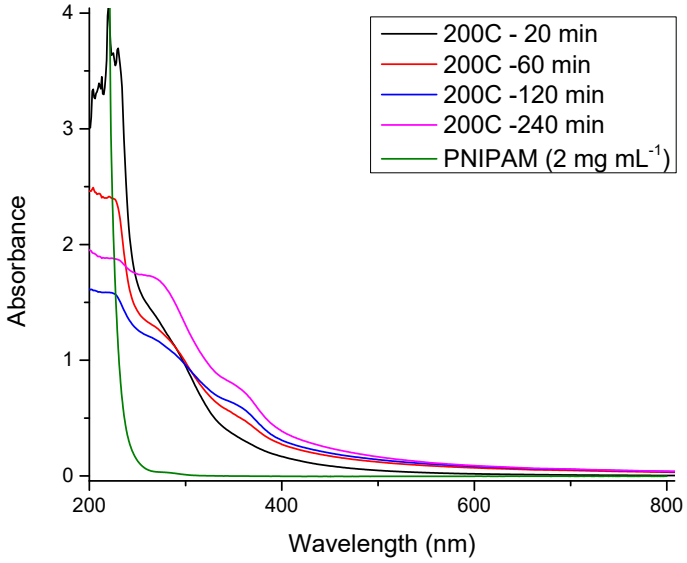


Figure S4. Optical absorption spectrum of CPDs-PNM dispersion at various carbonization process time.

HOMO-LUMO band gap calculation.

The optical band gap was calculated by Tauc plot, it shows the variation of (Ass hυ)$^{1/2}$ *versus* (hυ) for the CPDs-PNM. The data were obtained from the UV-Vis absorption spectrum reported in figure 1A.

The optical energy band gap for the direct allowed transitions was estimated to be about E_g=2.15 eV. This is in good agreement with N-doped nanomaterial containing a nitrogen dopant amount of about 5% as recently reported by Witjaksono [G. Witjaksono et al Molecules 2021, 26, 6424. https://doi.org/10.3390/molecules26216424] and by Lemes [Lemes, G.; Sebastián, D.; Pastor, E.; Lázaro, M.J. N-doped graphene catalysts with high nitrogen concentration for the oxygen reduction reaction. J. Power Sources 2019, 438, 227036].

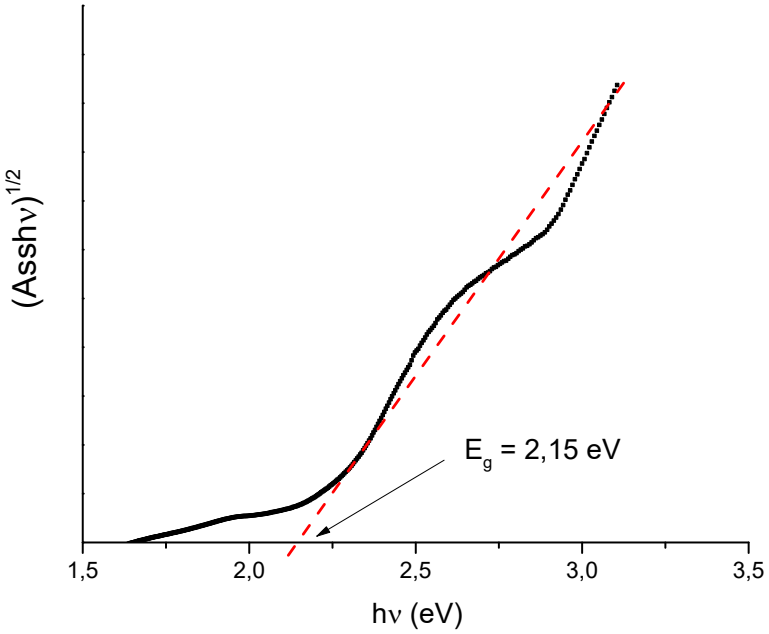


Figure S5. Tauc plot of CPDs-PNM.

Photothermal conversion efficiency (η).

Photothermal measurements were performed irradiating for 10 minutes a glass tube (diameter 3 mm) containing a volume of 100 µL of CPDs-PNM dispersion (16.2 mg mL^{-1}), using a continuous wave Laser 532 nm (power 230 mW). A Flirck infrared thermal imaging camera was used to measure the temperature of solution every 20 seconds, during the heating and cooling processes. The photothermal conversion efficiency (η) was calculated according to equation (1) introduced by Roper [1].

$$\eta = \frac{hA\,(T_{max} - T_{surr}) - Q_{Dis}}{I(1 - 10^{-A})} \quad (1)$$

Where Tmax (52.0 °C) and Tsurr (27.0 °C) represents the max photothermal temperature and the ambient temperature respectively. The absorbance (A) of CNPs-PNIPAM at 532 nm. The equations (2) and (3) were introduced to calculate the parameter hA.

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (2)$$

$$\tau = \frac{M_D\, C_D}{hA} \quad (3)$$

where MD and CD are the mass and the heat capacity of water, respectively, and τ_s is the time constant, calculated by the equation (4).

$$t = -\tau(ln\theta) \quad (4)$$

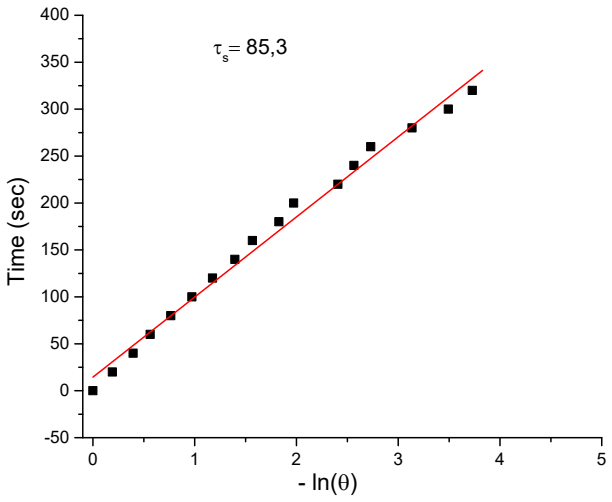


Figure S6. Linear relationship between time (sec) and ln(θ), the slope is the time constant (τ_s).

[1] Roper, D. K., Ahn, W., M. Hoepfner, Microscale heat transfer transduced by surface plasmon resonant gold nanoparticles, J. Phys. Chem. C 111 (2007) 3636–3641. doi: https://doi.org/10.1021/jp064341w].

Entrapment of curcumin in the CPDs-PNM. An excess of solid curcumin (3 mg/mL) was added to a phosphate buffered solution of dialyzed CPDs-PNM passed through a 0.2 µm GHP filter (19 mg in 2 mL). The mixture was stirred for 3 days. Then, it was centrifuged at 10,000 rpm for 15 min to give a clear yellow colloidal solution. As a control, curcumin alone was subjected to the same treatment.

Determination of loaded CUR. The amount of solubilized CUR in the presence and absence of CPDs-PNM was evaluated by UV–vis spectrophotometry (λ 427 nm) using a calibration curve in phosphate buffer:EtOH (1:1, *v*:*v*).

The total solubility enhancement of CUR was expressed as solubility enhancement factor (δ) calculated by the following equation:

$$\delta = \frac{S - S_0}{S_0} \times 100$$

where S_0 and S denote drug solubility in the absence and presence of CPDs-PNM respectively.

Release of curcumin from the CPDs-PNM. A sample of curcumin loaded CPDs-PNM (1 mL) was heated at 37 or 50 °C for 60 min. At time intervals the sample was centrifuged at 10,000 rpm for 15 min for removing the suspended released curcumin and the absorption spectra were recorded. Curcumin release % was calculated from the decrease of the curcumin absorption band over time.

Modelling Simulation. The interaction energy between a 15-mer chain of PNM and one molecule of curcumin was evaluated by means of density functional theory calculations which were carried out at the B3LYP/6-311G level [1-3] adopting the conductor-like polarizable continuum model (CPCM) to evaluate the solvent effect [4-6].

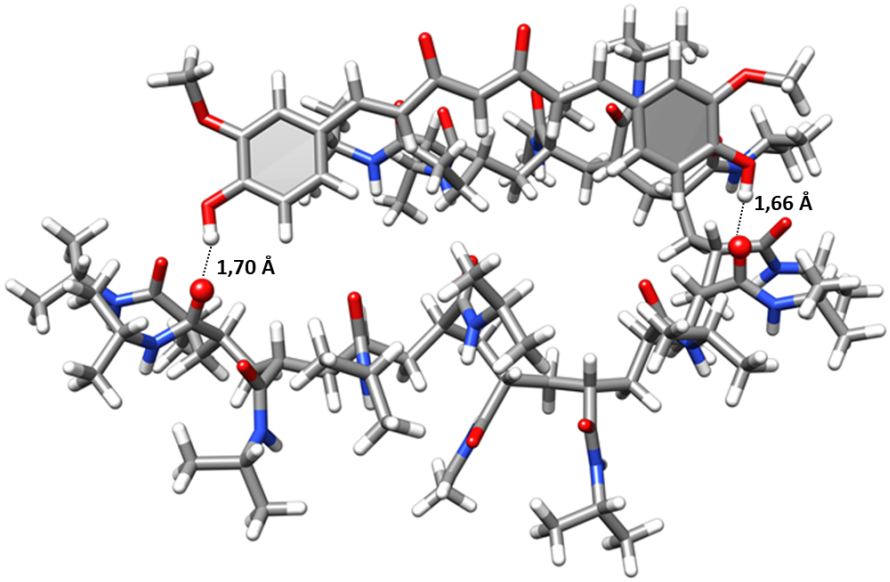


Figure S7. PNM/Curcumin adduct geometry after modelling simulation.

[1] A.D. Becke, , Physical Review A 38 (1988) 3098-3100.
[2] C. Lee, W. Yang, R.G. Parr, , Physical Review B 37 (1988) 785-789.
[3] A.D. Becke,. J. Chem. Phys. 98 (1993) 5648-5652.
[4] A. Klamt, G. Schuurmann, J. Chem. Soc. Perkin Trans. 2 (1993) 799-805.
[5] M. Cossi, N. Rega, G. Scalmani, V. Barone, J. Comp. Chem. 24 (2003) 669-681.
[6] Y. Takano, K.N. Houk,. J. Chem. Theory and Comp. 1 (2005) 70-77.

Cell viability. MTT Assay: The human neuroblastoma cell lines, SH-SY5Y, were maintained in DMEM-F12 (Gibco, Thermofisher) supplemented with 10% heat inactivated (HI) fetal bovine serum (Gibco, Thermofisher), 100 mg/mL penicillin and streptomycin (Gibco, Thermofisher), and 2 mM L-glutamine at 37 °C, 5% CO_2. One day before the experiment, cells were seeded onto 96-well plate at the density of $5x10^3$/ well. Before treatments, cells were washed with PBS buffer and the medium was replace with fresh DMEM-F12 with 3% of FBS. Cells were exposed with increasing dose of PDs (10 µg/mL, 20 µg/mL 50 µg/mL, and 100 µg/mL,). After 48 hr treatment, cell cultures were incubated with MTT (0.5 mg/mL) for 2 hr at 37 °C, then lysed with DMSO and the formazan production was evaluated in a plate reader through the absorbance at 570 nm. Cell images: After 48 hrs treatment cells were analyzed under a Leica DMI 6000B epifluorescence inverted microscope and representative images of different conditions were taken.

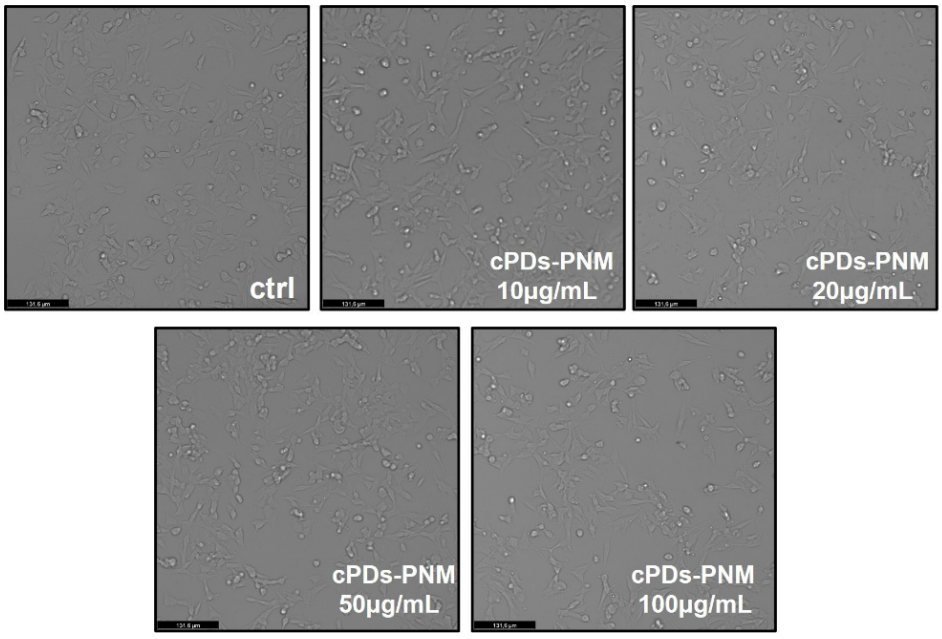


Figure S8. Representative images of cells after 48 hrs exposure with CPDs-PNM.