Supporting Information:

Triggered and Monitored Drug Release from Bifunctional Hybrid Nanocomposites

SUPPORTING EXPERIMENTAL

Materials and instrumentation

N-Ethyldiisopropylamine (99%), 11-hydroxyundecylphosphonic acid (HUPA, 95%), methanesulfonic acid (98+%), sarcosine (Sar, 98%) and triphosgene (>99%) were purchased from Alfa Aesar, UK and used as received. Doxorubicin hydrochloride (Dox·HCl, 95%) and γ -benzyl-L-glutamate (BLG, 97%) were purchased from Fluorochem, UK, and used as received. Acetate buffer solution at pH 5 and α -pinene (98%) were purchased from Thermo Fisher, UK and used as received. Acetate buffer solution at pH 5 and α -pinene (98%) were purchased from Thermo Fisher, UK and used as received. Acetate buffer solution at pH 5 and L-phenylalanine (Phe, 98.5+%) were purchased from Acros Organics, NJ, USA and used as received. 4-Aminobutylphosphonic acid (ABPA, ≥99%), calcium carbonate (CaCO₃, ≥99%), dialysis tubing (benzoylated, 2,000 Da MWCO), folic acid (≥97%), phosphate buffered saline (PBS) tablets, sodium hydroxide (NaOH) and triethylamine (≥99%) were purchased from Sigma-Aldrich (now Merck), UK, and used as received unless otherwise stated.

Fourier transform infra-red spectroscopy (FTIR) was obtained with a Perkin-Elmer Spectrum 100 with attenuated total reflectance accessory and sample mounting. Spectra were obtained between 4000 cm⁻¹ and 650 cm⁻¹ at 1 cm⁻¹ intervals. High performance liquid chromatography (HPLC) analyses were run on a C₁₈ column (Ascentis Express C₁₈, 2.7 μ m particle size, 50 mm x 2.1 mm) over five minutes. The mobile phase was water/acetonitrile (60:40 v/v) with 0.1% trifluoroacetic acid and a flow rate of 0.5 mL min⁻¹, similar to a previously reported procedure.¹

¹H nuclear magnetic resonance (NMR) spectroscopy at 400 MHz were recorded on a Bruker AVANCE III HD-400 spectrometer operating TOPSPIN and equipped with a 5 mm auto-tune BB/1H z-pfg probe. ¹H and ¹³C NMR conducted at 500 MHz were recorded on a Bruker AV4 NEO 11.75 T spectrometer operating TOPSPIN and equipped with a 5 mm Bruker C/H cryoprobe. ¹H and ¹³C NMR conducted at 600 MHz were recorded on a JEOL ECA600 series II spectrometer equipped with a 5 mm z-pfg BB/1H Royal Probe operating Delta 5.0 software. Cross-polarisation magic-angle spinning (MAS) (solid-state) ¹³C NMR spectra were obtained on a Bruker AVANCE II 400 MHz spectrometer with a double-bearing MAS probe head (BL4 type) and a Bruker MAS II control unit. Samples were packed into 4 mm diameter zirconium rotor tubes. Measurement parameters include a ¹³C resonant frequency of ~100.6 MHz, 90° proton pulse length of 2.5 μs, a 2 ms contact time, 5 s delay time and a spinning speed of 10 kHz.

Raman microscopy was conducted on glass substrates using a Reinshaw inVia Raman Microscope (785 nm laser) with a 50x objective using MS20 encoded sample stage. Data acquisition was undertaken with Reinshaw WiRE 3.4 with a laser intensity of 0.1% under three accumulated acquisitions between 1200 cm⁻¹ and 100 cm⁻¹. Scanning Electron Microscopy (SEM) was conducted on an FEI NanoSEM Nova 450. Samples were mounted on aluminium stubs with double-sided copper tape as a suspension and allowed to dry. Samples were coated with a 2 nm iridium conductive layer prior to analysis. Thermogravimetric analysis (TGA) was conducted on a TA Instruments SDT Q600 simultaneous TGA/DSC instrument from room temperature to 900 °C at a constant ramp rate of 5 °C min⁻¹ and with a N₂ flow rate of 100 mL min⁻¹. UV-vis absorbance spectrophotometry analyses were obtained on a Perkin-Elmer Lambda 35 spectrophotometer in quartz cuvettes. Powder X-Ray Diffraction (pXRD) was conducted in a Bruker D2 Phaser (LYNXEYE detector) where dry powdered samples were deposited onto a silicon wafer. Data was obtained between $2\theta = 10^{\circ}$ to 75° over 20 minutes on spinning samples.

Instrument control, data acquisition and conversion was conducted in DIFFRAC.SUITE software package.

Synthesis of Amino Acid NCAs

Phe-NCA was synthesised as reported previously.² Briefly, Phe (5.00 g, 30.3 mmol) was weighed into a 3-necked RB flask which had been flushed with nitrogen and dried in an oven. Under a stream of nitrogen, α -pinene (4.03 g, 29.6 mmol) and anhydrous tetrahydrofuran (THF) (80 mL) were added and the reaction mixture refluxed for 30 minutes. Triphosgene (4.02 g, 13.5 mmol) was dissolved in anhydrous THF (20 mL) and added dropwise to the reaction mixture using a dropping funnel and refluxed under nitrogen for three hours. Phe-NCA was evaporated to dryness, re-dissolved in THF and precipitated in ice-cold *n*-hexane (300 mL). Phe-NCA was recrystallised three times in THF/*n*-hexane (1:9 v/v) to yield a white amorphous powder (yield 71%). ¹H NMR (400 MHz, CDCl₃, δ ppm): δ 7.12-7.31 (m, 5H, aromatic), δ 5.46 (s, 1H, >N<u>H</u>), δ 4.46 (dd, 1H, CH₂C<u>H</u>(COO-)NH-), δ 3.08 (dd, 2H, PhCH₂C(COO-)NH-). FTIR v_{max} (solid): 3356 cm⁻¹ (m, phenyl), 1837 cm⁻¹ (m, cyclic conjugated anhydride) and 1770 cm⁻¹ (s, secondary amide). BLG-NCA was synthesised using the same method as above (yield 72%). ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm): δ 7.42-7.3 (m, 5H, aromatic), δ 5.10 (s, 2H, Ar-C<u>H</u>₂-OCO-), δ 4.52-4.42 (m, 1H, >NH-CHCOO(CH₂)-), δ 2.55 (s, 2H, -CH₂-COO-), δ 2.11-2.01 (m, 1H, >CH-CH₂-CH₂-), δ 1.93 (td, J = 15.1, 7.6 Hz, 1H, >CH-CH₂-CH₂-). FTIR v_{max} (solid): 3252, 3331 cm⁻¹ (w, N-H), 3088 cm⁻¹ (w, aromatic C-H), 2858, 2932 cm⁻¹ (w, alkyl C-H), 1859, 1880 cm⁻¹ (m, anhydride C=O), 1720, 1773 cm^{-1} (s, ester C=O) and 1606 cm^{-1} (m, amide C=O).

A similar procedure was employed for the synthesis of Sar-NCA as previously reported,³ yielding offwhite crystals (yield 50%). ¹H NMR (400 MHz, CDCl₃, δ ppm): δ 4.05 (s, 2H, >N-C<u>H₂</u>-COO-) and δ 2.99 (s, 3H, >NC<u>H₃</u>). FTIR v_{max} (solid): 1847 cm⁻¹ (m, anhydride) and 1760 cm⁻¹ (s, tertiary amide).

Synthesis of comparison polymers ABPA-PPhe₈-*b*-PSar₁₀₅, ABPA-PBLG₂₂-*b*-PSar₆₃ and HUPA-PSar₉₃, and self-assembly polymers HUPA-PSar₁₇ and HUPA-PSar₂₃

These procedures were similar to that of producing the grafting-from nanocomposites. ABPA-PPhe₈*b*-PSar₁₀₅: briefly, ABPA (10 mg, 0.07 mmol, 1 eq.) and Phe-NCA (25 mg, 0.13 mmol, 2 eq.) were dissolved in anhydrous DMF (20 mL) and stirred at room temperature with a flow of N₂ until FTIR analysis determined there was no more Phe-NCA remaining. Sar-NCA (455 mg, 3.96 mmol, 57 eq.) was dissolved in anhydrous DMF (7 mL) and added to the reaction mixture with stirring and a flow of N₂ at room temperature. Once all of the Sar-NCA had been exhausted, as determined by FTIR analysis, the amphiphilic block copolymer was precipitated and washed in ice-cold diethyl ether (200 mL), collected by centrifuge (4,500 r.p.m., 15 mins.), dialysed against DI water (2,000 Da MWCO) and lyophilised (yield 62%). ABPA-PBLG₂₂-*b*-PSar₆₃ was synthesised in a similar way: briefly, ABPA (10.6 mg, 0.07 mmol, 1 eq.) was dissolved in anhydrous DMF (20 mL) and BLG-NCA (0.45 g, 1.72 mmol, 25 eq.) in anhydrous DMF (10 mL) added. After ten days, Sar-NCA (0.80 g, 6.93 mmol, 99 eq.) in anhydrous DMF (10 mL) was added to the reaction mixture. After eleven days the polymer was precipitated in diethyl ether, collected by centrifuge, dialysed and lyophilised (yield 64%).

HUPA-PSar₉₅: into a N₂-flushed, oven-dried Schlenk tube, Sar-NCA (0.47 g, 4.07 mmol, 102 eq.) was dissolved in anhydrous chloroform (10 mL) with stirring. Methanesulfonic acid (0.01 g, 0.12 mmol, 3 eq.) was added together with HUPA (0.01 g, 0.04 mmol, 1 eq.) and the reaction mixture stirred with a flow of N₂ at 40 °C for 24 h. Propagation was then started by cooling the reaction mixture to 0 °C and

adding *N*-ethyldiisopropylamine (0.27 g, 2.09 mmol, 52 eq.). After being stirred at 0 °C with a flow of N₂ for four days the polymerisation reaction was complete as determined by FTIR. The polymer was precipitated by adding dropwise into ice-cold diethyl ether (1:5 v/v), collected by centrifuge, dialysed and lyophilised (yield 84%). HUPA-PSar₁₇ and HUPA-PSar₂₃ were synthesised in the same manner as HUPA-PSar₉₅ above.

Afterglow lifetime analysis

Afterglow lifetimes were estimated using a stroboscopic method. Videos of afterglow were obtained with a Canon EOS 7D SLR camera in video mode (25 fps) with a Canon ER 100 mm f/2.8 Macro USM lens. Whilst being recorded, the samples were irradiated with UV light at 365 nm, and the UV lamp switched off after a few seconds. The video recording continued for a few seconds after the UV lamp was switched off in order to capture afterglow. Individual frames of the video were obtained using VirtualDub software, and the frames analysed in ImageJ software. Afterglow lifetimes were obtained by plotting the mean grey values against time with a 0.04 s interval.

SUPPORTING FIGURES AND TABLES



Figure S1. (a) SEM micrograph of CND/CaNPs (inset: particle diameter distribution from this SEM micrograph calculated from 126 particles), scale bar = 200 nm. (b) Normalised afterglow lifetime of CND/CaNPs (inset: photos of blue fluorescence and green afterglow).



Figure S2. Raman spectrum of CND/CaNPs.



Figure S3. Powder XRD pattern of CND/CaNPs (red line) and commercially available calcite powder as a reference (black line). To remove shoulders on the peaks due to interference from the x-ray source, a K α 2 strip was performed on the data.



Figure S4. FTIR spectra of CND/CaNPs and ABPA- or HUPA-functionalised CND/CaNPs.



Figure S5. SEM micrograph of 4-ABPA-functionalised CND/CaNPs, scale bar is 200 nm.

	Initiator		Polymer				
	Label	Integral	Label	Integral	# of H		M _w (kg
Polymer/nanocomposite					atoms	Equivalence	mol⁻¹)
ABPA- PPhe₈- <i>b</i> -PSar ₁₀₅	d	6	а	37.58	5	8	8.8
ABPA-PPhe ₈ -b- PSar₁₀₅			с	314.70	3	105	
CND/CaNP-ABPA- PPhe 4-			2	20 52	E	4	
<i>b</i> -PSar ₁₆	d	6	d	20.53	5	4	1.9
CND/CaNP-ABPA-PPhe ₄ -			6	47 70	2	16	
b-PSar ₁₆			L	47.79	5	10	
ABPA- PBLG₂₂- <i>b</i> -PSar ₆₃	d	6	а	109.06	5	22	9.4
ABPA-PBLG ₂₂ -b- PSar₆₃			с	187.92	3	63	
CND/CaNP-ABPA- PBLG₁₈-				00 72	-	10	
<i>b</i> -PSar ₁₆	d	6	d	89.72	5	18	5.2
CND/CaNP-ABPA-PBLG ₁₈ -			_	46.02	2	16	
b-PSar ₁₆			Ľ	40.93	5	10	
HUPA- PSar 95	с	14	b	286.23	3	95	7.0
CND/CaNP-HUPA- PSar₈₂	с	14	b	246.95	3	82	6.1

Table S1. Comparison between the integrals of initiator protons to polymer protons to calculate polymer equivalents and molecular weight.



Figure S6. MAS ¹³C NMR (100 MHz, solid-state, δ ppm) of CND/CaNP-ABPA-PPhe₄-*b*-PSar₁₆ (bottom) and ¹³C NMR (125 MHz, DMSO-*d*₆) of ABPA-PPhe₈-*b*-PSar₁₀₅ (top).



Figure S7. Solid-state MAS ¹³C NMR (100 MHz, solid state, δ ppm) spectra of CND/CaNP-ABPA-PBLGA₁₈-*b*-PSar₁₆ (bottom) and ¹³C NMR (125 MHz, DMSO-*d*₆) spectra of ABPA-PBLGA₂₂-*b*-PSar₆₃ (top).



Figure S8. ¹³C NMR of CND/CaNP-HUPA-PSar₈₂ (125 MHz, DMSO- d_6 , δ ppm) (top) and HUPA-PSar₉₅ (125 MHz, CDCl₃) (bottom).



Figure S9. Raman microscopy spectra of CND/CaNP-ABPA-PPhe₄-b-PSar₁₆ (red line) and CND/CaNP-ABPA-PBLGA₁₈-b-PSar₁₆ (black line).



Figure S10. Raman microscopy spectra of the dried supernatant from washing CND/CaNP-ABPA-PPhe₄-b-PSar₁₆.



Figure S11. ¹H NMR spectra of HUPA-PSar₁₇ (a) and HUPA-PSar₂₃ (b) (500 MHz, CDCl₃, δ ppm).



Figure S12. ¹³C NMR of HUPA-PSar₁₇ (a) and HUPA-PSar₂₃ (b) (500 MHz, CDCl₃, δ ppm).



Figure S13. FTIR spectra of HUPA-PSar polymers.



Figure S14. FTIR spectra of the self-assembled nanocomposites CND/CaNP-HUPA-PSar_{17} and CND/CaNP-HUPA-PSar_{23}.



Figure S15. FTIR spectra of the dried supernatants collected from washing the CND/CaNP-HUPA-PSar₁₇ and CND/CaNP-HUPA-PSar₂₃ nanocomposites.



Figure S16. TGA thermograms of CND/CaNPs, some CND/CaNP-polymer nanocomposites, and polymer analogues.



Figure S17. Kinetic UV-vis absorbance of CND/CaNPs in deionised water (pH 7) and CND/CaNP-polymer nanocomposites over four hours. All suspensions/solutions were at a concentration of 0.1 mg mL⁻¹ and absorbencies were taken every 60 seconds at 650 nm.



Figure S18. UV-vis spectrum of an aqueous solution of the CNDs.



Figure S19. Dox calibration curves for samples in PBS buffer solution (a) and acetate buffer solution (b).

SUPPORTING REFERENCES

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4. D. C. Green, M. A. Holden, M. A. Levenstein, S. Zhang, B. R. G. Johnson, J. Gala de Pablo, A. Ward, S. W. Botchway and F. C. Meldrum, *Nature Communications*, 2019, **10**, 206.