SUPPORTING INFORMATION

Cross-linking Poly(caprolactone)−Polyamidoamine Linear Dendritic Block Copolymers for Theranostic Nanomedicine

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SYNTHESIS

70-PCL-G3 Structure

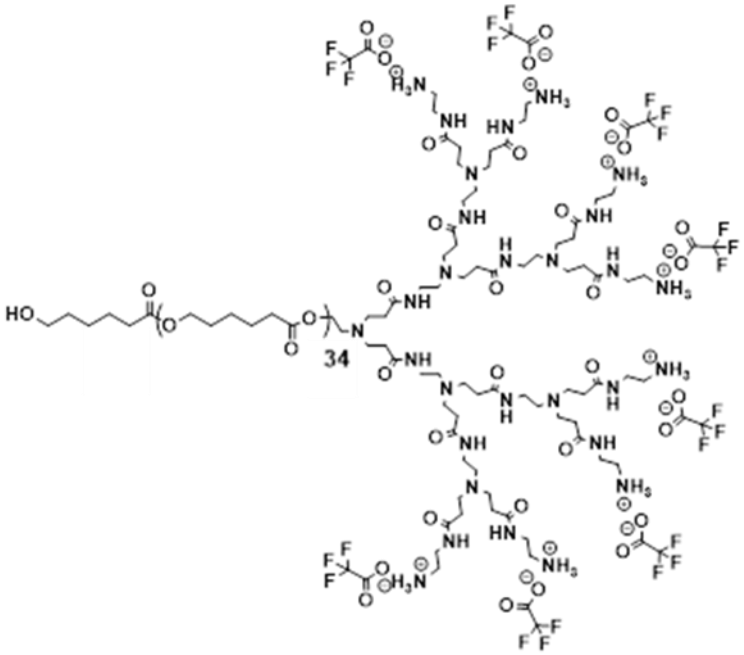
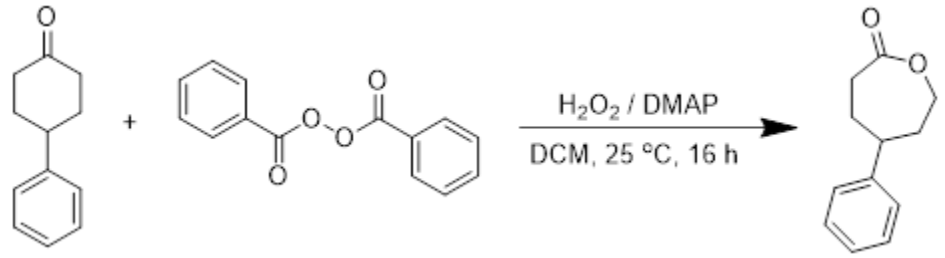


Figure S1. Structure of 70-PCL-G3 LDBC, consists of 70 wt% hydrophobic (PCL) and 30 wt% hydrophilic (PAMAM). [1] n = 34

The synthesis of 70-PCL-G3 is reported in our previous work. [1]

Ph-εCL Synthesis

Scheme S1. Synthesis of Ph-εCL[2]



Phenyl substituted ε-caprolactone was synthesized via Baeyer–Villiger oxidation of 4-phenylcyclohexanone.[33] (The synthesis was done according to the procedure described in the general synthesis section of the main text.) ^{1}H NMR (400 MHz, Chloroform-d) δ 7.37 – 7.15 (C**H**(Ph)- , m, 5H), 4.44 – 4.26 (-C**H$_2$**-O-, m, 2H), 2.91 – 2.70 (-C**H$_2$**-COO-, Ph-C**H**-(CH$_2$)$_2$-, m, 3H), 2.20 – 1.77 (-C**H$_2$**-CH$_2$-O-, -C**H$_2$**-CH$_2$-COO-, m, 4H).

5-PhPCL-G3

A mixture of PAMAM–G3–Boc (1) (1.0 g, 0.40 mmol), PhPCL (91.6 mg, 0.48 mmol) and ε-caprolactone (1.53 g, 13.4 mmol) in a mixture of chlorobenzene (15 ml) was heated to 90 °C. Tin(II) 2-ethylhexanoate (Sn(Oct)$_2$) (98.74 mg, 0.24 mmol) was then added under an ultra-high pure nitrogen environment, and the mixture was heated to 130 °C. The mixture was stirred at 130 °C for 10 h. The reaction mixture was allowed to cool down to room temperature, added dropwise into 250 ml of diethyl ether (Et$_2$O) while stirring. A precipitate was formed and settled own to the bottom of the flask after stirring stopped. The mixture was vacuum filtered and solid was separated. The resulting pale-yellow solid was redissolved in chloroform (5 ml) and added dropwise into 250 ml of Et$_2$O, similar to the previous step. The precipitation steps were repeated three times to get a

pure product. The resulting yellow solid was dried under a high vacuum at room temperature for 24 hours to obtain the pure and dried product with an 85.2 % yield (2.08 g).

10-PhPCL-G3

A mixture of PAMAM–G3–Boc (1) (1.0 g, 0.40 mmol), PhPCL (160 mg, 0.84 mmol) and ε-caprolactone (1.42 g, 12.5 mmol) in a mixture of chlorobenzene (15 ml) was heated to 90 °C. Tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) (98.82 mg, 0.24 mmol) was then added under an ultra-high pure nitrogen environment, and the mixture was heated to 130 °C. The mixture was stirred at 130 °C for 10 h. The reaction mixture was allowed to cool down to room temperature, added dropwise into 250 ml of diethyl ether (Et_2O) while stirring. A precipitate was formed and stuck on the wall of the flask. The mixture was kept stirring until a clear solution is observed (all the precipitate stuck onto the walls), and stirring was stopped. Then Et_2O was decanted from the mixture. The resulting yellow solid was redissolved in chloroform (5 ml) and added dropwise into 250 ml of Et_2O, similar to the previous step. The precipitation steps were repeated three times to get a pure product. The resulting yellow solid was dried under a high vacuum at room temperature for 24 hours to obtain the pure and dried product with an 83 % yield (2.03 g).

20-PhPCL-G3

A mixture of PAMAM–G3–Boc (1) (1.0 g, 0.40 mmol), PhPCL (319.4 mg, 1.68 mmol) and ε-caprolactone (1.28 g, 11.2 mmol) in a mixture of chlorobenzene (15 ml) was heated to 90 °C. Tin(II) 2-ethylhexanoate ($Sn(Oct)_2$) (98.8 mg, 0.24 mmol) was then added under an ultra-high pure nitrogen environment, and the mixture was heated to 130 °C. The mixture was stirred at 130 °C for 10 h. The reaction mixture was allowed to cool down to room temperature, added

dropwise into 250 ml of diethyl ether (Et_2O) while stirring. A precipitate was formed and stuck on the wall of the flask. The mixture was kept stirring until a clear solution is observed (all the precipitate stuck onto the walls), and stirring was stopped. Then Et_2O was decanted from the mixture. The resulting dark yellow sticky solid was redissolved in chloroform (5 ml) and added dropwise into 250 ml of Et_2O, similar to the previous step. The precipitation steps were repeated three times. As this reaction was not propagated to 100% conversion, the percentage yield was not calculated.

Table S1. Theoretical composition wt % of each block concerning the LDBC and wt% of each PCL type (i.e., PhPCL or PCL) concerning the hydrophobic block.[a]

	wt% of the hydrophilic block (PAMAM-G3)	wt% of the hydrophobic block (PCL + PhPCL)	PCL and PhPCL wt% concerning hydrophobic block weight	
			PhPCL wt%	PCL wt%
5-PhPCL-G3	30	70	5	95
10-PhPCL-G3	30	70	10	90
20-PhPCL-G3	30	70	20	80

[a] Actual composition qualitatively determined via spectroscopy and thermal analysis (Tables S2 and 3)

NMR SPECTRA

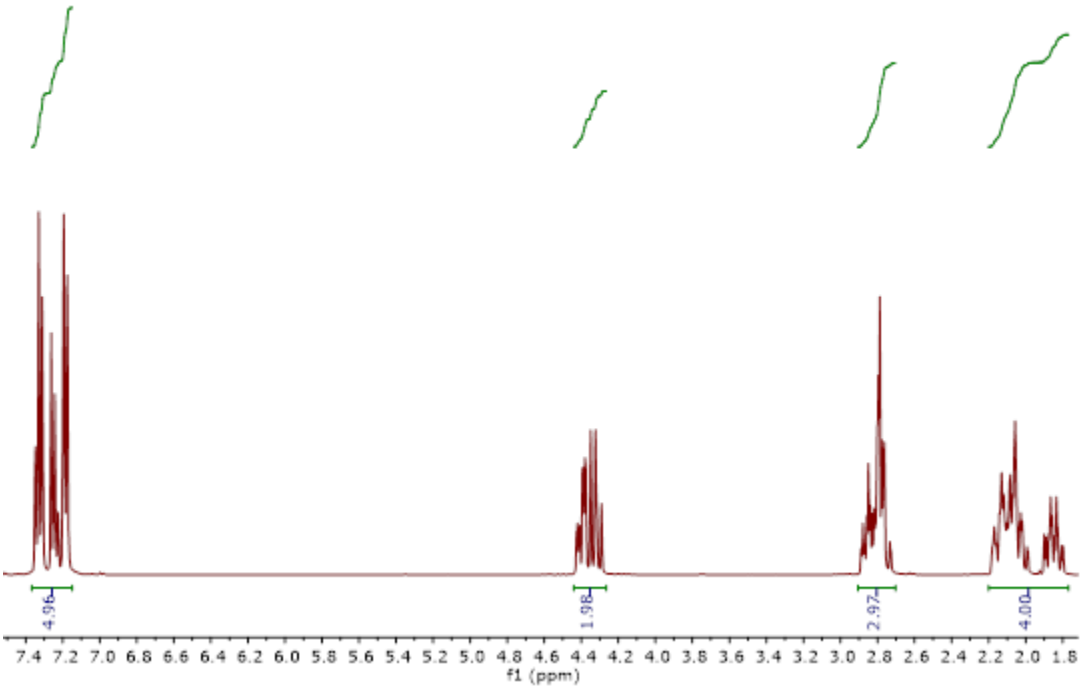


Figure S2. ^{1}H NMR for Ph-εCL (400 MHz, Chloroform-*d*) δ 7.37 – 7.15 (m, 5H), 4.44 – 4.26 (m, 2H), 2.91 – 2.70 (m, 3H), 2.20 – 1.77 (m, 4H).

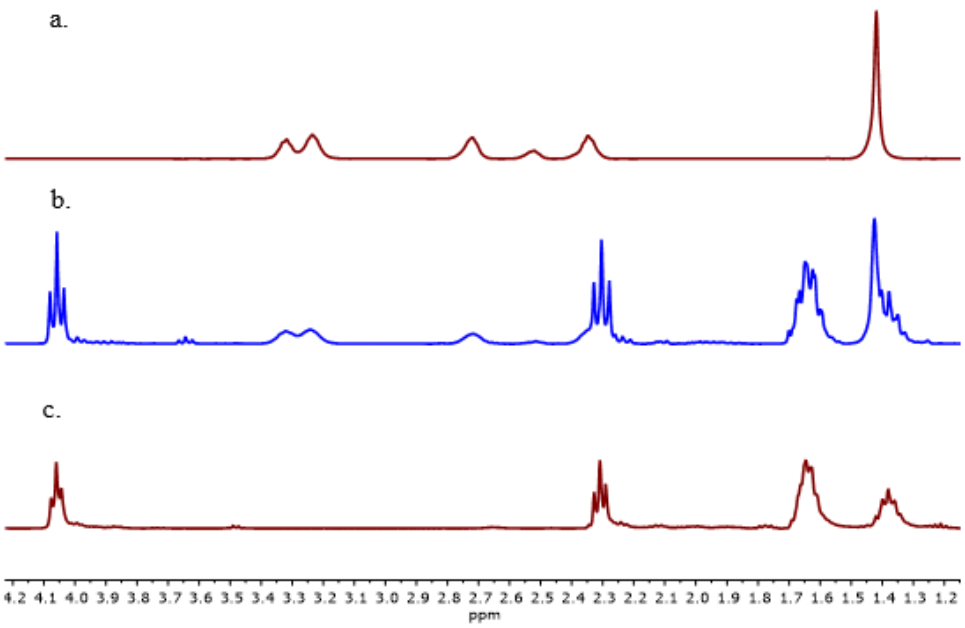


Figure S3. ^{1}H NMR overlay of the (a) macroinitiator (PAMAM-G3-Boc), (b) LDBC intermediate (10-PhPCL-G3Boc), and (c) LDBC after Boc deprotection (10-PhPCL-G3) in $CDCl_3$.

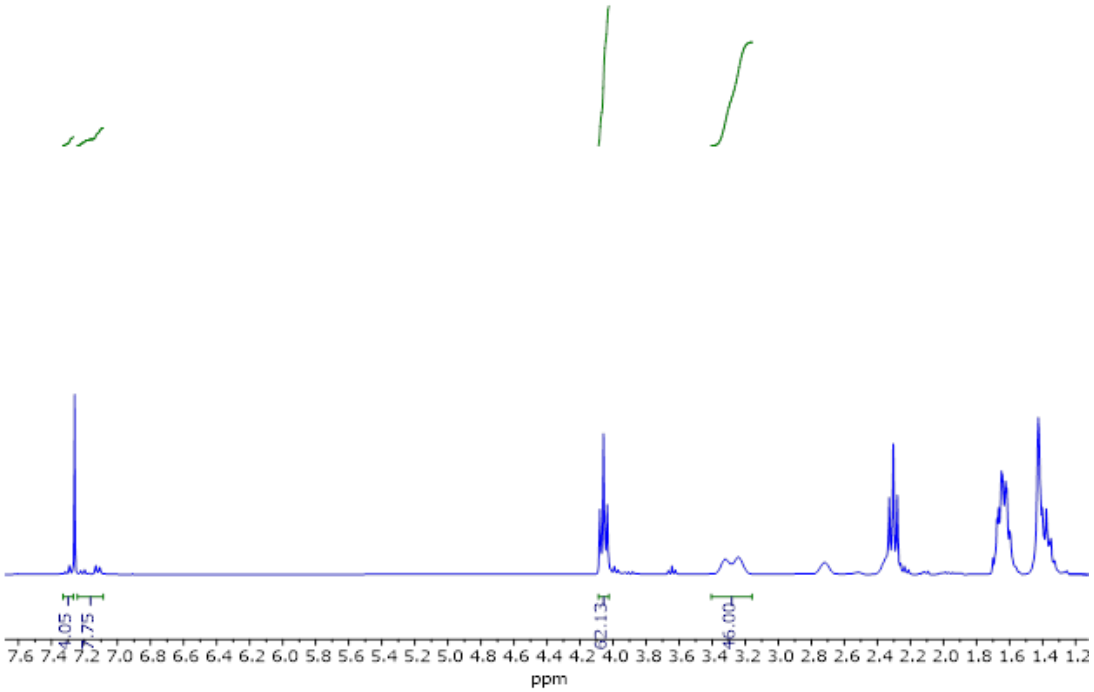


Figure S4. ^{1}H NMR for 10-PhPCL-G3Boc in $CDCl_3$

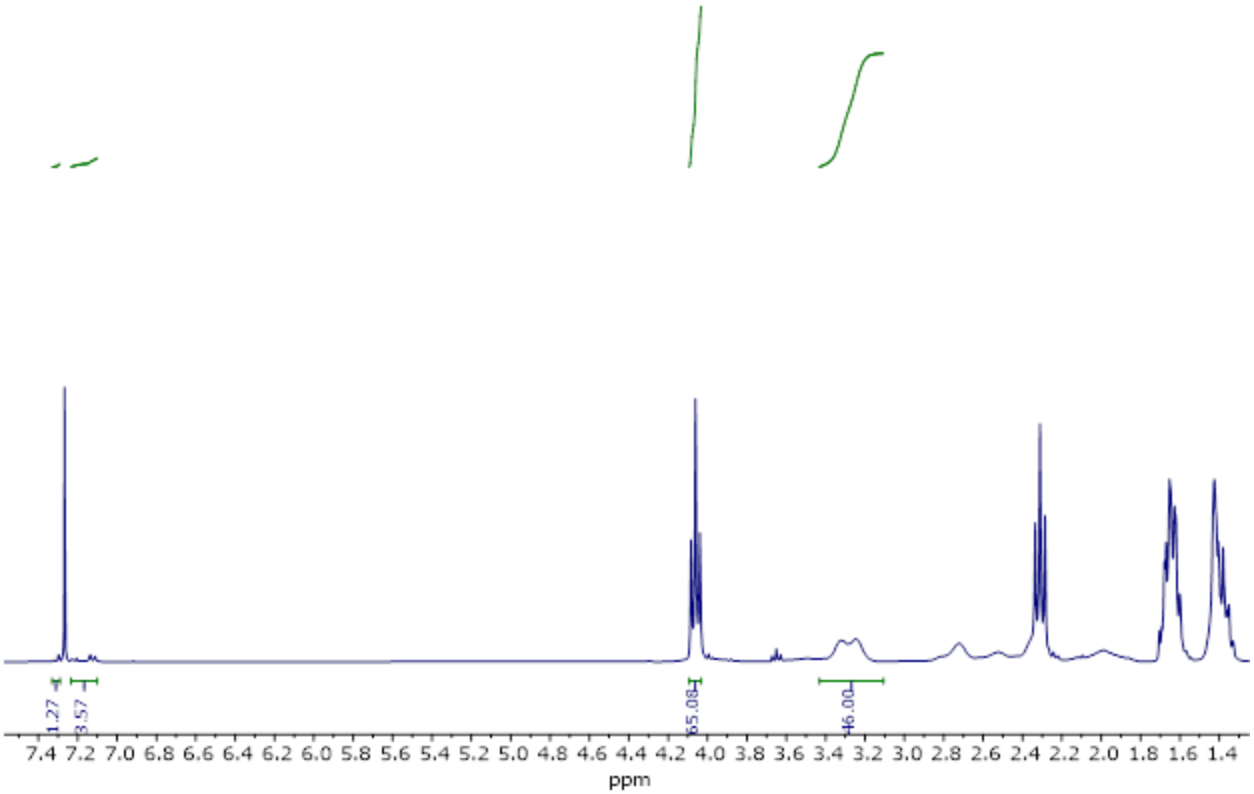


Figure S5. ^{1}H NMR for 5-PhPCL-G3Boc in $CDCl_3$

GPC ANALYSIS

Table S2. Molecular weight analysis of LDBCs by NMR spectroscopy and GPC.

	M_{th}	DP $_{th}$	nPhPCL $_{th}$	M_n NMR	DP NMR	nPhPCL NMR	%Ph	M_n	M_w	Đ
5-PhPCL-G3Boc	6350	34	1	6247	33	1	5.4	6143	7676	1.25
10-PhPCL-G3Boc	6350	34	2	6386	34	2	9.6	5280	6929	1.31

The notation of PhPCL-G3 denotes the LDBC composition, where phenyl substituted PCL connected to a G3 PAMAM with a weight percentage is in respect to the caprolactone monomer used; M_{th}, theoretical molar mass; DP_{th}, theoretical degree of polymerization; M_n, number average molar mass; M_w, weight-average molar mass; and Đ , dispersity.

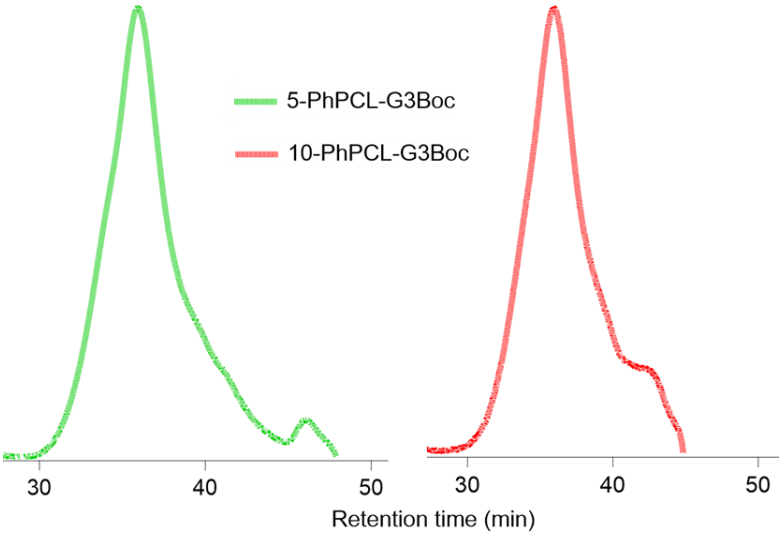


Figure S6. GPC chromatograms of PhPCL-G3Boc LDBC precursors.

THERMAL ANALYSIS

Table S3. Predicted and Experimental Thermal Analysis Data (TGA) Confirming the Weight Ratios for Each LDBC.

System	Composition			1st step		2nd step		3rd step	
	PAMAM	PCL + PhPCL	Ph substituents (wrt total molar mass)	T_d (°C)	ΔW (wt%)	T_d (°C)	ΔW (wt%)	T_d (°C)	ΔW (wt%)
10-PhPCL-G3	30	70	7	226	32.5	321	67.5	422	8
5-PhPCL-G3	30	70	3.5	220	33	314	67	410	NA

TGA

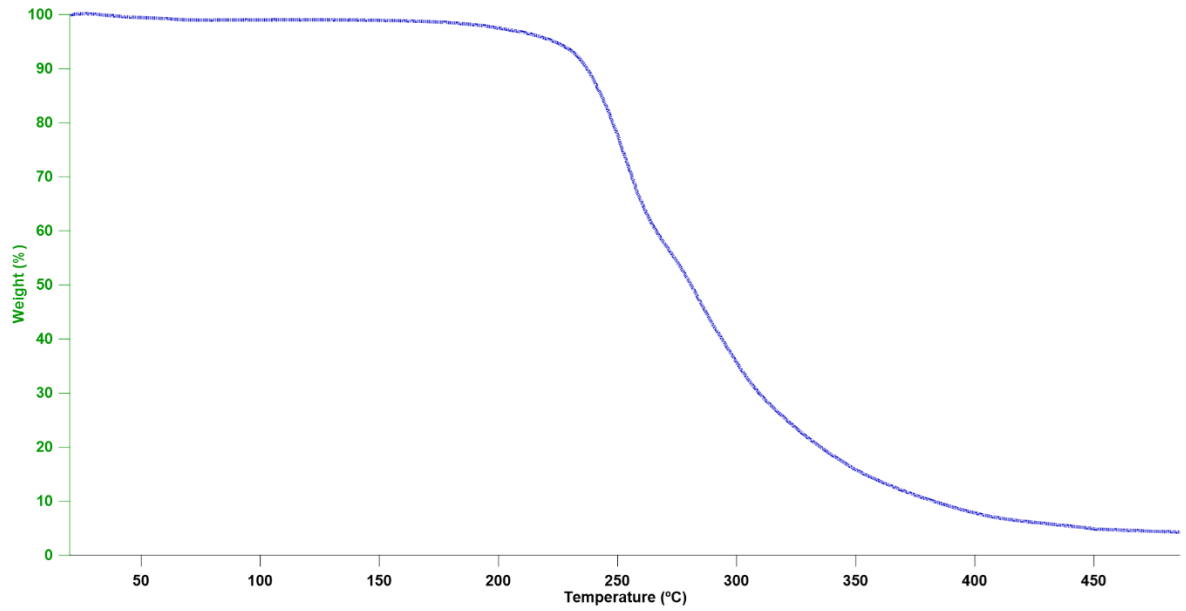


Figure S7. TGA of PAMAM-G3Boc; 95 % decomposition at 223.4°C

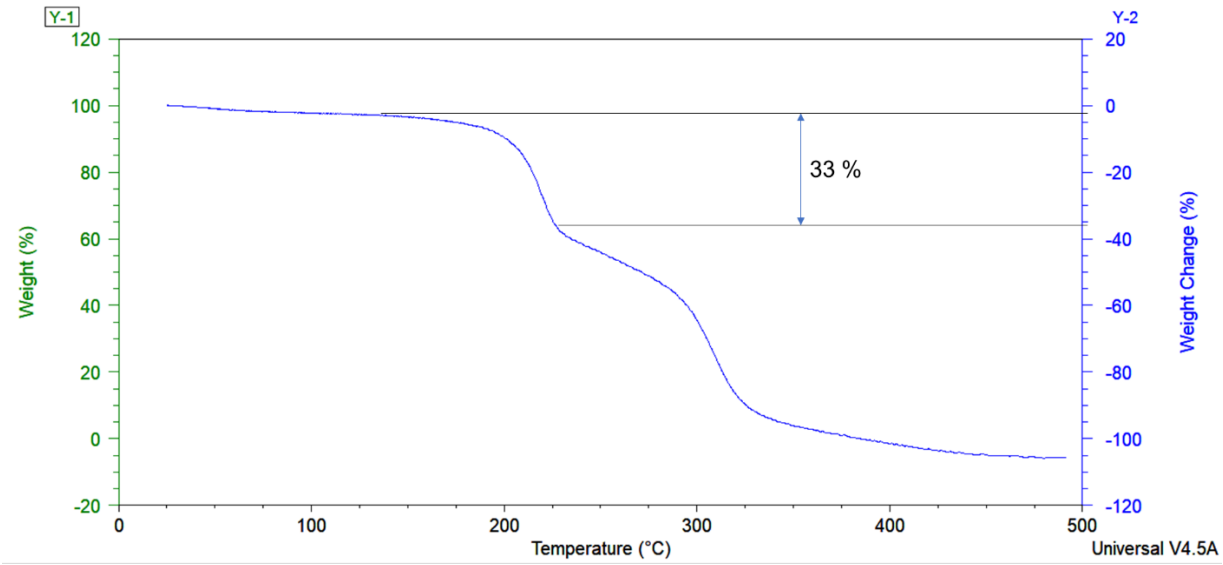


Figure S8. TGA of 5-PhPCL-G3

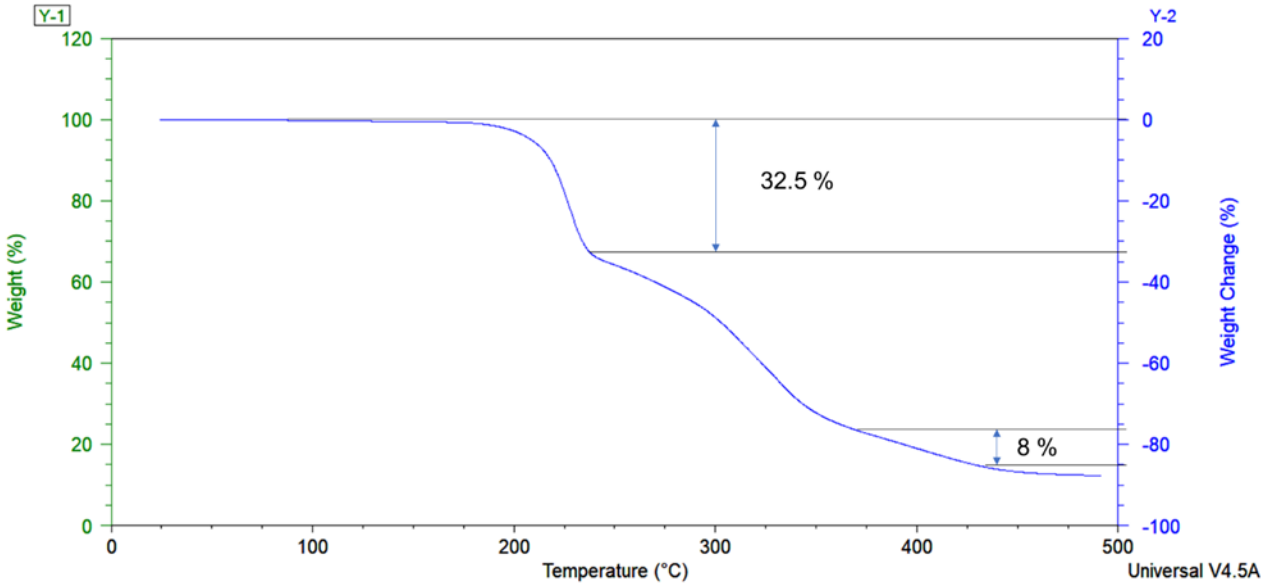


Figure S9. TGA of 10-PhPCL-G3.

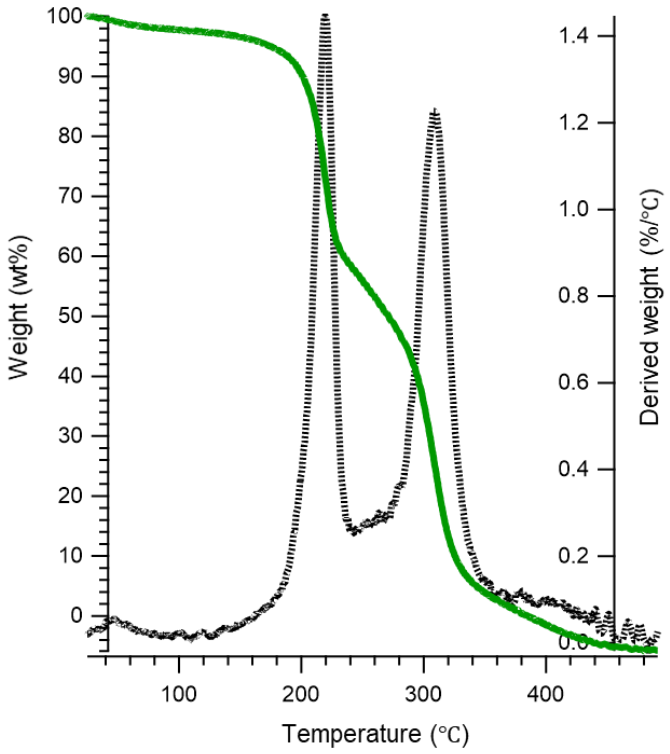


Figure S10. TGA and derived TG analysis of 5-PhPCL-G3. TGA (green) and derivative thermogravimetry (DTG) (black)

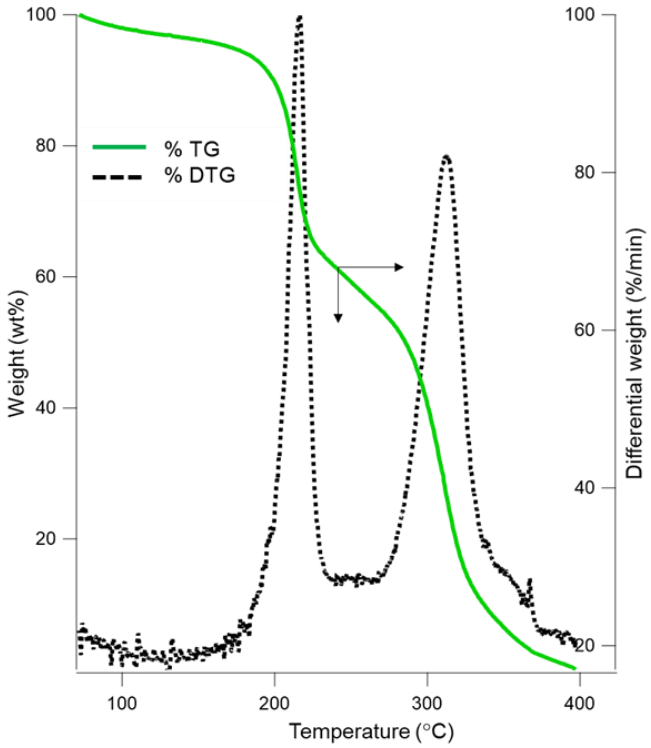


Figure S11. TGA and derived TG analysis of PCL-G3 (70-PCL-G3). TGA (green) and derivative thermogravimetry (DTG) (black)[1]

DSC

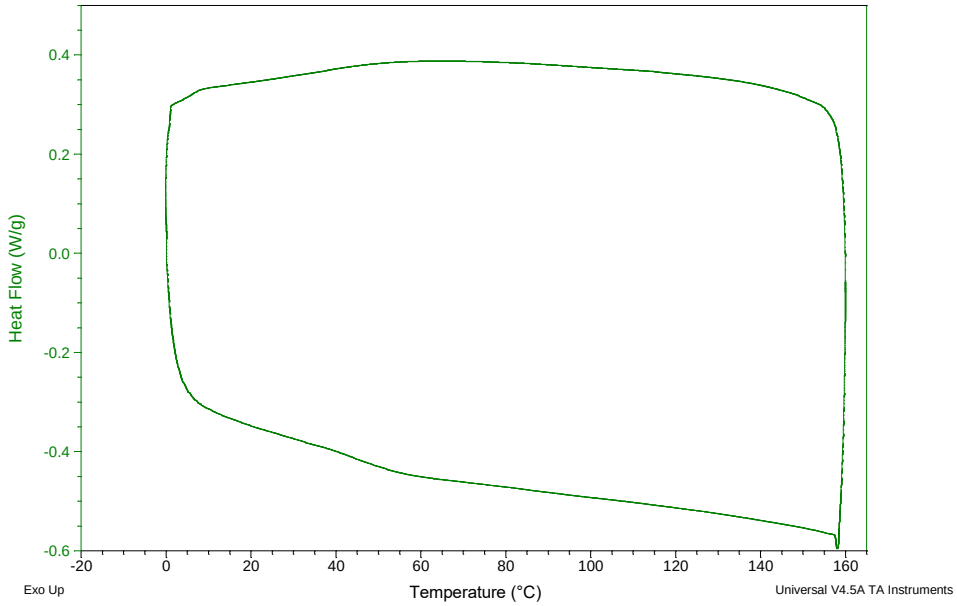


Figure S12. DSC thermogram for 5-PhPCL-G3; 3rd and 4th cycle

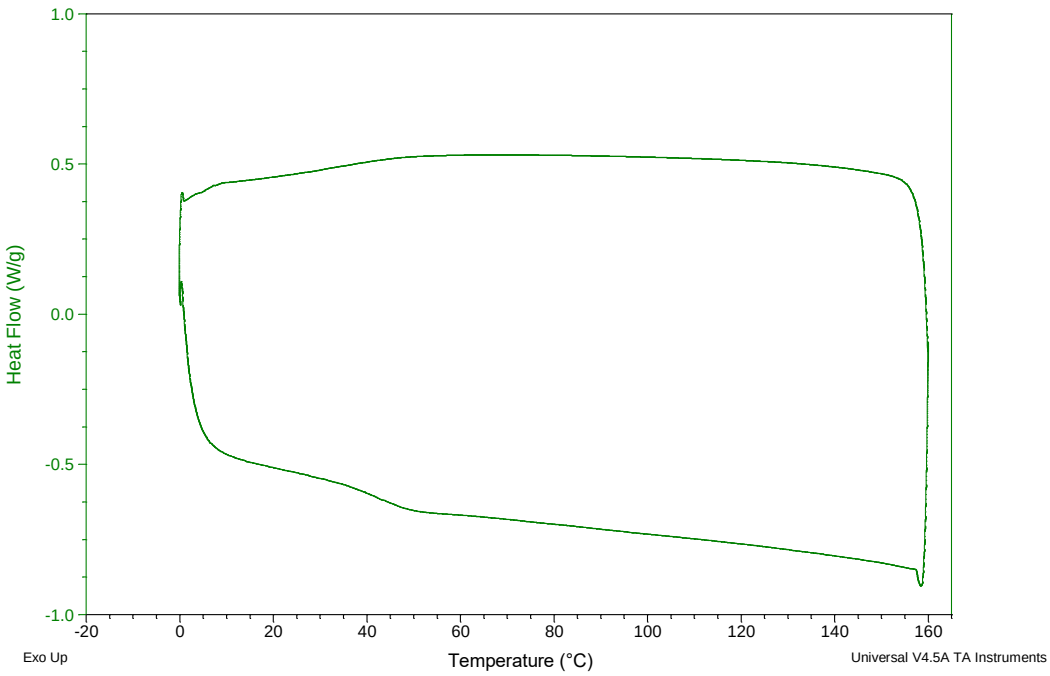


Figure S13. DSC thermogram for 10-PhPCL-G3; 3rd and 4th cycle

CRITICAL AGGREGATION CONCENTRATION (CAC)

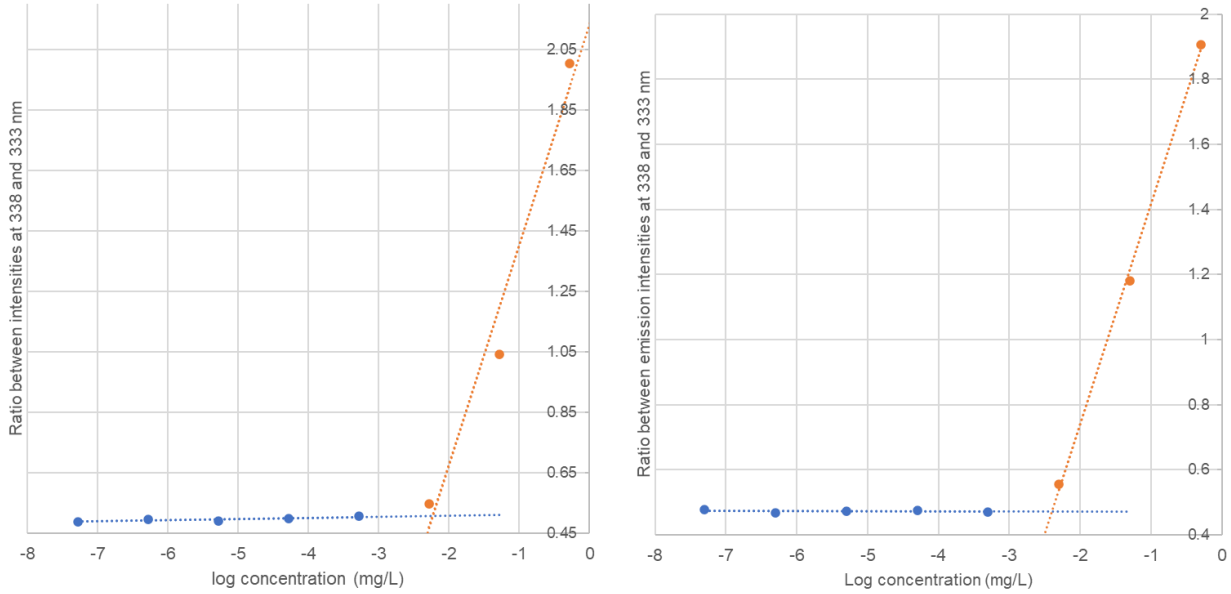


Figure S14. CAC plots of 5-PhPCL-G3 (left) 10-PhPCL-G3 (right). (Y axis - ratio of emission intensities at 338 and 333 nm)

DYNAMIC LIGHT SCATTERING (DLS) and TEM

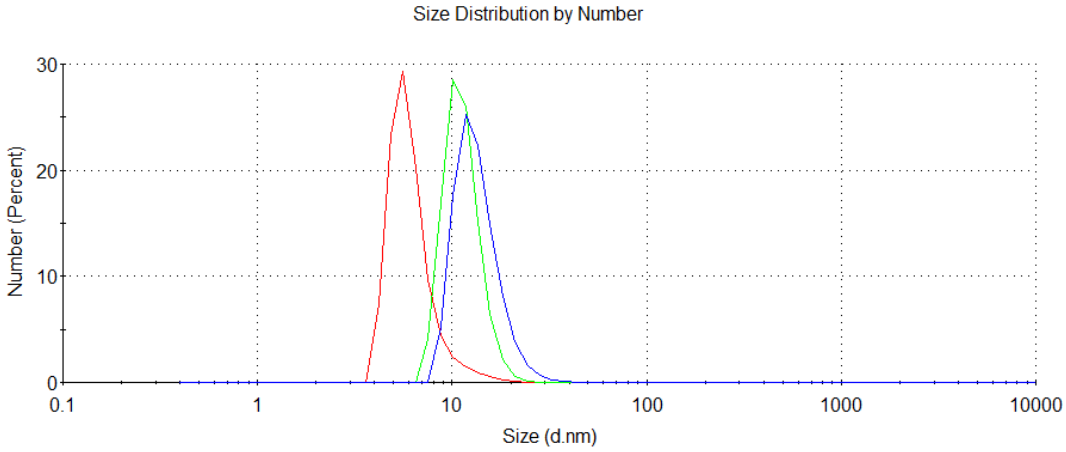


Figure S15. Number average size distributions for 5-PhPCL-G3.

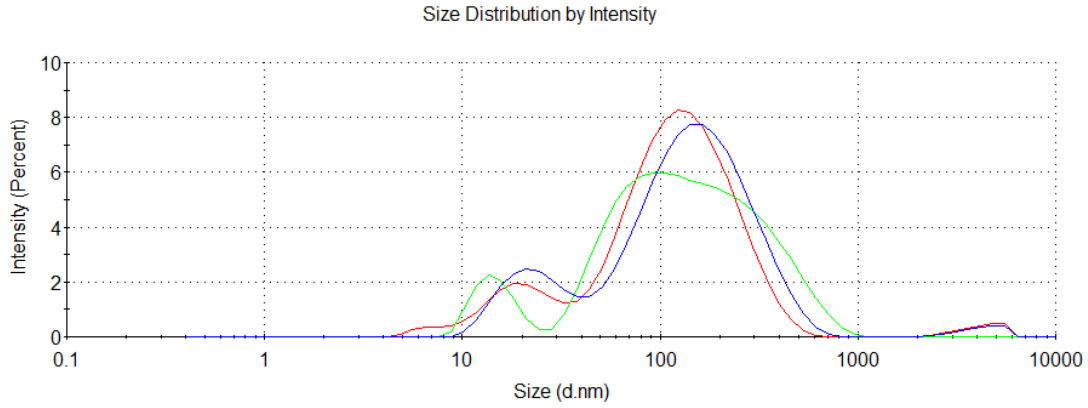


Figure S16. Intensity average size distributions for 5-PhPCL-G3.

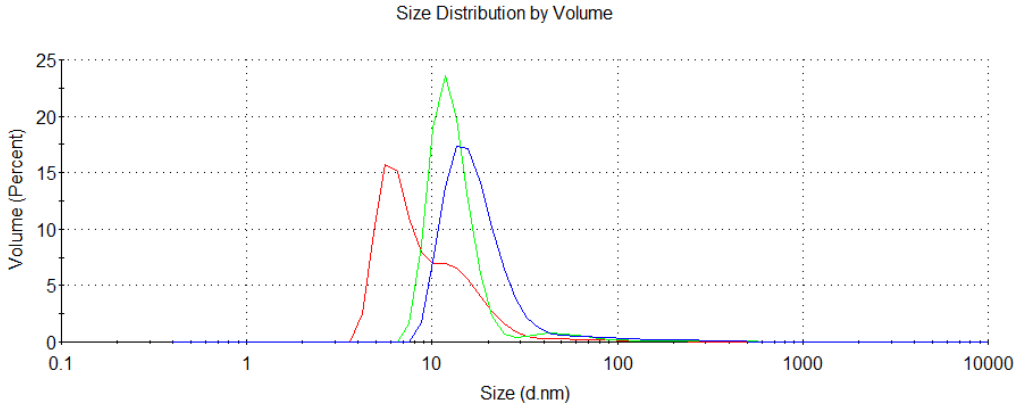


Figure S17. Volume average size distributions for 5-PhPCL-G3.

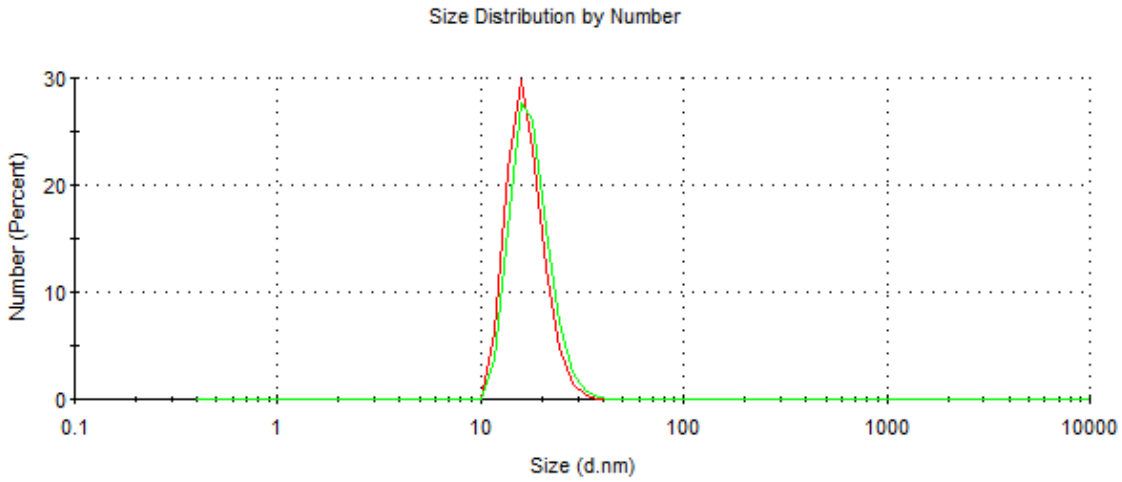


Figure S18. Number average size distributions for 10-PhPCL-G3.

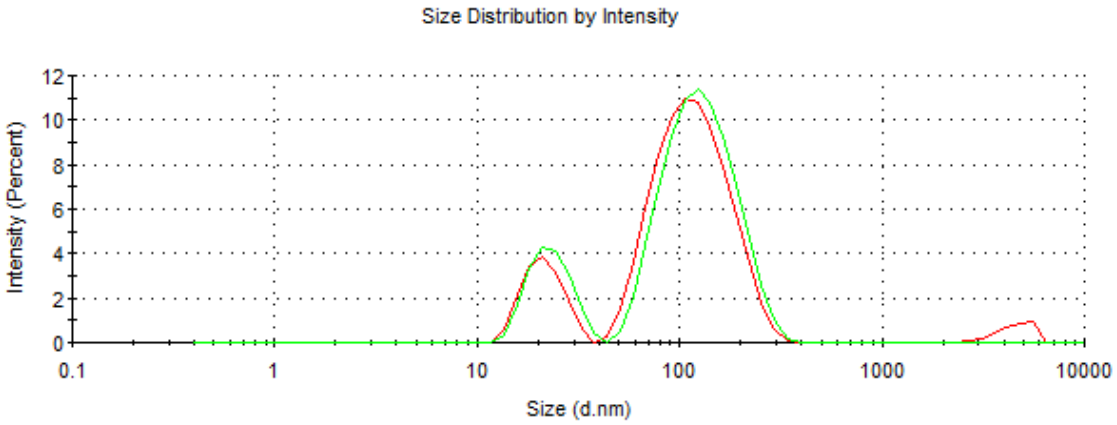


Figure S19. Intensity average size distributions for 10-PhPCL-G3.

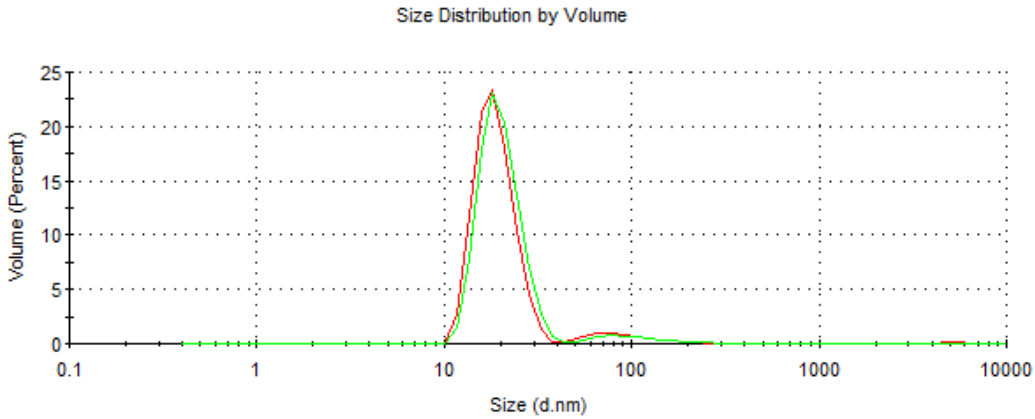


Figure S20. Volume average size distributions for 10-PhPCL-G3.

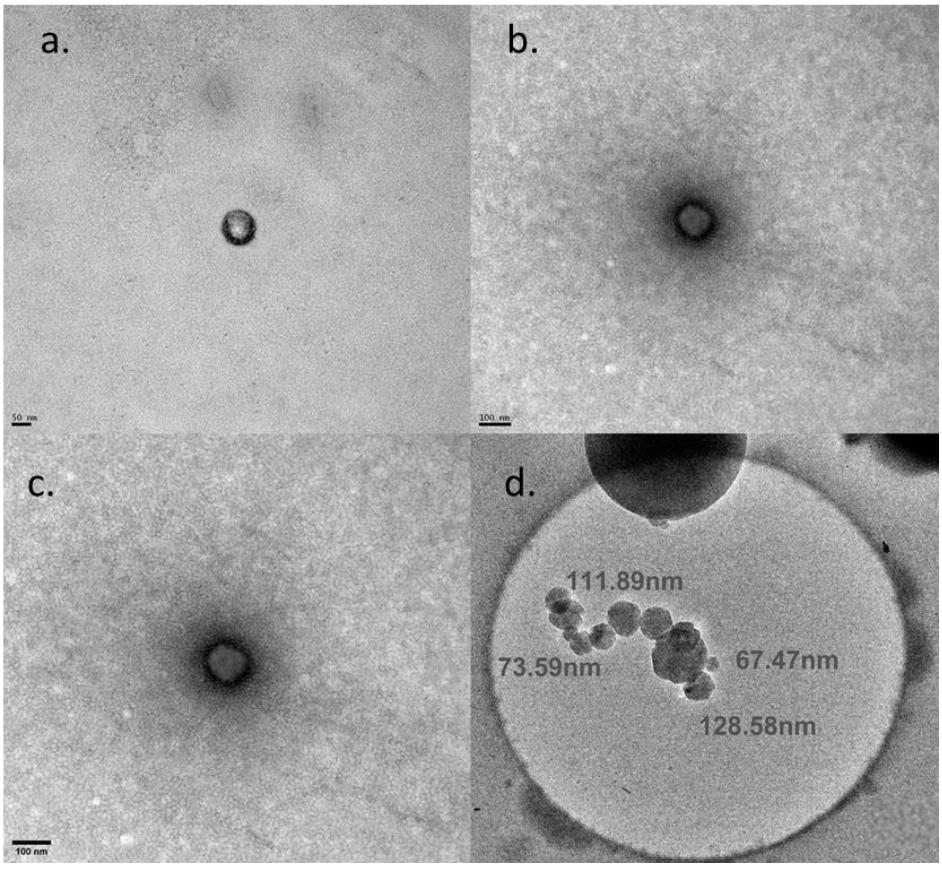


Figure S21. Additional TEM images that show evidence of bilayer vesicles and size distributions observed from DLS. Standard TEM (a-c) and cryo-TEM (d).

ENCAPSULATION STUDIES

Table S4. DL % comparison between phenyl substituted and non-substituted LDBCs formed via conventional nanoprecipitation.

	Encapsulation efficiency (DL %)		
	Curcumin	C3	C5
70-PCL-G3	13.70	1.70	2.58
5-PhPCL-G3	13.73	1.94	2.88
10-PhPCL-G3	17.50	2.40	3.41

EMISSION SPECTRA

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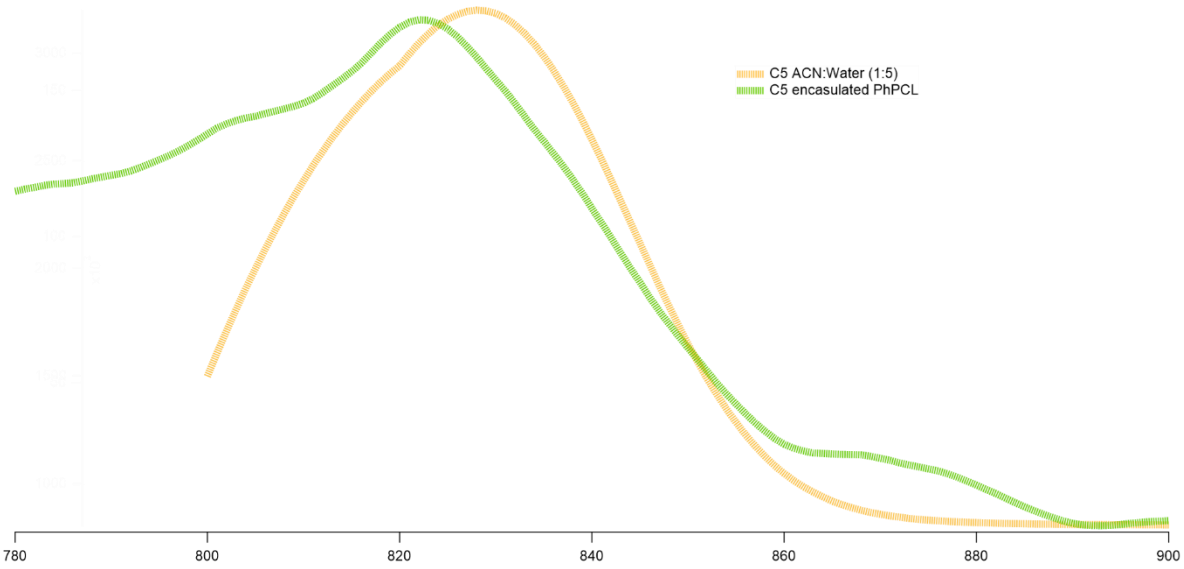

Figure S22. Emission spectra for free C5 in the solution and encapsulated in 10-PhPCL-G3 nanoparticles. Excitation wavelengths are 700 nm and 810 nm.

ABSORPTION SPECTRA AT DIFFERENT TIME INTERVALS

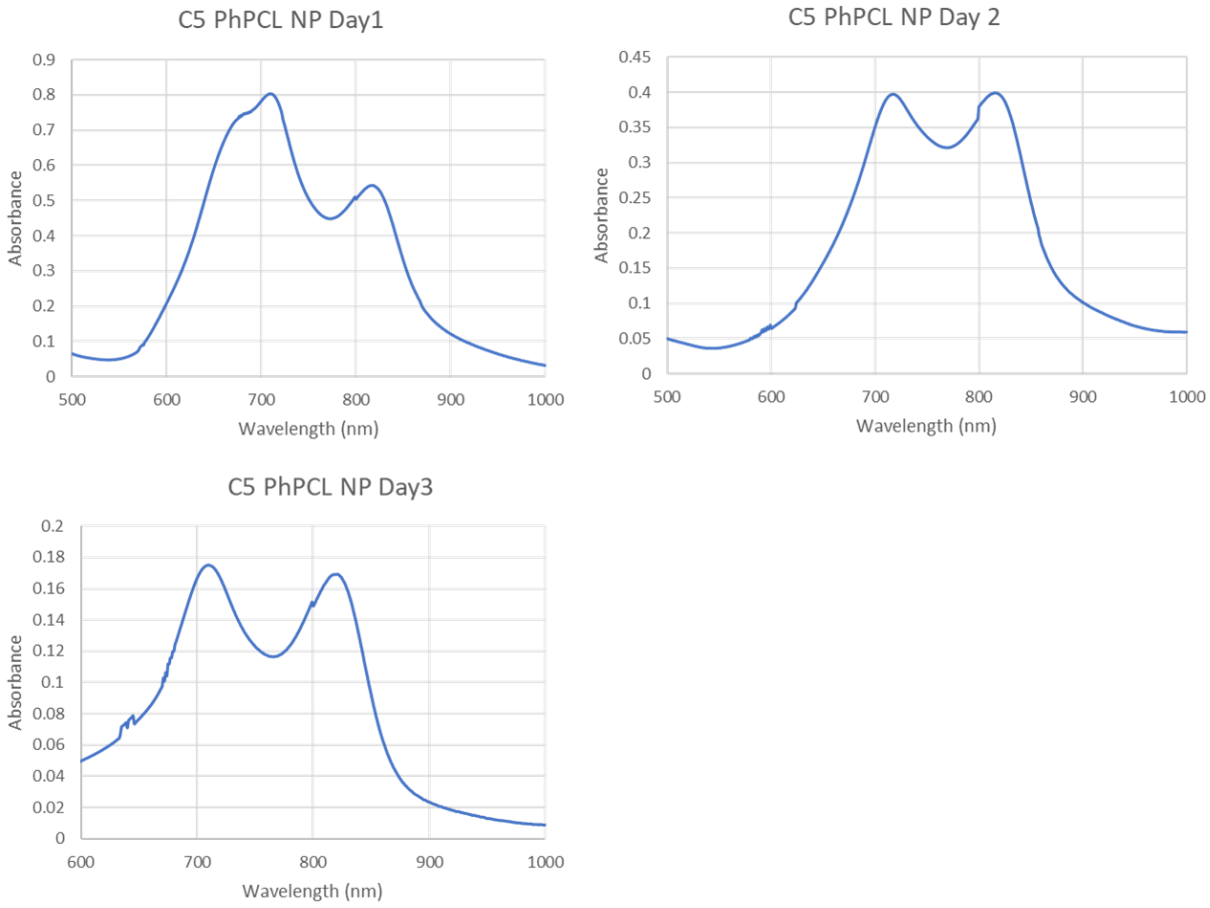

Figure S23. Absorption spectra of C5 loaded 10-PhPCL-G3 nanoparticles obtained in different time intervals via nanoprecipitation (NP)

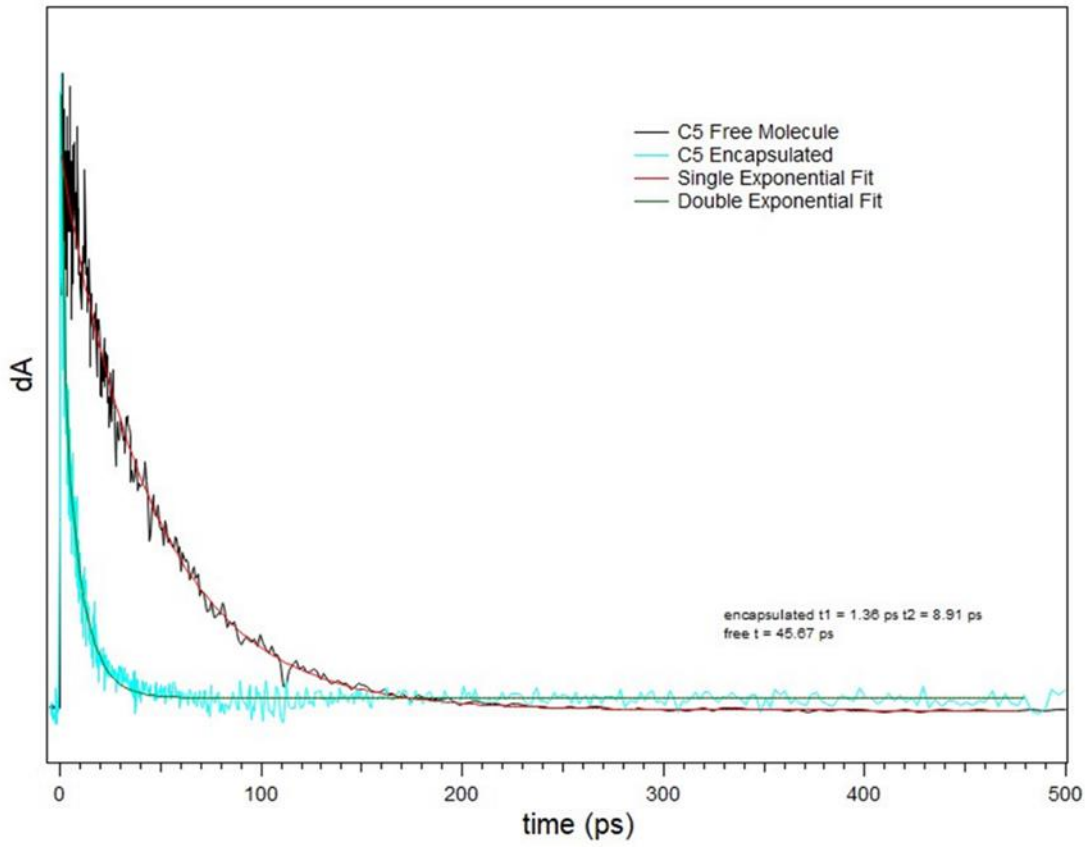

Figure S24. Excite state lifetimes for free C5 in the solution and encapsulated in 10-PhPCL-G3 nanoparticles, excitation wavelength 810nm.

PHOTOTHERMAL DATA

Table S5. Photothermal efficiency calculations

Sample	η	hA	hA $k_{t\sim}$ (s)	mc_p (J/K)	ΔT_{max} (K)	ΔT_{sol} (K)	I (J/sec)	A_λ (estimated)
C5-PhPCL	48.2%	0.011119	376.43	4.1855	43.4	2.2	0.95	9.555589
ICG-PhPCL	45.7%	0.010749	389.4	4.1855	42.6	2.2	0.95	27.17803

η: photothermal efficiency

h: heat transfer coefficient

A: surface area of the container

$k_{t\sim \ln\left(\frac{\Delta T}{\Delta T_{max}}\right)}$: slope obtained from the fitting of $t\sim \ln\left(\frac{\Delta T}{\Delta T_{max}}\right)$ during the naturally cooling process, ΔT is the difference between liquid temperature and room temperature

ΔT_{max}: the maximum temperature change of the liquid.

ΔT_{sol}: the maximum temperature change of MilliQ water irradiated by the 808 nm laser

I: irradiation power

A_λ: the absorbance of nanoparticles with photothermal agents loaded at 808 nm.

STORAGE STABILITY

Table S6. Storage stability of C5 loaded and empty 10-PhPCL-G3 nanoparticles.

day	Empty nanoparticles		C5 loaded nanoparticles	
	PDI	Size/nm	PDI	Size/nm
1	0.436	43.22	0.022	193.5
2	0.444	44.09	0.046	205.3
3	0.465	50.03	0.114	215.6
4	0.589	56.99	0.066	219.2
5	0.708	59.77	0.074	230.0
7	0.772	61.22	0.083	219.8
9	0.446	126.8	0.053	220.6
11	0.482	90.99	0.102	214.4

IN VITRO PHOTOTHERMAL EXPERIMENTS

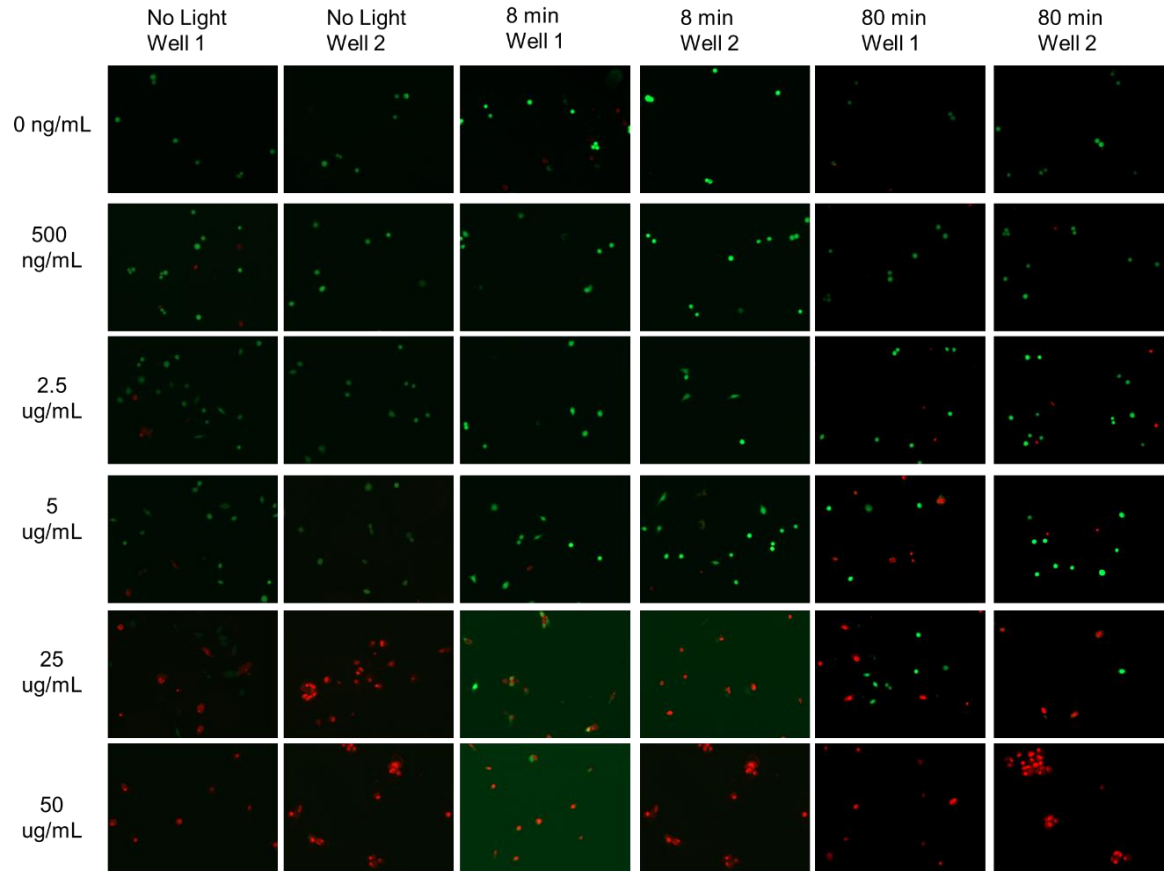

Figure S25. Representative trial experiments: cells treated with varying concentrations of the nanoparticle suspension where either irradiated with a 100W red LED lamp for 0, 8, or 80 mins.

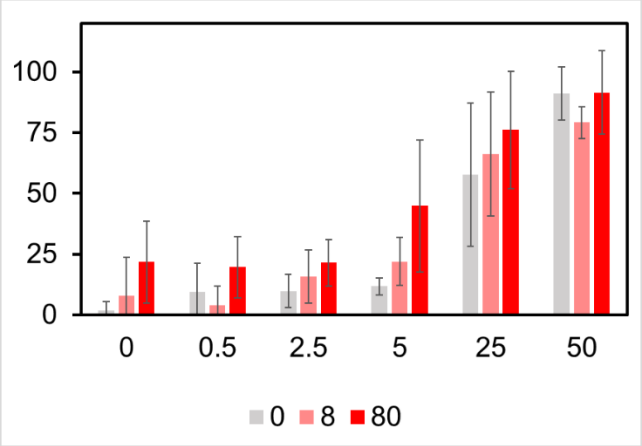

Figure S26. Quantified cell death upon irradiation time (x-axis concentration, y-axis dead cell %); cell death % determined as 100 x (Red/(Red+Green)) n = 4 technical replicates across 2 independent experiments error bars are SD; 0, 8, 80 = minutes

REFERENCES

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(2) Peris, G.; Miller, S. J. A Nonenzymatic Acid/Peracid Catalytic Cycle for the Baeyer−Villiger Oxidation. Org. Lett. 2008, 10 (14), 3049–3052. https://doi.org/10.1021/ol8010248.