SUPPORTING INFORMATION

Ultrasonic activation of polymer-drug conjugates for targeted and combinational pancreatic cancer therapy

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1. Chemistry

1.1. ^{1}H and ^{13}C NMR of prodrugs synthetic route

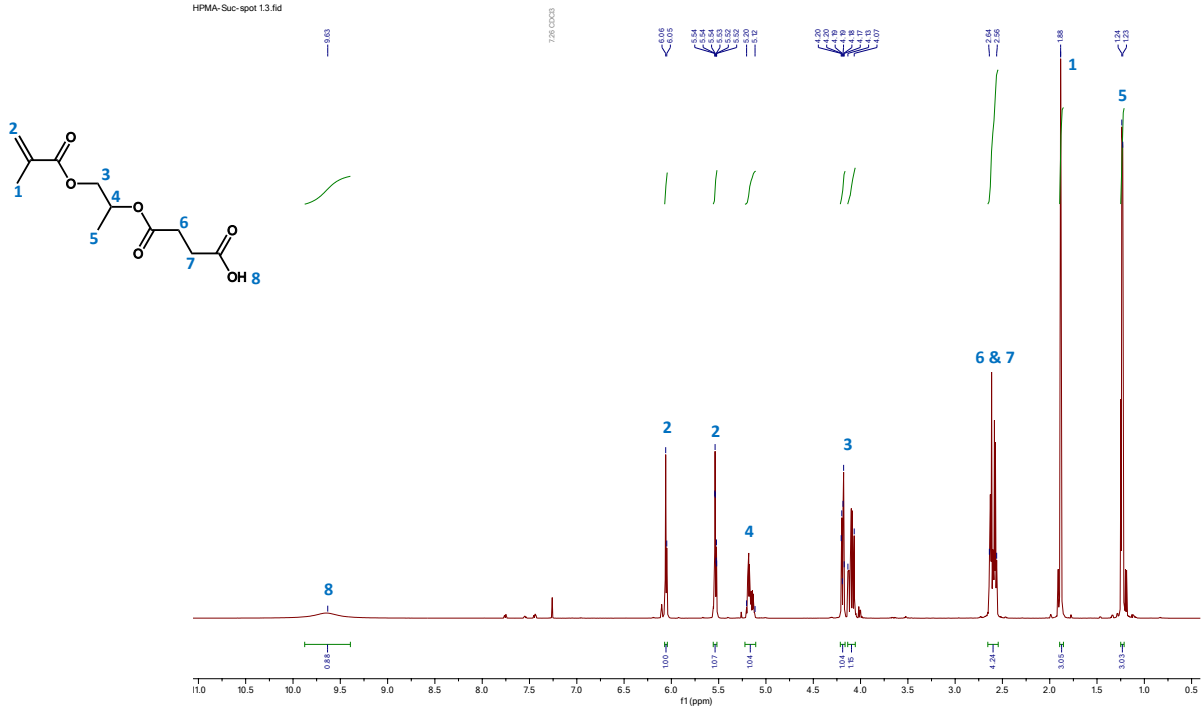

Figure S1: HPMA-Suc ^{1}H NMR spectra.

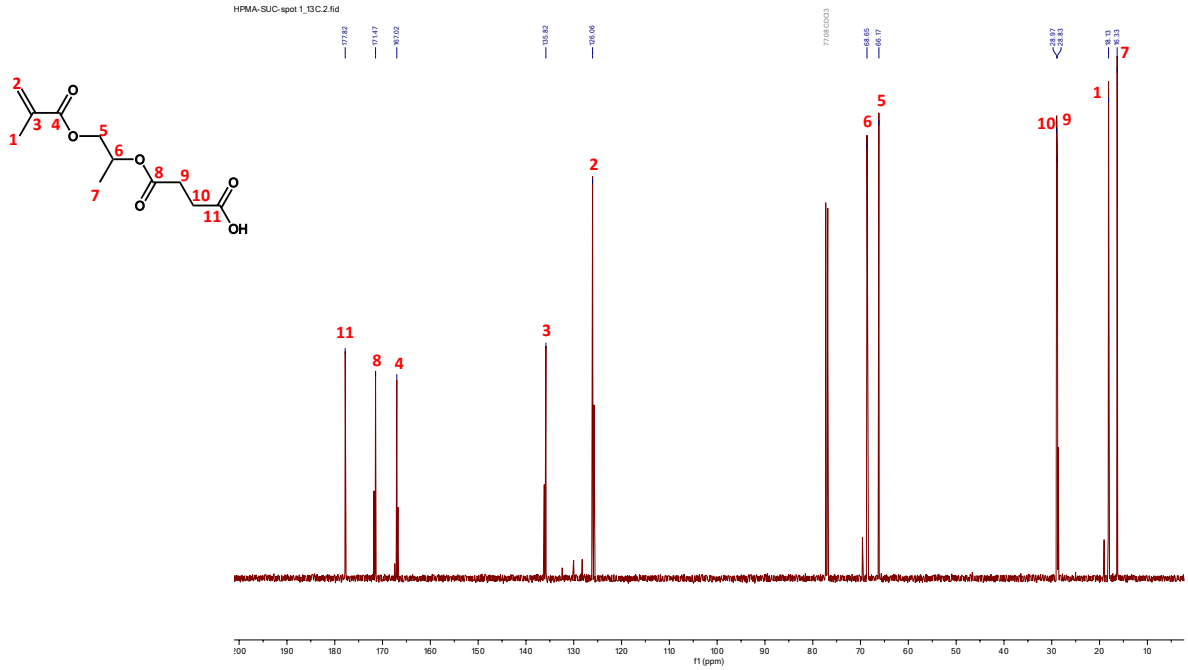

Figure S2: HPMA-Suc ^{13}C NMR spectra.

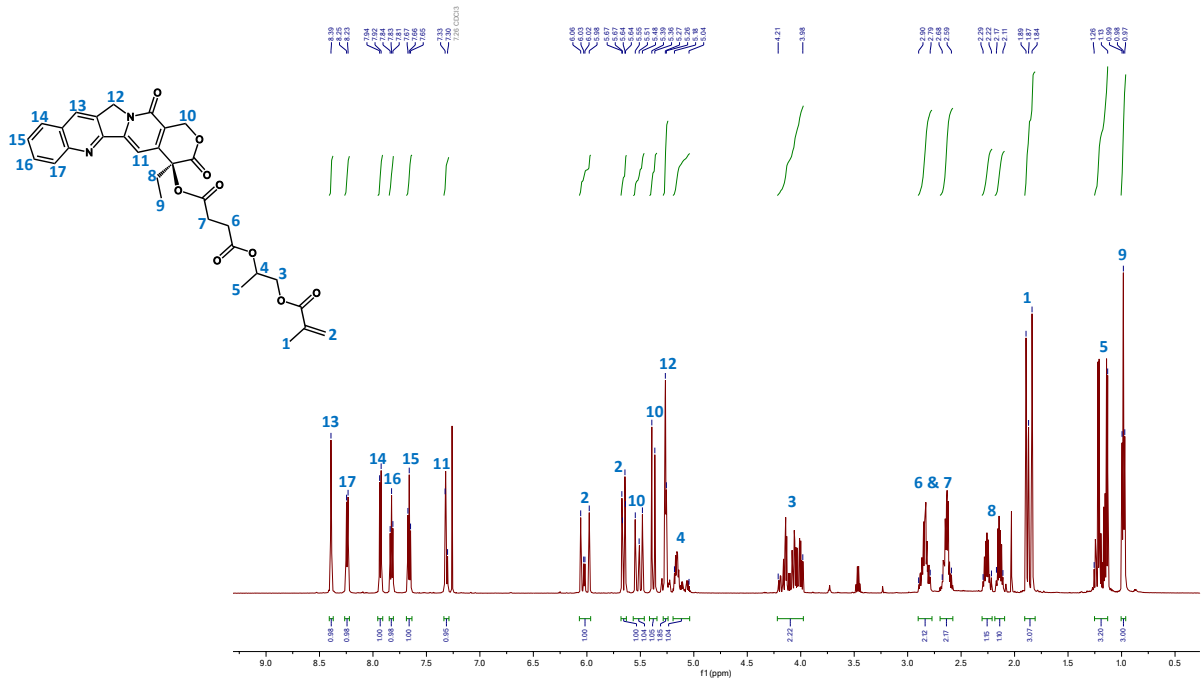

Figure S3: HPMA-Suc-CPT ^{1}H NMR spectra.

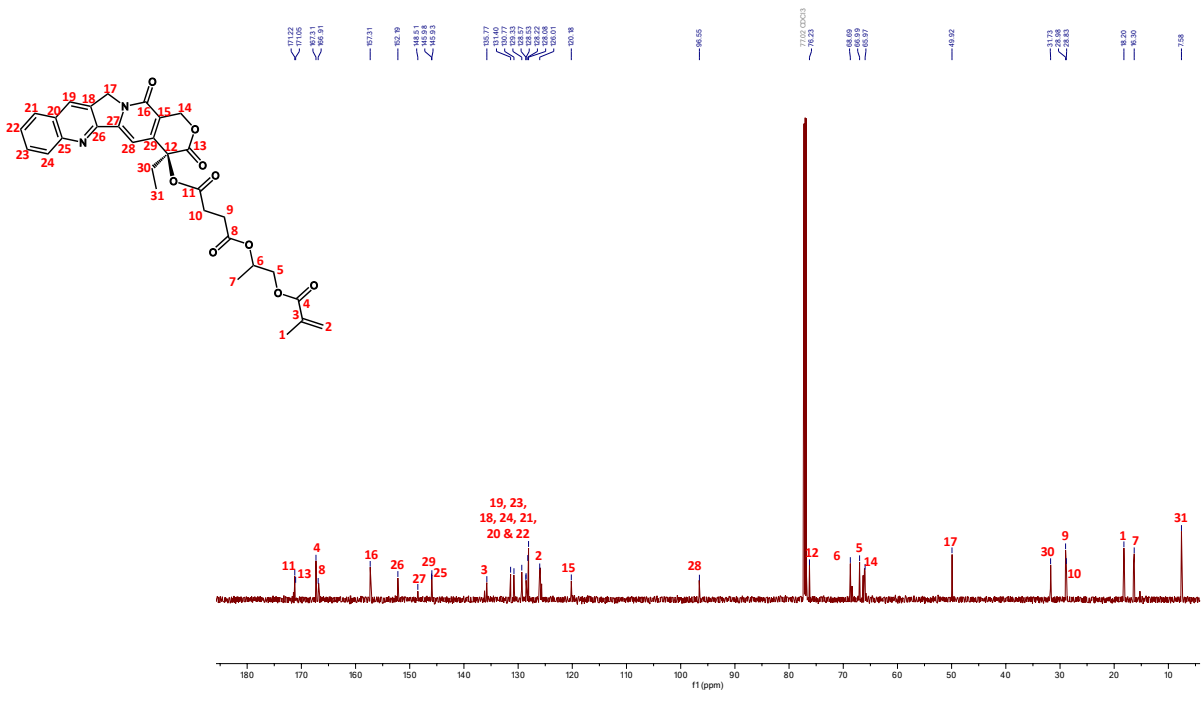

Figure S4: HPMA-Suc-CPT ^{13}C NMR spectra.

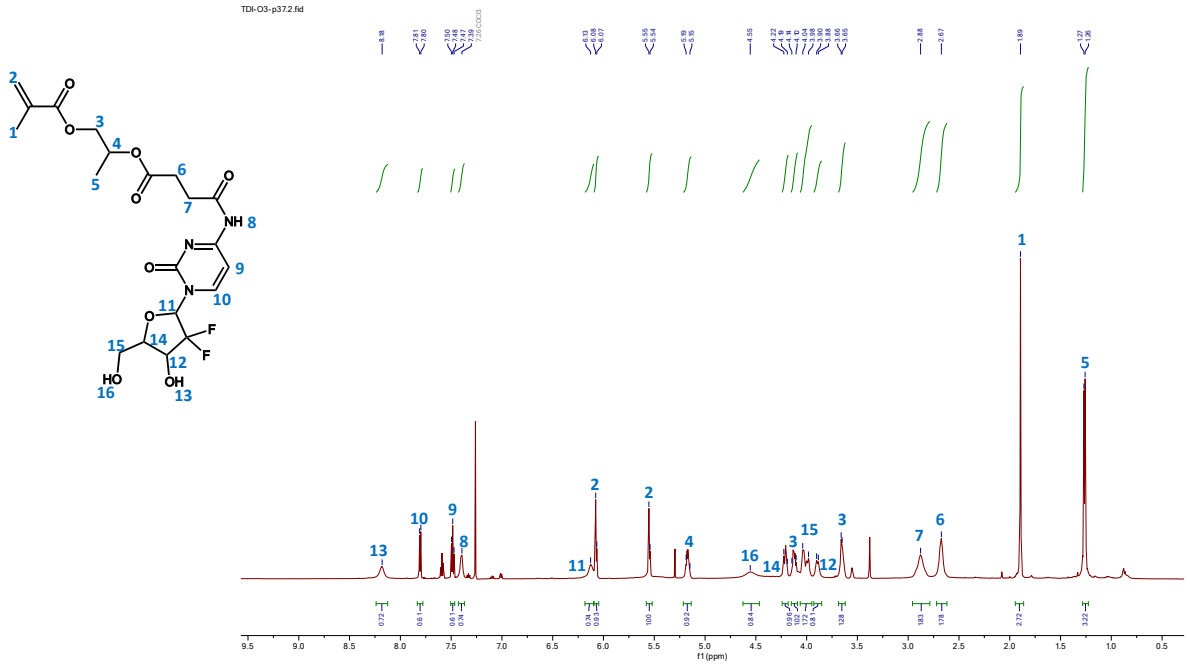

Figure S5: HPMA-Suc-GEM ^{1}H NMR spectra.

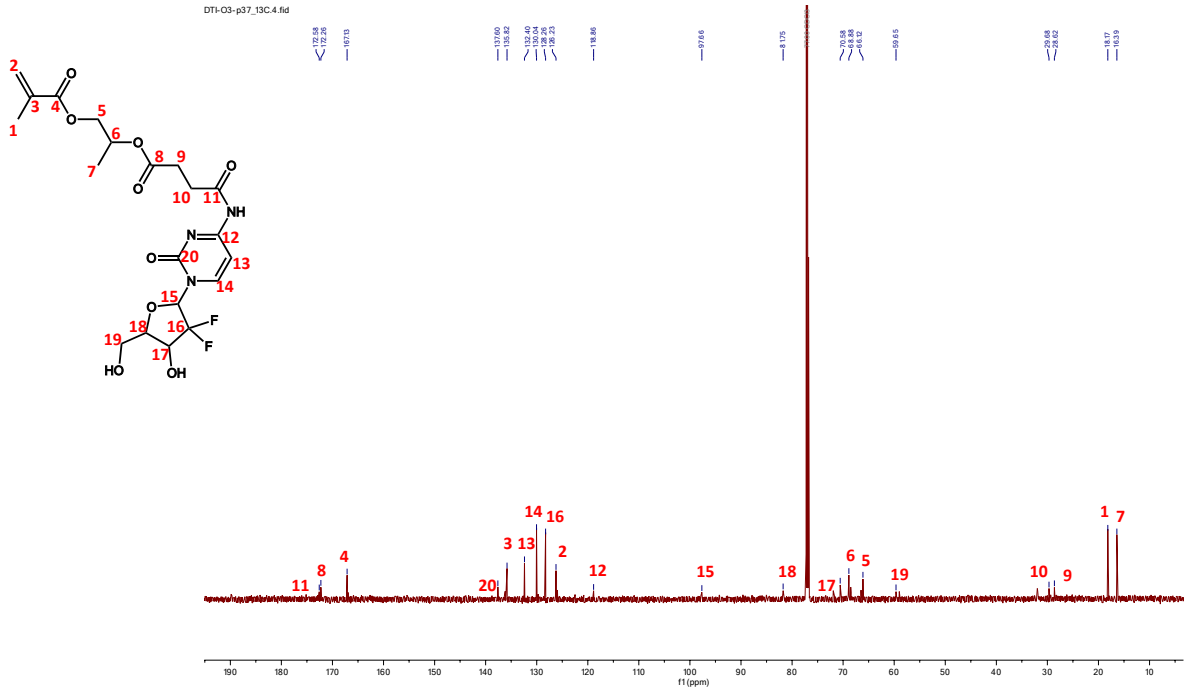

Figure S6: HPMA-Suc-GEM ^{13}C NMR spectra.

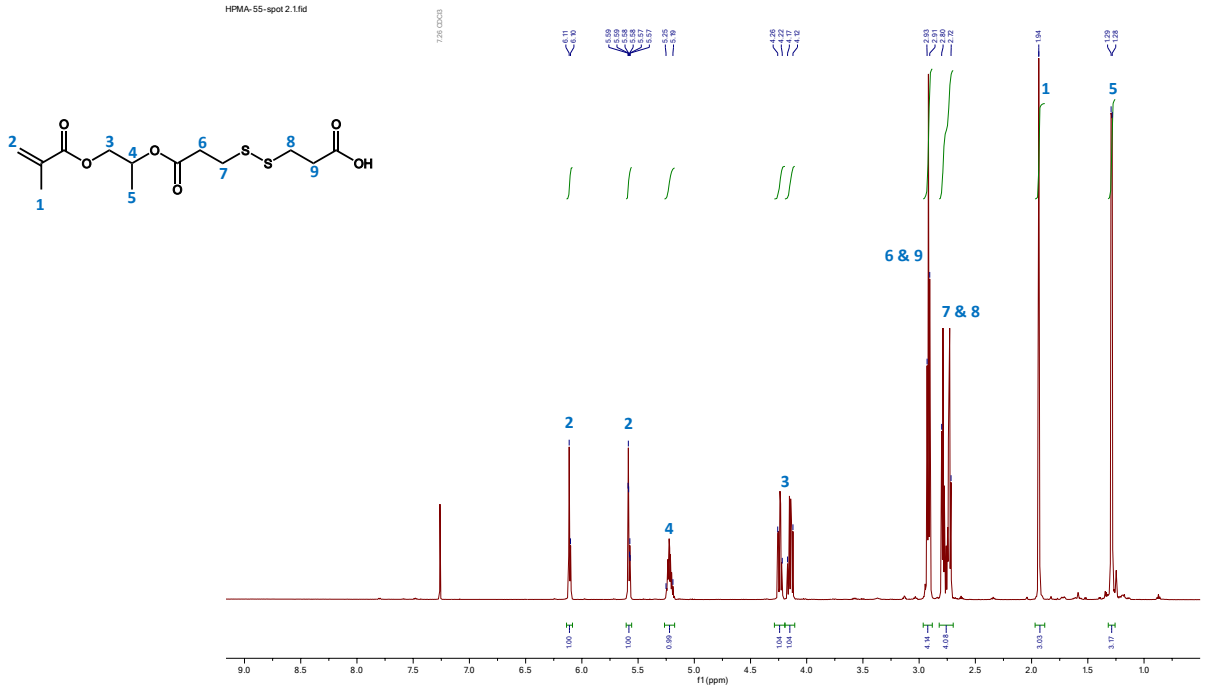

Figure S7: HPMA-SS ^{1}H NMR spectra.

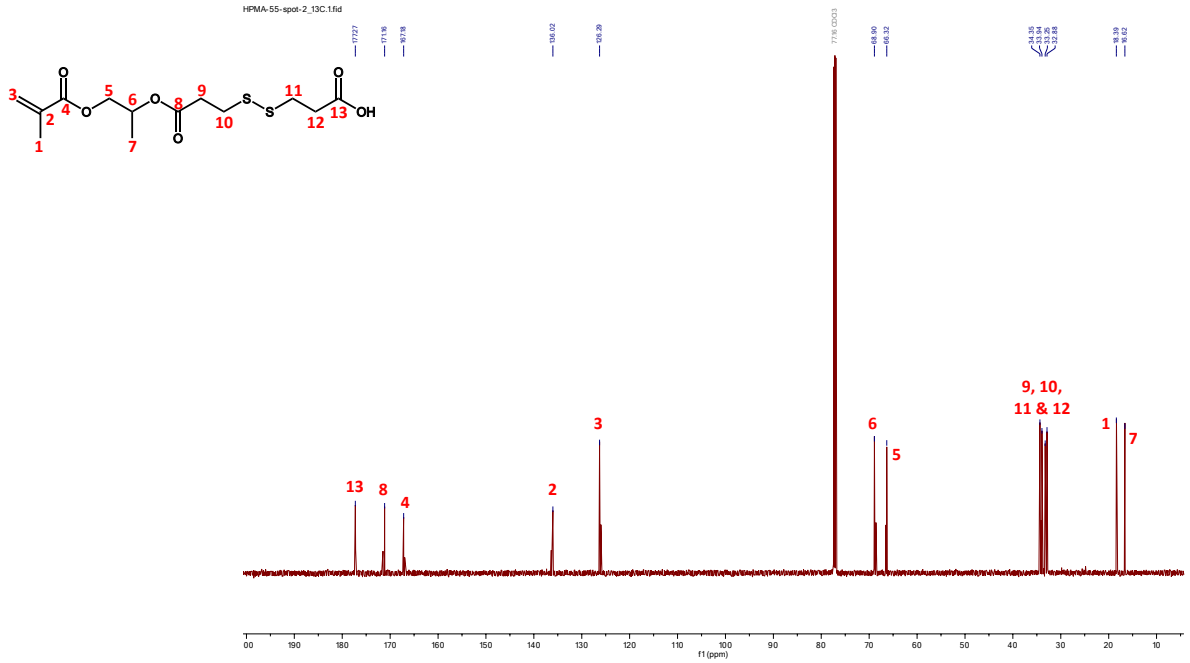

Figure S8: HPMA-SS ^{13}C NMR spectra.

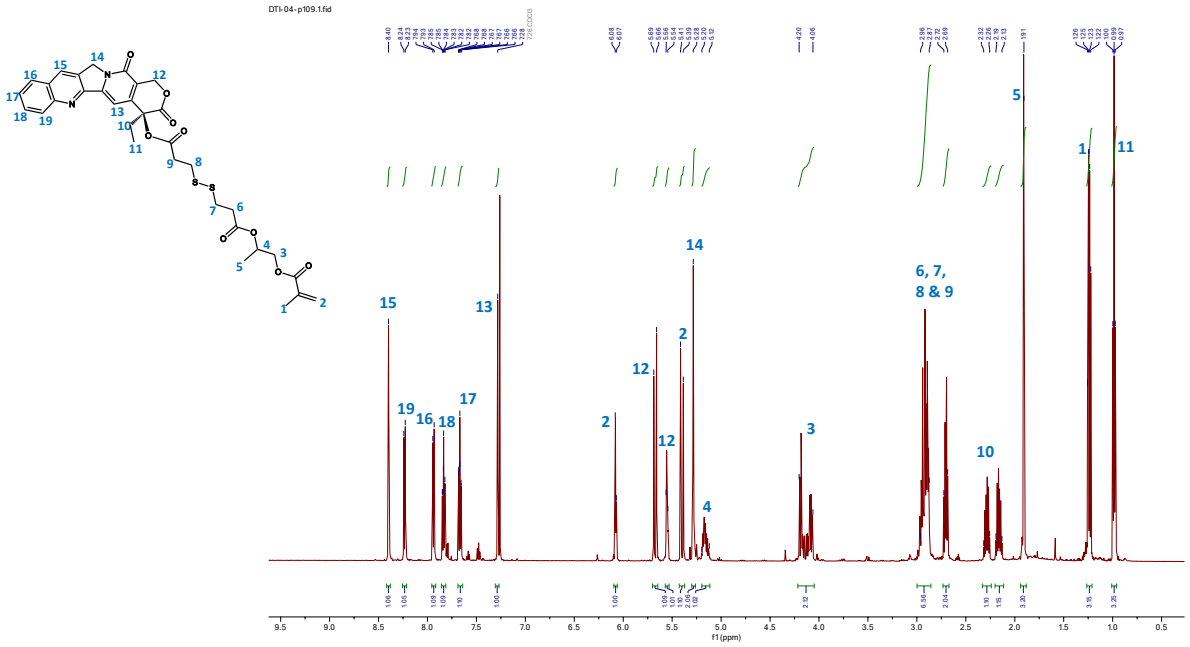

Figure S9: HPMA-SS-CPT ^{1}H NMR spectra.

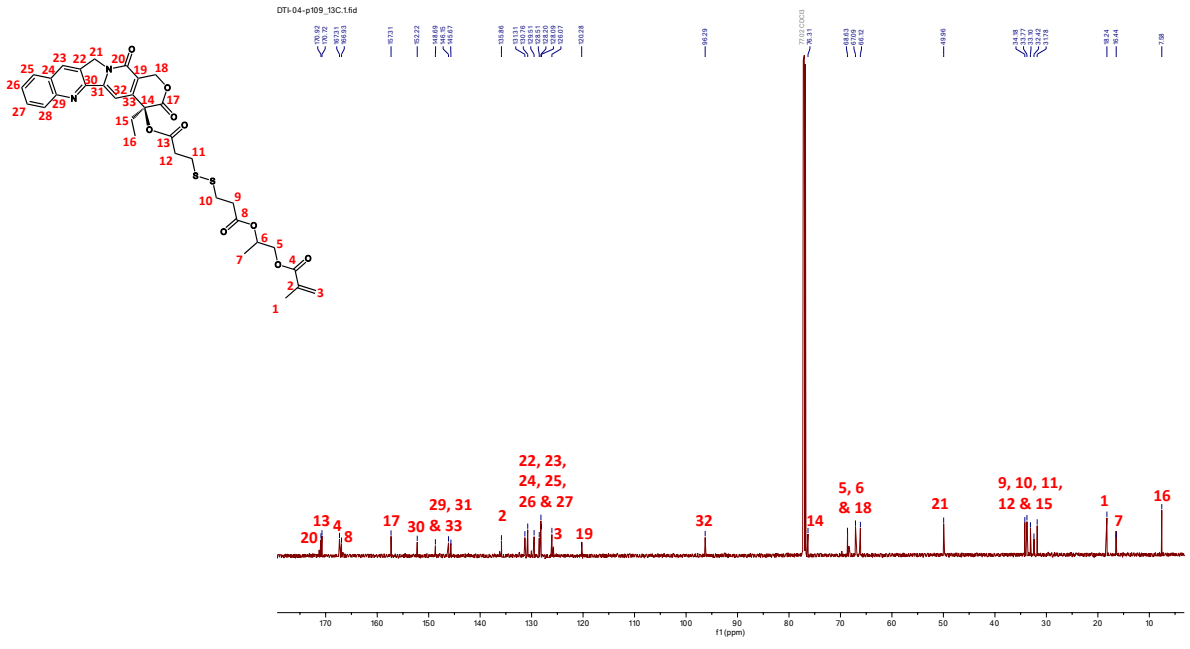

Figure S10: HPMA-SS-CPT ^{13}C NMR spectra.

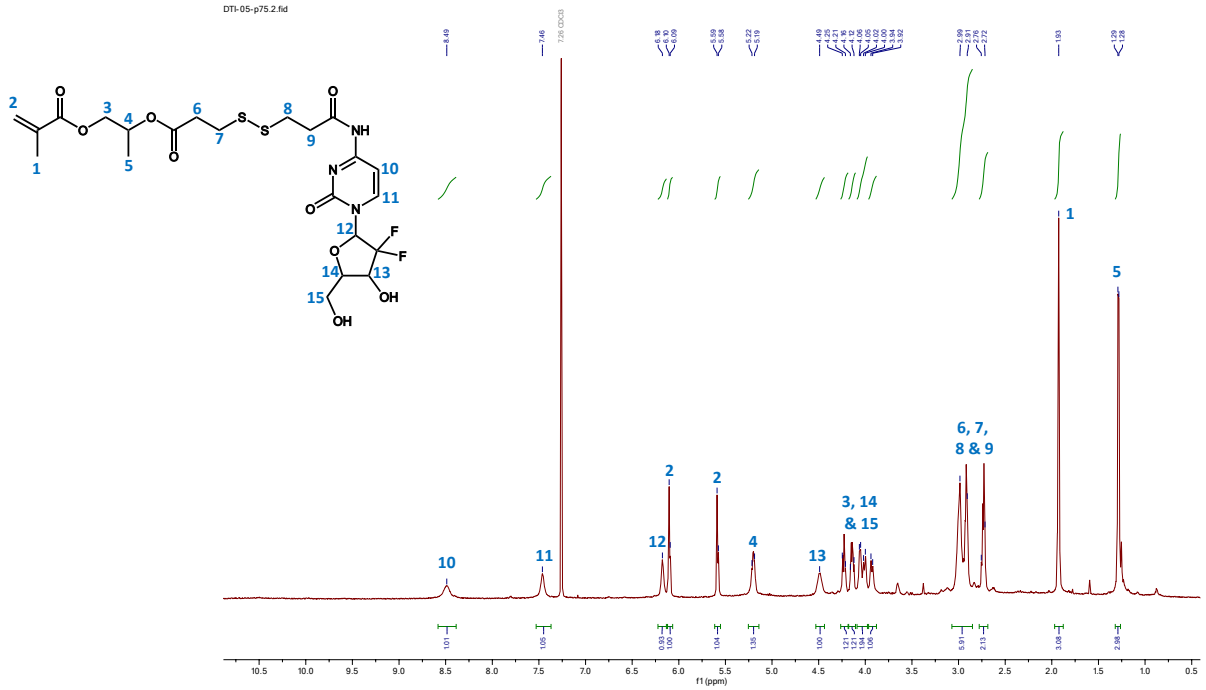

Figure S11: HPMA-SS-GEM ^{1}H NMR spectra.

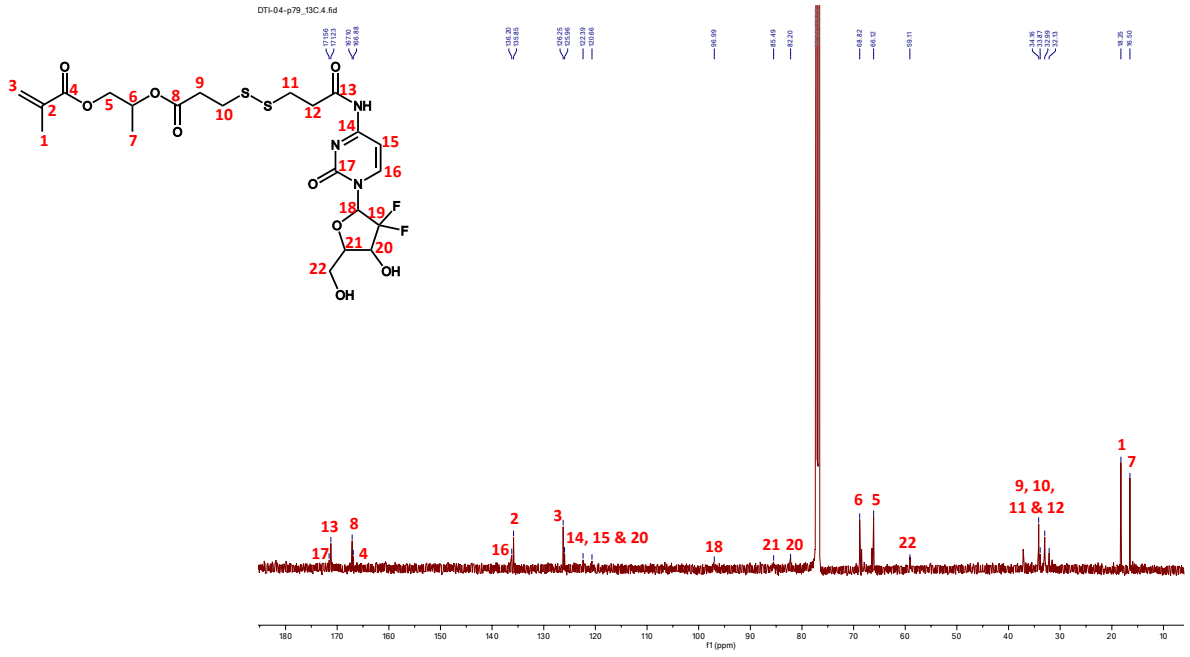

Figure S12: HPMA-SS-GEM ^{13}C NMR spectra.

1.2. ^{1}H NMR of the polymers

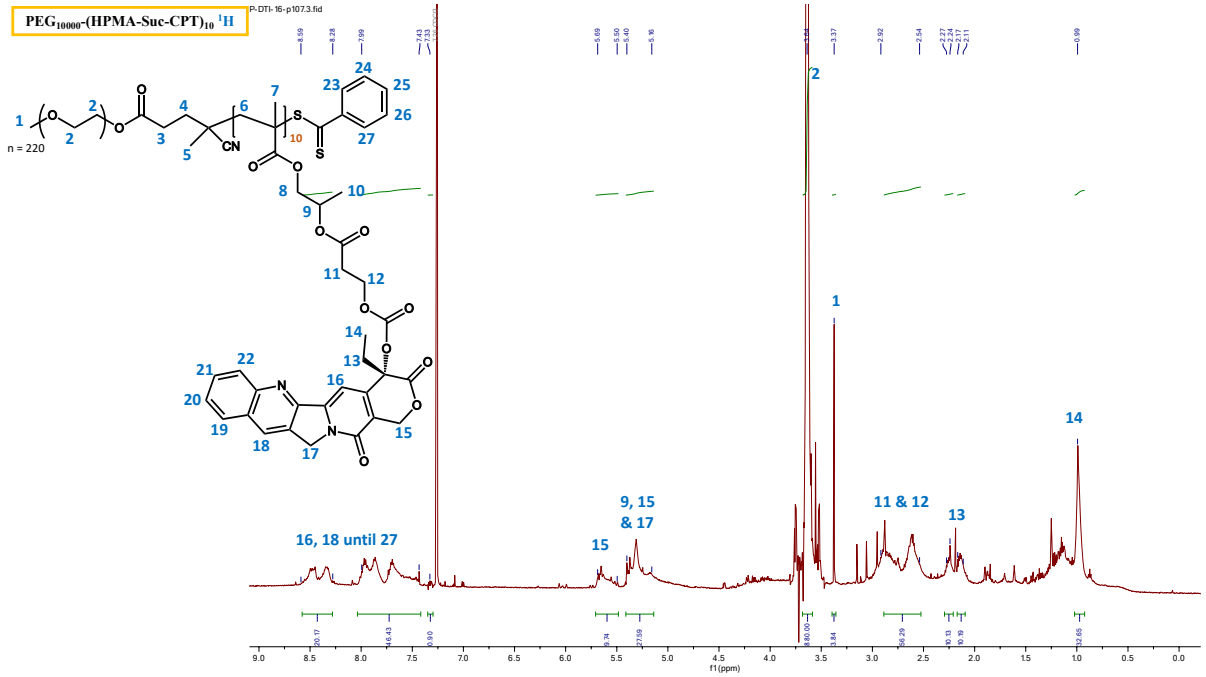

Figure S13: PEG_{10000}-$(HPMA\text{-}Suc\text{-}CPT)_{10}$ ^{1}H NMR spectra.

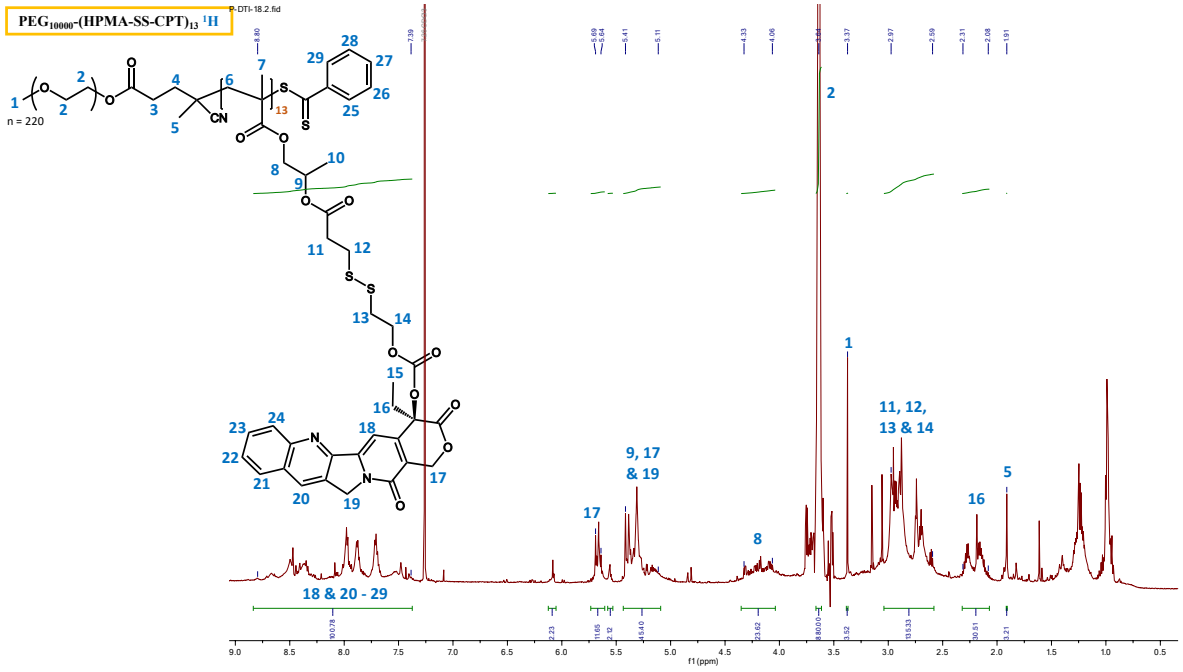

Figure S14: PEG_{10000}-$(HPMA\text{-}SS\text{-}CPT)_{13}$ ^{1}H NMR spectra.

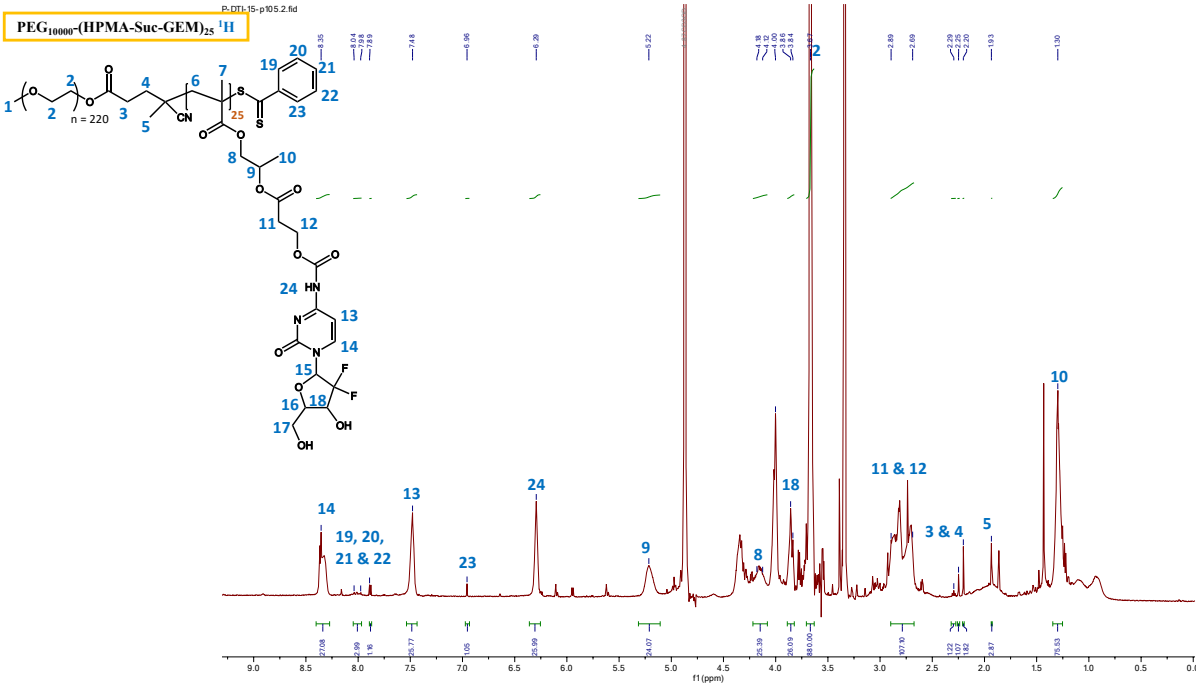

Figure S15: PEG_{10000}-$(HPMA\text{-}Suc\text{-}GEM)_{25}$ ^1H NMR spectra.

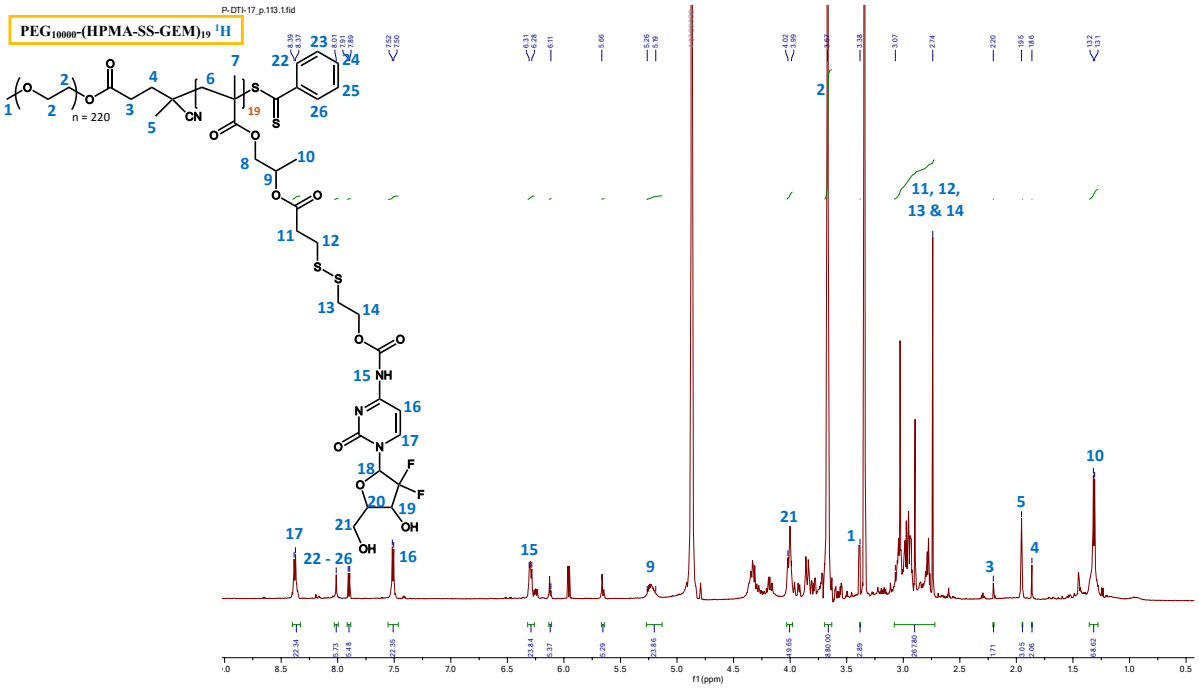

Figure S16: PEG_{10000}-$(HPMA\text{-}SS\text{-}GEM)_{19}$ ^1H NMR spectra.

1.3. SEC of the polymers

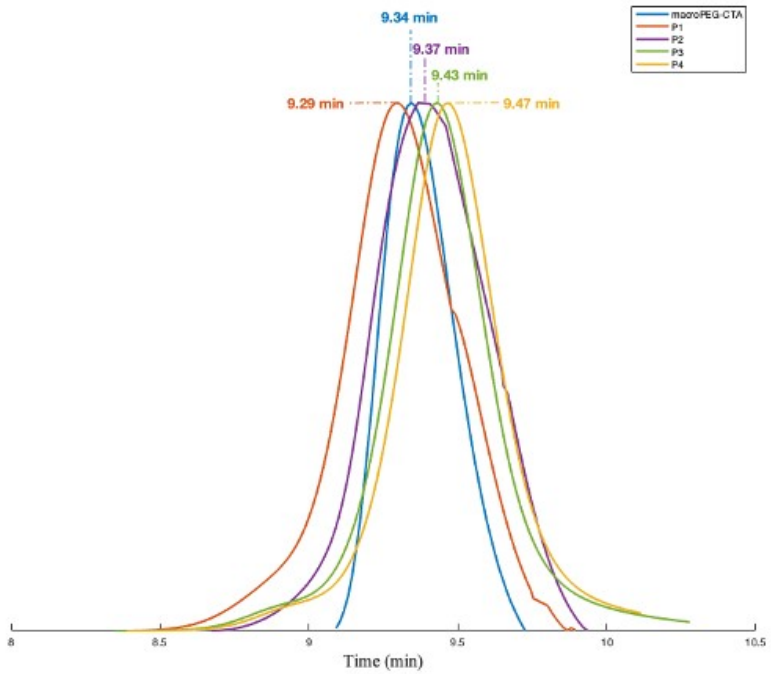

Figure S17: SEC chromatograms for the PDCs.

1.4. CAC of the polymers

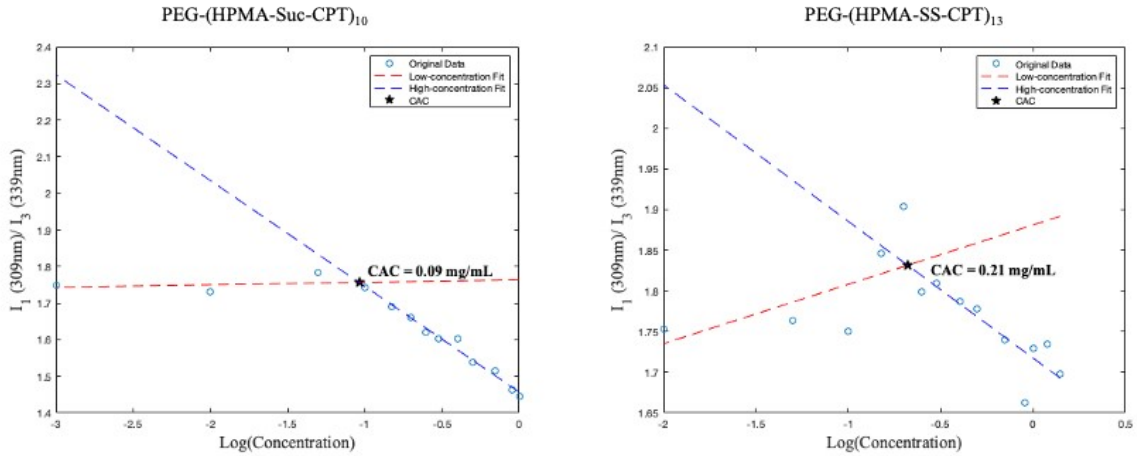

Figure S18: CAC graphs for the CPT-PDCs.

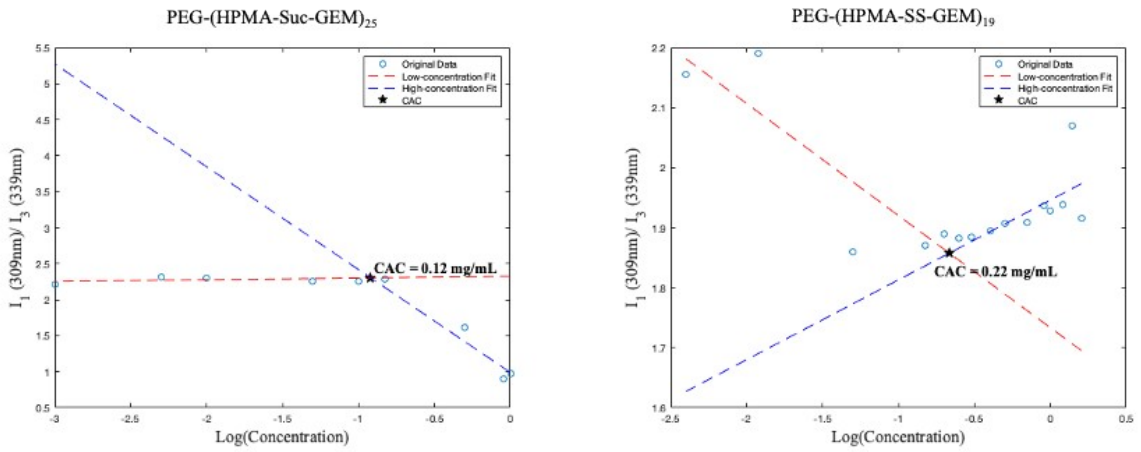

Figure S19: CAC graphs for the GEM-PDCs.

2. Nanoparticles (NPs)

2.1. DLS of the NPs

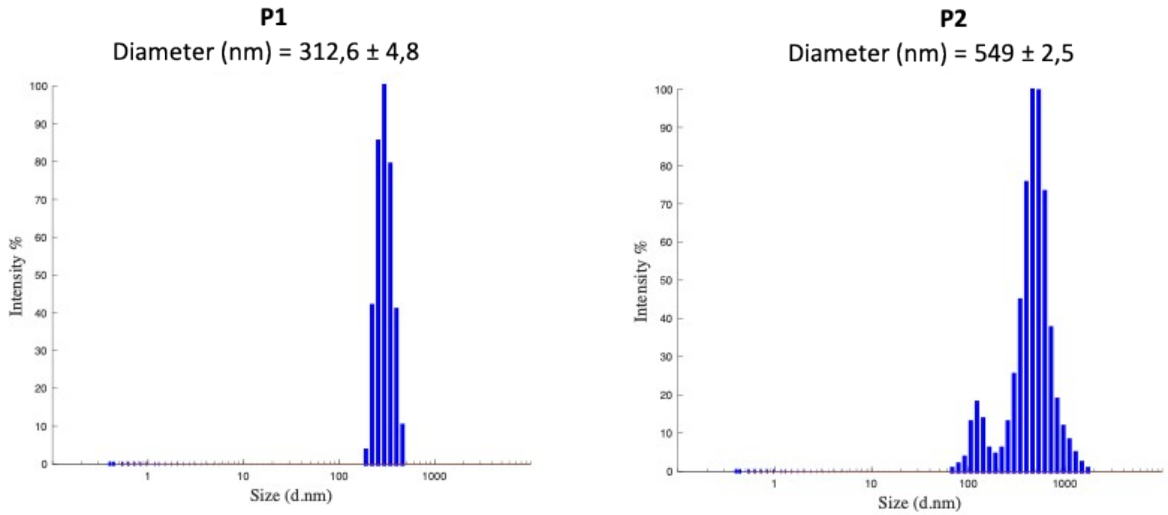

Figure S20: DLS for the CPT NPs.

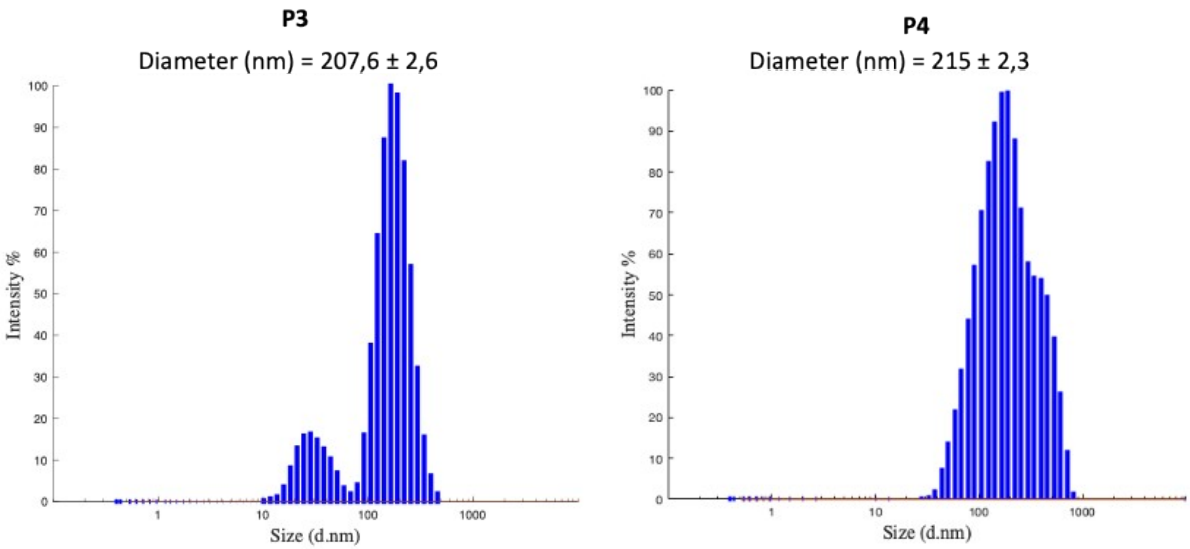

Figure S21: DLS for the GEM NPs.

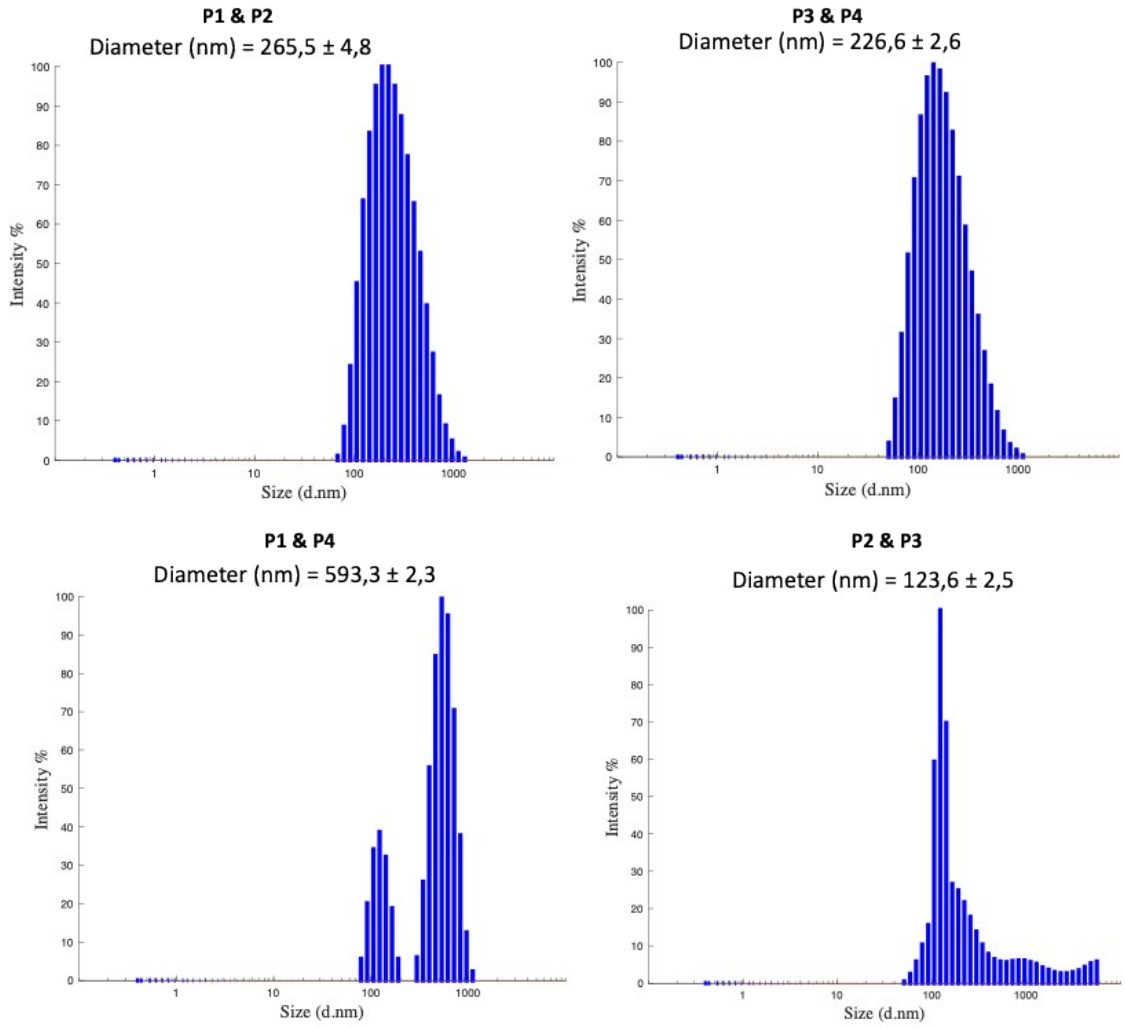

Figure S22: DLS of the NPs with combinations of the PDCs.

2.2. TEM of the NPs

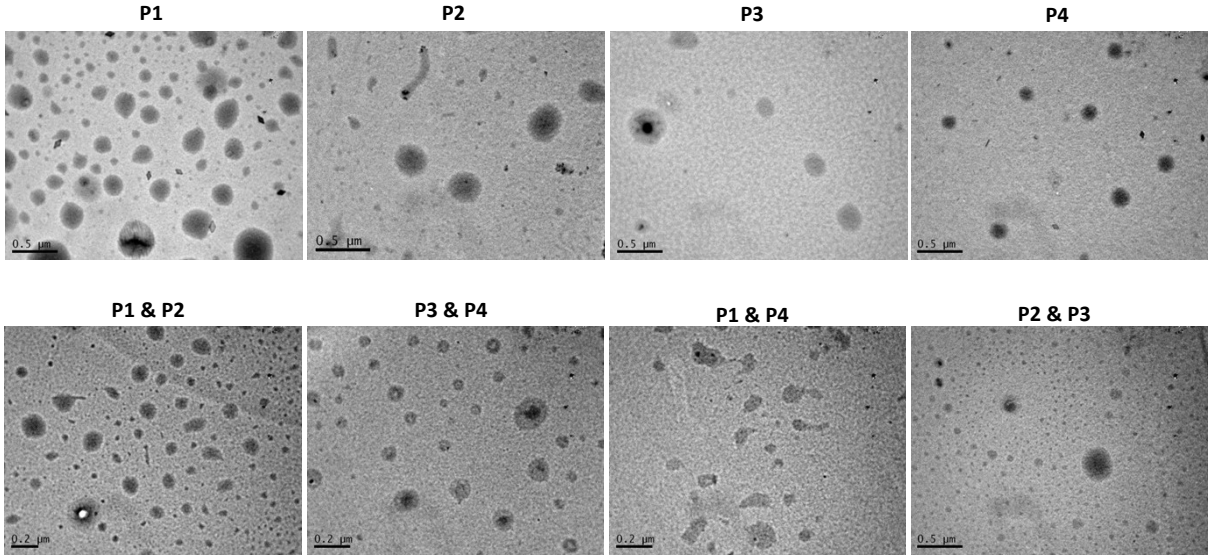

Figure S23: TEM of the NPs formulated from one type of PDC (first line) and their combinations (second line).

3. Release studies

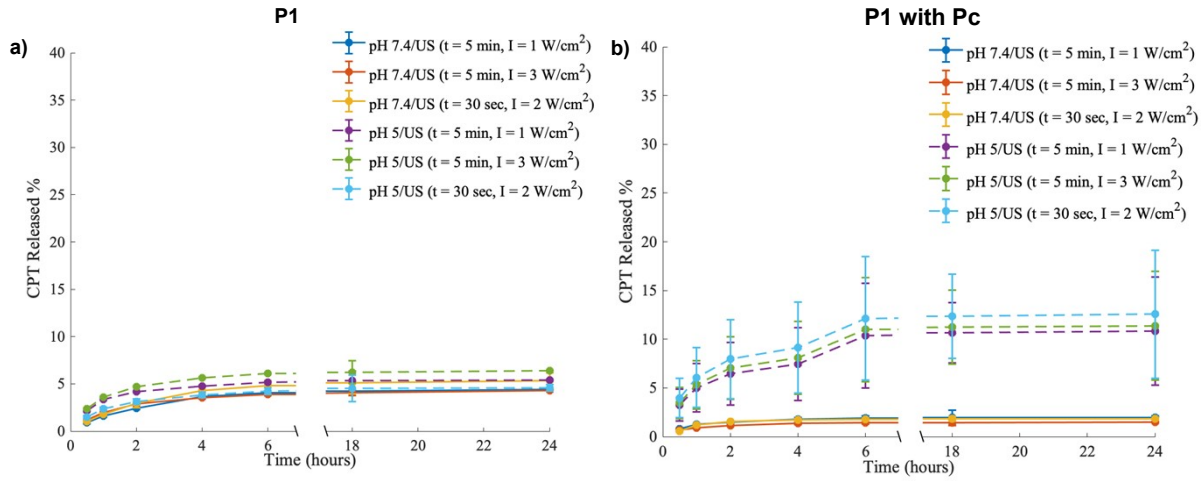

Figure S24: Release profile of **P1** NPs in different US and pH conditions a) without Pc, and b) with Pc.

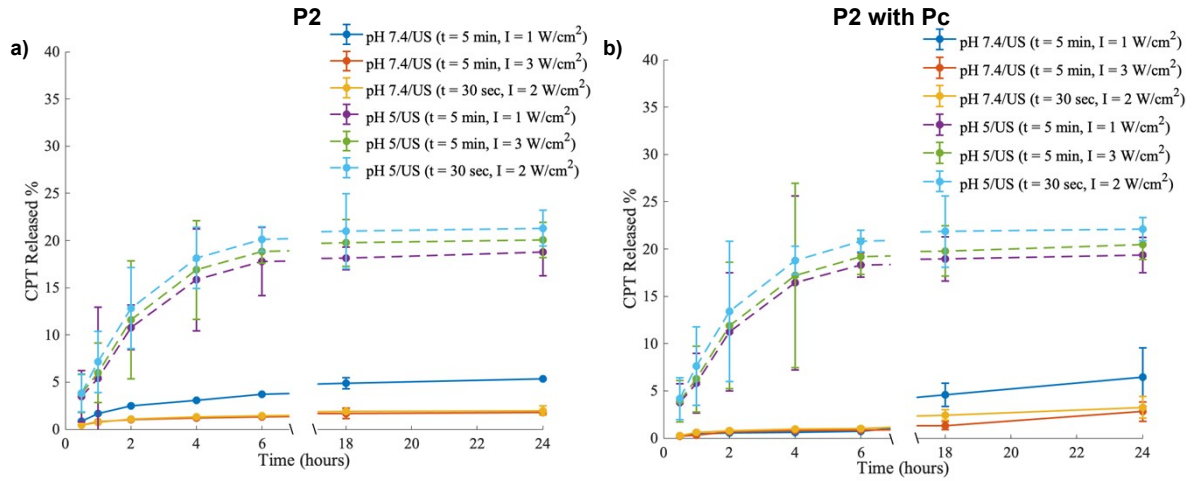

Figure S25: Release profile of **P2** NPs in different US and pH conditions a) without Pc, and b) with Pc.

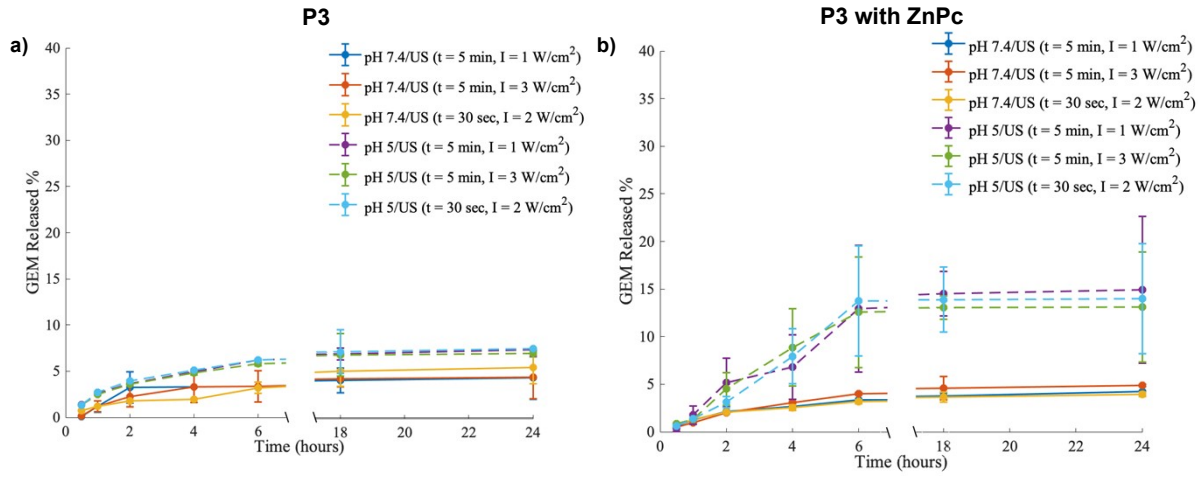

Figure S26: Release profile of **P3** NPs in different US and pH conditions a) without ZnPc, and b) with ZnPc.

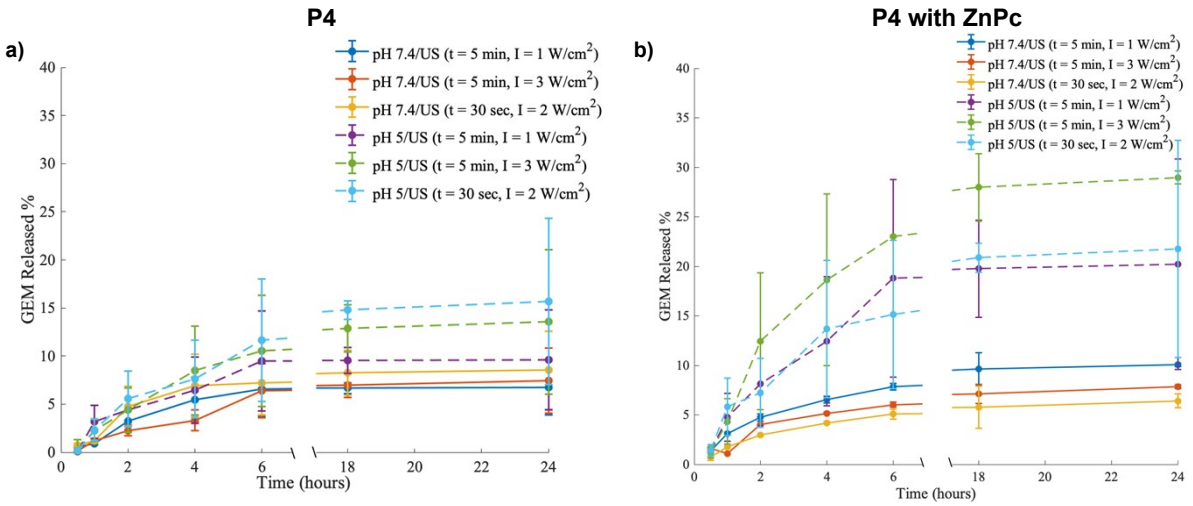

Figure S27: Release profile of **P4** NPs in different US and pH conditions a) without ZnPc, and b) with ZnPc.

4. XPS analysis of ZnPc

The C 1s spectra is deconvoluted into a total of 5 peaks:[1]

- C=C at 284.8 eV
- N-C-N at 286.0 eV
- N=C-N at 287.2 eV
- π-π* shake up transition peak present at 289.0 eV due to the aromatic ring
- COx surface species present due to contamination in the chamber at 286.3 eV.

The N 1s spectra deconvoluted into three peaks:[2]

- Pyrrolic N at 398.7 eV (quoted N_1)
- Bridging N at 399.0 eV (quoted N_2)
- The N_3 species peak is attributed to demetallized Pc centers, in combination with contribution from N satellite peaks.[3, 4]

The Zn 2p peak was also detected at 1022.2 eV and 1045.2 eV, for the $2p_{3/2}$ and $2p_{1/2}$ peaks respectively. These reported binding energies are in good agreement with previous studies into metalated ZnPc.[2]

The O 1s peak was also detected while scanning the sample.

Using relative sensitivity factors as mentioned above the atomic ratios are derived:

Element	Surface at %
C	75.8
N	15.7
Zn	1.6
O	6.9

This allows for the calculation of the ratios of N/C and Zn/N:

- N/C = 0.20 (Expected 0.25)
- Zn/N = 0.10 (Expected 0.12)

The results of the analysis are in good agreement with the expected values, however XPS spectra suggests not all Pc molecules are metalated.

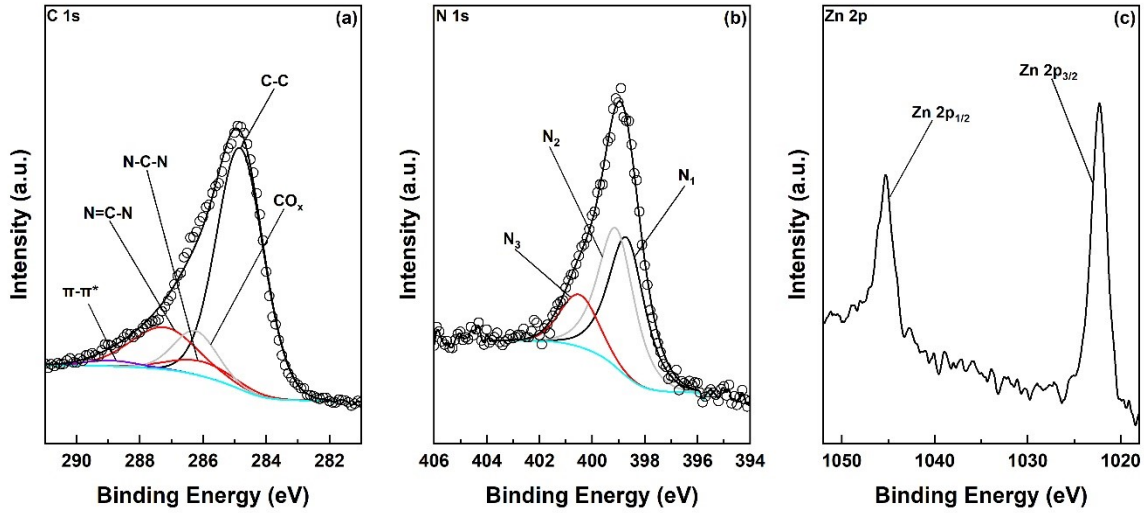

Figure S28: XPS spectra of ZnPc.

5. DPBF assay

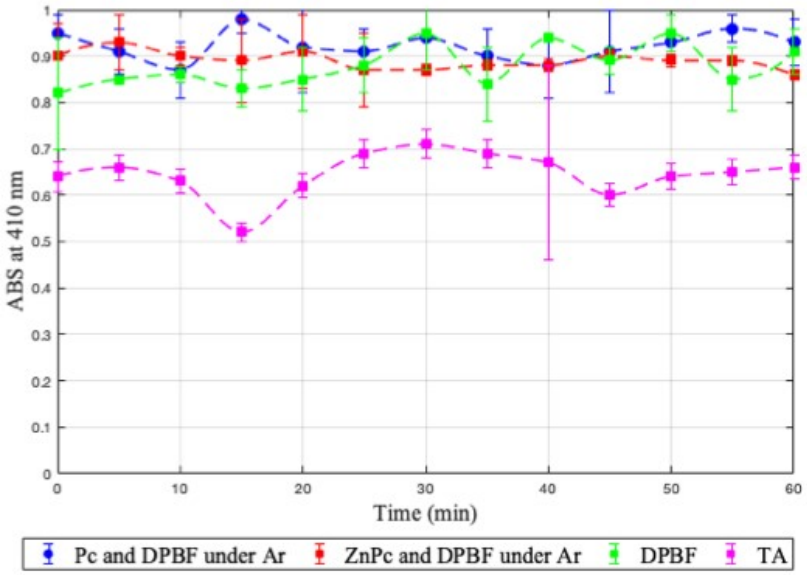

Figure S29: Controls of DPBF assay and terephthalic acid assay for Pc and ZnPc.

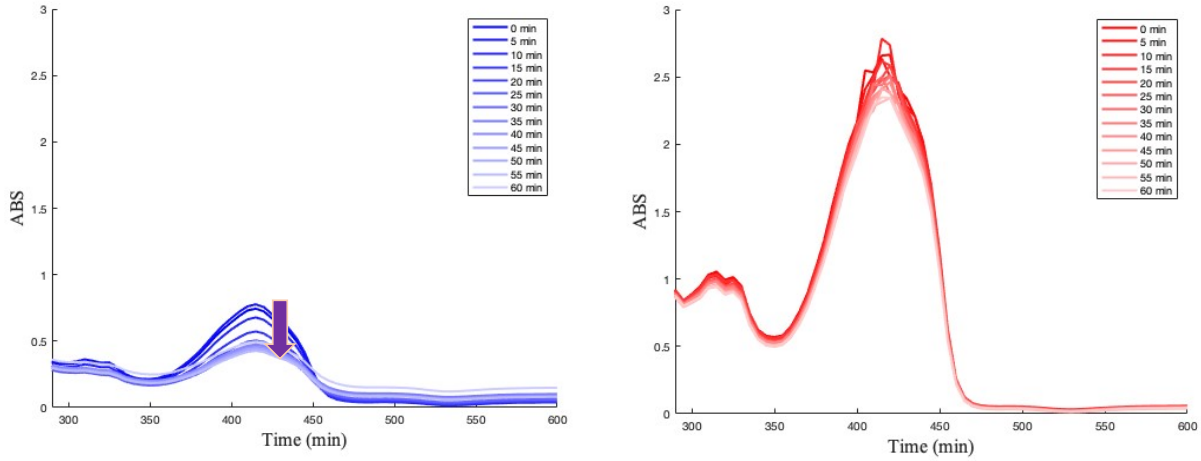

Figure S30: UV-Vis spectrum of DPBF assay for Pc and ZnPc.

6. *In vitro* studies

6.1. Cytotoxicity of US

To assess the cytotoxicity of the US, cell viability experiments (MTT assay) were performed on pancreatic cell line (PANC-1), in order to investigate which are the limits that US does not act a treatment by itself. Our goal is the therapeutic effect to be a result of the combination of the US with the nanoparticles, and not as a monotherapy. Thus, different time ranges were considered and in different intensity ranges. It was found that, after passing the limit of 1 min and intensity of 0.5 W/cm^2, the survival of the cells was dropping in almost half. Moreover,

passing these limitations, the cells seem to create resistance in US and survive no matter the intensity and the time it was applied. Note that with the apply of US for 1 min with an intensity of 0.5 W/cm^2 the viability of the cells was 92.31%, meaning that the cells were not affected from the therapeutic effect of US (Fig. S18).

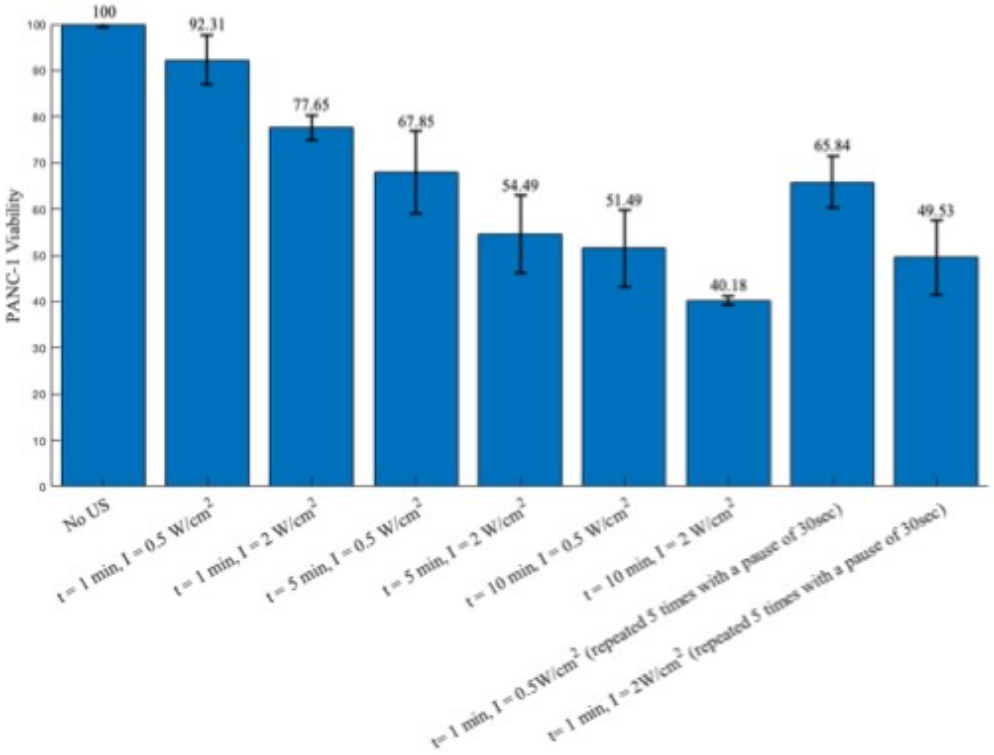

Figure S31: PANC-1 viability by the effect of US.

6.2. IC_{50} values for 48h

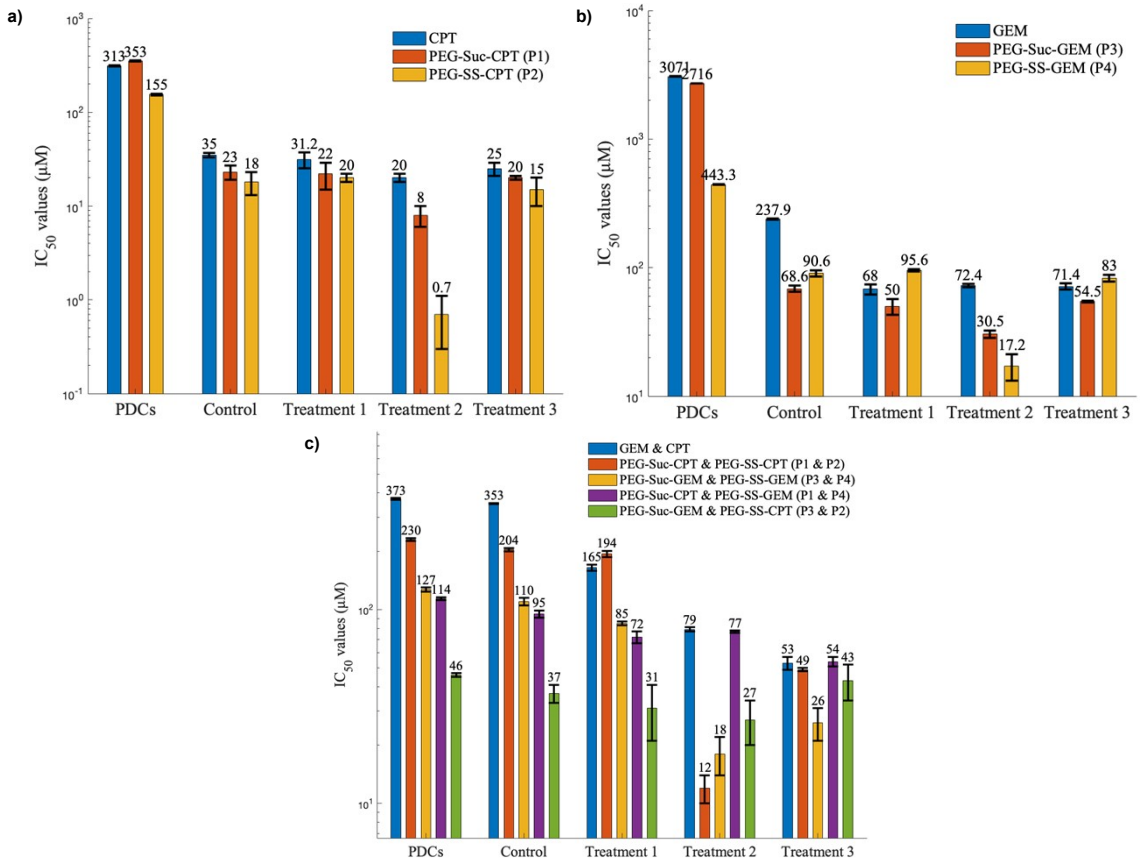

Figure S32: IC_{50} values of a) free CPT, NPs **P1** and **P2**, b) free GEM, NPs **P3** and **P4**, and c) there combinations: **GEM & CPT**, PEG-Suc-CPT & PEG-SS-CPT (**P1 & P2**), PEG-Suc-GEM & PEG-SS-GEM (**P3 & P4**), PEG-Suc-CPT & PEG-SS-GEM (**P1 & P4**), and PEG-Suc-GEM & PEG-SS-CPT (**P3 & P2**).

6.3. Controls for cytotoxicity

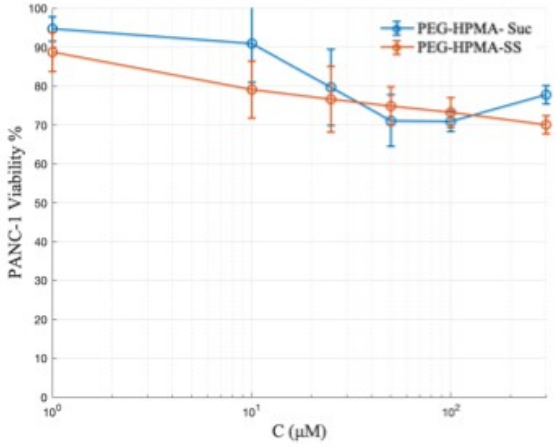

Figure S33: PANC-1 viability of PEG-HPMA-Suc and PEG-HPMA-SS.

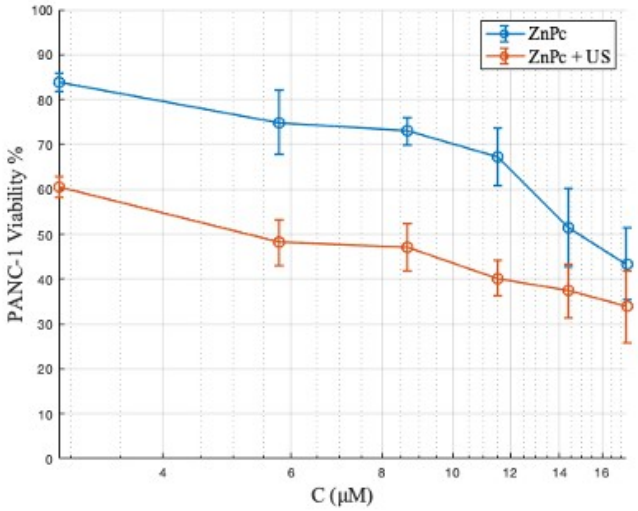

Figure S34: PANC-1 viability of ZnPc and the combination of ZnPc with US.

6.4. Synergism

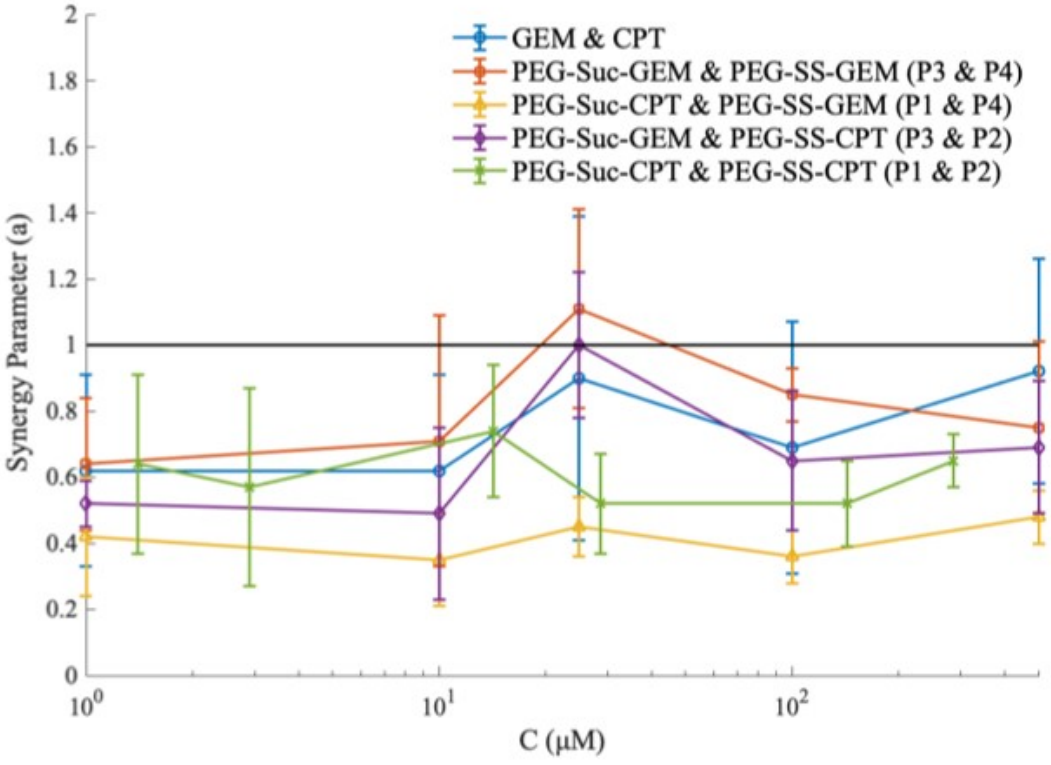

Figure S35: Synergy parameter for samples combinations alone.

6.5. Efficacy of the treatments

Table S1: IC_{50} and E_{max} values comparing the SDT treatments by drug class.

Drug	Treatment	IC_{50} (µM)	Folding improvement	E_{max} (% control) [±SE]	Efficacy change (fold)
CPT	US	20	+7.7	32 (±5)	+1.3
P1		10	+14.2	18 (±9)	+2.2
P2		7	+2.4	11 (±5)	+3.9
GEM		105	+2.4	12 (±1)	+3.3
P3		15	+42.1	12 (±1)	+4.2
P4		19	+53.3	31 (±1)	+1.6
GEM+CPT		5	+34.8	25 (±4)	+1.28
P1+P2		46	+3.3	24 (±2)	+1.7
P3+P4		4	+72.7	12 (±4)	+3.8
P1+P4		14	+3.7	25 (±2)	≡
P2+P3		1	+5	32 (±9)	≡
CPT	Treatment 1	23.7	+6.5	47 (±6)	-1.1
P1		9.7	+14.6	40 (±1)	≡
P2		5.6	+3	43 (±4)	≡
GEM		50	+5.1	45 (±2)	-0.8

P3		20	+32	39 (±8)	+1.3
P4		26	+39	41 (±2)	+1.2
GEM+CPT		4	+43.5	29 (±2)	+1.1
P1+P2		39	+3.4	30 (±1)	+1.4
P3+P4		4	+72.7	42 (±2)	+1.1
P1+P4		13	+4	38 (±2)	-0.65
P2+P3		0.3	+16.7	28 (±1)	+1.1
CPT	Treatment 3	19	+8.1	38 (±6)	+1.1
P1		5	+28.4	45 (±3)	-0.9
P2		2	+8.5	40 (±7)	+1.1
GEM		12	+21.2	40 (±4)	≡
P3		17	+31.1	30 (±7)	+1.7
P4		5.2	+195	38 (±2)	+1.3
GEM+CPT		0.75	+232	39 (±5)	-0.8
P1+P2		0.30	+507	28 (±4)	+1.5
P3+P4		0.7	+420	26 (±2)	+1.8
P1+P4		7	+7.4	42 (±9)	-0.6
P2+P3		1.1	+4.5	34 (±6)	-0.9

7. Literature

1. N. Kari, M. Zannotti, R. Giovannetti, D. Řeha, B. Minofar, S. Abliz and A. Yimit, *Nanomaterials (Basel)*, 2022, **12**.
2. D. Paoloni, G. Di Filippo, D. Cvetko, G. Kladnik, A. Morgante and A. Ruocco, *The Journal of Physical Chemistry C*, 2020, **124**, 22550-22558.
3. K. Eguchi, T. Nakagawa, Y. Takagi and T. Yokoyama, *The Journal of Physical Chemistry C*, 2015, **119**, 9805-9815.
4. Y. Bai, F. Buchner, M. Wendahl, I. Kellner, A. Bayer, H. Steinruck, H. Marbach and J. M. Gottfried, *Journal of Physical Chemistry C*, 2008, **112**, 6087-6092.