Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

pH-Triggered Release of Gemcitabine from Polymer Coated Nanodiamonds Fabricated by RAFT polymerization and Copper Free Click Chemistry

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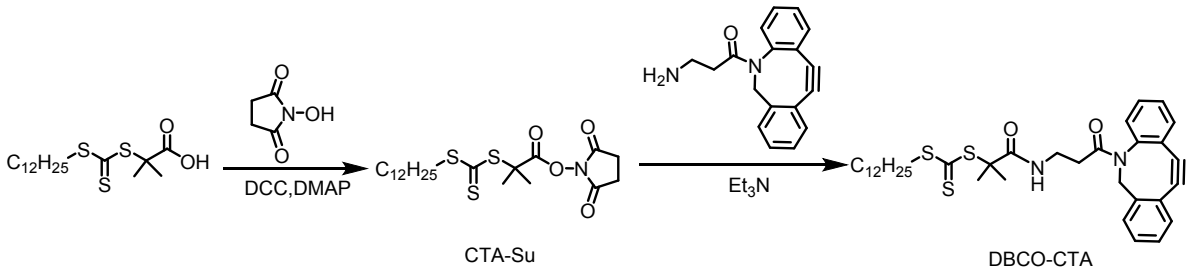
1. Synthesis.

1.1 RAFT agent synthesis.



Scheme S1. Synthesis of DBCO-CTA.

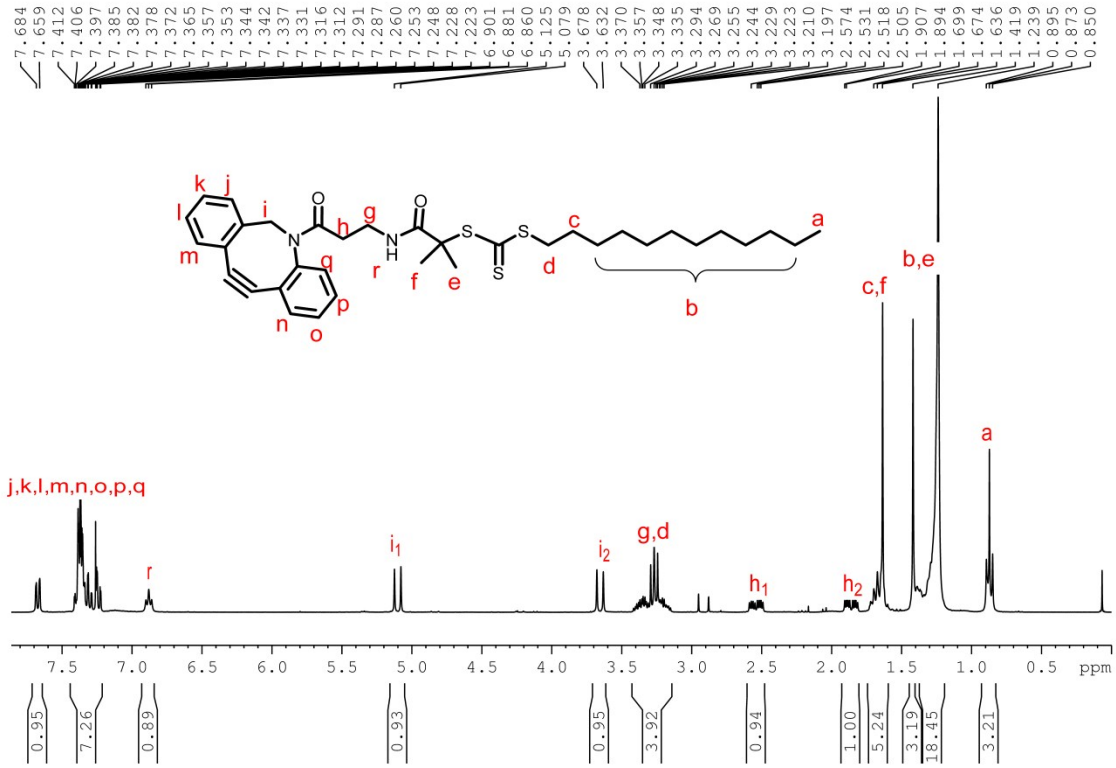


Figure S1. ^{1}H NMR spectrum of DBCO-CTA in $CDCl_3$.

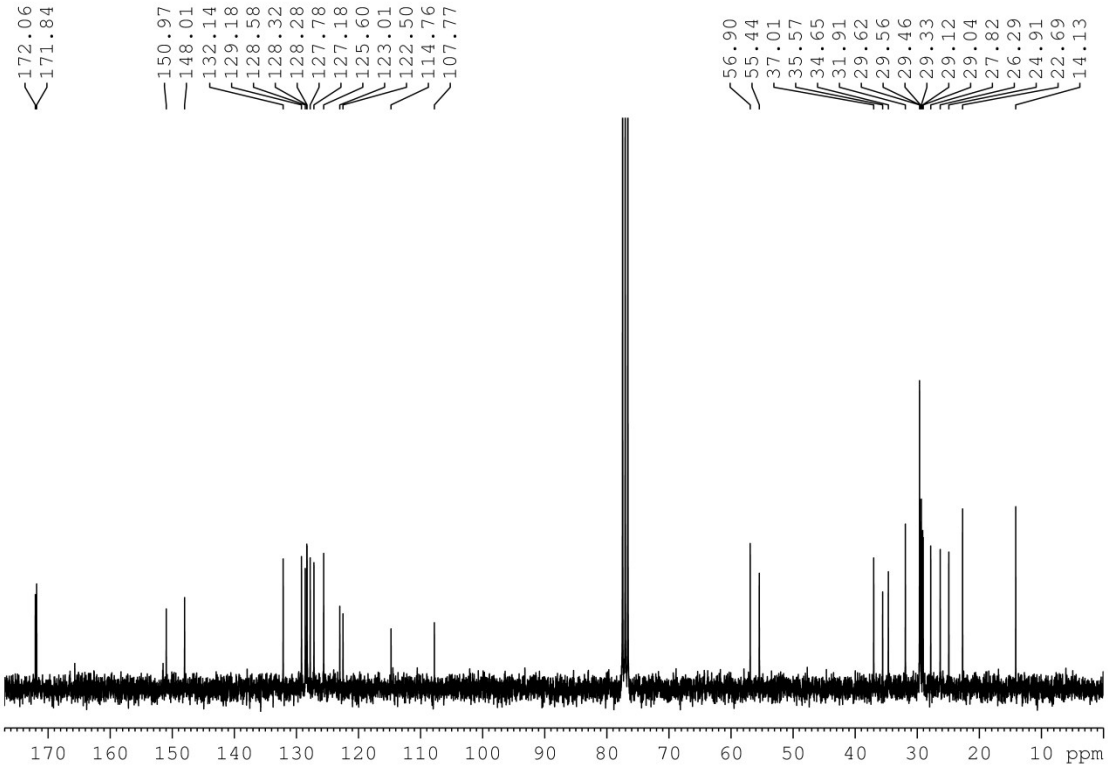


Figure S2. ^{13}C NMR spectrum of DBCO-CTA in $CDCl_3$.

1.2 Monomer synthesis.

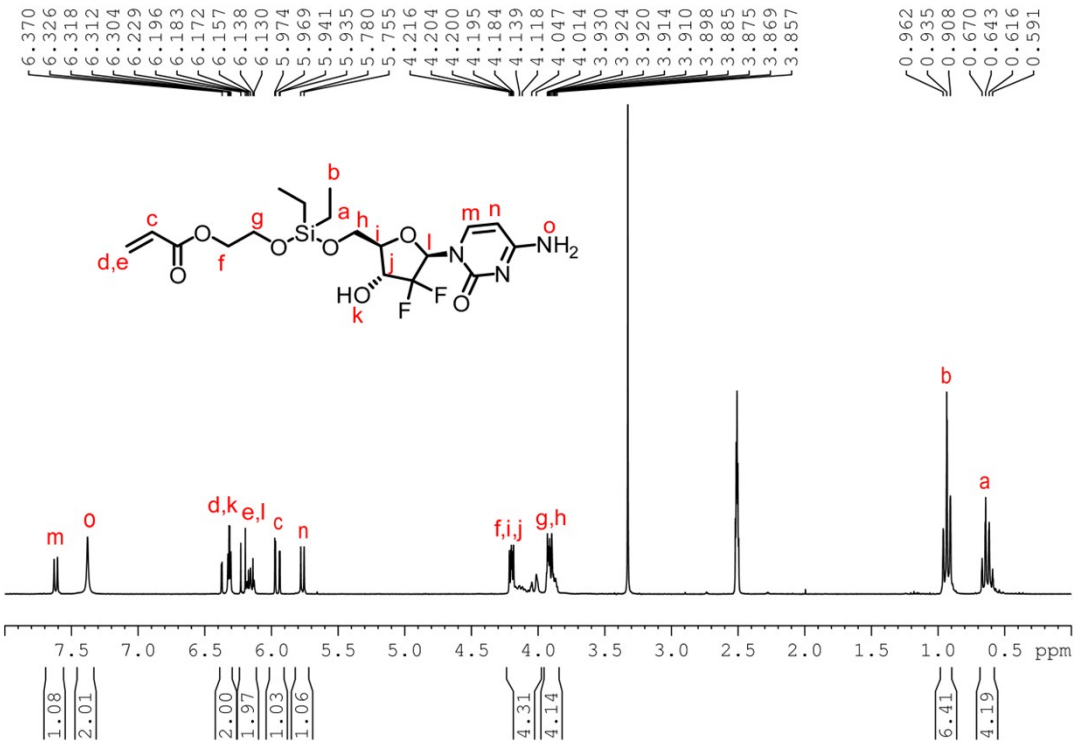


Figure S3. ^{1}H NMR spectrum of HEAGem monomer in d_6-DMSO.

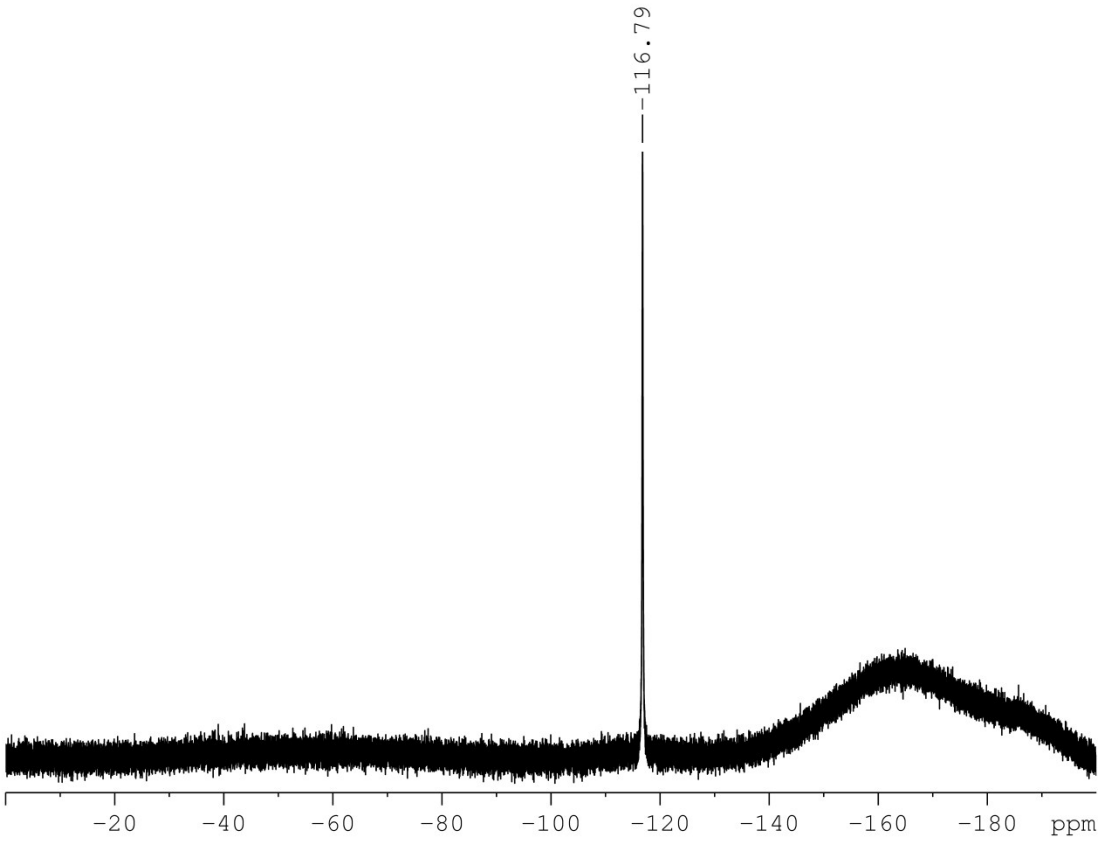


Figure S4. ^{19}F NMR spectrum of HEAGem monomer in d_6-DMSO.

1.3 Polymerization (Synthesis of prodrug polymers with DBCO end groups).

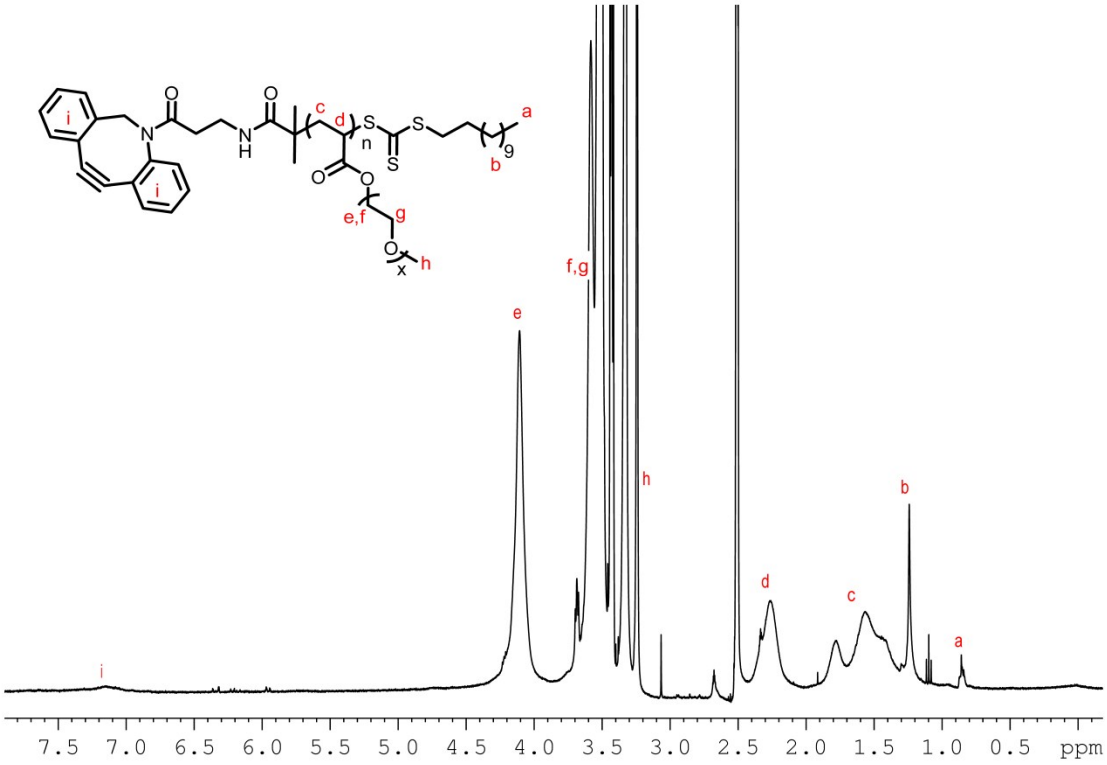


Figure S5. ^{1}H NMR spectrum of DBCO-POEGMEA$_{45}$ in d_6-DMSO.

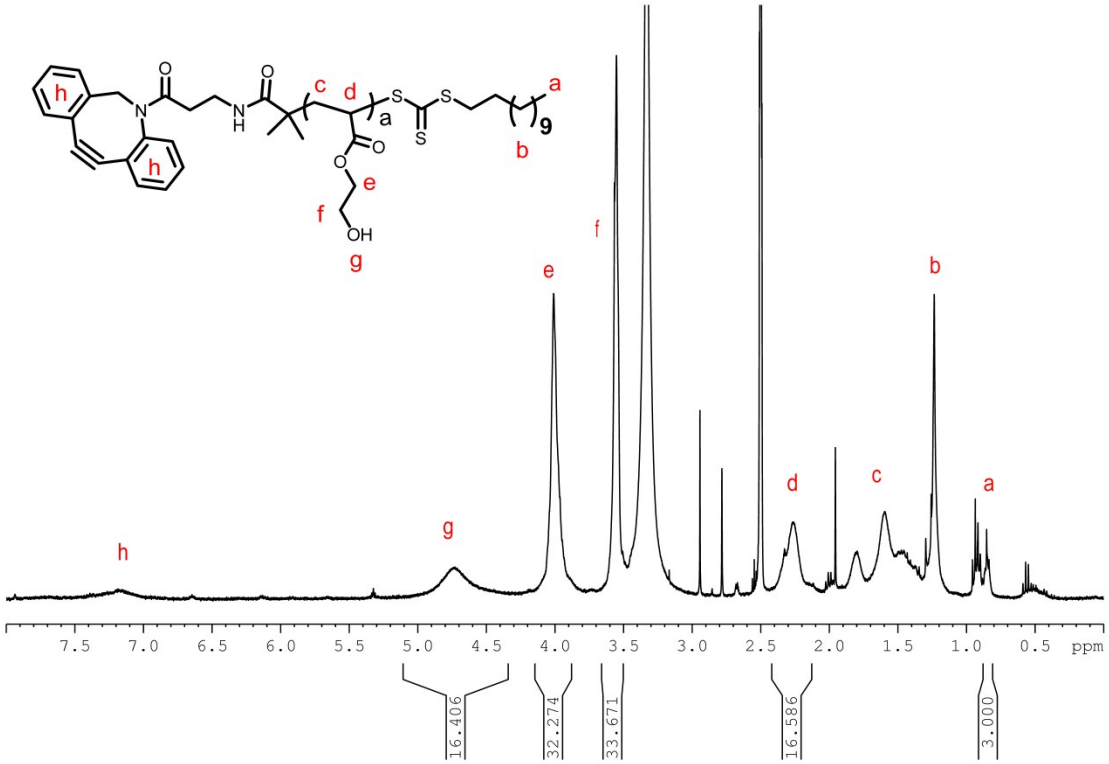


Figure S6. ^{1}H NMR spectrum of DBCO-PHEAGem$_{16}$ after hydrolysis to release all the drug (resulting in DBCO-PHEA$_{16}$) in d_6-DMSO.

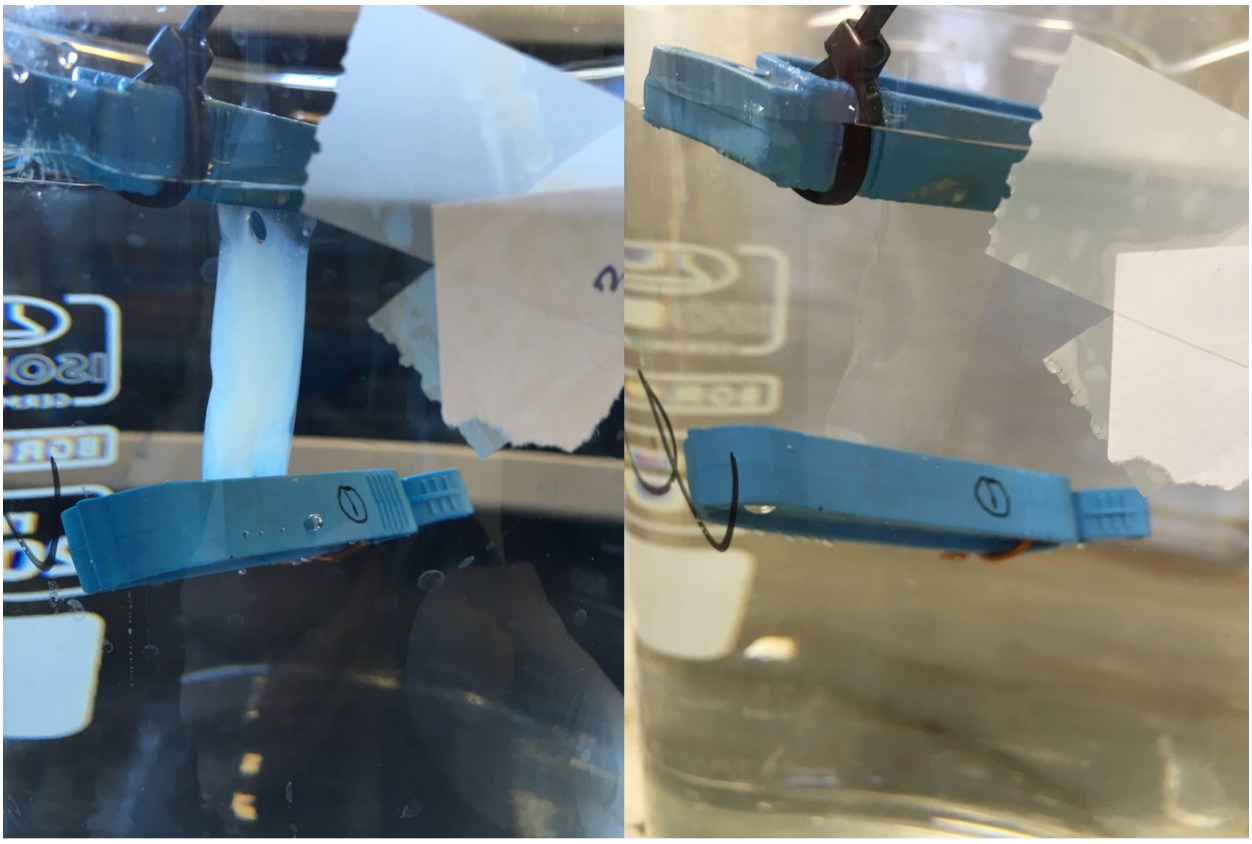


Figure S7. The turbidity of DBCO-PHEAGem$_{16}$ in dialysis bag against acidic water (pH 3) at begining and 16 h.

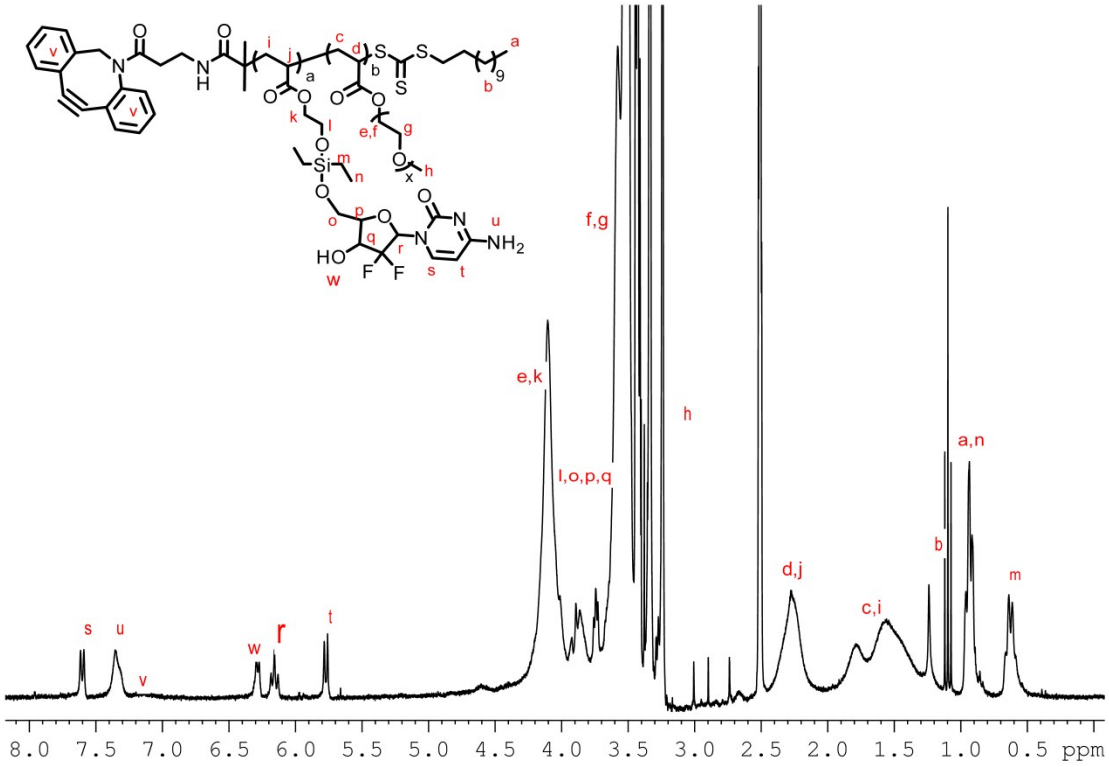


Figure S8. ^{1}H NMR spectrum of DBCO-PHEAGem$_2$-*co*-POEGMEA$_{18}$ in d_6-DMSO.

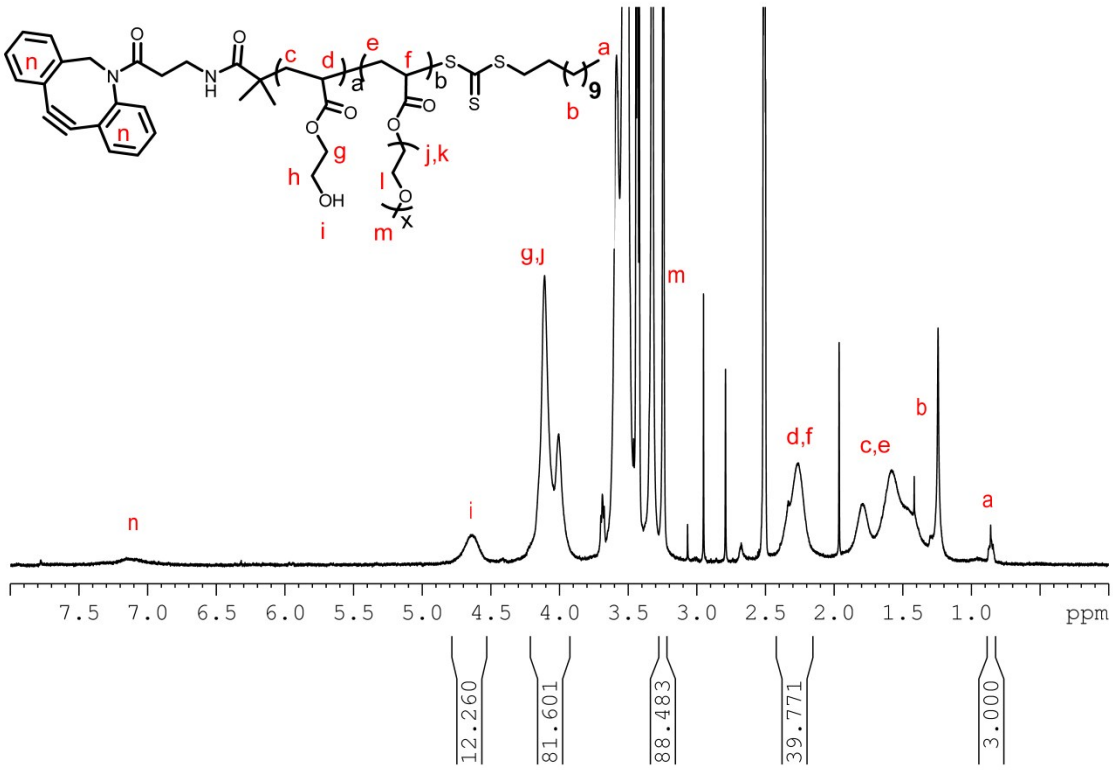


Figure S9. ^{1}H NMR spectrum of DBCO-PHEAGem$_{12}$-*co*-POEGMEA$_{28}$ after hydrolysis to release all the drug (resulting in DBCO-PHEA$_{12}$- POEGMEA$_{28}$) in d_6-DMSO.

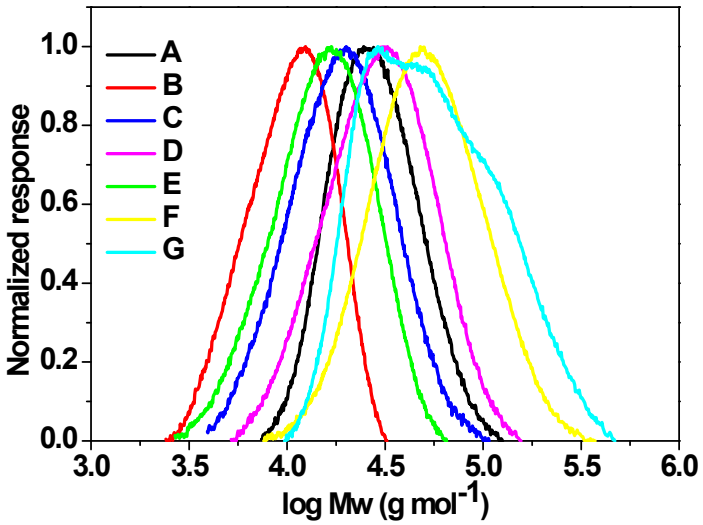
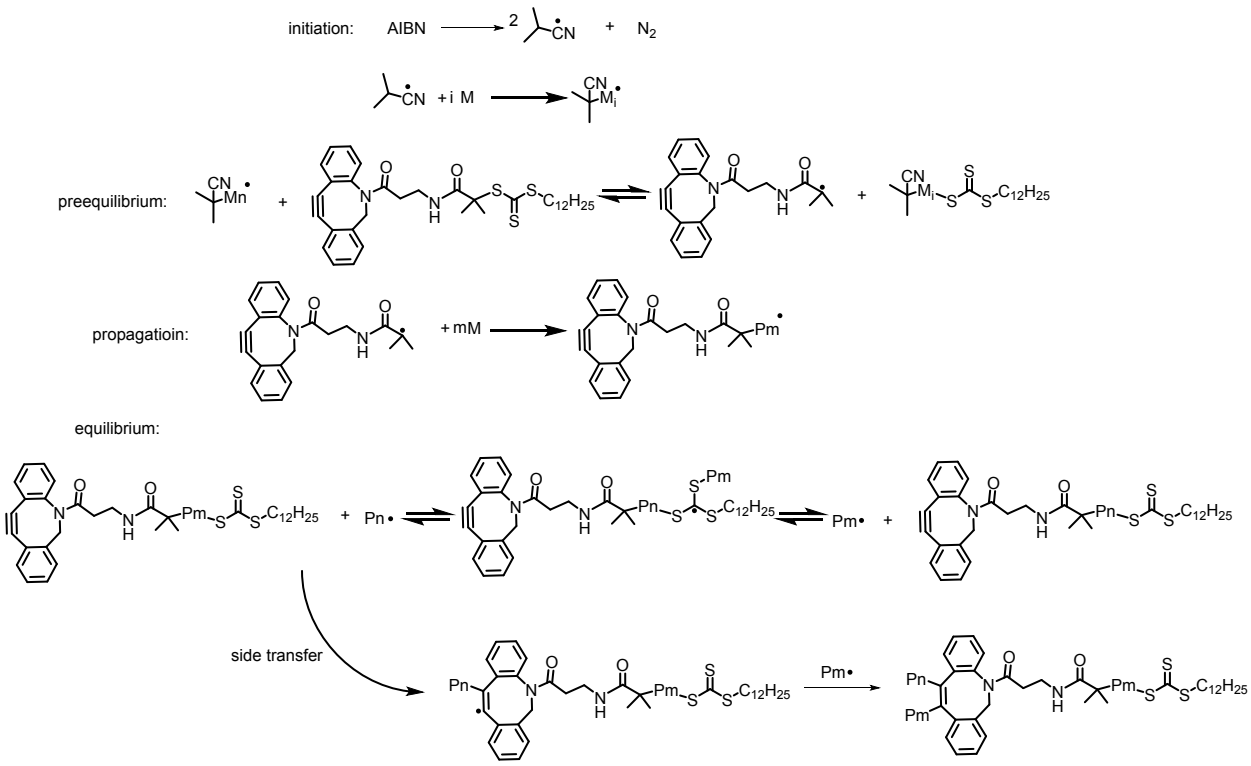
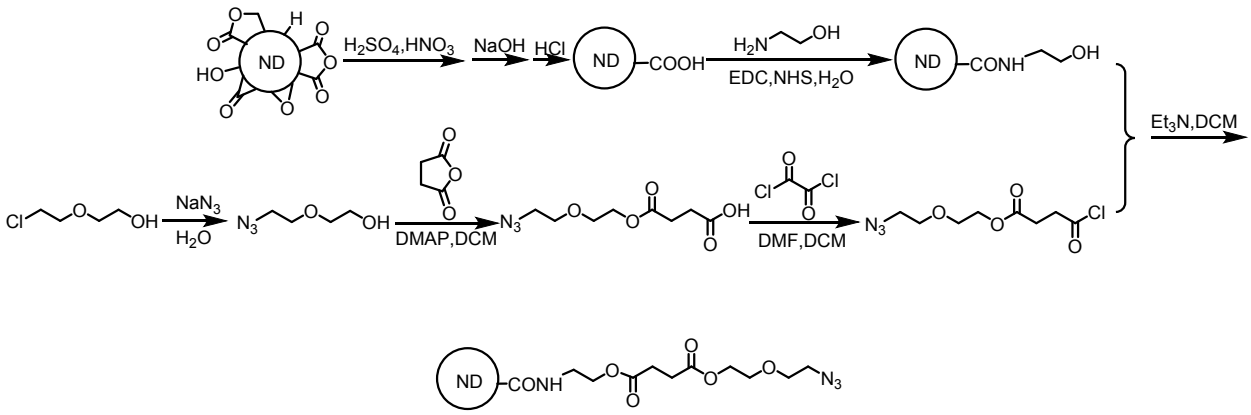


Figure S10. SEC (DMAc) traces of A) DBCO-PHEAGem$_{12}$-*co*-POEGMEA$_{28}$, B) DBCO-PHEAGem$_{16}$, C) DBCO-PHEAGem$_{10}$-*co*-POEGMEA$_{14}$, D) DBCO-POEGMEA$_{45}$, E) DBCO-PHEAGem$_{2}$-*co*-POEGMEA$_{18}$, F) DBCO-PHEAGem$_{20}$-*co*-POEGMEA$_{40}$, and G) DBCO-PHEAGem$_{16}$-*b*-POEGMEA$_{38}$.



Scheme S2. Proposed side reactions in DBCO-CTA mediated RAFT polymerization.

2. Surface functionalization of NDs.



Scheme S3. Surface functionalization of NDs.

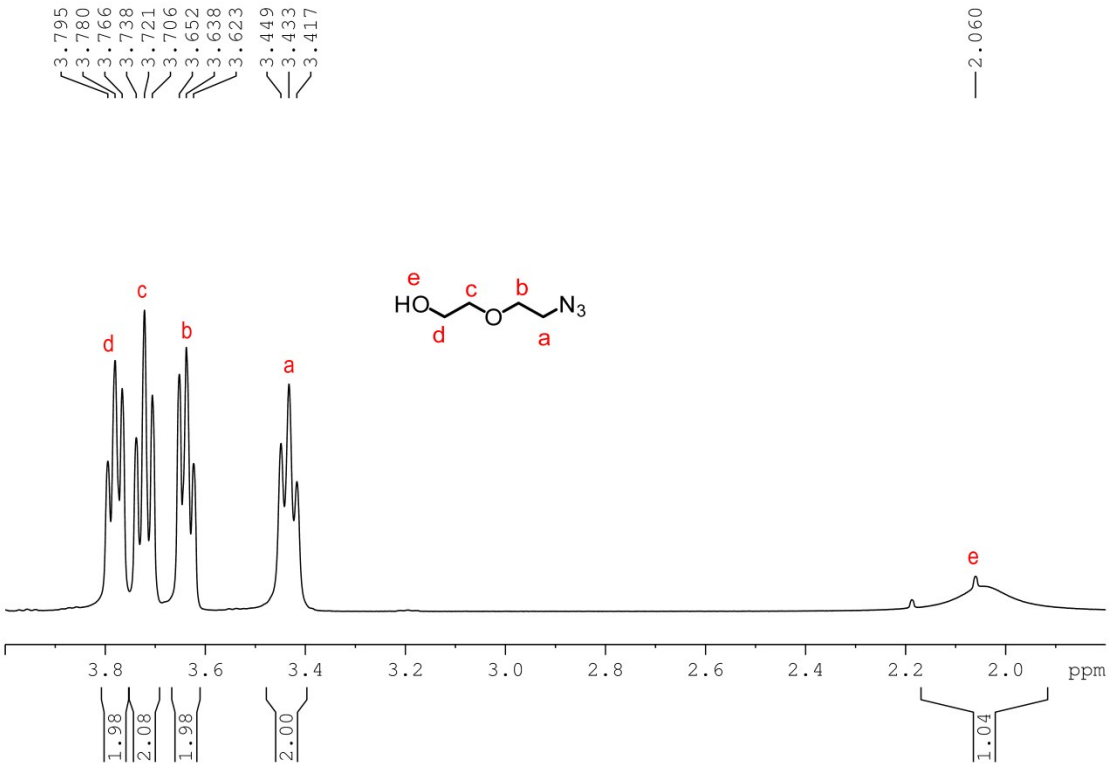


Figure S11. ^{1}H NMR spectrum of 2-azidethoxylethanol in $CDCl_3$.

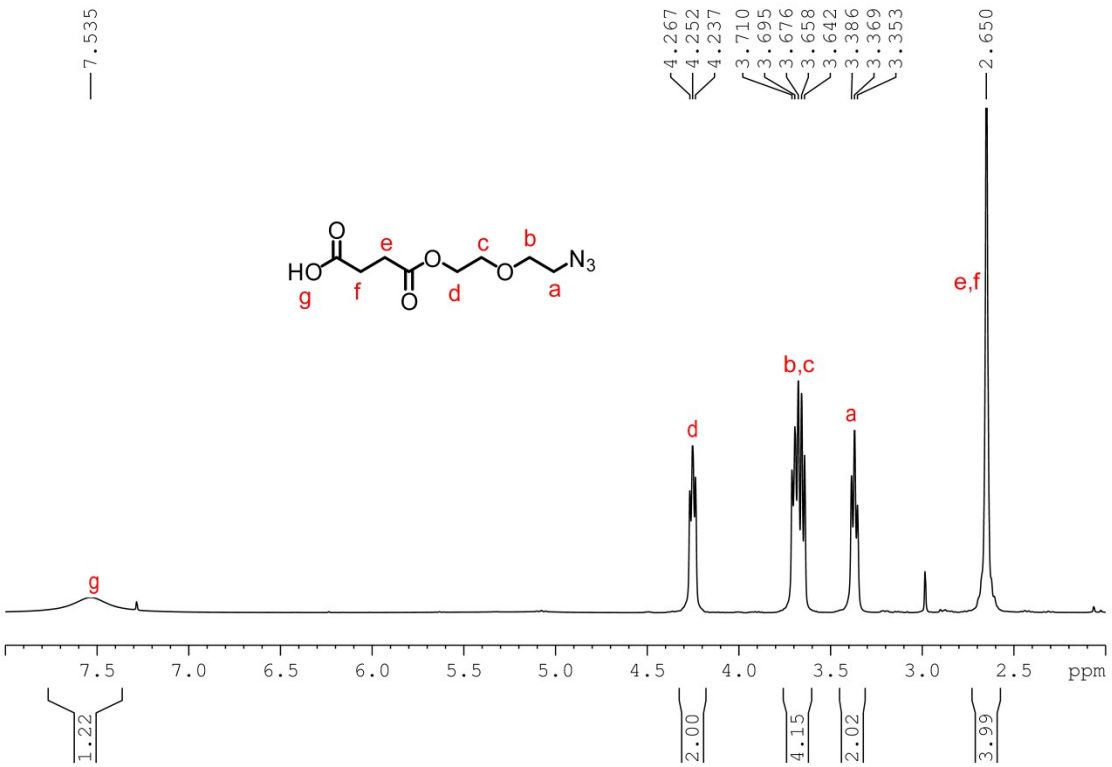


Figure S12. ^{1}H NMR spectrum of 4-(2-(2-azidoethoxy)ethoxy)-4-oxobutanoic acid in $CDCl_3$.

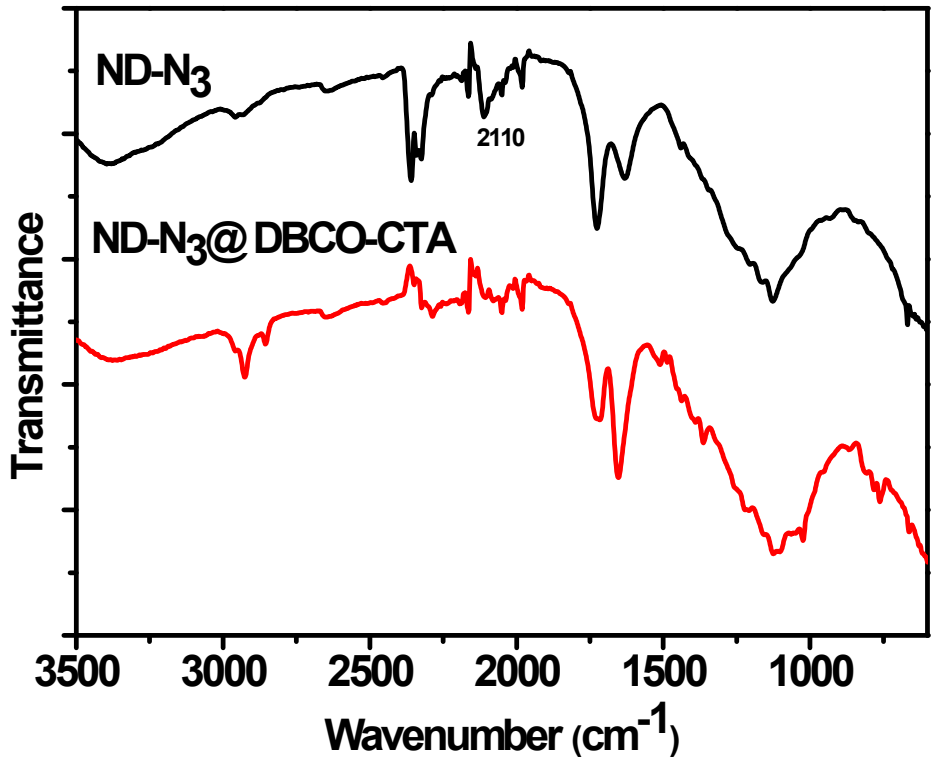
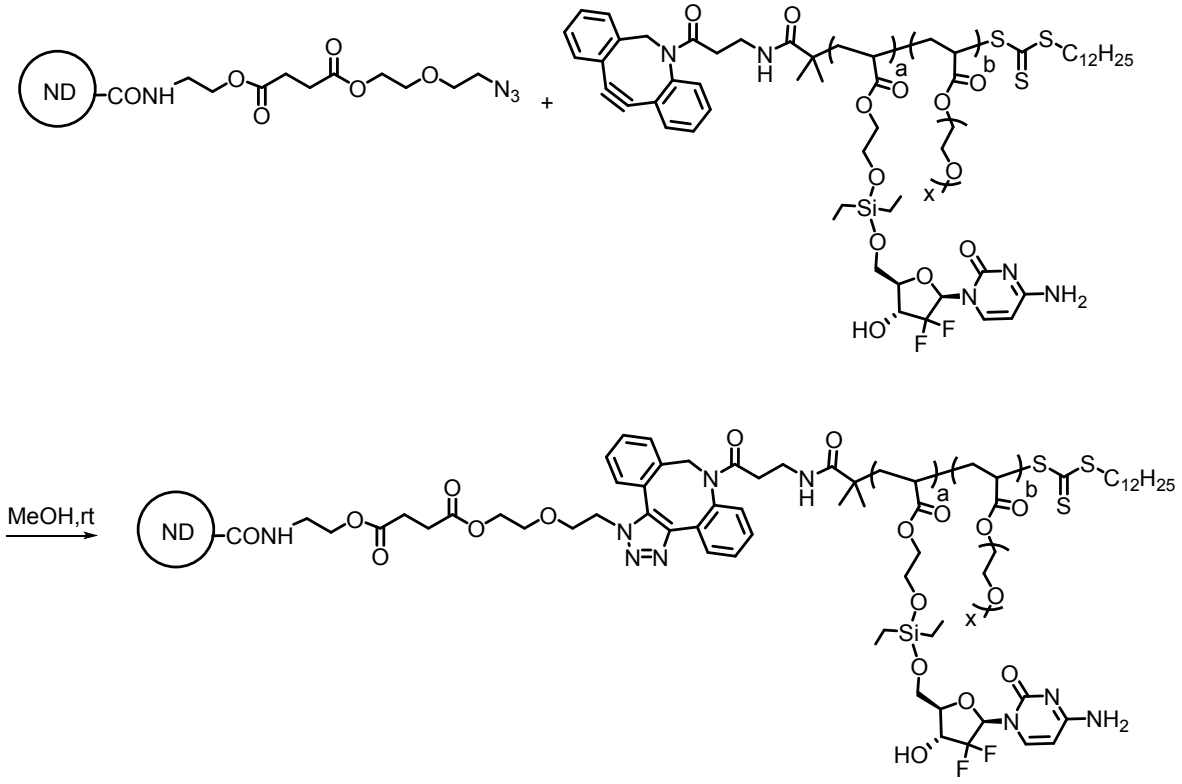


Figure S13. FTIR spectra of ND-N_3, and ND-N_3@DBCO-CTA.

3. Surface coating with polymer.



Scheme S4. Coupling the prodrug polymer to the surface of NDs using SPAAC click chemistry.

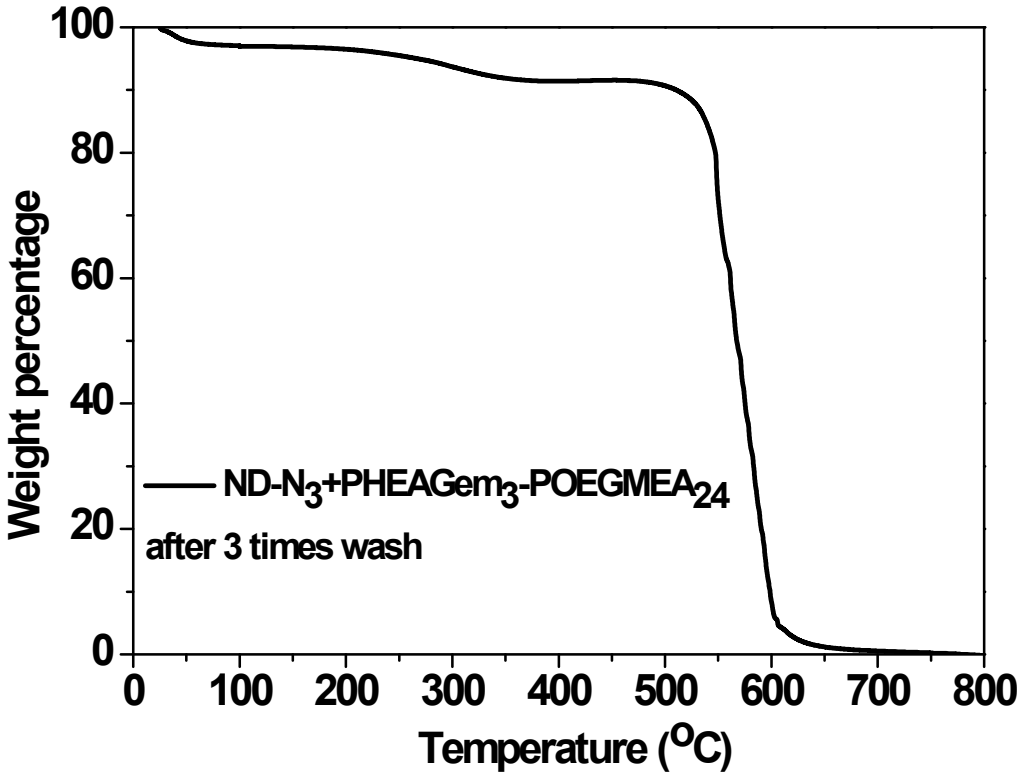


Figure S14. TGA graph of NDs obtained after mixing ND-N_3 and PHEAGem$_3$-*co*-POEGMEA$_{24}$ and 3 times of centrifuge and washing cycles with methanol.

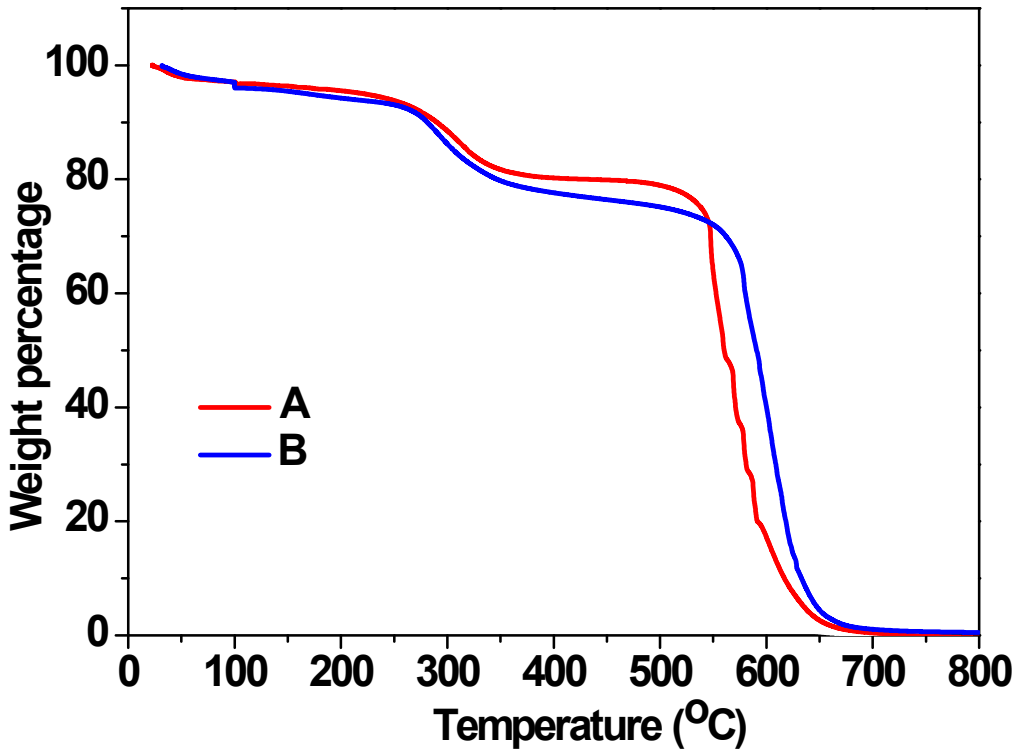


Figure S15. TGA curves of A) ND@PHEAGem$_{12}$-*co*-POEGMEA$_{28}$ and B) ND@PHEAGem$_{2}$-*co*-POEGMEA$_{18}$.

Table S1. Grafting densities of the prepared polymer coated NDs determined by TGA, molecular weight and particle sizes.

Samples	Polymer percentage on NDs [a]	Mn by NMR	Mn by GPC	D(nm)	Grafting density (chains nm^{-2})
ND@$POEGMEA_{45}$	17%	22200	23800	150	0.404
ND@$PHEAGem_{16}$-*b*-$POEGMEA_{38}$	20%	26300	34800	159	0.428
ND@$PHEAGem_{10}$-*co*-$POEGMEA_{14}$	18%	12000	16600	182	0.961
ND@ $PHEAGem_{20}$-*co*-$POEGMEA_{40}$	25%	28500	36700	165	0.512
ND@$PHEAGem_{2}$-*co*-$POEGMEA_{18}$	20%	10200	14000	272	1.87
ND@$PHEAGem_{12}$-*co*-$POEGMEA_{28}$	19%	19700	22800	226	0.775

Number of chains on NDs:

$$\text{Chains}=\frac{\Delta m}{Mn} * NA$$

Where Δm is the polymer percentage grafted on NDs, Mn is the molecular weight calculated from NMR and NA is Avogadro constant.

The specific surface are of a sphere:

$$SSA = \frac{6}{D * \rho}$$

$\rho=3.52\ g\ cm^{-3}=3.52\times10^{-21} g\ nm^{-3}$

Grafting density:

$$D=\frac{chains}{SSA}$$

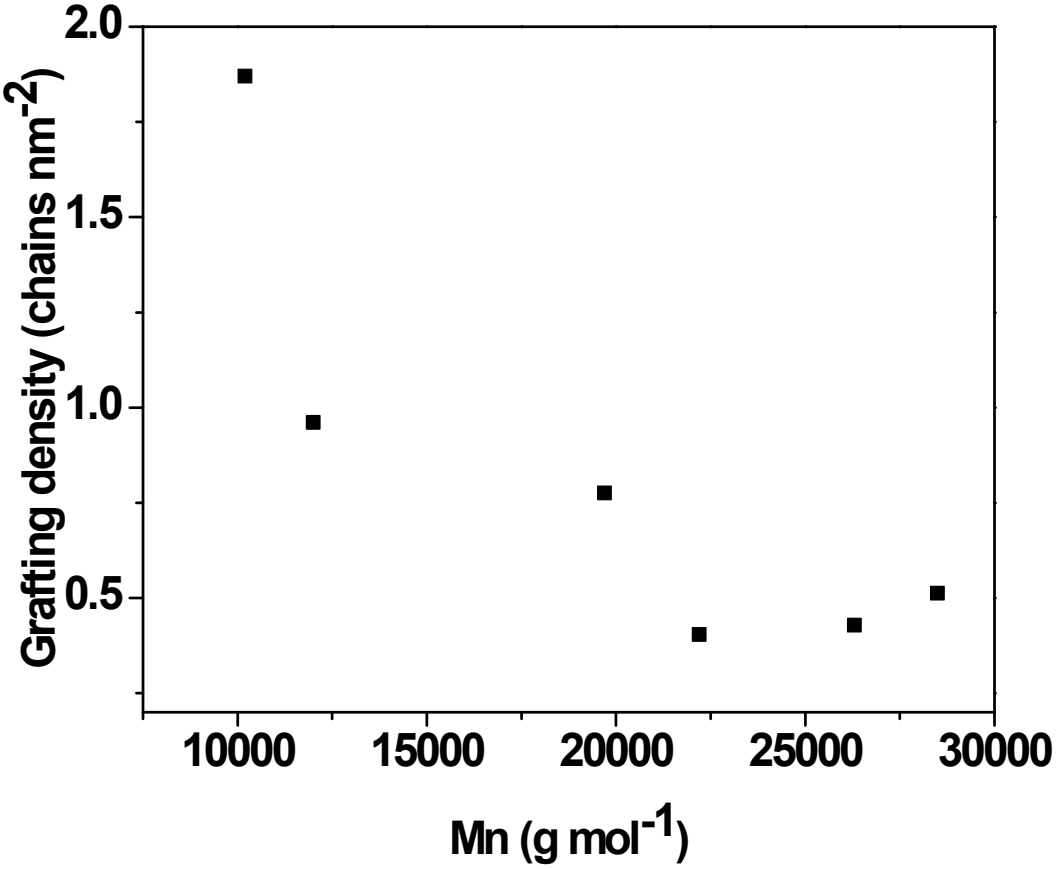


Figure S16. Correlation between grafting densities of polymer coated NDs determined by TGA and molecular weight.

4. Drug release.

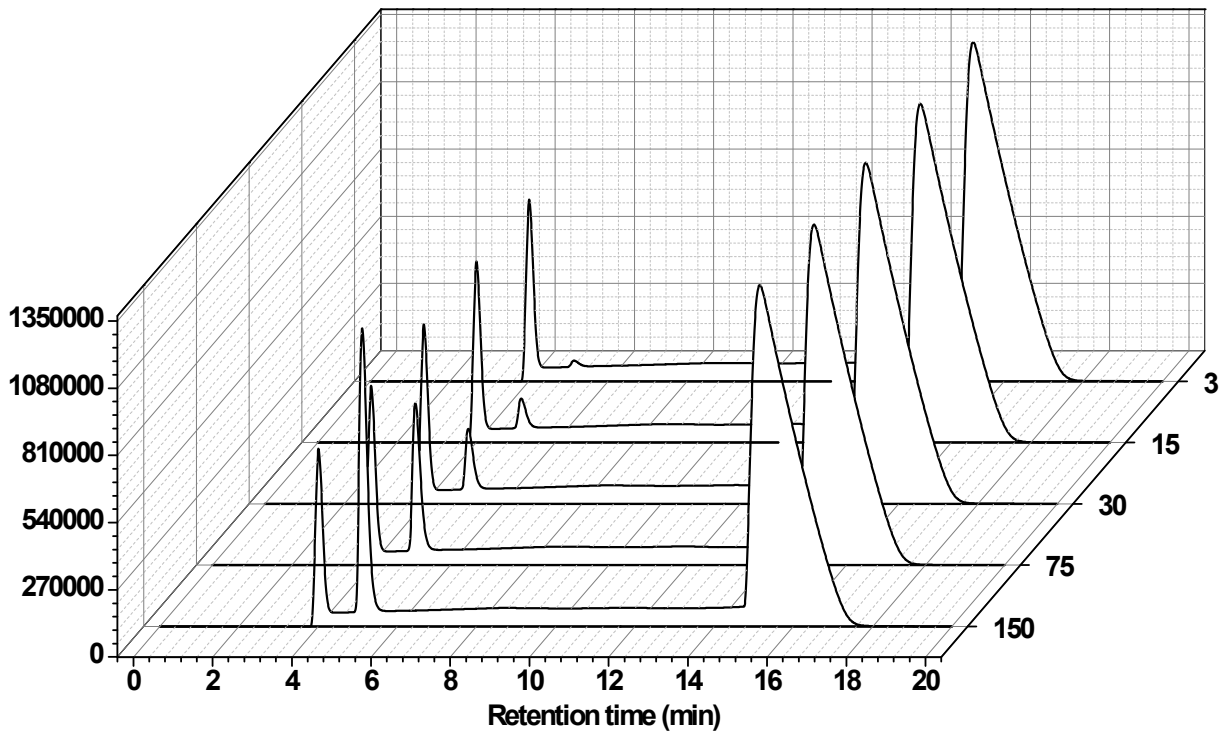


Figure S17. Chromatograms of gemcitabine hydrogen chloride standard in pH 5.0 buffer solution with concentration ranging from 3 μg mL^{-1} to 150 μg mL^{-1}.

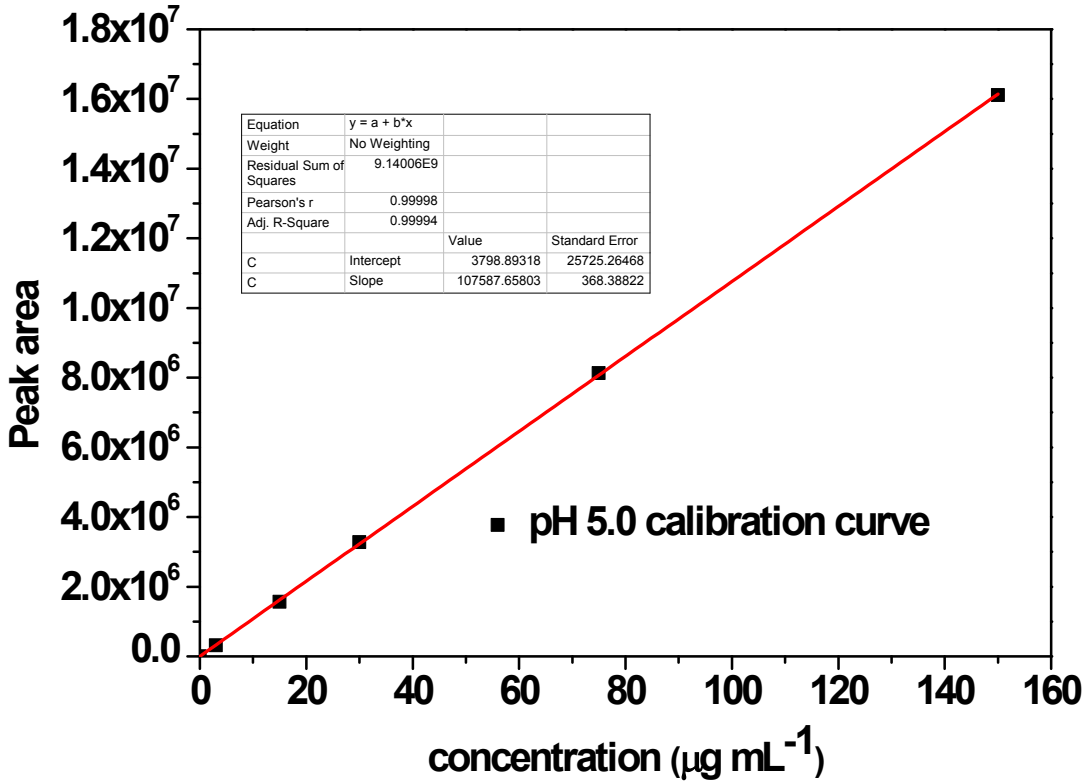


Figure S18. Gemcitabine calibration curve correlating the concentration of gemcitabine in pH 5.0 buffer solution with the area under the peak as analyzed by HPLC.

The equation for the fitted linear plot is:

$$Area=107587*concentration+3799$$

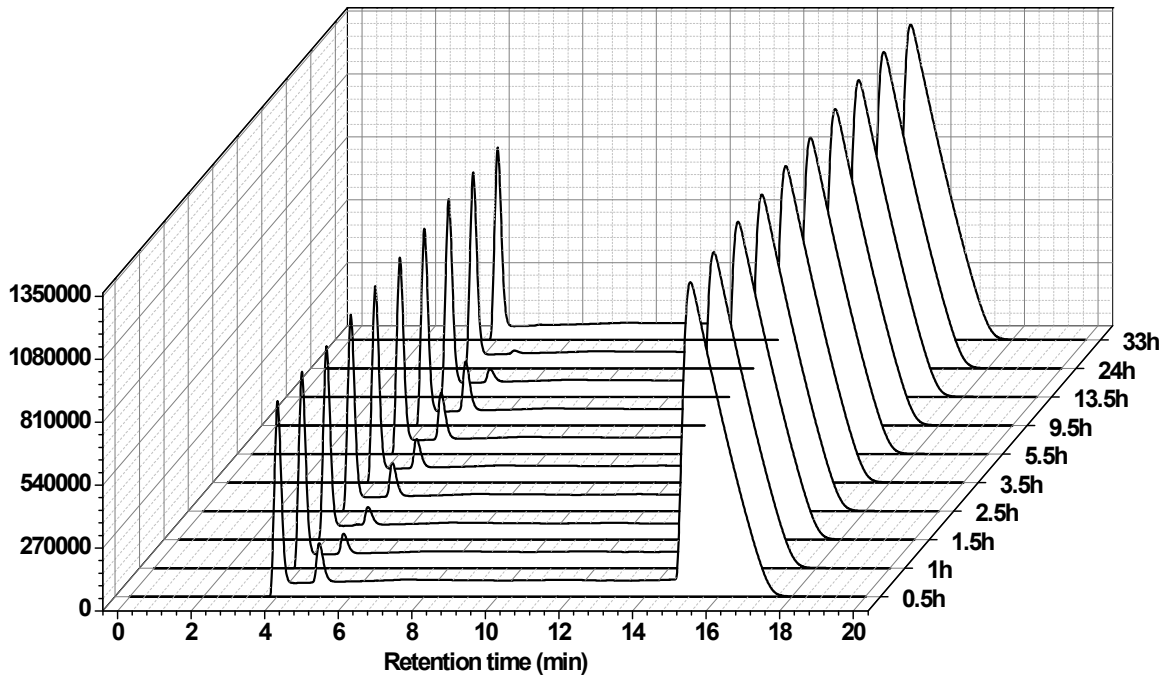


Figure S19. Chromatograms of gemcitabine released from ND@PHEAGem$_{20}$-*co*-POEGMEA$_{40}$ in pH 5.0 buffer solution at different time intervals.

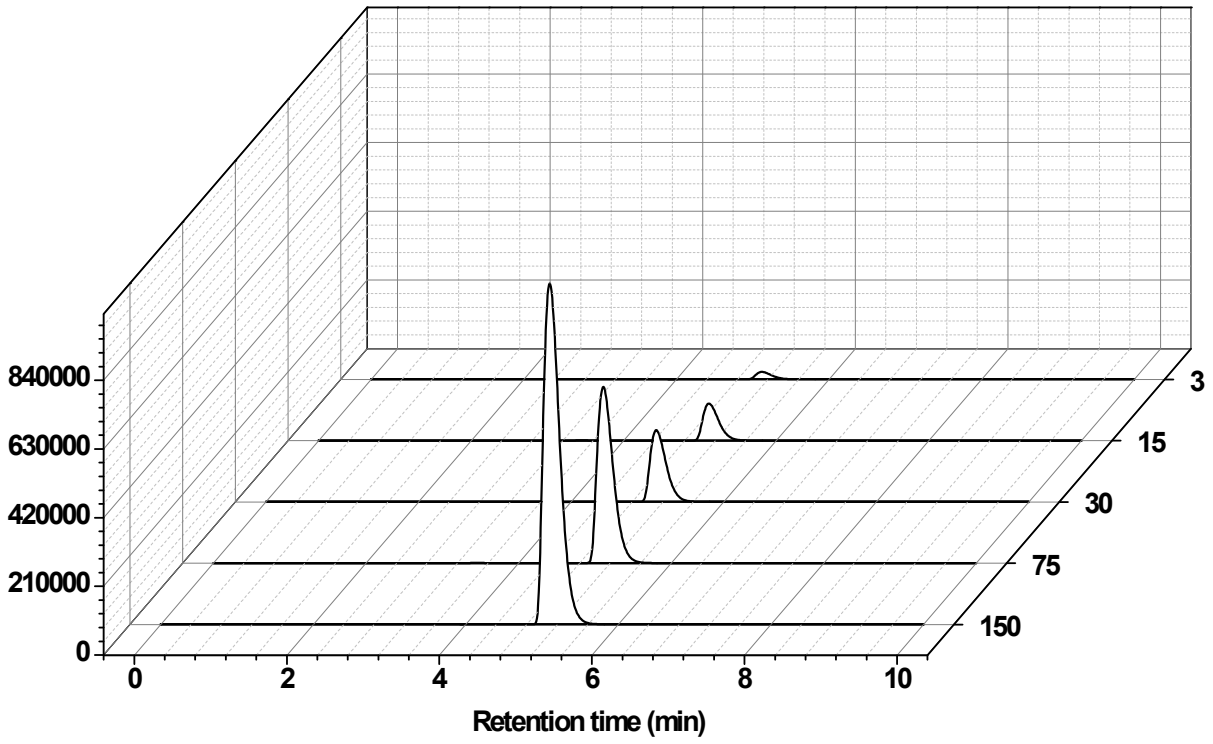


Figure S20. Chromatograms of gemcitabine hydrogen chloride standard in pH 7.4 buffer solution with concentration ranging from 3 μg mL^{-1} to 150 μg mL^{-1}.

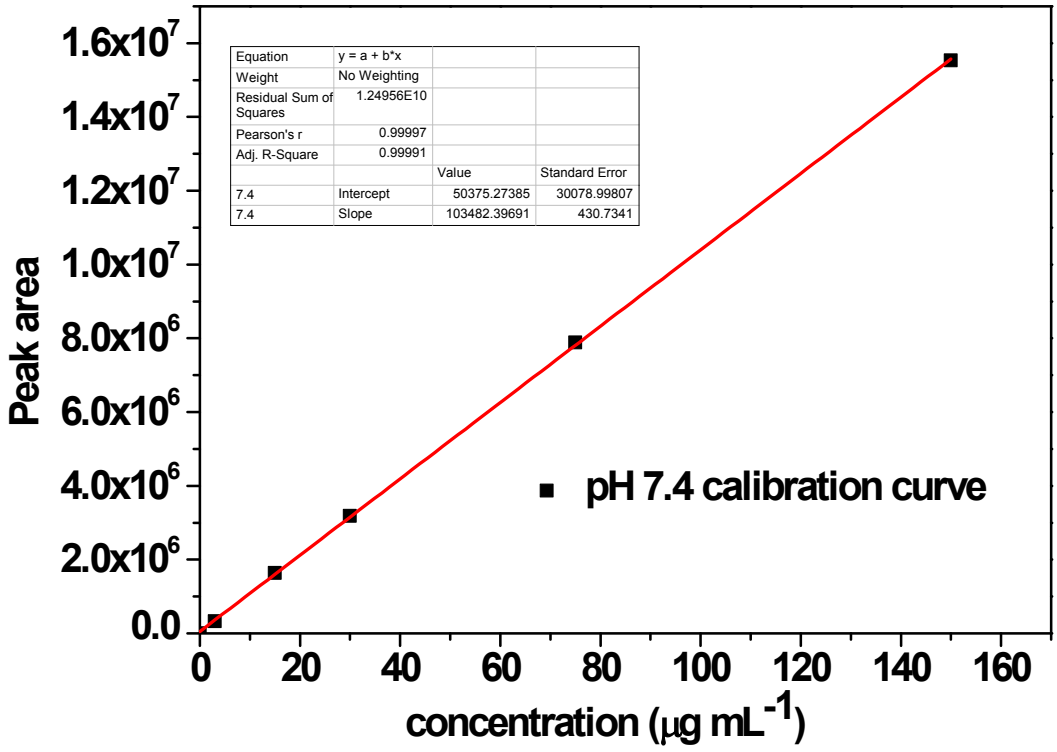


Figure S21. Gemcitabine calibration curve correlating the concentration of gemcitabine in pH 7.4 buffer solution with the area under the peak as analyzed by HPLC.

The equation for the fitted linear plot is:

$$Area = 103482 * concentration + 50375$$

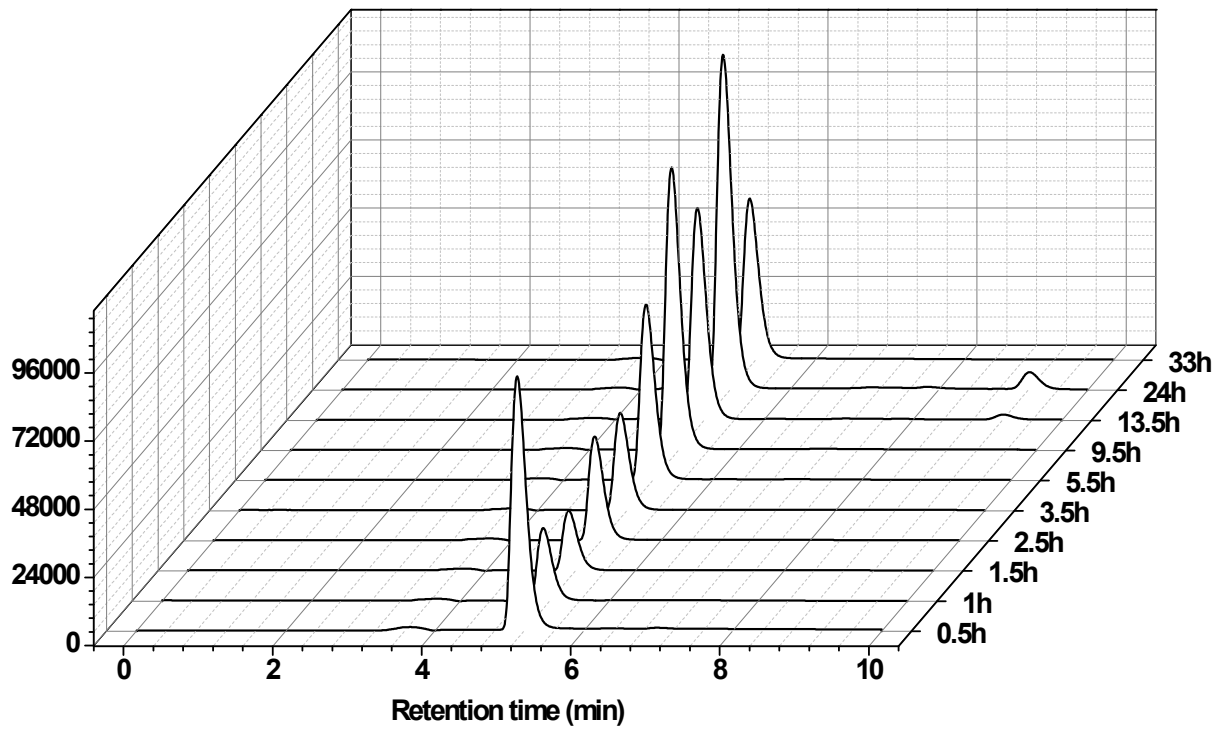


Figure S22. Chromatograms of gemcitabine released from ND@PHEAGem$_{20}$-*co*-POEGMEA$_{40}$ in pH 7.4 buffer solution at different time intervals.

Table S2. Peak areas at different time intervals and the corresponding concentration for drug release study of ND@PHEAGem$_{20}$-*co*-POEGMEA$_{40}$.

Time (h)	Area (pH 5.0)	Cumulative Area (pH 5.0)	Concentration (μg mL^{-1})	Area (pH 7.4)	Cumulative Area (pH 7.4)	Concentration (μg mL^{-1})
0.5	2105280	2105280	19.5	1259603	1259603	11.50
1	1047347	3152627	29.2	361081	1620684	15.0
1.5	924020	4076647	37.8	295647	1916331	17.8
2.5	1847349	5923996	55.0	511504	2427935	22.8
3.5	1585095	7509091	69.7	481643	2909478	27.4
5.5	2586274	10095365	93.8	866807	3776285	35.8
9.5	2647907	12743272	118.4	1400160	5176445	49.4
13.5	686341	13429613	124.7	1040583	6217028	59.5
24	146964	13576577	126.1	1656587	7873615	75.5
33	34409	13610986	126.4	808091	8681706	83.3
48	10012	13620998	126.5	766779	9448485	90.7
60	negligible	13620998	126.5	286077	9734562	93.5
84	negligible	13620998	126.5	353920	10088482	96.9
96	negligible	13620998	126.5	141799	10230281	98.3

Drug content calculation by HPLC:

ND@PHEAGem$_{20}$-*co*-POEGMEA$_{40}$

Final drug concentration: 126.5 μg mL^{-1}

Concentration of NDs: 2 mg mL^{-1}

Drug content in NDs: 126.5/2 μg mg^{-1}=63.3 μg mg^{-1}

Drug content calculation by TGA and monomer ratio in polymer:

Ratio of HEAGem:OEGMEA in polymer: 1:2

Weight percentage of HEAGem: $\frac{1 * 463}{1 * 463 + 2 * 480}$=0.32

Molecular weight of gemcitabine hydrogen chloride: 300 g mol^{-1}

Gemcitabine content if released completely: $\frac{0.25 * 0.32}{463} * 300 * 1000$=51.8 μg mg^{-1}

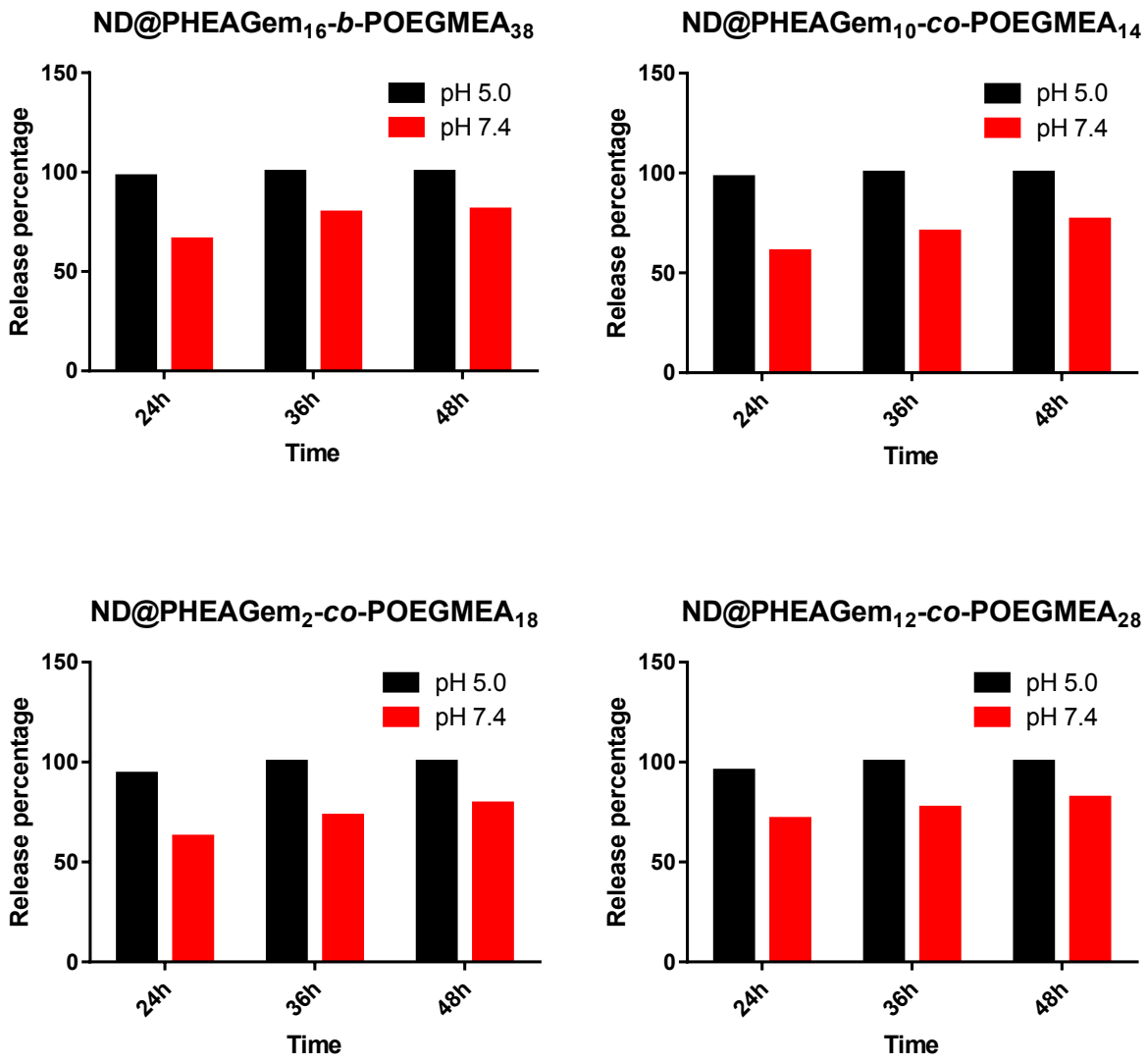
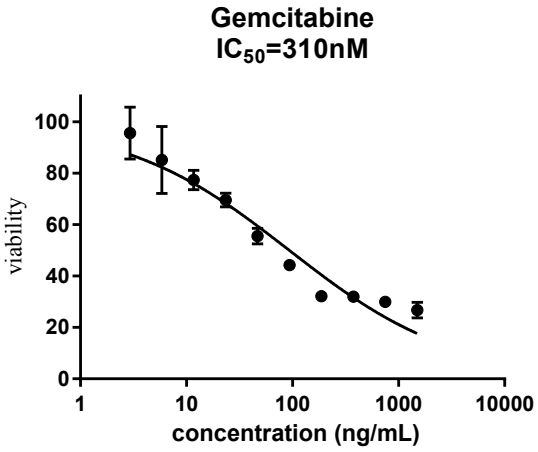
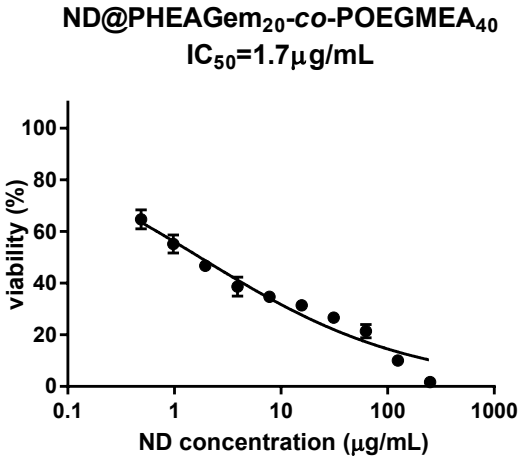
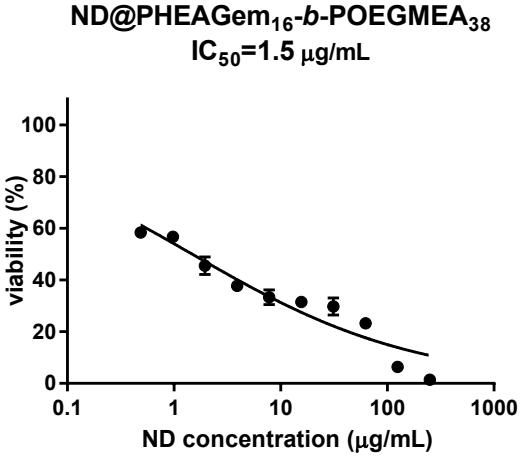


Figure S23. Cumulative release of gemcitabine versus time from ND@PHEAGem$_{16}$-*b*-POEGMEA$_{38}$, ND@PHEAGem$_{10}$-*co*-POEGMEA$_{14}$, ND@PHEAGem$_{2}$-*co*-POEGMEA$_{18}$ and ND@PHEAGem$_{12}$-*co*-POEGMEA$_{28}$ in buffer solution with pH 5.0 and 7.4 with concentration of 2 mg mL^{-1}.

5. Cytotoxicity Test







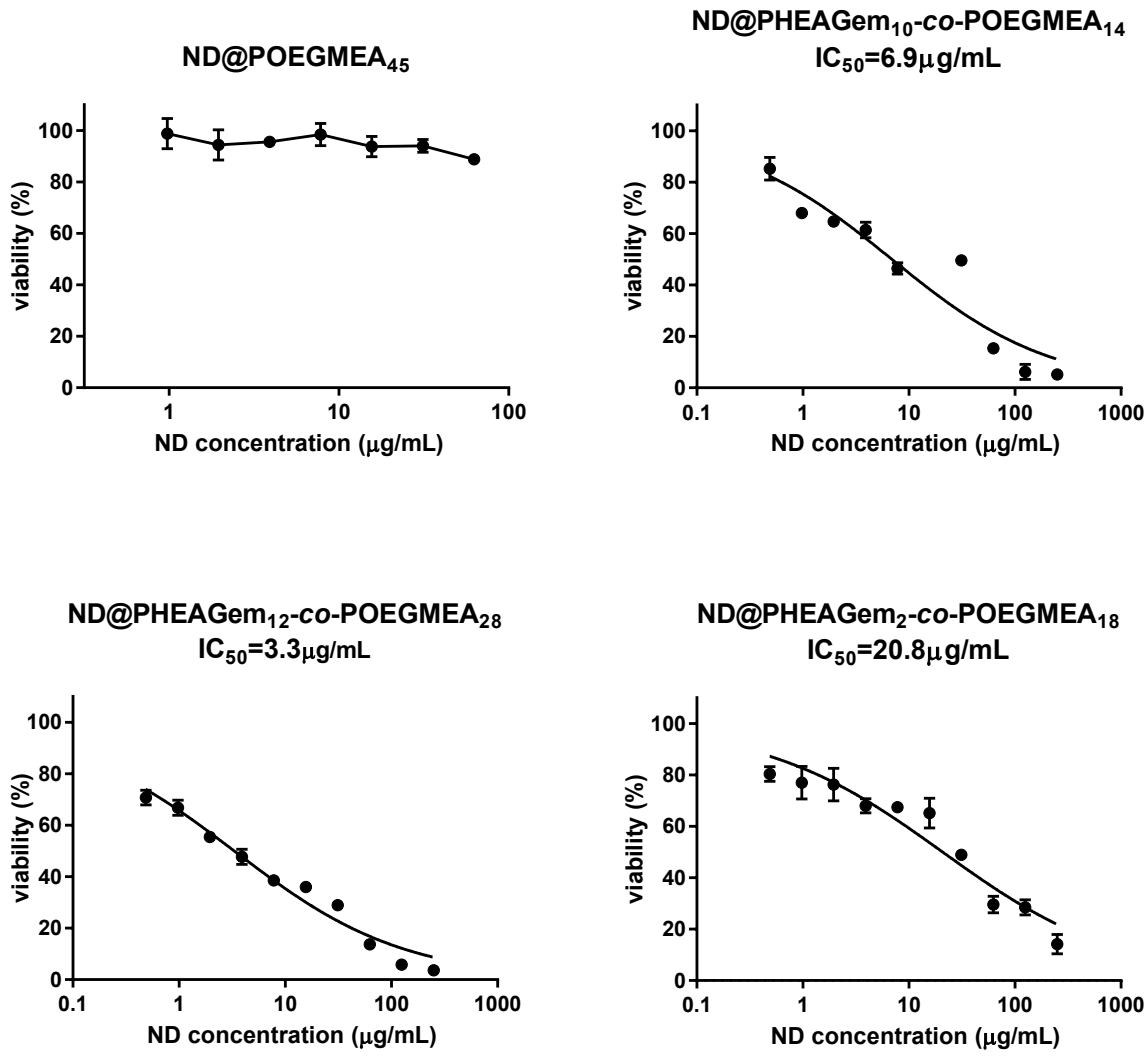


Figure S24. Cytotoxicity of Gemcitabine and polymer coated NDs.