Supplementary Information

Advanced pH-Responsive Copolymers for Stabilizing Lipid Nanoparticles and Manipulating their Internal Nanostructure

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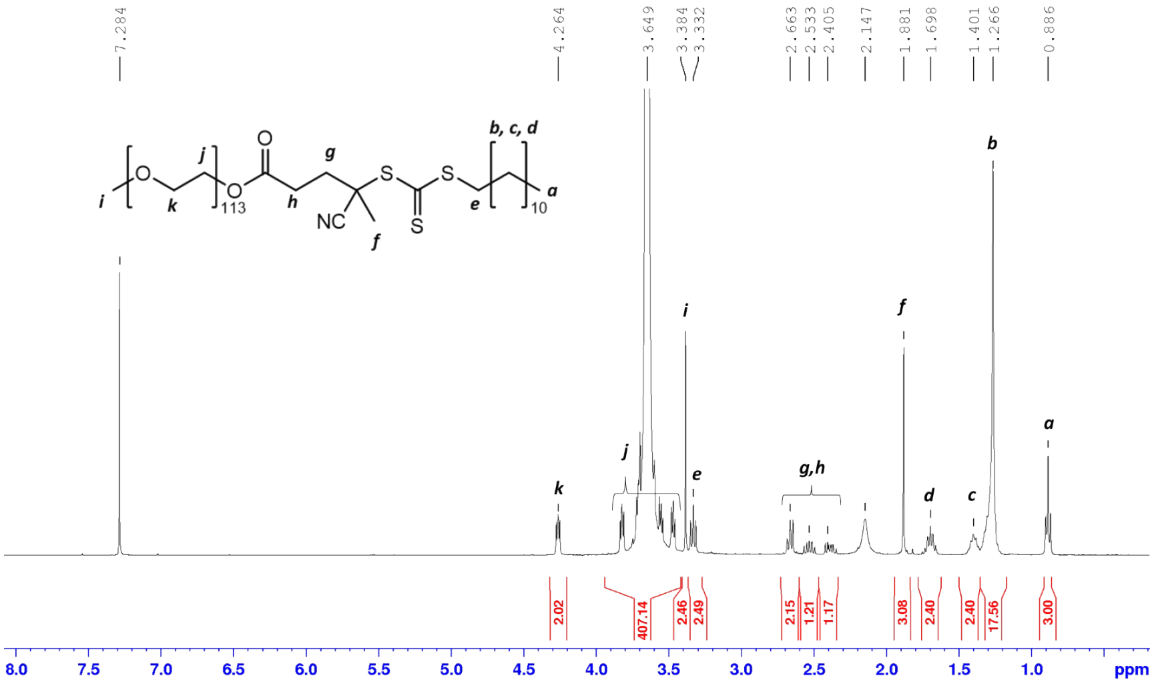

Figure S1. ^{1}H NMR of PEG_{113}-CDTPA in $CDCl_3$: δ 4.25 (t, 2H), 3.45-3.81 (m, 412H), 3.36 (s, 3H), 3.31 (t, 2H), 2.37-2.65 (m, 4H), 1.86 (s, 3H), 1.69 (m, 2H), 1.25-1.38 (b, 18H), 0.86 (t, 3H).

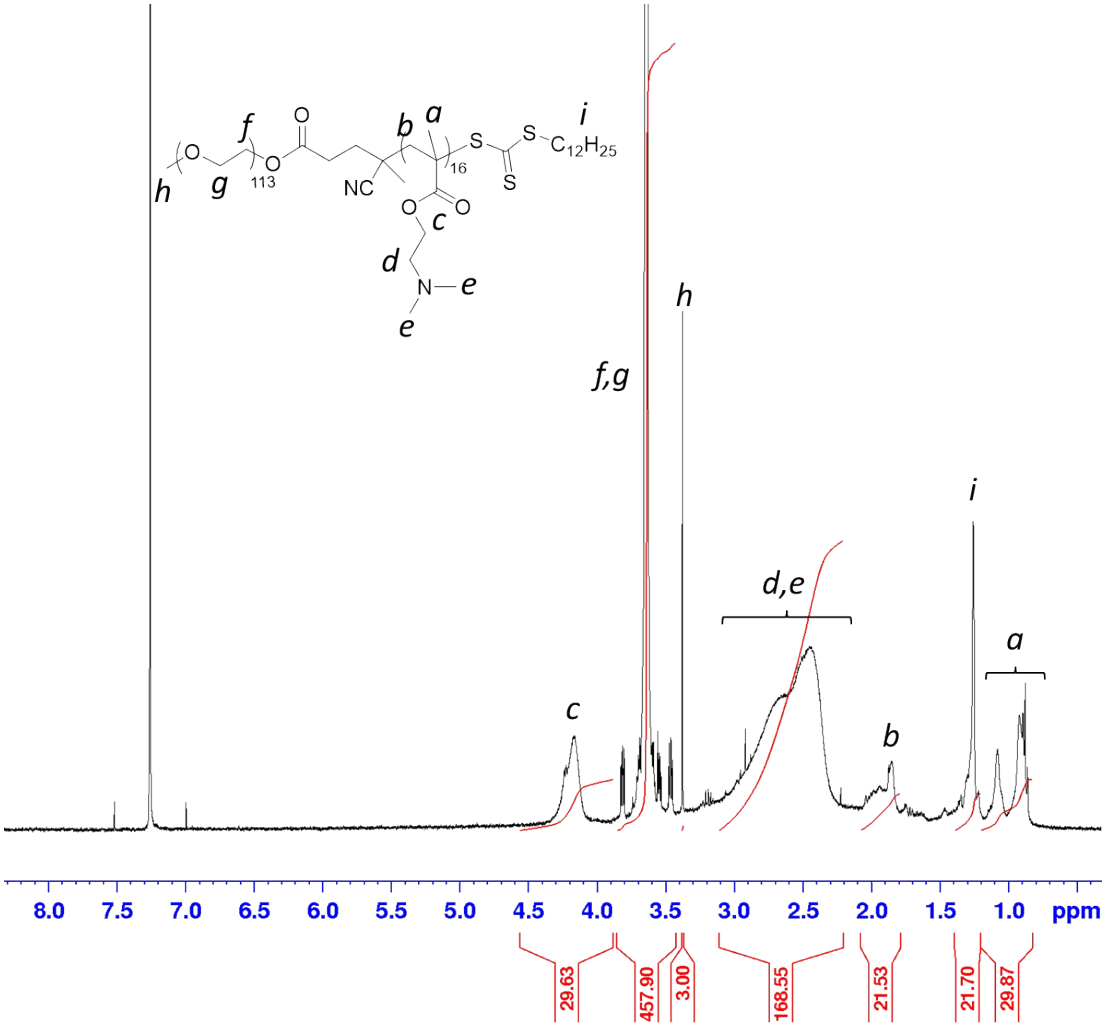

Figure S2. ^{1}H NMR of PEG_{113}-$PDMAEMA_{16}$-CDTPA in $CDCl_3$: δ 3.96-4.35 (b, 30H), 3.42-3.85 (b, 457H), 3.37 (s, 3H), 2.14-3.02 (b, 168H), 1.72-2.07 (b, 21H), 1.18-1.51 (b, 22H), 0.77-1.16 (b, 30H).

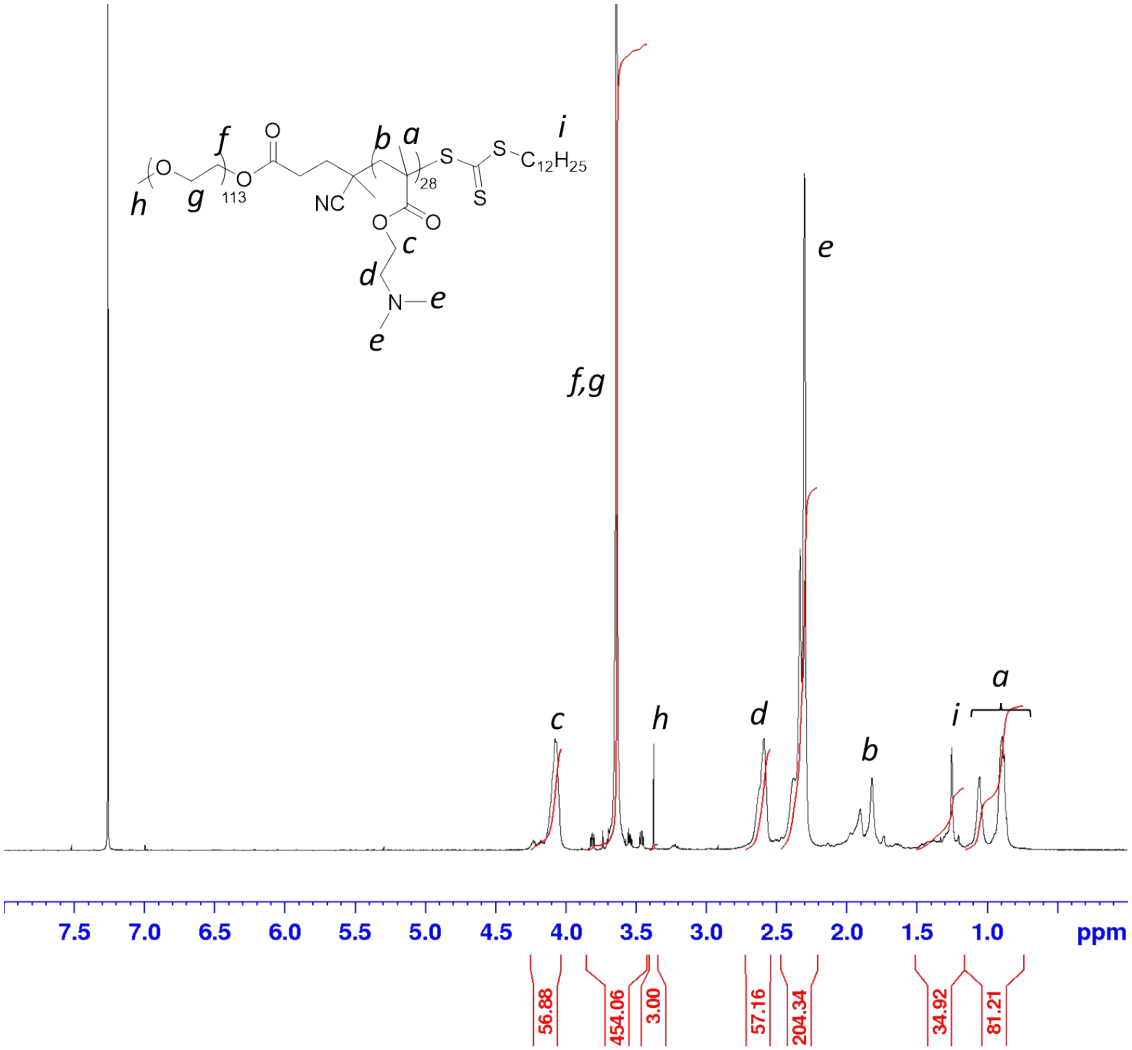

Figure S3. ^{1}H NMR of PEG_{113}-$PDMAEMA_{28}$-CDTPA in $CDCl_3$: δ 4.00-4.28 (b, 56H), 3.42-3.85 (b, 454H), 3.37 (s, 3H), 2.46-2.88 (b, 100H), 2.18-2.45 (b, 240H), 1.70-2.10 (b, 74H), 1.16-1.51 (b, 39H), 0.75-1.16 (b, 112H).

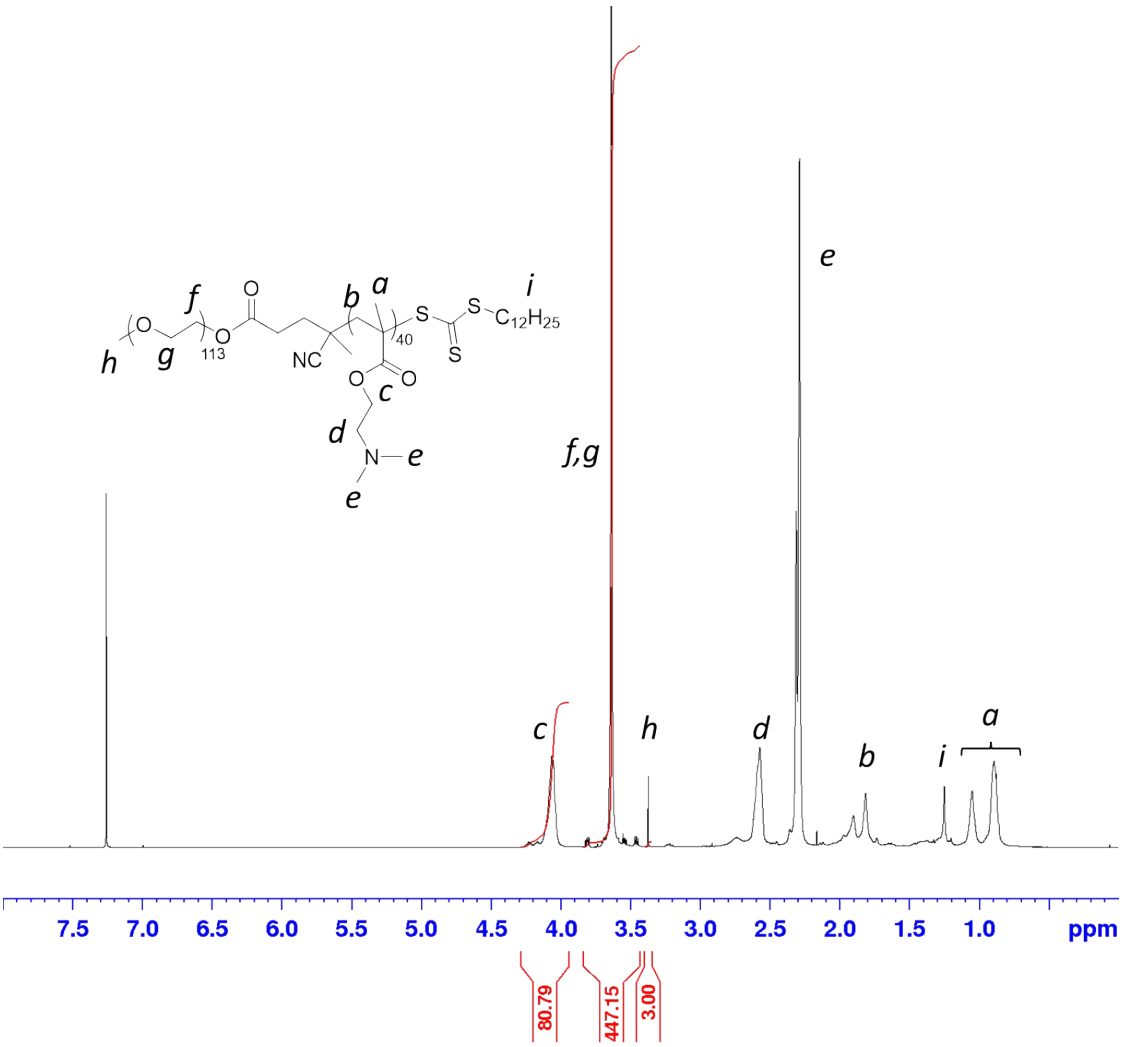

Figure S4. ^{1}H NMR of PEG_{113}-$PDMAEMA_{40}$-CDTPA in $CDCl_3$: δ 3.94-4.29 (b, 80H), 3.42-3.85 (b, 447H), 3.37 (s, 3H), 2.54-2.72 (b, 57H), 2.21-2.47 (b, 204H), 1.68-2.07 (m, 56H), 1.16-1.51 (m, 35H), 0.74-1.16 (m, 81H).

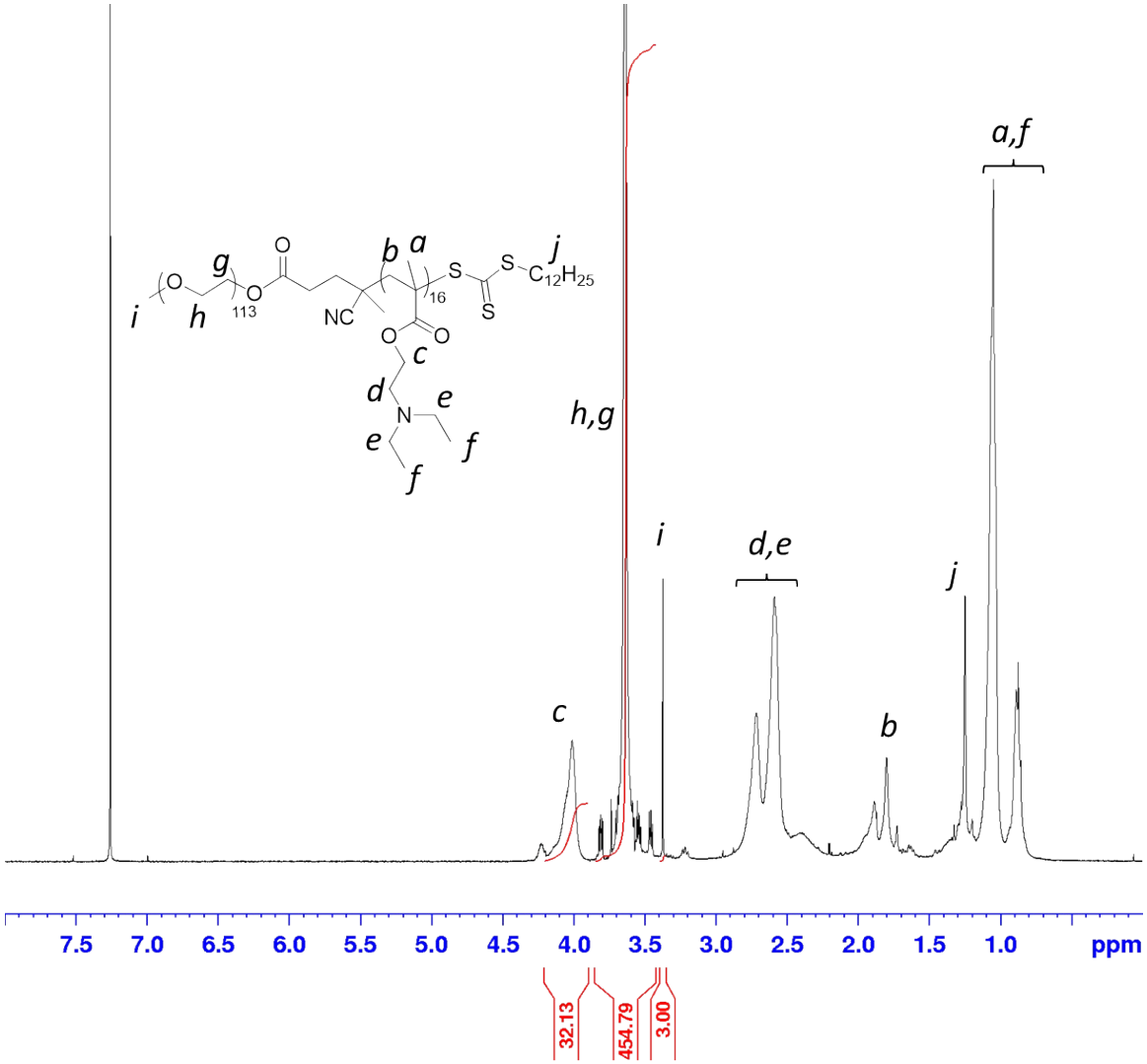

Figure S5. ^{1}H NMR of PEG_{113}-$PDEAEMA_{16}$-CDTPA in $CDCl_3$: δ 3.90-4.30 (b, 32H), 3.42-3.85 (b, 454H), 3.37 (s, 3H), 2.23-2.90 (b, 119H), 1.58-2.06 (b, 36H), 1.17-1.49 (b, 32H), 0.74-1.15 (b, 145H).

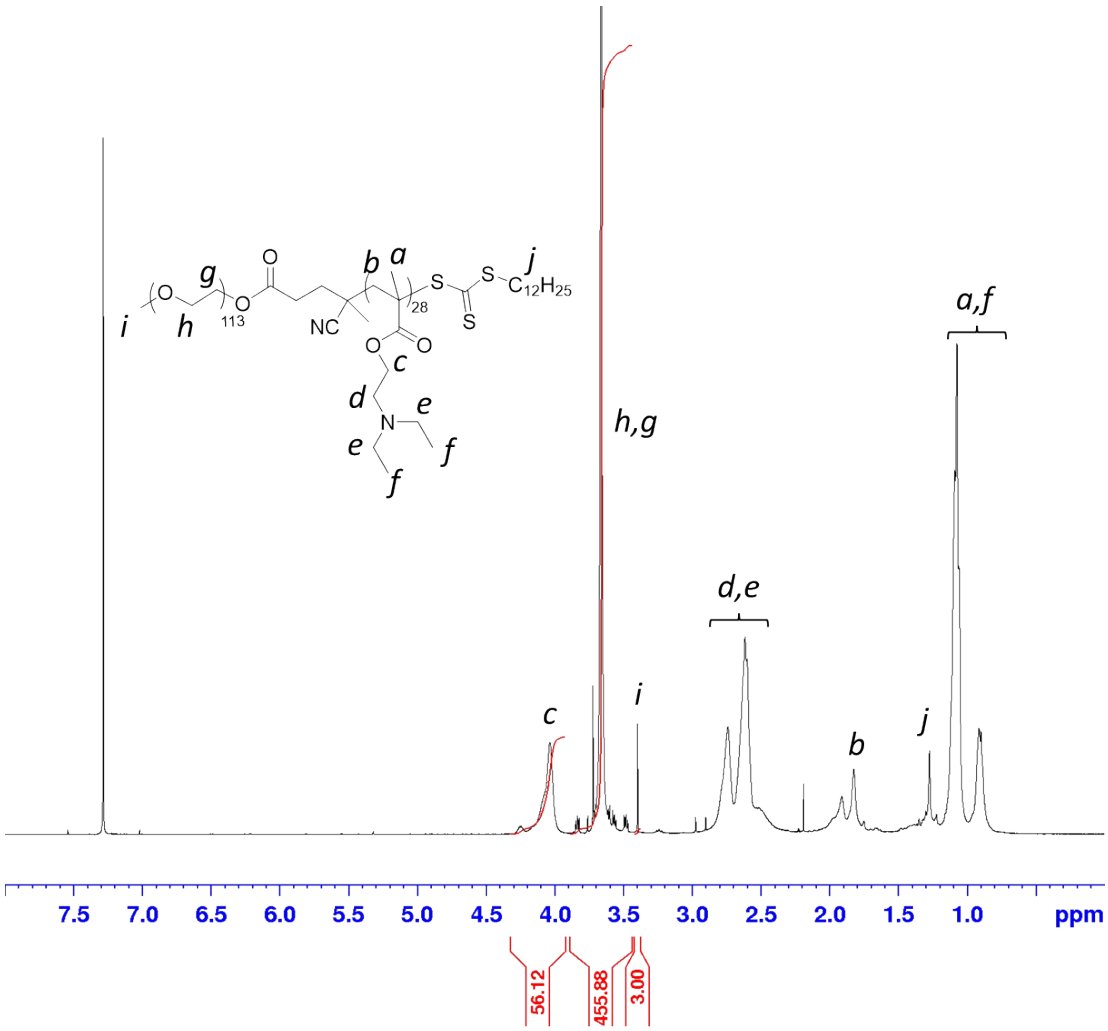

Figure S6. ^{1}H NMR of PEG_{113}-$PDEAEMA_{28}$-CDTPA in $CDCl_3$: δ 3.92-4.32 (b, 56H), 3.44-3.89 (b, 456H), 3.37 (s, 3H), 2.34-2.93 (b, 194H), 1.70-2.10 (b, 51H), 1.21-1.51 (b, 32H), 0.75-1.20 (b, 241H).

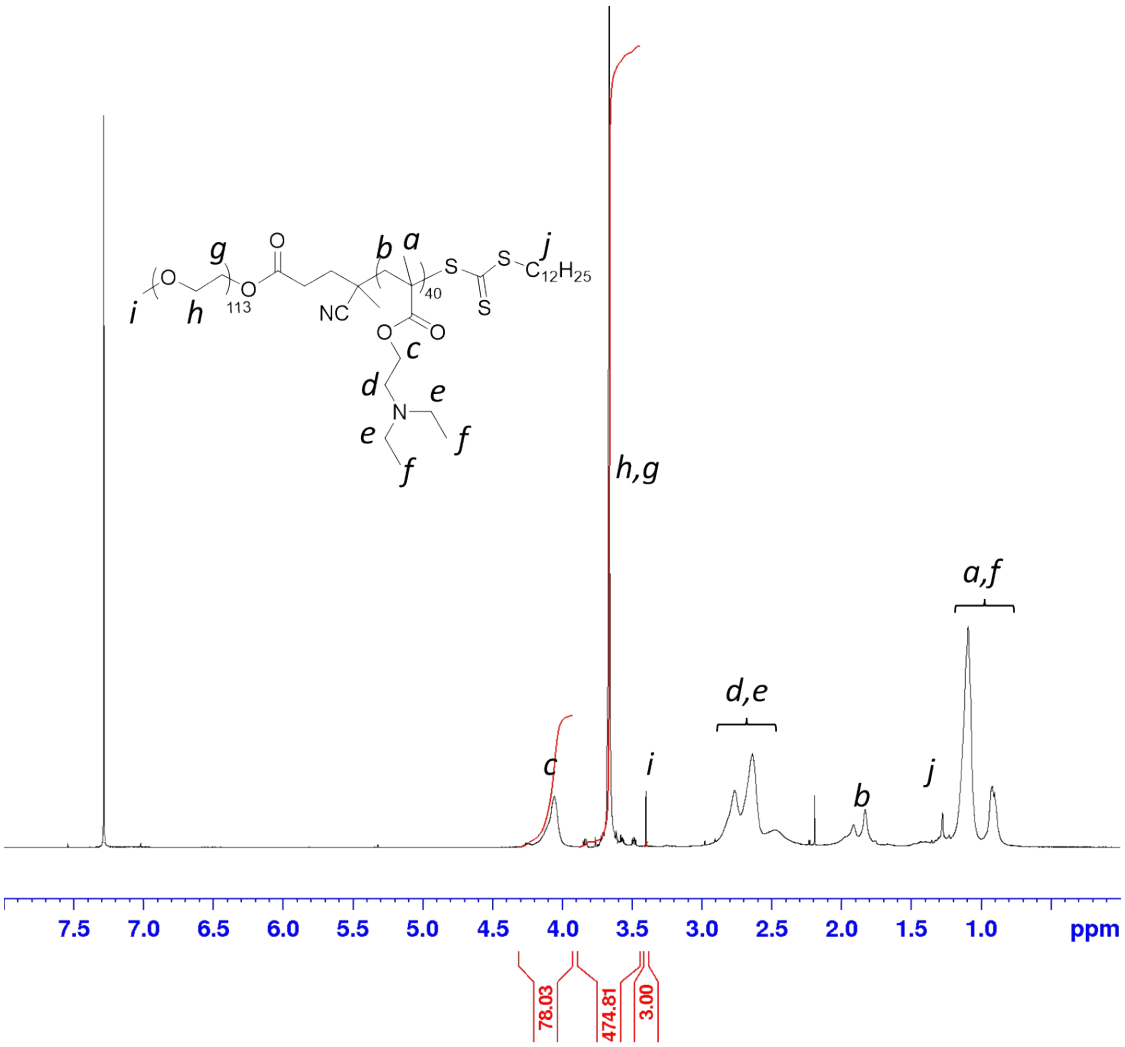

Figure S7. ^{1}H NMR of PEG_{113}-$PDEAEMA_{40}$-CDTPA in $CDCl_3$: δ 3.92-4.31 (b, 78H), 3.44-3.89 (b, 475H), 3.37 (s, 3H), 2.27-2.98 (b, 291H), 1.71-2.10 (b, 71H), 1.22-1.51 (b, 37H), 0.75-1.22 (b, 339H).

Note: The degree of polymerization of all samples with respect to their individual monomer units can be calculated by comparing the singlet proton peak at δ 3.37, which corresponds to the -O-CH_3 end group on the PEG_{113} moiety. This is compared with the broad proton peak at δ 3.96-4.35, which corresponds to the -CH_2-N- group on the DMAEMA or DEAEMA moiety. This comparison confirms the high conversion of all monomers into their respective polymers.

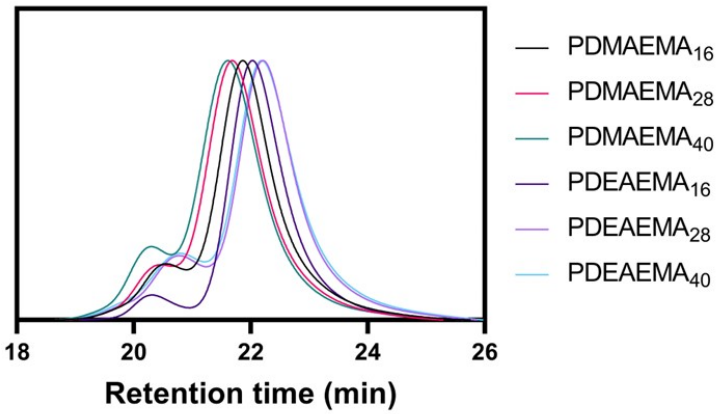

Figure S8. GPC curves of the synthesized copolymers: $PDMAEMA_n$ and $PDEAEMA_n$ (n = 16, 28 and 40).

Table S1. GPC and NMR data of the developed copolymers

	NMR	**GPC**		
Block copolymers	M_n	M_w	M_n	$Đ$
$PDMAEMA_{16}$: PEG_{113}-$PDMAEMA_{16}$-CDTPA	7919	15200	12800	1.19
$PDMAEMA_{28}$: PEG_{113}-$PDMAEMA_{28}$-CDTPA	9805	16200	13800	1.17
$PDMAEMA_{40}$: PEG_{113}-$PDMAEMA_{40}$-CDTPA	11692	17600	14600	1.21
$PDEAEMA_{16}$: PEG_{113}-$PDEAEMA_{16}$-CDTPA	8364	13100	11500	1.13
$PDEAEMA_{28}$: PEG_{113}-$PDEAEMA_{28}$-CDTPA	10584	13800	11300	1.22
$PDEAEMA_{40}$: PEG_{113}-$PDEAEMA_{40}$-CDTPA	12804	14000	11300	1.24

Note: PEG: polyethylene glycol **DMAEMA:** 2-(dimethylamino)ethyl methacrylate **DEAEMA:** 2-diethylaminoethyl methacrylate **CDTPA:** 4-cyano-4 (((dodecylthio) carbonothioyl) thio) pentanoic acid.

Table S2. The visual observation of LNPs stabilized by the developed copolymers at room temperature.

		$PDMAEMA_{16}$	**$PDMAEMA_{28}$**	**$PDMAEMA_{40}$**	**$PDEAEMA_{16}$**
MO-based LNPs	**0.4 mol%**	Milky +++	Milky +++	Milky +++	Milky +++
	0.8 mol%	Milky +++	Milky +++	Milky +++	Milky +++
	1.2 mol%	Milky +++	Milky +++	Milky +++	Milky +++
	1.5 mol%	Milky +++	Milky +++	Translucent milky +++	Translucent milky +++
	2.0 mol%	Milky +++	Milky +++	Translucent milky +++	Translucent milky +++
MO-based LNPs	**0.4 mol%**	Milky +++	Milky +++	Milky +++	Milky +++
	0.8 mol%	Milky +++	Milky +++	Milky +++	Milky +++
	1.2 mol%	Milky +++	Milky +++	Milky +++	Milky +++
	1.5 mol%	Milky +++	Milky +++	Milky +++	Milky +++
	2.0 mol%	Milky +++	Milky +++	Milky +++	Milky +++

+++: lipid nanoparticles are well dispersed without any lipid aggregates.

Table S3. The particle size (nm) and PDI of LNPs stabilized by the developed copolymers with various concentrations at room temperature.

		0.4 mol%	0.8 mol%	1.2 mol%	1.5 mol%	2.0 mol%
MO-PDMAEMA$_{16}$	**Particle size**	260±2	244±4	264±1	266±1	191±3
	PDI	0.22±0.01	0.19±0.04	0.15±0.01	0.19±0.01	0.22±0.03
MO-PDMAEMA$_{28}$	**Particle size**	281±1	247±4	268±6	207±6	139±2
	PDI	0.20±0.02	0.23±0.03	0.23±0.02	0.29±0.03	0.28±0.01
MO-PDMAEMA$_{40}$	**Particle size**	266±7	279±4	288±7	270±6	245±4
	PDI	0.21±0.03	0.26±0.03	0.16±0.03	0.22±0.03	0.23±0.01
MO-PDEAEMA$_{16}$	**Particle size**	308±4	304±4	328±2	301±4	321±3
	PDI	0.23±0.03	0.21±0.03	0.30±0.05	0.30±0.04	0.31±0.04
PT-PDMAEMA$_{16}$	**Particle size**	315±11	324±4	322±4	306±5	316±5
	PDI	0.21±0.01	0.19±0.05	0.19±0.05	0.17±0.02	0.12±0.06
PT-PDMAEMA$_{28}$	**Particle size**	306±6	350±5	356±11	359±64	355±5
	PDI	0.16±0.02	0.20±0.01	0.21±0.01	0.22±0.02	0.15±0.02
PT-PDMAEMA$_{40}$	**Particle size**	323±11	342±5	355±6	360±2	365±9
	PDI	0.18±0.01	0.26±0.02	0.21±0.02	0.23±0.01	0.20±0.05
PT-PDEAEMA$_{16}$	**Particle size**	308±9	322±8	379±9	381±10	401±36
	PDI	0.20±0.01	0.22±0.02	0.24±0.02	0.28±0.04	0.43±0.05

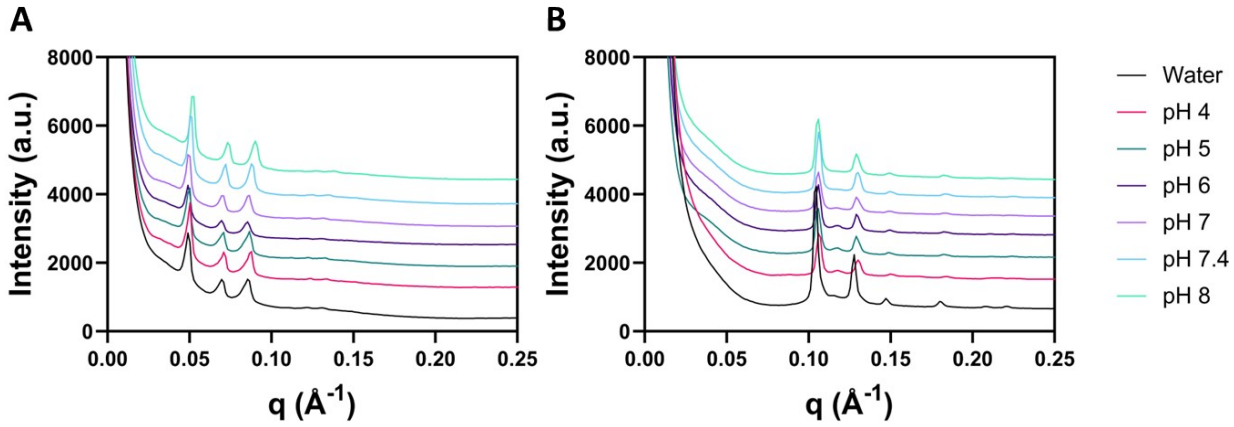

Figure S9. SAXS diffraction patterns of (A) MO-based LNPs stabilized by F127, and (B) PT-based LNPs stabilized by F127 in excess water and various pH conditions. All measurements were done at 37 ℃.

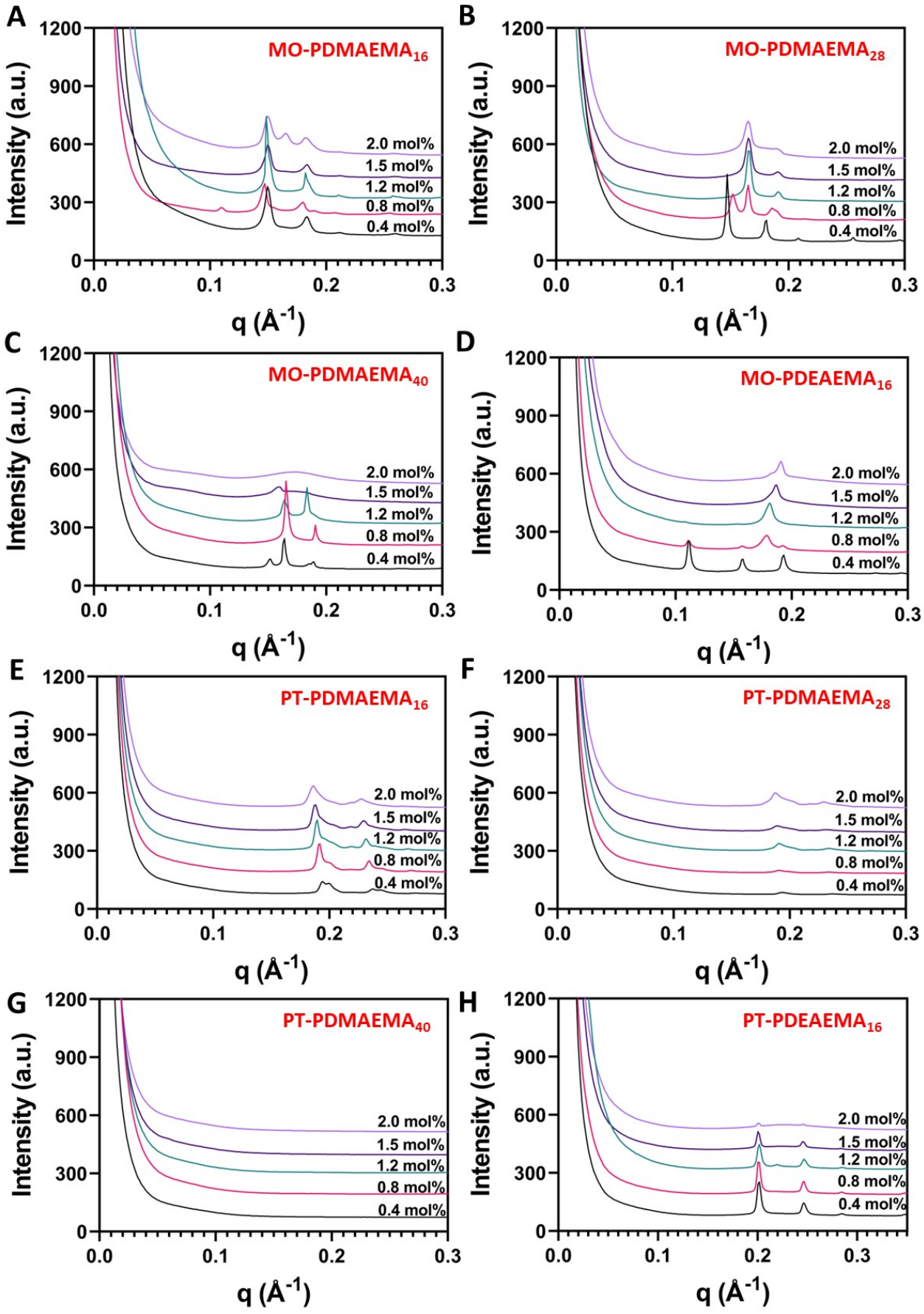
A
MO-PDMAEMA16
B
MO-PDMAEMA28
C
MO-PDMAEMA40
D
MO-PDEAEMA16
E
PT-PDMAEMA16
F
PT-PDMAEMA28
G
PT-PDMAEMA40
H
PT-PDEAEMA16
2.0 mol%
1.5 mol%
1.2 mol%
0.8 mol%
0.4 mol%
Intensity (a.u.)
q (Å-1)
0
300
600
900
1200
0.0
0.1
0.2
0.3

Figure S10. The stability study of the developed LNPs at day 7 after preparing them. SAXS diffraction patterns of MO based LNPs stabilized by the synthetic copolymer (A) $PDMAEMA_{16}$; (B) $PDMAEMA_{28}$; (C) $PDMAEMA_{40}$; (D) $PDEAEMA_{16}$; and PT-based LNPs stabilized by the synthetic copolymer (E) $PDMAEMA_{16}$; (F) $PDMAEMA_{28}$; (G) $PDMAEMA_{40}$ and (H) $PDEAEMA_{16}$ at various concentrations: 0.4, 0.8, 1.2, 1.5 and 2 mol% to MO or PT. All measurements were done at 37 °C.

Table S4. Mesophase identification and calculated lattice parameter (Å) of the MO/PT-based LNPs stabilized by the synthetic copolymers under different concentrations (at day 7 after preparation).

		$PDMAEMA_{16}$	**$PDMAEMA_{28}$**	**$PDMAEMA_{40}$**	**$PDEAEMA_{16}$**
MO-based LNPs	**0.4 mol%**	Pn3m; 60	Pn3m; 60	Pn3m; 58 Ia3d; 94	Im3m; 80
	0.8 mol%	Im3m; 82 Pn3m; 60	Pn3m; 58 Ia3d; 93	Ia3d; 93	Im3m; 80 H_2; 41
	1.2 mol%	Pn3m; 60	Ia3d; 93	Ia3d; 93 H_2; 54	Im3m; 83 H_2; 40
	1.5 mol%	Pn3m; 59	Ia3d; 93	H_2; 56 L_2	H_2; 39
	2.0 mol%	Pn3m; 59 H_2;44	Ia3d; 93	L_2	H_2; 38
MO-based LNPs	**0.4 mol%**	Pn3m; 46	Pn3m; 46	ND	Pn3m; 44
	0.8 mol%	Pn3m; 46	Pn3m; 47	ND	Pn3m; 44
	1.2 mol%	Pn3m; 47	Pn3m; 47	ND	Pn3m; 44
	1.5 mol%	Pn3m; 47	Pn3m; 47	ND	Pn3m; 44
	2.0 mol%	Pn3m; 48	Pn3m; 48	ND	Pn3m; 44 L_2

Note: Im3m refers to the primitive cubic phase with the symmetry group Im3m (Q_2^{Im3m}); Pn3m to the double-diamond cubic phase with the symmetry group Pn3m (Q_2^{Pn3m}); Ia3d to the inverse gyroid cubic phase with the symmetry group Ia3d (Q_2^{Ia3d}); H_2 to the inverse hexagonal phase; and L_2 to the inverse micellar phase. ND refers to no distinctive diffraction pattern detected.