

ELECTRONIC SUPPLEMENTARY INFORMATION

1.1. ¹H-NMR characterization of different hydrophobic modified pBAEs.

C32 polymer

¹H-NMR(400MHz, DMSO-*d*₆, TMS) (ppm): δ =6,32 (d, CH₂=CH-), 6,16 (d, CH₂=CH-), 5,94 (d, CH₂=CH-), 4.12 (br, CH₂-O-C(=O)-CH=CH₂), 4.01 (t, -CH₂-CH₂-O-), 3.35 (t, CH₂-CH₂-OH), 2.65 (br, -CH₂-CH₂-N-), 2.34 (br, -N-CH₂-CH₂-C(=O)-O), 1.63 (br, -O-CH₂-CH₂-CH₂-CH₂-O), 1.35 (br, -CH₂-CH₂-CH₂-CH₂-OH), 1.22 (br, N-(CH₂)₂-CH₂-(CH₂)₂-OH).

Table S1. Molar ratio of hexylamine/5-amino-1-pentanol of different C6 polymerizations.

| Polymer | Molar Ratio (Hexylamine/5-amino-1-pentanol) | Molar Ratio (Amine/Diacrylate) | 1,4- butanediol diacrylate | 5-amino-1- pentanol | Hexylamine |
|---------|--|-----------------------------------|----------------------------------|------------------------|------------|
| C6-100 | 1/0 | 1/1.1 | 9.1 | - | 8.3 |
| C6-50 | 0.5/0.5 | 1/1.1 | 9.1 | 4.1 | 4.1 |
| C6-25 | 0.25/0.75 | 1/1.1 | 9.1 | 6.2 | 2.1 |

C6-100 polymer

¹H-NMR(400MHz, Chloroform-*d*, TMS) (ppm): δ =6,45 (d, CH₂=CH-), 6,18 (d, CH₂=CH-), 5,88 (d, CH₂=CH-), 4.15 - 4.05 (br, CH₂-O-C(=O)-CH=CH₂), 3,99 (t, -CH₂-CH₂-O-), 2.62 (br, -CH₂-CH₂-N-), 2.32 (br, -N-CH₂-CH₂-C(=O)-O), 1.71 - 1.53 (br, -O-CH₂-CH₂-CH₂-CH₂-O), 1.36 - 1.12 (br, -CH₂-CH₂-CH₂-CH₂-OH, N-(CH₂)₂-CH₂-(CH₂)₂-OH), 0.83 (t, CH₂-CH₂-CH₃).

C6-50 polymer

¹H-NMR (400MHz, Chloroform-*d*, TMS) (ppm): δ =6,40 (d, CH₂=CH-), 6,10 (d, CH₂=CH-), 5,83 (d, CH₂=CH-), 4.18 (br, CH₂-O-C(=O)-CH=CH₂), 4,09 (t, -CH₂-CH₂-O-), 3.62 (t, CH₂-CH₂-OH), 2.78 (br, -CH₂-CH₂-N-), 2.45 (br, -N-CH₂-CH₂-C(=O)-O), 1.83 - 1.60 (br, -O-CH₂-CH₂-CH₂-CH₂-O), 1.40- 1.18 (br, -CH₂-CH₂-CH₂-CH₂-OH, N-(CH₂)₂-CH₂-(CH₂)₂-OH), 0.88 (t, CH₂-CH₂-CH₃).

C6-25 polymer

¹H-NMR(400MHz, Chloroform-*d*, TMS) (ppm): δ =6,40 (d, CH₂=CH-), 6,12 (d, CH₂=CH-), 5,83 (d, CH₂=CH-), 4.19 (br, CH₂-O-C(=O)-CH=CH₂), 4,09 (t, -CH₂-CH₂-O-), 3.61 (t, CH₂-CH₂-OH), 2.78 (br, -CH₂-CH₂-N-), 2.45 (br, -N-CH₂-CH₂-C(=O)-O), 1.83 - 1.60 (br, -O-CH₂-CH₂-CH₂-CH₂-O), 1.40- 1.18 (br, -CH₂-CH₂-CH₂-CH₂-OH, N-(CH₂)₂-CH₂-(CH₂)₂-OH), 0.88 (t, CH₂-CH₂-CH₃).

O), 1.80 – 1.57 (br, -O-CH₂-CH₂-CH₂-CH₂-O), 1.40- 1.18 (br, -CH₂-CH₂-CH₂-CH₂-OH, N-(CH₂)₂-CH₂-(CH₂)₂-OH), 0.88 (t, CH₂-CH₂-CH₃).

Table S2. Molar ratio of hexadecylamine/5-amino-1-pentanol of different C16 polymerizations.

| Polymer | Molar Ratio (Hexylamine/5-amino-1-pentanol) | Molar Ratio (Amine/Diacrylate) | 1,4-butanediol diacrylate | 5-amino-1-pentanol | Hexadecylamine |
|---------|--|-----------------------------------|------------------------------|--------------------|----------------|
| C16-50 | 0.5/0.5 | 1/1.1 | 9.1 | 4.1 | 4.1 |
| C16-25 | 0.25/0.75 | 1/1.1 | 9.1 | 6.2 | 2.1 |

C16-50 polymer

¹H NMR (400 MHz, Chloroform-*d*, TMS) (ppm): δ =6,40 (d, CH₂=CH-), 6,12 (d, CH₂=CH-), 5,83 (d, CH₂=CH-), 4.18 (br, CH₂-O-C(=O)-CH=CH₂), 4,09 (t, -CH₂-CH₂-O-), 3.61 (t, CH₂-CH₂-OH), 2.74 (br, -CH₂-CH₂-N-), 2.41 (br, -N-CH₂-CH₂-C(=O)-O), 1.88- 1.15 (m, -CH₂-CH₂-CH₂-CH₂-OH, N-(CH₂)₂-CH₂-(CH₂)₂-OH, -O-CH₂-CH₂-CH₂-CH₂-O, -CH₂-(CH₂)₁₄-CH₃), 0.85 (t, CH₂-CH₂-CH₃).

C16-25 polymer

¹H NMR (400 MHz, Chloroform-*d*, TMS) (ppm): δ =6,40 (d, CH₂=CH-), 6,12 (d, CH₂=CH-), 5,83 (d, CH₂=CH-), 4.18 (br, CH₂-O-C(=O)-CH=CH₂), 4,10 (t, -CH₂-CH₂-O-), 3.62 (t, CH₂-CH₂-OH), 2.76 (br, -CH₂-CH₂-N-), 2.41 (br, -N-CH₂-CH₂-C(=O)-O), 1.79- 1.18 (m, -CH₂-CH₂-CH₂-CH₂-OH, N-(CH₂)₂-CH₂-(CH₂)₂-OH, -O-CH₂-CH₂-CH₂-CH₂-O, -CH₂-(CH₂)₁₄-CH₃), 0.83 (t, CH₂-CH₂-CH₃).

Table S3. Different percentage of C32 polymer esterification using Cholesterol-COOH

| Polymer | C32 (mmol) | Cholesterol-COOH (mmol) | C32 (g) | Cholesterol-COOH (g) |
|------------|------------|----------------------------|---------|----------------------|
| Cchol-50 | 0.14 | 0.49 | 0.3 | 0.242 |
| Cchol-25 | 0.14 | 0.25 | 0.3 | 0.121 |
| Cchol-12,5 | 0.14 | 0.12 | 0.3 | 0.061 |

Cchol-50 polymer

¹H-NMR (400MHz, Chloroform-*d*, TMS) (ppm): δ =6,40 (d, CH₂=CH-), 6,12 (d, CH₂=CH-), 5,83 (d, CH₂=CH-), 5.37(s, -C=CH-C-), 4.69 – 4.55 (m, -O-CH(-C)-₂) 4.19 (br, CH₂-O-C(=O)-CH=CH₂), 4,09 (t, -CH₂-CH₂-O-), 3.62 (t, CH₂-CH₂-OH),

2.76 (br, -CH₂-CH₂-N-), 2.60 (t, -O-C(=O)-CH₂-CH₂-C(=O)-O), 2.42 (br, -N-CH₂-CH₂-C(=O)-O), 2.36 – 2.24, (m, from cholesterol), 2.09- 0.94 (m, - CH₂-CH₂-CH₂-CH₂-OH, -O-CH₂-CH₂-CH₂-CH₂-O, N-(CH₂)₂-CH₂-(CH₂)₂-OH, from cholesterol), 0.86 (dd, from cholesterol), 0.67 (s, -CH-(CH₂)₂).

Cchol-25 polymer

¹H NMR (400 MHz, Chloroform-d, TMS) (ppm): δ =6,40 (d, CH₂=CH-), 6,12 (d, CH₂=CH-), 5,83 (d, CH₂=CH-), 5.37(s, -C=CH-C-), 4.72 – 4.51 (m, -O-CH(-C-)₂) 4.19 (br, CH₂-O-C(=O)-CH=CH₂), 4,09 (t, -CH₂-CH₂-O-), 3.62 (t, CH₂-CH₂-OH), 2.76 (br, -CH₂-CH₂-N-), 2.60 (t, -O-C(=O)-CH₂-CH₂-C(=O)-O), 2.43 (br, -N-CH₂-CH₂-C(=O)-O), 2.35 – 2.22, (m, from cholesterol), 2.11- 0.89 (m, - CH₂-CH₂-CH₂-CH₂-OH, -O-CH₂-CH₂-CH₂-CH₂-O, N-(CH₂)₂-CH₂-(CH₂)₂-OH, from cholesterol), 0.86 (dd, from cholesterol), 0.67 (s, -CH-(CH₂)₂).

Cchol-12.5 polymer

¹H NMR (400 MHz, Chloroform-d, TMS) (ppm): δ =6,41 (d, CH₂=CH-), 6,12 (d, CH₂=CH-), 5,83 (d, CH₂=CH-), 5.37(s, -C=CH-C-), 4.66 – 4.55 (m, -O-CH(-C-)₂) 4.18 (br, CH₂-O-C(=O)-CH=CH₂), 4,09 (t, -CH₂-CH₂-O-), 3.61 (t, CH₂-CH₂-OH), 2.76 (br, -CH₂-CH₂-N-), 2.60 (t, -O-C(=O)-CH₂-CH₂-C(=O)-O), 2.43 (br, -N-CH₂-CH₂-C(=O)-O), 2.31, (m, from cholesterol), 2.09- 0.89 (m, - CH₂-CH₂-CH₂-CH₂-OH, -O-CH₂-CH₂-CH₂-CH₂-O, N-(CH₂)₂-CH₂-(CH₂)₂-OH, from cholesterol), 0.86 (dd, from cholesterol), 0.67 (s, -CH-(CH₂)₂).

1.2. ¹H-RMN characterization of oligopeptide-modified pBAE polymers

CR3-C6-CR3

¹H-NMR(400MHz, Methanol-*d*₄, TMS) (ppm): δ = 4.41-4.33 (br, NH₂-C(=O)-CH-NH-C(=O)-CH-NH-C(=O)-CH-NH-C(=O)-CH-CH₂-), 4.11 (t, CH₂-CH₂-O), 3.55 (t, CH₂-CH₂-OH), 3.22 (br, NH₂-C(=NH)-NH-CH₂-), OH-(CH₂)₄-CH₂-N-), 3.04 (t, CH₂-CH₂-N-), 2.82 (dd, -CH₂-S-CH₂), 2.48 (br, -N-CH₂-CH₂-C(=O)-O), 1.90 (m, NH₂-C(=NH)-NH-(CH₂)₂-CH₂-CH-), 1.73 (br, -O-CH₂-CH₂-CH₂-CH₂-O), 1.69 (m, NH₂-C(=NH)-NH-CH₂-CH₂-CH₂-), 1.56 (br, -CH₂-CH₂-CH₂-CH₂-OH), 1.39 (br, -N-(CH₂)₂-CH₂-(CH₂)₂-OH), 0.88 (t, CH₂-CH₂-CH₃).

CK3-C6-CK3

¹H-NMR(400MHz, Methanol-*d*₄, TMS) (ppm): δ = 4.38-4.29 (br, NH₂-(CH₂)₄-CH-), 4.13 (t, CH₂-CH₂-O-), 3.73 (br, NH₂-CH-CH₂-S-), 3.55 (t, CH₂-CH₂-OH), 2.94 (br, CH₂-CH₂-N-, NH₂-CH₂-(CH₂)₃-CH-), 2.81 (dd, -CH₂-S-CH₂), 2.57 (br, -N-CH₂-

$\text{CH}_2\text{-C(=O)-O}$), 1.85 (m, $\text{NH}_2\text{-(CH}_2\text{)}_3\text{-CH}_2\text{-CH-}$), 1.74 (br, $-\text{O-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-O}$), 1.68 (m, $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-(CH}_2\text{)}_2\text{-CH-}$), 1.54 (br, $-\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-OH}$), 1.37 (br, $\text{N-(CH}_2\text{)}_2\text{-CH}_2\text{-(CH}_2\text{)}_2\text{-OH}$), 0.88 (t, $\text{CH}_2\text{-CH}_2\text{-CH}_3$).

CH3-C6-CH3

$^1\text{H-NMR}$ (400MHz, Methanol- d_4 , TMS) (ppm): $\delta = 8.0\text{-}7.0$ (br $-\text{N(=CH)-NH-C(=CH)-}$), 4.61-4.36 (br, $-\text{CH}_2\text{-CH-}$), 4.16 (t, $\text{CH}_2\text{-CH}_2\text{-O-}$), 3.55 (t, $\text{CH}_2\text{-CH}_2\text{-OH}$), 3.18 (t, $\text{CH}_2\text{-CH}_2\text{-N-}$), 3.06 (dd, $-\text{CH}_2\text{-CH-}$), 2.88 (br, $\text{OH-(CH}_2\text{)}_4\text{-CH}_2\text{-N-}$), 2.82 (dd, $-\text{CH}_2\text{-S-CH}_2\text{-}$), 2.72 (br, $-\text{N-CH}_2\text{-CH}_2\text{-C(=O)-O}$), 1.75 (br, $-\text{O-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-O}$), 1.65 (m, $\text{NH}_2\text{-CH}_2\text{-CH}_2\text{-(CH}_2\text{)}_2\text{-CH-}$), 1.58 (br, $-\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-OH}$), 1.40 (br, $\text{N-(CH}_2\text{)}_2\text{-CH}_2\text{-(CH}_2\text{)}_2\text{-OH}$), 0.88 (t, $\text{CH}_2\text{-CH}_2\text{-CH}_3$).

CD3-C6-CD3

$^1\text{H-NMR}$ (400MHz, DMSO- d_6 , TMS) (ppm): $\delta = 4.56\text{-}4.44$ (br, $\text{NH}_2\text{-C(=O)-CH-NH-C(=O)-CH-NH-C(=O)-CH-NH-C(=O)-CH-CH}_2\text{-}$), 4.19 (br, $\text{NH}_2\text{-CH-CH}_2\text{-S}$), 4.00 (t, $\text{CH}_2\text{-CH}_2\text{-O}$), 3.34 (t, $\text{CH}_2\text{-CH}_2\text{-OH}$), 2.98 (dd, $-\text{CH}_2\text{-S-CH}_2\text{-}$), 2.57-2.48 (t, $-\text{CH-CH}_2\text{-COO-}$), 2.40 (br, $-\text{N-CH}_2\text{-CH}_2\text{-C(=O)-O}$), 1.60 (br, $-\text{O-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-O}$), 1.38 (br, $-\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-OH}$), 1.20 (br, $-\text{N-(CH}_2\text{)}_2\text{-CH}_2\text{-(CH}_2\text{)}_2\text{-OH}$), 0.88 (t, $\text{CH}_2\text{-CH}_2\text{-CH}_3$).

CR3-Cchol-50-CR3

$^1\text{H NMR}$ (400 MHz, Methanol- d_4 , TMS): $\delta = 5.40$ (s, $-\text{C=CH-C-}$), 4.54 – 4.33 (m, $-\text{O-CH(-C-)}_2$, $\text{NH}_2\text{-C(=O)-CH-NH-C(=O)-CH-NH-C(=O)-CH-NH-C(=O)-CH-CH}_2\text{-}$), 4.29-4.09 (br, $\text{CH}_2\text{-O-C(=O)-CH=CH}_2$), 4.09 (t, $-\text{CH}_2\text{-CH}_2\text{-O-}$), 3.60 (t, $\text{CH}_2\text{-CH}_2\text{-OH}$), 3.40-2.76 (m, $-\text{CH}_2\text{-CH}_2\text{-N-}$, $\text{NH}_2\text{-C(=NH)-NH-CH}_2\text{-}$, $-\text{CH}_2\text{-S-CH}_2\text{-}$, from cholesterol and peptide), 2.63 (t, $-\text{O-C(=O)-CH}_2\text{-CH}_2\text{-C(=O)-O}$), 2.38 – 2.25, (m, from cholesterol), 2.12- 0.99 (m, $-\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-OH}$, $-\text{O-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-O}$, $\text{N-(CH}_2\text{)}_2\text{-CH}_2\text{-(CH}_2\text{)}_2\text{-OH}$, from cholesterol and peptide), 0.90 (dd, from cholesterol), 0.74 (s, $-\text{CH-(CH}_2\text{)}_2$).

CK3-Chol-50-CK3

$^1\text{H NMR}$ (400 MHz, Methanol- d_4 , TMS): $\delta = 5.40$ (s, $-\text{C=CH-C-}$), 4.42 – 4.30 (m, $-\text{O-CH(-C-)}_2$, from peptide), 4.25-4.05 (m, $\text{CH}_2\text{-O-C(=O)-CH=CH}_2$, $-\text{CH}_2\text{-CH}_2\text{-O-}$), 3.60 (t, $\text{CH}_2\text{-CH}_2\text{-OH}$), 3.55-2.75 (m, $-\text{CH}_2\text{-S-CH}_2\text{-}$, $\text{CH}_2\text{-CH}_2\text{-N-}$, $\text{NH}_2\text{-CH}_2\text{-(CH}_2\text{)}_3\text{-CH-}$, from cholesterol and peptide), 2.62 (t, $-\text{O-C(=O)-CH}_2\text{-CH}_2\text{-C(=O)-O}$), 2.36 – 2.24, (m, from cholesterol), 2.10- 0.99 (m, $-\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-OH}$, $-\text{O-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-O}$, $\text{N-(CH}_2\text{)}_2\text{-CH}_2\text{-(CH}_2\text{)}_2\text{-OH}$, from cholesterol and peptide), 0.90 (dd, from cholesterol), 0.74 (s, $-\text{CH-(CH}_2\text{)}_2$).

CH3-Cchol-CH3

$^1\text{H NMR}$ (400 MHz, Methanol- d_4 , TMS): $\delta = 8.6\text{-}7.0$ (m $-\text{N(=CH)-NH-C(=CH)-}$), 5.41(s, $-\text{C=CH-C-}$), 4.75 – 4.49 (m, $-\text{O-CH(-C-)}_2$, from peptide), 4.20-4.08 (m, $\text{CH}_2\text{-O-C(=O)-CH=CH}_2$, $-\text{CH}_2\text{-CH}_2\text{-O-}$), 3.60 (t, $\text{CH}_2\text{-CH}_2\text{-OH}$), 3.55-2.70 (m, $-\text{CH}_2\text{-S-CH}_2\text{-}$,

CH₂-CH₂-N-, from cholesterol and peptide), 2.62 (t, -O-C(=O)-CH₂-CH₂-C(=O)-O), 2.32, (m, from cholesterol), 2.19-0.93 (m, -CH₂-CH₂-CH₂-CH₂-OH, -O-CH₂-CH₂-CH₂-CH₂-O, N-(CH₂)₂-CH₂-(CH₂)₂-OH, from cholesterol), 0.93-0.86 (dd, from cholesterol), 0.74 (s, -CH-(CH₂)₂).

CD3-Cchol-CD3

¹H NMR (400 MHz, DMSO-*d*₆, TMS): δ = 5.33(s, -C=CH-C-), 4.42 – 4.05 (m, -O-CH(-C-)₂, CH₂-O-C(=O)-CH=CH₂, -CH₂-CH₂-O-, from peptide), 3.63 (t, CH₂-CH₂-OH), 3.51-2.63 (m, -CH₂-S-CH₂, CH₂-CH₂-N-, -O-C(=O)-CH₂-CH₂-C(=O)-O, from cholesterol and peptide), 2.28 – 2.19, (m, from cholesterol), 2.05- 0.89 (m, -CH₂-CH₂-CH₂-CH₂-OH, -O-CH₂-CH₂-CH₂-CH₂-O, N-(CH₂)₂-CH₂-(CH₂)₂-OH, from cholesterol), 0.84 (dd, from cholesterol), 0.65 (s, -CH-(CH₂)₂).

CE3-Cchol-CE3

¹H NMR (400 MHz, DMSO-*d*₆, TMS): ¹H NMR (400 MHz, DMSO-*d*₆, TMS): δ = 5.33(s, -C=CH-C-), 4.44 – 4.06 (m, -O-CH(-C-)₂, CH₂-O-C(=O)-CH=CH₂, -CH₂-CH₂-O-, from peptide), 3.63 (t, CH₂-CH₂-OH), 3.50-2.62 (m, -CH₂-S-CH₂, CH₂-CH₂-N-, -O-C(=O)-CH₂-CH₂-C(=O)-O, from cholesterol and peptide), 2.27 – 2.18, (m, from cholesterol), 2.05- 0.89 (m, -CH₂-CH₂-CH₂-CH₂-OH, -O-CH₂-CH₂-CH₂-CH₂-O, N-(CH₂)₂-CH₂-(CH₂)₂-OH, from cholesterol), 0.84 (dd, from cholesterol), 0.65 (s, -CH-(CH₂)₂).

1.3. Biophysical characterization: Gel Retardation Assay

Polymer - nucleic acid binding capability was analyzed by gel retardation assay working at different polymer/nucleic acid weight ratios, ranging from 10:1 to 400:1. Polymers obtained by stoichiometric ratios of hydrophobic/hydrophilic amine and further end-modified with arginine were analyzed by gel retardation assay as a representative formulation of each hydrophobic modifications. Moreover, previously described C32-CR3 polymer was used as a control (Supplementary Figure 1).

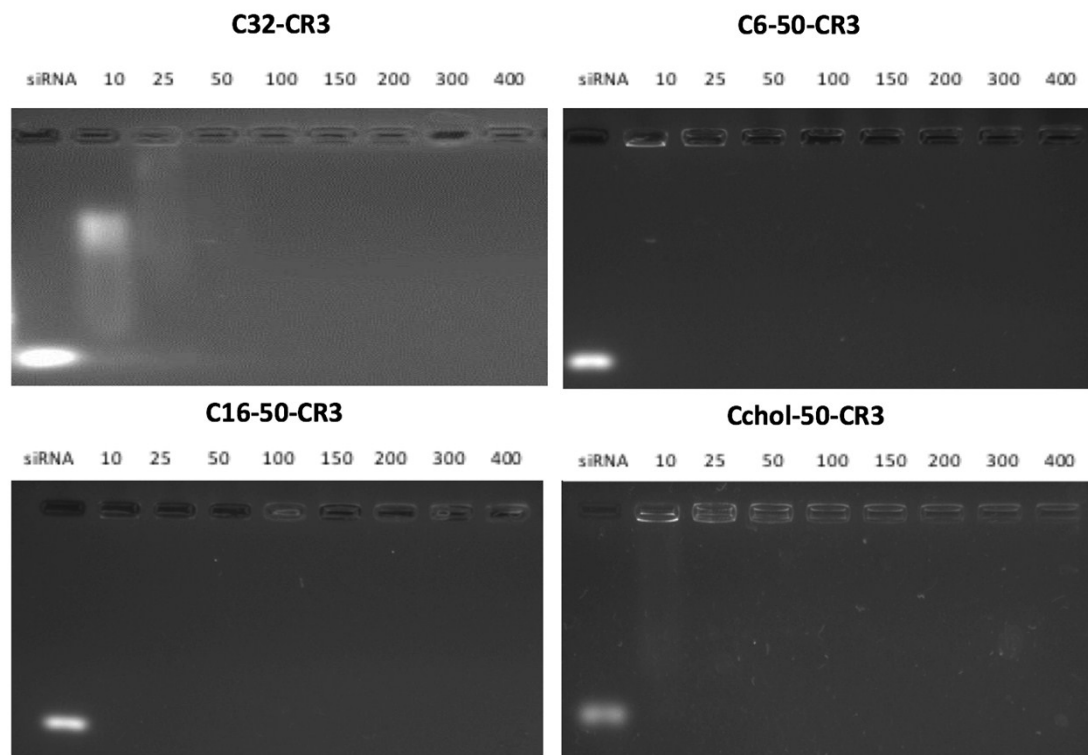


Figure S1. Agarose gel retardation assay of newly hydrophobic synthesized PBAES using siGFP siRNA at various weight ratios. Polymer:siRNA complexes were prepared at different ratios (w/w) ranging from 1:10 to 1:400. An intermediate hydrophobicity range, stequiometry hydrophobic/hydrophilic ratio, of different alkyl side chains was selected. Polymers were compared with previously well characterized C32 polymer. Polyplexes were freshly prepared prior the assay and agarose gel containing ethidium bromide loaded into 2,5% and was run 1 h at 80 V.

1.4. Oligopeptide-end modification of C6-50 and Cchol-50 polymers

C6-50 and Cchol-50 polymers were further modified using positive and negative oligopeptides and biophysically characterized by DLS.

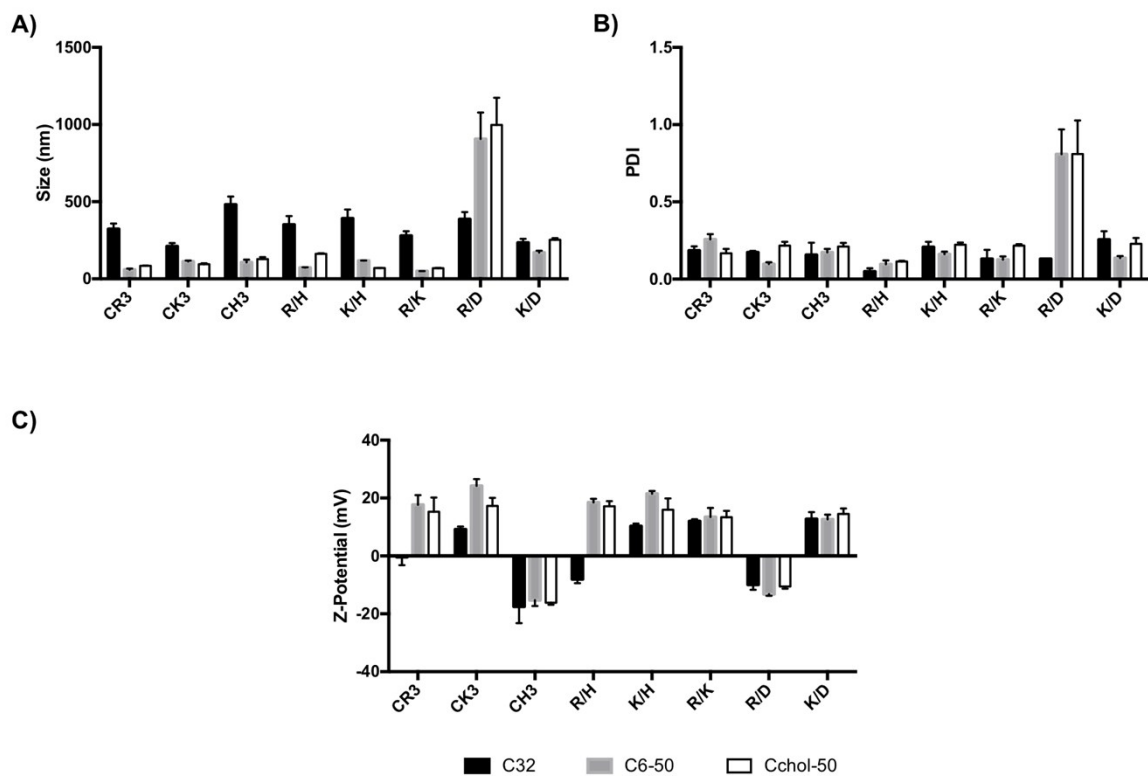


Figure S2. Biophysical characterization of oligopeptide end-modified C6-50 and Cchol-50 polymers. A) Average size, B) polydispersity, C) and zeta potential were determined by dynamic light scattering (DLS). Polyplexes were prepared using siRNA and different end-modified pBAE C6-50 and Cchol-50 polymers at 100:1 w/w ratio. 200:1 w/w ratio was used for C32 polymer. Results are shown as mean and standard deviation of triplicates.