Supplementary Material for Journal of Materials Chemistry

Influence of block sequences in polymer vectors for gene transfection *in vitro* and toxicity assessment of zebrafish embryos *in vivo*

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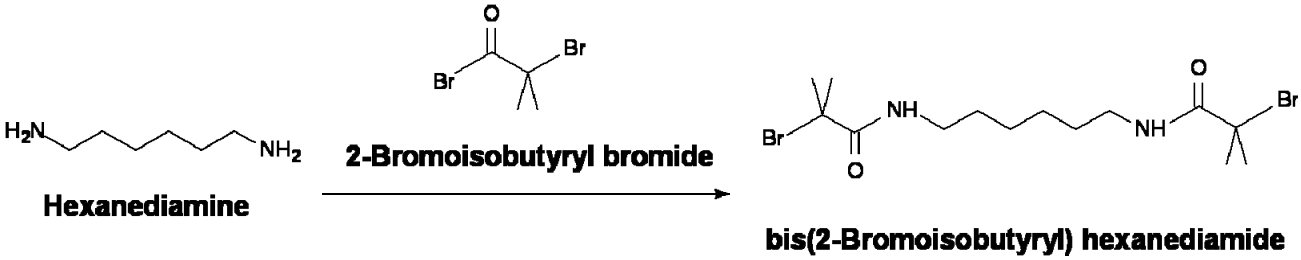
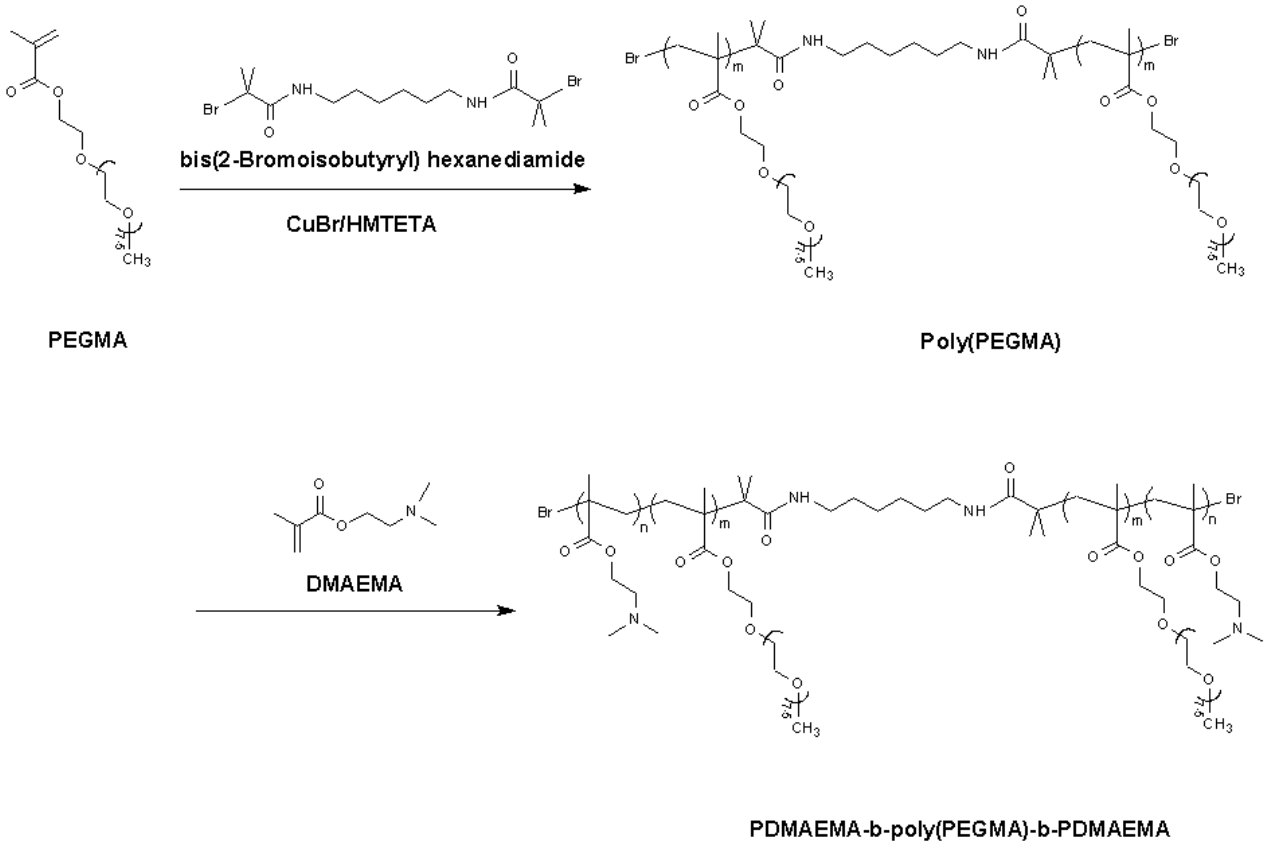
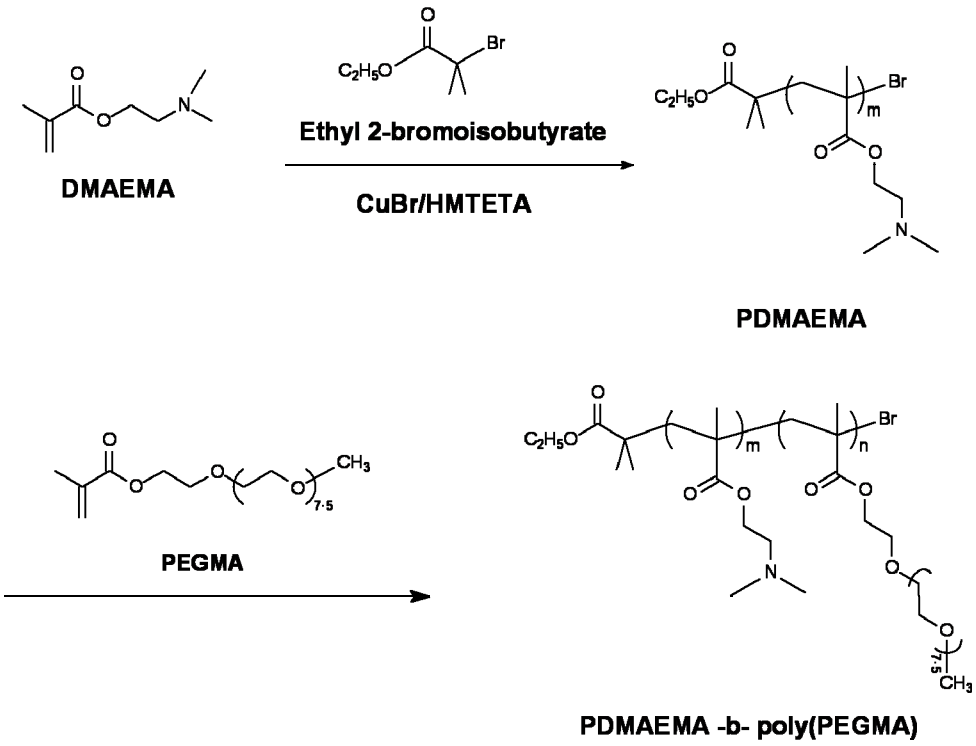


Scheme S1 Synthesis of initiator bis(2-bromoisobutyryl) hexanediamide (BiBH).



Scheme S2 Synthetic route for DED.



Scheme S3 Synthetic route for DE.

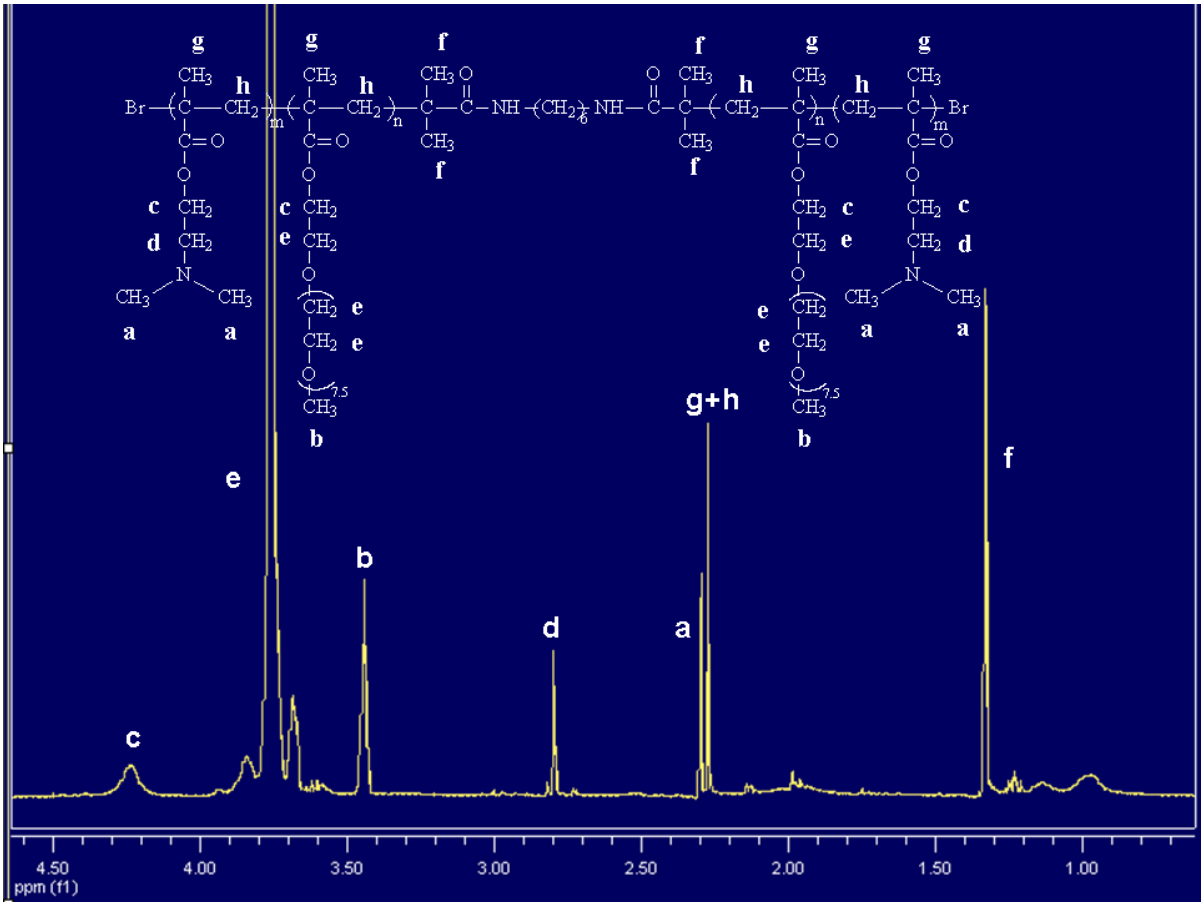


Fig S1 NMR spectrum of block copolymer DED.

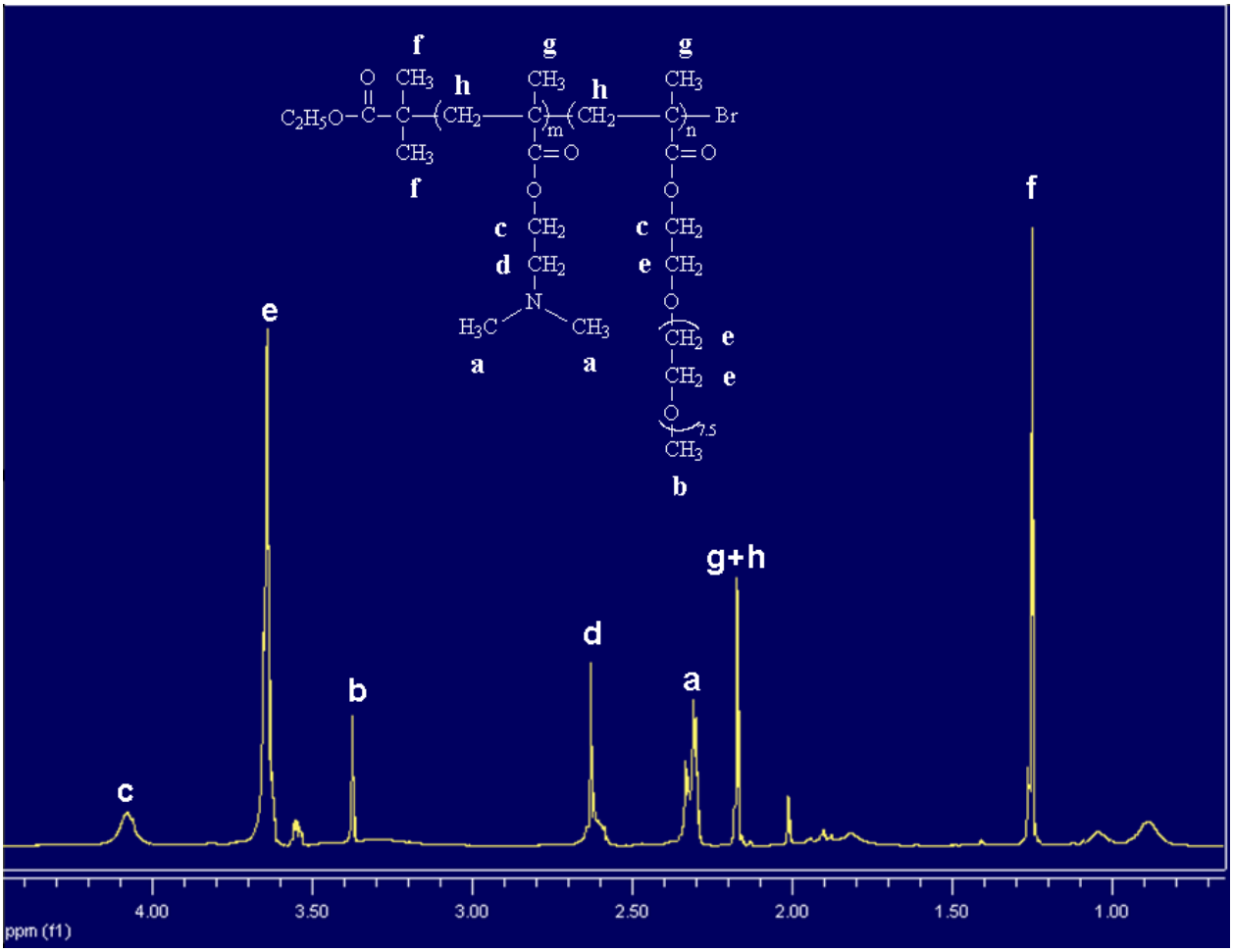


Fig S2 NMR spectrum of block copolymer DE.

GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Polydispersity
1		47113	64108	64144	84798	109115	1.360713

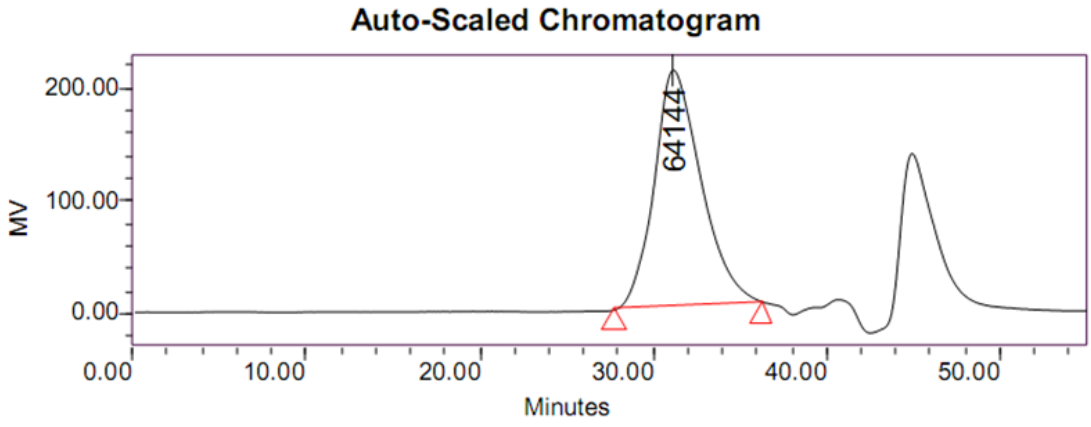


Fig S3 Gel permeation chromatogram (GPC) of block copolymer DED.

GPC Results

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Polydispersity
1		46730	65225	57742	89841	119306	1.395772

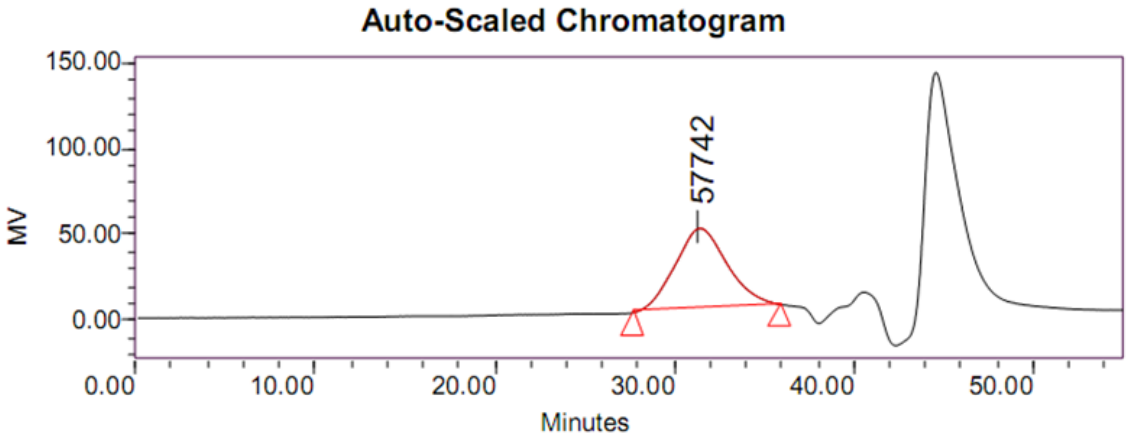


Fig S4 GPC curve of block copolymer DE.

Ethidium bromide displacement assay

The ability of the copolymers to condense DNA was studied using an EB displacement assay. Copolymer solution was added stepwise to plasmid pEGFP-C2 DNA solution (10 μg/mL) in PBS buffer containing EB (2 μg/mL). After each step, fluorescence intensity of the solution was monitored using a Hitachi F-4500 fluorescence spectrophotometer (Hitachi Scientific Instruments, Finchampstead, UK) at excitation and emission wavelengths of 510 nm and 590 nm, respectively, with slit widths set at 5 nm. The fluorescence intensity of the EB solution in the presence of free plasmid DNA corresponded to the maximum, whereas the fluorescence intensity of EB alone was used to correct for the minimal background fluorescence, according to the equation:

$$RF = 100 \times \frac{(F_P - F_{EB})}{(F_{DNA} - F_{EB})}$$

Where RF is the relative fluorescence intensity, F_P is the fluorescence intensity measured after addition of block copolymer. F_{DNA} and F_{EB} are the fluorescence intensities of DNA+EB and pure EB solutions respectively.

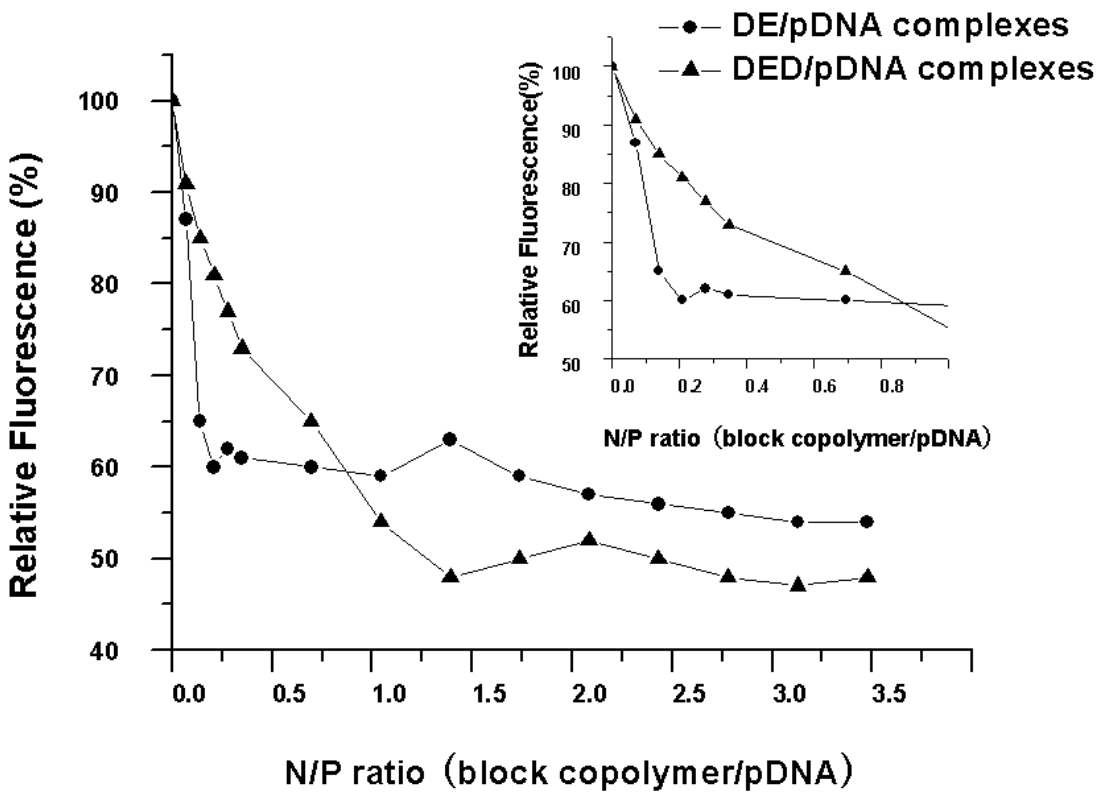


Fig S5 Fluorescence of ethidium bromide (EB) as a function of the N/P ration of the copolymers DE and DED. The fluorescent intensity decreases due to the competitive binding of DE and DED to pDNA in PBS buffer at room temperature, excitation 510 nm, emission 590 nm. Inset shows the data with an expanded x-axis.