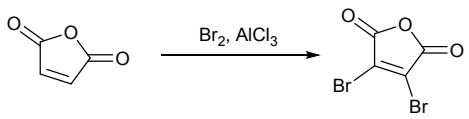
Electronic Supplementary Material (ESI) for Biomaterials Science.

Reducible, Dibromomaleimide-Linked Polymers for Gene Delivery

James-Kevin Y. Tan, Jennifer Choi, Hua Wei, Joan G. Schellinger, Suzie H. Pun
Department of Bioengineering, Molecular Engineering and Sciences, University of Washington

Supporting Information

Synthesis of Dibromomaleic Anhydride (3,4-Dibromofuran-2,5-dione)



Briefly, maleic anhydride (0.4 g, 4.1 mmol), aluminum (III) chloride (8.2 mg, 0.06 mmol), and bromine (0.42 mL, 8.2 mmol) were added to a pressure tube and heated at 120 °C for 16 hours. The tube was cooled and 20 mL of ethyl acetate was added. The solution was filtered and the solvent was azeotropically removed with chloroform under reduced pressure. The crude solution was redissolved in 20 mL of chloroform, washed twice with 20 mL of water, and dried with anhydrous sodium sulfate. The solution was filtered and the solvent was removed to yield a white powder (0.69 g, 64%). The product was analyzed by GC-MS on a Hewlett-Packard (Palo Alto, CA) 5971A GC-MSD.

δ_C (500 MHz; $CDCl_3$): 158.70 (2 C), 131.54 (2 C)
m/z: 258 (19%), 256 (M^+, 37), 254 (19), 214 (13), 212 (26), 210 (13), 186 (9), 184 (18), 182 (9), 133 (98), 131 (100); fragmentation follows bromine pattern

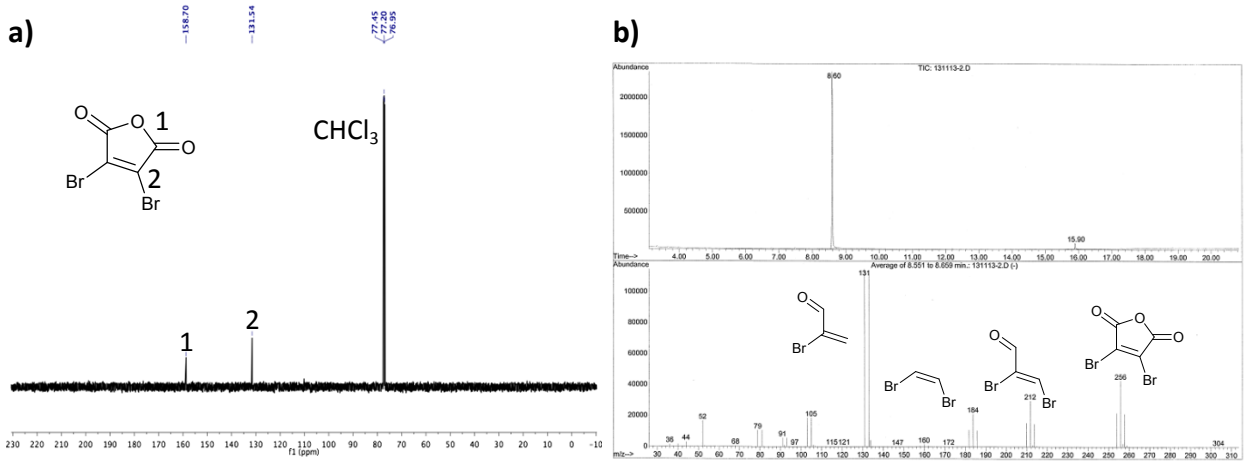
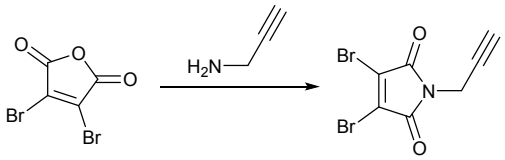


Fig. S1 Characterization of dibromomaleic anhydride by (a) ^{12}C NMR in $CHCl_3$ and (b) GC-MS in ACN.

Synthesis of Dibromomaleimide-alkyne (3,4-Dibromo-1-(prop-2-ynyl)-1H-pyrrole-2,5-dione)



m/z: 295 (9%), 293 (M^+, 18), 291 (9), 186 (95), 184 (100), 133 (51), 131 (52); fragmentation follows bromine pattern

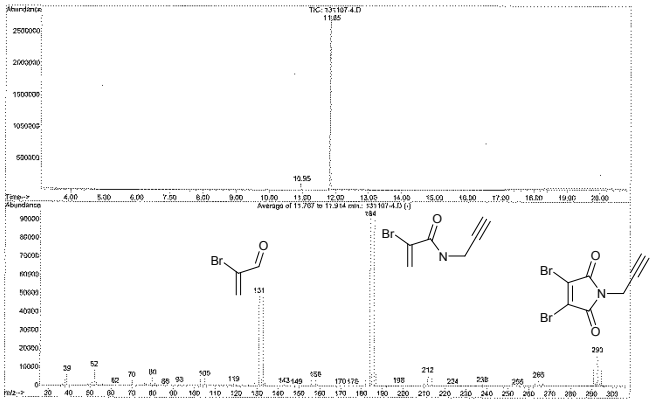
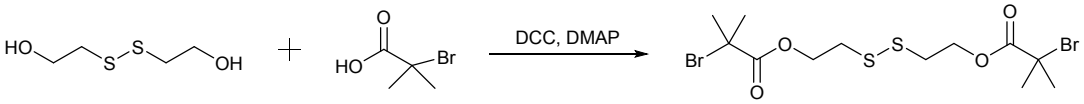


Fig. S2 Characterization of dibromomaleimide-alkyne by GC-MS in ACN.

Synthesis of double-headed ATRP initiator



2-hydroxyethyl disulfide (330 µL, 2.7 mmol), N,N'-dicyclohexyl carbodiimide (DCC) (2.5 g, 11.9 mmol), and 4-dimethylaminopyridine (DMAP) (0.10 g, 1 mmol) were dissolved in 25 mL of DCM and added dropwise to 2-bromo-2-methylpropionic acid (1.8 g, 10.8 mmol) dissolved in 25 mL of DCM in an ice bath with stirring. After all of the solution was added, the ice bath was removed and the solution was stirred for 30 more minutes. Afterwards, the solution was vacuum filtered and removed under reduced pressure. The crude product was isolated by a silica gel column (1:7 ethyl acetate:hexane) to yield the product as a semi-crystalline gel. ^{1}H NMR of the product was recorded on a Bruker (Billerica, MA) AV-500 MHz instrument.

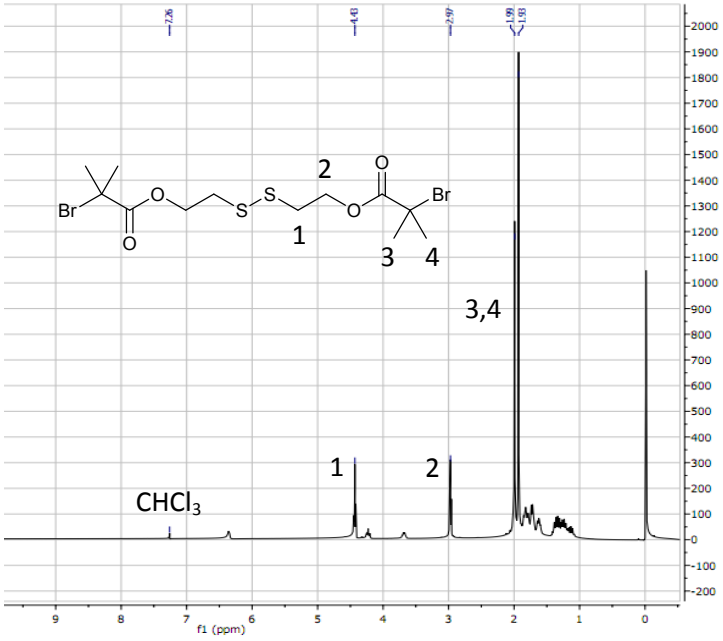
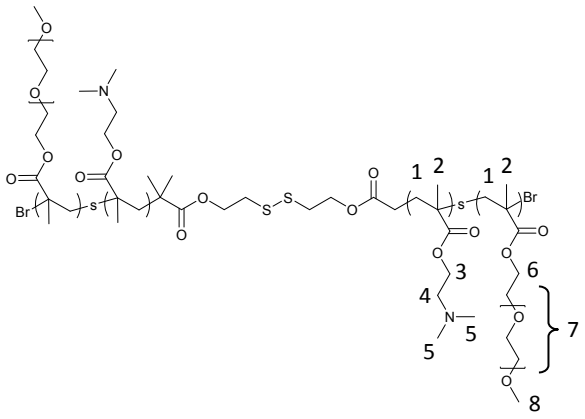


Fig. S3 ^{1}H NMR characterization of double-headed ATRP initiator in $CDCl_3$.

ATRP copolymerization of DMAEMA and OEGMA



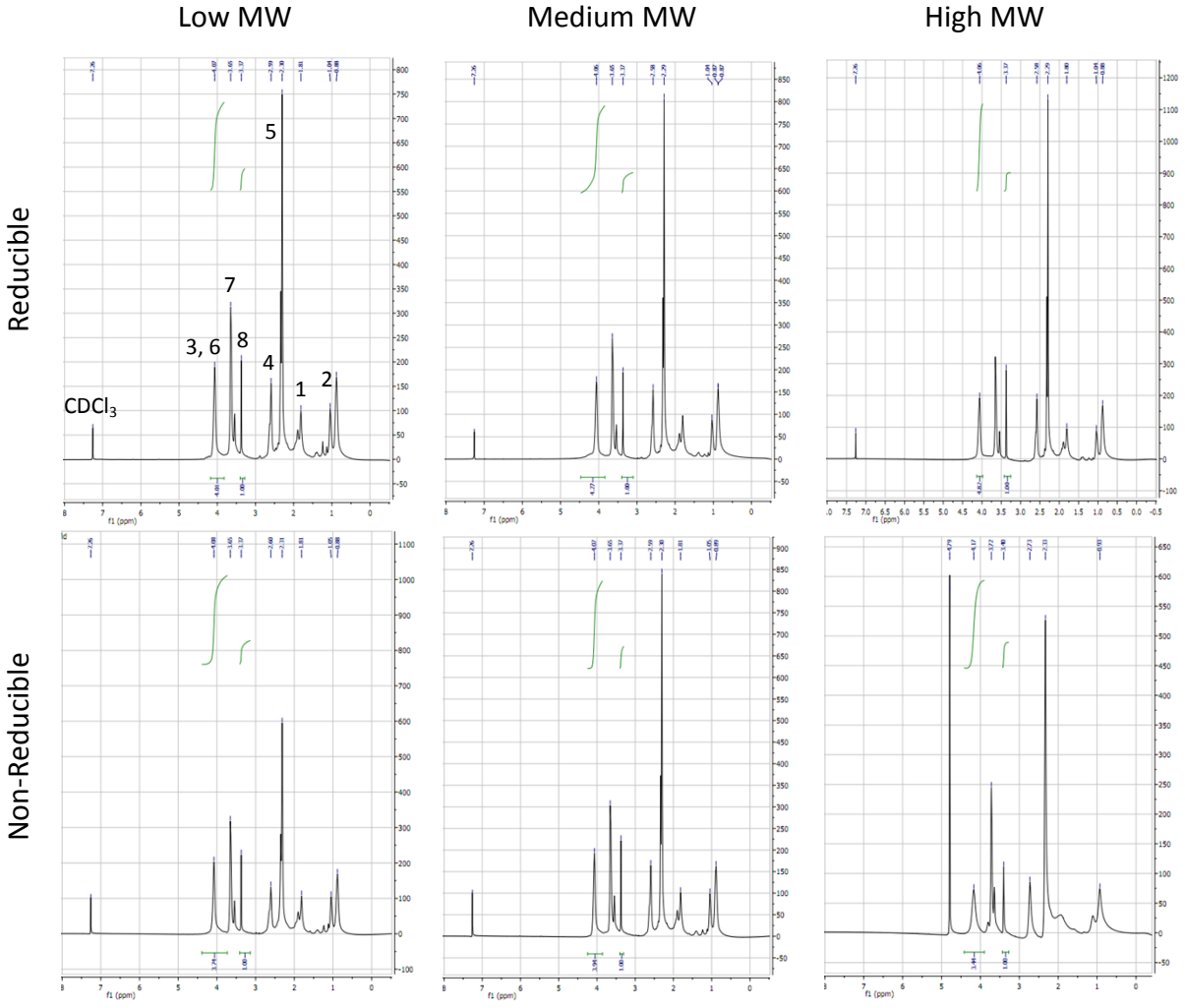


Fig. S4 ^{1}H NMR characterization of reducible, double-headed and non-reducible DMAEMA and OEGMA copolymers in $CDCl_3$. The ratio of DMAEMA to OEGMA was determined by comparing the ester methylene peaks of DMAEMA and OEGMA (3.9-4.2 ppm, peaks 3 & 6, 2 H's each) to the methoxy peak of OEGMA (3.3-3.4 ppm, peak 8, 3 H's) and the following formula: $\frac{peak\ f}{Peak\ b+d} = \frac{3x}{2x+2y}$, where "x" is number of OEGMA units and "y" is number of DMAEMA units.

DNA condensation by agarose gel retardation

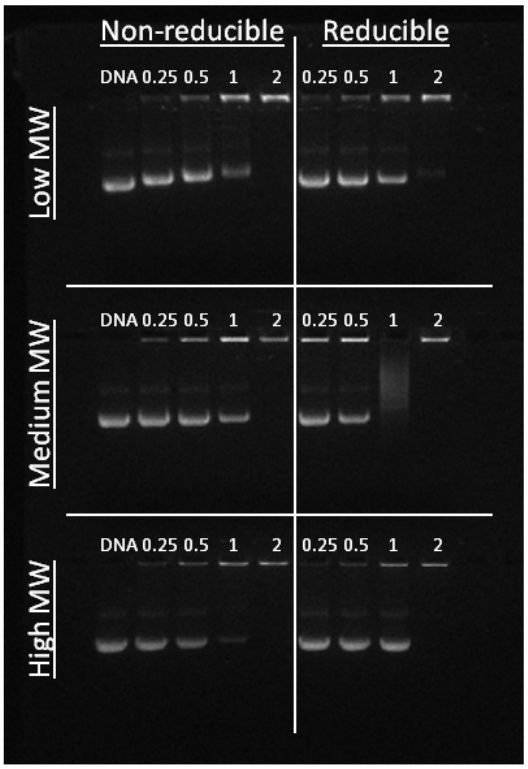


Fig. S5 Gel retardation assay for all polyplexes formulated at N/P ratios of 0.25, 0.5, 1, and 2 as labeled above. A moving band indicates incomplete complexation while an unmoved band indicates complete complexation with DNA. All polymers are able to fully complex DNA at N/P = 2.

Zeta Potential of Polyplexes

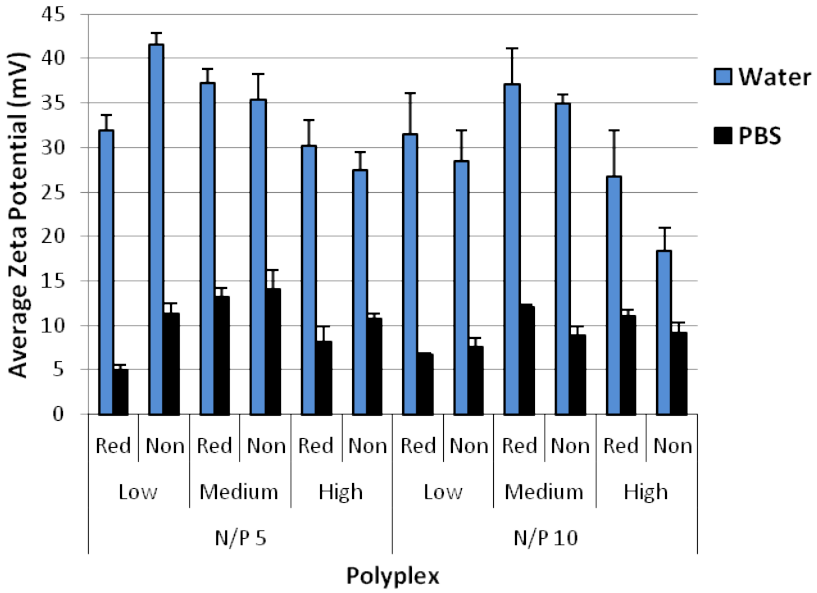


Fig. S6 Zeta potential of polyplexes in water and 10 mM PBS. Data is presented as mean ± SD, n = 3.

Polyplex Unpackaging

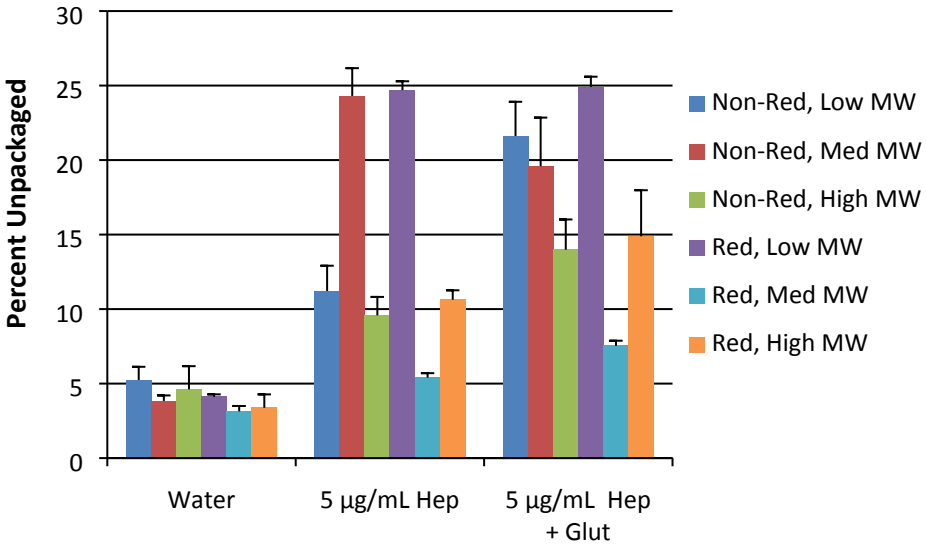


Fig. S7 Polyplex unpackaging in heparin sulfate and glutathione. Data is presented as mean ± SD, n = 3.