**Electronic Supporting Information**

# Rapid formulation of redox-responsive oligo-β–aminoester polyplexes with siRNA via jet printing

Tatiana Lovato[a†], Vincenzo Taresco[a†], Ali Alazzo[a], Caterina Sansone,[b] Snjezana Stolnik,[a] Cameron Alexander[a*] and Claudia Conte.[a,b*]

[a.] *Division of Molecular Therapeutics and Formulation, School of Pharmacy, University of Nottingham, NG7 2RD, UK.*
[b.] *Drug Delivery Laboratory, Department of Pharmacy, University of Napoli Federico II, Via Domenico Montesano 49, 80131 Napoli, Italy*

**Table of Contents**

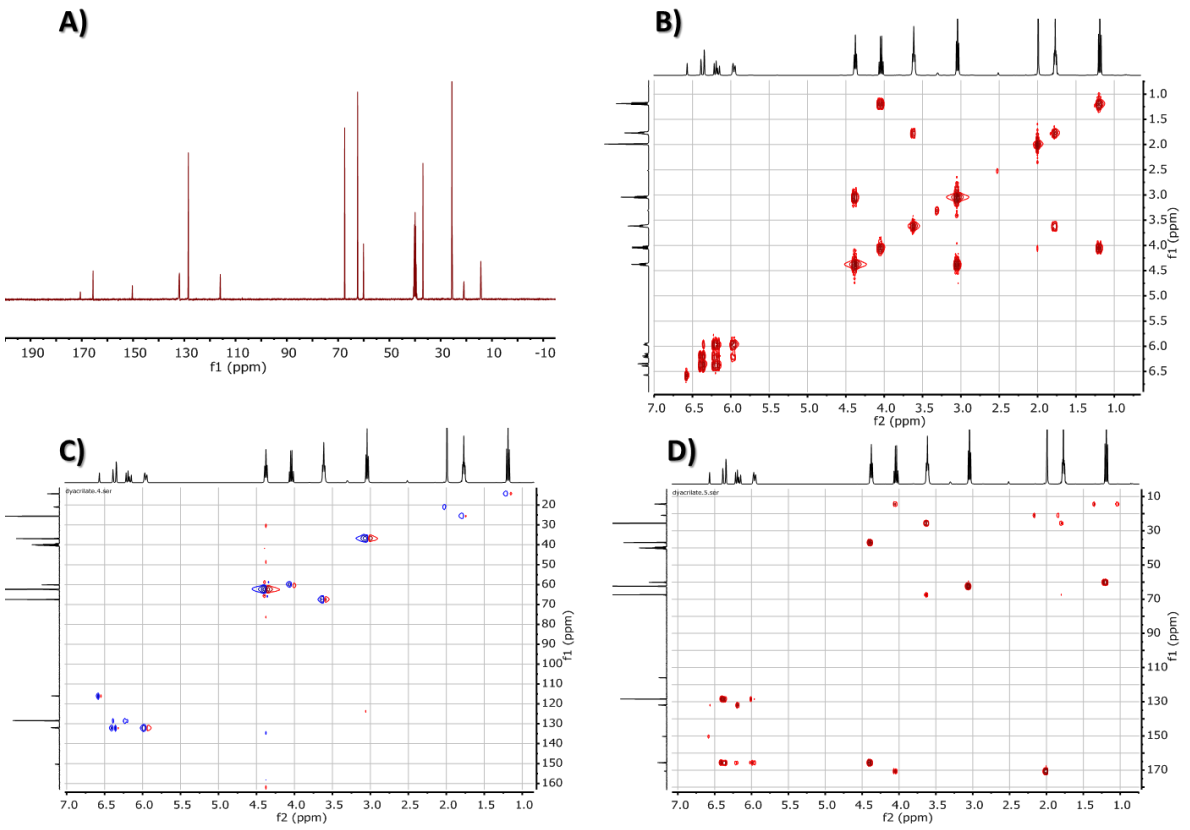


Figure S1. Characterisation of DSD starting material. A) $^{13}C$ NMR spectrum; B) $^{1}H$-$^{1}H$ COSY NMR spectrum; C) $^{1}H$-$^{13}C$ HSQC NMR spectrum and D) $^{1}H$-$^{13}C$ HMBC NMR spectrum. Spectra recorded at 500 MHZ in $d_6$-DMSO.

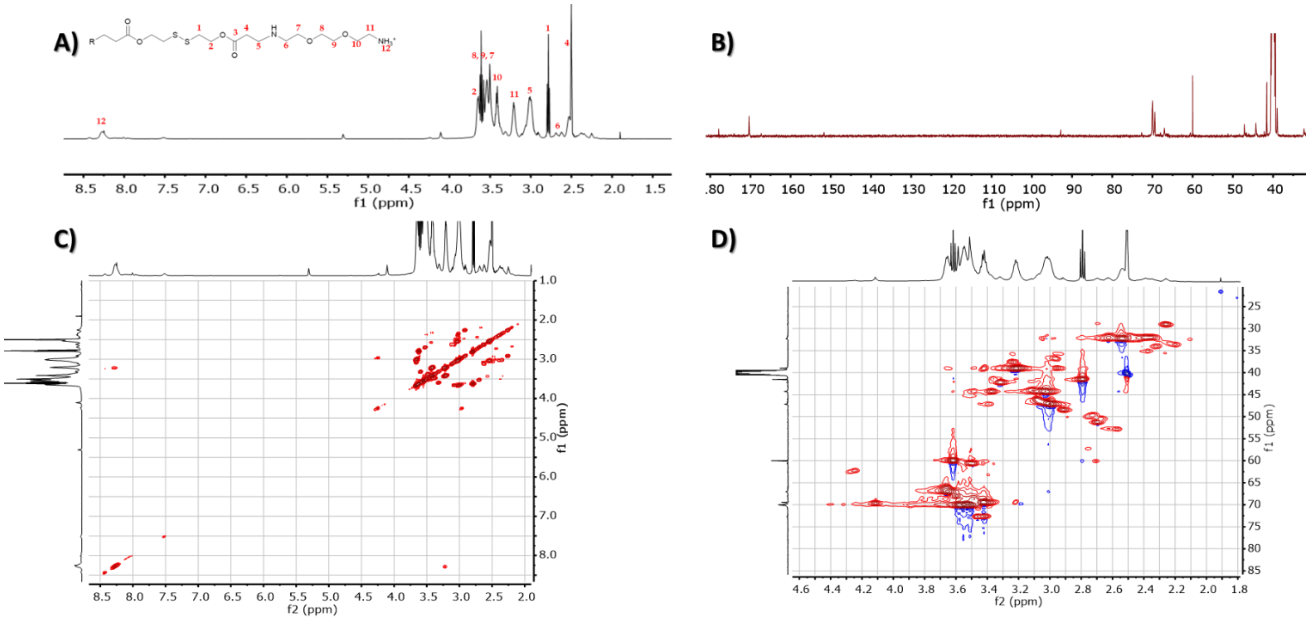


Figure S2. Characterization of OBAE A. A) $^{1}H$ NMR spectrum; B) $^{13}C$ NMR spectrum collected for 6h; C) $^{1}H$- $^{1}H$ COSY NMR spectrum and D) $^{1}H$- $^{13}C$ HSQC NMR spectrum. Spectra recorded at 500 MHZ in $d_6$-DMSO.

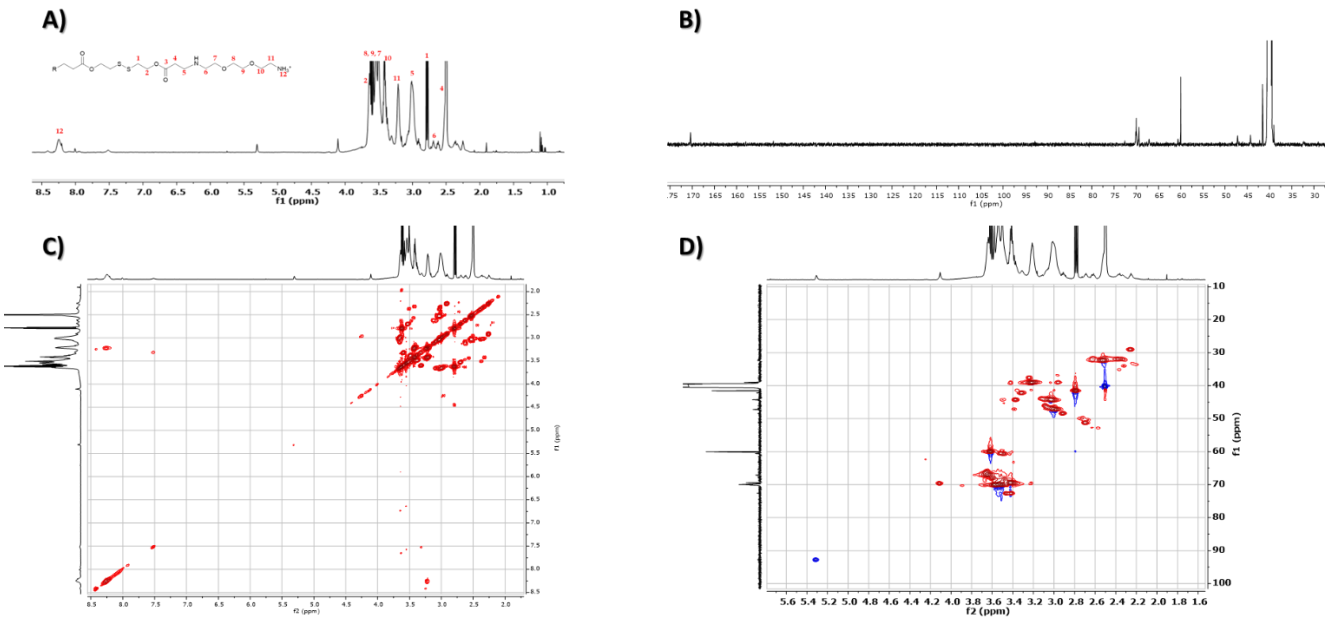


Figure S1. Characterization of OBAE B. A) $^{1}H$ NMR spectrum; B) $^{13}C$ NMR spectrum collected for 6h; C) $^{1}H$-$^{1}H$ COSY NMR spectrum and D) $^{1}H$- $^{13}C$ HSQC NMR spectrum. Spectra recorded at 500 MHZ in $d_6$-DMSO.

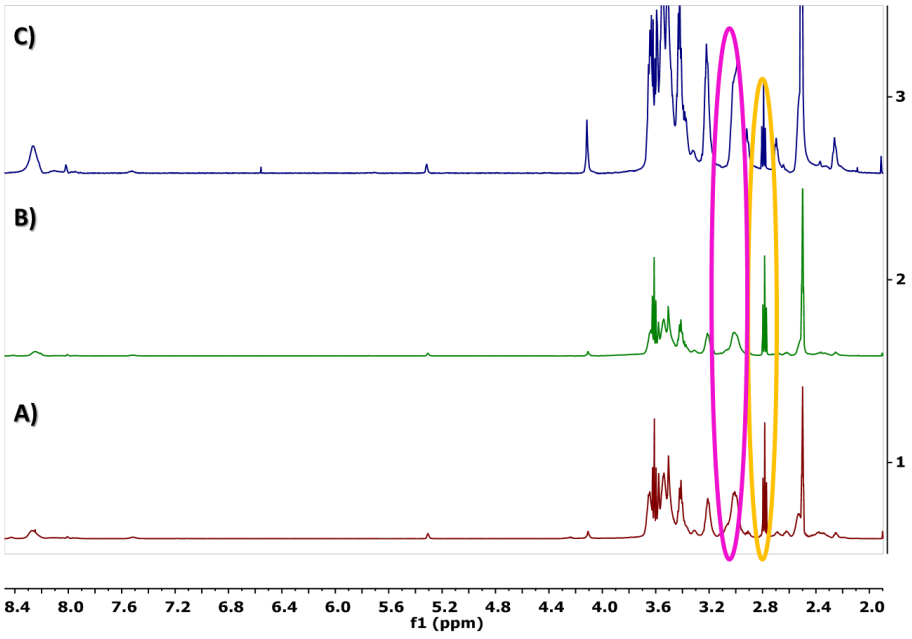


Figure S4. Comparison between $^{1}H$ NMR spectra of the three OBAE synthesised. A) $^{1}H$ NMR spectrum of OBAE A; B) $^{1}H$ NMR spectrum of OBAE B and C) $^{1}H$ NMR spectrum of OBAE C. Spectra recorded at 500 MHZ in $d_6$-DMSO.

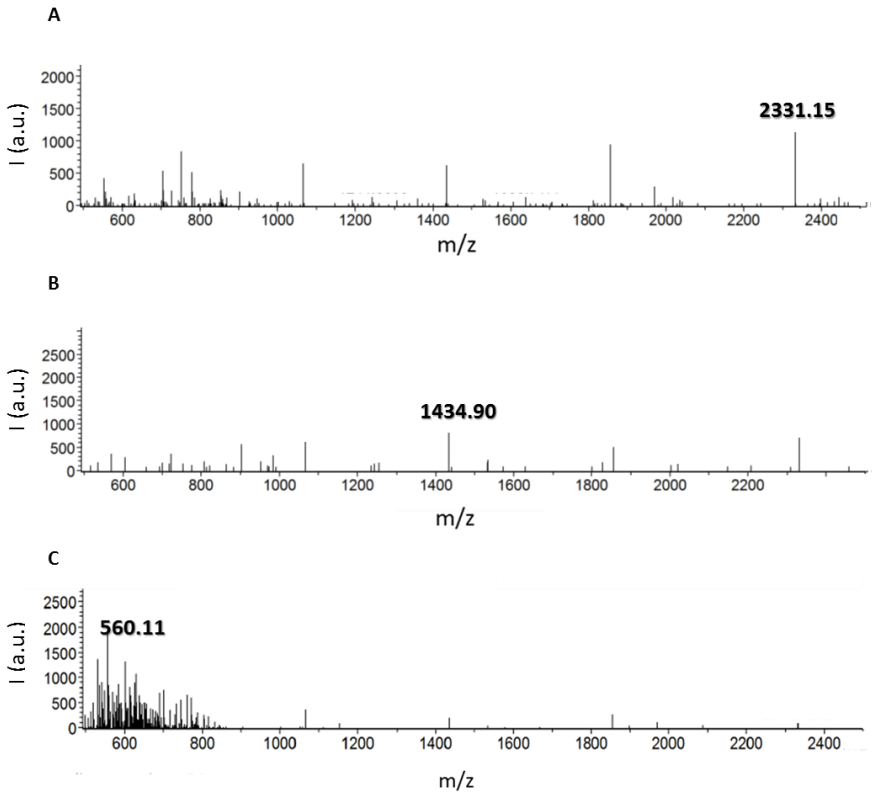


Figure S5. ESI mass spectra of OBAE A (A), OBAE B (B) and OBAE C (C) in water.

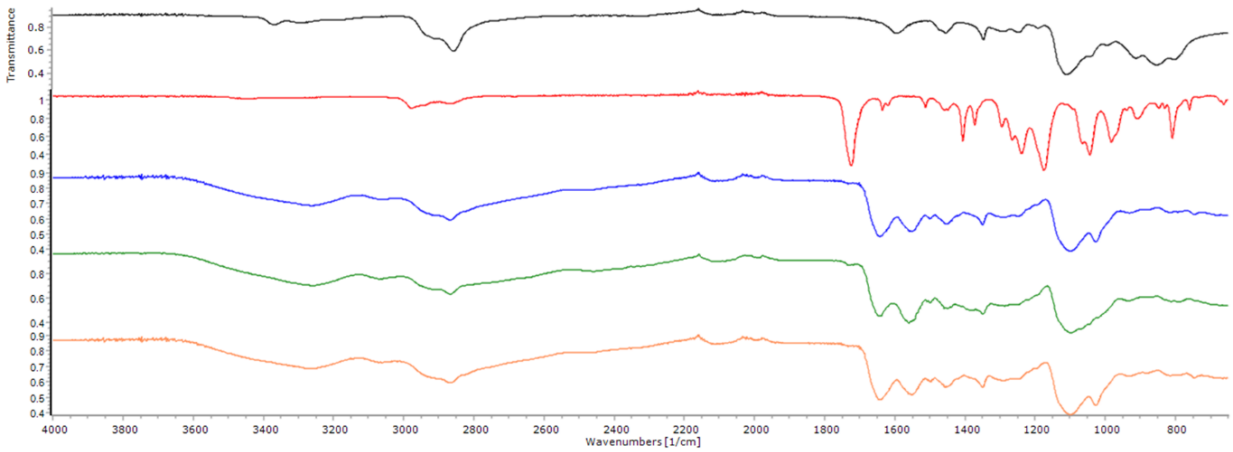


**Figure S6. FT-ATR-IR spectra** of OBAE A (blue), OBAE B (green) and OBAE C(orange) compared to the disulfide-based diacrylate (DSD in red) and ethlenedioxy-bis-ethylamine (in black). The starting material ethlenedioxy-bis-ethylamine (in black) presented two sharp peaks at around 3400 and 3200 $cm^{-1}$ typical of the solely presence of primary amine. On the contrary, OBAEs spectra (blue, green and orange) showed a broad signal at around 3250 $cm^{-1}$, due to an extended H-bonded network hinting at the formation of mix primary and secondary amine functionalities. Furthermore, it's possible to note the shift of the C=O stretching of the DSD from 1750 $cm^{-1}$ to 1643 $cm^{-1}$ in the spectra of the three OBAEs. A retention of some weak signal related to the C-S bond in the DSD were also observed throughout the OBAEs serie.

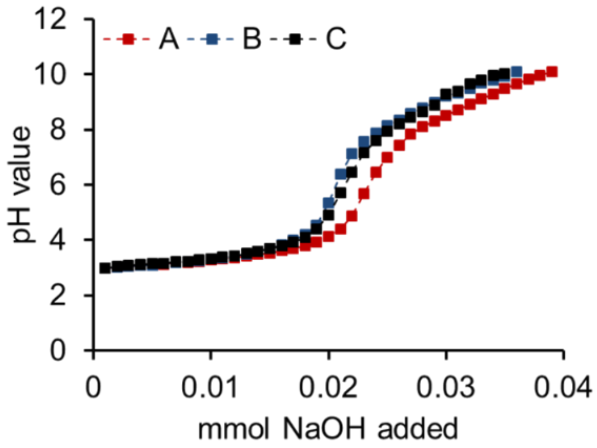


Figure S7. Acid−base titration curves for OBAEs. Measurements were taken using a Fisherbrand pH meter with a Hydrus 600 electrode. pH was adjusted to pH 3 with HCl and then titrated with NaOH.

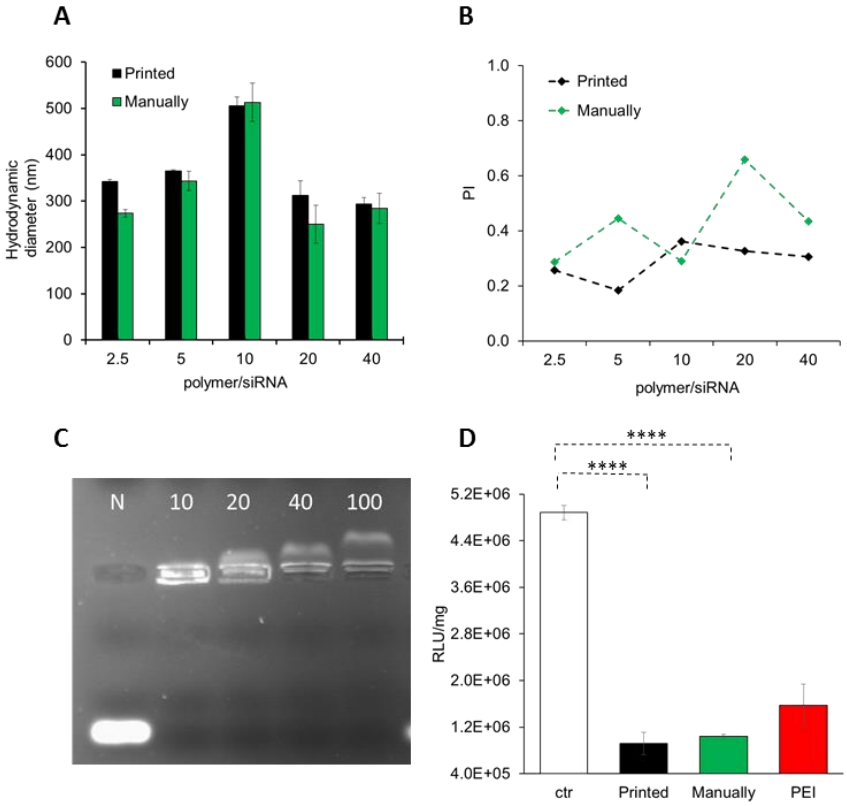


Figure S8. Comparison of size (A) and polydispersity index (B) of OBAE C/siRNA polyplexes at different polymer/siRNA weight ratios made through the classical manual method and the printing technique. C) siRNA condensation by C through the classical manual method at different polymer/siRNA weight ratios as evaluated by the gel retardation assay. N represents naked siRNA. D) In vitro luciferase siRNA transfection from OBAE C/siRNA polyplexes at 10 polymer/siRNA weight ratio made through the classical manual method and the printing technique polyplexes in A549-luciferase expressing cells after 4h of incubation. RLU= relative light units, a measure for luciferase expression. Results are expressed as mean ± SD of three experiments. ****P<0.0001, two-way ANOVA test.

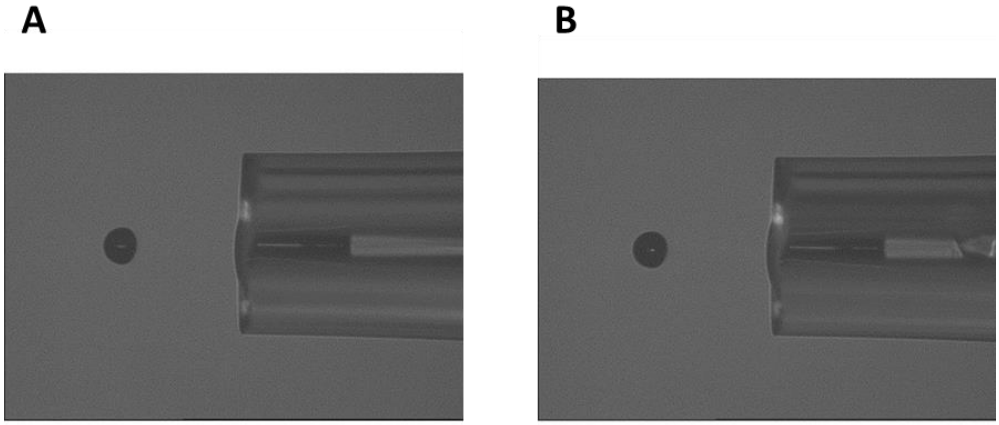


Figure S9. Droplet Size for pure water (a) (printing conditons: Voltage 105, Pulse 49, Drop Volume 270 pL) and siRNA aqueous solution (b) (printing conditons: Voltage 103, Pulse 55, Drop Volume 250 pL)

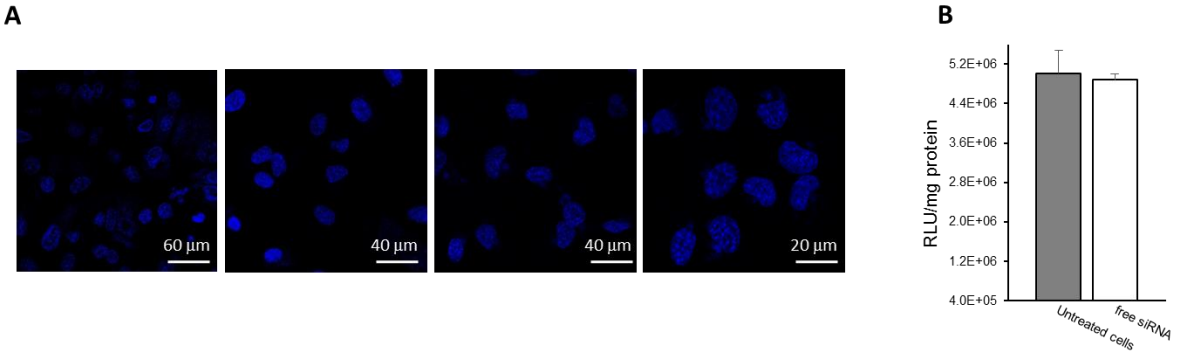


Figure S10. A) Confocal images of A549-luciferase expressing cells. Cell nuclei were stained with DAPI (blue) and the images were acquired with 545 nm excitation and LP 560 nm spectral filters. Zen 2009 image Software was utilized for image processing. B) Luciferase expression in A549-luciferase expressing cells untreated and treated with free Luciferase siRNA (1 μg) for 4h. RLU= relative light units. Results are expressed as mean ± SD of three experiments.