

Supporting information for:

Engineering immunostimulatory nanocarriers: TLR7-agonist conjugated poly(β -amino ester) mRNA delivery systems

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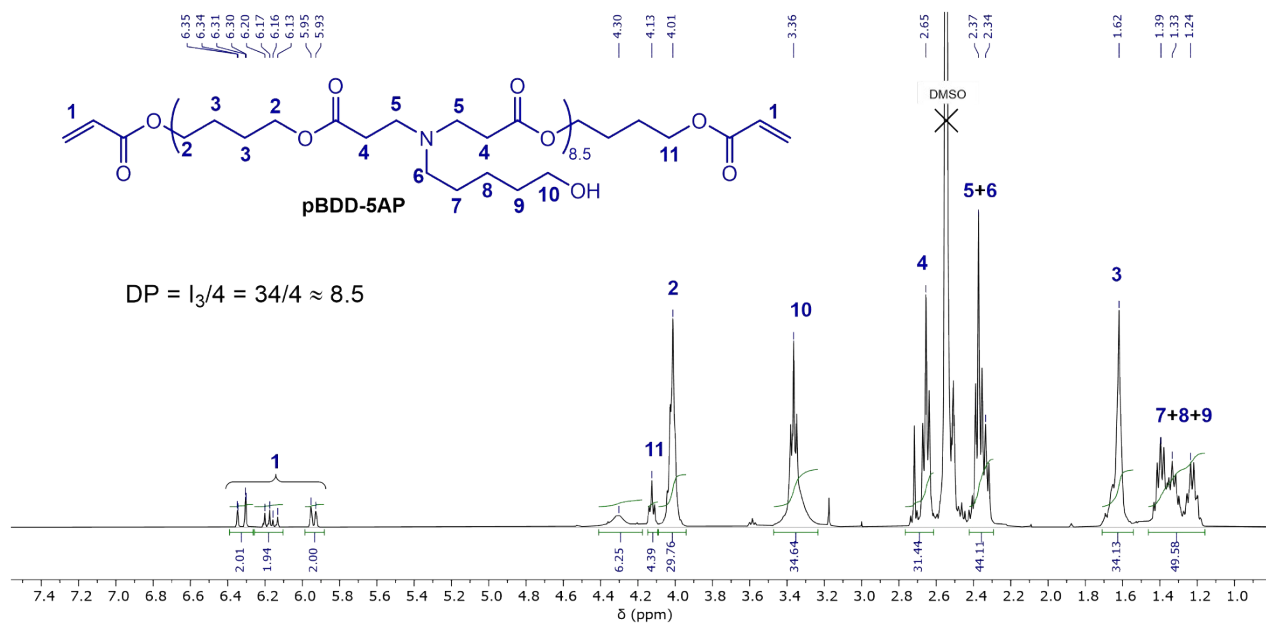
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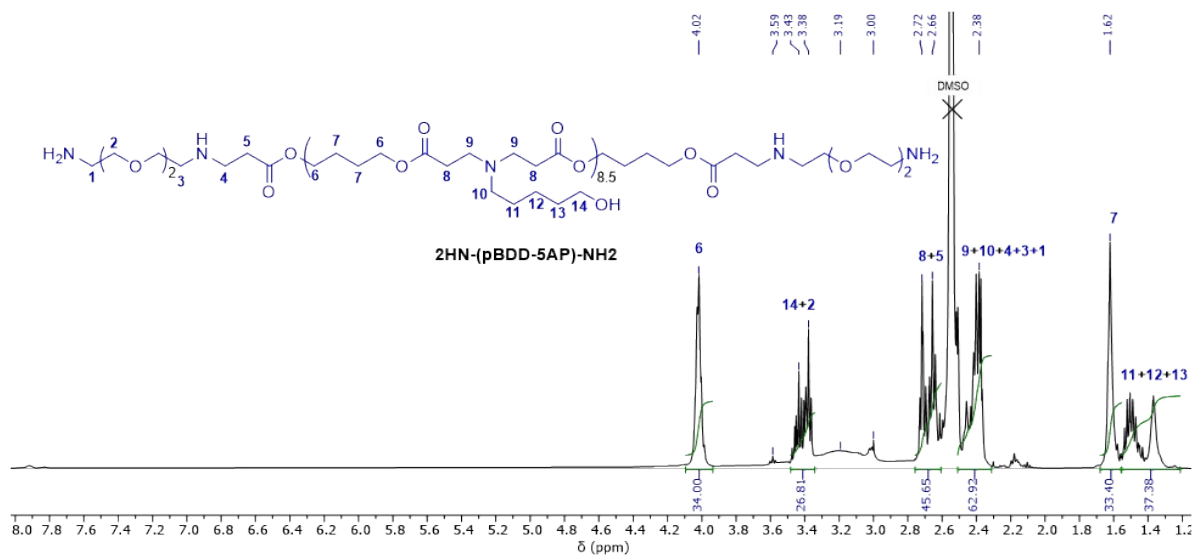
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Antibody	Supplier	Catalog number	RRID	Dilution
Anti-mouse B220 APC Cy7 (RA3-6B2)	BioLegend	103224	AB_313007	1:200
Anti-mouse CD4 APC Cy7 (GK1.5)	Thermo Fisher Scientific	12-0041-82	AB_465506	1:200
Anti-mouse CD11c APC (HL3)	BD Biosciences	550261	AB_398460	1:100
Anti-mouse CD45 BV605 (30-F11)	BioLegend	103155	AB_2650656	1:800
Anti-mouse CD86 PE Cy7 (GL1)	BioLegend	105014	AB_439783	1:400
Anti-mouse Ly6c APC Cy7 (HK1.4)	BioLegend	128025	AB_10643867	1:200
Anti-mouse Ly6G APC Cy7 (1A8)	BioLegend	127624	AB_10640819	1:200
Anti-mouse MHC II V510 (M5/114.15.2)	BioLegend	107636	AB_2561397	1:800
Anti-mouse NK1.1 APC Cy7 (PK136)	BioLegend	108724	AB_830871	1:200

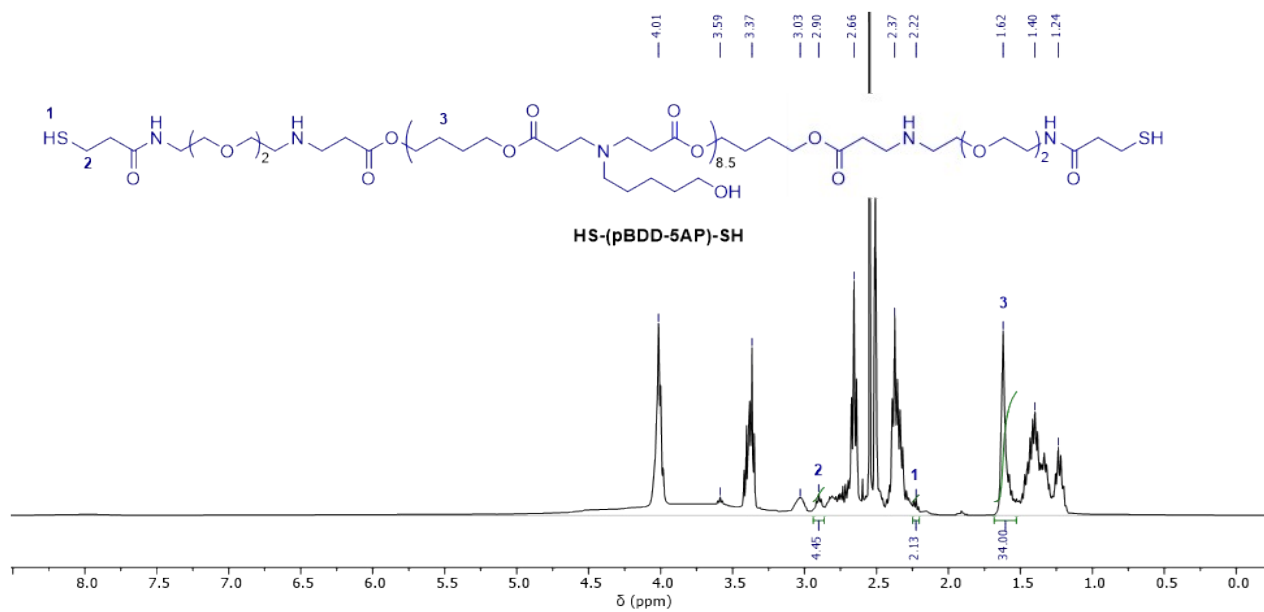
Table S1. Details on antibodies used.



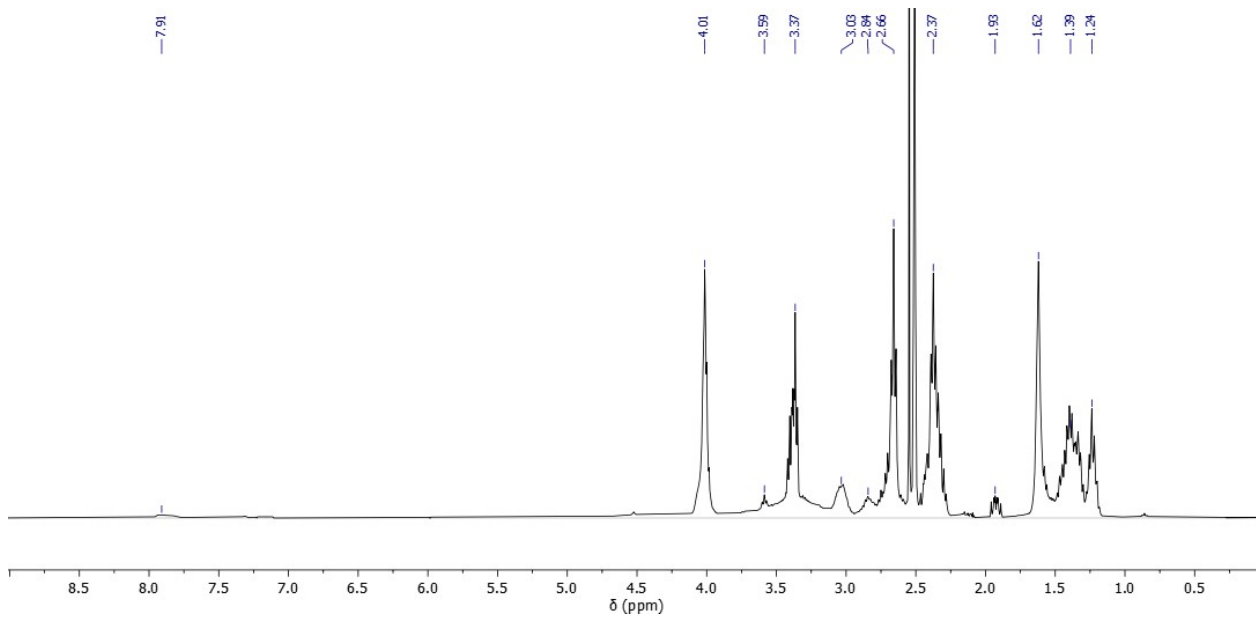
Spectrum S1. 1H NMR (400 MHz, DMSO-d₆) of pBDD-5AP.



Spectrum S2. 1H NMR (400 MHz, DMSO-d₆) of H₂N-(pBDD-5AP)-NH₂.



Spectrum S3. ^1H NMR (400 MHz, DMSO- d_6) of HS-(pBDD-5AP)-SH.



Spectrum S4. ^1H NMR (400 MHz, DMSO- d_6) of LOX-(pBDD-5AP)-LOX.

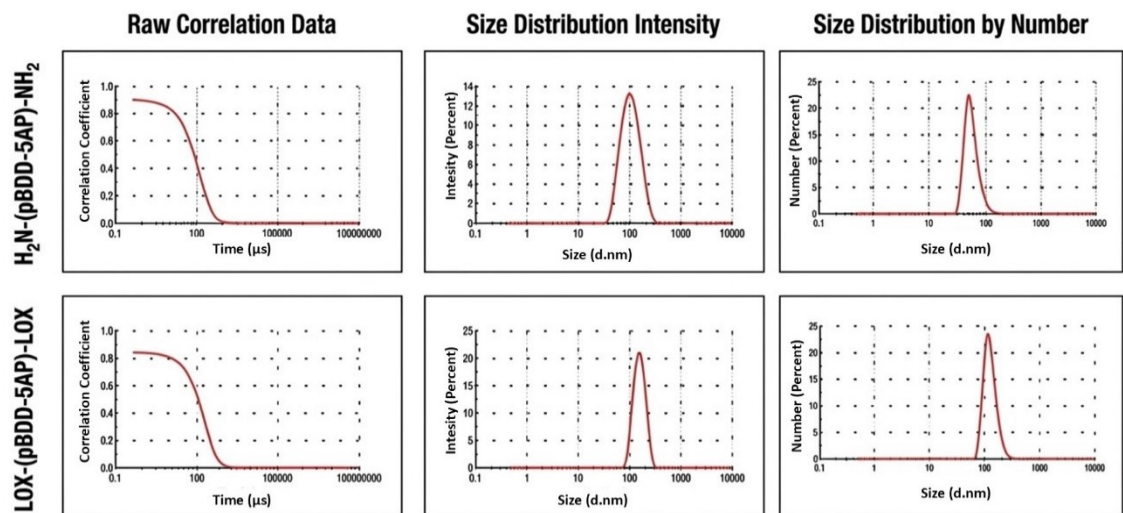


Figure S1. Representative Raw Correlation Data, Size Distribution Data and Size Distribution by Number Data measured by DLS for the nanoparticles $H_2N-(pBDD-5AP)-NH_2$ and $LOX-(pBDD-5AP)-LOX$.

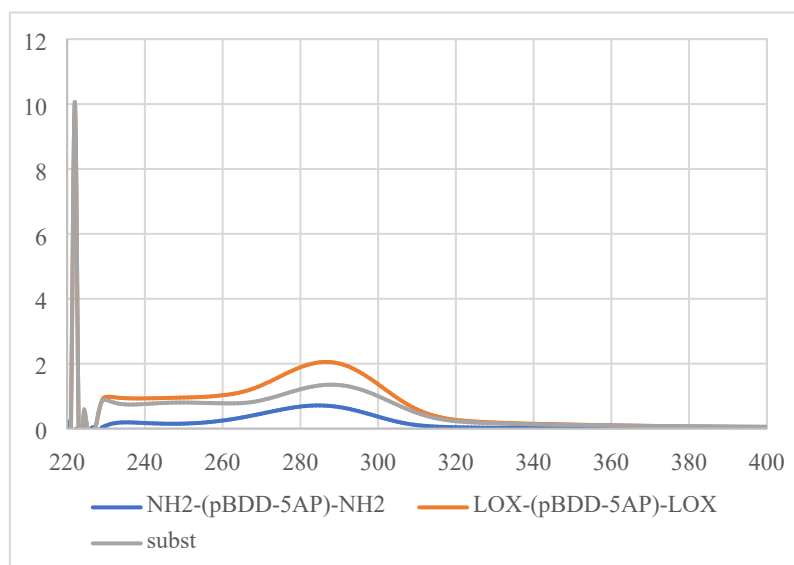


Figure S2. Determination of Drug Loading Using UV-vis spectroscopy.

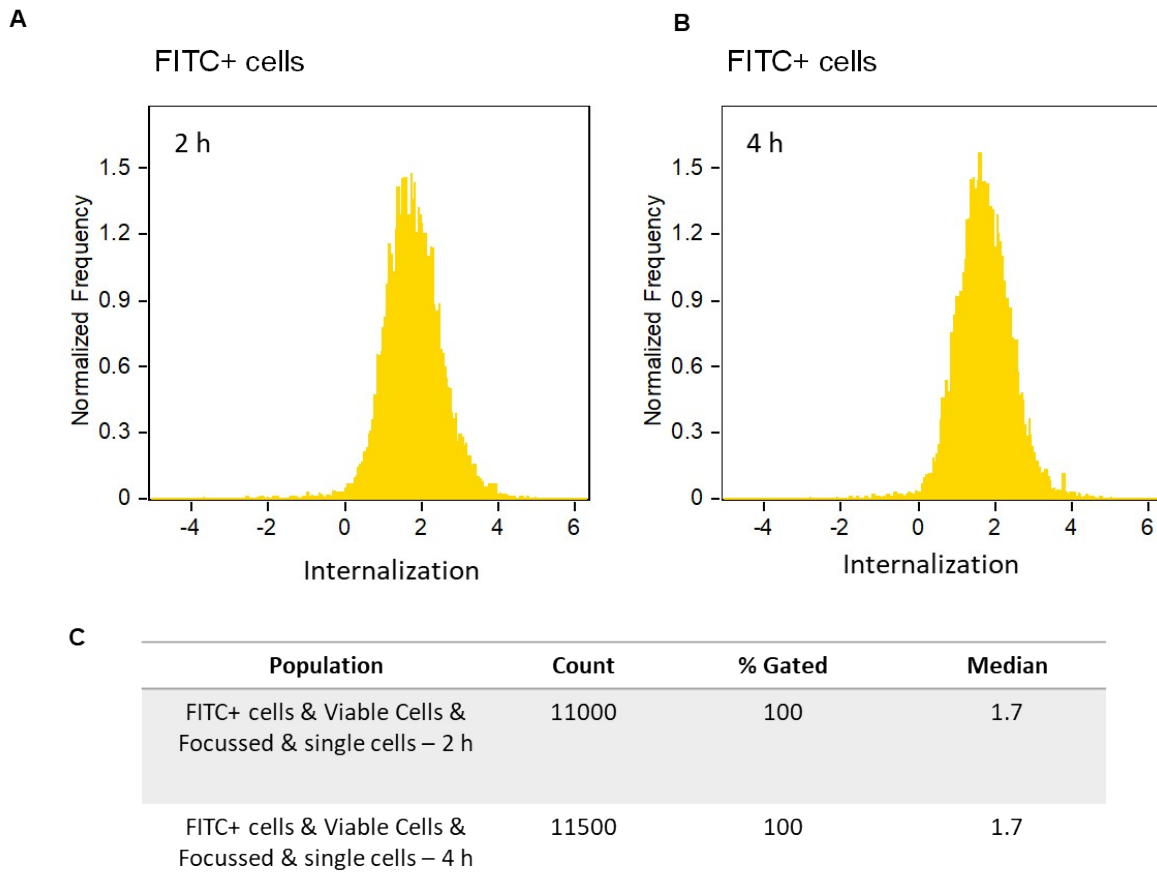


Figure S3. Cellular uptake of PBAE derived saRNA polyplexes. (A-B) Imaging flow cytometry internalisation histograms and (C) statistical values for DC2.4 cells after 2h and 4 h following treatment with FITC-labelled LOX-(pBDD-5AP)-LOX polyplexes at a PBAE/saRNA w/w ratio of 128 at a concentration of 500 ng/mL saRNA. Data were analysed using IDEAS software.

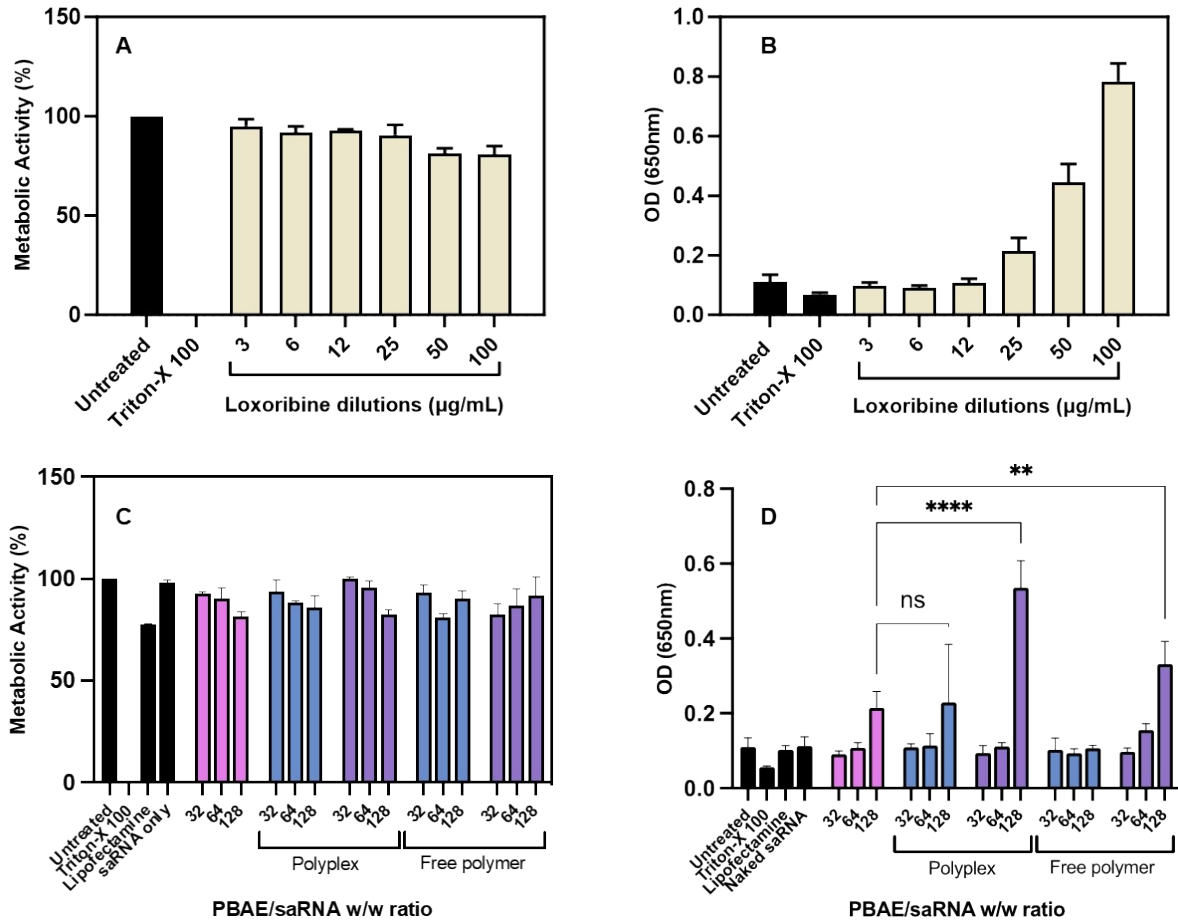


Figure S4. TLR7 Activation Assay using HEK-BLUE cells. (A) The determination of the TLR7 activation capacity of loxoribine dilutions (3 – 100 µg/mL) compared to controls in HEK-BLUE cells. (B) Metabolic activity of loxoribine dilutions (3 – 100 µg/mL) compared to controls in HEK-BLUE cells. (C) The determination of the TLR7 activation capacity of amine and loxoribine-end-capped polyplexes and free polymers (w/w 32, 64 and 128) compared to controls in HEK-BLUE cells. (D) Metabolic activity of the amine and loxoribine-end-capped polyplexes and free polymers (w/w 32, 64 and 128) compared to controls in HEK-BLUE cells.