Electronic Supplementary Information (ESI) for

Polyvalent Diazonium Polymers Provide Efficient Protection of Oncolytic Adenovirus Enadenotucirev from Neutralising Antibodies while Maintaining Biological Activity *In Vitro* and *In Vivo*

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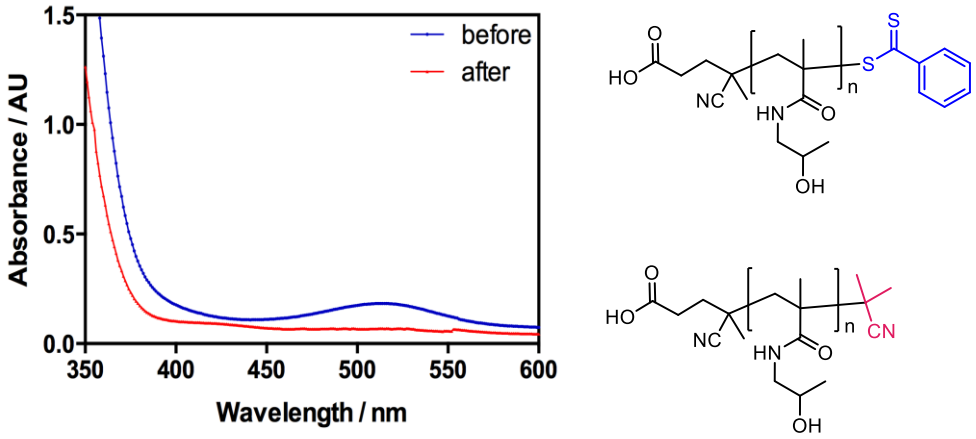
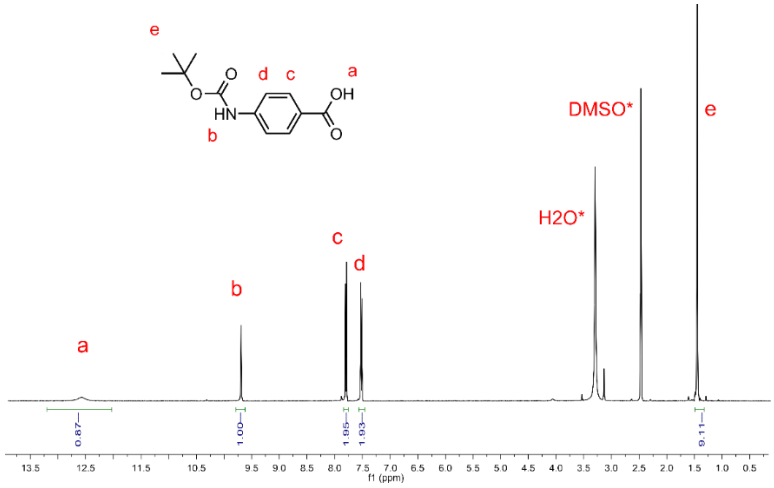


Figure S1. Representative UV/Vis spectra and chemical structures of PHPMA **P1** before (blue) and after (red) treatment with AIBN.

(a)



(b)

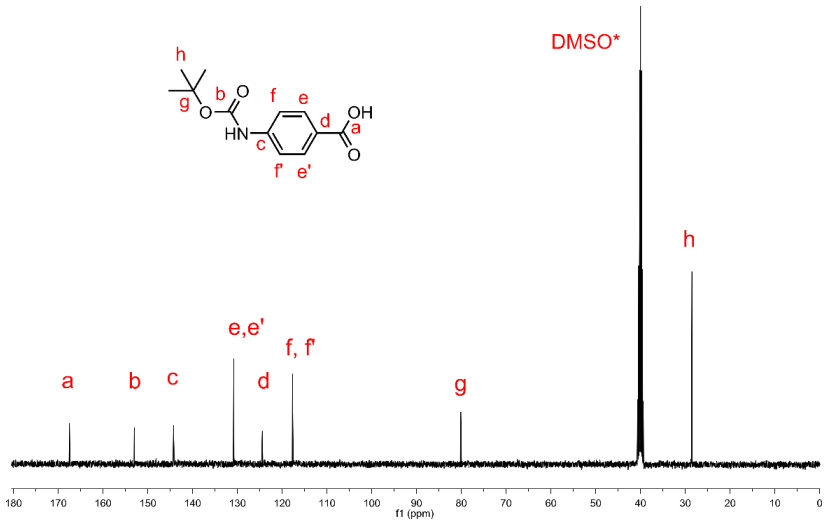


Figure S2 (a) ^{1}H and (b) ^{13}C NMR spectra of *p*-aminobenzoic acid-Boc protected (PABA-boc).

Conjugation to a BSA

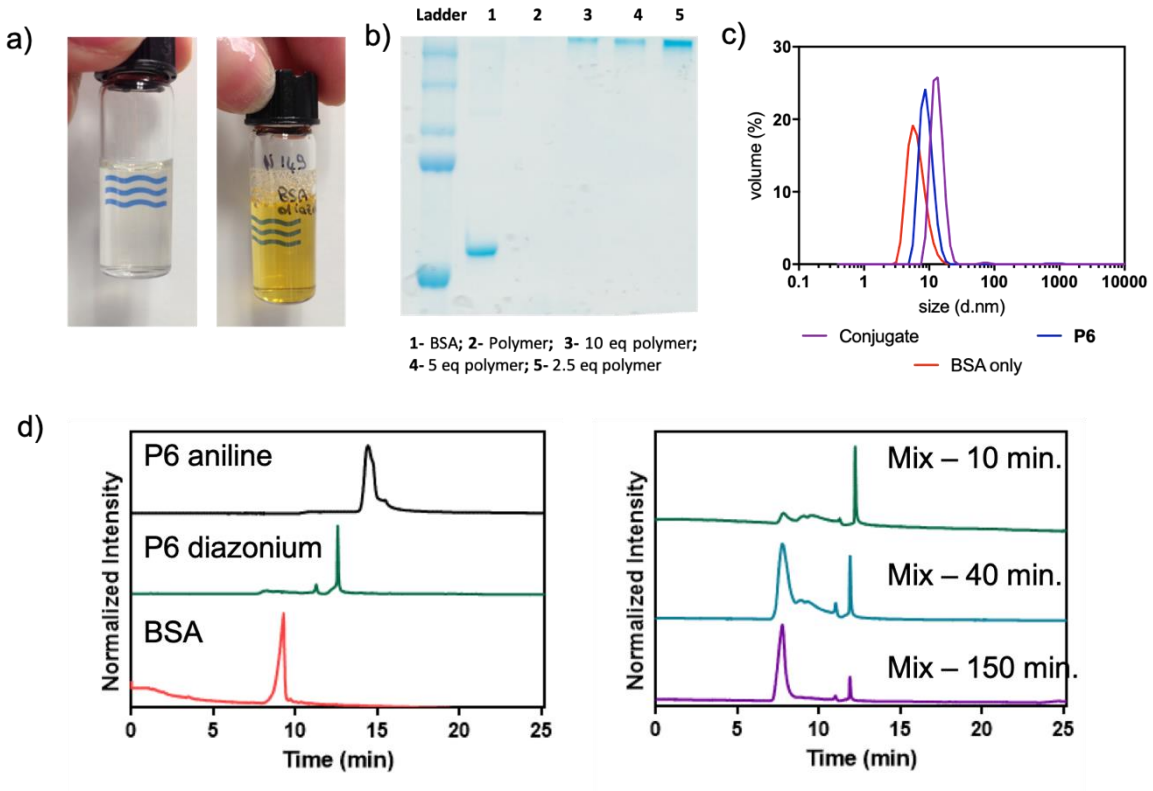


Figure S3 Polydiazonium polymer solutions before and after conjugation of **P6** with BSA (24 hours); b) SDS-PAGE, lane **1** ladder, **2** BSA, **3** P21 polymer , **4-6** P6-BSA conjugate at decreasing concentration. c) DLS of **P6**, BSA and P6-BSA conjugates in 10 mM HEPES pH 7.4; aqueous GPC of activiated P6, BSA and P6-BSA conjugate at different times post mixing.

Metabolic Activity Assay (MTS)

HT-29 cells were seeded into 96-well plate at a density of 25k cell/well in 100 µL of assay media. Cells were treated with 1 mg/mL dilutions of polymer-coated EnAd in assay media and incubated for 2 hours at 37°C, 5% CO_2 before replacing the media with fresh one. After 24 hours incubation, cells were washed with PBS and 120 µL of a solution of MTS in phenol-red free assay media (1:6 dilution) added to the well. The plate was incubated for 30 min at 37°C, 5% CO_2 and the absorbance measured at 40 nm using Wallac 1420 Victor multi-label counter or BioTek™ Synergy HT multimodal microplate reader.

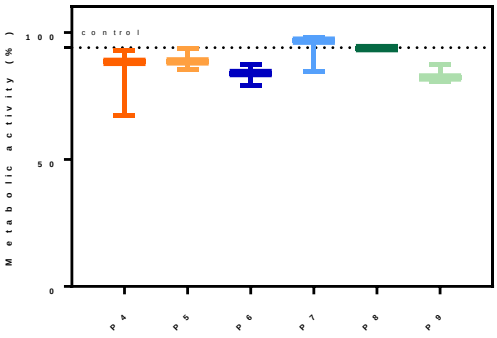


Figure S4. Metabolic activity of HT-29 cells measured by MTS assay 24 post-infection with P4 to P9 on HT-29 cell line. Polymer solution were prepared according to the concentrations required for polymer coating. (Means of triplicate values and standard deviation).

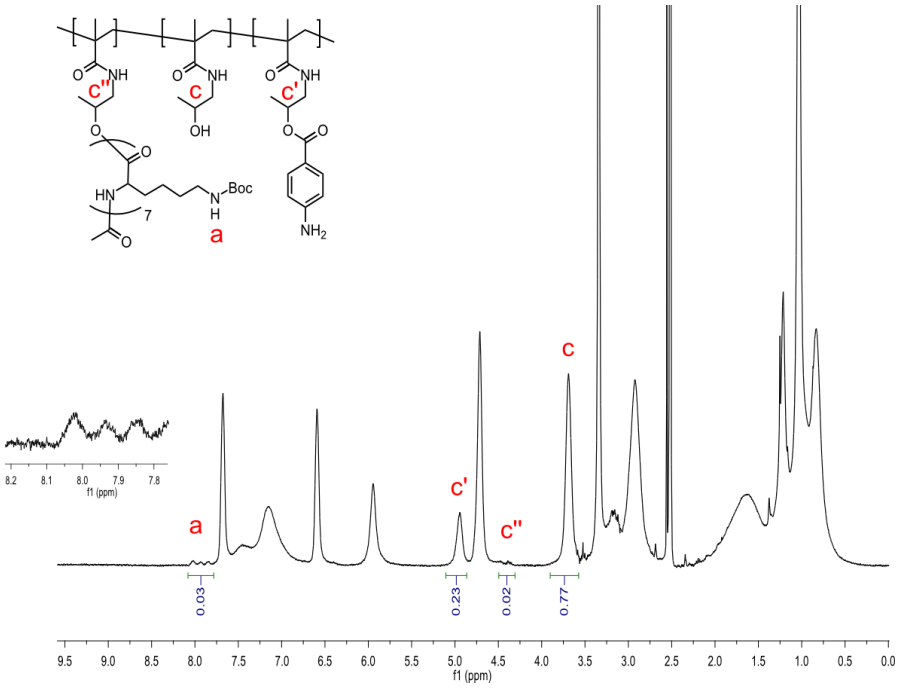


Figure S5. ^{1}H NMR of re-targeted labelled polydiazonium with K_7 (P9-K_7) after purification.

Live Confocal Microscopy

Uncoated EnAd was fluorescently labelled with a 20-fold molar excess per viral amine (approximately 1800 lysines per particle) of BODIPY-FL (Invitrogen) for 2 hours in 50 mM HEPES buffer at pH 7.4. Samples of polymer-coated EnAd were prepared according to the procedure described in paragraph 5.2.8, utilizing a 1 mg/mL final polymer concentration. Coating efficiency was tested by ELISA before conducting the experiments. 1×10^5 HT-29 cells were seeded on BD poly-L-Lysine glass bottomed 35 mm dishes and cultivated overnight in assay media. Cells were then washed with ice cold PBS, incubated in ice cold media (1.5 mL) before infection with 1×10^4 virus particles (uncoated EnAd or EnAd-P9- FL) for 1 hour over ice. After this time, cells were washed three times with ice cold PBS before addition of 1.5 mL of ice cold HBSS 20 mM HEPES buffer. The samples were stored on ice until they were placed in the incubation chamber for confocal imaging. Live cell imaging was performed on a LSM 710 (Carl Zeiss) confocal microscope using a 63× oil immersion objective and a CO_2-controlled incubator at 37 °C. Image analysis was performed using Zen Lite and ImageJ softwares. Images were digitized under constant exposure time, gain and offset.

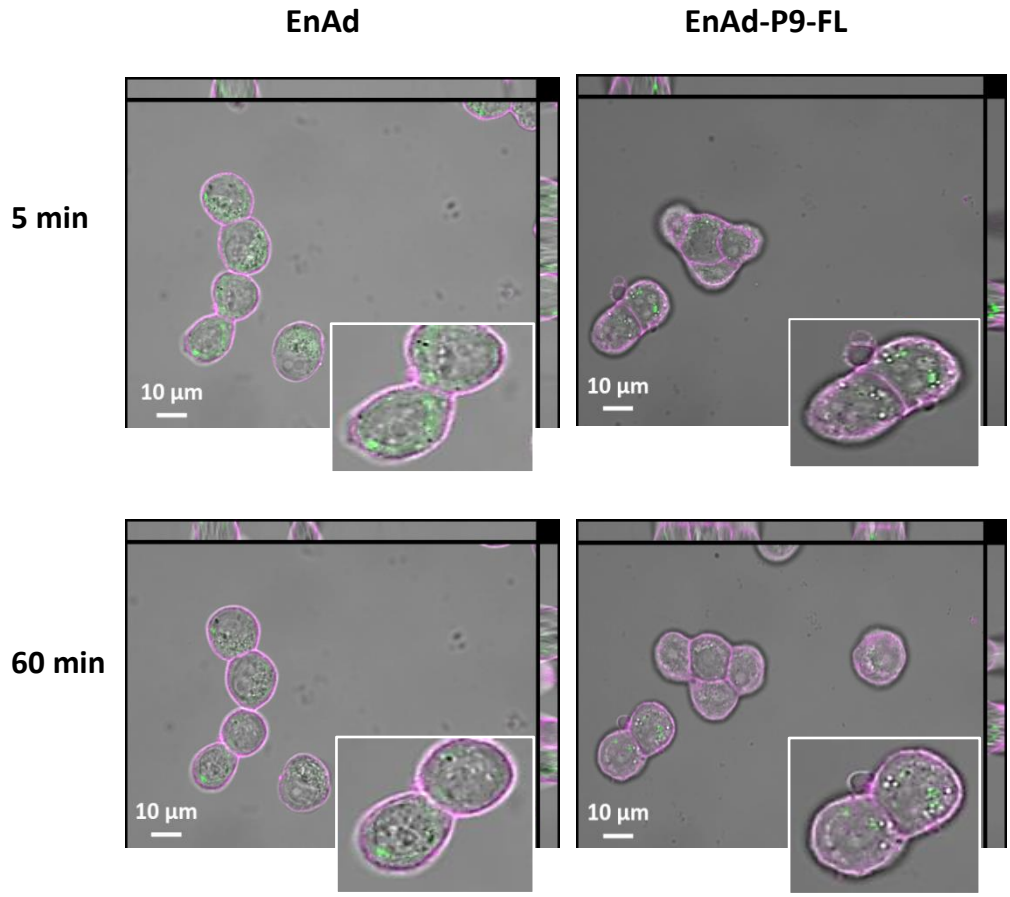


Figure S6. Merged confocal images of EnAd and EnAd-P9-FL at 5 and 60 minutes post-infection. Each ortho visualization was gained from a z-stack consisting of 10 slices, and was used to confirm that in both cases the virus is present within the cell and not at the membrane. Green = EnAd-FL or EnAd-P9-FL; Magenta = cell plasma membrane.