Supplementary Data

Anti-CD20 multivalent HPMA copolymer-Fab' conjugates for the direct induction of apoptosis

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1. Size exclusion chromatography (SEC) profiles of P-NH_2, P-mal, and P-Fab'

HPMA copolymers with pendant amino groups (P-NH_2) and conversion into maleimide-derivatized copolymers (P-mal) were analyzed by SEC using an ÄKTA FPLC system (GE Healthcare, Piscataway, NJ) (Figure S1, A-E). All P-mal demonstrated identical SEC profiles to corresponding P-NH_2 (data not shown). The average molecular weight and polydispersity of copolymers were determined using Superose 6 HR10/30 column (GE Healthcare) with sodium acetate buffer and 30% acetonitrile (v/v) (pH=6.5) in line with a MiniDawn (TREOS) light scattering detector, Opilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA), and UV detector. HPMA homopolymer fractions of different Mw were used for calibration. After conjugation with Fab', products were purified on a Superose 6 HR16/60 preparative column (GE Healthcare); the entire P-Fab' populations were collected as one fraction. Analytical Superose 6 column was used to analyze the profiles of the final products (sodium acetate buffer + 30% acetonitrile) (Figure S1, F-J).

2. Amino acid analysis for determination of valences (Fab' content per polymer chain)

Precisely quantified samples including HPMA homopolymer (PHPMA), Fab' fragment, and P-Fab' conjugates were hydrolyzed using 6 N HCl in a heating block (125 °C, 24 h), dried with rotary-evaporator, and re-dissolved in water. Then the samples were derivatized with o-phthalic dicarboxaldehyde (OPA) and 3-mercaptopropionic acid (MPA) immediately before loading onto an eclipse XDB-C8 column with an Agilent HPLC system (gradient elution with buffer A: 0.05 M sodium acetate, pH 6.0; buffer B: 70% methanol in buffer A). Glutamate and 1-amino-2-propanol (1-AP) were also analyzed with HPLC to further define the peaks shown in the results of Fab' and PHPMA samples (Figure S2). Calibrations of Fab' and PHPMA contents were performed by the peaks indicating glutamate and 1-AP, respectively. Analysis of Fab' content was also confirmed by other undefined peaks with good linear relationships between loading amount and area under curve (AUC).

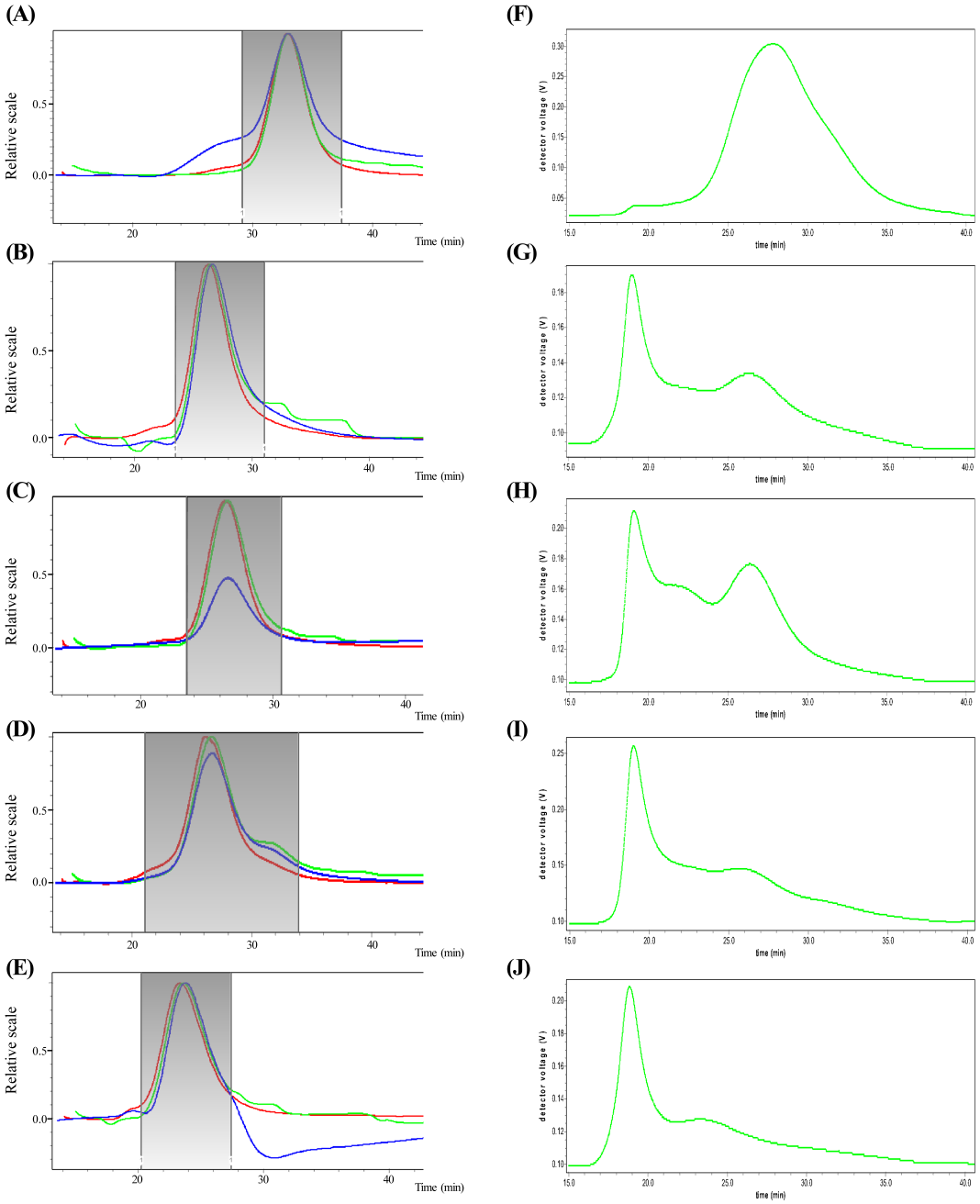
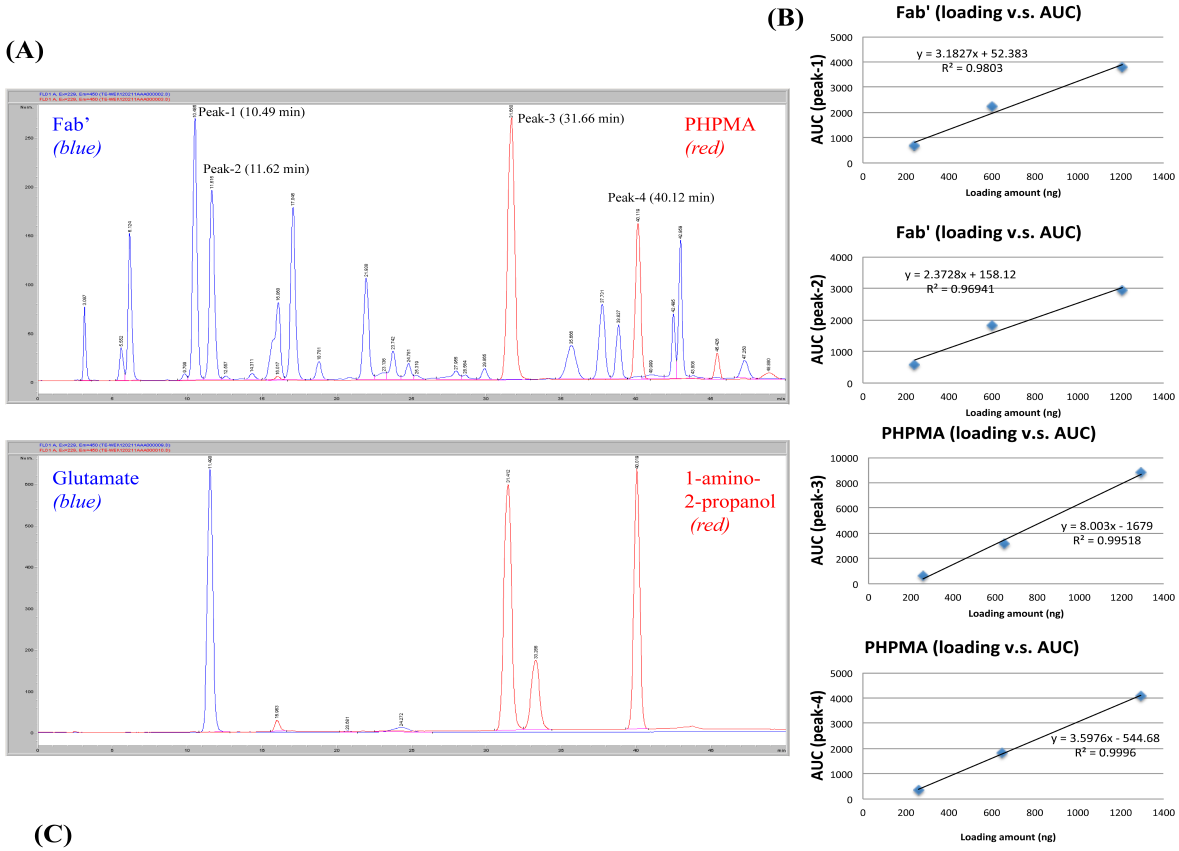


Figure S1. SEC profiles of the five P-NH_2 polymer precursors (**A-E** for P1, P2, P2a, P2b, and P3) and their corresponding P-Fab' multivalent conjugates (**F-J** for P1-Fab', P2-Fab', P2a-Fab', P2b-Fab', and P3-Fab') as analyzed by *ÄKTA FPLC* (Superpose 6 analytical column, acetate buffer + 30% acetonitrile v/v). For P-NH_2 **(A-E)** signals of UV at 280 nm (green), refractory index (blue) and light scattering (red) are shown; for P-Fab' **(F-J)** only the UV signal is shown.



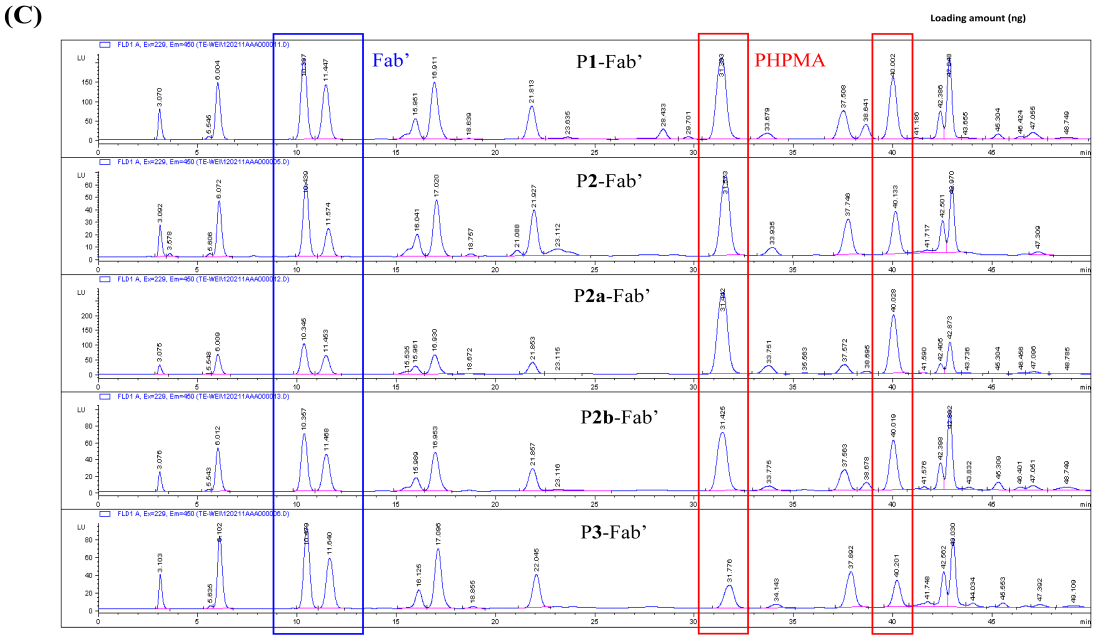


Figure S2. Determination of valence (Fab' content per polymer chain) of the five HPMA copolymer-Fab' conjugates by amino acid analysis (AAA). Calibration was performed by peak-2 (glutamate) for Fab' content and peak-3 (1-amino-2-propanol) for HPMA copolymers; and confirmed by peak-1 (undefined amino acid) and peak-4 (1-amino-2-propanol), respectively. **(A)** Overlaid images of Fab' fragment and HPMA homopolymer (top panel), and glutamate and 1-amino-2-propanol (bottom panel) as analyzed by HPLC eclipse XDB-C8 column. **(B)** Calibration of the four peaks shown as area under the curve (AUC) versus loading amount. **(C)** HPLC analyses of the five conjugates after acid hydrolysis.

3. Dynamic light scattering for measurement of effective diameters of P-Fab' conjugates

The hydrodynamic effective diameters of conjugates were analyzed by dynamic light scattering (DLS) using a Brookhaven BI-200SM goniometer and BI-9000AT digital correlator equipped with a He-Ne laser (λ=633 nm) at room temperature in PBS (pH 7.4). The scattering angle was 90°. A Nanosphere™ polystyrene size standard (diameter=102 nm ± 3 nm) (STD100nm) (Thermo Scientific, Waltham, MA) was measured in line. Conjugates in PBS (1, 0.5, 0.25 mg/mL; Fab'-equivalent concentration) were filtered through a 0.45 µm filter. All measurements were performed in at least duplicate.

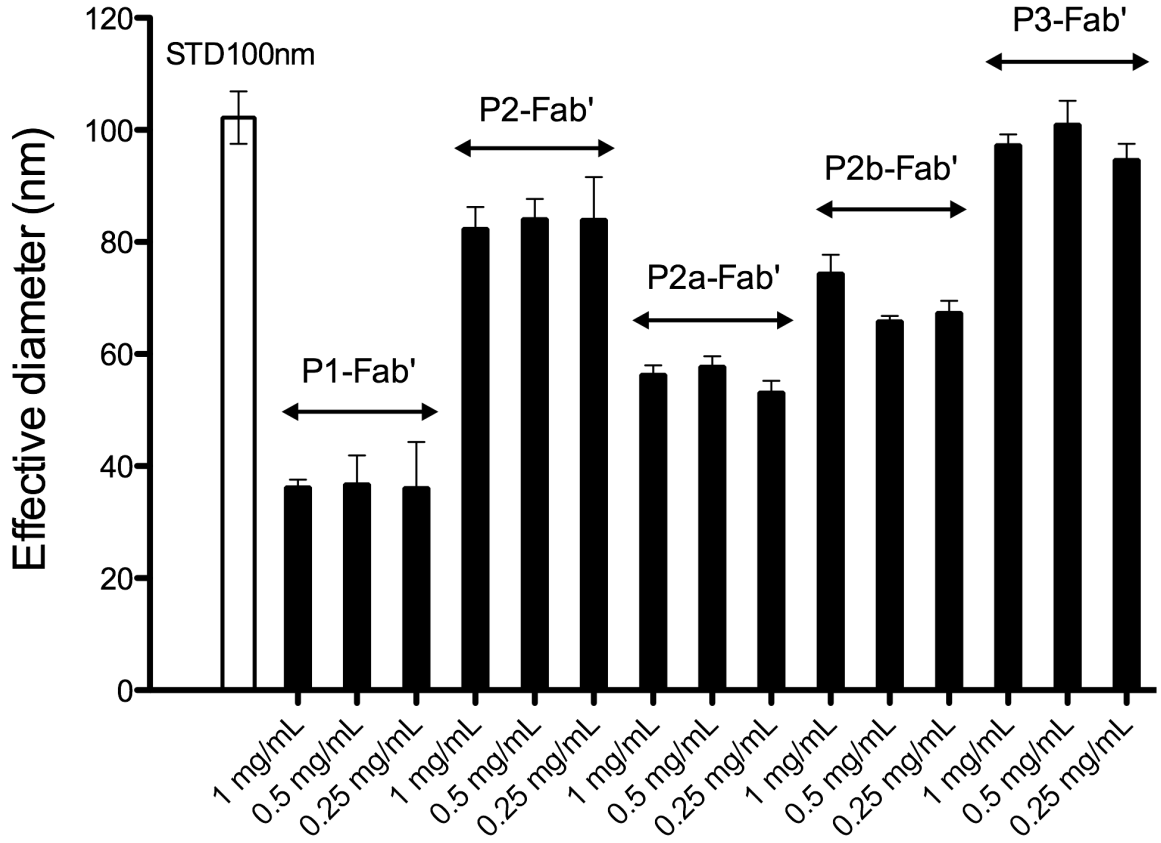


Figure S3. Hydrodynamic effective diameters of the five P-Fab' conjugates as analyzed by DLS. Samples were measured in different (Fab'-equivalent) concentrations as indicated, in line with a Nanosphere™ polystyrene size standard (STD100nm).