**Synthesis and evaluation of multivalent branched HPMA copolymer-Fab' conjugates targeted to the B-cell antigen CD20**

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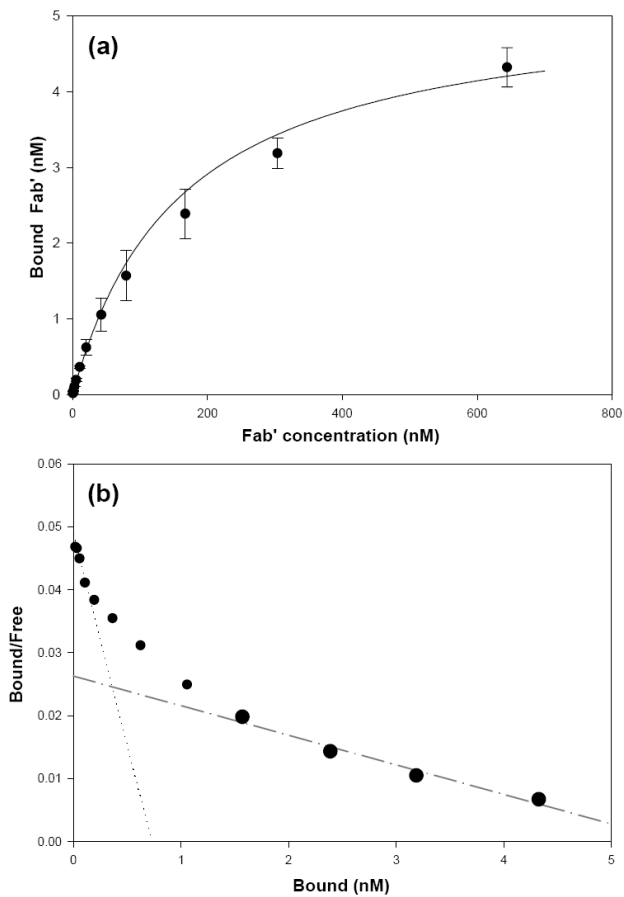
<u>Supporting Information</u>

Binding analysis of each multivalent conjugate was performed as described in the Experimental Procedures section. Briefly, Raji cells grown in RPMI medium containing 10% fetal bovine serum (FBS) were washed twice with Hank's balanced salt solution (HBSS). The cells were adjusted to 4×10^6 cells/ml and split into two volumes. To one of the volumes of Raji cells, unlabeled anti-CD20 IgG was added to a final concentration of 500 nM to block CD20 for a negative control. Serial dilutions of ^{125}I-labeled anti-CD20 IgG, Fab', and multivalent Fab' conjugates were made from 2000 nM to 1 nM of Fab' fragment concentration. Millipore 96-well filter plates, coated with a 1% solution of nonfat dry milk in distilled water and incubated at 4 °C overnight, were washed with HBSS. For each of the serial dilutions, 25 µL of negative control cells were added to a single well, and 25 µL of unblocked cells were added to 3 different wells; then 25 µL of the respective dilutions were added to each well. The filter-well plates were incubated for 90 min at 120 rpm on a shaker at 25 °C. Following incubation, the Raji cells were washed twice with 200 µL of cold HBSS with 10% FBS, and then collected on filters. The filters were allowed to air-dry, removed from the plate and counted. Negative control wells were used to correct for non-specific binding. Isotherms were evaluated with the Sips equation using SigmaPlot software (Systat Software, San Jose, CA).
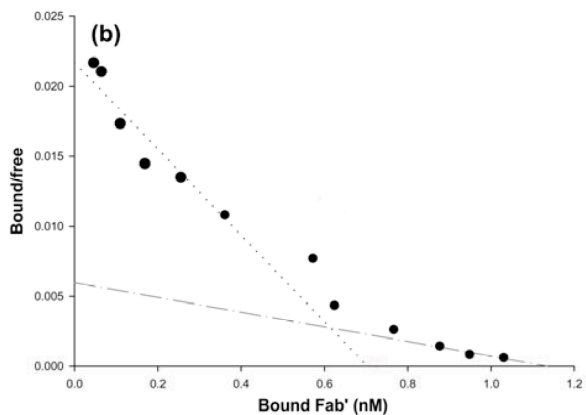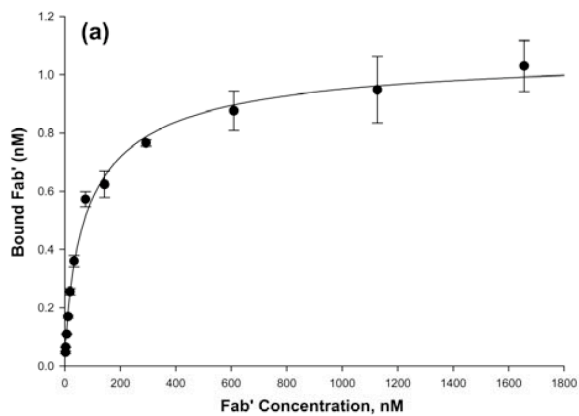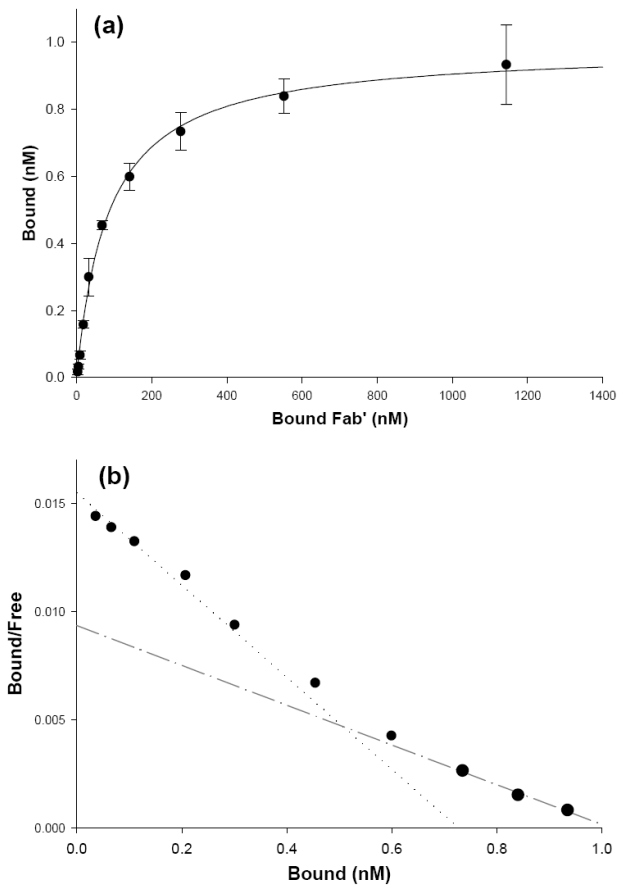
Suporting Information Figure 1. Binding analysis of conjugate *P-3Fab1.5*. (a) The isotherm for conjugate *P-3Fab1.5* was fitted with the Sips equation using the least squares method. (b) Scatchard plot of the binding data.
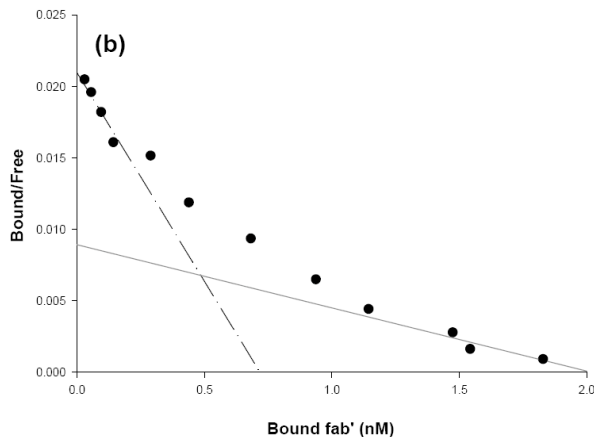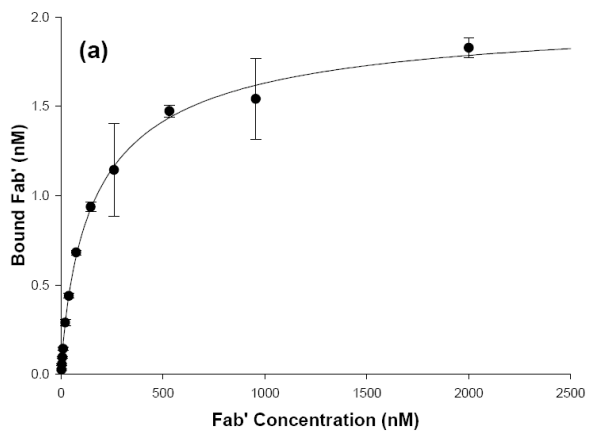
Suporting Information Figure 2. Binding analysis of conjugate *P-3Fab2.9*. (a) The isotherm for conjugate *P-3Fab2.9* was fitted with the Sips equation using the least squares method. (b) Scatchard plot of the binding data.

Suporting Information Figure 3. Binding analysis of conjugate *P-3Fab8.9*. (a) The isotherm for conjugate *P-3Fab2.9* was fitted with the Sips equation using the least squares method. (b) Scatchard plot of the binding data.

Suporting Information Figure 4. Binding analysis of conjugate *P-7Fab1.4*. (a) The isotherm for conjugate *P-3Fab2.9* was fitted with the Sips equation using the least squares method. (b) Scatchard plot of the binding data.