Supporting Information for:

Affinity-Based Precipitation via a Bivalent Peptidic Hapten for the Purification of Monoclonal Antibodies

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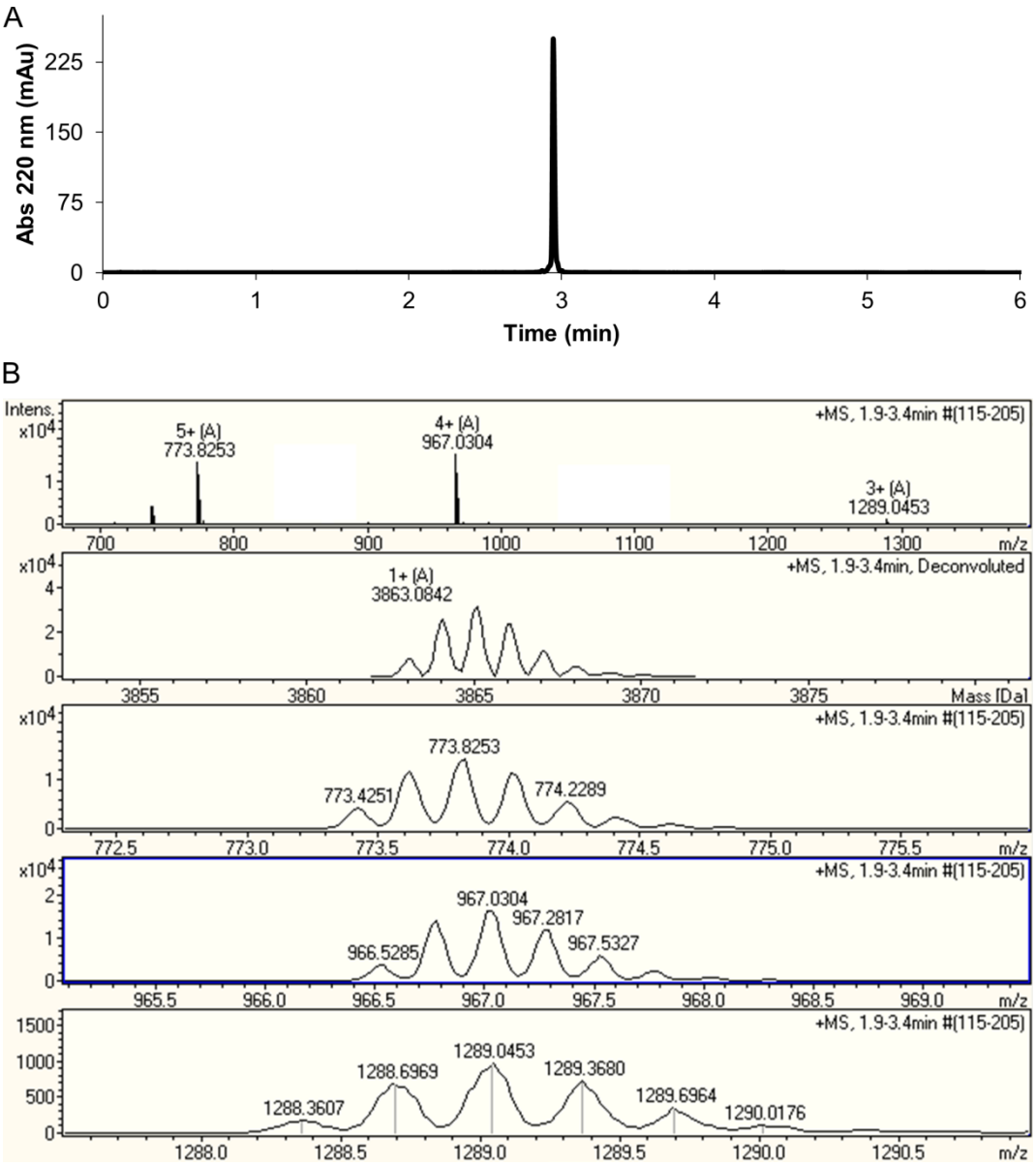

Figure S-1: Characterization of the Bivalent Hapten

(A) The calculated exact mass for the bivalent hapten ($C_{185}H_{272}N_{44}O_{47}$) was 3862.025 Da. Using a Bruker micrOTOFII mass spectrometer, the mass was found to be 3863.084 Da. (B) The purity of the bivalent hapten was determined using RP-HPLC with an analytical Agilent Poroshell 300SB-C8 column (2.1 mm x 75 mm) at 75˚C with a flow rate of 2 mL/min and a rapid gradient from 5% to 100% ACN in 5 minutes. The purity was estimated to be >97%.

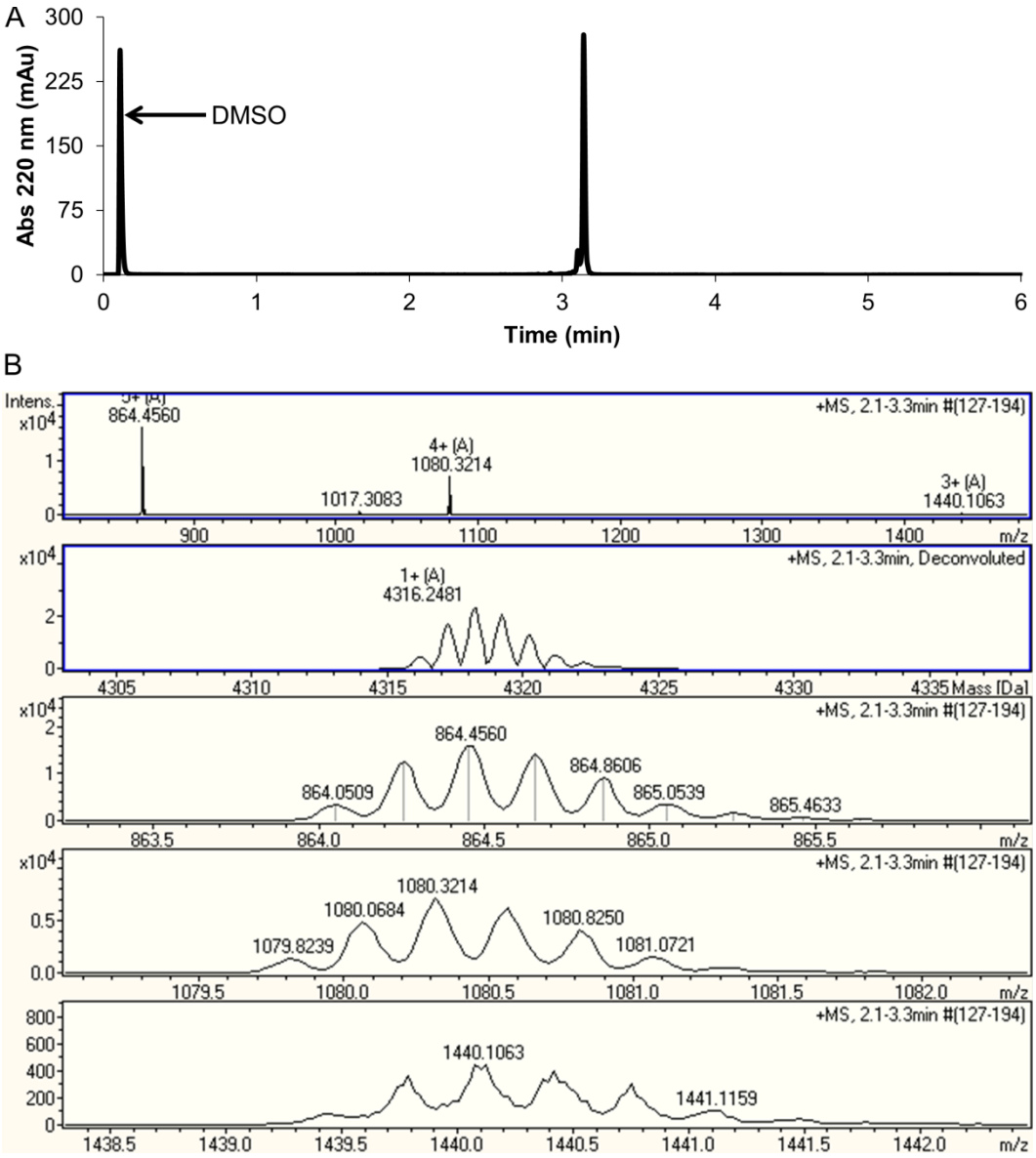

Figure S-2: Characterization of the DNP Labeled Bivalent Hapten

(A) The calculated exact mass for the DNP labeled bivalent hapten ($C_{204}H_{299}N_{49}O_{55}$) was 4315.211 Da. Using a Bruker micrOTOFII mass spectrometer, the mass was found to be 4316.248 Da. (B) The purity of the bivalent hapten was determined using RP-HPLC with an analytical Agilent Poroshell 300SB-C8 column (2.1 mm x 75 mm) at 75˚C with a flow rate of 2 mL/min and a rapid gradient from 5% to 100% ACN in 5 minutes. The purity was estimated to be >97%.

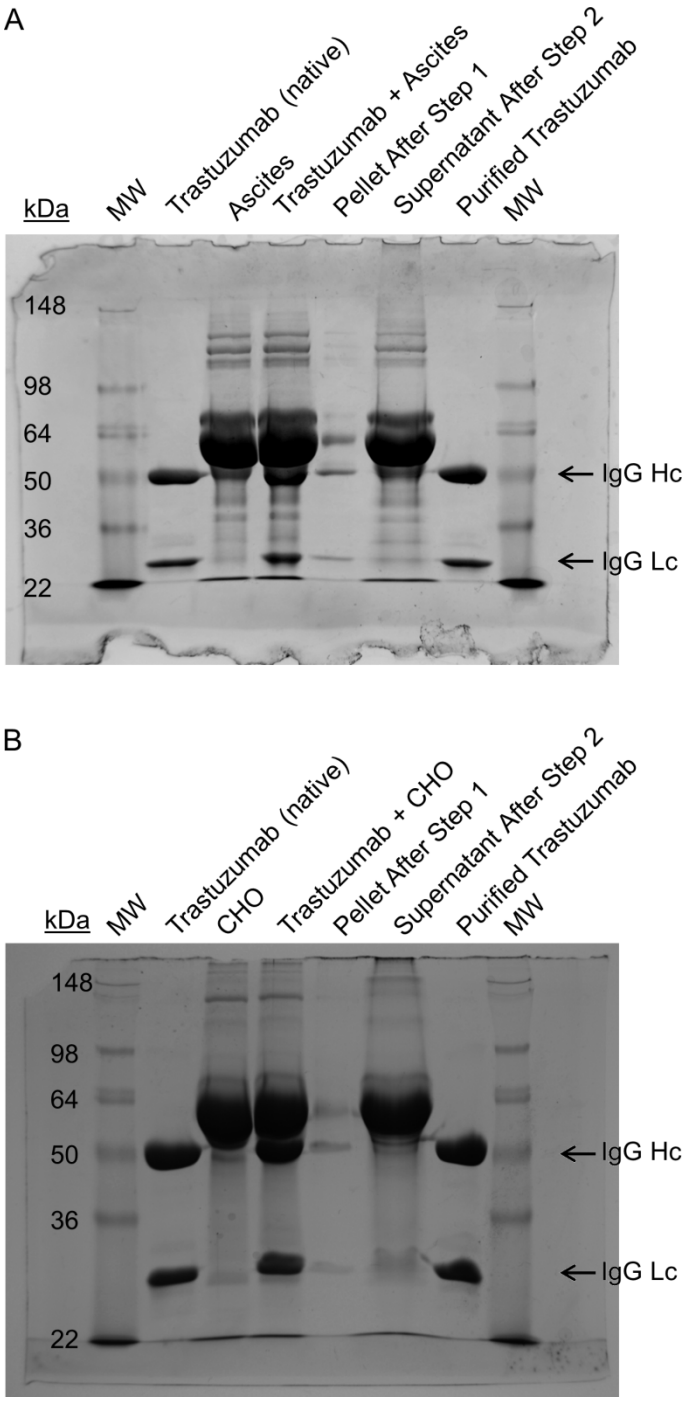

Figure S-3: Analysis of Antibody Purification

Samples from each step of the affinity-based precipitation method for the purification of trastuzumab from (A) ascites fluid and (B) CHO cell conditioned media were analyzed using SDS-PAGE. The purified antibody contained only 2 bands for the heavy and light chains demonstrating high purity.

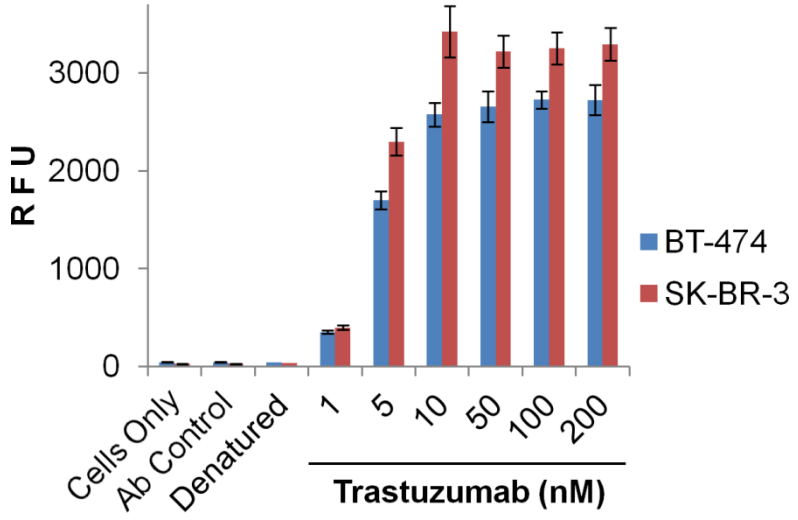

Figure S-4: Optimization of Trastuzumab Cell Binding Assay

Dose response of trastuzumab binding to HER2 expressing cell lines BT-474 and SK-BR-3 cells. The chemically denatured trastuzumab (5 nM) had complete loss of binding. Based on these results, a sub-saturating concentrating of 5 nM trastuzumab was used to evaluate antibody binding activity. The data represents means ± SD of triplicate experiments.