An Antibody CDR3-Erythropoietin Fusion Protein

Yong Zhang[1, 3], Danling Wang[2], Gus Welzel[2], Ying Wang[2], Peter G. Schultz[1, 2]* and Feng Wang[1, 3]*

[1]Department of Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines Road, La Jolla, CA 92037

[2, 3 (present address)] California Institute for Biomedical Research (Calibr), 11119 N. Torrey Pines Road, La Jolla, CA 92307

* Email: schultz@scripps.edu or fwang@calibr.org

Supporting Information

Table S1. List of primers used for generating expression vectors.

Ab N-terminal fragment	forward	5’ CAC ***GAA TTC*** GCA GGT CCA GCT GAG AG 3’
	reverse	5’ TGT GAG TTT TGT CGC AAG ATT TGG GTT CCA CTG CTT TG 3’
Ab C-terminal fragment	forward	5’ ACC CAA ATC TTG CGA CAA AAC TCA CAC ATG CCC ACC GTG 3’
	reverse	5’ CCA ***GCT AGC*** ACT TAT CAT TTA CCC GGA GAC AGG GA 3’
Ab-hEPO N-terminal fragment	forward	5’ CAC ***GAA TTC*** GCA GGT CCA GCT GAG 3’
	reverse	5’ GTG GGG CGC TTC CGC CAC CCC CGC TCT GGT ATT TCT TAG TTT CCT GGT GC 3’
Ab-hEPO hEPO fragment	forward	5’ CGG GGG TGG CGG AAG CGC CCC ACC ACG CCT CAT CTG 3’
	reverse	5’ ACT CCC ACC TCC GCC TCT GTC CCC TGT CCT GCA GG 3’
Ab-hEPO C-terminal fragment	forward	5’ GGA CAG AGG CGG AGG TGG GAG TTC TTA TAC CTA CAA TTA TGA ATG GCA TGT GGA TG 3’
	reverse	5’ CCA ***GCT AGC*** ACT TAT CAT TTA CCC GGA GAC 3’
hEPO	forward	5’ GTC AC***G AAT TC***G GCC CCA CCA CGC CTC ATC TGT 3’
	reverse	5’ CCA ***GCT AGC*** TCA CTA GTG ATG ATG ATG GTG ATG TCT GTC CCC TGT CCT GCA GG 3’
Light chain	forward	5’ CAC ***GAA TTC*** GCA GGC CGT CCT GAA CCA G 3’
	reverse	5’ CCA ***GCT AGC*** TCA CTA GGA GCA CTC GGA TGG 3’

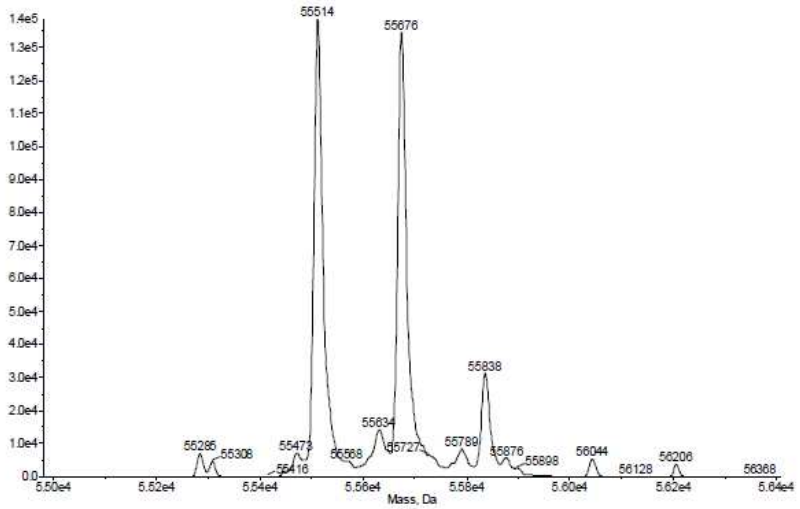

Figure S1. ESI-MS of the heavy chain of BLV1H12 full-length IgG (Ab) treated with DTT (Exp: 54225 Da; Obs: 55514 Da and 55676 Da (due to *N*-glycosylation on Fc region)).

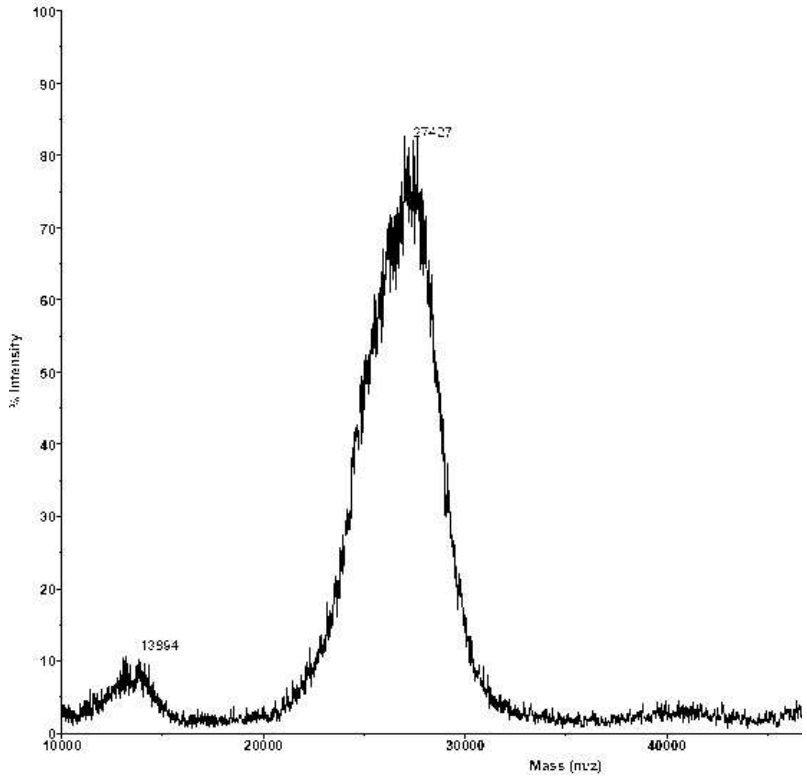

Figure S2. ESI-MS of the hEPO (Exp: 19219 Da; Obs: 22000~31000 Da (due to heterogeneous glycosylation)). The y-axis is shown in % intensity due to poor ionization caused by glycosylation.

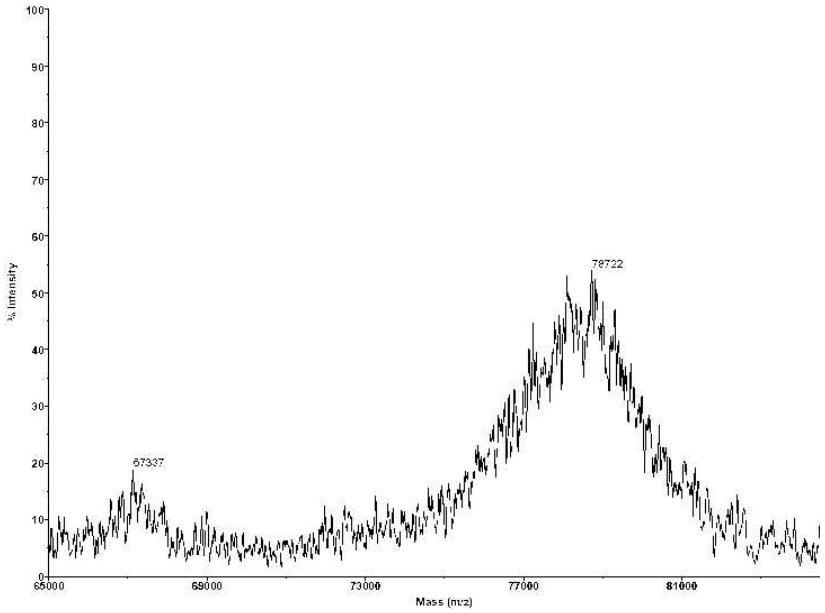

Figure S3. ESI-MS of the heavy chain of Ab-hEPO fusion protein treated with DTT (Exp: 69018 Da; Obs: 73000~82000 Da (due to heterogeneous glycosylation on hEPO)). The y-axis is shown in % intensity due to poor ionization caused by glycosylation.

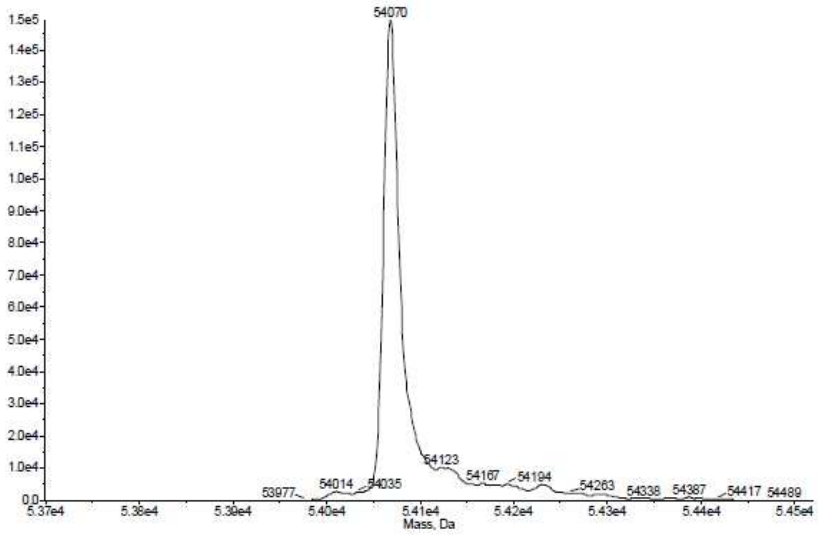

Figure S4. ESI-MS of the heavy chain of Ab treated with Peptide-*N*-Glycosidase and DTT (Exp: 54225 Da; Obs: 54070 Da (matching the mass of Ab without Gln1)).

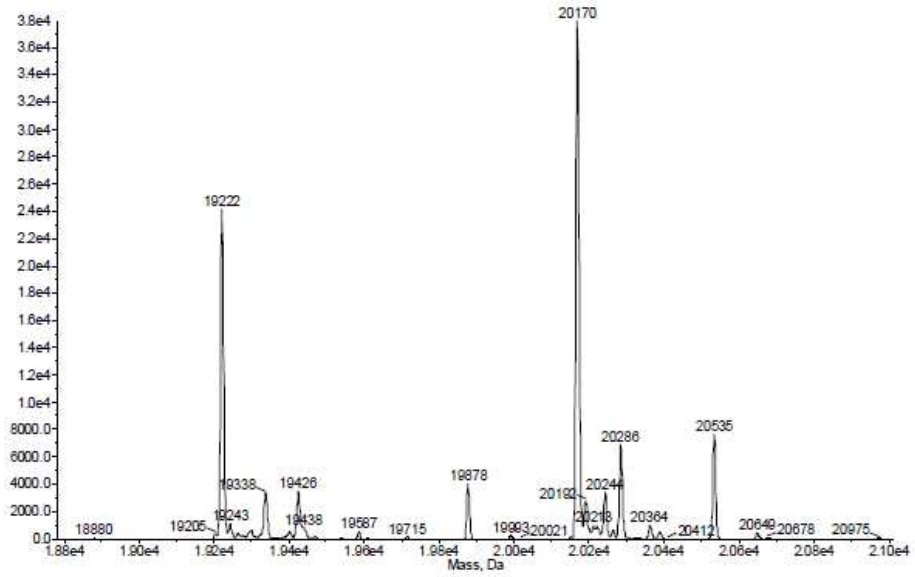

Figure S5. ESI-MS of the hEPO treated with Peptide-*N*-Glycosidase (Exp: 19219 Da; Obs: 19222 Da and 20170 Da (due to *O*-glycosylation)).

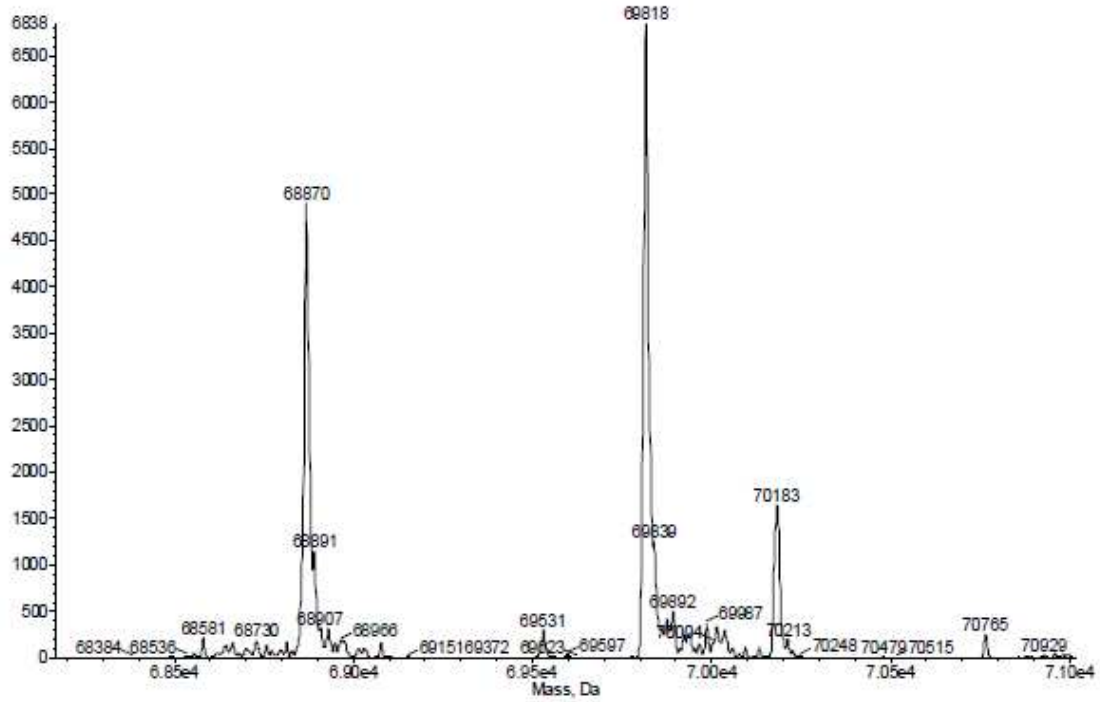

Figure S6. ESI-MS of the heavy chain of Ab-hEPO fusion protein treated with Peptide-*N*-Glycosidase and DTT (Exp: 69018 Da; Obs: 68870 Da (matching the mass of Ab-hEPO without Gln1) and 69818 Da (due to *O*-glycosylation on hEPO)).

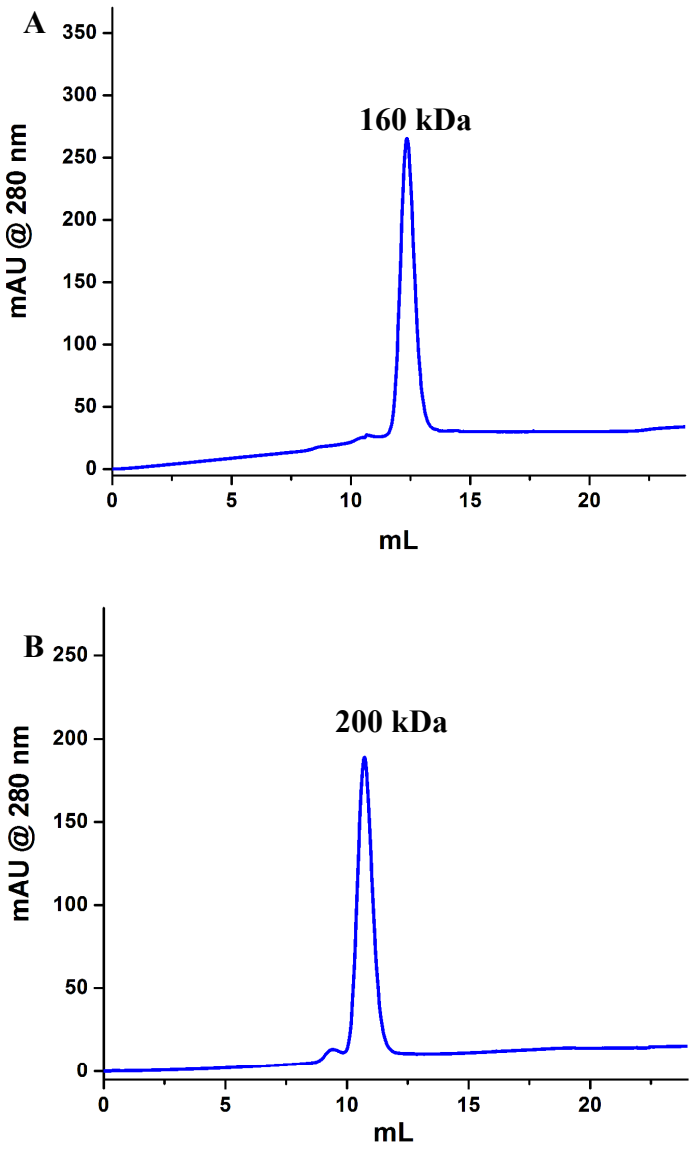

Figure S7. Gel filtration analysis of (A) Ab and (B) Ab-hEPO. 300 μL of Ab (1.4 mg/mL) or Ab-hEPO fusion protein (1.0 mg/mL) in PBS (pH7.4) was injected into Superdex 200 10/300 GL column (GE lifesciences), followed by elution using the same buffer. UV absorbance at 280 nm was plotted versus the elution volume. The apparent molecular weights of Ab and Ab-hEPO were estimated on the basis of their corresponding elution volume by referring to the elution profiles of a series of standard proteins provided in the manufacturer's instruction.