Supplementary Information For:

A Non-Chromatographic Method for the Purification of a Bivalently Active Monoclonal IgG Antibody from Biological Fluids

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SUPPLEMENTARY METHODS

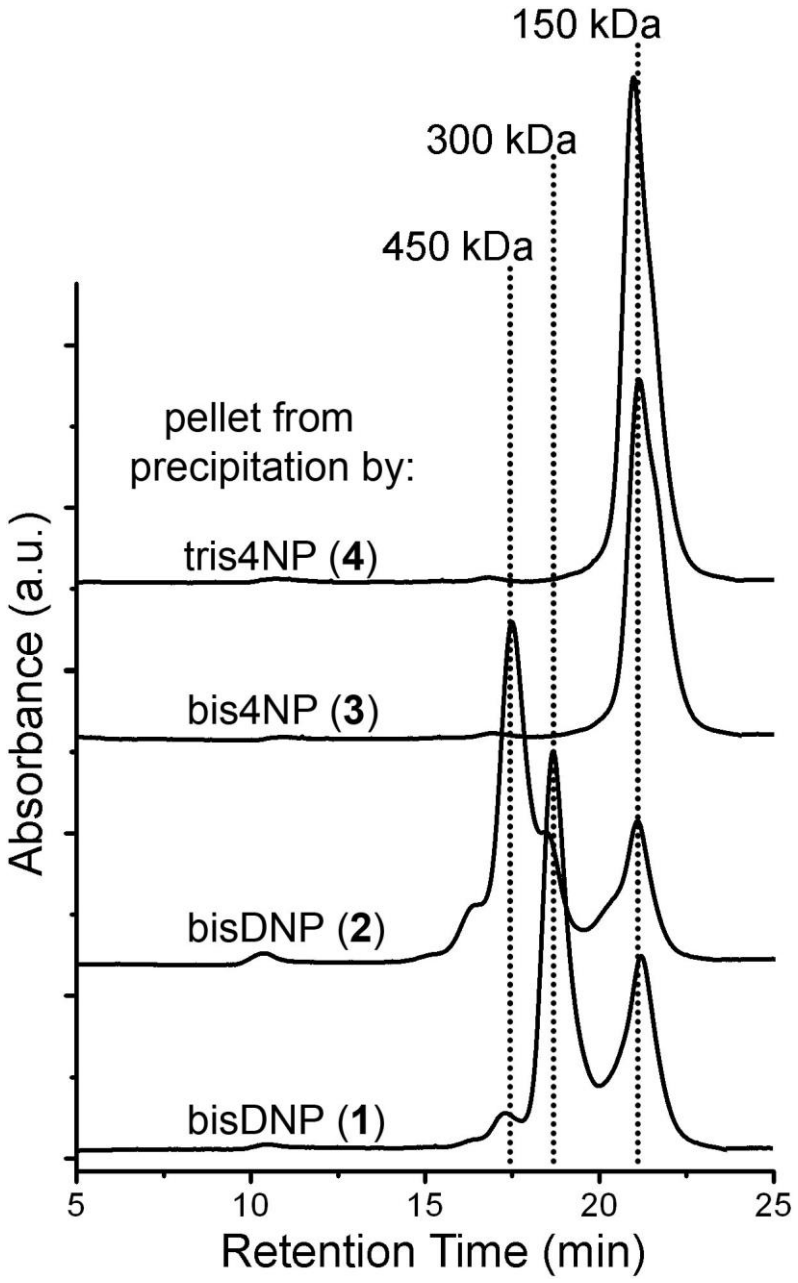
Chemicals. We purchased *N*-Fmoc-amido-dPEG$_8$^{TM}-acid from Quanta BioDesign, Ltd, HBTU from Novabiochem, *Tris*-succinimidyl aminotriacetate from Molecular Probes, Inc., *Nε*-DNP-Lysine from Sigma-Aldrich Co., *N*,*N*-diisopropylethylamine (DIEA) from Sigma, Monoclonal rat anti-2,4-DNP IgG antibodies (IgG^{DNP}) from Zymed, Inc (Invitrogen), and ascites fluid from rat containing monoclonal anti-DNP IgG from Technopharm, France. We purchased *N*,*N*-Dimethylformamide (>99.8%) and dimethyl sulfoxide (DMSO) (>99.8%) from EMD; and acetonitrile (>99.8%) from Mallinckrodt Chemicals. We used IgG^{DNP} without further purification. We estimate the purity of the commercial IgG^{DNP} to be >94%, but we did not determine its purity.

Synthesis of Multivalent Ligands. We dissolved 10 mg of *N*-Fmoc-amido-dPEG$_8$^{TM}-acid in 2 mL of *N*,*N*-dimethylformamide and used 1.2 equiv of 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU) to activate the carboxylic acid group with 2 equiv of *N*,*N*-diisopropylethylamine (DIEA). After 5 min, we added the activated *N*-Fmoc-amido-dPEG$_8$^{TM}-acid (purchased from Quanta Biodesign) to 3 equiv of *Nε*-DNP-Lysine (Sigma) dissolved in 1mL of dimethyl sulfoxide (DMSO). We let this reaction, which gave a quantitative yield (as determined by liquid chromatography), run for 2 h at room temperature before quenching it by the addition of 4 mL of 0.1% trifluoroacetic acid in water. We purified the product via reversed-phase high pressure liquid chromatography (RP-HPLC) as described in the next section. We removed the Fmoc group on the purified coupling product using 20% piperidine in DMF for 1 h, isolated the compound with the free amine using RP-HPLC, then lyophilized. We dissolved the lyophilized product in DMF in the presence of 2 equiv of DIEA,

and added it drop-wise, over a period of one hour, to a one-third equivalence of *Tris*-succinimidyl aminotriacetate (purchased from Pierce) dissolved in 1 mL of DMF. After running the reaction overnight, analytical HPLC showed the yield of ~60% (side products of this reaction are mono and bi substituted aminotriacetate). We purified the products (haptens **1** and **2**) via RP-HPLC and characterized it using MALDI-TOF. Synthesis of haptens **3** and **4** are carried out following the same procedure by using *Nε*-4-NP-Lysine as the starting compound.

Purification of multivalent ligands. We performed RP-HPLC purifications on a Vydac C18 column (10 mm x 250 mm, 300 Å pore size, 10 µm particle size), using linear solvent gradients of 1% per minute increments in acetonitrile concentration at 2.5 mL/min flow rate on a Dynamax Rainin system. We monitored the column eluant using UV absorbances at 218 nm and 360 nm with a dual wavelength UV detector, Dynamax model UV-D II.

Determination of the Dissociation Constants of Antibody for 2,4-DNP and 4-NP. The binding of DNP derivatives to IgG^{DNP} quenched the fluorescence of tryptophan residues of the antibody.(ref) Fluorescence titration of the IgG^{DNP} from clone (LO-DNP-2) with DNP-Lys, yielded a monovalent dissociation constant ($K_d^{affinity}$) of 0.80 ± 0.08 nM. The same analysis for association of 4-NP-Lys to this IgG^{DNP} yielded a $K_d^{affinity}$ value of 0.5 µM (supplementary material). Sigma reported the binding constant for anti-digoxin (K_d = 0.02 nM).



Supplementary Figure S.1. SE-HPLC chromatograms of re-dissolved pellets from step 2 of the procedure. The pellets from precipitation with bi- and trivalent DNP ligands (**1** and **2**) had significant amount of the complexes retained, while the pellets from precipitation with bi- and trivalent 4-NP ligands (**3** and **4**) eluted as a single peak of monomeric IgG.