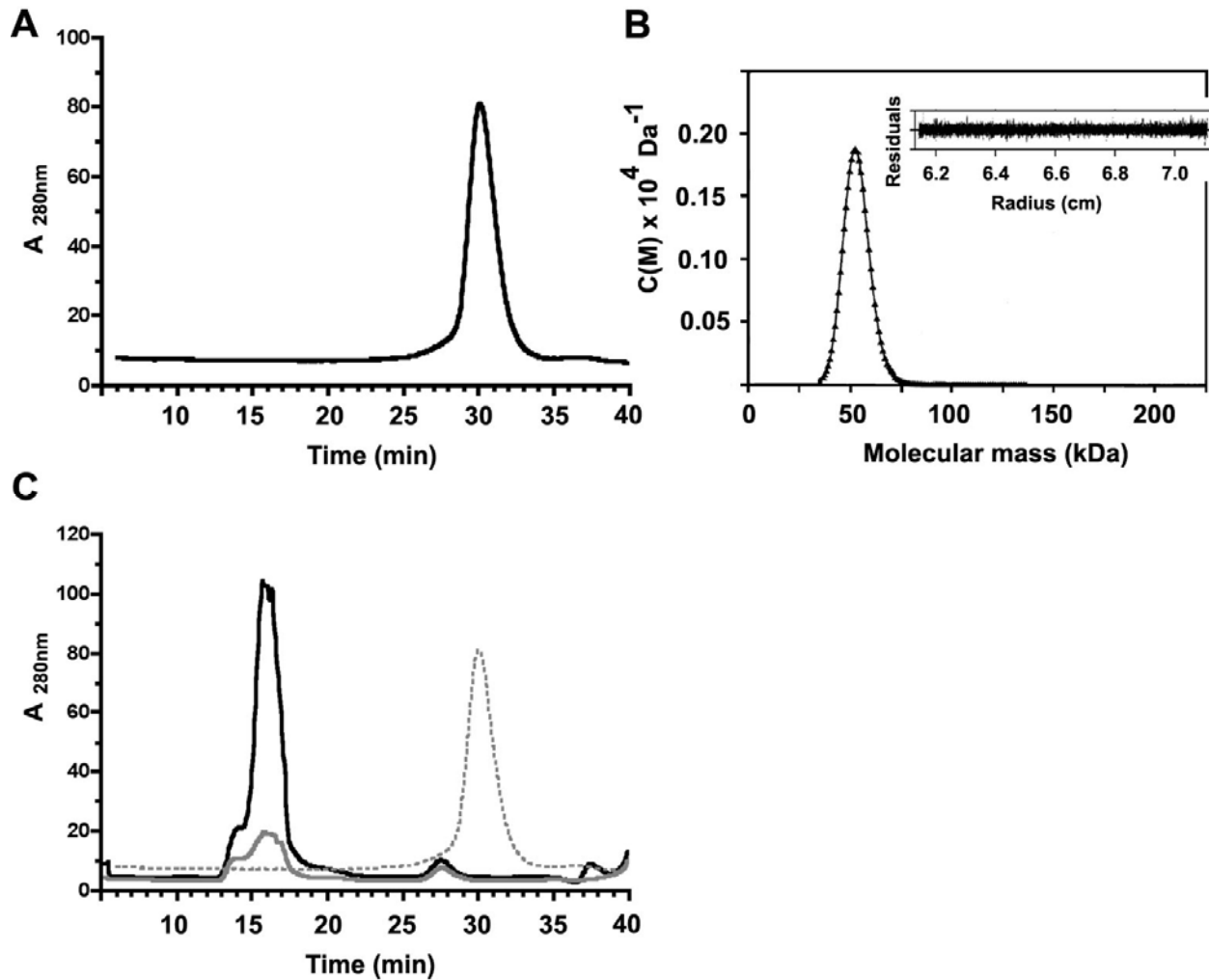
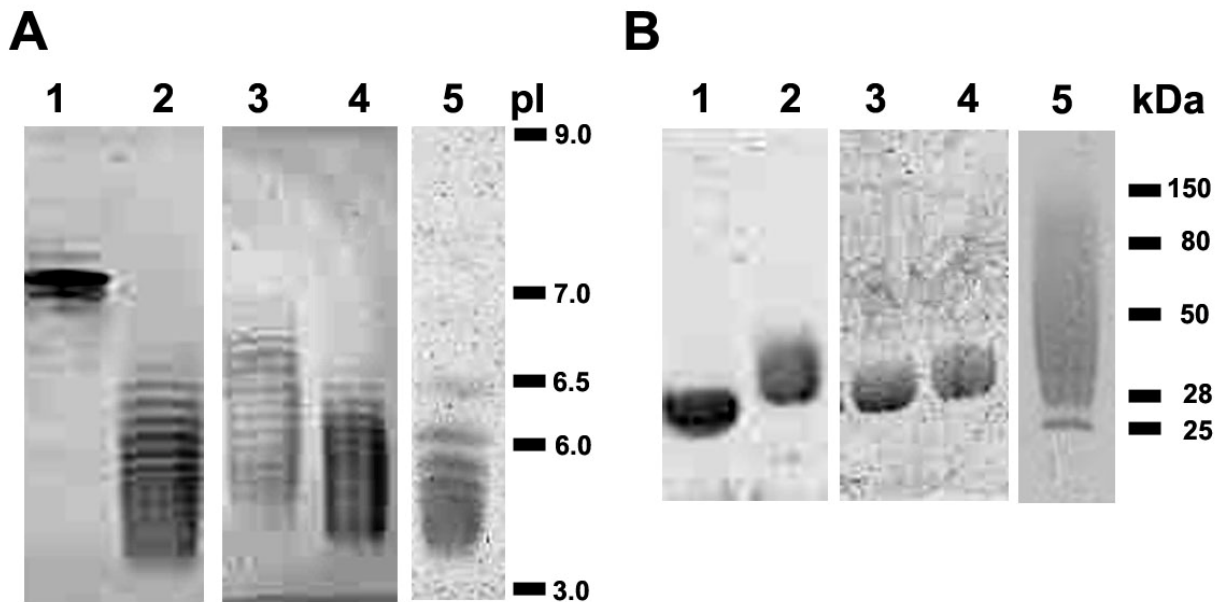


Supplemental Scheme 1. Synthesis of DOTA-PEG-Cys-VS. Fmoc-amido-PEG-acid was conjugated to S-t-butyl cysteine on Wang resin using standard activation chemistry (DCC/HOBt). The Fmoc was removed with piperazine and conjugated to DO3AtBuAc using standard activation chemistry (DCC/HOBt). The product was removed from the resin with TFA, purified by reverse phase HPLC, reacted with excess vinyl sulfone in DMF, and repurified by reverse phase HPLC.

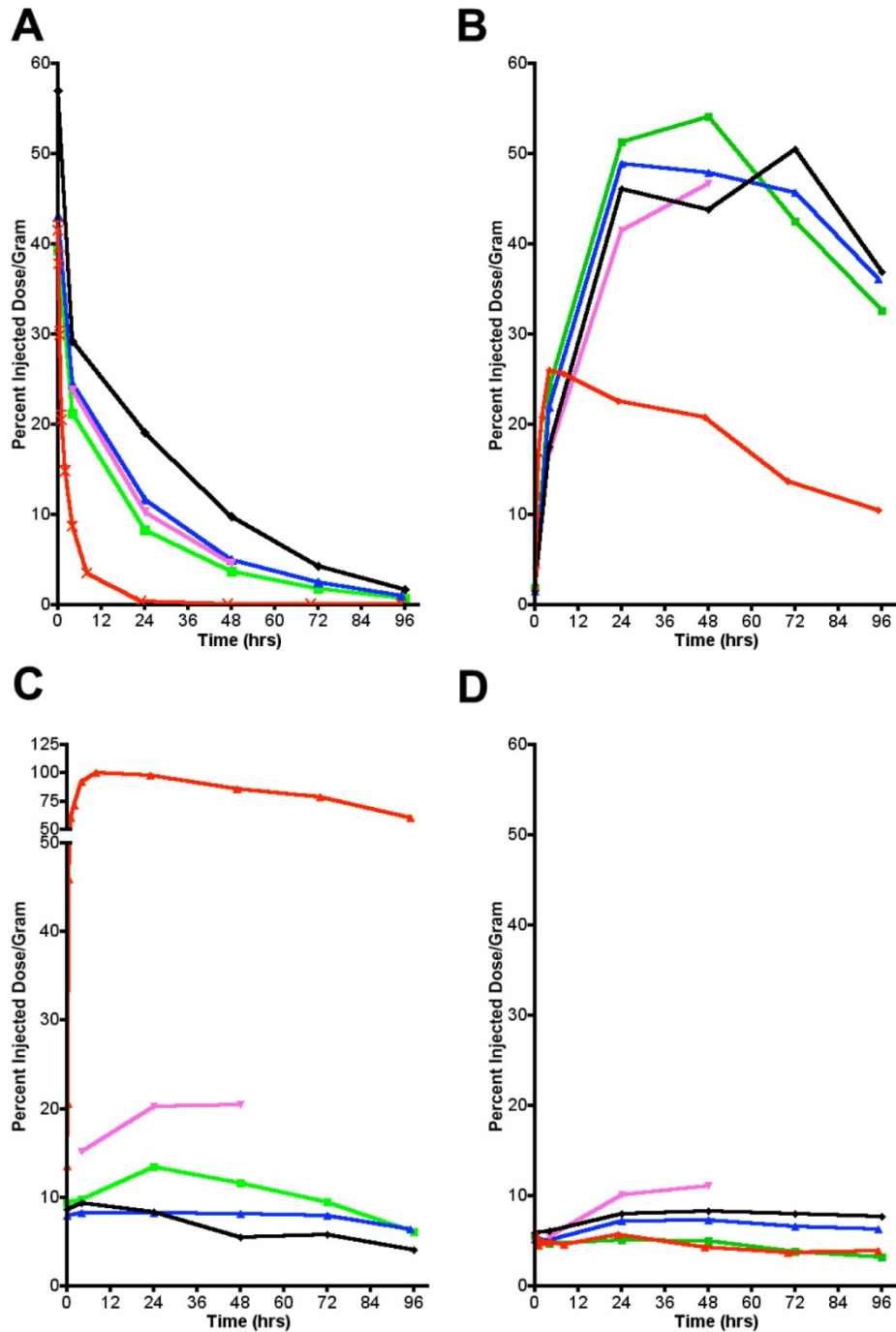


Supplemental Figure 1. Characterization of AVP04-07 Diabody.

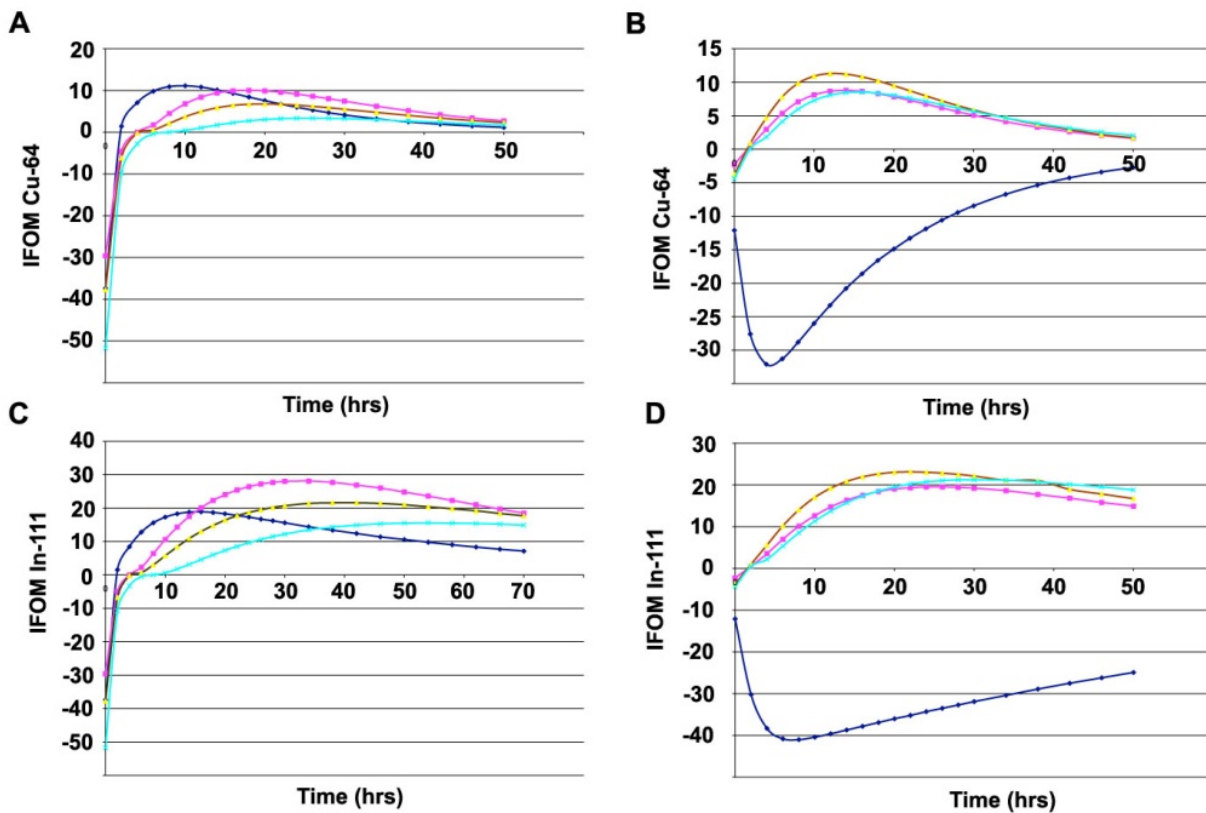
AVP04-07 expressed in *E. coli* was purified to homogeneity as indicated by a single elution peak on Superdex 200 gel filtration (**A**) and was further shown to be both dimeric and monodispersed by analytical ultracentrifugation (**B**), with an apparent molecular weight of 52 kDa. AVP04-07 diabody was able to bind antigen *in vitro* as shown by reduced elution time (i.e.; increases in apparent molecular weight) of diabody-antigen complexes (**C**, black solid line) when compared to AVP04-07 alone (**C**, grey dotted line) and antigen alone (**C**, gray solid line).



Supplemental Figure 2. Isoelectric focusing and SDS gel electrophoresis of AVP04-07 and its PEG conjugates. **A.** Isoelectric focusing: 1, non-PEGylated diabody; 2, PEG27 conjugate, 50:1 (ratio of PEGylating agent to diabody); 3, PEG12 conjugate, 20:1 (ratio of PEGylating agent to diabody); 4, PEG12 conjugate, 50:1 (ratio of PEGylating agent to diabody); 5, PEG3400 conjugate, 30:1 (ratio of PEGylating agent to diabody). **B.** SDS gel electrophoresis: 1, intact diabody; 2, PEG27 conjugate; 3, PEG12 conjugate; 4, PEG12 conjugate; 5, PEG3400 conjugate.



Supplemental Figure 3. Comparative blood, tumor, kidney and liver curves for ^{111}In -DOTA labeled PEGylated diabody derivatives and intact diabody. A. Blood clearance curves. B. Tumor uptake curves. C. Kidney uptake curves. D. Liver uptake curves. Red: intact diabody. Magenta: PEG3400 diabody conjugate, 15:1 molar ratio. Black: PEG3400 diabody conjugate, 60:1 molar ratio. Blue: PEG27 diabody conjugate. Green: PEG12 diabody conjugate.



Supplemental Fig 4. IFOM plots for tumor vs. blood and tumor vs. kidney for non-PEGylated diabody and its PEGylated conjugates.

A. Tumor vs. Blood, ^{64}Cu -labeled AVP04-07 and its derivatives. **B.** Tumor vs. kidney, ^{64}Cu -labeled AVP04-07 and its derivatives. **C.** Tumor vs. blood, ^{111}In -labeled AVP04-07 and its derivatives. **D.** Tumor vs. kidney, ^{111}In -labeled AVP04-07 and its derivatives. Dark Blue: unmodified diabody. Light Blue: PEG3400 diabody conjugate. Yellow: PEG27 diabody conjugate. Magenta: PEG12 diabody conjugate.