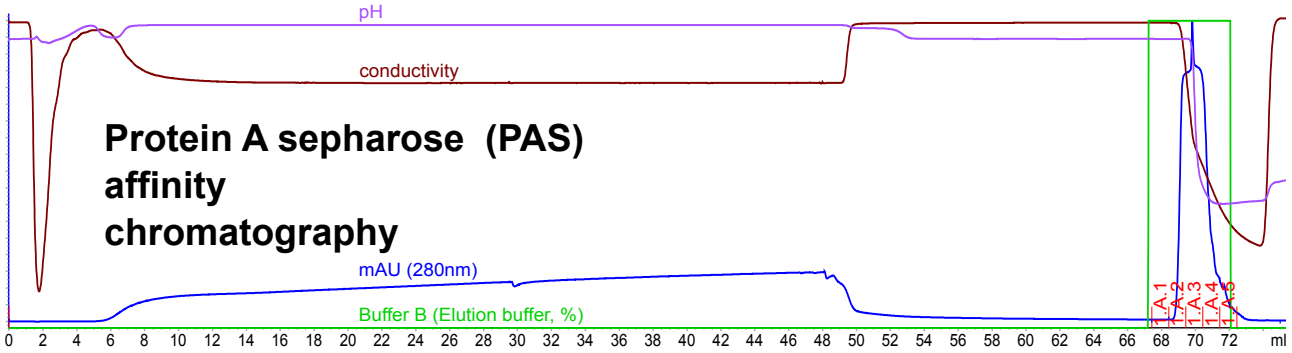
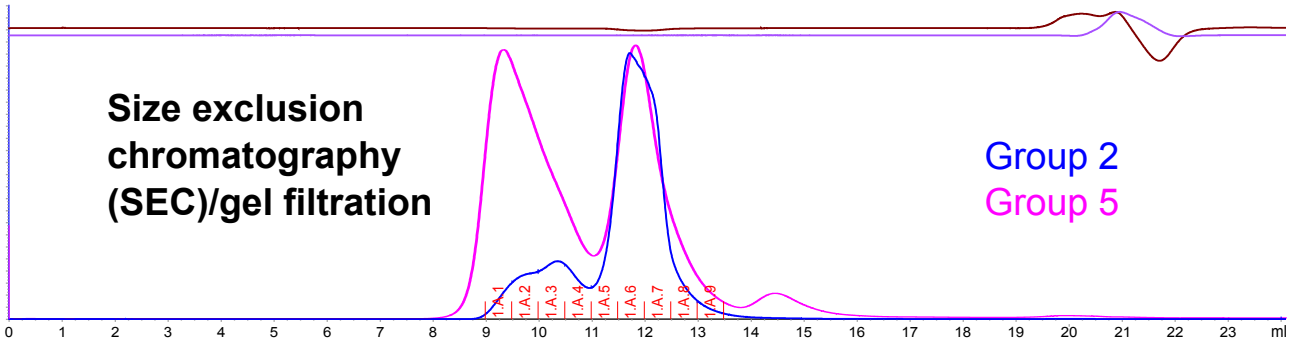
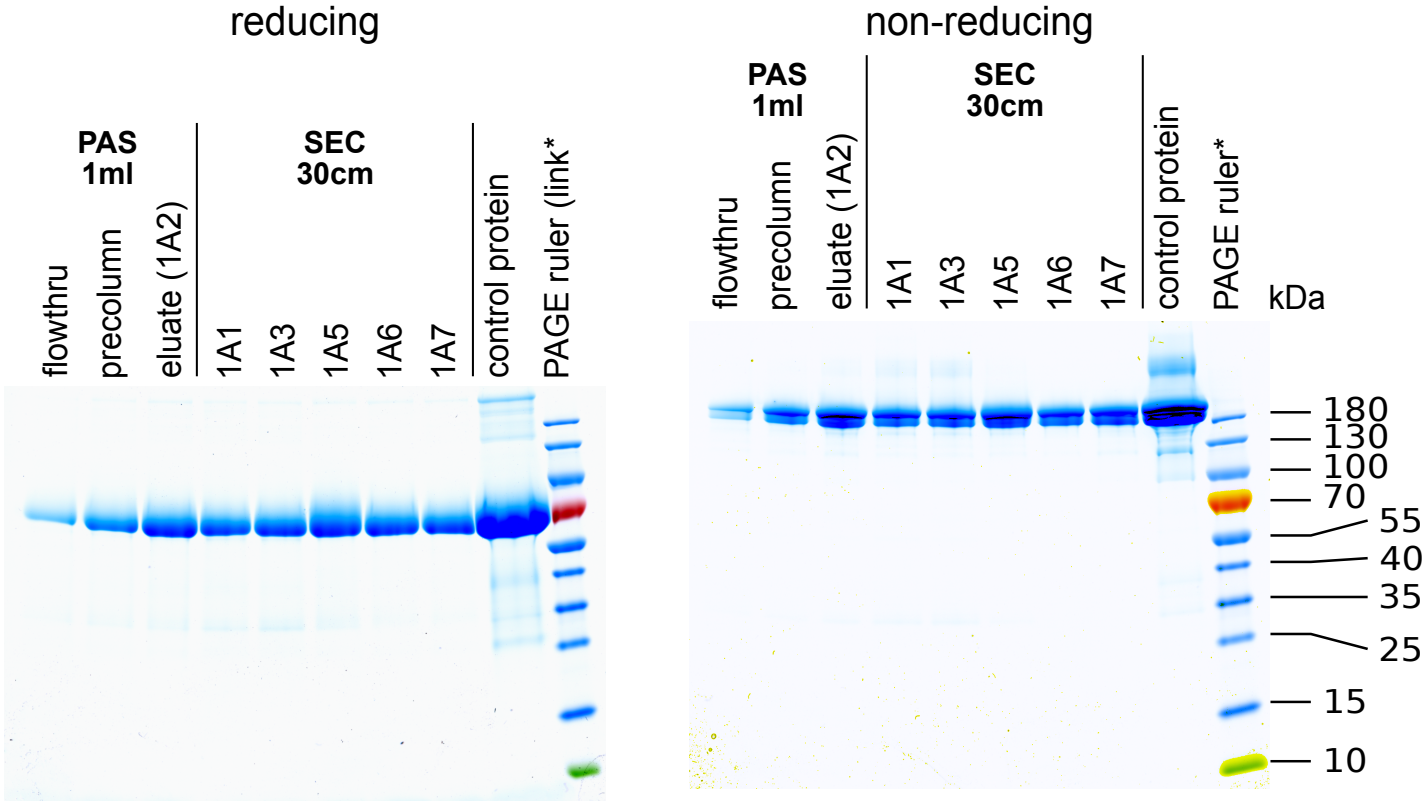
Purification of hVEGFR-3(ECD1-3)/hIgG$_1$Fc







Purification of a fusion protein (extracellular domains 1-3 of VEGFR-3 fused to the F_C portion of human IgG_1 (Jeltsch et al., JBC, 2006). Coomassie-stained 4-20% PAGE; PAS: 1 ml Baby A Protein A sepharose (BioWorks, equivalent to GE Healthcare 1 ml HiTrap rProteinA FF or similar), loading at pH 7.5, wash with PBS 0.05% Tween 20, step elution with 0.1M Na citrate pH 3.0, neutralization with 200 µl 1M Tris/HCl pH 8.5/ml eluate; SEC: Superdex 200 Increase 10/300 (GE Healthcare), buffer PBS, 0.5 ml/min

Despite the clear heterogeneity observed during the gel filtration run, the fractions are indistinguishable on both reducing and non-reducing Comassie-stained gels. Most likely they represent different aggrgated and oligo/multimeric forms, that are seperated by the sample preparation in Laemmli buffer. The higher the group number, the more aggregated and degraded protein was observed. This is likely due to the fact that we recycled the protein and that the neutralization step failed twice and thefore proteins were left o/n at pH 3.

*https://www.thermofisher.com/order/catalog/product/26616