Supplementary Figure 1 Gain in functional binding affinity due to multivalency. Antigen binding ELISA of designed constructs. Purified p185^{HER2-ECD} antigen was coated on microtiter plates, fusion proteins were applied and detection was performed using an anti-His-tag antibody⁵. All constructs have the same 4D5 scFv moiety and demonstrated specific binding. As every construct or assembly has only a single His-tag, the binding signal could be directly compared. The monovalent 4D5 scFv-barnase and 4D5 scFv-dibarnase fusions produced signals of similar intensities. The 4D5 scFv-barstar fusion demonstrated slightly higher signals, presumably due to aggregation of the construct. The highest binding was observed for the dimeric and trimeric barnase:barstar complexes. This translates to lowest apparent functional affinity for the monomers, intermediate affinity for dimeric complexes and highest functional affinity for the trimers.

