

Figure S1. Biosensor analysis of the interactions of ADAPT₆-ABD-PE25, ADAPT₆-ABD-PE38X8 and ADAPT₆-PE25 with HER2. Dilution series of the fusion toxins were injected over a flow cell with immobilized extracellular domain of HER2. All experiments were repeated once and each panel is an overlay of all concentrations in duplicates, for each fusion toxin. The numbers to the right of each panel indicates the concentration of the injected fusion toxin corresponding to each sensorgram. Chi² is the square of the average deviation of the overlayed experimental curves in each panel from the theoretical curves based on the calculated on- and off-rates in Table II. ADAPT, ABD-derived affinity protein; ABD, albumin binding domain; HER2, human epidermal growth factor receptor 2.

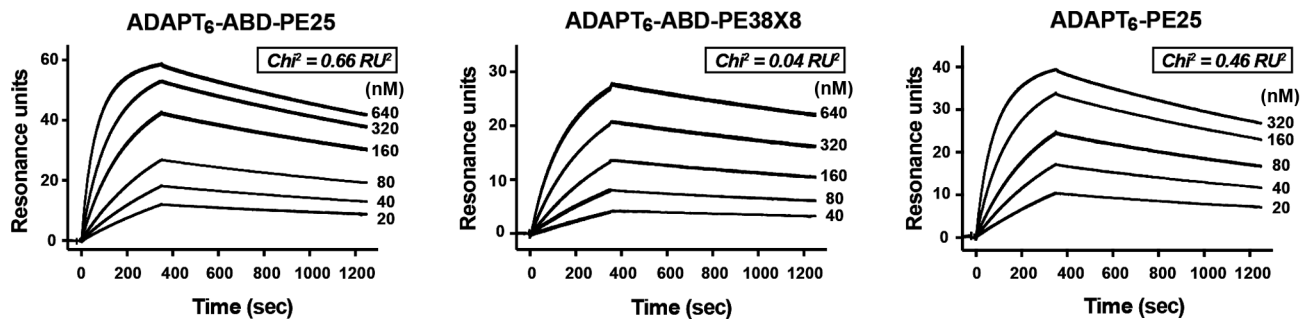


Figure S2. Biosensor analysis of the interactions of ADAPT₆-ABD-PE25, ADAPT₆-ABD-PE38X8 and Z_{Taq}-ABD-PE25 and serum albumins. Dilution series of the fusion toxins (Analyte) were injected over a flow cell with immobilized human or mouse serum albumin [ligand: (A) HSA or (B) MSA, respectively]. All experiments were repeated once and each panel is an overlay of all concentrations in duplicates, for each fusion toxin. The numbers in each panel indicates the concentration of the injected fusion toxin corresponding to each sensorgram. Chi² is the square of the average deviation of the overlaid experimental curves in each panel from the theoretical curves based on the calculated on- and off-rates in Table III. ADAPT, ABD-derived affinity protein; ABD, albumin binding domain.

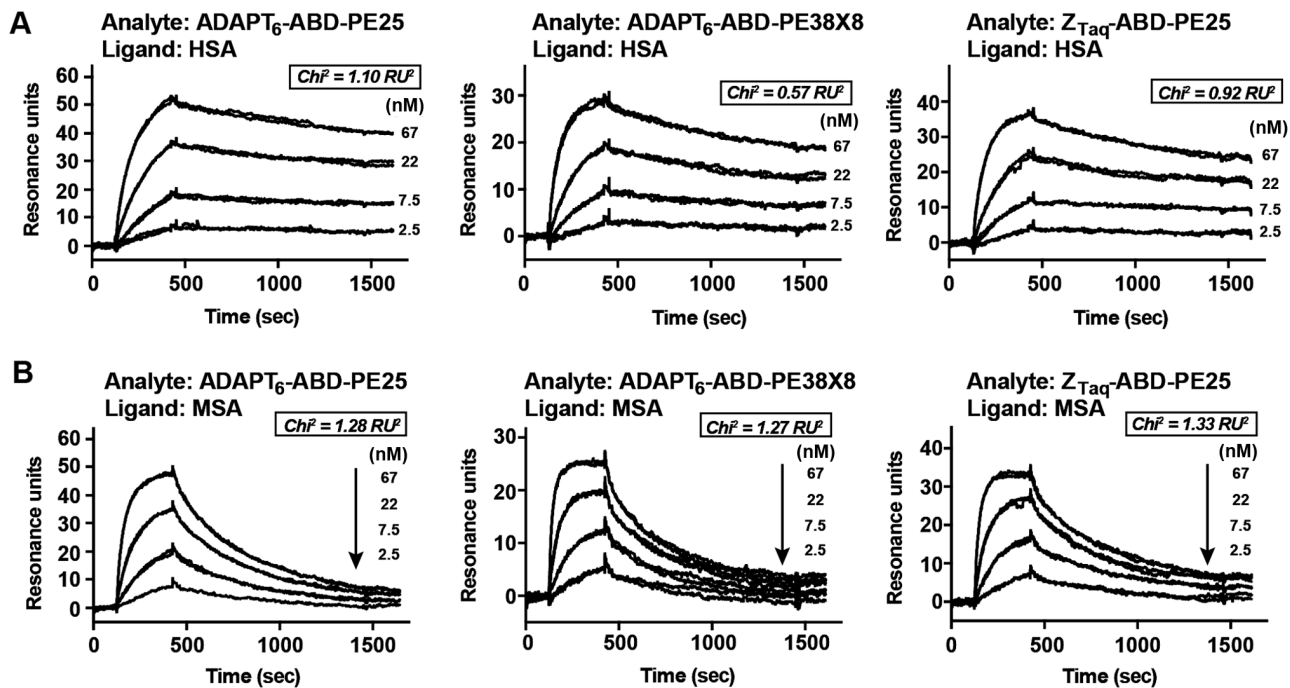


Figure S3. Analysis of the specificity of ADAPT₆-PE25 and Z_{Taq}-ABD-PE25. The two proteins were injected at a concentration of 320 nM over surfaces with immobilized extracellular domain of HER2, HSA or MSA. Each injection was performed twice. Each panel is an overlay of the four curves corresponding to the duplicate injection of ADAPT₆-PE25 and Z_{Taq}-ABD-PE25 in each flow channel. As expected, ADAPT₆-PE25 interacts with the HER2 surface, but does not interact with the HSA or MSA surfaces, since it lacks the ABD. Z_{Taq}-ABD-PE25 does not interact with the HER2 surface since it lacks an HER2 binding motif, but interacts with both the HSA and the MSA surfaces. ADAPT, ABD-derived affinity protein; ABD, albumin binding domain; HER2, human epidermal growth factor receptor 2; HSA, human serum albumin; MSA, mouse serum albumin.

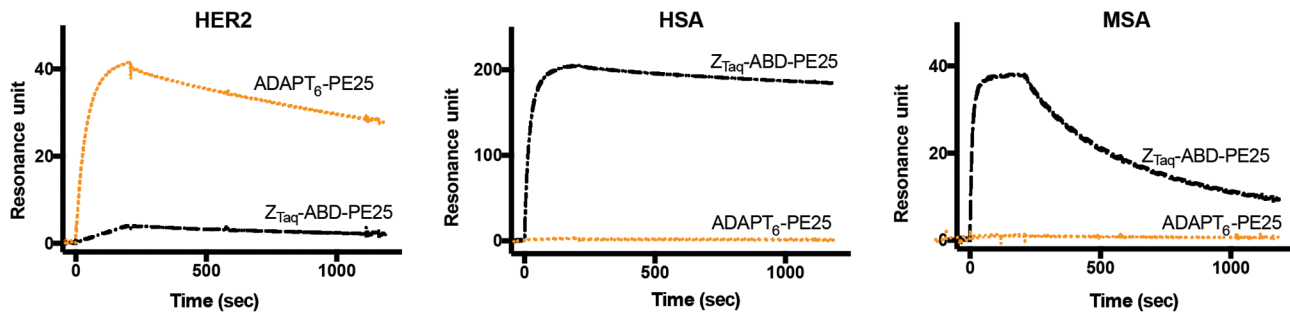


Figure S4. Analysis of relative expression levels of HER2. The SKBR3, AU565, SKOV3 and A549 cell lines were stained with the HER2-specific antibody trastuzumab, followed by an Alexa Flour 647 conjugated secondary antibody and analyzed by flow cytometry. Each panel is an overlay of three recorded histograms. The y-axis shows the number of recorded events and the x-axis shows the fluorescence of the cells. A large shift of median fluorescence intensity from the two controls to the cells stained with both trastuzumab and the secondary antibody was found for SKBR3, AU565 and SKOV3 cells, indicative of a high surface density of HER2. A moderate shift was recorded for A549, indicating a moderate HER2 expression level. For all panels, no shift in fluorescence intensity from the unstained cells (green) to the cells stained with secondary antibody only (red) was recorded, indicating that the secondary antibody did not stain the cells non-specifically at the concentration used. HER2, human epidermal growth factor receptor 2.

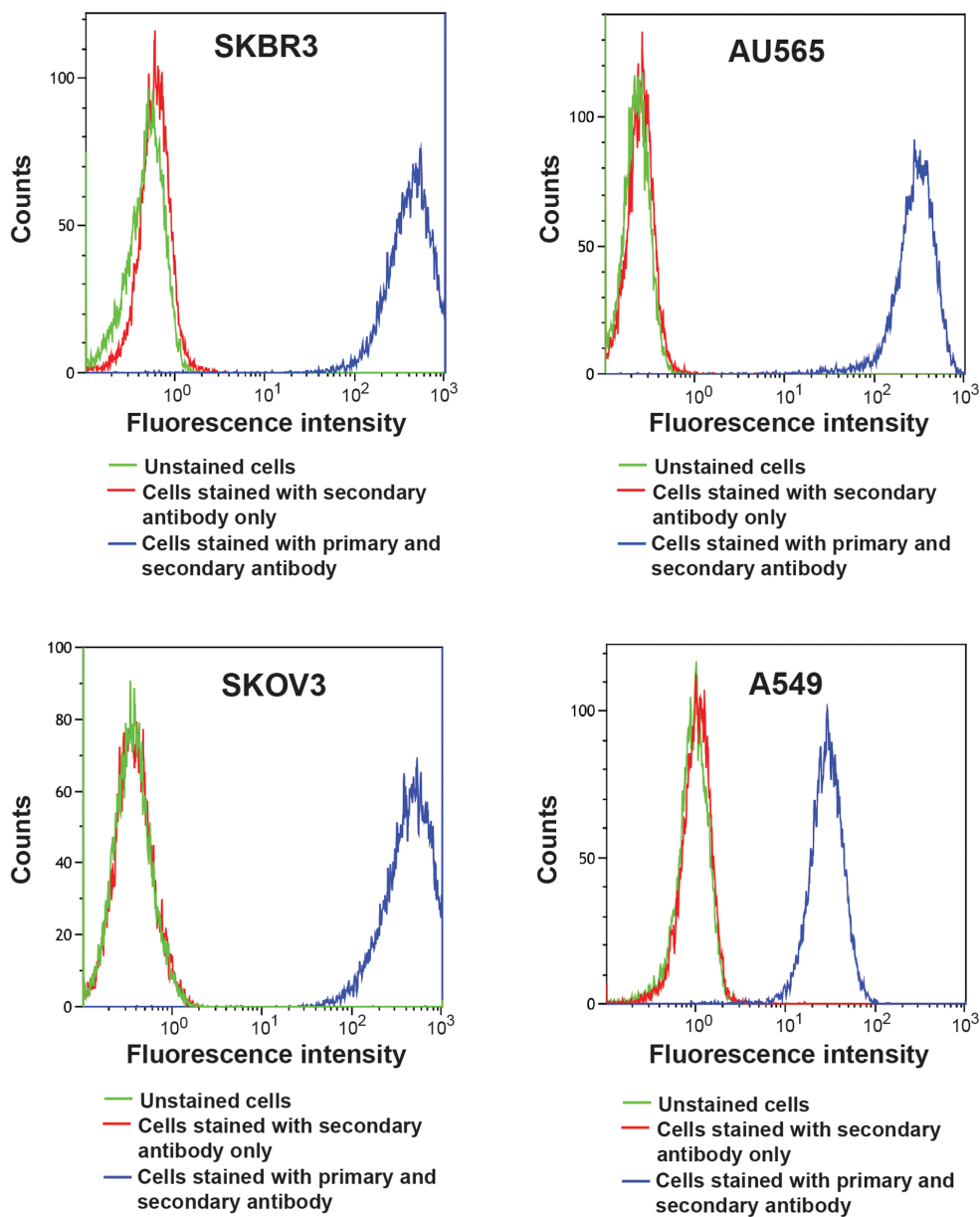


Table SI. Analysis of radiolabeling of the fusion toxins with ¹¹¹ In.			
	Yield (%) ^a	Radiochemical purity (%) ^a	Stability under 4 h EDTA challenge (%) ^a
ADAPT ₆ -ABD-PE25	65±3	97±0.5	96±1
ADAPT ₆ -ABD-PE38X8	99.5±1	N/A ^b	99.8±0.3
ADAPT ₆ -PE25	99±1	N/A	99±1
Z _{HER2:2891} -ABD-PE38X8	97±2	N/A	98.7±0.3

^aThe yield, radiochemical purity and stability under 4 h EDTA were determined by separating fusion-toxin-bound- and unbound- radioactivity using an instant thin layer chromatography plate impregnated with silica gel (ITLC-SG) as a stationary phase and 0.2M citric acid as mobile phase as described in experimental section. ^bNot applicable.