

# Figure S1 A & C

**A**



**C**

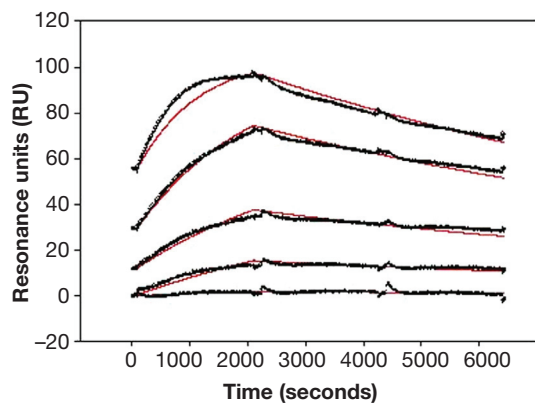
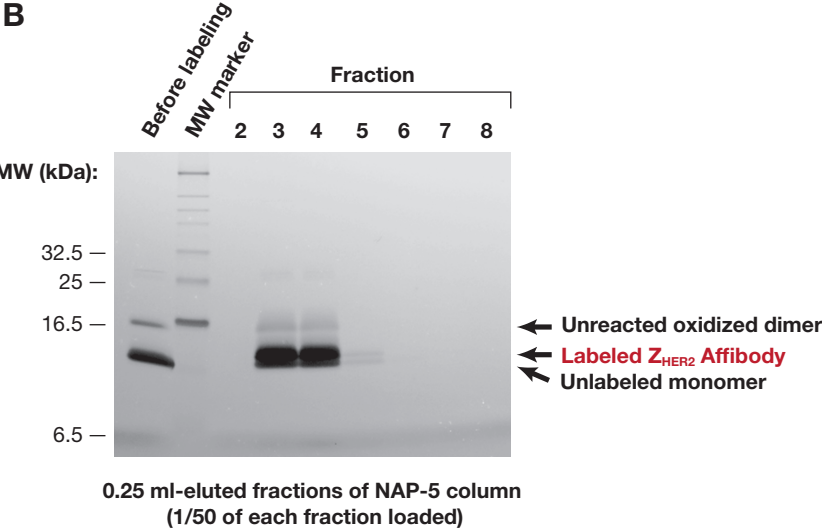


Figure S1 B



Type of file: table

Label: tables and legends

Filename: Supplementary Tables and Figure legend.pdf

**Supplementary Table S1.** FACS analyses after labeling with Affibody-Alexa Fluor 488 conjugate

<b>Labeling of SKBR-3 cells</b>	<b>% of control</b>
Alexa Fluor 488 Dye (—)	1.1 ± 0.5
5 nM Affibody-Alexa Fluor 488 conjugate (—)	94.6 ± 2.7
50 nM Affibody-Alexa Fluor 488 conjugate (—)	98.9 ± 0.6
5 nM conjugate + excess unlabeled Affibody (—)	0.8 ± 0.3
50 nM conjugate + excess unlabeled Affibody (—)	1.1 ± 0.7

<b>Labeling of U251 cells</b>	<b>% of control</b>
Alexa Fluor 488 Dye (—)	0.9 ± 0.4
5 nM Affibody-Alexa Fluor 488 conjugate (—)	2.2 ± 1.5
50 nM Affibody-Alexa Fluor 488 conjugate (—)	5.3 ± 2.2
5 nM conjugate + excess unlabeled Affibody (—)	1.0 ± 0.7
50 nM conjugate + excess unlabeled Affibody (—)	3.4 ± 1.1

NOTE: Percentages of cells under the gate designated as M1 in each sample shown in Fig. 2A are presented as mean ± SD from three independent experiments with duplicate samples.

**Supplementary Table S2.** FACS analyses after double labeling with Affibody-Alexa Fluor 488 and

Trastuzumab-Alexa Fluor 680 conjugates

<b>Double labeling of SKBR-3 cells</b>	<b>Both bound</b>	<b>Trastuzumab-Alexa Fluor 680 bound</b>	<b>Affibody-Alexa Fluor 488 bound</b>	<b>None bound</b>
1 nM Affibody-Alexa Fluor 488 + 10 nM Trastuzumab-Alexa Fluor 680 conjugates	98.3 ± 1.1	0.4 ± 0.3	1.1 ± 0.4	0.2 ± 0.1
Both conjugates + excess unlabeled Affibody	1.6 ± 0.5	97.0 ± 1.3	0.8 ± 0.1	0.5 ± 0.3
Both conjugates + excess unlabeled Trastuzumab	0.3 ± 0.2	0.5 ± 0.2	98.3 ± 0.9	0.8 ± 0.3
Both conjugates + excess Affibody/Trastuzumab	0.6 ± 0.2	0.4 ± 0.2	1.5 ± 0.4	97.4 ± 0.1
<b>Double labeling of U251 cells</b>				
1 nM Affibody-Alexa Fluor + 10 nM Trastuzumab-Alexa Fluor 680 conjugates	0.2 ± 0.1	0.3 ± 0.2	0.7 ± 0.3	98.7 ± 0.9
Both conjugates + excess unlabeled Affibody	0.2 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	99.2 ± 0.5
Both conjugates + excess unlabeled Trastuzumab	0.1 ± 0.1	0.2 ± 0.1	0.6 ± 0.4	99.0 ± 0.7
Both conjugates + excess Affibody/Trastuzumab	0.3 ± 0.1	0.1 ± 0.1	0.6 ± 0.5	98.9 ± 0.8

NOTE: Percentages of cells in each gate shown in Fig. 2B are presented as mean ± SD from three independent experiments with duplicate samples.

## Supplementary Figure Legend

**Supplementary Figure S1.** *A.* Schematic representation of conjugation chemistry. *B.* Typical analysis of conjugation reactions using SDS-PAGE and silver staining. *C.* Surface plasmon resonance analysis of Affibody  $Z_{\text{HER2:342}}$ -Alexa Fluor 750 binding to the extracellular domain of HER2 using the Biacore instrument to measure affinity and binding kinetics. Affibody  $Z_{\text{HER2:342}}$  and  $Z_{\text{HER2:342}}$ -Alexa Fluor 750 were diluted 2-fold serially from 500 to 7.8 pmol/L and injected, followed by two injections of buffer as described in “Materials and Methods”. Concatenated data from Affibody  $Z_{\text{HER2:342}}$ -Alexa Fluor 750 binding were shown here. The red lines represent the fitted lines according to a 1:1 binding model.