An Intein-Mediated Site-Specific Click Bioconjugation Strategy for Improved Tumor Targeting of Nanoparticle Systems

Drew R. Elias, Zhiliang Cheng, and Andrew Tsourkas

Department of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, 210 S 33rd St.. Philadelphia, Pennsylvania, 19104.

SUPPORTING INFORMATION

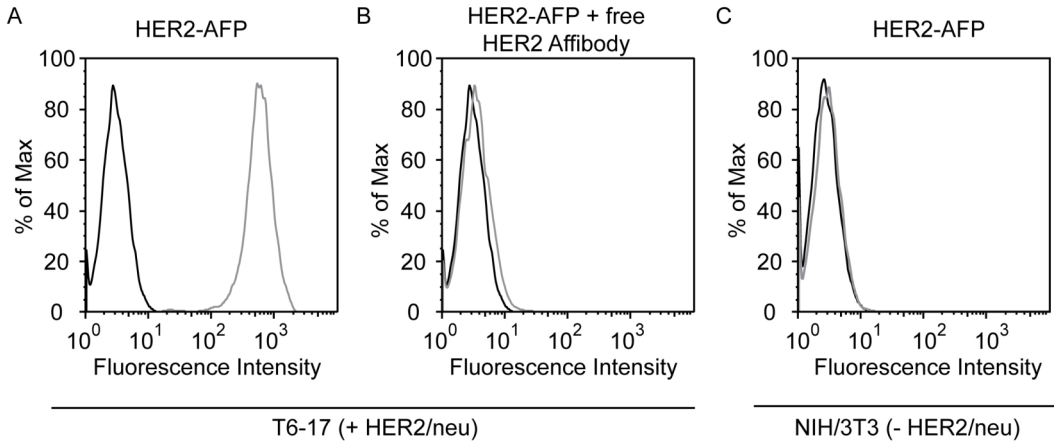

Figure S1. Flow cytometric analysis of cells incubated with HER2-AFP. (A) Flow cytometry histogram of HER2/neu-positive T6-17 cells incubated in the presence of HER2-AFP (grey line). (B) Flow cytometry histogram of HER2/neu-positive T6-17 cells incubated in the presence of HER2-AFP and an excess of free HER2-affibody (grey line). (C) Flow cytometry histogram of HER2/neu-negative NIH/3T3 cells incubated in the presence of HER2-AFP (grey line). Histograms of unlabeled cells are also shown (black line).

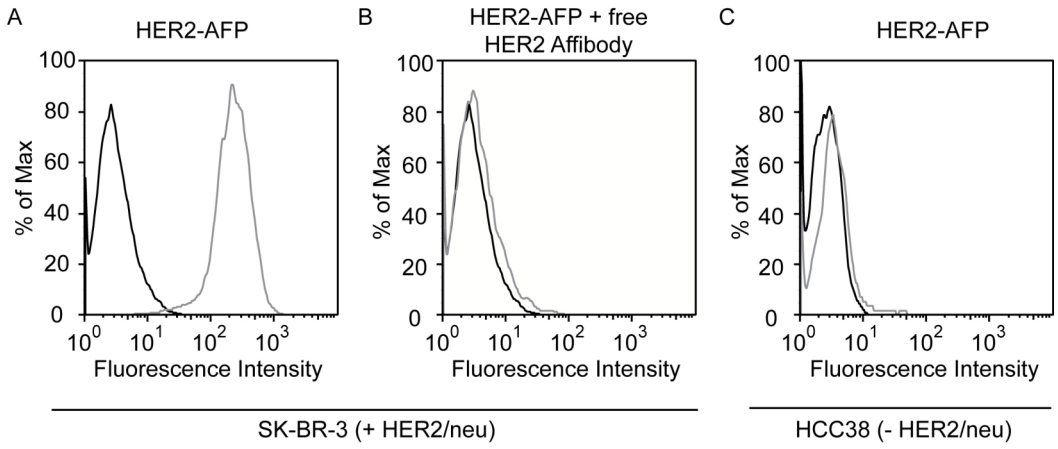

Figure S2. Flow cytometric analysis of cells incubated with HER2-AFP. (A) Flow cytometry histogram of HER2/neu-positive SK-BR-3 cells incubated in the presence of HER2-AFP (grey line). (B) Flow cytometry histogram of HER2/neu-positive SK-BR-3 cells incubated in the presence of HER2-AFP and an excess of free HER2-affibody (grey line). (C) Flow cytometry histogram of HER2/neu-negative HCC38 cells incubated in the presence of HER2-AFP (grey line). Histograms of unlabeled cells are also shown (black line).

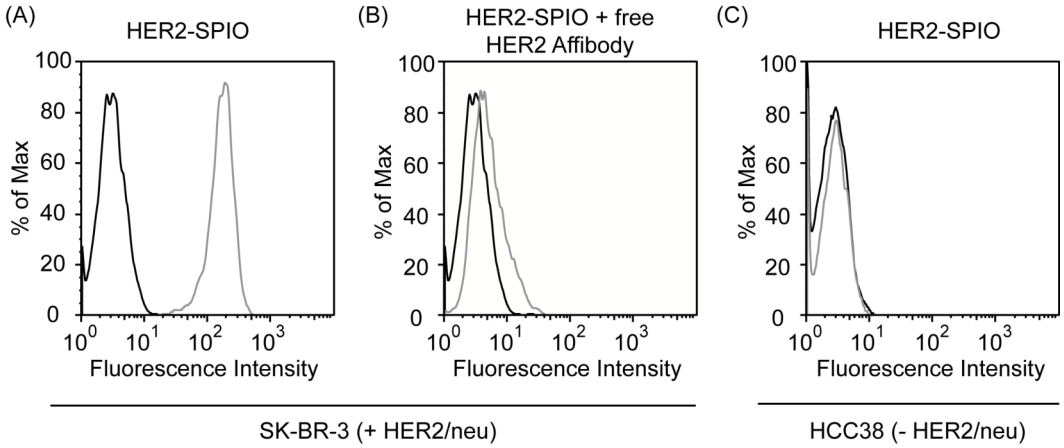

Figure S3. Flow cytometric analysis of cells incubated with HER2-SPIO. (A) Flow cytometry histogram of HER2/neu-positive SK-BR-3 cells incubated in the presence of HER2-SPIO (grey line). (B) Flow cytometry histogram of HER2/neu-positive SK-BR-3 cells incubated in the

presence of HER2-SPIO and an excess of free HER2-affibody (grey line). (C) Flow cytometry histogram of HER2/neu-negative HCC38 cells incubated in the presence of HER2-SPIO (grey line). Histograms of unlabeled cells are also shown (black line).

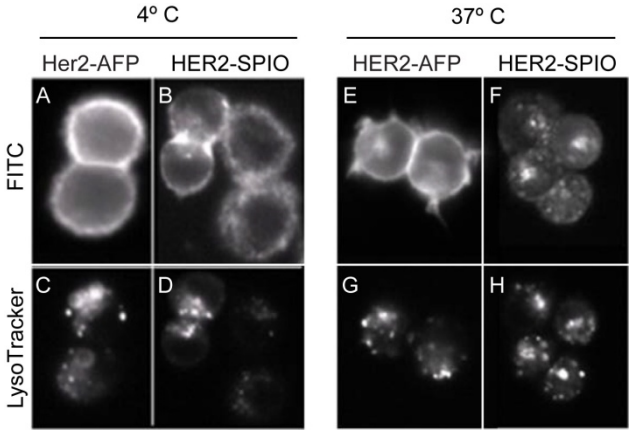

Figure S4. Fluorescence micrographs of SK-BR-3 cells labeled with 1 μM of AFP-Affibody and 125 μg Fe/mL of HER2-SPIO at 4°C and 37°C for 1 hour. Before images were collected, cells were incubated with LysoTracker Red, an acidotropic probe to track internalization through endosomes/lysosomes. The intracellular distribution of HER2-AFP and HER2-SPIO was followed by imaging the fluorescein (FAM5) dye present on the AFP. At 4°C, both (A) HER2-AFP and (B) HER2-SPIO were constrained to the cell membrane. There was no colocalization with lysosomes, (C) and (D), respectively. At 37°C, (E) the HER2-AFP was still on the outer cell membrane, while (F) HER2-SPIO appeared as punctate fluorescent spots likely emanating from within the cytoplasm. (G) HER2-AFP did not colocalize with the LysoTracker dye; however (H) the HER2-SPIO exhibited significant co-localization with LysoTracker Red, suggesting internalization through receptor mediated endocytosis.

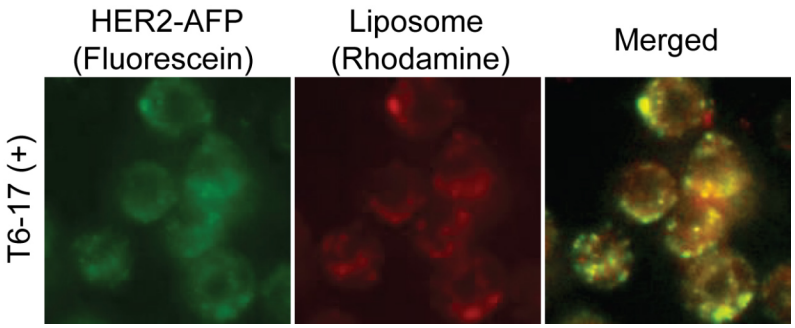

Figure S5. Fluorescence micrographs of T6-17 cells incubated with HER2-liposomes at 37°C for 1 hour. A) Fluorescent micrograph of the fluorescent label (fluorescein) on HER2-AFP. The HER2-AFP is coupled to the liposome surface B) Fluorescent micrograph of the Rhodamine-PE, which was doped into the liposome bilayer. C) Colocalization of HER2-AFP and Rhodamine-PE, indicating that the HER2-AFP remains coupled to the liposome surface.

```
GTG GAT AAC AAA TTT AAC AAA GAA ATG CGC AAC GCG TAT TGG GAA ATT
Val Asp Asn Lys Phe Asn Lys Glu Met Arg Asn Ala Tyr Trp Glu Ile

GCG CTG CTG CCG AAC CTG AAC AAC CAG CAG AAA CGC GCG TTT ATT CGC
Ala Leu Leu Pro Asn Leu Asn Asn Gln Gln Lys Arg Ala Phe Ile Arg

AGC CTG TAT GAT GAT CCG AGC CAG AGC GCG AAC CTG CTG GCG GAA GCG
Ser Leu Tyr Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala

AAA AAA CTG AAC GAT GCG CAG GCG CCG AAA ATG CGC ATG
Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys Met Arg Met
```

Figure S6. Nucleotide and corresponding amino acid sequence of the HER2-Affibody. The additional base pairs added to improve affinity column cleavage are shown in bold.