Labeling cell surface receptors with Ligand.BirA* bispecifics

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Keywords

BirA*, cell surface receptor discovery, bispecific, affibody, enzyme ligand fusion

Supporting information

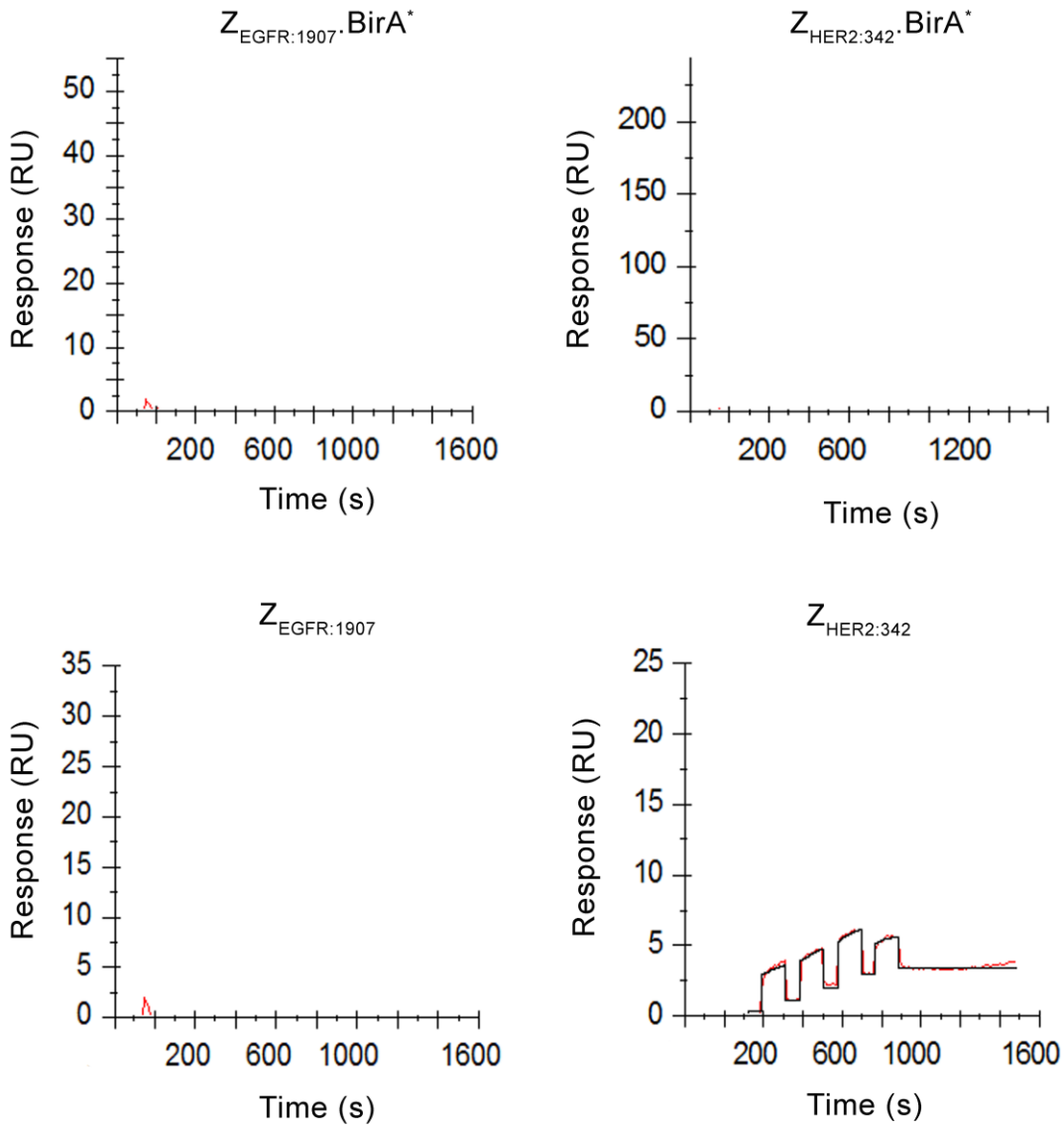


Figure S1 Single cycle kinetics sensorgrams derived from increasing concentrations of $Z_{EGFR:1907}$.BirA* and $Z_{EGFR:1907}$ interacting with immobilized HER2, as monitored using surface plasmon resonance (SPR). Similarly, sensorgram profiles of $Z_{HER2:342}$.BirA* and $Z_{HER2:342}$ interacting with immobilized EGFR. Concentration ranges covered the same ranges as described in Figure 3B. In all cases, no significant interaction could be detected between affibodies and their constructs and their unmatched receptors.

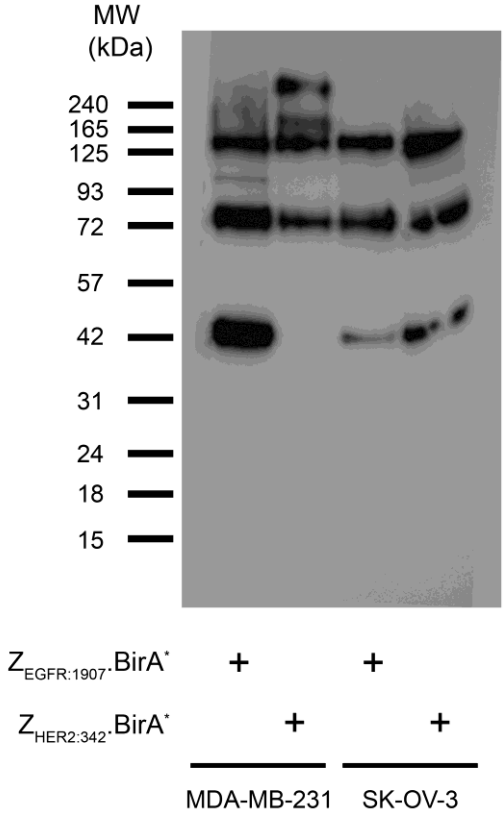
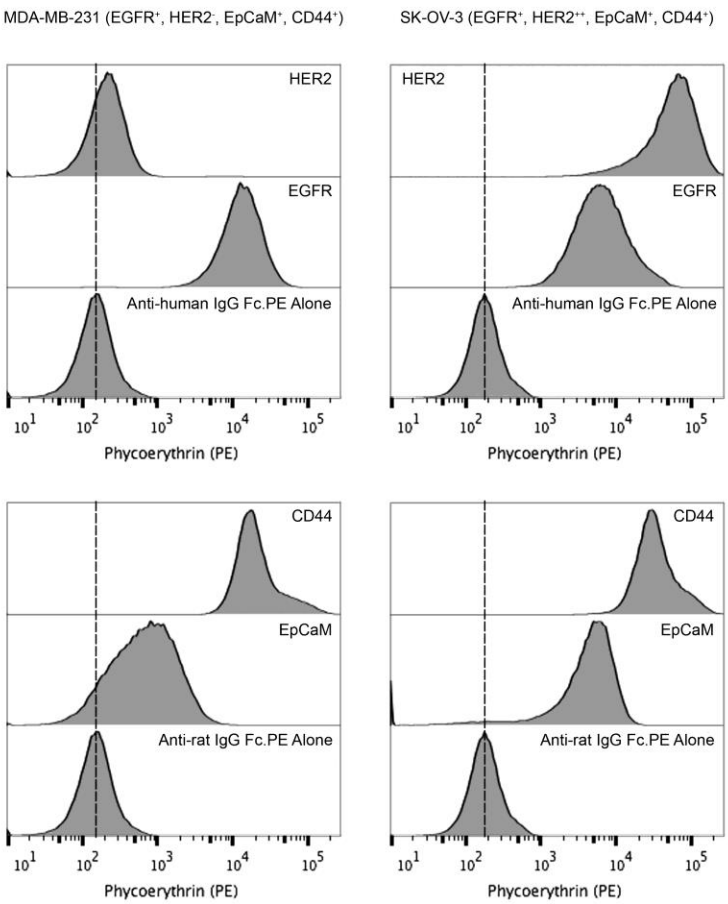


Figure S2 $Z_{EGFR:1907}$.BirA* and $Z_{HER2:34}$.BirA* are able to biotinylate the surface of human cancer cells MDA-MB-231 and SK-OV-3. Eluted fractions from complexes captured on streptavidin dynabeads (Figure 4C) were analyzed using a Streptavidin-HRP western blot, under non-reducing conditions.

A.



B.

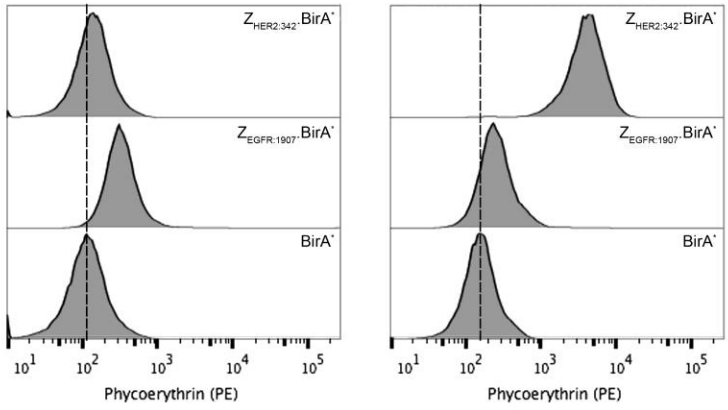


Figure S3 Flow cytometry analyses of Affibody.BirA* bispecifics binding to MDA-MB-231 and SK-OV-3 cells. A) The expression of EGFR, HER2, EpCaM and CD44 were monitored on these cells using antibodies (Cetuximab, trastuzumab, 9C4 and

IM7, respectively), followed by an anti-human IgG Fc.PE to track the anti-EGFR and anti-HER2 antibodies, and anti-rat IgG Fc.PE to track the anti-CD44. Anti-EpCaM mAb was directly labeled with PE. Cells stained with anti-human IgG Fc.PE alone served as a negative control. B) Binding of $Z_{EGFR:1907}$.BirA* and $Z_{EGFR:1907}$.BirA* to both cell lines were confirmed as these constructs are endogenously biotinylated during expression by the BirA*. Their binding to cells was directly confirmed using Streptavidin.PE. Cells exposed to BirA* alone and Streptavidin.PE served as negative controls to account for the nonspecific binding of BirA* domain of Affibody.BirA* constructs to cells. Representative histograms are shown of flow cytometric profiles performed in triplicate.

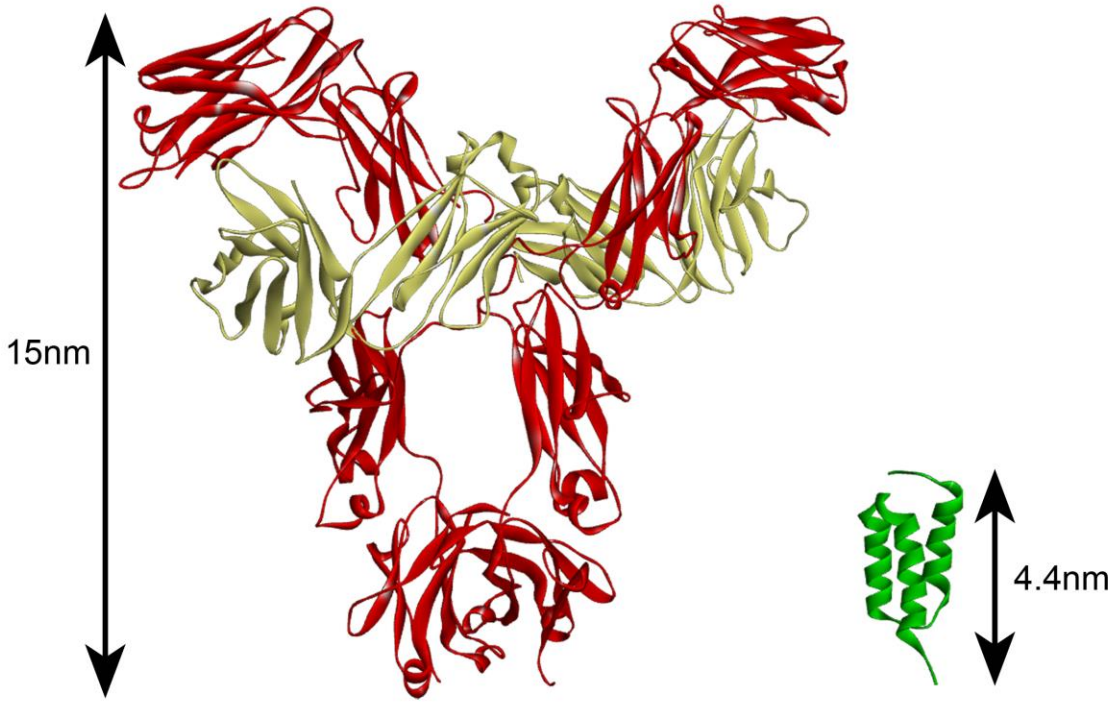


Figure S4 Ribbon representations of a human IgG antibody [25] (heavy chain in red, light chain in yellow) and of an affibody (green) emphasizing the difference in length along their long axis. Structures were generated from the I-TASSER server.[17]