A platform for the early selection of non-competitive antibody-fragments from yeast surface display libraries

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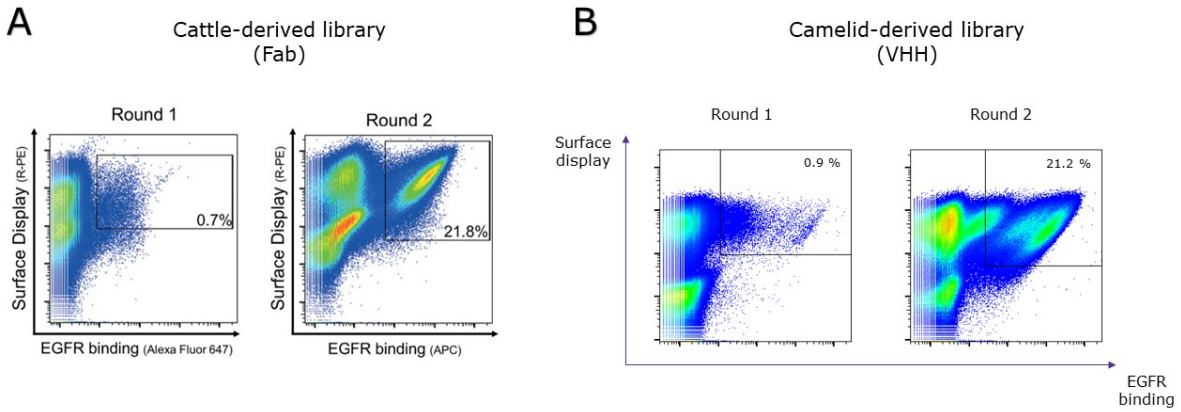
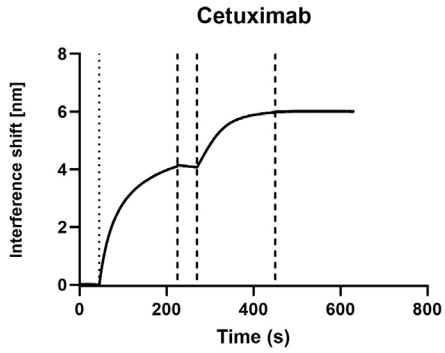
Supplemental material

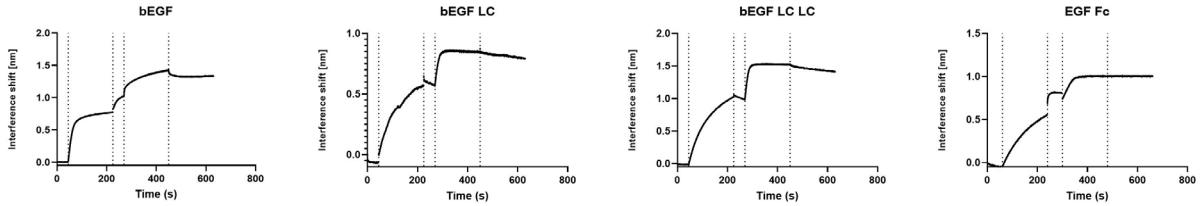


Supplemental data 1: Enrichment of EGFR binders. A: Enrichment of EGFR binders from cattle-derived yeast surface display library (adapted from Pekar et al, 2021). **B**: Enrichment of EGFR binders from camelid-derived yeast surface display library. Libraries were incubated with secondary detection antibodies and a two dimensional gate was applied to select simultaneously functional display and EGFR binding. Binding was detected via FACS and applied sorting gates and corresponding cell population (as % of total cells) are shown. Plots were generating with FlowJo.

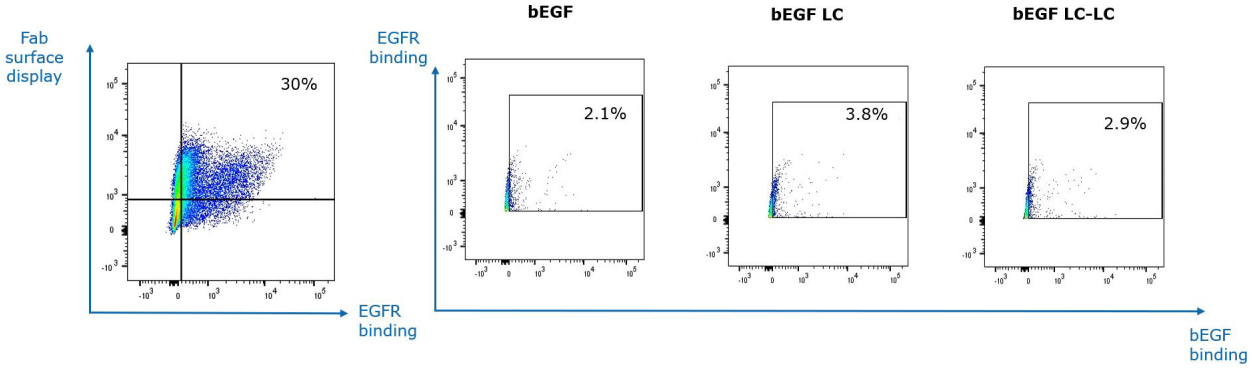


Supplemental data 2: Competition between cetuximab and EGF for the binding to EGFR determined by BLI. Cetuximab was loaded on AHC2 biosensors and after a baseline step incubated first with EGFR and subsequently with EGF Fc. No binding of

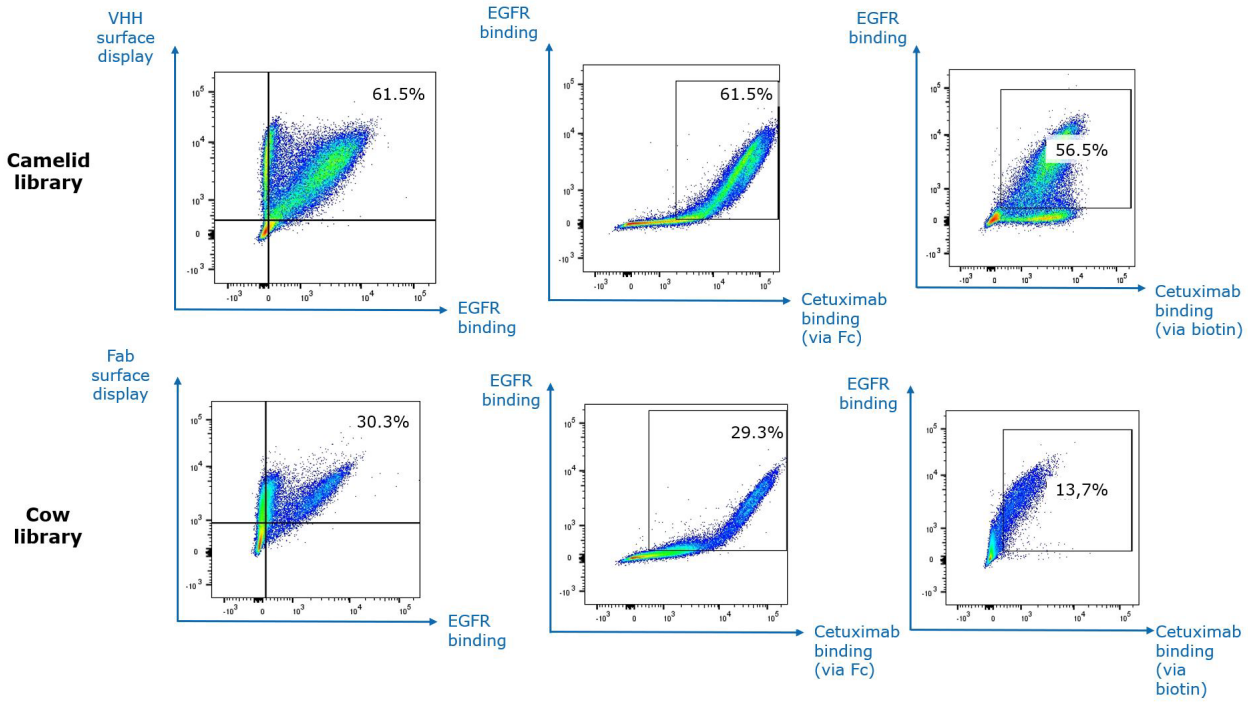
EGF Fc is detectable indicating competition between cetuximab and EGF for the binding to EGFR.



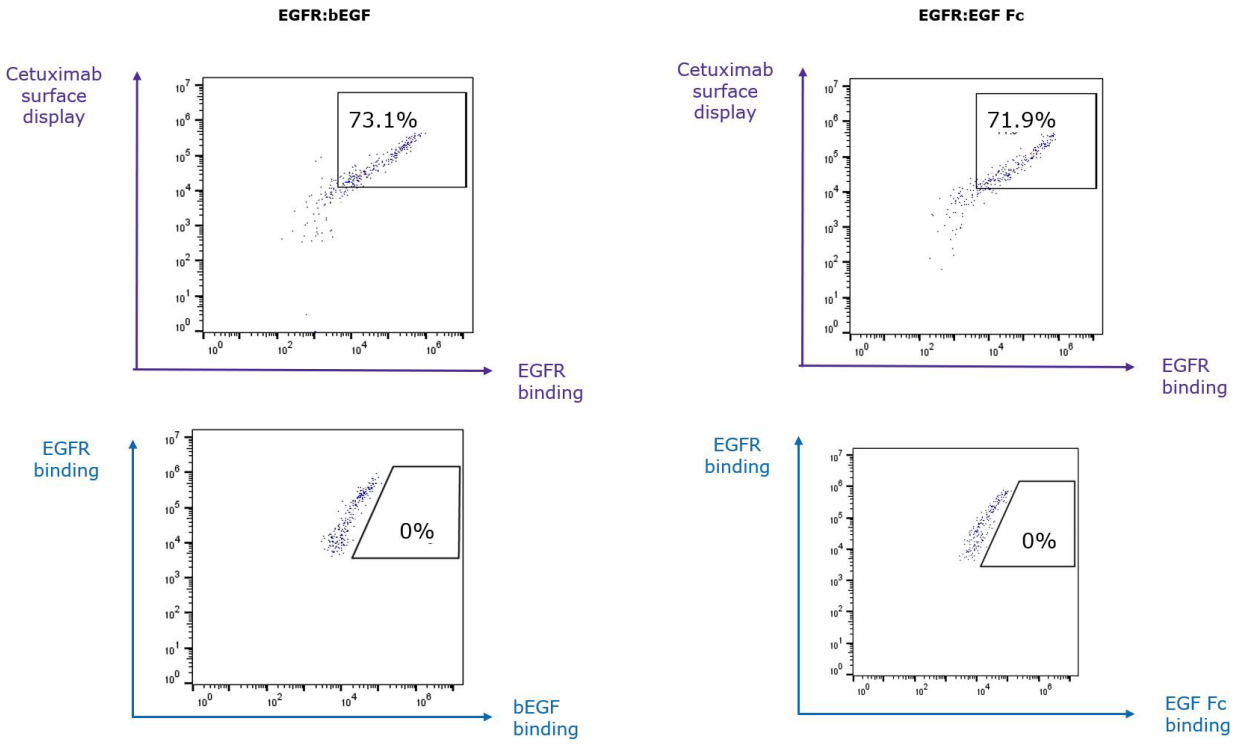
Supplemental data 3: QC binding of bEGF, bEGF LC, bEGF LC-LC and EGF Fc to EGFR determined by BLI. bEGF, bEGF LC and bEGF LC-LC were loaded on streptavidin biosensors (SA) and association with EGFR was tested and confirmed.EGF Fc was loaded on AHC biosensor and associated with EGFR tested and confirmed.



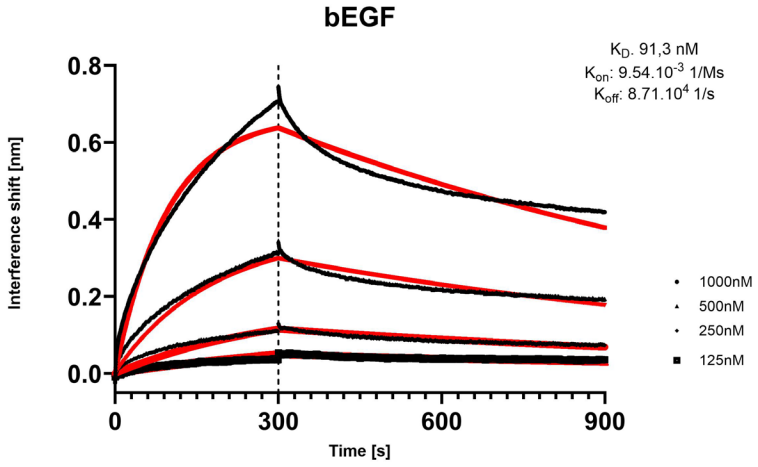
Supplemental data 4: Utilization of longer linkers for the biotinylation. Cattle-derived library was incubated with bEGF, long bEGF-LC (22,4 Å) or extra-long bEGF-LC-LC (30,5 Å) linkers. Secondary detection is subsequently incubated to detect the VHH surface display (anti-HA AlexaFluor® 488 conjugated antibody), EGFR (anti-His APC conjugated antibody) and biotinylated EGF (Streptavidin PE conjugate). Long linkers do not improve the detection of bEGF on the cells. Binding was detected via FACS and applied sorting gates and corresponding cell population (as % of total cells) are shown. Plots were generated with FlowJo.



Supplemental data 5: Investigation of the signal amplification. Camelid- and cattle-derived libraries were incubated with cetuximab (anti-EGFR antibody that is competing with EGF). Detection of cetuximab was performed either via its Fc portion (secondary detection antibody) or after biotinylation via streptavidin conjugate. VHH surface display was monitored using anti-HA AlexaFluor® 488 conjugated antibody, EGFR detection performed using anti-His APC conjugated antibody. For both libraries, the detection of cetuximab was improved by using a secondary detection antibody compared to streptavidin conjugate. Binding was detected via FACS and applied sorting gates and corresponding cell population (as % of total cells) are shown. Plots were generated with FlowJo.



Supplemental data 6: Negative control for the staining strategy. Yeast cells displaying the Fab fragment of cetuximab (EGF competing antibody) is displayed on the yeast surface. Cells are subsequently incubated with EGFR and bEGF (left panel) or EGFR and EGF Fc (right panel). No detection of EGF binding as expected. Binding was detected via FACS on an iQue 3 (Sartorius) and applied gates and corresponding cell population (as % of total cells) are shown. Plots were generating with FlowJo.



Supplemental data 7: K_D determination of bEGF to EGFR by BLI. Data was fitted to a 1:1 model (fitted values in red).