Characterization of a novel disulfide-stabilized scFv library

Niloufar Kazemi; Eeva-Christine Brockmann, PhD; Urpo Lamminmäki , Prof.
MOLECULAR BIOTECHNOLOGY AND DIAGNOSTICS
Department of Life sciences, University of Turku

BACKGROUND

Monoclonal antibodies have shown success in cancer therapy in recent years. Single-chain variable fragment (scFv) is the smallest antibody fragment that can bind to an antigen. ScFv is easily produced in *E.coli* and well suited for phage display, but it is less stable than larger antibody fragments or intact antibodies. Adding an extra disulfide bridge between the variable light and heavy chains improves the stability of scFv (1). We have previously built an antibody library based on a disulfide-stabilized scFv (ds-ScFv) (Fig. 1B)

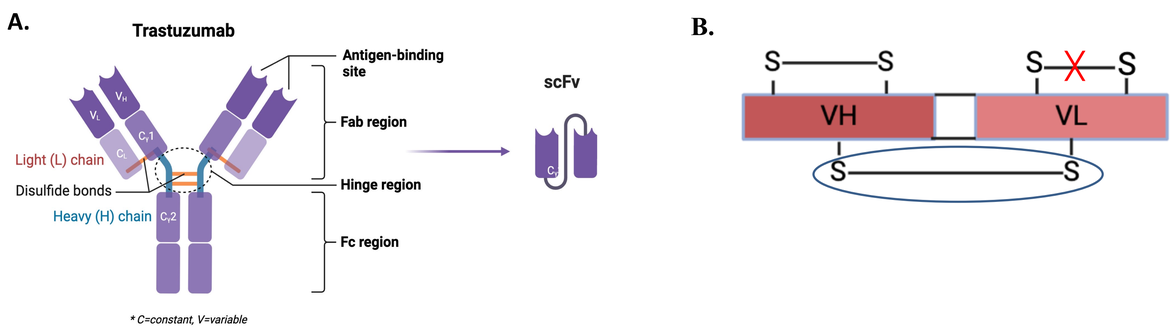


Figure1. A. *Framework of the disulfide-stabilized scFv library* ***B.*** *antibody library based on a disulfide-stabilized scFv . The red cross shows the eliminated disulfide bridge, and the circle shows the extra disulfide bridge that has been added.*

Ds-scFv library

- Framework: scFv of Trastuzumab, a therapeutic antibody used to treat breast cancer (Fig 1A)
- Disulfide bond was added to the CDR-L1 and CDR-H3 (Fig 1B) to improve stability.
- The native intra-domain disulfide bridge in VL was removed to establish phage display.
- The library has a varied 13 to 19 amino acid loop length in CDR-H3. (five sub libraries)

Aim

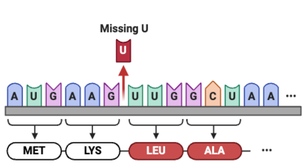
- Eliminate clones with frameshifts and improper folding.
- Selection of binders from libraries against protein targets (HER2, hTROP2, and Gremlin).
- Characterizing the isolated binders.

METHODS

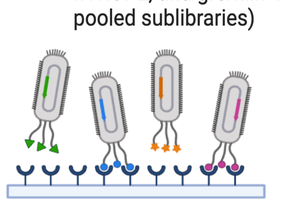
Phage display

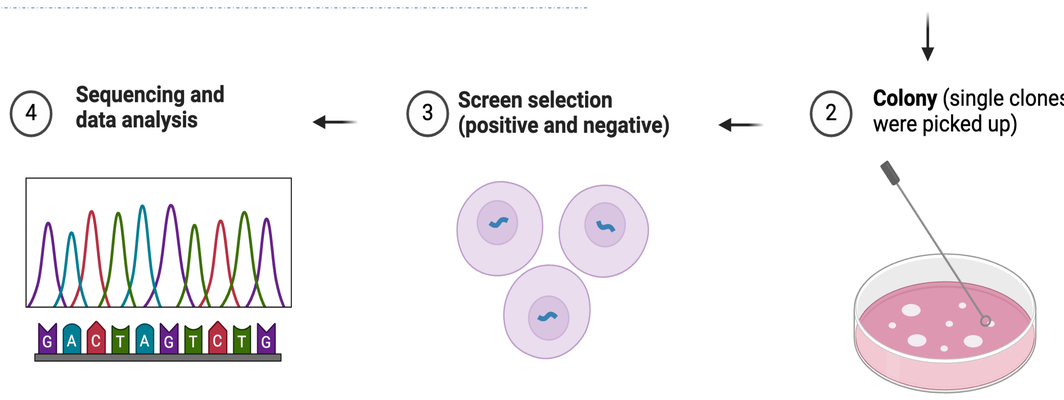
(A) Selection for folding

One panning round against protein L was carried out separately for each of the five sublibraries.



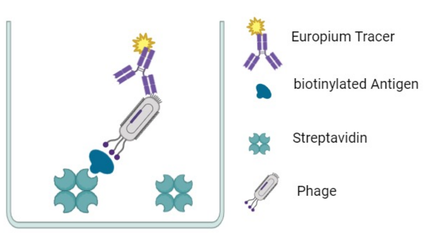
(B) Selection of new binders





C.

Phage immunoassay was done with biotinylated antigens to measure display level.



RESULTS

After protein L selection, all five loop libraries showed good display of ds-scFv and frameshifted clones had been eliminated (Fig. 3)

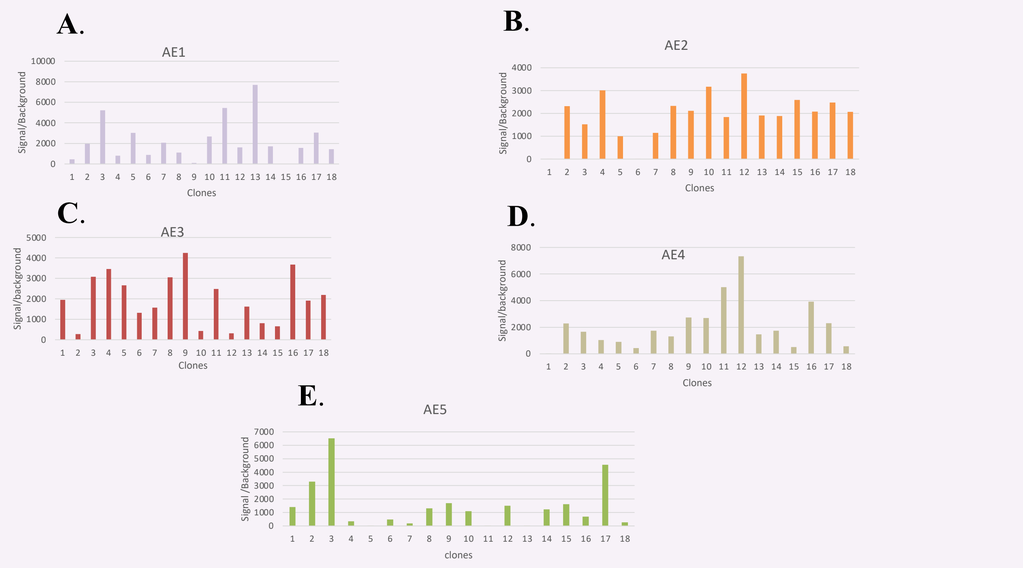


Figure3. screening *immunoassay results* ***A.*** *AE1: sublibrary with 13 aa* ***B.*** *AE2: sublibrary with 14 aa* ***C.*** *AE3: sublibrary with 15aa* ***D.*** *AE4: sublibrary with 17aa* ***E.*** *AE5: sublibrary with 19aa*

In phage display selection for HER2, hTROP2 and Gremlin-1, there was good enrichment based on phage immunoassay (Fig. 4).

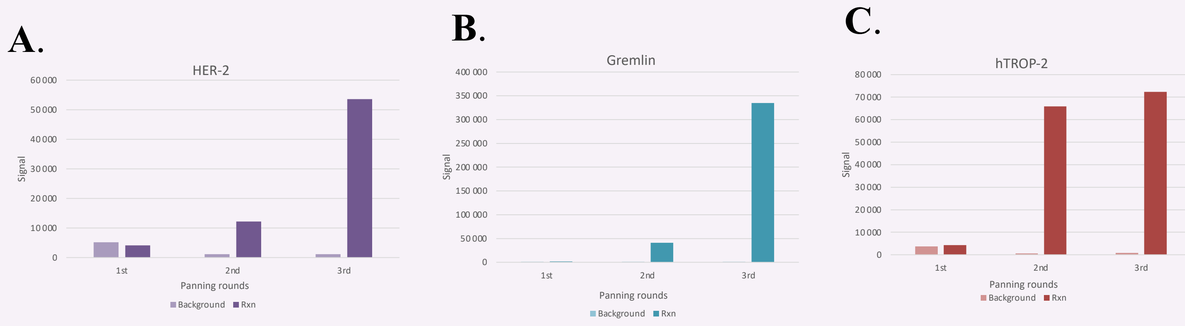


Figure4. *phage immunoassay results* ***A****.HER2* ***B****.hTROP2* ***C.****Gremlin*

After three rounds of panning, out of 90 clones that were screened from each antigen reaction, 56% of HER2, 48% of hTROP2, and 66% of Gremlin showed good binding to the target (signal-to-background ratio >10). However, for hTROP2, the selected two clones were not part of our designed dsScFv library.

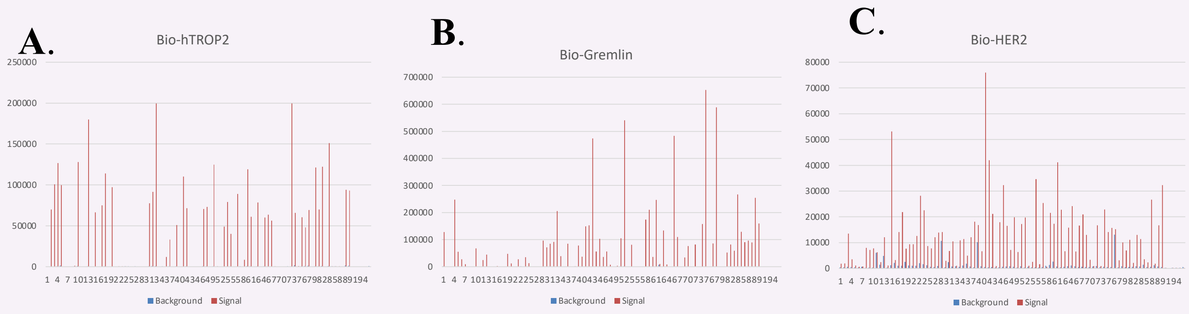


Figure5*. screening results.* ***A****.HER2* ***B****.hTROP2* ***C.****Gremlin*

Based on the sequencing, 6 unique binders for HER2 and 4 unique binders for Gremlin were found. For hTROP2, the selected clones were not from the dsScFv library.

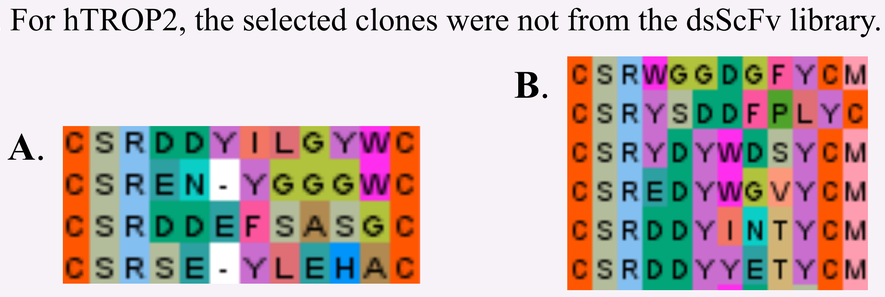


Figure6. *Amino acid sequencing of CDR-H3* ***A.*** *4 binders selected from Gremlin-1* ***B.*** *6 binders selected from HER2*

CONCLUSIONS

After selection against Pro L, a good number of active clones showed up.

Ds-ScFv library can be considered a source of superstable scFv fragments.

The next step is measuring the affinity and stability.

The final step involves determining whether replacing the missing intradomain disulfide bridge can maintain the binding characteristics of particular binders.

REFERENCES

(1) WoÈrn, Arne, and Andreas PluÈckthun. "Stability engineering of antibody single-chain Fv fragments." *Journal of molecular biology* 305, no. 5 (2001): 989-1010. (1)

Illustrations were created by biorender.com.