Protein therapeutics for cytoplasmic targets

Paulus Nyman[1], **Tuomas Huovinen**[1], **Ralf Paul**[2], and **Bryce Nelson**[2]

[1.] Department of Life Technologies, University of Turku,Turku, Finland

[2.] Protein and Antibody Engineering, Orion Corporation, Turku, Finland

Motivation

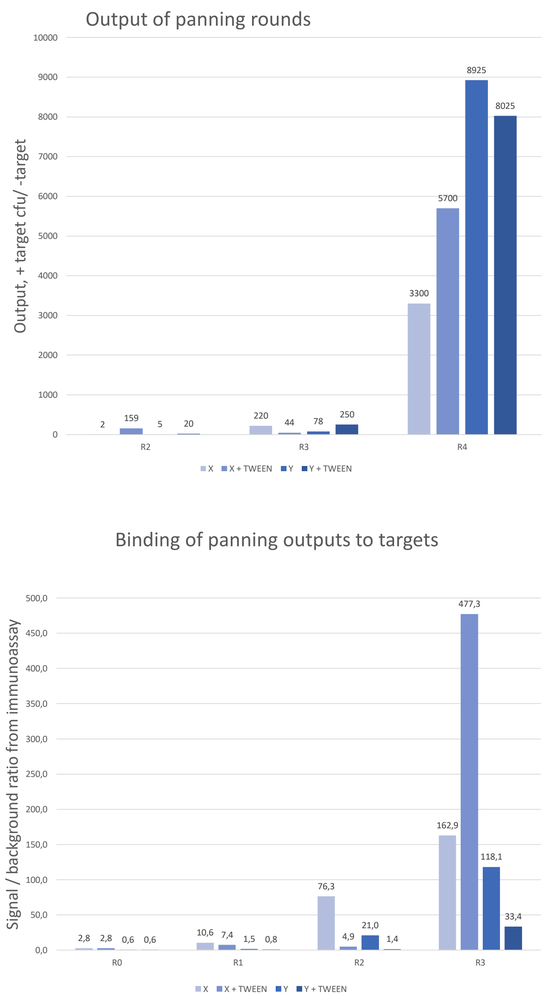
Antibody-based therapeutics have already proven the capabilities treating various diseases. Formation of disulfide bonds requires oxidative conditions and is not ideal when targeting intracellular proteins. Alternative binder proteins have been researched to tackle the challenges. DARPins (designed ankyrin repeat proteins) are small binder proteins. They stay functional even in cell cytoplasm. Other benefits are easy production in bacterial cells and good stability. These make DARPins an interesting research topic for therapeutic use.

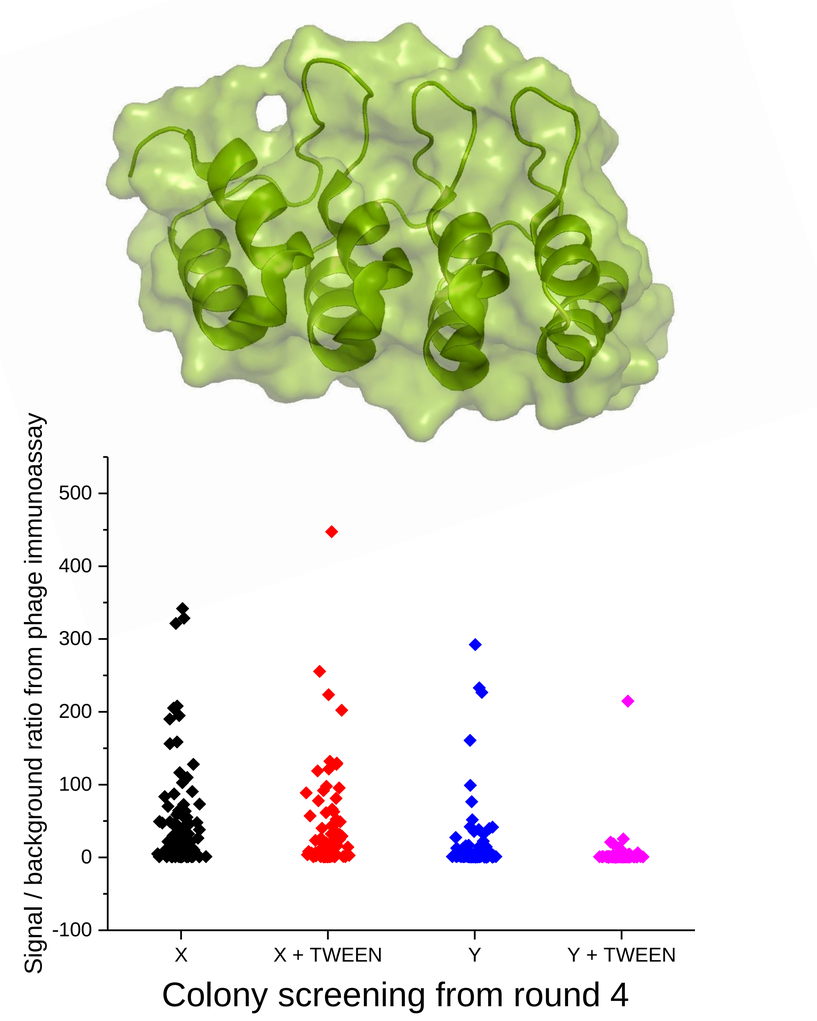
DARPin library

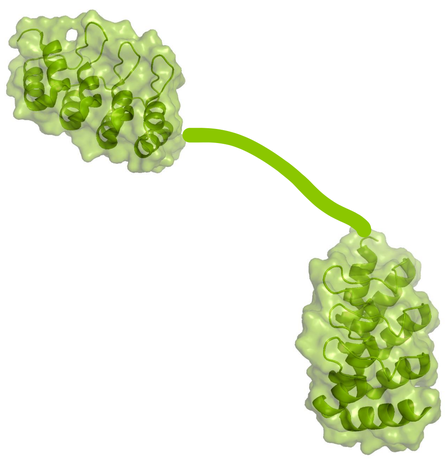
A DARPin library has been constructed in Turku University by randomizing variable amino acids in DARPins. As a result, the library has up to 2.4 x10^{11} binders with different binding properties.

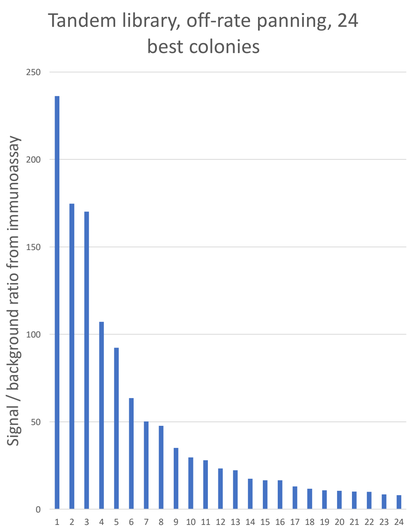
Biopanning

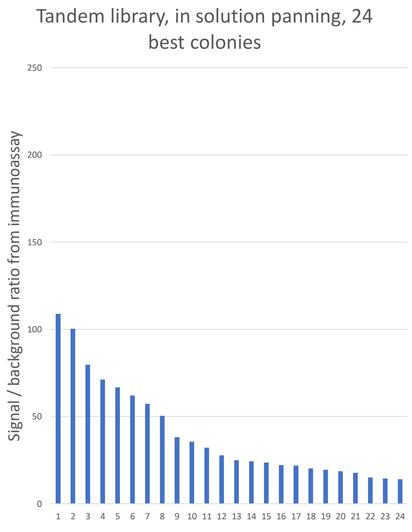
From the library, DARPins were selected against two undisclosed target proteins, X and Y using phage display. Bacteriophage contains DNA and expresses the corresponding DARPin at the surface. Only the phages expressing DARPin that binds to the provided target protein stay on immobilized target protein. Bacteria cells are infected with with phages eluted from the panning reaction. From cells, DNA can be extracted, or new phage clones can be produced. Panning rounds with and without detergent TWEEN were performed.











DARPin tandems

Bivalent binding has advantage over monovalent binding. For tandem library, DARPins were combined with linkers of variable lengths. The library was panned with and without off-rate method, by adding competitive target antigen after binding to biotinylated target.

Conclusions

Binders were found against both targets with both approaches. Off-rate panning was more efficient method for extracting binders with high binding capacity. The work was a proof of concept for using the DARPin library against cytoplasmic target proteins.

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