Effect of Linker Length on Protein Evolution Using Yeast Surface Display

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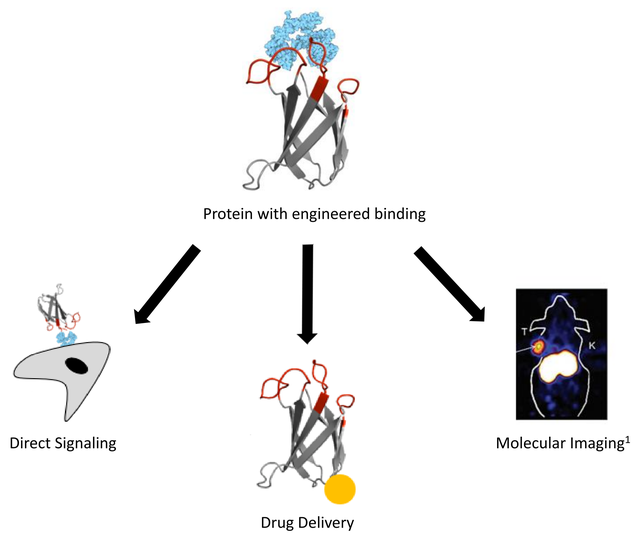
Background

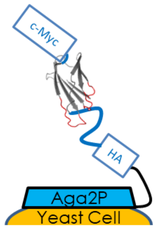
Motivation

Engineered proteins have a variety of uses in medicinal applications:



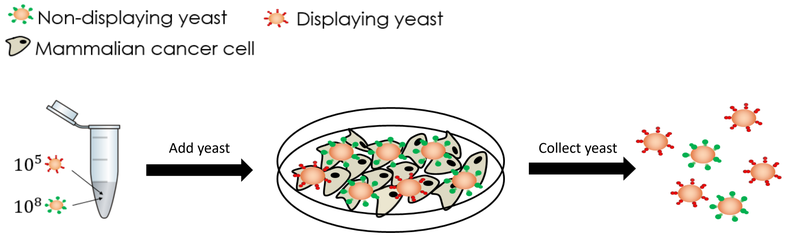
Yeast Surface Display

One of the platforms to discover proteins that bind to a cellular biomarker is yeast surface display (YSD). This technique links genotype and phenotype for high-throughput screening. YSD constructs consist of three parts: the anchor, which adheres the construct to the cell, the linker, which allows for flexible movement of the protein, and the surface-displayed protein.



Cell Panning Assay

This procedure uses target-expressing mammalian cells to expose proteins to native cellular target – a property that traditional recombinant selections lack.



This method has been previously used in conjunction with YSD to isolate binding proteins to a variety of cancer and blood-brain barrier targets.[2,3] However, this method is limited by its low recovery rates of binding yeast when the yeast are washed against the target-displaying mammalian cells.

Experimental Design

Objective

The goal of this study was to investigate the impact of varying YSD linker length on the effectiveness of selections by cell panning through examining the number of binding yeast recovered and enrichment ratio of binding to nonbinding yeast in systems of differing target expression.

Model Systems

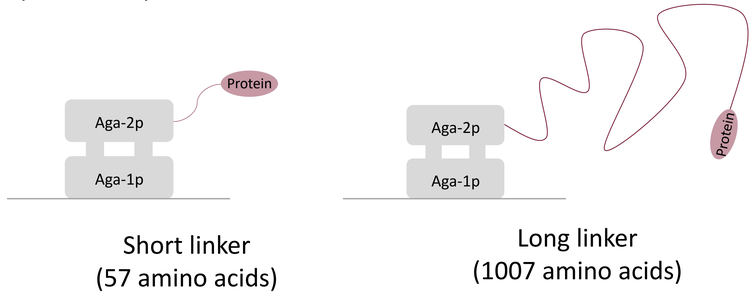
Two different model systems were used to simulate different biological conditions and test the generalizability of the results:

System 1: A431 cancer cells (2.7 ± 1.4 million EGFR/cell)
System 2: MB231 cancer cells (190,000 ± 50,000 EGFR/cell)[4]

In both cases, E6.2.6', a protein with 2 nM affinity to EGFR, was displayed on the yeast surface.

Linker Construction

We constructed a 1007 amino acid linker from the existing YSD linker (pCT 40) currently used for cell panning.[4] The longer linker (pCT 680) was constructed by a molecular biology method for generating long, repetitive sequences.

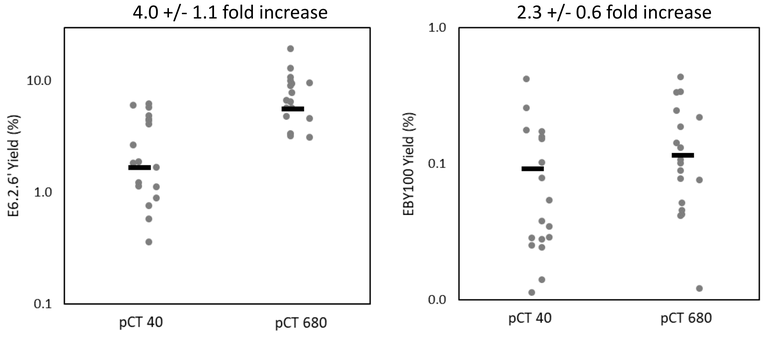


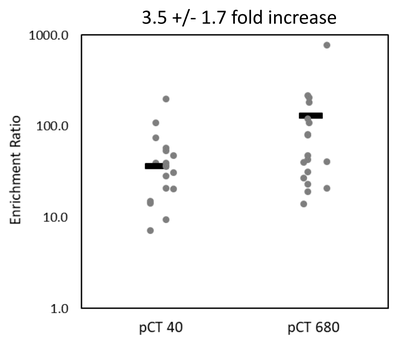
Hypothesis

The longer linker will increase the accessibility of the protein to the extracellular space, thus improving both enrichment and number of binding yeast recovered.

Results

High target expression system (A431, 2.7 ± 1.4 million EGFR/cell):

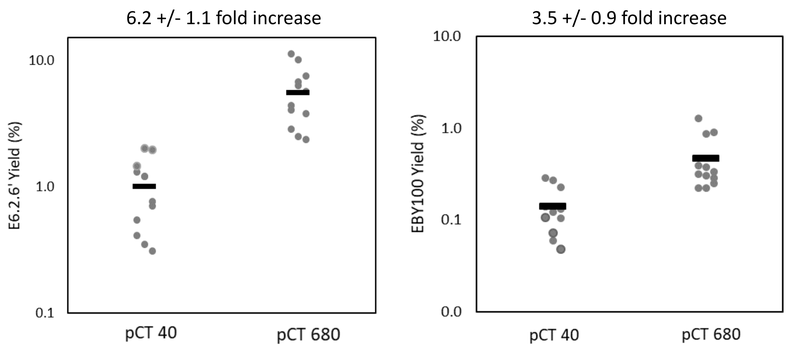


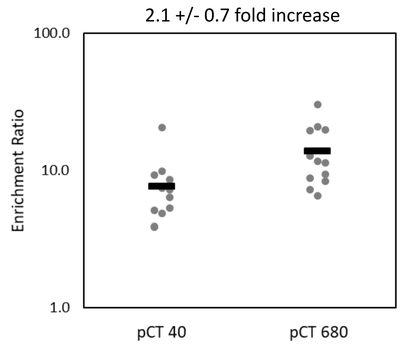


The E6.2.6' yield is the percent of binding yeast that was recovered after cell panning while the EBY100 yield is the percent of background yeast recovered. Enrichment ratio is important because it assesses how much the binding yeast is amplified in the post-assay population.

Confidence levels shown at 95%.

Medium Target Expression System (MB231, 190,000 ± 50,000 EGFR/cell):





Confidence levels shown at 95%.

Comparison between the two model systems show a continuity of superior performance of pCT 680 relative to pCT 40 even in lower biomarker environments.

Future Work

- Development of two more model systems:
  1. A lower affinity protein (AASV, >600 nM)[4] in place of the current high affinity protein (E6.2.6') to see how protein binding strength affects linker performance.
  2. A CD276-based system to test the generalizability of these results.
- We also hope to investigate the impact of the anchor protein on the display construct performance in order to determine the optimal linker/anchor combination for use in protein discovery.

Acknowledgements and References

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[1] Hackel, B. J. et al. *Radiology.* 2012.
[2] Tillotson, B.J., Cho, Y. K., & Shusta, E. V. *Methods* 2013.
[3] Zorniak, M. et. al. *Sci Rep* 2017
[4] Stern, L.A. et al. *Biotechnol and Bioeng.* 2016.