Engineered Yeast for Highly Efficient Fab Yeast Surface Display by Divergent Promoter, Molecular Chaperon, and ER Retention Optimizations

Meng Mei, Li Yi

Ph.D.

State Key Laboratory of Biocatalysis and Enzyme Engineering, Hubei Collaborative, School of Life Sciences, Hubei University, Wuhan, 430062, China

Email: meimeng@stu.hubu.edu.cn, liyi@hubu.edu.cn

Abstract

Yeast surface display (YSD) are widely used for antibody discovery and engineering. Currently, the most common format for antibody YSD is scFv, in which heavy and light chain variable domains (V_H and V_L) connect via a flexible, artificial linker. scFv lacks natural interactions between constant light and heavy domains, which may result in conformational variations on the antigen-binding sites, and questions for antibody affinity maturation. Comparably, antigen-binding fragment (Fab) can reserve V_H and V_L intact conformations, so Fab YSD holds great promises for developments of antibody discovery and engineering. Herein, using the highly economically valuable Adalimumab and Infliximab monoclonal antibodies as targets, systematic studies for Fab YSD were performed with focus on divergent *GAL1-GAL10* promoter, ER-associated molecular chaperon such as HSP70 family molecular chaperone Kar2 or ER-associated protein-disulfide isomerase 1 (Pdi1), and ER retention sequences (ERSs) for enhancing the pairing of V_H - C_H1 and V_L-C_L. Our results suggested the functional display of Fabs on yeast cell surface with expected sigmoidal binding responses, and co-expression of molecular chaperones or fusion with ERSs improved Fab display quality for up to 3-fold. Moreover, the feasibility of affinity maturation was further demonstrated by isolating a high affinity Fab clone from $1:10^3$ or $1:10^5$ spiked libraries. Given the importance of mAbs and their associated technologies, Fab YSD optimization performed in our studies will advance areas ranging from basic biochemical / biophysical researches to protein sciences and engineering, especially therapeutic antibody discovery and engineering from both academic and industrial settings.

Brief CV

Meng Mei, Ph.D.

School of Life Sciences, Hubei University, China

Education

B.S. Pharmacy Engineering, Huanggang Normal University, China, 2014

Ph.D. Bioengineering, Hubei University, China, 2019

Thesis: Study on the mechanism of protein retention in endoplasmic reticulum of *Saccharomyces cerevisiae* and its application in protein engineering, Advisor: Prof. Li Yi

Conference attended

1. The 11th National Symposium on Enzyme Engineering, October, 18-21, 2017, Wuhan, China (oral presentation)
2. The 15th Japan-China-Korea Joint Symposium on Enzyme Engineering, June 29 – July 3, 2018, Kyoto University, Japan (poster presentation)

Publications

1. **Mei, M.**, Li, J., Wang, S., Lee, B., Iverson B. L., Zhang, G., Ge, X., and Yi, L. *, " Highly Efficient Fab Yeast Surface Display by Divergent Promoter, ER Retention, and Chaperon Optimizations", ***Submitted***
2. Fan, X., Li, X., Zhou, Y., **Mei, M.**, Peng, W., Jiang, Z., Yang, S., Iverson, B. L., Zhang, G., and Yi, L. ***,** "Quantitative analysis of substrate specificities of the 3C protease of human rhinovirus and the exploration of its substrate recognition mechanisms ", ***In revision***
3. He, H., Zhai, C., **Mei, M.**, Rao, Y., Wang, F., Ma, L., Zhang, G., and Yi, L. *, " Functional expression of porcine interferon-α using a combinational strategy in *Pichia pastoris* GS115", ***Enzyme Microb. Technol.,*** 2019 Mar;122:55-63.
4. **Mei, M.**, Zhai, C., Li, X., Zhou, Y., Peng, W., Ma, L., Wang, Q., Iverson B.L., Zhang, G.*, Yi, L.*, Characterization of aromatic residue-controlled protein retention in the endoplasmic reticulum of *Saccharomyces cerevisiae*, ***J.Biol.Chem.***, 2017, 292, 20707-20719.
5. **Mei, M.**, Zhou, Y., Peng, W., Yu, C., Ma, L., Zhang, G.*, Yi, L.*, Application of modified yeast surface display technologies for non-antibody protein engineering, ***Microbiol Res.***, 2017, 196:118-128.