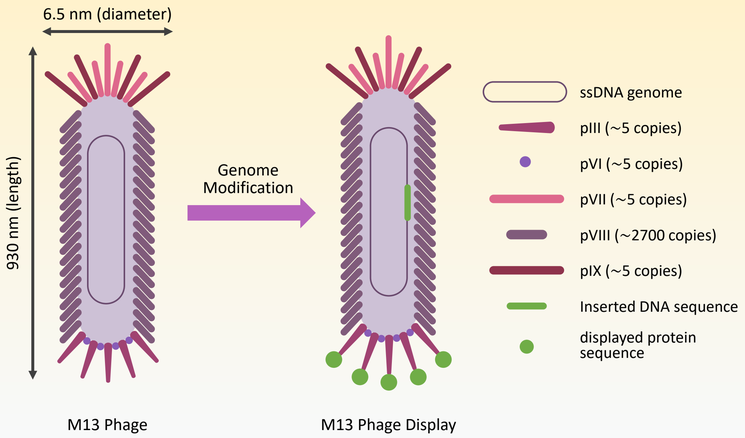
Protein Engineering

The primary objective of protein engineering is to identify specific changes in the amino acid sequence and to alter such sequence for desired functional properties. There are two methods in protein engineering, rational design and directed evolution, among which directed evolution is a protein engineering method that mimics natural evolution.

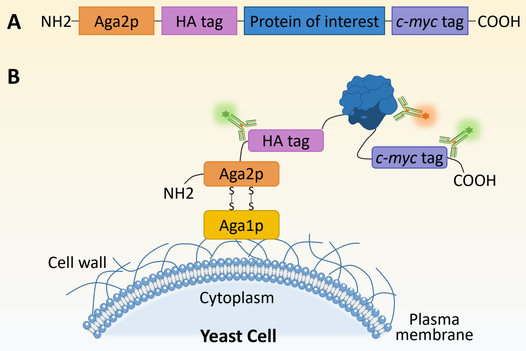
Phage Display

Phage display was the first directed evolution platform developed. Exogenous DNA sequences of interest are introduced into the phage genome at the location of the nucleotide sequence encoding one of the phage coat proteins. When phage infection occurs, phage gene expression is initiated inside the bacterial host, and the inserted peptide/antibody fragment is subsequently displayed on the phage surface as a combination product of the relevant genes encoding the coat protein and the cloned sequence. M13, fd and f1 belong to Ff (F-pilus specific filamentous) phages and share 98% identity at the DNA level, while T4 and T7 belong to tail phages. Compared with M13, T7 has several special advantages, including containing double-stranded DNA that is more stable and less prone to mutation during replication, foreign cDNA or bacterial genomic libraries can be directly inserted into the T7 ds DNA genome, independent of the bacterial protein secretion pathway. M13 can express five coat proteins (pIII, pVI, pVII, pVIII, and pIX) and a POI. POI is most often fused to the pIII due to its structural flexibility and ability to display large proteins with molecular weights up to 100 kDa.



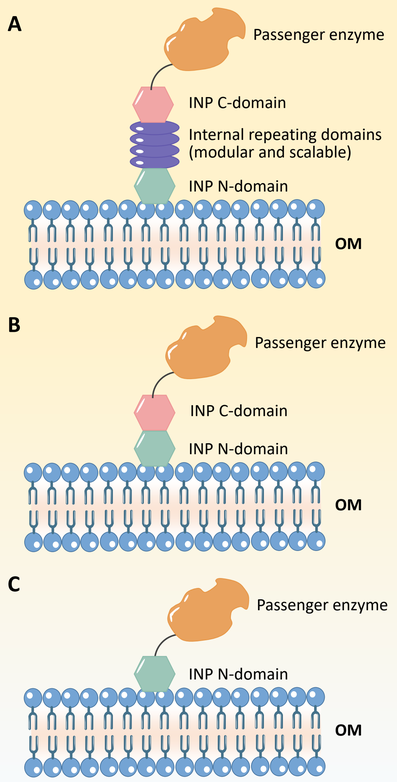
Yeast Display

The first yeast surface display system was developed by Boder and Wittrup in 1997. Yeast surface display is used for directed evolution of antibodies, peptides and proteins. Compared with other similar systems, yeast surface display technology has the following advantages: yeast strains such as *Saccharomyces cerevisiae*, *Pichia pastoris* and *Yarrowia lipolytica* have a GRAS status; post-translational modification, correct folding and glycosylation of proteins; easy cell culture and gene manipulation; compatibility with flow cytometry analysis. POI is fused to the C-terminus of the a-agglutinin Aga2p subunit in the most common yeast display system. Induction of protein expression results in surface display of the fusion protein through the formation of a disulfide bond between the Aga2p-POI fusion protein and the endogenous yeast cell wall protein Aga1p. Fusion protein expression can be quantified using the two epitope tags (an N-terminal HA tag and a C-terminal c-myc tag) present in this system, but information on POI fold or function needs to be studied using a ligand or antibody specific to the native fold of the displayed protein.



Escherichia coli Display

The Gram-negative bacterium *E. coli* is a common host for surface display of recombinant proteins and peptides because it can produce recombinant proteins in high yields and is easy to genetically manipulate. Since *E. coli* are larger than phages, selection, screening, and characterization can be performed directly using flow cytometry-based methods. Additionally, it has a lower stickiness and therefore results in lower background levels. *E. coli* can also be grown and concentrated to sufficiently high densities to represent large naïve and synthetic libraries. The display system must traverse the complex cell envelope of *E. coli* to display on the cell surface. The carrier protein contains a robust surface anchor, and the fusion of the POI to the carrier protein facilitates export across the cell envelope and anchors the POI to the bacterial cell surface. There are a variety of carrier proteins for displaying POIs on *E. coli*. Systems based on OMPs, surface appendages, virulence factors, and lipoprotein-derived systems are all typical surface display systems for *E. coli*, but these systems are mainly used for surface exposure of peptides and small proteins. Systems based on ATs and INP can be used to display large and complex POIs.



Creative BioMart

Creative BioMart provides a complete set of services to enable protein engineering for a variety of applications by combining multiple technologies. Thousands of researchers benefit from these services in improving or generating proteins with new and desired functions.

Creative BioMart has set up a protein engineering platform with unique features:

- Advanced bioinformatic tools and computational approaches for rational design
- Various strategies of library construction for directed evolution
- Development of *in vivo* and *in vitro* protein display systems
- High-throughput screening
- Characterization of screened/selected variants
- Quantitative sequence-structure relationship

Our Protein Engineering Platforms Include:

1. **Directed Evolution**
   - Phage display platform
   - *E. coli* display platform
   - Yeast display platform
   - Special cell-based display platform
   - Cell-free display platform
2. **Rational Design**
   - Sequence-based design
   - Structure-based design
   - *De novo* design
3. **Library Construction**
   - Random mutagenesis
   - Site directed mutagenesis
   - DNA recombination

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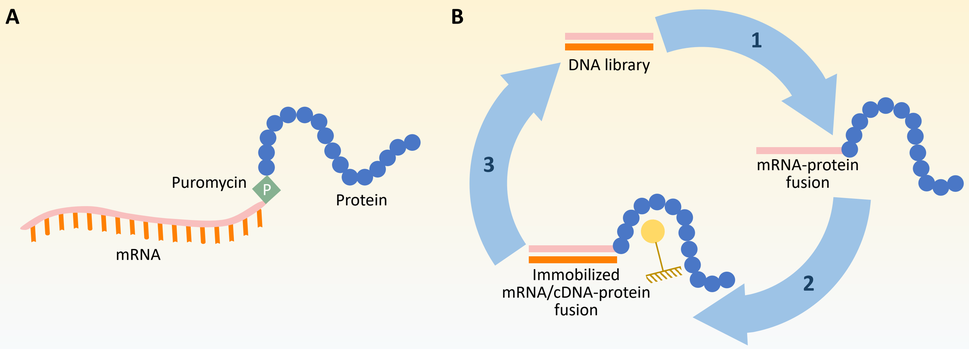
Cell-free Display

In vitro display and selection approaches involve three main steps: i) the generation of a large library of variants (library construction); ii) multiple rounds of enrichment of variants (biopanning) displaying the desired properties via the genotype–phenotype linkage provided by the display system used; and iii) functional screening and characterization of selected variants using appropriate assays. Many cell-free display methods have been developed, such as ribosome display, mRNA display, aptamer library display, IVC, CIS display and CAD.

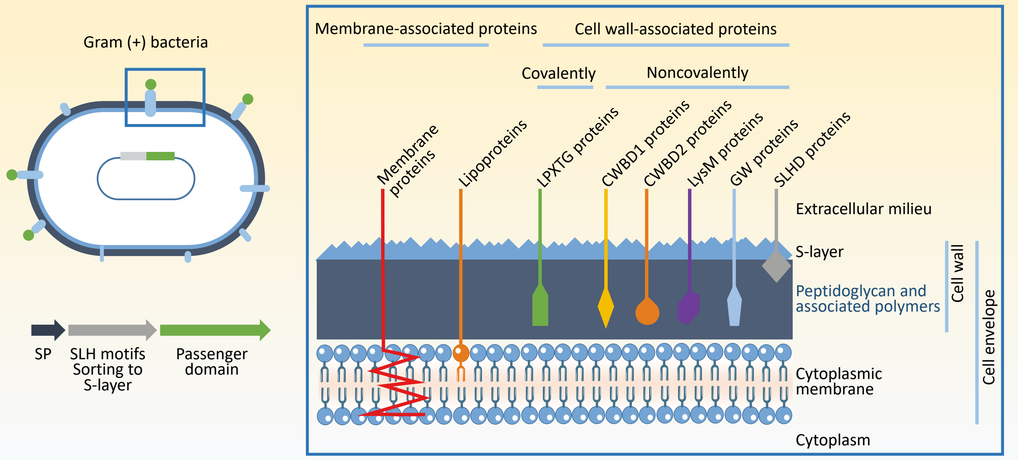
The ribosome display system utilizes the formation of stable PRM complexes to link nascent proteins to their corresponding mRNAs. In this system, since the vector does not contain a stop codon, the protein remains connected to the tRNA after synthesis. After biopanning, the complex dissociates and releases mRNA, which is then followed by in situ reverse transcription PCR to generate cDNA for additional rounds of screening. Ribosome display allows mutagenesis by error-prone PCR during screening, is not limited by host cell transformation efficiency, and also permits larger library sizes.

mRNA display is an *in vitro* selection technique similar to ribosome display, based on the formation of a covalent bond between a peptide and its encoding mRNA molecule during translation. The 3' end of the mRNA is modified with puromycin. During the final stage of translation, puromycin enters the A-site of the ribosome and covalently attaches to the C-terminus of the translated polypeptide. mRNAs display ease of incorporation of unnatural amino acids and can perform selection under a wide range of conditions.



Special Cell-based Display

Cell-surface display is the expression of peptides and proteins on the surface of living cells or their organelles by fusing them with functional components of cell exposed to the environment. Selection of cell-displayed libraries is typically performed using FACS, by labeling the antigen with a fluorophore and then incubating it with the cell-displayed protein library in solution. In addition to being widely used in *E. coli* and yeast, cell-surface display technology is also used on the surface of other bacteria, spores and even gas vesicles. There are various types of carrier proteins on the surface of Gram-positive bacteria, including membrane-associated proteins (membrane proteins, lipoproteins) and cell wall-associated proteins (LPXTG, CWBD1, CWBD2, LysM, GW and SLHD proteins). An LPXTG sequence motif, a stretch of ~23 hydrophobic residues and a tail of positively charged residues constitutes the sorting signal. Bacterial spores are also used in surface display technology. For example, the outer layer of the spore of *Bacillus subtilis* consists of more than 70 proteins, so its coat protein can be used to generate fusion proteins.



References:

Davydova, E.K. (2022) Protein Engineering: Advances in Phage Display for Basic Science and Medical Research. *Biochemistry Moscow.* 87 (Suppl 1): S146–S167.

Kizerwetter, M., *et al.* (2022) Empowering gene delivery with protein engineering platforms. *Gene Ther*.

Jaroszewicz, W., *et al.* (2022) Phage display and other peptide display technologies. *FEMS Microbiol Rev*. 46(2): fuab052.

Teymennet-Ramírez, K.V., *et al.* (2022) Yeast Surface Display System: Strategies for Improvement and Biotechnological Applications. Front. *Bioeng. Biotechnol*. 9: 794742.

Cherf, G.M., Cochran, J.R. (2015) Applications of Yeast Surface Display for Protein Engineering. *Methods Mol Biol*. 1319: 155-75.

Salema, V., Fernández, L.Á. (2017) *Escherichia coli* surface display for the selection of nanobodies. *Microb Biotechnol*. 10(6): 1468-1484.

Bloois, E., *et al.* (2011) Decorating microbes: surface display of proteins on *Escherichia coli*. *Trends Biotechnol*. 29(2): 79-86.

Galán, A., *et al.* (2016) Library-based display technologies: Where do we stand? *Mol Biosyst*. 12: 2342-2358.

Newton, M.S., *et al.* (2020) *In Vitro* Selection of Peptides and Proteins-Advantages of mRNA Display. *ACS Synth* Biol. 9(2): 181-190.

Lee, S.Y., *et al.* (2003) Microbial cell-surface display. *Trends Biotechnol.* 21(1): 45-52.

Abbreviation:

ATs, autotransporter proteins; CAD, covalent antibody display; CIS, cis-activity based; FACS, fluorescence-activated cell sorting; GRAS, generally recognized as safe; HA, hemagglutinin; INP, ice nucleation protein; IVC, *in vitro* compartmentalization; OM, outer membrane; OMPs, outer membrane proteins; POI, protein of interest; PRM, protein–ribosome–mRNA.