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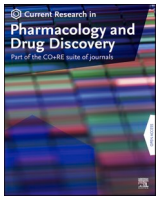

Development of Recombinant Antibody by Yeast Surface Display Technology

Mujahed I. Mustafa [a,*], Awad A. Alzebair [b], Ahmed Mohammed [b]

[a] Department of Biotechnology, College of Applied and Industrial Sciences, University of Bahri, Khartoum, Sudan
[b] Department of Biotechnology, School of Life Sciences and Technology, Omdurman Islamic University, Omdurman, Sudan

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ABSTRACT

Recombinant antibodies have emerged as powerful tools in various fields, including therapeutics, diagnostics, and research applications. The selection of high-affinity antibodies with desired specificity is a crucial step in the development of recombinant antibody-based products. In recent years, yeast surface display technology has gained significant attention as a robust and versatile platform for antibody selection. This graphical review provides an overview of the yeast surface display technology and its applications in recombinant antibody selection. We discuss the key components involved in the construction of yeast surface display libraries, including the antibody gene libraries, yeast host strains, and display vectors. Furthermore, we highlight the strategies employed for affinity maturation and optimization of recombinant antibodies using yeast surface display. Finally, we discuss the advantages and limitations of this technology compared to other antibody selection methods. Overall, yeast surface display technology offers a powerful and efficient approach for the selection of recombinant antibodies, enabling the rapid generation of high-affinity antibodies for various applications.

1. Introduction

The discovery and development of effective antibodies for therapeutic purposes has revolutionized the field of medical research and treatment. Antibodies are versatile proteins that can recognize and bind to specific targets making them valuable tools in diagnostics therapeutics and research applications (Weisser and Hall, 2009).

Traditionally, antibodies were obtained from immunized animals such as mice or rabbits. However, this approach has several limitations, including ethical concerns, batch-to-batch variability, and the potential for generating immune responses in patients receiving therapeutic antibodies. To overcome these challenges, recombinant antibody selection systems have emerged as a powerful alternative.

Recombinant antibody selection systems involve the generation of antibodies using molecular biology techniques rather than relying on animal immunization. These systems typically employ phage display or yeast surface display (YSD) technologies to create large libraries of antibody fragments that can be screened for binding to specific targets (Hoogenboom, 2005) (Fig. 1).

Phage display is one of the most widely used recombinant antibody selection systems. (Fig. 2).

YSD is another powerful recombinant antibody selection system that utilizes genetically engineered yeast cells instead of bacteriophages (Boder and Wittrup, 1997). In this approach, antibody fragments are displayed on the surface of yeast cells, allowing for the screening and isolation of specific binders(Chao, et al., 2006). (Fig. 3). YSD offers several advantages over phage display, including the ability to perform more complex selections and the potential for generating full-length antibodies.

Recombinant antibody selection systems have revolutionized the field of antibody engineering by enabling the rapid isolation of high-affinity antibodies against a wide range of targets(Saeed, 2017). These systems have been successfully used to generate therapeutic antibodies for various diseases, including cancer, autoimmune disorders, and infectious diseases.

In addition to their therapeutic applications, recombinant antibody selection systems have also facilitated advancements in diagnostics and research. They have been instrumental in developing diagnostic tests for infectious diseases, detecting biomarkers associated with various conditions, and studying protein-protein interactions (Pavlickova, et al., 2004).

Furthermore, these selection systems have paved the way for the

* Corresponding author.
E-mail address: mujahedimustafa@gmail.com (M.I. Mustafa).

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development of novel antibody-based therapeutics such as bispecific antibodies and antibody-drug conjugates. Bispecific antibodies can simultaneously bind to two different targets, offering enhanced therapeutic potential. Antibody-drug conjugates combine an antibody that specifically recognizes a target on cancer cells with a potent cytotoxic drug, resulting in targeted delivery and increased efficacy(Drago, et al., 2021).

2. Overview of YSD technology

YSD technology is a powerful tool used in molecular biology and protein engineering. It allows for the presentation of proteins or peptides on the surface of yeast cells, enabling the selection and isolation of desired variants through high-throughput screening techniques (Fig. 3). This technology has revolutionized various fields, including antibody engineering, vaccine development, enzyme optimization, and directed evolution (Cherf and Cochran, 2015).

The concept behind YSD is relatively simple. It involves genetically fusing the gene encoding the protein of interest with a gene encoding a yeast cell wall protein. This fusion gene is then expressed in yeast cells, resulting in the display of the protein on the cell surface. The most commonly used yeast species for this purpose, is *Saccharomyces cerevisiae*, a well-studied and easily manipulated organism.

The process of YSD involves several steps. First, the gene encoding the protein of interest is fused to a gene encoding a yeast cell wall protein, typically Aga2p or Flo1p. This fusion gene is then transformed into yeast cells using various methods such as electroporation or lithium acetate transformation. The transformed cells are then grown under selective conditions to ensure the expression of the fusion protein on the cell surface(Tanaka, et al., 2012).

Once the protein is displayed on the yeast cell surface, it can be screened or selected using different techniques. For example, fluorescence-activated cell sorting (FACS) can be used to isolate yeast cells displaying proteins with desired properties(Gera, et al., 2013). Alternatively, magnetic-activated cell sorting (MACS) can be employed by labeling the target protein with magnetic beads and isolating cells displaying these beads.

3. Methodology

3.1. Construction of antibody libraries for YSD

Antibodies are essential tools in various fields, including diagnostics, therapeutics, and research. Traditional methods of antibody generation, such as hybridoma technology, have limitations in terms of time, cost, and the ability to target specific antigens(Weaver-Feldhaus, 2004). To overcome these challenges, alternative approaches like YSDs have emerged as powerful tools for antibody engineering (Fig. 4).

3.1.1. Generation of antibody gene diversity

The first step in constructing an antibody library is to generate a diverse repertoire of antibody genes that can be displayed on the surface of yeast cells. This diversity is crucial to ensure that a wide range of antigen targets can be recognized by the antibodies (Fig. 4).

Advances in DNA synthesis technologies have enabled the creation of synthetic libraries with vast diversity. Combinatorial approaches such as phage display or ribosome display allow for the generation of large numbers (up to 10^11) of unique antibody variants by randomizing specific regions within the antibody gene sequences(Könning and Kolmar 2018).

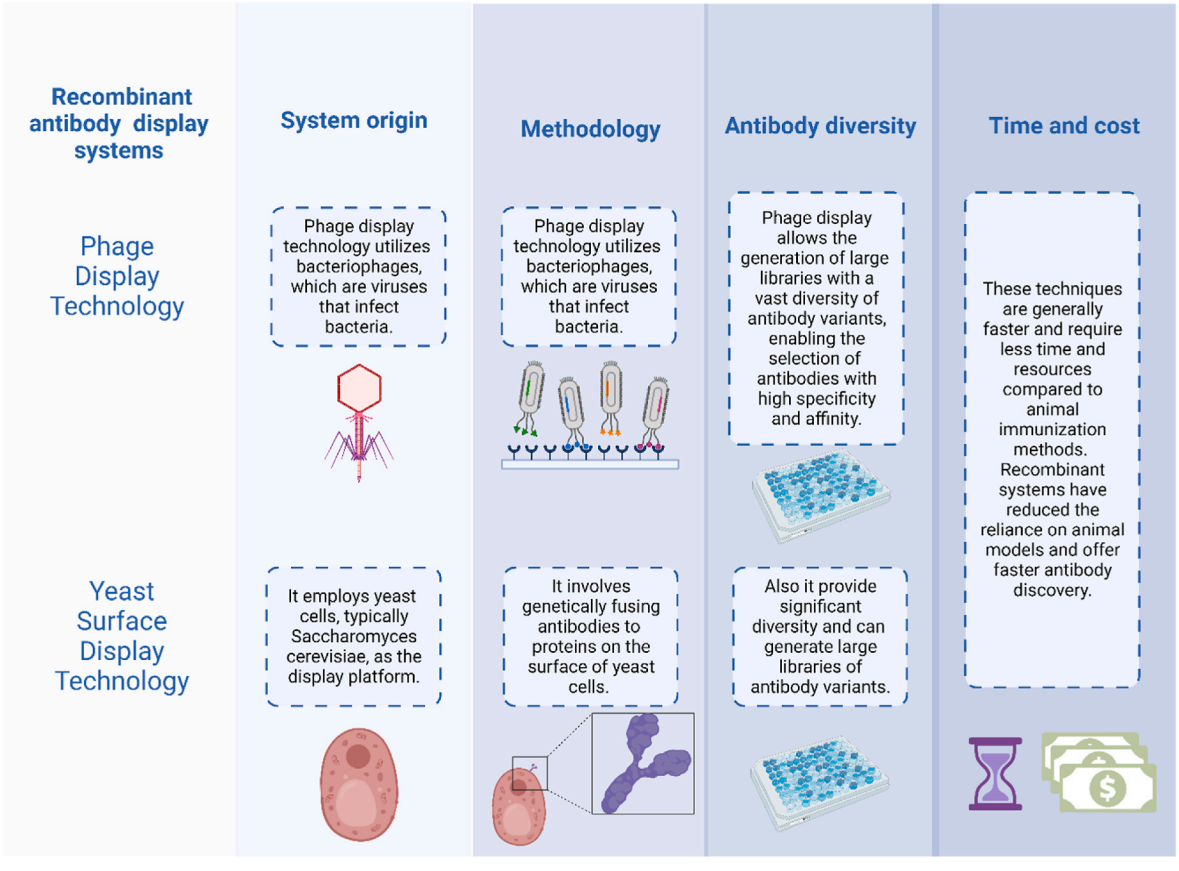

Fig. 1. The differences between phage display technology and YSD.

3.1.2. Cloning and expression of antibody genes in yeast

Once a diverse repertoire of antibody genes is obtained, the next step is to clone and express these genes in yeast cells for surface display. Yeast, particularly *Saccharomyces cerevisiae*, is an ideal host for this purpose due to its ease of manipulation, well-characterized genetics, and ability to properly fold and display functional antibodies on its surface (Mumberg, et al., 1995). The following steps are involved in this process:

a) Vector Design: A suitable yeast expression vector needs to be designed to facilitate the cloning and expression of antibody genes. The vector typically contains elements such as a yeast promoter, secretion signal sequence, antibody gene cassette, selectable marker, and replication origin(Frenzel, et al., 2013).
b) Transformation: The constructed vector is introduced into yeast cells using transformation techniques such as electroporation or lithium acetate treatment. This allows the integration of the vector into the yeast genome or its maintenance as an episomal plasmid.
c) Library Screening: Once transformed, yeast cells expressing antibody genes on their surface can be subjected to antigen-specific screening assays. These assays involve incubating the yeast library with the target antigen and selecting yeast cells that bind specifically to it. Positive clones can then be isolated for further analysis or downstream applications.
d) Antibody Production: After identifying specific antibody clones from the library, they can be further characterized and produced in larger quantities. This involves scaling up the culture of selected clones and purifying the antibodies using techniques like protein A/G affinity chromatography (Fig. 4).

4. Advantages of YSD

One of the key advantages of YSD is its ability to mimic the natural process of antibody selection that occurs in the human immune system. In this process, B cells produce a diverse repertoire of antibodies, which are then screened for their ability to bind to specific antigens. By using

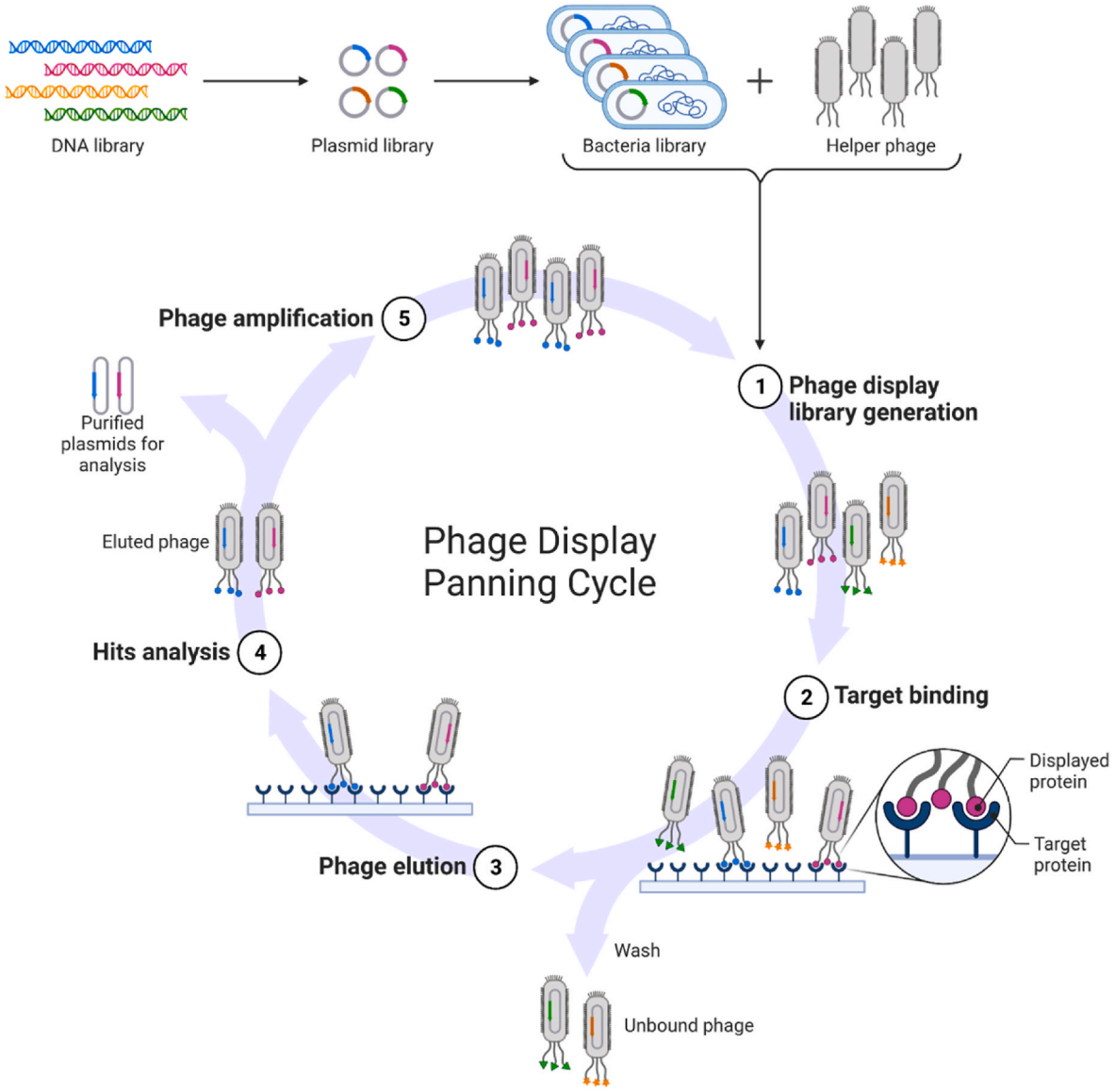

Fig. 2. Phage display panning cycle is a powerful technique used in recombinant antibody engineering. It involves the fusion of antibody fragments with coat proteins on the surface of bacteriophages. By introducing genetic diversity into the antibody genes through random mutagenesis or recombination techniques, vast libraries containing billions of different antibody variants can be generated. The phage display library is then subjected to multiple rounds of selection against the desired target antigen. During each round, non-binding phages are washed away while those that bind specifically to the target are retained. The bound phages can then be eluted and amplified for subsequent rounds of selection until highly specific antibodies are obtained.

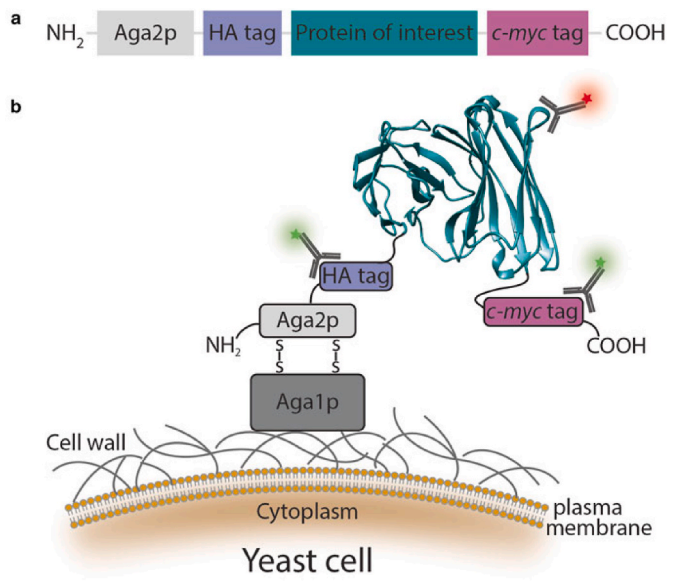

Fig. 3. Demonstrates the arrangement of YSDs in a schematic representation. In this display, the protein of interest is positioned between two epitope tags: a 9-amino acid hemagglutinin antigen (HA) tag and a 10-amino acid c-myc tag. These tags are fused to the C-terminus of the a-agglutinin Aga2p subunit. The process involves the translation of the fusion protein, where the 69-amino acid Aga2p subunit joins with the 725-amino acid a-agglutinin Aga1p subunit through two disulfide bonds. The resulting fusion protein is then secreted into the extracellular space. In this space, Aga1p is anchored to the cell wall by a 61,6-glucan covalent linkage. As a result, the protein of interest is displayed on the cell surface, where it can interact with soluble ligands. To detect the functional display of the protein, a fluorescently labeled antibody or ligand specific to the native fold can be employed (indicated by the red star). The epitope tags, on the other hand, are used to normalize protein function to surface expression level using labeled anti-HA or anti-c-myc antibodies (indicated by the green stars). These features enable the flow cytometric sorting of a diverse mixture of yeast cells. Each yeast cell displays up to 100,000 copies of an individual protein variant, and sorting can be based on the biophysical and biochemical properties of the displayed proteins (Cherf and Cochran, 2015). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

YSDs, researchers can create large libraries of antibody variants and screen them against a target antigen in a high-throughput manner (Tanaka, et al., 2012).

Another advantage of YSD is its compatibility with eukaryotic protein expression machinery. Unlike phage display, which relies on bacterial systems, yeast cells can correctly fold and assemble complex proteins like antibodies. This feature is particularly important when selecting antibodies against challenging targets such as membrane proteins or conformationally sensitive antigens.

Furthermore, YSD allows for the identification of antibodies with diverse binding modes. Unlike hybridoma technology which primarily generates monoclonal antibodies targeting a single epitope, YSD libraries can yield polyclonal populations with different binding specificities within a single experiment. This capability is particularly valuable when developing diagnostic assays or studying complex biological systems where multiple targets need to be simultaneously detected(McMahon, et al., 2018).

5. Limitations and challenges

One of the major limitations of YSD is the size constraint for displaying large proteins or protein complexes. The yeast cell wall has a limited capacity to accommodate large proteins, making it difficult to display them effectively. This can be particularly challenging when studying membrane proteins or multi-subunit complexes that are naturally larger in size. Researchers have attempted to overcome this limitation by using alternative display systems such as bacterial or mammalian cells, but these systems come with their own set of challenges.

Another challenge associated with YSD is the potential for misfolding or incorrect folding of displayed proteins. When a protein is displayed on the yeast cell surface, it may not fold correctly due to improper disulfide bond formation or lack of proper chaperones. This can lead to loss of function or altered binding properties compared to the native form of the protein. Researchers have developed strategies such as co-expression of chaperones or optimization of growth conditions to mitigate this issue, but it remains a challenge in certain cases(Linciano, et al., 2019).

Furthermore, there can be limitations in terms of library size and diversity when using YSD for directed evolution experiments. The number of variants that can be displayed on the yeast cell surface is limited by factors such as transformation efficiency and library complexity. This can restrict the ability to explore large sequence spaces and identify optimal variants efficiently. Researchers have attempted to address this limitation by developing strategies such as DNA shuffling or using alternative display systems with higher transformation efficiencies.

Another challenge associated with YSD is related to post-translational modifications (PTMs). Yeast cells may not possess the necessary machinery to perform certain PTMs, such as glycosylation or phosphorylation, which are critical for the proper function of certain proteins. This can limit the applicability of YSDs for studying proteins that require specific PTMs for their activity or binding properties. Researchers have explored alternative expression systems or engineered yeast strains to introduce specific PTM pathways, but this remains an ongoing challenge(Wang, et al., 2014).

Finally, there can be limitations in terms of scalability and throughput when using YSD technology. While it is a powerful tool for protein engineering and library screening, it may not be suitable for high-throughput applications due to the time-consuming nature of yeast cell culture and transformation. Additionally, scaling up the production of displayed proteins can be challenging, especially when large quantities are required for downstream applications.

6. Key factors to optimize yeast surface display technology

The Yeast Surface Display System (YSDS) has revolutionized the field of antibody engineering, allowing the display and selection of antibodies directly on the surface of yeast cells. This powerful technique enables the generation of high-affinity antibodies with desirable characteristics. However, to fully exploit the potential of the YSDS, several key components require optimization. (Fig. 5).

6.1. Protein of interest

Selecting the appropriate protein of interest is crucial for efficient antibody engineering. Factors to consider include the affinity, stability, and antigen-binding site accessibility. A proper understanding of the target antigen and its epitopes is essential for designing an optimized protein(Boder, et al., 2012).

6.2. Host cells

Different yeast species are employed as host cells in the YSDS, including *Saccharomyces cerevisiae* and *Pichia pastoris*. The choice of host should consider factors such as protein secretion, glycosylation patterns, growth rate, and expression levels. Each host has unique advantages and challenges, requiring careful assessment to determine the most suitable option for the specific antibody engineering project (Teymennet-Ramírez, et al., 2022).

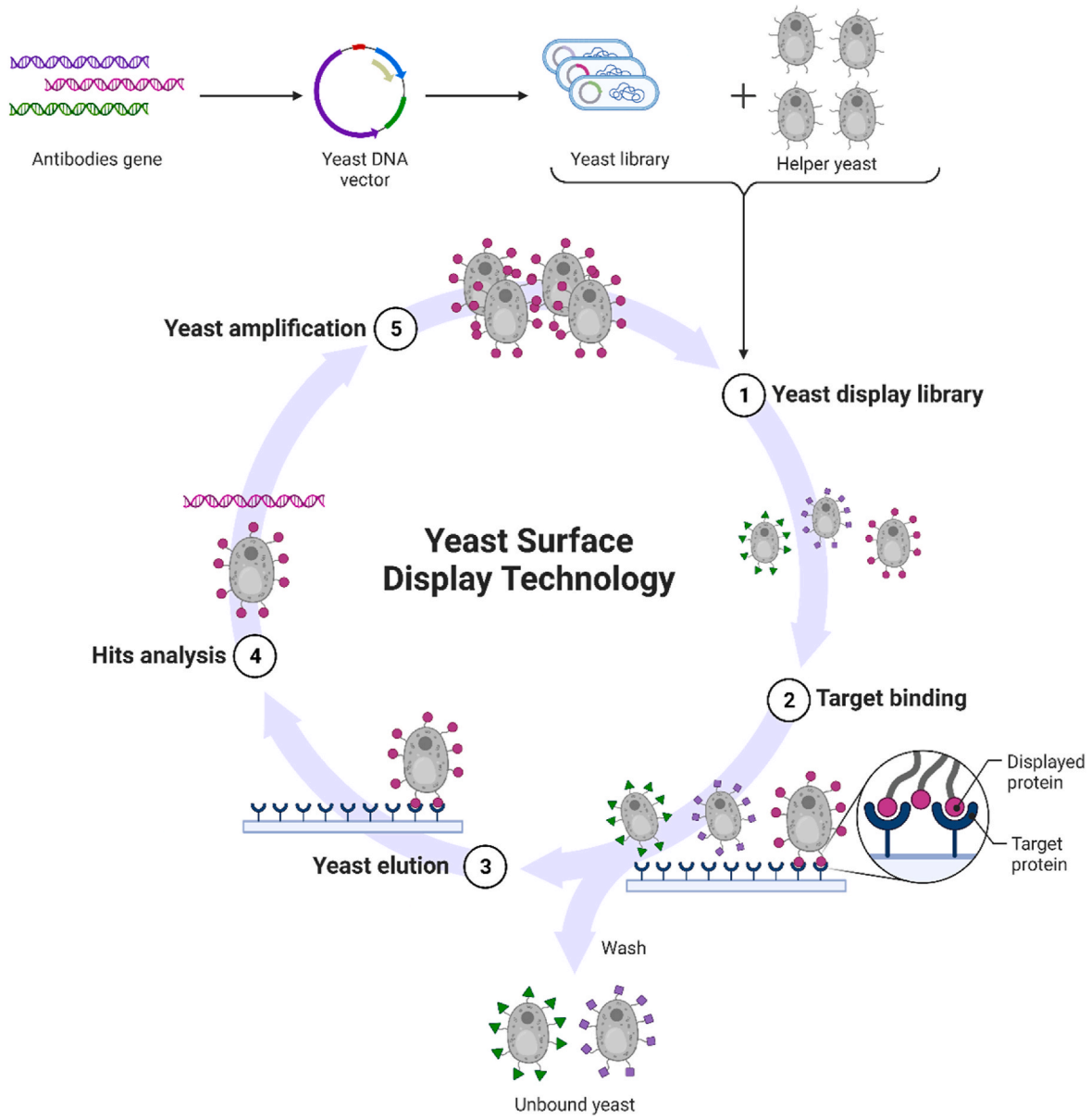

Fig. 4. A schematic representation of the most common method for recombinant antibodies production using YSD technology. DNA encoding the antibody of interest is synthesized and cloned into a yeast expression vector. The vector contains an inducible promoter, a signal peptide, and the gene of interest fused to the surface display protein (e.g., Aga2p). The yeast cell population expressing the recombinant antibody is subjected to different screening techniques to identify cells producing antibodies with desired characteristics, such as high affinity and specificity. The enriched yeast cell population is further evaluated for the binding affinity, specificity, and functional activity of the displayed antibodies.

6.3. Promoters

The selection of suitable promoters is crucial for driving the expression of the protein of interest. Promoters with varying strengths can significantly impact protein expression levels. Employing strong, inducible promoters can result in increased protein yields, while tightly regulated promoters can facilitate controlled expression. Careful promoter selection ensures optimal protein production without compromising host viability(Kim, et al., 2015).

6.4. Signal peptides

Signal peptides play a critical role in guiding proteins for secretion. Through optimization, one can enhance the efficiency of protein trafficking and secretion. Analysis of target protein characteristics, compatibility with the host's secretion pathway, and experimental considerations should guide the selection and engineering of signal peptides (Teymennet-Ramírez, et al., 2022).

6.5. Anchors

The YSDS relies on the anchoring of the protein of interest to the yeast cell surface. Various anchor systems, such as the AGα1, AGA2, SCÎL2, and Flo1p anchors, have been employed. Selection of the appropriate anchor system is essential for maximizing surface display efficiency, stability, and accessibility of the engineered antibodies (Teymennet-Ramírez, et al., 2022).

6.6. Culture conditions

Optimizing culture conditions, such as temperature, pH, media composition, and induction strategies, greatly impacts the efficiency of

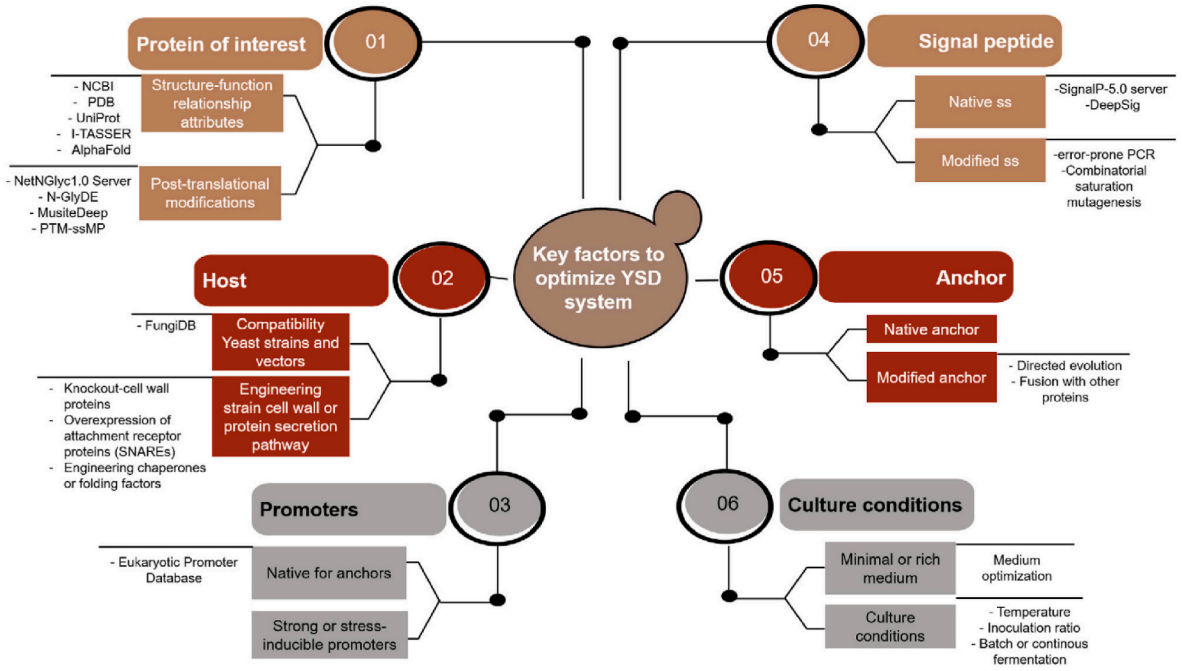

Fig. 5. Different strategies and tools available to improve the efficiency and selection of displayed proteins in the YSDS (Teymennet-Ramírez, et al., 2022).

the YSDS. Factors influencing cell growth, protein expression, and protein folding must be considered to achieve the desired antibody yields and quality(Tanino, et al., 2007).

Overall, the YSDS represents a powerful tool for antibody engineering, but its full potential can only be realized through strategic optimization of key components. By carefully considering the protein of interest, host cells, promoters, signal peptides, anchors, and culture conditions, researchers can maximize the efficiency, stability, and functionality of engineered antibodies. Continued advancements in these areas will undoubtedly drive innovation and unlock new opportunities for therapeutic antibody development and other biotechnological applications(Gai and Wittrup, 2007).

7. Conclusion and future directions

The literature review has provided valuable insights into the use of YSD technology as a recombinant antibody selection system. Firstly, it was found that YSD offers several advantages over other selection systems, such as phage display and yeast display. These advantages include high expression levels, proper folding of antibodies, and the ability to screen large libraries. Additionally, YSD allows for the selection of antibodies with desired binding affinities and specificities. Furthermore, this article highlighted various strategies for improving the efficiency and functionality of YSDSs. These strategies include optimizing library size and diversity, enhancing antibody expression levels, and developing novel screening methods.

Moving forward, future research in recombinant antibody selection systems using YSD technology should focus on several key areas. Firstly, there is a need to further optimize the library construction process to increase diversity and improve antibody quality. This can be achieved by exploring different mutagenesis techniques and incorporating synthetic biology approaches(Tanaka and Kondo, 2015). Secondly, efforts should be made to enhance the screening process by developing more efficient and high-throughput methods. This could involve the use of advanced imaging techniques or microfluidic devices to streamline the selection process. Additionally, there is a need for continued research into understanding the factors that influence antibody expression levels on yeast cell surfaces. This knowledge can help in designing strategies to improve antibody production and stability. Lastly, future studies should also explore novel applications of YSD technology beyond antibody selection. This could include using it for protein engineering or as a platform for drug discovery. Overall, with further research and development in these areas, YSD technology has great potential to revolutionize recombinant antibody selection systems and contribute to advancements in various fields such as therapeutics development and diagnostics.

Author contribution

MM Conceptualization, investigation, writing (original draft), and visualization. AA Conceptualization, investigation, writing (original draft).; AM Writing – review & editing, Supervision. All authors read and approved the final manuscript.

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Declaration of competing interest

None declared.

Data availability

No data was used for the research described in the article.

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