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Advances in Polymerase Engineering and Their Biotechnological Applications

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Abstract: DNA and RNA polymerases are essential enzymes responsible for nucleic acid replication, transcription, and amplification, and they play indispensable roles in molecular biology, biotechnology, and synthetic biology. However, the natural properties of polymerases are often limited by their evolutionary origins, resulting in constraints in catalytic efficiency, substrate compatibility, thermostability, and replication fidelity. In recent years, advances in protein engineering and structural biology have enabled the development of diverse strategies to enhance polymerase performance. This review summarizes recent progress in polymerase engineering aimed at structural optimization and functional enhancement. Major engineering strategies, including directed evolution, rational and semi-rational design, computational protein engineering, and domain fusion approaches, are discussed in terms of their principles and applications. In addition, the rapidly expanding applications of engineered polymerases are highlighted, particularly in molecular diagnostics, enzymatic DNA synthesis, artificial genetic systems, and medical and industrial biotechnology. Overall, the continuous integration of high-throughput screening technologies, structural analysis, and artificial intelligence–assisted design is expected to accelerate the development of next-generation polymerases with improved performance and expanded catalytic capabilities. These advances will further promote innovations in synthetic biology, nucleic acid diagnostics, genome engineering, and the development of novel biotechnological tools.

Keywords: Polymerase engineering; Directed evolution; Rational design; Synthetic biology; Molecular diagnostics

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1. Introduction

Polymerases are a class of essential enzymes involved in nucleic acid metabolism, playing critical roles in DNA replication, RNA transcription, and in vitro nucleic acid amplification. Owing to their catalytic capability in synthesizing nucleic acids with high specificity and efficiency, polymerases have been widely applied in basic molecular biology research, clinical diagnostics, genome sequencing, and synthetic biology [1–3]. For example, Taq DNA polymerase, which exhibits excellent thermostability, plays an indispensable role in PCR amplification [4]. Phi29 DNA polymerase, characterized by its strong strand-displacement activity and proofreading capability, has been extensively used in single-cell whole-genome amplification [5]. In addition, T7 RNA polymerase occupies an irreplaceable position in in vitro transcription systems due to its high transcription efficiency and promoter specificity [6].

However, the functionality of natural polymerases is often constrained by their evolutionary origins and physiological

environments. As a result, many polymerases exhibit limitations in substrate recognition range, thermal stability, catalytic rate, extension capability, and replication fidelity [7]. For instance, some polymerases are prone to thermal inactivation at elevated temperatures, restricting their applications in complex biochemical environments. Conversely, certain high-fidelity polymerases maintain replication accuracy at the expense of reduced catalytic speed. These trade-offs between structural stability and catalytic efficiency limit the broader application of polymerases in emerging biotechnological fields. Consequently, engineering polymerases to improve their catalytic properties has become an important research direction in protein engineering and synthetic biology. With the rapid development of structural biology, computational modeling, and high-throughput screening technologies, a variety of polymerase engineering strategies have been developed. Directed evolution enables high-throughput screening of polymerase variants by establishing a physical linkage between genotype and phenotype [8]. Rational and semi-rational design, guided by three-dimensional structural information, conserved sequence analysis, and energy calculations, allows precise prediction of beneficial mutation sites to improve catalytic efficiency or structural stability. In addition, domain engineering and terminal truncation strategies can modulate spatial conformation and interdomain interactions, thereby improving polymerase processivity and template-binding efficiency [9].

In this review, we summarize recent advances in the structural engineering and functional enhancement of polymerases. First, major strategies for polymerase engineering are discussed, including directed evolution, rational and semi-rational design, and structure-guided computational approaches. These strategies provide powerful tools for improving catalytic activity, stability, fidelity, and substrate adaptability. Subsequently, we highlight the emerging applications of engineered polymerases in molecular diagnostics, enzymatic DNA and XNA synthesis, and medical and industrial biotechnology. Understanding these advances not only provides insights into the structure–function relationships of polymerases but also offers guidance for the future design of high-performance enzymes for diverse biotechnological applications.

2. Major strategies for polymerase engineering

With the rapid development of protein engineering and bioinformatics, researchers have increasingly explored strategies to improve polymerase performance and expand their functional capabilities through structural optimization. Various approaches have been developed to engineer polymerases, among which directed evolution and rational or semi-rational design represent the two most widely used strategies [10]. Each method has its own advantages and limitations. In practical research, these strategies are often applied in combination to achieve more efficient and targeted polymerase engineering.

2.1. Directed evolution

Directed evolution is one of the most classical and widely used strategies for enzyme engineering. This approach introduces mutations into proteins lacking detailed structural information through methods such as random mutagenesis, DNA recombination, and saturation mutagenesis, thereby generating large mutant libraries from which improved variants can be selected through high-throughput screening [11,12]. Common mutagenesis techniques used in directed evolution include error-prone PCR, chemical mutagenesis, DNA shuffling, and saturation mutagenesis. One major advantage of directed evolution is its minimal dependence on structural knowledge. It enables the discovery of beneficial mutations that are often difficult to predict through rational design approaches, particularly for improving catalytic activity. However, directed evolution also has several limitations. The experimental workload can be substantial, screening efficiency may be relatively low, and beneficial mutations obtained through random mutagenesis are sometimes difficult to interpret mechanistically. Therefore, this strategy is often most suitable for random optimization of enzyme performance within established screening systems and experimental conditions.

In recent years, several innovative directed evolution methods have been developed, greatly accelerating polymerase engineering. Techniques such as compartmentalized self-replication (CSR) and orthogonal replication systems have significantly expanded the capabilities of polymerase evolution. Compartmentalized self-replication (CSR) is an efficient

in vitro directed evolution method that encapsulates individual genes and their expression products within water-in-oil emulsion droplets, forming microreactors. In this system, each gene encoding a polymerase variant is transcribed and translated within a separate droplet, and the resulting polymerase catalyzes the replication of its own encoding gene. Only polymerase variants with sufficiently high catalytic activity can produce enough copies of their genes to survive subsequent rounds of selection and amplification. Using isothermal CSR, Povilaitis et al. [13] successfully evolved phi29 DNA polymerase variants with improved catalytic activity and stability. Abil et al. [14] developed the compartmentalized partnered replication (CPR) strategy for directed evolution of genetic elements and circuits, providing a framework for evolving complex biological systems. CSR-based approaches have been widely applied to polymerase engineering, including improving the performance of Thermus thermophilus DNA polymerase I [15] and investigating the salt tolerance of phi29 DNA polymerase using microfluidic platforms [16]. In addition, in vitro CSR-based directed evolution of Thermus aquaticus DNA polymerase produced mutants capable of translesion synthesis, enabling DNA replication across damaged templates [17]. Subsequent studies by Naccache [18,19] and Dramé-Maigné [20,21] further demonstrated the enormous potential of CSR for ultra-high-throughput screening and evolution of strand-displacing polymerases and other enzymes.

To achieve continuous and autonomous evolution in complex cellular environments, researchers have also developed orthogonal replication systems. These systems operate within host cells but remain independent of the host genome, thereby providing a dedicated evolutionary platform for target genes. Diercks et al. [22] constructed an orthogonal T7 replication system that enables continuous hypermutation and rapid evolution in Escherichia coli, representing a new strategy for in vivo directed evolution. Similarly, Ma and Lin [23] achieved orthogonal RNA replication in mammalian cells, representing a major breakthrough that enables directed evolution within more complex eukaryotic cellular environments.

Directed evolution is increasingly being integrated with emerging genome-editing technologies such as CRISPR, enabling more precise and efficient mutagenesis and screening. For example, Chen et al. [24] developed a CRISPR–DNA polymerase-assisted targeted mutagenesis system that allows controllable laboratory evolution by introducing genetic diversity at specific genomic loci. In addition, Pu et al. [25] engineered split RNA polymerases through directed evolution to construct multifunctional biosensor platforms, demonstrating the potential of polymerase engineering in biosensing applications.

Directed evolution has led to remarkable advances in polymerase functional expansion and performance optimization, significantly influencing the development of synthetic biology and chemical biology [26]. For example, Maola et al. [27] successfully obtained an efficient threose nucleic acid (TNA) polymerase through homologous recombination and directed evolution, thereby expanding the chemical boundaries of genetic materials. Directed evolution has also been used to alter the promoter recognition specificity of bacteriophage RNA polymerases [28], improve the salt tolerance of phi29 DNA polymerase [16], and systematically expand the functionality of T7 RNA polymerase to meet diverse demands for in vitro nucleic acid synthesis [29].

In the future, as research into fundamental questions related to the origin of life—such as RNA replication and RNA polymerase ribozymes [30] continues to deepen, and as universal intracellular evolution platforms become more sophisticated, engineered polymerases are expected to play increasingly important roles in synthetic biology, disease diagnostics, drug development, and the exploration of fundamental biological principles.

2.2. Rational and semi-rational design

In contrast to directed evolution, rational and semi-rational design strategies rely on structural insights and computational analyses to guide targeted mutations. By integrating information from protein three-dimensional structures, bioinformatics analysis, and molecular dynamics simulations, researchers can predict mutation sites that are likely to improve enzyme performance. Through detailed analysis of polymerase structure and catalytic mechanisms, both residues near the active site and distal residues involved in structural stability can be rationally optimized. Common rational and semi-rational strategies used in polymerase engineering include structure- and energy-based computational design, domain fusion and terminal truncation engineering, and hotspot prediction based on sequence conservation analysis. These approaches

provide a systematic framework for improving polymerase activity, stability, fidelity, and substrate recognition.

2.2.1. Structure- and energy-based computational design

Computational approaches based on structural and energetic analyses not only provide powerful tools for mechanistic investigation but also actively guide polymerase engineering. By integrating three-dimensional protein structures with energy evaluation models, these strategies enable the prediction of how specific mutations influence conformational stability and catalytic performance. At the atomic level, such computational methods facilitate detailed analysis of the structure–function relationships of polymerases, their conformational dynamics, and their interactions with substrates or inhibitors. These insights provide a theoretical framework for the precise and efficient optimization of enzyme stability, catalytic activity, fidelity, and specificity.

Significant progress has been achieved using computational design for improving enzyme stability and activity. For example, by combining iterative mutagenesis with computational design, researchers successfully optimized both the expression level and thermal stability of terminal deoxynucleotidyl transferase (TdT). In another study, computational protein design was used to generate a TdT variant exhibiting both high thermostability and high catalytic activity, enabling the synthesis of long de novo DNA molecules. These studies complement previous computational investigations aimed at improving the stability and practical applications of thermophilic DNA polymerases. In addition, structure-guided engineering has been used to enhance the performance of polymerases in specific biotechnological applications. Based on detailed structural insights, researchers successfully engineered a novel DNA polymerase from Clostridium thermocellum, significantly improving its performance in loop-mediated isothermal amplification (LAMP) assays. Together with semi-rational evolution strategies and programmable site-directed mutagenesis techniques with single-amino-acid precision [9], these computational approaches constitute a powerful toolbox for the targeted engineering of polymerases.

2.2.2. Domain fusion and terminal truncation strategies

Domain fusion is a commonly used strategy in polymerase engineering, in which exogenous protein domains are introduced into the original polymerase structure to improve enzyme solubility, expression level, stability, and catalytic performance. One frequently used fusion partner is Sso7d, a small DNA-binding protein derived from Hyperthermophilic archaea. Fusion of Sso7d with high-fidelity Pfu DNA polymerase or the widely used Taq DNA polymerase has been shown to significantly enhance amplification efficiency and increase the length of amplified DNA products. Śpibida et al. fused the DNA-binding domain of *Pyrococcus furiosus* DNA ligase to Taq Stoffel polymerase, generating an engineered enzyme capable of efficiently amplifying complex DNA templates [31]. Similarly, Maksum et al. reported that fusion of an N-terminal maltose-binding protein (MBP) tag enabled soluble overexpression of Thermus thermophilus (Tth) DNA polymerase in *Escherichia coli* [32]. In addition, fusion with proteins possessing specific functional properties can generate multifunctional chimeric enzymes or significantly enhance polymerase performance. For example, Fei et al. demonstrated that fusion of Taq DNA polymerase with the CL7 protein from E. coli markedly improved DNA amplification efficiency [33].

Terminal truncation is another commonly used strategy for polymerase engineering. By removing regions at the N-terminus or C-terminus, truncation can improve protein expression levels, eliminate non-essential structural regions, enhance protein solubility, and, in some cases, increase polymerase fidelity. Villbrandt et al. generated two N-terminal truncation variants of Taq DNA polymerase: Δ288, lacking the first 288 amino acids, and Δ413, lacking the first 413 amino acids [34]. Both truncated enzymes retained polymerase activity; however, the Δ413 variant showed significantly reduced specific activity and thermostability. Sethy et al. engineered several terminal deoxynucleotidyl transferase (TdT) variants by deleting approximately 140 amino acids from the N-terminus [35]. This truncation significantly improved expression levels, increasing protein yield by 9.5-fold and 23-fold compared with the wild-type enzyme while maintaining similar catalytic efficiency. In another study, Barnes removed 235 amino acids from the N-terminal exonuclease domain of Taq polymerase, which resulted in a twofold increase in replication fidelity.

2.2.3. Hotspot prediction and conservation analysis

semi-rational polymerase engineering. This approach identifies residues that are either critical for enzyme function or tolerant to mutation by comparing evolutionary conservation patterns and structural energy features across homologous sequences. Through multiple sequence alignment, researchers can locate regions that play essential catalytic roles as well as regions that may accommodate mutations, thereby guiding targeted polymerase engineering and performance optimization.

Sequence analyses of multisubunit RNA polymerases have revealed that residues located in the catalytic center are highly conserved, whereas surrounding regulatory regions exhibit greater variability. Consequently, engineering efforts typically focus on low-conservation regions while avoiding highly conserved catalytic residues, thereby minimizing the risk of disrupting the enzyme's active center. Using customized multiple sequence alignment strategies, researchers have successfully improved the performance of Taq DNA polymerase. Conservation-based analysis has also been applied to study viral polymerases. Oany et al. identified highly conserved regions within the RNA polymerase of the Ebola virus through sequence alignment and conservation scoring. Molecular docking analyses further revealed that these conserved residues play key roles in substrate recognition and catalytic activity [36].

Similarly, comparative sequence analysis combined with molecular dynamics simulations has been used to investigate the structure–function relationships of DNA polymerases in pathogenic fungi. Satpati et al. demonstrated that conserved residues tend to form stable interaction networks at structural interfaces, whereas non-conserved regions are often involved in subunit regulation and conformational flexibility [37]. These variable regions can therefore serve as promising targets for protein engineering. In practice, conservation-based strategies have been successfully applied in polymerase engineering. For example, conservation-guided design has been used to engineer psychrophilic polymerases optimized for nanopore long-read sequencing, as well as thermophilic polymerases used in synthetic biology applications. These examples highlight the importance of understanding evolutionary conservation patterns when designing polymerase variants with improved performance and novel functionalities.

3. Applications of polymerase functional improvement

3.1. Molecular diagnostics and PCR technologies

With advances in polymerase engineering and structural optimization, the application of polymerases in molecular diagnostics has expanded from conventional PCR to a variety of highly sensitive nucleic acid detection platforms. The fidelity, thermostability, and processivity of polymerases directly affect the sensitivity and specificity of amplification systems. Therefore, recent studies have focused on developing engineered polymerases to support rapid detection, high accuracy, and simplified one-pot diagnostic reactions.

Polymerase stability and amplification efficiency are particularly important for miniaturized diagnostic platforms. Immobilization of polymerases on nanomaterials or mesoporous carriers can improve enzyme thermostability and reaction uniformity, thereby enhancing the robustness of PCR systems. Meanwhile, ultrafast PCR technologies based on plasma heating or microfluidic devices significantly shorten amplification time, enabling rapid nucleic acid detection suitable for point-of-care testing (POCT). In addition, nanoparticle–polymerase interactions can enhance signal amplification and improve detection sensitivity. For instance, polymerase-mediated end-labeling strategies have enabled single-molecule detection of DNA strand breaks, providing new approaches for studying DNA damage and developing ultrasensitive diagnostic methods.

Isothermal amplification technologies have also become important alternatives to conventional PCR because they eliminate the need for complex thermal cycling equipment. Loop-mediated isothermal amplification (LAMP) enables rapid and sensitive detection of bacterial, plant, and animal pathogens due to the strong strand-displacement activity of polymerases. To further improve specificity, probe-based detection strategies and high-resolution melting analysis have been introduced to distinguish single-nucleotide variations. Other isothermal methods, such as helicase-dependent

amplification (HDA) and recombinase polymerase amplification (RPA), further expand polymerase-based diagnostic systems and support rapid field detection.

In recent years, the integration of polymerase-based amplification with CRISPR detection systems has enabled highly sensitive nucleic acid detection without complex instrumentation. These platforms have been applied to the detection of viruses, parasites, and foodborne pathogens, demonstrating strong potential for public health surveillance and emergency diagnostics. In addition, polymerase-driven biosensors and one-pot RT-qPCR systems simplify workflows and enable low-copy nucleic acid detection. With the development of AI-assisted diagnostic platforms and automated detection technologies, polymerase-based molecular diagnostics is expected to become faster, more portable, and increasingly intelligent.

3.2. Enzymatic synthesis of DNA and RNA

With the rapid development of synthetic biology and artificial genetic systems, the functions of polymerases have expanded from conventional DNA replication to the synthesis of artificial DNA and xenonucleic acids (XNAs). Engineered polymerases obtained through rational design or directed evolution often exhibit broader substrate compatibility, improved catalytic efficiency, and enhanced template adaptability, providing important tools for de novo DNA synthesis and the construction of artificial genetic systems.

In enzymatic DNA synthesis, terminal deoxynucleotidyl transferase (TdT) has attracted considerable attention because of its template-independent catalytic activity. Structural analysis and mutagenesis studies have significantly improved the processivity and substrate selectivity of TdT, enabling efficient extension on complex 3′-end substrates. Protein engineering has further enabled TdT to incorporate non-natural nucleotides, expanding its applications in artificial gene synthesis and molecular encoding technologies. For example, directed evolution has produced high-activity TdT variants suitable for commercial enzymatic DNA synthesis, offering a more environmentally friendly alternative to conventional chemical synthesis methods.

Template-dependent polymerase systems have also been developed for controlled DNA synthesis. Polymerase-based strategies combined with modified nucleotides have enabled stepwise DNA synthesis with improved accuracy and efficiency. These approaches have also been applied in emerging areas such as DNA data storage, where high-fidelity polymerases ensure reliable information writing and reading processes. More recently, automated and high-throughput enzymatic DNA synthesis platforms have been developed, significantly improving DNA assembly efficiency and scalability for synthetic biology applications.

Polymerase reactions can also be coupled with cell-free transcription–translation systems to construct artificial replication cycles. Such systems enable repeated information replication and expression in vitro, providing experimental models for artificial cells and synthetic life systems. In addition, engineered thermophilic polymerases capable of incorporating modified nucleotides have enabled the synthesis of labeled DNA, artificial RNA molecules, and chemical biology probes.

Beyond natural DNA synthesis, engineered polymerases also play important roles in XNA synthesis. Enzymes such as engineered Phi29 polymerase and XNA polymerases can catalyze the synthesis of various artificial nucleic acids, demonstrating remarkable structural plasticity and substrate adaptability. These systems enable the creation of novel nucleic acid polymers with improved stability and functional diversity. Furthermore, polymerases capable of incorporating chemically modified nucleotides have significantly expanded the chemical diversity of nucleic acid synthesis reactions.

3.3. Applications in medical and industrial fields

Engineered DNA polymerases have shown broad potential in medical diagnostics and industrial detection. In clinical diagnostics, novel viral polymerases identified through metagenomic mining exhibit improved thermostability and broader substrate recognition, enabling the development of highly sensitive RT-PCR detection systems. Several diagnostic platforms have also been developed to simplify nucleic acid testing. For example, portable polymerase-based detection

systems have enabled rapid blood-based detection of the Ebola virus, while simplified RNA extraction and RT-qPCR workflows have significantly reduced the technical barriers for SARS-CoV-2 testing. In addition, single-enzyme RTX-PCR systems that integrate reverse transcription and amplification into one reaction have further improved detection efficiency and accuracy. Advances in detection devices have also enhanced polymerase-based diagnostic platforms. Photothermal temperature-control systems and microfluidic integrated detection platforms allow polymerase amplification reactions to operate under low-power conditions, making them suitable for point-of-care testing (POCT). Furthermore, digital bioluminescent POCT devices based on pyrophosphate signals released during polymerase reactions demonstrate the growing trend toward intelligent and integrated diagnostic technologies.

Protein engineering has further expanded polymerase applications in epigenetic analysis and complex environmental detection. For instance, engineered DNA polymerases capable of recognizing m^6A modifications provide new approaches for epigenetic sequencing. Charge-engineered polymerases with improved stability under high-salt conditions have also been developed for industrial detection environments. In addition, polymerase-based systems combined with magnetic nanoparticles enable automated signal amplification and detection, while polymerase inhibition effects have been used to develop probe-free methods for quantitative drug analysis, such as heparin detection. Polymerase-driven nanobiotechnology systems also show promise in biomedical research. DNA nanostructures generated through polymerase reactions have been used for long non-coding RNA imaging and cancer therapy, highlighting the potential of nucleic acid nanomaterials in medicine.

Beyond medical diagnostics, polymerase-based nucleic acid detection technologies play important roles in agriculture and environmental monitoring. Portable isothermal amplification systems have been widely applied for rapid detection of plant and animal pathogens in field conditions. Commercial diagnostic platforms and continued advances in polymerase engineering further support the large-scale commercialization of nucleic acid diagnostics. In addition, CRISPR-guided polymerase systems capable of generating targeted mutagenesis in vivo provide new opportunities for industrial biotechnology and genome engineering.

4. Conclusion

Polymerase engineering has become a central area of research in protein engineering and synthetic biology. Advances in structural biology, computational modeling, and high-throughput screening technologies have greatly improved our ability to modify polymerases with enhanced catalytic efficiency, stability, fidelity, and substrate compatibility. Strategies such as directed evolution, rational design, domain engineering, and conservation-based mutation analysis have provided powerful approaches for optimizing polymerase performance and expanding their functional diversity. These engineered polymerases have significantly broadened the application scope of nucleic acid technologies. In molecular diagnostics, improved polymerases have enabled highly sensitive PCR, isothermal amplification, and CRISPR-based detection systems. In synthetic biology, engineered polymerases facilitate enzymatic DNA synthesis, artificial genetic systems, and XNA replication. Furthermore, polymerase-based technologies are increasingly applied in medical diagnostics, industrial biotechnology, agriculture, and environmental monitoring. Despite these advances, challenges remain in achieving precise control over enzyme activity, substrate selectivity, and structural stability under complex reaction conditions. Future research combining artificial intelligence–assisted protein design, high-throughput evolution platforms, and structural mechanistic studies will further accelerate the development of next-generation polymerases. Such advances will continue to expand the boundaries of nucleic acid biotechnology and enable innovative applications across diverse scientific and industrial fields.

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References

[1] Ouaray Z, et al., 2020, Building Better Polymerases: Engineering the Replication of Expanded Genetic Alphabets. Journal of Biological Chemistry, 295(50): 17046–17059.

[2] Ishino S, Ishino Y, 2014, DNA Polymerases as Useful Reagents for Biotechnology – The History of Developmental Research in the Field. Frontiers in Microbiology, 2014: 5.

[3] Rk S, 1988, Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase. Science, 239: 487–491.

[4] Yamagami T, et al., 2014, Mutant Taq DNA Polymerases with Improved Elongation Ability as a Useful Reagent for Genetic Engineering. Frontiers in Microbiology, 2014: 5.

[5] Berman AJ, et al., 2007, Structures of Phi29 DNA Polymerase Complexed with Substrate: The Mechanism of Translocation in B-Family Polymerases. EMBO J, 26(14): 3494–3505.

[6] Borkotoky S, Murali A, 2018, The Highly Efficient T7 RNA Polymerase: A Wonder Macromolecule in Biological Realm. International Journal of Biological Macromolecules, 118: 49–56.

[7] Qing R, et al., 2022, Protein Design: From the Aspect of Water Solubility and Stability. Chemical Reviews, 122(18): 14085–14179.

[8] Abil Z, Ellington AD, 2018, Compartmentalized Self-Replication for Evolution of a DNA Polymerase. Current Protocols in Chemical Biology, 10(1): 1–17.

[9] Nikoomanzar A, et al., 2020, Programmed Allelic Mutagenesis of a DNA Polymerase with Single Amino Acid Resolution. ACS Synthetic Biology, 9(7): 1873–1881.

[10] Xu Z, et al., 2019, Recent Advances in the Improvement of Enzyme Thermostability by Structure Modification. Critical Reviews in Biotechnology, 40(1): 83–98.

[11] Wang YJ, et al., 2021, Directed Evolution: Methodologies and Applications. Chemical Reviews, 121(20): 12384–12444.

[12] Strack R, 2024, A Leap for Directed Evolution. Nature Methods, 21(3): 370.

[13] Povilaitis T, et al., 2016, In Vitro Evolution of Phi29 DNA Polymerase Using Isothermal Compartmentalized Self Replication Technique. Protein Engineering, Design and Selection, 29(12): 617–628.

[14] Abil Z, et al., 2017, Compartmentalized Partnered Replication for the Directed Evolution of Genetic Parts and Circuits. Nature Protocols, 12(12): 2493–2512.

[15] Aye SL, et al., 2018, Engineering of DNA Polymerase I from Thermus thermophilus Using Compartmentalized Self-Replication. Biochemical and Biophysical Research Communications, 499(2): 170–176.

[16] Sun Y, et al., 2023, Unraveling the Salt Tolerance of Phi29 DNA Polymerase Using Compartmentalized Self-Replication and Microfluidics Platform. Frontiers in Microbiology, 14: 1267196.

[17] Obeid S, et al., 2011, Learning from Directed Evolution: Thermus aquaticus DNA Polymerase Mutants with Translesion Synthesis Activity. ChemBioChem, 12(10): 1574–1580.

[18] Naccache J, 2023, Criblage et Évolution Dirigée des Polymérases à Déplacement de Brins par Autoréplication Compartimentée in Vitro à Ultra Haut Débit Screening and Directed Evolution of Strand-Displacing Polymerases Using Ultra-High-Throughput in Vitro Compartmentalized Self-Replication, thesis, Université de Paris.

[19] Naccache J, 2023, Screening and Directed Evolution of Strand-Displacing Polymerases Using Ultra-High-Throughput in Vitro Compartmentalized Self-Replication, thesis, Université Paris Sciences et Lettres.

[20] Dramé-Maigné A, 2018, Compartmentalized Directed Evolution of Enzymes Using Molecular Programs as Self-Selection Function, thesis, Université Sorbonne Paris Cité.

[21] Dramé-Maigné A, et al., 2021, Directed Evolution of Enzymes Based on in Vitro Programmable Self-Replication. bioRxiv, 2021.04.22.440993.

[22] Diercks CS, et al., 2025, An Orthogonal T7 Replisome for Continuous Hypermutation and Accelerated Evolution in E. coli. Science, 389(6760): 618–622.

[23] Ma L, Lin Y, 2025, Orthogonal RNA Replication Enables Directed Evolution and Darwinian Adaptation in Mammalian Cells. Nature Chemical Biology, 21(3): 451–463.

[24] Chen S, et al., 2025, CRISPR-DNA Polymerase Assisted Targeted Mutagenesis for Regulable Laboratory Evolution. Advanced Science, 2025: e11448.

[25] Pu J, Zinkus-Boltz J, Dickinson BC, 2017, Evolution of a Split RNA Polymerase as a Versatile Biosensor Platform. Nature Chemical Biology, 13(4): 432–438.

[26] Houlihan G, Arangundy-Franklin S, Holliger P, 2017, Exploring the Chemistry of Genetic Information Storage and Propagation Through Polymerase Engineering. Accounts of Chemical Research, 50(4): 1079–1087.

[27] Maola VA, et al., 2024, Directed Evolution of a Highly Efficient TNA Polymerase Achieved by Homologous Recombination. Nature Catalysis, 7(11): 1173–1185.

[28] Chao E, Dai L, Yu J, 2022, Switching Promotor Recognition of Phage RNA Polymerase in Silico Along Lab-Directed Evolution Path. Biophysical Journal, 121(4): 582–595.

[29] Shu L, et al., 2024, Advances in Evolved T7 RNA Polymerases for Expanding the Frontiers of Enzymatic Nucleic Acid Synthesis. ChemBioChem, 25(24): e202400483.

[30] Wachowius F, Holliger P, 2021, RNA Replication and the RNA Polymerase Ribozyme. In Ribozymes, 1: 359–386.

[31] Śpibida M, Krawczyk B, Zalewska-Piątek B, et al., 2018, Fusion of DNA-binding domain of Pyrococcus furiosus ligase with Taq Stoffel DNA polymerase as a useful tool in PCR with difficult targets. Applied microbiology and biotechnology, 102(2): 713–721.

[32] Maksum IP, Utami DF, Nurhakim EA, et al., 2022, Overexpression of soluble recombinant Thermus thermophilus (Tth) DNA polymerase in Escherichia coli BL21 (DE3) using an MBP fusion tag as a solubility enhancer. Journal of Applied Pharmaceutical Science, 12(9): 17–24.

[33] Li Z, Wang Y, Wang X, Niu S, Su Z, Wang F, Ni J, Gong Y, Rao B. A Fusion of Taq DNA Polymerase with the CL7 Protein from Escherichia coli Remarkably Improves DNA Amplification. Molecules. 29(5): 1145.

[34] Villbrandt B, Sagner G, Schomburg D, 1997, Investigations on the thermostability and function of truncated Thermus aquaticus DNA polymerase fragments. Protein Engineering, 10(11): 1281–1288.

[35] Sethy SK, 2023, Expression and purification of recombinant scaffold polymerase based on Φ29 DNA polymerase and terminal protein. Indian Institute of Science Education and Research Kolkata.

[36] Oany AR, Sharmin T, Chowdhury AS, et al., 2015, Highly conserved regions in Ebola virus RNA-dependent RNA polymerase may be act as a universal novel peptide vaccine target: a computational approach. In Silico Pharmacology, 3(1): 7.

[37] Satpati S, Manohar K, Acharya N, et al., 2017, Comparative molecular dynamics studies of heterozygous open reading frames of DNA polymerase eta (η) in pathogenic yeast Candida albicans. Scientific reports, 7(1): 41087.