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REVIEW

Systematic bio-fabrication of aptamers and their applications in engineering biology

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Abstract
Aptamers are single-stranded DNA or RNA molecules that have high affinity and selectivity to bind to specific targets. Compared to antibodies, aptamers are easy to in vitro synthesize with low cost, and exhibit excellent thermal stability and programmability. With these features, aptamers have been widely used in biology and medicine-related fields. In the meantime, a variety of systematic evolution of ligands by exponential enrichment (SELEX) technologies have been developed to screen aptamers for various targets. According to the characteristics of targets, customizing appropriate SELEX technology and post-SELEX optimization helps to obtain ideal aptamers with high affinity and specificity. In this review, we first summarize the latest research on the systematic bio-fabrication of aptamers, including various SELEX technologies, post-SELEX optimization, and aptamer modification technology. These procedures not only help to gain the aptamer sequences but also provide insights into the relationship between structure and function of the aptamers. The latter provides a new perspective for the systems bio-fabrication of aptamers. Furthermore, on this basis, we review the applications of aptamers, particularly in the fields of engineering biology, including industrial biotechnology, medical and health engineering, and environmental and food safety monitoring. And the encountered challenges and prospects are discussed, providing an outlook for the future development of aptamers.

Keywords Aptamer · SELEX · Engineering biology · Biosensors

Introduction

With the development and extensive application of biology, the concept of engineering biology came into being in recent years. In the application of engineering biology, diverse components and modules in life systems can be reprogrammed. Through this kind of transformation, the existing natural biological system is optimized so that it can achieve the predetermined function in the direction envisioned by a human. Engineering biology applications involve industrial biotechnology, environmental biotechnology, food and agriculture, health and medical care, etc., permeating and affecting every corner of daily life [1]. The development of intelligent detection and monitoring techniques can gain accurate and reliable parameters and information, providing a basis for guiding the engineering biology process. Through systematic transformation and precise regulation of microorganisms, cell factories can be constructed to produce specific products [2]. Analysis of microbial metabolites and products may help effectively grasp the growth and metabolism of microorganisms, and dynamically control the production process according to the parameters. In terms of environmental quality and food safety, new detection methods have been developed by means of chemical analysis to monitor potential pollution hazards and improve the quality of life [3]. In the face of stubborn diseases such as cancers, the combination of several biotechnologies can develop intelligent monitoring tools to master the development of diseases and conduct research and development of new treatments [4, 5]. Various biosensors have been developed and widely used in the above-mentioned applications. Enzymes and antibodies are conventional recognition elements for microbial detection [6], environmental and food safety testing [7], and early diagnosis of diseases [8]. However, such biologically active molecules are environment-sensitive, unstable, high

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cost and have certain difficulties in production and preservation, which restrict their development.

Aptamers are a class of DNA or RNA molecules that bind to specific targets. Similar to antigen–antibody binding, aptamers can also bind to their targets with great affinity and specificity, which makes them popular in the development of bioanalytical platforms, in vivo diagnosis and treatment, etc. Moreover, compared to antibodies, aptamers show some different characteristics and advantages, which are summarized in Table 1 [9, 10]. Due to the mature and automatic chemical synthesis technology, aptamers can be fast prepared, with high homogeneity between different batches [11]. The synthesized aptamers exist in the form of freeze-dried powder, which can be preserved for a long time, is insensitive to temperature and environment, and not easy to be denatured [12]. At the same time, modified with various chemical groups, the functions of aptamers can be customized according to their users [13]. For example, aptamers modified with a fluorescent group can be used for cell imaging, to better observe the changes in cell functions and traits, and understand the mechanism of a cellular process [12]. Particularly, aptamers can easily achieve endocytosis owing to their excellent biocompatibility and low molecular weight, which is convenient for the construction of intracellular biosensors, development of new drugs and other applications [9]. Aptamers have not only been used as analytical reagents but also combined with immunoassay, flow cytometry, imaging and mass spectrometry technology [14].

Aptamers are obtained in vitro based on the systematic evolution of ligands by exponential enrichment (SELEX) technology. Usually, targets are incubated with a random library of oligonucleotides during SELEX procedure to screen and enrich oligonucleotides with an affinity for the targets [12]. However, due to the differences in the physicochemical properties of different targets, there is no universal SELEX protocol applicable to all targets so far [15]. Therefore, it is important to choose an appropriate SELEX technology for screening of aptamers for specific targets. Meanwhile, screening of the optimal aptamers with an affinity for targets is also a great challenge. To screen aptamers with good performance from the aptamer candidates, primary structure analysis, secondary structure prediction, molecular docking simulation and other technology have been used to determine the possible binding sites and binding strength between aptamers and targets [16]. Moreover, to gain ideal aptamers for real application, a series of improvements and optimizations are generally required, including aptamer truncation and modification [17, 18].

This review focuses on the features of the SELEX techniques, aptamer truncation optimization and modification strategies to provide the technical and theoretical support for the systematic, platform-based and large-scale screening and optimization of aptamers. Study on the relationship between the structure and function of aptamers, and the folding law of nucleic acid aptamers is extremely helpful to the development of aptamers. In addition, the recent advances in the applications of aptamers in engineering biology are reviewed, and their prospects, opportunities and challenges are also discussed.

Table 1 Comparison of the characteristics of aptamers with antibodies

	Aptamer	Antibody
Screening speed	Weeks to months	Months
Mode of production	Artificial synthesis	Animal experiments or cell experiments
Production time	A few hours	Days to months
Production costs	Low	High
Batch difference	Small difference and high uniformity	Big difference
Storage time	At least one year	Weeks to years
Property characterization	Immature, affinity assay, specificity assay, secondary structure simulation, molecular docking simulation, etc	Mature, enzyme-linked immunosorbent assay (Elisa), surface plasmon resonance (SPR), peptide array, protein array, mass spectrometry, X-ray crystal, etc
Modification	Easy, a variety of modification ways	Difficult
Molecular weight	About 6~30 kDa	About 150 kDa
Thermal/chemical stability	High	Low (prone to irreversible change)
Immunogenicity	None/low	High
Tissue penetration	Easily internalized by cells	Low efficiency of internalization
Identifiable target	Widely recognized ions, peptides, proteins, viruses, bacteria, cells, tissues, etc	Identify only immunogenic targets

Primary screening of aptamers through SELEX technology

SELEX is a technology related to molecular biology, nucleic acid chemistry, material science and other disciplines, which is currently the only reported experimental technique (computational methods can serve as a supplement) to screen aptamers for various targets from the oligonucleotides library [19]. The process of SELEX technology includes several steps (Fig. 1). The first step is the construction of the initial nucleic acid library. The nucleic acid library is usually composed of an internal random sequence of 30~80 nucleotides and two terminal fixed primer binding sequences. The capacity of the library generally includes 10^{12}~10^{16} different DNA or RNA strands [20]. The second step is the combination of aptamer candidates with targets. Targets are incubated with the nucleic acid library for a certain period of time to ensure that the targets bind to the aptamer candidates [21]. The third step is the removal of unbound nucleic acids. Unbound oligonucleotides are separated from the library by repeated washing. And the elution method is customized according to the SELEX method to obtain aptamer candidates. The fourth step is aptamer candidate amplification. Aptamer candidates are amplified by PCR. And then ssDNA or RNA is regenerated to produce a secondary nucleic acid library. To remove the non-specific adsorption nucleic acids and increase the specificity of aptamer candidates, compounds with a similar structure to the target are often chosen to incubate with the aptamer candidate library to perform counter screening [22]. The screened aptamer candidates are repeatedly put into the next round of SELEX process. After 5~15 rounds of screening, aptamers with high affinity are enriched and then sequenced [23]. Finally, several optimal aptamers can be screened from the sequenced oligonucleotide candidates following special rules, which can be used in the development of a subsequent bioanalysis platform.

The emergence of SELEX technology provides a way to obtain aptamers. However, the conventional SELEX has problems such as low enrichment efficiency and long screening time. It cannot always exhibit the ideal performance when dealing with different targets [24]. Therefore, variant SELEX technologies have been developed to meet different needs. The characteristics of different SELEX technologies have been summarized and compared in Table 2. In can help researchers select the appropriate technology for

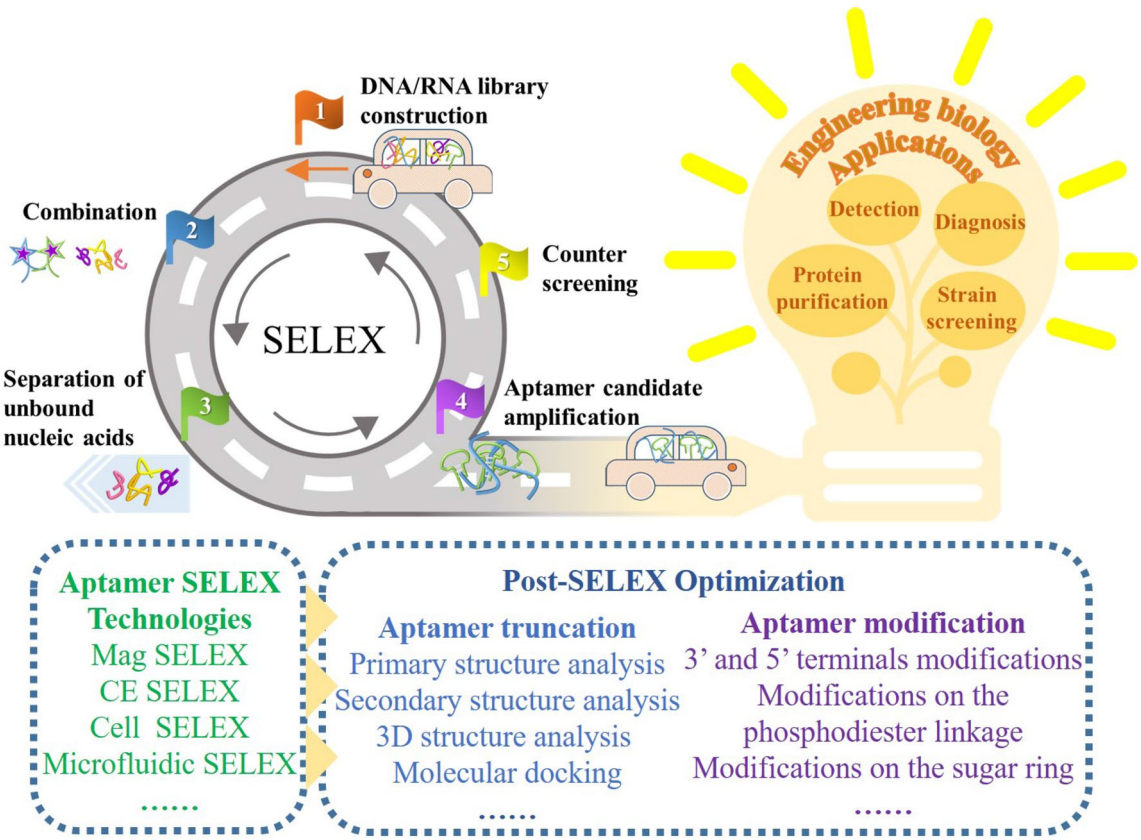


Fig. 1 General process of SELEX technology, the Post-SELEX optimization and the applications of aptamers in engineering biology

Table 2 Summary and comparison of various SELEX technologies

SELEX technology	SELEX principle	Characteristics	Targets
Mag SELEX	Immobilize targets on the surface of the magnetic beads; separation of the unbound sequences by the magnetic reaction	The unbound sequences can be separated easily	Proteins, cells
Capture SELEX	Immobilize the DNA library on the surface of the magnetic beads; separation only relies on the affinity between targets and aptamers	Avoid damage of immobilization to targets	Small-molecule targets, proteins, cells
CE SELEX	Separation according to different migration abilities in electrophoresis	High resolution and fast target separation rate	Not suitable for small-molecule targets
Cell SELEX	Select aptamers bound to characteristic proteins or markers on the cell surface	Simplify the process of cell-specific aptamer SELEX; improves the accuracy	Cells
Microfluidic SELEX	Miniaturize the aptamer SELEX laboratory onto a small chip; complete steps of combination, separation, and amplification in one microfluidic system	Save manpower and material resources Shorten the screening time; improve the efficiency	Small-molecule targets, proteins, cells

obtaining aptamers with good performance. At present, the commonly used SELEX technologies include magnetic bead SELEX (Mag SELEX), capillary electrophoresis SELEX (CE SELEX), cell SELEX, microfluidic SELEX, etc. Some other SELEX technologies have also been developed to complement the applications of SELEX in different scenarios, such as animal SELEX, atomic force microscopy-SELEX (AFM-SELEX), tissue-SELEX, biolayer interferometry SELEX (BLI-SELEX). This section mainly focuses on those widely used SELEX techniques.

Mag SELEX

The conventional SELEX conducts the binding step and the separation step in the reaction solution. However, the separation of unbound nucleic acid sequences is usually not easy. Separation can be carried out by affinity chromatography, cellulose membrane filtration or centrifugation, but this brings new problems such as high reagent consumption or non-specific adsorption [25–27]. Mag SELEX immobilizes targets on the surface of the magnetic beads. After incubation with the nucleic acid library, the unbound sequences can be easily separated by means of magnetic separation. Han et al. [28] screened a specific aptamer for tobramycin by Mag SELEX. Tobramycin was covalently immobilized on the surface of epoxy-modified magnetic beads. After incubation with the nucleic acid library, the sequences with affinity for the target were bound to the immobilized target, while unbound sequences were removed during elution. The aptamer candidates were enriched and amplified then, and multiple rounds of screening were performed to obtain aptamers with high affinity.

However, compared with large targets such as proteins or even cells, small-molecule targets have fewer available chemical groups and lower structural complexity. This makes them difficult to be fixed on the surface of the substrates. Introducing additional covalent bonds for immobilization may cause huge damage to the structure of small-molecule targets, leading to the failure of SELEX [29]. Even if the targets have appropriate chemical groups for immobilization, the specific binding sites may be masked because of the simple structure and the limited binding sites [15].

Capture SELEX technology is an improved version of Mag SELEX [30]. Instead of target-immobilization, capture SELEX screen aptamers by DNA library-immobilization. The entire or sometimes part of the region of the DNA sequences in the library is complementary to the sequences fixed on the surface of magnetic beads. When the affinity of sequences to targets is higher than that of their complementary sequences, a competitive replacement occurs. And aptamer candidates can be separated from the magnetic beads and enriched [31]. The screening process of capturing SELEX only relies on the affinity between targets and aptamers, avoiding the problems of reduced binding sites and changes in target conformation due to immobilization [32].

To visually compare the effect of target-immobilized Mag SELEX with DNA library-immobilized Mag SELEX on the screening of aptamers for small-molecule targets, our team took N-acetylneuraminic acid (Neu5Ac) as an example (Fig. 2A) [33]. It was found that the target-immobilized SELEX reached saturation at round 6~7, while the library-immobilized SELEX took only 4 rounds to reach saturation. This means that the screening efficiency of the library-immobilized SELEX is much higher. At the same

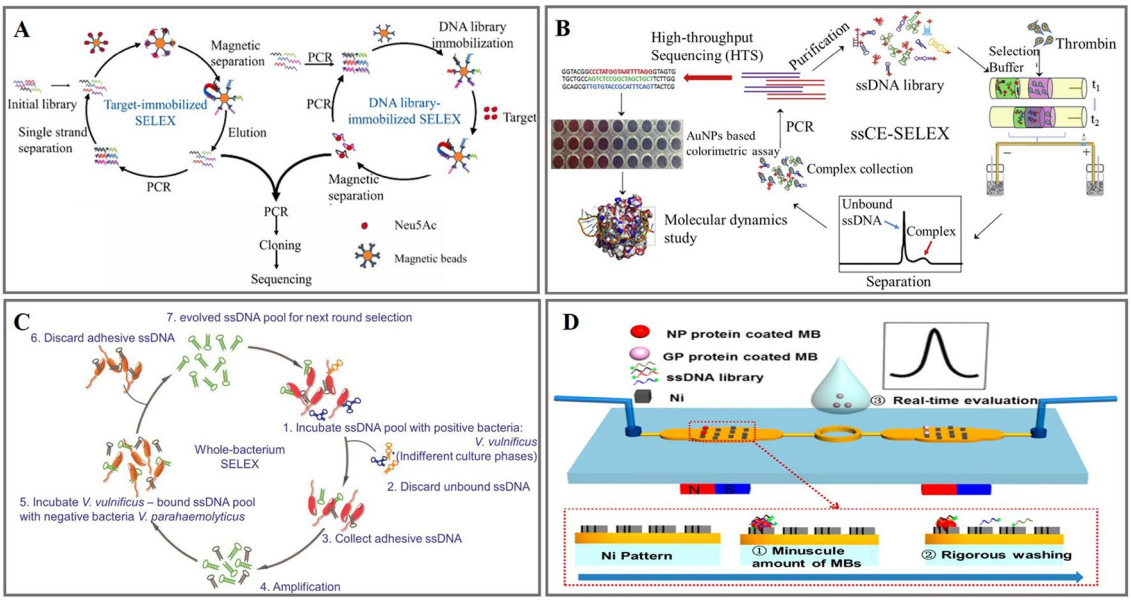


Fig. 2 **A** Schematic illustrations of target-immobilized MB-based SELEX and DNA library-immobilized MB-based SELEX, reprint permission was acquired from Elsevier [33]; **B** Schematic illustration of the ssCE-SELEX process, reprint permission was acquired from Elsevier [36]; **C** Scheme of whole bacterium aptamer SELEX technology, reprint permission was required from the Royal Society of Chemistry [38]; **D** Schematic illustration of magnetism-controlled selection chip and the overview of selection process on the magnetism-controlled selection chip, reprint permission was acquired from American Chemical Society [42]

time, through determining the average K_d of the enriched sequences obtained by different methods, aptamers screened by the library-immobilized SELEX showed a higher affinity for Neu5Ac. This may be due to the fact that the library-immobilized method allows targets to keep their natural state during the screening process, and all possible chemical groups for binding are retained. Besides, only when the affinity of aptamers to targets is higher than that to their complementary sequences, these aptamers can be replaced from the beads and enriched. However, in the target-immobilized SELEX, aptamers can bind to the beads and be enriched as long as there is an affinity between aptamers and targets. Thus, aptamers screened by library-immobilized SELEX are expected to possess higher affinity.

CE SELEX

Compared with the above two Mag SELEX methods that immobilize nucleic acid library or targets, CE SELEX has a higher resolution and faster target separation rate. Free targets, nucleic acids and nucleic acid-target complexes have different migration abilities in electrophoresis, thus they can be quickly separated by capillary electrophoresis, improving screening efficiency and accuracy [34, 35]. At the same time, it can also avoid the contamination of the aptamer candidate library caused by the nucleic acid residue during the elution process. Zhu et al. [36] developed an online reaction based on single-step CE SELEX (Fig. 2B). With this screening technology, mixing, incubation, reaction, separation, detection, and collection procedures can be finished within 15 min. Through batch analysis and monitoring of K_d of the library during the screening process, the affinity of the secondary library was improved by more than 1000 times compared with the primary library after two rounds of selection. This method speeds up the SELEX process, reduces the cost of SELEX, and can provide a convenient and effective SELEX method for a variety of targets, especially protein targets. However, when faced with aptamer screening for small-molecule targets, the complexes of the target and ssDNA confront little mobility change [22]. Therefore, it is quite challenging to precisely screen aptamers for small-molecule targets using the CE-SELEX method.

Cell SELEX

There are related protein markers on the surface of cells or bacteria, which can be used as targets for aptamer screening. However, the high-purity recombinant protein is sometimes difficult to obtain. In addition, due to the randomness of polypeptide folding, artificially obtained recombinant proteins may not form the correct structure of the target proteins [37]. This leads to difficulties in screening aptamers associated with cells or bacteria. Cell SELEX takes living cells as targets, and the aptamer candidates bind to characteristic proteins on the cell surface, thereby binding to the corresponding cell. In this process, there is no need to

know the specific proteins on the surface of bacteria or cells, which simplifies the process of cell-specific aptamer SELEX and improves the accuracy [32]. An aptamer was selected by cell SELEX for distinguishing *Vibrio vulnificus* which can cause rapid and deadly infection (Fig. 2C) [38]. The authors adjusted the rounds of positive and counter SELEX to screen aptamer candidates with high affinity and specificity, eliminating nonspecific sequences as much as possible and enriching candidate sequences. At the same time, the binding ability of candidate aptamers to the bacteria in different culture conditions, sources, and binding environments was investigated, and aptamers with better performance were finally obtained.

Cell SELEX technology simplifies the process of obtaining cell-specific aptamers. However, due to the unclear binding target of the obtained aptamers, it is difficult for the aptamers obtained by this method to predict the binding site by virtual technologies such as molecular docking like those aptamers for small molecules. This brings certain difficulties for the post SELEX of aptamers. To address this issue, Fellows et al. [39] developed the cross-over SELEX technology to screen aptamers that bind to cells expressing hCD4 (human Cluster of Differentiation 4) glycoprotein. The hCD4 protein and the cells expressing this protein were used as forward screening objects to conduct cross-screening of aptamers, so as to obtain aptamers for specific proteins on the cell surface.

Microfluidic SELEX

Microfluidic chip technology is a science and technology characterized by the manipulation of fluids in the micro-nano-scale space and has the characteristics of integration, miniaturization, automation and high throughput [40]. Microfluidic SELEX technology is equivalent to miniaturizing the aptamer SELEX laboratory onto a small chip. During the SELEX process, steps of combination, separation, and amplification can be completed in one microfluidic system, which greatly saves manpower and material resources [41]. Ebola virus (EBOV) is a haemorrhagic fever virus with a very high fatality rate. Hong et al. [42] used a magnetism-controlled selection chip to develop a multifunctional screening platform to screen EBOV aptamers (Fig. 2D). The high magnetic field gradient created by nickel pillars patterns on the chip surface was arranged into two regions in a cross-array. This special arrangement made it possible to obtain aptamer candidates for type I transmembrane protein (GP protein) and nucleoprotein (NP protein) in a single round of screening. The entire screening process was monitored using a fiber optic spectrometer, which was highly automated and had high bio-safety. After only 3 rounds of SELEX, high-performance aptamers were obtained with dissociation constants in the nanomolar range. This screening strategy greatly shortened the screening time, improving screening efficiency.

During the whole SELEX process, the affinity of the enriched candidate sequences for targets is generally monitored. As the rounds of screening increases, the affinity of the aptamer candidate library also increases and the screening reaches saturation when the measured affinity no longer increases [43]. The enriched pool is amplified and purified using PCR amplification techniques and the base information of the enriched sequences can be obtained by sequencing technology. Among them, cloning sequencing technology has relatively high accuracy and can detect longer target fragments [24], while high-throughput sequencing technology has greatly improved efficiency and can obtain comprehensive sequence information [44].

Post-SELEX optimization of aptamers

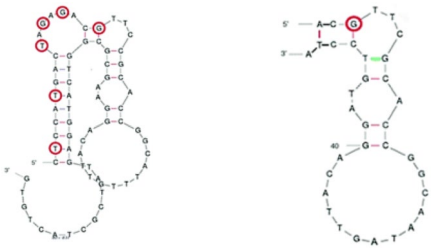
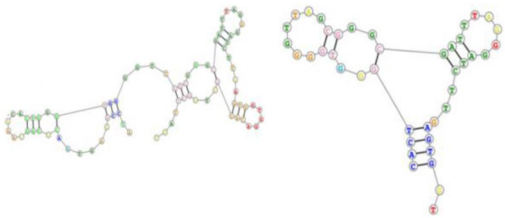
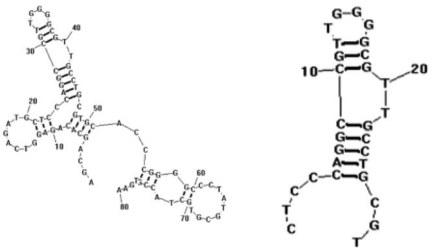
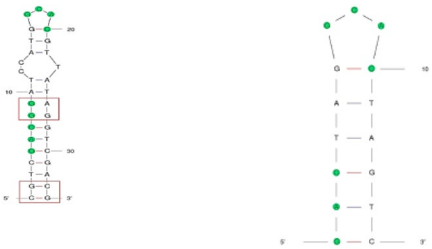
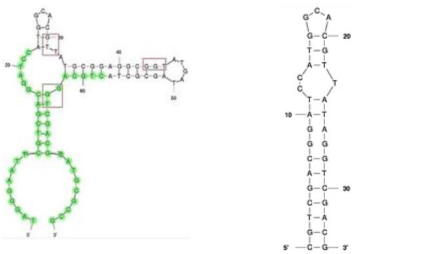
The full-length aptamer candidates primarily screened by SELEX technology are typical 50–100 nucleotides in length. There is no doubt that the synthesis and modification of such long sequences are comparably expensive and time-consuming. There is a view that in a certain condition the presence of non-essential nucleotides may interfere with the binding of aptamers to targets [45]. Therefore, reasonable truncation of aptamers is very helpful to improve the performance of aptamers, making it easy to manufacture biosensors with more variable and controllable mechanisms. Appropriate modification of aptamers can further improve the nuclease-resistance, affinity and specificity of aptamers, which helps promote the commercial application of aptamers.

The basis and method of aptamer truncation

The nucleotides in full-length aptamers can basically be divided into three functional regions: the region that interacts directly with targets, the supporting region helping aptamers interact with targets and the region containing non-essential nucleotides that do not bind to targets and do not play a supporting role [46]. Therefore, appropriate truncation of aptamers can remove unnecessary nucleotide sequences that may hinder target binding and effectively improve the affinity of aptamers [47]. Moreover, truncation shortens the length of aptamers, which not only facilitate the synthesis and modification of aptamers but also improves the flexibility and controllability of aptamer-contained devices.

Through the sequence alignment, secondary structure prediction of aptamer and molecular docking technology, the binding sites between aptamers and targets can be determined. Based on this, those non-essential nucleotides can be removed until the shortest optimal binding sequence is

Table 3 Typical examples of aptamer truncation and the corresponding methods

Aptamer name	Targets	Form of full-length aptamers	Form of truncated aptamers	Secondary structures of the aptamers before and after truncation	Truncation basis	References
XQ-2d	PL45 cell	80 nt 82.5 ± 7.8 nM	56 nt 55.02 ± 0.4 nM	Not mentioned	Nupack	[55]
NAC6853	Recombinant his-tagged human CD73	70 nt 9.94 ± 0.07 nM	34 nt 12.50 ± 1.14 nM		Mfold	[78]
R1.2	Membrane-bound IgM (mIgM)	79 nt 315 nM	41 nt 35.5 ± 8.94 nM		RNA structure Web	[56]
BB16-11f	*Bifidobacterium breve*	80 nt Not mentioned	29 nt 18.66 ± 1.41 nM		RNA structure 3.0	[153]
Ap4	Tobramycin	34 nt 48.40 ± 6.63 nM	15 nt 42.12 nM		Mfold Autodock	[77]
Ap32-2	Tobramycin	79 nt 56.9 ± 4.6 nM	34 nt 48.40 ± 6.63 nM		Autodock	[28]

found. Several typical aptamer truncation and methods are shown in Table 3.

Primary and secondary structure analysis

Primary structure analysis can provide insight into the

consensus nucleotides of aptamers to help identify the essential regions and the bases that can be deleted. Several existing software algorithms, including Clustal X and DNAMAN, can effectively perform multi-sequence alignment and derive consistent high-affinity binding motifs (Fig. 3A) [48]. According to the analysis results, aptamers can be divided into several families for further analysis [49, 50].

However, it is not enough to truncate aptamers only according to primary structural analysis. It has been found that the aptamers of different targets may contain similar sequence motifs. For example, the C-reactive protein aptamer screened by Lee et al. [51] and the ampicillin aptamer screened by Ban et al. [52] have significant sequence similarity. The two aptamers showed partial sequence overlap and achieved cross-recognition of the two targets [53]. Further analysis indicated that although the two aptamers contain similar sequence motifs, some different modules in their secondary structure still exist. These results reveal that the binding between aptamers and targets is related not only to the sequence of aptamers, but also to the formed secondary structures. Therefore, it is necessary to predict the secondary structure of aptamers and determine the possible binding sites, which can provide a useful starting point for aptamer truncation.

The specific motifs in DNA/RNA structure, including hairpin, pseudoknot, bulge and others (Fig. 3B), play very important roles in target binding [44]. The commonly used computer simulation programs, such as the UNAFold Web server (http://www.unafold.org/) [54], NUPACK web server (http://www.nupack.org) [55], RNA structure web (http://rna.urmc.rochester.edu/RNAstructureWeb) [56], ViennaRNA Web Services (http://rna.tbi.univie.ac.at/) [57] and some other services can be used to predict the secondary structures of DNA/RNA. These predictions can provide not only information about the conformation of the secondary structure of aptamers but also information on Gibbs free energy to judge the stability of the structure formed by aptamers. Typically, more structurally stable aptamers are selected for aptamer truncation and further applications [58]. Several researches have shown that the loop regions of aptamers are useful in most aptamer-target interactions. While the primer binding regions have been shown to occasionally be involved in binding, but more commonly, they have little effect [59, 60]. This could be due to the fact that primer binding regions of aptamers are the fixed sequences mainly used for PCR amplification. Compared with random sequences, the probability of fixed sequences participating in the binding is smaller [61]. Therefore, there is truncation strategy begins with the removal of primer binding regions in full-length aptamers. Heilkenbrinker et al. [62] removed the primer binding region from the 3' terminus of the aptamer and retained the core region of the aptamer. With the support and stabilization of the stem structure, the affinities of the two optimized aptamers were similar to the full-length aptamers in the low nanomolar range, while the affinities of the aptamers with disrupted stem structures decreased significantly or even disappeared. The detection limit of the developed ethanolamine biosensor using the optimized aptamer was as low as 10 pM. The stem-loop structure can stabilize and support the conformation of aptamers. Therefore, in case

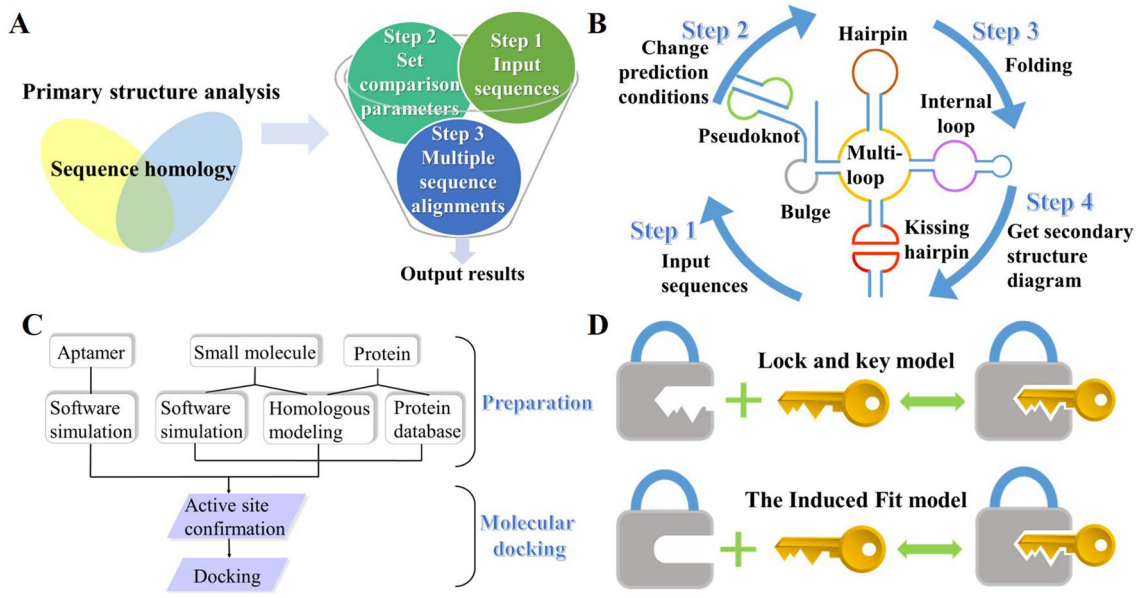


Fig. 3 The basis and method of aptamer truncation. **A** General steps for primary structure analysis; **B** general steps of DNA/RNA secondary structure prediction and specific motifs in DNA/RNA secondary structure; **C** basic process of molecular docking; **D** two docking models between aptamers and targets

the existence of the stem-loop structure, the primer binding region should not be removed directly to avoid destroying the conformation of aptamers [63, 64]. However, when the selected aptamers contain many stems or loops, the complex conformation may also affect the binding of aptamers and targets. Cutting off the stem or loop of the aptamers in turn is also a common truncation method. Ye et al. [65] used this method to truncate the lipopolysaccharide aptamers. After several rounds of truncation and determination, the length of the aptamer was reduced from 80 to 27 nt, and the K_d of the aptamer was improved from 102 ± 17 to 46.2 ± 9.5 nM. This may be due to the reduction of a steric hindrance after the aptamer is truncated [66]. By truncating aptamers, the stem-loop structure tightly bound to targets can be determined. The affinity between aptamers and targets can be further improved by combining the stem and loop sequences from different aptamers to form a chimeric aptamer. Soundy and Day [67] used this method to obtain aptamers for live *Pseudomonas aeruginosa* from the DNA library and truncated them. Finally, three truncated aptamers were chosen and their stem and loop structures were merged for generating three chimeric aptamers which showed enhanced binding. In addition to truncation optimization, the structure of aptamers can be stabilized by introducing point mutation or multi-point mutation in the stem-loop region of the secondary structure of aptamers [50, 68].

Primary and secondary structure analysis can be simulated on two-dimensional scale, providing information about the sequence Gibbs free energy. However, the prediction of the binding region between aptamers and targets only based on the homologous sequences and structurally similar regions of aptamers is not sufficient, which cannot directly know the spatial binding regions. Thus, the study of conformation and model is helpful to better understand the interaction between aptamers and targets.

3D structure analysis and molecular docking

Aptamers can be greatly truncated after secondary structure prediction. However, further truncation requires a large number of attempts due to the uncertainty of the binding sites. This seriously limits the efficiency of truncation. Through 3D structure analysis, the binding sites between the truncated aptamers and targets can be predicted to provide a basis for further truncation, so as to obtain aptamers with shorter strand length and higher affinity [69, 70].

Molecular docking is an in-silico strategy for intermolecular forces study, which predicts the binding mode and affinity between ligands and receptors. Exploration of the binding mechanism between aptamers and targets can provide evidence for aptamer truncation [71]. The process of molecular docking typically involves the construction of aptamer and target models, the determination of active sites and the analysis and optimization of docking results (Fig. 3C) [72]. It provides insights into how aptamer-target binding as a whole which can better avoid the local effect and poor overall binding in other methods [73]. The basis of molecular docking depends on the progress of X-ray crystal structure diffraction and nuclear magnetic resonance (NMR) which have provided more and more 3D structures of proteins and nucleic acids [74, 75]. Based on these 3D structures, the interaction between aptamers and targets can be determined more accurately. In particular, this simulation method plays an undeniable role in clarifying the mechanism at no cost. The commonly used molecular docking tools include Discovery Studios, AutoDock, AutoDock Vina, rDock, UCSF Dock and other softwares.

Similar to those of enzyme and substrate, the binding of aptamers and target molecules is usually described with the model of "key and lock". Aptamers and targets exhibit complementary specific conformations, allowing for a perfect fit. There is also another model: the induced fit model. Aptamers and targets undergo a conformational change in the binding process to achieve the combination of the two molecules (Fig. 3D). Molecular docking technique analyzes the interaction forces between aptamers and target molecules through a variety of computational methods, and reveals this binding form between aptamer and target molecule [76]. Compared with the secondary structure prediction, molecular docking technology may be more intuitive and reliable. Han et al. [28] screened the tobramycin aptamer by Mag SELEX and further predicted the binding motif of the aptamer to tobramycin by Autodock 4.0 software. On the basis of structural analysis and rational design, the aptamer was truncated by removing redundant sequences, and the K_d of the optimized aptamer was 48 ± 6.63 nM. On this basis, Nie et al. [77] further truncated the optimized aptamer according to the predicted binding sites and the simulated secondary structure. The nucleotides irrelevant to the binding sites and the unpaired nucleotides in the stem-loop structure were cutting off step by step to produce the shortest sequence. With the removal of the redundant nucleotides, the affinity of the aptamer increased continuously, and finally the tobramycin aptamer Ap4 with the length of only 15 nt was obtained with a dissociation constant of 42.12 nM.

Current challenges in aptamer truncation

Through structural analysis-based methods, the efficiency and accuracy of aptamer truncation can be greatly improved, which provides great advantages for the future application of aptamers [33, 77]. However, aptamer truncation has proved to be a double-edged sword. Aptamer truncation technology faces the challenges of the accuracy of structure prediction and renal filtration.

By predicting the secondary or tertiary structure of aptamers and judging the binding sites between aptamers and targets, shortened aptamer sequences can be obtained. This process mainly depends on the prediction of aptamer structure, but the accuracy of prediction is difficult to guarantee. The secondary and tertiary structure conformations of aptamers may change in their natural state and after binding to the targets, so it is difficult to determine which structures are necessary for the function of aptamers [78]. At the same time, the calculation and analysis of oligonucleotides is complicated by the high charge properties of the skeleton, the flexibility of oligomers and the huge conformational changes that may occur due to external triggers such as metal ions, temperature and ligands [74]. Therefore, similar predictive calculations can never be completely reliable, but this model can be used as a starting point for further experiments to guide future research [79]. In addition to the prediction models, relevant experimental methods such as NMR have also been used to reveal the spatial structure of DNA/RNA, which provide reasonable and reliable data for clarifying the binding properties of aptamers [80].

Due to a large number of bases, full-length aptamers bring many interference factors to construct DNA-based biosensors. Truncated aptamers not only save the cost of synthesis and modification but also improve the construction of various biosensors. At the same time, truncation can retain or enhance the affinity of aptamers by removing excess nucleotides. The molecular weight of truncated aptamers is usually low (less than 10,000 Da). Thus if the aptamers are administered into bloodstream, they will be excreted rapidly through renal filtration [81]. One solution is to combine aptamers with polymers to reduce renal filtration. Polyethylene glycol (PEG) is commonly used to increase the molecular weight of functional aptamers to prevent their excretion through renal filtration. Other substances, such as cholesterol, proteins, liposomes, organic or inorganic nanomaterials, have also been used to couple to aptamers and produce multivalent molecules with a higher molecular weight than the glomerular cut-off value [82].

Aptamer modification

Aptamers have many advantages over antibodies which makes it possible to achieve targeted drug delivery in organisms. However, these successes are mostly achieved at the laboratory level, and the commercial success rate of aptamers is still low [83]. The main factors leading to this delay are nuclease degradation and rapid renal filtration when the aptamers are used in vivo. Therefore, it is necessary to carry out appropriate chemical modifications of aptamers to solve these problems, and to improve the stability of aptamers. Common sites of the chemical modification of aptamers include 3' and 5' terminals of oligonucleotides, phosphodiester linkage, sugar ring, and nucleobases. Several typical modifications of aptamers are shown in Table 4.

Modifications on 3′ and 5′ terminals

The molecular weight of aptamers is generally within 6~30 kDa, which is lower than the filtration cut-off value of glomerulus (30~50 kDa). Thus the unmodified aptamers are easily excreted from the body by renal filtration,

Table 4 Typical modification types of aptamers

Modification types	Modification strategy	Aptamers	Function	References
Modifications on 3′ and 5′ terminals	PEG	AptA	Reduce renal filtration	[87]
		Polycation-nucleic acid complexes (polyplexes)	Resist to nuclease degradation	[154]
Modifications on the phosphodiester linkage	Phosphorothioate	Adipo8 aptamer	Enhanced biostability and prolonged binding time in biological fluids	[155]
		AF113-1	Improve target binding affinity	[94]
Modifications on the sugar ring	C2′-OMe	Macugen	Stable to nucleases	[101]
	2′-F-dG, 2′-OMe-dA/dC/dU	FmA12	Superior nuclease and serum stability	[102]
	LNA	ON6Z	Improve binding stability and resistance to nuclease	[106]
Modifications on the bases	Cy3-labeled 5-BzdU	AS1411-12	Increase the targeting affinity but no activity from normal healthy cells	[156]
	Pp-dC/Nap-dU	SL1063	Improve the aptamer binding affinity and potency	[157]
	Expanded genetic alphabets	LG5	Select high-affinity aptamer	[158]
	Expanded genetic alphabets	Not mentioned	Select high-affinity aptamer	[159]

which greatly weakens the role of aptamers in the body [84]. Appropriate chemical modification at the 5' and 3' terminals of DNA can effectively increase the size of aptamers, overcoming the effect of renal filtration, prolonging the circulation time [82]. Such modifications include PEG modification, cholesterol modification [85] and so on, of which PEG modification is the most commonly used. Research shows that longer PEG-modified aptamers can reduce renal filtration, while shorter PEG modification can improve the physicochemical properties of aptamers, making them resist nuclease degradation [86]. Pieve et al. [87] modified the 3′ mercapto terminal of anti-MUC1 aptamer with PEG20 kDa (BrPEG20) or PolyPEG17 kDa (PolyPEG17). It was found that the clearance rates of aptamers modified with different PEG in blood and in vivo were lower than those of unmodified aptamers. This confirmed the effect of PEG modification on the prolongation of the half-life of aptamers in vivo.

In addition to the renal filtration problems caused by molecular weight, the nuclease degradation of DNA is also a challenge for the application of aptamers in vivo. Seliger et al. [88] first put forward the concept of terminal 3′–3′ and 5′–5′ internucleotidic linkages. The terminal modified sequences can survive for 90 min in serum while unmodified sequences were degraded in 30 min. 3′ terminal modification is also a common modification method to resist 3' exonuclease attack. Chakravarthy et al. [89] compared the nucleases resistance of DNAzymes modified with inverted-dT, phosphorothioate backbone and LNA-nucleotides at 3′ terminals, respectively. DNAzyme modified with inverted-dT was found to provide more resistance to 3′–5′ exonuclease than other two modification methods. There was no obvious degradation even after incubation with phosphodiesterase for 1 h.

Modifications on the phosphodiester linkage

Phosphodiester linkage is the main site for the digestion of aptamers by nucleases, thus the modification of the phosphodiester linkage can effectively prevent the nuclease degradation of aptamers in vivo [90]. Thiolation [91] and methylation [92] are commonly used in aptamer modifications. Among them, replacing DNA phosphodiester linkage with phosphorothioate analog is the most commonly used modification method. BC15-31, a heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1)-specific aptamer, was chemically modified with a phosphorothioate backbone for its antitumor application in vivo. The unmodified BC15-31 aptamer was significantly degraded after 2 h incubation with 20% fetal bovine serum, while the all-site modified aptamer remained stable for 8 h [93]. The modification of aptamers with thiophosphate analogues can not only improve the nuclease stability of aptamers but also effectively improve the affinity between aptamers and targets. Abeydeera et al. [94] modified the single nucleotide of aptamer with phosphorodithioate, and the X-ray co-crystal structure of the α-thrombin: aptamer complex revealed a locally induced fitting rearrangement of this nucleotide, which significantly enhanced the affinity of aptamer by 1000-fold. However, some studies have shown that if the modified site and configuration of the modification are not appropriate, the formation of double strands with complementary DNA/RNA may be affected [95].

Modifications on the sugar ring

To avoid the degradation by nucleases, chemical modification of aptamers on the sugar ring is also a common modification method, including 2′-OMe, 2′-F, 2′-H, 2′-OH, 2′-NH_2 and so on [96–98]. Such modifications can improve the formation of double strands and the stability of secondary structures, and make the modified sequences more resistant to nucleases [99]. Macugen, approved by the FDA, is a nucleic acid drug that can target to vascular endothelial growth factor (VEGF) for the treatment of neovascular age-related macular degeneration [100]. Macugen was screened by the traditional SELEX method from the initial fYrR RNA library. After the SELEX process, the majority of purines in the aptamer was modified and replaced with 2′-F and 2′-OMe. This modification helps the aptamer resist nuclease degradation encountered in vivo, allowing it to be developed for the treatment of age-related macular degeneration [101]. Although successful cases have been reported, the steps of chemical modification and determination of affinity after SELEX are tedious and time-consuming, which are not conducive to the popularization of the method. The direct SELEX of aptamers from the 2′-fully modified DNA/RNA library can solve this problem. Friedman et al. [102] selected *Staphylococcus aureus* protein A (SpA) aptamers from the 2′-fully modified RNA library. After incubation in 10% mouse serum for more than 24 h, the selected 2′-fully modified aptamers were still viable while the unmodified aptamer showed unstable within 5 min. Aptamers obtained in this way have significantly higher serum stability and reduce the risk of post-SELEX modification for disrupting the structure and affinity.

Recently, besides 2′ modification, other sugar ring modification methods have been developed, such as locked nucleic acid (LNA) [103], unlocked nucleic acid (UNA) [104], 2′-deoxy-2′-fluoro-D-arabinonucleic acid (2′-F ANA) [105]. Among them, LNA modification has attracted particular attention. LNA is an RNA nucleotide with an extra linkage between 2′-O and 4′-C of the sugar ring, allowing the obtained hybrid to have a very stable conformation. A triazole-linked LNA (t-LNA) was synthesized and its biophysical properties were studied. Compared with the unmodified triazole-linked oligonucleotides, modification of LNA next

to triazole could effectively improve thermal stability, showing an increase in melting temperature (T_m) [106]. However, the modification of LNA may limit the flexibility of the sequences and affect the structure of the sequences to a certain extent. Most of the special loops and uplift regions in aptamers are related to the binding of aptamers and targets. When LNA bound to these regions, the affinity between aptamers and targets may be affected. As long as the regions modified by LNA avoid the binding areas of aptamers and targets, the LNA-modified aptamers can maintain the affinity while obtaining higher stability [107]. Unlike LNA, UNA has no bonds between C2′ and C3′ of the sugar ring, which is more flexible and can reduce the influence of modification on aptamer structure and affinity [104]. According to different requirements, the selection of appropriate modification methods may be of great help to the application of aptamers.

Challenges in chemical modifications of aptamers

Chemical modifications can significantly improve the ability of aptamers to resist nuclease degradation and renal filtration. They also have positive effects on the affinity and specificity of aptamers, which is essential for the development of aptamers. However, chemical modifications also bring some problems that should not be ignored, such as toxicity and allergic reactions.

Compared with antibodies, aptamers are generally considered to have low toxicity and low immunogenicity. However, only one aptamer drug (Macugen) has been approved by FDA so far, which raises people's doubts about the safety of aptamers [108]. The toxicological information of aptamers is very limited, and the control of their toxicity and behavior in vivo is still an outstanding issue. Several potential toxicities that may be applicable to aptamers can be identified by reference to the toxicology of antisense oligonucleotides, including polyanion effects, natural immune stimulation and tissue accumulation of oligonucleotides [109]. The binding of a high concentration of oligonucleotides to non-target proteins triggers the polyanion effect, which can cause the cardiovascular syndrome, mainly hypotension and tachycardia, leading to cardiovascular collapse or death in non-human primates [110]. In addition, non-natural nucleotides may be toxic and cause immune responses. For example, LNA-modified nucleic acids show strong hepatotoxicity, hepatocellular protein binding to toxic LNA gapmer may be one of the mechanisms of hepatotoxicity [111]. Study showed that 2′-methoxy-ethyl (2′-MOE) modified oligonucleotides also have certain nephrotoxicity [112]. Therefore, it should be careful to choose chemical materials for aptamer modification to avoid toxic problems in vivo.

The applications of aptamers in engineering biology

At present, aptamers specific for different targets have been screened by SELEX technology. Further truncation and modification can effectively improve the affinity and stability of aptamers and promote the wide applications of aptamers. Through the above-mentioned SELEX and post-SELEX process, aptamers with high affinity to the corresponding targets can be obtained. Combined with biotechnology and engineering technology, aptamers can be applied in many fields of engineering biology. For example, using aptamers to design high-throughput screening (HTS) platforms for strain selection [113], develop targeted drugs with better performance [114], and construct high-sensitive biosensors for the determination of food and environmental pollutants [115], etc.

Applications in industrial biotechnology

Industrial biotechnology is a technology that utilizes biochemical reactions for industrial manufacturing. Microorganisms accumulate metabolites through bio-oxidation during the growth process, and the desired products can be obtained through this characteristic. Since natural strains have the problem of low yield or poor environmental tolerance, it is necessary to screen strains to achieve a high production effect. At present, HTS technology has been widely used to obtain desired phenotypes in large mutant libraries of strains [113]. Combined with fast and sensitive biosensors, the efficiency and sensitivity of the technology can be further improved. Due to the high affinity, specificity and good biocompatibility of aptamers, corresponding aptasensors have been developed for monitoring the intracellular environment to guide HTS processes. Liu et al. [116] constructed a novel HTS platform based on a tryptophan (Trp)-specific aptamer biosensor for screening high Trp-producing strains (Fig. 4A). A conformational change occurred when the aptamer was bound to Trp. Subsequently, ribosome was bound to the exposed Trp-specific riboswitch (RBS), activating the downstream expression platform to produce a yellow fluorescent protein (YFP). The intracellular Trp level was converted into a fluorescent signal by this strategy, and the Trp titer of the mutant strain obtained by screening increased by 165.9% compared with the parental strain. However, this method is highly random, and mutations do not always proceed in the desired direction. At the same time, studies have shown that the genetic properties of high-yielding strains obtained by mutagenesis breeding may be unstable [117].

The regulation of microbial metabolism is a more purposeful strategy, and the target high-yielding strains can be customized according to the needs. However, changes

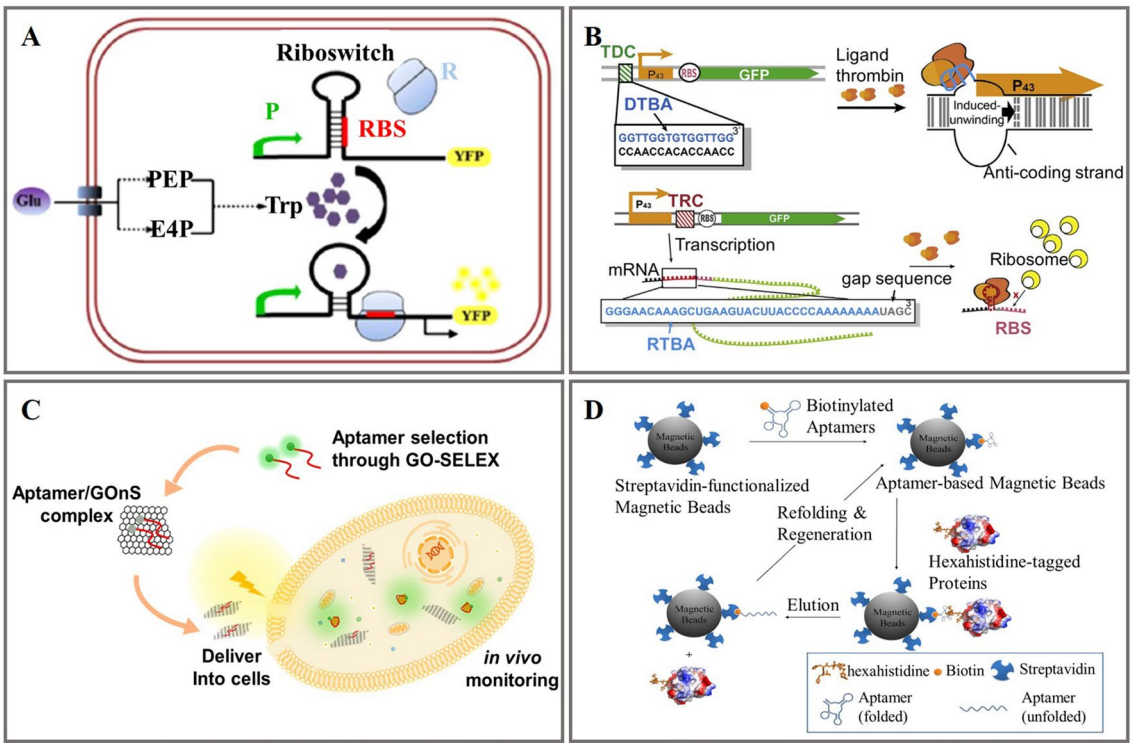


Fig. 4 **A** Schematic diagram of biosensor-based high-throughput screening platform for tryptophan overproduction, reprint permission was acquired from American Chemical Society [116]; **B** the working scheme of regulatory component TDC in BP1-TDCGFP and TRC in GFP expression regulation, reprint permission was acquired from Elsevier [120]; **C** schematic diagram of in vivo monitoring of intracellular metabolite in a microalgal cell using an aptamer/graphene oxide nanosheet complex, reprint permission was acquired from American Chemical Society [128]; **D** the schematic diagram of the principle of the aptamer-based affinity purification, reprint permission was acquired from Elsevier [130]

to the microbial genome often affect the natural growth of microorganisms, causing them to develop metabolic imbalances [118]. According to the characteristics of the natural regulation system of microorganisms, reprogramming cell metabolism is a milder and more effective metabolic regulation strategy, which can achieve high production of target products while maintaining the normal growth of cells [119]. Employing the thrombin aptamer, Deng et al. [120] constructed an intracellular dual-function regulatory biosensor (Fig. 4B). The regulation of the expression of enhanced green fluorescent protein (EGFP) was first taken as a model. In the up-regulated pathway, the binding of thrombin to the aptamer generated DNA bubbles upstream of the promoter which promoted the efficiency of promoter-mediated transcription. In the down-regulation pathway, the binding of thrombin to aptamers blocked the binding sites of ribosomes on mRNA, thereby hindering the expression of target proteins. Through this regulatory principle, they further developed a production strain expressing 2′-fucosyllactose (2′-FL). Dynamically up-regulated the expression of key genes fkp and futC for 2′-FL, and further down-regulated the expression of the lactose transport gene purR. The titer of 2′-FL was increased from the initial 24.7–674 mg/L. Through the development of this dual-function gene expression regulation mechanism or even multi-functional gene expression regulation mechanism, intracellular metabolic pathways can be reprogrammed to obtain engineered strains with high target yield and good growth status, which is a strategy with high potential.

Industrial microbial fermentation production needs to be carried out under certain environmental conditions. The concentrations of microorganisms, medium components and metabolites will change dynamically during the manufacturing processes. Monitoring the fermentation process and adjusting it in real-time according to its variables is of great significance for maintaining the ideal manufacturing conditions [121]. A variety of aptamer-based biosensors have been developed for the determination of bacteria, metabolites and other parameters [122, 123]. These biosensors can thus further be used to monitor the fermentation process. The determination of microbial concentration usually adopts the turbidimetric method, gravimetric method, plate counting method, etc. [124–126]. However, some of these methods have poor accuracy, some are very time-consuming, which makes it difficult to monitor the change of colony concentration in the process of fermentation production. Therefore,

the development of real-time, fast, and convenient biosensors for microbial detection is significant for fermentation process monitoring. An impedance-type biosensor based on an interdigital electrode (IDE) array was developed to detect the concentration of *E. coli* [127]. When the aptamer immobilized on the surface of IDE electrode was bound to *E. coli*, the electrode impedance showed significant changes. Further analysis was possible by connecting the chip to a smartphone or tablet, enabling the determination of *E. coli* in the sample. The detection scheme can be extended to the determination of other microorganisms by simply replacing the aptamer, which has broad application prospects. However, the quantification of intracellular metabolites using traditional detection methods is considered to be challenging. The content of intracellular metabolites varies with the fermentation process and changes in the environment. An aptamer/graphene oxide nanosheet complex was developed to detect β-carotene production in living cells. Due to the good biocompatibility and high affinity of aptamers, trace changes of target metabolites could be captured intracellularly (Fig. 4C) [128]. Through the measurement and analysis of intracellular metabolites, we can deepen the understanding of cellular metabolic pathways, and further control the fermentation process parameters according to the changes of metabolites to obtain better industrial production efficiency, which has broad prospects.

As another important module in industrial biotechnology, the key to exogenous protein expression and production lies on the development of simple, fast and efficient protein separation and purification strategies. Traditional protein purification techniques separate different proteins based on their solubility, molecular weight, charge properties and ligand specificity [129]. However, these methods involve a series of protein-based precipitation, dilution, filtration, purification and other processes, which are complicated and time-consuming. Wu et al. [130] obtained the hexahistidine-specific aptamer AptHis-C through SELEX technology and immobilized it on the surface of streptavidin magnetic beads for the separation and purification of target proteins in simple or complex systems (Fig. 4D). Magnetic separation eliminated the process of repeated centrifugation and filtration, which greatly simplified the process of protein purification. More importantly, the purification effect was comparable to traditional Ni-chelating column affinity chromatography. The use of aptamer-based protein purification techniques can even eliminate the process of affinity tagging the target protein. Beloborodov et al. [131] used magnetic beads-aptamer complexes to co-incubate with cell lysates. The captured target protein was obtained by elution with a high ionic strength buffer, and its protein activity was verified. This strategy enables proteins to maintain their original physicochemical properties and avoids the changes from additional affinity tags.

Applications in medical and health engineering

As mentioned above, aptamers are similar to antibodies which can specifically bind to target molecules, and are even more superior in some respects. They have the advantages of good stability, good biocompatibility, simple synthesis, small batch-to-batch variation and low cost. Currently, aptamers have been widely used in the fields of medical and health engineering, such as the early diagnosis of diseases, and the development of new drugs [12, 132].

Aptamers combined with signaling molecules can be used for disease diagnosis. Since 2019, the novel Corona Virus Disease (COVID-19) has swept the world, causing millions of infections and deaths. To avoid the rapid spread of the virus, the speed and quantity of testing need to be improved, as delays in testing could lead to a wider spread of the disease. However, traditional laboratory-based diagnostic testing methods have certain difficulties in the face of the huge number of samples for COVID-19 testing. Point-of-care testing abandons the tedious processes such as large-scale inspection equipment, data processing and transmission, and can obtain the detection results quickly and directly. Deng et al. [133] reported a one-step thermophoretic assay based on aptamer and PEG for the direct quantitative determination of viral particles (Fig. 5A). The complex formed by aptamer and the spike protein (S protein) of SARS-CoV-2 underwent thermophoretic movement in an infrared laser-induced temperature gradient. According to the fluorescence intensity of the enriched aptamer-virus complex, the rapid quantitative detection process could be completed within 15 min. At the same time, relevant reagents could be stored at ambient temperature for a long time, which provided a powerful tool for the rapid diagnosis of infectious diseases in resource-limited environments.

In addition to the above infectious diseases, early diagnosis of tumors is also of great significance. Malignant tumors may show non-specific symptoms in the early stage. However, once there are significant symptoms, the disease often has developed to the intermediate or advanced stage, and the best time for treatment is missed. Yang et al. [134] developed a ratiometric immobilization-free electrochemical sensor for the detection of tumor exosomes, which was promising for early tumor diagnosis (Fig. 5B). The dual-aptamer recognition system captured tumor exosomes by recognizing tumor CD63 and mucin (MUC1). Cholesterol-modified DNA probes were then bound to the surface of exosomes to activate the hybridization chain reaction (HCR), significantly improving the detection sensitivity, and achieving a detection limit of 3×10^4 particles/mL. This assay has high stability and accurate results and can directly target tumor exosomes, providing a promising detection method for non-invasive early cancer diagnosis.

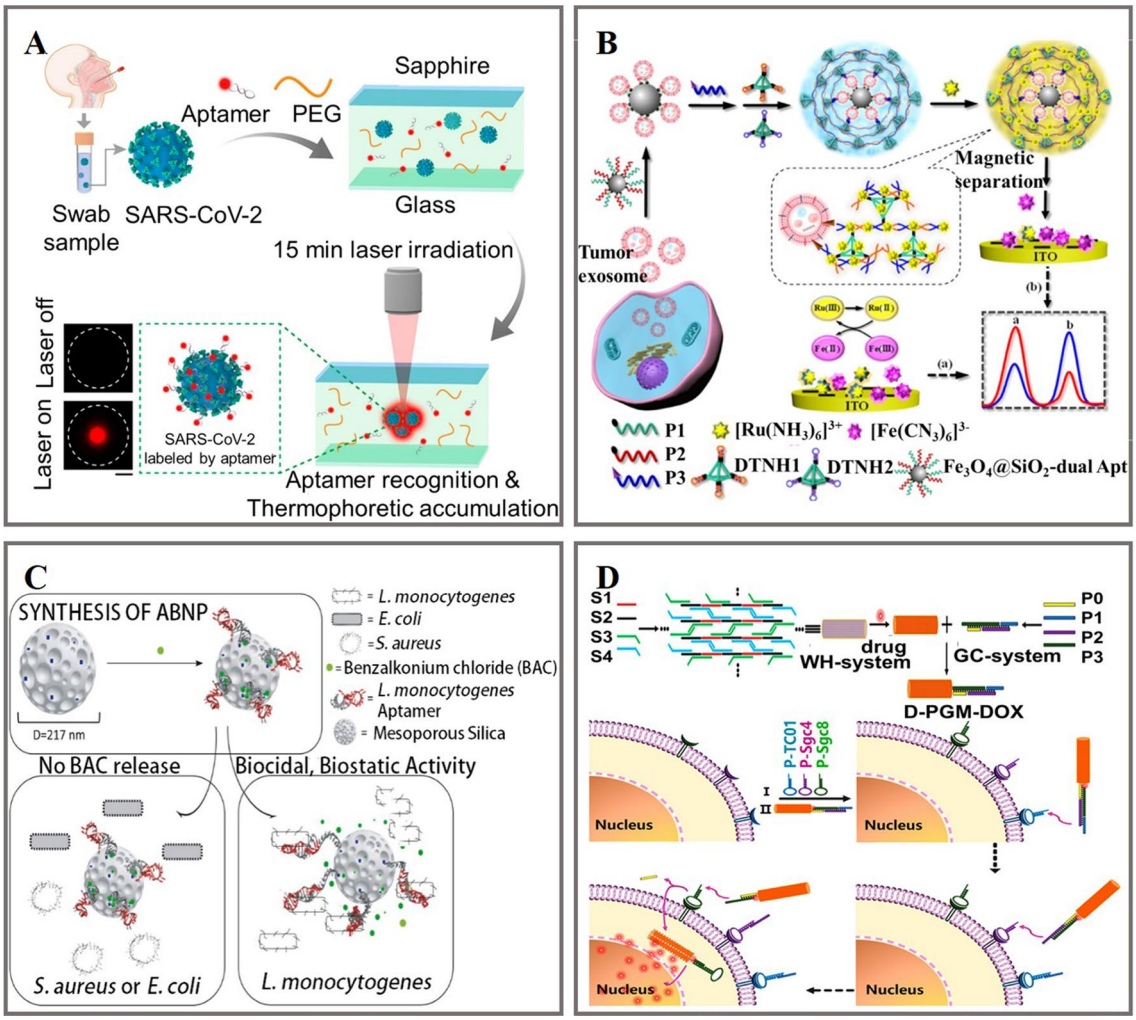


Fig. 5 **A** Schematic diagram of one-step aptamer-based thermophoretic assay for rapid detection of SARS-CoV-2 viral particles, reprint permission was acquired from American Chemical Society [133]; **B** schematic diagram of a ratiometric immobilization-free electrochemical sensing system for tumor exosome detection, reprint permission was acquired from American Chemical Society [134]; **C** schematic diagram of aptamer-gated-based nanocapsules for *S. aureus* inhibitory, reprint permission was acquired from the Royal Society of Chemistry [137]; **D** schematic diagram of D-PGM for Three-Receptor-Mediated cell identification and targeted cancer therapy, reprint permission was acquired from American Chemical Society [114]

The combination of aptamer and drug is equivalent to attaching satellite positioning to the drug so that it can accurately hit the corresponding target. According to this principle, a variety of aptamer-based targeted drugs have been developed to enhance the efficacy of related drugs [135, 136]. Antibiotics are the most commonly used drugs in infectious diseases. However, the excessive use of antibiotics will bring some problems, such as causing body adverse reactions or damage. More seriously, the abuse of antibiotics will lead to bacterial resistance, which is how superbug is produced. To avoid the overuse of antibiotics, an aptamer gated nanocapsule was developed (Fig. 5C) [137]. The aptamer-functionalized nanoparticles had a targeting effect on *Staphylococcus aureus* (*S. aureus*), greatly reducing the minimum inhibitory concentration (MIC) of vancomycin against *S. aureus*, thereby reducing the possibility of bacterial evolution to develop drug resistance.

In the treatment of tumors, the benefits of targeted drugs are even more significant. Targeted therapy drugs customized for tumor lesions or tumor cells can overcome the defects of large side effects and insignificant effects caused by conventional treatment methods, reducing the pain caused by treatment to patients, and improving the survival period of patients. Simulating modern precision-guided missiles, Ouyang et al. [114] developed a novel DNA nanostructure for efficient loading and precise transport of chemotherapeutic drugs to specific target cells (Fig. 5D). The warhead (WH) part of the DNA nanoscale precision-guided missile (D-PGM) was formed by four complementary DNA strands, and doxorubicin (DOX) was loaded into it by inserting a

"GC" site in the double-stranded DNA. The guidance/control (GC) part was formed by the self-assembly of three cancer cell-targeted aptamers (TC01, Sgc4f and Sgc8) with their partially complementary DNAs. After the D-PGM was injected into the body, the GC system first recognized and bound to the target cells to unlock the locked structure at the end of the WH system and promoted the internalization of DPGM. The special internal environment of tumor cells (such as lower pH and a certain concentration of nuclease) further promoted the decomposition of WH, which in turn released the drug DOX. The high specificity of aptamers makes it possible to develop this kind of targeted drug, which can significantly improve the therapeutic effect and reduce the cytotoxicity of the treatment to other non-target cells.

Applications in environmental and food safety monitoring

In traditional food fermentation engineering, food contamination may sometimes occur, resulting in poor product flavor, and even accumulation of some toxic and harmful pollutants that lead to food poisoning accidents [138]. Common environmental and food pollutants including pesticides, antibiotics, microorganisms and other harmful residues can bring serious threats to human health [139]. Aptamers have a high affinity for their specific targets, which can accurately identify contaminants in food and the environment, and convert the determination of contaminants into the determination of nucleic acids [140]. Combined with various signal amplification technologies such as nanotechnology and nucleic acid amplification technology, a variety of aptamer-based analysis and determination technologies have been widely used in the detection of various pollutants, providing a guarantee for the field of environmental and food safety [141, 142].

Pesticide and antibiotic contamination often occur in the collection stage of food raw materials. Excessive use of pesticides or antibiotics can easily cause residues in food raw materials, which have certain effects on the quality and safety of food products. The sensitive and convenient biosensing provides a promising analytical method for the determination of such contaminants in food. An aptamer-based lateral flow biosensor (LFB) was developed for the determination of isocarbophos pesticide in food (Fig. 6A) [115]. The sample was flowed from the sample pad to the binding pad by siphon effect and bound to the aptamer wrapped on the surface of the AuNPs. The exposed AuNPs continued to flow and were captured on the test line, showing a red band. Through this method, the pesticide residues in the sample could be distinguished with naked eyes in less than 1 min, and the determination was simple and easy to operate. However, LFBs developed based on colorimetry are susceptible to interference by the color of the sample. Fluorescence signal-based assays can reduce this kind of interference and broaden the application range of biosensors. An aptasensor based on water-soluble blue-emitting graphene quantum dots (GQD) was developed for robust and reliable detection of tetracycline in raw milk samples (Fig. 6B) [143]. This method utilized the ideal fluorescence resonance energy transfer effect of Pd NPs to quench the fluorescence signal of GQDs. After recognizing tetracycline, GQDs were released to enhance the fluorescence signal. Under the mild reaction conditions, samples containing tetracycline were reacted with Pd NPs/aptamers/GQDs solution for 5 min. The corresponding results could then be read by the instrument. The detection limit in spiked raw milk was as low as 45 ng/mL.

Microbial contamination is a common food and environmental pollutant, which easily occurs in the collection of food raw materials, food processing and product transportation. The common contaminating microorganisms include *S. aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Vibrio parahaemolyticus*, *Listeria*, and so on [144, 145]. A variety of aptamer-based microbial detection methods have been developed in recent years for the determination of contaminating microorganisms in the environment and food [146–148]. Our team developed an electrochemical biosensor based on *S. aureus* aptamers (Fig. 6C) [122]. After the aptamer was bound to the target, the partially complementary sequence was released, which triggered the strand displacement amplification, so that the signal DNA was synthesized and amplified in large quantities. The molecular switch on the electrode surface underwent a conformational change after binding to the signal DNA, enabling sensitive determination of *S. aureus*. By changing the aptamer, this assay had also been used to measure other microbial targets, which was a universal assay. In the construction of aptamer-based biosensors, nucleic acid amplification technology is introduced in the biosensor measurement process, which can effectively amplify the detection signal and significantly improve the measurement effect.

Food fermentation production is a complex system. If the raw materials and production process are not strictly controlled, harmful products can easily be produced. Common harmful substances in the process of food fermentation production include biogenic amines, acrylamide, ethyl carbamate, nitrite, etc., which can easily affect the flavor of food and have an impact on human health [149–151]. Since both tyramine (TYR) and β-phenethylamine (PHE) belong to aromatic biogenic amines with high structural similarity. Duan et al. [152] developed a one-pot SELEX strategy to simultaneously screen DNA aptamers that could bind to two targets (Fig. 6D). A pool of candidate aptamers bound to both TYR and PHE was first obtained by one-pot SELEX. On this basis, the magnetic bead separation technology was

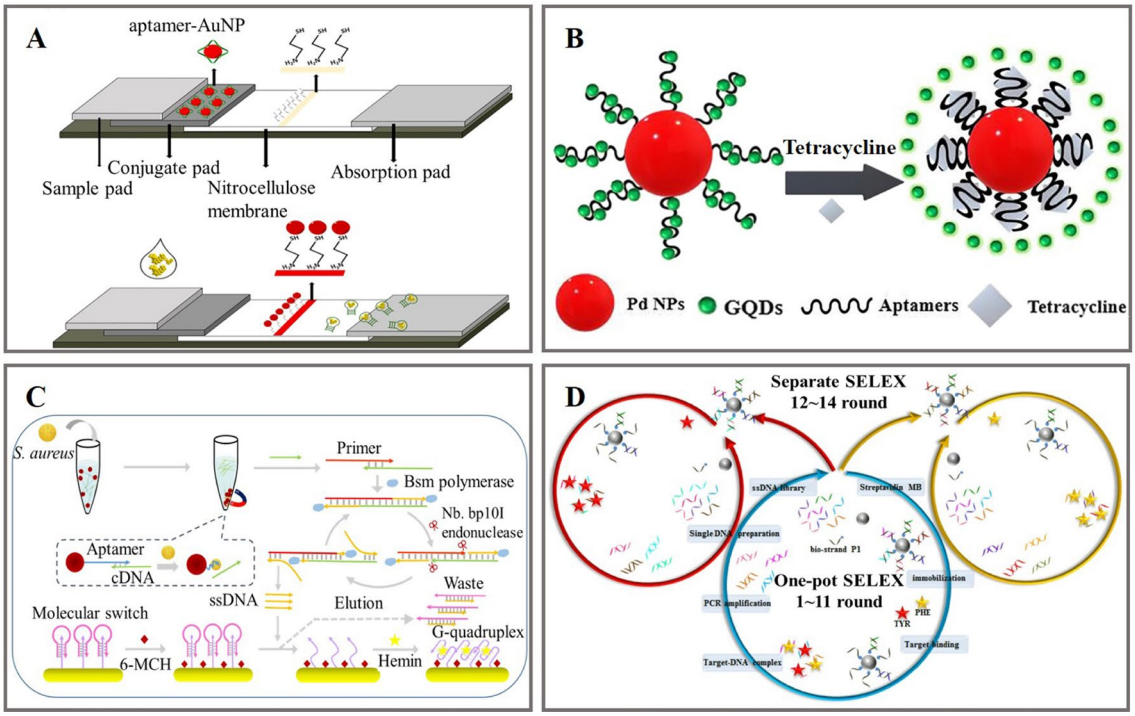


Fig. 6 **A** Schematic diagram of isocarbophos detection on lateral flow biosensor using AuNP-aptamers probe, reprint permission was acquired from Elsevier [115]; **B** Schematic diagram of the target specific aptamer-induced biosensor for sensitive detection of tetracycline in raw milk, reprint permission was acquired from Elsevier [143]; **C** Schematic diagram of the electrochemical detection of *S. aureus*, reprint permission was acquired from Elsevier [122]; **D** Schematic diagram of one-pot coupled with a separate SELEX selection process and the linear range of the calibration curve for TYR and PHE, reprint permission was acquired from American Chemical Society [152]

further used for separate SELEX, and TYR and PHE aptamers were obtained respectively. The aptamers obtained by this strategy were further applied to the determination of TYR and PHE in pork and beer samples with high accuracy.

Through the development of various biosensors based on aptamers, the detection of pesticide residues, antibiotic residues, microbial contamination, harmful products and other pollutants in the environment and food can be realized. The development of these technologies will help protect the natural environment and improve food quality, which has positive implications for people's healthy lives.

Conclusion and perspectives

Since the emergence of the concept of aptamer in 1990, the field of aptamers has grown steadily and the literature on aptamer applications have proliferated in the past three decades. The systematic bio-fabrication of aptamers and their applications in the emerging engineering biology are reported in this review, including SELEX technology for primary aptamer screening, post-SELEX optimization of aptamers, and the applications of aptamers in industrial technology, medical and health engineering, and environmental and food safety.

Aptamers with an affinity for specific targets can be obtained by SELEX technology through several rounds of screening. To improve the screening efficiency, the appropriate SELEX technology should be chosen according to different targets. Mag SELEX is a general technology to screen by immobilization of targets and magnetic separation. It can overcome the problem of low-affinity nucleic acid separation in traditional SELEX. As an upgraded version of Mag SELEX, library immobilization method is used in Capture SELEX, which can effectively avoid changes in target structure, especially those small molecules. CE SELEX separates complexes according to their different migration abilities in electrophoresis. Small sample loading and high screening efficiency make it an effective SELEX method. Cell SELEX simplifies the process of cell or bacteria aptamer screening by targeting cells directly. It can screen aptamers for corresponding target cells without obtaining proteins on the surface of cells or bacteria. The microfluidic SELEX is a highly integrated and automated screening technology. With the help of chips and microfluidic equipment, it can greatly

save manpower and material resources and reduce errors in the screening process.

To lower the cost in a scale-up application and facilitate subsequent design in biosensing configuration, further truncation of the selected full-length aptamer is helpful, which can also optimize aptamer sequences and effectively improve the affinity and stability of aptamers. However, the molecular weight of the truncated aptamers makes them easy to be excreted through renal filtration. A further modification is helpful to prolong the half-life of the aptamers and improve the nuclease resistance of the aptamer. Using appropriate truncation and modification technology, aptamers can be artificially improved to further put them into practical use from the research level.

With in-depth research on the structure and function, aptamers are expected to enter a new stage of development. Combined with biotechnology and engineering technology, aptamers have been applied in the fields of engineering biology. In industrial biotechnology, aptamers are combined with riboswitches, and HTS platforms with higher specificity are developed. Furthermore, by using aptamers to reprogram cell metabolism of microorganisms, high-yielding strains can be directly obtained. The use of aptamer-based biosensors to monitor fermentation processes and intracellular metabolism can deepen the analysis and understanding of cellular metabolic processes. The aptamer-based magnetic bead separation system can also be used for the separation of fermentation products, and the effect is even comparable to that of traditional nickel-chelating column chromatography. In the fields of medical and health engineering, aptamers have been used for the early diagnosis of diseases. Aptamers mounted on the surface of the drugs can more accurately hit the corresponding targets, reducing the dosage of the drugs, and improving their efficacy. In environmental and food safety monitoring, a variety of aptamer-based biosensors have been developed to detect various residues and contaminants in the environment and food, effectively ensuring food and environmental safety.

However, few aptamers have been put into use, and most of the work is still in the laboratory research stage. Due to the lack of in-depth research on the structure of nucleic acid aptamers, problems in the stability of aptamers appear during the application process. Therefore, the understanding of the structure–activity relationship of aptamers should be strengthened in the future, to establish a standard screening and post-optimization mechanism for the mass biomanufacturing process of aptamers, and use many evaluation methods to comprehensively analyze the overall performance of aptamers. On this basis, a variety of nanotechnology, micro/nano-manufacture and signal amplification technology can further be introduced to improve the performance of aptamers in the process of practical applications and broaden aptamer-based applications in engineering biology.

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Declarations

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