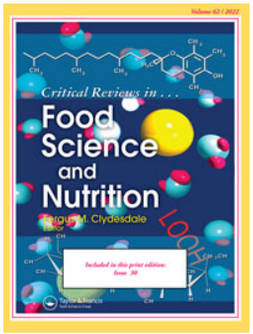

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Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Tianshun Li, Jia Wang, Longjiao Zhu, Chenwei Li, Qiaoying Chang & Wentao Xu (2022): Advanced screening and tailoring strategies of pesticide aptamer for constructing biosensor, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2022.2086210

To link to this article: https://doi.org/10.1080/10408398.2022.2086210

Published online: 14 Jun 2022.

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
https://doi.org/10.1080/10408398.2022.2086210

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REVIEW

Advanced screening and tailoring strategies of pesticide aptamer for constructing biosensor

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ABSTRACT

The rapid development of aptamers has helped address the challenges presented by the wide existed pesticides contaminations. Screening of aptamers with excellent performance is a prerequisite for successfully constructing biosensors, while further tailoring of aptamers with enhanced activity greatly improved the assay performance. Firstly, this paper reviewed the advanced screening strategies for pesticides aptamers, including immobilization screening that preserves the native structures of targets, non-immobilized screening based on nanomaterials, capillary electrophoresis-systematic evolution of ligands by exponential enrichment (CE-SELEX), virtual screening *in silico*, high-throughput selection, and rational secondary library generation methods, which contributed significantly to improve the success rate of screening, reduce the screening time, and ensure aptamer binding affinity. Secondly, the precise tailoring strategies for pesticides aptamers were modularly elaborated, containing deletion, splitting, elongation, and fusion, which provided various advantages like cost-efficiency, enhanced binding affinity, and new derived functional motifs. Thirdly, the developed aptamer-based biosensors (aptasensors) for pesticide detection were systematically reviewed according to the different signal output modes. Finally, the challenges and future perspectives of pesticide detection are discussed comprehensively.

KEYWORDS

Pesticide; aptamer; SELEX; tailoring; broad-spectrum; aptasensor

Introduction

Pesticides are valuable in preventing, eradicating and controlling diseases, pests and weeds that are harmful to agricultural and forestry production. However, extensive application and excess dosages can cause serious food and environmental pollution. The consumption of agricultural products containing pesticides can cause acute or chronic toxicity in humans. It is estimated that millions of people were poisoned by pesticides worldwide, and more than 300,000 people died from pesticide poisoning every year (Lekei, Ngowi, and London 2016; K. Liu, Dong, and Deng 2016). Therefore, methods for the efficient and rapid detection of pesticide residues are urgently needed to manage the safety of agricultural products.

The traditional strategies for pesticide residue detection commonly use instrumental methods, like high-performance liquid chromatography (HPLC) (Rejczak and Tuzimski 2017), liquid chromatography-mass spectrometry (LC-MS) (Bordin et al. 2017), and gas chromatography (GC) (M. Xu, Liu, and Lu 2014). These techniques are highly sensitive, accurate, and enabled multiple detection. However, these methods present several disadvantages, such as requiring expensive instruments and professional operators, complex assay procedures, and relatively costly materials and reagents, limiting their applications in rapid, large-scale, and on-site pesticide detection. Rapid detection techniques, such as the enzyme inhibition assay (EIA) (Najwa 2014), enzyme-linked immunosorbent assay (ELISA) (B. Liu et al. 2012) and capillary electrophoresis (CE) (Picó 2003), are convenient and time-saving, but they are highly susceptible to extreme environmental factors and hardly achieve on-site detection. Recent years, aptamer-based biosensors (aptasensors) have emerged and attracted much attentions owing to their rapidity, cost-efficiency, simple operation, high stability, sensitivity and specificity, greatly improved the assay performance for pesticides residues detection (Zhou et al. 2013).

Aptamers are short single-stranded oligonucleotides (RNA or DNA) selected via the systematic evolution of ligands by exponential enrichment (SELEX) method, which can selectively bind to their targets with high affinity. As promising alternatives to antibodies, aptamers are highly specific with low molecular weight and are simple to synthesize and modify. They are screened in vitro and can ideally be applied to any target of interest. When integrated into biosensors, aptamers exhibit unique characteristics like sequence programmability, stimulus responsiveness, and structural tailorability. Various aptasensors have been developed for pesticide detection, including electrochemical, fluorescent, and colorimetric sensors (M. Liu et al. 2019; Xie et al. 2022; Yan,

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Li, and Su 2018). To improve the pesticide detection performance, different nucleic acid amplification strategies (e.g. polymerase chain reaction, strand displacement amplification, loop-mediated isothermal amplification, rolling circle amplification, hybridization chain reaction, helicase-dependent amplification, recombinase polymerase amplification and cross-priming amplification) (Glökler et al. 2021), and superior nanomaterials (quantum dots (QDs), upconversion nanoparticles (UCNPs), graphene oxide (GO), etc) are usually adopted (Abdul Hakeem et al. 2021; D. Su et al. 2021; J. Wang, Zhu, et al. 2022; Wang et al. 2020a). Despite their improvement in assay sensitivity, these strategies play a limited role in resolving assay specificity and accuracy challenges when developing aptasensors. Therefore, this review attempts to provide more fundamental and useful solutions by enhancing the inherent properties and engineered functionality of aptamers.

Since the emergence of aptamers three decades ago, two approaches have been successfully employed to improve the inherent properties and engineered functionality of aptamers. The first involves the acquisition of a superior pre-selected aptamer library by optimizing the screening process. Since the early reported SELEX methods were commonly time-consuming and labor-intensive, the overall efficiency of SELEX is far from satisfactory, and the number of aptamers showing strong binding affinity and specificity remains limited. Advances in material science and bioinformatics prompted the development of SELEX variants, such as GO-SELEX and virtual screening *in silico*, to conserve processing time, ensure high aptamer to target affinity, and increase the screening success rate. Secondly, it is attractive to tailor aptamers to generate compact or specific structures with enhanced binding affinity and additional functions. This review collectively summarizes and analyses the advanced SELEX strategies and aptamer tailoring methods used for constructing pesticide biosensing platforms (Figure 1). Also, all of the aptamer sequences currently available for pesticides are listed (Table 1). Finally, the research progress and current challenges of pesticide aptasensors were systematically discussed, aiming to provide a beneficial reference for further research on pesticide monitoring.

Advanced SELEX strategies for pesticide aptamers

To develop more efficient aptasensors for pesticide residue detection, it is crucial to investigate and discuss screening strategies for pesticide aptamers. Traditional SELEX methods are commonly based on target-immobilization, the flow chart is shown in Figure 2(a). By immobilizing the target on a solid matrix, such as magnetic beads, agarose beads, composite resins, microfluidic chips, and glass slides (He et al. 2011; Hong and Sooter 2017; Williams et al. 2014b),

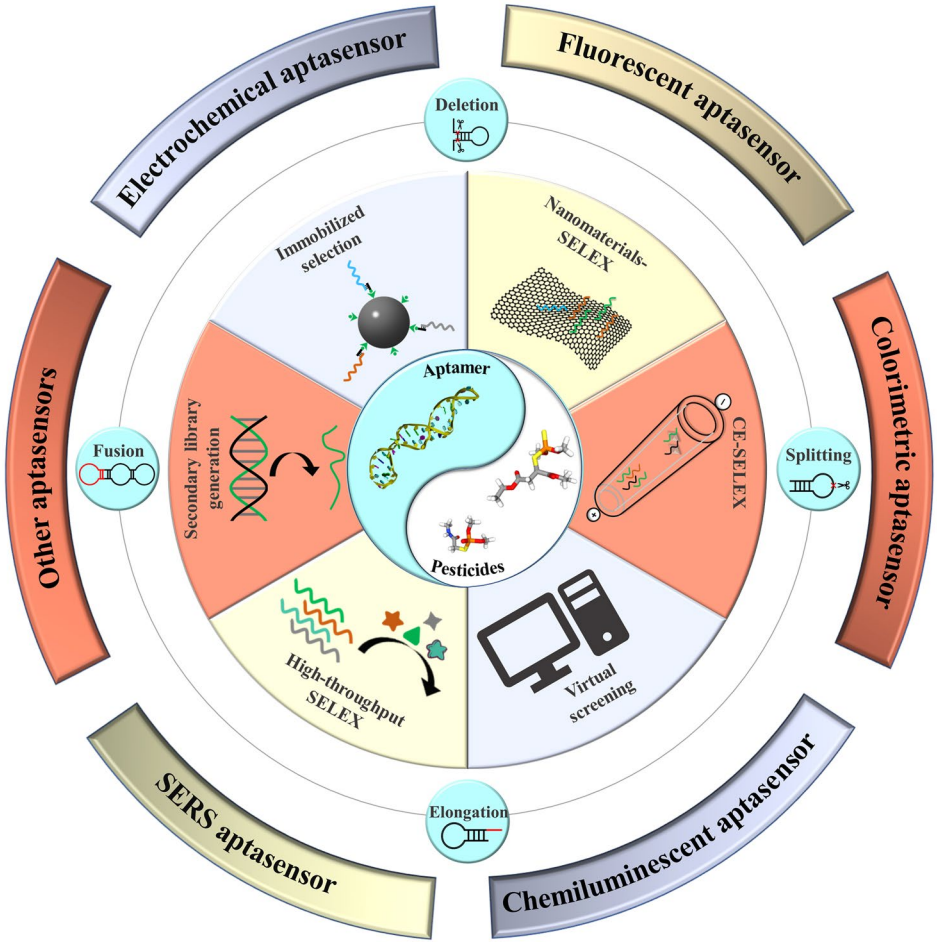

Figure 1. An illustration of the advanced SELEX strategies and aptamer tailoring methods used for constructing pesticide biosensing platforms.

Table 1. List of available aptamers specific to pesticides.

Target	Sequence	Screening strategy	Tailoring method	Affinity (K_d)	Reference
Fluoroacetamide	ACCTGCAGGCGCGAGTTTC AGATCAAAACTTGTCTGGCGT and GGCGTGTGCCCTGCGGA TGAAGACTGGTCT	Traditional method (Fix the target on microplate)	–	1 and 1.2 μM	(Cao et al. 2016)
Malathion	ATCCGTCACACCTGCTCTTATACA CAATTGTTTTTCTCTTAACTTC TTGACTGCTGGTGTTGGCTCCCGTAT	Traditional method (immobilize malathion on affinity column)	–	–	(Barahona et al. 2013)
	TGTACCGTCTGAGCGATTCGTA CTATGGTATCCGAGAGGCCT ACGGAATTGTTGTACAGCCA GTCAGTGTTAAGGAGTGC	Traditional method (Fix the target on magnetic bead)	–	2.43 nM	(Williams, Maher, and Sooter 2014)
Phorate Profenofos Isocarbophos Omethoate	AAGCTTTTTTGACTGACTGCA GCGATTCTTGATCGCCACGG TCTGGAAAAAGAG and AAGCTTGGTGTACTCACTGCA GCGATTCTTGATCGGTGAGTTA GACAACATGCGC	High-throughput selection (capture-SELEX)	–	0.8-2.5 μM	(L. Wang et al. 2012)
	AGCTTGCTGCAGCGATTCTTGA TCGCCACAGAGCT	–	Deletion Fusion	–	(C. Zhang et al. 2014)
Isocarbophos Omethoate	AAGCTTTTTTGACTGACTGC AGCGATTC-TTGATCGCCAC GGTCTGGAA AAAGAG	–	Splitting	–	(Liu, et al. 2020)
Paraoxon	AGCTTGCTGCAGCGATCCTTGA TCGCCACAGAGCT	Virtual screening	Mutation	around 1 μM	(Belinskaia et al. 2019)
Paraquat	AGGCTTACACCTGAAAAGCGGCTTA ATTTACACTACTGTAT	CE-SELEX	–	–	(Kuitio et al. 2021)
Iprobenfos Edifenphos	TAAGGGATTCCTGTAGAAG GAGCAGTCT	High-throughput selection (GO-SELEX)	Deletion	1.67 μM and 38 nM	(Kwon et al. 2015)
Bromacil	TGTACCGTCTGAGCGATTCGTA CTGTGGGCACCAATCGTACC CAATACTTGCGAATCAGCC AGTCAGTGTTAAGGAGTGC	Traditional method (Fix the target on magnetic bead)	–	9.6 nM	(Williams et al. 2014b)
Carbendazim	GGGCACACAACAACCGATGGT CCAGCCACCCGAATGACC AGCCCACCCGCCACCCCGCG	Traditional method (Fix the target on microtiter plate)	–	65nM	(Eissa and Zourob 2017)
Acetamiprid	TGTAATTTGTCTGCAGCGGTTCTT GATCGCTGACACCATATTATGAAGA	Capture-SELEX	–	4.98 μM	(He et al. 2011)
	CTGACACCATATTATGAAGA	–	Deletion	–	(Qi et al. 2016)
Atrazine	TGTACCGTCTGAGCGATTCGTACGAAC GGCTTTGTACTGTTTGCACTGGCGG ATTTAGCCAGTCAGTGTTAAGGAGTGC	Traditional method (Fix the target on magnetic bead)	–	0.62 nM	(Williams, Maher, and Sooter 2014)
	ACCGTCTGAGCGATTCGTACTTTATTCGG GAAGGGTATCAGCGGGG	Capture-SELEX	Deletion	3.7 nM	(Abraham et al. 2018)
Fipronil	TGTACCGTCTGAGCGATTCGTACAGTTTC TGGAGGACTGGGCGGGGTGACGGT TATGAGCCAGTCAGTGTTAAGGTGTGC	Traditional method (Fix the target on magnetic bead)	–	48 ± 8 nM	(Hong and Sooter 2017)
Tebuconazole Inabenfide and Mefenacet	CGTACGGAATTCGCTAGCCCCCCGGCAGGC CACGGCTTGGGTTGGTCCCACTGCGCGT GGATCCGAGCTCCACGTG	High-throughput selection(GO-SELEX)	–	1-100nM	(Nguyen et al. 2014)
Trichlorfon	CGCATCGAGAGTCGGAAAAGGCTAGTTCTTAG CGACGTCCGATATTTCGTTCGAA	–	–	–	(Feng et al. 2021)
Imidacloprid	TGTCGTCTACGGTTTTGGTTGTTGTTTGTTG GTGGGTGTA	GO-SELEX	–	–	(Bor et al. 2020)
O,p′- DDT	TCCAGCACTCCACGCTAACGATTGCGCTCATG CCCCTGCAGTGATGTGGAATTTGTTAGTG CGCGACGGTGAA	Capture-SELEX	–	412.3 ± 124.6 nM	(W. Zhang et al. 2020)
Thiamethoxam	CTCAGTTCGGGACGACGGCAAGGTAACGTAT GGGACCTTGGCACGAGTCCCGAA	Capture-SELEX	–	54.63 ± 1.09 nM	(Y. Luo et al. 2021)
	GACGGATCCACCGACCATGCAAAGATGCA CAAAAACG	GO-SELEX	Deletion	123.35 ± 29.80 nM	(Kong et al. 2022)
Dichlorvos	GGAAGAACATGTGAGCAAAAGGCCAGCAAA AGGCCAGGAACCGTAAAAAGGCCGCGTTG CTGGCG	Gold-SELEX	–	0.85 μM	(Chatterjee et al. 2020)
Diethyl thiophosphate	GACGGGAGCTTGACACAGTCATTCCTTTCTAG ATGGTGGTT	CE-SELEX	–	0.103 ± 0.014 μM	(Swainson et al. 2021)
Carbaryl	CTCAGTCGCTAGGGTTATGTTAATAAGATGAAC CCACGTTCCAGATGGCATCGCCTAGCGA	Capture-SELEX	Deletion Elongation	0.364 ± 0.055 μM	(Y. Liu et al. 2021)
λ-cyhalothrin	AGGGGAAGCACGGGCGGGCG	Capture-SELEX	Deletion	10.27 ± 1.33 nM	(Y. Yang et al. 2021)

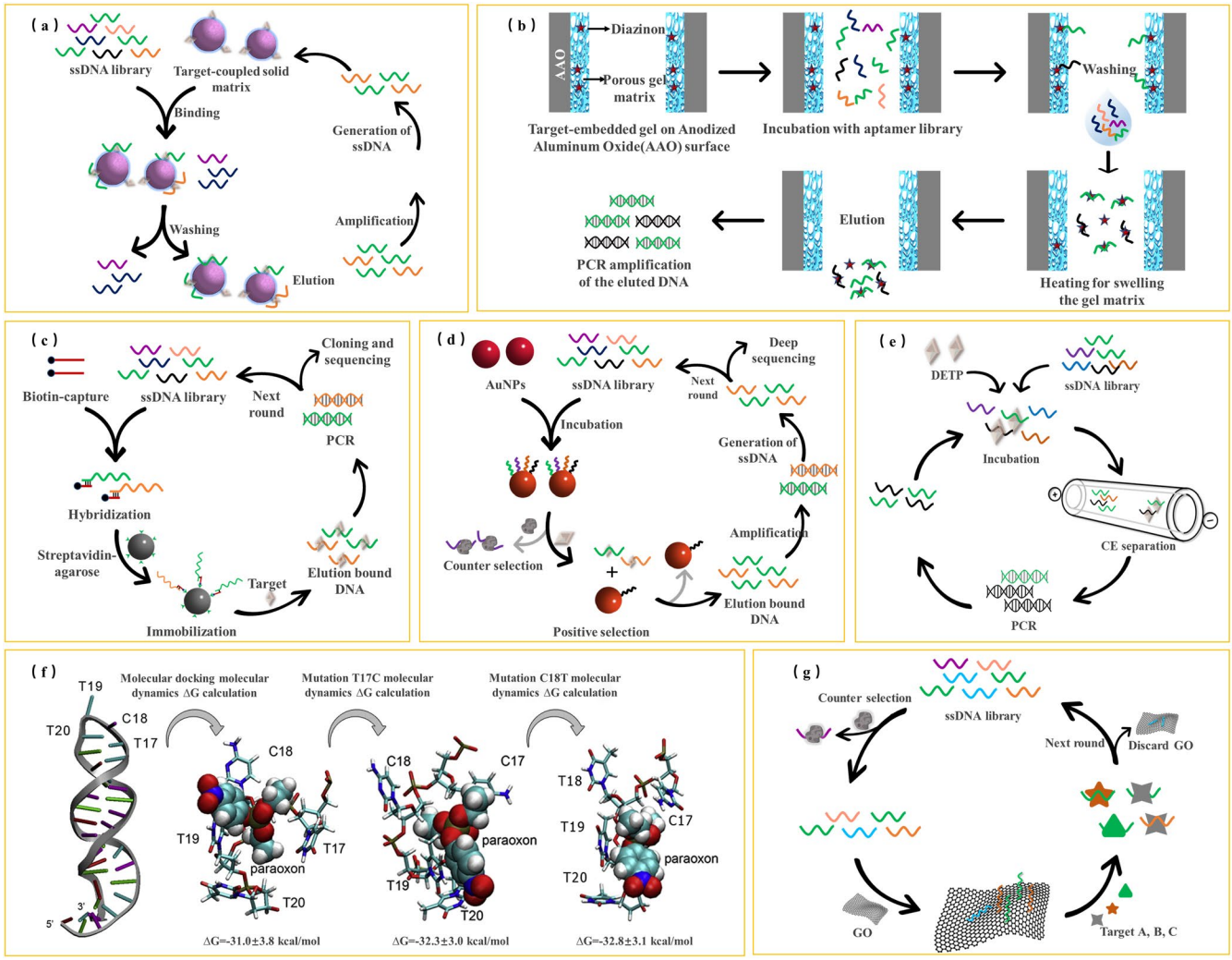

Figure 2. Schematic diagram of various aptamer screening strategies for pesticide detection. (a) A schematic diagram of the traditional SELEX method for target immobilization. (b) A schematic illustration of the SELEX process based on sol-gel nanocolumns (Lim et al. 2020). (c) A schematic illustration of the Capture-SELEX process for thiamethoxam. (d) A schematic diagram of GOLD-SELEX (Chatterjee et al. 2020). (e) A schematic diagram of the CE-SELEX process. (f) The paraoxon and aptamer complexes according to the molecular dynamics data (Belinskaia et al. 2019). (g) A schematic diagram of Multi-GO-SELEX (L. Wang et al. 2012).

the target-bounded oligonucleotides with affinity and specificity can be isolated by simple elution. While these methods also suffer from some disadvantages, first, small molecular targets such as pesticides are challenging to immobilize on solid substrates (Reinemann, Stoltenburg, and Strehlitz 2009). The process of target immobilization often requires chemical reactions, which will change its original structure (C. Chen et al. 2017). Therefore, the binding affinity of the aptamer screened by this method to the free-state target may be compromised (T. Wang et al. 2019b). Second, traditional aptamer selection mostly performed under simple and ideal circumstances, the screened aptamer may not function properly in real biosensor development. Third, these methods generally suffer from low resolution and separation efficiency, requiring multiple SELEX cycles (at least 10 rounds), which is time-consuming and may lead to the loss of potential sequences. Fourth, the experimental procedures of SELEX are usually labor-intensive, costly and blind "black box" operations. Fifth, the traditional SELEX mostly screened aptamers for single target, which cannot meet the demand of multiple or broad-spectrum aptamer screening. Sixth, the generation of high-quality ssDNA secondary libraries is crucial for the successful aptamers selection (Svobodová et al. 2012). Previously developed methods such as asymmetric PCR will increases the probability of nonspecific amplification (Gyllensten and Erlich 1988) and requires complex steps of gel separation and elution during secondary library generation. Therefore, novel SELEX strategies are needed to improve the development of high-affinity aptamers for pesticide detection. In this section, advanced SELEX strategies in six types are introduced to solve the above mentioned six disadvantages respectively.

Immobilized selection that preserves the natural structure of target

The target-immobilization method is likely to change the natural structure of target, affecting the recognition site.

However, in practical applications, the target maintains the native state, which may result in low binding affinity between the screened aptamer and the target, leading to false detection results. For example, the ampicillin aptamers were obtained by immobilizing ampicillin on tosyl-activated magnetic beads (Song et al. 2012). These aptamers have been used to develop different analytical methods and sensor configurations (Blidar et al. 2019; H. Wang et al. 2015). However, Bottari et al. (2020) employed isothermal titration calorimetry, native nanoelectrospray ionization mass spectrometry, and ^{1}H-nuclear magnetic resonance to evaluate binding between ampicillin and its aptamers. The results of these assays collectively demonstrate that these sequences do not show any specific binding with ampicillin. In addition, counter-selection with carriers as targets is required to reduce the effects of nonspecific adsorption between carriers and nucleic acid strands (Jo et al. 2011). And this method usually requires cumbersome elution steps, which may lead to the loss of ssDNA sequences and reduce the library diversity.

To overcome the shortcomings of target-immobilized SELEX, improvement strategies from two aspects have been developed. One is to immobilize the target without a chemical coupling reaction, the other is to immobilize the nucleic acid library instead of target. In terms of non-chemical immobilization, As shown in Figure 2(b), using the nanoscale cavities and micro-scale channels formed by sol-gel hydrolysis and condensation reactions to immobilize small molecular target (diazinon), ssDNA was bound to target via the surface nanopores in the cavities. The sequences bound to diazinon could be extracted from the sol-gel matrix by thermal elution. moreover, to maximize the binding efficiency, the nanoporous anodic aluminum oxide (AAO) membrane was selected as a sol-gel construction platform to increase the bonding surface. Innovatively, a sol-gel-coated AAO disk was mounted on the edge of the pipette tip, and the feasibility of the constructed platform for SELEX aptamer binding was confirmed. This proposed approach is expected to be employed as a pipette-tip-mounted SELEX platform for automated liquid handling systems (such as a pipetting robot) in the near future (Lim et al. 2020).

As for nucleic acid library immobilization, the Capture-SELEX was developed to select aptamers by target induced conformational change of the immobilized ssDNA library on the solid matrix. Figure 2(c) shows the general process of Capture-SELEX. First, the library is immobilized via hybridization between the docking sequences and complementary oligonucleotides fixed on the solid carrier. Docking sequences are usually located in the middle or on both sides of the random sequences and sometimes partially overlap with the primer-binding sequences (Lyu, Khan, and Wang 2021). Upon target addition, only sequences that bind to the target and produce conformational switches can be released from the solid-phase surface, while the weaker or unbound sequences remain. Then the released sequences are enriched and amplified, after which multiple rounds of selection are performed repeatedly until high-affinity aptamer is obtained. Y. Luo et al. (2021) obtained a DNA aptamer for thiamethoxam by immobilizing the ssDNA library on streptavidin agarose beads using a biotin-labeled capture strand. L. Wang et al. (2012) used a random single-stranded DNA library fixed on the filter column to screen the broad-spectrum aptamers of four organophosphorus pesticides: profenofos, phorate, isocarbophos, and omethoate. The dissociation constants were measured in the range of 0.8-2.5 μM using the inhibiting competition method. Recently, Trinh et al. (2021) isolated aptamers that could specifically bind to fenitrothion based on a similar principle. This screening method is highly efficient in maintaining target structure and developing structure-switching aptamers (Boussebayle et al. 2019; Coonahan et al. 2021).

Nanomaterials-based selection to simulate subsequent biosensing protocols

Most aptasensors are designed based on conformational changes or structural transformation when aptamers interact with targets. Among them, the GO-based fluorescent sensing platform and AuNPs-based colorimetric sensor represent two common strategies in biosensing (S. Wang et al. 2018c; T. Yang et al. 2020; H. Zhang et al. 2017). Nevertheless, due to the unpredictability of aptamer structural alterations, these sensing strategies may yield false or nonspecific signals. Nanomaterial-based aptamer screening methods, such as GO-SELEX and GOLD-SELEX, can significantly overcome these challenges. GO can adsorb free ssDNA or RNA through π-π stacking interaction and desorb oligonucleotides whose structures undergo target-induced transformation. By this mechanism, a simple, rapid and high-throughput GO-SELEX method was developed to screen aptamers against multiple pesticides (tebuconazole, inabenfide, and phenothiazole) (Nguyen et al. 2014). The random ssDNA library was first incubated with GO, then the adsorbed GO-ssDNAs were interacted with the target molecules, facilitating the release of candidate aptamer sequences with high affinity and specificity for the target. The desorbed ssDNA-target complexes in the solution were collected via centrifugation and enriched through PCR to accumulate a portion of the target-binding oligonucleotides for subsequent SELEX rounds. After only five rounds, ten ssDNA sequences with high affinity for the three pesticides were successfully isolated. Bor et al. (2020) also used the GO-SELEX to screen the imidacloprid aptamer and successfully applied for electrochemical detection. Another GO-SELEX screening mode choose to first incubated the random library with the target, the GO was then added to adsorb the unbounded ssDNA sequences. After centrifugation, the supernatant with bounded ssDNA sequences was collected for the subsequent steps (Kong et al. 2022). Obviously, the first model involved more stringent screening pressure. Similarly, based on the specific adsorption of AuNPs on ssDNA, GOLD-SELEX protocol as shown in Figure 2(d) was developed to screen the dichlorvos aptamers. This strategy purely relies on the affinity of the aptamers toward their target to detach from the AuNP surface after binding with target (Chatterjee et al. 2020).

The nanomaterials-based selection methods ensure the ability of aptamers to efficiently dissociate from the GO or gold surface upon specific binding to target in the real biosensing applications, avoiding false-negative results. Moreover, this non-immobilized screening method has no need for further structure modification of target, maintained high binding affinity. Also, fewer targets are required compared with the target-immobilized method, and the candidate aptamer sequences can be separated via simple centrifugation, which is rapid and cost-effective. It is noteworthy that these screened aptamers are also suitable for the development of other types of sensors (Qin et al. 2017; Shi et al. 2021).

Multi-functional CE-SELEX for the high-efficiency selection of aptamers

CE-SELEX is an attractive immobilization-free method. It can efficiently separate aptamer-target complexes, target and unbound sequences in a free solution based on the different mobilities generated by different charge-to-mass ratios (Mosing and Bowser 2009). As shown in Figure 2(e), the target and ssDNA library are first mixed and incubated for 10-30 min, after which the mixture is injected into a capillary and separated under a high voltage electric field (Zhu et al. 2019). The complex peak fraction was collected to obtain the target ssDNA complex. PCR amplification, secondary library generation, and high-throughput sequencing are then performed as in conventional SELEX. CE-SELEX has demonstrated versatility in the screening process: first, the target binds ssDNA in homogeneous solution, which eliminates linker and kinetic biases caused by stationary support preparation and washing (Y. Zhang, Lai, and Juhas 2019), and their natural structure is maintained. Second, CE-SELEX has the advantages of high partition coefficient and excellent screening efficiency, usually with only 1-4 rounds that shorten the selection process significantly from weeks to days (Berezovski et al. 2006). Third, CE enables qualitative and quantitative analyses that can be used to monitor ssDNA enrichment in each round of SELEX as well as target purity analysis and ssDNA quality evaluation before selection. At the end of each screening round, it is possible to quickly and visually calculate the affinity of the aptamer according to the peak area. Thanks to excellent screening efficiency and rapid affinity analysis, CE-SELEX is able to drastically reduce workload and study costs, providing the possibility of systematic studies to optimize the screening conditions. Fourth, CE-SELEX requires less sample and reagent depletion (<100 nL per round), whereas conventional SELEX requires ml or even more (Zhu et al. 2018).

CE-SELEX is generally more suitable for the aptamer screening of macromolecular targets than small molecular targets since the migration rate of the aptamer-macromolecular target complex can be more effectively distinguished from unbound sequences. However, many successful attempts have demonstrated that CE-SELEX can be applied for small molecule targets (Mendonsa and Bowser 2005; G. Yang et al. 2018; J. Yang and Bowser 2013). Sanchez (2012) first applied CE-SELEX for the aptamer selection of pesticides and obtained an atrazine-specific aptamer after six rounds. The fluorescence polarization technique was used to determine the binding affinity of the aptamer, and the K_d value was reported as 890 nM. The diethyl thiophosphate (DETP) aptamer was recently obtained using CE-SELEX. The colorimetric method revealed that the aptamer displayed a high affinity for DETP, with a mean K_d value of 0.103 μM (Swainson et al. 2021). It is further demonstrated the availability of CE-SELEX for small molecule targets, greatly reducing the molecular size requirements when using this technique.

Virtual screening in silico for precise and cost-effective aptamer selection

With the development of computer and bioinformatics in recent years, research has increasingly started to combine virtual screening *in silico* and *in vitro* SELEX for aptamer screening (Wondergem, Schiessel, and Tompitak 2017). Generally, *in silico* molecular docking programs include the secondary structure prediction of DNA, DNA tertiary structure determination, molecular docking, and molecular dynamics analysis. During this procedure, the nucleic acid library obtained from a particular round of SELEX procedure is sequenced, after which the various sequence classes are subjected to molecular docking utilizing a computer to compare the binding strength of each DNA sequence to the target (Navien et al. 2021). The DNA with the highest affinity to the target is selected as the potential aptamer, and subsequent experimental validation is performed to refine the conclusions and verify their plausibility.

Jokar et al. (2016) conducted molecular docking experiments using acetamiprid and aptamers. The Autodock results showed that the binding energy of acetamiprid aptamer was 6.812 ± 0.533 kcal/mol. The docking revealed the SS2-55 and SS4-54 loops as aptamer binding sites and confirmed the formation of an aptamer-acetamiprid complex. Then, this team used molecular docking and molecular dynamics simulation to screen the G-quadruplex DNA aptamer with the highest affinity to diazinon from 12 sequences (Jokar et al. 2017). In addition, As shown in Figure 2(f), Belinskaia et al. (2019) employed paraoxon to develop an organophosphorus pesticide aptamer design method via computer technology. A three-dimensional model was established based on the published aptamer sequences of organophosphorus pesticides (L. Wang et al. 2012). The possible binding sites of paraoxon and the aptamer were simulated by molecular docking and molecular dynamics. The binding site nucleotide was then mutated, and the free binding energy was calculated via the molecular dynamics trajectory and MM-PBSA method. Two sequences displaying high paraoxon affinity were selected according to the energy value, while the K_d value was about 1 μM.

In summary, molecular docking can provide a more significant understanding of the interaction between aptamers and targets, which is helpful for the subsequent optimization and rational design of aptamers. Various reliable molecular

docking simulation programs have been developed, such as RosettaDock, 3dRPC, FTDock, GRAMM, PatchDock (Marze et al. 2018), ZDock, HADDock, NPDock, AutoDockTools, AutoDock, and AutoDock Vina (Navien et al. 2021; Zavyalova et al. 2020), which allow modeling and prediction of target-aptamer interactions without performing experiments. Apparently, virtual screening is a more direct and rapid method for aptamer discovery than traditional experimental screening and offers the advantages of low-cost and efficient screening.

High-throughput selection strategies to screen broad-spectrum aptamers

Owing to the multiple residues of pesticides in food and environment, high-throughput detection is necessary for food safety and environmental health control. Compared to using multiple specific aptamers as detection probes, using broad-spectrum aptamer turns to be a low-cost and high-efficiency approach. Screening of broad-spectrum aptamer commonly adopted four types of strategies. First, as demonstrated in Figure 2(g), various pesticides are added simultaneously as positive targets for incubation with the ssDNA library, after which the broad-spectrum aptamers are isolated. Based on this principle, L. Wang et al. (2012) acquired two broad-spectrum aptamers able to recognize profenofos, phorate, isocarbophos, and omethoate. Nguyen et al. (2014) successfully screened 10 sequences displaying the affinity for tebuconazole, inabenfide, and mefenacet after only five rounds using the multiple GO-SELEX method. GO-SELEX has also been used to screen broad-spectrum aptamers that can identify iprobenfos and edifenphos (Kwon et al. 2015). Second, the aptamers with broad affinity can be screened by switching selection. The key of this method is that targets of interest are switched sequentially at each SELEX round, and the multiple targets usually need to have a common domain. Song et al. (2017) screened aptamers with affinity for six bacteria from different genera. *Escherichia coli* was used as target in the first round of SELEX. The amplicons were sequentially separated by incubation with *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Bacillus subtilis*, and *Staphylococcus epidermidis* in the subsequent screening. The sequences obtained using the last bacterial species were incubated again with the first bacterial species and this cycle was repeated two more times. The finally obtained aptamer had dissociation constants of 9.22-38.5 nM and had no affinity for other bacteria not included in SELEX. Third, the common metabolites of some pesticides were selected as positive screening targets, which were measured to obtain the exposure history of these contaminants. As the biomarker of organophosphorus pesticide exposure, DETP is one of the common metabolites of chlorethoxyphos, chlorpyrifos, coumaphos, diazinon, parathion, and sulfotep. Additionally, it can be generated via the metabolism of disulfoton, ethion, phorate, and terbufos (Sudakin and Stone 2011). Swainson et al. (2021) identified an aptamer specifically bound to DETP and incorporated it into optical and electrochemical sensors to measure DETP. Fourth, referring to the preparation method of broad-spectrum antibody, the common structure of pesticide molecules can be selected as hapten (target), so as to obtain the broad-spectrum aptamer of these pesticide molecules. This requires further research since no studies are currently available involving this issue.

Rational generation of secondary libraries to improve screening efficiency

The preparation of secondary libraries by converting PCR dsDNA products into ssDNA represents a critical SELEX step since the purity and quantity of ssDNA can significantly influence the success of the SELEX process (Svobodová et al. 2012). Therefore, choosing the appropriate method to generate single chains is necessary.

Streptavidin agarose resin/magnetic bead-based separation methods are commonly used to generate ssDNAs in pesticide aptamers screening (Williams et al. 2014a; Williams, Maher, and Sooter 2014). Biotinylated double-stranded DNA is produced by PCR amplification with biotin-modified reverse primers. The amplicons were captured by the streptavidin agarose column or streptavidin magnetic beads via streptavidin-biotin bond, then the sodium hydroxide is added to elute the non-biotinylated target strands (Ran and Wu 2019; Y. Yang et al. 2021). Since this method is simple to operate, takes a short time (usually only 20 min), and can generate ssDNA with high yield, it is particularly favored by researchers. Besides, the lambda exonuclease digest method is another high-efficiency secondary library preparation strategy. PCR amplification was performed by employing a standard forward primer and a 5′-phosphorylated reverse primer. The PCR product was subsequently digested by lambda exonuclease, which could selectively digest the 5 ‘phosphorylated dsDNA. Although simple to perform, this method presents several challenges, the insufficient enzymatic digestion may lead to poor ssDNA quality, and the additional exonuclease elimination step may affect the simplicity and yield of this technique.

Another effective ssDNA regeneration method is size-dependent separation by denaturing polyacrylamide gel electrophoresis. Eissa and Zourob (2017) performed PCR using a fluorescein-modified forward primer and a polyethylene glycol (PEG) spacer modified reverse primer to generate strands with distinguishing molecular weight. The relevant fluorescent ssDNA strands were subsequently separated from the dsDNA PCR product using 12% denaturing polyacrylamide gel electrophoresis. This method displays high separation resolution and minimal PCR by-product accumulation. However, this method is time-consuming because the traditional extrusion and soaking method require up to 12 h to extract ssDNA from the collected gel blocks. This issue can be resolved using electroelution, reducing the total elution time to 30 min (Rahimizadeh et al. 2017).

In summary, these methods are distinctly different in cost, product quality, and operational simplicity. So far, no ssDNA preparation method has been developed that

considers all the relevant aspects. For example, streptavidin agarose columns/magnetic beads are expensive and cannot be reused, lambda exonuclease can slowly digest ssDNA and non-phosphorylated substrates, reducing the amount of secondary library products, PEG-modified primers may adversely affect the PCR amplification efficiency. Therefore, the appropriate secondary library generation strategy should be selected according to individual needs and experimental conditions. In addition, studies have reported that the rational combination of the two methods displays better performance (Pagratis 1996; Svobodová et al. 2012). Svobodová et al quantitatively compared several secondary library generation methods, including asymmetric PCR, enzyme digestion, magnetic separation, and asymmetric PCR combined with enzyme digestion. The ssDNA generated via each method was quantified using an enzyme-linked oligonucleotide assay, and the quality of the generated secondary libraries was assessed using agarose gel electrophoresis. Results show that the asymmetric PCR and exonuclease digestion combination displayed the highest efficiency, producing the highest amount of ssDNA compared with other methods (Svobodová et al. 2012).

Aptamer tailoring for biosensors

Many studies have illustrated that original aptamers often contain some redundant sequences that are not involved in target recognition and structure maintenance (Xiao et al. 2019). These redundant nucleotides will increase the cost and lead to false-negative results, so it is necessary to tailor the aptamer. The schematic diagram of the principles of different aptamer tailoring strategies is shown in Figure 3 (using the malathion aptamer and characterization method of microscale thermophoresis (MST) as examples). The general aptamer tailoring protocols include structural analysis (to predict aptamer binding sites), sequence design (applying different tailoring strategies), and sequence characterization (to determine the optimum tailoring sequence) (J. Wang, Zhu, et al. 2022). Aptamer tailoring varied from different strategies, covering deletion, splitting, elongation, and fusion. The precise and rational aptamer tailoring help to enhance the binding ability, form specific functional motifs and improved the detection performance of pesticide biosensors.

Deletion of aptamers

The aptamers screened via SELEX vary from 60-100 nt in length and commonly contain redundant sequences which don't participate in both target binding and aptamer structure maintenance (C. Chen et al. 2017). Some scholars believe that deleting the redundant sequence of an aptamer can enhance its affinity and specificity to the target (S. Gao et al. 2016). Furthermore, the biosensor developed by truncated aptamer has been improved in reducing cost, eliminating false-negative results and enhancing sensitivity (J. Zhang et al. 2018). The most important procedure in deleting aptamers is to determine the target binding sequences. Generally, the constant primer-binding sequences of aptamer do not affect the binding properties. Therefore, these regions are usually deleted during sequence characterization, leaving only random regions for binding studies. For further binding sites identification, various methods, such as protein footprinting (Padlan et al. 2014) and nuclear magnetic resonance (Manimala et al. 2004) have been utilized. However, these methods are cumbersome, expensive and time-consuming. Currently, the preferred method is computer simulation-guided aptamer structure design combined with experimental verification. Researches use online structure prediction website (DNAMAN, ClustalW, Mfold, and RNAfold) to predict the secondary structure of aptamers, or use molecular docking software (Autodock, SYBYL, and Discovery studio) to simulate the binding interaction of aptamer to target, followed by site-directed mutagenesis and deletion, resulting in a series of structural derivatives. After comprehensively exploring the relationship between individual truncated sequences and aptamer function through binding/biological function experimental assessments, the shortest sequence required for the binding activity can be determined (Zheng et al. 2015).

Previous studies have shown that the regions where aptamers recognize targets usually display complex structures, such as G-quadruplex, three-way junction, and hairpin (stem-loop) structures. Therefore, aptamer deletion usually takes these special structures as the core for structural design and binding exploration. C. Zhang et al. (2014) examined a series of shortened aptamers with different stem-loop structures for searching the binding regions of four organophosphorus pesticides. They ultimately found that different loops in the aptamer structure contributed to different functions during target recognition. Further molecular docking and molecular dynamics simulations revealed three areas that referenced three respective binding sites. Forces such as hydrogen bonds and Van der Waals interactions seem to play an essential role in enabling and stabilizing the combination of the aptamer and the target. Y. Yang et al. (2021) conducted the molecular docking between λ-cyhalothrin and LCT1 aptamer, the docking results in Figure 4(a) showed that the binding sites between λ-cyhalothrin and LCT1 were primarily related to the A-5, C-6, C-28, A-29, C-30, G-31, and G-32 bases. Deleting other extraneous bases increased the binding stability between the truncated sequence and the target. The truncated aptamers have been successfully applied in pesticides biosensing, and significantly improved the assay performance. Tian et al. (2016) investigated the effect of shortened aptamer sequences for acetamiprid detection based on the AuNPs colorimetric assay. Excess flanking nucleotides were deleted from the parental 49-mer acetamiprid aptamer to produce truncated 37-mer and 25-mer aptamers. The results revealed that the assay sensitivity was improved by 3.3-fold using the shortened aptamers. This is because the target-bounded aptamer may still adhere to the AuNPs due to excess bases, resulting in partial aptamer dissociation from the AuNPs and suppressing the aggregation reactions. In conclusion, aptamer deletion not only reduces the synthesis cost and enhances the binding affinity, but also improves the assay sensitivity and eliminates the false results of developed biosensors.

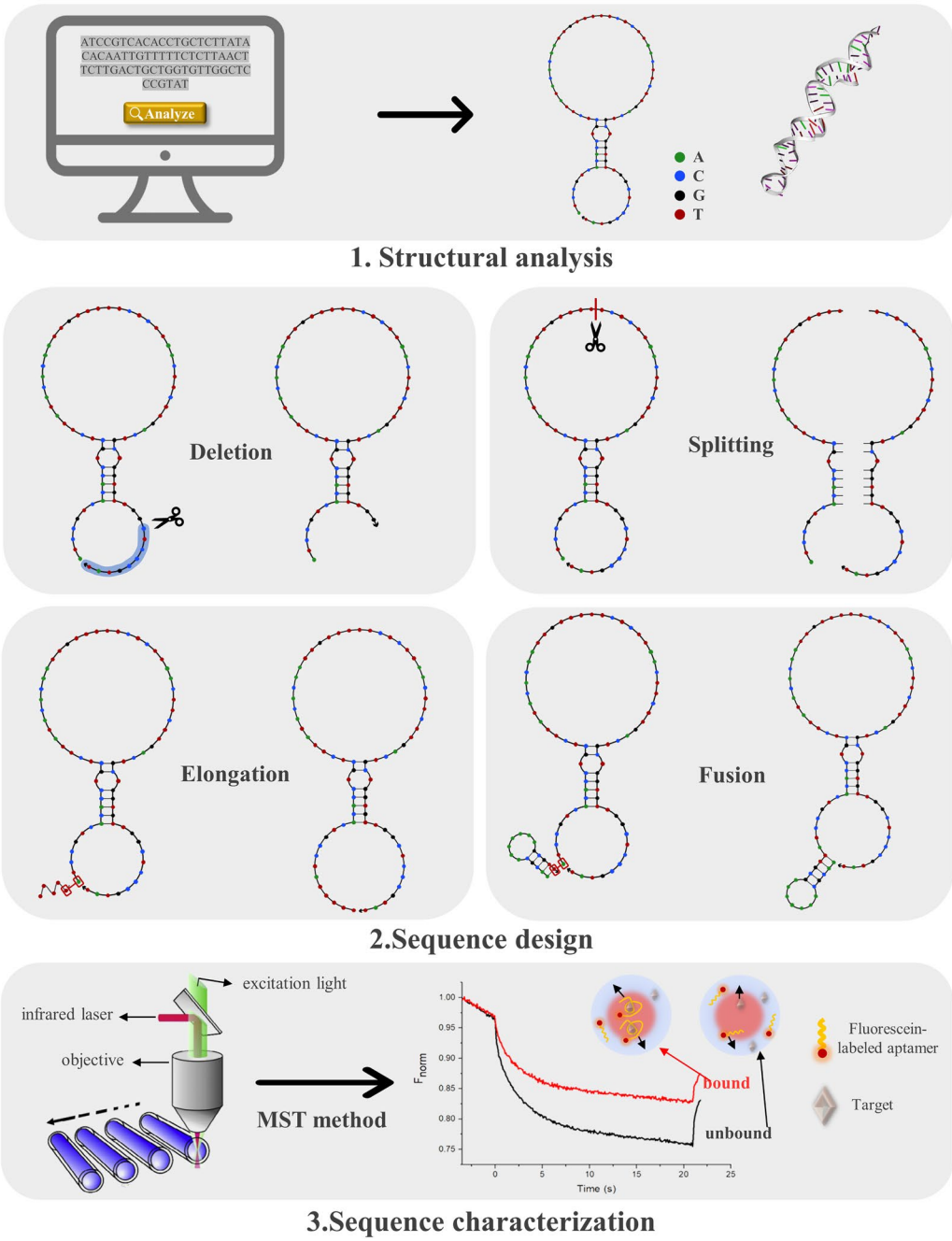

Figure 3. The protocols of different aptamer tailoring strategies.

Splitting of aptamers

Small molecular targets, such as pesticides, generally have a single epitope, which are detected in competitive reaction mode using single aptamer (A. Chen, Yan, and Yang 2016; R. Wang et al. 2019a). Considering the higher accuracy and sensitivity using dual recognition elements, aptamer splitting strategies came into mind. The split-aptamers not only have a similar target binding capacity to the parental aptamer, but also display less nonspecific adsorption and higher heat sensitivity. Successfully constructing split aptamers is challenging since the structure formed by combining the aptamer and the target is highly complex (A. Chen, Yan, and Yang 2016). The basic principle of splitting aptamers is to maintain the integrity of the binding areas. In the presence of target, there must be adequate complementary base pairs between the split nucleic acid chains to accommodate them. In the absence of target, the free nucleic acid strands do not bind (Sharma and Heemstra 2011). In other words, even though these sequences display certain complementarity with each other, the enthalpy gain of base pairs and base stacking is insufficient to overcome the entropic cost of assembly. After adding the target, adequate interaction between the target and the aptamer fragment generates enthalpy, inducing the thermodynamic equilibrium and pushing it toward assembly (Neves et al. 2010).

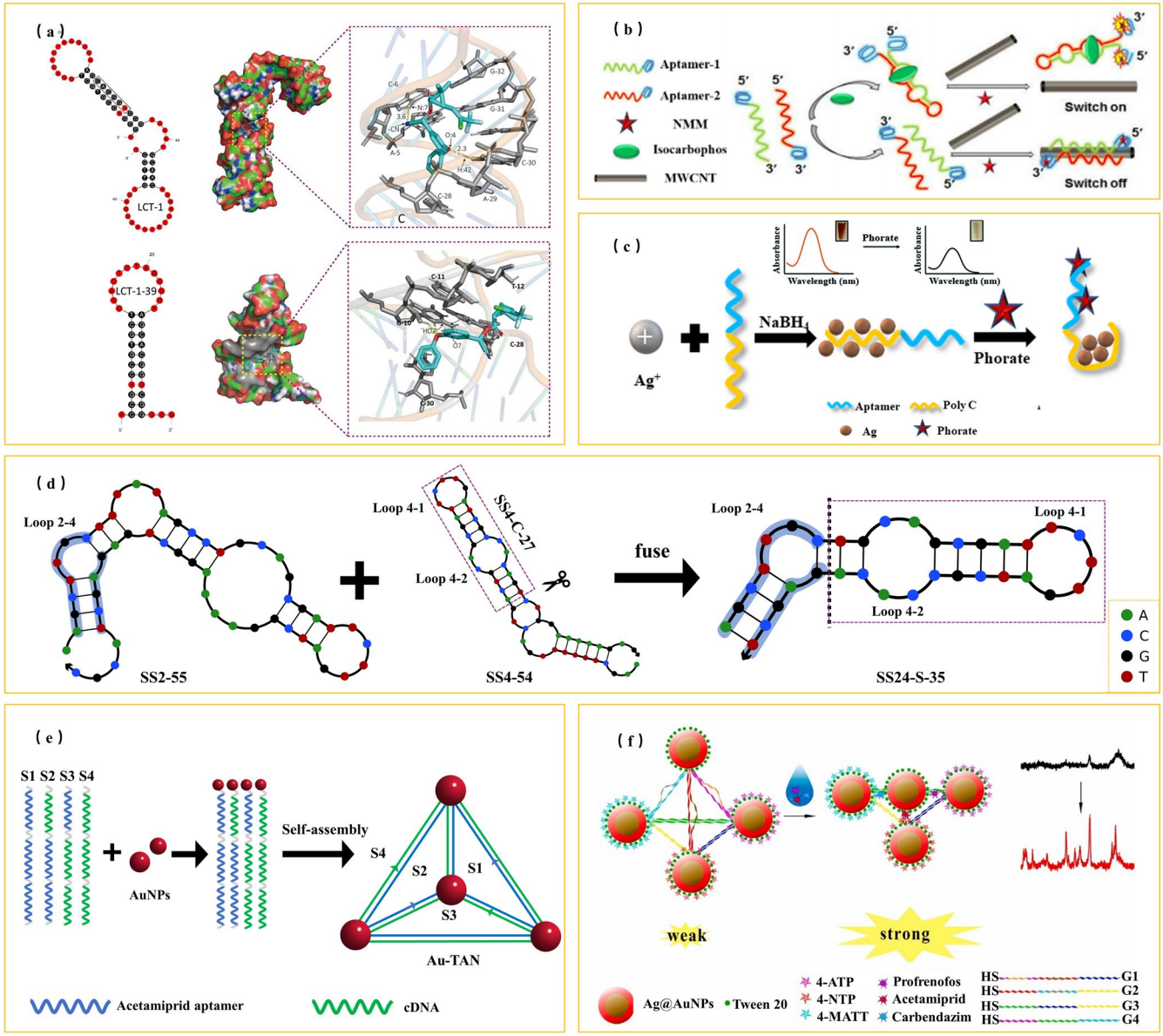

Figure 4. (a) The secondary structure and molecular docking with λ-cyhalothrin of the LCT-1 and LCT-1-39 aptamers (Y. Yang et al. 2021). (b) A schematic presentation of the label-free and enzyme-free fluorescent isocarbophos biosensor based on split aptamers (X. Li et al. 2018d). (c) A schematic diagram of phorate colorimetric detection based on elongated aptamers (X. Li et al. 2018c). (d) Schematic diagram of the formation of fusion aptamer SS24-S-35 (C. Zhang et al. 2014). (e) Schematic diagram of the construction process of Au-TAN (Y. Guo et al. 2021b). (f) A schematic diagram of the SERS sensor for detecting multiple analytes based on TAN (Lu et al. 2021).

Based on the predicted secondary structure of isocarbophos aptamer, X. Li et al. (2018d) selected the 24 G/25A site in the steam to split the aptamer into two shorter fragments. Then a fluorescent aptasensor has been developed based on split aptamers, multi-walled carbon nanotubes (MWCNTs) and G-quadruplexes, proposing a new label-free and enzyme-free method with the limit of detection (LOD) as low as 10 nM. The isocarbophos split aptamers were connected to a G-quadruplex at their respective ends. In the presence of the target substance, the split aptamers can undergo conformational changes to form a sandwich-like ternary complex, while increased steric hindrance prevents them from adsorbing to MWCNTs, significantly enhancing the fluorescence signal of the G-quadruplex probe N-methyl mesoporphyrin IX (NMM). In the absence of a target substance, the split aptamer exists in the form of single-stranded DNA, which is readily adsorbed by MWCNTs, quenching the NMM fluorescence signal (Figure 4(b)). The split aptamers enabled sandwich detection of small molecular target with high sensitivity and accuracy. And since the two independent nucleotide strands lack the secondary structure of the aptamer-target complex, false-positive or nonspecific signals can be avoided.

Elongation of aptamers

The elongation strategy artificially adds several nucleotides to the aptamer sequence, commonly at the terminal regions. X. Li et al. (2018c) added a polycytosine (poly C) sequence to the 5′-end of the phorate aptamer to develop a

colorimetric biosensor based on DNA-templated silver nanoclusters (DNA-AgNCs). As shown in Figure 4(c), the DNA-AgNCs were prepared using a poly C sequence as template and $NaBH_4$ as an Ag^+ reductant. In the absence of target, the system appears dark brown. After adding phorate, the aptamer specifically binds to the target and undergoes a structural transformation, leading to the aggregation of DNA-AgNCs, while the solution color changes from dark brown to colorless. As for thiamethoxam aptamer tailoring, Y. Luo et al. (2021) inserted an additional T-A base pair at the end of the stem-loop to stabilize the secondary structure. Then, five bases (CTCAG) were extended at the 5′ end of the aptamer to complement its quenching chain and develop the fluorescence detection method. In addition, Fu, An, et al. (2019) added a small oligonucleotide strand partially complementary to the aptamer at the 3′-end to successfully form the hairpin structure. Then the 5′- and 3′-ends of the hairpin aptamer were modified with amino groups and ferrocene respectively, to develop an electrochemical biosensor for detecting organophosphorus pesticides.

The purpose of aptamer elongation when developing pesticide biosensors is to form specific structures, generate signals, and enhance the structural stability of aptamers. Furthermore, some other functions of aptamer elongation have been reported, including recognition of target molecules and provision of spatial spacing, etc (Xiao et al. 2019).

Fusion of aptamers

Fusion of aptamers is vital in the biosensing field and plays a significant role in pesticide residue detection. This strategy can increase the number of binding sites and improve the efficiency of the detection system by connecting multiple sequences in series. The fusion of aptamers is not only the assembly or splicing of different individuals, but also may produce synergistic or other effects (Xiao et al. 2019). In 2012, two broad-spectrum aptamers of phorate, profenofos, isocarbophos, and omethoate named SS2-55 and SS4-54 were selected from an immobilized library (L. Wang et al. 2012). This team later performed a series of truncation and mutation experiments on these two aptamers to obtain the minimal functional structure for target binding. SS4-C-27 was identified as the critical functional segment of the parental aptamer SS4-54, while the loop2-4 in SS2-55 was found to be the shared binding site for the four pesticides (especially for omethoate, which showed stronger binding ability). As shown in Figure 4(d), C. Zhang et al. (2014) innovatively recombined loop 2-4 and SS4-C-27 into SS24-S-35. Compared to the parental aptamer, the fused aptamer was much shorter with only 35 nucleotides remaining, and surprisingly maintained broad specificity for all targets. Furthermore, this fused aptamer showed better affinity for profenofos and isocarbophos. As a novel recognition probe, tetrahedral aptamer nanostructure (TAN) was applied to develop ultrasensitive and selective electrogenerated chemiluminescece (ECL) aptasensors for acetamiprid (Y. Guo et al. 2021b). As shown in Figure 4(e), Six acetamiprid aptamers were fused to four DNA single strands and then self-assembled to form a tetrahedral nanostructure by pairing complementary bases. The tetrahedral spatial conformation of TAN ensures the uniform dispersion of biosensor molecules and improves the effective binding rate of the substrates to biosensors, thus developed aptasensor exhibits higher detection sensitivity (Huang et al. 2017). As can be seen from Figure 4(f), Lu et al. (2021) embedded the aptamers against profrenofos, acetamiprid, and carbendazim into the three edges of the DNA tetrahedral skeleton to generate new fused aptamer, and the tetrahedral corners were connected to modify the Ag@Au NPs with different Raman signal molecules. The specific binding between the target and aptamer modified the distance between the assembled Ag@Au nano-tetrahedron, producing a strong Raman enhancement effect. The changes of the Raman signals of the three beacon molecules corresponded to the concentrations of the three target pesticides, enabling simultaneous detection of multiple pesticides. Furthermore, Aptamer can also be fused with other types of functional nucleic acid to display diverse functions. W. Wang et al. (2020b) optimized the chlorpyrifos aptamer and lead DNAzyme and fused them using eight standard bases linkers, realizing ultrasensitive, two-in-one detection of chlorpyrifos and lead ions. The aptamer fusion strategy fulfills many functions when constructing pesticide sensing platforms, such as enhancing the affinity between aptamers and targets, increasing sensor sensitivity, and simultaneously detecting multiple targets.

Aptasensors for pesticide detection

Electrochemical aptasensor

Electrochemical analysis is widely used to detect pesticide residues due to its simplicity, miniaturization, high sensitivity and cost-efficiency. The electrochemical signal output modes can be classified into cyclic voltammetry (CV), alternating current voltammetry, electrochemical impedance spectroscopy (EIS), square wave voltammetry (SWV), differential pulse voltammetry (DPV) and photoelectrochemistry (S. Li et al. 2017; Xie et al. 2022). Nanomaterials, including graphene, single-walled carbon nanotubes (SWCNTs), MWCNTs, metal nanoparticles (MNPs), and conducting polymers (Mahmoudpour et al. 2021; W. Wang et al. 2020a), are often used in electrochemical strategies to provide biosensors with flexibility, stability, excellent conductivity, and compatibility. G. Xu et al. (2018) successfully prepared SWCNTs/Cupric oxide composites and modified the electrode, increasing its specific surface area and expanding the electron transfer rate on the electrode surface. The chlorpyrifos aptamer was immobilized on the electrode surface to achieve sensitive detection via its specific affinity with the chlorpyrifos target. This detection system can be recycled after treatment, significantly reducing detection time and cost. As shown in Figure 5(a), G. Xu et al. (2021) synthesized a zirconium-based metal-organic framework (MOF) that was covalently coupled with the complementary aptamer strand and carboxy-ferrocene to form nanocomposite probe. This probe was hybridized with the malathion aptamer on the electrode via complementary base-pairing and

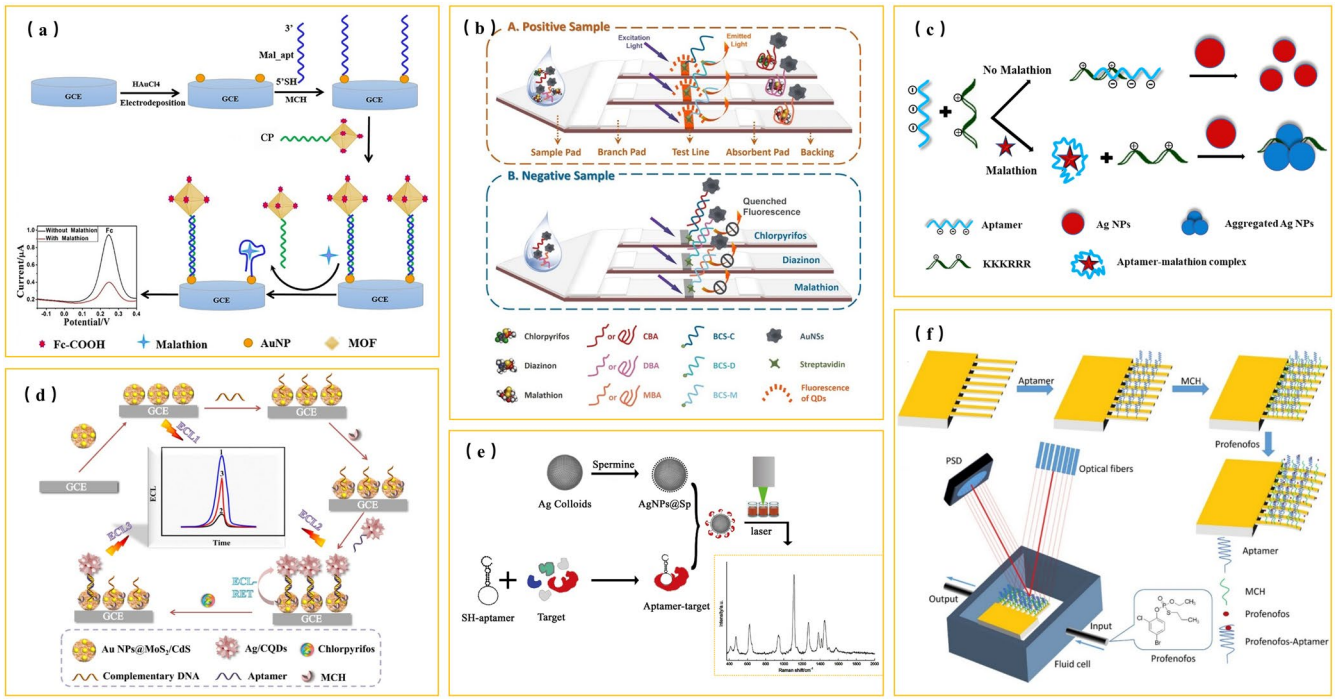

Figure 5. (a) A schematic diagram of the MOF-based electrochemical sensor used for malathion detection (G. Xu et al. 2021). (b) A design schematic diagram of the apta-LFB based on a novel fluorophore-quencher nano-pair (Cheng et al. 2018). (c) A schematic representation of the silver nanoparticle-based colorimetric aptasensor for malathion detection (Bala et al. 2018a). (d) A schematic representation of ECL aptasensor for chlorpyrifos detection based on resonance energy transfer (J. Liu, et al. 2020). (e) A schematic illustration of the label-free aptamer-based SERS sensor for malathion detection (Nie et al. 2018). (f) A schematic diagram of the aptamer-based microcantilever-array biosensor for profenofos detection (C. Li et al. 2018a).

competitively released in the presence of a target to attenuate the DPV signal. Due to the high specific surface area of the MOF, the prepared sensor showed excellent signal amplification capability with a LOD of 17.18 ng L^{-1}.

Fluorescent aptasensor

As one of the most widely used sensing platforms, fluorescent aptasensors have been applied for quantitative pesticide analysis with high sensitivity and simplicity. The fluorescence signal changes in the system have a certain relationship with the target concentration during aptamer-target binding to quantitatively detect molecules of interest. Mechanisms used to induce fluorescence changes include fluorescence resonance energy transfer (FRET) (Hu et al. 2016; X. Zhao et al. 2021b) and inner filter effect (IFE) (J. Wang et al. 2018b). Both methods require suitable fluorescent donors and acceptors. For FRET, the fluorescent donors commonly adopted various fluorescent dyes (L. Su et al. 2020), metal nanoparticles (Fan et al. 2020), QDs (Nair et al. 2021), carbon dots (CDs) (Y. Gao et al. 2022), carbon nanomaterials and UCNPs (Ouyang et al. 2021; Xie et al. 2022). As for fluorescent acceptors, nanomaterials, such as AuNPs (Hu et al. 2016; L. Su et al. 2020; J. Wang et al. 2018b), GO (Fan et al. 2020), MnO_2 (Ouyang et al. 2021; H. Wang et al. 2018a), MWCNTs (Lin et al. 2016; Talari et al. 2021), bimetallic nanoparticles (Q. Luo et al. 2018) and magnetic nanoparticles (Jiang et al. 2020) have been utilized. Lin et al. developed a FRET-based biosensor using aptamer-modified QDs and MWCNTs for quantification of acetamiprid (Lin et al. 2016). Without aminopyralid, the fluorescence of the probe was turned off by MWCNTs based on FRET between the aptamer-modified QDs and MWCNTs. After the introduction of aminopyralid, the specific binding of aptamer-aminopyralid kept the two materials away from each other, blocking the FRET effect. This sensing protocol was sensitive, selective and simple with a LOD of 0.7 nM. In addition, the fluorescence aptasensors can realize on-site and multiple detection without complex design. For example, our group developed a fluorescent aptamer-based lateral flow biosensor integrated with fluorophore-quencher nano-pairs and a smartphone spectral reader for the rapid on-site detection of chlorpyrifos, diazinon, and malathion. As shown in Figure 5(b), a novel fluorophore-quencher nano-pair (QDs and gold nanostars) was implemented for sensitive "signal on" detection. The feasibility of the platform for the on-site detection of pollutants in actual samples was further confirmed using 12 vegetable and fruit samples, in which the recoveries in the spiked vegetable samples were between 82.4% and 112.8% (Cheng et al. 2018). As for IFE, it refers to the absorption of the excitation and/or emission light of fluorophores by quenchers. Compared with FRET detection systems, IFE systems do not require modification of the quenchers and fluorophores to provide a specific distance or geometry for intermolecular interaction, which would facilitate the construction of flexible and simple fluorescent biosensors. J. Guo et al. (2016) reported a fluorescent aptasensor for acetamiprid detection based on the IFE of AuNPs on the fluorescent CdTe QDs, with a low

detection limit of 7.29 nM. Free acetamiprid-binding aptamer can wrap the surfaces of AuNPs to protect AuNPs from salt-induced aggregation, so the fluorescence of CdTe QDs would be quenched through the IFE of AuNPs effectively. Upon adding acetamiprid, the specific binding of aptamer sequences with target could release from the aptamers from the AuNPs surface. Therefore, the AuNPs tend to aggregate in the presence of salt and their absorption spectrum no longer overlaps with the fluorescence emission spectrum of the QDs, which leads to obvious fluorescence recovery of the quenched QDs.

When developing fluorescence biosensors, the fluorescence interference caused by the sample matrix can affect the accuracy of sensing. Two solutions can be used to solve these problems. The first approach is to use a fluorescent donor capable of distinguishing background fluorescence, such as red fluorescence. Second, ratiometric fluorescent methods can eliminate false signals emanating from environmental effects and improve sensitivity and accuracy (Tang et al. 2016; Yan et al. 2015).

Colorimetric aptasensor

Colorimetric method based on aptamer for pesticide detection is cost-efficient with a rapid response time. It is also possible to observe the results with the naked eye without the need for expensive analytical instruments. AuNPs, AgNPs, and nanozymes are often used to construct colorimetric aptasensors (Abnous et al. 2018; Bai et al. 2015; Bala et al. 2017; Bala et al. 2016; D. Liu, et al. 2020). Shi et al. (2013) reported a colorimetric detection method for acetamiprid based on the aggregation of AuNPs. The aptamer with a random coil structure was absorbed on the AuNPs surfaces by coordination bonds, increasing the AuNPs stability in a salt medium. Aptamers bound selectively to acetamiprid to produce aptamer-target complexes. Unprotected AuNPs aggregated in the presence of the proper quantity of salt, resulting in a color shift from red to blue. C. Wang et al. (2016) achieved the quantitative detection of acetamiprid based on the principle that AuNPs aggregation increased the intensity of resonance light scattering signals, while the LOD of this aptasensor was as low as 1.2 nM. When using this strategy, the interaction of the target with AuNPs needs to be avoided, otherwise false results may be obtained (Zong and Liu 2019).

Similar to AuNPs, AgNPs can also cause solution color changes and display a higher extinction coefficient at the same particle size. Therefore, they can be applied for the colorimetric detection of pesticides, too. As demonstrated in Figure 5(c), Bala et al. (2018a) established a highly sensitive, rapid, cost-efficient colorimetric detection method using the cationic hexapeptide KKKRRR and AgNPs. The AgNPs particles remained yellow in the absence of malathion due to the electrostatic binding between aptamer and hexapeptide. In the presence of malathion, the strong affinity of the aptamer toward malathion renders the peptides free, which then causes the particles to aggregate and turns them orange. This method displayed good selectivity with a LOD as low as 0.5 pM and was successfully applied to detect malathion in various water samples and apples.

Nanozymes with peroxidase mimetic activity has also been applied to biosensors to improve analytical performance (Weerathunge et al. 2019; Weerathunge et al. 2014; Z. Yang et al. 2015). Nanozymes can catalyze the oxidation and color development of substrates, such as 3,3′,5,5′-tetramethylbenzidine, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), and o-phenylenediamine to produce blue, green, and yellow oxidation products, respectively. Aptamers can regulate the catalytic performance of nanozymes to visually detect pesticide residues. X. Zhang et al. (2022) used an alkali-resisting Co-based metal-organic framework (Co-MOF) as a biomimetic nanoenzyme to convert acetamiprid content into glucose attenuation in seconds with a personal glucometer. The method is simple and provides a practical tool for the on-site monitoring of pesticide residues.

Chemiluminescent aptasensor

Chemiluminescence (CL) is a luminescence phenomenon based on multistep oxidation reaction. It has been an important analytical technique because of its high signal-to-noise ratio (almost no background signal), simple operation and high sensitivity (Ouyang et al. 2018; M. Zhao et al. 2021a). The Luminol-H_2O_2 system is the most commonly used substrate in CL detection. As demonstrated in Figure 5(d), Qi et al. (2016) developed a CL aptasensor for ultrasensitive and selective acetamiprid detection using the specific binding ability of aptamer to target and the catalytic effect of AuNPs to stimulate CL signal generation in the presence of H_2O_2 and luminol. The morphological transition (from dispersed to aggregated) of AuNPs caused by the acetamiprid-induced conformational shift of aptamers led to distinct CL signal changes. The proposed aptasensor was highly sensitive to acetamiprid, with a LOD of 62 pM, which was about 100-fold lower than that of other aptasensors for acetamiprid detection. Xiu et al. (2021) constructed a CL sensing platform for acetamiprid detection based on the synergistic co-catalysis of GO/AuNPs nanocomposites for the luminol CL reaction with a LOD of 8.9 pM. The aptamer can inhibit the co-catalysis of GO/AuNPs for the luminol CL reaction. The introduction of acetamiprid altered the spatial conformation of aptamer, restoring the synergistically catalytic amplification signal of GO/AuNPs. A signal amplification strategy can further improve detection sensitivity. Exonuclease-assisted dual signal amplification and G-quadruplex/hemin DNAzyme allowed the label-free, sensitive detection of malathion with a LOD of 0.47 pM (Wu et al. 2021).

Moreover, ECL sensors provide a promising analytical approach since they combine the advantages of electrochemistry and CL. Y. Guo et al. (2021b) developed an ultrasensitive ECL sensor with Au-TAN to detect acetamiprid in vegetables. Under electric excitation, luminol was oxidized to luminol radical. Then the free radical reacts with H_2O_2 to generate 3-aminophthalateion in the excited state. The excited state then released energy, generating 3-aminophthalate

ion and emitting light at 425 nm. AuNPs of the Au-TAN could catalyze H_2O_2 to generate intermediate reactive substance for signal amplification, leading to the enhanced luminescence performance of this aptasensor. In order to further improve the detection sensitivity, a "turn on" aptasensor was fabricated for chlorpyrifos detection by a highly efficient ECL resonance energy transfer between MoS_2/CdS nanospheres as a donor and silver/carbon quantum dots (Ag/CQDs) as acceptor (J. Liu, et al. 2020). As demonstrated in Figure 5(d), the complementary DNA of the chlorpyrifos aptamer was modified on AuNPs@MoS_2/CdS/GCE, then combined with Ag/CQDs-aptamer through base-complement pairing. Efficient energy resonance transfer between MoS_2/CdS and Ag/CQDs decreased the ECL intensity. The combination of chlorpyrifos and aptamer could destroy the double helix structure, resulting in the departure of Ag/CQDs-aptamer from the electrode surface. The ECL intensity of MoS_2/CDs was also recovered.

Surface-enhanced raman scattering-based aptasensor

Surface-enhanced Raman scattering (SERS) has emerged as a powerful technology for pesticide detection in foods (Y. Guo et al. 2021a). SERS uses the surface-sensitive resonance extension of standard Raman spectroscopy to detect trace molecules of interest when excited in the vicinity of or on rough metal substrates (Vendrell et al. 2013). Compared with ordinary Raman scattering, the SERS signal can be increased 10^4-10^8 fold (Bantz et al. 2011). The SERS-based aptasensors present advantages, such as ultrafast analysis, minimum photobleaching, high stability and nondestructive characterization (Bernat et al. 2019; Cialla-May et al. 2017). Pang, Labuza, and He (2014) established a SERS method capable of simply and rapidly detecting multiple organophosphorus pesticides. The thiolated aptamer was conjugated onto silver dendrites through Ag-thiol bonds, enhancing the Raman fingerprints of pesticides. The constructed SERS platform was mixed with each pesticide solution for 20 min and analyzed using DXR Raman microscope and TQ analysis software. The results confirmed the superior capability of the aptamer-based SERS method for rapidly detecting and discriminating multiple pesticides. Figure 5(e) shows a label-free aptamer-based SERS sensor, Ag colloids were modified with positive spermine to form highly stable suspension. The aptamer captured the malathion specifically and formed aptamer-malathion complex. The complex was adsorbed on the surface of Ag colloid by electrostatic interaction between negative phosphate backbone of aptamer and the positive spermine. SERS signal determination was then performed to establish the relationship between the characteristic peak intensity and malathion concentration (Nie et al. 2018).

Other aptasensors

In recent years, many other innovative methods have been used to develop pesticide aptasensors, such as microcantilever array, CE and microfluidic chip methods. As shown in Figure 5(f), C. Li et al. (2018a) combined the 5′-end of the profenofos aptamer with the gold surface of the microcantilever through Au-S bond to construct an aptamer-based microcantilever array sensor. When profenofos was examined, the structure of aptamer switched and the contact space with the surface of the microcantilever increased, so that the microcantilever was deflected. The average deflection value was positively correlated with the profenofos concentration. This sensor presents advantages, such as label-free, highly sensitive, one-step immobilization, and real-time detection. CE shows considerable promise for pesticide analysis due to its high separation efficiency, minimal reagent consumption, and rapid analytical speed (Tang et al. 2016). By combining the advantages of CE method, specific binding ability of aptamer and the accuracy of ratio detection, the developed aptasensor can significantly improve sensitivity and reduce detection time while providing a new strategy for trace pesticide detection. Microfluidic chips provide a more advanced and convenient technique for pesticide residue detection. Compared with traditional pesticide detection techniques, microfluidic chips present many advantages, such as portability, cost-efficiency, rapidity, ease of multiplexing and integration, and multiple and parallel sample detection (Kecskemeti and Gaspar 2018; Liao et al. 2018). For example, a pesticide vapor sensor was developed using an agarose gel-based chip containing a nanopore sensing system. In the presence of the target, the evaporated omethoate was absorbed into the gel. The aptamer and omethoate specifically bind to form a bulky complex, blocking the nanopores and providing a long-term blocking current signal. The constructed aptasensor displayed high specificity and sensitivity to omethoate with a LOD of 100 ppb as vapor and a detection time of about 60 s (Fujii et al. 2017).

Conclusions and future perspectives

To ensure food quality and safety, provide environmental protection, and protect human health, it is necessary to establish highly sensitive biosensing methods for monitoring pesticides residues. Accelerated aptamer advancement over the past decade have prompted the development of various aptasensors for the rapid, selective, sensitive, and on-site detection of pesticide residues. In order to further improve the performance of aptasensors, several strategies have been developed regarding advanced aptamer screening and precise aptamer tailoring. This paper reviews six types of advanced aptamer screening strategies for pesticides molecules, showing the ability to improve the binding affinity, increase the screening success rate, and reduce operational time. In addition, four kinds of tailoring strategies, covering deletion, splitting, elongation, and fusion have been systematically discussed with the advantages of lowering the cost, improving the binding affinity, and generating new functional structures. Finally, the current research progress in developing aptasensors for pesticides residues detection is comprehensively introduced Table 2. The following points may provide prospective areas for future research:

Table 2. Aptasensors for the detection of pesticide residues (in the last five years).

Assay type	Target	Real sample	Principle	Linear range	LOD	Reference
Electrochemical	Acetamiprid	Real water	EIS	10 pM-100 nM	1 pM	(Madianos et al. 2018)
Electrochemical	Atrazine	Real water	EIS	100 pM-1 μM	10 pM	(Madianos et al. 2018)
Electrochemical	Acetamiprid	Tea	SWV	0.1 pM-0.1 μM	71.2 fM	(Yi et al. 2020)
Electrochemical	Chlorpyrifos	Apple and celery cabbage	DPV	0.1-150 ng/mL	70 pg/mL	(Xu et al. 2018)
Electrochemical	Malathion	Cucumber and long bean	DPV	25–850 ng/L	17.18 ng/L	(Xu et al. 2021)
Electrochemical	Profenofos	Water	DPV	0.01-100 nM	0.003 nM	(Fu, et al. 2019)
	Phorate	Rape and spinach		1-1000 nM	0.3 nM	
	Omethoate	Water		0.1-1000 nM	0.3 nM	
	Isocarbophos	Water		1-500 nM	0.03 nM	
Electrochemical	Carbofuran	Vegetables and fruits	DPV	0.2-50 nM	67 pM	(S. Li et al. 2018b)
Electrochemical	Chlorpyrifos	Vegetables and fruits	DPV	0.1 nM-0.5 μM	0.178 nM	(Wang et al. 2020b)
Fluorescent	Acetamiprid	Tap water	FRET	5-50 nM	2.8 nM	(Bahreyni et al. 2018)
Fluorescent	Diazinon	Tea and apples	FRET	0.05-500 ng/mL	0.023 ng/mL	(Rong et al. 2020)
Fluorescent	Chlorpyrifos	Vegetables and fruits	FRET	100 pg/mL-10 μg/mL	0.73 ng/mL	(Cheng et al. 2018)
	Diazinon				6.7 ng/mL	
	Malathion				0.74 ng/mL	
Ratiometric fluorescent	Phorate	Fruits	FRET	0.6-10 μM	0.20 μM	(Tang et al. 2016)
	Profenofos			0.3-10 μM	0.10 μM	
	Isocarbophos			0.5-10 μM	0.17 μM	
	Omethoate			0.7-10 μM	0.23 μM	
Fluorescent	Malathion	Water, soil and orange juice	FRET	10 pM to 1 μM	4 pM	(Bala et al. 2018b)
Fluorescent	Acetamiprid	Apple and ginger	CuNPs as fluorescent signal sources	5-500 nM	2.37 nM	(Fan et al. 2019)
Fluorescent	Acetamiprid	Vegetable	IFE	5-100 μg/L	1.08 μg/L	(J. Wang et al. 2018b)
Fluorescent	Acetamiprid	Celery leaves and chinese green tea	IFE	0.025-1 μM	0.36 nM	(Yang et al. 2019)
Colorimetric	Acetamiprid	Agricultural soil, tap/river/agricultural runoff water, and apples matrices	Nanozymes	1-200 nM	0.42 nM	(Zhang et al. 2022)
Colorimetric	Malathion	Tap water, lake water and apples	AgNPs aggregation	0.01-0.75 nM	0.5 pM	(Bala et al. 2018a)
Colorimetric	Chlorpyrifos	River water	Ag-NanoZyme	35-210 ppm	11.3 ppm	(Weerathunge et al. 2019)
Colorimetric	Isocarbophos	Vegetables	AuNPs aggregation	50-500 μg/L	7.1 μg/L	(Wang et al. 2019a)
Colorimetric	Acetamiprid	Soil, wastewater and cucumber	AuNPs aggregation	8.7-920nM	0.56 nM	(Qi et al. 2020)
Colorimetric	Malathion	Human serum	AuNPs aggregation	5 pM-10 nM	1 pM	(Abnous et al. 2018)
Chemiluminescent	Acetamiprid	Wastewater, soil, cucumber and apple	GO/AuNPs' co-catalysis	0.21 pM-9 nM	8.9 pM	(Xiu et al. 2021)
Chemiluminescent	Malathion	Rice, wheat, *astragalus, codonopsis* and *angelica*	G-quadruplex/hemin DNAzyme	1 pM-100 nM	0.47 pM	(Wu et al. 2021)
ECL	Chlorpyrifos	Vegetables and fruits	resonance energy transfer between MoS_2/CdS nanospheres and Ag/CQDs	1 fM-10 nM	0.35 fM	(Liu, et al. 2020)
ECL	Acetamiprid	Lettuce, spinach and baby cabbage	Au-TAN was used in luminescent systems of luminol and H_2O_2	0.1 pM-10 nM	0.0576 pM	(Y. Guo et al. 2021b)
SERS	Malathion	Tap water	AgNPs@spermine as substrate	0.5-10 μM	0.5 μM	(Nie et al. 2018)
SERS	Acetamiprid	Apple juice	AgNPs@Si as substrate	25-250 nM	6.8 nM	(Sun et al. 2019)
SERS	Acetamiprid	Cabbage	Ag-PCR-sealing membranes	1 nM-10 μM	10 nM	(Zhou et al. 2022)
SERS	Profenofos	River water, soil, rice and apple	Ag@Au NPs-TAN as substrate	–	0.0021 ng/mL	(Lu et al. 2021)
	Acetamiprid				0.0046 ng/mL	
	Carbendazim				0.0061 ng/mL	
Microcantilever-array	Profenofos	Chinese chive	the deflection of microcantilever array	5-1000 ng/mL	1.3 ng/mL	(Li et al. 2018a)
Microarrays	Phoxim	Pear and radish	aptamer microarray fluorescent method based on thioflavin T	76.2-1000 ng/mL	25.4 ng/mL	(Wang, et al. 2022)
	Parathion			36-1000 ng/mL	12.0 ng/mL	
	Fensulfothion			23.1-1000 ng/mL	7.7 ng/mL	
	Isocarbophos			29.7-1000 ng/mL	9.9 ng/mL	
Microfluidic chip	Omethoate	Vapor	agarose gel-based chip containing a nanopore	–	4.8 nM	(Fujii et al. 2017)

1. Improving aptamer screening efficiency enhances the driving force for developing aptasensors. Future research can explore more efficient separation methods, simple real-time monitoring techniques, and automated selection equipment to reduce the workload and screening time.
2. The SELEX method based on doped library can rapidly obtain aptamers with superior binding properties. The doped library is constructed by randomizing partial sequences of parental aptamers obtained from previous SELEX analysis (Canoura et al. 2021). Specifically, a certain proportion of mutations are introduced into the parental aptamer to form the doped library, after which the doped-SELEX is performed following conventional selection procedures. This approach greatly reduces screening time and enhances aptamer-target affinity.
3. High-throughput detection represents the mainstream trend of pesticides analysis. The selection of broad-spectrum aptamers lays a foundation for multiple pesticide detection. Common structures of a series of pesticides can be selected as target (hapten) for broad-spectrum aptamer screening, based on the generation method of pesticide broad-spectrum antibody.
4. Molecular simulation technology can be introduced to comprehensively analyze the aptamer-target binding mechanism. So far, most of the analysis of the binding mechanisms of pesticides and aptamers still stays at the secondary aptamer structures. Future studies may consider combining techniques, such as molecular docking and molecular dynamics simulation, to design a series of mutating, deleting, and splicing experiments to probe the active sites of aptamers, which can guide the rational design and practical application of aptamers. Therefore, aptamers can be precisely tailored to further refine the structure and design different motifs according to the application requirements.
5. The development of a facile and portable, affordable, and real-time sensing platform for pesticide detection is a future development trend. Researchers may focus on developing on-site screening platforms for non-laboratory conditions by utilizing 3D printing technology or combining with smartphones. Furthermore, novel multiplex detection modes and matched devices need to be developed to improve the multi-analysis capability of aptasensors.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The numbers of these funds here have some errors and should be revised as: National Natural Science Foundation of China (No. 31671922 and No. 31871875); National Key Research and Development Program (No. 2018YFC1603704); Hebei Province key research and development program (21372801 D); 2115 Talent Development Program of China Agricultural University (No. 00109016).

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