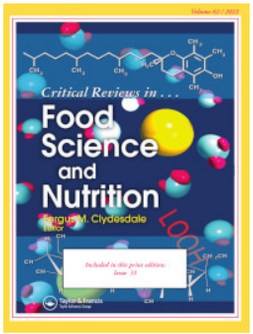

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Critical Reviews in Food Science and Nutrition

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

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To cite this article: Mingqi Zhao, Miao Wang, Xiaoguang Zhang, Yongan Zhu, Jing Cao, Yongxin She, Zhen Cao, Guangyue Li, Jing Wang & A. M. Abd El-Aty (2021): Recognition elements based on the molecular biological techniques for detecting pesticides in food: A review, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2021.2009762

To link to this article: https://doi.org/10.1080/10408398.2021.2009762

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REVIEW

Recognition elements based on the molecular biological techniques for detecting pesticides in food: A review

Mingqi Zhao[a,b*], Miao Wang[a,b*], Xiaoguang Zhang[c,d], Yongan Zhu[a,b], Jing Cao[a,b], Yongxin She[a,b], Zhen Cao[a,b], Guangyue Li[e], Jing Wang[a,b] and A. M. Abd El-Aty[f,g]

[a]Institute of Quality Standardization & Testing Technology for Agro-Products, Chinese Academy of Agricultural Sciences, Beijing, China; [b]Key Laboratory of Agrofood Safety and Quality (Beijing), Ministry of Agriculture and Rural Areas, Beijing, China; [c]Hebei Xiangzhi Testing Technology Co., Ltd, Shijiazhuang, China; [d]Core Facilities and Centers of Hebei Medical University, Shijiazhuang, China; [e]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China; [f]Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt; [g]Department of Medical Pharmacology, Medical Faculty, Ataturk University, Erzurum, Turkey

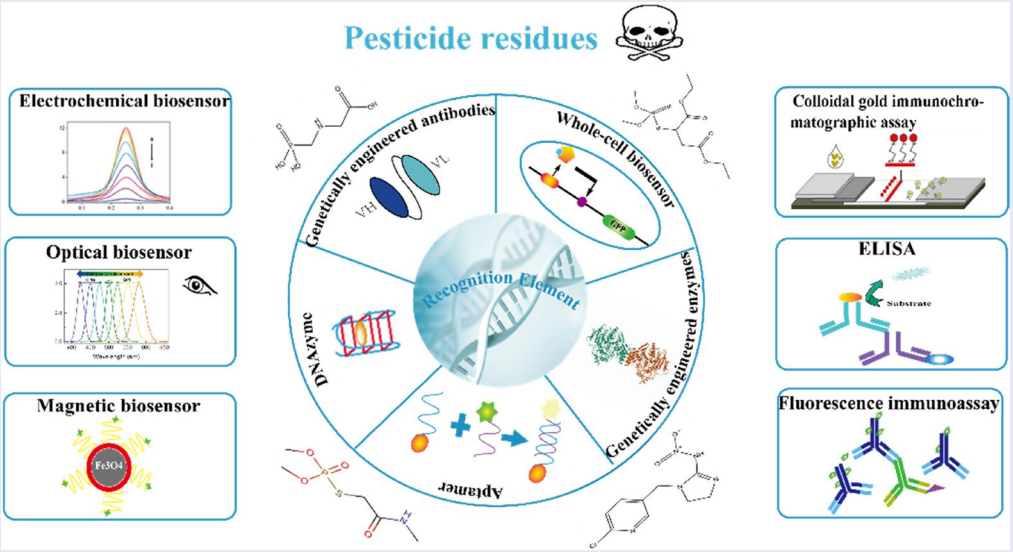
ABSTRACT

Excessive use of pesticides can cause contamination of the environment and agricultural products that are directly threatening human life and health. Therefore, in the process of food safety supervision, it is crucial to conduct sensitive and rapid detection of pesticide residues. The recognition element is the vital component of sensors and methods for fast testing pesticide residues in food. Improper recognition elements may lead to defects of testing methods, such as poor stability, low sensitivity, high economic costs, and waste of time. We can use the molecular biological technique to address these challenges as a good strategy for recognition element production and modification. Herein, we review the molecular biological methods of five specific recognition elements, including aptamers, genetic engineering antibodies, DNAzymes, genetically engineered enzymes, and whole-cell-based biosensors. In addition, the application of these identification elements combined with biosensor and immunoassay methods in actual detection was also discussed. The purpose of this review was to provide a valuable reference for further development of rapid detection methods for pesticide residues.

KEYWORDS

Molecular biology techniques; recognition elements; biosensor; immunoassay technology

GRAPHICAL ABSTRACT



Recognition elements based on the molecular biological technique and their application in pesticide detection.

CONTACT Miao Wang wm0510@126.com; Guangyue Li liguangyue@caas.cn; Jing Wang w_jing2001@126.com
*The first two authors are equally contributed to this study.

1. Introduction

Nowadays, the use of pesticides is expected to continue with population growth and accelerating urbanization. In modern agriculture, pesticides are essential for food production. They reduce diseases and insect pests, and make outstanding contributions to ensuring the output of farm products. Although pesticides are developed to function with reasonable certainty and minimal impact on human health and the environment, serious concerns have been raised regarding residues that would exceed the standard, affecting the quality and safety of agricultural products. At the same time, they might pose potential risks to the environment and human health (Van et al. 2018). Some human chronic diseases, such as cardiovascular, cerebrovascular, and nervous system diseases, are closely related to occupational exposure to pesticides (Govindasamy et al. 2018). In addition, leaching pesticides can move with the infiltrating water through the soil, immobilized through sorption onto organic matter and clay minerals, possibly causing adverse impacts on soil. The migration to the ground or surface water causes water pollution and eventually threatens human and animal health and ecosystems (Lozowicka et al. 2014).

With the widespread use of synthetic organic pesticides, the potention risks caused by their slow degradation, accumulation, toxicity, and residual hazards to the environment and organisms have gradually attracted people's attention. In this context, various conventional methods have been developed for detecting pesticide residues. These methods, such as liquid chromatography, gas chromatography, liquid chromatography-mass spectrum (LC-MS), and gas chromatography-mass spectrometry (GC-MS) (Kang et al. 2019; Weng et al. 2020; Ma et al. 2020; Caldas et al. 2016; Chen, Shi, et al. 2018; Golge 2021; Yamaki et al. 2021; Feng et al. 2021; Okubo and Yagi 2021), have high sensitivity, good selectivity, and accuracy. However, these methods suffered from the following shortcomings: high instrument costs, time-consuming detection procedures, and the need for skilled analytical technicians. Moreover, they hindered the real-time and on-site detection of pesticide residues.

Therefore, when multiple samples are involved and on-site inspection is required, the applications of some new sensitive and fast on-site inspection techniques are advantageous. Classical, rapid detection techniques with the characteristics of simplicity, such as enzyme inhibition and enzyme-linked immunoassay, have been extensively used in monitoring. However, there are still many challenges to be solved in recognition elements of these detection methods. For instance, the antibody has disadvantages of a complex preparation process, poor stability, and substantial differences between batches. There are also many problems with natural enzymes, such as complex expression and purification process, poor heat stability, differences among species. To improve their stability, detection sensitivity, and accuracy, we need to reform these recognition elements.

At present, with the gradual deepening of research in molecular biology, the molecular biology technique begins to be unlocked in various fields, such as medicine and the cosmetics industry. At the same time, it is also a necessary means to solve challenges existing in applying those recognition elements (Table 1). For example, we can modify the enzyme gene to improve catalytic activity, stability, and specificity in rapidly detecting pesticide residues. In addition, the antibody gene can be fused with a tag gene and expressed in microbial cells, or its gene can be redesigned to achieve a platform for the massive production of antibodies to function in different ways.

In this review, eligible studies were identified through an electronic search of Web of Science (1990–2020) (http://www.webofscience.com/wos/alldb/basic-search) and PubMed database (http://www.ncpi.nlm.nih.gov/pubmed). We used the primary search term "recognition elements" combined with terms "aptamers," "single-chain antibody," "single-chain antigen-binding fragment," "Nanobody," "antibody fusion protein," "genetically engineered enzyme," "whole-cell biosensor" "DNAzyme" and "pesticide detection" to find the relevant literature. We screened the titles, keywords, and abstracts of publications obtained from the database. If deemed appropriate, full-text articles were obtained for objective assessment.

Some reports have summarized the application and new trends of different recognition elements in the rapid detection of pesticide residues have been reviewed elsewhere. For instance, Seitz et al. focused on some molecular recognition elements, including Aptmers, MIPs, and antibodies. The figures of merit for equilibrium binding and strategies for preparing molecular recognition elements were also mentioned (Seitz et al. 2021). Another article dealt with new trends in biosensors for detecting pesticides using different recognition elements (Majdinasab, Daneshi, and Marty 2021). Here, we review studies focusing on molecular biology techniques, such as genetic engineering and DNA cloning, rather than chemical synthesis. Hence, the preparation of specific recognition elements, such as aptamer, genetic engineering antibodies, whole-cell biosensor, genetically engineered enzyme and DNAzyme, was reviewed. Some strategies using these recognition elements for pesticide sensing, including fluorescence biosensor, colorimetric biosensor, electrochemical sensor, magnetic biosensor, microcantilever-array sensor, SERS sensors, as well as immunoassay strategies, such as colloidal gold immunochromatography, enzyme-linked immunosorbent assay, and fluorescence immunoassay techniques were also addressed. We aim to provide new ideas for improving the sensitivity and stability of pesticide residues' rapid detection.

2. Preparation of recognition elements using molecular biology techniques

2.1. Nucleic acid aptamers

For a long time, DNA has been accepted as the carrier of genetic information. However, DNA and its analogs have made breakthroughs in many fields due to their unique structural characteristics. At the same time, aptamers, a type of single-stranded oligonucleotides, are the chemical equivalents of antibodies because of their high

Table 1. Recognition elements and their features.

Recognition elements		Features	Issues to be resolved
Aptamers		Good stability, easy preparation, and low cost	Poor stability, low yield, and complicated preparation procedure of antibodies and enzymes
Genetic engineering antibodies	VHH	High specificity, good stability, high yield, and tolerance to organic solvents	The process of antibody preparation is complex, time-consuming, and the difference between batches is significant.
	ScFV	scFv can be produced in batches and fused with a marker molecule for effective immunological detection	
	scFab	High expression efficiency, good stability, and strong affinity	
	Bispecific antibodies	High specificity, robust targeting, and simultaneous detection of two different substances	
	Antibody fusion protein	Fusion with other proteins or peptides and give antibodies specific functions	
Genetically engineered enzymes		Good catalytic activity, stability, and pesticide sensitivity	Enzymatic inactivation during immobilization, poor thermal stability, and poor repeatability due to different enzyme sources
DNAzyme		Well programmed and high stability	
Whole-cell biosensors	Regulation of transcription factors	The signal of the target concentration can be converted to an indication of the expression state of the target gene	Separation and purification of the enzyme is a complex and tedious process
	Preparation of reporting domain	Improve the sensitivity of detection by transforming genes in reporting sites	
	Display of cell surface	Display the target protein on the cell surface	

Table 2. The sequences of commonly utilized aptamers for pesticide detection.

Target	Sequence(5′→3′)	Reference
Isocarbophos	GACGTCGCTAAGAACTAGCCCACAGAGCT	(Xiang et al. 2019)
Acetamiprid	TGTAATTTGTCTGCAGCGGTTCTTGATCGCT GACACCATATTATGAAGA	(Su et al. 2021)
Carbaryl	ATTGGCACTCCACGCATAGGGTTATGTTAATAAGAT GAACCCACGTTCCAGATGGCATCGCCTATGCGTGCTACCGTGAA	(Liu, Yang, et al. 2021)
Alathion	ATCCGTCACACCTGCTCTTATACACAATTGTTTTTCTCTTAACTTCTTGACTGCTG GTGTTGGCTCCCGTAT	(Nguyen and Jang 2021)
Iprobenfos, Edifenphos	CGTACGGAATTCGCTAGCTAAGGGATTCCTGTAGAAGGAGCAGTCTGGATCC GAGCTCCACGTG	(Kwon et al. 2015)
Profenofos	AAG CTTGCT TTA TAG CCT GCA GCG ATT CTT GAT CGG AAA AGG CTG AGAGCT ACG C	(Li, Zhang, et al. 2018)
Dipterex, Glyphosate, Malathion, Phorate, Profenofos, Isocarphos, and Omethoate	AGCTTGCTGCAGCGATTCTTGATCGCCACAGAGCT	(Jiang et al. 2020)
Chlorpyrifos	CCTGCCACGCTCCGCAAGCTTAGGGTTACG CCTGCAGCGATTCTTGATCGCGCTGCTGGTAATCCTTCTTTAA GCTTGGCACCCGCATCGT	(Xu, Huo, et al. 2018)
Atrazine	TACTGTTTGCACTGGCGGATTTAGCCAGTCAGTG	(Madianos et al. 2018)
Phorate, Profenofos, Isocarbophos, and Omethoate	CTGCACAAGAATCGCTGCAG	(Zhang et al. 2014)
Imidacloprid	AGGAATTCAGATCTCCCTGCAGCTCGAGGAGCTCAGGATCCCG	(Bor et al. 2020)

affinity and selectivity (Stella and Marcel 2015; Tolle and Mayer 2012). A nucleic acid aptamer is a single-stranded oligonucleotide (DNA or RNA) screened in vitro, forming a specific tertiary structure through folding. It binds to the corresponding target with high affinity and/or high specificity (Ellington and Szostak 1990; Zhang, Lai, et al. 2019). Aptamers were used to identify a variety of targets, such as proteins (Park et al. 2012), metal ions (Zeng et al. 2017; Oroval et al. 2017), viruses (Labib et al. 2012), and small-molecule substances (Kim et al. 2011). Its emergence provides a new high-efficiency and rapid identification research platform for food safety, environmental testing, biomedicine, and other aspects. Compared with antibodies, it shows a good application prospect. First, the affinity of aptamers can reach the nanomolar and micromolar levels. Secondly, the aptamers are stable at room temperature and easy to transport and store in terms of stability. It is easy to be prepared without affecting its affinity with the target. Finally, in terms of synthesis, it is relatively easy and cheap. It takes 2–8 weeks of in vitro synthesis to obtain the appropriate aptamer (Chen and Yang 2015; Nan et al. 2018). Table 2 lists some commonly used aptamer sequences in pesticide residue detection.

2.1.1. The systematic evolution of ligands by exponential enrichment (SELEX)

The generation of aptamers was based on a selection method called the systematic evolution of ligands by exponential enrichment (SELEX). SELEX is a standard aptamer generation method, which includes five steps: library construction, incubation and binding, separation and elution, amplification, and single-strand preparation (Mansouri et al. 2020; Gu et al. 2018; Xing, Zhang, and Yang 2019; Kong et al. 2021). After several rounds of screening, sequences with high affinity in the library gradually become dominant.

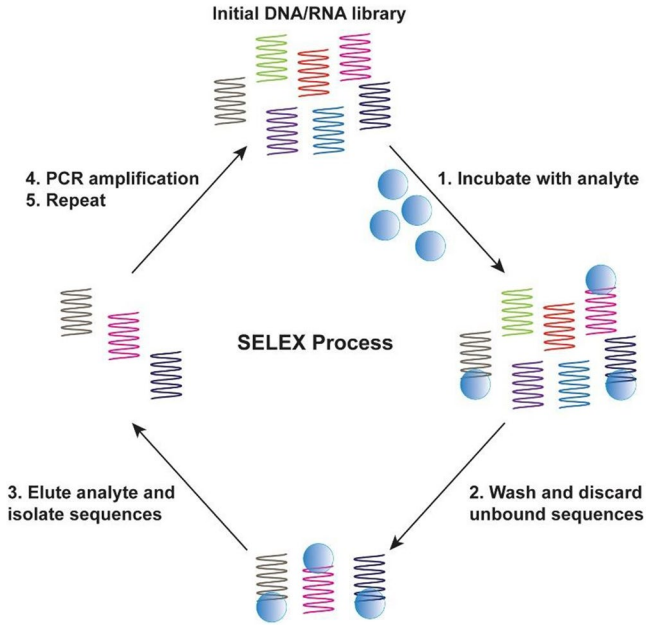


Figure 1. The systematic evolution of ligands by the exponential enrichment (SELEX) method (Crivianu-Gaita and Thompson 2016).

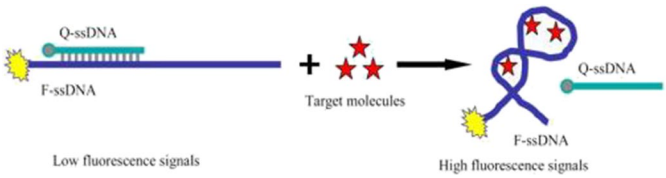


Figure 2. Schematic diagram of aptamer binding to isocarbophos (Wang et al. 2016).

Finally, aptamers with higher affinity and specificity can be obtained (Zhang, Lai, et al. 2019; Tuerk and Gold 1990). The process of SELEX is shown in Figure 1 (Crivianu-Gaita and Thompson 2016).

2.1.2. Immobilization of aptamers

In practical applications, nucleic acid aptamers are usually immobilized on the surface of different materials through a linker prepared at the 3' or 5' end (Balamurugan et al. 2008). Moreover, for different aptamers, the optimal position of the linker is different (Cho et al. 2006; Crivianu-Gaita and Thompson 2016). Standard immobilization methods mainly, include covalent bonding and non-covalent bonding. Covalent bonding, including the use of disulfide bonds and amide bonds, connects the aptamer to the material's surface. While non-covalent bonding commonly uses the affinity between biotin and streptavidin or the force of electrostatic adsorption.

The electrochemical biosensor based on aptamer uses the conformational change after the aptamer binds to the target, thereby changing the electron transfer efficiency between the redox molecules on the electrode surface and the reporter part (Schoukroun-Barnes, Glaser, and White 2015). In electrochemical biosensors, it is usually necessary to consider reference, working, and auxiliary electrodes. These electrodes should have properties such as chemical stability and conductivity. Therefore, graphite, gold, platinum, and silicide can be used as promising materials for electrodes (Grieshaber et al. 2008). In addition, to give the working electrode better performance, we can use some nanomaterials to modify the surface, aiming to obtain high conductivity, high surface-to-volume ratio, and easy chemical preparation. Ultimately, these can endow the sensor with higher sensitivity and lower pesticide detection limits (Khan 2016; Sharma et al. 2018)

To construct optical biosensing platforms using aptamers, different types of materials, such as fluorescent dyes, metal nanomaterials, carbon nanomaterials, semiconductor materials, and rare earth materials (Guo, Wang, and Zhuang 2019), have been introduced. Studies are using amino-functionalized magnetic nanoparticles and sulfhydryl-labeled aptamers to cross-link with the help of covalent bonds. After the target is introduced, the magnetic properties of the nanoparticles can be used to separate the aptamers from the fluorescence-labeled complementary strand. This method can effectively eliminate interference and improve sensitivity (Jiang et al. 2020).

2.1.3. Mechanism of the combination of aptamers with pesticides

In the presence of targets, most aptamers undergo adaptive conformational changes, such as forming three-dimensional structures (such as convex loops, hairpins, pseudoknots, and G-quadruplexes) and combine with the target through different forces, such as Van der Waals forces, hydrogen bond, hydrophobic interaction force, and base stacking force (Zhang et al. 2016; Peng et al. 2020). The combination of aptamers with pesticides is a dynamic process and eventually reaches a balanced state under the action of various bonds, that is, the aptamer-target complex. Therefore, the high affinity and specificity between the aptamer and the target molecule are based on the unique spatial structure of different nucleotide sequences (Kong et al. 2021).

For example, Wang et al. (2016) used aptamers to detect isocarbophos, and they labeled fluorescein FAM at the 5' end (Figure 2). When combined with complementary strands to form a double-stranded structure, the fluorescence signal weakened, and fluorescence resonance occurred. When the aptamer recognizes the target isocarbophos, it creates an aptamer-target complex; simultaneously, the complementary DNA strand was separated from the double-stranded structure, and the fluorescent signal became stronger.

2.2. Genetic engineering antibodies

With the gradual application of genetic engineering technology to the design and preparation of antibody molecules and the introduction of multiple construction modes and expression systems, some of the shortcomings of natural antibodies have been overcome. Further, some new biological functions have been given to them, making it a new trend to use genetic engineering antibodies to detect small molecules.

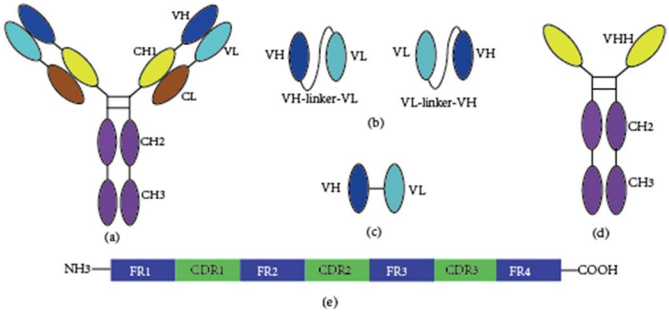


Figure 3. Structure diagram of genetically engineered antibody. (a) A monoclonal antibody, (b, c) single-chain antibody, (d) heavy chain antibody, and (e) nanobody (Wesolowski et al., 2009).

2.2.1. Types of genetic engineering antibodies

Obtaining antibodies with strong specificity, high sensitivity, and good stability is the key to establishing an immunoassay method. At present, traditional polyclonal antibodies and monoclonal antibodies have been widely used in routine immunoassays. However, the specificity and stability of polyclonal antibodies are poor. Although the performance of monoclonal antibodies meets the requirements, the preparation process is complicated and time-consuming (Zhao et al. 2021). It essentially limits the use of antibodies.

Due to the plasticity of the structure and function of genetically engineered antibodies, the types of recombinant antibodies are very abundant. It mainly includes humanized murine monoclonal antibodies, multivalent antibodies, bispecific antibodies, and antibody fusion proteins. The three major categories have application prospects in detecting pesticide residues, small molecule antibodies, bispecific antibodies, and antibody fusion proteins.

2.2.1.1. Small molecule antibodies. The binding site of the intact antibody and the antigen is in the V region. The amplification of the gene sequence of this region can be used to construct recombinant antibodies with smaller molecular weight and antigen-binding properties. Therefore, based on the structure of the antigen-binding site, it can be divided into single-chain antibodies (scFV), single-chain antigen-binding fragments (scFab), and nanobodies (VHH).

(1) Single-chain antibody (scFV)

Single-chain antibody (single-chain variable fragment, scFV) (Figure 3b and 3c) connect the VH and VL of the antibody through a linker or by mutating one amino acid in the VH and VL of the antibody to cysteine, forming a disulfide bond (Strachan et al. 2000). In addition to being used for biomolecular identification, scFV has also developed functional scFVs for small molecule pollutants, such as the insecticide parathion, chlorpyrifos, fenthion, and the fungicides fenazole, imazalil, thiabendazole (Li et al. 2012; Karsunke et al. 2012; Liu et al. 2016).

Some studies have constructed scFV that can simultaneously detect three pyrethroid metabolites based on hybridoma cells resistant to pyrethroid metabolites. By molecular docking, it showed that there were hydrogen bonds, hydrophobic interactions, CH-π interaction, and cation-π interaction between 3-PBA and its scFv. Using enzyme-linked immunosorbent assay results as evaluation indicators, the IC_{50} values of scFV for the three metabolites, 3-PBA, 3-PBAld, and 3-PBAlc, were 0.55, 0.59, and 0.63 μg/mL, respectively (Liu et al. 2020).

(2) Single-chain antigen-binding fragment (scFab)

The antigen-binding fragment (Fab) is a part of a complete antibody. It is composed of the VH and CH of the heavy chain and the whole light chain. Dimers are formed through disulfide bonds between chains, and a complete antigen-binding site is constructed. Because the Fab antibody is mainly expressed in the periplasmic cavity, it normally demonstrates low efficiency of expression and poor stability. These cause the light chain and the heavy chain to dissociate easily and the activity of scFab to be lost. Therefore, the use of Fab antibodies alone for pesticide detection faces particular challenges.

To fill the gap described above, Rao et al. (2016) prepared a single-chain antigen-binding fragment (scFab) composite antibody to detect organophosphorus pesticides to make up for the above shortcomings. Using the recombinant plasmid as a template, the antibody heavy (Fd) chain and light (k) chain genes were amplified and cloned in two different directions with (Gly4Ser)3 linker into the same plasmid template. The indirect competitive enzyme-linked immunosorbent assay was used to detect the immunological activity of two scFabs with different orientations. It is found that the Fd-linker-k with the reverse orientation has better sensitivity, and it also integrates the characteristics of scFV and Fab, which solves not only the problem of the low affinity of single-chain antibodies but also the obstacle of low expression efficiency of Fab antibody.

(3) Nanobody (VHH)

Nanobody (VHH), (Figure 3e) also known as the variable domain of the heavy chain of an antibody, is a type of genetically engineered antibody. It is the smallest functional antibody with a molecular weight of only 15 kDa. In 1933, Hamers Casterman first discovered a heavy chain antibody (HCAbs) with a particular structure and function in camelid animals (Figure 3d), then VHH began to be gradually applied. Because VHH has the advantages of strong specificity, high stability, high yield, and resistance to organic solvents, it has attrected extensive attention (Desmyter et al. 2015).

Some studies have screened a VHH with the most substantial specificity for Naphthylamine from 6 alpaca-derived heavy chain antibody libraries. Although its sensitivity in the enzyme-linked immunosorbent assay is not as good as that of monoclonal antibodies, it still can be sufficient to meet the maximum residue limit of Naphthylamine. The limit of detection in the system containing 10% methanol and 0.8% NaCl can reach 0.3 ng/mL, which is not possible for monoclonal antibodies (Liu et al. 2019).

2.2.1.2. Antibody fusion protein. The protein is endowed the antibody molecules with new functions, which is the fusion of genetically engineered antibody molecules with proteins or polypeptides with other specific functions. In pesticide detection, antibody molecules are usually expressed in fusion with alkaline phosphatase to enhance detection.

For example, Huo et al. cloned VH and VL in monoclonal antibody hybridoma cells, assembled the VH and VL genes through a (Gly4Ser) 3 linker to obtain an expression vector, who had the biologically functional of scFV, and alkaline phosphatase (AP). The obtained fusion protein retains the antigen-binding specificity and retains the AP enzyme activity. Based on the fusion protein, a fluorescent enzyme immunoassay method for rapid detection of pyrethroid pesticide metabolites was established. The LOD value can reach 0.11 ng/mL, and the IC_{50} value is nearly 10 times higher than that of the direct competition enzyme-linked immunosorbent assay through scFV, and showed good reliability (Huo et al. 2018).

2.2.2. Screening methods of genetic engineering antibodies

In recent years, with the rapid development of antibody engineering technology, the screening technology of recombinant antibodies has gradually become standardized and programmed. It mainly includes the technologies of phage display, ribosome display, mRNA display.

2.2.2.1. Phage display technology. Phage display technology refers to the introduction of exogenous genes into a phage vector, which can achieve the fusion expression of exogenous genes with phage surface protein and display entirely on the head of the phage. It is a powerful antibody screening technology (Li et al. 2017). Since the 1990s, phage display technology has been widely used to screen specific recombinant antibodies (Xu, Yang, et al. 2018).

Phage display technology can avoid the technical difficulties of hybridoma cells and imitate the mechanism of antibody diversity. The main principle is to extract total RNA from spleen B lymphocytes or peripheral blood lymphocytes of immunized or non-immunized animals, purify mRNA for reverse transcription, and amplify genes by RT-PCR technology, and randomly integrate them into phage vector. Then, we constructed an antibody library. After being infected with *Escherichia coli*, the antibody fragments Fab or scFV were proliferated and displayed on the surface of the phage. We can obtain the required specific antibody by searching the part that binds to the antigen (Zhao et al. 2021).

2.2.2.2. Ribosome display technology. Ribosome display technology is a process of simulating protein expression in vitro. Firstly, the scFV gene is obtained by RT-PCR technology as a template for in vitro expression. Then transcription and translation are performed in vitro to form a ribosome-mRNA-scFV complex. Finally, the complex of high-affinity scFV in the ribosome-mRNA-scFV is screened out with immobilized antigen molecules.

This technology is the first method to screen functional antibodies entirely in vitro, and it can overcome the shortcomings of other screening methods. First, ribosome display technology overcomes the problem of limited antibody library diversity due to low transformation efficiency in phage display technology; secondly, it avoids the difficulty of affecting the size of the antibody library, which is due to the growth disadvantage of the host bacteria and even the toxicity to *E. coli*. Finally, the elution process is challenging for some phages with very high antibody affinity (Luo and Xia 2012).

2.2.2.3. The mRNA display technology. As another display technology in vitro, mRNA display has the advantages of high screening efficiency and simple operation process. Its main principle is to connect the nucleic acid sequence with the protein it encodes through a covalent bond to form an mRNA-protein fusion. The sequences in the library are transcribed to form mRNA in vitro.

As a linker, puromycin with the function of amino tRNA enters site A of the ribosome. The 3′ end of mRNA is covalently bound to the carboxyl end of a polypeptide by inhibiting the protein translation process. Finally, the mRNA-protein fusion body is obtained. Then it was added to the solid phase carrier coated with antigen. The antibody with high specificity can be obtained by removing the unbound complex and eluting the specific complex from the stationary phase (Doshi et al. 2014).

2.3. Genetically engineered enzymes

Many types of enzymes can be used to determine pesticides. They mainly function by the hydrolysis or oxidation of the pesticides as the enzyme-substrates or the inhibition of the enzyme by the pesticides. At present, the principles of the detection of pesticides, based on the enzymatic method, mainly include the method of enzyme inhibition, enzyme-catalyzed hydrolysis reaction, and enzyme-catalyzed oxidation reaction.

When conducting large-scale screening of pesticides based on the above principles, the specificity, stability, and catalytic activity of enzymes are closely related to the relability of the test results. Natural enzyme sources are mainly isolated and purified from animals, plants, and microorganisms. Differences in sources will lead to poor reproducibility of the results in the detection process; in addition, natural enzymes will show a greater loss of activity during the immobilization process, poor thermal stability, pH value, and organic solvent resistance (Cao et al. 2020).

Because of the above reasons, it is necessary to improve the performance of some natural enzymes. With the development of genetic engineering technology, many researchers

are committed to modify enzymes through protein engineering to improve the catalytic activity, stability, and pesticide sensitivity of the enzyme to improve the detection accuracy and reduce the limit of detection.

Organophosphorus hydrolase (OPAA) has been proven to catalyze the hydrolysis of various organophosphorus compounds quickly. However, wild-type OPAA has a poor catalytic effect on methyl thiophosphate pesticides, resulting in poor sensitivity when detecting such pesticides. In this regard, Daczkowski, Pegan, and Harvey (2015) improved the catalytic efficiency by optimizing the OPAA substrate binding pocket. By mutating three consecutive amino acid positions in the pocket, the catalytic efficiency of racemic methyl thiophosphate pesticides was increased by 30 fold. In addition, OPHC2 is a heat-resistant organophosphate hydrolase belonging to the β-lactamase family. After mutating amino acids at positions 250 and 263, the H250I/I263W mutant has a positive effect on detecting methyl parathion. The catalytic efficiency of paraoxon and ethyl paraoxon can be increased by 6962 times and 106 times, respectively. It is confirmed that the thermal stability of the mutants has not been impaired (Luo et al. 2014).

Glutathione transferases (GSTs) can catalyze the nucleophilic attack of reduced glutathione on the electrophilic centers of heterologous compounds. GST isoenzymes have been successfully applied to the development of herbicides and pesticides detection methods. Molecular modeling showed that Phe117 is a key amino acid residue, which forms hydrophobic binding sites and regulates enzymes' affinity for pesticides. The catalytic efficiency of the Phe117Ile mutant can be increased by 3 times, and a higher affinity of α-endosulfan is obtained (Chronopoulou et al. 2019).

Acetylcholinesterase (AChE) is the core of enzyme inhibition detection technology. It has been derived from different sources, such as electric eels (Berman and Young 1971), fruit flies (Mutero and Fournier 1992), electric eels (Sato et al. 2009), humans (Soreq et al. 1990). AChE gene has been successfully expressed in various expression systems, but the amount of expression varies greatly. Protein disulfide isomerase (PDI) is an effective molecular chaperone, which plays a vital role in protein folding and isomerization in the endoplasmic reticulum. Studies have used PDI co-expression in *Pichia pastoris* to increase the yield of silkworm AChE, and the stability and detection sensitivity is not affected (Li, Cai, et al).

In summary, the specificity and sensitivity of pesticide detection based on enzymatic methods can be improved utilizing site-directed mutation of specific enzyme genes.

2.4. Whole-cell biosensor

Whole-cell biosensors (CBBs) are a platform that can be used for analysis and detection. The difference from other sensors is that they use microorganisms fixed on the sensor as signal receivers to detect compounds. The origin of the whole-cell biosensor can be traced back to the appearance of the polarographic oxygen electrode in 1960 and the first microbial sensor created by Isao Karube (Plekhanova and Reshetilov 2019). It was not until 1997 that he fixed bacteria on the Clark-type electrode to measure biological oxygen demand. It can also be said that this is the beginning of whole-cell biosensors and opens a new direction for cell biosensors based on genetic engineering.

Compared with enzyme biosensors, the main advantages of microbial sensors are: (1) There is no need to carry out the cumbersome process of enzyme separation, purification, and immobilization; (2) Some enzymes lose their activity during the process of separation and immobilization. Using the entire cell as a sensor, we can minimize this risk. (3) The activity, selectivity, and specificity of CBB can be further improved by mutating microorganisms (Plekhanova and Reshetilov 2019).

At present, the applications of whole-cell biosensors in pesticide residue detection are mainly based on transcription factor, preparation of reporting domain, and display of cell surface. Therefore, in the rest of this chapter, we will give a detailed introduction to these three aspects.

2.4.1. Transcription factors

Transcription factor-based whole-cell biosensors (TFBs) are an indicator that can convert the concentration signal of the target to be measured into the expression state of the target gene (Reberts et al. 2018; Wang, Tang, and Yang 2017). TFBs consist of two modules: sensor and reportor. The sensor module includes a transcription factor activated or inhibited by the substance to be tested. The homologous promoter in the transcription factor is fused with the reporter gene to form the reporter module. After the test substance activating the transcription regulator, the reporter gene will be expressed and output a measurable signal. Commonly available reporter genes include *lux*, GFP, which can generate luminescence or fluorescence signals (Chong and Ching 2016).

Under normal circumstances, TFBs can be divided into two categories: (1) repressor-based biosensors and (2) activator-based biosensors (Mannan et al. 2017) (Figure 4). When the inducer is present, the inhibitory function of the repressor can be activated or inhibited, resulting in non-expression or expression of the target gene (Xu 2018). On the contrary, when the inducer is present, the activation function of the activator will be activated or inhibited, resulting in the expression or non-expression of the target gene. The expression of the target gene can be used as an output signal, usually a detectable signal, such as luminescence, fluorescence (Hanko, Minton, and Malys 2018; Ding, Zhou, and Deng 2021).

Whangsuk et al. (2016) integrated *ChpR* and *ChpA* promoter-FGE-esterase fusion protein from *Sinorhizobium meliloti* into *E. coli* chromosome. The formylglycine-generating enzyme (FGE) and sulfate esterase were used as reporter genes, and FGE could activate sulfate esterase. The activity of sulfate esterase could be easily measured by small

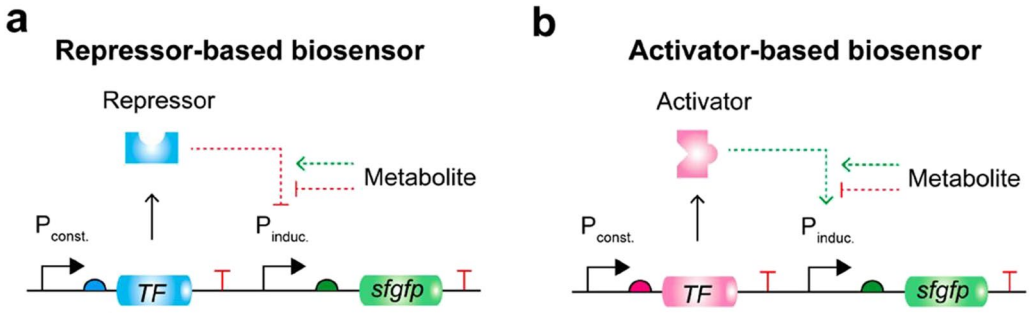


Figure 4. Response mechanism of TFBs in whole-cell sensor (Ding, Zhou, and Deng 2021).

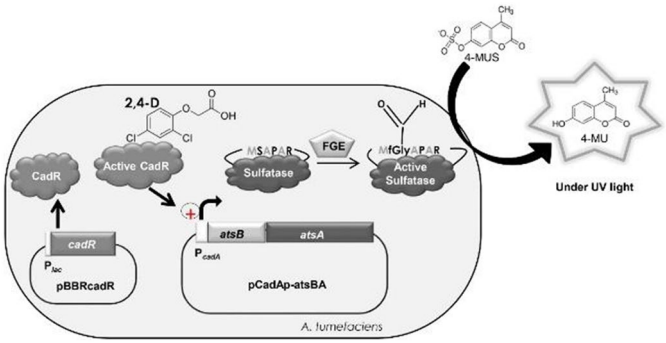


Figure 5. The architecture of a whole-cell genetic-based biosensor using sulfatase as a reporter system (Ritcharoon et al. 2020).

aromatic sulfate compound 4-methyl umbrella ketone sulfate (4-MUS). The system had been successfully applied to the specific detection of chlorpyrifos. Subsequently, Ritcharoon et al. (2020) of the team constructed a genetic-based biosensor for *Agrobacterium* tumefaciens (Figure 5). The sensor is based on cadA promoter and cadR regulator from *Brachyphyllum*. The use of random mutagenesis to evolve cadR improved the sensitivity of the biosensor to 2.4-D detection. It is a successful case of using TFBs to detect pesticide residues.

2.4.2. Preparation of reporting domain

As a part of the biosensor, the reporting domain can produce a measurable signal, including but not limited to the modulation of a fluorescent read-out (Moeyaert and Dedecker 2020). The critical challenge is how to engineer the reporting domain to improve the whole-cell sensor's sensitivity and reduce the detection limit.

The fluorescence of GFP is very sensitive to the pH in the system, wild-type GFP is quickly quenched by acidic pH, and some GFP mutants are more sensitive than wild-type. In addition, the fluorescence intensity of GFP displayed on the surface is more susceptible to pH changes outside the cell than inside. Therefore, to get accurate measurements, some researchers have developed an "off-type" fluorescent sensor based on these properties of fluorescent proteins for the detection of organophosphorus pesticides (Liu et al. 2013). The organophosphorus hydrolase (OPH) and methyl parathion hydrolase (MPH) was fused with a fluorescent protein and expressed together on the surface of *E. coli* cells. Due to the proton formation and the sensitivity of GFP to pH in the process of Ops hydrolysis, the fluorescence intensity of GFP can be used as a quantitative indicator of Ops concentration. In detecting parathion and methyl parathion, the correlation coefficients are 0.9912 and 0.9938, respectively, and the detection limit can reach 2 μM.

2.4.3. Display of cell surface

Cell surface display technology expresses exogenous polypeptides on the cell surface in the form of fusion proteins through genetic engineering methods. It maintains a relatively independent spatial structure and biological activity (Han et al. 2017, 2018). Using cells to immobilize enzymes and directly using whole cells as biocatalysts can avoid enzyme instability. At the same time, the complicated steps of protein purification can be eliminated, reducing costs and saving time.

The cell surface display system is usually composed of carrier protein, passenger protein, and receptor protein. The carrier protein guides and anchors the passenger protein to a specific part of the cell. The structure has anchor units and signal peptides, making the expressed fusion protein span the cell's plasma membrane. Bacterial carrier proteins can be divided into cell membrane porins, ice crystal nucleoproteins, cell wall-related proteins, and cell surface appendage proteins according to their structural positions and functions (Smith, Khera, and Wen 2015).

The most used carrier protein is ice crystal nucleoprotein, a secretory surface protein produced by the gram-negative bacterium *Pseudomonas syringae*. It has three functions of secretion, positioning, and anchoring at the same time. So that it has enough sequence to recombinant with foreign sequences, which makes it possible to display on the cell surface. Its anchor sequence is derived from glycosyl phosphate, which is mainly responsible for binding ice nucleoprotein to the cell surface. In this way, the expressed ice nucleoprotein and exogenous fusion protein can be fixed on the cell surface (Yang, Chen, and Wang 2015).

In recent years, displaying proteins or enzymes on the surface of microorganisms has become a new immobilization method using living cells as carriers. It has been widely used in biocatalysis, vaccine design, diagnosis and treatment of diseases, and environmental monitoring. In pesticide detection, we have successfully displayed acetylcholinesterase

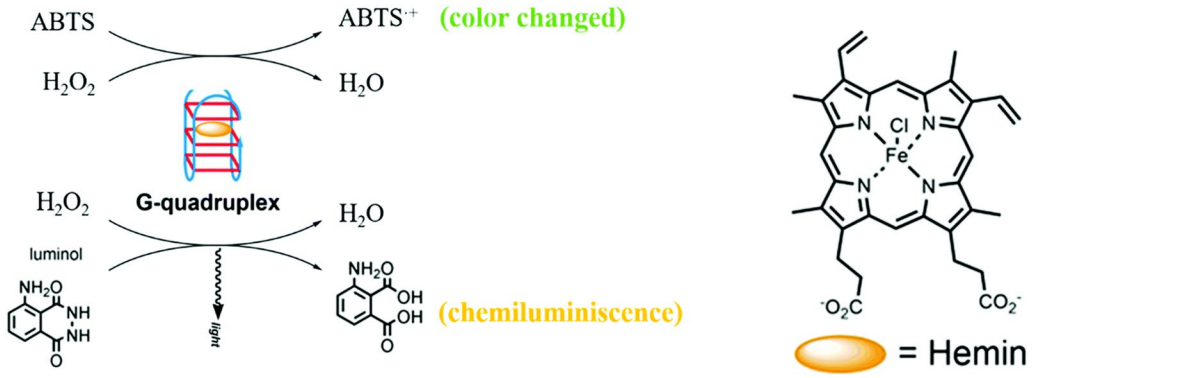


Figure 6. The structure and activity of G-quadruplex/hemin-based peroxidase-mimicking DNAzymes (also named HRP-mimicking DNAzyme or G4-DNAzyme) (Hu et al. 2020).

(Liang et al. 2019), organophosphorus hydrolase (Tang, Liang, et al. 2014), methyl parathion hydrolase-green fluorescent protein (GFP) fusion protein (Liu et al. 2013), and even nano-antibodies on the surface of microorganisms (Riangrungroj et al. 2019), and they all showed good application prospects.

2.5. Catalytic nucleic acid (DNAzyme)

DNAzyme is a nucleic acid that can exhibit catalytic activity in the presence of cofactors. In 1994, after Breaker and Joyce discovered DNAzyme for the first time (Breaker and Joyce 1994), many DNAzymes with different catalytic activities have been reported one after another, mainly including DNAzyme with RNA and DNA cleavage activity, DNAzyme with peroxidase mimic enzyme activity (Breaker and Joyce 1994; Lake et al. 2019). Due to it has the characteristics good programmability, high stability. DNAzyme has broad application prospects in environmental monitoring, food supervision, biosensing, and gene therapy (Hu et al. 2020; Zimmermann, White, and Kahn 2020).

G-tetrajoint/heme peroxidase mimetic enzyme (G4-DNAzyme) is the most widely used DNAzyme in pesticide detection. This G-tetramer is a DNA sequence rich in guanine (G), combined with hemin through hydrogen bonds to form a planar structure. Each planar structure is stably formed by the π-π stacking effect of G-quadruple structure (Mergny and Sen 2020). Chelating low valence cations can further stabilize the structure to reduce electrostatic repulsion (Hu et al. 2020).

The DNAzyme mechanism used in pesticide detection is mainly by simulating the catalytic activity of peroxidase oxidizing ABTS to produce color changes or catalyzing luminol/H_2O_2 to produce chemiluminescence. The reaction mechanism is shown in Figure 6 (Hu et al. 2020). For example: in the process of chlorpyrifos detection, G4-DNAzyme can be used as a catalyst to oxidize ABTS2- with H_2O_2 directly, and the product has apparent UV-visible absorbance at 418 nm; When chlorpyrifos exists, it can preferentially react with H_2O_2. All of these reduce the oxidation products $ABTS^{2-}$, so the UV absorbance at 418 nm will be changed (Liu et al. 2015). Therefore, the peroxidase activity of DNAzyme can be used in combination with an optical biosensor to be used in the rapid on-site detection of pesticides.

3. Analysis strategy based on biosensors

Biosensors are a type of signal equipment that converts biological or biochemical signals into measurable signals. They are valuable tools for detecting chemical and harmful substances in health products, food, and the environment (Goradel et al. 2018). A biosensor is a device that is sensitive to biological substances and converts their concentration into other signals, and amplifies them. It comprises immobilized biological identification elements (such as enzymes, antibodies, antigens, microorganisms, nucleic acids, and other biologically active substances) and signals amplification devices.

According to the sensing mechanism of the sensor, it can be divided into the optical sensor, quality sensor, temperature sensor, and electrochemical sensor. Among them, optical and electrochemical sensors are the most commonly used in pesticide detection. With the continuous improvement of detection sensitivity requirements, the two gradually occupy a dominant position (Schoukroun-Barnes, Glaser, and White 2015). Therefore, we will introduce optical and electrochemical sensing platforms using pesticide detection based on recognition elements prepared by genetic engineering, the application of aptamers, genetic engineering antibodies, genetically engineered enzymes, and whole-cell biosensors. Table 3 lists the representative examples of pesticide residue detection based on sensor strategy developed recently.

3.1. Electrochemical biosensor

The electrochemical biosensor is the most researched and developed pesticide detection due to its simplicity, low cost, fast response, high sensitivity, and compatibility with new micromachining technologies (Danesh et al. 2016; Madianos et al. 2018). This biosensor is divided into five types according to signal properties: conductivity, potential, ampere/volt-ampere, impedance, and field-effect transistor. The volt-ampere and impedance types are the most common in pesticide residue detection (Mahmoudpour et al. 2021).

Table 3. Representative examples of pesticide residue detection based on sensor strategy developed recently.

Sensor type	Strategy	Recognition element	Target pesticide	LOD	Linear range	Sample	Advantages	Reference
Fluorescence biosensor	Using fluorescence resonance energy transfer between NH_2-$NaYF_4$: Yb, Ho@ SiO_2(UCNPs), and gold nanoparticles (GNPs)	Aptamer	Acetamiprid	3.2 nM	50–100 nM	Tea	High selectivity to similar structure pesticides	(Hu et al. 2016)
Fluorescence biosensor	Fluorescence quenching induced by exonuclease, specific target recognition by an aptamer, and host-guest recognition was artfully integrated	Aptamer	Acetamiprid	3 nM	5 nM–1.2 μM	Honey, orange juice	High selectivity, sensitivity, safety, and well linear range.	(Lu and Fan 2020)
Colorimetric biosensor	Sensitive response of positively charged gold nanoparticles ((+) AuNPs) to the aptamer structure change	Aptamer	Acetamiprid	0.56 nM	0.9 nM–6.6 μM	Soil, wastewater, and cucumber	Outstanding practicability, sensitivity, and convenience	(Qi et al. 2020)y
Impedimetric biosensor	Employing the sputtering and e-beam lithography techniques, platinum nanoparticles (Pt NPs) were deposited in a bridge-like arrangement, in between interdigitated electrodes (IDEs).	Aptamer	Acetamiprid, Atrazine.	1 pM 10 pM	10 pM–100 pM 100 pM–1 μM	Water	Satisfactory selectivity, reproducibility, and accuracy	(Madianos et al. 2018)
Voltammetric electrochemical biosensor	The zirconium-based metal-organic framework (MOF) was synthesized by hydrothermal method, and was coupled with complementary probe (CP) and carboxy-ferrocene (Fc-COOH) by covalent bonds to form a CP-MOF-Fc nanocomposite probe.	Aptamer	Malathion	17.18 ng/L	25–850 ng/L	Cucumber and long bean	High selectivity, excellent repeatability, and good stability.	(Xu, Huo, et al. 2021)
Colorimetric biosensor	a protein disulfide isomerase (PDI) co-expression strategy in Pichia pastoris was employed to enhance the yield of recombinant Bombyx mori acetylcholinesterase 2 (rBmAChE2)	Genetically engineered enzymes	Methamidophos, Malathion, Dipterex, Dichlorvos, Profenofos, Omethoate, Monocrotophos	0.433 mg/kg, 2.725 mg/kg, 0.109 mg/kg, 0.016 mg/kg, 0.291 mg/kg, 1.500 mg/kg, 0.617 mg/kg.	–	Cabbage, baby cabbage, lettuce, leaf lettuce	High efficiency, simplicity, and low cost.	
Magnetic biosensor	Using chitosan to capture more graphene oxide@Fe_3O_4 and further amplify the current signal	Aptamer	Chlorpyrifos	0.033 ng/mL	0.1–105 ng/mL	Leafy vegetables, such as cabbage, lettuce, leek, pakchoi.	Good selectivity, reproducibility, and stability.	(Jiao et al. 2017)

Microcantilever-array sensor	The microcantilevers were functionalized with a profenofos-specific aptamer (SS2-55), and then the specific binding of profenofos to aptamer induced a deflection of the microcantilever,	Aptamer	Profenofos	3.5 nM	–	vegetable-soak solution	Highly sensitive, one-step immobilization method capable of quantitative and real-time detection.	(Li, Zhang, et al. 2018)
SERS	The silver nanoparticles prepared with positively charged spermine enhanced and captured reagents for the negatively charged aptamer.	Aptamer	Malathion	–	0.5 μM −10 μM	Tap water	Good selectivity, linearity, and recoveries	(Nie et al. 2018)
Voltammetric electrochemical biosensor	The genetically engineered *E.coli* surface-displayed mutant OPH (S5) with improved enzyme activity and favorable stability was constructed using a newly identified N-terminal of ice nucleation protein as an anchoring motif	CBBs	Paraoxon, Parathion, Methylparathion	9.0 nM 10 nM 15 nM	0.05–25 μM 0.05–25 μM 0.08–30 μM	Tap water, seawater, and sewage	Highly specific, sensitive, and rapid	(Tang, Zhang, et al. 2014)s
Fluorescence biosensor	Constructing a genetic-based *Agrobacterium tumefaciens* biosensor based on a *cadA* promoter and *cadR* regulator from *Bradyrhizobium sp.* strain HW13 (2,4-D degrader) with a formylglycine generating enzyme (FGE)sulfatase as the reporter gene	CBBs	2,4-D	1.56 μM	–	Environmental samples	Without encountering interference from other com-pounds well storage stability	(Ritcharoon et al. 2020)
Impedance electrochemical biosensor	Direct competition for binding to alkaline phosphatase-embedded nanobodies between free 3-PBA and a 3-PBA-bovine serum albumin conjugate covalently immobilized onto citric acid-decorated nylon nanofibers	VHH	3-PBA	0.64 pg/mL	0.8–1000 pg/mL	Human urine	Easy use, low-cost assay, reusability, and fast analysis	(El-Moghazy et al. 2020)
Colorimetric biosensor	A structure-based combinational approach was used for the design of an optimized mutant of *PvGmGSTUG*,	Genetically engineered enzymes	α-Endosulfan	–	0.625–30 μM	Water	The proposed biosensor provides a low-cost alternative to current chromatographic-based methods	(Chronopoulou et al. 2019)

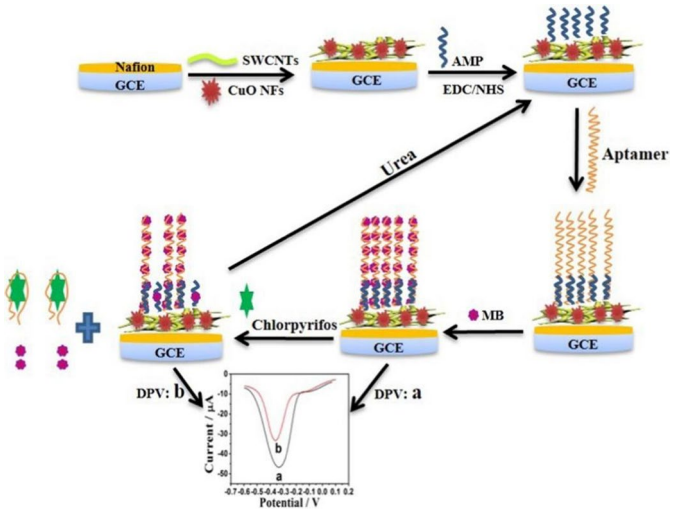


Figure 7. Schematic illustration of the regenerative electrochemical aptasensor for selective detection of chlorpyrifos (Xu, Huo, et al. 2018).

3.1.1. Voltammetric electrochemical biosensor

Using voltammetric electrochemical biosensors, many scientific attempts have been made for pesticide detection. Currently, the screen-printed carbon electrode is the most widely used small-size electrochemical platform. It comprises a reference electrode, a working electrode, and an electrochemical cell (Majdinasab, Daneshi, and Marty 2021). Some nanomaterials easily prepare the working electrode. For example, copper oxide nanoflowers exist in the form of three-dimensional nanostructures. Therefore, they have a large specific surface area. In addition, it has low cost and good chemical stability. It is a potential electrode material. As an excellent conductive medium, carbon nanotubes, which are nonmetallic materials, have excellent mechanical properties and at the same time have good biocompatibility and electrical properties that can amplify sensing signals and increase the electron transfer rate. Therefore, a composite material based on copper oxide nanoflowers and carboxylated single-walled carbon nanotubes has been developed. The synergistic effect between them can expand the electrode surface area and enhance electron transfer. At the same time, aminoated aptamer probes of chlorpyrifos can also be immobilized on their surfaces by covalent bonds (Figure 7). Methylene blue (MB) can be non-covalently bound to DNA (Gorodetsky, Buzzeo, and Barton 2008). When the aptamer binds to the target chlorpyrifos, MB will be released. At this time, the peak value of MB DPV will decrease as the concentration of chlorpyrifos increases. After the conditions are optimized, this sensor's linear range for chlorpyrifos detection is 0.1–150 ng/mL, and the LOD value is 70 pg/mL. It has been successfully applied to detecting apples and celery, which shows good selectivity and repeatability. In addition, another bright spot of this sensor is that it can easily be reused after urea regeneration treatment, which confirms the broad application prospects of nanocomposites in pesticide detection (Xu, Huo, et al. 2018).

In addition, a rapid detection method based on microbe-electrochemical biosensors has also been developed. We can anchor the organophosphorus compound hydrolase to the N-terminal of ice nucleoprotein using microbial surface display methods. And they are fused and expressed to form a whole-cell biosensor with a surface display function. The cell is fixed on the surface of ordered mesoporous carbon and hydrolyze organophosphorus with the p-nitrobenzene structure to produce p-nitrophenol. Rapid detection of p-nitrophenyl organic phosphorus can be realized by measuring the oxidation current of p-nitrophenol (Tang, Zhang, et al. 2014).

3.1.2. Impedance electrochemical biosensor

Impedance electrochemical biosensor is a highly sensitive electrochemical analysis technology. Its main principle is to apply a small amplitude sine wave with different frequencies at the electrode interface to interfere and then to determine the change of the ratio of alternating current potential to current (impedance) (Rotariu et al. 2016; Alatraktchi et al. 2016). Compared with the volt-ampere sensor, the impedance type has the advantage of not interfering with the polarization phenomenon of the electrode surface. It can realize the nondestructive testing of the sample and obtain stronger stability. At present, aptamer-based methods are mainly used for impedance-type electrochemical biosensors.

Madianos et al. developed an impedance biosensor based on aptamer-prepared nano-platinum microwires for the precise detection of acetamiprid. Nano-platinum particles are deposited between the interdigital electrodes (IDE) in a bridge-like arrangement. The platinum nanowires are chemically functionalized to allow the aptamer to be fixed on the sensor surface. This sensing platform is connected to the IDE through a micro-wire bridge and promotes charge transfer. When the immobilized aptamer binds to the target, the transfer of electrons will be hindered, increasing the battery's impedance. This method can detect acetamiprid; the limit can reach 1 pM (Madianos et al. 2018).

In recent years, MoS_2 nanosheets have attracted more and more attention because of their excellent capacitance. Their sheet-like morphology provides a large surface area for storing charges, making it an ideal choice for electrochemical capacitance spectroscopy applications (Chen, Park, et al. 2018). In addition, MoS_2 has a high affinity for aptamers, and they are adsorbed by van der Waals forces between them (Dalila et al. 2019). This makes MoS_2 nanosheets suitable for impedance-derived capacitive biosensors. Some studies have used Impedance-Derived Capacitance Biosensor to detect acetamiprid (Hamami, Raouafi, and Korri 2021). Through covalent adsorption with lipoic acid, a self-assembled layer is formed on the surface of MoS_2, which ensures the aptamer's stability on the nanosheet's surface. After interacting with acetamiprid, the aptamer will be desorbed from the nanosheet to form an aptamer-target complex. The separation of aptamers is

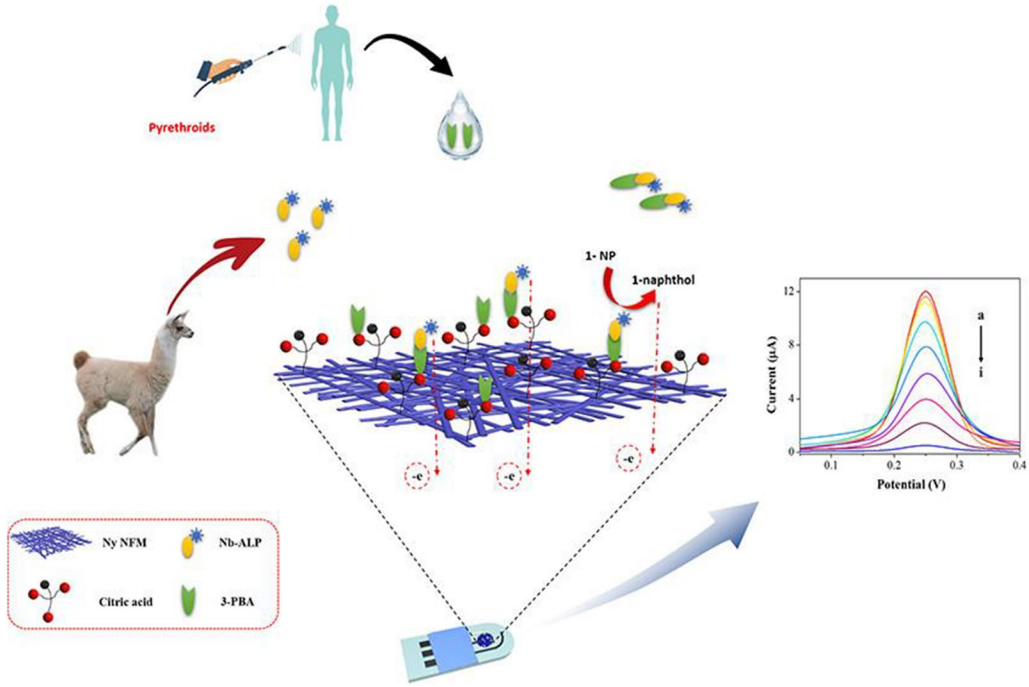


Figure 8. Fabrication process and sensing mechanism of nanobody-based electrochemical immunosensor for 3-PBA detection (El-Moghazy et al. 2020).

conducive to the ion diffusion of electrolytes. This method showed high specificity.

In addition to the application of aptamers to construct impedance-type electrochemical sensors, it has recently been reported that nanobody-alkaline phosphatase fusion protein (Nb-ALP) can also be used to build impedance-type electrochemical sensors (Figure 8) (El-Moghazy et al. 2020). The 3-PBA detection was via a direct competition for binding to alkaline phosphatase-embedded nanobodies between free 3-PBA and a 3-PBA-bovine serum albumin conjugate covalently immobilized onto citric acid-decorated nylon nanofibers, which were incorporated on a screen-printed electrode (SPE). Electrochemical impedance spectroscopy (EIS) was utilized to support the advantage of the employment of nanofibrous membranes and the success of the impedance electrochemical biosensor. The coupling between the nanofiber and nanobody technologies provided an ultrasensitive and selective immune sensor for 3-PBA detection in the range of 0.8 to 1000 pg mL^{-1} with a detection limit of 0.64 pg mL^{-1}.

3.2. Optical biosensor

Characteristics of pesticide detection methods based on optical biosensors are simple, fast, low-cost and sensitive. They are mainly based on strategies such as colorimetry, fluorescence, chemiluminescence, and Raman signals. The detection principle is that when the target specifically binds to the recognition element, it will change the optical signal (Majdinasab, Daneshi, and Marty 2021). This section will mainly introduce the application of optical biosensors in pesticide residue detection, including fluorescence, colorimetry, chemiluminescence, and surface-enhanced Raman spectroscopy (SERS) measurement.

3.2.1. Fluorescence biosensor

The fluorescence method is currently one of the most widely used biosensors and has been applied in many fields, such as biomedicine, food safety, and environmental detection (Xu, Li, et al. 2018). The main principle of fluorescence sensors is based on the phenomenon of fluorescence enhancement (on) and fluorescence quenching (off) caused by the binding of the target and recognition element (Zhang, Wu, et al. 2019). Therefore, based on the principle mentioned above, it is easy to realize the on-site detection of samples through fluorescent biosensors. This chapter introduces the application of fluorescent biosensors in pesticide detection from two aspects: "open type" and "closed type."

3.2.1.1. "Open type". Carbon nanomaterials have emerged as a promising tool for the development of enzyme-free biosensors. Single-walled carbon nanotubes (SWCNT), multi-walled carbon nanotubes, and various prepared graphene oxide (GO) have also been explored as "nanoquenchers" of fluorophores. Equipped with aptamer technology, these carbon nanomaterials can construct multifunctional fluorescent nanosensors for various target detection. Compared with SWCNs, MWCNTs have a larger diameter. It has been reported that the encapsulation and adsorption of ssDNA around SWCNTs are slower than that of MWCNTs (Li, Zhang, and Liao 2010). Therefore, some studies have designed a label-

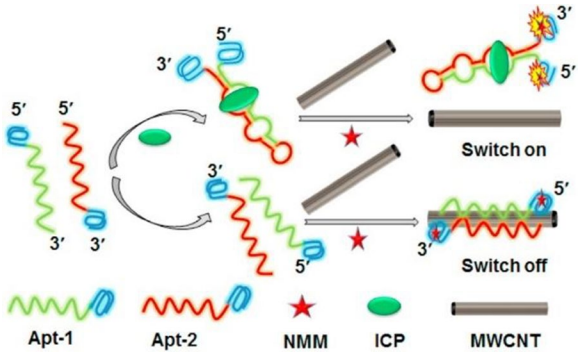


Figure 9. Schematic representation of fluorescent ICP biosensor using MWCNTs (Li, Tang, et al. 2018).

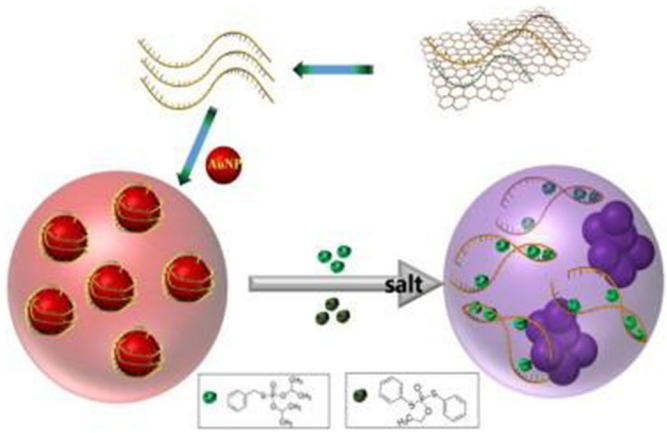


Figure 10. Schematic diagram of iprobenfos detection principle based on the colorimetric method (Kwon et al. 2015).

free detection strategy using MWCNTs (Li, Tang, et al. 2018) to detect the pesticide residues of isocarbophos (Figure 9). The ends of the single-stranded aptamers are connected to a G-tetramer sequence. When there is no target, the aptamers are adsorbed on both sides of the MWCNT in the form of two single-stranded aptamers, the fluorescence of G-quadruplex-fluorescent marker (NMM) will be quenched. When the target is present, the split aptamer will undergo a conformational change, forming a sandwich-like ternary complex. Due to the increase in steric hindrance, this will prevent the aptamer from adsorbing to the MWCNT, resulting in a significant increase in signal. According to the above principle, an "open type" fluorescent biosensor is constituted.

In addition, since the output signal of the whole-cell biosensor based on fluorescence detection is easy to visualize, it also has important application value in pesticide detection. Some studies have tried to change the expression level of homologous promoters by applying directed evolution technology on the transcription regulator DmpR, thereby reducing the LOD value of whole-cell biological slave sensors. Using the mutant of DmpR as the biosensing element of 4-nitrophenol, a whole-cell biosensor for detecting Ops with 4-nitrophenol as the hydrolyzate was developed in *E. coli*. Using red fluorescent protein as a reporter gene, 4-nitrophenol can be used as an activator. When it is present, transcriptional regulatory factors are activated, and red fluorescent protein is expressed. We can quantitatively detect Ops by detecting changes in fluorescence intensity (Chong and Ching 2016).

3.2.1.2. "Closed type" As a newly developed semiconductor fluorescent nanomaterial in the past 20 years, quantum dots (QDs) have good photochemical stability and spectral specificity and are very suitable as fluorescent markers. When used as a fluorescent probe to detect pesticides, the LOD value of the method can be significantly reduced, and the sensitivity and resolution of the technique can be improved. A new type of fluorescence sensing platform with high specificity based on CdTe@Cds quantum dots was developed to detect malathion. The design consists of quantum dots, poly(N-(3-guanidinopropyl) methyl acrylamide) polymer (PGPMA), and malathion aptamer. When malathion is not present, the cationic polymer binds to the aptamer through electrostatic interaction so that the quantum dot fluorescence is not affected. However, when the aptamer interacts with malathion, the fluorescence signal of the quantum dot is turned off due to the action of the cationic polymer. The detection limit of this method was 4 pM, and it maintains a good logarithmic correlation in the range of 0.01 nM–1 μM (Bala et al. 2018).

In addition, to enhance the convenience of detection, our laboratory proposed a novel detection strategy. We fused gene EPSPs with the GFP gene, and the obtained fusion protein can be used as both a recognition and signal amplification element. When glyphosate exists in the sample, it partially binds to EPSPs in the fusion protein, which destroys the structure of the fluorescent protein, and the fluorescence is quenched. We successfully constructed a closed biosensor based on the above principle, which provides a new idea for detecting glyphosate.

3.2.2. Colorimetric biosensor

The colorimetric analysis is considered a powerful technique for detecting pesticides. They are easy to read using fast visual inspection and have received widespread attention (Zhang, Wu, et al. 2019). The principle of most colorimetric biosensors is to change the dispersion state of AuNPs in a certain way, which in turn changes its color from red to blue. Based on the above principles, a colorimetric-based aptamer sensor was developed to detect iprobenfos (Figure 10). In the absence of a target, the aptamer binds to the gold nanoparticles, preventing their polymerization, and the system appears red. When the target exists, the target binds to the aptamer. Due to steric hindrance, the aptamer can no longer attach to AuNPs, and AuNPs spontaneously aggregate and appear blue. Especially in the presence of salt ions, the aggregation reaction is promoted. The aptamer sensor has been successfully applied to the spiked rice samples, and the recovery rate can reach about 80%–90% (Kwon et al. 2015).

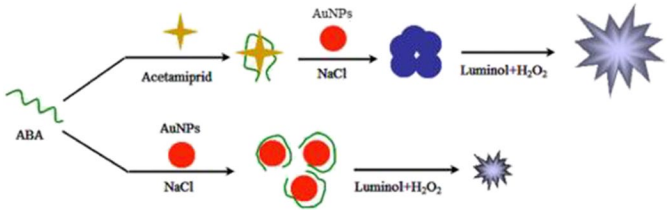


Figure 11. Schematic illustration of the proposed acetamiprid assay for acetamiprid detection (Qi et al. 2016).

In addition, enzyme inhibition methods are often combined with colorimetric biosensors for pesticide detection. Glutathione transferase (GST), belonging to the tau class, contains a crack in its structure, which has evolved to accommodate a wide range of hydrophobic substrates and non-substrate ligands. Non-substrate ligands are usually bound to L sites (ligand binding sites) located in different regions or overlapped with G sites and H sites. The binding of these compounds to the L site of GST affects the binding of normal substrates, thereby inhibiting the enzyme's catalytic activity (Reinemer et al. 1996). Based on these catalytic properties, the mutant enzyme Phe117Ile was studied to develop an optical biosensor for α-endosulfan. The enzyme was entrapped in the alkosixylane sol-gel system in two pH indicators (bromocresol purple and phenol red). The sensing signal was based on the inhibition of the sol-gel entrapped GST, with a subsequent decrease of released [H+] by the catalytic reaction, measured by sol-gel entrapped indicators. The assay response at 562 nm was linear in the pH range of 4–7. Linear calibration curves were obtained for α-endosulfan in the range of 0–30 μM (Chronopoulou et al. 2019).

3.2.3. Chemiluminescence biosensor

The chemiluminescence method is a mature detection technology. Since there is almost no background signal, it significantly improves the signal-to-noise ratio. This is an attractive method with the advantages of simple operation, good stability, and high sensitivity (Xu, Li, et al. 2018).

With the development of nanoscience, nanoparticles have been introduced into the Cl determination as a catalyst to improve the detection sensitivity. At the same time, the aptamer-Cl sensing platform involving nanoparticles has gradually attracted people's attention. Taking gold nanoparticles (AuNPs) as an example, in addition to the aforementioned colorimetric luminescence biosensors, their aggregation state can also affect the catalytic performance of Cl. In the Cl reaction system of luminol-H_2O_2, the catalytic activity of AuNPs in the aggregated state was higher than that in the dispersed form. Based on the above principles, an ultra-sensitive and selective acetamiprid chemiluminescence sensor was developed. In the presence of acetamiprid, the aptamer binds to the target AuNPs. AuNPs aggregated due to the lack of protection by the folded aptamer. It leads to changes in the catalytic performance of Cl, ultimately leading to changes in the chemiluminescence signal (Qi et al. 2016) (Figure 11). The detection limit can reach 62 pM, which is about 100 times lower than the LOD detected by other aptamer sensors, providing an effective method for the sensitive and selective detection of single-component pesticide residues.

3.2.4. Surface-enhanced Raman spectroscopy (SERS) sensor

Surface-Enhanced Raman Scattering (SERS) is a technique that enhances Raman scattering by molecules adsorbed on rough metal surfaces or by nano-scale rough metal surfaces supported by gold or silver. This technology can determine the chemical content of different molecular species by collecting molecular vibrations. It is a nondestructive analysis with high sensitivity (Xu, Li, et al. 2018).

Some studies have proposed a new type of unlabeled nucleic acid aptamer to detect trace malathion residues, a surface-enhanced Raman scattering (SERS) sensor. In this process, the binding of the malathion molecule and aptamer can be directly recognized. Silver nanoparticles prepared with positively charged spermine are used as capture reagents for negatively charged aptamers. Then, the aptamer-prepared silver nanoparticles were used to capture malathion precisely (Figure 12). The SERS background spectra of spermine, aptamer, and malathion were recorded and distinguished with the spectrum of malathion-aptamer. The selectivity of this method was verified in the mixed standard solutions of malathion,

Figure 12. Schematic illustration of label-free aptamer-based SERS sensor for detecting malathion (Nie et al. 2018).

chlorpyrifos, fluoride, and other pesticides. Moreover, this method has reasonable specificity and recovery rate, indicating that the SERS detection method can analyze malathion and other pesticides (Nie et al. 2018).

Another researcher has developed a new type of aptamer-based sensor for the detection of malathion. The aptamer was adsorbed on the surface of gold nanoparticles (AuNPs) to prevent AuNPs from aggregation in high-salt solutions. After adding malathion, the aptamer binds explicitly to malathion and is removed from the surface of AuNPs. The number of aptamers adsorbed on the surface of AuNPs was negatively correlated with the concentration of malathion. Unprepared AuNPs have different degrees of aggregation in NaCl solution. The different degrees of aggregation of AuNPs are catalytically reduced to Cu_2O of various sizes and exhibit different resonance scattering intensities. There is a good linear response between the intensity of the resonance scattering spectrum and the concentration of malathion. The detection limit of this method was 5.24 ng/L (15.86 pM), with satisfactory recovery and repeatability for apple samples (Lai et al. 2021).

3.3. Magnetic biosensor

3.3.1. Sensing system based on magnetic nanoparticles

Magnetic beads have been widely used in developing various biosensors in biomedical diagnosis, food safety, and environmental monitoring. Presently, reports on the use of magnetic beads ranging from nanometers to micrometers for separation and detection are not uncommon (Wang and Lin 2020). According to reports (Schaumburg, Carrell, and Henry 2019), 20–200 nm magnetic beads have more apparent advantages than those larger than 500 nm, such as larger specific surface area, faster reaction kinetics, less steric hindrance, and more uniform distribution. They have broad application prospects. For nano-scale magnetic beads, they usually play two roles in biosensors: (1) Used as a magnetic carrier to separate targets from complex samples to reduce background interference; (2) Used to amplify signals to improve the sensitivity of detection (Zou et al. 2019).

For example, Jiang et al. (2020) used functionalized magnetic nano-ions as a separation carrier. They used 6-carboxyfluorescein-labeled aptamers as probes to develop a rapid and sensitive detection method for organophosphorus pesticides using an "open type" fluorescence-magnetic biosensor. The aptamer hybridizes with the probe on the surface of the magnetic nanoparticle to form a double-stranded complex. When the aptamer binds to the target, it separates from the fluorescent probe, generating a fluorescent signal. This method's detection limits (LOD) for trichlorfon, glyphosate, and malathion of 72.20, 88.80, and 195.37 ng L^{-1} were consistent with gas chromatography results. This method has high sensitivity and provides an effective means for detecting a variety of organophosphorus pesticides.

In addition to the combination mentioned above of magnetic nanoparticles and optical biosensors to reduce the background signal as a magnetic carrier, there are still reports on the use of magnetic nanoparticles to amplify signals in pesticide detection to construct magnetic-electrochemical biosensors. Based on this, Jiao et al. (2017) developed a new type of magnetic-electrochemical sensor based on a new composite film (composed of carbon black and graphene oxide @ Fe_3O_4) to detect chlorpyrifos. This nanocomposite material produces a particular type of sensor film with a strong synergistic influence. On the one hand, the immobilization of the aptamer probe on the electrode can be significantly improved. On the other hand, graphene oxide can provide a larger surface area, and Fe_3O_4 is uniformly deposited on graphene oxide, which promotes electron transfer, thereby playing a role in signal amplification, and is used for sensitive detection of chlorpyrifos. The constructed biosensing platform has a linear range of 0.1–105 ng/mL for the detection of chlorpyrifos, and the detection limit was as low as 0.033 ng/mL. It shows good selectivity, reproducibility, and stability and can monitor chlorpyrifos in actual samples.

3.3.2. Sensing system based on magnetosomes

Magnetotactic bacteria, a type of bacteria that can move along a magnetic field, can synthesize membrane-coated magnetite (Fe_3O_4) or colloidal iron (Fe_3S_4) nanocrystalline particles in their cells, which are called magnetosomes (MTB) (Gareev et al. 2021). Scholars have always valued the research on magnetosomes synthesized by magnetotactic bacteria. Compared with artificial magnetic materials, magnetosomes have various excellent properties and can be widely used in multiple fields, such as immunomagnetic separation, detection technology, and targeted tumor therapy. The MTB particles are uniform, with stable crystal and magnetic properties, and the surface is covered by a lipid film (Ren et al. 2018). Because of these features, MTB can be used as a carrier for various compounds and biomolecules, and multiple groups on the lipid membrane can connect to compounds or anchor proteins (Mickoleit, Lanzloth, and Schuler 2020).

MamC and MamF are the two most abundant proteins in the magnetosome membrane. They exist in a transmembrane helical structure and are closely attached to the magnetosome. Therefore, MamC and MamF are often used in the magnetosome display of proteins with different functions. Presently, the widespread application of molecular biology techniques has promoted the binding of Nanobodies to MTB. Nanobodies chemically coupled to magnetosomes have proven effective tools for detecting environmental compounds (He et al. 2018). Therefore, it is also very promising to display Nbs on magnetosomes through genetic engineering to detect pesticide residues. Recently, Wu, Ma et al. (2021) declared nano-antibodies on magnetosomes through gene fusion for the first time, constructed functional magnetosomes for fipronil, and combined them with ELISA for detection. As a reaction site, MTB has shown vast application prospects in the field of pesticide detection.

Table 4. Representative examples of pesticide residue detection based on immunological techniques.

Analysis method	Recognition element	Target pesticide	LOD	IC50	Sample	Advantages	Reference
ELISA	Antibody fusion protein	Triazophos	–	6.6 ng/mL	Water, soil, and apple	Highly selective, high-affinity	(Wang et al. 2019)
ELISA	VHH	Carbaryl	0.3 ng/mL (10% methanol and 0.8% NaCl)	5.4 ng/mL (10% methanol and 0.8% NaCl)	Rice, maize, and wheat	Simple, sensitive, and cost-effective screening	(Liu et al. 2019)
Ic-ELISA	VHH	Carbofuran	0.65 ng/mL	7.2 ng/mL	Chinese cabbage, cucumber, and orange	Small size, high solubility, high stability, and strong resilience to organic solvents	(Zhang, Wang et al. 2019)
Ic-ELISA	scFV	Triazophos	–	1.73 ng/mL	Water	High affinity and good binding stability	(Liu et al. 2016)
ELISA	VHH	Cyantraniliprole, chlorantraniliprole		1.2 ng/mL, 1.5 ng/mL	soil and bok choy,	Satisfactory accuracy and precision	(Xu, Wang, et al. 2021)
Colloidal gold immunochromato-graphic assay	VHH	3-PBA	0.1 ng/mL		Lake water	High sensitivity, rapid detection, and low-cost	(Zhang et al. 2021)
FEA	VHH fusion protein	3-PBA	0.011 ng/mL	0.082 ng/mL	Urine	High sensitivity and fast testing velocity	(Huo et al. 2018)
FEA	VHH fusion protein	Parathion	0.2 ng/mL	1.6 ng/mL	Chinese cabbage, cucumber, and lettuce	Highly efficient in signal enhancement, simple, and readily available	(Zhang et al. 2018)
Ic-ELISA	Bispecific antibodies	Fluoroquinolones, sulfonamides	–	0.45 ng/mL,0.75 ng/mL	–	Highly affinity and specificity	(Chen et al. 2014)

4. Analytical strategy based on immunological techniques

With the continuous development of immunology technology, immunoassay methods based on radioisotope, fluorescein, and colloidal gold antibodies have been developed to quantify the analytes based on readily measurable signals (Jya et al. 2020). Therefore, immunological analysis can be classified according to the marker materials into radioimmunoassay, enzyme immunoassay, fluorescence immunoassay, and chemiluminescence immunoassay (Khan et al. 2021). For the detection of different samples, each method has its advantages. The most commonly used immunoassay techniques in pesticide detection are colloidal gold-based immunochromatography, enzyme-linked immunosorbent assay, and fluorescence immunoassay. Hence, this chapter highlights the detection methods based on genetic engineering and immunological analysis techniques from the abovementioned aspects. Table 4 lists the representative examples of pesticide residue detection based on immunological techniques.

4.1. Colloidal gold-based immunochromatographic assay

Colloidal gold-based immunochromatographic assay, also known as lateral flow immunoassay technology, is an immunoassay technology that uses colloidal gold as a tracer marker for the rapid quantitative or qualitative detection of analytes in samples. In addition, the technique is stable and sensitive.

Traditional immunochromatography techniques mostly use monoclonal antibodies or polyclonal antibodies. With the rapid development of genetic engineering technology, to overcome the shortcomings of poor thermal stability and weak accessibility of traditional antibodies, various small-size antibodies have gradually become a research hotspot in immune analysis technology. In this context, Zhang et al. developed a colloidal gold immunochromatography method based on nano-antibody to detect the metabolites of pyrethroid pesticides, 3-phenoxybenzoic acid (3-PBA). The detection limit of this method was 0.1 ng/L, which is suitable for the rapid qualitative detection of 3-PBA (Zhang et al. 2021).

Liu, Tang, et al. (2021) developed an aptamer-based lateral flow biosensor to overcome the stability of traditional antibodies in detection.(Figure 13) The aptamer was used as the recognition element of the alternative antibody. The aptamer could bind to the gold nanoparticles through electrostatic adsorption, and the conjugate was sprayed on the conjugate pad, and cysteamine was sprayed on the detection line (T). When the target isocarbophos was present in the sample solution, the aptamer would dissociate from the surface of AuNPs following specific binding to the target. When the exposed AuNP flows to the T line, the thiol group in cysteamine binds to AuNPs through the Au-S bond and forms a red band at the T line. When there were no isocarbophos in the sample, the binding sites on the surface of AuNPs were occupied by aptamers and could not bind to cysteamine. Thus no coloration was observed. The detection limit of this method was 2.48 μg/L, which can

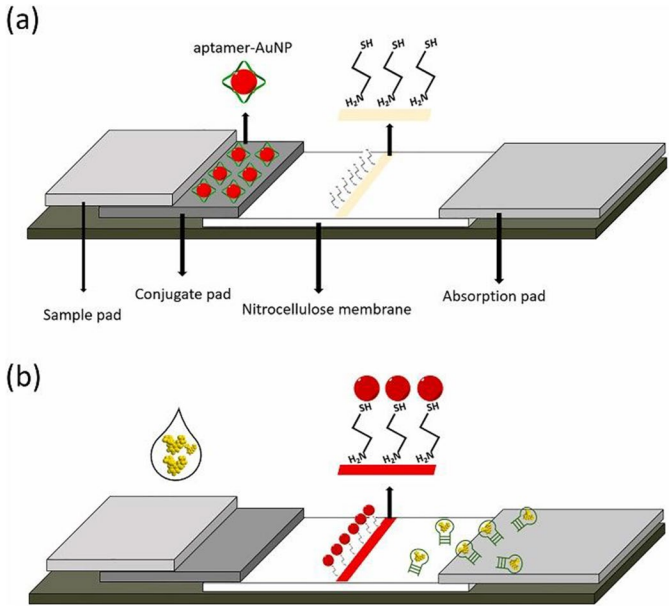


Figure 13. Schematic diagram of isocarbophos detection on the colloidal gold immunochromatographic assay. (a) The basic concept and description of negative control-no color change without isocarbophos. (b) Color change in T line in the presence of isocarbophos (Liu, Tang, et al. 2021).

distinguish whether the isocarbophos exceeds the standard within 1 min. It has good accuracy and practicability and has broad application prospects.

4.2. Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) is a highly sensitive immunoassay technology, which combines the specific reaction of antigen-antibody with the efficient catalytic effect of the enzyme on the substrate. Fix the antigen or antibody in the solid phase. After the antigen-antibody reaction was balanced, the excess free substance was washed out. The antigen-antibody reaction was detected by measuring the intensity of the chromogenic enzymatic response combined with the enzyme marker catalyzed chromogenic reaction on the solid phase.

Although single-chain antibodies largely compensate for the shortcomings of monoclonal antibodies, applications of single-chain antibodies are also limited by their low affinity. At present, there are many methods to improve the affinity of the single-chain antibodies, such as affinity maturation, penetration mutagenesis, and polymerization. One of the most convenient methods is to fuze single-chain antibodies with sensitive labels. Therefore, biotin-streptavidin is a promising option. In some studies, scFv was fused with the biotin receptor domain (BAD) and expressed at a high level in *E. coli* BL21 (DE3) in the form of inclusion bodies. Then, the protein was refolded by step dialysis with gradient urea solution and then biotinized by BirA enzyme. Finally, the single-chain antibody was purified. A sensitive, broad-specific competitive indirect enzyme-linked immunosorbent assay was developed using the obtained single-chain antibody to determine multiple residues of organophosphorus pesticides. The method can detect 30 organophosphorus pesticides, and the IC_{50} was between 19.4 and 515.2 ng/mL (Zhao et al. 2016).

In addition, a competitive direct enzyme-linked immunosorbent assay was developed to detect O, O-diethyl organophosphorus (O, O-diethyl OPs). In this method, a single-chain antibody (scFv) was used to connect alkaline phosphatase (AP) fusion protein, and VL and VH genes were cloned from hybridoma cells secreting monoclonal antibodies with broad specificity for O, O-diethyl OPs to prepare monoclonal antibodies. The linker ligated the amplified VL and VH regions to obtain the scFv gene, which was cloned into the expression vector pLIP6/GN containing AP gene to produce scFv-AP fusion protein in *E. coli* BL21. The obtained protein retains both antigen-binding specificity and AP enzyme activity. The analysis of standard addition, blind river water, and Chinese cabbage samples showed that the method had good sensitivity and reproducibility (Xu et al. 2012).

In addition to using antibodies as receptors for ELISA detection, Xiang et al. (2019) also developed an aptamer-based indirect competitive enzyme-linked immunosorbent assay for specific and sensitive detection of isocarbophos. The signal amplification was carried out by the affinity between aptamer and target substance and the catalytic color reaction of HRP enzyme, which showed that the method had good sensitivity and selectivity for detecting isocarbophos in water samples. The detection limit was 0.05 ng/L, and the sensitivity was better than the traditional ELISA method.

4.3. Fluorescence immunoassay (FIA)

Fluorescence immunoassay (FIA) requires the use of specific fluorescent substances to label antigens or antibodies. The antigen-antibody reaction is tracked and detected through the specificity of antigen-antibody reaction and the high sensitivity of fluorescence detection, which has the advantages of high sensitivity and easy labeling. The classical FIA was based on organic fluorescent molecules as markers, such as fluorescein isothiocyanate (FITC) and tetramethyl rhodamine isothiocyanate (TRITC). However, most organic fluorescent markers have a short fluorescence lifetime. In practical applications, due to the scattering of samples, actual self-fluorescence, and excitation light, the reliability and sensitivity of the influence measurement limit the application of FIA to a large extent (Zhang et al. 2020).

Nano-antibody has the advantages of high sensitivity, good stability, and easy expression, which can be used for immunoassay of pesticide residue detection. In some studies, six parathion VHHs were isolated from the phage display library, and a VHHH9 with the highest specificity and excellent thermal stability was screened out. The VHH9 was fused with alkaline phosphatase to establish a direct competitive fluorescent enzyme immunoassay (Zhang et al. 2018). The IC50 for the determination of parathion was 1.6 ng/mL, and the detection limit was 0.2 ng/mL. The sensitivity was 3–4 times that of direct competitive enzyme-linked

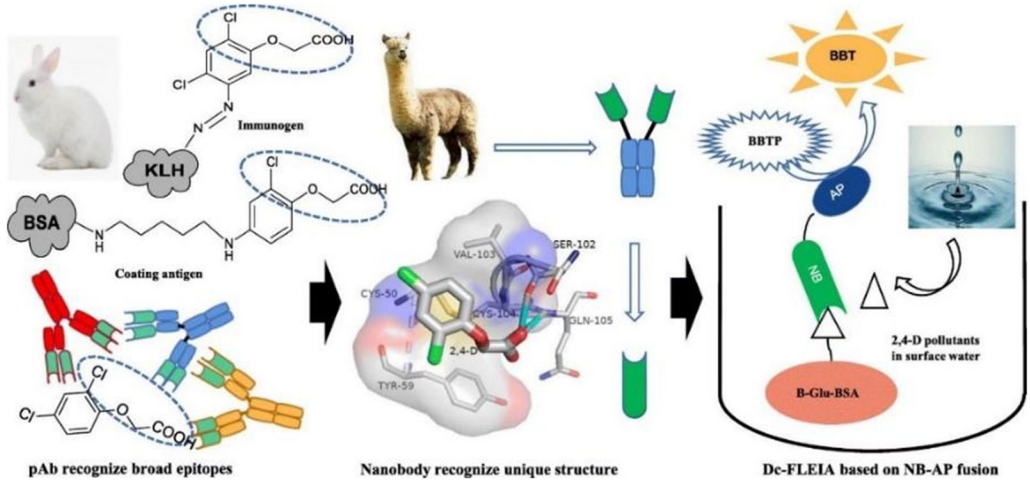


Figure 14. Schematic diagram of one-step FIA method for detecting 2,4-D by the nanobody-AP fusion protein (Li, Dong, et al. 2021).

immunosorbent assay and indirect competitive enzyme-linked immunosorbent assay.

In addition, to develop an immunoassay based on nanoparticles to monitor the trace level of 2,4-D, a step-by-step strategy was adopted to generate highly specific nanoparticles for this small molecule (Figure 14), A one-step fluorescent enzyme immunoassay was developed by hapten design, rabbit/camel antiserum evaluation, and 2,4-D high-specific nanobody-AP fusion expression. IC50 was 1.9 ng/mL, and the linear range was 0.4–8.6 ng/mL. Fluorescent enzyme immunoassay (FLEIA) and LC-MS/MS analyzed the environmental water samples, and the results were consistent. Therefore, the proposed step-by-step strategy from hapten design to nanobody-AP fusion production proved that nanobody-based FLEIA was a convenient tool for monitoring 2,4-D residues in environmental samples (Li, Dong, et al. 2021).

5. Conclusions and future perspectives

This paper reviewed the modification of recognition elements in pesticide detection based on genetic modification technologies. We listed the applications of these recognition elements from the perspectives of biosensors and immune analysis. At present, the molecular biology technique has been used in many disciplines and has made many breakthroughs. It can be used to address challenges identified with classical methods of pesticides detection. However, it is still in the primary stage of development in transforming identification components for rapid detection of pesticide residues. Therefore, there are still many challenges to make up for the shortcomings of recognition elements with the help of advanced molecular biology tools.

On the one hand, to make the rapid detection technology more effective, the use of directed evolution, surface display technology, and the introduction of particular amino acids to improve the activity, stability, and selectivity of recognition elements will become a complex and arduous task in the transformation of recognition elements. On the other hand, the sensitivity of the detection is closely related to the specificity of the recognition element. To further improve the specificity of the recognition element, we can encode the sensing and the reporting domain, combined with molecular modeling and calculation in related fields, which will become another challenge in this field.

Authors' contribution

Mingqi Zhao, Miao Wang, Jing Wang and Guangyue Li discussed the contents, wrote, and reviewed the manuscript. A. M. Abd El-Aty edited the manuscript. The final manuscript was read and approved by all authors.

Disclosure statement

The authors declare no conflicts of interest.

Funding

This research was funded by the National Key Point Research and Invention Program (No. 2019YFC1605600), Agricultural Science and Technology Innovation Program of CAAS (CAAS-ZDRW202011), National Natural Science Foundation of China (Nos. 32072313; 31772071); and Introduction of Foreign Intelligence Programs of Shijiazhuang City (No. 20210017).

ORCID

Zhen Cao http://orcid.org/0000-0003-1259-6107
A. M. Abd El-Aty http://orcid.org/0000-0001-6596-7907

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