# Optical Detection of Targets for Food Quality Assessment

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**Abstract**

Current technics used in food quality assessment require complex equipment and professional personnel, which has financially burdened the food industry. Optical devices are a powerful candidate which can provide simple, cost-effective, and rapid detection approaches with the possibility to be easily manufactured in a large scale to cover the vast need of this industry. This chapter will first present an overview regarding food safety and common analytes. Various optical sensing technics, including colorimetric, fluorescent, chemiluminescent, surface-enhanced Raman scattering (SERS), and surface plasmon resonance (SPR) methods will then be explored with examples of novel sensing platforms developed for food monitoring.

**Keywords**

Optical detection · Food quality · Biomarkers · Biological receptors · Analytical performance

## 5.1 Introduction

Continuous supply of healthy and quality food for citizens has always been one of the main concerns in all countries. Over the past three decades, hazard analysis, control of critical points, food production techniques, and standard health performance methods have aimed to meet this need. The identification of biological and

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chemical contaminants is of critical importance in order to ensure the healthy nutrition of consumers. In that regard, different analysis techniques for food safety analysis, preservation, and elevation of food quality have been developed which vary depending on the type of food. Despite the many efforts for preservation and quality control, countries are still plagued by the economical and health burdens of food fraud, the entry of substandard food into the market, and the resulting foodborne illness. To overcome these problems, food safety control and quality assessment are of vital significance which persist as a continuous discussion point for nations (Ragavan and Neethirajan 2019; Shams et al. 2020).

Food safety is the process of ensuring zero contamination, be it chemical or biological, during the preparation and storage of food. So far, suitable analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectrometry for chemical contaminants and cell culture methods, biochemical identification, and polymerase chain reaction (PCR) for pathogen analysis have been developed to analyze food contaminants as standard techniques. However, these approaches have many downfalls including being laborious and costly, requiring complex sample preparation steps, and needing the assistance of a professional technician and high-tech instrumentation (Narsaiah et al. 2012). Hence, developing rapid, simple detection methods is a dire requirement, especially in developing areas which do not have advanced laboratories for conventional detection. Biosensors offer selectivity and compact size, relatively high sensitivity, low cost, rapid response times, and are user friendly to operate (Chandra et al. 2012; Choudhary et al. 2016; Deka et al. 2018; Mahato et al. 2018; Verma et al. 2019).

Optical techniques, as a result of their simplicity, selectivity, and stability have dominated the world of sensing. Owing to their merits, they have become a popular approach for the identification of food analytes. Optical detection assays are techniques in which the identification of the target analyte is transduced to visible, ultraviolet or infrared (IR) radiations which can be detected by the naked eye or using spectroscopy methods (Damborský et al. 2016).

In this chapter, optical methods for food safety analysis will be discussed. We will first introduce the various kinds of food contamination including both chemical and biological contaminants. A scope of the developed optical sensing assays, classified in colorimetric, fluorescence, chemiluminescence, SERS, and SPR-based methods for the detection of these analytes will then be presented.

## 5.2 Food Safety Analytes

Acquiring nutritious food is very important to maintain a vigorous lifestyle and promote health. In the past, the significance of food contamination was underestimated due to the lack of reports related to food safety and the difficulty in analyzing the relationship between contaminated food and various diseases. There were also many shortcomings internationally in the knowledge, attitude, and behavior of food consumers suffering from serious diseases (Marklinder et al. 2020).

Finally, the significance of food safety in health was realized and in 2015, the World Health Organization (WHO) published an article estimating the global burden of foodborne diseases. This article showed that only 31 types of food contaminants lead to 32 diseases that cause 42,000 deaths per year (Griesche and Baeumner 2020). Compared to industrial countries, developing nations are harder afflicted by the repercussions of food contamination. Recently, food safety has attracted much attention due to the understanding of its heightened importance and also, the accumulation of knowledge regarding the presence of various contaminants in food samples and how they impact the health of the consumer. Some examples of such contaminants are heavy metal in the environment and how they pollute the water resources, improper use of pesticides and antibiotics, and other pollutants and toxins that accumulate in various plants and animals. Also, numerous pathogens including viruses, bacteria, and parasites can also contaminate food samples at various stages of agricultural plantation, production, processing, storage, and delivery (Lu et al. 2020).

In order to effectively develop sensing platforms for food monitoring, an understanding of common contaminants is necessary. Furthermore, the development of new receptors, including antibodies and aptamers, with a strong affinity toward specific molecules has been instrumental in developing sensitive and rapid diagnostic methods for hazardous substances in food samples (Caglayan et al. 2020). In this section, a brief description of the various food contaminants has been presented.

### 5.2.1 Pathogens

Microorganisms have always been present in food, drinking water, the sea and rivers, soil, and human intestines. Many of these microorganisms are quite beneficial for the environment and also our overall health like probiotic bacteria, but some are pathogenic and can cause many major or sometimes quite fatal problems. Pathogenic microorganisms include bacteria, viruses, and parasites.

Bacteria are among the most common of foodborne pathogens and exist in a variety of types, many of which are responsible for deadly food-related illnesses. Numerous pathogenic bacteria have been identified in food samples so far, with *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella typhimurium* being three of the most important and fatal pathogens (O'Kennedy et al. 2005; Jokerst et al. 2012). Also some bacterial pathogens generate spores with high resistance to heat treatments such as *Clostridium botulinum, Clostridium perfringens, Bacillus subtilis*, and *Bacillus cereus*, and some are able to secrete heat-resistant toxins such as *Staphylococcus aureus* and *Clostridium botulinum*. Although most bacteria are mesophilic and their growth is inactivated in temperatures below 20 °C, some species such as *Listeria monocytogenes*, and *Yersinia enterocolitica* can propagate in the refrigerator and contaminate food samples in colder temperatures (Bacon et al. 2003).

Viruses are another group of foodborne pathogens. To date, over 100 different enteric viruses have been reported to be the source of foodborne illnesses, among

which Hepatitis A and noroviruses are the most common. Most viruses are transmitted via bivalve molluscs such as clams, cockles, mussels, and oysters (Gosling 2008). Waters are also increasingly prone to virus contamination through sewage discharge and waste disposal from infected shellfish harvesters. These viruses accumulate for days in the shellfish digestive tract. Health problems arise as most shellfish are eaten raw, with their digestive tracts in place. The shellfish themselves also act as a protective barrier for the viruses against thermal inactivation (DiGirolamo et al. 1970).

Another important group of foodborne pathogens are parasites, which are known as single-celled microorganisms with an organized nucleus. Similar to viruses, the propagation of parasites is dependent on the host, thus they do not multiply in food samples. Cysts are the transmissible form of parasites which can be transmitted from animals or other humans to humans. These organisms live and procreate usually in the digestive tract of the infected host and are excreted in the feces, thus fecal transmission is quite common. A parasite infection can lead to severe malnourishment in the host. *Cyclospora cayetanensis*, *Toxoplasma gondii*, and *Trichinella spiralis* are the most common foodborne parasites (Bintsis 2017).

### 5.2.2 Toxins

Toxins are toxic chemicals produced by a living organism such as microorganisms, plants, and animals, which can pose serious health risks. Toxins are naturally present in food and can contaminate food during various stages, from production to processing and packaging, and even delivery. Although many foodborne illnesses are caused by bacteria, such as salmonellosis, campylobacteriosis, toxins secreted by bacteria during growth can also lead to severe health problems. *Staphylococcus aureus* and *Clostridium botulinum* are two bacterial species with the ability to produce heat-resistant toxins (Ligler et al. 2003; Hodnik and Anderluh 2009).

Mycotoxins, defined as toxins produced by various moulds (fungi), can contaminate a wide range of food samples from fruits and vegetables, to nuts and dry cereals. Aflatoxins are among the most poisonous kind which are generally produced by *Aspergillus flavus* and *Aspergillus parasiticus* on cereals, tree nuts, oilseeds, and even some spices. This toxin is also found in the milk of animals fed with contaminated feed. Aflatoxins are especially hazardous as they are known to damage the DNA which can in turn lead to cancer. Other mycotoxins include ochratoxins (produces by *Aspergillus* and *Penicillium* species), deoxynivalenol and zearalenone (produced by *Fusarium* fungi), and ergot alkaloids (produced by *Claviceps* species) (Abrunhosa et al. 2016).

Plants, as a defense mechanism against predators, insects, or microorganisms, are also known to create toxins (Yamane et al. 2010). Microscopic algae and planktons in oceans and in lakes create chemical compounds which are non-toxic to fish and shellfish, but can have an adverse effect on humans who eat seafood containing these toxins (Campàs et al. 2007).

### 5.2.3 Heavy Metals

With the development of countries, heavy metal industries ensued which led to their entrance in the human life. Heavy metals and their respective ions are metals with an atomic weight between 63.5 and 200.6 g $mol^{-1}$ and a specific gravity greater than 5 g $cm^{-1}$. Organisms need a minimum amount of specific heavy metals such as cobalt (Co), molybdenum (Mo), vanadium (V), zinc (Zn), and strontium (Sr). However, other highly toxic metals such as arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) have irreversible effects on the human health and ecosystems, even in very small amounts. Heavy metals are present in the earth's crust. Therefore, they become incorporated with food and water resources through agriculture and industrial processes, and as a result become consumed by humans and other organisms in various ways.

The toxicity of heavy metals derives from their interference in the body's biochemical and metabolic reactions, such as digestion. If heavy metal ions in drinking water are higher than the permitted amount, severe and dangerous diseases such as cancer, cardiovascular disease, brain damage, kidney failure, and nervous system complications will occur. Traditional heavy metal diagnostic methods such as atomic absorption spectroscopy have an acceptable detection limit but, have limitations such as their requirement of complex instrumentation and being costly (Wang et al. 2020a).

### 5.2.4 Pesticide Residues

Pesticides are widely used in agriculture to destroy or control various pests that can blemish the quality of crop. Pesticides are used as insecticides, fungicides, herbicides, and other types which help ensure the maximum quality of food. Based on their chemical structures and functionality, synthetic pesticides are classified into five classes: organochlorine, organophosphate, carbamate, neonicotinoid, and pyrethroid. Most pesticides are designed to attack pests, but they also adversely endanger humans, the environment, and wildlife and are considered among the most hazardous pollutants (Wang and Zhou 2014; Trojanowicz and Hitchman 1996). Furthermore, the more they perpetuate in the natural cycle, their concentration, and toxic intensity accumulates. Therefore, the study of different ways to diagnose pesticides is of crucial importance.

### 5.2.5 Veterinary Drug

Different types of veterinary drugs, including growth factors and antibiotics, are extensively used in livestock and poultry these days to prevent disease and help fight infections and also to induce growth. These drugs can accumulate in animal tissues and cause problems in humans in a variety of ways. 6051 ton of various active ingredients are used as veterinary medicines. Some of these drugs get excreted as

urine and feces from the animals' body and can then be used as fertilizers thus entering the environment. The continuous entry of these substances into the environment and long-term accumulations in the human body can cause persistent side effects such as allergies and bacterial resistance (Wang et al. 2020b).

### 5.2.6 Illegal Additives

In the food industry, certain substances known as preservatives are used to prevent microbial growth and make food seem pristine. For example, sodium and potassium salts of nitrite and nitrate are added to meat, fish, sausages, and cheese to preserve the products. They can also cause a better appearance by impacting the color and change the taste of these foods. However, excessive use of nitrite leads to immediate toxic reactions such as abdominal pain, vomiting, and decreased blood oxygenation. WHO has set a high daily intake of 3.7 mg $kg^{-1}$ of nitrate and 0.07 nitrite mg $kg^{-1}$ of body weight (Thongkam and Hemavibool 2020).

Melamine, which is another type of food additive, has become the focus of the food industry in the world. Melamine is added to milk and dairy products to increase their protein efficiency. Maximum residue levels (MRLs) are allowed to be added to milk powder in China and the United States at 1 and 2.5 mg $kg^{-1}$, respectively. Given the importance of dietary supplements and the adverse effect they can have when exceeding the permitted level, they should be identified and quantified by rapid and sensitive laboratory techniques (Boutillier et al. 2020).

## 5.3 Detection Based on Optical Sensors

The word sensor is derived from the Latin word *sentire*, meaning recognition. These devices are made of three sections including a sensing element, a transducing element, and a detector. The sensing element (antibody, enzyme, nucleic acid, cell, aptamer, bacteriophage, and microorganism) receives a physiological response and transfers it to the transducer (optical, electrochemical, mass-based), and finally, the signal is detected by the detector. Depending on the transducer, sensors can be divided into different groups including optical, electrochemical, acoustic wave, and piezoelectric sensors (Bahadır and Sezgintürk 2017). Biosensors are used for a large number of applications within the field of biotechnology, including medical diagnosis, pharmaceutical analysis, and food. One of the best types of sensors for the detection of contaminating microorganisms and substances in food samples are optical and electrochemical sensors (Huet et al. 2010).

Due to numerous advantages such as the possibility of remote control in hazardous environments, acceptable sensitivity and high stability, optical sensors are extensively used for the rapid detection of food contaminants. Furthermore, they enable the detection at different wavelengths and consequently provide a platform to simultaneously detect various parameters (Ohk and Bhunia 2013). The various optical geometries used in sensing assays include optical fibers, planar wave guides,

surface plasmon resonance, and microarrays. Optical biosensors measure changes in the phase, frequency, and amplitude of light (Narsaiah et al. 2012). They are divided into different types according to the transmission mechanism, including colorimetric, fluorescence, chemiluminescence, surface-enhanced Raman scattering (SERS), and surface plasmon resonance (SPR) (Silva et al. 2018).

Nanotechnology has impacted various fields of science and technology including the research for the development of efficient detection assays. NMs, with a high surface-to-volume ratio, demonstrate interesting optical and electrical properties which have led to their immense employment in the development of optical sensors. Compared to other nanomaterials, gold nanoparticles (AuNPs) have been tremendously used in many optical sensors due to their unique properties such as catalytic behavior, SPR, unique optical properties which results in the color change as they aggregate or dissociate, fluorescence emission (as seen in gold nanocrystals), fluorescence quenching, etc. To this day, and by taking advantage of the many merits of AuNPs, numerous sensing approaches have been proposed based on these nanomaterials for a multitude of reasons. The first reason is related to the compatibility of gold nanoparticles with various organic and metallic molecules, which leads to their natural reaction with molecules. Second, when gold nanoparticles are synthesized by the citrate method, due to their charge properties, the probability of their interaction with other molecules increases. The third is the high surface-to-volume ratio, which increases analyte detection. The last and fourth reason is associated with their ability to adjust to different conditions through creating a change in morphology, size, and synthetic environment (Naderi et al. 2018; Upadhyayula 2012).

In this section, we will examine various types of optical sensors for food analysis and present novel proposed sensing assays based on colorimetric, fluorescence, chemiluminescence, SERS and SPR methods for food safety analysis.

### 5.3.1 Colorimetric

Colorimetric sensors have been developed as an emerging and suitable sensing platform for food analysis and chemical screening. The colorimetric method has advantages over other approaches such as cost-effectiveness, facile portability and on-site application, repeatability, no need for a specialized operator, and the possibility to detect the results by the naked eye. Currently, colorimetric sensors play an important role in improving the safety and quality of food. In addition, it is possible to strengthen them through the development of nanotechnology, sample preparation and handling, and incorporation of more efficient reagents (Mesgari et al. 2020; Dehghani et al. 2019).

Colorimetric sensors determine sensitivity, response time, specificity, and signal-to-noise ratio by two essential factors:

1. Cognition elements that cause proper interaction between the analyte and the sensor targeting component.

2. Conductive materials or NMs with suitable optical properties that provide the appropriate color change response in the visible region (390–700 nm) after analyte recognition.

Colorimetric sensing approaches depending on the targeting technique can generally be classified in immunosensors, in which antibodies act as the target detection unit, and aptasensors, in which aptamers are used to capture the target; although less common targeting molecules are also used. Numerous approaches have been reported to generate the color readout signal in these sensors, the most predominant of which are NM-based sensors especially with Au or Ag nanoparticles, enzyme-based sensors such as horseradish peroxidase (HRP), DNAzyme-based sensors, and sensors using enzyme mimics. (Maduraiveeran and Jin 2017).

Many targeting elements can be used for specific detection on colorimetric assays, including antibodies, enzymes, nucleic acids and aptamers, receptor ligands, etc. Aptamers, defined as single-stranded oligonucleotides with a specific secondary structure which can bind to and detect various molecules, are gaining a lot of attention due to being more cost-effective and stable compared to antibodies (Hosseini et al. 2015).

AuNPs are used in colorimetric sensors due to their molecular interactions and visible color change which arises from the aggregation or dissociation of the NPs. In general, when AuNPs are dispersed, they appear red, but when they accumulate the solution color changes to purple. Based on this phenomenon, several colorimetric sensors have been designed to detect food pathogens. Acetamiprid in fruits and vegetables was detected in a simple and sensitive colorimetric method based on gold nanoparticles. Acetamiprid has a cyano group that induces accumulation of gold nanoparticles and changes the solution color from red to purple. The linear range of acetamiprid detection in this approach based on gold nanoparticles with a diameter of 15 nm and 22 nm is 6.6–66 μM and 0.66–6.6 μM, respectively (Yang et al. 2017).

Due to their high light absorption properties, silver nanoparticles also have many applications in colorimetric biosensors and, like with gold nanoparticles, the amount of light absorption by AgNPs can be adjusted by controlling their size, shape, and environmental conditions. Silver-based nanomaterials have unique electrical and catalytic properties and optical properties such as photoluminescence and SPR (Abou El-Nour et al. 2010). A rapid, sensitive, selective, and simple colorimetric assay based on AgNPs was reported to detect melamine in raw milk. Melamine can induce the aggregation of AgNPs via three amine groups in its structure and leading to solution color change from yellow to red. The proposed method can be used to detect melamine in raw milk, with a detection limit of 0.01 mM (Alam et al. 2017).

Numerous nanostructures have proven to possess peroxidase-mimicking activity; thus, they can be used to replace HRP in colorimetric sensors (Dehghani et al. 2019; Alam et al. 2017). Exploiting this phenomenon, a colorimetric biosensor was developed for the detection of *Campylobacter jejuni* with a detection limit of 100 CFU $mL^{-1}$. In this method, specific aptamers and Au@Pd nanoparticles were used. Interaction of free aptamers with the surface of the nanoparticle inhibits their catalytic activity and decreases 3,3′,5,5′-tetramethylbenzidine (TMB) oxidation;

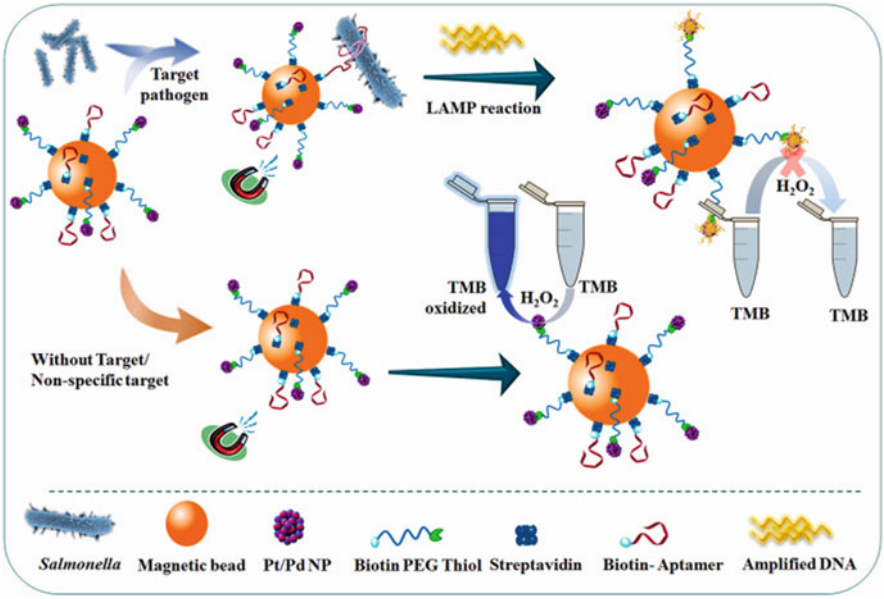


**Fig. 5.1** Schematic representation of a colorimetric aptasensor used for the detection of *Salmonella* based on loop-mediated isothermal amplification (LAMP) and Pt/Pd nanoparticles with peroxidase-mimicking activity (Dehghani et al. 2021)

whereas in the presence of the target bacteria, the aptamers attach to it, and TMB can be oxidized generating a blue-colored solution (Dehghani et al. 2018). In another study, magnetic beads modified with Pt/Pd nanoparticles and aptamers were used for the specific detection of *S. typhimurium* based on loop-mediated isothermal amplification (LAMP) (Dehghani et al. 2021). In the presence of the target bacteria, LAMP reaction based on *Salmonella*-specific primers led to the generation of amplified DNA sequences which, when absorbed on Pt/Pd NPs, inhibited their peroxidase-mimicking activity. In the absence of the target bacteria, Pt/Pd NPs created oxidized TMB, which led to a blue-colored signal (Fig. 5.1).

Another colorimetric method was developed to detect ochratoxin A (OTA) in wine based on a DNAzyme-aptamer sensing element. The DNAzyme part mimics the peroxidase properties of HRP enzymes and is connected to the OTA aptamer sequence through a hairpin loop. As shown in Fig. 5.1, when the OTA is in the environment, it interacts with the aptamer forming a complex. Thus, the hairpin opens, and the DNAzyme is free to create a blue color by oxidation of TMB to TMBox based on its peroxidase-mimicking property. The DNAzyme-based sensor is linearly correlated with the OTA concentration to 10 nM, showing a limit of detection of 2.5 nM (Yang et al. 2012).

### 5.3.2 Fluorescence

Fluorescence is very promising compared to other food analysis methods due to its low cost, high sensitivity, and simplicity, rapid hybridization kinetics, easy operation, and convenient automation, which have led to its immense employment in the design of sensors. Fluorescence emission is defined as the release of energy by specific molecules, known as fluorophores, at longer wavelengths and with a lower energy than the wavelength they receive upon excitation from the ground state to an excited state. The fluorescence process consists of three stages:

1. The electron of the fluorophore molecule is excited by energy absorption through photons at a specific wavelength.
2. An unstable state is created for the excited electron with a tendency to return to the ground state.
3. The electron emits energy in the form of light at a longer wavelength to return to the ground state.

In fluorescence, the two important spectra of excitation and emission are considered for each molecule. The excitation and emission spectra are taken simultaneously by determining a constant distance between the excitation and the emission wavelength (Ahmad et al. 2017).

The principles of fluorescence analysis depend on the analyte interaction with the sensing elements, which can alter the fluorescence properties. There are five major principles for fluorescence signal output as follows:

1. Fluorescence quenching.
2. Increased fluorescence intensity.
3. Shifts in the emission wavelength.
4. Fluorescence lifetime variations.
5. Fluorescence resonance energy transfer (FRET).

Many molecules display natural fluorescent, some of which may display fluorescence in one state and but be non-fluorescent in another. For example, the NADH molecule is fluorescent whereas $NAD^+$ lacks this property. Therefore, through inducing the change from NADH to $NAD^+$ and creating a change in the fluorescent behavior in the sensors, the target molecule can be detected.

Most analytes are non-fluorescent, thus labels must be used to detect them by fluorescence methods. Labels are materials that can be attached to the analyte by covalent bonding with the help of hydroxyl, amine, carboxyl, or sulfhydryl groups. Probes are another solution to create fluorescence for this purpose, but probes have a high response to environmental variations such as ions, pH, and oxygen. Organic dyes and nanomaterials like AuNPs, AgNPs, and carbon nanotube (CNTs) can be used as labels. Required criteria for selecting fluorescent labels include high excitability, the ability to generate a strong and recognizable signal, having a specific functional group for connection, high fluorescence quantum yield, and high molar

absorption coefficient. Fluorescent organic dyes such as rhodamine, fluorescein, cyanine, and coumarin also have some limitations for applications in sensing due to their low solubility, poor bioavailability, toxicity, and narrow excitation. Fluorescent nanomaterials such as metal nanoparticles and nanoclusters, quantum dots, and carbon and graphene dot have many merits over organic dyes but their wide applications for food analysis is also limited due to their complex synthesis, high toxicity, and low biocompatibility (Kermani et al. 2017; Borghei et al. 2017; Pebdeni et al. 2020; Dehghani et al. 2020).

In this sensing platform, as with others, a target molecule can increase the sensitivity and selectivity of the sensor by a multitude. In fluorescent biosensors, aptamers are an excellent option for targeting elements. Unlike antibodies, peptides and enzymes, aptamers have high flexibility for easy functionalization and chemical modification, and are more stable to environmental change. Therefore, fluorescent aptasensors to detect a wide range of analytes (Sharma et al. 2018). Aptabeacons, a new class of molecular beacons, have attracted a lot of attention in the development of fluorescent sensors. Aptabeacons consist of a hairpin structure attached to a fluorophore and a quencher. When the target is in the environment, it connects to the aptamer and activates the fluorescence signal by disrupting the energy transfer between the quencher and the fluorophore since the hairpin structure opens and the distance between the quencher and the fluorophore increases (Yamamoto and Kumar 2000).

Another type of aptamer-based fluorescence sensor can be designed based on an aptamer switch probe. An aptamer switch probe consists of an aptamer, a small DNA molecule that complements the end and a fluorophore is attached to the other end of the aptamer. When the analyte is in the environment, it binds to the aptamer leading to intramolecular displacement and converting the aptamer to a fluorescence probe (Tang et al. 2008). This technique was used in the detection of aflatoxin M1 in milk samples as depicted in Fig. 5.2 (Sharma et al. 2016).

Another approach to design fluorescent sensors is based on pyrene. Pyrene is a dye with little fluorescence, but if two monomer units come together to form an eximer, the fluorescence lifetime increases, which can be used to analyze the analyte. This approach has been used for the detection of $Hg^{2+}$ in samples (Wu et al. 2019).

Recently, most fluorescent sensing methods are based on FRET. FRET is a process in which an energy donor gives its energy (electrons) to the nearest acceptor (about 10 nm). The emission spectrum of the donor has to overlap with the excitation spectrum of the acceptor for FRET to take place. In a study, in an attempt to detect Aflatoxin B1(AFB) in rice and peanuts, a FRET biosensor was designed. In this method, specific aptamers, attached to quantum dot (QDs), are absorbed on AuNPs. When the target is not in the environment, emission of QDs and the absorption of AuNPs overlap, leading to fluorescence quenching by FRET. By attaching to the target, the aptamer is released from the gold nanoparticles, and the energy transfer is interrupted due to the increase in the distance between the QDs and AuNPs. Thus, the quantum dot's fluorescence emission is activated. The linear range of this method is 10–400 nM (Sabet et al. 2017).

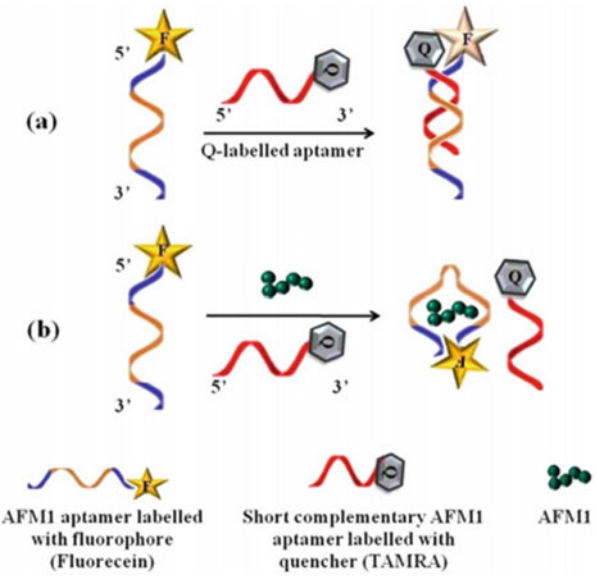


**Fig. 5.2** Schematic representation of structure switching signaling aptasensing platform for the detection of aflatoxin M1 (Sharma et al. 2016)

### 5.3.3 Chemiluminescence

Chemiluminescence (CL), defined as the process of light generation through a chemical reaction, has grown into a well-established optical detection technique for the analysis of various liquid phase samples. A typical chemiluminescent reaction is as follows:

$$[A] + [B] \rightarrow [\Diamond] \rightarrow [\text{Products}] + \text{light}$$

in which an interaction between A and B leads to the production of excited intermediate species ($\Diamond$), which emit light as they return to their grounded state. Compared to other optical techniques, CL has superior sensitivity as a result of reduced background noise due to the elimination of an external light source. Other advantages of this technique include controlled emission rate, rapidity, stability, and safety (Vacher et al. 2018).

The most commonly used CL reagent in luminol, which can be oxidized by hydrogen peroxide, permanganate, periodate, etc. in an alkaline medium to generate the excited 3-aminophthalate anion. The latter emits light upon its return to the ground state. Besides luminol, other reagents such as peroxyoxalate derivatives and tris(2,2-bipyridine) ruthenium(II) (Ru(bpy)3 2+) have also been used in the development of numerous sensors (Liu et al. 2010).

In order to gain higher efficiencies and quantum yields, various catalysts such as horseradish peroxidase (HRP) or alkaline phosphatase (ALP) are used in CL systems. In one study, HRP was used in a CL aptasensor for the detection of aflatoxin B1 (AFB1). In this approach, capture probes, which hybridize to selective

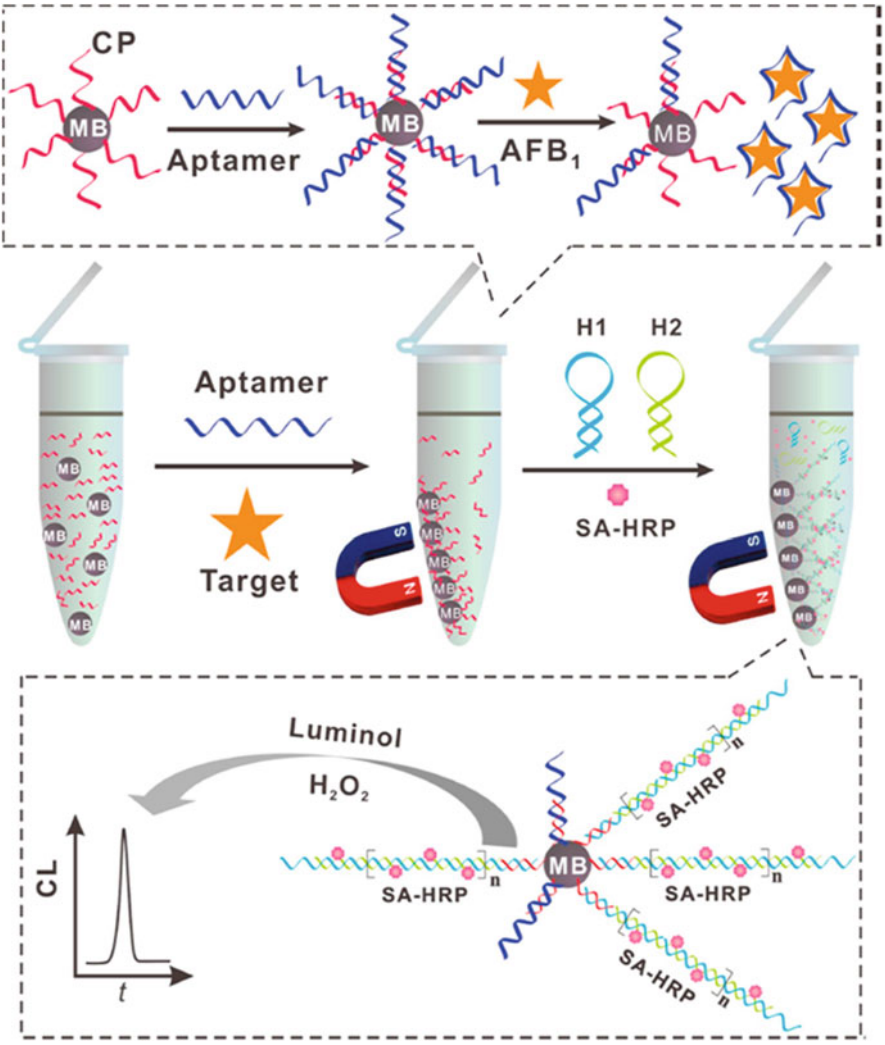


**Fig. 5.3** Schematic representation of a CL aptasensor for the detection of AFB1 based on HCR for signal enhancement (Xie et al. 2019)

aptamers for AFB1, were immobilized on magnetic beads in order to enable magnetic separation to reduce the background signal. Hybridization chain reaction (HCR) was also used for further signal enhancement. In Fig. 5.3, a schematic of the proposed sensor is presented (Xie et al. 2019).

As previously mentioned, nanomaterials possess unique optical and often catalytic features which has to an increased interest in employing them for signal enhancement in CL-assays. It has been proven that incorporating metal NPs or semiconductor crystals can significantly enhance the luminescence signal in CL

systems. AuNPs, AgNPs, platinum NPs (PtNPs), and CdTe nanocrystals have been reported to be able to enhance the sensitivity of CL sensors (Chen et al. 2011). Peroxidase-mimicking NMs have recently gained immense attention in CL-assays due to the unique features that arise from their large surface-to-volume ratio and their distinct advantages over conventional catalysts and enzymes such as being cheaper, more stable in varying conditions, and more tunable catalytic behavior. To date, numerous peroxidizing NMs have been discovered including iron oxide NPs ($Fe_3O_4$), AuNPs, graphene oxide and carbon dots, some quantum dots, and several nanoclusters (NCs) including PtNCs (Dehghani et al. 2018).

Chemiluminescence resonance energy transfer (CRET), similar to FRET, is a distance-dependent non-radiative energy transfer phenomenon in which a CL donor transfers its energy to a dye or fluorescence NMs. In comparison with FRET, CRET has higher sensitivity and lower noise interference. Also, owing to the elimination of an external light source for excitation, problems pertaining to autofluorescence or fluorescent bleaching are noticeably lessened. Taking advantage of CRET, a turn-on sensor was designed for the detection of melamine, an adulterant added to milk to increase its apparent protein content, in milk samples (Du et al. 2015). This sensing assay was based on the quenching CL effect of AuNPs on bis(2,4,6-trichlorophenyl) oxalate (TCPO)–hydrogen peroxide–fluorescein system because the absorption band of dispersed AuNPs overlaps with the CL spectrum. In the presence of melamine, the aggregation of AuNPs is induced, thus restoring the CL reaction in the system. Using this sensor, a detection limit of $3 \times 10^{-13}$ mol/L for melamine was attained.

### 5.3.4 Surface-Enhanced Raman Scattering

Spectroscopic methods have always been among the favored detection approaches, especially in the assessment of food quality as a result of their rapid and nondestructive nature. Vibrational spectroscopy, including infrared (IR) and Raman spectroscopy, are particularly highlighted as they are simple, fast, reliable, and require minimum sample preparation. In Raman spectroscopy, the sample is irradiated with monochromatic light in the visible or near-IR region, which elevated the vibrational energy levels to higher, short-lived states. Molecular relaxation can occur either by photon emission with the same wavelength (Rayleigh scattering), or by photon emission with a lower frequency (Raman scattering). Since only a small percentage of molecules undergo Raman scattering, the intensity of this technique is quite low (Thygesen et al. 2003). Surface-enhanced Raman scattering is an approach that aims to enhance Raman scattering of molecules either absorbed on metal surfaces or in vicinity of metal particles. Noble metals, such as Ag, Au, and Cu, due to their special plasmon resonance properties, are typically used for signal enhancement in SERS. A selection of SERS substrates, based on noble metals, are available which can be divided into colloidal and solid substrates. Samples are either dropped on a solid substrate, or put in a solution with colloidal substrates to be analyzed through SERS.

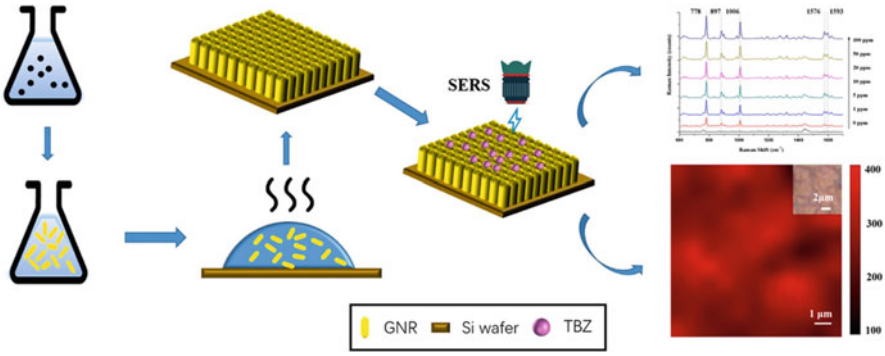


**Fig. 5.4** Schematic representation of a gold nanorod (GNR)-based SERS sensor for the specific identification of thiabendazole (Fu et al. 2019)

Nanotechnology has had a big impact on SERS, as noble metal NMs display higher plasmon resonance properties and can thus further enhance Raman scattering. Many strategies have been opted to improve the SERS signals through the optimization of the structure, shape, size, and components of noble metal NMs (Sharma et al. 2013). As Raman enhancement occurs at nanoscale gaps in between the NPs, decreasing metal particle size influences the particle density and increases SERS hotspots, thus generally increasing SERS signal (He et al. 2017). AgNPs are among the strongest signal enhancing substances used in SERS assays. Pertinent to these findings, Tang et al. hypothesized that a nonplanar SERS substrate can be more effective in signal enhancement. Using AgNP-coated glass beads, they designed a rapid sensor for the identification of two pesticides, chlorpyrifos and imidacloprid, in apple extracts, attaining a detection limit of 10 ng/mL and 50 ng/mL, respectively (Tang et al. 2019).

Various nanostructures containing gold have also been extensively used in SERS sensors for food analysis from simple AuNPs (Luo et al. 2018), to more complex structures such as densely arranged AuNPs templated from mesoporous silica film (MSF) as used for the detection of pesticides in food samples via SERS (Xu et al. 2020); and also $Fe_3O_4$@Au core-shell gold nanostructures as used in the SERS-based assessment of synthetic food colors such as acid orange II and brilliant blue (Xie et al. 2019). Au@AgNPs core-shell nanostructures combine the excellent enhancing properties of silver with the high stability of gold. This nanostructure was used for the simultaneous detection of thiram and dicyandiamide in liquid milk (Hussain et al. 2020). Gold nanorods (AuNRs) have also recently been under the spotlight for Raman enhancement as they display unique surface plasmon resonance and tunable aspect ratio, which can easily be adjusted for signal enhancement. As seen in Fig. 5.4, AuNRs were employed in an SERS-based sensing platform for the selective detection of thiabendazole, a pesticide, in apples with a detection limit of 0.037 mg/L (Fu et al. 2019).

### 5.3.5 Surface Plasmon Resonance

Surface plasmon resonance (SPR) is defined as the oscillation of the conductive electrons of a metal (commonly gold or silver) near the surface upon being excited by light with a specific angle of incidence. When this happens, a decrease in the intensity of the reflected light can be seen. The refractive index of the medium near the surface of the metal impacts the angle of light which triggers SPR. Hence, any variation in the refractive index, for instance caused because of the absorption of molecules to the surface, will affect SPR, thus making it possible to the molecule to be detected. Analyte identification in SPR occurs through the assessment of the intensity of the reflected light, or by analyzing the resonance angle shifts in a real-time manner (Zhu and Gao 2019). As SPR assays provide a label-free and real-time detection route and require minimum reagents, they have extensively been applied for the analysis of numerous analytes including food-related analytes.

In a typical SPR assay, targeting molecules such as antibodies or aptamers are absorbed on a gold or silver film. The presence of a target and its interaction with the respective target molecule lead to a mass change near the surface, which thus impacts the refractive index and the reflective light beam. This general approach was used to develop an SPR-based immunoassay for the specific detection of amantadine (AM), an antiviral drug, in animal-derived food samples with a detection limit of 4.0 ng $mL^{-1}$ (Pan et al. 2019). Multiplexed detection based on the SPR technique has also been extensively explored, as done in one study for the simultaneous detection of aflatoxin B1, ochratoxin A, zearalenone, and deoxynivalenol in cereal samples (Wei et al. 2019).

Localized surface plasmon resonance (LSPR) occurs in the interface of nanotechnology and SPR. In this approach, instead of a metallic film, metallic nanostructures, which have enhanced plasmon resonance and provide a bigger surface for functionalization, are employed. LSPR has been used in several novel sensing approaches for food monitoring. In one study, an LSPR-aptasensor was used for the recognition of *Staphylococcus aureus* in milk samples with a detection limit of $10^3$ CFU/mL (Khateb et al. 2020). In another study, gold nanorods were employed to develop an aptasensor for the in situ detection of ochratoxin A with a limit of detection of 12.0 pM (Lee et al. 2018). In a different approach, Au@Pt nanozymes were used in an LSPR-based sensor for the identification of silver ions $Ag^+$ with a detection limit of 500 nM. The presence of $Ag^+$ disrupts the peroxidizing effect of the nanozymes on $H_2O_2$. In this situation, and in the presence of a weak acid, the residual $H_2O_2$ leads to the reduction of the silver ions and a blue shift in the SPR spectrum (Fig. 5.5) (Tian et al. 2020).

## 5.4 Conclusion and Future Perspectives

Recent advances in optical biosensing assays have revolutionized the attempts for rapid analyte detection and can evidently provide many benefits in food safety control. Optical biosensors possess many profound advantages such as being able

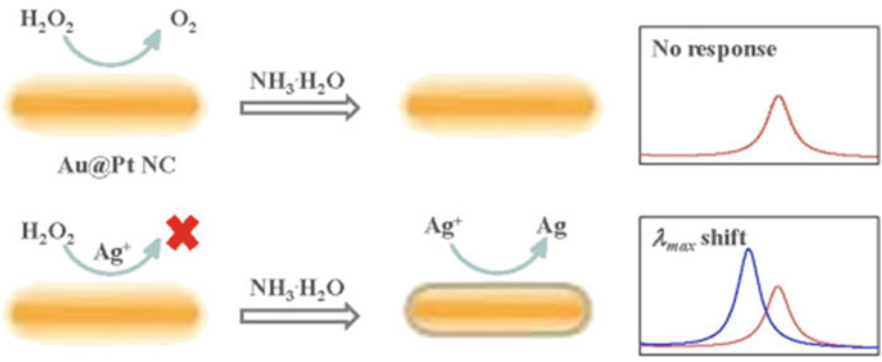


**Fig. 5.5** Schematic representation of an LSPR sensor based on the peroxidase-mimicking of Au@Pt nanozymes for the detection of silver ions (Tian et al. 2020)

to provide rapid, selective, and sensitive recognition methods and being able to provide more compact and portable sensors for in situ food analysis. The incorporation of functional nanomaterials, such as enzymes, DNAzymes, antibodies, and aptamers, can enhance the sensing performance through effective targeting or signal enhancing. Novel nanomaterials, due to their unique optical properties which arise from their increased surface-to-volume ratio, have also had a great impact on the improvement of optical strategies. Also, wireless-communication technologies and the advancement of smartphones can further help with the development of portable, in situ sensing platforms. Although the broad practical application of optical assays is still limited because of the numerous challenges mostly relating to sample preparation and creating compact systems for portability, optical sensors have proven to be among the most efficient detection routes for food safety monitoring.

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