Development of chromogenic detection for biomolecular analysis

Weipan Peng1,∗ | Wenna Li1,∗ | Houyu Han1 | Hao Liu2 | Ping Liu3 | Xiaoqun Gong1 | Jin Chang1

1 School of Life Sciences, Tianjin Key Laboratory of Function and Application of Biological Macromolecular Structures, Tianjin University, Tianjin, China
2 Tianjin Stomatological Hospital, Nankai University, Tianjin, China
3 Tianjin Enterprise Key Laboratory of Chemiluminescence and POCT Diagnostic Technology, Tianjin, China

Correspondence
Jin Chang and Xiaoqun Gong, School of Life Sciences, Tianjin Key Laboratory of Function and Application of Biological Macromolecular Structures, Tianjin University, Tianjin 300072, China. Email: jinchang@tju.edu.cn and gongxiaoqun@tju.edu.cn

Abstract
Chromogenic detection of target objects has attracted extensive attention in the academic field with high specificity and sensitivity. Based on color changes in chemical reactions, biomolecular analysis with simple and rapid signal readout in disease diagnosis can be realized. At present, three main mechanisms of chromogenic detection were proposed, including protease catalysis, enzyme mimics catalysis, and chemical reactions based on inorganic or organic for biomolecular analysis. With the unremitting efforts of scientific researchers, the evolution of chromogenic detection has been developed from the early qualitative detection to the current quantitative detection, accompanied by the improvement of sensitivity. Herein, this paper reviewed the mechanisms and relevant applications of chromogenic detection from the above three aspects, discussed the challenge, and provided a future perspective in this emerging field.

KEYWORDS
biomolecular analysis, chromogenic detection, enzyme mimics catalysis, inorganic or organic chemical reactions, proteases catalysis

1 | INTRODUCTION

Given the prevention, identification, and treatment of diseases are significant, the development of powerful diagnostic tools is crucial to molecular diagnosis.[1–4] Traditional detection techniques have been developed for biomolecular analysis, such as polymerase chain reaction,[5,6] enzyme-linked immunosorbent assay,[7] and biochip technology.[8] Although widely applied to biological detection, most of them suffered from complex operations, expensive experimental machines, and highly skilled operators.[9,10] In order to eliminate these
limitations, simple, inexpensive, and portable molecular detection technologies were required urgently.

Based on the development of chemistry and nanotechnology,[31] chromogenic detection,[12] which is also named color detection, was proposed and studied widely. As signal readout, the color changes of chromogenic substrates can be observed directly to analyze different targets.[13,14] This portable, timely and simple chromogenic detection strategy has been applied to molecular diagnosis, including detection of biomarkers, nucleic acid, and small molecules. However, the relatively low sensitivity, narrow dynamic range, and non-quantitative analysis limit the further application seriously.[15–18] Therefore, the researchers put lots of effort into exploiting highly sensitive, accurately quantitative chromogenic detection methods for biomolecular analysis.[19–22]

Although a number of excellent platforms based on color changes have been widely researched, the majority of them fasten on the aggregation of various nanoparticles (NPs), whereas chromogenic detection based on chemical color reactions is rarely reported. According to previous researches, this paper summarized different mechanisms and relevant applications of chromogenic detection. At present, there are mainly three mechanisms of chromogenic detection for biomolecules’ quantitative analysis.[23,24] The proteases catalyze specific chromogenic substrates and trigger color changes of substrates in reaction solutions for chromogenic detection. Moreover, the enzyme mimics,[25] such as peroxidase-mimicking DNAzymes,[26] nanozymes,[27] metal-organic frameworks (MOFs), and small molecule enzyme mimics exhibit superior enzyme-like characteristics,[28] which catalyze a wide range of chromogenic substrates for targets detection. Furthermore, some of the inorganic or organic chemical reactions can also analyze biomolecules for chromogenic detection. Herein, the advantages of chromogenic detection were summarized, and it proposed well-prospective applications in the emerging field.

2 | DIFFERENT MECHANISMS OF CHROMOGENIC DETECTION

2.1 | Chromogenic detection based on proteases catalysis

Proteases existing in the natural world play vital roles in biological processes because of high specificity and catalytic efficiency.[29–31] In particular, some proteases, such as horseradish peroxidase (HRP),[32] glucose oxidase (GOx), and alkaline phosphatase (ALP),[33] have synergistically catalytic effects. These proteases can be used in biomolecular analysis extensively by catalyzing chromogenic substrates to yield color changes.[34–36] In addition, proteases can also directly catalyze specific chromogenic substrates. Furthermore, most proteases collaborate in enzymatic cascade catalysis, which refers to the product of one enzyme being a substrate in another enzyme, and the detection signal can be amplified effectively.[37]

2.1.1 | Chromogenic detection based on synergistic catalysis of proteases

The classical chromogenic reaction mechanism was introduced systematically. Substrates can be catalyzed to produce color changes for chromogenic detection because of simple signal readout. There are various colorless substrates of peroxidase, including 3,3,5,5-tetramethylbenzidine (TMB), dopamine (DA), 2,2-azo (3-ethylbenzothiazolin-6-sulfonic acid) diammonium salts (ABTS), and 3,3-diaminobenzidine (DAB), which can be oxidized to different colored products.[38–44] At present, numerous HRP-based chromogenic detections have been constructed for biomolecular analysis. For example, Cao’s group reported a thrombin assay based on HRP-catalyzed TMB oxidation and proposed a simple electrophoresis titration-redox boundary model as shown in Figure 1A.[45] In this strategy, magnetic NPs-aptamer 1 and HRP-aptamer 2 could capture the target of thrombin, forming a sandwich complex. After being magnetically separated, the complex reacted with chromogenic substrate TMB and yielded a color change in the presence of H2O2 for thrombin analysis. Compared with the traditional thrombin detection methods, Cao’s strategy has high specificity and high sensitivity, and the limit of detection reached 0.04 nM based on synergistic catalysis of proteases. Another important example was reported by Zhou’s group. They designed an innovative nucleic acid detection platform that combined DNA-HRP- gold NPs (AuNPs) probes with the CRISPR/Cas system in test strips.[38] In the presence of Listeria monocytogenes, the CRISPR/Cas system was activated, and the HRP with the DAB catalyzed chromogenic reaction. Then, the test strips showed two colored bands. In the presence of TMB or DAB, DNA-HRP-AuNP probes will produce a color change for chromogenic detection with excellent specificity and sensitivity. Moreover, to further enhance the sensitivity of chromogenic detection, Liu’s group developed a sandwich enzyme-linked immunosorbent assay combined with an anti-human prostate-specific antigen (PSA) monoclonal antibody (anti-PSA).[46] When PSA was captured, HRP-conjugated antibody (HRP-IgG; where IgG refers to immunoglobulin G) reacted with o-phenylenediamine, which yielded color change and the low limit of detection reached 0.16 ng/mL.
The mechanism of ALP color detection was the same as HRP, so ALP was extensively adopted in biomolecular analysis because of high catalytic activity. For example, Sun's group constructed an easy one-pot synthetic method to detect PSA based on ALP. In the presence of PSA, ALP catalyzed hydrolysis of 4-aminophenol phosphate to AP (p-aminophenol), and then p-aminophenol and N-[3-(trimethoxysilyl)propyl] ethylenediamine could yield color change for PSA quantitative determination. The limit of detection was as low as 0.02 ng/mL. Therefore, chromogenic detection based on HRP and ALP was widely used in biomolecular analysis. In conclusion, chromogenic detection based on synergistic catalysis of proteases has high specificity and sensitivity, and the simultaneous but independent color change can significantly improve the accuracy. We believe that chromogenic detection with an easy sensing process and convincing results may hopefully contribute to a wider application in clinical diagnosis.

2.1.2 Chromogenic detection based on direct catalysis of proteases

Numbers of proteases catalyze specific chromogenic substrates directly and have been widely applied to biomolecular analysis over the past few years, including small molecules, protease activity, and nucleic acid. Recently, chromogenic reactions based on polyphenol oxidase catalysis have been proved to be a simple and portable phenolic compounds detection method. According to different chemical groups of substrates, enzymatic products exhibit various color changes. Wang's group developed a rapid chromogenic detection method to distinguish and detect phenolic compounds β2-agonists by oxidation catalysis of tyrosinase. The catalytic oxidation products of the enzyme exhibited color changes based on the heterogeneous substituent groups of substrates. The tyrosinase-catalyzed reaction system had excellent characteristics of good linear output and lower detection limit and had a great application in the actual sample analysis. In addition, catalysis of chromogenic substrates by proteases can also be applied to the evaluation of protease activity. Johnson and Schaeper constructed a simple, portable chromogenic detection platform for human leukocyte elastase (HLE) activity determination by 3-[(N-tosylalaninyl) oxy]-5-phenylpyrrole and 4-sulfophenolquinone 1-diazide. The 3-[(N-tosylalaninyl) oxy]-5-phenylpyrrole was hydrolyzed by HLE, generating 3-hydroxyl-5-phenylpyrrole, and the catalytic reaction between 3-hydroxyl-5-phenylpyrrole and colorless 4-sulfophenolquinone 1-diazide occurred subsequently to produce the colored products. The color change of chromogenic substrates as signal readout was observed directly to detect the catalytic activity of HLE. This method based on lactic acid colorimetry was proposed for the first time in this research, which was significant for the
subsequent research of other enzyme activity determination methods.

In addition to the above detection of small molecules and protease activity, chromogenic detection platforms based on direct catalysis of proteases were also proposed to detect a nucleic acid. Recently, the proximity proteolysis reaction has been applied to nucleic acid detection. Park and Yoo designed a proximity proteolysis reaction platform in which the enhanced reaction between Tobacco Etch Virus protease and β-lactamase zymogen occurred owing to the existent of nucleic acid molecules. Two proteins were modified by single nucleic acids which all hybridized with the target nucleic acid, causing the Tobacco Etch Virus to hydrolyze proximal zymogen, and then the activated zymogen catalyzed specific colorimetric substrate to generate a color signal (Figure 2A). Furthermore, the target nucleic acid could be detected rapidly (<1 h) at nanomolar concentrations (Figure 2B). Moreover, the proximity proteolysis reaction combined with an isothermal nucleic acid amplification method to realize higher detection sensitivity (10 fM) (Figure 2E–I). The results indicated that direct catalysis was an efficient and simple method for the determination of target nucleic acid, and had the potential to construct a new detection platform in the future.

2.1.3 Chromogenic detection based on enzymatic cascade catalysis

In addition to the catalysis of the single enzyme above-mentioned, recently, the enzyme-cascade reaction as a significant molecular biological technology also has been used for molecular diagnosis. Enzymatic-cascade amplification, which performs multiple enzymes and generates effective amplification signals, has been broadly applied to biosensors and other fields. Zhao et al. designed a chromogenic detection method based upon enzyme-cascade reaction to determine the activity of ALP and tyrosinase. ALP catalyzed the transformation of p-aminoethyl-phenyl phosphate disodium salt into intermediate tyramine, which was subsequently catalyzed to DA.
by tyrosinase. Eventually, DA reacted with resorcinol to make the solution exhibit an obvious color change from colorless to bright-colored yellow. Therefore, the enzyme cascading system provided a new strategy for further research of cascading biocatalysis mechanisms and wider application in the field of biosensors. In reality, current chromogenic detection involved in the enzyme-cascade reaction is almost triggered by HRP or peroxidase, and the characterization and quantization of the reaction are shown through specific color changes of enzyme substrates. Lu et al. developed a multifunctional ultrasensitive chromogenic detection platform by designing two hairpin DNA strands which were modified by GOx and HRP, respectively. Owing to a hybridization chain reaction-triggered signal amplification and the enzymatic cascade reaction-triggered catalysis enhancement, the chromogenic analysis platform showed excellent sensitivity. Analogously, Xiang et al. constructed an intricate enzyme-cascade reaction system which provided an excellent method to detect glucose or lactose through encapsulating GOx and β-galactosidase into DNAzyme hydrogel. In brief, this highly efficient enzymatic cascade catalysis provides a promising platform for the construction of multi-enzyme and nucleic acid detection systems.

In addition to the above enzyme-cascade catalysis based on multiple proteases, the enzyme-cascade reaction of proteases and enzyme mimics has also been extended to construct chromogenic strategies in the past few years. Guo et al. constructed an innovative chromogenic detection platform to detect acid phosphatase (ACP) activity sensitively through cascades of ACP and photoresponsive enzyme mimic. The principle is shown in Figure 3D. ACP hydrolyzed pyrophosphate to release the chelated copper ion (Cu$^{2+}$), and Cu$^{2+}$ was used to trigger photoresponsive enzyme mimic C$_3$N$_4$ nanosheet to endow the capability for the efficient catalytic oxidation of TMB (Figure 3E). As shown in Figure 3F, this innovative enzyme-cascade detection platform had an excellent characteristic of signal amplification, in which the ACP could be detected as low as 8.8 × 10$^{-3}$ U/L. Moreover, the enzyme-cascade detection platform combined natural enzymes with enzyme mimics to construct a simple and sensitive detection strategy. In summary, we reviewed promising biotechnology based on enzymatic-cascade amplification to construct an exquisite chromogenic detection platform for target analysis.

### 2.2 Chromogenic detection based on enzyme mimics catalysis

Compared with proteases, the activity of enzyme mimics is very stable in harsh environments. The use of enzyme mimics for biomolecular analysis causes
extensive attention due to several reasons. Their construction, morphology, and function are not easily damaged in harsh environments and they are prepared relatively easily. Therefore, the enzyme mimics based on the activity of peroxidase have proposed excellent applications in developing advanced biomedical molecular sensors.\[63\] In this research, we concluded four kinds of enzyme mimics, which exhibited catalytic activities of peroxidase-like or oxidase-like, such as peroxidase-mimicking DNAzyme, nanomaterials with enzyme-like activities (nanozymes),[64,65] MOFs, and small molecule enzyme mimics. Furthermore, they can efficiently catalyze substrates (i.e., TMB, DAB, DA, and ABTS) to generate color changes for chromogenic detection.[66]

2.2.1 Chromogenic detection based on peroxidase-mimicking DNAzymes

DNAzymes, single-stranded DNA molecules that have the characteristics of peroxidase-like activity, have drawn wide attention recently in biology, chemistry, and materials science.[67] DNA chains frequently form a G-quadruplex structure to ensure the catalytic property, combining with hemin molecules to accelerate the oxidation of chromogenic substrates and show color change as a detection signal in solution.[68] Peroxidase-mimicking DNAzymes have been extensively applied to molecular diagnosis.[69,70] Plenty of various approaches to nucleic acid detection through enzymatic catalysis of peroxidase-mimicking DNAzymes have been proposed.[71,72] Based on DNAzyme molecular beacon (DNAzymeMB) with free hairpin which has characteristics of peroxidase activity, Fu et al. designed an innovative method for chromogenic detection of DNA.[73] In this research, the catalytic activity of DNAzymeMB was blocked because of hybridization with the blocker DNA. When the target DNA was present, DNAzymeMB separated from the hybridization to recover peroxidase activity, which subsequently catalyzed chromogenic substrates to exhibit color signal readout. Similarly, Li et al. also designed a new both-end blocked molecular beacon approach based on DNAzymes to chromogenic detection of microRNA.[74] In conclusion, the DNAzymeMB strategy is capable of highly sensitive microRNA detection in sub-picomolar and has shown preeminent identification ability to distinguish single-base mismatch microRNA. At present, based on the catalysis of peroxidase DNAzyme, highly sensitive chromogenic detection of DNA as the target also has received increasing interest recently.

Wu’s group developed an innovative chromogenic sensor that depended on DNAzyme and nucleic acid signal amplification methods for ultrasensitive DNA detection.[75] The study took the p53 gene as the target to verify the principle. First of all, the authors designed a palindrome-based molecular beacon consisting of complementary C-riched sequences with DNAzyme, nicking sites, primers, and polymeric templates. In the presence of the target DNA, the palindrome-based molecular beacon was opened because of the complementary pairing with the target, releasing the palindrome sequences and leading to intermolecular hybridization. With the action of polymerase, the primers were extended backward to form the double strand, releasing target DNA to combine with another hairpin probe in the next cycle. At the same time, the double-strand was cleaved by the DNA double-strand endonuclease (Nb. BbVCI) to produce a large number of G-riched DNAzyme structures. Moreover, the G-riched DNAzyme fragments assembled with hemin molecules to form the complex which exhibited peroxidase-like activity and catalyzed ABTS to be the colored ABTS•- with \( \text{H}_2\text{O}_2 \). To further improve sensitivity, the DNAzyme amplification technology combined with nicking enzyme signaling amplification and the detection limit was up to 10 pM (Figure 4D). This technique with high sensitivity, specificity, and operation convenience showed a broad application prospect in the detection of related biomarkers.

2.2.2 Chromogenic detection based on nanozymes catalysis

Research on nanozymes is rapidly underway due to the merits of cost-effectiveness, stability, and portability. And they can oxidize substrates such as TMB, DAB, and ABTS to produce color changes because of their excellent peroxidase-like catalytic activity. Therefore, many nanozymes have been extensively used in chromogenic detection.[76] Among these nanozymes, Gao et al. first reported in 2007 that magnetic \( \text{Fe}_3\text{O}_4 \) with peroxidase-like activity has been widely researched.[77] Later, Tang’s group designed a DNA-engineered nanozyme, which could detect oral bacteria in saliva rapidly and sensitively based on the chromogenic reaction in Figure 5A.[69] They developed a DNA-engineered nanosystem based on \( \text{Fe}_3\text{O}_4 \) NPs, which could catalyze TMB to turn blue for detecting oral bacteria and the low detection limit achieved 12 CFU/mL. In this work, DNA-engineered nanozymes showed a rapid and inexpensive method to detect directly oral bacteria which made a great contribution to clinical diagnosis and prevention. Another example is the work of Yang’s group, as they prepared \( \text{Fe}_3\text{O}_4 / \text{carbon nanodots hybrid NPs} \) that perform similar peroxidase-like catalytic activity for sensitively detecting glutathione (GSH).[78] GSH could make the blue solution of TMB-\( \text{H}_2\text{O}_2-\text{Fe}_3\text{O}_4/\text{carbon} \)
nanodots hybrid NPs system fade. Therefore, GSH could be rapidly and selectively determined based on the colorimetric method which had extremely high sensitivity as low as 0.058 μM. In addition to Fe₃O₄ nanozymes, Pt nanozymes are widely used for chromogenic detection.\(^{79}\) For example, Pompa’s group developed an excellent detection method of the total antioxidant capacity (TAC) in saliva and blood.\(^{79}\) In this method, substrates were catalyzed by Pt nanozymes to generate color changes and then the color of the solution could fade in the presence of TAC. So, the Pt nanoenzyme-enabled strategy is used in rapid (5 min) and accurate detection of the body’s TAC. Therefore, chromogenic detection based on nanozymes catalysis offers a simple and novel method and it has great potential for the development of biomolecular analysis. In summary, we believe that the color detection method will be extended to medical diagnostics and environmental monitoring in the future.

2.2.3 Chromogenic detection based on the MOFs

MOFs are synthesized by self-assembling metal ions and organic adhesive, which have been widely used in many fields because of their large surface area, macrovoid, good adsorption, and excellent stability.\(^{80}\) With peroxidase-like activity, MOFs can oxidize substrates in the presence of H₂O₂ to yield color change, exhibiting the great potential of MOFs in chromogenic detection.\(^{81}\) Yao’s group built a color reaction platform based on Cu-MOF NPs to detect *staphylococcus aureus* (*S. aureus*) as shown in
Figure 6A[82] Cu-MOF NPs processed peroxidase-like activity that could catalyze TMB to produce blue substances with \( \text{H}_2\text{O}_2 \). In this work, Cu-MOF modified with *S. aureus* aptamers could specifically recognize *S. aureus*. When different concentrations of *S. aureus* were captured, Cu-MOF NPs could catalyze color changes and show a good linear trend by measuring absorbance, and the sensitivity was up to 20 CFU/mL. Therefore, this strategy could detect *S. aureus* sensitively and selectively. Similarly, Tan’s group proposed a new strategy to detect corresponding antigen (mIgG) via connecting rabbit antimouse IgG antibody with Cu-MOF based on chromogenic reaction.[83] The mIgG could be captured by rabbit antimouse IgG antibody-labeled Cu-MOFs with capture antibody to form the sandwiched immunocomplex, catalyzing TMB to become blue oxidative products (oxTMB) with \( \text{H}_2\text{O}_2 \). The limit of mIgG detection reached 0.34 ng/mL. Therefore, Cu-MOFs which have peroxidase-like activities were used in chromogenic detection for biomolecular analysis. Moreover, Ce-MOFs also exhibited the activities of peroxidase and oxidase. Wang’s group reported a mixed-valence Ce-MOF, which was applied to chromogenic detection by catalyzing the chromogenic substrates to yield color changes.[84] Therefore, in this work, Ce-MOFs were used to detect ascorbic acid (AA) sensitively because of the high activity of enzyme mimics and great stability, which exhibited the low limit of detection (0.28 \( \mu \text{M} \)). In brief, a novel enzyme mimic based on MOFs was developed, which could extensively be used in rapid, sensitive, and accurate biomolecular analysis field by catalyzing substrates color changes based on enzyme-like activities, showing broad application prospects in the construction of biosensors.

### 2.2.4 Chromogenic detection based on small molecule enzyme mimics

Small molecule enzyme mimics have characteristics of oxidase and peroxidase and can catalyze chromogenic substrates. He’s group found that 9-mesityl-10-methylacridinium ion (Acr\(^{+}\)-Mes) had oxidase-like activity, which could effectively catalyze some small molecule substrates and protein biomacromolecule (e.g., cytochrome c) to produce chromogenic reaction under visible light irradiation in Figure 7.[85] Due to color changes based on this strategy two biothiols (L-cysteine [Cy] and L-GSH) were detected sensitively and highly selectively. The lowest detectable limit was 100 nM. Therefore, based on small molecule enzyme mimics, a low-cost, convenient, and highly specific chromogenic detection strategy is extensively applied to the biomolecular analysis.

In summary, the detection system based on enzyme mimics catalysis has merits of high sensitivity and specificity which is developed for biomolecular analysis.
2.3 Chromogenic detection based on inorganic or organic chemical reactions

During the past few years, numerous chemical chromogenic reactions based on inorganic or organic molecules, have also been used for small molecules detection. Kumar et al. proposed a novel chemical chromogenic reaction platform to detect sulfur mustard by synthesizing 3,6-bis(dimethylamino)-9(10H)-acridine thione probe. Due to labeling the sulfur nucleophilic on the probe, the reaction between the probe and sulfur mustard triggered the regain of conjugation with the color change of probe from yellow to orange within 1 min. Furthermore, Cheng et al. designed a ratiometric chemo dosimeter for analysis of CN anion based on the azobenzene structure and the special nucleophilicity of cyanide toward the acceptor moiety. The color of the azobenzene molecule changed from red to yellow with the presence of the target. As expected, the detection limit was 1.1 μM, and the synthesized probe was more selective to cyanide than other common anions. Similarly, He’s group designed a method to detect aflatoxin by the chromogenic reaction. In the first step, aspergillus flavus (AFs) was designed to react with sodium hydroxide to produce coumaric acid. In the second step, the generated substituted coumaric acid reacted with 2,6-dichloroquinone-4-chloroimide (DBQC) to produce green products. Based on this chromogenic method, the limit detection of AFs could reach 3.9 μg/kg. This strategy is expected to detect total AFs in rice. In addition,
chromogenic platforms based on inorganic or organic chemical reactions can also be applied to detect proteins and nucleic acid.

### 2.3.1 Chromogenic detection based on complex reactions

The complex can react with chemical molecules (such as AA) leading to color changes in the solution. Therefore, chromogenic detection based on complex reactions can be widely used in biomolecular analysis. A chromogenic reaction between AA and Fe(BPT)$_3^{3+}$ can generate a colored complex. On the basis of this strategy, Chen et al. established a chromogenic enzyme-linked immunosorbent method as shown in Figure 8A to detect alpha-fetoprotein.\[^{89}\] When alpha-fetoprotein was captured, the sandwich immunoreactions were finished and ALP-labeled antibodies absorbed on the microporous plates hydrolyzed substrates to produce AA. AA reacted with colorless ferro-red phenanthroline complex Fe(BPT)$_3^{3+}$ to produce red Fe(BPT)$_3^{2+}$ eventually. The reduction-oxidation cycle was generated in the system under the catalysis of enzymatic products, and a large amount of Fe(BPT)$_3^{2+}$ was accumulated, and the color intensity was significantly amplified. The limit of detection was 5 pg/mL, which was lower than the established detection methods. The chromogenic method is more sensitive and other biomarkers can be efficiently detected based on this method. Analogously, Sheng’s group detected carcinoembryonic antigen (CEA) based on complex reactions.\[^{90}\] As illustrated in Figure 8C, in the presence of CEA, AA2-phosphate (AAP) was hydrolyzed to AA, and then AA reacted with Fe$^{3+}$ to produce orange-red iron(II)-phenanthroline ([Phen)$_3$Fe]$^{2+}$) complex. Therefore, the concentration of CEA can be determined by the color change of ([Phen)$_3$Fe]$^{2+}$ complex, and the detection limit could reach 21.1 pg/mL.

Similarly, the starch-iodine complex was extensively used in biomolecular analysis based on color change, attributing to the different charge-transfer.\[^{91,92}\] Kawai et al. designed a functional DNA system to detect single nucleotide polymorphisms (SNPs) based on the iodine starch reaction which could yield a purple complex.\[^{93}\] In this process, I-I covalent bonds were formed by converting the absorbed photon energy into chemical energy, and each functional DNA generated more than 100 I$_2$ molecules. The photon energy conversion efficiency was measured for SNPs detection in genomic sequences. As the mismatched DNA of SNPs in the genome sequence prevented the process of charge transfer, and it significantly affected color change which was used to detect mismatched DNA of SNPs based on the iodine-starch reaction (Figure 8H). Analogously, Grinsven’s group also developed a simple and efficient method for phosphate detection.\[^{94}\] Phosphate levels in urine samples could be quickly and
accurately reported, suggesting that the chromogenic reaction could be used as a prescreening test for hyperphosphatemia, chronic kidney, and other diseases. In summary, chromogenic detection based on complex reactions can not only specifically capture targets, but also trigger an amplified colorimetric signal, which allows detecting targets selectively and sensitively. We believe that this method will exhibit a wider application in bioanalysis.

2.3.2 Chromogenic detection based on chemical indicators

At present, chromogenic detection based on various chemical indicators has attracted increasing interest and exhibited extensive application prospects in biomolecular analysis, such as proteins, small molecules, and ions. Some researches constructed more efficient, convenient,
and simple platforms based on chemical indicators with final color signal readout which was more obvious with a low background signal. As a chemical dye indicator, the ABTS can be used in concentration nitric oxide detection in cells. Based on the color change of ABTS in the gas flow. Busch’s group designed an excellent method for rapid solid catalyst DeNOx detection. The filter paper was impregnated with ABTS and placed in the NO airflow combining with obvious color change from colorless to green for higher sensitivity.

In addition, methylene blue (MB), an aromatic heterocyclic compound, also works as the chemical indicator dye and staining agent in the field of biochemistry. The aqueous solution of MB appears blue in an oxidizing environment and it can reduce to colorless in the presence of zinc, ammonia, and other reducing agents, so the color change can be observed to detect target objects. Satoh et al. designed a portable strategy to qualitatively analyze hydroxyl radicals depending on the bleaching of MB. Moreover, Zhang et al. constructed a chromogenic platform for GSH and Cy detection, which was on the basis of AuNPs catalysis in the bell reaction between MB and hydrazine. With the catalysis of AuNPs, the blue MB was reduced by hydrazine to colorless leuco-MB, and it was quickly oxidized to blue MB by oxygen subsequently. GSH or Cy could inhibit the formation of gold hydride, and the catalytic activity of AuNPs decreased subsequently, resulting in a longer color reaction time (Figure 9B–E). So, this chromogenic method can intuitively realize the detection of GSH/Cy with a limit as low as 8 and 10 μM respectively (Figure 9G and H). Therefore, this novel technology based on chemical indicators will exhibit great potential in clinical diagnosis with high sensitivity and specificity.

3 | CONCLUSIONS AND FUTURE PERSPECTIVES

Chromogenic detection based on various types of color reactions has been widely reported which solves the problems of complex operation and expensive experimental machines in the detection process. Herein, we summarize several types of chromogenic detections based on three mechanisms. The chromogenic detection based on various color reactions has significant advantages. First, the various types of reactions used for chromogenic detection are classical chemical color reactions, which cannot produce false-positive and are applied to ultrasensitive detection under complex matrices. Moreover, the detection sensitivity can improve significantly through
different signal amplification experiments. Finally, the detection process can be extremely simple and portable as the detection signal can be directly observed through color changes. Therefore, chromogenic detection is also widely applied to food safety analysis, environmental pollution detection in resources-limited countries.

However, several critical problems still need to be solved before chromogenic sensors are more widely used. Since chromogenic platforms are mainly established on account of various color changes of chromogenic substrates, it is crucial to utilize optically and chemically stable chemical materials, such as nanozymes, MOFs, and hemin/G-quadruplexes. However, preparation methods of chemical materials are still intricate, which greatly limits the potential application of chromogenic sensors. Besides, there is a lack of theoretical calculation of the relationship between color changes of substrates and the morphology, size, or composition of NPs. Therefore, the extensive application of chromogenic detection sensors still faces great challenges.

In practical applications, quantitative detection of target molecules is the key to establishing a chromogenic detection platform. Although chromogenic detection can almost meet the primary requirements of detection, the detection sensitivity remains to be improved. Therefore, the chromogenic detection platform can be used in combination with intelligent devices to improve sensitivity and extend the application in many fields. In a word, compared with traditional diagnostic methods, chromogenic detection platforms show obvious advantages. A portable, timely, and simple biomedical testing strategy is an inevitable requirement for future development.

ACKNOWLEDGMENTS
This work was supported by the National Key Research and Development Plan nanotech key project (2017YFA0205104), and the Natural Science Foundation of Tianjin, China (19ZXDBSY00070, 20YFZCSY00990).

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ORCID
Jin Chang https://orcid.org/0000-0002-6752-8526

REFERENCES
1. G. X. Li, R. B. Zhang, M. J. Wei, C. S. Yin, J. Sun, Y. C. Zhang, ACS Sensors 2020, 5, 10.
2. E. Jordana-Lluch, B. Rivaya, C. Marco, M. Gimenez, M. D. Que-sada, A. Escobedo, M. Batlle, E. Martro, V. Ausina, J. Infect. 2016, 74, 2.
3. K. Yin, X. Ding, Z. Y. Li, H. Zhao, K. Cooper, C. C. Liu, Anal. Chem. 2020, 92, 12.
4. D. Lee, Y. Shin, S. Chung, K. S. Hwang, D. S. Yoon, J. H. Lee, Anal. Chem. 2016, 88, 24.
5. C. Platteau, M. De Loose, B. De Meulenaer, I. Tavermers, J. Agric. Food Chem. 2011, 59, 21.
6. M. Roder, K. Kleiner, A. Sachs, N. Keil, T. Holzhauser, J. Agric. Food Chem. 2013, 61, 25.
7. I. M. Lopez-Calleja, S. de la Cruz, N. Pegels, I. Gonzalez, T. Garcia, R. Martin, Food Control 2013, 30, 2.
8. Y. J. Zhan, J. J. Yan, M. Wu, L. H. Guo, Z. Y. Lin, B. Qiu, G. N. Chen, K. Y. Wong, Talanta 2017, 174, 365.
9. C. C. Zou, M. F. Foda, X. C. Tan, K. Shao, L. Wu, Z. C. Lu, H. S. Bahlol, H. Y. Han, Anal. Chem. 2016, 88, 14.
10. M. Roder, K. Kleiner, A. Sachs, N. Keil, T. Holzhauser, J. Agric. Food Chem. 2013, 61, 25.
11. D. Ramos, M. Arroyo-Hernandez, E. Gil-Santos, H. D. Tong, C. Van Rijn, M. Calleja, J. Tamayo, Anal. Chem. 2009, 81, 6.
12. B. Hu, Y. Zhao, H. Z. Zhu, S. H. Yu, ACS Nano. 2011, 5, 4.
13. L. Wu, S. H. Yang, H. Xiong, J. Q. Yang, J. Guo, W. C. Yang, G. F. Yang, Anal. Chem. 2017, 89, 6.
14. S. El Sayed, L. Pascual, M. Licchelli, R. Martinez-Manez, S. Gil, A. M. Costero, F. Sancenon, ACS Appl. Mater. Interfaces 2016, 8, 23.
15. L. Feng, C. J. Musto, K. S. Suslick, J. Am. Chem. Soc. 2012, 134, 12.
16. J. J. Xu, N. Bao, X. H. Xia, Y. Peng, H. Y. Chen, Anal. Chem. 2005, 76, 23.
17. A. Milcamps, S. Rabe, R. Cade, A. J. De Framond, P. Henriksson, V. Kramer, D. Lisboa, S. Pastor-Benito, M. G. Willis, D. Lawrence, G. Van den Eede, J. Agric. Food Chem. 2009, 57, 8.
18. J. W. Judge, V. L. Mcguffin, Anal. Chem. 1991, 63, 22.
19. R. Qu, L. L. Shen, Z. H. Chai, C. Jing, Y. F. Zhang, Y. L. An, L. Q. Shi, ACS Appl. Mater. Interfaces 2014, 6, 21.
20. Y. Ni, H. Liu, D. Dai, X. Q. Mu, J. Xu, S. J. Shao, Anal. Chem. 2018, 90, 10152.
21. J. Zhang, X. Y. Wang, Y. H. Wang, D. D. Wang, Z. Song, C. D. Zhang, H. S. Wang, Anal. Chem. 2020, 92, 18.
22. S. Tong, B. B. Ren, Z. L. Zheng, H. Shen, G. Bao, ACS Nano. 2013, 7, 6.
23. B. Garcia-Acosta, F. Garcia, J. M. Garcia, R. Martinez-Manez, F. Sancenon, N. San-Jose, J. Soto, Org. Lett. 2007, 9, 13.
24. A. Singh, S. Kaur, A. Kaur, T. Aree, N. Kaur, N. Singh, M. S. Bakshi, ACS Sustainable Chem. Eng. 2016, 2, 4.
25. J. Kosman, B. Juskowiak, Anal. Chim. Acta 2011, 701, 1.
26. S. Cho, S. M. Lee, H. Y. Shin, M. S. Kim, Y. H. Seo, Y. K. Cho, J. Lee, S. P. Lee, M. I. Kim, Analyst 2018, 143, 5.
27. A. M. Brynskikh, Y. L. Zhao, R. L. Mosley, S. Li, M. D. Boska, N. L. Klyachko, A. V. Kabanov, E. V. Batrakova, Nanomedicine 2010, 5, 3.
28. W. Song, B. Zhao, C. Wang, Y. Ozaki, X. F. Lu, J. Mater. Chem. B 2019, 7, 6.
29. C. Q. Zhang, X. D. Xue, Q. Luo, W. Y. Li, K. N. Yang, X. X. Zhuang, Y. G. Jiang, J. C. Zhang, J. Q. Liu, G. Z. Zou, X. J. Liang, ACS Nano. 2014, 8, 11.
30. P. K. Saini, D. W. Webert, J. C. Judkins, J. Vet. Diagn. Invest. 1995, 7, 4.
31. C. B. Ma, Y. Zhang, Q. Liu, Y. Du, E. K. Wang, Anal. Chem. 2020, 92, 7.
AUTHOR BIOGRAPHIES

**Weipan Peng** received her BSc degree from Cangzhou Normal University in 2016. Since September 2016, she joined Professor Chang’s group to pursue her Master degree in Tianjin University. In 2019, she continued to study for PhD degree in Tianjin University. Her research interest mainly focuses on developing different nano-biosensors for tumor biomarkers detection.

**Xiaoqun Gong** received her Master’s and Doctor’s degree in School of Materials Science and Engineering, Tianjin University in 2009 and 2013, respectively. She worked as a visiting student in John Hopkins University in 2012. Then, she started her career in School of Life Sciences, Tianjin University. Her research interests are in designation, construction and application of different IVD devices for the detection of various biomarkers, such as the protein, microRNA and so on.

**Professor Jin Chang** received his bachelor’s and master’s degrees from Tianjin Medical University in 1986 and 1991, respectively. And he got his Doctor’s degree in school of polymer chemistry and physics, Institute of Polymer Chemistry, Nankai University in 1994. Currently, he is the director of the Institute of Nanobiomedicine Sciences, School of Life Sciences, Tianjin University, and the director of the Engineering Technology Center of Micro-Nano Biomaterials and Theranostics in Tianjin. His research interests include the application of nanomaterials and technologies in targeted-regulating release of genes, drug delivery systems and medical diagnostics.

**How to cite this article**: W. Peng, W. Li, H. Han, H. Liu, P. Liu, X. Gong, J. Chang, *VIEW*. 2022, 3, 20200191. https://doi.org/10.1002/VIW.20200191