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REVIEW

# Peroxidase-like nanozyme sensing arrays for versatile analytes

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**Abstract** Nanozyme biosensors are generally constructed by using the conventional lock-and-key strategy, which may not be available for some emerging analytes without specific recognition elements. In addition, it is difficult to carry out multiplex detection. To address these challenges, array-based sensing platforms have been constructed by using peroxidase-like nanozymes (i.e., "nanozyme sensor arrays"). In this review, we highlight the newly developed nanozyme sensor arrays and their applications for detection and discrimination of various analytes, including bioactive small molecules, proteins, and cancer cells. Moreover, we discuss the use of nanozyme sensor arrays for monitoring the enzymatic hydrolysis process by tracking patterns. They were successfully used for probing the hydrolytic processes of adenosine 5′-triphosphate disodium salt (ATP) and pyrophosphate (PPi) catalyzed by apyrase and PPase, respectively. To further enhance the robustness of output signals, ratiometric fluorescent sensor arrays were also constructed by using $C_3N_4$-based fluorescent nanozymes. Finally, some insights on future development for nanozyme sensor arrays were provided.

**Keywords** Nanozymes · Sensor array · Peroxidase-like activity · Biosensing

## Introduction

Nanomaterials with enzyme-like catalytic activities (i.e., "nanozymes") have recently been developed to address the limitations of high cost and low stability of enzymes (Huang et al. 2019; Wang et al. 2016; Wu et al. 2019). Until now, several types of enzymes, such as peroxidase, oxidase, catalase, and superoxide dismutase, have been mimicked by various nanomaterials including metal, metal oxide, and carbon (Wang et al. 2019a; Wu et al. 2019). Among them, great efforts have been devoted to developing peroxidase-like nanozymes because of their broad applications (Gao et al. 2007; Wu et al. 2019). Peroxidase-mimicking nanozymes have been extensively used for constructing biosensing platforms for various analyte detections (Wang et al. 2018a). While breakthroughs in the field of nanozyme biosensing have been achieved, current nanozyme biosensors are generally

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constructed by using a conventional lock-and-key strategy (Fig. 1a). There are two important processes involved in current nanozyme biosensing: analyte-specific recognition and signal transduction (Fig. 1a). The specific recognition is usually achieved by pre-selected bioligands (e.g., an oxidase, an aptamer, or an antibody), while the signal transduction is commonly achieved by catalyzing a colorless or non-fluorescent substrate to the corresponding colorimetric or fluorescent product (Wang et al. 2017; Zhang et al. 2017). However, some emerging analytes without specific ligands may not be detected by these nanozyme biosensors. In addition, the conventional "one-to-one" nanozyme biosensor can only detect one analyte, which is difficult to perform multiplex detection. To address these challenges, cross-reactive sensor arrays have been constructed by using peroxidase-like nanozymes (Diehl and Anslyn 2013).

Sensor arrays (the so-called artificial noses/tongues) are array-based sensing platforms using materials or/and molecules to mimic the mammalian gustatory and olfactory systems (Diehl and Anslyn 2013; Geng et al. 2019; Hizir et al. 2017). To identify an odorant or tastant molecule, the mammalian gustation and olfaction use cross-reactive receptors to differently interact with them, resulting in a characteristic pattern for that odor or taste in the brain (Diehl and Anslyn 2013). Similar with the gustatory and olfactory systems, sensor arrays can also generate distinct cross-reactive signals for each analyte, enabling multiplex detection and non-specific event recognition (Fig. 1b). Using peroxidase-like nanozymes for constructing sensor arrays can not only address the limitations of conventional "one-to-one" nanozyme biosensor but also provide catalytically amplified signal for transduction originated from the peroxidase-like activities.

In this review, we focus on the array-based sensing of versatile analytes using peroxidase-like nanozymes, demonstrating its unique advantages compared with traditional nanozyme biosensing method. We first review the use of nanozyme sensor arrays for discrimination of various analytes, including bioactive small molecules, proteins, and cells (Huang et al. 2017; Li et al. 2019; Li et al. 2012; Qiu et al. 2018; Wang et al. 2018b;

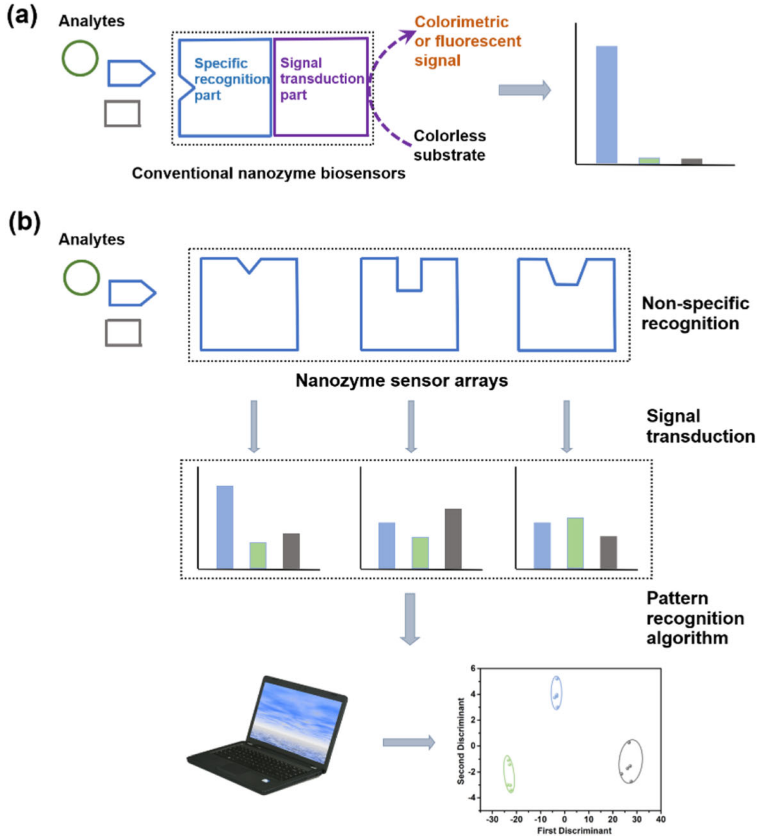

**Fig. 1** **a** Nanozyme biosensing platform based on lock-and-key strategy. **b** General outline of array-based sensing using peroxidase-like nanozymes for discrimination of analytes

Wei et al. 2017). Subsequently, we discuss the use of nanozyme sensor array for probing enzymatic hydrolysis reaction (Qin et al. 2018). Moreover, to enhance the robustness of output signals, ratiometric fluorescent nanozyme sensor arrays were constructed (Rana et al. 2016; Wang et al. 2019b). Finally, we will provide some insights on future development for array-based sensing using peroxidase-like nanozymes.

## Nanozyme sensor arrays for detecting versatile analytes

Rotello group first reported that array-based sensing of proteins could be achieved by using peroxidase-mimicking $Fe_3O_4$ nanoparticles (Fig. 2a) (Li et al. 2012). In their study, two nanoparticles (Dop-$Fe_3O_4$ and TMA-$Fe_3O_4$) were obtained by functionalizing $Fe_3O_4$ with dopamine (Dop) and trimethylammonium (TMA), respectively (Fig. 2b). They found proteins could affect the availability of reaction substrates to the $Fe_3O_4$ surface, which would in turn lead to the differential inhibitory effect on the peroxidase-like activity of $Fe_3O_4$ nanoparticles (Fig. 2a). Therefore, these two $Fe_3O_4$ nanoparticles with different modifications could be used as receptors for probing the difference in protein size and surface hydrophobicity. In addition, the $Fe_3O_4$ nanoparticles could give signal transduction by catalyzing the oxidation of the colorless 2,2′-azinobis (3-ethyl benzothi-azoline-6-sulfonic acid) ammonium (ABTS) to a green product with the help of $H_2O_2$ (Fig. 2a). Response patterns for proteins could be obtained by the OD value at 420 nm (Fig. 2c). Subsequently, response patterns were processed by linear discriminant analysis (LDA). Ten proteins with 50 nM were efficiently discriminated with no misclassification (Fig. 2d).

To further extend the use of nanozyme sensor arrays for other analyte detection, we recently constructed universal cross-reactive sensor arrays using peroxidase-mimicking platinum (Pt), ruthenium (Ru), and iridium (Ir) nanoparticles (Wang et al. 2018b). Analytes including biothiols, proteins, and cancer cells were successfully identified

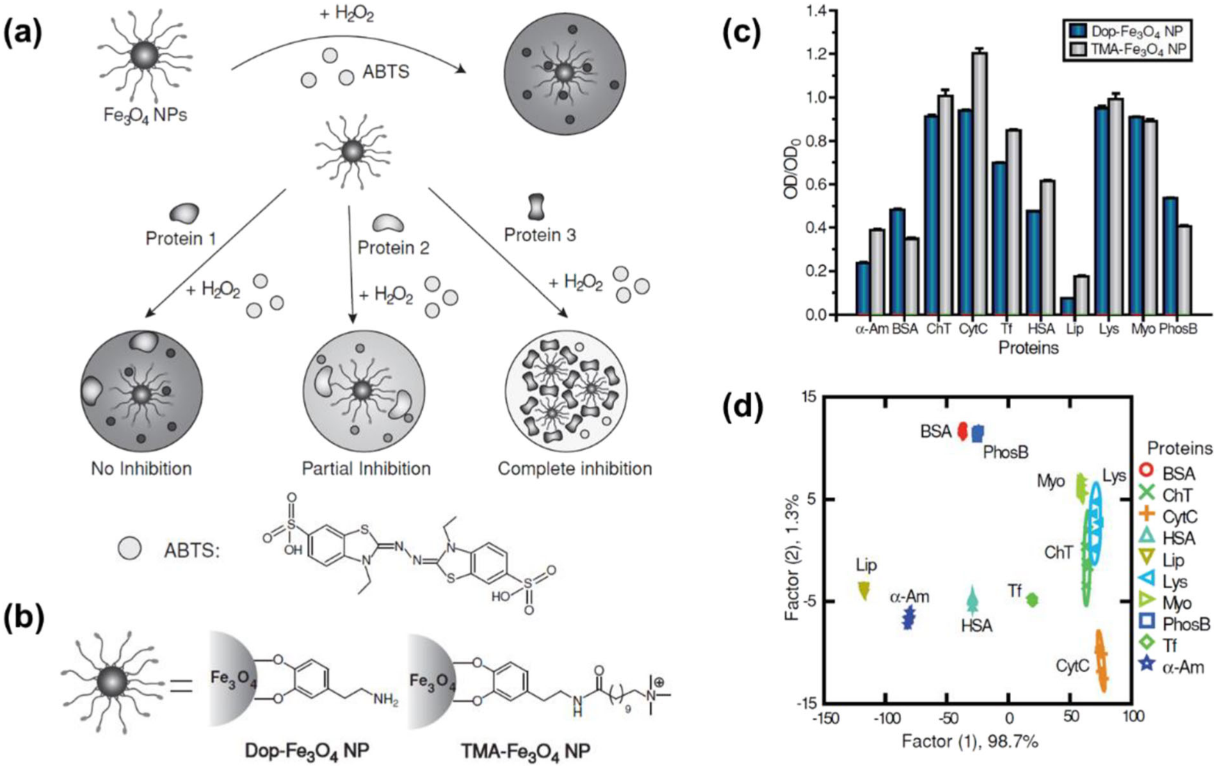

**Fig. 2** Array-based sensing of ten proteins. **a** Schematic of the $Fe_3O_4$ nanoparticles with peroxidase-mimicking activities for colorimetric sensing of proteins. **b** Structure of Dop-$Fe_3O_4$ and TMA-$Fe_3O_4$. **c** Response patterns in the presence of proteins at 50 nM. Each error bar shows the standard deviation of five independent measurements. **d** Canonical score plot for the response patterns as obtained from LDA with 95% confidence ellipses (reprinted with permission from John Wiley and Sons (Li et al. 2012))

(Fig. 3) (Wang et al. 2018b). Biothiols could be discriminated because their differential competitive effect on the catalytic activities of Pt, Ru, and Ir nanozymes. The differentiation principle for proteins was the same with Rotello's work (Li et al. 2012). The identification of cancer cells was conducted by interfacing nanozymes with cell lysate solution. Cancer cells from different tissue origins possessed differential components in cell lysate solutions, resulting in the differential effect on the catalytic activities of nanozymes. In this study, the universal sensor arrays were successfully used for discriminating various analytes, including six biothiols, nine proteins, and five cancer cells (Fig. 3) (Wang et al. 2018b). Moreover, biothiols in serum and proteins in human urine were successfully discriminated, demonstrating the practical applications of the nanozyme sensor arrays.

In another study, we fabricated two-dimensional metal-organic framework (2D-MOF) nanozyme-based sensor arrays (Fig. 4) (Qin et al. 2018). Three 2D-MOFs were prepared by coordination of divalent metal ions (i.e., $Zn^{2+}$, $Co^{2+}$, or $Cu^{2+}$) and a heme-like metalloporphyrin ligand Fe(III) tetra(4-carboxyphenyl)porphyrin chloride (TCPP(Fe)) (Cheng et al. 2017; Qin et al. 2018). Further studies suggested that phosphates could interact with the metal node ions of 2D-MOFs to trigger the collapse of the 2D structure, which in turn promoted the binding between phosphates and iron catalytic activity sites in TCPP(Fe) monomer (Fig. 4a). Therefore, different phosphates could differentially modulate the peroxidase-like activities of 2D-MOF nanozymes, which formed the basis for phosphates discrimination (Fig. 4b). Five phosphates, including adenosine 5′-monophosphate sodium (AMP), adenosine 5′-diphosphate sodium salt (ADP), ATP, PPi, and phosphate (Pi), were successfully discriminated not only in aqueous solutions but in serum samples (Fig. 4c). Thirty unknown samples containing phosphates were accurately identified, demonstrating the practical applications of 2D-MOF nanozyme sensor arrays (Qin et al. 2018).

## Nanozyme sensor arrays for probing enzymatic hydrolysis

In addition to analytes detection, real-time biologically important events were also probed by using the sensor arrays (Hatai et al. 2017; Pode et al. 2017; Qin et al. 2018). As discussed above, different phosphates exhibited differential inhibitory effect on the peroxidase-like activities of 2D-MOFs (Fig. 4). Based on this principle, 2D-MOF-based nanozyme sensor arrays could also be used for monitoring the hydrolytic reactions catalyzed by phosphatase-related hydrolases (Fig. 4a) (Qin et al. 2018). Two important hydrolases (i.e., apyrase and PPase) involved in ATP and PPi hydrolysis reactions were studied. These hydrolases could catalyze the hydrolysis of phosphoester bonds in ATP and PPi, which in turn resulted in the decreased inhibitory effect on the catalytic activities of 2D-MOFs (Qin et al. 2018). As shown in Fig. 5, PPase was taken as a sample to demonstrate the power of nanozyme sensor arrays for monitoring the enzymatic hydrolytic process (Qin et al. 2018). As the hydrolysis reaction of PPi proceeded, the values of $A/A_0$ increased because the hydrolytic product (i.e., Pi) possessed a weaker inhibitory effect on nanozymes' catalytic activities (Fig. 5a). Moreover, the hydrolytic process was also demonstrated by the conventional molybdenum-blue

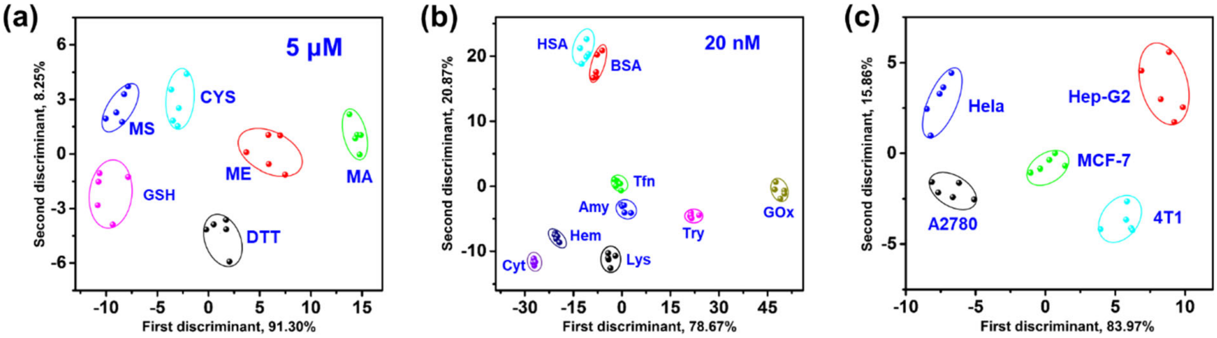

**Fig. 3** Nanozyme sensor arrays for detecting versatile analytes including **a** biothiols, **b** proteins, and **c** cancer cells (reprinted with permission from American Chemical Society (Wang et al. 2018b))

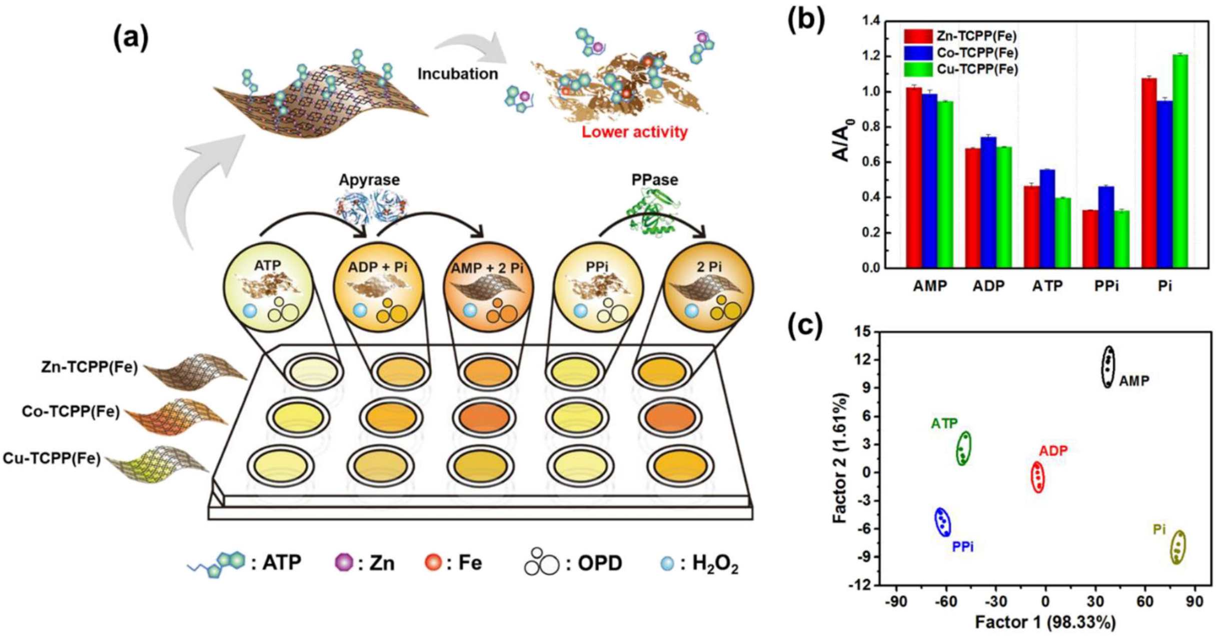

**Fig. 4** **a** Schematic of 2D-MOF-based nanozyme sensor arrays for discrimination of five phosphates. **b** Colorimetric response patterns of nanozyme sensor arrays toward 10 μM phosphates. Each error bar shows the standard deviation of six independent measurements. **c** Two-dimensional canonical score plot for the first two factors of the colorimetric response patterns obtained against 10 μM phosphates (reprinted with permission from American Chemical Society (Qin et al. 2018))

colorimetric method (Fig. 5b). Increased concentration of Pi was detected over time (Fig. 5b). As the hydrolysis reaction proceeded, the cluster of canonical patterns at different reaction time points moved from the position of PPi to the Pi position, demonstrating the whole hydrolytic process could be monitored by tracking the fingerprint patterns (Fig. 5c).

## Ratiometric sensor arrays with enhanced robustness

To enhance the robustness of detection, very recently, we developed ratiometric fluorescent sensor arrays (Wang et al. 2019b). In this study, we reported that three different fluorescent $C_3N_4$-based nanozymes (i.e., $C_3N_4$-Ru, $C_3N_4$-Cu, and $C_3N_4$-hemin) possessed

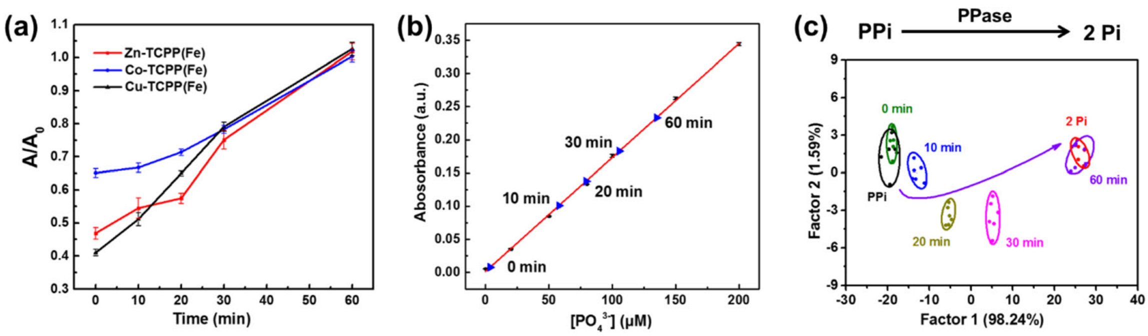

**Fig. 5** Probing PPase-catalyzed hydrolytic reaction by nanozyme sensor array. **a** The $A/A_0$ responses of hydrolytic process catalyzed by PPase over time, where A was the absorbance of oxidized OPD (OPDox) at 450 nm in the presence of phosphates, and $A_0$ was that of the blank one without phosphates. Each error bar shows the standard deviation of six independent measurements. **b** Molybdenum-blue colorimetric method quantification of hydrolysates catalyzed by PPase. **c** Two-dimensional canonical score plot for the first two factors of response patterns obtained against PPi, 2 Pi, and assay samples from different time points in PPi hydrolytic process catalyzed by PPase. The canonical scores were calculated by LDA for the identification and discrimination of phosphates in catalytic reactions (reprinted with permission from American Chemical Society (Qin et al. 2018))

excellent peroxidase-like activity. Ruthenium and copper ions could be incorporated into $C_3N_4$ nanosheets by the coordination of metal ions with pyridinic nitrogen moieties to form $C_3N_4$-Ru and $C_3N_4$-Cu, while $C_3N_4$-hemin could be obtained by the π-π interaction between the $C_3N_4$ nanosheets and hemin. The excellent fluorescent properties of $C_3N_4$-based nanozymes enabled radiometric signal output. As shown in Fig. 6, these $C_3N_4$-based nanozymes possessed fluorescent peaks at 438 nm when exciting at 385 nm (Wang et al. 2019b). In the presence of $H_2O_2$ and *o*-phenylenediamine (OPD), $C_3N_4$-based nanozymes could catalyze the oxidation of OPD to OPDox. OPDox could emit a new fluorescent peak at 564 nm. Moreover, it could also quench the fluorescence of $C_3N_4$-based nanozymes by photoinduced electron transfer effect. Therefore, the fluorescence intensity ratio between 564 and 438 nm enabled the ratiometric nanozyme bioanalysis. Moreover, phosphates exhibited differential inhibitory effects on the peroxidase-like activities of $C_3N_4$-based nanozymes. On the basis of this principle, ratiometric fluorescent sensor arrays were constructed and five

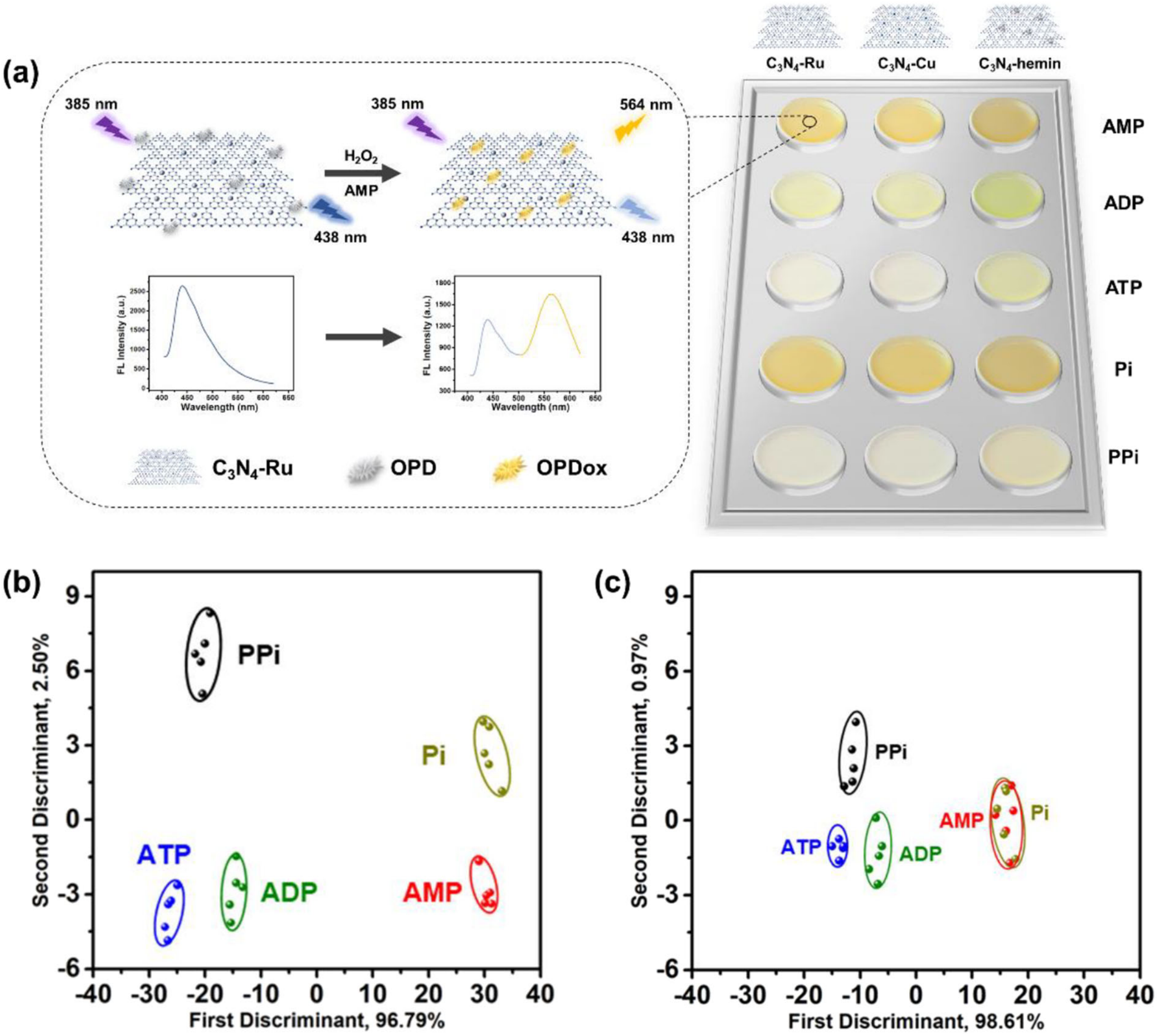

**Fig. 6** **a** Ratiometric fluorescent sensor arrays constructed using peroxidase-like $C_3N_4$-based nanozymes for discrimination of five phosphates. Two-dimensional canonical score plot for the first two factors of phosphates obtained from the **b** ratiometric fluorescence-response patterns and **c** colorimetric response patterns toward 10 μM phosphates (reprinted with permission from American Chemical Society (Wang et al. 2019b))

phosphates (i.e., AMP, ADP, ATP, PPi, and Pi) were successfully discriminated (Fig. 6). Compared with the ratiometric fluorescent sensor arrays, array-based sensing using single colorimetric signal produced overlapped clusters and had a narrower distance between five clusters, demonstrating the unique advantages of ratiometric signal output (Fig. 6b, c).

## Summary

In this review, peroxidase-like nanozyme sensing arrays for versatile analytes were discussed. The unique physicochemical properties of nanozymes make them ideal sensing elements for interacting with non-specific analytes. Moreover, the peroxidase-like activities of nanozymes endow them catalytically amplified ability for transduction of the recognition events. Therefore, constructing array-based sensing method using peroxidase-like nanozymes could be promising for analyte detection. Versatile analytes, including bioactive small molecules, proteins, and cells, were detected and discriminated by nanozyme sensor arrays. Moreover, nanozyme sensor arrays were also used for monitoring the enzymatic hydrolysis process. Despite these substantial successes, several challenges still need to be addressed for advancing further research of array-based nanozyme biosensing. First, the analytes detected by nanozyme sensor arrays are still limited. More receptors should be carefully designed for other emerging and biologically important analytes detection. Second, highly specific nanozyme sensor arrays should be constructed, enabling to track patterns to follow real-time biologically important events. Finally, more advanced designs are needed to promote nanozyme sensor arrays into clinical diagnosis by coupling paper-based sensing systems and mobile reading technologies, etc.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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