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TRENDS

Functional nucleic acid-based fluorescence polarization/anisotropy biosensors for detection of biomarkers

Yunlong Bai[1] · Tong Shu[1,2] · Lei Su[1] · Xueji Zhang[1,3]

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Abstract

The sensitive and selective detection of biomarkers plays a crucial role in disease diagnostics, drug discovery, and early screening of cancers. The achievement of this goal highly depends on the continuous development of biosensing technologies. Among them, fluorescence anisotropy/polarization (FA/FP) analysis receives increasing interest due to the advantage of simple operation, fast response, and no background interference. In recent decades, great progress has been achieved in FA/FP sensors thanks to the development of functional nucleic acids (FNAs) including aptamers and nucleic acid enzymes. This review focuses on FNA-based FA/FP sensors for the quantitative detection of biomarkers, such as nucleic acid, small molecules, and proteins. The design strategies, recognition elements, and practical applications are fully highlighted. The article also discusses the challenges of applying FNA-based FA/FP sensors in the next generation and the potential solutions along with future prospects.

Keywords Fluorescence anisotropy · Fluorescence polarization · Biosensor · Functional nucleic acids · Biomarker

Introduction

Biomarkers including nucleic acids, proteins, and small metabolic molecules are biochemical indicators that reflect the change of structure or function of human systems, organs, tissues, and cells [1–4]. The quantitative detection of diverse pathological biomarkers in biofluids (e.g., blood or urine) plays vital roles in the field of clinical monitoring, drug discovery, and early screening of cancers. However, it is still a challenge to precisely measure biomarkers because of the complexity of the sample and the high accuracy requirements. Therefore, there is great demand for the rapid development of sensitive and specific sensors for the analysis of target biomarkers in complex matrixes [5–8]. Among many available analysis methods, fluorescence anisotropy/polarization (FA/FP) sensors receive increasing interest due to their advantages of high sensitivity, simplicity, rapidity, and robustness [9–13]. Typically, FA/FP sensors employ antibodies as the key recognition elements, which have been used for quantitative detection of low mass molecules with the availability of rapidly developed instruments. In such immunoassays, homogeneous analysis without separation can be performed owing to the large different FA/FP values of the macromolecule antibody and the antigen, which greatly shortens the measurement time [14–16]. Nowadays, this method has been expanded to biological analysis, food contaminants detection, and environmental monitoring [17, 18].

The discovery and synthesis of a large number of functional nucleic acids (FNAs), such as aptamers and nucleases, have provided great convenience for the construction of biosensors and made FNA-based sensors widely used in assay development [19–21]. Nucleic acid aptamers, single-stranded nucleic acids selected by in vitro selection, can sense and bind a range of targets, including inorganic ions, small molecules, proteins, and cells. Some aptamers have certain enzyme-like activities upon specific binding of the targets [22–26]. Compared with antibodies, aptamers have promising features, such as good stability, easy labeling with functional groups, facile

✉ Tong Shu
shutong@ustb.edu.cn

✉ Xueji Zhang
zhangxueji@ustb.edu.cn

[1] Research Center for Bioengineering and Sensing Technology, School of Chemistry and Biological Engineering, University of Science and Technology Beijing, Beijing 100083, China

[2] Guangdong Provincial Key Laboratory of Luminescence from Molecular Aggregates, South China University of Technology, Guangzhou 510640, Guangdong, China

[3] School of Biomedical Engineering, Shenzhen University Health Science Center, Shenzhen 518060, Guangdong, China

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production, low cost, remarkable specificity, and great affinity for targets [26, 27]. In recent years, smart FP/FA sensors have attracted much attention due to the effective integration of FNAs and FP/FA technology [28–30]. Although some reviews have highlighted the utility of FNA-based FA/FP sensors in sensing many target analytes, topics of FNA-based sensors specifically targeting disease-related biomarkers are not well organized. The present review focuses on the introduction of principles and advantages of the FA/FP sensors and details the applications of various types of FNA-based FP/FA sensors to achieve quantitative detection of different kinds of biomarkers including DNA, miRNA, small molecules, and proteins. Challenges and potential solutions are also discussed.

Principle of FA/FP

FP/FA, a powerful signaling transduction approach, provides quantitative information on molecular interaction based on the change of the rotational diffusion of a fluorophore. Figure 1 shows a schematic diagram of FA/FP. Polarized light is used to excite the fluorophore. If the fluorophores rotate slowly, the emitted light is still greatly polarized. On the contrary, if the fluorophores rotate quickly, depolarized light will be observed. In general, FP/FA values can be easily acquired by determining the fluorescence intensities from two polarization planes [10, 17, 18]. The FA value can be defined by the following equation: $FA = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$, where $I_{\parallel}$ represents the fluorescence intensity with polarization parallel to the excitation plane and $I_{\perp}$ stands for the fluorescence intensity with polarization perpendicular to the excitation plane. Similarly, the FP value is given according to the equation: $FP = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$. Accordingly, the FP and FA values are interchangeable according to the following equation: $FA = 2FP/(3 - FP)$ or $FP = 3FA/(2 + FA)$ [9, 17]. FA and FP are not only related to innate properties of the fluorescent molecules including molecular volume, shape, fluorescence lifetime, and rotation speed but also to the environmental factors, such as the solution viscosity and temperature. In particular, fluorophores with fast rotation rates usually exhibit low FA/FP signals, while fluorophores with low rotation rates display high FA/FP signals. This approach does not require separation of fluorescent molecules from the sample, making the FA/FP sensors simple and rapid, which can be applied in high-throughput analysis [31–34]. So far, FA/FP sensors have been extensively utilized for the detection of various targets in a variety of application fields including clinical diagnosis, environmental monitoring, and food safety.

FNA-based FA/FP biosensors for detection of biomarkers

The detection of diverse biomarkers attracts much research interest due to its widespread applications in biomedical, clinical, and biochemical fields. It is essential to develop simple, cost-effective, sensitive, and selective detection methods for biomarkers. FNA-based FA/FP biosensors with advantages of rapidness, high sensitivity, and selectivity can be used for sensing different kinds of biomolecules including nucleic acids, proteins, and small molecules, which are summarized in Tables 1, 2, and 3, respectively, along with the design methods, response type, detection limit, and dynamic range.

How to obtain a large FA/FP change in the presence of target is the key step in the design of FA/FP sensors. Generally, the strategies used for FNA-based FA/FP biosensors are divided into three types: (1) Direct FA/FP assays. Fluorescently labeled aptamers can be used to generate FA/FP signal changes upon interaction with target molecules, especially for detection of proteins, which will be discussed in "FNA-based FA/FP biosensors for detection of proteins" in detail. This change of FA/FP signal is achieved through the binding-induced changes of local rotation of fluorophores. (2) Competitive FA/FP approach. A fluorescently labeled target molecule (also called fluorescent tracer) and a label-free

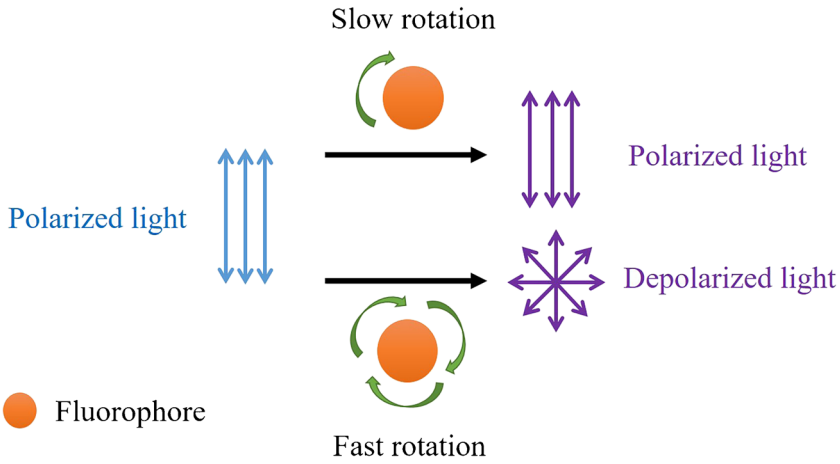

Fig. 1 Principle of FA/FP

aptamer are needed. The presence of analytes induces the displacement of fluorescent tracer from the aptamer–tracer complexes, showing a decreased FA/FP signal due to the smaller molecular volume. Aptamer-based competitive FA/FP assay is suitable for the detection of small molecules, which will be discussed in "FNA-based FA/FP biosensors for detection of small molecules" in detail. (3) Signal amplification of FA/FP assays. A lot of magnification methods based on the application of DNAzymes, endonucleases, exonucleases, and nanoparticles have been successfully used for development of FA/FP sensors, which greatly improve their sensitivity. Among them, unique nanomaterials including silica nanoparticles, gold nanoparticles (AuNPs), and carbon nanomaterials have been successfully used to amplify FA/FP signals. FNAs can be adsorbed by nanomaterials, which will increase the size and affect the fluorescence lifetime of fluorophores caused by fluorescence quenching in the close distance between nanoparticle and fluorescent dyes. Hence, change of FA/FP signal enables the detection of targets. On the other hand, quenching effects may cause false positive test results. Changes of experimental conditions including the concentration of nanomaterials, amounts of fluorescent probes etc., and rational design strategy will greatly improve the detection effect [35]. FA assays with nanomaterials as signal amplifiers for detection of nucleic acids, proteins, and small molecules have been successfully achieved and the design strategies will be discussed in the following section in detail.

FNA-based FA/FP biosensors for detection of nucleic acid

It is essential to develop simple and sensitive detection assays for specific nucleic acids, such as DNA and miRNA, the concentration of which are related to some diseases. FNA-based FA/FP biosensors can be used to detect specific nucleic acid sequences, which are summarized in Table 1. Li et al. reported a sensitive FP sensor for detection of miRNA based on streptavidin-coated polystyrene nanospheres (SA-PS NPs) and T7 exonuclease (T7 Exo)-assisted dual-cycle signal amplification [41]. As demonstrated in Fig. 2, in the presence of target miRNA, the hairpin probe hybridized with miRNA, forming a double-stranded DNA (dsDNA) structure, which was then recognized and digested by T7 Exo, releasing some single-stranded DNA fragments and miRNA. The released target miRNA could trigger the next cycle of hybridization and digestion, releasing more non-degraded fragments from the hairpin probe, which will hybridize with a signal probe with fluorescein and biotin modification at 5′- and 3′-ends. The formed signal probe was then recognized and degraded by T7 Exo, releasing the fragments and the fluorophore. The free fluorescein could not connect with SA-PS NPs, which were used as the FP signal amplifier. Therefore, there was a big change of FP signal after adding miRNA into the detection system and the limit of detection (LOD) of this method was about 0.001 nM.

Liang et al. achieved an FP gene sensor for human immunodeficiency virus (HIV) DNA detection based on the use of AuNPs acting as signal amplifiers [42]. AuNPs were modified with DNA sequences that were partially matched with the target DNA. In the presence of HIV DNA, the AuNPs–DNA and dye-labeled DNA probe combined with HIV DNA in a sandwich shape to form a conjugate. This reaction slowed down the rotational speed of the dye-decorated DNA probe as a result of the increase in molecular volume and weight, resulting in an increased FP signal. The LOD of this FP sensor was 73 pM DNA.

2D nanosheet with distinctive advantages can be used to amplify FA/FP signals [5]. Using graphene oxide (GO) as a mass enhancer, Wang et al. reported a turn-on FP sensor for detection of HIV DNA [37]. T7 Exo-assisted target recycling amplification was used to amplify the detection signal. The presence of the target DNA led to target recycling with the

Table 1 Comparison of FNA-based FA/FP sensors for detection of nucleic acids in homogeneous assays

Signal	Targets	Design methods	Dynamic range	Response type	Detection limit	Ref.
FP	DNA	SiNPs–AgNCs–DNA	0.001–10 nM	On	0.02 nM	[36]
FP	DNA	GO–hairpin DNA	50 pM–2 nM	On	38.6 pM	[37]
FP	DNA	GO–hairpin DNA–T7 Exo	50 pM–8 nM	On	9.12 pM	[38]
FA	miRNA	CHA–GO–DNA	47 pM–16 nM	Off	47 pM	[39]
FP	DNA	HCR–SiNPs	35 pM–2.5 nM	On	35 pM	[40]
FP	miRNA	DNA–T7 Exo–DNA–PS NPs	0.001–10 nM	Off	0.001 nM	[41]
FP	DNA	AuNPs–DNA	150 pM–6 nM	On	73 pM	[42]
FA	DNA	GO–DNA	8–40 nM	Off	4.6 nM	[43]

DNA deoxyribonucleic acid, *SiNPs* SiO_2 nanoparticles, *AgNCs* silvernanocluster, *Exo* exonuclease, *CHA* catalyzed hairpin assembly, *HCR*hybridization chain reaction, *PS NPs* polystyrene nanoparticles, *GO* grapheneoxide.

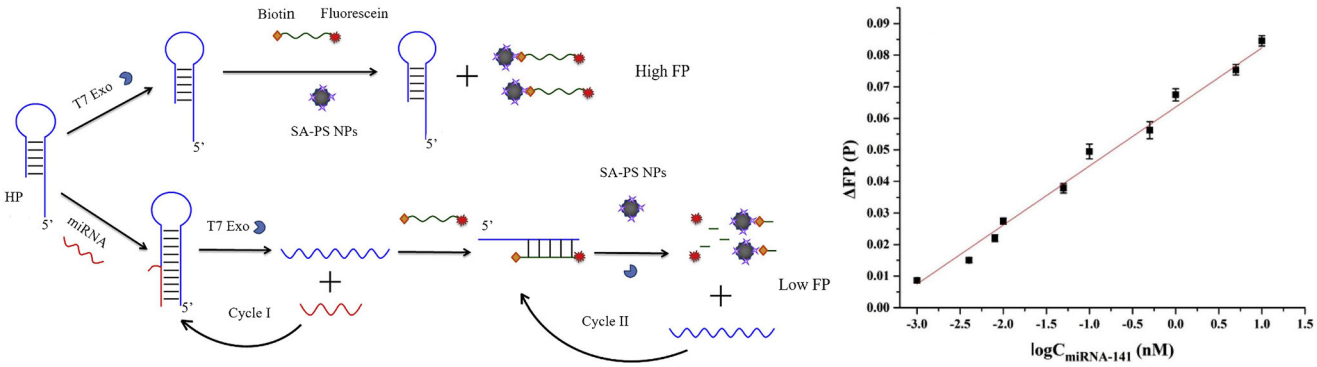

Fig. 2 Schematic of FP sensor for detection of miRNA based on PS NPs and T7 Exo-assisted dual-cycle signal amplification. PS NPs polystyrene nanospheres, SA-PS NPs streptavidin-coated polystyrene nanospheres, T7 Exo T7 exonuclease. Reprinted with permission [41], 2019 Elsevier B.V.

assistance of T7 Exo. Furthermore, the amplification products were adsorbed onto the surface of GO, leading to a higher FP value. The LOD of this FP biosensor was 38.6 pM. In addition, the same group described a quadratic recycling amplification FP sensor for HIV DNA sequence detection [38]. The assay used T7 Exo-assisted two-round recycling amplification to enlarge the nuclear acid signals and used GO as an amplifier of FP. The LOD of this FP sensor was 9.12 pM. In order to improve the detection sensitivity, Zhen and co-workers reported a target-catalyzed hairpin assembly (CHA)-assisted GO-amplified FA strategy for miRNA detection [39]. This approach featured a pair of designed hairpins which could not initially interact with each other but could catalytically form a duplex in the presence of an initiator single-stranded nucleic acid input and dye-labeled double-stranded probes adsorbed onto GO. The presence of target miRNA triggered the CHA reaction, which induced the release of the dye-labeled double-stranded probes from GO. The decrease FA signals allowed sensitive detection of miRNA with a LOD of 47 pM.

SiO_2 nanoparticles (SiNPs) were another widely used amplifier in FP/FA assays. Chen et al. reported a turn-on FP biosensor based on the SiNPs–DNA–silver nanocluster (AgNCs) sandwich structure for amplified detection of hepatitis B virus DNA [36]. The biotin–DNA probe was attached to the surface of SiNPs through the high-affinity interaction of streptavidin and biotin and another AgNC-decorated DNA probe was also added. This system showed a small FP signal due to the small molecular volume of DNA–AgNCs. In the presence of target DNA, the sandwich structure forms at the surface of SiNPs, resulting in a substantial increased FP signal due to the enlargement of the molecular volume of the formed SiNP–functionalized DNA–AgNCs sandwich structure with slow rotation. Zhao and co-workers reported a FP sensor for detection of nucleic acid based on the amplification of hybridization chain reaction (HCR) and SiNPs as illustrated in Fig. 3 [40]. This platform contained three parts: biotin-labeled hairpin probe H1, fluorescein-modified hairpin probe H2, and streptavidin-coated SiNPs (SA-SiNPs). In the presence of target nucleic acid, probe H1 and probe H2 formed long fluorescent double-stranded polymers. The rotation rate of fluorescein slowed down by the formation of long HCR products with a larger molecular mass, resulting in a high FP value. After introduction of SA-SiNPs, the double-stranded HCR products and SA-SiNPs were combined via the interactions of biotin and streptavidin, generating much larger fluorescent complexes. Thus, a further amplified FP signal was obtained. The LOD of this HCR and nanoparticle-based biosensor was 35 pM DNA.

FNA-based FA/FP biosensors for detection of proteins

Protein plays a critical role in regulating various physiological functions. Extremely sensitive and accurate detection of protein at ultra-trace level is essential to disease diagnostics and clinical therapy. Table 2 summarizes FNA-based FA/FP sensors for detection of proteins in homogeneous assays. Single dye-labeled aptamer-based turn-on FA/FP sensors have been used for detection of target proteins including human neutrophil elastase [56], PDGF-BB [49], IgE [52], angiogenin [53], and lysozyme [50]. In general, the molecular weight of the aptamer was smaller than that of the protein; thus, the molecular size and weight of an aptamer dramatically increased upon binding of the target protein and consequently the rotation of the aptamer slowed down. If the aptamer was fluorescently labeled with a small dye molecule, the slowly tumbling aptamer displayed an increased FA/FP signal and the changed FA/FP value allowed the sensitive detection of proteins without the need for separation. On the other hand, Wang's group reported a turn-off FA sensor for thrombin detection [44], which used a dye-labeled internal base of the aptamer as recognition element. The authors also comprehensively compared the influence of different types of fluorophore (TMR, FAM, Cy3, and Cy5) and metal in the binding buffer on the change of FA signal. This turn-off sensor showed a LOD of 0.1 nM. Thrombin is a multifunctional serine protease and involved in various physiological coagulations including

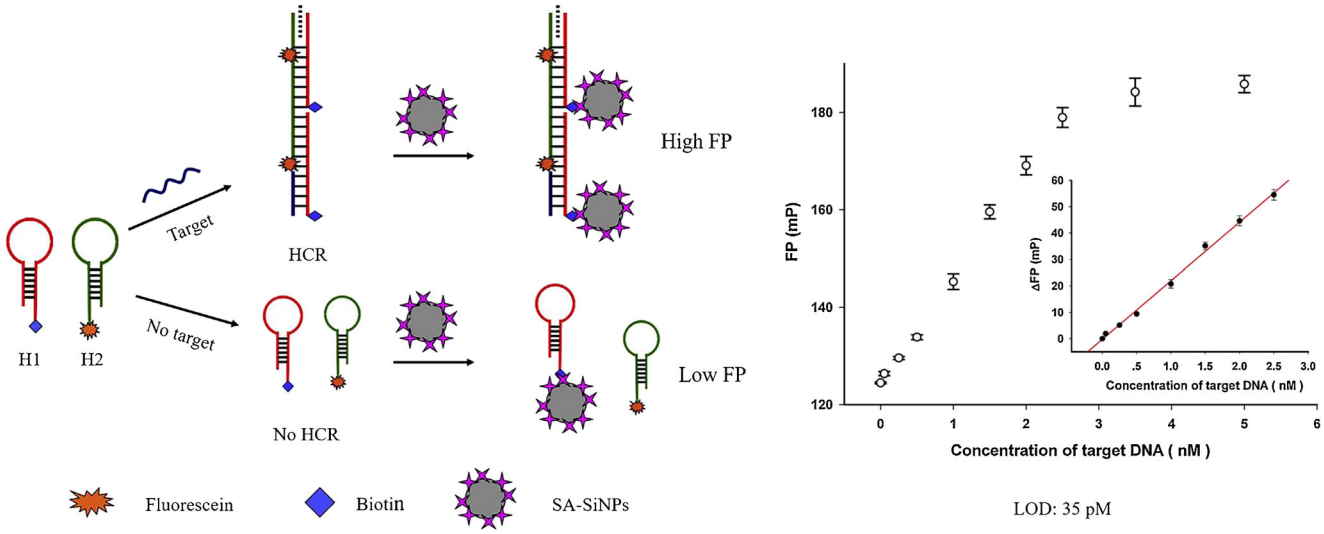

Fig. 3 Schematic illustration of the amplified FP sensor based on HCR and SiNPs. HCR hybridization chain reaction, SA-SiNPs streptavidin-coated SiO_2 nanoparticles. Reprinted with permission [40], Copyright 2014 Elsevier B.V.

regulation of platelet aggregation and conversion between fibrin monomer and fibrinogen. The occurrence of many diseases, such as cancers, Parkinson's disease, and diabetes, is related to thrombin, the concentration of which changed from picomolar to micromolar levels during the coagulation process [57].

Effective signal enhancement in a simple manner is most desirable for FA/FP sensor development. A terminally labeled dsDNA strategy was designed for DNA recognition-based applications [45]. The FA of dsDNA could be regulated by the molecular volume, dye types, and the end structure of dsDNA. By properly designing the end structure of dsDNA, the authors successfully fabricated turn-on and turn-off FA sensors for thrombin detection. In addition, the application of dyes with a positively charged center could induce higher FA signal due to the confinement of the segmental motion of dyes through electrostatic interactions.

Nanomaterials have been widely used as amplifiers in FP/FA sensors for detection of proteins. For instance, Yue et al. reported a SiNP-enhanced FP sensor for thrombin detection as shown in Fig. 4 [46]. Biotin-labeled thrombin aptamers were attached to SA-SiNPs. In the presence of target thrombin, it

Table 2 Comparison of FNA-based FA/FP sensors for detection of proteins in homogeneous assays

Signal	Targets	Design methods	Dynamic range	Response type	Detection limit	Ref.
FA	Thrombin	GO–aptamer	0.5–4 mg/L	Off	0.5 mg/L	[43]
FA	Thrombin	Internal dye-labeled aptamer	0.1–2 nM	Off	0.1 nM	[44]
FA	Thrombin	Terminal dye-labeled aptamer	51–40 nM 31–15 nM	On Off	51 nM 31 nM	[45]
FP	Thrombin	Dye–aptamer–SiNPs	0.6–100 nM	On	0.2 nM	[46]
FP	FR Thrombin	TiS_2 nanosheet–folate–DNAzyme TiS_2 nanosheet–aptamer–DNAzyme	0.01–20 ng/mL 0.05 pM–100 nM	Off	0.003 ng/mL 0.01 pM	[47]
FP	Thrombin	GO–aptamer–nicking enzyme	2 fM–200 nM	Off	2 fM	[48]
FA	PDGF-BB	Terminal dye-labeled aptamer	About 2–200 nM 0.22–1.25 nM	On	2 nM 0.22 nM	[49]
FA	Lysozyme	Terminal dye-labeled aptamer	12.5–300 nM	On	4.9 nM	[50]
FA	Angiogenin	Terminal dye-labeled aptamer	1–60 nM	On	1 nM	[51]
FA	IgE	Terminal dye-labeled aptamer	1–100 nM	On	0.35 nM	[52]
FP	PDGF-BB	PS NPs–aptamer–exonuclease	75 aM–100 nM	Off	75 aM	[53]
FP	Thrombin VEGF	Aptamer–CSDA–PS NPs	50 aM–100 pM 150 aM–1000 pM	On On	28 aM 86 aM	[54]
FP	Lactoferrin	Aptamer–AgNPs	0.2 ng/mL–25 μg/mL	On	0.1 ng/mL	[55]

FR folate receptor, *TiS_2* titanium disulfide, *PDGF-BB* platelet-derived growth factor BB, *IgE* immunoglobulin E, *VEGF* vascular endothelial growth factor, *CSDA* cascade strand-displacement amplification, *PS NPs* polystyrene nanoparticles

was captured by aptamer-functionalized SiNPs. When another terminal fluorescein-labeled aptamer was added to the system, a sandwich structure could form at the surface of SiNPs. The enhanced FP value enabled the quantification of thrombin. The sensor provided a linear range from 0.6 to 100 nM for thrombin with a LOD of 0.2 nM. Li et al. presented a TiS_2 nanosheet-enhanced FP biosensor for ultrasensitive detection of biomolecules including folate receptor (FR) and thrombin [47]. The working principle of this turn-off FP biosensor is demonstrated in Fig. 5. Folate-linked probe 1 could be digested by exonuclease I (Exo I) without the protection of target FR. Therefore, the absorption of FAM-linked probe 2 onto the TiS_2 nanosheet led to a high FP value. On the other hand, the presence of target FR could protect probe 1 from degradation by Exo I, which triggered Zn^{2+}-dependent DNAzyme-assisted signal amplification. The dye-labeled short DNA could thus not be adsorbed onto the TiS_2 nanosheet, resulting in a low FP signal. This assay showed a LOD of 0.003 ng/mL FR. The same analytical method was also applied to detect thrombin with good accuracy and sensitivity. A turn-off FA sensor using GO as amplifier for detection of target molecules was achieved [43]. TMR-labeled aptamers were adsorbed onto GO through a double-stranded region with the capture DNA, which restricted the rotation of TMR and showed a high FA signal. The presence of target thrombin triggered toehold-mediated strand exchange reaction and TMR-labeled aptamers were released from GO. A decreased FA signal was acquired for thrombin detection. Huang and coworkers developed a FP sensor based on T7 Exo and PS NPs signal amplification [53]. The presence of target enabled the immobilization of aptamer–target complex to dye-labeled DNA-functionalized PS NPs, which leads to the cyclic T7 Exo-catalyzed digestion of the dye-labeled DNA. A decreased FP value was measured for the sensing of PDGF-BB, an important protein regulating tumor cell growth and division.

FNA-based FA/FP biosensors for detection of small molecules

The determination of small molecule plays a vital role in clinical and biomedical fields. Table 3 summarizes the FNA-based FA/FP sensors for detection of different small molecules. Aptamer-based competitive FA/FP assays are used for small molecule detection. This approach requires a fluorescent tracer and a label-free aptamer that can bind to the target. The binding of aptamer and fluorescent tracer increases the molecular volume, giving a higher FA/FP signal. In the presence of small molecule analyte, fluorescent tracers are displaced from the aptamer–tracer complexes, showing a lower FA/FP signal due to the smaller molecular volume. Thus, changes of FA/FP values enable the quantitative determination of targets in the sample. The aptamer-based competitive assay has been applied to the detection of adenosine triphosphate (ATP) as shown in Fig. 6 [59]. ATP has biological functions in regulation of metabolism, energy cycle, transportation of ions, signal transduction, and building of large molecules. In order to increase the FA signal through changing the complex size, Li et al. presented an FA sensor using an extended aptamer sequence or a biotinylated aptamer that bound to streptavidin as recognition elements. The competitive binding between ATP in the test buffer and fluorescent tracer–aptamer complex led to larger changes of FA values. Hence, a higher sensitivity was achieved for the detection of target biomarkers [59].

Nanomaterials and proteins are often used as amplifiers for FA/FP sensor development. Zhu et al. reported a turn-off FP sensor based on the use of single-stranded DNA (ssDNA) binding protein as a signal enhancer [58]. The fluorophore-labeled aptamer was able to bind with ssDNA binding proteins, which showed high FP signal due to the large molecular size of aptamer–protein complex. The presence of target adenosine triggered the release of aptamer from the protein,

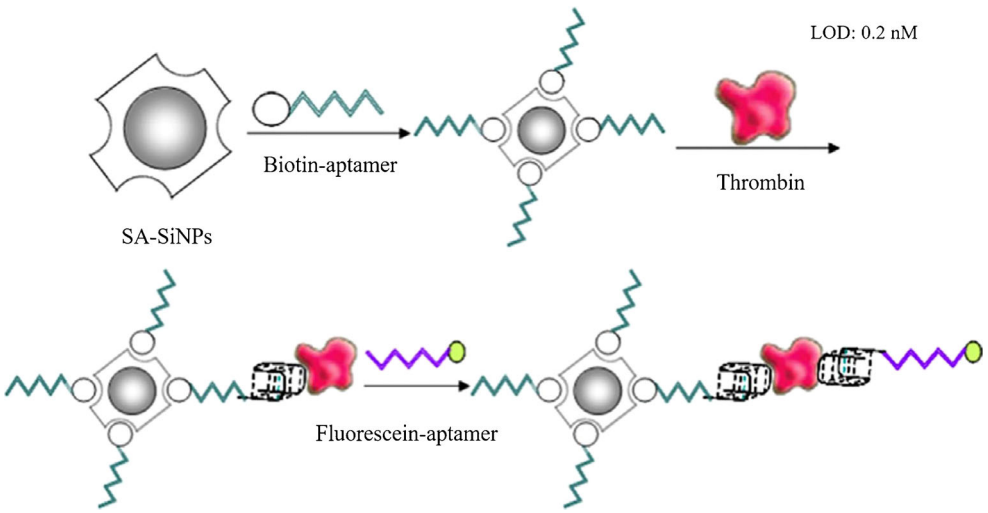

Fig. 4 Working principle of SiNPs-enhanced FP sensor for detection of thrombin. Reprinted with permission [46], Copyright 2014 Elsevier B.V.

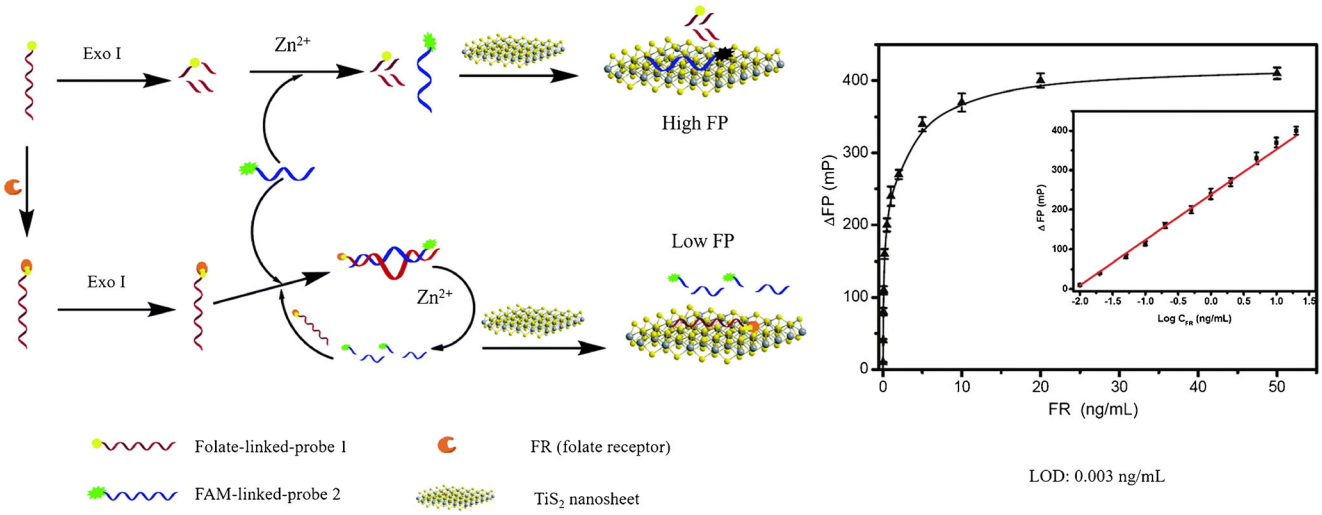

Fig. 5 Working principle of TiS_2 nanosheet-enhanced FP biosensor for FR detection via terminal protection of small-linked DNA and DNAzyme assisted signal amplification. Reprinted with permission [47], Copyright 2016, Royal Society of Chemistry

resulting in a lower FA signal. The measurement of FA change enabled the selective detection of target. Yang and co-workers built an HCR-based turn-on FA assay for detection of ATP [60]. Target–aptamer recognition released one blocking DNA strand, which served as an initiator to trigger enzyme-free autonomous cross-opening of hairpin probes. The biotin-functionated HCR product could combine with streptavidin. The larger size of the resultant complex showed a higher FA value and the change of FA signal enabled the sensitive detection of ATP. A molecular mass amplifying strategy to construct FA aptamer probes for ATP sensing was developed by Cui et al. [61]. This approach used a dye-labeled aptamer sequence containing a thrombin-binding domain and analyte-binding domain as probe. The presence of target ATP activated the thrombin-binding domain and the binding of thrombin further amplified the FA signal as a result of the large size of the ATP–thrombin complex. This sensor showed a LOD of 0.5 μM ATP. An FA sensor for detection of ATP was developed by using fluorophore-labeled aptamers containing a designed hairpin sequence as the probes and DNA-functionalized SiNPs as signal amplifiers [64]. Fluorophore-labeled aptamer could not open the hairpin in the absence of target ATP, which did not interact with DNA-modified SiNPs. However, the introduction of ATP

Table 3 Comparison of FNA-based FA/FP sensors for detection of small molecules in homogeneous assays

Signal	Targets	Design methods	Dynamic range	Response type	Detection limit	Ref.
FA	Adenosine	GO–aptamer	60 μM–40 μM	Off	22 μM	[44]
FP	Adenosine	GO–aptamer–nicking enzyme	4 pM–10 μM	Off	2 pM	[49]
FP	Adenosine	PS NPs–aptamer–exonuclease	10.2 fM–10 μM	Off	10.2 fM	[54]
FA	Adenosine	Aptamer–ssDNA protein	2 μM–400 μM	Off	1 μM	[58]
FA	ATP	Aptamer–ATP–dye	0.5 μM–1 mM	Off	0.5 μM	[59]
FA	ATP	Aptamer–HCR–streptavidin	0.2 μM–500 μM	On	0.1 μM	[60]
FA	ATP	Aptamer–thrombin	1 μM–1000 μM	On	0.5 μM	[61]
FA	ATP	Internal dye-labeled aptamer	1 μM–1000 μM	On/off	1 μM	[62]
FA	Vasopressin	Internal dye-labeled aptamer	0.2–200 μM	Off	0.2 μM	[63]
FP	ATP	SiNPs–aptamer	40 pM–10 μM	On	20 pM	[64]
FA	Adenosine Tyrosinamide	SYBR Green	0.8 μM–1 mM 19.5 μM–1 mM	On	0.8 μM 19.5 μM	[65]
FA	Adenosine	“Kiss complex”–aptamer	1–40 μM	Off	1 μM	[66]
FA	Vasopressin	“Kiss complex”–aptamer	2.3–150 nM	On	8 nM	[67]
FA	Adenosine	“Kiss complex”–aptamer	2 μM–15 μM	On	2 μM	[68]
FP	L-Histidine	DNAzyme	0.6 μM–5 μM	Off	0.6 μM	[69]

ATP adenosine triphosphate

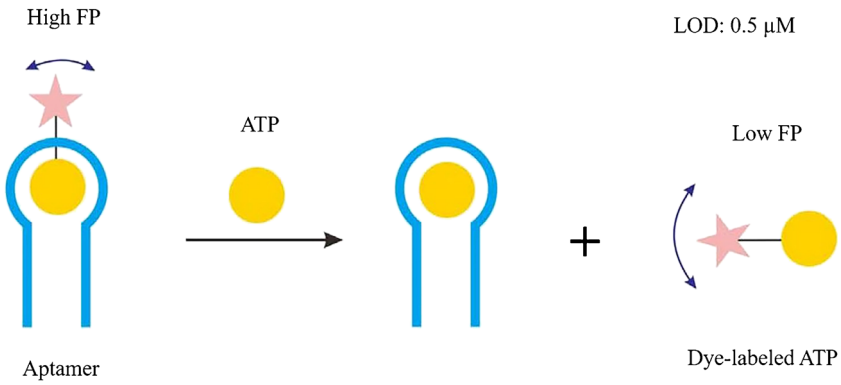

Fig. 6 Working scheme of aptamer-based competitive aptamer FA assay for ATP using aptamer and fluorescently labeled ATP. Reprinted with permission [59], 2017 Elsevier B.V. All rights reserved

triggered a structure change of the probe, which hybridized with the DNA functionalized SiNPs, resulting in an increased FA signal due to the increased molecular size. The detection of FA change gave a LOD of 20 pM ATP.

Fluorescently labeled aptamers can be used to directly generate anisotropy signal changes upon binding to small molecules. Zhao et al. reported a direct FA strategy for ATP detection using TMR-labeled aptamers based on the binding-induced change of intramolecular interactions between guanosine (G) bases of the aptamer and TMR [62]. Figure 7 illustrates the TMR-labeled aptamer-based FA sensing of ATP. The addition of target induced the conformation change of aptamer to form a hairpin structure, which altered the interaction between TMR and G bases, resulting in the increased local rotation of labeled TMR. Thus, FA of TMR decreased with addition of ATP (Fig. 7a). In another example, the binding of ATP led to an enhanced TMR–G interaction and an increased FA could be observed as the strengthened interaction caused a decrease of local rotation of TMR (Fig. 7b). The decreasing and increasing response of FA could be used for quantitative detection of ATP. Similarly, such a TMR–G interaction-based FA strategy had also been used to detect vasopressin, a antidiuretic hormone and related to physiologic and pathologic processes such as adjusting the balance of glucose, salt, and water in the blood [63].

A "kissing complex"-based FA strategy was developed for amplifying anisotropy signals [66]. The addition of target molecule induced a hairpin loop of the unique aptamer, which was recognized by a second hairpin through loop–loop interactions termed a "kissing complex". The assembly of one aptamer onto a fluorescent labeled hairpin effectively improved the FA signal owing to the increased molecular volume. Detection of adenosine [66, 68], guanosine triphosphate [66, 70], and vasopressin [67] could be achieved by using different kinds of aptamers and hairpin nucleic acid sequences.

DNAzyme which performed an enzymatic function in the presence of specific cofactors has also been used to fabricate FA/FP sensors. For example, an FA sensor using DNAzyme as identification element was used for sensing L-histidine [69]. The oligo substrate of a DNAzyme was labeled with a fluorophore at one end. In the absence of the cofactor L-

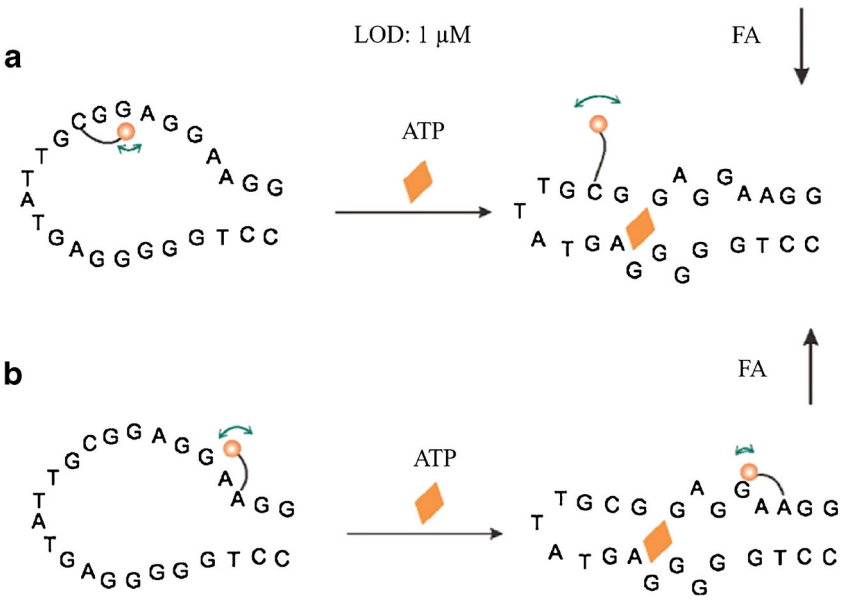

Fig. 7 Schematic of detection of ATP by TMR-labeled aptamer sensor with FA-decreasing response (**a**) or FA-increasing response (**b**). Reprinted with permission [62], Copyright 2015 Elsevier B.V.

histidine, the DNAzyme did not cleave the oligo substrate and the relatively large substrate–DNAzyme complex gave a high FA. In the presence L-histidine, the activated DNAzyme cleaved the fluorescently labeled substrate into a short fluorescent fragment. Hence, a decreased anisotropy signal was observed.

Outlook

FP/FA sensors based on FNA recognition are being used for reliable and efficient detection of diverse biomarkers including large molecular proteins, small biological molecules, and nucleic acids, which have broad application prospects in biological analysis, medical diagnosis, and biological monitoring owing to their advantages of high sensitivity, selectivity, and high throughput. In addition, the efficient FP/FA magnification methods based on HCR, CHA, endonucleases, exonucleases, DNAzymes, and nanoparticles significantly improve the sensitivity of such devices, which enlarge the application range of FNA-based FP/FA sensors. Although such great progress in the area has been made, there are still challenges needed to be overcome. Firstly, even though the in vitro selection techniques have facilitated the selection of nucleic acid aptamers for diverse targets, only limited good aptamers with high affinity and selectivity are available for biomarkers. New methods for the selection and design of aptamers are essential for the diverse applications of aptamers. Secondly, the use of DNAzymes often requires metal ions as cofactors. Therefore, rational designs and complicated steps are required to achieve quantitative detection of biomarker. Thirdly, it is still a rather difficult task to measure multiple target analytes in a single solution simultaneously. Until now, there are few papers achieving the goal of multiple biomarker detection in one assay using FNA-based FP/FA sensors. It is pivotal to create more advanced sensors with different output signals to detect diverse biomarkers. Fourthly, the application of FP/FA sensors in living cells is still rare. A future development trend will be to detect the target biomolecules in living cells, tissues, or even the living body. Fifthly, nearly all the established FNA-based FA/FP sensors so far still remain at the proof-of-principle stage, let alone reaching the commercial level. This is because of the existence of some non-specific interactions between biological/chemical molecules in the real complex biofluids and recognition elements of FA/FP sensors during the testing procedure. Nowadays, the fabrication of high-quality FNA-based FP/FA sensors with advantages of convenience, rapidness, sensitivity, and selectivity is greatly demand to meet the needs of applications. Along with the great progress in the field of sensor design, we believe that FNA-based FP/FA sensors will play a critical role in various applications in the future.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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