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Overcoming Major Barriers to Developing Successful Sensors for Practical Applications Using Functional Nucleic Acids

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Abstract

For many years, numerous efforts have been focused on the development of sensitive, selective, and practical sensors for environmental monitoring, food safety, and medical diagnostic applications. However, the transition from innovative research to commercial success is relatively sparse. In this review, we identify four scientific barriers and one technical barrier to developing successful sensors for practical applications, including the lack of general methods to (*a*) generate receptors for a wide range of targets, (*b*) improve sensor selectivity to overcome interferences, (*c*) transduce the selective binding to different optical, electrochemical, and other signals, and (*d*) tune dynamic range to match thresholds of detection required for different targets; and the costly development of a new device. We then summarize solutions to overcome these barriers using sensors based on functional nucleic acids that include DNAzymes, aptamers, and aptazymes and how these sensors are coupled to widely available measurement devices to expand their capabilities and lower the barrier for their practical applications in the field and point-of-care settings.

1. INTRODUCTION

Sensors play important roles in various applications, including environmental monitoring, food safety, and medical diagnostics, and they have been under constant development for more than 60 years (1). In general, a sensor is an analytical device that detects the presence or measures the concentrations of specific targets and typically includes two basic components: a receptor that binds its target and a transducer that converts the receptor binding into a detectable signal (2) (**Figure 1**). Based on the capability of the receptor, they can recognize a wide range of targets, such as pH, metal ions, organic metabolites, nucleic acids, and proteins, whereas different transducers can produce different signals such as fluorescence, colors, electrochemical currents, or potentials.

Despite many years of efforts by thousands of scientists and engineers spending hundreds of millions of dollars, only a limited number of sensors have achieved global commercial success, especially at the consumer level. In this review, we identify four major scientific barriers and one technical barrier to the successful development of sensors for practical applications. We summarize approaches to overcome these barriers using functional nucleic acids (FNAs) that include DNAzymes (DNA molecules with enzymatic activity), DNA aptamers (DNA molecules that can bind targets selectivity, like an antibody), and aptazymes (a combination of an aptamer and a DNAzyme in which the binding of the aptamer's target can enhance or inhibit the DNAzyme activity) (3–9). We also provide our assessment of the future trajectory of these technology advancements and illustrate the potential routes to translate these new chemical sensors into practical applications.

2. MAJOR FUNDAMENTAL SCIENTIFIC BARRIERS TO DEVELOPING SUCCESSFUL SENSORS FOR PRACTICAL APPLICATIONS

In the past decade, the field of sensors has been revolutionized by rapid advancements in synthetic molecular recognition units, which has paved new ways to remarkably improve the analytical

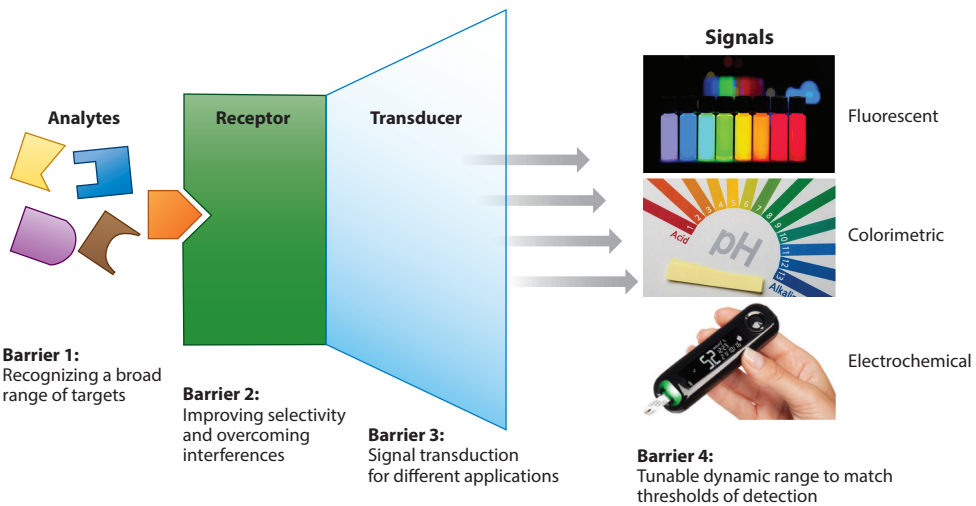


Figure 1

The general design of a sensor and the four barriers to developing a sensor for practical applications. These include (1) the lack of a general and simple receptor to recognize a wide range of targets, (2) the lack of a general method to improve selectivity of a receptor to overcome interferences, (3) the lack of a general method to translate the receptor binding event to measurable signals, and (4) the lack of a general method to tune the dynamic range of a sensor to match a specific detection threshold.

performance of sensors and realize commercial success for a variety of practical applications. In this section, we briefly introduce the scientific barriers that current sensing technologies are facing from the perspective of practical applications.

2.1. Scientific Barrier 1: Lack of a General Method to Obtain Receptors that Can Recognize a Broad Range of Targets

An essential component of the sensors is the receptor molecule for selective target recognition, and such a feature is especially important to meet the demand of applications. This is because new targets can emerge constantly, such as new pathogens in epidemic monitoring, new adulterants in food safety, and novel biomarkers in medical diagnostics and imaging. Currently, the receptors commonly employed in various sensors are protein bioreceptors, such as enzymes and antibodies, which originate from various organisms. Enzymes deployed in biosensors can selectively recognize their substrates and generate measurable products, such as protons, electrons, light, and heat, with fast kinetics; however, suitable enzymes are limited in number and enzymes for new targets are difficult to obtain. Additionally, antibodies have been used as receptors for biosensors to broaden the range of targets (10) with some inherent limitations, such as the difficulty of obtaining antibodies for highly toxic targets that can kill animals or cells before antibodies can be generated (11), instability under harsh environments and limited shelf life (12), and resource consumption for isolation and production. Besides the protein bioreceptors, entities such as cells or even tissues have been used for certain biosensing applications.

In addition to biological receptors, synthetic (small-molecule) receptors and polymers have also been developed, especially for metal-, anion-, and carbohydrate-sensing applications (13). Although small-molecule-based synthetic receptors hold the promise of being designed in silico owing to their simpler structure, most synthetic receptors are designed based on a small number of organic scaffolds, such as crown ethers, cyclodextrin, and pinwheels, to recognize a specific target based on a lock and key model (14). However, these scaffolds also limit the capabilities of the synthetic receptors, such as selectivity and affinity. In addition, synthesizing a new receptor is a laborious process with high uncertainties. Hence, other polymeric synthetic receptors, such as molecularly imprinted polymers, as well as some combinatorial methods to obtain small-molecule synthetic receptors, have also been explored (15). However, most of these methods are based on trial and error processes where success in obtaining a receptor for one target can rarely be generally applied to obtaining another target.

Owing to the limitations imposed by the availability of natural bioreceptors and the difficulties of obtaining synthetic receptors, a general method to obtain molecules to recognize any target specifically remains a major barrier in developing sensing applications.

2.2. Scientific Barrier 2: Lack of a General Method to Improve Selectivity to Overcome Interferences

Selectivity describes a sensor's ability to differentiate between targeted and nontargeted entities in a sample (16). Although most sensors perform well in clean laboratory samples, they often fail when used in real samples, such as contaminated water or soil in the environment, or adulterated food or blood, as testing in these complex matrixes requires sample pretreatment. The presence of abundant background species in these complex media poses a significant challenge to attaining necessary selectivity (17). As a result, only a limited number of these sensors have been commercialized for consumer applications. For instance, despite the remarkable success of immunoassays for therapeutic drug monitoring of various immunosuppressants (e.g., cyclosporine), the cross-reactivity against various metabolites of the parent drug remains a critical challenge (18). Although

the literature has reported improving antibody specificity and affinity via various mutagenesis approaches (19), it is normally laborious and technically complicated even for well-characterized antibodies. Similar issues have also been observed in synthetic receptors because many of them have a limited interaction with their targets and a small surface area for Van der Waals interactions. Furthermore, their performance can be drastically influenced by components in complex sample matrices. For example, crown ethers have been known for their ability to coordinate alkali metal ions with high sensitivity, but they still suffer from unsatisfactory selectivity due to the similarity between different metal ions such as Na^+ and K^+ (20). Although it is possible to rationally design and improve the selectivity and affinity of these small-molecule synthetic receptors, incorporation of multiple modes of interactions in a small molecule has proven to be highly challenging and laborious.

2.3. Scientific Barrier 3: Lack of a General Method to Transduce Selective Binding to Different Signals

Selective binding of the target is only one component of the sensors. The binding needs to transduce into detectable signals, such as optical, thermal, or electrochemical signals (21). One common strategy for signal transduction is to place the signal reporter, such as a fluorophore or electrochemical tag, into the target-binding site so that the reporter could be competitively replaced by the target, resulting in changes in the fluorescent or electrochemical signal. While this strategy seems generally applicable in principle, it is difficult to apply in practice. For this strategy to work well, one needs to know the three-dimensional (3D) structure of the target-binding site; however, for many receptors, the 3D structure is difficult to obtain. Even for those receptors with known binding sites, the placement of the reporter close to the target-binding site needs to be determined carefully. If the reporter is too close to the receptor-binding site, it would interfere with the binding; if the reporter is placed too far from the receptor-binding site, it would not transduce the receptor binding well. As a result, many iterations of trial and error are required to optimize between target binding and signal generation. Furthermore, the same placement of a reporter for one receptor may not apply to other receptors, because the 3D structure of receptors and transduction mechanism can be very different. Finally, because the reporter is intimately involved in the binding of the target, it is often quite difficult to substitute one reporter group with another, making it nearly impossible to transduce the same binding event into multiple signals (e.g., from fluorophore to chromophore). Other signal transduction strategies include conjugating of the catalytic reporter to the receptor, which is commonly employed for immunoassays (22); generating a reporter using a receptor with enzymatic activity, which is often used for enzymatic assays (23); and designing a receptor with signaling capability, for example, bioluminescence change upon target binding, which is employed for semisynthetic receptors (24). Although there are examples of applying the same signal transduction strategies for different receptors and targets (25), such instances are limited, especially in comparison to the enormous number of targets demanded for detection. Therefore, a general signal transduction strategy that can be applied to sensors for almost any target of interest without compromising the binding affinity and selectivity while still efficiently converting the target recognition event into a physical detectable signal is highly desirable. Yet, it remains another barrier to the successful development of consumer-level sensors.

2.4. Scientific Barrier 4: Lack of Tunable Dynamic Range to Match Thresholds of Detection

For practical sensing applications, an important but often overlooked attribute is the sensor's dynamic range, as the concentration of most targets can vary considerably under different

circumstances. For example, in environmental monitoring, the US Environmental Protection Agency (EPA) defined the toxic threshold for lead in paint as 2 μM, whereas the threshold for lead in drinking water is 72 nM (4, 26). Therefore, the development of a sensor with a tunable dynamic range to match the threshold of different applications without a redesign will have a significant impact. Simply altering sensor concentration is not an effective way to tune dynamic range, as it often changes dramatically the signal intensity and thus detection limits. Additionally, the dynamic range of sensors based on the single-molecule binding process is limited by the inherent binding affinity and kinetics. Toward this goal, various strategies have been developed to tune the dynamic range of sensors for the detection of nucleic acids, small molecules, heavy metal ions, and proteins (27, 28). However, these strategies are target specific and difficult to apply to other sensor designs. Therefore, it is still challenging to develop a general method for tuning a sensor's dynamic range.

3. ADVANCES IN FUNCTIONAL NUCLEIC ACID–BASED TECHNOLOGY TO OVERCOME THE MAJOR FUNDAMENTAL SCIENTIFIC BARRIERS

In the past three decades, nucleic acids have emerged as a new class of bioreceptors due to newly discovered functions, including catalysis and molecular recognition (4). The catalytic DNA, or DNAzyme, can act as enzymes to catalyze various reactions, including hydrolysis or formation of the phosphodiester bond, phosphorylation, and peroxidation. Additionally, DNA aptamers, similar to antibodies, can bind their target molecules with high affinity and selectivity (29). Furthermore, aptamers and DNAzymes can be rationally combined to form aptazymes so that the binding of targets can either inhibit or enhance the catalytic activities. Collectively, DNAzymes, aptamers, and aptazymes are called FNAs. The discovery of FNAs has contributed to the development of sensors for practical applications (3, 30–35).

3.1. Using In Vitro Selection to Overcome Scientific Barrier 1

FNAs are discovered by and known as the systematic evolution of ligands by exponential enrichment (SELEX) or in vitro selection (4). SELEX is based on the combinatorial chemistry principles in which the winner (DNAzyme or aptamer) is isolated from an initial random nucleic acid library with approximately 10^{15} different sequences through cycles of target binding, separation, and amplification (36–39) (**Figure 2*a***). Currently, the most commonly used selection method includes gel-based selection for metal-dependent DNAzymes (**Figure 2*b***) and column-based selection for aptamers (**Figure 2*c***). In gel-based selection, the nucleic acid library with an RNA cleavage site is first incubated with a target metal ion, followed by the separation of cleaved sequences from uncleaved sequences by denaturing polyacrylamide gel electrophoresis (PAGE). These separated sequences are amplified by polymerase chain reaction (PCR) and subjected to further rounds of selection. In column-based selection, the nucleic acid library is incubated in a column containing targets that are preimmobilized on a solid-state matrix such as agarose beads. The nucleic acid sequences having affinity to the target molecule can be captured in the column, while the unbound sequences will be discarded. The bound sequences are then eluted from the target molecule and amplified by PCR to generate a new pool of enriched sequences. After several rounds of selection, enriched DNA sequences emerge as the candidates binding specifically to the target of interest. Based on the selection conditions, this process can produce FNAs that are highly specific for their targets in a range of concentrations. In addition, in vitro selection using highly diverse DNA/RNA libraries can deliver artificial FNAs for almost any desired target of interest. Moreover, the selection process can be performed in many challenging conditions, such as in organic solvents (40) and at a high temperature of 90°C (41). Therefore, the major advantage of this system is that the

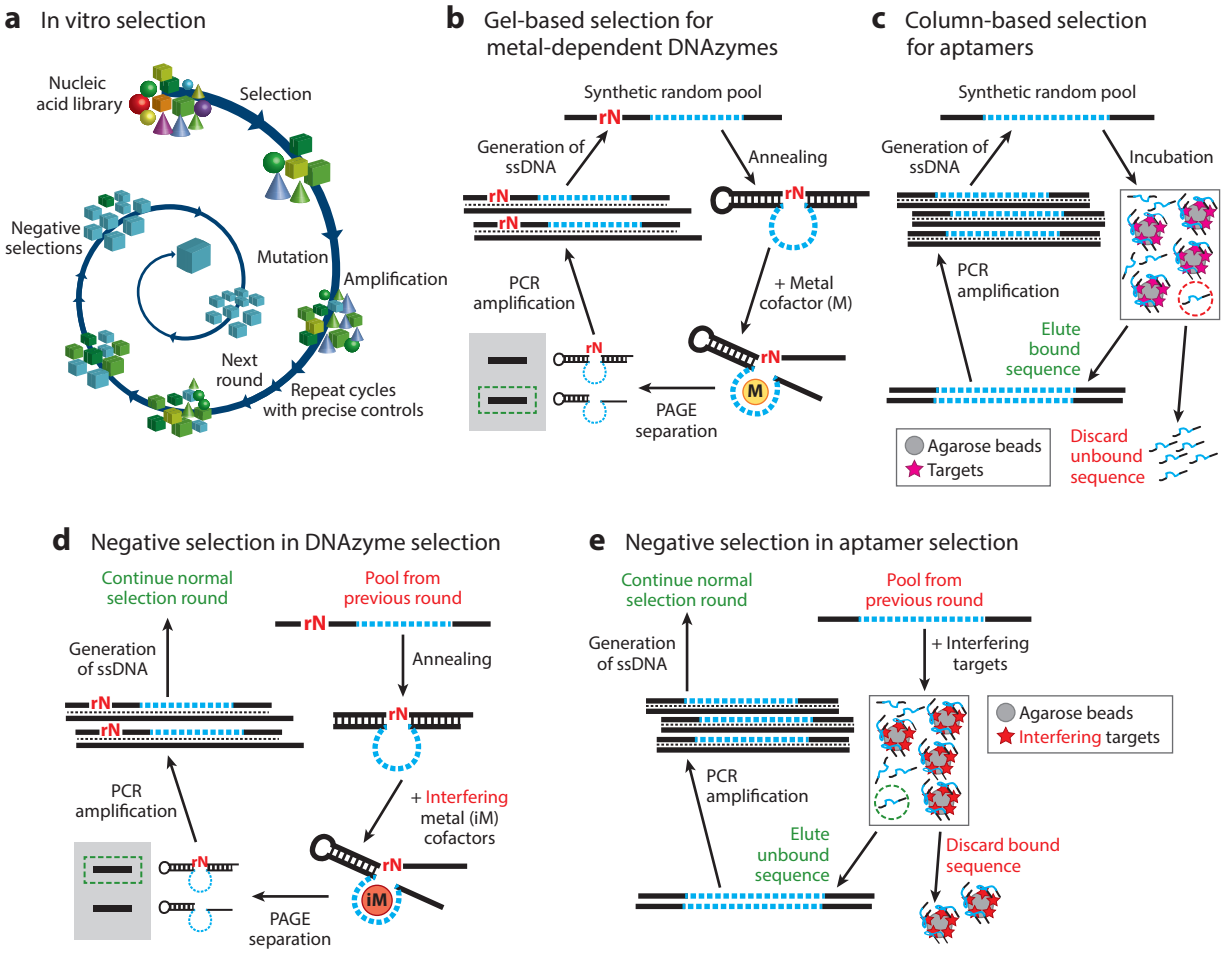


Figure 2

(*a*) In vitro selection as a general method for receptor isolation. (*b*) Gel-based DNAzyme selection. (*c*) Column-based aptamer selection. (*d*) Negative selection for DNAzyme. (*e*) Negative selection for aptamer. Abbreviations: PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; rN, RNA base at the cleavage site; ssDNA, single-stranded DNA.

generality of the in vitro selection process is then carried over to the synthesis of FNAs; in other words, it provides a general method for artificial bioreceptor synthesis and to thereby overcome the first barrier in developing successful chemical sensors.

3.2. Using Negative Selection to Overcome Scientific Barrier 2

Target selectivity is critical for any sensor, and several approaches have been developed to ensure high selectivity (42). However, for small-molecule receptors and antibodies, target selectivity is often difficult to improve with a general method. To overcome this barrier, for FNAs obtained via in vitro selection, the target selectivity can be improved evolutionarily during SELEX or in vitro selection by employing a negative selection strategy, also termed counter-selection, (38) which can overcome our limited knowledge about the ligand-binding affinity and selectivity. For example, to isolate a DNAzyme with minimal interference from other metal ions or the matrix, negative

selection cycles can be added during in vitro selection, in which the DNA pool is incubated with any potential interfering metal ions or the matrix (e.g., serum), followed by discarding the active DNA pool and retaining the inactive DNA pool for further positive selection using the target (**Figure 2*d***). Considering that the negative selection can be generally applied to the isolation of FNAs for nearly any target, it could be a general method to improve the selectivity of the final FNAs obtained for a variety of metal ions (36–38, 43). Similarly, negative selections can also be applied in aptamer isolation (39) (**Figure 2*e***), in which the pool of DNA sequences will be incubated with interfering targets, and any sequences that bind to these undesired targets will be discarded. The remaining sequences are subjected to incubation with the desired target, and the successfully captured sequences are separated and amplified for the next round of selection (39). This negative selection process can be introduced multiple times as needed to remarkably eliminate nonspecific sequences.

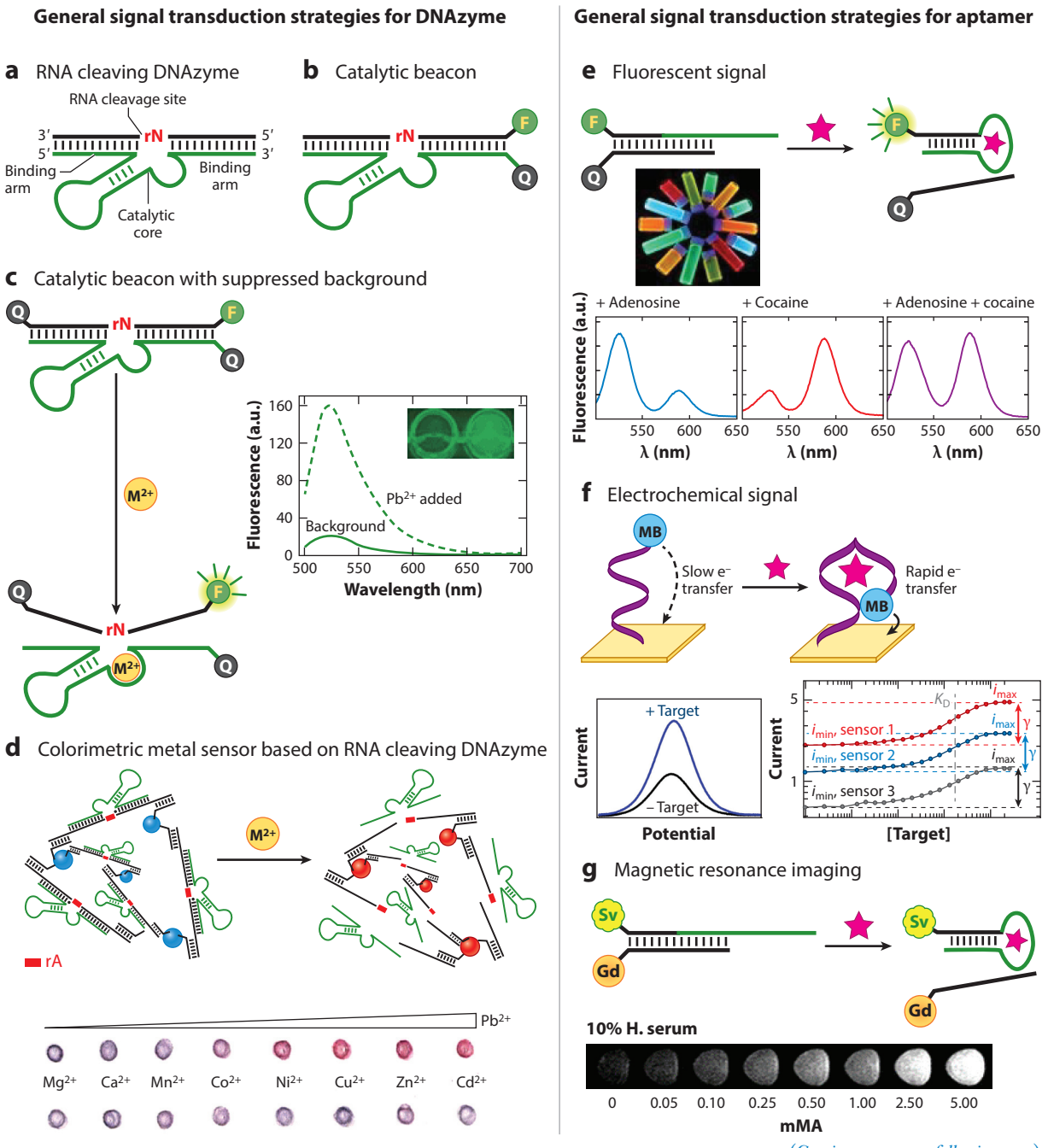
3.3. Using Differences in Melting Temperatures Before and After Binding to Targets to Overcome Scientific Barrier 3

To overcome the third barrier, the similar secondary structures shared between different FNAs can be utilized to develop a general signal transduction strategy. For example, DNAzymes generally consist of an enzyme and a substrate strand partially hybridized with two arms and a single-stranded loop or hairpin (catalytic core) in between (3–5, 26, 44–57) (**Figure 3*a***). The catalytic core is the conserved region responsible for catalysis, while the sequences in the binding arms can vary as long as the hybridization is stably maintained. Hence, controlling the stability of the binding arms plays a key role in signal transduction. In 2000, we (44) reported the design of a catalytic beacon, in which the melting temperatures of the binding arms before and after metal-specific cleavage of the substrate is rationally designed (**Figure 3*b***). In the absence of a target, the melting temperature for the binding arms is above room temperature, so the arms remain hybridized. The fluorophore and quencher conjugated to the enzyme and substrate are in close proximity, resulting in low fluorescent signal. In the presence of its metal cofactor, the substrate strand is cleaved into two segments, each with a melting temperature below room temperature. As a result, the two shorter DNA strands dehybridize, releasing the fluorophore away from the quencher and leading to an enhanced fluorescent signal. By using an intramolecular quencher (**Figure 3*c***), background fluorescence can be further reduced (46). The Li group (58, 59) has independently developed another catalytic beacon, where the fluorophore and quencher pair are located near the cleavage site.

The same catalytic beacon design can also be used to develop a colorimetric sensor. By replacing the fluorophores and quenchers with gold nanoparticles (AuNPs), colorimetric metal ion sensors can be developed by using the DNAzyme and selective metal ion–induced disassembly of AuNPs (45) (**Figure 3*d***). Here, the cleaved substrate will dissociate from the DNAzyme-substrate complex due to its lower melting temperature, triggering the disassembly of cross-linked AuNPs, which leads to a color change. The melting temperature–guided, rationally designed DNAzyme sensor for metal ions has become a general strategy, and these sensors have been widely demonstrated for in vitro diagnosis, cellular imaging, and in vivo studies (33, 60–68).

In order to transduce aptamers' target binding to a signal, the Nutiu & Li (69) first introduced a structure-switching method. In this design, a DNA duplex consisting of a fluorophore-labeled aptamer strand with an extended sequence partly hybridized to a complementary strand of quencher-labeled DNA (**Figure 3*e***) was constructed with a low fluorescent signal because of the close proximity of the fluorophore and quencher. However, the presence of a target resulted in the formation of the aptamer-target complex and subsequently disassembled the DNA duplex due to the decreased melting temperature, generating a high fluorescence signal. Using a similar

design, researchers have expanded structure-switching signaling aptamers to detect a variety of targets (28, 70–73) and, more importantly, these aptamers are versatile and able to function with other signal transduction methods, including electrochemical (**Figure 3*f***) and magnetic resonance imaging (MRI) platforms (**Figure 3*g***).



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Sensor designs based on DNAzymes and aptamers. (*a*) General structure of an RNA-cleaving DNAzyme (*green*) bound to its substrate (*black*); the cleavage site is indicated by rN. The binding arms are responsible for substrate binding through Watson-Crick base pairing, and the catalytic core is responsible for catalysis in the presence of a cofactor (commonly a metal ion). (*b*) Catalytic beacon based on DNAzyme with a pair of fluorophores (F) and quenchers (Q). (*c*) Catalytic beacon with extra Q for additional background suppression. (*d*) Colorimetric DNAzyme sensor based on a metal ion (e.g., Pb^{2+}) induced disaggregation of gold nanoparticles. (*e*) Aptamer-based fluorescent sensor. F is attached to a single-stranded aptamer (*green*) and, in the absence of target, the aptamer is hybridized with a short oligo conjugated with a Q, thus resulting in low-fluorescence signal. Target binding to the aptamer triggers a conformational change of the aptamer, which weakens and disrupts the hybridization with the Q-labeled short oligo, resulting in an increased fluorescent signal. (*f*) Aptamer-based electrochemical sensor. An electroactive tag, such as methylene blue (MB), is attached to one end of the aptamer. The MB-functionalized aptamer is then conjugated on an electrode surface. In the absence of target, the aptamer assumes a random structure, resulting in a less-efficient electron transfer (with an applied potential), and in the presence of target, the aptamer forms a more rigid and defined structure, resulting in a more-efficient electron transfer. (*g*) Aptamer-based magnetic resonance imaging sensor. Similar to a fluorescent aptamer sensor, the F-Q pair is replaced with a large protein (streptavidin, Sv) and gadolinium (Gd) tag. Target binding and release of the Gd tag from the aptamer-Sv conjugate results in a detectable T2 signal.

3.3.1. Representative examples of DNAzyme-based biosensors. By adopting the general method described above, a broad spectrum of DNAzyme-based sensors has been developed for a wide range of applications, including environmental monitoring, in vitro diagnosis, and intracellular and in vivo imaging.

DNAzyme-based colorimetric, fluorescent, electrochemical, and electrochemiluminescent sensors have been developed for diagnostic applications (36, 65, 67, 74). Among these sensors, one of the earliest and best-established DNAzyme sensors is based on the catalytic molecular beacon for the fluorescent detection of various metal ions (65, 75). These catalytic beacon sensors have been commercially available for monitoring drinking water systems in public schools. Other catalytic beacons have also been developed using quantum dots (76) and metal nanoclusters (77) instead of traditional fluorophores. Besides fluorescent sensors, colorimetric sensor designs have also been extensively explored by researchers (45, 47, 52), as well as electrochemical sensors using carbon nanomaterials and electroactive labels (78–81) and DNAzyme sensors designed for MRI using the gadolinium complex (82) as reporter groups. Recently, to facilitate the practical application of DNAzyme-based sensors, inkjet printing technology has been applied to develop DNAzyme-based printed paper sensors (83, 84). Printing fluorogenic DNAzyme probes on food packaging allows real-time monitoring of food contamination. The inkjet printer offers the advantage of rapid, covalent, large-scale printing and, more importantly, control over the amount of DNAzyme being applied (84).

Besides in vitro applications, only a few DNAzyme-based sensors have been demonstrated for cellular or in vivo applications. One major challenge is the precise delivery of DNAzyme into desired locations in cells while maintaining its catalytic activity. In 2013, using an AuNP as the carrier, Lu and coworkers (85) reported the first DNAzyme-based sensor for UO_2^{2+} in living cells. This team subsequently developed three other DNAzyme sensors for intracellular Zn^{2+} (86), Na^{+} (87), and Mg^{2+} (88). In addition, Zhang et al. (89) went a step further and designed a split DNAzyme nanodevice for multiplexed intracellular microRNA imaging with logic operation. Later, Li et al. (90) developed a two-color DNAzyme-modified AuNP probe for the simultaneous imaging of Zn^{2+} and Cu^{2+} in living cells. Another significant advancement in intracellular imaging was the introduction of a photocaging group on either the RNA active site (91) or the nucleobases essential for the regulation of DNAzyme activity (92), allowing for light-controlled activation of DNAzymes. This elegant strategy could minimize the nonspecific cleavage of DNAzymes during their delivery into cells. More importantly, by combining the photocage and upconversion nanoparticle, Yang et al. (93) reported a near-infrared (NIR) light-controlled in vivo sensor for tracking metal ions in zebrafish by conjugating a photocaged Zn^{2+}-specific DNAzyme

to upconversion nanoparticles that achieved upconversion of a deep tissue–penetrating NIR 980-nm light into 365-nm emission, which decages the cleavage site and activates the DNAzyme.

3.3.2. Representative examples of aptamer-based biosensors. Similar to DNAzyme, the fluorescent sensor based on structure-switching strategies first reported by the Li group was among the first aptamer sensors reported (69, 94). The structure-switching as a result of secondary or tertiary structure change as aptamer binding to its target remains the main principle for signal transduction (61, 95–97). To circumvent the requirement for a fluorimeter, the Lu group (98) developed a colorimetric dipstick sensor by conjugating aptamers to AuNPs, a commonly used colorimetric tag in lateral flow devices (LFDs). In this design, adenosine-responsive AuNP aggregates containing two kinds of DNA-functionalized AuNPs and an adenosine aptamer were synthesized and deposited on an LFD. The addition of adenosine would disassemble the AuNP aggregates to produce dispersed biotin-modified DNA–AuNP conjugates owing to the binding of adenosine by the aptamer, followed by the migration along the membrane and capture by streptavidin to form a red-colored line for visual detection. A fluorescent and colorimetric bimodal aptasensor has also been reported by Hui et al. (99). Besides these optical aptasensors, several research groups have reported electrochemical aptasensors by using electroactive tags (100–104). Finally, aptamers also can be converted to MRI sensors based on the relaxation time change induced by the conformational alteration of aptamers before and after the binding to their targets (105, 106). For in vitro diagnostic and other routine monitoring, aptamer-based sensors have been developed for the detection of various analytes, including proteins, drugs, ions, and small molecules in environmental and biological samples (60, 100, 107, 108).

Intracellular and in vivo imaging has also been demonstrated with various aptamer-based sensor platforms. For instance, Liu et al. (109) reported the intracellular detection of ATP using an aptamer sensor covalently linked to graphene oxide to reduce nonspecific probe displacement. Using a similar method, Chen et al. (110) developed an aptameric nanosensor for fluorescence activation imaging of cytochrome c (Cyt c), which provides a method for real-time visualization of the Cyt c release kinetics as well as the direct identification of the regulators for apoptosis. To allow imaging in live animals, Zhang et al. (111) designed a DNA aptamer–based photoacoustic imaging probe that provides a target-dependent photoacoustic signal change, enabling selective quantification and visualization of the protein target in living animals. The sensor system is based on the efficient contact quenching between an NIR dye and its dark quencher labeled in close proximity to two DNA strands, assembled together through aptamer-mediated duplex formation. The controlled release and activation of sensor probes in vivo have also been demonstrated by using a photocaged aptamer and upconversion nanoparticle for controlled probe activation using NIR (112).

3.3.3. Representative examples of aptazyme-based biosensors. Because aptamers and DNAzymes are best suited for recognizing different classes of targets, by combining the two, the resultant aptazyme possesses unique ability to directly transduce the aptamer's molecular recognition into a quantitative catalytic event (6, 113). After the initial demonstration by Tang & Breaker (114), various fluorescent, colorimetric, and electrochemical sensors have been developed for a wide range of targets (4, 52, 115–117). Additionally, intracellular imaging (118) and even mammalian cell genome editing (119) have also been demonstrated with aptazyme systems.

3.4. Using a Functional Nucleic Acid Variant to Overcome Scientific Barrier 4

The first FNA-based sensors with a tunable dynamic range was rationally designed by Liu & Lu (45), where the sensor dynamic range can be tuned by varying the amount of an inactive DNAzyme

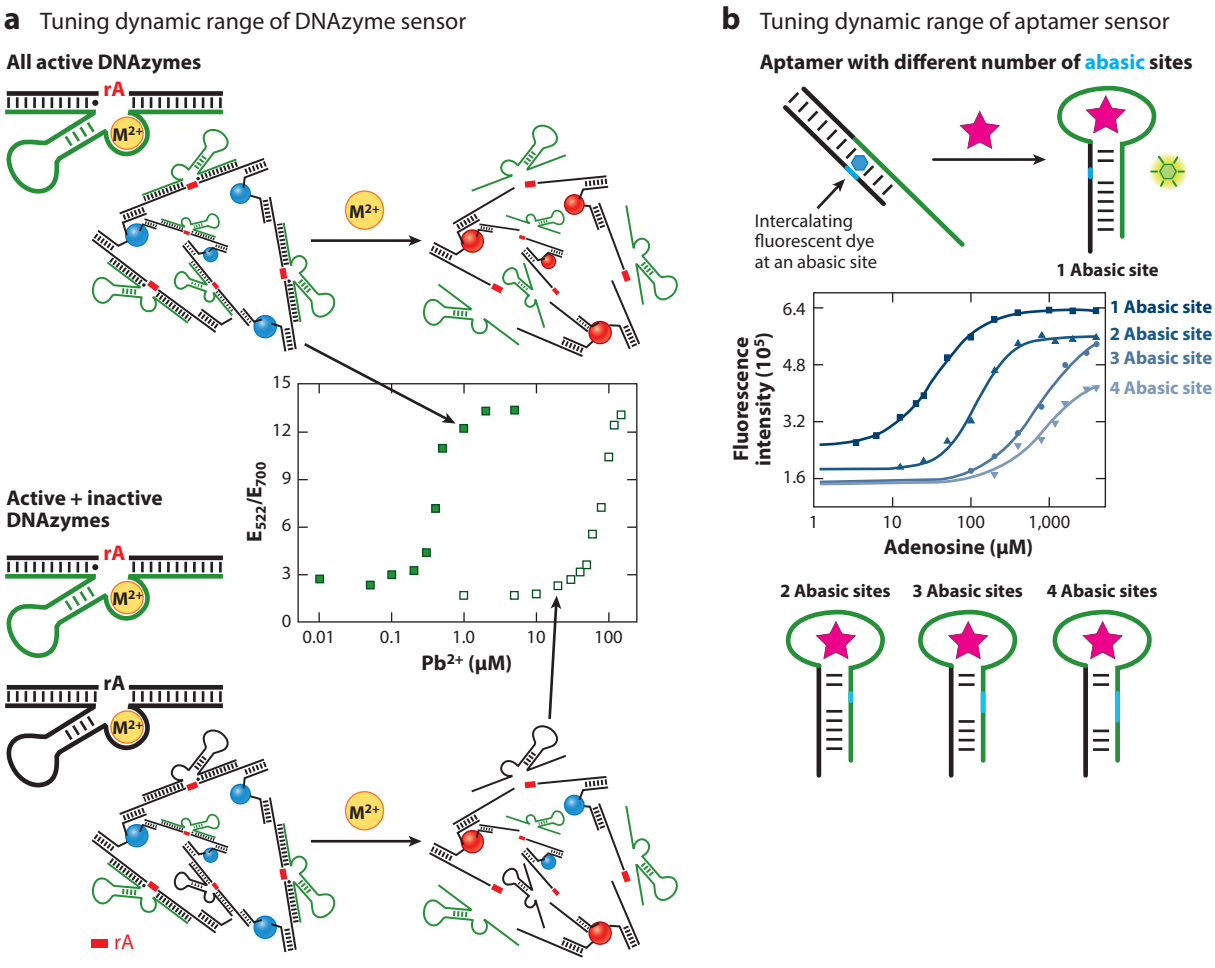


Figure 4

Tuning the dynamic range of (*a*) a DNAzyme sensor using inactive, fully complementary DNAzymes, which are capable of binding a targeted metal ion but cannot catalyze the cleavage at the RNA base. By varying the ratio of active and inactive DNAzymes, the dynamic range of the DNAzyme sensor can be changed to match a specific detection threshold. (*b*) Aptamer sensors using abasic sites within the stem region of an aptamer sensor. Response of the aptasensor changes, e.g., the threshold of detection shifts toward higher concentration, as the number of abasic sites increases, which allows matching the dynamic range with a detection threshold required by a specific application. Abbreviation: rA, adenosine ribonucleotide.

variant mixed with the active DNAzyme sensor. The inactive lead-dependent DNAzyme has a point mutation that changes the G•T wobble pair to a G-C canonical base pair (**Figure 4*a***), which results in the loss of catalytic activity, but it can still bind Pb^{2+}. For an AuNP-based colorimetric sensor, both the active and inactive DNAzyme-functionalized AuNP can form an aggregate; however, based on the amount of inactive DNAzyme present in the aggregate, the disassembly of the aggregate can occur at different concentrations of Pb^{2+}. Hence, the dynamic range can be tuned by varying the ratio between active and inactive DNAzymes. This strategy can be used as a general scheme for tuning the dynamic range of other DNAzyme-based colorimetric (54) or fluorometric (120) sensor systems.

Other than DNAzyme-based sensors, the tunable dynamic range can also be rationally designed in aptamer-based sensors. As reported by Xiang et al. (26), the tunable dynamic range on an aptamer sensor can be achieved by using an abasic site in the aptamer duplex (**Figure 4*b***). The effect of the hybridized base-pair number on the efficiency of structure switching of aptamers upon adenosine binding is the basis to tune the dynamic range of an adenosine sensor from the micromolar to millimolar range. Because more aptamers that are specific for other targets can be obtained through in vitro selection, this strategy could be further expanded to other aptamer-based sensors for the detection of a wide range of analytes with tunable dynamic range. Another innovative method has been reported by Porchetta and colleagues (121, 122), in which distal site mutation and allosteric control were used to modulate the folding of the aptamer to achieve a tunable dynamic range. In addition, the integration of nanomaterials with FNAs also provided new approaches for designing sensors with tunable dynamic ranges (28, 123).

Finally, other strategies to tune the dynamic range of FNA-based sensors were also developed by using a gold-decorated single-walled carbon nanotube random network in electrical DNA sensors through switching the measurement mode between real-time mode and static mode (124). Other studies also used quantum dots as donors in fluorescence resonance energy transfer (125), a DNA/ligand/ion-based ensemble in florescent sensors (126), and DNA aptamer engineering (127) by adjusting the relative ratio of the two types of quantum dot–probe conjugates, the concentration of metal ions, and the aptamer affinities, respectively.

4. OVERCOMING THE TECHNOLOGICAL DEVELOPMENT BARRIERS TO PRACTICAL APPLICATIONS BY REPURPOSING SUCCESSFUL PORTABLE ANALYZERS

Although it has been well recognized that portable devices play an essential role in on-site applications, such as point-of-care (POC) medical tests, a tremendous number of resources are required for their development. One reason for such a high developmental cost is that although a sensor can meet only some of the requirements, such as low detection limit, high sensitivity, high selectivity, tunable dynamic range, simple operation, and cost-effectiveness in order to be worthy of publication, it has to meet all the above requirements to be commercially available. Even some commercially successful sensors, such as pH paper and glucose meters, are often dedicated to detecting only a single analyte. Furthermore, the form factors, operations, and user interface designs of these commercially successful sensors have been meticulously designed and improved over a long time for their targeted end users. Hence, the costly product development demands that a sensor be versatile so that once it is developed for one target, it can be readily applied for a wide range of other targets with minimal changes to the sensor components. This versatility is particularly important for medical in vitro diagnostic tests, as there is an additional regulatory burden (128). In addition, instead of developing a new POC device for every new target, the Lu group (129–131) and others have demonstrated how to repurpose small and widely available analyzers, such as the glucose meter, thermometer, and pH meter, for detecting a wide range of targets by developing interchangeable strips for these meters.

4.1. Blood Glucose Meter

The blood glucose meter (BGM) is likely the most popular POC device that allows determination of glucose levels from a single drop of blood for people with diabetes. Today's BGM is rapid, affordable, portable, and easy to use, making it an ideal general purpose platform. Previous assays for nonglucose analytes had to be integrated with the device designed for only glucose detection (132). A major challenge in using a BGM as a general portable meter is to find a general method to

link the concentration of nonglucose targets with a glucose signal while avoiding major changes to the BGM. Moreover, the blood concentration glucose is relatively high (micromolar levels) compared to other targets at much lower concentrations (below nanomolar). Therefore, an even bigger challenge is signal amplification. To solve these challenges, the Xiang and Lu labs pioneered the conjugation of a receptor, such as an FNA, to an enzyme, invertase, which can catalyze the conversion of BGM-inert sucrose into glucose (129, 131, 133–142). By using invertase as a bridge between a nonglucose target and the BGM, and a glucose signal amplifier (143), the BGM can be transformed into a general meter to detect a wide range of targets. An initial demonstration with an aptamer and DNAzyme has shown that the BGM can be used to quantify small molecules (cocaine, adenosine), a protein marker (interferon-gamma), and a metal ion (UO_2^{2+}) with excellent sensitivities. Follow-up studies have further demonstrated the quantification of small molecules (139), metal ions (135), cancer biomarkers (134), viral DNA (133, 142), and bacteria (144) using other bioreceptors, such as antibodies and nucleic acids that are widely used in today's in vitro diagnostic tests. Furthermore, to simplify the system and avoid enzyme conjugation, a target-responsive sweet hydrogel was further developed by using a glucoamylase-trapped aptamer cross-linked hydrogel (145). In the presence of a target, the hydrogel collapses to release glucoamylase, which could catalyze the hydrolysis of amylose to glucose. Similarly, for simpler operation, Zhang et al. (139) have demonstrated the integration of all assay procedures in solution on an LFD for the quantification of cocaine. Other applications, such as a BGM-based biocomputation platform, have also been reported by Zhang & Lu (140).

Besides invertase, other enzymes, such as alkaline phosphatase that converts glucose-1-phosphate to glucose, have been used to generate glucose signals for BGM-based sensors. Zhang et al. (146) have reported a simple method to link the enzymatic activity of galactose-1-phosphate uridyltransferase to the production of glucose, which allows POC diagnosis of galactosemia in authentic human clinical samples. Given the presence of alkaline phosphatase in numerous enzymatic assays for clinical diagnostics, this method can be adapted to quantify a wide range of enzyme activities using a BGM. In addition, other glucose-generating enzymes such as glycosidases, esterases, phosphatases, and proteases have also been quantified using a BGM by using artificial enzyme substrates covalently linked to glucose (147).

A recent discovery by Zhang et al. (148) found that some BGMs have a dose-dependent response to nicotinamide coenzyme (NADH), which is involved in numerous enzymatic reactions. Some of these enzymatic reactions are widely used in diagnostic enzymatic tests, such as lactate dehydrogenase reaction. Hence, many of these clinically important enzymatic tests can also be monitored using the BGM.

4.2. Other Portable Meters

The thermometer is the most widely available, easy-to-use, and cheapest quantitative device for portable measurements and plays a vital role in environmental monitoring and medical diagnostics. However, a major challenge in using a thermometer for the detection of other targets beyond temperature is to develop a method that can transform the binding of any target into a temperature change so that the presence and concentration of a target can be detected using a thermometer. To address this issue, Zhang et al. (130) developed an innovative example for repurposing the thermometer for practical applications. They translated molecular detections into a simple temperature test using a target-responsive smart thermometer. The sensor system is based on the target-induced release of DNA–phospholipase A_2 enzyme conjugate from a functional DNA duplex immobilized on magnetic beads, which subsequently catalyzes the hydrolysis of liposome-indocyanine green to produce a change of temperature signal under NIR-laser irradiation. With the advantage of low cost and facile incorporation with suitable FNAs to recognize

many targets, the system makes the thermometer an affordable and pocket-size device for sensing diverse targets.

Another widely used portable device is the potentiometric pH meter, which can precisely monitor a subtle pH change. A series of pH-based biosensors have been developed by combining the biological component (e.g., antibodies, DNAzymes, aptamers) with a proton-generating or proton-depleting enzyme (e.g., glucose oxidase, urease, acetylcholinesterase), which produces a pH signal readout that can be measured using a portable pH meter for POC testing (149).

5. CONCLUSIONS AND PERSPECTIVES

In summary, today's demands for sensing applications, such as environmental monitoring, food safety, and medical diagnostics and imaging, require many sensors capable of detecting a wide number of targets, including emerging targets such as new viruses and pathogens, with high selectivity and sensitivity. However, several major barriers have prevented the transformation of many results from research laboratories into devices and methods that can be used by the general public. Because sensing different targets often requires the design and synthesis of different receptors, sensors for one target can be difficult to apply straightforwardly into sensors for other targets, often relying on trial and error for success. In addition, while selectivity for the target against interfering species in real samples is critical to practical applications, few methods can be customized to overcome the interference by increasing the selectivity. Moreover, while some receptors can recognize targets with good enough selectivity, it is difficult to transform the binding into detectable physiochemical signals without compromising the selectivity. Finally, most targets exist only in certain concentration ranges at different locations or time periods, and few methods have been developed that can tune the dynamic range of detection to match the concentrations of the targets.

Over the last 20 years, the emergence of FNAs as a new platform for designing sensors has helped researchers overcome the major barriers discussed in this review. The use of in vitro selection techniques has led to a general method to isolate highly selective and sensitive FNAs as receptors for almost any target, including emerging ones, and to the use of a negative selection method as a general way to improve selectivity and overcome interference. More importantly, the innovation of using differences in melting temperatures of the FNAs before and after target binding and the ready conjugation of many signaling moieties (e.g., fluorophores, AuNPs, and electrochemical agents) to the FNAs make FNAs an extremely versatile platform to transform the molecular recognitions into detectable signals. Finally, the use of mutant FNAs to tune the dynamic range of detection is also generally applicable. These advantages, together with other traits, such as high stability, low cost, biocompatibility, and biodegradability, make FNAs an excellent choice to serve as universal receptors and rival antibodies in sensor development. The substantial advancement of FNA-based sensors, as highlighted in this review, further validates the idea that FNAs can be easily integrated with various signal transduction modalities, such as the BGM, pH meter, and thermometer, to achieve practically useful applications. The generality of these techniques means that they can usually be applied in a variety of detection methods and different sample conditions, including both environmental and clinical samples.

To realize the full potential of FNAs for practical applications, much effort should be devoted to selecting FNAs for more targets with affinities similar to those of antibodies. To achieve this goal, introducing more functional groups into FNAs, such as those from peptides (150) and CRISPR technology (151), is required to increase the repertoires of FNAs to detect a wide variety of targets with very high affinity and sensitivity. Future research should also include the integration of chemical sensor technology with automation, wireless technology, and miniaturization.

Considering the current trend of decentralized testing, personalized and precision medicine, POC testing, and at-home smart testing devices now play vital roles in lowering healthcare costs by providing more frequent disease marker monitoring, precise drug selection, accurate drug dosing, and agile dose management at a lower cost. There will be significant market opportunities in addition to the existing multibillion-dollar markets for these devices. With their unique advantages, biosensors and future diagnostic devices based on FNAs can ultimately have a significant and positive impact on society.

DISCLOSURE STATEMENT

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