Synthetic and Systems Biotechnology 10 (2025) 543–554

Contents lists available at ScienceDirect

Synthetic and Systems Biotechnology

journal homepage: www.keaipublishing.com/en/journals/synthetic-and-systems-biotechnology

Review Article

The advancement of biosensor design and construction utilizing biomolecular motors

Jinhong Zhao [a], Fangyuan Guo [a], Mengqi Wang [a], Jie Zhang [b], Sanjun Ying [a], Ying Gao [c], Gensheng Yang [a], Weiyong Hong [d,*]

[a] College of Pharmaceutical Science, Zhejiang University of Technology, 310032, Hangzhou, China
[b] Taizhou Technician College, 318000, Taizhou, China
[c] Zhejiang Moda Biotech Co., Ltd, 310018, Hangzhou, China
[d] Department of Pharmacy, Municipal Hospital Affiliated to Taizhou University, 318000, Taizhou, China

ARTICLE INFO

Keywords:
Biomolecular motors
Biosensors
Detection

ABSTRACT

Biomolecular motors have been extensively studied as efficient molecular machines in detection systems owing to their unique signal conversion mechanisms and high energy conversion efficiencies. The application of these motors in the detection of pathogenic microorganisms is particularly promising. Through reasonable design and optimization, biomolecular motors can enable precise and efficient detection, enhancing clinical diagnostics. This paper reviews recent advances in detection systems utilizing various biomolecular motors, including kinesin, dynein, myosin, DNA polymerase, F_oF_1-ATPase, and flagellar motors. Detection mechanisms involving these motors are also introduced. Furthermore, the review covers recent progress in detecting antigens, antibodies, bacteria, and small molecules using biomolecular motors. Finally, the challenges and future prospects of biomolecular motor-based detection systems for pathogenic microorganisms are discussed, highlighting their potential as rapid and efficient tools for applications in food safety and medicine.

1. Introduction

Biosensors are highly specialized analytical devices designed to detect specific chemicals and bioactive substances, offering transformative potential in medical diagnostics, drug discovery, environmental monitoring, food safety, and defense applications [1]. These devices typically comprise biosensor elements [2], which are constructed using a bioreceptor (such as an enzyme, protein, DNA, antibody, antigen, biofilm, microorganism, or cell), signal transducer, and electronic system [3]. Biosensors are selective to specific chemicals and bioactive substances. Compared with conventional biochemical detection methods such as immunoassays and optical analyses, biosensors offer greater selectivity, higher sensitivity, higher detection speed, improved accuracy, and simpler operation, making them indispensable across a variety of disciplines. Over the past few decades, biosensors have significantly advanced and evolved through three distinct generations (Fig. 1). The first generation of biosensors utilized natural media for electron transfer, enabling direct detection of an increase in products or decrease in reactants. The second generation introduced artificial redox media, which enhanced the reproducibility and sensitivity of biosensors by using organic dyes, ferrocene, and its derivatives. In the third generation, redox enzymes are immobilized on the electrode surface, facilitating direct electron transfer between the enzyme and sensor. Immobilization not only enables the necessary proximity between the biomolecule and sensor but also stabilizes the biomolecule, making the biosensor reusable [4].

Based on differences in signal transduction, biosensors can be classified into six categories: electrochemical, electronic, optical, mass-sensitive, thermal, and magnetoelastic [6]. Among these, electrochemical biosensors are particularly favored for their cost-effectiveness, portability, and notable sensitivity, whereas optical biosensors excel in real-time, highly sensitive detection and are extensively employed in both clinical and industrial applications. Depending on their recognition elements, biosensors can be further categorized into enzyme sensors, protein-receptor sensors, immunosensors, DNA sensors, and whole-cell sensors. Enzyme and DNA aptamer-based biosensors are more specific

Peer review under the responsibility of Editorial Board of Synthetic and Systems Biotechnology.
* Corresponding author. Department of Pharmacy, Municipal Hospital Affiliated to Taizhou University #581 Shifu Avenue East, 318000, Taizhou, China.
E-mail address: weiyongh@126.com (W. Hong).

https://doi.org/10.1016/j.synbio.2025.02.007
Received 3 October 2024; Received in revised form 4 February 2025; Accepted 13 February 2025
Available online 18 February 2025

and sensitive than whole-cell biosensors. Notably, whole-cell biosensors—which often utilize microorganisms such as bacteria, fungi, viruses, or algae—provide distinct advantages, including self-replication and cost-efficient recognition component production [7].

Recent technological breakthroughs have propelled the development of advanced biosensors. For example, Grattieri et al. introduced self-powered biosensors that do not require a constant potentiostat, offer high sensitivity, and feature a simpler electrode design than traditional electrochemical biosensors [8]. Magnetoresistance-based biosensors have been widely employed for detecting proteins and DNAs as well as mapping cardiovascular and brain signals [9]. Cell-free biosensors have also been developed, providing high safety, stability, tolerance to toxicity/chemicals, sensitivity, robustness, and selectivity [10]. Advances in materials science, such as the use of gold nanoparticles, quantum dots, and carbon nanotubes, have significantly improved the analytical performance of electrochemical sensors [11]. With the integration of microfluidics, wireless data communication, and positioning technologies, wearable biosensors are have gradually become more prevalent. Owing to their fast response, specificity, and sensitivity, wearable biosensors have become increasingly valuable for home self-testing and first aid, offering breakthroughs in remote health monitoring [12].

Biomolecular motors are highly specialized nanomachines that perform mechanical work by converting chemical energy derived from ATP hydrolysis into directional motion. Among these molecular machines, kinesin facilitates chromosome segregation during mitosis and the intracellular transport of vesicles, signaling molecules, and proteins [13]. Similarly, myosin converts the chemical energy of ATP into mechanical energy through its interaction with actin filaments, resulting in muscle contraction [14]. In the context of biosensing, biomolecular motors can be applied to *in vitro* assays, which enables target-specific analyte capture, proton transport, and energy conversion, thereby amplifying detection signals with notable sensitivity. Some biosensors utilize the sensitivity and specificity of biomolecular motors for straightforward complex bioanalytical measurements [15]. *In vitro* biomolecular motor sensor detection is intuitive, accurate, sensitive, and convenient. Moreover, biomolecular motors can be sourced at low cost from specific organisms, such as thermophilic bacteria. This technology has advanced diagnostic applications, including the detection of viruses, bacteria, and small molecules. In contrast to non-motor devices, motor systems can simultaneously operate numerous agents with high energy efficiency and notable miniaturization [16]. This review explores recent analytical applications of biomolecular motors.

2. Biosensors constructed on biomolecular motors

Biomolecular motors are proteins or nucleic acids widely distributed in cells, responsible for various essential biological processes such as cell transport, division, and movement [17]. These motors hydrolyze low concentrations of ATP into ADP and inorganic phosphate, efficiently converting the free energy generated into mechanical energy [18]. The energy conversion efficiency exceeds 40 % [17], and they exhibit high substrate specificity [19]. Biomolecular motors can be categorized into linear and rotating types, based on their motion modes [17]. Linear biomolecular motors include kinesins, dyneins, myosins, and DNA/RNA polymerases, while rotating biomolecular motors include ATP synthases and cell flagella (Fig. 2) [16,20]. In eukaryotic cells, the cytoskeleton consists of three types of protein polymer filaments: actin, microtubules (MTs), and intermediate filaments [21]. In addition to providing mechanical stabilization, actin and MTs serve as the "tracks" for biomolecular motors (such as myosin, kinesin, and dynein) to transport macromolecular cargo and organelles. Biomolecular motors have become promising biosensors because of their high energy conversion efficiency, specificity, and strong motility. Currently, approaches of constructing biomolecular motor sensors involve connecting the motors to specific probes, utilizing the motor's inherent motion characteristics or sequential connections to achieve the enrichment of target analytes and signal amplification were made.

2.1. Kinesins and dyneins

2.1.1. Construction

Kinesins are a family of biomolecular motors in eukaryotes involved in mitosis, meiosis, and intracellular transport. Based on the position of the kinesin motor domain on the polypeptide chain, kinesins are classified into three categories: 1) those with an amino-terminal motor domain, moving toward the plus end of the microtubule; 2) those with a carboxy-terminal motor domain, moving toward the minus end; and 3) those with a central motor domain, which is immobile and destabilizes the microtubule. Most kinesins belong to the first category [22,23]. Kinesin-1 primarily facilitates the transport of molecular cargo from the cell center to the periphery along microtubules. It consists of two heavy chains and two light chains. Each monomer has a motor head, neck linker, long coiled region, and globular tail domain. The head of the heavy chain binds to the microtubule, generating the force to move along a protofilament toward the plus end, while the light chain binds to the cargo. The current model of movement along microtubules involves

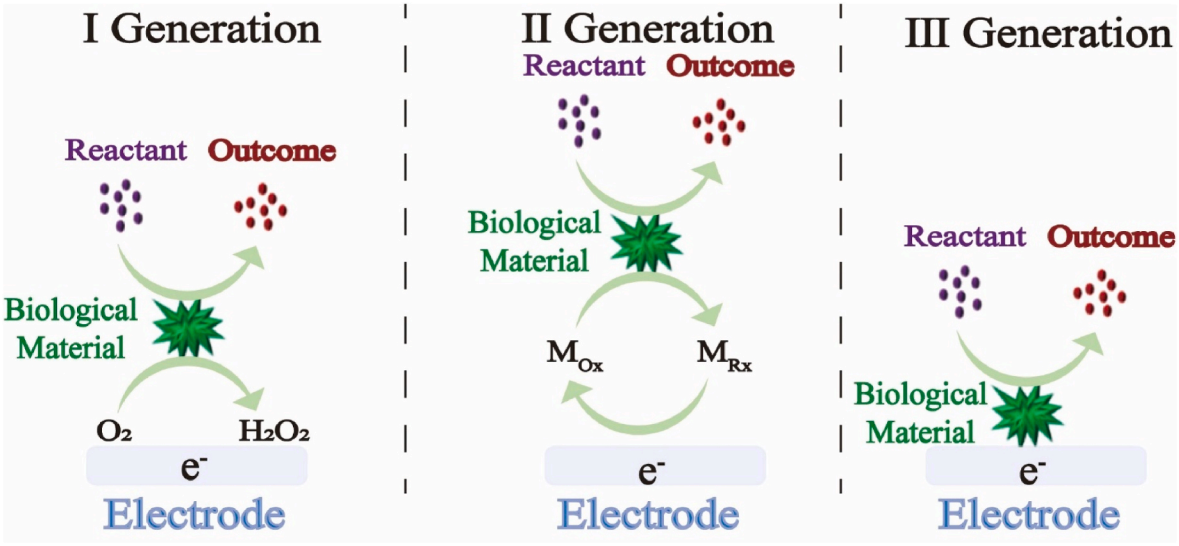

Fig. 1. Development of biosensors. Reprinted with permission from Ref. [5].

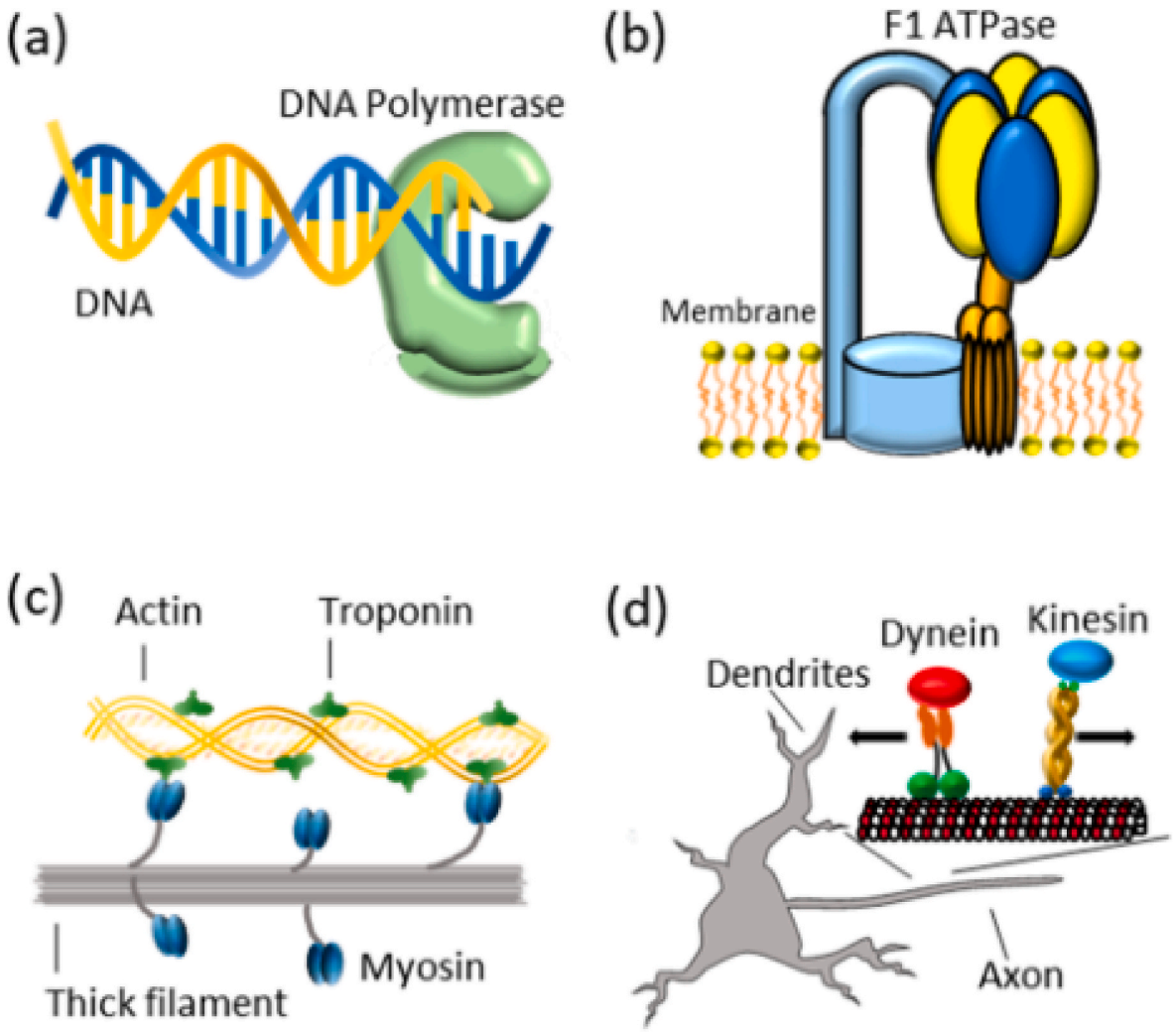

Fig. 2. Illustrations of biomolecular motors. (a) DNA polymerase synthesizing DNA. (b) The structure of F1 ATPase. (c) Myosin moving along an actin filament. (d) Kinesin and dynein "walking" on a microtubule in a neuron. Reprinted with permission from Ref. [17]. Copyright 2018, American Chemical Society.

a hand-over-hand mechanism, with each hydrolyzed ATP enabling a step of approximately 8 nm, facilitating cargo transport along the light chain [24].

Dyneins are large molecular motor complexes ranging from 1 to 2 MDa, categorized into flagellar and cytoplasmic dyneins. The coordinated actions of flagellar dyneins generate specific waveforms necessary for cell movement in solution, while cytoplasmic dyneins participate in various intracellular motility processes, including the regulation of mitotic spindle localization and assembly, maintenance of the Golgi apparatus, and transport of membranous vesicles and other intracellular particles [25]. Human dynein is composed of six distinct polypeptides belonging to the dynein heavy chain (DHC), intermediate chain (DIC), light intermediate chain (DLIC), and three dynein light chain (DLC) families. The DHC features a C-terminal motor domain and an N-terminal tail domain. The last helical bundle of the tail is linked to the motor domain, which consists of a linker, a loop of six AAA + domains, and a C-terminal domain that binds to its track via a microtubule-binding domain (MTBD) at the end of a coiled-coil stalk emerging from the AAA + loop. The first four domains (AAA1–AAA4) contain conserved ATP-binding/hydrolysis motifs, while the MTBD is situated between AAA4 and AAA5. Single-molecule studies indicate that dynein walks similarly to the hand-over-hand model; however, its walking behavior and directionality are less consistent than those of kinesins [26,27].

Both kinesin and dynein contain an ATP-catalytic site and an MT-binding site. Moreover, both proteins are powered by ATP hydrolysis and are involved in cell division and transportation [28,29]. Some viruses exploit these functions to accelerate transport from the cell periphery to the nucleus by "hijacking" these proteins [30]. However, compared with kinesin-1, dynein moves ten times slower [16], and its movement along MTs is more complex, whereas kinesin is relatively straightforward in terms of transport direction and energetic efficiency [31]. Consequently, research on dynein has focused more on intracellular processes, such as cargo transport [32], rather than their potential applications in biosensing. Kinesins utilize conformational changes in the motor head to produce various motor properties, such as direction reversal [33] and polymerization/depolymerization with MTs. Based on the principles and characteristics of these functions, the MTs transport system is designed as an efficient biomolecular motor for biological detection. These results have been confirmed by numerous studies [34–36].

2.1.2. *Kinesin-MT biosensors*

Biomolecular motors are utilized in two modes, based on the mechanism of the microtubule transport system [28]. First, minimal reconstruction of the microtubule transport system is achieved by immobilizing MT on the surface of specific materials and adding a purified movement protein that can move along the MT. However, the restricted size of MT tracks and the difficulty in organizing them into complex structures necessary for practical applications limit the use of

such molecular motors for complex detection. To address this issue, MT transport systems are commonly applied in a second mode [37]. In this mode, the protein is fixed to the surface of the solid material, and MT bind to and move over the protein. This inverted mode employs a surf-like approach to drive MT motion. The advantages of the reverse MT transport system are as follows: (1) The transport track of protein construction can be extended and controlled to form a specific path. Microtubules can be modified and controlled using external magnetic or electric fields to achieve more advantageous molecular motor-detection techniques. (2) MT are easier to modify than proteins owing to their structure, and functionalized MT modified with specific substances can readily capture analytes. (3) Fluorescent labels, such as fluorescent dyes and proteins [38], can be attached to microtubules for detection and observation, enabling real-time visual detection during the process [36, 39].

Detection methods based on kinesin–MT systems include biotin–streptavidin binding, antigen–antibody reactions, and redox reactions (Table 1). Among these, the sandwich method, which is based on double antibodies, is commonly used. For example, in the model introduced by Bachand et al. [40,41], kinesin was immobilized on an inorganic surface, and a specific transport track was formed using microfluidic technologies. The tomato mosaic virus (ToMV) served as the antigen target. Anti-ToMV antibodies were linked to MTs via covalent crosslinking, enabling the functionalization of the antibody-modified MT (Ab-MT) and enhancing its stability (Fig. 3A). In this assay, anti-ToMV antibody-coated fluorescent microspheres were used as reporters for the capture effect. The detection process is divided into three steps: first, functionalizing MTs to capture the antigen in the "capture" zone; second, entering the "tagging" zone to bind to fluorescent microspheres and form a double-antibody sandwich structure; and finally, moving the MTs to the "detection" zone for optical detection. Experimental results indicate that the detection limit is 1 ng/mL, confirming the analytical specificity of the antibody-functionalized microtubules. Compared to routine ELISA, the new method enhanced the detection signal and improved sensitivity.

The advantages of the dual anti-sandwich model are as follows: (1) Multiple antibody-antigen bonds increase the stability of the sensor, and (2) Signal amplification is achieved when multiple fluorescent microspheres are attached to the antigen and enriched following microtubule movement.

The effectiveness and accuracy of detection were significantly affected by the random movement of the MTs. Therefore, the artificial control of biomolecular motor trajectories has become a primary research direction. A major challenge in utilizing protein biomolecular motors in integrated nanoscale systems is their ability to modulate motor function *in vitro*. Several artificially controlled biomolecular motor sensors have been developed, and establishing control through external fields enables multifunctional detection of complex samples [48–51].

The original design utilized a specific orbital shape to guide the motion of MTs, allowing them to passively perform directional movements along designated tracks. With various modifications to the MTs, electric, magnetic, and other external fields were designed to control their motion trajectories [52]. The accuracy of the directional movements of the MTs was further enhanced by applying an electric field. After MTs are modified with different substances, more complex samples can be analyzed and detected in specifically shaped orbits under external fields, with both electric and magnetic fields controllable via a computer-programmed system [53,54], thereby enabling more intelligent detection.

The introduction of an external field can distinguish between functionalized MTs with different properties, allowing for the simultaneous detection of multiple analytes and greatly increasing the versatility of detection. However, biomolecular motor sensors still rely on biofuels (ATP), and for sensors containing multiple molecular motors, ATP only drives certain types of molecular motors. A previous study reported a general mechanism for reversibly controlling the function of kinesin biomolecular motors, independent of fuel supply (ATP) [55]. Schola introduced a chemical switch to create a Zn^{2+} binding site by genetically engineering three histidine residues. The movement of MTs was inhibited by changes in the kinesin neck junction region when Zn^{2+} binds to the site. These results demonstrate that the mutant kinesins were successfully inhibited by concentrations greater than 10 mM Zn^{2+}. MTs successfully restored mobility by removing zinc using multiple chelators. Munmun et al. proposed a simpler way to regulate MT movement using the natural osmolyte trimethylamine-N-oxide (TMAO), modulating the MT-kinesin system in a concentration-dependent manner without relying on the modulation of fuels, cofactors, or relevant physicochemical parameters. Furthermore, this regulation can be rapidly reversed and repeated over multiple cycles. As the concentration of TMAO increased, the kinesin-driven motility of MTs gradually decreased *in vitro*, and when the concentration was further increased to 3000 mM or more, the motility of MTs on kinesins was completely halted; however, when the concentration of TMAO decreased, the motility gradually increased [48]. The introduction of a chemical switch represents a major breakthrough in sensor development, as the sensor can now be controlled not only by ATP but also designed to achieve expected timing, fixed points, and quantitative detection through manual design.

An intriguing class of experiments involves studying the population motion of biomolecular motors [56,57]. Keya et al. [58,59] focused on the collective motion of biomolecular motors and designed a biomolecular motor sensor that could be reversibly controlled via DNA signaling. A feasible scheme was devised for the collective motion of the

Table 1
Different types of Kinesin-MT biosensors.

Detection principal	Model analyte	Detection time (min)	Sensitivity	Advantage (significance)	Ref.
Biotin–streptavidin system (biotinylated microtubules combined with streptavidin labeled targets)	Tetramethylrhodamine (TMR)	45	14 fM	Two orders of magnitude higher detection sensitivity than typical existing immunoassays employing ELISA	[42]
	IL-2, TNF-α	–	–	Simultaneous detection of multiple analytes in a sample, eliminating the need for bulky pumping systems or large power	[43]
Antigen–antibody reactions	$CD45^+$ microvesicles	30	0.1 nM	Label-free detection, comparable to that of commercial ELISA-based IgG detection kits	[36]
	Staphylococcal enterotoxin B	30	0.5 ng/mL	The first demonstration of the use of a gliding MT carrying a capture antibody for the detection of SEB in a sandwich assay format	[44]
	Tomato mosaic tobamovirus	45	1 ng/mL	Approaching the standard ELISA sensitivity and published detection limits	[40]
Redox reaction (MT immobilized)	Ferrocene monocarboxylic acid-kinesin conjugates	–	0.38 nM	The sensitivity of the designed biosensor or its ability to record such events can be controlled at any given time.	[34]

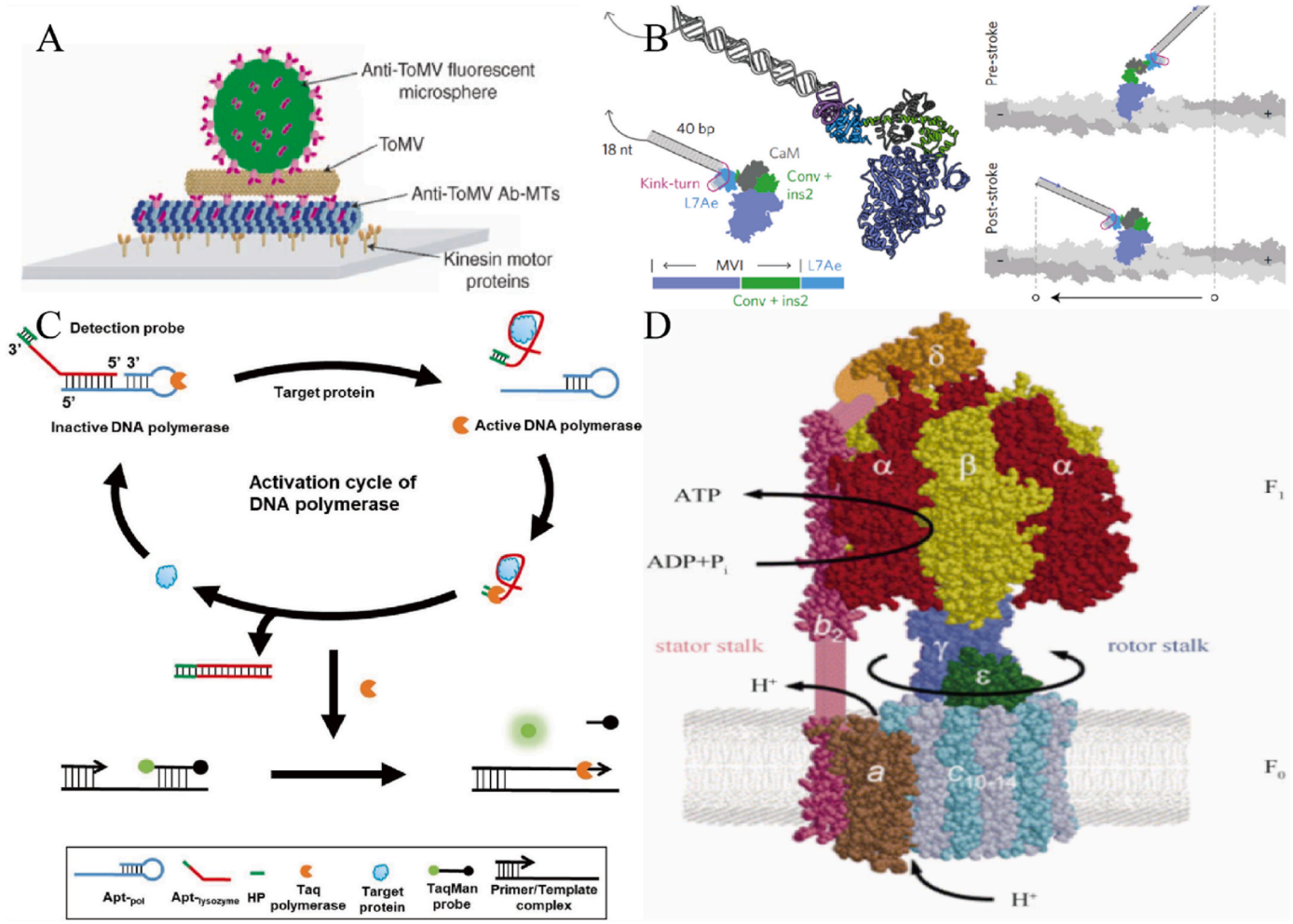

Fig. 3. (A) Graphical representation of the Ab-MT scheme for transporting ToMV virus particles: viruses can be captured and detected by a double-antibody sandwich on a microtubule. Reprinted with permission from Ref. [41]. (B) Engineered myosin with an RNA lever arm: An annotated schematic is shown alongside a larger 3D ribbon diagram for M6-RB:ktL and the movement of the lever arm tip from the pre-stroke to the post-stroke state. Reprinted with permission from Ref. [45]. (C) The protein detection method based on target-responsive DNA polymerase activity: The detection probe is created through the hybridization of two engineered DNA aptamers (the target protein, Apt-$_{lysozyme}$, and DNA polymerase, Apt-$_{pol}$). When lysozyme is present, it interacts with Apt-$_{lysozyme}$, causing the destabilization of the detection probe by detaching Apt-$_{lysozyme}$ from Apt-$_{pol}$. This interaction activates DNA polymerase, which initiates a primer extension reaction at the 3′ end of Apt-$_{lysozyme}$ with the helper primer (HP), displacing lysozyme to trigger another activation cycle of DNA polymerase. Additionally, the activated DNA polymerase, with 5′→3 exonuclease activity, catalyzes the primer extension on the primer/template complex alongside a TaqMan probe labeled with a fluorophore (FAM) and a quencher (BHQ1) at each end, resulting in significantly enhanced fluorescence intensities. Reprinted with permission from Ref. [46]. (D) Structure of F_oF_1-ATPase. Reprinted with permission from Ref [47].

biomolecular motors. First, ssDNA binds to MTs that capture analytes to form an ssDNA-MT complex, which then binds to complementary ssDNA with multiple binding domains to form a dsDNA-MT complex. The motion velocity of the dsDNA-MT complex is 0.5 1 ± 0.02 μm/s, which is close to that of an individual MT. The speeds of the MTs were unaffected. After the MTs were enriched with the DNA strands, they were moved to the detection region, where the MTs that did not enter the detection area were reduced, amplifying the detection signal. This design allows biomolecular motor sensors to achieve lower errors and detection limits while further improving detection accuracy.

In summary, kinesin-MT biomolecular motors are typical linear motors, and the detection process involves three key steps: (1) analyte capture, (2) analyte transfer, and (3) signal detection. Probe selection is critical for analyte detection by MTs. Certain substances, such as antibodies and nucleic acids [60], have been used as probes for capturing analytes [43]. These probes capture antigens, viruses, bacteria, and small molecules [41] and they have been used to construct various basic biomolecular motor sensors with good detection performances. On this basis, studies on the three key steps have enabled continuous optimization and increased intelligence of molecular motor sensors. However, biomolecular motor sensors do have certain limitations.

The first concern is protein longevity. The bioactivity of a protein is influenced by its temperature, pH, and ionic strength [61,62]. Studies on the effects of pH on the movement of MTs have demonstrated a loss of MT mobility due to pH values being too high or low. This loss of mobility is primarily attributed to reduced kinesin activity caused by the pH. Furthermore, it is well known that excessively high or low ionic strength and pH can lead to protein degeneration, which affects sensor detection. Temperature is another critical factor that influences the actual application of a sensor. Kinesin ATPase activity increases steadily up to approximately 37 °C. To ensure detection consistency in various environments, sensors must address the problem of reduced protein activity at low temperatures.

Second, the separation of kinesin and MT during *in vitro* assays is a possible risk. Studies show an increased propensity for MTs to separate from kinesin at temperatures below 30 °C or above 40 °C. If the analytes cannot reach the detection area, the detection signal can be reduced or even lost, resulting in false-negative results. Third, the slower movement

speeds of kinesin and MTs partly affect the detection speeds of the sensors.

In summary, biomolecular motors based on protein-MT systems have great research potential in the field of *in vitro* assays. A mathematical model indicates that more than 40 % of active kinesin is required to continuously glide molecular shuttles, which is much less than the 80 % needed for active myosin. Therefore, kinesin microtubule systems may be more stable and advantageous for the preparation of biosensors [63]. Proteins, small molecules, bacteria, and viruses can serve as target analytes for detection. However, kinesin-MT biomolecular motors have various limitations that need to be addressed. Therefore, developing more intelligent and controllable biomolecular motor sensors is the direction of future research.

2.2. Myosin

Myosin moves along actin filaments (AFs) [64], converting ATP into mechanical energy to power muscle contraction, cell division, cell crawling, and changes in cell morphology. Myosin is a hexamer consisting of two heavy chains and four light chains, with the following structural components: (1) Myosin heavy chains (MHCs) that contain actin and ATP-binding sites, located in the N-terminal globular head or motor domains; (2) A neck region that acts as a lever arm and contains a varying number of IQ motifs, to which myosin light chains (MLCs)—also called calmodulin (CaM) or CaM-like proteins—bind. The MLCs stabilize the lever arm and amplify the motion generated during the ATP hydrolysis cycle; (3) A variable-length carboxy-terminal tail region that typically includes an α-helical stretch responsible for MHC dimerization and a globular domain with functional motifs responsible for cargo binding and myosin localization [65].

The hydrolytic state of the nucleotide controls the affinity of the motor for the track and the conformational changes within the motor domain that lead to the lever arm swinging. In the absence of nucleotide and during the ADP-binding state, the head interacts strongly with actin. In contrast, when ATP or ADP bound to inorganic phosphate (ADP-pi) binds to the catalytic domain, the affinity of the myosin head for actin is significantly reduced. Additionally, the duty ratio affects the actin binding time. Muscle contraction is driven by the relative sliding of myosin and actin filaments, with this movement realized by the myosin lever arm. This amplifies the conformational change of the catalytic domain, thus producing a step size that is directly dependent on the length of the lever arm [66].

Proof-of-principle devices based on the microtubule-kinesin motor system do not match the speeds of existing methods [67]. An attractive solution to overcome this limitation is the use of myosin-driven propulsion for AFs, which offers motility that is one order of magnitude faster than that of the kinesin microtubule system. Lard et al. [68] used HMM to capture the actin filaments, the filaments were tranported to the detector area after ATP added. By optimizing the device, they achieved extensive enrichment of actin filaments driven by myosin on a detector area of less than 10 μm^2, with a concentration halftime of approximately 40 s. Myosin is also similar to kinesin, making the myosin-AF system a suitable choice for sensor preparation. The methods and principles for building such sensors are largely consistent with those for kinesin-MTs. This section describes an engineered biomolecular motor for controlling motor transport using custom components. The engineered motor provides new methods for exploring structure-function interactions and offers ideas for the development of biomolecular motor sensors.

M6-RB (myosin VI-RNA-binding) [45] contains myosin and an RNA lever arm that form hybrid assemblies through an oriented rigid connection (Fig. 3B). Angular changes in myosin are transmitted to the RNA via rigid junctions, amplifying and redirecting conformational changes in the protein motor domain. The speed and direction of motor transport are determined by the geometry of the RNA lever arm and can be dynamically controlled using a chemical switch in the lever arm structure. Detection using biomolecular motor sensors is thus a controlled process.

Myosin-AFs are transported ten times faster than kinesin-MTs; therefore, sensors constructed with myosin-AF systems exhibit significantly faster detection speeds [21]. Furthermore, AFs have lower bending stiffness, allowing them to be designed as smaller and more intelligent biomolecular motor sensors [68]. However, myosin AFs present several challenges: (1) High-speed movement results in relatively low actin affinity for myosin, and high bending flexibility leads to a lower number of myosin-binding sites per micron in AFs [21]. This increases the likelihood of AFs separating from the surface of the myosin layer. (2) Because AFs are thought to rotate around their long axes, it is likely that the analytes captured by the AFs will collide with the dense myosin layer, potentially causing them to fall off and resulting in false-negative results. (3) The binding of certain proteins to actin filaments alters their structure and interferes with their motility [69]. In contrast, MTs are generally more stable than the AFs [70]. (4) As AFs aggregate, collisions between them increase, raising the risk of AFs leaving the detection area [68]. Additionally, both the myosin and kinesin systems are inhibited by complex body fluids, which can partially address these concerns [21].

2.3. DNA polymerase

DNA polymerases are a class of enzymes that utilize parental DNA as a template to catalyze the polymerization of substrate dNTP molecules, forming daughter DNA. They can replicate DNA strands at a high rate and with high fidelity, displaying an error rate for single-base substitutions due to proofreading-defective DNA polymerases that ranges from only 10^{-3} to 10^{-6} [71].

Some studies on DNA polymerase biosensors are related to PCR technology. DNA polymerase primarily functions in cleavage, catalysis, and signal amplification. Wang et al. [72] reported the first one-step, general-purpose PCR-based sensor for detecting the sizes of nucleic acids and proteins. This sensing system employs DNA-modified AuNPs (AuNPs-DNA) as TaqMan-like signaling probes. During PCR extension, the DNA on the surfaces of the AuNPs is cleaved by Taq polymerase, resulting in the aggregation of AuNPs and a detectable change in size via DLS. Compared with conventional colorimetric methods, this approach offers higher sensitivity, with a detection limit of 1.2 fg/μL. Zhang et al. [73]constructed a polymerase-based electrochemical luminescence (ECL) DNA sensor using a cyclic chain displacement polymerization technique combined with a target mRNA cycle and quantum dot signal amplification for label-free detection of leukemia marker mRNA (miR-16), with a detection limit of 4.3×10^{-7} mol/L. Jung et al. [46] described a novel method for the detection of target proteins based on target-responsive DNA polymerase activity, combining two different types of DNA aptamers ($Apt\text{-}_{lysozyme}$ and DNA polymerase ($Apt\text{-}_{pol}$)) as a detection probe. This probe inhibits DNA polymerase activity; however, when the target protein is present, lysozyme interacts with $Apt\text{-}_{lysozyme}$, detaching it from $Apt\text{-}_{pol}$ and thereby activating the DNA polymerase. The activated polymerase then couples to a TaqMan probe labeled with a fluorophore and quencher at each end, catalyzing the primer extension reaction on the primer/template complex and resulting in a significant increase in fluorescence intensity (Fig. 3C). This principle enables selective detection of the model target lysozyme down to 0.80 nM, allows for the detection of other target proteins through a simple replacement of the probe, and provides better sensitivity at a lower cost compared to previously developed methods. Similarly, He et al. [74] developed a double-amplification sensor for DNA detection using only one enzyme: DNA polymerase. The sensing system consists of a hairpin probe (HP), double-stranded DNA (DSD) labeled with a quencher and a fluorophore, and DNA polymerase in the presence of the target DNA. A series of reactions, including hybridization and conjugation, forms a dual reaction cycle that significantly enhances detection sensitivity (detection limit: 0.38 nM).

In summary, DNA polymerase biosensors offer high specificity and

sensitivity for nucleic acid targets. They also provide a versatile platform for amplification-based detection, enabling detection at low concentrations of the target DNA. In contrast to other molecular motor sensors, including those based on rotary or linear motors such as kinesin or myosin, DNA polymerase sensors are particularly suitable for DNA-related applications and enable straightforward signal amplification through enzyme-driven processes.

2.4. F_oF_1-ATPase

2.4.1. Construction

ATP synthase (F_oF_1-ATPase) is widely distributed in mitochondria, chloroplasts, and bacterial plasma membranes. Compared to other motors, ATP synthase has a high energy conversion efficiency (near 100 %). However, under high external torque due to mechanical slip, this efficiency can drop from 80 % to 40 % [75]. Fig. 3D illustrates the structure of F_oF_1-ATPase.

F_oF_1-ATPase primarily consists of two parts: the proton transport domain, called F_o, and the catalytic synthesis domain (F_1). The F_o, serving as a stator, is composed of three subunits: a, b, and c, which are embedded in the membrane. F_1, functioning as a rotor, consists of five subunits: α, β, γ, ε, and δ [76–78]. The F_oF_1-ATPase is a typical rotating molecular motor. Rotary ATPases couple ATP turnover with ion translocation across membranes and are central to biological energy conversion. The F_oF_1-ATPase synthesizes ATP from ADP and inorganic phosphate when F_1 rotates in the clockwise direction. The energy for synthesis is derived from the transport of H^+ along the electrochemical gradient created by protons across the membrane. Energy is released, and F_1 rotates counterclockwise during ATP hydrolysis [79]. The rotation of F_oF_1-ATPase labeled with a special probe has been observed using single-molecule imaging techniques [80]. Iino et al. [19] demonstrated the rotational motion of the F_1 motor probed with a polystyrene bead duplex. The three catalytic sites for ATP synthesis/hydrolysis are located at the interfaces of α and β subunit heterodimers. Theoretically, during ATPase-driven rotation, each 120° power stroke occurs due to the binding of ATP to an empty catalytic site, causing three pauses [81]. These cycles consist of periods without motion (pauses) and abrupt motion (steps) along the trajectory. Such cycles of pauses and steps are fundamental behaviors of motor proteins. During the pauses, the motor protein waits for the elementary steps of chemical reactions, such as substrate binding, covalent bond breakage, and product release.

2.4.2. F_oF_1-ATPase biosensors

Owing to its unique energy conversion mechanism and endogenous driving force, F_oF_1-ATPase can be designed as a sensor (Fig. 4). The construction of a sensor utilizing F_oF_1-ATPase as the molecular motor can be divided into the following main steps: (1) Biotin first reacts with the monoclonal antibody of the subunit (β or ε subunit) and then binds to the subunit via antibodies to yield biotinylated F_oF_1-ATPase. (2) Biotin modifies the detection probes. (3) The biotinylated probe is linked to the biotinylated F_oF_1-ATPase using streptavidin. (4) Fluorescent substances or quantum dots are employed as signal probes to mark chromatophores.

The F_oF_1-ATPase biomolecular motor sensor is also mass-sensitive. The weight of the side chain increases when the analyte binds to the probe. Subsequently, the rotational speed of the rotor decreases, and the rate of ATP synthesis, along with the concentrations of protons inside and outside the membrane, are altered. The load is reflected by detecting changes in ATP content or pH. Thus, chromatophores with F_oF_1-ATPase are considered suitable sensors in principle. These sensors have proven useful for detecting viruses, bacteria, and even small molecules in medicine, food, and other fields (Table 2) [84].

Considering the ability of antibodies to undergo specific immune reactions with antigens, antibodies are first designed as probes for biosensors. Zhao et al. [85] synthesized a novel biosensor (immunorotation biosensor) utilizing a deoxynivalenol (DON) monoclonal antibody as a

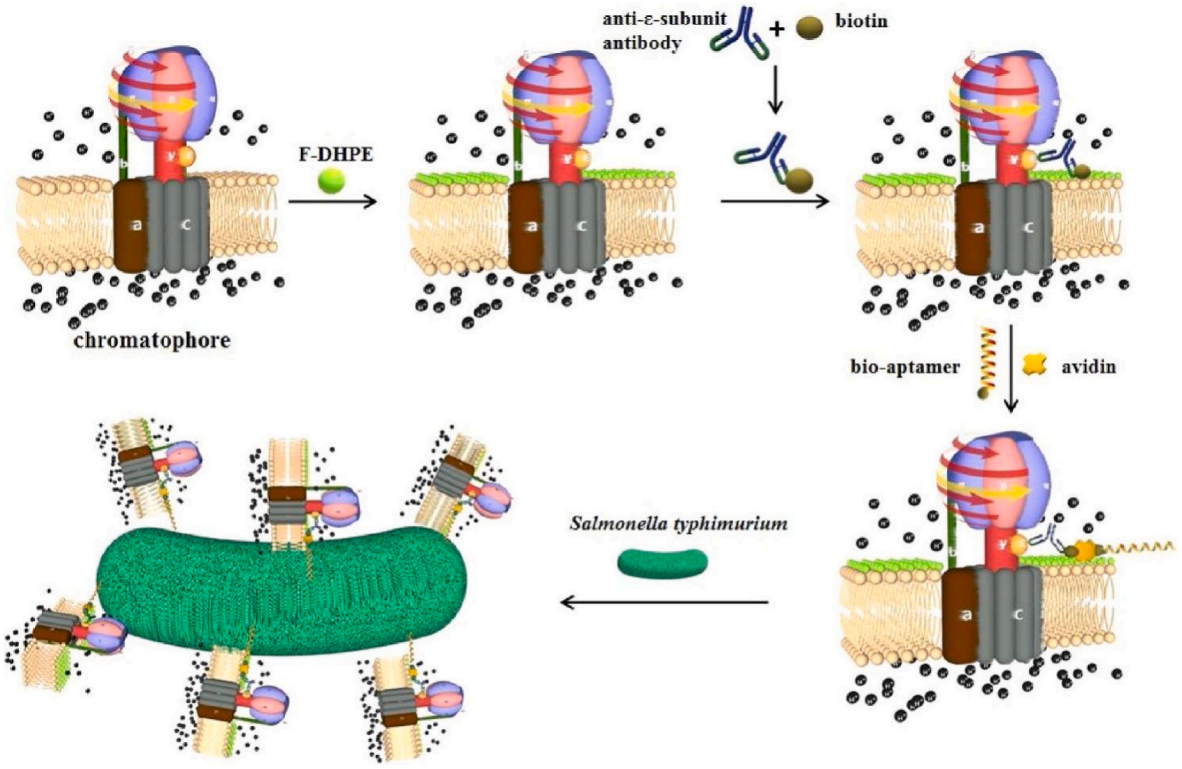

Fig. 4. F_oF_1-ATPase biomolecular motor sensor construction process. The chroma loaded with F_oF_1-ATPase is fluorescently labeled. By designing suitable probes, the analyte is captured via the biotin–streptavidin system, which subsequently alters the motor rotation speed, resulting in changes in the proton concentration across the membrane and variations in the fluorescence intensity. Reprinted with permission from Ref. [82].

Table 2
Different types of F_oF_1-ATPase biosensors.

Detection probe	Detection method	Loading sites	Detection metrics	Sensitivity	Detection time	Ref.
Deoxynivalenol (DON) monoclonal antibody	Luciferin–luciferase system	ε subunits	ATP synthetic activity	10^{-7} mg/mL	20 min	[85]
NT-proBNP	Luciferin–luciferase system	ε subunits	ATP synthetic activity	15 pg/mL	30 min	[86]
2-dodecylcyclobutanone (2-DCB) monoclonal antibodies	Luciferin–luciferase system	ε subunits	ATP synthetic activity	10^{-8} μg/mL	10 min	[87]
H9 influenza antibodies	Fluorescence probe F1300	β subunits	Fluorescence intensity (due to pH change)	–	10 min	[88]
Norovirus aptamer	Fluorescence probe F-DHPE	ε subunits	Fluorescence intensity (due to pH change)	0.005 ng/mL	1 h	[89]
Salmonella typhimurium aptamer	Fluorescence probe F-DHPE	ε subunits	Fluorescence intensity (due to pH change)	10 cfu/mL	1 h	[82]
V. parahaemolyticus aptamer	Fluorescent probe F1300	ε subunits	Fluorescence intensity (due to pH change)	15 cfu/mL	1 h and 40 min	[90]
V. parahaemolyticus aptamer	Quantum dots	ε subunits	Fluorescence intensity (due to pH change)	7 cfu/mL	1 h and 40 min	[91]
miR2-HRP and miR1	Luciferin–luciferase system and HRP	ε subunits	ATP synthetic activity and fluorescence intensity	5 nmol/L	15 min	[83]

probe. The probe is linked to the ε subunit through a biotin-avidin system, enabling the detection of DON via an antibody-antigen reaction. The detection range of the biosensor spanned from 10^{-1} mg/mL to 10^{-7} mg/mL, with the entire detection process taking approximately 20 min, significantly shorter than most clinical tests.A series of biomolecular motor sensors based on the same principle has led to breakthroughs in food safety detection, such as the rapid detection of 2-dodecylcyclobutanone (2-DCB) in γ-irradiated beef [87]. The detection limit was 10^{-8} μg/mL, and the detection time was 10 min. In another study, a highly sensitive F_oF_1-ATPase biomolecular motor sensor was developed to detect the influenza virus [88]. Unlike the previously mentioned sensors, the β subunits were used as binding sites, and three detection probes were assembled for each sensor. Changes in fluorescence intensity due to ATP synthesis were directly observed via fluorescence microscopy within 10 min. The incorporation of three probes significantly enhanced the sensor's ability to capture viruses, while its high pH sensitivity improved signal conversion. Ultrasensitivity is a major advantage of this sensor, and its capability for single-molecule detection makes it superior to other virus detection methods.

Using an antibody as a probe is fast, easy, and suitable for large-scale screening. However, antibody stability is poor and is susceptible to temperature and pH changes [92]. Additionally, the antibody preparation process is complex, and preservation conditions can be harsh. The detection accuracy of antibody probes based on sequence pairing is lower than that of nucleic acid probes, with erroneous nucleotides added to the extended ends occurring at a frequency of only 1 in 10,000 nucleotides. Therefore, the use of nucleic acid probes has become a trend for high-precision detection. RNA, single-stranded DNA, and double-stranded DNA probes can be easily obtained through the repeated screening of a random oligonucleotide sequence library synthesized *in vitro* using the ligand index enrichment method (SELEX) [93]. Different aptamers have been used for detection [82,89,90].

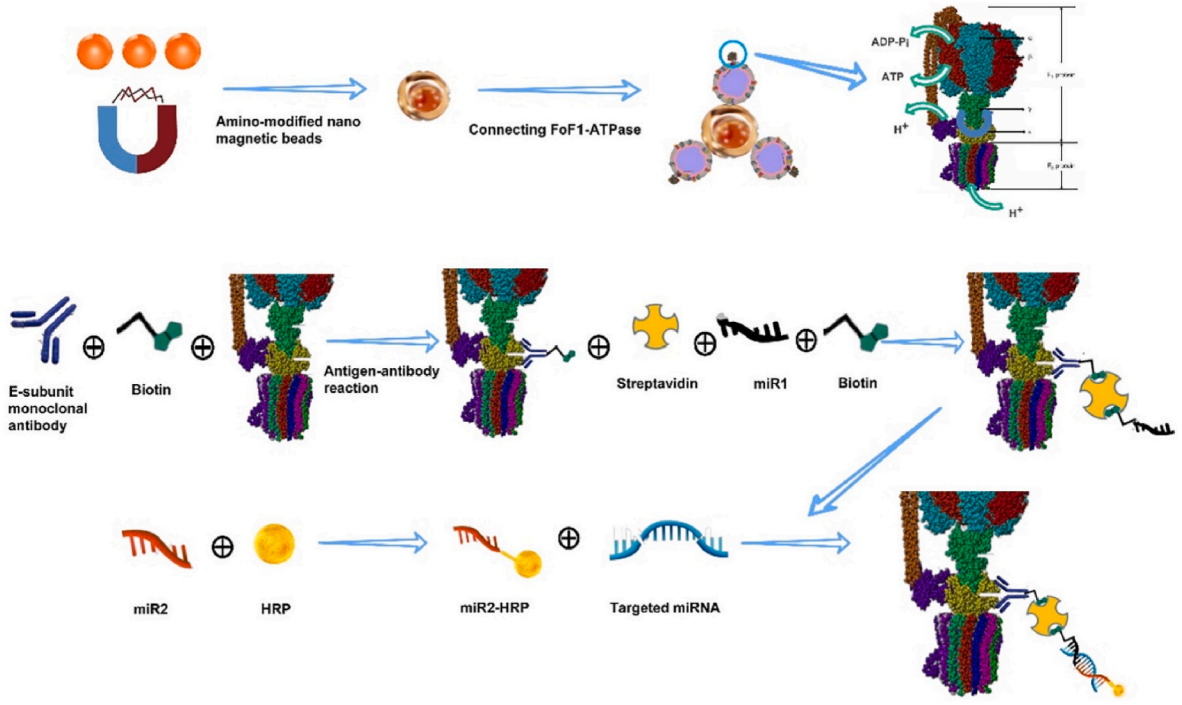

Fig. 5. Detection diagram of the F_oF_1-ATPase molecular motor biosensor. The F_oF_1-ATPase molecular motor is immobilized on magnetic beads and binds to the capture probe via the biotin–streptavidin system. Moreover, the signal probe miR2-HRP can bind to the remaining sites of the target, achieving dual-signal detection. Reprinted with permission from Ref. [83].

Subsequently, Duan et al. [91] replaced the original fluorescent probe with a quantum dot-labeled chroma, further expanding its application in the sensor field and reducing its detection limit for *V. parahaemolyticus* to 7 CFU/mL. A recent study combined miRNA with an F_oF_1-ATPase molecular motor to construct a capture probe for the detection of colon cancer-related miR-17 (Fig. 5) [83]. Additionally, another HRP-labeled miRNA was designed as a signal probe to bind to the free sequence of miR-17. This approach not only increased the motor load but also enabled dual-signal characterization, including ATP synthesis and fluorescence signal detection. The results demonstrated that the designed sensor exhibited a good linear relationship between the chemiluminescence intensity and OD 450 nm with an miR-17 concentration in the range of 5–200 nmol/L, enabling the detection of miR-17 in serum at levels comparable to those obtained via PCR.

2.4.3. *Other detection principles of F_oF_1-ATPase biosensors*

Krah et al. found that the R103A/R115A double mutant of the ε subunit from thermophilic Bacillus PS3 exhibits strong binding ability to ATP [94]. Therefore, the ATP synthase ε subunit can directly function as an ATP sensor. Zhou et al. [95] developed a hybrid-type ATP optical sensor (ATPOS) that consists of an ATP-binding protein, specifically the *Bacillus* F_oF_1-ATP synthase ε subunit, combined with fluorescent dyes Cy3 and Alexa Fluor 488 (Alexa488). This sensor enabled *in vivo* fluorescence imaging of extracellular ATP dynamics in the brain. In addition to the mass-sensitive sensor, a nanoflow sensor can be developed by coupling it to nano-sized tag beads whose rotational motion is affected when the flow near the surface exerts hydrodynamic forces on the F1 beads. Based on this principle, Lee et al. developed a microfluidic testbed chip to precisely control fluid motion on the F1 bead. Experiments showed that the rotational velocity of the F1-ATPase decelerated linearly with an increase in the flow rate, successfully demonstrating the potential of biomolecular motors as nanoflow sensors [96].

Compared with linear biomolecular motors, the F_oF_1-ATPase biomolecular motor has unique advantages. The motion of the rotating motor does not depend on MTs/AFs, eliminating the possibility of detachment phenomena, such as kinesin's separation from MTs, which can result in undetectable signals. Consequently, the likelihood of false negatives may be lower, and the test results are highly credible. Constructing rotary motor sensors is simpler than building linear motor sensors, and because of their inherent rotation, F_oF_1-ATPase does not need to be fixed, similar to kinesin. After capturing the analyte using F_oF_1-ATPase, the change in rotational velocity is immediately converted into a fluorescent signal. Additionally, F_oF_1-ATPase does not need to transfer from the capture region to the detection region, resulting in a relatively fast detection rate.

In conclusion, the preparation of biomolecular motor sensors is relatively advanced. For detecting proteins, nucleic acids, and small molecules, the F_oF_1-ATPase biomolecular motor sensors offer high specificity, sensitivity, and shorter detection times. An interesting study utilized F_oF_1-ATPase as a power engine to drive artificial supramolecular colloidal motors, significantly extending the application scenarios of conventional colloidal motors and providing new ideas for applications in the biosensing field [97]. F_oF_1-ATPase is ideal for sensor preparation and is expected to play an important role in many fields in the future.

2.5. *Bacterial flagella biosensors*

The bacterial flagellum is a macromolecular protein complex that is more than five times the length of the bacterial cell. Flagella are responsible for bacterial motility and are unique rotating molecular machines embedded on the surface of bacteria (Fig. 6). They consist of several concentric rings that can spin at hundreds of revolutions per second. Generally, the bacterial flagellum includes a bidirectional rotary motor, a universal joint, and a propeller. Similar to a macroscopic motor,

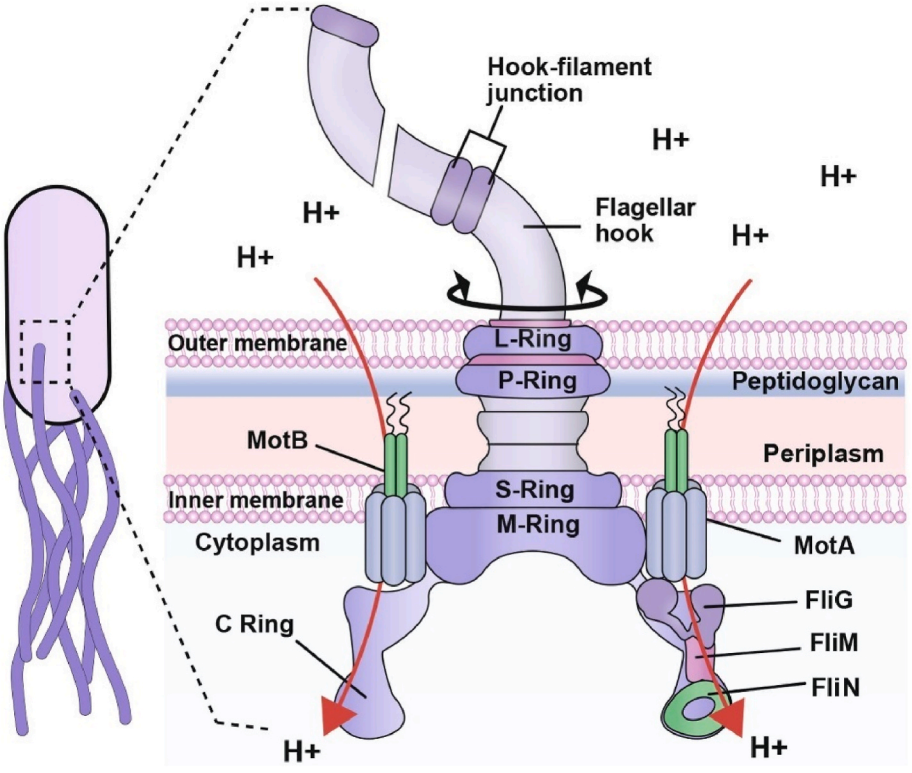

Fig. 6. Structure of the bacterial flagellar motor. The bacterial flagellar motor comprises a basal body, flagellum filament, and hook. The basal body anchors the motor to the cell, with key components such as the M, S, and C rings; the latter is a stack of multiple copies of FliG, FliM, and FliN proteins, each being arranged in a ring. MotA is a part of the torque generating MotA5MotB2 units, where MotB subunits anchor the stator to the cell wall (peptidoglycan layer). Reprinted with permission from Ref. [99].

the bacterial flagellar motor contains a rotor and a stator. The rotor comprises cytoplasmic proteins, including FliG, FliM, and FliN, as well as membranous/supramembranous (M, S) rings. Each stator unit is composed of five MotA proteins and two MotB proteins [98]. These structures are crucial for the rotation of bacterial flagella. The movement of bacterial flagella is coordinated by chemical signals, enabling the flagella to sense changes in the environment and drive bacteria to different locations. Based on this principle, the bacterial flagellum can be utilized as a molecular motor biosensor for *in vitro* detection.

One study monitored the tropism of flagellar movement and explored the potential for designing flagellar motor sensors based on this principle [100]. The tropism of motors to L-aspartate (Asp) and L-leucine (Leu) was observed using fluorescence microscopy. Researchers collected hundreds of wild-type chemotactic *E. coli* motor responses to Asp and Leu dilutions and trained *E. coli* to estimate aspartate concentrations. Another *E. coli* strain was trained to distinguish between aspartate and leucine with 83 % accuracy. This method represents a step toward the development of chemotaxis-based biosensors and also attempts to incorporate chemotactic bacteria as front-end biomolecular motor sensors for microbiorobotics.

The bacterial flagellar motor (BFM) acts as a chemosensor. Depending on the presence or absence of chemical signals *in vivo*, *Escherichia coli* transmits signals from methyl-accepting chemotactic proteins to bacterial flagellar motors through interactions involving phosphorylated CheY proteins with motor complex switches. The MCPs span the inner membrane and the periplasmic space between the two membranes, extending into the cytoplasmic space to transmit information about specific chemicals in the extracellular environment. This mechanism allows them to sense low concentrations of specific chemicals [99].

Another application of the BFM is in mechanosensors. To investigate the mechanosensing mechanism of flagellar motor proteins, Pushkar et al. increased the motor speed from a low load to a high load by significantly increasing the viscous drag on the motor. When the motor was under a low load, the stator rotated the motor at a high speed. As the load increased, the stator drove the motor at a lower rate while delivering higher torque or force. At this point, remodeling was likely to occur, increasing the likelihood of stator element attachment. As additional stator elements were absorbed, the speed gradually increased until it stabilized. Thus, their study suggested that the stator itself likely functions as a mechanosensor, while the conformation of the FliG ring and FliL is unlikely to play a role in stator remodeling and signal sensing [101]. Hug et al. introduced a second-messenger-mediated tactile response using a bacterial rotary motor, where the flagellar motor acted as a sensor [102]. Ma et al. utilized a nucleotide messenger to identify flagellar motors. In this study, c-di-GMP was found to control the dynamic exchange of flagellar stator units, regulating motor torque and speed as well as modulating the frequency of flagellar motor switching through chemosensory signaling pathways [103].

As a biosensor, the bacterial flagellum can sense nanomolar concentrations of specific chemicals within seconds, achieving rapid detection. In contrast to artificial motors, bacterial flagellar motors rotate faster and exhibit higher energy-conversion efficiency [104]. Both flagella and F_oF_1-ATPase are rotating motors, making it feasible to prepare sensors using bacterial flagella as templates while utilizing F_oF_1-ATPase [99].

3. Conclusions

Currently, immunization, optical analysis, and PCR are commonly used for biochemical assays. The disadvantages of these methods include high time consumption, the need for high purity, high costs, and significant equipment requirements. Recently, biomolecular motors have gained popularity as materials for sensor preparation owing to their unique properties. Biomolecular motors are a special class of proteins with endogenous dynamics that efficiently convert chemical energy into mechanical energy, including F_oF_1-ATPase, kinesin, dynein, myosin, and flagella. Biomolecular motor sensors have low preparation costs, fast detection speeds (with detection times as low as 1 h), and high accuracy. Therefore, these sensors have been extensively studied for various detection applications. Biomolecular motors are attractive options for qualitative and quantitative detection in food quality assessment, clinical disease monitoring, and many other areas. However, molecular motor biosensors face several challenges. In addition to longevity, factors such as stability (protein oxidative stress degradation and microtubule depolymerization), cost, cross-reactivity, production scale, storage, and transport significantly impact biomolecular motor sensors. Developing more stable, efficient, and intelligent biomolecular motor sensors is expected to become an important research direction in the field of detection.

CRediT authorship contribution statement

Jinhong Zhao: Writing – original draft. **Fangyuan Guo:** Writing – review & editing, Investigation. **Mengqi Wang:** Investigation, Writing – review & editing. **Jie Zhang:** Visualization, Investigation. **Sanjun Ying:** Writing – original draft. **Ying Gao:** Validation, Investigation. **Gensheng Yang:** Writing – review & editing, Conceptualization. **Weiyong Hong:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Funding sources

This work was financially supported by the National Natural Science Foundation of China (Grant number 22078297), Natural Science Foundation of Zhejiang Province (Grant number LTGC23H200002), Science and Technology Plan Project of Taizhou (Grant number 23ywa37), and Special Research Fund of Hospital Pharmacy of Zhejiang Pharmaceutical Association (Grant number 2020ZYY46).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ying Gao is currently employed by Zhejiang Moda Biotech Co., Ltd.

Acknowledgement

The authors appreciate the inputs from Dr. Bang Lou.

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