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Review Article

Smart Polythiophenes: Pioneering imprinted and functionalized materials in biosensor technology

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ABSTRACT

Smart designs beyond the limitations of the biosensors present fascinating opportunities for portable, flexible, versatile, and effective performance that allow for the rapid in-vivo, and real-time detection of potential targets. Conjugated polythiophenes (CPTs) are particularly valuable as biosensors because of their remarkable brightness, excellent photostability, and low toxicity. CPTs potentially molecularly self-assemble using an imprinted method resulting in imprinted conjugated polythiophenes (ICPTs). ICPTs combined the distinctive characteristics of CPTs with the excellent selectivity arising from robustly particular binding sites of molecular imprinting. ICPT-based biomimetic sensors represent a specialized subset within this extensive field. An overview of various types of CPT-based sensors was described to achieve a systematic analysis. These included biosensors based on printing technologies, microfluidic systems, film transistors, colorimetric methods, and electrochemical approaches. Additionally, we discussed the optical-electrical properties, and sub-types of polythiophene derivatives examining their specific applications and advantages in biosensor technology. The final section provided an in-depth exploration of the imprinted techniques employed in developing ICPTs-based sensors, with particular emphasis on applications in biochemical sensing.

1. Introduction

The development of smart biosensors is critical for biomedicine and healthcare due to its diversity of various biofluids such as blood, serum, saliva, or urine. For these reasons, affinity and catalytic sensors have been employed recently [1,2]. Biosensors fall into five categories: visual, thermal, semiconductor, bioelectrode, and piezoelectric, depending on the kind of transducer parts. Sensitive analytical signal transduction can be rendered possible by specific physical techniques, but achieving reliable selectivity for these techniques remains highly challenging. Innovative materials and methods have been invented to determine analytes to overcome the issue [2,3].

Molecular imprinted polymers (MIPs), represent a significant advancement in sensor technology by emulating the recognition capabilities of natural biological systems [4]. Unlike biosensors, which rely on biological elements like enzymes, antibodies, or nucleic acids for detection, biomimetic sensors use synthetic materials designed to mimic these natural receptors. Compared to biosensors, biomimetic sensors offer advantages in terms of robustness, longer shelf life, and the ability to function in harsh environmental conditions, making them suitable for a broader range of applications [5]. Various polymers are applicable in creating MIPs, yet conjugated polymers exhibit particularly appealing attributes for molecular imprints across diverse chemical compounds [1]. Importantly, conjugated polymers, including but not limited to polythiophene, have considerable potential for various applications. The process of over oxidizing polythiophene serves to enhance the selectivity of MIPs constructed with polythiophene as the base material. Imprinted conjugated polythiophenes (ICPTs) leverage the unique properties of polythiophenes combined with molecular imprinted technology to create specific recognition sites within a conductive polymer matrix.

Conjugated polythiophenes (CPTs) are primarily used in the development of sensing platform for various targets [6,7]. CPTs possess strong

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signaling features, excellent biocompatibility, good conductivity, and extraordinary photochemical stability [8,9]. Additionally, biomolecules that may selectively bind certain analytical targets, such as DNA [10], antigens [10], antibodies [11] and enzymes [12], could be immobilized on CPTs as an immobilization matrix. Moreover, monomers can be functionalized with various functional groups, thus granting the polymer two advantages. MIPs have specific regions or template that are specifically designed to match the dimensions, form, and functional groups of the desired molecules [13]. This template is usually created when the target is incorporated into the polymer matrix during the polymerization process, and then the target is removed using desorption techniques [14,15]. While biosensors often excel in sensitivity and specificity due to their biological components, the durability and versatility of biomimetic sensors position them as a powerful alternative in the field of chemical and biological sensing. It should be mentioned that similar designs could be constructed using conjugated polymers, which can offer better sensitivity than other nanomaterials thanks to the signal amplification feature [16,17]. Thiophene monomers can be polymerized in the presence of a template molecule, or pre-polymerized CPTs can self-assemble into larger nanostructures in the presence of a template molecule to generate ICPTs [18]. Imprinted conjugated polythiophenes, which integrate the unique properties of conjugated polymers such as intrinsic signal transduction, providing high sensitivity and high selectivity resulting from highly specific molecular imprinted binding sites, demonstrate significant potential for biomimetic sensor applications [17].

To meet the increasing demands for effectiveness and feasibility, biosensors need to be more functional and integrated. To promote the development of biosensors, several techniques are used, such as printing approaches, microfluidics, organic thin film transistors (OTFT), paper strips and smartphone [19–22]. Utilizing these technologies, biosensors for biological material detection can be equipped with modern features like microprocessors, wireless connection, and auto-calibration capabilities, in addition to being able to be integrated with other equipment [23,24]. It's interesting to note that certain biosensors were developed to fit over mouth guards or connect to smartphones, enabling in-mouth installation and direct measurement or analysis of biotargets in the oral cavity for initial integration attempts [25,26]. These biosensors could set aside space or achieve straightforward biosensor-to-external device integration. In order to be a potentially effective technology for significantly enhancing the use of point-of-care (POC) diagnostics in a timely, economical, and user-friendly manner [27].

This review aims to provide a comprehensive examination of ICPT-based sensors and position them within the broader context of CP-based sensors. CPs have been successfully applied in developing various sensors, including electrochemical, fluorescence, and colorimetric types. ICPT-based biomimetic sensors represent a specialized subset within this extensive field. An overview of various types of CP-based sensors will first be discussed to achieve a systematic analysis. This will include biosensors based on printing technologies, microfluidic systems, film transistors, colorimetric methods, and electrochemical approaches (Fig. 1). Additionally, we will discuss optical-electrical properties, and sub-types of polythiophene derivatives examining their specific applications and advantages in sensor technology. The final section will provide an in-depth exploration of the imprinted techniques employed in developing ICPTs-based sensors, with particular emphasis on applications in biochemical and environmental sensing. Through this comprehensive review, we aim to elucidate the unique contributions of ICPT-based biomimetic sensors, highlight their integration within the wider array of CP-based sensors, and identify future opportunities for innovation and development in the field of imprinted conjugated polymer-based sensing technologies.

2. Biosensors

A biosensor is a device that detects biological materials and turns their concentration into signals that can be detected. A biosensor is a combined receptor-transducer system that uses bio-sensitive substances as a bio-identification component, a physical/chemical component, an amplifier component, and other components to give specific quantitative analysis regarding a certain target [28–31]. Biosensors are classified depending on the type of identification components to the enzyme, nucleic acid, microbial, cell, tissue, and immunosensors. Biosensors have also been categorized as catalytic, or affinity biosensors based on the kind of recognition mechanisms [32,33]. Currently, technologies such as organic thin film transistors, microfluidic chips, digital printing, paper devices integrated with smartphones, and wearable technology in biosensors have been increasingly used to achieve biosensors' adaptability, accessibility, and miniaturization [27].

2.1. Biosensors based on electrochemical methods

Electrochemical approaches have been widely researched and have laid the foundation for various commercially available sensors due to their numerous advantages, including low test costs, simple instrument operation, rapid data acquisition, and high sensitivity. Conjugated polymers (CPs), owing to their high electrical conductivity, three-dimensional nanostructures, and large specific surface area, have been

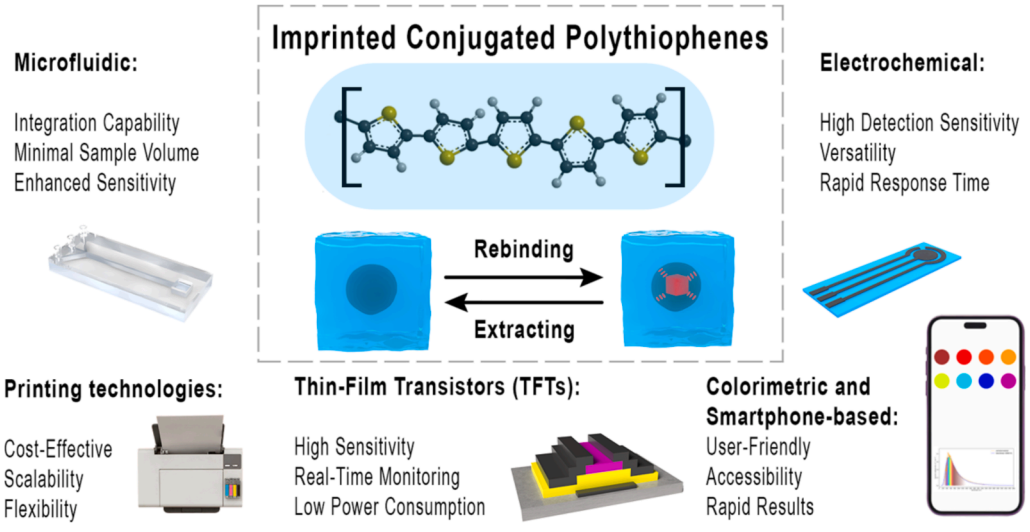

Fig. 1. Key advantages of utilizing imprinted conjugated polythiophenes in biosensors technology.

extensively utilized as foundational materials for the preparation of electrochemical sensors [34].

Furthermore, CP-based electrochemical sensors have demonstrated significant potential in various applications, such as medical diagnostics, environmental monitoring, and industrial process control. Their ability to provide real-time, accurate measurements makes them indispensable in these fields. The integration of CPs with advanced nanomaterials and novel fabrication techniques continue to enhance the performance and functionality of these sensors, opening new avenues for research and development. Recent research work by Lili Yang et al. explores the development of a novel PANI/PA hydrogel with exceptional antifouling properties and high electrochemical performance [35]. The hydrogel consisting of CP showed good resistance to nonspecific protein adsorption, which is crucial for accurate biosensing in clinical diagnostics. This hydrogel is synthesized through a one-step copolymerization process, resulting in a material with a hierarchical porous structure suitable for biosensing applications. The PANI/PA hydrogel-based biosensor demonstrated high sensitivity and specificity in detecting miRNA24, an important cancer biomarker, even in complex biological media. In contrast to the hydrogel-based biosensor, Elif Burcu Aydın et al. coated disposable electrodes with CP film for the development of a label-free immunosensor of resistin, an obesity biomarker [36]. Unlike the hydrogel biosensor that employed PANI and phytic acid, this immunosensor utilizes a double epoxy group-substituted thiophene (TdiEpx) monomer, electrochemically polymerized on a disposable indium tin oxide (ITO) sheet. The resulting immunosensor achieves a wider linear detection range 0.0125 to 15 pg/mL and a significantly lower detection limit calculated to be 4.17 fg/mL, enhancing its sensitivity for clinical applications. Furthermore, the immunosensor demonstrates excellent stability, repeatability, and reproducibility, contrasting with the hydrogel-based sensor's focus on antifouling in complex biological media.

Although CP-based sensors conjugated with either DNA or antibodies are sensitive, they have several disadvantages, such as high cost and stability issues. In contrast, ICPs allow for overcoming these issues. For example, recent work by Maleeha Saeed et al. reports a novel approach using molecularly imprinted polythiophene (miPTh) nanofibers combined with graphitic carbon nitride (g-C3N4) nanosheets [6]. This hybrid sensor demonstrates excellent sensitivity and selectivity for creatinine detection, with a sub-nanomolar detection limit and a high recovery rate in human saliva samples. Overall, synthetic recognition sites in ICP-based sensors demonstrate similar sensitivity and selectivity to biological recognition sites but are more cost-effective and stable.

2.2. Biosensors based on printing technology

The recent advancement of the printing process has been used in biosensing thanks to advancements in current technology and printing devices [38–40]. Regarded as a straightforward and fundamental technique, it allows for the simultaneous, large-scale, and slightly inexpensive fabrication of planar or flexible electrodes. Printing the required biosensor on various surfaces, such as polymeric materials, semiconductors, paper, textile materials, etc., provides a portable biosensor and allows for the integration of smart electrical components [41].

2.2.1. Screen-printed electrode (SPE)

The screen-printing approach is one of the many printing techniques that is widely employed in biosensors because of its merits, which include easy fabrication, cost-effective large-scale production, and superior electrochemical properties [42,43]. Several studies have been conducted using screen printing techniques for biosensor applications up to date [44,45]. Technology for screen printing may expand to develop adaptable electrodes on flexible surfaces, such as paper or other besides the rigid substrates that are usually employed to make SPEs. This would enable the development of inexpensive, and disposable biosensors. For example, a potentiometric and enzyme-free poly(3-aminophenyl boronic acid-co-3-alkylthiophene) was used to fabricate the glucose biosensor [46]. The octyl thiophene group working as a separate layer and the phenylboronic group as a multifaceted layer were used to describe glycose sensing. Similarly, utilizing poly(terthiophene benzoic acid) (PTTBA) as the recognizing component, a reusable finger prick blood biosensor-based amperometry glycated hemoglobin (HbA1C) was described [47]. A PTTBA was electrodeposited onto a screen-printed electrode decorated with Au NP to construct the biosensor. Aminophenyl boronic acid (APBA) was then chemically attached to PTTBA to function as an anchor for the recognition of HbA1C.

To develop an electrochemical biosensor that can detect many neurotransmitters simultaneously, including uric acid (UA), dopamine (DA), and ascorbic acid (AA), the 1-butyl-3-methylimidazolium chloride ionic liquid (IL) was spin-coated onto an electrodeposited PEDOT-coated SPCE [48]. Owing to the unique interaction between PEDOT and IL, the distinct pulse voltametric tests using the PEDOT/IL demonstrated excellent stability and electrocatalytic performance if compared with those using the SPCE/IL. PEDOT-having polydopamine (PDA) was used to determine DA selectively. The mechanisms for the high selectivity have been attributed to the hydrophobic reaction of the PEDOT-PDA with DA and the electrostatic reaction with the other compounds, such as AA or UA [49].

2.2.2. Inkjet printing technology

The advantages of digital printing using inkjet technology include rapid printing, excellent quality, affordability, adaptability, less waste products and contamination [38]. Since the shape of it is drawn using computer software and is more controllable because of the simplicity of the deposition technique than screen printing [50]. For example, a biotin-polythiophene films were printed on gold electrodes to work as a biosensing area for the streptavidin target [51]. The results show that the streptavidin and tetraethylene-glycol polythiophene (TEGPT) robust supramolecular biosensing films were effectively generated by inkjet printing on a paper-based ultrathin gold film (UTGF) (Fig. 2). While the strong affinity between biotin and streptavidin is responsible for the reliability of the streptavidin film, the excellent thiophene's affinity to UTGF is likely ascribed to the chemical bond between sulfur and gold.

Fluorescent ICPTs for straightforward protein biomarkers detection using optical signals are investigated in diagnostic testing. For instance, Tawfik and coworkers reported the nontoxic imprinted polythiophenes for specific biosensing of AFP biomarker in human serum [18]. By modifying the polythiophene structure, the emission characteristics of the polythiophenes can be improved. These ICPTs can be used to fabricate a point-of-care paper test device for diagnosis by printing them using inkjet technology on filter paper (Fig. 3).

2.3. Biosensors based on microfluidics devices

Microfluidic is a type of technology that involves the precise control and manipulation of small volumes of fluids through micro-scale channels and devices. The integration of such technology in a micro-scale sensor device, also referred to as a "lab-on-a-chip" (LOC) technique, enables healthcare monitoring, including preparation, reaction, segregation, and evaluation of samples [52,53]. Portable, minimal sample consumption, and simple-to-apply are some of its advantages. The integration of microfluidics with its numerous advantages into sensors makes them highly suitable for performing point-of-care and on-site analysis [54]. For example, an optical microfluidic biosensor for the multiple detection of various protein biomarkers in saliva was developed in 2017 [55]. The validation studies demonstrated a strong relationship with ELISA; however, the observed higher results may have originated from variations in the viscosity of the real saliva and artificial saliva used for calibration. The LOD for interleukin 8 (IL-8), interleukin-1 beta (IL-1β), and matrix metalloproteinase-8 (MMP-8) were also shown by the authors to be between 80 and 120 pg/mL. The device is

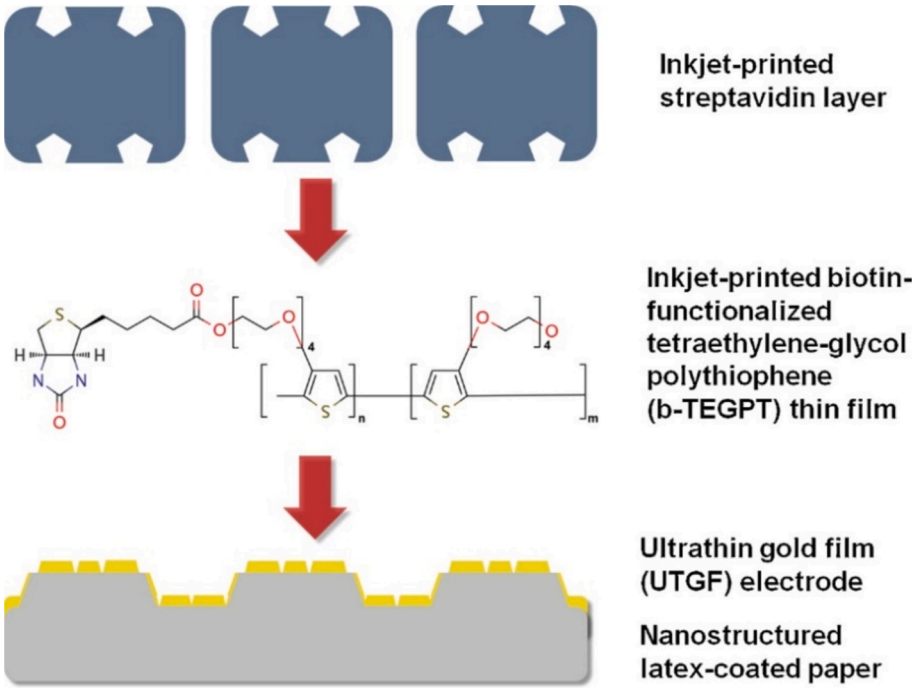

Fig. 2. An illustration showing the various components and production processes of bio-detection devices using inkjet printing. Reprinted with permission from Ref. [51].

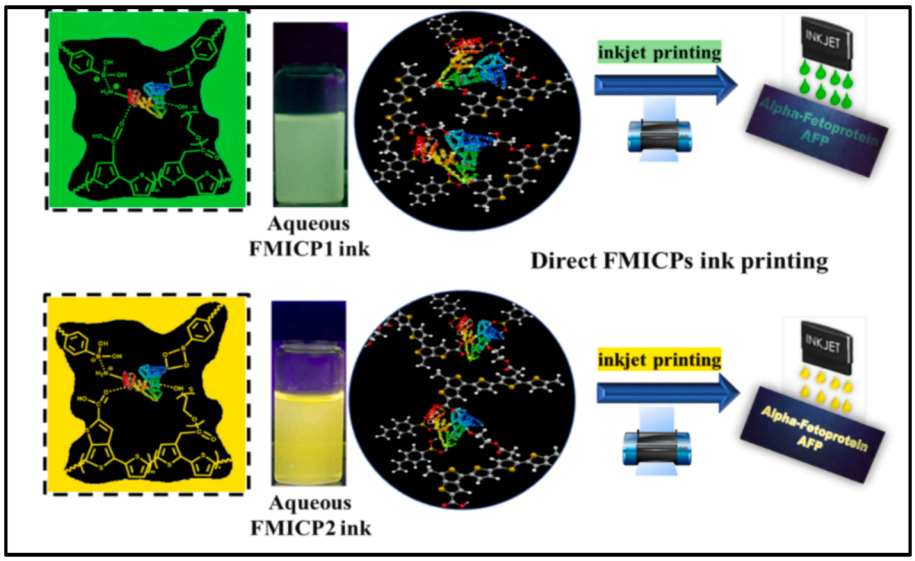

Fig. 3. Preparation of α-fetoprotein biosensors using inkjet-printed paper assay Reprinted with permission from Ref. [18].

appropriate for the early detection of oral cancer. Among them, the LOD of IL-8 was significantly below the medical level (600 pg/mL).

Another investigation established an innovative, enzyme-free, inexpensive, and user-friendly technique employing FMICPs to detect CEA and AFP biomarkers [18]. Here, they combined the FMICP1 and FMICP2 microfluidic paper layers to construct FMICP1-μPAD and FMICP2-μPAD microfluidics for AFP detection in the saliva samples. The paper-based microfluidics is constructed from layered sheets of paper with a wax pattern serving as a barrier to produce circular zones composed of hydrophobic and hydrophilic (Fig. 4). Three zones compose each device: the sample injection zone, the buffer loading zone, and the detecting zone. The detection zone was deposited with FMICP1/FMICP2, while the buffer loading zone was filled with PBS. The three layers were assembled and stuck together using a conventional laminator. A portable prototype device was also developed to measure RGB colors, combining two filters, a very sensitive photodiode, and UV LEDs (365 nm). Utilizing FMICP1-μPAD and FMICP2-μPAD, the estimated LODs for AFP were 1.04 pg/mL and 1.10 pg/mL, respectively.

In a another study [12], quantum dots/conjugated polythiophene (CdTe/CP) hybrids based on a fluorescent μPAD have been employed to detect acetylcholinesterase (AChE). This is achieved by triggering the emission of the CdTe/CP hybrids through the interactions of CP with thiocholine generated by ATCh hydrolysis and aggregation-induced emission enhancement (AIEE). The μPAD system possesses four distinct zones (sample, substrate, buffer, and detection). A 365-nm UV light can be utilized for recognizing variations in color intensity as a response to the injection of various AChE concentrations into the μPAD. The change in the emission intensity varies with the concentration of

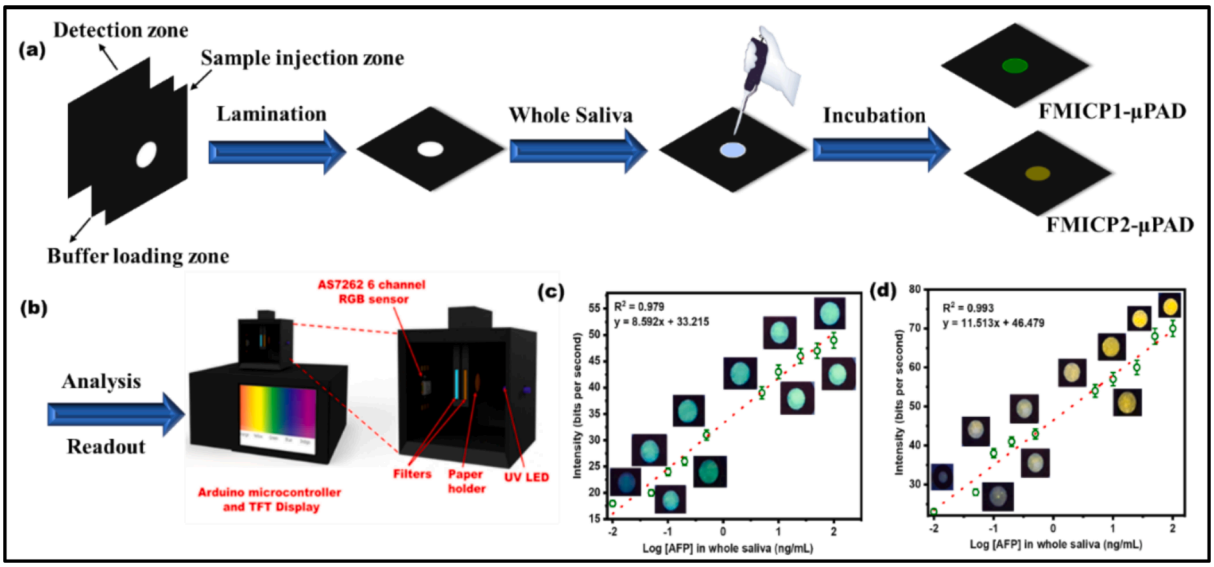

Fig. 4. (a) Test protocol and microfluidics development. (b) An inexpensive and portable instrument for fast AFP diagnosis. (c, d) The linear correlation between the concentration of AFP and the intensity of color throughout the photodiode. Reprinted with permission from Ref. [18].

AChE. Upon the injection of AChE into the detection zone at concentrations ranging from 0 to 0.1000 U/mL, the color intensity of the CdTe/CP progressively enhanced. Interestingly, when AChE was injected into the detection zone at a higher level (> 0.1000 U/mL), a reverse effect was observed; the color intensity was quenched as the amount of AChE increased. The color in the sample injection zone on the μPAD system was captured using a smartphone camera and analyzed.

2.4. Biosensors based on organic thin film transistors

In recent years, organic thin-film transistors (OTFT) have been utilized in the field of biosensing [24]. OTFT, like the traditional field effect transformer, comprises semiconductor layers and three electrodes (source, gate, and drain). The semiconductor could be regulated by manipulating the gate voltage, thereby altering the current flowing between the source and drain electrodes [56]. OTFTs can significantly enhance the signal generated by the interaction between the biomaterials and the target, effectively converting it into an electrical signal. OTFTs have gained significant attention for analyzing biomolecules in biological fluids, such as saliva, due to their advantageous features, including excellent flexibility, cost-effectiveness, easy-to-prepare, and diverse fabrication approaches [57].

Polythiophenes are commonly employed in fabricating organic field-effect transistor (OFET) biosensor devices because of their superior performance and ability to interact with receptors. An example of this is the DNA sensor that has been developed, which utilizes an OFET and active P3HT nanochannel [58]. The DNA biosensors were conjugated to the P3HT sensor through covalent bonding. The output currents of the OFET device exhibited a distinct disparity following the DNA hybridization processes. The researchers study the enhancement of the Debye length and reduce the off-current during DNA hybridization. Additionally, this device can operate at extremely low voltages (less than 1 V) due to forming an electric double layer. However, developing CP-based OFET biosensors remains challenging, as CPs tend to deteriorate upon contact with moisture. Addressing this issue is crucial for enhancing the durability and reliability of CP-based OFET biosensors in practical applications. Organic electrochemical transistors (OECTs) are an exciting alternative with improved stability and sensitivity. They could detect multiple targets, such as ions, small molecules, biomolecules, and viruses. One of the main advantages of OECTs is their simplicity of integration into wearable and flexible devices for on-the-spot diagnostics and monitoring. An instance of this is the development of an OECT biosensor reported by Y. Miyahara team [59]. The biosensor utilizes a PEDOT: PSS conjugated to trisaccharide composed of Sia-α2,6′-Gal-Glu to detect the human influenza A virus with exceptional sensitivity and selectivity in aqueous environments. This biosensor system could detect influenza A virus antigens with label-free identification and selection. The drain current of the OECT was altered following the adsorption of the virus onto the channel. The mechanism was hypothesized to be influenced by the doping effect. The findings demonstrated that the OECT biosensor exhibited superior efficacy in detecting the influenza virus compared to commercial techniques in a short timeframe. Additionally, due to its low power consumption and printing processability, the OECT device may have been incorporated into the wearable system designed to monitor the influenza virus.

In another study [60], a PEDOT:PSS OECT was incorporated into an easy-to-operate microfluidic platform to create a DNA biosensor that is highly sensitive and does not require labeling. The OECT was fabricated on a PET substrate, and a PDMS microfluidic device was attached to the upper surface of the device (Fig. 5**a**). The OECT-microfluidic demonstrated exceptional stability and reliability, allowing all sides to be effortlessly bent without compromising charge transport features (Fig. 5**b**). Moreover, integration into the microfluidic channel significantly increased the analytical performance by enhancing the LOD of this OECT device from 1 nM to 10 pM, enabling it to detect DNA targets at these concentrations. In another research, Fu's team has established a highly sensitive RNA biosensor using a smartphone-OECT system. This biosensor can detect viruses and diseases at an early stage [61]. The portable sensor consists of three primary components: a miRNA OECT biosensor, a meter-readout circuit, and a smartphone, as shown in Fig. 5**c**. This biosensor was remotely operated through a smartphone using Bluetooth communication. The gate of the biosensor was altered and treated with miRNA, and DNA biomolecules. This modification process was facilitated by the presence of H_2O_2 in the aqueous solution, as shown in Fig. 5**d**. The overall schematic illustration of a gate modification procedure is presented in Fig. 5**e**. The devices exhibited excellent sensitivity in detecting miRNA cancer biomarkers, even in small sample volumes and at low concentrations, thanks to their inherent amplification capability.

PEDOT:PSS OECTs have also been successfully applied for sensing sodium ions via synthesized MIPs by incorporating sodium ions as templates into PEDOT:PSS [62]. These MIPs were used as the channel active layer material OECTs for the specific sensing of sodium ions. Because of the specificity of MIPs, their functions as a membrane that

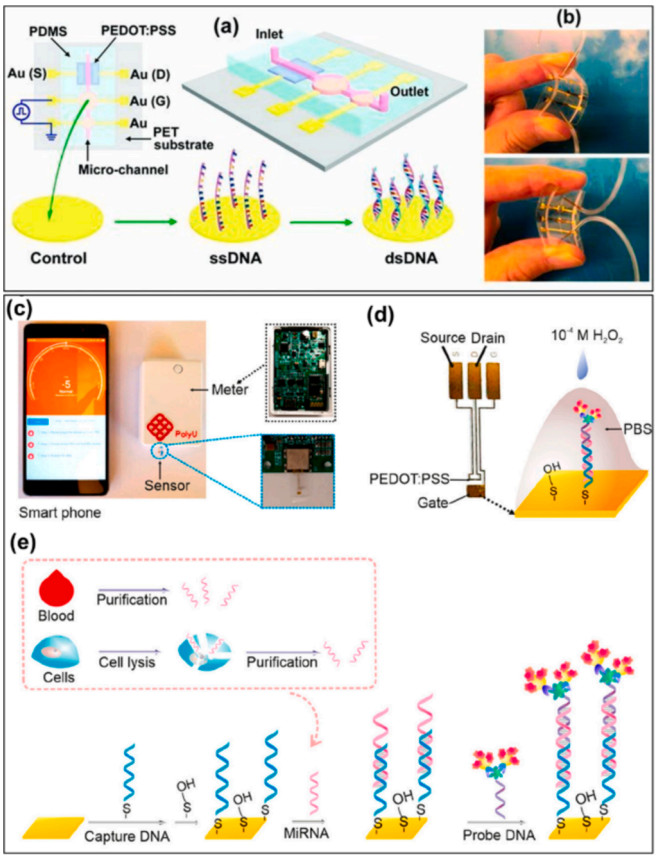

Fig. 5. (a) A simplified representation of an OECT integrated into an adaptable microfluidic platform for DNA recognition. (b) An ideal representation of the versatile biosensor [60]. (c) The OECT-based smartphone and Bluetooth. (d) A photo of an OECT miRNA biosensor with three electrodes. (e) Example of a gate modification procedure. Reprinted with permission from Ref. [61].

selectively allows certain ions to pass through while blocking others, even when the concentration of interfering ions is below 1 mM. The utilization of MIPs effectively addresses the issue of insufficient selectivity towards electrolyte ions in previous OECTs.

The integration of imprinted technology and FET can also be valuable for sensing HSA biomarker [63]. An inverse opal polythiophene was synthesized on SiO_2 nanoparticles through electro-polymerization, using a semi-covalent imprinted approach. The FET-sensor demonstrated the capability to detect HSA at levels as low as sub-clinical femtomolar concentrations. This offers an alternative to the current gold standard method, which necessitates the extraction of blood from patients. An albumin test is typically not performed independently but rather as part of a comprehensive analysis of a patient's blood sample. Consequently, conducting a standalone albumin test does not hold commercial appeal. Nevertheless, the study offers compelling evidence for the identification of proteins in blood and urine, and the potential to present a commercially viable alternative to conventional laboratory tests.

2.5. Biosensors based on colorimetric paper strips and smartphone

Paper substrate is a substance most frequently used in everyday activities and due to its merits regarding excellent accessibility, hydrophilic nature, low toxicity, and versatility, it is used in biosensing applications [64]. These features align well with the requirements of point-of-care testing in an aging society [65]. Within this biosensor, the fluorescent materials that have been pre-loaded onto the paper device can interact with the target analytes, thereby causing a noticeable alteration in the color of the paper. The amount of the analyte will be calculated using colorimetric analysis, which relies on the intensity of color. However, the sensing process becomes more complicated and stationary when a computer is needed to analyze images. With the advancement of technology, smartphones are becoming portable equipped with cameras that enable the on-site analysis of colorimetric paper strips [66]. The biosensing procedures can be conducted at home using smartphones by analyzing the color intensity of fluorescent spots or lines. This simple procedure enhances healthcare availability and accessibility.

Recently, a paper-coated MOFs biosensor for the 1-hydroxypyrene (1-HP) biomarker detection have been demonstrated [67]. The biosensors were prepared by integrating nonionic polythiophenes (PLQY up to 65 %) into MOFs to yield CP1-Eu-MOF and CP2-Eu-MOF. To design a simple and portable biosensor, 1-HP paper-based biosensors were fabricated by applying a layer of CP1-Eu-MOF and CP2-Eu-MOF onto wax-printed paper. For visual detection purposes, various concentrations of c (0.05–10 nM) were applied to the modified paper. The addition of 1-HP resulted in a color change of the CP1-Eu-MOF and CP2-Eu-MOF paper sensors, which could be observed by exposing them to 256 nm UV light. Inspired by the noticeable changes in color on the paper, which indicate different levels of 1-HP in urine, a smartphone was employed to analyze the color images using the "PAD Analysis" application to calculate the RGB values (Fig. 6). The R/B ratio versus different concentrations of 1-HP was plotted to obtain curves that can be presented by the equations: $y = 0.785x + 1.064$ ($R^2 = 0.9912$) and $y = 0.892x + 1.2731$ ($R^2 = 0.9903$). The limits of detection for 1-HP using CP1-Eu-MOF and CP2-Eu-MOF biosensors were determined to be 38.21 pM and 33.63 pM, respectively.

3. Conjugated polythiophenes based biosensors

Thiophene polymers are a class of conjugated polymers known for their remarkable optical and electrochemical properties. These properties have made them of significant interest in various applications, including organic electronics, sensors, and photovoltaics. Recent advancements have paved the way for numerous exciting opportunities in both experimental and theoretical research, particularly concerning the application of polythiophene-based materials in various devices. These materials are highly regarded for their affordability, ease of solution processing, and tunable conductivity, which facilitate the creation of three-dimensional stacking devices. Notable applications include solar cells, field-effect transistors (FETs), light-emitting diodes (LEDs), hydrogen storage systems, water purification technologies, and DNA detection methods. Consequently, polythiophene-based materials present extensive potential for future study and practical implementation in various technological fields [68].

This review explores the unique optical and electrochemical characteristics of thiophene polymers, discussing their underlying mechanisms and practical implications in molecular imprinted technology.

3.1. Cpts immobilization properties

Thiophene polymers exhibit strong absorption in the visible region of the electromagnetic spectrum due to their conjugated π-electron systems. The extensive delocalization of π-electrons lowers the band gap, allowing for efficient absorption of visible light. This property is essential for applications in organic photovoltaics where light absorption is critical [69]. Upon excitation by light, thiophene polymers can emit fluorescence. The fluorescence properties, including emission wavelength and intensity, can be tuned by modifying the polymer structure [70]. For example, substituents on the thiophene ring can significantly alter the electronic properties and, consequently, the fluorescence behavior [71]. This tunability makes thiophene polymers attractive for use in organic light-emitting diodes (OLEDs) and fluorescence-based sensors.

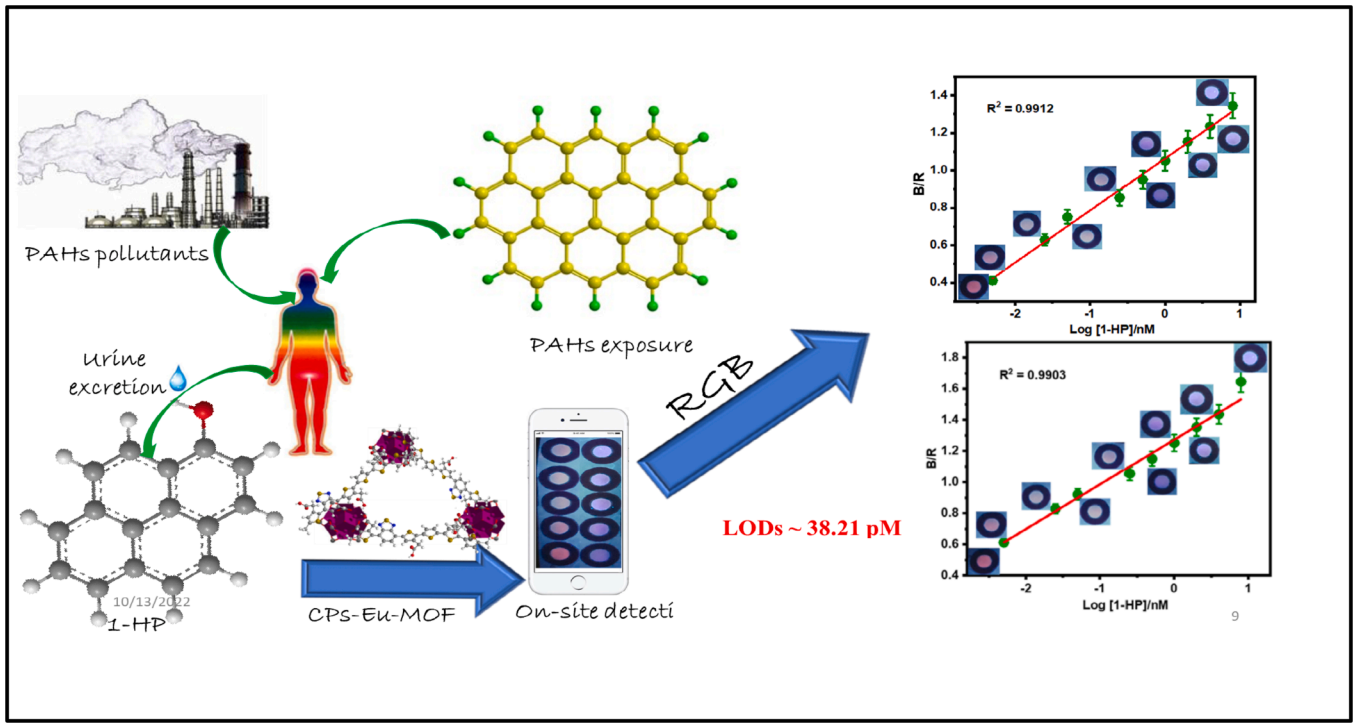

Fig. 6. Fabrication of paper biosensors coupled with a smartphone for 1-HP detection. Reprinted with permission from Ref. [67].

Thiophene polymers are known for their photostability, which refers to their ability to maintain optical properties under prolonged exposure to light [72]. This stability is crucial for practical applications where materials are exposed to light for extended periods, such as in solar cells and display technologies. Conjugated polythiophenes typically exhibit absorption and emission in the visible to near-infrared range. The absorption spectra of hybrid polythiophene derivatives with modified side chains show strong absorption bands between 350–380 nm. This corresponds to the π-π* electronic transition properties. Photoluminescence quantum yields (PLQYs) in water around 61–78 % have been reported [9].

Thiophene polymers are highly redox-active, meaning they can undergo reversible oxidation and reduction processes [73]. This redox activity is attributed to the conjugated π-electron system, which facilitates the transfer of electrons. The redox behavior of thiophene polymers can be exploited in various electrochemical applications, including batteries, supercapacitors, and electrochemical sensors. Upon doping, thiophene polymers can become highly conductive. Doping involves the introduction of charge carriers into the polymer, which enhances its electrical conductivity to 71.7 S/cm [74]. This property is particularly useful in applications such as organic field-effect transistors (OFETs) and conductive coatings. Interestingly, self-doping strategy is also well-known for thiophene polymers rendering them peculiar materials that synergistically improves the battery performance, ideally mitigating the dissolution and the reduction and oxidation peaks were observed at 1.17 V and 1.27 V [75]. Thiophene polymers exhibit good electrochemical stability, which is their ability to maintain performance over multiple redox cycles [76]. This stability is essential for the longevity and reliability of devices such as rechargeable batteries and electrochromic displays. The redox activity and conductivity of thiophene polymers make them suitable candidates for use in electrochemical sensors. These sensors can detect various analytes based on changes in the polymer's electrical properties in response to chemical interactions. As research continues to advance, the potential for thiophene polymers in new and emerging technologies remains promising, highlighting their importance in the field of materials science.

Thiophene-based conducting polymers are highly useful as substrates for the immobilization of biomolecules due to several key features: (i) Thiophene-based conducting polymers facilitate rapid electron transfer, which is essential for electrochemical biosensors. This property ensures efficient signal transduction when biomolecules are immobilized on these substrates [77]. (ii) These polymers can form strong covalent bonds with biomolecules through functional groups such as –COOH, $–NH_2$, and –SH, leading to stable and robust immobilization which enhances the durability and reliability of the biosensor system [78]. (iii) Their ability to provide a biocompatible environment that preserves the activity of immobilized biomolecules. Their stability under different conditions also makes them suitable for long-term applications [79]. A variety of biomolecules, including enzymes and antibodies, have been successfully immobilized onto functionalized poly (thiophene-pyrrole) matrices, demonstrating the versatility of these polymers for different applications [60,80].

The characteristics of conjugated polythiophenes (CPTs) are altered by the conjugation of functional groups at the thiophene. Thiophenes can undergo functionalization with a wide range of substituents that have distinct effects on electron withdrawal or donation, as well as varying sizes and shapes. This process leads to the production of substituted CPTs with diverse properties that differ from those of unsubstituted CPTs. CPTs that have been functionalized with cationic, anionic and zwitterionic groups have been employed in biosensor applications. Various water-soluble derivatives of CPTs have garnered interest as biosensor materials due to their beneficial characteristics, such as signal amplification and ease of production (Fig. 7) [81]. The observed optical responses to the targets were ascribed to the alterations in the structure or clustering of the conjugated backbone, resulting from the interaction with the targets.

3.2. Functionalized CPTs

The poly(3-(4-methyl-30-thienyloxy)propyl trimethyl ammonium) (PMTPA) was employed to detect cysteine (Cys) and homocysteine (Hcys) using fluorescence detection methods [82]. PMTPA exhibits low affinity for biomolecules including amino acids and peptides, due to physical interactions. The modification of Cys with 2-cyano-6-

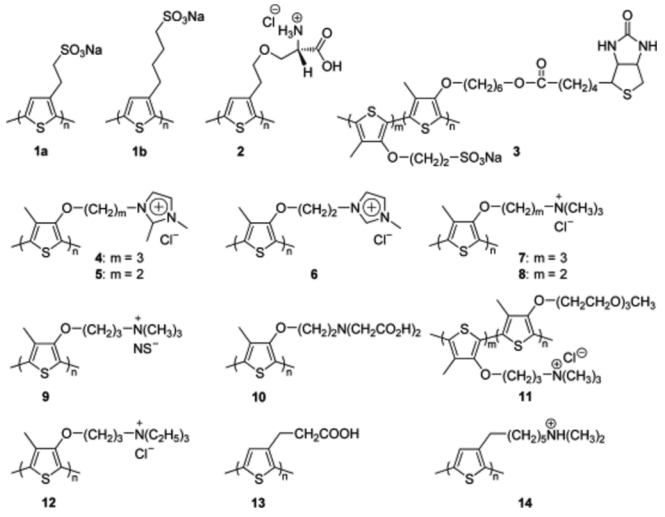

Fig. 7. Structures of some functionalized CPTs: sodium salts of poly(3-thiophene-β-ethanesulfonate) (1a), poly(3-(thiophene-δ-butanesulfonate) (1b), poly (3-[(S)-5-amino-5-carboxyl-3- oxapentyl]-2,5-thiophene hydrochloride) (2), biotinylated copolymer of water-soluble poly(3-alkoxy4-methylthiophenes) (3), a series of cationic water-soluble poly(3-alkoxy-4-methylthiophenes) (4–12), 2,5-poly(thiophene-3-propionic acid) (13), 2,5-poly (3-(6-N,N-dimethylhexylammonium thiophene) (14). Reprinted with permission from Ref. [81].

methoxybenzothiazole (CBT) was conducted to improve its interaction with Cys. The PMTPA solution, which was initially optically inactive, exhibited a significant optical emission in the π–π* transition area of PMTPA upon the addition of CBT. A sensor capable of distinguishing between 15 nucleotide-based phosphates (XNPs) was created utilizing PMTPA [83]. The distinct interaction of PMTPA with various XNPs was caused by modifications in the structure and the way PT backbones came together. Specifically, the creation of an organized PT phase was influenced by the ionic interaction between the ammonium groups on the CP and the phosphate units of the XNPs. In addition, the presence of numerous negative charges (triphosphate) and purine nucleotide moiety promoted the development of PMTPA particles. Nucleotide binding involves a distinct signal mechanism that relies on the interaction between hydrophilic and hydrophobic contacts, and the generation of PMTPA particles with varying chemical arrangement. The interactions between the ordered structures of functionalized PTs across different chains lead to the formation of tiny clusters. These clusters are responsible for spreading light across a wider spectrum and serve as the foundation for an alternative transduction process. In a another study, an optical biosensor was developed for folic acid utilizing PMTPA [84]. When folic acid binds to PMTPA, it causes PMTPA to adopt a flatter shape and generate stronger connections between its molecules through π–π interaction. Similarly, poly(1-ethyl-3-(2-((4-methylthiophen-3-yl)oxy) ethyl-1 h-imida-zol-3-ium bromide) (PEMTEI) was selectively confined to lysosomes and has been employed as an optical indicator to identify ATP in cells via Ca^{2+} controlled exocytosis triggered by conventional lysosomal medicines [85].

New water-soluble cationic polythiophene derivatives have been used by Mario Leclerc's group that facilitate the detection of oligonucleotide hybridization using a 20-mer capture probe. This method produces clear optical signals, either colorimetric or fluorometric, without needing chemical reactions between probes and analytes. It relies on electrostatic interactions and conformational differences between the cationic poly(3-alkoxy-4-methylthiophene)s and the nucleic acids, whether single-stranded or double-stranded [86].

In another work [87], a flow-through optical biosensor that detects microRNA (mir21) and hepatitis B virus DNA (HBV-DNA) in plasma have been developed. This biosensor avoids the need for sample pre-preparation and clean-up procedures. With a LOD of about 2 nM in plasma, the biosensor exhibits optical sensitivities at nanomolar levels of mir21 and HBV-DNA from 1 nM to 10 mM. The colorimetric biosensor platform has the potential to be employed for both point-of-care disease diagnostics and the detection of several viral biomarkers in plasma. These investigations' findings showed that polythiophenes and their derivatives are promising choices for the development of optical biosensors that may be used to quickly and easily identify virus biomarkers [88].

Recently, a study focused on cationic conjugated polymer (CCP)-based binding assays, utilizing the conformational changes in aptamers upon binding to their targets to induce fluorescence changes in CCPs. Poly(3-(3′-N,N,N-triethylamino-1′-propyloxy)-4-methyl-2,5-thiophene hydrochloride) (PMNT) was the model CCP used, with optimal buffer conditions close to physiological (100 mM NaCl, 10 mM $MgCl_2$). Four aptamers were characterized for K^+, adenosine, cortisol, and caffeine. For cortisol and caffeine, quantification was based on the reduction in 580 nm peak intensity, while for K^+ and adenosine, the fluorescence ratio at 580/530 nm was used. The longer stem-loop structure of the aptamers enhanced target binding and signal detection. The method demonstrated high specificity, and equal or superior sensitivity compared to SYBR Green I dye staining. This label-free, cost-effective, and quick-response assay is suitable for general aptamer binding evaluations and can function as a biosensor for target detection [89].

A novel multifunctional test and portable device for polythiophene-based fast detection of creatine kinase (CK), a commonly used biomarker for colorectal and cardiac cancer [90]. The cationic polythiophene (PMNT) exhibits remarkable visibility and can effectively detect CK based on unique structural, optical, and electrical activity. Without utilizing any antibodies or enzyme-coupled processes, which are commonly utilized in conventional approaches, fluorescence turns on the ability to recognize. This tactic demonstrates the promise of polythiophenes-based medical diagnostic tools for early disease detection and biological activity research (Fig. 8).

To detect protamine poly(2-(2-(4-methylthiophen-3-yloxy)ethyl) malonate acid) (PMTEMA) was synthesized [91]. Numerous electrostatic affinities between the positively charged protamine and PMTEMA generated a complex that changed the backbone shape of PT from a random coil to planar phase. The quenching of fluorescence and a shift in the color of solution resulted from these modifications. Using anionic poly[2-(3-thienyl) ethyloxy-4-butylsulfonate] (PTEBS) for colorimetric detection of H_2O_2 was studied. PTEBS can catalyze the reactivity of the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) because it has inherent peroxidase-like action [92]. Through electrostatic interactions, PTEBS captured TMB. The electron transfer from lone-pair radicals in the amino groups of TMB to the electrically delocalized backbone of PTEBS essentially causes an increase in electron density and mobility of PTEBS. As a non-oxidative voltametric glucose sensor, the APBA-functionalized poly-5,2′:5,2″-terthiophene-3′-carboxylic acid (PTTCA) was designed [93]. The amine portion of APBA and the –COOH group of PTTCA interacted to generate the biosensor through electro-polymerization of PTTCA and the tetrahedral boronate ester was formed.

A straightforward fluorescent biosensor based on zwitterionic conjugated polythiophene (ZCPT) for the detection of *Escherichia coli* (*E. coli*) have been developed [8]. Water-soluble thiophene monomers were oxidatively polymerized in water to generate the ZCPT biosensor. ZCPT facilitates the recognition of *E. coli* in milk and water samples, with strong repeatability (n = 3, [RSD] = 1.78 %) as well as excellent recoveries of 96.94–100.83 % and 93.27–116.75 %, respectively.

3.3. Biofunctionalized CPTs

It has been accomplished to biofunctionalize CPTs or modify their structures with biomolecules, to optimize their characteristics and investigate the possibilities for biosensor applications. In certain situations, covalently attaching a biomolecule or physically trapping

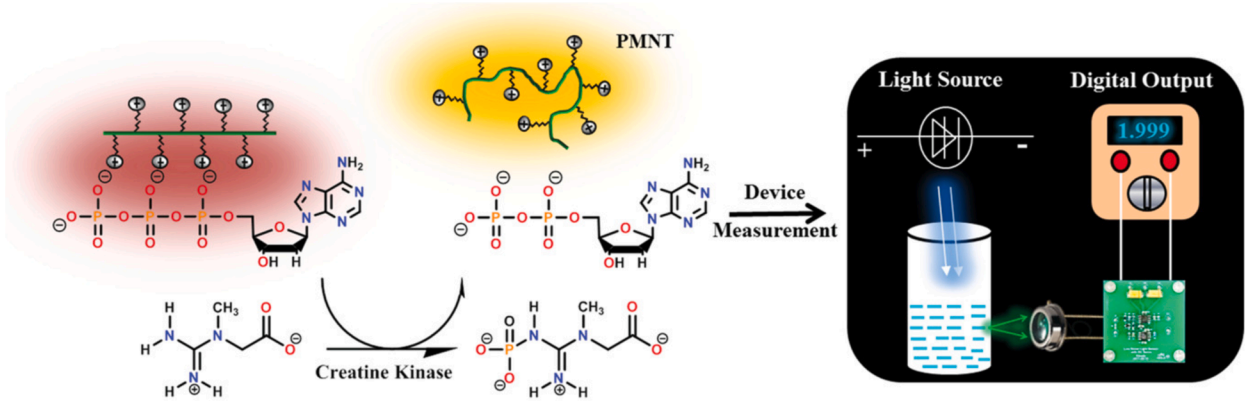

Fig. 8. Illustration of the various methods based cationic polythiophene PMNT to measure creatine kinase activity. Reprinted with permission from Ref. [90].

enzymes to the structure of CPT has been explored for biosensing enhancement. For instance, a poly(3-(3-bromopropoxy)-4-methylthiophene) modified with MB, was employed to detect ODNs hybridization [94]. The electrostatic interactions between PMT-MB and ODNs were responsible for the enhanced current signal of PMT-MB. Using a dendritic poly(propyleneimine)-polythiophene (G1PPT-co-PEDOT) and biotin–avidin complexes, an aptamer has been developed for the measurement of endocrine disrupting chemical (EnDC) 17β-estradiol [95]. The interaction of biosensor with 17β-estradiol was determined by observing a reduction in the voltammetry current after EnDC bound to the ssDNA aptamer. Using the PEDOT-organic electrochemical transistor, a DNA biosensor was fabricated by immobilizing ssDNA sensors on the Au gate electrode's surface [60]. The detection mechanism was identified as the adsorption of DNA biomolecules. The LOD of the organic transistor was enhanced to 10 pM, allowing it to recognize DNA targets at levels as low as 1 nM. Similarity, using the glycol-surface chemistry of the PT containing quinone units, the PT with carbonate units were functionalized and employed as label-free biomarkers for the recognition of bacteria (Fig. 9) [96]. The polyvalent coupling of quinone groups to carbohydrates and the electrochemical absorption of PT units improved the sensitivity and precision of the biosensor.

3.4. Hybrid structures of CPTs

An electrochemical paper biosensor-based PEDOT: polystyrene sulfonate (PSS) and Fe_2O_3 nanocomposite for CEA sensing was developed [97]. In this instance, the anti-CEA antibody could bind to active parts of the nanocomposites. Due to the combination of Fe_2O_3 NPs and PEDOT/PSS composite, the CEA biosensor showed an improvement in analytical performance. Aptasensor utilizing PEDOT: PSS-coated paper was fabricated for CEA detection. This CEA biosensor demonstrated exceptional linearity throughout a broad range of 0.76–14 ng/mL with an LOD of

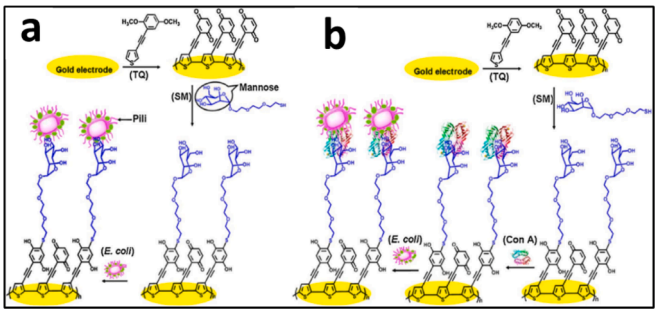

Fig. 9. (a) A fast E. coli biosensing using pili-mannose affinity. (b) Con A-mediated E. coli biosensing utilizing LPS-Mannose interaction. Reprinted with permission from Ref. [96].

0.45 ng/mL. This low-cost and reusable paper biosensor showed possibilities for application as an early cancer detection point-of-care (POC) tool [98]. Similarly, the CS-AuNPs-PEDOT-PB biosensor for CEA detection was recently published [99]. It was found that the conductive PEDOT improved Prussian blue's electrochemical response and durability in the system. In this research, the LOD of 5.05×10^{-5} pg/mL and the broad linear ranges from 1.0×10^{-4} to 1.0×10^{3} pg/mL were achieved.

The detection of spermine and heparin using two turn-off–on fluorescent biosensors, P1QD and P2QD, constructed from polythiophenes/CdTe hybrids, with LOD of 1.66 nM and 0.88 nM, respectively, have been established [7] (Fig. 10). Because of their surface passivation, the CdTe QDs exhibited enhanced emission after coating with amphiphilic polythiophenes. Following the addition of heparin, the P1QD and P2QD assemble over the heparin, resulting in a turn-off mechanism. This was caused by both heparin's hydrogen bonding formation with the NH and OH groups of the polythiophenes existing on the P1QD and P2QD surface and the effective electrostatic interaction between anionic heparin and the positive charge on the P1QD and P2QD surface. Furthermore, the strong electrostatic interaction between spermine and heparin makes it possible for the QD-heparin complexes, which in turn allows for the fluorescence recovery of the coated CdTe QD with the introduction of cationic spermine to the system.

In a another study AuNPs and the conductive poly (3, 4-ethylene dioxythiophene) (PEDOT) were immobilized using 3D graphene aerogel [100]. With the incorporation of graphene aerogel, exceptional physical and electrochemical characteristics of PEDOT are prevented from aggregating polymers and generating conductivity reduction. The resultant composite immobilizes biomolecule with its huge surface area and significant conductive properties. Under ideal conditions, this biosensor captured PSA with a LOD of 0.03 pg/mL and linearity of 0.0001–50 ng/mL. This biosensor retained 86.56 % of its initial value after being stored for 14 days.

Two thiophene monomers to use as a functionalizing agents for $NaLuF_4$:Yb^{3+}/Er^{3+} UCNPs were synthesized via *in-situ* polymerization [101]. This material was employed as an optical biosensor to detect Alprenolol (1-(o-allylphenoxy)-3-(isopropylamino)-2-propanol), a drug that is frequently employed for the treatment of high blood pressure. The biosensor exhibits satisfactory analysis efficiency, with a low limit of detection (LOD = 0.22 nM) and acceptable linearity within the 0.5 nM to 75 μM range. Additionally, with good recoveries of 97–94 %, this biosensor was effectively used to measure the amount of Alprenolol in human serum and urine samples.

In a recent study, blue graphene quantum dots (GQD) acted as a pH-sensitive method for detecting cancer cells. Hydrophilic polythiophene worked as the donor in these donor–acceptor energy transfer-based nanoparticles. This technology might be used to produce broad-range, customizable GQD-loading contents and fast acid-triggered

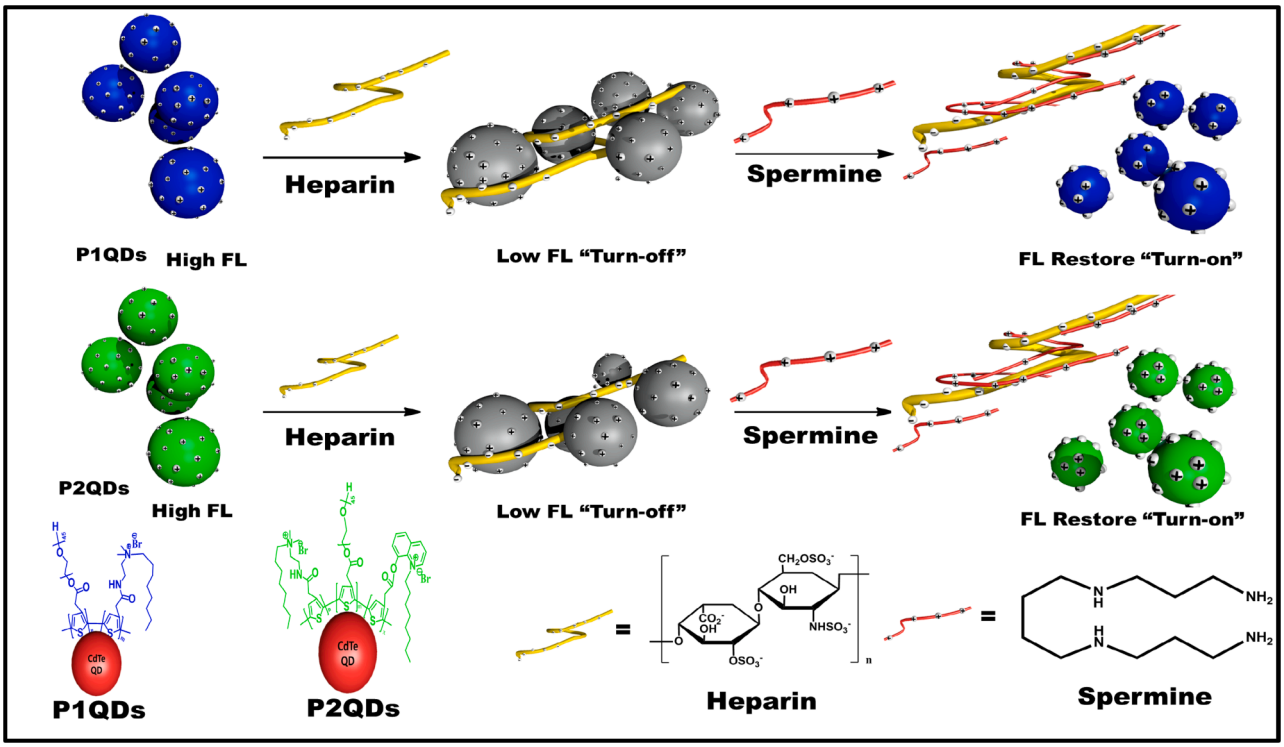

Fig. 10. Principle of heparin and spermine detection using polythiophene caped CdTe QDs. Reprinted with permission from Ref. [7].

fluorescence activity under acidic pH, which could be used for the quick detection of cancer cells [102].

4. Imprinted conjugated polythiophenes-based biosensors (ICPTs)

Within the realm of nature, the process of molecular recognition is predominantly steered by many different non-covalent interactions, encompassing hydrogen bonding, ionic interactions, hydrophobic features, and van der Waals forces. Identification of molecules is contingent upon the establishment of a thermodynamically advantageous combination between a building block and receptor that is based on a stereochemical and electrostatic relationship. The conceptual framework of "lock and key, "originally formulated by Emil Fischer over a century ago, aptly characterizes this intricate molecular interplay, elucidating the precise molecular inherent in recognition processes. Concerning MIPs, the "lock" is meticulously engineered through the copolymerization of monomers and a crosslinker within a carefully chosen solvent. This controlled synthesis occurs concomitantly with the introduction of the analyte, designated as the "key," imparting specificity to the molecular imprint. Subsequently, the template is extracted from the resultant polymeric layer utilizing an appropriate procedure.

Table 1
Analytical performance metrics of biosensors based on imprinted conjugated Polythiophenes.

Analyte	Method	Time	Linear range	LOD	Matrix	Reusability	Ref.
Creatinine	Electrochemical	NA	200–1000 nM	340 pM	saliva	14 days	[6]
Tumor factor-α	Field- transistor	NA	0.001–0.25 ng/mL	0.55 pg/mL	serum	six cycles	[129]
Chlorpyrifos	Electrochemical		0.02 to 1000 nM	4.0 pM	tap water	5 cycles	[130]
Ethionamide	Electrochemical	25 min	0.04 to 0.1 mM	0.005 μg/mL	ETA tablet	five consecutive measurements	[131]
Staphylococcus	Electrochemical	10 min	10 to 10^8 CFU/mL	2 CFU/mL	milk	13 times	[123]
Dopamine	Electrochemical	NA	0.05 μM to 250 μM	20 nM	NA	14 days	[132]
Matrix metalloproteina	Electrochemical	10 min	1.0 pg/mL up to 10.0 pg/mL	1.0 fg/mL	A549 cancer culture	6 cycles	[128]
Ovalbumin	Electrochemical	10 min	0.001–100 ng/mL	0.82 pg/mL	egg	15 days	[133]
Acrylamide	Electrochemical	14 min	0.08–100 ng/mL	5.1 pg/mL	food	15 days	[134]
Tyramine	Electrochemical	NA	290 μM to 2.64 mM	159 μM	food	NA	[135]
α-fetoprotein	Fluorescence	15 min	0.001–25 ng/mL	15 fg/mL	serum	90 days	[18]
Carcinoembryonic antigen	Fluorescence	15 min	0.005–15 ng/mL	3.5 fg/mL	serum	90 days	[18]
Cysteine/homocysteine	Colorimetric and fluorescence	NA	0.05—0.25 mM	0.1 nM	aqueous media	NA	[82]
Protamines	Colorimetric and fluorescence	NA	0.1 to 30 μg/mL	10^{-7} g/m	serum	NA	[91]
Cholinesterase	Fluorescence	30 min	0.001–0.0023 U/mL	0.14 U/L	serum		[12]
Escherichia coli	Fluorescence	15 min	1.15×10^8 –1.15×10^9 cfu/mL	NA	tap water and milk	NA	[8]
Spermine	Fluorescence	15 min	1–12 μM	1.66 nM	serum	90 days	[7]
Heparin	Fluorescence	15 min	1–10 μM	0.88 nM	serum	90 days	[7]

This process leaves a distinctive binding template that mirror the analyte regarding structure, dimensions, and functional units. The resulting three-dimensional polymeric structure is finely tuned to recognize and selectively bind the target analyte due to the tailored imprinted process. Thus, these materials can mimic and consequently function as substitutes for natural antibodies in applications such as adsorption, sensors, and medical devices [103,104]. Importantly, conjugated polymers including but not limited to polythiophene and poly(3,4-ethylenedioxythiophene) have considerable potential for various applications. The process of over oxidizing polythiophene serves to enhance the selectivity of MIPs constructed with polythiophene as the base material (Table 1). This capability makes polythiophene stand out as a highly fitting choice for constructing MIP-based structures with molecular imprints capable of accommodating analytes across a spectrum of molecular weights.

In this review we focused on the key aspects highlighting their potential: (i) Since thiophene polymers can be modified with a variety of substituents that influence electron-donating or electron-withdrawing capabilities and come in different sizes and shapes. This modification results in substituted conjugated polythiophenes (CPTs) that exhibit unique applications compared to either their unsubstituted counterparts or conventional polymers. Comparative characteristics of various biosensors utilizing CPs are illustrated in Table 2. Functionalizing CPTs with cationic, anionic, and zwitterionic groups has shown significant potential in molecularly imprinted polymers (MIPs) that enable the creation of highly specific and selective recognition sites that are complementary to the target molecules in shape, size, and functional groups. This specificity, which relies on a wide range of interactions [105], is crucial for the accurate detection of biological molecules in complex samples. (ii) The extreme conjugated nature of polythiophenes provides excellent electrical conductivity, which enhances the sensitivity of the biosensors. The imprinted sites within its polymer matrix facilitate the binding of target molecules, leading to measurable changes in the electrical properties of the material [106]. (iii) Thanks to functionalizing CPTs with cationic, anionic, and zwitterionic groups, ICPs can be tailored to detect a wide range of biomolecules, including proteins, nucleic acids, and small molecules. This versatility makes them suitable for various applications such as disease diagnostics, environmental monitoring, and food safety testing [107]. (iv) ICPs exhibit high stability under different environmental conditions, and their robustness allows for multiple uses without significant loss of sensitivity or selectivity. This makes them cost-effective and practical for long-term applications [108]. Comprehensively, the combination of molecular imprinted with conjugated polythiophenes offers a powerful approach for the development of next-generation biosensors with high specificity, sensitivity, and stability.

Achieving signal transduction is notably enhanced through the adoption of conducting polymers (CPs). The synergistic integration of a MIP with a CP strategically leverages the unique properties inherent in each material. The dual goals of achieving both high specificity and sensitivity in a biomimetic sensor are met through the integration of molecularly imprinted polymers with CPs. The subsequent paragraphs detail how imprinted conjugated polythiophenes (ICPTs) can be employed to fabricate materials acting as transducers, facilitating selective recognition in real-time biosensing applications. Additionally, the application of theoretical calculation methods in the design of MIPs has enabled the anticipation of optimum monomer selection, and the affinity between monomers and templates [109].

Due to the conjugated backbones, the ICPs exhibit excellent electrical conductivity, which enhances the sensitivity of biosensors and other electronic devices. On the other hand, conventional imprinted polymers typically do not possess intrinsic conductivity, limiting their application in electronic sensing without additional conductive components [110]. This conjugated nature allows for better signal

Table 2
Comparative characteristics of electrochemical, fluorescence, and colorimetric biosensors utilizing conjugated polymers.

Sensing method	Recognition method	Polymer type	Target	Linear range	LOD	Ref.
Electrochemical	Molecular imprinting	Polythiophene	Creatinine	200–1000 nM	340 pM	[6]
	Molecular imprinting	Thiophene- carbazole	Cilostazol	50–923.6 nM	15 nM	[136]
	Molecular imprinting	Polythiophene	Ethionamide	0.04—0.1 nM	0.005 μg/mL	[131]
	Sandwich enzyme-linked immunosorbent	Polythiophene derivative	Resistin	0.0125–15 pg/mL	4.17 fg/mL	[36]
	Sandwich enzyme-linked immunosorbent	Polythiophene derivative	Lung carcinoma biomarker	0.03–90 pg/mL	4.7 fg/mL	[37]
	DNA recognition	PANI/PA	microRNA24	1.0 fM-1 pM	0.34 fM	[35]
	DNA recognition	PFBT-COOH	microRNA155	10 aM to 5 pM	3.3 aM	
	Immunoassay	PPV derivative	AFP	0.1–1 × 10^2 ng/mL	0.03 ng/mL	[137]
	DNA recognition	PFN	SARS-CoV-2 RdRp gene	100 aM-100 pM	39 aM	[138]
Fluorescence	Electrostatic forces, hydrophobic interactions, and hydrogen bonding	Tetraphenyl-ethylene derivatives	Pathogenic microbes	NA	NA	[139]
	Electrostatic and hydrophobic interaction	cationic polythiophene	ATP	0 to 3 μM	90 nM	[140]
	Electrostatic and hydrophobic interaction	1,4-dithienylbenzothiadiazole (DBT)	HSA	NA	1.8 μg/mL	[141]
	Electrostatic interaction	PFP derivative	Thrombin	3—54 nmol/L	11 pM	[142]
	Electrostatic interaction	PFEP and fluorescein-HA complex	CD44	0 to 0.1 μg/mL	0.23 ng/mL	[143]
	Molecular imprinting	Polythiophene derivative	AFP	0.001 to 25 ng/mL	15 fg/mL	[18]
			CEA	0.001 to 200 ng/mL	3.5 fg/mL	
	NA	Poly(p-phenyleneethynylene)	Telomerase on live cells	NA	Three HeLa cells in 400 μL	[144]
	Electrostatic interaction	Polyfluorene derivative	E. coli	NA	NA	[145]
	Molecular interactions	PFBZ	Bilirubin	NA	6.9 pM	[146]
Colorimetric	DNA recognition	Polydiacetylenes	Bacillus thuringiensis	NA	3 × 0^7 CFU/mL	[147]
	Immunoassay	Polydiacetylenes	SARS-CoV-2 spike protein	1 to 100 ng/mL	1 ng/mL	[148]

transduction, providing higher sensitivity and more precise detection of target molecules [111]. Secondly, due to their functionalizing with diverse substituents, ICPs are considered suitable candidates for a wide range of applications including biosensors, environmental monitoring, and electronic devices due to their unique combination of electrical and chemical properties. While, Conventional imprinted polymers are primarily used in chemical sensing and separation technologies, with limited use in electronic applications [112]. On the other side, removing the template molecule from the conjugated polythiophene matrix without affecting the polymer's conductive properties can be challenging which is not such an issue for the conventional imprinted polymers [110]. Yet, conventional imprinted polymers are more easily scalable with well-established industrial processes [113].

Polythiophenes are particularly effective in this context due to their structural versatility. The various side substitutes on polythiophenes, which can either donate or withdraw electrons, along with the intrinsic properties of the thiophene ring, facilitate a wide range of non-covalent interactions. These interactions include hydrogen bonding, ionic interactions, dipole–dipole interactions, hydrophobic features, and van der Waals forces. This diversity in interaction capabilities makes polythiophenes a preferred alternative in the development of imprinted biosensors, allowing for greater specificity and binding affinity in the detection of target molecules.

4.1. *Icpts-based fluorescent biosensors*

ICPTs biomimetic sensor for the tobacco necrosis virus (TNV) on polythiophene nanofilms has been developed [114]. These nanofilms

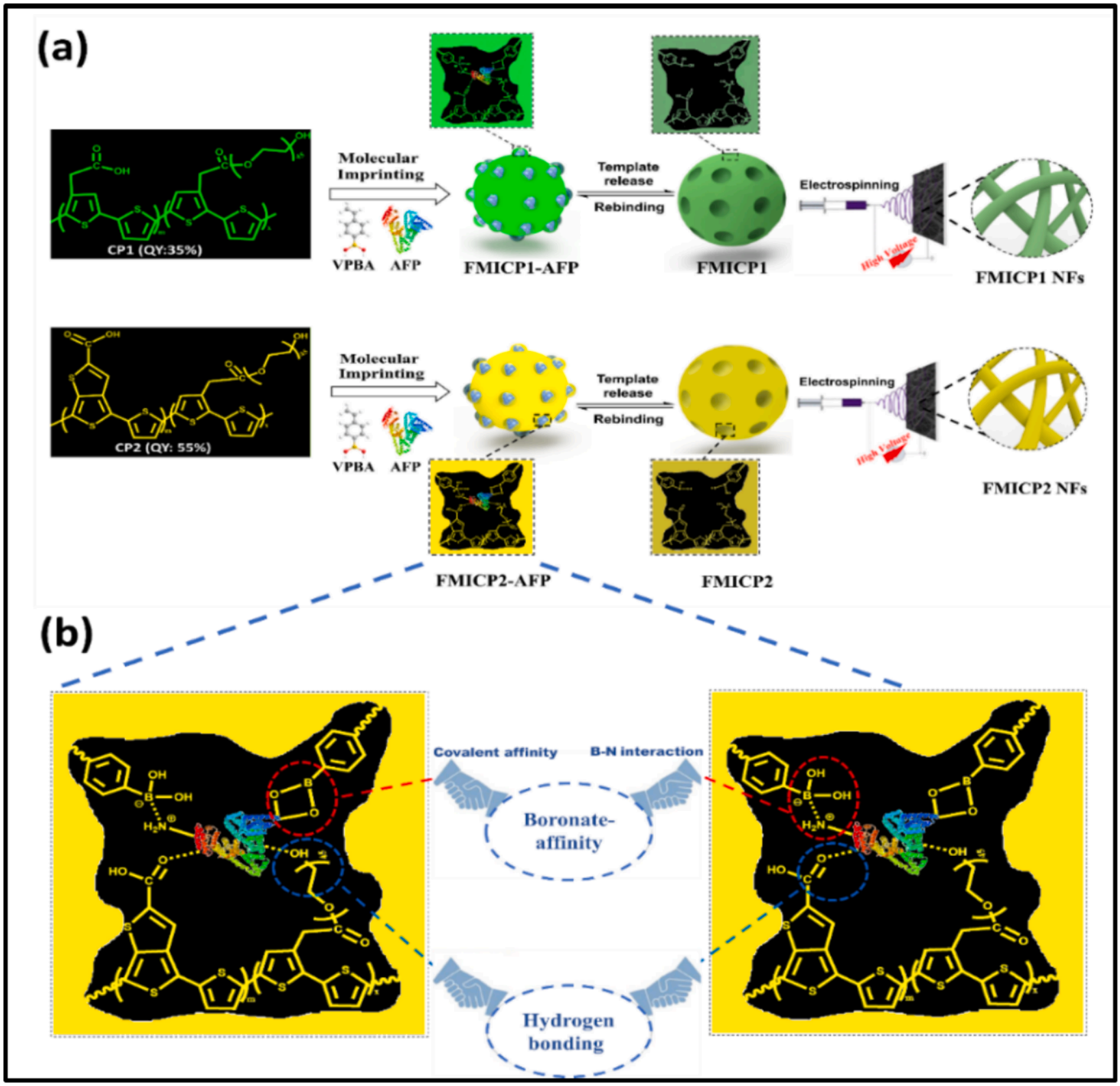

Fig. 11. (a) Synthesis procedures for FMICPs and their nanofibers structures. (b) Mechanism based boronate and hydrogen bonding interaction for AFP detection. Reprinted with permission from Ref. [18].

were electrochemically synthesized on conductive gold surfaces. The interaction between TNV and polythiophene led to changes in the fluorescence intensity of the nanofilm upon rebinding with the cavities within the polymeric matrix. The result demonstrated that the emission intensity at 410 nm was directly correlated to the concentration of TNV. The detection limit and liner range were determined to be 2.29 ng/L and 0.1–10 ng/L, respectively.

A pioneering and cost-effective assay-based fluorescent molecular imprinted conjugated polythiophenes (FMICPs) for detecting AFP and CEA proteins have been developed [18]. Notably, they introduced the electrospinning technique to prepare nanofibers of fluorescent molecularly imprinted conjugated polythiophenes (FMICP NFs) (Fig. 11.). The highly promising structure of conjugated polythiophenes, boasting a PLQY reaching 55 %, offers a straightforward and enzyme-free method. The unique FMICP NFs exhibit a sensitivity 80-fold greater than FMICPs. Both FMICP1 NF and FMICP2 NF showed LODs of 15 fg/mL and 3.5 fg/mL for AFP and CEA, respectively. Additionally, the recoveries of spiked serum fell within the ranges of 99.0 % to 101 % and 97.6 % to 110 %, respectively, showcasing exceptional accuracy. Furthermore, the developed biomimetic sensors proved effective in swiftly diagnosing AFP in serum samples. The results obtained with FMICP and FMICP NFs exhibited good concordance with clinical ELISA results.

In recent study, graphitic carbon nitride nanosheets (gCN) combined with nanofibers meticulously crafted from creatinine-imprinted polythiophene (miPTh) [6], showcases a harmonious integration of advanced materials for precise and efficient diagnostics. Examinations at the microscopic level unveil the porous nanofibrous of miPTh/gCN biomimetic. The incorporation of imprinted gCN materials significantly diminishes charge transfer resistance and enhances electron exchange at the interface between the electrolyte and nanozyme. The biomimetic sensor exhibits a linear range from 200 to 1000 nmol/L with sub-nanomolar LOD (340 pmol/ L), and exceptional specificity over commonly found salivary targets. To validate its practical applicability, the miPTh/gCN biomimetic sensor exhibits an outstanding 94.8 % recovery of added creatinine amount saliva. This reusable biomimetic sensor offers a significant potential for point-of-care (POC) tests that are accurate and efficient for healthcare.

4.2. Icpts-based electrochemical biosensors

An array of thiophene derivatives was employed to enable supplemental attraction with a designated analyte via the imprinted technique [115]. For instance, to detect melphalan, an ICPTs sensor was prepared by electropolymerizing 3-thiophene acetic acid (3-TAA) on the surface of a gold electrode for melphalan imprinted [116]. The interaction between melphalan molecule and the ICPTs involves non-covalent interaction with 3-TAA. Moreover, the exploration of employing modified monomers was also undertaken to enhance specificity against the target analyte in ICPTs. In the case of a folic acid target, the MIP was fabricated through potentiodynamic polymerization of a bisterthiophene-modified monomer, utilizing an acetonitrile solution of tetrabutylammonium hexafluorophosphate [117]. Following the preparation, the folic acid analyte was extracted using a suitable solvent. The good linearity of 0 – 100 μM and the detection limit of 15.4 μM were determined. Regrettably, the folic acid-imprinted polymer exhibited limited cross-selectivity against interferents like pteroic acid, caffeine, and theophylline. This suggests substantial cross-interaction between the template of the MIP and the interferent compounds.

The development of a ICPTs through *in-situ* electro-polymerization employing modified and crosslinking monomers, specifically terthiophene and carbazole was studied [118]. This process also included physical interactions with the naproxen, paracetamol, and theophylline

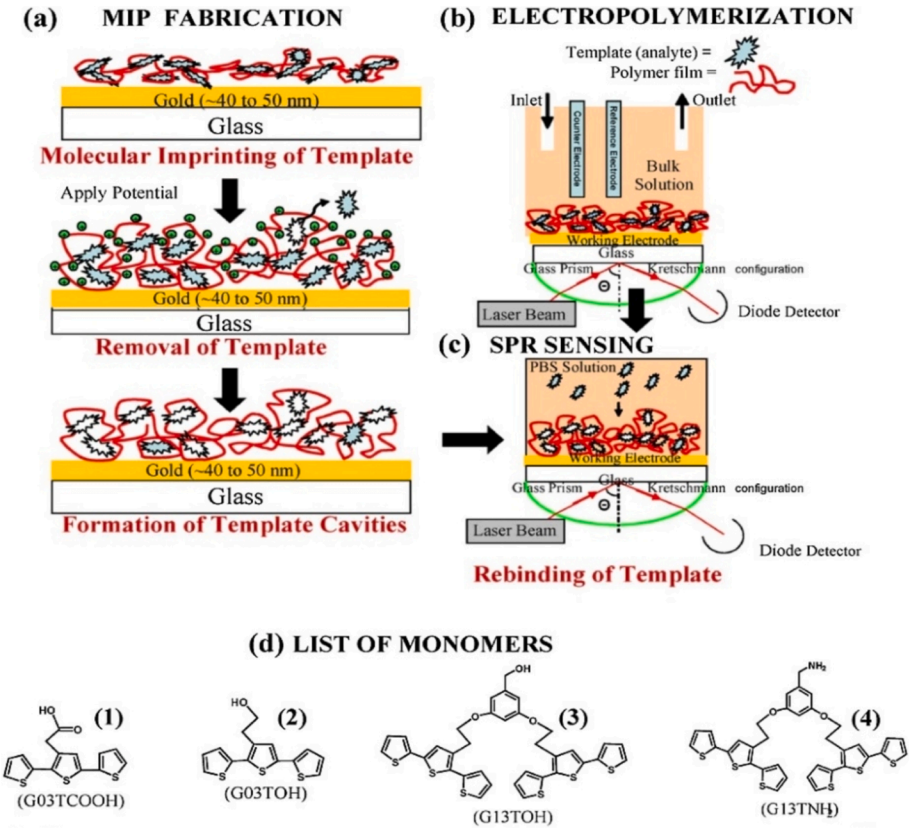

Fig. 12. (a) The process for developing biomimetic sensor films involves imprinted molecules and removing templates using a constant potential wash at 0.4 V (compared to Ag/AgCl). (b) Electropolymerization. (c) Detection of the imprinted host molecule. (d) Various poly(terthiophene) monomers, G0 3 T – COOH (1), G0 3 T – OH (2), G1 3 T – OH (3), and G1 3 T-NH_2 (4). Reprinted with permission from Ref. [118].

drugs (Fig. 12). Additionally, a comprehensive investigation of the terthiophene monomers was conducted to understand the formation of conjugated polymer network (CPN) films through quantitative electrochemical and electrochromic analyses.

A novel and reusable impedimetric monitoring system is proposed for the accurate and fast detection of harmful microorganisms, utilizing a microorganism-imprinted polythiophene layer (ICPTs). *Staphylococcus aureus* (*S. aureus*) is employed as an example microorganism in this study. The MIL detection layer is efficiently coated on a glassy electrode through an eco-friendly process via electro-polymerization of the *S. aureus* and TE monomer. The reassembling of *S. aureus* onto the MIL induces an increase in impedance. The ICPTs biomimetic sensor exhibits quantitative determination of *S. aureus* over a broad linear range from 10 to 107 CFU/mL, and very low LOD of 4 CFU/mL. Importantly, the ICPTs detection platform can be regenerated to five cycles while maintaining strong signal persistence [119].

An approach for the biosensing of progesterone utilizing differential pulse voltammetry (DPV), creating a 3-thiopheneacetic acid (3-TAA)-based film via molecular imprinted technique was established [120]. In this investigation, progesterone served as the target compound, and a working electrode-based carbon fiber paper (CFP) was chosen. A molecular imprint of progesterone was constructed through electropolymerization on the surface of the electrode (Fig. 13). Following the modification, the electrodes underwent investigation employing impedance spectroscopy and cyclic voltammetry electro-techniques. The inclusion of Pd nanoparticles in the modification enhanced the performance of the biomimetic sensor and lowered the LOD. The application of molecular imprinted further heightened its selectivity, resulting in a LOD of 0.05 nmol/L with a linearity ranging from 0.1 nmol/L to 110 nmol/L. Pd NPs played a crucial role in enhancing detection efficiency owing to their outstanding conductivity.

Conducted exemplary research, developing a series of electro-synthesized ICPTs by utilizing thiophene-derived monomers [96]. The objective was to incorporate specific units in the polythiophene structure, facilitating targeted interactions with the desired molecules. In certain instances, functionalized the target molecule, HSA, with bithiophene to prepare an electrochemical biomimetic sensor for HSA detection. Leveraging the amino and carboxylic groups on the surface of the protein, they introduced polymerizable functional groups, allowing the synthesis of a strong specific MIP tailored for the selected analyte. The MIP-HSA was fabricated on the electrode through electropolymerization in the presence of monomers and targets. In this work, the researchers employed an imprinted approach for protein, involving the chemical binding of functional monomer with the template. Following template extraction and cleavage of covalent bonds, in contrast to covalent imprinting, semi-covalent imprinted exclusively relies on non-covalent reactions during the rebinding step. The resultant biomimetic sensor facilitated impedimetric sensing of the analyte within the concentration range of 4–80 μg/mL. The research included a comprehensive investigation of selectivity. Nevertheless, the presence of glucose should not impact the sensing signal, emphasizing that a blood sample must undergo a 1000-fold dilution before HSA determination.

Employed terthiophene (G03TCOOH) to construct an ICPTs biomimetic sensor via electro-polymerization on an Au-coated QCM surface for dengue virus recognition was studied [121]. Because terthiophene molecules possess a limited propensity for oxidation, they are suitable for functionalization and modification through electrochemistry. For NS1 protein, a limit of detection was attained to be 0.056 μg/mL; however, real-world specimens were unavailable to test the real application of biomimetic sensor. The biomimetic sensor presented remarkable selectivity, sensitivity, and long-term stability.

A highly sensitive ICPTs biomimetic sensor to bind *L. monocytogenes* with the GCE, 3-thiopheneacetic acid was electropolymerized in this investigation [122]. The LOD value for this study was 6 CFU/mL. This

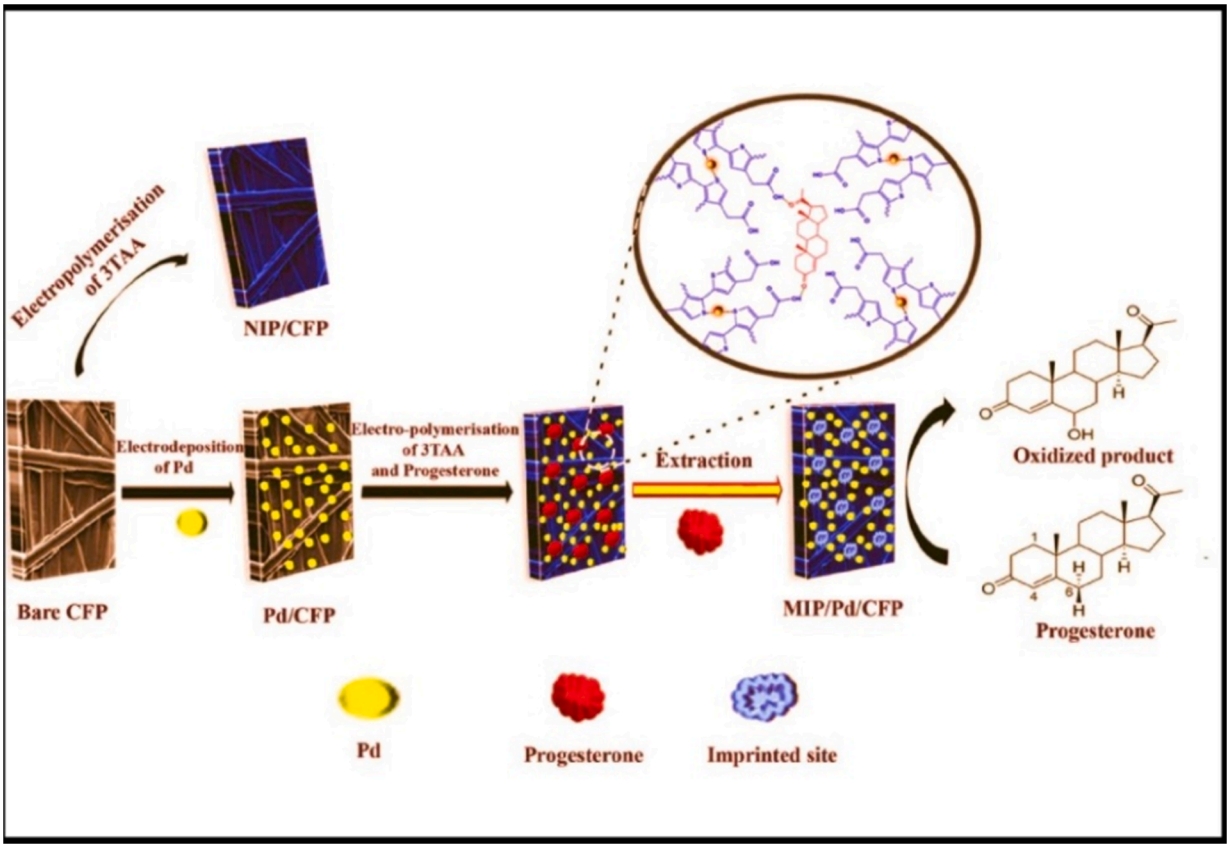

Fig. 13. Fabrication of MIP/Pd/CFP biomimetic sensor for progesterone detection. Reprinted with permission from Ref. [120].

biomimetic sensor also showed selectivity towards *Salmonella enteritidis*, *Escherichia coli*, *Shigella*, *Vibrio parahaemolyticus*, and *staphylococcus aureus*.

One of the most dangerous organisms for humans is staphylococcus aureus (S. aureus), which can cause serious bloodstream infections with a high risk of morbidity and death. Utilizing the conjugated poly (3-thiopheneacetic acid) that was in situ coated on the surface of gold electrode, a new impedimetric *biomimetic* sensor was presented to selectively recognize S. aureus. When applied to real milk samples, the suggested *biomimetic* sensor demonstrated an extremely rapid detection (10 min), a linearity range of 10–108 CFU/mL, and selectivity of more than two kinds of organisms: L. monocytogenes or Escherichia coli O157, depending on how the analytes were prepared [123].

Two distinct biomimetic sensors based ICPTs film were developed for adenosine 5′-triphosphate (ATP) detection [124]. Three various thiophene derivatives were employed as functional monomers for ICPTs preparation. An imprinted factor of 9.47 ± 0.2 was achieved using potentiodynamic electro-polymerization of this complex to produce MIP films. Piezoelectric microgravimetry (PM) and capacitive impedometry (CI) were used to determine ATP under flow-injection analysis (FIA) factors using a test signal transduced with a 10-MHz EQCM resonator and a Pt electrode, respectively. An order of magnitude less than the ATP content in biofluids, the PM and CI biomimetic sensors exhibited detection limits of 0.1 and 0.2 μM, respectively. In addition, cross-specificity was proven using ATP selectivity and adenosine-5′-diphosphate (ADP) imprinting.

Due to the small physiological levels of dopamine, high-performance biomimetic sensors are required. To meet this need the polymerization of thiophene and 3-thienylboronic acid via cyclic voltammetry onto pencil graphite, generating a PT material bearing boronic acid units was performed [125]. These units changed the devices' impedimetric response by immobilizing dopamine compounds at the normal pH in dopamine solution, facilitating specific dopamine recognition in real samples with high linearity (7.8–125 μM) and excellent LOD (0.3 μM).

Theophylline, a bronchodilator drug, was detected using a novel electrochemical biomimetic sensor [126]. The ICPTs were electropolymerized to produce molecular binding sites, and theophylline target was bonded to the electrode via thiophene-3-acid acid. The researchers showed that the ICPTs −based biomimetic sensor was able to recognize theophylline ranging from 0.03 nM to 30 μM with an impressive LOD of 11 pM using differential pulse voltammetry (DPV).

A disposable voltametric biomimetic sensor to reliable and specific measure UA amounts at ambient temperature and pH 7.4 has been recently established [127]. The biomimetic sensor binds and recognizes UA reversibly by using a specific polythiophene material that is deposited on disposable screen-printed electrodes (Fig. 14). Comprehensive electrochemical analyses reveal outstanding efficacy of UA monitoring throughout the broader concentration range of 1–500 μM and LOD as low as 354 nM and significant specificity over conventional interferents. The biomimetic sensor can recover 90.4 % of the spiked UA concentration in human saliva.

Poly(triphenylaminerhodanine-3-acetic acid) (pTPARA) and EDOT have been utilized to construct a metalloproteinase-1 (MMP-1) biomimetic sensor with MMP-1 coated onto the surface of MoS_2/ITO electrode [128]. It has been observed that MoS_2 improves the performance of the biomimetic sensor. The preparation cost was decreased by using this procedure instead of purchasing any costly peptides or antibodies. Furthermore, a fabrication technique like this exhibit improved biomimetic sensor reliability. The detection range of the PIP-coated electrodes can be improved from 1.0 pg/mL to 10.0 pg/mL by using MoS_2 cML coating with PIPs. The MMP-1 content in the A549 cell line culture media was found to be 800 ng/mL using the MoS_2 cML electrode. A precision of 95 ± 5 % was found when compared to data from an ELISA kit.

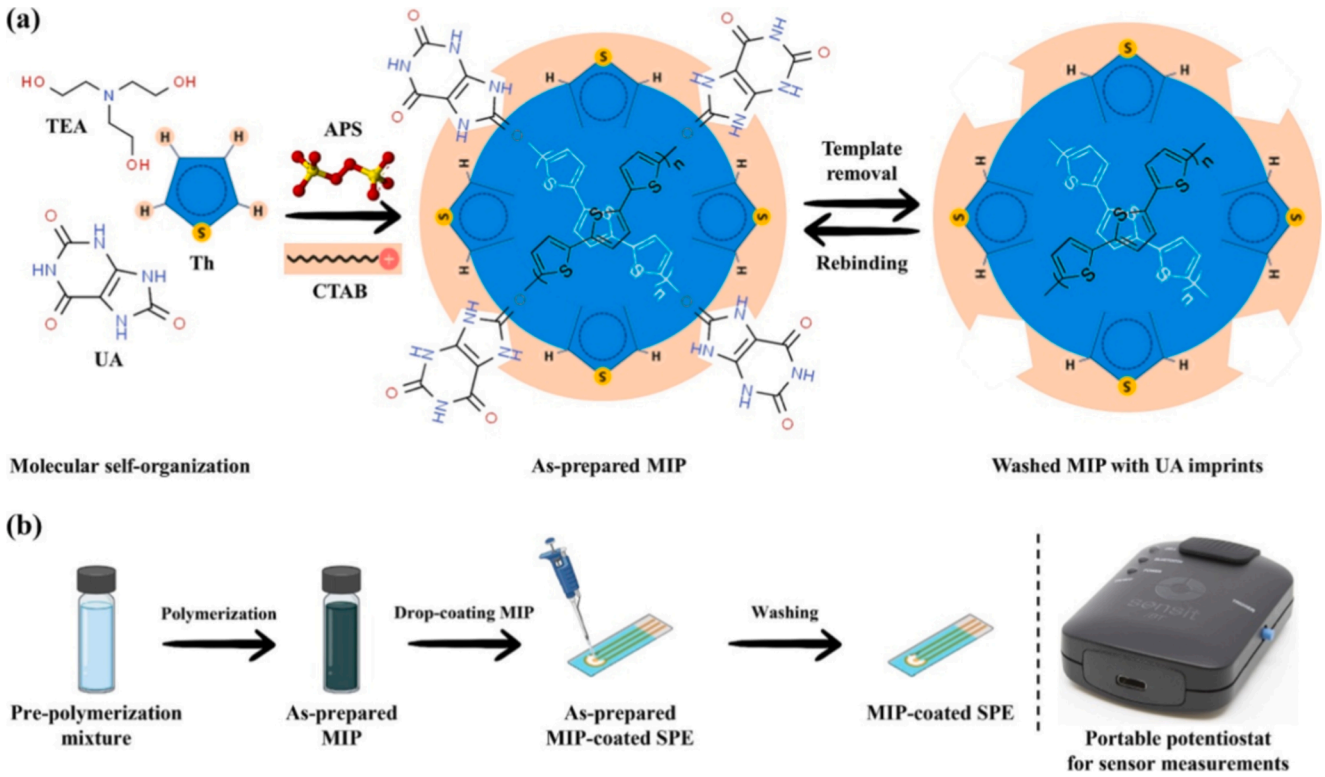

Fig. 14. A schematic illustration of the procedures involved in the generation of molecularly imprinted polythiophene (MIP) and drop-coated screen-printed electrodes (SPE). Additionally, a portable, wireless potentiostat called Sensit BT, is utilized for data collection and electrochemical sensor measurements. Reprinted with permission from Ref. [127].

5 . Conclusion

This review summarizes the state-of-the-art in ICPTs, situating them within the broad field of CP-based sensors and discussing both general and novel fabrication strategies, as well as signal transduction in sensing procedures. The first two sections introduce the impact of various functionalization techniques on the optical and electrical properties of CPTs. Using a variety of functionalization techniques, optical and electrical characteristics of CPTs can be significantly modified or adapted. These CPTs have enhanced biosensor effectiveness, rendering them one of the most promising substrates for biosensor and biomimetic sensor development. The effective translation of biological occurrences, or transduction, is one of the main obstacles in the construction of biosensors. Biofunctionalized CPTs convert signals that are optical, gravimetric, electroluminescent, or electrochemical from the analyte's attraction to biological molecules. The CPT backbone of biosensors increases the number of bioreceptor groups and functions as a signal component, reducing the recognition threshold of the analyte and enhancing the sensitivity. The fabrication of biosensors benefits from the functional combination of biomolecules with CPTs. Biosensors have been constructed via a variety of techniques, including chemical functionalization of CPTs with the biomaterials, doping the CPTs with biomolecules during polymerization, and post-functionalization of the bioanalytic onto the CPTs structure. However, biosensors are still fragile and expensive due to the biological component that plays a main recognition function.

On the other hand, advanced fabrication methods, such as printing technologies, microfluidic chips, OTFTs, and colorimetric paper strips combined with smartphones, have addressed various challenges faced by biosensors. For instance, printing technologies not only enhance the reproducibility of sensors across batches but also enable the fabrication of wearable sensors using minimal materials. Additionally, microfluidic chips tackle challenges such as automation of sensing with minimal sample volumes. These fabrication methods, combined with CPTs, bring us closer to developing ideal biosensors with notable advantages including portability, flexibility, multifunctionality, and ease of use. The integration of such biosensors with AIBN will allow users to send detection results to the network and immediately receive a remote health assessment from a doctor thanks to the combination of smartphones and wireless transmission with biosensors.

Finally, integrating the selective recognition capabilities of imprinted technology with the unique electrical and optical properties of CPTs into a single platform offers an excellent opportunity to enhance both specificity and sensitivity while overcoming common biosensor drawbacks such as instability and high costs. Additionally, the intrinsic signal transduction of ICPTs addresses some of the challenges associated with MIP biomimetic sensors that use non-conjugated polymer backbones. By carefully selecting a functional group that interacts within an ICPT for a specific target and optimizing the ICPT and its signal transduction, it is anticipated that a high-performance biomimetic sensor with excellent specificity and sensitivity can be developed. This optimized biomimetic sensor would also be processable in different solvents, broadening its applicability.

6. Challenges and future prospective

The key benefit of ICPTs over other MIPs lies in their intrinsic electrical conductivity, enabling direct electrochemical sensing without additional conductive components. This characteristic facilitates the creation of highly sensitive and selective electrochemical sensors. ICPTs also demonstrate remarkable stability and can be readily synthesized via electropolymerization, allowing precise control over film thickness and structure. Nevertheless, ICPTs face certain limitations. The narrow range of available conjugated monomers may constrain the diversity of template molecules suitable for imprinting. Moreover, the rigid backbone of conjugated polymers could restrict binding site flexibility, potentially diminishing binding affinity for certain analytes.

High-performance biosensor devices will be greatly impacted by the synthesis of innovative biosensing substances based on multicomponent ICPTs or ICPTs nanocomposites, which will help in the resolution of current issues with sample volume, multianalyte detection, extending the lifetime of biosensors, reaction time, signal generation, and processing associated with transduction methods. We are currently in the early stages of developing biomimetic sensors using imprinted polythiophenes. Many of these biomimetic sensors require further optimization for stability and must be evaluated in more complex matrices before they can be widely deployed for daily use. Moreover, like conventional MIPs, imprinted polythiophenes need further research to address defects associated with the imprinted process, such as mass transfer resistance, clogged recognition sites, and inaccessible recognition sites. Overcoming these defects in the imprinted process may further improve the sensitivity of these sensors, making them more effective and reliable for various applications. Although there are many challenges to overcome, we believe that the development of commercial products rather than proof-of-concept level models will allow us to solve current problems. To accomplish superior integrated assessment for a range of data, for instance, the device might be further miniaturized, linked with numerous functions like automation, artificial intelligence, the internet of things, and various biosensor types integrated with the recognizing device. Future work is expected to focus on developing wireless biosensors for medical use, multi-sensing lab-on chips for developing biosensors with multiple functional options, and ICPT-based detection systems to achieve short-time and on-site detection of analytes. It is important to note that the recent COVID-19 pandemic showed the importance of biosensors and biomimetic sensors with high-throughput assays to meet increasing test requirements in medical facilities and multi-sensing lab-on chips for outpatients.

CRediT authorship contribution statement

Salah M. Tawfik: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Mirkomil Sharipov:** Writing – original draft, Investigation. **Mohamed R. Elmasry:** Writing – original draft, Investigation. **Shavkatjon Azizov:** Visualization, Data curation. **Dong-Hwan Kim:** Writing – review & editing. **Abbaskhan Turaev:** Writing – review & editing. **Yong-Ill Lee:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Hoon Eui Jeong:** Writing – review & editing, Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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