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ARTICLE

Antifouling strategies in advanced electrochemical sensors and biosensors

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Electrochemical biosensors have been applied in a broad range of clinical applications for pathogen biomarker detection and medical applications and diagnosis due to the sensitivity of electrochemical methods and the bioselectivity of the components. The complexity of clinical conditions with various biofoulants (proteins, cells, polysaccharides and lipids) severely influences the reliability and stability of sensors for direct detection or immersion under changing conditions. Therefore, designing an antifouling sensing platform that can effectively reduce undesired binding to maintain biosensor performance in optimized analysis is necessary. For this purpose, the fundamental mechanisms of fouling materials and commonly used biocompatible antifouling components have been discussed, and the relevant effective modification strategies are introduced in this review. Recent advances in these strategies are demonstrated in examples with analysis of essential modification methods for reliable sensing in non-specific binding solutions or complex biofluids. The challenges and future perspectives of modification strategies for current clinical application are also discussed in this review.

1. Introduction

Biosensors have been the most active field in analytical chemistry for years and have drawn great attention due to their potential to be rapid diagnostic tools for various clinical applications. Numerous detection methods have been developed, including surface plasmon resonance (SPR)[1], silicon nanowire field-effect transistor (SiNWD-FET)[2-4], quartz crystal microbalance (QCM)[5] and electrochemical methods[6]. The electrochemical method is a surface detection technique that provides several advantages for biosensing detection, including easy processing, low cost and accuracy. It can also achieve a low detection limit and only requires a small reaction volume during measurements[7]. These features provide an advantage for disease determination in clinical applications that require high sensitivity and low sample volume in the analysis process. An electrochemical sensing system contains recognition elements that are immobilized on transducers (electrode) and selectively bind to target analytes, generating an electrical signal that depends on the concentration of the analyte in clinical complex solutions (blood, urine and pleural effusion). Such electrical signals often encounter serious fouling issues when biofoulant molecules attach to the electrode surface through nonspecific binding, causing the background signal to increase and negatively affecting the sensitivity and reproducibility of the sensors. This fouling phenomenon significantly disturbs the reliability and performance of electrochemical sensors, especially in complex environments.

Biofouling is involved in a broad range of mechanisms that mostly depend on the type of fouling agent, including the matrix of clinical samples (i.e., proteins, cells, lipids, polysaccharides), other analytes and side products of electrochemical reactions[8-10]. These undesirable molecules tend to attach to the platform surface through several interactions, such as hydrophobic, electrostatic and intermolecular forces, resulting in a dense layer that passively forms on the electrode and blocks recognition elements and electrodes. The selectivity and binding affinity of biological recognition elements are strongly influenced by the fouling layer, resulting in poor signal-to-noise ratio and high background signal. This fouling layer not only inhibits physical contact of the analyte with the electrode but also directly impedes electron transfer through electrochemical responses. In recent years, the requirement of detecting analytes in real samples has driven the study of antifouling performance in biosensors. For example, the concentration of protein in human blood ranges from 60 to 80 g/L[11]. To maintain reliable analysis results in the presence of numerous interference factors in clinical use or other complex particle conditions, antifouling treatment of the electrode is necessary for the sensing platform. Construction of an antifouling biosensor can strongly improve sensitivity, selectivity, reproducibility and reliability in electrochemical devices. Therefore, the development of a high sensitivity system with an antifouling ability is critical for electrochemical biosensors in expanding further reliable clinical detection.

Recently, a valid strategy for alleviating fouling issues is integrating antifouling materials on an electrode surface to

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effectively minimize nonspecific attachment. These antifouling materials must be biocompatible, naturally inert and able to survive in a complex environment, such as sweat, urea, wastewater, tissue fluid and blood. Cooperating with the electrode surface, antifouling reagents include a broad range of materials, most of which attempt to improve the antifouling resistance of the electrodes[12-14]. According to different types of materials, proper modification strategies are incorporated into an analysis platform either by changing the chemical properties or creating a physical topological structure[15-17]. Both antifouling materials and interactions between the sensing platform and modification layer are crucial for antifouling efficiency. Because generally used antifouling materials have been explained in several excellent reviews, we will only briefly mention some of the most commonly used materials and mainly focus on related fouling mechanisms and recent developments in modification strategies for electrochemical biosensors.

2. Surface antifouling mechanism

The attachment of foulants can be minimized by changing a surface-modified electrode with hydrophilic and zwitterionic molecules. For clinical applications, these antifouling coatings are designed to be biologically ''inert''; i.e., they do not cause an immune response and/or chemical reaction and do not eliminate fouling proteins or cells directly. With great biocompatibility, these anti-fouling materials inhibit foulants from attachment via mechanisms including hydrophilic interactions, steric repulsion, electrostatic repulsion and low surface energy (Fig. 1).

2.1 Hydration Layer

In electrochemical biosensors, most electrodes, such as gold, ITO and other carbon-based electrodes, tend to have hydrophobic surfaces that cause hydrophobic foulants to adhere to the electrode[18-21]. Hydrophobic interactions between foulants and electrodes are quite stable in aqueous environments. Two hydrophobic materials favorably adhere together, pushing the hydrophilic molecules in the surrounding out, resulting in a reduction in the entropy of the system. Under the generally mild conditions for biomedical application, such entropy-driven hydrophobic interactions are irreversible. While solving fouling issues, studies attempt to avoid hydrophobic interactions and instead reconstruct a hydrophilic electrode surface that can effectively minimize the adhesion of foulants. Unlike hydrophobic surface conditions in which water pushes foulants onto the surface, water competes with the foulants and binds to the hydrophilic surface, strongly displacing foulants through dehydration mechanisms[22,23]. In this case, hydrophilic components, such as polyethylene glycol (PEG) /OEG, are incorporated on the electrode surface. Each PEG (poly(ethylene oxide))/OEG (oligo(ethylene glycol)) backbone can strongly bind water molecules, connecting each chain through the ether oxygen and forming a tight hydration layer that physically blocks out foulant adsorption[24-26].

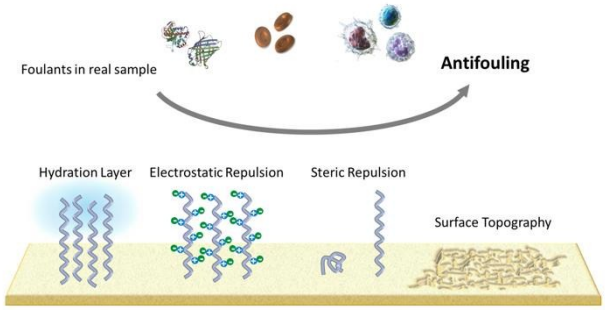

Fig. 1 Illustration of antifouling properties on an electrode surface in a real sample.

2.2 Steric Repulsion

Almost all water-soluble polymers have the ability to resist protein, but the best materials can achieve both a hydration layer and steric repulsion characteristics. For long chain polymers, steric repulsion is involved in antifouling resistance due to the flexibility of the chain[27,28]. When foulants approach the sensing platform, grafting polymers configurationally compress through volume change, and this dehydration process is an unfavorable change in free energy. Due to the entropy effect, polymers tend to expand and repel foulants. Such steric force prevents foulants from reaching the electrode surface. For polymers, both repulsion forces and hydrophilic features are important for antifouling properties in polymer strategies.

2.3 Electrostatic Repulsion

Similar to PEG/OEG-based materials, molecules containing cationic or anionic moieties also have the ability to form hydration layers through ionic solvation interactions with water. This dipole arrangement of water is more effective for water bonding than hydrogen bonding in the case of PEG/OEG material. However, a large amount of ion charges accumulated on the electrode surface attracts macromolecules with opposite charges, causing serious foulant adsorption. To avoid charge aggregation and impart protein resistance to the substrate, a neutralized molecule carrying a 1:1 positively and negatively charged residue is a method to overcome electrostatic attraction since all proteins contain both charged residues. Zwitterionic materials contain separate anions and cations that can be corporately synthesized or naturally formed, such as phosphocholine (PC), sulfobetaine (SB) and carboxybetaine (CB). While the composition ratio might be varied, the overall charge is neutral. In this case, both negatively and positively proteins can be resisted by a brush film forming a denser and thicker hydration layer than PEG/OEG materials. Studies have demonstrated the antifouling performance of zwitterionic materials over traditional PEG/OEG-based materials. A study by Wu et al. demonstrated that PEG indeed exhibits weak interactions with adsorption proteins, while the interaction between poly(sulfobetaine methacrylate) (pSBMA) and protein was undiscovered[29]. Other studies also revealed differences in the hydration strength ability of electrostatic interactions and hydrogen bonding in PEG/OEG polymers and zwitterionic materials[28,30,31].

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2.4 Surface Topography

Surface topography has been used to affect foulant adsorption on sensing platforms. It provides a certain degree of influence on the fouling level, protein conformation and binding affinity, especially on the nanoscale, i.e., in the same scale range as protein size[32]. Engineering the surface topography is another way to influence biomolecular fouling in exceedingly broad strategies[33-36]. Without changing bulk properties and altering surface chemistry, the topography changes the behavior of surface wetting, resulting in control of protein adsorption. According to the Wenzel and Cassie-Baxter equation[37], increasing facial roughness makes a hydrophilic surface more hydrophilic and a hydrophobic surface more hydrophobic when there is entrapment of air in the surface structure. Such topology restricts the contact of proteins and cells, limiting foulant attachment.

3. Modification strategies for antifouling electrodes

There are two strategies for improving the antifouling resistance of an electrode, by altering the surface morphology (physical) and immobilizing additive component (chemical) on the electrode. A suitable method is determined by the physical and chemical properties of both the electrode and antifouling reagent and has been developed for years. In the following paragraph, we will briefly discuss the physical and chemical mechanisms and methods that lower the adsorption of foulant molecules.

3.1 Physical strategies

3.1.1 Physical Adsorption Physical methods of antifouling usually do not change chemical properties and instead steadily

Table 1 Comparison of various antifouling strategies of for electrochemical sensors and biosensors

Modification Method	Component	Detection Method	Result	Ref
		Physical		
Physical	GCE/ PDA/GSSG	CV	Linear range 0.1 pM - 0.01 µM, with a detection limit of 0.01 pM of EGF; GSSG as blocking agent	[40]
Physical	Carbon electrode/ methacrylate polymer	CV	pH-sensitive coating (pH>6 and pH<5) H2O2 sensor 100% recovery sensitivity in blood and saliva for 2 h	[42]
Surface topology	Vertical graphene/nanorods (vG/NRs)	CV	Fluorinated vG/NRs 2 to 15 mM of H_2O_2 with a detection limit of 0.32 mM and high stability in 1% serum	[44]
Surface topology	Nanoporous Au	DPV	High recovery rate (95.1% and 97.3%) for AA and UA detection in 10% FBS	[50]
		Chemical		
SAM	Au/Cys-PEG/peptide	SWV	PEG spacer dynamic range of 0.1-100 nM and LOD of 90-800 pM for targeting trypsin. PEG-6 has the minimized nonspecific response in 100 nM BSA.	[56]
SAM	Au/peptide/MCH	EIS	For MCH blocking, zwitterionic peptide for BRAC1 detection prevents antifouling in 10% FBS.	[57]
SAM	Au/ thiolated SB/thiolated apatamer	EIS	Good antifouling resistance for PSA detection in 100 µM serum (variance< 1%).	[61]
Polymer brush (graft-to)	Pt/PSBEDOT/GOx	Amperometric response	GOx trapped in PSBEDOT retained 90% signal in human blood plasma.	[68]
Polymer brush (graft-to)	GCE/PEDOT/PEG/AuNPs	EIS	Detection of AFP in 10% human serum; 99.91% signal recovery	[69]
Polymer brush (graft-to)	GCE/PANI-PThi/phytic acid	SWV	CA125 antigen showed a range from 0.0001U/mL to 1kU/mL with a LOD of 0.00125 U/mL with great antifouling resistance in human serum.	[70]
Polymer brush (graft-to)	GCE/PANI/PEG nanofibers	DPV	BRAC1 DNA detection in 1-5% serum was unaffected. 92.17% signal remained in 100% serum.	[71]
Polymer brush (graft-from)	Pt/PANI/GOx/SBMA	Amperometric response	94% sensitivity remained after incubation for 15 days in 100% FBS.	[73]
Polymer brush (Electrodeposition)	Au/aniline-SB	CV	Resists nonspecific binding through rapid surface construction	[78]

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cover a material with a layer of blocking components that reduce direct contact between foulants and electrodes. Adsorption is a facile method that generally modifies electrodes with inactive proteins via intermolecular forces, mainly through polar interactions, hydrogen bonding, ionic bonds and van der Waals forces. BSA is the most commonly used blocking reagent in biosensors but has several flaws, including a nonuniform layer and a potential steric hindering effect on small molecules[38,39].Lin et al. demonstrated a small inactive peptide, oxidized glutathione (GSSG), as a blocking reagent, which can significantly lower the linear calibration range and detection limit, especially for targeting small molecules[40]. In this immunosensor, a layer of polydopamine (PDA) is deposited on a GCE, which interacts with epidermal growth factor (EGF), atarget analyte. The remaining sites of the PDA film are covered with GSSG or BSA and subsequently detected with anitibody-AgNPs via CV and EIS. The GSSG blocking strategy responds to the detection range from 0.1 pM to 0.01 μM, with a detection limit of 0.01 pM, which is much more sensitive thanthe BSA blocking method. Such physical adsorption ensures that the recognition elements bind to the analytes specifically under real sample demonstration. However, the immobilized mechanism reacts through weak intramolecular interactions that can easily be reversed by conditional changes through environmental variance, resulting in an influence on the strength of the interactions (pH value, temperature, ionic strength and solvent polarity)[41]. Such an adsorption layer may be involved in cross-reactivity, high variance and changing surface behavior.

3.1.2 Mechanical Coating Another electrode modification method is physically coating antifouling reagents on electrodes by directly forming polymer films. The advantages of physical coating (spin-coating, dip coating and spray coating) are that it can quickly fabricate uniform films with controllable film thicknesses. Additionally, the easy coating process is conducive to mass manufacturing with time savings and low cost. However, the physical adsorption of antifouling material on an electrode is relatively unstable because the coating might physically degrade during application under complex conditions. Montiel et al. utilized such features to develop a pH-responsive electrochemical sensor array that controlled delayed detection approaches by coating a film of pH-sensitive methacrylate polymer[42]. These commercial polymers, Eudragit® L100 and Eudragit®EPO, will dissolve under certain pH value conditions, pH>6 and pH<5, respectively. After coating the protective polymers, electrochemical sensors were sheltered from the complex biofluid with a certain time sequence depending on the coating density and thickness. When exposed to a specific pH environment during controlled delay times, fresh electrodes are available for electrochemical detection without fouling interference. They demonstrate an impressive approach for antifouling resistance by coating a glucose oxidase (GOx)-Prussian blue graphite electrode for targeting glucose in biofluid. After incubating 2 hours in saliva or blood, the coated sensor presented the best sensitivity (100%) over the uncoated electrode, reaching only 29.6% and 34.6% sensitivity.

3.1.3 Superhydrophobic Engineering the surface morphology is another way to reduce electrode fouling in exceedingly broad strategies without changing the chemical properties of an electrode. There are two main strategies that are devoted to antifouling resistance. One is the lotus effect, creating a superhydrophobic surface that causes water to pick up contaminants, preventing any biomolecular attachment and contributing to the self-cleaning effect[43]. In the other way, construction of a surface morphology that has a special structure prevents protein and cell attachment. For example, when the structure is smaller than the size of the foulant, foulant binding can be reduced by decreasing the effective contact area. In the following paragraph, we will discuss two different antifouling mechanisms.

A superhydrophobic surface minimizes water droplet adhesion due to the nanoscopic architecture on the surface, providing self-cleaning properties. When water drops are on a superhydrophobic surface, dirt particles can be picked up and taken away freely. Such a natural lotus effects inspired the construction of sensing platforms that can be applied in biomedical and sensing systems. Some studies mimic the hierarchical micro/nanoscale structure on leaves that creates low surface energy and roughness, resulting in excellent water-repelling and self-cleaning properties. Hang et al. reported a H_2O_2 electrochemical sensor based on a hierarchical vertical graphene(vG)/nanorod(NR) structure with antifouling and self-cleaning properties[44]. The hierarchical structure was fabricated through plasma-enhanced chemical vapor deposition, atomic layer deposition and hydrothermal growth building ZnO NRs on vG. Such a structure showed even greater water resistance and anti-adhesion after fluorination. After detecting the H_2O_2 concentration in diluted blood serum (1% v/v), the electrodes were reused again for testing biocontamination in real samples. Compared with untreated vG/NRs, fluorinated vG/NRs obtained almost the same current response as fresh vG/NRs-F, confirming the antifouling resistance and complex biosample sensing ability. vG/NRs-F sensors revealed a linear detection range from 2 to 15 mM of H_2O_2 with a detection limit of 0.32 mM. Additionally, in the detection stability test, the RSD was 2.21% in PBS buffer and 2.78% in diluted blood for detecting 1 mM H_2O_2 5 times. Several studies have also directly fabricated similar hierarchical nanostructures on gold or other electrodes for superhydrophobic modification[45,46].

3.1.4 Nanoporous structure Recent studies have shown that an adequate electrode topography can minimize electrode fouling. Nanoporous electrodes have been revealed to have a greater antifouling performance than planar electrodes because the inner porous structure restricts the mass transport speed, and small molecules are permitted from efficient exchange (Fig. 2A). Additionally, the resistance efficiency is strongly dependent on the sizes of the pores and foulant molecules[47]. Daggumati et al. reported nanoporous gold electrodes that enable the detection of target DNA molecules with a detection range from 10 to 200 nM under complex conditions with BSA and fetal bovine serum (FBS). The electrode pore size plays an important role in foulant adsorption and transport[48]. Additionally, Collinson and his group

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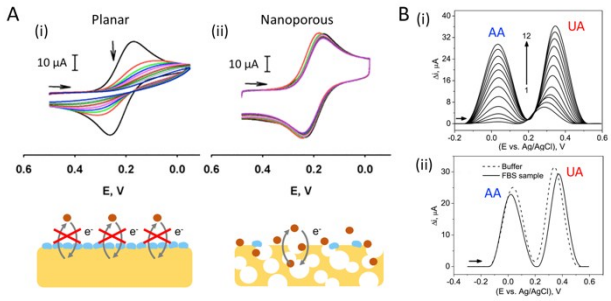

Fig. 2 (A) Cyclic voltammetry in planar and nanoporous gold surfaces in a BSA solution. Reproduced from ref. 49 with permission from American Chemical Society, copyright 2013. (B) Schematic of direct electrochemical sensing of ascorbic acid and uric acid. Nanoporous structures provide stable signals under fibrinogen and BSA conditions. Reproduced from ref. 50 with permission from Elsevier, copyright 2019.

demonstrated nanoporous gold electrodes with great fouling protection with only slight variance in the current change[49,50]. They also presented an NPG-based electrochemical biosensor that determined the voltammetric current of ascorbic acid (AA) and uric acid (UA) under biofouling conditions (Fig. 2B). AA and UA were simultaneously analyzed with LODs of 63.0 and 9.0 μM, respectively, by DPV. The average recoveries were also detected under dilute FBS conditions, and 95.1% and 97.3% were achieved for AA and UA, respectively.

3.2 Chemical strategies

Chemical strategies for antifouling surface construction are a relatively stronger immobilization method compared with physical modification and are most widely used in antifouling electrochemical biosensors by incorporating antifouling polymers on transducers[51,52]. According to the type of target analyte, the detecting environment and the type of platform, chemical modification methods vary and can be strategically applied in one device to optimize the sensing and antifouling performance. In terms of constructing the structure, there are two construction methods: self-assembled monolayers (SAMs) and polymer brushes (Fig. 3).

3.2.1 SAMs SAMs have been widely used to build electrode surfaces in sensing systems. The method involves spontaneous formation of an arranged structure through passive reactions and can be designed for suitable molecules, conditions and driving forces[53]. Such a monolayer is grafted by chemical molecules constructing a layer with dense packing and uniform thickness, approximately thinner than 10 nm depending on the molecular size[54]. In many self-assembled systems, alkanethiols for gold electrodes are the most commonly used derivatives in

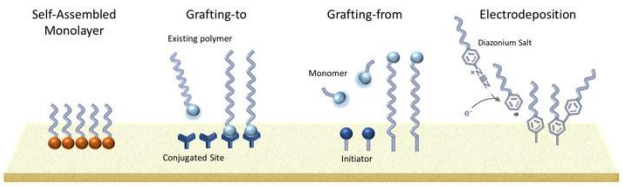

Fig. 3 Strategies of antifouling materials exploited on electrodes through chemical modification.

biosensing systems, providing reliable formation. The interaction is quite stable due to the strong bonding of S-Au and van der Waals forces between molecules. Several advantages of using thiol-Au SAM include easy preparation of the reaction process and accessibility of varied thiol derivatives with antifouling functional groups[55]. The generally used antifouling materials, including PEG- and OEG-based materials and zwitterionic and peptide-based molecules, are discussed below.

PEG and OEG materials are commonly used in antifouling SAM methods due to their stability in biological environments. The thickness of the monolayer can be easily controlled by the length of repeated chain in PEG. Studies have shown that the antifouling performance of PEG-based materials depends on their surface hydration ability, which is strongly related to the length of the molecular chain. Some shorter PEG materials present a less orderly arrangement and low coverage, resulting in diminished antifouling properties. In Fig. 4, Bradley and his research group modified a PEG-based polymer with a thiolated anchor group and a short peptide head for protease detection on a gold electrode surface[56]. They also synthesized different spacer lengths of PEG to study the length effect on the sensitivity and antifouling efficiency. The analytical results were good for all lengths of the PEG spacer with a dynamic range of 0.1-100 nM and LOD of 90-800 pM. Interestingly, PEG-6 showed the minimum response under nonspecific binding conditions, revealing that adjusting the spacer length can optimize the antifouling efficiency of the sensing platform. To reduce the weakly adsorbed molecules and to help the arrangement of thiolated materials, the electrode surface is immobilized with a hydroxyl head group for optimal hybridization and partially resolved nonspecific binding[57]. These small-sized thiolated alcohol components (i.e., 6-mercaptohexanol (MCH) and 11-mercaptoundecanol) occuppy the empty space of Au, stabilize the SAM conformation and further improve the antifouling resistance of the system[58-60]. The Luo group presented an electrochemical DNA hybridization sensor targeting BRCA1 breast cancer via EIS measurements[57]. Zwitterionic peptides containing several glutamic acid (E) and lysine (K) molecules as the antifouling substrate were immobilized on the electrode through SAM followed by a modified MCH blocker being added on the gold. The BRCA1-specific oligonucleotides were covalently bonded with an antifouling peptide for target sensing. The electrochemical DNA biosensor showed analytical performance with a linear range of 1.0 to 10.0 pM with an LOD of 0.3 fM. MCH was also fabricated on a sensing system to compare the antifouling effect with that of thiolated sulfo-betain (SB). Jolly et al. reported an apatamer biosensor for detecting prostate specific antigen (PSA), a cancer biomarker, using two types of antifouling bases, MCH and thiolated SB, to test the sensitivity, selectivity and antifouling resistance in EIS sensors[61]. For better detection sensitivity, the SB-based apatmer sensor showed a low detection limit of 1 ng/ml and negligible variance (<1%) when changing the buffer to 100 μM human serum, indicating good fouling resistance. In contrast, MCH revealed a 12.5% increase in signal upon replacement of PBS with human serum, resulting in a decrease in PSA levels.

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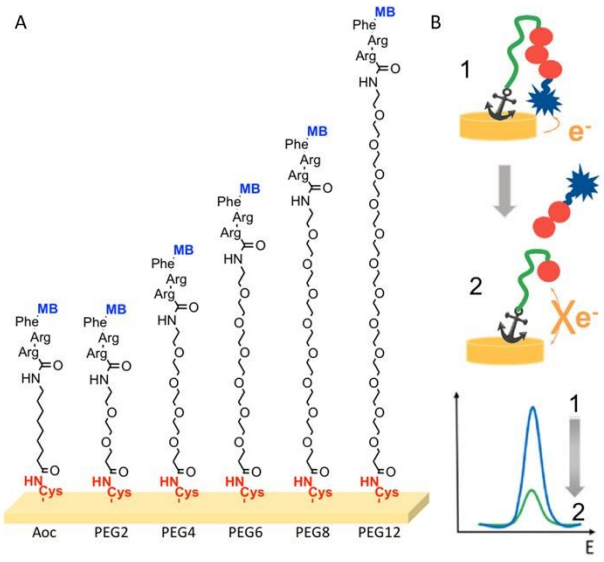

Fig. 4 (A) The length-dependent probe coating on PEG and an alkyl-based spacer on a gold electrode through SAM formation. (B) Schematic of the peptide-based electrochemical platform; the enzyme cleaves the label peptide, releasing the redox probe into solution, resulting in a decrease in the SWV signal. Reproduced from ref. 56 with permission from Elsevier, copyright 2018.

With the self-recognizing property, antifouling molecules can be modified meticulously under mild conditions, forming a high-packing-density layer. Despite the fact that SAM provides several advantages for improving the sensing system, many disadvantages result in the limited application in electrochemical sensing systems, including limitations of available grafting molecule types, restriction of transducer materials, long modification times and low stability under complex conditions.

3.2.2 Polymer brushes Polymer brushes are a soft polymer hybrid structure in which one chain end of a polymer is tethered on substrates. The grafted surface extends nanometers of thickness with vertical development of the polymer chain extension. They have attracted great attention due to utilizing electrodes other than gold, such as carbon, silver, and ITO, adjusting the space and ratio of the polymer, and conjugating to various additional functional groups[62-64]. Most types of polymers form brush layers through grafting methods, leading to large freedom for customization by immobilizing the required functional terminals. In the features of antifouling polymers, a large degree of hydration can be easily delaminated from the sensing platform and carried away by water flow in aqueous environments, when it is not strongly anchored to the transducer. Therefore, the modification method for anchoring these polymers to the substrate surface is crucial for the fundamental antifouling efficiency.

The typical strategies for coating polymer brushes are 'grafting-to' and 'grafting-from' that optimize the antifouling performance for material immobilization[65,66]. The grafting-to approach describes existing polymers covalently conjugated to anchor substrates on a reactive surface, while the modification method might involve SAMs, which we mentioned in the former paragraph. The grafting-from approach builds up polymer brushes via polymerization from the initiative substrate on a scaffold surface. The coupling reactions of polymer brushes are relatively complex and restricted to certain types of substrates and materials.

Grafting-to Recent studies have presynthesized antifouling materials conjugated with conducting polymers before grafting to a platform surface. Cooperating with a conducting polymer (i.e., PEDOT) could enhance the sensitivity of electrochemical sensors and compensate for the poor conductivity of most antifouling molecules. The Miyahara group synthesized a series of zwitterionic poly(3,4- ethylenedioxythiophene) (PEDOT) molecules that conjugated PC, CB and SB on thiol-functionalized EDOT (Fig. 5A)[67]. The monomers were modified on the electrode through electrodeposition, subsequently providing both conducting and antifouling properties. Among the layers investigated, EDOTPC showed the minimum interference and the best resistance to cell adhesion and protein adsorption. Wu et al. also synthesized PEDOT-based zwitterionic molecules (PSBEDOT) and introduced them to an electrode as the enzyme matrix for trapping GOx on the electrode[68]. PSBEDOT presented high stability and low fouling properties when stored in 100% human plasma for 14 days, and over 90% of the current signal remained (Fig. 5B). This direct linking strategy provides a facile single-step electrografting method that significantly reduces the modification time and simplifies the fabrication process of biosensors with good sensitivity improvement in electrochemical biosensors.

Primary layer An alternative grafting method for antifouling polymer attachment is to generate an anchoring film that reacts

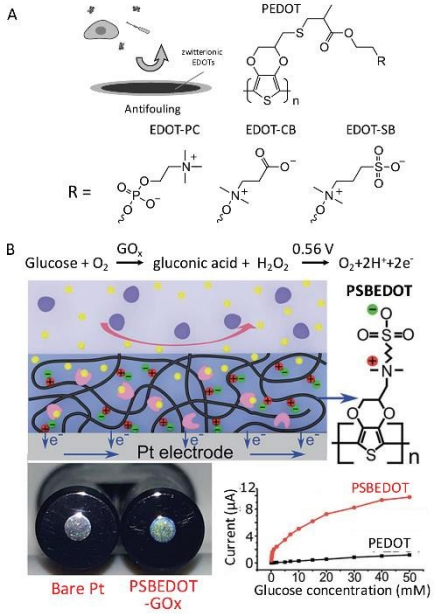

Fig. 5 (A) Presynthesis zwitterionic polymer conjugated with EDOT forms an antifouling layer. Reproduced from ref. 67 with permission from American Chemical Society, copyright 2018. (B) Zwitterionic PSBDOT was modified on the Pt electrode for glucose detection with long-term stability in blood. Reproduced from ref. 68 with permission from Royal Society of Chemistry, copyright 2018.

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with both the electrode and functional polymer. When deposited on the substrate, the primary layer provides stable films that allow for target antifouling polymer formation. It provides more variability for surface construction that can properly utilize different components, and more importantly, it opens up application on platform substrates by adding specific functional groups for certain polymer reaction initiation. An example of a PEDOT-based layer for an antifouling molecule on an electrode was developed by Cui et al[69]. A highly biocompatible composite of PEDOT doped with PEG is deposited on a GCE through electrodeposition, and the terminal group of PEG contains a thiol group (Fig. 6A). Gold nanoparticles are added into the sensing system for immobilizing alpha fetoprotein (AFP) antibody along with an enhancement of conductivity. The system showed good linear detection of 0.001 fg/mL to 10 fg/mL with a detection limit of 0.0003 fg/mL. Such an AFP sensor could be used in 10% human serum with antifouling resistance by analyzing a 0.001 fg/mL AFP summary with 99.91% recovery and 0.71% RSD.

Similar to PEDOT, PANI is also a common conducting polymer used as a primary layer that helps enhance stability and sensitivity or antifouling for electrochemical biosensing. A 3D porous network hydrogel deposited by PANI and polythionine (PThi) was synthesized on a GCE, and the fabrication process is shown in Fig. 6B[70]. Such a porous microstructure provides a large effective area for doping of phytic acid, a crosslinker agent with outstanding hydrophilicity, in the layer to form a conducting, hydrated protective layer. After immobilizing as-prepared AuNPs on PANI-PThi gel, the H_2O_2 electrocatalytic ability was detected with a wide linear range from 0.0001 U/mL to 1 kU/mL and an LOD of 0.00125 U/mL. The antifouling analysis test in 0 - 100% human serum showed that the protein immunoassay only slightly changed with a fluctuation variance less than 4 μA during SWV measurements. Another example of using PANI as the base layer for antifouling molecule immobilization was demonstrated by Luo group[71]. The research team presented antifouling nanofibers where PEG was covalently grafted to PANI nanofibers followed by direct hybridization of the BRCA1 DNA probe. DPV monitored the change in the methylene blue signal while the DNA probe targeted the BRCA1 gene. Such a device showed a linear range from 0.01 pM to 1 nM with a detection limit of 0.0038 pM. The PEGylated PANI nanofibers were capable of resisting complex conditions, while the same current responses were shown after incubating the electrode in 1% and 5% human serum and even retained 92.17% of the initial current response under 100% serum conditions. The primary layer provides a solution to overcome the combination restriction between the polymer brush and electrode, giving more variety and flexibility for different environments. Additionally, combining different materials allows complementing flaws in the characteristics of functional materials.

Grafting-from In the grafting-from approach, polymer brushes can be prepared via surface-initiated polymerization (SIP) that enables control of the grafting density, film thickness, charge distribution and copolymer ratio[72]. Ye et al. fabricated an

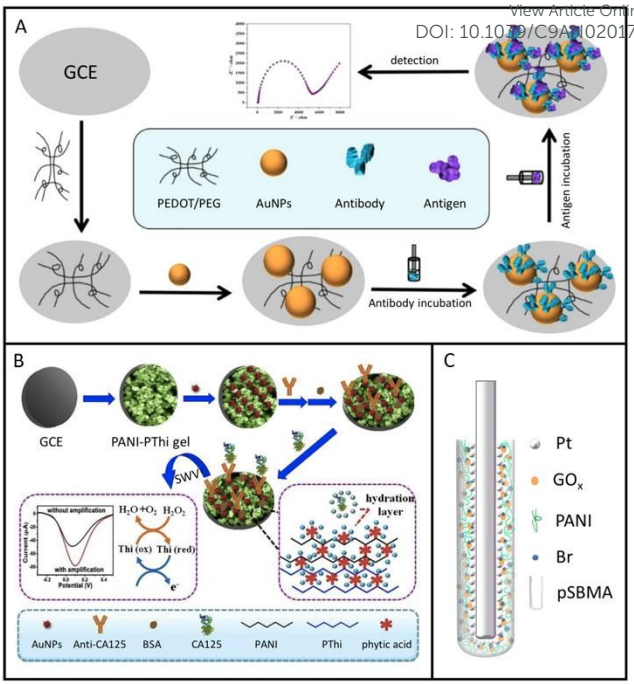

Fig. 6 (A) Illustration of the fabrication procedure of the AFP biosensor based on the PEDOT/PEG layer. Reproduced from ref. 69 with permission from Elsevier, copyright 2016. (B) Schematic diagram of the fabrication process of the label-free immunosensor by AuNPs/PANI-PThi gel for antifouling. Reproduced from ref. 70 with permission from Elsevier, copyright 2018. (C) For preparing an enzyme-based glucose detector, GOx was immobilized on the PANI layer, and electrochemically surface-induced ATRP was applied to polymerization forming a zwitterionic polymer brush SBMA. Reproduced from ref. 73 with permission from American Chemical Society, copyright 2016.

enzyme-absorbed electrode with good antifouling properties via zwitterionic polymers and sulfobetaine methacrylate (SBMA)[73]. Glucose oxidase (GOx) was first physically immobilized on a polyaniline layer deposited on a Pt electrode, as shown in Fig. 6C. While adding BIBB into the system for overall bromination, electrochemically induced surface-induced ATRP was applied to SBMA polymerization soon after presenting a stable enzyme-based sensor. After testing the antifouling performance and stability by immersing the biosensors in 100% bovine serum for 15 days, the GOx-based sensor showed that 99% of the adsorption protein was blocked from the SBMA brushes, and the sensitivity remained 94%. Homola et al. also performed SIN grafting to create antibody-functionalized carboxybetaineacrylamide (pCBAA) coatings with an initiator that anchors to the surface through SAM following monomer brush polymerization[74]. This optimal method compared with standard OEG-based SAM exhibits good antifouling behavior in complex food samples.

Diazonium Electrodeposition Although these results produce good antifouling efficiency, for developing an advanced electrochemical platform, the process of polymer brush grafting is relatively complex for electrode fabrication. The diazonium salt electrografting method provides another immobilization method with advantages including a rapid and single-step process and high density[75-77]. Polymer brushes are built through

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the reduction of azydiazonium salts while radicals attack the electrode surface, resulting in strong covalent bonding between the molecule and transducer. Various diazonium derivatives are available, giving multiple choices for improving the antifouling performance for different analytes and transducers. Booksh et al. demonstrated a series of diazonium derivatives with different antifouling coatings that could be utilized on gold. The electrografting zwitterionic 4-phenylalanine diazonium chloride (4-APhe) showed the lowest fouling performance in comparison with 11-MUA and 16-MHA (SAM). Li et al. demonstrated a rapid construction antifouling surface through a high yield aniline-based zwitterionic molecule (AP-SB) (>70%) via quick electrodeposition (Fig. 7)[78]. For comparison of the antifouling resistance with literature values, SAM-based SB was also synthesized. High-density coverage was also shown in this study, with 99% of the surface efficiently modified by electrodeposition in 3 minutes, compared to 70% coverage with the SAM modification method over 3 days. AP-SB not only showed a short modification time but also revealed excellent antifouling performance in diluted FBS that was 15 times (0.04 μg/cm^2) higher than that of SAM-based SB (0.6 μg/cm^2). Both studies showed a higher fouling resistance with electrodeposition in comparison to SAM modification, indicating the surface coverage is extremely important for such zwitterionic polymer brushes as antifouling layers. The modification method is crucial in affecting factors that severely change polymer antifouling efficiency, including layer thickness, brush density, flexibility of the chains and the type of terminal groups. However, a contrary result was presented in a study by Taufik et al[79]. They also compared two different approaches, SAM and electrodeposition, for antifouling performance on gold electrodes. The SAM method showed a rather good antifouling

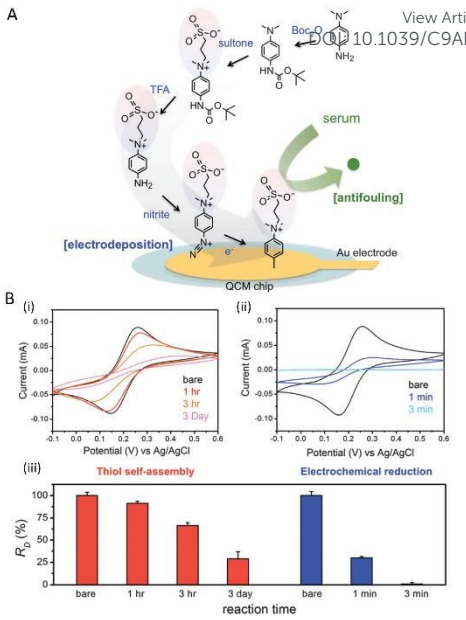

Fig. 7 Rapid construction of zwitterionic molecules (SB) on a gold electrode through diazonium electrodeposition. (A) Illustration of the electrodeposition process of the aniline-based SB. (B) Diazonium electrografting showed a higher surface coverage in CV (i) than the SAM method (ii) and had a shorter modification time (iii). Reproduced from ref. 78 with permission from Royal Society of Chemistry, copyright 2014.

behavior with less protein adsorption than diazonium electrodeposition. While the traditional SAM method was linked with a thiol-based OEG on Au, diazonium electrodeposition was applied to the OEG polymer covalently linked to the prereduction aryl diazonium salt layer for polymer grafting, resulting in a relatively lower grafting density than SAM. Although the construction method seems the same, the

Table 2. Comparison of the different properties of antifouling modification strategies

	Physical		**Chemical**			
	Blocking/Coating	Surface topology	SAM	Polymer Brush		
				Graft-to	Graft-from	Electrodeposition
Molecular type	Protein/polymer	Metal	Mainly thiolated compounds	Almost all types of polymers	Almost all types of polymers	Diazonium compounds
Transducer material	charged surfaces, hydrophobic hydrophilic surface	All electrode	Au, Ag, Cu, Pt	All electrodes	All electrodes	All electrodes
Thickness	Thin	Depends on the methods	Thin: monolayer	Thick: can be adjusted by chain length and reaction time	Thick: can be adjusted by chain length and reaction time	Thin to thick: can be controlled by potential[80]
Density	Low	-	High	Low	High	High
Stability	Low stability: easily affected by charge variance	High stability	Relatively low	High stability	High stability	High stability
Modification time	Quick	Slow	Slow	Medium	Medium	Quick

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ultimate result is highly influenced by the structural spaces between layers decided by the behavior of the polymer brushes. The diazonium modification method has great potential in preparing electrochemical biosensing systems due to its one-off usage. Rapid, simple construction methods are necessary to avoid electrode fouling problems and are easier to apply in real conditions.

4. Conclusion and perspective

Electrochemical biosensors have made significant progress in the past decade for determination-related bioanalytes in clinical applications in complex environments. Strategies for engineering antifouling electrochemical sensing systems have been developed and reported recently. Numerous studies have elaborated on the antifouling performance for biomedical applications, implant electronics and disease detection, and electrochemical biosensors are still in the beginning of converting these platforms for commercial applications. With this purpose, further challenges remain that must be overcome. Effective electrode modification strategies with a wide range of modification methods and appropriate related antifouling materials have been discussed in this review, and the comparison of the modification method is presented in Table 2. One challenge is that most of the antifouling materials offer good biocompatibility for the bioenvironment but reduce the electrochemical sensitivity due to the poor conductivity of the polymer backbone. Conducting polymers can be added into the system with other materials or as a primary layer for antifouling molecules, further increasing the electron transfer efficiency for electrochemical sensitivity improvement. However, the stability and antifouling efficiency are highly dependent on the structural strategies, detection of analytes and the environment. With some modification methods, such as redox reactions for deposited polymers, low mechanical stability and low cyclic life are observed in complex environments. Another apparent problem is that the performance in recent studies must minimize the antifouling efficiency while testing targets in practical situations. Most studies have demonstrated the sensing process in pure or relatively simple solutions in the lab. In practical applications, irrelevant molecules are numerous in complex biofluids and severely affect the analysis sensitivity. Additionally, in the presence of mostly foulants in an environment, the concentration of the target analyte, such as the biomarker, is relatively low and demands a high level of biorecognition.

For a better solution to these challenges, we expect more progress in practical applications through further understanding the fundamental aspects of every fouling phase and antifouling phenomena in interface chemistry and utilizing an appropriate modification method, from coating a layer of thin film to chemically grafting antifouling materials on an electrode surface, that is highly dependent on the analytes, environmental conditions and type of electrode. Additionally, the properties of the bio-recognition elements (antibody, nucleotide, apatamer) would reduce the antifouling efficiency under different circumstances and the effect between recognition and antifouling probes are worth to considered. Multifunctional probe including both antifouling and recognizing effect may be another balancing design in solving such problems.[81] Research should also strive to achieve success not through forced optimization of biosensors in certain conditions but by expanding the detection range for a variety of complex biofluids.

In the future, seeking a proper and effective combination of antifouling materials and modification approaches is worth devoting efforts. With the enhanced understanding of antifouling advances, electrochemical biosensors could be realized for applications in the practical world.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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References

1 P. Singh, *Sensors and actuators B: Chemical*, 2016, **229**, 110-130.
2 M.-Y. Shen, B.-R. Li and Y.-K. Li, *Biosensors and Bioelectronics*, 2014, **60**, 101-111.
3 B.-R. Li, Y.-J. Hsieh, Y.-X. Chen, Y.-T. Chung, C.-Y. Pan and Y.-T. Chen, *Journal of the American Chemical Society*, 2013, **135**, 16034-16037.
4 B.-R. Li, C.-C. Chen, U. R. Kumar and Y.-T. Chen, *Analyst*, 2014, **139**, 1589-1608.
5 P.-H. Lin, S.-C. Huang, K.-P. Chen, B.-R. Li and Y.-K. Li, *Sensors*, 2019, **19**, 28.
6 N. J. Ronkainen, H. B. Halsall and W. R. Heineman, *Chemical Society Reviews*, 2010, **39**, 1747-1763.
7 A. Warsinke, A. Benkert and F. W. Scheller, *Fresenius' journal of analytical chemistry*, 2000, **366**, 622-634.
8 P. Singha, J. Locklin and H. Handa, *Acta biomaterialia*, 2017, **50**, 20-40.
9 T. Bertok, L. Klukova, A. Sediva, P. Kasák, V. Semak, M. Micusik, M. Omastova, L. Chovanová, M. Vlček and R. Imrich, *Analytical chemistry*, 2013, **85**, 7324-7332.
10 M. Hasanzadeh and N. Shadjou, *TrAC Trends in Analytical Chemistry*, 2016, **80**, 167-176.
11 S. N. Islam, K. J. Hossain, A. Ahmed and M. Ahsan, *British Journal of Nutrition*, 2002, **88**, 507-513.
12 N. Liu, Z. Xu, A. Morrin and X. Luo, *Analytical methods*, 2019, **11**, 702-711.
13 S. Campuzano, M. Pedrero, P. Yáñez-Sedeño and J. M. Pingarrón, *International journal of molecular sciences*, 2019, **20**, 423.
14 B. Liu, X. Liu, S. Shi, R. Huang, R. Su, W. Qi and Z. He, *Acta biomaterialia*, 2016, **40**, 100-118.
15 B. L. Hanssen, S. Siraj and D. K. Wong, *Reviews in Analytical Chemistry*, 2016, **35**, 1-28.

16 V. B. Damodaran and N. S. Murthy, *Biomaterials research*, 2016, **20**, 18.
17 S. Zhang, R. Geryak, J. Geldmeier, S. Kim and V. V. Tsukruk, *Chemical reviews*, 2017, **117**, 12942-13038.
18 G. Valette, *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*, 1982, **139**, 285-301.
19 H.-J. Kim, M.-H. Piao, S.-H. Choi, C.-H. Shin and Y.-T. Lee, *Sensors*, 2008, **8**, 4110-4118.
20 C.-S. Lee, S. Yu and T. Kim, *Nanomaterials*, 2017, **8**, 17.
21 L. Jing, Y.-e. Shi, J. Cui, X. Zhang and J. Zhan, *RSC Advances*, 2015, **5**, 13443-13450.
22 E. A. Vogler, *Advances in colloid and interface science*, 1998, **74**, 69-117.
23 S. Megelski, J. S. Stephens, D. B. Chase and J. F. Rabolt, *Macromolecules*, 2002, **35**, 8456-8466.
24 S. Lüsse and K. Arnold, *Macromolecules*, 1996, **29**, 4251-4257.
25 Y. He, Y. Chang, J. C. Hower, J. Zheng, S. Chen and S. Jiang, *Physical Chemistry Chemical Physics*, 2008, **10**, 5539-5544.
26 D. Yao, W. Zhao, L. Zhang and Y. Tian, *Analyst*, 2017, **142**, 4215-4220.
27 S. Jeon, J. Lee, J. Andrade and P. De Gennes, *Journal of colloid and interface science*, 1991, **142**, 149-158.
28 S. Chen, L. Li, C. Zhao and J. Zheng, *Polymer*, 2010, **51**, 5283-5293.
29 J. Wu, C. Zhao, R. Hu, W. Lin, Q. Wang, J. Zhao, S. M. Bilinovich, T. C. Leeper, L. Li and H. M. Cheung, *Acta biomaterialia*, 2014, **10**, 751-760.
30 C. Leng, H.-C. Hung, S. Sun, D. Wang, Y. Li, S. Jiang and Z. Chen, *ACS applied materials & interfaces*, 2015, **7**, 16881-16888.
31 F. Deng, Y. Li, M. J. Hossain, M. D. Kendig, R. Arnold, E. M. Goldys, M. J. Morris and G. Liu, *Analyst*, 2019, **19**, 5682-5690
32 E. Luong-Van, I. Rodriguez, H. Y. Low, N. Elmouelhi, B. Lowenhaupt, S. Natarajan, C. T. Lim, R. Prajapati, M. Vyakarnam and K. Cooper, *Journal of Materials Research*, 2013, **28**, 165-174.
33 S.-T. Wu, C.-Y. Huang, C.-C. Weng, C.-C. Chang, B.-R. Li and C.-S. Hsu, *ACS Omega*, 2019, **15**, 16292-16299.
34 S.-T. Wu, C.-C. Weng, B.-R. Li and C.-S. Hsu, *Journal of Materials Science*, 2019, **54**, 10179-10190.
35 F. Yan, X. Lin and B. Su, *Analyst*, 2016, **141**, 3482-3495.
36 D. Rana and T. Matsuura, *Chemical reviews*, 2010, **110**, 2448-2471.
37 D. Murakami, H. Jinnai and A. Takahara, *Langmuir*, 2014, **30**, 2061-2067.
38 B. Jeong, R. Akter, O. H. Han, C. K. Rhee and M. A. Rahman, *Analytical chemistry*, 2013, **85**, 1784-1791.
39 V. Rodovalho, G. Araujo, E. Vaz, C. Ueira-Vieira, L. Goulart, J. Madurro and A. Brito-Madurro, *Biosensors and Bioelectronics*, 2018, **100**, 577-582.
40 Y. Lin, K. Liu, C. Wang, L. Li and Y. Liu, *Analytical chemistry*, 2015, **87**, 8047-8051.
41 J. Y. Lichtenberg, Y. Ling and S. Kim, *Sensors*, 2019, **19**, 2488.
42 V. c. Ruiz-Valdepeñas Montiel, J. R. Sempionatto, B. Esteban-Fernández de Ávila, A. Whitworth, S. Campuzano, J. M. Pingarrón and J. Wang, *Journal of the American Chemical Society*, 2018, **140**, 14050-14053.
43 Y. T. Cheng, D. Rodak, C. Wong and C. Hayden, *Nanotechnology*, 2006, **17**, 1359.
44 T. Hang, S. Xiao, C. Yang, X. Li, C. Guo, G. He, B. Li, C. Yang, H.-j. Chen and F. Liu, *Sensors and Actuators B: Chemical*, 2019, **289**, 15-23.
45 L. Wang, S. Guo, X. Hu and S. Dong, *Electrochemistry Communications*, 2008, **10**, 95-99.
46 S. Lee, W. Kim, C. Yim, K. Yong and S. Jeon, *RSC advances*, 2019, **9**, 761-765.
47 L.-C. Sang, A. Vinu and M.-O. Coppens, *Langmuir*, 2011, **27**, 13828-13837.
48 P. Daggumati, Z. Matharu, L. Wang and E. Seker, *Analytical chemistry*, 2015, **87**, 8618-8622.
49 J. Patel, L. Radhakrishnan, B. Zhao, B. Uppalapati, R. C. Daniels, K. R. Ward and M. M. Collinson, *Analytical chemistry*, 2013, **85**, 11610-11618.
50 T. A. Silva, M. R. K. Khan, O. Fatibello-Filho and M. M. Collinson, *Journal of Electroanalytical Chemistry*, 2019, **846**, 113160.
51 J.-G. Wu, J.-H. Chen, K.-T. Liu and S.-C. Luo, *ACS applied materials & interfaces*, 2019, **11**, 21294-21307.
52 S. Krishnan, C. J. Weinman and C. K. Ober, *Journal of Materials Chemistry*, 2008, **18**, 3405-3413.
53 Z. Matharu, A. J. Bandodkar, V. Gupta and B. D. Malhotra, *Chemical Society Reviews*, 2012, **41**, 1363-1402.
54 A. Mesnage, 2011.
55 V. Ganesh, S. K. Pal, S. Kumar and V. Lakshminarayanan, *Journal of colloid and interface science*, 2006, **296**, 195-203.
56 E. González-Fernández, M. Staderini, N. Avlonitis, A. F. Murray, A. R. Mount and M. Bradley, *Sensors and Actuators B: Chemical*, 2018, **255**, 3040-3046.
57 M. Cui, Y. Wang, H. Wang, Y. Wu and X. Luo, *Sensors and Actuators B: Chemical*, 2017, **244**, 742-749.
58 I. Zaccari, B. G. Catchpole, S. X. Laurenson, A. G. Davies and C. Wälti, *Langmuir*, 2014, **30**, 1321-1326.
59 T. M. Herne and M. J. Tarlov, *Journal of the American Chemical Society*, 1997, **119**, 8916-8920.
60 K. Malecka, A. Stachyra, A. Góra-Sochacka, A. Sirko, W. Zagórski-Ostoja, H. Radecka and J. Radecki, *Sensors and Actuators B: Chemical*, 2016, **224**, 290-297.
61 P. Jolly, N. Formisano, J. Tkáč, P. Kasák, C. G. Frost and P. Estrela, *Sensors and Actuators B: Chemical*, 2015, **209**, 306-312.
62 W. Kim and J. Jung, *BMB reports*, 2016, **49**, 655.
63 W. Yang and F. Zhou, *Biosurface and Biotribology*, 2017, **3**, 97-114.
64 Y. Higaki, M. Kobayashi, D. Murakami and A. Takahara, *Polymer Journal*, 2016, **48**, 325.
65 B. A. Laurent and S. M. Grayson, *Journal of the American Chemical Society*, 2011, **133**, 13421-13429.
66 B. Zhao and W. J. Brittain, *Progress in Polymer Science*, 2000, **25**, 677-710.
67 T. Goda and Y. Miyahara, *Langmuir*, 2018, **35**, 1126-1133.
68 H. Wu, C.-J. Lee, H. Wang, Y. Hu, M. Young, Y. Han, F.-J. Xu, H. Cong and G. Cheng, *Chemical science*, 2018, **9**, 2540-2546.
69 M. Cui, Z. Song, Y. Wu, B. Guo, X. Fan and X. Luo, *Biosensors and Bioelectronics*, 2016, **79**, 736-741.
70 L. Zhao and Z. Ma, *Analytica chimica acta*, 2018, **997**, 60-66.
71 N. Hui, X. Sun, S. Niu and X. Luo, *ACS applied materials & interfaces*, 2017, **9**, 2914-2923.
72 S. Edmondson, V. L. Osborne and W. T. Huck, *Chemical society reviews*, 2004, **33**, 14-22.
73 Y. Hu, B. Liang, L. Fang, G. Ma, G. Yang, Q. Zhu, S. Chen and X. Ye, *Langmuir*, 2016, **32**, 11763-11770.
74 H. Vaisocherová-Lísalová, I. Víšová, M. L. Ermini, T. Špringer, X. C. Song, J. Mrázek, J. Lamačová, N. S. Lynn Jr, P. Šedivák and J. Homola, *Biosensors and Bioelectronics*, 2016, **80**, 84-90.
75 D. Belanger and J. Pinson, *Chemical Society Reviews*, 2011, **40**, 3995-4048.
76 T. Menanteau, E. Levillain and T. Breton, *Chemistry of Materials*, 2013, **25**, 2905-2909.
77 J. Pinson and F. Podvorica, *Chemical Society Reviews*, 2005, **34**, 429-439.
78 B.-R. Li, M.-Y. Shen, H.-h. Yu and Y.-K. Li, *Chemical Communications*, 2014, **50**, 6793-6796.
79 S. Taufik, A. Barfidokht, M. T. Alam, C. Jiang, S. G. Parker and J. J. Gooding, *Journal of Electroanalytical Chemistry*, 2016, **779**, 229-235.

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DOI: 10.1039/C9AN02017A

80 L. Pichereau, I. López, M. Cesbron, S. Dabos-Seignon, C. Gautier and T. Breton, *Chemical communications*, 2019, **55**, 455-457.
81 N. Liu, N. Hui, J. J. Davis, and X. Luo, *ACS Sensors*, 2018, **3**, 1210-1216.

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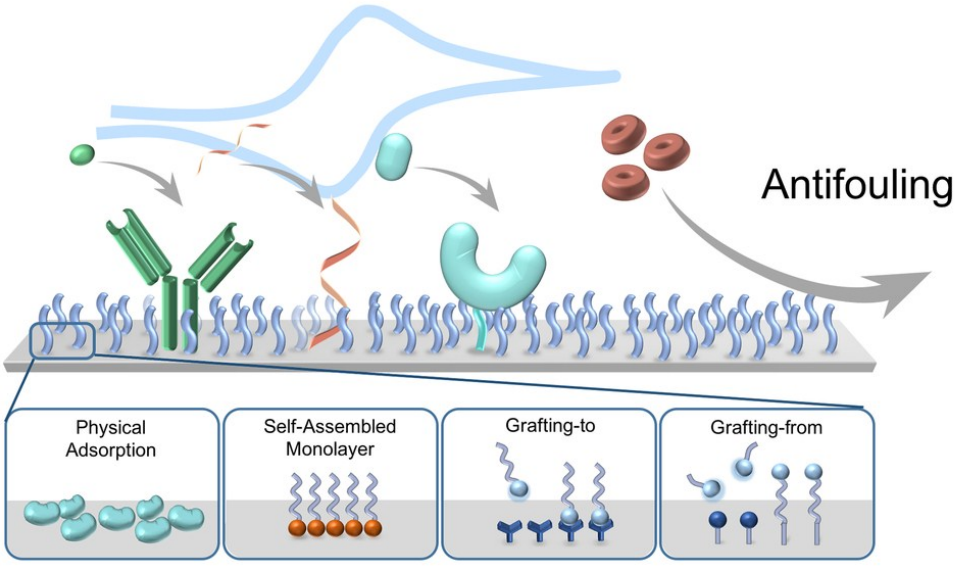
DOI: 10.1039/C9AN02017A



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