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Historical Perspective

Rational design of smart nano-platforms based on antifouling-nanomaterials toward multifunctional bioanalysis

Mansour Mahmoudpour [a,b], Abolghasem Jouyban [a,c], Jafar Soleymani [a,*], Mahdi Rahimi [d,e,**]

[a] Pharmaceutical Analysis Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Liver and Gastrointestinal Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Faculty of Pharmacy, Near East University, PO BOX: 99138 Nicosia, North Cyprus, Mersin 10, Turkey
[d] International Center for Research on Innovative Biobased Materials (ICRI-BioM)—International Research Agenda, Lodz University of Technology, Lodz, Poland
[e] Lodz University of Technology, Institute of Polymer and Dye Technology, Stefanowskiego 16, 90-537 Lodz, Poland

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ABSTRACT

The ability to design nanoprobe devices with the capability of quantitative/qualitative operation in complex media will probably underpin the main upcoming progress in healthcare research and development. However, the biomolecules abundances in real samples can considerably alter the interface performance, where unwanted adsorption/adhesion can block signal response and significantly decrease the specificity of the assay. Herein, this review firstly offers a brief outline of several significances of fabricating high-sensitivity and low-background interfaces to adjust various targets' behaviors induced via bioactive molecules on the surface. Besides, some important strategies to resist non-specific protein adsorption and cell adhesion, followed by imperative categories of antifouling reagents utilized in the construction of high-performance solid sensory interfaces, are discussed. The next section specifically highlights the various nanocomposite probes based on antifouling-nanomaterials for electrode modification containing carbon nanomaterials, noble metal nanoparticles, magnetic nanoparticles, polymer, and silicon-based materials in terms of nanoparticles, rods, or porous materials through optical or chemical strategies. We specially outline those nanoprobes that are capable of identification in complex media or those using new constructions/methods. Finally, the necessity and requirements for future advances in this emerging field are also presented, followed by opportunities and challenges.

1. Introduction

Nowadays, one of the most important challenges in clinical diagnosis is the progress of medical devices to screen the biochemical or biological procedures in “real-life” samples. As a portable and economical analytical technique, electrochemical biosensors remove the classical analytical matters since they deliver highly selective and sensitive detection with the rapid signal response. Electrochemical biosensors are increasingly important in biological assays to monitor the mechanisms that regulate diverse metabolic pathways and any possible changes in physiological conditions [1,2]. Nevertheless, in many biological and clinical diagnoses, they should be utilized in real multifaceted media for direct detection of targets, which examines with these tools difficult as a result of the intense biofouling. Biofouling is a phenomenon that generally arises from unwanted adsorption of the coexisting materials on the device surface, and therefore unfavorably affects the analytical efficiency via impeding the target's transport and signal transduction. Lipids, proteins, polysaccharides, and cells are the main fouling substances in which protein adsorption is a determined challenge for any engineered surfaces that are exposed to biological samples. Such unwanted protein adsorption can lead to decreased sensor performances. To address this issue, it is important to interpret the interfaces and proteins interactions. As is well known, when fabricated interfaces were subjected to biological media, the biofouling procedure could be triggered with deleterious properties [3]. The foulant adhered onto the platform surfaces arises from superficial nucleation, electronic attraction, and hydrophobic effects, which result in a dense layer that passively creates on the designed interface and blocks detection units on the electrode surfaces. The binding affinity and selectivity of biological sensing units were intensely affected via the fouling layer, resulting in

* Corresponding author.
** Corresponding author at: Lodz University of Technology, Institute of Polymer and Dye Technology, Stefanowskiego 16, 90-537 Lodz, Poland.
E-mail addresses: soleymanij@tbzmed.ac.ir (J. Soleymani), mahdi.rahimi@p.lodz.pl (M. Rahimi).

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false positives, impaired specific recognition/response, and lower signal to noise [4]. This fouling layer either prevents physical contact between the targets and interfaces or immediately blocks the signal responses through electron transfer inhibitions.

Therefore, the need to detect target molecules in real samples motivated the researchers to investigate surface functionalization with antifouling materials spanning from biomedical to bioanalysis applications including human disease biomarkers monitoring, hormone detection, determination of drug concentrations in serum, plasma or blood, and so on [169]. To preserve reliable examination results in the presence of various interference elements in clinical use or other complicated real samples, different antifouling interface buildings, have been reported [5]. For the construction of antifouling nanoprobes, one of the most actual approaches is incorporating antifouling nanomaterials on an electrode interface that can reduce fouling matters and decrease nonspecific binding. These antifouling reagents should be naturally inert, biocompatible, and stable in a complex matrix like wastewater, food matrix, urea, sweat, blood, tissue, and other biological fluids [6,7]. Different antifouling functional materials like acrylamides, polysaccharides, and oligomers or polymers using ethylene glycol via various antifouling strategies have been suggested to meet these requirements. Moreover, the reliability, reproducibility, selectivity, and sensitivity of sensing devices can also be influenced via antifouling strategy. Once assessing an antifouling strategy for a specified sensing substrate, it is imperative to select the suitable target and sample matrix to evaluate the fouling influence. The application of sole-molecule fouling solution like bovine serum albumin, clean buffer solutions like phosphate-buffered saline (PBS) with neutral pH levels, and even diluted-pretreated biological media were appropriate for proof-of-concept antifouling capabilities [8]. Besides, the charge density of various polymer-based antifouling layers could be affected by the sample pH, where more basic/acidic solutions can defect in the antifouling layer and decrease the overall coating stability [9]. Incubation/rinse techniques are also used to evaluate antifouling ability in electrochemical biosensing. These methods normally contain a primary incubation stage, where an antifouling-treated system is exposed with fouling solution for a given period. Then, this platform is rinsed with buffer solution or water to eliminate the fouling media. Finally, the surface is exposed to the analyte solutions, followed by electrochemical measurement [10–13]. According to reagent varieties, different modification strategies have been conducted onto the substrate either by altering the chemical characteristics or generating a physical topological construction [14–16]. In sum, to suppress unwanted adsorption on the electrode surface, the designing and expansion of various nanocomposite probes based on antifouling-nanomaterials that show high resist fouling is urgently needed to enhance the sensitivity and accuracy of biosensors. Various nanocomposite probes based on antifouling-nanomaterials for electrode modification including carbon nanomaterials, noble metal nanoparticles (NPs), magnetic NPs, polymer, and silicon-based materials in terms of NPs, rods, or porous materials through optical or chemical strategies.

Considering the aforementioned concept, first of all, this review summarizes the most widely used antifouling materials along with antifouling strategies and describes their syntheses and structural characteristics. Then, it is described the most current progress in the construction of solid sensory interfaces based on antifouling-nanomaterials for electrode modification containing carbon nanomaterials, noble metal NPs, magnetic NPs, polymer, and silicon-based materials in terms of NPs, rods, or porous materials through optical or chemical strategies. Finally, insights were given on the future applications and prospects of antifouling materials in the context of biomedical applications.

2. The antifouling strategies in constructing of bioactive interface

Numerous research has been reported concerning the improvement of cell adhesion modified interfaces. For instance, a sequence of a peptide could be attached to different bioactive interfaces in order to obtain a well-defined fundamental adhesion platform. Arg-Gly-Asp (RGD) sequence containing peptide is an example that has been used vastly for immobilization on the desired surface via physical or chemical coating approaches [17–20]. Some investigations revealed that the chemically bonded RGD on the coatings shows much better anti-wash-out ability and an effective way to favor the interaction of cells in comparison to physical adsorption coatings [21]. As a consequence, there is a need for effective linkers which can connect the peptide directly to the surface of the substrate. The most common linker used in recent studies is 3-aminoalkyltriethoxysilane and poly(ethylene glycol) diamine with various lengths [22,23]. It has been determined that once proteins are attached to the substrate models, the anchored ligands might be blocked and different proteins ligands might enter the surface, thus changing the composition of the entire surface. Therefore, inhibition of adsorption/adhesion nonspecific cell and protein in designing bioactive interface is extremely imperative to guarantee that the active biomolecules onto the surface can effectively demonstrate their basic biological functions.

The development of antifouling strategies in fabricating bioactive interfaces has attracted increasing attention because of their potential in considering the issues listed above. In this section, we will accentuate the progress of antifouling approaches in constructing bioactive interfaces via two main categories; physical and chemical modifications.

2.1. Bovine serum albumin blocking approach

Bovine serum albumin (BSA) is the broadly studied protein because of its inexpensive, safe, ready availability, well-stability, and ideal ligand binding properties. It can also reduce the steric hindrance of specifically binding proteins [24]. As this protein can prevent adsorption/adhesion nonspecific protein and cell, BSA surface passivation “blocking” was frequently used in various bioassays to decrease background noise [25]. The interface substrate is treated with blocker BSA solution in which the protein biomolecules are adsorbed non-covalently and subjected to a structural alteration (“denature”) on the surface to produce an ultra-thin monolayer of proteins. The BSA blocking approach has several flaws, including a nonuniform layer and a potential steric hindering effect on small molecules [26]. The performance coating layers are sensitive to different material parameters like nano-curvature effects, surface roughness, and atomic composition, as well as environmental elements such as ionic strength and pH solution [27–29]. Besides, in a complex serum medium, BSA can be substituted by some other proteins as they are typically attached through a non-covalent bonding on the surface. Additionally, a major disadvantage associated with the use of BSA is that it lacks the diversity which is required to block adsorption sites on different surfaces such as hydrophobic or hydrophilic surfaces. Most importantly, BSA has intrinsic limitations like susceptibility to proteolysis and denaturation under certain conditions, which can decrease the service life of bioactive surfaces and antifouling efficiency.

2.2. Self-assembled monolayers approach

Self-assembled monolayer (SAM) is a common approach for fabricating of target adhesion interface with a high-density of monolayer surface-bound functional biomolecules. In this method, molecules could be grafted via chemical interaction for constructing a layer with dense packing and uniform thickness. The thickness of SAMs is usually thinner than 10 nm depending on the molecular size of deposition molecules [30]. The deposition of a monolayer in the form of a thin film on a solid surface was first studied by Blodgett in 1935 [31]. An appropriate

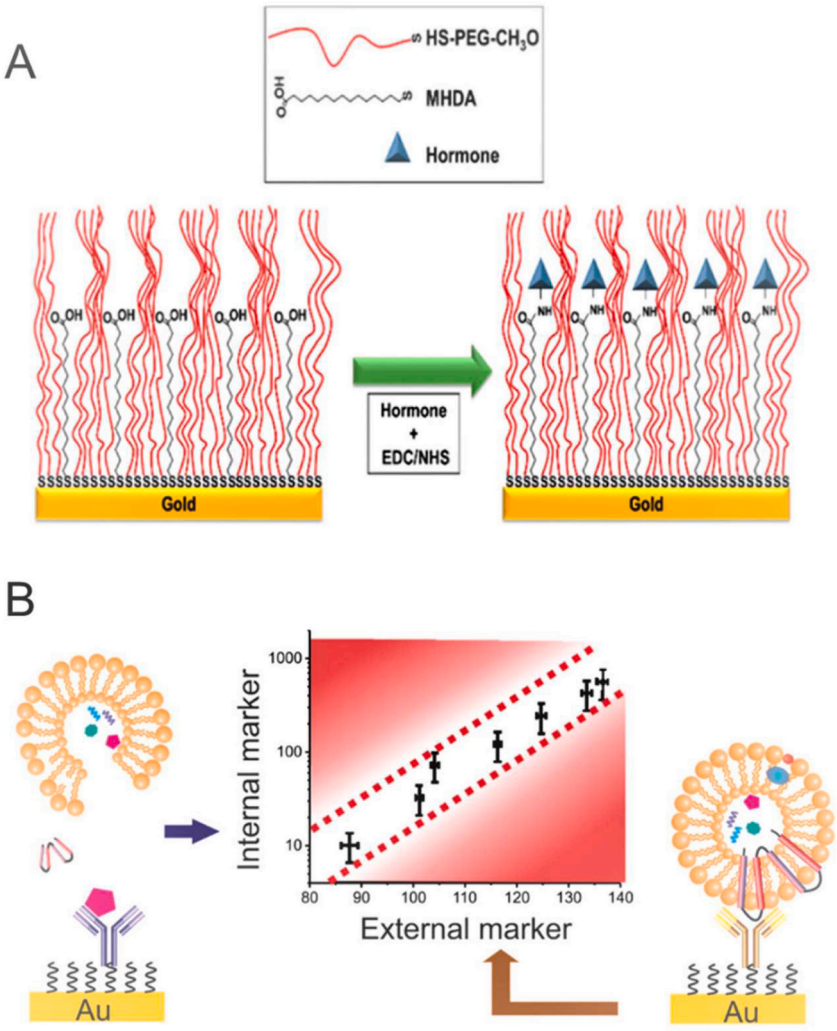

Fig. 1. (A) Schematic diagram for the surface functionalization of thin Au films with a mix SAM of thiolated PEG as a spacer and antifouling element and 16 mercaptohexadecanoic as a linker (Reprinted with permission from [57], Copyright 2018 American Chemical Society). (B) Approaches diagram to quantify both internal and external exosome-specific markers based on electrochemical impedance spectroscopy (Reprinted with permission from [63], Copyright 2017 American Chemical Society).

substrate is chosen to react with the head group of antifouling molecules and it can be planar surfaces, such as silicon and metals (gold or silver), or curved surfaces, as well as NPs. Alkanethiolates as a commonly used compound can be self-assembled on a gold substrate to make a well-organized monolayer depending on the quality of the functional groups exposed to the surface [30]. Using this method, researchers can design a well-defined chemical interface to evaluate particular analyte-platform interactions, though, selecting the type of head group depends on the application of the SAM. Another advantage of the SAM approach is introducing desired molecules (target biomolecules and functional elements) on the surface before or after the formation of the monolayer via facile surface modification processes. For instance, one strategy for modification of Au substrate to obtain antifouling properties is forming a SAM by using a series of oligo(ethylene glycol)-terminated alkanethiol amides and/or their analogous esters [32,33]. Poly(ethylene glycol) and oligo(ethylene glycol)-based materials are commonly used in anti-fouling SAM methods due to their stability in biological environments. This technique has been widely used in biological studies to analyze the factors that influence the minimization of protein resistance [34,35]. Although the SAM approach usually reveals considerably better anti-fouling qualities than others like the BSA "blocking", however, it has some inherent shortcomings. Instability of prepared SAM over the long term is the crucial drawback of this method, so, the designed SAM interface must be immediately used and cannot be exposed in the air for a long time. [36].

2.3. Polymeric coating approach

The polymeric coating strategy is a more extensive approach for fabricating bioactive interfaces. The surface modification can be either obtained via a physical or chemical modification. Surface modification of a substrate with a polymer via physical absorption is the simplest way leading thermally and shear forces unstable modified surface with easily displaceable coating by chemicals or proteins and cells [37]. The typical strategies for coating polymer via chemical modifications on the substrates are 'grafting-to' and 'grafting-from' approaches. The grafted substrate extends nanometers of thickness with the vertical development of the polymer chain extension. These strategies have attracted great attention due to utilizing electrodes other than gold, such as carbon, silver, silica, etc. [38]. In the grafting-to method, polymer chains are grafted on the surface activated previously. However, the polymer chains can be grown from the surface via a grafting-from method [37,39,40]. The grafting method is preferred for fabricating bioactive interfaces since it is possible to achieve higher grafting density and film thickness. Because grafting-to method has some limitations due to steric hindrance between polymeric chains, which makes it difficult to tether chain ends at short intermolecular distances [37]. In this regard, the desired substrate can be functionalized with various specific molecules to initiate a polymerization reaction on its surface. Several polymerization methods have been developed for grafting polymer on a surface, including ring-opening polymerization (ROP), ring-opening metathesis

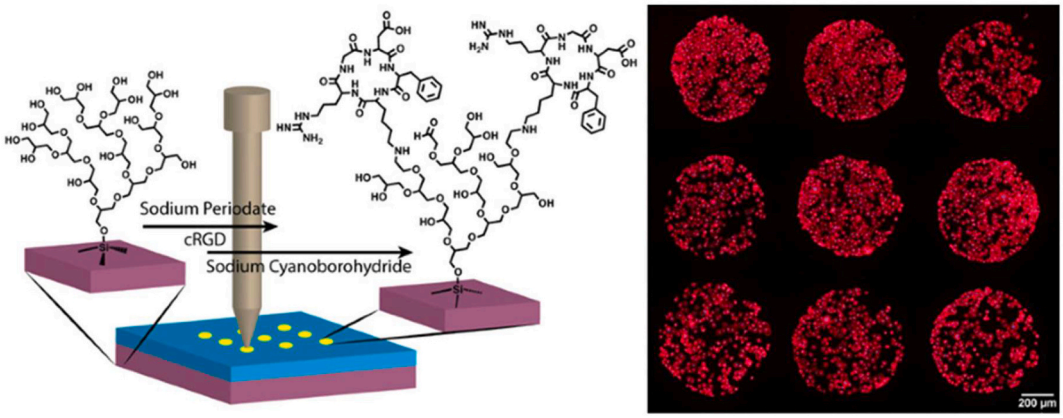

Fig. 2. Functionalization and patterning of antifouling hyperbranched polyglycerol coatings on glass and silicon platforms (Reprinted with permission from [68], Copyright 2014 American Chemical Society).

polymerization (ROMP), or radical polymerization [41]. On the other hand, well-established techniques for controlled polymerization are also available such as atom transfer radical polymerization (ATRP), nitroxide mediated radical polymerization (NMP), or reversible addition-fragmentation chain transfer (RAFT) [43,168].

Previous investigations proved the antifouling activity of hydrophilic polymers and hydrogels against some sessile organisms, cells, and proteins [44,45]. Surface decorating of polymeric coating with biomolecules (peptide, lipid, carbohydrate) could enhance the antifouling properties. As a consequence, the fabrication of an antifouling layer based on polymeric coating can usually provide tremendous resistance to fouling. Although many factors are involved in the antifouling properties, however, the density of biologically active molecules strongly affects the target adhesion.

3. Selection and immobilization of antifouling agents

Over the past years, an extensive range of molecular systems was suggested for the construction of low fouling interfaces, including polyethylene glycol (>10 ethylene glycol units) and its derivatives, zwitterionic species (carboxybetaine, sulfobetaine, and phosphorylcholine), peptides with alternating or randomly mixed negatively charged using aspartic acid or glutamic (D/E) and the positively charged arginine (R) and lysine (K) [46–50]. Some other polymers, like hyperbranched polyglycerol, polyoxazolines, polysaccharides are also used for this purpose [51–53]. In this section, we will introduce antifouling agents which are vastly used in constructing bioactive interfaces.

3.1. Polyethylene glycol as antifouling agents

Owing to the highly biocompatible, good hydrophilic, and nontoxic, polyethylene glycol (PEG) was extensively applied to decrease unwanted protein adsorption, and it has often been named the "gold standard" of anti-biofouling materials [6,54]. Meantime, the protein resistance of PEG coatings is associated with two key mechanisms; (i) water barrier and (ii): steric repulsion. This is an entropic result strongly related to the adverse alteration of free energy associated with the dehydration and entrapment of soft polymer chains. Moreover, the PEG antifouling efficiency is usually depending on the grafting method along with the polymer construction. For example, the "grafting from" process needs somewhat intricate chemical production and caused brushes without satisfactory density [55,56]. Nevertheless, "grafting to", as another alternative approach, was demonstrated to be a surprisingly operative technique to form inert surfaces toward various bio-molecular adsorption. PEG could be anchored to interfaces via covalent bonds formation using "grafting to" techniques to create well-defined polymer brushes. For instance, PEG chains were coupled to a polylysine backbone with a high density, which was assembled through electrostatic interactions on different oxide surfaces, and the PEG branches have required to be extended into the solution. Grafting of long-chain antifouling polymers directly on the surface of the electrode, like PEG, is not conducive, however, can cause layer creation with high impedance and consequently in electrode sensitivities. It was hoped that the combination of PEG polymer with determined conductive soft nanomaterials like conducting polymers, will be useful to solve this problem.

Recently multiplex SPR sensors using PEG-based SAMs were developed for the simultaneous determination of somatostatin, glucagon, and insulin as three human pancreatic islets peptide hormones [57]. In this study, an antifouling substrate contained a combined self-assembly monolayer (SAM) of 16-mercaptohexadecanoic acid and CH_3O-PEG-SH for immobilization of antibody has been designed. Antibody functionalized spots on the chip surface exhibited minimal interference from 1 mg mL^{-1} lysosome or BSA with excellent specificity to the corresponding hormones (Fig. 1A). While it has not been proven that this platform can be operated in real-life samples, the simultaneous sensing of diverse biomarkers is of importance in clinical diagnosis. In another work, Xu and his coworker introduced a label-free and facile impedimetric platform based on the SAM of PEG containing thioland carboxylic acid to determine insulin as simultaneous antibody attaching support and antifouling layer. They revealed that this method can identify insulin in 50% serum with negligible interference (3%), a clinically linear range of 5 pM-50 nM, and a limit of detection (LOD) of 1.2 pM [58]. Similar platforms have also been suggested based on at anti-CRP-EG-SAMs modified for the identification of C-reactive protein (CRP), a significant biomarker of inflammation and cardiac stress), where an immittance function method was engaged to extract the most sensitive analytical factor [59]. Recently, the same group was utilized electrochemical impedance spectroscopy (EIS) analysis at EG-SAMs-Au substrate to examine both internal (syntenin) and external (tetraspanin) with high sensitivity in the exosomal lysate (Fig. 1B). Regardless of widespread applications as an anti-fouling nanomaterial, it is worth noting that PEG chemistry has many insuperable defects like those related to the chemical tailor-ability, sensitivity to oxidative destruction, and decomposition in the existence of transition metal ions and oxygen (hindering its functionality/long-term stability) [60–62].

3.2. Polyglycerols as antifouling agents

Hyperbranched polyglycerols (HPGs) are the new-fangled emerging polymers including linear, dendritic, and terminal repeating glycerol units. HPG with branched and linear constructions is regarded as a substitute to PEG because this polymer has better resistance to oxidation

and heat along and high functionality with an equivalent antifouling performance with PEG [64,65]. Furthermore, bioconjugation and activation of HPG can be conducted simply through a one-pot reaction. These properties induce HPG as one of the most promising polymers broadly utilized in different biomedical applications [66,67]. HPG has been reported and confirmed to have superior antifouling features. In this regard, Voelcker and coworkers have engaged an ultra-low fouling HPG as the coating layers on glass and silicon platforms, thereby designing them with RGD for evaluation of cell adhesion (Fig. 2). The results proved that the decorated interface exhibited high-contrast fibroblast microarrays that could be feasibly utilized in microarray [68]. Likewise, Haag and colleagues synthesized a soft and robust 3D dendritic HPG-based hydrogel peptide array toward antibody determination with enhanced selectivity and sensitivity. This hydrogel substrate is adjustable for peptide and antibody diffusion and can covalently bind each peptide for specifically identifying its resultant antibody. This property has been explored through two different types of peptides: GSH peptide-anti GSH antibody and FLAG peptide-anti FLAG antibody and the obtained results revealed the high specificity of this platform [69].

In comparison to PEG which contains only terminal hydroxyl groups for functionalization, HPG has more hydroxyl groups as functionalization positions. This is the main benefit of HPG application as an interface coating for more adjustment by functional biomolecules. Nevertheless, the anchored ligands could be present heterogeneously in the HPG construction, which limits the progress of additional quantitative analyses. In sum, the suitable choice of HPG configuration and the density of functional biomolecules should be considered and sensibly adjusted once based on HPG as the anti-fouling biomolecule to adapt diverse interfaces.

3.3. Zwitterionic polymers as antifouling agents

Zwitterion compounds are a type of electrically neutral compound containing both cations and anions in their molecular structures (an equal number of positively- and negatively-charged functional groups). Besides, zwitterionic polymers, including polyampholytes and polybetaines, are polymers that consist of oppositely charged cationic and anionic groups along the chain or side chain [70,71]. In the first type, the zwitterionic electrolyte polymer has the same amount of positive and negative charges generated from different monomers. In the second one, both negative and positive moieties are presented in a single monomer, such as poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), poly(sulfobetaine) (PSB), poly(carboxybetaine) (PCB), and poly[2-(methacryloyloxy)ethyl choline phosphate] (PMCP). Although the history of preparation and investigation of synthetic polyzwitterion compounds (polyampholytes and polybetaines) is known since 1950, however, novel polymerization approaches (such as ATRP, RAFT ROP, etc.) have been established and new materials would be generated based on such monomers via the facile and tunable methods [72].

As a consequence, zwitterionization is emerging as a powerful strategy to design advanced biomaterials for the diagnostic and treatment of various diseases that are envisioned to result in better clinical outcomes [73]. A large number of studies have shown that zwitterionic polymers are polar and can combine with water molecules to form a hydration layer through strong ionic solvation leading to leads to “superhydrophilicity” and high protein resistivity. They can block effectively the adhesion of proteins, platelets, other biomolecules, and bacteria to the surface, and improve the blood compatibility of materials [73]. Therefore, these materials have tremendous potential as vehicles to aid in drug and gene delivery, or as anti-fouling coatings and stabilizers for NPs and proteins, and as hydrogel for self-healing materials, etc. [74–77].

The outer surface of non-thrombogenic erythrocyte cell membranes has zwitterionic phospholipids which show significant antifouling properties [15]. Thus, researchers have explored the potential utility of zwitterionic molecules and their structural analogs in a variety of biomedical applications [78]. Like many carboxybetaines, densely packed poly(sulfobetaine methacrylate) (polySBMA)-grafted surfaces have also been found to be completely resistant to the adsorption of several plasma proteins including human serum albumin, gamma globulin, fibrinogen, and lysozyme, even at low ionic concentrations [79]. Poly(carboxybetaine methacrylate) (polyCBMA) is also an interesting polymer for making blood-compatible surfaces, because of its structural comparability with the naturally occurring glycine betaine. Furthermore, the surface carboxyl groups polyCBMA provide a suitable platform for covalent immobilization of bioactive species such as monoclonal mouse antibodies for specifically binding to human chorionic gonadotropin (hCG) while retaining the nonspecific protein repellency [80–82]. This dual functional behavior of polyCBMA makes it useful for the design of antifouling surfaces for biosensors and diagnostic applications.

3.4. Peptides as antifouling agents

Synthetic peptides have become a prospective candidate, not only owing to their inherently exceptional biocompatibility that is favorable to maintain biomolecules activity, but also as a result of their flexible configurations that are appropriate to attach diverse monomers [83]. Peptides by suitable charge neutrality interface and hydrophilicity could be designed via changing the types and number of amino acid monomers. Excellent hydration of the surface could significantly inhibit the adsorption of protein via hydrophobic force. The recent research claimed that hydrophilic peptides realized surface hydration by hydrogen bonding, and mixed-charged peptides like mixed charged SAMs, coatings, or hydrogels realized their surface hydration by ionic solvation [84]. Indeed, these mixed-charged groups, once equally dispersed at the molecular level, were comparable to zwitterionic groups and show extreme resistance to undesirable various proteins adsorption. Likewise, in another hopeful study, a substrate was synthesized to monitor peptides presenting the capability of removing protein binding [85]. According to that study, it was perceived that the combination of charged residues of amino acid nearby in neutral charge possesses the lowest fouling degrees. So, similar to other antifouling reagents, electric neutrality, and strong hydrophilicity are also two imperative elements that can influence the antifouling ability of peptides. Optimistically, such two issues can be easily predicted and adjusted via the accommodation and selection of diverse amino acids. Peptides are mainly categorized into amphipathic and zwitterionic peptides. Zwitterionic peptides exhibited superior antifouling performing to the amphiphilic, nonionic peptides. Two types of peptides with amphiphilic CYSYSYS and zwitterionic CRERERE sequences were anchored via Au—S bonds onto gold platforms, and their antifouling efficiency has been examined. Even though the negligible protein adsorption (1.97–11.78 ng cm^{-2}) insole protein samples have been observed in both peptide self-assembled monolayers, the zwitterionic type was indicated good antifouling performance in natural protein media owning to the higher surface hydration of zwitterionic constituent in the peptide skeleton [86]. Besides, the zwitterionic peptides with hydrophobic properties can prevent the approach of biofouling targets to the surface of the electrode via hydrophobic force, while the zwitterionic peptide with electro-neutrality of positive/negative charges can prevent the approach of biofouling targets by the absorbing of opposite charges. Moreover, in comparison to other reported zwitterionic nanomaterials like sulfobetaine, phosphorylcholine, and carboxybetaine, zwitterionic peptides with the specific

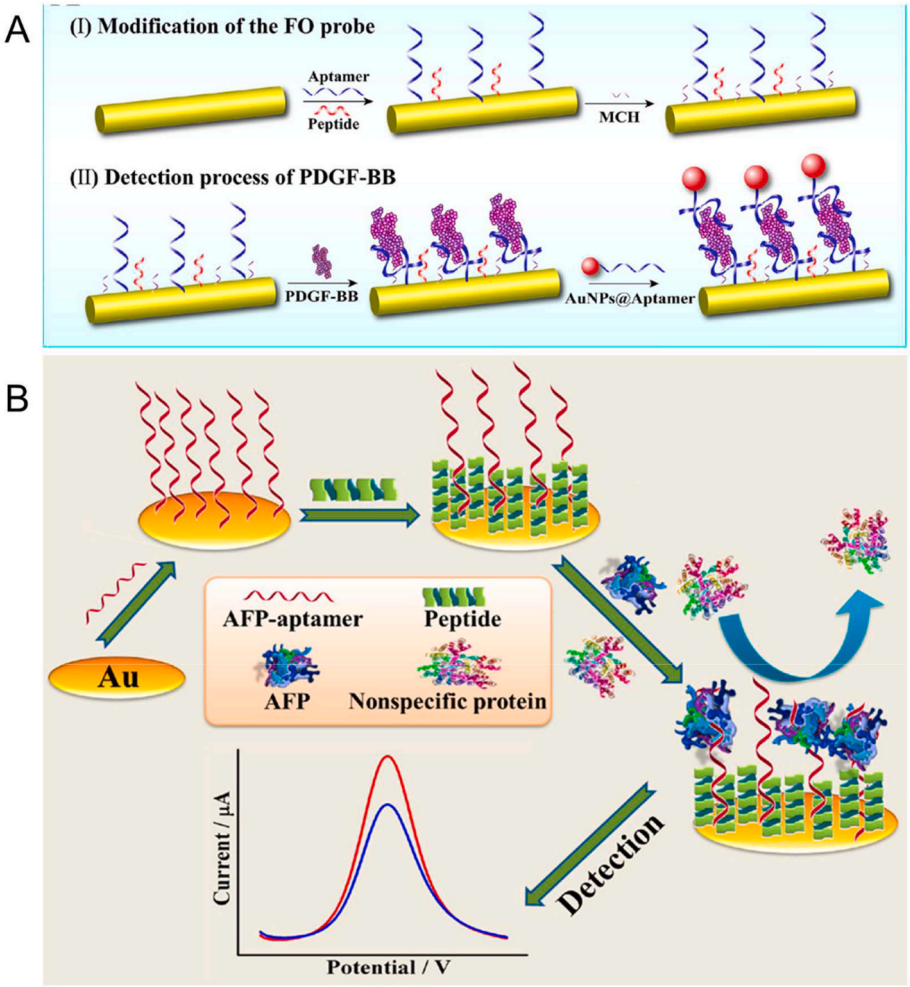

Fig. 3. (A) Construction step of FO-SPR biosensing detection system using antifouling peptide monolayer and self-assembled aptamer (Reprinted with permission from [93], Copyright 2019 Elsevier with license code: 5244250224845). (B) Schematic diagram of the self-assembly of short-chained peptides and long-chained AFP-specific aptamers onto AuE surface for alpha-fetoprotein detection (Reprinted with permission from [98], Copyright 2017 American Chemical Society).

feature of adjustable sequence, facile synthesis, excellent hydrolytic stability, high oxidative resistance, and low cost have garnered a lot of attention as hopeful candidates to PEG in designing of antifouling surfaces with high performance [87–89].

These beneficial properties lead to the emergence of various zwitterionic antifouling peptides that can be incorporated into sensor substrates as protective coatings [90,91]. The construction manners between recognition probes and antifouling peptides are generally considered two types: covalent binding and self-assembly. In light of the significant antifouling ability of EKEKEKE-PPPPC peptide, many nanoprobe substrates were suggested for the quantification of biomarkers [92]. Herein, Qian and colleagues introduced a home-built fiber optic surface plasmon resonance (FO-SPR) nanoprobe to determine platelet-derived growth factor (PDGF-BB) as an imperative tumor biomarker [93]. The EKEKEKE-PPPPC peptide and AuNPs-aptamer attached to the FO probe via Au—S binding to form antifouling surfaces. To explore the antifouling capability, the sole proteins of LYS and BSA have been engaged as test species to incubate with FO probes (Fig. 3A). Also, the SGKGSSGSST sequence, wherein glycine (G) was not hydrophilic but imperative for the creation of the helical building, was demonstrated superb ability in inhibiting unwanted proteins adsorption [94]. White et al. have introduced the peptide capabilities with glutamic acid has and lysine (K) or alternating poly EK in nonspecific adsorption resistance. According to the key fabricate rules of uncharged and hydrophilicity, the EESKSESKSGGGGC peptide sequence was synthesized and its protein-resistance quality was revealed via designing a sensing interface.

In general, one current method of usual sensing substrates, incorporating both the antifouling reagents and the bio-recognition unit at a single layer, suffers the shortcoming of relatively low loading of a molecule, which can inhibit the signal response due to improper biomolecules penetration in the highly packed brush film [95]. Direct grafting of the recognition elements to the antifouling layers is another technique, which could induce high analyte binding, however, it could also cause high non-specific adsorption, owing to the loose polymeric construction [96]. In this regard, a two-layer engineered scaffold has been reported for high target loading and low protein fouling by Jiang et al., and Yu et al. suggested a general concept of mixed self-assembled monolayers containing fructose probe and antifouling polymer [97]. Likewise, Cui et al. described a two-layer engineered platform for quantitative analysis of alpha-fetoprotein (AFP) via mixed self-assembled of EESKSESKSGGGGC peptide sequence and specific aptamer (Fig. 3B) [98]. These engineered layers exhibit ultra-high protein loading and ultra-low biofouling via the differential pulse voltammetry (DPV) method in various human serum solutions (*V*/V) and human serum albumin (HSA) concentrations. With such a low fouling peptide, the sensor responded to the analyte with a striking linear range from 10.0 fg mL^{-1} to 100.0 pg mL^{-1} and a LOD at 3.1 fg mL^{-1},

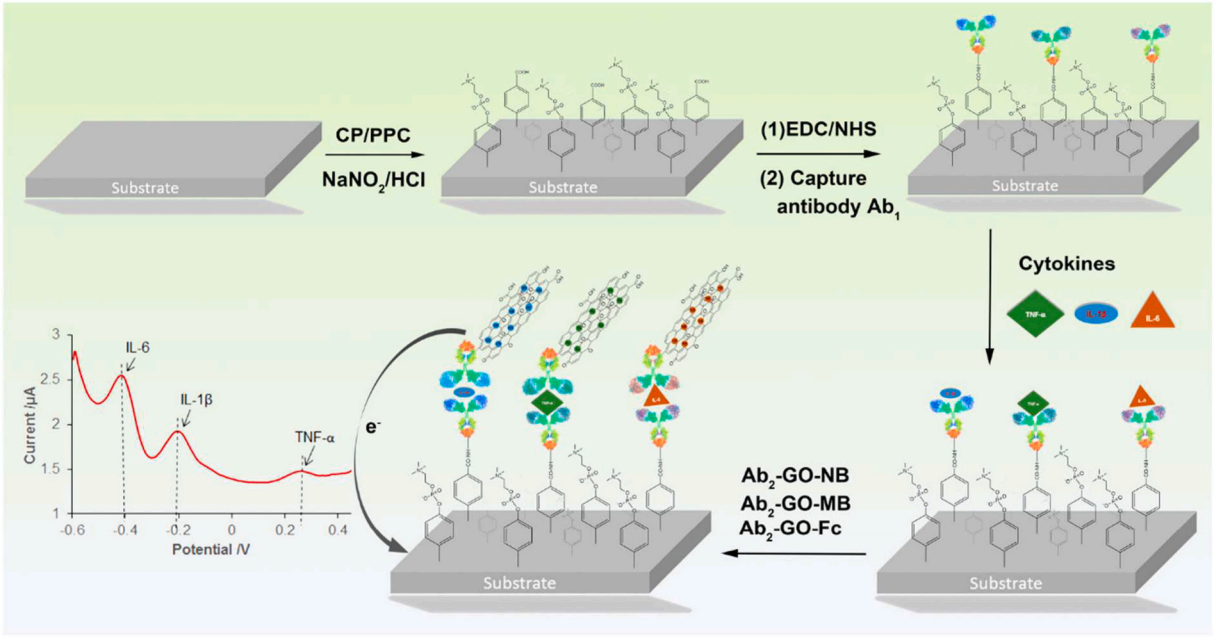

Fig. 4. Construction of the immunosensor for simultaneous determination of three cytokines IL-6, IL-1β, and TNF-α (Reprinted with permission from [110], Copyright 2018 American Chemical Society).

presenting hopeful feasibility in selective and sensitive AFP identification.

In light of their brilliant antifouling abilities, few zwitterionic materials are industrially accessible that require complex fabrication and purification of these high-charged molecules. Additionally, it can be assumed that the electric field particularly in electrochemical biosensing and the external pH can be influenced the overall status of the interfacial charge and thus the antifouling behavior.

4. Materials for antifouling sensing

Recently, there is a growing trend in the electrochemical biosensors field, particularly, in the advancement of new substrates which integrate antifouling strategies with high-performance scaffolds like those containing nanoporous- or nanocarbon-based surfaces (i.e., CNTs and graphene oxide) and/or nanoparticle-based metals. In light of that, the next sections emphasize the recent progress of various nanostructures based on nanomaterials and antifouling reagents in bioanalysis (See Table 1).

4.1. Combination of carbon-based nanomaterials with antifouling reagents

Carbonaceous nanomaterials were proposed as appropriate choices in diverse biosensing applications, because of their extraordinary characteristics like aspect ratio, robust mechanical strength, biocompatibility, high chemical stability, and electrical conductivity [99–101]. Their brilliant electrochemical features generally depend upon the different synthesis procedures. Hence, many morphological carbon variants (for example zero to three-dimensional scales) have been effectively employed as a well-organized electrode nanostructure for biosensing applications. Besides, their capability as non-quenchers or transducers along with tunable bandgap and high elasticity facilitate their amalgamation in sensing devices with high performance. Additionally, because of the hydrophobicity of their surface, various therapeutic targets like ssDNA molecules can be preferably attached to the carbon nanostructures. It could be implemented via π-stacking forces between the side-walls of the carbon materials and oligonucleotides bases [102]. The anti-biofouling characteristics of carbonaceous nanomaterials permitted their utilization in many biomedical applications. Until now, various researchers have suggested carbon nanotubes (CNTs) as ideal candidates for the improvement of fouling-release coatings, as a result of their antifouling and antimicrobial attributes, they could also be used in water and wastewater treatment, and as adsorbents for chemical and biological contaminants [103–105].

In this perspective, the high-performance antifouling interfaces can be realized via incorporating carbonaceous nanostructures with zwitterionic materials as promising antifouling reagents [106]. For example, electrochemical sensors were developed by Jiang and coworker [107,108], who modified chip surfaces with low-impedance and short binary aryl layers fabricated via the aryldiazonium salts electrodeposition which comprises phenylphosphorylcholine (PPC), a zwitterionic molecule, for attaching of bio-recognition elements. Additionally, Qi et al. [109] were introduced a label-free nanoprobe for the TNF-α sensing using an rGO-ph-AuNP-aryldiazonium salt modified surface. Initially, AuNPs loaded reduced graphene oxides nanostructures (rGO-ph-AuNP) have been fabricated, then the modified surface was further decorated with mixed carboxyphenyl groups and zwitterionic PPC via reduction of diazonium which anti-TNF-α has been attached. Following amperometric identification of molecules was then conducted via a sandwich technique whereby the employed targets have subjected to ferrocene and anti-TNF-α-modified rGO. This electrochemical immunosensor has been effectively applied for the determination of TNF-α secreted from BV-2 cell lines (in cell media).

Recently, the same group was designed a nanoprobe able to quantify cytokines interleukin (IL)-6, IL-1β, and TNF-α in serum media [110]. As seen in Fig. 4, the sensing interface has been synthesized by the electrodeposition of mixed layers of PPC and 4-carboxylic phenyl onto the surface of the electrode via a C—C covalent bond. Afterward, capture monoclonal antibodies for these biomarkers have been anchored by amide bonding to the -COOH terminated biosensing. At last, a sandwich test was established. Indeed, in this scaffold, target identification was performed amperometrically with the rGO both antibody and redox reporter (Ferrocene (Fc) methylene blue (MB), or Nile blue (NB))-modified. Owing to the different redox peaks related to these reporters (−0.4 V, − 0.2, and 0.2 V, respectively), a simultaneous determination of all three targets in undiluted mouse serum was considered feasible. By

rational assessment and selection of redox labels, this multianalyte sensing platform has a hopeful future in complex media. Liu et al. [111] applied PEG diazonium salts as an antifouling reagent to modify the glassy carbon electrode (GCE) surface for the troponin-I (cardiac biomarker) determination. Troponin-I quantification was carried out through an immunosensor Fc-GO-detection Ab/Troponin-I/Ab1/GO-ph-AuNPs-ph-PEG/ph-amine modified electrode with LOD of 0.05 ng mL^{-1} and linear range of 0.05 to 3 ng mL^{-1}. The antifouling effect of PEG could successfully improve the sensitivity of the developed method in human plasma samples. He et al. [112] developed another scaffold using a self-assembled microgel with abilities of bio-recognition element and antifouling attachment which the surface of GCE was modified with glycidyl methacrylate (GMA)-anti-streptomycin/GO and 1-propyl-3-vinyl imidazole sulfonate (PVIS) as the sensing and antifouling reagents, respectively. Similarly, PVIS as a usual zwitterionic species could be utilized for zwitterionic group integrations on the shell of the microgels. Moreover, for more conjugation with biomolecules, GMA as a functional monomer was applied to insert epoxy groups inside of the microgels [113]. Consequently, by surfactant-free emulsion polymerization, they can be prepared two kinds of modified microgels by using 3-(Methacrylamide) propyl trimethyl ammonium chloride (MPTC) and acrylic acid (AA) based on P(NIPAm-MPTC-GMA) and P(NIPAm-AA-PVIS). Indeed, the zwitterionic group can bind water molecules through strong ionic solvation or hydrogen bonding, a tight hydration layer has been created which can act as a physical obstacle to inhibit the nonspecific proteins' adsorption [114]. As compared, the sensitivity of the immunosensor is 1.7 pg mL^{-1} which provides at least 100 times better detection ability.

PEG and its derivatives have been enormously applied as antifouling reagents in various sensory systems. Choi et al. [115] utilized diamine-PEG to modify the surface of carbon dots (CDs). In their work, PEG and amine groups act as antifouling support and active groups for further functionalization, respectively. Folate functionalized CDs showed high selectivity to the folate receptor on the surface of overexpressed cancer cells. In another work, Iverson et al. [116] reported the combination of DNA segments with PEG to the recognition of NO in mouse veins. DNA-PEG was utilized for the modification of the single-walled carbon nanotubes (SWCNTs) surface. While it was not verified the capability of operating the sensory system in real samples, however, a LOD of about 1 μM was obtained.

Filtration methodologies are other shapes of antifouling which a physical approach such as membrane filters is used. For example, a graphene nanoribbons probe was applied to Nafion-modified chip for the specific cysteine recognition in serum samples [117]. The fabricated graphene-based nanoribbons provide high electrical conductivity and electrocatalytic activity to electrochemical cysteine detection. The negatively charged Nafion substrate can either enhance the dispersion of graphene particles or decrease the electroactive activity of coexisting molecules i.e. uric acid and ascorbic acid, permitting in vitro determination of cysteine without any interfering effect of them.

"Off-surface filter" is another approach to reduce biofouling on the surface of electrochemical electrodes which has been introduced by Ferguson et al. [118] Off-surface filter approach was utilized to determine doxorubicin (DOX) through an aptamer-based amperometric detection method using a carbon-fiber electrode. This sensor allows continuous monitoring of DOX in live rats with acceptable sensitivity and specificity performance. The reduced biofouling effect can ultimately enhance about 100 times the diffusivity of the DOX as compared with coexisting non-target reagents such as HSA, Immunoglobulin G (IgG), and fibrinogen (Fib).

4.2. Combination of noble metal nanomaterials with antifouling reagents

Noble metal nanomaterials like gold (Au), silver (Ag), palladium (Pd), platinum (Pt) consistent core-shell NPs, and bi- or tri-metallic alloys have been broadly amalgamated with bio-recognition units for monitoring of various targets in terms of their exclusive electronic, physical and chemical properties. Besides, outstanding catalytic and conductive attributes make them "electronic wires" while increasing e-transfer between the redox centers in the surface of protein and electrodes [119,120,170]. Until now, nanoprobes based on noble metals/EG-SAMs as an antifouling reagent provided a high potential for improvement of both sensitivity and selectivity by increasing the tuned signal. Therefore, a particular emphasis on these platforms has led to the development of various innovative analytical methodologies, which can be utilized in quality control programs for the effective determination of various analytes like small molecules, cells proteins, metal ions, antibiotics, and mycotoxins. On this note, Arroyo-Currás and collaborator presented off-surface filter to develop an electrochemical aptasensor for small molecules detection [121]. Nevertheless, the simplicity of the off-surface filter antifouling approach, this method might be appropriate to the recognition of a wide variety of small molecules. In their study, the developed Au nanowire-based antifouling approach was used to the continuous detection of DOX, kanamycin, gentamicin, and tobramycin in blood flow by inserting a catheter into the jugular vein. The catheter is composed of a polysulfone membrane (as filtering agent) and Au nanowire-modified electrode as sensing segment of the developed approach. Xie et al. [122] reported a PEG-based antifouling magnetic Au nanoflower to develop an absorbance-based nanoprobe for in vivo sensing of matrix metalloproteinase (MMP) expression. MMP is a protease that is secreted on large scale by malignant tumor cells. The produced magnetic AuNPs were functionalized with a Cy5.5-labeled GPLGVRG peptide which already contains tridihydroxyphenylalanine (TDOPA)/(Cy5.5-GPLGVRGTDOPA), and PEG5000-SH moieties. Cy5.5 is a NIR dye that is attached to the enzyme-based GPLGVRG spacer. The emission of Cy5.5 was quenched by the Au substrate, however, the fluorescence of Cy5.5 is turned on upon cleavage of the GPLGVRG peptide by MMP molecules. The Cy5.5–based nanoprobe was introduced intravenously in SCC-7 mouse cells (neck and head squamous cell carcinoma) as models, and later NIR bio-imaging was captured. From the obtained results, it is obvious that the fluorescence of SCC-7 cells was about 10-times stronger than control groups. Remarkably, the fluorescence signal of control particles without PEG molecules (as nonfouling molecule) were enhanced due to the nonspecific attachment of the nanoprobe upon fouling.

PEG-based antifouling material was utilized by Xu and co-workers to fabricate a label-free impedimetric electrochemical sensor for the recognition of insulin [58]. In the work, SAM layers of PEG containing thiol and insulin-specific antibodies were attached on the Au electrode surface to induce simultaneous antifouling and immunosensing agents, respectively. This immunosensor is sufficient capacity to perform specific assessments of insulin in complex biological fluids with negligible interference (3%) from coexisting molecules. The LOD of the nanoprobe is about 1.2 pM which can cover clinically relevant concentration of insulin in serum (5 pM to 50 nM). Especially, the developed probe can be reused after washing with a buffer. Other impedimetric nanoprobes using PEG chemistries were also introduced by the Davis groups in which α-synuclein antibodies (biomarkers for Parkinson's disease) were measured in a blood sample through modification of the surface of gold electrodes with the corresponding antigen [123]. They utilized the SAM approach for electrode surface modification. This platform was then utilized to determine α-synuclein antibodies contents of serum samples

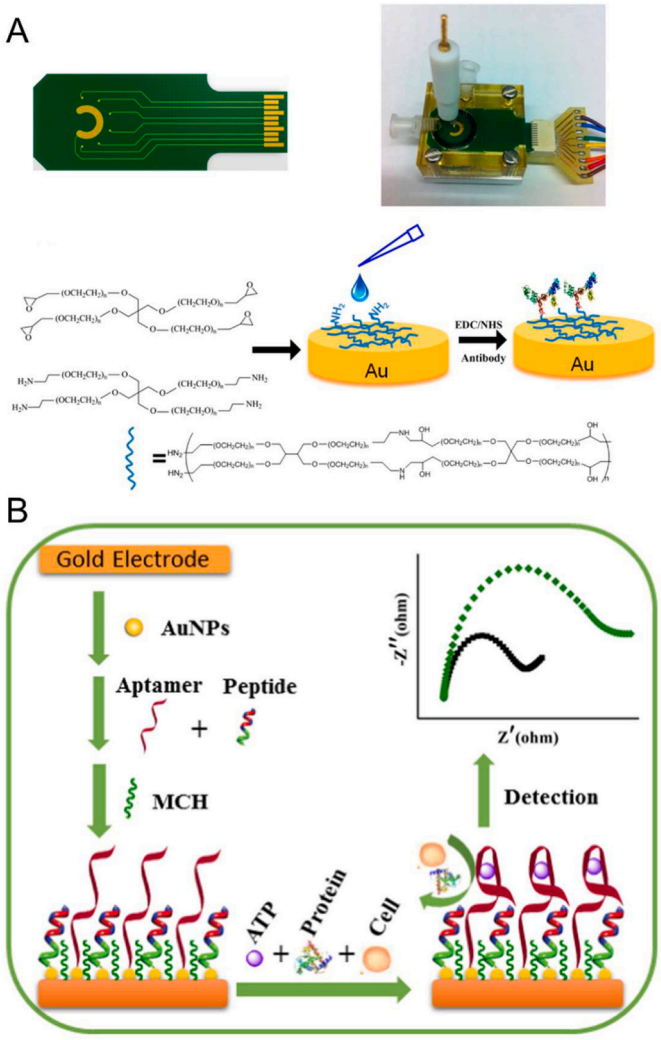

Fig. 5. (A) The preparation mechanism of multiplexed EIS-based nanoprobe for insulin and CRP detection using 4-armed PEG-epoxide and 4-armed PEG-amine modified gold electrode (Reprinted with permission from [127], Copyright 2014 American Chemical Society). (B) Diagram procedure for the preparation of the ATP aptasensor based on mixed zwitterionic peptide and self-assembled aptamer (Reprinted with permission from [131], Copyright 2018 Elsevier with license code: 5245311333801). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of healthy controls and Parkinson's disease, confirming the high specificity and sensitivity of the developed platform in real samples. A similar antifouling reagent and SAM approach were utilized by Bryan et al. [124] to modify the Au electrode and then quantification of α-synuclein. The response of the reported sensor is linear from 0.5 to 10 nM and has a LOD of about 55 pM. Favorable features of the reported provide a reliable platform for monitoring α-synuclein to determine disease stage and to monitor the effects of prescribed pharmaceuticals on the disease stage and modifying possible interventions.

Likewise, some nanoprobes based on similar antifouling reagents i.e. EG-SAMs were designed for the identification of C-reactive protein (CRP) as a cardiac marker) through an immunosensing platform. Firstly, EG-SAMs and anti-CRP were anchored on the interface of the Au electrode, respectively. Then, impedance techniques have been utilized to acquire CRP-related immittance signals [59]. Using SAM, interferences caused by unwanted molecules' adsorption on the chip surface were mainly reduced, enhancing the sensitivity of the probe. Bryan groups introduced a dynamic range of 0.5–50 nM for CRP sensing through an impedance method, however, the reported LOD is 176 pM. They utilized PEG-thiol HS-C_{11}-$(EG)_3$-OCH_2-COOH used as an antifouling reagent. Significantly, the LOD is better than the currently used CRP clinical protocols, in addition, the developed assay is actually inexpensive, reusable, portable, and relatively automated [125]. Lately, the same team has utilized electrochemical impedance spectroscopy and modified Au surface electrodes with EG-SAMs and Au bead to detect the exosomal proteins of CD 9 and Syntenin in exosomal lysate with high sensitivity at picomolar level [63].

Sharma's group has evaluated the antifouling ability of PEGs by employing monothiol-alkane-PEG-acid to attach to the surface of a typical Au electrode. This probe was used to determine IL-8 in human serum samples through a specific interaction between cystatin protein and IL-8. Due to the high formation constant ($K_f = 35 \pm 10$ nM) and antifouling effect of monothiol-alkane-PEG-acid, this probe provides LOD of 90 pg L^{-1}, which is considerably lower than the basal clinical levels of 5–10 ng L^{-1}. As with most of the developed electrochemical approaches, this method has a high potential for designing of point-of-care (POC) device for IL-8 detection [126]. Luo and coworkers have developed the multiplexed EIS-based nanoprobe for insulin and CRP detection using PEG-modified Au electrodes [127]. In their work, 4-armed PEG-epoxide and 4-armed PEG-amine were attached to the surface of the Au electrode and then antibodies were tethered (the immunosensor designing steps are presented in Fig. 5A). This probe can detect insulin and CRP in whole serum samples with LODs of fM and pM levels, respectively. The exhibited LODs can comfortably cover clinically required concentrations of both analytes and linear dynamic ranges of the probed were 0.5 pM to 100 pM and 0.5 nM to 50 nM for insulin and CRP, respectively. The same 4-armed PEG-amine was also utilized by Hui et al. [128] for alpha-fetoprotein (AFP) biosensing. The electrode modification was done via electrochemically polyaniline (PANI) synthesis on the substrate followed by PANI functionalization via AuNPs. Then, AuNPs-PEG can be formed via binding of -NH_2 groups of the PEG. Subsequently, anti-AFP-PEG conjugations allowed selective AFP determination. The redox current has been reduced by antigen-antibody binding as a result of electron transfer insulating biomolecule behaviors. The PEG functionalized AuNPs/PANI composite provide high hydrophilicity which may effectively decrease nonselective attachment of biomolecules onto the GCE surface through the produced electrostatic hydrophobic interaction, and thus presenting a favorable substrate for the construction of AFP anti-fouling biosensor. Using DPV mode, AFP sensing was carried out in the range of 0.01 pg mL^{-1} to 1.0 ng mL^{-1} with LOD of 0.007 pg mL^{-1}.

To use in biological media, more biocompatible antifouling reagents are needed. In this context, Cui et al. [98]. established an electrochemical alpha-fetoprotein nanoprobe using zwitterionic peptides antifouling reagent. Besides the biocompatibility of these types of antifouling reagents, the enhanced antifouling influence mainly arises from their high hydrophilicity and low charged nature. This aptasensor shows some beneficial features of exceptional protein-resistant performance in biological serum samples and feasibility to quantitative detection of alpha-fetoprotein in patient's samples. As is well known, the poly-(carboxybetaine methacrylate) (PCBMA) as zwitterionic polymers, constitute superb antifouling nanomaterials [129]. So, their suitable assembling onto the transducing surfaces in a manner conducive to extremely sensitive electroanalytical sensing is of importance. Similarly, the integration of non-Faradaic EIS underpins with surface chemisorbed and assembled PCBMA polymer offer scaffold with negligible electroactive interference or nonspecific response and extraordinary sensitivity. To present and highlight the effectiveness of this approach, which could be strongly used to the determination of each biomarker for which there is a receptor. In this perspective, Luo et al. [130] engaged PCBMA as a zwitterionic polymer to the surface functionalization of the Au electrode where Ins-Ab is attached to the terminals of the polymer to specify the nanoprobe for insulin identification. This platform response is LOD of 42.6 fM with a wide linear of 0.1–200 pM range in unprocessed

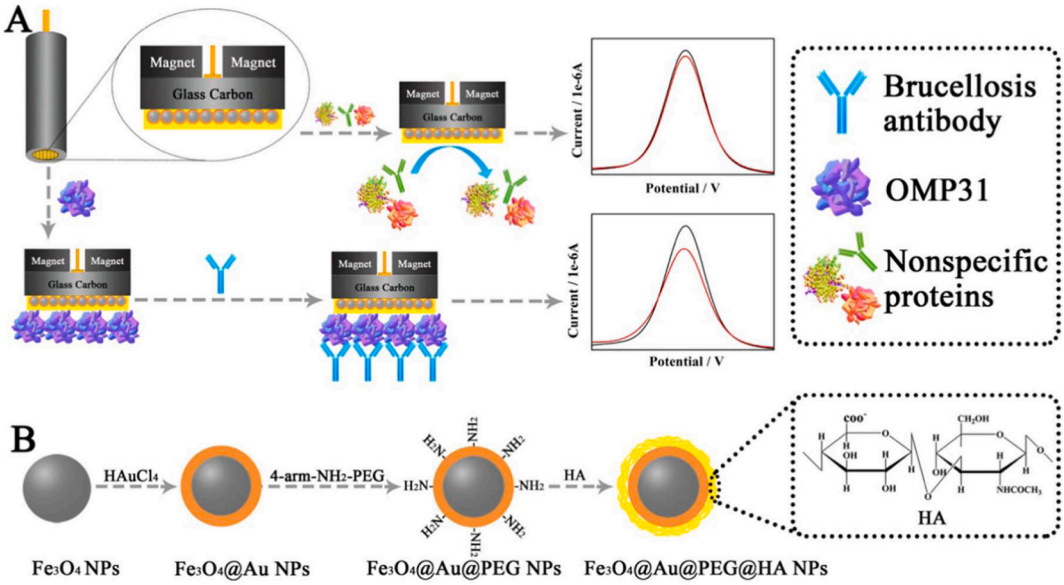

Fig. 6. Construction diagram for the low-fouling electrochemical brucellosis immunosensor based on Fe_3O_4@Au@PEG@HA NPs (Reprinted with permission from [142], Copyright 2019 Elsevier with license code: 5244690422698).

human serum. The presented electrochemical method represents a specific manner that could be extended as a POC device for label-free electrochemical recognition of protein-based analytes in biological fluids. The combination of peptides and Au materials provides surfaces with tunable hydrophilicity, charge, and functional groups to do secondary modifications of the surface. As compared with PEG and EG-based antifouling reagents, peptide-based ones are more applicable, especially in aqueous media. Through self-assembly, diverse bioactive molecules could be anchored onto suitable nanoprobe surfaces to form a uniform and ordered interface. Based on the significant antifouling ability of EKEKEKE-PPPPC peptide [92], numerous biosensing scaffolds were suggested for biomarker determinations. Wang's group has evaluated EKEKEKE-PPPPC zwitterionic peptide to provide an antifouling effect on the aptamer probe to accurate and sensitive ATP detection in complex media. The AuNPs modified Au electrode determined ATP without any significant interference from the available reagents, indicating the high antifouling effect of zwitterionic peptide (Fig. 5B) [131]. In another example, Wang and colleagues used a zwitterionic peptide to modify the surface of the macroporous Au electrode for selective recognition of immunoglobulin E (IgE). The combination of zwitterionic peptide and physical antifouling effects can acceptably decrease the nonspecific adsorption on the surface of Au electrodes [132]. Another fascinating antifouling interface using self-assembly mode is an electrochemical enzyme-based substrate with a self-designed peptide of CGGGGDKDKDKDK and sacrificial iron metal-organic framework (Fe-MOF) for quantification of T4 polynucleotide kinase (T4 PNK) [44]. As a general rule, antifouling reagents can decrease current of an electrochemical half-reaction and using such a high electro conductive metal (i.e. Fe) can extremely enhance the sensitivity of the developed probes along with antifouling effect of the reagents. With the help of peptide, the electrochemical DNA probe demonstrated sufficient analytical performance toward T4 PNK with a detection limit of 3.5×10^{-4} U mL^{-1} and a broad linear range of 1.0×10^{-3}-10 U mL^{-1}. Additionally, the nanoprobe was capable of T4 PNK determination in 5% human serum solutions.

Qi and colleagues described three antifouling reagents i.e. dithiothreitol (DTT), anti-thrombin binding aptamer (TBA), and 6-mercapto-1-hexanol (MCH) to produce a ternary mono-layer on the surface of Au electrodes. [133] The developed interface provides high selectivity and direct sensing of thrombin at attomole levels. Inspired by using multi-antifouling reagents, this probe can detect target thrombin in human serum samples. In another study, MCH was used to enhance the specificity of the detection of thrombin in an aptamer-based probe. MCH has been used to modify the polydopamine(PDA)/AgNPs-GCE electrode. The PDA embedded AgNPs composited provided a more sensitive detection system with LOD as low as 36 fM. Despite various favorable features, the application of this aptasensor was not checked in biological media [134]. Likewise, aptamer-DTT-MCH was used as a ternary monolayer for the modification of gold electrodes for lysozyme (Lys) detection. The fabricated sensor is able resistant to the nonspecific adsorption of interfering reagents. The antifouling effect of the reported sandwich typed enzyme-based Au/aptamer-DTT/MCH/Lys/horseradish peroxidase (HRP)-AuNP-LBA electrochemical sensor confirmed in serum samples, showing g the potential of the apta-sensor in complex biological media [135].

In sum, in addition to the previously reviewed biosensors that depend on extremely hydrated interfacial chemistries to resist unwanted adsorption, substitute approaches to improve the impediments along with interfacial fouling were also considered. Direct physical sensory interfaces manipulation is one such methodology. For instance, the application of membrane filters or the manufacturing of nanoporous chips is promising tactic to reduce sediment problems via large molecules or cells by offering a diffusional barrier (sieving) that limits the access of large biomolecules to the underlying nanoprobe while also allowing diffuse access to smaller targets.

4.3. Applications of antifouling molecules-functionalized magnetic nanomaterials

Recent developments in magnetic NPs (MNPs) are one of the most hopeful progress for synergistic application in electrochemical sensors. Magnetic nanostructures usually have an iron oxide core and superparamagnetic nanomaterials of different three-dimensional (3D) shapes [136,137]. The size of MNPs can range from the nanometer (nm) to micrometer (μm) scale renders extraordinary flexibility for optimal monitoring of biomolecules with diverse size ranges. These materials can also offer an assay benefit in multianalyte sensing platforms by taking simply amendable surface coatings/chemistries for anchoring various analyte-binding molecules like aptamer, antibodies, and nucleic acids [138]. In summary, with the ongoing advancement in the synthesis

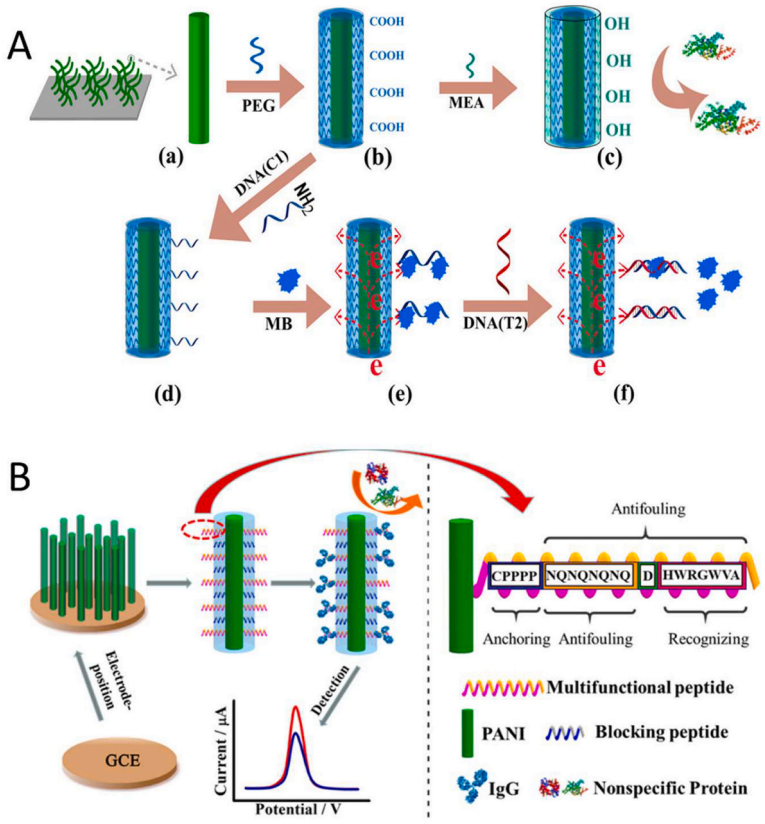

Fig. 7. (A) Fabrication process of PANI/PEG nanofibers antifouling interface and its application in low fouling and ultrasensitive DNA probes (Reprinted with permission from [149], Copyright 2017 American Chemical Society). (B) Multifunctional PANI supported peptides-based nanowire-based probe for the IgG detection (Reprinted with permission from [154], Copyright 2017 American Chemical Society).

of cutting-edge MNPs and related innovative applications in bioanalysis, there is considerable potential in integrating these research areas to tackle remaining issues in small molecules biosensing.

Recently, the aptamer-magnetic solid supports were utilized for purification of numerous biological targets along with a precursor for analyte enrichment from complicated sample mixtures to prevent the interference of the other probable interactive analytes with nanoplatforms. Indeed, magnetic matrices can isolate pre-concentration different small and macromolecules from biological media to remove interference ingredients in the samples. The acrylic-based nanostructures have also been applied for magnetic support materials preparation and arranged in requisite geometry and size. Moreover, they could be synthesized with a large surface area, required pore size, and functionalized with the desired elements. In this regard, Bayramoglu and coworkers have immobilized specific thrombin aptamer on the magnetic particles to determine thrombin in serum samples by quartz crystal microbalance (QCM) techniques [139]. Ethylene glycol dimethacrylate (EGDMA) has been applied as an antifouling reagent. Magnetic poly(2-hydroxyethyl methacrylate-ethylene glycol dimethacrylate-vinylene carbonate) Mp(HEMAEGDMA-VC) microbeads showed favorable antifouling effect where thrombin was selectively separated from serum samples in the presence of HSA, immunoglobulin G (IgG), and elastin,). The high specificity of this probe mainly arose from the antifouling effect of EGDMA and the high dissociation constant of aptamer and thrombin (68.2 nM) molecules. Magnetic core-shell Fe_3O_4@Au NPs are widely applied to design bio-electrochemical interfaces for sensors. One case is that Yao et al. developed a highly specific and ultrasensitive electrochemical aptasensor based on Fe_3O_4@Au NPs toward thrombin sensing [140]. Also, Hao and colleagues successfully fabricated a new electrochemiluminescence immunosensor using glucose oxidase (GOD)-labeled antigen and Fe_3O_4@Au NPs, which can be easily detected of *Bacillus thuringiensis Cry1Ac* with varied linear range and excellent sensitivity [141]. The outstanding conductivity of the gold shell is recognized. Meantime, the superb biocompatibility of Fe_3O_4@Au NPs renders this nanostructure with chemical modification, consequently, lays the foundation of its brilliant anti-fouling characteristics. Herein, based on PEG-coated Fe_3O_4@Au NPs, hyaluronic acid (HA) modified Fe_3O_4@Au@PEG NPs have been developed and utilized to design *brucellosis* immunosensor (Fig. 6) [142]. The fabricated Fe_3O_4@Au@PEG@HA NPs-based immunosensor showed detection ability as low as 0.36 fg mL^{-1} in the presence of VirB5, BSA, and BP26 proteins. The exceptional specificity of the probe may be aided by the antifouling property of PEG on the probe and the robust affinity between the corresponding antibody and antigen. Although, the reported aptamer- and antibody/antigen-based sensors showed excellent analytical performances, however, the ability of the PEG and EGDMA to suppress interfering reagent is more pointed out when these biomolecules have not been utilized. Also, Kongsuphol et al. [143] separated tumor necrosis factor (TNF) or TNF-α using its antibody/antigens. In contrast with the reported approaches, in this work, a biological reagent i.e. antibodies were used to remove interferents from TNF-α. Contrary to the chemical and physical strategies for antifouling, this approach is high cost and not applicable for in vivo sensing. In the reported method, the captured TNF-α molecules were eluted from the magnetic beads and determined by EIS at about fM sensitivity levels in whole serum samples. This type of separation is more efficient if the separation process could be done within a fluidic device which is reported by Oleschuk et al. [144]. They designed a microfluidic device that can simultaneously deplete three types of biomolecules i.e. anti-HSA, protein A, and protein G through a device. This device enhanced

sensitivity at least four times. Magnetic particles are candidates for this type of antifouling strategy. Biological molecules-based antifouling strategies provide advantageous features of substantial depletion of interfering reagents from small sample volume, however, low depletion ability, limited throughput, and high cost of biological molecules are the main problems which limits their uses in biological media.

4.4. Combination of polymer-based materials with antifouling reagents

Polymeric antifouling reagents have been extensively utilized in the fabrication of sensing platforms through different approaches. Facile control of physical and chemical properties of polymer-based antifouling reagents provides a favorable substance to decrease the effects of possible interfering reagents in biological media via self-filtration. However, biomaterial-based antifouling polymers not only are effective to decrease bio-fouling effects, but also their functionalization is easy due to the presence of various functional groups. For example, Ma et al. [146] produced HPG functionalized poly3,4-ethylene dioxythiophene (PEDOT-HPG) through electrochemical polymerization. PEDOT-HPG functionalized electrochemical sensor can detect alpha-fetoprotein in the proteins and cells presences as low as 0.035 pg mL^{-1}. This sensitivity mainly arises from HPG which has many active functional groups that are helpful for antifouling power of the PEDOT-HPG polymer. To fabricate the label-free biosensor interface, Cui and coworkers suggested an electrochemical nanoprobe by using PEDOT for GCE modification [147]. PEDOT is an ideal candidate to fabricate electrochemical sensors owing to features of moderate bandgap, stability, low redox potential, and modification capability with various functional elements. The thiol-terminated 4-arm PEG has been doped into PEDOT polymer to enhance negatively charged -SH groups followed by AuNPs binding the surface through Au—S bond. Existence of the Au enhanced stability and conjugations of antibodies on the sensor interface. Lastly, GCE/PEDOT/PEG/AuNPs/anti-AFP chip has been applied for the AFP determination via EIS method with a detection limit of 0.0003 fg mL^{-1}.

Feng et al. proposed a new nano-platform using PANI for dopamine identification [148]. PANI as conducting polymers have benefits like fast direct electron transfer, biocompatibility, and high stability. They were utilized polymerized tannic acid-PANI composite to graft to the surface of the carbon-fiber electrode (CFE) as an efficient antifouling material for in vivo sensing of dopamine. Despite the favorable results, the application of nanoporous structures encounters various challenges in regulating cavity size and maintaining the active spots on the electrode surface. Grafting of antifouling polymers onto electrochemical working electrodes can cause the construction of high-impedance layers, resulting in decreased sensitivities. Grafting of certain conductive soft materials (such as PANI) can be useful to solve this issue. Hui et al. [149] grafted PEGs onto PANI nanofiber to fabricate PEGylated PANI nanofibers for the detection of breast cancer. In this work, PANI was added to provide both sensitivity (by decreasing impedance of the elected ode) and specificity (in the presence of PEG/PANI) at the same time. Fig. 7A demonstrates the sensor fabrication steps and applying the PEG for decreasing the fouling effect from the serum matrix. After attaching capture nanoprobes through NHS/EDC coupling on their PEGylated PANI nanofibers, sensitive DNA electrochemical sensors have been established for the breast cancer susceptibility gene (BRCA1). This nanoplatform presented excellent sensitivity with a wide linear range from 0.01 pM to 1 nM. In another work, the surface of screen-printed graphene electrode (SPE) electrodes have been electro-polymerized via polyaniline (PANI) to selectively detect C-reactive protein in plasma samples [150]. As an advantage of the production of antifouling via electro-polymerization, permits facile regulation of the surface properties of the polymers, modulating the specificity, fouling effect, and sensitivity of the probe.

PEDOT polymers, like PANI, were also used to modification of zwitterionic EKEKEKE peptides as antifouling reagents for the detection

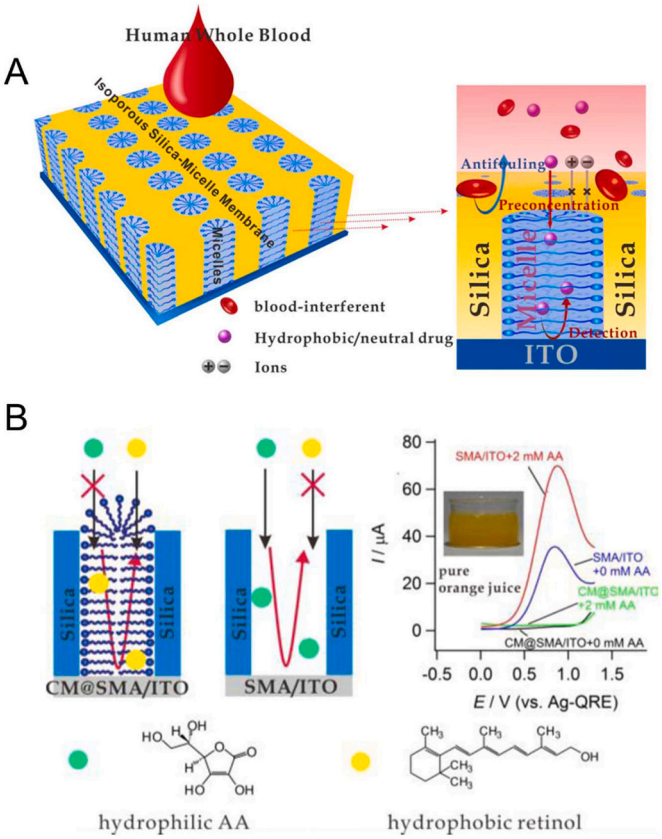

Fig. 8. (A) Drawing of the isoporous silica micelle membrane, emphasizing the capability to preconcentrate drug compounds with inhibiting fouling in whole blood samples (Reprinted with permission from [157], Copyright 2016 American Chemical Society). (B) Electrochemical detection of fruit juices antioxidants based on silica mesochannel array (SMA) on the ITO electrode (Reprinted with permission from [156], Copyright 2016 American Chemical Society).

of DNA of the BRCA marker. Initially, a high PEDOT conductive layer with plentiful -COOH groups has electrodeposited onto the surface of the electrode to improve response signal, sensitivity, stability, and biocompatibility of advanced surfaces. Secondly, nickel cations have been presented to further bridge -COOH from the PEDOT surface and peptide by the formation of Ni—O coordination bonds. Afterward, the capture DNA probe terminated with the -COOH group was attached with peptide via covalent binding. The antifouling quality was confirmed by the electrochemical current changes after soaking in various concentrations of human plasma and HSA solutions. Although, the developed sensor showed high analytical performances the functionalization of the probe surfaces with peptides antifouling reagents is too hard and usually needs to provide interfaces with high surface density and well-ordered building [151].

Surface hydrophilization is one of the main strategies to limit nonspecific adsorption. HA is a hydrophilic polysaccharide which extensively used as an antifouling reagent in biological media due to its high compatibility and hydrophilicity. Wang et al. [152] fabricated a carcinoembryonic antigen (CEA) immunosensor in which HA showed an exceptional antifouling effect and the fabricated sensor detected CEA at aM levels. Filtering-interface of mesoporous materials can also decrease fouling from proteins and other species. In another strategy that was introduced by Wang et al. [153] in which PEDOT was used to dope the HA layer for electrochemical immunoassaying of CEA. HA has carboxylic groups which could be co-electropolymerized with PEDOT wherein HA and PEDOT enhance the interfacial hydration and conductivity, providing favorable analytical performances for the fabricated CEA sensor.

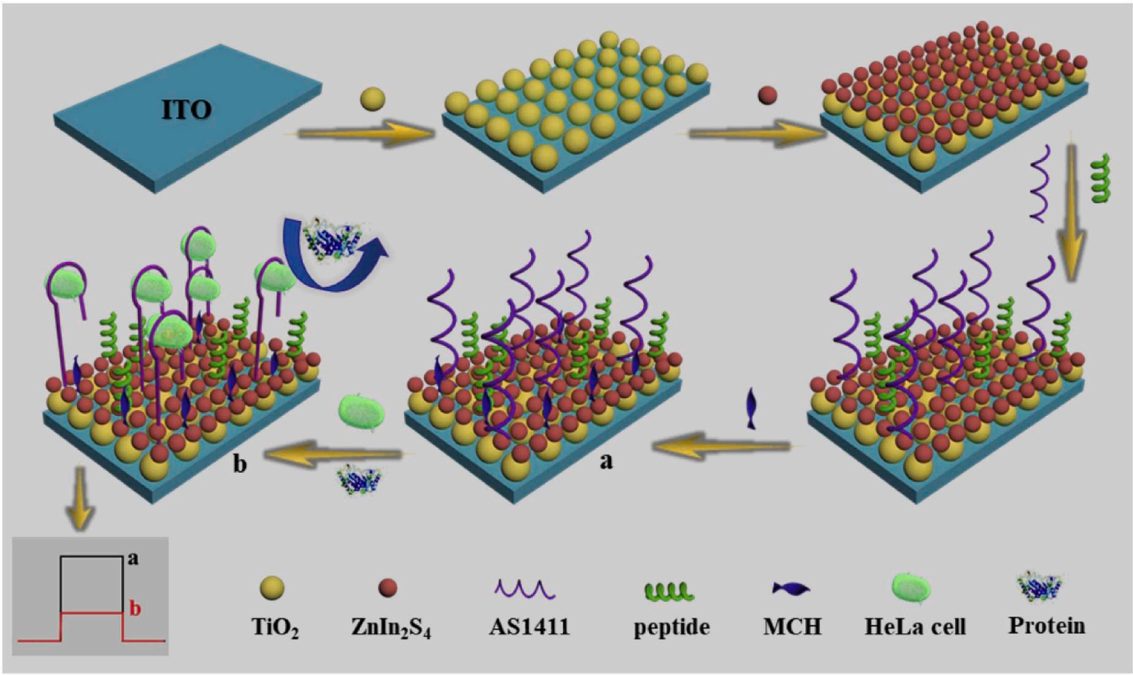

Fig. 9. Fabrication procedure of the antifouling PEC cytosensor using zwitterionic peptide (Reprinted with permission from [163], Copyright 2019 Elsevier with license code: 5245270081661).

In a study reported by Liu et al. [154], PANI was used as a surface modifier of the GCE, in which peptide-based antifouling reagents were attached to the amine ends. As seen in Fig. 7B, the human immunoglobulin G (IgG) was detected as low as 0.26 ng mL^{-1} in which the antifouling effect was further reinforced by PANI molecules. The antifouling capability has also been explored with fluorescence microscopy image and DPV mode when incubated in diverse FBS concentrations with different incubation time intervals. All results exhibit brilliant antifouling characteristics of this multifunctional peptide. This peptide-based nanoprobe can detect IgG in neat serum and clinical samples. Despite the sensitivity, the reported method suffers from low stability as a result of using a peptide-based antifouling reagent.

4.5. Combination of silicon-based materials with antifouling reagents

The antifouling reagents can be significantly enhanced the impeditive effect of the surface toward redox spices or mediators. How to engineer a probe surface to eliminate and/or decrease the biofouling effect but concurrently not to affect the surface activity of the probe is of great importance in the sensor design process. Nanopores layers that cover the surface of the probe are one solution to provide a sensor with the antifouling ability and unaffected sensitivity. It has also been demonstrated by some studies that silica nano-channel membranes (SNM) could function as an effective antifouling layer to inhibit the surface of the electrode from unwanted species adsorption.

Hence, the direct electrochemical monitoring of numerous target biomolecules in diverse complex media like urine, human serum, fruit juice, and even whole blood has been realized without sample pretreatments [155,156]. For instance, in human plasma samples, not only the analytical sensitivity for the chloramphenicol detection can be enhanced but also the current stability is meaningfully amplified, as clarified in Fig. 8A. Indeed, the antifouling ability of mesoporous silica-micelle membrane arises from the nanosized microchannels and the lipophilicity of the micellar layer. The redox current of chloramphenicol at the modified indium tin oxide (ITO) electrode with SNM depleted only 21.0% after 45 min, while for the bare ITO electrode was 75.5%. [157]. Besides, the same silica mesochannels material attached to the ITO electrode has been used to determine lipophilic and hydrophilic antioxidants, such as retinol and ascorbic acid. As shown in Fig. 8B, the nanocomposite comprising silica mesochannel array can entirely modify the ITO electrode without cracks, wherein silanol groups provided the channel negative charges and hydrophilicity resulting in the noticeable current individual antioxidant responses (10–2000 mM for ascorbic acid and 1–60 mM for retinol). This approach was also applied to modify the GCE surface for Alzheimer's biomarkers detection [158]. Also, Yan et al. [159] used silica perm-selective membranes for the detection of small organic analytes. In this work, another form of silica layer i.e. vertically ordered silica mesochannels and ordered surfactant micelles was applied to provide the antifouling effect. The molecular permeability of the nanoporous layer is the main adjustable factor to regulate the sensitivity and specificity of the probe. Moreover, the SNM antifouling capabilities are also superb in the in vivo examination. As is well known under the in vivo state the surface biofouling via proteins along with the foreign-body response because of innate immunity, is more severe. Both foreign body response and biofouling hamper or entirely avoid target reaching the surface of electrode. Additionally, in vivo analysis frequently needs long-term activity and stability of nanoprobe in order to investigate the ethology of living animals and time dependent physiological procedures. Therefore, construction antifouling surfaces and inhibiting surface from biofouling become tremendously imperative yet challenging. A most updated study was discovered that in vivo condition SNM could successfully impede the surface carbon fiber microelectrode (CFME) biofouling and meantime remain permeable to molecular oxygen, thus allowing the long-term constant identification of oxygen in the brain of live rat [160]. Compared with the bare CFME, the CFME-SNM modified electrode can preserve its sensitivity to oxygen after implantation in the live rat brain. What is more, the implanted chip can continuously monitor oxygen under the in vivo state, revealing a fast response (up to 2 h) and outstanding current stability. We believe the SNM antifouling capabilities are chiefly a result of the size exclusion

effect, hydrophilic, and electrostatic repulsion. Furthermore, SNM is biocompatible and the foreign body response may be less.

4.6. Combination of other materials with antifouling reagents

Due to the high impedance of the PEG-based layer on the electrochemical electrode, Jiang et al. [13] employed small chain zwitterionic reagents as antifouling components. Small chain zwitterionic molecules not only induce an antifouling effect but they have a very low passivation effect on the sensor surface. In this study, TNF-α was quantified in whole blood samples from 0.01 ng mL^{-1} to 500 ng mL^{-1}. Besides the favorable sensitivity, the obtained results are as good as the commercial ELISA kit, proposing the application of the developed immunosensor clinic uses.

The photoelectrochemical (PEC)-based sensory systems have received attention owing to the inherited properties that combine the beneficial features of both electrochemical and spectroscopic methods including simplicity, ease to operate, and high sensitivity. TiO_2 NPs are the most used nanomaterials for the fabrication of PEC-based biosensors with high photoelectric efficiency [161]. Fan and coworkers have modified the surface of ITO electrodes with PEG and HA as a hybrid antifouling component for the detection of CD44 bio-receptor. The developed PEC-based sensor suffers from the UV response of TiO_2 responses which could be hazardous to the biomolecules [162]. In another work, the zwitterionic peptide was employed as an antifouling reagent to modify the surface of ITO for HeLa cell a biomarker of cervical carcinoma [163]. Generally, MCH or BSA was employed as a blocking reagent in PEC biosensors to reduce biofouling influence. However, the antifouling capability of these reagents in real biological media is limited. Zwitterionic peptides can be an efficient antifouling reagent in biological media that prevent the probe surface from nonspecific adsorption due to their hydrophobic and electro-neutrality properties. Based on this peptide, a PEC cytosensor has been established to HeLa cell which can precisely couple to the aptamer AS1411. For anchoring preferred peptide and specific aptamer AS141, $ZnIn_2S_4$ nanocrystals and TiO_2 NPs were fixed on an ITO electrode (Fig. 9). In light of the excellent antifouling feature of the preferred peptide, the designed PEC cytosensor showed a high sensitivity toward HeLa cell in biological environment.

Table 1
Construction of nanoprobes based on the different antifouling molecular systems and their in vitro sensing performance.

Substrate	Anti-fouling Agent	Detection Method	Analytes	Sensing performance	Ref.
GO	PPC	SWV	IL-6, IL-1β, and TNF-α	LOD = 0.21 pM, 0.16 pM, 0.285 pM LR = 0.21–6.2 pM, 0.16–6.4 pM, 0.285–11.4 pM	[110]
GO	PVIS	DPV	streptomycin	LOD = 1 3 pM LR = 0.09–171 nM	[112]
Au	PEG	EIS	Insulin	LOD = 1.2 pM LR = 0.09–171 nM	[58]
Au	PEG	EIS	α-Synuclein	LOD = 55 pM LR = 0.5–10 nM	[124]
Au	PEG	EIS	CRP	LOD = 176 pM LR = 0.5–50 nM	[125]
Au	PEG	EIS	IL-8	LOD = 8.1 fM LR = 81 fM − 81 nM	[126]
Au	PEG	EIS	Insulin, CRP	LOD = 171 fM, 150 pM LR = 0.5 − 100pM, 0.5–50 nM	[127]
Cysteamine/Au	PCBMA	EIS	Insulin	LOD = 42.6 fM LR = 0.1–200 pM	[130]
AuNPs	Peptide	EIS	ATP	LOD = 0.1 pM LR = 0.1 pM − 5 nM	[131]
PANI/AuNPs	PEG	DPV	AFP	LOD = 103 aM LR = 147 aM−14.7 pM	[128]
rGO/AuNPs	zwitterionic PPC	SWV	TNF-α	LOD = 5.7 fM LR = 5.7 fM-8.55 pM	[109]
GO/AuNPs	PEG	Amperometric	Troponin-I	LOD = 0.05 ng mL^{-1} LR = 0.05–3 ng mL^{-1}	[111]
PEDOT/AuNPs	PEG	EIS	AFP	LOD = 0.0003 fg mL^{-1}	[147]
GCE/PEDOT-AuNPs	Peptide	CV, DPV	Human IgG	LOD = 32 pg mL^{-1}	[164]
GCE/Ti_3C_2Tx-MXene-Methylene Blue-AuNPs	Peptide	DPV	Prostate- Antigen	LOD = 0.83 pg mL^{-1}	[165]
Fe-MOF/AuNPs	Peptide	DPV	T4 PNK	LOD = 3.5×10^{-4} U mL^{-1} LR = 1.0×10^{-3}-10 U mL^{-1}	[166]
Magnetic Microbeads	EGDMA	QCM	Thrombin	LOD = 1 nM LR = 1–100 nM	[139]
Fe_3O_4/Au/ HANPs	PEG	DPV	Brucellosis Ab	LOD = 18 aM LR = 50 aM−500 fM	[142]
PEDOT	HPG	CV, DPV	MCF-7	LOD = 0.035 pg mL^{-1}	[146]
GCE/PANI Nanowire	Peptide	DPV	COVID-19 Nucleic Acid	LOD = 5.5 fM	[167]
PANI Nanofibers	PEG	DPV	BRCA1	LOD = 18 aM LR = 0.01 pM - 1 nM	[149]
HA-PDA/TiO_2	PEG	PEC	CD44	LOD = 5.5 fM LR = 0.06 pM − 6.25 nM	[162]
MCH/ $ZnIn_2S_4$/TiO_2	Peptide	PEC	Hela cell	LOD = 34 cell mL^{-1} LR = 100–106 cells mL^{-1}	[163]

5. Conclusions and perspective

Nowadays, the progress of non-fouling nano-platforms for the monitoring of target biomolecules in complex matrices has witnessed excessive advancement. Herein, we aim to offer a comprehensive review discussing concepts and current progress in the development of biosensing platforms using diverse antifouling and multifunctional nanomaterials to decrease or remove biofouling to further improve convenience, accuracy, selectivity, and sensitivity of the assays. Firstly, we introduced the significance of fabricating a high-sensitivity and low-background interface to adjust various targets behaviors induced via bioactive molecules on the surface. Besides, some important strategies to resist non-specific protein adsorption and cell adhesion, followed by imperative categories of antifouling reagents utilized in the construction of high-performance solid sensory interfaces, are discussed. The next section explained the various nanocomposite probes based on antifouling-nanomaterials for electrode modification containing carbon nanomaterials, noble metal NPs, magnetic NPs, polymer, and silicon-based materials in terms of NPs, rods, or porous materials through optical or chemical strategies. We specially outlined those nanoprobes that are capable of identification in complex media or those using new constructions/methods.

So far, various signal transduction techniques were suggested for the construction of antifouling-based nanoprobes. Thereinto, the amalgamation of different antifouling strategies with diverse nanomaterials offers enhanced specificity and sensitivity in biosensors that can be directly used in patient serum sample assays, signifying their hopeful potential in clinical diagnosis. Although incredible efforts were devoted to the proposals of nanocomposite probes based on antifouling-nanomaterials, only part of these new concepts are being transformed into practical applications and commercial products. It is also worth noting that these issues are particularly intensified when long-term and continuous monitoring is conducted or derived probes are deemed to have a tenable shelf life. The investigation of hybrid synergic systems or new chemical architectures as antifouling platforms may also be very imperative as real world applications become more and more relevant. This becomes even more apparent once we consider that in most applications these interfaces must be diagnostically well and non-toxic. For instance, polyelectrolytes containing mixed charge polymers (polyampholytes) as a substitute for traditional zwitterions are both highly adjustable and potentially very potent. Additionally, zwitterionic organic/inorganic hybrid polymers with superior stability to degradation were progressively studied in the material sciences but have not yet been used in a (bio)sensor field. Physical methodologies like bioinspired topographies, for instance, mollusc shell mimics or shark skin patterns, have been utilized to control cell adhesion and resist marine fouling but have, again, rarely found application in sensors. Moreover, with the arrival of stronger computational approaches the simulation-assisted design of chemically complex or chemical-physical antifouling sensing probes will become more common in supporting extremely particular sensor design. There are various emerging technologies using cutting-edge micro/nanofabrication, soft electronic methods, self-powered devices, and 3-D printing with important potential for a numerous of sensing applications. Herein, the incorporation of the antifouling technologies detailed can significantly improve their utilization and long-term stability most remarkably for in vivo and in vitro theranostics/diagnostics. Such combined methods will also be important in stimuli-responsive bio-interfaces, like cell-based nanoprobes, organ-on-a-chip and enhanced wearable substrates for monitoring of drug.

In summary, with the rational design of smart nanostructures based on flexible/biocompatible antifouling materials and two-dimensional (2D) materials as well as the progress of lab-on-a-chip and 3D printed devices, significant commercial nanoprobes indirect testing of disease biomarkers in complex environments can come true in the near future.

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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