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Do not let electrode fouling be the enemy of bioanalysis

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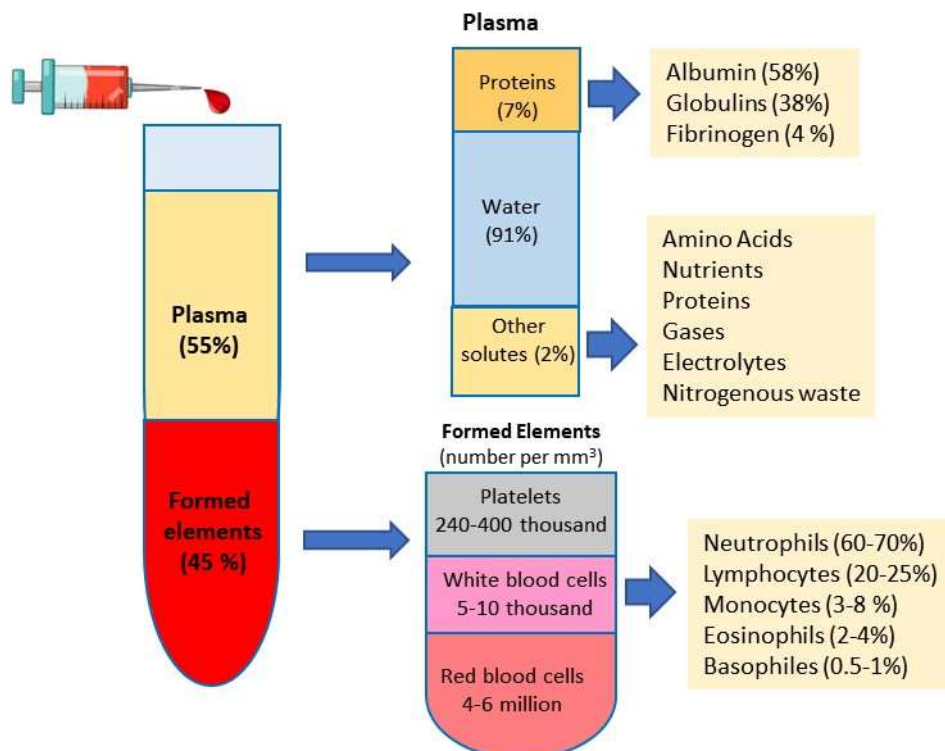
Abstract

Electrochemical biosensors are one of the best choices for the sensing of biomarkers due to their high performance, low cost, miniaturization, and wide applicability. However, like for any sensing process, electrode fouling affects severely the analytical performance of the sensor, such as sensitivity, detection limit, reproducibility, and overall reliability. Fouling arises from nonspecific adsorption of different components present in the sensing medium and is in particular several in complex biofluids such as full blood. The complex composition of blood where biomarkers are present at extremely low concentrations compared to the rest of the fluid composition makes electrochemical biosensing challenging. Direct biomarker analysis within full blood samples remains however central for the future development in electrochemical-based diagnostics. Herein, we aim to provide short discussion of past and more recent strategies and concepts employed to diminish background noise due to surface fouling and overcome current hurdles for the implementation and commercialisation of electrochemical-based biosensors for medical diagnostics of protein biomarkers in a point of care format.

1. Introduction

The dosing of different protein biomarker concentrations in human serum samples has become a standard means for screening the health state of a patient as their change in expression can be related to a specific diseases state. Blood drawings in conjunction with laboratory blood analysis have evolved into effective tools for healthcare providers and medical professionals in their decision making. While a large amount of blood testing is based on enzyme-linked immunosorbent assay (ELISA), electrochemical sensors have gained large popularity and are by far the most frequently employed today [1]. However, their use for the sensing in full blood or complex biological biofluids (saliva, sweat, sputum, nasopharyngeal) remains challenging. The recent advances in surface science and nanoscience generated new opportunities to tackle these hurdles in a more comprehensive way [2].

Blood is a complex biofluid being composed of plasma, the liquid portion of blood, red and white blood cells as well as of cell fragments called platelets (Schematic 1). The main component of blood i.e. plasma consists of 91 % water and contains proteins (hormones, antibodies), lipids, ions, nutrients, dissolved gases and other metabolites. Human serum albumin (HSA) accounts for 60 % of the total plasma proteins ($35\text{-}50\text{ mg mL}^{-1}$) with a half-life of 21 days. Together with IgG ($6\text{-}16\text{ mg mL}^{-1}$) and fibrinogen (2 mg mL^{-1}) they are mainly responsible for decreasing the sensitivity of the analytical approaches due to sensor' surface fouling. Furthermore, low platelet adhesion is essential to achieve improved compatibility for accurate blood analysis [3] .



Schematic 1: Blood Constituents: Blood is composed of blood cells suspended in blood plasma, which constitutes 55% of blood fluid, mostly water, but contains biomarkers and fouling biomolecules mostly in form of albumins.

While pre-analytical sample preparation approaches such as sample pre-purification aims at reducing the concentration of interfering proteins and biomolecules, these processes remain time-consuming and greatly reduce the clinical relevance of these sensors. To circumvent this hurdle, antibody-modified magnetic beads can be employed to directly capture and remove abundant coexisting proteins in blood, greatly reducing the fouling capability of the sample [4]. Beside remaining a two-step process, one of the drawbacks is that magnetic-based purification requires large quantities of antibody-functionalized nanostructures.

The adsorption of proteins on metallic surfaces such as gold electrodes depends on numerous physical and chemical aspects, such as protein concentration, pH, salt concentration, ionic strength of the medium, surface charge of the substrate, isoelectric point, and electrostatic interaction at the protein/metal interface. Soluble proteins are hydrophilic at their outer-part, while the core is commonly hydrophobic to maintain protein folding. Most electrode surfaces tend to be hydrophobic and promote species adsorption possessing hydrophobic components. These hydrophobic interactions are entropically favourable in an aqueous electrolyte because water molecules are released from the solvation shell around hydrophobic compounds or components and are typically irreversible in an aqueous electrolyte under mild conditions [5, 6]. Many antifouling strategies aim thus at reducing fouling by increasing the hydrophilicity of the electrode surface and thus limiting or even preventing direct contact of the target analyte with the electrode anchored bioreceptors and to minimize false positive responses [7, 8]. Although such strategies can be quite effective, they are inappropriate for systems in which the analyte itself is also the fouling agent. Recent developments in materials science have largely moved the field forward in proposing novel approaches for inhibiting surface fouling process to occur.

This mini-review is a short journey from poly(ethyleneglycol) (PEG) modified electrodes to the use of porous and nanomaterial modified architectures to allow sensing in full blood.

2. From poly(ethylene) glycol to hydrogels and anti-fouling peptides

The most widely used strategy to prevent protein to adsorb onto electrical interfaces and the analyte of interest to make physical contact with the electrode for electron transfer to elicit an electrochemical response is the use of chemical modifications that render the surface hydrated. The use of highly polar and hydrated chemical groups/materials such as oligo(ethylene glycol) (OEG), poly(ethyleneglycol) (PEG, >10 ethylene glycol unit (EG) units), hyaluronic acid, poly(2-hydroxyethyl methacrylate) as well as self-assembled monolayer (SAM) bearing terminal COOH and OH functions (mercaptohexanol, 2-mercaptoethanol, 4-mercaptobutan-1-ol, 2-mercaptoundecanoic acid, 11-mercaptoundecanol, etc) have been at the forefront of use over decades (Figure 1A).

In the case of SAMs formed from alkanethiols, irreproducibility and often incomplete coverage of the electrode surface results in most cases in limited long-term anti-fouling stability [9]. Long-term stability of PEG-SAMs coatings is furthermore limited due to easy oxidation of the SAMs in air [10] Highly packed and more stable SAMs to extend the operational life of electrochemical sensors notably in *in vivo* setting sensing from hours to days were lately proposed by Arroyo-Curras and co-workers [11], considering hydrophobicity arguments. They showed that modification of gold electrodes using methyl-terminated alkanethiols (e. g. 1-

hexanethiol) reduces not only the charging currents compared to electrodes modified with 6-mercapto-1-hexanol (MCH) (Figure 1B), but improves monolayer packing and SAM stability under continuous voltammetric interrogation. Improved SAM stability could be also achieved by using mixed SAMs [12] In all these works, not much attention was paid to the anti-fouling character of the SAMs and the short and long time stability.

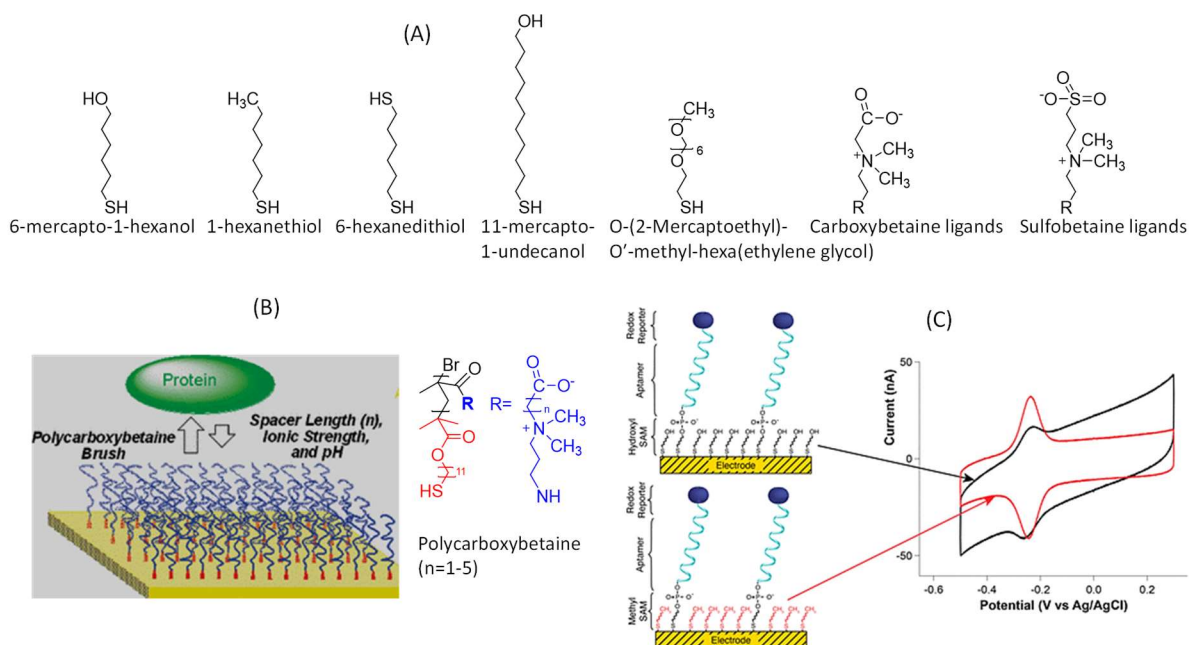


Figure 1: Functional self-assembled monolayer strategies: (A) Chemical structures of widely used functional thiols. (B) Anti-fouling architecture using polycarboxybetaine grafted surfaces (reprint permission from Ref.[13]). (C) Methyl-terminated SAMs for achieving a tighter packing and longer stability relative to hydroxyl-terminated SAMs with no desorption over time even under continuous electrochemical interrogation (reprint with permission form Ref. [11]).

It was with SAMs carrying terminal oligo(ethylene glycol) (OEG) moieties that enhanced resistance to proteins adsorption was observed. This work dates back to Prime and Whitesides report, [14] being among the first to demonstrate that OEG-SAMs reduce protein adsorption onto surfaces. The main driving force behind the decreased protein adsorption on PEG-modified electrodes is based on the compression of the PEG chains upon approaching of the protein to the surface [15]. Each EG unit can strongly bind to one water molecule, bridging the ether oxygen along the OEG/PEG chain thus, resulting in a hydrated layer, where compression, when a protein approaches the hydration layer barrier, leads to the biomolecule repulsion [16]. The strength of protein repulsion is thus increased on PEGylated-SAMs compared to CH₃- and OH-terminated SAMs [17].

Surface plasmon resonance studies allowed a direct comparison of the adsorption among different anti-fouling SAMs on gold, with polymeric brushes made out of carboxybetaine methacrylate (CBMA) showing the best anti-fouling behaviour [3]. Gold surfaces modified by zwitterionic carboxybetaine derivatives (polycarboxybetaine methacrylate, polycarboxybetaine acrylamides) by a “grafting from” strategy using surface-initiated atom transfer radical

polymerization (Figure 1C) showed indeed the expected antifouling properties against fibrinogen adsorption over a wide pH and ionic strength, although protein adsorption tends to increase at low ionic strength and low pH values [13]. The response-dependency to ionic strength and pH was attributed to the protonation/deprotonation properties of polycarboxybetaines, respectively. In line with this work, mixed SAMs of thiolated sulphobetaine (SB) and 11-mercaptopundecanoic acid (MUA) for covalent lectin immobilization was employed to allow for glycoprofiling of serum samples [18]. Despite the attractiveness of PEG as antifouling agent, limited long-term applications due to susceptibility to oxidative damages is a major drawback.

Biopolymers such as cellulose nanocrystal coatings [19], bovine serum albumin [20], and more lately small chain amino acid peptides have been considered as antifouling coatings. Peptides based on alternating positively charged lysine (K) and negatively charged glutamic acid (E) groups proved efficient anti-fouling ligands due to the zwitterionic character of the EK sequenced peptides [21, 22]. Luo and co-workers [22] pushed this field further by constructing peptide YVEYHLCREKEKEKEKEKAKAKAKAK (Figure 2A). The AKAKAKAK domain is for surface anchoring as the positively charged sequence interacts with the negatively charged poly(3,4-ethylenedioxythiophene) (PEDOT) citrate films. To this was added an antifouling part (EKEKEKEK) and the recognition sequence YVEYHLC with nanomolar affinity for aminopeptidase N, a cancer biomarker for early diagnosis of cancer [22].

A few teams have investigated DNA as anti-fouling coatings [23, 24] without focusing on its utility in the development of electrochemical sensing. The team of Wang and co-workers bridged this gap recently by proposing poly-adenine (polyA) coated electrodes in combination with a CN14 specific peptide for sensing of rituximab in lymphoma patient's plasma[24]. The intrinsic adhesion ability of polyA onto gold with an affinity comparable to Au-S chemistry as well as the interactions of the adenine bases with carbon, leaving the hydrophilic phosphate groups thoroughly exposed on the electrode surface increase the hydrophilicity of the electrode surface, making it a potential anti-fouling coating against protein adsorption?

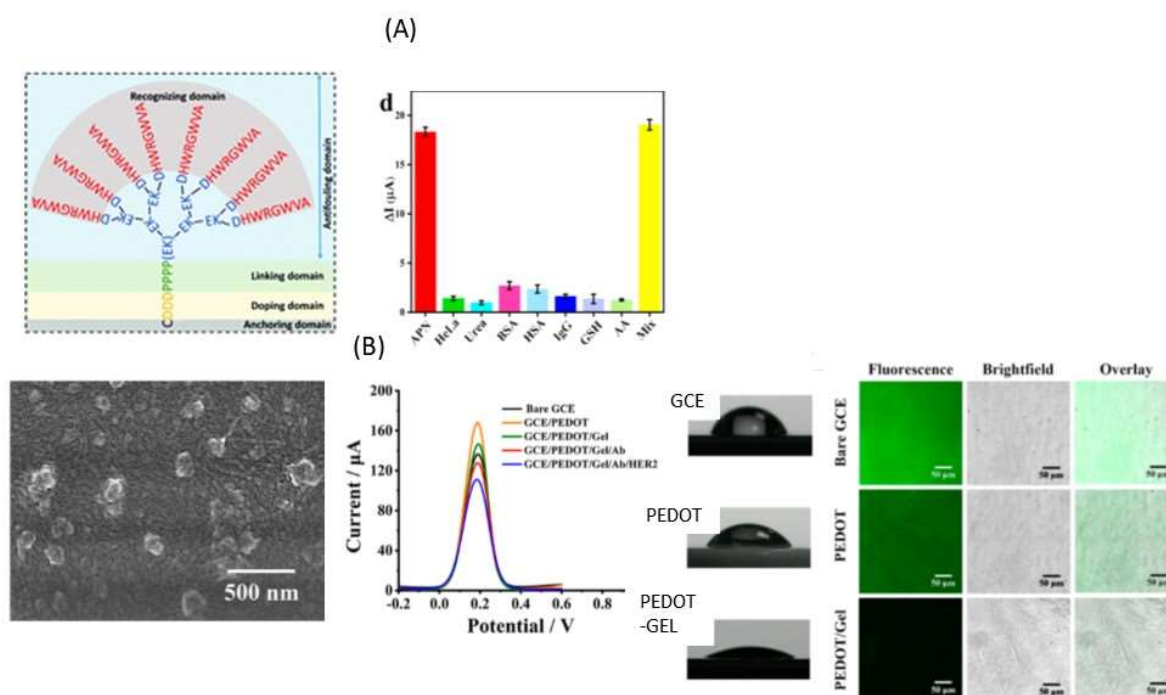


Figure 2: From anti-fouling peptides to conducting hydrogels : (A) Designed Three-in-One peptides with anchoring, antifouling, and recognizing capabilities for highly sensitive and low-fouling electrochemical sensing in complex biological media together with current change (ΔI) of the sensor toward $5 \mu\text{g}\cdot\text{mL}^{-1}$ APN, $1.0 \times 10^4 \text{ cells}\cdot\text{mL}^{-1}$ (HeLa cells), $1 \text{ mg}\cdot\text{mL}^{-1}$ urea, BSA, HSA, IgG, GSH, AA, and their mixture (Mix) (reprint permission from Ref.[22]). (B) SEM image of PEDOT/peptide-gel modified interface together with DPV responses of the different electrodes in PBS (10 mM, pH 7.4) containing $5 \text{ mM Fe(CN)}_6^{3-/4-}$ and 0.1 M KCl as well as contact angle characterisation and fluorescence of FITC-BSA-treated surfaces (reprint with permission from Ref. [25]).

The nonconductive nature of PEG-containing SAMs as well as anti-fouling peptide layers leads, however, mostly to interfaces of high impedance and decreased sensing sensitivity. Incorporation of PEG or peptides moieties with conducting materials, polymers and hydrogels addresses this issue [26, 27]. Using PEDOT and a biocompatible peptide hydrogel, prepared from a Phe-Glu-Lys-Phe peptide, human epidermal growth factor receptor 2 (HER2) could be sensed on this hydrophilic surface (water contact angle of 25°) (Figure 2B). Using confocal microscopy (Figure 2B), the ability of the peptide hydrogel modified surface to resist nonspecific BSA protein adsorption was validated. The use of PEG hydrogels conjugated with PEDOT atop gold or ITO electrodes and functionalized with monoclonal antibodies allowed also for the sensitive sensing of interferon- γ molecules in whole blood samples [27].

3. The future of nanostructured electrodes for antifouling

Next to the electrodes relying on highly hydrated interface chemistries to inhibit non-specific adsorption of proteins and cells, a large effort has been devoted over the years into the design of nanostructured electrodes. The use of porous electrodes as diffusion filters to alleviate fouling of proteins, while allowing the diffusive access to smaller analytes has been demonstrated in several research papers. In 2013, Patel et al. demonstrated experimentally that porous gold electrodes had better anti-fouling properties than planar ones [28]. In this work, cyclic voltammograms in the presence of $\text{Fe(CN)}_6^{3-/4-}$ as redox mediator on planar, macroporous (pores of $1.2 \mu\text{m}$) and nanoporous (pores $<50 \text{ nm}$) gold electrodes in the presence of serum albumin (2 mg mL^{-1}) and fibrinogen (1 mg mL^{-1}) were recorded (**Figure 3a**) [28]. From the time dependent cyclic voltammograms, it became clear that the nanoporous electrode offered the greatest protection. The anti-fouling property is due to the high surface area of the porous electrode and the 2D structures. While proteins could readily adsorb on planar gold electrodes, fouling of the porous is expected to be slower due to restricted mass transport of larger species through small pores. Indeed, the macroporous electrodes had an antifouling character more closely related to the planar configuration (Figure 3A). A similar finding was reported by Summerlot et al. using fragmented amyloid- β proteins where after 30 min incubation, the current of a nanoporous gold electrode decreased only by 13% whereas the current of the planar one declined by 96.5 % [29]. Using DNA hybridization as model reaction, the structure-property relationship between pore size and antifouling character was lately revisited and the importance of the sample composition was underlined (Figure 3B) [30]. It was found that the accessibility of target DNA increased with bigger pores, with small pores featuring excellent biofouling but less sensitivity to DNA hybridisation. A pore size of 25-30 nm was considered optimal in terms of sensing performance and anti-fouling properties.

These works formed the base for the current approach proposed by Ingber and co-workers to enable affinity-based electrochemical biosensing in complex biological fluids [31]. It was shown that coating an electrode with a porous matrix of glutaraldehyde (GA) cross-linked BSA supported by a network of conductive gold nanowires prevented non-specific interactions, while enhancing electron transfer to the electrode surface allowing for the sensitive quantification of anti-interleukin 6 in plasma (Figure 3C).

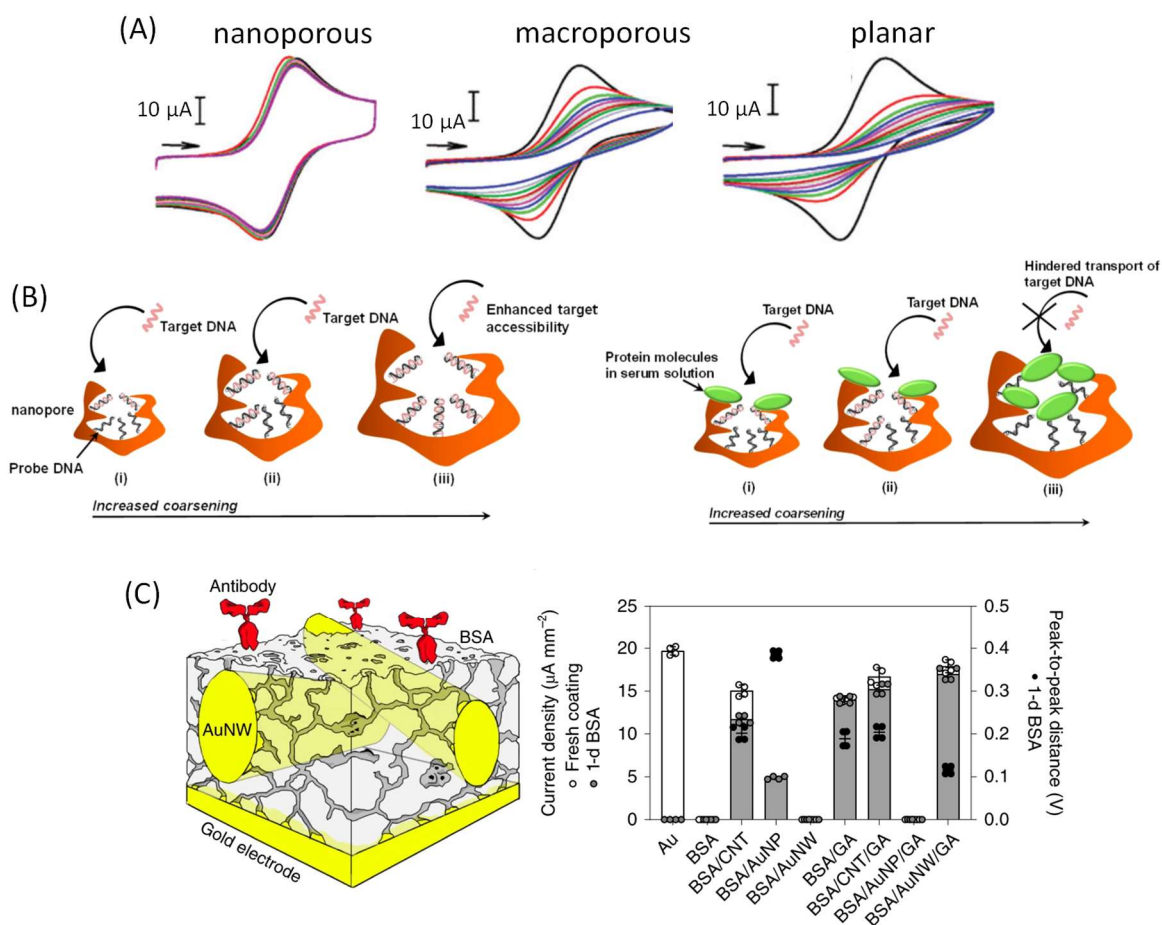


Figure 3: Nanostructured electrodes for antifouling (A) Cyclic voltammograms at nanoporous, macroporous, and planar gold electrodes using $\text{Fe}(\text{CN})_6^{3-/4-}$ redox probe and bovine serum albumin (2 mg mL^{-1}). Cyclic voltammograms were obtained between ~ 1 and 60 min (Reproduced with permission from Ref. [28]). (B) Schematic presentation of a hybridization event in uncoarsened and coarsened nanoporous Au electrodes (Reproduced with permission from Ref. [30]). (C) Schematic of BSA/AuNW/GA coating functionalized with antibodies on a gold electrode and electrochemical characterization of various electrode coatings. Bars are the mean values of the current densities of various nanocomposite-coated electrodes before (white) and after (grey) 1 day exposure to 1% BSA; black circles denote the final mean value of peak-to-peak distances ($n = 4$ independent electrodes) (Reproduced with permission from Ref.[31]).

Reduced graphene oxide (rGO) has further generated great expectations as anti-fouling matrix next of being a transducing platform in electrochemical sensing, owing to its high electrical conductivity, and fast charge transfer kinetics. Coating of electrodes by electrophoretic deposition with rGO/polyethyleneimine (rGO/PEI) [32-34] resulted in smooth coatings (Figure 4A) with improved anti-fouling properties, as evidenced by immersion of the interfaces for 24h

into saliva, serum and full unprocessed blood (Figure 4A). Modification with pyrene-PEG molecules via π - π stacking and H-bonding interactions improved the anti-fouling properties. Porosity could be integrated into rGO via treatment with hydrogen peroxide (H_2O_2) at elevated temperature where the generated oxidizing free radicals (OH^\bullet , O^\bullet , HO_2^\bullet) lead to the formation of an increasing amount of epoxy groups on rGO, which upon hydrolysis to hydroxyl groups result in a subsequent breaking of C-C bond, generating uniformly distributed nanopores (4-6 nm in diameter) (Figure 4A) [35]. Interestingly such surface exhibited comparable anti-fouling behaviour than that achieved on pyrene-PEG modified Au-rGO [36]. Drop-casting of glutaraldehyde (GA) crosslinked BSA in the presence of aminated rGO or pentaamine-modified graphene nanoflake had also proven great potential as antifouling electrode coating [37, 38] (Figure 4B). The antifouling properties of the BSA/rGO_x/GA nanocomposite were confirmed when exposing the coated electrodes to serum, plasma, and whole blood, with no significant changes in current density observed after 60 min. incubation. An additional benefit of the BSA/rGO_x/GA nanocomposite is the presence of amino acids, naturally present in the BSA protein, that could be applied for chemical conjugation and integration of different antibodies for the sensing of three different sepsis biomarkers [37].

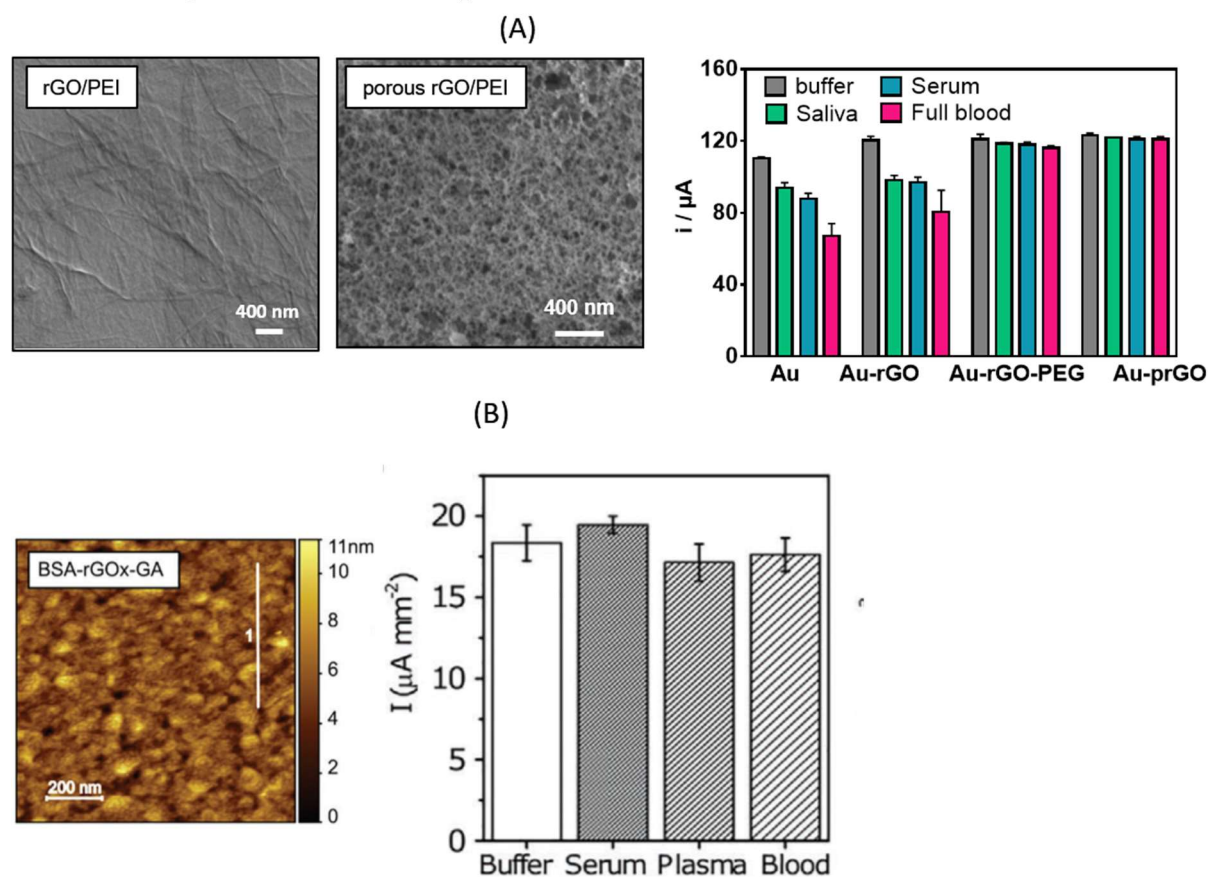


Figure 4: Development of antifouling nanocomposites based on reduced graphene oxide and derivatives: (A) Scanning electron microscopy (SEM) micrograph of Au electrodes after electrophoretic coating with rGO/PEI or prGO/PEI nanoflakes upon application of 15 V_{DC} for 4 min. and oxidation currents of ferrocenmethanol in PBS and after 24h exposure to undiluted saliva, serum and full blood samples. (B) AFM topography of BSA-rGO_x-GA coated gold electrode and oxidation currents of $\text{Fe}(\text{CN})_6^{3-/4-}$ couple obtained after exposure of nanocomposite coated electrodes to PBS, human serum, plasma, and whole blood.

Conclusion and Perspectives

Paving the way to more robust electrochemical sensors remains one of the challenges in current electrochemical-based diagnostics. This challenge is not only related to satisfy the requirement of high sensor performance, but also to lead to future point-of-care (POC) testing systems for continuous monitoring of the health status of the person without signal degradation. This mini-review article looked at past and future of anti-fouling strategies notably for full blood-based sensing. Translation of technological advancements and multidisciplinary knowledge in the nanomaterial field seems to be well-adapted to bridge the gap between laboratory-based sensing and commercial blood analysis products. While different anti-fouling strategies have been successfully put in place, a detailed account on the stability target of anti-fouling coatings from short term for disposable sensors to long terms (e.g. glucose monitoring for 7 days) for continuous measurements are missing in most reports. While some research teams looked at long-term stability if antifouling counting,[39, 40] reports on the long-term stability of anti-fouling layers under electrical bias are rare. For an antifouling coating to become technically feasible, it should fulfil several including long-term stability. Use of efficient, scalable as well as long-term anti-fouling surface chemistry remains a critical requirement for commercializing reliable electrochemical biosensors for diagnostic applications. Donald Ingber and his team contributed to this question lately by showing the long-term (9-weeks) efficiency of pentaamine-modified graphene nanoflakes an anti-fouling coating. However, the approaches discussed are adaptable to other complex biological media such as saliva, sweat, sputum, or nasopharyngeal samples. Integration of such concepts into electrochemical biosensors development will pave the way to future point-of-care (POC) testing systems due to more robust sensor interfaces.

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References

- [1] Mahato, K., and Wang, J. (2021) Electrochemical sensors: From the bench to the skin, *Sensors and Actuators B: Chemical* 344, 130178.
- [2] Barbosa, A. I., Rebelo, R., Reis, R. L., Bhattacharya, M., and Corelo, V. M. (2021) Current nanotechnology advances in diagnostic biosensors, *Med. Devices Sens.* 4, e10156.
- [3] Zhang, Z., Zhang, M., Chen, S., Horbett, T. A., Ratner, B. D., and Jiang, S. (2008) Blood compatibility of surfaces with superlow protein adsorption, *Biomaterials* 29, 4285-4291.
- [4] Kongsuphol, P., Ng, H. H., Pursey, J. P., Arya, S. K., Wong, C. C., Stulz, E., and Park, M. K. (2014) EIS-based biosensor for ultra-sensitive detection of TNF- α from non-diluted human serum, *Biosens. Bioelect* 61, 274-279.
- [5] Thevenot, P., Hu, W., and Tang, L. (2008) Surface chemistry influence implant biocompatibility, *Curr. Top. Med. Chem.* 8, 270.
- [6] Grinnell, F., and Feld, M. K. (1981) Adsorption characteristics of plasma fibronectin in relationship to biological activity, *J. Biomed. Mater. Res.* 15, 363-381.
- [7] Jiang, C., Wang, Hein, R., Liu, N., Luo, X., and Davis, J. J. (2020) Antifouling Strategies for Selective In Vitro and In Vivo Sensing, *Chem. Rev.* 120, 3852-3889.

- [8] Russo, M. J., Han, M., Desroches, P. E., Manasa, C. S., Dennaoui, J., Quigley, A. F., Kapsa, R. M. I., Moulton, S. E., Guijt, R. M., Greene, G. W., and Moraes Silva, S. (2021) Antifouling Strategies for Electrochemical Biosensing: Mechanisms and Performance toward Point of Care Based Diagnostic Applications, *ACS Sensors* 6, 1482-1507.
- [9] Campuzano, S., Kuralay, F., Lobo-Castañón, M. J., Bartošik, M., Vyavahare, K., Paleček, E., Haake, D. A., and Wang, J. (2011) Ternary monolayers as DNA recognition interfaces for direct and sensitive electrochemical detection in untreated clinical samples, *Biosens. Bioelectron.* 26, 3577–3583.
- [10] Schoenfish, M. H., and Pemberton, J. E. Air Stability of Alkanethiol Self-Assembled Monolayers on Silver and Gold Surfaces, *J. Am. Chem. Soc.* 120, 4502–4513.
- [11] Shaver, A., Curtis, S. D., and Arroyo-Currás, N. (2020) Alkanethiol Monolayer End Groups Affect the Long-Term Operational Stability and Signaling of Electrochemical, Aptamer-Based Sensors in Biological Fluids, *ACS Appl. Mater. Interfaces* 12, 11214–11223.
- [12] Kuralay, F., Campuzano, S., and Wang, J. (2012) Greatly extended storage stability of electrochemical DNA biosensors using ternary thiolated self-assembled monolayers, *Talanta* 99, 155-160.
- [13] Zhang, Z., Vaisocherová, H., Cheng, G., Yang, W., Xue, H., and Jiang, S. (2008) Nonfouling Behavior of Polycarboxybetaine-Grafted Surfaces: Structural and Environmental Effects, *Biomacromolecules* 9, 2686–2692.
- [14] L. Prime, L., and Whitesides, G. M. (1991) Self-Assembled Organic Monolayers: Model Systems for Studying Adsorption of Proteins at Surfaces, *Science* 252, 1142-1167.
- [15] Jeon, S. I., Lee, J. H., Andrade, J. D., and De Gennes, P. G. (1991) Protein—surface interactions in the presence of polyethylene oxide: I. Simplified theory, *J. Colloid Interface Sci.* 142, 149-158.
- [16] Aray, Y., Marquez, M., Rodríguez, J., Vega, D., Simón-Manso, Y., Coll, S., Gonzalez, C., and Weitz, D. A. (2004) Electrostatics for exploring the nature of the hydrogen bonding in polyethylene oxide hydration, *J. Phys. Chem. B* 108, 2418–2424.
- [17] Zheng, J., Li, L., Tsao, H.-K., Sheng, Y.-J., Chen, S., and Jiang, S. (2008) Strong repulsive forces between protein and oligo (ethylene glycol) self-assembled monolayers: a molecular simulation study, *Biophys J* 89, 158-166.
- [18] Bertok, T., Klukova, L., Sediva, A., Kasák, P., Semak, V., Micusik, M., Omastova, M., Chovanová, L., Vlček, M., Imrich, R., Vikartovska, A., and Tkac, J. (2013) Ultrasensitive impedimetric lectin biosensors with efficient antifouling properties applied in glycoprofiling of human serum samples, *Anal. Chem.* 85, 7324–7332.
- [19] Yang, W., Pan, M., Zhang, J., Zhang, L., Lin, F., Liu, X., Huang, C., Chen, X.-Z., Wang, J., Yan, B., and Zeng, H. (2022) A Universal Strategy for Constructing Robust and Antifouling Cellulose Nanocrystal Coating, *Adv. Funct. Mater.* 32, 2109989.
- [20] Li, Y., Han, R., Chen, M., Yang, X., Zhan, Y., Wang, L., and Luo, X. (2021) Electrochemical Biosensor with Enhanced Antifouling Capability Based on Amyloid-like Bovine Serum Albumin and a Conducting Polymer for Ultrasensitive Detection of Proteins in Human Serum, *Anal. Chem.* 93, 14351–14357.
- [21] Wang, G., Han, R., Li, Q., Han, Y., and Luo, X. (2020) Electrochemical Biosensors Capable of Detecting Biomarkers in Human Serum with Unique Long-Term Antifouling Abilities Based on Designed Multi- functional Peptides, *Anal. Chem.* 92, 7186–7193.
- [22] Song, Z., Chen, M., Ding, C., and Luo, X. (2020) Designed Three-in-One Peptides with Anchoring, Antifouling, and Recognizing Capabilities for Highly Sensitive and Low-

- Fouling Electrochemical Sensing in Complex Biological Media, *Anal. Chem.* *92*, 5795–5802.
- [23] McQuistan, A., Zaitouna, A. J., Echeverria, E., and Lai, R. Y. (2014) Use of thiolated oligonucleotides as anti-fouling diluents in electrochemical peptide-based sensors†, *Chem. Commun.* *50*, 4690.
- [24] Hunga, S., Tang, R., Zhang, T., Zhao, J., Jiang, Z., and Wang, Q. Anti-fouling poly adenine coating combined with highly specific CD20 epitope mimetic peptide for rituximab detection in clinical patients' plasma, *Biosens. Bioelect.* *71*, 112678.
- [25] Wang, W., Han, R., Chen, M., and Luo, X. (2021) Antifouling Peptide Hydrogel Based Electrochemical Biosensors for Highly Sensitive Detection of Cancer Biomarker HER2 in Human Serum, *Anal. Chem.* *93*, 7355-7361.
- [26] Hui, N., Sun, X., Niu, S., and Luo, X. (2017) PEGylated polyaniline nanofibers: Antifouling and conducting biomaterial for electrochemical DNA sensing, *Appl. Mater. Interfaces* *9*, 2914–2923.
- [27] Shin, D.-S., Matharu, Z., You, J., Siltanen, C., Vu, T., Raghunathan, V. K., Stybayeva, G., Hill, A. E., and Revzin, A. (2016) Sensing Conductive Hydrogels for Rapid Detection of Cytokines in Blood, *Adv. Healthcare Mater.* *5*, 659-664.
- [28] Patel, J., Radhakrishnan, L., Zhao, B., Uppalapati, B., Daniels, R. C., Ward, K. R., and Collinson, M. M. (2013) Electrochemical properties of nanostructured porous gold electrodes in biofouling solutions, *Anal. Chem.* *58*, 11610–11618.
- [29] Summerlot, D., Kumar, A., Das, S., Goldstein, L., Seal, S., Diaz, D., and Cho, H. J. (2011) Nanoporous gold electrode for electrochemical sensors in biological environment, *Proc. Eng.* *25*, 1457–1460.
- [30] Matharu, Z., Daggumati, P., Wang, L., Dorofeeva, T. S., Li, Z., and Seker, E. (2017) Nanoporous-gold-based electrode morphology libraries for investigating structure-property relationships in nucleic acid based electrochemical biosensors, *ACS Appl. Mater. Interfaces* *9*, 12959–12966.
- [31] Del Río, J. S., Henry, O. Y. F., Jolly, P., and Ingber, D. E. (2019) An antifouling coating that enables affinity-based electrochemical biosensing in complex biological fluids, *Nat. Nanotechnol.* *17*, 1143-1149.
- [32] Subramanian, P., Niedziolka-Jonsson, J., Adam Lesniewski, A., Qian Wang, Q., Musen Li, M., Boukherroub, R., and Szunerits, S. (2014) Preparation of reduced graphene oxide–Ni(OH)₂ composites by electrophoretic deposition: application for non-enzymatic glucose sensing, *J. Mater. Chem. A* *2*, 5525-5533
- [33] Subramanian, P., Lesniewski, A., Kaminska, I., Vlandas, A., Vasilescu, A., Niedziolka-Jonsson, J., Pichonat, E., Happy, H., Boukherroub, R., and Szunerits, S. (2013) Lysozyme detection on aptamer functionalized graphene-coated SPR interfaces, *Biosens. Bioelectron.* *B50*, 239-243.
- [34] Jijie, R., Kahlouche, K., Barras, A., Yamakawa, N., Bouckaert, J., Gharbi, T., Szunerits, S., and Boukherroub, R. (2018) Reduced graphene oxide/polyethylenimine based immunosensor for the selective and sensitive electrochemical detection of uropathogenic Escherichia coli, *Sensors and Actuators B: Chemical* *260*, 255-263.
- [35] Boulahneche, S., Jijie, R., Chekin, F., Teodorescu, F., Singh, S. K., Bouckaert, J., Medjram, M. S., Kurungot, S., Boukherroub, R., and Szunerits, S. (2017) On demand release of drugs from porous reduced graphene oxide modified electrodes flexible electrodes, *J. Mater. Chem. B*, *2017*, *5*, 6557-6565
- [36] (unpublished results).
- [37] Zupančič, U., Jolly, P., Estrela, P., Moschou, D., and Ingber, D. E. (2021) Graphene Enabled Low-Noise Surface Chemistry for Multiplexed Sepsis Biomarker Detection in Whole Blood, *Adv. Funct. Mater.* *31*, 2010638.

- [38] Timilsina, S. S., Durr, N., Yafia, M., Sallum, H., Jolly, P., and Ingbe, D. E. (2022) Ultrarapid Method for Coating Electrochemical Sensors with Antifouling Conductive Nanomaterials Enables Highly Sensitive Multiplexed Detection in Whole Blood, *Adv. Healthcare Mater.* *11*, 2102244.
- [39] Jiménez-Pardo, I., Van der Ven, L. G. J., Van Benthem, R. A. T. M., De With, G., and Esteves, A. C. C. (2018) Hydrophilic Self-Replenishing Coatings with Long-Term Water Stability for Anti-Fouling Applications, *Coatings* *8*, 184.
- [40] Maan, A. M. C., Hofman, A. H., de Vos, W. M., and Kaperman, M. (2020) Hydrophilic Self-Replenishing Coatings with Long-Term Water Stability for Anti-Fouling Applications, *Adv. Funct. Mater.* *30*, 2000936.