ImmunoFET feasibility in physiological salt environments

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Field-effect transistors (FETs) are solid-state electrical devices featuring current sources, current drains and semiconductor channels through which charge carriers migrate. FETs can be inexpensive, detect analyte without label, exhibit exponential responses to surface potential changes mediated by analyte binding, require limited sample preparation and operate in real time. ImmunoFETs for protein sensing deploy bioaffinity elements on their channels (antibodies), analyte binding to which modulates immunoFET electrical properties. Historically, immunoFETs were assessed infeasible owing to ion shielding in physiological environments. We demonstrate reliable immunoFET sensing of chemokines by relatively ion-impermeable III-nitride immunoHFETs (heterojunction FETs) in physiological buffers. Data show that the specificity of detection follows the specificity of the antibodies used as receptors, allowing us to discriminate between individual highly related protein species (human and murine CXCL9) as well as mixed samples of analytes (native and biotinylated CXCL9). These capabilities demonstrate that immunoHFETs can be feasible, contrary to classical FET-sensing assessment. FET protein sensors may lead to point-of-care diagnostics that are faster and cheaper than immunoassay in clinical, biotechnological and environmental applications.

Keywords: biosensing; immunoFET; protein detection; immunoassay; AlGaN; CXCL9

1. Introduction

Biosensing modalities can be of pivotal utility in clinical settings. Biosensors detecting appropriate analytes, with sensitivities and modes of operation, can potentially detect incipient disease prior to manifestation of clinical symptoms,

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reducing patient morbidity and mitigating mortality. Optimally, such sensors should operate non- or minimally invasively, such that the act of sensing itself does not impose significant morbidity on the patient.

Biosensors couple a bioelement (receptor that provides recognition of a specific analyte) with a transducer element to convert a biological event (e.g. binding of analyte to receptor, production of an enzymatic reaction product, consumption of an enzyme substrate, etc.) into an electrically measurable signal [1]. This general paradigm has been manifest in electrochemical biosensors since the earliest attempts to sense biologically important small molecules or macromolecules, and was followed in the first electrochemical biosensor (the glucose enzyme electrode [2]). The glucose enzyme electrode was followed relatively quickly (in 1970 [3]) by the development of the earliest ion selective field-effect transistors (ISFETs). ISFET design was significantly refined within a decade of inception of the sensing modality [4,5]. ISFETs feature integration of bioaffinity/catalytic elements (in ISFETs, typically an enzyme) deployed in the place of a gate on the capacitance layer of a metal oxide semiconductor field-effect transistor (MOSFET). Basic ISFET operation is broadly similar to operation of conventional FETs, excepting that ISFET current modulation is provided by ions produced by the enzyme and not by an electrical bias on a gate electrode. This coupling of biological affinity elements (receptors, antibodies, other proteinaceous affinity elements, nucleic acids of various descriptions, etc.) with the field-effect modulation principle of semiconductor devices was enticing, offering the promise of rapid, highly sensitive detection using a sensing platform (i.e. the MOSFET) that had been highly developed in the electronics industry. The ability of ISFETs to detect ionic charges further suggested the possibility that charged biological macromolecules (e.g. proteins, nucleic acids) might also be detectable by FETs.

The measurement of proteins via their intrinsic charges was first proposed by Schenk [6] when he envisioned deploying a receptor layer of antibodies with specific affinity for an antigen of interest (i.e. protein analyte) on the gate region of a FET: an immunoFET. One of the earliest immunoFET-like sensors featured immobilized anti-human serum albumin (anti-HSA) IgG on a polyvinylbutyral (PVB) membrane overlying an ISFET-sensing channel [7]. This sensor was capable of detection of HSA in solution (pH 7.0, 37°C) with a 2 mV shift in \( I_{ds} \) (current, drain to source). Sensor signal was linear with analyte concentration over a range of 0.01–1.0 mg ml\(^{-1}\), with a lower detection limit of 0.01 mg ml\(^{-1}\). While these experiments demonstrated that proteins could indeed be detected via immunoFET, the devices were not satisfactorily sensitive for many medical, biological and biotechnological applications. Limited sensitivity was a key limitation of many early immunoFETs [8–12], which impeded their translation to clinical devices.

The goal of optimizing immunoFET sensitivities to be comparable to those offered by existing immunoassays (i.e. enzyme-linked immunosorbent assays (ELISAs)) was elusive. Lack of a sensitive immunoFET led to promulgation of a hypothetical, theoretical rationale for the sensitivity limitations of the platform [13,14]. This model purported to define the limited sensitivity as being unavoidable and, in fact, intrinsic to all immunoFETs. Bergveld et al. [14] opined that ‘it should be concluded that a direct detection of protein charge is impossible’ and, further, that the idea that a layer of
charged proteins at a FET surface could modulate the electric field in the semiconductor ‘should definitely be forgotten’ [15]. This is the classical immunoFET assessment [13,15–18].

Classical assessment asserts that buffer counter-ions between the bound analyte and sensing surface shield analyte charges to the extent that those bound charges cannot influence current flowing through the FET semiconductor channel. The counter-ions were hypothesized to result in the formation of an electric double-layer between the analyte and the sensing surface having the thickness of the Debye length (inversely proportional to ionic strength). In physiological solutions (approx. 150 mM Na\(^+\)), Debye length is short: of the order of a couple of nanometres. Since some proteins (specifically, antibodies) have dimensional aspects of the order of 10–12 nm, it was argued that charges of proteins bound to antibody receptors would be held at distances greater than the Debye length from the sensor surface, making electrostatic detection of such charges impossible. Figure 1a shows a schematic of the classical view.

The classical model was readily accepted to rationalize immunoFET failures, and has long held sway in discussions of immunoFET protein sensing. Unfortunately, the classical model is marred by multiple misconceptions about the properties of antibodies deployed on synthetic surfaces. Classical analysis misrepresents not only antibody adsorption properties, but also antibody structure and dynamics. These misconceptions render the classical model incapable of accurately describing the actual behaviour of immunoFETs.

Classical analysis hinges on the distance between the extreme ends of antibody variable domains and C3 domains (figure 2). The distance is about 10 nm, substantially greater than the predicted Debye length under physiological salt concentrations. Bergveld reasoned that charges of analytes bound to antibody receptors would be shielded from the sensing channel of the FET by buffer counter-ions, and therefore not be detectable.
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Figure 2. Schematic of an IgG antibody. Heavy and light chains of the variable region are labelled as $V_H$ and $V_L$, respectively. The heavy chains of the constant region are labelled as $C_{H1}$, $C_{H2}$ and $C_{H3}$, while the light chain is $C_L$. The hinge region is indicated with an arrow. Analyte (or antigen) binding interaction occurs at the variable domain.

The reasoning is immunologically unsound for multiple reasons, though we focus primarily on considerations that are applicable to all immunoFET sensors. For classical analysis to be valid, antibodies must (i) be rigid bodies that (ii) adsorb to the sensing surface solely through their terminal C3 domain (figure 2). Logically, failure of antibodies to conform to either of these conditions calls classical analysis into question. In fact, antibodies adsorb to surfaces via a nearly random distribution of their structural domains [19], depicted in figure 1b. Moreover, antibodies are highly flexible, able to bend through arcs of 180° or more, with additional flexibility provided by the so-called ‘molecular ball and socket’ region occurring between the V and C1 domains [19]. These facts are discussed in most immunology textbooks (for example [19]), and have been for multiple years. These facts have implications for assessment of immunoFET function.

The combination of antibody adsorption in variable orientations relative to the surface and antibody flexibility causes bound analytes to be held in a distribution of orientations and distances from the sensing surface. Some of the analyte charges should thus be expected to be held within the Debye length, and therefore analyte electrical fields should be detectable by FET. That antibody adsorption to surfaces is not typically oriented, and that antibodies are indeed highly flexible, is not only immunological orthodoxy but also provides a rationale for successful immuno- and bioFETs [20–30]. These facts also indicate that, when immunoFETs fail, the mechanism is probably not as described by the classical model. This is important because understanding the mode of failure can potentially drive remediation of the immunoFET design. These misconceptions are pivotal in classical assessment, but their invalidity has been widely appreciated in immunology for years [19].

Classical assessment further ignores potential interfacial design approaches to minimize the distance between bound analyte charges and immunoFET sensing surfaces to maximize sensitivity. Neither does classical assessment consider
differentiable ion permeability of various FET platforms. Ion permeation alters electrical properties of MOSFETs, impeding accurate sensing [31]. Less ion-permeable FETs (e.g. AlGaN/GaN HFETs) have long been available and were used in the studies presented herein.

Beyond the above issues, the classical assessment depicts proteins as point-charges. In reality, proteins are space-filling nanoscale objects with fixed or highly constrained three-dimensional conformations whose charges are tightly associated with individual amino acid (aa) residues. Specific distribution of charge is determined by both analyte primary aa sequence and analyte conformation. Furthermore, antibodies recognize specific epitopes of their cognate protein antigens. Epitopes typically span regions of 10–12 aas, such that a protein of 100 aas is expected to have at least 10–12 distinct epitopes which could be bound individually or simultaneously [19]. This fact may have interesting potential implications for further development of immunoFET technology to detect charges of proteins independent of one another for purposes of protein identification and characterization (see §3).

While FET-based sensing is well accepted and widely used for the detection of various macromolecules, its use for the detection of proteins is not. This is due in part to the previous assertions that direct, label-free protein detection was infeasible [13–15], and the frustrating history of immunoFET development. This is unfortunate, in light of the fact that immunoFET assay detects an analyte attribute directly (i.e. analyte charge or electrical field), without need for secondary reagents (e.g. enzymes, chromogenic or radioactive substrates). This could make immunoFET assay potentially rapid, convenient and desirable, providing that immunoFETs operate with adequate sensitivity in physiological salt environments. In contrast, optical and other detection modalities that are available for proteins are often cumbersome, requiring multiple, time-consuming steps followed by the addition of secondary fluorescent labels or radioisotopically labelled compounds. In addition to providing real-time, direct detection, FET platforms are made by standard semiconductor processing methods, and are conveniently size-scalable.

ImmunoFET sensing has clear biomedical application in multiple disease states (in the presented work, for transplant applications), and could be applied to any biotechnology or environmental use where detection of a protein analyte is required. For example, immunoFETs might be used to detect proteinaceous environmental toxins and agents for biodefence applications (e.g. detection of biological warfare substances or proteins modified by them). Accurate consideration of the properties of the biological and polymeric components of immunoFETs, as well as immunoFET sensing data, shows clearly that genuine immunoFETs are not infeasible as a class [20–22]. We show here multiple functional immunoFETs operating physiological buffers, contrary to predictions of classical analysis.

Our analytes here are monokine induced by interferon-γ (MIG, CXCL9) of humans and mice. In both species, MIG is a pro-inflammatory chemokine that is a chemoattractant for cytotoxic T-cells. MIG increases during inflammatory responses, rising from a normal concentration of 40–100 pM [31], to 1–2 orders of magnitude higher during acute inflammation [32]. In transplant biology, rising graft MIG concentrations precede allograft rejection and can be used as an early indicator of imminent rejection [33,34]. Human and murine
MIG (huMIG, muMIG) have about 80 per cent protein sequence identity, similar isoelectic points and perform similar physiological functions, but are immunologically differentiable. Antibodies (IgG) specific for huMIG and muMIG \[35,36\] were used to build species-selective immunoHFETs. Results show that receptor/analyte properties define immunoFET specificities in predictable ways, a finding that is difficult to rationalize, unless planar immunoHFETs can in fact function in physiological buffers. This report extends our previous paper demonstrating feasibility of bioFETs \[20\] into the arena of immunoFET detection by demonstrating selective and sensitive immunoFETs. Multiple planar immunoHFETs detecting muMIG and huMIG \[37\] in physiological buffer provide proof.

2. Material and methods

(a) Chemicals

The silane polymer used was triethoxysilane aldehyde (TEA; United Chemical Technologies, Bristol, PA), and aminopropyl triethoxysilane (APTES; Gelest, Inc., Morrisville, PA). Polyclonal anti-muMIG IgG was from R&D Systems, Inc. (Minneapolis, MN). Polyclonal anti-huMIG IgG, biotinylated anti-huMIG IgG and recombinant huMIG and muMIG are from Peprotech, Inc. (Rocky Hill, NJ). EZ-Link Sulfo-NHS biotin was purchased from Pierce, Inc. (Rockford, IL). Streptavidin (SA), SA conjugated to horseradish peroxidase (SA-HRP) and Dulbecco’s phosphate-buffered saline (PBS) containing 150mM NaCl, pH 7.4, are from Invitrogen, Inc. (Carlsbad, CA). o-Phenylenediamine dihydrochloride (OPD) tablets were purchased from Sigma-Aldrich, Co. (St. Louis, MO).

(b) Transistor fabrication

AlGaN/GaN HFETs were constructed as previously described \[20,30,38\]. AlGaN/GaN heterostructures were purchased from CREE, Inc. (Raleigh, NC) and surface oxidized via ion-coupled plasma treatment (ICP) by oxygen plasma \[30\]. One hundred micrometres of the AlGaN barrier was recessed in a Cl-based ICP plasma so that the threshold voltage of the device was shifted to the −0.5 to +0.5V range. The conducting channel of the HFETs varied from 50 to 100μm in width and length. The device reservoir with an average height of 10–20μm allowed the conducting channel access by the samples. The chemical gate formed on the oxide was functionalized with receptors (antibodies or SA) for specific analyte binding.

(c) Surface preparation and device sample exposure

For the huMIG and muMIG receptor-specificity experiments, AlGaN HFETs were surface functionalized with 5 per cent TEA in ethanol (following the APTES deposition protocol \[39\]) and subsequently 1μg ml\(^{-1}\) anti-huMIG or anti-muMIG IgGs. The sample reservoir was exposed to 15μl of 5μg ml\(^{-1}\) (0.43, 0.41μM, respectively) huMIG or muMIG.

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For experiments comparing detection of mixed biotinylated and native MIG samples (bMIG and huMIG, respectively), two AlGaN HFETs were used. One device was functionalized with 5 per cent APTES in ethanol, biotinylated using 1 mg ml\(^{-1}\) biotin at 37°C and subsequently treated with 5 µg ml\(^{-1}\) SA. This device was used for bMIG detection using SA as the specific recognition element to bind biotin on bMIG. The second device was functionalized as described above with 5 per cent TEA in ethanol and anti-huMIG IgG and used to detect MIG (both MIG and bMIG).

\(d\) Electrical measurements

Three-terminal (source, drain and gate) current–voltage characteristics of AlGaN/GaN HFETs were measured using an Agilent 4156C semiconductor parameter analyser at room temperature. The source/drain current is modulated by the gate bias to the order of 1 µA mm\(^{-1}\) for detections, so that the device is working in the subthreshold regime for best device performance [28,29]. A device source/drain characteristic was first measured with PBS only (baseline measurement). The protein solution was then applied with a micro-pipette and incubated for 5 min, after which second source/drain characteristics were measured for comparison. The charges introduced by the binding of analyte to surface receptors modulate the source/drain current.

\(e\) Immunosorbent assay

ELISAs were performed in 96-well Nunc Maxisorb ELISA plates to corroborate electrical sensor data. For huMIG versus muMIG detection, wells were incubated with 1 µg ml\(^{-1}\) anti-huMIG or anti-muMIG IgG for 1 h at 37°C; background wells were incubated with PBS. Wells were then blocked with 5 per cent bovine serum albumin (BSA) in PBS for 2 h at 37°C before exposure to 10 ng ml\(^{-1}\) huMIG or muMIG for 10 min at 37°C. Subsequently, wells were incubated with 1 µg ml\(^{-1}\) biotinylated anti-huMIG or anti-muMIG IgG for 1 h at 37°C. Plates were then incubated with 1 µg ml\(^{-1}\) SA-HRP in PBS for 1 h at 37°C. OPD was freshly prepared according to the manufacturer’s directions and added to each well for 20 min; the reaction was stopped with 3 M H\(_2\)SO\(_4\), and the absorbance of the reacted OPD solutions were measured in a Victor X3 Plate Reader (spectrophotometer from Perkin-Elmer) at 490 nm. Wells were rinsed five times in 0.1 per cent Tween-20 in PBS between each step.

For confirmation of bMIG/MIG electrical sensing data, ELISAs similar to those described above were performed. In these tests, wells were treated as above with anti-huMIG IgG for MIG detection. Treatment for these tests varied in that the wells were exposed to solutions of bMIG/MIG in varying ratios as opposed to solely huMIG. Wells were also treated for detection of bMIG using SA as the detection agent. Wells were incubated with 500 ng ml\(^{-1}\) SA for 2 h at 37°C followed by exposure to 1 ng ml\(^{-1}\) bMIG/MIG solutions (0–100% bMIG in 20% increments) for 30 min at room temperature. Wells were then incubated in 1 µg ml\(^{-1}\) SA-HRP and exposed to OPD as above. Absorbance of reacted OPD solutions for all wells was measured in the Victor X3 Plate Reader at 490 nm.
3. Results and discussion

huMIG and muMIG in PBS were quantified and the species selectivity of anti-huMIG and anti-muMIG IgGs were corroborated by ELISA (figure 3). huMIG and muMIG are positively charged (+19 charges/muMIG, +20 charges/huMIG, pH 7.4 [37]) and HFETs are n-type: their charge carriers are electrons. Therefore, as expected, binding of huMIG or muMIG to sensing channels increased current drain to source ($I_{ds}$; figure 3). However, the immunologically distinct MIG species are detected differentially by immunoHFETs with corresponding species-specific antibodies on their channels (figure 3).

Both murine and human-specific MIG immunoHFETs exhibited unchanged $I_{ds}$ after exposure to PBS. Anti-huMIG immunoHFETs exposed to muMIG gave responses similar to PBS background, and anti-muMIG immunoHFETs exposed to huMIG also gave responses comparable to background. Conversely, immunoHFETs decorated with anti-huMIG IgG exhibited approximately 22 per cent $I_{ds}$ increase on exposure to huMIG, and sensors with anti-muMIG IgG exposed to muMIG exhibited approximately 15 per cent $I_{ds}$ increase (figure 3). huMIG and muMIG minimal detection limits were similar (approx. 1 fM; electronic supplementary material, figure S1). Analyte detection specificities of immunoHFETs reflect ELISA-demonstrated antibody-binding specificities (figure 3), the though the assays are different (ELISA incorporates multiple
secondary reagents that the immunoHFET assay does not). Having demonstrated that homologous but immunologically distinct analytes are differentially detected by immunoHFET, we next tested whether immunologically similar but distinct analytes in single samples could be discriminated.

Native (unbiotinylated) and biotinylated huMIG were mixed at varying stoichiometries and assayed by bioFET (SA as receptor), and immunoFET (anti-huMIG IgG as receptor). Neither the bioFET nor the immunoFET responded to PBS, nor did the SA bioFET respond to native MIG (electronic supplementary material, figure S2). However, immunoHFETs with anti-huMIG on their sensing channels detected huMIG regardless of biotinylation state (reflecting total huMIG, bioinylated and native; figure 4), while streptavidin bioFETs detected only biotinylated analyte, differentially detecting the varying concentrations of biotinylated huMIG in samples (figure 4a, b).

Immuno/bioHFETs detect MIG at biologically meaningful concentrations in physiological buffers and can be configured to detect analyte mixtures, or single constituents of mixtures (figure 4). Results, though contrary to classical assessment [13,15–18], are congruent with recent HFET protein-sensing results [20,21,24,25,29,30,40]. Nonetheless, the notion that some immunoHFETs are feasible in physiological environments is not widely accepted, motivating direct demonstration of the proposition here.

Classical analysis [13,15–18] cannot model actual behaviour of immunoHFETs because it misrepresents antibody properties. Both assumptions that antibodies are rigid and uniformity of antibody orientation on immunoFET channels (adhering to surfaces solely by C3 domains) are critical to classical immunoFET analysis: negation of either assumption invalidates the central argument of classical analysis. Immunologists have long known both assumptions are incorrect [19], and our results reflect the invalidity of those assumptions.

IgG hinge region (between C2 and C1 domains) flexibility, known since immunoglobulin structure was determined, allows individual arms of antibodies (consisting of C1 and variable domains) to bend through arcs of up to 180° [19]. This conformational freedom is critical to antibody biology. Hinge flexibility allows binding of multi-valent antigens, even if relative positions/orientations of individual epitopes of antigens are irregular, facilitating formation of aggregates for phagocytic uptake and elimination [41]. In immunoFETs using intact IgGs as receptors, hinge flexibility should allow positioning of bound analytes in a distribution of orientations/proximities relative to the rest of the antibody and to the immunoFET sensing channel. This should occur whether antibodies are consistently oriented on sensing channel surfaces or not.

Individual antibodies bind specific single epitopes of protein antigens, typically 10–12aa long, often contiguous in antigen sequence. Through the use of antibody fragments, to remove flexible hinge regions and reduce overall size, it may be possible to preferentially position specific charged regions of analytes proximal to sensing surfaces. This may allow for the detection of specific charged regions of analytes as opposed to detection of analyte net charge. Use of epitope-specific orientation would lend itself to the detection of net neutral proteins via exploitation of more highly charged regions.

Also, in the absence of specific affinity elements or chemoselective conjugation, surface adsorption of antibodies is not consistently oriented. No biochemical process forces antibodies to adsorb exclusively via the C3 (or any other) antibody...
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Figure 4. Differential detection of native and biotinylated huMIG in mixed samples by (a) immunoassay and (b) immuno- and bioFET assays. (a) Primary analyte receptor (anti-huMIG or SA, respectively) was deployed on ELISA plates followed by the secondary affinity element (biotinylated anti-huMIG or SA-HRP, respectively). Hatched bars indicate biotinylated huMIG content of samples; black bars represent total huMIG content of samples. (b) Per cent change in current is from baseline to the exposed analyte source/drain characteristic. Note that part (a) compares the absolute magnitude of absorbance, while part (b) compares the trend of device signal. (a) Bars with horizontal lines, bMIG; filled bars, MIG. (b) Squares with continuous line, bMIG; diamonds with dashed line, MIG.

domain: consistent alignment on surfaces requires modification of antibodies or surfaces [42–44]. As for most immunoFETs, alignment of antibodies relative to sensing channels was not attempted here, but may have potentially interesting consequences.

Since adsorption does not occur exclusively at any specific antibody domain, the calculated distance between the sensing surface and bound charges, determined assuming uniform antibody adsorption to the surface by antibody C3 domains, and comparison of that distance with the predicted Debye length (the distance over which counter-ion shielding should occur) in physiological buffer cannot be relevant to immunoFET feasibility. However, the comparison (bound analyte charges to sensing surface distance to Debye length) is the crux of the
classical infeasibility argument. Hence, the infeasibility argument as originally formulated is not germane to behaviour of immunoHFETs made as ours are. That said, interfacial film structure is a primary determinant of sensitivity [39], though not in the manner classical immunoFET assessment suggests.

Film morphology determines analyte charge to sensing channel distance, and, thus, immunoFET sensitivity [39,45,46]. Consistent with theory [47], previous data [20,29,30,39,40,48] show that immuno- and bioFET signal magnitude is highly dependent on analyte charge to sensing channel proximity. Israelachvili [47] states that FET signal magnitude varies with the sixth power of charge-to-surface distance. We cannot determine the mean distance of analyte charge to HFET surface sufficiently accurately to empirically validate Israelachvili’s prediction with our immunoFETs, though it is clear that nanometre-scale changes in film thickness, and in position of bound analytes in interfaces, profoundly influence signal magnitude [29,30,39,40,48].

The sensors here were not engineered to minimize analyte-to-channel distance and maximize sensitivity, but this critical parameter can be addressed by multiple means. First, the sensor interfaces presented in this work use a trivalent silane (TEA) that is similar to APTES. Therefore, we expect the interfacial height of the TEA layer to be higher than the minimal height achieved using a monovalent silane derivative (i.e. as with aminopropyl dimethylethoxy silane (APDMES) [49]). In comparison with films made with trivalent silanes, monovalent silanes form thinner polymer films with more regular (smoother) surfaces, shown in figure 5 [49]. Based on differential sensitivity observed using APTES (trivalent) and APDMES (monovalent) interfacial polymers [26,48], we expect that the sensitivity of the presented devices will be enhanced by depositing more ideal (i.e. thinner, more regular) interfaces built using monovalent silanes. That said, the sensitivity of the unoptimized immunoHFETs demonstrated here was sufficient to allow us to demonstrate immunoHFET feasibility in a physiological environment.

Shielding of charges of bound analytes by buffer ions indeed occurs in immunoFETs [20,29,30,39,40,48], but the key issue for immunoHFET feasibility is whether charges of any given analyte bound to antibody receptors of an immunoHFET are shielded by ions in physiological buffers beyond detection by the underlying FET. This is a complex consideration (encompassing analyte charge density, charge distribution, specific receptor and analyte three-dimensional structures, specific receptor epitope recognition properties, receptor bioconjugation conditions, interfacial film morphology, sensor dielectric thickness, specifics of sensor operation, etc.), dependent on particulars of the immuno- or bioFET at hand. We demonstrate multiple affinity elements (two antibodies and SA) used to make functional HFET protein sensors and there is no reason to believe that the affinity elements used here are special per se, allowing configuration of multiple immuno/bioHFETs for diverse sensing tasks in diverse environments.

Orientation of affinity elements might drive differences in HFET performance should differential orientation influence positions of analyte charges relative to the sensing channel. Interfaces with regular affinity element orientation can be constructed [42–44]. Assuming the validity of theoretical exponential charge-to-surface distance relationships [47], it may be possible to use oriented, rigid affinity elements to build immunoFETs that detect specific analyte charges.
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or regions of charge in preference to or exclusion of others. ImmunoHFETs using intact antibodies, and perhaps even some antibody fragments, as receptor may not allow for detection of specific charged regions of analytes owing to antibody (IgG) conformational freedom [19,41]. Intact IgGs would be expected to hold analytes in a distribution of orientations and distances relative to the surface, even if antibody binding to the FET surface was exclusively via the IgG antibody C3 domain, as was originally, but inaccurately, assumed in classical immunoFET analysis.

huMIG and muMIG are highly charged: analytes with lower net charges may be less sensitively detected by immunoFET. It is unknown whether immunoFETs detect net molecular charges or, as seems more likely, individual charges within a critical distance of the sensor regardless of charge affiliation with individual analyte molecules. The relationship of protein size, charge distribution and conformation to detection by immunoFETs is also largely uninvestigated. We have begun to manipulate the charge, multimerization state and size of huMIG and other analytes by recombinant DNA and bioconjugate means to differentiate these possibilities.

Differing FET architectures provide differing device electrical stabilities in high salt environments [20], potentially affecting their amenability to in vivo use. FET architecture also influences other immunoFET/bioFET properties, as improvements in sensitivity from incorporation of a control gate show [29,30,39,40,48]. AlGaN/GaN HFETs may be particularly suited to use in high osmolarity environments (as in vivo) because of limited AlGaN permeability to buffer ions and high-electron current drive properties [20]. Other FETs with, or engineered to have, similar properties may be as efficacious and more economical [39]. ImmunoFET economy and ease of fabrication may be important, as potential for immunoFET sensors in clinical applications is large.
4. Conclusions

The history of immunoFETs is illustrative of potential negative consequences of dogmatism to technological progress. The classical assessment is flawed in such a way that it can provide neither explanation nor rationale for limitations of immunoFETs, nor, more importantly, guide solutions to technical challenges for development of functioning immunoFETs. We provide proof for immunoHFETs operating in physiological solution, using an ion-impermeable platform (AlGaN/GaN HFETs) and show signal magnitude for a fixed analyte charge to be directly related to charge proximity to the sensing channel [20–22,30,39,40,44,48]. We believe recognition of the significance of these nuances to immunoFET function will facilitate development of a myriad of immunoFETs directed to other analytes working in high salt environments. Given economical FET platforms, immunoFET assay could potentially supplant more laborious, time-consuming and expensive immunoassays in clinical and laboratory settings.

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References

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