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TRENDS

Critical aspects of biointerface design and their impact on biosensor development

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Abstract The stable integration of a biological recognition element on a transducing substrate surface is the single most important step in the creation of a high-functioning sensor surface. The key factors affecting biotic and abiotic functionalities at the biointerface are both chemical and physical. Understanding the interactions between biomolecules and surfaces, and their emergent complexity, is critical for biointerface implementation for sensing applications. In this overview, we highlight materials and methods typically used for biosensor development. Particular emphasis has been given to the experimental evaluation of biointerfacial properties and functionality. Promising research directions for application of biointerfaces to biosensing are suggested.

Keywords Biointerface · Biosensor · Surface topography · Bio-immobilization · Surface functionalization · Surface characterization

Introduction

Biosensors are detection/measurement devices that transform a biological recognition event, for example a ligand-binding event or enzymatic catalysis, into an optical, thermal, electrical, or chemical signal that can be measured in real-time. Biosensor development is an interdisciplinary process, encompassing biology, chemistry, physics, mathematics, information technology, and engineering. In the past 10–20 years, biosensors have had a significant impact on environmental and industrial monitoring, medicine, food safety, biotechnology, and national security and defense. Annual worldwide investment in biosensor R&D is estimated to be $300 US million [1]. Between 1998 and 2004, over 6000 articles and 1100 patents/pending relating to biosensors have been published. Interestingly, besides the blood glucose and lactate biosensors (85% of the biosensor market) and a few other hand-held devices, only a minimal number of biosensors have successfully transitioned to the commercial sector for use outside the laboratory. This slow technology transfer is because of the relatively high development cost for a single analyte system and some key technical barriers associated with biointerface development.

Critical to biosensors, irrespective of their application, is the biointerface consisting of a biological or biologically-derived recognition element integrated or associated with an abiotic transducing platform. It is at this biointerface that retention of biosensor functionality is most often decided in terms of biological activity and platform response. A successful marriage of the biotic and abiotic components is essential to the development of a stable, sensitive, accurate, and ultimately marketable biosensor. Therefore, the importance of understanding the physicochemical properties of the abiotic layer and how they affect the bio-functionality of the biotic layer has been widely recognized by many in the biosensing field [2–4]. The choice of core material provides unique opportunities for tailoring biomolecule binding and activities that are application- and platform-specific. Preferred substrates for biosensing include metal and metal oxides, silica-based materials, polymers, or a

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combination of these materials. Additional surface modifications in (bio)chemical composition and/or topographical structures increase the functional complexity of the biointerface. One must consider, for example, Van der Waals and electrostatic forces, hydrophobic and hydrophilic interactions, solvation, valence, and surface structure and topography. These may be critical for determining immobilization strategies and optimizing biointerface functionality.

The design process proceeds iteratively. Preparation and characterization of the chemical and physical properties of each layer is essential to ensure proper biosensor operation and optimization of biosensor performance. Key elements of the biointerface design process are illustrated in Fig. 1. Combinations of surface characterization techniques have been used for more than 20 years in the development of biomaterials. However, it is only recently that these methods and strategies have been more commonly utilized by the biosensing community, allowing the relationships between structure and function, the biotic and abiotic, to be determined.

In this paper, we highlight materials and methods typically used for biosensor development with particular emphasis on the evaluation of biointerfacial properties and functionality. More recent thrusts in biosensor development, including the use of polymers and other novel materials as emerging substrates and platforms, the increasing push toward nanoscale architectures, and the introduction of unconventional and engineered biosensor recognition elements are also discussed.

Overview of substrate materials and surface chemistry

Metal and metal oxides

Metal and metal oxide substrates have long been used as platforms for biosensors, and their surface chemistries are well known. Typically, the biointerface on metals (e.g., Au, Ag, Cu, or Pt) is based on covalent bond formation (i.e., chemisorption) of self-assembling organosulfur compounds [5]. Of the chemisorbed self-assembled monolayers (SAMs), alkylthiols on gold surfaces have been studied the most and used for various bio-applications, including biolabeling, bioimaging, and modeling biological surfaces and biosensors through the addition of molecular recognition elements. The main compounds used for modifying metal oxide surfaces, for example titanium oxide and aluminium oxide, are silanes and phosphates/phosphonates [5]. Silanes are commonly used to modify metal oxide surfaces because of their ease of use and commercial availability. They can bear different functional or reactive groups capable of subsequent covalent coupling reactions. Alkyl phosphates/phosphonates have also been shown to form SAMs on a number of transition metal oxide surfaces. In contrast to silanes, phosphates/phosphonates undergo reactions only with surface hydroxyl groups and are not prone to self-condensation reactions, minimizing multilayer formation and deposition of aggregates on the surface. Incorporation of alkyl SAMs and other insulating materials into electrochemical sensors can promote direct, unmediated electron transfer between immobilized biomolecules and electrodes (metal substrate), and enhance sensor performance [6].

Some challenges have been associated with the use of metal and metal oxides as stable substrates in biointerfaces. For metal surfaces these include oxidation of the thiol functionality to a sulfate or sulfonate, decrease of metal–sulfur interaction because of ageing or biological media, and desorption of organosulfur molecules. For metal oxides the effectiveness and strategies for silanization depend on the type of oxide. In contrast with titanium oxide and aluminium oxide, certain metal oxides, for example copper oxide and zinc oxide, form unstable surface oxides while the precious metals (e.g., gold and nickel) typically do not form oxides. In addition, the use of chloro- and alkoxysilanes often leads to corrosive and reactive chemical byproducts that can degrade metal and metal oxide materials.

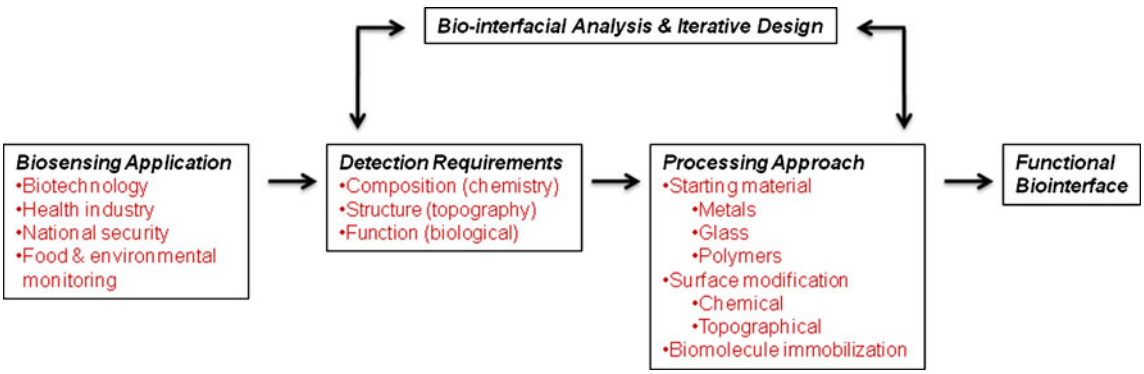

Fig. 1 Biointerface design

Silica-based materials

Silica-based substrates (fused silica, glass, and quartz) are favorable materials for sensor development because of their low intrinsic fluorescence, high insulation, and excellent optical properties, and their inertness in the presence of a variety of different solvents. Substantial efforts have been made in recent years to improve bio-immobilization on modified silica-based surfaces. Methods include poly- L-lysine coating, formation of SAMs after gold-coating, and addition of three-dimensional matrices (e.g., polymers, agarose, and acrylamide-based hydrogels), but glass surface modification methods continue to rely primarily on silanes [7]. Silane chemistry is a robust approach to surface modification and bio-functionalization. Silanes undergo hydrolysis and subsequent condensation reactions to form siloxane bonds with surface hydroxyl groups yielding a modified surface with pendant functional or reactive groups.

Self-assembled silane monolayers can promote surfaces with well-defined topographic features for enhanced bio-immobilization and control of the distance between sensor surface and immobilized biomolecule to localize binding events within the region of highest optical intensity. However, a number of physical and chemical factors at the abiotic-biotic interface can result in irregular surface morphology and non-planar orientation of functional/reactive moieties. These include the density and type of surface hydroxyl groups (Si–OH, Me–OH, where Me=Al, Ti, Cu, etc.), surface energies, and the physical dimensions of the substrate. Even after silane monolayer assembly, most siloxane bonds can be hydrolyzed at high pH and, therefore, may not provide long term stability.

Polymers

In recent years, there has been remarkable growth in the use of polymers for bio-applications, because of their relatively low cost and desirable bulk physicochemical characteristics, such as high strength-to-weight ratio, resistance to corrosion, and excellent optical and mechanical properties. In contrast to glass and metal substrates, polymers are hydrophobic and often lack appropriate chemical moieties for functionalization, thereby restricting bio-immobilization without pre-treatment. The distinct surface chemistries and electrokinetic properties of polymeric materials necessitate the use of diverse approaches for surface modification that are typically non-transferable from one material to another. In general, polymers are bio-functionalized using physisorption, which is a simple, high capacity approach to bio-immobilization. Covalent attachment schemes are sometimes preferred to ensure stable, functional and oriented display of the biomolecule of interest. This often requires surface modification of the polymer substrate, which can be accomplished by techniques such as wet chemical treatments, plasma (ionized gas) treatments, and UV irradiation. Additionally, initial polymer modification can be coupled with a secondary functionalization step such as silanization or plasma polymerization or plasma deposition and functionalization.

In the past, many surface-modification strategies for covalent attachment of biomolecules to polymeric substrates have relied primarily on variable thickness coatings applied through thin film deposition. Ideally, the objective of surface modification approaches should be to introduce no more than a single monolayer of a desired functional group to a substrate surface. Yet, in these cases, bio-functionalization has little to do with the core substrate but rather the deposited film or coating. Wet chemistry approaches, on the other hand, often require extensive treatment in corrosive solutions that result in non-specific biomolecule attachment, irregular surface etching, and the production of a wide range of oxygen-containing functional groups (e.g., C=O, O–C=O, CO_3). These methods are, therefore, less useful for applications that require more specific functionalities. Plasma treatments and UV irradiation are alternative methods that produce hydrophilic polymer surfaces. However, these treatments also tend to be harsh, physically altering the surface. In addition, they have their own issues with surface stability; with plasma treatments, for example, initial hydrophilicity is often reduced over time. Goddard and Hotchkiss [8] have reviewed various techniques and criteria to be considered in the process of surface functionalization for attachment of bioactive compounds to the polymer surface.

Biointerface design considerations and production

Surface topography (inherent or engineered) has profound effects on the immobilization, presentation, and activity of biomolecules and biomolecular assemblies and on the binding events that occur at the biointerface. The effects of surface topography are several, from a straightforward increase in surface area that results in increased loading capacity, to more complex effects related to changes in the biophysical and biochemical behavior of the substrate surface and/or the immobilized biomolecule [9–13], including cytocompatibility, surface energy, specific and non-specific binding of target analytes, and the valence of attachment of biomolecules. The “Gulliver Effect”, an economic term describing a large target incapacitated by attack from many smaller entities, has also been applied to the field of biosensors [14] where the valence of attachment of the biotic and abiotic components can significantly affect biointerfaces with surface features on the same scale as the biological

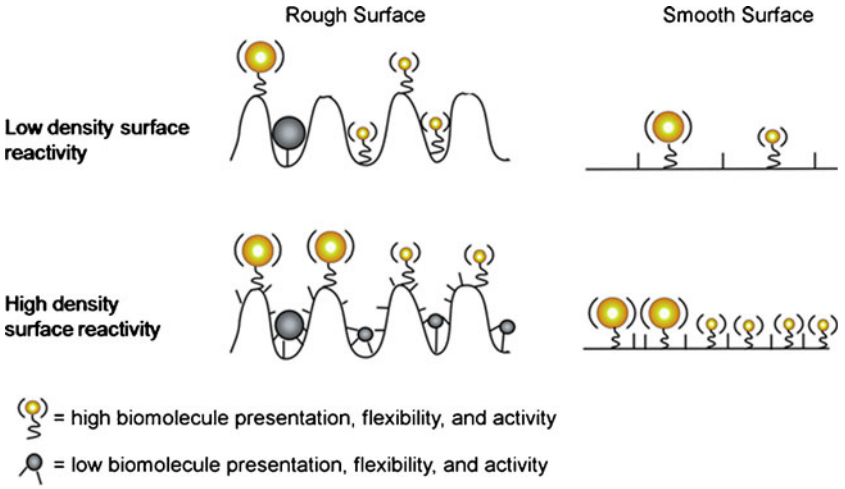

Fig. 2 Surface roughness, density of reactive groups, and size of the attached biomolecule are important factors in overall bio-immobilization efficacy, biomolecule presentation and activity, and target analyte binding capacity of the biointerface

component (Fig. 2). Furthermore, the incorporation of dense three-dimensional structures (sol–gels, hydrogels) produces diffusional barriers that affect the movement of target analyte to appropriate recognition sites and alter K_d values of affinity sensors or K_m values of catalytic sensors [3, 15–17].

Because many surface modification methods alter surface chemistry and surface topography simultaneously, it becomes difficult to identify the contribution of each to the efficacy of bio-immobilization and bioactivity. Independent control of these surface properties would enable better understanding and optimization of the biosensor. We have begun to explore the use of an electron beam-generated plasma source developed at the Naval Research Laboratory, which has the ability to modify the chemical functionalities on the surface *without significantly altering the surface morphology* [18]. Unlike most plasmas, this plasma delivers very low energy ions to adjacent surfaces and has a low production of photons. Typically, ion kinetic energies are at or below the binding energy of polymeric materials [19]. Complex surface chemistries can be achieved simply by varying the gas that is used (e.g., argon, nitrogen, oxygen, water vapor, carbon dioxide, ammonia, etc.), which provides the opportunity to endow the surface with specific and non-specific performance features without surface roughening. In essence, this approach combines the benefits of a high-temperature wet-chemistry approach with all the benefits of dry methods.

Topographical surface structures can be designed specifically to match biological components or induce biological responses independent of surface chemistry [10, 20, 21]. The creation of nano-structured topography has evolved from the use of mechanical methods and wet chemical etching to more refined "top-down" approaches such as photolithography and "bottom-up" approaches based on self-assembly. Photolithography is a well-established patterning technique that generates reproducible, high-resolution (submicron) features. However, a wet photoresist development step is often required and may be incompatible with some materials, resulting in structural damage or collapse of features. The organic solvents, high temperatures, and high radiation intensities can denature biomolecules that are attached to the surface prior to patterning. Furthermore, the use of conventional lithography for nanofabriation is limited by its spatial resolution and choice of patterned material, and it is not compatible with aqueous conditions [22]. Microcontact printing is a simple alternative to photolithography that is particularly suited for patterning of gold surfaces. An advantage of microcontact printing is that it can be used with curved and other three-dimensional surfaces. Unconventional nanofabrication techniques lie in areas of molding and embossing, edge lithography, and scanning probe lithography (dip-pen nanolithography, scanning probe contact printing). Direct, *non-contact* biopatterning techniques include ultrasonic micropipetting, capillary-free jetting, and ink-jet printing.

The bottom-up nanofabrication methods use self-assembly to arrange nanoparticles into various arrangements [23]. This approach requires an understanding the chemical and physical properties of the nanoparticles to properly manipulate them to self-assemble. Nanoparticles can be organized by drying-mediated assembly or chemically assisted assembly. Alternatively, magnetic nanoparticles can be arranged in 1D, 2D, or 3D structures using strong magnetic fields or through interfacial assembly. The advantage of the bottom-up approach is that it can achieve heterogeneous manufacture of a variety of structures and materials with high spatial resolution. However, it is limited in its practical applications because of difficulties in precise nanoparticle assembly and the formation of supramolecular structures. Currently, combined strategies have aimed at merging the two approaches to enable the bottom-up creation of nanoscale structures with the precision and robust fabrication provided by top-down technologies. With the ability to spatially and temporally control surface chemistry,

the patterned geometry will continue to provide new insights into the fundamental aspects of biomolecule/cell-surface interactions.

An example of the inextricable tie between structure, chemistry, and function in biointerfaces was observed in our laboratories. We recently encountered difficulties in achieving reproducible high-density antibody immobilization on glass microscope slides used in an immunosensor. Upon conducting a survey of many commercial slides, we found that the quality of the silane monolayer, responsible for the immobilization chemistry, was correlated with high magnesium content, irrespective of manufacturer or lot [24]. The presence of high magnesium at the surface (and high bio-immobilization) was further correlated with elevated surface roughness. Although this relationship has not yet been fully elucidated, the physicochemical complexities of this relationship serve as an illustration of the interdependency of surface topography, chemistry, and biofunctionalization (Fig. 3).

Challenges of biointerface characterization

Optimum biosensor performance is determined by the ability of the biointerface to generate a detectable signal when a target of interest is present. Therefore it is critical that functionality of both the biotic and the abiotic layers of the interface is preserved. The physicochemical properties that define the surface-specific nature of a substrate (e.g., adhesion, optical clarity, conductivity, chemical resistance/reactivity) determine the analytical method by which quantitative or semi-quantitative information is obtained, the functionality of the attached biomolecule and the ability of the analyte to interact with the biointerface (Fig. 1).

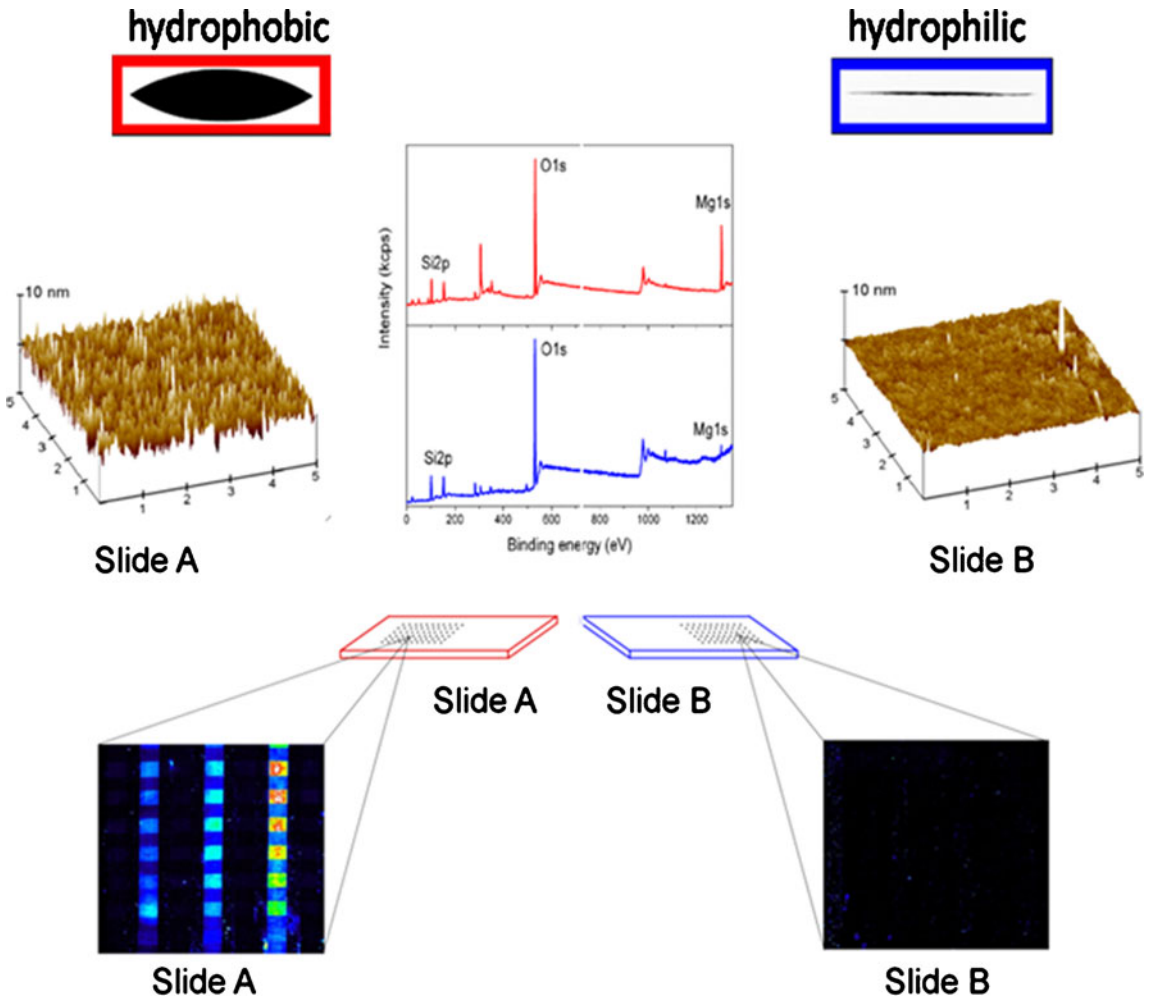

Fig. 3 Surface analysis of soda lime microscope slide A (*red*; high magnesium) and slide B (*blue*; low magnesium). Characterization of slides by contact angle measurements (*top level*), X-ray photoelectron spectroscopy (*center*), atomic force microscopy (*middle level*), and functional binding assay (*lower level*) reveal a significant correlation between magnesium content, surface roughness, and bioimmobilization efficacy. Reprinted in part with permission from Ref. [24]. Copyright 2010, American Chemical Society

Furthermore, the chemistry and structural/topographical properties (e.g., curvature, porosity, roughness) of the surface are subject to change over time under ambient conditions or in aqueous environments, resulting in modification of surface energy, local pH, material dissolution, and restructuring of the interface layer. Because of the inherently complex nature of a biointerface, detailed surface analysis should be performed at each step of its preparation, e.g., determining the surface properties of the untreated substrate, the density and type of surface modifications, and the quality of bio-functionalization.

Table 1 summarizes some of the surface analysis techniques based on surface properties of interest, including but not limited to: surface composition, surface topography, surface electronic structure, surface vibration and reaction dynamics, and surface thermodynamics. It should be noted that each characterization technique has its limitations and the information obtained must be critically evaluated with regard to the surface being analyzed. For example, detailed characterization of the chemical composition of materials is provided by time-of-flight secondary-ion mass spectroscopy (ToF SIMS) with a penetration depth of 1 to 2 nm and spatial resolution down to 0.1 μm [25]. Depending on the take-off angle, X-ray photoelectron microscopy (XPS) provides information on atomic composition in the range 0.5 to 10 nm with a minimum spatial resolution of the order of 5–30 μm, whereas Fourier-transform infrared (FTIR) spectroscopy has a sampling depth and spatial resolution of the order of microns. Depending on the thickness and complexity of the abiotic and biotic overlayers, it is only through the complementary use of each of these analytical techniques that one obtains a comprehensive understanding of the biointerface. The proper technique must consider the scale of modified surface (macro, micrometer, or nanometer) and the biological species of interest (tissue, cells, proteins, nucleic acids) for determining the size of the

Table 1 Surface properties with corresponding characterization techniques

Surface Properties	Surface Characterization Techniques	Specific Examples
Surface composition	• Auger electron spectroscopy (AES)	Chemical environment
	• X-ray photoelectron spectroscopy (XPS)	Composition, depth profiles, imaging
	• Time of flight secondary ion mass spectroscopy (ToF-SIMS)	Composition, depth profiles, imaging
Surface structure	• Low energy electron diffraction (LEED)	Surface crystallography
	• Reflectrion high-energy electron diffraction (RHEED)	Surface crystallography
	• X-ray diffraction (XRD)	Surface crystallography
	• Transmission electron microscopy (TEM)	Defect density, interface structure
	• Scanning tunneling microscopy (SEM)	Morphology, crystal quantity
	• Atomic force microscopy (AFM)	Structure, force measurements
	• Scanning tunneling microscopy (STM)	Electronic structure
	• Near-edge X-ray absorption fine structure (NEXAFS)	Structure
Surface electronic structure	• Ultraviolet photoelectron spectroscopy (UPS)	Molecular orbitals/valence region
	• X-ray emission spectroscopy (XES)	Density of electronic states
	• Capacitance-voltage measurements (C-V)	Carrier density profile
	• Deep level transient spectroscopy (DLTS)	Electronic defect distribution
	• Photoluminescence (PL)	Electronic states, defects, interface roughness
Surface vibration and reaction dynamics	• High resolution electron energy loss spectroscopy (HREELS)	Chemical structure, bonds
	• Surface extended X-ray absorption fine structure (SEXAFS)	Bond lengths, coordination numbers
	• Fourier Transform Infrared spectroscopy (FTIR)	Chemical structure
	• Raman/Surface Enhanced Raman spectroscopy (SERS)	Chemical structure, imaging
	• Sum-frequency generation spectroscopy (SFG)	Surface structure, composition
Surface thermodynamics	• Microcalorimetry	All of these techniques provide
	• Thermal desorption spectrometry (TDS)	Adsorption/desorption kinetics.
	• Laser desorption spectrometry (LDS)	
	• Molecular beam scattering (MBS)	

analyzed area and the vertical and horizontal spatial resolution. Furthermore, when characterizing hard and soft materials (i.e., metals, polymers, biological materials), the force exerted on the sample must be adjusted to limit surface deformation induced by the analysis [26].

One of the main challenges in analyzing biomolecules on surfaces has been that conventional surface-science analytical techniques require high vacuum and a non-aqueous environment. However, current trends in characterization techniques development are directed toward analysis of biotic–abiotic interactions in more natural environments and in real time. For example, high-pressure chemical composition analyses are allowed by the development of high pressure XPS and scanning-tunnel microscopy (STM). Advances in atomic-force microscopy (AFM), surface-enhanced Raman scattering (SERS), scanning-probe microscopy (SPM), ToF SIMS, and transmission electron microscopy (TEM) further enable imaging of biomolecular surface interactions. In particular, AFM-based methods have the capability to analyze a wide range of biological systems from single molecules to live cells [27]. The use of TEM cryomicroscopy enables unstained biomolecule and intercellular structure imaging at the sub-nanometer level [28]. This information, combined with data processing, enables imaging of molecular topographies of single biomolecules in conformational states not accessible through X-ray diffraction. Furthermore, real-time determination of the interaction forces and the dynamics of a variety of cell-surface proteins and bacterial adhesion, specific binding forces of antibodies, and distribution mapping of individual receptors of cells is allowed by current advances in the microscopic technologies.

Outlook

Major areas of research in biosensing include definition of physicochemical relationships on the nanometer scale and the integration of new materials with potential for improving (or modifying) performance. Nanotechnology is making possible the development of novel interface materials that provide superior transducing capabilities.

Within the burgeoning field of nanomaterials research, colloidal nanocrystalline quantum dots (QDs) and graphene have received particular attention for their unique properties and potential for use in biosensing. QDs have a number of advantages over conventional fluorophores for optical biosensing, including higher fluorescence quantum yields and better chemical and photoluminescence stability. With narrow, size-dependent photoluminescence emissions (from the UV to the near-IR spectral region) and a broad excitation spectrum, QDs offer tremendous promise for multiplexed detection [29–32] and for FRET-based, “reagentless” sensing [29, 33]. Graphene, a monolayer of carbon laid out in a hexagonal structure, has long been a model for carbon-based electronic materials and the building block for fullerenes and carbon nanotubes. It has extraordinary thermal, mechanical, and electrical properties and is extremely sensitive to changes in its local environment (i.e., adsorbate binding). These properties, associated with its high surface area, have made graphene a desirable platform for biochemical sensing systems[34].

New advances in nanoparticle synthesis and modification have enabled ever more detailed design and engineering of these nanomaterials for biosensor applications [35]. However, physical and chemical behavior and forces guiding the biotic–abiotic interactions at biointerfaces on the nanoscale cannot be predicted from microscale analogs. For example, the radius of curvature of spherical nanoparticles of different sizes can significantly affect immobilization density. As surface variations occur on the same size scale as the biomolecular component, the effects of surface properties, for example surface charge, wetting, interfacial energies, non-specific adsorption, and surface structure/topography become significantly more pronounced because of the high surface-to-volume ratio, and thus impose thermodynamic challenges for sensing and detection applications [28, 36].

Although improvements in materials engineering may help address the issue of interfacial stability and function, limited biosensor lifetime is often dictated by the stability of the biological recognition element. Many researchers are therefore addressing the issue of biological stability by exploring bio-derived, biomimetic, and bio-inspired materials. Aptamers and catalytic aptamers (aptazymes; [37]) are bio-derived materials whose unique structural motifs allow them to recognize ligands and catalyze reactions. Because their functionality is based on structure, and not complementary base-pairing, aptamers and aptazymes have no naturally evolved analogs, but can be engineered using rational design and integrated into biosensing platforms [38–40]. The development of molecularly imprinted polymers (MIPs), on the other hand, illustrates a trend towards fully biomimetic, artificial recognition elements [41, 42]. With selective binding sites formed by molecular templating, MIPs offer the possibility of a stable non-biological alternative to traditional biosensing elements [43, 44]. Substantial emphasis is now placed on development of bio-inspired materials that utilize biological building blocks as a basis, but with significantly improved function and overall resistance to degradation. Bio-inspired peptide nucleic acids (PNAs) [45, 46] and locked nucleic acids (LNAs) [47, 48] are structurally and functionally analogous to naturally evolved nucleic acids, but with structural modifications that endow them with enhanced interactions and stability in various biosensor platforms, in some cases leading to superior discrimination [49–51].

The capability to model and design many of these biomimetic and bioinspired materials is rapidly evolving [52, 53], with the prospect of angstrom-scale prediction of structural information in the near future. This capability offers the potential to engineer—using rational design—new biomimetic and bioinspired materials with specific chemical and physical properties. These new materials can, in turn, be integrated with abiotic substrates that are custom-tailored with a given roughness, chemical functionality, and valence. The chemical, physical, and functional properties of the resulting biointerfaces can be used to determine how different nano-scale physicochemical forces and behavior at biointerfaces (e.g., fluid hydrodynamics, energy dissipation and heat transfer, mass transport, capillary forces, electrostatic and van der Waals interactions) fit together, and, for biosensing purposes, how they affect the functionality of the biointerface. Of course, this is predicated on the ability to accurately characterize the biointerface. Just as advances in surface diagnostics have enabled better understanding of the biointerface, emerging diagnostic methods and strategies must reflect these developments away from conventional biosensing systems. While downscaling and novel biointerfaces require a change in emphasis, the iterative process involving proper characterization of the biotic and abiotic components remains essential in the design of novel and non-traditional biointerfaces for maximum biosensor performance.

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