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Biosensor Regeneration: A Review of Common Techniques and Outcomes

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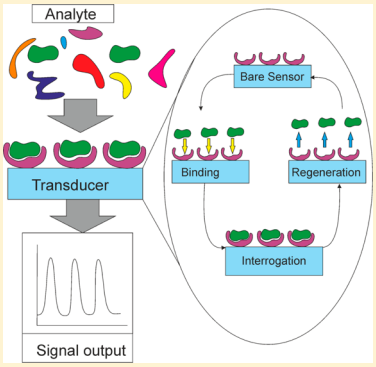
ABSTRACT: Biosensors are ideally portable, low-cost tools for the rapid detection of pathogens, proteins, and other analytes. The global biosensor market is currently worth over 10 billion dollars annually and is a burgeoning field of interdisciplinary research that is hailed as a potential revolution in consumer, healthcare, and industrial testing. A key barrier to the widespread adoption of biosensors, however, is their cost. Although many systems have been validated in the laboratory setting and biosensors for a range of analytes are proven at the concept level, many have yet to make a strong commercial case for their acceptance. Though it is true with the development of cheaper electrodes, circuits, and components that there is a downward pressure on costs, there is also an emerging trend toward the development of multianalyte biosensors that is pushing in the other direction. One way to reduce the cost that is suitable for certain systems is to enable their reuse, thus reducing the cost per test. Regenerating biosensors is a technique that can often be used in conjunction with existing systems in order to reduce costs and accelerate the commercialization process. This article discusses the merits and drawbacks of regeneration schemes that have been proven in various biosensor systems and indicates parameters for successful regeneration based on a systematic review of the literature. It also outlines some of the difficulties encountered when considering the role of regeneration at the point of use. A brief meta-analysis has been included in this review to develop a working definition for biosensor regeneration, and using this analysis only ∼60% of the reported studies analyzed were deemed a success. This highlights the variation within the field and the need to normalize regeneration as a standard process across the field by establishing a consensus term.



1. INTRODUCTION

Biosensors are often described as a three-element system consisting of a bioreceptor, a transducer, and a signal-processing unit;[1] when the analyte interacts with the bioreceptor, a quantifiable signal is generated. Sensors have been developed for a variety of analytes spanning the fields of medicine,[2] food testing,[3] and environmental sensing[4] as well as process control monitoring for research and industry.[5] These sensors have been developed to replace traditional testing procedures that are often technical in nature, requiring specific expertise and time, therefore representing a significant cost in their respective industries.[6−8]

Although some more expensive sensors are being used in research environments,[9] cheaper sensors have the potential to penetrate wider markets. The current high costs are typically attributed to the specialized nature of instrumentation required as well as the reliance on high-grade analytical reagents and materials; a standard system may require many thousands of dollars of upfront capital investment, additionally each sensor transducer assembly can cost up to $80.[6]

There are a number of strategies currently being pursued to bring down the costs of biosensors. On the one hand, the development of low-cost disposable transducers and biosensor assemblies is using techniques such as advanced printing using conductive polymer inks.[10] This has had some success in displacing more expensive components and bringing down system costs. These disposable biosensors may be useful, particularly in medical application where cross contamination and hygiene may present a concern.[11] For some systems, however, disposable sensors are unsuitable; if, for example, time course measurements need to be taken, then chip-to-chip variance may become a source of error. Equally, for some applications very accurate high-grade transducers are required, and the associated costs cannot be avoided. In such situations, regeneration may be a key technique in lowering the cost per test.

Cost reduction is particularly important when considering the development of biosensors that address the needs of the developing world.[12,13] Proven examples of biosensor catering, particularly toward these needs, include biosensors to assess food safety,[14] water sanitation,[15,16] and environmental testing.[17−19] Another key need in the developing world is in healthcare and diagnostic tools for preventable diseases that currently cause high rates of mortality and morbidity.[12]

More recently, there has been an emerging trend toward the development of multianalyte arrays of biosensors.[20] The analysis of multiple biomarkers can, in principle, provide a higher

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certainty in diagnosis. However, one problem presented by multianalyte arrays is the inherent need for more complex transducer systems and data analysis, making cost a significant barrier to their commercialization. These multianalyte arrays may present a particular difficulty when regenerating because each receptor/analyte pair will have its own discrete binding physics[20] and buffer systems that are optimized for one receptor/analyte pair may be a poor choice for others.

By enabling biosensor regeneration, an accessible method for multiple sampling is permitted. In doing so, sensor-to-sensor variance is removed, which is particularly useful when measuring over a time course or interrogating similar levels of analyte. One area in which the chip-to-chip variance still represents an important barrier is in the development of impedimetric immunosensors.[21] By enabling regeneration, this issue may be eliminated entirely.

While appraising the literature, it became apparent that there was no easy method of comparing the success of different regeneration schemes. This was primarily due to the varying definitions of regeneration across the literature. In our conclusions, we propose a number of criteria to determine biosensor regeneration to develop an accepted definition within the field and ensure that this is applicable across all areas of biosensor research.

2. BIOSENSOR CLASSIFICATION

Biosensors may be classified in two ways: according to the signal transduction method (optical, mechanical, or electrochemical) or according to the bioreceptor type. Classifying the uby bioreceptor generates two broad categories: catalytic sensors that use enzymes[22] and affinity sensors that use binding proteins or nucleotides, a category that includes immunosensors. Immunosensors are affinity sensors that use antibodies or their derivatives to detect the target analyte. When considering the regeneration of biosensors, it is important to consider the molecular interaction of the bioreceptor and the analyte by mediating a particular reaction. Catalytic sensors use enzymes as the bioreceptor and process an analyte in order to generate a signal. In the context of regeneration, some of these enzymatic sensors need not be actively regenerated because the analyte is consumed and the baseline signal is eventually restored. Though some studies have reported the reuse of these biosensors,[23,24] this is not active regeneration, which is an important distinction to make within the field. Sometimes this process is referred to as passive regeneration. Another important distinction to make between biosensors is whether assays measure an analyte directly or in an assay such as a competitive assay. Competitive-assay-based sensors do not directly generate data from the analyte itself but work on the competitive binding or inhibition of a secondary process. When considering regeneration, one must consider the inhibition of analyte detection alongside the inhibition of other steps in the assay technique, which may also affect the signal.

3. MECHANISMS OF BIOSENSOR REGENERATION

Regeneration has been demonstrated in a number of systems, though the techniques, reagents, and conditions employed vary significantly throughout the literature. In what follows, the various mechanisms of regeneration are discussed, and the most successful regeneration agents are evaluated. In all cases, regeneration is achieved by overcoming the attractive forces between the bioreceptor and analyte. If these forces are considered in terms of thermodynamics, then both an enthalpic and an entropic contribution must be considered. These forces are subject to the solvent environment and can therefore be altered using a regeneration buffer.

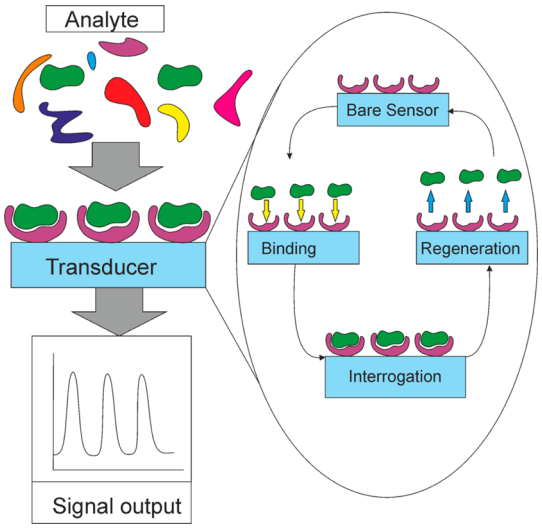

Figure 1. Schematic of biosensor operation (left) and regeneration (right). After analyte binding and interrogation, regeneration is executed in order to return the sensor and bioreceptor to their original configuration.

3.1. Enthalpic Interactions. Enthalpy is defined as the total energy of a thermodynamic system.[25] This energy can be distributed in a number of ways, including heat (kinetic energy) and potential energy that can take on many forms such as chemical bonds or as ionic or polar charges. According to the first law of thermodynamics, a system will equilibrate to reduce the total potential energy.

When considering interactions involved in biosensor operation, the potential energy differences are often a major force in bioreceptor/analyte binding. The interactions are commonly mediated by charge−charge interactions. At a given solution pH, various amino acids may be either positively or negatively charged, depending on the isoelectric point (pI) of the amino acid residue. Taking the physiological example of blood (around pH 7.4), in this environment there are acidic, positively charged amino acids such as asparagine and glutamine, as well as corresponding basic or negatively charged residues such as lysine, arginine, and histidine. These charged side groups are integral in forming the tertiary structure of the bioreceptor binding region. The interaction of charges is spontaneous and tends toward the minimum potential energy of the system.

Because charge is dependent on the solvent environment, factors such as the ionic strength, pH, and presence of competitor ions within the solvent can alter the relative strength of the charge interactions to screen enthalpic interactions effectively between the analyte and the bioreceptor assisting in biosensor regeneration.[26] Typical changes in enthalpy upon antibody/antigen binding range from changes as low as -26 kJ mol^{-1} down to more enthalpically driven interactions where the change may be -130 -kJ mol^{-1} in the most extreme examples.[27] This is a considerable change in enthalpy when compared to typical values for covalent bonds that range from 200 to 400 kJ mol^{-1}.[25] It is important to note that at very low ionic strengths the binding of an antibody can be promiscuous as any charge differential mediates less specific binding that may lower the overall stringency of the binding species. Conversely, high-ionic-

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strength environments may screen the antigen antibody interaction and reduce binding.[28]

3.2. Entropic Interactions. Entropy is defined as the inherent chaos or disorder of a system.[25] The second law of thermodynamics states that the entropy of a system will always increase, creating a more disordered system. This acts to lower the potential energy of the system overall. According to Gibbs' law, a process will be spontaneous if the Gibbs free energy is negative. The Gibbs free energy is the change in enthalpy minus the change in entropy.[25] Though analyte binding may be assumed to cause a decrease in the entropy of a system, there is also entropic compensation by processes such as solvent displacement. To explain this, we must consider the role of solvent molecules in the system. In most systems, the unbound state is the high-entropy system because the free analyte is highly disordered; although there is a decrease in entropy when the analyte binds, this is outweighed by the change in enthalpy and overall there is a negative Gibbs energy change that explains why this is a spontaneous process. Though less frequent, there are certain systems in which the entropy is increased upon binding, particularly when dealing with hydrophobic analytes. This is due to the fact that hydrophobic analytes lead to ordered water caging at the solvent interface. Upon binding, these interactions are interrupted, and the solvent molecules are then free in solution, thus leading to a rise in entropy overall.

Certain amino acids are known for their hydrophobic properties, including tryptophan, valine, leucine, methionine, phenylalanine, cysteine, and isoleucine. In some systems, hydrophobic interactions are key to the antibody/antigen interaction, and it has been identified that apolar surfaces are often buried at the binding interface, which may be critical to analyte binding and the subsequent regeneration of a biosensor.[27] At the protein level, biosensors have been developed for the detection of hydrophobic analytes such as fibrin, which have been subsequently regenerated.[29]

If an analyte in solution has any hydrophobic regions, for instance, due to clusters of hydrophobic amino acids, then hydration shells must be formed. Hydration shells are arrangements of water molecules around a solute.[25] In hydrophobic systems, the water is arranged to minimize the surface contact between the hydrophobic areas and the polar water, often by the lateral mediation of charge between water molecules across the interface and the coalescence of the hydrophobic species.[30] The formation of hydration shells leads to a highly ordered low-entropy system with respect to the solvent, in particular, at the interface where any less order would be energetically unfavored. In these cases, the minimal reduction in entropy with respect to the analyte and receptor is outweighed by the increase in entropy from liberated water molecules. To reverse these interactions, entropically driven binding must be minimized by negating the effects of hydrophobic regions; consequently, aliphatic detergents are often used. In aqueous solution, this allows the interruption of water caging and the minimization of the hydrophobic effect[31] at the interface of the analyte and bioreceptor to enable regeneration.

3.3. Chemical Regeneration. As discussed above, the solvent environment at a sensor interface is a key parameter that determines analyte/bioreceptor binding. The most widely used approach for regenerating biosensors is therefore to alter the solvent environment chemically. This can be accomplished readily by removing the transducer from any assembly and submerging it in a regeneration buffer. Because such regeneration solutions are often composed of common reagents, this

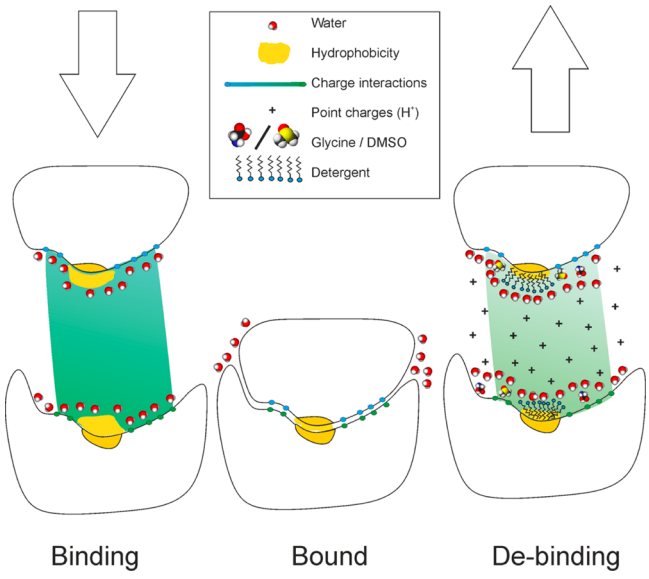

Figure 2. Schematic of biosensor regeneration showing relevant forces in binding and debinding. The role of hydrophobicity and the formation of hydration cages can be seen (left). The screening of these by detergent molecules can be observed (right), as well as enthalpic interaction screening mediated by the increasing ionic strength of the solvent environment.

represent an inexpensive method for sensor regeneration. Though it is a crude technique, it can be refined by the use of a fluidic control system or a computerized control module, something that has limited demonstration currently. This approach may prove vital in the development of a field-use regenerable biosensor. Below is an evaluation of the common chemical approaches that have been demonstrated for biosensor regeneration

3.3.1. Acid/Base-Mediated Regeneration. In many reports, regeneration has been achieved by the application of high-[32,33] or low-pH[34−37] buffers to the system. Typically, a low-pH buffer will go no lower than pH 2 in order to prevent irreversible damage of the bioreceptor. Conversely, a high-pH buffer will often be limited to a pH of around 11 for the same reason.[38] This has a twofold effect on the system.

First, a change in pH alters the enthalpic state of the system by changing the relative charges between the analyte and the bioreceptor. As the side groups become ionized,[39,40] the charge distributions that maintain the tertiary structure of the bioreceptor are also altered. This structural denaturing aids the decoupling of the analyte from the bioreceptor.[41]

Second, the change in pH contributes to a change in the ionic strength of the environment and the screening of receptor/analyte interactions.[39,40] Another way of altering the ionic strength is to use strong electrolytes such as Ca^{2+}[39] and NaCl.[42] If a system is particularly sensitive to a change in pH, then this may offer a preferable alternative to prevent irreversibly denaturing components of the sensor such as the bioreceptor or altering the electronic state of the transducer.

Though the use of acidic/basic regeneration has been widely reported, one disadvantage is that it can be used only in systems where the altered pH will not interfere with the sensor signal. This makes it particularly difficult to use pH regeneration in electrochemical systems where charge may affect the baseline signal of the sensor itself. Another key area that is unsuitable for pH regeneration is where particularly fragile bioreceptor proteins

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are used. If they are easily denatured, then this would be a poor method because it would irrevocably damage the bioreceptor. The major advantage of using acidic or basic regeneration is the low cost and general utility.

3.3.2. Use of Detergents. Detergents are often used at low concentrations in the regeneration of biosensors.[38,43,44] Structurally, detergents are conventionally heterobifunctional molecules that comprise two distinct regions: a polar head that is highly soluble owing to its charge and an aliphatic nonpolar tail. Because the tail regions are hydrophobic, they coordinate with similar regions of the bioreceptor or analyte in an entropically driven process. The polar headgroup then extends into the aqueous phase, minimizing repulsion and encouraging the solubility of the analyte.[45] In certain biosensor systems, hydrophobicity may be a key force in the interaction of the bioreceptor with the analyte such as in the detection of hydrophobic analytes including 2-naphthol and 3-isobutyl-2-methoxypyrazin.[31,46] Therefore, detergents may be a key component of a regeneration buffer. Typically, mild detergents such as Tween are used for this,[38,47] although low concentrations of harsher detergents such as SDS have been used.[17,43,44] Although detergents are useful at low concentration and avoid extremes in pH, they may interrupt systems such as self-assembled monolayers (SAMs) and so should be used only in systems with a solid transducer interface.

3.3.3. Glycine. One regeneration agent that is widely used is the amino acid glycine,[4,48−53] which is useful for a number of reasons that aid separation and minimize damage to the bioreceptor. Glycine is a widely available low-cost regent that has a buffering range of pH 2−7.[54] This buffering range makes it ideal for an acidic buffer that avoids localized extremes in pH. Because it is the simplest amino acid with both positively and negatively charged regions, glycine dissolves well in both aqueous and more hydrophobic environments and can readily mediate forces at a particularly hydrophobic interface, thus reducing the entropic favorability of the bound state. In solution, glycine is zwitterionic and acts as a mild screening agent for charges at the interface, again helping to reduce enthalpic forces between the bioreceptor and analyte. Glycine tends to bind to the surface of the bioreceptor and analyte because this is thermodynamically favored. During exposure to a regeneration buffer, the bioreceptor is therefore partially protected from damage caused in the altered pH environment. Although useful for optical and mechanical sensor systems, glycine may have limited application in electrochemical sensor systems because the use of low pH may affect the sensor signal permanently.

3.3.4. Urea. Another widely demonstrated chaotrope is urea,[55−57] which is often employed in pH-sensitive environments to maintain a neutral pH in solution. In publications by both Yang et al. and Liu et al., urea was used to regenerate sensors that obtained their data using cyclic voltammetry methods, which avoided the alteration of the tethering layer and any disturbance in signal.[55,57]

Other chaotropes that have been successfully used for the regeneration of biosensors are dimethyl sulfoxide (DMSO) by Tedeschi et al. for the regeneration of the human serum albumin sensor;[58] formidamide for an oligonucleotide sensor;[59] EDTA in the case of IgE sensors;[60] and finally potassium thiocyanate by Karaseva et al. that was used to regenerate sensors detecting chloramphenicol.[61]

3.4. Thermal Regeneration. The structure and behavior of biological molecules such as proteins and oligonucleotides are often affected by changing the temperature. Elevated temperatures give molecules increased kinetic energy, which may allow binding forces to be overcome.[62] Although for most proteins overheating causes irreversible denaturing and aggregation,[63] certain groups of proteins and more broadly oligonucleotide base pairs can be decoupled by raising the temperature in a process known as melting.[54] At ambient temperature, double-stranded DNA dsDNA is held together by base pairing between nucleotides.

The number of base pairs involved in bonding the strands together translates directly to an associated temperature at which the individual DNA strands gain enough kinetic energy to overcome the base pairing and separate.[64] The use of DNA melting has been previously demonstrated to be a viable method for the regeneration of nucleic acid biosensors.[62] Double-stranded DNA and other DNA structures such as aptamers can be transiently denatured. This has been demonstrated for sensor regeneration using DNA/protein interaction-based biosensors.[65] Though thermal regeneration has been proven to be successful when using nucleotide-based bioreceptors, it is practically limited to this type of sensor because heating would cause the destruction or denaturing of the biological components of many other sensors.

3.5. Electrochemical Regeneration. In a limited number of studies, biosensors have been regenerated using direct electrochemical methods. In these studies, the reductive desorption of surface species has been achieved by applying a negative potential to the sensor surface.[29,66] Though presently under-represented in the literature, perhaps because of its limited applicability, this is an elegant solution for the problem of regeneration because it provides a highly localized regeneration environment that can be precisely controlled. One example of the electrochemical regeneration of a biosensor demonstrated by Liron et al.[67] subjected indium tin oxide electrodes to an electroreductive current in order to regenerate the antibodies on the sensor surface.

4. BIOSENSOR ARCHITECTURE AND CONSTRUCTION

4.1. Transducer Surface. When constructing a biosensor, the transducer surface is a primary consideration because this is the physical substrate on which the sensor is constructed and to which the bioreceptor is attached. The choice of transducer often has an influence on the regeneration technique employed. Below is a brief description of the common transducer materials used.

Silica, SiO_2 (more commonly glass), is a frequently used transducer in a range of biosensor systems.[68] Silica facilitates regeneration particularly well because it is chemically robust, etching only usually at high pH, and though limited etching can occur even at neutral pH, this is unlikely to be an issue over the few hours to days that would represent normal use.

Silica is also particularly useful because it can be fabricated to have surfaces that are flat on the microscale. Its inert chemical nature prevents the reaction of the regeneration buffer with the transducer surface, and the flatness ensures that the buffer can be easily washed from the sensor surface.

Electrochemical sensors require an electrically conductive substrate. Many current examples achieve this through the screen-printing of carbon or metallic electrodes, which, although economic, presents potential quality issues that are due to the diverse array of micro- and nanotopologies generated in the printing process.[69] This local variation becomes problematic upon attempted regeneration, particularly when rinsing the electrodes, with rougher regions proving more difficult to regenerate.[70] Other methods for electrode fabrication have been

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undertaken in order to generate flatter surfaces; these include sputtering and vapor deposition of the conductive layer that generate layers that are flat on the nanoscale.[6] Such additional preprocessing inevitably results in additional costs.

Though some types of biosensor allow the direct conjugation of the bioreceptor to the transducer surface, there is often an associated loss in biological activity that is a particular problem when dealing with metal surfaces.[71] To prevent this, the bioreceptor is usually separated from the sensor substrate. This is commonly achieved through the use of a thin tethering layer that provides accessible functional groups for chemical coupling; examples of tethering layers include self-assembled monolayers (SAM),[72] polymers,[73] and silanes.[68]

After attaching a suitable tethering layer, the next key consideration is the conjugation method used to attach the bioreceptor to the tethering layer: it must be robust enough to withstand regeneration. If the bioreceptor is noncovalently tethered to the transducer surface, for instance, via weak charge interactions, the alteration of ionic strength or pH when regenerating the sensor may induce the dissociation of the bioreceptor from the sensor surface itself. Therefore, covalent tethering of the bioreceptor to the transducer surface is desirable.

Many optical biosensors are constructed on silica fiber optic surfaces, which are often functionalized to enable the conjugation of the bioreceptor through silinization. This is a particularly robust covalent method for the attachment of the bioreceptor and has been extensively proven to be resilient to the regeneration of biosensors, particularly on silica substrates[74] where the available surface silicon atoms provide an accessible chemical route for conjugation, which makes it a viable method for use in the quartz crystal microbalance (QCM) as demonstrated by Bunde et al.[75]

QCM may also operate using gold-coated quartz wafers. Similarly, a gold surface is also used in surface plasmon resonance (SPR) as well as in electrochemical methods. Gold is used because its atoms will spontaneously form a dative bond with any sulfhydryl group,[34,52,59] which is particularly resilient against damage often caused by regeneration techniques. These dative bonds can be exploited for the construction of SAMs and mixed SAMs (mSAMs) that may be attached to the surface of the sensor before being stabilized by noncovalent forces that, though relatively weak individually, provide a substantial matrix for the conjugation of the bioreceptor.[29,32,43,76] Care must be taken when regenerating SAM-based sensors because the molecular components may be susceptible to reactions with certain components in the regeneration buffer itself, for example, detergents.[76,77]

4.2. Bioreceptor. Biosensors are often categorized according to the type of bioreceptor used. Catalytic biosensors use enzymes as the bioreceptor, whereas affinity-based sensors typically use antibodies, other affinity proteins such as aptamers, engineered receptors, or nucleic acids. Enzyme-type biosensors are used commonly for the detection of small metabolites, the most well-known of which is the glucose biosensor often used in the management of diabetes.[78] As previously discussed, reuse has been reported with these enzyme-based sensors.[23,24] However, there is only one example of an active regeneration approach having been used where a regeneration step was used rather than allowing the catalysis of the residual analyte to restore the baseline signal. In the study by Lu et al., 8 M urea was applied to the sensor in order to obtain the baseline signal more rapidly.[55]

The principal protein bioreceptor group used in affinity biosensors is antibodies. They are particularly useful for biosensor applications because they can be raised against a vast array of analytes. They have predictable binding physics[1,45,79] that have been widely demonstrated to be reversible;[27] antibodies have an isoelectric point of around pH 7,[80] and by changing the pH, dissociation may be induced. This can be a delicate procedure because antibodies cannot be exposed to extremes in pH for very long without causing irreversible denaturing and a loss of function as side chains become ionized and the specific three-dimensional structure of the binding region of the bioreceptor is lost.[40] This denaturation will lead to a reduction in the specificity and sensitivity across a population of antibodies and can sometimes be seen as a loss in signal of the biosensors over regeneration cycles as some of the antibodies become damaged.[1]

As an alternative to antibodies, semisynthetic routes have been achieved for the development of artificial bioreceptors for sensor applications, and these are collectively known as non-antibody-binding proteins (NABPs). Typically these use a stable protein motif such as a conserved loop or fold from a natural structure to which a paratope-like complementary determining region (CDR) can be evolved or engineered using high-throughput techniques.[20] Such bioreceptors are useful because they are often very small and stable and have regular binding physics. Examples include Nanobodies,[81] DARPins,[82] lipocalins,[83] and adhirons.[84] One key advantage of using these binding proteins is that they do not require periodic animal sources and allow for continual production from bacterial fermentation, consequently ensuring consistent binding physics from batch to batch. In addition, NABPs tend to be much more stable across pH, temperature, and time ranges.[84] This exceptional stability makes NABPs beneficial for regeneration applications as well as more widely applicable in biosensor research. Though initial studies have assessed the suitability for NABP bioreceptors in biosensor applications, there is as yet limited work studying the regeneration of these sensors. A notable example is the regeneration of nanobody-based biosensors.[85]

Other affinity biosensors operate using nucleic acid-based bioreceptors where biorecognition is mediated either through direct interaction by complementary base pairing of a linear sequence (i.e., a target strand binds to a recognition strand) or through a non-nucleic acid analyte such as a protein binding to a 3D epitope presented by a folded nucleic acid aptamer.[36,65,86] These bioreceptors can be quickly evolved in bacteria using viral display technologies. Because the nucleotide binders are held by their charges, they have a wide variety of potential structures that can easily be denatured, and because this structure is self-encoded, their complex structures are easily reformed after regeneration.[42]

A variant to DNA-based biosensors are sensors that use protein nucleic acid (PNA) as a bioreceptor; PNA has a similar structure to DNA, with the phosphate−sugar backbone replaced by protein, and has been shown to demonstrate higher affinities than DNA upon base pairing and has been demonstrated to be a strong candidate for genetic biosensors.[87−89]

4.3. Binding Valency. An important consideration when considering analyte/receptor binding is the role of valency. If a multivalent analyte is to be detected, then each analyte molecule will be bound at more than one location.[20] Each of these locations or epitopes may have a discretely different binding affinity, and the analyte will be bound with the collective force of the different binding events. This may occur when a relatively large analyte is bound or a mixed batch of receptors is used, as in the case of polyclonal antibodies.[1] Another instance where

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Table 1. Regeneration Studies in Optical Biosensors[a]

author	ref	biosensor system	analyte	regeneration conditions	repeats	signal loss	data
Absorbance/Fluorescence-Based Biosensors							
Bright et al. (1990)	37	silane-based mSAM on fiber optic probe	HSA	0.1 M PBS + 0.1 M phosphoric acid	12	5%	fluorescence intensity
Hilton and Nguyen (2011)	65	fluorescence-tagged DNA aptamer	cocaine	temperature cycled from operational 22 to 37 °C	3	0%	fluorescence data
Kandimalla et al. (2004)	51	antibodies on silinated glass beads; antigen was HRP conjugated and optical density (450 nm) was recorded	ethyl parathion	glycine/HCl pH 2.3 + 1% DMSO	14	2%	optical density in ELISA-style assay
Tedeschi et al. (2003)	59	many different schemes of antibody tethering on fiber optic probe	HSA	DMSO	5	10%	binding capacity (defined by fluorescence)
Wijesuriya et al. (1994)	91	GMBS cross-linked to anti-TNB antibody on fiber optic probe	TNB	100 mM glycine/HCl in 50% ethylene glycol pH 1.75	5	40%	% fluorescence signal
SPR-Based Biosensors							
Albrecht et al. (2008)	44	reflectometric interference spectrometry, NHS- cross-linking	CRP	0.5% SDS, 6 mM NaOH, 0.6% ethanol	100	N/A	change in optical thickness
Anderson et al. (1999)	47	Biacore SPR platform with various binding proteins conjugated using NHS/EDC	GST	multivariate cocktail approach to screen various buffers	20	1%	cycle 1 response −cycle 20 response
Choi and Chai (2009)	29	mSAM in SPR setup, mSAM desorbed before reformation and reinterrogation	fibrinogen	reductive desorption (30 s, −0.9 V)	15	0%	SPR angle shift ($d\theta$)
Dillon et al. (2005)	92	Biacore SPR sensor with NHS-EDC cross-linking to anti-M3G antibodies	M3-G	100 mM glycine/HCl pH 2.2	15	1.5%	binding rate (RU.sec-1)
Dillon et al. (2003)	52	Biacore sensors to M3G	M3-G	40 mM HCl + 40 mM NaOH	51	13.5%	response units
Drake and Klakamp (2011)	53	Biacore chip with nonspecified monoclonal antibody using NHS/EDC cross-linking	nonspecified "antigen" protein	10 mM glycine pH 2.5	200	50%	response units
ForteBio Inc./Octet (2007)	38	octet, SPR on fiber optic probe with protein A covalently bound	IgG	5% Tween-20, 10 mM phosphoric acid pH 2.0	10	1.2%	binding, wavelength shift (nm)
Indyk et al. (2004)	93	Biacore Q Optical biosensor with CM5 sensor substrate	lactoferrin	10 mM glycine−HCl, pH 1.75 at 50 mL min^{-1}	>500	0.1%	response units

[a]Abbreviations: EDC, ethyl-dimethyl(aminopropyl)carbodiimde; GMBS, gamma-maleimidylbutyryl succinimide; GST, glutathione-*S*-transferase; HRP, horseradish peroxidase; HSA, human serum albumin; M3-G, morphine 3-glucoronide; mSAM, mixed self-assembled monolayer; NHS, *N*-hydroxysuccinimide; TNB, trinitrobenzene; CRP, C-reactive protein.

multivalent binding may be encountered is when binding a large analyte with a repeating epitope motif. In this instance, the same paratope may bind at a number of locations, once again providing a greater overall binding energy.

Regenerating sensors in which the analyte has bound multivalently may prove more difficult because some epitope/paratope pairs may be dissociated more easily than others. This could lead to uneven dissociation and damage across the sensor surface. It has also been reported that multivalently bound analytes may have a tendency to denature when exposed to common regeneration buffers. This may lead to the formation of insoluble protein on the biosensor surface.[90] This is another limitation of biosensor regeneration.

5. CASE STUDIES

5.1. Optical Biosensors. Optical sensors have been demonstrated to be particularly successful in terms of regeneration studies because the bioreceptor is often tethered to a chemically robust surface, such as a plastic ELISA plate,[4] a fiber optic,[37,51,58,65,91] or a gold SPR wafer.[29,38,47,53,92] Optical sensors have been regenerated over 500 times with a minimal loss in signal[93] (Table 1).

Although regeneration has been extensively demonstrated in these systems, the large capital expense associated with necessary hardware means that they tend to be restricted to research and a few high-throughput medical applications.

There are portable low-cost alternatives emerging such as the Texas Instruments Spreeta chip, a relatively low cost SPR platform.[94] Another drawback of some optical devices is their reliance on sample processing, which requires the addition of reagents such as fluorescent markers, substrates, or enzymes requiring a certain level of technical skill. It is important to note that some single-use planar waveguides have been developed specifically with ease of use in mind;[95] however, they have not been demonstrated to be regenerable. There are some high-throughput methods that have been widely demonstrated to be regenerable,[38] and because of this decrease in cost, they are being adopted more widely in both research and diagnostic fields.

5.2. Acoustic. The regeneration of acoustic biosensors such as QCM-based sensors has also been successfully demonstrated. These sensors operate by propagating a harmonic oscillating wave that is varied as the mass on an oscillating surface is altered.[96] These sensors have been shown to achieve regeneration after a similar number of cycles as optical sensors,[34,35] and a variety of different regeneration buffers have been used to achieve biosensor regeneration[33,60,61,97−100] (Table 2). These sensors include devices such as the quartz crystal microbalance (QCM) and other piezoelectric oscillators. In such systems, the mechanical properties of the interface such as elasticity are key, and it is important that the regeneration does not affect them because this would interfere with measurement. Because the mechanical properties are relatively difficult to alter, any loss in signal can be attributed to the gradual degradation of the bioreceptor. This would explain the gradual loss across a large number of regenerative cycles that is commonly seen.[75] Much like optical sensors, acoustic sensors require expensive

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Table 2. Regeneration Studies in Acoustic Biosensors[a]

author	ref	biosensor system	analyte	regeneration conditions	repeats	signal loss	data
			Nucleic Acid Aptamer-Based Sensors				
Mannelli et al. (2003)	100	DNA probe for GMO's	ssDNA	1 mM HCl	20	0%	frequency shift (Hz)
Tadeschi et al. (2005)	59	ssRNA on gold QCM, SMCC cross-linker	oligonucleotides	1:1 formidamide/water solution	N/A	0%	frequency shift (Hz)
Lazerges et al. (2006)	34	disulfide- ssDNA on gold QCM Chip	ssDNA	NaOH	>100	0%	frequency shift (Hz)
Hong et al. (2009)	48	SAM on QCM chip SAM with N- or C-terminal tethering of IgG	*E. tarda*	0.2 M tris-glycine, 0.6 M NaCl 1% DMSO, pH 2.3	10	0%	frequency shift (Hz)
Yao et al. (2009)	60	DNA aptamer tethered to mSAM using biotin/avidin interaction	IgE	30 mM EDTA	10	20%	relative frequency response (%)
Chen et al. (2010)	99	thrombin DNA aptamers with sandwich to gold nanoparticle secondary aptamer conjugates for signal amplification	thrombin	50 mM NaOH + 1 M NaCl + tris-HCl	5	0%	relative frequency response (%)
			Protein-Based Sensors				
Hao and Wang (2009)	76	mSAM on gold electrode cross-linked to protein A with bound anti-*B. Anthracis* IgG	*Bacillus Anthracis*	PBS pH 1	N/A	N/A	frequency shift (Hz)
March et al. (2009)	35	mSAM with EDC/NHS cross-linking to antibodies	various metabolites and pesticides	0.1 M HCl	150	45%	frequency shift (Hz)
Michalzik et al. (2005)	32	cystamine-glutaraldehyde SAM with protein A binding of IgG	BMP-2	0.05 M NaOH	7	0%	frequency shift (Hz)
Mattos et al. (2012)	43	gold QCM mSAM cross-linked to anti-troponin monoclonal antibody	troponin	0.1 M NaOH + 1% SDS	4	0%	frequency shift (Hz)
Steegborn (1997)	33	silinized gold chip with glutaraldehyde cross-linker to anti-atrazine-IgG	atrazine	0.1 M NaOH	25	0%	frequency shift (Hz)
Karaseva (2012)	61	polypyrrole base layer with glutaraldehyde activation that was NHS/EDC cross-linked to CAP-STI binding protein	chloramphenicol	0.04 M KCNS	12	5%	frequency shift (Hz)

[a]Abbreviations: BMP-2, bone morphogenic protein-2; CAP-STI, CAP protein soybean trypsin inhibitor; GMO, genetically modified organism; ssDNA, single-stranded DNA; SMCC, succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate.

Table 3. Regeneration Studies in Electrochemical Biosensors[a]

author	ref	biosensor system	analyte	regeneration conditions	repeats	signal loss	data
			Cyclic Voltammetry-Based Biosensors				
Bhalla et al. (2010)	66	mSAM on interdigitated sensor, capacitance directly measured in a drop	SAM formation	oxidative desorption (1 min at +1.4 V)	N/A	N/A	CV, capacitance, SEM inspection
Yang and Chang (2009)	57	gold/CHITcomposite surface with adsorbed anti-HCG antibodies	HCG	4 M urea	5	1.35%	CV data (A)
Liu et al. (2010)	56	antibodies on Nafion film SAM	β-glucans	5 M urea	10	37%	CV peak height (μA)
			Amperometric Biosensors				
Vidal et al. (2004)	23	amperometric sensor using cholesterol oxidase on FAD cofactor monolayer	cholesterol	N/A (substrate is consumed)	~1000	N/A	sensitivity (nA mM^{-1})
Manso and Mena (2008)	24	alcohol dehydrogenase colloidal gold/CHIT matrix	alcohol	N/A (substrate is consumed)	9	0%	current (μA)
Lu and Liu (2011)	55	polycysteine on gold plus nanocomposite−anti MPO IgG	MPO	8 M urea	5	10%	current (μA)
			Impedimetric Biosensors				
Bryan et al. (2013)	106	mSAM on gold linked to goat anti-CRP antibody using EDC, impedance measurements on gold	CRP	6 mM NaOH + 0.6% EtOH	7	3%	sensitivity x, sensitivity 0
Xu and Luo (2013)	50	SAM w/EDC-linked anti-insulin antibodies, impedance measurements on gold	insulin	100 mM glycine-HCl pH 2.0 + 1% DMSO	4	0%	ΔRctx/Rct0 (%)
Yun et al. (2013)	108	gold electrode + MPA then EDC-NHS cross-linked to antiketamine antibody	ketamine	NaOH−H_3PO_4 pH 12	5	~5%	Rct
Huang and Yang (2010)	49	glassy carbon electrode plus IgG-coated nanoparticle	*Campylobacter jejuni*	10 mM glycine−HCl pH 2.8	10	4.8%	ΔRct
Querios and de Los Santos (2013)	36	DNA aptamer-based impedimetric immunosensors	*E. coli*	2 M HCl	1	0%	(Rct,1 − Rct,0)/(Ret,0)

[a]Abbreviations: CHIT, chitosan; FAD, flavin adenine dinucleotide; HCG, human chorionic gonadotropin; MPA, mercaptopropionic acid; MPO, myeloperoxidase.

instrumentation, often with a dedicated fluidics control system. This means that they are often unsuitable for field deployment and require considerable effort to realize a portable, affordable device for biosensing. In spite of the above drawbacks, acoustic sensors have been used in precision-critical applications, notably, HIV detection.[101]

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5.3. Electrochemical. Although electrochemical biosensors are frequently stated as having a significant potential impact in a range of analytical fields,[6] as the success of the glucose biosensor has demonstrated, many electrochemical sensors have yet to achieve widespread success. Regeneration may assist in improving the commercial viability of these sensors, yet investigations to date have been limited. Despite limited research, there have been some successes, with amperometric and potentiometric sensors shown to be regenerated successfully.[57,66] In the case of the amperometric sensor, this has been reported to be reused 1000 times with minimal signal loss, although as previously mentioned this is not regeneration per se. In potentiometric sensors, the current[102] or potential[103] is altered in the presence of the analyte, therefore allowing a calibration. In the most successful regeneration study by Liu et al., urea, a strong chaotrope, was used to regenerate the sensor through 10 cycles with minimal signal loss.[56] Further examples of regeneration in potentiometric biosensors are given in Table 3. Urea has been employed in many examples[56,57] in order to avoid the effect of harsh acids or bases that could alter the electrochemical properties of the sensor irreversibly. However, the use of urea may have affected the signal over time by subtly changing the charge characteristics of the biosensor surface. This has led to limited success in the regeneration of electrochemical biosensors.[56]

Electrochemical immunosensors can be developed for a much wider array of analytes because of their reliance on binding proteins that have a much wider repertoire than enzymes.[104,105] Often they are interrogated impedimetrically,[21] a method that is very sensitive and depends on a combination of the capacitive and resistive properties of the transducer surface.[21] These sensors can either directly look at the change in these properties upon analyte binding (reagentless sensors) or use reagents such as HRP-tagged secondary antibodies or nanoparticles to enhance the signal observed on analyte binding. Either way, the charge-transport properties are crucial in this technique, and any regeneration buffers used may alter these charge-related aspects. There have, however, been a few reported examples of successful regeneration that has avoided irreversible alteration of the biosensor, using mildly acidic glycine[49,50] or mildly alkali regeneration buffer[106−108] before neutralizing to restore a stable baseline signal.

6. CONCLUSIONS

Although many different biosensors have demonstrated regeneration successfully, optical sensors have achieved the greatest success. Optical sensors are most successful because it has proven relatively simple to devise regeneration techniques that do not affect the optical properties of the sensor. Though this is also true of acoustic biosensors, regeneration has been successful on a lesser scale, both in the number of studies and the extent to which sensors can be reused. It can be seen that the use of pH is the most widely used example for the regeneration of protein/protein-interaction-based sensors and low-pH glycine buffer is the most widely used agent. Low/high pH pulses are strong candidates for regenerating optical and acoustic sensors.

Other regeneration protocols such as altering the temperature and ionic strength and the use of strong detergents have been shown to regenerate some sensors. These must be chosen only in systems where the particular biophysics of recognition is a driving force such as in the cases of hydrophobic or highly ionic analytes. A comparison of different regeneration techniques provides valuable insight into how other types of biosensors may be regenerated.

One issue encountered across the literature is that there are currently no established criteria for determining successful regeneration. Different biosensor techniques have led to data sets that cannot be easily compared. This makes it difficult to assess the validity of different regeneration procedures across studies. Table 4 outlines a set of criteria that the authors propose as parameters for determining whether successful regeneration of a biosensor has been achieved. Although not exhaustive, it can be unilaterally implemented across all types of biosensors. The authors invite discussion surrounding these criteria, with the aim of developing a robust working definition in the field.

Table 4. Criteria for the Successful Regeneration of a Biosensor

attribute	criteria
signal loss between interrogation cycles	<5%
number of continual cycles achieved	>10
restoration of baseline signal	<±5%
biosensor/transducer reconstruction	avoided
signal loss profile	linear in order to allow accurate calibration
regeneration conditions	explicitly listed with time of incubation and full buffer components

Using these criteria to critically appraise the studies considered in this review, overall only ∼60% of the reported regeneration successfully meet these criteria. This is particularly noticeable within the electrochemical biosensors where only a third of regeneration studies meet the listed criteria. We must therefore make an effort to report regeneration as a standardized process across the field.

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Notes

The authors declare no competing financial interest.

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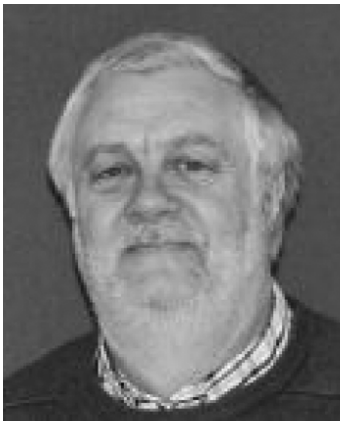
Jo Rushworth received her Ph.D. in structural molecular biology at Leeds University, during which she studied the interaction between the prion protein and Aβ oligomers. She worked in a postdoctoral position in the Leeds Bionanotechnology Group on the development of biosensors for amyloid proteins. She has taught chemistry for several years and is now a lecturer in biomedical science and biochemistry at De Montfort University, Leicester, U.K.



Paul Millner directs the Bionanotechnology group at the University of Leeds. The group's interests can be divided broadly into two main themes: biosensors and nanotechnology. He initially trained as a biochemist and obtained his Ph.D. in plant sciences from the University of Leeds. After around 12 years of working on plant cell signaling, his research shifted direction and he is now a leader in biosensors and bionanotechnology. Paul is also Director of the School of Biomedical Sciences and delivers lectures on a range of topics.

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