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Guide to Selecting a Biorecognition Element for Biosensors

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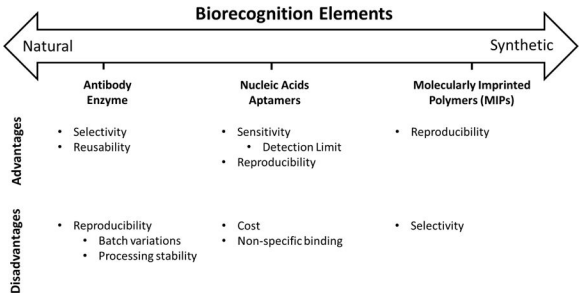
Abstract

Biosensors are powerful diagnostic tools defined as having a biorecognition element for analyte specificity and a transducer for a quantifiable signal. There are a variety of different biorecognition elements, each with unique characteristics. Understanding the advantages and disadvantages of each biorecognition element and their influence on overall biosensor performance is crucial in the planning stages to promote the success of novel biosensor development. Therefore, this review will focus on selecting the optimal biorecognition element in the preliminary design phase for novel biosensors. Included is a review of the typical characteristics and binding mechanisms of various biorecognition elements, and how they relate to biosensor performance characteristics, specifically sensitivity, selectivity, reproducibility and reusability. The goal is to point towards language needed to improve the design and development of biosensors towards clinical success.

Graphical Abstract



Introduction

The first biosensor, developed by Leland Clark over 55 years ago, combines glucose oxidase with an amperometric oxygen sensor.[1] Since then, there has been an outbreak of activity and progress towards using biosensor technology in diagnostic applications, specifically towards point-of-care analysis of biomarkers.[2–5] Biosensors are defined as having both a biorecognition element and a transducer. The biorecognition element is used for the specific sequestration of the target bioanalyte, and the transducer then creates a measurable signal for analysis. The first blood glucose biosensor has been a staple of the community, setting a standard of success for the development of novel biosensors, because of its simplicity, high sensitivity, selectivity, and reproducibility.[1]

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Since the original glucose biosensor, many researchers across disciplines have advanced bioanalyte sensing using novel paradigms with improved biorecognition elements or transducer technology.[6–10] Many biosensor review articles focus on signal transduction methods, or provide a detailed overview of biosensing capabilities and mechanisms of each biorecognition element paradigm.[11–13] Whereas, this review will serve as a guide for the optimal selection of a biorecognition element in the initial design phase based on the biosensor characteristics: selectivity, sensitivity, reproducibility and reusability.

Traditionally, a researcher will first select a biorecognition element design based on their training, and then apply the biosensor towards appropriate applications for the selected paradigm. Rather than relying on previous training, we propose this review as a guide for researchers to select a biosensor paradigm based on the desired application. An early emphasis on the clinical application during the biosensor design phase has the potential to enhance patient-centric point-of-care devices and device accessibility in low-resource regions.

Within this review we focus on the advantages and limitations of each biorecognition element defined by the following biosensor characteristics: selectivity, sensitivity, reproducibility, and reusability. High sensitivity is a large measurable change in biosensor signal as a function of small changes in bioanalyte concentration. High selectivity indicates the sensor will only respond to the target bioanalyte, ignoring all potential competing analytes in the sample. High reproducibility indicates the ability to fabricate multiple identical sensors, with each sensor providing the same predictable response. High reusability indicates the ability to reuse a single sensor multiple times with a consistent sensor response. These biosensor characteristics where chosen to provide a framework to understand the capabilities of each biorecognition element, and ultimately, how the biosensor performance is influenced by the selection of biorecognition element. Ideally, it is best to have high sensitivity, selectivity, reproducibility, and reusability; however, typically biosensor development requires a tradeoff on these characteristics. Therefore, understanding the fundamental limitations of each biorecognition elements is needed to better inform decisions for biorecognition element selection in the preliminary design phase leading to the development of more robust biosensors.

Biorecognition Elements

The primary purpose of a biorecognition element is to provide analyte specificity for a biosensor. Specificity requires a strong and selective affinity between the biorecognition element and target bioanalyte. Several classes of biorecognition elements exist, giving rise to distinct structures that uniquely influence biosensor performance characteristics. Therefore, a fundamental understanding of the inherent characteristics of each biorecognition element is first needed before an in-depth analysis of biosensor performance may occur.

Numerous biorecognition elements exist ranging from naturally occurring to synthetic constructs. Naturally occurring biorecognition elements, such as antibodies and enzymes, are biologically derived constructs that take advantage of naturally-evolved physiological interactions to achieve analyte specificity. Synthetic biorecognition elements are artificially

engineered structures developed to mimic physiologically defined interactions. However, at the cross roads of natural and synthetic biorecognition elements, there are pseudo-natural modalities possessing characteristics of both natural and synthetic recognition approaches. Pseudo-natural biorecognition elements are artificially engineered supramolecular structures using natural subunits. Each class of biorecognition element is comprised of several different types of recognition structures, all of which cannot be discussed within this review. Instead, prominent biorecognition elements from each category will be briefly summarized to serve as a representative of each category.

Antibody

Antibodies are naturally occurring 3D protein structures, typically ~150 kDa in size, that can be identified within biochemical pathways and purified for biosensor applications.[14] The 3D protein structure of antibodies creates a unique recognition pattern with high specificity and accuracy for the bioanalyte. Antibodies share a general structural trend of a “Y” shaped 3D conformation, each comprised of a light chain and a heavy chain, with analyte binding domains located on the arms, seen in Figure 1. Antibody biorecognition elements can be classified as affinity-based, the biosensor signal is dependent on the binding event to form an antibody-antigen immunocomplex. The binding event is often monitored using colorimetric or piezometric transduction methods.[15–18] Typically, antibodies are immobilized via covalent linkage to a sensor surface, forming a brush-like array.[19,20]

Antibodies remain a staple in the biosensor community despite the widely known and accepted limitations of this biorecognition element. For example, antibody production requires experimentation with animals which is a costly and time-consuming process, which limits the discovery of new antibodies.[4,21] Further, once an antibody is discovered, the isolation and purification procedures can be costly.

Enzymes

Enzymes achieve bioanalyte specificity with binding cavities buried within their 3D structure, using hydrogen-bonding, electrostatics, and other non-covalent interactions to form recognition patterns.[22] Enzymatic biosensors are biocatalytic in nature, meaning the enzyme captures and catalytically converts the target bioanalyte to a measurable product, frequently monitored via amperometric or electrochemical transduction methods.[23] Following bioanalyte sequestration an intermediate complex is formed before release of the measurable end product, shown in Figure 2.[23,24] Enzymes are often embedded within surface structures, allowing for short diffusion pathways between biorecognition element and transducer.[25–27]

Nucleic Acid

Nucleic acid biosensors, termed genosensors, take advantage of the complementary binding motif of DNA to achieve bioanalyte specificity.[28] Once a DNA target sequence has been identified, a DNA fragment can be artificially designed and immobilized at the sensor surface as a biorecognition element.[29–31] Specificity is achieved through the unique complementary recognition pattern between the immobilized DNA fragment and the target sequence.[28,32] Recent advances in using nucleic acid recognition elements include locked

nucleic acids (LNA) and peptide nucleic acids (PNA).[33–35] LNA "locks" the ribose in the 3'-endo confirmation, which reduces the conformational flexibility and therefore improves binding with the complementary nucleic acid target. PNA is a synthetic oligonucleotide, comprised of a repeating aminoethyl-glycine unit linked by peptide; PNA is uncharged, and therefore, creates a higher stability in nucleic acid binding. Overall, nucleic acid biorecognition elements are extremely limited in their range of applications as their use is only optimal for biosensor applications targeting nucleic acids.[36–39]

Aptamer

Aptamer biorecognition elements, and other pseudo-natural modalities, provide a much wider range of biosensor applications with the ability to target various bioanalytes, including metal ions, small molecules, proteins, and more complex targets (*e.g.* whole cells).[40] Aptamers are single-stranded oligonucleotides designed through a combinatorial selection process called Systemic Evolution of Ligands by Exponential Enrichment (SELEX).[40] SELEX is an iterative process to search a library of randomly generated oligonucleotide sequences for strong binding affinities between the target analyte and oligonucleotide sequences, ensuring a selective and strong interaction pair shown in Figure 3. The SELEX cycle starts with incubation of the target bioanalyte with an oligonucleotide library containing all potential aptamer sequences. Unbound aptamer sequences are then removed only retaining bound aptamer sequences for polymerase chain reaction (PCR) amplification to regenerate the oligonucleotide library for the next SELEX round. Aptamers are typically 100 base pairs in length compromised of a 20–70 randomized base pair binding region in the center with constant primer binding regions at both ends.[40]

SELEX is a beneficial biorecognition element discovery tool providing researchers with the ability to tailor a sequence for a target bioanalyte. A major drawback is that the SELEX method is costly, requiring multiple iterations using a large library of oligonucleotides each time. However, cost is an obstacle that could be mitigated with further research and development.[14,41]

Molecularly Imprinted Polymers

Molecularly imprinted polymers (MIPs) are synthetic biorecognition elements using a templated polymer matrix to achieve analyte specificity through patterns of non-covalent bonding, electrostatic interactions, or size inclusion/exclusion.[42] Tunability of MIPs comes from the choice of functional monomer, crosslinker, target bioanalyte, and solvent.[43] MIPs are designed to encapsulate the target bioanalyte, effectively forming synthetic recognition patterns between the bioanalyte and polymer matrix shown in Figure 4. Unlike natural biorecognition elements, MIP biorecognition elements are synthetically fabricated for each unique target bioanalyte. In other words, the synthetic polymer-based biorecognition element is designed, often *in situ*, around the bioanalyte.[44] Therefore, a major benefit of MIPs is that a specific biorecognition element-bioanalyte pairing does not need to be biochemically identified.

Other Sensor Paradigms

The biggest obstacle for natural biorecognition elements (*e.g.* enzyme, antibody) paradigms is the initial identification of a biorecognition element paired to a target analyte. In addition to aptamers, many researchers use protein-based phage display as a pseudo-natural modality to identify protein-protein interactions.[45,46] Phage display inserts a DNA fragment that encodes the desired protein target into a phage coat protein gene. A random-peptide library is used to search for high affinity interactions to the target protein sequence.[47,48] Eventually the phage DNA can be extracted and encoded to be grown recombinantly in bacteria.[48,49] With phage display, the potential for discovering new biorecognition elements is growing, but it can be limited similarly to other natural biorecognition element paradigms.

Another approach to detecting bioanalytes is to use synthetic surface nanostructures to catalytically activate a bioanalyte without the need of a natural biorecognition element.[50–55] As an example, nanozymes mimic enzymatic catalytic function by interfacing with a recognition element mimic and an inorganic catalysts.[25,50,56] The nanostructured surface can have high sensitivity but poor specificity, as multiple bioanalytes can be simultaneously catalytically activated.[57–59] Even though progress has been made to improve the selectivity of biorecognition element mimics with nanozymes, this sensor paradigm remains less selective than the other discussed paradigms within this review.[25] One promising application of nanostructured surfaces includes integrating multiple catalytic surfaces to create a cross-reactive (e.g. profile-based) sensors.[60–63] However, clinicians struggle with profile-based sensing because it does not identify specific analytes in the traditional biosensor paradigm and definition.[64]

Other recognition elements, beyond those described in this review, do exist, and it is helpful to investigate alternatives prior to designing a biosensor. For example in protein detection, a protein biorecognition element can be immobilized on a surface, and protein-docking can be detected through the affinity interactions, similar to enzymes and antibodies.[65] An advantage of protein specific recognition elements, for example maltose binding proteins which bind with glucose, is that they can be grown recombinantly in E. coli which reduces the costs of development and production.[66] Also, some binding proteins have the ability to transport across cellular walls creating live-cell-based biosensors.[67] Alternatively, proteins recognition elements can be engineered via phage display or SELEX aptamer processes. Yet, for the purposes of this review, we keep to the basic antibody, enzyme, nucleic acids (natural), aptamer (pseudo-natural), and MIP (synthetic) biorecognition elements as we compare and contrast biosensor characteristics.

Biosensor Characteristics

Selectivity

Selectivity is the ability of a biosensor to generate a positive result only from interactions with the target bioanalyte. A false positive is defined as a positive biosensor result generated from a negative sample; meaning the target bioanalyte is not present in the sample, but an inaccurate positive result is generated. Biosensors with poor selectivity tend to have high false positive rates preventing success in clinical applications. Selectivity is essential for the

development of robust point-of-care biosensors especially because biological samples are complex and comprised of numerous competing analytes. Selectivity is often first published with more common competing analogs in a simplified buffered solution, which is used to artificially to mimic a biological matrix. However, this simplified approach still does not fully capture the impact of the matrix on biosensor selectivity. Therefore, we encourage skepticism until publications demonstrate selectivity of competing analogs in complex biofluid matrices from patient samples.

Naturally occurring biorecognition elements, such as antibodies or enzymes, are optimal for biosensor applications when selectivity is the most important biosensor characteristic. Antibodies achieve specificity through binding domains located on the arms of their "Y" conformational shape.[68–70] Enzymes have binding pockets with unique hydrogen bonding and electrostatic biorecognition patterns to achieve analyte specificity.[71,72] The shared high selectivity of antibody and enzyme biorecognition elements stems from their biologically evolved role requiring high specificity for the success of immunological and other physiological processes.[73]

The selectivity of nucleic acid or aptamer biorecognition elements is hindered by non-specific electrostatic interactions. The inherent negative charge of DNA causes non-specific electrostatic interactions with competing analytes, which can be overcome to some degree using peptide nucleic acids.[32,35] Aptamer biorecognition elements are also comprised of oligonucleotide subunits which can also results in non-specific binding. However, post-synthesis chemical modifications of aptamers can help to reduce non-specific binding to improve biosensor selectivity.[4,74]

Synthetic modalities, such as MIPs, are beneficial biorecognition elements in terms of cost, stability, and ease of development but result in poor biosensor selectivty.[43,44] MIP selectivity is hindered by non-specific binding of bioanalytes with similar structures and functional groups due to the heterogeneity of interactions within the binding cavity. Non-specific binding is more common when investigating large molecules, specifically for analytes greater than 1.5 kDa.[75–77] Increasing the amount of polymer crosslinking can both preserve the binding cavity and reduce non-specific binding.[78,79] However, extensive crosslinking can create a highly dense polymer construct, leading to the permanent entrapment of the bioanalyte template and reduction in diffusion of the bioanalyte through the construct.[75] Therefore, highly crosslinked MIPs typically result in effectively low binding affinities and slow response times.[79,80]

Despite the tunability in the design for MIPs, their success in clinical applications is severely hindered by the poor selectivity of this class of biorecognition elements. Polymer chemists continue to employee techniques to improve the selectivity of MIPs, but often, the sensing paradigm is approached with a negative stigma that is often too difficult to overcome. Despite the history of MIPs in the biosensor community with commercial applications and products already available, many publications are limited to proof-of-concept applications feeding this stigma.[75] Two major improvements are needed towards this biorecognition paradigm: (1) more MIP products tested in complex matrices and real bioanalyte samples, (2) more literature on the optimization point of all the variables involved in MIP

development.[77,81] Other synthetic modalities, such as nanozymes, are just starting to be tested in complex biofluid matrices,[25] and we encourage careful development with discussion on the optimization selectivity in biofluid matrices for these evolving technologies.

Sensitivity

Biosensor sensitivity is defined by the relationship between changing bioanalyte concentration and transduced signal intensity. Highly sensitive biosensors can generate a biosensor response with small fluctuations in bioanalyte concentrations. Sensitivity is also often related to the biosensor range defined by the upper and lower limits of detection, indicating the maximum and minimum bioanalyte concentrations that can be accurately measured. Many publications solely push for the lowest possible detection limit, but improved biosensor sensitivity within physiological relevant concentration ranges is more desirable.[82–85] Typically, the desired physiologically relevant bioanalyte concentration range is often in the picomolar to nanomolar scale.[86–89] Improvements in biosensor sensitivity facilitates the ability to accurately measure small variations in biomarker concentrations to allow for earlier disease diagnosis and treatment intervention.

Biosensor sensitivity and range is primarily dictated by the number of available binding sites per surface area, equilibrium dissociation constant, and steric hindrances. A high surface loading density of a biorecognition element is desired to maximize the number of available analyte binding sites. A major advantage of aptamer biorecognition elements for biosensor sensitivity is their small size. For example, antibodies are ~10–15 nm in size compared to aptamers which are only ~1–2 nm in size.[14] Therefore, aptamers can achieve higher density surface coverage with more available binding sites per surface area of the biosensor.[14]

Surface loading is not the sole influence on biosensor sensitivity; steric hindrances from adjacent biorecognition elements must be considered, especially for antibodies. Steric effects from adjacent antibodies result in a conformational change resulting in inaccessibility of the binding site, shown in Figure 5.[90–93] To mitigate some of the obstacles associated with antibody biorecognition elements, single chain formats of antibodies, referred to as nanobodies, are growing increasingly popular.[94] Discovered in 1993 in camels, camelid heavy-chain antibodies (HcAb) are devoid of the light chain.[95,96] Therefore, nanobodies are smaller than conventional antibodies and have simplified “Y” conformational shape binding domains.

Sensitivity range of traditional nucleic acid biorecognition elements is limited due to steric hindrances, which is overcome with the use of locked nucleic acids.[30,31,33] The influence of surface loading and steric effects on aptamers has been recently studied, which leads to greater accessibility of binding domains, decreasing the possibility of sensor signal saturation, and increasing the functional concentration biosensing range.[31,97–100]

Enzyme and MIP biorecognition elements are integrated differently into a signal transduction platform. Instead of covalent linkage to a surface, enzyme and MIP biorecognition elements are often imbedded in a surface construct. The depth and density of the surface structure dictates the biosensor sensitivity, range, and response time.[25,81,101]

Thicker surface constructs allow for more biorecognition elements per unit area, and therefore, more binding sites for the target bioanalyte. However, the increase in thickness can also result in a loss of sensitivity, lower concentration limits and, poor response time.[102] Therefore, sensitivity and range for enzyme and MIP biorecognition elements can be optimized with alterations to the surface construct.

Reproducibility

Reproducibility is defined as the ability to fabricate multiple identical biosensors that will produce the same response to a target bioanalyte. An understanding of the biorecognition element structure, behavior and production process can improve biosensor reproducibility. Reproducibility can be limited through various development processes, for example, in the construction of the recognition element (*e.g.* batch-to-batch variability), attachment of the biorecognition element to surface (*e.g.* surface loading), or variability in surface constructs. Reproducibility is often left unreported, and we propose that biosensor publications should include how many different sensors were fabricated and used in the publication or show data over multiple sensors to demonstrate reproducible variability.

Although antibody and enzyme based biosensors are the most represented on the clinical market, the production of these biosensors needs to be carefully controlled to prevent irreproducible results.[103,104] Antibodies and enzymes are sensitive to degradation, from pH or temperature variations, making industrial processing and production difficult, especially in low-resource regions.[21,105] Activity of the biorecognition element is dependent on storage and transport conditions, making a consistent biosensor response difficult to achieve.[106] Despite their prevalence in the community, quality control and environmental instability are known and well defined problems for antibodies and enzymes.

Aptamers are optimal biorecognition elements to ensure highly reproducible biosensors.[107–109] Chemical synthesis of aptamers is a well-defined and highly reproducible process leading to the manufacture of robust biorecognition elements. Post-synthesis modifications of aptamers is easily achieved to enhance stability and decrease non-specific binding to improve biosensor reproducibility and selectivity.[4] Typically, aptamers interface with the sensor surface through thiol chemistry, which is a well understood and characterized process.[31]

MIP biorecognition elements experience similar reproducibility to aptamer biorecognition elements due to their well characterized chemical synthesis process.[110–112] Synthetic polymer constructs (*e.g.* MIPs) are predictable and well understood reproducible paradigms. Additionally, translation from benchtop to point-of-care applications can easily be achieved due to the predictable nature of polymer processing, stability, and extended self-life of MIPs compared to natural biorecognition elements.[110–113] Industrial production of MIPs is simple, cost-effective, and typically lacks batch-to-batch variations resulting in highly reproducible biosensors.[113]

Reusability

Reusability is not often quantitatively evaluated in biosensor literature. Tied to the stability and binding kinetics of the biorecognition element, reusability is defined as the ability to

reuse a sensor multiple times.[114,115] Instead of studying reusability, single-use biosensors, most commonly paper-based biosensors, are growing more popular in funding and literature.[116–120] While single-use sensors have their applications and advantages, reusable biosensors have potential to improve accessibilities of biosensor technologies in low-resource regions.

Typically, the dissociation equilibrium constant is reported for bioanalyte-biorecognition complex kinetics, but biosensor use over multiple assays is unclear unless the literature directly specifies the result. Therefore, we encourage researchers to publish details of how often a sensor needs to be replaced to obtain the desired results. In addition, we encourage researchers to also discuss the off-kinetics of the bioanalyte-biorecognition complex or details to regenerate the biorecognition element. Regeneration of a biosensor occurs when the target bioanalyte dissociates from the biorecognition element reopening the binding site. Improved characterization of the off-kinetics, part of the dissociation constant, will provide a better understanding of how to effectively regenerate the biosensor surface. Further, regeneration of a biosensor surface should be considered for future publications, including chemical, thermal, or electrochemical regeneration methods of various biosensor paradigms.[65,121–123]

Understanding the forces mediating the bioanalyte-biorecognition element interaction is important when considering biosensor regeneration and reusability. Enzymatic biosensors are a prime candidate for reusable biosensors, as enzymes are not consumed or altered during catalytic reactions, therefore, the binding site is preserved maintaining activity after use.[24] This process is often referred to as passive regeneration, as no extra action is needed to initiate regeneration and it is a spontaneous process. Though not extensively reviewed in the literature, regeneration of antibody, aptamer, or DNA biorecognition elements can achieve biosensor reusability.

Affinity based interactions, common for many biorecognition elements, are dominated by thermodynamic forces in the form of enthalpy and entropy. Charge mediated interactions are dominated by enthalpy forces while entropy drives hydrophobic interactions.[121] The solvent environment influences these forces, and therefore, can be altered to achieve biosensor regeneration for reuse. For example, the charge state of amino acids will differ depending on the solvent pH, and placing the biosensor in a different pH buffer could disrupt protein based interactions.[121] The goal of biosensor regeneration is to successfully unravel the bioanalyte-biorecognition element complex while maintaining the integrity of the biorecognition element. Biosensor reusability is challenged by the complex nature of numerous symbiotic forces mediating bioanalyte-biorecognition element interactions.

Choosing a Biorecognition Element

Blood glucose monitoring continues to set a standard of success within the biosensor community due to the simplicity of design, ease of use, accuracy, and quick response time. While blood glucose monitoring remains a gold standard for biosensors, this sensing paradigm is not appropriate for all bioanalytes and applications. Instead, there are countless biosensor configurations, each with a unique combination of biorecognition element, transducer, and target bioanalyte. One method to perpetuate the standard of success set by

glucose monitoring is through greater care in the selection of the correct biorecognition element paradigm during the preliminary design phase.

Understanding the advantages and disadvantages of each biorecognition element in terms of biosensors performance is important during developmental stages. Very rarely will a biosensor paradigm lead to the most sensitive, selective, reproducible, and reusable sensor. Instead, a trade-off on biosensor characteristics is necessary for specific applications. For some applications, sensitivity and a low limit of detection is necessary to monitor a specific analyte. In alternative applications, selectivity is very important to distinguish the analyte from other similar competing analogs, and therefore, a small sacrifice in sensitivity can occur. However, emphasizing one biosensor characteristics does not indicate that other biosensor characteristics are unimportant. For these reasons, it is important to first understand the bioanalyte target and intended biosensor application prior to building your biosensor. The provided decision map, Figure 6, serves as a simplified guide for the selection of a biorecognition element in the initial design phase after the target bioanalyte and biosensor performance characteristics are clearly defined for the intended application.

The decision map, Figure 6, is only used as a starting point for further investigation of the selected biorecognition element. This decision map also serves as a tool for biosensor redesign if obstacles arise during preliminary testing of the first design. Based on observations during preliminary biosensor tests, a different biorecognition element may be selected using the decision map to assist in biosensor redesign. Overall this systematic approach to the development and evaluation of new biosensors will promote the success of creative biosensor designs and facilitate the translation from bench-top to point-of-care applications.

Summary and Conclusions

Over the past 10 years, biosensor technology has significantly grown from the integration of different, interdisciplinary, scientific disciplines, leading to unique and novel biosensor designs and applications.[124–127] Understanding the optimal role of each type of biorecognition element in the community is important to ensure successful advancements in novel biosensor technology. Novice biosensor researchers and collaborators can benefit from a brief overview of multiple paradigms in selecting which biorecognition element and transducer will best target the desired bioanalyte and clinical goals. The language used herein is proposed as a guide in collaborative discussions to understand the advantages and limitations of each biorecognition element when designing a new biosensor.

Further, published literature often uses quantitative evaluation of sensitivity and selectivity of a biosensor as the singular metric of success. As the community grows, a set standard of communication and evaluation, beyond sensitivity and selectivity of biosensor success, is important. The full potential of currently researched biorecognition elements is limited due to the lack of reproducibility and reusability biosensor performance characteristics in literature. Not openly discussing the limitations of reusability, reproducibility, and selectivity in complex matrices limits successful transition of biosensors technologies from bench-top to clinical applications. Often, the lack of reporting on reproducibility and reusability leads

to false promises and over-exaggeration of existing technology. For these reasons we encourage researchers to publish results on reproducibility and reusability, even if the results are not positive, demonstrating the ability to generate the same results over multiple sensors and the ability to regenerate a singular sensor for reuse. The development of an evaluation standard for reproducibility and reusability is needed to fully grasp the potential of biosensor technologies. A guide for the optimization of biorecognition element selection, as well as, subsequent characterization will help push the community toward the development of more robust biosensor technologies.

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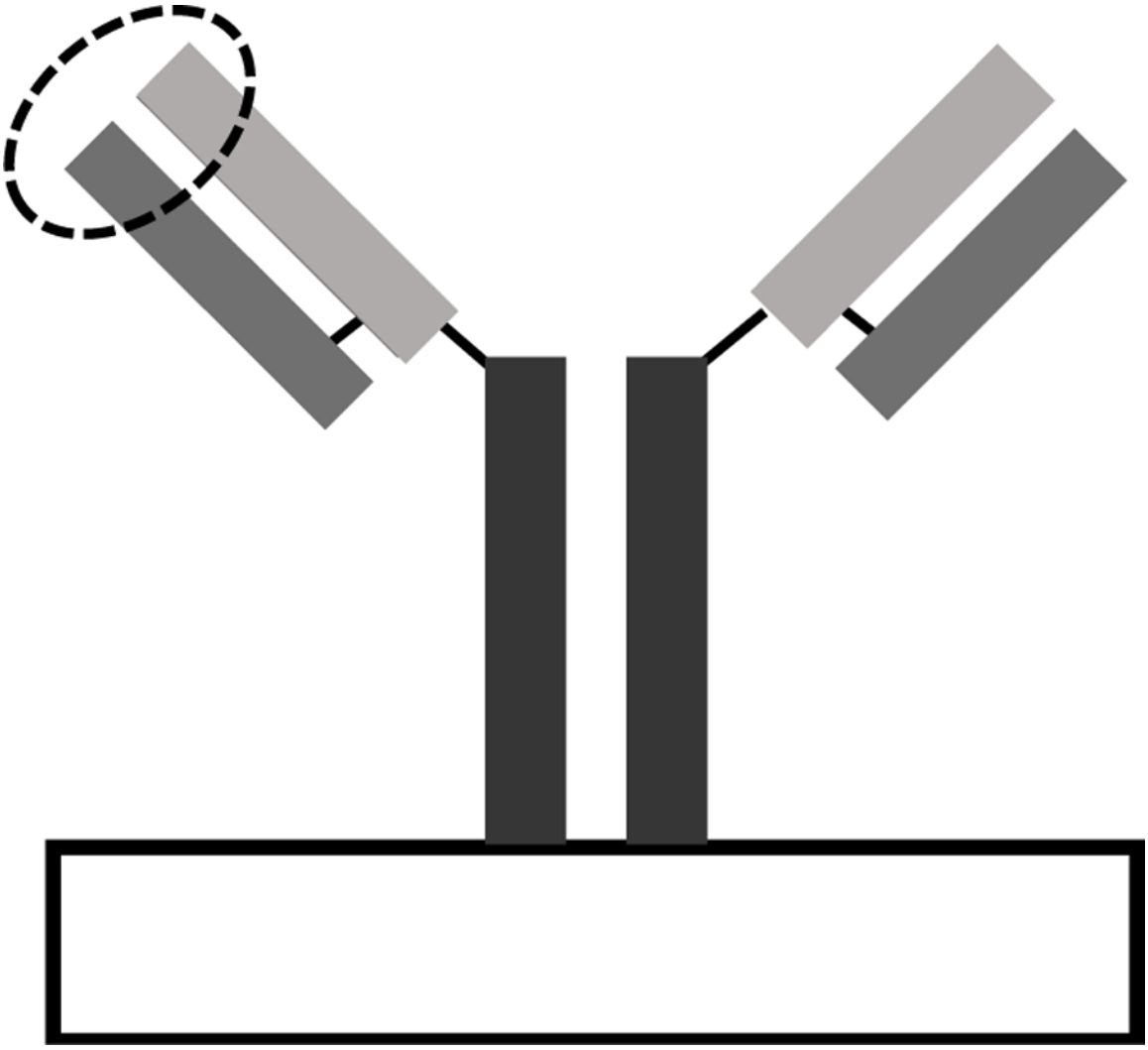

Figure 1:
3D confirmation of antibodies is “Y” shaped with binding domains, circled above, typically located on the distal end.

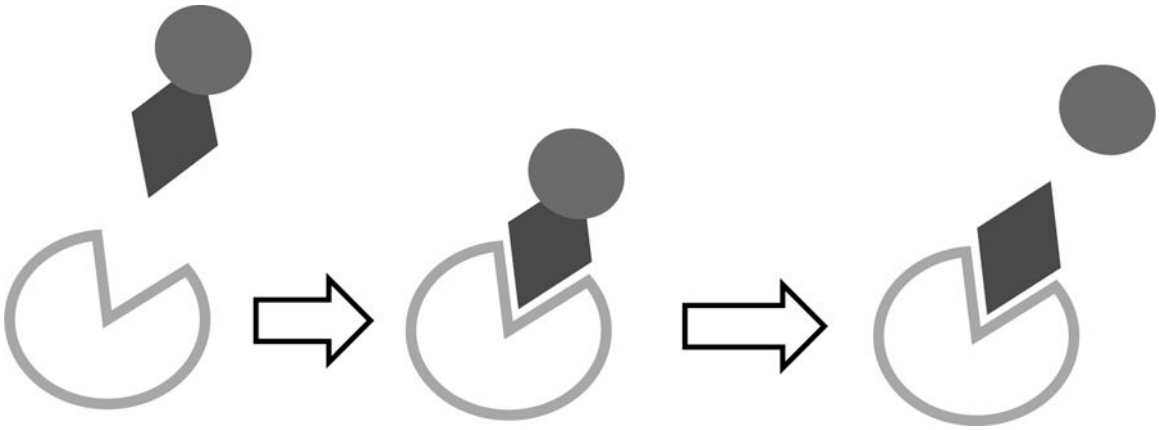

Figure 2:
Target bioanalyte binds active site (left) to form bioanalyte-enzyme complex (middle) where a catalytic reaction converts target bioanalyte to measurable reaction product (dark circle, right).

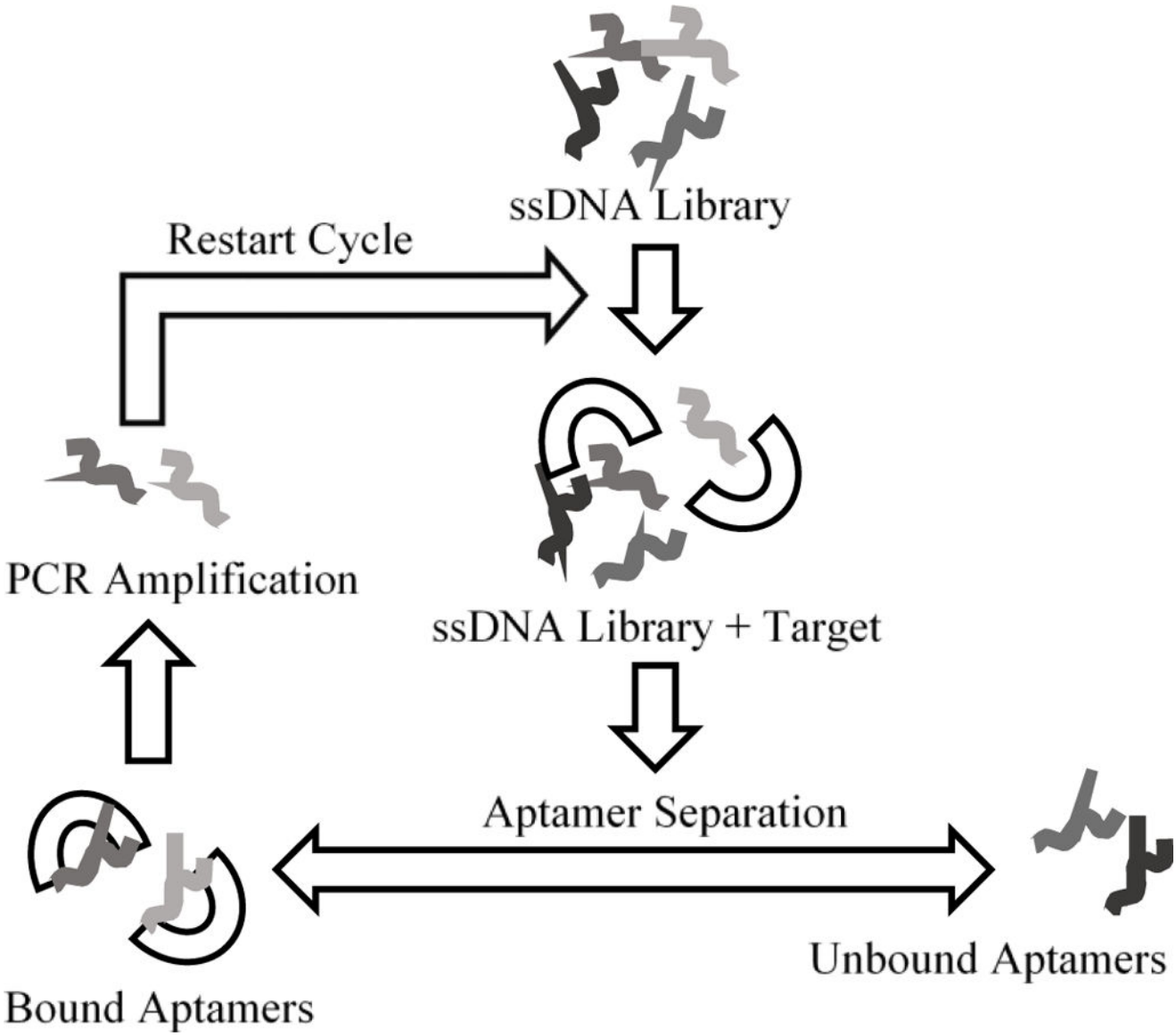

Figure 3:
SELEX cycle starts with incubation of target bioanalyte with oligonucleotide. Unbound aptamers are removed retaining only bound aptamer sequences for PCR amplification to regenerate the oligonucleotide library and restart cycle.

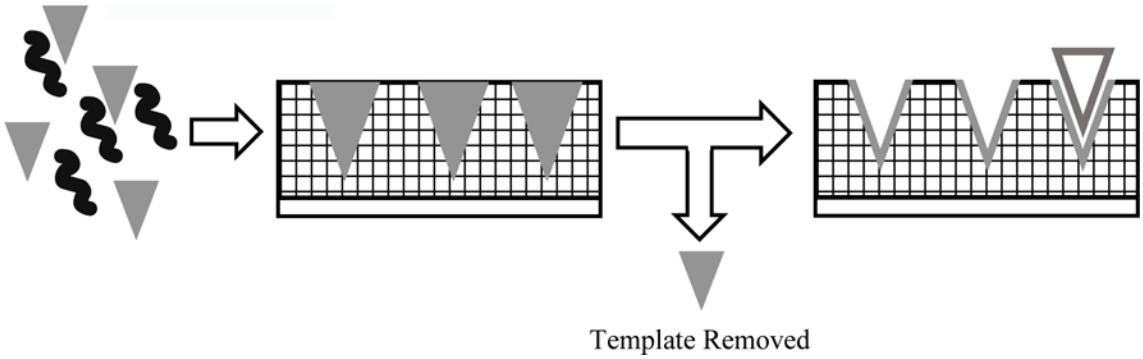

Figure 4:
Synthetic polymerization encapsulating the target bioanalyte forms an analyte binding site.

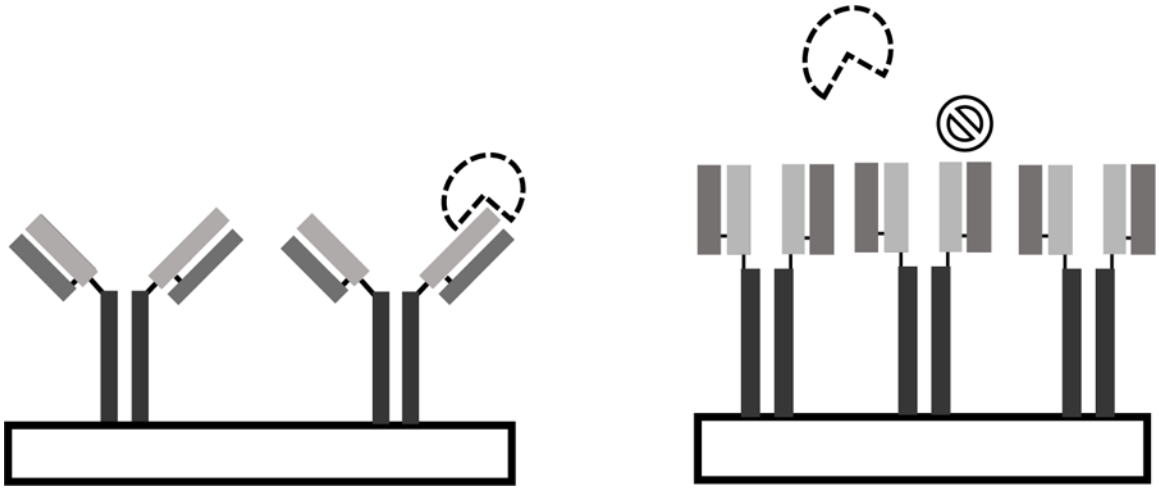

Figure 5:
Steric hinderances occur when antibody recognition elements are too closely packed resulting in a conformational change making binding sites inaccessible.

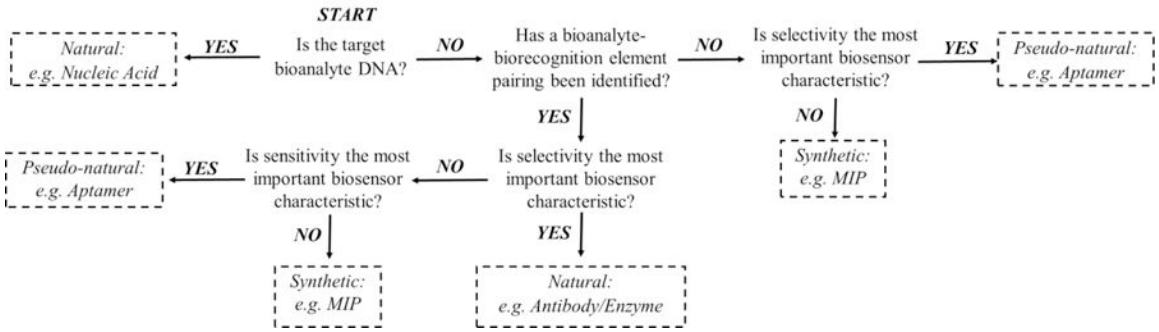

Figure 6:

Decision map that can be used to choose a recognition element in the initial design of biosensors.