Lecture 5 - 4B03 Biosensor - 400138679

Lecture 5 of ENGPHYS 4B03 Biosensors covers the topic of nonspecific binding (NSB). There are numerous terms in the literature such as nonspecific absorption, bio-fouling, selectivity and specificity, which all generally refer to the phenomena of NSB. NSB refers to unintended binding of different compounds that does not elicit the desired response. In contrast, specific binding (SB) represents an interaction between a molecule and its target with a defined end state. In biosensing, NSB is undesirable as it negatively impacts the performance of a sensor. NSB is also undesirable in biomaterials as NSB causes undesired additional reactions, which can cause issues with devices such as implants.

Biosensing is limited by nonspecific binding due to larger concentration of interfering species compared to the target species. Due to nonspecific binding compacting for receptor sites, in bulk it can reduce the effective target concentrations, block the receptor sites for target binding, and can cause false positives if captured on the receptor or transducer surface.

A model for NSB can be conceptualized as there being empty spaces available on the transducer or binding at a nonbinding site of the receptor. These empty spaces are locations where the transducer surface does not have any biorecognition elements. The empty spaces exist due to a maximum limit of density of approximately 50% of the available surface area due to the randomness of binding size and ligands that span multiple receptors.

There are a variety of strategies to reduce NSB. One potential strategy is to use smaller biorecognition elements. Smaller elements allow for more optimal space usage and have reduced overall NSB action. Another strategy to reduce nonspecific binding is via blocking. At equilibrium there are still about half of binding sites available and blocking impedes NSB by ensuring that these unoccupied sites are not able to participate in binding. It is important that blocking agents have minimal reactivity with other assay components, act to stabilize biomolecules, exhibit low enzymatic activity, and be reproducible. The most common blocking agent is bovine serum albumin. Blocking agents are added immediately after the functionalization with the capture antibody. Thus, any nonreacted surface will then be blocked by the agent. The blocking agent has a higher affinity for nonspecific binding compared to the target so as to be able to out compete the target for those nonspecific binding locations. Additionally, the biosensor surface can undergo a variety of different treatments to render it inert thereby preventing the NSB. These surfaces all share a common strong binding for water. The final way to prevent NSB is by treatment of the sample to reduce the number of nonspecific components and amplify the desired components. Dilution reduces the concentration of all binders within the system, both specific and nonspecific. Depletion reduces the concentration of high abundance proteins. Enrichment creates fractions of the sample where the proteins of interest are corrected specifically.

There are several ways in which NSB can be corrected. One of the most common is washing, which can release NSB while leaving SB intact, however this process can take a very long time due to the slow rates of NSB removal. Discrimination by force is another method of releasing NSB by using a force (electrical, magnetic) to induce the release of NSB while the curated SB molecules are kept on the sensor surface. A final method of NSB removal is by discrimination of space where a signal and a reference sensor are utilized, the NSB is removed by taking the differential of the sensors. In theory, because both of the sensors should have the same NSB, the differential will remove the NSB contribution, however in reality, the noise is additive and increases the probability distribution, which can complicate the process, or render this NSB reduction technique less effective.