Design for Biosecurity

Prof. Mainak Das

Department of Design

Indian Institute of Technology, Kanpur

Lecture 36

Electrochemical Biosensors

Welcome to this week's lecture, where we will focus on electrochemical sensors, a topic that will span the next two to three weeks. If we reflect on the history of this field, it was around the 1960s when research on electrochemical biosensors truly started to gain momentum. A key milestone was the development of the Clark electrode, named after its inventor. This electrode was initially designed to measure oxygen levels, and from that point onwards, the field of electrochemical sensors has only advanced. Over the past 60 years or so, we have seen a remarkable evolution in this area of research.

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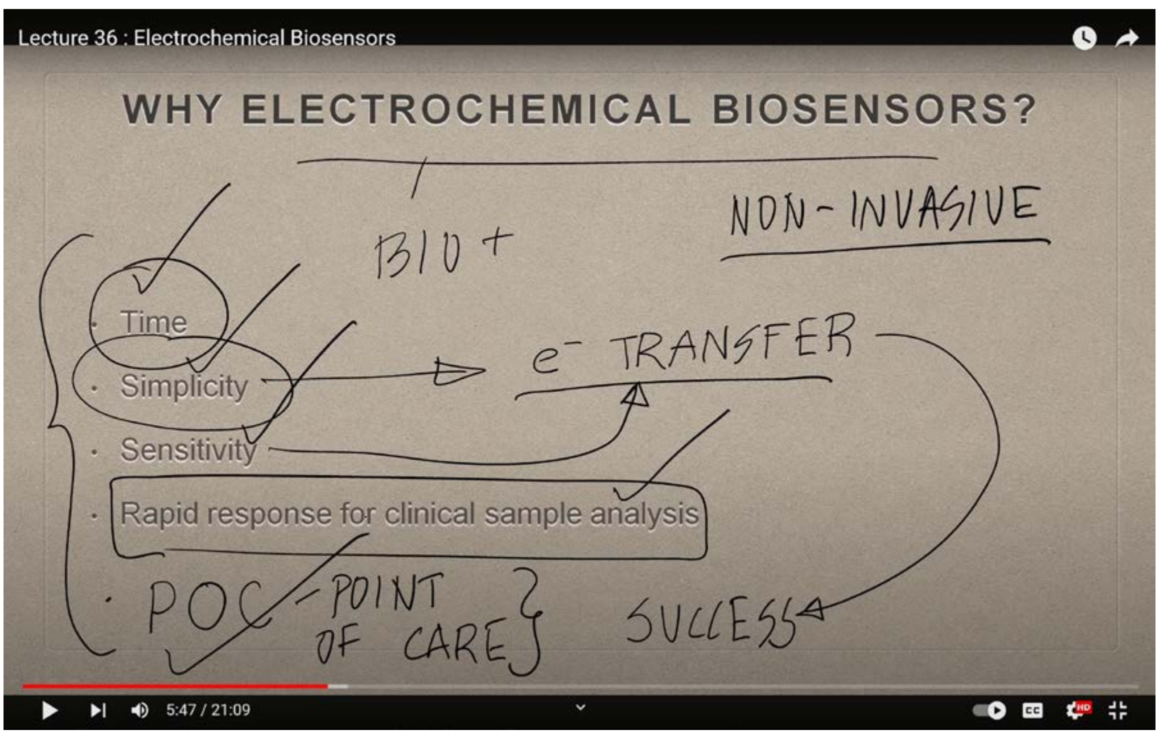

There are several factors contributing to this progress, which we will delve into as we move forward. To maintain continuity, let's connect today's discussion with what we covered last week. We explored insulin regulation, glucose metabolism, and the malfunctioning of insulin regulation that leads to diabetes. Interestingly, the first biosensor ever developed was a glucose sensor, which ties directly into last week's topic. The complex interplay between insulin, glucose, and diabetes is still fresh in your minds, and we even touched upon the controversial notion of insulin being potentially weaponized in the context of murder.

Building on that, today's focus will continue with glucose biosensors. Following that, we will discuss the exact design of Clark's electrode, providing an overview of its creation. We will also journey back to the early roots of electrochemistry, tracing its beginnings to the early 20th century, specifically between 1901 and 1910. During that time, Debye-Hückel's theory laid a foundational framework. Additionally, we will cover notable works such as Havrosky's mercury electrode, the development of two-electrode and three-electrode systems, along with their respective advantages and disadvantages.

In later discussions, we will examine topics like polarimetry, voltammetry, and other related techniques. However, for now, we'll kick off this week by exploring a few examples to give you a clearer understanding of the significance and potential of electrochemical biosensors. By grasping these concepts, I hope you'll be inspired to experiment with these biosensors in your own laboratory, perhaps even developing bioelectrodes.

Now, when we discuss electrochemical biosensors, there are four critical factors that contribute to their widespread popularity, likability, and the reason they are often the first choice in many applications.

Most electrochemical biosensors, often referred to as bio-biosensors, operate at a remarkably fast pace, allowing you to obtain data almost instantaneously. One of the key advantages of these biosensors is their simplicity. This simplicity arises from the fundamental process that governs them: electron transfer. Essentially, the efficiency of electron transfer directly influences the sensitivity of the biosensor. The higher the sensitivity in detecting this electron transfer, the more effective and successful the biosensor will be.

These rapid response times are especially beneficial in clinical sample analysis, making electrochemical biosensors highly popular in point-of-care devices. Think of handheld glucose monitors or serotonin sensors, just a single droplet of blood is enough to provide quick and reliable results. Although non-invasive models are emerging, we won't delve into those at this moment. However, the critical factors that make electrochemical biosensors stand out in research, commercialization, and practical applications are their rapid response time, simplicity, sensitivity, and adaptability for clinical use.

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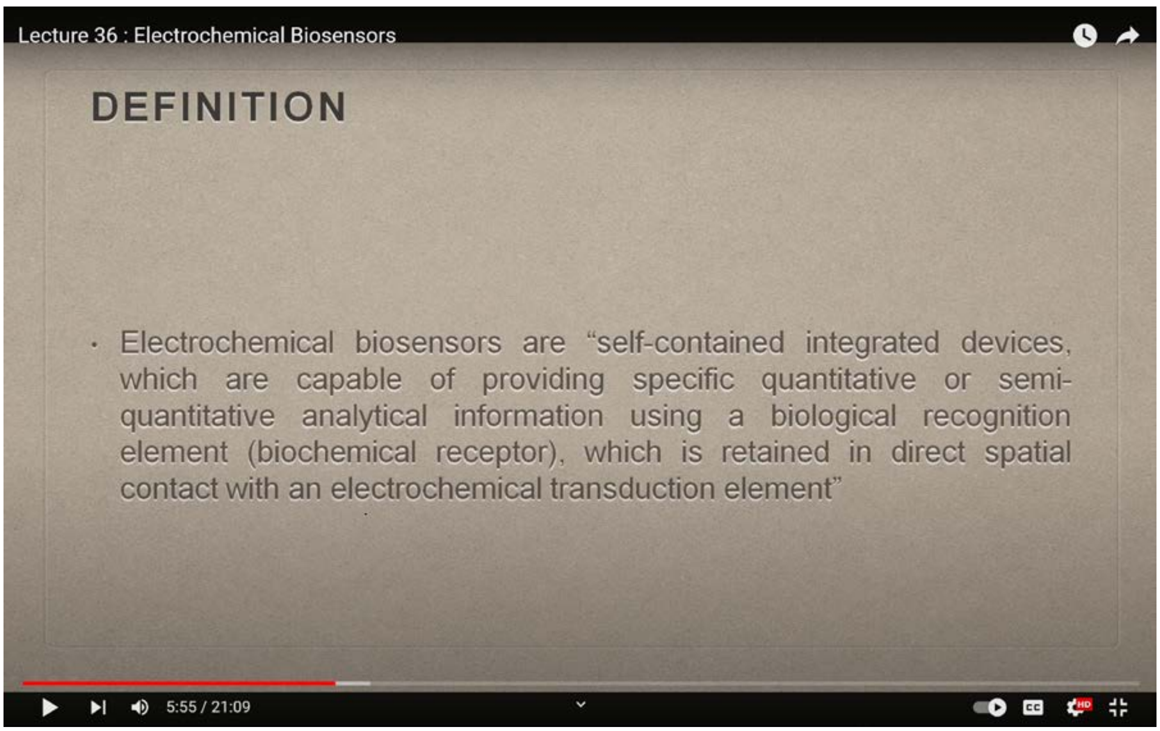

According to the IUPAC (International Union of Pure and Applied Chemistry) definition, an electrochemical biosensor is a self-contained, integrated device capable of providing specific quantitative or semi-quantitative analytical information. This is achieved through the use of a biological recognition element, known as a biochemical receptor. These receptors are responsible for detecting specific elements, whether it's glucose, NO_2, or CO_2. The biochemical receptor is situated in direct spatial contact with the electrochemical transducer element. Once the receptor binds to its target, a detectable electrical activity is generated. The transducer element then captures these electrical signals, completing the process of detection.

This is the simplest explanation of how electrochemical biosensors function, encompassing all possible variations. When it comes to their applications, the scope is broad. In environmental monitoring, for instance, bioelectrochemical sensors are used to monitor river or pond water, mines, undersea environments, and other ecological niches. You'll encounter sensors for carbon dioxide, oxygen, NO_2, glucose, pesticides, and insecticides, among others. These sensors also play a significant role in agriculture, helping monitor food safety and pathogens.

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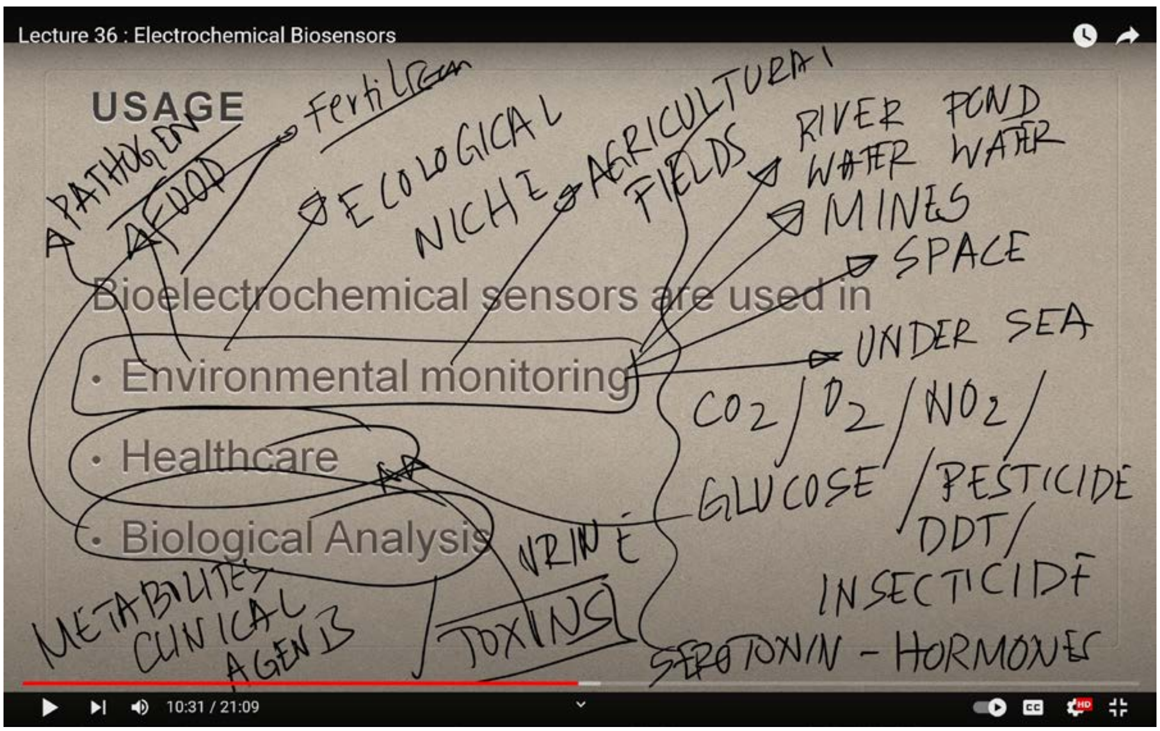

Another major area where electrochemical biosensors excel is healthcare. They are used to monitor glucose levels, detect toxins, serotonin, hormones, various clinical agents, and metabolites. Their range of applications even extends to analyzing urine samples. The third important field is biological analysis. Many laboratories rely on bioelectrochemical sensors for a variety of analytical tasks, such as detecting fertilizers, volatile organic compounds (VOCs), or the by-products of fermentation. Wherever you look, these sensors are embedded in a variety of processes, demonstrating their indispensability across different industries and research fields.

Now, how do we classify biosensors? What are the broad categories? There are multiple ways to classify them, and what I'm about to present is just one broad classification. However, this is not the only way to categorize biosensors. As you dive deeper into this field and expand your knowledge, you'll encounter various classification schemes based on different criteria. Your experience will help you develop an understanding of how these classifications can vary. That being said, biosensors can generally be divided into two main groups based on the recognition process.

When we refer to the recognition process, it's central to the definition of electrochemical biosensors as self-contained, integrated devices capable of providing specific quantitative or semi-quantitative analytical information via a biological recognition element, or biochemical receptor. The recognition process, which is key to how these devices work, can be categorized into two distinct types: affinity sensors and biocatalytic sensors.

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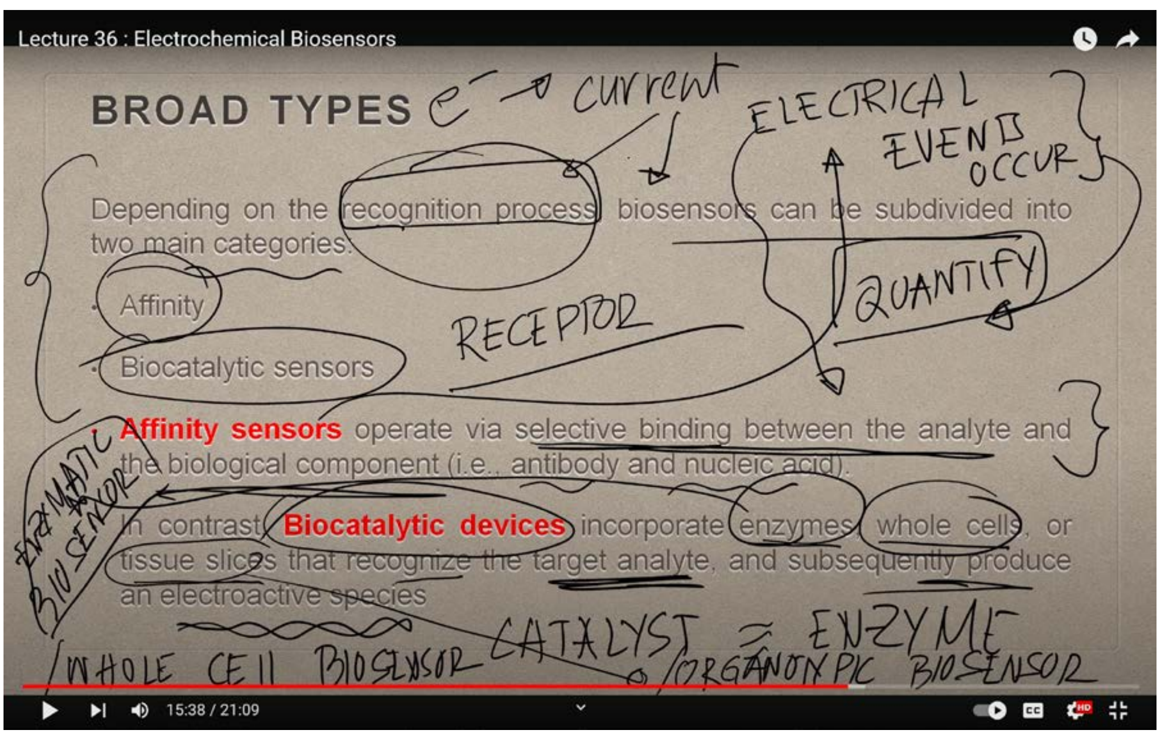

Let's start with affinity sensors. These sensors function through selective binding between the analyte and the biological component of the biosensor. This biological component could be an antibody, nucleic acid, aptamer, or even a nanobody. You may recall we've already covered aptamers. A nanobody, for instance, is a small fragment of an antibody. Essentially, affinity sensors are characterized by their ability to bind to a specific target due to a natural affinity. Once this binding occurs, certain electrical events are triggered, and it is these electrical events that we measure and quantify.

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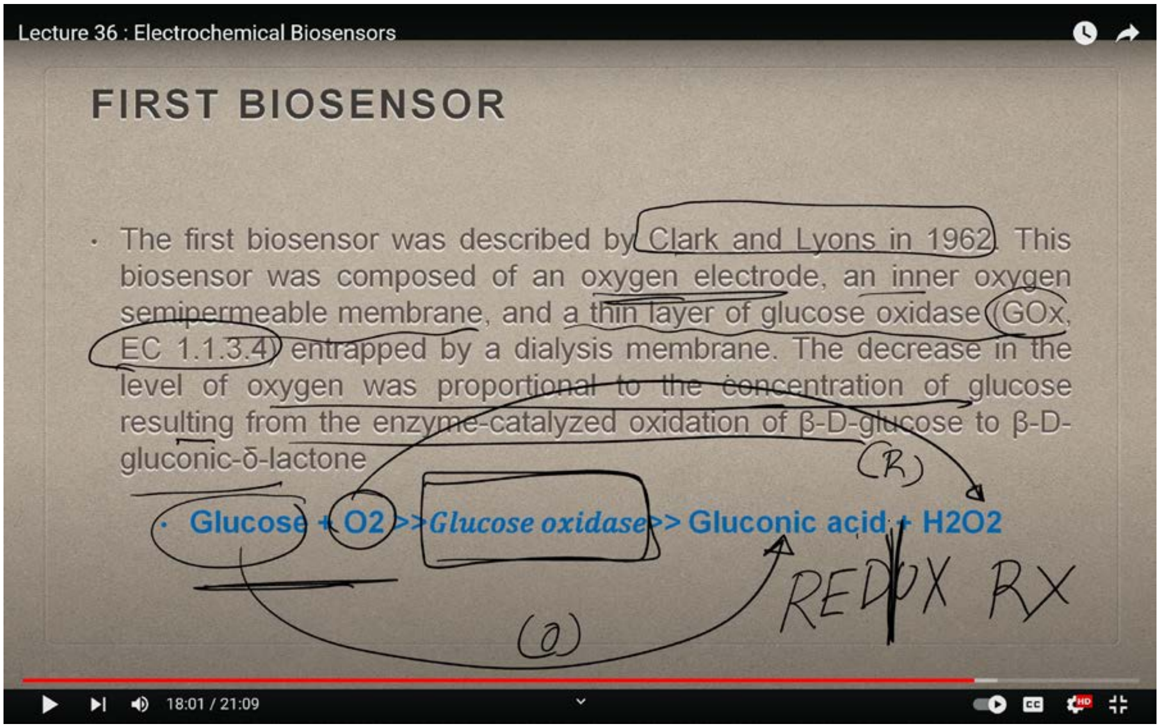

In contrast, biocatalytic sensors rely on a catalytic process. As we know from inorganic chemistry, a catalyst is something that speeds up a reaction without being consumed in the process. In the biological context, this catalyst is often an enzyme. Biocatalytic devices incorporate enzymes, whole cells, tissues, or tissue slices to recognize the target analyte and generate an electroactive species. This generation of an electroactive species is critical to the process. These sensors are also referred to as whole-cell biosensors or organotypic biosensors when tissues or organ-like structures are involved.

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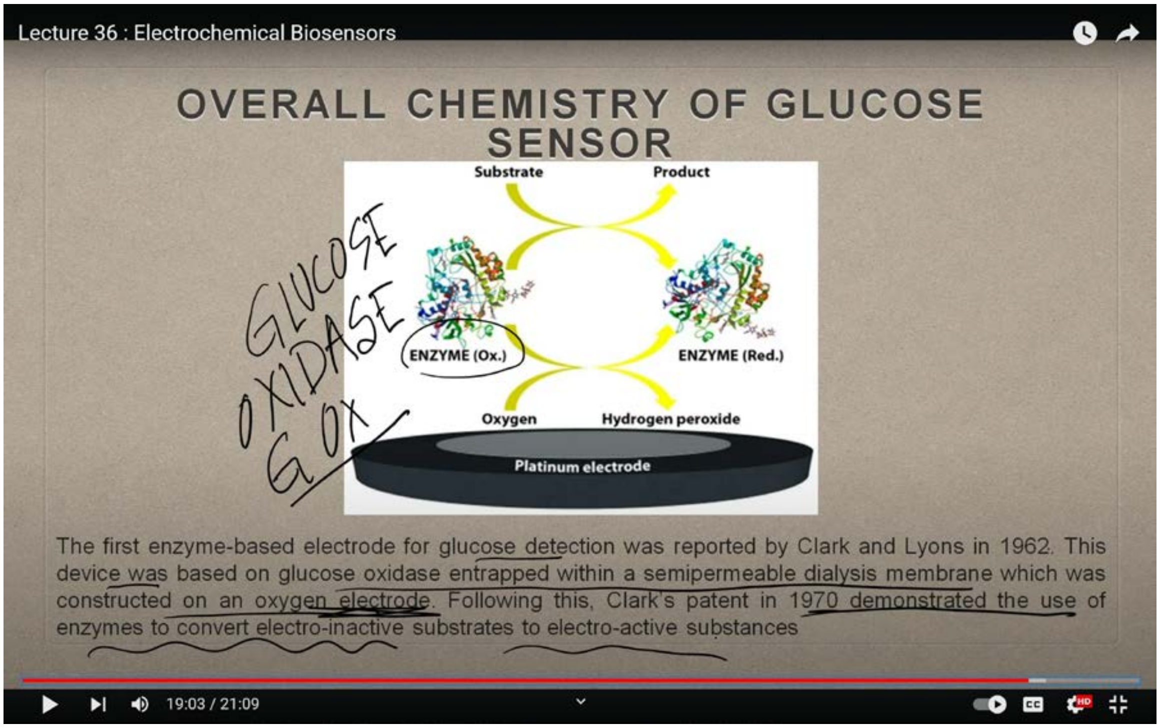

Since whole-cell and organotypic biosensors will be discussed later, today's focus will primarily be on enzymatic biosensors. Enzymes, as we know, catalyze reactions without being consumed in the process. What they do is facilitate either the oxidation or reduction of a molecule, meaning they either allow the molecule to donate an electron or release an electron. This transfer of electrons is crucial because it can be translated into an electrical current. The uniqueness of this electron transfer process is what helps us identify the specific analyte, as each compound has its own distinct electron transfer behavior.

To give you a bit of historical context, the first biosensor was developed by Clark and Leon in 1962, as I mentioned earlier. This field has been thriving for almost 60 years now and continues to grow, offering numerous innovations and employment opportunities. The original biosensor designed by Clark and Leon consisted of an oxygen electrode, an inner semipermeable oxygen membrane, and a thin layer of glucose oxidase. This fundamental design laid the groundwork for the electrochemical biosensors we see.

Let's revisit the concept of a biocatalytic device. In this case, we're referring to a biocatalytic device because it involves an enzyme, glucose oxidase (commonly abbreviated as GOx). The enzyme's catalogue number, E.C. 1.3.4, helps identify it within the enzyme classification system. The enzyme is encapsulated within a dialysis membrane, and the decrease in glucose levels is directly proportional to the glucose concentration. This happens as a result of the enzyme-catalyzed oxidation of β-D-glucose to β-D-gluconolactone.

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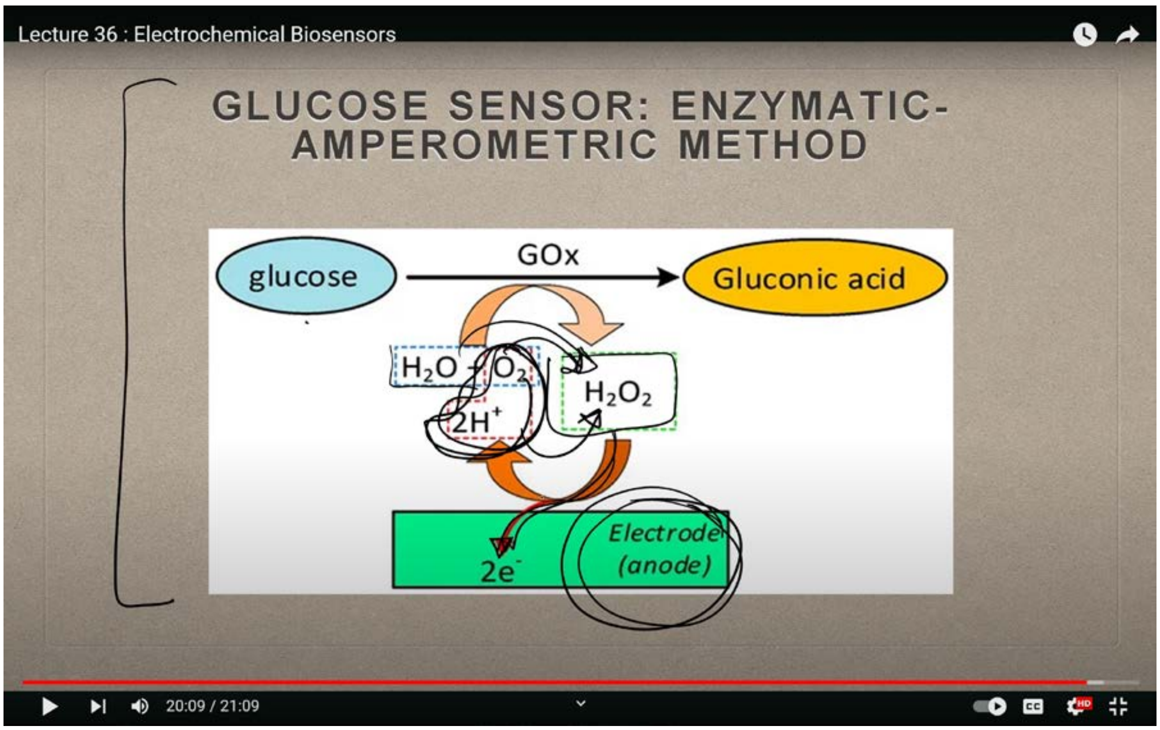

The reaction can be summarized as follows: glucose, in the presence of oxygen, reacts with glucose oxidase, which catalyzes the process. This results in glucose being converted into gluconic acid, while oxygen is reduced to hydrogen peroxide. Essentially, we are witnessing a redox reaction, both reduction and oxidation occur simultaneously. Specifically, the glucose undergoes oxidation, and oxygen is reduced to peroxide.

Clark and Leon ingeniously leveraged this redox chemistry to create the first glucose sensor. Let's break down what's happening in the glucose sensor from a chemical standpoint. The key element here is glucose oxidase, the enzyme that plays a central role.

The first enzyme-based glucose sensor was reported by Clark and Leon in 1962. In their design, glucose oxidase was encapsulated within a semi-permeable dialysis membrane that was mounted on an oxygen electrode. Subsequently, Clark's patent in 1970 took this concept further, demonstrating how enzymes could convert an electro-inactive substrate into an electro-active one.

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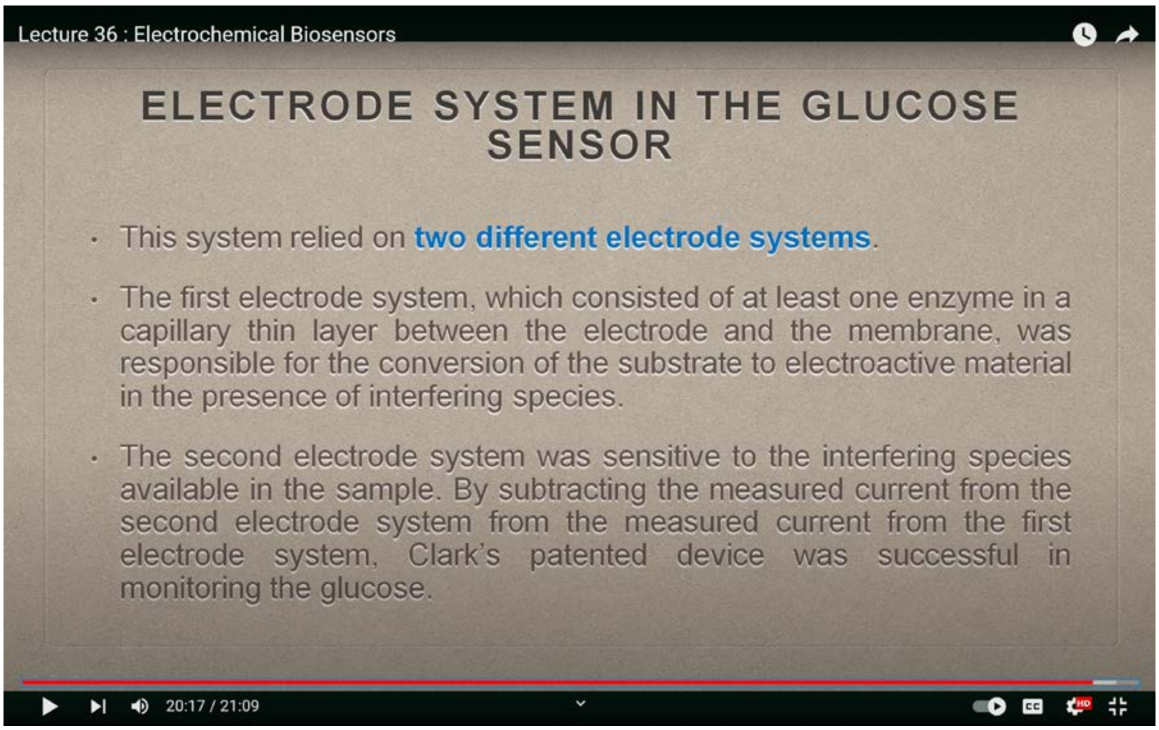

As I described earlier, the reaction involves glucose being converted into gluconic acid, and oxygen being reduced to peroxide. To visualize this: glucose transforms into gluconic acid under the action of glucose oxidase. Concurrently, H_2O and oxygen are reacting to form peroxide. During this process, two electrons are liberated at the anode. This redox reaction is measured by detecting these liberated electrons, which provides a quantitative indication of glucose concentration. Now, the process seems straightforward, but there’s more to it than meets the eye, there’s a crucial aspect or a “catch” that we’ll explore soon. Before we dive into that, we need to take a closer look at the electrode systems involved in glucose sensors. In the next session, we will delve deeper into the enzymatic amperometric method used for glucose sensing. Thank you, and we will resume this in the next class.