Design for Biosecurity

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Lecture 39

Glucose Sensor - Part 1

Welcome back to the fourth lecture of this week. So far, we have primarily focused on enzymatic glucose sensors. This discussion follows from our previous week's topics, where we explored the dual nature of insulin, both as a therapeutic agent and, intriguingly, as a potential murder weapon, and the challenges faced in managing diabetes, which present two very contrasting situations. This week, however, we are delving into the fundamentals of glucose sensors.

To give a quick recap, the reason we begin with glucose sensors is their foundational role in the world of biosensors. Historically, the first biosensor was developed by Leland Clark, and it was an oxygen sensor. We will examine the design and workings of the Clark electrode in detail in the upcoming week. This will include a discussion on electrode fabrication, configuration of different electrode types, such as reference, working, and standard electrodes, the role of the anode and cathode, and the electrocatalytic reactions that transform these components into a functional device.

But before we get into the intricate details of electrochemical sensors and the underlying electrochemistry, this week serves as a case study on glucose sensors. This is because the glucose sensor was one of the first biosensors developed, with its origins tracing back almost 60 years.

Now, let's focus on the basic principles of glucose sensors. Essentially, glucose detection can be carried out in any sample, whether it’s a urine sample, blood sample, soil sample, or even a plant sample. However, one of the key challenges lies in the fact that these samples contain a myriad of other molecules that can interfere with the detection of glucose. Therefore, it becomes crucial to isolate glucose selectively, and one common approach is the use of a membrane. In the previous class, we discussed polycarbonate membranes, which were initially designed to minimize interference from other molecules that might slow down glucose detection, especially in the presence of oxygen.

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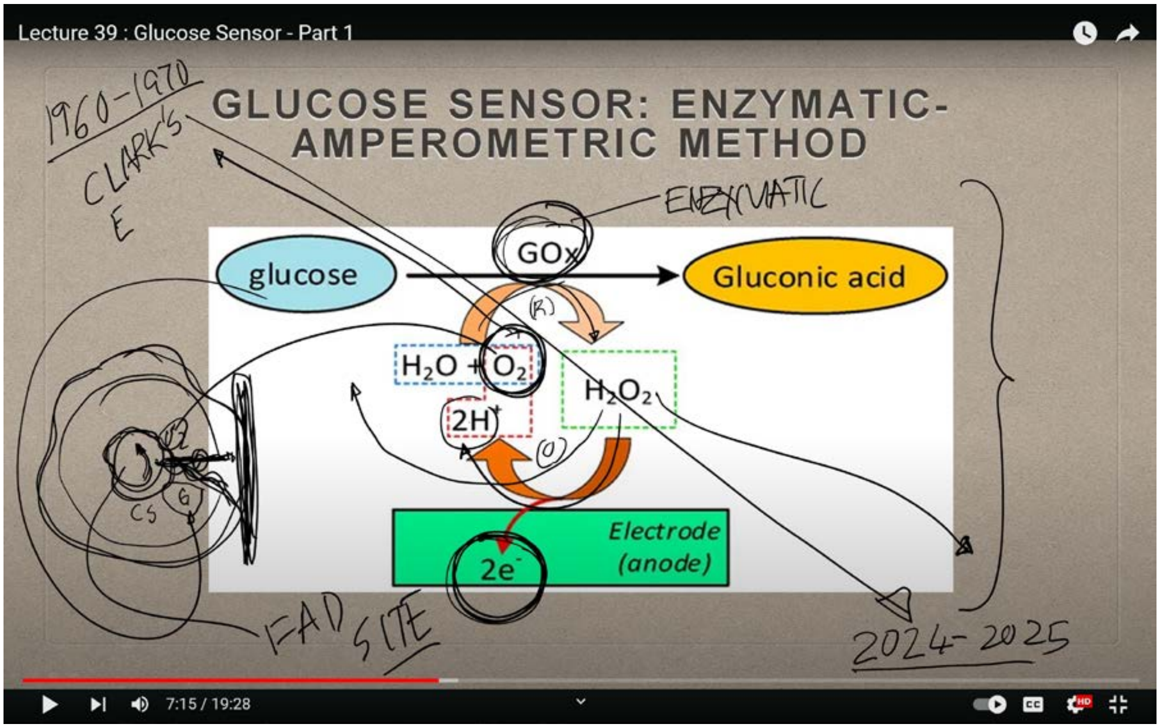

The detection process involves an enzyme called glucose oxidase, which catalyzes the conversion of glucose into gluconic acid. Simultaneously, oxygen is reduced to hydrogen peroxide (H_2O_2). This reaction forms the basis for the detection process, and it can be measured in multiple ways, as summarized in the slides we've reviewed. The enzymatic reaction is straightforward: glucose, in the presence of glucose oxidase, is converted into gluconic acid. During this reaction, oxygen plays a crucial role by being reduced to peroxide, which can then be further oxidized. This chain of reactions forms the crux of the glucose detection mechanism, and it is through quantifying these reactions that glucose levels are determined.

One of the primary challenges in glucose sensing lies within the structure of the enzyme glucose oxidase itself. This enzyme has a catalytic site that is deeply embedded within its molecular structure, which adds complexity to the sensor design and operation.

If we consider the catalytic site of the enzyme, there are two key regions: the glucose binding site and the oxygen binding site. The glucose binding site is where the electron transfer takes place. One of the primary challenges we discussed in the last class concerns the spatial limitations. If you observe the reaction in three-dimensional space, the reaction happens deep inside the enzyme's pocket, and the electrode sits far outside. Due to this spatial separation, much of the signal is lost before it reaches the electrode. This means a substantial amount of glucose must be converted to generate a detectable signal.

Now, when the glucose concentration in your sample is low, the sensor's sensitivity is compromised. To address this issue, many advancements have been made. But before we dive into those developments, it's important to recall that the very first glucose sensor was based on measuring oxygen consumption with the Clark electrode. Essentially, the sensor detected how much oxygen was being converted or how much peroxide was being produced. Another method involved measuring how much electron release occurred when peroxide was oxidized, resulting in the release of two protons and oxygen. This step-wise monitoring forms the core of glucose detection.

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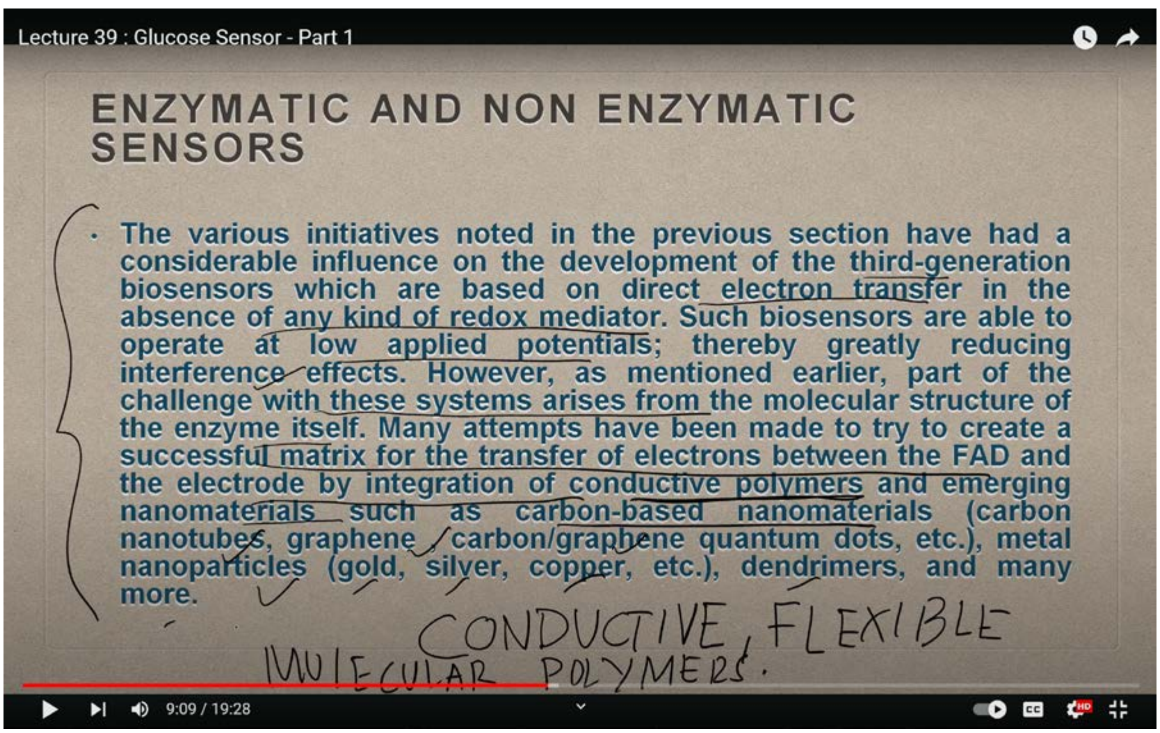

So far, most of the assays we’ve discussed fall under the category of enzymatic assays. While effective, enzymatic assays come with their own set of challenges, particularly because of the enzyme's complex architecture. For instance, the catalytic site we referred to earlier is called the FAD (flavin adenine dinucleotide) site, which we have already discussed in detail.

Advancements in sensor design have focused on overcoming these architectural challenges. One approach has been to couple the FAD site to an electrode through a linker, or even have the electrode in direct contact with the site. Other developments involve flexible electrodes that can penetrate the enzyme's structure like a wire, or wiring the entire enzyme to the electrode. These are some of the key challenges facing enzymatic assays today.

However, sensor technology has come a long way since the Clark electrode was introduced in the 1960s and 1970s. Today, in 2024-2025, we have seen nearly six decades of progress, yet detecting a seemingly simple molecule like glucose remains a difficult task. A tremendous amount of work has been done, and today we will look into some of the most recent advancements in this area.

To begin with, let’s differentiate between enzymatic and non-enzymatic sensors. The innovations mentioned earlier have significantly influenced the development of third-generation biosensors, which are based on direct electron transfer without the use of a redox mediator. These biosensors can operate at low applied potentials, which dramatically reduces interference from other molecules.

However, as mentioned earlier, one of the major challenges is still the enzyme's molecular structure. Many attempts have been made to design an efficient matrix that facilitates electron transfer between the FAD site and the electrode. One solution involves incorporating conductive polymers into the system. These polymers, along with emerging nanomaterials, have shown great promise. Among the most notable materials are carbon-based nanomaterials such as carbon nanotubes, graphene, and carbon-graphene quantum dots, as well as metal nanoparticles like gold, silver, and copper. Additionally, dendrimers and other novel materials have been explored.

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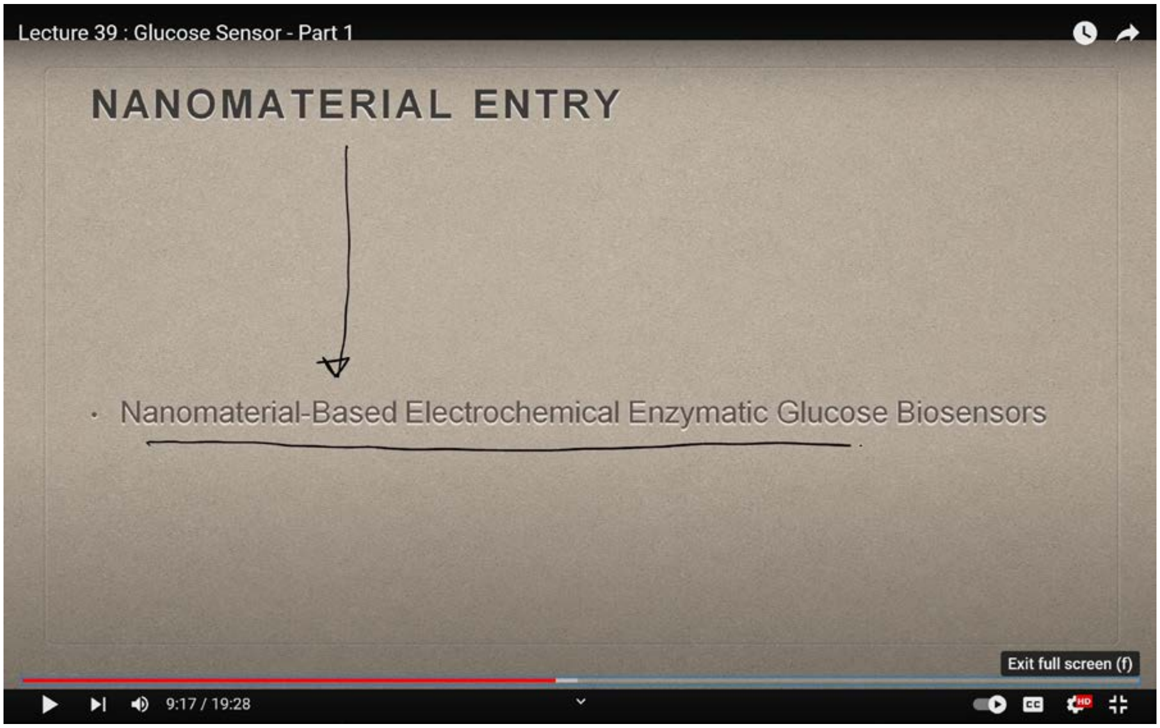

These approaches, developed over the last 25 years, represent the cutting-edge advancements in the field of biosensors. Let's delve into the revolutionary impact of nanomaterials in glucose biosensing. One of the earliest and most significant developments involves coupling the FAD (flavin adenine dinucleotide) site with gold nanoparticles. This is a case study worth discussing in detail.

Here, we are focusing on electrically wiring the glucose oxidase enzyme. This involves reconstituting the apo-glucose oxidase, which is the enzyme without the FAD, as the FAD usually resides in a specific pocket within the enzyme. When FAD is removed, the resulting enzyme, now devoid of its active site, is called apo-glucose oxidase or apo-FAD. In this setup, FAD is functionalized onto gold nanoparticles, which are then linked to the electrode surface via a dithiol bridge. The dithiol bridge serves as a sulfur bridge, with the thiol groups facilitating the connection. This setup enables direct electrical contact between the FAD site and the electrode.

Once this system is established, a cyclic voltammogram can be generated, which provides valuable data on the sensor's performance across varying glucose concentrations. We'll explore voltammograms in more detail in the following weeks, examining how these graphs are generated and interpreted.

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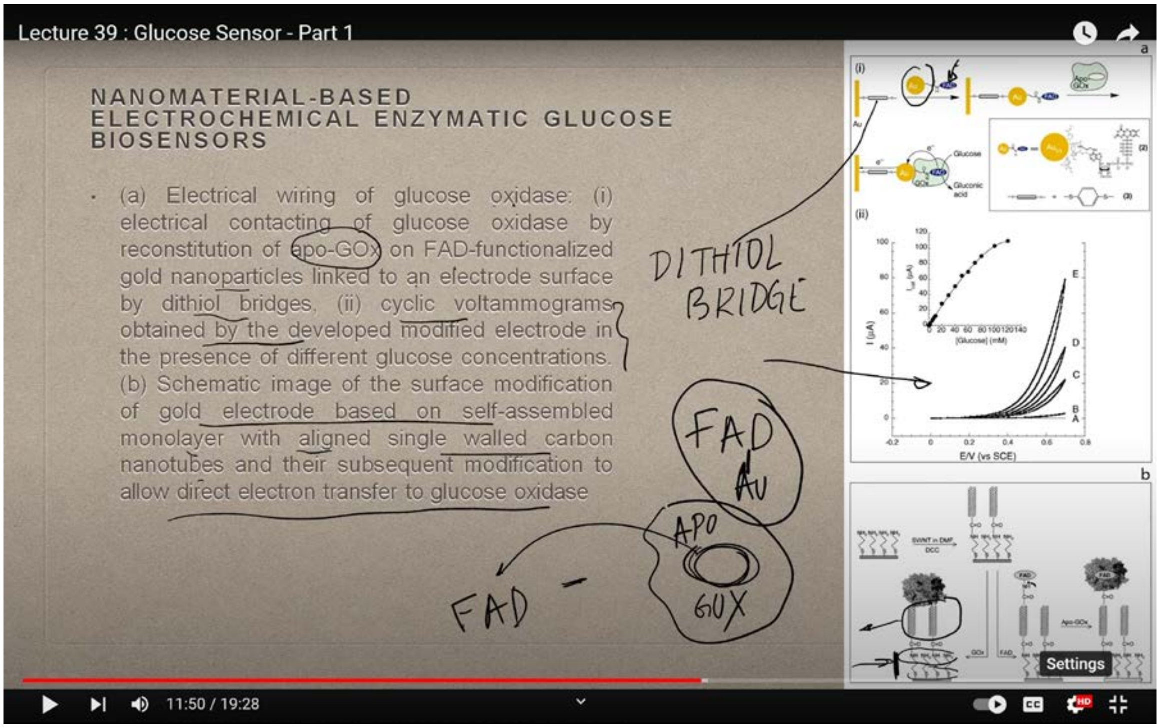

In this particular system, the surface modification of the gold electrode is achieved through the self-assembly of aligned single-wall carbon nanotubes. These nanotubes are subsequently modified to enable direct electron transfer to glucose oxidase. Once again, the dithiol bridge is key here, with sulfur atoms playing a crucial role in the bonding process. Additionally, silanes, particularly sulfur-containing silanes, assist in this process, while carbon sheets linked to FAD ensure efficient electron transfer.

This is one of the most promising advancements in glucose biosensor technology. Moving forward, let's examine the use of gold nanostructures in hybrid glucose biosensors. Gold nanostructures, whether used alone or in combination with other nanomaterials, have been extensively studied to create optimal matrices for biomolecule immobilization, particularly for glucose oxidase. One area of great interest is the development of nanoporous gold electrodes.

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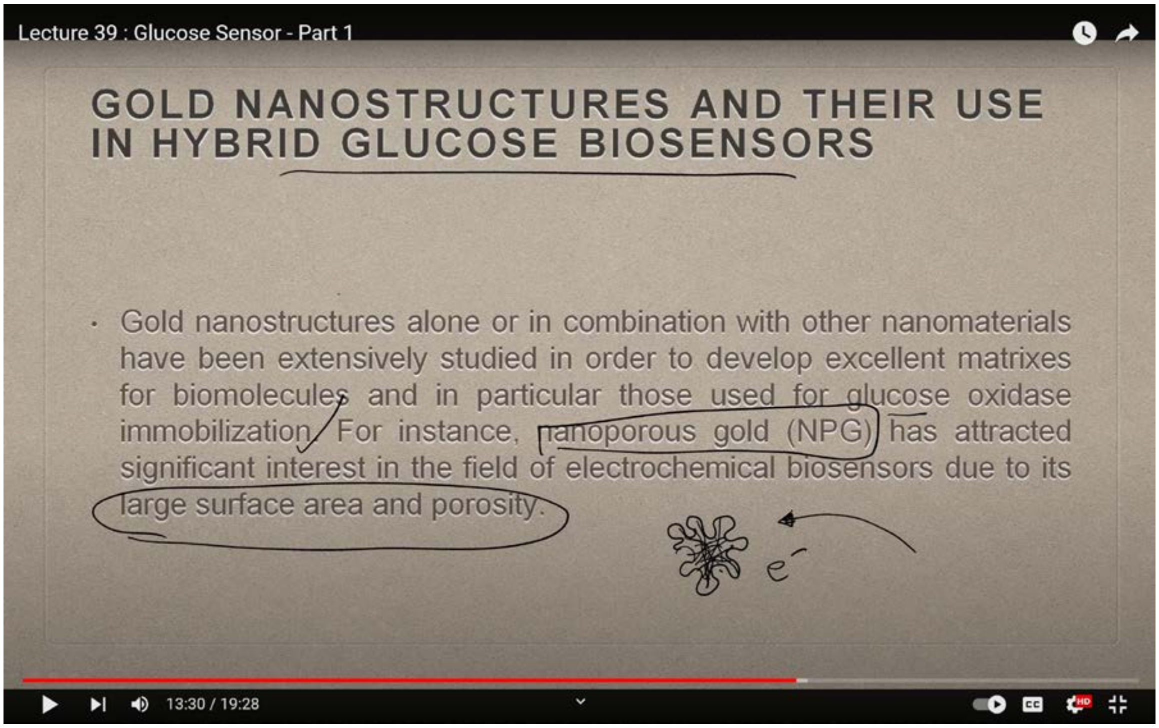

Why nanoporous gold? There are several reasons. First, gold electrodes offer superior conductivity, which is critical for high-performance sensors. Additionally, gold is highly stable, with minimal leaching, making it a durable choice for biosensor applications. The nanoporous structure itself offers a large surface area and high porosity, with interconnected channels forming an intricate network. This structure provides ample space for conjugating electron transfer sites, allowing for the capture of significantly enhanced signals due to the increased surface interaction with the electrode.

Next, let's discuss some specific work from Wu in 2015. We've journeyed through the development of glucose sensors from the early days of 1960 to the late 1980s, and now we're looking at more recent advancements. In 2015, Wu reported the development of a glucose biosensor based on a porous gold enzyme combination. In his work, nanoporous gold (NPG) was prepared by immersing 12-karat white gold leaves in concentrated nitric acid (HNO_3).

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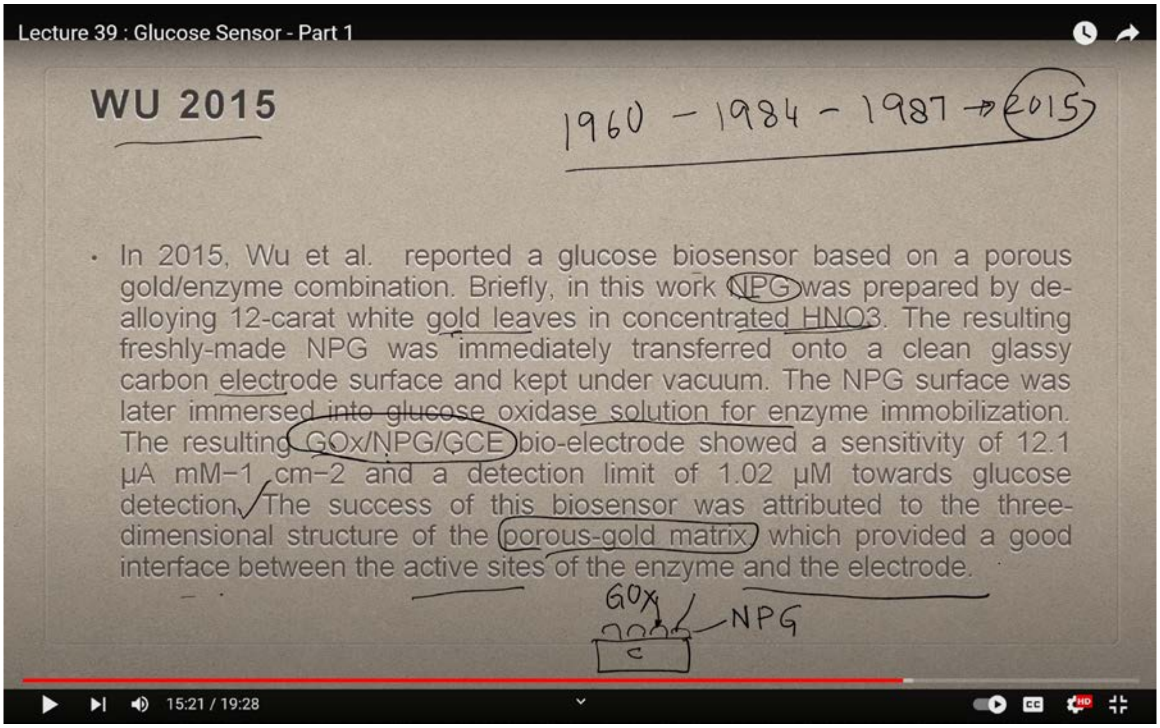

The freshly prepared nanoporous gold (NPG) was immediately transferred onto a clean glassy carbon electrode (GCE). So, imagine you have a carbon electrode with a layer of porous gold, the NPG. This entire assembly was carefully placed onto the glassy carbon electrode surface and kept under vacuum conditions. Once stabilized, the NPG surface was immersed in a glucose oxidase (GOX) solution to facilitate enzyme immobilization. This resulted in a complex structure composed of GOX, NPG, and GCE, essentially a combination of nanoporous gold, glucose oxidase, and the glassy carbon electrode. The resulting biosensor demonstrated a sensitivity of 12.1 µA with a detection limit of 1.02 µM for glucose detection.

The success of this biosensor is attributed to the three-dimensional structure of the nanoporous gold matrix, which provided an excellent interface between the active site of the enzyme and the electrode surface. This work stands out as one of the first significant achievements in successfully reaching the site of electron transfer for glucose sensing.

Following this, Riva's group conducted further groundbreaking work between 2015 and

2018. They demonstrated the use of spherical gold nanoparticles as part of a nanohybrid structure. By applying a series of chemical modifications, they established an exceptionally efficient immobilization matrix for glucose oxidase. The key to their approach was immobilizing a greater amount of glucose oxidase on the gold surface. To achieve this, they functionalized the gold nanoparticles with 3-mercaptophenylboronic acid.

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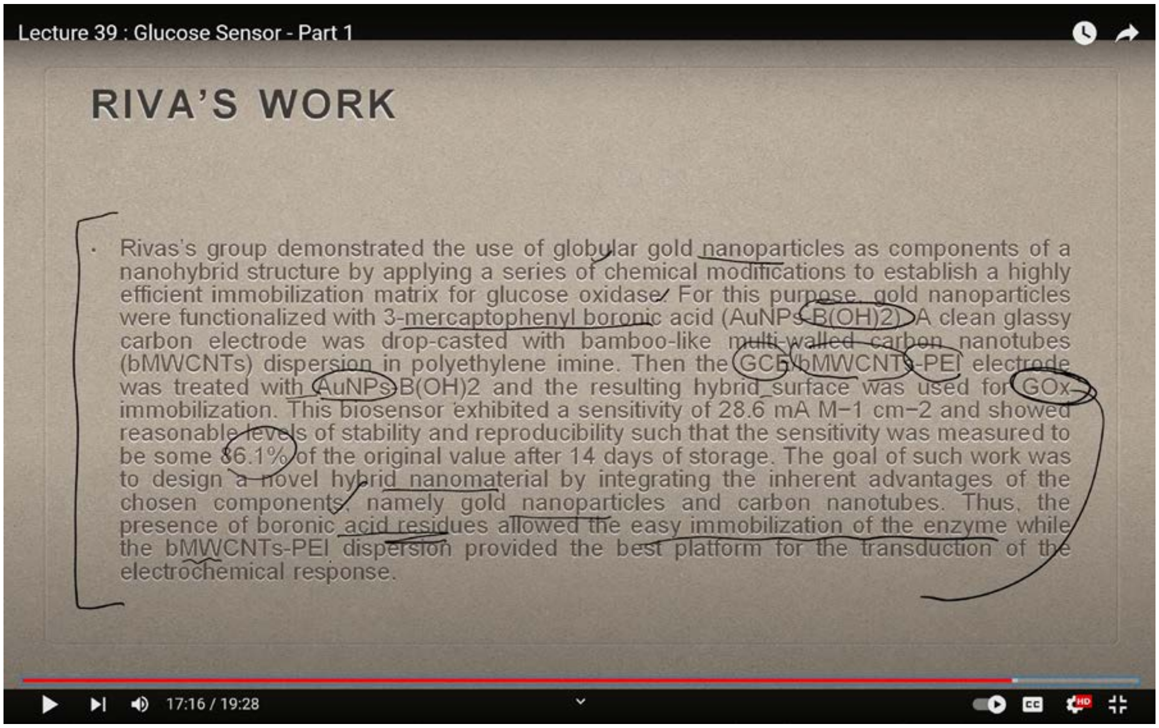

Their setup involved a clean glassy carbon electrode, which was drop-cast with a bamboo-like multi-walled carbon nanotube (MWCNT) dispersion in polyethyleneimine (PEI). After this, the glassy carbon electrode, multi-walled carbon nanotubes, and PEI were treated with gold nanoparticles and 3-mercaptophenylboronic acid, creating a hybrid surface ideal for glucose oxidase immobilization.

The resulting biosensor exhibited an impressive sensitivity of 28.6 mA, along with notable stability and reproducibility. In fact, after 14 days of storage, the biosensor maintained 86.1% of its original sensitivity. The goal of this work was to design a novel hybrid nanomaterial that integrated the unique advantages of its components, specifically gold nanoparticles and carbon nanotubes. The boronic acid residues allowed for the easy immobilization of enzymes, while the MWCNT-PEI dispersion provided an optimal platform for the transduction of electrochemical signals.

As you can see, the process of developing glucose biosensors has become increasingly complex over time. Yet, all of these advancements are driven by a single goal: improving sensitivity. This ongoing evolution highlights the dedication of researchers who have been pursuing this objective for more than 40 years. And remember, this is just for glucose detection! When you think of other potential applications, such as biosensors for detecting bioterrorism agents, the scope of this work becomes even more significant.

As I mentioned at the start of the course, there is immense opportunity in this field for those who develop a holistic understanding of the science behind biosensors. So, we will follow up on this in the next class, where we will talk a little bit more about the enzymatic sensors and move on to the non-enzymatic sensors. Thank you.