Design for Biosecurity

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Lecture 40

Glucose Sensor - Part 2

In our last class, we discussed Riva's work, where we explored the complex hybrid structure involving 3-mercaptophenyl boronic acid, gold nanoparticles, and glassy carbon electrodes that were drop-cast with bamboo-like multi-walled carbon nanotubes. The glucose oxidase enzyme was immobilized on this hybrid structure, resulting in a highly efficient biosensor. That was a significant milestone in the development of glucose sensors, but today we are going to shift our focus to another remarkable work, Turner's work. Turner adopted a much more innovative and strategic approach in his research.

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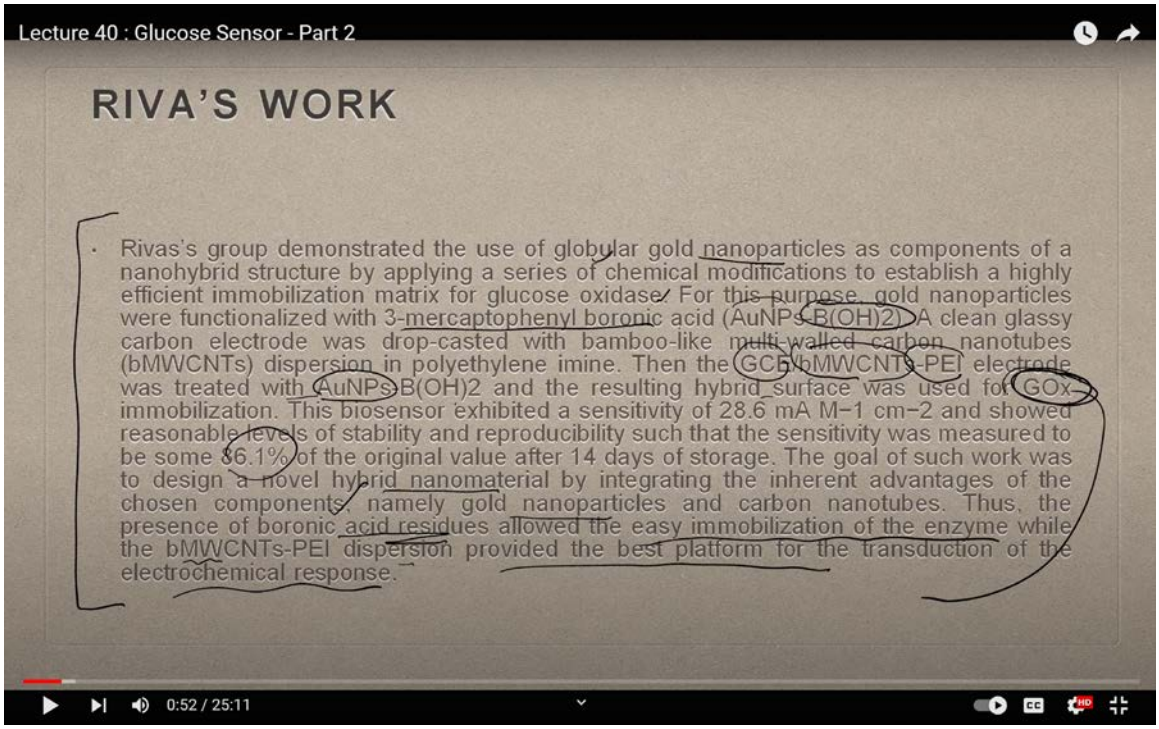

Instead of relying on the previous complex hybrid models, Turner turned to molybdenum

sulfide (MoS_2), one of the emerging two-dimensional (2D) materials with unique properties. For those of you familiar with 2D materials, graphene often comes to mind due to its exceptional characteristics. Molybdenum sulfide, however, is a close relative of graphene and has shown great potential, especially in the field of biosensing.

Turner's group capitalized on the planar structure of MoS_2 to create a highly structured layer of gold nanoparticles on the surface of a two-dimensional molybdenum sulfide nanosheet. This innovative matrix has recently garnered considerable attention as an ideal candidate for electrochemical biosensors.

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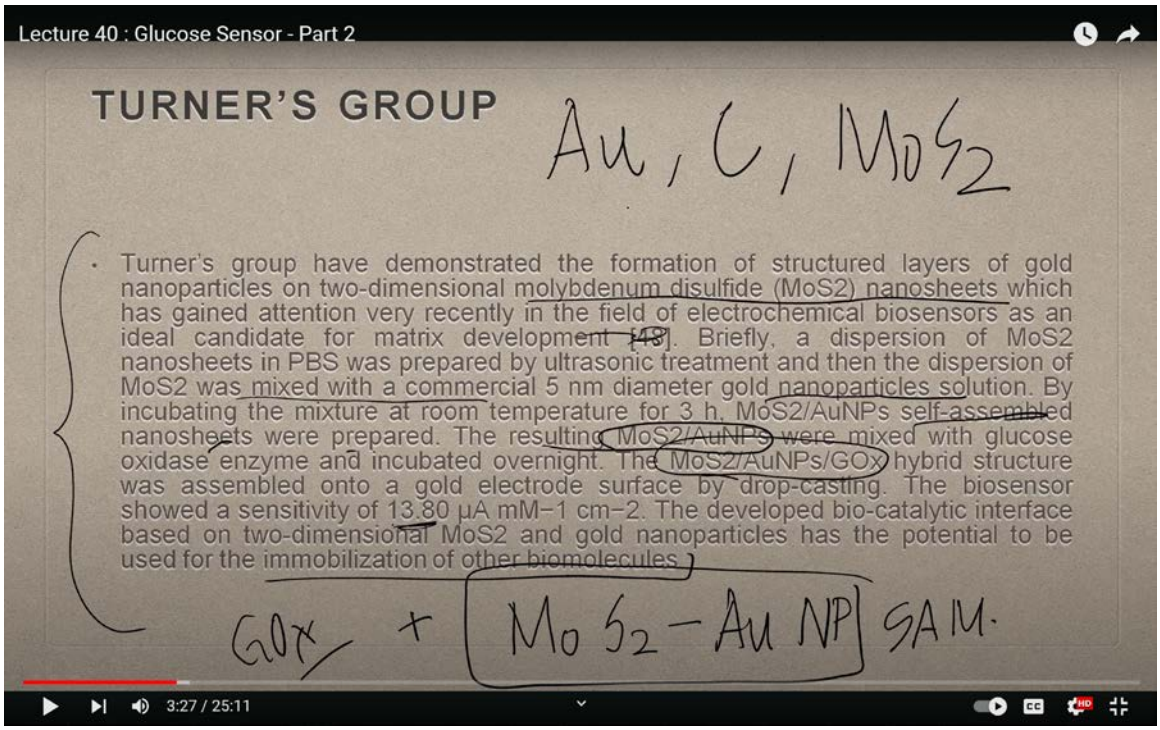

To give you a brief overview of Turner's methodology: A dispersion of MoS_2 nanosheets in phosphate-buffered saline (PBS) was prepared through ultrasonic treatment. This MoS_2 dispersion was then mixed with a commercial solution containing gold nanoparticles with a diameter of approximately 5 nanometers. After incubating this mixture at room temperature for three hours, the molybdenum sulfide and gold nanoparticles self-assembled into a structured nanosheet. This MoS_2-gold nanoparticle assembly was then

combined with glucose oxidase, and the mixture was incubated overnight.

The final MoS_2-gold nanoparticle and glucose oxidase hybrid structure demonstrated a sensitivity of 13.8 μA, which is quite significant. This biocatalytic interface, based on the two-dimensional MoS_2 and gold nanoparticles, has shown tremendous potential for the immobilization of biomolecules and further advancements in biosensing. This is a key part of Turner's contributions to the field.

If you observe the trend, we initially started with gold-based systems, transitioned to carbon-based technologies, and now we find ourselves exploring molybdenum sulfide. This journey of innovation and material exploration continues, pushing the boundaries of what is possible in biosensor design.

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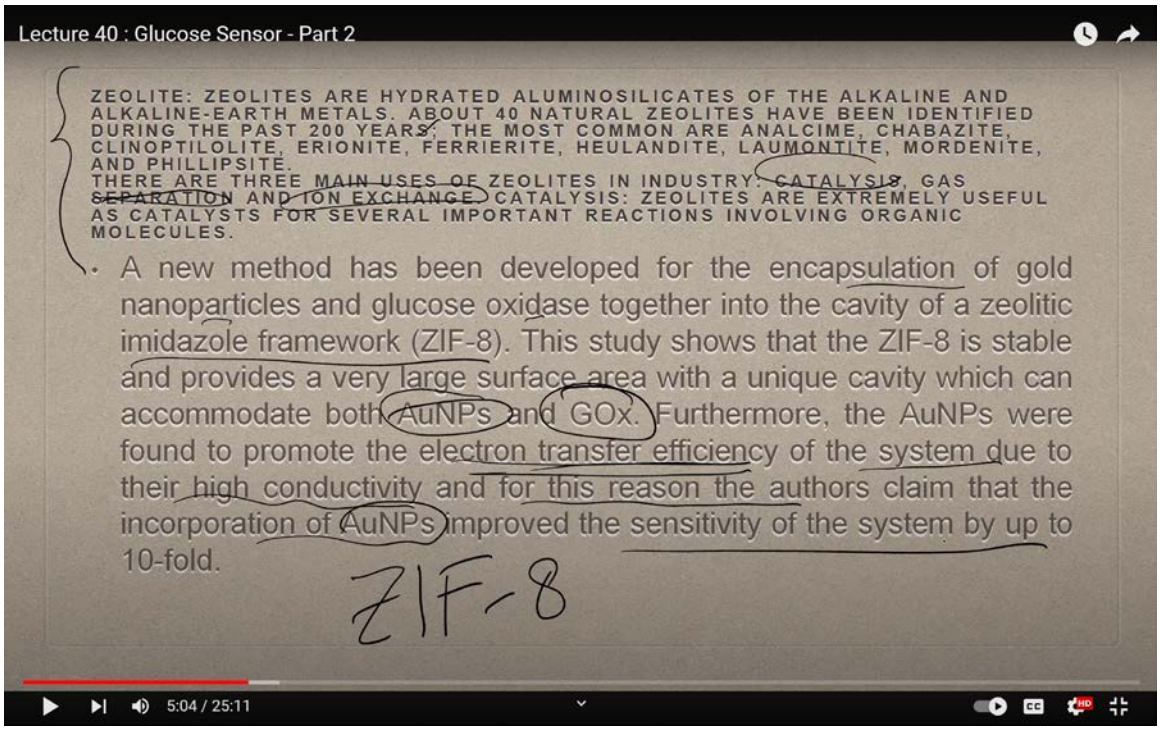

Moreover, there’s another fascinating advancement that I want to introduce, zeolites. What are zeolites, you might ask? Zeolites are hydrated aluminosilicates composed of alkaline and alkaline earth metals. Over the past 200 years, approximately 40 natural zeolites have been identified. These materials have opened new doors in various fields, including

biosensing, due to their unique properties.

In the field of material science, zeolites, particularly the natural ones like analcime, chabazite, aronite, and perrarite, have become vital components in various industrial applications. These materials have three primary uses: catalysis, gas separation, and ion exchange. In fact, many of the ion exchangers found in household water purification systems contain zeolites. Recently, a novel technique has emerged for encapsulating gold nanoparticles and glucose oxidase within the cavity of a zeolitic imidazole framework (ZIF-8).

ZIF-8 has proven to be a stable material, boasting an exceptionally large surface area and a unique cavity capable of housing both gold nanoparticles and glucose oxidase. The gold nanoparticles facilitate efficient electron transfer, which significantly enhances the performance of the biosensor. The authors of this study claimed that incorporating gold nanoparticles increased the system's sensitivity by a factor of ten, mainly due to their high conductivity.

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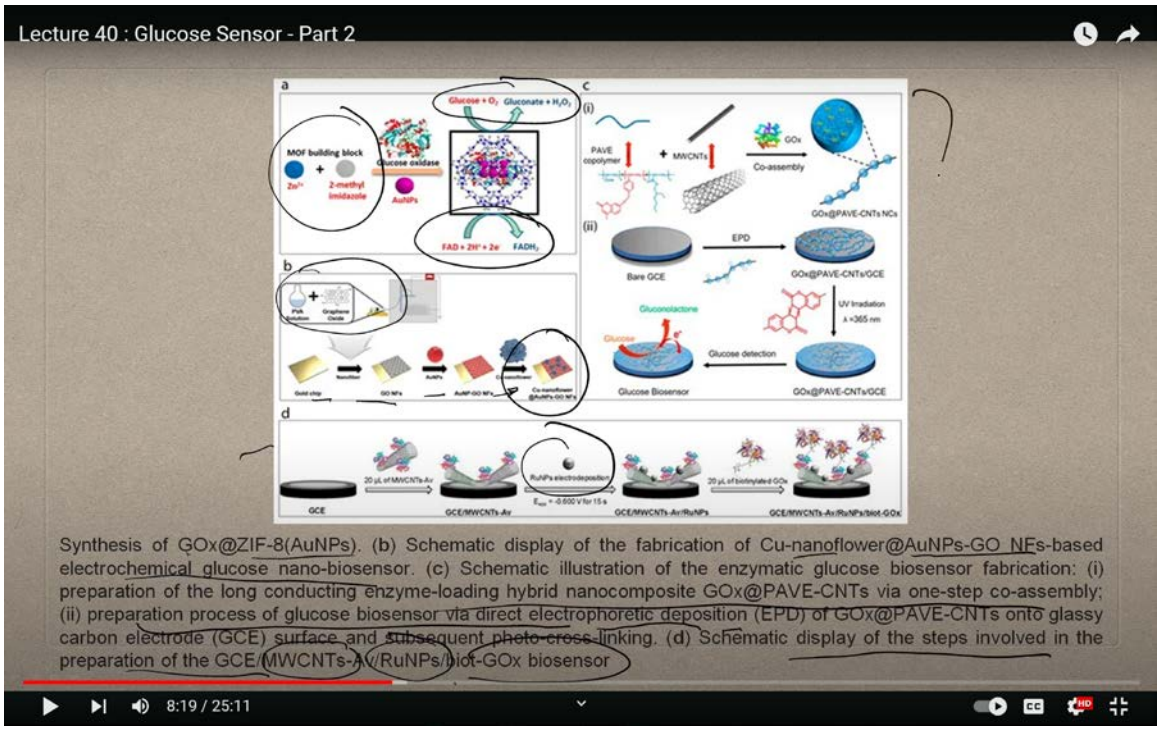

To this already impressive lineup of materials, carbon nanotubes, graphene, and carbon quantum dots, we now add zeolite. These materials are not only known for their excellent electrical conductivity but also for their increasing use in biosensor research. The trend you can observe is that advancements in biosensor technology are closely linked to developments in material science, engineering, and chemistry. Materials that offer superior electrical conductivity are particularly favored. We have seen the use of gold, silver, and eventually, we'll delve into copper, nanocarbon, zeolite, and molybdenum sulfide (MoS_2), with the added advantage of porosity. Porous structures provide the higher surface area needed for enzymatic assays, which allows for greater immobilization of glucose oxidase and, in turn, increased system sensitivity.

Now, let's move on to some schematics that illustrate how biosensor technology is evolving. First, we revisit the reaction site of FAD and see the synthesis of glucose oxidase within ZIF-8, using zinc and 2-methyl imidazole. There's also a schematic display of copper nanoflower-based glucose nanobiosensors, and we see an illustration of glucose biosensor fabrication. The immobilization process of glucose oxidase is shown in a step-by-step manner, eventually leading to the completion of the biosensor chip.

Section C of the schematic delves into the fabrication of enzymatic glucose biosensors. Part 1 details the creation of a long conductive enzyme-loading hybrid nanocomposite, while Part 2 discusses the preparation process of glucose biosensors through direct electrophoretic deposition onto a glassy carbon electrode surface, followed by photo-linking. In Section D, we explore the one-step fabrication process involving a glassy carbon electrode, multi-walled carbon nanotubes, ruthenium nanoparticles, and a GeoXBioSensor.

A recent development worth noting is the creation of a hydrogel chitosan-based glucose biosensor. Hydrogels made from chitosan are typically used in wound dressings, tissue engineering, and controlled-release systems. Chitin, a polymer found in the exoskeleton of insects, is broken down by the enzyme chitinase. Chitosan is derived from chitin through a process of controlled N-deacetylation. Despite being insoluble in water, chitosan becomes soluble in acidic solutions with a pH lower than 6.5 due to its amino groups. Most importantly, chitosan can form hydrogels, which makes it possible to produce thin hydrogel

films of chitosan on solid electrode surfaces. This unique property has opened up new avenues in biosensor development.

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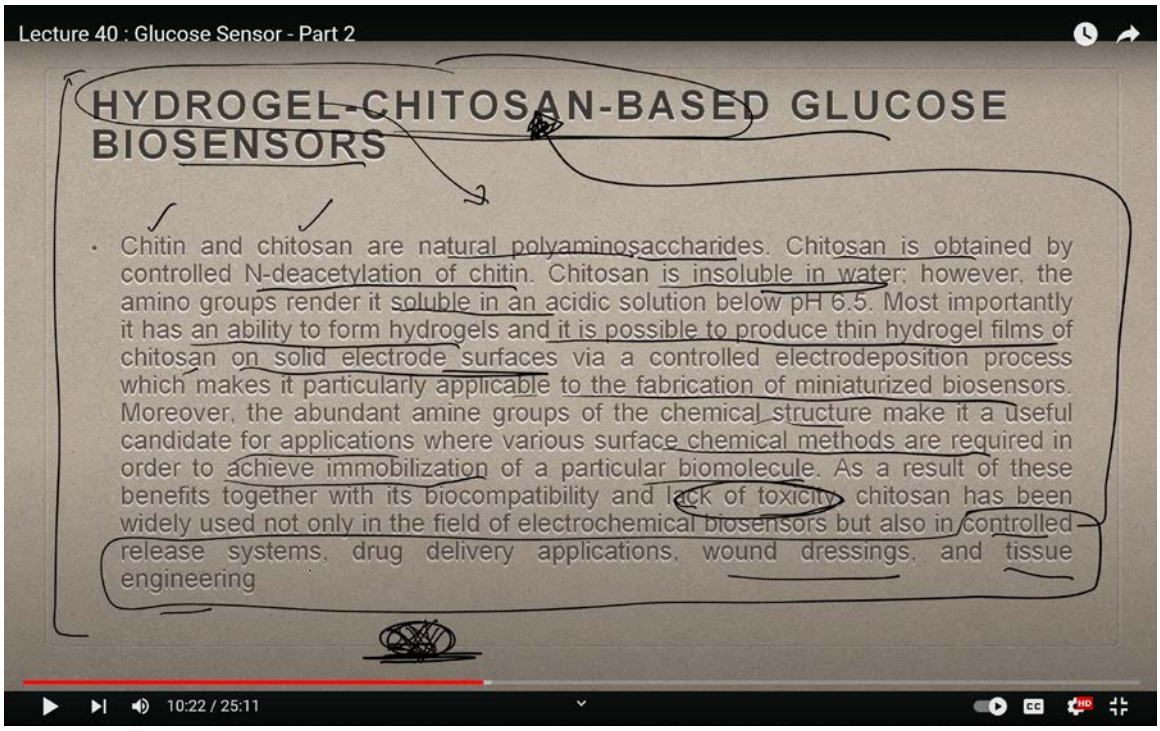

The electrode surface, coated with a hydrogel layer, plays a pivotal role in the development of miniaturized biosensors due to its highly porous structure and vast interconnected network. This porosity significantly enhances its surface area, making it ideal for biosensor fabrication. Additionally, the chemical structure of chitosan, with its abundant amine groups, allows for versatile surface modification techniques, enabling the immobilization of specific biomolecules. Chitosan's biocompatibility and non-toxic nature further add to its appeal, leading to widespread use not only in electrochemical biosensors but also in drug delivery systems, wound dressings, and tissue engineering.

One study worth mentioning is Krishnan's work, which utilized chitosan as a matrix for immobilizing the glucose oxidase enzyme. In this research, palladium nanocubes with an average edge length of 30 nm were synthesized, followed by the deposition of a platinum shell on their surface, forming a palladium-platinum complex. Glucose oxidase (GOX) was

covalently immobilized on the surface of these chitosan-coated nanocubes through a reaction with glutaraldehyde. The resulting GOX-immobilized nanocubes were then deposited onto a glassy carbon electrode surface. The sensor sensitivity was measured at 6.82 µA, marking a significant step forward in glucose sensing technology.

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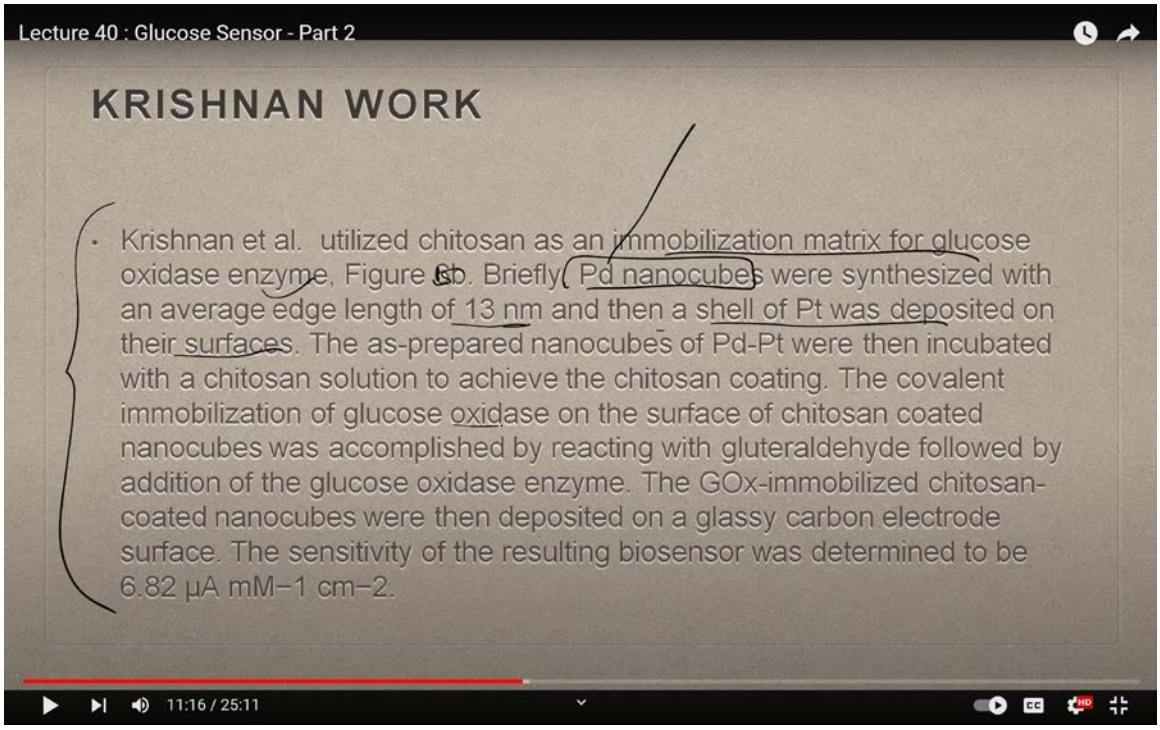

This process highlights some of the recent advancements in glucose detection, but it also underscores the ongoing challenges in the field. One of the primary hurdles involves the complex nature of the sample itself. Typically, a sample contains a multitude of compounds, and detecting one or two specific molecules, such as glucose, becomes difficult due to interference from other components. For example, in a urine sample, various ions, proteins, and other compounds may be present, making glucose detection particularly challenging.

The core challenge is ensuring that glucose reacts efficiently with oxygen in the presence of glucose oxidase, leading to the reduction of oxygen to peroxide and subsequent oxidation, which generates measurable electrons. For this reaction to take place, glucose

needs to reach the enzyme's active site, and oxygen must also be present. Even after successful binding, monitoring the electron transfer reactions occurring at the enzyme's binding site remains a critical obstacle.

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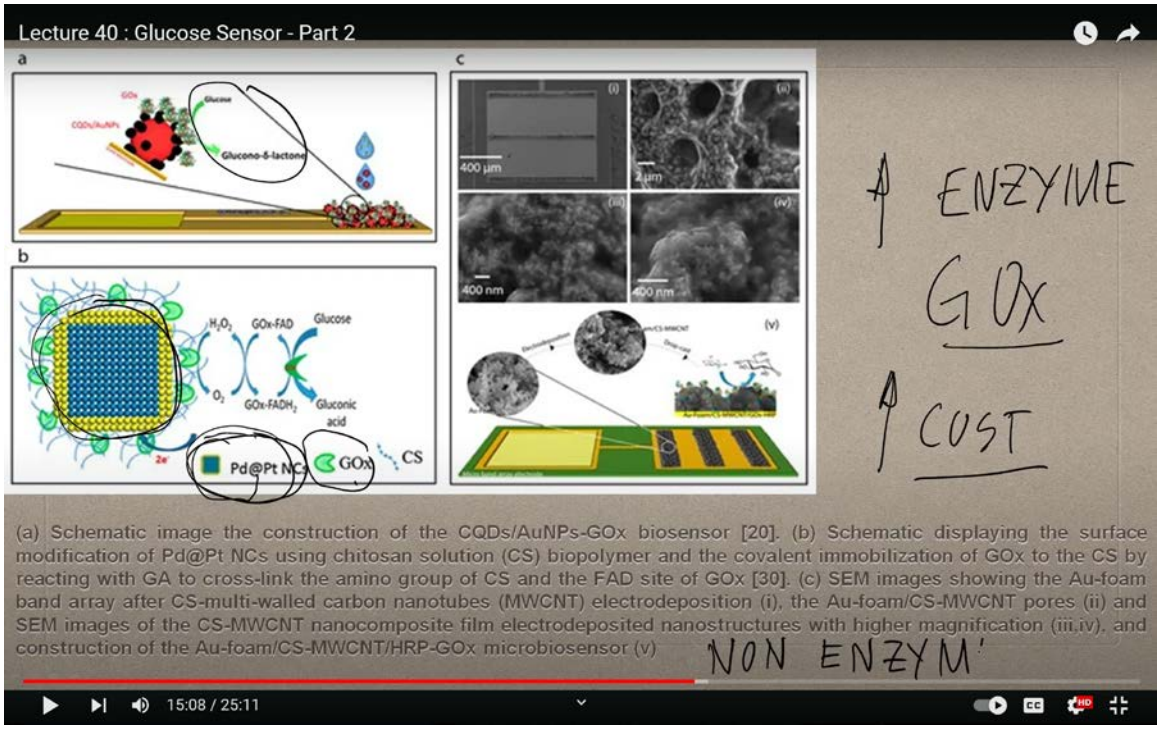

(a) Schematic image the construction of the CQDs/AuNPs-GOx biosensor [20]. (b) Schematic displaying the surface modification of Pd@Pt NCs using chitosan solution (CS) biopolymer and the covalent immobilization of GOx to the CS by reacting with GA to cross-link the amino group of CS and the FAD site of GOx [30]. (c) SEM images showing the Au-foam band array after CS-multi-walled carbon nanotubes (MWCNT) electrodeposition (i), the Au-foam/CS-MWCNT pores (ii) and SEM images of the CS-MWCNT nanocomposite film electrodeposited nanostructures with higher magnification (iii,iv), and construction of the Au-foam/CS-MWCNT/HRP-GOx microbiosensor (v)

Throughout our exploration of this field, we've encountered several strategies. These include creating apoenzymes, directly connecting the electron transfer site (such as FAD) to the electrode, using coupling molecules, or encapsulating the entire enzyme. However, one major issue persists: the need for large amounts of purified enzyme, such as glucose oxidase, drastically increases the cost of the system.

This leads us to the concept of non-enzymatic sensors. The basic philosophy behind non-enzymatic sensors is worth considering, especially when revisiting the fundamental reaction at the start of our journey. This reaction forms the basis for understanding the potential and uniqueness of non-enzymatic approaches to biosensing, where the reliance on enzymes like glucose oxidase can be reduced, thus lowering costs and simplifying the process.

If we understand this core concept, the rest will be straightforward. So, what exactly is happening in this reaction? Let’s break it down. When glucose reacts with oxygen in the presence of glucose oxidase, the products formed are gluconic acid and hydrogen peroxide. Essentially, in this reaction, oxygen is gaining electrons and undergoing reduction, while glucose is being oxidized to gluconic acid. This is a redox reaction, a reduction-oxidation process. In this context, the oxidation process liberates two electrons and two protons, and we measure these two electrons. That's the core mechanism of this biosensor.

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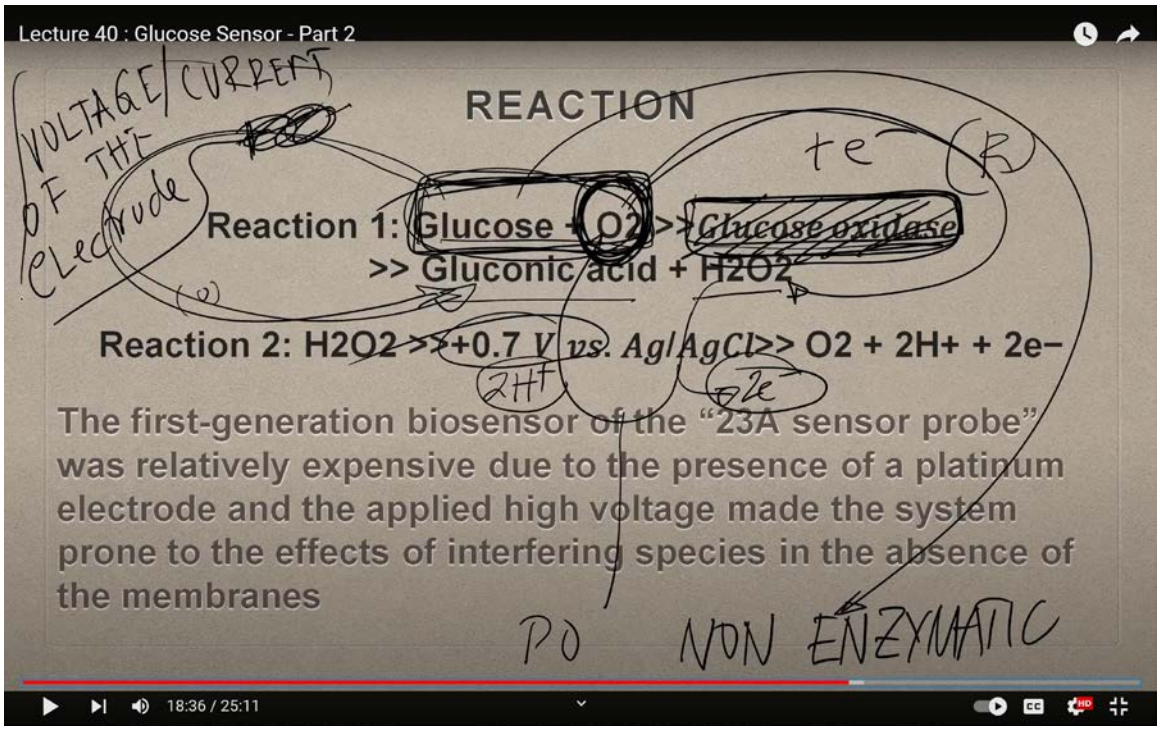

Additionally, the second part of the reaction occurs on the electrode, where we apply a specific potential, typically around 0.7 volts. Now, imagine removing glucose oxidase from this system. What happens then? Can glucose and oxygen still react without the enzyme? This scenario is the foundation of a non-enzymatic sensor. If we can bring the glucose and oxygen molecules close enough and create the right conditions, such as a reducing environment for oxygen, the reaction can still occur without glucose oxidase. This environment can be established by manipulating the voltage or electrical parameters of the electrode on which the sensor is built.

By altering the electrode's properties, either by donating electrons or withdrawing them, we can control the reaction. In non-enzymatic glucose detection, or direct glucose electro-oxidation, the process involves oxidizing glucose and reducing oxygen, leading to the formation of gluconic acid and peroxide. The challenge with glucose oxidase-based biosensors, which have been widely studied, is the potential loss of catalytic activity during the immobilization process. This affects both the performance and long-term stability of the sensor.

We've discussed several cutting-edge biosensors utilizing materials like molybdenum sulfide, gold, silver, copper, zinc, carbon nanotubes, and carbon quantum dots. While these materials offer significant advancements, their activity often diminishes when embedding molecules or enzymes into such structures. This decrease in activity lowers the detection efficiency, requiring more enzymes to maintain performance, which in turn increases costs. As enzyme activity declines, so does the sensitivity and efficiency of the sensor, which are directly correlated.

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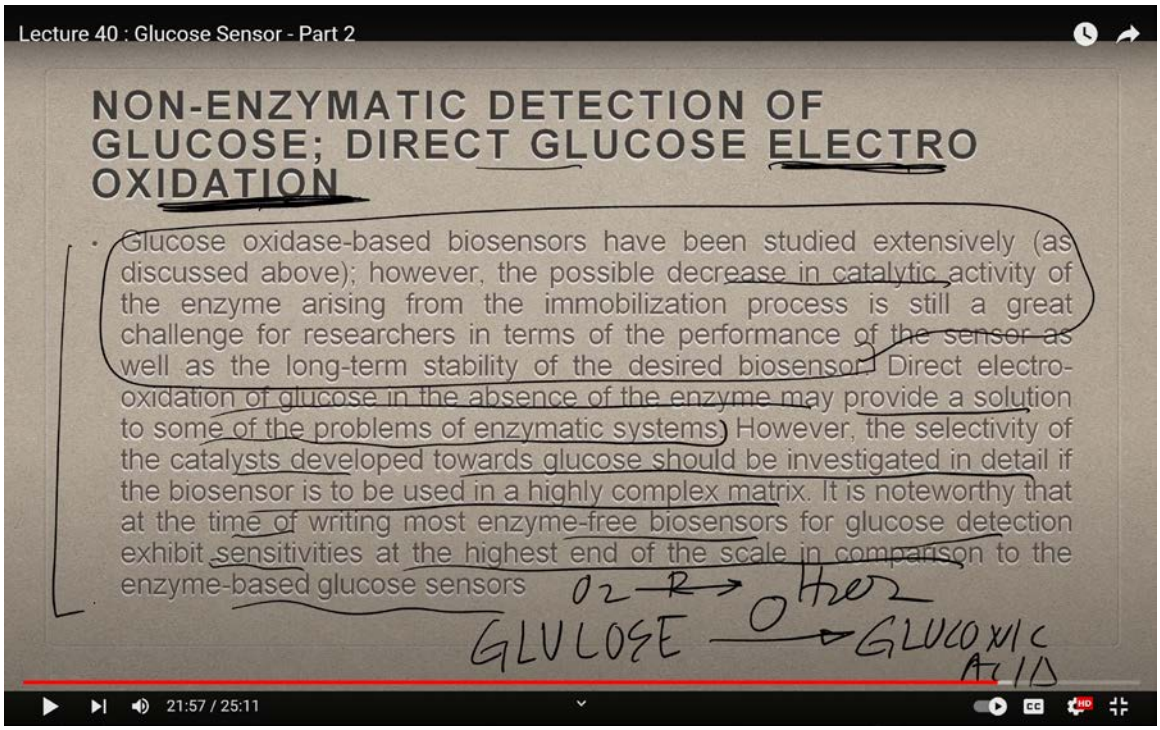

Direct electro-oxidation of glucose without the use of enzymes could address some of the challenges associated with enzymatic systems. This is where the fundamentals of non-enzymatic detection come into play. However, for these biosensors to function effectively in complex environments, the selectivity of the catalyst toward glucose needs to be rigorously investigated. At the time of writing, it is important to note that most enzyme-free biosensors for glucose detection are demonstrating sensitivities at the higher end of the spectrum compared to their enzyme-based counterparts. This is where the future of the field is heading, towards non-enzymatic detection. If we can develop a parallel technology that is enzyme-free, cost-effective, and matches the detection limits of enzymatic systems, it would be a significant breakthrough. The goal is to create a system that is error-free and highly efficient. This is where much of the current glucose sensing research is focused, on non-enzymatic methods. Instead of relying on glucose oxidase, we can use electro-oxidation or, in some cases, electro-reduction, depending on the approach. Essentially, the electrode does the work for us. Once this concept is understood, the rest of the electrochemistry behind these processes will become much clearer.

In the next session, we'll delve deeper into the electrochemical aspects of biosensors, exploring their journey in greater detail. We will cover biosensor fabrication, design, and the intricate chemistry between electrodes, voltammograms, amperometry, and the selection of appropriate electrodes. I must point out that over the past 60 to 70 years, many of the advancements in sensor technology have drawn inspiration from oxygen and glucose biosensing mechanisms. This is why I am emphasizing this area so heavily.

Materials like molybdenum sulfide, gold nanoparticles, carbon nanotubes, carbon quantum dots, graphene oxide layers, nitrogen-doped graphene oxide layers, boronic acid, and chitosan-based materials have all been critical in advancing sensor technology. These same materials have been adapted and employed in various other sensors as well. Once you thoroughly understand a few sensors, two, three, or four, the rest will become far easier to grasp. This is why we are focusing on the foundational principles, so you can extend your thinking beyond just these examples. That is why we are devoting time to helping you understand the basics so that you can think beyond this. So, with this, I will close the class. Thank you.