Design for Biosecurity

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Lecture 41

Non Enzymatic Glucose Sensor - Part 1

Welcome back to this week of our course. In the previous session, we delved deeply into the concept of glucose sensors. We focused on the fundamental reaction where glucose is converted into gluconolactone, and oxygen plays a crucial role in this process. In the presence of the enzyme glucose oxidase, this reaction leads to the formation of hydrogen peroxide. Hydrogen peroxide is then oxidized, and the key point here is that during this process, electrons are generated. It is these electrons that are evaluated, and many glucose sensors operate based on this very principle. So far, all the sensors we've discussed have been enzymatic glucose sensors, relying on the glucose oxidase enzyme.

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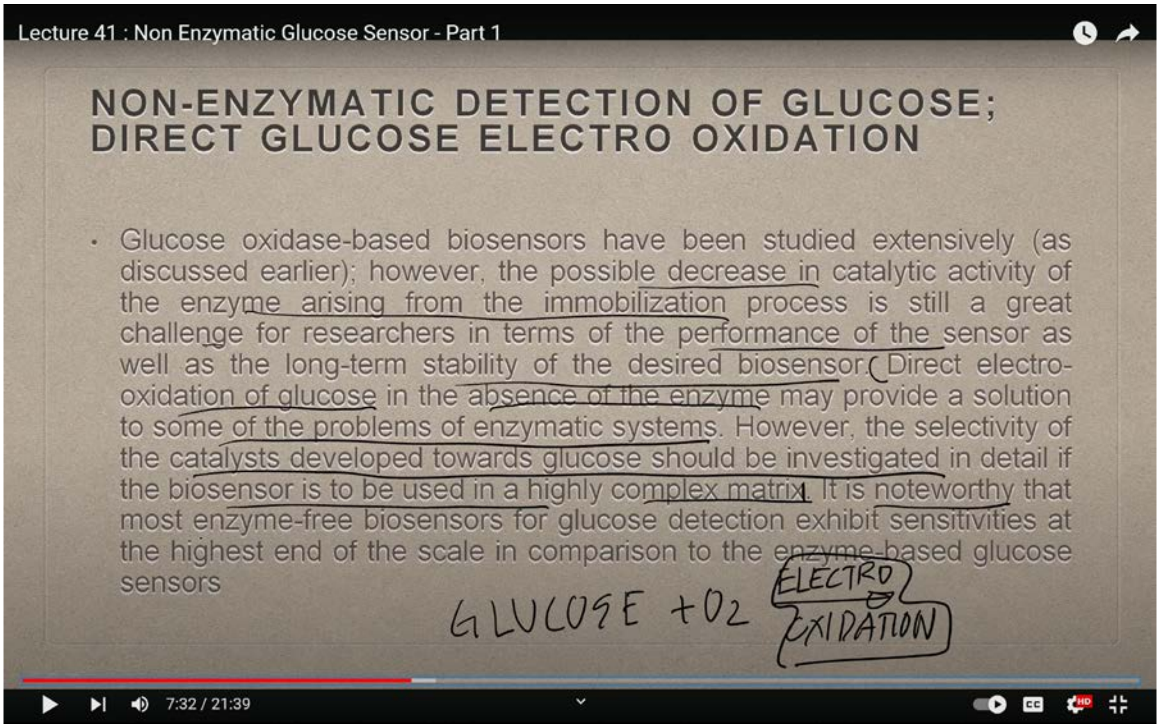

NON-ENZYMATIC DETECTION OF GLUCOSE;
DIRECT GLUCOSE ELECTRO OXIDATION

- Glucose oxidase-based biosensors have been studied extensively (as discussed earlier); however, the possible decrease in catalytic activity of the enzyme arising from the immobilization process is still a great challenge for researchers in terms of the performance of the sensor as well as the long-term stability of the desired biosensor. Direct electro-oxidation of glucose in the absence of the enzyme may provide a solution to some of the problems of enzymatic systems. However, the selectivity of the catalysts developed towards glucose should be investigated in detail if the biosensor is to be used in a highly complex matrix. It is noteworthy that most enzyme-free biosensors for glucose detection exhibit sensitivities at the highest end of the scale in comparison to the enzyme-based glucose sensors

GLUCOSE $+O_2$ ELECTRO OXIDATION

Now, let's take a closer look at the reaction itself: glucose reacts with oxygen to produce gluconolactone and hydrogen peroxide. Essentially, this is an oxidation reaction, where the glucose molecule is oxidized. The enzyme, glucose oxidase, is driving this oxidation process. But here’s an interesting thought, what if we remove the enzyme? Is it still possible to achieve this reaction? Theoretically, the answer is yes, as long as we can create an oxidizing environment.

To create an oxidizing environment, we need to understand what we mean by oxidation and reduction. Oxidation refers to the removal of electrons, while reduction involves the addition of electrons. Using the fundamental concepts of current electricity, we can manipulate these electron exchanges. By creating an excess of electrons, we induce reduction, whereas removing electrons from a surface initiates oxidation. This is the core principle behind the development of non-enzymatic glucose sensors, a technology that has evolved significantly over the past 20 to 30 years.

Today, non-enzymatic glucose sensors are at the forefront of glucose monitoring technology, particularly for diabetes management. These sensors are not only capable of detecting glucose in blood but also in other body fluids like interstitial fluid, sweat, and even tears. They are becoming integral to non-invasive, point-of-care devices, offering a more convenient and less intrusive method for glucose monitoring.

In our previous session, we also discussed some of the challenges with enzymatic glucose sensors. First, there is the need for a high-quality enzyme, and second, the reactants must come into very close proximity to the enzyme for the reaction to occur effectively.

When working with heterogeneous samples such as sweat, blood, urine, or tears, one of the key challenges is the presence of numerous interfering molecules. These molecules can act as barriers, making it difficult for glucose to reach its enzyme, glucose oxidase. Imagine the enzyme as a target, and these interfering molecules as obstacles preventing the glucose from reaching it. This creates significant hurdles for glucose detection, which is why scientists, developers, and technologists have been motivated to explore alternative approaches. Today’s focus is on non-enzymatic glucose sensors, an area that has gained substantial attention as a promising alternative.

Enzyme-based glucose sensors, particularly those relying on glucose oxidase, have been extensively studied. However, one of the key issues discussed previously is the reduction in the enzyme’s catalytic activity due to the immobilization process. This process, whether through chemical means or adsorption, involves trapping the enzyme in a specific location, like placing it in a pocket. When the enzyme is immobilized, it can no longer remain in its native, optimal state. The changes in its structure, even down to the bond angles at the angstrom level, inevitably affect its efficiency. While immobilization is one of the best techniques available, it still leads to slightly less efficient systems, which presents ongoing challenges for researchers, especially when it comes to the performance and long-term stability of enzymatic biosensors.

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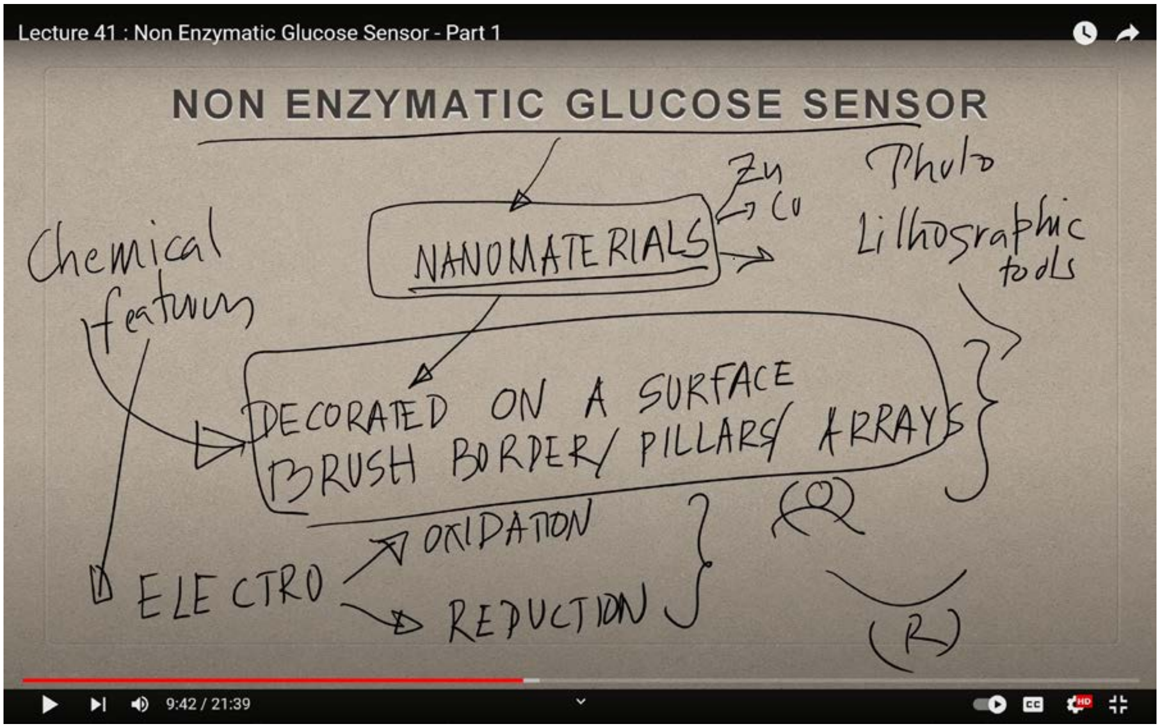

As we’ve previously discussed, glucose can be oxidized in two primary ways: either through enzymatic reactions or by direct electro-oxidation, which forms the basis of non-enzymatic glucose sensors. By eliminating the need for enzymes, some of the challenges associated with enzymatic systems may be resolved. However, developing a catalyst that is highly selective for glucose remains a critical issue when dealing with complex matrices like biological fluids.

Interestingly, many enzyme-free glucose biosensors have demonstrated sensitivity at the higher end of the scale compared to enzyme-based systems. This is one of the fundamental reasons why non-enzymatic glucose sensors are gaining traction. A key area of research in this field involves the use of nanomaterials, which play a pivotal role in enhancing sensor performance. These nanomaterials can be strategically decorated on surfaces in various forms, such as brush borders, pillars, or arrays, created using techniques like photolithography. The surfaces are designed with chemical properties to enable both electro-oxidation and electro-reduction, sometimes incorporating multiple layers to facilitate both processes.

A wide variety of nanomaterials, including zinc and cobalt, have been explored for these purposes. One notable case study involves vertically aligned zinc oxide (ZnO) nanorods grown on fluorine-doped tin oxide (FTO) electrodes. These zinc oxide nanorods, hydrothermally grown on the electrode surface, were then modified with copper oxide (CuO) nanostructures through a dip-coating and annealing process. To further optimize the sensor, the hybrid material was coated with Nafion, which helps reduce potential fouling and interference, while improving ion mobility and conductivity. This hybrid sensor exhibited a remarkable sensitivity of 2961.7 μA, demonstrating excellent reproducibility, repeatability, stability, and selectivity.

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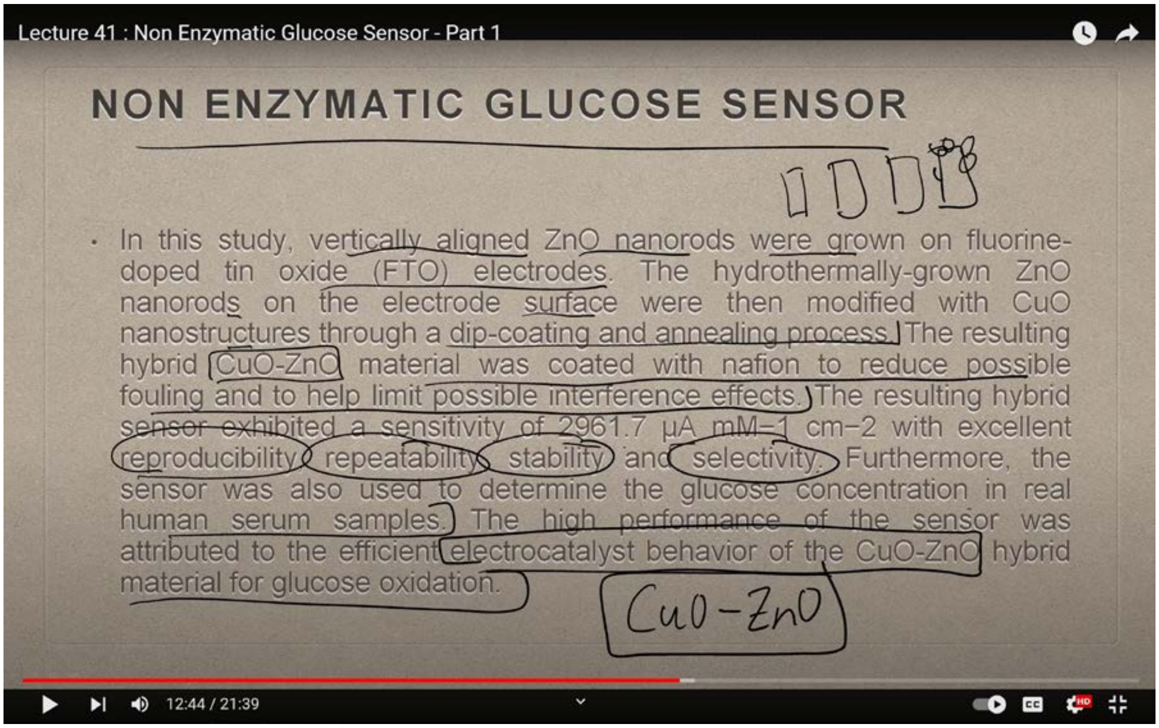

NON ENZYMATIC GLUCOSE SENSOR

- In this study, vertically aligned ZnO nanorods were grown on fluorine-doped tin oxide (FTO) electrodes. The hydrothermally-grown ZnO nanorods on the electrode surface were then modified with CuO nanostructures through a dip-coating and annealing process. The resulting hybrid CuO-ZnO material was coated with nafion to reduce possible fouling and to help limit possible interference effects. The resulting hybrid sensor exhibited a sensitivity of 2961.7 μA mM−1 cm−2 with excellent reproducibility, repeatability, stability and selectivity. Furthermore, the sensor was also used to determine the glucose concentration in real human serum samples. The high performance of the sensor was attributed to the efficient electrocatalyst behavior of the CuO-ZnO hybrid material for glucose oxidation.

CuO-ZnO

These are critical attributes, reproducibility, repeatability, stability, and selectivity, that are essential for any high-performing sensor. This hybrid sensor also successfully determined glucose concentrations in real human serum samples, with its outstanding performance attributed to the synergistic electrocatalytic behavior of the CuO and ZnO materials used for glucose oxidation. This combination of materials is currently being explored by numerous research groups and industries around the world as a promising approach for non-enzymatic glucose sensors.

In another example, a flexible, non-enzymatic glucose biosensor was recently developed using a laser-induced graphene electrode, modified with copper nanoparticles. In this case, the graphene base was used as a foundation, with copper nanoparticles layered on top to enhance sensor performance. This type of sensor design continues to push the boundaries of what is possible in glucose detection technologies.

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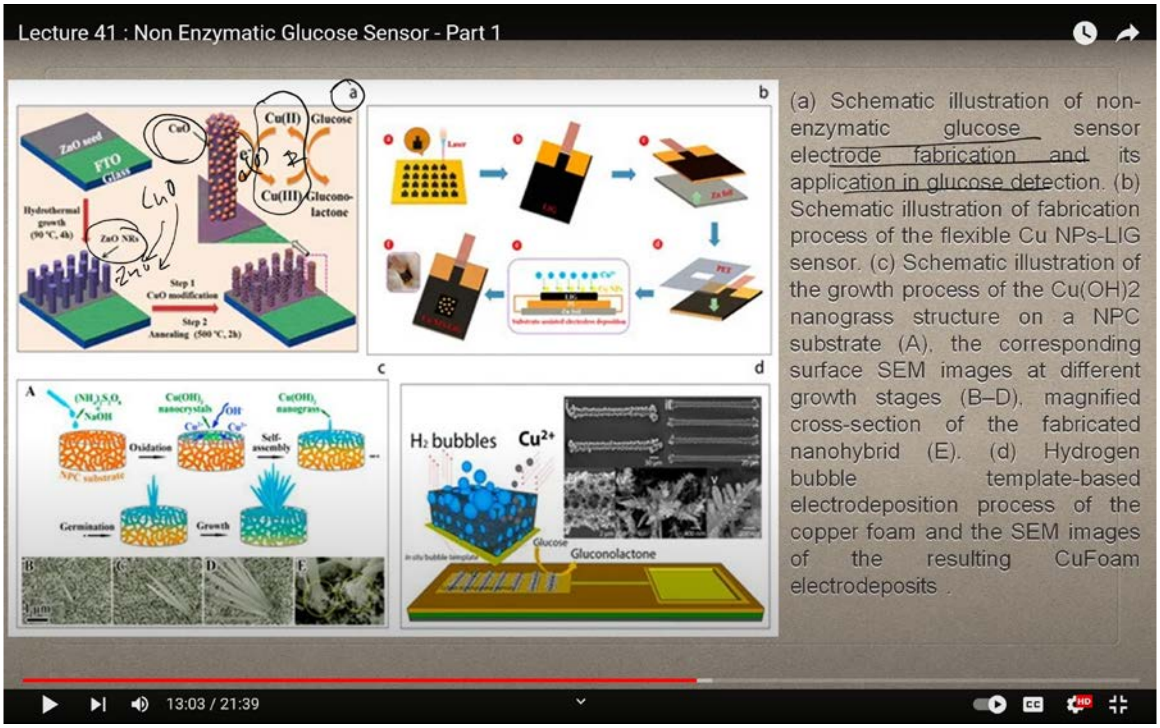

Lecture 41 : Non Enzymatic Glucose Sensor - Part 1

(a) Schematic illustration of non-enzymatic glucose sensor electrode fabrication and its application in glucose detection. (b) Schematic illustration of fabrication process of the flexible Cu NPs-LIG sensor. (c) Schematic illustration of the growth process of the Cu(OH)2 nanograss structure on a NPC substrate (A), the corresponding surface SEM images at different growth stages (B–D), magnified cross-section of the fabricated nanohybrid (E). (d) Hydrogen bubble template-based electrodeposition process of the copper foam and the SEM images of the resulting CuFoam electrodeposits .

The reason for using graphene, as most of you know, is due to its remarkable electrical and optical properties as a single or monolayer material. On top of these properties, graphene can be further enhanced by decorating it with copper nanoparticles. In this specific example, flexible graphene electrodes were prepared by subjecting a polyamide sample to laser irradiation, as shown in Figure B. The high-intensity laser radiation essentially depolymerizes the polyamide, leading to carbonization and ultimately graphitization. This process results in the formation of three-dimensional porous laser-induced graphene (LIG), which was subsequently modified with copper nanoparticles.

Earlier, we discussed the ZnO-CuO hybrid; now, we are focusing on copper nanoparticles and laser-induced graphene. This approach has shown great promise, with the resulting sensor demonstrating a glucose sensitivity of 495 μA. The authors of this study suggest that this straightforward fabrication method could be highly appealing for developing the next generation of flexible diagnostic devices. This brings us into the emerging field of flexible bioelectronics or biosensors, which is gaining significant traction worldwide. However, the requirement for high-intensity laser irradiation raises questions regarding cost-effectiveness since such high-end technologies naturally increase manufacturing costs. This, of course, leads to a compromise between performance and cost.

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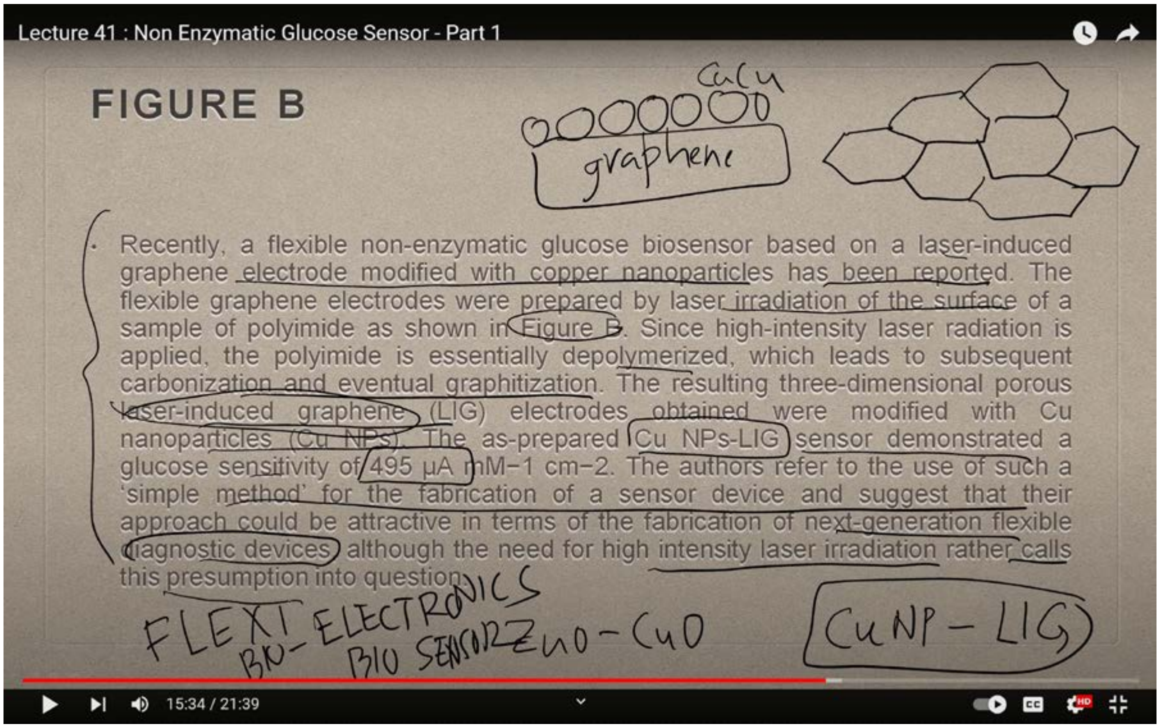

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FIGURE B

- Recently, a flexible non-enzymatic glucose biosensor based on a laser-induced graphene electrode modified with copper nanoparticles has been reported. The flexible graphene electrodes were prepared by laser irradiation of the surface of a sample of polyimide as shown in Figure B. Since high-intensity laser radiation is applied, the polyimide is essentially depolymerized, which leads to subsequent carbonization and eventual graphitization. The resulting three-dimensional porous laser-induced graphene (LIG) electrodes obtained were modified with Cu nanoparticles (Cu NPs). The as-prepared Cu NPs-LIG sensor demonstrated a glucose sensitivity of 495 μA mM−1 cm−2. The authors refer to the use of such a 'simple method' for the fabrication of a sensor device and suggest that their approach could be attractive in terms of the fabrication of next-generation flexible diagnostic devices although the need for high intensity laser irradiation rather calls this presumption into question.

Let's break down the process: laser irradiation creates the surface, followed by doping that surface with copper, which sets the stage for the reaction. This is essentially the schematic for copper nanoparticle and laser-induced graphene sensors. Now, moving on to the third sensor: a novel hybrid non-enzymatic glucose sensor composed of free-standing copper hydroxide nanograss.

We started with the CuO-ZnO hybrid, then progressed to copper nanoparticles and laser-induced graphene, and now, we are discussing free-standing copper hydroxide nanograss. As mentioned earlier, there are various ways to arrange nanomaterials, like brush borders, pillars, arrays, dots, and even "grass." These terms refer to how the nanomaterials are arranged or "decorated" on the surface of the electrode or electrocatalyst, with the goal of maximizing surface contact.

The key idea behind these arrays is to increase the surface area available for the reactants to interact with, as the electrode lies beneath. The larger the surface area, the higher the chances of successful detection; conversely, the smaller the surface area, the lower the detection efficiency. Therefore, the efficiency of these sensors is directly correlated with the extent of contact between the reactant and the surface where the reaction occurs. In this case, we are dealing with a non-enzymatic electrocatalyst surface, whether it's a hybrid of copper oxide and zinc oxide, a copper oxide laser-induced graphene substrate, or, in this example, a copper hydroxide nanograss standing surface.

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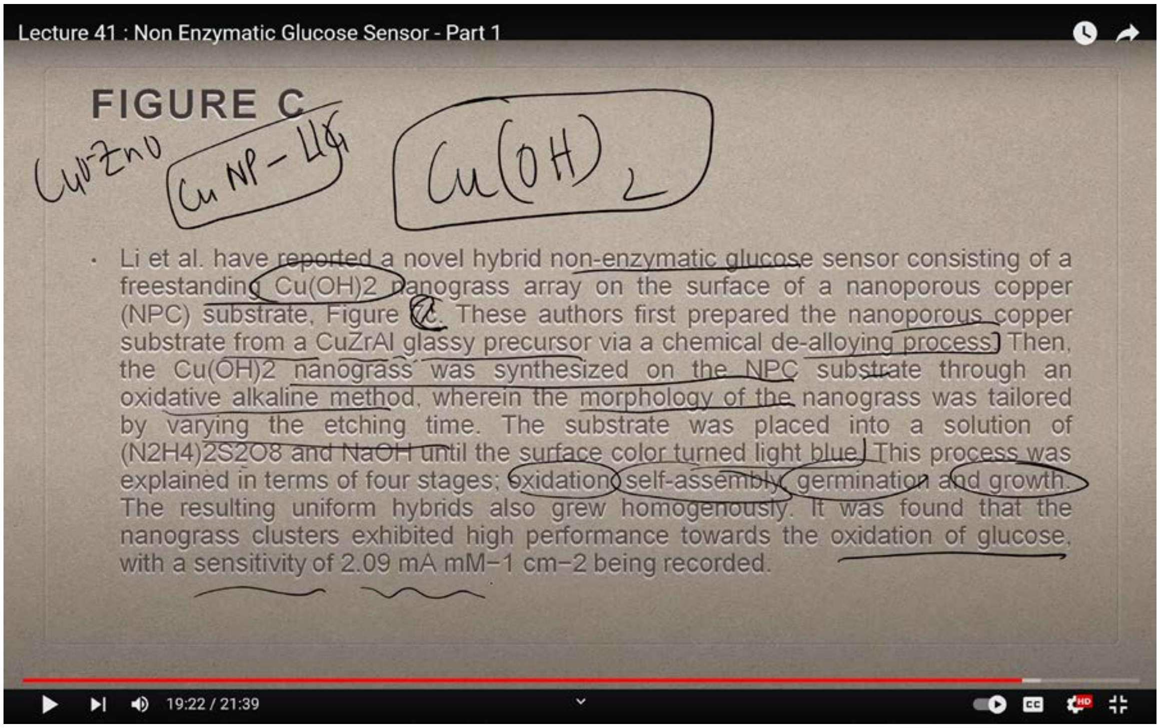

FIGURE C

- Li et al. have reported a novel hybrid non-enzymatic glucose sensor consisting of a freestanding Cu(OH)2 nanograss array on the surface of a nanoporous copper (NPC) substrate, Figure C. These authors first prepared the nanoporous copper substrate from a CuZrAl glassy precursor via a chemical de-alloying process. Then, the Cu(OH)2 nanograss was synthesized on the NPC substrate through an oxidative alkaline method, wherein the morphology of the nanograss was tailored by varying the etching time. The substrate was placed into a solution of (N2H4)2S2O8 and NaOH until the surface color turned light blue. This process was explained in terms of four stages; oxidation, self-assembly, germination and growth. The resulting uniform hybrids also grew homogenously. It was found that the nanograss clusters exhibited high performance towards the oxidation of glucose, with a sensitivity of 2.09 mA mM−1 cm−2 being recorded.

Figure C provides further insights into this process. The researchers first prepared a nanoporous copper substrate (NPC) from a glassy precursor composed of copper, zirconium, and aluminum via a chemical de-alloying process. Subsequently, copper hydroxide nanograss was synthesized on the NPC substrate using an oxidative alkaline method. The morphology of the nanograss was carefully tailored by adjusting the etching time. The substrate was immersed in a solution containing $H_4S_2O_8$ and NaOH until the surface turned light blue. This process unfolded in four key stages: oxidation, self-assembly, germination, and growth.

The resulting uniform hybrid structure grew homogeneously, and the nanograss clusters exhibited excellent performance in glucose oxidation, with a recorded sensitivity of 2.09 mA, the highest value achieved so far. If you examine the entire process, it begins with the initial reaction set occurring on the NPC substrate. Following that, copper hydroxide nanocrystals form, which then undergo a self-assembly process that leads to the emergence of "nanograss." This growth phase represents the final stage, where the reaction takes place on this highly efficient substrate.

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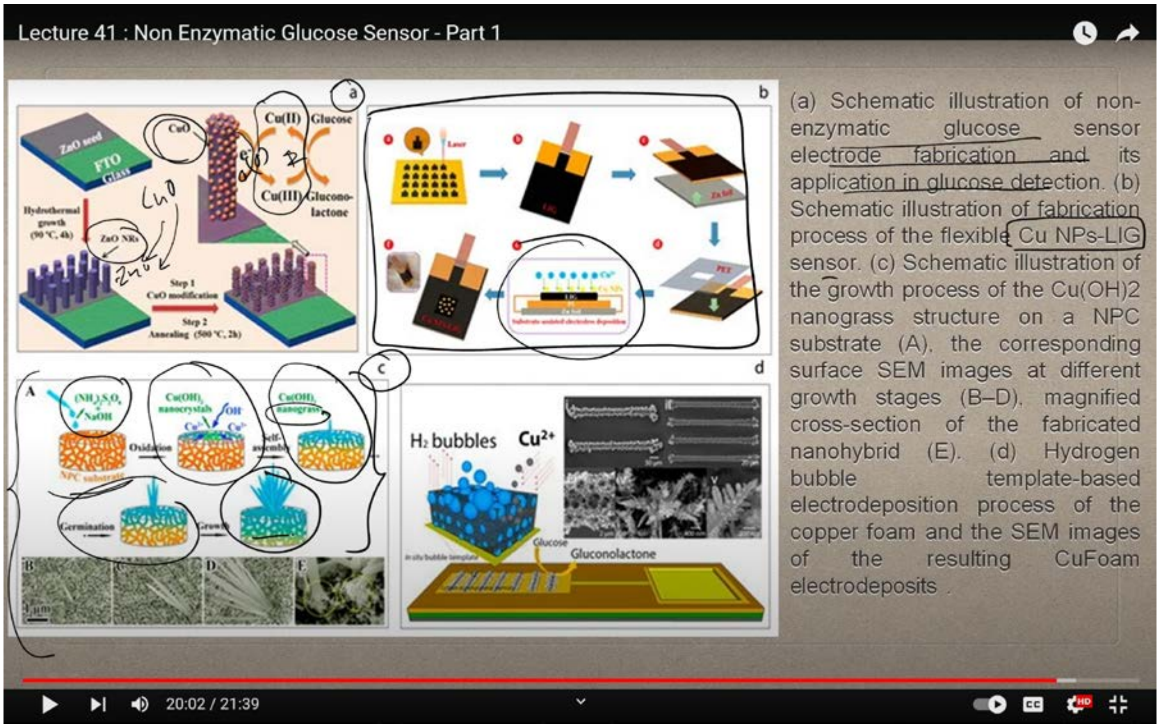

(a) Schematic illustration of non-enzymatic glucose sensor electrode fabrication and its application in glucose detection. (b) Schematic illustration of fabrication process of the flexible Cu NPs-LIG sensor. (c) Schematic illustration of the growth process of the $Cu(OH)_2$ nanograss structure on a NPC substrate (A), the corresponding surface SEM images at different growth stages (B–D), magnified cross-section of the fabricated nanohybrid (E). (d) Hydrogen bubble template-based electrodeposition process of the copper foam and the SEM images of the resulting CuFoam electrodeposits .

These advancements highlight the cutting-edge developments in nanomaterial engineering and biosensors. We've moved from traditional enzymatic assays to non-enzymatic detection methods, which represent a significant shift in this field. As we conclude today’s session, we'll explore another example in our next class and then summarize the different detection tools we’ve discussed. After that, we'll delve deeper into the intricacies of electrochemical sensors. Thank you.