Design for Biosecurity

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Lecture 30

Developing Biosensor for Botulinum Toxin

Welcome back to the fifth class of the sixth week, our final class for this week. Today, we will be concluding this segment by exploring a few more biosensors developed for the detection of botulinum toxin. In the previous class, we delved into electroactive sensors, and we've organized this topic into three main aspects.

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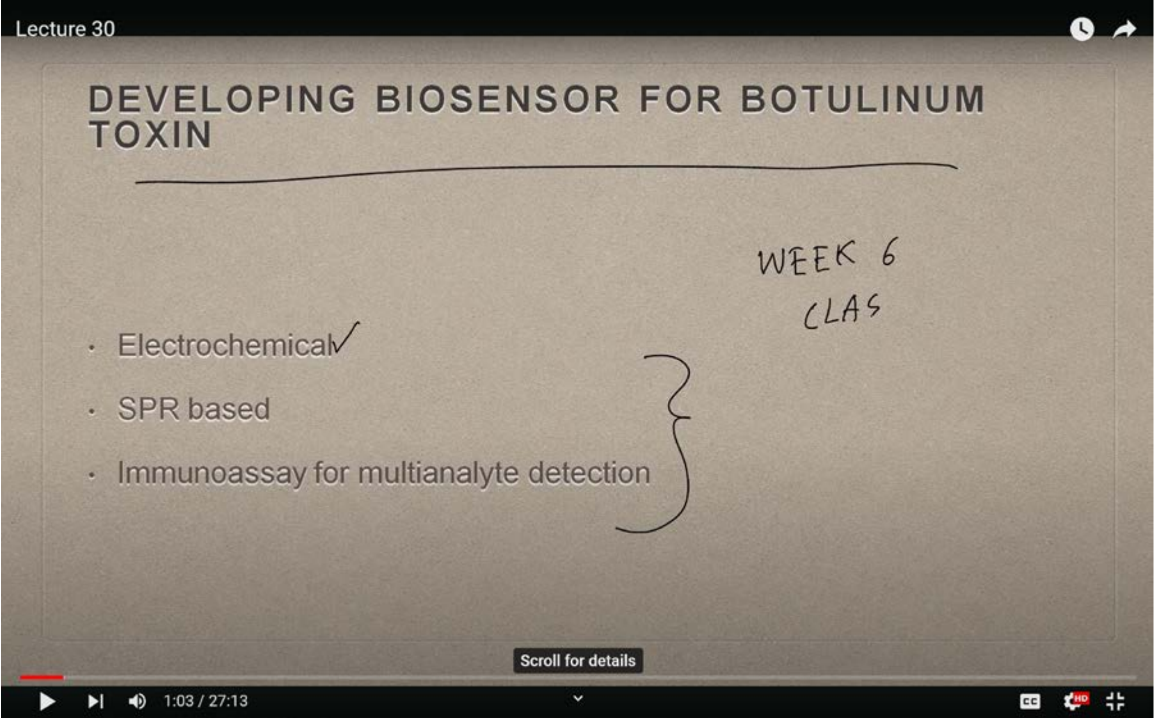

We've already covered electrochemical sensors, and today, we'll focus on surface plasmon resonance (SPR)-based sensors, as well as immunoassays designed for multi-analyte detection. So, to clarify, this is Week 6, Class 5, and the title of today's lecture is "Optical Biosensor Assay for Rapid Dual Detection of Botulinum Toxins A and E."

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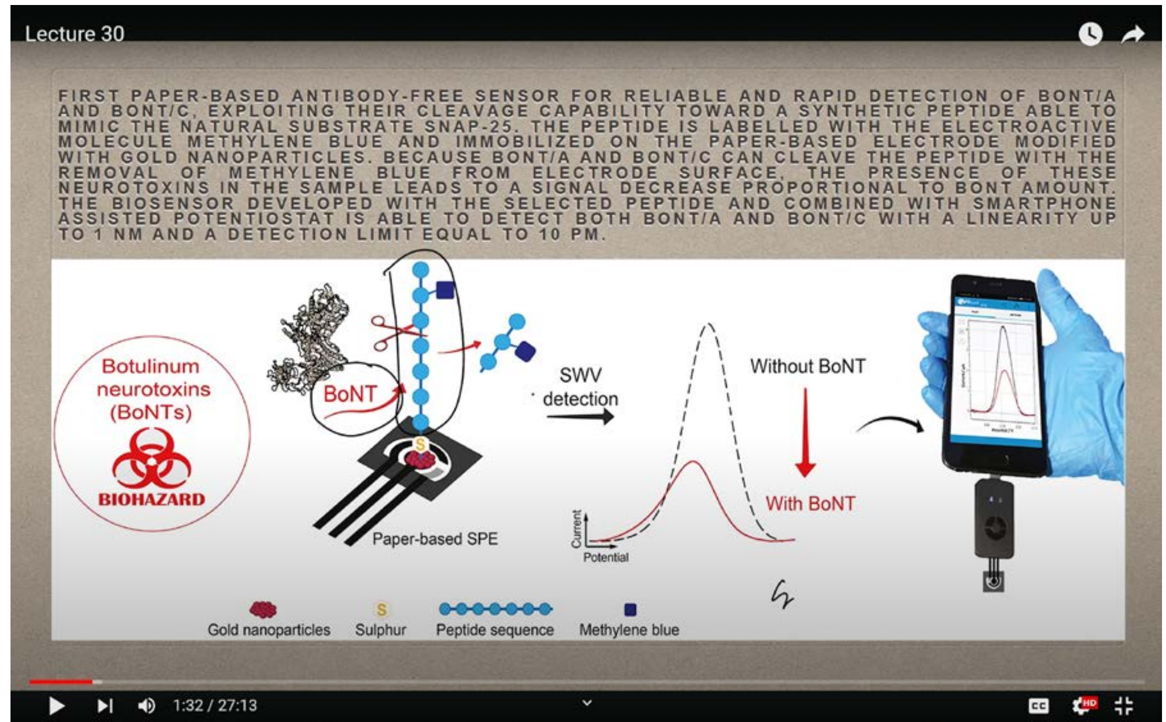

To quickly recap what we discussed last time: we talked about how botulinum toxin cleaves a peptide that is derived from the SNAP-25 protein. Just to refresh your memory, SNAP-25 is the protein to which the neurotoxin binds, ultimately cleaving it. The peptide in question is developed based on this cleavage event. As the neurotoxin performs its function, the electrical signal measured in the assay decreases, which is how we confirm the activity of the botulinum toxin.

Today, I'll also briefly revisit the SPR (Surface Plasmon Resonance) technique since it's central to our discussion. For those who may need a refresher, SPR captures any interactions that occur on a sensor surface. At a specific incident or resonance angle, plasmons are excited and resonate with the light, leading to the absorption of light at that particular angle. This results in the appearance of a dark line in the reflected light beam, a feature illustrated in the figure we've shown before. The dark line represents the excitation of surface plasmons, and its angular position shifts as molecules bind to the sensor surface.

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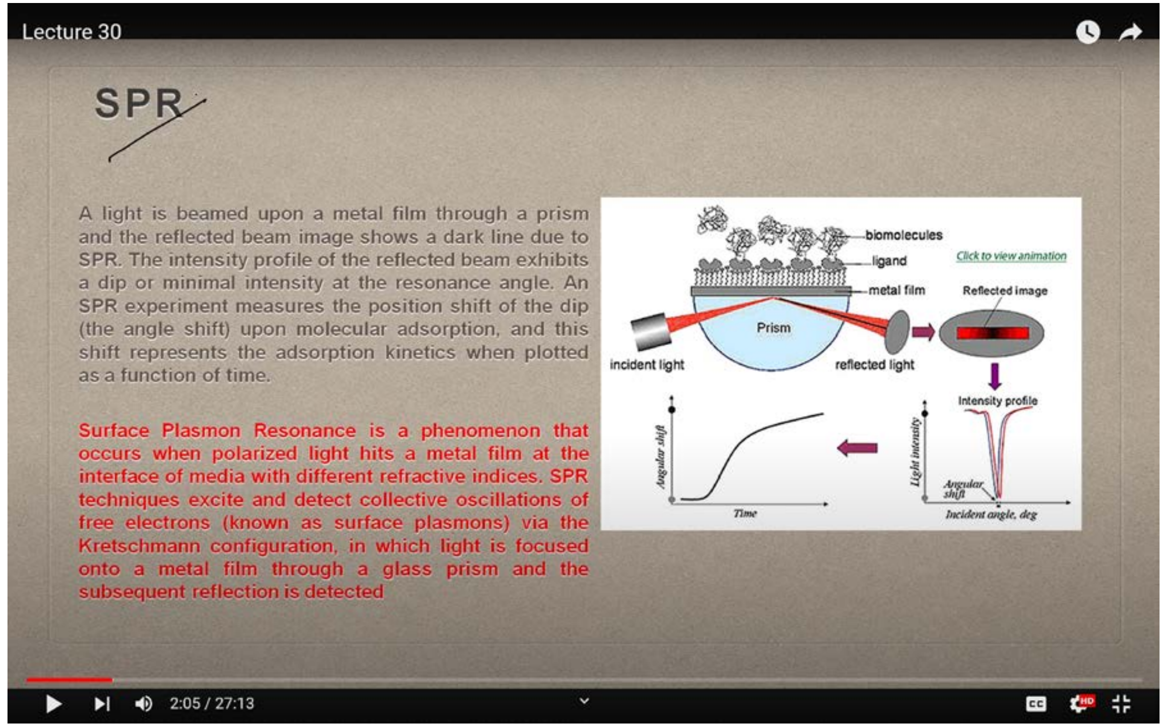

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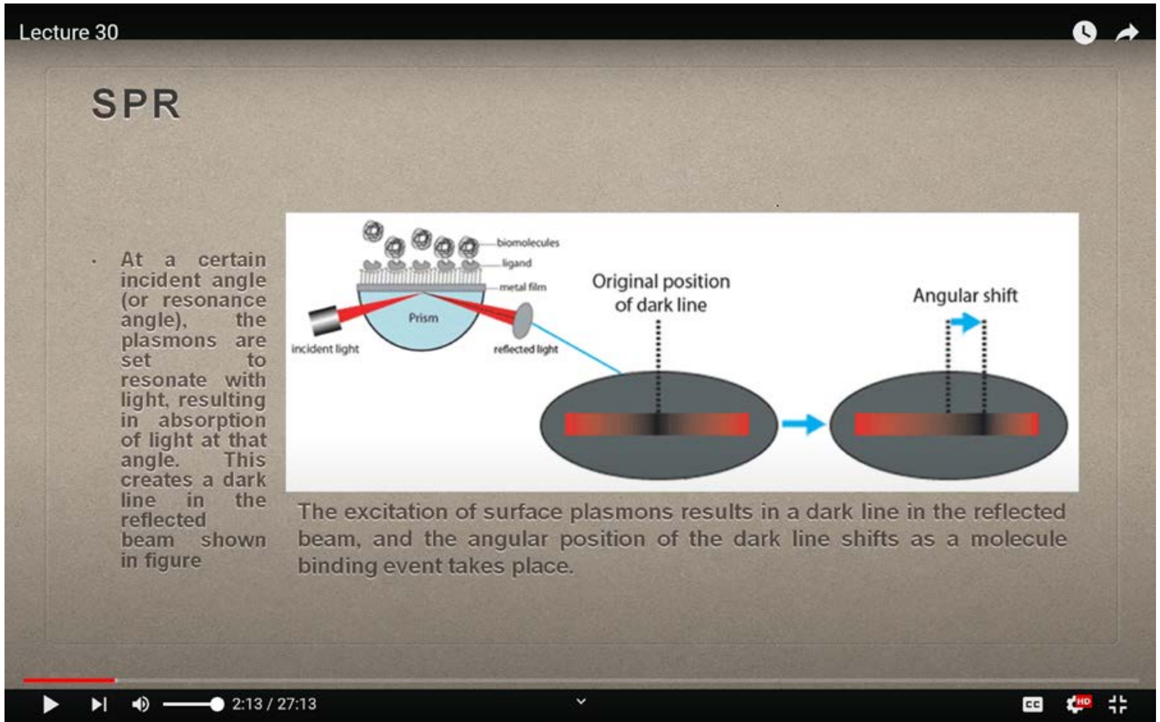

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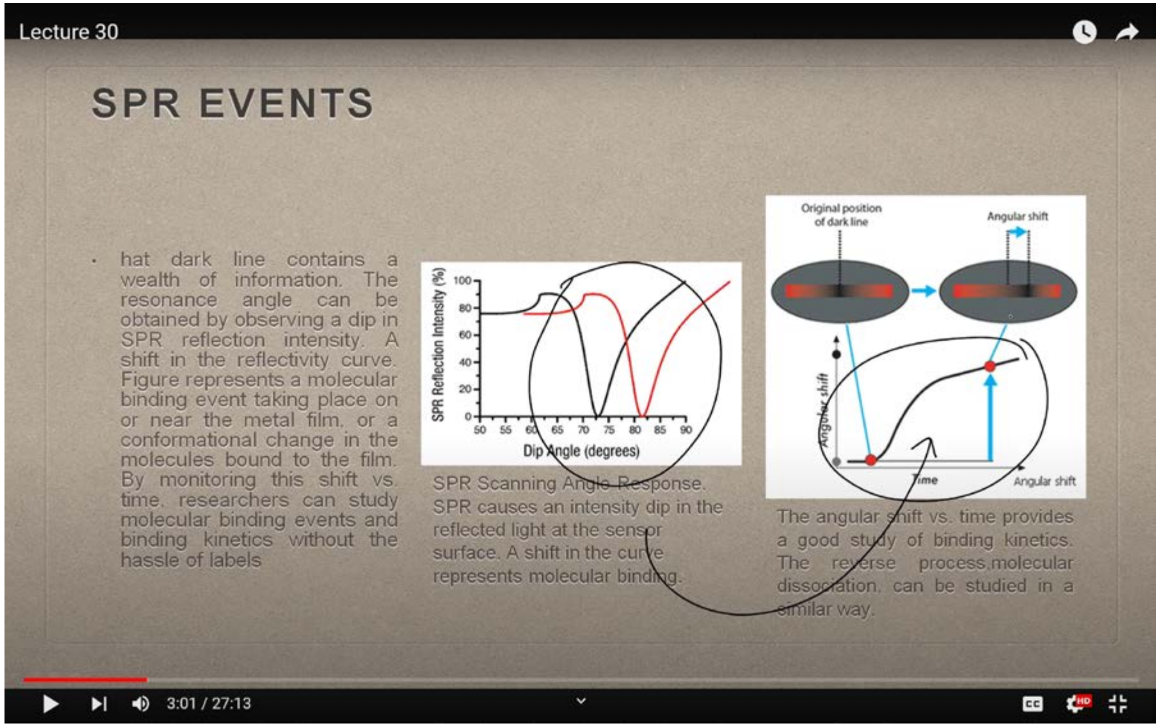

Any type of molecular binding assay will cause a shift in this dark line. For example, a shift may look like this (refer to the figure). These shifts can occur in either direction, depending on the molecular events taking place. The angular shift provides quantitative information about these molecular interactions. Now, today, you will see how this fascinating technology is applied to the detection of botulinum toxin.

Let's go over a brief recap to clarify the concepts further. Here, we are dealing with the interaction between botulinum toxin and the SNAP-25 protein. What happens is that when botulinum toxin types A and E come into contact with the SNAP-25 protein, this interaction leads to the cleavage of the protein, generating new molecular fragments. These newly created fragments are detected by an SPR (Surface Plasmon Resonance) signal.

Now, let's dive into the process more closely. We begin with the C-terminal sequence of SNAP-25. This is the region of the protein we’re discussing, the C-terminal. The arrows you see here represent where different isoforms of botulinum toxin cleave the SNAP-25 protein. For example, botulinum toxin type C cleaves the protein at this specific location, while type A cleaves it here, and type E cleaves it at this position.

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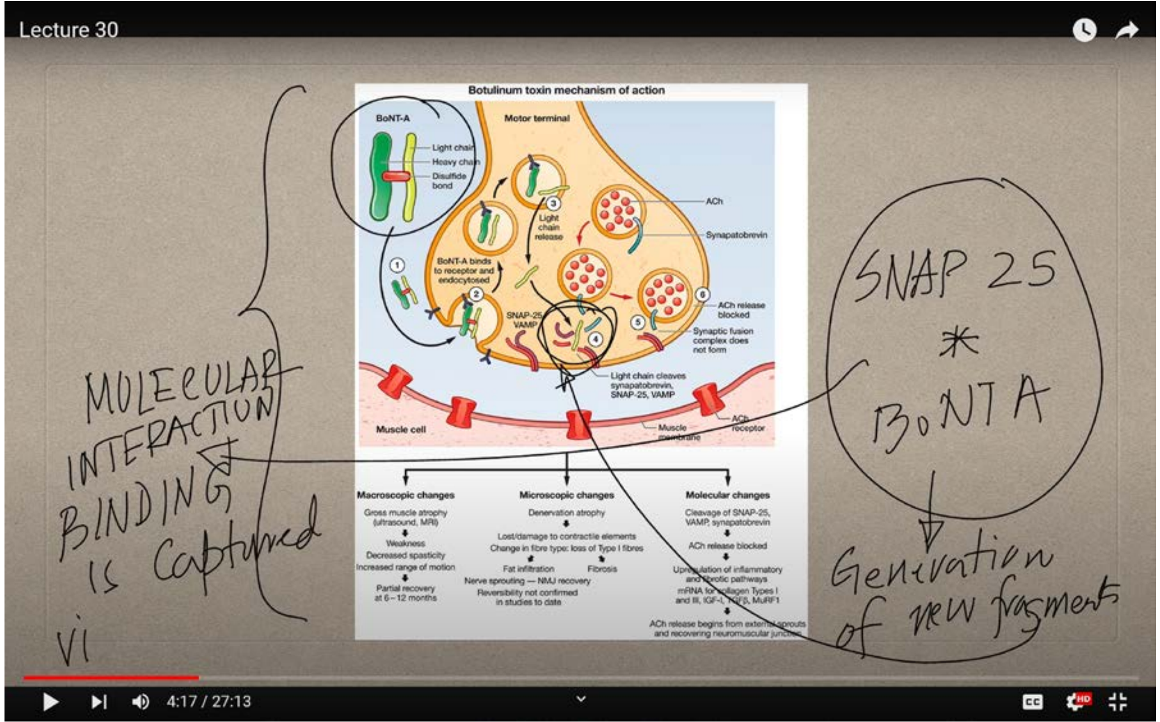

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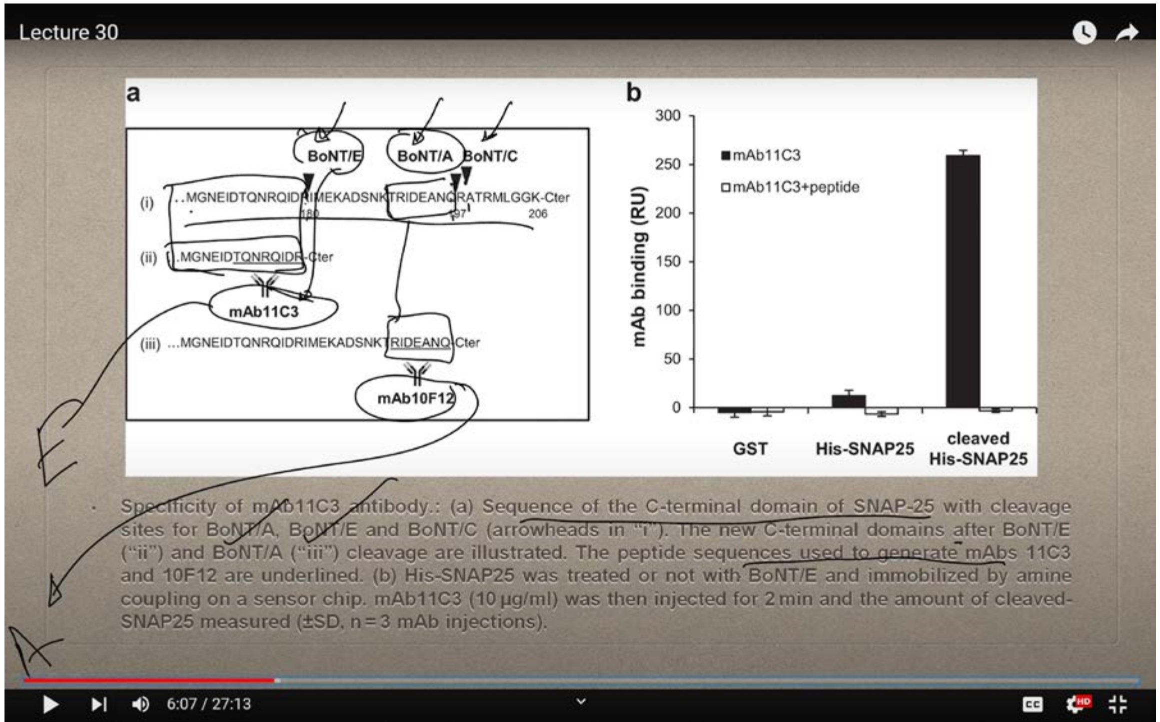

Now, focusing on the segment that gets cleaved, this is the part where antibodies are developed. For botulinum toxin type E, a specific monoclonal antibody (MAB) is created to bind to the cleaved fragment. Similarly, for botulinum toxin type A, another monoclonal antibody is generated to target that specific cleavage site. However, for botulinum toxin type C, no antibody is shown in this instance. The new C-terminal domains produced by the cleavage of botulinum toxin types A and E are illustrated here, with the peptide sequences used to generate the monoclonal antibodies, such as MAB 11C3 for type E and a different monoclonal antibody for type A.

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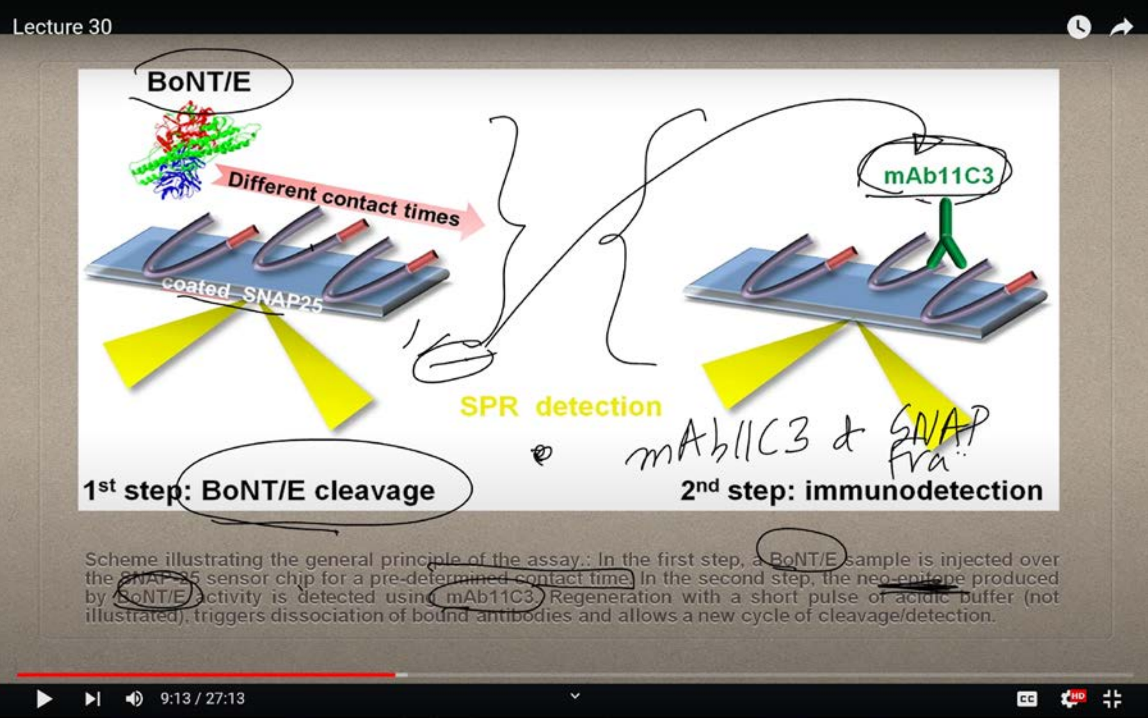

With these monoclonal antibodies now available, they can be used to bind to the toxin fragments. To better explain the logic behind this, imagine replacing the botulinum toxin fragment with monoclonal antibodies that are specific to different toxin types, A, E, or C, for instance. This binding interaction is then detected on an SPR chip. Remember, in SPR, these assays are label-free, meaning there is no need for additional labeling because the interaction between the antibody and the toxin fragment is what is being measured and quantified directly.

Now, moving forward, let's discuss how this all comes together. Here we have monoclonal antibody MAB 11C3, which is specific to botulinum toxin type E, binding to the cleaved peptide fragment. This interaction is visualized as the cleavage points of the peptide become clear. When the SNAP-25 protein is coated onto the sensor chip and allowed to interact with the antibody, the toxin will cleave the protein at specific residues, in this case, at the 180th residue.

To summarize the assay's general principle: in the first step, a sample containing botulinum toxin type E is injected over a SNAP-25 sensor chip and is allowed to interact for a predetermined contact time, this timing is critical. In the second step, the newly produced epitope resulting from the toxin's activity is detected using the monoclonal antibody MAB 11C3, which binds to the cleaved fragment, providing us with a measurable response.

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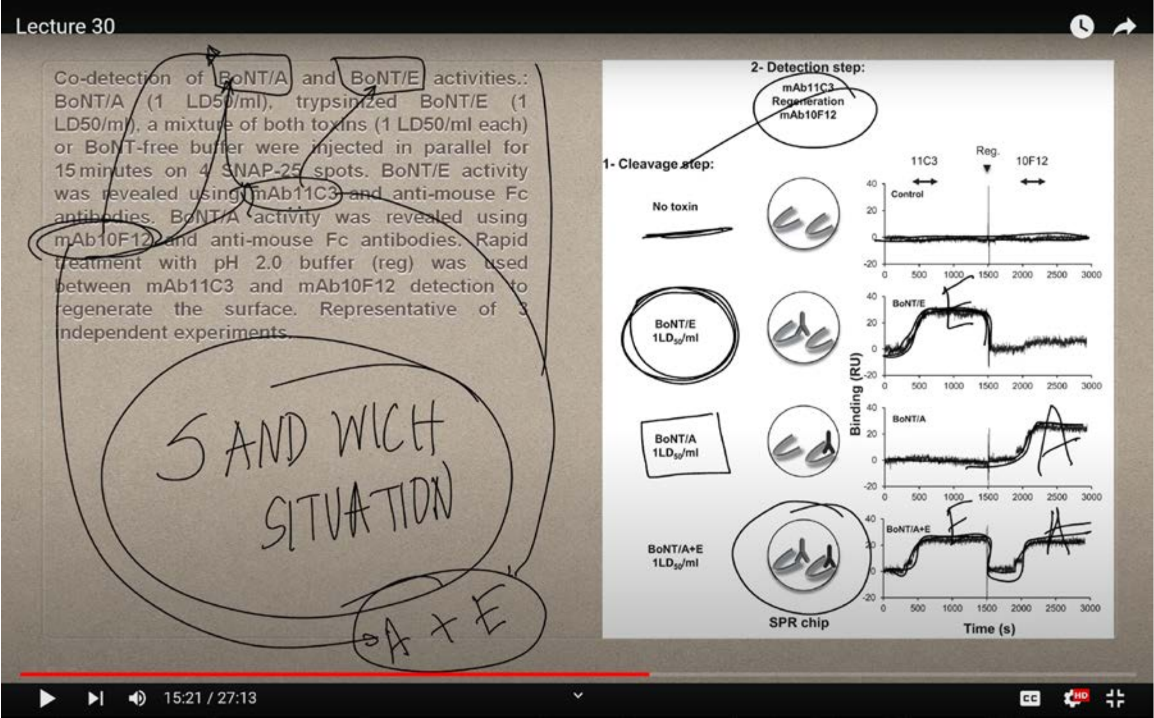

What's happening here is that the botulinum toxins are cleaving SNAP-25 into fragments. These fragments are specific to different isoforms of botulinum toxin, and antibodies have been raised against them. For instance, one such antibody, MAB 11C3, is designed to bind

specifically to the fragment generated by botulinum toxin E. This binding interaction between MAB 11C3 and the SNAP fragment is what you will observe during the SPR cycle.

Let’s break this down further. Each isoform of botulinum toxin cleaves the SNAP-25 protein at distinct points, as shown in the diagram. For example, botulinum toxin A cleaves at one site, botulinum toxin E at another, and so on. This specificity means that each antibody, like MAB 11C3 for botulinum toxin E, will only bind to the fragment that results from the cleavage by that specific isoform. Other antibodies, such as MAB 10F12, are developed for botulinum toxin A and will only bind to the corresponding fragment generated by toxin A. This precise matching of antibody to fragment is what drives the detection process in SPR.

Now, let's look at how this assay works. Initially, with no toxin present, you have a baseline signal. Once you introduce the toxin, botulinum toxin E, for instance, you start to observe molecular interactions, and the signal begins to register. If you also introduce botulinum toxin A, a signal will appear at a different point, indicating a dual detection. This means you need both antibodies: MAB 11C3 for toxin E and MAB 10F12 for toxin A. This setup allows for the simultaneous detection of both toxins within the same assay, a concept known as a sandwich assay.

Here’s how this works on a larger scale: In synaptic vesicles, the SNAP-25 protein is cleaved by different isoforms of botulinum toxin, including A, E, C, B, and others. Each isoform cleaves the protein at a unique site, producing distinct peptide fragments. For example, botulinum toxin A will cut SNAP-25 at a specific site, producing a fragment that will then be used to generate the monoclonal antibody MAB 10F12. Likewise, the fragment produced by botulinum toxin E will be used to develop MAB 11C3. In this sandwich assay, if your sample contains both toxin A and toxin E, you need both antibodies, 10F12 for A and 11C3 for E, on your SPR chip.

During the assay, the first signal you see corresponds to botulinum toxin E, detected by MAB 11C3. The second signal corresponds to botulinum toxin A, detected by MAB 10F12. These distinct signals represent the binding events of the respective antibodies to

their cleaved peptide fragments. In the SPR chip, you will observe two shifts in the signal, one for E and one for A, depending on which botulinum toxin is present in the sample.

Now, as we transition to a real-world application, this principle remains consistent. Without the need for any labeling, you can detect different fragments of SNAP-25 using monoclonal antibodies specifically raised against the cleaved peptides. In essence, this enables you to simultaneously detect both botulinum toxin A and E on a single SPR chip, because two molecular binding events are occurring: the fragment cleaved by botulinum toxin E binds to MAB 11C3, and the fragment cleaved by botulinum toxin A binds to MAB 10F12.

Thus, the entire assay revolves around the interaction between the peptide fragments cleaved from SNAP-25 and the monoclonal antibodies raised against these fragments. The role of the botulinum toxin is solely to cleave the SNAP-25 peptide, thereby allowing these molecular interactions to be detected in the assay.

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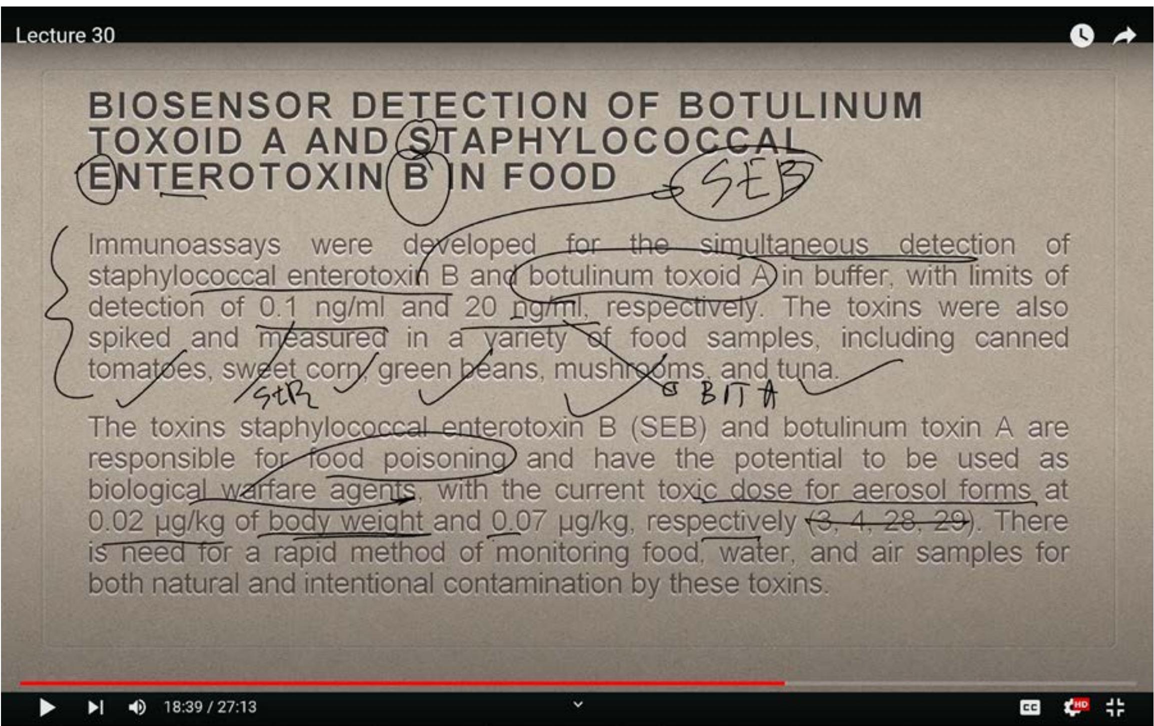

Now, let’s revisit this process one more time to reinforce the concept.

Here's what's happening: In the first step of the assay, the botulinum toxin E sample is injected over the SNAP-25 sensor chip for a predetermined contact time. During this period, the toxin interacts with the sensor. In the second step, the new epitope generated by the activity of botulinum toxin E is detected using monoclonal antibody 11C3. Now, imagine if the chip also contained another antibody, such as MAB 10F12, which is specific to botulinum toxin A. In that case, if botulinum toxin A were present, it would bind to MAB 10F12, allowing for its detection as well. This setup enables simultaneous detection of different botulinum toxin isoforms on the same chip.

This represents the cutting edge of botulinum toxin detection. As I mentioned, SPR (Surface Plasmon Resonance) is one of the most widely adopted methods for detecting botulinum toxins around the world.

Moving forward, we enter the next level of detection techniques: multi-modal detection. One of the most challenging areas in biosensor technology is the detection of foodborne toxins, such as botulinum toxoid A and staphylococcal enterotoxin B (often abbreviated as SEB). These toxins can contaminate food and pose serious health risks, sometimes even being considered for use in biological warfare. The immunoassays developed for the simultaneous detection of these toxins in buffers have shown impressive sensitivity, with detection limits as low as 0.1 nanograms per milliliter for SEB and 20 nanograms per milliliter for botulinum toxin A.

These assays have also been tested by spiking various food samples, including canned tomatoes, sweet corn, green beans, mushrooms, and tuna fish, with the toxins. Both staphylococcal enterotoxin and botulinum toxin are notorious for causing food poisoning and have the potential to be weaponized. The toxic doses for these agents in aerosol form are extremely low, approximately 0.02 micrograms per kilogram of body weight for SEB and 0.07 micrograms per kilogram for botulinum toxin. Given this, there is an urgent need for rapid and accurate methods to monitor food, water, and air samples for both natural and intentional contamination by these toxins.

Now, let's discuss the immunoassay chip technology in more detail. You might recall that we previously talked about sandwich assays, this is an example of that approach.

Biotinylated antibodies are captured on phosphate-buffered saline containing Tween (a detergent), which is patterned onto neuroavidin-functionalized slides using PDMS (polydimethylsiloxane) flow cells. This microfluidic setup is commonly used to create chambers for various assays. Each slide is patterned with two columns for chicken immunoglobulin Y as a positive control, five columns for botulinum toxin A, and five columns for SEB. A sandwich immunoassay is then performed over a 20-minute period.

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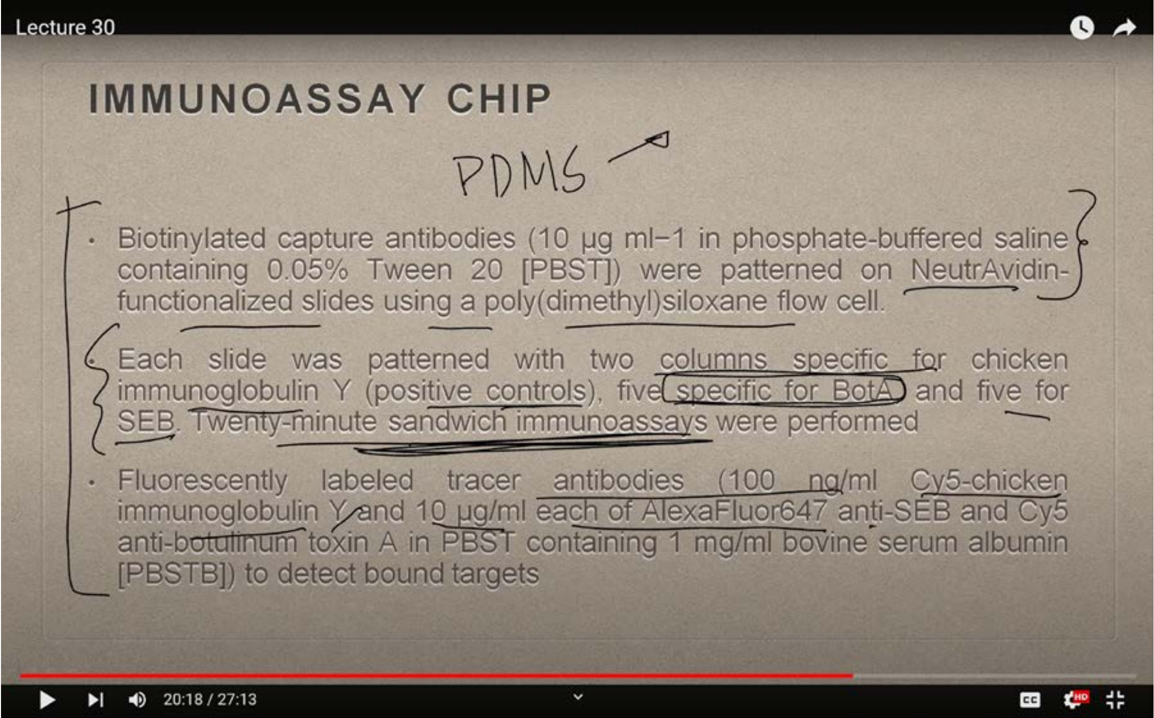

For detection, fluorescent tracer antibodies are employed, specifically, Psi-5-conjugated chicken immunoglobulin Y, as well as Alexa Fluor 647-labeled antibodies targeting SEB and Psi-5-labeled antibodies targeting botulinum toxin A. These antibodies are used at a concentration of 10 micrograms per milliliter in PBS (phosphate-buffered saline) containing 1 milligram per milliliter of bovine serum albumin. The bound targets are then detected via fluorescence.

The results are captured as images from charge-coupled devices (CCDs), showing the assay’s sensitivity to different concentrations of botulinum toxin, ranging from 0 to 500

nanograms per milliliter. The strongest signal is seen at the highest concentrations, while fainter signals are detected at concentrations as low as 10 nanograms per milliliter. Similarly, for SEB, the assay detects concentrations ranging from 0 to 7.5 nanograms per milliliter, with the strongest signal at 7.5 nanograms and diminishing signals as the concentration decreases.

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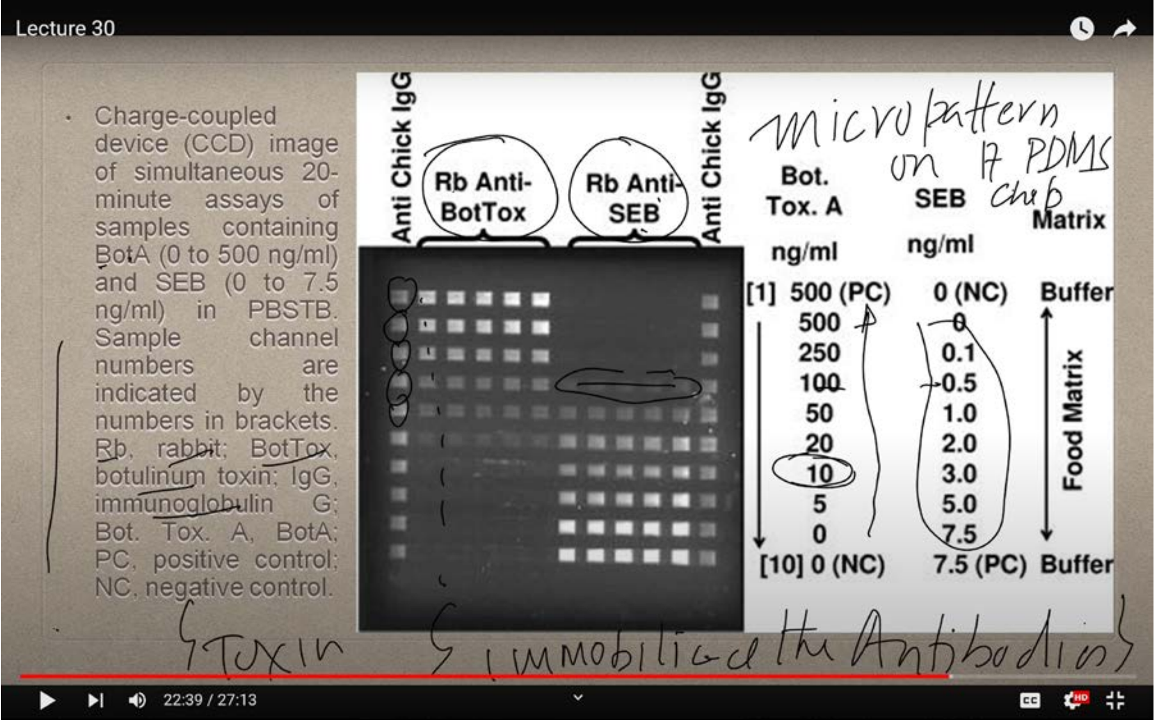

The sample channel numbers are indicated in brackets, and abbreviations like "RB" stand for rabbit botulinum toxin IgG (immunoglobulin G). These cutting-edge assay systems utilize micro-patterned PDMS chips, with immobilized antibodies, toxins, or substrates strategically positioned within the flow cells. These positions are defined through advanced micro-printing, micromachining, or micro-spraying techniques. The data generated from these assays is normalized based on the concentration, allowing for a clearer interpretation of the results.

If we examine the detection limits here, you can see how these vary across different pH levels, this is crucial, as the pH plays an integral role in the accuracy of detection. When

measuring in different food samples, such as 20 nanograms, 50 nanograms, or 20 nanograms, these limits will vary based on the type of food. For example, tomato juice, whole tomatoes, mushrooms, sweet corn, sweet corn juice, green beans, green bean juice, and tuna fish all show different detection ranges. Tuna, often being canned, presents a particularly high risk for contamination. So, these are the kinds of numbers we are focusing on in our analysis.

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Lecture 30

MATRIX	PH[A]	BOTA LOD (NG/ML)[C]	SEB LOD (NG/ML)[C]
PBSTB	7.5	20	0.1
TOMATO JUICE (UNBUFFERED)	4.5	50	0.5
TOMATO JUICE (BUFFERED)	7.0	20	0.1
WHOLE TOMATOES[B]	6.0	50	0.1
WHOLE TOMATOES[B]	7.5	50	0.1
MUSHROOMS[B]	7.0	100	0.5
SWEET CORN[B]	7.0	50	0.5
SWEET CORN JUICE	7.0	50	0.5
GREEN BEANS[B]	7.0	250	0.5
GREEN BEAN JUICE	6.5	100	0.1
TUNA[B]	7.0	500	0.5

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In the previous class, I emphasized that the detection limit is paramount, and it must be unequivocal. If we state the detection limit is 0.1 nanograms, then it must be that, at least 99% of the time, it must register as 0.1 nanograms or lower. This precision is critical, especially at the commercial level, where accuracy is non-negotiable.

This leads us to multi-analyte detection. These assays are not only simple to perform but also rapid, show no cross-reactivity, and often require little to no sample pretreatment or pre-concentration. One of the primary advantages of array biosensors over older technologies is their ability to detect multiple analytes across multiple samples

simultaneously. The speed and specificity of these sensors are absolutely critical. While multi-analyte sensors are becoming more prevalent in the literature, many still haven't tackled more complex sample matrices beyond simple buffers. However, being able to carry out multi-analyte detection in real-world complex samples, whether food, water, or air, offers a huge advantage when screening for hazards, whether naturally occurring or deliberately introduced.

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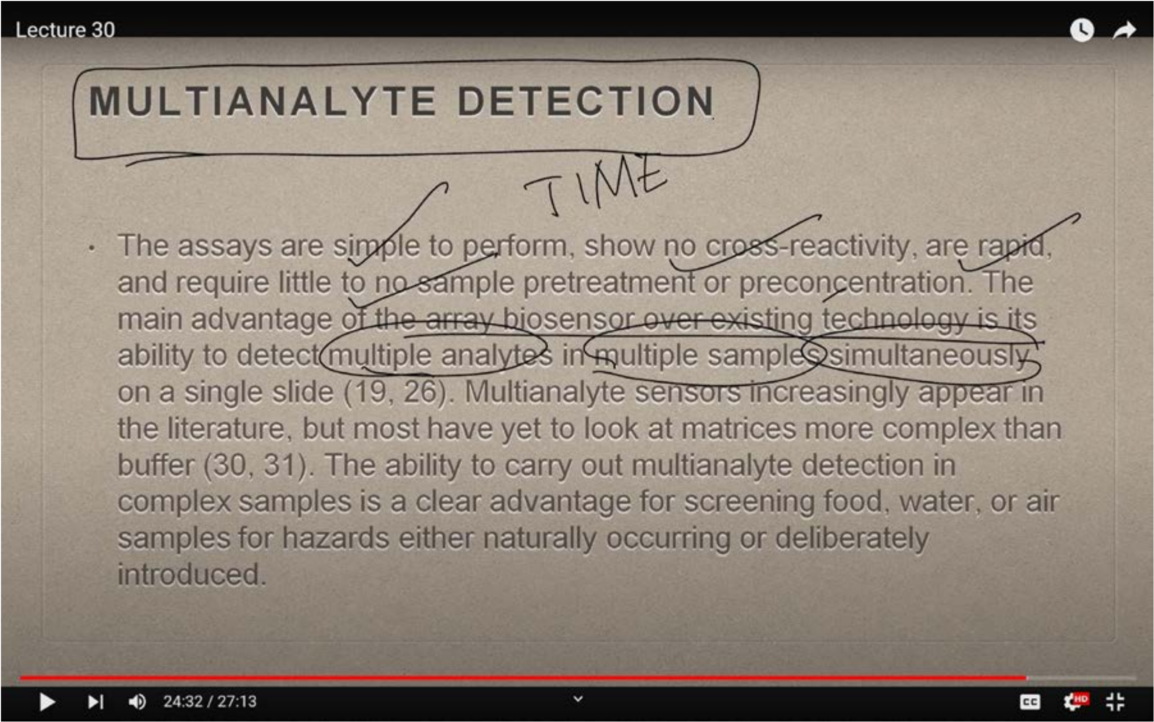

As I've mentioned, much of the global biosensor development now focuses on integrating multiple techniques and multiple analytes simultaneously. This technology is evolving rapidly, and we are beginning to see a shift towards AI-driven systems. In the near future, we will likely be using AI algorithms to manage complex samples with multiple contaminants. These systems will feature multiple modalities to detect various threats and contaminants, requiring advanced computational power to process the enormous volumes of data generated. AI will not only help in detection but will also guide decision-making, enabling professionals to swiftly and accurately assess how to handle any given sample.

With that, we conclude the sixth week of our course. As we move forward, we will explore additional types of analytes and detection systems. So far, we've discussed botulinum toxin and other significant toxins, but we'll soon expand our focus to encompass a wider range of detection technologies. I've given you a solid foundation for understanding the critical areas you must grasp to become proficient in the development and design of biosensors. Thank you.