Design for Biosecurity

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Lecture 24

Recognition Elements ScFvs

Welcome back once again in the fifth week of this course. Today, we are in lecture 24 about the recognition elements, specifically ScFvS. What are ScFvS? So, in terms of our definition, if you look at ScFv, what is it? Schematic representation of a full-length monoclonal antibody and a single chain variable domain FV fragment; this is called ScFv, ok. So, I told you that most of these antibodies have variable heavy chains and light chains. So, this is the variable region. You look at it in this picture.

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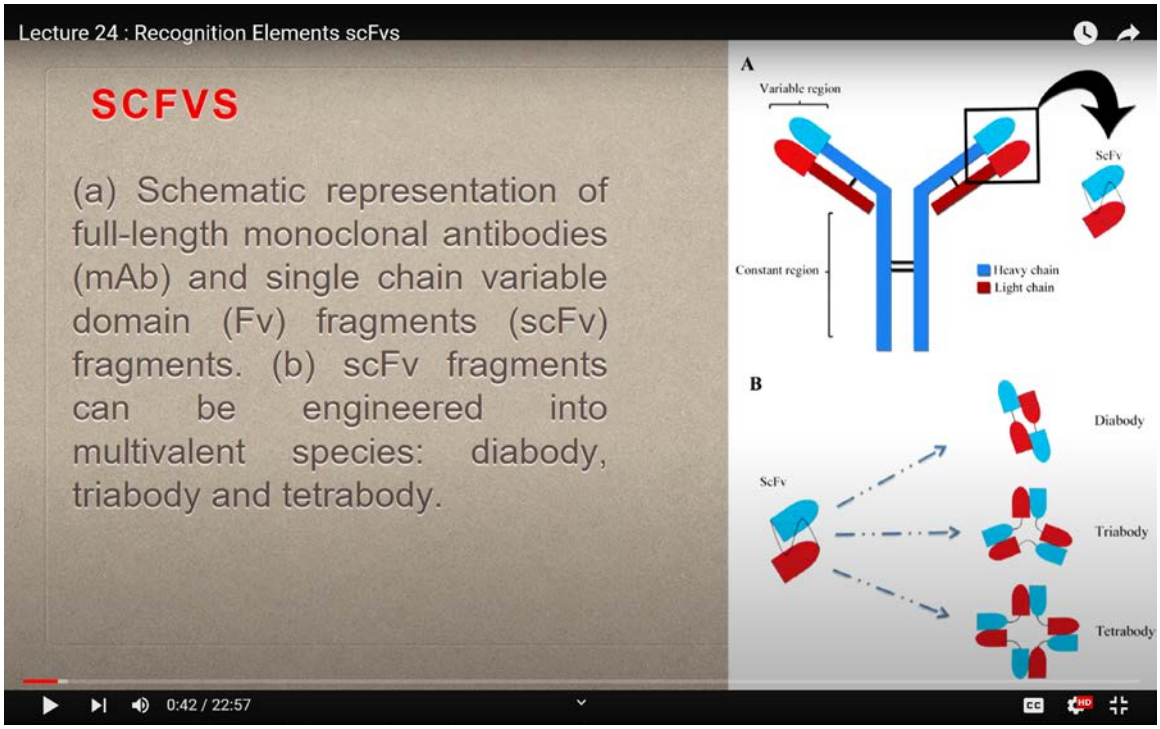

This is the variable region. The heavy chain is blue, and the light chain is red; this particular part is called SC. So, if you remember, in the last class, I mentioned that each one of these

parts is coded by specific genes or DNA sequences, okay? Now, if you could isolate that particular sequence of DNA, which codes for this particular part, and you express it in some say bacteria parts protein surface protein then on the top of that particular animal. So, it is to visualize the situation.

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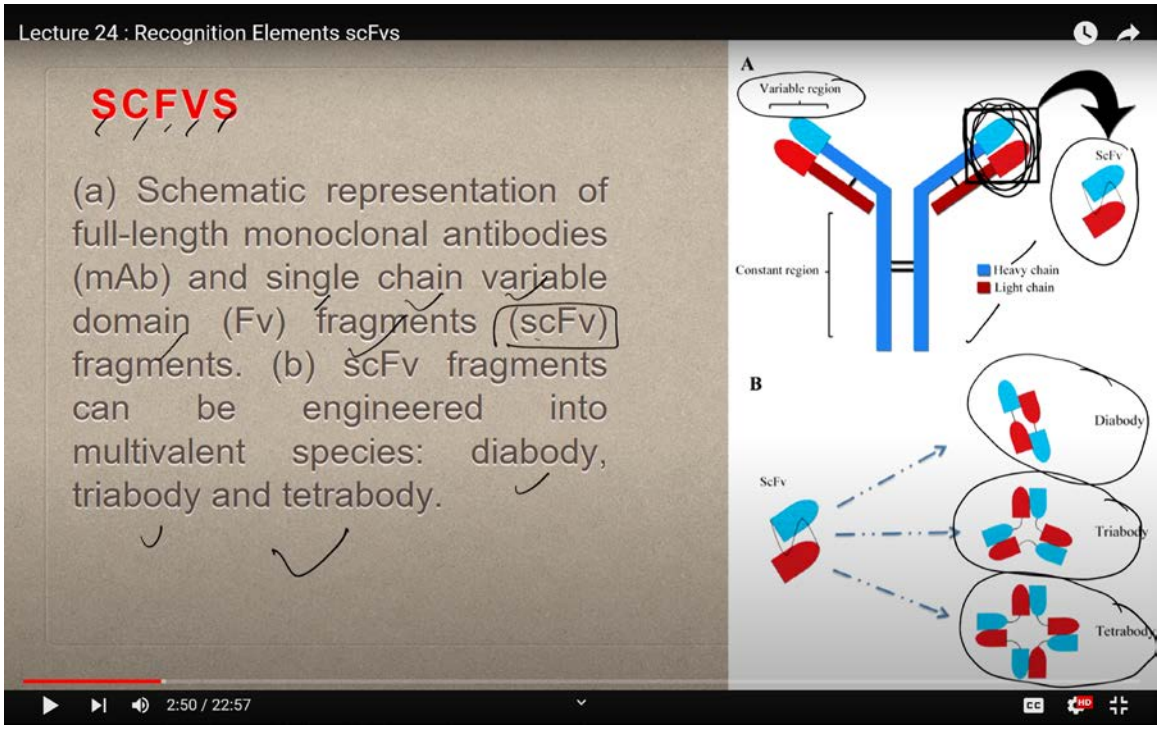

So, you have DNA for this particular part, and you couple this DNA with something that will be expressed on a surface protein of a specific cell. Now, what will happen on the surface of that particular cell? You will see the expression of this kind of molecule. So, this is what SCFVS is: a single-chain variable domain FV fragment, a single-chain variable domain FV fragment. It can be engineered into a multivalent species, diabody, triabody or tetrabody. So you have the diabodies, two triabodies and tetrabodies, and four of them.

Tetra, di, tri, tetra, you could even have more of them configuration, but these are the common configurations. So, do not think that these are the only configurations. Now, SCFVs have a wide range of uses. So, the chain, single-chain variable domains, represented by FV variable domain fragments, are powerful tools in research and clinical settings. They

have better pharmacokinetic properties than the parent monoclonal antibodies and the relative ease of producing them in large quantities at low cost.

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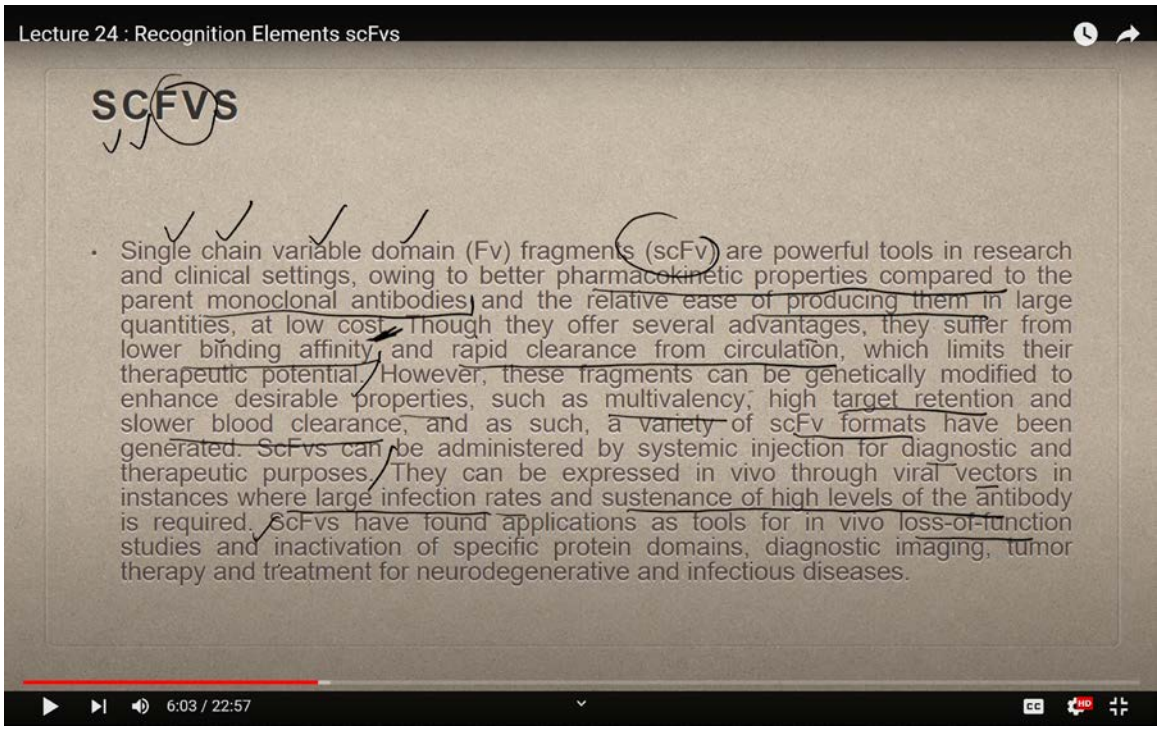

Unlike monoclonal antibody production, where we talk about the hybridoma technique and phage display technique, the production of this single-chain variable domain fragment is far more economical because you are just taking a small part of the DNA; you are coupling it with a surface protein of the bacterial phage. You are producing them in a large number. Though they offer several advantages, they suffer from lower binding affinity. Why is there a lower binding affinity? Think of it when we look at the structure in the normal case of a monoclonal antibody: this whole thing is going, and there are multiple binding sites where it is binding to the host, contrary to that when we are only expressing this much part. So, automatically, your binding area is reduced.

So binding may happen at a higher affinity, but then you need a lot, a lot of this. So it's kind of a trade-off. If you have a lot of these, then of course, the binding will happen much better. But if you have a lesser amount, the binding will be lesser because your site of

contact and site of bindings is less, right? Because these are very specific sites where it binds. It's not that everywhere they will be binding.

So if this whole antibody is a monoclonal antibody that has many binding sites, then this will have only these many binding sites. So try to realize in a physical frame of your mind why SCFVs have a lesser binding. Though they offer several advantages, they suffer from lower binding affinity and rapid clearance from circulation, which limits their therapeutic potential. However, these fragments can be genetically modified to enhance desirable properties such as multivalency, high target retention, and slower blood clearance. As such, a variety of ScFv formats have been generated.

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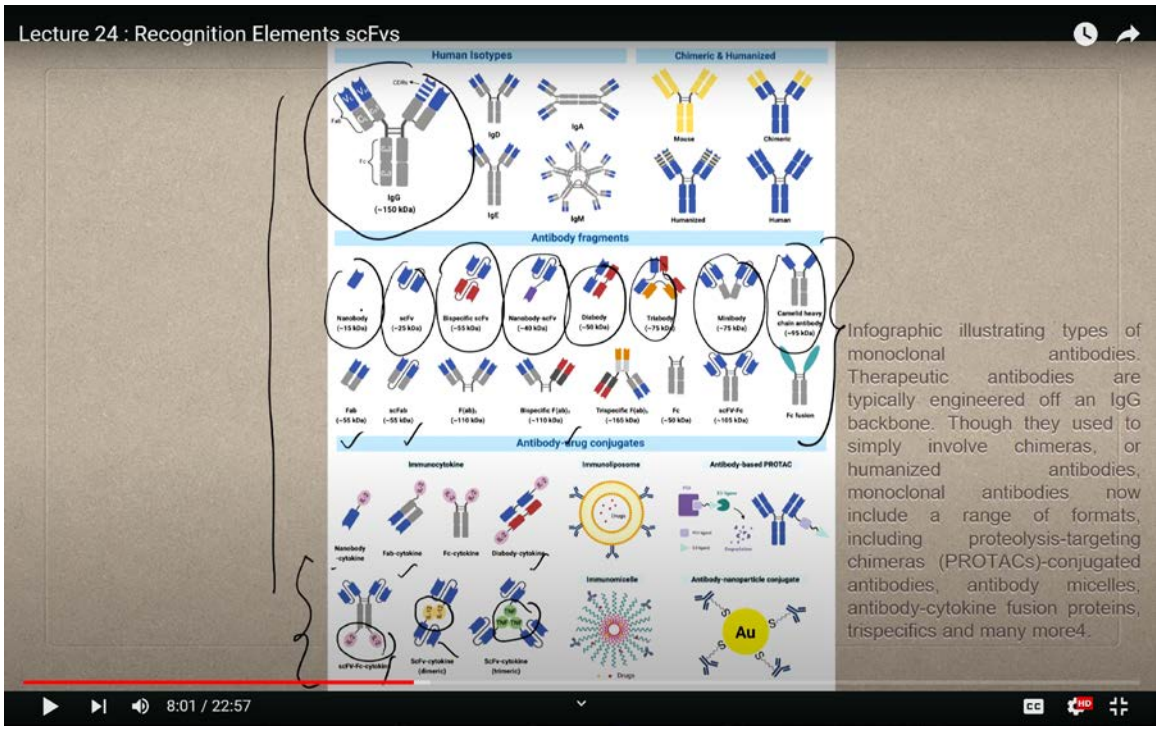

ScFv can be administered by systemic injection for diagnostic and therapeutic purposes. They can be expressed in vivo through viral vectors in instances where large infection rates and sustenance of a high level of antibody are required. SCFVs have been found to be useful tools for in vivo loss of function studies and the inactivation of specific protein domains, diagnostic imaging, tumour therapy, and the treatment of neurodegenerative and

infectious diseases. So now, having said this, it must be clear why there are more preferences for these dye bodies, dry bodies, and tetra bodies. And I will take you back to this picture, what I was telling you in the last class.

Look, these are the ones. All these different kinds of configurations are present there; these are all getting generated because of, so look at it, where they are binding. So their binding sites are out here with the cytokines where they are binding. Again, here are the cytokines binding. Here again, this TNF alpha or tumour necrosis factor binding.

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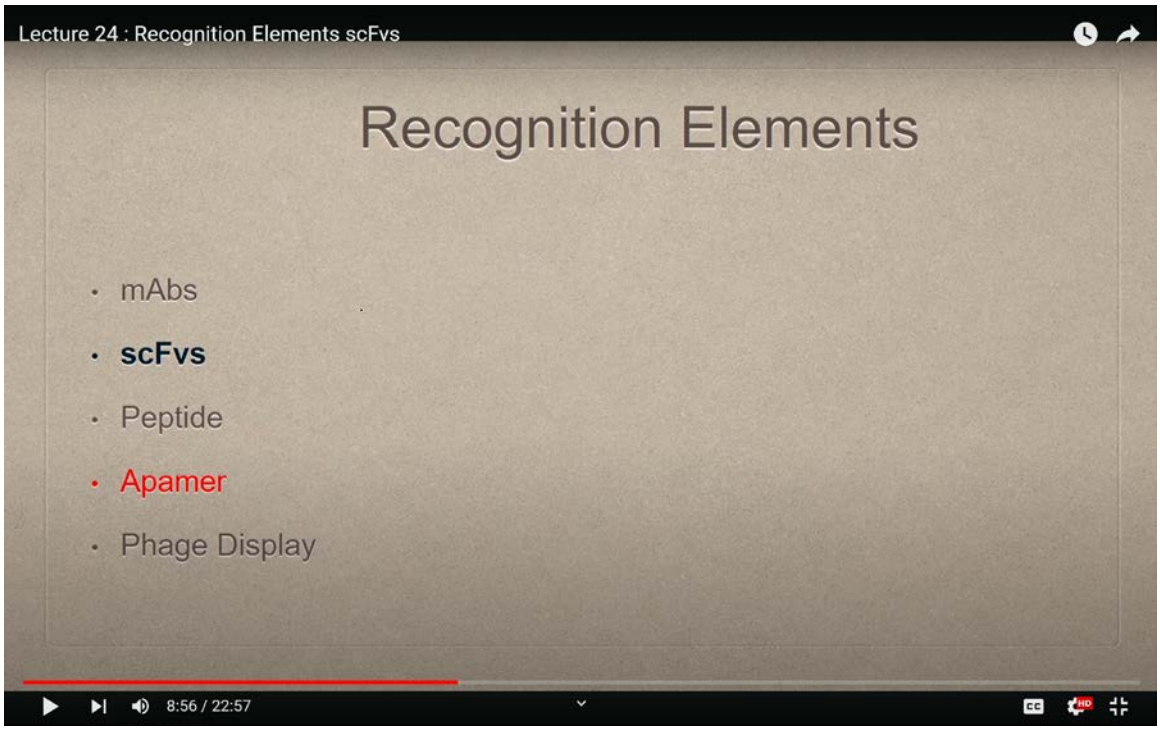

So you see, the number of binding sites has become much smaller. Still, they could be genetically modified as it's being mentioned that they could be genetically altered further in order to slow their blood clearance and have desirable properties like multivalency and high target retention. The ease of genetic modification of these fragments makes them a popular choice for futuristic therapeutic potential, and they're far more specific as compared to monoclonal antibodies. So, if you remember back in the 80s, When you used

to get polyclonal antibodies, you were very happy. Then came the whole era of mass production of monoclonal antibodies.

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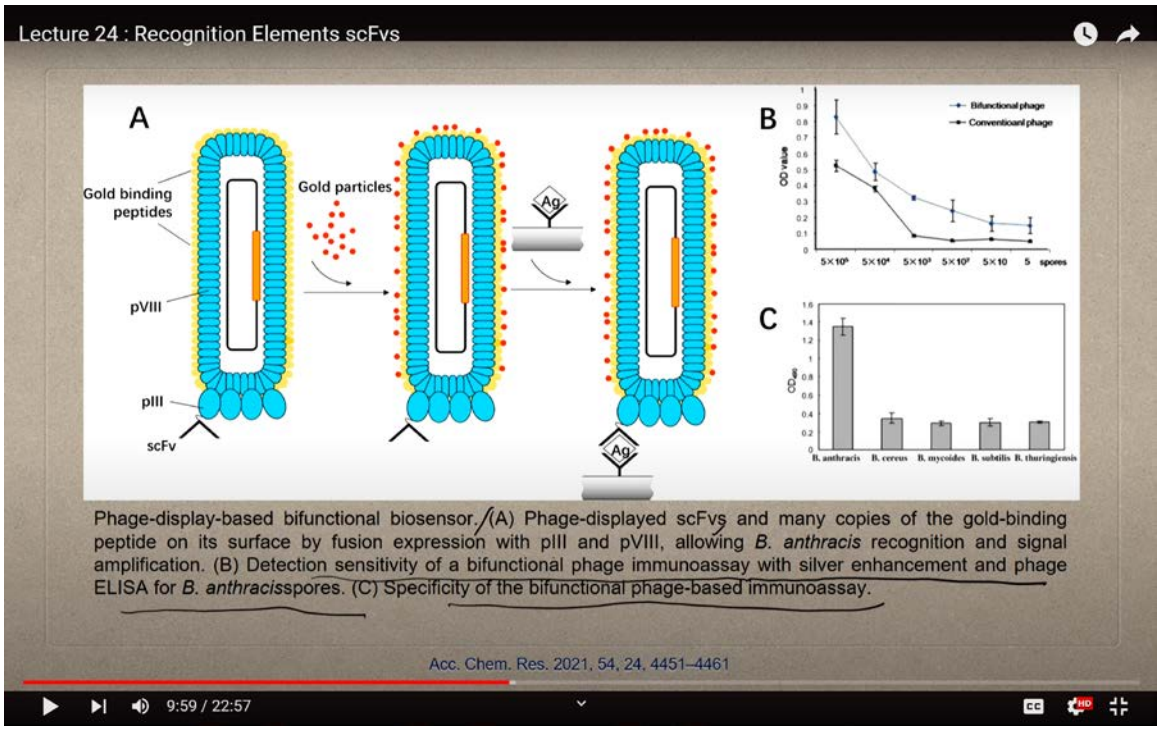

So things started to change, and everybody was asking, okay, let's get monoclonal antibodies for immunohistochemistry. Following that, there was the next era which came, SCFVs. You get even much more. And you started working on the nanobodies. So nowadays, I see people who work on GPCR and all others; they work on these nanobodies, even much smaller fragments.

They're very specific. But whenever this kind of thing happens, always remember, in order to recognize them, the kind of fluorescent signature you need, the clear imaging you need, those are also equally challenging because when you are picking up such low signals, you are also picking up a lot of noise. In practical life, while these are extremely potent and potentially path-changing discoveries, they come with their own set of challenges in terms of signal-to-noise ratio, binding, and further detection using other technologies. So, this is how the SCFBS looks overall. From here, we move to the next, called aptamers.

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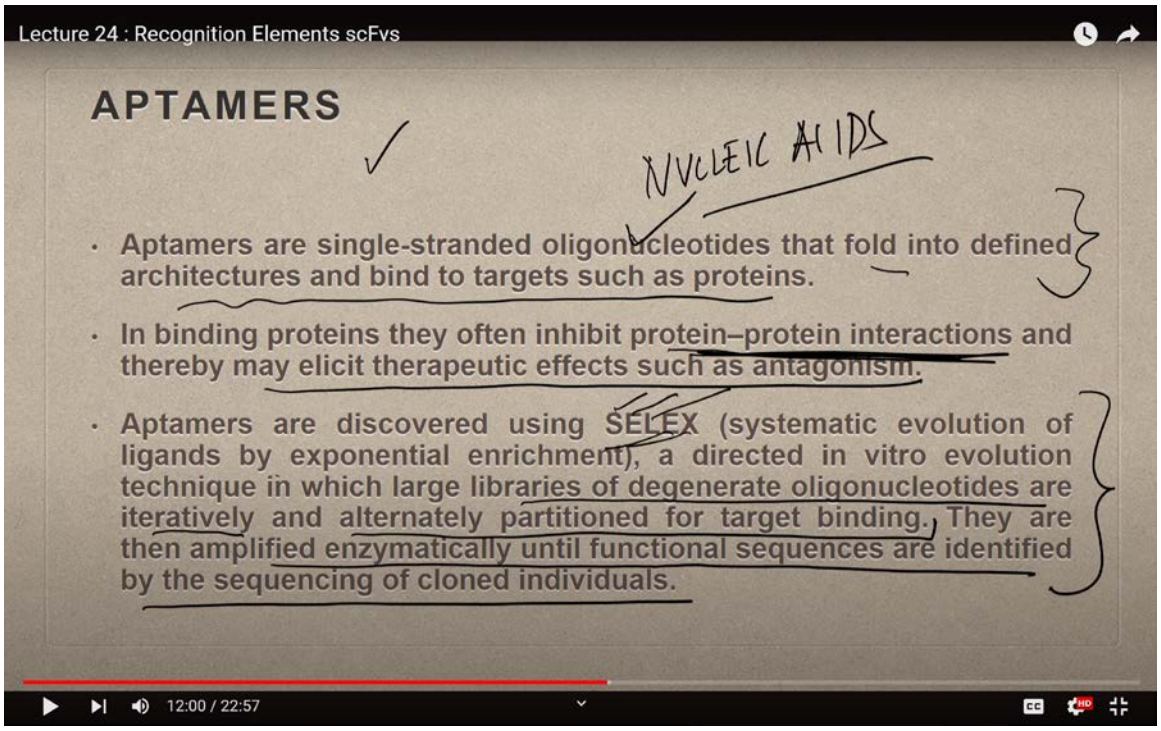

So before I move on to Aptamer, if you look at the FOS display based on bifunctional sensors, these are the sensors when you talk about are the kind of detection level that is happening. So you have the gold-binding peptides. You have the gold, which is getting bound on the gold binding. Then you have the antibodies, which are binding on top of it. And then you do a display.

This is used for Bacillus anthraxus, part display of SCFVS and many copies of the gold-binding peptide on its surface by fusion expression with P3 and P8, allowing B anthraxus recognition and signal amplification. Detection sensitivity of bifunctional phage immunoassay with silver enhancement and phage ELISA of bacillus anthrax spores. Specificity of bifunctional phage display immunoassay. So, these are the practical applications where bacillus anthrax is detected using the technologies we have just covered. So now, from here, I move to Aptamers.

This class is further from SCFVS; now, move to the aptamers part. Aptamers are single-stranded oligonucleotides.

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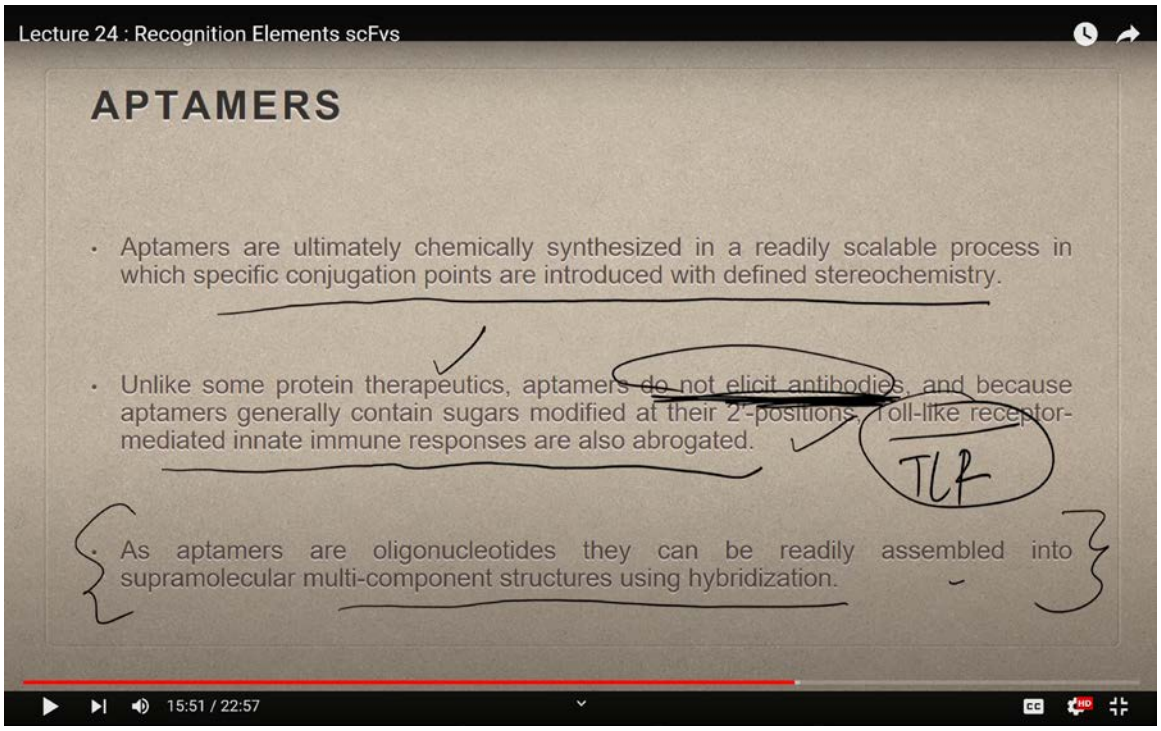

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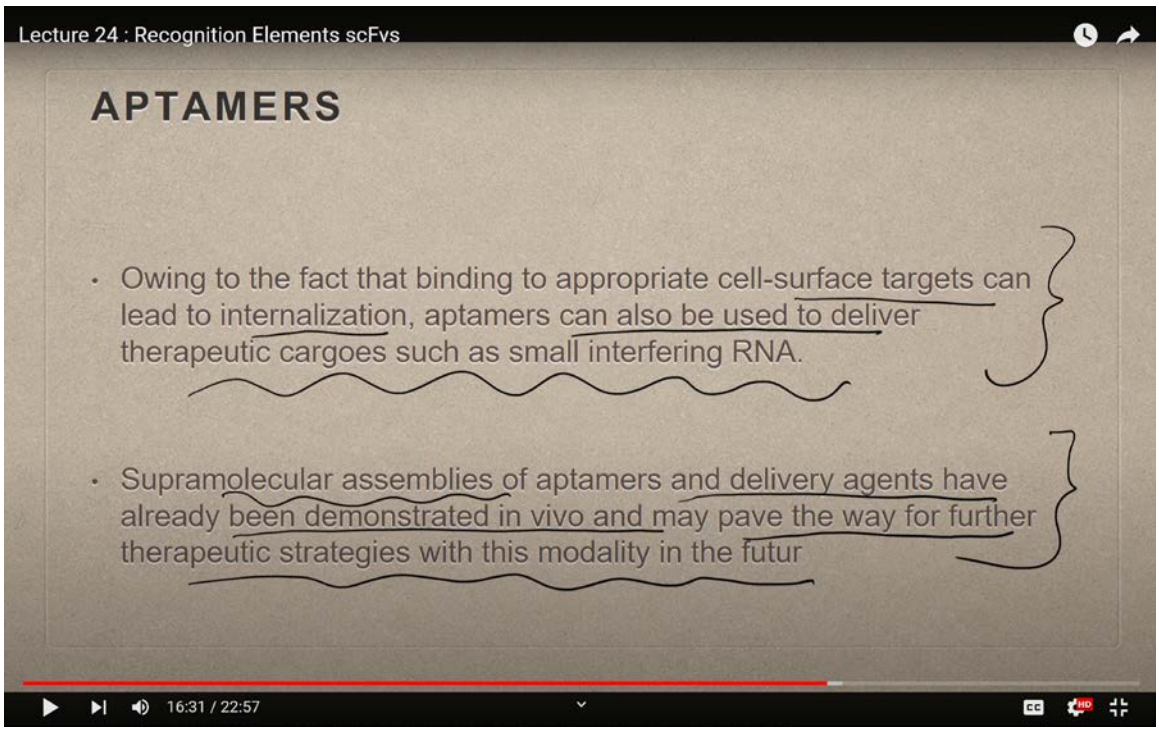

So, now we are talking about nucleic acids that fold into a defined architecture and bind to targets such as proteins. So, aptamers are a sequence of oligonucleotides that bind to proteins. So now we are venturing into nucleic acid-protein binding.

So here, nucleic acids are recognizing specific proteins. Binding proteins often inhibit protein-protein interaction and elicit therapeutic effects such as antagonism. Optomer therapy prevents the protein-to-protein interaction and protein-to-protein signalling. Thereby, it elicits therapeutic effects such as antagonism. Optomers are discovered using SELEX, the systemic evolution of ligands by exponential enrichment.

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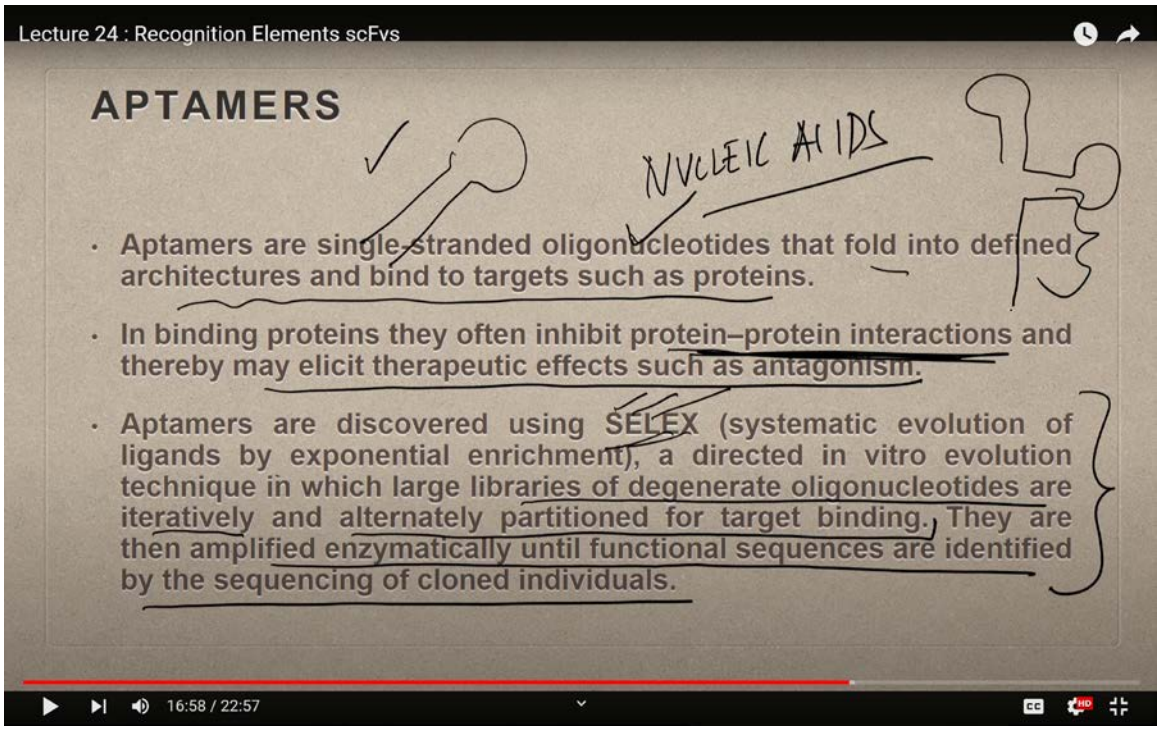

Systemic evolution of ligands by exponential enrichment. A directed in vitro evolution technique in which large libraries of degenerate oligonucleotides are iteratively and alternatively partitioned for target binding. They are then amplified enzymatically until the sequencing of the cloned individual identifies functional sequences. So, this is what aptamer technology is about. For most therapeutic purposes, aptamers are truncated to reduce synthesis cost, modified at the sugars, capped at their termini to increase nucleus

resistance, and conjugated to polyethene glycol or another entity to reduce the renal filtration rate.

So, nuclei are all over the body, which can chop off the nucleic acid because it breaks it. So, there are techniques by which you can prevent nucleus activity by pairing it with polyethene glycol or another entity to reduce renal filtration. So, you are retaining, you are increasing the retaining time within the body. The first aptamer approved for therapeutic application was pegaptinib sodium, approved in 2004 by the US Food and Drug Administration for macular degeneration. This is the first aptamer that has been approved.

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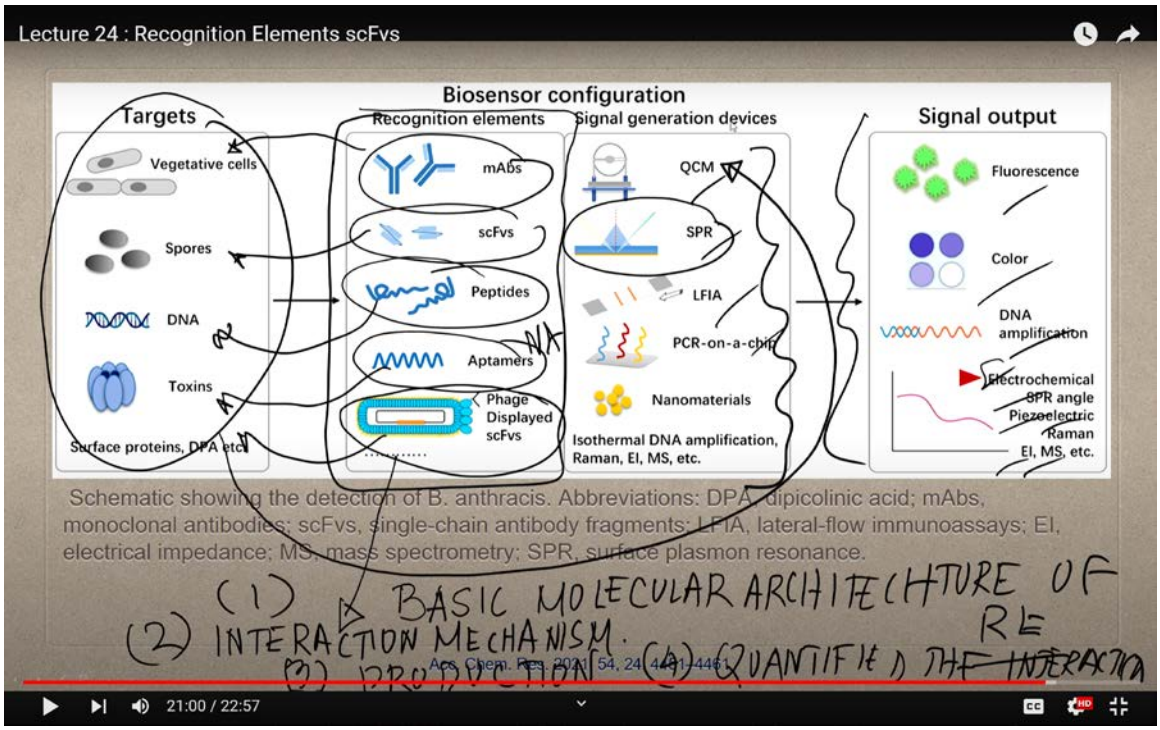

Eight other aptamers are undergoing clinical evaluation for various haematology, oncology, ocular, and inflammatory indications. Aptamers are ultimately chemically synthesized in a readily scalable process in which specific conjugation points are introduced into the defined stereochemistry. Unlike some protein therapeutics, aptamers do not elicit antibodies. Remember this. And because aptamer generally contains sugar

modified at the two prime positions, toll-like receptor-mediated innate immune responses are also repealed.

So when you talk about TLR or toll-like receptors, in short, these toll-like receptors are innate immune systems. That means TLR receptors are present across the surface of your body, across the orifices of your body, and across every entry port. It's like a toll. You all have heard about the word called toll. You have to pay tolls while travelling on the road—toll tax.

It is exactly the same word that has been used in immunology in innate immunity. The surface of the body has a lot of toll-like receptors. So whenever you introduce anything into your body, the first line of defence is the toll-like receptor. They prevent the stuff from getting into your body. There are a lot of efforts that have been made that are still underway to prevent the entry of the AIDS virus by activating the toll-like receptors.

Toll-like receptors are the ones that elicit a huge amount of the innate immune response, but it has been seen by modifying the sugar at the two prime positions. Toll-like receptor-mediated innate immune responses are also abrogated. So, it means you are giving the aptamers a free hand to act on the specific protein and abrogate their interaction with another protein. So, in a way, you are striking at their signalling platform, right? As aptamers are oligonucleotides, they can be readily assembled into a supramolecular multi-component structure using hybridization.

These are pretty small structures. They are just some small thread-like nucleic threads, okay? So, they could be made into a complex supramolecular assembly as a therapeutic agent. Since binding to appropriate cell surface targets can lead to internalization, aptamers can also deliver therapeutic cargo, such as a small interfering RNA. For the supramolecular assembly, as I mentioned, of aptamer, our delivery agent has already been demonstrated in vivo and may prove the way for further therapeutic strategies with the modality in the future. So, if we look at it in terms of aptamers, they are single standard oligonucleotides, and they fold into a defined architecture and bind to targets such as proteins. So, they could fold something like this, have structures like this, and have a unique three-dimensional geometry.

They form supermolecular assemblies that can bind to any of these proteins, and molecular manipulation of aptamers is far easier than molecular manipulation of bigger protein molecules like antibodies. If we look at it regarding the recognition elements, we start with toxins. Hence, if I go back to the recognition elements where we started our journey, as we are almost about to complete all the recognition elements we discussed, it is worth visiting them here. So these are our vegetative cells, dormant, spores, and so on and so forth. These are those elements, toxins, DNA, surface proteins, BPA, and others.

And now if you look at all the recognition elements out here, this is what we covered. Monoclonal antibodies, SCFVS. I did not talk much about the peptides because we will talk about it a little bit. These are simple. We can say 2,2,2 anti-amino acid chains and small peptides are there.

Then, aptamer, which is a nucleic acid, nucleotide and then the FAC display using SCFBS. So, the reason I devoted these four classes to these recognition elements is, first of all, you have to understand the basic molecular architecture of recognition elements. I just put RE. Second, their interaction mechanism. So, for example, proteins interact differently, like MABs and SCFvs.

These are protein fragments versus aptamers. Second, how could these be produced on a large scale? And further, how these could be quantified in the interaction. That's why understanding of these are important. These key elements will recognize these targets when making a biosensor. And when you talk about biosensors, once they recognize this target, you need a way to measure these interactions using the QCM, SPR, LFIA, PCR, nanomaterials, etc. And the signal output of all these different interactions could be the electrochemical, SPR angle, piezoelectric, Raman, EI, MS, DNA amplification, colour, and fluorescence.

So, that's why you must understand these recognition elements as efficiently as the target elements. If you understand both of them, you can design a better biosensor at your disposal because, with this, I will move on to the next class with the SPR, which is the signal generation device. So far, we are trying to talk about monoclonal antibodies, where we talk about the fundamentals of monoclonal antibodies. We talked about the difference between

monoclonal antibodies and polyclonal antibodies. We discussed the two critical monoclonal antibody production techniques: hybridoma technology and phage display technology.

Then, we spoke of B cells, using B cells and protein sequencing technology. Then we talked about SCFVS, how there are smaller units of variable heavy chains, which could all be utilized, and how they could be made more potent to retain them in the body. They are not neutralized by the nucleases and all the molecular manipulations that could be done on them. After that, we talked about the aptamers and how they are being used, and in between, we spoke of the phage display of scfvs and how They are being isolated.

They are being screened. And finally, we talked about different techniques for quantifying these kinds of interactions. So with this, I will close the 24th class. In the next class, in the 25th class, we'll talk about the SPR. Thank you.