**Nanobio Technology Enabled Point-of-Care Devices**

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**Lecture - 05**
**Signal Amplification for Ultrasensitive Biosensors**

Dear students, today I will teach you the Signal Amplifications for Ultrasensitive Biosensors. Last class I taught you how to modify the sensor surface with nanomaterials. Now, let us use this sensor surface for ultrasensitive detections. So, this class is really important for point of care device development.

Because I will let you know the ultrasensitive way modifications of the sensing method, sensing technology. Why it is important? What is ultrasensitive this keyword you should know first time. Let us show you what I will cover today.

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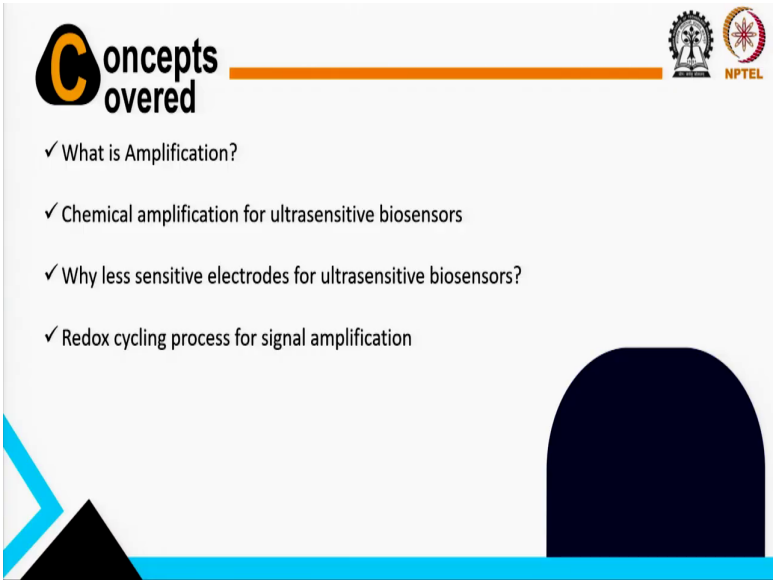


Today mainly I will cover the signal amplifications and I will let you know that really amplification is necessary and why. So, the technique like we can use the chemical amplifications, we can use different kind of like electrochemical amplifications, those technology I can mentions today. And what is the ultrasensitive biosensors? And for this ultrasensitive biosensor development, we need some electrodes.

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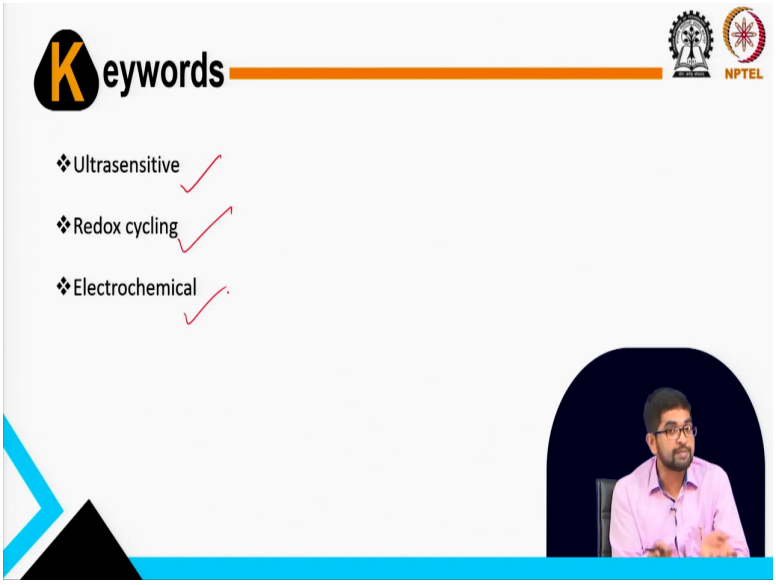


So, what electrode we need? Suppose I told you like different nanoparticle modified electrode. Sometime this nanoparticle modified electrode can be very much conductive, highly conductive. It can cause also some drawback; it can cause us some problem. Why? Because some other reaction, some other side reaction also can happen. So, we have to normalize, you have to optimize the sensor surface, those optimizations method I will let you know.

And why we need a optimum sensing surface that I will teach you today. And redox cycling process. This is the main theme of today's lecture redox cycling. This can help you the signal amplifications. So, what is redox cycling? How we can design the different kind of the redox cycling? How it can help to unlisted these diagnosis? I will show you with a real example.

So, the main keyword for today's lecture is ultrasensitive. So, if you can source this all the keywords also, you will get lots of other report also. So, you should know this one redox cycling and electrochemical.

So, mainly these lectures I will emphasize on the electrochemical. So, why we need the electrochemical at very first class I told you know that electrochemical technique is very much user friendly, it is very cost effective technology, easily you can make the miniaturized sensor, right. That is why I mainly focus on the electrochemical.

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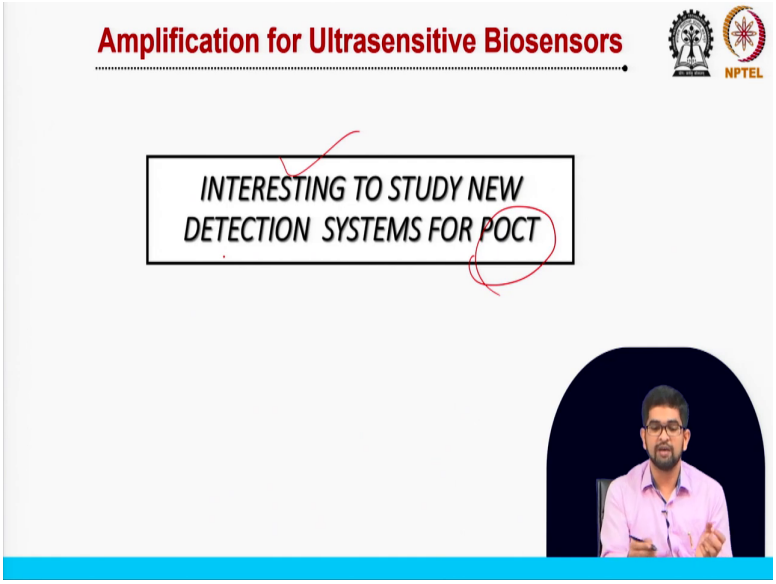


Let us come the amplifications. So, we are always focus on some interesting technology, some new technology, new detections method that can be useful for point of care.

So, point of care means that near the patient. So, that patient can handle by themselves or it can be no need the expert persons, no specialized person do not need, right. So, for point of care we always focus on some new technology that can be useful. It is very interesting to develop some new technology in this nano biosensing course.

It is very interesting you will get so many information here. I mean I will teach you very generic concept like this way that way this way can be developed. Now, similar knowledge you can try for other sensor development.

So, if you can understands like one sensor properly here, these generic concept you can apply for other diagnosis. This is the main important part of this course. This is not like if you learn this is a very suppose want cancer detections, if this technology same time can be useful for like the kind of kind of virus detections or maybe kind of other disease detections like the generic concept. So, that is why it is very interesting to study some new detection protocol to understands to develop a new ultra sensitive biosensor.

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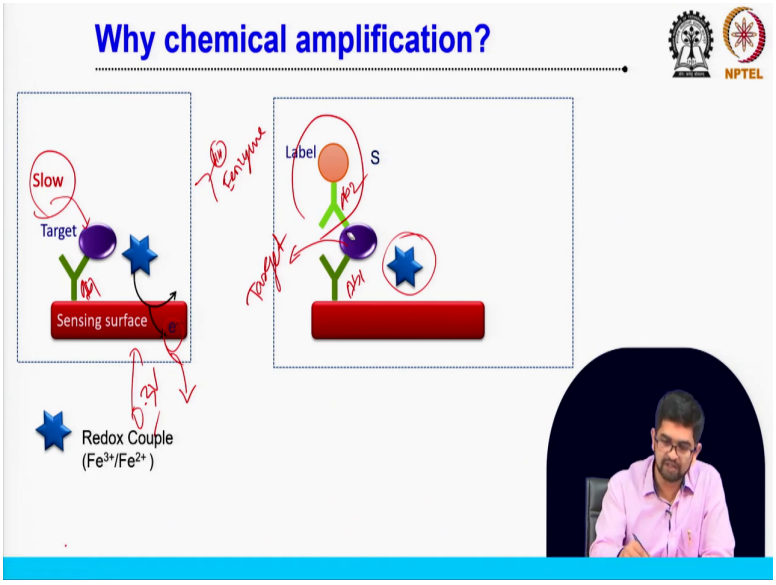


Let us show you. So, why chemical amplification? Generally, people developed a sensing surface with primary antibody, suppose this is the primary antibody we want and we immobilize that is means drop the target and here we are using a redox couple. You know what is redox, that also I will show you in the next slide. Suppose want redox couple that can help actually signal generations. So, it will release electron that electron will measure like based on number of the electron we can get the signal.

So, this is a very simple technique like after adding your target solutions and you are if it is applying some suppose you are applying some potential like 0.3 volt and you are getting some electron. But there is no any other technology no other amplification you are using just a very basic method. We can try for development of biosensor.

But this is a slow technique where as you can say the slow detections. You can you cannot achieve very ultra sensitive detections here. How we can go for the ultrasensitive then? We can detect I mean we can take a help from another antibody second that is called the secondary antibody. This is the primary antibody; this is secondary antibody and this is the target.

Now, this secondary antibody can be labeled that I told you know last class it can be labeled with antibody, it can be labeled it can be labeled with like other antibody that conjugated with some gold nanoparticles. Also, you can use some enzyme also here as a tag. That can react with some chemical.

So, for getting a amplifications generally we are using some chemical. That chemical will be reacting with the label and it will form some certain product and that product will react with this kind of redox couple that act as a mediator and it will help the signal amplifications.

So, I will teach you more details way. This is the main thing see suppose one S this is one chemical I am using. So, this chemical will help let us remove this. So, very basic for now I will show you see. Suppose you have one S. So, this is a chemical I am using.

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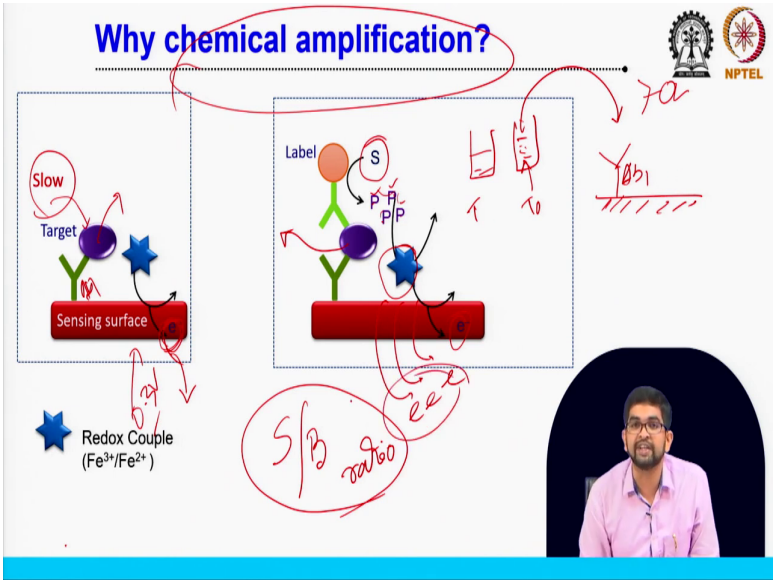


So, this chemical say is reacting with the label is form the many P, P means some from s you are getting some product. This product will reacting with this mediator or this it is also one chemical and we are getting the electron. So, here number of the electrons that we are getting from the surface will be much higher than these number of the electron.

So, necessarily if you are getting here is with the see here also one target here also one target. But with the one target here we are getting one electron, but here one, two three, four product and they will release and may be here also here we will get almost four electron.

So, we are amplifying the signal that is called amplification. So, although you have one target, but you can amplify the signal because of these chemical reactions. So, that is why these chemical reaction amplifying your reactions. So, we are amplifying basically signal to background ratio. I will come this topic. What is signal to background? See when so this is

your sensor surface you have the primary antibody. This is your sensor surface ready to detect.

Now, when suppose you have two sample two sample solution you have one is with target may be like cancer patient, we took some sample it has some cancer biomarker. And some sample like normal patient it does not have any target it is T 0 no target, it is T it is the with target. So, you add these solutions here as it does not contain any target.

So, any necessarily there is no antigen or any biomarker. Now, when you add the secondary antibody with level. So, it cannot bind here because it does not had any target. And we are actually after adding the secondary antibody, we are washing the circuits.

So, as you do not have the target so, naturally secondary antibody not binding. So, if you add the substrate in this sensor solution surface then naturally as you do not have label means these kind of reaction will not happen. So, you will not get these kind of signal amplifications.

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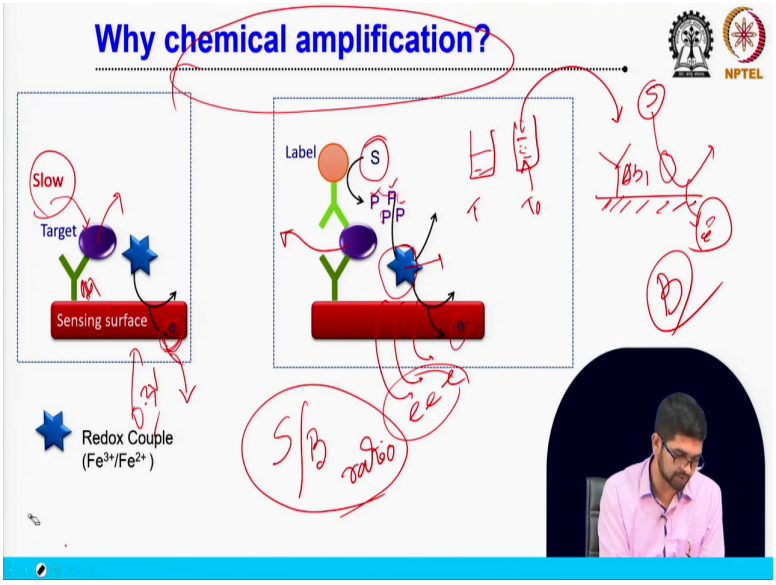


So, without target you are not getting any signal amplification. So, without target that is so, let us remove this there is no secondary antibody with label. So, this is the reactions you are getting means here S only and you have only this mediator that is ferrosoferric cyanide mediator. And with these reactions we are getting only very very low number of electron. So, this is called background, this is the background.

Background so simply we can say background means without target whatever say means electrons we are getting transfer we are getting on the sensor surface that is the background. Now, now let us come to the second solutions with target, right.

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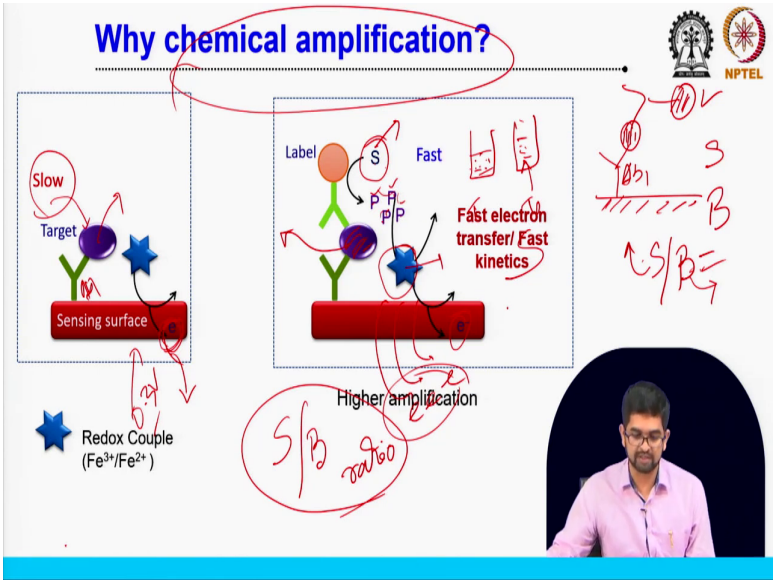


So, now if you have target like if you have some cancer antigen right, it will necessarily it will bind here then when you add the secondary antibody with level and see. Now, it is here so, now if you now say it is similar like this. So, we add the substrate from product you will get so many electrons.

So, you are naturally you are getting the very high signal you are getting here. So, this is actually called the signal, with target signal, without target is the background. So, S by B it is this ratio is called signal to background ratio. So, that is why if you want to make the ultrasensitive biosensor your background should be very very low and signal should be very very high then only you can amplify the signal and very low number of the target you can detect, ok.

So, if it is close to background then that is difficult because they will be almost overlap signal and background. So, although you have various although you have only one target I mean one antigen, but still if the signal to background ratio is very high then easily necessarily you can detect them. So, that is why the selections of these kind of chemical also very important. I will show you now next slide how we can select those chemicals.

So, naturally this that is why this is a fast electron transfer process and it is very very fast technique, ok. This is called amplification.

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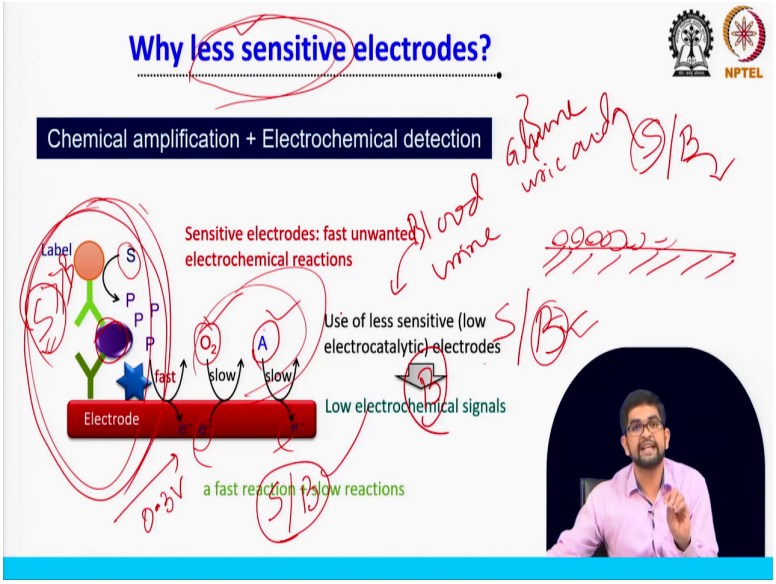


Just now I told like signal to background ratio you know. So, we have to get the this value high this value low then only we can achieve ultra sensitivity. So, for that we have to choose not highly sensitive your electrode surface. So, we are selecting some electrode where we are modifying some nanomaterial whatever I taught you in the last few classes for that you have

to select some electrode and that electrode should be not too much sensitive with other chemical.

Other chemical mean when you use some very complex sample like blood some complex sample or urine, they contain many other species that is the interfering species blood. Suppose you are going to detect a cancer marker from blood samples. So, this is your marker from blood, but it also contain blood contain glucose it cause the it contain uric acid it also got fructose, galactose so many things.

So, when you are detecting some cancer marker and you are applying some potential suppose 0.3 volt you are applying here. So, other species they are also getting the same potential they are getting and they also can oxidize also surface, they also can generate some electron and they can generate the current. Then naturally they are actually contributing to the your signal then actually your signal then getting some current from this kind of species that is called interfering species.

So, basically your background current actually increasing. See your electrode A means some interfering species like uric acid, ascorbic acid, glucose they can also oxidize O 2 your sample content also dissolve oxygen they also can take part they can release the electron.

So, they cause some extra background. So, if you want to get the high signal to background. So, your background should be low. But your other interference species as I mentioned like ascorbic acid, glucose they if they cause then this value will increase, naturally S by b will going to (Refer Time: 14:15) decrease your sensor will not be ultrasensitive.

So, for that you can choose a some less sensitive electrode towards those interference species. So, that they should react very very slow, you cannot avoid basically totally 100 percent you cannot avoid them, but you can decrease their oxidation on the sensor surface they mean come almost tends to 0 you can make them.

But you have to select a electrode so that your main reaction that I taught you last class sorry, last slide like your last slide I taught you know like by using secondary antibody and label.

So, substrate will react with your label will from the product and that will generate the electron that we can measure.

So, this is very specific reaction actually. These reactions we can measure and this is signal and this should be very high this is should be very very high then background. So, if we use some less sensitive electrodes towards the interference species, but your signals should not be hampered it should be high and we can get very high signal to background and we can achieve the ultra sensitivity, clear.

Now, next slide I will teach you, how this part like chemical reaction part easily we can design and we can get the high I mean we are getting fast reactions. Because this is this part is important also signal part is important you have to focus here.

But why we are focusing signal at the same time you have to take care of the side reactions. So, that like other side reactions will be low. I will show you next slide now. So, these all the background reactions should be low, this should you should keep in mind so that you get a slow reactions with background and fast reactions will be your expected reactions, ok.

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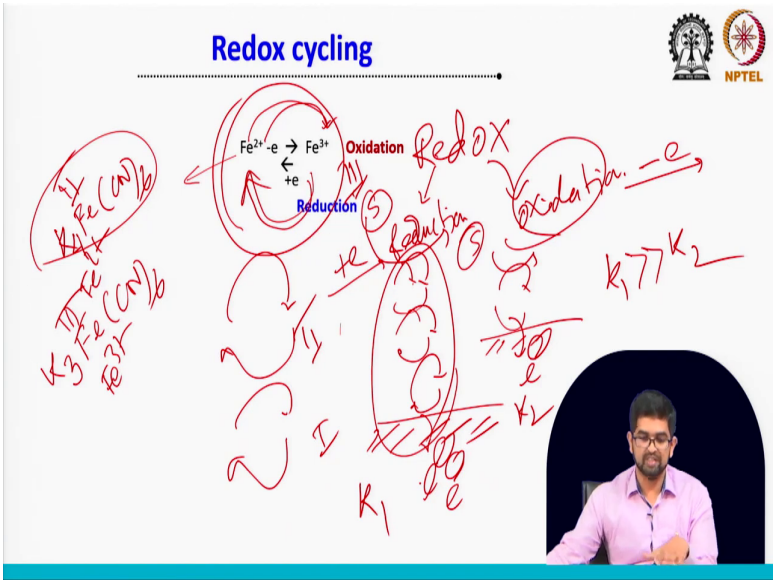


Now, I will come to the redox cycling. What is redox cycling that can help the signal amplifications? So, redox cyclings it is the two reactions one is the redox means red means reduction, ox means oxidation. So, you know reductions means we have to provide some electron right, and oxidations means it will release electron.

You can see this step Fe 2 plus Fe 2 plus means this iron we can get potassium ferrocyanide K 4 Fe CN whole 6. So, we just mention Fe 2 plus here oxidation state iron 2. So, it is actually Fe 2 plus. So, that I just put the Fe 2 plus. So, Fe means Fe 2 plus means it is means like single like this some mediator or it is a complex iron complex and Fe 3 plus means K 3 Fe CN 6 here iron oxidization state 3 it is Fe 3 plus, ok.

See if it release the one electron then it will form Fe 3 plus. So, it means it is the oxidation say one electron releasing. So, when next slide I will say oxidation. So, you should keep in

mind that one electron it is releasing. Now, if we give one electron then Fe 3 plus will form the Fe 2 plus.

So, these oxidations and reductions we can make it cyclically on the sensor surface not only on redox I mean here is just for example, iron we can make many redox couple on the sensor surface and they can react each other like this way ha they can react each other. Like I cycle, II cycle, III cycle they can form on the sensor surface.

If the sensor surface like this way like to many like 2 or 3 cycle if we can make. So, then so, one substrate can take part. So, these electron transfer will be very very fast on the sensor surface if there is a this kind of redox cycling happen. But if the sensor surface only one redox cycling from substrate this electron transfer if we compare with the here electron transfer suppose here kinetics if we mention here kinetics 1 here kinetics 2 this k 1 will be very very high than the k 2.

So, that is why now we will focus on how we can make this kind of a redox cycle on the biosensor surface. So, that we can amplify the signal and we can achieve ultra sensing biosensor, ok.

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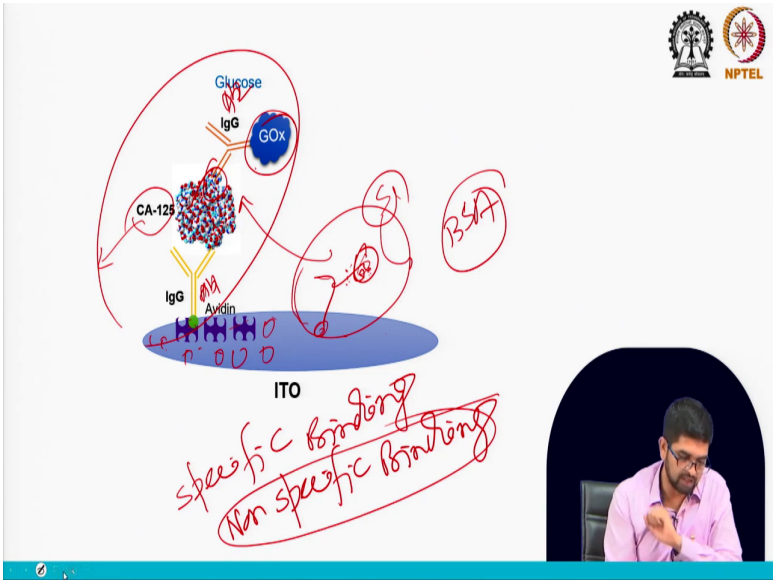


So, just now I told oxidations and reductions. Now I will come a real example with redox cycling. I think this part I already taught you last few slides you have a primary antibody; this is primary antibody on the sensor surface that is Avidin coated ITO electrode that is the bi functionalization, I already taught. This is the primary antibody and Avidin and this primary antibody already conjugated with biotin Avidin biotin they have strong interaction like this way I form a sensor surface clear.

So, this sensor surface is ready for CA-125 detection, this is a cancer antigen just for example, a cancer antigen I want to detect. So, I have to use a specific cancer antibody. So, generally I will tell you the brief procedure how we are developing this kind of sandwich biosensor on the sensor surface. So, up to this your sensor surface is up to primary antibody your sensor surface is ready for detections. Now, we will draw your target solutions.

So, if it has target then it will bind and then we will wash the surface. Because there is some other places where there is no antibody, but target still may be there we have to clean them otherwise the secondary antibody also can bind here and there because it is the specific side where other portion is the non specific side.

So, two terms come here one is specific binding another is non specific binding. So, what is specific binding? Specific binding means this is the like the antigen antibody binding here this is the specific binding. So, if antigen there then this secondary antibody it will bind here.

So, we need these portions because this is the expected your path. But sometime when you add the secondary antibody that is conjugated with some tag or label that suppose here glucose oxidase one enzyme, we tagged it the secondary antibody. And it also can bind here also sometime it should bind with the target.

But sometime there may be some your surface may behave such a way they can attract your secondary antibody that is conjugated. But that is not expected, why? Because as it is tagged it also can react with the some chemical it will form the like a signal.

Means you should get the signal once you have the target. But see here there is no target, but still if the secondary present, then you will get the signal because they can increase the background current. So, this is actually non specific binding and non specific binding can increase the background current, you do not have target still secondary antibody binding.

That is why after adding the secondary antibody you have to watch the surface very carefully. Otherwise, secondary antibody also can be here and there and they can react with the substrate it can generate the electron or current that is actually can actually it cannot count with the signal.

So, we have to be very careful that is why. How we can be careful means this is how we can avoid the non specific binding then? We can very carefully we can clean the surface after

adding the secondary antibody. So, that it should bind only here. Because they have the strong interaction they can easily bind.

But this non specific binding is very loosely bind this one. So, we can easily we can remove them by washing. Otherwise see there is some bare surface this bare surface sometime can help to bind this non specific binding.

So, that bares bare part we can add some small small protein molecule that is called BSA Bovine Serum Albumin the small small protein molecule if we can cover the surface then they actually will hinder the to not to bind this kind of secondary antibody on the surface. So, we can avoid the non specific binding, ok.

So, I just told you the like this. So, we can form a sandwich method on the sensor surface. Now, we can add here some substrate. So, ok so, now, I will show you the actual redox cycling reactions. So, let us remove the basic part of the sensor formation now. Now, I will show you only chemical amplification. So, how we can amplify, ok. So, I will add some substrate. So, here substrate is the glucose.

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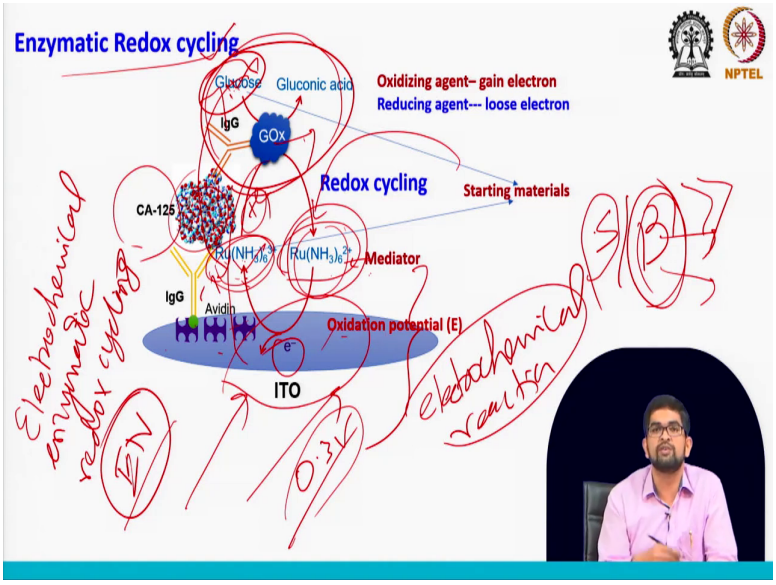


So, glucose will react with the glucose oxidase it will form the gluconic acid. So, here actually electrons we are getting glucose oxidase has the label it is also catalyst. And this will help to oxidize the glucose and we will use some mediator I told last time I use the iron based here I use the ruthenium based mediator.

Why it is mediator? Because it will help to take the electron from glucose that oxidize on the glucose oxidase and it will form ruthenium 3 plus ruthenium hexamine 3 plus ruthenium hexamine 2 plus. So, it will reduce because this electron we are getting from glucose and it will oxidize glucose oxidase it will help to transfer the electron. So, this reaction happen. Basically, this is what it is getting on electron, right. So, this is the reductions.

So, then we can apply some potential on the sensor circuit so that we can oxidize it, because we have to form a cycle. So, we can oxidize suppose we are applying here as a 0.3 volt we are

applying. So, it can oxidize it can release the electron. So, again it will form the ruthenium 3 plus.

So, this ruthenium 3 plus again it will get the electron from glucose after oxidizing on glucose oxidase. So, this cycle reaction happen again and again on the sensor surface. So, this sense these reactions is happening again and again and it show the ruthenium hexamine 3 to 2, 2 to 3. So, kind of it is reproducing.

So, redox cycling basically helping to make the kinetics faster by reproducing the mediator on the sensor surface, clear. And you will get the faster electron transfer. So, so, this is the mediator and just now you can understands here in this sensor surface what is the starting material. So, we are using glucose and you are using ruthenium hexamine 3 plus. These 2 is the starting material for this kind of signal amplification strategy.

See if you just thing if you have target then only glucose oxidase will be there, right. If you do not have target there is no glucose oxidase, right. And if glucose oxidase present then only glucose will oxidize and then only ruthenium hexamine 3 plus will form the ruthenium hexamine 2 plus.

So, you are using glucose and ruthenium hexamine 3 plus. So, to generate the high signal to background ratio your glucose and ruthenium hexamine 3 plus should not react to each other, there should not be any chemical reactions or very slow chemical reactions.

Because they are starting reagent, they will produce the background current. So, if there is no target like there is no target. So, only these and these will present because there is no glucose oxidase, ruthenium hexamine 2 plus also cannot form because they are not getting electron.

So, and ruthenium hexamine 3 plus because it is already oxidized form, if we want to oxidize it again ruthenium hexamine 4 plus is not stable much. We cannot get make the faster reactions. That is why background will be very very slow. So, we have to choose such a

mediator in the higher oxidation state. So, that it will not react very fast on the electrode surface.

So, it naturally we will get the low background and point target present that you have the CA-125 target, then it will bind with the secondary and with glucose oxidase and glucose will react then ruthenium hexamine 3 plus immediately transfer to a ruthenium hexamine 2.

Now, these reduced form should be highly reactive on the surface when we apply some potential. This is the electrochemical reaction. On this electrode surface we are applying some potential and it is highly active and it will release the electron. So, this part is the electrochemical part electrochemical reaction, ok.

This part electrochemical reaction and this part it is actually enzymatic reaction. See glucose reacting on the glucose oxidase enzyme form the gluconic acid release the electron. But it is kind of chemical reaction, but as we are using enzyme that is why we are saying it is the enzymatic reaction.

So, we can put some nomenclature in this kind of reaction. So, here it is a redox cycling reactions, but electrochemical reaction, electrochemical redox cycling and this part enzymatic. So, we can put a name. So, this is a redox cycling happening here. So, we can put a name enzymatic redox cycling. Because here enzymatic reaction and here some redox cycling electrochemical. So, we can mentions also it is electrochemical enzymatic redox cycling electrochemical enzymatic redox cycling

It has a one abbreviation we are saying it is EN, EN we can mention. So, let us clear this concept today. Next class I will bring again more redox cycling. This is just one example. This is one example I taught you today like enzymatic redox cycling. So, we can add some redox cycling procedure to amplify the signal and for that we can we have to use a mediator and we have to use some starting reagent some substrate.

So, here in this case glucose. So, your glucose and ruthenium hexamine 3 plus is a starting material and they should not react each other very fast. And also ruthenium hexamine 3 plus

glucose this mixture also should not electrochemical when we are apply some potential on the surface of they should not react fast. So, that we will get very low background, ok. So, background part clear.

So, we will get less current here. Now, when we will add the target then you will get actually your label, your tag your catalyst. Then your substrate will react with this then your mediator will start reactions and this should be highly active on the electrode surface when we apply some potential.

Then redox cycling will form and we will get very high current. Just keep in mind in the presence of target it should be. So, that you will get the very high signal. So, naturally your signal to background ratio will be very very high and will and this will be a very highly sensitive biosensors. This is just one example.

Next class I will teach you again like different kind of redox cycling. This is EN now I will change the tag we will incorporate some many other chemical and why I choose ruthenium here, why not any other mediator? So, we can incorporate many mediators also that I just I last slide I taught you know. So, like two or three cycle also make to make the reaction much faster to get high signal that also possible.

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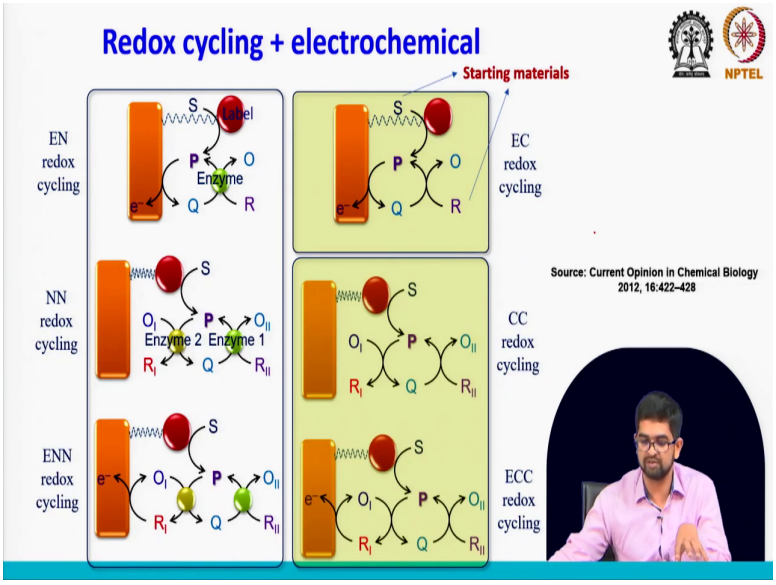

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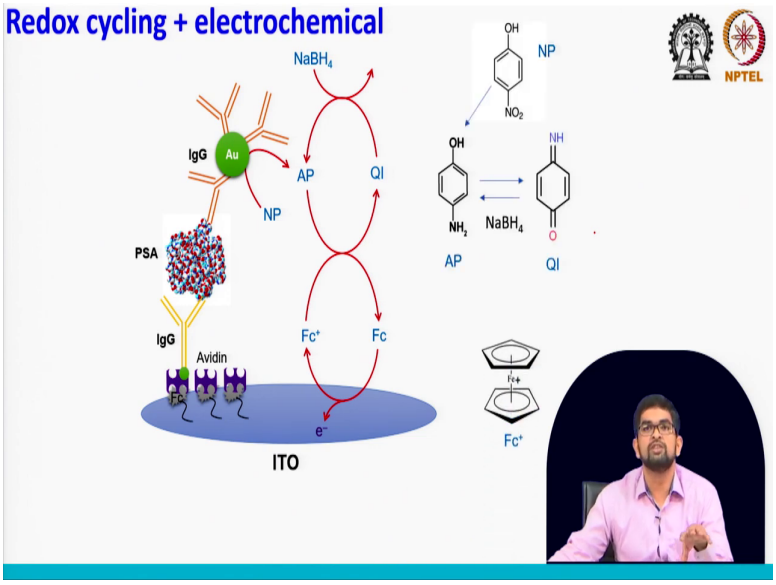

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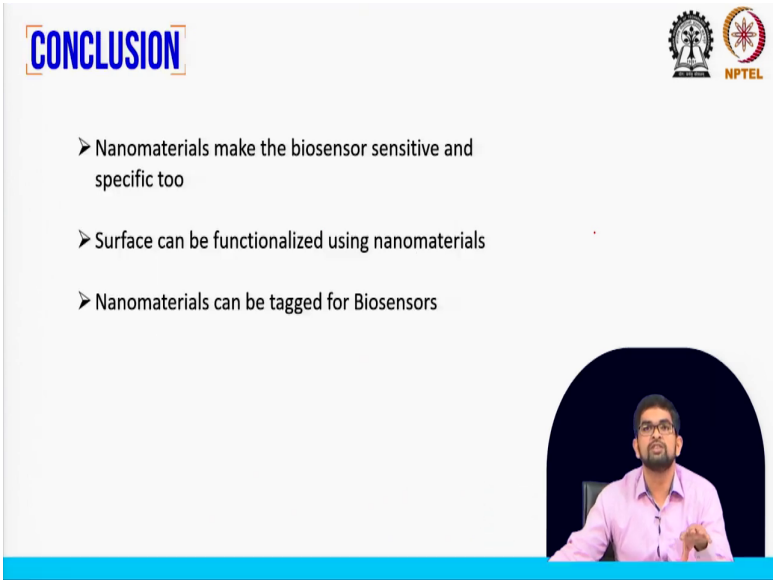


So, that things I will teach you in the next class. So, this is the different different redox cycling that I will teach you the teach you. And I will give you more example like we can bring some more tag not only the glucose oxidase we can make some other label based on nanoparticle that I will teach you in the next class, ok.

So, this is the things sorry. So, this is the conclusion spot the main conclusions here that nanoparticles can be useful for biosensor development and we can functionalize the surface and we can use this kind of fabrication technology by using some extra chemical for development a ultrasensitive biosensors. So, let us wait for the next class again I will teach you some more technology for getting the ultrasensitive biosensors.

Thank you. Thank you all.