Nanobio Technology Enabled Point-of-Care Devices
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Lecture - 06
Signal Amplification for Ultrasensitive Biosensors (Continued)

Dear students; so, today I will start again the Signal Amplifications and this amplification strategy for biosensing applications. So, last class I taught you how to amplify the signals using different kind of signal amplification strategy using some chemical. So, let us now show you different kind of amplification strategy and use them for biosensing applications.

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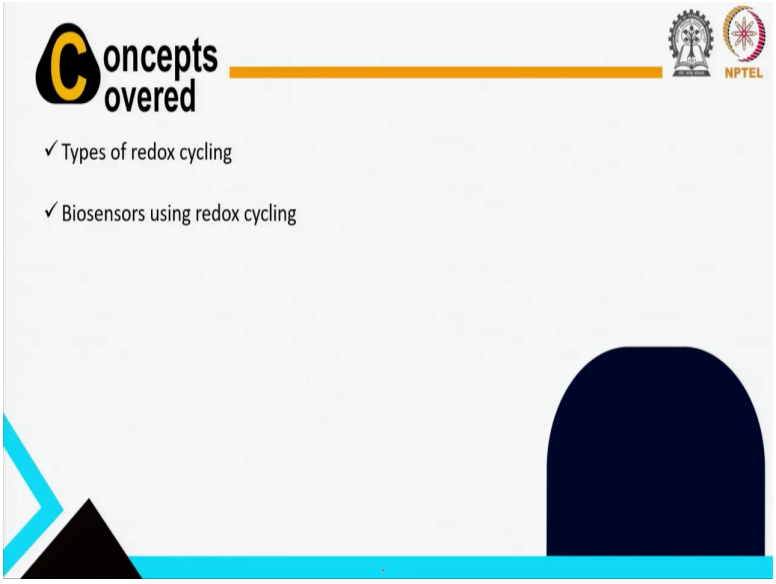

So, main concept I will cover today mainly the types of redox cycling that we want to use and we will cover the biosensors using the redox cycling amplifications.

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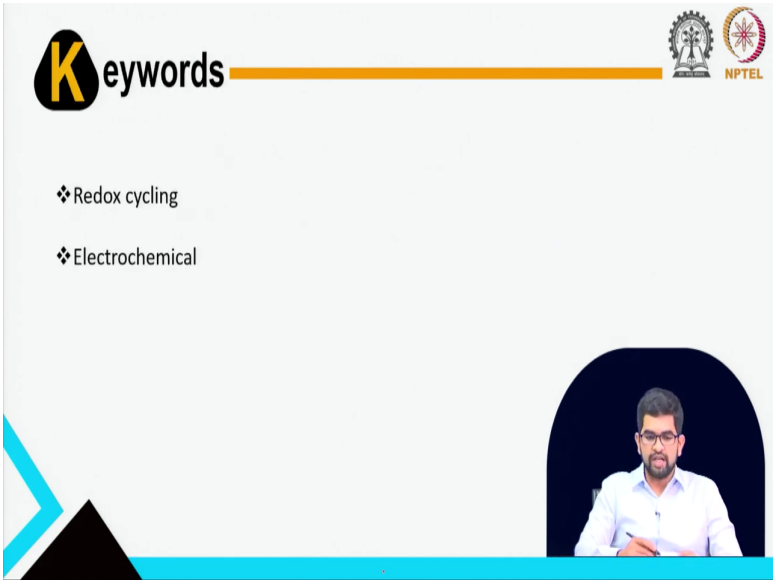

So, main keywords for these lectures you can search also like redox cycling, electrochemical redox cycling, signal amplifications. So, they are the main keyword for this lecture.

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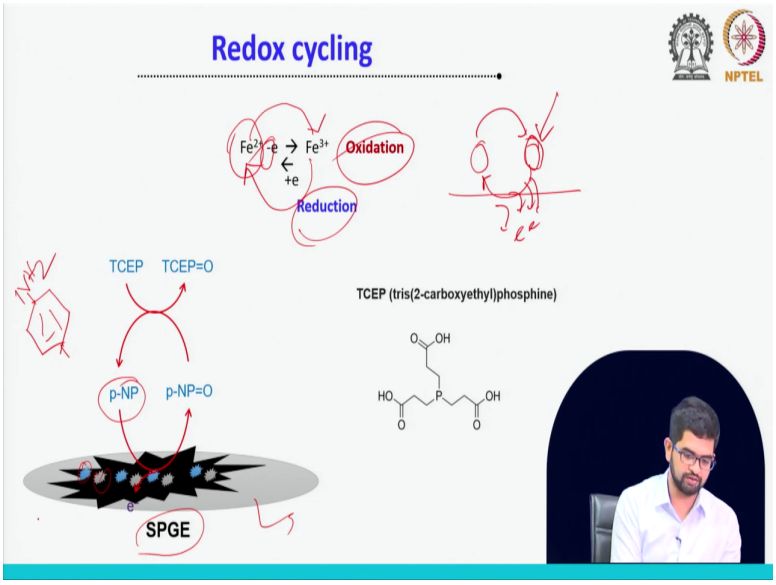

So, the redox cycling what is the oxidation, what is the reduction reactions that I already told you at the last class. But just I want to summarize say if you have a redox mediator like if you see that Fe 2 plus then if you want to oxidize it to Fe 3 plus means you have to means release one electron right. So, for that see e minus then this process called the oxidations.

Now, if Fe 3 plus to form the Fe 2 plus then it can accept one electron and form the reductions. So, when this oxidations and reduction process happens simultaneously on your electrode surface that is called the redox reactions. And redox cycling means; so, on your surface this species oxidized species or reduced species will be reproduced cycling way and you will get the signal amplifications.

Because, this cycling reactions can reproduce and can and means accumulate this on redox active species on the surface and it will help the many electron transfer on the surface ok. So,

let us that concept already told; now, let us come to a example after giving some example then I will come the names of the different redox cycling.

So, that you can interpret or you can design a how redox cycling you can use and you can design by yourself like this cycling or this reactions can be useful for signal amplifications for biosensor that you can try ok. So, here you can see this is a this surface you can see this is a skin printed glassy carbon electrode. So, on that this is just a transducer a electrode surface; on that surface say; so, you can see just to be modified using some nanomaterial.

So, this surface on the surface we want to use some redox cycling reactions. You can see here this p-NP this is just nothing but a Para Nitrophenol, para nitrophenol means this one this is NH 2. So, let us remove this shown right; so, see; so, we are using a redox species that is the para nitro phenol and this para nitro phenol will take part in the redox cycling reactions.

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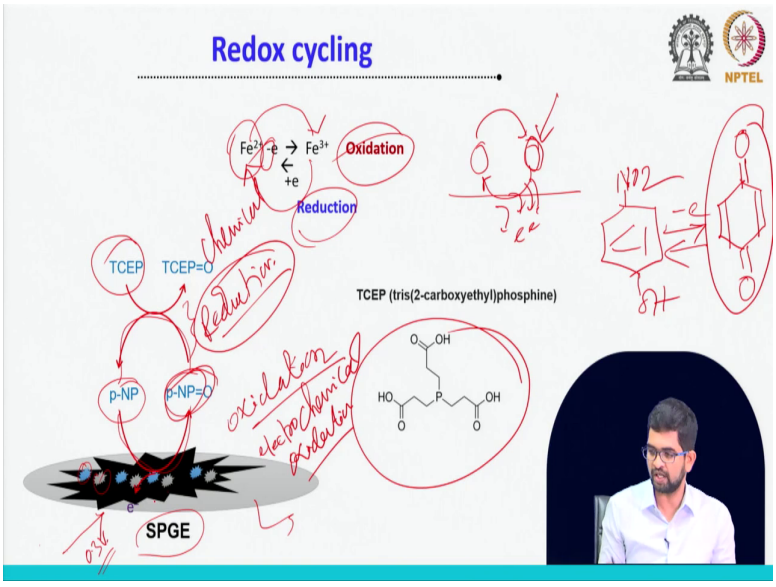

So, para nitro phenol nitro, nitro means NO 2 right NO 2 and nitro phenol means OH right. So, this species can form this is like NO2 could this para NP nitro phenol this can be this is actually oxidizing on the surface; means nitro phenol will form like the oxidized species it will form on the surface.

So, it will be o this one means it can form like quinine or maybe it can form also in the oxidized species in the intermediate and this kind of the intermediate step also it can form. Means in this means oxidized and reduced form we need a reducing agent, what reducing agent we can use?

We can use like a TCEP, TCEP is a reducing agent this is the structures of the TCEP and see it is reducing then again come back to this nitro phenol. So, this here is a chemical reduction process this is a chemical reduction ok and here on the surface we are actually oxidizing right; so, here oxidation happen on the electrode surface.

See so, this oxidation actually happen not by using any chemical right, we are applying here some potential; on the electrode surface we can apply some oxidation potential. Suppose you can apply some positive potential like 0.3 volt we can apply; so, that it can release the electrons; so, it can release the electrons and it will form the oxidized species.

So, this is actually electro because we are oxidizing this on electrochemically; so, it is electrochemical oxidations ok this is called electrochemical oxidation and here reductions, but this is chemical reduction and this is chemical reductions. But here oxidations and this oxidation is the electrochemical oxidations. So, by this way we can cyclically form this redox see this is consuming and then again reproducing consuming again reproducing right.

This kind of cycling reactions can be possible on the surface and it can be useful for signal amplification. So, I will show you some basic example like how we can use this one for biosensor development that I can show you.

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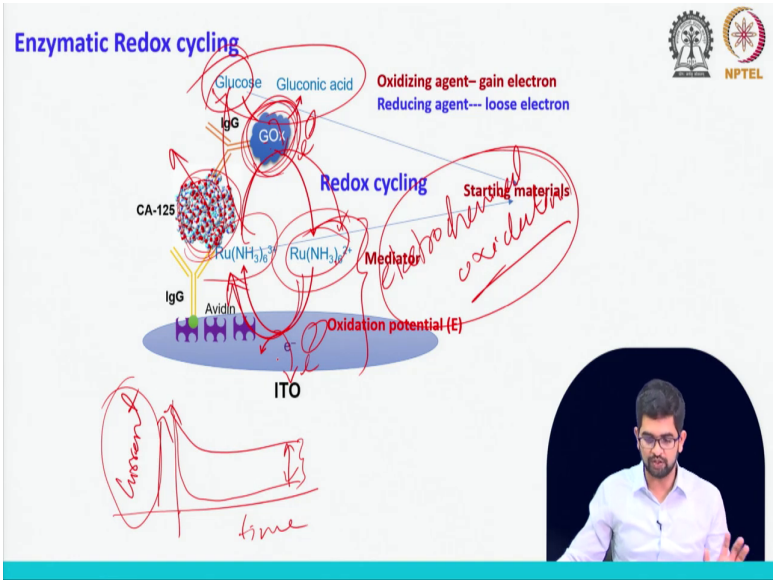

So, this is a very basic sandwich biosensor that I taught you last class, just I want to show you again to like glucose it will react with the glucose oxidase form a gluconic acid. And so, it is kind of oxidizing agent right and then we are using some electron we are using some electron mediator like you think its I mean 3 plus and that will reduce to ruthenium hexamine 2 plus based on this.

Based on this like oxidation say glucose oxidizing it will be releasing the electron that electron actually getting ruthenium hexamine 3 plus is getting right and from the ruthenium hexamine 2 plus. So, now we will again this one we will oxidize right on the surface we will oxidize it and it will release one electron.

So, again that I am saying; so, these oxidations again what oxidation reaction this one this is the electro chemical oxidation right, electro chemical oxidation ok. And here this reaction is

the enzymatic reaction, this ruthenium hexamine 3 plus we use at the very beginning at the starting region and glucose also we use at the starting region.

So, this ruthenium hexamine 3 plus actually cannot react faster with the glucose, but if we can if we use glucose oxidase then only this reaction can happen means ruthenium hexamine 3 plus can form the ruthenium hexamine 2 plus. So, glucose oxidase necessary, how you will get the glucose oxidase? If your sensor surface already have the target, this is the target.

So, then secondary antibody can only bind if it has target only secondary when you will bind and glucose oxidase may come on the surface. So, it is totally proportional to the target concentrations right, if you have more target on the surface means more glucose oxidase may come on the surface because the secondary antibody will bind on the target.

So, more glucose oxidase will react and this reductions from the ruthenium hexamine 3 to ruthenium hexamine 2 will be faster right. So, many ruthenium hexamine 3 plus will form, many ruthenium hexamine 2 plus; so, then this electron transfer rate this oxidation the electrochemical oxidation rate will be increased right.

So, where number of ruthenium hexamine 2 plus concentrations will be increased necessarily you will get the more current right. So, like this way we can determine the concentrations of the target right; so, if we get like this way. So, there is some output like if we can measure some that different technique, I will teach you again after some class. So, like I just for example, if you want to measure right just current current we are going to measure with respect to time.

So, if you have the less concentration ruthenium hexamine 2 plus, suppose you are getting this much current. And now, if you more target we more glucose oxidase and more ruthenium hexamine will form, then we will get the more current. So, you can see the difference between the lower I mean low concentration glucose (Refer Time: 09:44) high concentration (Refer Time: 09:45)

So, easily we can compare the number of target present on the surface; so, it will help you to predict the number of target. So, that again I will teach you when I will teach you that this kind of technique this is called actually chrono amperometry this technique that I will teach you after some class like which technique we can use for biosensor this kind of signal detections ok.

So, now it is pretty clear reducing agent oxidizing agent and the we are applying some oxidation potential right. So, here ruthenium we are using as a mediator and what is the starting material; so that is that is I told you right. And this is the redox cycling happening in between on the surface and this is the actually enzymatic redox cycling right that I told you also in the last class.

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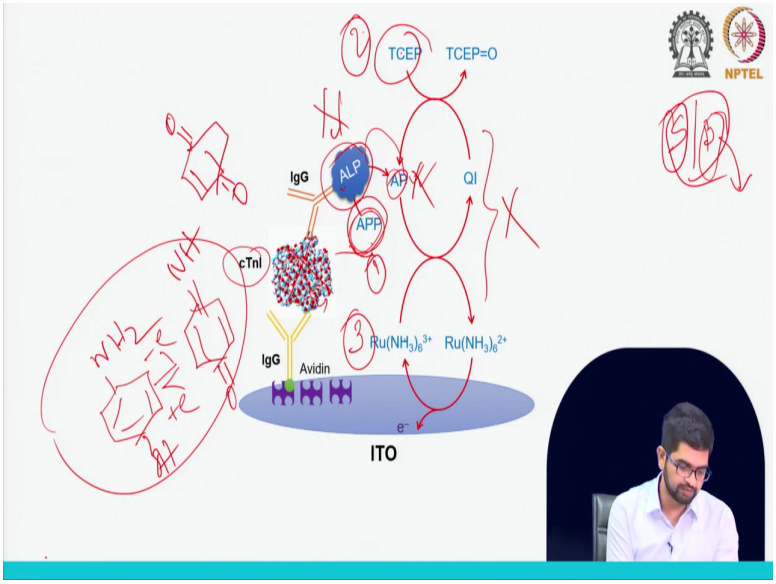

Now, I will come the another example right see this example; like, not only the one cycle like in the previous slide I told there is one cycle right ruthenium hexamine 3 plus from the ruthenium hexamine 2 plus. And this cycling happening and you will get the signal amplifications. But we can we can amplify its better way; if we can incorporate one more cycle, see here you can see in this scheme.

So, CTNI means Carbonic Troponin I this is just a target. So, if you have this target the secondary antibody can bind and secondary antibody this IGG actually we conjugate it with a enzyme that is called ALP Alkaline Phosphate ok. So, if in your sensor surface if you have target, then the secondary antibody will bind right that just I told you. Now, for this enzyme we need a substrate right, because enzyme will not directly participate in the redox cycling.

So, some electron you can say that some active material we form by using this enzyme and that material will take part in the redox cycling ok. So, we will use a substrate like APP amino Alkaline Phosphate and this one amino phenyl this is a for this is just a substrate for ALP. Now, when this substrate will react with ALP, it will form the AP, AP is the Amino Phenol.

So, this is amino phenol it will it can read easily it can form quinonimine the O NH or maybe it is sometimes it is the intermediator sometime it can form like this quinone. So, this is the electron transfer process like it can release one electron it can get one electron and this kind of redox reactions can happen; so, these are highly active species.

So, this ALP basically helping from APP to generate a highly electro-active species; so, AP here highly electro-active species but, APP no it is not a highly electro-active species ok. So, in this scheme now you have to tell right what is the starting material right; so, AP should not be starting material, if it is the starting material for this biosensor.

Starting material means that I told know like you have to think about signal to background ratio; so, signal means in the presence of target right. So, in the presence of target then ALP present, then APP will react then AP will form and then this reaction will start that is signal.

So, this all the reaction corresponds to the signal reactions and what is the background? Background means if you do not have this target this target you do not have. Then you then if there is no target no ALP right; if no ALP, then no AP AP will not form. So, if no AP if no AP, then this reactions will not happen ruthenium hexamine 3 2 2; so, these all the reaction will not happen on the surface.

So, your starting agent in the case of background, what is your starting agent? Reagent in this biosensor scheme APP, because the ALP not there say APP and this TCEP you are using a reducing agent. So, only in the presence target AP will be form; so, then thing we which one will be your starting agent? One APP, two TCEP and three your ruthenium hexamine 3 plus, these 3 will be your starting agent clear.

So, your background should be very low in this biosensor ok. So, let us remove again everything; so, I am just removing; so, that I can show you again the all the background signal. So, just keep in mind that your background signal should be very very low to design the ultra-sensory biosensor. So, in this case as I told your starting agent APP TCEP ruthenium hexamine 3 plus.

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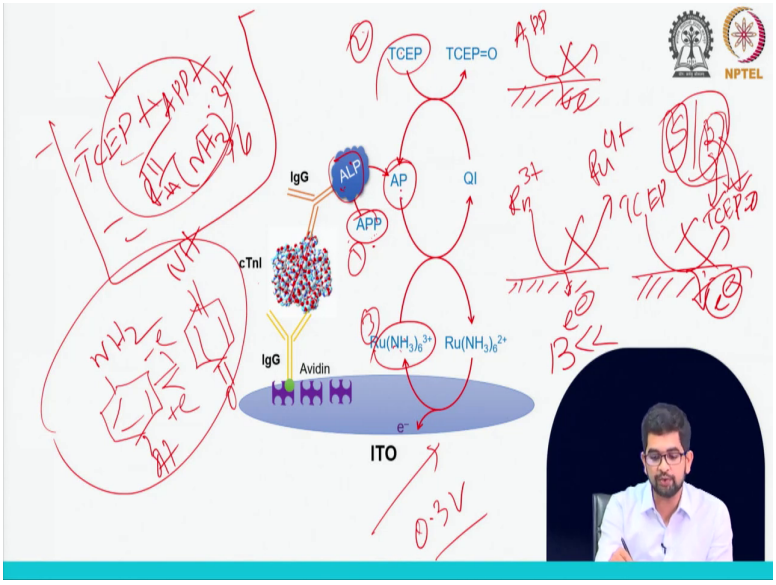

So, necessarily if you want to get low background current; so, these starting agent 1, 2, 3 they should not react each other; if they react each other means, they will generate the high signal. So, if you want to get low background and high signal then these two should react very very slow their kinetics reaction kinetics should be very very slow. One thing, these three together or you can like thing like separately also in the ruthenium hexamine 3 plus at the starting it present.

Because at the end of the reaction scheme I will show you that we are applying here some potential suppose 0.3 volt we are applying right from the outside. So, when you apply some potential, in that potential this starting reagent also can be oxidized on the surface. So, they should not also oxidize very fast on the surface right, then only we can generate very low background current.

So, ruthenium hexa I can draw now like ruthenium hexamine 3 plus it should not release the electron very far. I think you know also ruthenium hexamine 3 to 4 this is also not favourable process; so, anyway; so, this reaction rate will be very very low because this is not favourable process. And TCEP see TCEP also can be oxidized on the surface right that is also your starting agent TCEP, TCEP can be oxidized TCEP O, because it is reducing agent.

So, it can also easily oxidized, but it should not generate it should be very very slow or they should react a no reaction almost you know, this electron release almost it should be stopped; if it is slow then it is very good sensor ok. So, this is also should be slow process, another APP this is also starting agent you show your sensor surface your APP also should not react.

So, these conditions you have to keep in mind to get low background current and you are using these three mixture actually on the sensor surface right. So, you are using TCEP plus APP plus ruthenium NH 3 whole 6 3 plus right here oxidation state 3; so, you are taking this one in a sample tube right, then you are putting on the sensor surface. So, there should not be any chemical reactions also right, in this mixture no chemical reactions should happen; otherwise, the chemical they can change and they can behave something different.

So, you may get some current right; so, that also you should keep in mind this should not chemical (Refer Time: 17:50) should not react ok. So, then if this satisfy then your background will be very very low right your background will be very very low. And let us get to the signal part; so, your signal part is ALP there, because your target there ALP there, APP reacting with the ALP and AP form.

So, this AP is highly electrochemically active, immediately AP will react with ruthenium hexamine 3 plus. So, you have to keep in mind let us erase it and everything here; so, what is the signal part right. So, which part there should be the high reactions and which part there should be the very very low reaction rate ok, ok let us show you here now.

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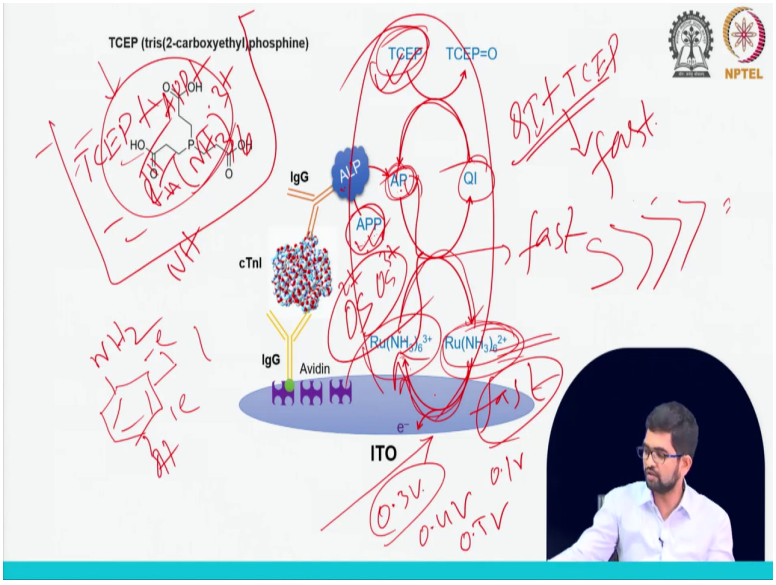

So, this AP and ruthenium hexamine 3 plus; so, they should react very fast right. Then only ruthenium hexamine 3 plus can generate ruthenium hexamine 2 plus very easily, because this will finally, will oxidize on the surface, it will generate the electron you will get the current. So, these reactions should be very very fast and when AP react with ruthenium hexamine 3 plus it form the oxidized species quinonimine.

So, this quinonimine should reduce to amino phenol immediately, why? Because then again amino phenol can react with ruthenium hexamine 3 plus so, that this cycle will be very fast. So, quinonimine plus TCEP this reaction should be very fast; so, you have to choose; so, I am telling this as generic idea. So, even you can change this reducing agent you can change reducing agent, you can change something else also instead of using AP, APP something different.

ALP has so many substrate, you can use something different substrate now you can produce the another electro active species. So, you can optimize which reducing agent is the best, which substrate is the best that we can optimize, then we can get the best redox cycling method. So, in this redox cycling case we found that quinonimine can easily form amino phenol with TCEP that is why we choose the TCEP.

But there is some other reducing agent also like sodium borohydride that also you can try lots of scope also there ok; so, this process done. Now, as you say as you seen you have seen that ruthenium hexamine 3 plus already form ruthenium hexamine 2 plus. So, ruthenium hexamine 2 plus should react on the electrode surface very fast, because this is the final species that we are going to oxidize on the surface right; so, this should be react on the surface very very fast.

If ruthenium hexamine 2 plus itself if it is reaction means electrochemical oxidation reaction is very slow, then you will get the lower current your signal will not be very high. So, you will get a very very high signal if your ruthenium hexamine 2 plus will oxidize on the sensor surface very fast; so, this reaction should be again very very fast clear.

So, these things just you will keep in mind who is electron mediator will you use; here I use ruthenium hexamine 3 plus maybe you can try something else also, if you see like periodic table in the same there is the osmium also. So, osmium 2 plus osmium 3 plus this redox couple also we can try.

Let us see if they are showing the high amplification or lower amplification by comparing this then you can try which one is the best. So, just this is the example, but you have lots of scope you can change also by using different different chemical. So, it is that is why I am saying it is as a generating concept, but you can change any chemical you can try there is still scope like you can change the substrate, you can change the reducing agent, you can change the mediator, and you can get the best one and another thing.

So, here as I am always saying we have to apply some potential like finally, suppose you are applying 0.3 volt, but you can apply 0.4 volt, you can apply 0.5 volt, 0.1 volt; but, which potential is the best to oxidize this ruthenium that also we can optimize. So, which potential we can apply like this way we can predict that this redox cycling is the best or not. So, you can find out the best redox cycling and that you can try for your target or biomarker detections ok.

So, this is one example that is why I am just describing you very very clearly; so, that you can understand the all the steps. So, in this picture I think it is very clear that I describe everything that how we can design a redox cycling and how we can change the material; so, that is a structure you know now let us come here.

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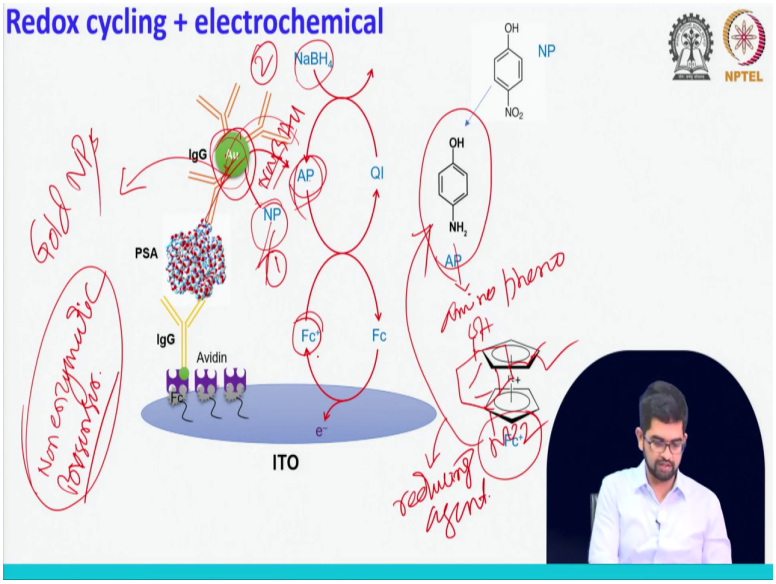

So, see in the previous slide you have seen that ALP I used as a tag, this slide I am showing you here that in the last slide you have seen the tag is the ALP. But here I am using gold nanoparticle Au means gold nanoparticle. So, here I am using gold nanoparticles as the tagging agent; here it its role similar like ALP, but it is non-enzymatic material right.

So, this is the utility here that we can use some nanomaterial also as a tag that can help to generate the similar kind of signal, what is the utility that we are not using here enzyme? So, it is non-enzymatic it is non-enzymatic biosensor. So, what is the benefit because enzyme is the protein; so, if you want to develop a point of care device; so, sometime you have to store your sensor at the room temperature the high temperature.

So, at that temperature as enzyme is protein can be denatured easily your sensor may not work. So, there is some drawback using the enzyme and what is the benefit of using enzyme? Because enzyme is very very specific that is the utility of the enzyme and reaction kind of also very fast. But if you can eliminate the enzyme by a nanomaterial that will be very good for your sensor design because you can store it even at the high temperature.

So, this non-enzymatic sensor will very much useful for point of care device development ok. So, here I use the golden nanoparticle and NP just a nitro phenol NP means; so, this is the nitro phenol no; so, NP is the amino phenol means NH2Oh present amino phenol we are using. So, this amino phenol now amino phenol ok; so, let us clarify this one amino phenol amino phenol and this NP is the nitro group nitro phenol.

So, this nitro group will be reduced to will be reduce to amino group; so, we need some reducing agent ok, we need some reducing agent. So, what is the reducing agent? Sodium borohydride basically here, NaBH BH 4 can be used for a reducing agent. But for this nitro phenol nitro phenol I mean this one can be reduced to amino phenol in the presence of sodium borohydride, but we need a catalyst this catalyst is the gold.

If your sensor suppress present gold nanoparticle, then only amino phenol will form otherwise this amino phenol will not form ok. So, you need gold nanoparticle I mean this tag like similar way like previous case you need the ALP always then only amino phenol will form.

So, in this case you always need gold nanoparticle then only sodium borohydride can reduce the nitro phenol to amino phenol clear. But this gold not directly participating in the redox cycling; just keep in mind this one not-daily participating, it is forming a very active material electroactive you can say or maybe chemically active material it is forming that is the amino phenol it is very active, but nitro phenol is not active right.

So, in this case what is the starting material then you can easily find out nitro phenol NP the starting material, sodium borohydride is reducing is in the starting material and Fc plus this is a this is just like the ferrocene. So, I will show you here yes; so, you can see here ferrocenes huh; so, this ferrocene is the starting material. So, ferrocene is like the structure here; so, five membered ring and irons here the you can see the iron; so, this is the this is actually we are using as the starting material.

So, naturally they should not; so, ferrocene plus as I said before like this ferrocene plus, nitro phenol, sodium borohydride they should not react because they are causing the background current clear. And as in the presence of gold nano particle amino phenol form; so, amino phenol should react very fast with the ferrocene and it will form the ferrocene 0. And it can react easily on the sensor surface it will generate the electron that is the very fast electron transfer.

And ferrocene plus should not react the sodium borohydride this reaction should be slow, but this sodium borohydride should react with the quinonimine very fast; so, that amino phenol will regenerate again see. So, in the previous slide I showed only if you go back to the previous slide let us see. So, here like 2 cycle, here 1 cycle; so, but here you can see again the 2 cycle, here 1 cycle, here 1 cycle like 2 cycle.

So, more cycle means the electron transfer rate also will be very faster. Number of cycle can improve the electron transfer rate and your signal naturally will be very very high.

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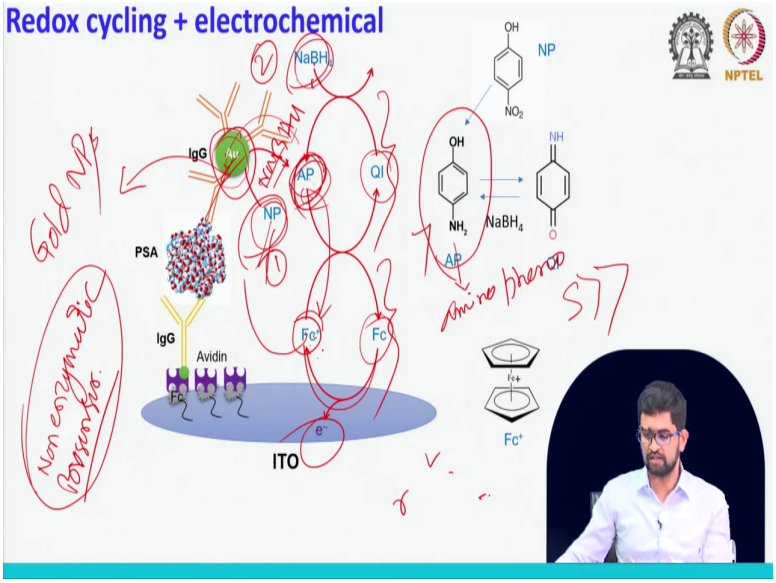

That is why sometime we are incorporating more number of cycle to enhance the signal ok. So, this things that this is this things just you have to remember that how they are reacting.

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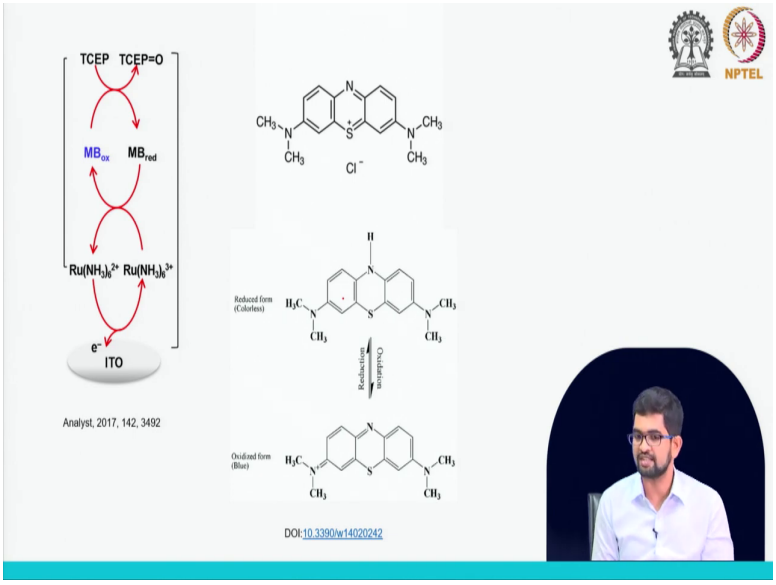

And another things here I am I just showing you like methylene blue also can be used for the redox cycling reactions, but that I will teach you again in the next class, methylene blue one kind of the chemical. So, this kind of also chemical also can be used for the redox cycling reactions, but that I will teach you again the next class.

So, this is the utility means I am teaching slowly like enzyme to non-enzymatic method. So, that we can tag the antibody with some now first I showed you enzyme then I showed you nanomaterial, now I will show you some with some chemical we can tag, slowly we can increase the stability of the sensor ok.

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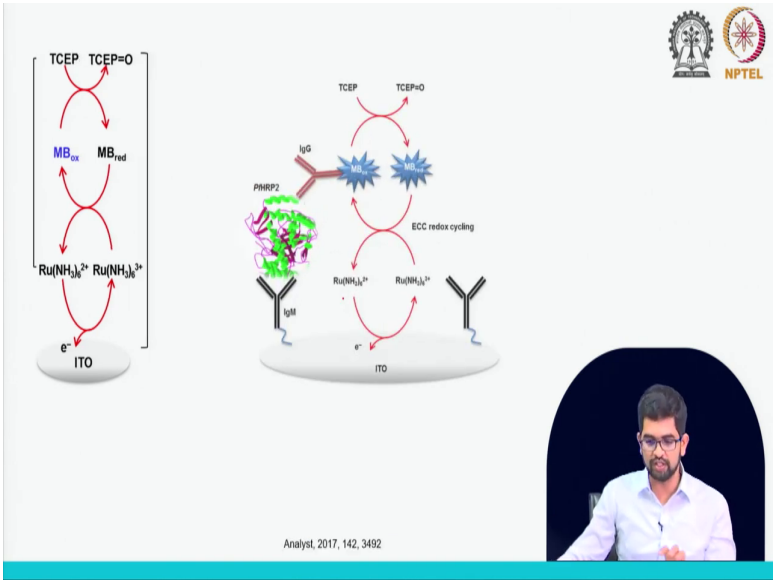

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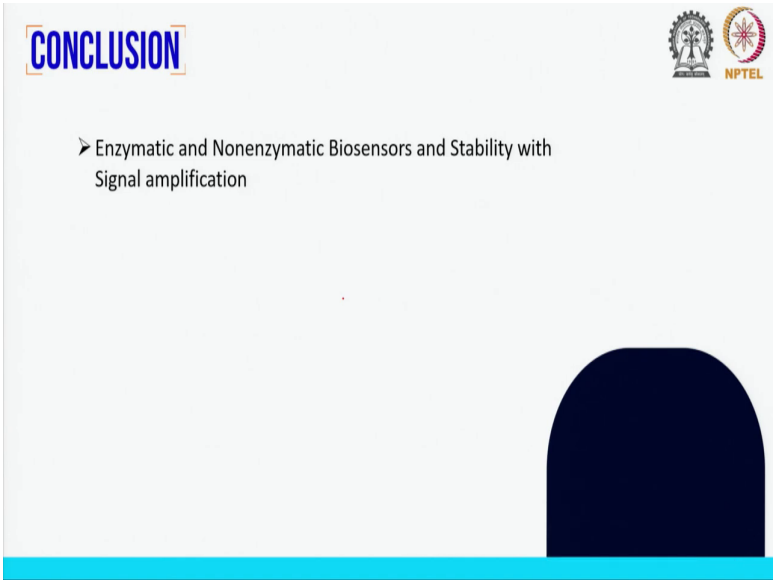

That I will show you slowly in the next class; so, the conclusion. So, the enzymatic non-enzymatic biosensor we can improve the stability with the signal amplification. So, that we can use this one for biosensing applications; so, we can detect very low amount of biomarker; so, that is all for this class.

Thank you. Next class I will teach you again this signal amplification.