Nanobio Technology Enabled Point-of-Care Devices

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Lecture - 07
Signal Amplification for Ultrasensitive Biosensors (Continued)

Dear students so last class I taught you different strategy of the signal amplifications again I will continue because there is different class of the signal amplification there. So, I will continue few classes like this way using different example, then only you can understand the whole story because there is lots of design you can make.

So, you should know like one by one all the design, then I will show you how to make the nomenclature of the all the design. So, there is some nomenclature also make the nomenclature and then I will finally, show you the how you can use this one for biosensing like device applications ok.

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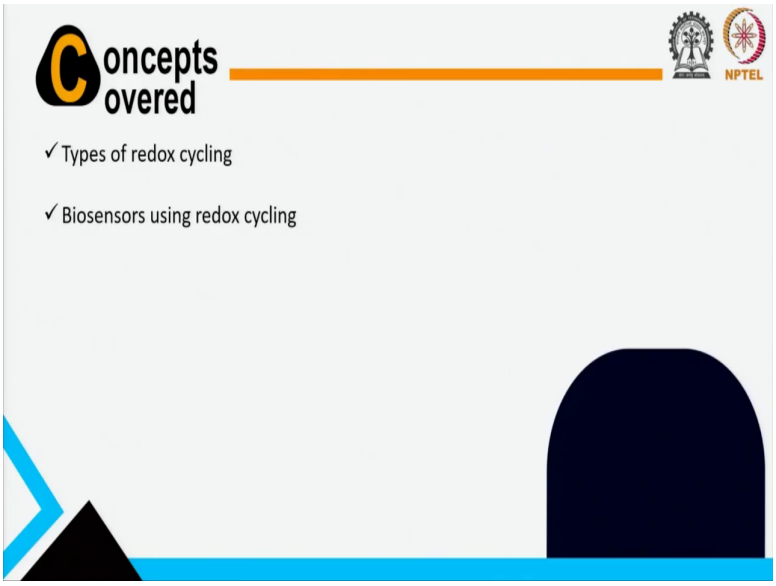

So, let us come to the next slide. So, today I will mainly concept that I will cover that is the types of redox cycling. So, different types of the redox cycling we can cover. So, it can be that I showed that we are using actually chemical reactions and with combinations of electrochemical reactions right.

So, there can be chemical reactions, chemical reaction, then electrochemical reaction like similar kind. So, we can put different different nomenclatures that is why today actually I mainly I will focus of the types of the redox cycling. Then biosensors some biosensors where we can use this kind of redox cycling.

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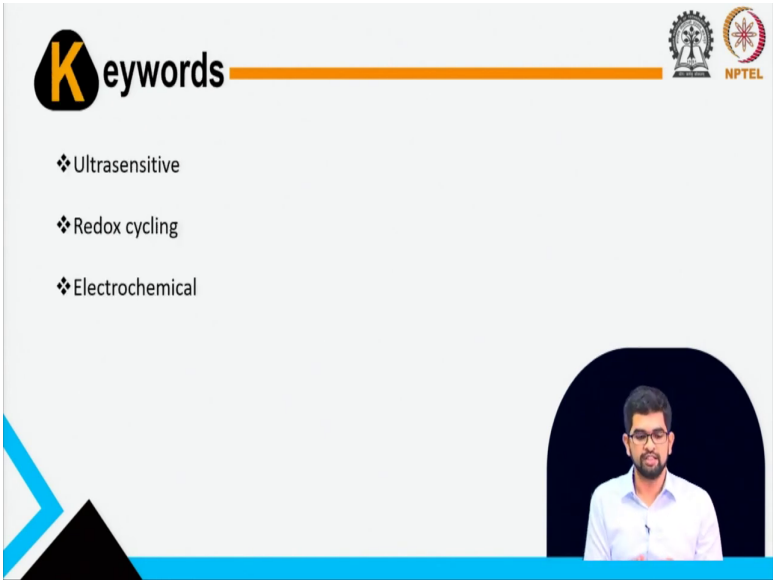

So, our main topic of this all the classes is the ultra sensitive biosensors development using redox cycling and this is mainly electrochemical Also chemical we are using their story I will tell you now.

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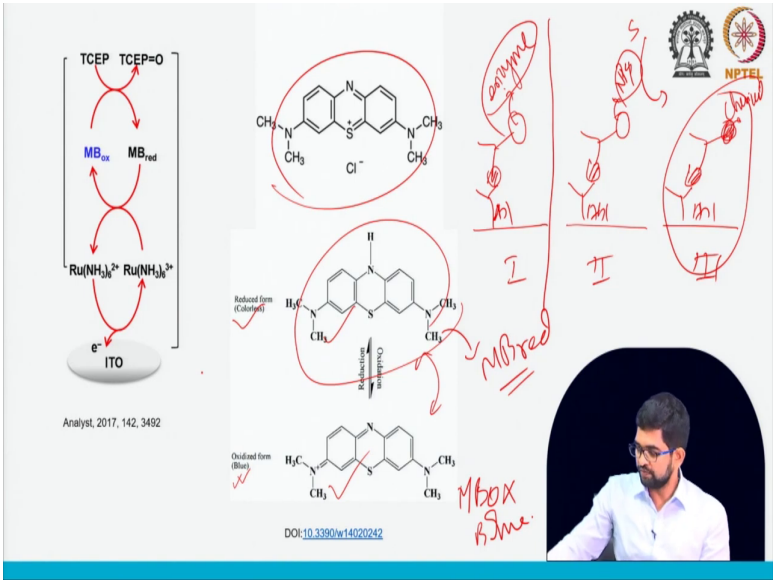

See last class I stop here that we can tag the antibody with different material right. So, let us summarize first. So, like three biosensors I am sorry I am going to show you I, II, III. So, I. So, see every cases we are we can immobilize the antibody for as for the receptor we are using just a primary antibody 1, antibody 1, antibody 1. So, if you add the target here, so it will immobilize.

Now, the secondary antibody we can tag, secondary antibody we can tag now we can label right. So, this label can be enzyme, this label can be some nanoparticles or this label can be any chemical ok. So, there is some utility as I just mentioned last class. So, enzyme they are very much specific is very good not much like side reactions because very very specific, but nanoparticle case you have to be very much careful.

So, you have to design or you have to develop a nanoparticle that should be very specifically it should react with the substrate. So, that is why you have to choose a very specific nanoparticles and chemical skills also you can use some chemical that can react with some substrate and it will generate some signal that part now I will show you the chemical part. So, enzyme part I showed you already like ALP you can use nanoparticle case like gold nanoparticle you can use that with some nitrophenol last class I showed you.

Now, we are going to show you the chemical you can use as a tag. So, this chemical is a methylene blue you can see the structure of the methylene blue. Methylene blue has two species; one is oxidized form and the reduced form see this is the reduced form it is the color less and oxidized form it is the blue form. So, your so that is why in the next couple of slides I will show you like methylene blue ox it is the blue color and methylene blue red it is the colorless.

So, these chemical can directly take because it is a oxidized form reduced form. So, it can take part in the redox cycling process. See one positive part here like few class last few like last class as few slides I showed you like enzyme or nanoparticles they are not directly taking part in the redox cycling. But in this case as it is a chemical and it is formed see like one is the reduced form and the oxidized form they can directly take part in the redox cycling.

So, we do not need any some extra chemical we can reduce the number of the chemical right. So, sometime you may ask if we can if we use the redox cycling this kind of amplifier process, we need many chemicals yes that is I completely agree. But slowly we are trying how we can reduce the number of chemicals. So, that, but still, we will get the similar implications slowly I will teach you this one. At first, I showed you that like we want chemical now I slowly I want to show you that how we can reduce the number of chemicals also, but we still get the similar amplifications.

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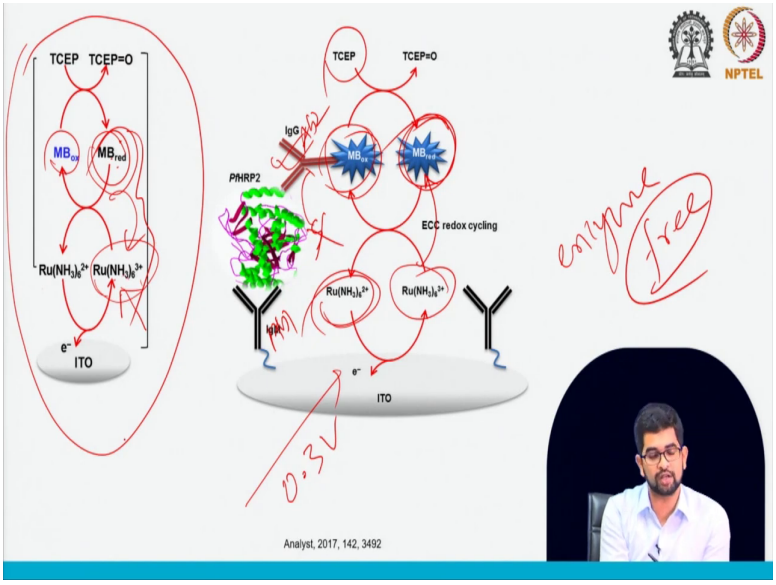

See so let us show you first actual sensor. So, at the last class I showed you that this is the ALP was used or gold nano particle was used that is separable right it was separate and it was reacting with some substrate and it was formed some active material that one that participated in the redox cycling.

But see in this case what is the new thing? See in this case the tag that I used with the nano with the secondary antibody right AB 2 antibody 2 this is antibody 1. This is that directly participated in the redox cycling right. So, last time we use some substrate right we do not need this substrate in this case.

So, naturally we are reducing one chemical here hm. See so methylene blue oxidized form we used. So, these oxidized form can react with the TCEP, TCEP reducing as a it can reduce the methylene blue red reduced form and we used here ruthenium hexa amine 3 on the sensor

surface. And these ruthenium hexa amine 3 can react with methylene blue red mean reduced from the methylene blue and form the methylene blue oxidized form again.

So, we are generating ruthenium hexa amine 2 plus on the surface because it is a highly active material right. So, electrochemical when again we will apply some potential it can easily oxidize on the surface, we will get the very high current. So, our problem solved, but we do not want any extra substrate. So, we do not want any extra substrate and this is at the same time enzyme free right here there is no enzyme.

So, this is see you can see the left hand side the complete reactions here when you can design like this way. So, methylene blue ox that is an oxidized form you are using with the secondary antibody that can react with the TCEP from the methylene blue red and this remains reduced form and this reduced form of the methylene blue reacting with ruthenium hexamine 3 plus.

So, this reduced form, so it should get the oxidized form. So, you can ask sir why we are not using ruthenium hexamine 2 plus because your starting agent should be higher oxidation state. So, that it can react with the methylene blue reduced form right. So, if it is already ruthenium hexamine 2 plus it is reduced form this is also reduced form. So, both reduced form cannot react because this is the chemical reactions.

So, this kind of electrochemical or chemical reactions case when you are generating the high signal in the redox cycling. So, certain part is the chemical reaction certain part is the electrochemical reactions, so that we can put some nomenclature for that. So, that I will come now first I will show you the example then I will come the nomenclature.

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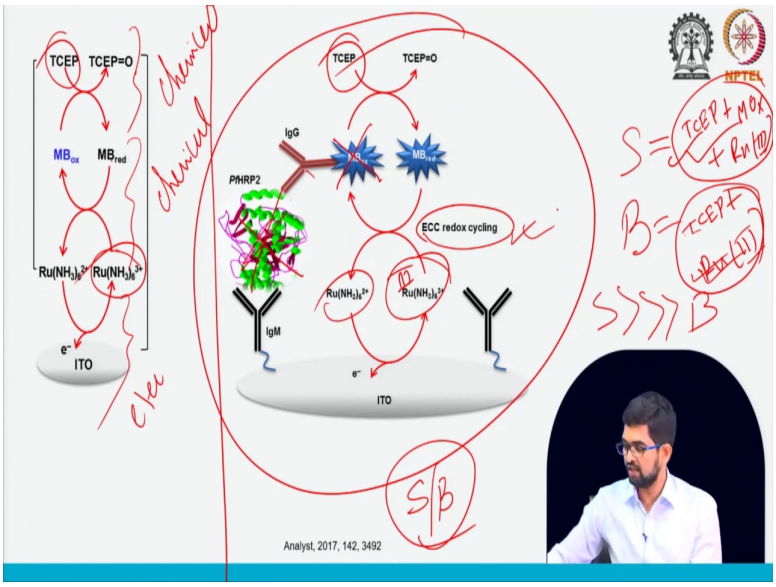

So, here one chemical reaction right, here one chemical reactions and this part electrochemical reaction right this is chemical here also chemical and this part electrochemical right. Clear so, like this way you have to choose the chemical all the reagent also which reagent is I mean which oxidation state reagent you should try that you can decide by yourself.

So, in this case what is the starting reagent then ruthenium hexamine 3 plus starting reagent right and TCEP these two is the starting reagent why these two starting reagent? See last cases there are extra starting reagent was there, but in this case, we are using less number of the starting reagent.

So, we are reducing the number of the chemical right. So, see the actual biosensor so, see this this image. So, in this biosensor say what is the signal and what is the background? This

should be clarified. So, signal means in the presence of target so, this is the PfHRP2 a malaria antigen. So, if it is present then only methylene blue that conjugated with the antibody that will come right otherwise it will not be here. So, let us remove it for the background case, but signal case let us keep it.

So, if methylene blue ox there, then it will react to the TCEP, then this one will react to the ruthenium hexamine 3 plus it will come to 2 plus. So, this TCEP plus methylene blue ox plus ruthenium 3. So, I am writing ruthenium 3 because this one is the ruthenium 3 right ruthenium hexamine, I am writing the ruthenium hexamine 3 second one I am writing this is the signal and background case. So, let us leave this one leave this one because its background. So, this case TCEP and 3 this will be there 2 will be generated right.

So, TCEP plus ruthenium 3 so, this is the background. So, naturally you have to think about these reagents should not react each other, but these reagents should react first they should react first, but they should not react very fast. Then signal will be very very high then background and will necessarily your signal to background is very very high.

So, again I am saying this one this is a non-enzymatic biosensors will develop using some redox cycling. So, this redox cycling is the ECC redox cycling I mentions here this is the nomenclature that I will come now. So, how we can I mean give a nomen name of this reaction right let us come this slide.

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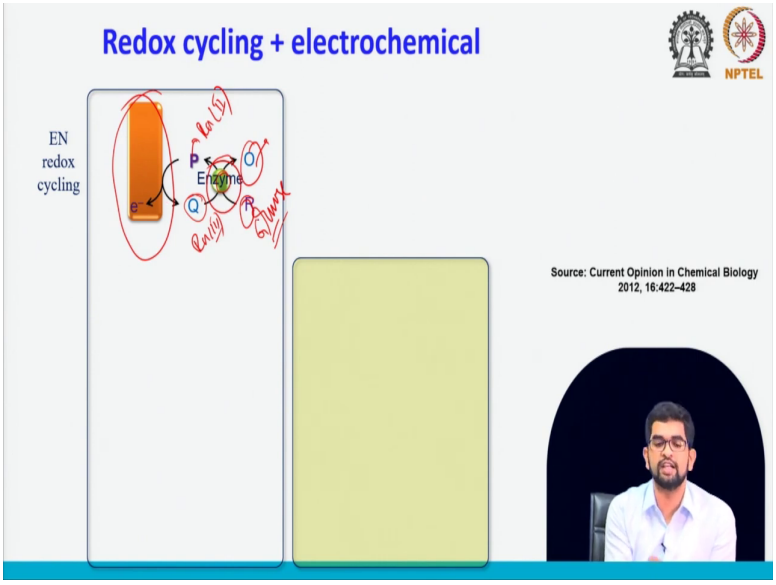

So, redox cycling plus electrochemical this two see. So, here I showed you one this is just a transducer this one just the electrode surface. So, here you use the enzyme something like glucose-oxidase as I mentioned know. So, R means the reducing agent suppose.

So, in this case like last time I use the glucose right glucose is the R here and it from the gluconic acid. So, O is the gluconic acid for example. Then here I use the which one? This Q; Q is the oxidized form suppose it is the ruthenium 3 P is the reduced form suppose is the ruthenium 2 you can remember last slides I told showed you that with the enzyme hm. So, because here enzyme directly taking part in this redox cycling.

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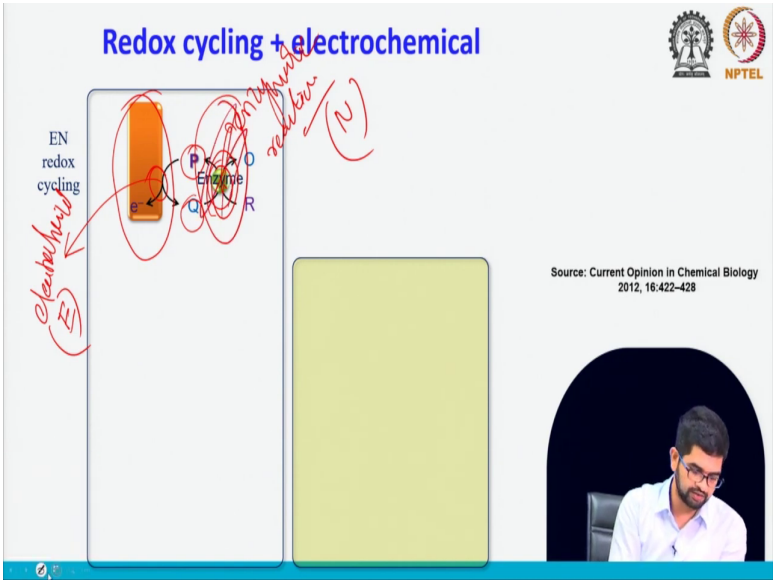

So, I can mention this this one here and why it is redox cycling reactions, why it is redox cycling reactions? Because P actually generating; P generating here from the Q with the help of enzymatic reaction. So, enzyme directly taking part and so, we are oxidizing here right here we are applying some electrochemical potential or some electrochemical chemical when electrochemical behaviour that is we can see here on the electrode surface. So, this is the electrochemical reaction right.

So, P can be oxidized it will form the Q that is the electrochemical part. So, this is electrochemical part and this part enzyme. So, it is enzymatic reaction not chemical only it is chemist chemical reaction definitely, but enzymatic reaction; enzymatic reaction ok. So, that is how we can keep a nomenclature.

So, electrochemical if it is the electrochemical reactions, we will put E this abbreviation enzymatic case we will put N this abbreviation ok. So, as it is enzymatic so, this case enzymatic ok let us clear these things and again I will show you. So, I will put the nomenclature.

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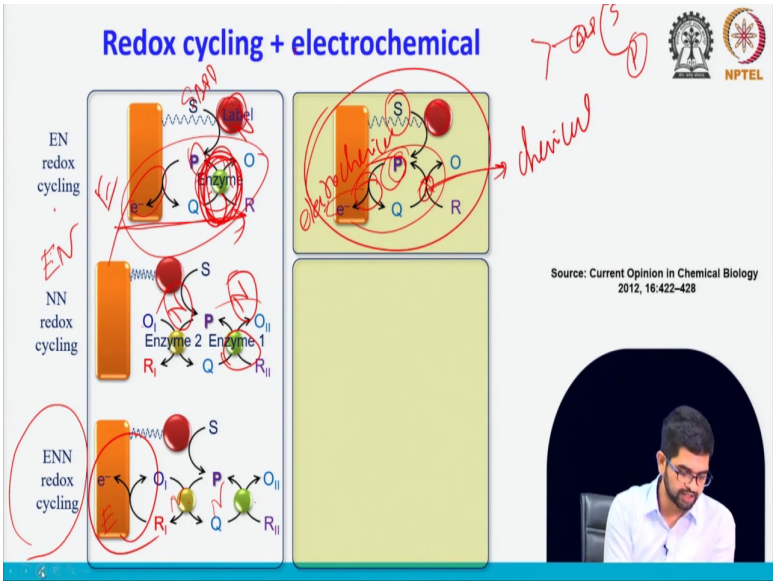

So, electrochemist when it is electrochemical reactions, we put E when enzymatic reaction we put N. So, naturally we can give a nomenclature this one EN means from bottom means from electrode surface to bulk reactions. So, electrochemical and enzymatic so, it is called EN redox cycling ok. So, now, you can this if you can increase the more enzymatic reactions.

So, here actually I see here I can use some label that label means some like I mentioned know like ALP you can use. So, here you can use the APP some substrate it can form the AP that is

also possible. So, something like this like any kind of reaction you can use that is why I showed you first some example.

But anyway, in the redox cycling, enzymatic reactions and electrochemical reaction that is all. Now, see here I will show you the another two example where we can use more number of enzyme and they will directly take part in the redox cycling reaction.

See enzyme 1 and enzyme 2 two enzyme, so two redox cycling here one redox cycling and then this one can be redox cycling if you apply electrochemical reactions mean some potential we can apply, but that is we still not applied yet. So, we did not apply any electrochemical we still not apply here any potential. So, here two enzyme range took part in the redox cycling. So, here one N here another N right, then see N now if we apply here electrochemistry part I mean electrochemi chemical part see here.

So, we applied here see what you can say then here then you can say that this is E this is N, this is N right. So, we can say this is ENN redox cycling clear. So, why this is E N redox cycling? Because here we applied two enzymatic reaction one electrochemical reaction. Now, now as I told you that slowly we want to develop some biosensor where we do not want enzyme right let us slowly remove one by one the enzyme.

You can see here in this scheme right hand side in this scheme you see here once reactions, but here no enzyme see it is just a chemical reaction last cases we use enzyme right this is we use enzyme, but here no enzyme just a chemical reactions ok. So, here we use just a chemical reactions. So, this is chemical.

And here we just use see here electrochemical right. So, P form the Q after applying some potential. So, this is electrochemical; electrochemical right. So, here electrochemical this is chemical and this P actually form using some reactions side reaction where we use some another substrate that can react with some tag that tag can come from the antibody.

So, if you go back, I mean I showed you last few if you go back like your last class and in today's lecture some other slide. So, we conjugated your antibody with some tag right, it can

be some label like ALP or even you do not want ALP you can use the gold nanoparticle and with some substrate it will form some PP means some product that can be participate in the redox cycling it is that way and this can directly participate here. So, here one cycle, but one chemical part one is electrochemical part right.

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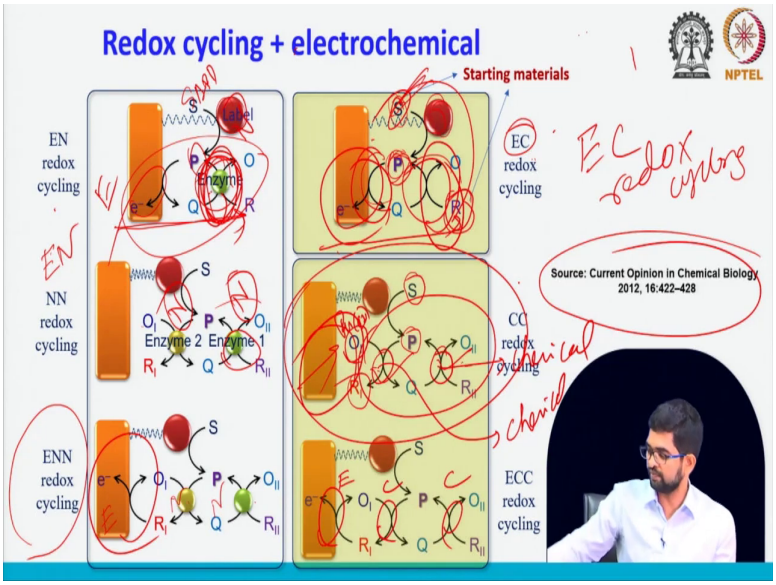

So, let us put the nomenclature then now we can put some nomenclature here ok what nomenclature you can put here. So, here we can put the nomenclature it is ECC redox cycling, EC redox cycling we can put EC redox cycling. Why EC? Because here electrochemical and here chemical that is why it is EC redox cycling.

And here we use which one is the starting material? See reducing agent R is the starting material and your P form basically with using some S and that S react with this label and form that P, then it is it participate in the redox cycling that is why S is the starting material.

Otherwise, P can be your starting material, but we actually P form indirectly using some label and where substrate actually react why indirectly form? Because P is the very active material. So, but S is the very low active material. So, it will create low background that is why we dint use P as the starting material we use S as the starting material.

So, S and R is the starting material in this case and this is the E C redox cycling because it is see here chemical here electrochemical. So, electrochemical chemical EC redox cycling. Now, come to the another how means slowly we want to improve the signal amplification.

So, more number of cycling slowly will incorporate here. See here again you can see in this second example here one chemical again chemical reaction here again one chemical reaction right here one more chemical reactions. So, there is two chemical reaction. So, how you can incorporate these two chemical reactions?

Again, see so, we use S is the starting material that form the P this P last cases is directly oxidized, but here we will not directly oxidize on the surface we will use another material O I, I mean suppose like the ruthenium 3 plus like ruthenium 3 plus.

So, that one will react with P and form the R I like ruthenium 2 plus. So, like this way. So, one more cycle we can bring some extra cycle we can bring and more amplification we will get. So, one chemical reaction one chemical reaction. So, this so, here CC one chemical one chemical you can say CC redox cycling right. So, it is CC redox cycling now bring. So, if we apply now here electrochemist chemical like some potential. So, here it will be electrochemical reaction.

So, here electrochemical, here chemical, here chemical, then tell me what is the name of these reactions; what will be the name of this reaction? It will be ECC redox cycling right ECC. So, it will be ECC redox cycling reaction. So, like this way we can give the nomenclature ok. So, I put some reference here please go through this publications also you will get more details, but I already described everything very little so and now I will give you again some example based on this nomenclature.

Now, please try to find out the name nomenclatures of the redox cycling. I mean next class again I will give you some example you can find out the name.

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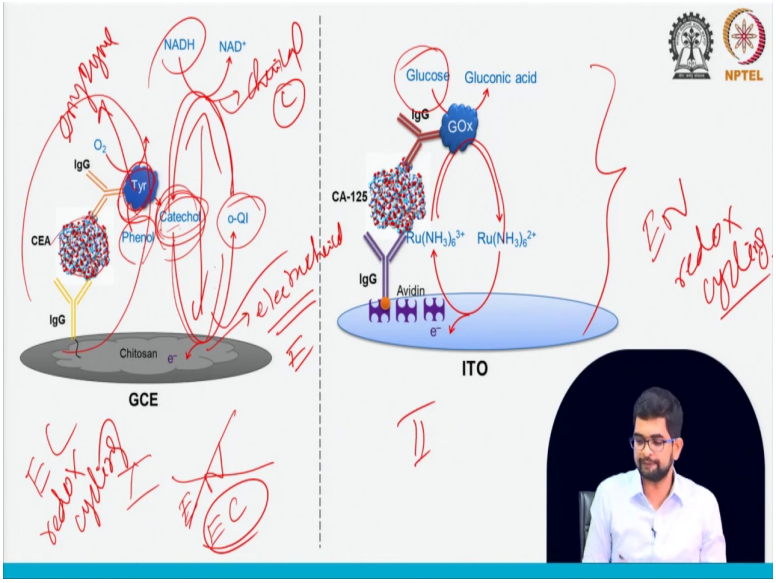

Let us show you some different example. See here two example I put here; see you have to try to mention the nomenclature. So, in this biosensor where we use CEA this is a target. So, that is I have mentioned many times and tyrosine this the enzyme ok. So, this enzyme will react with phenol and form the catechol. And this catechol can be oxidized easily on the sensor surface and it will form the oxidized form of the catechol. It can reduce the electron and this catechol can be reduced by using NADH.

So, is it enzymatic redox cycling? We are we use the enzyme see this is the enzyme right tyrosine it is the enzyme and so, I mentioned like en redox cycling and EC redox cycling these two redox cycling right. So, because see this one chemical reaction right and here

electrochemical. So, here electrochemical E this is chemical C and this is the enzyme these enzymatic reaction tyrosine reacting with phenol form the catechol this is the enzymatic reaction.

But here catechol participating in this redox cycling right. Catechol participating in redox cycling not tyrosine participating in redox cycling. So, last time you can remember the glucose-oxidase directly participating in the redox cycling that is why it is enzymatic redox cycling. So, in this case it is not enzymatic redox cycling right.

So, it would be EC redox cycling chemical electrochemical. So, here this is the example. See here glucose oxidase directly participating right ruthenium hexamine 3 plus form of 2 plus that glucose oxidase actually helping with the reaction with the glucose hm.

But in this case tyrosine is not actually reacting in this case catechol actually this product actually reacting on the surface that is why it is EC redox cycling and it is en redox cycling ok.

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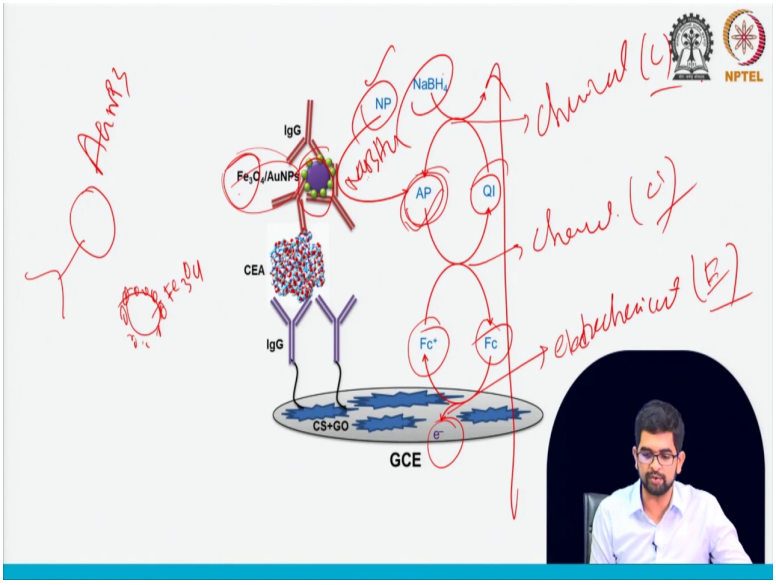

So, this is pretty clear now here; here one more example say. Here we use the secondary antibody with conjugated some nanomaterial. See this nanomaterial is the not only gold nanoparticle it has a core of the iron oxide. It means last time we use only gold nanoparticles. Now, here we use some core that is iron oxide Fe 3 O 4 that is decorated with gold nanoparticles.

So, it has some different activity. So, that is why you can try different different nanomaterial and you have to choose some substrate that can specifically react with this and form some active material ok. So, in this case naturally you can say that it is enzymatic not enzymatic means enzyme based redox.

Redox cycling or not is the enzyme based biosensor, it is a enzyme free biosensor right. This is the nanomaterial we use and we use here sodium borohydride as a reducing agent and this

sodium borohydride only can reduce nitrophenol aminophenol if this Fe 3 O 4 gold nanoparticle this composite present hm.

So, this one specifically actually nitrophenol can be oxidized sorry nitrophenol can be form amino phenol I mean it can reduce to amino phenol if this Fe 3 O 4 and gold nanoparticles present. Now, this gold amino phenol can react with the FC plus that is ferrocene. This will react with the ferrocene plus and it will form FC ferrocene and then now it will oxidize on the sensor surface and it will get the current.

So, you see this is the electrochemical again electrochemical and this one again chemical because this amino phenol reacting with the FC plus and it will form the quinonimine this is chemical and sodium borohydride react with the quinonimine ion. So, this part again chemical. So, chemical C, chemical C and electrochemical E. So, just put the nomenclature form say surface to bulk reactions like ECC, clear like this. So, you can put the nomenclature of this redox cycling ok.

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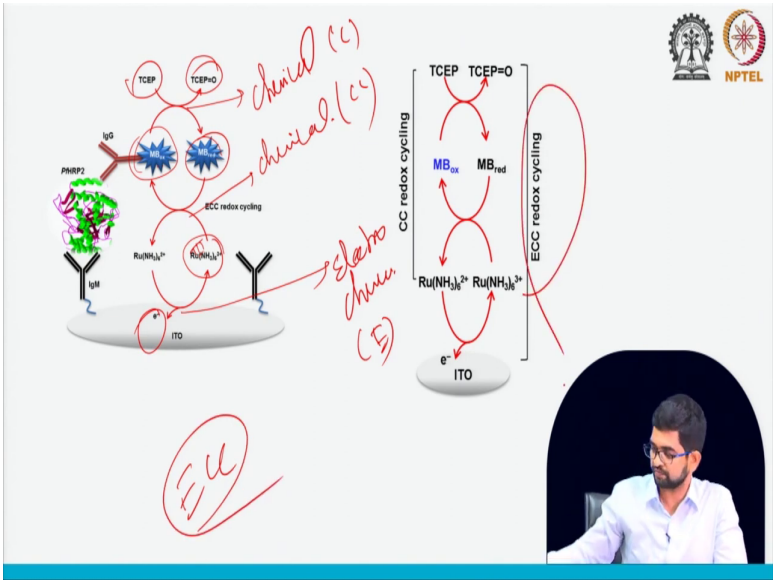

So, this reaction just I showed you the last like previous slides also like what is the nomenclature of this redox cycling right. What is the nomenclature of this redox cycling? Now, you can easily interpret last time I told it is ECC redox cycling why it is ECC? See here no enzyme right here methylene blue itself is participating. So, we conjugated methylene blue with the secondary antibody it is directly participating in the chemical reactions.

So, methylene ox reacting with TCEP red form the oxidized species and this one methylene blue become colorless methane blue red. So, this is chemical again methylene blue red means reduced form is reacting with the methylene hexane 3 plus means it is oxidized form of the ruthenium. So, this is again chemical and here we are applying some potential and we are getting the electron this is electrochemical. So, this is C, this is C and this is. So, it is this is a

ECC redox cycling clear. So, why you put the ECC redox cycling for this (Refer Time: 26:41) set?

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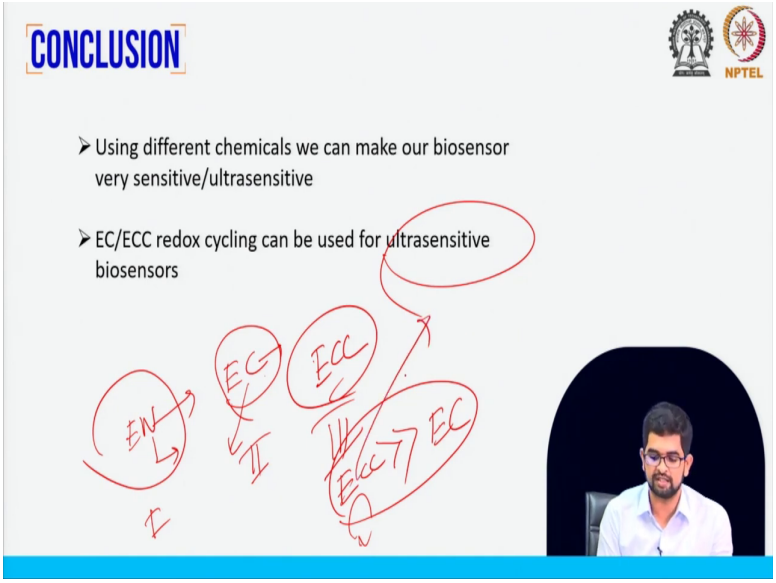

So, in the conclusions of this up to now we I just today I taught you the nomenclatures right how you can put different different name for the biosensor where we are use the signal amplifications like. So, I taught you today like en redox cycling where we can use enzyme and these enzymes should directly participate in the redox cycling, then EC redox cycling; EC means electrochemical and chemical. So, here you can use enzyme, but that enzyme should not participate directly.

So, in this case this redox cycling is the enzyme free, but this redox cycling is not enzyme free. Even you can but EC redox cycling can be enzymatic biosensor may be non-enzymatic

biosensor. Like suppose if we use the gold nanoparticle in this case it is enzyme free, but if you use ALP for generating the ALP with active species then it is not be enzyme free.

And I taught you today ECC redox cycling in electrochemical chemical redox cycling. So, if you see many examples again, I will show you the next classes few more examples and more practical applications where we can use this en ECC EC and ECC redox cycling I will give some practical example in the next class where you can see that easily we can amplify the signal and there is lots of applications.

So, and from all the application case we can summarize that ECC actually always much much better than the EC redox cycling why because here is a more number of cycle actually we are using and we can enhance the electron transfer rate on the sensor surface. So, this can be useful for the that is why very much ultrasensitive biosensor by using ECC redox cycling ok.

So, that is all for today. Thank you very much for the this ultrasensitive biosensor using different cycling next class I will teach you more applications using this redox cycling reaction where we can develop more biosensor.

Thank you.