Bio-electrochemistry

Prof. Mainak Das
Department of Biological Science & Bioengineering and Design Programme
Indian Institute of Technology, Kanpur

Lecture – 20
Redox Proteins, Metallo Proteins & Cyclic Voltammetry

Welcome back to the twentieth lecture on bio electrochemistry. So, we have talked about different kind of electrodes, ion selective electrodes, metal electrodes, detection of analytes like oxygen using amperometry technique, detection of glucose using amperometry technique that we talked about detection of potassium ions using ion selective electrode, where valinomycin has been impregnated onto the ion selective membrane which has special affinity or very specific affinity for potassium ions.

We talked about p H detection based on the point 2 volt potential generated across a glass membrane, when on one side you have the acid, and other side you have a salt solution. Then we talked about a metal electrode example, where we measured the silver ion concentration. Now we will be closing in on this course, today we will talk little bit about the redox proteins, biology is kind of a almost redox chemistry all over the place. Most of the proteins mostly the metal proteins, they are involved in transfer of electron they are getting reduced oxidized.

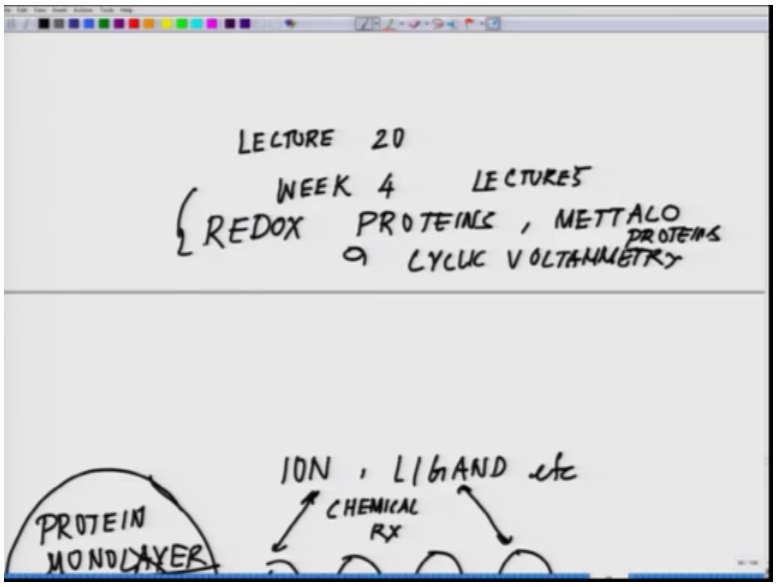
You all have heard about it NADP p H it is getting reduced getting oxidized NADH one of the strong electron donor, a very potent electron acceptor like oxygen, we have already talked about oxygen detection using Clarks electrode.

So, different proteins could be studied like, if you think of the proteins which are embedded in the mitochondria. The proteins which are embedded on chloroplasts look for system 1 and for system 2. Similarly series of hemeproteins which are present series of metal proteins which are present. How these their electron transfer, or electron carrier ability or their reduction potentials could be studied. So, think in terms of the electrochemistry, if 1 could isolate these proteins, and embed them on electrodes, and such proteins can be studied and of course, we have already talked about.

The major electrodes which are being used in this situation will be the metal electrodes like platinum, or maybe gold because gold is the most preferred once, as we have already

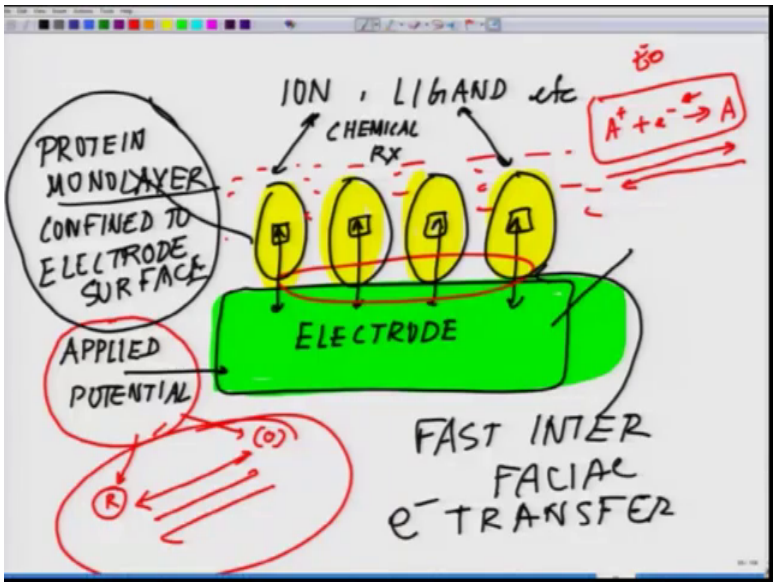
discussed about it. And nowadays people are using grapheme, or carbon, electrodes they are also very powerful tool in terms of allowing the electron transfer to happen. So, one of the strategies which remain is that having a monolayer of such proteins redox protein on surface of the electrode it is something like.

(Refer Slide Time: 03:53)



Put it this is our lecture 20, week 4, lecture 5. So, today we will talk about the redox proteins, metallo proteins, and cyclic voltammetry. So, this is the kind of a strategy.

(Refer Slide Time: 04:39)

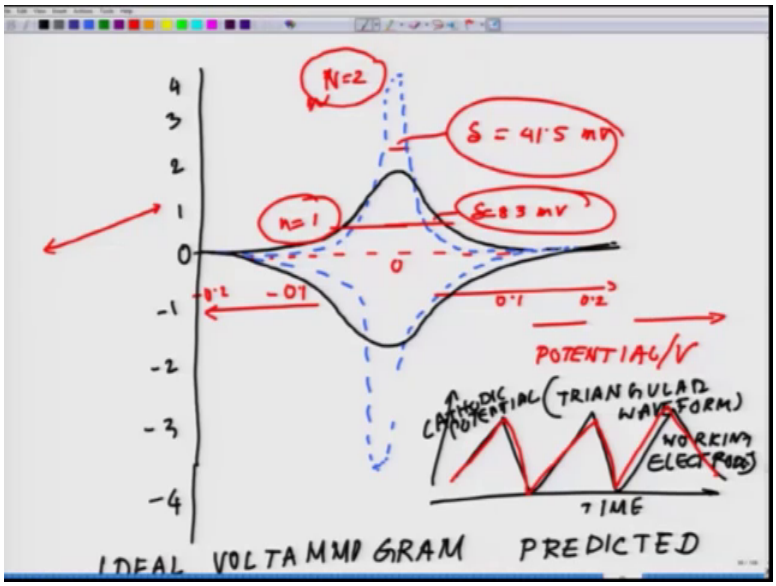


So, you have a protein mono layer confined to the electrode surface the electrode mostly they use gold electrode. So, this is the protein mono layer, which is all these proteins are electrically active proteins, they have the power either to accept an electron or donate an electron. And the green is showing your electrode surface, you are confining your protein on top of the electrons electrode surface.

Now you have access, you can do two things here, you can apply a potential 1 second let me that you can apply a potential, and either you can oxidize the protein, or you can reduce the protein, because you can allow you can flooded with excess electrons if it is an electron acceptor, or you can make a deficit of it. Because you can change the polarity of it. So, think of it for a minute say for example, whenever we write this reaction A plus electron making A plus plus an electron becomes A. Now and we give A value of the E 0 here out here.

Now, think of it you can do this reaction in both direction, you can balance it you can take the reaction forward, you can break the reaction opposite, you just have to keep on changing cycling it, from anode to cathode, cathode to anode, anode to cathode, cathode to anode. And by reversing the polarity, you can actually do this. That is precisely what is being exploited, when we talk about the cyclic voltammetry techniques could be used for such things. So, these proteins before I get into that. So, such proteins you can study their electron transfer, how many electrons are getting transfer at what potential they are showing those kind of traces, you really can use something like a cyclic voltammetry technique.

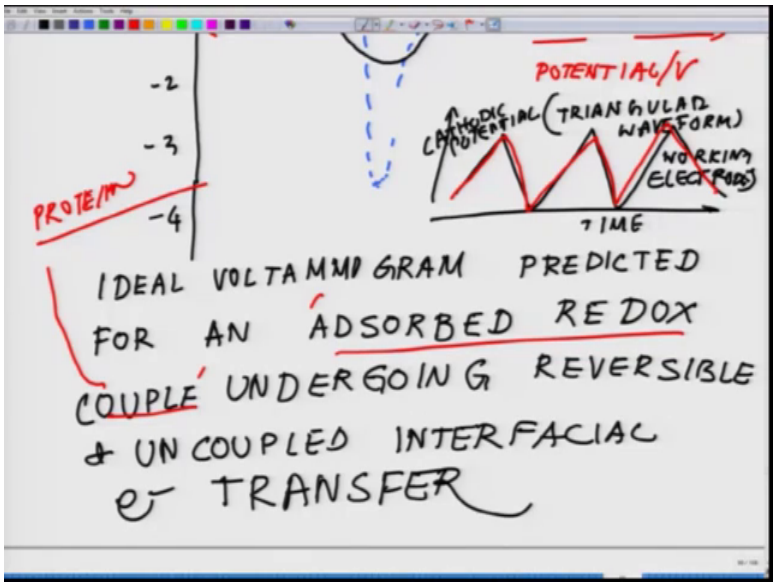
(Refer Slide Time: 06:55)



Where you are measuring the current based on the applied potential, and you are giving a cathodic potential something called a triangular pulse, I am not getting into the technical details of it because that is the beyond the scope of this course.

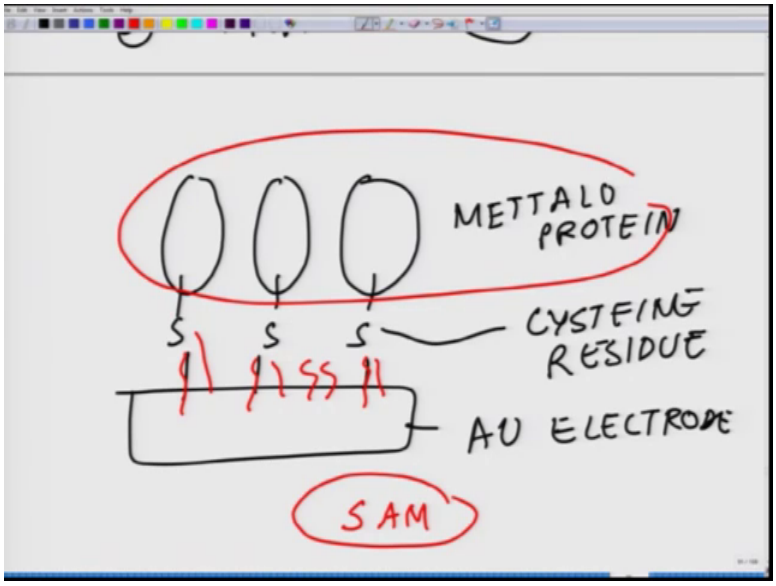
But based on that you can switch from anode to cathode, you can allow the oxidation any reduction of a particular analyte happening in an cyclic fashion. And based on the path, and based on the width, or based on the width of or the height of the current, you can figure out how much electrons are being accepted or donated. This N out here actually should be small n, N represent the number of electrons. And the weight tells you the voltage. So, what you are having is something like, you are cycling your analyte.

(Refer Slide Time: 07:59)



So,this is an essentially, this is an ideal voltano graham predicted for an absorbed redox couple. So, here redox couple is here, is your protein or whatever in this case we are talking about the protein. Undergoing reversible uncoupled interfacial electron transfer. So, the electron transfer is happening out here, and this applied potential could allow it to go to reversibly in the forward as well as in the reverse direction. So, these kind of techniques could be used, and how we are attaching them.

(Refer Slide Time: 08:35)



Some of these metallo proteins use a strategy, if they have a cysteine residue, which is a sulfur containing residue it could bound to the gold electrode. There are self-assembled monolayers SAMs which are commonly called, which are used to interface with the gold electrode to connect to it. So, there are several strategies, you can use conducting polymers you can use several ways, by which you can actually get to the point where you can study, the reaction kinetics and you can change the milieu out here under what condition.

Say for example, this protein works at a certain p H differently as compared to different another p H. So, you can really figure all those out, by using these simple electrochemistry techniques. So, the point what I wanted to highlight here, in this class is as of now, we have talked about the basic fundamental of Nernst equation that is where we all started the Nernst equation which I repeatedly told you that is the basis of much of these kind of things.

Then we talked about the redox potential of different compounds, or different molecules. So, every protein which is sitting say for example, let us take an example of respiratory chain of mitochondria, where NADH is funneling an electron to the oxygen. While it funnels. So, there are 3 different complexes which are present there, each complex had a different electron binding capacity, as a matter of fact oxygen is one of the potent acceptor, but other 3 complexes of proteins are have a different binding affinity.

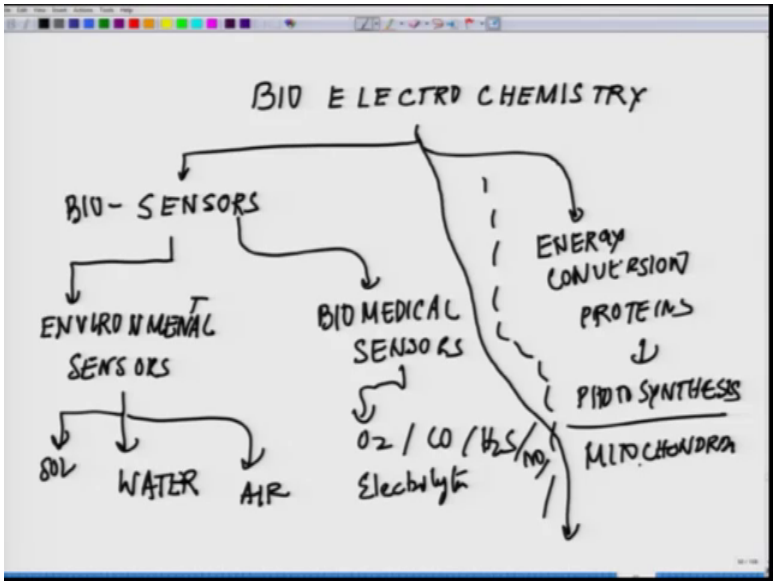
How you test that. Now if you could isolate those proteins on top of an electrode, you actually can figure out what is the binding affinity of these kind of things. So, similarly if we look at the photo systems, where you have series of complexes like VF complex Quinone's, you have iron sulfur clusters, you have series of them sitting there, and each one of them are sitting if you see the photo system, you see each one of them are sitting at different redox potential. And that essentially means, each one of them have a different electron acceptance power.

So, if we know these things how we really can like you know utilize them, could we really understand the kinetics at the electrode surface. How the electron transfer is mediated. So, all these different kind of studies, falls under one of the very potentially challenging area which is coming up is a bio electrochemistry, where electrochemistry is taking a forefront in most of the biological measurements.

So, it has 2 aspects while on a concluding note, one is the bio sensor aspect, how what we have talked about the glucose sensor. We talked about oxygen electrode, we talked about potassium measurements, we talked about silver measurements, we talked about the p H, there are urea sensors which could be used in milk, and as well as in urine, and several other things wherever the urea is one of the analytes.

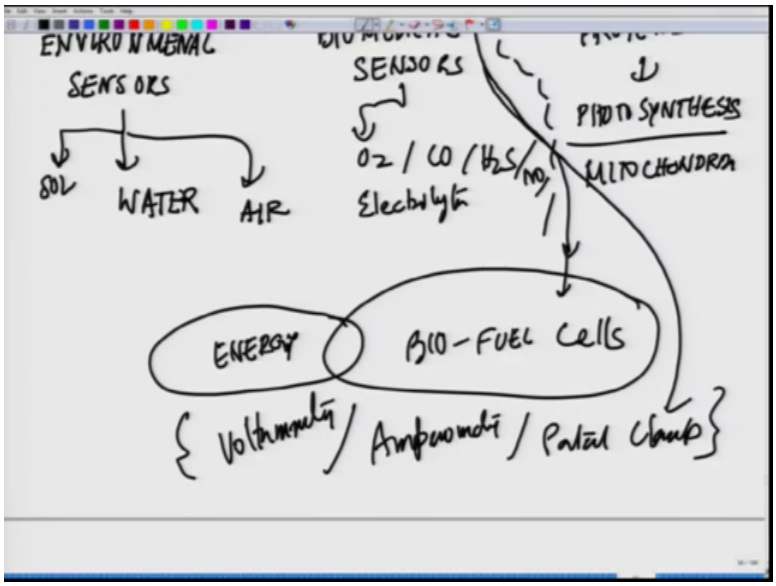
So, that is one key area. Similarly we can detect hydrogen sulfide, we can detect N O, we can detect carbon monoxide. Similarly several vapor, or volatiles, could also be detected using electrochemical tools, like electric nose we talked about it. So, in a sense bio electro chemistry has 2 aspects, 1 aspect is the biomedical or bio sensor approach. So, how to put it like this, what are the different potential areas where this area can move.

(Refer Slide Time: 13:17)



Bio electro chemistry one of the potential area is biosensors. Biosensors could be further divided it into environmental sensors, it could be soil, water, as well as air. Similarly there is a bio medical sensors, which includes oxygen, carbon monoxide, electrolytes H 2 S likewise so on and so forth. And you have another area of bio electrochemistry which deals with energy conversion proteins that is in photosynthesis OH by the way N O synthesis, and mitochondrial oxidative phospho relation apart from it.

(Refer Slide Time: 15:06)



It is another area which we have not discussed, which is the area of bio fuel cells is beyond the scope, because in 20 lectures you really cannot cover from the fundamental basics bio fuel cells. Another area which is more on the energy sector.

So, if you realize it that bio electrochemistry is an is a old technique, yet it always have a scope from fundamental studies to all the way to the industrial application, it is spectrum is pretty wide ranging application it has, and that necessitates a reasonable knowledge or you know knowledge of analytical chemistry, acid base titration, oxidation reduction, little bit knowledge about electrochemistry, little bit knowledge of physical chemistry, and a very sharp insight where all such techniques could be utilized in biology.

Whether it is an action potential measurements, which is a case of concentration cell, whether it is a channel studies, whether it is a environmental sensors, by the way that part I have forgotten to mention, whenever we talk about biological sensors. We talk about you know patch clamp, amperometry, voltammetry, all these different techniques which of course, we could not really cover in depth detail because of positive time.

But it is a very interesting area, where what I have tried all throughout is to get the basics. Once the basics are kind of clear in your brain, then you always can apply it for more better understanding of the instrumentation. So, with this I will close in the course and I hope it has helped you, to understand some of the basic fundamentals of course, I

could not really cover as much wider spectrum as I could, but at least I try to ensure that the basics are clear out there.

Thank you.