Chemical and Biological Thermodynamics: Principles to Applications

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Lecture - 38
Thermodynamics in Systems of Biological Interest

We have already discussed the fundamentals of chemical thermodynamics. We have discussed in details the various fundamental quantities which are important in thermodynamics and now time has come to discuss about the possible applications of those thermodynamic principles and thermodynamic quantities in various systems of chemical and biological interest.

In fact, in the form of numerical problems I have already discussed several cases where the applications of these thermodynamic quantities in chemical processes was demonstrated and now I will take several examples which are of biological interest.

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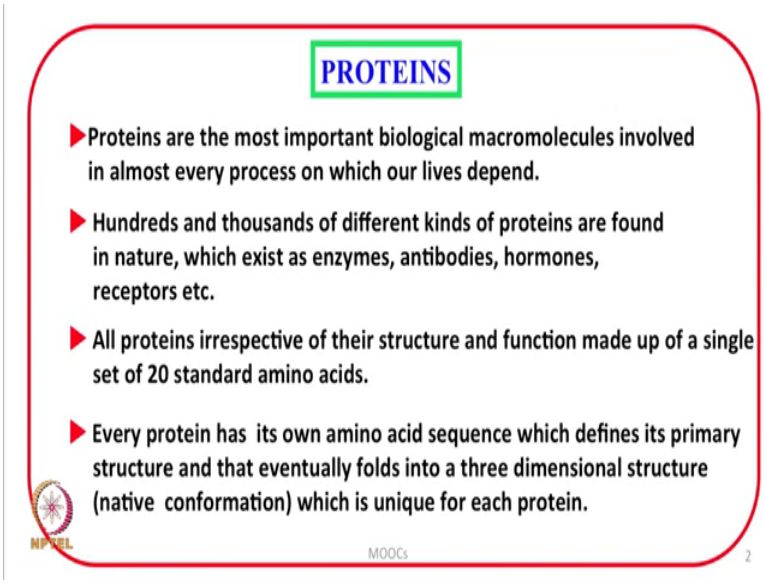

So, that is why I gave a title to this lecture thermodynamics in systems of biological interest proteins are most important molecules when it comes to the living systems let us discuss a little bit about proteins. As I just mentioned that proteins are the most important biological macro molecules involved in almost every process on which our lives depend.

So, therefore, it becomes very important to discuss about the various processes in which proteins are involved there are hundreds and thousands of different kinds of proteins which are found in nature which exist as enzymes antibodies hormones receptors etcetera and all proteins irrespective of their structure and function are made up of a single set of twenty standard amino acids.

Every protein has its own amino acid sequence which defines its primary structure that eventually folds into a three dimensional structure which is called the native conformation and that is unique for each protein.

I will spend some more time on the discussion on proteins although our main goal in this lecture is to connect the thermodynamics with proteins, but we will be able to appreciate this connection more if we understand that why we need to connect this thermodynamic information with the proteins let us discuss more.

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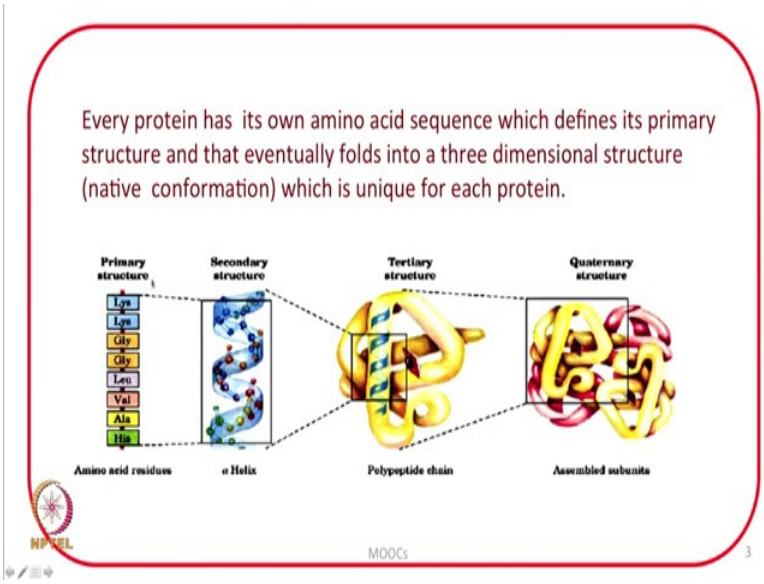

As I just mentioned that every protein has its own amino acid sequence and that amino acid sequence defines its primary structure let us take a look at the slide this is the primary structure of a protein and it has its own amino acid sequence. So, the primary structure having its own amino acid sequence this is the first level of protein structure and the next level of protein structure is secondary structure.

Secondary structure can be either alpha helix or it can be beta sheet there are several secondary structural motives and the details of protein structure can be can be obtained from several bio chemistry textbooks, but here we will briefly describe the various levels of protein structure.

After the secondary structure the next level of protein structure is the tertiary structure and beyond tertiary structure it can be quaternary structure after the protein is synthesized from ribosome it will have its own primary structure it will have its own amino acid sequence. Then at the next level of protein structure that is in the secondary structure most important interactions which drive the formation of secondary structure are hydrogen bonding interactions.

Hydrogen bonding interactions are involved in the formation of helix or sheets in the next level of protein structure that is the tertiary structure there are four type of interactions which are involved one is hydrogen bonding interaction. Second is ionic interactions third is disulfide bonds and the forth type of interaction is the most important interaction which leads to the formation of a tertiary structure of a protein and that is hydrophobic interactions.

Once the tertiary structure is formed several proteins can remain in the monomeric form; however, some of the proteins may even go to a higher order formation in which the folded monomers can be further organized into a higher order subunit structure which forms the quaternary structure.

As we can see here in this slide you have assembled sub units this is the formation of a dimer from a monomer.

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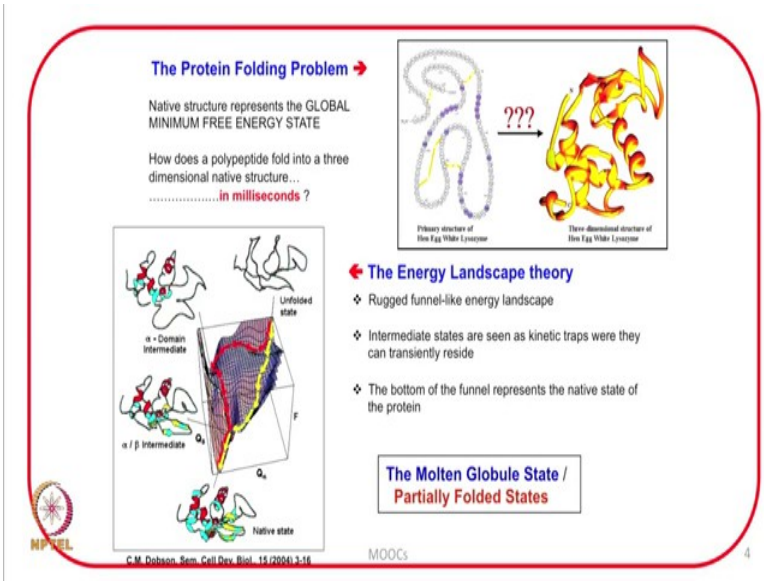

And there can be higher orders assemblies which will form the quaternary structure of a protein as I just mentioned that after the protein is synthesized from a ribosome it will have its own primary structure which is defined by its amino acid sequence.

Now, take a look at the slide that primary structure or a given sequence of amino acids it takes a unique three dimensional tertiary structural fold in other words a given amino acid sequence will result into a unique three dimensional conformation and why that particular amino acid sequence folds into a unique three dimensional structure is called the protein folding problem.

So, what is protein folding problem that if there is a unique primary structure with a unique sequence of amino acid it will take up only a specific tertiary structural fold not any structure it will take up only a unique tertiary structural fold and why it folds into that unique tertiary structure is called the protein folding problem.

For several years scientist have been working to understand that why a given primary amino acid sequence takes up a unique final fold and we have not been able to answer. So, far in other words if we call these if we look at the slide the question mark that is the protein folding problem and if I call it as what is the protein folding code.

In spite of a work of several years we have not been able to decode the protein folding code in other words we have we are we do not still have an answer for the protein

folding problem the address to understand in the protein folding problem has been from many directions experimentalists have been working on it by using a variety of experimental methods computational chemists and theoretical chemists have also been trying to find an answer to this protein folding code.

I will soon discuss with you that what is the current status of the protein folding code, but one of our focus in this course is to understand that how thermodynamics has contributed to the understanding of protein folding problem and how thermodynamics contributes to understand the conformational stability of a protein under the given environmental condition.

Now, let us go back to slide and look at the comments over there the native structure represents global minimum free energy state I am talking about this native structure this is a global minimum free energy state and the question is how does a polypeptide fold into a three dimensional native structure and that too on a fast time scale that is in millisecond.

Now, as I just explained that scientist have been working for a long time to understand the protein folding code several experiments have been done and lot of computational or theoretical efforts have been dedicated. But one of the most important theory or one of the most popular theory which has been used to understand the protein folding problem or to describe the protein folding process is called the energy landscape theory and that is shown in this slide this is the energy landscape theory this is also called a funnel model I will show another slide on the funnel model because this looks like a funnel that is what is commented over here rugged funnel like energy landscape.

So, if this is the primary structure in order to achieve or in order to arrive at the final minimum energy native conformation it can go through the yellow path it can go through the red path or it can go through any other path which is physical.

In other words there are several intermediate states where there are local minimas. So, therefore, it becomes very important to understand the protein folding pathways and our focus will be how to apply the concepts of thermodynamics in understanding these processes in detail.

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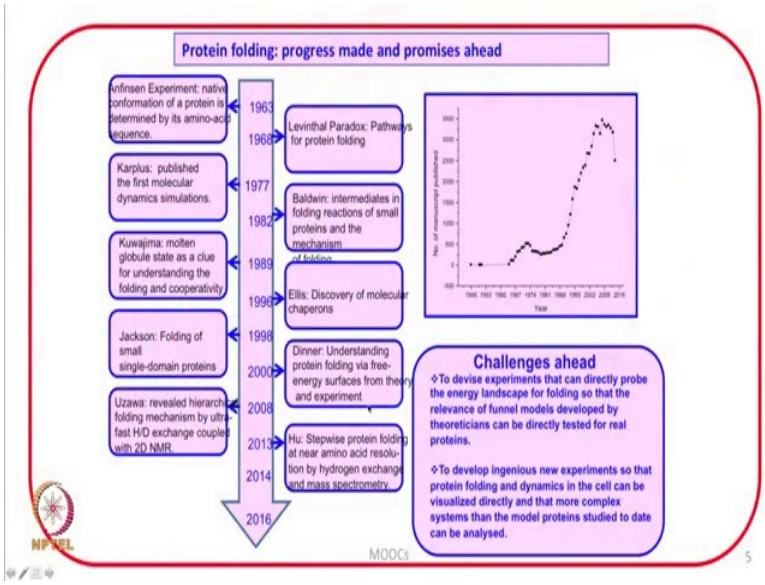

Let us take a look at how the protein folding problem has evolved take a look at the slide starting from nineteen sixties nineteen sixty three we can see some experiment demonstrating that native conformation of a protein is determined by its amino acid sequence. And over the years you see several experimental and theoretical or computational efforts have been made by different scientist and in 2016 or 2017s what do we know understanding protein folding problem has always been a thrust area of research because we understand that the proteins play very important functions in the living systems.

Proteins are used in the drug delivery therefore, proteins become very very important for pharmaceutical industries also it will be of immense interest to both academicians and industrialist biologist to understand protein folding problem to understand the protein folding to understand the protein conformation under different environments and to understand whether a given protein can be used for drug delivery or what modification should be done in the drug and vice versa for an improved drug delivery we will discuss these issues later on.

But just pointing out on from the research perspective just look at this figure which is the number of research publications verses the year particular year and you see here one or two publications in the protein folding area started appearing in 1940s and then in 1960s this area gained some momentum and in 1990s you see there was a big rush or if this

area became very very popular a lot of publications started work appearing in it and in 2014 2015 if we reach that it is reaching near saturation.

So, what does it mean does it mean that this area is no more important or does it mean that it is becoming very very difficult to find more information on protein folding protein folding means when the protein is folding there are several intra molecular interactions which lead to the folding of the protein.

If we want to understand the protein folding we must identify those interactions which are holding the protein together and we also must understand what leads to the disturbance or weakening of those interactions what we will discuss that how thermodynamics has helped in understanding the various forces which are responsible for holding the three dimensional conformation of a protein intact.

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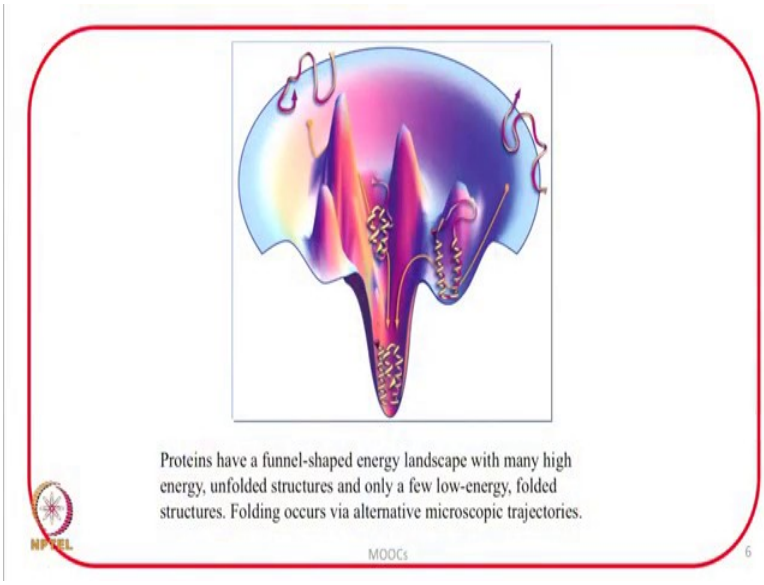

Let us take a look at this figure this is what I was calling as the funnel model according to this funnel model this is as I said this is one of the most widely used model that describes the protein folding problem. Or protein folding pathway that is the polypeptide which represents the primary structure it has to reach the final minimum energy three dimensional conformation and it can go through various processes some of the pathways are shown over here.

Some way there are local minimas and we can always define the Gibbs energy over there, but the global minima is here at the bottom. So, if we are able to understand these intermediate structures and if we are able to understand the energetics that drives the primary sequence primary structure into a three dimensional unique fold that is a contribution to the protein folding problem and thermodynamics has a lot of role to play in it.

We have already discussed what is the protein folding problem the protein folding problem was formally recognized in the year 1962.

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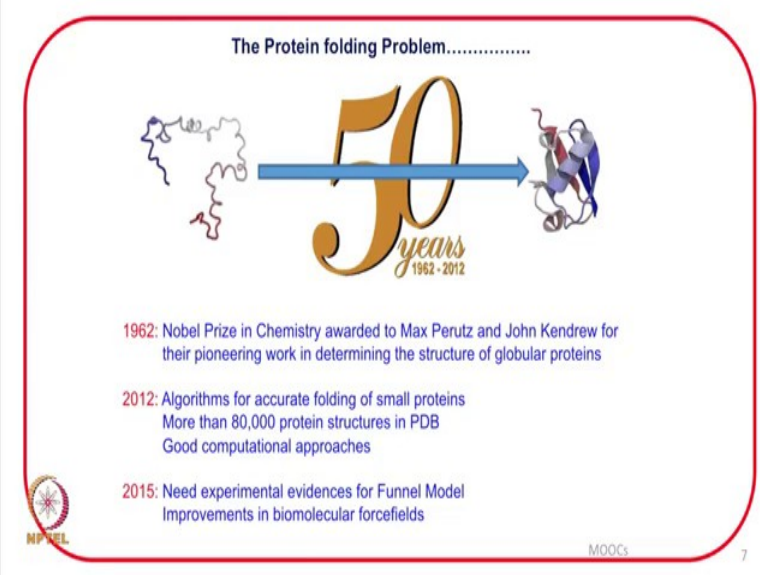

And in 2012, the 50 years of the recognition of protein folding problem was celebrated so; obviously, it makes a sense to turn back after 50 years and see what progress has been made in this field and what kind of efforts have let to a proper understanding of the protein folding problem or the associated processes let us take a look at.

As I just said 1962 was the year when this protein folding problem was formally recognized and in recognition to that a Nobel Prize in chemistry was awarded to Max Perutz and John Kendrew for their pioneering work in determining the structure of globular proteins that was a land mark recognition.

Then in 2012 what did we have in 2012 in 2012 what we have what we had was algorithms for accurate folding of small proteins more than 80,000 protein structures in protein data bank and good computational approaches.

When it comes to understand the energetics when it comes to understand the thermodynamics the approach can be both experimental and both can approach can also be theoretical or computational in understanding the protein folding code or protein folding problem computational chemists or computational approaches have been quiet successful.

Today the computational chemists claim that they can give you the folding code for small peptides monomeric small peptides; however, when it comes to large proteins they do not have the protein folding code.

In 2015 a comment was made that there is a need for experimental evidences for funnel model because you remember that when I was talking about funnel models we talked about the local Gibbs energy minimas and the final global Gibbs energy minima. There is need to understand there is need to have experimental understanding or evidences for the funnel model and for there for that several experiments need to be designed which should address or which should characterize various intermediate states and assign energetics to that.

Then let us take a look at that there is a need for improvements in bio molecular force fields. So, even in fifty years the progress in understanding the protein folding problem has been limited to an understanding of the protein folding code of very small peptides or folding code of very small peptides or very small monomeric protein, but when it comes to large proteins there is no protein folding code available and that is why he has commented over here there is a need for experimental evidences for funnel model and there is a need for improvements in bio molecular force fields.

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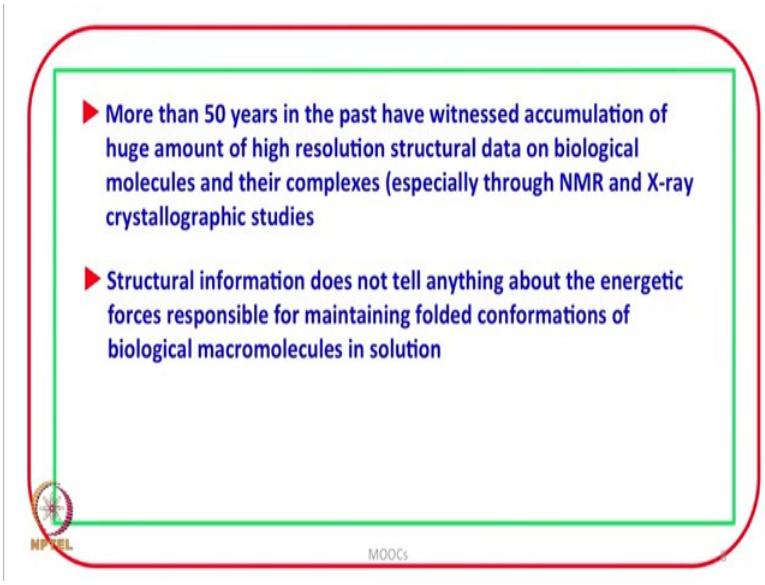

So, in more than fifty years in the past there has been a huge accumulation of a large amount of high dissolution structural data on biological molecules and their complexes and this structural data comes from NMR and X-Ray crystallographic studies, but the structure information alone does not tell anything about the energetic forces which are responsible for maintaining folded conformation of biological macromolecules in solution.

For energetic information we need thermodynamics we need to understand the thermodynamics of the processes.

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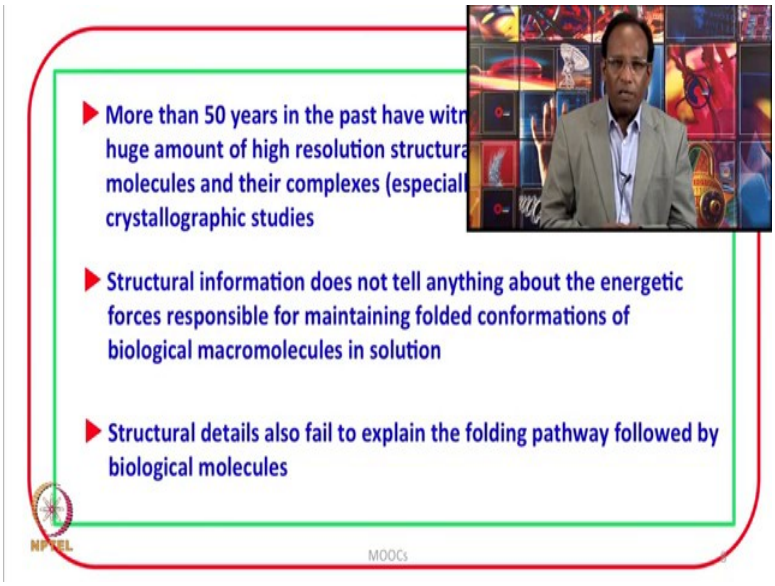

So, therefore, we shall also recognize that only the structural details cannot explain the folding pathway followed by biological molecules if you recall the funnel model again only understanding the structural detail is not sufficient we need to understand what is the folding pathway what drives deformation of particular local minima or the global minima in free energy.

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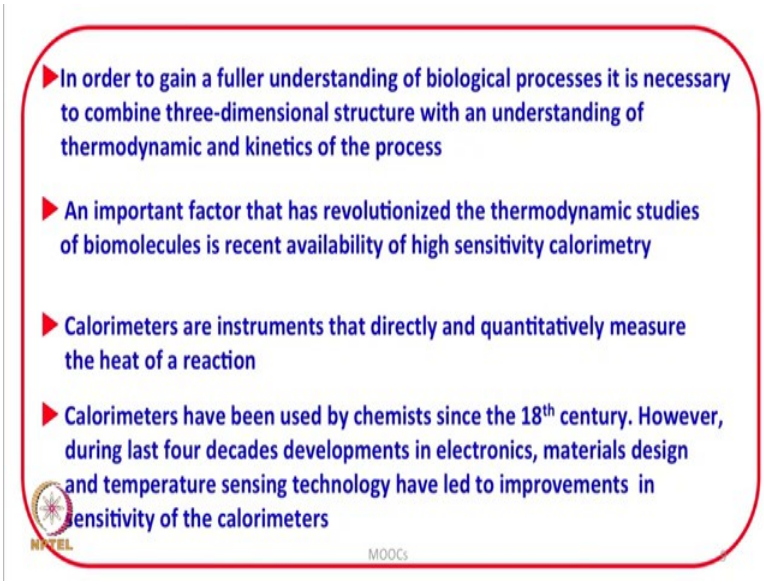

Therefore in order to give a fuller understanding of biological processes it is necessary to combine three dimensional structure with an understanding of thermodynamic and

kinetics of the process. So, what we need is information on the structure three dimensional structure we need information on the kinetics of the process and we need information on thermodynamic aspects and that is what we are going to discuss in detail the thermodynamics aspect.

Now, an important factor that has revolutionized the thermodynamic studies of biomolecules is recent availability of high sensitivity calorimetry and we will be discussing about calorimetry or calorimeters in details; what are calorimeters.

Calorimeters are instruments that directly and quantitatively measure heat of reaction and calorimeters have been used by chemists since the eighteenth century; however, during the last four decades developments in electronics materials design and temperature sensing technology have left to improvements in sensitivity of the calorimeters.

See some reactions can produce large amount of heat and some reactions for examples for example, reactions in the living systems reactions of biological significance do not produce large amount of heat. So, therefore, if we are interested in understanding the processes which are taking place in the living systems or the processes which involve very weak interactions we need very very sensitive calorimeters and that is what is commented that during the last couple of decades there has been tremendous improvement in electronics material design and the sensing technology.

And today we have calorimeters which can detect very very small amount of heat changes and we will be discussing those in details and since the purpose of the next few lectures is not only to cover the basic fundamentals, but also apply these fundamentals to research problems of thrust areas as well as to industrial problems of chemical and pharmaceutical interest.

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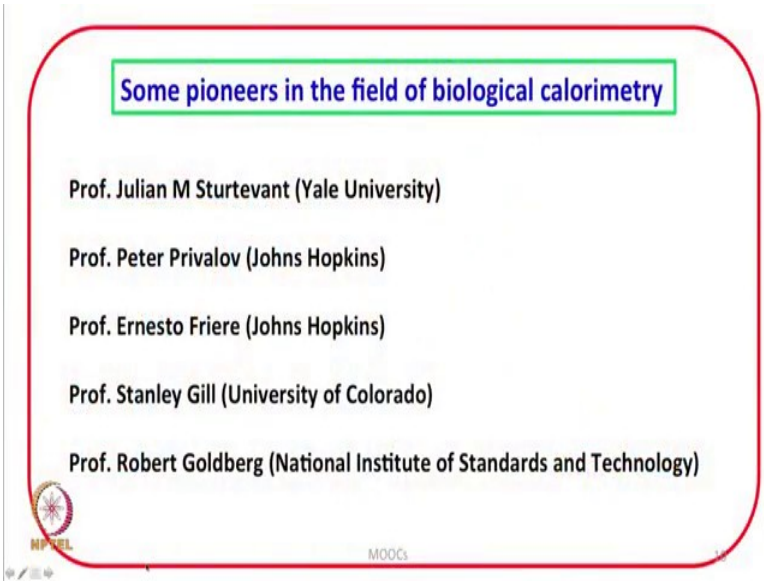

I will just provide a list of some pioneers in the field of biological calorimetry where or in their laboratory the calorimetry was extensively used in the application of biological systems and they gave us directions that in what way this information can be used to describe the biological processes and; what is the need for future.

Some of the names are Professor Julian Sturtevant at Yale University, Professor Peter Privalov at John's Hopkins, Professor Ernesto Friere at John's Hopkins University, Professor Stanley Gill; University of Colorado, Professors Robert Goldberg at National Institute of Standards and Technology. Their laboratories have used calorimetry in addressing the biological systems in details and therefore, if you are interested in understanding their work and how they applied to various biological problems please look at their publications.

We will continue our discussion on calorimetry and how thermodynamics of the system is important for various biological processes in the next lecture.

Thank you very much.