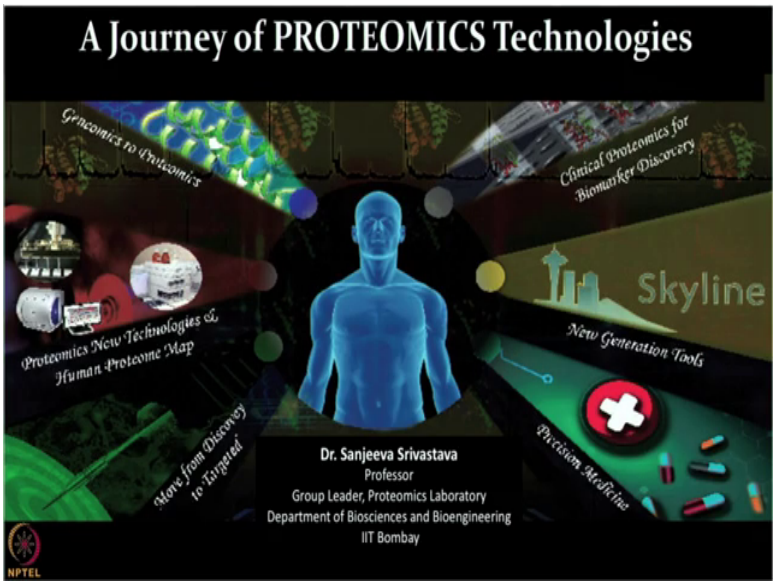
Interactomics Basics and Applications
Prof. Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay

Lecture - 01
Introduction to Proteomics

Welcome to MOOC Intractomics course. Hello students and participants, this course is essentially going to give you a more detail understanding of how various type of bio molecular interactions work. While the major focus of the course will be to look at different type of technologies which measures protein-protein and protein other bio molecular interactions.

(Refer Slide Time: 00:57)



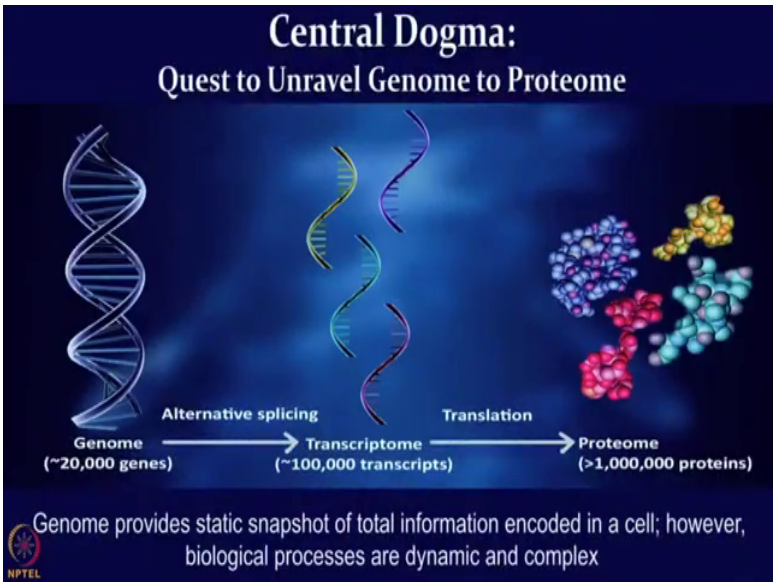
But today what I thought, I am going to talk to you about a journey of entire proteomics field and different technologies which are part of the field of proteomics. This is going to give you

a much broader overview of how different technologies are playing an important role to study the very complex proteome, almost all the proteins which constitutes any organism. And of course, to understand this, we need much more breadth of the technologies as well as the depth of the technologies. So, we cannot just achieve all the questions by looking at single technology platform.

(Refer Slide Time: 01:22)

So, in today's lecture outline will be; first I am going to give you an overview of proteomic technologies and then I am going to talk to you about various opportunities which big data essential offers to us and which are the challenges which are part of this field. As you are aware the central dogma is starts from the basic bio molecular work flow; is starting from the genes to the transcripts and the protein.

(Refer Slide Time: 01:55)



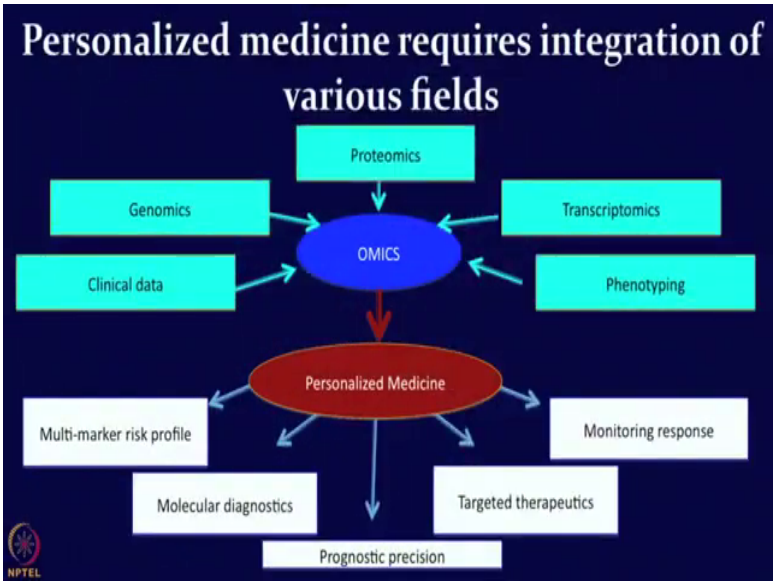
The entire field which aims to study all the genes of a given organism is known as genomics. And all the genes is constitute the organism is known as genome. And likewise all the transcripts we study with transcriptome in the field of transcriptomics and all the proteins we study in the field of proteomics.

As you can see in the slide that genome provides only the snapshot of the total information which is encoded in a cell, but the biological processes are very dynamic and very complex. And they are governed along with the transcripts and the proteins which make the changes in response to the dynamic behavior of the cell.

When it comes for the medical field, we cannot just simply rely on one set of technologies and looking at only single type of bio molecule of interest; because the human physiology or any other organism physiology depends on the intricate relationship of various bio molecules.

So, if we are looking at the complex physiological systems, we have to look at all the possibilities of different type of bio molecules.

(Refer Slide Time: 03:03)



For example, if we are trying to capture the actual physiological consequence, then we should look at the genome, the transcriptome, the proteome as well as the clinical information and the phenotyping of the individuals all of this comes under the field of omics. So, far what we are doing in the field of medicine, is essentially we are generalizing the medicine and giving the same treatment to all the patients; but the field of personalized medicine aims to look at each individual as a unique entity.

So, can we look at a given individual and all of its bio molecule and try to make an educated sense of that what should be the best drug given to this individual, how to monitor the response of a given drug or therapy, what should be the best way of diagnosing the disease for

the individual, how multi marker risk assessment could be analyzed, how one could eventually offer the therapy which are very very effective for a given individual based on our comprehensive understanding of the omics system of that individual.

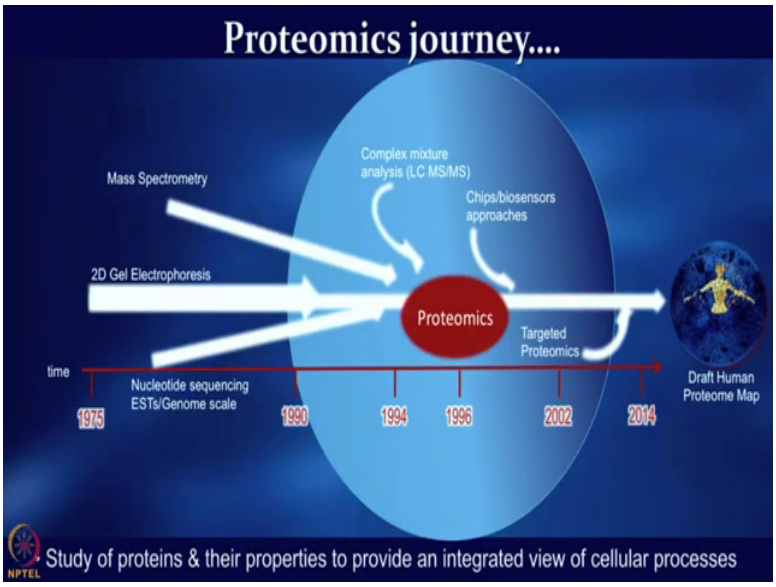
All of these things are revolutionary changes which are happening in the field of biology; that is good idea through this course you will be exposed for many of these technologies which should be interesting for all the life systems including human.

(Refer Slide Time: 04:31)

So, the entire field of omics is started from the very ambitious and very comprehensive human genome project. Along with many other genome projects which actually laid the foundation of the whole field of omics. It was not only that 15 years of effort in a very disciplined manner, which lead first time they draft genome map of human.

But also it also gives us an ability that we have now gone in the field of life sciences in biology to which paradigm shift where we can start looking at all the possible genes of a given system. So, genomics which was you know the studies published in Nature and Science in 2001, 2002; those really laid the foundation for the whole omics field including the field of proteomics which got inspiration from the field of genomics.

(Refer Slide Time: 05:40)



As soon as people unraveled all the possibilities of the genes which constitutes human and other organism; they realize their biological systems are very complex, but the number of genes which are present they are not that many and they are not very different in different organism.

So, the overall genes range was around 20000 to 30000 for different model organism including human. So, what makes human different, what makes different organism different

and what gives them the unique ability to cope up with the environment to cope with the specific physiological context and that is where the whole field shifted towards more functional molecules, more towards transcriptomics and proteomics.

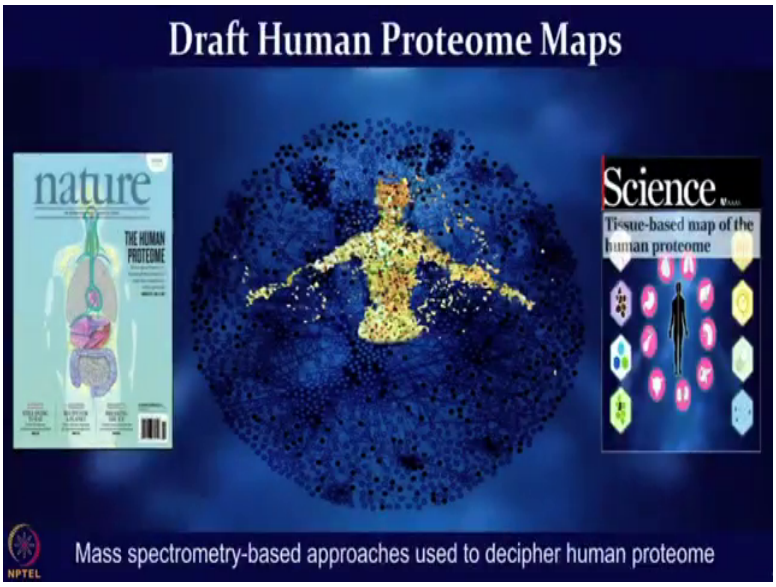
Here I am depicting you the proteomics journey, which is started from 1970s and started with simple experiment of how to separate the complex protein mixture using two different properties their molecular weight and isoelectric point which made the 2 dimension electrophoresis. And then along with that, there were different developments were happening in the field of mass spectrometry and various type of sequencing projects E steel and various type of NiCo tight sequencing these were all also happening parallel. And together all of those made huge progress in the field of omics around 1990 to 2000.

When many of these things started getting more mature, on the side we see the advent of electrospray ionisation and MRD and the initial sources for the mass spectrometers. So, all of them were really giving the foundation for the new field of proteomics start; but only after the completion of the human genome project, scientists started moving towards the entire field of proteomics and thought now, we can start understanding the functional molecules which are the proteins.

Different other technologies including various intractomics based technologies, protein micro arrays, various type of immuno precipitation, followed by mass spectrometers and label free bio sensors all of them is started coming together and then started making the field of functional proteomics and intractomics.

Further there are many constraints were there to validate the biomarkers validate the proteins. So, new field of target proteomics emerged around 2012, 2014 and then as we moved along, then first time the scientists were started realizing the full potential of mass spectrometers and the first draft of human proteome maps are published.

(Refer Slide Time: 07:51)

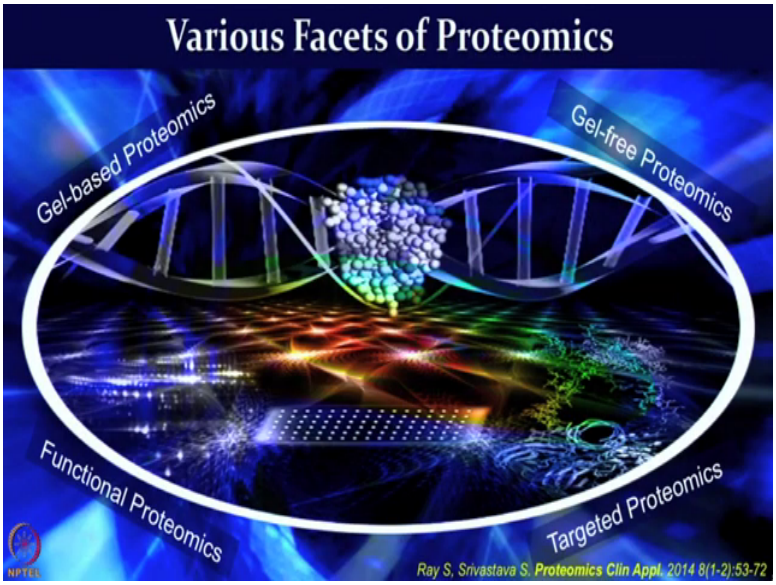


So, to see here that the cover page images of the nature which first time showed in 2014, the human proteome map the draft was actually published. While the whole proteome was not unraveled, but it still majority of the proteins were discovered and then idea was that first time we have now the evidence that the genes make the protein and we are now able to measure those peptides using mass spectrometers. The two different independent groups publish these studies first time and show the draft human proteome maps.

Additionally another group worked on the antibody based approaches and they also showed first time in 2015 a study publish in science, which is a tissue based map of the human proteome. First time they showed that where the proteins are localized and there is evidence that proteins not only exist, but how it is localized and they also provided evidence at the

tissue level and looking at the various type of immune histo chemistry and tissue arrays based approaches

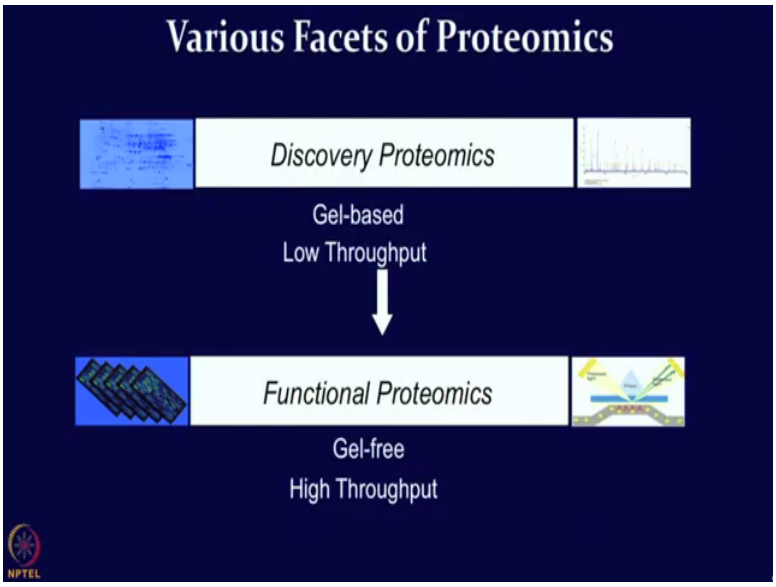
(Refer Slide Time: 09:06)



So, there are various facets of proteomics; one could think about gel based proteomics, which essentially looks for variety of you know gels to separate the proteins. Gel free proteomics which essentially could be mass spectrometry or even various type of interactomic technologies.

Functional proteomics which again involves various technologies which looks at the bio molecular interactions, look at the functional consequence of these proteins, how they interact, how they are part of the different networks in pathways and targeted proteomics which essentially looks at validating the protein targets.

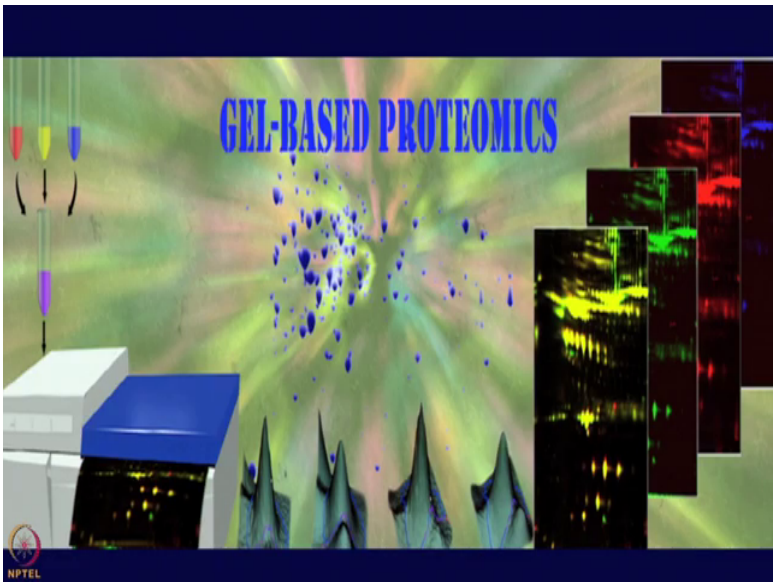
(Refer Slide Time: 09:38)



So, broadly we can divide the whole field into two broad categories; one is discovery proteomics and second could be functional proteomics. Different technologies which are part of the gel based proteomics as well as different type of mass spectrometers could be covered under discovery proteomics. Various technologies which are more high throughput in nature essentially protein micro arrays, label free myosin sets which constitute the interactomic analysis, they are more part of the functional proteomics.

So, I am going to give you an overview of different technologies in a very nutshell, because the focus of this whole course will be mainly on interactomics; but it is good idea for you to appreciate that where interactomics field fits into the broad field of proteomics and which are other technologies which are all going to help each other to understand the complex bio molecules.

(Refer Slide Time: 10:26)

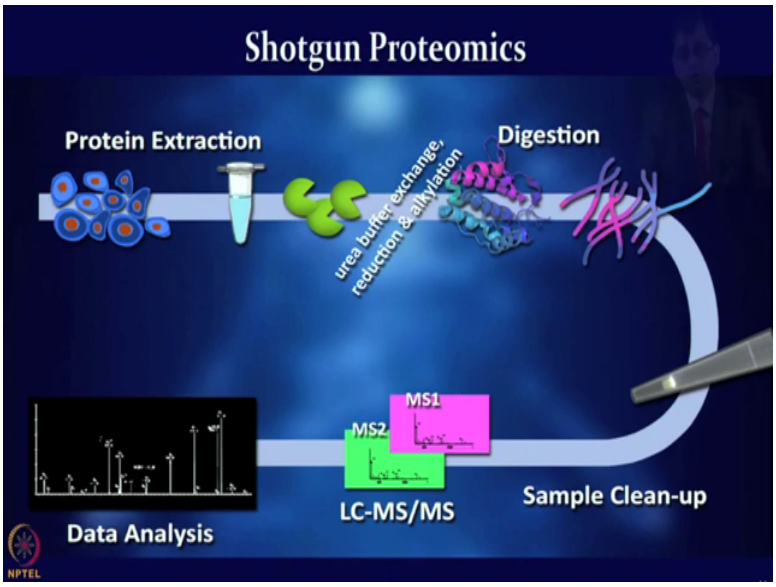


So, gel based proteomics as the image conveys that you can separate the proteins based on either only their molecular weight which you do in the standard SDS page electrophoresis or you can also separate them based on the ISO electric point in the isoelectric focusing units. Or you can combine the two and then separate proteins in the 2 dimensional which is known as 2 dimensional electrophoresis. Or you can make it more advanced, you can do some quantity proteomics; then you can add the dice to a control samples I 3 die, your treatment samples I 5 dice and then you mix even the various control and treatment samples in one tube, label them I 3 die mix all of them together.

Now, separate them in the first dimension with the ISO electric focusing, second dimension with the molecular weight SDS page and then now you are able to get not only that you know the protein separation in two dimension, but also the protein expression changes you can start monitoring. So, this whole thing is itself is very vast and heavily used which we will talk in a

different course in different platform. But I just thought to give you a reminder again that there are many technologies which are equally important, which we need to appreciate in the whole field of proteomics.

(Refer Slide Time: 11:37)

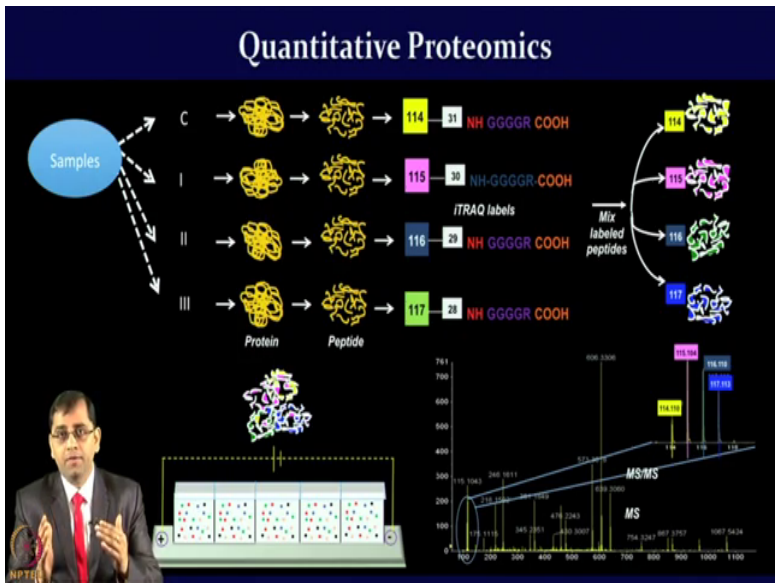


Then comes the field of shotgun proteomics, which is essentially driven with the mass spectrometry. The complex protein sample you can make the lysates, you can digest them using line LysC Trypsin, do reduction alkylation and digestion, followed by the cleanup of the peptides from the complex proteins.

Now, you have made the peptides which could be clean up and now these peptides can be separated using mass spectrometers for MS or MS MS analysis which is going to provide us the peptide sequences and the protein information using the different database search.

But what if you want to quantify the proteins using shotgun approaches where you do not want to separate them for different gels. But directly you want to separate in the mass spectrometers and also you want to look at their expression changes.

(Refer Slide Time: 12:34)



So, therefore, the quantity proteomics using mass spectrometers have become very attractive a way of proteome analysis. Where all the proteins which are not digested in the peptide forms, you want to label those peptides on the n terminal sequence and a different type of reporter ions where different type of tags available, which could be used to label these peptides and that could be used for the field of quantity proteomics

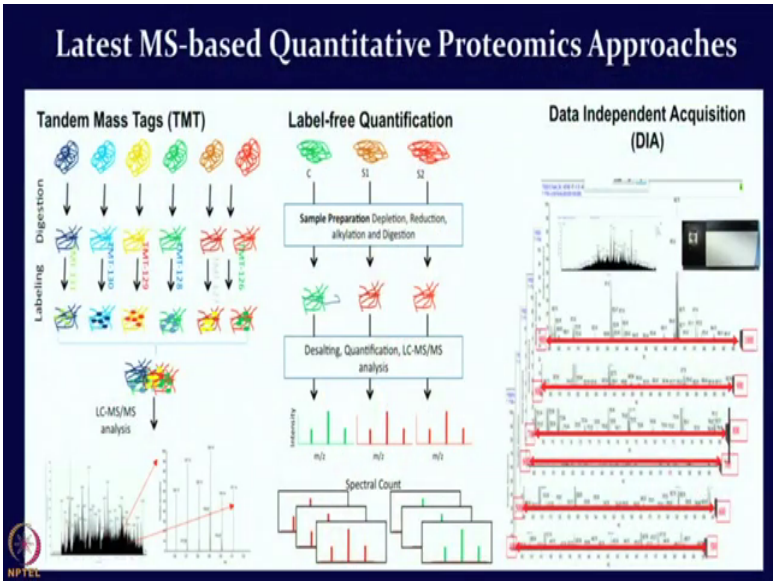
I have shown you here one example of using isobaric tags which are known as i TRAQ or isobaric tag for relative and absolute quantification. Let us take a simple situation, when we have control samples along with three different grades of disease and we want to analyze that

what are the changes happening for each protein or each peptide from the healthy individual to the patients which are having the low grade of any cancer middle grade and high grade.

So, then we are labeling all the four different conditions using four different type of these i TRAQ labels, which gives you the numbers which are reporter ions from 114 to 117 and these reporter ions are going to analyze and look at in the mass spectrometer which will be further used for the peptide quantification and protein quantification.

This whole field of mass spectrometry based proteomics is really evolving very rapidly and while you will have much more detail of this whole field in a different course; but it is good idea for you to appreciate there are lot of advancements are happening in the mass spectrometry based proteomics.

(Refer Slide Time: 14:02)



For example I have shown on the slide different approaches both based on the label based and label free analysis using mass spectrometry for looking at quantitative proteome changes. For example, one could use like i TRAQ even the TMT tag which are another isobaric tag called as tandem mass tag and one could do 6 plates, 10 plates or even 14 plates analysis now with the recent tandem mass tag which are available. Or you want to not label your proteins and you still want to look at their quantification in the mass spectrometers that is known as label free quantification.

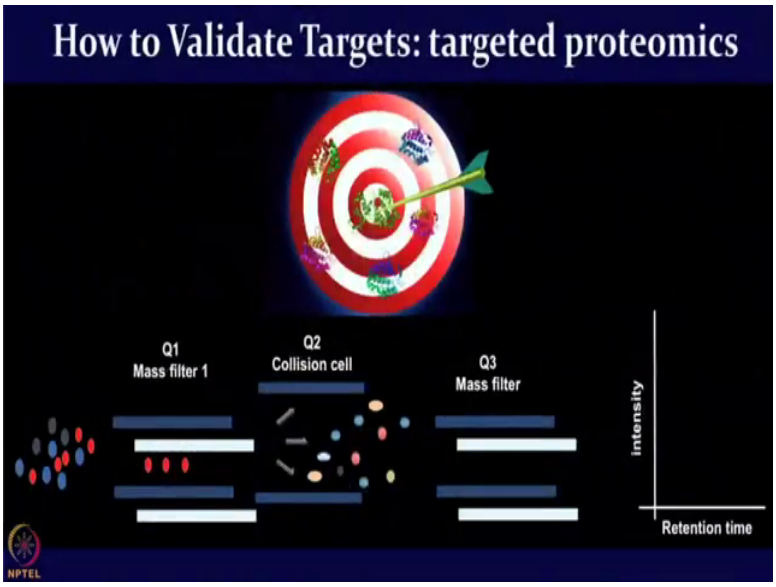
And in this middle part which you see; what you are looking at, you are looking at the spectral count of peptide. So, idea is that, if you given let us say you know we are comparing control with the disease; for a given protein if that protein expression is very high in the disease sample as compared to the control, then many peptides will be formed for that particular protein and then the spectral count will be much more or different numbers will be obtained for the same protein. So, can we measure those spectral count and then that information we can use for the label free quantification.

Additional approaches to also look at data independent acquisition, where each of the window of your master charge separation you want to really obtain a very high revolution image of that and analyze the protein using label free manner.

All of these things are very recent advancement. Of course I am not talking in detail right now, because that is not the focus of the whole course right now. But you should appreciate lot of advancement which has happened in the field of proteomics is starting from the gel based moving to the mass spectrometers and various type of quantitative proteomics methods.

The whole scientific community has been struggling how to validate our targets. We have identified many proteins; whether using micro arrays or using the mass spectrometers or gels. But now if you want to validate your proteins, then you are relying simply on the antibody basis; either western blots or immune assays, some way you need some antibody or some biochemical assay to confirm that what you identified is correct. Therefore, a new field of a vast spectrometry based validation strategy has emerged which is known as target proteomics.

(Refer Slide Time: 16:17)



Here idea is you have identified set of peptides which are differentially expressed from your control versus disease. Now, you want to validate these peptides using triple quadrupole based mass spectrometers. You will set up the various parameters, so that software and hardware will only recognize the specific set of peptides and their transitions. And you are only going to monitor, then the intensity of these peptides to find out are they really up regulated or down regulated when you compare the expression changes in different samples.

So, you can now set up this assay more high throughput manner and if you really want to go even to look at more accurate quantification of the protein. So, you can synthesize the synthetic peptides which could be heavy labeled. And now first you run in your mass spectrometers only synthetic peptides and look at their MS MS spectrum pattern, then you run

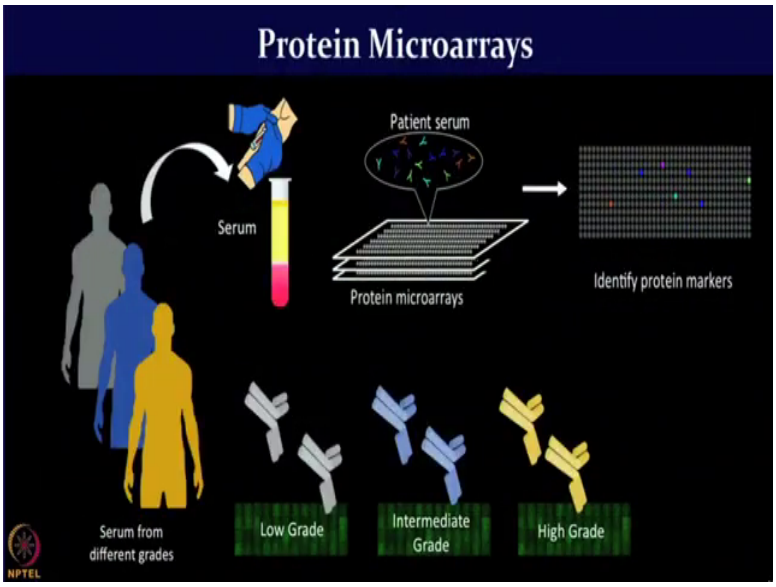
your unknown sample and if it is matching then you are very confident and you can also compare the intensity with the heavy labels.

So, this is known as accurate quantification of the protein, in this way you are able to validate your targets and also you are able to measure that what will be the concentration of these peptides in the given sample.

So, while variety of these technologies have been emerging; scientists have been still exploring further ways more high throughput to manner where we can with very small volume of the sample with very you know; a lot of clinical samples are precious, so when the drug molecules are precious, a lot of biological samples are very precious, your protein and peptide which you extract on the samples are very precious. How best we can conserve the sample and they still obtain the meaningful information in a very high throughput manner.

And also most of the previous technique which I have taught are able to give you lot of leads; but how to now determine the functional consequence of these proteins, how to assign some function to these proteins. As a result different field has emerged known as intractomics, which relies on protein microarrays and different type of label free bio sensors.

(Refer Slide Time: 18:19)



I have shown you here one of the work flows for using protein micro arrays for auto antibody screening in the human patient samples. So, let us assume that you know the patient's blood sample followed by serum has been separated and now you are looking at which are the auto antibodies produced in the serum and can we detect that at the proteome wide manner.

So, in this case on the protein chips the very small volume of the patient serum which is diluted with the buffer will be floated. And now then with a very small volume you know let us say we are talking about 50 to 100 micro litre of the volume of the sample which is sufficient to cover your glass slide which is already having the entire proteome of a given organism.

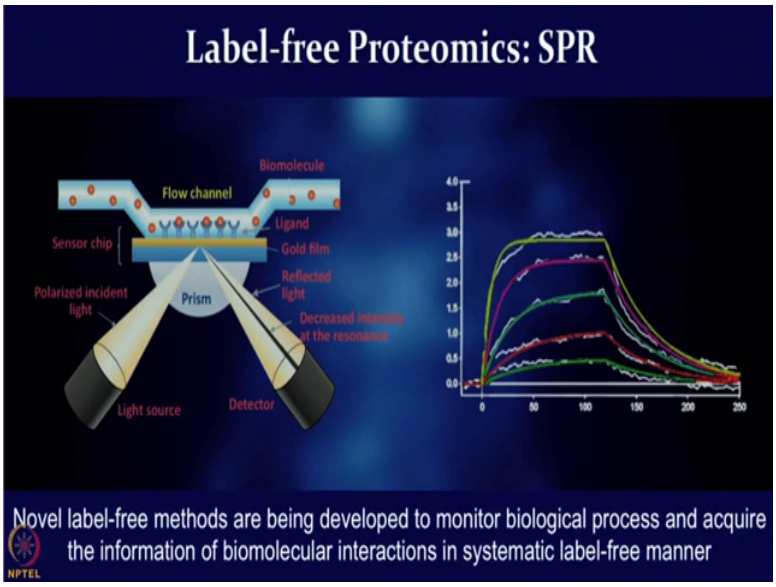
Let us say all the 20000 proteins of human you are able to screen with a very very small volume of the given sample. And wherever you see the signal which you can detect using

secondary antibodies which is anti human igGs labeled with psi 3 or psi 5, then you can find out the potential biomarker candidate. Or you take another context you want to look for the an interactor of a given protein and you have the entire chip which is having all the 20000 human proteins.

So, this protein which is your you know the target protein of interest; how this protein goes and binds with the many other proteins in the entire chip, that will give you idea for the potential interactors of these proteins. And likewise there are many applications which can be done using protein microarrays.

But what if you want to determine that you know the not only there is a protein x bunch of the protein y; but also what is the strength of this binding, what is the on rate of binding, what is the off rate of binding, how strongly these are associated and how strongly or weakly that is dissociated.

(Refer Slide Time: 20:08)



So, then you have to move on to a label free bio sensor platform and surface plasma resonance or SPR is one of the very strong platform to offer this kind of approach. So, as you can see in the slide here. Now, we have a gold slide on which the antibodies are printed; when you flow through your protein of interest, the right antigen will bind to the right antibody pair. And likewise you can have even the you know various other type of molecules printed on the gold slide and then if a small molecule is coming, then protein will bind to the small molecule of the drug and you are looking at protein drug binding interactions.

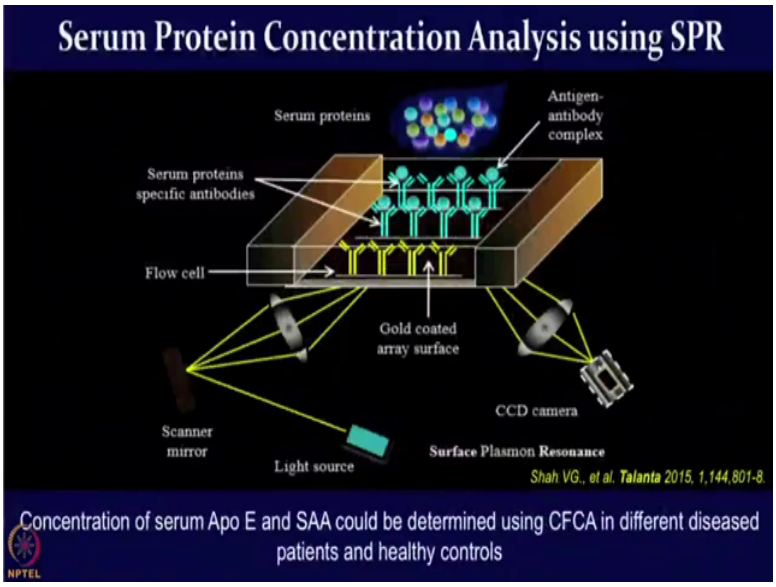
So, many type of interaction studies could be planned and designed using these kind of label free bio sensors; and what you obtain is essentially the sensodram which gives an idea for the association and dissociation rate of these binding. And eventually you can also calculate the

capital K, capital D which is the decision constant and the kinetics of this binding which is happening in the system.

So, the many novel label free methods are currently being used; of course, I have only showed one of them, but many of them are relying on different type of physical principles to measure the proteins and different bio molecules with the physical principles without having any tag which is associated with the these molecules to be detected. So, you are not adding any kind of fluorescent tag or radioactive tags to measure the binding interaction.

But you are simply looking at the change in the reflectance or looking at the conductance change, various type of you know even interferometry based change those are all going to help us to determine what is the binding happening in the label free manner. So, this is probably most you know natural most closest thing which can happen without giving any artifact signal. So, even our own group and others have also started using some of these technologies for even some very interesting applications which are beyond the standard workflow of these you know instruments.

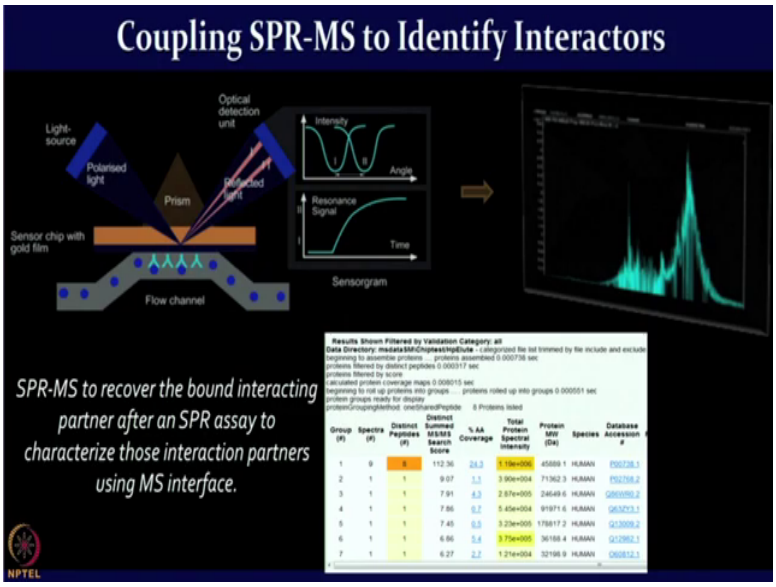
(Refer Slide Time: 22:13)



For example, even a sphere we have used to measure the protein concentration from the serum sample. If there are some serum biomarkers of interest rather than relying on the Eliza kit, can we measure their binding on the gold chip by passing the complex serum samples and looking at how unique this binding interaction is happening and one could actually start developing these assays much faster. And we have also come up with some very innovative ways of measuring these interactions using CFCA, which is calibration free concentration analysis of different type of serum protein measurement.

As you go along with the course you will be exposed to many technologies; but you should also keep in mind how best to integrate different technologies and make best use of each other technology platform.

(Refer Slide Time: 22:59)

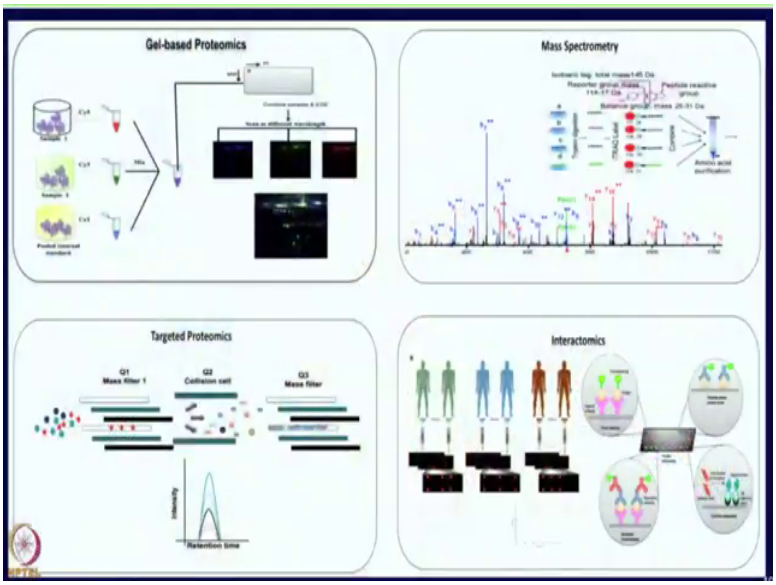


For example SPR could be used to identify the interactors of interest, right. Let us say you have an antibody and you are passing a complex lysate. Now, many proteins will be there in the complex lysate which will probably bind to the antibodies; antibody will bind to an antigen of interest and antigen may have even the complex which is having many other proteins.

So, now, if you enrich this particular interaction by running the same reaction in multiple wells, multiple channels. And now you can elute out all the potential interactions which you have identified from these reactions followed by if you can digest these proteins make a peptide form and run in the mass spectrometer. So, you can actually identify now the potentially interactors.

So, in many ways you are now able to look for the new potential interactors, which was not known; but you have to apply coupling the different technology platforms. So, we have utilized here surface plasma resonance with mass spectrometry to identify some new interactors.

(Refer Slide Time: 24:01)



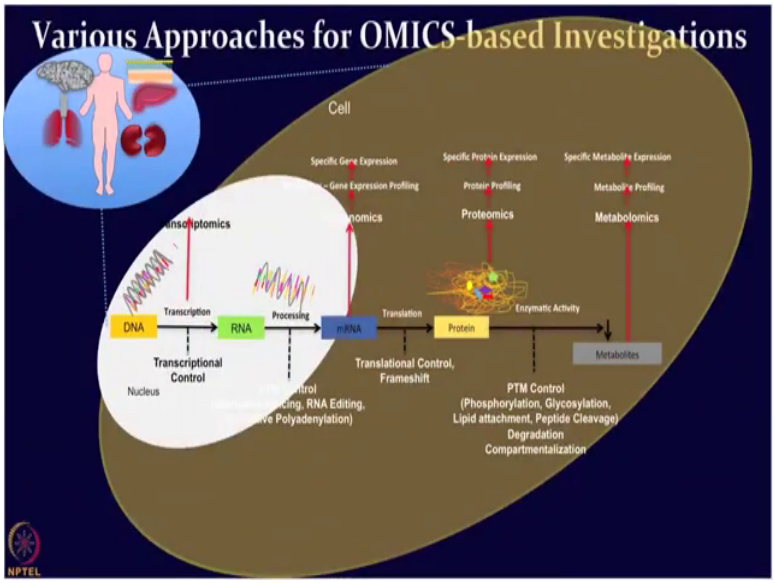
So, in a nutshell if you look at the slide, I try to convey you the idea that there are many technologies which are a part of the field of proteomics is starting from the gel based proteomics to mass spectrometry based platform, target proteomics, interactomics.

Of course, each field itself is very vast and deserve a full course on its own. So, we have dedicated this particular course on the field of interactomics which is much more functional understanding functional consequence of the proteins to understand and that is probably much

more relevant for that of you know biologist and people working in the field of you know life sciences.

Then it comes the looking at any kind of complex biological problem. I think you have to be very unbiased and think about any technology which can answer the question in a more efficient manner.

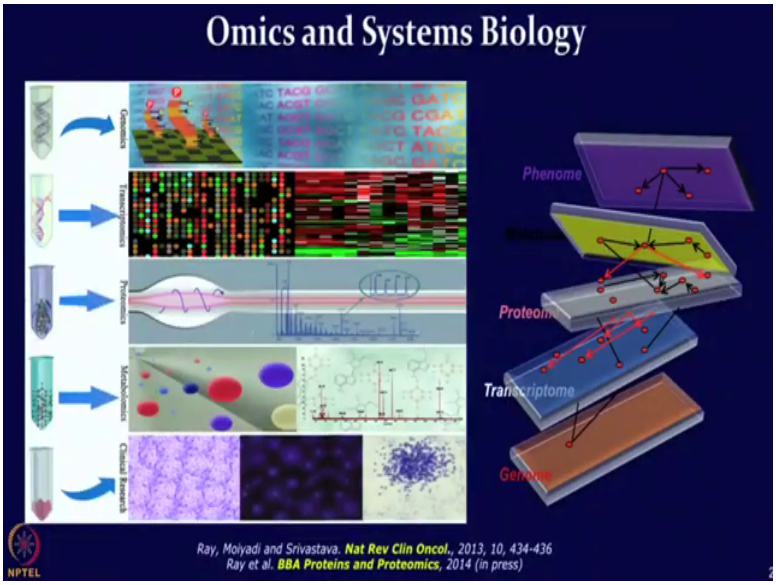
(Refer Slide Time: 24:53)



So, therefore, even when people are looking at the biomarkers for a given disease; it is good idea to look at various bio molecules and then try to integrate the information. As you can see the complex systems biology, the complex central dogma of the life is starting from DNA, RNA, mRNA protein and then followed by metabolites.

All of this offers you know the various type of novel molecules and you never know that the most of the clues are coming from which type of bio molecule. If our resources, our time, if our technologies are available and we could apply that it is actually a good idea to take the same samples and try to understand different level of information is starting from the genome, transcriptome, proteome and metabolome and then try to integrate the information to find the most meaningful data set.

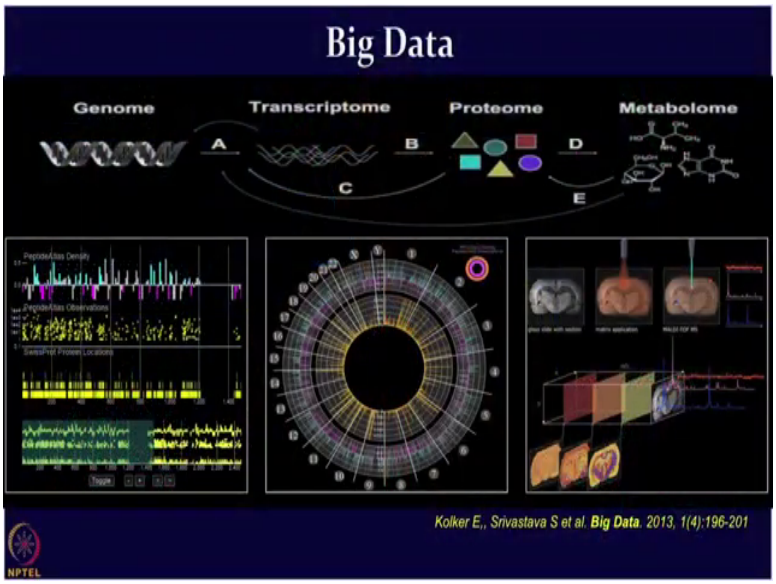
(Refer Slide Time: 25:37)



This is what is conveyed in the slide that the actual physiological context is very complex. We have to build the layers of information for the same organism. For any organism we have to start looking at the entire cascade of the events happening from the genes to transcripts to the proteins to metabolites and along with their phenotype, their environmental effect all of them is only going to give the complete picture.

And if you are looking at more on the human biology or medicine, then we have to add the clinical details and clinical information to make it more comprehensive; and then only I think we should be able to get some very very right answer for the question which we wanted to address.

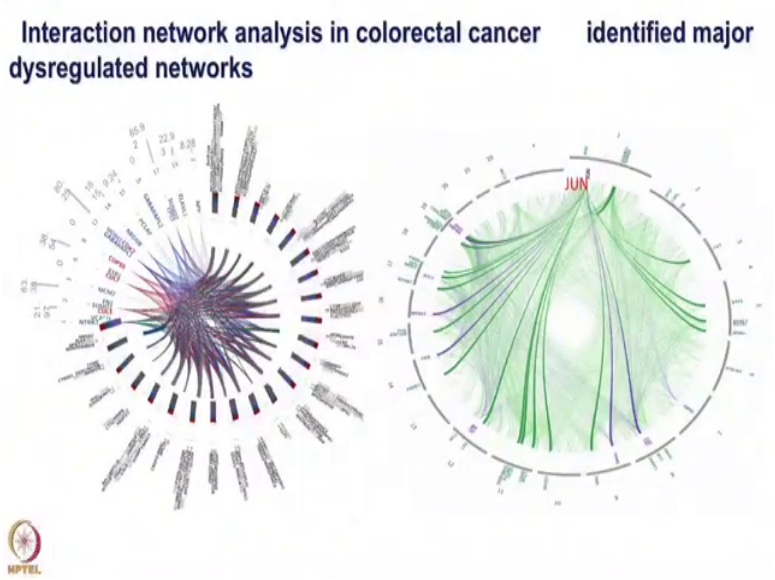
(Refer Slide Time: 26:27)



So, all of this technologies which we talked starting from the genomics and of course, genomics have really taken the big leads with the net generation sequencing technology platforms. And lot of things are still you know very revolutionary happening in the field of genomics. And then field also progressed toward transcriptomics and proteomics and metabolomics, all of them are generating the big data set; along with lot of medical information, the radiology, immunohistochemistry and different types of other datasets in life sciences.

If you think about now the life science field and omics field is generating a huge amount of data and that data is going to be very very revolutionary nature, very it is going to transform our understanding of biology.

(Refer Slide Time: 26:59)



Because I showed a slide here for a given disease which we were looking at in the colorectal cancer; how different type of data set when we started analyzing them in the system network level, what we are identified was that it was a set of proteins were quite distinct in the colorectal cancer patients as compared to the you know the peritumoral regions or the normal regions. And then some of these associated candidate proteins we are trying to map them as a part of the interaction analysis.

And finally, with the system that work analysis we found that, most of these are actually mapping towards certain nodes and which are those nodes, how they are going to regulate the

complex disease this is kind of excitement which one could try to obtain by looking at this is a big data set and then try to understand that there are many changes happening.

But what can be the root cause of many other changes, which are the major nodes which are governing all of these associated protein changes. So, then I think you know looking at data in much more comprehensive manner and then trying to identify the systems level analysis is going to be very powerful.

(Refer Slide Time: 28:05)

However the big data are also offered a lot of challenges; how are we going to link the big data which we obtain from variety of these technology I have discussed to the real life innovation and the new knowledge and ultimately how it can really transform the societal development.

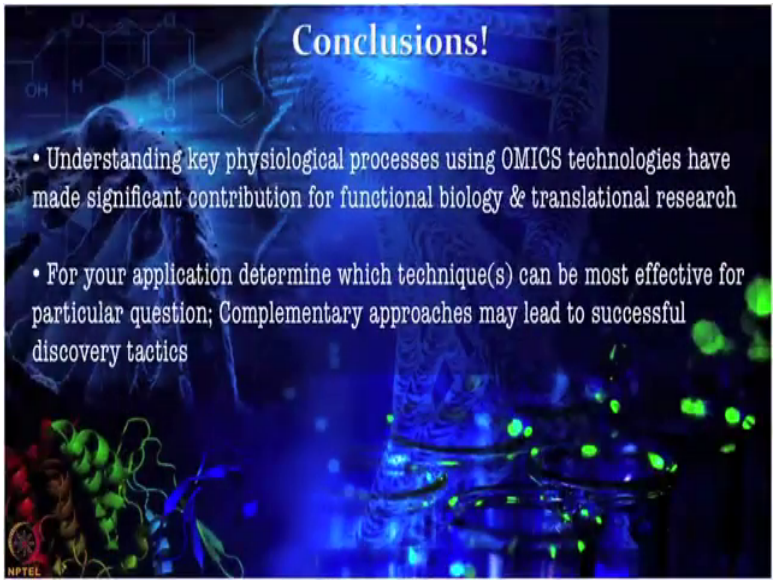
These are the questions in front of us and of course, the big data which is coming very rapidly also opens many question; what are the guidelines of the good data set which we are obtaining for the big data.

And of course, field it is evolving for each of the kind of instrument and technology Now, there are set of guidelines coming forward which one of you should keep in mind and keep an eye on those papers which defines the guideline for what is accepted as a best data set.

Next question comes where the data should be stored. So, much data terabytes and terabytes of data every day, we are generating; how are you going to store the data which can also be retrieved easily from the other users.

Data accessibility; are we going to just simply share with everybody or we are going to have at least you know some way of coding the data or some way of coding the patient information and then start utilizing that and share that information in more meaningful way. What could be the ethical consequence of sharing this data? There are many things which are part of the regulations and guidelines of the big dataset. I must appreciate that Department of Biotechnology Government of India has they simply taken of this matter seriously and they have also not defined the certain guidelines and regulations for the big data. And likewise the whole OMICS field is also keep deliberating what should be the best guideline of looking at a Big Data and sharing the Big Data sets.

(Refer Slide Time: 29:36)



All right just to summarize the lecture; I hope I conveyed you that there are many technologies many promising technologies and approaches are part of the field of OMICS. And specifically I spoke about the proteomics and variety of platforms are available which you can utilize to address different type of biological questions. Each of these technologies have a lot of potential; but you need not to get influenced with these technologies, rather you think about what is the biological question you want to address and accordingly you can choose the right type of technology for your application type.

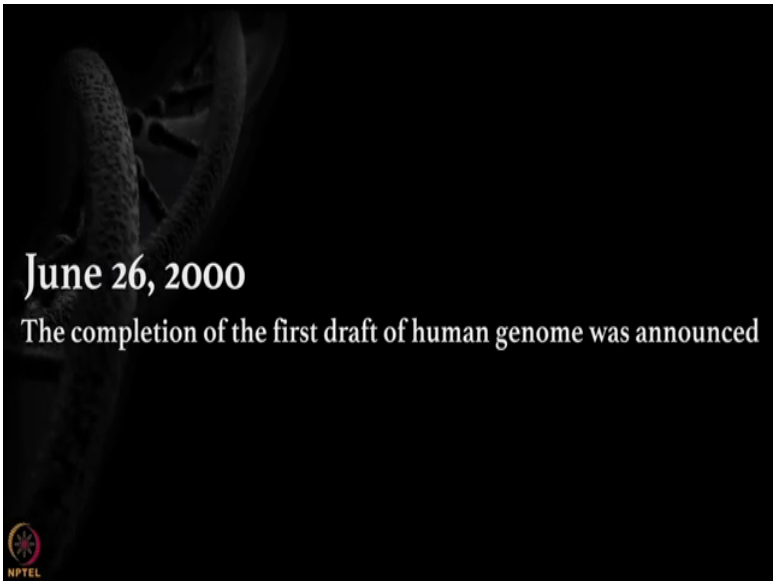
I hope this sets up a good platform for you to appreciate the vast field of proteomics and several excitements and challenges which are part of this field. And we are going to focus more on it specifically or interactomics from the subsequent lectures. But I am going to leave you with this video which you will watch on journey of proteomics field and how the

proteomics can translate the code of life. So, we made this documentary and please watch this.

Thank you very much for attending the lecture and finish this video and then be ready to interact with you in the next lecture on interactomics.

Thank you.

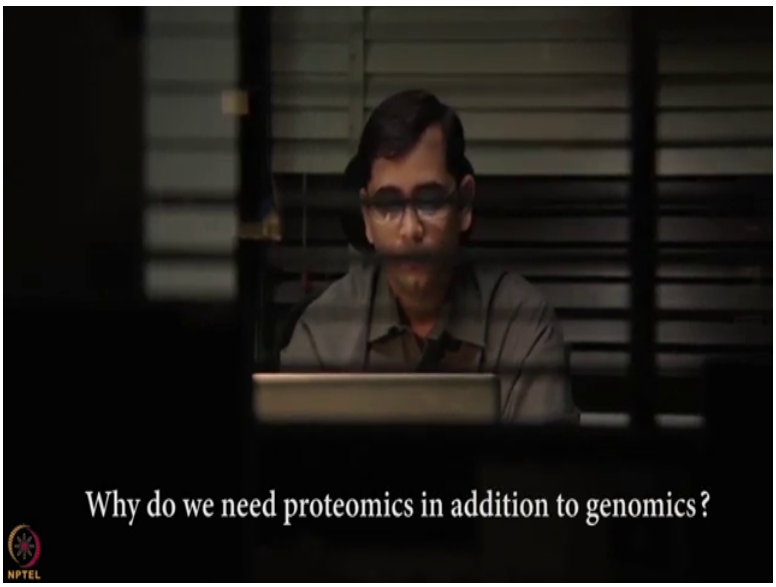
(Refer Slide Time: 30:44)



Most important most wondrous map ever produced by humankind.

With genomics setting a foundation in the quest to uncover the mysteries of life; what lies next I wonder. Perhaps time has now come to appreciate the significance of OMIC technologies such as proteomics.

(Refer Slide Time: 31:17)



Proteins and proteomics are central to connect genomes with phenotypes with normal biological processes.

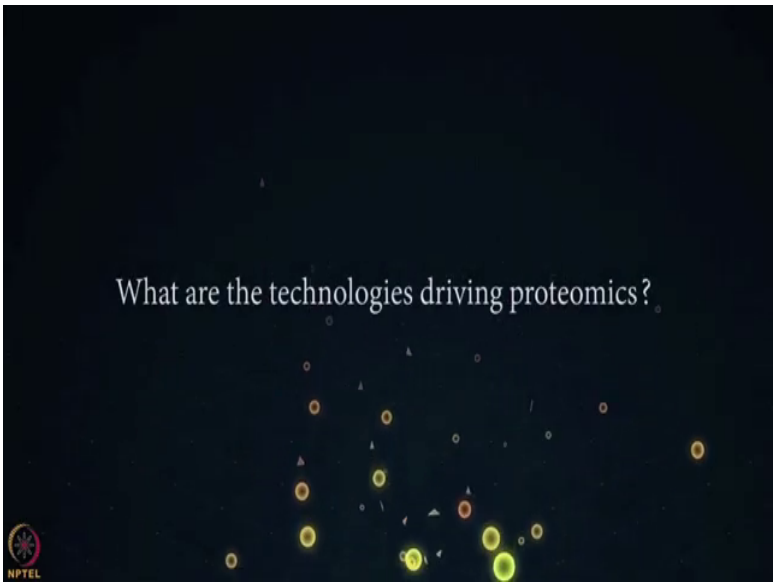
There is no way to predict from the genome itself, the crucial features of proteins.

The real key here is that genomics can only tell you of the snapshot of the organism; whereas, proteomics is will enable us to look at the organism dynamically.

Proteomics is the science where we want to know about all the proteins that are coded for by the genome.

Proteomics addresses whole systems and uses a broad unbiased approach to derive new findings.

(Refer Slide Time: 31:58)



What are the technologies driving proteomics?

(Refer Slide Time: 32:01)

Several techniques like mass spectrometry protein, microarrays.

(Refer Slide Time: 32:04)

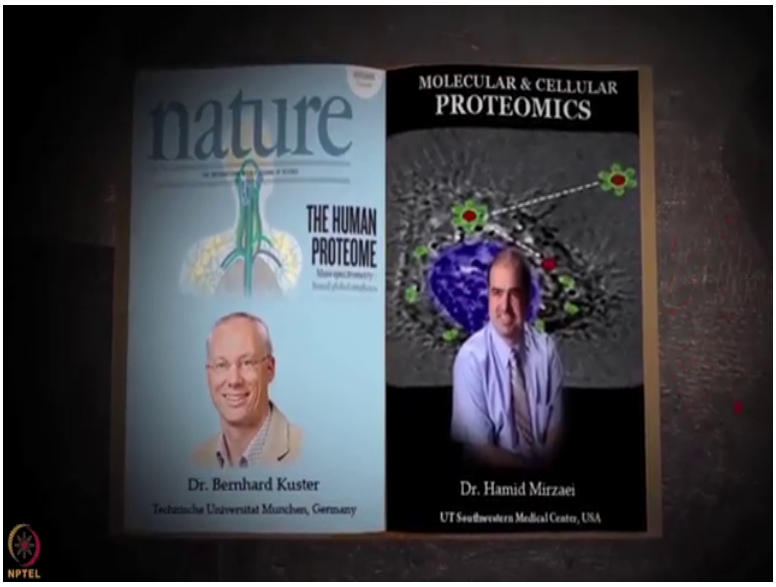
(Refer Slide Time: 32:06)

And label free bio sensors have been used in proteomics to elucidate the expression, localization and interaction of proteins.

(Refer Slide Time: 32:14)

Unraveling the proteome is a very onerous and complex journey that demands the efforts and insights of an interdisciplinary team of scientists.

(Refer Slide Time: 32:25)

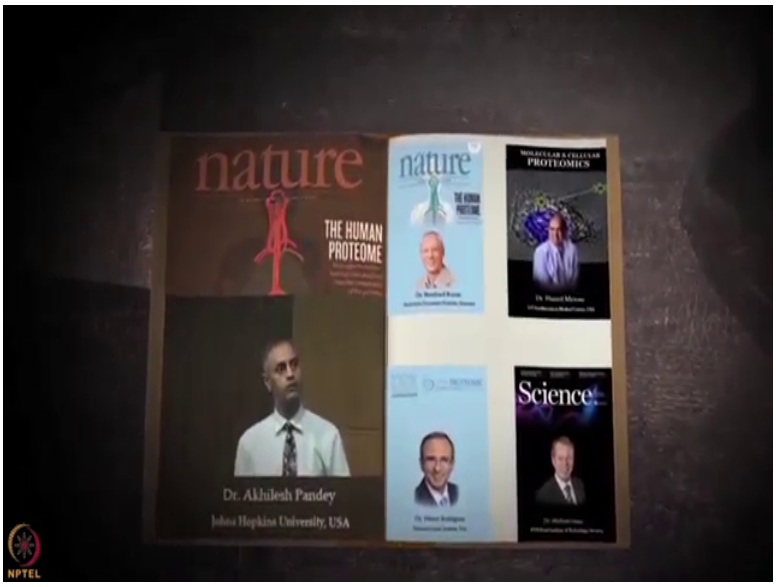


14 years after the release of the first draft of the human genome, scientists are working on the map of the human proteome.

(Refer Slide Time: 32:30)

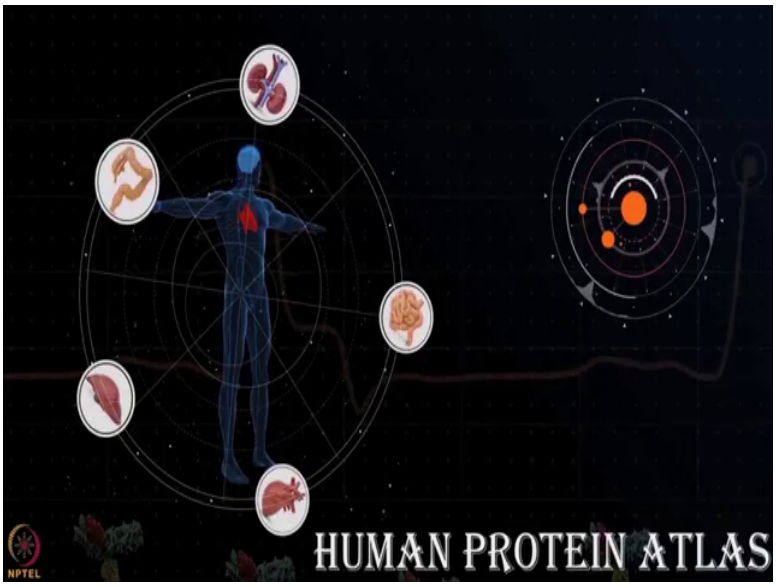
Using high resolution mass spectrometry combined with high credence infomatics.

(Refer Slide Time: 32:34)



And other methods including antibody based protein microarrays.

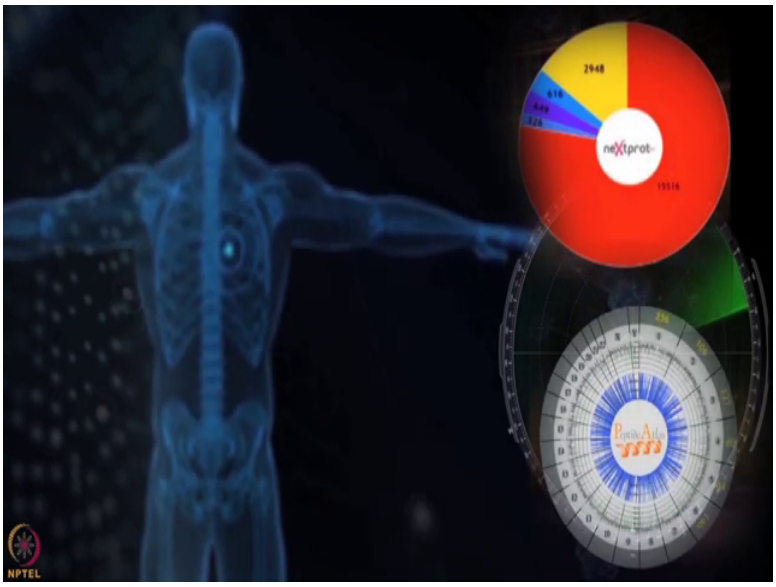
(Refer Slide Time: 32:42)



In addition to mass spectrometry based protein maps, scientists used tissue array platforms to specially locate proteins in human cells and tissues and map their expression levels in various biological tissues. One major effort in this area has resulted in the human protein atlas.

(Refer Slide Time: 33:00)

(Refer Slide Time: 33:05)



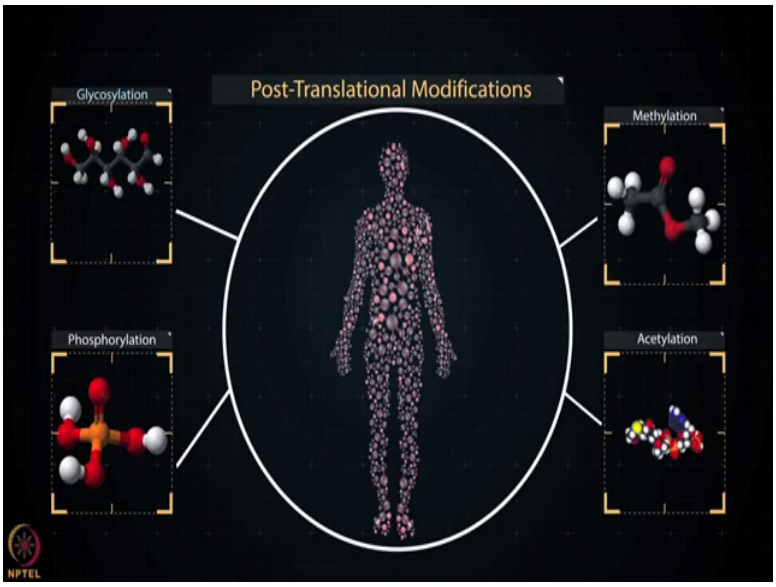
Despite this progress there exist missing proteins which are yet to be annotated. Scientists hypothesize that the missing proteome could be a result of relatively low abundance, tissue type, development or stress specific expression of proteins; with the road ahead requiring comprehensive proteogenomic analysis and consideration of many additional sources of biological information.

(Refer Slide Time: 33:28)

What is the applicability of proteomics in healthcare?

Researchers are using novel approaches for biomarker discovery by studying the differential expression of alternatively spliced ISO forms in cancer, low abundance blood based biomarkers for disease diagnosis, studying cell secretome, analyzing human immune response to develop vaccine, improving proteomic methods to study the host pathogen interactions and so on. And hopefully some of the biomarkers that we discover will be useful for identifying disease easily, for helping doctors identify the best prognosis for patients.

(Refer Slide Time: 34:05)



Proteomics has the unique ability to analyze protein production, degradation and post translational modifications which are highly pertinent for biomarker discovery and translation.

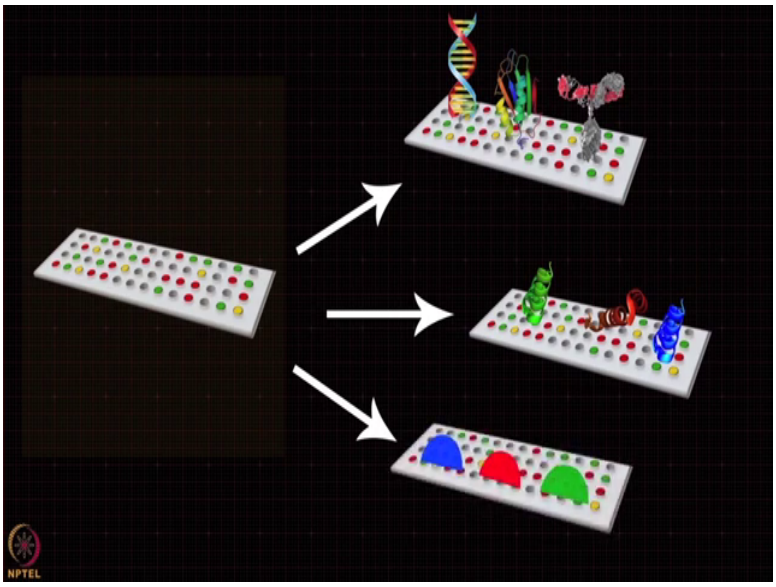
In critical solution specific form of protein will give us a more specific and sensitive approach to detect the disease in a tissue and body fruit.

(Refer Slide Time: 34:29)

Several novel techniques such as multiplex protein microarrays and beet based arrays are also contributing to biomarker discovery.

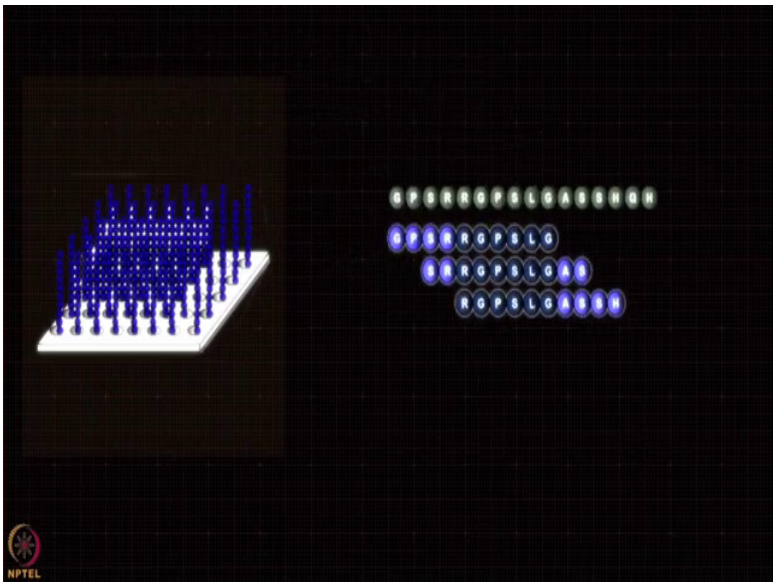
Protein micro array contains sources of proteins in one chip which allow us to grow protein functions is a certain molecular problem.

(Refer Slide Time: 34:48)



A recent innovation in this field is the development of ultra high density peptide arrays comprising of millions of peptides.

(Refer Slide Time: 34:57)

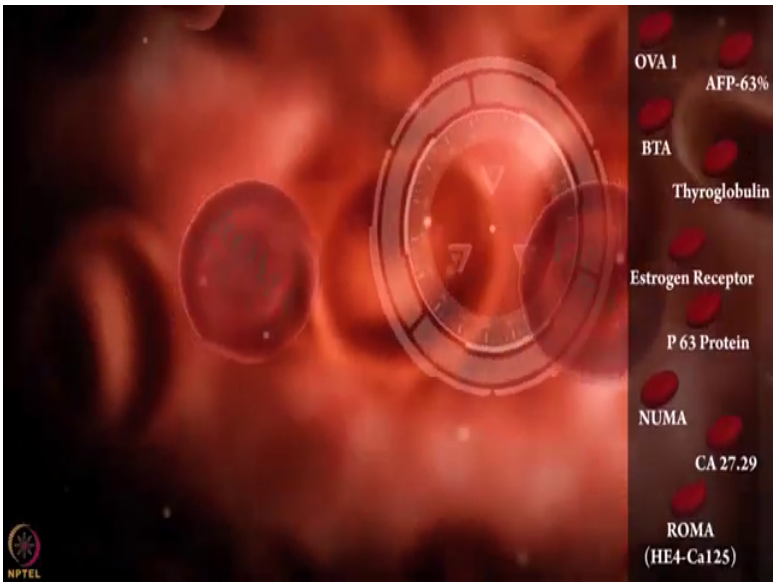


Thereby allowing large scale probing of biological samples.

(Refer Slide Time: 35:04)

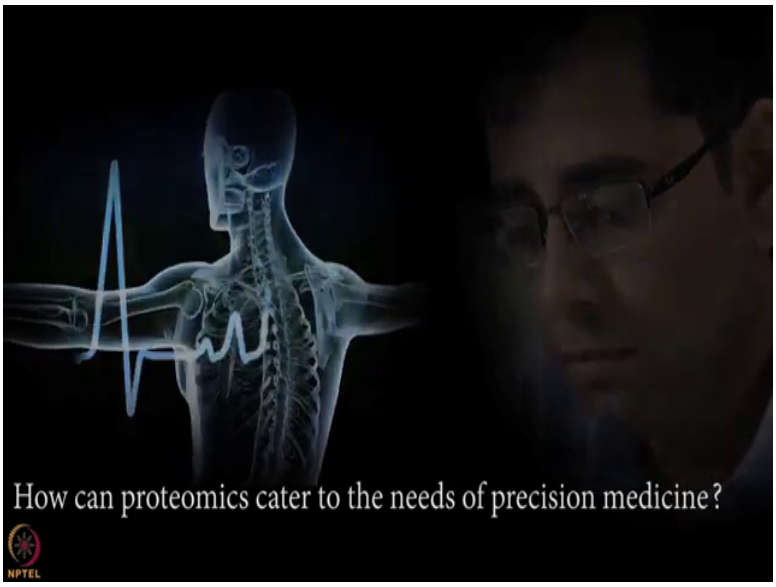
Present diagnostic as well as therapeutic strategies that are existing in clinical scenario, clinical setting are all a result of the biology discoveries that have gone on over the last 50 years.

(Refer Slide Time: 35:27)



The proteomics community has kept up it is expectation of being a complementary approach alongside genomics, thus contributing to diagnostic assays in clinics.

(Refer Slide Time: 35:40)



How can proteomics cater to the needs of precision medicine?

What if figuring out the right dose of medicine was as simple as taking our temperature and that is the promise of precision medicine.

The precision medicine means, one patient, one drug at a time and therefore, if you can detect specific target for any given cancer type; we can then develop treatment based on that target information and therefore, the precision medicine is going to be the future up on quality.

My opinion that to be able to figure out precision medicine both at the diagnostic level as well as to be able to determine the pathways in which drugs should be targeted too and you have to be able to really quantify well all the premium.

(Refer Slide Time: 36:33)

How is proteomics moving towards targeted validation?

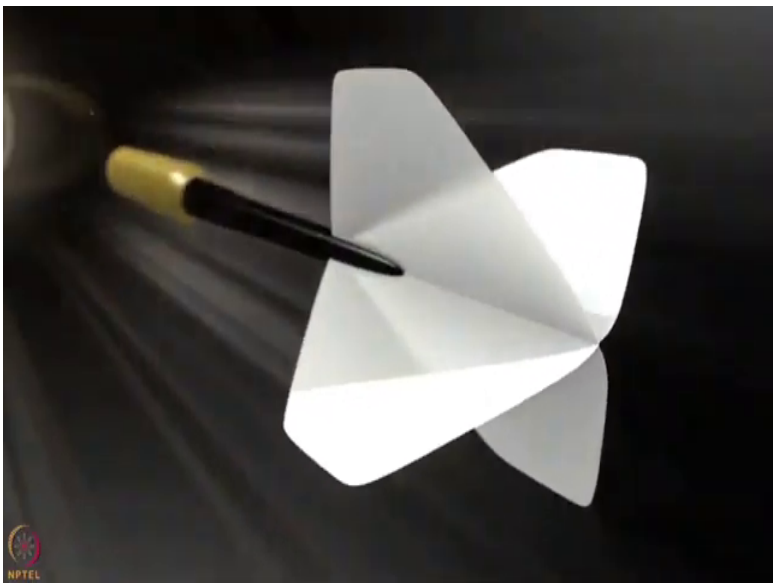
One of the challenges facing proteomics is being able to get from the discovery all the way to validated markers they are useful in the clinic.

So, that is why targeted proteomics is so important by sacrificing a little bit in terms of depth of coverage; but selecting analytes that we can very deeply about we can design proteomic assays, that will be able to measure analytes whether their proteins or peptides each and every time that we do an experiment.

More specifically this assay have been now widely accepted in the community as PRM or a perform on triple quadrupole instrument.

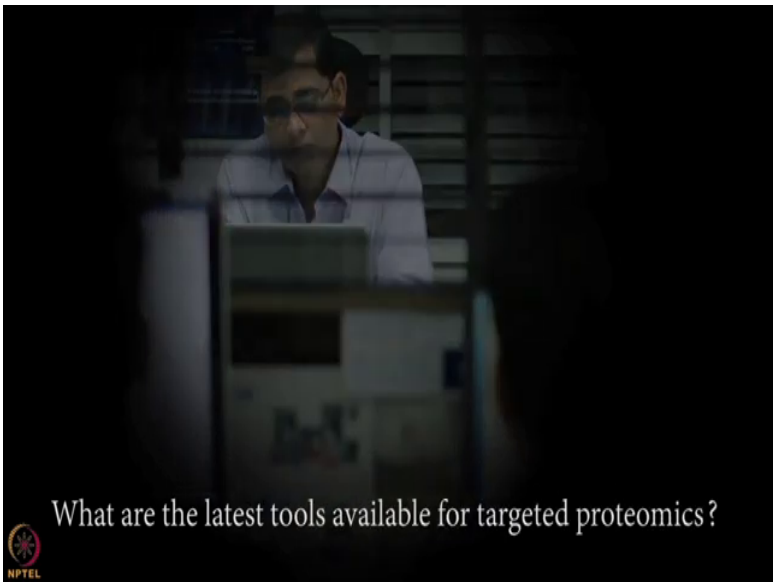
And I think it is become very clear, that those methods including multiple reaction monitoring and PRM and allied methods are going to have a major impact on the developments of clinical diagnostics and then ultimately on patients health unlock.

(Refer Slide Time: 37:31)



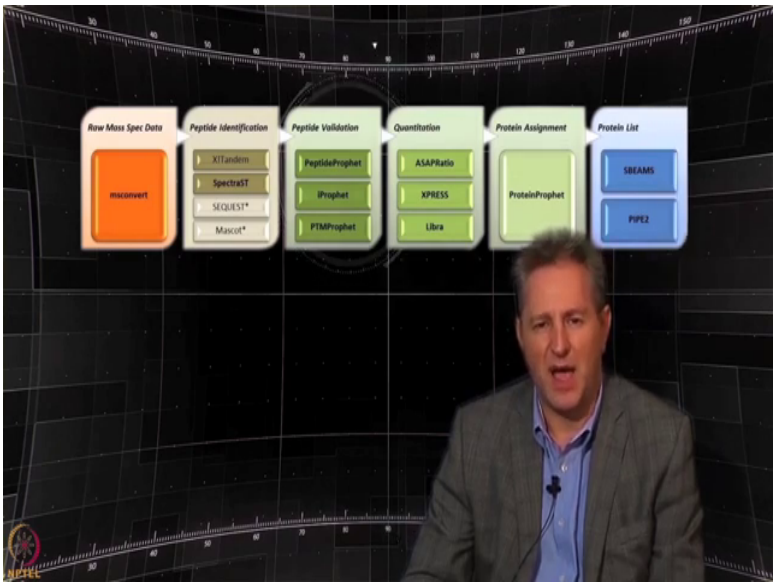
Innovations and technologies like targeted proteomes have allowed researchers to validate interesting targets overcoming the bottleneck of availability of reliable analytical reagents.

(Refer Slide Time: 37:42)



(Refer Slide Time: 37:47)

.

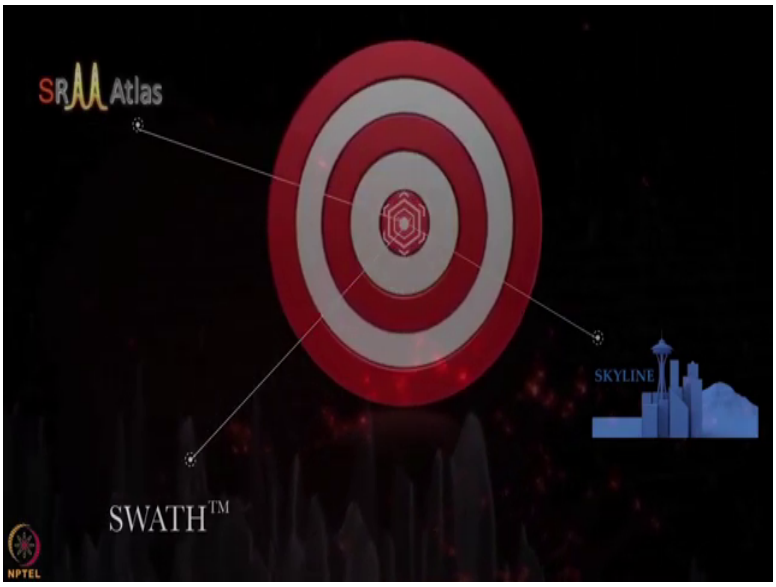


The transferring partners has given us the capability to analyze proteomics in an unprecedented manner; but there is also given us the capability to understand which proteins are being expressed, which proteins can be analyzed by mass spectrometry and we can utilize that in a targeted approach.

I have been developing SKYLINE for the past 8 years and it is grown incredibly; it is been used around the world and it is exciting to see that the proteomics researchers really seemed to feel that it helps them with their research.

SWATH analysis method was to expand the SRM type approach to much larger number of proteins potentially 100s to 2000 of proteins.

(Refer Slide Time: 38:29)



The advent of new age techniques like SWATH with ability to identify and quantify proteins or peptides over a dynamic range and powerful tools like SKYLINE and SRM atlas have added to the arsenal of targeted proteomics.

How is the global proteomics community poised?

(Refer Slide Time: 38:47)

The human proteome organization is an international scientific organization that promotes international scientific cooperation, educational training and technology breakthroughs in proteomics.

Hippo was started in February 2001 in Versailles France. Around the time when the first draft of the human genome was released; the human proteome project was launched nearly 10 years later in Sydney at our annual world congress. Hippo coordinates it is flagship scientific project it is called the human proteome project and that is broken down into three main thrusts; chromosomal effect, a biology effect and a disease effect.

(Refer Slide Time: 39:32)

And it has three supporting technology pillars; bioinformatics, mass spectrometry and antibodies that support these thrusts.

Must take a comprehensive proteome wide view of cell functions connect the genome, proteome and the phenotypes.

Whether you are an early researcher or a citizen veteran seeking to make important new discoveries; I encourage you to engage in this vibrant and really exciting project.

The journey of proteomics has been promising, my belief is that the proteomics community can contribute immensely to functional biology and decipher innovative solutions. Persistent efforts from communities like Hippo has helped proteomics establish an undeniable

international prestige. This has established the broader theme of research directions thus translating the code of life.