Introduction to Proteogenomics

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Supplementary Lecture – 11
Introduction to Targeted Proteomics

Hello. So, over the next two lectures we will be talking about selected reaction monitoring also called multiple reaction monitoring.

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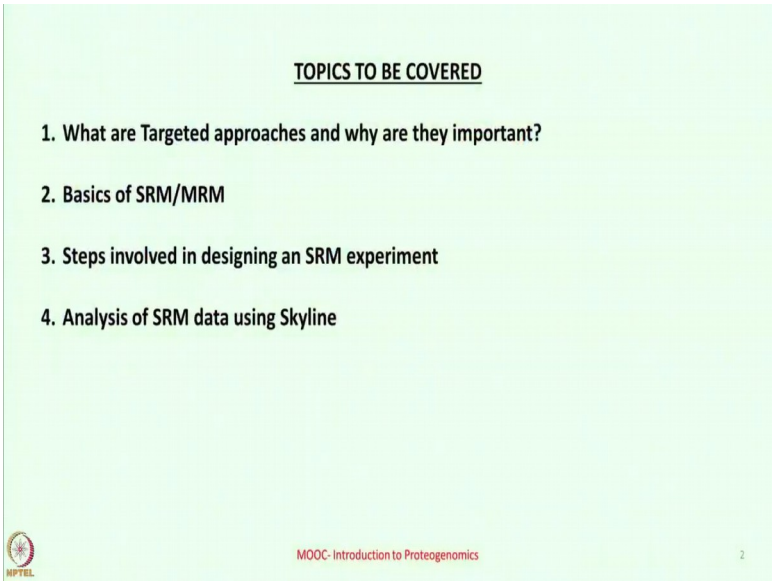

So, the topics that would be broadly covered would involve what are Targeted Proteomics approaches and why are they important? The basics of SRM or MRM, the various steps that are involved in designing a successful targeted proteomics experiment and the analysis of SRM data using skyline. The analysis of the generated data set would be covered in the next lecture and this lecture would broadly cover the first three points.

So, let us now dive deep into what are targeted approaches and why do they play such an important role in the field of proteomics. So far you have learnt about the shotgun proteomic approaches where in the abundance of the peptides that are detected at the MS1 level determines which peptides get fragmented at the MS2 level. Upon fragmentation at the MS2

level the identity of the peptide can be known. Subsequently this these are mapped to the proteins they come from.

So, it can be argued that the shotgun proteomic approaches largely rely on the abundance of the peptides. So, one inherent problem of the shotgun approaches has been that the peptides which are of low abundance usually go undetected. Hence, the identity of all the proteins may or may not be possible using shotgun approaches. So, just to give an idea this is how the shotgun approach looks like.

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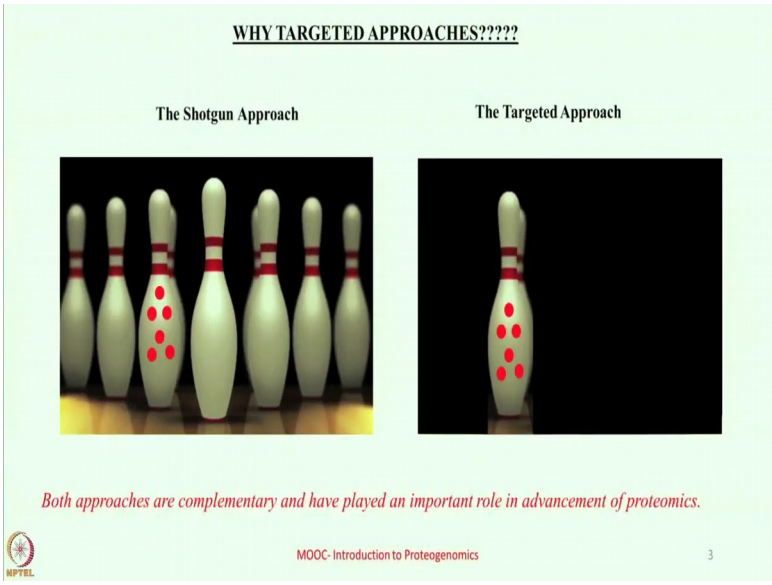

You have different targets. One of the targets which bears the red dots is the actual target which you wish to hit, but you may or may not always succeed in hitting this target and more often than not you are likely to hit all the other targets along with the target of interest. This pretty much sums up how a shotgun approach works.

On the contrary the targeted approach relies very heavily on the information of the protein and its peptides and a few other parameters about which we would discuss in the slides to come. It is to be noted that the targeted approaches are very specific and hence, have become the choice of interest for most proteomics labs around the world. The targeted approaches are largely hypothesis driven. So, one can just look at those proteins of interest on which the hypothesis has been built upon.

However both the shotgun approaches and the targeted approaches are equally important and have played an important role in the development of proteomics. So, while we do not advocate any one particular approach over the other, it is to be noted that the shotgun approaches have long served the proteomics field in the discovery phase whereas, the targeted approaches are now increasingly replacing the conventional validation approaches which involved the use of antibodies like ELISA or western blotting. So, this is again the overview of the shotgun proteomics workflow.

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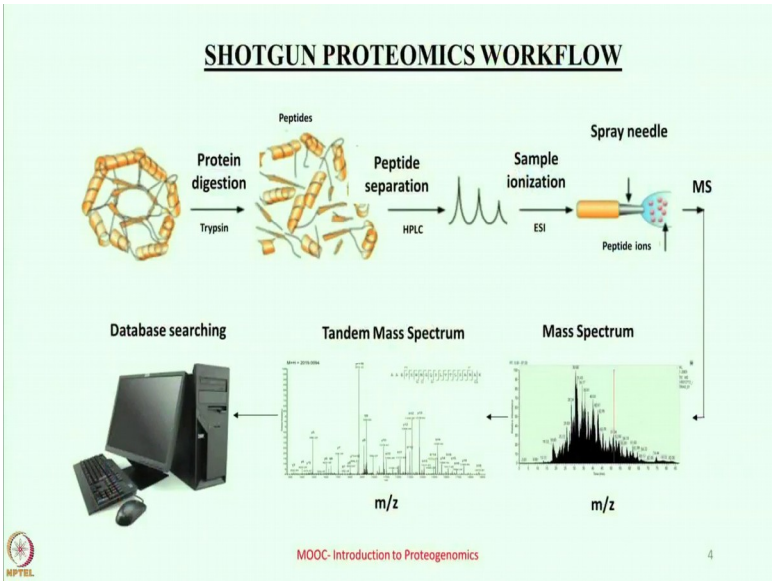

I am sure that by now you are very well acquainted with the steps involved in a shotgun experiment, but I would quickly walk through the major steps that are involved. So, we start with say the protein lysate on which the experiment is to be performed and then using a protease, we digest these proteins to give rise to peptides. The digested peptides are then separated on column and using electrospray ionization, the peptides get charged.

So, by now you must be aware that only charged species get detected inside a mass spectrometer. So, ESI plays a very crucial role in mass spectrometry since it imparts charges on the peptides enabling them to fly inside a mass spectrometer. The next step is the identification of the peptides and database searching. This is the conventional shotgun proteomics workflow.

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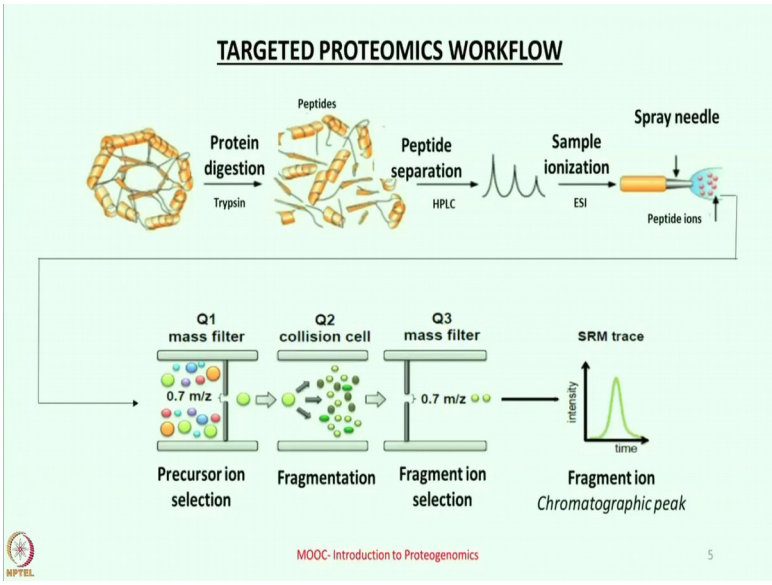

So, the targeted proteomics workflow follow pretty much the same steps except for the fact that the mass spectrometer is instructed to monitor specific peptides and their resulting fragments. The slide that you see currently differs from the previous slide in the fact that the instrument here has been much better instructed to selectively monitor only a few peptides. So, before we look at the principle of selected reaction monitoring and how it works, I would want to spend some time on the different targeted approaches that are known to us.

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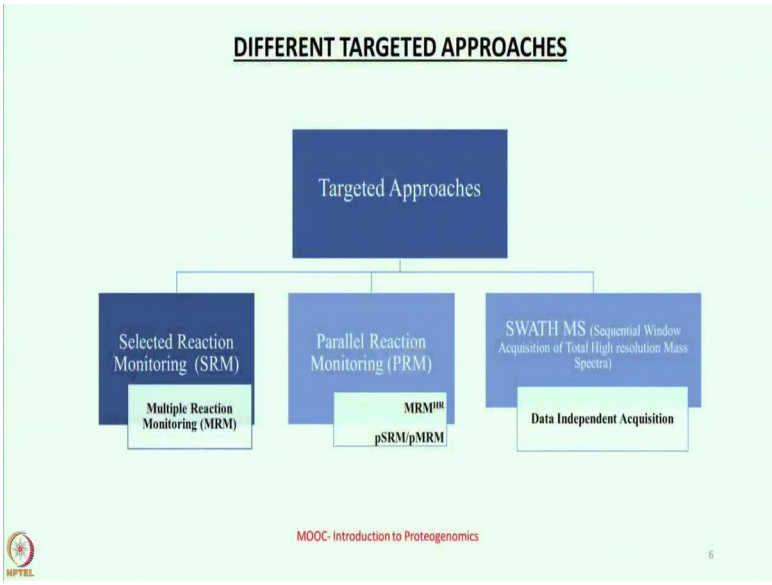

So, as we can see in the slide there are three main branches for the targeted approaches wherein the first is selected reaction monitoring, the second is parallel reaction monitoring and the third is SWATH MS. While the SWATH MS is beyond the scope of this lecture? This topic has been extensively covered by Mr David Campbell from Institute for Systems Biology.

The selected reaction monitoring approach is also called multiple reaction monitoring. This technique involves the use of a triple quadrupole mass spectrometer. The first quadrupole and the third quadrupole of the instrument act as mass filters allowing us to monitor a peptide and its corresponding fragment alone. The parallel reaction monitoring approach is an advancement over selected reaction monitoring, contrary to the use of a triple quadrupole mass spectrometer as seen in selected reaction monitoring. The parallel reaction monitoring approach involves the use of mass spectrometers with very high resolution.

So, these mass spectrometers usually contain orbitrap or the time of flight mass analyzers. So, it can be argued that parallel reaction monitoring approaches will offer much higher resolution as compared to selected reaction monitoring. So, the parallel reaction monitoring approaches are also called MRM HR or SRM HR with HR standing for High Resolution. So, we now look at the crucial steps that are involved in designing a targeted proteomics experiment.

So, I would like to spend some time on this slide because it is very crucial to make a note of all those parameters that one needs to optimize for the success of a targeted proteomics experiment.

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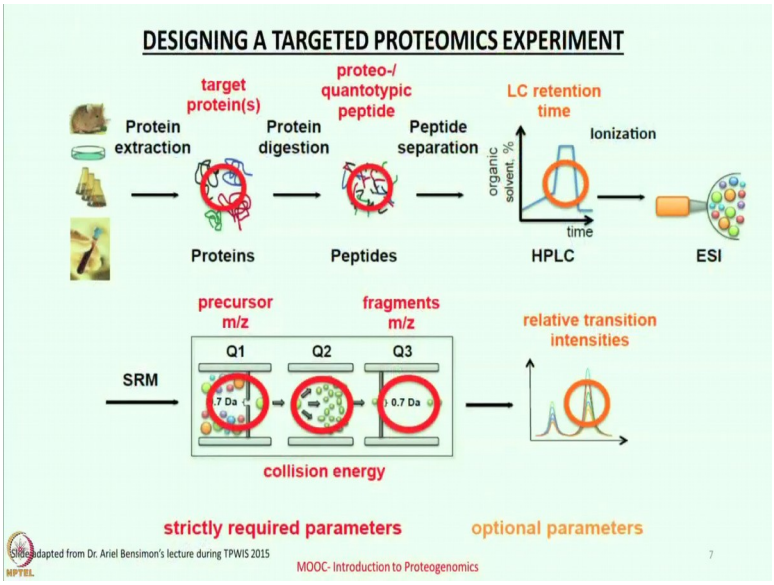

Since the targeted experiments are usually hypothesis driven or the researcher has an idea as to the proteins that here she wishes to monitor, the target proteins. The information on the target proteins plays a very important role. The sample processing involved in a targeted proteomics experiment is no different from that seen in a conventional shotgun experiment. The proteins are first digested using a protease and this would result in the formation of peptides.

The digested product would now contain peptides corresponding to the proteins of interest. A few of these peptides may or may not be proteotypic or quantotypic. So, quantotypic peptides are the ones which are relatively stable over a period of time and can be used for quantification. So, one can narrow down on these peptides through repeated experiments and through repeated optimization experiments and these peptides can then be taken forward at a later stage when the goal is to quantify or to find out the amount of the proteins of interest.

So once the peptides are formed, they are separated over a liquid chromatography set up and one of the more crucial parameters for the success of a targeted proteomics experiment is to have an idea of the retention time of the peptides. By now you are aware that peptides differ in their behavior based on their amino acid composition and their hydrophobicity.

So, it is very much likely that the peptides elute at different times from a column. So, one needs to have an idea of the LC gradient that is to be used for efficient elution of the peptides

of interest. The next step is the parameter called collision energy. Collision energy is that energy which is needed to break the bond between two amino acids.

So, on applying correct collision energy the peptides give rise to different fragments. So, one needs to have a very clear idea of the collision energy that is to be used to fragment the peptide of interest thereby giving rise to the different fragments which will then be monitored in the mass spectrometer.

So, as you can see from the slide that one needs to have an idea of the precursor mass to charge ratio and the fragment mass to charge ratio. The precursor mass to charge ratio corresponds to the mass to charge value of the peptide of interest. Subsequently the fragment mass to charge corresponds to the mass to charge of the fragment that results from fragmentation of the precursor peptide.

So, having an idea of each of these parameters would largely determine the success of an experiment and I urge you to look at different tutorials and lectures that are already available which cover these topics in much more detail. We now see the principle of selected reaction monitoring.

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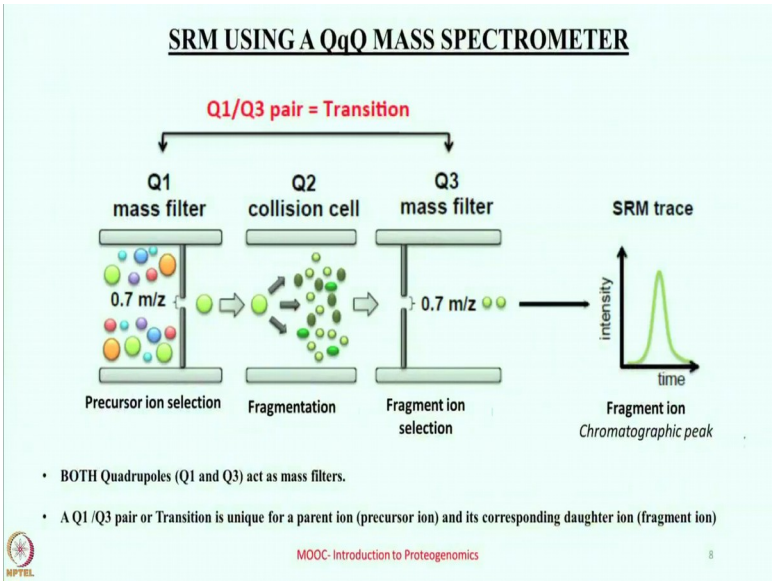

So as you can see from the slide, selected reaction monitoring uses a triple quadrupole mass spectrometer and in this current slide we can see that there are three quadrupoles quadruple 1

is a mass filter and quadrupole 3 is also a mass filter, quadrupole 2 on the other hand is a collision cell where the fragmentation of the peptides takes place.

So, the quadrupole 1 here has been assigned a particular mass to charge ratio. This would mean that only the species which fall in this mass to charge range would be allowed inside the mass spectrometer. Ideally, you would want the mass spectrometer to be as much selective as possible. So, the success of the experiment would largely depend on each of the parameters that were discussed in the slide before. Once the peptides have entered into the collision cell, they will get fragmented based on the collision energy value that has been already provided to the instrument.

This fragmentation would randomly result in the formation of different fragments and it is at this stage that the third quadrupole acts as a selective mass filter again wherein it would led through only a few fragments to pass through. So, it is to be noted that the method file that is provided to the instrument for acquisition contains all the relevant information and that is how the instrument is able to monitor the peptides and the fragments of interest. So to sum up the slide, both the quadrupoles of the instrument act as a mass filters thereby filtering out all the other unwanted peptides and the fragments and an important term that needs to be discussed here is the term Transition.

So, you would come across this term very frequently when you read papers on selected reaction monitoring and a transition is basically a quadrupole 1 to quadrupole 3 pair. So, the value of quadrupole 1 and quadrupole 3 for a peptide and its corresponding fragment is called a transition. So, let me just quickly walk you through an example to help you understand this concept better.

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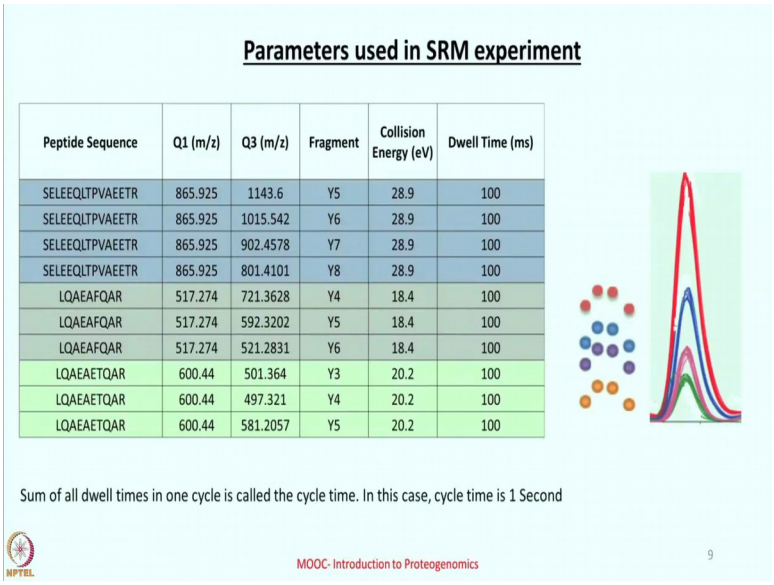

In this slide you can see that there are 3 peptide sequences and the table contains the information of the peptide that is the peptide sequence. It also contains the quadrupole 1 mass to charge and the quadrupole 3 mass to charge. It also contains information of the fragment which results after cleavage of the peptide bond, the collision energy information and another column called the dwell time about which we will talk in a few minutes from now.

So, as you can see each of these 3 peptides has different values fed in the table. So, let us consider the first four rows of the column wherein we are talking about peptide 1. So, as you can see the quadrupole 1 mass to charge for this peptide is the same in all the four rows, however what is different is the quadrupole 3 mass to charge which means that while the quadrupole 1 is selectively allowing only the one peptide of interest, so the quadrupole 3 has a job to let four different fragments arising from peptide 1 to pass through.

The collision energy of this peptide is the same and the same would apply for all the other peptide sequences in the table. Subsequently the table also contains information of two other peptides wherein a quadrupole 1 and quadrupole 3 charges have been mentioned and their respective collision energies. Now, the time that the instrument takes to monitor a transition in a particular cycle is called dwell time. So as you can see each of these fragments has been acquired over a period of 100 milliseconds.

So, the instrument does the job of acquiring individual fragments for peptide 1, then this is a brief pause time after which it starts collecting the information for peptide 2 again a pause

time and then it would collect the information of peptide 3. So, the sum total of all these dwell times that you see in the table would give rise to a cycle time.

The cycle time is that time where is the instrument needs to collect at least one data point for every transition that is a part of the transition list. So, it is safe to assume that the data points would be picked largely depending on the chromatographic behavior of the peptide of interest and the end result would be a chromatogram as you see on the slide. So, this chromatogram is formed by joining all the data points that the instrument was able to collect over the retention time of the peak.

With this we conclude the basic principle and the steps that are required to design a selected reaction monitoring experiment. In the next slide, we will look at a data that was generated in the lab and we would use the popular software skyline to analyze a data set.

Thank you.

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