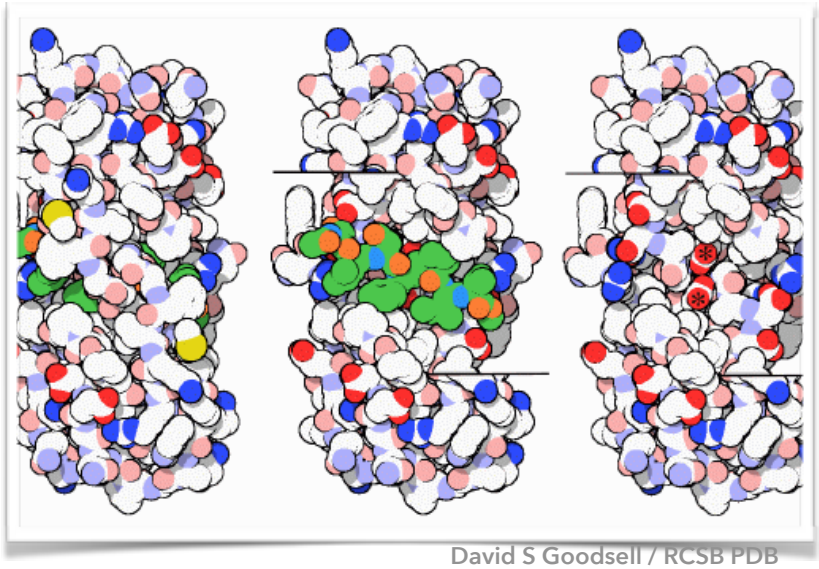

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Protease: Advances in Proteomics

Advances of proteomics and technologies applied to protease have contributed tremendously to the discovery of protein as well as understanding the complexity of the biological system

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The involvement of proteases in physiological functions of all living organisms is necessary given their key roles in many important biological processes. Among the essential processes include progress of cell-cycle, digestion of food proteins, apoptosis, blood-clotting cascade, signal transduction, tissue remodelling and immune responses. In fact, they are thoroughly studied to investigate their structure-function relationships and interactions with substrates and inhibitors. Nowadays, the studies are focusing towards on improving their efficiency and changing the specificity by protein engineering to cater industrial and therapeutic purposes.

Protease serves a broad range of research applications and one of the prominent areas that are rapidly developing is proteomics. Aimed at identifying, characterizing and quantifying proteins, proteomic studies offer a great deal of information in understanding the dynamics of cellular processes. While genomics have provided a wealth of data on DNA and protein sequences, it is the proteomics that have fundamentally delineate the functional units of a cell, proteins and their complex interactive networks and signalling pathways in the biological system.

The advent of mass spectrometry (MS) as powerful analytical tool for biological analysis is timely and many areas have benefited from this technology. MS-based proteomics is one absolute evident where the central life science technology has generated great advance towards the comprehensive study of protein. Furthermore, major breakthrough in MS and its application to proteomics has led to the discovery of thousands of both known and novel proteins. This allows identification of boundaries

in biological networks, investigation and measuring changes of perturbation-induced network and correlation between dynamic networks with cellular phenotype.

Basically, proteins consisted of large fragments and present abundantly in a sample. In order to guarantee an efficient and accurate analysis of the proteomes, proteins of interest are first converted into peptides before they are subjected for identification and quantification. This first step of proteomics pipeline is also known as proteolytic digest which yields the smaller fragments of peptides. These small fragments of peptides are then separated and ionized by liquid chromatography (LC) coupled to electrospray ionization upon their analysis by MS which resulted in spectra that led to detection, identification and quantification of the proteomes (Walther and Mann, 2010).

Liquid chromatography-mass spectrometry (LC-MS) has been used as powerful method for the aforementioned purposes. It is highly regarded as high performance due to the mass accuracy, sensitivity and analytical robustness. However, even a powerful analytical tool has its own limit in terms of reproducibility. Proteins and peptides are entities of complex biological system that exhibit a wide range of physicochemical properties. Fragmentation of the proteins by universal means proved to be a mounting task due to the variations in solubility and hydrophobicity and these give rise to large differences in MS responses.

Discrimination of the proteases for certain amino acid residues has been identified as one of the limitations to a successful coverage of a protein sequence. One of the optimization strategies towards MS-based proteomics which is the bottom-up approach using different proteolytic enzymes such as trypsin has been proven as a successful application. In addition, Fonslow et al. (2013) have cited the exploitation of classic Michaelis-Menten enzyme kinetics to equalize proteomes in an unbiased fashion. The digestion of abundant proteins into peptides is made selectively with a molecular weight cutoff spin-filter. For example, a combination of both highly selective and non-selective proteases is used on protemic samples with low complexity whereas complementary peptides are created by utilizing a combination of highly selective proteases for complex proteomic samples (Swaney et al., 2010).

Currently, the enzymatic technologies are applied on the bottom-up proteomics to improve efficiency of the digestion and coverage of the proteomes. Kinetics of tryptic digestion is improved with the immobilization of trypsin within microreactors (Freije et al., 2005; Ethier et al., 2006), microparticles (Guo et al., 2011) and nanoparticles (Qin et al., 2012) which further improve the speed and digestion time of the proteomes. Apart from that, conditions of the enzymatic digestion of the proteomes are also optimized by microwave heating of proteins under acidic conditions as well as increasing the pressure of proteolytic digestion.

As of late, researches focus are trending towards application of advanced proteomics especially for clinical purposes, an area that could further information on disease, pathways, targets and drug effects. Sakurai et al. (2013) have recently developed an advanced method to identify metabolizing enzyme in human kidney by proteomic correlation profiling. The team has

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successfully identified alkaline phosphatase from a drug metabolizing enzyme with potential benefits to treat autoimmune diseases. Cravatt (2014), on the other hand, quoted the discovery of functionally annotate enzyme activities in mammalian physiology and disease through the application of activity-based protein profiling (ABPP) and complementary proteomic methods.

ABPP is a chemical strategy to determine the functional state of enzymes in complex proteomes by utilizing active site-directed probes where several distinct classes of enzymes have been successfully identified. Among the enzymes include serine proteases, metalloproteases, aldehyde dehydrogenases and glutathione S-transferases that have been proposed to play a role in cancer. A great help can be benefited from the proteomic profiling based on enzyme activity especially in drug discovery where targets of diagnosis and therapy can be identified as well as revealing the fundamental mechanisms of action in disease-sustaining proteins (Sanchez-Carbayo, 2010).

In Conclusion

advances of proteomics and technologies applied to protease have contributed tremendously to the discovery of protein members in cellular context as well as understanding the complexity of the biological system. Technology-driven tools like MS have not only helped generating amass amount of information that had led to significant findings especially in clinical development. Further progress can be made with the integration of other members in OMICS (i.e. genomics, transcriptomics and metabolomics) in an effort to unlock the mysteries underlying the complex of biological system.

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