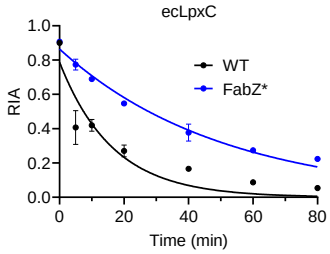
Abstract

Title: Drug Discovery: The use of Mass spectrometry and other analytical tools

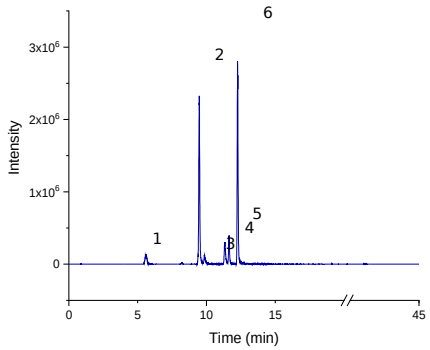
Drug discovery is considered as a three element equation where bioactive compounds are developed against a molecular target and thereby modulate a disease phenotype. Recent advances in omics technologies and chemical biology are revolutionizing the analytical toolbox available to support drug discovery. Proteins are important biochemical components in both drug target and viral/bacterial associated infections. They are regulated at multiple levels which include expression, post-translational modification, turn-over, subcellular localization and interactions with other biomolecules. The recent developments of multifaceted cell biological and biochemical approaches coupled to MS-based proteomics have also made it possible to measure multiple functional properties of proteins.

In the first part of the talk, using MS based proteomic technique, target turn-over(degradation and re-synthesis of a target) has been explored as one of the factors that effect vulnerability of a target. Target vulnerability is defined as the fraction of the target that is required to be occupied to elicit a desired pharmacodynamic response. The degree of target occupancy (inhibition) required to block bacterial growth, or the target vulnerability may vary widely across different targets. Low vulnerability targets must remain continuously occupied at high levels, whereas a high vulnerability target require low levels of occupancy to evoke a response. Therefore, continuous doses of drug must be administered to keep a low vulnerability target inhibited, whereas for a high vulnerability target, the dosage requirements would be reduced. Consequently, the therapeutic index is improved. Cell-wash out experiments can provide insight into target vulnerability. Therefore, if the lifetime of a drug-target complex is much longer than the elimination time of the drug, prolonged occupancy on a highly vulnerable target will have sustained effects. Kinetic selectivity on a high vulnerability target thus contributes to the widening of the therapeutic index. In antibacterial space the cell-wash out experiments, or post-antibiotic effect (PAE) is assessed by diluting the cells exposed to drug into fresh media and then monitoring regrowth. Target turn-over can tune vulnerability of a target, a high turn-over target is less vulnerable as more doses of drug would be required for desired occupancy. In this work, pulse-Chased SILAC has been used to probe turn-over of a bacterial enzyme target UDP-3-O-acyl-N-acetylglucosamine deacetylase (LpxC) and linking it to cell-wash out PAE(1).



In the second part of the talk, LC MS/MS using multiple reaction monitoring (MRM) has been leveraged to study a new drug modality called oncolytic virus (OV)- Vesicular Stomatitis Virus (VSV). Replication-competent oncolytic viruses (OVs) are emerging as promising cancer

immunotherapies with demonstrable clinical efficacy. The precise and accurate quantification of viral associated proteins are prerequisite for the successful development of this modality. We have leveraged the power of MRM technique along with heavy-surrogate peptide standard to obtain precise quantification of VSV proteins. With this assay we have answered an important question, that remains a knowledge gap in the field: what the level of full-length GPC processing in the virus is. Our results demonstrate that this assay can monitor the processing of GPC to its components GP1 and GP2 over time, providing a novel edge to our assay(2, 3).



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