1.1

The End of the End: High through-put discovery and analysis of degrons

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The stability of proteins is controlled in large part by the ubiquitin-proteasome system, UPS, whereby certain polyubiquitinated proteins are recognized and selectively degraded by the proeasome. Recognition of proteins for ubiquitination by E3 ubiquitin ligase complexes occurs through the recognition of degron motifs on the proteins. These degron motifs are often small linear amino acid stretches that provide docking sites for the E3 complexes to bind and ubiquitinate the target protein. We have developed a high through-put genetic proteomic screening platform called Global Protein Stability (GPS) analysis to profile and identify degron sequences in proteins as well and their the cognate E3 ligase. Using this system and peptide libraries covering the entire human proteome we have identified 1000s of degron containing peptides. Among these we identified multiple classes of C-end degrons and 11 families of E3 ligases including Cul2-KLHDC2, Cul2-KLHDC3, Cul2-KLHDC10, Cul2-FEM1C, Cul4-DCAF12, Cul4-TRCP4AP and others that recognize some of these. We also performed stability profiling of the human N-terminome, and uncovered multiple additional features of N-degron pathways. In addition to uncovering extended specificities of UBR E3 ligases, we characterized two related Cullin-RING E3 ligase complexes, Cul2-ZYG11B and Cul2-ZER1, that act redundantly to target N-terminal glycine. N-terminal glycine degrons are depleted at native N-termini but strongly enriched at caspase cleavage sites, suggesting roles for the substrate adaptors ZYG11B and ZER1 in protein degradation during apoptosis. Furthermore, ZYG11B and ZER1 participate in the quality control of N–myristoylated proteins, wherein N-terminal glycine degrons are conditionally exposed following a failure of N–myristoylation. Thus, an additional N-degron pathway specific for glycine regulates the stability of metazoan proteomes. We will discuss these findings and additional applications of the GPS system for degron analysis.

1.2

Drug effects on protein homeostasis

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Protein degradation plays important roles in biological processes and is tightly regulated. Further, targeted proteolysis is an emerging research tool and therapeutic strategy. Mass spectrometry-based proteomics techniques enable the investigation of the causes and consequences of protein degradation in biological systems. We recently developed 'multiplexed proteome dynamics profiling', mPDP, combining dynamic-SILAC labelling with isobaric mass tagging for the multiplexed analysis of drug effects and stimuli on protein degradation and synthesis. When applied in combination with other quantitative proteomics approaches such as chemoproteomics and thermal protein profiling, mPDP provides unique insights in drug mechanism-of-action. The presentation will focus on the MoA of targeted protein degraders (PROTACs) and other compounds affecting protein stability.

1.3

Development, use, application of novel TIMS-Tof system

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Adding an ion mobility separation capability to quadrupole time of flight (TOF) instruments has the principal attraction that its time scale of about 100 ms fits perfectly between the chromatographic (about 5 s) and the TOF time scales (0.1 ms). The fact that ion mobility and mass are correlated is turned into an advantage in the 'parallel acquisition serial fragmentation' principle. In PASEF, the quadrupole isolation window is placed in rapid succession at the m/z of the peptides eluting from the trapped ion mass spectrometry (TIMS) device in a Bruker instrument. In this way, PASEF increases the fragmentation speed by an order of magnitude without any loss of sensitivity. This is especially attractive in conjunction with short gradient, high throughput analyses and in conjunction with metabolic labeling strategies such as the EASI-tag. The PASEF principle can also be extended to data independent analysis (diaPASEF), as will be shown for a number of applications. Furthermore, the ion mobility values turn out to be highly reproducible – within the one percent range – and they can also be readily predicted from the peptide sequence using deep neural networks. Finally, we show that the PASEF principle directly carries over to the analysis of small molecules such as lipids. A major interest of our group is the translational or clinical use of proteomics. In this regard, the robustness of the PASEF tims-TOF instrument is particularly promising. This will be illustrated with measurements of macrodissected FFPE material from many cancer patients. We will also introduce a novel 'single cell proteomics' approach called 'deep visual proteomics', in which we employ deep learning to classify and isolate cancer cells of interest from pathology slides, followed by high sensitivity analysis by PASEF tims TOF.

1.4

Lysine-targeted covalent inhibitors and chemoproteomic probes

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Most targeted covalent drugs have been designed to react with cysteine. However, many ligand binding sites in protein targets of interest lack an accessible cysteine. An alternative strategy is to target lysine, which is more prevalent yet less reactive than cysteine. Here, I will describe our efforts to design chemoproteomic probes that selectively modify catalytic and noncatalytic lysines in living cells. We have developed a chemoproteomic workflow that enables direct identification of probe targets and modification sites by mass spectrometry. Our lysine-targeted probes have shown utility in cell biological and target engagement experiments.

1.5

Mapping proteolysis at the surface of living cells

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Proteolysis of cell surface proteins impacts a broad range of biological activities, including adhesion, migration, cell-cell communication, and signal transduction. However, few tools are available to identify protease substrates at specific subcellular locations, such as the plasma membrane. We developed a strategy for spatially restricted N-terminal tagging that enables global analysis of proteolytic cleavage events at the plasma membrane with single amino acid resolution. Our method uses the designed peptide ligase subtiligase to install a biotin handle onto N termini on the surface of living cells in which membranes, protein complexes, and spatial relationships remain intact. Using this technique, we have sequenced proteolytic cleavage sites in >500 human membrane proteins. Analysis of these cleavage events shows that the vast majority occur in between domain boundaries and are likely to be of high functional importance. By combining this method with pharmacological protease inhibitors and CRISPRi protease knockdowns, we have begun to define the proteases responsible for specific cleavage events.

1.6

Characterization and turnover of RNA-binding proteins: novel insights into ribosome maintenance

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To understand the functional interplay of protein and RNA, we have recently developed a methodology to globally identify proteins that interact with RNA, independent of the RNA-biotype. The method, termed XRNAX, purifies protein-RNA complexes, serving as a starting point for detailed exploration of the proteins and RNA they contain. Specifically, we have used this to globally characterize RNA-interactomes in various cell lines, to determine dynamic changes in interaction networks upon cellular stress, and to identify protein-RNA interaction sites. In addition, we used pulsed-SILAC labelling to investigate if binding to RNA influences protein stability. Indeed, we found that in MCF7 cells the half-life of RNA-bound proteins was on average 1.6 fold longer than the same proteins in the overall proteome, and up to 5-fold for individual cases, suggesting that protein stabilization can be a general function of RNA when associating with protein. Likewise, ribosomal proteins are overall stabilized on RNA, however half-lives within the ribosome span more than one order of magnitude, suggesting protein-specific exchange from the complex. To investigate the dynamics of ribosome maintenance and turnover in more detail, we combined pulsed-SILAC labelling with sucrose gradient fractionation to determine half-lives of ribosomal proteins in the 40S, 60S, 80S and polysome fraction, demonstrating profound differences in protein stability between these subunits, especially when translation is inhibited. Collectively these data demonstrate ribosome maintenance by exchange of individual proteins from the ensemble, thereby adding a novel dimension to the classical model describing ribosome production at a defined stoichiometry and destruction by subunit.

1.7

Deciphering gene expression regulation in health and disease using integrative omics approaches

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The intestinal epithelium displays remarkable cellular plasticity. Studying gene expression regulation in adult intestinal stem cells and their differentiated progeny has been quite challenging, mainly due to a lack of in vitro culture models for these cells. However, recently, it was shown that isolated Lgr5+ adult stem cells from the mouse gut can form "miniguts" or organoids in vitro in a well-defined semi-solid culture medium supplemented with essential growth factors. These organoids self-organize into an epithelial structure that is phenotypically and functionally reminiscent of the in vivo situation in the intestine of an adult mouse. Most importantly, all major intestinal cell types are continuously generated from the proliferative stem cell/progenitor pool followed by differentiation and migration toward their correct positioning along the crypt–villus axis. Intestinal organoids have great potential to study cell-type specification in the intestine, but their inherent multicellular heterogeneity represents a major drawback when combined with systems biology approaches to study gene expression regulation per cellular lineage. However, we and others have recently shown that minor modifications of the organoid culture medium to generate cell-type enriched mouse intestinal organoids, e.g., stem cell or enterocyte, can be used in combination with a multi-omics framework to decipher the molecular mechanisms that drive cell fate changes in small intestinal organoids. In my lecture I will present our recent findings on this topic.

1.8

Molecular interaction networks controlling gene expression programs

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Regulation of gene expression involves interactions between a large number of proteins including DNA binding transcription factors, co-activators/repressors, chromatin remodeling and modifying complexes and the general transcription machinery, all in the context of chromatin. While many of the proteins involved in transcriptional regulation are now known, our understanding of how they interact with one another, both physically and in regulatory networks to control gene expression programs is less advanced. To address these challenges, my laboratory has been developing and applying mass spectrometry (MS)-based proteomics approaches to study the architecture of transcriptional regulatory complexes and to quantify the transcription factor proteome during dynamic processes such as cell differentiation. In this talk, I will describe how we are using structural proteomics technology along with a number of other approaches, to elucidate the structure and function of large transcription complexes. I will also discuss our efforts to build transcriptional regulatory networks underlying human erythropoiesis via integration of quantitative proteomics and RNA expression data. I will describe the challenges that we encountered during the course of these studies and how we are attempting to overcome these challenges to improve our understanding of the molecular interactions underlying gene regulation.