Proteomics Propels Protein Degradation Studies in San Diego*

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Exquisite in vitro biochemical examinations of protein ubiquitylation and degradation have historically been the dominant methods for unraveling the mechanisms of protein destruction. The study of protein abundance alterations and protein modifications, a cornerstone of protein degradation pathways, naturally lends itself to global and systematic proteomic methods to decipher the emerging complexity of protein degradation pathways. Advances in proteomic technologies have fueled an explosion of systematic and quantitative studies aimed at understanding how the proteome is shaped and regulated by ubiquitin-dependent processes. These types of studies, as well as targeted analyses of cellular pathways, have revealed that alterations in protein degradation function can have a severe impact on human health and disease. The fusion of these two themes was the focus of the January 2012 conference on proteomics of protein degradation and ubiquitin pathways (PPDUP) held in San Diego. To gain insights into both the current state-of-the-art proteomic methods to investigate protein turnover, and how protein degradation function is altered within a range of human disorders a variety of speakers revealed the many connections between altered protein degradation function and human disease. Many of the sessions were framed by a consistent focus aimed at the discovery and development of novel therapeutics targeting protein turnover, and providing a future prospectus on the use of proteomics toward more systematic investigations of protein modifications and their link to protein turnover and human health and disease.

Participants of the second Proteomic in Protein Degradation and Ubiquitin Pathways (PPDUP) conference, most of whom were escaping winter, met near San Diego, California to share their viewpoints and new results in the field. Keynote speakers Alfred Goldberg (Harvard Medical School, MA) and Mike Tyers (University of Montreal, Canada) opened the meeting and provided a comprehensive tour of the various biochemical activates associated with ubiquitin-dependent protein turnover. Goldberg presented an update to their work unraveling the biochemical processes associated with the proteasome to facilitate protein degradation, and Tyers reported on their efforts to characterize and pharmacologically inhibit ubiquitin conjugating enzymes. These early talks, together with Ralph Bradshaw (UCSF, CA) and Al Burlingame (UCSF, CA), provided insightful perspectives on previous work studying protein degradation pathways and their links to human disease. The conference was bookended by two closing keynote addresses from Ben Cravatt (Scripps Research Institute, CA) and Wade Harper (Harvard Medical School) who together demonstrated the power of proteomic methodologies to characterize and identify substrates for proteases and various ubiquitin-related enzymes as well as identify new roles for the complex array of enzymes that mediate ubiquitylation of proteins. These talks highlighted several important themes and topics that resonated throughout many of the talks providing a future prospectus on the use of proteomics toward more systematic investigations of protein modifications and their link to protein turnover and human health and disease.

Identification of Ubiquitylated Proteins and the Sites of Modification—One of the largest challenges in the field of ubiquitin-dependent regulation has been the mapping and characterization of endogenous protein ubiquitylation and the identification of the exact lysine residues modified by ubiquitin. This is mainly because of the low abundance and stoichiometry of the fraction of the proteome that is ubiquitylated at any given time. However, studies from various labs over the past year have traversed this barrier through the use of antibody-based peptide enrichment strategies that use an antibody that specifically recognizes the diGlycine (diGly) remnant of ubiquitin that remains linked to modified lysine residues after trypsinolysis (1). Steve Gygi (Harvard Medical School, MA), in collaboration with Wade Harper's group (Harvard Medical School, MA) and Cell Signaling Technologies (CST, Danvers, MA) detailed their studies using a monoclonal diGly-specific antibody to enrich for modified peptides followed by quantification and identification by mass spectrometry. They reported the identification of over 17,000 diGly-
modified lysine residues spread over ~5000 proteins and characterized the alterations in abundance of many of these sites in response to proteasome inhibition. Using the same approach, Don Kirkpatrick (Genentech, CA) reported the identification of more than 4500 unique ubiquitin-modified peptides from mouse brain homogenates. Lastly, using an independent diGly-specific monoclonal antibody, Chunaram Choudhary (University of Copenhagen, Denmark) reported the identification of greater than 11,000 diGly-modified sites from tissue culture studies. Altogether, these studies illustrated a powerful new approach in which thousands of ubiquitin-modified lysines can be examined in a single experiment. This represents a great expansion in the scale of experiments that can be accomplished toward more global analyses of protein degradation. One of the caveats in using this strategy to capture potentially ubiquitylated peptides is that, because of the similarity in their C-terminal sequence, proteins that are modified by the ubiquitin-like proteins NEDD8 or ISG15 will leave identical diGly-remnants attached to lysines following trypsin digestion making the unequivocal identification of ubiquitylated proteins difficult. Gygi and colleagues extended their analysis to carefully measure the impact of other ubiquitin-like (UBL) modifications and estimate that over 94% of the identified sites likely are the result of ubiquitylation versus other UBL modifications. Choudhary presented a comparison of these diGly modified lysine residues to those identified to be acetylated revealing that diGly-modified lysine residues are more likely to be acetylated compared with unmodified lysines in the proteome; a finding that was also reported from the Gygi and Harper study. This suggests potential cross-talk between these two lysine post-translational modifications (PTMs) although careful studies interrogating individual lysine residues are needed to understand the functional significance of this overlap. Choudhary also reported preliminary studies comparing two distinct diGly-specific antibodies suggesting that, although they identify largely overlapping sets of modified peptides using the two different antibodies, there appeared to be a slight difference in the amino acid sequence surrounding the modified lysine residue recognized by either antibody. However, it should be noted that neither study was able to identify a specific ubiquitylation motif sequence, even with the large increase in the number of identified ubiquitin-modified peptides, suggesting a large plasticity in site-utilization by individual ubiquitin ligases. Using these new data sets of known sites of ubiquitin modification, Yaakov Levy (Weizmann Institute, Israel) took a bioinformatics approach to study the structural and evolutionary nature of ubiquitylated proteins and lysines. Levy reported that, surprisingly, ubiquitylated lysines were more often found in structured regions of the proteins and 38% of diGly-modified proteins do not contain an unstructured region within their entire coding sequence. However, because the used data sets contain experiments in which proteasome inhibitors were used it is uncertain if the identified proteins represent the fully folded and native portion of the proteome and caution should be taken when analyzing the structural characteristics of modified sequences. Indeed, studies from the Gygi and Harper groups suggest that a large fraction of the ubiquitin-modified proteome arises from newly translated proteins that may have not reached their final folded native state. Further, Levy reported that ubiquitin-modified lysines were more evolutionarily conserved compared with all lysines in the human proteome suggesting a possible selective pressure to maintain protein ubiquitylation over evolution. As an alternative to peptide-based enrichment methods, Manuel Rodriguez (Bizkaia, Spain) reported on the use of tandem repeated ubiquitin binding entities (TUBES) to isolate ubiquitin-modified proteins prior to digestion with trypsin. Rodriguez and colleagues have further developed this strategy to capture and identify sumoylated (another UBL modification) proteins as well, an often difficult task because of the lack of peptide enrichment methods for sumo-modified proteins. These studies set the stage for analysis of in vivo protein ubiquitylation in both mouse models of human disease as well as from human patient tissues to uncover new links between altered protein degradation and the onset of a myriad of human disorders.

Strategies for the Identification of Ubiquitin Ligase and Protease Substrates—Another large, and often daunting, challenge to the field is the identification of substrates for ubiquitin ligases and deubiquitylating enzymes (DUBs) known to have roles in critical cellular pathways. As many cellular pathways are regulated by the precise degradation of components within crucial signaling hubs, identification of ubiquitin pathway components that govern this regulation would reveal potential candidates for therapeutic intervention. Use of the diGly-specific antibodies allows for the detection of the endogenously modified proteins which provide an opportunity to directly quantify the exact population of the proteome regulated by ligases and DUBs. Gygi and colleagues presented data using the diGly-capture approach to identify substrates for a family of multicomponent ubiquitin ligases known as cullin–RING ligases (CRL). Using a pharmacological inhibitor of the NEDD8 E1 enzyme, MLN4924, to specifically ablate the ubiquitylation of CRL targets, they reported the identification of more than 200 novel CRL substrates. Cellular apoptosis is another critical signaling pathway known to be regulated by the ubiquitin proteasome system. Don Kirkpatrick, as well as Min Zhuang from the laboratory of Jim Wells (UCSF, CA), reported on two distinct approaches to identify substrates for IAP (inhibitor of apoptosis) family of ubiquitin ligases. The Kirkpatrick group used the diGly-antibody enrichment approach in combination with a SMAC-mimetic to specifically inhibit the ubiquitylation of IAP targets in response to apoptosis stimulation. By using cell lines treated with the SMAC-mimetic followed by general caspase inhibition, the Kirkpatrick group identified a group of mitochondrial proteins that are regulated by IAP ligases XIAP and CIAP in response to apoptotic stimuli. Zhuang took a protein engineering approach...
toward the identification of IAP targets by fusing the NEDD8
specific E2, UBC12 (UBE2M), to the substrate binding domain
of either XIAP or CIAP. Incubation of cell lysates with His-Bio-
tagged (containing a poly-histidine and peptide sequence for
biotinylation) NEDD8 in the presence and absence of these
engineered ligases followed by purification of His-Bio-neddy-
lated proteins allowed for the differential identification of pro-
teins that were modified by the engineered ligase. Among the
potential candidates identified by this approach, Zhuang iden-
tified the mitochondrial localized protein PGAM5 as a new
substrate for XIAP. Interesting, only the proteolytically cleaved
product of PGAM5 was a substrate for XIAP suggesting a
two-step process in targeting PGAM5 for destruction by IAP
family ligases. The faithful segregation of the genetic material
during cell division via the separation of sister chromatids
during mitosis is regulated by APC (anaphase promoting
complex)-mediated ubiquitylation of a host of mitotic sub-
strates. To identify new APC substrates, Hanno Steen (Chil-
dren’s Hospital Boston, MA) used a quantitative mass spec-
trometry approach in which cell lysates from various time
points during mitosis were differentially labeled, postdiges-
tion, using tandem-mass tags (TMT). Then the abundance of
thousands of proteins were monitored and quantified relative
to time points at the onset of mitosis. This approach identified
known APC substrates, like securin, as well as novel sub-
strates like the kinesin KIFC1. The advantage of this approach
is that it interrogates protein abundance directly and does not
require metabolic labeling of cell lines enabling this approach
to be applied to a wide range of protein samples including
human patient tissue. Proteomics encompasses a variety of
approaches toward the systematic study of protein function.
Although mass spectrometry based approaches were the
dominant theme of the conference, Daniela Rotin (SickKids
Hospital, Canada) presented data using protein arrays toward
the identification of substrates for HECT E3 ligases. Rotin and
colleagues identified FGFR1 as a substrate for the HECT
domain ligase Nedd4–1 and characterized the mechanism of
Nedd4–1 mediated regulation of FGFR1 signaling. Deletion of
the non-canonical Nedd4 binding motif in FGFR1 (i.e. dissimi-
lar to the PY motif) resulted in constitutive activation of
FGFR1 signaling and phosphorylation of downstream targets
demonstrating the critical role of Nedd4–1 mediated ubiqui-
tylation in controlling FGFR1 activity and animal development.
Moving away from protein-based approaches, Theo Kline
from the laboratory of Chris Overall (University of British Co-
lumbia) detailed preliminary studies using targeted peptide-
based arrays to probe the sequence specificity of various
dubiquitylating enzymes. Although identification of sub-
strates for proteolysis via proteasome-mediated catalysis was
a major focus of the conference, Ben Cravatt (Scripps Re-
search Institute, CA) reported on their efforts to map sites of
proteolytic cleavage arising from nonproteasomal catalysis.
Cravatt presented the PROTOMAP method to identify both
the protein substrates that are proteolyzed and the site of
cleavage by using SDS-PAGE followed by mass spectrome-
ty. Interestingly, Cravatt and colleagues observed that the
vast majority of the proteolytic fragments remain intact during
apoptosis, indicating that the physical separation of functional
domains rather than the full proteolysis of the target proteins
is required for this process. PROTOMAP also provided evi-
dence for functional crosstalk between caspases and kinases
during apoptosis. The richness of methods detailed during the
conference underlines the resourcefulness in the field, and
exemplifies that examinations of particular ubiquitin-regulated
pathways may require the integration of different proteomic
approaches.

Proteomic Analysis of In Vivo Protein Degradation Rates—
Metabolic labeling studies have historically been the method
of choice to directly examine the degradation rates of pro-
teins. However, these studies have been mostly limited to
experiments using simple unicellular eukaryotes or tissue cul-
ture systems. Robert Beynon (University of Liverpool, UK)
reported on their studies using whole animal metabolic label-
ing to examine the in vivo degradation rates of a large fraction
of the proteome within both chicken and mouse models. These
studies allowed for the simultaneous measurement of the
degradation rates of hundreds of proteins. Data from the
chick studies indicated that in order to build body mass during
development, protein degradation rates are slowed suggest-
ing that global protein degradation pathways may be devel-
opermently regulated. Beynon noted two important findings
from the totality of their metabolic labeling studies, including
cells grown in culture. First, degradation rates did not depend
upon the identity of the N-terminal amino acid suggesting that
the N-end rule does not dictate global degradation rates but
instead may be a specialized pathway to target specific un-
stable proteins. Second, Beynon noted that small changes in
degradation rates are expected to be the dominant mode of
regulation for most biological pathways instead of the all or
none examples that have been previously studied. Rapid and
large changes in the degradation rates of particularly critical
signaling proteins have been clearly demonstrated, but higher
precision measurements of degradation rates for the majority
of proteins are needed to better understand the impact of
altering protein degradation pathways. Jeffrey Savas from the
lab of John Yates (Scripps Research Institute, CA) reported on
the discovery of extremely long-lived proteins that were iden-
tified using whole animal 15N pulse-chase labeling followed by
mass spectrometry analysis. Near complete labeling was
achieved by a two generational scheme. First a female
breeder rat was labeled via feeding with a specialized 15N-
labeled food source. The subsequent pups derived from the
breeder were labeled for one month post weaning, and then
switched to an unlabeled food source for 6 or 12 months.
Examination of the number of heavy-labeled proteins that
remained in the brain after 6 or 12 months revealed that 25
proteins contained heavy peptides in the brain even after 12
months of normal feeding. Apart from the expected long-lived
myelin basic protein, Savas and colleagues found that histones and the core of the nuclear pore complex also retained the heavy label indicative of an extremely low degradation rate. These types of whole animal labeling studies exemplify the transformative nature of mass-spectrometry based proteomics on the study of protein degradation pathways.

Proteasome Dynamics and Regulation—The 26S proteasome has been the subject of intensive research over the past 25 years and Alfred Goldberg offered perspectives on both historical and contemporary issues related to proteasome biology. Notably, Goldberg reported that the proteasome in cells does not normally degrade proteins ubiquitylated with a K63 chain because several ubiquitin-binding proteins involved in cell trafficking can block in vitro the binding of K63-linked polyubiquitin chains to the proteasome. Although K63 and K48-linked ubiquitin chains bind to the purified proteasomes equally well, the presence of cell extract blocks the binding of K63 chains, thereby providing specificity. In addition to substrate docking, deubiquitylation, and unfolding, the 19S proteasome subunit plays a major role in regulating the gate opening of the 20S barrel that is required for substrate entrance and proteolysis. Goldberg’s group demonstrated that the six 19S ATPase subunits function coordinately, and that the binding of ATP on two opposing subunits of the 19S ATPase hexameric ring is optimal to ensure the proper opening of the 20S gate. Interestingly, gate opening and ATP hydrolysis are further activated upon ubiquitin conjugate binding. These processes are also coordinated with substrate deubiquitylation. Although most proteasome substrates are targeted in a ubiquitin-dependent manner, a few talks reminded the audience of other pathways that are regulated via proteasomal degradation in a ubiquitin-independent manner. Michal Sharon (Weizmann Institute, Israel) showed that a double negative feedback loop exists between NQO1 (NADH quinone oxidoreductase 1) and the 20S proteasome, whereby NQO1 inhibits the proteolytic activity of the 20S proteasome and the 20S proteasome degrades the apo form of NQO1 in a ubiquitin-independent manner. Robert Kalejeta (University of Wisconsin-Madison, WI) reported on the pp71 viral protein that can mediate the ubiquitin-independent degradation of several other proteins. Intriguingly, in this case the 19S subunit was still required. Assembly of the 19S subunit has been increasingly scrutinized in the past years, and understanding the exact function of each proteasome subunit remains a major challenge. Michael Glickman (Technion, Israel) presented data on proteasome integrity in response to oxidative stress or mitochondrial malfunction. A mutation in the C-terminal of yeast Rpn11 subunit affects the assembly of other subunits like Rpn12 and Rpn3 that are distantly located on the reported 19S structure (2). Remarkably, addition of the C-terminal domain of Rpn11 alone was sufficient to restore the integrity of the 19S in presence of the mutant. Glickman and colleagues also observed major defects in mitochondrial organization and respiratory function within this Rpn11 mutant background. Further analysis in higher eukaryotes confirmed that inhibition of the proteasome (both chemically or by RNAi) induces mitochondrial fragmentation and an increase of reactive oxygen species (ROS) in the cell. This increase in ROS then further impacts proteasome activity by causing disassembly of intact 19S from the 20S subcomplex, as suggested by work in the Huang laboratory (3). Systemic characterization of both proteasome activity and composition, as well as the consequences of its inhibition, is a pressing topic because of the increased usage of proteasome inhibitors as therapeutics.

Drugging the Ubiquitin Proteasome System—The development and successful application of the proteasome inhibitor bortezomib (Velcade) toward the treatment of multiple myeloma was the first indication that the ubiquitin proteasome system (UPS) could be successfully targeted to treat human disease. Because the UPS is comprised of a hierarchy of enzymatic cascades that control both the ubiquitylation and deubiquitylation of substrates, it has long been suggested that the UPS represents a pharmacologically target-rich pathway. The past five years has witnessed a large increase in the effort toward the development of new small-molecule inhibitors targeting various components of the pathway (4). Ken Anderson (Dana-Farber Institute, MA) provided both a perspective on the use of proteasome inhibitors to treat multiple myeloma, and an update on recent clinical data on use of newly developed proteasome inhibitors to treat multiple myeloma. Anderson suggested that combination therapies with other drugs targeting UPS components might be an attractive strategy to treat a variety of human malignancies. Mike Tyers (University of Montreal, Canada) suggested that small molecules need not target the catalytic site of ubiquitin pathway enzymes in order to be effective. Building upon previous work from Tyers’ group and others demonstrating the successful targeting of the SCF(Skp1, Cullin-1, F-box containing complex)CDC4 ubiquitin ligase via allosteric inhibition of substrate binding, Tyers reported on their development of the first small-molecule inhibitor of an E2 enzyme, CDC34, which acts in conjunction with SCF and other CRLs. Akin to what was observed for the SCF(CDC4) inhibitor, the CDC34 inhibitor seems to operate through allosteric mechanisms rather than direct targeting of the catalytic cysteine residue. The molecular target of the drug thalidomide was eventually identified to be a CUL4 ubiquitin ligase component cereblon, providing another example of a drug-targeted UPS component. Rajesh Chopra (Celgene, NJ) provided new data on two other drugs, lenalidomide and pomalidomide, which also target cereblon, and demonstrated that resistance to lenalidomide arises from a down-regulation of cereblon protein levels. Pomalidomide on the other hand was less sensitive to cereblon levels suggesting that there may be other partners for these immunomodulatory drugs. Yihong Ye (NIH, MD) presented data on the eeyearestatin ERAD (Endoplasmic Reticulum Associated Degradation) inhibitor that is composed of two functional groups: one inhibits the retrotranslocation-mediating ATPase p97/
cdc48, and the second specifically localizes the compound to the membrane to impart specificity. Finally, Benedikt Kessler (Oxford, UK) reported on their proteomic approach to probe the specificity of newly developed DUB inhibitors. By comparing the DUBs that are modified and immunopurified by the well-established DUB active site probes HA-UbVME and HA-UbBr2 in the presence and absence of the putative DUB inhibitors, Kessler’s group was able to pinpoint the DUBs that were targeted by these drugs. One of the drugs appears to primarily target USP7, a DUB that has previously been the target of small-molecular inhibitor development efforts. This quantitative proteomics approach provides a facile method, when used in combination with active site probes that can be affinity captured, to establish the selectivity and specificity of newly developed therapeutics targeting the UPS. These talks highlight the excitement and intense effort now being invested into the development of new inhibitors of UPS enzymes that will serve not only as useful molecular tools to probe UPS functionality, but also as putative therapeutics for the treatment of a variety of human diseases.

Protein Degradation and Links to Human Diseases—As the ubiquitin system is implicated in most cellular pathways, it is not surprising to find it associated with numerous diseases, and many presentations nicely illustrated this link. Ze’ev Ronai (Sanford-Burnham MRI, CA) presented studies in which inhibition of the Siah2 ubiquitin ligase reduces the rate of metastasis in mice. Siah2 regulates the hypoxia response and cooperates with the transcription factor FoxA2 to sustain the expression of three major HIF1α targets (Hes6, Sox9, and Jmj1a) that are sufficient to induce metastasis. Future efforts will focus on identifying small molecule inhibitors of Siah2 for potential therapeutics. Poul Sorensen (University of British Columbia, Canada) reported on their recent characterization of Hace1, a ubiquitin ligase tumor suppressor downregulated in 75% of Wilms’ tumors. Sorensen’s group found that ROS levels were elevated in cells derived from Hace1−/− knock-out animals, a phenomenon that was accentuated in tumor cells derived from these mice. Moreover, Hace1 expression and localization are both regulated in response to increasing oxidative conditions within cells. Using mass spectrometry, Sorensen and colleagues identified the Hace1 substrate Rac1 that is targeted for proteasome degradation. Lower superoxide levels in Hace1−/− cells were observed when Rac1 was down-regulated by RNAi, or upon re-expression of the wild-type Hace1. Altogether, Sorensen proposes that higher ROS levels may play an important role in the proliferation rate that is driven by higher levels of Cyclin D in these tumor cells. Although cancer biology has often been at the center of attention in the ubiquitin field, other pathological pathways were discussed. For instance, Rama Mallampalli (University of Pittsburgh, PA) proposed that the SCF-based ubiquitin ligase components could be involved in the cellular release of the toxic phospholipid cardiolipin during bacterial infection in the severe lung disorder, acute respiratory distress syndrome (ARDS). SCF ligase components may regulate the level of CLS1 (cardiolipin synthase 1), which is required to maintain mitochondrial integrity and cardiolipin within the inner mitochondrial membrane. Using protein pull-down followed by mass spectrometry, Brian Raught (University of Toronto) analyzed the interactome of the Rad6A E2, which is found mutated in a X-link mental retardation (XLMR) syndrome. Interestingly, one of the mutations associated to XLMR affected the interaction with several novel interacting proteins, including a putative ubiquitin ligase, but not other well-characterized Rad6 E3-partners like Rad18 or Ubr1. Chang-Wei Liu (University of Colorado-Boulder, CO) presented new findings related to spinal muscular atrophy (SMA), a disorder that arises from deletion or mutation of the survival motor neuron gene SMN2. Using mass spectrometry, his laboratory identified that the SMN complex interacted with a DUB, Usp9x that regulates the stability of SMN, likely via deubiquitylation of SMN to protect it from ubiquitin-dependent degradation. Proteopathies and neurodegenerative diseases are often associated to an impairment of the ubiquitin system (5). Junmin Peng (St Jude Children’s Research Hospital, TN) presented data on the profiling of ubiquitin linkages in different stress conditions and upon expression of disease-specific protein aggregates. He demonstrated that the impact of heat-shock on the ubiquitin system is reminiscent to changes observed in tissues derived from post-mortem tissue from Alzheimer’s patients. So far, therapeutic efforts linked to the UPS have mainly focused on inhibitory approaches, especially for treating cancer. Conversely, studies linked to proteopathies suggest that the activation of the proteasome could be beneficial in other conditions. For instance, Xuejun Wang (University of South Dakota, S.D.) presented data showing that overexpression of the PA28 proteasome subunit within mice expressing mutant CryAB that normally aggregates and results in cardiomyopathy, resulted in a decreased rate of protein aggregation and a 20% increase in life expectancy in these mice. Wade Harper also detailed a striking phenotype for mice in which UBA6 was conditionally deleted. UBA6 is the alternative ubiquitin E1 activating enzyme that specifically uses the E2 conjugating enzyme Use1 (6). These mice displayed a reduction in lifespan and major developmental defects in two brain regions that manifest in behaviors reminiscent of autism spectrum disorder typified by an increased fear response and altered social interactions. These studies underline the need to further explore the function of genes implicated in the ubiquitin system to broaden our understanding of disease mechanisms.

Interaction Network Analysis and Bioinformatics Tools to Study Protein Degradation Pathways—The “en masse” identification of ubiquitylated proteins and other recent progresses must be accompanied by the development of novel approaches to study the ubiquitin system. Several computational approaches were highlighted in the last session on bioinformatics. Alfred Vertegaal (Leiden University, Nether-
lands) demonstrated the feasibility of mapping SUMO acceptor lysines in endogenous target proteins by mass spectrometry and reminded the audience about the interplay between ubiquitylation and sumoylation. Nobel Zong from the laboratory of Peipei Ping (UC Los Angeles, CA) presented data on the COPaKB a knowledgebase with a spectral library search engine that enables on-line analysis of large-scale mass spectrometry data. Christian Behrends (Frankfurt University, Germany) further explored the ATG8 (a ubiquitin-like modifier regulating autophagy) protein interaction network and reported data on the TBC1D5 Rab GAP protein that contains two LIR motives. Surprisingly, one of LIR motives, which normally mediates the interaction with ATG8 (7), preferably binds to a component of the retromer complex expanding the interaction range of this motif. Wade Harper, in collaboration with Steve Gygi, also presented a new strategy to multiplex protein pull-downs using TMT isobaric labeling that allows for the interrogation of multiple complexes with a single run on the mass spectrometer greatly accelerating the laboratory’s interaction network studies. Finally, Ben Major (University of North Carolina, NC) presented data on the new SPOTLIGHT software platform developed in his lab to analyze data from affinity precipitation followed by mass spectrometry analysis (AP-MS). SPOTLIGHT offers an advantage over previous platforms in that it takes into account baits that are closely associated within the hierarchy of interactions from curated protein interaction databases when scoring a potential interaction. This allows for the detection of additional interactions that would otherwise not be considered.

On the whole, it was a stimulating meeting that connected major players in the ubiquitin field who rely on proteomics. There was a sense that antibody-based enrichment of ubiquitylated peptides will deeply impact our understanding of the ubiquitin system in cellular pathways in the near future, and that the probable discovery of additional small-molecules inhibitors will maintain ubiquitin in the spotlight. More reports are anticipated in the next PPDUP meeting that will be organized in Los Angeles in 2013.

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