A Special Symposium to Celebrate the Opening of CeTPD in Dundee

19th-22nd May 2024

CeTPD Journal Club – Special Edition

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FOREWORD

| *Charlotte, Valentina & Alessio*

Dear Readers,

It is our pleasure to present to '*Protein Degradation in Focus'* CeTPD Journal Club Special Issue, produced by CeTPD volunteer Editors: Angus Cowan, Aitana de la Cuadra Baste, Selma Gulyurtlu, Alex Hallatt, Tessa Harzing, Andreas Holmqvist, Dylan Lynch, Giorgia Kidd, Mokhitli Morake, Gajanan Sathe, David Zollman and Alessandra Salerno.

In May 2024, we held "**Protein Degradation in Focus: A Special Symposium to Celebrate the Opening of CeTPD in Dundee**" at the University of Dundee, between the School of Life Sciences and the new Centre for Targeted Protein Degradation. This meeting was organised between the Scientific Organising Committee (Charlotte Crowe, Valentina Spiteri, Alessio Ciulli, Brenda Schulman and Craig Crews), and the Local Organising Committee (Charlotte Crowe, Valentina Spiteri, Louise McGreavey, Diane Purves, Pamela Johnstone, Ailsa Roy and Alessio Ciulli).

It is almost a decade since the reports of CRBN- and VHL-based small-molecule PROTACs and the elucidation of the mechanism of action of thalidomide; pivotal papers that marked a tipping point ushering the field of targeted protein degradation (TPD). Fast-tracking today, the phenomenal success of PROTACs and molecular has transformed drug discovery, with well over 40 investigational drugs that work as degraders currently in clinical trials across the globe, giving hopes to patients with no other options. Academic and translational chemical biology have been taken by storm by this revolution, and beyond TPD, novel induced-proximity modalities have begun to emerge. The Symposium marked a celebration of the science and the people that have led to the establishment of TPD, while offering a glimpse of the directions of travel of where the field is going today and forward-looking to the next 10 years.

To best enable the contributions that Dundee can continue to make to the field, the University established our new Centre, for which Alessio has the privilege to be the Founder and Director. It all started 5 years ago when the idea first emerged during a Friday afternoon lunch. Those of you who have been following our CeTPD Journal Club will recall that Alessio wrote a brief piece on this in the February 2023 Issue. Since the beginning of 2023 we have been based our new laboratories in 1 James Lindsay Place. The past few years have been extraordinarily hard work in setting up our new home and come together and grow as a team - a success in which everyone, past and present, has played their part. Today, CeTPD houses ~70 people, of which ~60 are scientists. Funded from a mix of public, private and charity sources, it is expected to grow to more than 100 over the next 2-3 years. We are grateful to all our funders and donors for their generosity and alignment to our vision to innovate, collaborate and inspire. Being part of Dundee's School of Life Sciences means we not only share collaborative ethos but also key infrastructure, resources and expertise – all critical to success. It is vital that we continue to work together to bring the best of Dundee to the world.

The idea of the Symposium therefore came about as an opportunity to bring the field together to mark the opening of our new Centre at Dundee. First announced through the grapevines at the

BioMed Induced proximity conference in Barcelona in May 2023, and then officially advertised in October 2023, we were overwhelmed by the positive response from the scientific community to our Symposium which came to life thanks to the incredible reception and input from all its participants.

We were honoured to welcome 300 delegate scientists from all over the world, from academia, and industry all active and interested in TPD and beyond. We explored the latest scientific advancements through a vibrant programme arranged over 4 days, including 3 Keynote lectures, 22 invited lectures, 14 short talks (selected from the abstracts), and 3 poster sessions with a remarkable 130 poster presentations. For those who were unable to attend, or who would like to remind themselves of the event, a summary of each of these talks is provided here in this **Journal Club Special Issue**. A Nature Chemical Biology meeting report has also been provided by David Zollman and Kirsten McAulay, both Team Leaders in the CeTPD, distilling some of the topics discussed and the exciting outlook for science in the TPD space.

Outside of the scientific programme, the Symposium provided a platform for networking and interactions across disciplines and organisations. In particular, we were delighted to welcome Ollie Hsia, Bekky Feltham, Ingrid Wertz and Dan Nomura to the stage for a Careers in Academia & Industry Network Panel, hosted by CeTPD PhD student Tom Webb. We also hosted informal discussion sessions for early-career development and mentorship opportunities with the Careers in Academia & Industry Round Tables, where we welcomed representatives from across academia, pharma, biotech, non-research based organisations and publishing: Angela Cacace (Sr. Vice President, Arvinas), Emanuela Cuomo (Senior Director, AstraZeneca), William Farnaby (Principal Investigator, University of Dundee), Dafydd Owen (Senior Scientific Director, Pfizer), David Peters (Managing Director, Tocris Bioscience), Kristin Riching (R&D Group Leader, Promega), Irene Serrano (Associate Editor, Nature Communications), and Andrea Testa (Senior Director, Amphista Therapeutics). We were also delighted to welcome the Women in TPD Network by supporting a Breakfast Networking Social, organised by Danette Daniels, Aileen Frost and Chiara Maniaci.

Our Symposium was held within the School of Life Sciences and the Centre for Targeted Protein Degradation, at the University of Dundee itself, located in the historic city Dundee with breath-taking

views overlooking the estuary of the River Tay. One of the aspects we prioritised while planning this Symposium was that we deeply wanted to be able to share our city, Dundee, with our delegates. One area where delegates were able to notice this was with our goodie bags, which represented three industries for which Dundee was historically famous: "Jute, Jam and Journalism". During the Monday evening CeTPD Celebration, we had the pleasure of sharing some of our local CeTPD favourites: drinks by 71 Brewing, a local brewery that's so local that you can see it from the CeTPD car park, and Fallone's pizza and gelato, that pops up at events regularly around Dundee! At the Symposium Gala Dinner, we selected a menu that showed off Scottish cuisine, and delegates enjoyed the fun of Scottish party dancing run by a local ceilidh band, ending the night with Auld Lang Syne, traditionally played at midnight of the New Year or at the end of weddings. Those who arrived earlier on Sunday were able to join a trip to St Andrews, visiting a local whisky distillery followed by a round of golf, organised by Roberta Ibba and Maria Rodriguez-Rios.

We would also like to take this opportunity to thank the School of Life Science Dean,
Julian Blow, and current interim Deans Mike Ferguson and Paul Davies for financial support; the University's Facilities and operations managers, Estates, Catering and Web Design services for their cooperation in enabling us to arrange all events and activities; Alessandra Salerno for her contributions as editor to our conference Booklet which was very well-received; and the Editors of this Special JC Issue for their input to this Issue. Thanks goes to All the members of the Centre for Targeted Proteins Degradation who volunteered their time to help make this event run smoothly, All of whom made important contributions to bring this Symposium to life!

We would also like to take this opportunity to thank again the generous Sponsors and Donors who supported this Special Symposium: Bio-Techne, Promega, Nanotemper, Thermo Fisher Scientific, GenScript, Almirall, Amphista Therapeutics, Arvinas, BioAscent, Boehringer Ingelheim, Foghorn Therapeutics, Oxeltis, Proxygen, and Vernalis Research.
We hope everyone had a memorable stay in Dundee, and we hope to see you again back in Dundee in the not too distant future.

Charlotte Crowe
PhD Student, CeTPD
University of Dundee

Valentina Spiteri
PDRA, CeTPD
University of Dundee

Alessio Ciulli
Professor, Founder and Director, CeTPD
University of Dundee

SYMPOSIUM 2024 - SOCIAL EVENTS

| *Roberta & Maria*

The conference festivities kicked off early on Sunday, the 19th, with social events and registration. We organized a group trip to St Andrews, an enchanting town close to Dundee. On Sunday morning, we set off from the Centre for Targeted Protein Degradation to **Kingsbarns Distillery**. We stopped at **St Andrews** at first where a small group got off the bus to stroll around the beauties of the ancient town. We walked across the Cathedral and saw the Est sands; leaving the St Andrews Pier on the right we walked down to the Castle ruins coasting the cliffs and the Castle sands. We then headed to the city centre where the group had breakfast together and chatted and networked while waiting for the second group to re-join.
The second group was dropped off at the Kingsbarns Distillery for an unforgettable whiskey tour. Enveloped in the iconic "haar" (Scottish sea fog), the distillery welcomed us into a world where the art of crafting the world's finest Scotch whiskey was revealed. Our tour guide, brimming with wit and wisdom, made the experience both educational and entertaining, leaving us all with smiles and newfound whiskey knowledge. The tour was a vibrant experience, complete with a whiskey tasting that left us all in high spirits. Next, we ventured to St. Andrews for a taste of another **Scottish tradition—golf**!
We split in groups and everyone tried to bring out the hidden talent in golf! The competition was fierce, and laughter filled the air.

Following our golf adventures, we strolled through the historic town, soaking in its charm before settling down for a delightful lunch. The day wrapped up back at CeTPD, giving everyone a chance to freshen up for an evening of social drinks and networking.

The symposium started on the 20th and on the first day, after the scientific session concluded, the attendees moved across the road from the SLS building to the new CeTPD. The centre was officially opened with **an inauguration ceremony** led by Alessio, the director, along with some colleagues and collaborators. It was a momentous occasion, marking the beginning of a new chapter for the centre.

Following the inauguration, they had the opportunity to take a **rapid guided tour** of the centre. It was great to see people learning about the science we carry at the centre and showcase the research facilities we are so proud of.

Photos by Mark Nakasone and Kevin Haubrich

The celebrations continued under a marquee, where we all enjoyed a casual and lively atmosphere with pizzas, gelatos, and drinks. DJ Angus provided the music, creating a festive vibe that had everyone in high spirits. It was a perfect way to unwind and connect with fellow attendees after a day of intense scientific discussions.

The second day of exciting science was concluded with **a gala dinner**. The organizers thoughtfully mixed speakers and attendees at the tables to enhance networking opportunities and foster new acquaintances. The elegant setting and delicious food provided the perfect backdrop for engaging conversations and the exchange of ideas.
Following the dinner, we were treated to a ***Ceilidh***, a traditional Scottish social gathering featuring folk music and dancing. The lively tunes of fiddles and accordions filled the air, and a caller guided us through the steps of various Scottish dances. It was a joyous and energetic affair, with everyone enthusiastically participating, regardless of their dancing skills. The Ceilidh was a fantastic way to experience Scottish culture firsthand, and it brought a sense of camaraderie and fun to the evening. The laughter and cheers echoed through the hall as we danced the night away, creating memories that will last.

Overall, the social events added a wonderful dimension to the symposium, making it a memorable experience both professionally and personally.

THERAPEUTIC DEVELOPMENT

DISCOVERY CHEMISTRY & DESIGN

MOLECULAR MECHANISMS & BIOLOGY

Session: Therapeutic Development I

| *David*

***Craig Crews**, Yale University, New Haven, USA*

Keynote - **Tik-TAC-Toe: A Heterobifunctional for Every Need**

Professor Craig Crews gave a fantastic keynote talk that incorporated three new, or recent stories coming out of his lab. A key theme that echoed across the whole conference was that the TPD field is currently achieving great things from a highly restricted starting point. There are approximately 647 E3 ligases to choose from, some of which display organ-, cell cycle stage- or disease-specific expression profiles, potentially allowing for exquisitely targeted PROTAC interventions by selection of an optimal E3 ligase. Despite that, the field is currently restricted to only a small handful of E3 ligases, due to the lack of appropriate binders for the vast majority of ligases. MAGE-A3 is expressed in testes and many tumour cells so would be an attractive ligase to recruit. The Crews lab used a combination of DEL screens and *in silico* methods to identify a novel binder for MAGE-A3 with 8 µM affinity. They were subsequently able to convert this binder into a PROTAC with 2.5 µM DC50 and a Dmax of ~50%, providing proof-of-concept that POI degraders that recruit MAGE-A3 can be developed in the future.
Professor Crews also presented on a recently published proof-of-concept story aimed at validating a PhosTAC approach for modulating protein phosphorylation. PhosTACs recruit a phosphatase to a POI (in this case FKBP12), selectively dephosphorylating the target protein. This can be particularly powerful for modulating the activity of transcription factors and could have implications for e.g. the accumulation of Tau plaques.
The final project presented concerned the recently reported RIPTAC approach. RIPTACs are heterobifunctional molecules that combine an inhibitor of an essential protein with a binder for a protein that is selectively expressed at a high level in a tumour cell. Through binding to the tumour-specific protein, the RIPTAC reaches super-occupancy in the tumour cell, resulting in the essential protein being inhibited, ultimately killing the cell.

| *Dylan*

***Mikihiko Naito,** University of Tokyo, Japan*

Invited Speaker - **Protein degradation induced by IAP-based PROTACs (SNIPERs)**

In this short talk, Mikihiko detailed the recent advancement in Specific and Nongenetic IAP-dependent Protein Erasers (SNIPERs).). IAPs are different from CRLs in their nature to generate K63- and K48-

linked poly-ubiquitin chains while CRLs primarily generate K48-linked poly-ubiquitin chain for proteasomal degradation. Miki showed some data that SNIPER can induce mitophagy and lysosomal degradation when mitochondrial proteins and transmembrane receptors are ubiquitinated by SNIPER treatment, respectively. In the second portion of the presentation, Livin-based SNIPERs were discussed; Livin is yet another IAP-family protein which is not ubiquitously expressed, but is expressed in melanoma and thus offers a unique avenue for cancer treatment. It was highlighted that Livin-based SNIPERs do not degrade proteins of interest in livin-negative A375 melanomas.

Shaomeng Wang, *University of Michigan, USA*
Invited Speaker - **"Targeted undruggable proteins through degradation"**

Shaomeng is well known for his work on degrading androgen receptor with low nanomolar ligands, and drugging SH2 domain proteins. The focus of this short talk was in degrading STAT proteins – namely STAT3 – within a cancer therapy context. The Wang group had hypothesised that dimerization of STAT3 was essential for its activity, however Shaomeng disclosed that this is actually not true, and that monomeric STAT3 is also transcriptionally active.
Shaomeng went on to showcase both classical phosphotyrosine (pY) and F2pmp binders of the STAT3 SH2 domain, including those with unmasked phosphate groups, avoiding the use of prodrugs. One such example that was discussed was SI-109, a 9 nM STAT3 inhibitor featuring an unmasked phosphate F2pmp-type functionality. Over 300 STAT3-CRBN and 100 STAT3-VHL PROTACs were investigated in the course of this work, and Shaomeng highlighted an elegant STAT3-HiBiT assay to monitor degradation. Their optimized STAT3 degrader is capable of inducing long-lasting and selective STAT3 degradation in vivo and is capable of achieving complete and permanent tumour regression with a single dose of administration. Shaomeng also showed that STAT3 degrader is effective against triple-negative human cancer models and can be developed as a new class of immuno-therapy.

Session: Discovery Chemistry and Design I

| *Gajanan*

Dan Nomura, *University of California, Berkeley, USA*

Invited Speaker - **Reimagining Druggability using Chemoproteomic Platforms**

In this talk, Dan initially discussed the undruggable proteome, including K-RAS, C-myc, β-catenin, and AR. He explored how to find ligandable sites for these proteins and suggested that ABPP (Activity-Based Protein Profiling) could be a more useful strategy. He then shifted to the rational design of molecular glue degraders, noting the lack of a rational approach for their discovery. They converted protein-targeting ligands into molecular glue degraders by identifying a chemical handle that transforms ligands into molecular glues. Using the CDK4/6 inhibitor ribociclib as a prototype, they discovered a covalent handle (a solvent-exposed piperazine) that, when appended to ribociclib, induced the proteasome-mediated degradation of CDK4 in cancer cells. They then used the same covalent handle on several protein ligands to degrade BRD4, BCR-ABL, c-ABL, PDE5, AR and AR-V7, BTK, LRRK2, HDAC1/3, and SMARCA2/4. They confirmed the interaction between the compound and RNF126 using gel-based ABPP. Global proteomics were carried out to see the effect of these molecular glues on the proteome, finding they degrade target proteins but also alter the expression of several others. Dan mentioned that more chemical tuning is needed for specificity. Another study discussed was related to MYC, an intractable target due to its intrinsically disordered nature. They performed a cysteine-reactive covalent ligand screen to identify compounds disrupting MYC's binding to its DNA consensus sequence in vitro and impairing MYC transcriptional activity in cells. This identified a covalent ligand, EN4, targeting cysteine 171 (C171) of MYC within its disordered region. Following this, he discussed another non-druggable target, β-catenin. They conducted a screen using a library of cysteine-reactive covalent ligands and identified a monovalent degrader, EN83, that depletes CTNNB1 in a ubiquitin-proteasome-dependent manner. Using covalent chemoproteomic approaches, they found EN83 directly engages CTNNB1 in cells by covalently targeting cysteines—C466, C520, and C619—contributing to CTNNB1 degradation. Overall, his talk was very interesting and resonated with me as he addressed critical aspects for the Targeted Protein Degradation (TPD) community: the rational design of molecular glues and the use of covalent compound screening for undruggable targets, leveraging chemoproteomics to discover involved E3 ligases.

| *Gajanan*

Fleur Ferguson, *University of California, San Diego, USA*

Invited speaker **- Depletion of ZBTB11 Targets Metabolic Vulnerabilities in K-RAS Inhibitor Resistant PDAC**

In her talk, Fleur discussed her unpublished preprint "ZBTB11 Depletion Targets Metabolic Vulnerabilities in K-RAS Inhibitor Resistant PDAC." She explained the prevalence of K-RAS mutations in PDAC patients and the ineffectiveness of existing drugs due to rapid K-RAS inhibitor resistance. She highlighted that this resistance is often mediated by mutations and activation in other pathways, underscoring the need for alternate strategies to combat resistance. Adaptive K-RAS inhibitor resistance involves metabolic reprogramming, including PI3K/AKT pathway hyperactivation, increased TCA cycle flux, Myc amplification, and upregulated mitochondrial biogenesis, leading to reliance on oxidative phosphorylation (OXPHOS). With impaired glycolysis, cancer cells depend more on OXPHOS for energy. This resistance mechanism affects all K-RAS-targeting drugs. Combining K-RAS inhibition with OXPHOS inhibition is an attractive strategy for treating PDAC and other RAS-addicted malignancies. She also discusses the limitation of OXPHOS inhibitors like insufficient potency, poor selectivity, and dose-limiting toxicity, as seen with the

mitochondrial complex I inhibitor IACS-010759. To find alternatives, they evaluated public datasets for targets meeting four criteria: 1) Upregulated during K-RAS inhibitor resistance in PDAC cells; 2) Essential for survival in glycolysis suppression media; 3) Not a complex I component, to avoid affecting high-OXPHOS cells; 4) Chemically tractable. This led to identifying Zinc Finger and BTB Domain Containing 11 (ZBTB11) as a promising anti-OXPHOS target for molecular glue degrader development. ZBTB11 harbors the CXXCG beta-hairpin motif found in many CRBN molecular glue targets. Using CRISPR/Cas9 to tag endogenous ZBTB11 with HiBiT, they profiled a library of CRBN-binding molecular glue candidates. This led to identifying JWJ-01-306, which degrades ZBTB11 by up to 60% at 10 µM via HiBiT quantification and up to 90% by immunoblot. JWJ-01-306-mediated ZBTB11 degradation was rescued by pre-treatment with the proteasomal inhibitor carfilzomib, NAE1 inhibitor MLN4924, and CRBN binder lenalidomide. In human iPSC-derived neuronal models, ZBTB11 degradation had minimal effects on neuronal viability and function at relevant time points (3 days), unlike IACS-010759. While in vivo studies are essential to establish the therapeutic window of ZBTB11 degradation in PDAC, this work marks a milestone in identifying a new strategy for targeting high OXPHOS cancer states. This validates ZBTB11 as a vulnerability in K-RAS inhibitor-resistant PDAC and provides molecular glue degrader tool compounds to investigate its function.

Key Literature

Tran NL et al. ZBTB11 Depletion Targets Metabolic Vulnerabilities in K-Ras Inhibitor Resistant PDAC. BioRxiv, **2024**. doi: 10.1101/2024.05.19.594824.

| *Andreas*

William Farnaby, *Centre for Targeted Protein Degradation, UK*

Invited speaker - **Pursuing targeted protein degradation for investigating CNS disease**

The potential of bifunctional molecules for treating central nervous system (CNS) diseases has sparked significant debate, particularly regarding their ability to cross the blood-brain barrier. In his presentation, William Farnaby discussed their efforts to develop a high-throughput direct-to-biology (D2B) synthesis platform, using GSK3 as a model system. This platform employs orthogonally reactive linkers to create extensive libraries of bifunctional molecules with diverse properties, with the ultimate goal of understanding the physicochemical space required for brain permeable bRo5 compounds. William highlighted the inclusion of basic centres within their linkers, which allows for ion-exchange as the sole purification step, thereby eliminating the need for preparative HPLC purification. He pointed out that this approach has led to the identification of 27 hits with DC50 values below 100 nM for GSK3b degraders and provided a preliminary structure-activity relationship (SAR) within their library. This demonstrates the significant potential of their innovative D2B platform for discovering effective CNS-active degraders.

Key Literature

Wagner FF et al. Exploiting an Asp-Glu "switch" in glycogen synthase kinase 3 to design paralog-selective inhibitors for use in acute myeloid leukemia. *Sci Transl Med*, **2018**, 10(431):eaam8460

Doble BW et al. Functional Redundancy of GSK-3α and GSK-3β in Wnt/β-Catenin Signaling Shown by Using an Allelic Series of Embryonic Stem Cell Lines, *Dev Cell*, **2007**, 12 (6) 957-971

Hurtado D et al. Selectively Silencing GSK-3 Isoforms Reduces Plaques and Tangles in Mouse Models of Alzheimer's Disease. *J. Neurosci.* **2012**, 32 (21) 7392-7402

Qu L et al. Discovery of PT-65 as a highly potent and selective Proteolysis-targeting chimera degrader of GSK3 for treating Alzheimer's disease, *Eur J Med Chem.* **2021**, 226, 113889.

| Dylan

Laura Serini, *University Medical Center Utrecht, The Netherlands*

Short Talk - **A predictive screening platform for targeted degradation of cell surface proteins using SureTACs**

Laura provided an excellent overview of the Surface Removal Targeting Chimeras (SureTACs), using membrane ubiquitin ligases for the degradation of transmembrane proteins, which can also provide tissue selectivity. Laura discussed how these SureTACs can recruit transmembrane E3 ligases and utilise them for the ubiquitination, endocytosis and subsequent lysosomal degradation of membrane-bound proteins, by binding their extracellular domains. The established screening platform is crucial for identification of optimal target:E3 ligase combinations. Laura disclosed excellent degradation of PD-L1, an important immune checkpoint protein.

| Dylan

Matthias Hinterndorfer, *Research Center for Molecular Medicine (CeMM), Austria*

Short Talk - **Targeted protein degradation via intramolecular bivalent glues**

Matthias presented a story which we are very familiar with at CeTPD, following a fruitful collaboration with Ollie and Angus. This short talk began with discussing the molecular glue E7820, an aryl sulfonamide, which degrades the mRNA splicing factor RBM39 *via* DCAF15. It was then identified that a heterobifunctional compound composed of E7820 and JQ1 acts as a new type of TPD modality, connecting two adjacent domains of target protein in *cis*, rather than the classical *trans* engagement of protein and ligase seen with PROTACs. Terming these intramolecular bivalent glues (IBGs), Matthias highlighted the key experiments that elucidated this discovery, and reported this novel IBG modality as a mechanism through which protein domains can be bridged and their surface complementarity with E3 ligases enhanced. IBG1 glues BRD4 to DCAF16, and boasts an excellent DC_{50} of 0.15 nM; I'm excited to see how the intramolecular bivalent glue space grows in the future, and how this new modality can be leveraged for TPD.

| Dylan

Katherine McPhie, *The Francis Crick Institute, UK*

Short Talk - **Chemical Biology Approaches to Explore the Ligandability of TRIM E3 Ligases**

Katherine delivered a short talk on expanding the E3 toolbox with the discovery of new ligands – work which is close to my own heart, as I also work in the "new E3 ligand" space. Using a classical fragment-based approach, they tackled the TRIM family of E3 ligases. Katherine disclosed the numerous fragment approaches used to explore the PRYSPRY domain of two TRIM E3s. Katherine went on to discuss Direct-to-Biology (D2B) methods used in this discovery, a method which is continually gaining traction in the TPD space and beyond. Ending the talk with a roundup of the TRIM25 ligand discovery story, potent binders (including some covalent) were disclosed.

Session: Molecular Mechanisms & Biology I

| *Gajanan*

Yifat Merbl, *The Weizmann Institute of Science, Israel*
Invited speaker - **Proteasome Profiling: global views of protein degradation**

In her talk, Yifat presented a unique perspective that captivated the conference audience. She began by explaining the basics of the proteasome mechanism and its crucial role in maintaining protein balance within cells, ensuring proper function and stability. Disruptions in proteasomal activity and protein breakdown have been linked to various human diseases. However, the changes in the degradation landscape under physiological and pathological conditions remain largely unexplored. Using mass spectrometry analysis of proteolytic peptides (MAPP), her team examined proteasomal pathological states. They have developed a mass spectrometry analysis and a bioinformatics platform to identify these peptides from proteosome in native condition. Using MAPP, integrated with bioinformatics the group has uncovered novel roles for proteasome- cleaved peptides. Their findings reveal a novel function of cellular proteomes in the context of innate immunity.

| *Tessa*

Ronald Hay, *University of Dundee, UK*
Invited speaker - **Targeted degradation of the Promyelocytic Leukaemia protein by arsenic**

In this talk, Ron discusses on a mechanistic level how treatment with arsenic trioxide cures patients with acute promyelocytic leukemia. He explains that arsenic targets promyelocytic leukemia (PML) protein, which induces SUMOylation, followed by ubiquitination. This clears the promoters on DNA which then leads to cell differentiation and apoptosis, after which the patient is cured.
Ron discusses how arsenic induces B-box 2 trimerisation via interactions with C213. This is confirmed by studies in which C213 was mutated to alanine, in these cases, PML is unresponsive to arsenic. Similarly, in patients that are resistant to treatment with arsenic trioxide, mutations surround this C213 in PML. He specifically discusses the L217F mutation, in which SUMO1 cannot be recruited, this ultimately leads to failure to recruit p97 segregase. Both proteins seem to be required for the effective degradation of PML.

If you would like to read more on this subject, the following paper published last year discusses how p97 is essential for degradation of PML: Jaffray et al. "The p97/VCP segregase is essential for arsenic-induced degradation of PML and PML-RARA" *J Cell Biol,* **2023** 222 (4): e202201027

Key Literature
Background on the discovery of arsenic trioxide as treatment for acute promyelocytic leukemia:
Wang et al. Acute promyelocytic leukemia: from highly fatal to highly curable. *Blood*, **2008,** 111(5), 2505-15

| *Gajanan*

Keriann Backus, *University of California, Los Angeles, USA*

Invited speaker - **Chemoproteogenomic approaches to stratify the functional and therapeutically relevant proteome**

Keriann discussed the importance of chemical probes for characterizing protein function, especially for essential genes and post-translational processes. Most human proteins (>90%) lack selective probes, and many remain 'undruggable.' While many studies have identified ligandable cysteine residues, their functional impact is unclear. Her talk focused on detecting and annotating these cysteines, discovering novel covalent degraders, and synthesizing custom reagents for better multiplexing and quantification. Her group has developed a unified cysteine-focused database to aid global and targeted analyses of the cysteinome. This database would incorporate large-scale study datasets, include information on cysteine reactivity, ligandability, and protein druggability, and integrate data from sources like UniProtKB, CGC, ClinVar, HPA, ChEMBL, DrugBank, and PDB to prioritize targets. They presented CysDB, an interactive database for 62,888 cysteines and 11,621 proteins, with a method for adding future datasets to support the growth of cysteine chemoproteomics. Protein homeostasis is regulated by proteasome and autophagosome pathways. Targeted protein degradation technologies exploit these processes to manipulate protein abundance. Cysteine-reactive molecules have been added to the degrader toolbox, unlocking 'undruggable' targets. The proteome-wide impact of these molecules is not fully understood. Using chemical proteomics, they identified a cysteine-reactive degrader of SARS-CoV-2 nsp14 that modifies cysteines in nsp14 and host chaperones, activating stress response pathways. These electrophiles increase protein ubiquitylation, trigger proteasome activation, and cause aggregation and depletion of host proteins, including the nuclear pore complex. Stress granule formation is common with cysteine-reactive compounds. The study highlights the complexities and opportunities in manipulating proteostasis via cysteine-centric stress response pathways.

| *Mokhitli*

Martin Volker Schmiedel, *Boehringer Ingelheim, Austria*

Short Talk - **Homo-BacPROTAC-induced degradation of ClpC1 as a strategy against drug-resistant mycobacteria**

This talk covered the work undertaken by BI through their Research Beyond Borders initiative for the proof-of-concept (PoC) targeted protein degradation (TPD) against bacteria. With increasing antimicrobial infections and protracted treatment regimens for bacterial infections such TB, the talk highlighted the novel drug modality that may not be prone to current resistance. Bacterial PROTACs (BacPROTACs) were developed from cyclomarin which bind ClpC1 and chaperone it for degradation. While the monomeric cyclomarin A lacked degradation efficiency, dimeric homo-BacPROTACs demonstrated degradation of ClpC1 with improved binding affinity and better MICs. BacPROTACs also targeted the intra-macrophage bacteria and had activity against drug-resistant strains of TB. These also showed efficacy *in vivo*. This PoC study demonstrate the potential of TPD in infectious diseases and, is a starting point as well as a good reference for future application in this area.

Key Literature

Junk L , Schmiedel VM., Guha S. et al. Homo-BacPROTAC-induced degradation of ClpC1 as a strategy against drug-resistant mycobacteria. *Nat Commun,* **2024***,* 15, 2005

| *Tessa*

Ella Livnah, *Weizmann Institute of Science, Israel*

Short Talk - **Rationally designed bifunctional ligands induce intracellular precipitation of homomeric protein targets**

In this talk, Ella describes the work done during their PhD on developing Polymerization Inducing Chimeras (PINCH). These compounds are homomeric, consisting of two POI ligands connected by a linker. Rather than recruiting an E3 ligase to induce degradation, this system creates large, symmetric supramers of homomers of the target protein, thus causing it to precipitate and removing it from the soluble fraction of the cell. Ella presents two case studies in which PINCH have successfully led to precipitation of the target proteins BCL6 or KEAP1. Ella argues that this approach may be beneficial in those cases where there is a lack of space for recruiting the E3 ligase complex to the target protein. Additionally, this approach is independent of the function of the protein.

Key Literature

Garcia-Seisdedos H, Empereur-Mot C, Elad N., et al. Proteins evolve on the edge of supramolecular self-assembly. *Nature,* ***2017***, 548, 244–247.

Słabicki M, Yoon H, Koeppel J., et al. Small-molecule-induced polymerization triggers degradation of BCL6. *Nature,* ***2020****,* 588, 164–168..

Teng M, Ficarro SB et al. Rationally designed covalent BCL6 inhibitor that targets a tyrosine residue in the homodimer interface. *ACS Medicinal Chemistry Letters* **2020** *11* (6), 1269-1273.

PDB entries mentioned: 5X4Q (BCL6); 4CXT (KEAP1)

| *Tessa*

Alejandro Rojas-Fernandez, *Universidad Austral de Chile, Chile*

Short Talk - **Specific proteolysis mediated by a p97-directed proteolysis-targeting chimera (PROTAC)**

In their short talk, Alejandro described a method of using alpaca-derived nanobodies to recruit proteins for degradation. Nanobodies are the antigen-binding fragments of alpaca heavy-chain antibodies. Rather than recruiting an E3 ligase system to ubiquitinate the protein of interest, Alejandro explained how they can fuse a nanobody with picomolar affinity for the protein of interest to the UBX domain on the p97 protein. This then leads to p97-mediated degradation. At the end of his talk, Alejandro made sure to not only thank his scientific team, but also the vast team of alpacas that was essential for this research.

If you would like to stay up to date on Alejandro and the team of alpacas, you can follow them on Instagram **@alpaca_buddha**

If you would like to read more on this system, you can have a look at the available preprint:.

Key Literature

Salinas-Rebolledo C, et al. Specific proteolysis mediated by a p97-directed proteolysis-targeting chimera (PROTAC). *boRxiv* **2024** doi: 10.1101/2024.03.08.584142

Session: Molecular Mechanisms and Biology II

| Angus

***Brenda Schulman**, Max Planck Institute of Biochemistry, Germany*

Keynote - **Signaling through the ubiquitin-proteasome system**

Brenda Schulman set the scene for this talk by describing the mystery that spawned a 20-year career: how do Cullin-RING E3 ligases (CRLs) bridge a large gap between substrate and E2 enzyme to facilitate ubiquitin transfer? One missing part of the picture from initial structures of CRL complexes was a small ubiquitin-like protein NEDD8, which is covalently ligated onto the Cullin WHB domain. Brenda went on to describe structures revealing that various NEDD8-modified CRLs partner with various ubiquitylating enzymes for both priming substrates with an initial ubiquitin and extending a ubiquitin chain, both essential and distinct events for polyubiquitination and hence proteasome recognition and degradation of substrates. The broad variety of CRL neo/substrates are collectively accommodated by various E2s or ARIH-family E3s that achieve optimal activity depending on interactions with NEDD8, the cullin, RBX family member, substrate receptor and/or substrate. Chain extension by UBE2R-family E2s is achieved through multiple features of these E2s including a synergy loop co-activated (directly and indirectly) by the neddylated CRL and donor and acceptor ubiquitins. While the extender E2 uses its synergy loop to position the RING, and the donor and acceptor ubiquitins.
Deep structural and mechanistic investigation of mechanisms in ubiquitin transfer is Benda's signature, helping us better understand the most widely used and successful TPD modality: hijacking of the ubiquitin-protease system.

Key Literature

Liwocha, J. et al. Mechanism of millisecond Lys48-linked poly-ubiquitin chain formation by cullin-RING ligases. *Nat. Struct. Mol. Biol.*, **2024**, 31,378–389

Li J *et al.* Cullin-RING ligases employ geometrically optimized catalytic partners for substrate targeting. *Mol. Cell.*, **2024,** 84(7), 1304-1320.e16

| Angus

Eric Fischer, Dana-Farber Cancer Institute & Harvard Medical School, USA

Invited Speaker - **Lessons from protein design and directed evolution for degrader discovery**

In this talk, Eric Fischer described the application of continuous and non-continuous evolution platforms to evolve a small degron tag that forms a high affinity ternary complex with CRBN and a thalidomide derivative. While zinc finger (ZF) domains represent attractive degron tags due to their small size and known chemical matter (IMiDs), sparing known neosubstrates (e.g. GSPT1, ZF transcription factors) from degradation and reaching high potency has been challenging. Starting from a IMiD derivative PT-179 that binds CRBN but does not degrade known pomalidomide neosubstrates and a 60 aa 'super-degron' tag, the team developed a platform for phage-assisted continuous and non-continuous evolution and performed iterative rounds of experiments, mutagenesis and truncation to arrive at a 36 amino acid tag 'SD40' which was degraded in cells by PT-179 with a DC50 of 4.5 nM. The small size of the tag makes it amenable for prime editing, which has a lower by-product knock-in rate than CRISPR, and global proteomics showed no significant reduction in any protein other than SD40 upon treatment with PT-179. Structural characterisation of the ternary complex was a nice way to cap off this story, and it was interesting to see the more extensive protein-protein interactions SD40 makes with CRBN compared to

the parent ZF fold, including interactions with the LON domain. The platform was also used evolve a degron tag that works with mouse CRBN opening the door to *in vivo* target validation with this degron system. The application of directed evolution to the TPD field has yielded a nice new tool, and it will be interesting to see what other problems Eric and co. apply it to in future.

Key Literature

Mercer JAM., DeCarlo SJ, Burman SSR, et al. Continuous evolution of compact protein degradation tags regulated by selective molecular glues. *Science*, **2024** 383(6688)

| *Angus*

Bekky Feltham*, The Walter and Eliza Hall Institute for Medical Research, Australia*

Invited Speaker - **Unlocking the Potential of Tag-targeting PROTACs**

In the spirit of the conference, Bekky Feltham's talk included three unpublished stories with a focus on providing data and tools for the TPD and wider ubiquitin community. Keeping on theme with the previous talk, Bekky described work exploring *in vivo* degradation in mice of GFP reporter construct tagged with Halotag, NLuc and FKBP for degradation using HaloPROTAC, NanoTAC and dTAG degraders, respectively. Looking at degradation in different tissues, each tagging system performed differently, demonstrating systemic degradation or more tissue-specific degradation. This information could be useful for target validation, and Bekky posed the question "can it be used to discover new biology?". She answered her own question quickly, sharing some interesting new data about ubiquitin chain types and branching induced by different degraders and how they affect degradation. Our familiar friend K48 is of course a key player, but there seems to be more going on than initially thought. The final part of the talk focused on the E3 ligasome, with the nebulous and familiar number of 600 E3 ligases we see quoted so often being thoroughly scrutinised. Bekky identified the lack of a consensus resource of E3 ligases as a problem for the field, with many of the existing lists showing major discrepancies in the number and identities of E3s. This community focused project involved computational analyses and curation by experts in the field to arrive at a more complete and higher confidence list for the community to use. This will be a great for everyone working the TPD and ubiquitin fields and I look forward to seeing it out there soon!

Session: Discovery Chemistry & Design II

| *Alex*

Ivan Đikić, *Goethe University Frankfurt, Germany*

Invited Speaker - **Identification of novel molecular glue degraders**

Ivan kicked off the afternoon session with a hilarious and heartfelt opening address, which included proof that Dundee is in fact "the sunniest city in Scotland" and that 'TPDers' love a good selfie! The conversation then turned back to science and Ivan gave a great overview of the ProxiDrugs consortium (https://www.proxidrugs.de) and its research focuses. The bulk of Ivan's talk was dedicated to a case study from the iGlue arm of the ProxiDrugs portfolio, which involved a comprehensive story of a new Cereblon (CRBN) construct to enable the rapid discovery of new molecular glues, as covered in their recent preprint.
The new CRBN construct was devised to combat the poor-yielding and costly expression of WT CRBN due to its requirement to be expressed as a complex with its binding partner, DDB1, in insect cells. The approach of the Đikić group was to truncate the hydrophobic region of the helical bundle domain (HBD) and replace it with a soluble linker, so that this new CRBN_ΔHBD construct could be expressed in E. coli and without DDB1. CRBN_ΔHBD is functionally equivalent to WT CRBN with respect to its interaction with thalidomide and other IMiD-based compounds.
This was exemplified by the high-throughput screening of over 4,000 IMiD-derived small molecules using fluorescence polarisation (FP), which led to the identification of >100 compounds which showed greater affinity for CRBN than the clinical candidate, Iberdomide (Carfilzomib®, EC50 = 150 nM). The rapid screening of compounds, enabled by this new CRBN construct, allowed novel CRBN chemotypes to be identified and will aid the development of new CRBN-based ligands and degraders in the future.

Key Literature

Bailey HJ, Eisert J. et al., Engineering CRBN for rapid identification of next generation binders. BioRxiv **2024** https://doi.org/10.1101/2024.01.18.576231

| *Alex*

Christina Woo, *Harvard University, USA*

Invited Speaker - **Chemical biology studies of the thalidomide-binding domain of cereblon**

Christina's talk centred around updates to a story which has been published in Nature (2022) and more recently in Cell Chemical Biology (2024) regarding the elucidation and subsequent utilisation of the endogenous CRBN degrons, cyclimids, now on preprint (bioRxiv, 2024).
Work from Christina's lab used the specificity of the glutarimide ring of CRBN ligands to elucidate that the cyclisation of C-terminal glutamine and asparagine residues afforded the cyclic degrons, glutarimide (cQ) and aspartimide (cN) respectively. These motifs are generated in vivo as a result of protein damage and so are recognised by CRBN as a means of removing the damaged protein.
Christina went on to explain the investigations her group have made into the mechanism by which these cyclimids are generated. This work focused on the 5m aspartimide that is formed most readily *in vivo* due to the increased rate of cylisation for 5-membered rings (Baldwin's Rules).
Work from the Woo lab has since shown that these native degrons can be utilised in bifunctional degraders to recruit CRBN. Using BRD4 as an example, they showed that degradation potency and selectivity of degraders bearing cyclimid warheads can be effectively tuned by the choice of adjacent peptidic residues.

Overall, Christina provided a great story which covered the interrogation of biological functions and mechanisms, and how this might be leveraged for chemical biology and drug discovery applications.

Key Literature
Ichikawa S, Flaxman HA., Xu W et al., Nature, **2022**, 610, 775-782.
Ichikawa S, Payne CN et al., *Cell Chem. Bio.,* **2024**, 31, 1-14.

| *Alex*

Michael Erb, *Scripps Institute, USA*

Invited Speaker - **Systematic remodeling of protein-ligand surfaces for the prospective discovery of molecular glues**

For the next talk in the session, Michael decided to change gears a little and provide a summary of a collaborative project with Benjamin Cravatt's group (Scripps Institute) to use chemoproteomics to identify covalent small molecules that target the transcription factor, FOXA1.
FOXA1 is a pioneer transcription factor (pTF) with critical roles in gene regulation due to its ability to bind directly to, and decompact, chromatin-bound DNA. Despite its critical function, mechanistic insights into pTFs had been historically limited to *in vitro* systems due to a lack of small-molecule modulators. Erb and Cravatt have used activity-based protein profiling (ABBP) to identify tryptoline acrylamide probes that site-selectively and stereoselectively bind to Cys258 of FOXA1 in prostate cancer models.
Interestingly, the interaction between recombinant FOXA1 and the stereogenic probes was dependent on the presence of DNA that contained the canonical FOXA1 binding sequence (GTAAACA) and that treatment with benzonase led to complete perturbation of reactivity. Treatment of FOXA1 with the active stereoisomer strengthened the interaction with DNA and led to changes in its pioneering activity with chromatin. This work highlights the need for selective chemical probes in order to probe complex biological systems.

Key Literature
Won SJ, Zhang Y, et al, Redirecting the pioneering function of FOXA1 with covalent small molecules BioRxiv, **2024,** https://doi.org/10.1101/2024.03.21.586158

| *Giorgia*

Johanna Huchting, *Fraunhofer Institute for Translation Medicine and Pharmacology, Germany*

Short Talk - **Integrated screening and knowledge platform for molecular degrader discovery**

In this talk, Johanna showed utilisation of a positive selection assay where degradation of your target protein of interest prevents cell death. This is due to the presence of a fusion of POI to DCK*, where addition of a "suicide substrate" which will be transformed by DCK* to a cytotoxic product leads to cell death. Degradation of the POI and concurrently DCK* will prevent the turnover of the suicide substrate and thus the cells will survive and proliferate. This will give a positive read out related to the efficacy of the POI degrader used.
This assay was used to screen for small molecule degraders, namely molecular glues, targeting IKZF1. To test the setup, ~1000 molecules were screened, in a set including positive controls and frequent hitters, and covering a range of chemical space.

The assay requires 5 days but can be expanded to a 384 well plate so is still moderate throughput. For comparison, the same set of compounds was tested in parallel using a TR-FRET-based assay, and the cell death prevention assay was seen to give far fewer false positive results.
This assay was also applicable to screen a less well investigated compound set, where hits could again be validated *via* TR-FRET.
Overall, this assay seems like a useful addition to the screening toolbox used by TPD scientists, giving an orthogonal assay for screening new chemical matter.

Key Literature
Koduri et al. Targeting oncoproteins with a positive selection assay for protein degraders *Sci. Adv.* **2021**; 7, eabd6263

| Giorgia

***Mikołaj Słabicki**, Dana-Farber Cancer Institute, USA*

Short Talk - **Expanding the human zinc finger degrome targeted by glutarimide analogs through CRBN**

Glutarimide analogs redirect the CRL4CRBN E3 ubiquitin ligase to induce ubiquitination and proteasomal degradation of transcription factors containing zinc finger (ZF) domains. Mikołaj described how they assembled a comprehensive ZF library, encompassing C2H2 ZF domains as well as other variants such as RING, PHD, C3H1, C4, and MYM domains. This library comprised 9,098 reporters from 1,656 ZF-containing proteins. They screened the activity of 29 glutarimide analogs across the ZF library, leading to the identification and validation of 38 ZF reporters that are efficiently degraded by at least one molecule. To elucidate the role of individual amino acids contributing to drug-induced degradation, they conducted systematic alanine mutagenesis scans on both the ZFs and CRBN and integrated the functional studies with structural analyses. Their findings elucidate that: (1) subtle modifications in the glutarimide analogs can broaden the scope of target degradation and define target selectivity, (2) residues neighbouring the principal interacting ZF decisively influence degradability of the ZF, and (3) a single ZF amino acid can determine drug specificity. This study provides a roadmap for the rational design of glutarimide analogs, expanding the range of ZF targets and providing insights into mitigation of toxicity.
Mikołaj also highlighted work on development of molecular glues from binders, a method that was used before for the degradation of cyclin K and BCL6. More can be read about this in: Nature, 2020, 585, 293–297 and Nature, 2020, 588, 164–168.

Session: Molecular Mechanisms and Biology III

| Giorgia

***Georg Winter**, Center for Molecular Medicine, Austria*
Invited Speaker - **Identification and characterization of small-molecule degraders**

Georg showed us utility in understanding how an inhibitor might destabilise the target protein, leading to apparent degradation, for example, ATP competitive binders will block kinase activity, with examples seen in JAK, Pi3Ks and LRRK.
The question at hand was whether this is a more general effect and if it could be utilised. To probe this, 100 cell lines with kinase–nanoLuc–GFP–EGFP fusions expressed were produced. The use of these cell lines revealed 160 selective inhibitors induced destabilisation effects. The data given was checked for known hits, such as HSP90 which was shown to be destabilised in Taipale et al., Cell, 2012, 150, 5, 987-1001.
This study showed that some proteins are more frequently destabilised, with HER2 the most frequently destabilised protein. The hits were mapped out and this implied direct correlations between some of the proteins. To probe the degradation via destabilisation, the protein levels were compared to the T1/2 given by treatment with cyclohexamide, however there was no correlation between degradability and degradation by PROTACs.
The cases for BLK, LYK, and RIPK2 were shown in more detail. In the case of RIPK2, an immediate destabilisation effect was seen which wasn't copied by HSP90 inhibition so can be through direct binding to the target. Any hits can be validated in a CRISPR screen, showing whether or not an E3 ligase is implicit. Control experiments such as whether the destabilisation effect was rescued by Bafilomycin (showing FIP200 dependancy), or the effect of a FIP 200 knock out (probing degradation through the lysozyme) were performed.

| Giorgia

***Nicolas Thomä,** Swiss Federal Institute of Technology (EPFL), Switzerland*
Invited Speaker - **Nucleosomal gate access of cofactors to chromatin-bound P53**

P53, a tumour suppressor which is regulated by the UPS, directly binds to chromatin to function, but what about all its interactors and regulators – in particular the UPS? This was the question Nicolas and team aimed to address, and to do this, DUB USP7 was used with E6/E6AP, leveraging previously acquired structures of P53 by the Pavletich lab. Selective engagement on the nucleosomal sequence SeEN-Seq used to define where on nucleosomes p53 bound, revealing multiple possibilities. Multiple structures of P53 bound to chromatin were solved and showed preferred binding to nucleosomal at the DNA entry/exit site on the nucleosome, revealing different interfaces between P53 and the chromatin/nucleosome, with P53 TET (tetramiseration) domain engaged to DNA and P53 DBD.
The solving of the structures was aided by cross-linking mass spectrometry, and comparison to another system (DDB code 7XZZ), showing P53 in 3 positions. They then showed that
USP7 bound to P53 on the nucleosome in CryoEM structures, and USP7 was found to be active when bound to p53 with or without nucleosomes. Another E3 ligase, E6AP, did not recognise p53-bound nucleosomes, arguing that chromatin binding restricts the binding space for p53 interactors. This also provided a general model how TF interactor with transcriptional regulators.

| Giorgia

Gopal Sapkota, *University of Dundee, UK*

Invited Speaker - **Targeted dephosphorylation of phospho-proteins through induced-proximity**

Phosphatases are hard to target, which is thought to be due to their promiscuous nature in dephosphorylating >90% of phosphorylation events. Some however are more selective, like PPM1H, which dephosphorylates pRABs. However, many are not well understood. Thus, instead of targeting the inhibition of phosphatases, an alternative approach that could be more advantageous would be to harness and redirect the phosphatase activity through proximity-induction to dephosphorylate target proteins in cells. This can be achieved via PhosTACs, a concept which is emerging as a new druggable approach but is limited due to lack of effective phosphatase ligands.

Proof of concept for the use of PhosTACs at the endogenous level was demonstrated using a BromoTAG–dTag system (c.f. Nabet et al. Nat Chem Biol, 2018, and Bond, Craigon, et al. J Med Chem, 2021), where the Sapkota lab developed a small bivalent bromoTAG-dTAG Proximity-inducing chimera (BDPIC).

The first target which was investigated was TFEB, a highly phosphorylated transcription factor. Dephosphorylation of TFEB leads to its translocation to the nucleus, where it drives the transcription of target genes. In cells knocked in with dTAG-TFEB and bromoTAG-PPP2CA, 100 nM BDPIC induced dephosphorylation of TFEB within 5 minutes and was shown to be reversible by a washout after 1 hour. Targeted dephosphorylation of TFEB triggered its nuclear translocation and transcriptional activity (Zhao et al., 2024). Similarly, BDPIC triggered dephosphorylation of dTAG-SMAD3 by recruiting bromoTAG-PPM1H (Brewer et al., 2024).

These studies showed the application of dTAG and BromoTAG to further targets can allow not only the ability to see whether PROTACs may be useful for the degradation of a target, but also whether other induced-proximity mechanisms are applicable in cells.

| Tessa

Charlotte Crowe, *University of Dundee, UK*

Short Talk - **Mechanism of degrader-targeted protein ubiquitinability**

In this short talk, Charlotte describes how she was able to obtain a CryoEM structure of the VHL cullin 2 RING E3 ligase complex, with degrader MZ1 and the target protein BRD4^{BD2}. She does this by recombinantly neddylating the E3, and then adding the E2-ubiquitin conjugate to form the full degrader-mediated ubiquitination complex. A video of the cryoEM structure shows the flexibility of the ligase complex. Additionally, she showed that only the lysines facing the catalytic site of the complex were ubiquitinated, and those which appeared to be geometrically and spatially optimal by cryoEM were ubiquitinated in higher levels.

If you would like to read more about this project, you can find the preprint on BioRxiv:

Key Literature

Crowe C., et al. Mechanism of degrader-targeted protein ubiquitinability. *BioRxiv,* **2024,** https://doi.org/10.1101/2024.02.05.578957

| Tessa

Jiho Park, *Dana-Farber Cancer Institute, USA*

Short talk - **Polymerization of ZBTB transcription factors regulates chromatin occupancy**

In this short talk, Jiho presented several case studies from his work on the polymerization of ZBTB transcription factors (TFs). Inspired by the fact that BCL6 forms polymers of homodimers in presence of a molecular glue, he and his colleagues at the Dana-Farber Cancer Institute screened a large library of other ZBTB TFs. His talk focused on the structural and biochemical characterization of ZBTB3, ZBTB5, and ZBTB9, which form punctate nuclear foci in cells. For ZBTB5, Jiho was able to obtain a 3.7 Å cryo-EM structure, illustrating the interactions between the homodimers required to form foci in cells. These interactions are mostly electrostatic, with positively charged residues on one homodimer interacting with the negatively charged residues on the other homodimer. The 8.2 Å cryo-EM structure of ZBTB9, on the other hand, demonstrated that formation of these filaments is based more on hydrophobic, rather than electrostatic interactions. While Jiho did not obtain a cryo-EM structure for ZBTB3, he demonstrated filamentation using negative-stain EM and modelling with AlphaFold-Multimer. Lastly, with ChIP-seq, Jiho and his colleagues showed that polymerization of these proteins enhanced DNA binding and downstream gene repression.
Jiho's work was published in the July 11th issue of *Molecular Cell* as part of a back-to-back series with a research article from the group of Marcin Suskiewicz at CNRS Orléans.

| *Selma*a

David Nie*, University of Toronto, Canada*

Invited speaker - **Recruitment of FBXO22 for Targeted Degradation of NSD2**

In this short talk, David talked about Nuclear Receptor-Binding Set Domain Protein 2 (NSD2), an important target in cancer whereby they were able to show that its small molecule induced degradation was mediated by E3 ligase FBXO22. NSD2 is an epigenetic regulator that regulates transcription through histone methyltransferase activity, nominally dimethylating histone H3 at its lysine 36 residue. David showed the optimisation efforts to design their more potent second generation NSD2 chemical degrader, UNC8732, with a DC50 of 350 nM. Using the miniTurbo fusion protein to NSD2 in an unbiased proximity-dependent biotin identification (BioID) assay, they were able to identify F-Box Only Protein 22 (FBXO22) ubiquitin ligase as the mediator of this degradation pathway through its association with the SKP1-CUL1-F-box (SCF) E3 ligase complex. This was confirmed by a knockdown of SCFFBXO22 components demonstrating rescue of NSD2 levels. This was further confirmed by looking at ternary complex formation between NSD2 and FBXO22 using NanoBRET, and using Hydrogen Deuterium Exchange-Mass Spectrometry (HDX-MS) to identify the interacting interface as the FIST_C domain of FBXO22. Interestingly, David also presented that UNC8732 was not the active compound, but in fact in the presence of FBS it forms an aldehyde species (identified by mass spectrometry) that is needed for binding. This phenomenon was emphasized when NSD2 was not able to be degraded in the absence of FBS, as well as in the presence of the amine oxidase inhibitor, aminoguanidine (AG). As the active aldehyde was difficult to purify, David then described an aldehyde bisulfite adduct, UNC10088, that was synthesized to further investigate the phenomenon. This compound was able to degrade NSD2 in the absence of FBS, and achieve maximal degradation faster compared to UNC8732. Furthermore, bio-layer interferometry was used to confirm ternary complex formation between NSD2 and SKP1-FBXO22, and HDX-MS confirmed binding to the FIST_C domain. Lastly, to finish their talk, they showed this active aldehyde species phenomenon was transferable to another SCFFBXO22 mediated degradation pathway, using a chemical

degrader for Inhibitor of Apoptosis, X-linked (XIAP). Similarly, FBS and AG presence also prevented XIAP degradation, and lent support to the hypothesis that a primary amine prodrug metabolisation is required for FBXO22 recruitment.
If you want to read more about David's work on NSD2 and XIAP, have a look at his paper:

Key Literature
Nie DY, et al. Recruitment of FBXO22 for Targeted Degradation of NSD2. *Nat Chem Biol*, **2024** https://doi.org/10.1038/s41589-024-01660-y.

Session: Discovery Chemistry and Design III

| Aitana

Alessio Ciulli, *Centre for Targeted Protein Degradation, University of Dundee, UK*

Keynote - **We keep degrading! CeTPD's past, present and future**

Alessio walked us through the establishment and evolution of the TPD field, from the initial discoveries within the group to the ever-developing field as we know it today. He started by paying homage to MZ1, one the key molecules that started it all. He talked about the characteristics of this compound that make it a great degrader: cooperativity, stability, and a long-lived ternary complex. Structure-guided mutagenesis work led to the discovery that a single amino acid at the neo-PPI interface drives the specificity of MZ1; and coupling MZ1 to the bump-and-hole approach led to the development of BromoTAG. Both these studies highlighted the importance of structure-guided PROTAC design.
Since then, the CeTPD has solved many ternary complexes by X-ray crystallography, the great majority of which are based on VHL. However, the field of TPD has shifted to a CRBN-based preference due to its oral bioavailability. Crystallography using CRBN has traditionally been limited due to the challenges regarding the production of a suitable recombinant CRBN protein. Alessio introduced a new CRBN construct: $CRBN^{midi}$, which expands the toolbox for degrader characterization. He excitingly disclosed that $CRBN^{midi}$ has been used to solve several ternary complex co-crystal structures at high-resolution.
Alessio then moved on to talk about the E3 ligase SOCS2. SOCS2 recognises phosphorylated tyrosines *via* its SH2 domain and has historically been considered undruggable. Interest in SOCS2 from the TPD field has grown since the first crystal structure for this protein was solved in 2006. In 2022 Alessio's group published a structure-guided design campaign leading to the discovery of a chemical probe for SOCS2. Initially grown from a phosphotyrosine fragment, compound MN551 covalently engages cysteine 111 of the SOCS2-SH2 domain. The co-crystal form of MN551-modified SOCS2 and adaptor protein complex EloBC was found to be highly reproducible, it consistently diffracts to high resolution and is stable at high concentrations of DMSO. This was leveraged to perform XChem crystallographic fragment screening at Diamond Light Source to search for non-orthosteric binding sites. Several new ligandable sites were discovered, with fragment expansion and follow-up focused on a protein-protein interface between SOCS2 and EloC. Chemist assisted robotics (CAR) was used to rapidly generate follow-up compounds and crude reaction products were subjected to a second round of crystallographic screening, leading to the discovery of a robust binding pharmacophore for this previously unliganded site.
Alessio's talk was very enlightening, providing us with a chance to hear a variety of interconnected stories from the group and witness the impressive advancements in the field.

Key Literature

Ciulli A; Trainor N., A beginner's guide to PROTACs and targeted protein degradation. *Pharmacol Ther.*, **2017,** 174, 138-144.
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| *Andreas*

Cristina Mayor-Ruiz, IRB Barcelona, Spain

Invited Speaker - **Unlocking drug discoveries and navigating drug resistance challenges**

In this talk, Cristina highlighted the challenges and potential of rational design in developing molecular glues to navigate drug resistance. She presented their innovative work using their glueE3 virtual screening pipeline. The goal of this pipeline is to identify molecular glues that enhance weak, preexisting target-E3 interactions through computational mapping, aiding in rationalising molecular glue discoveries. Cristina illustrated their approach with a case study targeting Cyclin K (CycK) using a specific E3 ligase. The team employed their pipeline to initiate the discovery of molecular glue degraders. Starting with a library of 20 million small molecules, they screened and identified 44 candidates, ultimately discovering 9 new molecular glues. This virtual approach enables the discovery of molecular glue degraders for specific targets, moving beyond the traditional reliance on serendipitous findings. Cristina's work demonstrates the power of the glueE3 pipeline in facilitating targeted molecular glue discoveries in a rational manner, offering a promising solution to address drug resistance through rational design of molecular glue degraders.

Key Literature

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Nabet B, Roberts JM., Buckley DL et al. The dTAG system for immediate and target-specific protein degradation. *Nat Chem Biol, **2018***, 14, 431–441

| *Andreas*

Stefan Knapp*, Goethe University and SGC Frankfurt, Germany*

Invited Speaker - **Development and validation of PROTACs and E3 ligase ligands as selective chemical tools**

In this talk, Stefan Knapp emphasized the rigorous quality control essential for developing small molecule degraders, such as PROTACs and molecular glues. These probes are designed to be drug-like small molecules that selectively modulate specific proteins in cells, thus opening new research avenues. Knapp highlighted their commitment to providing these chemical probes for research without restrictions on use, promoting open scientific exploration. Key quality criteria for degraders were outlined: a DC_{50} of less than 100 nM and a D_{max} of over 70%. However, he pointed out that proper validation of chemical probes was lacking, with the majority of studies failing to use appropriate controls at the right concentrations to confirm on-target, on-mechanism activity. To address these issues, Knapp's team developed a comprehensive toolbox for CRL-based degrader development, encompassing E1 inhibitors, Neddylation inhibitors, E3 handles, and proteasome inhibitors. They have established concentration recommendations for these control compounds to ensure accurate degradation rescue assays. Overuse of such compounds can kill cells and produce false readouts, underscoring the importance of precise application. This

meticulous approach, combined with their stringent quality control measures, ensures the reliability and effectiveness of their chemical probes, advancing biological research through high-quality tools.

Key Literature

Picaud S, Fedorov O , Thanasopoulou A , Leonards K et al. Generation of a Selective Small Molecule Inhibitor of the CBP/p300 Bromodomain for Leukemia Therapy. *Cancer Res*, **2015**, 75 (23), 5106–5119.

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Session: Therapeutic Development II

| *Selma*

***Ingrid Wertz**, Lyterian Therapeutics, USA*

Invited Speaker - **Co-opting the Ubiquitin System for Therapeutic Benefit**

Ingrid gave an insightful overview of how to enhance efficacy in generating complex compounds that hijack the ubiquitin system, how they affect proteostasis, with desirable pharmacological properties, using their work on the Estrogen Receptor (ERα) as an example. Here, Ingrid also introduces the nomenclature of Chemical Inducers of Degradation, or CIDEs, to describe their degraders. It was outlined how ERα is an important target in breast cancer and selective ER degraders (SERDs) such as Fulvestrant and Giredistrant are approved or clinical therapeutics, respectively. Ingrid went onto describe their most recent development of a pan-IAP antagonist based ERα degrader (pan-IAP/ERα-CIDE). Interestingly, as well as degrading ERα, it also induces degradation of IAP itself, which in turn activates TNFα production and promotes cell death via apoptosis in TNF-producing cells. The mechanism here was described as the pan-IAP/ERα-CIDE harnessing XIAP within tumour cells to mediate ERα degradation, and also activating cIAP1/2 within tumour and immune cells to induce TNFα expression to drive cell death. Tumour cells that don't produce TNFα, such as BT474-M1 cells show reduced ERα levels and slowed proliferation upon pan-IAP/ERα-CIDE treatment but fail to activate cell death. It was emphasized when BT474-M1 cells are exposed to co-culture with PBMCs that do produce TNFα, and the co-cultures are treated with the pan-IAP/ERα-CIDE, the TNFα produced by the PBMCs plus ERα degradation induce BT474-M1 cell death. Lastly, this mechanism was demonstrated in vivo using the NF1-deficient rat model. NF1 is a tumour suppressor gene that encodes neurofibromin and when it is absent in rats, heterogenous ERα-dependent tumours form. Ingrid showed a dose-dependent reduction in ERα staining with pan-IAP/ERα-CIDE treatment, as well as a reduction in tumour volume that was rescued by ENBREL (TNF inhibitor). These results present how modifying proteostasis using heterobifunctional compounds that hijack both degradation pathways and cytokine signalling pathways can have a complex systemic effect that functionally combine to promote tumour cell death, and presents a great example of how this can be utilised for therapeutic benefit.

| *Aitana*

***Danette Daniels,** Foghorn Therapeutics, USA*

Invited Speaker - **Targeting chromatin Regulatory Proteins with Therapeutic Degraders**

Dannette started her talk introducing EP300 and CBP, histone acetyltransferases which are important cancer targets. These proteins have multiple common domains, which makes achieving selectivity with a small molecule challenging.

The existing dual inhibitors cause thrombocytopenia, so they set out to develop a selective degrader by leveraging an inhibitor and an E3 ligase. She revealed that the E3 ligase they chose to utilise is VHL and disclosed this for the first time in the 'House of VHL', referring to the CeTPD. Their initial series of degraders showed good dual degradation, but cell proliferation assays showed that these degraders killed the majority of cell types, even if the cell line is not CBP or EP300-dependent. They were able to solve a very flexible structure of VHL ELOBC and the bromodomain of CBP of 2.56Å which showed a minimal interaction interface between VHL and CBP.

To avoid dual inhibition within the context of the dual degrader, they weakened the inhibition binding from nM to mM, yet they were still able to develop a very selective and fast degrader of CBP. This was taken forward for xenograft studies with showed CBP degradation and tumour regression in CBP-

dependent cancers. They developed a long-acting injectable formulation which provides 2 weeks of substantial CBP degradation after 1 single dose. Importantly, the CBP selective degraders do not induce thrombocytopenia at efficacious doses.
Danette shared a fantastic story that captured my curiosity, specifically in how they achieved selectivity through strategically weakening the affinity of the dual inhibitor.

Key Literature

Weinert BT et al. Time-Resolved Analysis Reveals Rapid Dynamics and Broad Scope of the CBP/p300 Acetylome. *Cell*, **2018**, *174* (1), 231-244.

| Andreas

***Angela Cacace**, Arvinas, USA*

Invited Speaker - **Advancements in PROTAC technology: breaking down barriers in Neuroscience targeted drug development**

In this presentation from Arvinas, Angela discussed their work on the discovery and development of ARV-102, a brain-permeable degrader targeting LRRK2, highlighting the feasibility of CNS-active proteolysis-targeting chimeras (PROTACs). Arvinas has compiled a comprehensive dataset of approximately 1,575 different PROTAC, which includes detailed pharmacokinetic (PK) including intravenous (I.V.) and oral (PO) data measurements in rats. They routinely administer an oral dose of 30 mg/kg to wild-type mice and analyze the outcomes after 24 hours. (This is based on detailed dose response time course studies to investigate warhead and PROTAC PK/PD to determine the timepoint for maximal pharmacodynamic effect for target engagement in the brain). In their findings, about 42% of their PROTAC library exhibits a brain-to-plasma (B/P) ratio greater than 0.3. Theses data suggests that a considerably larger proportion of these bifunctional molecules penetrate the brain than was previously assumed. Additionally, Arvinas conducted a comparative study of their LRRK2-targeting PROTAC against a traditional LRRK2 inhibitor. The results were noteworthy, showing that the PROTAC demonstrated a two-order magnitude increase in the potency of the free drug in the brain. These findings underscore the potential of PROTACs in developing effective treatments for CNS diseases. They challenge prior assumptions regarding the ability of these molecules to traverse the blood-brain barrier and highlight a promising avenue for future research and therapeutic development in CNS disorders. This advancement could pave the way for more effective and targeted treatments, addressing a critical need in the field of CNS disease management.

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| Mokhitli

***Andrea Testa**, Amphista Therapeutics, UK*

Short Talk - **Degradation of BRD9 by a novel "targeted glue"**

Bromodomain-containing protein 9 (BRD9) is an essential component of a chromatin remodelling complex named non-canonical BAF complex. Targeted degradation of BRD9 is an emerging therapeutic avenue for potentially transformative treatments for synovial sarcoma, malignant rhabdoid tumour and leukemias. Amphista discovered a potent and selective, reversibly covalent BRD9 degrader, compound 1. Detailed mechanistic studies involving proteomics, genetics and biomolecular assays pointed to a "targeted glue mechanism" were presented. BRD9 degradation was also achieved in vivo, demonstrating that this new mechanism may be a viable therapeutic strategy.

| *Mokhitli*

Vesna Vetma, *University of Dundee, UK*

Short Talk - **Targeting cancer with small molecule pan-KRAS degraders**

The talk highlighted the discovery of the novel panKRAS degrader with high affinity, degrading 13 out of 17 KRAS mutations. The biophysics-driven SAR optimization of the VHL engaging PROTACs led to the discovery of compound 3, whose crystal structure showed possible π-stacking interaction of the exit vector with Tyr112. This compound had improved DC50, was selective for KRAS, and had increased rate of degradation. Its optimization led to the discovery of compound 4 which had unfavourable PK, high plasma protein binding and thus, insufficient exposure. Incorporation of the triazole and hydroxymethyl in ACBI3, the lead panKRAS degrader, increased the binding which resulted in increased ternary complex stability, efficient ubiquitylation and better degradation. This compound is identified as the first potent and fast proof-of-concept panKRAS degrader.

Key Literature

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| *Mokhitli*

Rebecca Stevens*, GSK, UK*

Short Talk - **Direct-to-Biology for High-Throughput PROTAC Synthesis and Biological Evaluation**

The talk described a new 'direct-to-biology high-throughput-chemistry' approach which is aimed at streamlining the synthesis efforts of PROTACs while expediting the test of new PROTACs for rapid hit and lead identification. In this platform, 100s of PROTACs are screened as crude mixtures in cellular assays and their purity is determined by HPLC. The synthetic approach was based on Pd-mediated multistep synthetic platform for PROTAC synthesis, using chemical transformations such as C(sp2)-C(sp3) cross-couplings and metallaphotoredox chemistry. The new platform had >80% success rate and this enabled more PROTACs to be synthesized in one day. This further accelerates SAR exploration to within 3 weeks. The findings showed that the CRBN-based PROTACs were more potent, sp3 linkage was important for activity and 12-15 atoms were optimum. There was strong correlation with purification method, with no requirement for cartridges. There was no impact on DC50/Dmax and the platform provided a valuable workflow for rapid synthesis and testing of PROTACs.

Key Literature

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