CeTPD Journal Club

Targeted protein degradation, medicinal chemistry, chemical structural biology & cell biology

Journal Club

February 2023

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

Content

Meet this Month's Editors

This month's editors are (from left to right): Zoe Rutter, Conner Craigon, and Andreas Holmqvist

"The JC is great resource for keeping up to date with the field of TPD."

Zoe completed her undergraduate degree in biochemistry at Newcastle University followed by a PhD in Structural Biology in the lab of Prof. Rick Lewis. In April 2021 she joined the ACBI team as a structural biologist/biophysicist.

"The journal club is a great reminder of the ever-burgeoning field of TPD and helps highlight the best research of this field."

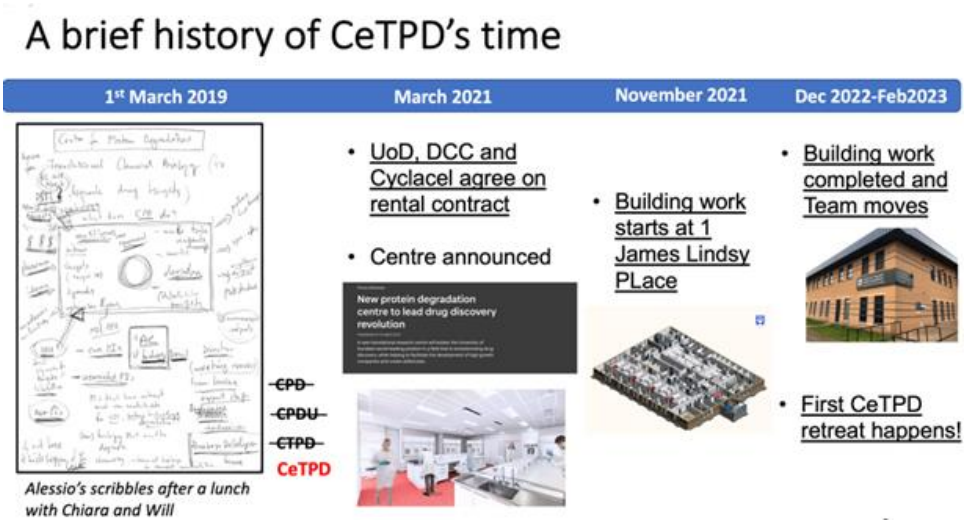
Conner completed his MRes and recently his PhD at the Univeristy of Dundee. He is currently as of 2023 working in an academic postdoctoral position in the Ciulli group as a cell biologist.

"The journal club is a great place to find inspiration of novel ideas and research within the field of proximity-based drug design"

Andreas is a first year Ph.D. student who obtained his M.Sc. in organic and medicinal chemistry from University of Gothenburg, Sweden. He joined the CeTPD autumn 2022 to pursue his Ph.D. where he is working the development of CNS active degraders.

The CeTPD project and Labs moves: Past, Present and Future

Contributor: Alessio



On Monday 9th January 2023, the whole CeTPD group moved into our brand-new laboratory space at 1 James Lindsay Place. This event was the culmination of a long project of establishing CeTPD, taking almost 4-years. It all started on 1st March 2019 when the idea first emerged and was drafted on an A4 page of scribbled notes after a lunch I had with Chiara and Will. Following much internal discussions and planning, it was not until March 2021 that the University, Dundee City Council and Cyclacel (the former tenant of the building) agreed on a rental contract at 1 James Lindsay Place. The refurbishment of the laboratories started on 1st November 2021, and was completed in mid-December 2022. Hence, January 9th marked a big day all of us at CeTPD, the day where a long hatched and much awaited project and aspiration eventually became a reality. Constraints in the previous lab spaces at the main research complex meant we all spent the last few years spread around various labs. At one point we occupied up to 7 different labs to accommodate the fast growth of the group (doubled from 30 to 60 in just the last 12 months). Therefore, the move was very important to us since this would be very first time that we all find ourselves to be sharing the same Labs and space. It marked the beginning of a new era for us all at CeTPD! University Press Release.

The much-awaited Lab move went smoothly and worked out so effectively. It reminded me how very fortunate I am to be at the helm of such a fantastic team. The move was well-planned and executed in all major areas (Chemistry, Biology, Biophysics), and it was fun to watch it all come together. The video posted by Valentina on Twitter conveyed some of the excitement of the team moving into the new space from the very first day!

It felt like an opportunity to take stock of the amazing journey we had all been through until then and to ride the wave of a fresh new start. I was amazed by how quickly we all got the labs up and running, with no major hiatus in scientific and research activities experienced.

None of this would have been possible without the fantastic teamwork of everyone involved. A big shoutout must go to our amazing CeTPD Operation Manager Louise McGreavey and Lab Managers Debbie Murray and their team, as well as our formed BCDD Lab Managers Shona and team and Letty Gibson, for their unabated support and their hard work throughout the past 4 years. Diane Cassidy and Adam Wisher, our Senior Technicians for Biology and Chemistry, respectively, worked relentlessly as the move was 110% of their focus during the past few months. There are way too many people to thank individually, but I do want to mention everyone in our *CeTPD Working Group*: Will (who's kind of been my partner-in-crime since those scribbled notes), David, Diane, Adam, Louise, Anne, Diane Purves, project manager David Stewart and Roddy MacLeod and so many others. In the two months since that day, we have come a long way already. All key facilities are now up and running and we continue to grow and develop them further, we even have signage out on the building already! None of this could have been happened without the support and contributions from many in the University and School, right the way down from the Principal's and Dean's Offices to the many people who contributed to the project, its fundraising, marketing, logistics, operations and communication. These activities build on investments of >£8M in equipment, lab space and refurbishments at our new premise, and we are so grateful to our many funders, donors and partners - without their support we could not be here. Last but not least, a wholehearted thank you must go to my wife Chiara, who over the past 4 years has helped to keep me real and grounded, especially through the difficult times. Coincidentally, on the same day as CeTPD moved labs, Chiara also moved her group (slightly longer distance, from Newcastle to Dundee!), and opened her new lab at the MRC-PPU.

I would like to close with a big shoutout to all the groups' scientists: without your commitment, drive, brilliance, hard-work, and scientific accomplishments, none of this would be even imaginable. We have come a very long way since the Ciulli group moved from Cambridge to Dundee, and we will soon be celebrating the 10th anniversary of the group in Dundee! Everyone has taken part and contributed to getting us where we are today. A truly exciting new journey now begins for CeTPD as a brand-new Institute within the SLS. We are soon to advertise recruitment of up to 5 new PIs, and 3 new Heads of Discipline (Chemistry, Structural Biology/Biophysics and Cell Biology). These are unique and long-term opportunities for outstanding individuals to contribute to the Centre's growing diverse and supportive leadership team, while driving forward innovative, collaborative and inspiring science with wide-reaching impact. Do get in touch with me directly via DM on email or social media for informal enquiries.

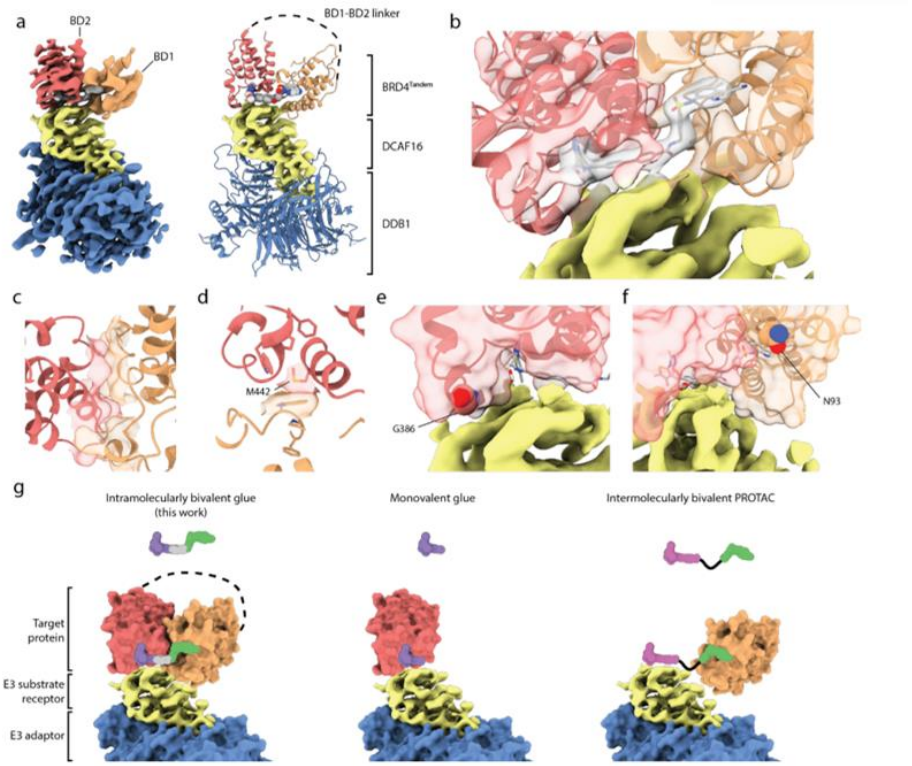
Target Protein Degradation

Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Zoe

An intramolecular bivalent degrader glues an intrinsic BRD4-DCAF16 interaction

Oliver Hsia[§], Matthias Hinterndorfer[§], Angus D. Cowan[§], …, Georg E. Winter* & Alessio Ciulli*
BioRxiv, **2023**, DOI: https://doi.org/10.1101/2023.02.14.528511



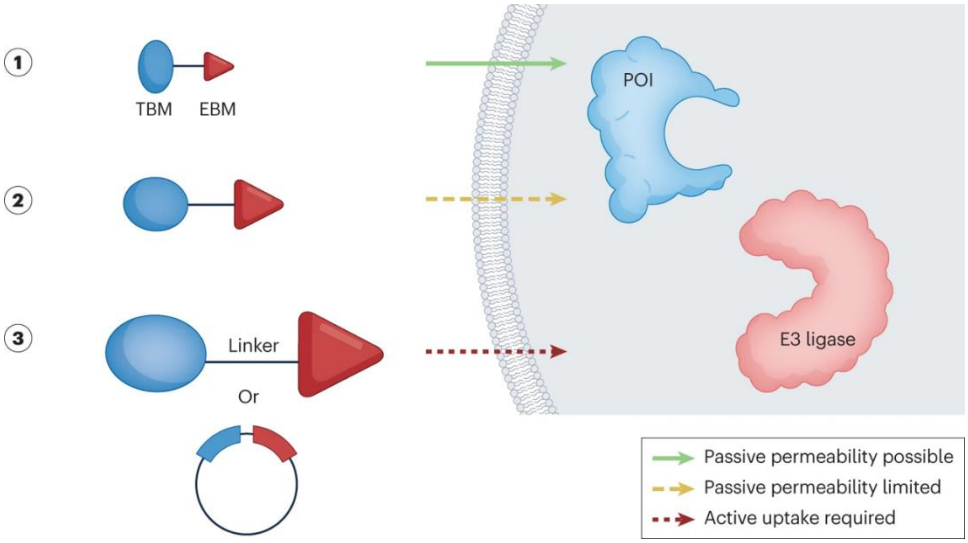
This paper is a prime example of combining multidisciplinary techniques to discover and validate a novel degradation mechanism. The authors used an array of experiments in chemistry, cell biology and structural biophysics to show that compound 1 acts a bivalent glue stabilising the intrinsic interaction between BRD4 (BD1 and 2) and DCAF16 to promote degradation. Compound 1, a BET bromodomain ligand tethered to E7820 (an aryl sulfonamide molecular glue) was previously reported as a potent BRD4 degrader. In this study it was shown that the action of compound 1 is through proteasomal degradation and it engages BRD4 via it's BET bromodomain ligand. Knockdown/out tests demonstrated that BRD4 degradation was DCAF15 independent and a time-resolved, FACS-based CRISPR/Cas9 screen revealed CRLDCAF16 was recruited by compound 1. Ternary complex formation involving 2 bromodomains of BRD4, compound 1 and DCAF16 was shown using size exclusion chromatography, alphaLISA and TR-FRET (BD1 and 2) The cell biology and biophysics results were ultimately explained by the 4 Å ternary complex cryoEM structure that showed one molecule of compound 1 glues at the interface between DCAF16, BD1 and BD2, mediating numerous inter and intramolecular interactions. This discovery opens up a new exploration space in the field of TPD to productively glue by modulating protein conformation and surface topology via engaging multiple regions at the protein interface.

Cell Biology | Chemistry | Modelling/Simulation

Contributor: Zoe

Delivering on the promise of protein degraders

Matthew N. O'Brien Laramy*, Suman Luthra*, Matthew F. Brown* & Derek W. Bartlett*
Nat. Rev. Drug Discov, **2023**



This detailed review outlines the current workflow and considerations in the design and delivery of protein degrader drugs. The authors emphasise how most clinical candidates are designed for the more preferred oral administration route and an up-to-date table of current PROTACS in clinical trials is provided. The 4 stages of a PROTAC drug discovery project are detailed and 3 categories of degraders that sit on a drug design to drug delivery spectrum is proposed. The first category, just beyond Ro5 (Lipinski's Rule of 5) are PROTACS with smaller molecular weights (MW) that could have sufficient solubility and permeability for oral administration. Category 2: beyond Ro5 are PRTOACS with a broader range of POI and E3 ligands that are potentially bigger in MW and are unlikely to have sufficient intestinal permeability or achieve oral bioavailability. Category 3: far beyond Ro5 are peptide, protein or oligonucleotide-based binders that require drug delivery approaches to enable absorption, distribution and cellular entry to engage the POI. The authors then go on to discuss current approaches in the enhancement of permeability solubility in oral formulations and detail the advantages and disadvantages of parenteral delivery of category 2 and 3 degraders, stressing the importance of PK-PD modelling in decision making. The take home message is that the full scope of targets amenable to TPD cannot be accessed through oral administration alone and design constraints such as focusing on oral delivery could exclude possible degrader design space.

Structural Biology/Biophysics

Contributor: Zoe

Breaking free from the crystal lattice: Structural biology in solution to study protein degraders

Kevin Haubrich[§], Valentina Spiteri[§], William Farnaby, Frank Sobott & Alessio Ciulli*

Curr. Opin. Struct. Biol., **2023**, 79, 102534

This compact review nicely summarises the current in solution structural biology techniques used in relation to the TPD field. The advantages and disadvantages of NMR, structural MS, SAXS and cryoEM are explored with specific examples of how these techniques are used to study protein degraders. X-ray crystallography is highly regarded in the TPD field to visualise ternary complexes and design better degraders, it is also the most accessible structural biology technique. The authors stress the importance of in solution structural biology for dynamic systems or situations where X-ray crystallography is unsuccessful. An important point raised is that for a non-expert user these in solution structural biology techniques need to be more accessible, have increased throughput and be easier to visualise and interpret results. Implementing this will lead to more impact from these techniques like what is currently available with X-ray crystallography.

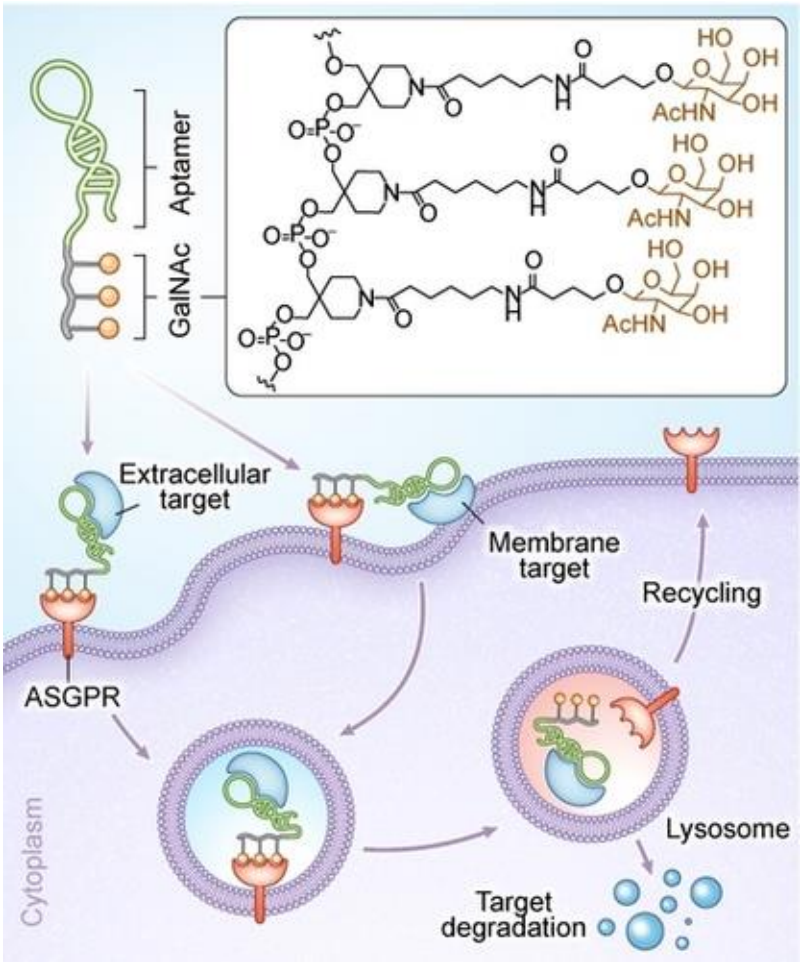
Cell Biology | Structural Biology/Biophysics | Chemistry

Contributor: Conner

Aptamer-LYTACs for Targeted Degradation of Extracellular and Membrane Proteins

Wu Y§, Lin B, Lu Y, Li L, Deng K, Zhang S, Zhang H, Yang C, and Zhu Z*

Angew. Chem. Int. Ed., 2023 DOI: 10.1002/anie.202218106



Wu and colleagues present the first example of aptamer-based LYTACs capable of inducing the degradation of extracellular and membrane proteins PDGF and PTK7 through the lysosome degradation pathway. Many disease-causing proteins lack accessible binding sites precluding their targeting via the traditional inhibitor approach. PROTACs can bridge this gap, but PROTACS cannot target extracellular and membrane proteins. LYTACS are a novel strategy to target these classes of proteins as they can recruit proteins to the lysosome degradation pathway through cell-surface lysosome-shuttling receptor proteins. However, the primary disadvantage of this strategy is that current LYTAC approaches to date require N-acetyl galactosamine conjugated antibodies, which have a considerable molecular weight, high immunogenicity, and are expensive. Wu and colleagues have circumvented this by developing the first in a series of aptamer-based LYTACs. Aptamers are single-stranded small DNA or RNA with complex three-dimensional structures which exhibit high binding affinity and specificity for their targets; compared to antibodies, aptamers can be rapidly synthesised, exhibit low immunogenicity and have a lower molecular weight. First, Wu and colleagues developed a PDGF targeting GalNAc-AptPDGF-PDGF that demonstrated PDGF degradation in HepG2 cells. Following this, they created a PTK7 targeting apt-LYTAC, GalNAc-AptPTK7; upon a 24 h incubation of GalNAc-AptPTK7 with HepG2 cells, they were able to show a 50% reduction in surface expression levels of PTK7. This paper stands out because it shows the progression of the LYTAC field towards a more accessible design. While Wu and colleagues could not reach complete degradation using their approach, this work is reminiscent of early work on PROTACs. With this paper's help, it could lead to an expansion of the LYTAC field for the rapid and efficient degradation of extracellular and membrane-bound proteins.

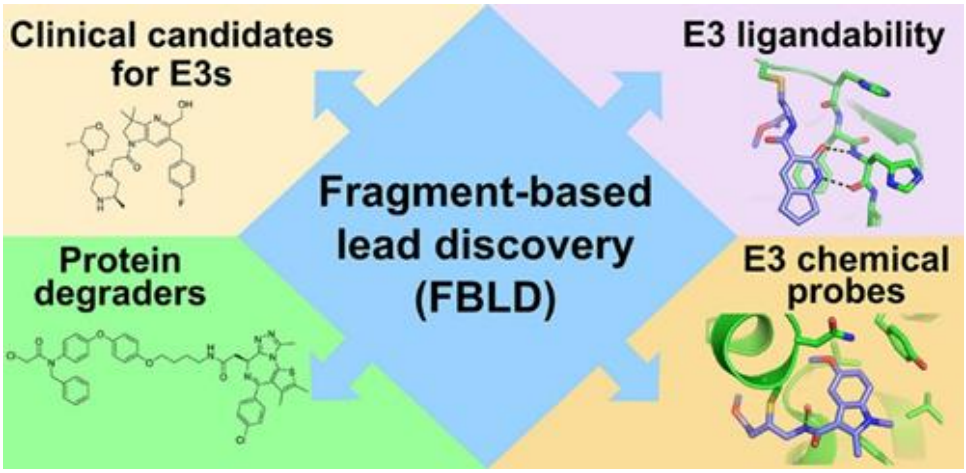
Cell Biology | Structural Biology/Biophysics | Chemistry

Contributor: Andreas Holmqvist

E3 Ligases Meet Their Match: Fragment-Based Approaches to Discover New E3 Ligands and to Unraval E3 Biology

Iacovos N. Michaelides§ and Gavin W. Collie§

J. Med. Chem. 2023



When it comes to target protein degradation, ubiquitination is the key post-translational protein modification for successful target degradation. However, the human genome is estimated to be equipped with 600 – 700 different E3-ligases, representing ~5% of the human genome. And when it comes to TPDs, only a handful of these ligases are utilized for successful degradation. In this paper, the authors have compiled some very interesting Fragment Based Lead Discovery (FBLD) methods that has been used by different groups to identify new E3-ligandability, E3 chemical probes, TPDs and clinical candidates for some E3-ligases. They highlight what type of libraries that are most commonly used for these types of screenings which consists of small molecules, also known as fragments, that are within the rule-of-three chemical space. The fragments that are identified as binders could then be built on to further increase the selectivity towards the desired target. The authors also provide several examples on where different FBLD methods have been used to identify both new E3-ligands, as well as identifying E3-biology. One example the author highlights is the covalent fragment screen carried out by Kathman and co-workers, who managed to identify a NEDD4-1 by screening an electrophilic fragment library, identifying two different fragments. This then led to the development of ligand which could be used to help investigate and understand the mechanism by which NEDD4-1 catalyses the formation of polyubiquitin chains, displaying the importance of fragment-based methods for unveiling E3 ligase biology. This paper is of great importance and may prove to be a great source if the reader wants to learn more about different FBLD methods that has been used since the authors have managed to compile a quite the impressive paper.

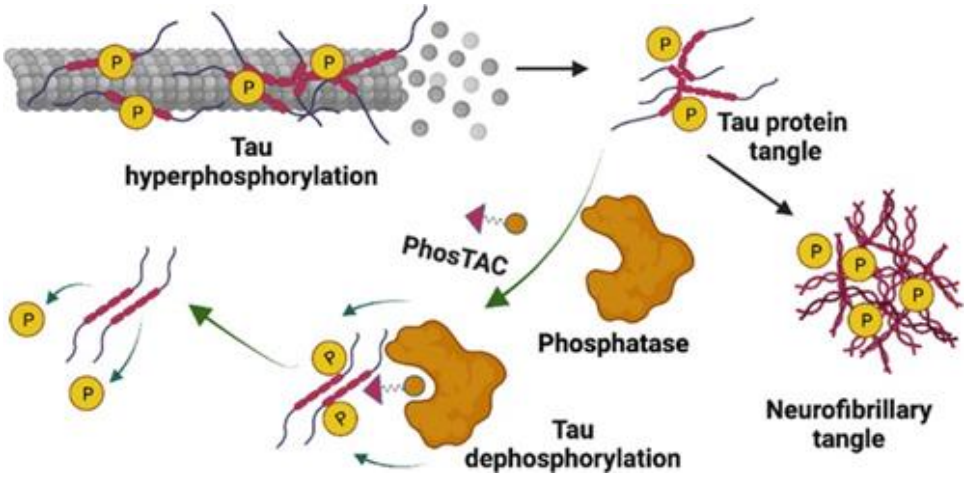
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Andreas Holmqvist

Targeted Dephosphorylation of Tau by Phosphorylation Targeting Chimeras (PhosTACs) as a Therapeutic Modality

Zhenyi Hu [§], Po-Han Chen [§], Wenxue Li, Todd Douglas, John Hines, Yansheng Liu, and Craig M. Crews*

Nat. Rev. Drug Discov, **2023**



Neurofibrillary tangles, which are abnormal accumulated proteins made up of hyperphosphorylated tau proteins within neurons are a pathological hallmark of Alzheimer's and other tau related diseases. In this article, Chen et al. utilise the bifunctional molecule approach to investigate whether it is possible to induce dephosphorylation of tau proteins as a potential treatment of Alzheimer's disease. Their lab has previously demonstrated the pharmacological potentials of phosphorylation targeting chimeras (PhosTAC), where they proved that they could successfully dephosphorylate both PDCD4 and FOXO3a by the use of these types of bifunctional molecules. In this article, they took advantage of the PP2A phosphatase, which has been reported to be responsible for roughly 70% of tau dephosphorylation. What they did first was to investigate the ternary complex formation between tau and PP2A by using a halo-tagged tau system together with a FKBP12^{F36V}-PP2A fusion protein, allowing recruitment using the

FKBP ligand PhosTAC7. To validate the ternary-complex they used of a pull-down assay, where they successfully managed to demonstrate ternary complex formation between tau and PP2A. Once proof of ternary-complex was established, they investigated whether the ternary complex formation could modulate tau phosphorylation levels by treating cells with both a PhosTAC7 and a PhosTAC7-fluorine as a negative control. Here they observed that PhosTAC7 could induce target dephosphorylation of tau via the ternary complex formation and the PP2A phosphatase activity. This study is built upon the framework of PROTAC technology and the potential in targeting PP2A to dephosphorylate tau, expanding the toolbox in both the use of bifunctional molecules to target highly complex diseases, as well as providing new insight into the potential in PhosTACs future.

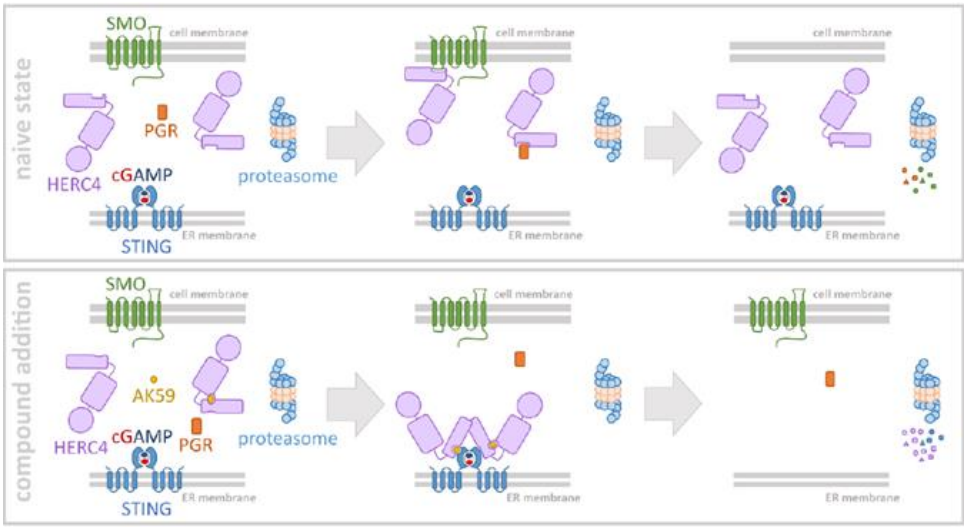
Cell Biology | Chemistry | Structural Biology/Biophysics

Contributor: Andreas

A novel HERC4-deoendent glue degrader targeting STING

Merve Mutlu, Isabel Schmidt, Andrew Morrison, Benedikt Goretzki, Felix Freuler, Damien Begue, Nicolas Pythoud, Erik Ahrne, Sandra Kapps, Susan Roest, Debora Bonenfant, Delphine Jeanpierre, Thi-Thanh-Thao Tran, Rob Maher, Shaojian An, Amandine Rietsch, Florian Nigsch, Andreas Hofmann, John Reece-Hoyes, Christian N. Parker*, Danilo Guerini*

BioRxiv, **2023**, DOI: https://doi.org/2023.02.08.527642



Stimulator interferon gene (STING) is a highly important transmembrane protein that is connected to the innate immune system and the response to aberrant intracellular DNA. In its native state, the STING protein is located in the endoplasmic reticulum (ER) transmembrane, which dimerizes in the presence of cGAMP, translocating STING from the ER membrane triggering interferon signalling. In this article, Mutlu et al., have successfully identified a novel compound that exhibits molecular glues properties, triggering proteasomal degradation of STING protein. Here, the authors used a portion of the Novartis compound collection, which was screened to identify compounds that inhibits the type 1 interferon response, identifying AK59-51TB (AK59) as a potential compound of interest due to its significant STING inhibition. Thereafter, the authors carried out a wester-blot analysis of THP1 cells treated with AK59, where they noticed a drastic decrease of STING protein concentration, while compounds with similar structure and properties of AK59 did not cause the same effect. To demonstrate what caused the decrease of STING concentration upon treatment of AK59, Mutlu et al. added bortezomib, a proteasome inhibitor, prior of the treatment of AK59. Here, they noticed that the downregulation of STING concentration was stopped, indicating that AK59 was triggering proteasomal degradation of STING. To further validate this, they carried out a pull-down assay which showed STING-ubiquitination upon AK59 treatment, leading to the theory that AK59 might act as a molecular glue towards STING. Furthermore, do understand what caused this ubiquitination of STING and which E3-ligase was involved, Mutlu et al. carried out a pooled, genome-wide CRISPR/Cas-9 knockout screen, with the aim to identify genes that was connected to the reduced levels of STING protein upon compound treatment. From this, they identified that loss of HERC4 inhibited the effect of AK59 on STING concentration. For AK59 to act as a molecular glue, the physical interactions between HERC4 and STING had to be investigated and proved. In pursuance of HERC4 and STING interactions, Mutlu et al. investigated compound-dependent PPI interactions between HERC4 and STING protein by another pull-down assays where they noticed that only in presence of AK59 was STING pulled down together with HERC4. To support the pull-down data, they developed a NanoBiT complementation assay (Promega), which showed that LgBiT and SmBiT interaction only occurred in presence of AK59, further strengthening the hypothesis of AK59

acting as a molecular glue between HERC4 and STING. All the experiments carried out by Mutlu et al. where consistent with the activity of AK59 being a novel STING molecular glue degrader. This article highlights a beautiful execution of a step-by-step approach to identify, validate and determine the mechanism of action of a novel STING degrader, which the reader can use as a guideline on how to work to on similar projects to identify other novel molecular glue degraders.

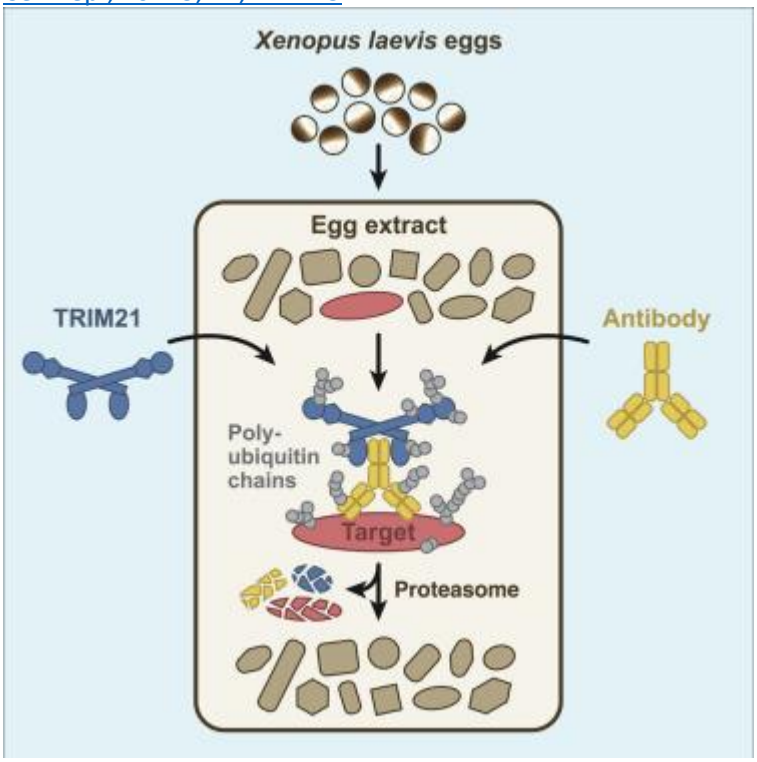
Cell Biology | Structural Biology/Biophysics

Contributor: Conner

TRIM21-dependent target protein ubiquitination mediates cell-free Trim-Away

Tycho E T Mevissen[§], Anisa V Prasad[§], Johannes C Walter*

Cell Rep., 20223, 42, 112125



Tripartite motif-containing protein 21 (TRIM21) is an intracellular immunoglobulin receptor and E3 ubiquitin ligase that functions in the innate immune response. When non-enveloped viruses enter a cell, they bring capsid-bound serum antibodies. TRIM21 binds to these antibodies, activating its E3 ubiquitin ligase activity, resulting in the degradation of the virus. TRIM21 has been repurposed to allow antibody-mediated degradation of endogenous proteins, a method called Trim-Away. In Trim-Away, antibodies directed against a protein of interest are delivered into cells via micro-injection or electroporation. Cellular extracts are robust systems to study complex biochemical processes that have not been reconstituted with purified components. *Xenopus laevis* egg extracts are exceptionally versatile, leading to significant advances in our understanding of many cellular processes, including DNA replication, DNA repair, mitosis, cell-cycle progression, organelle assembly, apoptosis, and cytoskeletal dynamics. In this paper, Mevissen et al. demonstrate rapid and efficient Trim-Away in cell-free extracts derived from Xenopus eggs.

In contrast to cell-based systems, where the delivery of Trim-Away components can be technically challenging, extract systems are highly tractable. Upon addition of recombinant human TRIM21, Mevissen et al. observed complete degradation of multiple proteins in less than 12 min. Cell-free trim-away contrasts with immunodepletion, which takes hours and requires 50-fold more antibodies than cell-free Trim-Away. Due to the tractability of cell-free systems, degradation can be observed with a high temporal resolution, which enabled detailed characterization of antibody-mediated target degradation by TRIM21. Interestingly, Mevissen et al. demonstrated that p97 - an E3 ligase - inhibition delayed target proteolysis suggesting that p97 may play a general role in Trim-Away, that had not been seen previously.

In this paper, Mevissen et al. clearly and precisely demonstrates a novel application of the somewhat limited Trim-away degron technology to improve the ability to degrade proteins in a highly tractable in-vitro system, thereby not only advancing upon the span of possible utilities of the Trim-away technology but also providing researchers with a more rapid and reliable approach to protein degradation in the *Xenopus leavis* cell-free system.

Other Paper Highlights

Chemistry

Contributor: Jeff

Organogel delivery vehicles for the stabilization of organolithium reagents

Petr Slavík[§], Benjamin R. Trowse, Peter O'Brien* & David K. Smith*
Nat. Chem. **2023**, DOI: 10.1038/s41557-023-01136-x

Organolithium reagents are essential tools in modern organic synthesis, which allows the formation of various C-C bonds. However, these reagents are often unstable and pyrophoric, therefore inert atmosphere and anhydrous solvents are necessary for their use. The shelf live of organolithiums are very limited because they are commonly storage as solutions. The overcome this issue, the authors reported a novel method to suspend organolithiums into low-molecular-weight organogelators (LMWGs), this allows the stabilisation of these reagents by encapsulating them into the gel network.

Remarkably, these organolithium gels can be handled under air for significant amount of time without any degradation. They can also be divided up into different pieces for use in multiple reactions. The authors have tested the gelation system for various commercially available organolithium and -magnesium reagents (n-BuLi, s-BuLi, PhLi, vinylMgBr and PhMgBr) and even activated organolithiums such as n-BuLi/TMEDA. They have also successfully demonstrated the synthetic utility of these gels by employing them into nucleophilic addition, Li/Br exchange and deprotonative lithiation-trapping.

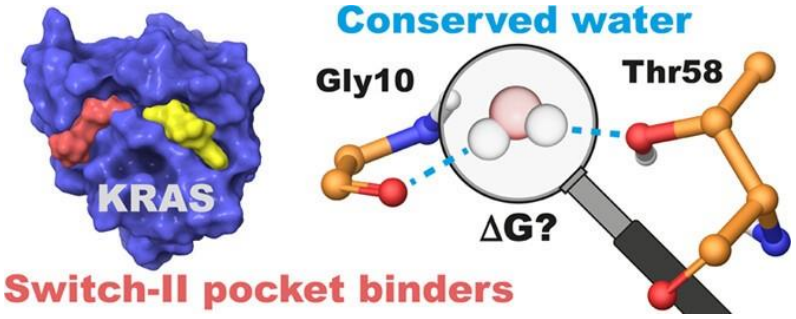
I believe the discovery of organometallic gels is ground-breaking and could change how we organic chemist use these organometallic reagents in the future. I also anticipate these gels will be on the commercial market soon and I personally cannot wait to get my hands on them!

Computational Chemistry | Modelling/Simulation

Contributor: Sohini

In Silico Evaluation of the Thr58-Associated Conserved Water with KRAS Switch-II Pocket Binders

Renne Leini[§], …, Corresponding Author(s)*
J. Chem. Inf. Model **2023**, DOI: 10.1021/acs.jcim.2c01479



Mutated KRAS is one of the key targets in anti-cancer drug discovery. Available data suggests targeting switch-II pocket (SII-P) is the most promising approach to inhibits this oncoprotein. In this study, the authors analysed the thermodynamic profile of a conserved water that forms H-bonds with Gly10 on β1 sheet and Thr58 on switch-II in SII-P of several KRAS structures. They used the WaterMap technology and further performed microsecond timescale MD simulations. Their investigation reveals that the conserved water and its neighbouring sites are high energy sites which if displaced, could be expected to boost the ligand's potency. None of the known noncovalent binders displaces this conserved water. However, the most potent Mirati KRAS (G12D) binder is noted to displace the adjacent high-energy water, and a similar trend is observed with G12C inhibitors. Therefore,

it could be beneficial to displace at least the high-energy hydration sites adjacent to the conserved water. Further, shielding of the conserved water (if it is not displaced) could be helpful for efficient SII-P binding.

The authors have rightly emphasized, targeting high-energy hydration sites is one of the many possibilities to achieve better potency and it is not a trivial task. Nonetheless, in my opinion, this study is an important guideline for KRAS drug hunters to consider targeting SII-P water molecules and explore the possibilities of improving ligand potency.

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