Ciulli Lab

Targeted Protein Degradation

Targeted protein degradation, medicinal chemistry & chemical structural biology literature highlights

Journal Club

February 2022

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): Angus Cowan, Claire Whitworth and Yuting Cao

"The almost exponential growth of TPD literature makes it difficult to stay on top of everything, but the Journal Club has been extremely useful in keeping up to date with the latest developments"

Angus completed his PhD on the structural biology of pro-apoptotic Bcl-2 family proteins at WEHI in Melbourne, Australia. After a 2 year postdoc working as part of multidisciplinary drug development team, he moved to Dundee to join the Ciulli Lab. Angus is a Marie Skłodowska-Curie Postdoctoral Fellow focusing on structural biology of E3 ligase substrate receptors.

"The Journal Club is a great resource to keep up to date with the ever-evolving TPD field and apply the latest advancements in TPD research to our drug discovery approach."

Claire is a senior drug discovery scientist and cell biologist in the AC-BI PROTAC collaboration. She joined the group in November 2016 and led the establishment of the AC-BI bio labs and cellular profiling in Dundee. Prior to joining the group, she trained as a cell and molecular bioscientist at Newcastle University for 9 years, with a particular focus on studying the biological mechanisms underpinning oncology.

"Journal Club provides us with an opportunity which enables us to follow the TPD field efficiently and quickly."

Yuting completed her master's degree in medicinal chemistry at Nankai University. Her master's project consisted of synthesis of novel PROTACs that could be used to degrade CDK2/4/6. Then, she moved to the Artemisinin Research Centre where she worked as a research assistant. Since October 2021, she joined the Ciulli lab as a PhD student on the China Scholarship Council (CSC) Programme.

Charles and Greta Keller visit the CeTPD

Contributor: Valentina

This last month we were fortunate to welcome Dr Charles Keller and his daughter Greta Keller for a visit all the way from the Children's Cancer Therapy Development Institute (cc-TDI), Oregon, US. Charles' work and centre have the noble focus of trying to make childhood cancers universally survivable. During his visit Charles highlighted this motivation in an incredibly poignant way when speaking of Shane, a little boy, who passed away at 19 months from alveolar rhabdomyosarcoma, a rare cancer that impacts muscle and predominantly affects children. Charles' purpose is clear, he starts his seminar by telling his audience that there are twelve drugs approved to treat adult cancers annually but there have only been twelve drugs approved for the specific treatment of childhood cancer in the last 44 years. He wants to change this, and he has set out to do this with his cc-TDI, a non-profit biotech.

One of the cc-TDI's objectives is to de-risk proof-of-concept pre-clinical candidates, with extensive and cutting edge *in vitro* and *in vivo* studies to make the therapies attractive candidates for pharmaceutical companies to take on to clinical trials. In the cc-TDI's first 49 months the team was able to push two drugs into clinical trials, entinostat and panobinostat. The team at the cc-TDI have their hands full with an ambitious pipeline and partnerships with several industrial leaders.

During Charles' visit it also became obvious that the cc-TDI is not just a place for fantastic science but also a very cool place to work. First and foremost, due to its team culture, it was clear from interacting with Charles that fostering a happy and curious team was a matter close to his heart. Moreover, the cc-TDI comes complete with a well-stocked bar, DJ booth and a cute corgi running around. No doubt this sets a precedent for our own CeTPD (hint, hint Alessio).

Charles has collaborated with Alessio over the last couple of years and has recently published a study that utilised ACBI1 (a degrader developed as part of the Dundee-Boehringer Ingelheim collaboration) as a probe to demonstrate that SMARCA4 is implicated in alveolar rhabdomyosarcoma (covered in this issue). Therefore, it was very fitting that Charles was able to visit us, only a few days after this work was published, to share the science with all of us at Dundee. Indeed, impact in science can sometimes be intangible so it was very special to have someone whose science has been directly impacted by your lab's work come all the way across an ocean to tell you the story.

Charles' visit is also a special one for us as it is the first in our CeTPD seminar series. Over the past few months, we have been quietly cooking up ideas for a CeTPD seminar series that was a little different from the norm. We wanted a seminar series that was community-led, with members of the centre nominating speakers, and that went beyond a talk but rather a day long visit that would allow members of the Centre to really interact with our invitee. We are thrilled that Charles fully embraced our action-packed itinerary including a lunch with the centre's PhD students, coffee with the post docs and several rounds of what was described as "science speed dating" (short one-on-one meetings with several School of Life Sciences faculty members). We wanted everyone to have the chance to form connections with our invited speaker. What was apparent in these more informal meetings was Charles' interest in our young researchers, asking questions and offering encouragement. He was clearly thrilled to be meeting everyone and the feeling was mutual, everyone in the centre left the day feeling happy to have had met Charles and Greta.

Considering Greta's patience of coming to visit us at Dundee on her holidays, we were keen that she also had the opportunity to take something from the visit, so we planned for Greta to spend time in the lab with three of our scientists, Sarah, Selma, and Alena. Greta interviewed Sarah about her perspective on being a woman in STEM. Greta kindly agreed to write a short piece on her experience in Dundee on the next page.

Greta's Perspective

Contributor: Greta Elizabeth Keller, age 17, High School Senior, Portland, Oregon USA

I had the incredible opportunity to be a Scottish molecular biologist for the day at the Centre for Targeted Protein Degradation. My dad was the first speaker to start a lecture series hosted by the University of Dundee and Prof. Alessio Ciulli and I was lucky enough to tag along and spend time with three amazing young women in their lab. My day started with Sarah Chandler. Sarah is a second year PhD student researching molecular chemistry; specifically, she studies a new form of drug called a PROTAC (protein targeted degraders). With only three years of high school sciences under my belt, I understand PROTACs as the drug-like bridge structure that connects two proteins, one of which is intended to be destroyed in order to relieve suffering of a disease. Sarah's research is so important because PROTACs address the end product of genetic mutations that cause disease, like cancer. After finishing a small experiment in the lab, we hung up our lab coats and sat down for a cup of tea. I hope to pursue a career in medicine, and so I was eager to ask Sarah about her experience being a woman in STEM. Inspiration is what drives us to do what we do — and do it well. The cc-TDI inspiration is rooted in giving back childhood to kids that might not otherwise survive cancer. I began here with Sarah. Sarah dedicated her inspiration to her biology teacher who pushed her to pursue molecular biology. Sarah said she was slightly intimidated by the field (as anyone might be at the beginning), but it really appealed to her. Sarah also gave credit to her mother for being a role model. She was a nutritionist. Her mother actually pushed her to pursue *art* along with science.

Walking through the lab with Sarah I noticed an abundance of women. When I asked Sarah about her peers and mentors, she noted that there were majority female PhD students which was opposite of the number of women who held higher leadership roles. Sarah noted the lack of women as lab leaders because of maternity leave setback, impostor syndrome among female researchers and lack of confidence in academic settings. All of these things seemed hard to discuss, and do not always apply to every young woman scientist - but can be factors. The best advice Sarah gave me to counter feelings of inadequacy as a woman in science was to not compare my achievements with others. To know that you had earned your place and deserved to be there, you were accepted for a reason and have more to offer than you think.

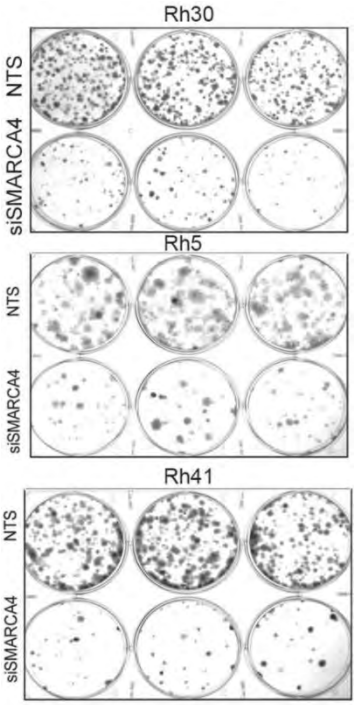
Cell Biology

Contributor: Valentina

SMARCA4 biology in alveolar rhabdomyosarcoma

Narendra Bharathy [§], Megan M. Cleary[§], …, Alessio Ciulli*, Charles Keller*

Oncogene **2022**, DOI: 10.1038/s41388-022-02205-0



Rhabdomyosarcoma (RMS) makes up 5-10% of all paediatric malignancies, making it the most common soft tissue sarcoma in children. The alveolar subtype (ARMS) has the poorest prognosis with only an 8% five-year progression-free survival for metastatic ARMS. Moreover, there is a great unmet need for novel therapies. This study delineates the role of SMARCA4 in ARMS using several *in vitro* and *in vivo* techniques.

Utilising CRISPR screening of 192 epigenetic targets, SMARCA4 was identified as essential in ARMS cell lines and this was validated in SMARCA4 depletion studies that demonstrated impaired cell survival. Thirty-nine archival ARMS tumour samples were analysed using tissue microarray for SMARCA4 expression levels, which were found to have positive, strong uniform expression. SiRNA knockdown of SMARCA4 in human PAX3:FOXO1 + ARMS cell lines showed that in the short-term SMARCA4 did not play a central role. However, the long-term depletion of SMARCA4 impaired anchorage-independent colony formation at 21 days by 30-70%. This mirrored *in vivo* murine orthotopic allograft models, where SMARCA4 knockdown delayed tumour growth. To translate these findings to the clinic, the team used ACBI-1, a dual SMARCA4/SMARCA2 degrader. Cell viability was not impaired 3 days after treatment with ACBI-1 but became increasingly impaired after 8-11 days of treatment. In *in vivo* models ACBI-1 delayed tumour formation and growth.

Identifying SMARCA4 as a key driver of ARMS presents researchers with new therapeutic opportunities for treating ARMS. Moreover, the robust and integrative approach applied here reinforces the application potential for using degraders as probes.

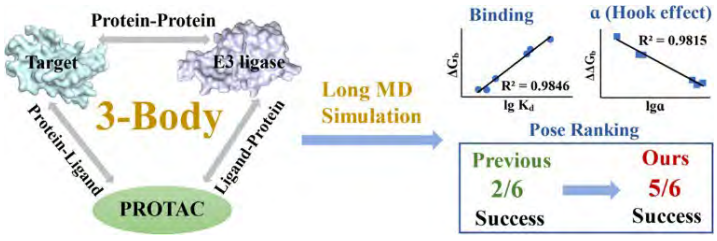
Computational Chemistry | Structural Biology/Biophysics

Contributor: Angus

Importance of Three-Body Problems and Protein–Protein Interactions in Proteolysis-Targeting Chimera Modeling: Insights from Molecular Dynamics Simulations

Wenqing Li[§], Jiabin Zhang, Li Guo, Qiantao Wang*

J. Chem. Inf. Model **2022**, *62*, 523



Computational studies of ternary complex formation between target protein, PROTAC and E3 ligase are building iterative momentum, but the complexity of predicting interactions between 3 components remains a challenge. Of particular interest is the prediction of the cooperativity factor, α, of a given ternary complex.

Using a set of VHL-recruiting, BRD4-degrading PROTACs where the cooperativity has been measured experimentally and for which some ternary crystal structures have been solved, Li and colleagues use molecular dynamics simulations and molecular mechanics generalized Born surface area (MM/GBSA) calculations to predict and rank ternary complex poses and to predict cooperativity. They find good correlation between experimentally determined and predicted α values, and improved pose ranking in comparison to docking-based prediction in PRosettaC.

Prediction of cooperativity remains a key challenge in TPD and a reliable computational method for cooperativity prediction would be of immense benefit to the field. This paper makes a good start towards this goal, however, as the authors acknowledge, the scope and impact of computational studies is currently limited by the scarcity of ternary complex structures and experimentally determined α values in the literature.

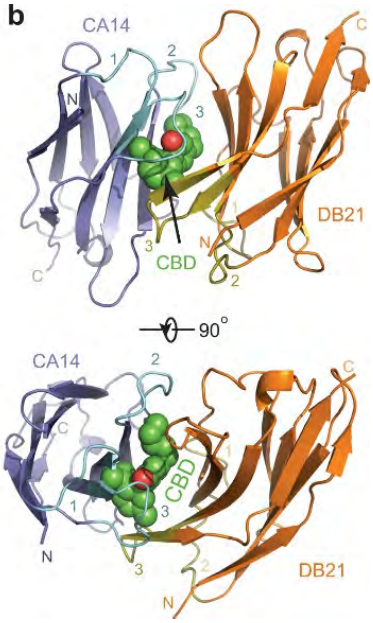
Structural Biology/Biophysics

Contributor: Angus

Defining molecular glues with a dual-nanobody cannabidiol sensor

Shiyun Cao[§], Shoukai Kang, Haibin Mao, Jiayu Yao, Liangcai Gu & Ning Zheng*

Nat. Commun. **2022**, *13*, article 815



The description of the plant hormone auxin as a molecular glue between the E3 ligase TIR1 and its substrate IAA7 is one of the field defining moments in TPD. Here, the Zheng lab continue their structural and biophysical characterisation of molecular glues with a *de novo* engineered cannabidiol sensing system where cannabidiol acts as a molecular glue between two nanobodies. They measure cooperativity of binding in this system, a feature of three-body binding systems that in the context of TPD has primarily been investigated in PROTACs alone, and expand the scope of the study to look at cooperativity in two other molecular glue systems, auxin and immunomodulatory drugs (IMiDs).

Interestingly, they find intrinsic affinity of the two protein binding partners in the absence of the molecular glue in all cases. These "non-specific" interactions are enhanced by the glue leading to a functional outcome of ubiquitination that does not occur in the absence of the glue compound. They also provide a mathematical model for molecular glue systems. This is an important paper in furthering our conceptual understanding of molecular glues in TPD and in wider biology.

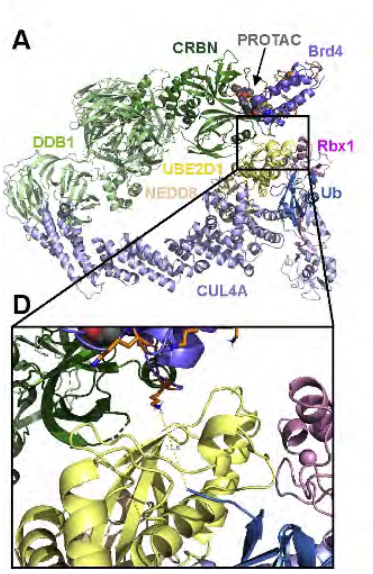
Cell Biology | Computational Chemistry | Structural Biology/Biophysics

Contributor: Angus

Modeling the CRL4A ligase complex to predict target protein ubiquitination induced by cereblon-recruiting PROTACs

Nan Bai[§]*, Kristin M. Riching[§]*, …, Sara C. Humphreys*

J. Biol. Chem. **2022**, DOI: 10.1016/j.jbc.2022.101653



Formation of a ternary complex between E3 ligase, PROTAC and neosubstrate is necessary, but not sufficient, to induce neosubstrate ubiquitination and degradation. An important factor that has been under-researched is the requirement of a lysine residue on the neosubstrate that is structurally accessible for ubiquitination. The authors of this paper address this lack of information by creating a computational workflow to generate ensembles of ternary complex models consisting of the $CRL4A^{CRBN}$ E3 ligase, a PROTAC or molecular glue and their corresponding neosubstrate. They validate the ensembles generated from the workflow using 3 reported crystal structures of CRBN ternary complexes and then apply the workflow to prediction of productive complexes for ubiquitination with a set of cyclin-dependent kinases (CDK) and a previously reported pan-kinase PROTAC. Finally, they attempt to predict which specific lysine residues are ubiquitinated on these CDKs based on proximity to ubiquitin in the ensembles.

This paper addresses a long-neglected aspect of TPD in the key event of neosubstrate ubiquitination that occurs after to the more heavily studied event of ternary complex formation. The workflow will be very useful in PROTAC design, and I look forward to seeing its practical application in other studies.

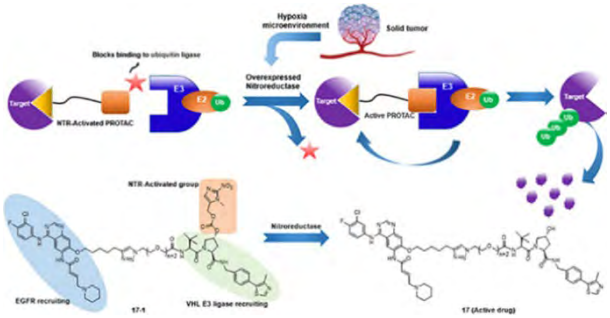
Cell Biology | Chemistry

Contributor: Angus

Rational Design for Nitroreductase (NTR)-Responsive Proteolysis Targeting Chimeras (PROTACs) Selectively Targeting Tumor Tissues

Shi Shi[§], Yu Du[§], …, Yungen Xu*, Qihua Zhu*

J. Med. Chem. **2022**, DOI: 10.1021/acs.jmedchem.1c02221



Tissue specificity is a highly desirable trait in drug discovery that can reduce undesirable on- and off-target effects of a given therapeutic. Here, the authors take advantage of the elevated levels of nitroreductase (NTR) found in hypoxic solid tumours compared to most normal tissue to design a prodrug PROTAC. The PROTAC is activated once a caging nitroimidazole group is removed from the VHL-binding moiety of the PROTAC by NTR, releasing the PROTAC to degrade EGFR in hypoxic cells and tumours.

Along with another "pro-PROTAC" reported this month (DOI: 10.31635/ccschem.022.202101529), this study is an interesting example of how to exploit differences in diseased and normal tissue to ensure PROTAC molecules work only where they are required rather than systemically.

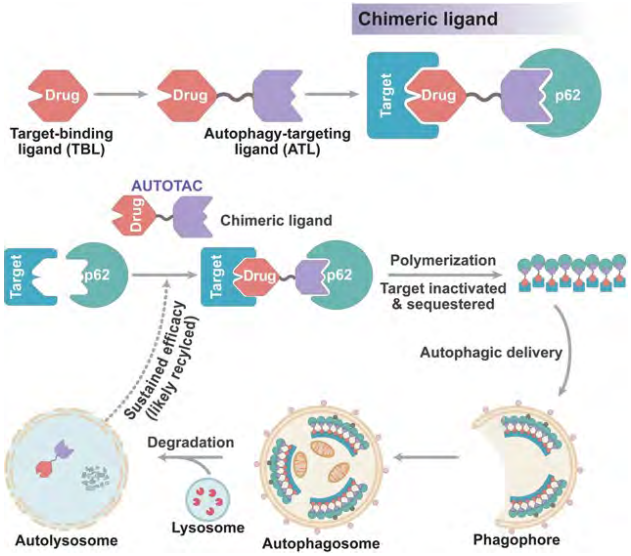
Cell Biology | Chemistry | Computational Chemistry

Contributor: Yuting

The AUTOTAC chemical biology platform for targeted protein degradation via the autophagy-lysosome system

Chang Hoon Ji[§], Hee Yeon Kim[§], Min Ju Lee[§], …, Yun Kyung Kim*, Bo Yeon Kim*& Yong Tae Kwon*

Nat. Commun. **2022**, *13*, 904



In this paper, the authors developed a novel degrader system called AUTOphagy-TArgeting Chimeras (AUTOTACs). AUTOTACs bind the ZZ domain of the otherwise dormant autophagy receptor p62/Sequestosome-1/SQSTM1, which is activated into oligomeric bodies in complex with targets for their sequestration and degradation. The AUTOTACs can efficiently degrade not only oncoproteins but also oligomeric species of aggregated proteins in which Ub-dependent degraders may not show ideal potency due to the diameter of the proteasome. Furthermore, AUTOTACs can mediate the eradication of tau aggregates from mouse brains. Unlike Ub-dependent degraders that require specific linker lengths and types for ternary complex formation, AUTOTACs may not be critically reliant upon a specific linker length and do not require ubiquitination of the target substrate for its degradation. However, the authors also admit that the pharmacological and mechanistic properties of AUTOTACs are unclear. Besides, the off-target and selectivity issues of the AUTOTAC platform have yet to be fully investigated and should be addressed in follow-up studies.

Although the general efficacy of AUTOTACs has yet to be fully evaluated, their research verified that activating the p62-ZZ domain in an Arg/N-degron-dependent manner for targeted proteolysis provides a complementary approach to the current TPD therapies.

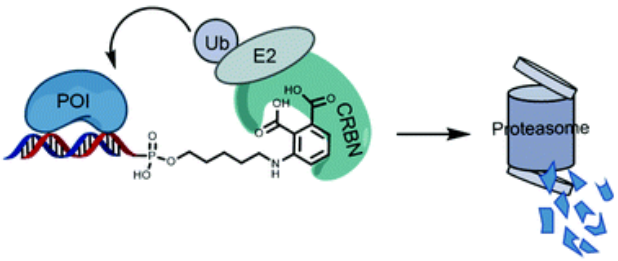
Cell Biology | Chemistry | Computational Chemistry

Contributor: Yuting

3-Aminophthalic acid, a new cereblon ligand for targeted protein degradation by O'PROTAC

Yuqian Yan[§], Jingwei Shao[§], …, Hong-yu Li* and Haojie Huang*

Chem. Commun. **2022**, *58*, 2383



Oligonucleotide-based PROTACs (O'PROTACs) are a class of noncanonical PROTACs in which a TF-recognizing double-stranded oligonucleotide is incorporated as a binding moiety of a POI. Through mass spectrum analysis, the authors found that the reverse strand of pomalidomide-based ERG-degrading O'PROTACs (ERG OPs) was not of the expected mass. Since the molecular weight of pomalidomide-based ERG OPs generated through post-synthesis conjugations were correct, they reasoned that the pomalidomide moiety was hydrolyzed to 3-aminophthalic acid during the deprotection of all protecting groups of the ERG OP-C1 reverse strand. Thus, they hypothesized that phthalic acid can be a new E3 ligase recruiter of O'PROTACs that are effective in proteolytic degradation of a target protein.

To verify their hypothesis, they designed and synthesized a phthalic acid-based ERG O'PROTAC which can significantly degrade the ERG protein and impair the ERG function in cell growth and cell invasion. This new phthalic acid-based ERG O'PROTAC shows activity comparable to the parent pomalidomide-based ERG O'PROTAC. Moreover, phthalic acids are more chemically stable and economical than classical immunomodulatory drugs (IMiDs). Their research provides an alternative new ligand CRBN PROTACs, especially O'PROTACs.

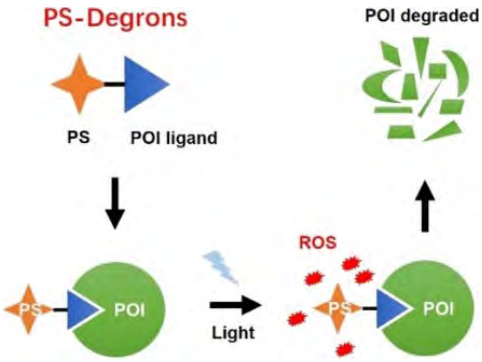
Cell Biology | Chemistry

Contributor: Yuting

Activable Targeted Protein Degradation Platform Based on Light- triggered Singlet Oxygen

Silong Zhang[§]*, Yuanyuan Li[§], …, Juan Xu*, and Huan He*

J. Med. Chem **2022**, *65*, 3632



TPD therapies are emerging as novel and powerful approaches in chemical biology and drug discovery. Despite the tremendous advantages over traditional inhibitors, they also have some challenges, one of them is the risk of off-tissue toxicity when applied systematically. Photodynamic therapy (PDT) is a new technology by using visible or infrared light to activate the photosensitizer which can recruit cytotoxic singlet oxygen ($^1O_2$) then causing the oxidative damage of biomolecules, leading to cell death. PDT has emerged as a selective and non-invasive cancer-therapy modality with high spatiotemporal precision.

To reduce the risk of off-tissue toxicity, the authors described a novel TPD platform composed of photosensitizers and protein ligands (PS-Degrons), which allows controllable knock down of the target protein with high spatiotemporal precision. Their lead compound PSDalpha induces acute ERα degradation in MCF-7 cells with an EC_{50} of 17 nM using visible light and enables an excellent anti-proliferation performance.

Interesting research by the combination of PDT and TPD which offers an opportunity for localizing the protein degradation in desired tissues and cells. PS-degrons may open a chapter for TPD.

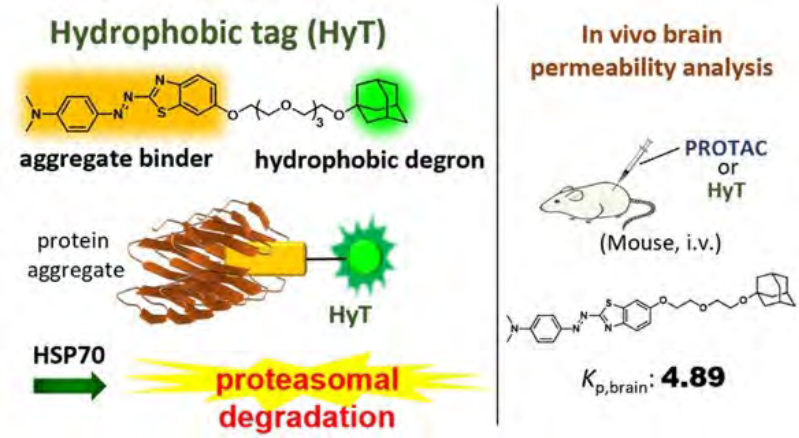
Cell Biology | Chemistry

Contributor: Yuting

Conversion of a PROTAC Mutant Huntingtin Degrader into Small- Molecule Hydrophobic Tags Focusing on Drug-like Properties

Keigo Hirai[§], …, Shusuke Tomoshige*, and Minoru Ishikawa*

ACS Med. Chem. Lett. **2022**, DOI:10.1021/acsmedchemlett.1c00500



Neurodegenerative disorders (NDs), such as Alzheimer's disease, Parkinson's disease, and various polyglutamine (polyQ) diseases, are an array of progressive diseases causing impairments of mobility and/or cognitive functions. The onset of neurodegenerative disorders (NDs) is associated with the accumulation of aggregates of misfolded proteins. Previously, research from this group showed that chemical knock down of ND-related aggregation-prone proteins can be achieved by PROTACs/SNIPERs. However, these PROTACs showed poor permeability into the central nervous system where NDs are located. In this paper, by replacing the E3 ligand moiety with an adamantyl group they successfully converted the PROTACs into hydrophobic tags (HyTs). Different researchers may hold different views about drug-like properties of PROTACs since the ternary complex is of great importance. But their new HyT molecules with a lower molecular weight and a smaller number of HBDs/HBAs show higher brain permeability and comparable degradation potency to parent PROTACs.

Moreover, the compounds developed in this study are the first small-molecule HyTs targeting aggregation-prone protein. The authors also found that the adamantane moiety is a potent hydrophobic degron, providing insight into the relationship between the bulkiness and degradation activity. Further studies of HyTs are underway in their laboratory. Let's look forward to their further research updates.

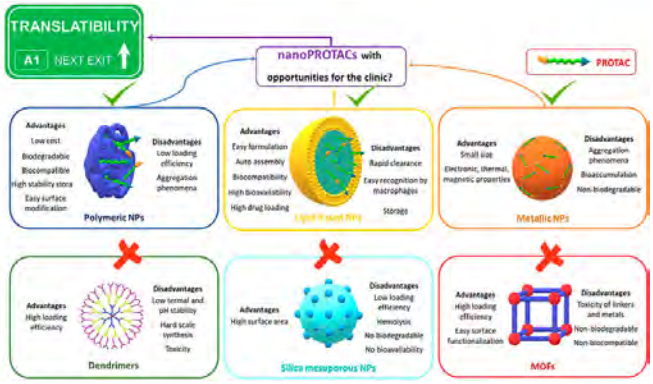
Cell Biology

Contributor: Claire

Options to Improve the Action of PROTACs in Cancer: Development of Controlled Delivery Nanoparticles

Alberto Juan[§], María del Mar Noblejas-López, María Arenas-Moreira, Carlos Alonso-Moreno*, Alberto Ocaña*

Fron. in Cell and Devel. Bio. **2022**, *9*, 805336



Drug delivery systems may be utilised to improve PROTAC physiochemical properties towards more favourable in vivo profiles. Encapsulating PROTACs in nanoparticles (nanoPROTACs) could offer reduced PROTAC metabolism, improved permeability and allow for controlled or guided delivery of high concentrations of PROTAC which could in turn reduce off-target toxicity issues. As the majority of PROTACs in clinical trials are orally administered, applying

recent developments in oral delivery systems to the generation of nanoPROTACs could offer improved absorption and oral bioavailability of these degraders.

An array of nanoparticles of varying properties are now available, allowing for tailoring to compound properties and function, thereby converting PROTACs to nanoPROTACs could be rapidly translated to the clinic. This review captures the details of properties related to the different drug delivery systems available, comparing the pros and cons of each approach. The authors discuss how lipid-based or polymeric nanoparticles offer the most promise for nanoPROTAC development in addition to guided PROTAC delivery, for example via vectorised nanocarriers with surface ligands for target-cell specific receptors. Examples of developed nanoPROTACs are highlighted, including MZ1 PROTAC-loaded trastuzumab-conjugated nanoparticles and PLGA–PEG polymeric nanoparticles of ARV-825, both of which showed promising anticancer activity compared to free compound. The authors do however conclude that optimisation of chemical and manufacturing control procedures for nanomedicines will be key for their future clinical development.

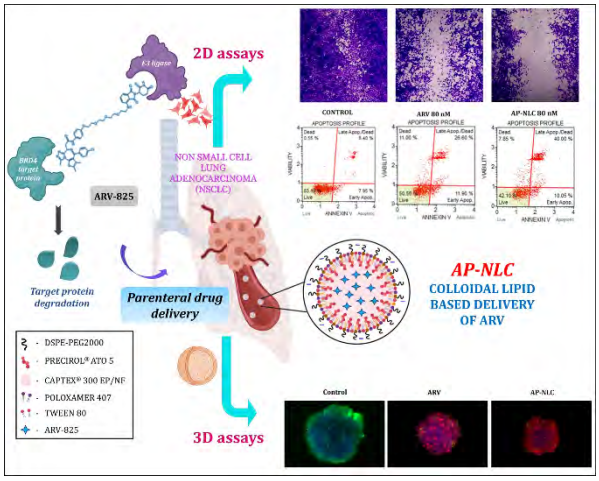
Cell Biology

Contributor: Claire

Susceptibility of Lung Carcinoma Cells to Nanostructured Lipid Carrier of ARV-825, a BRD4 Degrading Proteolysis Targeting Chimera

Richa Vartak[§], Aishwarya Saraswat, Yuqi Yang, Zhe-Sheng Chen, Ketan Patel*

Pharm. Res. **2022**, DOI: 10.1007/s11095-022-03184-3



Formulation development and delivery are key aspects to consider during the development of drug candidates. Advancements in nanotechnology for drug delivery offers a means to improve the in vivo properties of a drug. Lipid based colloidal nanoparticles such as solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) and liposomes offer such favourable properties, including bio-acceptability, biodegradability, sustained-release behaviour and amenability to large scale production, as well as delivery of therapeutic drugs to tumour targets.

ARV-825, a CRBN-based BRD4 PROTAC, is known to be highly lipophilic with poor aqueous solubility. Here, the authors explored the cytotoxic potential of ARV-825 against NSCLC and characterised ARV-825 loaded PEGylated (AP)-NLCs for parenteral delivery. Lipid matrix selection can influence drug loading, stability and release, therefore solid and liquid lipids were evaluated and different stabilizing fatty acids were also assessed. AP-NLCs protected ARV-825 from microsomal degradation, thereby improving half-life without compromising the ability of ARV-825 to degrade BRD4 or affecting its cytotoxicity against A549 and H460 cell lines. In fact, AP-NLCs increased apoptosis and reduced cell migration and colony formation compared to free ARV-825. AP-NLCs also demonstrated an antiproliferative effect on tumour spheroids similar to that of ARV-825.

These results suggest that development of PROTAC loaded NLCs could overcome unfavourable PROTAC physiochemical properties thereby improving PROTAC translation to the clinic.

Cell Biology

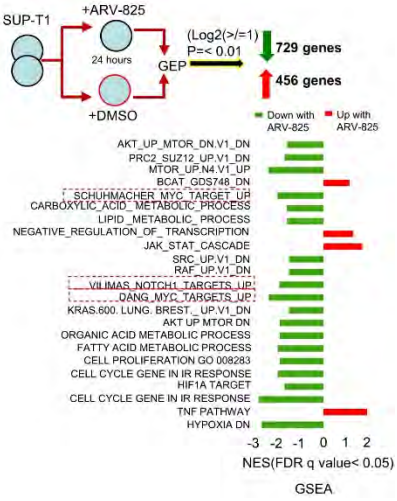
Contributor: Claire

Targeting the NOTCH1-MYC-CD44 axis in leukemia-initiating cells in T-ALL

Sujan Piya[§]*, Yaling Yang[§], …, M. James You*, Gautam Borthakur*

Leukemia 2022, DOI: 10.1038/s41375-022-01516-1

Relapsed T-cell acute lymphoblastic leukemia (T-ALL) is difficult to treat and is believed to be driven by leukaemia-initiating cells (LICs), which are characterised by high CD44 expression and low levels of reactive oxygen species (ROS). Myc, Notch1 and CD44 are known to be associated with LIC persistence in T-ALL. As BRD4 has a role in regulation of Myc and Notch1 oncogene transcription, here BRD4 degradation was explored as a potential therapy for T-ALL.



In addition to downregulation of Myc, Myc target genes and Notch1 target HES1, degradation of BRD4 with ARV-825, a CRBN-based PROTAC, was found to downregulate CD44 expression and trigger a functionally related increase in ROS levels and reduced migration of T-ALL cells. ARV-825 inhibited proliferation and induced apoptosis of Notch1-mutated human and mouse T-ALL cells and demonstrated anti-leukaemic activity associated with improved survival of mouse models with Notch1 activated PDX and PTEN tumour suppressor deleted T-ALL. The authors also use single cell proteomics and secondary transplantations to show that ARV-825 depletes T-ALL LICs by dismantling NOTCH-MYC-CD44 regulatory circuits.

This work highlights that BRD4 degradation has therapeutic potential for T-ALL with clinically relevant mutations.

Cell Biology | Chemistry

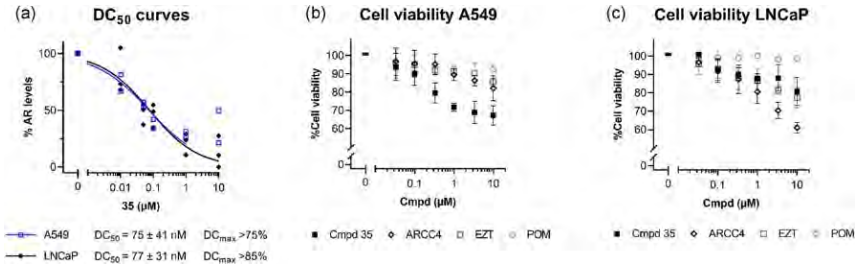
Contributor: Claire

Design, synthesis, and characterization of PROTACs targeting the androgen receptor in prostate and lung cancer models

Lukas M. Gockel[§], Vladlena Pfeifer[§], …, Christian Steinebach*

Arch. Pharm. **2022**, DOI: 10.1002/ardp.202100467

The androgen receptor (AR) gene is a commonly mutated gene in castration-resistant prostate cancer thereby making AR an attractive drug target. Indeed, a variety of AR-targeting PROTACs have been developed and the first PROTACs to enter clinical trials were AR degraders for prostate cancer. The AR also represents an attractive target for other malignancies.



Here, the authors describe the synthesis of novel enzalutamide-based CRBN-recruiting PROTACs with three series of exit vector modifications to degrade AR in lung cancer models. Compounds were profiled for physiochemical properties and AR degradation in the A549 lung cancer cell line. Frontrunner compounds had logD values of ~5 and all exhibited high plasma protein binding of >95%. Further optimisation using the best exit vector and rigidifying the linker yielded a more potent AR degrader in initial screens that was taken forward for further profiling. The lead compound was a potent (~80 nM) degrader of AR in both lung and prostate cancer cell lines though reduced cell viability was observed only in lung cancer cells. PROTAC effect on the protease TMPRSS2 was also investigated since its expression is known to be regulated by AR in prostate cancer and it enables host entry of SARS-CoV-2 in lung tissue. However,

CRBN- and VHL-based AR PROTACs did not alter TMPRSS2 levels in lung cancer cell lines and therefore do not appear to be suitable for application in COVID-19 therapy.

Cell Biology | Chemistry

Contributor: Claire

Proteolysis targeting chimeras in antiviral research

Jenny Desantis[§], Laura Goracci*

Fut. Med. Chem. **2022**, DOI: 10.4155/fmc-2022-0005

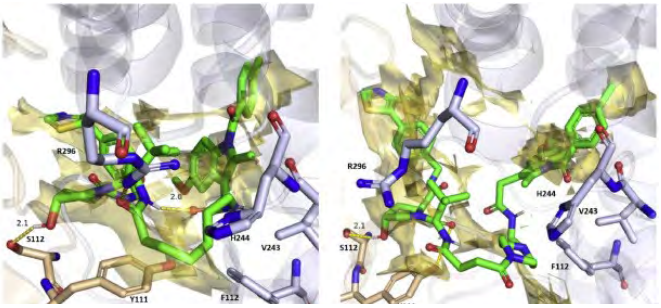


Image from previous publication by authors: Indomethacin-based PROTACs as pan-coronavirus antiviral agents, Eur. J. of Med. Chem. ***2021***

Thus far, the PROTAC field has given substantial focus to developing PROTACs that target oncoproteins. Given the catalytic and sub-stochiometric nature of PROTACs, which offers advantages over classical inhibitors, the scope for therapeutic application of PROTACs is vast. This editorial piece highlights the opportunity to use PROTACs to target pathogens, specifically in the field of antivirals. The development of peptide-based PROTACs to target hepatitis B virus and hepatitis C virus proteins are discussed. Given the recent emergence of COVID-19, concepts relating to the development of PROTACs targeting SARS-CoV-2 proteases or envelope proteins are also highlighted. Alternatively, host proteins could be targeted by degraders to prevent SARS-CoV-2 replication, as has been demonstrated in the context of CDK9 PROTACs showing anti-human cytomegalovirus activity as well as inhibition of SARS-CoV-2 replication, in addition to oseltamivir-based PROTACs with demonstrated anti-influenza activity. Generation of PROTACs for use against an array of pathogens therefore offer potentially huge clinical benefit for a range of pathogenic diseases. Further mechanistic research and application of PROTAC technology within antiviral research is needed to understand the antiviral therapeutic potential.

Other Paper Highlights

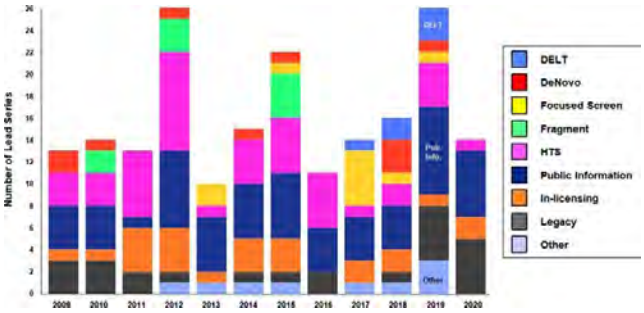
Chemistry | Computational Chemistry

Contributor: Tasuku

Small-Molecule Lead-Finding Trends across the Roche and Genentech Research Organizations

Peter S. Dragovich[§]*, Wolfgang Haap*, Melinda M. Mulvihill, Jean-Marc Plancher, and Antonia F. Stepan

J. Med. Chem, **2022**, DOI: 10.1021/acs.jmedchem.1c02106



A variety of new technologies for identifying seed compounds, such as DNA-encoded library and fragment-based drug design, are available and these technologies as well as other standard methodologies are being applied to projects in pharmaceutical companies. However, it's not ideal to utilize all technologies for projects because of resource limitations. The authors therefore analyzed the historical records of Roche and Genentec to find which methodology was used for identifying each seed compound. They collected and analyzed data from 2009 to 2020 to determine trends in Roche and Genentec. Surprisingly, the most frequently used method to identifying seed compounds was from public information. In addition, DELs and FBDD, which are relatively new technologies than others, contributed to obtaining seed compounds but no clinical candidate compound was obtained from these technologies. Based on the comparison between the two institutes, they also stressed the effectiveness of a focused screening approach over conventional HTS campaigns. There are a lot of ways to understand these data and also some missing information, but their analysis should help us to navigate what technologies should be implemented in-house and which methods would be suitable for each early project.

Must read for medicinal chemists! I was really impressed with this sentence; "In particular, we observed that it was typically difficult for a project team to shift resources away from an established chemical series to progress another (less advanced) lead that subsequently became available."

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