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

Ciulli Lab
Targeted Protein Degradation

University of Dundee

Journal Club

September 2021

Content

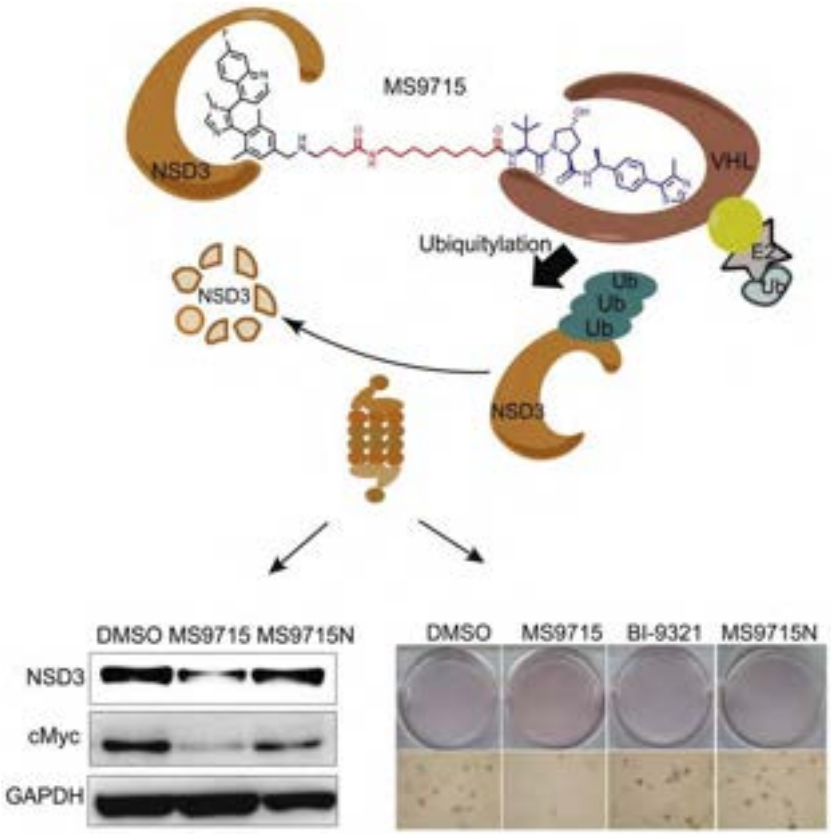
Targeted Protein Degradation

Contributor: Sarah

A NSD3-targeted PROTAC suppresses NSD3 and cMyc oncogenic nodes in cancer cells

Chenxi Xu[§], …, Jian Jin*, Gang Greg Wang*

Cell Chem. Biol., **2021**, DOI: 10.1016/j.chembiol.2021.08.004



The dysregulation of chromatin-regulatory machineries contributes to various oncogenic pathways. Therefore, targeting chromatin modulators such as the nuclear receptor binding SET domain protein 3 (NSD3) is an attractive anti-tumour strategy. NSD3 is a methyltransferase that mono- and di-methylates histone H3 lysine 36 (H3K36), which it interacts with *via* its H3K36me3/2 binding domain (PWWP1). Despite frequent NSD3 alterations in various human tumours the selective antagonist of the NSD3 PWWP1 domain, BI-9321 does not lead to effective killing of cancer cells. In this study the authors discovered MS9715, an NSD3 targeted-PROTAC. They used a long alkyl linker to join BI-9321 to the E3 ligase VHL binder (*S*,*R*,*S*)-AHPC-Me (VHL1-Me). In a range of haematological cancer cell lines, MS9715 (2.5, 5 μM) degrades NSD3 after 48 hrs. The authors establish that this effect is ubiquitin-proteasome system-dependent and use proteomic analysis to demonstrate NSD3-selectivity. Crucially, they show that MS9715 but not BI-9321, effectively inhibits cancer cell proliferation and colony formation. They attribute this response to the concurrent degradation of the oncoprotein, cMyc, which is observed with MS9715 treatment. This study is strengthened by the consistent use of a negative control MS9715N, identical to MS9715 except the VHL binding portion is changed to its diastereomer to abolish VHL binding.

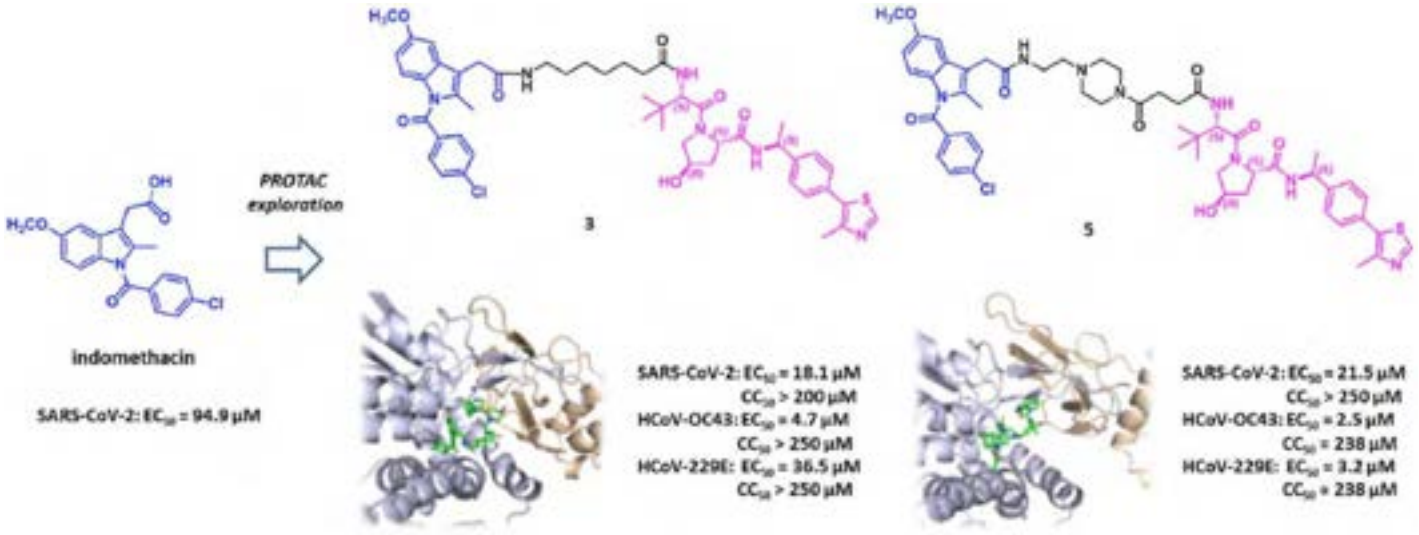
This paper provides evidence that a targeted degradation strategy to treat NSD3-dependent cancers may be optimal compared to the pharmacological blocking of NSD3 due to the concurrent degradation of cMyc.

Contributor: Sarah

Indomethacin-based PROTACs as pan-coronavirus antiviral agent

Jenny Desantis[§], Beatrice Mercorelli[§], …, Arianna Loregian*, Laura Goracci*

Eur. J. Med. Chem., **2021**, DOI: 10.1016/j.ejmech.2021.113814



In the quest to repurpose drugs for the treatment of SARS-CoV-2, researchers identified that indomethacin (INM), a non-steroidal anti-inflammatory drug (NSAID) displayed antiviral activity. The mechanism of action for both its NSAID and antiviral properties remain unclear. However, it is known to inhibit both phospholipase A2(PLA-2), and microsomal prostaglandin E synthase type 2(mPGES-2). The anti-SARS-CoV-2 potency of INM is limited in infected cells, being in the range of ~100 µM. Here, the authors hypothesise that exploiting INM for PROTAC design could enhance its antiviral activity. They design and synthesise four INM-based PROTACs by conjugating INM with the Von Hippel Lindau (VHL) E3 ligase ligand *via* aliphatic and polyethylene glycol (PEG)ylated linkers. The antiviral activity of the four PROTACS was evaluated in simian Vero E6 cells infected with SARS-CoV-2. The authors report that compounds **3** (bearing a 6-methylene unit linker) and **5** (bearing a piperazine-based linker) were the most potent (EC_{50} values of 18.1 µM and 21.5 µM, respectively). However, there was no confirmation that this response was VHL-dependent or ubiquitin-proteasome system-dependent. Despite the molecular models generated for compounds **3** and **5** in a predicted ternary complex formation with VHL and PGES-2 there was little evidence of targeted substrate degradation in this paper.

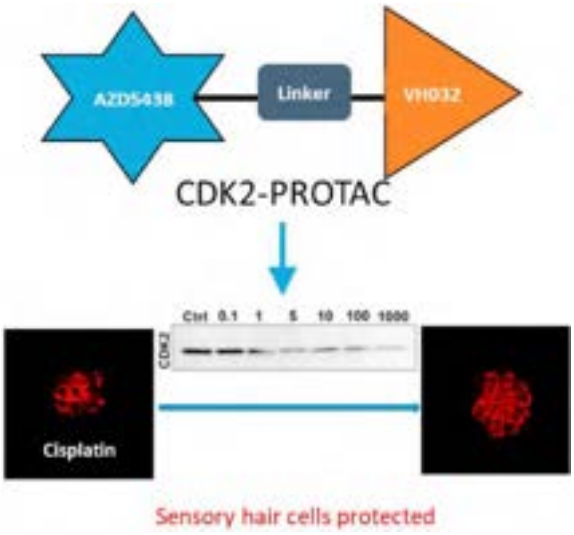
There is undoubtedly a massive need for a COVID-19 treatment and all potential avenues must be explored, therefore it's important that the application of PROTACs in the context of coronavirus inhibition has been examined in this paper.

Contributor: Sarah

AZD5438-PROTAC: A selective CDK2 degrader that protects against cisplatin- and noise-induced hearing loss

Santanu Hati[§], Marisa Zallocchi[§], Robert Hazlitt[§], …, Jian Zuo*

Eur. J. Med. Chem., **2021**, DOI: 10.1016/j.ejmech.2021.113849



The use of the chemotherapeutic agent, cisplatin, causes permanent high-frequency hearing loss in 40-80% of treated cancer patients. Currently, there are no FDA-approved drugs available to prevent acquired or age-related hearing loss. The authors previously identified cyclin-dependent kinase 2 (CDK2) as a potential therapeutic target for cisplatin-induced ototoxicity. The absence of CDK2 in a knock-out mouse model provides resistance to cisplatin-induced hair cell loss. Here, the authors design and screen for selective CDK2 degraders to prevent cisplatin induced hair cell loss. The most effective CDK2 degrader (PROTAC-8) induced around 50% CDK2 degradation at 100 nM concentration. PROTAC-8 consists of the CDK2 ligand, AZD5438, linked to E3 ligase ligand, VH-032. Degradation was dependent upon proteasomal activity and was selective for CDK2 over closely related CDKS. They then tested this compound *in vivo* for its ability to prevent hair loss using a zebra fish lateral line model. The zebrafish lateral line is a sensory system comprised of clusters of mechanosensory hair cells called neuromasts. PROTAC-8 offered marginally more protection against hair cell loss induced by cisplatin and kainic acid damage (noise-induced damage) than AZD5438.

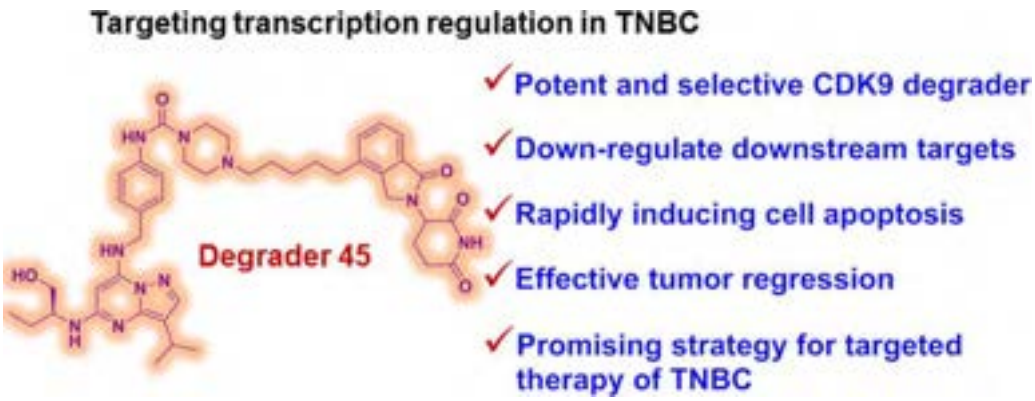
This paper provides evidence that a targeted degradation approach can induce physiological effects *in vivo* comparable to those observed in genetic knock-out studies.

Contributor: Sarah

Discovery of potent and selective CDK9 degraders for targeting transcription regulation in triple-negative breast cancer

Dan Wei[§], Hanlin Wang[§], Qinghe Zeng[§], …, Ruimin Huang*, Jia Li*, Xiao-Hua Chen*

J. Med. Chem., **2021**, DOI: 10.1021/acs.jmedchem.1c01350



Developing a targeted treatment for the highly aggressive, triple-negative breast cancer (TNBC) remains an elusive clinical challenge. The dysregulation of the transcriptional regulator, cyclin-dependent-kinase 9 (CDK9) is prominent in multiple cancer cells. In the venture to find a targeted TNBC treatment the authors screen and optimise a series of CDK9-targeted degraders in TNBC cells. Initial molecular docking simulations identify four nitrogen-containing fused heterocycles as the CDK9 ligands. These ligands were linked to CRBN E3 ligase binders, either thalidomide or lenalidomide. The optimal CDK9 ligand, linkage site and linker length were determined, identifying thalidomide -containing compound **28**, which effectively induced degradation of CDK9 in 4 hours at 100 nM in MDA-MB-231 cells. The authors demonstrate that compound **28** downregulates the downstream targets, such as MYC, at the transcriptional level, resulting apoptosis in TNBC cells. However, due to low plasma exposure of **28** they optimise to generate the lenalidomide based, compound **45**. To improve solubility compound **45** has a piperazine group within the linker and free hydroxyl group at the side chain. Compound **45** displayed similar efficiency in the degradation of CDK9 compared to degrader **28** and inhibited the growth of MDA-MB-231 cells at 13.6 nM. In a TNBC xenograft mouse model degrader **45** induced effective tumour regression at a tolerated dosing schedule.

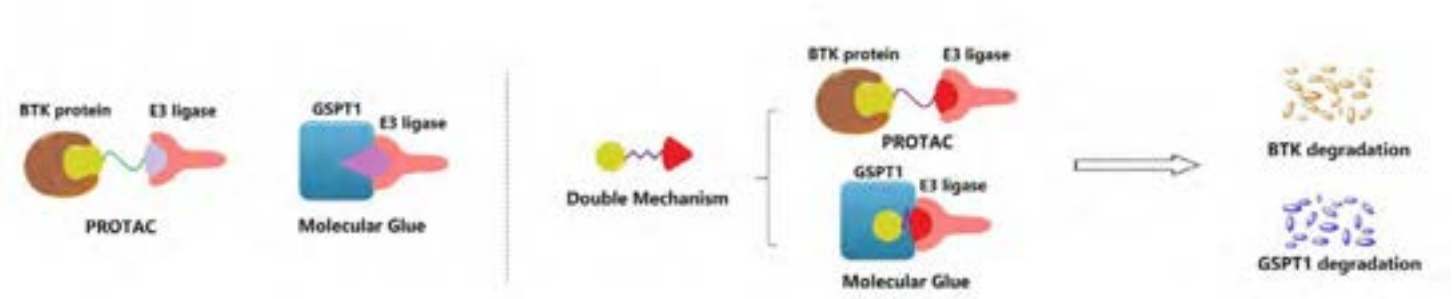
Despite limited evidence for the mechanism of action of compound **45**, this paper details a comprehensive optimisation strategy for potent CDK9-degraders. Leading to the first reported *in vivo* therapeutic potential evaluation of CDK9 degraders for the treatment of TNBC.

Contributor: Sarah

Merging PROTAC and molecular glue for degrading BTK and GSPT1 proteins concurrently

Zimo Yang[§], …, Yu Rao*

Cell Res., **2021**, DOI: 10.1038/s41422-021-00533-6



BTK (Bruton Tyrosine Kinase), is a tyrosine kinase found to be overactive in lymphoma cells. The authors previously developed BTK targeted degraders, however they had limited efficacy for refractory diffuse large B cell lymphoma and acute myeloid leukaemia cells. GSPT1 (G1 to S phase transition 1) is a translation termination factor that can be degraded by certain molecular glues. The downregulation of GSPT1 can inhibit proliferation in diverse tumour cells. Here, the authors aim to improve the efficacy of BTK-PROTAC, L18I, by promoting the concurrent degradation of BTK and GSPT1. They attempt to recapitulate the features of a molecular glue within L181 by shortening the linker from 16 atoms to 6-12. The binding of these designed compounds to GSPT1 and CRBN was simulated and 'potential glues' were identified. Compounds with a linker length of 7-9 carbons induced degradation of both BTK and GSPT1. GBD-9 was the most effective, degrading BTK and GSPT1 by 80% and 90%, respectively. The binding pocket of BTK was blocked with ibrutinib but GBD-9-induced degradation of GSPT1 was unperturbed, whereas BTK was no longer degraded. This indicated that the degradation of GSPT1 is not fully dependent upon binding to the ibrutinib-containing end of GBD-9. The authors also established that GBD-9 is superior to ibrutinib and L18I at inhibiting cancer cell survival.

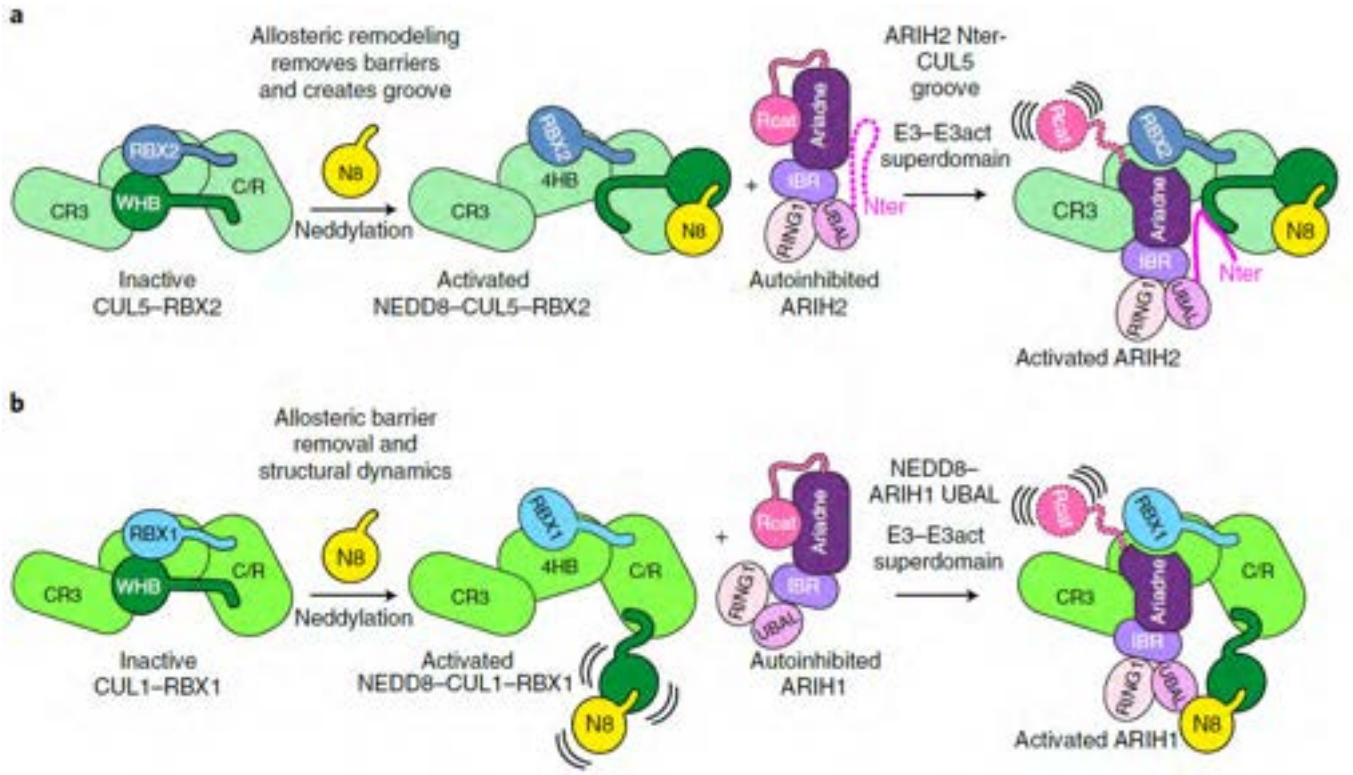
Both PROTACs and molecular glues have uniquely favourable properties, such as the small MW of glues and the broad range of targets available for PROTACs. This letter demonstrates that combining the best of both could be a promising strategy to generate efficacious targeted degraders.

Contributor: David

CUL5-ARIH2 E3-E3 ubiquitin ligase structure reveals cullin-specific NEDD8 activation

Sebastian Kostrhon[§], …, Brenda A. Schulman*

Nat. Chem. Biol., **2021**, *17*, 1075–1083



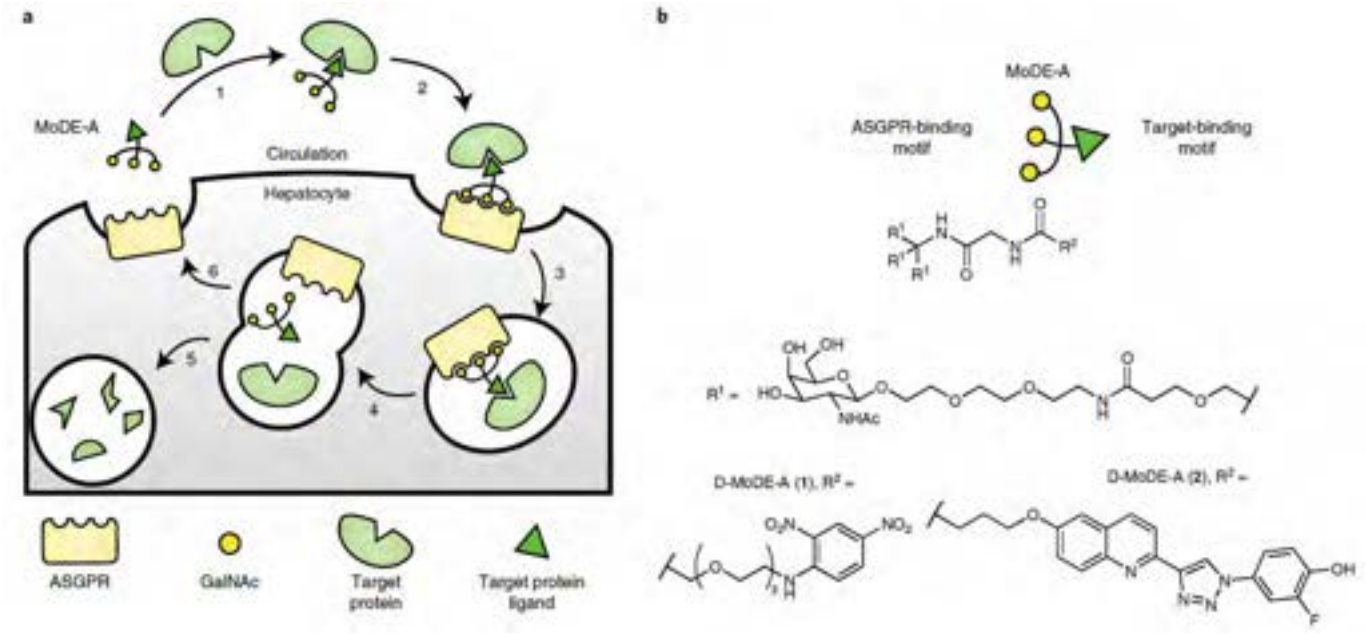
In a paper investigating the molecular mechanism of the neddylation cascade, Kostrhon *et al.* provide structural insights into the CUL5-ARIH2 E3-E3 complex, revealing an allosteric mechanism for binding partner specificity. Using a combination of X-ray crystallography and cryo-EM the authors were able to compare structures of closely related E3-E3 assemblies neddylated CRL5-ARIH2 and neddylated CRL1-ARIH1, as well as the E3 proteins alone. By comparing these structures, the authors were able to distinguish an indirect, allosteric mechanism by which NEDD8 induces binding of CUL5 to ARIH2. To the authors knowledge this represents the first structurally defined protein-protein interaction to be driven by conformational changes induced by a ubiquitin(/-like) protein, without directly binding itself. The authors postulate that allosteric specificity of ubiquitin-like protein modifications such as those revealed in this study may offer novel opportunities for therapeutic targeting.

Contributor: David

Bifunctional small molecules that mediate the degradation of extracellular proteins

David F. Caianiello[§], …, David A. Spiegel*

Nat. Chem. Biol., **2021**, *17*, 947 – 953



Most targeted protein degradation (TPD) systems function by redirecting the ubiquitin-proteasome system to degrade target proteins and are therefore limited to intracellular proteins. Caianiello *et al*. present an exciting potential advancement of the possibilities of the TPD field in the form of extra-cellular protein degraders named MoDE-As (molecular degraders of extracellular proteins through the asialoglycoprotein (ASGPR) receptor). MoDE-As comprise an ASGPR-binding motif and a target-binding motif connected by a (in this case PEG-based) linker. By bringing the target protein and ASGPR into close proximity, the protein of interest is endocytosed and degraded by lysosomal proteases. Caianiello *et al*. synthesised example MoDE-As molecules and demonstrated they were able to form desired ternary complexes using flow cytometry, and found they were able to induce endocytosis and lysosomal degradation of target proteins *in vitro* in a MoDE-A-dependent manner.

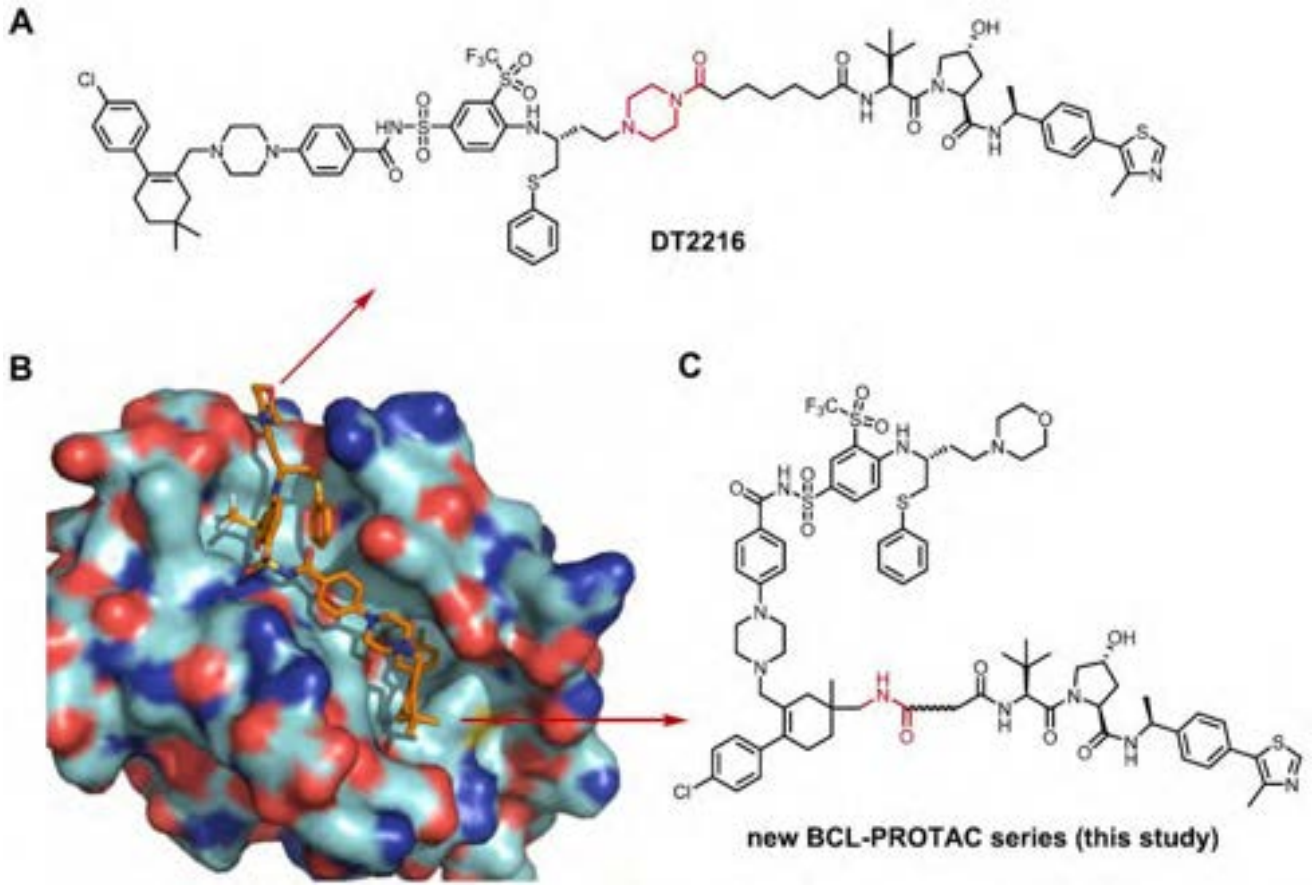
While other technologies for depleting extra-cellular proteins, such as LYTACs have previously been discussed, the authors point out that MoDE-As have advantages in being relatively small, non-protein-based and monodisperse, as well as functioning by directing proteins of interest to hepatocytes, which are equipped to catabolise large amounts of endocytosed proteins.

Contributor: David

Discovery of a novel BCL-X_L PROTAC degrader with enhanced BCL-2 inhibition

Pratik Pal[§], Dinesh Thummuri[§], …, Daohong Zhou*, Guangrong Zheng*

J. Med. Chem., **2021**, DOI: 10.1021/acs.jmedchem.1c00517



BCL-X_L and BCL-2 are well known targets for anti-cancer therapeutics. Inhibition of BCL-X_L is associated with on-target platelet toxicity, therefore PROTACs have previously been developed to circumvent this toxicity by degrading BCL-X_L. Unfortunately, the PROTACs presented in previous studies showed minimal effects in cancers dependent on BCL-2 or co-dependent on BCL-2 and BCL-X_L. Pal *et al.* present a novel series of PROTACs including PZ703b, which is able to induce highly potent degradation of BCL-X_L, while inhibiting, but not degrading BCL-2. This hybrid function is thanked for the molecules high potency at killing BCL-X_L-dependent, BCL-2-dependent, and BCL-X_L/BCL-2 co-dependent cell lines in a VHL-dependent manner.

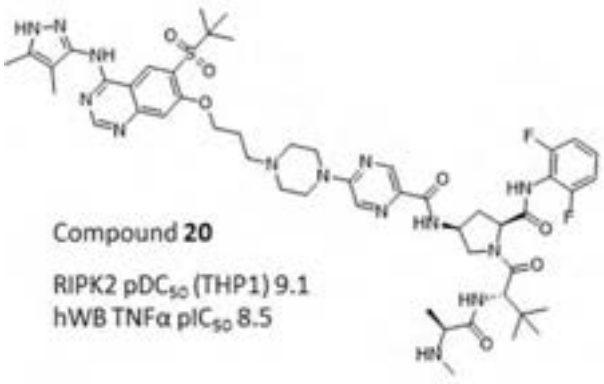
Interestingly, despite degrading BCL-X_I, but not BCL-2, PZ703b was shown to be able to form cooperative (2-5-fold) ternary complexes with either protein *in vitro* and *in cell* in AlphaLISA and NanoBRET assays, respectively. The authors suggest that this distinct dual inhibition/degradation mechanism of action may allow for a pharmacokinetic-pharmacodynamic profile advantageous for clinical development.

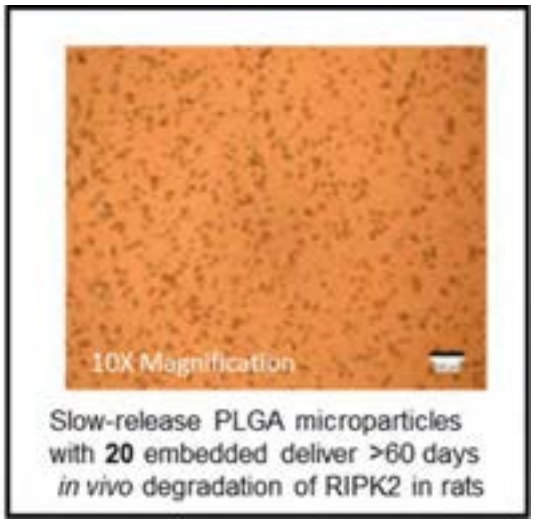
Contributor: David

Optimization of a series of RIPK2 PROTACs

Afjal H. Miah[§], …, John D. Harling*

J. Med. Chem., **2021**, *64*, *17*, 12978-13003





Miah *et al.* present a project aimed at degrading RIPK2, a kinase important to the immune response system, by designing a series of PROTACs that utilize the IAP family of E3 ligases. The bulk of this paper describes optimizations based on PROTAC **3**, a potent IAP-based RIPK2 degrader previously disclosed by the authors which displayed high lipophilicity and poor microsomal stability. The approach the authors use in this paper focusses on minimizing lipophilicity during the early stages of PROTAC design, enabling the prioritization of analogues displaying improved solubility and microsomal stability. Furthermore, the authors present a proof-of-concept slow-release matrix PROTAC delivery mechanism, demonstrating the potential for long-acting parenteral formulation with >1 month duration. Compound **20** displayed the best overall profile with good solubility, and potent degradation of RIPK2, delivering ~60 days of *in vivo* degradation of RIPK2 (>90%) in rats when administered using slow-release PLGA microparticles.

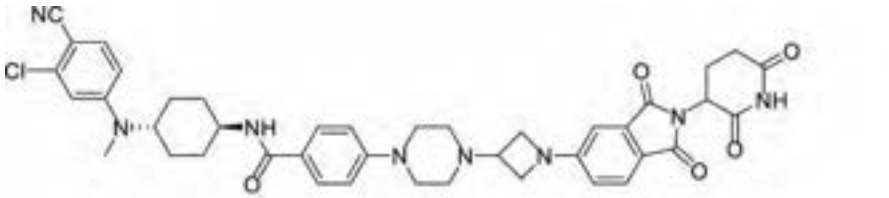
While many PROTAC drug programs have focused on obtaining orally bioavailable PROTACs, Miah *et al.* suggest the slow-release subcutaneous depot formulation may be a beneficial alternative dosing approach in chronic disease settings to facilitate compliance, while also avoiding high C_{max} drug concentrations and associated toxicities.

Contributor: Tasuku

Discovery of ARD-2585 as an exceptionally potent and orally active PROTAC degrader of androgen receptor for the treatment of advanced prostate cancer

Weiguo Xiang[§], Lijie Zhao[§], Xin Han[§], …, Shaomeng Wang*

J. Med. Chem., **2021**, DOI: 10.1021/acs.jmedchem.1c00900



ARD-2585:An exceptionally potent and orally efficacious AR degrader
(DC_{50} = 0.04-0.1 nM in VCaP and LNCaP cells and Oral Bioavalability in Mice = 51%)

The androgen receptor (AR) is one of the most well-known biological targets for the treatment of prostate cancer. Recently, Arvinas reported that their AR-targeted PROTAC, ARV-110, showed good clinical response and acceptable toxicity in Ph I/II clinical trials. In this paper, the authors investigated the development of novel AR-targeted PROTACs based on a known AR inhibitor and thalidomide motif. They found that structurally rigid linkers gave better DC_{50} and D_{max} values, possibly because the structural restriction facilitated the formation of a stable ternary complex. After fine-tuning of the linker and AR ligand moiety, the authors finally identified ARD-2585 and found that it showed excellent degradation activity in a variety of prostate cancer cell lines. In fact, it showed great AR degradation activity in MDA-PCa-2b cell line, which has a double mutation on AR and is not very sensitive to ARV-110. In terms of PK profile, it has good oral availability and a favourable tissue distribution profile. In xenograft model studies, ARD-2585 was administrated orally and inhibited tumour growth strongly at 10 mg/kg/day, QD. Their findings are not only great examples of orally-available PROTACs but also showcase the importance of linker design.

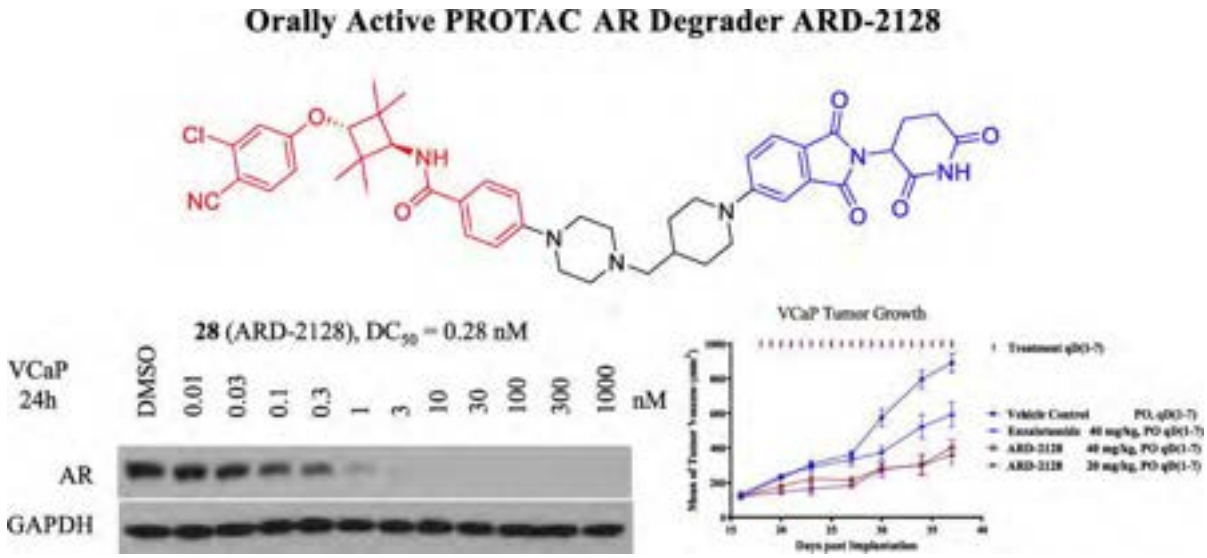
They didn't mention some important data, e.g., unbound drug concentration in plasma, and solubility, so the reason for the unusual PK profile is unclear (plasma concentration at 24 hours was higher than that at 6 hours, etc.) and non-linear dose-dependency in xenograft models. In addition, I'm not sure exactly what they mean by 'an excellent volume of distribution'.

Contributor: Tasuku

Strategies toward discovery of potent and orally bioavailable proteolysis targeting chimera degraders of androgen receptor for the treatment of prostate cancer

Xin Han[§], Lijie Zhao[§], Weiguo Xiang[§], …, Shaomeng Wang*

J. Med. Chem., **2021**, DOI: 10.1021/acs.jmedchem.1c00882



The authors previously developed ARD-61, a VHL-based PROTAC, which showed significant degradation of androgen receptor (AR) in the mouse xenograft model. However, possibly because of its high molecular weight, large polar surface area, and high lipophilicity, ARD-61 has no oral bioavailability in mice. To improve drug-like properties, they chose cereblon ligands for PROTAC development due to their lower molecular weights. They found that the introduction of a piperidine ring to the linker improved plasma drug concentration by oral administration. Then, a variety of linkers with conformational restrictions were tested and they found that some compounds, including ARD-2128, showed excellent AR degradation activity in VCaP and LNCaP cells. Finally, they identified that ARD-2126 has the most favourable PK profile and decided to use it for further studies. In a VCaP bearing xenograft mouse model, mice were administered ARD-2126 at 10, 20, or 40 mg/kg/day, po, QD and strong tumour growth inhibition was observed in each case, without severe body weight loss. Unfortunately, ARD-2126 was the compound Arvinas disclosed on their patent in 2018 as one of the orally available PROTACs, but their methodology could be useful for designing highly potent PROTACs with good oral availability.

The tumour growth inhibition between 20 and 40 mg/kg/day was almost the same, and that implies the drug absorption from the intestine was saturated at 20 mg/kg/day and aqueous solubility is not great (they didn't mention it, though). Also, they noted that the plasma-protein binding ratio is extremely high (99.8%). Further optimization might be needed to move it forward to clinical trials.

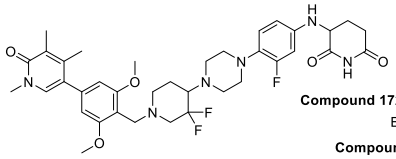
Contributor: Tasuku

Compounds for targeted degradation of BRD9

Christopher G. Nasveschuk[§], …, Jeremy L. Yap*

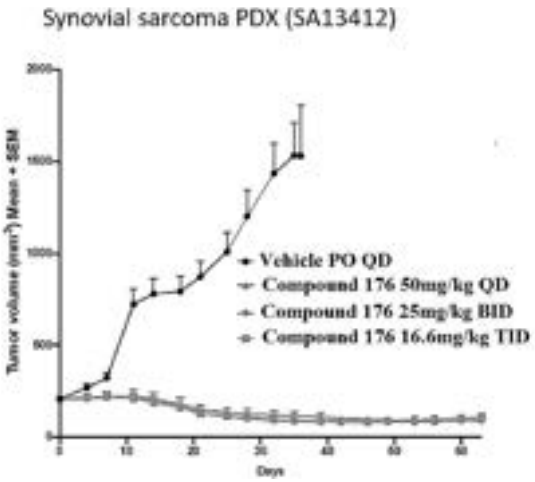
World Intellectual Property Organization, 2021/178920, 10 September 2021.

The mammalian switch/sucrose nonfermentable (SWI/SNF) complexes regulate gene expression, DNA replication, and DNA repair. It's subunit, BRD9, was known to be required for proliferation of AML and to be overexpressed in some cancers. The authors divulged novel BRD9-targeted PROTACs based on BRD9 ligands that were developed by researchers in Boehringer Ingelheim, and cereblon ligands. One of the best compounds in this patent, **compound 172**, as well as **compound 176**, which is one of four isomers of **compound 172**, were shown to degrade BRD9 efficiently in a 293T.166 BRD9-HiBiT assay. Four cell lines (A204, HS-SY-II, SW982, and Yamato-SS) were treated with **compound 176** and the DC_{50} value for BRD9 in each cell line appeared to be lower than 10 nM (exact values not mentioned). **Compound 176** has a great PK profile, no inhibition against hERG, and showed great tumour growth inhibition not only in the Yamato-SS xenograft model but also in the synobial sarcoma PDX model. Interestingly, **compound 176** only degrades BRD9 and does not affect to BRD7 and BRD4. The molecular weight of **compound 176** is only 710.80 and has a great PK profile with oral bioavailability, so I'm presuming they're planning to proceed with this compound in clinical trials.



Compound 172 (diastereo and enantio mixture)
BRD9 DC_{50}: <100 nM

Compound 176 (one of four isomers)
BRD9 DC_{50}: <100 nM



Contributor: Tasuku

Harnessing the E3 ligase KEAP1 for targeted protein degradation

Jieli Wei[§], Fanye Meng[§], …, Ümit Kaniskan*, Jian Jin*

J. Am. Chem. Soc., **2021**, *143*, 15073-15083

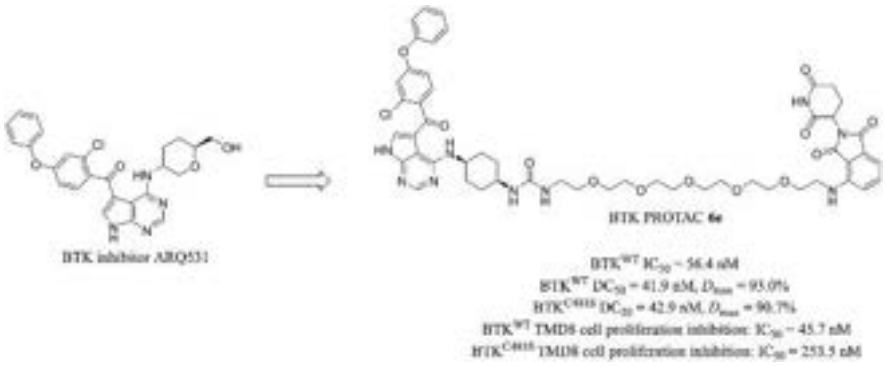
Expanding the toolbox of E3 ligases is one of the key factors in expanding the application of PROTACs. Recently, KEAP1-recruiting PROTACs based on a KEAP1-binding peptide or covalent binding motif were reported, but these PROTACs have some drawbacks that need to be improved (e.g. permeability, non-specific binding). In this paper, the authors developed a novel type PROTAC, MS83, which has a chemical motif of KEAP1-L, a non-covalent KEAP1 binder. They connected JQ-1 from the methoxy group of KEAP1-L based on the cocrystal structure of KEAP1 with KEAP1-L. Because KEAP1-L has a carboxylic acid moiety which limits cell permeability, the corresponding ester form was used for the degradation assay as a prodrug. It was found that MS83 showed strong degradation activity against BRD3 and BRD4, but it took a longer time to show a great degradation effect (24-36 hours), possibly because hydrolysis of the ester is not fast in cells. Interestingly, a strong hook effect was observed in MDA-MD-438, but not in MDA-MD-231. In contrast, MS83 only degraded the short form of BRD4 in MDA-MD-231, and this might have weakened the growth inhibitory effect. I guess it might be possible to improve the degradation effect by modifying the ethyl ester moiety to others.

Contributor: Tasuku

Discovery of novel BTK PROTACs for B-Cell lymphomas

Yunpeng Zhao[§], Yongzhi Shu[§]…, Nannan Sun*, Yonghui Wang*

Euro. J. Med. Chem., **2021**, *225*, 113820



Recently, Bruton's tyrosine kinase (BTK)-targeted PROTACs were reported, but these PROTACs which have BTK binding motifs with covalent binding warheads did not show a significant degradation effect, possibly because of a lack of catalytic turnover. In this paper, the authors developed novel types of BTK-targeted PROTACs based on ARQ531, a non-covalent BTK inhibitor. In the cocrystal structural analysis, the hydroxymethyl moiety on the tetrahydropyran ring is exposed to the solvent exposure region, so they anchored the pomalidomide moiety to this part. After optimization of the linker part, they found that **4g**, which has a simple 6-PEG linker, and **6e** showed excellent degradation activity not only for BTK^{WT} but also for BTK^{C481S}, a resistant mutation against covalent BTK inhibitors. Interestingly, **4g** had a weak inhibitory activity against BTK (IC_{50}: 1390.5 ± 205.8 nM, DC_{50}: 8.3 ± 0.3 nM), but showed stronger degradation activity than that of **6e** (IC_{50}: 56.4 ± 6.9 nM, DC_{50}: 41.9 ± 5.3 nM). Their new PROTAC **6e** has a great degradation activity against BTK^{C481S} and a good physicochemical profile, so their findings might be useful for developing non-covalent BTK-targeted PROTACs

---

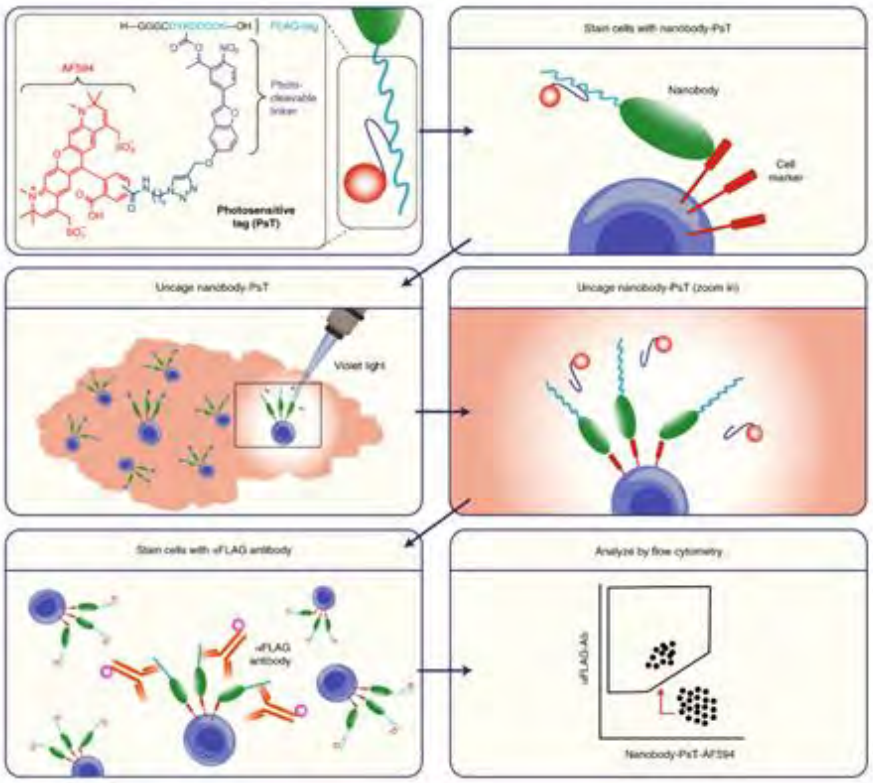
Other Paper Highlights

Contributor: Sarah

Single-cell analysis of regions of interest (SCARI) using a photosensitive tag

Anne M. van der Leun[§], , Mirjam E. Hoekstra[§], …, Sander I. van Kasteren*, Ton N. Schumacher*

Nat. Chem. Biol., **2021**, DOI: 10.1038/s41589-021-00839-x



The characterisation of single cells using proteomics and transcriptomics provides insights into how cells function within human tissues. However, any information regarding spatial localisation, which largely influences cell state, is lost upon tissue disaggregation. Laser tagging of tissue regions has been combined with fluorescence-activated cell sorting (FACS) to isolate cells, but this requires genetically encoded photoactivable fluorescent proteins which are incompatible with primary human samples.

Here, a new spatial biology method called Single Cell Analysis of Regions of Interest (SCARI) is described. This approach allows specific cells to be isolated from primary tissue without the need for genetic manipulation. Cells are labelled with a photosensitive FLAG-tagged nanobody that targets universal surface proteins. This nanobody has an AF594 fluorochrome attached *via* a photocleavable linker, 4-nitrophenyl(benzofuran). The fluorochrome shields the FLAG-tag of the nanobody to prevent antibody binding. The regions of interest within the tissue are excited with a 405 nm violet laser this cleaves the linker, releases the reference fluorophore, and leaves the FLAG-tag available for antibody recognition. These cells are referred to as being 'uncaged' and they can be distinguished from cells that were not exposed to the laser ('caged') using FACS. Uncaged cells are AF594^{low} αFLAG^{high} and caged AF594^{high} αFLAG^{low}. The authors use this approach with single-cell RNA-sequencing of spatially defined CD8+ T cells to reveal location-dependent cell states.

The application of low-intensity violet light rather than potentially phototoxic ultraviolet light makes SCARI suitable for sensitive cell types and tissues. The approach described here is exciting because it allows live primary cells to be recovered from selected tissue zones, which can then be probed with functional assays to dissect how the local microenvironment influences a cells state.

Contributor: Tasuku

Complexation of polyethyleneglycol containing small molecules with magnesium chloride as a purification and isolation strategy

Bin Zheng*, …, Martin D. Eastgate*

Org. Process Res. Dev., **2021**, DOI: 10.1021/acs.oprd.1c00174

PEG-linkers are commonly used for a wide range of biochemical tools and drugs, including photoprobes, bioconjugations, anti-drug conjugations (ADCs), and PROTACs. However, almost all PEG-rated linker intermediates are polar and viscous oils, so these properties may cause problems in purification, handling, and storage. In this paper, the authors investigated the chelate formation of PEG-containing compounds and inorganic salts in non-polar solvents and found that magnesium chloride ($MgCl_2$) with small amounts of THF in DCM gave favourable solid complexes with good recovery. These magnesium-PEG complexes can be applied to reactions, like peptide synthesis, and $MgCl_2$ in the complexes can be easily removed by simple DCM-H_2O separation. Boc and *tert*-butyl esters were partially decomposed, probably because of the Lewis acidity of $MgCl_2$, and were then supressed by addition of organic bases, such as pyridine, triethylamine, and diisopropylethylamine. This new method would be especially useful for large-scale synthesis of PEG containing derivatives.

One of the great advantages of their method is the easy-handling of magnesium complexes, so I hope a variety of PEG-magnesium complexes will be commercially available in the near future.

University of Dundee

Ciulli Laboratory
School of Life Sciences
Dow Street, Dundee,
DD1 5EH
United Kingdom

lifesci.dundee.ac.uk/groups/alessio-ciulli/publications/journal-club

@alessiociulli @CharlCrowe

23281