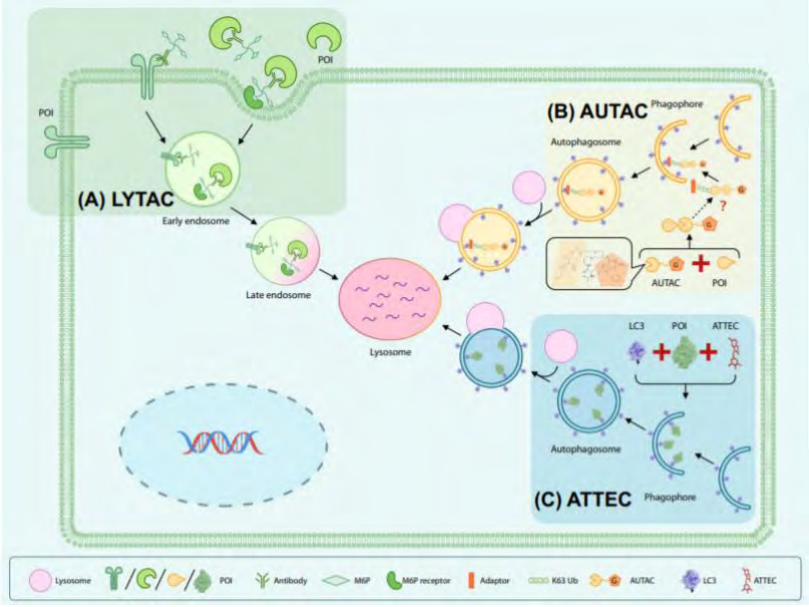

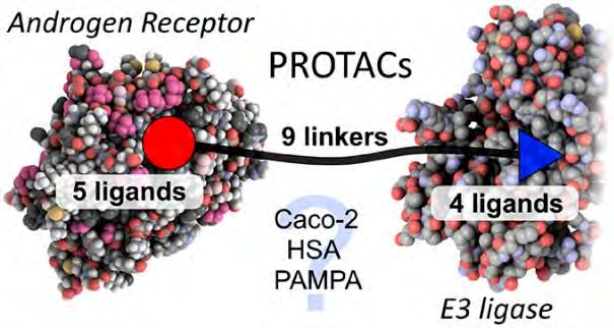

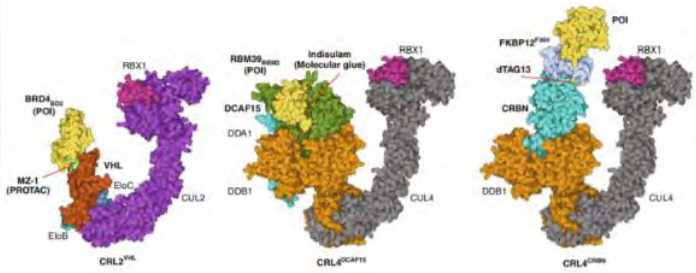

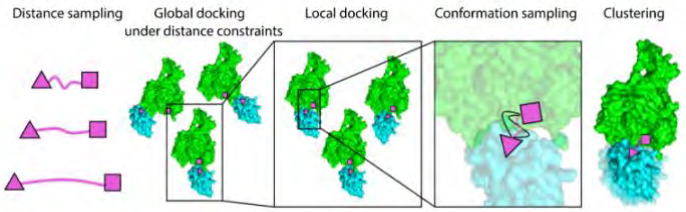

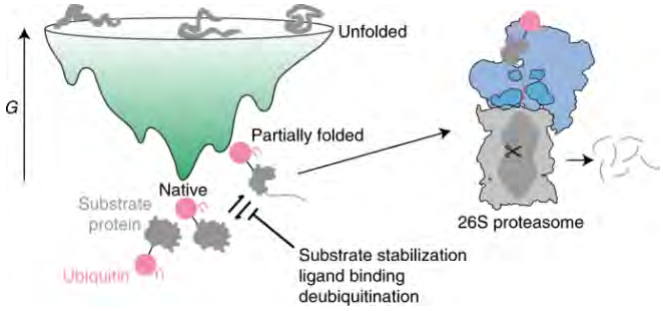

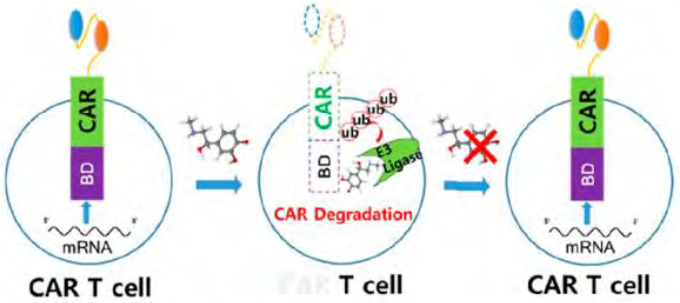

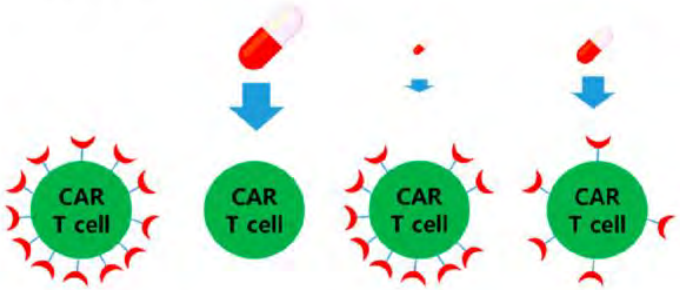

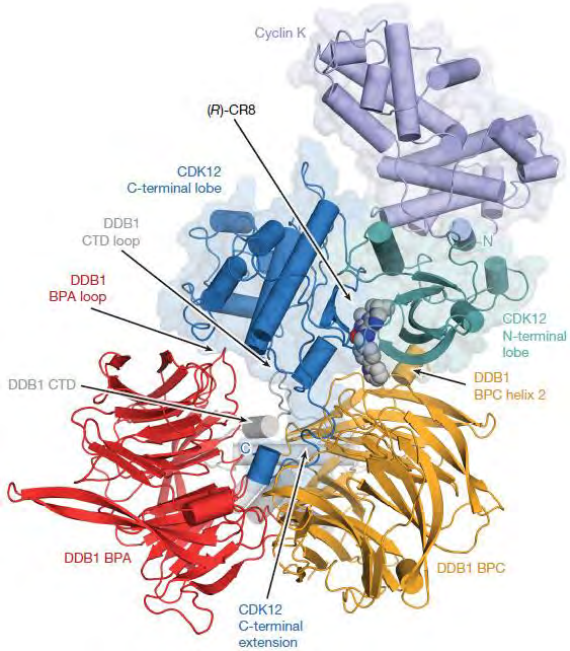

Ciulli Group Journal Club

Targeted Protein Degradation,

Medicinal Chemistry and

Chemical Structural Biology

Literature Highlights

June 2020 Edition

Ciulli Group Journal Contents

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Special Feature: Equality, Diversity and Inclusion: We all have a role to play

Contributors: Alessio and Will

The events that have unfolded over the past few weeks have brought right in front of everyone's eyes the deep, visceral problems with respect to equalities, diversity and inclusion, that impact the daily lives of many members of our communities.

The brutal killings caught on camera of George Floyd and Rayshard Brooks, by police officers, is a tragic reminder of the terrible reality of the systemic racism and insidious discrimination that are still, unfortunately, rooted in our societies. This led to worldwide protests, including from the Black Lives Matter movement. Black people continue to be subjected to institutionalised racism, overlooked and deprived of equity of opportunities across the globe.

Amid this backdrop, the scientific community, and in particular the wider chemistry field, watched with dismay one of its "pinnacle journal", Angewandte Chemie, publishing an appalling essay by a senior faculty member from Brock University (Ontario, Canada) who alleged that:

1. efforts to promote diversity in the field and increase the representation of women and minorities were hurting science;
2. fraud and improper publication protocols are common amongst Chinese scientists;
3. the relationship of the student to the supervisor should follow unconditional submission of an apprentice to the master.

As the accepted article was quickly shared on social media, it prompted near unanimous expressions of anger and condemnation. Shortly after appearing online, the journal made the article disappear, without a retraction note. Events rapidly unfolded, with 16 members of the journal's international advisory board resigning from their roles while denouncing the essay. The journal later suspended two of its own senior editors and removed two referees from their selection of peer reviewers.

Many reactions and statements from leaders and more widely across the community have followed, to reinforce the values we stand for, and to pledge to oppose racism, hate and discrimination. Let us be clear. We (AC and WF) condemn these tragic deaths and so stand in solidarity with members of the Black community. We forcefully reject the mind-set and ugly sentiments expressed in that essay. These events have deeply disturbed and outraged us.

Words of condemnation are a beginning, as silence or indifference would be much worse and could be mistaken for endorsement. Yet just speaking out is not enough. Words will need to be followed by action, with recent events demonstrating we as scientific and social communities are not doing enough.

Before we can even begin to think of doing something about a problem, we must first acknowledge that we have a problem. We must recognize that these events would be outrageous if they were isolated incidents, but sadly they are not: they evidence long-standing issues that are more pervasive than we think. For example, one should not be naïve to think that there are not others out there who quietly approve of some of the statements or views that were shared in that essay. After all, it did go through the journal's peer review process.

Pointing fingers and placing blame however, is not a solution in itself. We must start by recognizing that we all have responsibilities, that we must be accountable for our actions. Indeed, responsibility should be taken up first by those who have more opportunities to do something about a problem, especially leaders and

representatives of majorities. In a skewed and dominant society, "club", or group, unconscious bias surfaces readily and it is difficult to control. When that society, club or group is over-represented by white-male human beings, the burden of responsibility must therefore fall primarily on white-male leaders (*disclaimer: both AC and WF are white-males*).

Leaders have an important responsibility for the actions and words that they use to convey the values that they stand for. Respect, honesty, compassion, solidarity, generosity, openness, integrity are just some amongst the many values we aspire to live by. As ourselves leaders in our own different roles and with different remit, we embrace our responsibility by aspiring to act and to live by those values as best as we can, and by striving to inspire others to do so too.

To begin to address imbalances in representation underrepresented groups must be ''seen'', and it must be understood that they have likely had to overcome hurdles that do not present to other groups, such as our own (WF and AC). We believe that we must value people for who they are and what they do and the values they themselves live by, regardless of gender, colour of skin, nationality, amongst other categories that put us into boxes. Yet we must recognise that we all have these characteristics.

Our diversity, tolerance and respect of different opinions and those who are different from us are great strengths. In fact, there is documented evidence that diversity improves science, society and lives more widely – and that diverse teams are more innovative and better at creative thinking, producing higher impact research (see Hofstra *et al.*, 2020 The Diversity-Innovation Paradox in Science; How diverse teams produce better outcomes; Teams solve problems faster when they're more cognitively diverse; How diversity makes us smarter - for just some evidence). Most importantly though, being tolerant and respectful is the right way to treat people.

So what actions can and must we take?

Angewandte have taken the much needed step to begin a process towards increasing inclusiveness and representation across the board. Read more in their Editorial here.

Some examples of the steps we can all actually start taking to help, have been in our opinion very succinctly put in a recent Editorial by the American Chemical Society.

We all have a role to play in creating professional, cultural and social environments where everyone feel safe and valued, starting with checking our behaviours and attitudes on a day-to-day basis. We (AC and WF) believe in promoting transparency over secrecy, particularly in decision-making processes; taking up responsibility and accountability for our actions; encouraging kindness over hostility; and keeping an open mind to actively challenge our own assumptions and biases.

We can all do better. We must all do better. Even more so today, given the unprecedented circumstances we find ourselves in. None of us is perfect. We can all make mistakes, but by learning and listening we can endeavour to be better and do better. Together, we can meet this challenge.

Acknowledgements: We thank Melissa D'Ascenzio and Lesley Pearson from the University of Dundee Athena Swan team for critical comments.

Targeted Protein Degradation

Contributor: Claire

Site-specific ubiquitination affects protein energetics and proteasomal degradation

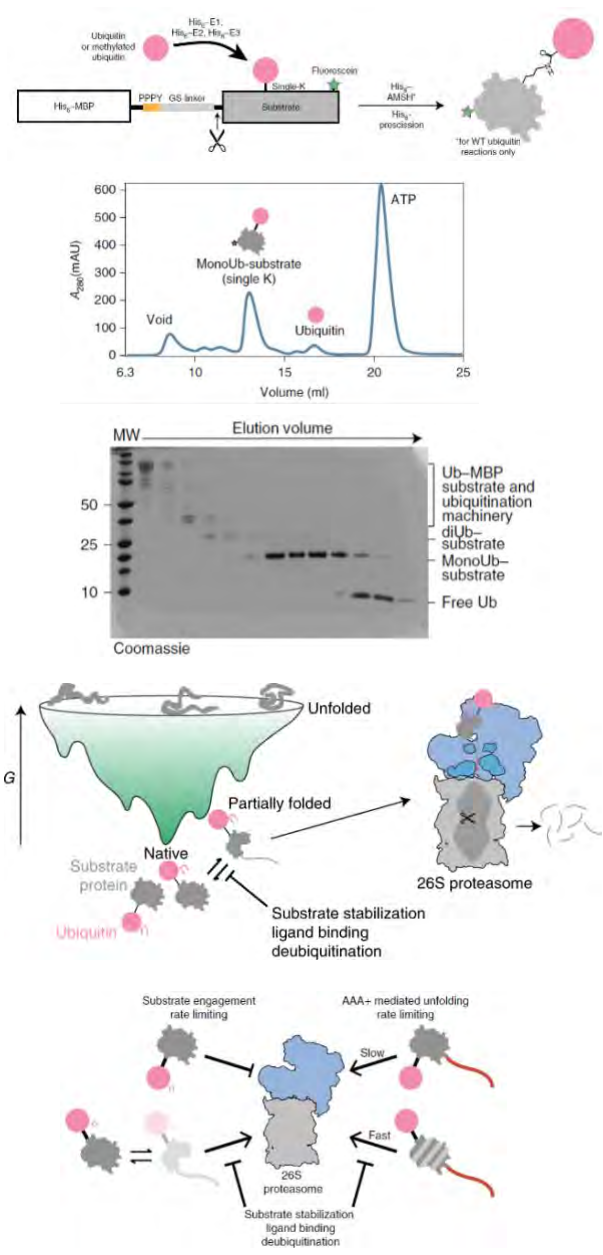
Emma C. Carroll, …, Andreas Martin*, Susan Marqusee*

Nat. Chem. Biol., **2020***,* DOI: 10.1038/s41589-020-0556-3

Ubiquitination and conformational properties of a protein that determine its energy landscape affect a protein's susceptibility for degradation via the proteasome. Purification of ubiquitin-conjugated substrates with native isopeptide bonds has previously hindered the ability to investigate ubiquitin-induced protein destabilisation. Here the authors developed a biochemically reconstituted enzymatic ligation and deubiquitination strategy to produce homogenously mono-ubiquitinated protein via isopeptide-linkage of ubiquitin within structured regions of the target protein. Stability changes could then be detected using chemically induced equilibrium unfolding monitored by intrinsic fluorescence. The authors observed variable effects on the energy landscape of the target protein that were dependent on the protein and specific site of ubiquitination. The energetic modulations caused by site-specific ubiquitination could destabilise the native protein structure which affected proteasome engagement and enhanced the rate of proteasomal degradation.

The summarised model (bottom figure) is as follows: Ubiquitin-modification on a sensitive (versus a non-sensitive) lysine causes the otherwise well-folded substrate to be sufficiently destabilized to populate partially unfolded, proteasome-engageable conformations and be successfully degraded.

Proteasome engagement and degradation kinetics thus appear dependent on the changes to the protein energy landscape on ubiquitination.



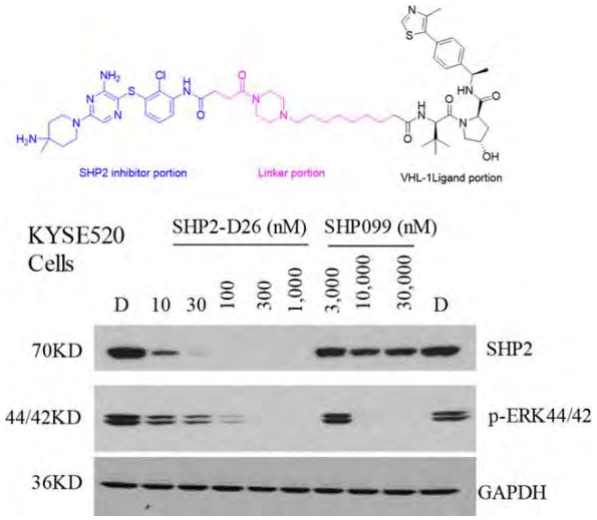
The article introduction provides a nice overview/refresher on the details of ubiquitin-proteasomal degradation. Given that the potential energetic effects of substrate ubiquitination on proteasomal degradation were previously unknown, it is interesting to see the authors establish a connection between ubiquitin-induced changes in substrate energetics and proteasomal processing. It will be important to consider the impact of modulating substrate energy landscapes by site-specific ubiquitination when reviewing the potential of a given target for UPS-induced degradation using PROTACs or other TPD technologies.

Contributor: Adam

Discovery of SHP2-D26 as a First, Potent, and Effective PROTAC Degrader of SHP2 Protein

Mingliang Wang[§], Jianfeng Lu[§], …, Shaomeng Wang*

J. Med. Chem., **2020**, DOI: 10.1021/acs.jmedchem.0c00471



To target SHP2, the authors modified a SHP2 inhibitor, SHP099, which increased potency to SHP2 and allowed for an effective tethering site via an amide bond. They chose to use VHL-1 ligand to recruit the VHL E3 ligase and systematically varied the linker length. They found that degraders with short linkers (2-7 methylene groups) demonstrated little or no degradation whereas compounds with 8-14 methylene groups exhibited 80% to >95% degradation in a KYSE520 cell line. Upon optimisation of linker composition, they discovered that incorporation of a piperazinyl group led to degraders which showed near complete degradation of SHP2 at 100 nM in both KYSE520 and MV4:11 cell lines. They further evaluated SHP2-D26 in a dose dependant manner and found that it achieves DC_{50} values of 6.0 nM and 2.6 nM towards SHP2 in KYSE520 and MV4:11 cell lines respectively. They next evaluated the kinetics of SHP2-D26 induction of SHP2 degradation in KYSE520 and MV4:11 cell lines. In KYSE520 cells, SHP2-D26 at 100 nM effectively degrades SHP2 after 4h and complete degradation after 8 h. Similar results were seen in MV4:11 cells.

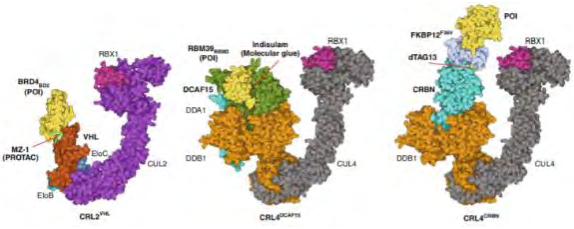
In addition to assessing the degradation profile of SHP2-D26, they also assessed its inhibitory abilities. They found that SHP2-D26 is >30 times more potent than SHP099 (existing SHP2 inhibitor) at inhibition of ERK phosphorylation.

Contributor: Nikolai

Targeted protein degradation as a powerful research tool in basic biology and drug target discovery

Tao Wu, . . ., Eric S. Fischer*

Nat. Struct. Mol. Biol., **2020** DOI: 10.1038/s41594-020-0438-0



Another excellent paper in the growing collection of TPD reviews, with a focus on the application of degraders as research tools in order to answer complex biological questions. This well-written and well-illustrated review covers small molecule–induced degradation, genetically encoded degradation tags and the use of protein conjugates. In addition, the common validation methods for targeted protein degradation are briefly discussed.

This comprehensive review covers the recent developments in the TPD field. Worth reading!

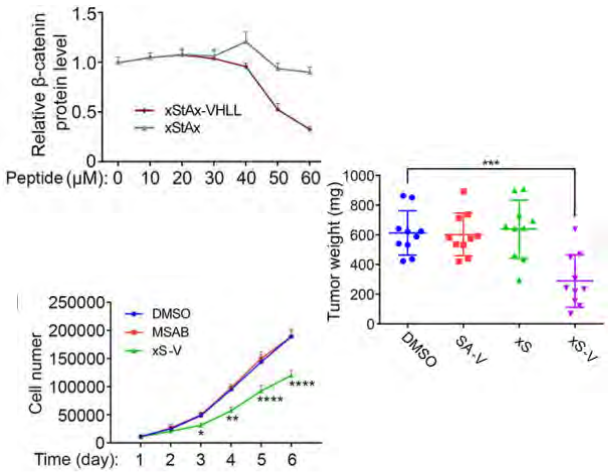
Contributor: Claire

A PROTAC peptide induces durable β-catenin degradation and suppresses Wnt-dependent intestinal cancer

Hongwei Liao[§], Xiang Li[§], Lianzheng Zhao[§], . . ., Hong-Gang Hu*, Ye-Guang Chen*

Cell Discov., **2020**, *6:35,* DOI: 10.1038/s41421-020-0171-1

Wnt/β-catenin signalling plays a key role in cell survival pathways and β-catenin, which is controlled by a destruction complex containing APC, is an attractive target for cancer therapy. SAHPA1 and xStAx peptides, known to affect β-catenin activity through activation and inhibition respectively, were used to make VHL-based peptidic PROTACs. The xStAx PROTAC peptide was shown to degrade β-catenin and inhibit Wnt signalling in cancer cell lines. In vivo activity against APC defective tumours was also observed and the xStAx PROTAC peptide reduced proliferation of colorectal cancer organoids to a greater extent than the xStAx peptide alone.



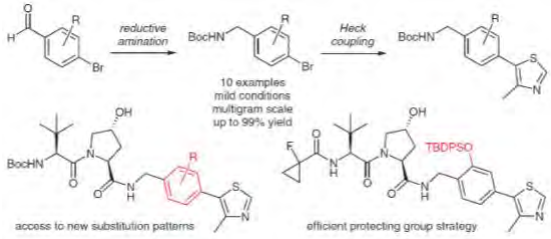
β-catenin is a perfect example of a target that needs druggability through degradation given that it has proved extremely difficult to target by small molecule inhibition alone. Although degradation of β-catenin has previously been achieved with small molecules e.g. methyl 3-{[(4-methylphenyl)sulfonyl]amino}benzoate (MSAB), this is the first published example of β-catenin-degrading PROTACs albeit via using VHL binding peptides. However, since the xStAx PROTAC peptide induced degradation at high concentrations (≥50 μM) over long treatment times (≥12 h), optimisation towards improved potency and degradation rate will be necessary to prove therapeutic viability of the xStAx PROTAC peptide approach for targeting β-catenin.

Contributor: Adam

A Facile Synthesis of Ligands for the von Hippel–Lindau E3 Ligase

Christian Steinebach, . . ., Michael Gütschow*

Synthesis, **2020***,* DOI: 10.1055/s-0040-1707400



The VHL E3 ligase plays a central role in the PROTAC field with a vast number of degraders consisting of a VHL recruiting ligand. The authors have developed an efficient synthetic protocol to VH032 based VHL inhibitors and handles for PROTAC design. They describe a diverse series of Boc-protected 4-bromobenzylamines accessed by an efficient reductive amination with triethylsilane and trifluoroacetic acid. This, followed by a palladium catalysed Heck coupling allows access to new substitution patterns on the central phenyl group of the VHL ligand. They also provide a new TBDPS O-protecting group strategy for the synthesis of phenolic VHL ligands with overall yields of 16-22%.

Previous syntheses of the VHL ligand started from a 4-bromobenzonitrile and subsequent low yielding Heck coupling followed by nitrile reduction strategies. This new strategy provides an improved alternative.

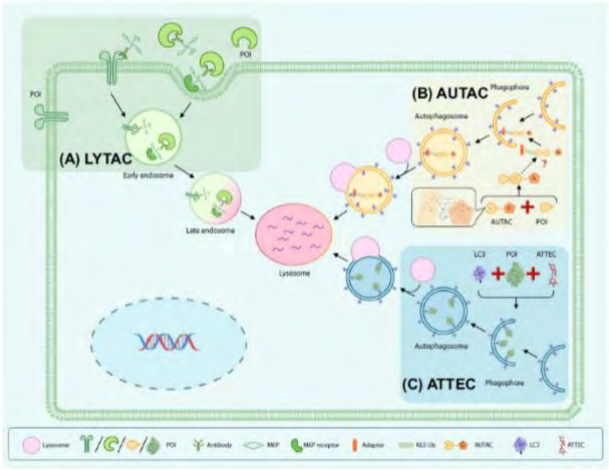
Contributor: Claire

Emerging New Concepts of Degrader Technologies

Yu Ding*, Yiyan Fei*, Boxun Lu*

Trends Pharmacol. Sci., **2020***, 41:7,* 464*,* DOI: 10.1016/j.tips.2020.04.005

This review article highlights the importance of degrader technologies and gives an overview of the PROTAC field in the context of MDM2, cereblon and VHL-based PROTACs while also touching on the development of light-controlled PROTACs. It goes on to discuss emerging chimeric degraders and the details of their mechanisms of action. LYTACs utilise lysosome-mediated degradation and have been shown to degrade extracellular and plasma membrane-associated targets. AUTACs can induce ubiquitination of cytosolic proteins and organelles and degradation via the autophagy pathway. ATTECs trigger degradation by the autophagosome, independent of ubiquitination and have potential for the degradation of non-protein targets such as DNA/RNA. Potential applications of degrader technologies are also highlighted, such as targeting protein aggregates in neurodegenerative diseases and microbial pathogens to overcome antibiotic resistance.



A nice overview of PROTACs and the latest developments in the targeted protein degradation field! The table in particular provides a great summary on the pros, cons and applications of each technology.

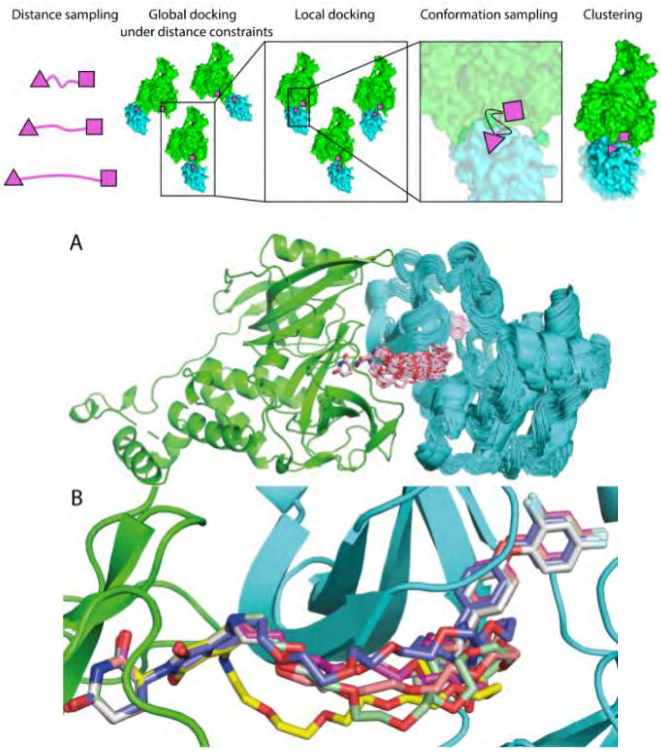
Contributor: Adam

PRosettaC: Rosetta based modelling of PROTAC mediated ternary complexes

David Zaidman, Nir London*

bioRvix, **2020***,* DOI: 10.1101/2020.05.27.119354

Due to the flexibility of linkers present in most PROTACs, and the many possible protein-protein interactions that they can mediate between the E3 ligase and target protein, accurate prediction and modelling of ternary complexes is difficult. In this preprint, the authors have developed a promising multi-step protocol which helps to model and predict PROTAC mediated ternary complexes. Their so-called PRosettaC protocol combines several computational docking software's such as: PatchDock, RosettaDock and Rosetta Packer. They were able to recapitulate 6/10 known ternary complexes which they chose to validate their method.



One of the ternary complexes they applied their protocol to was Brd4:dBET23:CRBN. They were able to rank the near native complex top with the modelled protein-protein interactions and thalidomide and Brd4 binder moieties accurately matching the crystal data. Interestingly, the structure with the highest score observed a flip of the thalidomide phthalimide which forced the linker to exit from the opposite atom compared to the original structure. With this finding, the authors hypothesised a potential for

macrocyclisation and have placed a nice figure in their supplementary information highlighting this. They sought to predict unknown ternary complexes of BTK-targeting PROTACs and CRBN. Their findings recapitulated experimental trends found by Zorba et al. in 2018 and led to high confidence predictions of a PROTAC-induced BTK-CRBN ternary complex.

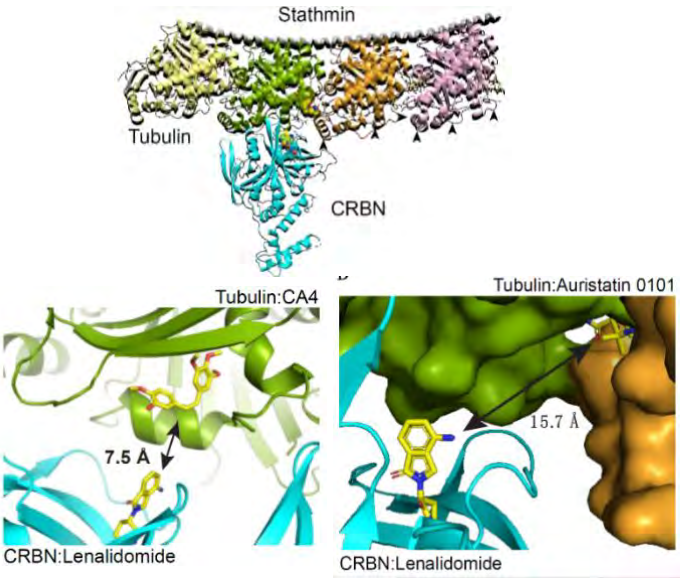
A really nice paper with very promising results. Predicting and modelling ternary complexes is very challenging and this new protocol has us moving in the right direction! It was also nice to see that they applied their protocol to the macrocyclic PROTAC developed by our group and ranked the near native cluster third which recapitulated the ligand binding moieties to sub-angstrom accuracy.

Contributor: Claire

Tubulin Resists Degradation by Cereblon-Recruiting PROTACs

Ivana Gasic[§], Brian J. Groendyke[§], . . ., Nathanael S. Gray*, Timothy J. Mitchison*

Cells, **2020***, 9,* 1083*,* DOI: 10.3390/cells9051083



The microtubule cytoskeleton consists of a network of filaments of α- and β-tubulin that is responsible for intracellular transport and organisation of the cytoplasm. Microtubule dysregulation is associated with human diseases such as neurodegenerative disorders and cancer. Tubulins have been successfully targeted in cancer therapy but this has been ineffective in neurodegenerative diseases. Here, using molecular docking to predict optimal linker length, a panel of covalent PROTACs, based on microtubule-destabilizing agents, monomethyl auristatin E (MMAE) and combretastatin A-4, were engineered in an attempt to degrade α- and β-tubulin. Tubulin ligands that were previously reported to degrade α- and β-tubulin were used to benchmark the PROTACs. However, these cereblon-recruiting PROTACs did not degrade tubulin even at high concentrations (up to 10 μM) yet the MMAE PROTAC did retain the microtubule-destabilising properties of the parent inhibitor.

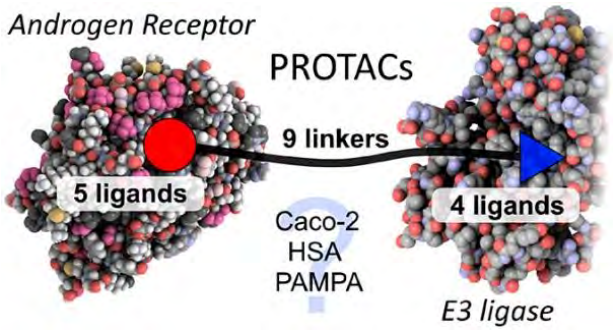
Given that tubulins are in high abundance and have complex homeostasis, cycling between soluble and polymerized states, they represent a tricky target for PROTAC development. Additionally, cell proliferation can affect tubulin levels independent of degradation, therefore requiring short time course degradation assays (here performed at 5 h). Observing PROTAC activity at short time points requires efficacious compounds with fast target degradation kinetics, driven by slow ternary complex dissociation kinetics. The extent to which the authors have investigated ternary complex characteristics is unclear. Further PROTAC optimisation towards improving ternary complex stability could push these compounds into the realms of at least partial degradation within short treatment times.

Contributor: Adam

Systematic Investigation of the Permeability of Androgen Receptor PROTACs

Duncan E. Scott, . . ., John Skidmore*

ACS Med. Chem. Lett., **2020***,* DOI: 10.1021/acsmedchemlett.0c00194



PROTACs are rule-breaking with respect to traditional medicinal chemistry guidelines as most possess high molecular weights, high TPSA and so go beyond the rule of 5. Despite this, PROTACs still show impressive in cellulo activity and potency.

The authors hypothesised that PROTAC permeability is mediated by either linker conformational collapse in water or that the two ligands of the PROTAC pass through the membrane in a pseudo-independent fashion.

They set out to evaluate the impact of linker, protein-ligand and E3 ligase ligand on the permeability of a series of AR targeting PROTACs. They firstly designed a series of PROTACs which systematically varied the three PROTAC components. Five SARM based AR-ligands were chosen which had a wide range of permeability from low (1.4 x 10-6 cm/s) to high (13.3 x 10-6 cm/s) PAMPA Papp values. These were connected by a PEG linker to either CRBN ligands lenalidomide and pomalidamide, or a hydrophobic adamantyl tag.

A series of VHL recruiting PROTACs were also developed which focused on varying linker composition and length. For the first set of PROTACs, a panel of ADMET properties: PAMPA permeability, HSA binding and chromLogD7.4, were selected for their potential to impact cell activity. Almost all PROTACs in the PAMPA assay resulted in very low permeability, or below the limit of quantification of the assay. Interestingly, PROTAC ***19****, comprised of the most permeable AR ligand and the hydrophobic adamantyl tag, showed moderate PAMPA permeability (2.3 × 10−6 cm/s).* ***19****, helped by the lipophilic adamantyl group, gave a high LogD and this is presumed to have assisted in the permeability. Caco-2 permeability was assessed for the VHL PROTAC set which varied the linker composition. Although low passive permeability in PAMPA is observed with PROTACs, they found that Caco-2 permeability was much better at comparing the efflux ratios for linkers, and ligand structural influences.*

They conclude that due to low PAMPA and Caco-2 A2B permeability, it is clear that PROTAC permeability is not "rule-breaking". However, they do recommend to use Caco-2 permeability assay as they found that it sheds more light on the permeability profiles of some PROTACs. As PROTACs are catalytic in nature, only a small amount is required to pass into the cell for effective degradation.

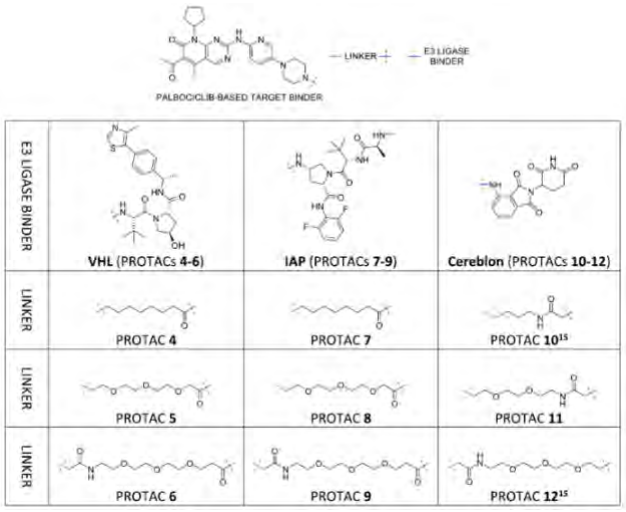
Contributor: Claire

Selective CDK6 degradation mediated by cereblon, VHL, and novel IAP-recruiting PROTACs

Niall A. Anderson[§], Jenni Cryan[§], . . ., Andrew B. Benowitz*

Bioorg. Med. Chem. Lett., **2020***, 30:9,* 127106*,* DOI: 10.1016/j.bmcl.2020.127106

FDA-approved CDK4 and CDK6 inhibitors, including palbociclib, are used for breast cancer treatment. Here, palbociclib-based PROTACs are made using binders for three different E3 ligases (VHL, cereblon and a novel IAP-binder) and employing a selection of linkers between the target binder and the E3 ligase binder. All PROTACs similarly and effectively degrade CDK4 and CDK6 and exhibit preferential CDK6 vs. CDK4 degradation.



Previous CDK4/6 palbociclib-based PROTACs targeting cereblon have also shown preferential degradation of CDK6 over CDK4, despite palbociclib having simliar inhibitory potency. This study shows that this selectivity is maintained with other E3 ligases and is another example of how PROTACs can confer selective degradation with non-selective target binders, likely due to ternary complex stability and productive conformation.

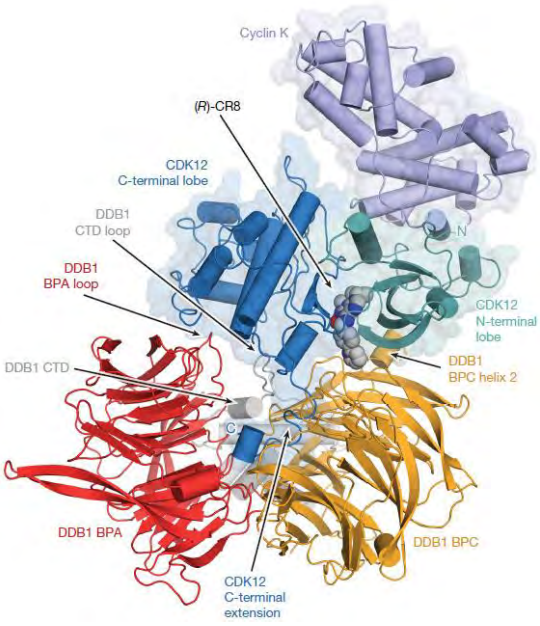
Contributor: Tasuku

The CDK inhibitor CR8 acts as a molecular glue degrader that depletes cyclin K

Mikołaj Słabicki[§], Zuzanna Kozicka[§], Georg Petzold[§], . . ., Nicolas H. Thomä*, and Benjamin L. Ebert*

Nature, **2020***,* DOI: 10.1038/s41586-020-2374-x

Molecular glues and PROTACs induce protein–protein interactions between a substrate protein and E3 ligase to facilitate ubiquitination and subsequent degradation of the substrate. Molecular glue degraders are clinically attractive due to their physicochemical profile. However, there are no systematic approaches to find this class of compounds. In this paper, the authors analysed cytotoxicity data of 4518 clinical and preclinical small molecules against 578 cancer cell lines to show correlation between cytotoxicity and expression level of 499 E3 ligase mRNA. They identified that CR8, a pan CDK inhibitor, induced proteasomal degradation of Cyclin K via CDK12-mediated recruitment to DDB1-containing cullin-RING ubiquitin ligase. They then obtained a co-crystal structure of the Cyclin K-CDK12-CR8-DDB1ΔBPB complex revealing that CR8 induces protein-protein interactions between CDK12 and DDB1 without any substrate receptors and Cyclin K coordinated to CDK12, not DDB1. Their results implied that modification of solvent-exposed regions of known small molecular binders could be converted to molecular glue degraders of target proteins.



This is a third structural report of molecular glue degraders following IMiDs and sulfonamides. Surprisingly, CR8 induced protein-protein interaction between CDK12 and the CRL adaptor DDB1 without substrate recognition proteins, not like the other two cases. In addition, they also observed that minor modification of solvent exposed region of CR8 affected formation of the ternary complex. Their findings could not only allow expanding the

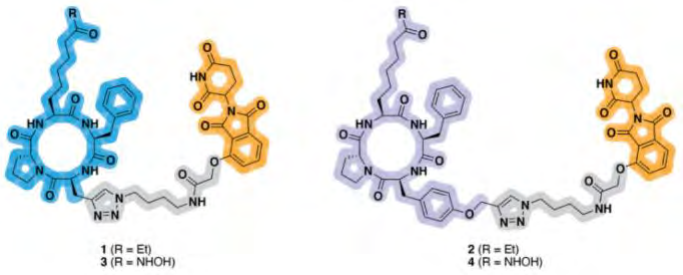
repertoire of E3 ligases for PROTACs but also provide novel way to develop molecular glues to induce degradation of proteins of interest from small molecular binders.

Contributor: Adam

Proteolysis-Targeting Chimeras (PROTACs) Based on Macrocyclic Tetrapeptides Selectively Degrade Class I Histone Deacetylases 1–3

Martin Roatsch, …, Christian Adam Olsen*

ChemRvix, **2020***,* DOI: 10.26434/chemrxiv.12416303.v1



The authors chose to target class I HDACs for degradation by using a trapoxin based, macrocyclic peptide class I HDAC inhibitor, conjugated to a CRBN ligand via a clickable thalidomide-azide building block. They set out to make a small series of four PROTACs which would address the effects of both linker length and zinc-binding strength. Two containing a strong hydroxamic acid zinc-binder, and two containing a weaker ethyl ketone zinc binder. Position 3 of the macrocycle was chosen for the linker attachment point via alkyne containing amino acids, L-propargylglycine and propargylated tyrosine which underwent a Cu(I)-catalysed Huisgen [3+2] cycloaddition with a thalidomide-azide linker.

*They first assessed the selectivity and inhibition of each compound to HDACs from different classes in vitro. All compounds were selective to class I HDACs 1-3 with the most selective compounds containing the weaker zinc-binder and the most potent compounds possessing a longer linker. They next assessed the inhibition kinetics towards class I HDACs and found that compounds with an ethyl ketone (**1** and **2**) exhibited fast-on/off rates whereas compounds with a hydroxamate (**3** and **4**) bound more tightly.*

*Preliminary degradation studies in HEK293T cells revealed that only compounds **2** and **4** which contained the longer tyrosine-based linkers decreased HDAC2 levels with the ethyl ketone-bearing compound **2** showing more pronounced degradation. The authors comment on the findings saying "Slow, tight-binding kinetics can be beneficial for occupancy-driven pharmacology (classical enzyme inhibitors), but might be unfavourable for event-driven pharmacology (PROTACs)". Compound **2** was further investigated in a time dependant manner and was found to efficiently degrade HDAC2 after 2-4 hours at 300 nM treatment. Long term exposure resulted in HDAC2 levels returning to 'normal'. Optimal degradation was also seen for HDAC1 and HDAC3 after 4 h at 100-300 nM. Finally, the authors showed that compound **2** exhibits selective degradation for HDAC1-3 with no significant degradation of HDAC4-9 and 11.*

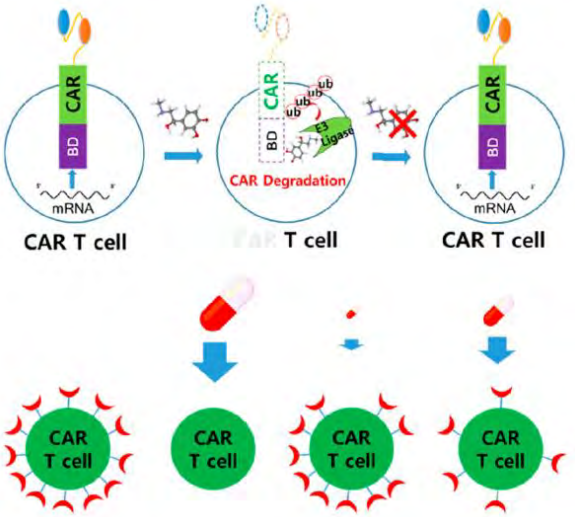
During the preparation of this work, another group reported other class I HDAC degraders which were based on a different HDAC targeting scaffold. Although efficient degradation was observed for HDAC1-3, the effects on other HDACs was not reported. This is a first example of a PROTAC employing a macrocyclic peptide as a target protein ligand.

Contributor: Claire

A Chemical Switch System to Modulate Chimeric Antigen Receptor T Cell Activity through Proteolysis-Targeting Chimaera Technology

So Myoung Lee, . . ., Chi Hoon Park*

ACS Synth. Biol., **2020**, *9*, 987, DOI: 10.1021/acssynbio.9b00476



Chimeric antigen receptor (CAR) T cell therapy redirects T cells to recognise surface antigens on cancer cells and destroy them. Whilst a huge advancement in the immune-oncology space that have shown excellent efficacy, there are safety concerns related to aberrant upregulation of CAR T cell activity that can trigger a lethal cytokine storm response i.e. they work so well at annihilating cancer cells that the body has an overwhelming systemic inflammatory response to the amount of tumour-specific cytotoxicity. Clinical trials are underway to test an iCas9 system for overcoming this but this irreversible destroys the CAR T cells therefore reinfusions are required. To overcome this, the authors modify the CAR construct to bear a bromodomain (BD) which did not affect CAR function. They then target the BD-CAR protein with BD-degrading PROTACs ARV771 (VHL targeting) and ARV825 (cereblon targeting). Both PROTACs induced degradation of BD-CAR protein and suppressed CAR T cells lytic activity against a leukaemia cell line in a concentration-dependent and reversible manner. This approach has potential for use as a safety system in which CAR T cells can be "reversibly" controlled by a PROTAC.

Given the substantial expense of CAR T cell therapy, reversibly targeting CAR T cells via PROTAC-induced suppression of activity is a very interesting concept. Being able to modulate the CAR T response in this way could allow a patient to receive multiple rounds of CAR T activity from only one infusion and in a controlled manner after recovery from any signs of adverse effect, thereby potentially avoiding a severe cytokine storm response. Here, partial BD-CAR degradation and reduced BD-CAR T cell lytic activity are shown after 12 h of treatment. How feasible this is to suppress adverse immune responses, which often manifest and worsen rapidly, remains to be seen. Given that this is the first publication of this nature, it will be interesting to see how this approach fares in future clinical development. Watch this space!

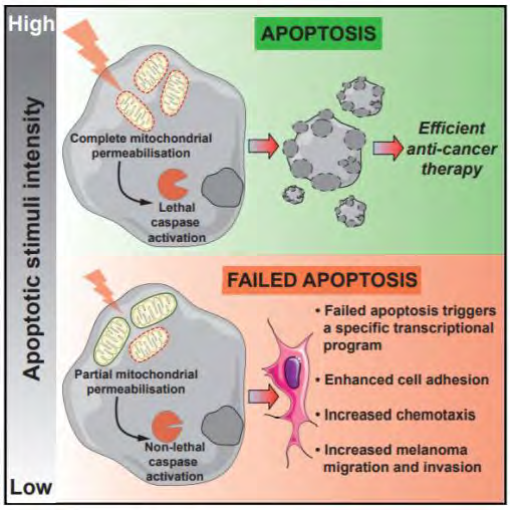
Others

Contributor: Vesna

Failed Apoptosis Enhances Melanoma Cancer Cell Aggressiveness

Kevin Berthenet, . . ., Gabriel Ichim*

Cell Rep., **2020**, *31,* 107731, DOI: 10.1016/j.celrep.2020.107731



Apoptosis is one type of cell death than can be triggered in cancer cells. The central event in apoptosis is permeabilisation of outer mitochondrial membrane (MOMP), often referred to as a point of no return. However, some exception to the rule have been identified, such as incomplete MOMP and minority MOMP, where the apoptotic stimuli is not strong enough to cause full permeabilisation and death. Therefore, the cells don't die, but the apoptotic execution machinery has been activated. This can lead to genomic instability, oncogenic transformation or damaged DNA. In this study, the authors wanted to characterise changes in cell behaviour after caspases have been sub-lethally activated. They have found that melanoma cancer cells show a change in transcriptomic signature associated with focal adhesions, migration and modifications to the actin cytoskeleton. Also, these cells gained migration and invasion properties in vitro and in vivo. The authors have connected this new behaviour to be regulated via the JNK pathway which was found to be upregulated in metastatic melanoma. This study shows how a death stimulus can act as a double edged sword and the cells that survive it are the ones responsible for metastasis, drug resistance and relapse.

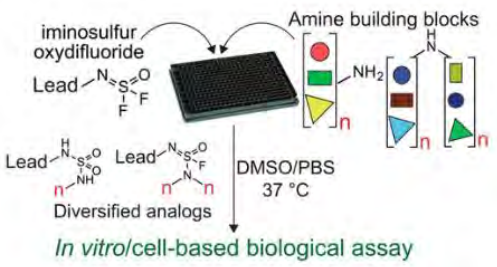
It is known that sub-lethal doses of chemotherapy can result in increased metastatic potential and resistance to drugs but not many studies have been done to characterise that fully. Although chemotherapy isn't the first line of treatment for melanoma anymore, it is important understand why it only worked in less than 10% of patients and why should our focus be on developing other types of therapies like targeted therapy or immunotherapy.

Contributor: Ross

Sulfur(VI) Fluoride Exchange (SuFEx)-Enabled High-Throughput Medicinal Chemistry

Seiya Kitamura[§], Qinheng Zheng[§], . . ., K. Barry Sharpless*, Dennis W. Wolan*

J. Am. Chem. Soc., **2020**, DOI: 10.1021/jacs.9b13652



Optimisation of small molecule probes and drugs is a long, challenging and expensive process. This paper utilises a biocompatible sulfur(VI) fluoride exchange (SuFEx) click chemistry to generate a HTS library, imparting chirality into the generated compound (rather than the planar triazole in the classic 'click' reaction) performed on picomolar scales.

*From a moderately potent initial hit (**1**, IC_{50} = 14 µM), two iminosulfur oxydifluoride derivatives were generated (**2** and **3**) and subsequently converted into 460 analogues through overnight incubation and directly screened for inhibition, directly leading to drug-like inhibitors with far higher potency (**5**, K_i = 18 nM), and activity against SpeB (**7**,*

K_i = 67 nM). Molecules derived from secondary amines were shown to exhibit higher potencies than from primary amines.

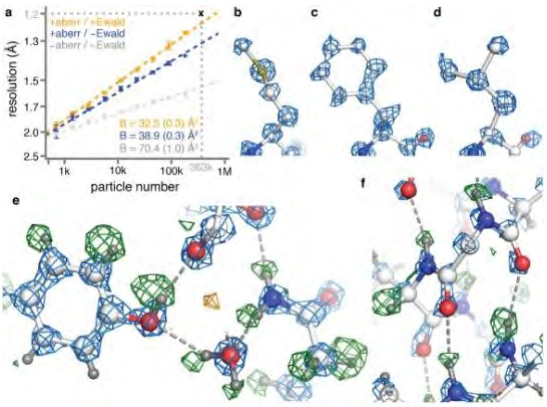
This paper highlights the use of SuFEx chemistry for rapidly generating diversified molecules for hit to lead application, alongside the efficiency of 'click' chemistry and immediate subsequent biological testing. This method could prove useful for speedy diversification of planar hit compounds.

Contributor: Angus

Single-particle cryo-EM at atomic resolution

Takanori Nakane[§], Abhay Kotecha[§], Andrija Sente[§], . . ., A. Radu Aricescu*, Sjors H.W. Scheres*

bioRxiv, **2020**, DOI: 10.1101/2020.05.22.110189



Structural information increases with resolution, and cryo-EM has experienced a steady increase in maximum and routinely achievable resolution since the advent of direct electron detectors. The previous record for maximum resolution of 1.54 Å was achieved with apoferritin. Here, using a new cold field emission electron gun, the latest generation Falcon detector and a new energy filter, the authors managed to smash the old record and achieve atomic resolution with a 1.2 Å structure of mouse apoferritin. Concurrently and using similar tech, another group also managed to reach atomic resolution, with a 1.25 Å structure of the same protein (https://doi.org/10.1101/2020.05.21.106740). At this resolution, individual atoms, as well as some hydrogen atoms, are resolved. Using the new tech, the authors also obtain a 1.7 Å structure of the clinically relevant $GABA_A$ receptor, an exceptional improvement on previous results (2.5-3 Å).

The cryo-EM train rolls on, smashing through the atomic resolution barrier with this paper and a second one released on the same day on bioRxiv from Holger Stark's group (https://doi.org/10.1101/2020.05.21.106740). This result would have been unthinkable a decade ago (or even more recently!) and represents and incredible leap forward for cryo-EM. Of course, there are some caveats to these results. As with x-ray crystallography, most proteins are unlikely to be stable enough to achieve atomic resolution like the rock-like apoferritin. Apoferritin also has 24-fold symmetry which reduces the amount of data collection required in cryo-EM. Proteins with lower or no symmetry are generally much more challenging and require far more data to reach high resolution. Still, the new technological advancements presented in these two papers will improve the routinely achievable resolution range for more difficult and medically relevant proteins. As cryo-EM resolution continues to improve, structure-based drug design using cryo-EM becomes a more feasible prospect.

Contributor: Emelyne

Quest for a COVID-19 Cure by Repurposing Small-Molecule Drugs: Mechanism of Action, Clinical Development, Synthesis at Scale, and Outlook for Supply

Chris De Savi*, David L. Hughes*, and Lisbet Kvaerno

Org. Process Res. Dev., **2020**, DOI: 10.1021/acs.oprd.0c00233

The outbreak of the COVID-19 pandemic has spurred an intense global effort to repurpose existing approved drugs for its treatment. In this review, the authors highlight the development of seven small-molecule drugs that are currently being assessed in clinical trials for the treatment of COVID-19. Three sections are presented for each drug: (1) history, mechanism of action, and status of clinical trials; (2) scalable synthetic routes and final forms; and (3) outlook for supply should clinical trials show treatment efficacy. A brief overview of diagnostic testing and vaccine development is also presented.

Drug repurposing has gathered increased interest, particularly as one of the fastest ways to fight against COVID-19 (Nature Biotech. 2020, 38, 379-381). It has also been of interest in other fields such as oncology (metformin, thalidomide), neurodegenerative and rare diseases. For recent reviews on examples, opportunities and challenges see: Eur. J. Med. Chem. 2020, 195, 112275 and Expert Opin. Drug Dis. 2020, 15 (4), 397-401.

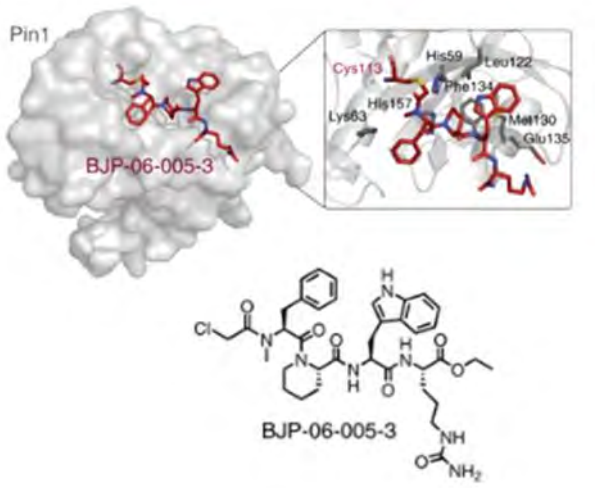
Contributor: Siying

Identification of a potent and selective covalent Pin1 inhibitor

Benika J. Pinch[§], . . ., Kun Ping Lu* and Nathanael S. Grey*

Nat. Chem. Biol., **2020**, DOI: 10.1038/s41589-020-0550-9

Pin1, a peptidyl-prolyl isomerase (PPIase) which interconverts amide conformations (between cis and trans) of phosphorylated Serine and Threonine-Proline, is overexpressed in cancers, such as pancreatic ductal adenocarcinoma (PDAC). Previously reported Pin1 inhibitors suffer from poor cell permeability & selectivity. In this paper, structural guided modifications of a cell-impermeable, non-covalent inhibitor (d-PEPTIDE) led to BJP-06-005-3, a chloroacetamide-based covalent peptidic Pin1 inhibitor that is potent (apparent Ki of 15 nM measured by FP assay), selective and cell permeable.



The covalent binding mode is validated by intact mass spectrometry, pull-down experiments using a desthiobiotin-labelled BJP-06-005-3 derivative, and a high-resolution X-ray structure of a BJP-06-005-3 derivative covalently bound to Pin1. Selective cellular engagement of Pin1 is demonstrated by Covalent Inhibitor Target Site Identification (CITe-Id) studies. Using the potent inhibitor as a tool compound together with genetic approaches and dTAG, the role of Pin1 in PDAC was interrogated. Pin1 overexpression was found to cooperate with $KRAS^{G12V}$ to promote transformation in PDAC and the inhibitor reduced PDAC cell viability in a Pin-1 dependent manner. Upregulation of c-Myc (which is crucial in Ras-driven cancer growth) led the authors to investigate the effect of Pin1 inhibition on the antiproliferative effect of $FKBP12^{F36V}$-$KRAS^{G12V}$ degradation in PDAC cells. Compared to treatment of dTAG and inhibitor alone, cotreatment of the inhibitor and dTAG increases the degradation of $FKBP12^{F36V}$-$KRAS^{G12V}$ (and the antiproliferative effect). However, cotreatment of a negative control compound (which lacks the chloroacetamide moiety and therefore cannot inhibit Pin1) and dTAG also amplifies $FKBP12^{F36V}$-$KRAS^{G12V}$ degradation. Therefore, it is

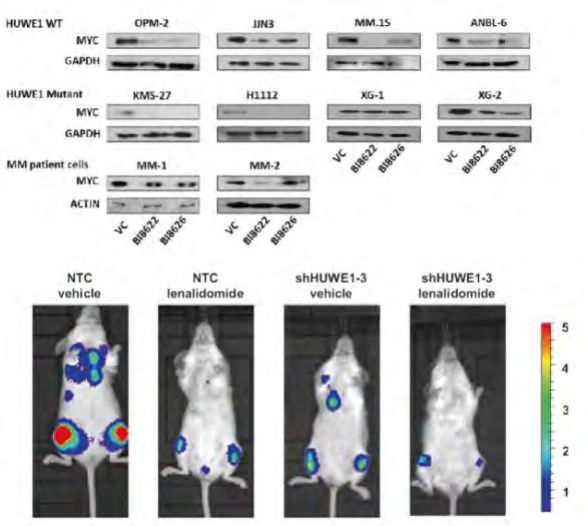
not clear whether inhibition of Pin1 using BJP-06-005-3 amplifies the degradation of the mutant KRAS construct (and therefore the antiproliferative effect).

Contributor: Oliver

The E3 ligase HUWE1 inhibition as a therapeutic strategy to target MYC in multiple myeloma

Lisa J. Crawford*, . . ., Alexandra E. Irvine

Oncogene, **2020***,* DOI: 10.1038/s41388-020-1345-x



This work supports and builds upon earlier findings which delineate an important role for the HECT E3 ligase HUWE1 in multiple myeloma (MM) signalling pathways through direct regulation of c-MYC levels. The researchers demonstrate that HUWE1 levels are elevated in MM cell lines compared to normal cells, and this expression positively correlated with MYC expression in MM bone marrow cells. Genetic and biochemical knockdown of the ligase caused a concomitant reduction in MYC levels which mirrored findings that small-molecule inhibition with a compound called BI8622 inhibited growth in MM cells preferentially over normal cells. The pharmacokinetic properties of the compound are not currently favourable to in vivo studies, however shRNA targeting HUEW1 in a mouse model was shown to act synergistically with existing MM drugs including lenalidomide to reduce tumour burden.

This paper provides compelling evidence for the role of HUWE1 as a positive regulator of c-MYC levels, where previous data has been conflicting. From a targeted protein degradation standpoint, the fact that HUWE1 levels are increased in MM cells and the fact that these can be inhibited using a small molecule indicate that this HECT E3 ligase may be amenable to degradation using a homo-PROTAC-like compound based upon the existing inhibitor known as BI8622. There is no biophysical information currently for the HUWE1/BI8622 complex, however there is some data for related HECT E3 ligases in complex with various inhibitors (reviewed in https://doi.org/10.1002/cbic.201800321) which may provide some general biophysical insight and aid in optimisation of the HUWE1 inhibitor. To date, HECT-based E3 ligases have not been used to make bivalent degrader molecules but perhaps in future they might be of interest.

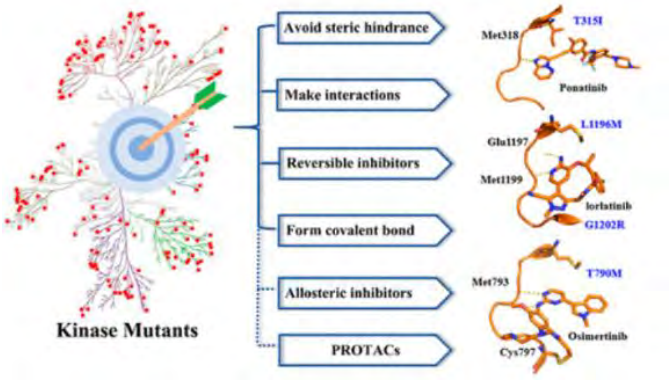
Contributor: Aileen

Medicinal Chemistry Strategies for the Development of Kinase Inhibitors Targeting Point Mutations

Xiaoyun Lu*, …, Ke Ding*

J. Med. Chem., **2020***,* DOI: 10.1021/acs.jmedchem.0c00507

Resistance to small molecule kinase inhibitors can occur after 1-2 years of clinical use and is a serious problem in cancer therapy. This perspective utilises case studies to outline medicinal chemistry strategies for overcoming drug resistance arising from mutations of kinase drug targets. Highlighted approaches include: redesign of molecules to avoid steric hindrance, seeking additional specific interactions with mutated residues, making reversible inhibitors irreversible (or vice versa), and targeting allosteric sites. The potential for targeted protein degradation to address drug resistance issues is acknowledged, and a discussion of BTK PROTACs is included.



The authors provide a thoughtful overview of both current success stories in overcoming kinase point mutation resistance, and of the remaining challenges facing the field. The article is well written and clearly set out: the figures, which include docked images of the inhibitors discussed for clarity, are particularly noteworthy.

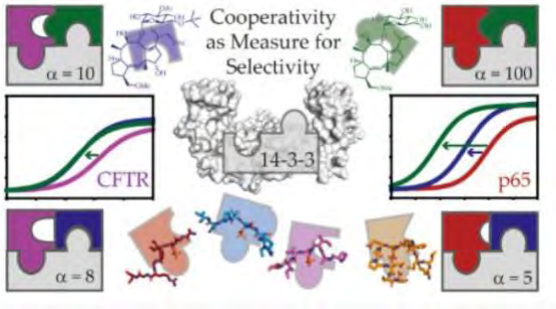
Contributor: Alessio

Selectivity via Cooperativity: Preferential Stabilization of the p65/14-3-3 interaction with Semi-Synthetic Natural Products

Madita Wolter, . . ., Christian Ottmann*

J. Am. Chem. Soc., **2020**, DOI: 10.1021/jacs.0c02151

Wolter et al. make a strong biophysical case for monitoring cooperativity in the protein-protein interaction between 14-3-3 proteins and their "client" phosphorylated substrate peptides, as mediated and stabilized by small molecules. They provide comprehensive biophysical data (fluorescence anisotropy and ITC) and structural understanding (X-ray crystallography and NMR spectroscopy) of ternary complexes that reveal some tight structure-activity relationship (SAR). They establish that a synthetic analogue of the natural compound fusicoccin aids highly cooperative stabilization between 14-3-3-gamma subunit and peptides from the p65 subunit of NF-kB. This leads to selective binding of p65, when compared to other combinations between 14-3-3 and client peptides.



Cooperativity is a well described phenomenon for ternary complex formed by PROTAC and molecular glue degraders, as well as other inducers/stabilizers of protein-protein interactions such as the 14-3-3 model system studied here. It evidences an example of remarkable cooperativity (~120 fold) – that is in line with what is observed for known cooperative molecular glues and PROTAC compounds.

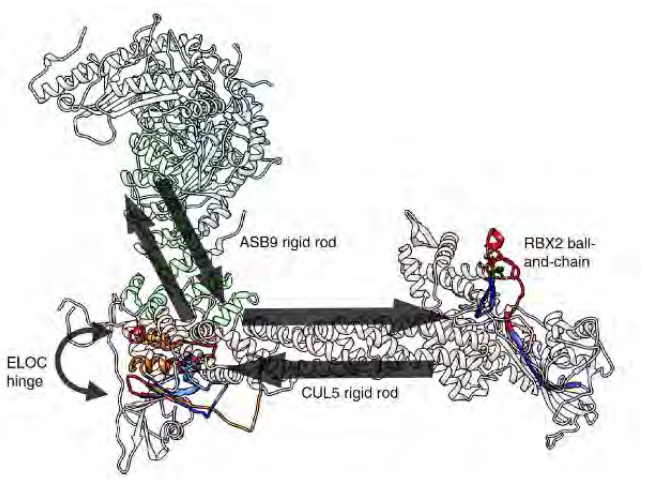
Contributor: Sarath

Structure and dynamics of the ASB9 CUL-RING E3 Ligase

Ryan J. Lumpkin, …, Elizabeth A. Komives*

Nat. Commun., **2020**, *11,* 2866, DOI: 10.1038/s41467-020-16499-9

The Cullin 5 (CUL5) Ring E3 ligases (CRL5) use adaptors, Elongins B and C (ELOB/C), to bind different SOCS-box-containing substrate receptors, determining substrate specificity. The 18-member ankyrin and SOCS box (ASB) family is the largest substrate receptor family of CRL5. Here the authors report cryo-EM data for the substrate, creatine kinase (CKB) bound to ASB9-ELOB/ C, and for full-length CUL5 bound to the RING protein, RBX2, which binds various E2 conjugating enzymes. To date, no full structures were available for a substrate-bound ASB or for CUL5. Hydrogen–deuterium exchange (HDX-MS) mapped onto a full structural model of the ligase revealed long-range allostery extending from the substrate through CUL5. The authors propose a revised allosteric mechanism for how CUL-E3 ligases function. ASB9 and CUL5 behave as rigid rods, connected through a hinge provided by ELOB/C transmitting long range allosteric crosstalk from the substrate through CUL5 to the RBX2 flexible linker.



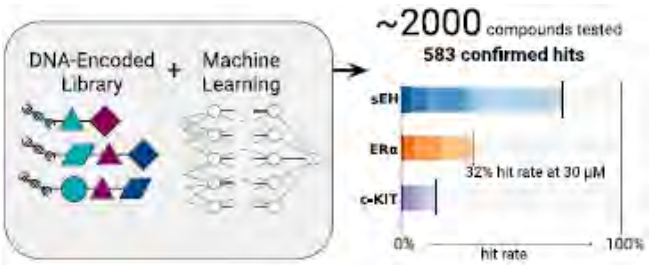
Contributor: Will

Machine Learning on DNA-Encoded Libraries: A New Paradigm for Hit Finding

Kevin McCloskey[§], Eric A. Sigel[§], . . ., Patrick Riley*

J. Med. Chem., **2020**, DOI: 10.1021/acs.jmedchem.0c00452

Here in, the authors use experimental DEL selection data as the basis for training machine learning methods, which in turn, are used to perform virtual screening of commercially available compounds. Automated filters are used to prioritise molecules for screening, producing high hit rates (in the best case 29% at 1 μM). They apply this approach to three protein targets sEH (a hydrolase), ERα (a nuclear receptor), and c-KIT (a kinase) and in each case identify hits that are structurally diverse and are novel with respect to the original DEL libraries themselves.



I read this as an interested observer, rather than in any way an expert reader, but the potential utility is clear and nicely presented. A good example of how machine learning can be used to complement and potentially enhance established methods.
