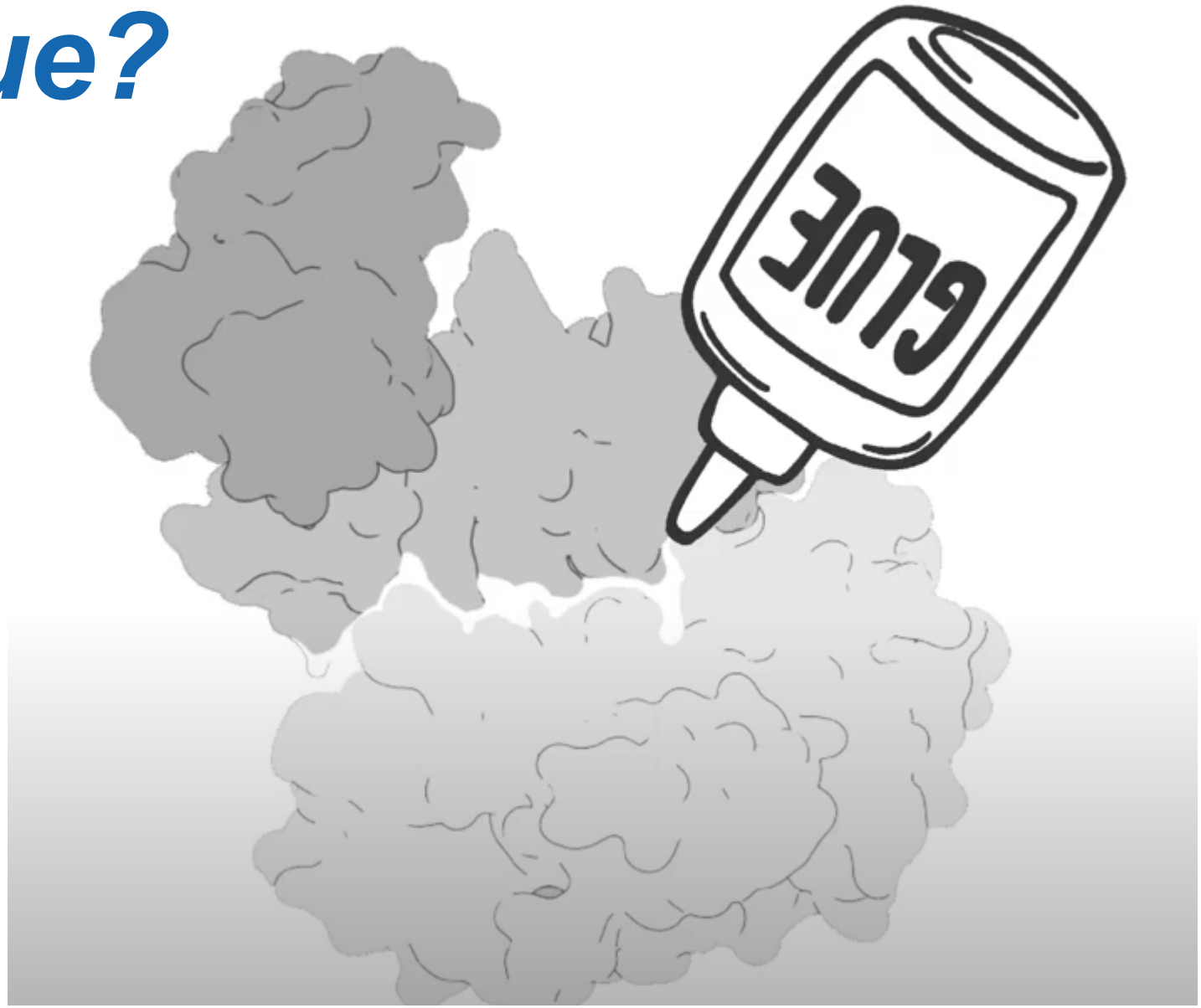
Haven’t got a glue?

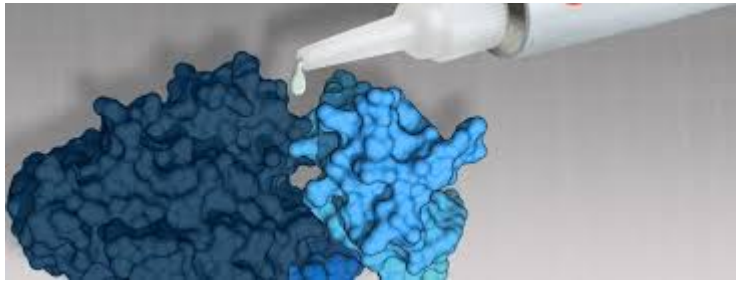
Small molecule glue for protein interaction

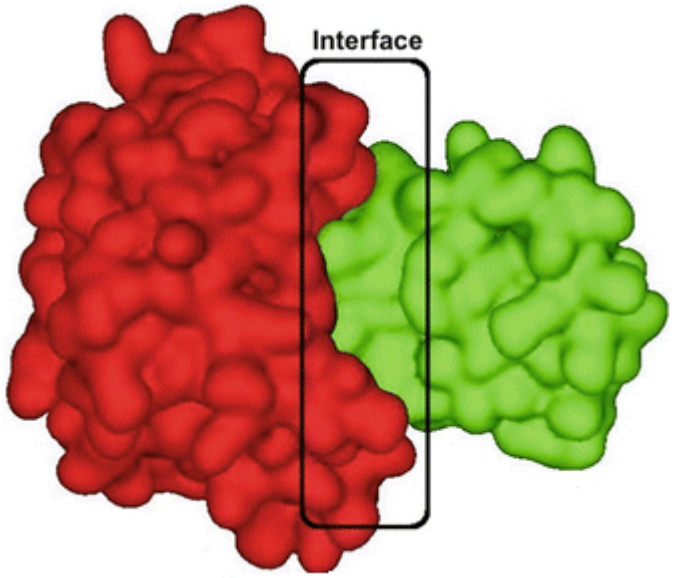


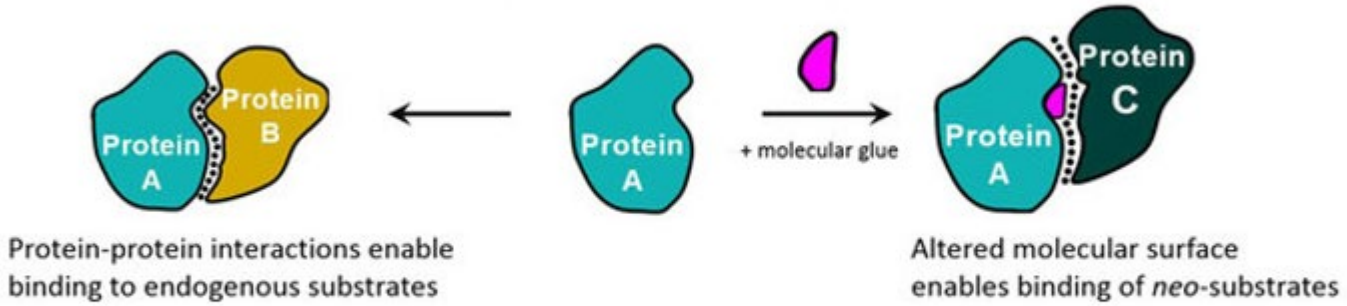
2021.06.01

HUI ZHANG

What is molecule glue?







Protein-protein interactions enable binding to endogenous substrates

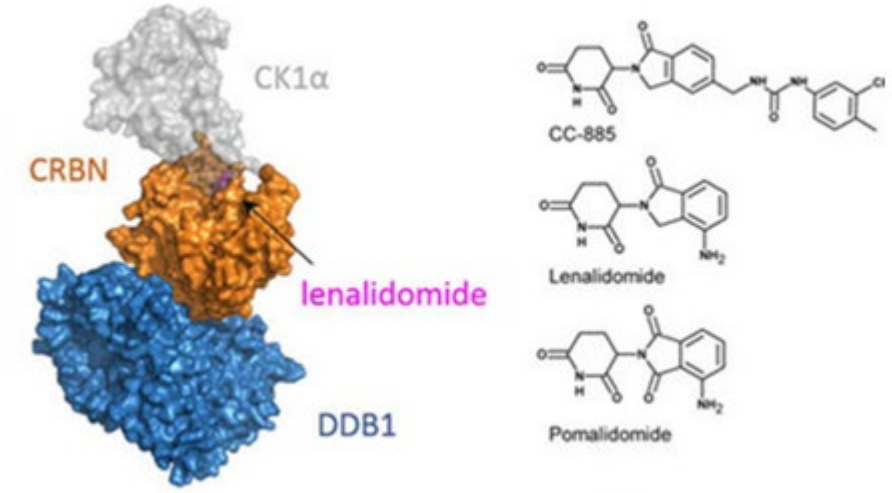
Altered molecular surface enables binding of *neo*-substrates

- Receptor blocker
- Protein degrader

Essays in Biochemistry 61(5):505-516

Thalidomide analogues

Revlimid® (lenalidomide) capsules
2.5 · 5 · 10 · 15 · 20 · 25 mg



- Effective treatment for Multiple myeloma;
- Molecular target: CRBN, substrate receptor of the cullin-RING E3 ubiquitin ligase CUL4A/B–RBX1–DDB1–CRBN ($CRL4_{CRBN}$)12

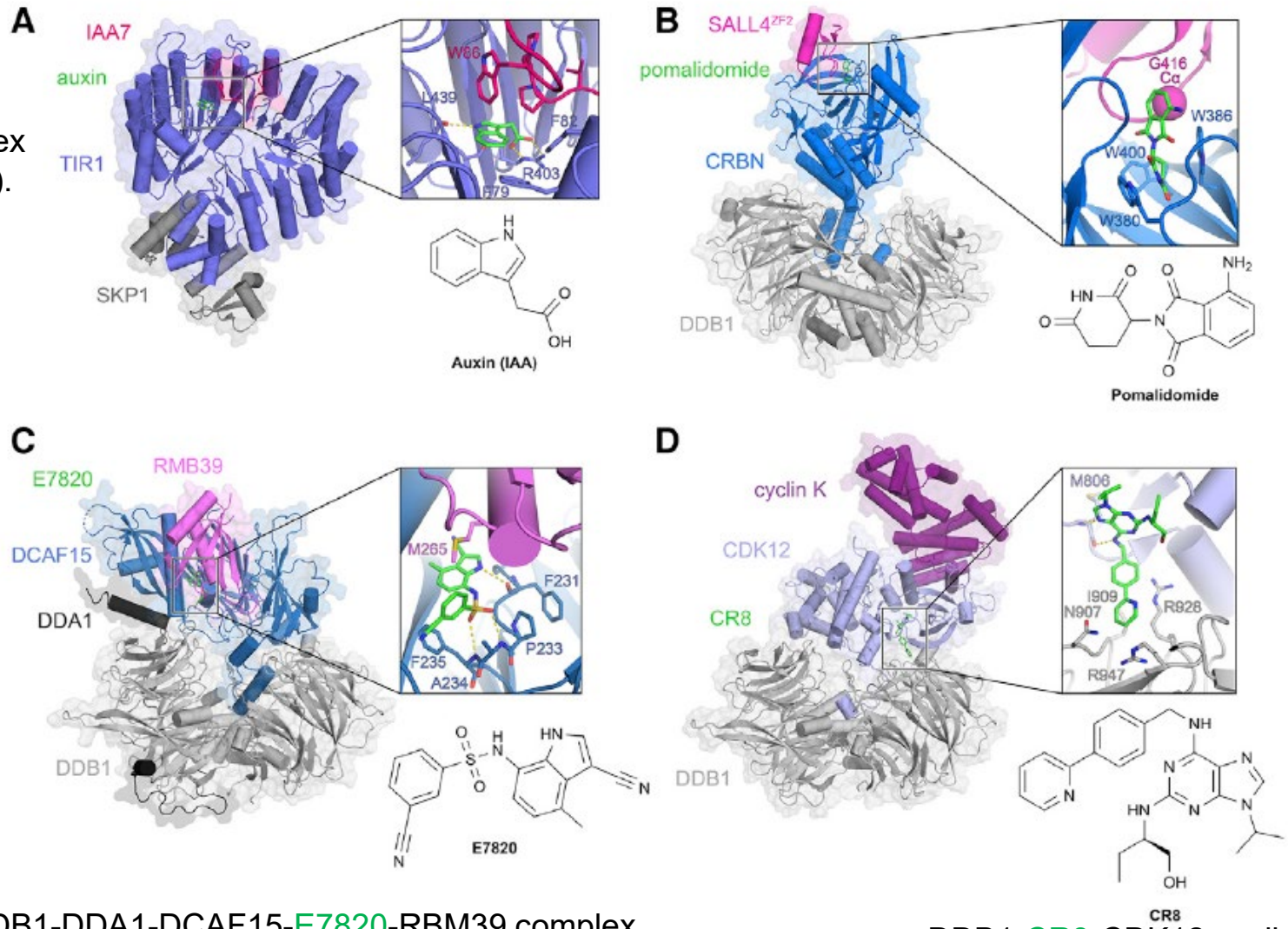
    - Novel PPI with CK1α
    - CUL4–Rbx1–DDB1–CRBN E3 ubiquitin ligase complex

Essays in Biochemistry 61(5):505-516

Overview of degrader-induced interfaces

SKP1-TIR1-auxin-IAA7 complex (PDB: 2P1Q) (Tan et al., 2007).

DDB1-CRBN-pomalidomide-SALL4ZF2 complex (PDB: 6UML) (Matyskiela et al., 2020a).



DDB1-DDA1-DCAF15-E7820-RBM39 complex (PDB: 6PAI) (Du et al., 2019).

DDB1-CR8-CDK12-cyclin K complex (PDB: 6TD3) (S1abicki et al., 2020a).

Article

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

https://doi.org/10.1038/s41586-020-2374-x

Received: 9 November 2019

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Article

Small-molecule-induced polymerization triggers degradation of BCL6

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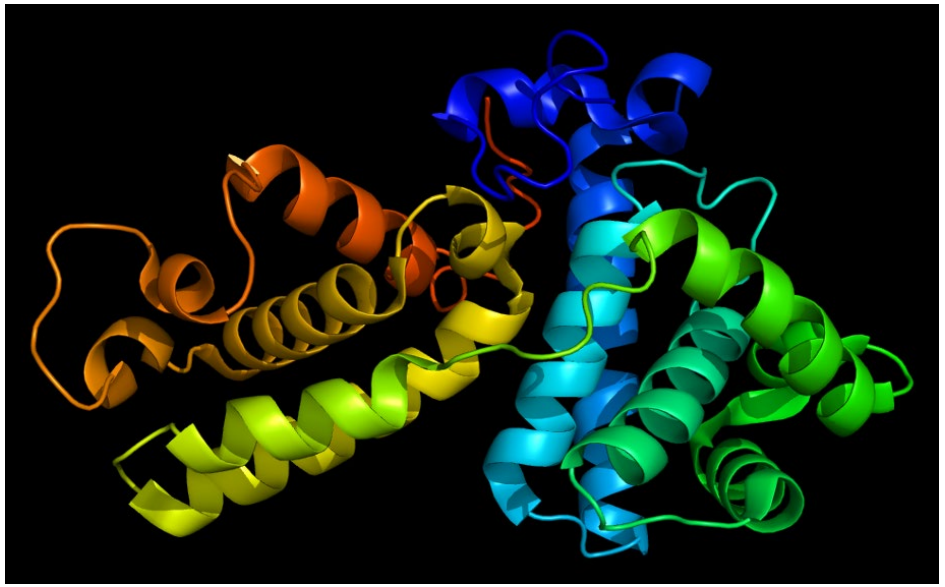
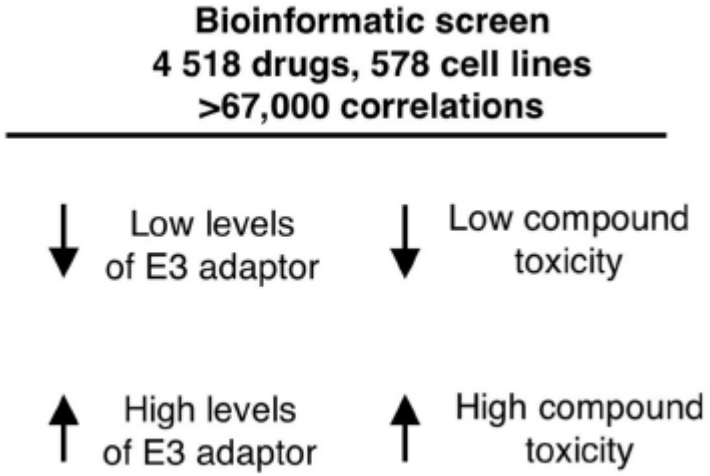
Cyclin K

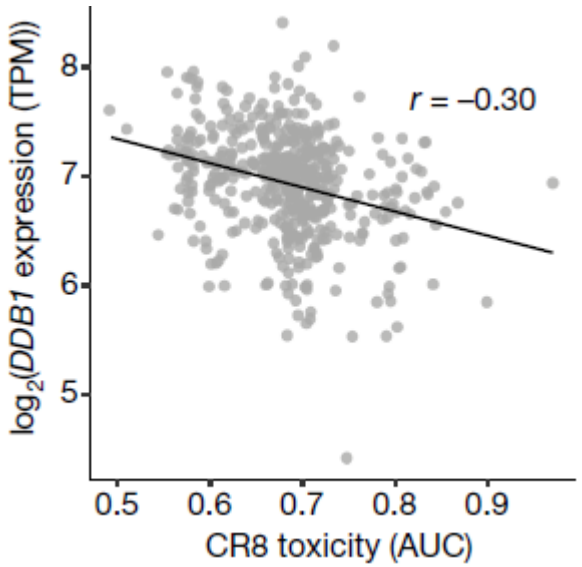
- **Cyclin-K** is a protein that in humans is encoded by the CCNK gene and a member of the transcription cyclin family.
- These cyclins may regulate transcription through their association with and activation of cyclin-dependent kinases (CDKs) through conformational changes.
- Interaction with multiple CDKs including CDK9 and latest CDK12 and CDK13, HIV nef protein.
- Indispensable for Leukemia growth.



Screen for small molecules that mediate protein degradation through an E3 ubiquitin ligase



Drug sensitivity data
mRNA levels of 499 E3 ligase components

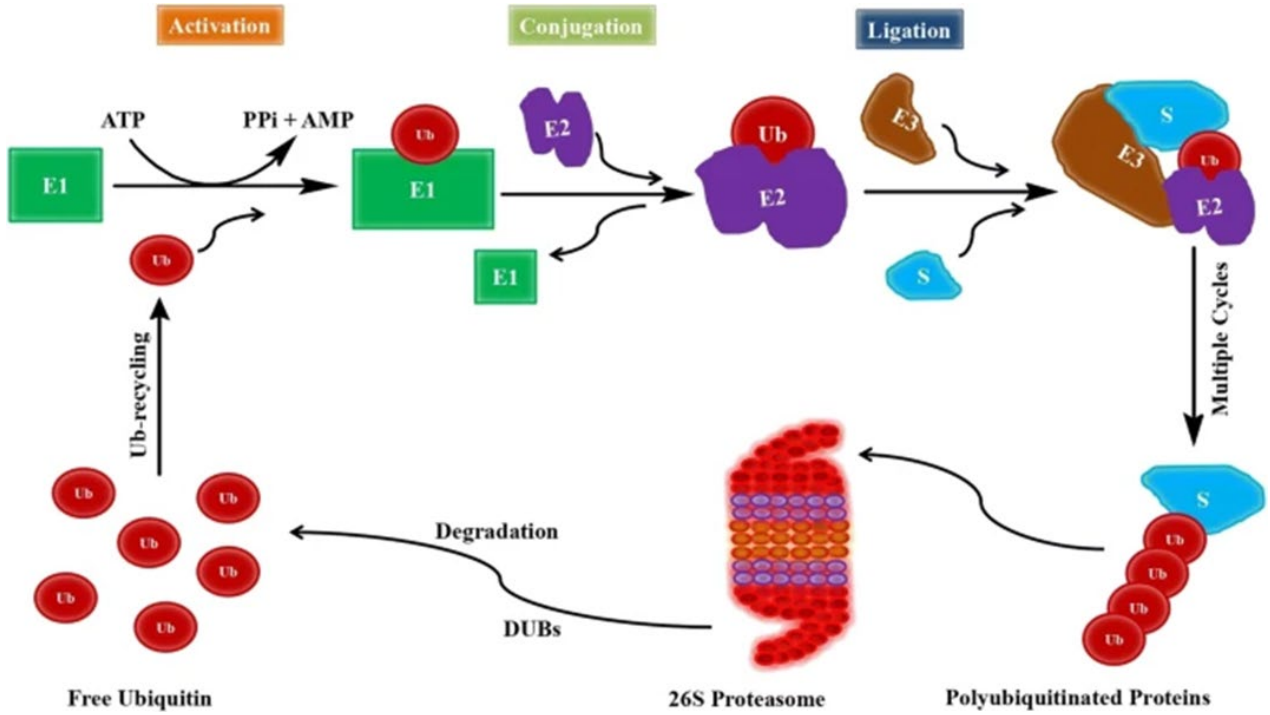


CR8: CDK inhibitor

Ranking	Gene	Drug
1	DCAF15	indisulam
2	DDB1	CR8
3	DCAF15	tasisulam

DDB1: CUL4 adaptor protein

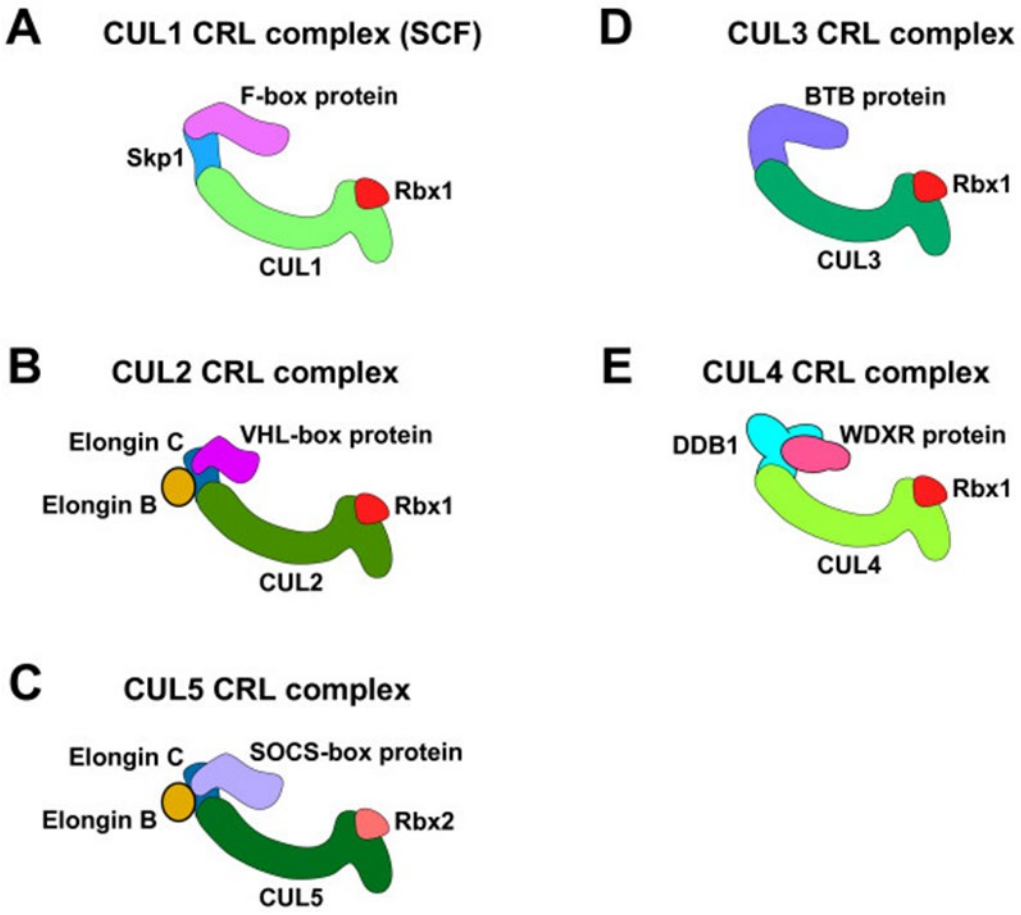
The ubiquitin-proteasome system



Ubiquitin ligases provide the substrate specificity for ubiquitination (ubiquitylation) reactions.

Neurotoxicity Research **volume 38**, pages8–17 (2020)

cullin-RING finger ligases (CRLs)

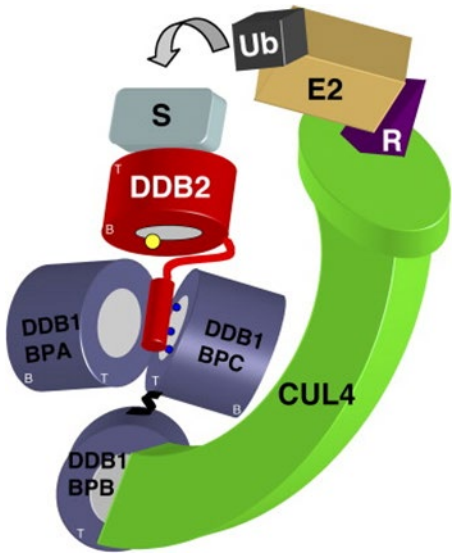


Multisubunit complexes:

- A cullin,
- A RING finger protein,
- A substrate-recognition subunit (SRS), an adaptor subunit that links the SRS to the complex.

Cell Div. 2008; 3: 7

CUL4-DDB1 Ubiquitin Ligase



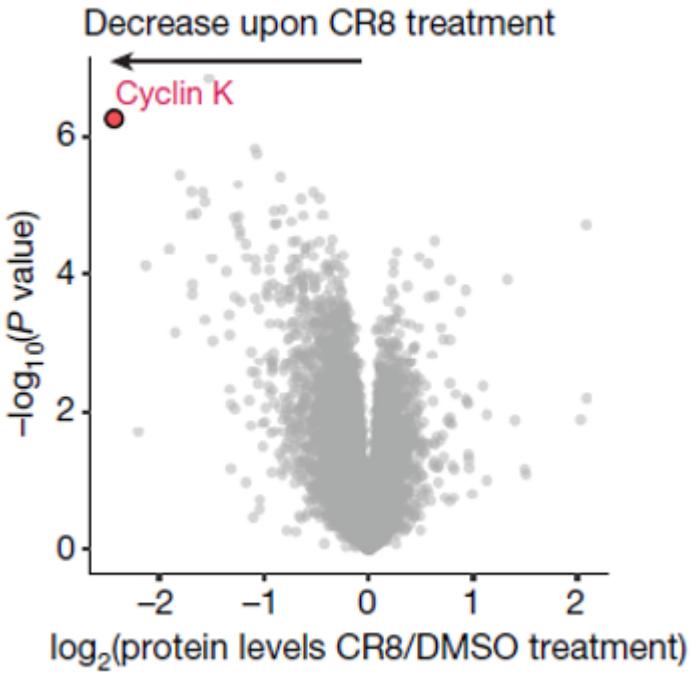
- CUL4A ----a rigid scaffold
- N-terminal ----DDB1-BPB
- C-terminal ----Rbx1 and the E2 ubiquitin-conjugating enzyme.

- The DDB1-BPA and DDB1-BPC double propeller folds into a clam-shaped structure that binds DCAFs and regulators of the CUL4 CRL.

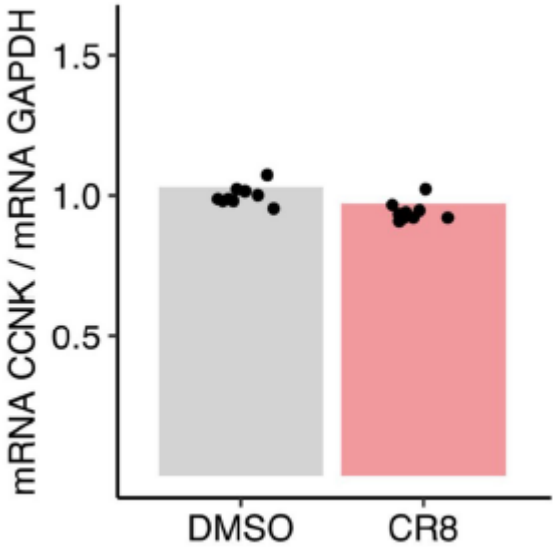
BPB: β propeller B
DCAFs: DDB1-CUL4-associated factors

Jennifer Lee, 2007

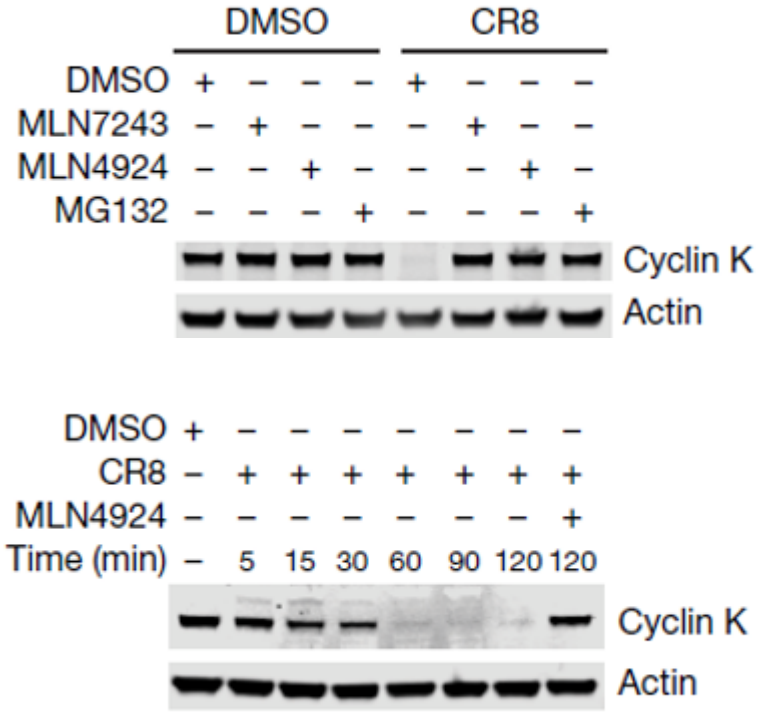
Quantitative proteome-wide mass spectrometry



Cyclin K was the only protein that consistently showed a decrease in abundance after addition of CR8



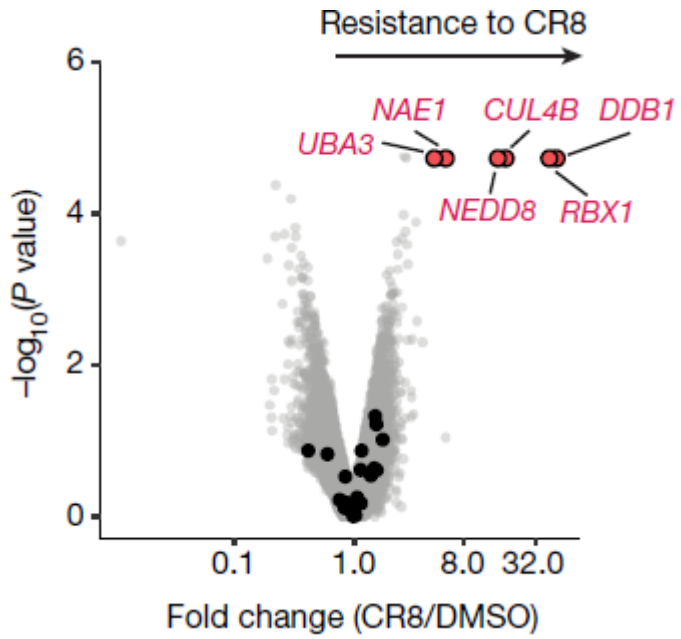
CR8 did not alter the levels of cyclin K mRNA



Rescued by inhibition of the E1 ubiquitin-activating enzyme (MLN7243), inhibition of cullin neddylation (MLN4924) and inhibition of the proteasome (MG132) CR8 triggers rapid proteasomal degradation of cyclin K through the activity of a DDB1-containing cullin-RING ubiquitin ligase.

Dissection of the molecular machinery

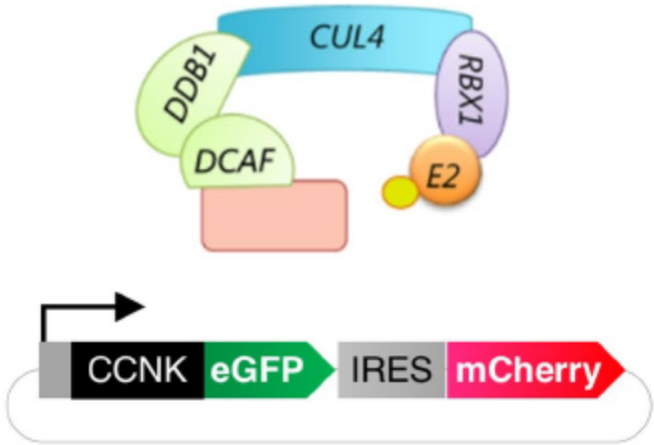
Genome-wide and E3 ubiquitin ligase-focused CRISPR–Cas9 resistance screens



DDB1, CUL4B, RBX1, the cullin-RING activator NEDD8 and the NEDD8-activating enzyme (NAE1 and UBA3) were substantially enriched in the CR8-resistant cell population. CUL4–RBX1–DDB1ubiquitin ligase complex is involved in CR8 cytotoxicity.

No substrate receptors like DCAFs were identified

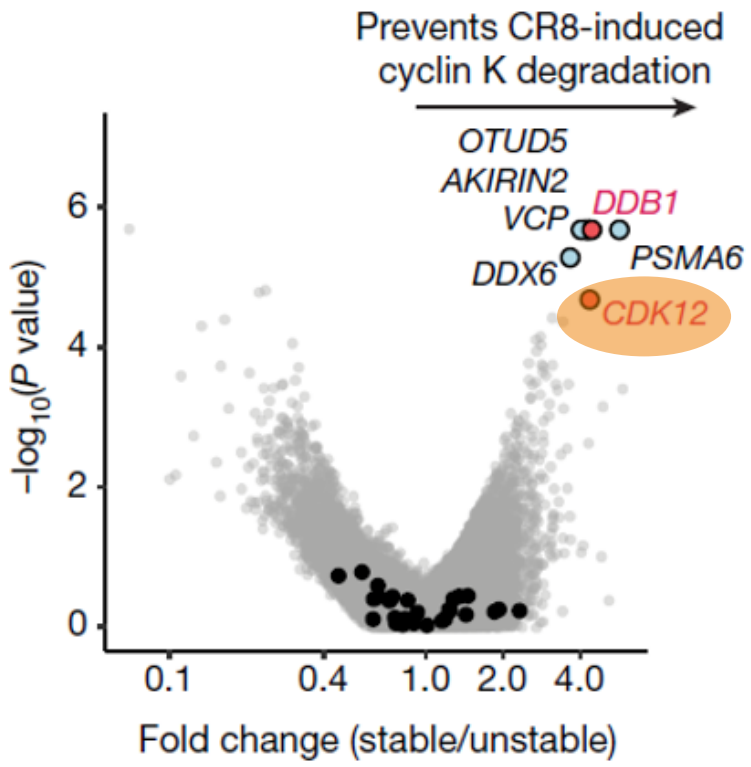
DCAFs: DDB1-CUL4-associated factors



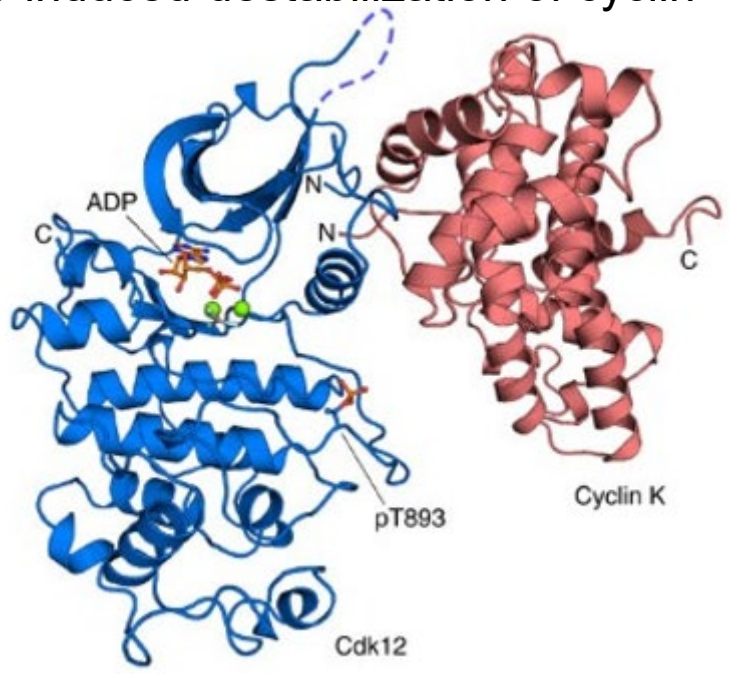
Reporter of cyclin K stability

The extent of degradation can be determined by measuring the levels of cyclin KeGFP normalized to mCherry expression

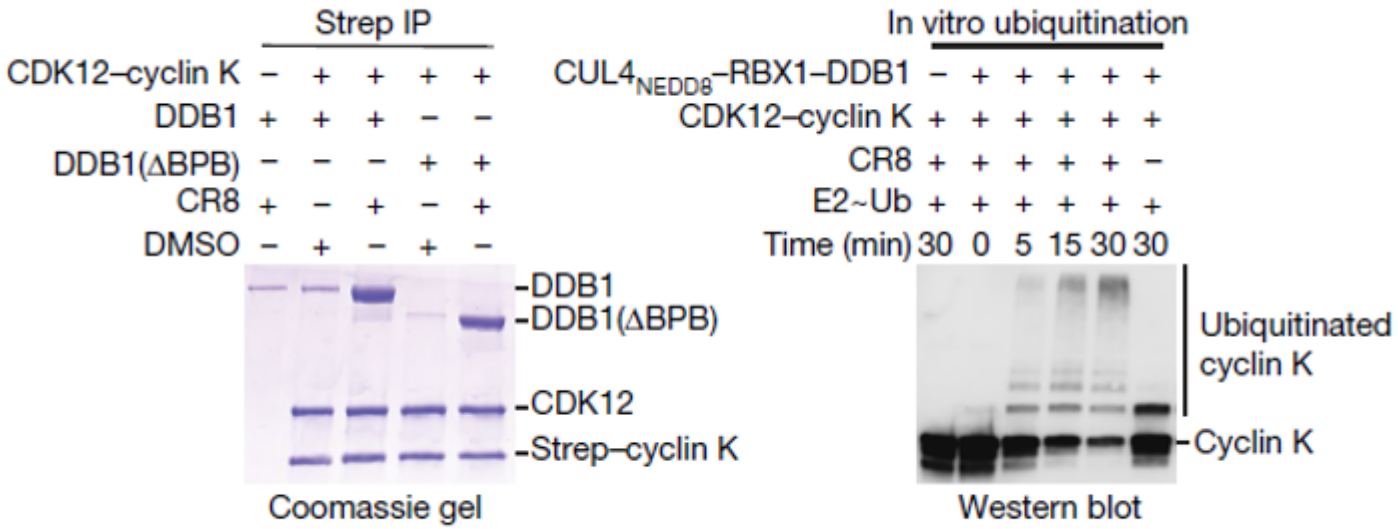
CRISPR–Cas9 screen for genes involved in cyclin K reporter stability



- Genome-wide CRISPR–Cas9 screen for genes involved in cyclin K reporter stability and validated the involvement of DDB1 in CR8-mediated, but not CR8-independent, degradation of cyclin K.
- CDK12—a known target of CR8 that depends on the interaction with cyclin K for its activity—as a crucial component for CR8-induced destabilization of cyclin KeGFP.



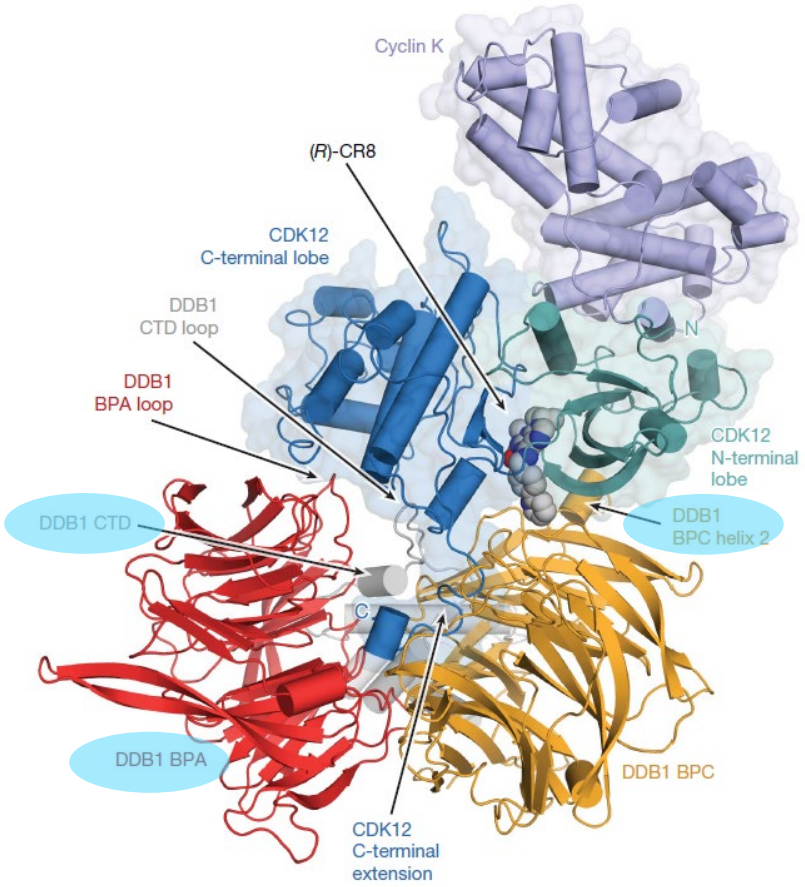
CR8-bound CDK12 binds DDB1 in a DCAF-like manner



CDK12 bound to cyclin K did not markedly enrich DDB1 over the bead-binding control in the absence of CR8, whereas equimolar amounts of CR8 led to stoichiometric complex formation.

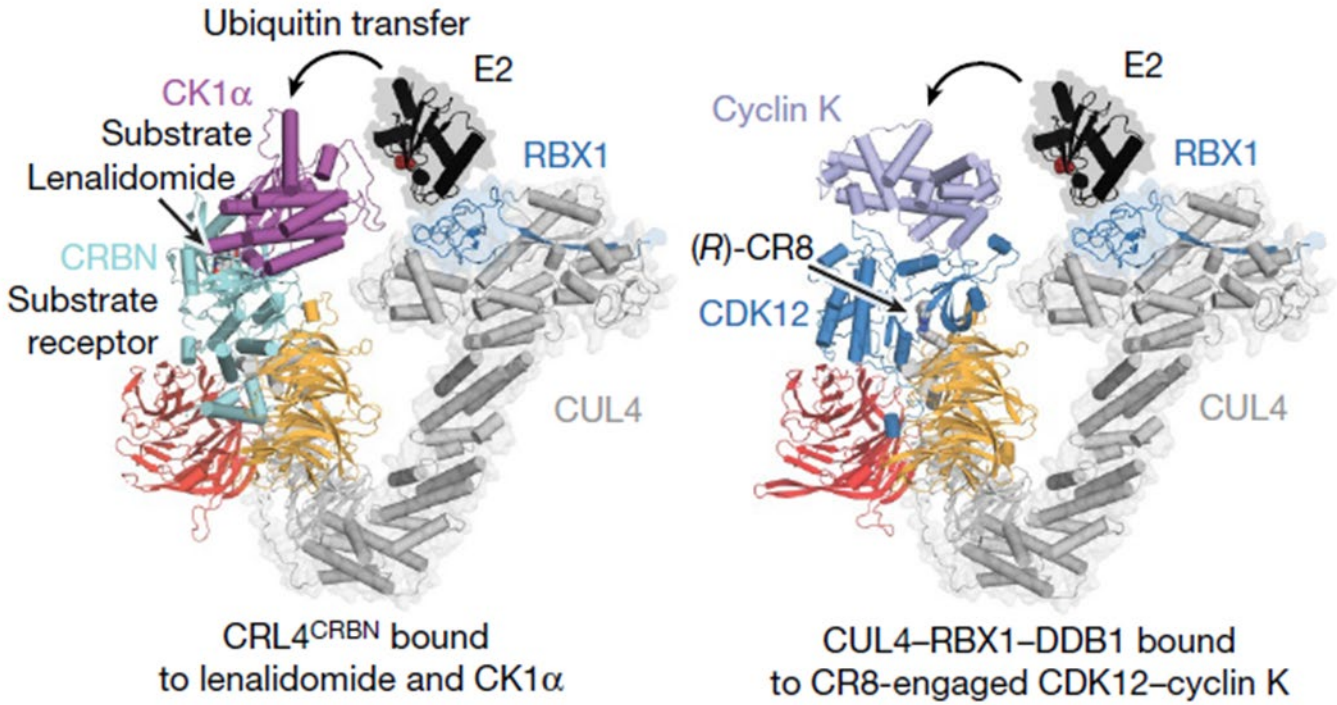
CUL4A–RBX1–DDB1 ligase core alone is sufficient to drive robust ubiquitination of cyclin K.

Crystal structure of DDB1-CR8-CDK12-cyclin K

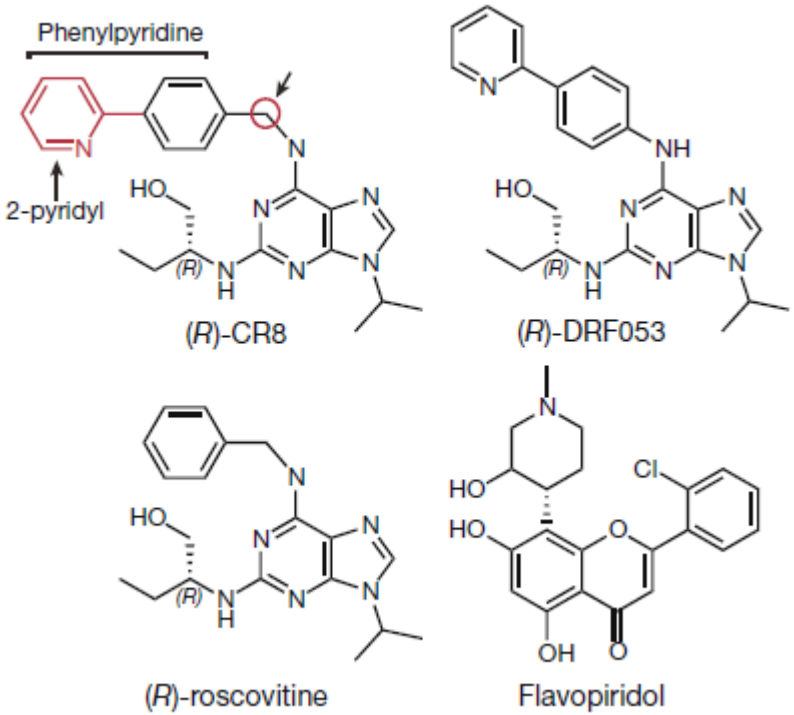


- CDK12 forms extensive protein–protein interactions with DDB1.
- CR8 binds the active site of CDK12 and bridges the CDK12–DDB1 interface, whereas cyclin K binds CDK12 on the opposite side and does not contact DDB1.
- The N-terminal and C-terminal lobes of CDK12 are proximal to DDB1 residues located in a loop of the BPA domain (amino acids 111–114), helix 2 of the BPC domain (amino acids 986–990) and a loop in the C-terminal domain (amino acids 1078–1081), which are otherwise involved in DCAFs binding.

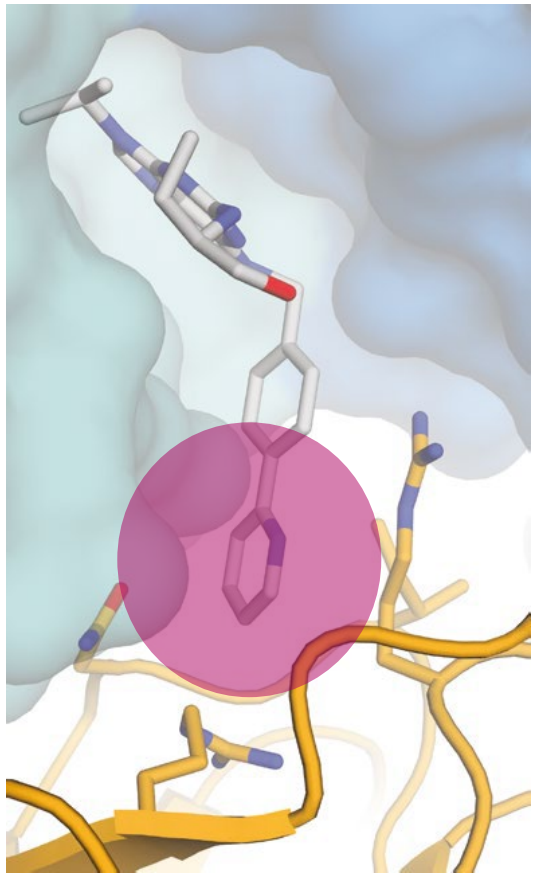
Crystal structure model of lenalidomide and CR8



Chemical structures of CDK inhibitors

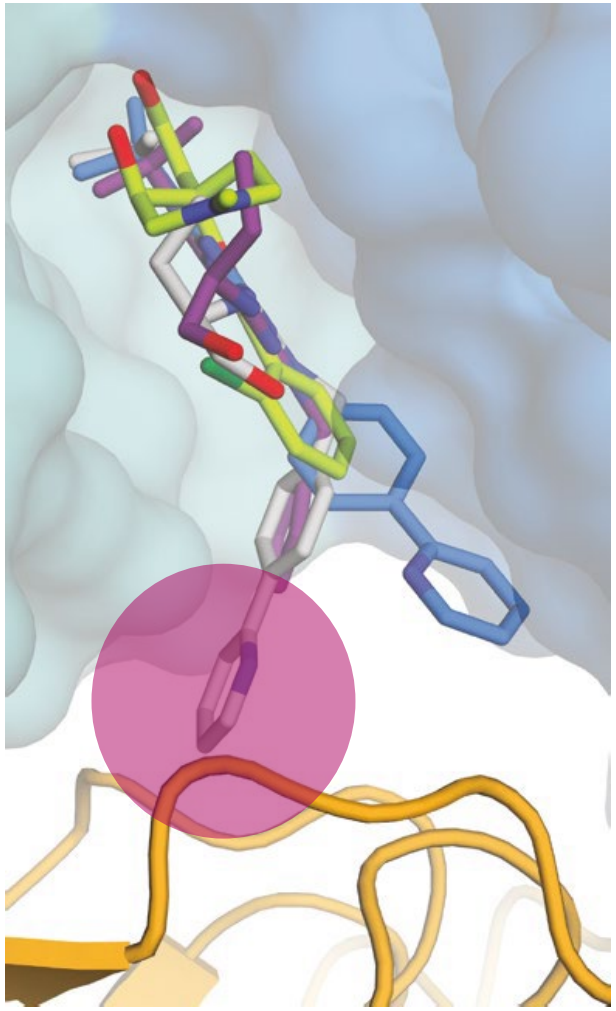


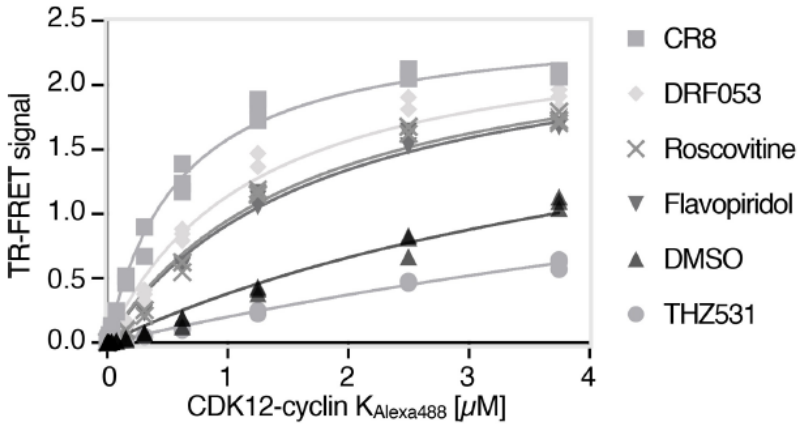
DDB1–CR8–CDK12 interface



A surface-exposed 2-pyridyl moiety of CR8 confers glue degrader activity

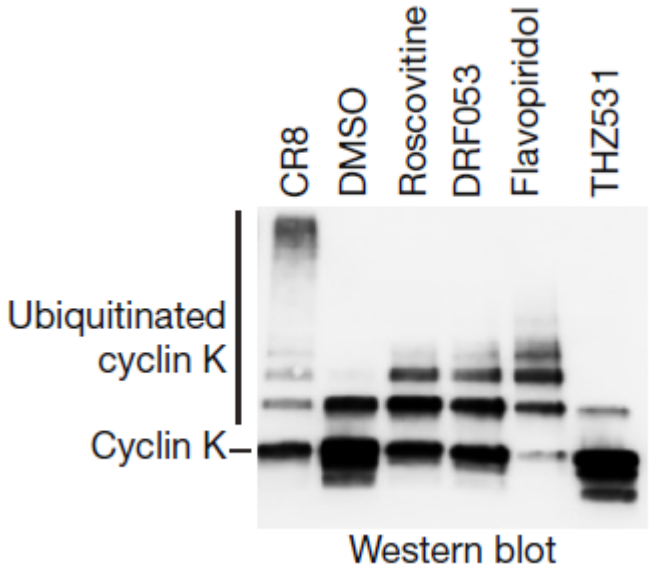
Other CDK inhibitors showed lower affinity than CR8

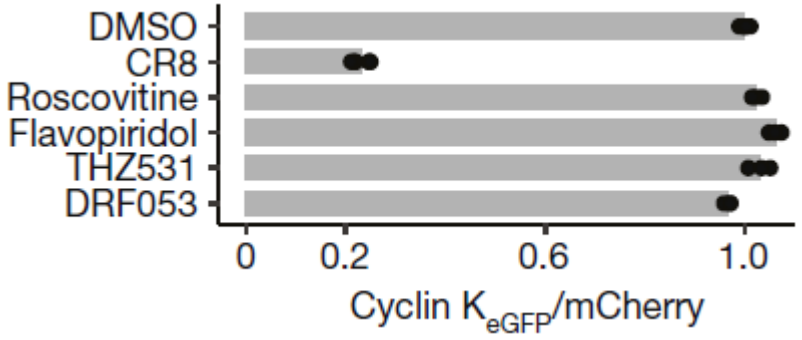




Compound	$K_{apparent}$ [μM]
CR8	0.51±0.03
DRF053	0.98±0.12
Roscovitine	1.44±0.18
Flavopiridol	1.53±0.17
DMSO	n.d.
THZ531	n.d.

CR8 phenylpyridine confers glue activity





- Ubiquitination assay and cyclin KeGFP reporter in cells confiamation.
- The presence and correct orientation of the 2-pyridyl moiety on the surface of CDK12 confer the gain-of-function activity of CR8 that leads to cyclin K degradation.

Summary

- A bioinformatics screen for compounds whose cytotoxicity correlates with ligase mRNA levels across different cell lines pointed to CR8, a preclinical cyclin-dependent kinase (CDK) inhibitor, as a possible degrader. CR8 induces proteasomal cyclin K degradation.
- The compound was found to be a novel type of a molecular glue degrader that binds the heterodimeric target complex CDK12-cyclin K and recruits the DDB1-CUL4-RBX1 E3 ligase core to ubiquitinate cyclin K.
- Through structural elucidation, CR8 was shown to bind the active site of CDK12, with a phenylpyridine moiety extending out of the pocket and into the interface, making contacts with several DDB1 residues
- Recruitment of a traditionally undruggable neosubstrate (cyclin K) to the ligase via a ligandable protein partner (CDK12) is a viable strategy.

Article

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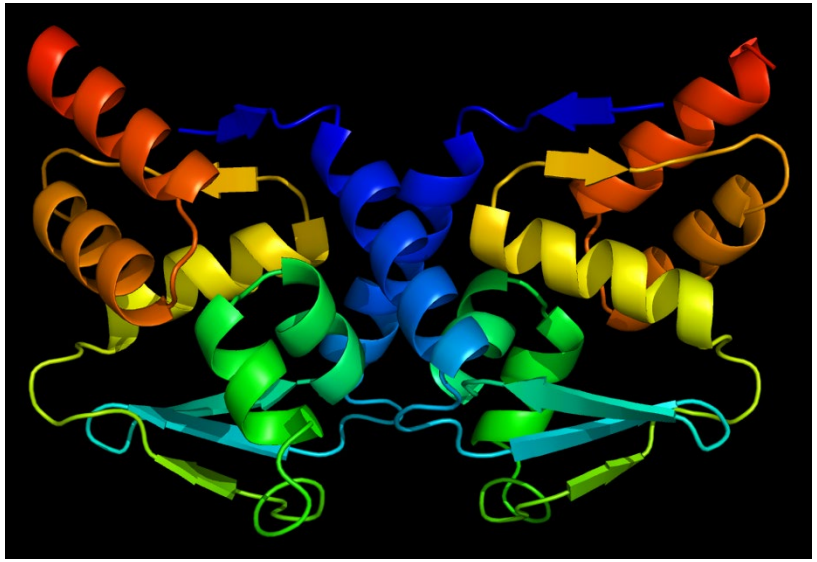
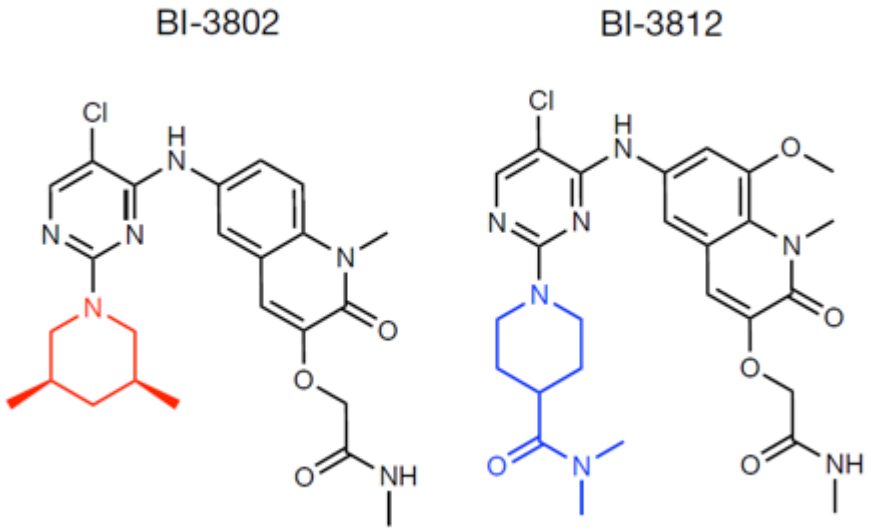
BCL6: B-cell lymphoma 6 protein



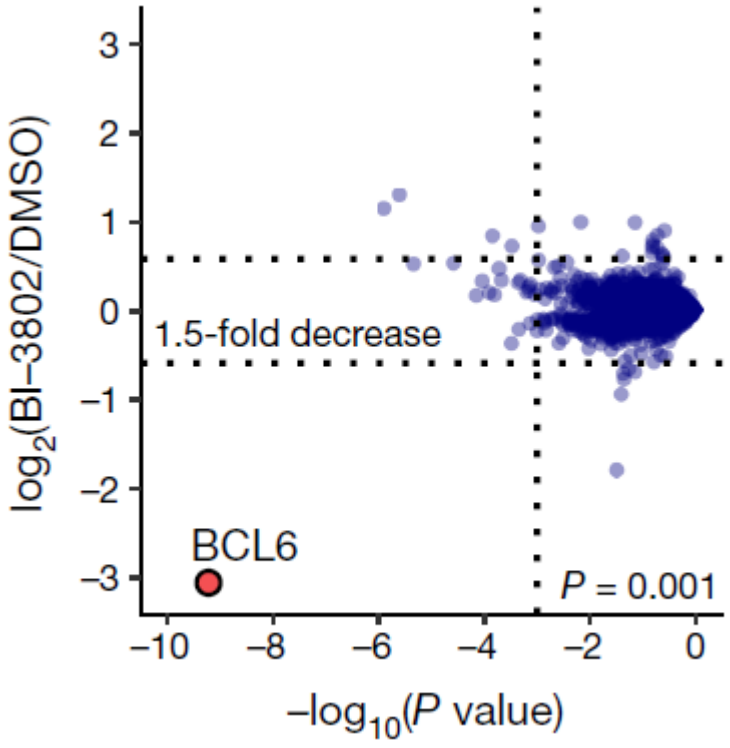
- Pathologically increased expression of BCL6 (somatic BCL6 translocation, exonic mutation, promoter mutation or mutations in regulatory pathways) is a common driver of B cell malignancies.
- Overexpression of BCL6 is sufficient to drive lymphoma development.
- BCL6 acts as a master transcriptional repressor that enables the rapid expansion of germinal centre B cells and tolerance to the genomic instability that is caused by hypermutation of the immunoglobulin genes and class-switch recombination.
- BCL6 represses a broad range of genes involved in the DNA damage response18, cell cycle checkpoints19 and differentiation.
- knockout of *BCL6* in lymphoma cells results in tumour stasis.

Screens for novel BCL6 inhibitors→BI-3802



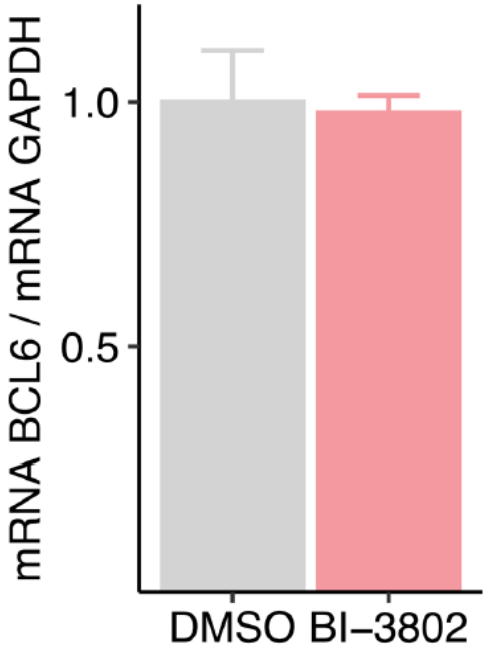
- BI-3802 binds to the BCL6 BTB domain that mediates the homodimerization of BCL6 and its interactions with co-repressor proteins.
- Treatment with BI-3802 induces rapid ubiquitination and degradation of BCL6.
- The effects of BI-3802 are comparable to those that result from a genetic knockout and are more pronounced than those induced by non-degrading BCL6 inhibitors (such as BI-3812) or by heterobifunctional BCL6 degraders.

BI-3802 induces specific degradation of BCL6

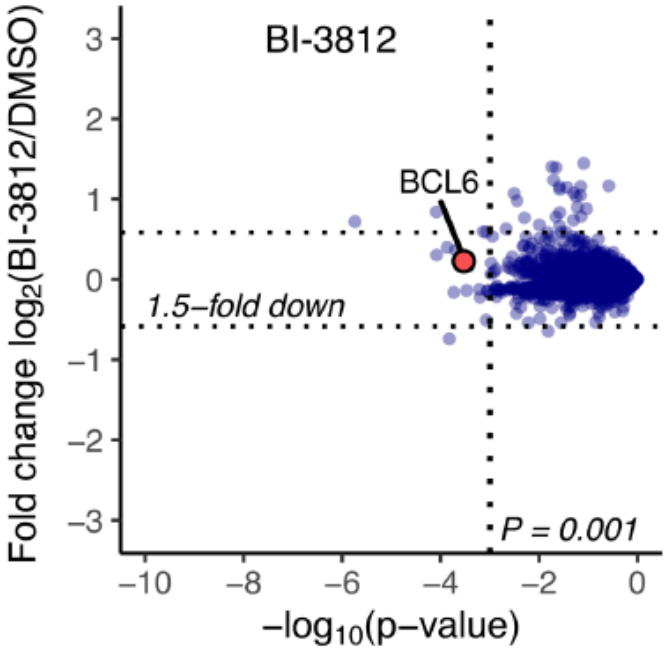


- Quantitative-mass-spectrometry based proteomics
- SuDHL4 cells (a DLBCL-derived cell line)
- Treatment with BI-3802 for 4 hours
- BCL6 was the only protein with significantly decreased abundance

BI-3802 induces specific degradation of BCL6

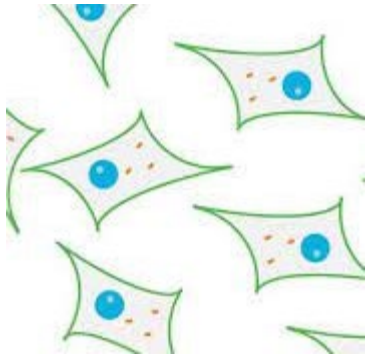


BI-3802 did not alter the expression of *BCL6* mRNA

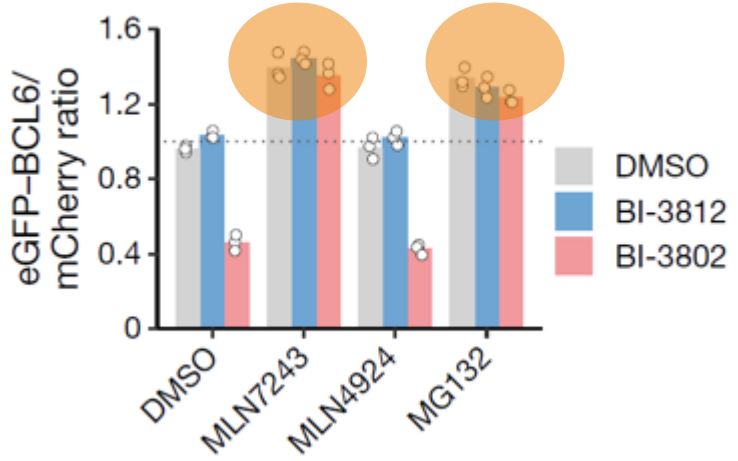


Treatment with the structurally similar BCL6 inhibitor BI-3812 did not alter the abundance of any protein

BI-3802 induces specific degradation of BCL6



HEK293T cells

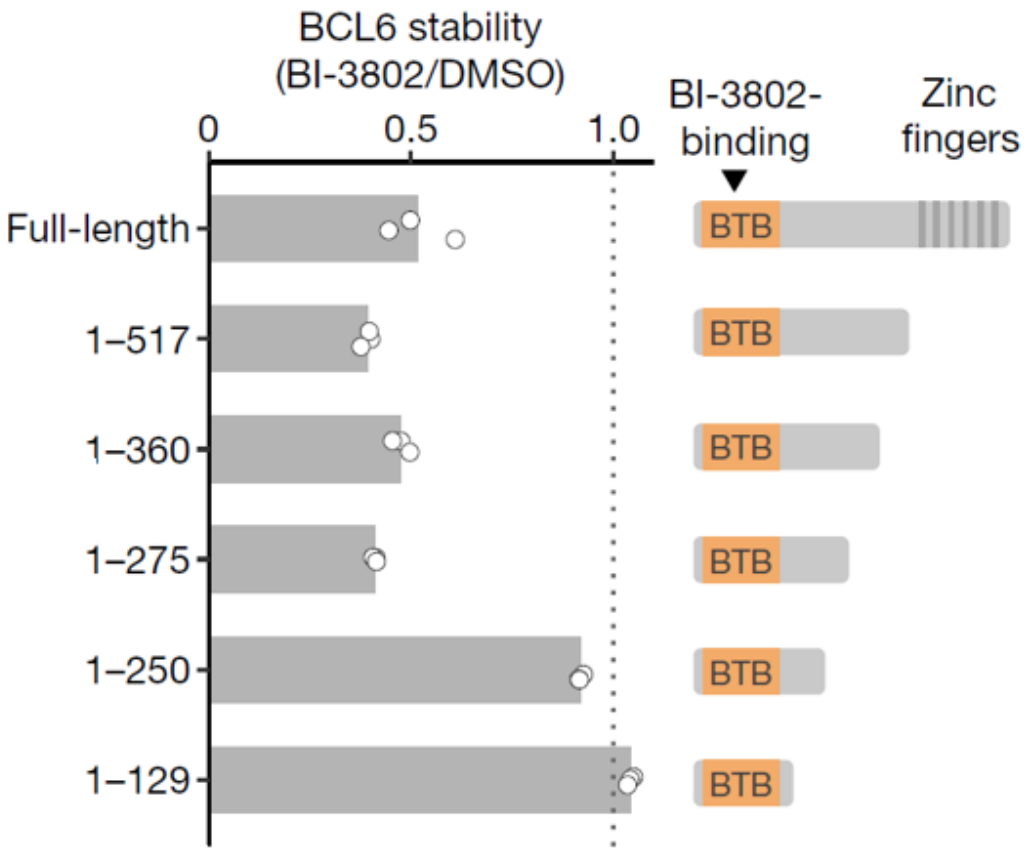


To identify the critical region of BCL6 that mediates drug-induced degradation.
Treatment with BI-3802 led to the degradation of eGFP–BCL6.
Whereas treatment with BI-3812 did not alter the stability of the reporter.

- **MG132**: 26S proteasome inhibitor;
- **MLN7243**: ubiquitin-activating enzyme UBA1 inhibitor;
- **MLN4924**: the neddylation pathway inhibiyor which is required for activity of the cullin-RING family of E3 ubiquitin ligases

BI-3802 induced degradation via non-cullin E3 ubiquitin ligase!

BI-3802 induces specific degradation of BCL6

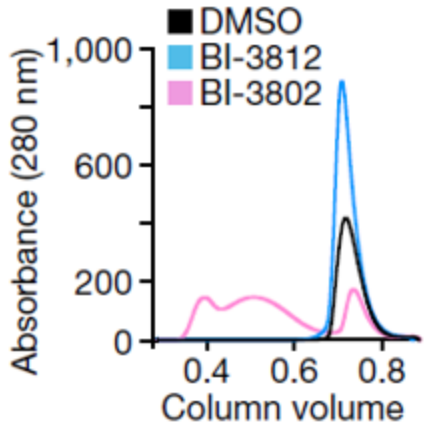


BCL6 stability calculated as eGFP–BCL6/mCherry

- Stepwise C-terminal truncations.
- First 275 amino acids, which include the drug-binding BTB domain, are sufficient for BI-3802-mediated degradation.

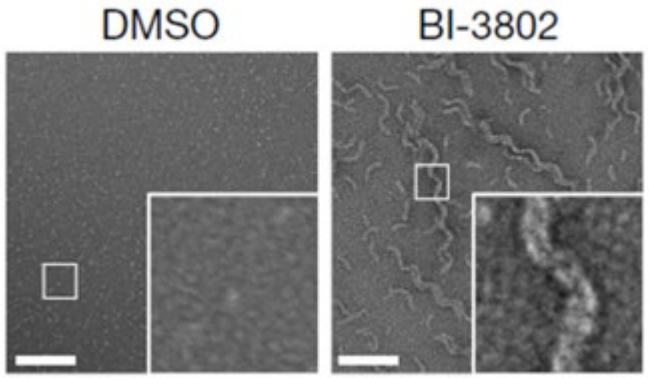
Region of 275 AA within BCL6 is sufficient for drug-dependent degradation.

BI-3802 induces the polymerization of BCL6



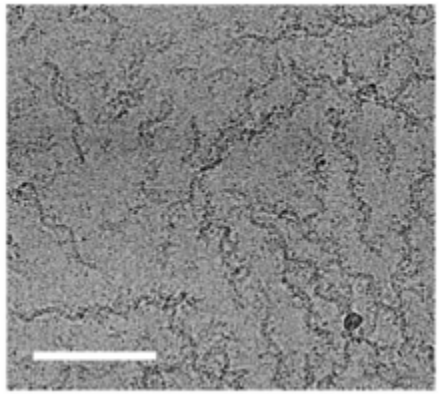
- During purification of the BCL6 recombinant protein, the presence of BI-3802—but not BI-3812—led to species of BCL6 of a higher molecular weight than was observed without drug treatment.
- Hypothesis: BCL6 forms regular higher-order structures upon binding to BI-3802.

BI-3802 induces the polymerization of BCL6



Negative-stain electron microscopy

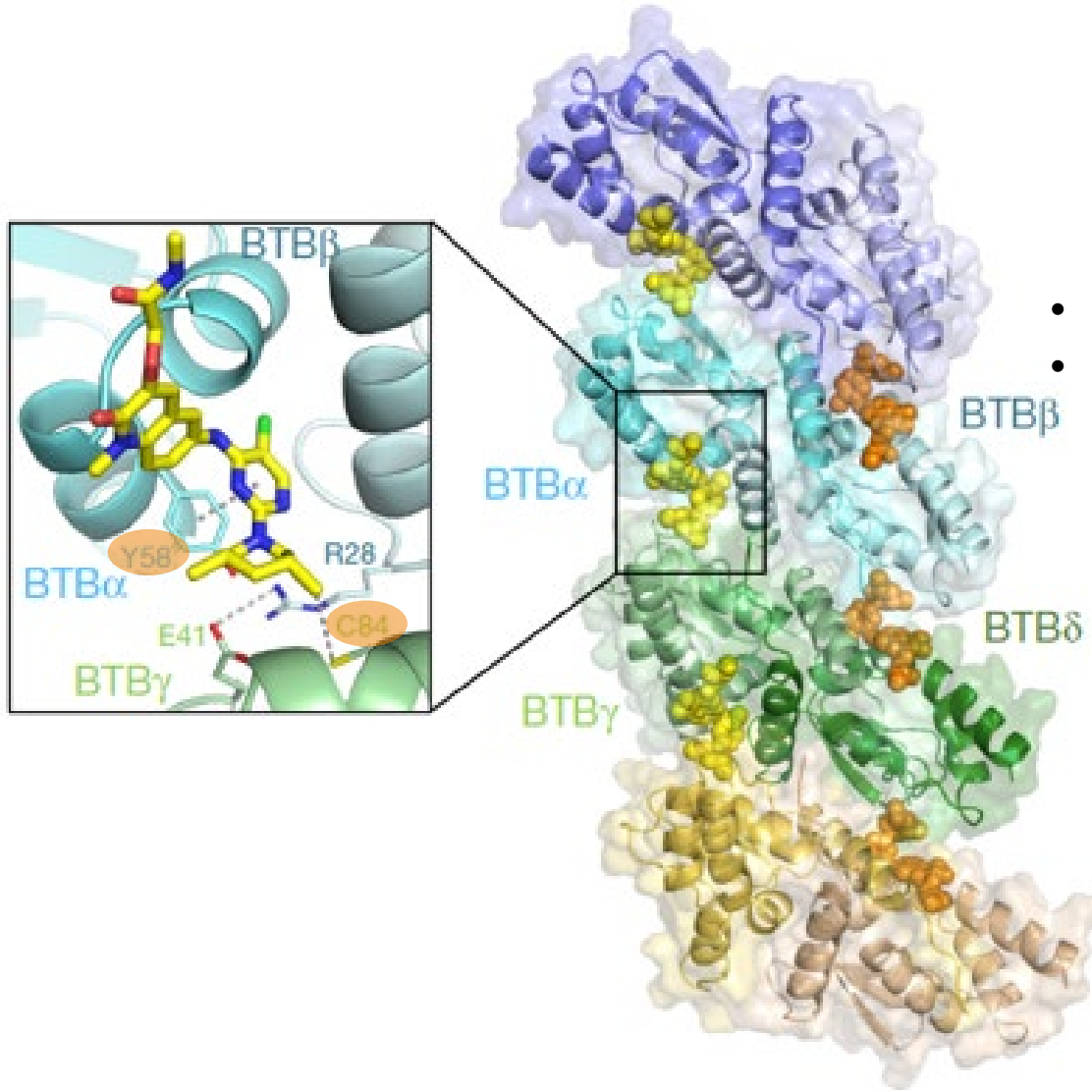
In the absence of BI-3802, BCL6 is present as monodisperse particles.
After incubation of BCL6 with BI-3802, the formation of regular structures with a sinusoidal shape appeared.



Cryo-electron microscopy

Consistent with the modelling and negative-stain data, the cryo-EM micrographs showed well-dispersed helical filaments.

Cryo-EM model of the BCL6 BTB filament with BI-3802



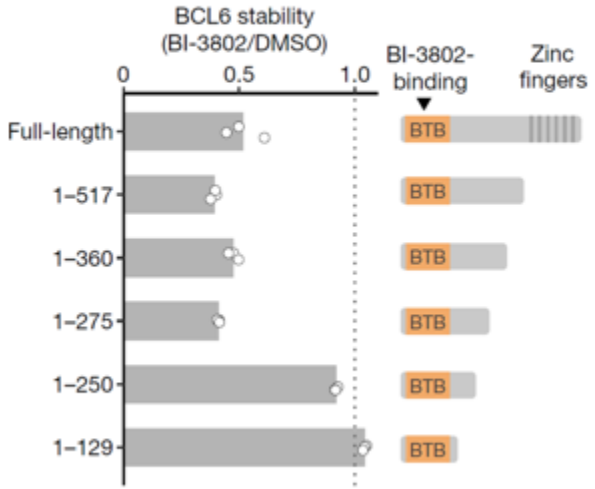
- Each BCL6 dimer is labelled in a distinct color.
- BI-3802 binds at a groove between BCL6 dimers, directly in contact with Tyr58 of BTBα and facilitates higher-order assembly through hydrophobic interactions of the compound with Cys84 on an adjacent BCL6 dimer.

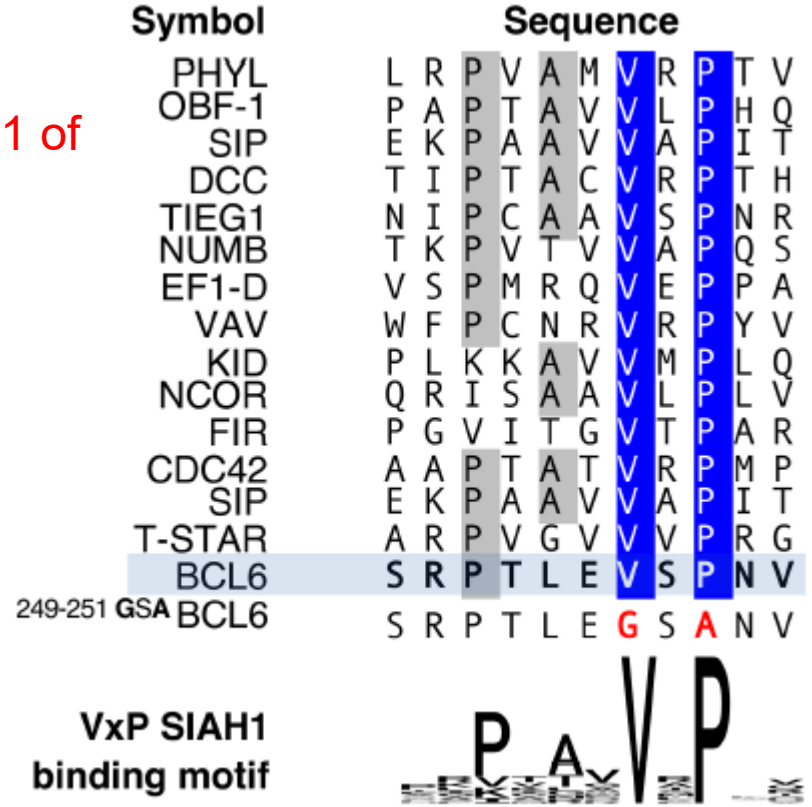
SIAH1 degrades polymerized BCL6

- Two complementary, genome-scale CRISPR–Cas9 genetic screens to interrogate the mechanism of drug-induced BCL6 degradation.
- Flow-cytometry-based BCL6 reporter screen in HEK293T cells.
- BI-3802 resistance screen in SuDHL4 cells.

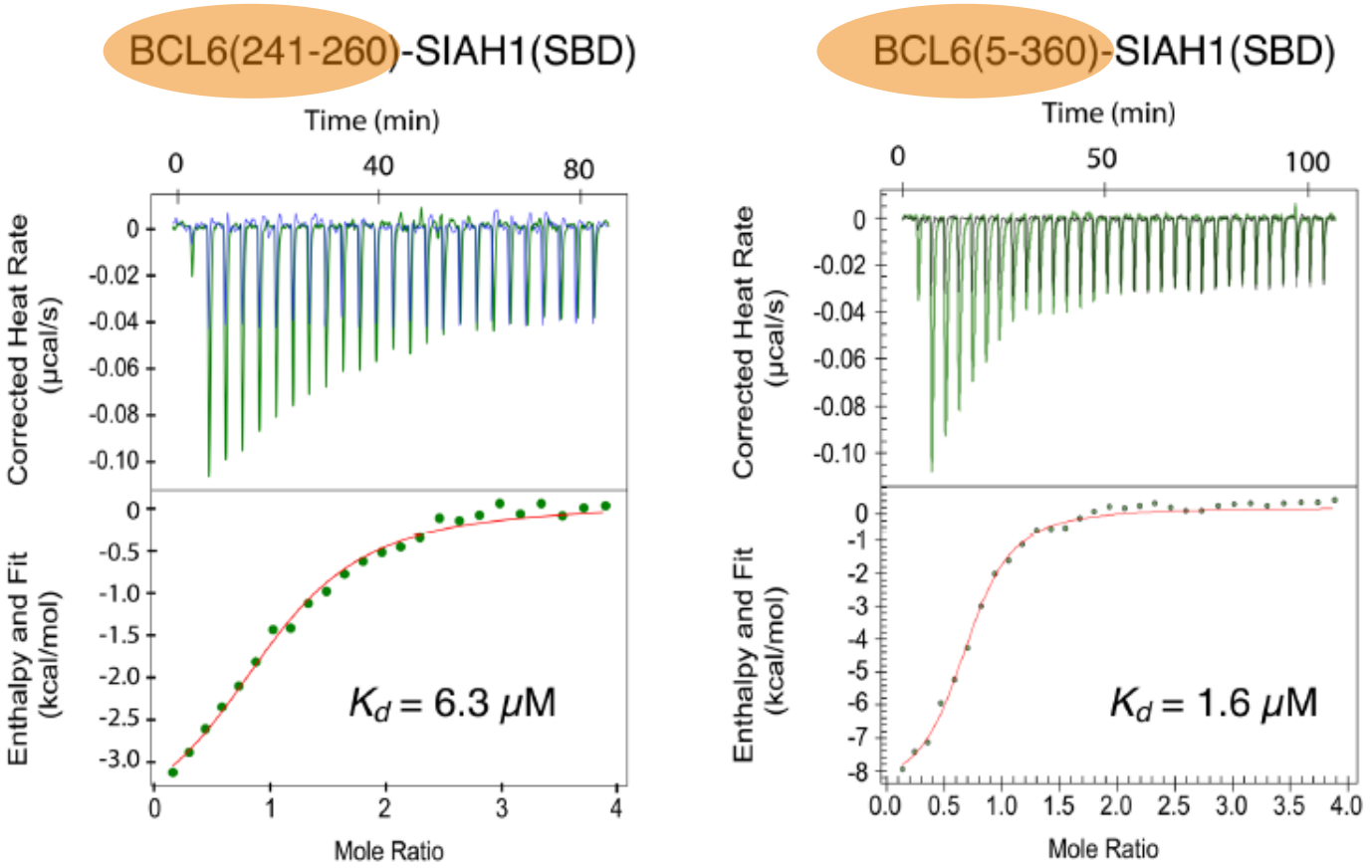
Machinery of SIAH1

The SIAH1 E3 ligase recognizes a VxP present in residues 249–251 of BCL6.





Peptide alone is sufficient for SIAH1 interaction



Summary

- Binding of BI-3802 to the BTB domain of BCL6 triggers the higher-order assembly of BCL6 into filaments.
- Polymerization promotes the ubiquitination of BCL6 by SIAH1, an E3 ligase that recognizes a VxP motif distal to the drug-binding site, and proteasomal degradation.
- These findings represent a novel mechanism of targeted protein degradation, in which a small molecule inactivates a target protein through specific drug-induced polymerization and subsequent degradation.

- **CRISPR based screening for small molecule glues could be promising in therapeutic applications.**

Thanks for your attention!