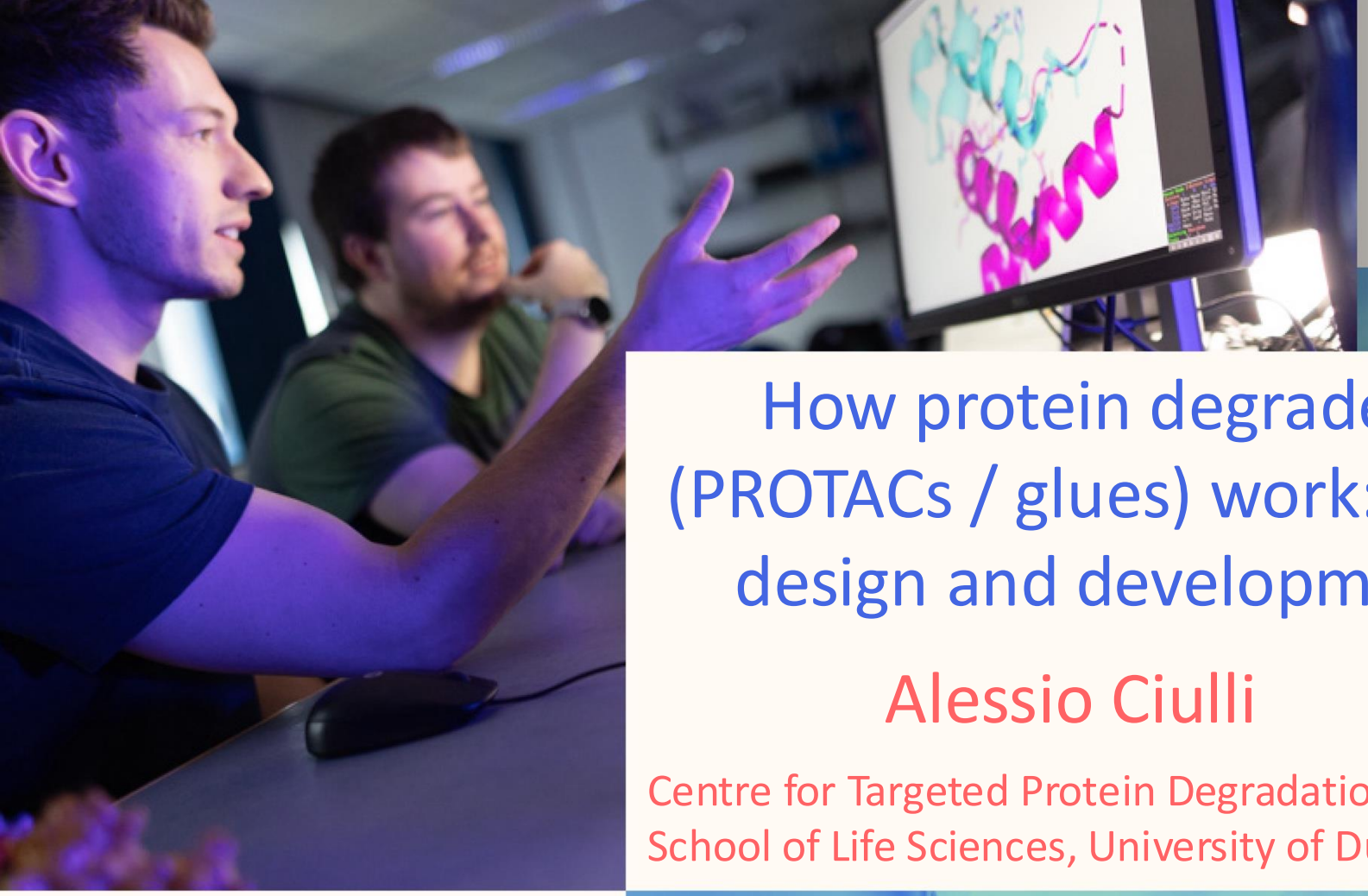

Centre for Targeted Protein Degradation
University of Dundee

innovate
collaborate
inspire

How protein degraders (PROTACs / glues) work: Drug design and development

Alessio Ciulli

Centre for Targeted Protein Degradation
School of Life Sciences, University of Dundee

Ecole Polytechnique Fédérale de Lausanne (EPFL)

Thursday, 20th March 2025

@alessiociulli

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Key concepts: Target Inhibition

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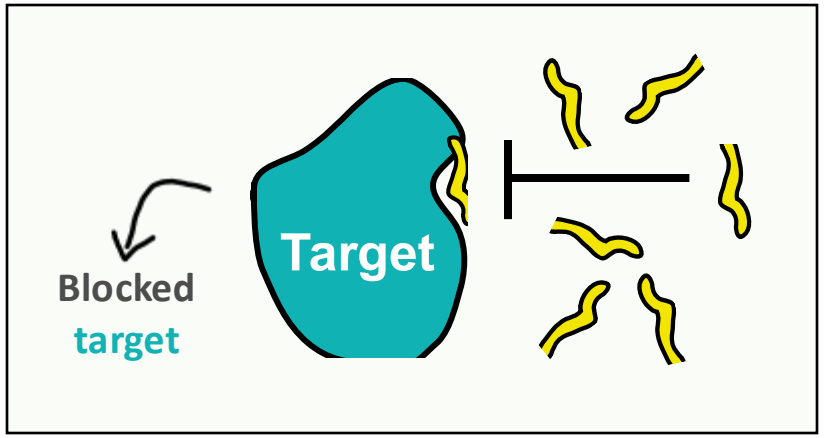
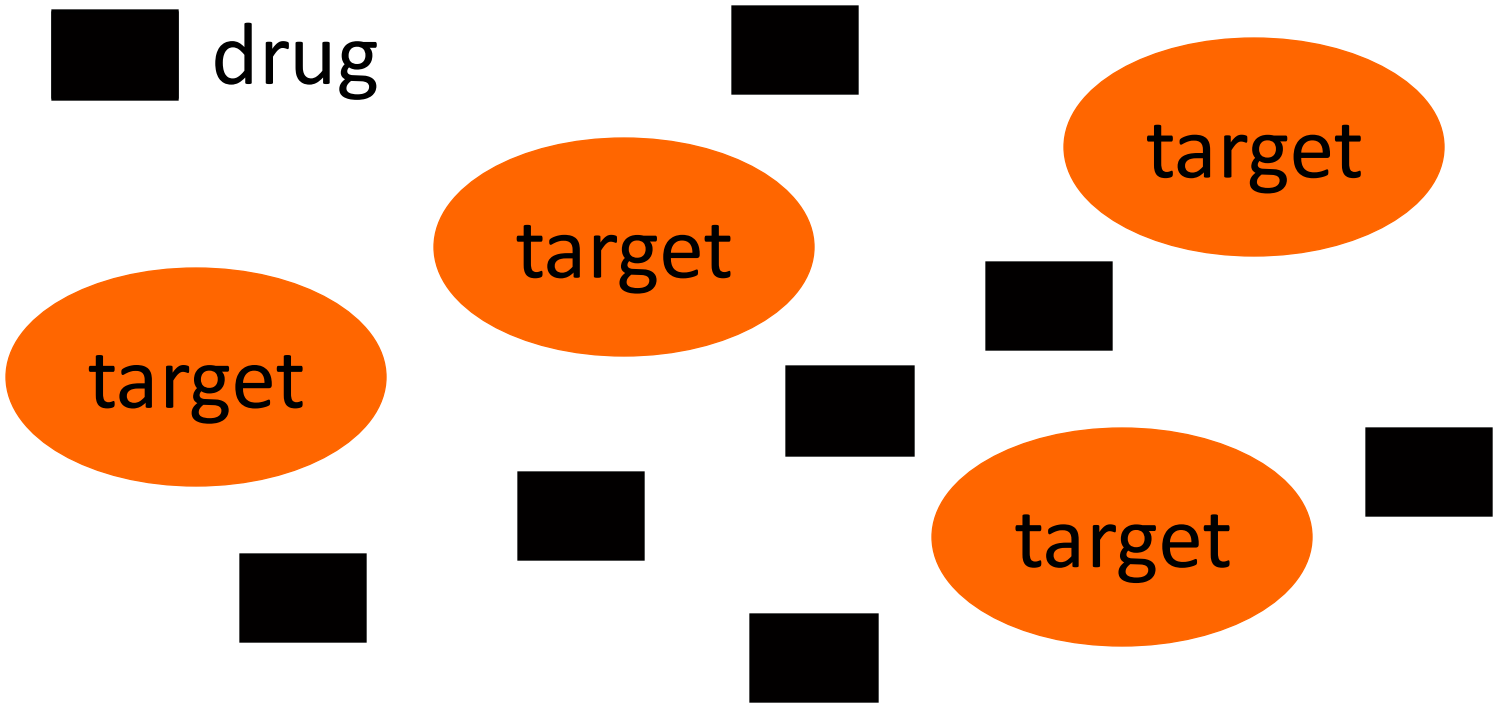
innovate collaborate inspire





- Need **potency** and high conc.
- **Drug** must affect target function
- **Target** remains inside cell

- **Occupancy-driven, stoichiometric, blocks single site**

Hughes, S.J., Ciulli, A. *Essays Biochem.* **2017**, *61,* 505-516.

Key concepts: Target Degradation

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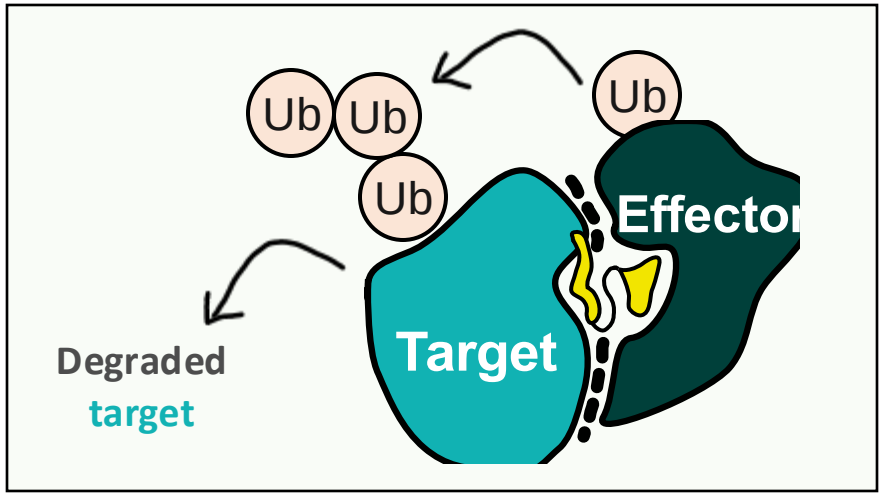

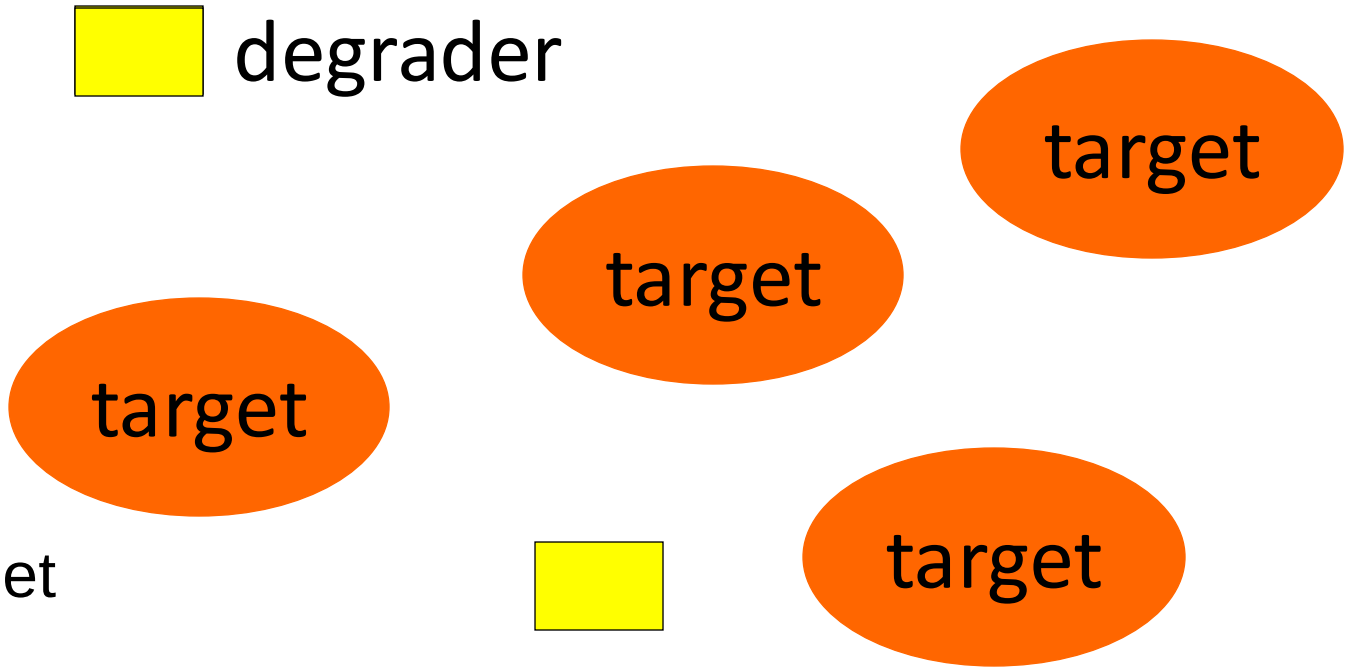

- **Catalytic:** active at low conc.
- **Drug** can bind anywhere on the target
- **Target** is depleted by the cell

- **Event-driven, sub-stoichiometric, catalytic, via the ternary complex**

Hughes, S.J., Ciulli, A. *Essays Biochem.* **2017**, *61,* 505-516.

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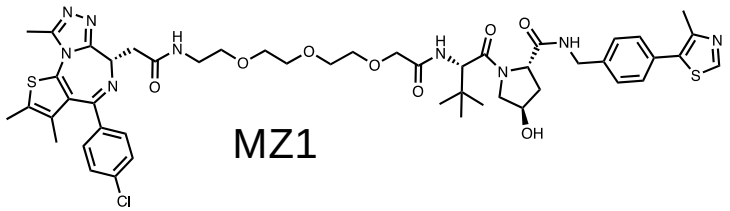
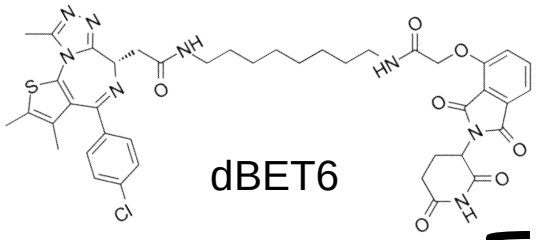
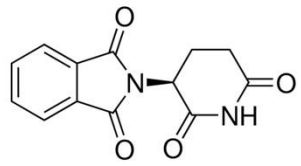
Degraders recruit target proteins to E3 ligases

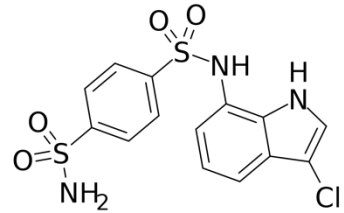


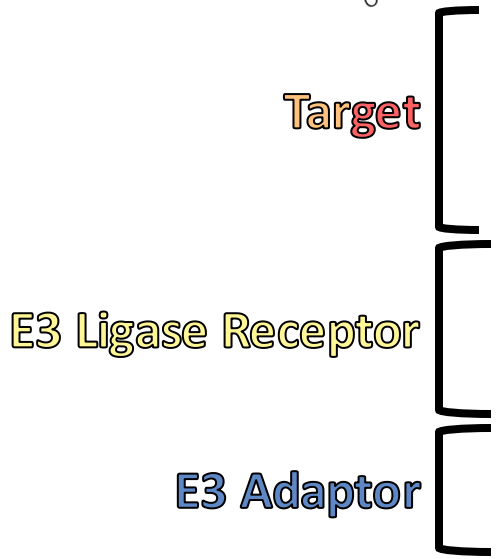


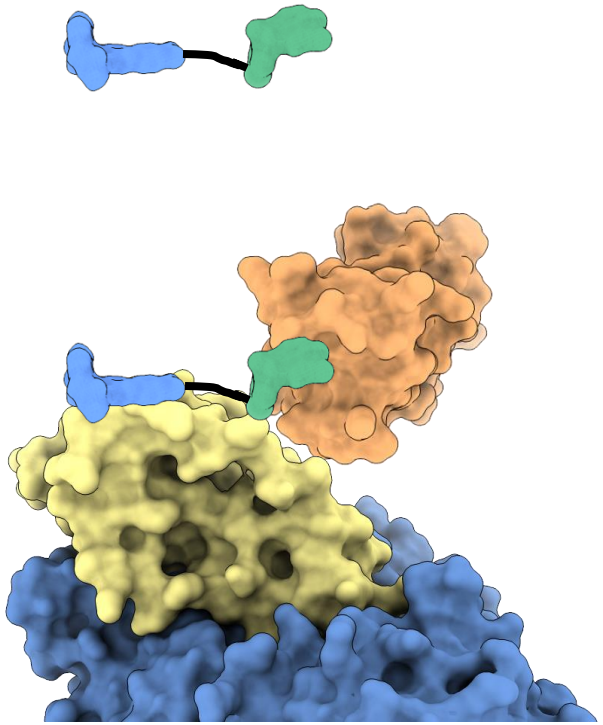
PROTAC (bivalent)

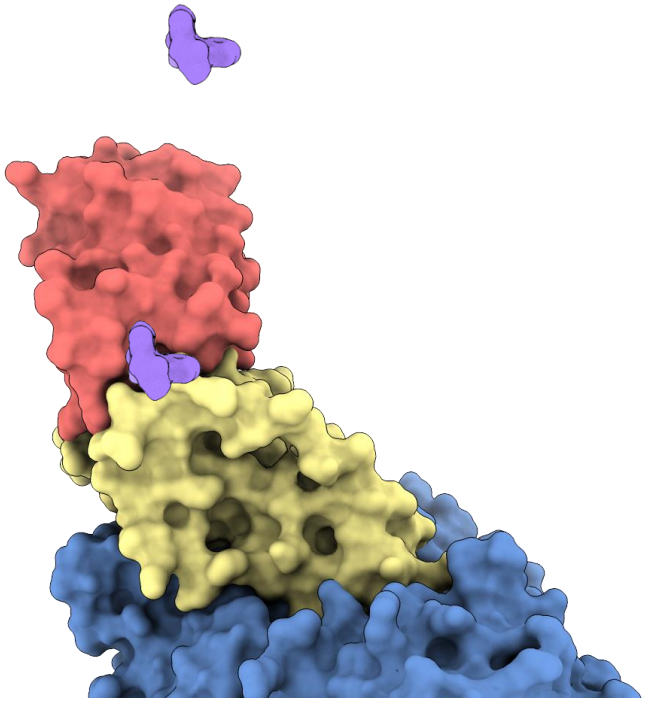
Molecular Glue (monovalent)









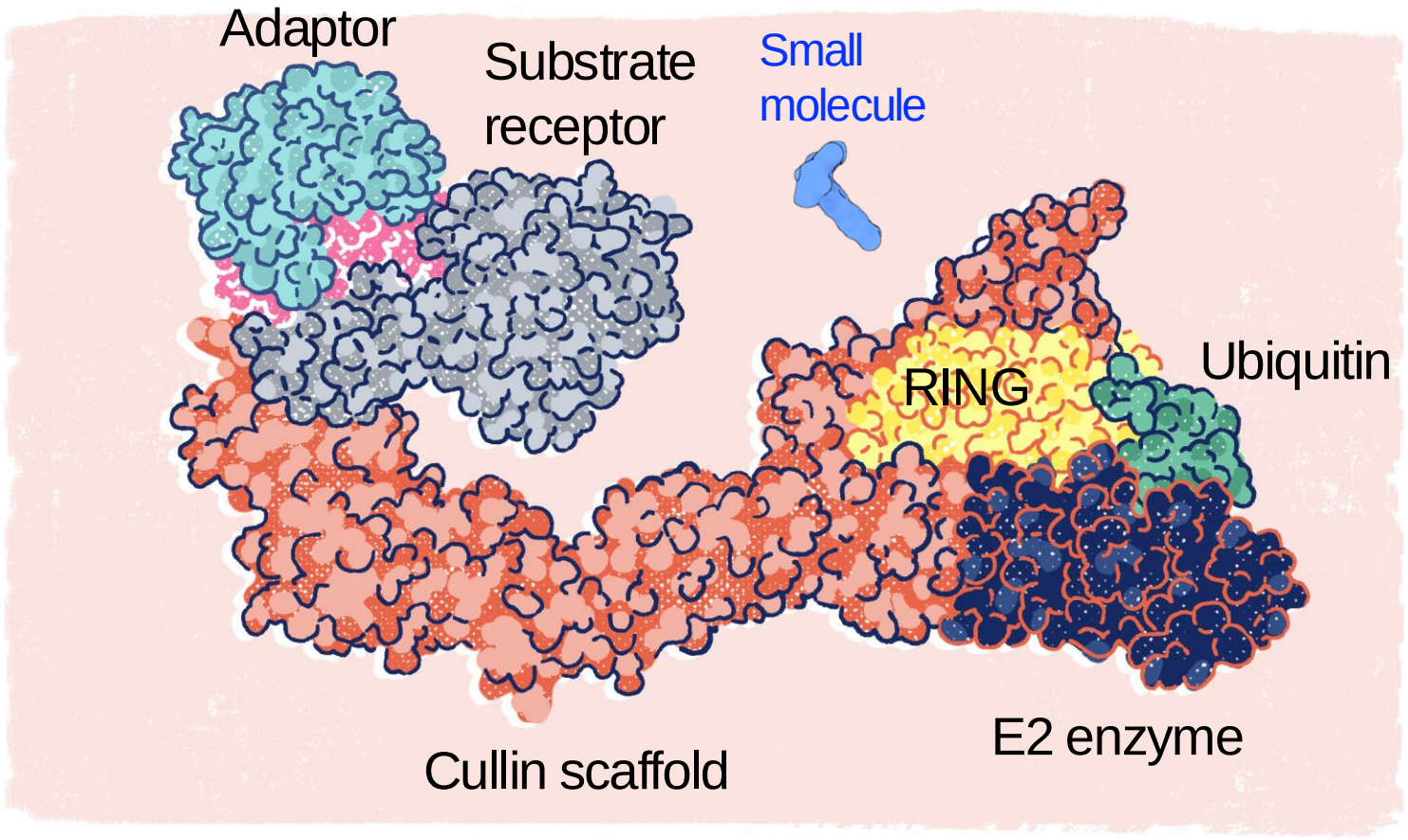


Target protein

E3 ligase

Target depletion	=>	removes all function, phenocopies genetics
Catalytic MoA	=>	sub-stoichiometric, works at lower doses
Proximity-inducers	=>	binding anywhere, much more selective

Cullin RING E3 ubiquitin ligases

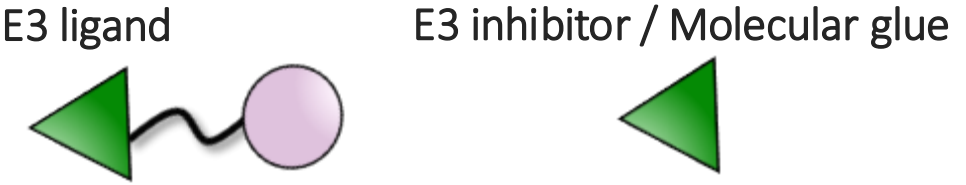


- Multi-subunit enzymes
- Catalyze ubiquitin transfer
- Specific substrate recognition via protein-protein interactions (PPIs)
- Challenging targets for small molecules

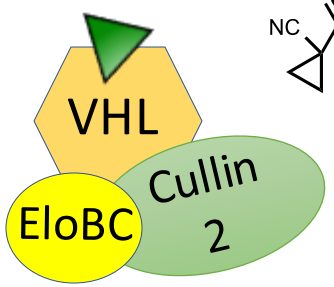
- Cullin RING ligase complexes (>230 in humans)
- Early PROTACs (Crews, Deshaies) targeted CRLs (β-TRCP, **VHL**)

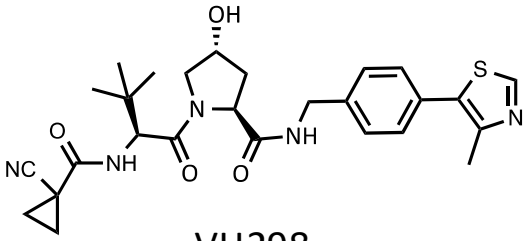
Zheng, Schulman et al. *Nature* **2002**
Oleinikovas, *Annu Rev Pharmacol Toxicol.* **2024**
Bulatov & Ciulli, *Biochem J.*, **2015**

Drug-like E3 ubiquitin ligase ligands

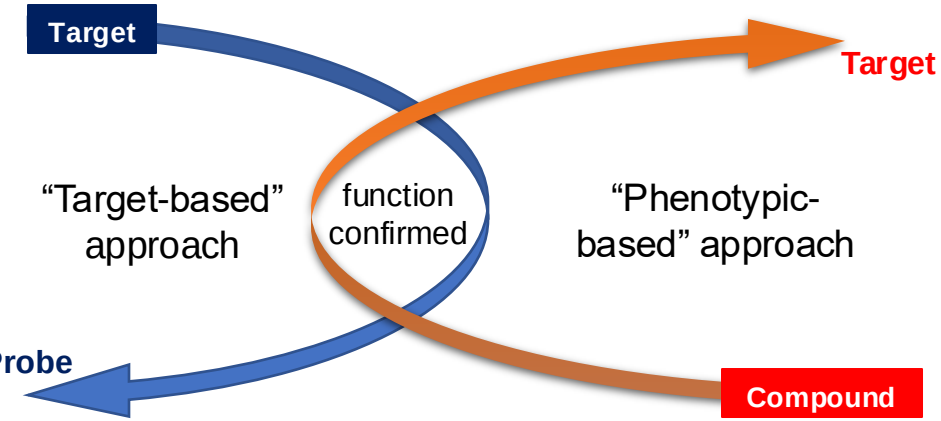


Fragment-based design of VHL ligands

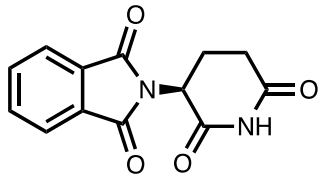




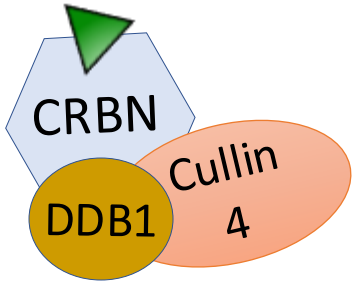
VH298
K_d = 90 nM

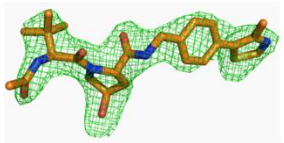


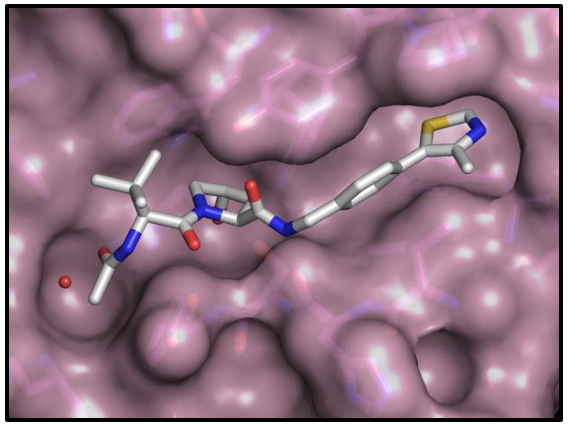
CRBN drug target ID

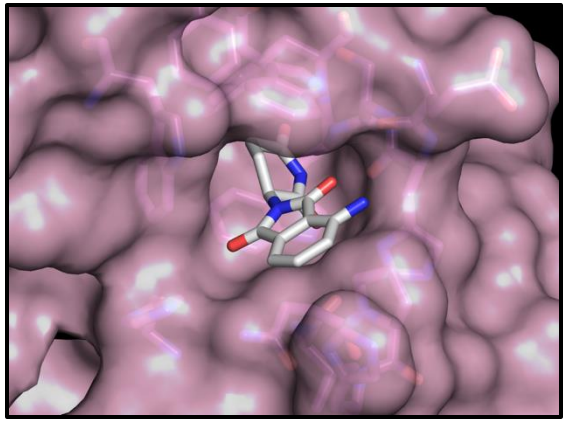


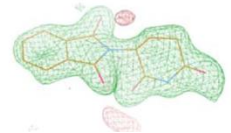
thalidomide
K_d ~ 250 nM











J. Am. Chem. Soc. **2012**; *Chem. Biol.* **2012**; *Angew. Chem. Int. Ed.* **2012**; *ACS Med. Chem. Lett.* **2014**; *J. Med. Chem.* **2014**; ; *Nat. Commun.* **2016**; *J. Med. Chem.* **2018**

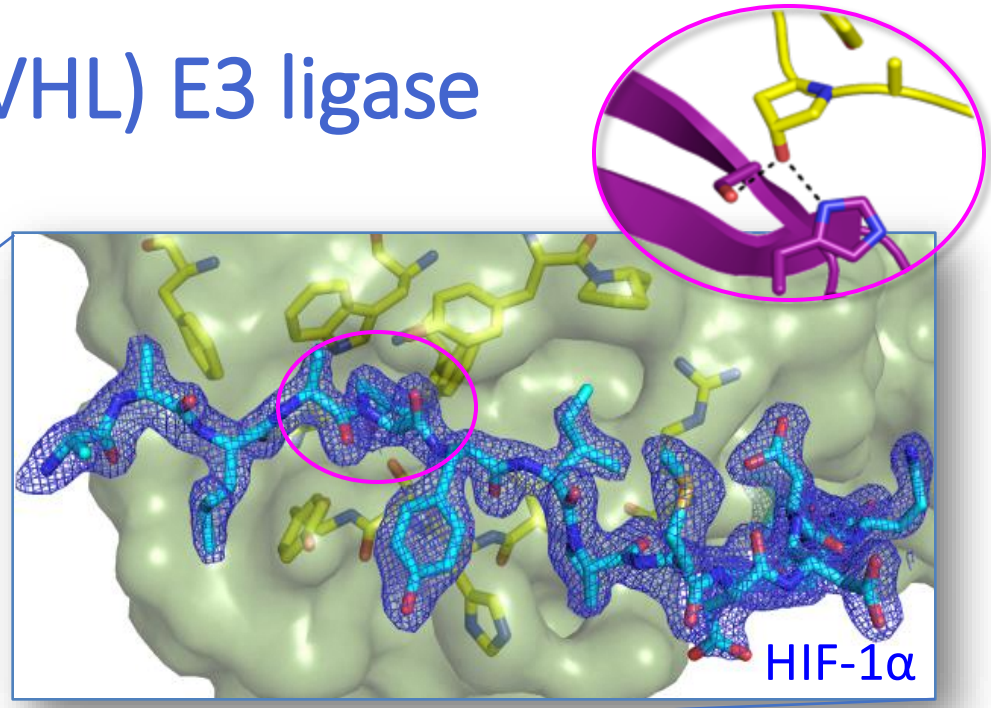
Reviewed in: Diehl & Ciulli, *Chem. Soc. Rev.*, **2022**

Ito et al. Science **2010**; Fischer et al. *Nature* **2014**; Chamberlain et al. *Nat. Struct. Mol. Biol* **2014**

Reviewed in: Yamamoto & Handa, *Chem. Soc. Rev.*, **2022**

von Hippel-Lindau (VHL) E3 ligase

Model from X-ray structure of
VHL-EloBC-Cul2-Rbx1
(Cardote *et al. Structure* **2017**)



substrate
HIF-1α

VHL

Rbx1

EloC/EloB
adaptor

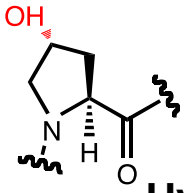
Cullin2

Min et al. *Science* **2002**
Hon et al. *Nature* **2002**

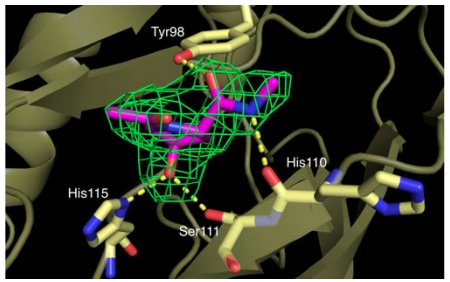
- Ubiquitously expressed
- Recognises hydroxylated Hypoxia Inducible Factor 1 alpha subunit (HIF-1α)
- Constitutively active
- Critical in oxygen sensing
- Mutations associated with VHL disease

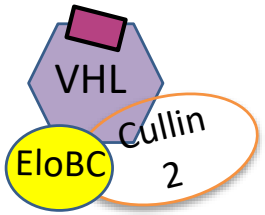
Fragment-Based Design of VHL ligands

Targeting the von Hippel-Lindau (VHL) ligase with small molecules

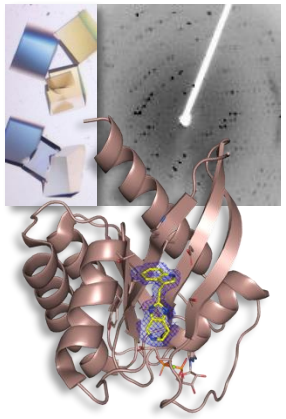


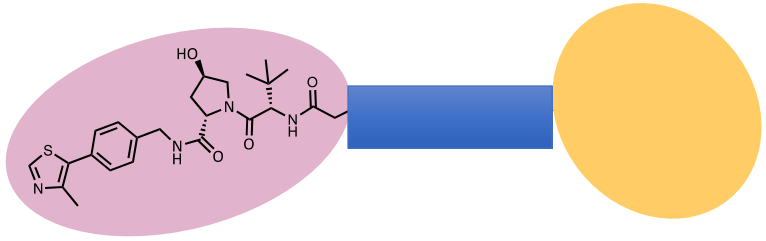
Hydroxyproline
(K_d ~10 mM, LE = 0.22)





X-ray
Crystallography



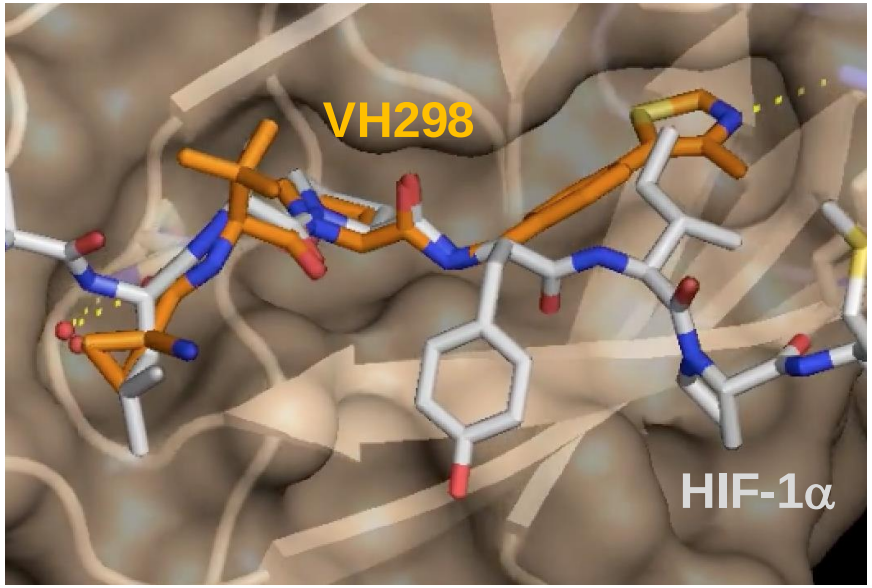


E3 ligase ligand
Rationally designed *de novo*

Dennis Buckley & Inge van Molle
Crews Lab (Yale) & Ciulli Lab (Cambridge)
Later optimized into VHL inhibitor VH298 by Ciulli Lab (Dundee)

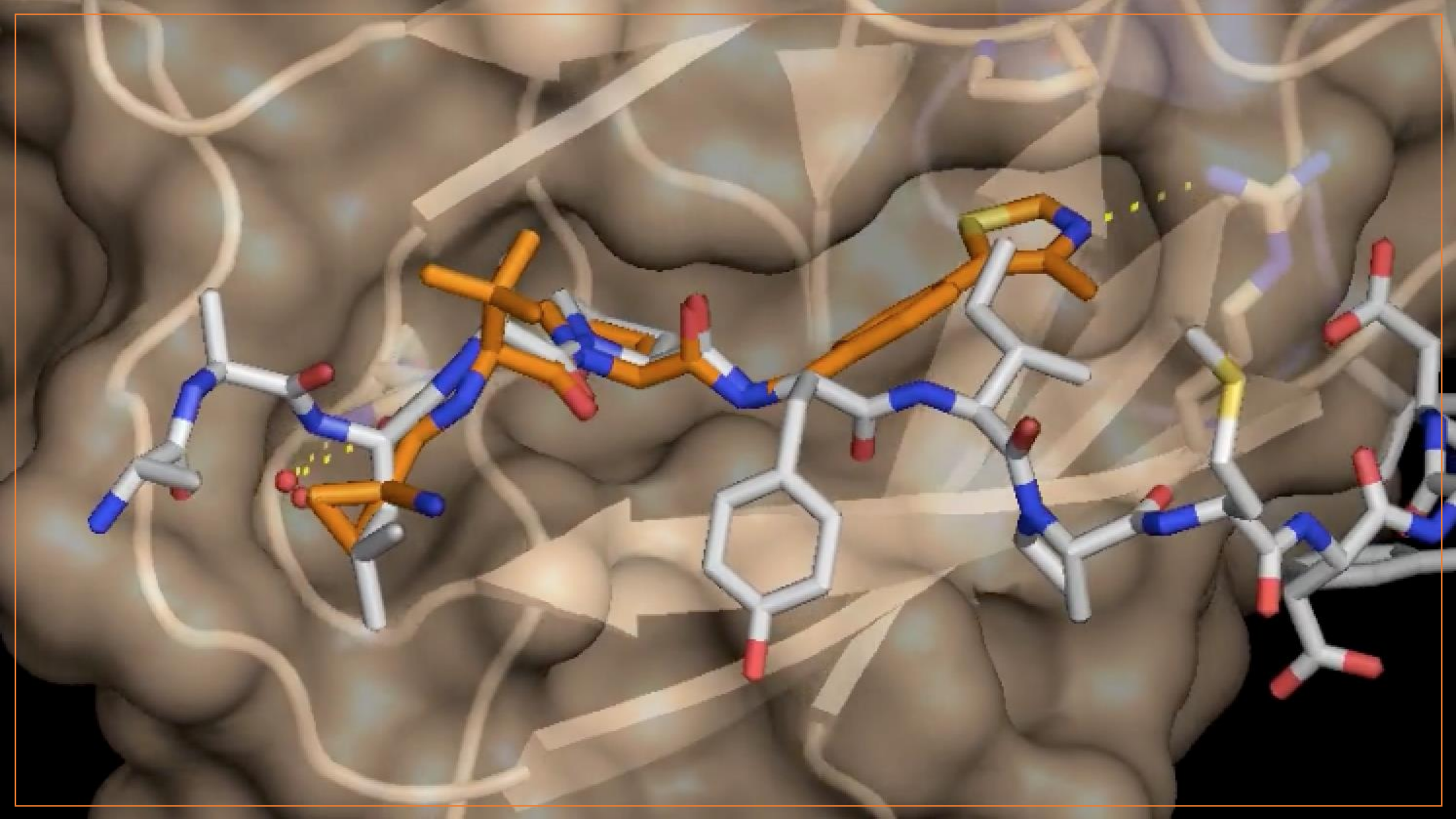
>200s compounds made
>50s structures solved

VHL inhibitor VH298
(K_d 80 nM, LE = 0.28)

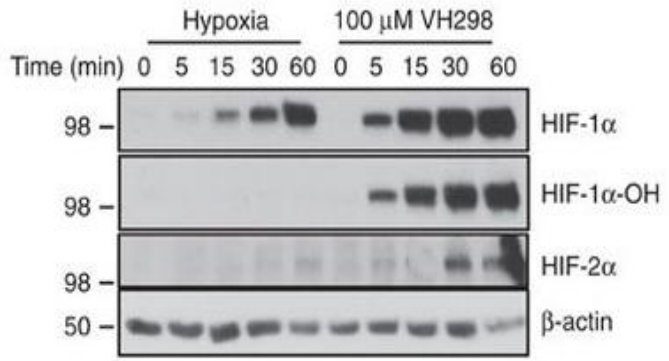


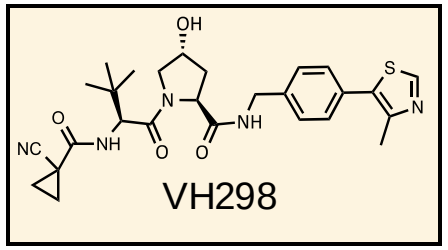
J. Am. Chem. Soc. **2012**; *Chem. Biol.* **2012**; *Angew. Chem. Int. Ed.* **2012**; WO2013/106643/6; *ACS Med. Chem. Lett.* **2014**; *J. Med. Chem.* **2014**; *Nat. Commun.* **2016**; *J. Med. Chem.* **2018;** *J. Biol. Chem.* **2021**

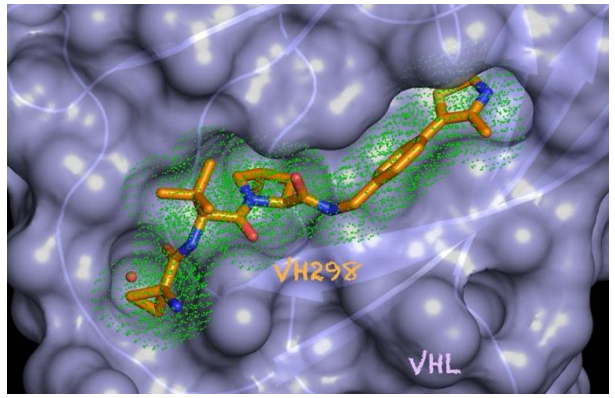
Led to founding of Degradation DPU at GSK (2012) and Crews-founded Yale spin-off Arvinas (2013)

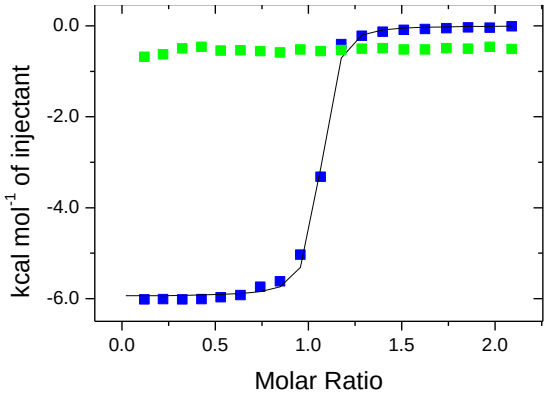


Discovery of VH298, a potent and selective VHL inhibitor









Cell permeable, non-toxic, potent and selective VHL inhibitor

nature COMMUNICATIONS

ARTICLE

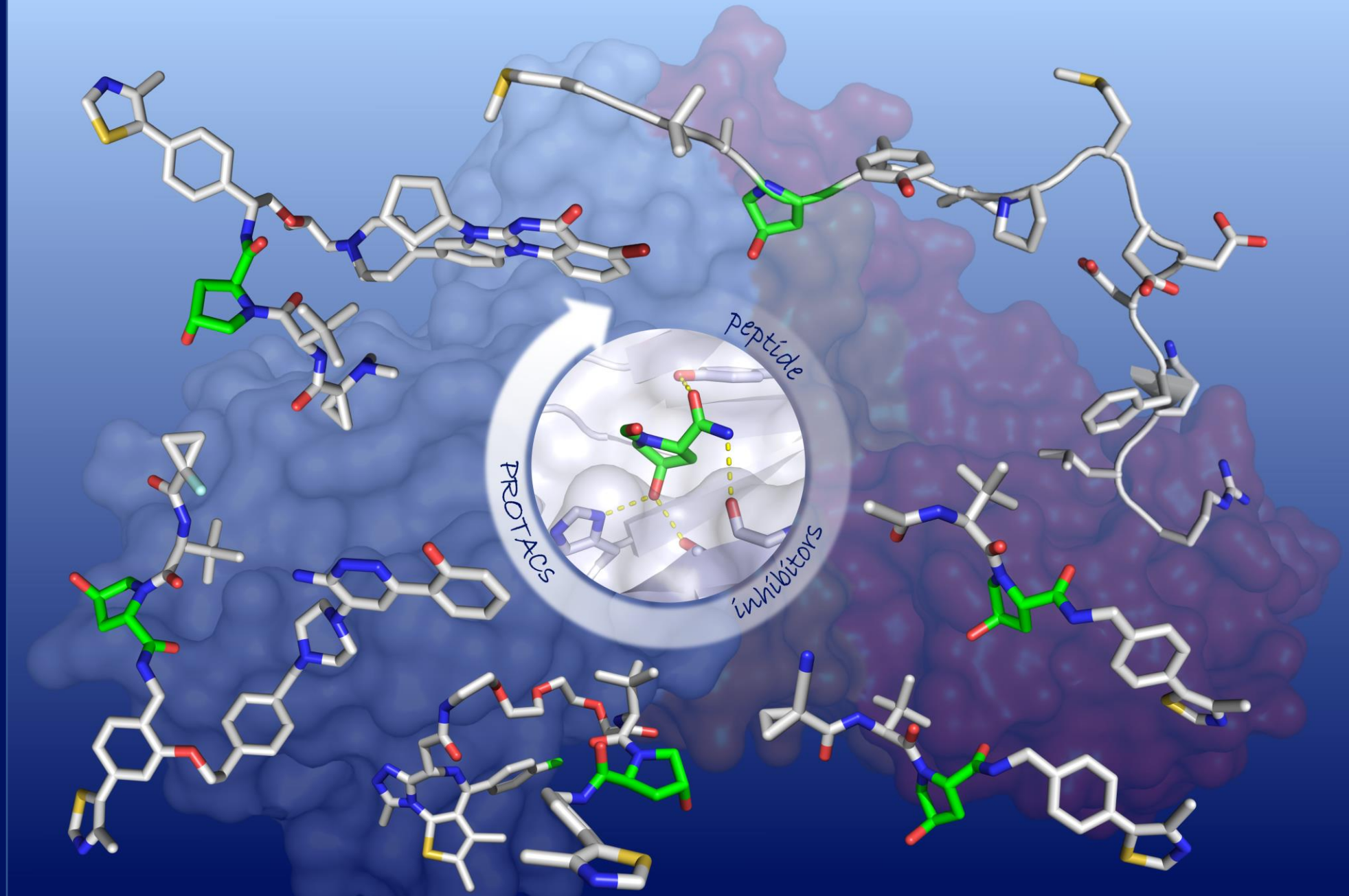
Received 21 Sep 2015 | Accepted 22 Sep 2016 | Published 4 Nov 2016 DOI: 10.1038/ncomms13312 OPEN

Potent and selective chemical probe of hypoxic signalling downstream of HIF-α hydroxylation via VHL inhibition

Julianty Frost[1,*], Carles Galdeano[1,*,†], Pedro Soares[1], Morgan S. Gadd[1], Katarzyna M. Grzes[2], Lucy Ellis[1], Ola Epemolu[1], Satoko Shimamura[3], Marcus Bantscheff[3], Paola Grandi[3], Kevin D. Read[1], Doreen A. Cantrell[2], Sonia Rocha[4] & Alessio Ciulli[1]

Collaboration with Sonia Rocha, Doreen Cantrell and Kevin Read (SLS Dundee) and Paola Grandi (Cellzome-GSK)

Frost et al., *Nature Commun.* **2016**, 7:13312
Soares et al. *J. Med. Chem.* **2018**, *61*, 599

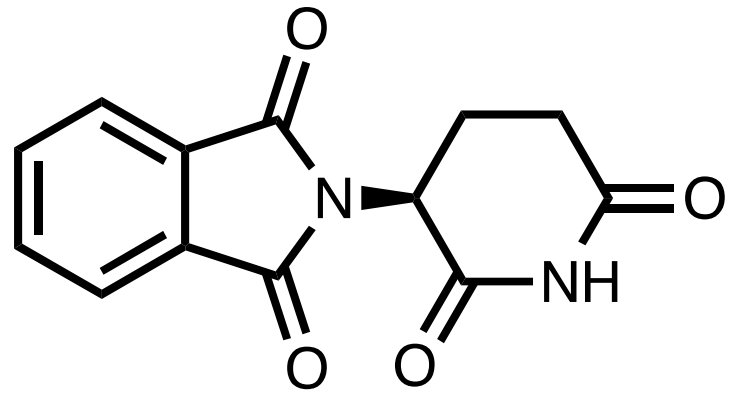


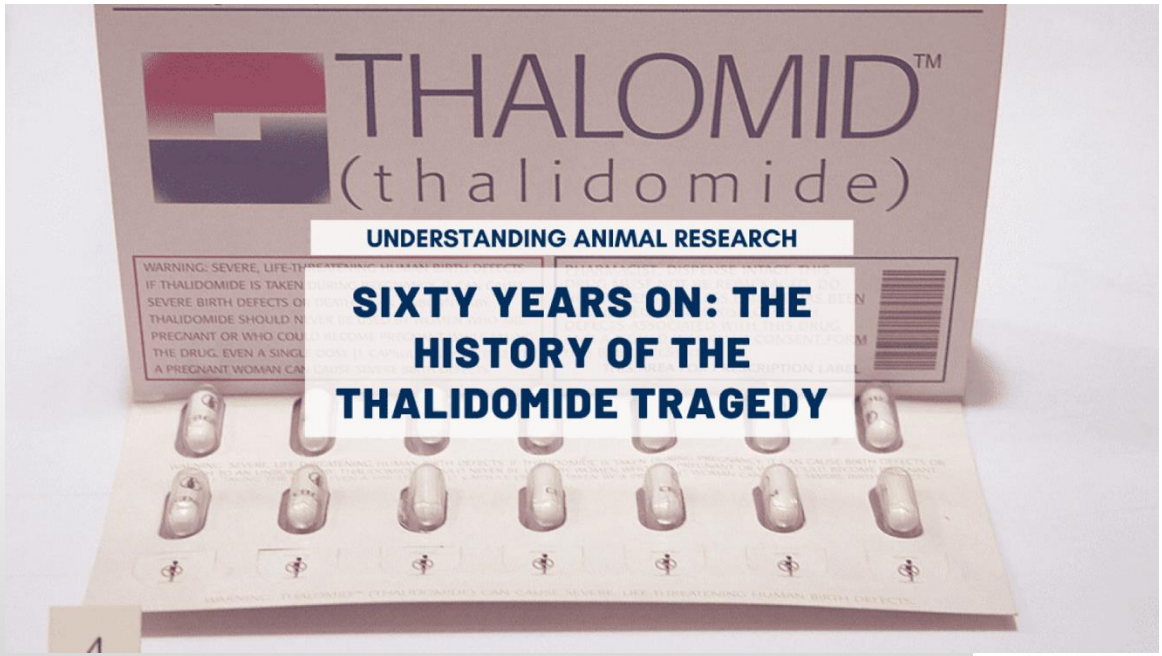
Reviewed in: Claudia J. Diehl and Alessio Ciulli, *Chem. Soc. Rev.*, **2022**, *51 (19)*, 8216-8257

The history of thalidomide

Thalidomide

-1957 to 61: Treatment of morning sickness during pregnancy
>10,000 children born with phocomelia
-1991: TNF inhibitor
-1994: Antiangiogenesis: first trials in cancer
-2006: Approved for multiple myeloma

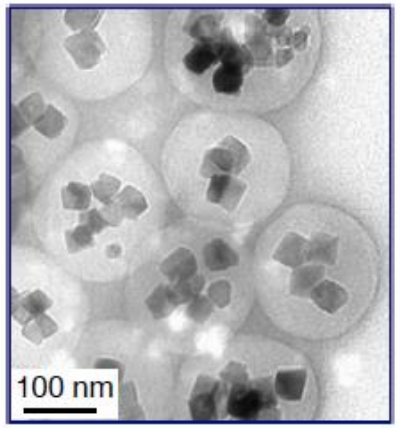


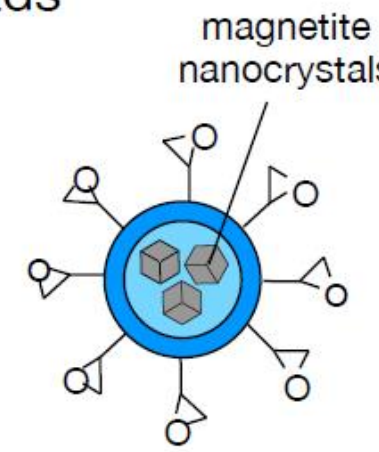


Preparation of Thalidomide-fixed FG Handa beads

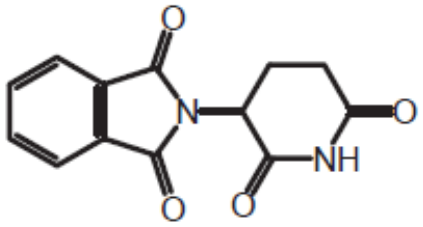
FG beads

Handa beads

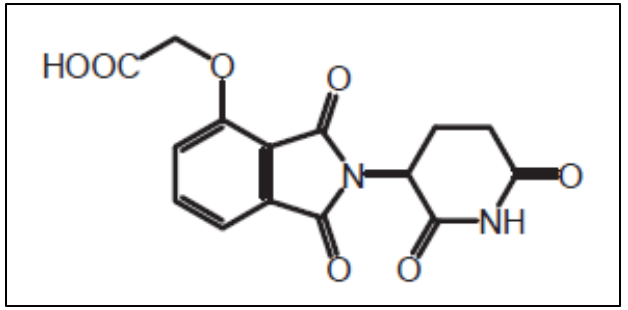




Nishio *et al.*, ***Colloids Surf B Biointerface*** (2008)

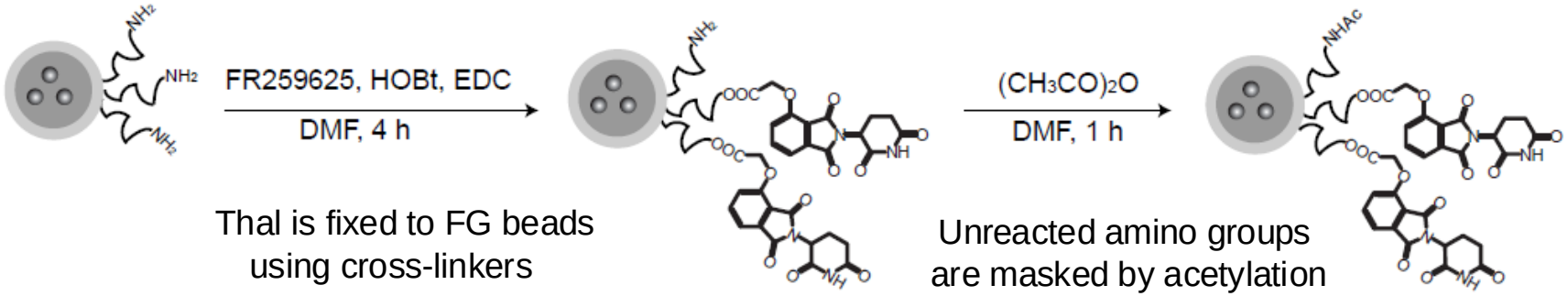


Thalidomide (Thal)



FR259625
(carboxylated derivative)

Hiroshi Handa



Thal is fixed to FG beads using cross-linkers

Unreacted amino groups are masked by acetylation

FG beads

Thal-fixed FG beads

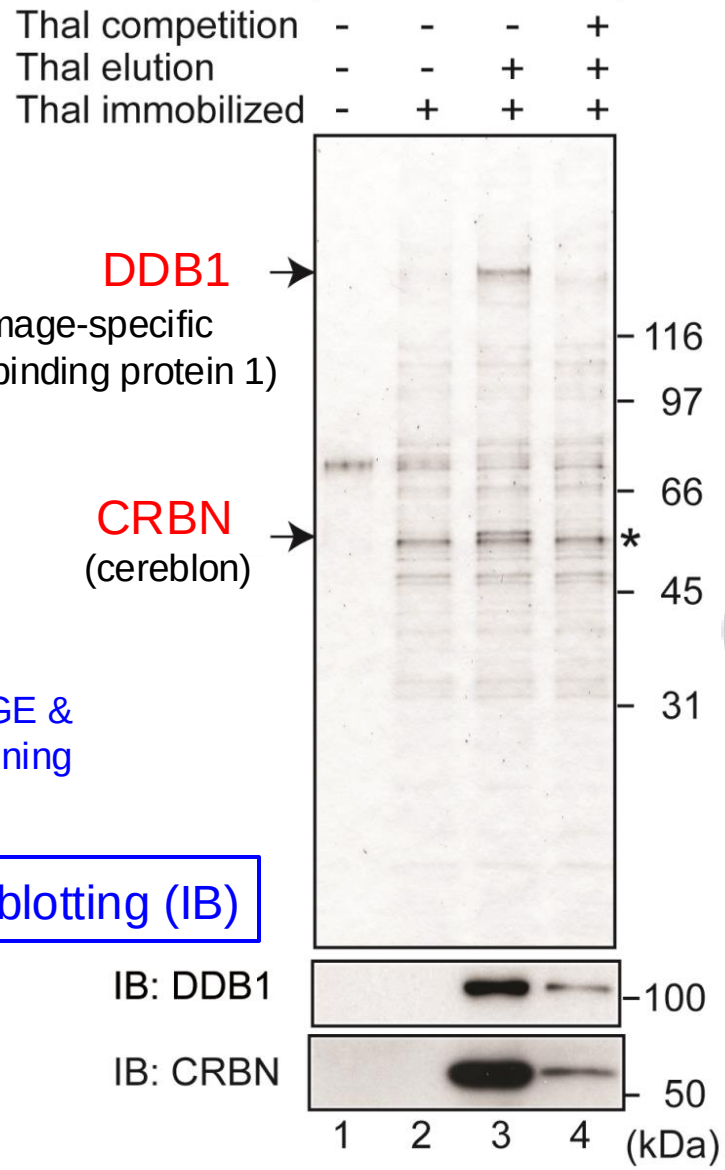
Ito *et al.*, ***Science*** (2010)

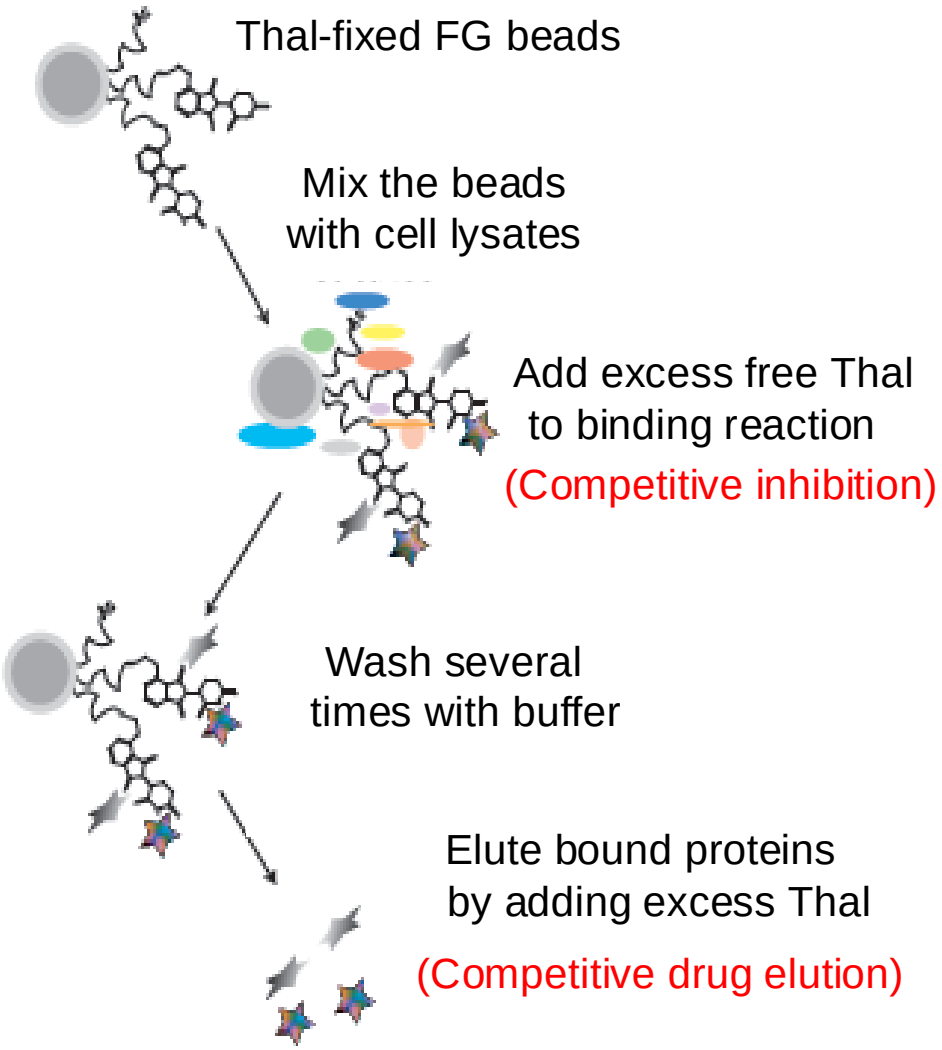
Credit: Hiroshi Handa

Centre for Targeted Protein Degradation | University of Dundee
innovate collaborate inspire

Identification of CRBN and DDB1 as Thal-binding proteins

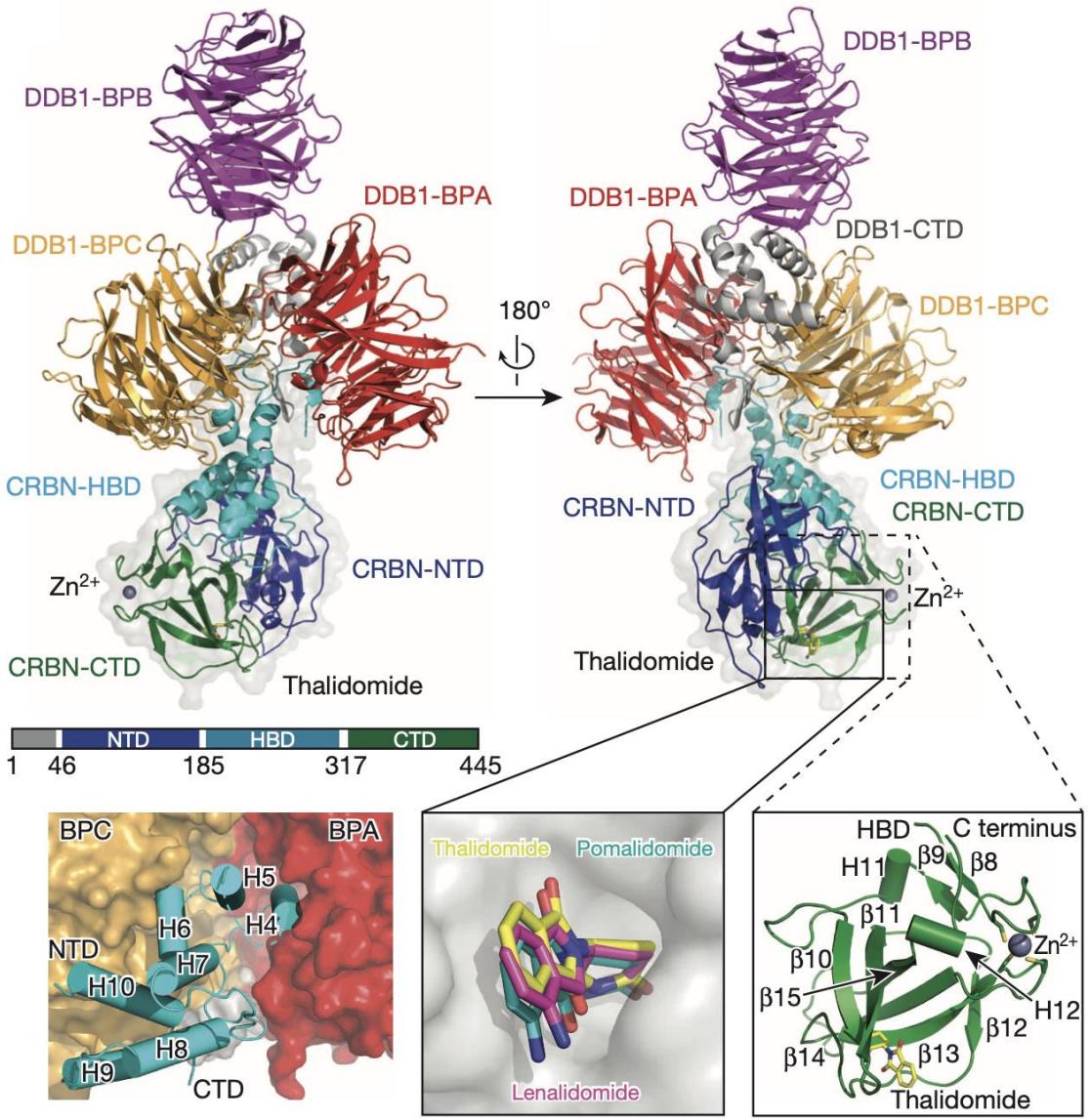
Affinity isolation



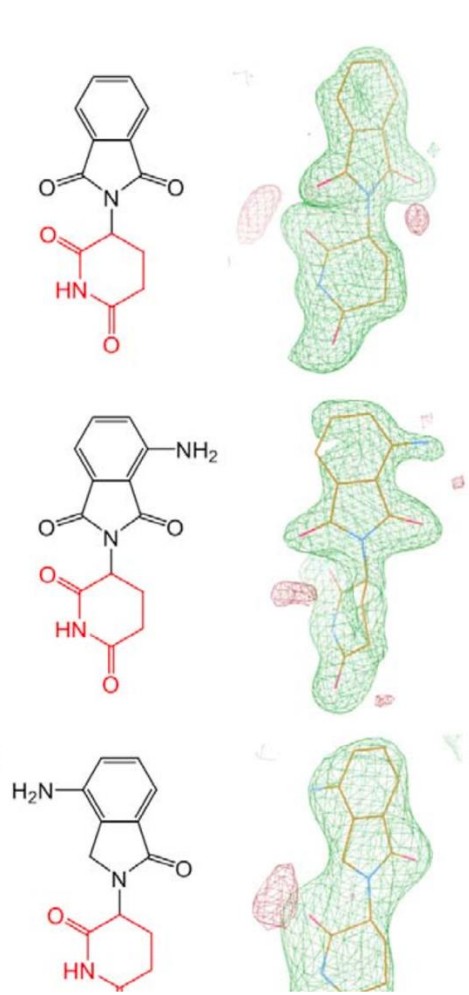


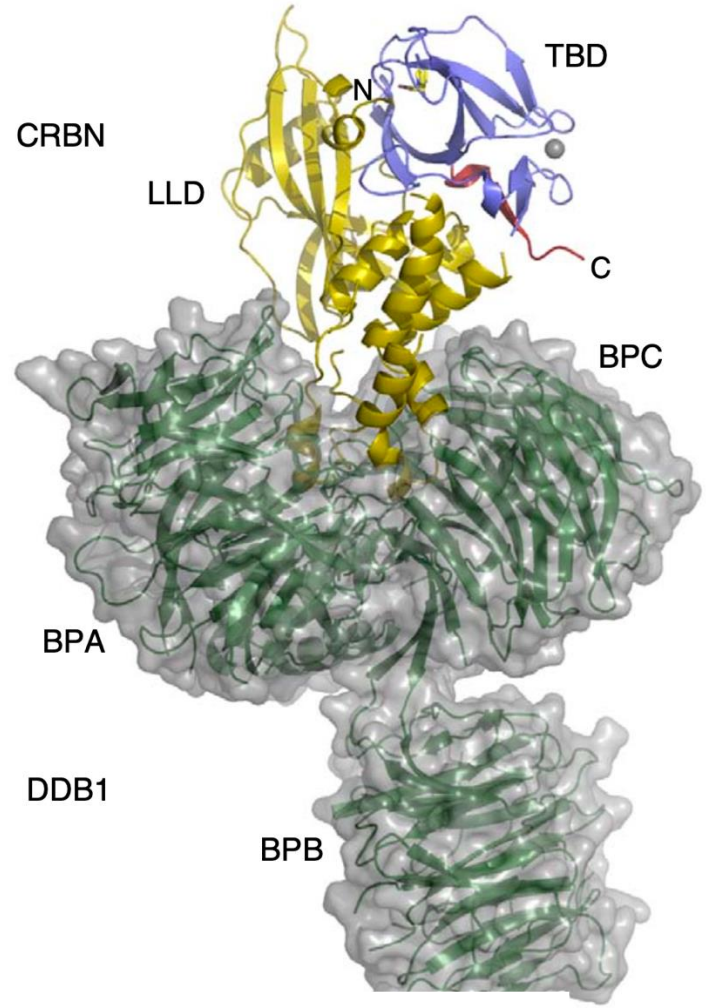
Credit: Hiroshi Handa

Structures of CRBN with bound Thalidomide



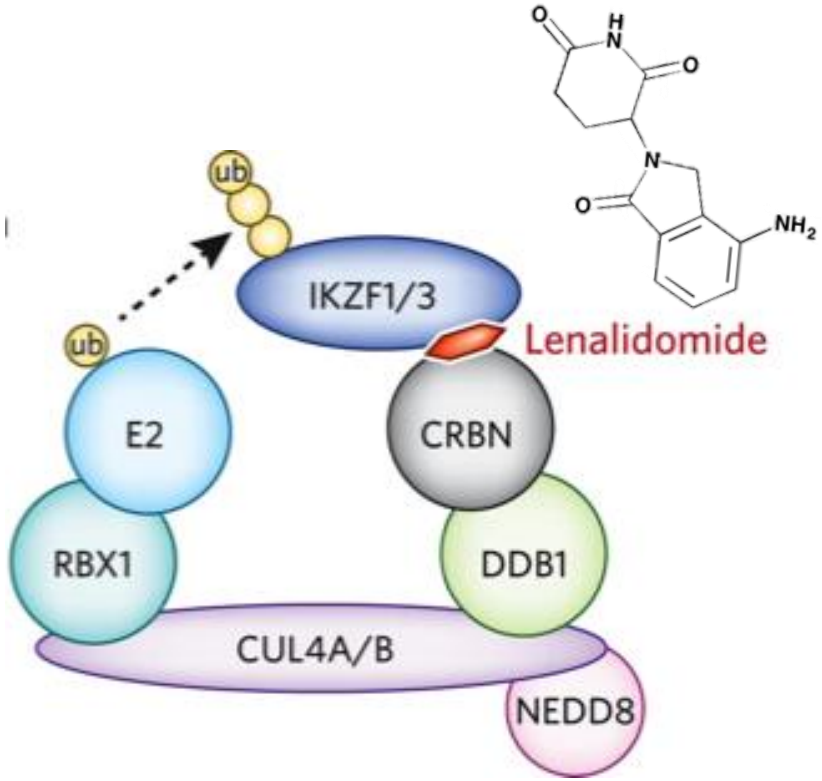
Fischer et al. *Nature* **2014**, *512*, 49





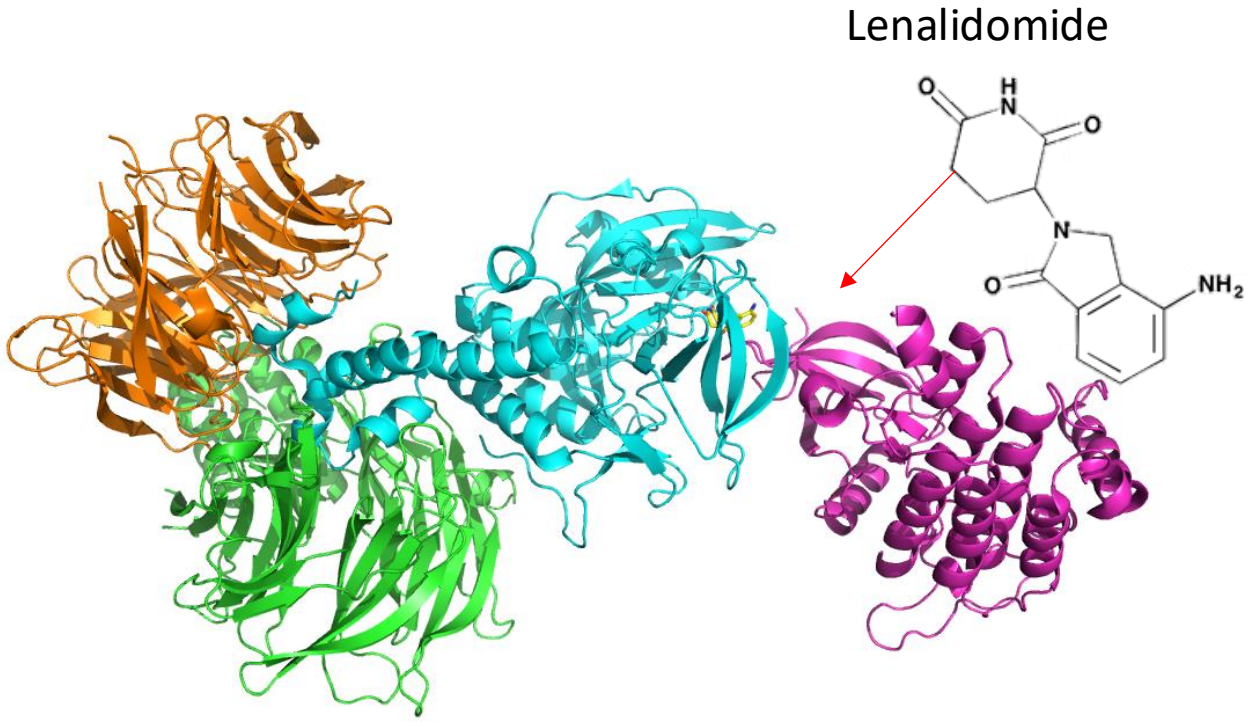
Chamberlain et al. *Nat. Struct. Mol. Biol.* **2014**, *21*, 803

Hijacking CRBN Cul4 E3 ligases with small molecule drugs



IKZF1/3 – Ikaros and Aiolos transcription factors

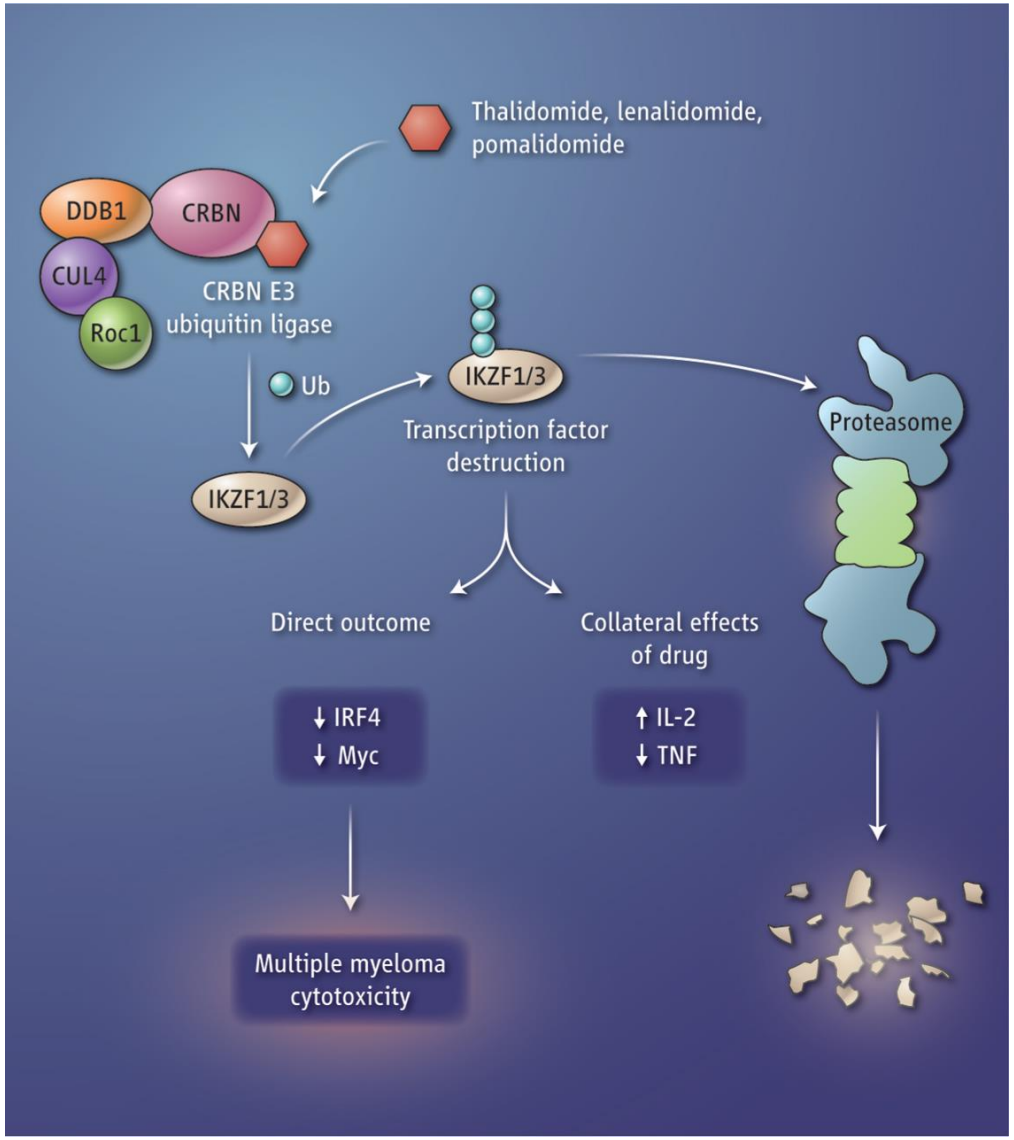
Lu et al. *Science* **2014**, *343*, 305
Krönke et al. *Science* **2014**, *343*, 301



CK1a - Wnt signaling, DNA repair, DNA transcription etc.

Petzold *et al.* Nature **2016** volume 532, pp 127–130

3. External validation – from drugs!

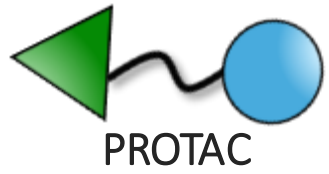


Source: *Science* **2014**, *343*, 256

- Thalidomide and other ImIDs bind to the E3 ligase cereblon (CRBN)
- Binding to CRBN hijacks the ligase to ubiquitinate and degrade new substrate proteins

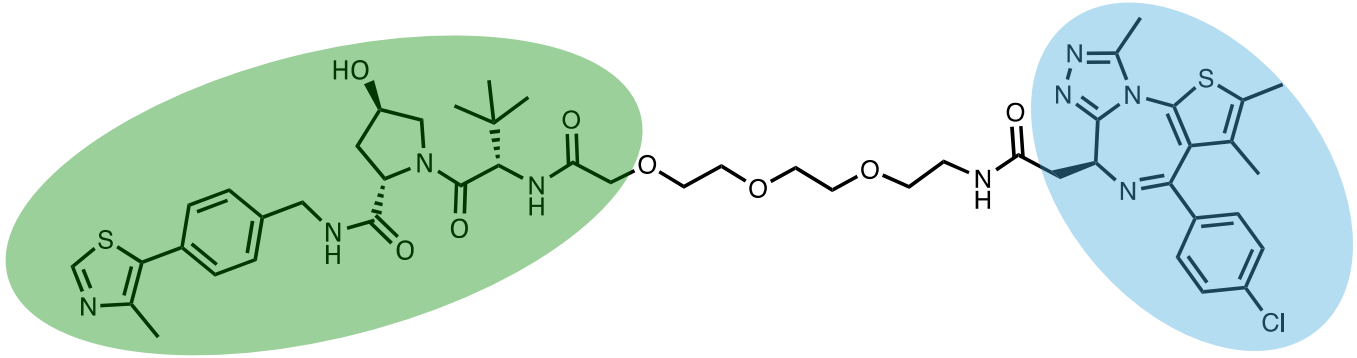
Lu et al. *Science* **2014**, *343*, 305
Krönke et al. *Science* **2014**, *343*, 301
Fischer et al. *Nature* **2014**, *512*, 49
Kronke et al. *Nature* **2015**, *523*, 183
Petzold et al. *Nature* **2016**, *532*, 127

Potent small-molecule PROTAC degraders - 2015



BRD4 degrader MZ1

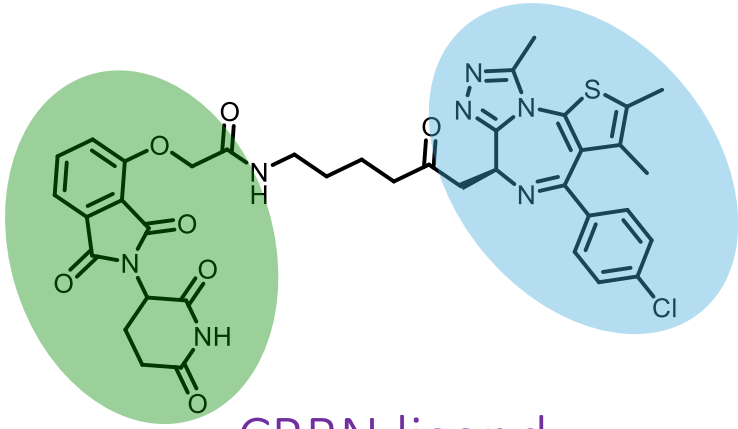
Zengerle et al. *ACS Chem Biol* 2015



VHL ligand

BET degrader dBET-1

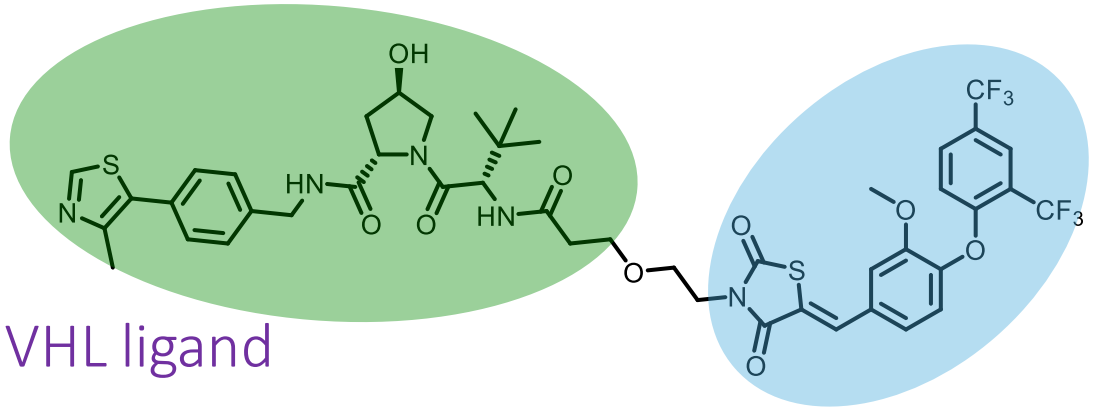
Winter et al. *Science* 2015



CRBN ligand

Kinase ERRα degrader

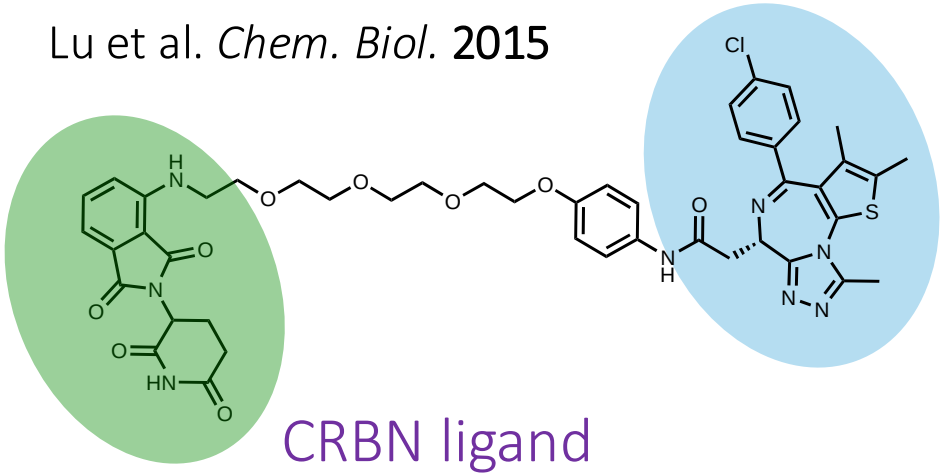
Bondeson et al. *Nat Chem Biol* 2015



VHL ligand

BET degrader ARV-825

Lu et al. *Chem. Biol.* 2015



CRBN ligand

>10 Molecular Glue degraders are in the clinic

(as of March 2024)

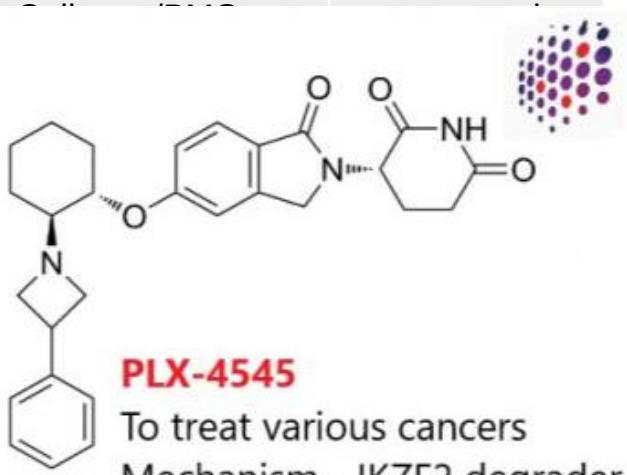
Drug	Target	E3 ligase	Disease	Company	Phase
Thalidomide	IKZF1/3, ZNFs..	CRBN	Lymphoma, Multiple Myeloma, others	Cellgene/BMS	approved
Lenalidomide	IKZF1/3, CK1α, ZNFs..	CRBN	Myelodysplastic Syndrome, Lymphoma, Multiple Myeloma, others	Cellgene/BMS	approved
Pomalidomide	IKZF1/3, ZNFs..	CRBN	Lymphoma, Multiple Myeloma, others	Cellgene/BMS	approved
Iberdomide CC-220	IKZF1/3, ZNFs..	CRBN	Lymphoma, Multiple Myeloma	Cellgene/BMS	Phase 2
Avadomide CC-122	IKZF1/3, ZNFs..	CRBN	Lymphoma, Melanoma	Cellgene/BMS	Phase 2
Tasisulam LY573636	RBM39	DCAF15	Melanoma, NSCLC, Sarcoma, other solid tumours	Eli-Lilly	Phase 3 (suspended)
Indisulam E7070	RBM39	DCAF15	CRC, Leukemia, Melanoma, other solid tumours	Eisai	Phase 1 (sus.)
E7820	RBM39	DCAF15	AML, Colorectal, Lymphoma, other solid tumours	Eisai	Phase 2
DKY709	IKZF2, SALL4	CRBN	Colorectal, Melanoma, NSCLC, Nasopharyngeal, TNBC	Novartis	Phase 1
CC-90009	GSPT1	CRBN	AML, Myelodysplastic Syndromes	Cellgene/BMS	Phase 1
Mezigdomide CC-92480	IKZF1/3	CRBN	Multiple Myeloma	Cellgene/BMS	Phase 1
CC-99282	IKZF1/3	CRBN	Lymphoma	Cellgene/BMS	Phase 1
CFT7455	IKZF1/3	CRBN	Lymphoma, Multiple Myeloma	C4 therapeutics	Phase 1
MRT2359	GSPT1	CRBN	DLBCL, NSCLC, MYC Amplified Solid Tumours	Monte Rosa therapeutics	Phase 1

Ciulli et al., *ChemMedChem* **2023**, e202300464.

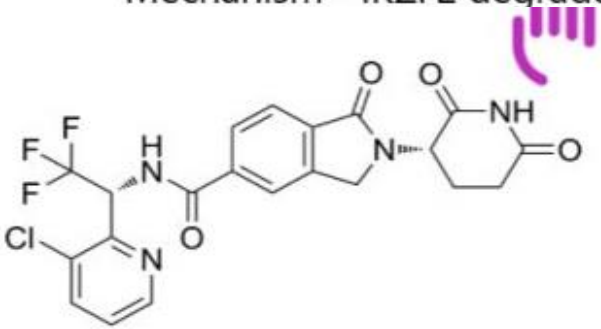
>10 Molecular Glue degraders are in the clinic

(as of March 2024)

Drug	Target	E3 ligase	Disease	Company	Phase
Thalidomide	IKZF1/3, ZNFs..	CRBN	Lymphoma, Multiple Myeloma, others	Cellgene/BMS	approved
Lenalidomide	IKZF1/3, CK1α, ZNFs..	CRBN	Myelodysplastic Syndrome, Lymphoma, Multiple Myeloma, others		
Pomalidomide	IKZF1/3, ZNFs..	CRBN	Lymphoma, Multiple Myeloma, others		
Iberdomide CC-220	IKZF1/3, ZNFs..	CRBN	Lymphoma, Multiple Myeloma		
Avadomide CC-122	IKZF1/3, ZNFs..	CRBN	Lymphoma, Melanoma		
Tasisulam LY573636	RBM39	DCAF15	Melanoma, NSCLC, Sarcoma, other solid tumours		
Indisulam E7070	RBM39	DCAF15	CRC, Leukemia, Melanoma, other solid tumours		
E7820	RBM39	DCAF15	AML, Colorectal, Lymphoma, other solid tumours		
DKY709	IKZF2, SALL4	CRBN	Colorectal, Melanoma, NSCLC, Nasopharyngeal, TNBC		
CC-90009	GSPT1	CRBN	AML, Myelodysplastic Syndromes		
Mezigdomide CC-92480	IKZF1/3	CRBN	Multiple Myeloma		
CC-99282	IKZF1/3	CRBN	Lymphoma		
CFT7455	IKZF1/3	CRBN	Lymphoma, Multiple Myeloma		
MRT2359	GSPT1	CRBN	DLBCL, NSCLC, MYC Amplified Solid Tumours		



PLX-4545
To treat various cancers
Mechanism - IKZF2 degrader

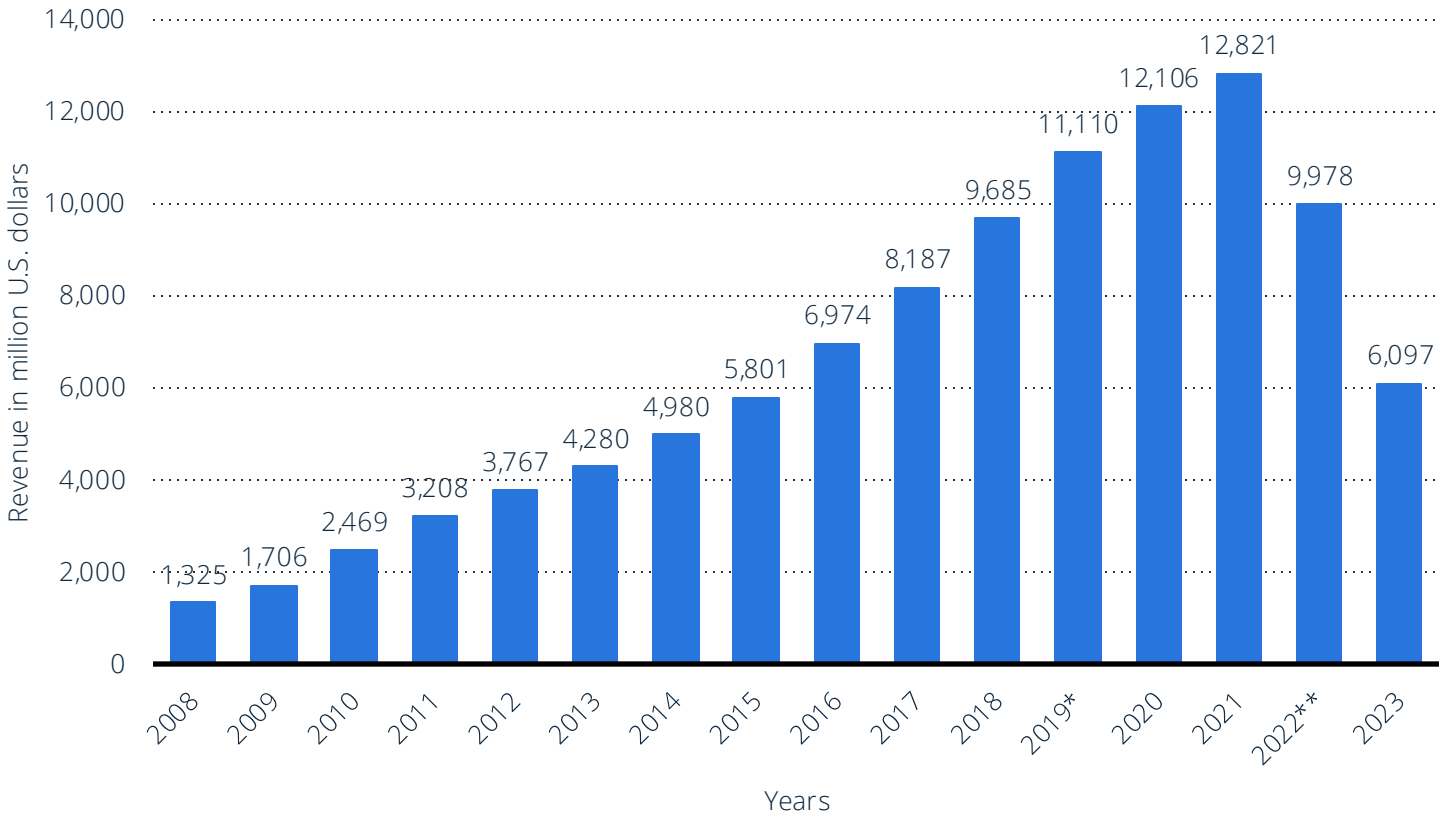


BMS-986397
To treat AML
Mechanism - CK1α degrader

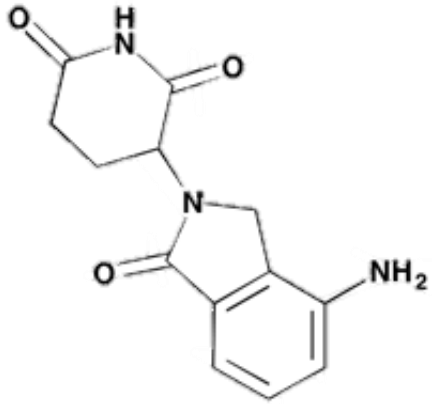
Ciulli et al., *ChemMedChem* **2023**, e202300464.

Revenue of top product Revlimid from 2008 to 2023 (in million U.S. dollars)

Bristol Myers Squibb's Revlimid revenues 2008-2023



Lenalidomide, sold under the brand name **Revlimid**, is a medication used to treat multiple myeloma, smoldering myeloma, and myelodysplastic syndromes (MDS)



Note(s): Worldwide
Further information regarding this statistic can be found on page 8.
Source(s): Celgene Corporation; Bristol-Myers Squibb; ID 1269411

>25 PROTAC degraders are in the clinic

(as of October 2024)

CRBN | VHL | undisclosed

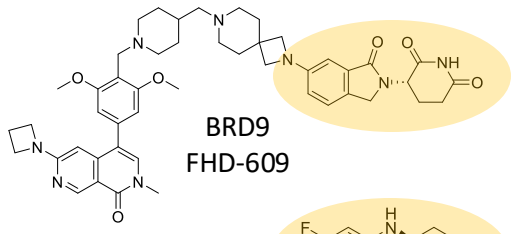
Drug	Target	Disease	Company
AC176	AR	Prostate Cancer	Accutar Bio
ARV-766	AR	Prostate Cancer	Arvinas
Bavdegalutamide (ARV-110)	AR	Prostate Cancer	Arvinas
GT20029	AR	Acne, Acne Vulgaris, Androgenic alopecia,	Suzhou Kintor Pharmaceutical Inc
HP518	AR	Prostate Cancer	Hinova
CC-94676 (AR-LDD)	AR	Adenocarcinoma of the Prostate, Castration Resistant Prostate Cancer, etc	Bristol-Myers Squib
NX-2127	BTK	B-Cell Malignancies	Nurix
NX-5948	BTK	B-Cell Malignancies	Nurix
BGB-16673	BTK	B-Cell Malignancies; Lymphoma	BeiGene
HSK29116	BTK	B-Cell Malignancies; Lymphoma	Haisco Pharmaceutical
ABBV-101	BTK	ALK Positive Anaplastic Large Cell Lymphoma,	Abbvie
BTX-9341	CDK4/6	HR+/HER2- Breast Cancer	Biotheryx
CG001419	TRK	Advanced Solid Tumors, etc	Cullgen Inc
CG428	TRK	Alopecia, Breast Cancer, Colorectal Carcinoma, etc	Cullgen Inc

Drug	Target	Disease	Company
AC0682	ER	Breast Cancer	Accutar Bio
ARV-471	ER	Breast Cancer	Arvinas
FHD-609	BRD9	Synovial Sarcoma	Foghorn Therapeutics
CFT8634	BRD9	Advanced Cancers, Chordoma, etc	C4 Therapeutics
KT-413	IRAK4	B-Cell Malignancies; Lymphoma	Kymera Therapeutics
KT-474	IRAK4	Eczema; Hidradenitis Suppurativa; etc	Kymera Therapeutics
KT-253	MDM2	AML	Biognosys, Kymera Therapeutics
CFT1946	BRAF (V600X)	Anaplastic Thyroid Cancer, BRAF Mutant Cancers, etc	C4 Therapeutics
ASP-3082	KRAS	Lung and other cancer	Astellas
PRT3789	SMARCA2/4	Advanced Solid Tumors, etc	Prelude Therapeutics
PRT7732	SMARCA2	Advanced pr Metastatic SMARCA4 Solid Tumors	Prelude Therapeutics
DT2216	Bcl-xL	Hematological Malignancies; etc	Dialectic Therapeutics
HSK40118	EGFR	Advanced Non-small Cell Lung Cancer (NSCLC), etc	Haisco pharmaceutical
KT-333	STAT3	Solid Tumours; Lymphoma	Kymera Therapeutics

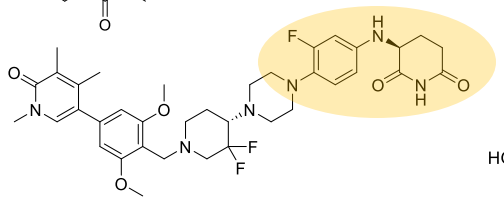
Vetma, O'Connor, & Ciulli, *Annu. Rev. Cancer Biol.*, 30 October 2024

CRBN VHL

Synovial sarcoma

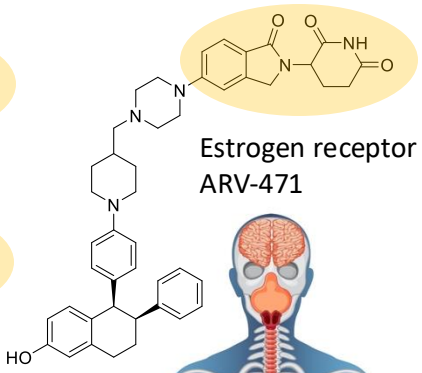


BRD9
FHD-609



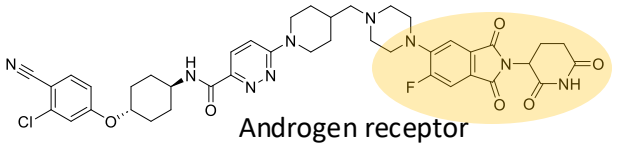
BRD9
CFT 8634

Breast cancer

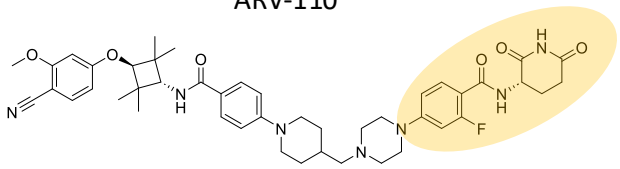


Estrogen receptor
ARV-471

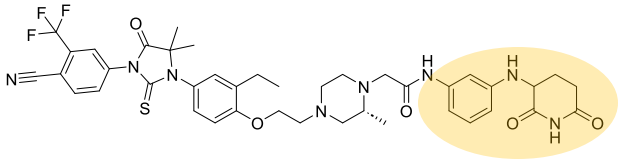
Prostate cancer



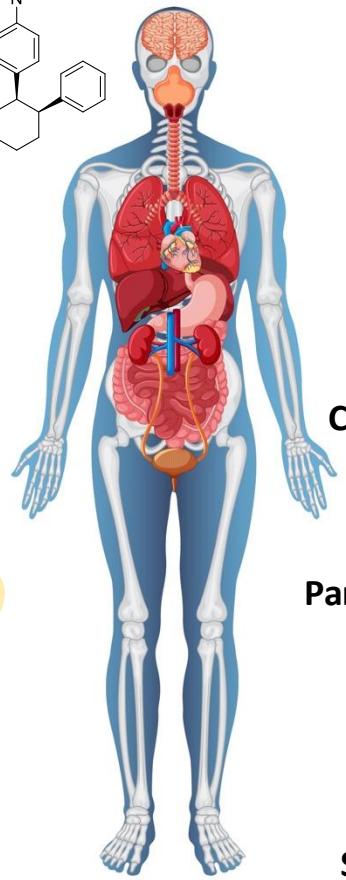
Androgen receptor
ARV-110



Androgen receptor
ARV-766



Androgen receptor
BMS-986365



Lung cancer

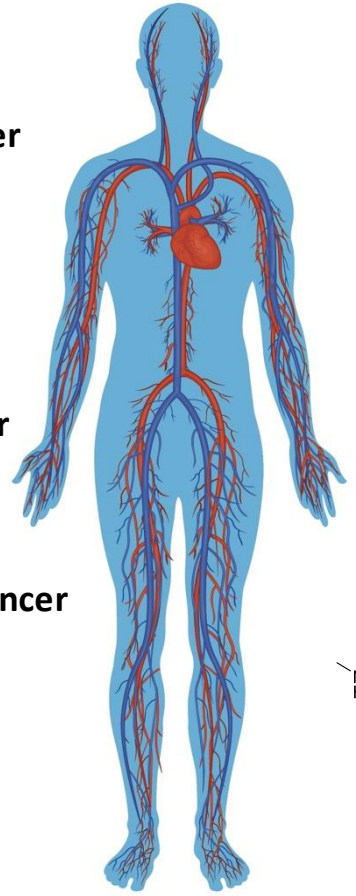
SMARCA2
$KRAS^{G12D}$
$BRAF^{V600E}$
EGFR

Colon cancer

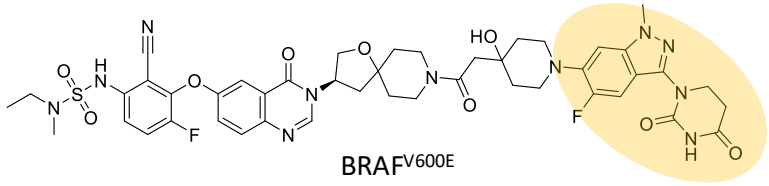
$KRAS^{G12D}$
$BRAF^{V600E}$

Pancreatic cancer

SMARCA2
$KRAS^{G12D}$
$BRAF^{V600E}$
EGFR



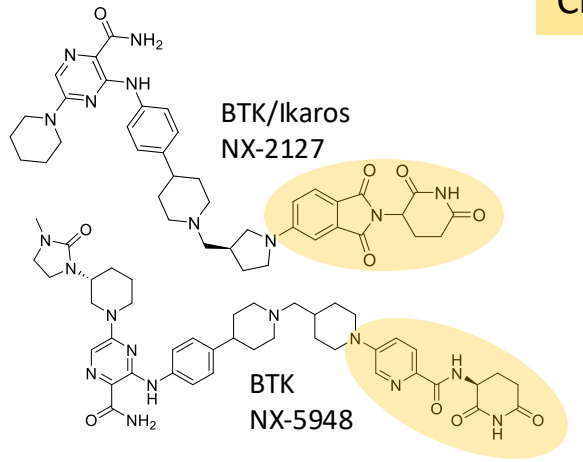
Skin cancer



$BRAF^{V600E}$
CFT-1946

B-cell malignancies

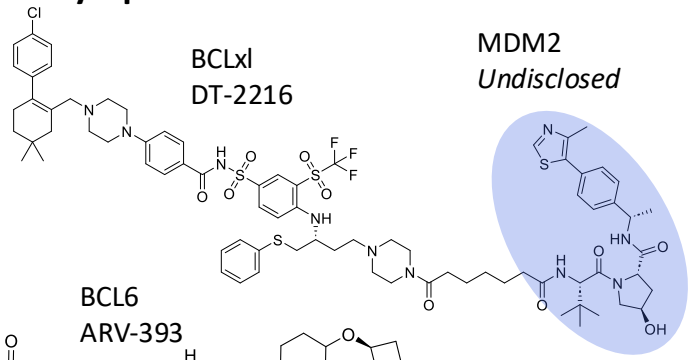
IRAK4
Undisclosed



BTK/Ikaros
NX-2127

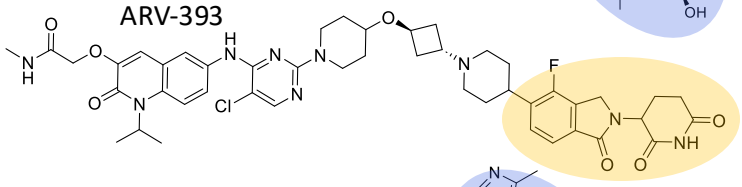
BTK
NX-5948

Lymphoma and Leukemia

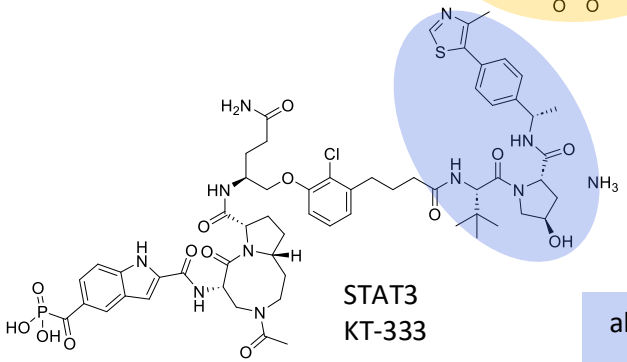


BCLxl
DT-2216

MDM2
Undisclosed



BCL6
ARV-393



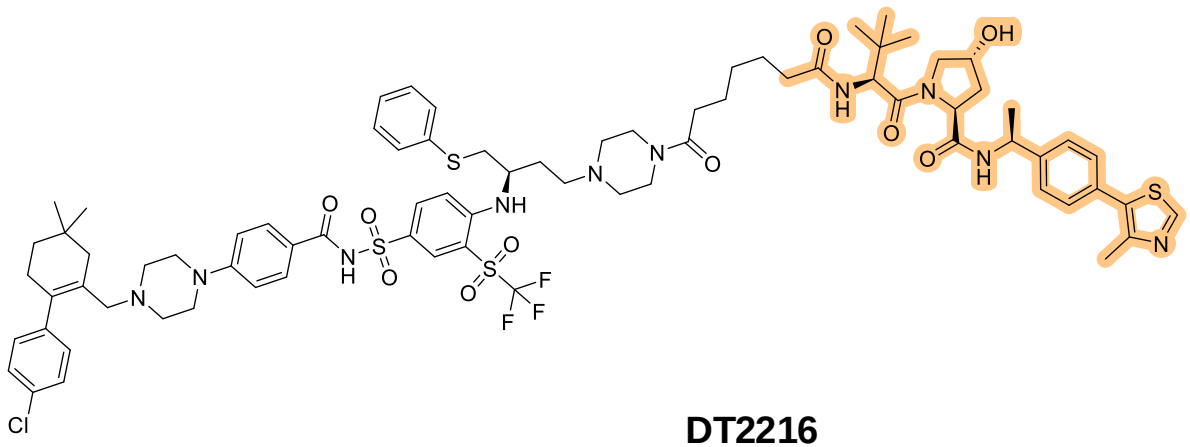
STAT3
KT-333

also SMARCA2 and KRAS PROTACs

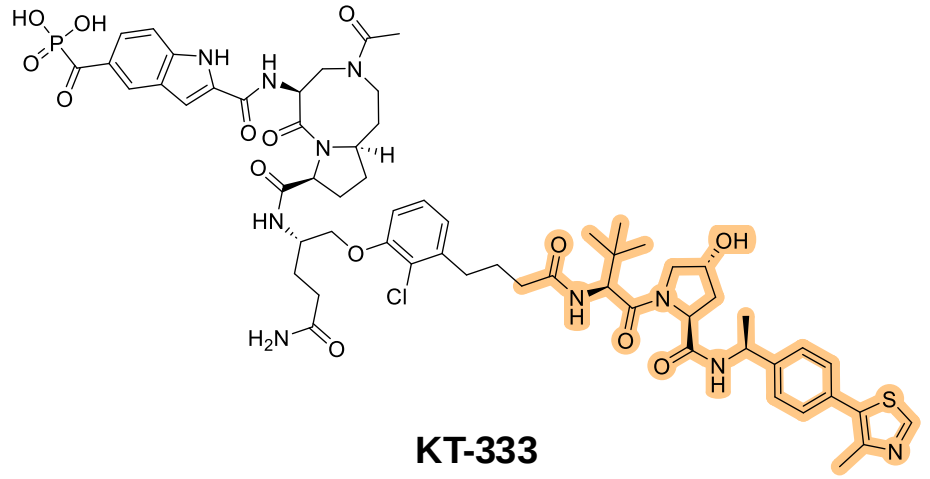
Vetma, O'Connor, & Ciulli, *Annu. Rev. Cancer Biol.*, 30 October 2024

Four VHL based PROTAC degraders in the clinic

Degrader	Company	Target	Clinical Trial	Refs
DT2216	Dialectic Therapeutics	BCL-xL	NCT04886622	1, 2
KT-333	Kymera Therapeutics	STAT3	NCT05225584	3
ASP3082	Astellas Pharma.	$KRAS^{G12D}$	NCT05382559	4
PRT3789	Prelude Therapeutics	SMARCA2/4	NCT05639751	5



DT2216



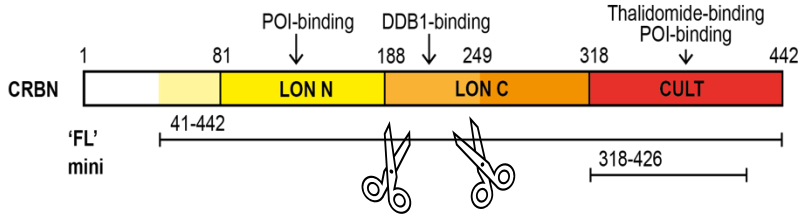
KT-333

1. *Nat Med* **2019**, *25* (12), 1938–1947.
2. *Dialectic Therapeutics Announces First-in-Human Dose in Phase 1 Clinical Trial Evaluating DT2216*. PR Newswire.
3. *Journal of Clinical Oncology* **2024**, *40* (16_suppl), TPS3171–TPS3171.
4. *Journal of Clinical Oncology* **2023**, *41* (4_suppl), TPS764–TPS764.
5. *Annals of Oncology* **2024**, *35*, S483–S484.

Urbina, Hallatt, Robertson & Ciulli, *Expert Opin. Ther. Pat.* **2025** Feb 6:1-42.

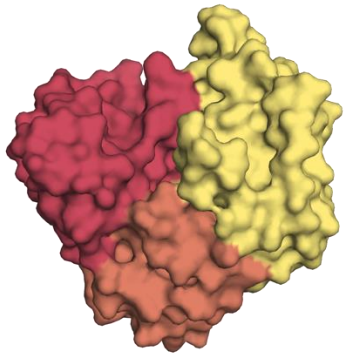
CRBN-Midi accelerates structural enablement

Centre for Targeted Protein Degradation
University of Dundee
innovate collaborate inspire



Alena Kroupova

David Zollman



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CRBN^{midi} is available on www.addgene.org/Alessio_Ciulli

nature communications

Article

https://doi.org/10.1038/s41467-024-52871-9

Design of a Cereblon construct for crystallographic and biophysical studies of protein degraders

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Check for updates

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Kroupova et al. *Nat. Commun.*, 2024 October 15

Bailey et al. *Cell Chem Biol*, 28 Nov 2024

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His6-TEV-CRBN-midi
(Plasmid #215330)

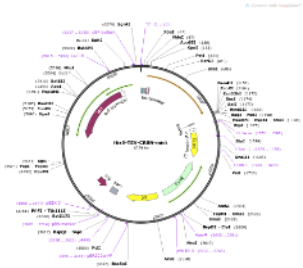
Print

Purpose
CRBN midi construct for use in crystallography and biophysical studies enabling ligand and degrader design

Depositing Lab
Alessio Ciulli

Publication
Alena et al bioRxiv 2024.01.17.575503
(How to cite)

Sequence Information
Sequences (1)



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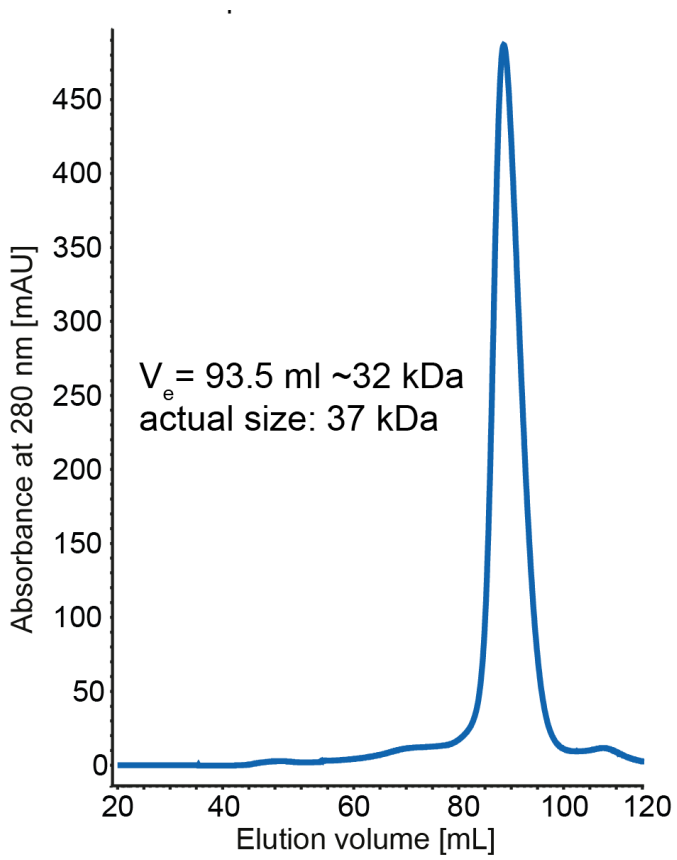
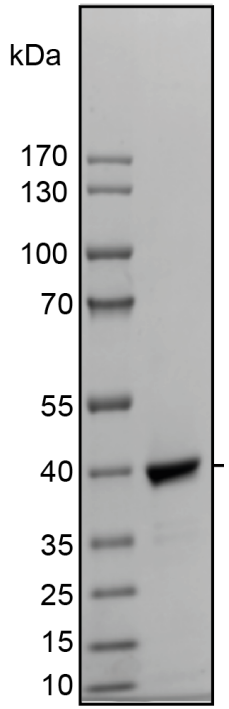
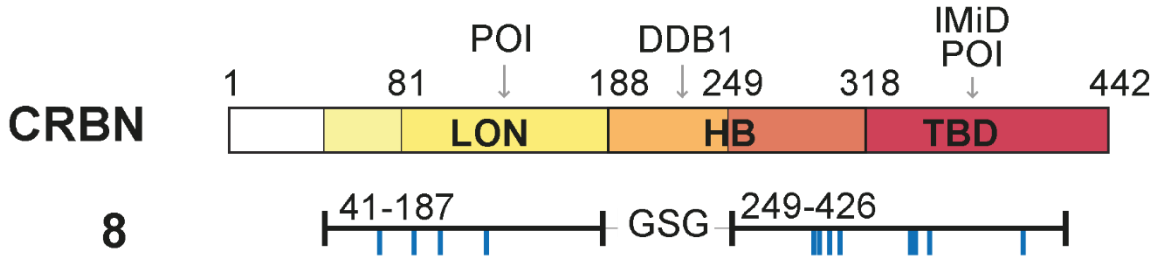
Convenient purification from *E. coli*

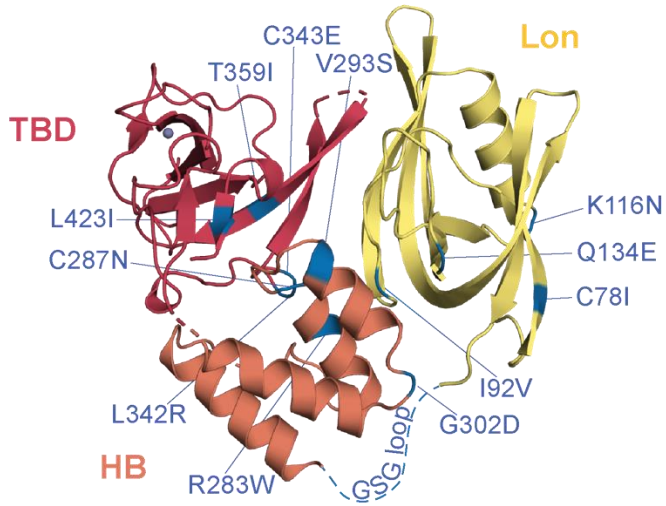
Centre for Targeted Protein Degradation
University of Dundee
innovate collaborate inspire

Size-exclusion chromatography









☑ Expressed in *E.coli*
☑ Soluble
☑ Monomer

Construct **8** = CRBN^{midi}

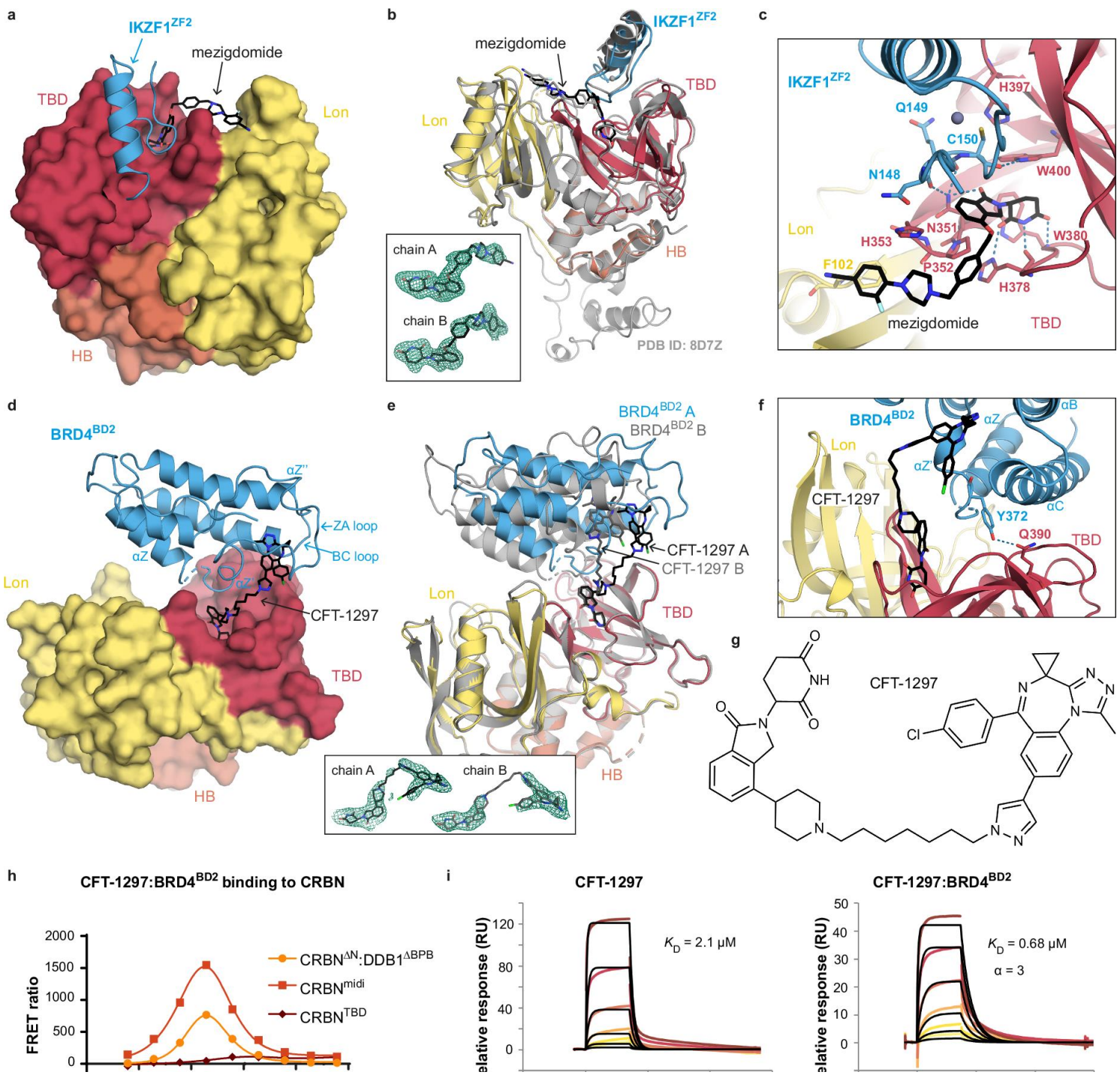
Yield = 4 mg/L LB media

Alena Kroupova
Julie Pethe

Kroupova et al. *Nat. Commun.*, **2024** October 15

Ternary liganded CRBN-Midi structures

The CeTPD-CRBN Taskforce Team



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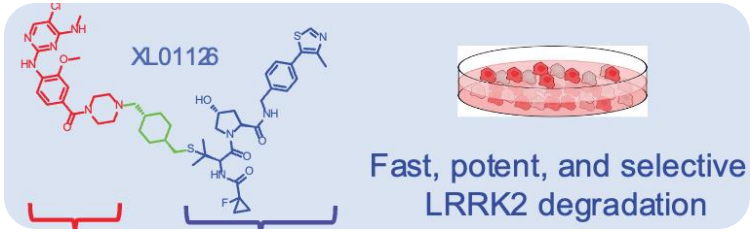
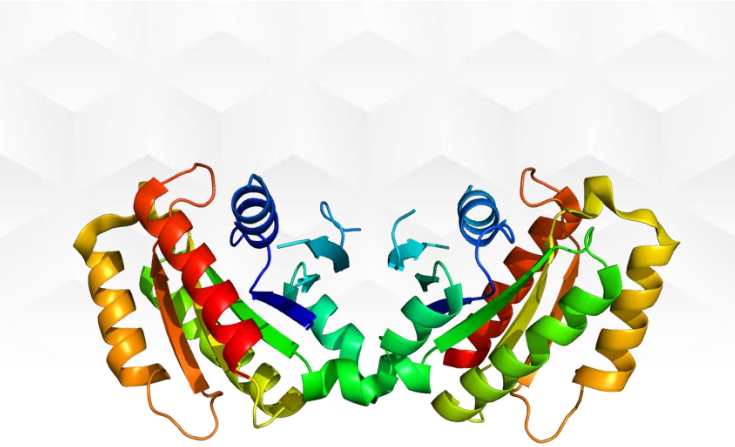
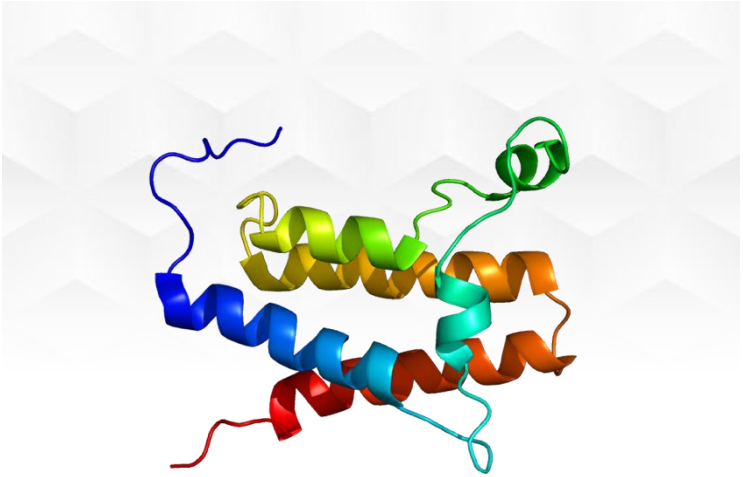
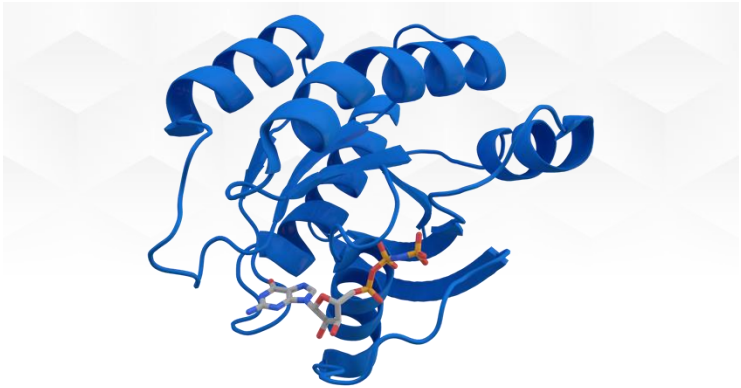
Our successfully prosecuted targets

innovate collaborate inspire | Centre for Targeted Protein Degradation University of Dundee









LRRK2

XL01126 / Liu et al.
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SMARCA2

ACBI1 / Farnaby et al.
Nat Chem Biol 2019
ACBI2 / Kofink et al.
Nat Commun 2022

KRAS

ACBI3 / Popow et al.
Science, 2024

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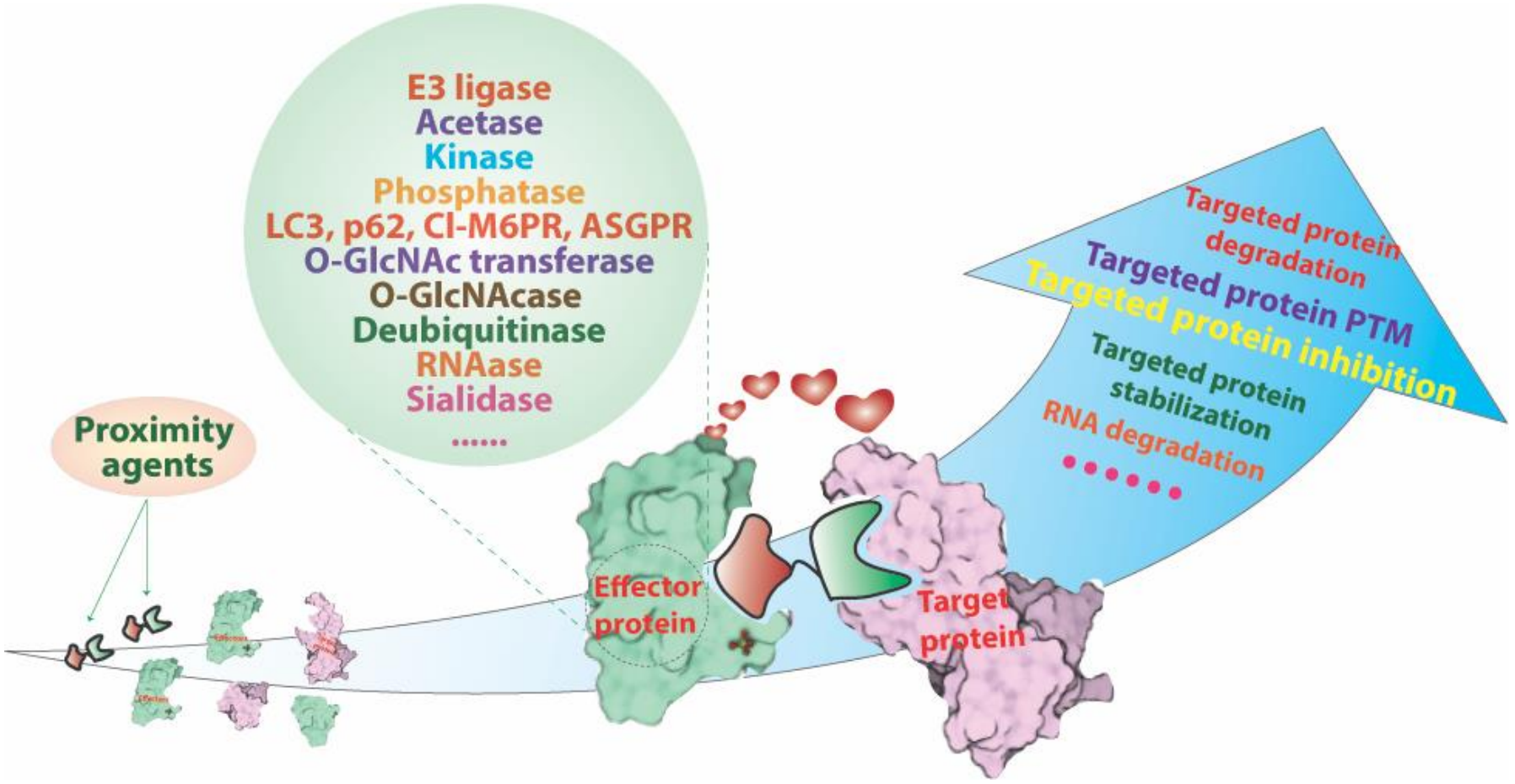

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