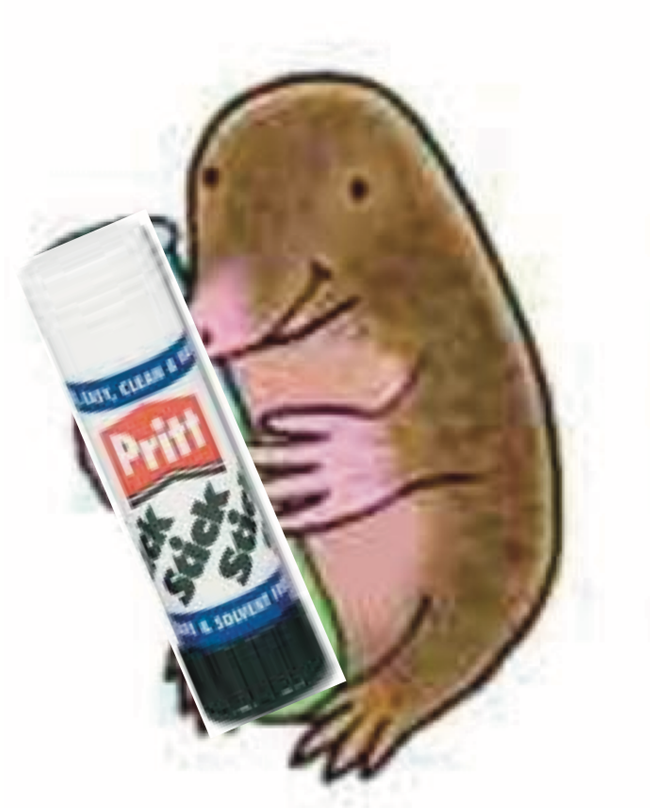
Molecular Glues



Ciaran P. Seath

MacMillan Group Meeting

4/20 2021

Outline

What is a molecular glue?

Why are they important

Background/History

Case study: Hijacking the ubiqutination pathway

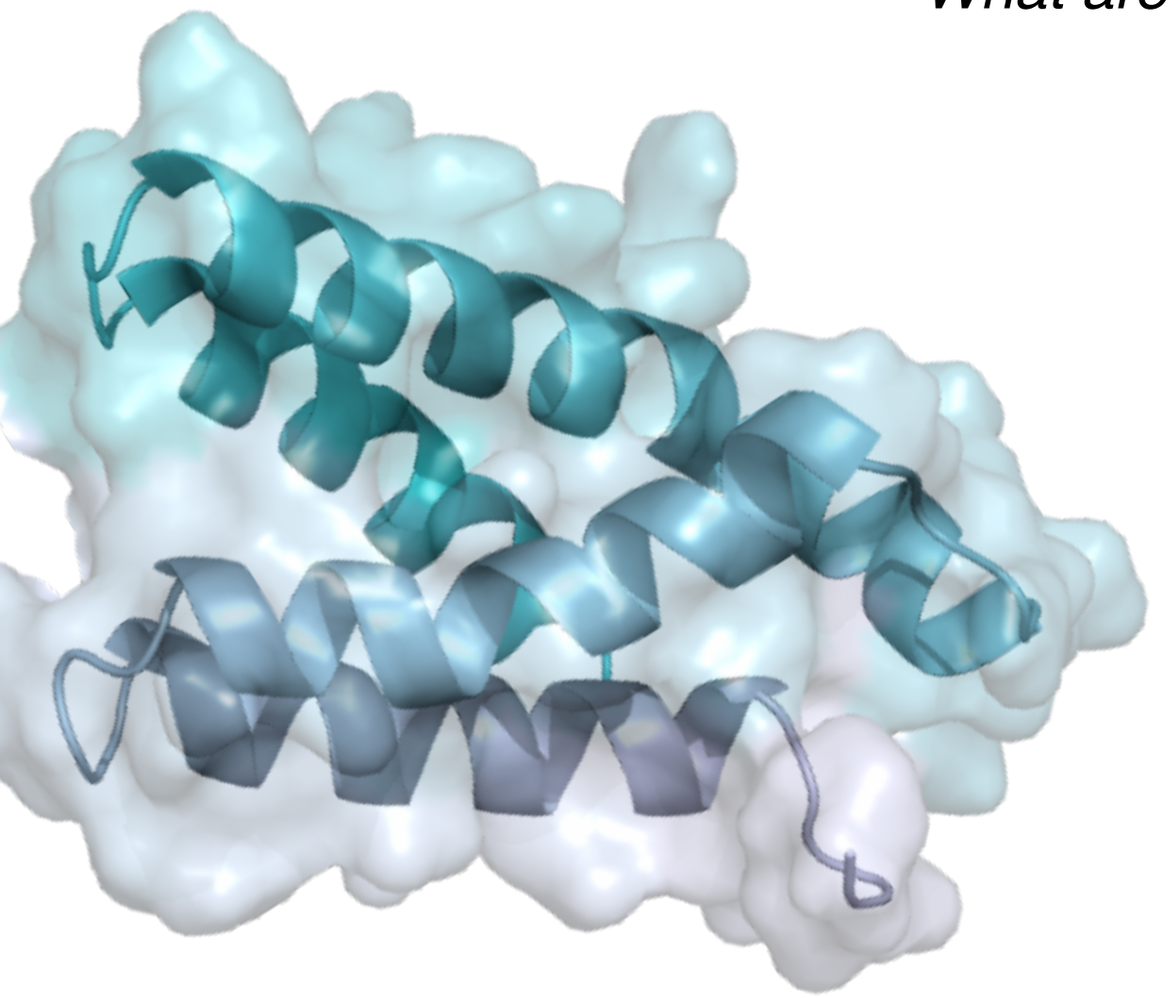
Highlights in molecular glue research

Conclusions

What are Molecular Glues?

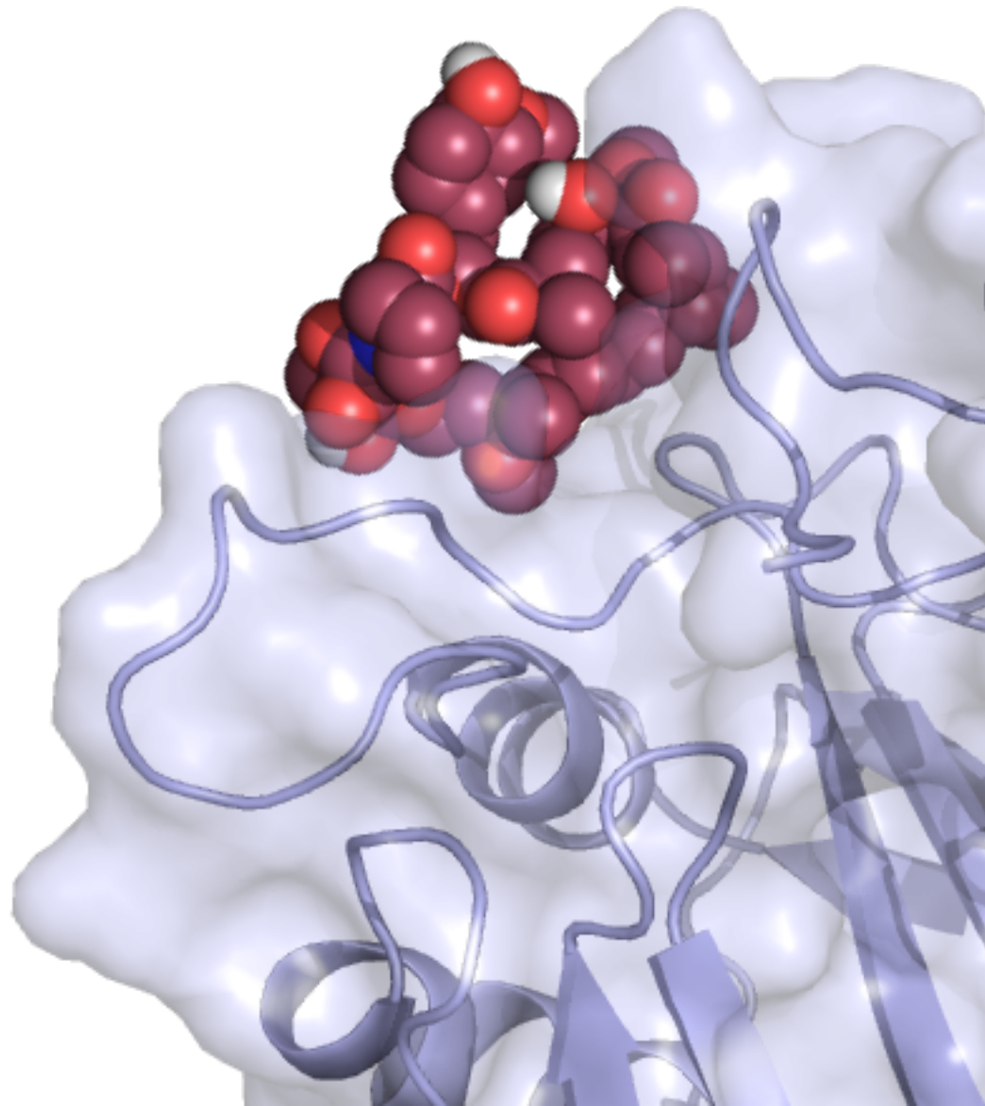
Molecular glues are small molecules that induce protein-protein interactions

What are Molecular Glues?



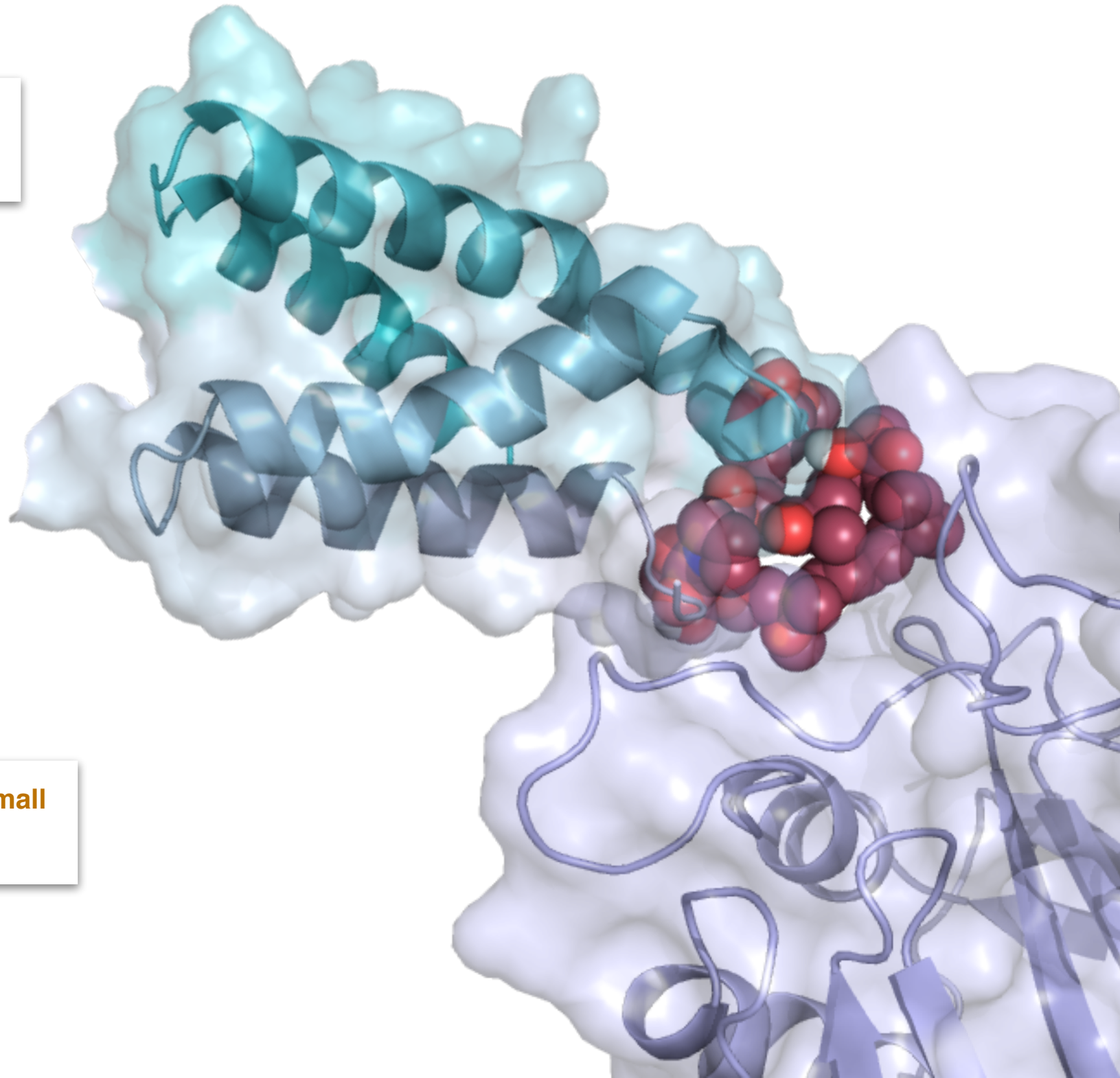
two proteins without binding affinity

binding is initiated through a small molecule ligand



What are Molecular Glues?

a new protein-protein interaction is initiated



binding is initiated through a small molecule ligand

What are Molecular Glues?

Cell
Leading Edge

CellPress

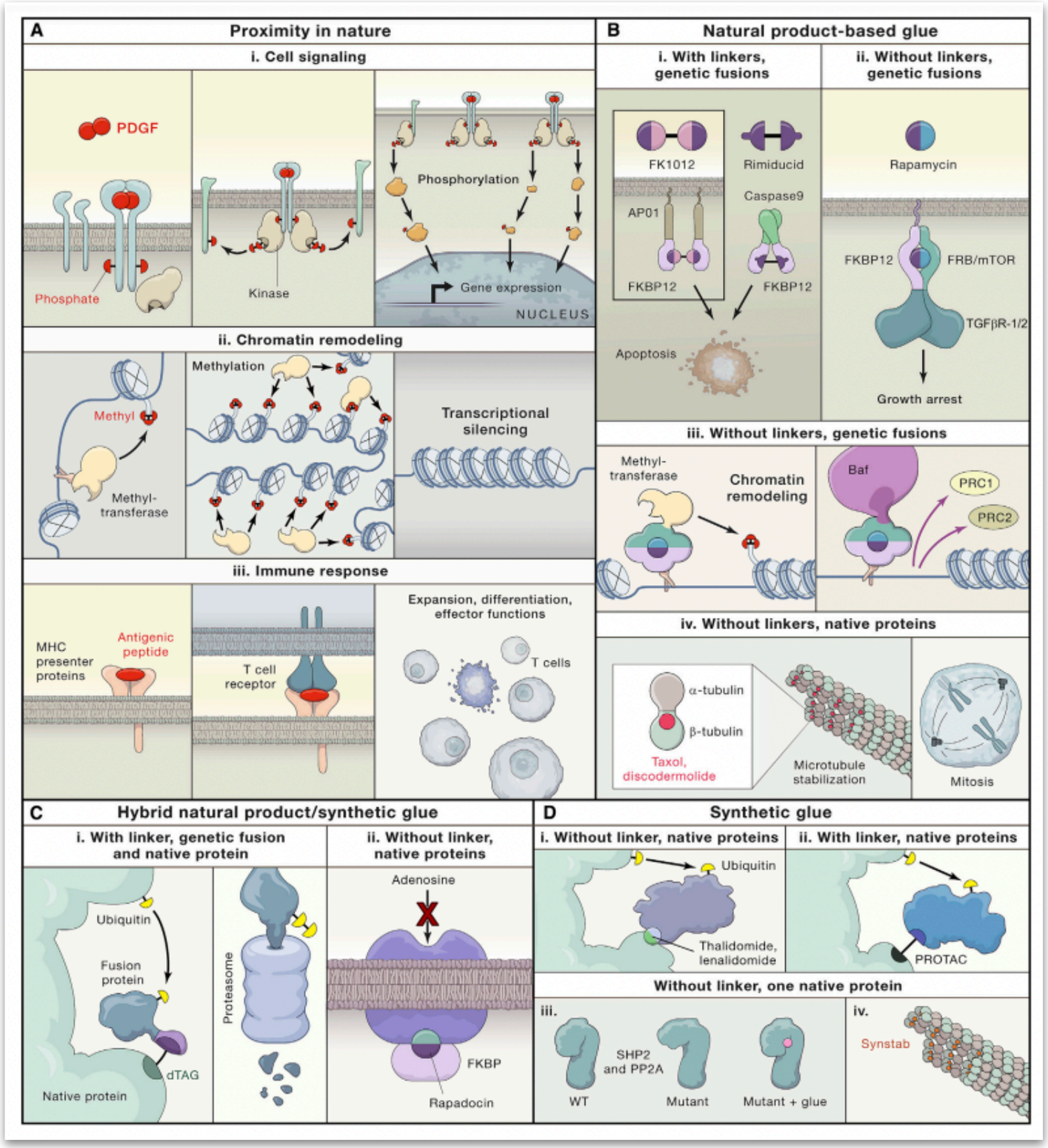
BenchMarks

The Rise of Molecular Glues

Stuart L. Schreiber[1,2,*]
[1]Department of Chemistry & Chemical Biology, Harvard University, 12 Oxford St, Cambridge, MA 02138, USA
[2]Chemical Biology & Therapeutics Science Program, Broad Institute, 415 Main St, Cambridge, MA 02142, USA
*Correspondence: stuart_schreiber@harvard.edu
https://doi.org/10.1016/j.cell.2020.12.020

2021 marks the 30th anniversary of the revelation that cyclosporin A and FK506 act in a way previously not seen—as "molecular glues" that induce neo-protein–protein associations. As a torrent of new molecular-glue probes and medicines are fueling interest in this field, I explore the arc of this story.

What are Molecular Glues?



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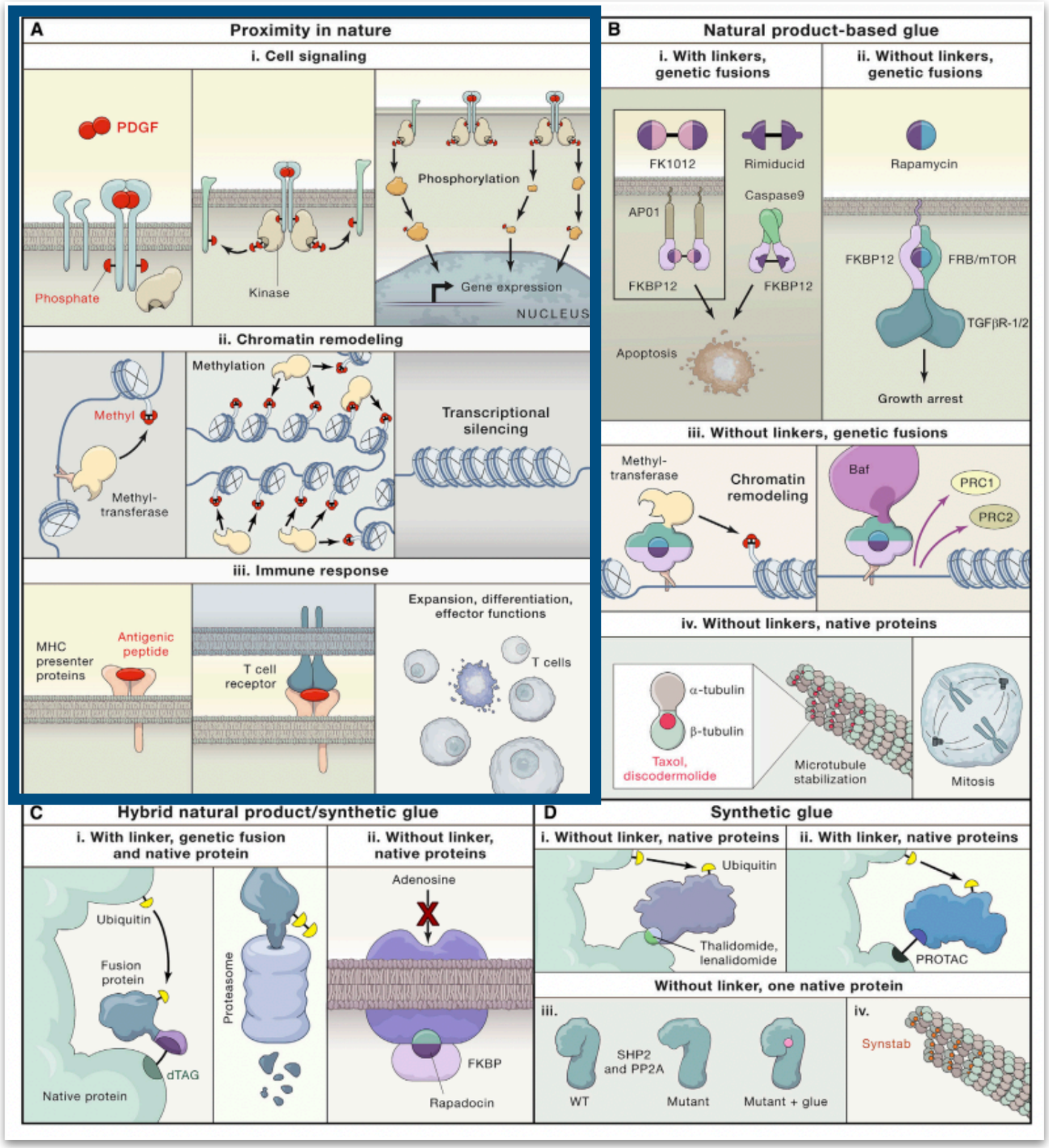
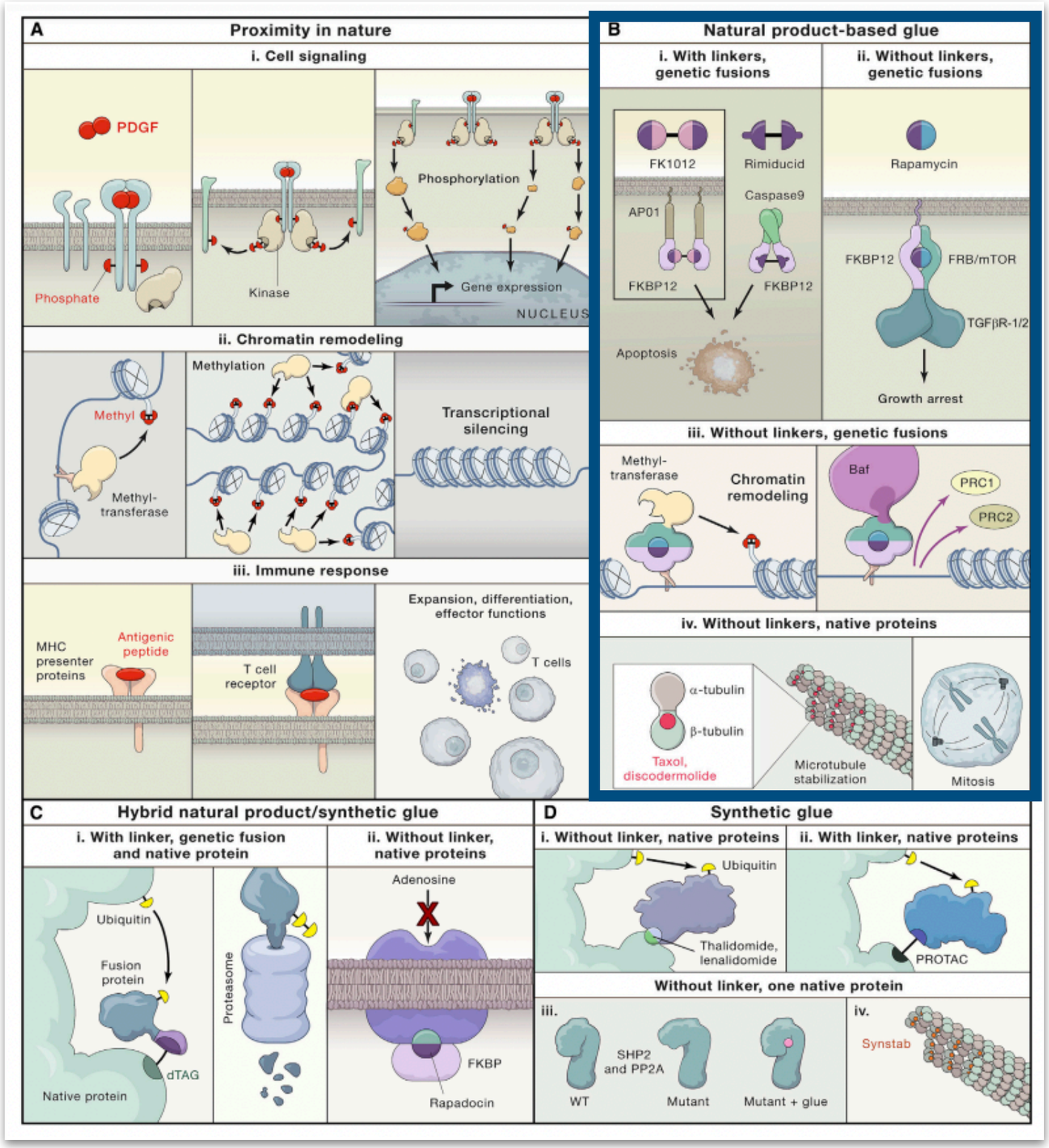


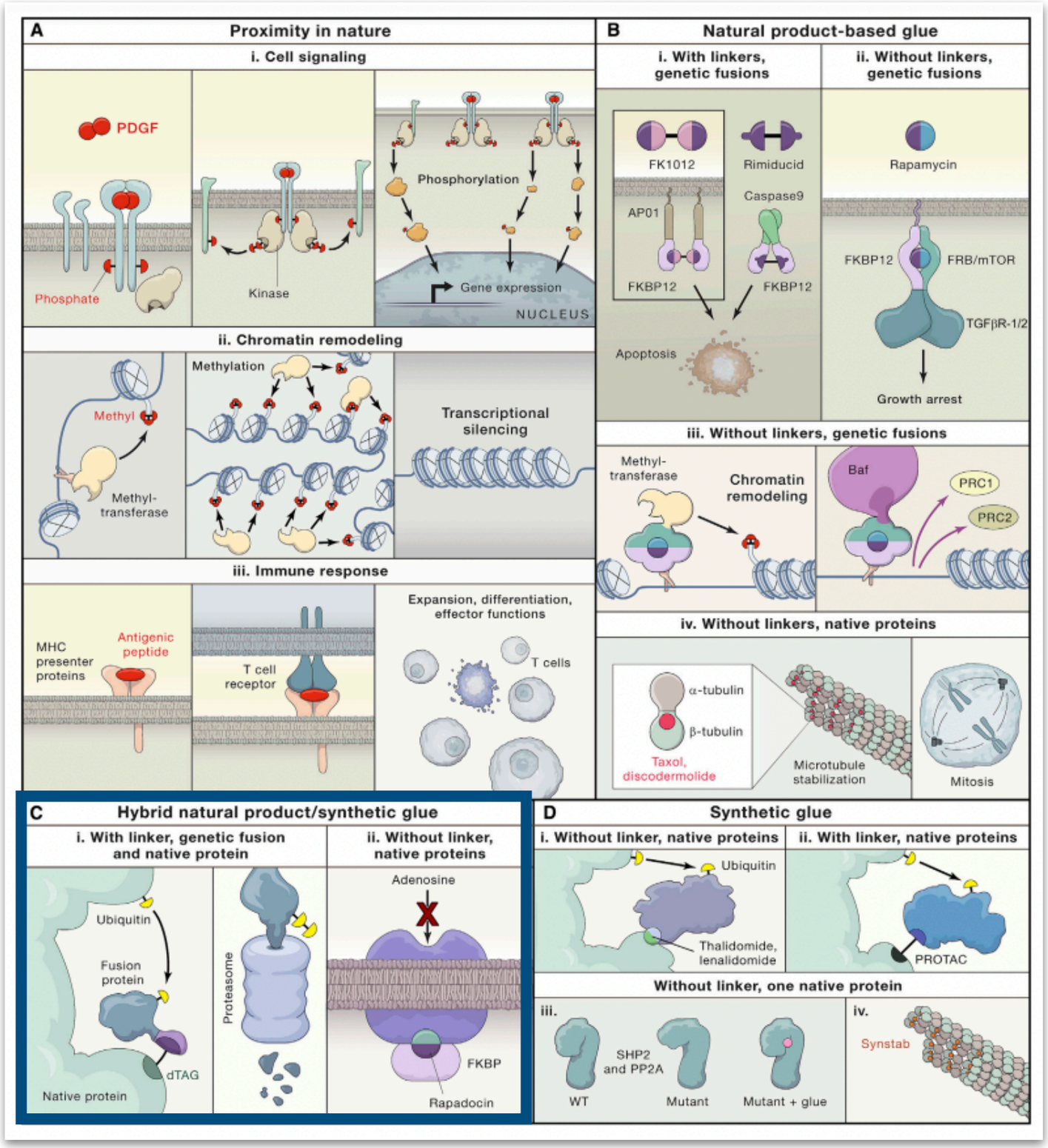
Hormones, antigenic peptides and PTMS all induce PPIs in nature

What are Molecular Glues?



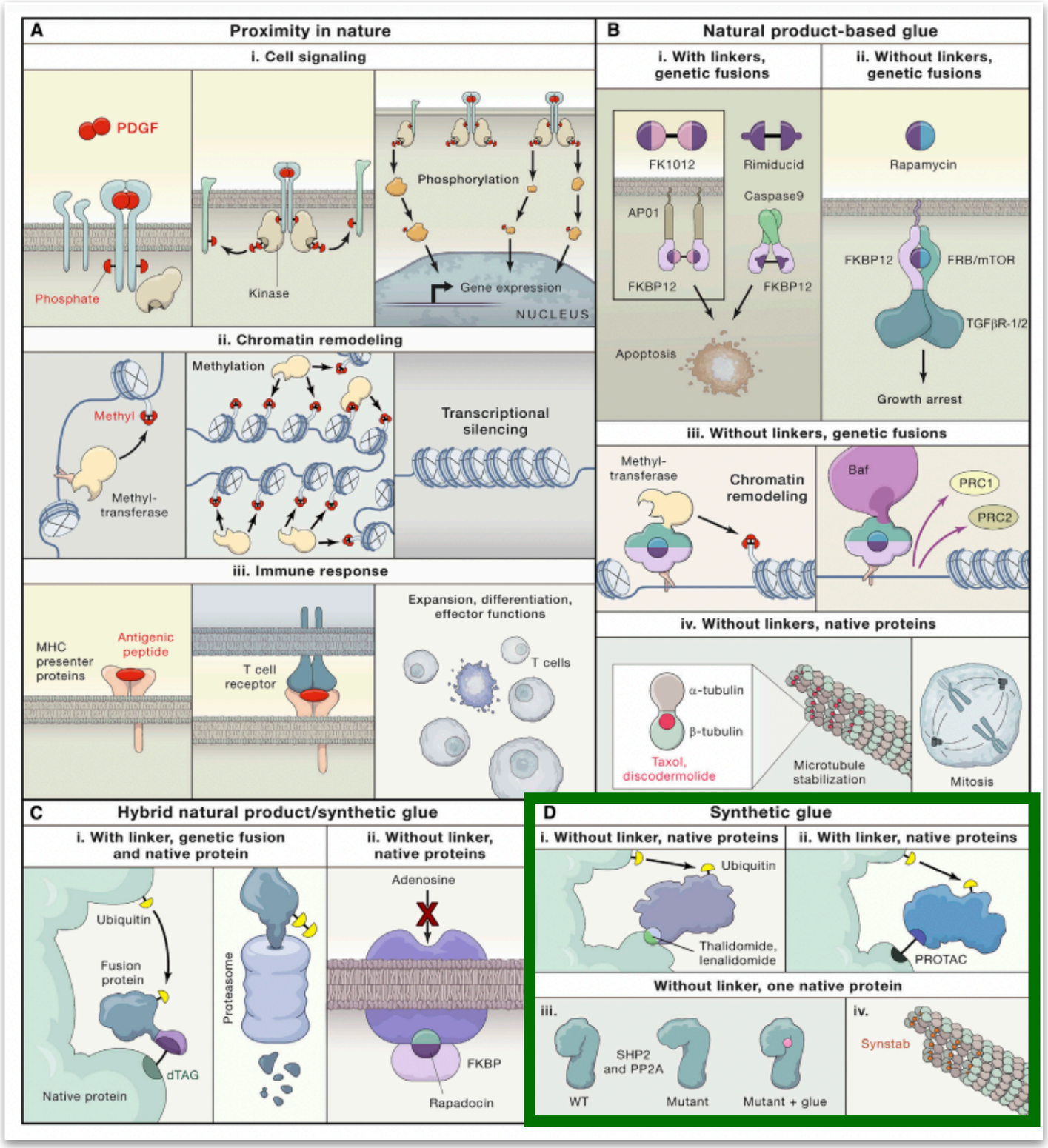
Natural products are known to induce protein dimerization and can be exploited through genetic fusions

What are Molecular Glues?



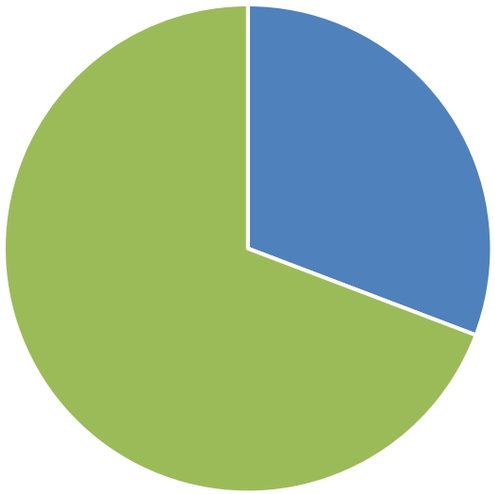
Synthetic hybrids have been used as tools to control biology

What are Molecular Glues?

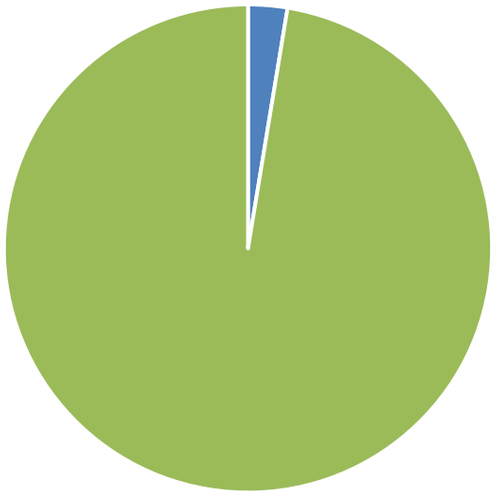


Molecular glues for therapeutic intervention

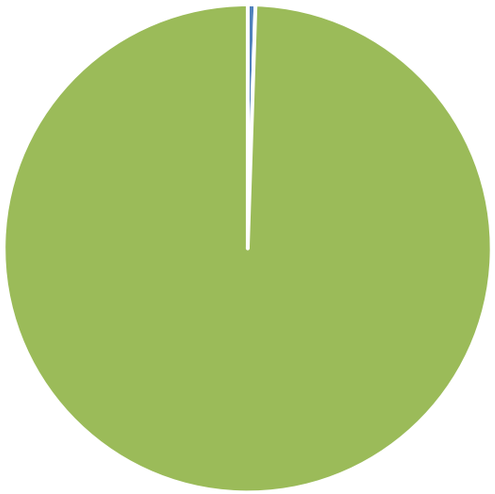
Why are they Important?



44% of the “druggable” proteome currently have FDA approved compounds

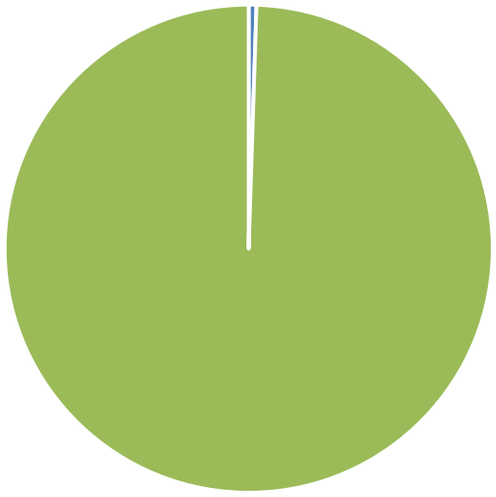


this accounts for 3% of the total proteome



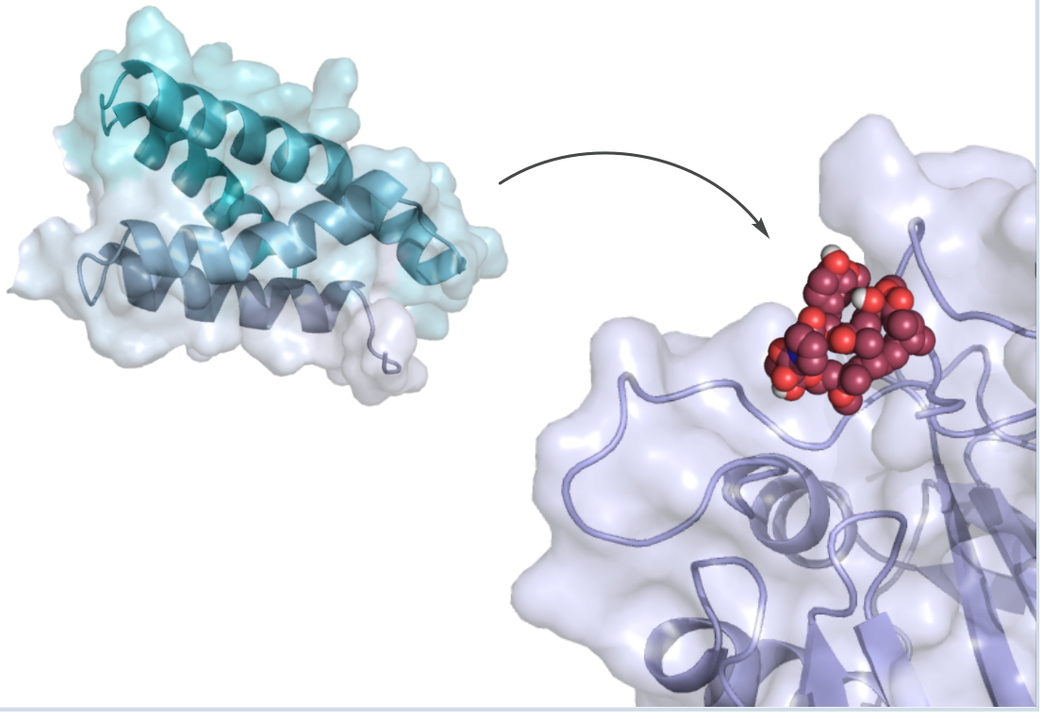
or 0.5% when you include protein-protein interactions

Why are they Important?

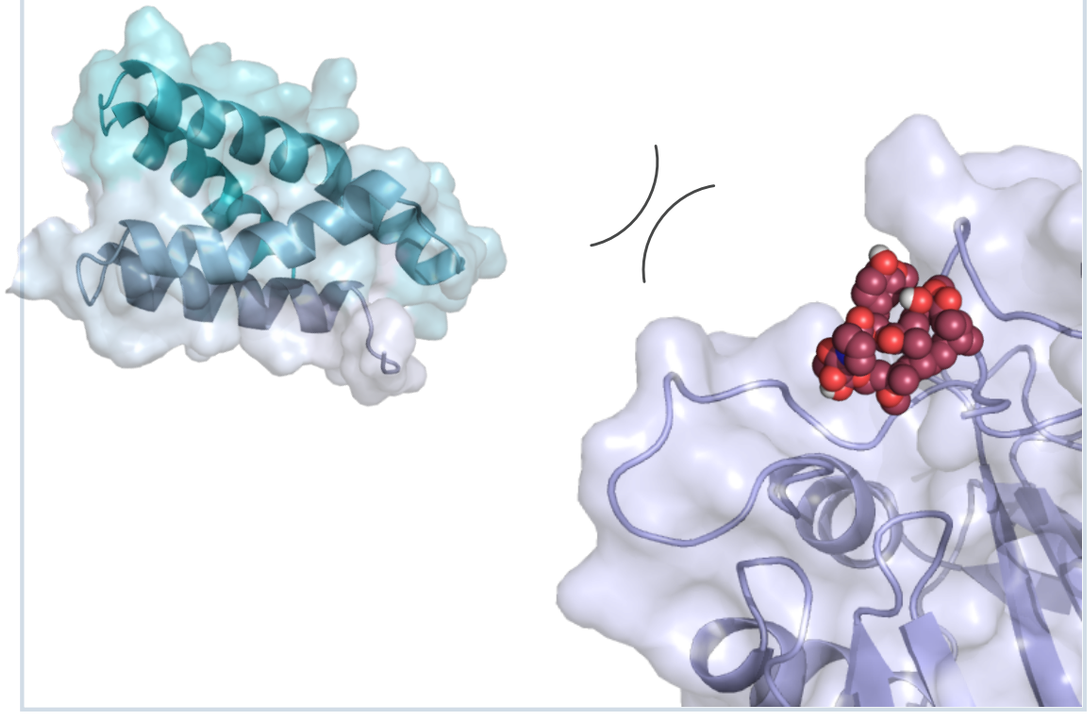


Accessing these PPIs in drug discovery would be extremely powerful

enabling interactions for *gain of function*



blocking interactions for *loss of function*



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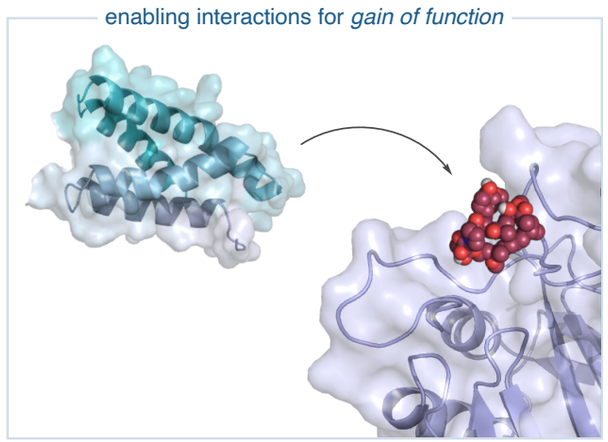
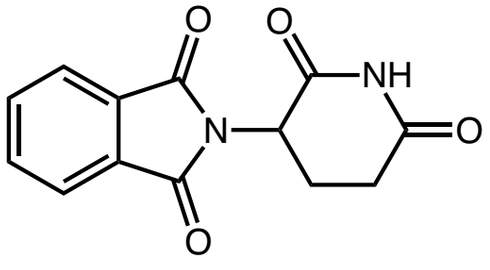


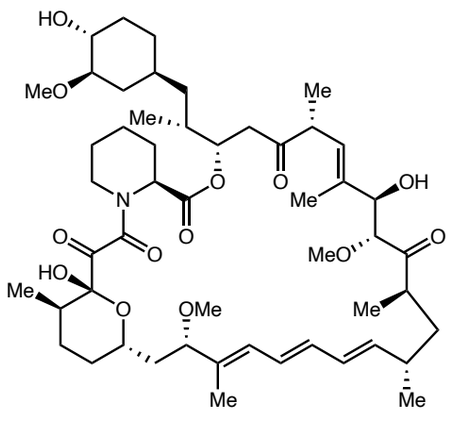
Most glues provide gain-of-function (new interaction)

Molecular Glues (this presentation)

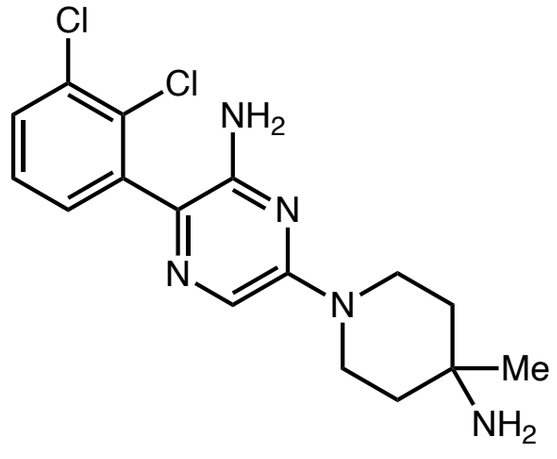
- Small molecules
- Numerous mechanisms of action
- Several blockbuster drugs and developing area
- New research area



thalidomide derivatives

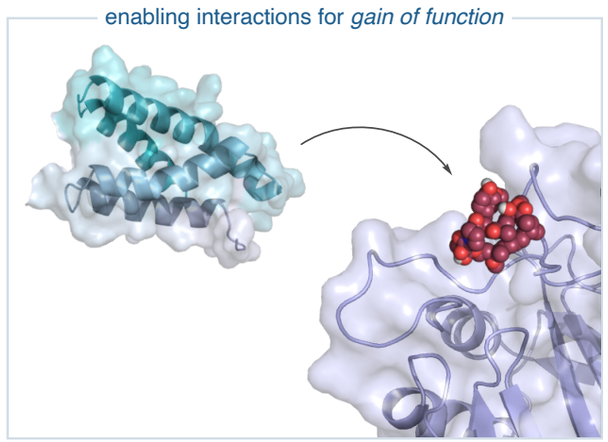


rapamycin analogs



SHP099

Why are they Important?



Most glues provide gain-of-function (new interaction)

Molecular Glues (this presentation)

- Small molecules
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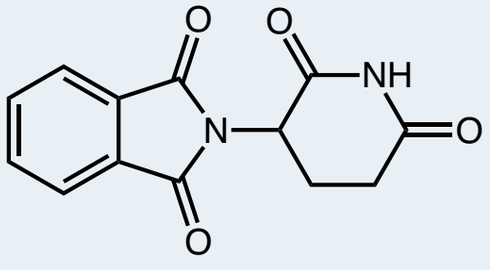
- New research area

Degraders of undruggable proteins



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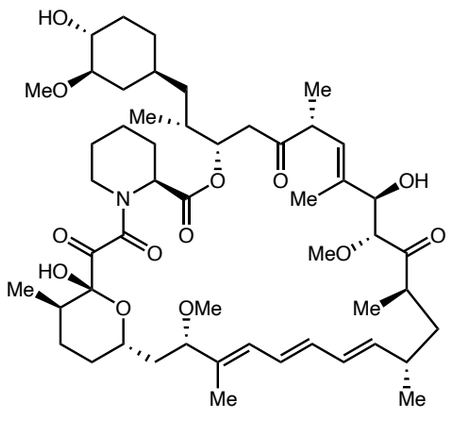

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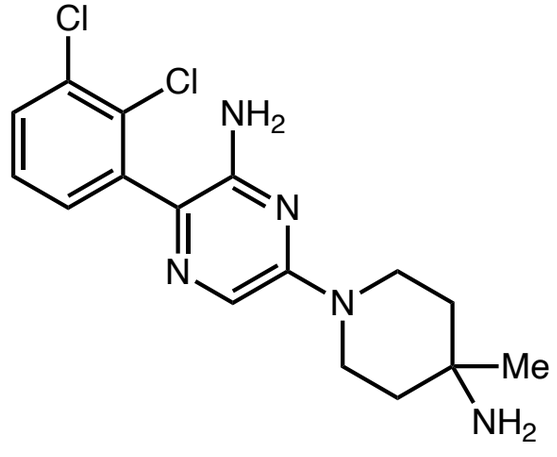
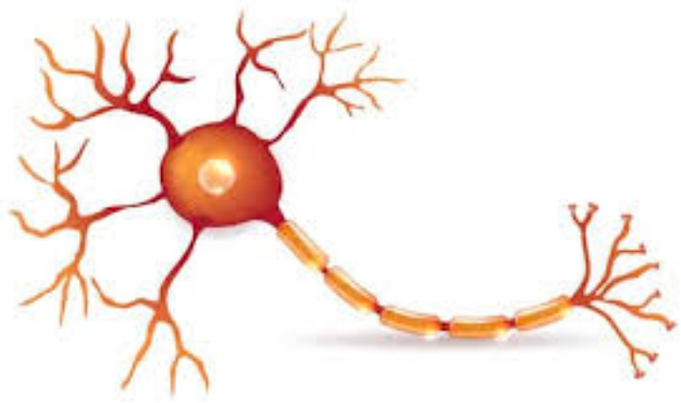


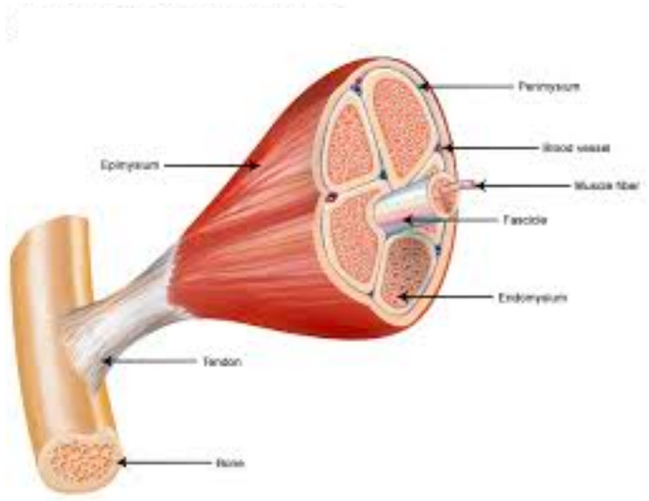
SHP099

A Quick Look at the Ubiquitin Proteosome System

Nerve cell



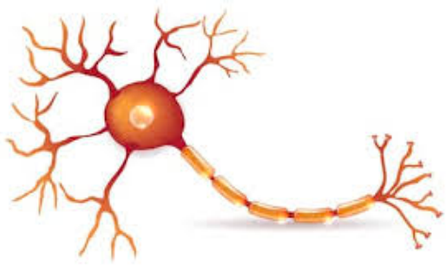
Muscle cell



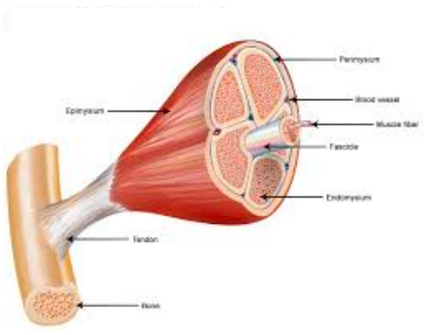
Cells have same genome - but vastly different constituents

A Quick Look at the Ubiquitin Proteosome System

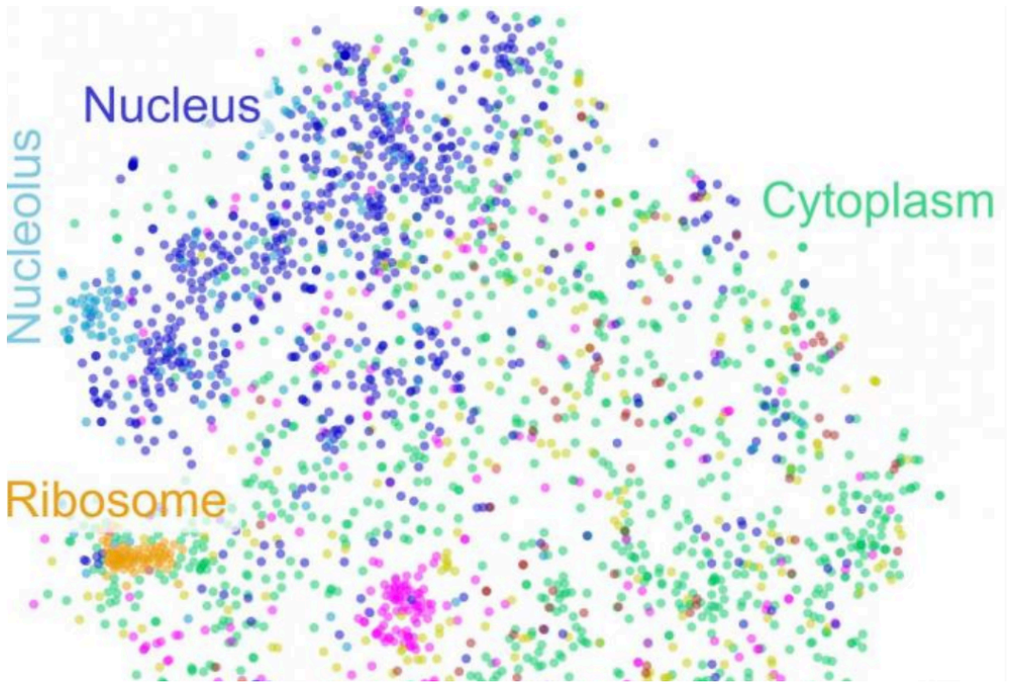
Nerve cell



Muscle cell



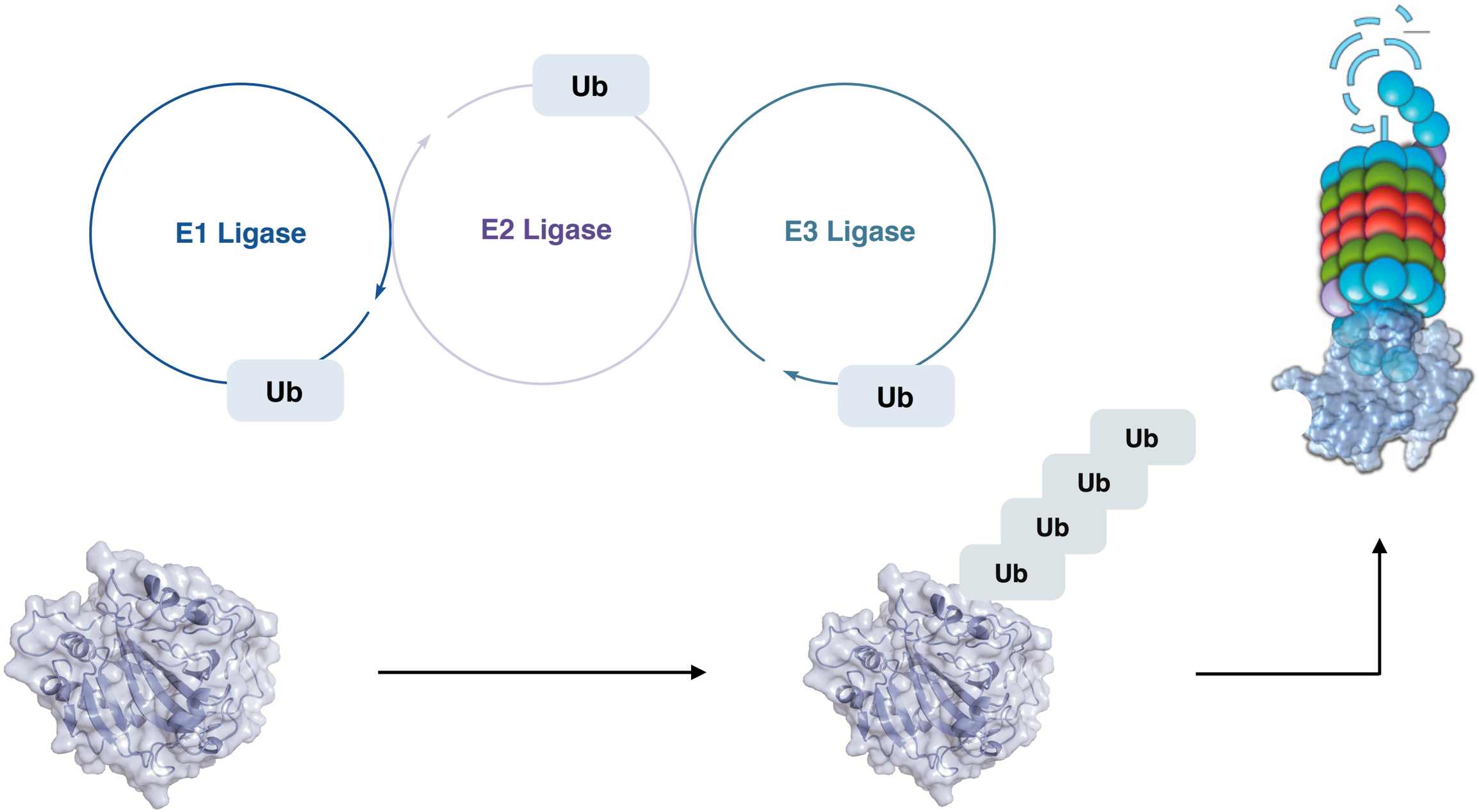
Proteome



different cells have different proteomes

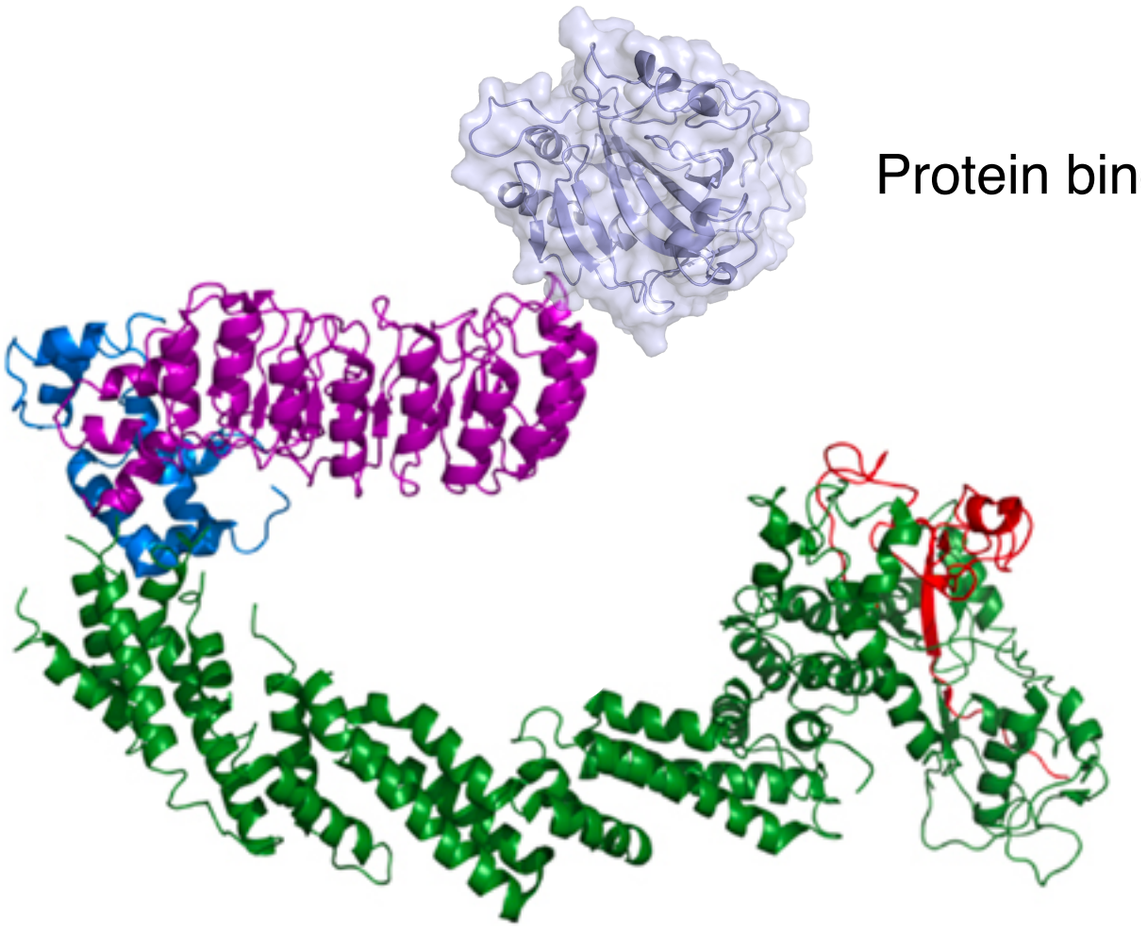
they *make* different proteins and *degrade* different proteins

A Quick Look at the Ubiquitin Proteosome System



Ubiquintation by sequential E1/2/3 ligases leads to altered function or degradation

A Quick Look at the Ubiquitin Proteosome System

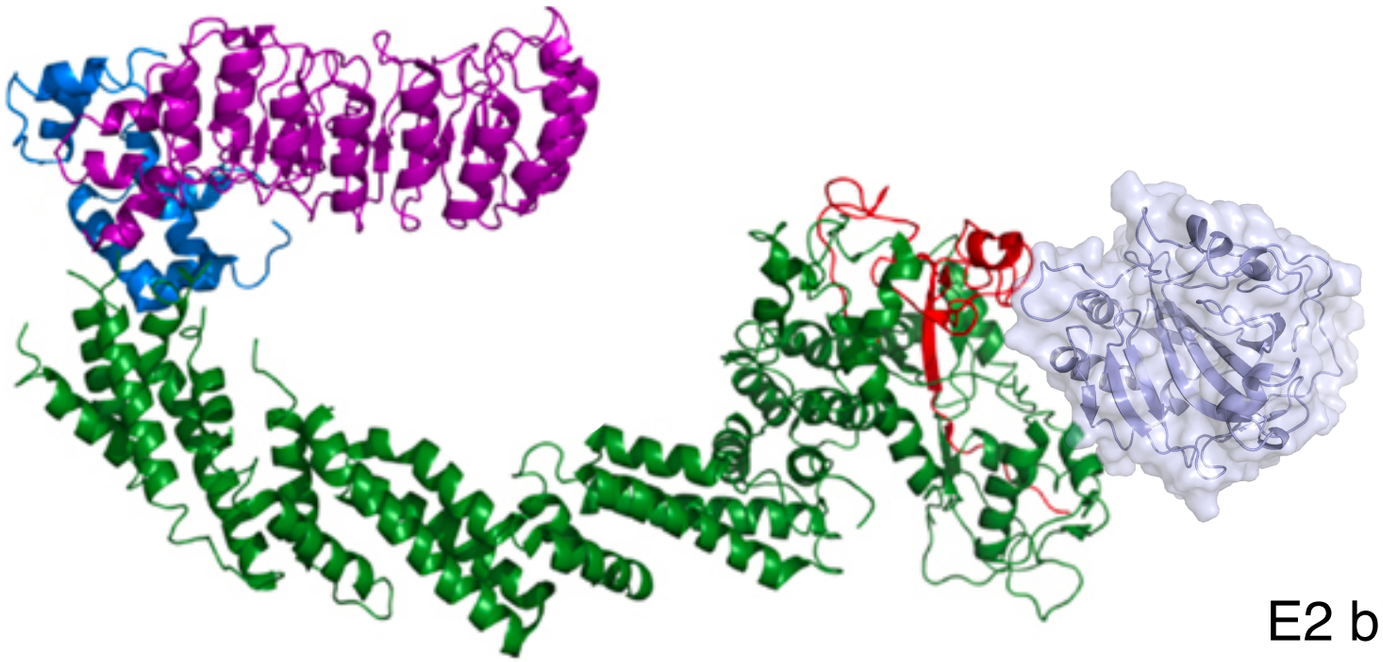


Protein binding site

Ubiquintation by sequential E1/2/3 ligases leads to altered function or degradation

A Quick Look at the Ubiquitin Proteosome System

At least 600 known E3 ligases - combinations allow discrete function



E2 binding site

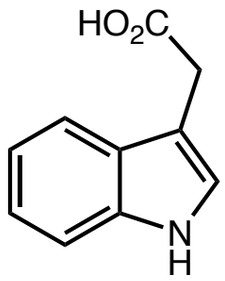
Ubiquintation by sequential E1/2/3 ligases leads to altered function or degradation

A Historical Perspective

E3 ligases and molecular glues were first linked in 2007 in the context of plant biology

Nature **2007**, *446*, 640.

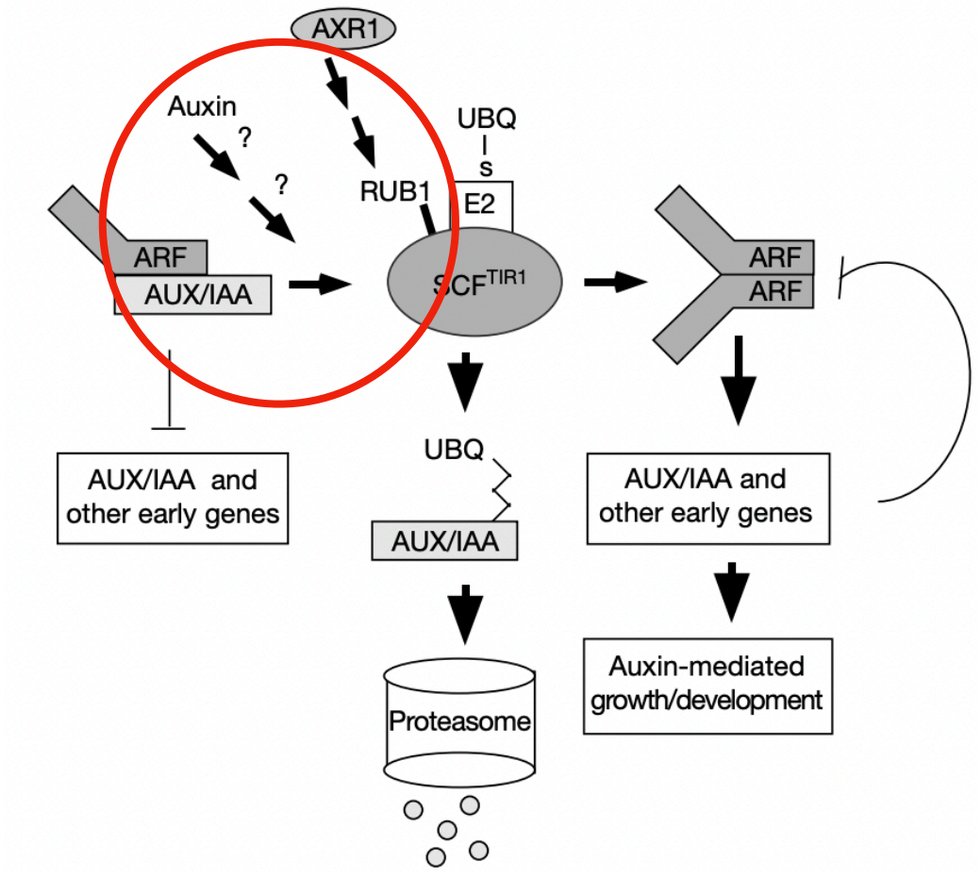
A Historical Perspective



auxin

A plant hormone that controlled plant development

Implicated in degradation of transcription factors

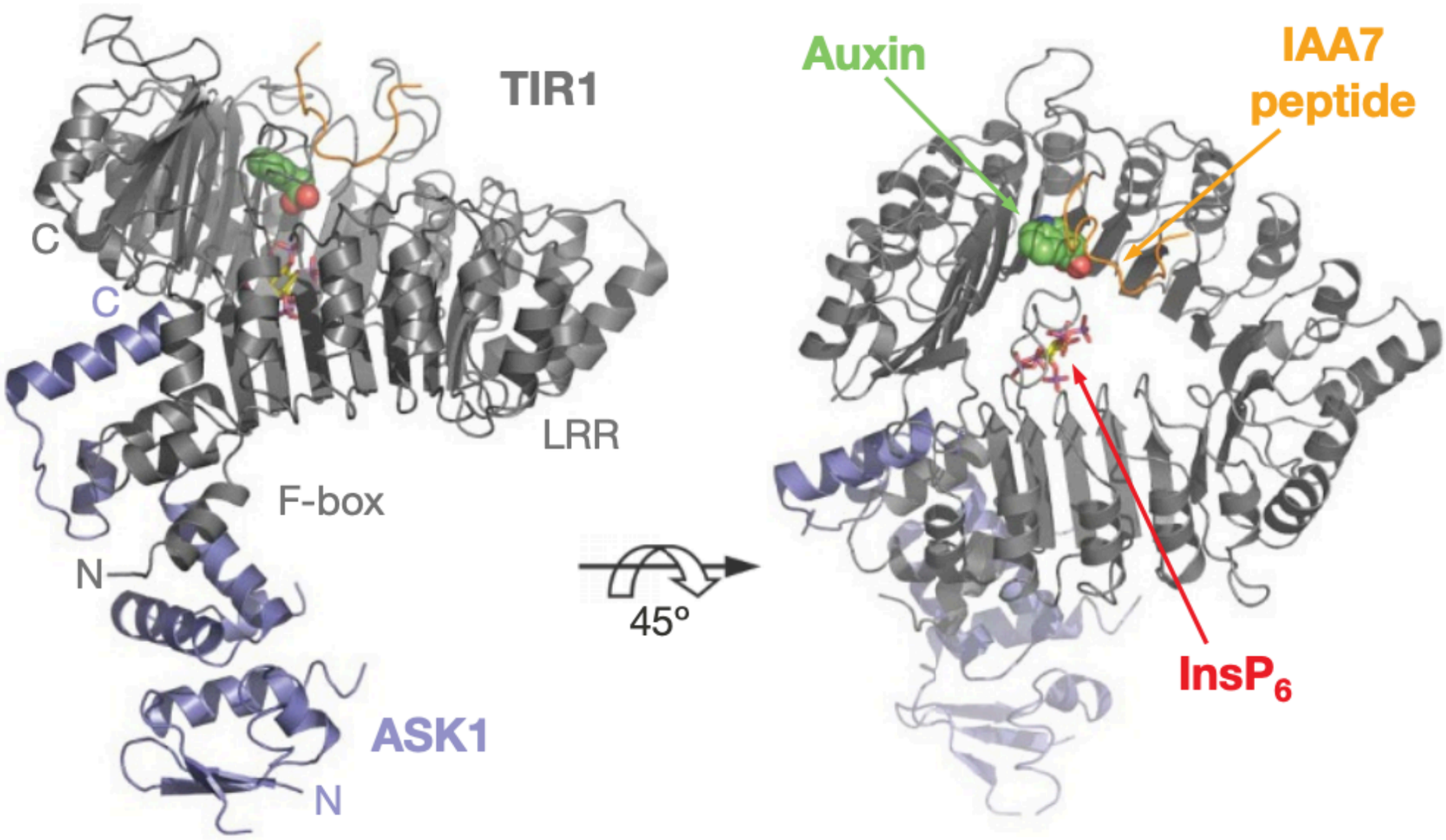


A report in 2001 showed that auxin was key for inducing SCF^{TIR1} degradation of AUX/IAA

Still no molecular basis for this - SAR not instructive

Nature **2001**, *414*, 271–276.

A Historical Perspective

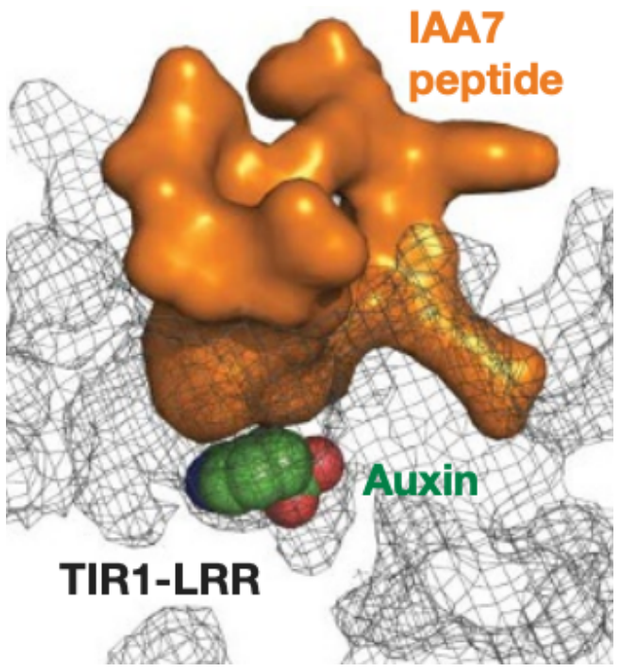


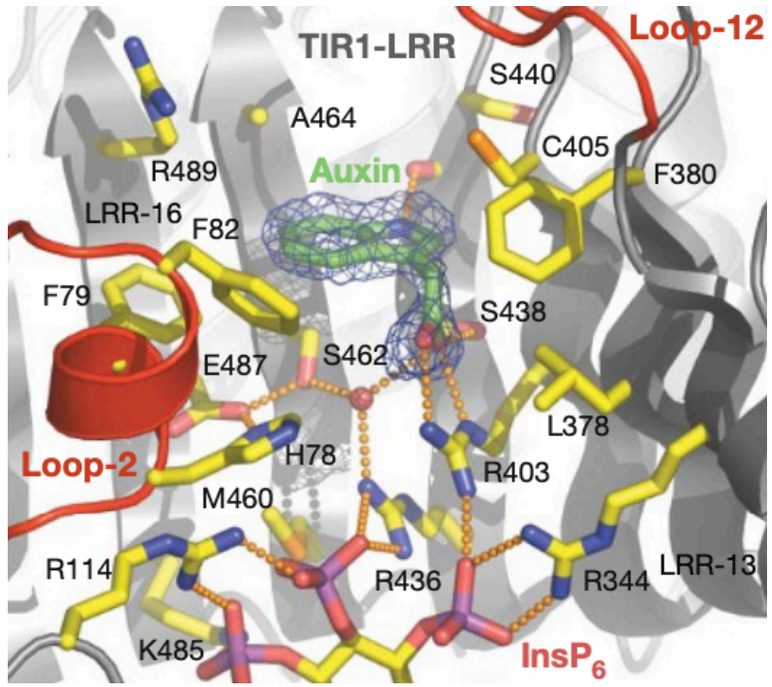
Structural biology from the Zheng group revealed the answer

Nature **2007**, *446*, 640.

A Historical Perspective

Auxin ligand binds to peptide recognition pocket, promoting a PPi





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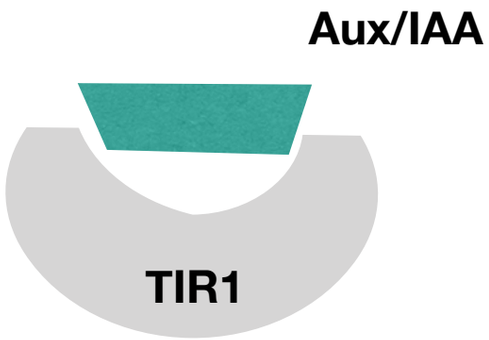


no auxin - no binding - no degradation

Structural biology from the Zheng group revealed the answer

Nature **2007**, *446*, 640.

A Historical Perspective

Auxin ligand binds to peptide recognition pocket, promoting a PPi

Auxin binding - recruits IAA/AUX - degradation

Structural biology from the Zheng group revealed the answer

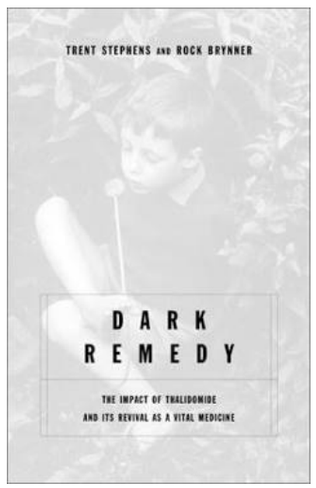
Nature **2007**, *446*, 640.

This discovery prompted a closer look at this mechanism in humans

Case Study - Thalidomide as a molecular glue

Case Study: The Thalidomide Story - From Villain to Hero

How did thalidomide turn around its image? Once a poster child for bad Pharma, now the genesis of the hottest area in small molecule drug discovery.



2000

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

~$10 Billion/year

2021

15 years of world class structural and molecular biology

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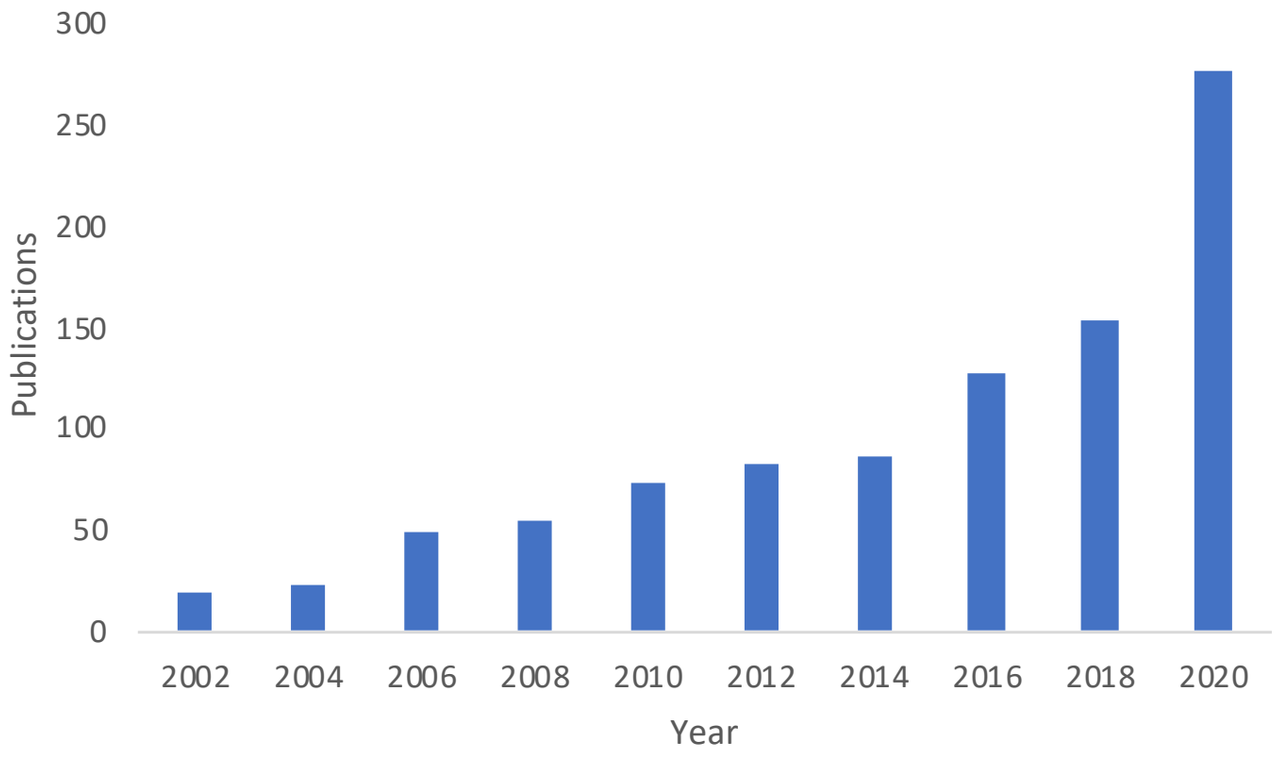
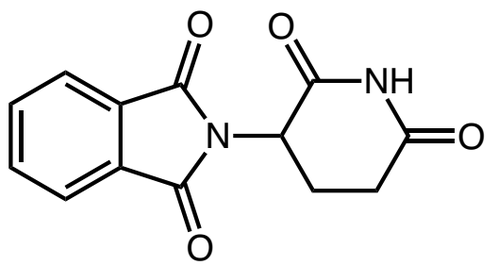


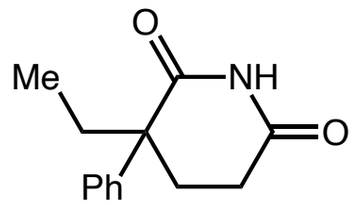
15 years of world class structural and molecular biology

Scifinder search “molecular glues”

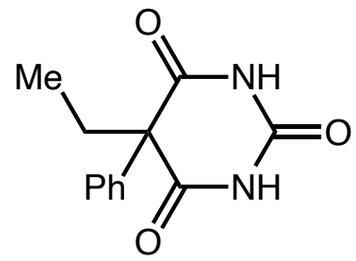
Case Study: The Thalidomide Story - Highjacking the Ubiquitination pathway



Thalidomide



Glutethimide

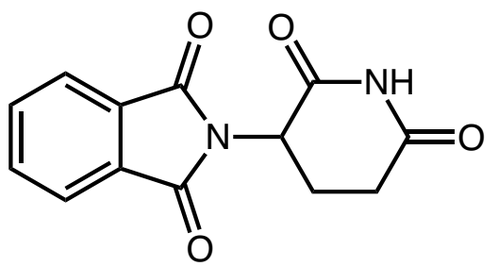


Phenobarbitol

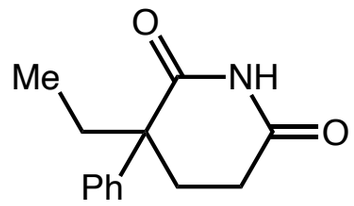
Developed by Chemie Grunenthal in 1953 as a safer alternative to barbiturates

Marketed heavily the drug was widely prescribed, including to pregnant women

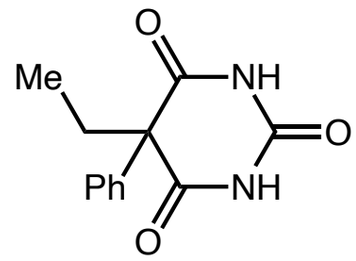
Case Study: The Thalidomide Story - Highjacking the Ubiquitination pathway



Thalidomide



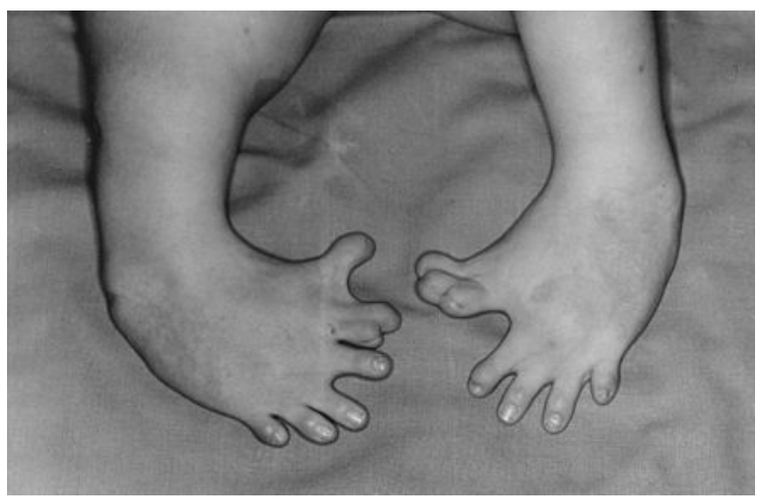
Glutethimide



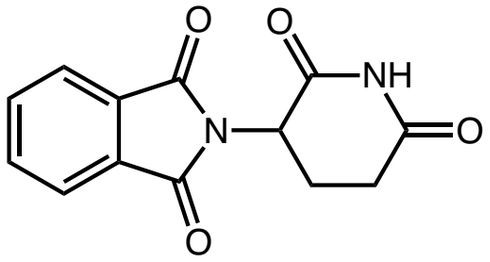
Phenobarbitol

Developed by Chemie Grunenthal in 1953 as a safer alternative to barbiturates

This led to the a terrible medical tragedy as 80,000 babies died of teratogenic effects



Case Study: The Thalidomide Story - Highjacking the Ubiquitination pathway



Thalidomide

Dr Folkman at his Harvard lab

Given a new lease of life by Dr Judah Folkman who found it was an effective treatment for multiple myeloma

at the time multiple myeloma was incurable by conventional chemotherapy

33% of patients responded to the therapy

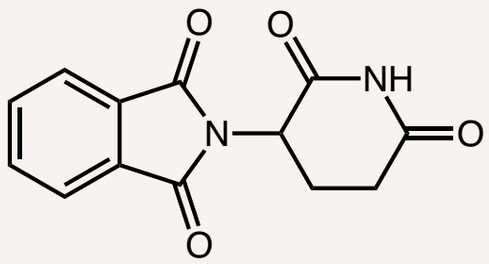
New Engl. J. Med. **1999**, *341*, 1565.

New Engl. J. Med. **1971**, *285*, 1182.

Case Study: The Thalidomide Story - From Villain to Hero

- *Following thalidomide, two more IMiDs were approved for MM and other indications*

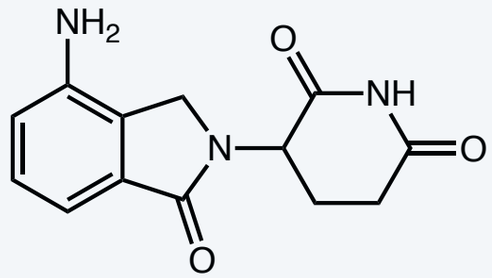
Thalidomide



Celgene, 2006

ENL, MM

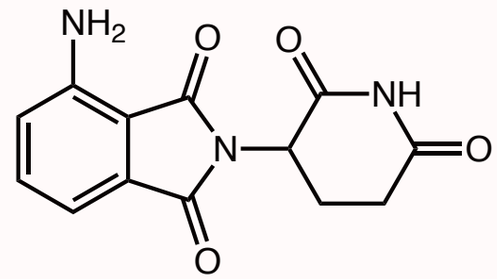
Lenalidomide



Celgene 2006

MM, amyloidosis, mantle cell lymphoma. In the clinic for many other cancers

Pomalidomide

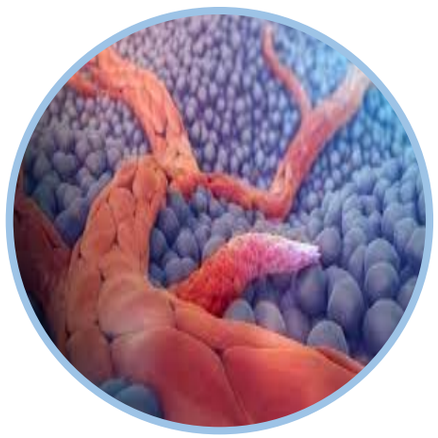


Celgene, 2013

Primary myelofibrosis, MM

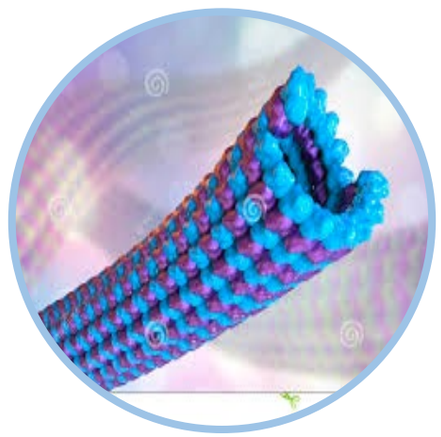
Case Study: The Thalidomide Story - Mechanistic Hypothesis

Older reviews (into early 10's) provide numerous differing hypotheses to explain the therapeutic effects of thalidomide (FDA approval 2006)



Anti-angiogenesis

FGF and Shh signaling decreased, modulates expression growth factors and immune-modulatory proteins. TNF-α inhibition.



Tubulin binding

Thalidomide metabolites bind tubulin altering cell division

O_2

ROS lead to abnormal growth

ROS

Induces oxidative stress. Upregulation of Bmp and dkk1 both prevented by spin trapping agents

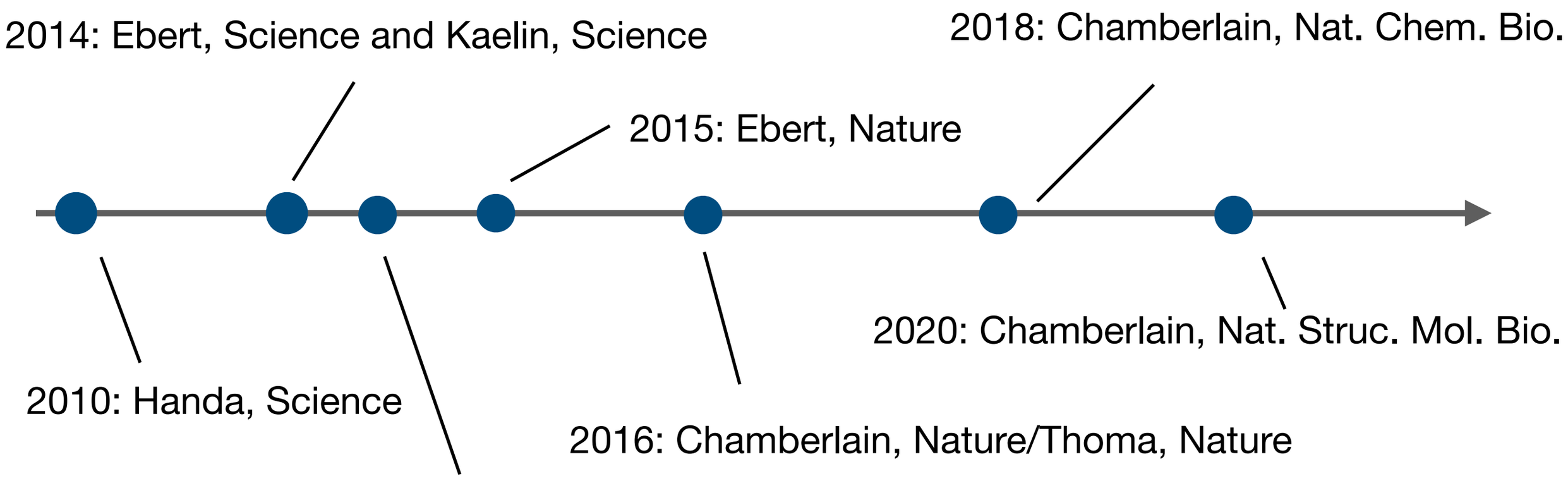
Birth Defects Res. C Embryo Today. **2015**, *105*, 140–156.

Mechanistic target and molecular mechanism were unknown until recently

Birth Defects Res. C Embryo Today. **2015**, *105*, 140–156.

Case Study: The Thalidomide Story - CRBN

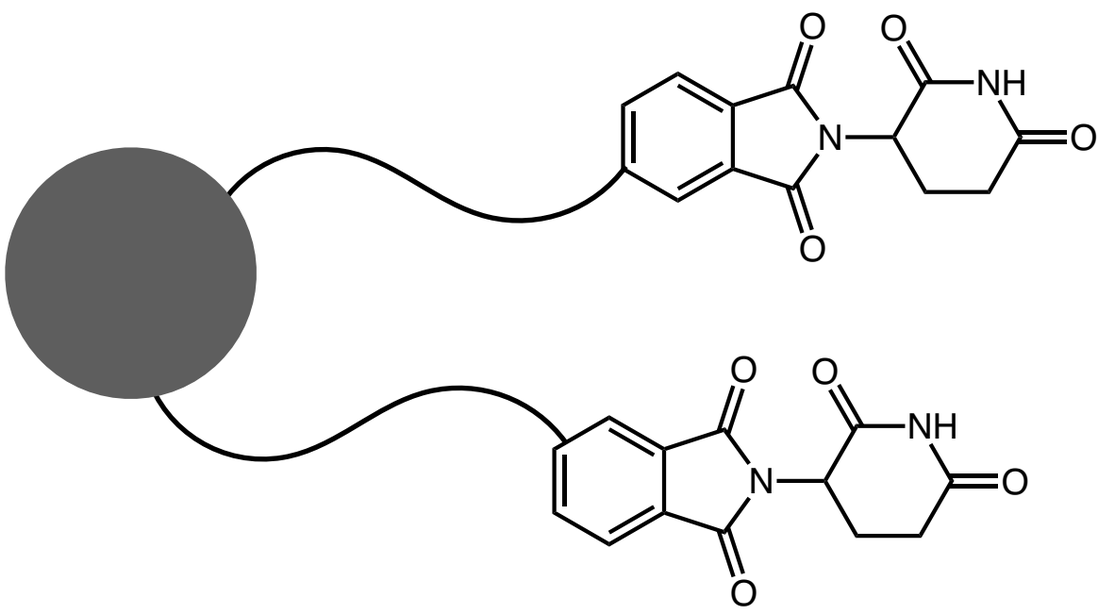
A race to understand Thalidomide

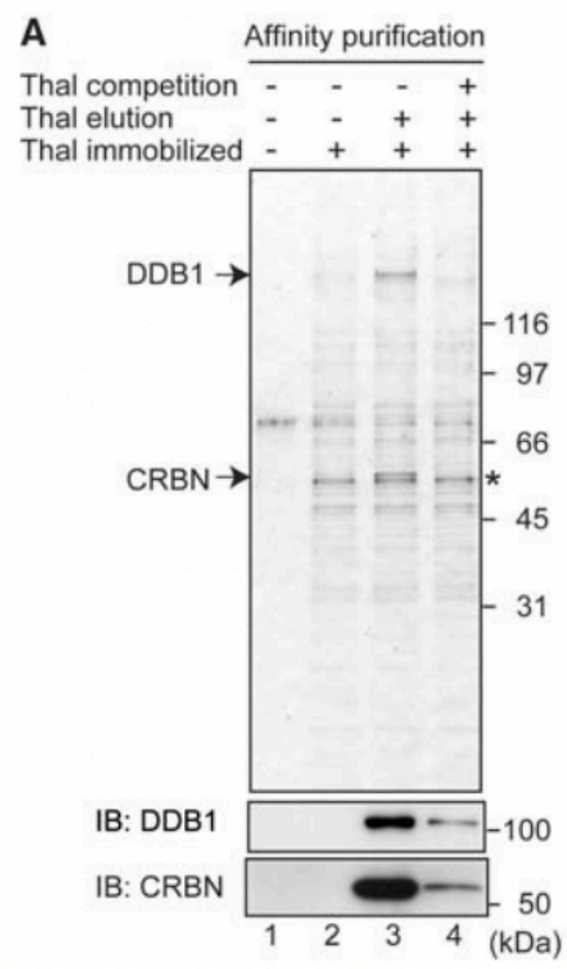


Most of the key data gathered at Celgene or Dana-Farber

Case Study: The Thalidomide Story - CRBN

2010: Hiroshi Handa and co-workers show CRBN is the primary target of thalidomide



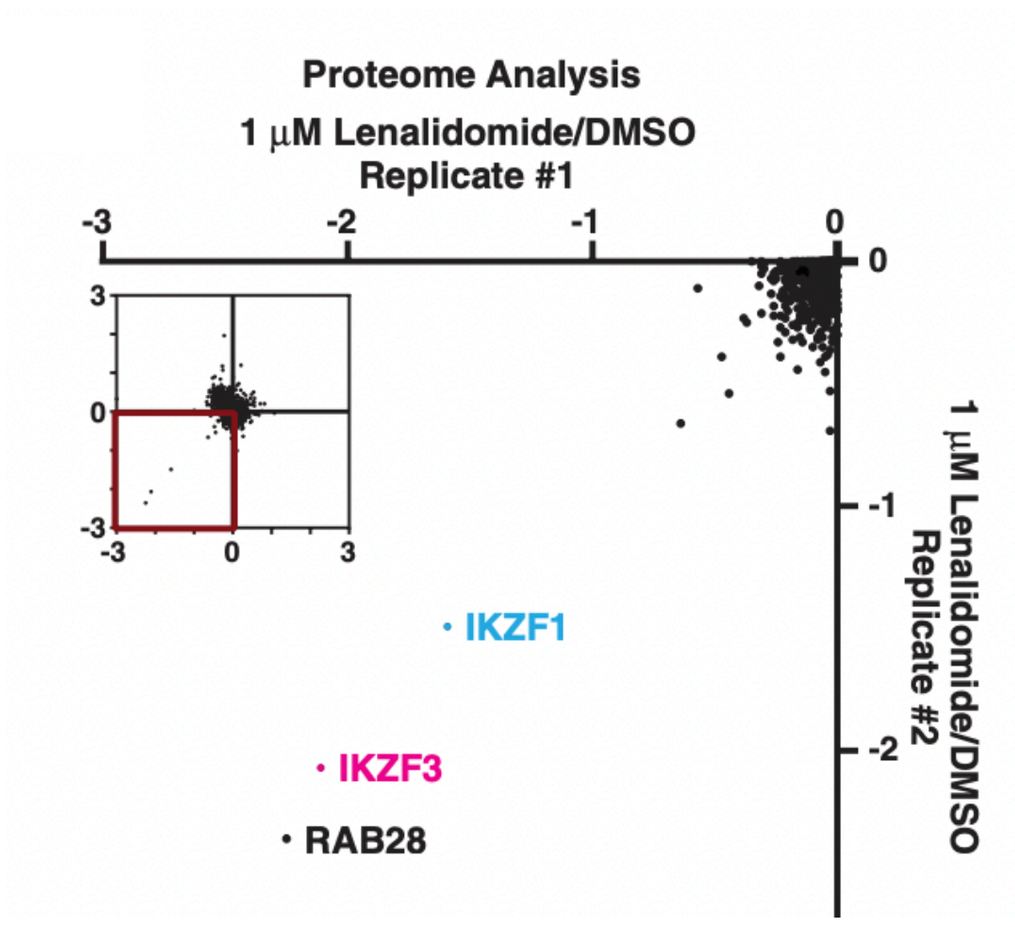


Hypothesis: Thalidomide blocks natural degradation pathways

Science **2010**, 327, 1345–1350

Case Study: The Thalidomide Story - CRBN

2014: IMiD binding to CRBN leads to degradation of Ikaros transcription factors



Previously undruggable TFs

MS analysis (SILAC) of MM1S cells after treatment with Lenalidomide shows significant degradation of Ikaros and Aiolos

Hypothesis: CRBN binders also provide a gain-of-function, a new MOA in cancer DD

Ebert/Kaelin: *Science* **2014**, 343, 301–305, and *Science* **2014**, 343, 305–309

Case Study: The Thalidomide Story - CRBN

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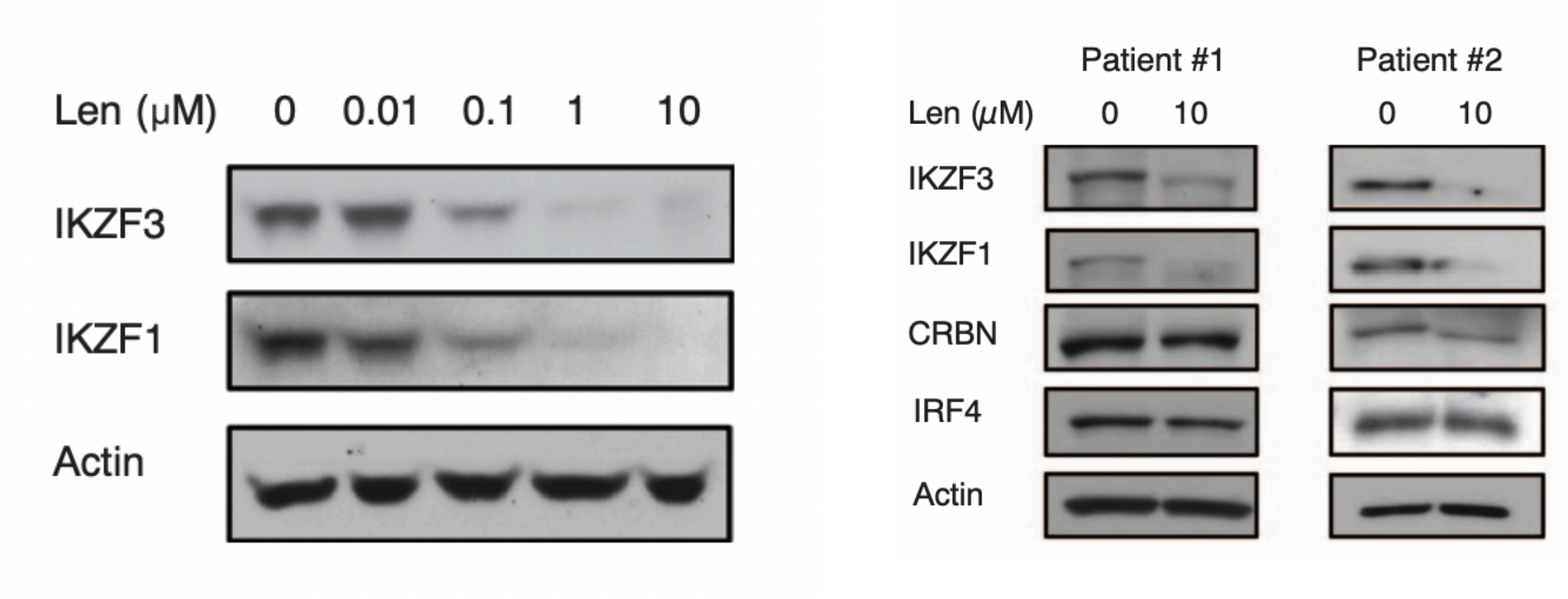


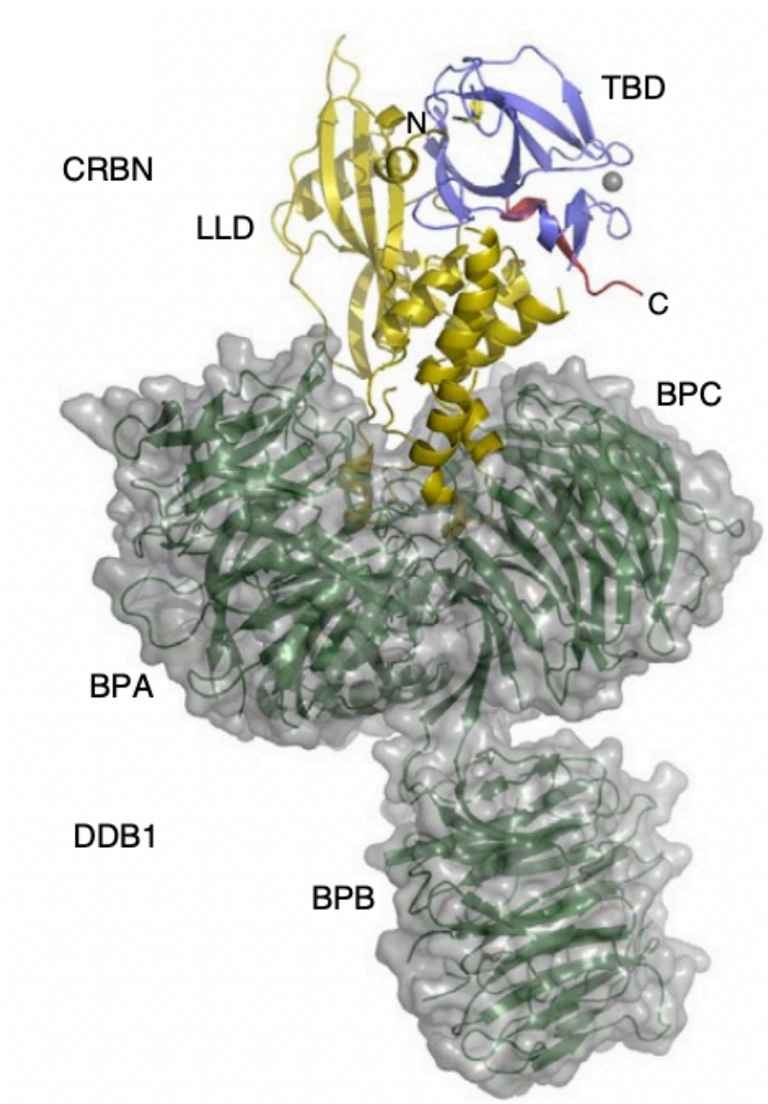
Confirmed by WB in MM1S cells and in cancer tissue

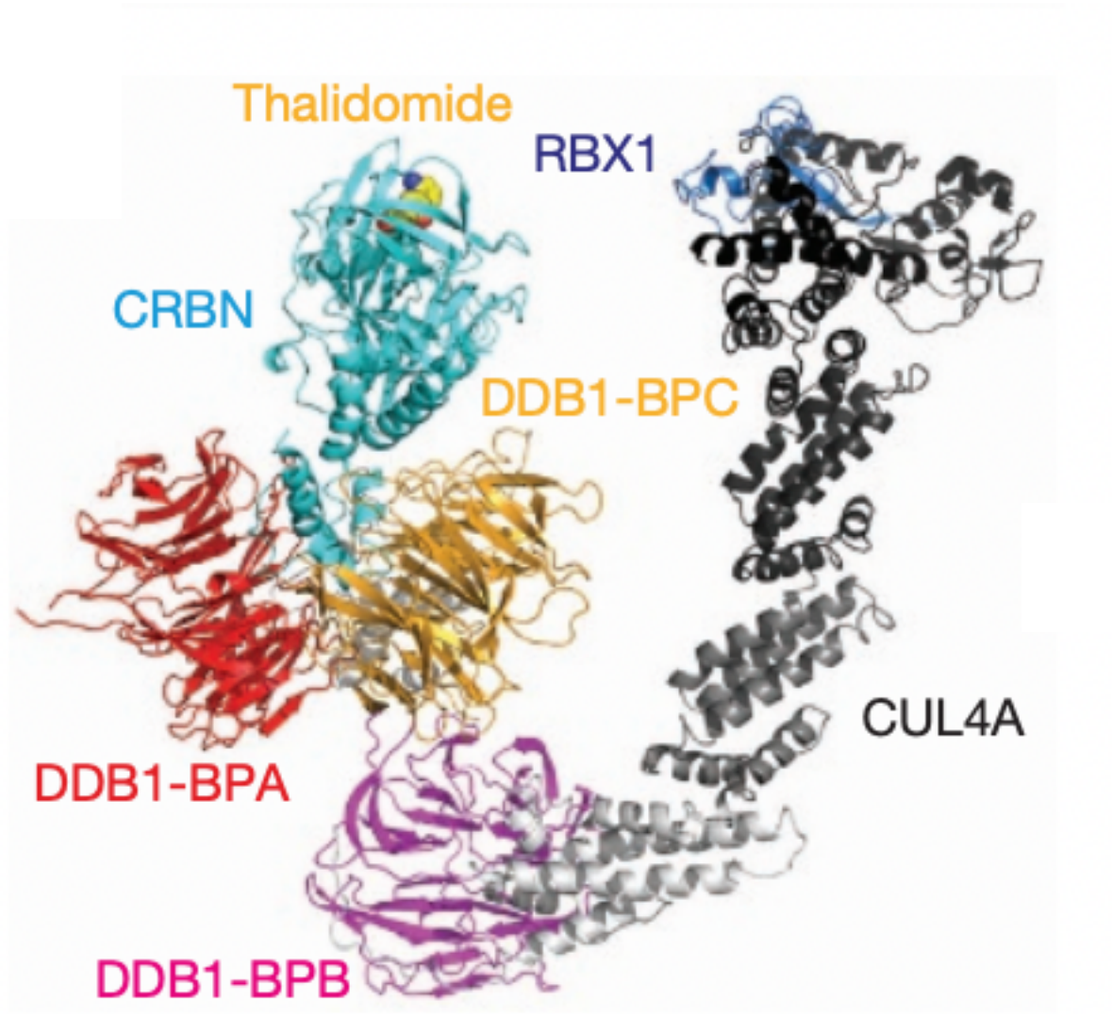
Hypothesis: CRBN binders also provide a gain-of-function, a new MOA in cancer DD

Ebert/Kaelin: *Science* **2014**, 343, 301–305, and *Science* **2014**, 343, 305–309

Case Study: The Thalidomide Story - CRBN

2014: Thalidomide binds the surface of CRBN - stabilizing PPIs





Fischer/Chamberlain: *Nature* **2014**, 512, 49–52 and *Nat Struc. Mol. Bio*. **2014**, *21*, 803–808

Case Study: The Thalidomide Story - CRBN

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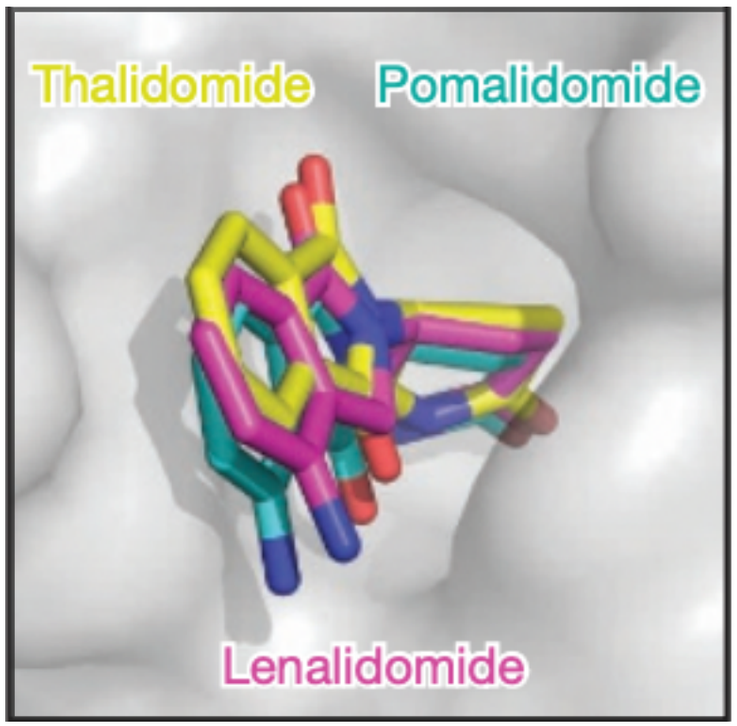

IMiDs all bind the same surface of CRBN

Fischer/Chamberlain: *Nature* **2014**, 512, 49–52 and *Nat Struc. Mol. Bio*. **2014**, *21*, 803–808

Case Study: The Thalidomide Story - CRBN

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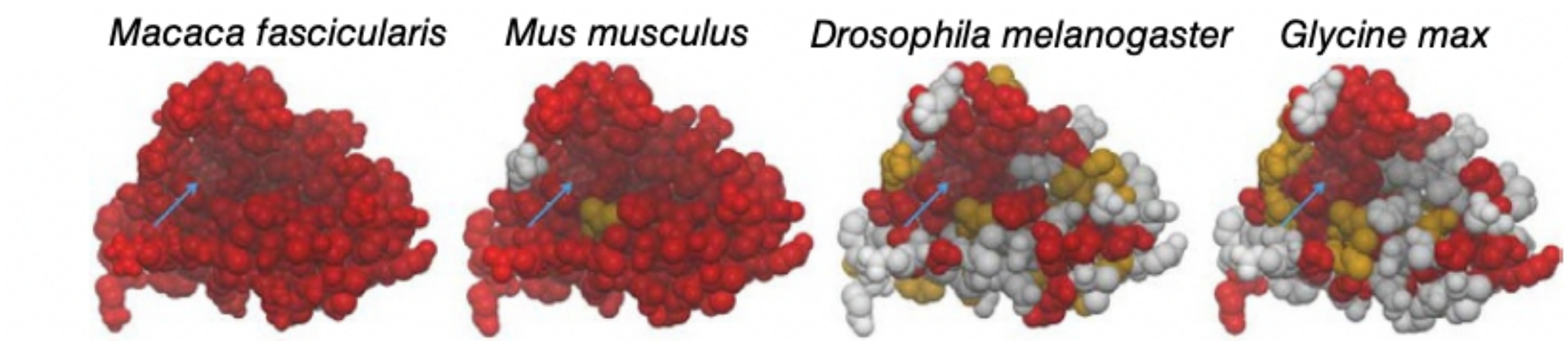

Macaque Mouse Fly Soybean

IMiDs binding surface is conserved across species

Fischer/Chamberlain: *Nature* **2014**, 512, 49–52 and *Nat Struc. Mol. Bio*. **2014**, *21*, 803–808

Case Study: The Thalidomide Story - CRBN

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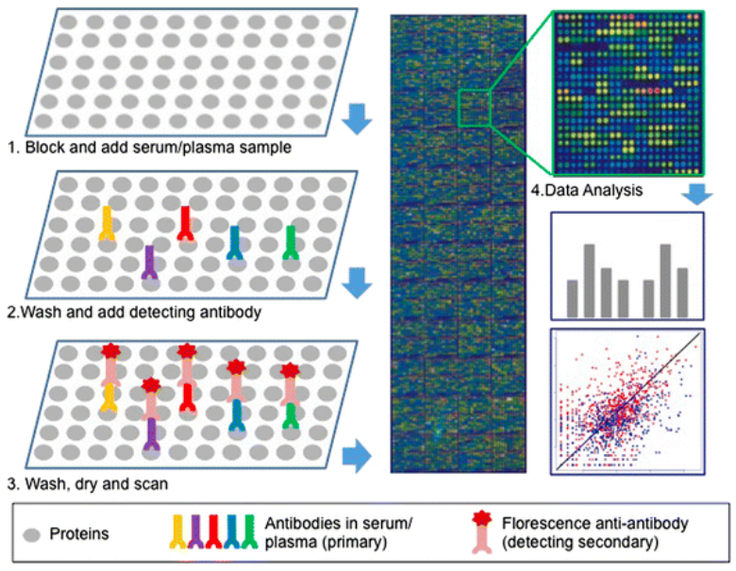

Protein microarrays suggest MEIS2 as a native substrate for CRBN (no ligand).

MEIS2 involved in transcriptional regulation – important for human development

Fischer/Chamberlain: *Nature* **2014**, 512, 49–52 and *Nat Struc. Mol. Bio*. **2014**, *21*, 803–808

Case Study: The Thalidomide Story - CRBN

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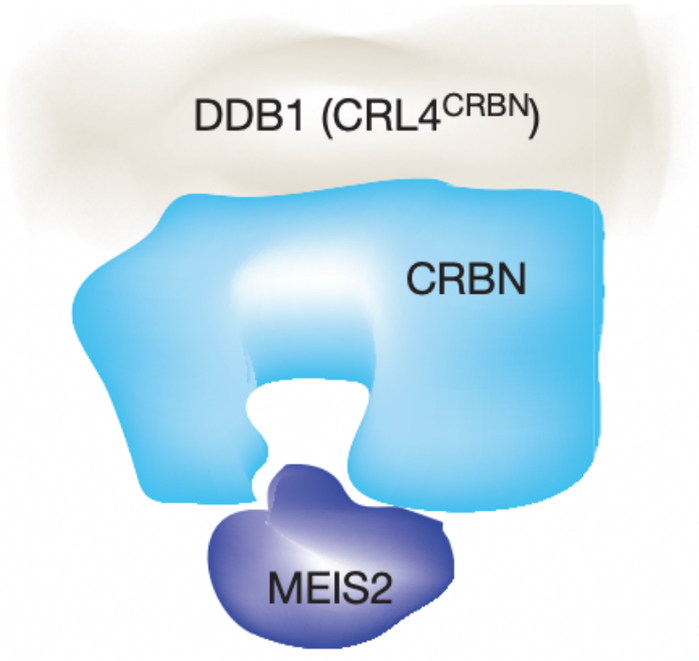

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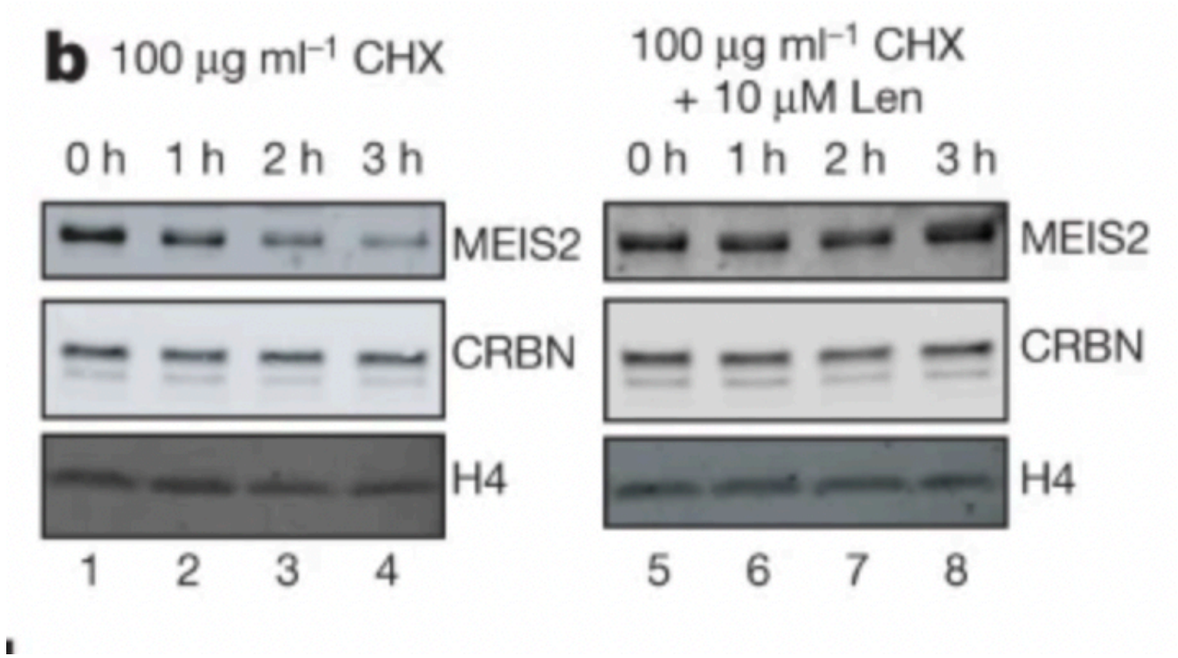


MEIS2 levels are stabilized by Len exposure

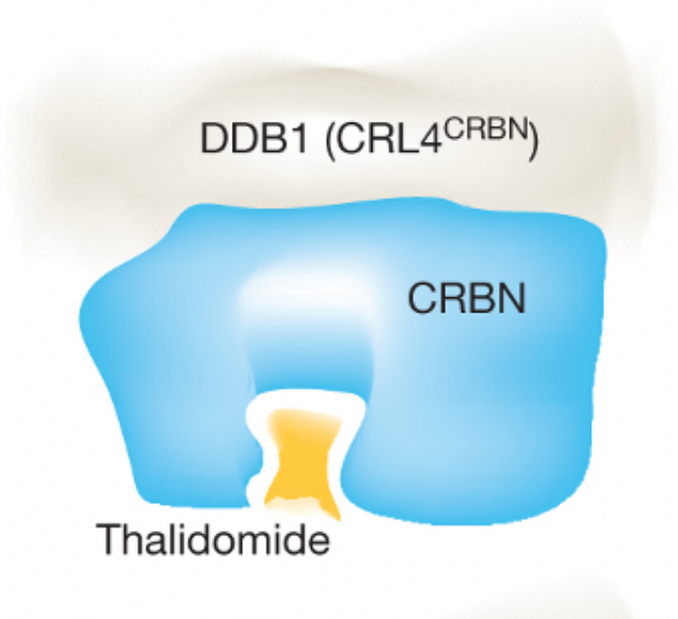
MEIS2 involved in transcriptional regulation – important for human development

Fischer/Chamberlain: *Nature* **2014**, 512, 49–52 and *Nat Struc. Mol. Bio*. **2014**, *21*, 803–808

Case Study: The Thalidomide Story - CRBN

Hypothesis: IMiDs act as both agonists and antagonists

MEIS2 upregulated

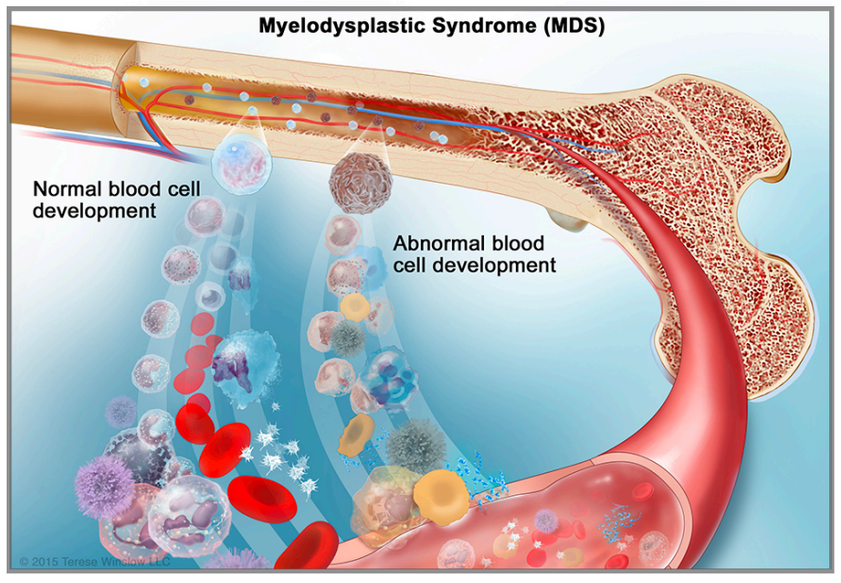


Ikaros/Aiolos downregulated

Fischer/Chamberlain: *Nature* **2014**, 512, 49–52 and *Nat Struc. Mol. Bio*. **2014**, *21*, 803–808

Case Study: The Thalidomide Story - CRBN

2015: Lenalidomide provides GoF over other IMiDs, and cancer specific degradation



Lenalidomide also effective treatment for MDS with chromosome 5q deletion

Small changes in IMiD structure can have dramatic effects

Ebert: *Nature* **2015**, 523, 183–188

Case Study: The Thalidomide Story - CRBN

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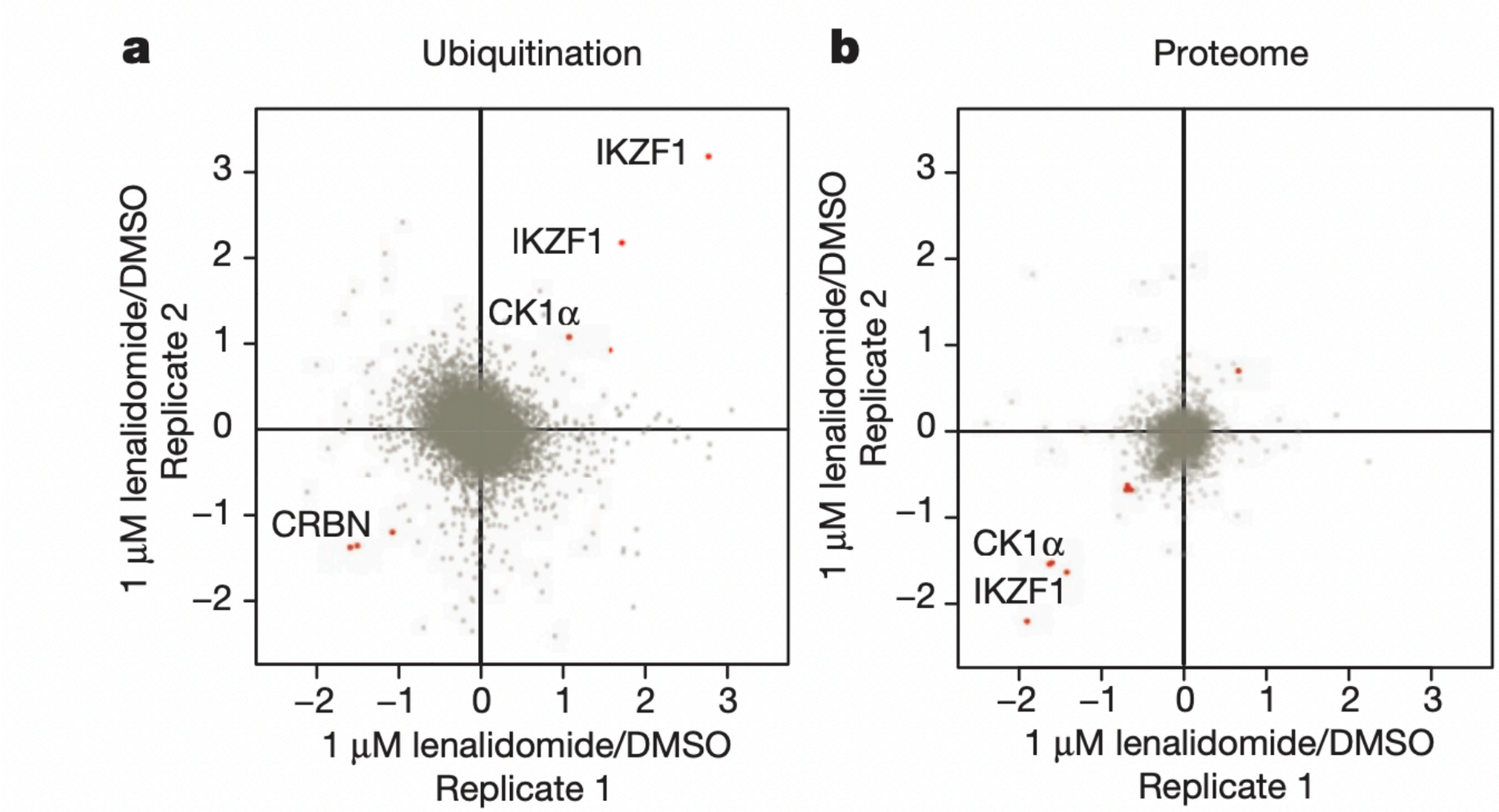

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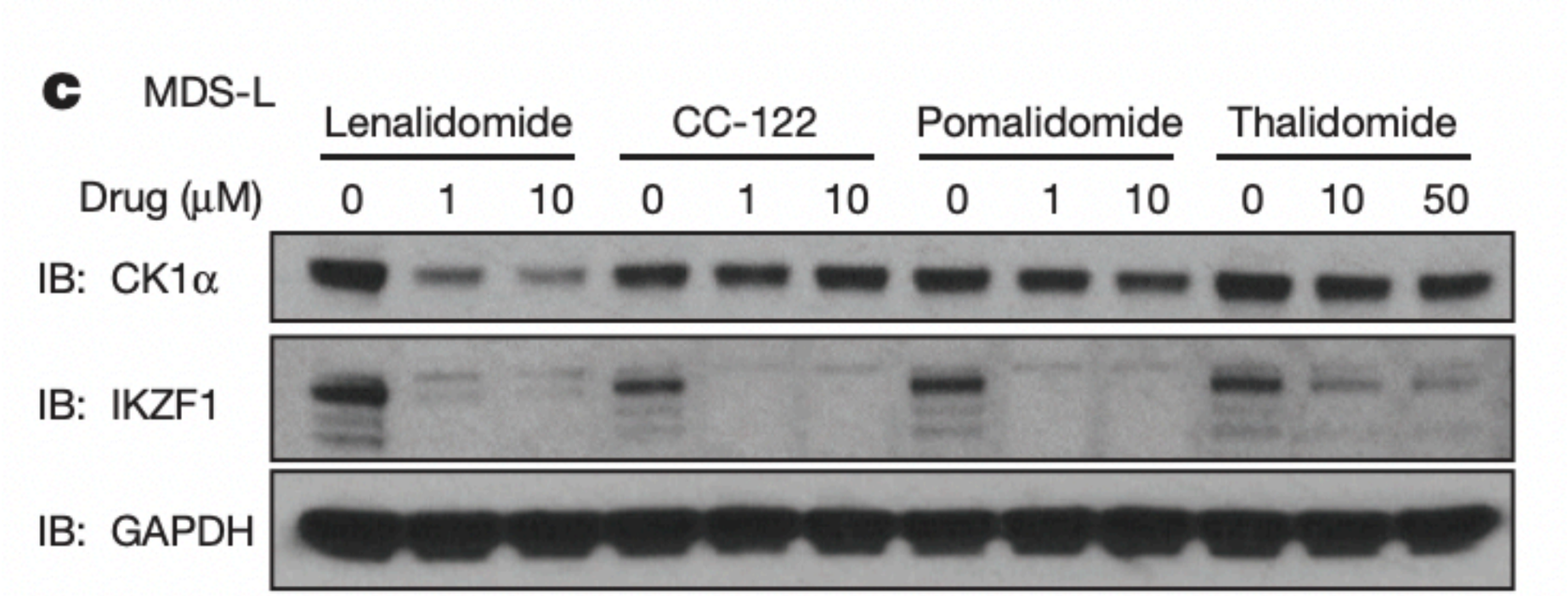


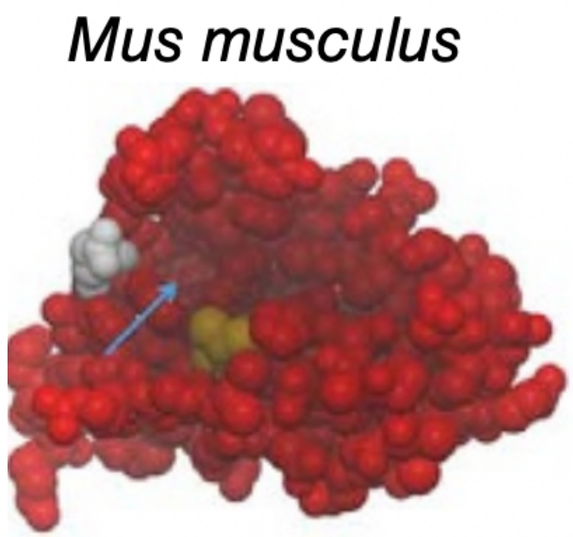
Lenalidomide and not pomolidomide or thalidomide degrades CK1α

Ebert: *Nature* **2015**, 523, 183–188

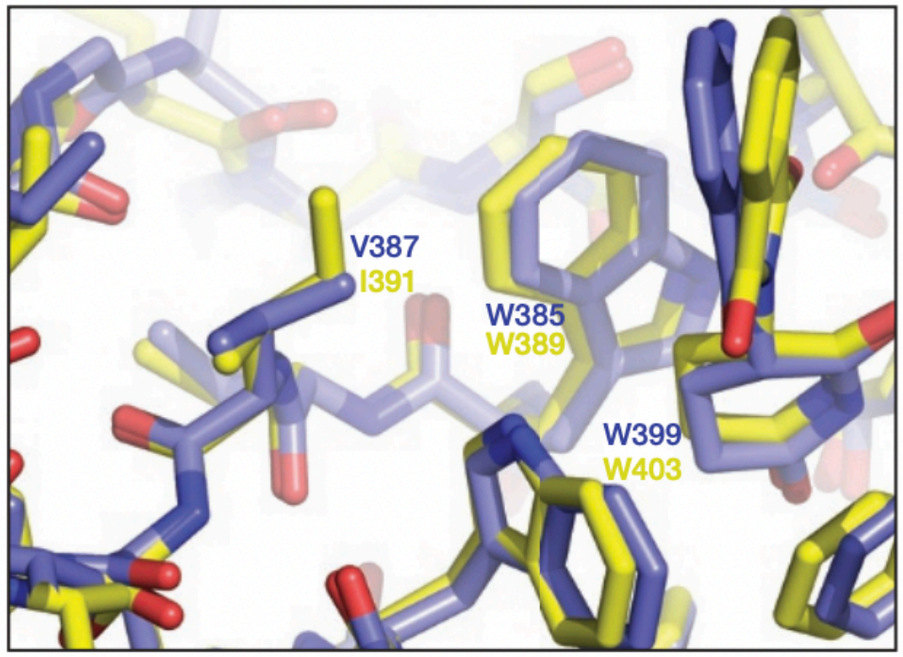
When designing mouse models the authors observed some interesting differences

Case Study: The Thalidomide Story - CRBN

2015: Lenalidomide provides GoF over other IMiDs, and cancer specific degradation



Mouse



Mouse CRBN does not respond to Lenalidomide

Valine to isoleucine change shown to be responsible

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Case Study: The Thalidomide Story - CRBN

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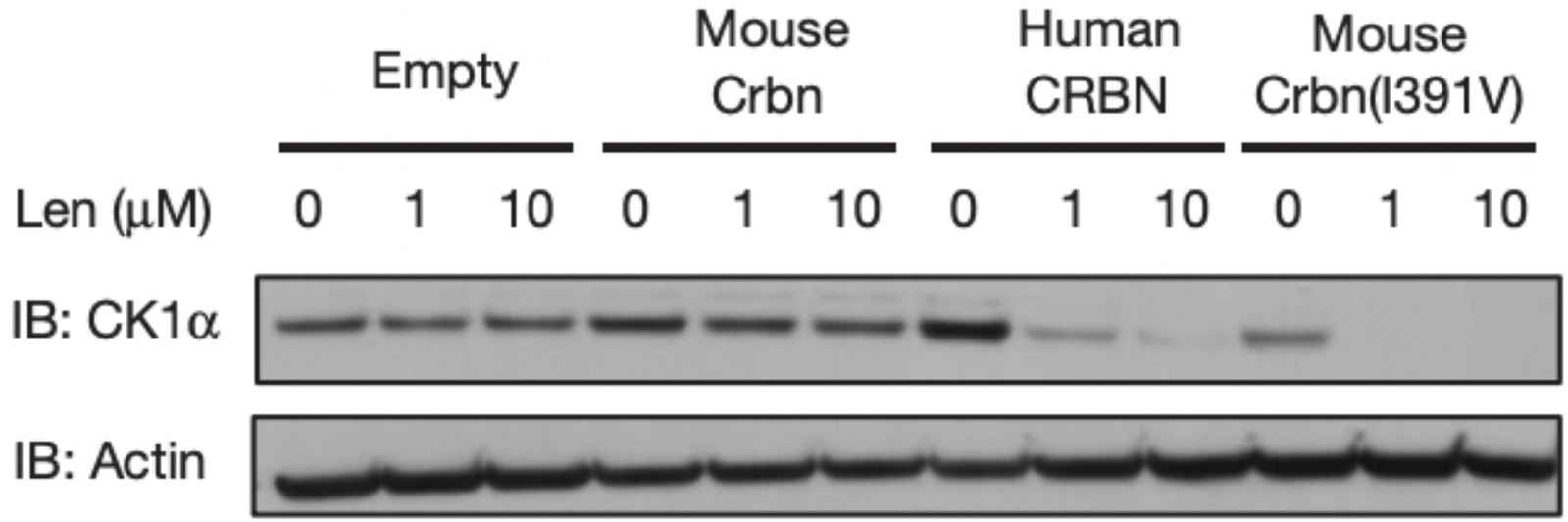

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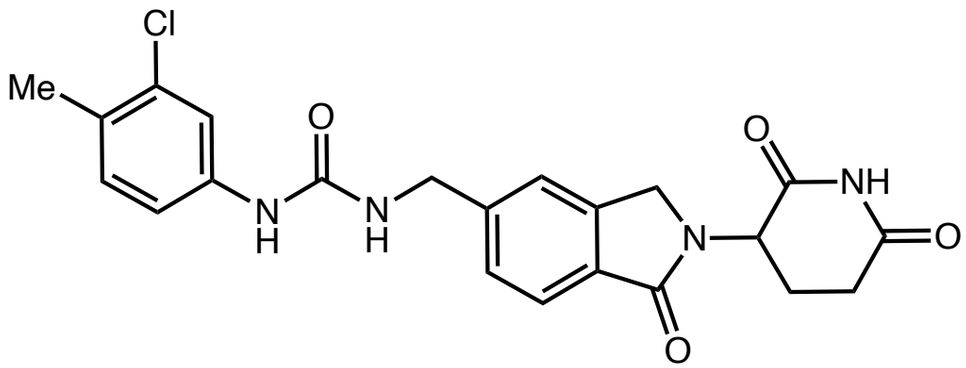
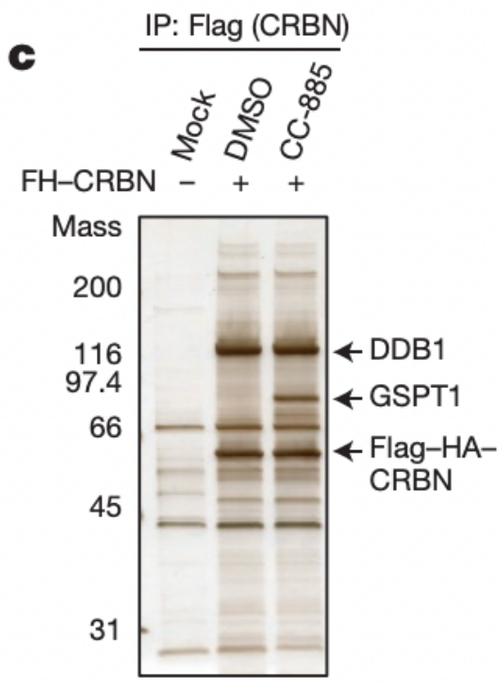


CC-885

Potent anti-cancer effect vs Acute Myeloid Leukemia

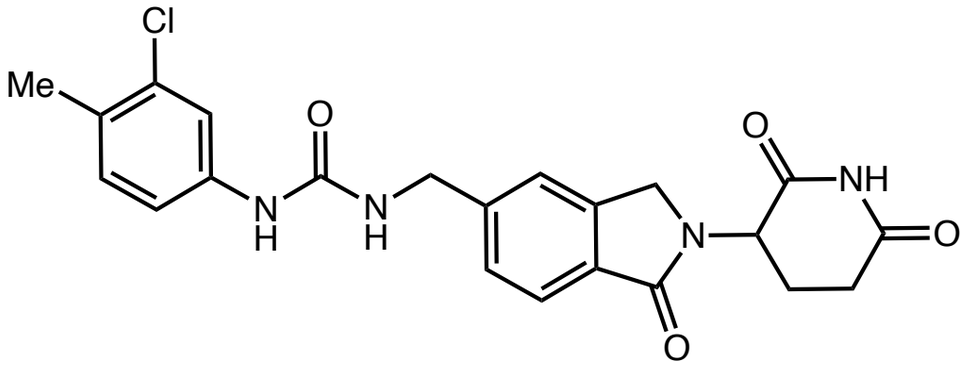
CC-885 shown to have clinical potential in phenotypic screens



GSPT1 shown to be degraded by CC-885

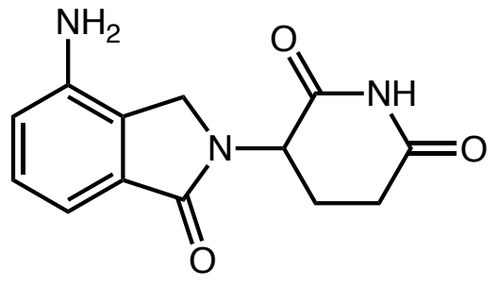
Thoma/Chamberlain: *Nature* **2016**, 535, 252–257 and *Nature* **2016**, *532*, 127–130

Case Study: The Thalidomide Story - CRBN



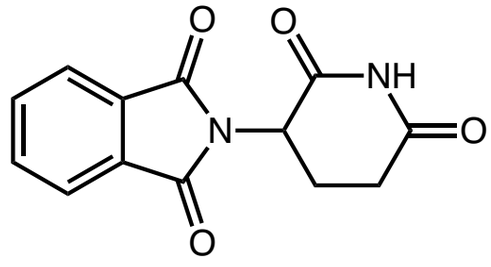
CC-885

GSPT1



Lenalidomide

CK1-α, Ikaros



Thalidomide

Ikaros

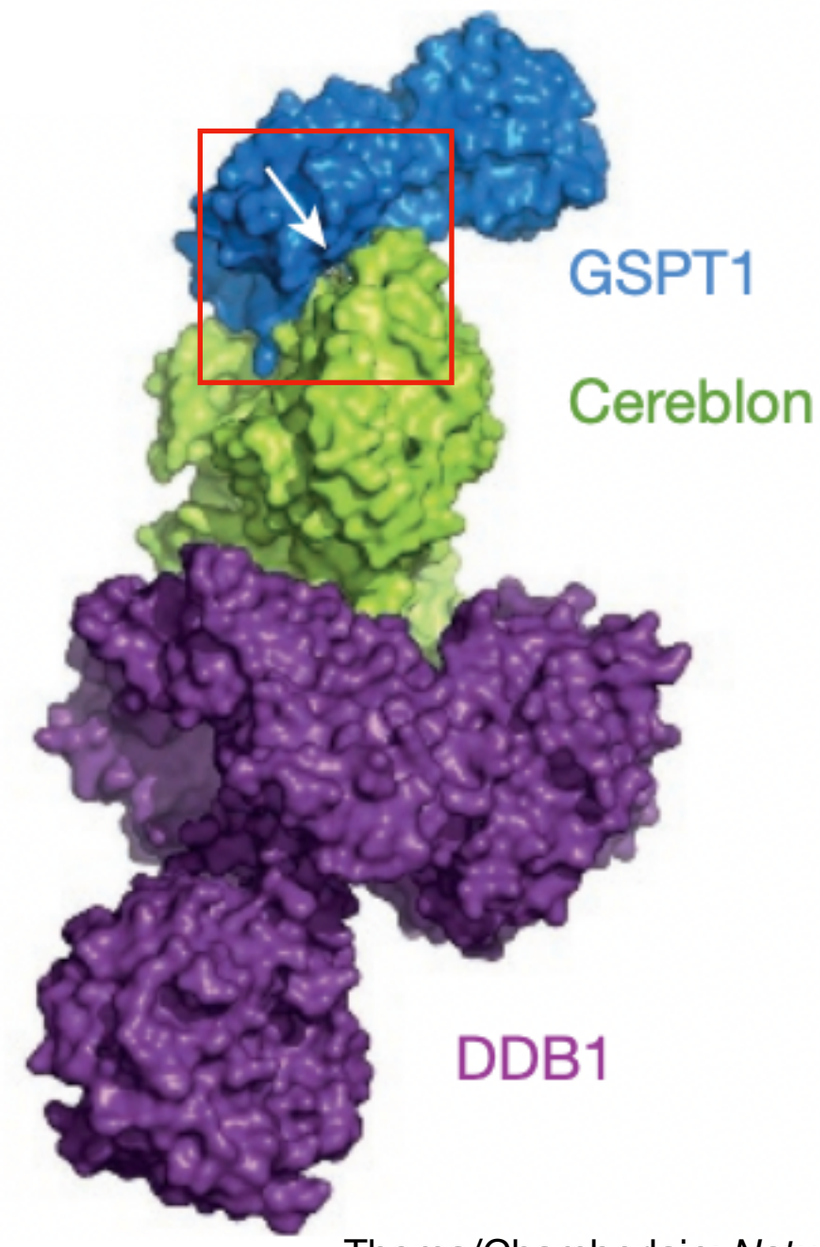
4 Proteins now identified as Neo-substrates – no identifiable sequence homology

What is going on?

Thoma/Chamberlain: *Nature* **2016**, 535, 252–257 and *Nature* **2016**, *532*, 127–130

Case Study: The Thalidomide Story - CRBN

Structural biology reveals the loop required for degradation

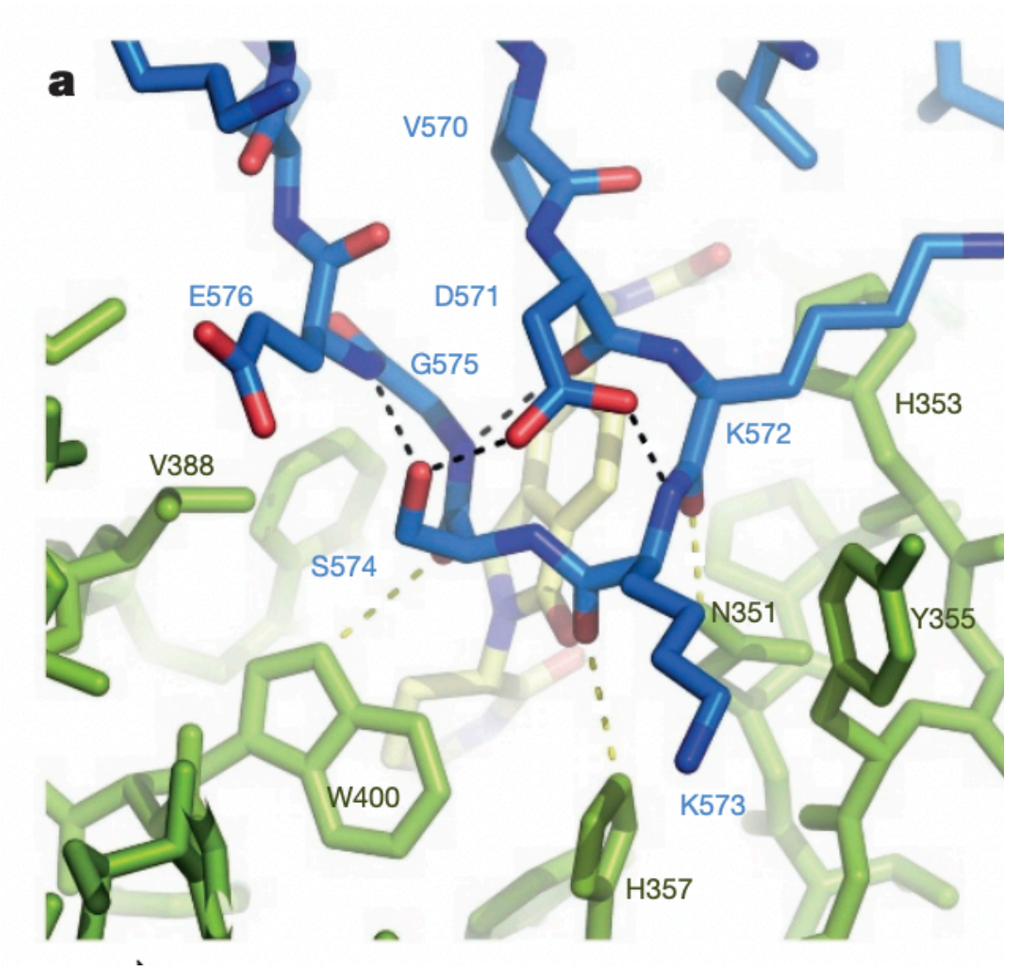


Xray of CRBN/DDB1 in complex with CC-885 and GSPT1

Thoma/Chamberlain: *Nature* **2016**, 535, 252–257 and *Nature* **2016**, *532*, 127–130

Case Study: The Thalidomide Story - CRBN

Structural biology reveals the loop required for degradation



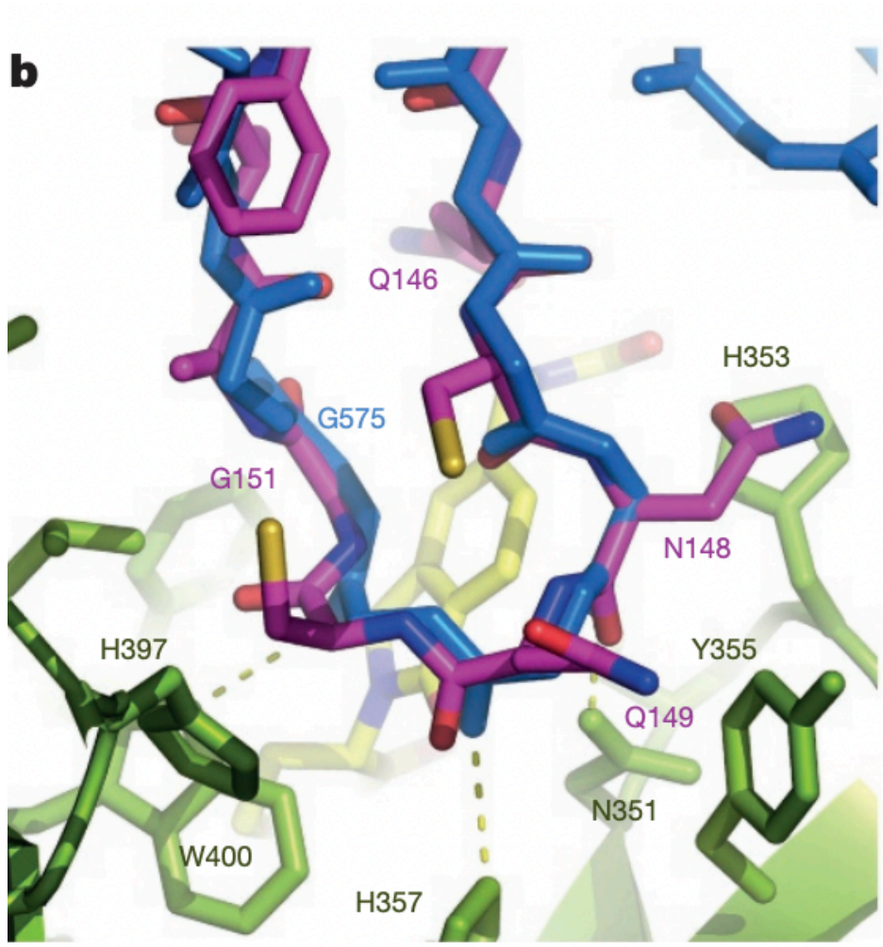
Interactions with W400, H357, N351 are key

Thoma/Chamberlain: *Nature* **2016**, 535, 252–257 and *Nature* **2016**, *532*, 127–130

Case Study: The Thalidomide Story - CRBN

Structural biology reveals the loop required for degradation

Overlay with Ikaros shows same loop.
Only homologous AA G141.



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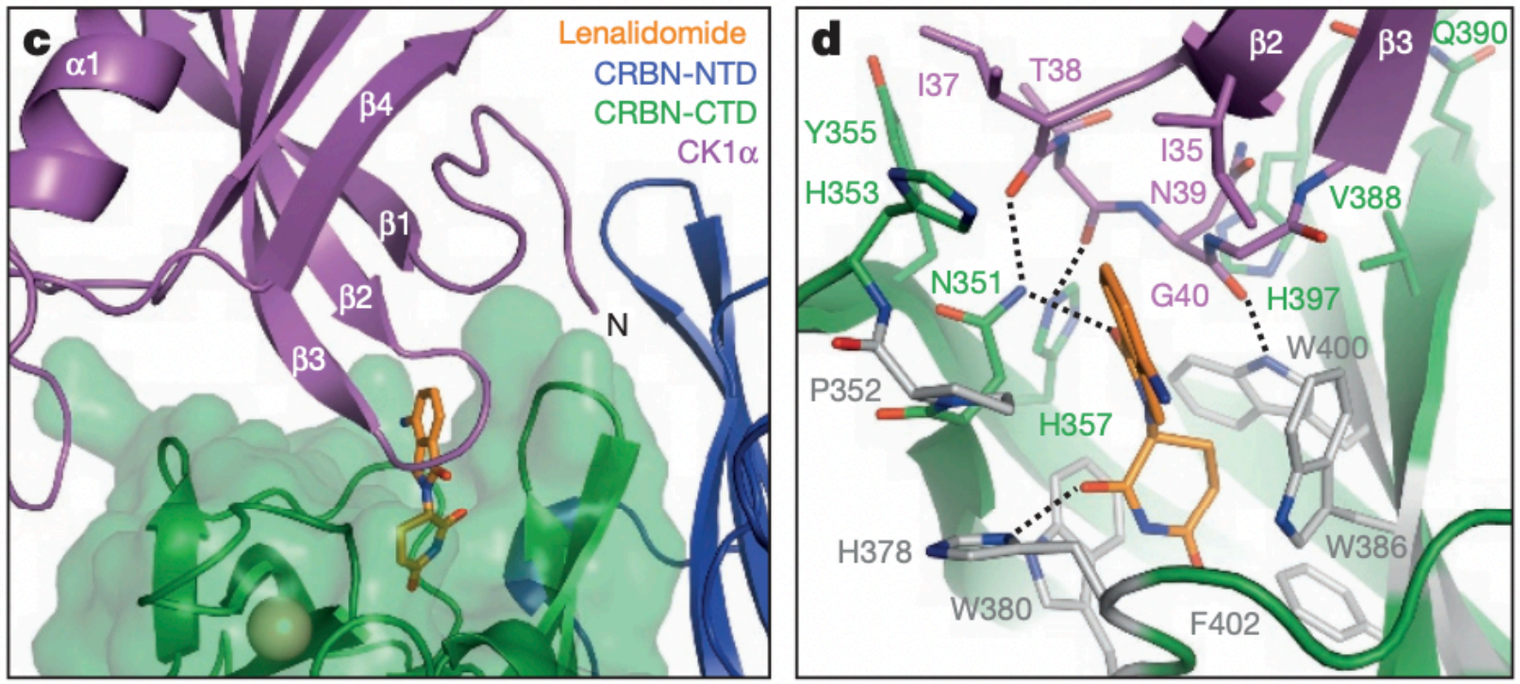

This degron hypothesis was further strengthened by the structure of CK1α with CRBN and lenalidomide

Thoma/Chamberlain: *Nature* **2016**, 535, 252–257 and *Nature* **2016**, *532*, 127–130

Case Study: The Thalidomide Story - CRBN

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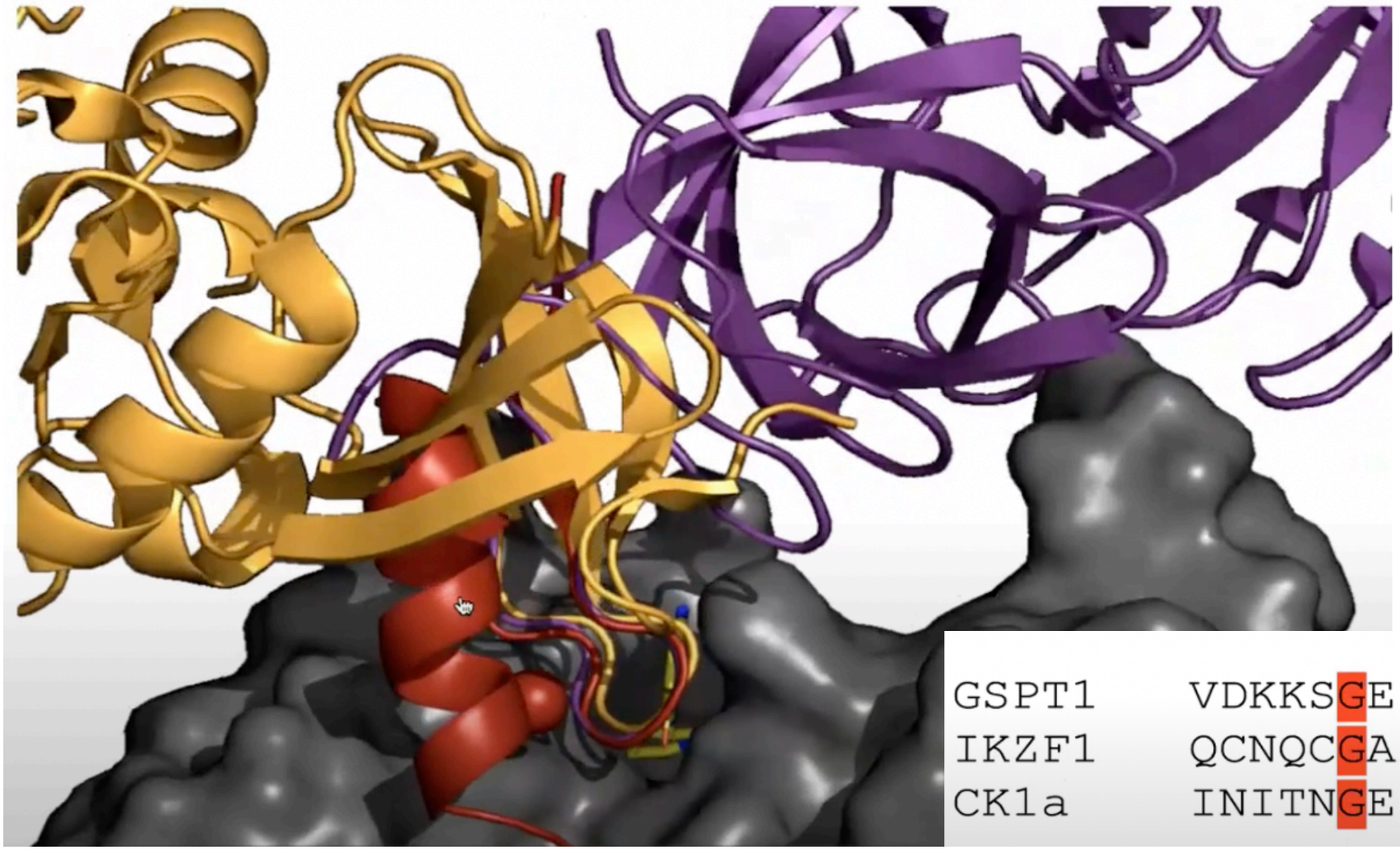


Thoma/Chamberlain: *Nature* **2016**, 535, 252–257 and *Nature* **2016**, *532*, 127–130

Case Study: The Thalidomide Story - CRBN

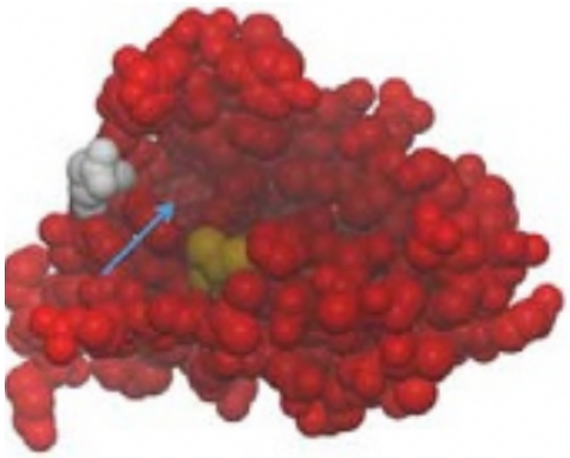
Back to the beginning - how does Thalidomide cause teratogenic birth defects?

Chamberlain: *Nat. Chem. Bio.* **2018**, 535, 252–257

Case Study: The Thalidomide Story - CRBN

Back to the beginning - how does Thalidomide cause teratogenic birth defects?

Mus musculus



Mouse CRBN

Mice are resistant to thalidomide teratogenic effects

Chamberlain: *Nat. Chem. Bio.* **2018**, 535, 252–257

Case Study: The Thalidomide Story - CRBN

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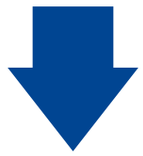

Mouse CRBN does not induce degradation or Ikaros etc with IMiDs

Chamberlain: *Nat. Chem. Bio.* **2018**, 535, 252–257

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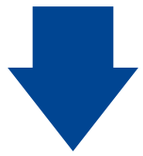

Mouse CRBN does not induce degradation or Ikaros etc with IMiDs

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Maybe Ikaros degradation is responsible?

Chamberlain: *Nat. Chem. Bio.* **2018**, 535, 252–257

Case Study: The Thalidomide Story - CRBN

Do humanized mice respond to thalidomide?

Mouse expressing hCRBN

hCRBN mouse displays degradation of Ikaros/Aiolos but remains resistant to teratogenicity

Ikaros is not the neosubstrate that leads to teratogenic effects!

Chamberlain: *Nat. Chem. Bio.* **2018**, 535, 252–257

Case Study: The Thalidomide Story - CRBN

SALL4 was considered as a possible target

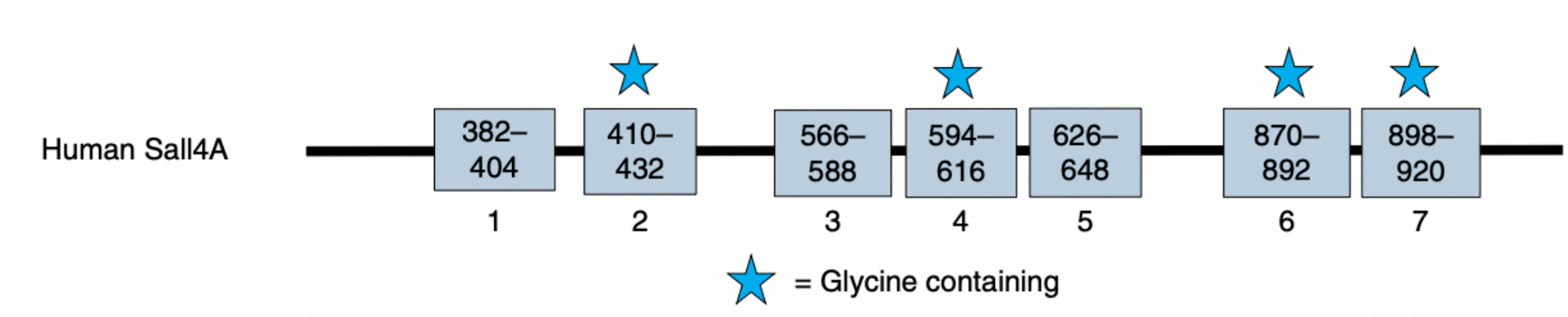
ORIGINAL ARTICLE

Mutations at the *SALL4* locus on chromosome 20 result in a range of clinically overlapping phenotypes, including Okihiro syndrome, Holt-Oram syndrome, acro-renal-ocular syndrome, and patients previously reported to represent thalidomide embryopathy

J Kohlhase, L Schubert, M Liebers, A Rauch, K Becker, S N Mohammed, R Newbury-Ecob, W Reardon

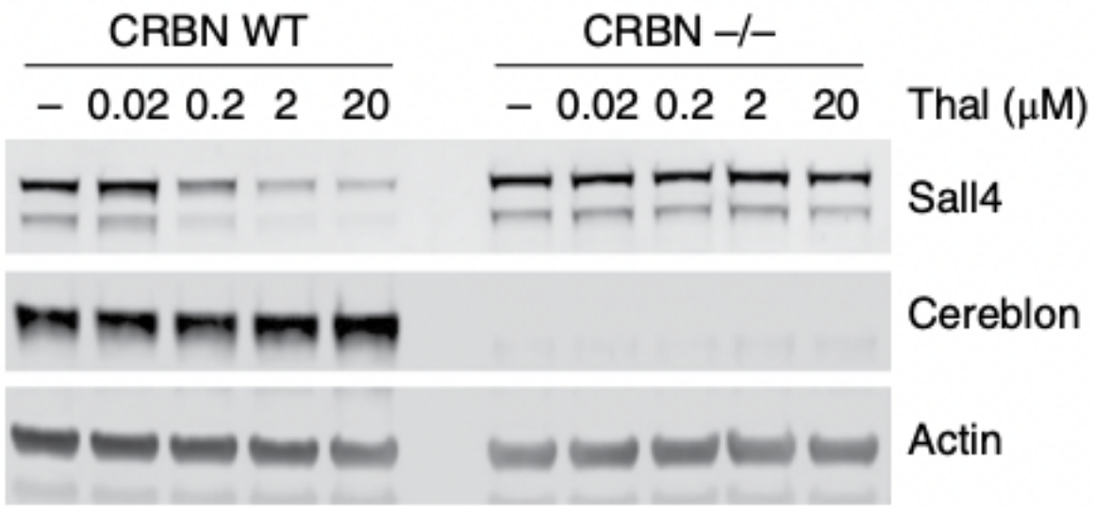
J Med Genet 2003;**40**:473–478

TF that displays correct degron motif



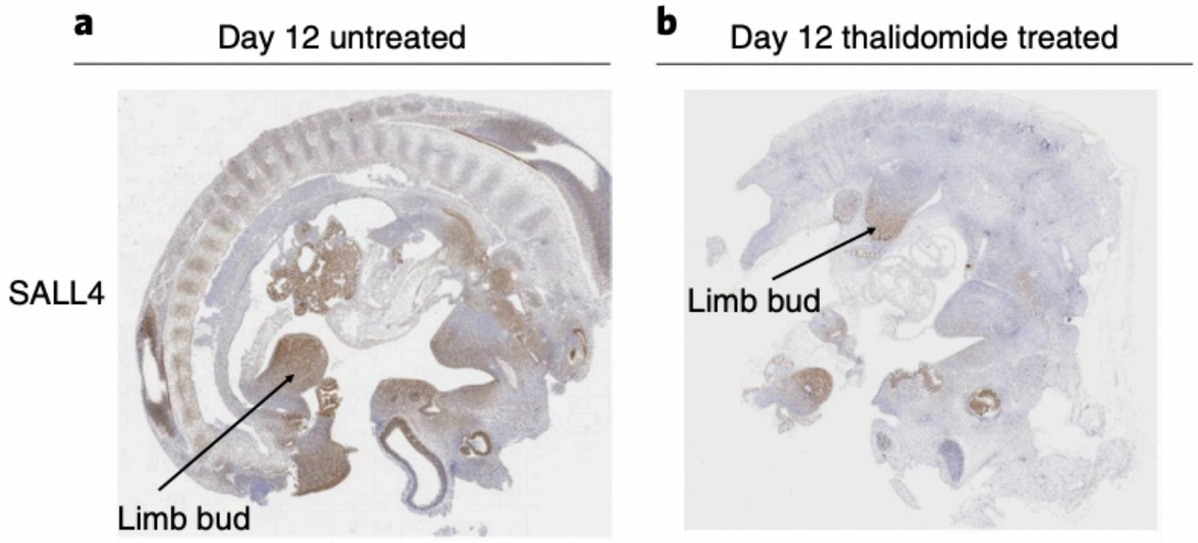
Chamberlain: *Nat. Chem. Bio.* **2018**, 535, 252–257

Case Study: The Thalidomide Story - CRBN



Thalidomide degrades SALL4 in a CRBN dependent manner

SALL4 localizes to limb buds decreases with thalidomide treatment

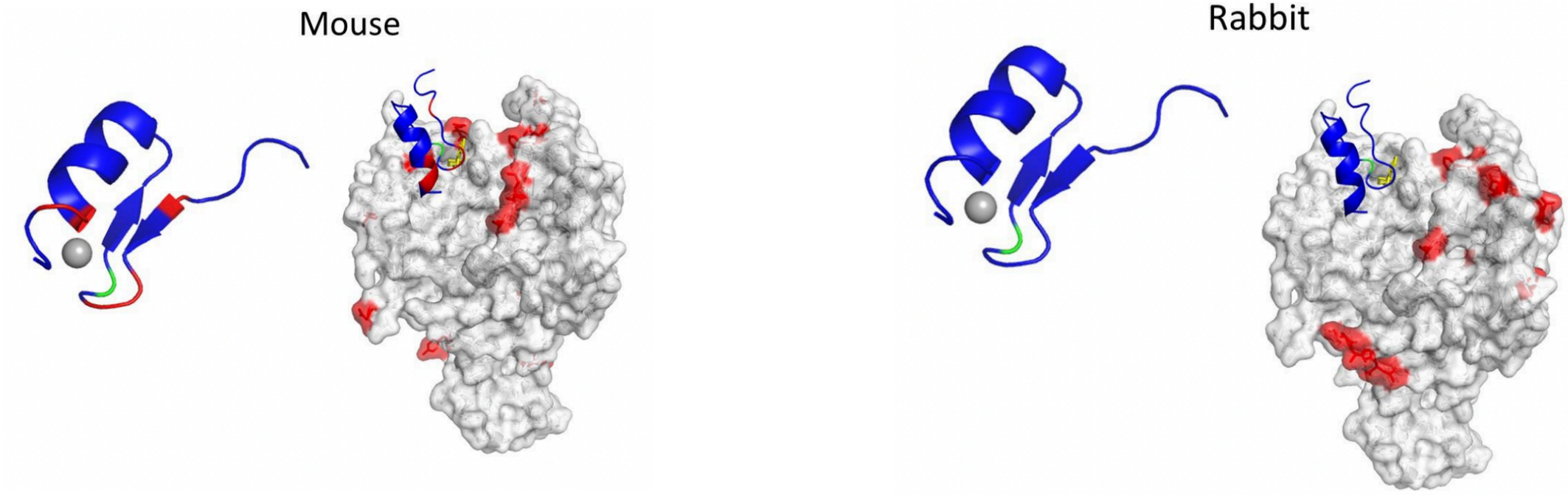


Chamberlain: *Nat. Chem. Bio.* **2018**, 535, 252–257

Case Study: The Thalidomide Story - CRBN

Mouse immunity can be explained by SALL4 sequence homology models

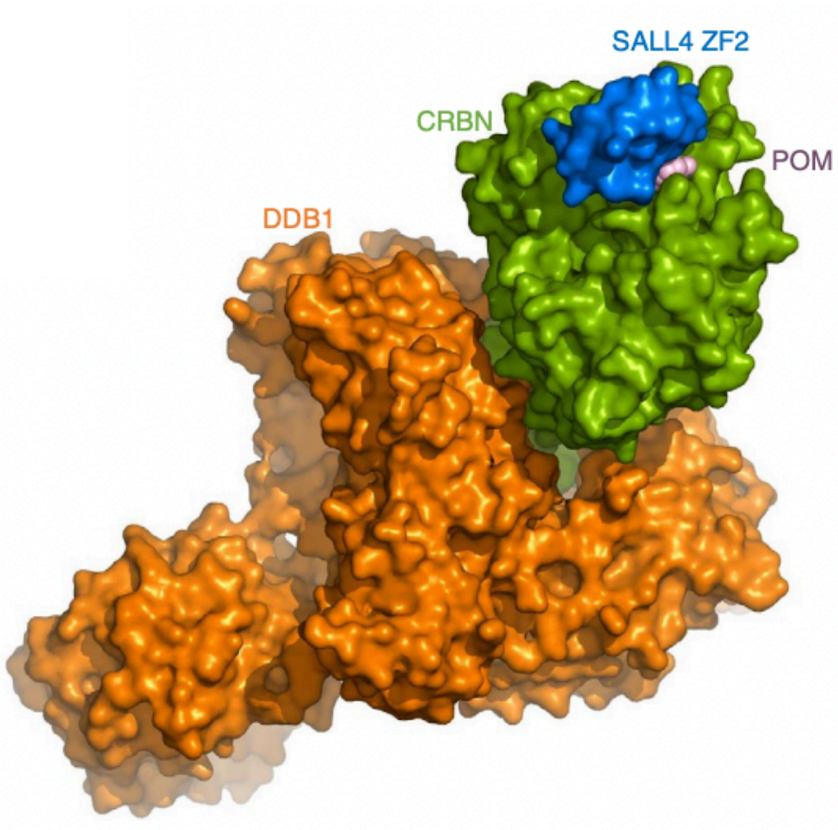
4 AA changes in ZF2

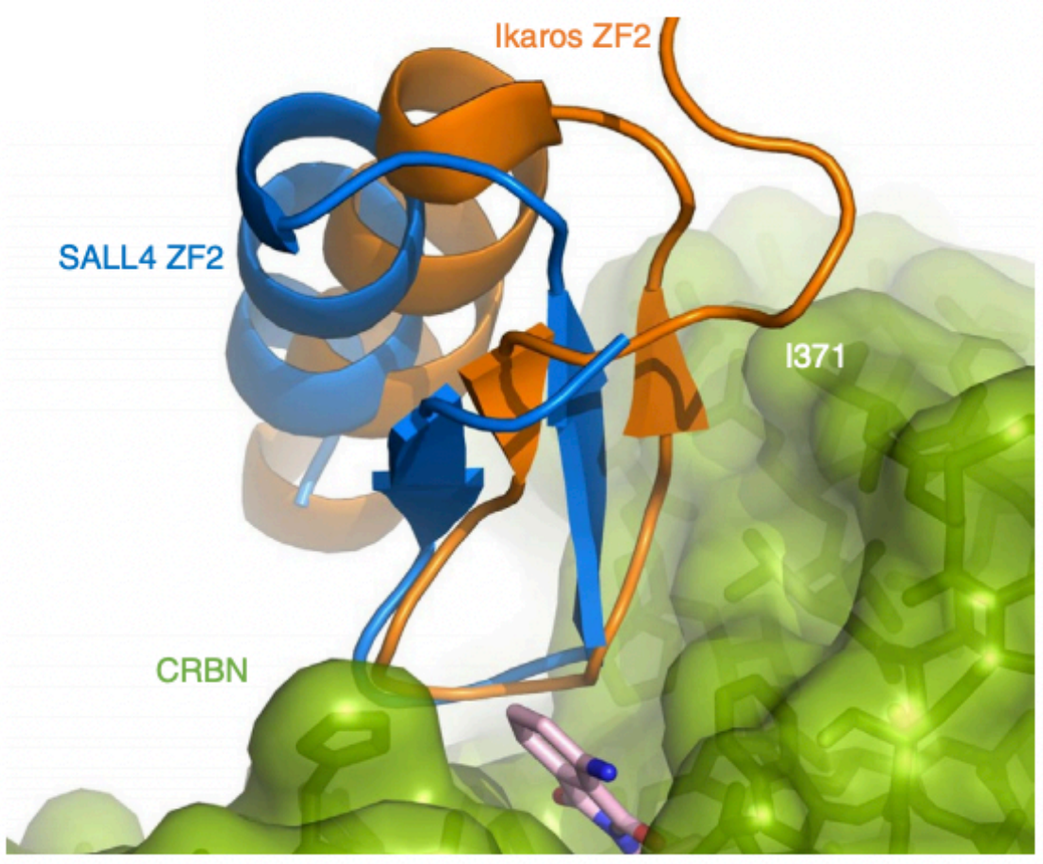


Chamberlain: *Nat. Chem. Bio.* **2018**, 535, 252–257

Case Study: The Thalidomide Story - CRBN

X-ray consistent with all of the work performed so-far





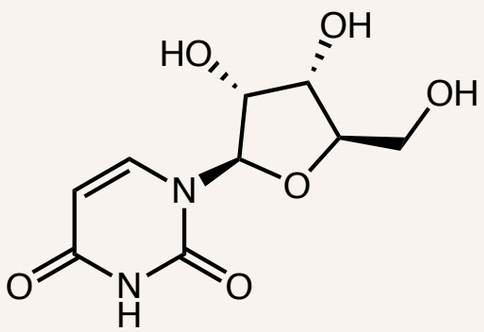
Nat. Struc. Mol. Bio. **2020**, 27, 319–322

Case Study: The Thalidomide Story - CRBN

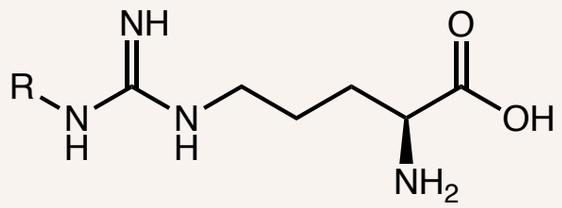
What’s coming next in the Thalidomide story?

Case Study: The Thalidomide Story - CRBN

Native ligands for CRBN?

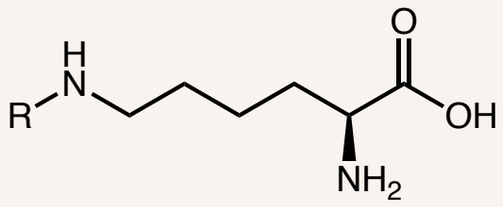


uridine



arginine

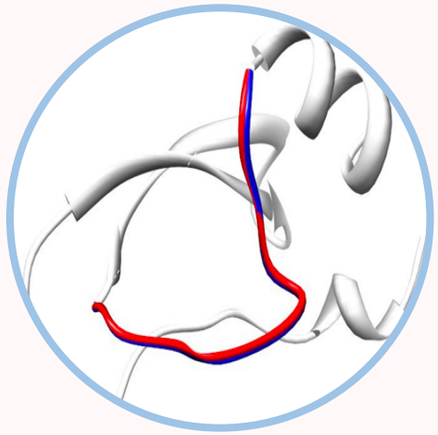
R = Me_n



lysine

R = Ac, Me_n

Other proteins containing degron



Methods for discovery of new glues

Science **2018**, 362, eaat0572

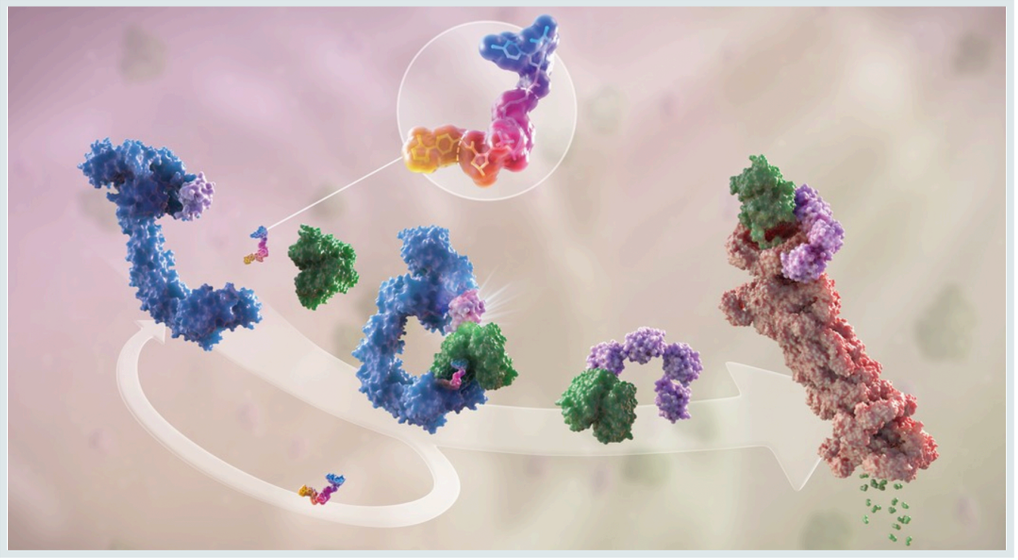
Case Study: The Thalidomide Story - CRBN

Thalidomide has changed the industry

Case Study: The Thalidomide Story - CRBN

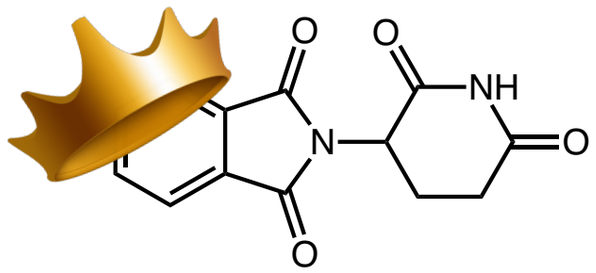
Clinical regulation changed overnight

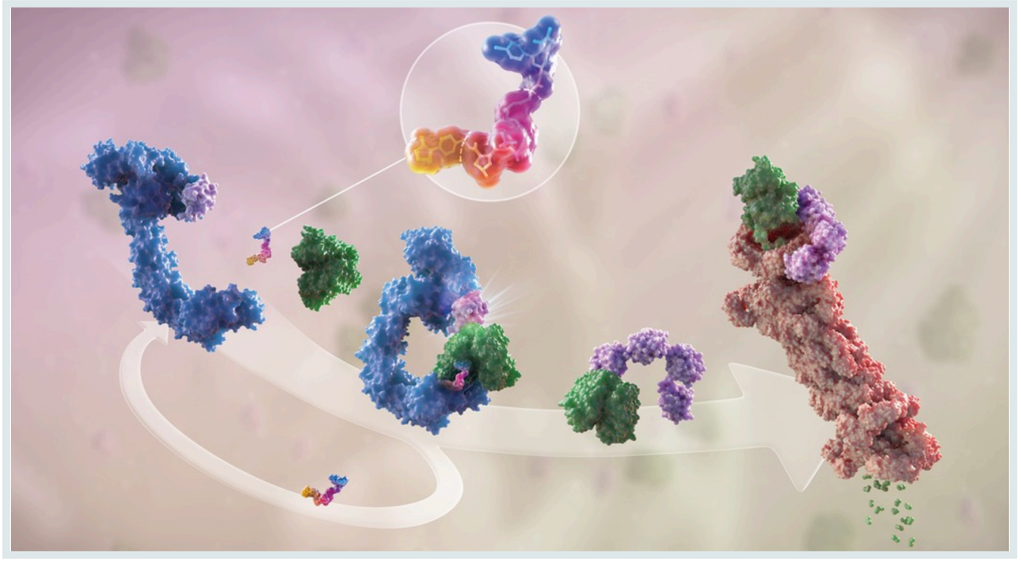
Thalidomide has changed the industry



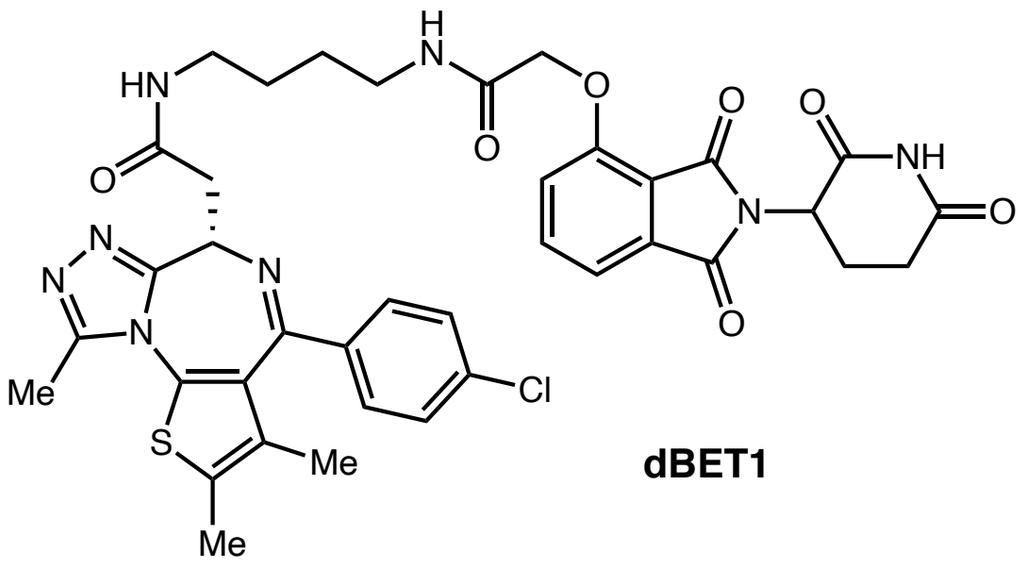
Case Study: The Thalidomide Story - CRBN

Clinical regulation changed overnight

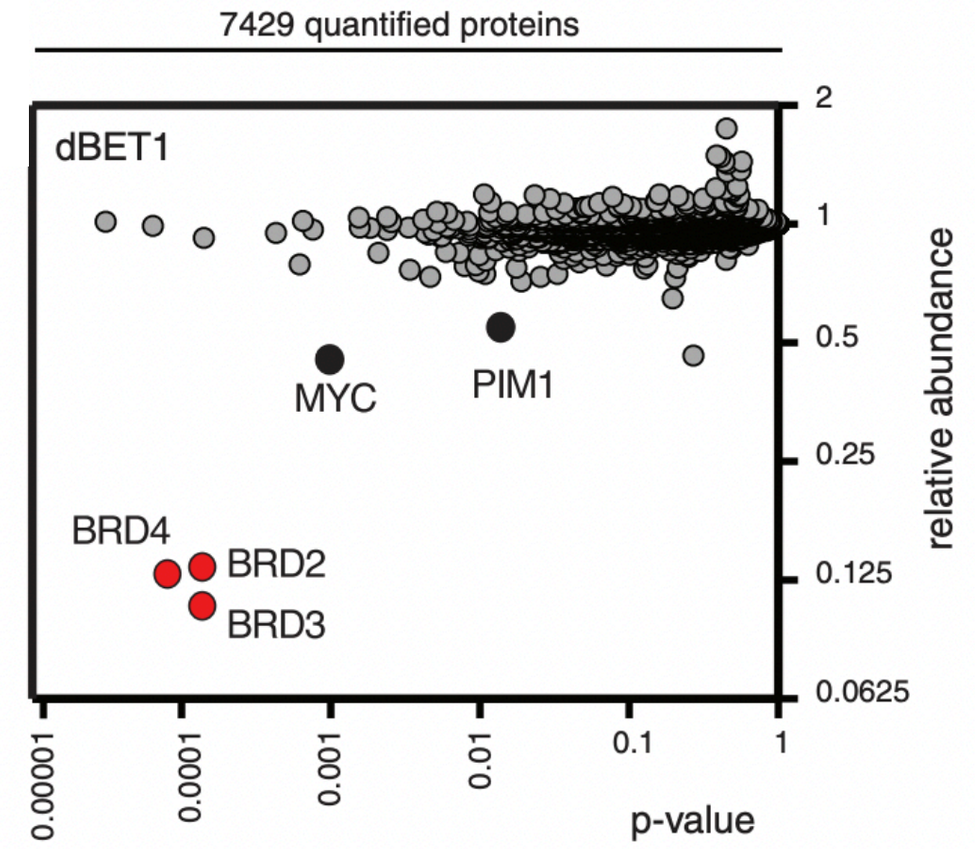




PROTACS



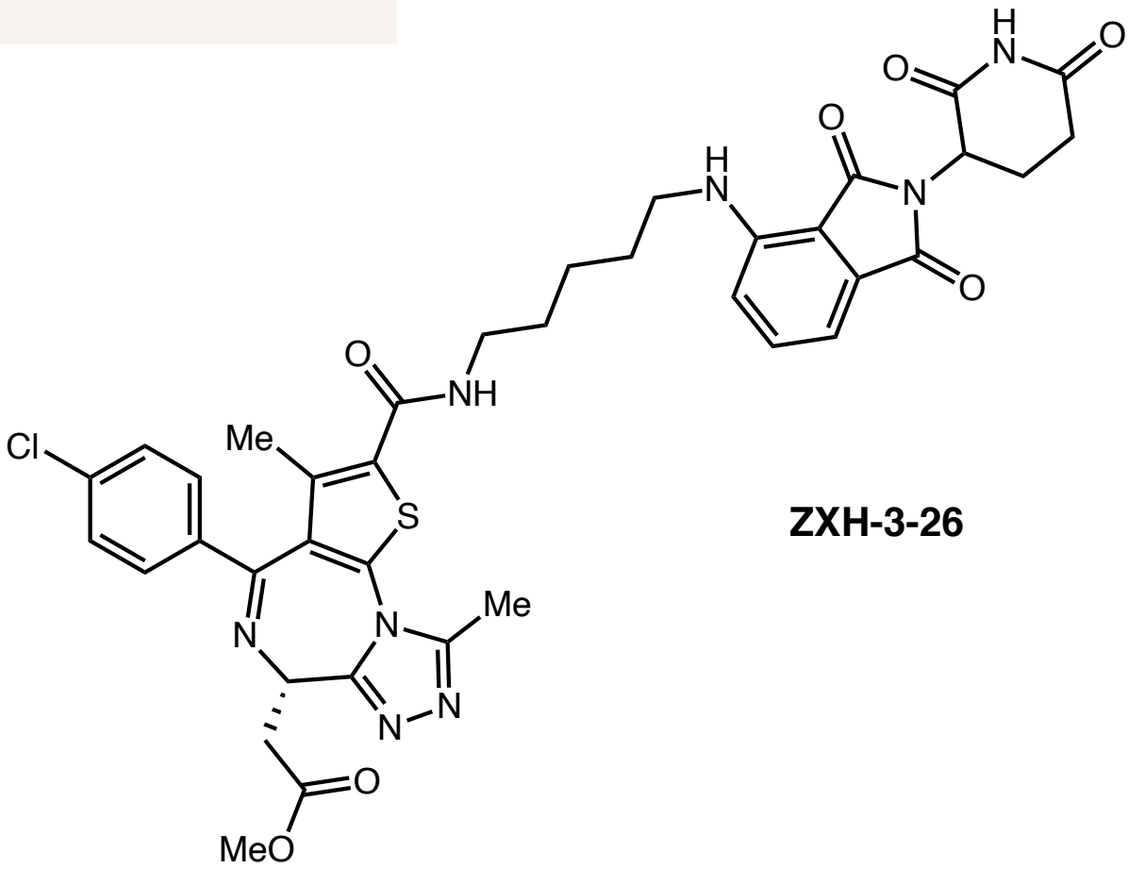
dBET1



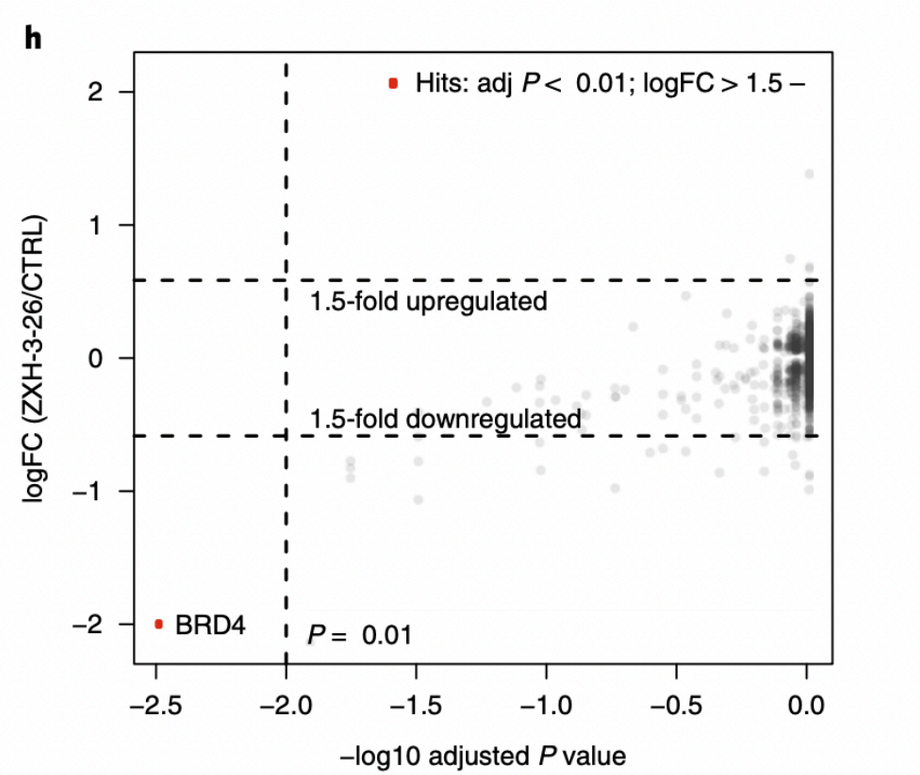
The Bradner lab describe the quintessential PROTAC

Science **2015**, *348*, 1376–1381.

PROTACS



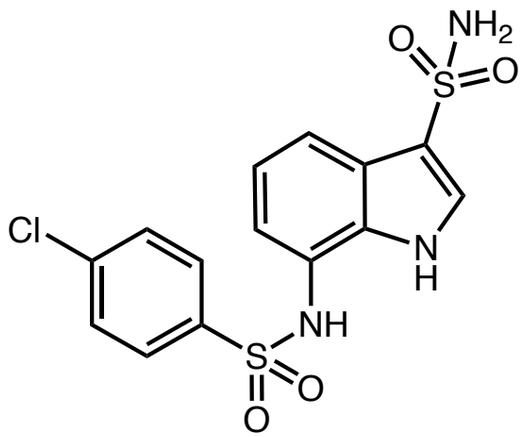
ZXH-3-26



Bradner and Fischer lab report the first BRD4 selective degrader

Nat. Chem. Bio. **2018**, *348*, 1376–1381.

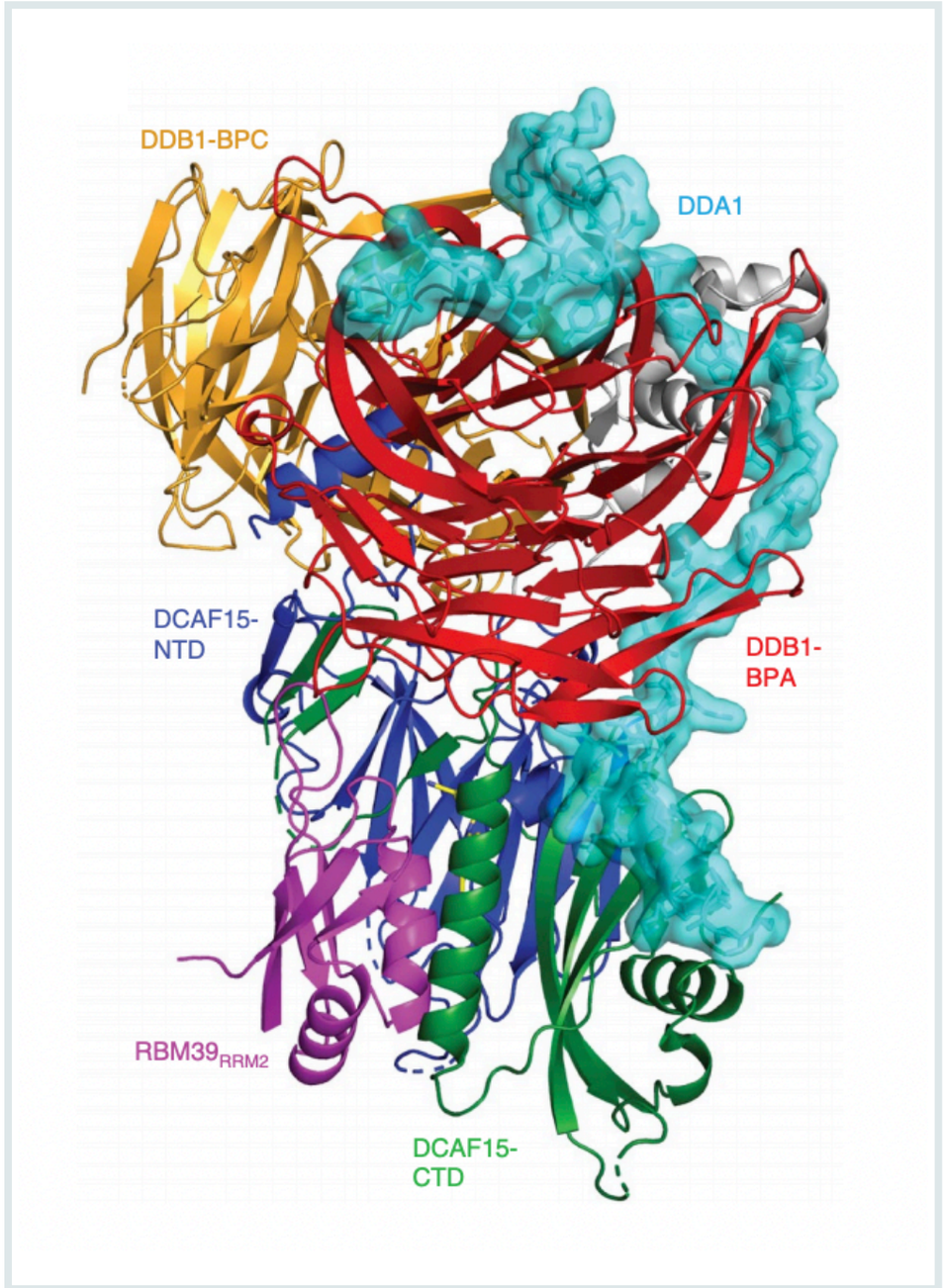
New E3 Ligases



indisulam

Indisulam recruits RBM39 to the DCAF15 E3 ligase complex

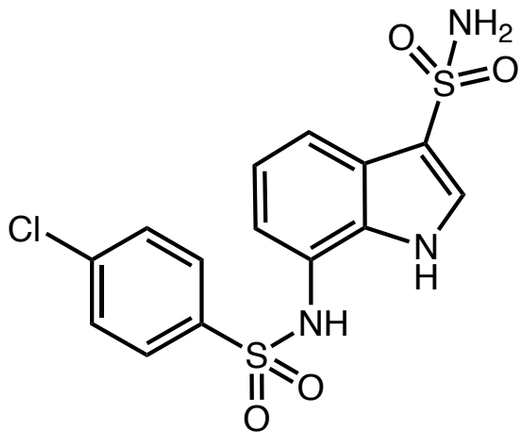
(In clinic vs AML and MDS)



Science **2017**, *356*, eaal3755; *Nat. Chem. Bio.* **2020**, *16*, 15–23; and *Nat. Chem. Bio.* **2020**, *16*, 7–14.

Highlights in Molecular Glue Research

New E3 Ligases



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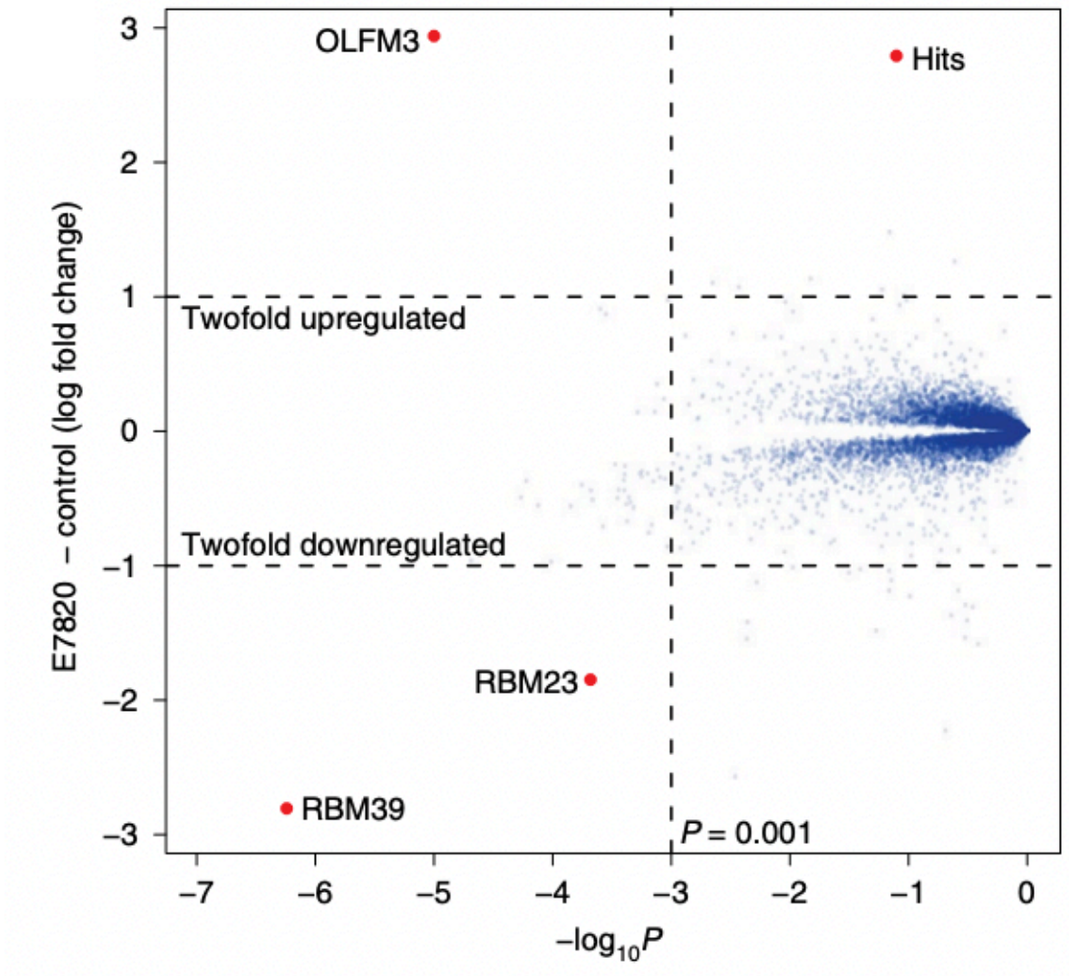
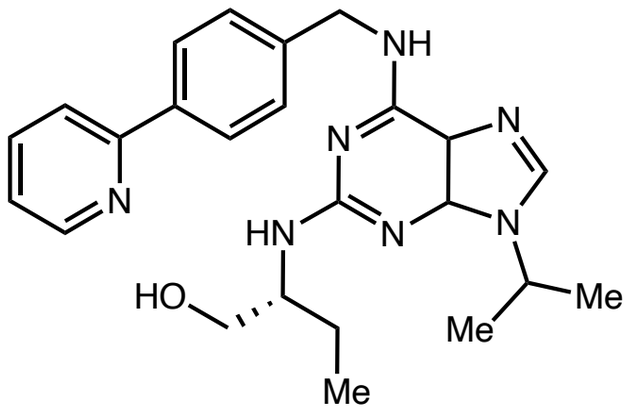
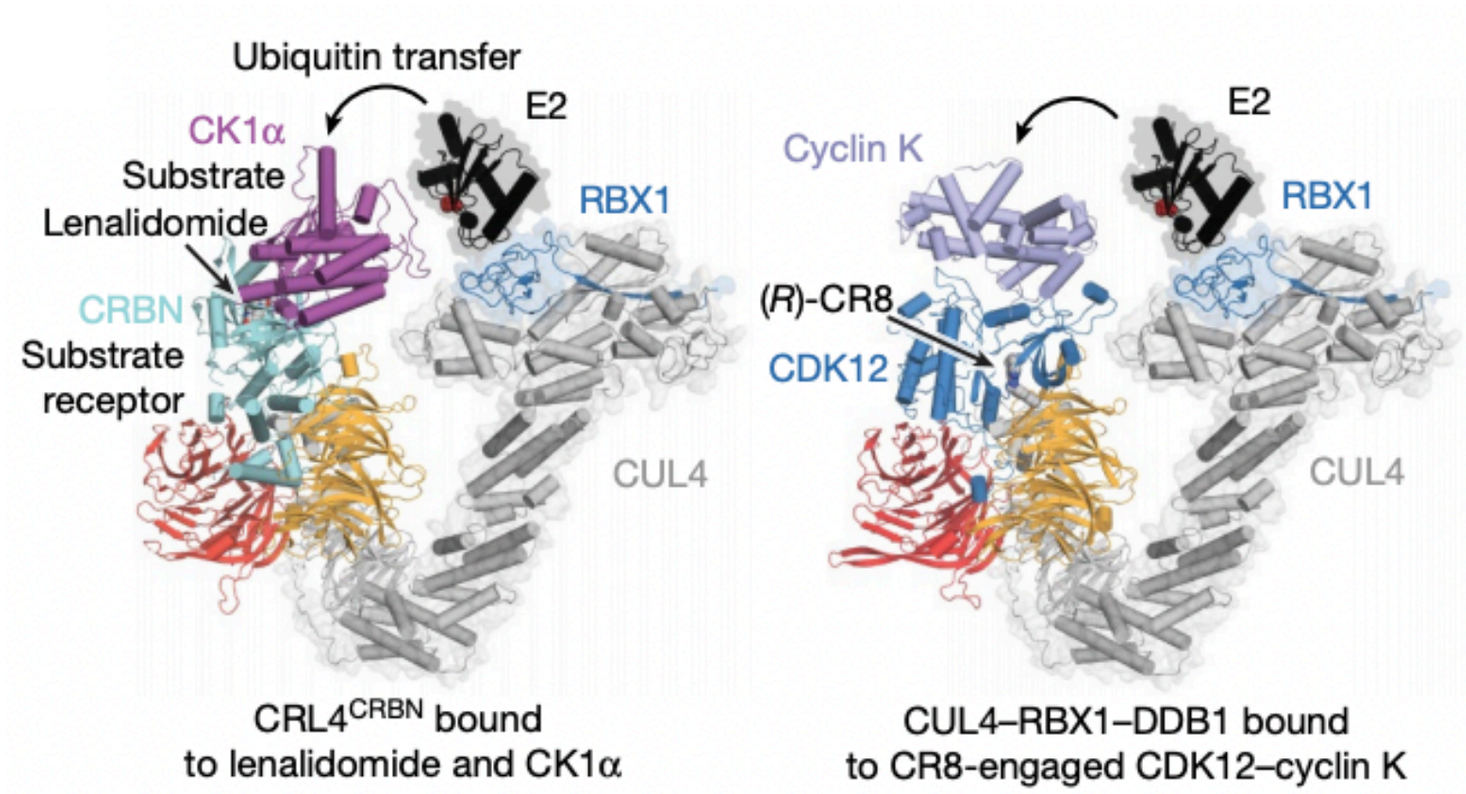


Science **2017**, *356*, eaal3755; *Nat. Chem. Bio.* **2020**, *16*, 15–23; and *Nat. Chem. Bio.* **2020**, *16*, 7–14.

New E3 Ligases



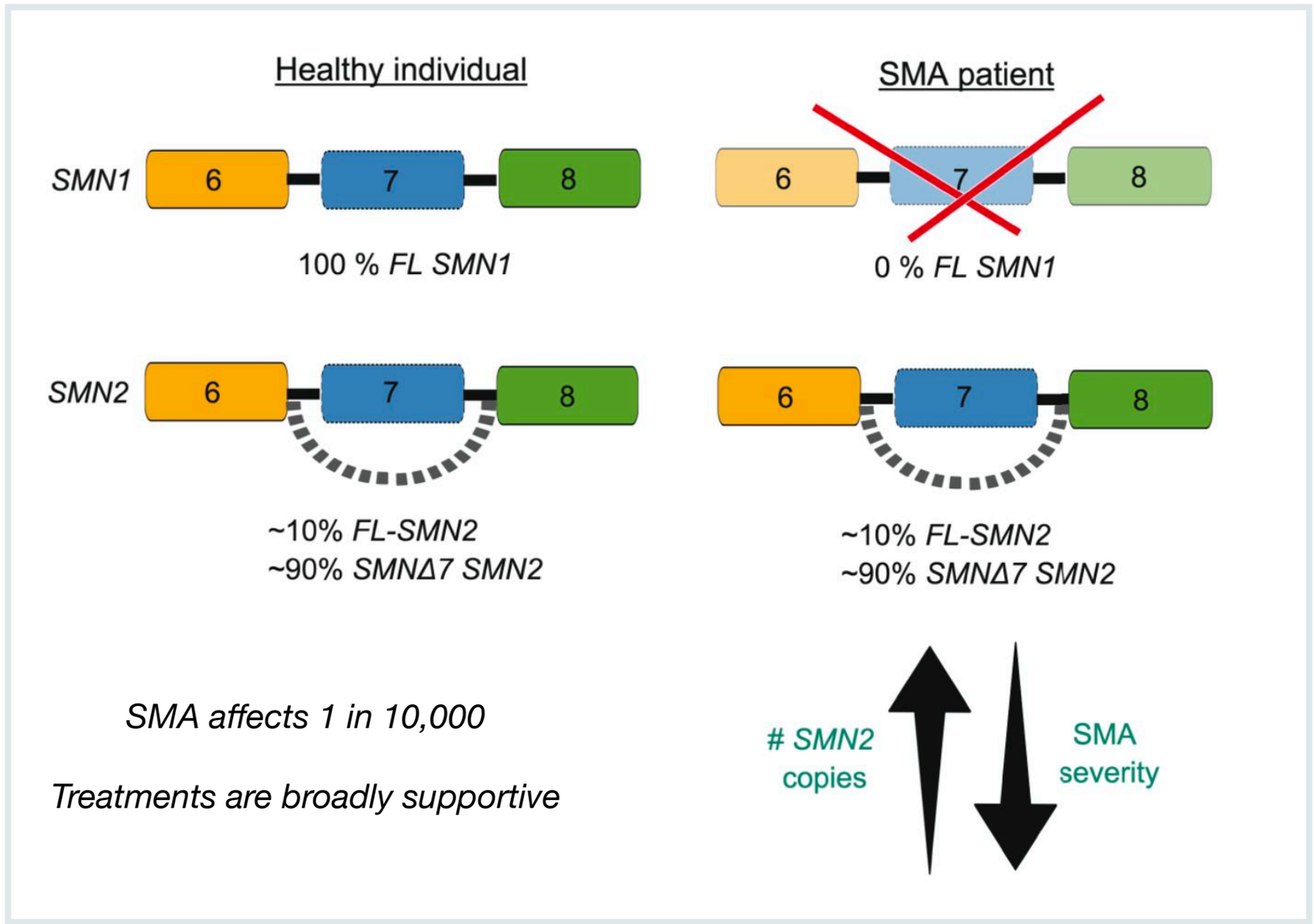
(R)-***CR8***



CR8 recruits CDK12/Cyclin K complex to CRL4 E3 ligase (without CRBN)

Nature **2020**, *585*, 293–297

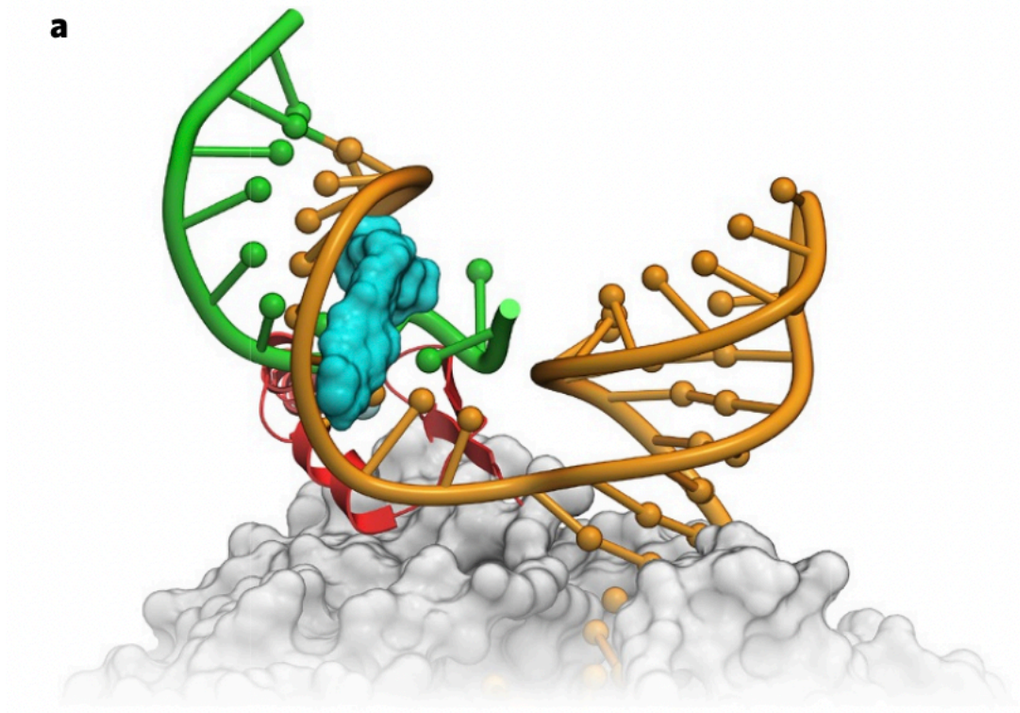
Protein-RNA Molecular Glues: A treatment for Spinal Muscular Atrophy

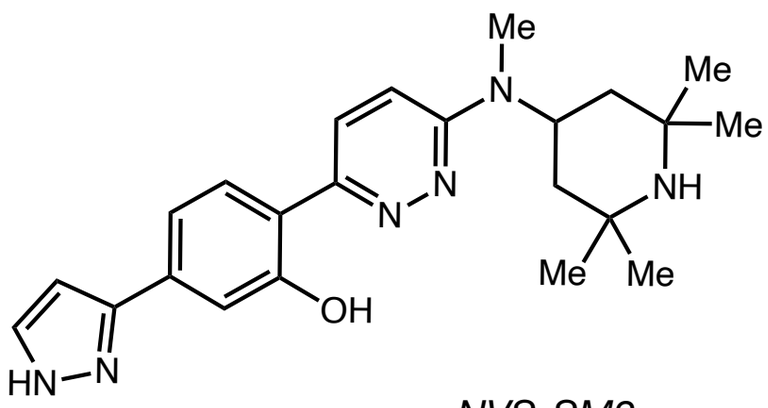


Nat. Chem. Bio. **2015**, *11*, 511–517

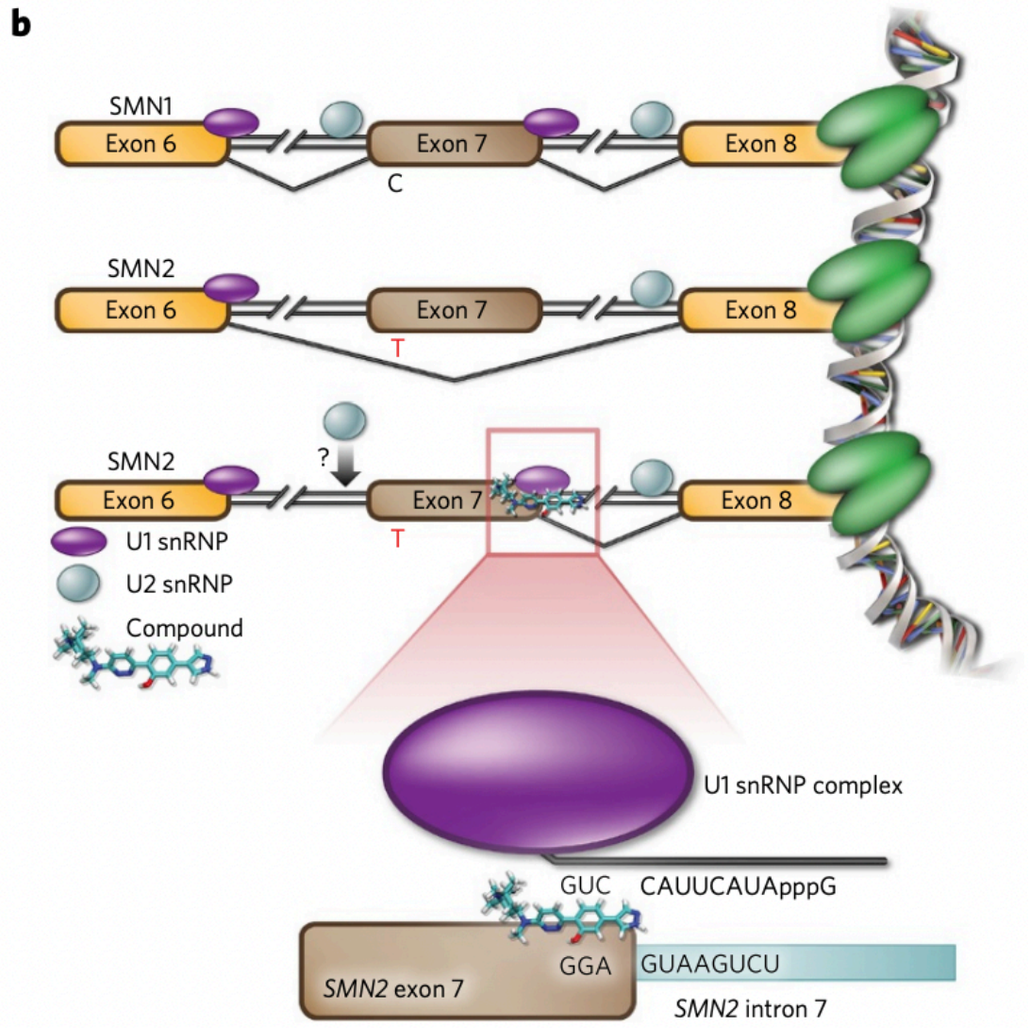
Highlights in Molecular Glue Research

Protein-RNA Molecular Glues: A treatment for Spinal Muscular Atrophy





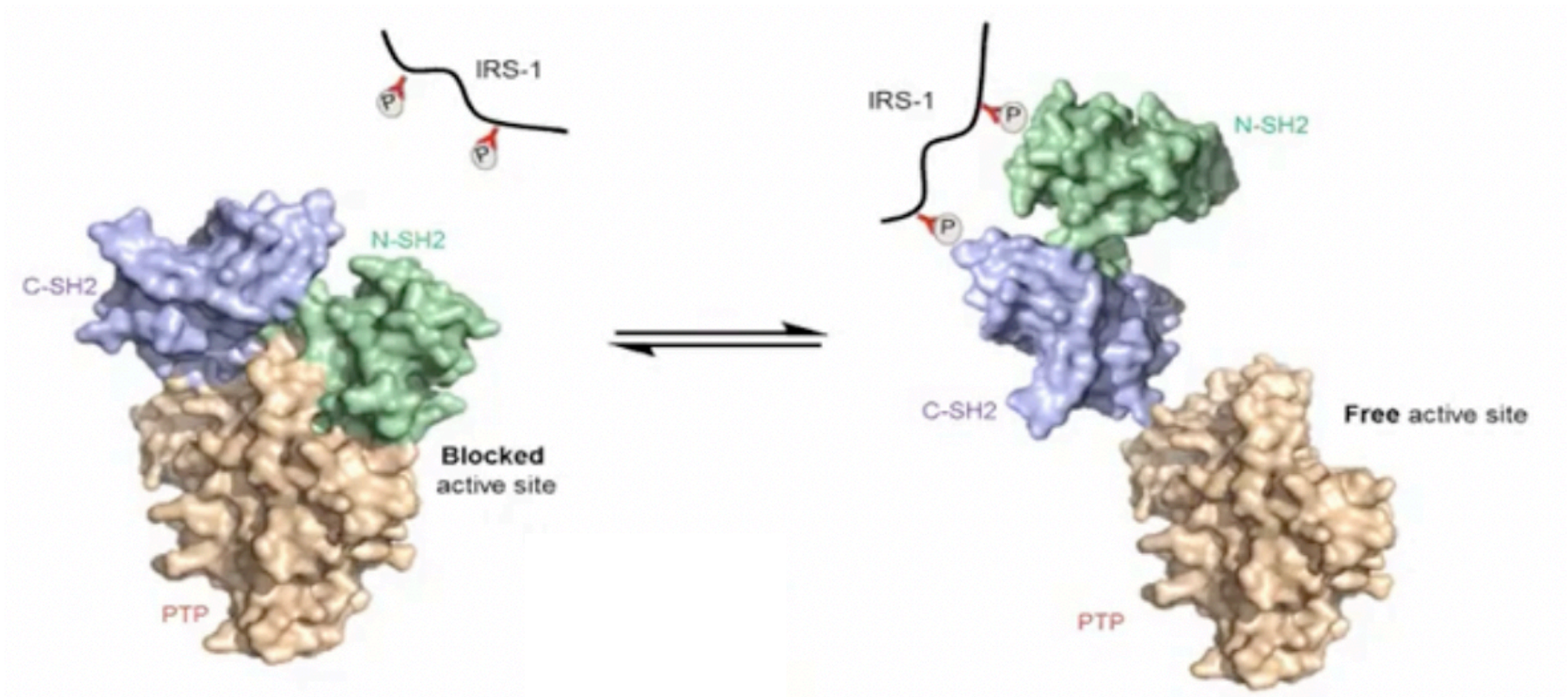
NVS-SM2



Nat. Chem. Bio. **2015**, *11*, 511–517

Highlights in Molecular Glue Research

Intramolecular Glues: SHP2



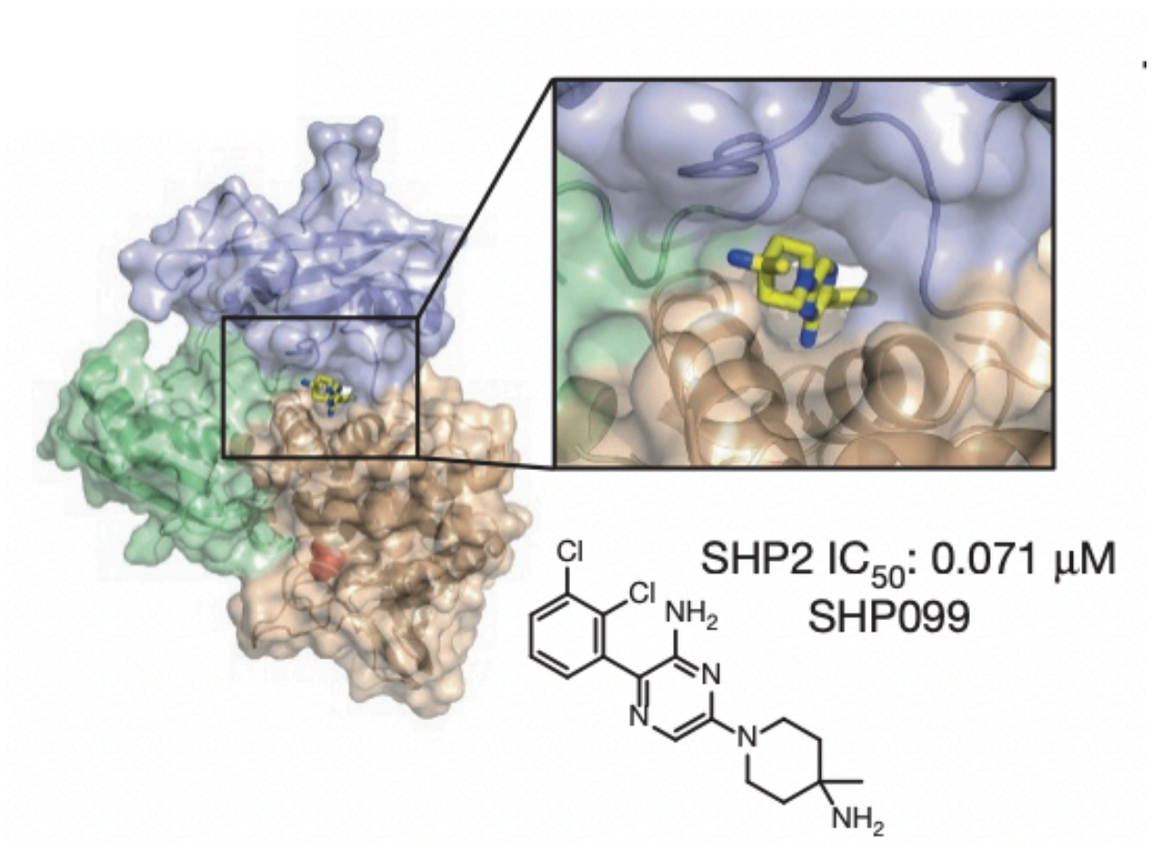
Auto-inhibited conformation

Open/active conformation

Phosphatases “undruggable targets” - anionic molecules make poor medicines

Nature **2016**, *535*, 148–152

Intramolecular Glues: SHP2



SHP099 locks SHP2 in closed conformation

First clinical phosphatase inhibitor

6 ongoing clinical trials for advanced solid tumors

Nature **2016**, *535*, 148–152

Conclusions

- This fast moving topic isn't finished, the best is yet to come
- Proteomics has been at the forefront of this research, new innovations will accelerate discovery
- Industry driven fundamental research
- Has the potential to unlock 1000's of "undruggable" targets

Resources

Schreiber Cell 2021

Dana-Farber Targeted Degradation Webinar Series: Bradner, Chamberlain, Koduri and others

novartis.com

Evolution of Cereblon-Mediated Protein Degradation as a Therapeutic Modality

ACS. Med. Chem. Lett. **2019**, *10*, 1592

Thanks for listening! Questions?